80. Methylated DNA: The Influence of 7-Deaza-7-methylguanine on the Structure and Stability of Oligonucleotides

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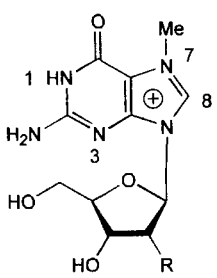
The 7-deaza-2′-deoxy-7-methylguanosine (**2b**) [9], which is the glycosylic-bond-stable, noncharged analogue of 2′-deoxy-7-methylguanosine (**1b**), was incorporated in DNA by solid-phase synthesis. As building blocks, the protected phosphonate **3a** and the phosphoramidite **3b** were prepared. The 7-methyl group of **2b** stabilizes the B-DNA duplex compared to 7-deaza-2′-deoxyguanosine but does not induce a B-Z transition as it is known from compound **1b**. The stabilization by the 7-deaza-7-methylguanine moiety is sequence-dependent, and the nearest-neighbor influence is different from that of 7-deazaguanine. Homooligonucleotides of **2b** show sigmoidal melting indicating a highly ordered single-stranded structure. In general, oligonucleotides containing **2b** are very stable against hydrolysis with calf-spleen phosphodiesterase (CS-PDE, 5′ → 3′ exonuclease), while phosphodiester hydrolysis with snake-venom phosphodiesterase (SV-PDE, 3′ → 5′ exonuclease) is only slightly reduced.

Introduction. – 7-Methylguanine is an integral part of the cap structure of messenger RNA necessary for the attachment of the nucleic acids to the ribosome [1]. In DNA, it is formed by exo- and endogenous methylating agents [2]. A B–Z transition is induced when the guanine residues of poly d(G–C) become methylated [3]. The 2′-deoxy-7-methylguanosine (**1b**) is a labile nucleoside. The 7-methyl group generates a positive charge on the imidazole moiety making the molecule sensitive to *N*-glycosylic bond hydrolysis even under neutral conditions [3–5]. This is used in the *Maxam-Gilbert* sequencing [6]. The instability of m^7G_d precludes its use in chemical oligonucleotide synthesis. Recently, incorporation of a single 2′-deoxy-7-methylguanosine residue in the *Dickerson-Drew* dodecamer was reported using the nucleoside triphosphate and performing a combination of enzymatic reactions [7] [8].

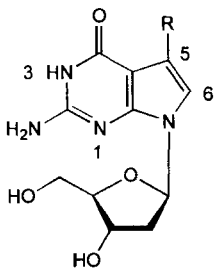
From model building of a B-DNA duplex, it is apparent that the 7-substituent of a purine base located in the major groove of the nucleic acid has steric freedom, similar to that of the Me substituent in thymine. Furthermore, the Me group hydrophobizes the major groove of the DNA molecule, thereby acting oppositely to the positive charge of 7-methylguanine which increases hydration. The guanine and the 7-deazaguanine heterocycles are isosteric, and the latter stays uncharged if methylated at position 7. The 7-deaza-2′-deoxyguanosine (**2a**) as well as its 7-methyl derivative **2b** have been synthesized previously [9] [10]. It has been shown earlier that 7-bromo or 7-iodo substituents in 7-deaza-2′-deoxyguanosine derivatives stabilize the DNA duplex structure [11]. Recently, it was observed that the same is true for oligonucleotides containing 7-halogeno-substituted 8-aza-7-deaza-2′-deoxyguanosine residues. The T_m values of $d(Br^7c^7z^8G–C)_4$ (T_m 88°) and $d(I^7c^7z^8G–C)_4$ (T_m 84°) are significantly higher than that of $d(c^7z^8G–C)_4$

(T_m 72°) and much higher than that of the parent d(G–C)$_4$ (T_m 61°) [12]. Similar findings have been observed on oligonucleotides containing 7-substituted 7-deazaadenine residues [13]. Moreover, the X-ray structure of 7-deaza-2′-deoxy-7-iodoadenosine shows that the sugar residue prefers the *anti* conformation, similar to regular purine nucleosides, and does not form *syn*-orientation as induced by bulky 8-substituents [14].



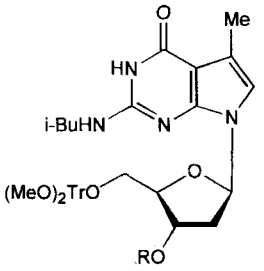
1a R = OH
b R = H

Purine numbering



2a R = H
b R = Me

Systematic numbering



3a R = P(O)HO$^-$ Et_3NH^+

b R = P[(i-Pr)$_2$N](OCH$_2$CH$_2$CN)

c R = COCH$_2$CH$_2$CONH 

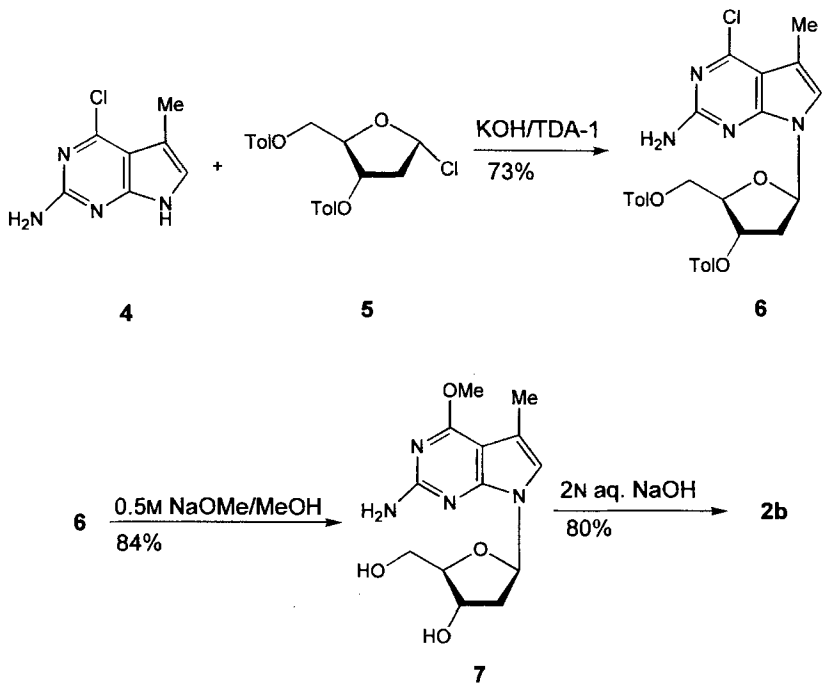
i-Bu = $Me_2CHC(O)$, $(MeO)_2Tr$ = 4,4′-dimethoxytriphenylmethyl

The steric effect of a purine 7-Me group on the properties of the DNA can be studied separately from charge phenomena when 7-deaza-7-methylguanine is replacing guanine within an oligonucleotide. In the following, the synthesis of oligonucleotides containing 7-deaza-2′-deoxy-7-methylguanosine (**2b**) residues will be described, and the base pairing as well as the thermodynamic stability of duplexes will be studied. Furthermore, the exonuclease degradation of oligonucleotides containing **2b** will be compared with that of the parent oligomers containing non-methylated 7-deaza-2′-deoxyguanosine (**2a**) as well as of those containing 2′-deoxyguanosine. Finally, the single-stranded structure of oligonucleotides containing consecutive 7-deaza-2′-deoxyguanosine or 7-deaza-2′-deoxy-7-methylguanine residues will be discussed.

Results and Discussion. – *Monomers.* Earlier, 7-deaza-2′-deoxy-7-methylguanosine (**2b**) was synthesized by nucleobase-anion glycosylation using liquid-liquid phase-transfer glycosylation [9]. As the total yield of the protocol was only 22% based on the starting material 4-methoxy-5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine, the 4-chloro-5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (**4**) was used, and the conditions were changed. The nucleobase-anion glycosylation of compound **4** was performed using the halogenose **5** and employing solid-liquid conditions (MeCN, KOH, and tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1)) [15] (*Scheme 1*). The glycosylation proceeded stereoselectively, and the pure β-D-isomer **6** was isolated in 73% yield which is significantly higher for the β-D-nucleoside than described before (α-D isomer 47%, β-D-anomer 40%) [9]. Compound **6** was treated with 0.5M NaOMe in MeOH to give the methoxy compound **7** (84% yield). The latter was hydrolyzed with 2N aqueous NaOH furnishing the nucleoside

2b in 80% yield compared to 60% yield by using $AlCl_3$/DMF [9]. The total yield of the reaction sequence was 48% based on compound **4** as starting material.

Scheme 1



When *H*–C(8) of compound **2b** was saturated in a ^{1}H-NMR experiment, a NOE of 1.7% was observed on *H*–C(1′) (see *Table 1*). From this value, the *syn*/*anti* ratio was determined using a graph which was recently published [16]. The population of the *anti*-conformer was found to be 86% which is significantly higher than that for 2′-deoxyguanosine (70% *anti*) [17]. Unfortunately, we were not able to determine the *syn*/*anti* ratio of 7-deaza-2′-deoxyguanosine (**2a**) due to an overlap of the signals of H–C(7) and H–C(1′). We also examined the sugar puckering on the basis of vicinal ^{1}H,^{1}H-coupling constants using the PSEUROT program (version 6.2) [18]. From these data, the N/S ratio was found to be 28:72 for compound **2b**, which was the same as found for **2a**. Also the pK_a values were measured. For compound **2b**, pK values of 1.3 and 10.8 were determined which were slightly different to those of **2a** (pK = 1.1 and 10.3). The site of protonation is expected to be N(3) (purine numbering), as this position was identified to be the protonation site of compound **2a** [19]. This is different to 2′-deoxyguanosine which shows pK values of 1.6 and 9.2 with N(7) as protonation site.

For the solid-phase oligonucleotide synthesis, the phosphonate **3a**, the phosphoramidite **3b** as well as silica-bound **3c** were prepared. For this purpose, compound **2b** was blocked at the 2-NH_2 group with an isobutyryl (i-Bu) residue using the protocol of transient protection [20]: the derivative **8** was obtained crystalline (89%) (*Scheme 2*). The

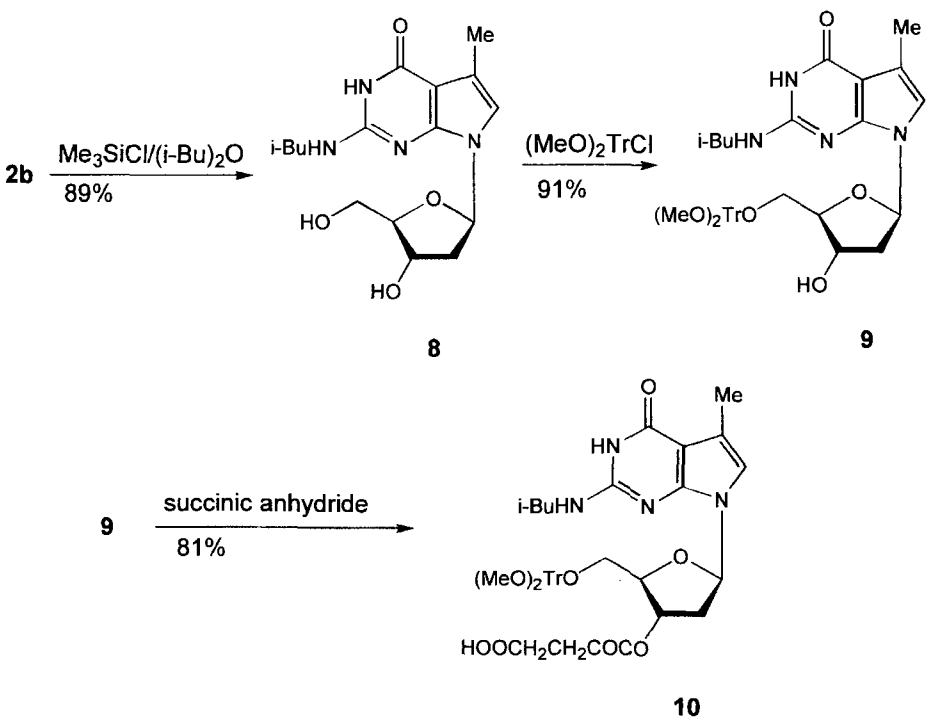
Table 1. *^{1}H-NMR NOE Data of Compounds* **2a, b**[a])[b])

	Proton irradiated	NOE
c^7G_d (**2a**)	H–C(8)	H–C(1') and H–C(7) (11.9%), H_β–C(2') (4.1%), H–C(5') (1.2%), H–C(3') (1.0%)
$m^7c^7G_d$ (**2b**)	H–C(8)	H–C(1') (1.7%), H_β–C(2') (5.9%), H–C(5') (1.2%), H–C(3') (1.4%), OH–C(5') (1.0%), OH–C(3') (0.7%), CH_3 (4.1%)

[a]) Taken from spectra measured in (D_6)DMSO at 23°.
[b]) Purine numbering.

stability of the protecting group was determined UV-spectrophotometrically by hydrolysis in 25% aqueous NH_3 solution at 40°. The half-life of the deprotection was found to be 93 min for **8** compared to 109 min for isobutyrylated **2a** [11]. Next, the 4,4'-dimethoxytriphenylmethyl ($(MeO)_2Tr$) group was introduced at the 5'-OH position using the standard protocol which furnished **9** in 91% yield. This derivative was converted into the phosphonate **3a** (PCl_3/*N*-methylmorpholine/1*H*-1,2,4-triazole; 72% yield) as well as into the phosphoramidite **3b** (chloro(2-cyanoethoxy)(diisopropylamino)phosphane; 85% yield). Succinylation of **9** gave **10** (81% yield), which was activated to the 4-nitrophenyl

Scheme 2



i-Bu = $Me_2CHC(O)$, $(MeO)_2Tr$ = 4,4'-dimethoxytriphenylmethyl

ester with dicyclohexylcarbodiimid (DCC) and linked to amino-functionalized *Fractosil* furnishing the polymer support **3c**. The ligand concentration is 70 μmol/g calculated according to [21]. The monomeric compounds described above were characterized by ^{1}H-, ^{31}P-, and ^{13}C-NMR (*Table 2*) spectra as well as by elemental analyses.

Table 2. *^{13}C-NMR Chemical Shifts of Pyrrolo[2,3-d]pyrimidine 2'-Deoxyriboucleosides*[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	Me_2CHCO
2a	152.5	158.5	100.0	102.1	116.7	150.5		
2b	152.5	159.2	99.5	113.8	113.6	150.6	11.3	
3a	146.7	157.2	103.3	114.6	116.2	147.5	11.0	179.8
4	151.0[d])	159.1[d])	108.0	108.7	120.4	155.0	11.4	
6	151.6[d])	159.3[d])	108.4	110.8	119.7	154.6	11.5	
7	159.1	163.6	97.7	109.9	116.2	154.2	11.4	
8	146.7	157.3	103.4	114.6	116.5	147.5	11.2	180.0
9	146.7	157.3	103.5	114.6	116.4	147.5	11.1	180.0
10	146.8	157.1	103.6	114.7	116.1	147.7	10.9	

	C(1')	C(2')	C(3')	C(4')	C(5')	Me_2CH	CO
2a	82.2	–	70.8	86.9	61.9		
2b	81.8	39.2	71.0	86.2	62.1		
3a	82.3	–	73.0	85.6	63.9	55.0	
6	82.3	35.8	75.3	81.1	64.3		
7	81.9	39.7	70.9	86.6	62.0		
8	82.2	–	71.0	87.1	62.1		
9	82.3	–	70.8	85.3	64.3	55.1	
10	82.8	34.7	74.9	85.7	64.0	55.0	171.8, 173.4, 179.8

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

In the ^{13}C-NMR spectra, a 10-ppm downfield shift of C(7) is observed for the methyl compound **2b** compared to **2a**. A similar shift is observed for **6** and **7** when compared to the non-methylated compounds [10]. Generally, gated-decoupled spectra were used for the unambiguous assignment of the ^{13}C-NMR chemical shifts.

Oligonucleotides. Solid-phase oligonucleotide synthesis was performed on an automated synthesizer using H-phosphonate as well as phosphoramidite chemistry [22] [23]. The oligonucleotides were purified on oligonucleotide-purification cartridges from *Ap-*

Table 3. *T_m Values, Thermodynamic Data, and DNA Structure of Self-complementary Oligonucleotides* **11**–**15**

	T_m [°][a])	ΔH [kcal/mol]	ΔS [cal/K · mol]	Structure (0.01M and 4M NaCl)
d(G–C)$_4$ (**11**)	61	–82	–247	B-DNA
d(c^{7}G–C)$_4$ (**12**)	53	–62	–190	B-DNA
d(m^{7}c^{7}G–C)$_4$ (**13**)	58	–82	–250	B-DNA
d(C–G)$_4$ (**14**)	59	–84	–251	B/Z-DNA
d(C–m^{7}c^{7}G)$_4$ (**15**)	56	–81	–247	B-DNA

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

Table 4. T_m *Values, Thermodynamic Data, and DNA Structure of Non-self-complementary Oligonucleotides*[a])

	T_m [°]	ΔH [kcal/mol]	ΔS [cal/K · mol]	Structure
d(G–T)$_4$ · d(A-C)$_4$ (**16** · **17**)	38	–62	–231	B-DNA
d(c^{7}G–T)$_4$ · d(A–C)$_4$ (**18** · **17**)	37	–57	–218	B-DNA
d(m^{7}c^{7}G–T)$_4$ · d(A–C)$_4$ (**19** · **17**)	41	–60	–225	B-DNA
d(T$_4$G$_4$T$_4$) · d(A$_4$C$_4$A$_4$) (**20** · **21**)	52	–108	–333	B-DNA
d[T$_4$(c^{7}G)$_4$T$_4$] · d(A$_4$C$_4$A$_4$) (**22** · **21**)	45	–85	–266	B-DNA
d[T$_4$(m^{7}c^{7}G)$_4$T$_4$] · d(A$_4$C$_4$A$_4$) (**23** · **21**)	47	–88	–278	B-DNA
d[(c^{7}G)$_5$-G] · d(C$_6$) (**24** · **25**)	27	–48	–157	A-DNA
d[(m^{7}c^{7}G)$_5$-G] · d(C$_6$) (**26** · **25**)	23	–45	–147	A-DNA
d[(c^{7}G)$_5$-G] · poly(C)[b])	42			A-type
d[(m^{7}c^{7}G)$_5$-G] · poly(C)[b])	41			A-type

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

Table 5. T_m *Values of Homooligonucleotides*[a])

	c [μM]	T_m [°]
d[(c^{7}G)$_5$–G] (**24**)	60	-[b])
d[(c^{7}G)$_9$–G] (**27**)	16	21
d[(c^{7}G)$_9$–G] (**27**)	60	27
d[(m^{7}c^{7}G)$_5$–G] (**26**)	28	16
d[(m^{7}c^{7}G)$_5$–G] (**26**)	90	27

[a]) 60 mM Na-cacodylate, 100 mM $MgCl_2$, 1M NaCl, pH 7.0.
[b]) T_m value cannot be determined.

plied Biosystems. Tables 3–5 contain the oligonucleotides which were synthesized and give their T_m values together with the thermodynamic data of duplex formation. The base composition of the oligonucleotides was confirmed by enzymatic hydrolysis. *Fig. 1* shows representative examples of the HPLC profiles.

From *Table 3*, it is obvious that the duplex d(c^{7}G–C)$_4$ (**12**) is less stable than that of d(G–C)$_4$ (**11**). This destabilization was already recognized on shorter oligomers [24] as well as on polynucleotide duplexes having the same composition [25]. The 7-methylated octanucleotide d(m^{7}c^{7}G–C)$_4$ (**13**) shows a significantly increased stability compared to that of d(c^{7}G–C)$_4$ (**12**) coming close to the values of the parent purine oligonucleotide d(G–C)$_4$ (**11**) [26]. The same trend was found for d(C–m^{7}c^{7}G)$_4$ (**15**) compared to d(C–G)$_4$ (**14**) [27]. According to *Table 3*, the comparably low duplex stability of oligonucleotide **12** is caused by an unfavorable reaction enthalpy due to weaker H-bonding and/or stacking interactions. The entropy of **12** is more favorable for duplex formation compared to **11** or **13**. The 7-methylated oligomers **13** and **15** show about the same enthalpic and entropic changes during duplex formation as the corresponding oligomers **11** and **14**.

Oligonucleotides containing alternating d(C–G) undergo a B-Z transition when the NaCl concentration is increased from 1M to 4M. Corresponding oligonucleotides containing m^{7}G form a Z-DNA already under physiological conditions. This B-Z transition

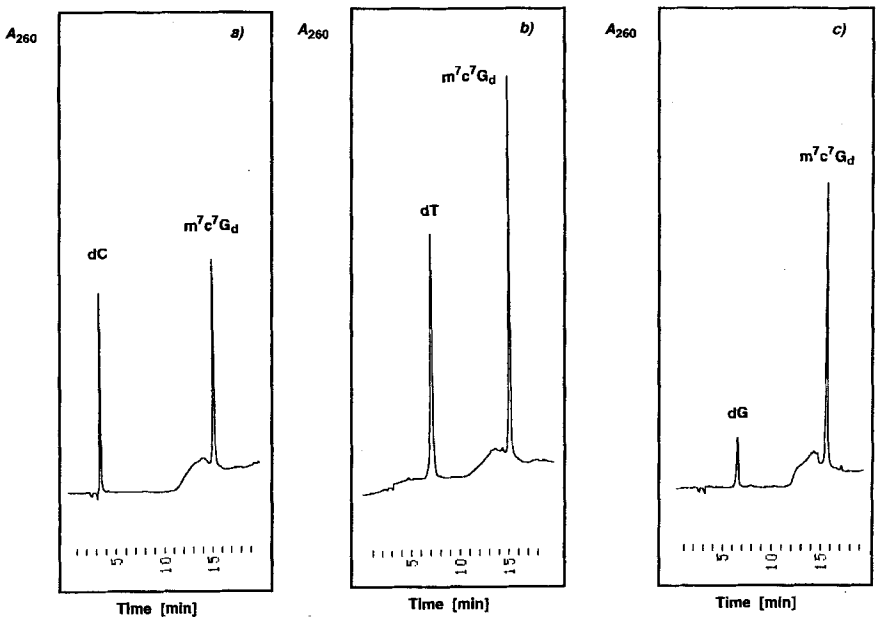


Fig. 1. *HPLC Profiles:* a) *oligomer* **13**, b) *oligomer* **19**, *and* c) *oligomer* **26**, *after enzymatic hydrolysis with snake-venom phosphodiesterase followed by alkaline phosphatase in 0.1*M Tris-*HCl buffer* (pH 8.3). For details, see *Exper. Part.*

produces a dramatic change in the CD spectrum [28]. As the shape of the CD spectra is diagnostic for a particular DNA structure, the CD spectra of compounds **11**–**15** were measured. At low salt concentration, the CD spectra of the oligomers **11**–**15** are similar. As those of **12** and **13** are in accordance to a B-like DNA structure, a structural change is not observed when the 7-deazaguanine moiety carries a 7-Me group. Only the oligonucleotide $d(C-G)_4$ (**14**) forms a Z-DNA at 4M NaCl similar by to poly [$d(C-m^7G)$] under physiological conditions [3]. The oligomer **15** containing 7-deaza-7-methylguanine maintains still a B-form duplex structure, even at high salt concentration (*Fig. 2*). As only the 7-methylated guanine base facilitates the transition from B- to Z-DNA but not 7-deaza-7-methylguanine, it was suggested that the transition is induced by the charge and not by the spatial effect of the Me group [3] [27].

According to observations on non-modified oligonucleotides, the stability of a base pair depends on the nearest neighbors. This was shown by the oligonucleotide duplex $d(c^7G-T)_4 \cdot d(A-C)_4$ (**18** · **17**). In contrast to the alternating self-complementary duplex of $d(c^7G-C)_4$ (**12**) which was significantly destabilized compared to the parent **11**, the duplex $d(c^7G-T)_4 \cdot d(A-C)_4$ (**18** · **17**) was as stable as the parent $d(G-T)_4 \cdot d(A-C)_4$ (**16** · **17**). The duplex $d(m^7c^7G-T)_4 \cdot d(A-C)_4$ (**19** · **17**) shows a 3° higher T_m value than that of the parent **16** · **17** (*Table 4*). Entropic and enthalpic data of the c^7G_d-modified duplexes are quite similar to those containing guanine; c^7G_d reduces the duplex stability of the base pair in alternating d(G–C) but not in d(G–T). From the thermodynamic data of the duplexes with alternating $d(m^7c^7G-C)$ (see **13**), it is apparent that the Me group stabilizes the duplex by a more favorable reaction enthalpy, while the effect

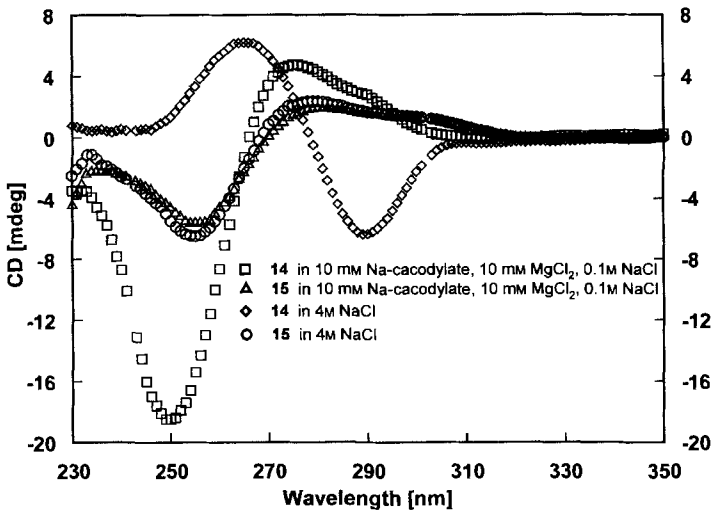


Fig. 2. *CD Spectra of the oligonucleotide duplexes d(C–G)$_4$* (**14**; 10 μM) *and d(C-m^{7}c^{7}G)$_4$* (**15**; 10 μM), *measured at 20°*. pH 7.0.

on d(m^{7}c^{7}G–T) is only minor. Furthermore, all the CD spectra of those duplexes are similar showing a B-like DNA structure.

Next, oligonucleotide duplexes were investigated containing four consecutive 7-deazaguanine residues (*Table 4*). The duplex d[T$_4$(m^{7}c^{7}G)$_4$T$_4$] · d(A$_4$C$_4$A$_4$) (**23** · **21**) containing four consecutive 7-deaza-7-methylguanine residues (T_m 47°) is slightly more stable than that missing a 7-substituent (d[T$_4$(c^{7}G)$_4$T$_4$] · d(A$_4$C$_4$A$_4$) (**22** · **21**; T_m 45°), and both are less stable than the parent oligomer d(T$_4$G$_4$T$_4$) · d(A$_4$C$_4$A$_4$) (**20** · **21**; T_m 52°) [29]. From thermodynamic data, it is apparent that this destabilization is caused by an unfavorable term of enthalpy which is, however, partially compensated by a more favorable entropy term. The CD spectrum of the methylated duplex d[T$_4$(m^{7}c^{7}G)$_4$T$_4$] · d(A$_4$C$_4$A$_4$) (**23** · **21**) shows characteristics of B-DNA with a negative B_{1u} transition at 246 nm and a positive B_{2u} transition at 276 nm, which is similar to those of the non-methylated duplex **22** · **21** as well as of the parent duplex **20** · **21** [29].

A main characteristic of homooligonucleotides containing 7-deaza-2′-deoxyguanosine is their inability to form tetraplex structures by *Hoogsteen* base pairing as it is found for oligonucleotides containing 2′-deoxyguanosine. As a result, the structure of homooligomers can be studied without taking such aggregates into account. When the hexamer d[(m^{7}c^{7}G)$_5$–G] (**26**) is hybridized with d(C$_6$), a duplex was formed with a T_m value of 23°. The T_m was higher in the case of the duplex d[(c^{7}G)$_5$–G] · d(C$_6$) (**24** · **25**; T_m 27°). However, when the hexamers d[(m^{7}c^{7}G)$_5$–G] (**26**) or **24** were hybridized with poly(C) much more stable duplexes were formed (*Table 4*). This is in accordance with findings on DNA · RNA hybrids which often show higher stability over corresponding DNA · DNA duplexes [30]. Both duplexes d[(m^{7}c^{7}G)$_5$–G] · d(C$_6$) (**26** · **25**) and d[(m^{7}c^{7}G)$_5$–G] · poly(C) show A-type DNA/RNA CD spectra with a positive *Cotton* effect around 270 nm (*Fig. 3*). Therefore, it can be concluded that the modified oligonucleotides may nicely hybridize with RNA.

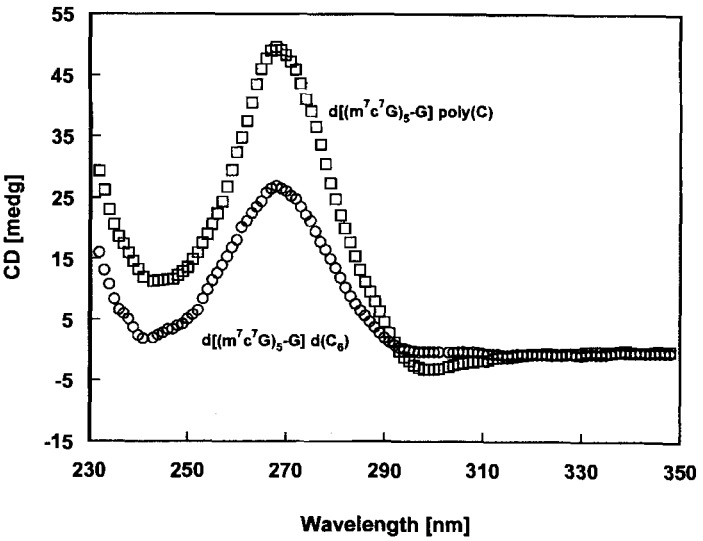


Fig. 3. *CD Spectra of oligonucleotide duplexes* $d[(m^7c^7G)_5{-}G] \cdot d(C_6)$ (**26** · **25**; ○) *and* $d[(m^7c^7G)_5{-}G] \cdot poly(C)$ (□). Measured in 10 μM Na-cacodylate, 10 μM $MgCl_2$, 0.1M NaCl, pH 7.0 at 5°.

It was also of interest to study the behavior of the homooligonucleotides **24**–**27**. Therefore, we measured their melting curves UV-spectrophotometrically. From the T_m profile of hexamer **24** containing consecutive 7-deaza-2′-deoxyguanosine residues, a sigmoidal melting was observed, but the T_m value was too low to be determined. When the chain length was increased to the decamer **27**, a complete sigmoidal melting profile was observed leading to a T_m value of 21° (*Table 5*). This T_m value was increased to 27° by enlarging the oligonucleotide concentration from 16 μM to 60 μM (*Fig. 4, a*). In the case of 7-deaza-2′-deoxy-7-methylguanosine, the hexamer **26** was already stable enough to obtain sigmoidal melting curves showing T_m values of 16° for 28 μM of **26** and 27° for 90 μM (*Fig. 4, b*). The T_m values were also determined CD spectrometrically and were found to be 1 to 2° higher than those measured by UV (*Fig. 5*).

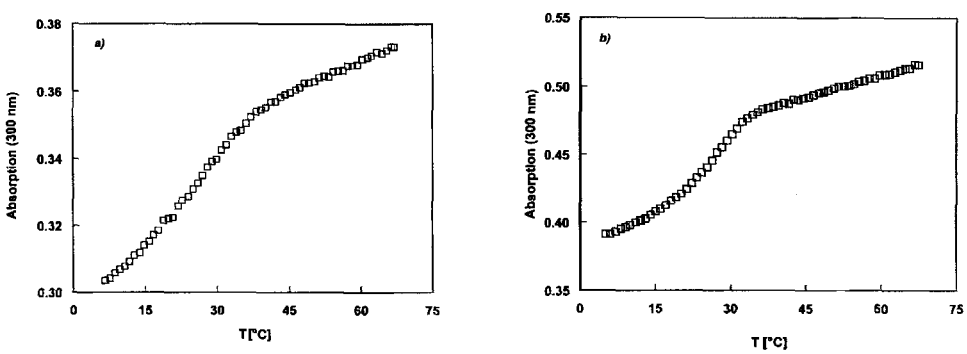


Fig. 4. *Melting curves of oligonucleotides:* a) $d[(c^7G)_9{-}G]$ (**27**; 60 μM) *and* b) $d[(m^7c^7G)_5{-}G]$ (**26**; 90 μM). Measured in 60 μM Na-cacodylate, 100 μM $MgCl_2$, 1M NaCl, pH 7.0.

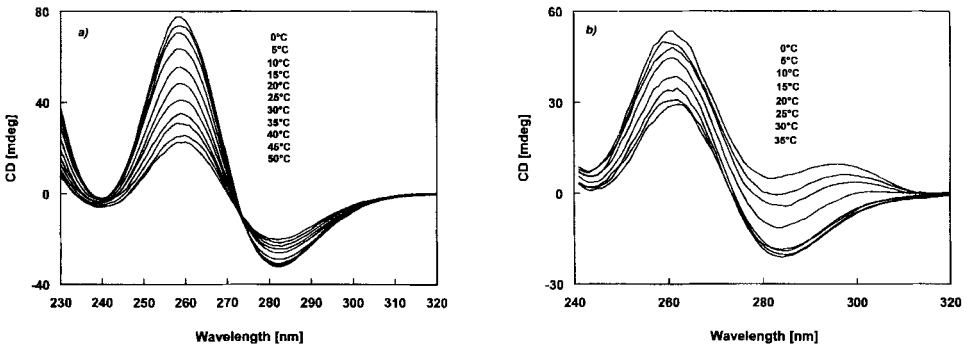


Fig. 5. *Temperature-dependent CD spectra:* a) *d[(c⁷G)₉–G]* (**27**; 16 μM) *and* b) *d[(m⁷c⁷G)₅–G]* (**26**; 28 μM). Measured in 60 μM Na-cacodylate, 100 μM $MgCl_2$, 1M NaCl, pH 7.0.

Both oligonucleotides **26** and **27** have similar CD spectra showing a negative trough at 285 nm and a positive lobe at 262 nm (*Fig. 5*). These are distinctly different from the spectra of the duplexes d[$(m^7c^7G)_5$–G] · $d(C_6)$ (**26** · **25**) and d[$(c^7G)_5$–G] · $d(C_6)$ (**24** · **25**) [11] showing a positive *Cotton* effect around 270 nm (*Fig. 3*). Both monomeric nucleosides **2a** and **2b** exhibit very weak CD spectra, but they become pronounced in the case of oligonucleotides indicating the ordered structure. The hypochroism observed on UV measurements clearly indicates that these oligomers are heavily stacked, especially the 7-methylated oligomer **26**.

The behavior of modified oligonucleotides to exonuclease hydrolyses is an important property which determines their use as antisense oligonucleotides as well as their ability to undergo exonucleolytic cleavage used in the single-molecule sequencing of oligonucleotides. It is known that low biostability of naturally occurring oligonucleotides has been improved by phosphodiester backbone modification [31] [32]. To test the biostability of the oligonucleotides containing c^7G or m^7c^7G residues, the phosphodiester hydrolysis of the oligonucleotides was tested against snake-venom phosphodiesterase (SV-PDE, 3′ → 5′ exonuclease) as well as calf-spleen phosphodiesterase (CS-PDE, 5′ → 3′ exonunclease). Enzymatic phosphodiester hydrolysis of methylated oligonucleotides $d(m^7c^7G–C)_4$ (**13**), $d(m^7c^7G–T)_4$ (**19**) and d[$(m^7c^7G)_5$–G] (**26**) towards SV-PDE was only *ca.* 10-fold slower than for the unmethylated oligomers (*Table 6*). The unmethylated oligonucleotides containing compound **2a** or 2′-deoxyguanosine showed very similar hydrolysis rates. This makes 7-substituted 7-deazaguanines favorable to be used in the single-molecule sequencing of DNA as the 3′-exonuclease can liberate such nucleotide residues without great difficulty. The oligonucleotides containing compounds **2a** (c^7G_d) or **2b** ($m^7c^7G_d$) were hydrolyzed significantly slower by CS-PDE than the parent oligonucleotides (see *Table 6*) which is an advantage for the use as antisense oligonucleotides.

In conclusion, oligonucleotide duplexes containing 7-deaza-7-methylguanine are stabilized compared to those containing unmethylated 7-deazaguanine. This is different from the zwitterionic 2′-deoxy-7-methylguanosine residues which destabilize the duplex [7]. Although, the B-DNA duplex structure is retained during the replacement of a d(G · C) base pair by those of $d(c^7G \cdot C)$ or $d(m^7c^7G \cdot C)$, the nearest-neighbor influences in duplexes are significantly different in duplex structures. As expected, each replacement of a regular by a modified nucleotide leads to an autonomous DNA. However,

Table 6. *Half-life Values* (τ) *of Enzymatic Phosphodiester Hydrolyses of Oligonucleotides by Snake-Venom* (SV-PDE) *and Calf Spleen Phosphodiesterase* (CS-PDE)[a])

	Exonuclease τ [min]	
	SV-PDE[b])	CS-PDE[c])
d(G–C)$_4$ (**11**)	3	82
d(c^{7}G–C)$_4$ (**12**)	1	*ca.* 1000
d(m^{7}c^{7}G–C)$_4$ (**13**)	16	>1200
d(G–T)$_4$ (**16**)	2	–
d(c^{7}G–T)$_4$ (**18**)	1	–
d(m^{7}c^{7}G–T)$_4$ (**19**)	15	>1200
d[(c^{7}G)$_5$–G] (**24**)	3	>1200
d[(m^{7}c^{7}G)$_5$–G] (**26**)	35	>1200

[a]) Measured in 0.1M *Tris* · HCl, pH 8.3, at room temperature.
[b]) SV-PDE: 0.009 u.
[c]) CS-PDE: 0.04 u.

these changes are comparably small with regard to the overall DNA structure. Furthermore, oligonucleotides containing 7-deaza-7-methylguanine show higher stability against 5′-exonucleases, whereas the stability against 3′-exonucleases is only slightly increased.

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Experimental Part

General. See [13]. TLC: Al 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 16:4:1 (*E*), CH_2Cl_2/AcOEt 3:1 (*F*). CD Spectra: *Jasco-600* spectropolarimeter; thermostatically controlled 1-cm cuvettes with a *Lauda-RCS-6* bath. The enzymatic hydrolysis of the oligomers was carried out as described [33].

*4-Chloro-7-[2-deoxy-3,5-di-*O*-(*p*-toluoyl)-β-*D*-*erythro*-pentofuranosyl]-5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine* (**6**). To a suspension of **4** [9] (500 mg, 2.7 mmol) in anh. MeCN (40 ml), powdered KOH (600 mg, 8.91 mmol) and TDA-1 (= tris[2-(2-methoxyethoxy)ethyl]amine; 100 µl, 0.31 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 (**5**) [34] (1.16 g, 2.98 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 (silica gel, 8 × 3 cm, *A*) and the content of the main zone crystallized from MeOH: colorless needles (1.05 g, 73%). M.p. 156–157°. TLC (silical gel, *A*): R_f 0.4. UV (MeOH): 320 (4600), 239 (58600). ^{1}H-NMR ((D$_6$)DMSO): 2.16 (*s*, Me); 2.37, 2.39 (2*s*, 2 Me); 2.85 (*m*, H–C(2′)); 4.46 (*m*, 2 H–C(5′)); 4.60 (*m*, H–C(4′)); 5.67 (*m*, H–C(3′)); 6.52 (*t*, *J* = 6.7, H–C(1′)); 6.70 (*s*, NH_2); 6.99 (*s*, H–C(6′)); 7.3–7.9 (*m*, arom. H). Anal. calc. for $C_{28}H_{27}ClN_4O_5$ (535.00): C 62.86, H 5.09, N 10.47; found: C 62.91, H 5.12, N 10.42.

*7-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-4-methoxy-5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine* (7). A soln. of **6** (1.0 g, 1.87 mmol) in 0.5M NaOMe/MeOH (40 ml) was heated under reflux for 2 h. The soln. was cooled and adsorbed on silica gel and the mixture evaporated. The silica gel was located on the top of a column (silica gel, 9 × 4 cm, *B*). The substance of the main zone was isolated and crystallized from H_2O: colorless needles (460 mg, 84%). M.p. 204–205° ([9]: 201–203°).

*2-Amino-7-(2-deoxy-β-*D-erythro-*pentofuranosyl)-3,7-dihydro-5-methyl-4*H-*pyrrolo[2,3-*d*]pyrimidin-4-one* (**2b**). A suspension of **7** (1.0 g, 3.4 mmol) in 2N aq. NaOH (100 ml) was heated under reflux for 10 h. Upon neutralization with 20% aq. HCl soln., **2b** crystallized in colorless needles (0.76 g, 80%). M.p. 260° (dec.) ([9]: 263° (dec.)).

*7-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-3,7-dihydro-2-(isobutyrylamino)-5-methyl-4*H-*pyrrolo[2,3-*d*]pyrimidin-4-one* (**8**). Compound **2b** (500 mg, 1.78 mmol) was dried three times by co-evaporation with dry pyridine and then suspended in dry pyridine (9 ml). Chlorotrimethylsilane (1.2 ml, 9.0 mmol) was added at r.t. After stirring for 15 min, the soln. was treated with isobutyric anhydride (1.5 ml, 9.0 mmol) and maintained at r.t. for 3 h. The mixture was then cooled in an ice bath, and H_2O (1.8 ml) was added. After 5 min, 25% aq. NH_3 soln. (1.8 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 crystals (555 mg, 89%). TLC (silica gel, *C*): R_f 0.7. M.p. 172–173°. UV (MeOH): 299 (12000), 275 (10000), 223 (16500). ^{1}H-NMR ((D$_6$)DMSO): 1.10 (*d*, *J* = 6.5, 2 Me); 2.11, 2.28 (2*m*, 2 H–C(2′)); 2.23 (*s*, Me); 2.73 (*q*, *J* = 6.6, CH); 3.48 (*m*, 2 H–C(5′)); 3.75 (*m*, H–C(4′)); 4.29 (*m*, H–C(3′)); 4.85 (br., OH–C(5′)); 5.20 (br., OH–C(3′)); 6.36 (*t*, *J* = 6.7, H–C(1′)); 6.94 (*s*, H–C(6)); 11.42, 11.67 (2*s*, 2 NH). Anal. calc. for $C_{16}H_{22}N_4O_5$ (350.38): C 54.85, H 6.32, N 15.99; found: C 54.76, H 6.46, N 16.01.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D-erythro-*pentofuranosyl]-3,7-dihydro-2-(isobutyrylamino)-5-methyl-4*H-*pyrrolo[2,3-*d*]pyrimidin-4-one* (**9**). Compound **8** (390 mg, 1.1 mmol) was dried by repeated co-evaporation with dry pyridine and then dissolved in dry pyridine (8 ml). At r.t., 4,4′-dimethoxytrityl chloride (448 mg, 1.3 mmol) was introduced while stirring, and stirring was continued for 4 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 (Na_2SO_4) and evaporated, and the residue submitted to FC (silica gel, 4 × 8 cm, *B* containing traces of Et_3N): colorless powder (654 mg, 91%). TLC (silica gel, *B*): R_f 0.3. ^{1}H-NMR ((D$_6$)DMSO): 1.10 (*d*, *J* = 6.7, 2 Me); 2.16 (*s*, Me); 2.20, 2.40 (2*m*, 2 H–C(2′)); 2.74 (*q*, *J* = 6.8, CH); 3.12 (*m*, 2 H–C(5′)); 3.72 (*s*, 2 MeO); 3.89 (br. 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 $C_{37}H_{40}N_4O_7$ (652.76): C 68.08, H 6.18, N 8.58; found: C 68.25, H 6.29, N 8.50

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D-erythro-*pentofuranosyl]-3,7-dihydro-2-(isobutyrylamino)-5-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, 7.57 mmol) was added at r.t. After 30 min, the mixture was cooled to 0°, and a soln. of **9** (300 mg, 0.46 mmol) in CH_2Cl_2 (14 ml) was added slowly. After stirring for 30 min at r.t., the mixture was poured into 1M (Et_3N)HCO_3 (30 ml), shaken, and separated. The aq. layer was extracted with CH_2Cl_2 (3 × 40 ml), the combined org. extract dried (Na_2SO_4) and evaporated, and the residue submitted to FC (silica gel, 3 × 7 cm, *D*). The crude product was dissolved in CH_2Cl_2 and washed with 0.1M (Et_3N)HCO_3 (5 × 15 ml) and the org. layer dried (Na_2SO_4) and evaporated: colorless foam (270 mg, 72%). TLC (silica gel, *D*): R_f 0.5. ^{1}H-NMR ((D$_6$)DMSO): 1.16 (*m*, 5 Me); 2.19 (*s*, Me); 2.30 (*m*, H–C(2′)); 2.74 (*q*, *J* = 6.3, CH); 3.00 (*q*, *J* = 6.4, 3 CH_2); 3.13, 3.18 (2*m*, 2 H–C(5′)); 3,75 (*s*, MeO); 4.09 (br., H–C(4′)); 4.77 (*m*, H–C(3′)); 6.43 (*d*, *J*(P,H) = 346, PH); 6.45 (*t*, *J* = 6.7, H–C(1′)); 6.8–7.4 (*m*, arom. H, H–C(6)); 11.67, 11.69 (2*s*, 2 NH). ^{31}P-NMR ((D$_6$)DMSO): 0.94 (^1J(P,H) = 584, ^3J(P,H–C(3′)) = 8.1). Anal. calc. for $C_{43}H_{56}N_5O_9P$ (817.93): C 63.14, H 6.90, N 8.56; found: C 63.06, H 6.88, N 8.51.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D-erythro-*pentofuranosyl]-3,7-dihydro-2-(isobutyrylamino)-5-methyl-4*H-*pyrrolo[2,3-*d*]pyrimidin-4-one 3′-(2-Cyanoethyl-*N,*N-Diisopropylphosphoramidite)* (**3b**). To a soln. of **9** (500 mg, 0.77 mmol) in anh. CH_2Cl_2 (4 ml), (i-Pr)$_2$EtN (0.43 ml, 2.45 mmol) and chloro(2-cyanoethoxy)(diisopropylamino)phosphane (0.3 ml, 1.33 mmol) were added at r.t. After stirring for 30 min, the soln. was diluted with CH_2Cl_2 (10 ml), and 5% aq. $NaHCO_3$ soln. (5 ml) was added. The mixture was extracted with CH_2Cl_2 (3 × 20 ml), the combined org. layer dried (Na_2SO_4) and evaporated, and the residue applied to FC (silica gel, 8 × 2 cm, *E*): colorless foam (560 mg, 85%). TLC (silica gel, *F*): R_f 0.6. ^{31}P-NMR ($CDCl_3$): 148.1, 148.5.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D-erythro-*pentofuranosyl]-3,7-dihydro-2-(isobutyrylamino)-5-methyl-4*H-*pyrrolo[2,3-*d*]pyrimidin-4-one 3′-(3-Carboxypropanoate)* (**10**). To a soln. of **9** (200 mg, 0.31 mmol) in anh. CH_2ClCH_2Cl (0.7 ml), 4-(dimethylamino)pyridine (43.2 mg, 0.35 mmol), succinic anhydride (45.9 mg, 0.46 mmol), and Et_3N (0.1 ml, 0.72 mmol) were added at r.t. The mixture was stirred for 30 min at 50°. The soln. was diluted with CH_2ClCH_2Cl (10 ml), washed with an ice-cold 10% aq. citric acid soln. (3 × 8 ml) followed by H_2O (10 ml). The org. layer was dried (Na_2SO_4) and evaporated. The residue was submitted to FC (silica gel, 8 × 3 cm, *B*): colorless powder (190 mg, 81%). TLC (silica gel, *B*): R_f 0.5. ^{1}H-NMR ((D$_6$)DMSO): 1.13 (*d*, *J* = 6.3, 2 Me); 2.18 (*s*, Me); 2.36 (*m*, H–C(2′)); 2.54 (*m*, CH_2); 2.75 (*m*, CH, H–C(2′)); 3.17 (*m*, CH_2); 3.29 (*m*, 2 H–C(5′)); 3.74 (*s*, 2 MeO); 4.06 (br. H–C(4′)); 5.30 (*m*, H–C(3′)); 5.75 (*s*, COOH); 6.39 (*t*, *J* = 6.7, H–C(1′)); 6.8–7.4 (*m*, arom. H, H–C(6)); 11.44, 11.73 (2 br., 2 NH). Anal. calc. for $C_{41}H_{44}N_4O_{10}$ (752.83): C 65.41, H 5.89, N 7.44; found: C 65.29, H 5.89, N 7.49.

7-[2-Deoxy-5-O-(4,4'-dimethoxytrityl)-β-D-erythro-pentofuranosyl]-3,7-dihydro-2-(isobutyrylamino)-5-methyl-4H-pyrrolo[2,3-d]pyrimidin-4-one 3'-[3-(N-Fractosilcarbamoyl)propanoate] (**3c**). A soln. of **10** (100 mg, 0.13 mmol) in 1,4-dioxane containing 5% pyridine (1 ml) was treated with 4-nitrophenol (33 mg, 0.24 mmol) and dicyclohexylcarbodiimide (50 mg, 0.24 mmol) at r.t. The mixture was stirred for 2 h at r.t. and dicyclohexylurea filtered off. The filtrate was added to a suspension of amino-linked *Fractosil 200* (200 mg) and dry DMF (1 ml). Then, Et_3N (0.2 ml) was introduced and the suspension shaken for 4 h at r.t. Ac_2O (60 µl) was added, and shaking was continued for another 30 min. The *Fractosil* derivative was filtered off, washed with DMF, EtOH, and Et_2O, and dried *in vacuo*. The ligand concentration was determined according to [21] and was found to be 70 µmol/g.

Synthesis of the Oligonucleotides **13**–**27**. Oligonucleotide synthesis was performed on DNA synthesizers, model *380 B* for phosphonates and model *392* (*Applied Biosystems*, Weiterstadt, Germany) for phosphoramidites on a 1-µmol scale. The oligomers **13**–**27** were purified as described [13]. Retention times, composition, and yields are shown in *Table 7*.

Enzymatic Oligonucleotide Hydrolysis. The oligonucleotides (*ca.* 0.8 A_{260} units) in 1 ml 0.1M *Tris* · HCl (pH 8.3) were hydrolyzed by snake-venom phosphodiesterase (0.009 units) or calf-spleen phosphodiesterase (0.04 units). The reaction was followed UV-spectrophotometrically at 260 nm. For details, see *Table 6*.

Table 7. *Data of the Oligonucleotides* **13**–**27**

	t_R [min][a])	Yield [A_{260} units]	Nucleoside composition
13	18.7	27	$m^7c^7G_d\ C_d$ 1:1.13
14	17.6	24	$G_d\ C_d$ 1:1.09
15	19.0	17	$m^7c^7G_d\ C_d$ 1:1.18
16	16.6	10	$G_d\ T_d$ 1:1.03
17	16.1	24	$A_d\ C_d$ 1:1.16
18	16.8	9	$c^7G_d\ T_d$ 0.95:1
19	17.0	24	$m^7c^7G_d\ T_d$ 1:1.12
23	17.8	11	$m^7c^7G_d\ T_d$ 1:2.04
26	15.5	16	$m^7c^7G_d\ G_d$ 5.12:1
27	17.5	10	$c^7G_d\ G_d$ 10.20:1

[a]) Retention times. For details, see [13].

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