N^7-DNA: Base-Pairing Properties of N^7-(2′-Deoxy-β-D-*erythro*-pentofuranosyl)-Substituted Adenine, Hypoxanthine, and Guanine in Duplexes with Parallel Chain Orientation

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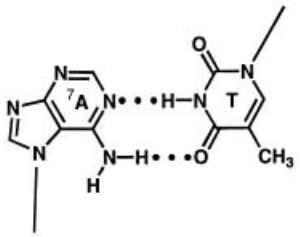
Oligonucleotides containing N^7-(2′-deoxy-β-D-*erythro*-pentofuranosyl)adenine (**1**), -hypoxanthine (**2**), and -guanine (**3**) were synthesized on solid-phase using phosphonate and phosphoramidite chemistry. As part of the synthesis of compound **2**, the nucleobase-anion glycosylation of various 6-alkoxypurines with 2-deoxy-3,5-di-*O*-(4-toluoyl)-α-D-*erythro*-pentofuranosyl chloride (**5**) was investigated. The duplex stability of oligonucleotides containing N^7-glycosylated purines opposite to regular pyrimidines was determined, and thermodynamic data were calculated from melting profiles. Oligodeoxyribonucleotide duplexes containing N^7-glycosylated adenine · T_d or N^7-glycosylated guanine · C_d base pairs are more stable in the case of parallel strand orientation than in the case of antiparallel chains.

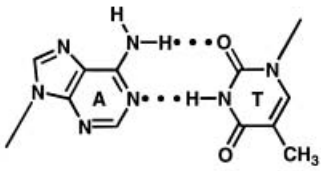
Introduction. – Hydrogen bonding plays an important role in the double-helix stabilization of DNA, and it was postulated that the base recognition which is manifested in the two *Watson-Crick* pairs $A_d \cdot T_d$ and $G_d \cdot C_d$ determines the structure of nucleic acids [1]. Later, intrastrand base stacking was recognized, and it became apparent that also several other factors are influencing base recognition in duplex, triplex, or tetraplex DNA [2]. Modified backbones, such as in hexose-DNA/RNA, *xylo*-DNA, or PNA, form base pairs with altered stability which indicates that the base recognition is changed when the backbone is varied [3–5]. Recently, it has been reported that duplex stability depends on the chain orientation. So, the A_d and T_d residues can form *Watson-Crick* as well as reverse *Watson-Crick* base pairs resulting in duplexes with antiparallel or parallel chain orientation depending on an appropriate sequence selection [6]. Moreover, any single-stranded natural DNA can form a duplex with parallel chain orientation when the second strand contains the four bases adenine, thymine, isoguanine, and isocytosine [7]. Based on the ambiguity of the adenine · thymine pairing, it is expected that not only this regular DNA constituents can lead to parallel hybridization but also those with N^7-glycosylated purines. The *Watson-Crick* base pair of the N^7-glycosylated adenine with thymine is shown by motif **I** and that of the reverse *Watson-Crick* base pair of the N^9-counterpart by motif **II**, both being in the parallel pairing modes (parallel strands (ps)).

The N^7-glycosylated purines have been already incorporated into oligonucleotides [8]. The first report on this matter appeared in 1992 [9]. The self-pairing of the oligonucleotide d[$(N^7A)_8$-T_8][1]) was described [10]. Later, the N^7-glycosylated guanine

[1]) The shortened names 2′-deoxyaden-7-osine (**1**; N^7A_d), 2′-deoxyin-7-osine (**2**; N^7I_d), and 2′-deoxyguan-7-osine (**3**; N^7G_d) stand for N^7-(2′-deoxy-β-D-*erythro*-pentofuranosyl)adenine (**1**), N^7-(2′-deoxy-β-D-erythro-pentofuranosyl)hypoxanthine (**2**), and N^7-(2′-deoxy-β-D-erythro-pentafuranosyl)guanine (**3**), respectively.

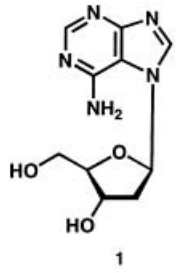


Watson-Crick base pair **I** (ps)



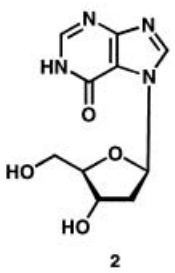
Reverse *Watson-Crick* base pair **II** (ps)

was studied, and base pairing between N^7G_d[1]) and C_d was observed [11][12]. From these investigations, an antiparallel strand orientation (aps) was anticipated. Nevertheless, in the case of d(N^7G-C)$_6$, a parallel chain orientation was discussed [11]. In the meantime, several communications appeared which focus on ps-DNA (ps = parallel stranded) (see *e.g.*, [13]). The work presented here reports on ps duplexes containing the N^7-glycosylated purines **1**–**3**, in particular the base derivatives of adenine, hypoxanthine, or guanine. The thermodynamic stability of such duplexes is investigated and compared with those showing aps-chain orientation. The experiments will present evidence that the base pairing between compounds **1**–**3** and T_d or C_d is favored in oligonucleotides with ps-chain orientation compared to those with antiparallel chains.



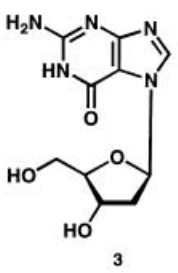
1

2′-Deoxyaden-7-osine[1])(N^7A_d)



2

2′-Deoxyin-7-osine[1])(N^7I_d)



3

2′-Deoxyguan-7-osine[1])(N^7G_d)

Results and Discussion. – *Monomers.* The 2′-deoxyaden-7-osine[1]) (**1**, N^7A_d) was prepared according to [8]. The synthesis of 2′-deoxyguan-7-osine[1]) (**3**, N^7G_d) followed a route described earlier by us [11] and that of 2′-deoxyin-7-osine[1]) (**2**; N^7I_d) has been carried out earlier by nucleobase-anion glycosylation of 6-methoxypurine (**4a**) or from 6-chloropurine *via* the ribonucleoside [14][15].

During the synthesis of 2′-deoxyguan-7-osine (**3**), it was found that the ratio of regioisomers formed during nucleobase-anion glycosylation can be shifted towards the N^7-isomer when 6-alkoxypurin-2-amines are used carrying bulky alkoxy groups instead of small substituents as in 6-methoxypurin-2-amine [12]. Consequently, the glycosylation of various 6-alkoxypurines was studied with the aim to increase the yield of the N^7-isomer. The alkoxy derivatives **4b**–**e** were obtained from 6-chloropurine and sodium alkoxides [16] and glycosylated with 2-deoxy-3,5-di-*O*-(4-toluoyl)-α-D-*erythro*-pentofuranosyl chloride (**5**) *via* the nucleobase anions [17–19] (*Scheme 1*). The

obtained nucleosides **6b–e** and **7b–e** were separated by flash chromatography. According to *Table 1*, the yield of the N^7-isomers increased with increasing size of the alkoxy substituent (from **4a** to **4d**); however, the effect is moderate. Compounds **6b–e** and **7b–e** were deblocked (NaOMe/MeOH) furnishing the alkoxy-nucleosides **8b–e** and **9b–e**, respectively. Further treatment of **9d** with 2N NaOH at 50° displaced the isopropoxy group and yielded the nucleoside **2**.

Scheme 1

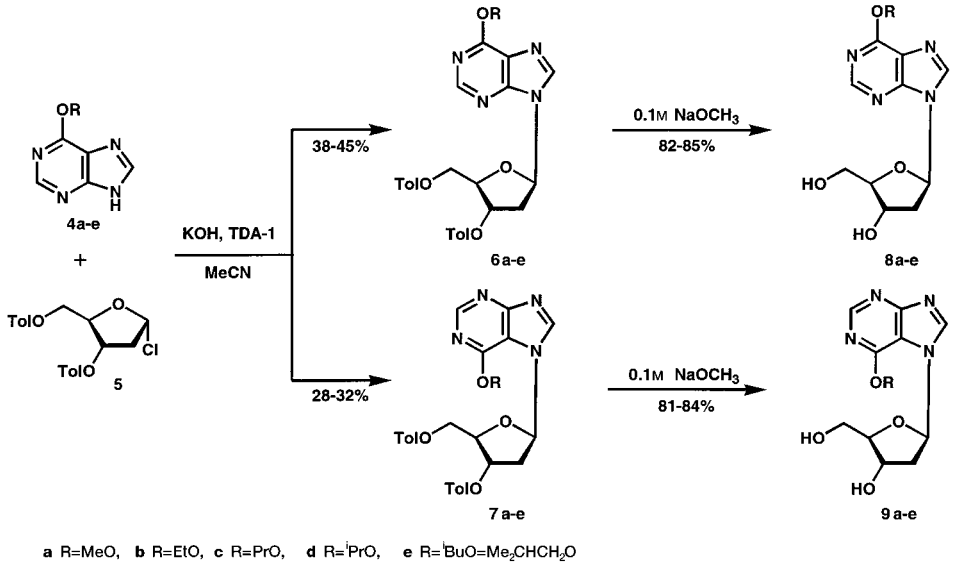


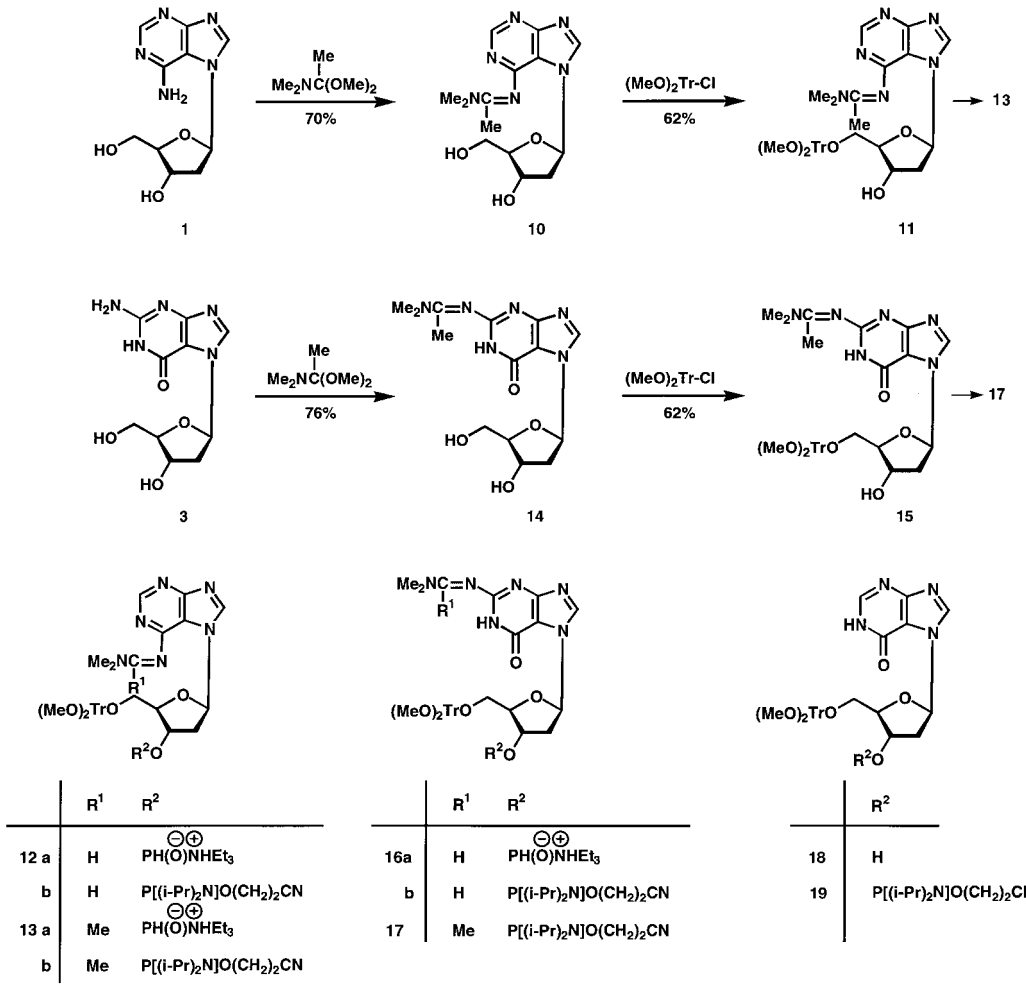
Table 1. *Reaction Yields and Ratios of Regioisomers Obtained on Nucleobase-Anion Glycosylation of 6-Alkoxypurines* **4**[a])

	N^9 [%]	N^7 [%]	N^7/N^9	Yield [%]
4a [14]	48	24	0.50	72
4b	45	28	0.62	73
4c	42	30	0.71	71
4d	38	32	0.84	70
4e	43	38	0.65	71

[a]) Determined after flash column chromatography.

Next, the building blocks for the oligonucleotide solid-phase synthesis were prepared. The phosphonates and phosphoramidites **12a**,**b** and **16a**,**b** of compounds **1** and **3** have already been described [10][11]. As their formamidine (*i.e.*, (dimethylamino)methylidene) protecting groups show rather fast deprotection in alkaline solution, the (dimethylamino)ethylidene residue was introduced as an alternative. Therefore, building blocks **13a**,**b** and **17** were synthesized from **1** and **3**, respectively (*Scheme 2*). The intermediate acetamidine derivatives **10** and **14** as well as **2** were protected at the 5′-OH group with 4,4′-dimethoxytrityl chloride, yielding **11**, **15**, and **18**,

Scheme 2



respectively. From the latter, the phosphoramidites **13b**, **17**, and **19** were prepared by treatment with 2-cyanoethyl diisopropylphosphoramidochloridite. Starting with **11**, also the phosphonate **13a** was prepared.

Unfortunately, only in the 2′-deoxyguan-7-osine (**3**) series, the (dimethylamino)-ethylidene group was suitable for protection, whereas in the 2′-deoxyaden-7-osine (**1**) series the half-life of deprotection in alkaline solution was too long for its efficient use in oligonucleotide synthesis (see **14** and **10**, resp., in *Table 2*). Regarding glycosylic-bond stability, 2′-deoxyaden-7-osine (**1**) is less stable than that of A_d. This is different to the N^7-nucleosides **2** and **3** which are more stable than their N^9-counterparts (*Table 3*).

All monomers were characterized by ^{1}H- and ^{13}C-NMR spectra (*Table 4* and *Exper. Part*). The assignment of the ^{13}C-NMR chemical shifts was based on gated-decoupled spectra (*Table 5*). The change of the glycosylation position from N^9 to N^7 resulted in a

Table 2. *Half-lives of Deprotection of the Amino-Protecting Groups of Compounds* **10** *and* **14** *in Conc. Aq. NH_3 Solution*[a]). $m_2$fa = (dimethylamino)methylidene; $m_2$aca = (dimethylamino)ethylidene.

	$(m_2fa)^2N^7G_d$ [11]	$(m_2aca)^2N^7G_d$ (**14**)	$(m_2fa)^6N^7A_d$ [10]	$(m_2aca)^6N^7A_d$ (**10**)
τ [min]	6	200	17	150[b])
Wavelength [nm]	284	284	310	315

[a]) Measured at 40°. [b]) Measured at 60°.

Table 3. *Half-lives of Glycosylic-Bond Hydrolysis of the* N^7*-Isomers* **1**–**3** *and of Their* N^9*-Counterparts*

	3 (N^7G_d) [11]	G_d [11]	**2** (N^7I_d)	I_d [20]	**1** (N^7A_d) [8]	A_d [21]
Half-life [min]	35[a]) [c])	11[a]) [c])	35[a]) [d])	27[b]) [d])	4.7[b]) [d])	95[a]) [d])

[a]) Determined by HPLC at 30°. [b]) Determined UV-spectrophotometrically at 30°. [c]) In 0.5N HCl. [d]) in 0.1N HCl.

Table 4. *^{13}C-NMR Chemical Shifts of Purine 2′-Deoxyribonucleosides*[a])[b]). Purine numbering.

	C(2)	C(4)	C(5)	C(6)	C(8)	OR		C(1′)	C(2′)	C(3′)	C(4′)	C(5′)
4b	151.2	155.0	118.1	158.9	142.4	62.2	**6b**	84.3	35.6	74.9	81.9	64.0
4c	151.7	156.9	119.3	159.8	144.4	68.5	**7b**	86.4	38.0	74.7	81.9	64.2
4d	152.1	156.1	119.3	159.5	143.6	70.1	**6c**	84.1	35.5	74.8	81.7	63.8
4e	152.1	156.2	118.9	160.0	143.6	73.0	**7c**	86.4	37.9	74.7	81.9	64.0
6b	151.8	151.7	121.5	160.2	142.7	62.7	**6d**	84.1	39.0	74.8	81.7	63.9
7b	151.9	161.9	111.6	156.2	144.7	63.0	**7d**	86.4	38.2	74.6	81.8	64.1
6c	151.6	151.5	121.3	160.2	142.4	68.0	**6e**	84.2	35.5	74.8	81.9	64.0
7c	151.7	161.5	111.6	156.3	144.1	68.2	**7e**	86.3	37.6	74.9	82.0	64.0
6d	151.6	151.6	121.5	159.7	142.3	69.6	**8b**	83.9	[d])	70.7	88.0	61.6
7d	151.7	161.7	111.7	155.8	144.1	70.3	**9b**	86.5	41.4	69.9	88.0	61.1
6e	151.8	151.7	121.4	160.1	142.6	73.4	**8c**	83.9	[d])	70.8	88.0	61.5
7e	151.6	161.3	111.7	156.3	144.8	73.4	**9c**	86.5	41.4	69.8	88.0	61.0
8b	151.5	151.5	121.1	160.0	142.0	62.5	**8d**	83.9	[d])	70.6	88.0	61.6
9b	151.5	161.5	111.5	156.1	144.2	62.7	**9d**	86.6	41.8	70.0	88.0	61.0
8c	151.5	151.5	121.4	160.0	142.1	68.0	**8e**	83.9	[d])	70.8	88.0	61.0
9c	151.4	161.4	111.5	156.3	144.1	68.1	**9e**	86.4	41.2	69.6	87.8	60.8
8d	151.5	161.6	121.2	159.7	142.0	69.6	**10**	86.0	42.0	70.0	87.7	61.7
9d	151.4	161.6	111.6	155.9	144.0	69.7	**11**	85.6[c])	41.1	70.0	85.5[c])	63.9
8e	151.5	151.5	121.3	159.8	142.0	73.4	**13a**	85.7[c])	[d])	71.9	85.6[c])	63.5
9e	151.3	161.3	111.4	156.3	143.9	73.4	**14**	85.6	41.3	70.4	87.9	61.6
10	152.0	160.0	116.8	154.5	142.9	–	**15**	85.2	[d])	70.5	86.0	64.3
11	152.2	160.1	116.9	154.7	142.5	55.0	**18**	85.5[c])	40.7	70.2	86.0[c])	63.9
13a	152.3	160.0	116.9	154.7	142.4	55.0	**19**	85.7[c])	41.8	73.0	86.5[c])	63.0
14	155.4[c])	159.5[c])	109.7	155.8[c])	141.4	–						
15	155.4[c])	159.5[c])	109.9	155.9[c])	141.0	55.1						
18	144.7	157.5	114.4	154.0	141.2	55.1						
19	144.3	158.3	114.5	155.5	141.0	55.0						

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

Table 5. *J(C,H) Coupling Constants* [Hz] *of Purine 2′-Deoxyribonucleosides*[a])[b])

	8b	**9c**	**9d**	**10**	**14**
J(C(2), *H*–C(2))	204	203	204	197	–
J(C(4), *H*–C(8)) or *J*(C(4), *H*–C(2))	*ddd*	12	13	10	–
J(C(4), *H*–C(8)) or *J*(C(4), *H*–C(2))	*ddd*	13	12	12.4	–
J(C(4), *H*–C(8))	–	–	–	–	8.8
J(C(4), *H*–C(1′))	*ddd*	–	–	–	–
J(C(5), *H*–C(8))	12	–	–	–	–
J(C(5), *H*–C(8)) or *J*(C(5), *H*–C(1′))	–	4.2	4.3	3.9	n.r.[d])
J(C(6), *H*–C(2))	6.3	11.8	12	11.4	–
J(C(6), CHO)	2.9	n.r.[d])	2.9	–	–
J(C(8), *H*–C(8))	213	212	213	210	211
J(C(8), *H*–C(1′))	4	3.8	3.5	3.6	4.4
J(C(1′), *H*–C(1′))	166	169	170	169	161
J(C(2′), *H*–C(2′))	[c])	132	134	133	[c])
J(C(3′), *H*–C(3′))	148	152	148	148	149
J(C(4′), *H*–C(4′))	147	146	147	147	147
J(C(5′), *H*–C(5′))	139	139	140	139	140
J(*C*HO–C(6), C*H*O–C(6))	140	144	149	–	–
J(Me*C*=N, *Me*C=N)	–	–	–	*m*	*m*

[a]) From ^{13}C-NMR spectra measured in (D_6)DMSO at 23°. [b]) Purine numbering. [c]) Superimposed by DMSO. [d]) n.r., not resolved.

significant downfield shift of C(4) (10 ppm) and an equivalent upfield shift of C(5) which is in line with data reported earlier for the corresponding 6-alkoxypurin-2-amine nucleosides [12]. In case of the 6-alkoxypurine bases **4b**–**e**, the ^{13}C-NMR fusion-site signals did not coincide with the signals of either the N^7- or the N^9-nucleosides, indicating that a tautomeric mixture of the bases was present in DMSO solution. The ^{1}H,^{13}C coupling of C(8) with the anomeric proton indicated N^7- or N^9-glycosylation, ruling out the N^3-isomer. The coupling constants of the sugar C-atoms were used to differentiate between the C(1′) and C(4′) signals.

Oligonucleotides: Synthesis and Characterization. The synthesis of the oligonucleotides **20**–**57** was performed using either the phosphonates **12a**, **13a**, and **16a** or the phosphoramidites **12b**, **13b**, **16b**, **17**, and **19** (see *Scheme 2*) [9][10]. The methodology followed the standard protocols of solid-phase technique using the trityl-on mode [22–24]. The coupling efficiency of the modified building blocks was similar to that found for the regular compounds. The oligonucleotides were detritylated and purified in one step using oligonucleotide-purification cartridges [25]. The composition of the oligonucleotides was confirmed by tandem hydrolysis with snake-venom phosphodiesterase and alkaline phosphatase as described [26]. Representative examples of the composition pattern are shown in *Fig. 1*. Also the MALDI-TOF mass spectra were taken to determine the molecular mass (*Table 6*).

Oligonucleotides Containing $N^7A_d \cdot T_d$ *Base Pairs.* It has been reported that oligonucleotides containing N^7A_d (**1**) form base pairs with T_d [10][11]. In the case of the 'homooligomer' 5′-d[(N^7A)$_{11}$-A]-3′ with 5′-d(T_{12})-3′, a duplex with a relatively low T_m value (22°) was obtained. From examination of the block oligomer 5′-d[(N^7A)$_8$-T_8]-3′ (T_m 35°), an antiparallel strand orientation was suggested. To study the base-pair selectivity, nucleoside **1** was incorporated into both strands of the non-self-comple-

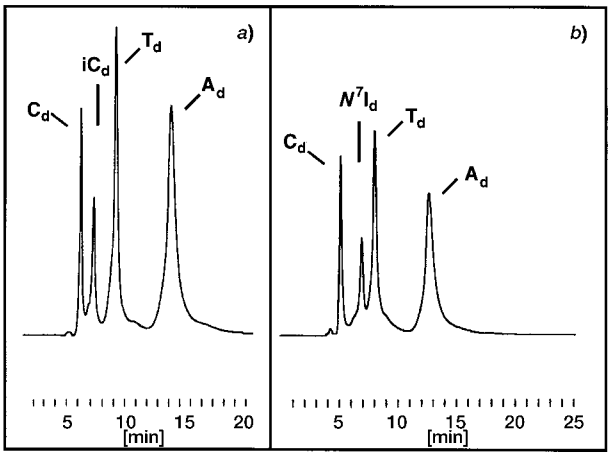


Fig. 1. *Reversed-phase HPLC* (*RP-18*) *profiles of the reaction products obtained after enzymatic hydrolysis of the oligonucleotides* a) **47** *and* b) **43** *by snake-venom phosphodiesterase at 37° after subsequent addition of alkaline phosphatase in* I*M Tris·HCl buffer* (pH 8.3). Solvent system for HPLC, *I*; for details, see *Exper. Part*.

Table 6. *Molecular Mass Oligonucleotides Determined by MALDI-TOF Mass Spectra*[a])

	M^+ (calc.)	M^+ (found)
5′-d(TAGGTCAATACT)-3′ (**20**)	3646.5	3645.6
5′-d(TAGGTC^{7}A^{7}ATACT)-3′ (**22**)	3646.5	3647.7
3′-d(AT^{7}A^{7}AAGTTATGA)-5′ (**23**)	3692.4	3693.6
5′-d(ATiCiCAGTTATGA)-3′ (**27**)	3672.4	3670.9
5′-d(TAGGTiC^{7}A^{7}ATAicT)-3′ (**28**)	3672.4	3673.4
5′-d(T^{7}AGGTiC^{7}A^{7}AT^{7}AiCT)-3′ (**29**)	3672.4	3673.9
5′-d(T^{7}AGGTC^{7}A^{7}AT^{7}ACT)-3′ (**31**)	3646.5	3650.5
5′-d(ATCC^{7}AGTT^{7}ATGA)-5′ (**32**)	3646.5	3648.3
5′-d(AA^{7}A^{7}AiGCCC^{7}A^{7}AAA)-3′ (**35**)	3640.5	3645.4
5′-d(AA^{7}A^{7}AGCCC^{7}A^{7}AAA)-3′ (**38**)	3640.5	3643.4
5′-d(TA^{7}I^{7}ITCAATACT)-3′ (**43**)	3614.4	3614.1
5′-d(AT^{7}I^{7}IAGTTATGA)-5′ (**44**)	3694.4	3694.3
5′-d(AT^{7}G^{7}GAGTTATGA)-5′ (**49**)	3724.4	3722.6
5′-d(TA^{7}G^{7}GTiCAATAiCT)-3′ (**50**)	3672.4	3673.0
5′-d(ATiCiCA^{7}GTTAT^{7}GA)-3′ (**51**)	3672.4	3672.5

[a]) The short forms ^7A_d, ^7I_d, and ^7G_d are used for N^7A_d, N^7I_d, and N^7G_d, respectively; iC_d = 2′-deoxy-5-methylisocytidine (m^5iC_d), iG_d = 2′-deoxyisoguanosine.

mentary oligonucleotides 5′-d(TAGGTCAATACT)-3′ (**20**) and 3′-d(ATCCAGTTATGA)-5′ (**21**). Mismatches were formed by incorporating G_d, C_d, and A_d opposite to **1**. According to *Table 7*, compound **1** paired selectively with T_d and not with the other regular nucleosides, as suggested by the T_m values as well as by the thermodynamic data.

As mentioned earlier, A_d is able to form *Watson-Crick* as well as reverse *Watson-Crick* base pairs, thus yielding duplexes with antiparallel or parallel strand orientation (aps and ps, resp.). The same can be expected for the base pair N^7A_d (**1**) with T_d

Table 7. T_m *Values and Thermodynamic Data of Duplex Formation of Hybrids Derived from 5′-d(TAGGTCAATACT)-3′* (**20**) · *3′-d(ATCCAGTTATGA)-5′* (**21**) *Containing* N^7A_d *Opposite to* T_d, G_d, C_d, *or* A_d[a]). $^7A_d = N^7A_d$.

	T_m [°]	$\Delta H°$ [kcal/mol]	$\Delta S°$ [cal/mol · K]	$\Delta G°_{298}$ [kcal/mol]
5′-d(TAGGTC^7**A**^{7}**A**TACT)-3′ (**22**) 3′-d(ATCCAG**T T**ATGA)-5′ (**21**)	35	– 71	– 206	– 7.7
5′-d(TA**G G**TCAATACT)-3′ (**20**) 3′-d(AT^7**A**^{7}**A**AGTTATGA)-5′ (**23**)	21	– 45	– 129	– 5.4
5′-d(TA^7**A**^{7}**A**TCAATACT)-3′ (**24**) 3′-d(AT**C C**AGTTATGA)-5′ (**21**)	19	– 41	– 113	– 5.4
5′-d(T**A**GGTC**A A**T**A**CT)-3′ (**20**) 3′-d(A^7**A**CCAG^7**A**^{7}**A**A^7**A**GA)-5′ (**25**)	no sigmoidal melting			

[a]) Measured at 260 nm in 0.1M NaCl, 10 mM $MgCl_2$, and 10 mM Na-cacodylate (pH 7.0) at 5 μmol of single-strand concentration.

resulting in ps or aps duplexes (see **I** and **III**). For this purpose, two sets of oligonucleotide duplexes were prepared (*Table 8*). The hybrids of the upper part of *Table 8* which were derived from 5′-d(TAGGTiCAATAiCT)-3′ (**26**) · 5′-d(ATiCiCAGTTATGA)-3′ (**27**) showed parallel chain orientation, whereas those of the lower part being derivatives of 5′-d(TAGGTCAATACT)-3′ (**20**) · 3′-d(ATCCAGTTATGA)-5′ (**21**) had antiparallel chains. The direction of the strands within the duplex

Table 8. T_m *Values and Thermodynamic Data of Parallel and Antiparallel Duplexes Containing* $N^7A_d \cdot T_d$ *Base Pairs*[a]). $^7A_d = N^7A_d$, $iC_d = m^5iC_d$.

		T_m [°]	$\Delta H°$ [kcal/mol]	$\Delta S°$ [cal/mol · K]	$\Delta G°_{298}$ [kcal/mol]
ps Series:	5′-d(TAG G TiCAATAiCT)-3′ (**26**) 5′-d(ATiCiCAG T TATGA)-3′ (**27**)	36 (39)	– 70 (– 68)	– 202 (– 195)	– 7.7 (– 8.4)
	5′-d(TAG G TiC^7**A**^{7}**A**TAiCT)-3′ (**28**) 5′-d(ATiCiCAG **T T**ATGA)-3′ (**27**)	32 (35)	– 57 (– 60)	– 159 (– 169)	– 7.2 (– 7.4)
	5′-d(T^7**A**G G TiC^7**A**^{7}**A**T^7**A**iCT)-3′ (**29**) 5′-d(A**T**iCiCAG **T T** A **T** GA)-3′ (**27**)	32 (33)	– 63 (– 61)	– 182 (– 175)	– 7.0 (– 7.1)
	5′-d(TAG G **T**iCAA **T** AiCT)-3′ (**26**) 5′-d(ATiCiC^7**A**G T T^7**A** T GA)-3′ (**30**)	34 (35)	– 61 (– 59)	– 173 (– 166)	– 7.6 (– 7.7)
	5′-d(T^7**A**G G T iC^7**A**^{7}**A** T ^{7}**A**iCT)-3′ (**29**) 5′-d(A**T**iCiC^7**A**G **T T** ^{7}**A** T GA)-3′ (**30**)	24 (27)	– 65 (– 66)	– 221 (– 195)	– 5.5 (– 5.9)
aps Series:	5′-d(TAGGTCAATACT)-3′ (**20**) 3′-d(ATCCAGTTATGA)-5′ (**21**)	47 (50)	– 82 (– 90)	– 230 (– 252)	– 10 (– 12)
	5′-d(TAGGTC^7**A**^{7}**A**TACT)-3′ (**22**) 3′-d(ATCCAG **T T** ATGA)-5′ (**21**)	35 (38)	– 71 (– 81)	– 206 (– 235)	– 7.7 (– 8.8)
	5′-d(T^7**A**GGTC^7**A**^{7}**A**T^7**A**CT)-3′ (**31**) 3′-d(A**T**CCAG **T T** A **T** GA)-5′ (**21**)	26 (29)	– 56 (– 55)	– 162 (– 157)	– 5.8 (– 6.6)
	5′-d(T^7**A**GG **T** C^7**A**^{7}**A** **T** ^{7}**A**CT)-3′ (**31**) 3′-d(A**T**CC^7**A**G **T T** ^{7}**A** **T** GA)-5′ (**32**)	(20)	(– 65)	(– 194)	(– 4.3)

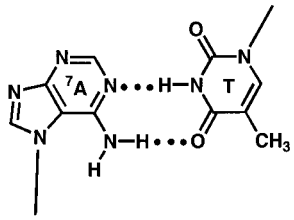
[a]) Measured at 260 nm in 0.1M NaCl, 10 mM $MgCl_2$, and 10 mM Na-cacodylate (pH 7.0) at 5 μmol of single-strand concentration. Data in parentheses measured at 260 nm in 1M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.0) at 5 μmol single-strand concentration.

structures is controlled by the sequence and the fact that fully matched duplexes can be only obtained when they contain $G_d \cdot C_d$ base pairs in the antiparallel case and $iC_d \cdot G_d$ or $iG_d \cdot C_d$ base pairs when they are parallel ($iC_d = m^5iC_d$, $iG_d = 2'$-deoxyisoguanosine). Within one series of oligonucleotides (aps or ps) either one strand or both were modified with N^7A_d (**1**) (*Table 8*). The duplexes **20**·**21** and **26**·**27** were used as references for duplex stability. For comparison, the T_m values were measured at low salt (0.1M NaCl, 10 mM $MgCl_2$, 10 mM Na-cacodylate) and high salt conditions (1.0M NaCl, 100 mM $MgCl_2$, 60 mM Na-cacodylate). Before the data of *Table 8* are compared, it has to be born in mind that aps duplexes are generally more stable than those with ps-chain orientation (**20**·**21** *vs.* **26**·**27**). According to *Table 8*, the $N^7A_d \cdot T_d$ base pair when incorporated in one strand of a ps duplex showed a similar stability as that of an $A_d \cdot T_d$ pair. The situation was different when the $N^7A_d \cdot T_d$ base pair was part of an aps duplex. In this case, a substantial loss of stability was observed compared to the reference duplex **20**·**21** indicating an unfavorable situation. In both series (aps and ps), the incorporation of N^7A_d residues in both strands of a duplex had a more serious effect than when only one strand was modified. The changes of the ΔG values of duplex formation showed a much larger change in the case of the aps duplexes than in the case of the ps chains. An interpretation of enthalpic and entropic data is difficult. Apart from the oligonucleotides shown in *Table 8*, another set of compounds was studied which contained four stable $G_d \cdot C_d$ or $iG_d \cdot C_d$ base pairs in the center and the less stable $A_d \cdot T_d$ or $N^7A_d \cdot T_d$ pairs at the termini (*Table 9*). Again the $N^7A_d \cdot T_d$ base pair was more stable in duplexes with ps orientation than in the aps hybrids. It is interesting that even a combination of N^7A_d (**1**) and A_d residues in one strand of the duplex was tolerated. Regarding the thermodynamic data, the enthalpic changes induced by the incorporation of compound **1** were small in the case of ps duplexes compared to the aps ones. According to the results discussed above, the $N^7A_d \cdot T_d$ base pair (base pair **I**) is well accommodated in a ps duplex, whereas a hybrid with an aps orientation (base pair **III**) becomes much more labile. Apart from the glycosylation position, the base-pair

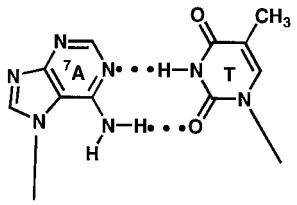
Table 9. T_m *Values and Thermodynamic Data of Duplex Formation of Hybrids Derived from 5′-d(AAAAiGCCCAAAA)-3′ · 5′-d(TTTTCiGiGiGTTTT)-3′ or 5′-d(AAAAGCCCAAAA)-3′ · 3′-d(TTTTCGGGTTTT)-5′ Containing* N^7A_d *or/and* $A_d \cdot T_d$ *Base Pairs*[a]). $^7A_d = N^7A_d$, $iG_d = 2'$-deoxyisoguanosine.

	T_m [°]	$\Delta H°$ [kcal/mol]	$\Delta S°$ [cal/mol·K]	$\Delta G°_{298}$ [kcal/mol]
5′-d(AAAAiG C C C AAAA)-3′ (**33**) 5′-d(T T T T C iGiGiG T T T T)-3′ (**34**)	50	−58	−152	−10.6
5′-d(AA^7**A**^{7}**A**iG C C C ^{7}**A**^{7}**A**AA)-3′ (**35**) 5′-d(T T **T T** C iGiGiG **T T** T T)-3′ (**34**)	47	−60	−162	−9.5
5′-d(AAAAG C C C AAAA)-3′ (**36**) 3′-d(T T T T C G G G T T T T)-5′ (**37**)	52	−94	−262	−12.3
5′-d(AA^7**A**^{7}**A**G C C C ^{7}**A**^{7}**A**AA)-3′ (**38**) 3′-d(T T **T T** C G G G **T T** T T)-5′ (**37**)	39	−65	−182	−8.2
5′-d(AA^7**A**^{7}**A**iG C C C ^{7}**A**^{7}**A**AA)-3′ (**35**) 3′-d(T T **T T** C iGiGiG **T T** T T)-5′ (**39**)	34	−61	−175	−7.2

[a]) Measured at 260 nm in 1M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.0) at 5 μmol of single-strand concentration.



Watson-Crick base pair **I** (ps)



Reverse *Watson-Crick* base pair **III** (aps)

motif **I** is actually the motif which is found in aps-DNA with a H-bond of the amino group of adenine to the 4-keto group of thymine (*Watson-Crick* base pair).

Oligonucleotides Containing $N^7I_d \cdot C_d$ Base Pairs. Earlier, the pairing mode of N^7I_d (**2**) embedded in the third strand of an oligonucleotide triplex was investigated [27–29]. Similar work with N^7G_d (**3**) has been reported before [30][31]. One outcome of these investigations was the formation of stable triplexes when neutral N^7G_d (**3**) replaced the protonated C_d [30][31] and even N^7I_d which is expected to form only one classical H-bond resulting in rather stable triplex structures [28]. Base-pairing studies of N^7I_d (**2**) with G_d, c^7G_d (7-deaza-2′-deoxyguanosine), or N^7G_d (**3**) in duplexes with antiparallel chains have already been performed in our laboratory [32][33].

As it is known from a number of studies, the normal I_d shows ambiguous base-pairing properties [34–36]. Nevertheless, the base pair with C_d is significantly more stable than those with the other 3 common bases. This is due to the fact that I_d acts as deamino analogue of G_d. When N^7I_d (**2**) was replacing I_d in duplexes with antiparallel chain orientation, they became strongly destabilized (upper part of *Table 10*). This is due to a much less favorable term of enthalpy. As mentioned in the chapter before, also in this case, the T_m values of duplex formation were measured under low salt and high salt conditions. Other bases which were located opposite to N^7I_d (**2**) or I_d are G_d, c^7G_d (7-deaza-2′-deoxyguanosine) and iC_d ($= m^5iC_d$; 2′-deoxy-5-methylisocytidine) [37][38] (lower part of *Table 10*). When N^7I_d (**2**) was located opposite G_d, c^7G_d, or iC_d, more stable duplexes were observed than when I_d was positioned opposite to G_d. The $N^7I_d \cdot G_d$ base pair was enthalpically more stable than the $I_d \cdot G_d$ base pair (*Table 10*).

The situation is different when N^7I_d (**2**) was incorporated to parallel duplexes. To obtain the parallel chain orientation, the two $G_d \cdot C_d$ base pairs of the standard aps duplex **20**·**21** were replaced by $I_d \cdot iC_d$ pairs, and two $G_d \cdot C_d$ are replaced by two $G_d \cdot iC_d$ pairs, yielding the duplex **27**·**48** (*Table 11*). This resulted in the formation of an already rather labile hybrid. Now the N^7-nucleoside **2** was introduced, and the two $I_d \cdot iC_d$ base pairs were replaced by two $N^7I_d \cdot C_d$ pairs (see **44**·**47**). Compared to the reference hybrid **27**·**48** the decrease of the T_m value was only 6° in the ps case (*Table 11*), whereas the aps duplexes **41**·**44** and **41**·**42** showed a T_m difference of 17°. These findings are in line with those found for N^7A_d (**1**). The most likely pairing motifs for N^7I_d (**2**) and I_d are **IV**–**VII**. Only in the case of motiv **IV** (ps chains) and motif **VII** (aps chains), strong base pairs can be expected (two H-bonds), whereas the motifs **V** and **VI** should be rather labile. This agrees with the enthalpic data shown in *Table 11* (see $\Delta\Delta H$ of **27**·**48** and **44**·**47** *vs.* **41**·**42** and **41**·**44**).

Table 10. T_m *Values and Thermodynamic Data of Duplex Formation of Hybrids Derived from 5′-d(TAGGTCAATACT)-3′* (**20**) · *3′-d(ATCCAGTTATGA)-5′* (**21**) *Containing* $N^7I_d \cdot C_d$ *or* $I_d \cdot C_d$ *Base Pairs*[a]). $^7I = N^7I_d$, $iC_d = m^5iC_d$.

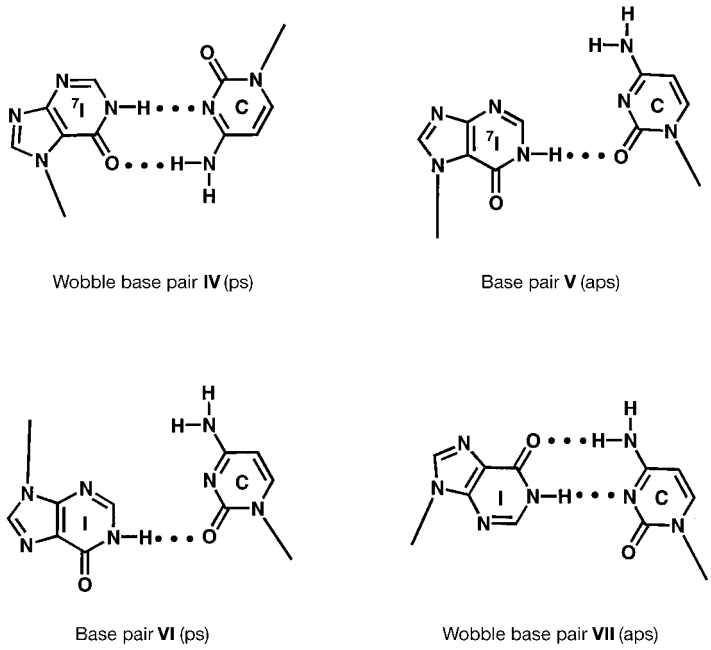
	T_m [°]	$\Delta H°$ [kcal/mol]	$\Delta S°$ [cal/mol · K]	$\Delta G°_{298}$ [kcal/mol]
5′-d(TA**I I**TCAATACT)-3′ (**40**) 3′-d(AT**CC**AGTTATGA)-5′ (**21**)	36 (n.d.)	– 79 (n.d.)	– 231 (n.d.)	– 7.6 (n.d.)
5′-d(TA**CC**TCAATACT)-3′ (**41**) 3′-d(AT**I I**AGTTATGA)-5′ (**42**)	35 (41)	– 95 (– 93)	– 280 (– 272)	– 7.6 (– 9.0)
5′-d(TA^7**I**^{7}**I**TCAATACT)-3′ (**43**) 3′-d(AT**CC**AGTTATGA)-5′ (**21**)	20 (27)	– 42 (– 52)	– 117 (– 174)	– 5.3 (– 6.0)
5′-d(TA**CC**TCAATACT)-3′ (**41**) 3′-d(AT^7**I**^{7}**I**AGTTATGA)-5′ (**44**)	19 (26)	– 49 (– 54)	– 140 (– 154)	– 5.2 (– 6.0)
5′-d(TA**GG**TCAATACT)-3′ (**20**) 3′-d(AT**I I**AGTTATGA)-5′ (**42**)	21 (24)	– 41 (– 45)	– 114 (– 127)	– 5.8 (– 6.2)
5′-d(TA**GG**TCAATACT)-3′ (**20**) 3′-d(AT^7**I**^{7}**I**AGTTATGA)-5′ (**44**)	27 (31)	– 52 (– 50)	– 149 (– 140)	– 6.1 (– 7.0)
5′-d(TA**c**^{7}**G**^{7}**G**TCAATACT)-3′ (**45**) 3′-d(AT ^{7}**I** ^{7}**I** AGTTATGA)-5′ (**44**)	26 (29)	– 52 (– 51)	– 149 (– 142)	– 6.0 (– 6.8)
5′-d(TA**iCiC**TCAATACT)-3′ (**46**) 3′-d(AT^7**I** ^{7}**I**AGTTATGA)-5′ (**44**)	28 (30)	– 63 (– 55)	– 184 (– 154)	– 6.1 (– 6.9)

[a]) Measured at 260 nm in 0.1M NaCl, 10 mM $MgCl_2$, and 10 mM Na-cacodylate (pH 7.0) at 5 μmol of single-strand concentration. Data in parentheses measured at 260 nm in 1M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.0) at 5 μmol single-strand concentration; n.d. not determined.

Table 11. T_m *Values and Thermodynamic Data of Parallel and Antiparallel Duplexes with Base Pairs Containing* N^7I_d *or* I_d[a]). $^7I_d = N^7I_d$, $iCd = m^5iC_d$.

		T_m [°]	$\Delta H°$ [kcal/mol]	$\Delta S°$ [cal/mol · K]	$\Delta G°_{298}$ [kcal/mol]
ps Series:	5′-d(TAGGTiCAATAiCT)-3′ (**26**) 5′-d(ATiCiCAGTTATGA)-3′ (**27**)	36 (36)	– 70 (– 65)	– 202 (– 185)	– 7.7 (– 7.8)
	5′-d(TA**I I**TiCAATAiCT)-5′ (**48**) 5′-d(AT**iCiC**AGTTATGA)-3′ (**27**)	26 (n.d.)	– 64 (n.d.)	– 189 (n.d.)	– 5.7 (n.d.)
	3′-d(TA**CC**TiCAATAiCT)-5′ (**47**) 3′-d(AT^7**I**^{7}**I**AGTTATGA)-5′ (**44**)	20 (25)	– 46 (– 46)	– 132 (– 125)	– 4.9 (– 6.0)
aps Series:	5′-d(TA**GG**T**C**AATA**C**T)-3′ (**20**) 3′-d(AT**CC**A**G**TTAT**G**A)-5′ (**21**)	47 (50)	– 82 (– 90)	– 230 (– 252)	– 10 (– 12)
	5′-d(TA**CC**TCAATACT)-3′ (**41**) 3′-d(AT**I I**AGTTATGA -5′ (**42**)	36 (n.d.)	– 85 (n.d.)	– 250 (n.d.)	– 7.6 (n.d.)
	5′-d(TA**CC**TCAATACT)-3′ (**41**) 3′-d(AT^7**I**^{7}**I**AGTTATGA)-5′ (**44**)	19 (26)	– 49 (– 54)	– 140 (– 154)	– 5.2 (– 6.0)

[a]) Measured at 260 nm in 0.1M NaCl, 10 mM $MgCl_2$, and 10 mM Na-cacodylate (pH 7.0) at 5 μmol of single-strand concentration. Data in parentheses measured at 260 nm in 1M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.0) at 5 μmol single-strand concentration; n.d. not determined.



Wobble base pair **IV** (ps)

Base pair **V** (aps)

Base pair **VI** (ps)

Wobble base pair **VII** (aps)

Oligonucleotides Containing $N^7G_d \cdot C_d$ *Base Pairs.* Earlier, triplex formation of N^7G_d recognizing a $G_d \cdot C_d$ base pair of a duplex structure was described [30][31]. Our laboratory studies the base pairing of N^7G_d (**3**) with various partners in oligonucleotide duplexes with antiparallel chains [12]. Also the base pairing of compound **3** with C_d in the 'self-complementary' duplex 5′-d(N^7G-C)$_6$ was described [11]. In this case, it was not clear whether the duplex showed strands with parallel or antiparallel chain orientation. As shown in the cases of N^7A_d (**1**) and N^7I_d (**2**), also N^7G_d (**3**) was incorporated into the standard duplexes **20** · **21** and **26** · **27** with antiparallel or parallel chains, respectively. At first, the duplex stability of N^7G_d (**3**) with C_d was studied in ps hybrids (see *Table 12*, upper part). The duplex **26** · **27** was chosen as reference and the data determined under high and low salt conditions. When the two iC_d residues were replaced by residue **3**, and C_d was located opposite to N^7G_d (**3**), the resulting ps duplexes **47** · **49**, **50** · **52**, or **20** · **51** showed a similar stability as duplex **26** · **27**. As proof for the efficient base pairing of N^7G_d (**3**) with C_d, another hybrid was examined which was also derived from the duplex **26** · **27** but contained four G_d residues opposite to C_d (duplex **20** · **52**). In such a case, G_d cannot pair with C_d due to a parallel chain orientation. Indeed, no sigmoidal melting was observed for **20** · **52** excluding duplex formation. When iG_d (2′-deoxyisoguanosine) was located opposite to N^7G_d (**3**) (see duplexes **49** · **53** or **54** · **55**), the $N^7G_d \cdot iG_d$ base pair became as stable as that of N^7G_d (**3**) with C_d (*Table 12*). In this case, iG_d is considered to be in the 3*H*-tautomeric form.

Next, duplex stability was studied with hybrids showing antiparallel chain orientation. Compound **3** was located opposite to C_d, iC_d, or G_d. The aps reference duplex (**20** · **21**) showed a T_m value of 47°; when G_d was replaced by N^7G_d (**3**) (duplexes

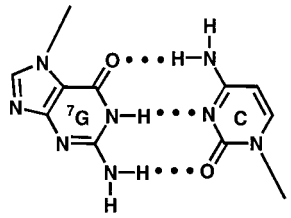
Table 12. T_m *Values and Thermodynamic Data of Parallel and Antiparallel Duplexes with Base Pairs Containing* N^7G_d *or* G_d[a]). $^7G_a = N^7G_d$, $iC_d = m^5iC_d$, $iG_d = 2'$-deoxyisoguanosine.

		T_m [°]	$\Delta H°$ [kcal/mol]	$\Delta S°$ [cal/mol·K]	$\Delta G°_{298}$ [kcal/mol]
ps Series:	5′-d(TA**G G** T**iC**AATA**iC**T)-3′ (**26**) 5′-d(AT**iCiC**A**G**TTAT**G**A)-3′ (**27**)	36 (36)	−70 (−65)	−202 (−185)	−7.7 (−7.8)
	3′-d(TA**C C** TiCAATAiCT)-5′ (**47**) 3′-d(AT^7**G**^{7}**G**AGTTATGA)-5′ (**49**)	34 (36)	−49 (−56)	−135 (−156)	−7.3 (−8.1)
	5′-d(TA^7**G**^{7}**G**TiCAATAiCT)-3′ (**50**) 5′-d(AT**C C**AGTTATGA)-3′ (**52**)	29 (32)	−39 (−47)	−102 (−127)	−7.0 (−7.3)
	5′-d(TAGGT**C**AATA**C**T)-3′ (**20**) 5′-d(ATiCiCA^7**G**TTAT^7**G**A)-3′ (**51**)	35 (35)	−68 (−63)	−193 (−177)	−7.8 (−7.9)
	5′-d(TA**GG**T**C**AATA**C**T)-3′ (**20**) 5′-d(AT**CC**A**G**TTAT**G**A)-3′ (**52**)	no sigmoidal melting			
	3′-d(TA**iGiG**TiCAATAiCT)-5′ (**53**) 3′-d(AT^7**G**^{7}**G**AGTTATGA)-5′ (**49**)	n.d. (35)	n.d. (−38)	n.d. (−100)	n.d. (−7.2)
	5′-d(TAGGT^7**G**AATA^7**G**T)-3′ (**54**) 5′-d(ATiCiCA**iG**TTATi**G**A)-3′ (**55**)	n.d. (32)	n.d. (−32)	n.d. (−80)	n.d. (−7.0)
aps Series:	5′-d(TA**GG**T**C**AATA**C**T)-3′ (**20**) 3′-d(AT**CC**A**G**TTAT**G**A)-5′ (**21**)	47 (50)	−82 (−90)	−230 (−252)	−10 (−12)
	5′-d(TAGGT**iC**AATA**iC**T)-3′ (**26**) 3′-d(ATCCA^7**G**TTAT^7**G**A)-5′ (**56**)	43 (45)	−94 (−91)	−274 (−266)	−9.4 (−9.5)
	5′-d(TA**iCiC**TCAATACT)-3′ (**46**) 3′-d(AT^7**G**^{7}**G**AGTTATGA)-5′ (**49**)	40 (42)	−57 (−47)	−156 (−122)	−8.4 (−9.0)
	5′-d(TAGGT^7**G**AATA^7**G**T)-3′ (**54**) 3′-d(ATCCA**G**TTAT**G**A)-5′ (**21**)	34 (38)	−62 (−67)	−176 (−188)	−7.3 (−8.1)
	5′-d(TA**G G**TCAATACT)-3′ (**20**) 3′-d(AT^7**G**^{7}**G**AGTTATGA)-5′ (**49**)	36 (42)	−61 (−64)	−172 (−179)	−7.9 (−8.7)
	5′-d(TA^7**G**^{7}**G**TCAATACT)-3′ (**56**) 3′-d(AT**C C**AGTTATGA)-5′ (**21**)	25 (27)	−45 (−44)	−127 (−120)	−5.8 (−6.5)
	5′-d(TAGGT**C**AATA**C**T)-3′ (**20**) 3′-d(ATCCA^7**G**TTAT^7**G**A)-5′ (**57**)	21 (23)	−40 (−44)	−112 (−125)	−5.5 (−5.5)

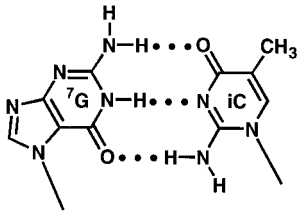
[a]) Conditions, see *Table 11*.

21·**56** and **20**·**57**), the T_m value was decreased by more than 20° (*Table 12*, lower part). The hybrids became again stable when iC_d was located opposite to N^7G_d (**3**) (duplexes **26**·**56** and **46**·**49**); and even in the case of G_d, a base pair with N^7G_d (**3**) was formed (duplexes **21**·**54** and **20**·**49**). In the case of the $N^7G_d \cdot G_d$ base pair (base pair **XIII**), **3** should be in the 3*H*-tautomeric form.

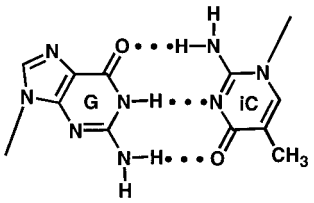
Regarding the various base-pair motifs, strong base pairs were formed when N^7G_d (**3**) paired with C_d or iG_d in duplexes with parallel chain orientation (**47**·**49** and **20**·**51** or **49**·**53** and **54**·**55**, resp.; base-pair motifs **VIII** and **XII**). On the other hand, when N^7G_d (**3**) was located opposite to iC_d, the duplexes became stable when the chain orientation was antiparallel (**26**·**56** and **46**·**49**; base-pair motif **IX**). A stable duplex was also formed when N^7G_d (**3**) was located opposite to G_d (**21**·**54** and **20**·**49**; base-pair motif **XIII**). The aps hybrids containing N^7G_d (**3**) opposite to C_d (duplexes **21**·**56** and **20**·**57**) showed a rather low stability due to unfavorable H-bonding. In nearly all



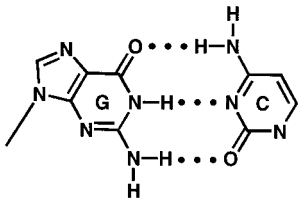
Watson-Crick base pair **VIII** (ps)



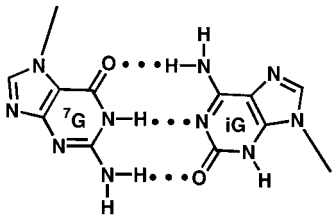
Reverse *Watson-Crick* base pair **IX** (aps)



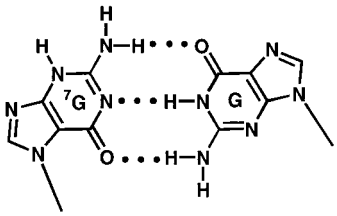
Reverse *Watson-Crick* base pair **X** (ps)



Watson-Crick base pair **XI** (aps)



Watson-Crick purine · purine base pair **XII** (ps)



Reverse *Watson-Crick* – purine · purine base pair **XIII** (aps)

cases, the $N^7G_d \cdot X$ ($X = C_d$, iC_d) base pairs were enthalpically less stable than those of the $G_d \cdot X$ ($X = C_d$, iC_d) counterparts.

From the duplexes containing either N^7- and N^9-glycosylated purines, CD spectra were measured. According to *Fig. 2,a*, the duplexes with antiparallel chain orientation showed the typical spectra of a B-DNA. The CD spectra of duplexes with parallel chain orientation differed significantly (*Fig. 2,b*).

Conclusion. – The N^7-glycosylated nucleobases adenine, hypoxanthine, and guanine, *i.e.*, residues **1**–**3**, respectively, form stable base pairs with the naturally occurring pyrimidine nucleosides T_d or C_d when they are embedded in a duplex with parallel chain orientation. In such ps duplexes, also another strong base pair of N^7G_d (**3**), with iG_d, is observed. When the chain orientation is changed into antiparallel, the duplex with C_d opposite to the residue **3** is labile. Such an aps duplex becomes stabilized when **3** is located opposite to G_d or iC_d. According to this, the N^7-glycosylated purines show a somewhat similar base-pairing behavior as purine nucleosides in which the

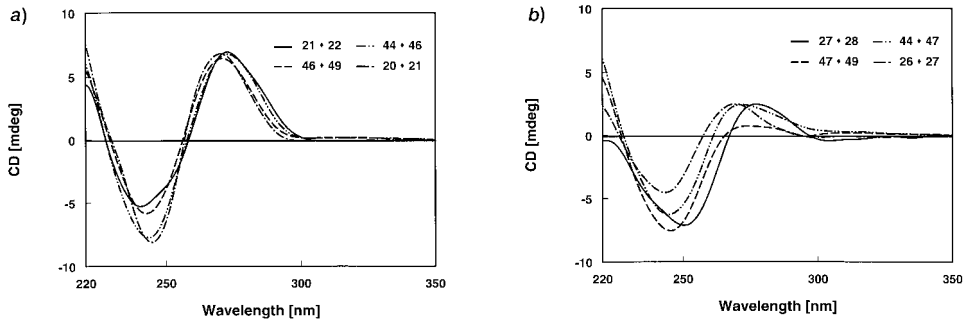


Fig. 2. a) *CD Spectra of aps duplexes and* b) *CD-spectra of ps duplexes*

substituent pattern is altered, *e.g.*, the replacement of guanine by isoguanine. Nevertheless, the base-pair motif **VIII** is less stable than motif **XI** which can be seen from the T_m values of the ps duplex 5′-d(N^7G-C)$_6$-3′ which is 40° compared to that with aps orientation d(G-C)$_3$ (46°) [11][39]. A chain length of 12 with 11 base pairs is necessary for the ps duplex to reach about the stability of the aps hexamer 5′-d(G-C)$_3$ with only 6 base pairs (*Fig. 3*). According to the results described above, the N^7-glycosylated purine nucleosides extend the base-pairing modes of the naturally occurring nucleic acids and form more stable duplex structures when incorporated in parallel DNA (motif **VIII**) than in duplexes with aps chain orientation.

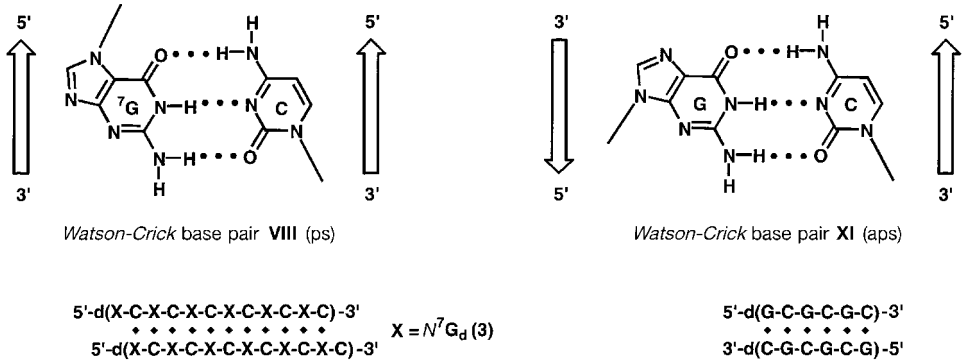


Fig. 3. Watson-Crick *base-pair motif* **VIII** *for ps duplex containing* C_d *and* N^7G_d (**3**) (dodecanucleotide) vs. Watson-Crick *base-pair motif* **XI** *for aps duplex containing* C_d *and* G_d (hexanucleotide)

Financial support by the *Deutsche Forschungsgemeinschaft* is gratefully acknowledged. We thank Mrs. *E. Feiling* for the synthesis of the 5-methylisocytidine cyanoethyl phosphoramidite.

Experimental Part

General. See [10]. 6-Chloropurine was obtained from *Pharma Waldhof*, Germany. The phosphonates were prepared as described, and the controlled-pore-glass (CPG)-immobilized nucleosides (30–50 μmol/g solid support) were purchased from *PerSeptive*, Wiesbaden, Germany. Oligonucleotide synthesis was performed on a DNA synthesizer, model *380 B* and model *392*, *Applied Biosystems*, Weiterstadt, Germany. The enzymatic

hydrolysis of the oligonucleotides was performed as described [26] using the following extinction coefficients ε_{260}: N^7G_d 2700, T_d 8800, C_d 7300, A_d 15400, G_d 11700. N^7A_d 6500, N^7I_d 7500, iC_d 6300. 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*, Germany. Solvent systems for flash chromatography (FC) and TLC: 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/MeOH/Et_3N 88:10:2 (*E*), CH_2Cl_2/AcOEt/Et_3N 49:49:2 (*F*), petroleum ether/AcOEt 1:1 (*G*), petroleum ether/AcOEt 4:6 (*H*), 0.1M (Et_3NH)OAc (pH 7.0)/MeCN 95:5 (*I*). Melting curves: *Cary-1/3* UV/VIS spectrophotometer (*Varian*, Australia) equipped with a *Cary* thermoelectrical controller; the actual temp. was measured in the reference cell with a Pt-*100* resistor. UV Spectra: *150-20* spectrometer (*Hitachi*, Japan). MALDI-TOF spectra were provided by Mrs. *S. Hahner* and Mrs. *J. Groß* (Prof. *Hillenkamp*, Institute of Medicinal Physics and Biophysics, Westfälische-Wilhelms-Universität Münster, Germany).

6-Alkoxypurines **4b**–**e** [16]: *General Procedure*. 6-Chloropurine (1.0 g, 6 mmol) was suspended in the appropriate alcohol/alkoxide soln. (50 ml, 30 mmol), and the resultant mixture 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 aq. layer was acidified to pH 5 with AcOH and the solid removed by filtration and crystallized from H_2O.

6-Ethoxypurine (**4b**): Colorless crystals (891 mg, 90%). M.p. 223–224° [16]. UV (pH 1): 254 (10450). ^{1}H-NMR ((D$_6$)DMSO): 1.38 (*t*, *J* = 7.0, *Me*CH_2O); 4.48–4.57 (*q*, *J* = 7.0, MeC*H*$_2$O); 8.01 (*s*, H–C(2)); 8.26 (*s*, H–C(8)).

6-Propoxypurine (**4c**): Colorless needles (982 mg, 90%). M.p. 180–181° [16]. UV (pH 1): 254 (11180). ^{1}H-NMR ((D$_6$)DMSO): 1.00 (*t*, *J* = 7.0, *Me*CH_2CH_2O); 1.75–1.89 (*m*, MeC*H*$_2$CH$_2$O); 4.48 (*t*, *J* = 7.0, MeCH$_2$C*H*$_2$O); 8.29 (*s*, H–C(2)); 8.43 (*s*, H–C(8)).

6-Isopropoxypurine (**4d**): Colorless needles (957 mg, 90%). M.p. 192–193° [16]. UV (pH 1): 255 (11900). ^{1}H-NMR ((D$_6$)DMSO): 0.99 (*t*, *J* = 7.0, *Me*$_2$CHO); 2.04 (*m*, Me$_2$C*H*O); 8.28 (*s*, H–C(2)); 8.41 (*s*, H–C(8)).

6-Isobutoxypurine (**4e**): Colorless needles (865 mg, 75%). M.p. 185–186° [16]. UV (MeOH): 245 (10700). ^{1}H-NMR ((D$_6$)DMSO): 0.99, 1.02 (2*s*, *Me*$_2$CH); 2.14 (*m*, Me$_2$C*H*); 4.30 (*t*, *J* = 6.8, CH$_2$O); 8.35 (*s*, H–C(2)); 8.46 (*s*, H–C(8)). Anal. calc. for $C_9H_{12}N_4O$ (192.22): C 56.24, H 6.29, N 29.15; found: C 56.38, H 6.24, N 29.20.

Other data of compounds **4b**–**e**: identical with those reported earlier [16].

*Glycosylation of 6-Alkoxypurines with 2-Deoxy-3,5-di-*O*-(4-toluoyl)-α-*D-erythro-*pentofuranosyl Chloride* (**5**): *General Procedure*. Powdered KOH (1 g, 17.8 mmol) and tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1; 60 µl, 0.18 mmol) were suspended in anh. MeCN (40 ml). The suspension was stirred for 15 min. Then, the 6-alkoxypurine (5 mmol) was added, and stirring was continued for another 15 min. Then, the halogenose **5** [17] (2.3 g, 6 mmol) was 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, *G* (500 ml), than *H*) and separated into two main zones in all cases. The faster migrating zone was always the N^9-isomer and the slower migrating one the N^7-isomer.

*9-[2-Deoxy-3,5-di-*O*-(4-toluoyl)-β-*D-erythro-*pentofuranosyl]-6-ethoxy-9*H*-purine* (**6b**): Colorless foam (1.16 g, 45%). TLC (*H*): R_f 0.6. UV (MeOH): 241 (42900). ^{1}H-NMR ((D$_6$)DMSO): 1.40 (*t*, *J* = 7.0, *Me*CH_2O); 2.35, 2.39 (2*s*, 2 Me); 2.77 (*m*, H$_\beta$–C(2′)); 3.40 (*m*, H$_\alpha$–C(2)); 4.52–4.66 (*m*, MeC*H*$_2$O, H–C(4′), 2 H–C(5′)); 5.84 (*m*, H–C(3′)); 6.59 (‘*t*’, *J* = 6.8, H–C(1′)); 7.28–7.94 (arom. H); 8.45 (*s*, H–C(2)); 8.57 (*s*, H–C(8)). Anal. calc. for $C_{28}H_{28}N_4O_6$ (516.55): C 65.11, H 5.46, N 10.85; found: C 65.26, H 5.56, N 10.86.

*7-[2-Deoxy-3,5-di-*O*-(4-toluoyl)-β-*D-erythro-*pentofuranosyl]-6-ethoxy-7*H*-purine* (**7b**): Colorless foam (720 mg, 28%). TLC (*H*): R_f 0.2. UV (MeOH): 240 (37000). ^{1}H-NMR ((D$_6$)DMSO): 1.40 (*t*, *J* = 7.0, *Me*CH_2O); 2.34, 2.39 (2*s*, 2 Me); 2.84 (*m*, H$_\beta$–C(2′)); 3.03 (*m*, H$_\alpha$–C(2′)); 4.54–4.62 (*m*, MeC*H*$_2$O, H–C(4′), 2 H–C(5′)); 5.69 (*m*, H–C(3′)); 6.67 (‘*t*’, *J* = 6.5, H–C(1′)); 7.24–7.94 (arom. H); 8.56 (*s*, H–C(2)); 9.06 (*s*, H–C(8)). Anal. calc. for $C_{28}H_{28}N_4O_6$ (516.55): C 65.11, H 5.46, N 10.85; found: C 65.23, H 5.52, N 10.73.

*9-[2-Deoxy-3,5-di-*O*-(4-toluoyl)-β-*D-erythro-*pentofuranosyl]-6-propoxy-9*H*-purine* (**6c**): Colorless foam (1.11 g, 42%). TLC (*H*): R_f 0.6. UV (MeOH): 241 (42600). ^{1}H-NMR ((D$_6$)DMSO): 0.99 (*t*, *J* = 7.4, *Me*CH_2CH_2O): 1.81 (*m*, MeC*H*$_2$CH$_2$O); 2.36, 2.39 (2*s*, 2 Me); 2.81 (*m*, H$_\beta$–C(2′)); 3.41 (*m*, H$_\alpha$–C(2′)); 4.49 (*t*, *J* = 6.6, MeCHC*H*$_2$O); 4.59 (*m*, 2 H–C(5′)); 4.66 (*m*, H–C(4′)); 5.85 (*m*, H–C(3′)); 6.62 (‘*t*’, *J* = 6.7, H–C(1′)); 7.27–7.96 (arom. H); 8.47 (*s*, H–C(2)); 8.58 (*s*, H–C(8)). Anal. calc. for $C_{29}H_{30}N_4O_6$ (530.58): C 65.65, H 5.70, N 10.56; found: C 65.75, H 5.75, N 10.72.

*7-[2-Deoxy-3,5-di-*O*-(4-toluoyl)-β-*D-erythro-*pentofuranosyl]-6-propoxy-7*H*-purine* (**7c**): Colorless foam (800 mg, 30%). TLC (*H*): R_f 0.2. UV (MeOH): 240 (38000). ^{1}H-NMR ((D$_6$)DMSO): 1.01 (*t*, *J* = 7.4, *Me*CH_2CH_2O); 1.82 (*m*, MeC*H*$_2$CH$_2$O); 2.39, 2.43 (2*s*, 2 Me); 2.87 (*m*, H$_\beta$–C(2′)); 3.00 (*m*, H$_\alpha$–C(2′)); 4.52 (*t*, *J* = 6.3, MeCH$_2$C*H*$_2$O); 4.60–4.66 (*m*, H–C(4′), 2 H–C(5′)); 5.73 (*m*, H–C(3′)); 6.72 (‘*t*’, *J* = 7.2,

H–C(1′)); 7.30–7.96 (arom. H); 8.59 (*s*, H–C(2)); 8.81 (*s*, H–C(8)). Anal. calc. for $C_{29}H_{30}N_4O_6$ (530.58): C 65.65, H 5.70, N 10.56; found: C 65.71, H 5.88, N 10.69.

9-[2-Deoxy-3,5-di-O-*(4-toluoyl)-β*-D-erythro-*pentofuranosyl]-6-isopropoxy-9*H-*purine* (**6d**): Colorless foam (1.01 g, 38%). TLC (*H*): R_f 0.6. UV (MeOH): 241 (42400). ^{1}H-NMR ((D$_6$)DMSO): 1.41, 1.42 (*d*, *Me*$_2$CHO); 2.39, 2.42 (2*s*, 2 Me); 2.82 (*m*, H$_\beta$–C(2′)); 3.42 (*m*, H$_\alpha$–C(2′)); 4.58 (*m*, 2 H–C(5′)); 4.68 (*m*, H–C(4′)); 5.60 (*m*, Me$_2$C*H*O); 5.87 (*m*, H–C(3′)); 6.63 (‘*t*’, *J* = 6.8, H–C(1′)); 7.30–7.98 (arom. H); 8.48 (*s*, H–C(2)); 8.58 (*s*, H–C(8)). Anal. calc. for $C_{29}H_{30}N_4O_6$ (530.58): C 65.65, H 5.70, N 10.56; found: C 65.82, H 5.81, N 10.44.

7-[2-Deoxy-3,5-di-O-*(4-toluoyl)-β*-D-erythro-*pentofuranosyl]-6-isopropoxy-7*H-*purine* (**7d**): Colorless foam (850 mg, 32%). TLC (*H*): R_f 0.2. UV (MeOH): 240 (38000). ^{1}H-NMR ((D$_6$)DMSO): 1.41 (*d*, *Me*$_2$CHO); 2.35, 2.40 (2*s*, 2 Me); 2.83 (*m*, H$_\beta$–C(2′)); 2.95 (*m*, H$_\alpha$–C(2′)); 4.59–4.65 (*m*, H–C(4′), 2 H–C(5′)); 5.53 (*m*, Me$_2$C*H*O); 5.70 (*m*, H–C(3′)); 6.69 (‘*t*’, *J* = 6.5, H–C(1′)); 7.26–7.94 (arom. H); 8.56 (*s*, H–C(2)); 8.75 (*s*, H–C(8)). Anal. calc. for $C_{29}H_{30}N_4O_6$ (530.58): C 65.65, H 5.70, N 10.56; found: C 65.77, H 5.70, N 10.43.

9-[2-Deoxy-3,5-di-O-*(4-toluoyl)-β*-D-erythro-*pentofuranosyl]-6-isobutoxy-9*H-*purine* (**6e**): Colorless foam (1.16 g, 43%). TLC (*H*): R_f 0.6. UV (MeOH): 241 (42500). ^{1}H-NMR ((D$_6$)DMSO): 1.00 (*d*, *Me*$_2$CH); 2.10 (*m*, Me$_2$C*H*); 2.39, 2.42 (2*s*, 2 Me); 2.82 (*m*, H$_\beta$–C(2′)); 3.42 (*m*, H$_\alpha$–C(2′)); 4.40 (*m*, CH$_2$O); 4.58 (*m*, 2 H–C(5′)); 4.68 (*m*, H–C(4′)); 5.87 (*m*, H–C(3′)); 6.63 (‘*t*’, *J* = 6.8, H–C(1′)); 7.30–7.98 (arom. H); 8.46 (*s*, H–C(2)); 8.55 (*s*, H–C(8)). Anal. calc. for $C_{30}H_{32}N_4O_6$ (544.60): C 66.16, H 5.92, N 10.29; found: C 66.12, H 5.89, N 10.26.

7-[2-Deoxy-3,5-di-O-*(4-toluoyl)-β*-D-erythro-*pentofuranosyl]-6-isobutoxy-7*H-*purine* (**7e**): Colorless foam (762 mg, 28%). TLC (*H*): R_f 0.2. UV (MeOH): 240 (38000). ^{1}H-NMR ((D$_6$)DMSO): 0.99 (*d*, *Me*$_2$CH); 2.08 (*m*, Me$_2$C*H*); 2.36, 2.40 (2*s*, 2 Me); 2.85 (*m*, H$_\beta$–C(2′)); 2.95 (*m*, H$_\alpha$–C(2′)); 4.31 (*m*, CH$_2$O); 4.57–4.64 (*m*, H–C(4′), 2 H–C(5′)); 5.70 (*m*, H–C(3′)); 6.71 (‘*t*’, *J* = 6.5, H–C(1′)); 7.28–7.94 (arom. H); 8.56 (*s*, H–C(2)); 8.81 (*s*, H–C(8)). Anal. calc. for $C_{30}H_{32}N_4O_6$ (544.60): C 66.16, H 5.92, N 10.29; found: C 66.12, H 5.94, N 10.36.

General Deprotection Procedure. Compound **6** or **7** (1.0 mmol) was dissolved in 0.1M NaOMe/MeOH (20 ml) and stirred for 30 min at r.t. The mixture was adsorbed on silica gel (10 g) and applied to FC (silica gel; column 10 × 5 cm, *B* (300 ml), then *C*): **8** or **9**, resp.

9-(2-Deoxy-β-D-erythro-*pentofuranosyl)-6-ethoxy-9*H-*purine* (**8b**): Colorless foam (240 mg, 85%). TLC (*C*): R_f 0.5. UV (MeOH): 249 (10500). ^{1}H-NMR ((D$_6$)DMSO): 1.38 (*t*, *J* = 7.0, *Me*CH$_2$O); 2.32 (*m*, H$_\beta$–C(2′)); 2.73 (*m*, H$_\alpha$–C(2′)); 3.56 (*m*, 2 H–C(5′)); 3.87 (*m*, H–C(4′)); 4.41 (*m*, H–C(3′)); 4.56 (*q*, *J* = 7.0, MeC*H*$_2$O); 4.59 (*t*, *J* = 5.5, OH–C(5′)); 5.29 (*d*, *J* = 4.0, OH–C(3′)); 6.41 (‘*t*’, *J* = 6.8, H–C(1′)); 8.48 (*s*, H–C(2)); 8.55 (*s*, H–C(8)). Anal. calc. for $C_{12}H_{16}N_4O_4$ (280.28): C 51.42, H 5.75, N 19.99; found: C 51.56, H 5.85, N 19.86.

7-(2-Deoxy-β-D-erythro-*pentofuranosyl)-6-ethoxy-7*H-*purine* (**9b**): Colorless foam (230 mg, 81%). TLC (*C*): R_f 0.3. UV (MeOH): 259 (8000). ^{1}H-NMR ((D$_6$)DMSO): 1.42 (*t*, *J* = 7.0, *Me*CH$_2$O); 2.42 (*m*, H$_\beta$–C(2′)); 2.54 (*m*, H$_\alpha$–C(2′)); 3.60 (*m*, 2 H–C(5′)); 3.91 (*m*, H–C(4′)); 4.37 (*m*, H–C(3′)); 4.56 (*m*, MeC*H*$_2$O); 4.65 (*t*, *J* = 5.5, OH–C(5′)); 5.32 (*d*, *J* = 4.0, OH–C(3′)); 6.51 (‘*t*’, *J* = 6.1, H–C(1′)); 8.54 (*s*, H–C(2)); 8.80 (*s*, H–C(8)). Anal. calc. for $C_{12}H_{16}N_4O_4$ (280.28): C 51.42, H 5.75, N 19.99; found: C 51.46, H 5.73, N 19.96.

9-(2-Deoxy-β-D-erythro-*pentofuranosyl)-6-propoxy-9*H-*purine* (**8c**): Colorless foam (241 mg, 82%). TLC (*C*): R_f 0.5. UV (MeOH): 249 (10700). ^{1}H-NMR ((D$_6$)DMSO): 0.98 (*t*, *J* = 7.3, *Me*CH$_2$CH$_2$O); 1.78 (*m*, MeC*H*$_2$CH$_2$O); 2.23 (*m*, H$_\beta$–C(2′)); 2.61 (*m*, H$_\alpha$–C(2′)); 3.55 (*m*, 2 H–C(5′)); 3.85 (*m*, H–C(4′)); 4.37 (*m*, MeCH$_2$C*H*$_2$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′)); 8.50 (*s*, H–C(2)); 8.57 (*s*, H–C(8)).

7-(2-Deoxy-β-D-erythro-*pentofuranosyl)-6-propoxy-7*H-*purine* (**9c**): Colorless foam (247 mg, 84%). TLC (*C*): R_f 0.3. UV (MeOH): 259 (7200). ^{1}H-NMR ((D$_6$)DMSO): 1.01 (*t*, *J* = 7.3, *Me*CH$_2$CH$_2$O); 1.80 (*m*, MeC*H*$_2$CH$_2$O); 2.32 (*m*, H$_\beta$–C(2′)); 2.54 (*m*, H$_\alpha$–C(2′)); 3.56 (*m*, 2 H–C(5′)); 3.86 (*m*, H–C(4′)); 4.34 (*m*, H–C(3′)); 4.35 (*t*, *J* = 6.3, MeCH$_2$C*H*$_2$O); 5.03 (*t*, *J* = 5.2, OH–C(5′)); 5.27 (*d*, *J* = 4.1, OH–C(3′)); 6.50 (‘*t*’, *J* = 6.1, H–C(1′)); 8.52 (*s*, H–C(2)); 8.80 (*s*, H–C(8)).

9-(2-Deoxy-β-D-erythro-*pentofuranosyl)-6-isopropoxy-9*H-*purine* (**8d**): Colorless foam (250 mg, 85%). TLC (*C*): R_f 0.5. UV (MeOH): 250 (10700). ^{1}H-NMR ((D$_6$)DMSO): 1.41 (*d*, *Me*$_2$CHO); 2.33 (*m*, H$_\beta$–C(2′)); 2.75 (*m*, H$_\alpha$–C(2′)); 3.60 (*m*, 2 H–C(5′)); 3.91 (*m*, H–C(4′)); 4.45 (*m*, H–C(3′)); 5.01 (*t*, *J* = 5.0, OH–C(5′)); 5.32 (*d*, *J* = 3.7, OH–C(3′)); 5.60 (*m*, Me$_2$C*H*O); 6.44 (‘*t*’, *J* = 6.4, H–C(1′)); 8.51 (*s*, H–C(2)); 8.57 (*s*, H–C(8)). Anal. calc. for $C_{13}H_{18}N_4O_4$ (294.31): C 53.05, H 6.16, N 19.04; found: C 53.19, H 6.08, N 18.98.

7-(2-Deoxy-β-D-erythro-*pentofuranosyl)-6-isopropoxy-7*H-*purine* (**9d**): Colorless foam (244 mg, 83%). TLC (*C*): R_f 0.3. UV (MeOH): 259 (7400). ^{1}H-NMR ((D$_6$)DMSO): 1.41 (*d*, *Me*$_2$CHO); 2.42 (*m*, H$_\beta$–C(2′)); 2.48 (*m*, H$_\alpha$–C(2′)); 3.62 (*m*, 2 H–C(5′)); 3.91 (*m*, H–C(4′)); 4.36 (*m*, H–C(3′)); 5.03 (*t*, *J* = 5.3, OH–C(5′));

5.32 (d, J = 4.2, OH–C(3′)); 5.54 (m, Me_2CHO); 6.50 ('t', J = 6.2, H–C(1′)); 8.53 (s, H–C(2)); 8.79 (s, H–C(8)). Anal. calc. for $C_{13}H_{18}N_4O_4$ (294.31): C 53.05, H 6.16, N 19.04; found: C 52.91, H 6.11, N 18.92.

*9-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-6-isobutoxy-9*H*-purine* (**8e**): Colorless foam (244 mg, 79%). TLC (*C*): R_f 0.5. UV (MeOH): 249 (10600). ^{1}H-NMR ((D$_6$)DMSO): 1.06 (d, Me_2CH); 2.12 (m, Me_2CH); 2.33 (m, H_β–C(2′)); 2.75 (m, H_α–C(2′)); 3.62 (m, 2 H–C(5′)); 3.93 (m, H–C(4′)); 4.45 (m, H–C(3), CH_2O); 5.05 (t, J = 5.0, OH–C(5′)); 5.32 (d, J = 3.7, OH–C(3′)); 6.45 ('t', J = 6.4, H–C(1′)); 8.51 (s, H–C(2)); 8.57 (s, H–C(8)). Anal. calc. for $C_{14}H_{20}N_4O_4$ (308.34): C 54.54, H 6.54, N 18.17; found: C 54.55, H 6.28, N 18.27.

*7-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-6-isobutoxy-7*H*-purine* (**9e**): Colorless foam (237 mg, 77%). TLC (*C*): R_f 0.3. UV (MeOH): 259 (6700). ^{1}H-NMR ((D$_6$)DMSO): 1.04 (d, Me_2CH); 2.10 (m, Me_2CH); 2.39 (m, H_β–C(2′)); 2.57 (m, H_α–C(2′)); 3.57 (m, 2 H–C(5′)); 3.89 (m, H–C(4)); 4.34 (m, H–C(3′), CH_2O); 5.03 (t, J = 5.3, OH–C(5′)); 5.33 (d, J = 4.2, OH–C(3′)); 6.53 ('t', J = 6.2, H–C(1′)); 8.53 (s, H–C(2)); 8.82 (s, H–C(8)). Anal. calc. for $C_{14}H_{20}N_4O_4$ (308.34): C 54.54, H 6.54, N 18.17; found: C 54.68, H 6.43, N 18.08.

*9-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-1,7-dihydro-6*H*-purin-6-one* (**2**). Compound **9d** (200 mg, 0.68 mmol) was treated with 2N aq. NaOH (40 ml) at 50° for 2 h. The soln. was cooled, neutralized with AcOH, diluted with H_2O (250 ml), and applied to a *Serdolite-AD-4* column (15 × 5 cm). The column was washed with H_2O (300 ml), and **1** was eluted with ^{i}PrOH/H_2O 9 : 1: **2** in 80% yield. Anal. data: identical with those reported [10].

*7-{5-*O*-[Bis(4-methoxyphenyl)phenylmethyl]-2-deoxy-β-*D*-*erythro*-pentofuranosyl}-1,7-dihydro-6*H*-purine-6-one* (**18**). Compound **2** (300 mg, 1.19 mmol) 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 (509 mg, 1.5 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. phase was dried (Na_2SO_4) and evaporated and the residue chromatographed (silica gel, column 15 × 3 cm, *B*). The main zone afforded a colorless foam (540 mg, 82%). TLC (*C*): R_f 0.7. UV (MeOH): 249 (10000). ^{1}H-NMR ((D$_6$)DMSO): 2.41 (m, H_α–C(2′)); 2.61 (m, H_β–C(2′)); 3.22 (m, 2 H–C(5′)); 3.74 (s, MeO); 4.00 (m, H–C(4′)); 4.36 (m, H–C(3′)); 5.37 (m, OH–C(3′)); 6.65 ('t', J = 6.0, H–C(1′)); 6.64–7.37 (3m, arom. H); 8.02 (s, H–C(2)); 8.38 (s, H–C(8)). Anal. calc. for $C_{31}H_{30}N_4O_6$ (554.60): C 67.14, H 5.45, N 10.10; found: C 67.11, N 5.74, N 10.42.

*7-{5-*O*-[Bis(4-methoxyphenyl)phenylmethyl]-2-deoxy-β-*D*-*erythro*-pentofuranosyl}-1,7-dihydro-6*H*-purin-6-one 3′-[(2-Cyanoethyl) Diisopropylphosphoramidite]* (**19**). To a soln. of **18** (100 mg, 0.18 mmol) and (^{i}Pr)$_2$EtN (50 µl, 0.28 mmol) in anh. CH_2Cl_2 (2 ml), 2-cyanoethyl diisopropylphosphoramidochloridite (40 µl, 0.17 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, *F*): **19** (75 mg, 55%). Colorless foam. TLC (*F*): R_f 0.4, 0.5. ^{1}H-NMR ($CDCl_3$): 1.03, 1.06 (2s, 2 Me_2CH); 2.39 (t, J = 6.5, CH_2CH_2CN); 2.39 (m, H_β–C(2′)); 2.55 (m, H_α–C(2′)); 3.12, 3.18 (2s, Me_2N); 3.32–3.36 (2 Me_2CH); 3.49–3.70 (m, 2 H–C(5′), CH_2O); 3.76 (s, MeO); 4.22 (m, H–C(4′)); 4.56 (m, H–C(3′)); 6.80 ('t', J = 6.3, H–C(1′)); 6.77–7.42 (3m, arom. H); 8.28 (d, J = 11.6, H–C(2)); 8.83 (s, H–C(8)). ^{31}P-NMR ($CDCl_3$): 149.8, 149.6.

N′*-[7-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-7*H*-purin-6-yl]-*N,N*-dimethylethanimidamide* (**10**). A soln. of **1** (250 mg, 1.0 mmol) in dry MeOH (30 ml) was treated with *N,N*-dimethylacetamide dimethyl acetale (2 ml, 12 mmol). The mixture was stirred overnight and evaporated and the residue adsorbed on silica gel (10 g) and applied to FC (silica gel, column 20 × 6 cm, *C*): **10** (224 mg, 70%). Colorless solid. TLC (*C*): R_f 0.3. UV (MeOH): 310 (30000). ^{1}H-NMR ((D$_6$)DMSO): 2.21 (s, Me); 2.35 (m, 2 H–C(2′)); 3.14 (2s, Me_2N); 3.59 (m, 2 H–C(5′)); 3.65 (m, H–C(4′)); 4.31 (m, H–C(3′)); 5.04 (t, J = 5.0, OH–C(5′)); 5.26 (d, J = 3.5, OH–C(3′)); 6.32 ('t', J = 6.3, H–C(1′)); 8.44 (s, H–C(2)); 8.67 (s, H–C(8)). Anal. calc. for $C_{14}H_{20}N_6O_3$ (320.35): C 52.49, H 6.29, N 26.23; found: C 52.33, H 6.37, N 26.12.

N′*-{7-{5-*O*-[Bis(4-methoxyphenyl)phenylmethyl]-2-deoxy-β-*D*-*erythro*-pentofuranosyl}-7*H*-purin-6-yl}-N,N-dimethylethanimidamide* (**11**). As described for **18**, with **10** (250 mg, 0.78 mmol), anh. pyridine, dry pyridine (2 ml), 4-(dimethylamino)pyridine (10 mg, 0.08 mmol), and bis(4-methoxyphenyl)phenylmethyl chloride (329 mg, 0.97 mmol): **11** (301 mg, 62%). Colorless foam. TLC (*C*): R_f 0.7. UV (MeOH): 310 (29500). ^{1}H-NMR ((D$_6$)DMSO): 2.20 (s, Me); 2.64 (m, 2 H–C(2′)); 3.15 (2s, Me_2N); 3.47 (m, 2 H–C(5′)); 3.72 (2s, MeO); 3.96 (m, H–C(4′)); 4.31 (m, H–C(3′)); 5.34 (d, J = 3.6, OH–C(3′)); 6.65 ('t', J = 6.3, H–C(1′));

6.60–7.38 (3*m*, arom. H); 8.43 (*s*, H–C(2)); 8.46 (*s*, H–C(8)). Anal. calc. for $C_{35}H_{38}N_6O_5$ (622.72): C 67.51, H 6.15, N 13.50; found: C 67.42, H 6.51, N 13.31.

N′-*{7-{5-*O-*[Bis(4-methoxyphenyl)phenylmethyl]-2-deoxy-β-*D-erythro-*pentofuranosyl}-7*H-*purin-6-yl}-*N,N-*dimethylethanimidamide 3′(Triethylammonium Phosphonate)* (**13a**). To a soln. of PCl_3 (225 µl, 2.5 mmol) and 4-methylmorpholine (2.7 ml) in CH_2Cl_2 (10 ml) was added 1*H*-1,2,4-triazole (600 mg, 8.7 mmol). The soln. was cooled to 0°, then **11** (310 mg, 0.5 mmol) in CH_2Cl_2 (10 ml) was added dropwise, and stirring was continued for 30 min at r.t. The mixture was poured into 1M $(Et_3NH)HCO_3$ (TBK, pH 8.0, 25 ml), the aq. layer extracted with CH_2Cl_2 (3 × 20 ml), the combined org. phase dried (Na_2SO_4) and evaporated, and the residue applied to FC (silica gel, column 15 × 3 cm, *E*). The obtained colorless oil was dissolved in CH_2Cl_2 and the soln. extracted with aq. 0.1M TBK (10 × 20 ml), dried, and evaporated: **13a** (250 mg, 63%). Colorless foam. TLC (*E*): R_f 0.4. UV (MeOH): 310 (29000). ^{1}H-NMR ((D_6)DMSO): 1.06 (*t*, *J* = 7.2, *Me*CH_2N); 2.26 (*s*, MeC=N); 2.55 (*m*, 2 H–C(2′)); 2.79 (*q*, *J* = 7.1, MeC*H*$_2$N); 3.14, 3.20 (2*s*, Me_2N); 3.70 (*m*, 2 H–C(5′), MeO); 4.11 (*m*, H–C(4′)); 4.70 (*m*, H–C(3′)); 6.60 (*d*, *J* = 585, PH); 6.82–7.25 (2*m*, arom. H); 7.38 (‘*t*’, *J* = 6.9, H–C(1′)); 8.45 (*s*, H–C(2)); 8.46 (*s*, H–C(8)). ^{31}P-NMR ((D_6)DMSO): 2.78 (*dd*, *J* = 585, 8.9).

N′-*{7-{5-*O-*[Bis(4-methoxyphenyl)phenylmethyl]-2-deoxy-β-*D-erythro-*pentofuranosyl}-7*H-*purin-6-yl}-*N,N-*dimethylethanimidamide 3′-[(2-Cyanoethyl) Diisopropylphosphoramidite]* (**13b**). As described for **19**, with **11** (100 mg, 0.16 mmol), $(^iPr)_2$EtN (50 µl, 0.28 mmol), CH_2Cl_2 (2 ml), and 2-cyanoethyl diisopropylphosphoramidochloridite (40 µl; 0.17 mmol): **13b** (86 mg, 65%). Colorless foam. TLC (*F*): R_f 0.4, 0.5. ^{1}H-NMR ($CDCl_3$): 1.03, 1.06 (2*s*, 2 *Me*$_2$CH); 2.39 (*t*, *J* = 6.5, CH_2C*H*$_2$CN); 2.39 (*m*, H_β–C(2′)); 2.55 (*m*, H_α–C(2′)); 3.12, 3.18 (2*s*, Me_2N); 3.32–3.36 (2 Me_2C*H*); 3.49–3.70 (*m*, 2 H–C(5′), C*H*$_2$CH$_2$CN); 3.76 (*s*, MeO); 4.22 (*m*, H–C(4′)); 4.56 (*m*, H–C(3′)); 6.80 (‘*t*’, *J* = 6.3, H–C(1′)); 6.77–7.42 (3*m*, arom. H); 8.28 (*d*, *J* = 11.6, H–C(2)); 8.83 (*s*, H–C(8)). ^{31}P-NMR ($CDCl_3$): 149.6, 149.5.

N′-*{7-[2-Deoxy-β-*D-erythro-*pentofuranosyl]-6,7-dihydro-6-oxo-1*H-*purin-2-yl}-*N,N-*dimethylethanimidamid* (**14**). A soln. of **3** (250 mg, 0.94 mmol) in anh. amine-free DMF (10 ml) was treated with *N,N*-dimethylacetamide diethyl acetal (1.5 ml, 8 mmol). The suspension was stirred for 18 h at r.t. The solvent was evaporated and the oily residue co-evaporated with toluene (10 ml, twice) and applied to FC (silica gel, column 10 × 6 cm, *C* (400 ml), then *D*). **14** (240 mg, 76%). Amorphous white powder. TLC (*D*): R_f 0.5. UV (MeOH): 281 (11000). ^{1}H-NMR ((D_6)DMSO): 2.16 (*s*, Me); 2.22 (*m*, 2 H–C(2′)); 3.56 (*m*, 2 H–C(5′)); 3.83 (*m*, H–C(4′)); 4.31 (*m*, H–C(3′)); 4.95 (*t*, *J* = 5.3, OH–C(5′)); 5.27 (*d*, *J* = 4.2, OH–C(3′)); 6.51 (‘*t*’, *J* = 6.3, H–C(1′)); 8.33 (*s*, H–C(8)); 11.30 (br. *s*, NH). Anal. calc. for $C_{14}H_{20}N_6O_4$ (336.35): C 49.99, H 5.99, N 24.99; found: C 50.14, H 5.95, N 24.77.

N′-*{7-{5-*O-*[Bis(4-methoxyphenyl)phenylmethyl]-2-deoxy-β-*D-erythro-*pentofuranosyl}-6,7-dihydro-6-oxo-1H-purin-2-yl}-*N,N-*dimethylethanimidamide* (**15**). As described for **18**, with **14** (500 mg, 1.48 mmol), anh. pyridine, dry pyridine (2 ml), 4-(dimethylamino)pyridine (10 mg, 0.08 mmol), and bis(4-methoxyphenyl)phenylmethyl chloride (542 mg, 1.60 mmol): **15** (585 mg, 62%). Colorless foam. TLC (*C*): R_f 0.7. UV (MeOH): 281 (11000). ^{1}H-NMR ((D_6)DMSO): 2.16 (*s*, Me); 2.50 (*m*, 2 H–C(2′)); 3.03 (2*s*, Me_2N); 3.47 (*m*, 2 H–C(5′)); 3.71 (*s*, MeO); 4.20 (*m*, H–C(4′)); 4.31 (*m*, H–C(3′)); 5.33 (*d*, *J* = 3.6, OH–C(3′)); 6.56 (‘*t*’, *J* = 6.3, H–C(1′)); 6.80–7.33 (3*m*, arom. H); 8.16 (*s*, H–C(8)); 11.30 (br. *s*, NH). Anal. calc. for $C_{35}H_{38}N_6O_6$ (638.72): C 65.82, H 6.00, N 13.16; found: C 65.39, H 6.18, N 13.01.

N′-*{7-{5-*O-*[Bis(4-methoxyphenyl)phenylmethyl]-2-deoxy-β-*D-erythro-*pentofuranosyl}-6,7-dihydro-6-oxo-1*H-*purin-2-yl}-*N,N-*dimethylethanimidamide 3′-[(2-Cyanoethyl) Diisopropylphosphoramidite]* (**17**). As described for **19**, with **15** (100 mg, 0.16 mmol), $(^iPr)_2$EtN (50 µl, 0.28 mmol), CH_2Cl_2 (2 ml), and 2-cyanoethyl diisopropylphosphoramidochloridite (40 µl, 0.17 mmol): **17** (80 mg, 65%). Colorless foam. TLC (*F*): R_f 0.4, 0.5. ^{1}H-NMR ($CDCl_3$): 1.03, 1.06 (2*s*, 2 *Me*$_2$CH); 2.39 (*t*, *J* = 6.5, CH_2C*H*$_2$CN); 2.50 (*m*, 2 H–C(2′)); 3.12, 3.18 (2*s*, Me_2N); 3.32–3.36 (2 M2$_2$C*H*); 3.49–3.70 (*m*, 2 H–C(5′), CH_2O); 3.76 (*s*, MeO); 4.22 (*m*, H–C(4′)); 4.77 (*m*, H–C(3′)); 6.80 (‘*t*’, *J* = 6.3, H–C(1′)); 6.77–7.42 (3*m*, arom. H); 8.16 (*s*, H–C(8)); 11.30 (br. *s*, NH). ^{31}P-NMR ($CDCl_3$): 149.6, 149.5.

Solid-Phase Synthesis of Oligodeoxyribonucleotides. The synthesis of the oligonucleotides **20**–**57** was carried out on a 1-µmol scale using the 3′-phosphonates of $[(MeO)_2Tr]T_d$, $[(MeO)_2Tr]bz^6A_d$, $[(MeO)_2Tr]bz^4C_d$, $[(MeO)_2Tr]ibu^2G_d$, $[(MeO)_2Tr]fami^{5m}C_d$ [38] and $[(MeO)_2Tr]fam^6N^7G_d$ [11], as well as the 3′-phosphoramidites of $[(MeO)_2Tr]T_d$, $[(MeO)_2Tr]bz^6A_d$, $[(MeO)_2Tr]bz^4C_d$, $[(MeO)_2Tr]ibu^2G_d$, $[(MeO)_2Tr]ibu^2c^7G_d$ [33], $[(MeO)_2Tr]fam^5iC_d$ [38], $[(MeO)_2Tr]fam^6N^7A_d$ [10], $[(MeO)_2Tr]acam^6N^7A_d$ and $[(MeO)_2Tr]fam^6N^7G_d$ [11], following the regular protocols of the DNA-synthesizer for 3′-hydrogen phosphonates and 3′-phosphoramidites [22–24] (bz = benzoyl, ibu = isobutyryl, fam = Me_2NCH=, c = deaza, acam = $Me_2NC(Me)$=). The crude oligonucleotides were purified and detritylated on an oligonucleotide-purification cartridge following the standard protocol for purification [25].

REFERENCES

[1] J. D. Watson, F. H. C. Crick, *Nature (London)* **1953**, *171*, 737.
[2] E. T. Kool, *Chem. Rev.* **1997**, *97*, 1473.
[3] K. Groebke, J. Hunziker, W. Fraser, L. Peng, U. Diederichsen, K. Zimmermann, A. Holzner, C. Leumann, A. Eschenmoser, *Helv. Chim. Acta* **1998**, *81*, 375.
[4] H. Rosemeyer, F. Seela, *Helv. Chim. Acta* **1991**, *74*, 748.
[5] M. Engholm. O. Buchardt, P. E. Nielsen, R. H. Berg, *J. Am. Chem. Soc.* **1992**, *114*, 1895.
[6] K. Rippe, N. B. Ramsing, R. Klement, T. M. Jovin, *J. Biomol. Struct. Dyn.* **1990**, *7*, 1199.
[7] F. Seela, C. Wei, *Helv. Chim. Acta* **1997**, *80*, 73.
[8] F. Seela, H. Winter, *Bioorg. Med. Chem. Lett*. **1993**, *3*, 273.
[9] F. Seela, H. Winter, '10th International Roundtable', Park City, USA, 1992.
[10] F. Seela, H. Winter, *Helv. Chim. Acta* **1994**, *77*, 597.
[11] F. Seela, P. Leonard, *Helv. Chim. Acta* **1996**, *79*, 477.
[12] F. Seela, P. Leonard, *Helv. Chim. Acta* **1997**, *80*, 1301.
[13] H. Sugijama, S. Ikeda, I. Saito, *J. Am. Chem. Soc*. **1996**, *118*, 9994.
[14] F. Seela, H. Winter, *Nucleosides Nucleotides* **1995**, *14*, 129.
[15] J. Marfurth, S. G. Parel, C. Leumann, *Nucleic Acids Res.* **1997**, *25*, 1875.
[16] G. Huber, *Chem. Ber.* **1957**, *90*, 698.
[17] M. Hoffer, *Chem. Ber*. **1960**, *93*, 2777.
[18] F. Seela, B. Westermann, U. Bindig, *J. Chem. Soc., Perkin Trans. 1* **1988**, 697.
[19] H.-D. Winkeler, F. Seela, *J. Org. Chem.* **1983**, *48*, 3119.
[20] F. Seela, Y. Chen, U. Bindig, Z. Kazimierczuk, *Helv. Chim. Acta* **1994**, *77*, 194.
[21] M. Krecmerova, F. Seela, *Nucleosides, Nucleotides* **1992**, *11*, 1393.
[22] Applied Biosystems, 'User Manual of the DNA Synthesizer *380 B*'.
[23] Applied Biosystems, 'User Manual of the DNA Synthesizer *392*'.
[24] B. C. Froehler, in 'Protocols for Oligonucleotides and Analogs', in 'Methods in 'Molecular Biology', Ed. E. S. Agrawal, Humana Press, Totowa, N. J., 1993, Vol. 20, p. 63.
[25] Applied Biosystems, 'Use Manual for Oligonucleotide Purification Cartridges'.
[26] F. Seela, S. Lampe, *Helv. Chim. Acta* **1991**, *74*, 1790.
[27] J. Marfurth, J. Hunziker, C. J. Leumann, *Bioorg. Med. Chem. Lett*. **1996**, *6*, 3021.
[28] C. Leumann, *Angew. Chem.* **1998**, *110*, 184.
[29] J. Marfurt, S. P. Parel, C. J. Leumann, *Nucleic Acids Res.* **1997**, *25*, 1875.
[30] J. Hunziker, E. S. Priestley, H. Brunar, P. B. Dervan, *J. Am. Chem. Soc.* **1995**, *117*, 2661.
[31] H. Brunar, P. B. Dervan, *Nucleic Acids Res.* **1996**, *24*, 1987.
[32] F. Seela, P. Leonard, *Nucleosides Nucleotides* **1997**, *16*, 669.
[33] F. Seela, H. Driller, *Nucleic Acids Res.* **1985**, *13*, 911.
[34] Y. Kawase, S. Iwai, E. Ohtsuka, *Chem. Pharm. Bull*. **1989**, *37*, 599.
[35] M. D. Topal, J. R. Fresco, *Nature (London)* **1976**, *263*, 289.
[36] S. C. Case Green, E. D. Southern, *Nucleic Acids Res.* **1994**, *22*, 131.
[37] Y. Tor, P. B. Dervan, *J. Am. Chem. Soc.* **1993**, *115*, 4461.
[38] F. Seela, C. Wei, A. Melenewski, E. Feiling, *Nucleosides Nucleotides* **1998**, *17*, 2045.
[39] F. Seela, G. Becher, *Chem. Commun*. **1998**, 2017.

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