98. Studies on the Base-Pairing Properties of N^7-(2-Deoxy-β-D-*erythro*-pentofuranosyl)guanine (N^7G_d)

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Dedicated to Prof. *W. Pfleiderer* on the occasion of his 70th birthday

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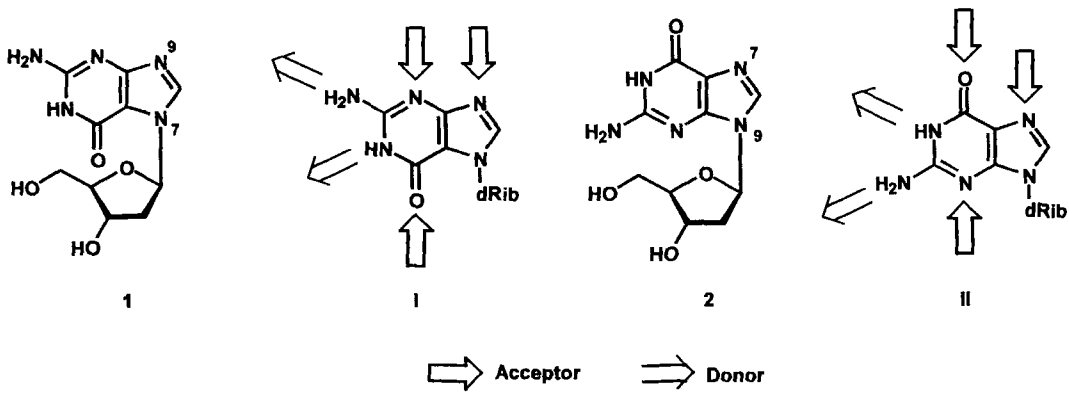
The base-pairing properties of N^7-(2-deoxy-β-D-*erythro*-pentofuranosyl)guanine (N^7G_d; **1**) are investigated. The nucleoside **1** was obtained by nucleobase-anion glycosylation. The glycosylation reaction of various 6-alkoxypurin-2-amines **3a–i** with 2-deoxy-3,5-di-*O*-(4-toluoyl)-α-D-*erythro*-pentofuranosyl chloride (**8**) was studied. The N^9/N^7-glycosylation ratio was found to be 1:1 when 6-isopropoxypurin-2-amine (**3d**) was used, whereas 6-(2-methoxyethoxy)purin-2-amine (**3i**) gave mainly the N^9-nucleoside (2:1). Oligonucleotides containing compound **1** were prepared by solid-phase synthesis and hybridized with complementary strands having the four conventional nucleosides located opposite to N^7G_d. According to T_m values and enthalpy data of duplex formation, a base pair between N^7G_d and dG is suggested. From the possible $N^7G_d \cdot$ dG base pair motives, *Hoogsteen* pairing can be excluded as 7-deaza-2′-deoxyguanosine forms the same stable base pair with N^7G_d as dG.

Introduction. – The duplex formation of nucleobases with an unusually linked sugar moiety can lead to new base-pairing modes and eventually to new DNA structures. In 1988, it was shown by our laboratory that N^8-linked 8-aza-7-deazapurine nucleosides form base pairs with regularly linked pyrimidine nucleosides [1]. This work was later extended to N^7-linked purine nucleosides. The base pairing of N^7-(2-deoxy-β-D-*erythro*-pentofuranosyl)adenine with dT within an oligonucleotide duplex was the first report on the duplex formation of an N^7-oligonucleotide [2–4]. Later, another unusually linked nucleoside (P1) was incorporated into the third strand of a triplex DNA [5]. Previously, the N^7-(2-deoxy-β-D-*erythro*-pentofuranosyl)guanine (N^7G_d; **1**) was shown to form a duplex of considerable stability when introduced in an alternating self-complementary N^7G_d-dC sequence [6]. Also, triplex formation of oligonucleotides containing N^7G_d (**1**) was reported [7] [8].

The glycosylation of guanine at position 7 (see **1**) does not change the overall donor-acceptor pattern of the base compared to 2′-deoxyguanosine (**2**) (see **I** *vs.* **II**). However, different atoms are contributing to the pairing mode altering the steric requirements of the base pair. Also the *Hoogsteen* site does not exist in compound **1** impairing this type of base pairing. On the other hand, the nucleoside **1** has a new base-pairing region (NH_2, N(3), N(9)). Consequently, new base-pairing modes can be expected, and unusual DNA structures can be realized. This communication reports on the incorporation of N^7G_d (**1**) residues into various positions of oligonucleotide duplexes. Their stability is investigated and compared with the non-



modified duplex structures. From this work, evidence of new base-pair motives is given.

Results and Discussion. – *Monomers.* Earlier, it has been observed that N^9-substituted purines are the main products formed during the nucleobase-anion glycosylation of purines with 2-deoxy-3,5-di-*O*-(4-toluoyl-α-D-*erythro*-pentofuranosyl)chloride (**8**) [9]. The N^7-isomers are usually obtained as minor components. Nevertheless, it was found that the isomer ratio can be shifted towards the minor isomer when 6-methoxypurine instead of 6-chloropurine was used [10]. A similar observation was made during the synthesis of N^7-(2-deoxy-*erythro*-pentofuranosyl)guanine **1** [6]. Whereas the yield of the N^7-isomer was only 16% when the glycosylation was performed on 6-chloropurin-2-amine [11] [12], it was increased to 24% when 6-methoxypurin-2-amine was used [6] [13]. As we wanted to increase the yield of the N^7-isomer further, we undertook a comparative study of the glycosylation reaction using various 6-alkoxypurin-2-amines.

In all cases, the starting 6-alkoxypurin-2-amines **3a–i** were obtained from 6-chloropurin-2-amine [12] which was treated with the corresponding sodium alkoxide. Normally, the alkoxy derivatives were sufficiently pure after crystallization. Compounds **3a–e** and **3i** have been prepared earlier [13] [14]. Compounds **3f–h** are new. The glycosylation was carried out at room temperature as described using MeCN/powdered KOH and TDA-1 as catalyst [15] [16] (*Scheme 1*). The glycosylation products **4** and **5** were separated by flash chromatography. *Table 1* summarizes the results of this study. It is obvious that alkoxy derivatives with very short and very long alkyl chains change the ratio in favor of the N^9-isomer **4**. The yield of the N^9-product was particularly high when the 2-methoxyethoxy compound **3i** was used. This result is similar to alkylation experiments performed on compound **3i** [17]. We obtained the highest yield of the N^7-isomer **5** when 6-ethoxypurin-2-amine (**3b**) or 6-isopropoxypurin-2-amine (**3d**) were used in the glycosylation reaction.

The isomers **4a–i** and **5a–i** were deprotected with NaOMe/MeOH to give the nucleosides **6a–i** and **7a–i**. Then compound **7d** was treated with 2N NaOH giving N^7-(2-deoxy-*erythro*-pentofuranosyl)guanine **1** in 82% yield [6]. The N^9-isomers **6a–e** have already been prepared by another route starting from 2′-deoxyguanosine [18–20]. In the

Scheme 1

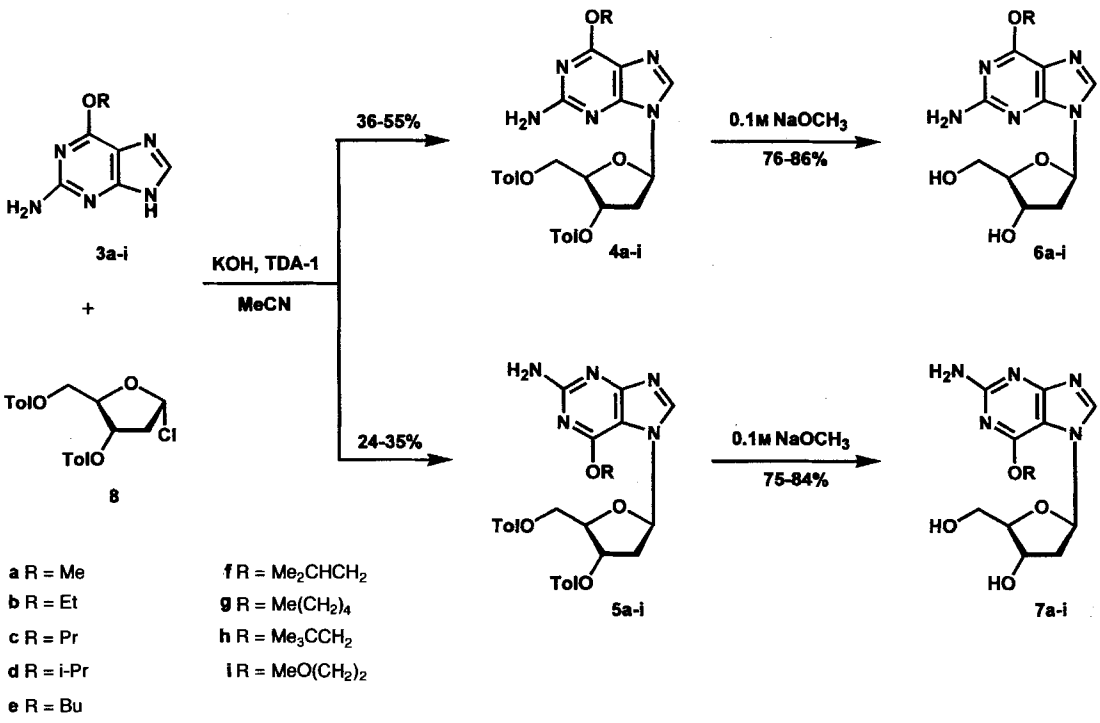


Table 1. *Yields and Ratios of Regioisomers of the Glycosylation of 6-Alkoxypurin-2-amines* **3**[a])

	N^9-Isomer **4** [%]	N^7-Isomer **5** [%]	**4**/**5** (N^9/N^7)	Yield [%]
3a [6]	48	24	2.0	72
b	37	30	1.2	67
c	43	28	1.5	71
d	36	35	1.0	71
e	39	30	1.3	69
f	37	32	1.2	69
g	38	29	1.3	67
h	40	28	1.4	68
i	55	30	1.8	85

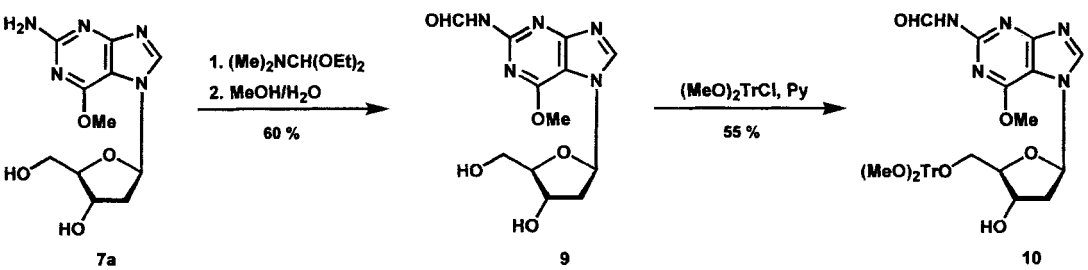
[a]) Determined after flash column chromatography.

case of bulky alkoxy substituents, the reaction time for the displacement reaction was significantly longer than it was found for the MeO compound **7a**. Nevertheless, the final yield obtained after displacement was the same. Other synthetic routes described for the synthesis of isobutyryl-protected **1** are more laborious and give a lower yield [8] [21].

The glycosylic-bond stability of **6a** and **7a** as well as of **6d** and **7d** was determined by exposure to HCl solution followed by HPLC analysis. The N^7-nucleosides **7a** and **7d** (0.5N HCl, 25°; HPLC (280 nm): $t_{1/2}$ 15 and 27 min) were found to be more stable as their N^9-counterparts **6a** and **6d** (0.1N HCl, 25°; HPLC (280 nm): $t_{1/2}$ 13 and 11 min) [4] [6]. The O^6-alkyl residues were not hydrolyzed under these conditions. The higher glycosylic-bond stability of the N^7-nucleosides **7a** and **7d** is in agreement with results reported for N^7G_d (**1**) compared to dG (**2**) [6].

Earlier, the phosphoramidite **12** has been prepared [6]. Now, the phosphoramidite **11** was synthesized from nucleoside **7a** which was treated with dimethylformamide dimethyl acetal to give the (dimethylamino)methylidene derivative, together with the formyl compound **9** (*Scheme 2*). The formation of formyl derivatives from amidines was already reported in the case of 6-methoxy-7-deazapurin-2-amine 2′-deoxyribofuranoside [22]. It was necessary to complete the transformation of the (dimethylamino)methylidene derivative to **9** by treatment of the reaction mixture with MeOH/H_2O at 50°. The half-life value of the deformylation of **9** was determined UV-spectrophotometrically at 275 nm (25% aqueous NH_3 solution at 40°). The half-life (35 min) was short enough to avoid displacement of the 6-methoxy group by ammonia. This reaction was observed in the case of the N^9-nucleoside which carries an isobutyryl protecting group [20]. Compound **9** was protected at the 5′-OH group with 4,4′-dimethoxytrityl chloride to give derivative **10**. The phosphoramidite **11** was prepared by treatment of **10** with chloro(2-cyanoethoxy)(diisopropylamino)phosphine in the presence of diisopropyl(ethyl)amine.

Scheme 2



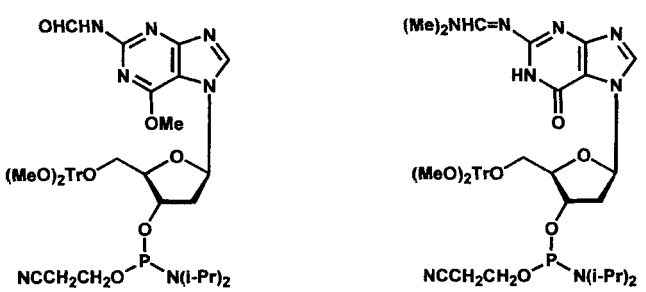


Table 2 shows the ^{13}C-NMR chemical shifts of the monomers **3**–**7**, **9**, and **10**. Some ^{13}C-NMR data of the N^9-isomers **6b,c** have been published earlier [19]. However, in these cases, the assignment of C(1′) and C(4′) had to be reversed according to our data. Our attributions were based on the observation that the coupling constant ^1J(C,H) of C(1′) is larger (162–168 Hz) than that of C(4′) (148–153 Hz) (see *Table 3*) [23]. The ^{13}C-NMR data of the alkoxy derivatives followed that of the methoxy compound [6]. The assignment of the C(2) and C(4) signals was made on the basis of [^{1}H,^{13}C] gated-decoupled spectra. The anomeric configuration of the sugar moiety was confirmed by ^{1}H-NOE data and found to be β-D in all cases. Furthermore, the *syn*/*anti*-population of *N*-glycosylic bond can be determined by a calibration graph described earlier [24]. The N^9/N^7-guanine isomers **1** and **2** show a similar population of *syn*/*anti*-conformers (*Table 4*). In the case of the alkylated N^7-nucleosides **7a**–**d**, it is

Table 2. *^{13}C-NMR Chemical Shifts of Purine 2′-Deoxyribonucleosides*[a])[b])[c])

	C(2)[d])	C(4)	C(5)	C(6)[d])	C(8)	CHO		C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	C=O
3c	159.6	156.0	112.8	160.0	138.4	66.9	**4b**	83.2	35.5	75.7	81.5	64.2	
f	159.7	155.0	113.5	160.3	137.5	71.5	**c**	83.2	35.5	75.3	81.5	64.2	
g	159.7	155.1	113.6	159.8	137.6	65.4	**d**	83.1	35.4	75.1	81.4	64.1	
h	159.7	155.0	113.5	160.6	137.4	74.7	**e**	83.1	35.5	75.1	81.5	64.1	
4b	159.9	153.9	114.2	160.5	137.7	61.7	**f**	83.1	35.5	75.1	81.5	64.1	
c	160.0	154.0	114.2	160.7	137.7	67.3	**g**	83.1	35.4	75.1	81.4	64.1	
d	159.8	153.9	114.3	160.0	137.4	68.3	**h**	83.0	35.5	75.1	81.4	64.1	
e	159.8	153.9	114.1	160.7	137.5	71.7	**i**	83.1	35.4	75.1	81.5	64.1	
f	159.8	153.9	114.1	160.6	137.5	71.7	**5b**	86.0	37.2	75.7	81.4	64.3	
g	159.8	153.8	114.1	160.5	137.5	65.7	**c**	86.0	37.2	74.8	81.5	64.0	
h	159.8	153.9	114.0	160.9	137.5	75.1	**d**	86.1	38.0	74.7	81.4	64.3	
i	159.7	154.0	114.0	160.3	137.7	70.0	**e**	86.0	37.5	74.9	81.6	64.1	
5b	159.9	164.0	104.9	156.5	143.5	62.0	**f**	85.9	37.4	75.0	81.7	64.1	
c	159.8	164.0	105.0	156.5	142.7	67.3	**g**	86.0	37.5	74.8	81.6	64.1	
d	160.0	164.3	105.0	156.1	143.1	69.1	**h**	85.9	37.1	75.2	82.0	64.1	
e	159.8	164.0	105.0	156.5	142.7	65.5	**i**	86.2	37.7	74.7	81.4	64.2	
f	159.8	163.8	105.1	156.6	142.2	71.8	**6b**	82.9	[e])	70.8	87.6	61.6	
g	159.8	164.0	105.0	156.5	142.7	65.8	**c**	82.8	[e])	70.8	87.6	61.2	
h	159.9	163.7	105.3	156.8	141.9	75.2	**d**	82.8	[e])	70.8	87.6	61.2	
i	159.7	164.5	104.6	156.2	143.8	69.8	**e**	82.8	[e])	70.8	87.6	61.7	
6b	159.8	153.8	114.0	160.4	137.7	61.8	**f**	82.8	37.4	70.7	87.6	61.7	
c	159.7	153.8	114.0	160.5	137.6	67.1	**g**	82.8	[e])	70.8	87.6	61.8	
d	159.7	153.8	114.1	159.9	137.5	70.8	**h**	82.8	[e])	70.8	87.6	61.7	
e	159.7	153.8	114.0	159.7	137.6	65.3	**i**	82.8	[e])	70.8	87.6	64.6	
f	159.7	153.9	114.1	160.6	137.6	71.7	**7b**	86.0	41.1	70.1	87.7	61.3	
g	159.7	153.8	114.0	160.5	137.6	65.7	**c**	86.0	41.1	69.9	87.6	61.2	
h	159.7	153.8	113.9	160.8	137.6	74.9	**d**	86.2	41.5	69.9	87.7	61.2	
i	159.6	153.9	113.9	160.2	137.7	70.0	**e**	86.0	41.1	69.9	87.6	61.2	
7b	159.8	164.0	105.0	156.5	143.0	61.8	**f**	86.0	41.1	69.8	87.6	61.1	
c	159.7	163.9	105.0	156.5	143.0	67.2	**g**	85.9	41.0	69.9	87.6	61.2	
d	159.8	164.1	105.1	156.2	142.8	68.8	**h**	85.9	41.0	69.8	87.6	61.0	
e	159.6	163.9	105.0	156.6	142.7	65.4	**i**	86.1	41.1	70.0	87.7	61.2	
f	159.6	163.8	105.0	156.7	142.5	71.7	**9**	86.2	40.9	70.1	87.9	61.2	163.5
g	159.6	163.8	105.0	156.5	142.7	65.6	**10**	85.4	[e])	70.2	85.9	64.0	163.2
h	159.6	163.7	105.0	156.9	142.3	75.1							
i	159.6	164.1	104.9	156.4	143.1	70.0							
9	152.2	162.4	108.7	156.9	145.0	54.3							
10	152.3	162.7	108.6	156.9	144.8	54.5							

[a]) Spectra were measured in (D_6)DMSO rel. to $SiMe_4$ at 23°. [b]) From [^{1}H,^{13}C] gated-decoupled spectra. [c]) Purine numbering. [d]) Tentative. [e]) Superimposed by DMSO.

obvious that with a more bulky alkoxy substituent, a rise of the *anti*-population is observed. This is not the case for the N^9-nucleosides **6c,d,h**. Steric and stereoelectronic effects are influencing also the sugar conformation. The N-conformer population of the sugar moiety is increased in the case of the N^7-nucleosides **1** and **7a** compared to 2′-deoxyguanosine (**2**) (see *Table 5*). The data were obtained by PSEUROT 6.0 measurements [25].

Table 3. *J(C,H) Coupling Constants* [Hz] *of Purine 2′-Deoxyribonucleosides*[a])[b])

	4f	**4i**	**5f**	**5g**	**5i**	**6c**	**6d**	**7c**	**7d**	**9**
J(C(4),*H*–C(8))	*m*	*m*	12.9	12.8	12.8	*m*	*m*	12.8	13.0	13.0
J(C(4),*H*–C(1′))	*m*	*m*	–	–	–	*m*	*m*	–	–	–
J(C(5),*H*–C(8))	11.8	11.8	4.1	*m*	3.0	11.8	11.8	4.0	4.4	3.7
J(C(8),*H*–C(8))	213	213	211	210	211	213	213	211	211	213
J(C(8),*H*–C(1′))	3.7	3.7	4.5	3.6	3.6	4.4	4.4	4.0	4.0	3.8
J(C(1′),*H*–C(1′))	165	166	168	168	162	167	164	168	168	168
J(C(2′),*H*–C(2′))	139	134	138	134	135	–	–	–	–	–
J(C(3′),*H*–C(3′))	160	159	160	158	160	146	150	148	150	150
J(C(4′),*H*–C(4′))	153	153	143	152	153	149	149	148	148	148
J(C(5′),*H*–C(5′))	149	141	150	148	141	140	140	140	140	140
J(OCH_2)	148	148	149	149	141	149	149	142	145	–
J(*C*HO,N–*H*)	–	–	–	–	–	–	–	–	–	201

[a]) From ^{13}C-NMR spectra measured in (D_6)DMSO at 23°.
[b]) Purine numbering.

Table 4. *^{1}H-NOE Data* [%] *of Alkoxypurin-2-amine 2′-Deoxyribofuranosides*[a])[b])

	Irradiated proton	NOE [%]
1	H–C(1′)	H_α–C(2′) (5.3); H–C(4′) (1.6); H–C(8) (3.7)
	H–C(8)	H–C(1′) (3.4); H–C(3′) (1.1)
2 [24]	H–C(1′)	H_α–C(2′) (5.6); H–C(4′) (1.6); H–C(8) (3.1)
	H–C(8)	H–C(1′) (3.1); H_β–C(2′) (3.7); H–C(3′) (1.1)
6c	H–C(8)	H–C(1′) (3.9); H_β–C(2′) (3.7)
d	H–C(1′)	H_α–C(2′) (6.9); H–C(4′) (2.3); H–C(8) (4.1)
	H–C(8)	H–C(1′) (4.6); H_β–C(2′) (4.1); H–C(3′) (1.2)
h	H–C(1′)	H_α–C(2′) (6.6); H–C(4′) (1.1); H–C(8) (3.2)
	H–C(8)	H–C(1′) (3.6); H_β–C(2′) (3.6); H–C(3′) (1.2)
7a [6]	H–C(1′)	H_α–C(2′) (7.2); H–C(4′) (2.4); H–C(8) (3.9)
	H–C(8)	H–C(1′) (4.3); H_β–C(2′) (3.9); H–C(3′) (1.6); OH–C(5′) (1.8)
b	H–C(1′)	H_α–C(2′) (5.6); H–C(4′) (2.0); H–C(8) (3.1)
	H–C(8)	H–C(1′) (3.6); H_β–C(2′) (3.4); H–C(3′) (1.4); OH–C(5′) (1.1)
c	H–C(1′)	H_α–C(2′) (6.1); H–C(4′) (2.8); H–C(8) (3.0)
	H–C(8)	H–C(1′) (3.2); H_β–C(2′) (3.0)
d	H–C(1′)	H_α–C(2′) (5.2); H–C(4′) (2.0)
	H–C(8)	H–C(1′) (2.5); H_β–C(2′) (3.3); H–C(3′) (1.3)

[a]) In (D_6)DMSO at 23°. [b]) Purine numbering.

Table 5. $^3J(H,H)$ *Coupling Constants of the Sugar Moieties and N/S-Conformer Populations of Compounds* **1**, **2**, *and* **7a** *at 303 K*[a])

	J(H–(1'), H–(2'))	J(H–(1'), H–(2''))	J(H–(2'), H–(3'))	J(H–(2''), H–(3'))	J(H–(3'), H–(4'))	Conformation	
						% N	% S
1	6.60	6.25	6.20	3.40	3.70	34	66
2 [33]	7.30	6.50	6.30	3.60	3.20	29	71
7a	6.65	6.30	6.45	4.80	4.30	44	56

[a]) Solvent, D_2O; r.m.s., ≤ 0.4 Hz; $|\Delta J_{max}| \leq 0.5$ Hz. N = $^3T_{2'}$; S = $_3T^{2'}$.

Oligonucleotides. The synthesis of oligonucleotides **13**–**38** shown in the *Tables 6*–*8* was performed using either phosphonate or phosphoramidite chemistry [26] [27]. The methodology followed the standard protocols, and the efficiency of coupling was similar for the modified building blocks as found for the regular compounds. The oligonucleotides were detritylated and purified using oligonucleotide-purification cartridges. The composition of the oligonucleotides was confirmed by tandem hydrolysis with snake-venom phosphodiesterase and alkaline phosphatase as described [28]. Representative examples of composition pattern are shown in *Fig. 1*. Also the MALDI-TOF spectra were taken in a few cases.

Table 6. T_m *Values of 5'-d(TTTTTXTTTTTT)-3' · 5'-d(AAAAAAYAAAAA)-3' and 5'-d(TTTTTXXTTTTT) · 5'd(AAAAAYYAAAAA)*

	X · Y	T_m [°C][a])		XX · YY	T_m [°C][a])
13 · 14	T · A	37 (44)	**13 · 14**	TT · AA	37 (44)
15 · 16	G · C	39 (45)	**22 · 23**	GG · CC	39 (46)
17 · 18	N^7G · G	30 (35)	**24 · 25**	N^7GN^7G · GG	28 (36)
17 · 19	N^7G · c^{7}G	27 (33)	**24 · 26**	N^7GN^7G · c^{7}Gc^{7}G	26 (32)
17 · 20	N^7G · N^7G	28 (33)	–	–	–
17 · 16	N^7G · C	28 (35)	**24 · 23**	N^7GN^7G · CC	34 (39)
17 · 21	N^7G · T	22 (30)	**24 · 27**	N^7GN^7G · TT	15 (23)
17 · 14	N^7G · A	21 (27)	**24 · 14**	N^7GN^7G · AA	14 (26)
15 · 18	G · G	< 10 (19)	**22 · 25**	GG · GG	< 10 (< 10)

[a]) Measured at 260 nm in 0.1M NaCl, 10 mM $MgCl_2$, and 10 mM Na-cacodylate (pH 7.0) at 5 μmol single-strand concentration; values in parentheses were obtained at 260 nm in 1M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.0) at 5 μmol single-strand concentration.

In a first series of experiments, the base-pairing capability of N^7G_d (**1**) with the four conventional bases was investigated (*Table 6*). For this purpose, a series of dodecamers were synthesized which are derived from $d(A)_{12}$ (**14**) or $d(T)_{12}$ (**13**). Either one or two dT residues were replaced in the center of $d(T)_{12}$ by N^7G_d. For comparison, the dT residues were also substituted by 2'-deoxyguanosine dG (**2**). Hybrids were formed with complementary $d(A)_{12}$ strands containing one or two dA, dT, dC, dG, or 7-deaza-2'-deoxyguanosine (c^7G_d) residues located opposite N^7G_d (**1**). Also a duplex with a dG · dC

Table 7. T_m *Values and Thermodynamic Data of Duplex Formation of 5'-d(TAXXTCAATACT)-3' · 3'-d(ATYYAGTTATGA)-5'*

	XX · YY	T_m [°C][a])	ΔH [kcal/mol][a])	ΔS[cal/mol K][a])
28 · 29	GG · CC	47 (50)	– 94 (– 98)	– 292 (– 304)
28 · 30	GG · N^7GN^{7}G	37 (40)	– 83 (– 76)	– 287 (– 239)
31 · 30	c^{7}Gc^{7}G · N^7GN^{7}G	39 (40)	– 84 (– 75)	– 271 (– 271)
32 · 30	N^7GN^{7}G · N^7GN^{7}G	37 (38)	– 61 (– 72)	– 196 (– 239)
28 · 33	GG · (m$^6N^7$G)$_2$	27 (32)	– 69 (– 71)	– 228 (– 241)
31 · 33	c^{7}Gc^{7}G · (m$^6N^7$G)$_2$	30 (35)	– 75 (– 56)	– 249 (– 184)
32 · 33	N^7GN^{7}G · (m$^6N^7$G)$_2$	30 (32)	– 54 (– 63)	– 181 (– 209)
34 · 30	(m$^6N^7$G)$_2$ · N^7GN^{7}G	32 (35)	– 24 (– 48)	– 81 (– 157)
34 · 33	(m$^6N^7$G)$_2$ · (m$^6N^7$G)$_2$	23 (29)	– 60 (– 64)	– 199 (– 216)
28 · 35	GG · GG	24 (30)	– 58 (– 60)	– 197 (– 199)
28 · 29	GG · CC	47 (50)	– 94 (– 98)	– 292 (– 304)
32 · 29	N^7GN^{7}G · CC	23 (27)	– 54 (– 57)	– 182 (– 188)
34 · 29	(m$^6N^7$G)$_2$ · CC	18 (23)	– 29 (– 55)	– 97 (– 184)

[a]) Determined at 260 nm in 0.1M NaCl, 10 mM $MgCl_2$, and 10 mM Na-cacodylate (pH 7.0) at 5 μmol single-strand concentration; values in parentheses were obtained at 260 nm in 1M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.0) at 5 μmol single-strand concentration.

Table 8. T_m *Values and Thermodynamic Data of Duplex Formation of 5'-d(TAGGTXAATAXT)-3' · 3'-d(ATCCAYTTATYA)-5'*

	X...X · Y...Y	T_m [°C][a])	ΔH [kcal/mol][a])	ΔS [cal/mol · K][a])
28 · 29	C...C · G...G	47 (50)	– 94 (– 98)	– 292 (– 304)
38 · 29	N^7G...N^7G · G...G	37 (39)	– 85 (– 83)	– 270 (– 266)
36 · 37	N^7G...N^7G · c^{7}G...c^{7}G	35 (39)	– 81 (– 75)	– 262 (– 240)
36 · 38	N^7G...N^7G · N^7G...N^7G	32 (34)	– 81 (– 72)	– 264 (– 234)
28 · 38	C...C · N^7G...N^7G	19 (23)	– 61 (– 58)	– 196 (– 197)

[a]) Determined at 260 nm in 0.1M NaCl, 10 mM $MgCl_2$, and 10 mM Na-cacodylate (pH 7.0) at 5 μmol single-strand concentration; values in parentheses were obtained at 260 nm in 1M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.0) at 5 μmol single-strand concentration.

base pair (**15** · **16**) and one with a dG · dG mismatch (**15** · **18**) was formed. The T_m measurements were performed in 0.1M NaCl containing 10 mM $MgCl_2$ as well as in 1M NaCl (data in parenthesis). The melting curves were determined UV-spectrophotometrically and are sigmoidal melting profiles in all cases. Two typical profiles are shown in *Fig. 2a* and *2b*.

The T_m values of these duplexes are listed in *Table 6*. For comparison the T_m values of non-modified d(T)$_{12}$ · d(A)$_{12}$ (**13** · **14**) are included. From the T_m values of *Table 6* it is apparent that the incorporation of two N^7G_d residues in the dT strand of the duplex d(T)$_{12}$ · d(A)$_{12}$ reduces the T_m values by only 9° (**24** · **25**) compared to the unmodified duplex **13** · **14**. The duplex **22** · **25** with two dG-dG mismatches shows a T_m decrease of more than 25°. Also a clear discrimination between the other bases located opposite to N^7G_d (**1**) is observed (*Table 6*). The duplexes in which N^7G_d is facing dG or 7-deaza-2'-deoxyguanosine show significantly higher T_m values as those facing dA or dT. Also the

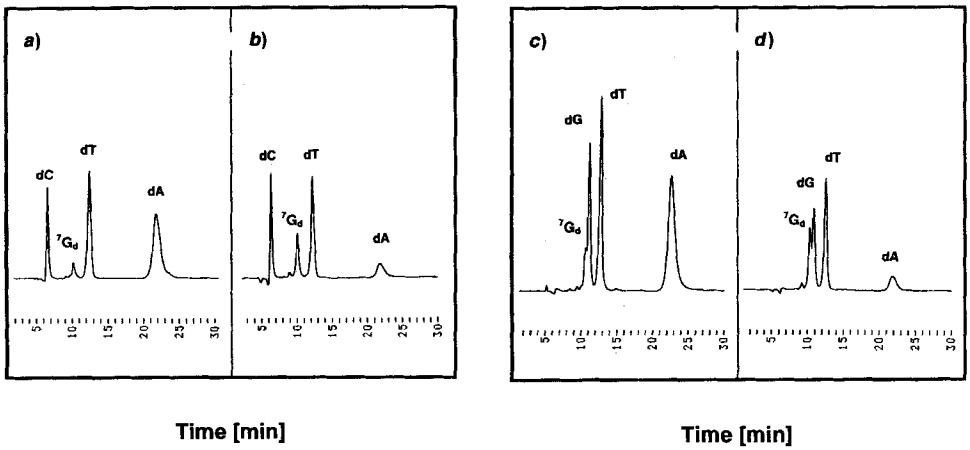


Fig. 1. *HPLC Profiles of the oligonucleotide* **32** *and* **30** *after enzymatic hydrolysis with snake-venom phosphodiesterase followed by alkaline phosphatase in* 1M Tris · *HCl buffer* (pH 8.3): a) *from* **32** *measured at 260 nm*, b) *from* **32**, *measured at 280 nm*, c) *from* **30**, *measured at 260 nm, and* d) *from* **30**, *measured at 280 nm.* $^7G_d = N^7G_d$.

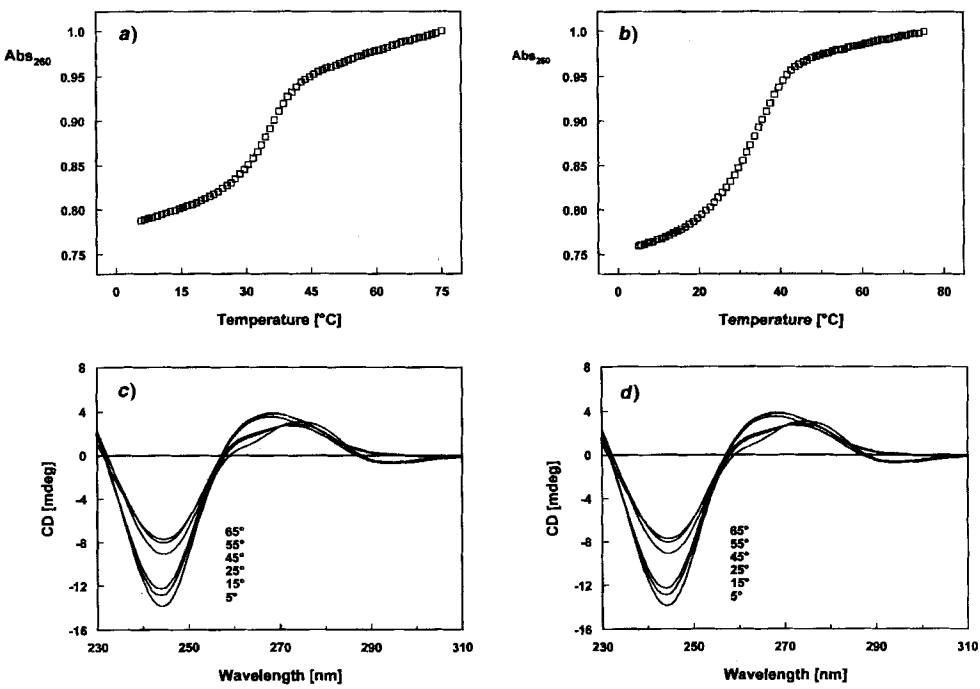


Fig. 2. *Normalized melting profiles of the duplexes* a) **17** · **18** *and* b) **24** · **25** *measured at 260 nm in* 1M *NaCl, 100* mM $MgCl_2$, *and 60* mM *Na-cacodylate* (pH 7.1) *at 5* μM *single-strand concentration.* c) *Temperature-dependent CD spectra of the duplexes* $d(A_{12}) \cdot d(T_{12})$ (**13** · **14**) *and* d) $d(T_5N^7G_2\,T_5) \cdot d(A_5G_2A_5)$ (**24** · **25**)

hybrids with N^7G_d opposite to dC (**17** · **16** and **24** · **23**) give a relatively high T_m value. From these observations, it is concluded that N^7G_d pairs with dG and 7-deaza-2′-deoxyguanosine and eventually with dC and N^7G_d, but not with dA or dT. The CD spectra of duplexes, *e.g.* **17** · **18** (N^7G_d · dG) and **24** · **25** ($N^7G_dN^7G_d$ · dGdG) were similar to that of **13** · **14** (*Fig. 2, c* and *d*). No significant changes of the CD spectra were observed when one or two N^7G_d residues were introduced into the duplex structure.

In a further set of experiments, the base pairing of N^7G_d (**1**) with dG (**2**) and with 7-deaza-2′-deoxyguanosine was investigated on another duplex which is derived from the dodecamers 5′-d(TAGGTCAATACT)-3′ (**28**) and 5′-d(ATCCAGTTATGA)-3′ (**29**). This study was undertaken to determine thermodynamic data and to avoid complications which may result from the hybridization of $d(A)_{12}$ with $d(T)_{12}$. In the latter case, it is possible that triplexes or even parallel-stranded structures are formed which make the interpretation of data difficult. This is particularly the case, when the modified nucleoside **1** is introduced which shows a tendency to form *Hoogsteen* base bairs in triplex DNA [8]. The unmodified dodecamers **28** and **29** form a duplex with a T_m value of 47° (0.1M NaCl containing 10 mmol of $MgCl_2$ and 10 mmol of Na-cacodylate). The T_m value in 1M NaCl is slightly increased (*Table 7*). In the following experiments, two consecutive dC residues of **29** were replaced by two N^7G_d residues (→ **30**). Also the two dG residues of **28** were substituted by two 7-deaza-2′-deoxyguanosine residues (→ **31**). Finally, the two dG residues of **28** were replaced by two N^7G_d residues (→ **32**). The duplexes **28** · **30** (dGdG · $N^7G_dN^7G_d$), **31** · **30** ($c^7G_dC^7G_d$ · $N^7G_dN^7G_d$), and **32** · **30** ($N^7G_dN^7G_d$ · $N^7G_dN^7G_d$) were obtained by hybridization. Typical melting curves of the hybrids **28** · **30** and **32** · **30** are shown in *Fig. 3, a* and *b*. According to *Table 7* the T_m values of these duplexes are in the range between 37° and 39° compared to 47° for the parent oligomer.

From the T_m values of *Table 7* (duplex **28** · **30** *vs.* duplex **28** · **29**), a base pair of N^7G_d with dG was considered, as it was already discussed on the basis of the T_m values of the modified homooligonucleotides **17** and **24** (*Table 6*). Also base pairing can be discussed for N^7G_d with N^7G_d (duplex **32** · **30**). These observations are supported by the enthalpy data of duplex formation of N^7G_d (**1**) with dG. The replacement of two dC residues by two N^7G_d (**1**) in the parent duplex **28** · **29** gives rise to an enthalpy change of *ca.* 10 kcal (low salt) for the duplex **28** · **30** (dGdG · $N^7G_dN^7G_d$) compared to *ca.* 35 kcal in the case of a dGdG · dGdG (**28** · **35**) mismatch. This supports N^7G_d-dG base pairing. In order to test the participation of the 6-oxo group in the N^7G_d · dG base pair, a series of duplexes were formed containing N^7-(2-deoxy-*erythro*-pentofuranosyl)-O^6-methylguanine ($m^6N^7G_d$; **7a**) instead of N^7G_d. The oligomers **33** and **34** containing two $m^6N^7G_d$ residues were hybridized with compound **28** (dGdG), **31** ($c^7G_dc^7G_d$), and **32** ($N^7G_dN^7G_d$). All of them showed considerably reduced stability with regard to the non-methylated counterparts.

A strongly decreased stability is observed for the duplex **32** · **29** ($N^7G_dN^7G_d$ · dCdC) compared to that of **28** · **29** (dGdG · dCdC), and a further decrease is found in the case of **34** · **29** ($m^6N^7G_dm^6N^7G_d$ · dCdC) and also of **34** · **33** ($m^6N^7G_dm^6N^7G_d$ · $m^6N^7G_d$-$m^6N^7G_d$). The low stability of these O^6-methylated duplexes is similar to that found for the base pairing of 2′-deoxy-O^6-methylguanosine (m^6G_d, **6a**) with dC in regular oligonucleotides [29]. This base pair was under intensive studies as mutagenesis occurs when DNA is treated with methylating carcinogens [30] [31]. The melting experiments per-

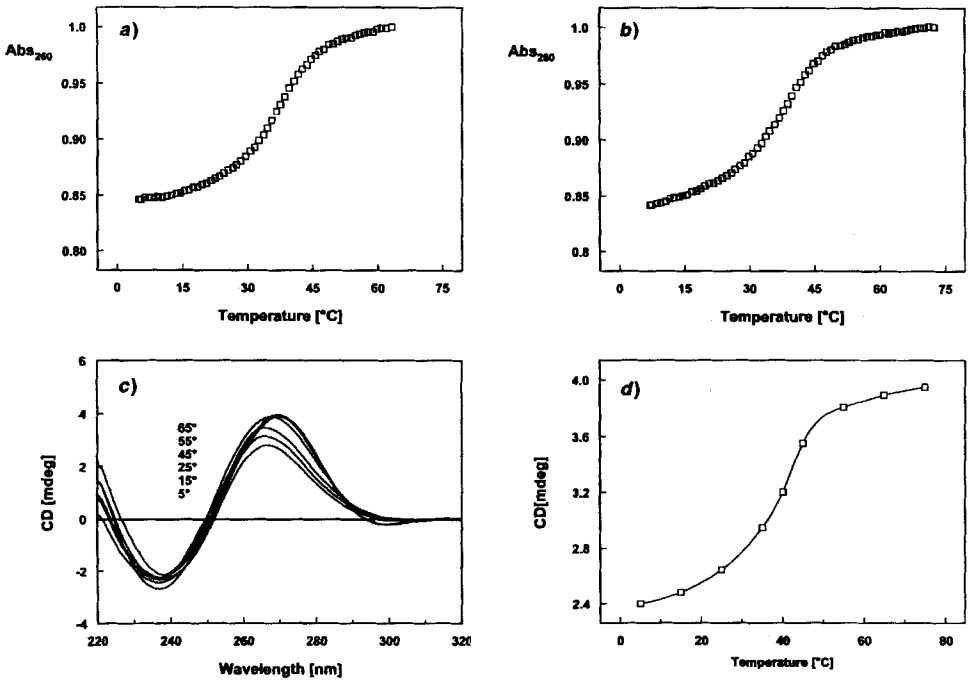
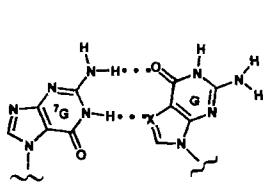


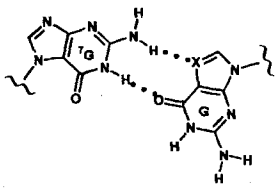
Fig. 3. *Normalized melting profiles of the duplexes* a) *5'd(TAGGTCAAATACT)-3'* · *5'-d(ATN*^{7}*GN*^{7}*GAGTTATGA)-3'* (**28** · **30**) *and* b) *5'd(TAN*^{7}*GN*^{7}*GTCAATACT)-3'* · *5'-d(ATN*^{7}*GN*^{7}*GAGTTATGA)-3'* (**32** · **30**) *measured at 260 nm in 0.1*M *NaCl, 10* mM $MgCl_2$, *and 10* mM *Na-cacodylate* (pH 7.1) *at 5* μM *single-strand concentration.* c) *Temperature-dependent CD spectra of the duplex* **28** · **30** *and* d) *temperature-dependent* B_{1u} *transition* (270 nm) *of the duplex* **28** · **30** *measured at 5* μM *single-strand concentration.* Solvent system for HPLC (*F*).

formed in 0.1M NaCl were also carried out in 1M NaCl. These conditions increased the T_m values but led to the same trends with regard to duplex stability as found under low salt concentration conditions (*Table 7*). Opposite to the observation made on the homooligomer duplexes **17** · **16** (N^7G_d · dC) and **24** · **23** ($N^7G_dN^7G_d$ · dCdC) implying a N^7G_d · dC base pair (*Table 6*), this phenomenon is not observed in the case of the strictly antiparallel duplex **32** · **29** ($N^7G_dN^7G_d$ · dCdC). This indicates that such a base pair does not exist in antiparallel duplex DNA. A base pair of N^7G_d with dC as it was recently found in the duplex d(N^7G_d–C)$_6$ might have been formed under parallel chain orientation. However, this phenomenon needs further investigation. *Table 8* summarizes data of duplexes in which two N^7G_d residues are not nearest neighbors as in *Table 7* but are separated by four regular nucleosides. By comparing the stability of the duplexes shown in *Table 8* with those of *Table 7*, similar trends of base-pair stabilities are observed.

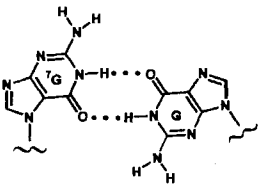
As it was of interest to establish the structural motives for the base-pairing mode of N^7G_d with dG, conceivable base pairs were constructed and placed in the center of the DNA duplex. The model building followed the following principles: The duplex is in the B-form, the most favored conformation of the *N*-glycosylic bond is *anti*, and the distance between the glycosylated N-atoms is similar to that of regular base pairs. From this examination, the pairing modes **III**–**VIII** can be considered for a N^7G_d · dG base pair.



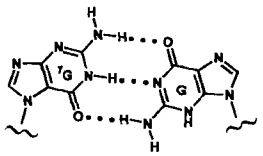
III a: X = N
b: X = CH



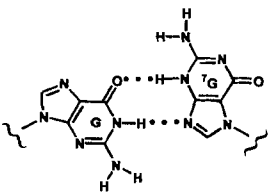
IV a: X = N
b: X = CH



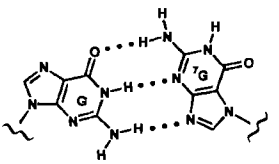
V



VI

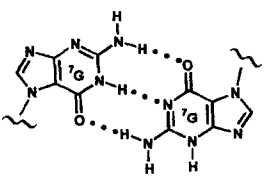


VII

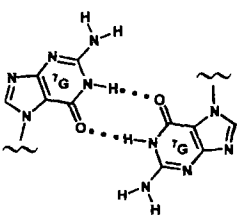


VIII

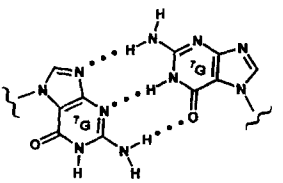
$^7G = N^7G$



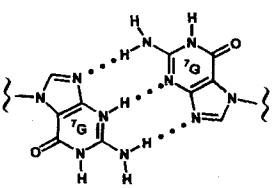
IX



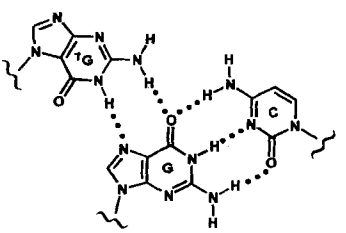
X



XI



XII



XIII

$^7G = N^7G$

Hoogsteen pairs such as **III** or **IV** were excluded as the replacement of 2′-deoxyguanosine by 7-deaza-2′-guanosine opposite to N^7G_d does not change the duplex stability significantly (*Tables 6–8*). The other base-pairing modes **V–VIII** are all conceivable, and no decision can be made on the most favorable motive.

Apart from the $N^7G_d \cdot dG$ base pair, base-pair motives **IX–XII** were built up for the self-pairing of N^7G_d residues. Motives **IX** and **X** are expected to form parallel chains. The

motives **XI** and **XII** should lead to antiparallel duplexes. A parallel chain orientation has already been proposed for the $N^7G_d \cdot$ dG base pair (motive **XIII**) in triplex DNA [8].

From the experiments described above, it can be concluded that N^7G_d can form a base pair with dG within an antiparallel duplex structure. This base-pair motive is definitely different from that of a *Hoogsteen* mode found in triplex DNA [8]. Earlier work on d(N^7G_d-C)$_6$ [6] has shown that this oligonucleotide forms a fairly stable duplex suggesting a base pair between N^7G_d and dC. As this base pair is not formed in the case of an antiparallel DNA (see *Table 7*), the duplex formed by oligonucleotides containing alternating N^7G_d-dC might have parallel chains.

We thank Dr. *H. Rosemeyer* for the pseudorotational analyses of nucleosides. We also appreciate the help of Mrs. *E. Feiling* and Mrs. *C. Mittelbach* during oligonucleotide synthesis. Financial support by the *Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie* is gratefully acknowledged.

Experimental Part

1. *General.* See [4]. The regular phosphonates were prepared by Mrs. *E. Feiling*, and the CPG-immobilized nucleosides (30–50 μmol/g solid support) were from *PerSeptive*, Wiesbaden, Germany. Oligonucleotide synthesis was performed on a DNA synthesizer, model *380 B*, *Applied Biosystems*, Weiterstadt, Germany. Snake-venom phosphodiesterase (EC 3.1.15.1, *Crotallus durissus*) and alkaline phosphatase (EC 3.1.3.1, *E. coli*) were generous gifts from *Boehringer Mannheim GmbH*, Germany. The enzymatic hydrolysis of the oligomers was performed as described [28] using the following extinction coefficients: ε_{260}: N^7G_d 2700, dT 8800, dC 7300, dA 15400, dG 11700. Solvent systems for flash chromatography (FC), TLC, and HPLC: CH_2Cl_2/MeOH 98:2 (*A*), CH_2Cl_2/MeOH 95:5 (*B*), CH_2Cl_2/MeOH 9:1 (*C*), CH_2Cl_2/MeOH 4:1 (*D*), CH_2Cl_2/AcOEt/Et_3N 45:45:10 (*E*), 0.1M (Et_3NH)OAc (pH 7.0)/MeCN 95:5 (*F*), MeCN (*G*). Gradient *I*: 40 min 0–40% *G* in *F*. Melting curves were measured with a *Cary*-1/3 UV/VIS spectrophotometer (*Varian*, Australia) equipped with a *Cary* thermoelectrical controller. The actual temperature was measured in the reference cell with a Pt-100 resistor. UV Spectra: *150-20* spectrometer (*Hitachi*, Japan). MALDI-TOF spectra were provided by Mrs. *S. Hahner* (Prof. *Hilgenkamp*, Institute of Medicinal Physics and Biophysics, University of Münster, Germany).

2. *6-Alkoxypurin-2-amines* **3f–h**: *General Procedure*: A suspension of 6-chloropurin-2-amine (1.0 g, 6 mmol) [12] in the alcohol/alkoxide soln. (50 ml, 30 mmol) was heated under reflux for 18 h. The solvent was evaporated and the residue dissolved in H_2O (200 ml) and extracted with Et_2O (100 ml, twice). The H_2O layer was acidified to pH 5 with AcOH. The solid was removed by filtration, dissolved in MeOH, adsorbed on silica gel (10 g), and applied to FC (silica gel, column 15 × 3 cm, *A*): **3f–h**.

6-Isobutoxypurin-2-amine (**3f**): Colorless foam (850 mg, 69%). TLC (*B*): R_f 0.5. UV (MeOH): 281 (8800). ^{1}H-NMR ((D$_6$)DMSO): 0.98 (*d*, *J* = 6.60, Me); 2.07 (*m*, CH); 4.18 (*d*, *J* = 6.7, CH_2O); 6.16 (*s*, NH_2); 7.81 (*s*, H–C(8)); 12.38 (br. *s*, NH).

6-(Pentyloxy)purin-2-amine (**3g**). Colorless powder (940 mg, 70%). TLC (*B*): R_f 0.7. UV (MeOH): 281 (8500). ^{1}H-NMR ((D$_6$)DMSO): 0.88 (*t*, *J* = 6.8, Me); 1.33 (*m*, 2 CH_2); 1.73 (*t*, *J* = 6.5, CH_2); 4.38 (*t*, *J* = 6.4, CH_2O); 6.14 (*s*, NH_2); 7.80 (*s*, H–C(8)); 12.38 (br. *s*, NH). Anal. calc. for $C_{10}H_{15}N_5O$ (221.26): C 54.28, H 6.83, N 31.65; found: C 54.36, H 6.88, N 31.41.

6-(2,2-Dimethylpropoxy)purin-2-amine (**3h**): Colorless foam (860 mg, 65%). TLC (*B*): R_f 0.7. UV (MeOH): 281 (8600). ^{1}H-NMR ((D$_6$)DMSO): 1.02 (*s*, Me); 4.12 (*s*, CH_2O); 6.19 (*s*, NH_2); 7.79 (*s*, H–C(8)); 12.37 (br. *s*, NH).

3. *Glycosylation of 6-Alkoxypurin-2-amines with 2-Deoxy-3,5-di-*O*-(4-toluoyl)-α-*D*-erythro-pentofuranosyl Chloride* (**8**) [9]: *General Procedure.* Powdered KOH (650 mg, 11.6 mmol) and TDA-1 (60 μl, 0.18 mmol) were suspended in anh. MeCN (40 ml). The suspension was stirred for 15 min. Then the 6-alkoxypurin-2-amine (2.6 mmol) [13] [14] was added, and stirring was continued for another 15 min. The halogenose **8** [9] (1.2 g, 3.1 mmol) was then added in portions. After 20 min, insoluble material was filtered off and the solvent evaporated. The resulting oil was applied to FC (silica gel, column 15 × 6 cm, *A* (500 ml), than *B*) and separated in two main zones in all cases. The faster migrating zone was always the N^9-isomer and the slower migrating the N^7-isomer.

*9-[2-Deoxy-3,5-di-*O*-(4-toluoyl)-β-*D*-erythro-pentofuranosyl]-6-ethoxy-9*H*-purin-2-amine* (**4b**): Colorless foam (511 mg, 37%). TLC (*B*): R_f 0.3. UV (MeOH): 281 (10800). ^{1}H-NMR ((D$_6$)DMSO): 1.34 (*t*, *J* = 7.0, Me); 2.35, 2.38 (2*s*, 2 Me); 2.69 (*m*, H_α–C(2')); 3.19 (*m*, H_β–C(2')); 4.51 (*t*, *J* = 6.7, CH_2O); 4.51 (*m*, H–C(4'),

2 H–C(5')); 5.73 (*m*, H–C(3')); 6.37 ('*t*', *J* = 7.1, H–C(1')); 6.46 (*s*, NH_2); 7.28–7.94 (arom. H); 8.06 (*s*, H–C(8)). Anal. calc. for $C_{28}H_{29}N_5O_6$ (531.57): C 63.27, H 5.50, N 13.17; found: C 63.27, H 5.65, N 13.24.

*7-[2-Deoxy-3,5-di-*O*-(4-toluoyl)-β-*D*-*erythro*-pentofuranosyl]-6-ethoxy-7*H*-purin-2-amine* (**5b**): Colorless foam (415 mg, 30%). TLC (*C*): R_f 0.4. UV (MeOH): 294 (6000). ^{1}H-NMR ((D$_6$)DMSO): 1.36 (*t*, *J* = 7.0, Me); 2.35, 2.38 (2*s*, 2 Me); 2.73 (*m*, H_α–C(2')); 2.91 (*m*, H_β–C(2')); 4.50 (*m*, CH_2O); 4.50 (*m*, H–C(4'), 2 H–C(5')); 5.65 (*m*, H–C(3')); 6.20 (*s*, NH_2); 6.50 ('*t*', *J* = 6.8, H–C(1')); 7.26–7.93 (arom. H); 8.36 (*s*, H–C(8)). Anal. calc. for $C_{28}H_{29}N_5O_6$ (531.57): C 63.27, H 5.50, N 13.17; found: C 63.17, H 5.58, N 13.14.

*9-[2-Deoxy-3,5-di-*O*-(4-toluoyl)-β-*D*-*erythro*-pentofuranosyl]-6-propoxy-9*H*-purin-2-amine* (**4c**): Colorless foam (606 mg, 43%). TLC (*B*): R_f 0.5. UV (MeOH): 281 (10800). ^{1}H-NMR ((D$_6$)DMSO): 0.96 (*t*, *J* = 7.4, Me); 1.74 (*m*, CH_2); 2.36, 2.39 (2*s*, 2 Me); 2.68 (*m*, H_α–C(2')); 3.20 (*m*, H_β–C(2')); 4.34 (*t*, *J* = 6.7, CH_2O); 4.53 (*m*, 2 H–C(5')); 4.65 (*m*, H–C(4')); 5.71 (*m*, H–C(3')); 6.38 ('*t*', *J* = 7.8, H–C(1')); 6.46 (*s*, NH_2); 7.28–7.94 (arom. H); 8.15 (*s*, H–C(8)). Anal. calc. for $C_{29}H_{31}N_5O_6$ (545.60): C 63.84, H 5.73, N 12.84; found: C 64.02, H 5.86, N 12.78.

*7-[2-Deoxy-3,5-di-*O*-(4-toluoyl)-β-*D*-*erythro*-pentofuranosyl]-6-propoxy-7*H*-purin-2-amine* (**5c**): Colorless foam (395 mg, 28%). TLC (*C*): R_f 0.4. UV (MeOH): 294 (6000). ^{1}H-NMR ((D$_6$)DMSO): 0.98 (*t*, *J* = 7.4, Me); 1.76 (*m*, CH_2); 2.37, 2.41 (2*s*, 2 Me); 2.75 (*m*, H_α–C(2')); 2.93 (*m*, H_β–C(2')); 4.38 (*m*, CH_2O); 4.57 (*m*, H–C(4'), 2H–C(5')); 5.66 (*m*, H–C(3')); 6.18 (*s*, NH_2); 6.54 ('*t*', *J* = 6.0, H–C(1')); 7.30–7.94 (arom. H); 8.38 (*s*, H–C(8)). Anal. calc. for $C_{29}H_{31}N_5O_6$ (545.60): C 63.84, H 5.73, N 12.84; found: C 63.94, H 5.82, N 12.75.

*9-[2-Deoxy-3,5-di-*O*-(4-toluoyl)-β-*D*-*erythro*-pentofuranosyl]-6-isopropoxy-9*H*-purin-2-amine* (**4d**): Colorless foam (511 mg, 36%). TLC (*B*): R_f 0.5. UV (MeOH): 281 (10500). ^{1}H-NMR ((D$_6$)DMSO): 1.14, 1.35 (2*s*, 2 Me); 2.38, 2.41 (2*s*, 2 Me); 2.71 (*m*, H_α–C(2')); 3.21 (*m*, H_β–C(2')); 4.55 (*m*, 2 H–C(5')); 4.65 (*m*, H–C(4')); 5.49 (*m*, CHO); 5.75 (*m*, H–C(3')); 6.39 ('*t*', *J* = 6.0, H–C(1')); 6.40 (*s*, NH_2); 7.31–7.95 (arom. H); 8.05 (*s*, H–C(8)). Anal. calc. for $C_{29}H_{31}N_5O_6$ (545.60): C 63.84, H 5.73, N 12.84; found: C 63.87, H 5.92, N 12.90.

*7-[2-Deoxy-3,5-di-*O*-(4-toluoyl)-β-*D*-*erythro*-pentofuranosyl]-6-isopropoxy-7*H*-purin-2-amine* (**5d**): Colorless foam (496 mg, 35%). TLC (*C*): R_f 0.4. UV (MeOH): 294 (6100). ^{1}H-NMR ((D$_6$)DMSO): 1.34, 1.36 (2*s*, 2 Me); 2.35, 2.38 (2*s*, 2 Me); 2.60 (*m*, H_α–C(2')); 2.86 (*m*, H_β – C(2')); 4.58 (*m*, H–C(4'), 2H–C(5')); 5.43 (*m*, CHO); 5.65 (*m*, H–C(3')); 6.18 (*s*, NH_2); 6.49 ('*t*', *J* = 7.0, H–C(1')); 7.26–7.93 (arom. H); 8.33 (*s*, H–C(8)). Anal. calc. for $C_{29}H_{31}N_5O_6$ (545.60): C 63.84, H 5.73, N 12.84; found: C 63.76, H 5.66, N 12.80.

*6-Butoxy-9-[2-deoxy-3,5-di-*O*-(4-toluoyl)-β-*D*-*erythro*-pentofuranosyl]-9*H*-purin-2-amine* (**4e**): Colorless foam (568 mg, 39%). TLC (*B*): R_f 0.4. UV (MeOH): 281 (10000). ^{1}H-NMR ((D$_6$)DMSO): 0.97 (*t*, *J* = 7.4, Me); 1.45 (*m*, CH_2); 1.78 (*m*, CH_2); 2.41, 2.44 (2*s*, 2 Me); 2.73 (*m*, H_α–C(2')); 3.25 (*m*, H_β–C(2')); 4.45 (*t*, *J* = 6.6, CH_2O); 4.56 (*m*, 2 H–C(5')); 4.68 (*m*, H–C(4')); 5.79 (*m*, H–C(3')); 6.43 (*dd*, *J* = 6.4, H–C(1')); 6.46 (*s*, NH_2); 7.34–7.99 (arom. H); 8.09 (*s*, H–C(8)). Anal. calc. for $C_{30}H_{33}N_5O_6$ (559.63): C 64.39, H 5.94, N 12.51; found: C 64.50, H 5.90, N 12.57.

*6-Butoxy-7-[2-deoxy-3,5-di-*O*-(4-toluoyl)-β-*D*-*erythro*-pentofuranosyl]-7*H*-purin-2-amine* (**5e**): Colorless foam (437 mg, 30%). TLC (*C*): R_f 0.4. UV (MeOH): 294 (5700). ^{1}H-NMR ((D$_6$)DMSO): 0.83 (*t*, *J* = 7.3, Me); 1.42 (*m*, CH_2); 1.70 (*m*, CH_2); 2.35, 2.39 (2*s*, 2 Me); 2.75 (*m*, H_α–C(2')); 2.91 (*m*, H_β–C(2')); 4.38 (*t*, *J* = 6.3, CH_2O); 4.55 (*m*, H–C(4'), 2 H–C(5')); 5.63 (*m*, H–C(3')); 6.18 (*s*, NH_2); 6.37 ('*t*', *J* = 5.9, H–C(1')); 7.27–7.93 (arom. H); 8.57 (*s*, H–C(8)). Anal. calc. for $C_{30}H_{33}N_5O_6$ (559.63): C 64.39, H 5.94, N 12.51; found: C 64.42, H 6.04, N 12.45.

*9-[2-Deoxy-3,5-di-*O*-(4-toluoyl)-β-*D*-*erythro*-pentofuranosyl]-6-isobutoxy-9*H*-purin-2-amine* (**4f**): Colorless foam (538 mg, 37%). TLC (*B*): R_f 0.4. UV (MeOH): 281 (10800). ^{1}H-NMR ((D$_6$)DMSO): 0.98, 0.99 (2*s*, 2 Me); 2.08 (*m*, CH); 2.37, 2.41 (2*s*, 2 Me); 2.73 (*m*, H_α–C(2')); 3.22 (*m*, H_β–C(2')); 4.20 (*d*, *J* = 6.5, CH_2O); 4.55 (*m*, 2H–C(5')); 4.65 (*m*, H–C(4')); 5.75 (*m*, H–C(3')); 6.40 (*s*, NH_2); 6.44 ('*t*', *J* = 7.5, H–C(1')); 7.30–7.95 (arom. H); 8.07 (*s*, H–C(8)). Anal. calc. for $C_{30}H_{33}N_5O_6$ (559.63): C 64.39, H 5.94, N 12.51; found: C 64.57, H 5.83, N 12.53.

*7-[2-Deoxy-3,5-di-*O*-(4-toluoyl)-β-*D*-*erythro*-pentofuranosyl]-6-isobutoxy-7*H*-purin-2-amine* (**5f**): Colorless foam (466 mg, 32%). TLC (*C*): R_f 0.4. UV (MeOH): 294 (6000). ^{1}H-NMR ((D$_6$)DMSO): 0.94, 0.97 (2*s*, 2 Me); 2.02 (*m*, CH); 2.35, 2.39 (2*s*, 2 Me); 2.75 (*m*, H_α–C(2')); 2.90 (*m*, H_β–C(2')); 4.18 (*d*, *J* = 6.0, CH_2O); 4.55 (*m*, H–C(4'), 2 H–C(5')); 5.65 (*m*, H–C(3')); 6.19 (*s*, NH_2); 6.53 (*dd*, *J* = 5.7, H–C(1')); 7.27–7.92 (arom. H); 8.37 (*s*, H–C(8)). Anal. calc. for $C_{30}H_{33}N_5O_6$ (559.6): C 64.39, H 5.94, N 12.51; found: C 64.24, H 6.03, N 12.43.

*9-[2-Deoxy-3,5-di-*O*-(4-toluoyl)-β-*D*-*erythro*-pentofuranosyl]-6-(pentyloxy)-9*H*-purin-2-amine* (**4g**): Colorless foam (578 mg, 38%). TLC (*B*): R_f 0.4. UV (MeOH): 281 (9900). ^{1}H-NMR ((D$_6$)DMSO): 0.88 (*t*, *J* = 6.8, Me); 1.35 (*m*, 2 CH_2); 1.78 (*t*, *J* = 6.7, CH_2); 2.36, 2.39 (2*s*, 2 Me); 2.67 (*m*, H_α–C(2')); 3.19 (*m*, H_β–C(2')); 4.38 (*t*, *J* = 6.6, CH_2O); 4.50 (*m*, 2 H–C(5')); 4.62 (*m*, H–C(4')); 5.71 (*m*, H–C(3')); 6.43 ('*t*', *J* = 6.6, H–C(1')); 6.44 (*s*, NH_2); 7.29–7.94 (arom. H); 8.05 (*s*, H–C(8)). Anal. calc. for $C_{31}H_{35}N_5O_6$ (573.66): C 64.91, H 6.15, N 12.21; found: C 65.30, H 5.85, N 12.00.

*7-[2-Deoxy-3,5-di-*O*-(4-toluoyl)-β-*D*-erythro-pentofuranosyl]-6-(pentyloxy)-7*H*-purin-2-amine* (**5g**): Colorless foam (441 mg, 30%). TLC (*C*): R_f 0.4. UV (MeOH): 294 (5800). ^{1}H-NMR ((D$_6$)DMSO): 0.79 (*t*, *J* = 7.3 Me); 1.25, 1.38, 1.73 (3*m*, 3 CH$_2$); 2.37, 2.41 (2*s*, 2 Me); 2.75 (*m*, H$_\alpha$–C(2′)); 2.94 (*m*, H$_\beta$–C(2′)); 4.41 (*t*, *J* = 6.3, CH$_2$O); 4.57 (*m*, H–C(4′), 2 H–C(5′)); 5.66 (*m*, H–C(3′)); 6.18 (*s*, NH$_2$); 6.52 ('*t*', *J* = 7.7, H–C(1′)); 7.29–7.94 (arom. H); 8.38 (*s*, H–C(8)). Anal. calc. for $C_{31}H_{35}N_5O_6$ (573.66): C 64.91, H 6.15, N 12.21; found: C 64.73, H 5.88, N 12.18.

*9-[2-Deoxy-3,5-di-*O*-(4-toluoyl)-β-*D*-erythro-pentofuranosyl]-6-(2,2-dimethylpropoxy)-9*H*-purine* (**4h**): Colorless foam (609 mg, 41%). TLC (*B*): R_f 0.4. UV (MeOH): 281 (8900). ^{1}H-NMR ((D$_6$)DMSO): 1.0 (*s*, Me); 2.38, 2.41 (2*s*, 2 Me); 2.80 (*m*, H$_\alpha$–C(2′)); 3.00 (*m*, H$_\beta$–C(2′)); 4.10 (*m*, CH$_2$O); 4.57 (*m*, H–C(4′), 2 H–C(5′)); 5.69 (*m*, H–C(3′)); 6.20 (*s*, NH$_2$); 6.59 (*dd*, *J* = 5.4, H–C(1′)); 7.31–7.92 (arom. H); 8.04 (*s*, H–C(8)). Anal. calc. for $C_{31}H_{35}N_5O_6$ (573.66): C 64.91, H 6.15, N 12.21; found: C 65.18, H 6.38, N 12.00.

*7-[2-Deoxy-3,5-di-*O*-(4-toluoyl)-β-*D*-erythro-pentofuranosyl]-6-(2,2-dimethylpropoxy)-7*H*-purin-2-amine* (**5h**): Colorless foam (426 mg, 28%). TLC (*C*): R_f 0.4. UV (MeOH): 294 (5500). ^{1}H-NMR ((D$_6$)DMSO): 1.0 (*s*, Me); 2.38, 2.41 (2*s*, 2 Me); 2.80 (*m*, H$_\alpha$–C(2′)); 3.00 (*m*, H$_\beta$–C(2′)); 4.10 (*m*, CH$_2$O); 4.57 (*m*, H–C(4′), 2 H–C(5′)); 5.69 (*m*, H–C(3′)); 6.20 (*s*, NH$_2$); 6.59 (*dd*, *J* = 5.4, H–C(1′)); 7.31–7.92 (arom. H); 8.40 (*s*, H–C(8)). Anal. calc. for $C_{31}H_{35}N_5O_6$ (573.65): C 64.90, H 6.15, N 12.21; found: C 64.92, H 6.22, N 12.10.

*9-[2-Deoxy-3,5-di-*O*-(4-toluoyl)-β-*D*-erythro-pentofuranosyl]-6-(2-methoxyethoxy)-9*H*-purin-2-amine* (**4i**): Colorless foam (803 mg, 55%). TLC (*B*): R_f 0.3. UV (MeOH): 281 (9500). ^{1}H-NMR ((D$_6$)DMSO): 2.36, 2.38 (2*s*, 2 Me); 2.72 (*m*, H$_\alpha$–C(2′)); 3.20 (*m*, H$_\beta$–C(2′)); 3.29 (*s*, MeO); 3.69 (*t*, *J* = 4.4, CH$_2$O); 4.52–4.65 (*m*, H–C(4′), 2 H–C(5′), CH$_2$O); 5.74 (*m*, H–C(3′)); 6.38 ('*t*', *J* = 6.8, H–C(1′)); 6.48 (*s*, NH$_2$); 7.28–7.94 (arom. H); 8.07 (*s*, H–C(8)). Anal. calc. for $C_{29}H_{31}N_5O_7$ (561.60): C 62.02, H 5.56, N 12.47; found: C 62.26, H 5.62, N 12.45.

*7-[2-Deoxy-3,5-di-*O*-(4-toluoyl)-β-*D*-erythro-pentofuranosyl]-6-(2-methoxyethoxy)-7*H*-purin-2-amine* (**5i**): Colorless foam (438 mg, 30%). TLC (*C*): R_f 0.4. UV (MeOH): 294 (5900). ^{1}H-NMR ((D$_6$)DMSO): 2.34, 2.39 (2*s*, 2 Me); 2.72 (*m*, H$_\alpha$–C(2′)); 2.96 (*m*, H$_\beta$–C(2′)); 3.24 (*s*, MeO); 3.70 (*t*, *J* = 4.3, CH$_2$O); 4.56 (*m*, H–C(4′), 2 H–C(5′), CH$_2$O); 5.63 (*m*, H–C(3′)); 6.22 (*s*, NH$_2$); 6.48 ('*t*', *J* = 6.9, H–C(1′)); 7.25–7.98 (arom. H); 8.36 (*s*, H–C(8)). Anal. calc. for $C_{29}H_{31}N_5O_7$ (561.60): C 62.02, H 5.56, N 12.47; found: C 62.22, H 5.68, N 12.47.

4. *Deprotection of Compounds* **4a**–**i** *and* **5a**–**i**: *General Procedure.* A soln. of **4a**–**i** or **5a**–**i** (0.50 mmol) in 0.1M NaOMe/MeOH (20 ml) was stirred at r.t. for 30 min. The mixture was adsorbed on silica gel (10 g) and applied to FC (silica gel; column 10 × 5 cm, *B* (300 ml), then *C*).

*9-(2-Deoxy-β-*D*-erythro-pentofuranosyl)-6-ethoxy-9*H*-purin-2-amine* (**6b**): (117 mg 79%). TLC (*C*): R_f 0.5. UV (MeOH): 247 (9400), 281 (8900). ^{1}H-NMR ((D$_6$)DMSO): 1.36 (*t*, *J* = 6.9, Me); 2.24 (*m*, H$_\alpha$–C(2′)); 2.61 (*m*, H$_\beta$–C(2′)); 3.56 (*m*, 2 H–C(5′)); 3.86 (*m*, H–C(4′)); 4.38 (*m*, H–C(3′)); 4.47 (*q*, *J* = 7.0, CH$_2$O); 5.0 (*t*, *J* = 4.9, OH–C(5′)); 5.27 (*d*, *J* = 3.2, OH–C(3′)); 6.23 ('*t*', *J* = 6.7, H–C(1′)); 6.36 (*s*, NH$_2$); 8.09 (*s*, H–C(8)). Anal. calc. for $C_{12}H_{17}N_5O_4$ (295.30): C 48.81, H 5.80, N 23.72; found: C 48.91, H 5.97, N 23.42.

*7-(2-Deoxy-β-*D*-erythro-pentofuranosyl)-6-ethoxy-7*H*-purin-2-amine* (**7b**): Colorless foam (120 mg, 81%). TLC (*D*): R_f 0.5. UV (MeOH): 294 (5600). ^{1}H-NMR ((D$_6$)DMSO): 1.35 (*t*, *J* = 7.0, Me); 2.30 (*m*, H$_\alpha$–C(2′)); 2.42 (*m*, H$_\beta$–C(2′)); 3.52 (*m*, 2 H–C(5′)); 3.82 (*m*, H–C(4′)); 4.29 (*m*, H–C(3′)); 4.45 (*m*, CH$_2$O); 4.97 (*t*, *J* = 5.0, OH–C(5′)); 5.31 (*d*, *J* = 3.8, OH–C(3′)); 6.12 (*s*, NH$_2$); 6.31 ('*t*', *J* = 6.2, H–C(1′)); 8.38 (*s*, H–C(8)). Anal. calc. for $C_{12}H_{17}N_5O_4$ (295.30): C 48.81, H 5.80, N 23.72; found: C 48.71, H 6.01, N 23.66.

*9-(2-Deoxy-β-*D*-erythro-pentofuranosyl)-6-propoxy-9*H*-purin-2-amine* (**6c**): Colorless foam (118 mg, 76%). TLC (*C*): R_f 0.5. UV (MeOH): 247 (9400), 281 (8900). ^{1}H-NMR ((D$_6$)DMSO): 0.98 (*t*, *J* = 7.3, Me); 1.78 (*m*, CH$_2$); 2.23 (*m*, H$_\alpha$–C(2′)); 2.61 (*m*, H$_\beta$–C(2′)); 3.55 (*m*, 2 H–C(5′)); 3.85 (*m*, H–C(4′)); 4.37 (*m*, CH$_2$O); 4.37 (*m*, H–C(3′)); 5.0 (*t*, *J* = 5.4, OH–C(5′)); 5.26 (*d*, *J* = 3.7, OH–C(3′)); 6.23 ('*t*', *J* = 7.3, H–C(1′)); 6.37 (*s*, NH$_2$); 8.08 (*s*, H–C(8)). Anal. calc. for $C_{13}H_{19}N_5O_4$ (309.33): C 50.48, H 6.19, N 22.64; found: C 50.58, H 6.31, N 22.52.

*7-(2-Deoxy-β-*D*-erythro-pentofuranosyl)-6-propoxy-7*H*-purin-2-amine* (**7c**): Colorless foam (127 mg, 82%). TLC (*D*): R_f 0.4. UV (MeOH): 294 (5900). ^{1}H-NMR ((D$_6$)DMSO): 0.80 (*t*, *J* = 7.4, Me); 1.78 (*m*, CH$_2$); 2.27 (*m*, H$_\alpha$–C(2′)); 2.34 (*m*, H$_\beta$–C(2′)); 3.58 (*m*, 2 H–C(5′)); 3.83 (*m*, H–C(4′)); 4.35 (*m*, CH$_2$O); 4.35 (*m*, H–C(3′)); 4.96 (*t*, *J* = 5.3, OH–C(5′)); 5.27 (*d*, *J* = 4.2, OH–C(3′)); 6.10 (*s*, NH$_2$); 6.33 ('*t*', *J* = 6.3, H–C(1′)); 8.38 (*s*, H–C(8)). Anal. calc. for $C_{13}H_{19}N_5O_4$ (309.33): C 50.48, H 6.19, N 22.64; found: C 50.48, H 6.25, N 22.40.

*9-(2-Deoxy-β-*D*-erythro-pentofuranosyl)-6-isopropoxy-9*H*-purin-2-amine* (**6d**): Colorless foam (118 mg, 76%). TLC (*C*): R_f 0.5. UV (MeOH): 247 (9400), 281 (8900). ^{1}H-NMR ((D$_6$)DMSO): 1.31, 1.33 (2*s*, 2Me); 2.19 (*m*, H$_\alpha$–C(2′)); 2.58 (*m*, H$_\beta$–C(2′)); 3.55 (*m*, 2 H–C(5′)); 3.82 (*m*, H–C(4′)); 4.34 (*m*, H–C(3′)); 5.01 (*t*, *J* = 5.3, OH–C(5′)); 5.26 (*d*, *J* = 3.7, OH–C(3′)); 5.47 (*m*, CHO); 6.20 ('*t*', *J* = 6.2, H–C(1′)); 6.35 (*s*, NH$_2$); 8.05 (*s*, H–C(8)). Anal. calc. for $C_{13}H_{19}N_5O_4$ (309.33): C 50.48, H 6.19, N 22.64; found: C 50.32, H 6.19, N 22.47.

*7-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-6-isopropoxy-7H-purin-2-amine* (**7d**): Colorless foam (128 mg, 83%). TLC (*D*): R_f 0.5. UV (MeOH): 294 (5600). ^{1}H-NMR ((D$_6$)DMSO): 1.33, 1.35 (2*s*, 2Me); 2.28 (*m*, H$_\alpha$–C(2')); 2.44 (*m*, H$_\beta$–C(2')); 3.57 (*m*, 2 H–C(5')); 3.83 (*m*, H–C(4')); 4.29 (*m*, H–C(3')); 5.0 (*t*, *J* = 5.2, OH–C(5')); 5.30 (*d*, *J* = 4.1, OH–C(3')); 5.42 (*m*, CHO); 6.10 (*s*, NH$_2$); 6.30 ('*t*', *J* = 6.2, H–C(1')); 8.37 (*s*, H–C(8)). Anal. calc. for $C_{13}H_{19}N_5O_4$ (309.33): C 50.48, H 6.19, N 22.64; found: C 50.31, H 6.19, N 22.39.

*6-Butoxy-9-(2-deoxy-β-*D*-*erythro*-pentofuranosyl)-9H-purin-2-amine* (**6e**): Colorless foam (124 mg, 77%). TLC (*C*): R_f 0.5. UV (MeOH): 247 (9400), 281 (8500). ^{1}H-NMR ((D$_6$)DMSO): 0.93 (*t*, *J* = 7.3, Me); 2.21 (*m*, H$_\alpha$–C(2')); 2.57 (*m*, H$_\beta$–C(2')); 3.53 (*m*, 2 H–C(5')); 3.98 (*m*, H–C(4')); 4.40 (*m*, H–C(3'), CH$_2$O); 4.99 (*t*, *J* = 5.5, OH–C(5')); 5.25 (*d*, *J* = 3.8, OH–C(3')); 6.21 ('*t*', *J* = 7.0, H–C(1')); 6.61 (*s*, NH$_2$); 8.06 (*s*, H–C(8)).

*6-Butoxy-7-(2-deoxy-β-*D*-*erythro*-pentofuranosyl)-7H-purin-2-amine* (**7e**): Colorless foam (130 mg, 80%). TLC (*D*): R_f 0.5. UV (MeOH): 294 (5800). ^{1}H-NMR ((D$_6$)DMSO): 0.95 (*s*, *J* = 7.3, Me); 1.45, 1.76 (2*m*, 2 CH$_2$); 2.32 (*m*, H$_\alpha$–C(2')); 2.45 (*m*, H$_\beta$–C(2')); 3.59 (*m*, 2 H–C(5')); 3.85 (*m*, H–C(4')); 4.32 (*m*, H–C(3')); 4.42 (*m*, CH$_2$O); 4.97 (*t*, *J* = 5.1, OH–C(5')); 5.28 (*d*, *J* = 3.9, OH–C(3')); 6.10 (*s*, NH$_2$); 6.34 ('*t*', *J* = 6.1, H–C(1')); 8.40 (*s*, H–C(8)).

*9-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-6-isobutoxy-9H-purin-2-amine* (**6f**): Colorless foam (126 mg, 78%). TLC (*C*): R_f 0.5. UV (MeOH): 247 (9400), 281 (9200). ^{1}H-NMR ((D$_6$)DMSO): 0.98, 0.99 (2*s*, 2 Me); 2.21 (*m*, C–H); 2.24 (*m*, H$_\alpha$–C(2')); 2.61 (*m*, H$_\beta$–C(2')); 3.57 (*m*, 2 H–C(5')); 3.84 (*m*, H–C(4')); 4.20 (*m*, H–C(3'), CH$_2$O); 4.99 (*t*, *J* = 5.2, OH–C(5')); 5.25 (*d*, *J* = 3.7, OH–C(3')); 6.23 ('*t*', *J* = 6.5, H–C(1')); 6.37 (*s*, NH$_2$); 8.08 (*s*, H–C(8)). Anal. calc. for $C_{14}H_{21}N_5O_4$ (323.35): C 52.00, H 6.55, N 21.66; found: C 52.12, H 6.52, N 21.57.

*7-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-6-isobutoxy-7H-purin-2-amine* (**7f**): Colorless foam (136 mg, 84%). TLC (*D*): R_f 0.5. UV (MeOH): 294 (5900). ^{1}H-NMR ((D$_6$)DMSO): 1.00, 1.01 (2*s*, 2 Me); 2.10 (*m*, H–C); 2.33 (*m*, H$_\alpha$–C(2')); 2.44 (*m*, H$_\beta$–C(2')); 3.60 (*m*, 2 H–C(5')); 3.86 (*m*, H–C(4')); 4.20 (*m*, CH$_2$O); 4.33 (*m*, H–C(3')); 4.97 (*t*, *J* = 5.1, OH–C(5')); 5.28 (*d*, *J* = 3.7, OH–C(3')); 6.11 (*s*, NH$_2$); 6.37 ('*t*', *J* = 6.1, H–C(1')); 8.41 (*s*, H–C(8)). Anal. calc. for $C_{14}H_{21}N_5O_4$ (323.35): C 52.00, H 6.55, N 21.66; found: C 51.91, H 6.27, N 20.98.

*9-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-6-(pentyloxy)-9H-purin-2-amine* (**6g**): Colorless foam (133 mg, 79%). TLC (*C*): R_f 0.5. UV (MeOH): 247 (9400), 281 (8600). ^{1}H-NMR ((D$_6$)DMSO): 0.89 (*t*, *J* = 7.0, Me); 1.35, 1.73 (2*m*, 3 CH$_2$); 2.24 (*m*, H$_\alpha$–C(2')); 2.59 (*m*, H$_\beta$–C(2')); 3.55 (*m*, 2 H–C(5')); 3.85 (*m*, H–C(4')); 4.40 (*m*, H–C(3'), CH$_2$O); 5.01 (*t*, *J* = 5.2, OH–C(5')); 5.27 (*d*, *J* = 3.7, OH–C(3')); 6.23 ('*t*', *J* = 6.1, H–C(1')); 6.36 (*s*, NH$_2$); 8.08 (*s*, H–C(8)).

*7-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-6-(pentyloxy)-7H-purin-2-amine* (**7g**): Colorless foam (127 mg, 75%). TLC (*D*): R_f 0.5. UV (MeOH): 294 (5700). ^{1}H-NMR ((D$_6$)DMSO): 0.91 (*t*, *J* = 7.0, Me); 1.40, 1.77 (2*m*, 3 CH$_2$); 2.32 (*m*, H$_\alpha$–C(2')); 2.44 (*m*, H$_\beta$–C(2')); 3.55 (*m*, 2 H–C(5')); 3.85 (*m*, H–C(4')); 4.10 (*m*, CH$_2$O); 4.31 (*m*, H–C(3')); 4.96 (*t*, *J* = 5.1, OH–C(5')); 5.27 (*d*, *J* = 3.8, OH–C(3')); 6.10 (*s*, NH$_2$); 6.34 ('*t*', *J* = 6.3, H–C(1')); 8.40 (*s*, H–C(8)).

*9-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-6-(2,2-dimethylpropoxy)-9H-purin-2-amine* (**6h**): Colorless foam (133 mg, 79%). TLC (*C*): R_f 0.5. UV (MeOH): 247 (9400), 281 (8800). ^{1}H-NMR ((D$_6$)DMSO): 0.89 (*t*, *J* = 7.0, Me); 1.35, 1.73 (2*m*, 3 CH$_2$); 2.24 (*m*, H$_\alpha$–C(2')); 2.59 (*m*, H$_\beta$–C(2')); 3.55 (*m*, 2 H–C(5')); 3.85 (*m*, H–C(4')); 4.40 (*m*, H–C(3'), CH$_2$O); 5.01 (*t*, *J* = 5.2, OH–C(5')); 5.27 (*d*, *J* = 3.7, OH–C(3')); 6.23 ('*t*', *J* = 6.1, H–C(1')); 6.36 (*s*, NH$_2$); 8.08 (*s*, H–C(8)).

*7-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-6-(2,2-dimethylpropoxy)-7H-purin-2-amine* (**7h**): Colorless foam (127 mg, 75%). TLC (*D*): R_f 0.5. UV (MeOH): 294 (5900). ^{1}H-NMR ((D$_6$)DMSO): 0.91 (*t*, *J* = 7.0, Me); 1.40, 1.77 (2*m*, 3 CH$_2$); 2.32 (*m*, H$_\alpha$–C(2')); 2.44 (*m*, H$_\beta$–C(2')); 3.55 (*m*, 2 H–C(5')); 3.85 (*m*, H–C(4')); 4.10 (*m*, CH$_2$O); 4.31 (*m*, H–C(3')); 4.96 (*t*, *J* = 5.1, OH–C(5')); 5.27 (*d*, *J* = 3.8, OH–C(3')); 6.10 (*s*, NH$_2$); 6.34 ('*t*', *J* = 6.3, H–C(1')); 8.40 (*s*, H–C(8)).

*9-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-6-(2-methoxyethoxy)-9H-purin-2-amine* (**6i**): Colorless foam (140 mg, 86%). TLC (*C*): R_f 0.5. UV (MeOH): 247 (9400), 281 (8600). ^{1}H-NMR ((D$_6$)DMSO): 2.23 (*m*, H$_\alpha$–C(2')); 2.59 (*m*, H$_\beta$–C(2')); 3.31 (*s*, MeO); 3.70 (*m*, CH$_2$O); 3.58 (2 H–C(5')); 3.70 (*m*, CH$_2$O); 3.84 (*m*, H–C(4')); 4.37 (*m*, H–C(3')); 6.23 ('*t*', *J* = 5.9, H–C(1')); 6.40 (*s*, NH$_2$); 8.09 (*s*, H–C(8)). Anal. calc. for $C_{13}H_{19}N_5O_5$ (325.32): C 48.00, H 5.89, N 21.53; found: C 48.10, H 5.95, N 21.39.

*7-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-6-(2-methoxyethoxy)-7H-purin-2-amine* (**7i**): Colorless foam (132 mg, 81%). TLC (*D*): R_f 0.5. UV (MeOH): 294 (5700). ^{1}H-NMR ((D$_6$)DMSO): 2.33 (*m*, H$_\alpha$–C(2')); 2.46 (*m*, H$_\beta$–C(2')); 3.31 (*s*, MeO); 3.72 (*m*, CH$_2$O); 3.55 (2 H–C(5')); 3.72 (*m*, CH$_2$O); 3.85 (*m*, H–C(4')); 4.31 (*m*, H–C(3')); 6.13 (*s*, NH$_2$); 6.33 ('*t*', *J* = 6.2, H–C(1')); 8.41 (*s*, H–C(8)). Anal. calc. for $C_{13}H_{19}N_5O_5$ (325.33): C 48.00, H 5.89, N 21.53; found: C 48.03, H 5.88, N 21.50.

5. *2-Amino-7-(2-deoxy-β-*D*-*erythro*-pentofuranosyl)-7H-purin-6-one* (**1**): Compound **7d** (170 mg, 0.55 mmol) was treated with 2N aq. NaOH (40 ml) at 50° for 100 h. The soln. was cooled, neutralized with AcOH, diluted with

H_2O (250 ml), and applied to *Serdolite AD-4* (column 15 × 5 cm). The column was washed with H_2O (300 ml) and **1** eluted with i-PrOH/H_2O 9:1. Evaporation afforded **1** (82%). Anal. data: identical with those reported [6].

6. N-*[7-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-6-methoxy-7H-purin-2-yl]formamide* (**9**): A soln. of **7a** (300 mg, 1.07 mmol) in MeOH (30 ml) was stirred with *N,N*-dimethylformamide dimethyl acetal (2 ml, 12 mmol) at 50° for 4 h. Then H_2O (20 ml) was added and stirring continued for another 24 h (50°). The soln. was evaporated and the oily residue co-evaporated with acetone (10 ml, twice) and applied to FC (silica gel, column 15 × 4 cm, *D*), Evaporation of the main zone afforded an amorphous powder (199 mg, 60%). TLC (*D*): R_f 0.6. UV (MeOH): 294 (7500). ^{1}H-NMR ((D$_6$)DMSO): 2.37 (*m*, H$_\alpha$–C(2')); 2.55 (*m*, H$_\beta$–C(2')); 3.55 (*m*, 2 H–C(5')); 3.88 (*m*, H–C(4')); 4.35 (*m*, H–C(3')); 4.96 (*t*, *J* = 5.0, OH–C(5')); 5.33 (*d*, *J* = 3.7, OH–C(3')); 6.41 ('*t*', *J* = 6.3, H–C(1')); 8.70 (*s*, H–C(8)); 9.40 (*d*, *J* = 9.2, CHO); 10.72 (*d*, *J* = 9.7, NH). Anal. calc. for $C_{12}H_{15}N_5O_5$ (309.29): C 46.60, H 4.89, N 22.64; found: C 46.70, H 4.81, N 22.53.

7. N-{*7-{5-O-[Bis(4-methoxyphenyl)phenylmethyl]-2-deoxy-β-*D*-erythro-pentofuranosyl}-6-methoxy-7H-purin-2-yl}formamide* (**10**): Compound **9** was dried by repeated co-evaporation with anh. pyridine and suspended in dry pyridine (2 ml). The soln. was stirred in the presence of 4-(dimethylamino)pyridine (10 mg, 0.08 mmol) and bis(4-methoxyphenyl)phenylmethyl chloride (329 mg, 0.97 mmol) for 5 h. The mixture was diluted with 5% aq. $NaHCO_3$ soln. (20 ml) and extracted with CH_2Cl_2 (3 × 20 ml). The combined org. layer was dried (Na_2SO_4), the solvent evaporated, and the residue chromatographed (silica gel, column 15 × 3 cm, *B*): colorless foam (269 mg, 55%). TLC (*A*): R_f 0.6. UV (MeOH): 294 (7600). ^{1}H-NMR ((D$_6$)DMSO): 2.39 (*m*, H$_\alpha$–C(2')); 2.67 (*m*, H$_\beta$–C(2')); 3.16 (*m*, 2 H–C(5')); 3.72 (2*s*, MeO); 4.0 (*m*, H–C(4'), MeO); 4.34 (*m*, H–C(3')); 5.40 (*d*, *J* = 4.6, OH–C(3')); 6.44 ('*t*', *J* = 6.3, H–C(1')); 6.78–7.34 (3*m*, arom. H); 8.55 (*s*, H–C(8)); 9.42 (*d*, *J* = 9.8, CHO); 10.76 (*d*, *J* = 9.9, NH). Anal. calc. for $C_{33}H_{29}N_5O_5$ (575.62): C 64.80, H 5.44, N 11.45; found: C 64.37, H 5.62, N 11.50.

8. N-{*7-{5-O-[Bis(4-methoxyphenyl)phenylmethyl]-2-deoxy-β-*D*-erythro-pentofuranosyl}-6-methoxy-7H-purin-2-yl}formamide 3'-[(2-Cyanoethyl)* N,N-*Diisopropylphosphoramidite]* (**11**). To a soln. of **10** (100 mg, 0.16 mmol) and (i-Pr)$_2$EtN (50 µl, 0.28 mmol) in anh. CH_2Cl_2 (2 ml), chloro(2-cyanoethoxy)(diisopropylamino)phosphine (133 µl, 0.51 mmol) was added at r.t. After stirring for 30 min, the mixture was diluted with CH_2Cl_2 (10 ml) and quenched by adding 5% $NaHCO_3$ soln. (20 ml). Then the aq. layer was extracted with CH_2Cl_2 (3 × 20 ml), the combined org. layer dried (Na_2SO_4) and evaporated, and the colorless oil applied to FC (silica gel, column 9 × 3 cm, *E*): **11** (64 mg, 52%). Colorless foam. TLC (*E*): R_f 0.4, 0.5. ^{1}H-NMR ($CDCl_3$): 1.08, 1.09 (2*s*, 2 *Me*$_2$CH); 2.43 (*t*, *J* = 6.5, CH_2CH_2CN); 2.59–2.79 (*m*, H$_\alpha$–C(2'), H$_\beta$–C(2')); 3.37–3.63 (*m*, 2 H–C(5'), 2 Me$_2$C*H*); 3.76 (*s*, MeO); 3.87 (*t*, *J* = 6.6, CH_2O); 4.00 (*s*, MeO); 4.29 (*m*, H–C(4')); 4.60 (*m*, H–C(3')); 6.54 ('*t*', *J* = 6.3, H–C(1')); 6.78–7.40 (3*m*, arom. H); 7.85 (*s*, H–C(8)); 8.21 (*d*, *J* = 7.8, CHO); 9.53 (*d*, *J* = 7.9, NH). ^{31}P-NMR ($CDCl_3$): 149.8, 149.6.

9. *Solid-Phase Synthesis of Oligodeoxyribonucleotides.* The synthesis of the oligonucleotides **13**–**38** was carried out on a 1-µmol scale using the 3'-phosphonates of [(MeO)$_2$Tr]T$_d$, [(MeO)$_2$Tr]bz^{6}A$_d$, and [(MeO)$_2$Tr]fam^6*N*^{7}G$_d$ [6], as well as by the 3'-phosphoramidites of [(MeO)$_2$Tr]T$_d$, [(MeO)$_2$Tr]bz^{6}A$_d$, [(MeO)$_2$Tr]bz^{4}C$_d$, [(MeO)$_2$Tr]ibu^{2}G$_d$, [(MeO)$_2$Tr]ibu^{2}c^{7}G$_d$ [32] and [(MeO)$_2$Tr]fam^6*N*^{7}G$_d$ [6], following the regular protocols of the DNA synthesizer for 3'-(hydrogen phosphonates) and 3'-phosphoramidites [26] [27]. The crude oligonucleotides were purified and detritylated on an oligonucleotide-purification cartridge from *Applied Biosystems* following the standard protocols. Selected data of the modified oligonucleotides are shown in *Table 9*.

Table 9. *Selected Data of Modified Oligonucleotides*

	17	**24**	**30**	**32**
Retention time [min][a]	29.5	28.4	26.2	26.5
Yield [%][b]	20	10	43	45
m/z calc.	3613.9	3638.9	3722.5	3646.5
m/z found	3612.9	3637.9	3725.7	3648.7

[a]) The retention times refer to gradient *I*.

[b]) The yields were calculated on the basis of silica-gel-bound nucleosides.

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