The Base-Pairing Properties of 7-Deaza-2′-deoxyisoguanosine and 2′-Deoxyisoguanosine in Oligonucleotide Duplexes with Parallel and Antiparallel Chain Orientation

by **Frank Seela*** and **Changfu Wei**

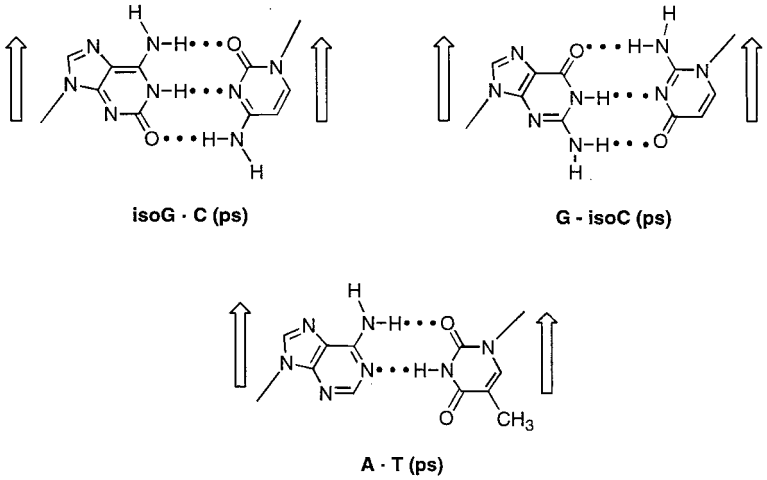
Laboratorium für Organische und Bioorganische Chemie, Institut für Chemie, Universität Osnabrück, Barbarastr. 7, D-49069 Osnabrück, Germany

Oligonucleotides with parallel (ps) or antiparallel (aps) chain orientation containing 7-deaza-2′-deoxyisoguanosine (**1**) or 2′-deoxyisoguanosine (**2**) were prepared. The phosphoramidite and phosphonate building blocks **3**–**6** were synthesized and used in solid-phase synthesis. The diphenylcarbamoyl (dpc) residue was used for the 2-oxo group protection and the isobutyryl (iBu = ib) residue for the amino function. Hybridization experiments were performed with oligonucleotides containing 7-deazaisoguanine or isoguanine. Regarding 7-deazapurine-containing oligonucleotides, the 7-deazaisoguanine · cytosine base pair was the strongest in ps-duplexes, while that of 7-deazaisoguanine · 5-methylisocytosine was the most stable one in aps-DNA. Ambiguous base pairing of 7-deazaisoguanine with cytosine, 5-methylisocytosine, thymine, and guanine was observed in the case of aps-duplexes, whereas in ps-duplexes, the ambiguity was extended to adenine. The 7-deazaisoguanine-containing duplexes showed almost identical base-pair stabilities as those containing isoguanine. According to this, various base-pair motifs are proposed. The 7-deaza-2′-deoxyisoguanosine was found to be an effective substitute of 2′-deoxyisoguanosine.

1. Introduction. – Parallel-stranded DNA can be constructed from oligonucleotides containing reverse *Watson-Crick* base pairs of the adenine and thymine residues, accompanied by a sequence selection which allows the hybridization of both strands in the $5' \rightarrow 3'$ direction [1]. The situation is more complex when the oligonucleotides contain bases other than adenine and thymine. Guanine and cytosine cannot be arranged in a way that a stable tridendate base pair is formed to give duplexes with parallel chains. In order to achieve this goal, the *Watson-Crick* dC · dG base pair has been altered into a reverse *Watson-Crick* base pair. Guanine was replaced by isoguanine and cytosine by isocytosine.

The first report on a parallel duplex with an isoguanine · cytosine base pair has been published in 1993 [2]. Later, also parallel duplexes with isocytosine and guanine pairs were reported [3][4]. Meanwhile, any naturally occurring single-stranded DNA can be hybridized to a duplex with parallel chains when one strand contains the four bases adenine, thymine, isoguanine, and isocytosine [3][4].

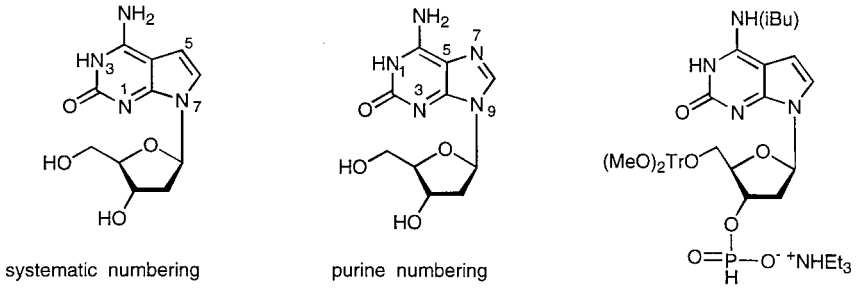
Also, tetraplex DNA formed by oligonucleotides containing short runs of 2′-deoxyisoguanosine (**2**) has been identified by our laboratory [5][6]. However, compound **2** has a rather labile *N*-glycosylic bond (τ 14 min in 0.1N HCl at 25°) [5]. It is hydrolysed 10 times faster than 2′-deoxyguanosine: its stability is in the range of 2′,3′-dideoxyguanosine [7]. To avoid depurination during oligonucleotide synthesis, as well as during subsequent detritylation, it was decided to make use of the more stable 2′-deoxyisoguanosine analogue 7-deaza-2′-deoxyisoguanosine (**1**) (purine numbering is



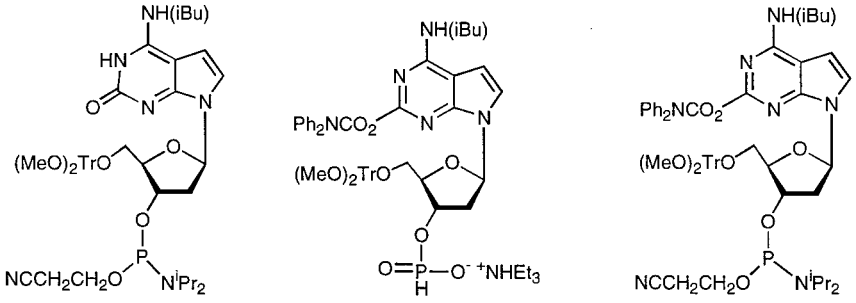
used throughout the *General Part*). In general, the 7-deazapurine 2′-deoxyribonucleosides, including compound **1**, are very stable under acidic conditions [8]. They have been used for many purposes in molecular biology [9][10]. This prompted us to use compound **1** as a 2′-deoxyisoguanosine substitute. We now report on the synthesis of compound **1** and of its building blocks **3** and **5** (phosphonates) as well as **4** and **6** (phosphoramidites). Furthermore, oligonucleotides containing nucleoside **1** were prepared and the base-pairing properties of compound **1** in parallel and antiparallel duplexes were studied and compared with those of the parent 2′-deoxyisoguanosine **2** in corresponding oligonucleotides.

2. Results and Discussion. – 2.1. *Monomers.* Earlier, 7-deaza-2′-deoxyisoguanosine (= 4-amino-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-1,7-dihydro-2*H*-pyrrolo[2,3-*d*]pyrimidin-2-one; **1**) has been synthesized by either the glycosylation of 2-methoxy-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine with 2-deoxy-3,5-di-*O*-(4-toluoyl)-α-D-*erythro*-pentofuranosyl chloride followed by hydrolysis of the MeO group of the nucleobase [8] or by photochemical conversion of the Cl substituent of 2-chloro-7-deaza-2′-deoxyadenosine [11]. Both methods are laborious and not applicable to a multi-gram scale synthesis. Now, a more efficient route was used, starting with 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine [12]. This base was reacted with 2-deoxy-3,5-di-*O*-(4-toluoyl)-α-D-*erythro*-pentofuranosyl chloride under the conditions for nucleobase-anion glycosylation to yield the nucleoside **7** stereoselectively in 67% yield (*Scheme 1*). Ammonolysis (conc. ammonia/dioxane) in an autoclave (120°, 3 d) furnished 7-deaza-2′-deoxyadenosin-2-amine (**8**) [13]. The detoluoylated compound **9** was obtained when the reaction was performed at lower temperature in conc. ammonia/dioxane (60°, 3 d). Selective deamination of compound **8** with sodium nitrite in 20% $AcOH/H_2O$ furnished the nucleoside **1**.

The crystal structure of compound **1** was determined by a single-crystal X-ray analysis. It is, to the best of our knowledge, the first solid-state structure of an isoguanine 2′-deoxyribonucleoside. The data revealed that 7-deaza-2′-deoxyisoguano-

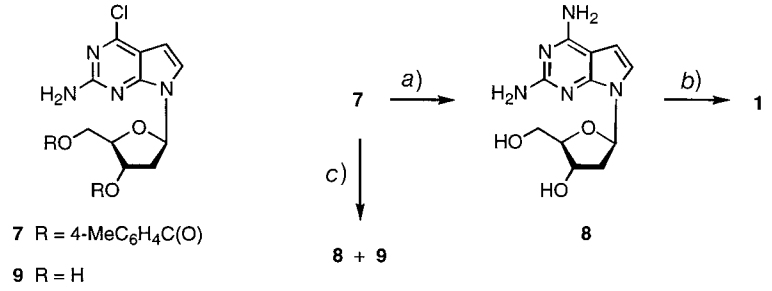


1 **2** **3**



4 **5** **6**

Scheme 1



7 R = 4-$MeC_6H_4C(O)$

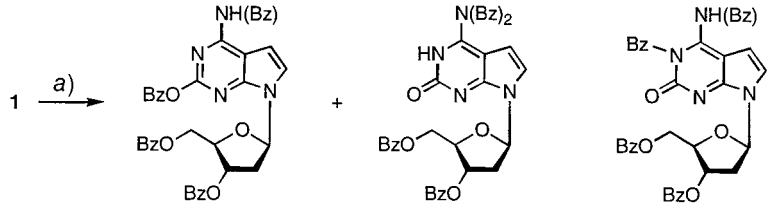
9 R = H

a) 25% NH_3 soln./dioxane 1:1 (*v*/*v*), 120°, 3 d; 88% of **8**. *b*) $NaNO_2$, 20% AcOH/H_2O; 67% of **1**. *c*) 25% NH_3 soln./dioxane 1:1 (*v*/*v*), 60°, 3 d; 33% of **8** and 47% of **9**.

sine adopts the N(1)-H/6-NH_2/keto tautomeric form, and the sugar conformation is C(1′)-*exo* [14].

In the case of 2′-deoxyisoguanosine (**2**), it was found that the substituent reactivity differs significantly from that of 2′-deoxyguanosine [15]. The 2-oxo group of compound **2** is much more reactive towards electrophiles, while the 6-amino-acylated derivatives are rather difficult to obtain due to the low glycosylic bond stability of these reaction

Scheme 2



10 **11** **12**

a) **1**/BzCl 1:6, pyridine, r.t. 6 h; 23% of **10** and 39% of **11** or **12**.

Table 1. *^{13}C-NMR Chemical Shifts of 7-Deaza-2′-deoxyisoguanosine Derivatives*[a])

	C(2)[b])[d]) C(2)[c])	C(4)[b])[d]) C(6)[c])	C(4a)[b]) C(5)[c])	C(5)[b]) C(7)[c])	C(6)[b]) C(8)[c])	C(7a)[b])[d]) C(4)[c])	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)
1 [8]	153.9	156.3	92.6	100.8	118.9	152.6	83.4	[e])	71.1	87.2	62.0
10	159.2	156.3	101.5	101.1	121.5	151.3	81.1	36.1	75.3	83.0	64.5
11 (or **12**)	154.6	153.9	107.8	104.6	124.6	152.9	81.2	36.0	75.1	83.1	64.3
13	158.8	155.8	100.9	100.1	121.6	150.7	82.7	[e])	71.0	87.2	62.0
14	158.9	156.0	100.7	100.6	120.8	150.8	82.1	[e])	70.7	83.8	65.9
15[g])	156.1	155.8	99.4	101.3	118.9	155.7	82.9	[e])	71.2	87.1	62.3
16[g])	156.1	156.0	98.4	102.1	119.5	152.5	82.6	37.7	71.0	87.0	62.1
17	154.3	153.6	106.3	104.4	119.5	152.5	82.6	[e])	70.9	87.4	61.9
18	158.0	157.9	113.1	105.2	121.1	[f])	81.7	[e])	70.6	85.4	64.1
19	158.0	153.6	106.2	104.6	123.8	151.4	82.2	[e])	70.6	85.5	64.0
3	158.0	157.9	113.1	105.1	121.1	[f])	81.9	38.1	72.8	85.5	63.8
5	158.0	153.7	106.2	104.6	123.3	151.4	82.3	38.3	72.6	85.5	63.7

	C=O	arom. C	CH (of iBu)	Me (of iBu)
10	167.4; 165.6 165.4; 164.4	134.3 – 128.6		
11 (or **12**)	165.8; 165.4 165.2; 164.3	134.5 – 128.1		
13	151.7	129.2 – 126.7		
14	153.9; 151.8	129.3 – 126.5		
15	175.3	139.5 – 127.7		
16	183.7		36.5	19.9; 19.7
17	175.8; 152.1	141.7 – 127.0	34.3	19.2

[a]) Measured in (D_6)DMSO at 303 K. [b]) Systematic numbering. [c]) Purine numbering. [d]) Assignment tentative. [e]) Superimposed by DMSO. [f]) Not detected. [g]) Measured in 0.4M NH_4OAc/(D_6)DMSO.

Table 2. *UV Data of Substituted 7-Deaza-2′-deoxyisoguanosine Derivatives in MeOH*

	$\lambda_{max}(\varepsilon)$ [nm]			$\lambda_{max}(\varepsilon)$ [nm]	
1	256 (7900),	305 (7200)	**15**	238 (29100),	360 (10300)
10	228 (53600),	270 (15400)	**16**	236 (25400),	341 (6420)
11 (or **12**)	231 (58900),	362 (12200)	**17**	233 (36500),	294 (7200)
13		269 (12900)	**18**	236 (52900),	346 (8000)
14		265 (13200)	**19**	233 (56100),	294 (7600)

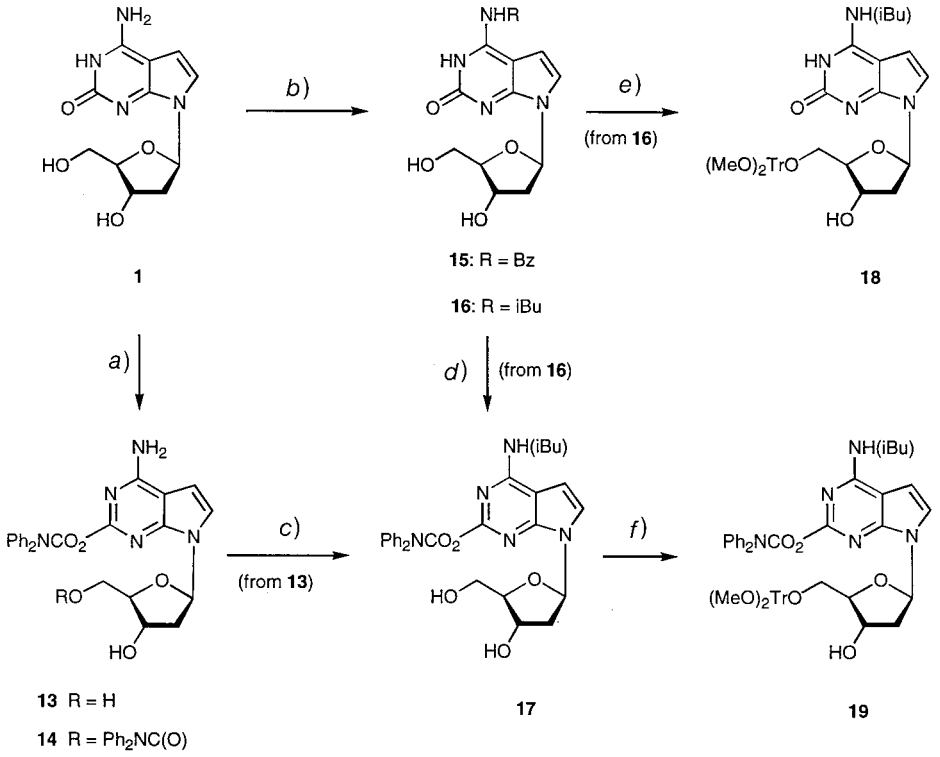
products. However, similar to findings on 7-deaza-2′-deoxyguanosine, the glycosylic bond stability of **1** is increased. Benzoylation of 7-deaza-2′-deoxyisoguanosine with an excess of benzoyl chloride (BzCl) in pyridine at room temperature yielded two tetrabenzoylated derivatives (*Scheme 2*), one faster migrating (23%; λ_{max} 270 nm) and one slower migrating isomer (39%; λ_{max} 362 nm). Both compounds were characterized by ^{1}H- and ^{13}C-NMR spectra (see *Exper. Part* and *Table 1*) and elemental analysis. The UV spectrum (*Table 2*) of the compound with λ_{max} 270 nm was similar to that of 2′-deoxy-2-methoxyadenosine (λ_{max} 267 nm). Therefore, this faster migrating isomer is assigned to structure **10**. The slower-migrating isomer can not carry a benzoyl residue at the 2-oxo group (λ_{max} 362 nm). Therefore, the structure **11** or **12** has to be considered; however, structure **11** is more likely.

Next, the selective protection of the 6-NH_2 group was performed using the protocol of transient protection [16]. This route furnished compound **15** upon treatment of compound **1** with BzCl in presence of trimethylsilyl chloride (room temperature, pyridine). The N^6-isobutyrylated nucleoside **16** was prepared under the same conditions with isobutyryl chloride (iBuCl = ibCl) (*Scheme 3*). The introduction of the isobutyryl group was superior to the benzoyl protecting group, which was found to be too stable (**15**: τ 180 min; **16**: τ 60 min; 25% ammonia, 40°)

Similarly to 2′-deoxyisoguanosine, the 2-oxo group of **1** needed to be protected when an oligonucleotide synthesis was performed on solid-phase using the phosphoramidite chemistry. In the case of phosphonates, *O*-protection was found to be unnecessary. Protection of compound **1** with diphenylcarbamoyl chloride (dpc-Cl) in pyridine gave compound **13** (80%; *Scheme 3*) [5]. As a by-product (8%), the 2,5′-bis(diphenylcarbamoyl) derivative **14** was formed. The dpc-protected intermediate **17** was prepared to form the building blocks **5** and **6**. Reaction of **13** with iBuCl in the presence of trimethylsilyl chloride furnished this material. Compound **17** can also be prepared by diphenylcarbamoylation of **16** with dpc-Cl in pyridine. However, the total yield from **1** *via* **13** to **17** (64%) was higher than that from **1** *via* **16** to **17** (38%). Therefore, the first route was chosen for further experiments. Compound **17** was converted into the 5′-*O*-(dimethoxytrityl)-protected **19** employing standard conditions. The final reaction of **19** with PCl_3/4-methylmorpholine/1*H*-1,2,4-triazole in CH_2Cl_2 furnished the 3′-phosphonate **5** (93%), which was purified chromatographically and isolated as triethylammonium salt. The phosphoramidite **6** (85%) was prepared by treatment of **19** with 2-cyanoethyl diisopropylphosphoramidochloridite (= chloro(2-cyanoethoxy)(diisopropylamino)phosphine) in the presence of diisopropylethylamine (^{i}Pr$_2$NEt). Alternatively, the 2-oxo-unprotected phosphonate **3** (70%) and the phosphoramidite **4** (95%) were synthesized from compound **16** by the same protocol as described above.

2.2. *Oligonucleotides.* 2.2.1. *Synthesis.* The oligodeoxyribonucleotide synthesis was performed on an *ABI-380B* synthesizer (for phosphonates) or an *ABI-392-08* apparatus (for phosphoramidites). The phosphonates **3** and **5** and the phosphoramidites **4** and **6** were used. The coupling yields of the modified phosphonates **3** and **5** were somewhat lower, and the 2-oxo-unprotected phosphoramidite **4** resulted in inefficient coupling. Therefore, the fully-protected phosphoramidite **6** was used for further experiments which gave higher coupling yields (> 90%). The 2′-deoxy-5′-*O*-(dimethoxytrityl)-N^2-[(dimethylamino)methylidene]-5-methylisocytidine 3′-(2-cyanoethyl phosphoramidite) and the 3′-(triethylammonium phosphonate) were also prepared

Scheme 3



a) Ph_2NCOCl, $^iPr_2EtN$, pyridine, r.t., 2 h; 80% of **13** and 8% of **14**. *b*) 1. Me_3SiCl, BzCl, pyridine, r.t., 3 h; 75% of **15**; 2. Me_3SiCl, iBuCl, pyridine, r.t., 3 h; 84% of **16**. *c*) Me_3SiCl, iBuCl, pyridine, r.t., 2 h; 80% of **17**. *d*) Ph_2NCOCl, $^iPr_2EtN$, pyridine, r.t., 3 h; 46% of **17**. *e*) $(MeO)_2TrCl$, $^iPr_2EtN$, pyridine, r.t., 3 h; 58% of **18**. *f*) $(MeO)_2TrCl$, pyridine, r.t., 3.5 h; 75% of **19**.

as described [3][17][18] and employed in solid-phase oligonucleotides synthesis. Incorporation of iG_d was achieved using the 5′-*O*-(dimethoxytrityl)-O^2-(diphenylcarbamoyl)-N^6-[(dimethylamino)ethylidene]-2′-deoxyisoguanosine 3′-(2-cyanoethyl phosphoramidite) and the 3′-(triethylammonium phosphonate) [5].

The oligonucleotides were deprotected in concentrated ammonia solution at 60° for 16 h and purified by reversed-phase HPLC. The unmodified oligodeoxynucleotides were purified using *OPC* cartridges (*Applied Biosystems*). The nucleoside composition of oligodeoxyribonucleotides was determined by tandem hydrolysis with snake-venom phosphodiesterase and alkaline phosphatase and identified on reversed-phase HPLC. Selected examples are given in the *Figure*. Some oligonucleotides were also characterized by MALDI-TOF mass spectra.

2.2.2. *Base-Pairing Properties. Preamble.* Previously, the isoguanine · cytosine and the isoguanine · isocytosine base pairing have been investigated on oligonucleotide duplexes with parallel (ps) and antiparallel (aps) strand orientation [2–5][17][19]. It has been observed that isoguanine shows base-pairing selectivity for either cytosine in ps-hybrids or isocytosine in aps-DNA. Furthermore, our laboratory has reported on the

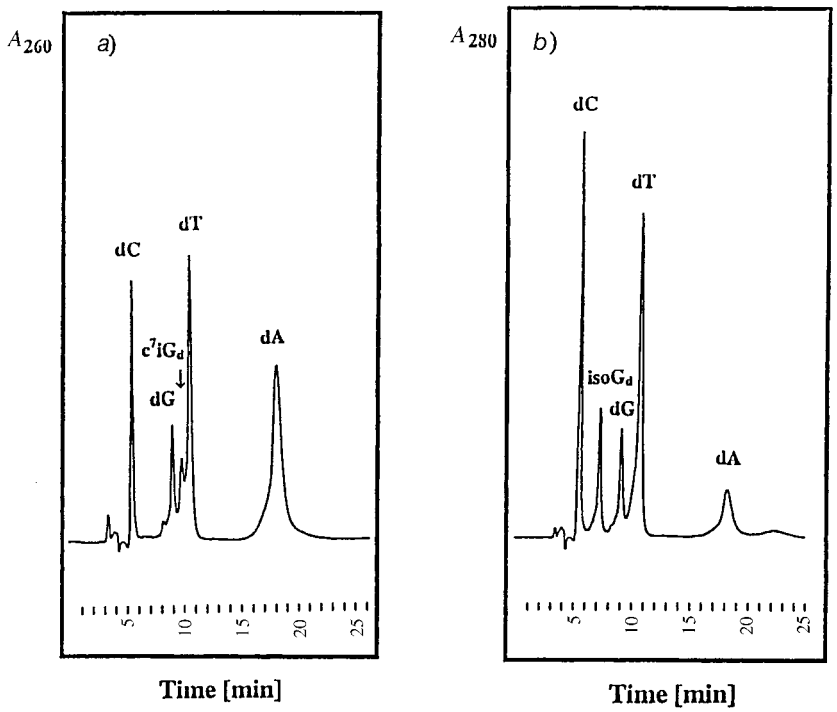


Figure. *Reversed-phase HPLC profiles of hydrolysis products of* a) *5′-d(TAGc*^{7}*iGTCAATACT)-3′ and* b) *5′-d(TAGiGTCAATACT)-3′ formed by snake-venom phosphodiesterase followed by alkaline phosphatase*

alternating oligonucleotide containing the 7-deaza derivative of 2′-deoxyisoguanosine (**1**) incorporated in a self-complementary duplex [20] or in a quadruplex forming a tetrad structure [21]. To investigate the pairing properties of 7-deaza-2′-deoxyisoguanosine, a series of hybridization experiments were performed in which oligonucleotides were hybridized either under parallel or antiparallel chain orientation. The stability of the duplexes was examined by temperature-dependent UV spectra. The melting temperatures (T_m) were determined, and the thermodynamic data of duplex formation were calculated with the program MeltWin [22].

Base-Pairing Properties of 7-Deazaisoguanine with Cytosine, 5-Methylisocytosine, Thymine, Guanine, and Adenine in Oligonucleotide Duplexes with Parallel or Antiparallel Chain Orientation. For this study, a duplex was chosen which had been employed in our laboratory in many cases and which was used as a reference compound to study the influence of modified bases on the duplex stability. According to *Table 3*, the duplex with one 7-deazaisoguanine opposite to 5-methylisocytosine (**22** · **23**) gives the most stable base pair. Its stability is higher than that of the duplex **20** · **21** containing the base pair guanine · cytosine. The high T_m values observed for the duplex **22** · **23** underlines the formation of a tridendate base pair. Next, oligonucleotide duplexes were studied containing 7-deazaisoguanine in one strand and cytosine, thymine, guanine, or adenine opposite to it in the second strand. A decrease of the T_m value by *ca.* 7° was observed for a single mismatch in the case of the duplexes **22** · **21**, **22** · **24**, and **22** · **25**.

Table 3. T_m *Values and Thermodynamic Data of the aps-Hybrids Formed by Oligonucleotides Containing* c^7iG_d *and* m^5iC_d [a])

	aps-Duplexes	T_m [°]	$\Delta H°$ [kcal/mol]	$\Delta S°$ [cal/mol·K]	$\Delta G°_{298}$ [kcal/mol]
20·21	3′-d(TCATAACT**G**GAT)-5′ • • • • • • • • • • • • 5′-d(AGTATTGA**C**CTA)-3′	51	– 91	– 256	– 11.4
22·23	3′-d(TCATAACT**c⁷iG**GAT)-5′ • • • • • • • • * • • • 5′-d(AGTATTGA **iC** CTA)-3′[b])	52	– 89	– 247	– 12.4
22·21	3′-d(TCATAACT**c⁷iG**GAT)-5′ • • • • • • • • * • • • 5′-d(AGTATTGA **C** CTA)-3′	43	– 76	– 214	– 9.8
22·24	3′-d(TCATAACT**c⁷iG**GAT)-5′ • • • • • • • • * • • • 5′-d(AGTATTGA **G** CTA)-3′	44	– 75	– 209	– 10.0
22·25	3′-d(TCATAACT**c⁷iG**GAT)-5′ • • • • • • • • * • • • 5′-d(AGTATTGA **T** CTA)-3′	44	– 77	– 216	– 9.8
22·26	3′-d(TCATAACT**c⁷iG**GAT)-5′ • • • • • • • • * • • • 5′-d(AGTATTGA **A** CTA)-3′	31	– 58	– 163	– 7.1
28·27	3′-d(TCATAA **iC** TGGAT)-5′[b]) • • • • • • * • • • • • 5′-d(AGTATT**c⁷iG**ACCTA)-3′	54	– 92	– 253	– 13.0
20·27	3′-d(TCATAA **C** TGGAT)-5′ • • • • • • * • • • • • 5′-d(AGTATT**c⁷iG**ACCTA)-3′	43	– 76	– 213	– 9.7
29·27	3′-d(TCATAA **G** TGGAT)-5′ • • • • • • * • • • • • 5′-d(AGTATT**c⁷iG**ACCTA)-3′	42	– 66	– 181	– 9.4
30·27	3′-d(TCATAA **T** TGGAT)-5′ • • • • • • * • • • • • 5′-d(AGTATT**c⁷iG**ACCTA)-3′	42	– 72	– 203	– 9.4
31·27	3′-d(TCATAA **A** TGGAT)-5′ • • • • • • * • • • • • 5′-d(AGTATT**c⁷iG**ACCTA)-3′	29	– 48	– 132	– 6.9

[a]) Measured by UV in 1M NaCl, 0.1M $MgCl_2$, 60 mM Na-cacodylate buffer, pH 7.0; the oligonucleotide concentration is 5 μM (single strands). [b]) d(iC) = m^5iC_d = 2′-deoxy-5-methylisocytidine.

Only the duplex **22·26** showed a much more decreased stability ($\Delta T_m = -20°$). Similar results reported for these series of duplexes were observed when a duplex with another sequence was chosen, but containing the same base composition (see duplex **28·27** as standard and the duplexes **20·27** and (**29**–**31**)·**27** as hybrids with mismatches).

Next, the base-pair stability of 7-deazaisoguanine with cytosine, 5-methylisocytosine, guanine, thymine, and adenine was investigated on duplexes with a parallel chain orientation. For this purpose, the oligonucleotides shown in *Table 4* were synthesized, and hybridization experiments were performed as described for the antiparallel chains.

The oligonucleotides which were used in these cases contain two $iG_d \cdot dC$ and two $m^5iC_d \cdot dG$ base pairs in order to induce parallel chain orientation. Among those duplexes, the duplex with the 7-deazaisoguanine · cytosine base pair (**32 · 21**) represents the most stable structure. The T_m values of the other ones are *ca.* 10° lower. The base-pair discrimination within parallel duplexes shows similarities to that having antiparallel chains in the cases of 7-deazaisoguanine pairing with guanine, thymine, and also 5-methylisocytosine. Now, even the duplex with adenine opposite to 7-deazaguanine (**32 · 26**) shows almost the same stability as the others. By choosing the duplex **33 · 20** instead of **32 · 21** having another sequence, a similar base discrimination was observed (see the bottom part of *Table 4*).

Comparison of the Thermodynamic Stability of Duplexes Containing 7-Deazaisoguanine or Isoguanine in ps- or aps-Duplexes. In view of the results discussed above, it was of interest to compare the base-pairing properties of 7-deazaisoguanine and isoguanine with the four common DNA constituents as well as with 5-methylisocytosine. Investigations on the pairing properties of the regular isoguanine have already been performed [3][17]. As we wanted to compare the data of 7-deazaisoguanine and isoguanine substitution with respect to the T_m values as well as to the thermodynamic parameters, the set of oligonucleotides shown in *Tables 5* and *6* were synthesized. The

Table 4. T_m *Values and Thermodynamic Data of the ps-Hybrids Formed by Oligonucleotides Containing* c^7iG_d *and* m^5iC_d[a])

	ps-Duplexes	T_m [°]	$\Delta H°$ [kcal/mol]	$\Delta S°$ [cal/mol · K]	$\Delta G°_{298}$ [kcal/mol]
32 · 21	5′-d(TiCATAAiCT**c⁷iG**c⁷iGAT)-3′[b])				
	• • • • • • • • * • • •	44	−72	−199	−9.9
	5′-d(AGTATTGA **C** C TA)-3′				
32 · 24	5′-d(TiCATAAiCT**c⁷iG**c⁷iGAT)-3′[b])				
	• • • • • • • • * • • •	33	−49	−132	−7.7
	5′-d(AGTATTGA **G** C TA)-3′				
32 · 25	5′-d(TiCATAAiCT**c⁷iG**c⁷iGAT)-3′[b])				
	• • • • • • • • * • • •	31	−46	−125	−7.3
	5′-d(AGTATTGA **T** C TA)-3′				
32 · 26	5′-d(TiCATAAiCT**c⁷iG**c⁷iGAT)-3′[b])				
	• • • • • • • • * • • •	32	−48	−131	−7.3
	5′-d(AGTATTGA **A** C TA)-3′				
32 · 23	5′-d(TiCATAAiCT**c⁷iG**c⁷iGAT)-3′[b])				
	• • • • • • • • * • • •	37	−55	−151	−8.2
	5′-d(AGTATTGA **iC** C TA)-3′				
33 · 20	5′-d(ATiCiCA**c⁷iG**TTATc⁷iGA)-3′[b])				
	• • • • • * • • • • • •	39	−51	−135	−8.7
	5′-d(TAGGT **C** AATA C T)-3′				
33 · 30	5′-d(ATiCiCA**c⁷iG**TTATc⁷iGA)-3′[b])				
	• • • • • * • • • • • •	29	−39	−102	−7.3
	5′-d(TAGGT **T** AATA C T)-3′				
33 · 31	5′-d(ATiCiCA**c⁷iG**TTATc⁷iGA)-3′[b])				
	• • • • • * • • • • • •	29	−41	−109	−7.0
	5′-d(TAGGT **A** AATA C T)-3′				

[a]) [b]) See *Table 3*.

Table 5. T_m *Values and Thermodynamic Data of the aps-Hybrids Formed by Oligonucleotides Containing* iG_d *and* m^5iC_d [a])

	aps-Duplexes	T_m [°]	ΔH° [kcal/mol]	ΔS° [cal/mol·K]	ΔG°_{298} [kcal/mol]
20·21	3′-d(TCATAACT**G**GAT)-5′ • • • • • • • • • • • • 5′-d(AGTATTGA**C**CTA)-3′	51	– 91	– 256	– 11.4
34·23	3′-d(TCATAACT**iG**GAT)-5′ • • • • • • • • ∗ • • • 5′-d(AGTATTGA**iC**CTA)-3′ [b])	54	– 102	– 286	– 13.5
34·21	3′-d(TCATAACT**iG**GAT)-5′ • • • • • • • • ∗ • • • 5′-d(AGTATTGA **C** CTA)-3′	44	– 73	– 205	– 9.6
34·24	3′-d(TCATAACT**iG**GAT)-5′ • • • • • • • • ∗ • • • 5′-d(AGTATTGA **G** CTA)-3′	44	– 71	– 199	– 9.6
34·25	3′-d(TCATAACT**iG**GAT)-5′ • • • • • • • • ∗ • • • 5′-d(AGTATTGA **T** CTA)-3′	45	– 79	– 223	– 10.1
34·26	3′-d(TCATAACT**iG**GAT)-5′ • • • • • • • • ∗ • • • 5′-d(AGTATTGA **A** CTA)-3′	35	– 58	– 161	– 7.6
20·35	3′-d(TCATAA **C** TGGAT)-5′ • • • • • • ∗ • • • • • 5′-d(AGTATT**iG**A**C**CTA)-3′	44	– 67	– 183	– 9.8
29·35	3′-d(TCATAA **G** TGGAT)-5′ • • • • • • ∗ • • • • • 5′-d(AGTATT**iG**ACCTA)-3′	44	– 68	– 189	– 9.8
30·35	3′-d(TCATAA **T** TGGAT)-5′ • • • • • • ∗ • • • • • 5′-d(AGTATT**iG**ACCTA)-3′	42	– 62	– 171	– 9.1
31·35	3′-d(TCATAA **A** TGGAT)-5′ • • • • • • ∗ • • • • • 5′-d(AGTATT**iG**ACCTA)-3′	32	– 42	– 113	– 7.2
28·35	3′-d(TCATAA**iC**TGGAT)-5′ [b]) • • • • • • ∗ • • • • • 5′-d(AGTATT**iG**ACCTA)-3′	54	– 95	– 263	– 13.4

[a]) [b]) See *Table 3*.

difference between the two series (*Table 5 vs. Table 3* and *Table 6 vs. Table 4*) is the presence of isoguanine in one series and 7-deazaisoguanine in the other. Also duplexes with parallel or antiparallel chain orientation were compared.

If one compares the data of *Table 5* with those of *Table 3* (aps-duplexes), it is apparent that isoguanine and 7-deazaisoguanine behave very similarly. This is true for the T_m values as well as for the thermodynamic data. The most stable aps-duplexes are those with isoguanine·5-methylisocytosine or 7-deazaisoguanine·5-methylisocytosine base pairs. The stability of the isoguanine-containing duplex **34·23** (*Table 5*) is slightly higher than that of the 7-deazaisoguanine-containing one **22·23** (*Table 3*), and the

Table 6. T_m *Values and Thermodynamic Data of the ps-Hybrids Formed by Oligonucleotides Containing* iG_d *and* m^5iC_d [a])

	ps-Duplexes	T_m [°]	$\Delta H°$ [kcal/mol]	$\Delta S°$ [cal/mol·K]	$\Delta G°_{298}$ [kcal/mol]
36·21	5′-d(TiCATAAiCT**iG**iGAT)-3′[b])				
	• • • • • • • • * • • •	44	−85	−242	−10.3
	5′-d(AGTATTGA**C** CTA)-3′				
36·24	5′-d(TiCATAAiCT**iG**iGAT)-3′[b])				
	• • • • • • • • * • • •	36	−56	−154	−8.2
	5′-d(AGTATTGA**G** CTA)-3′				
36·25	5′-d(TiCATAAiCT**iG**iGAT)-3′[b])				
	• • • • • • • • * • • •	33	−60	−170	−7.5
	5′-d(AGTATTGA**T** CTA)-3′				
36·26	5′-d(TiCATAAiCT**iG**iGAT)-3′[b])				
	• • • • • • • • * • • •	34	−57	−161	−7.6
	5′-d(AGTATTGA**A** CTA)-3′				
36·23	5′-d(TiCATAAiCT**iG**iGAT)-3′[b])				
	• • • • • • • • * • • •	40	−63	−175	−8.8
	5′-d(AGTATTGA**iC** CTA)-3′[b])				
37·20	5′-d(ATiCiCA**iG**TTATiGA)-3′[b])				
	• • • • • * • • • • • •	39	−74	−211	−8.8
	5′-d(TAGGT**C**AATACT)-3′				
37·30	5′-d(ATiCiCA**iG**TTATiGA)-3′[b])				
	• • • • • * • • • • • •	31	−51	−140	−7.3
	5′-d(TAGGT**T**AATACT)-3′				
37·31	5′-d(ATiCiCA**iG**TTATiGA)-3′[b])				
	• • • • • * • • • • • •	34	−55	−151	−7.6
	5′-d(TAGGT**A**AATACT)-3′				
37·28	5′-d(ATiCiCA**iG**TTATiGA)-3′[b])				
	• • • • • * • • • • • •	30	−45	−121	−7.2
	5′-d(TAGGT**iC**AATACT)-3′[b])				

[a]) [b]) See *Table 3*.

enthalpy of duplex formation is *ca.* 100 kcal/mol for **34·23** (*Table 5*) instead of 90 kcal/mol for **22·23** (*Table 3*). In the other examples containing base mismatches, the thermodynamic data are almost identical, even for the hybrid having dA located opposite 7-deazaisoguanosine or isoguanosine. These findings are not only valid for a particular sequence but are of general applicability; see data shown in *Tables 3* and *5*.

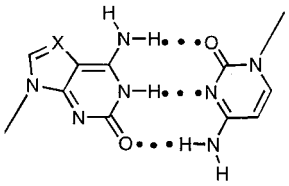
Next, the behaviour of isoguanine and 7-deazaisoguanine was studied in the case of duplexes with parallel chain orientation. In this case, data of *Table 4* were compared with those of *Table 6*. Also in these cases, very similar results were found, regarding the T_m values and thermodynamic data, between isoguanine- and 7-deazaisoguanine-containing oligonucleotides. The most stable duplexes with the most favourable enthalpies were **32·21** with the 7-deazaguanine·cytosine base pairs or **36·21** with a base pair isoguanine·cytosine (see *Tables 4* and *6*). Unfavourable enthalpy and entropy data were observed when 7-deazaisoguanine is located opposite to the bases guanine, thymine, adenine, and 5-methylisocytosine (*Table 4*). This tendency can also be seen in *Table 6* in which isoguanine replaces 7-deazaisoguanine.

2.2.3. *Proposed Motifs for the Base Pairs of 7-Deazaisoguanine or Isoguanine with Cytosine, 5-Methylisocytosine, Thymine, Guanine, and Adenine. Preamble.* Base-pair motifs of isoguanine with various other bases have already been proposed over the years by various laboratories [2][5][19][23–28]. Their work mainly focuses on duplexes with antiparallel chain orientation. As very little is known on the base pairing of isoguanine with other common bases, our study reports on the stabilities of the duplexes containing isoguanine or 7-deazaisoguanine with various other bases. The most likely base-pair motifs are constructed on the basis of duplex stabilities. These include the base pairs formed by duplexes with parallel and antiparallel chain orientation.

Duplexes with Parallel Chain Orientation. Oligonucleotide duplexes containing only adenine · thymine base pairs have been studied before [1]. The first report on a parallel duplex with an isoguanine · cytosine pair was published by our laboratory [2]. The stability of ps-duplexes formed by oligonucleotides containing 5-methylisocytosine and isoguanine with DNA or RNA oligomers have been examined by *Sugiyama et al.* [3]. The base-pair motif between 5-methylisocytosine and guanine was proposed and that between isoguanine and cytosine was established [3]. According to *Tables 4* and *6*, rather stable duplexes are formed when isoguanine is located opposite to adenine, guanine, thymine, or 5-methylisocytosine. The individual base-pair motifs of 7-deazaisoguanine or isoguanine with those bases are now discussed.

Duplexes with isoguanine · cytosine pairs show parallel chain orientation [2][5]. The base pair of isoguanine and cytosine is particularly stable [5][15]. The 7-deazaisoguanine · cytosine pair shows a similar duplex stability to that of isoguanine · cytosine. The absence of N(7) excludes *Hoogsteen* pairing. Apart from the oligonucleotides described in *Tables 4* and *6*, alternating self-complementary duplexes and also non-self-complementary duplexes containing isoguanine · cytosine base pairs have been studied [5][20]. Also the base pair **Ib** leading to duplexes with parallel chain orientation is included in a paper describing the duplex stability of pyranosyl-RNAs [26]. The ps-duplexes containing the isoguanine · cytosine pair have also been investigated by *Sugiyama et al.* [3] and have been determined by an NMR study [28]. The base pair of isoguanine and cytosine as well as of 7-deazaisoguanine and cytosine follows the motifs **Ia** or **Ib** with a tridendate pairing mode.

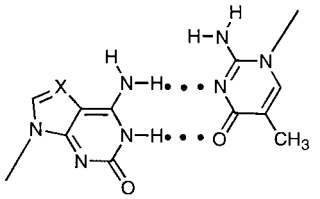
Parallel duplexes with 7-deazaisoguanine · 5-methylisocytosine and isoguanine · 5-methylisocytosine base pairs are significantly less stable than those containing cytosine (see *Tables 4* and *6*). The motifs **IIa** and **IIb**, which are wobble pairs, represent the most likely pairing modes for $c^7iG_d \cdot m^5iC_d$ and $iG_d \cdot m^5iC_d$. *Hoogsteen* base pairs can be excluded, as duplexes containing 7-deazaisoguanine show a comparable stability to those containing isoguanine. According to the T_m values in *Tables 4* and *6*, the base-pair motifs of 7-deazaisoguanine with thymine and isoguanine with thymine are expected to be identical. *Hoogsteen* pairing cannot occur. Therefore, the base pairs **IIIa,b**, representing wobble pairs, are the most likely ones for $c^7iG_d \cdot dT$ and $iG_d \cdot dT$. Moreover, the tridendate motifs **IVa,b** with isoguanine in the enol form has also to be considered. For the isoguanine · guanine as well as for the 7-deazaisoguanine · guanine pair, the same motifs are expected as duplexes containing these pairs show similar stability (*Tables 4* and *6*). According to the various tautomeric forms of isoguanine and guanine, one wobble base pair is conceivable (see **Va,b**), as well as one *Hoogsteen* motif (see



Ia **c^7iG·C** (X = CH)

b **isoG·C** (X = N) [2]

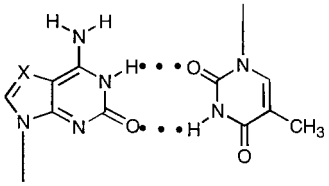
reverse *Watson-Crick* base pair



IIa **c^7iG·me^5iC** (X = CH)

b **isoG·me^5iC** (X = N) [3]

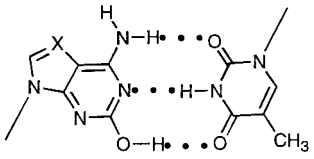
Wobble base pair



IIIa **c^7iG·T** (X = CH)

b **isoG·T** (X = N)

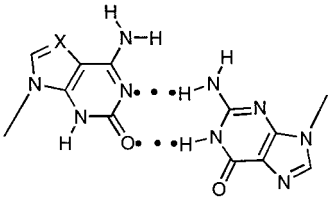
Wobble base pair



IVa **c^7iG·T** (X = CH)

b **isoG·T** (X = N)

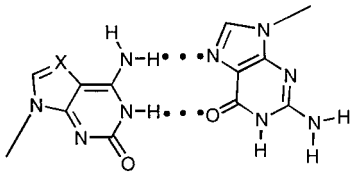
reverse *Watson-Crick* base pair



Va **c^7iG·G** (X = CH)

b **isoG·G** (X = N)

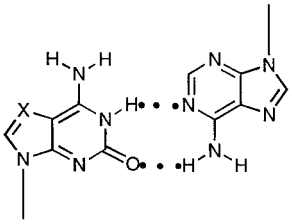
Wobble base pair



VIa **c^7iG·G** (X = CH)

b **isoG·G** (X = N)

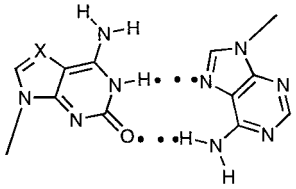
reverse *Hoogsteen* base pair



VIIa **c^7iG·A** (X = CH)

b **isoG·A** (X = N)

purine · purine base pair



VIIIa **c^7iG·A** (X = CH)

b **isoG·A** (X = N)

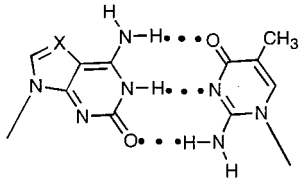
reverse *Hoogsteen* base pair

VIa,b). In the case of oligonucleotide duplexes containing isoguanine · adenine or 7-deazaisoguanine · adenine base pairs, a reverse purine · purine (see **VIIa,b**) as well as the *Hoogsteen* (see **VIIIa,b**) motifs are proposed. The stability of duplexes containing the 7-deazaisoguanine · adenine or isoguanine · adenine base pair is very similar and, therefore, the same pairing modes are suggested.

Duplexes with Antiparallel Chain Orientation. The most stable base pairs in antiparallel-stranded duplexes are those of 7-deazaisoguanine or isoguanine with 5-methylisocytosine (see *Tables 3* and *5*); the 7-deazaisoguanine pairs with the common DNA bases resulting in duplex destabilization. The pairs of 7-deazaisoguanine with cytosine, guanine, and thymine make very equal contributions to the duplex stability. The least stable aps-duplexes in *Table 3* are those having 7-deazaisoguanine opposite adenine (aps-**22** · **26** and aps-**31** · **27**). Their T_m values which are *ca*. 20° lower than that of the parent hybrids aps-**22** · **23** and aps-**28** · **27** indicate that no base pair is formed. Therefore, we do not suggest any base-pair motif in this case.

The tridentate isoguanine · isocytosine base pair, as well as the isoguanine · 5-methylisocytosine pair, have been investigated by several laboratories [4][17][24][27]. According to *Tables 3* and *5*, the 7-deazaisoguanine · 5-methylisocytosine pair shows a similar stability as the isoguanine · 5-methylisocytosine pair. The tridendate base pairs **IXa** and **IXb** represent the most likely structures. *Hoogsteen* base pairing can be excluded.

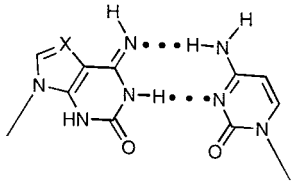
In the case of the isoguanine · cytosine base pair, a non-standard *Watson-Crick* motif **Xb** has been proposed by *Roberts et al*. [27]. It was considered that a *trans*-imino oxo isoguanine tautomer is favoured according to their theoretical calculation. However, the wobble pair **XIb** and a base pair **XIIb**, in which isoguanine adopts the enol form, can also be formed [27]. According to the similar stability of duplexes containing either isoguanine or 7-deazaisoguanine, identical pairing modes are expected for the purine and the 7-deazapurine bases (see **Xa,b**, **XIa,b**, and **XIIa,b**). The base pairing of isoguanine and thymine in duplexes with aps-orientation has been under intensive investigation [17][18][27]. It was shown that 2′-deoxyisoguanosine triphosphate is incorporated opposite to thymine when DNA polymerases were used for chain elongation [19][24]. Also, an X-ray structure has recently been published [28]. The two base pairs **XIIIb** and **XIVb** have been suggested in the *Dickerson-Drew* dodecamer in different locations of the duplex [28]. These motifs are also suitable for base pairs between 7-deazaisoguanine and thymine (see **XIIIa** and **XIVa**). The driving force for the *Watson-Crick* pair **XIV** is the hydrophobic environment of the duplex which induces the formation of the enol. These findings are similar to those observed for the monomeric nucleoside, which forms the enol in less polar solvents [15][29]. In the case of the isoguanine and guanine base pair, a tridendate H-bonding pattern has been proposed for the duplex formation of pyranosyl-oligonucleotides [26]. This purine · purine base pair can also be formed in normal DNA duplexes. In this case, the isoguanine base adopts the keto/N(3)−H tautomer **XVb**. However, the *Hoogsteen* motif **XVIb** has also to be considered. Due to the similar duplex stabilities of the isoguanine- and 7-deazaisoguanine-containing oligonucleotides (see *Tables 3* and *5*), similar motifs **XVa** and **XVIa** are expected.



IXa c^7**iG·me**^{5}**iC** (X = CH)

b **isoG·me**^{5}**iC** (X = N) [17]

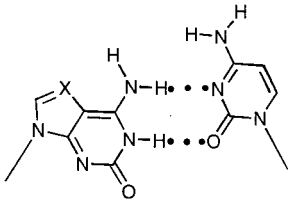
Watson-Crick base pair



Xa c^7**iG·C** (X = CH)

b **isoG·C** (X = N) [27]

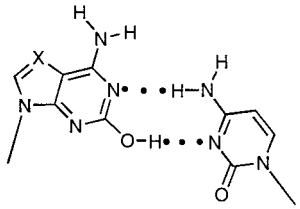
Watson-Crick base pair



XIa c^7**iG·C** (X = CH)

b **isoG·C** (X = N) [27]

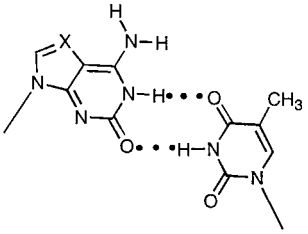
Wobble base pair



XIIa c^7**iG·C** (X = CH)

b **isoG·C** (X = N) [27]

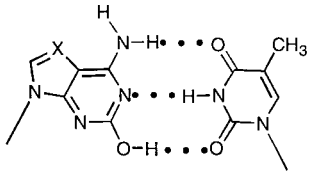
Wobble base pair



XIIIa c^7**iG·T** (X = CH)

b **isoG·T** (X = N) [28]

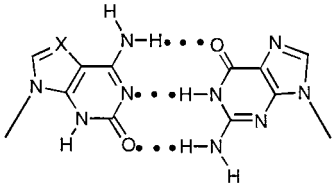
Wobble base pair



XIVa c^7**iG·T** (X = CH)

b **isoG·T** (X = N) [28]

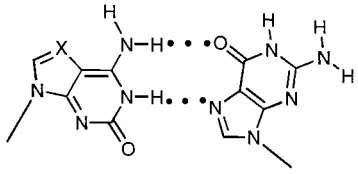
Watson-Crick base pair



XVa c^7**iG·G** (X = CH)

b **isoG·G** (X = N) [26]

Watson-Crick base pair



XVIa c^7**iG·G** (X = CH)

b **isoG·G** (X = N)

Hoogsteen base pair

3. Conclusion. – From the results described above, it is concluded that 7-deazaisoguanine shows rather similar base-pairing properties compared to isoguanine. This is valid for both parallel and antiparallel duplexes and shows that the purine N(7) of isoguanine does not take part in base pairing. The most stable ps-DNA duplex is formed by 7-deazaisoguanine · cytosine or isoguanine · cytosine (ps-**32** · **21**, *Table 4*; ps-**36** · **21**, *Table 6*). Correspondingly, the 7-deazaisoguanine · 5-methylisocytosine pair is the most stable base pair in antiparallel-stranded DNA (aps-**28** · **27**, *Table 3*) as it was found already for the base pair of isoguanine with 5-methylisocytosine (aps-**28** · **35** and aps-**34** · **23**, *Table 5*). The stability of the duplex decreases towards other bases such as thymine, adenine, or guanine. As the base pairs of 7-deazaisoguanine with cytosine, guanine, or thymine show almost the same stability within aps-DNA and with guanine, thymine, or adenine within ps-duplexes, the base-pairing properties of 7-deazaisoguanine with other bases fulfills the requirement of a universal nucleoside. In this case, base pairing is ambiguous against four different bases. In this regard, the modified base behaves similarly to 2′-deoxyinosine [30] as well as its 7-deazapurine analogue [31].

The rather low glycosylic-bond stability of 2′-deoxyisoguanosine (**2**) against acid, which causes problems during oligonucleotide synthesis or reactions which require acidic conditions, was overcome with 7-deaza-2′-deoxyisoguanosine (**1**). As **1** shows almost the same base-pair stability and base-pair selectivity as **2**, it can be considered to be an ideal substitute.

We thank Drs. *N. Ramzaeva*, and *H. Rosemeyer* for NMR spectra and Mr. *Y. He* for supplying us with the phosphoramidite of 2′-deoxy-5-methylisocytidine. Financial support by the *Deutsche Forschungsgemeinschaft* and *Roche Diagnostics GmbH* is gratefully acknowledged.

Experimental Part

General. See [5]. The solid-phase synthesis of oligonucleotides was carried out on an automated DNA synthesizer (*Applied Biosystems*, model *ABI 380 B* for phosphonate synthesis and *ABI 392-08* for phosphoramidite chemistry). Snake-venom phosphodiesterase (EC 3.1.4.1., *Crotallus durissus*) and alkaline phosphatase (EC 3.1.3.1., *E. coli*) were generous gifts from *Boehringer Mannheim GmbH*, Germany. All other reagents are commercially available and used as received. The solvents were purified and dried according to standard procedures. Alkaline hydrolysis of nucleosides followed the reported protocol [5]. NMR Spectra: *Avance-DPX-250* and -*AMX-500* spectrometer (*Bruker*, Germany); δ values in ppm downfield from internal $SiMe_4$ (^1H, ^{13}C) or external 85% H_3PO_4 (^{31}P).

Synthesis and Purification of Oligonucleotides **20**–**37**. The synthesis was carried out on a 1-μmol scale with the 3′-phosphoramidites of $[(MeO)_2Tr]T_d$ $[(MeO)_2Tr]ib^2G_d$, $[(MeO)_2Tr]bz^6A_d$, and $[(MeO)_2Tr]bz^4C_d$, together with the dpc-protected phosphoramidite of 2′-deoxyisoguanosine [5], the building block **6**, and the solid supports CPG-dT or CPG-dA. The synthesis of **20**–**37** followed the regular protocol of the DNA synthesizer for phosphoramidites [32]. After cleavage from the solid-support, the oligonucleotides were deprotected in 25% NH_3/H_2O (12–18 h at 60°).

The purification of oligonucleotides was performed by reversed-phase HPLC (*RP-18*); the following solvent gradients were used (*A*, 0.1M $(Et_3NH)OAc$ (pH 7.0)/MeCN 95:5; *B*, MeCN); gradient *I*, 3 min 15% *B* in *A*, 12 min 15–40% *B* in *A*, 5 min 40–15% *B* in *A* with a flow rate of 1.0 ml/min; gradient *II*, 20 min 0–20% *B* in *A* with a flow rate of 1.0 ml/min; gradient *III*, 20 min 100% *A* with a flow rate of 0.6 ml/min.

Thus, the 5′-(dimethoxytrityl)-oligomers were purified on a 250 × 4 mm *RP-18* column (gradient *I*), and the 4,4′-dimethoxytrityl residues were removed by treatment with 2.5% $CHCl_2COOH/CH_2Cl_2$ for 5 min at r.t. The detritylated oligomers were purified by reversed-phase HPLC (gradient *II*). The oligomers were desalted on a short column (*RP-18*, silica gel) using H_2O for the elution of the salt, while the oligomers were eluted with $MeOH/H_2O$ 3:2. The oligonucleotides were lyophilized on a *Speed-Vac* evaporator to yield colourless solids which were dissolved in 100 μl of H_2O and stored frozen at −18°.

Nucleoside-Composition Analysis. The analyses were performed as described [5]. Extinction coefficients of the nucleoside constituents: ε_{260}: c^7iG_d 7400, iG_d 4300, dA 15400, dT 8800, dG 11700, dC 7600, m^5iC_d 6300; ε_{280}: c^7iG_d 3330, iG_d 7300, dA 2400, dT 6337, dG 7980, dC 6900, m^5iC_d 1600).

Determination of T_m *Values.* The UV melting temperatures (T_m) of all hybrids containing 7-deazaisoguanine or isoguanine were measured, as well as those of the corresponding unmodified control duplex, by means of a *Cary-1E*-UV/VIS spectrophotometer (*Varian*, Australia), equipped with a *Peltier* block which was connected to a *Cary* temp. controller (*Varian*, Australia). The oligonucleotides were dissolved in a buffer consisting of aq. NaCl (1M), $MgCl_2$ (0.1M), and sodium cacodylate (60 mM) at pH 7.0. The concentration of each strand was 5 μM. The sample was heated to 80° and stabilized for 10 min at this temp. A cooling rate of 1°/min was used throughout the measurement. The melting profile was read at 260 nm.

Synthesis of Monomers. The starting material 7-deazaguanine (= 2-amino-7*H*-pyrrolo[2,3-d]pyrimidin-4(3*H*)-one) was prepared as reported [33][34]. It was converted to the nucleoside **7** *via* 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine following a previously published protocol [13].

*7-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-7*H-*pyrrolo[2,3-*d*]pyrimidine-2,4-diamine* (**8**). A suspension of **7** (3.00 g, 5.77 mmol) in dioxane (60.0 ml) and 25% NH_3/H_2O (80.0 ml) was introduced into an autoclave and stirred at 120° for 70 h. The solvent was evaporated and the residue applied to flash chromatography (FC; column 4 × 16 cm, CH_2Cl_2/MeOH 9 : 1 → 8 : 2). From the main zone, crude **8** was obtained after evaporation as pale yellow powder (1.35 g, 88%) which was not purified further. NMR Data: identical to those reported [13].

*4-Amino-7-(2-deoxy-β-*D-erythro-*pentofuranosyl)-1,7-dihydro-2*H-*pyrrolo[2,3-*d*]pyrimidin-2-one* (**1**). To a soln. of **8** (1.32 g, 4.98 mmol) in 20% AcOH/H_2O (*v*/*v*; 80 ml), a soln. of $NaNO_2$ (0.73 g, 10.58 mmol) in H_2O (15 ml) was added dropwise at r.t. under stirring. Stirring was continued for 110 min, and the pH of the dark soln. was adjusted to 6.0 (25% aq. NH_3 soln.). The soln. was applied to a *Serdolit AD-4* column (4 × 20 cm, resin 0.1–0.2 mm; *Serva*, Germany), the column washed with H_2O (500 ml), and the product eluted with H_2O/^{i}PrOH 95 : 5. Compound **1** crystallized from the solvent: **1** (0.88 g, 67%). Colourless needles. M.p. 230°–232° (dec.). UV, ^{1}H- and ^{13}C-NMR Spectra: identical with published data [8][11].

*4-(Benzoylamino)-7-(3,5-di-*O*-benzoyl-2-deoxy-β-*D-erythro-*pentofuranosyl)-7*H-*pyrrolo[2,3-*d*]pyrimidin-2-ol Benzoate* (**10**) *and 4-(Dibenzoylamino)-7-(3,5-di-*O*-benzoyl-2-deoxy-β-*D-erythro-*pentofuranosyl)-1,7-dihydro-2*H-*pyrrolo[2,3-*d*]pyrimidin-2-one* (**11**) *or 3-Benzoyl-4-(benzoylamino)-7-(3,5-di-*O*-benzoyl-2-deoxy-β-*D-erythro-*pentofuranosyl)-3,7-dihydro-2*H-*pyrrolo[2,3-*d*]pyrimidin-2-one* (**12**). To the stirred suspension of **1** (266 mg, 1 mmol) in pyridine (3 ml), benzoyl chloride (0.7 ml, 5.96 mmol) was added. The mixture was stirred at r.t. for 6 h. A few pieces of ice were added, and the mixture was poured into ice-water (20 ml) and extracted with CH_2Cl_2 (3 × 12 ml). The combined org. layers were washed with a 5% aq. $NaHCO_3$ soln. (10 ml) and H_2O (15 ml), dried (Na_2SO_4), and evaporated, and the residue was applied to FC (silica gel, column 2 × 16 cm, CH_2Cl_2 (100 ml), CH_2Cl_2/Me_2CO 95 : 5 (200 ml)): more polar **10** (157 mg, 23%) and less polar **11** or **12** (268 mg, 39.3%).

Data of **10**: Colourless powder. TLC (CH_2Cl_2/MeOH 98 : 2): R_f 0.3. UV (MeOH): 228 (53600), 270 (15400). ^{1}H-NMR ((D$_6$)DMSO): 2.70 (*m*, 1 H–C(1′)); 3.01 (*m*, 1 H–C(2′)); 4.51–4.64 (*m*, H–C(4′), 2 H–C(5′)); 5.72 (*m*, H–C(3′)); 6.58 (*t*, *J* = 6.3, H–C(1′)); 6.68 (*d*, *J* = 3.5, H–C(5)); 7.38 (*d*, *J* = 3.5, H–C(6)); 7.51–8.15 (*m*, 20 arom. H). Anal. calc. for $C_{30}H_{30}N_4O_8$ (682.7): C 68.61, H 4.43, N 8.21; found: C 68.52, H 4.63, N 8.15.

Data of **11** *or* **12**: Colourless foam. TLC (CH_2Cl_2/MeOH 98 : 2): R_f 0.7. UV (MeOH): 231 (58900), 362 (12200). ^{1}H-NMR ((D$_6$)DMSO): 2.82 (*m*, 1 H–C(2′)); 3.11 (*m*, 1 H–C(2′)); 4.57–4.67 (*m*, H–C(4′), 2 H–C(5′)); 5.78 (*m*, H–C(3′)); 6.79–6.82 (*m*, H–C(1′), H–C(5)); 7.52–8.18 (*m*, H–C(6), 20 arom. H); 11.46 (*s*, NH). Anal. calc. for $C_{30}H_{30}N_4O_8$ (682.7): C 68.61, H 4.43, N 8.21; found: C 68.36, H 4.89, N 8.61.

*4-(Benzoylamino)-7-(2-deoxy-β-*D-erythro-*pentofuranosyl)-1,7-dihydro-2*H-*pyrrolo[2,3-*d*]pyrimidin-2-one* (**15**). Chlorotrimethylsilane (0.5 ml, 4.1 mmol) was added to a soln. of **1** (133 mg, 0.5 mmol) in dry pyridine (3 ml). After the mixture was stirred for 35 min, benzoyl chloride (0.2 ml, 1.7 mmol) was introduced and the mixture maintained at r.t. for 3 h. The mixture was cooled in an ice bath and diluted with H_2O (0.6 ml). After 5 min, 25% NH_3/H_2O (0.2 ml) was added, the mixture stirred at r.t. for 0.5 h and evaporated, and the residue applied to FC (column 2 × 10 cm, CH_2Cl_2/MeOH 95 : 5 → 85 : 15); **15** (139 mg, 75%). Slightly yellow powder. TLC (CH_2Cl_2/MeOH 8 : 2): R_f 0.45. UV (MeOH): 238 (29100), 360 (10300). ^{1}H-NMR ((D$_6$)DMSO): 2.16 (*m*, 1 H–C(2′)); 2.37 (*m*, 1 H–C(2′)); 3.54 (*m*, 2 H–C(5′)); 3.81 (*m*, H–C(4′)); 4.33 (*m*, H–C(3′)); 5.26 (*m*, OH–C(5′), OH–C(3′)); 6.40 (*t*, *J* = 6.75, H–C(1′)); 6.58 (*d*, *J* = 3.5, H–C(5)); 7.26 (*d*, *J* = 3.3, H–C(6)); 7.49–8.07 (*m*, 5 arom. H); 11.40 (br. *s*, NH). Anal. calc. for $C_{18}H_{18}N_4O_5$ (370.37): C 58.37, H 4.90, N 15.13; found: C 58.40, H 5.07, N 15.16.

*7-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-1,7-dihydro-4-[(2-methylpropanoyl)amino]-2*H*-pyrrolo[2,3-*d*]-pyrimidin-2-one* (**16**). Chlorotrimethylsilane (2.0 ml, 16.7 mmol) was added to the soln. of 7-deaza-2′-deoxyisoguanosine (**1**; 532 mg, 2 mmol) in dry pyridine (12 ml). After stirring for 30 min, isobutyryl chloride (0.8 ml, 7.6 mmol) was added and stirring continued for another 3 h. The mixture was cooled in an ice bath and diluted with H_2O (2.4 ml). After 5 min, a conc. aq. NH_3 soln. (2.0 ml) was added. The mixture was stirred for 20 min at r.t., evaporated and co-evaporated twice with MeOH. The residue was submitted to FC (column 4×10 cm, CH_2Cl_2/MeOH $95:5 \rightarrow 80:20$): **16** (570 mg, 84%). Pale-yellow powder. TLC (CH_2Cl_2/MeOH 3:1): R_f 0.56. UV (MeOH): 236 (25400), 341 (6420). ^{1}H-NMR ((D$_6$)DMSO): 1.11 (2*s*, 2 Me); 2.07 (*m*, 1 H–C(2′)); 2.10 (*m*, 1 H–C(2′)); 2.91 (*m*, CH); 3.50 (*m*, 2 H–C(5′)); 3.77 (*m*, H–C(4′)); 4.30 (*m*, H–C(3′)); 5.26 (br. *s*, OH–C(5′), OH–C(3′)); 6.34 (*t*, $J = 7.25$, H–C(1′)); 6.69 (*d*, $J = 3.5$, H–C(5)); 7.14 (*d*, $J = 3.6$, H–C(6)); 11.38 (br. *s*, NH). Anal. calc. for $C_{15}H_{20}N_4O_5$ (336.36): C 53.56, H 5.99, N 16.66; found: C 53.93, H 5.96, N 16.71.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-1,7-dihydro-4-[(2-methylpropanoyl)-amino]-2*H*-pyrrolo[2,3-*d*]pyrimidin-2-one* (**18**). Compound **16** (483 mg, 1.4 mmol) was dried by repeated co-evaporation from anh. pyridine and dissolved in anh. pyridine (10 ml). ^{i}Pr$_2$EtN (0.24 ml, 1.34 mmol) and 4,4′-dimethoxytrityl chloride ((MeO)$_2$TrCl; 542 mg, 1.6 mmol) were added at r.t., and the soln. was stirred for 3 h. To this soln. MeOH (2 ml) was added, and after 5 min, the soln. was poured into 5% aq. $NaHCO_3$ soln. (15 ml) and extracted with CH_2Cl_2 (3×8 ml). The org. layer was dried (Na_2SO_4) and evaporated and the residue submitted to FC (silica gel, column 10×2.5 cm, prewashed with CH_2Cl_2/acetone 8:2, then CH_2Cl_2/MeOH $95:5 \rightarrow 90:10$): **18** (526 mg, 58%). Colourless foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.44. UV (MeOH): 236 (52900), 346 (8000). ^{1}H-NMR ((D$_6$)DMSO): 1.14 (*m*, 2 Me); 2.21 (*m*, 1 H–C(2′)); 2.41 (*m*, 1 H–C(2′)); 2.85 (*m*, CH); 3.15 (*m*, 2 H–C(5′)); 3.91 (*m*, H–C(4′)); 4.33 (*m*, H–C(3′)); 5.30 (*d*, $J = 3.3$, OH–C(3′)); 6.43 (*t*, $J = 6.70$, H–C(1′)); 6.62 (*d*, $J = 3.8$, H–C(5)); 7.07 (*d*, $J = 3.7$, H–C(6)); 6.81–7.39 (*m*, arom. H); 10.85 (br. *s*, NH); 11.30 (br. *s*, NH). Anal. calc. for $C_{36}H_{38}N_4O_7$ (638.73): C 67.70, H 6.00, N 8.77; found: C 67.83, H 6.11, N 8.61.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-1,7-dihydro-4-[(2-methylpropanoyl)-amino]-2*H*-pyrrolo[2,3-*d*]pyrimidin-2-one 3′-(Triethylammonium Phosphonate)* (**3**). To a mixture of 1*H*-1,2,4-triazole (287 mg, 4.16 mmol) and 4-methylmorpholine (1.3 ml) in CH_2Cl_2 (8 ml), PCl_3 (108 µl, 1.20 mmol) was added under stirring. Stirring was continued 30 min, the mixture then cooled to 0°, and **18** (150 mg, 0.23 mmol) in CH_2Cl_2 (8 ml) added dropwise. The mixture was stirred at r.t. for 50 min and then poured into 1M (Et_3NH)HCO_3 (14.4 ml). The aq. layer was extracted with CH_2Cl_2 (3×4 ml) and the combined org. phase dried (Na_2SO_4) and evaporated. The residue was submitted to FC (silica gel, column 2×8 cm, CH_2Cl_2/MeOH/Et_3N $94:4:2 \rightarrow 88:10:2$): **3** (129 mg, 70%). Colourless powder. TLC (CH_2Cl_2/MeOH/Et_3N 88:10:2): R_f 0.5. UV (MeOH): 236 (58700), 344 (8700). ^{1}H-NMR ((D$_6$)DMSO): 0.95 (*m*, 3 *Me*CH$_2$); 1.09 (*m*, 2 Me); 2.41 (*m*, 1 H–C(2′)); 2.60 (*m*, 1 H–C(2′)); 2.79 (*m*, CH); 2.81 (*m*, 3 MeC*H*$_2$); 3.14 (*m*, 2 H–C(5′)); 3.71 (*s*, 2 MeO); 4.04 (*m*, H–C(4′)); 4.73 (*m*, H–C(3′)); 5.44, 7.81 (2*s*, H–P); 6.38 (*t*, $J = 6.60$, H–C(1′)); 6.56 (*d*, $J = 3.3$, H–C(5)); 6.93 (*d*, $J = 3.3$, H–C(6)); 6.76–7.37 (*m*, 13 arom. H). ^{31}P-NMR ((D$_6$)DMSO): –0.47 (^1J(P,H) = 600, ^3J(P,H) = 9.8). Anal. calc. for $C_{42}H_{54}N_5O_9P$ (803.89): C 62.75, H 6.77, N 8.71; found: C 62.63, H 6.51, N 8.61.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-1,7-dihydro-4-[(2-methylpropanoyl)-amino]-2*H*-pyrrolo[2,3-*d*]pyrimidin-2-one 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**4**). A soln. of **18** (319 mg, 0.50 mmol) in CH_2Cl_2 (14 ml) was flushed with Ar. Then 2-cyanoethyl diisopropylphosphoramidochloridite (0.33 ml, 1.5 mmol) was added, together with ^{i}Pr$_2$EtN (0.35 ml, 2.0 mmol) under Ar. After stirring for 40 min at r.t., 5% aq. $NaHCO_3$ soln. (14 ml) was added and the mixture extracted with CH_2Cl_2 (3×7 ml). The combined org. layer was dried (Na_2SO_4) and evaporated to give an oil. This was co-evaporated with toluene and the residue submitted to FC (silica gel, column 2.5×10 cm, CH_2Cl_2/MeOH/Et_3N 95:4:1): **4** (400 mg, 95%). Colourless foam. TLC (CH_2Cl_2/MeOH/Et_3N 95:4:1): R_f 0.46. ^{31}P-NMR ($CDCl_3$): 149.6, 149.7.

*4-Amino-7-(2-deoxy-β-*D*-*erythro*-pentofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl Diphenylcarbamate* (**13**) *and 4-Amino-7-[2-deoxy-5-*O*-(diphenylcarbamoyl)-β-*D*-*erythro*-pentofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl Diphenylcarbamate* (**14**). Compound **1** (1.15 g, 4.3 mmol) was dried by repeated co-evaporation from anh. pyridine and then suspended in anh. pyridine (22 ml). Diphenylcarbamoyl chloride (dpc-Cl; 1.54 g, 6.4 mmol) and ^{i}Pr$_2$EtN (1.1 ml, 6.4 mmol) were added, and the mixture was stirred for 2 h at r.t. The excess of dpc-Cl was destroyed with crushed ice. The mixture was poured into 5% aq. $NaHCO_3$ soln. (30 ml) and extracted with CH_2Cl_2 (3×30 ml), the combined org. phase dried (Na_2SO_4), and evaporated, and the residue submitted to FC (silica gel, column 2×17 cm, CH_2Cl_2/Me$_2$CO 85:15 (400 ml), then CH_2Cl_2/MeOH 90:10): more polar **13** (1.58 g, 80%) and less polar **14** (0.23 g, 8%).

Data of **13**: Colourless foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.40. UV (MeOH): 269 (12900). ^{1}H-NMR ((D)$_6$DMSO): 2.15 (*m*, 1 H–C(2′)); 2.42 (*m*, 1 H–C(2′)); 3.53 (*m*, 2 H–C(5′)); 3.80 (*m*, H–C(4′)); 4.33 (*m*, H–C(3′)); 4.96 (*t*, J = 5.5, OH–C(5′)); 5.25 (*d*, J = 4.0, OH–C(3′)); 6.39 (*t*, J = 6.8, H–C(1′)); 6.59 (*d*, J = 3.5, H–C(5)); 7.25–7.48 (*m*, H–C(6), NH_2, 10 arom. H). Anal. calc. for $C_{24}H_{23}N_5O_5$ (461.48): C 62.47, H 5.02, N 15.18; found: C 62.63, H 5.17, N 15.08.

Data of **14**: Pale yellow foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.56. UV (MeOH): 265 (13200). ^{1}H-NMR ((D)$_6$DMSO): 1.98 (*m*, 2 H–C(2′)); 3.96 (*m*, H–C(4′)); 4.16–4.30 (*m*, 2 H–C(5′), H–C(3′)); 5.40 (*d*, J = 4.2, OH–C(3′)); 6.33 (*t*, J = 6.3, H–C(1′)); 6.54 (*d*, J = 3.6, H–C(5)); 6.62 (*d*, J = 3.6, H–C(6)); 7.20–7.43 (*m*, NH_2, 20 arom. H). Anal. calc. for $C_{37}H_{32}N_6O_6$ (656.70): C 67.67, H 4.91, N 12.80; found: C 67.55, H 4.99, N 12.88.

*7-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-4-[(2-methylpropanoyl)amino]-7*H-*pyrrolo[2,3-*d*]pyrimidin-2-yl Diphenylcarbamate* (**17**). a) *From* **13**. To a soln. of **13** (1.03 g, 2.23 mmol) in dry pyridine (20 ml), chlorotrimethylsilane (2.0 ml, 16.7 mmol) was added. After the mixture was stirred for 25 min, isobutyryl chloride (0.8 ml, 7.6 mmol) was introduced and stirring continued for 2 h. The insoluble material was filtered off and the filtrate cooled in an ice bath. Upon addition of H_2O (2.8 ml), three portions of 25% NH_3/H_2O (12 ml) were added within 2.5 h under stirring at r.t. The mixture was poured into 5% aq. $NaHCO_3$ soln. (10 ml) and extracted with CH_2Cl_2 (4 × 30 ml), the combined org. phase dried (Na_2SO_4) and evaporated, and the residue submitted to FC (silica gel, column 3 × 16 cm, CH_2Cl_2/MeOH 98:2 → 95:5): **17** (0.95 g, 80%). Colourless foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.52. UV (MeOH): 294 (7200), 233 (36500), 221 (40000). ^{1}H-NMR ((D)$_6$DMSO): 1.11, 1.14, (2*s*, 2 Me); 2.21 (*m*, 1 H–C(2′)); 2.42 (*m*, 1 H–C(2′)); 2.87 (*m*, CH); 3.54 (*m*, 2 H–C(5′)); 3.83 (*m*, H–C(4′)); 4.36 (*m*, H–C(3′)); 4.92 (*t*, J = 5.3, OH–C(5′)); 5.31 (*d*, J = 4.2, OH–C(3′)); 6.55 (*t*, J = 6.5, H–C(1′)); 6.84 (*d*, J = 4.0, H–C(5)); 7.30–7.63 (*m*, H–C(6), 10 arom. H); 11.0 (*s*, NH). Anal. calc. for $C_{28}H_{29}N_5O_6$ (531.57): C 63.27, H 5.50, N 13.17; found: C 63.19, H 5.60, N 13.07.

b) *From* **16**. To a soln. of **16** (183 mg, 0.54 mmol) in anh. pyridine (3.0 ml), dpc-Cl (181 mg, 0.75 mmol) and ^{i}Pr$_2$EtN (130 µl, 0.75 mmol) were added. The mixture was stirred at r.t. for 3 h. The excess of dpc-Cl was destroyed with ice and the mixture then poured into 5% aq. $NaHCO_3$ soln. (10 ml) and extracted with CH_2Cl_2 (4 × 8 ml). The combined org. phase was dried (Na_2SO_4) and evaporated and the residue submitted to FC (silica gel, column 2 × 14 cm, CH_2Cl_2/Me_2CO 9:1 → 7:3): **17** (132 mg, 46%). Colourless foam.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D-erythro-*pentofuranosyl]-4-[(2-methylpropanoyl)amino]-7*H-*pyrrolo[2,3-*d*]pyrimidin-2-yl Diphenylcarbamate* (**19**). Compound **17** (531 mg, 1.0 mmol) was dried by repeated co-evaporation from anh. pyridine and dissolved in anh. pyridine (10 ml). This soln. was treated with 4,4′-dimethoxytrityl chloride (373 mg, 1.1 mmol) at r.t. under stirring (3.5 h). MeOH (5 ml) was introduced and stirring continued for 15 min. The mixture was poured into 5% aq. $NaHCO_3$ soln. (15 ml) and extracted with CH_2Cl_2 (3 × 15 ml). The org. layer was dried (Na_2SO_4) and evaporated and the residue submitted to FC (silica gel, column 2 × 14 cm, with CH_2Cl_2, CH_2Cl_2/Me_2CO 9:1 containing traces of Et_3N): **19** (622 mg, 75%). Colourless foam. TLC (CH_2Cl_2/Me_2CO 9:1): R_f 0.70. UV (MeOH): 221 (55700), 233 (56100), 294 (7600). ^{1}H-NMR ((D)$_6$DMSO): 1.13, 1.15 (2*s*, 2 Me); 2.30 (*m*, 2 H–C(2′)); 2.88 (*m*, CH); 3.16 (*m*, 2 H–C(5′)); 3.72 (*s*, 2 MeO); 3.95 (*m*, H–C(4′)); 4.38 (*m*, H–C(3′)); 5.37 (*d*, J = 4.1, OH–C(3′)); 6.58 (*t*, J = 6.3, H–C(1′)); 6.58 (*d*, H–C(5)); 7.22–7.46 (*m*, H–C(6), 23 arom. H). Anal. calc. for $C_{49}H_{47}N_5O_8$ (833.95): C 70.57, H 5.68, N 8.40; found: C 70.29, H 5.65, N 8.33.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D-erythro-*pentofuranosyl]-4-[(2-methylpropanoyl)amino]-7*H-*pyrrolo[2,3-*d*]pyrimidin-2-yl Diphenylcarbamate 3′-(Triethylammonium Phosphonate)* (**5**). As described for **3**, with 1*H*-1,2,4-triazole (615 mg, 8.9 mmol), 4-methylmorpholine (2.8 ml), CH_2Cl_2 (16.8 ml), PCl_3 (232 µl, 2.5 mmol), and **19** (428 mg, 0.51 mmol) in CH_2Cl_2 (16.8 ml; 35 min at r.t.). Workup with 1M $(Et_3NH)HCO_3$ (31 ml), and CH_2Cl_2 (3 × 15 ml). FC (silica gel, column 2 × 12 cm, CH_2Cl_2/MeOH/Et_3N 96:2:2) afforded **5** as a colourless foam (480 mg, 93%). This material was redissolved in CH_2Cl_2 (18 ml) and the soln. washed with 0.1M $(Et_3NH)HCO_3$ (5 × 24 ml), dried (Na_2SO_4), and evaporated: **5** (384 mg, 74%). Colourless foam. TLC (CH_2Cl_2/MeOH/Et_3N 88:10:2): R_f 0.48. UV (MeOH): 220 (47100), 233 (47400), 273 (8600), 294 (7200). ^{1}H-NMR ((D)$_6$DMSO): 0.95 (*m*, 3 *Me*CH_2); 1.11 (2*s*, 2 Me); 2.26 (*m*, 2 H–C(2′)); 2.57 (*m*, Me*C*H_2); 2.87 (*m*, CH); 3.54 (*m*, 2 H–C(5′)); 3.70 (*s*, 2 MeO); 4.09 (*m*, H–C(4′)); 4.75 (*m*, H–C(3′)); 5.47, 7.81 (2*s*, H–P); 6.55 (*t*, J = 6.5, H–C(1′)); 6.80–7.45 (*m*, H–C(5), H–C(6), 10 arom. H); 10.93 (br. *s*, NH). ^{31}P-NMR ((D)$_6$DMSO): 0.82 (^1J(P,H) = 583, ^3J(P,H) = 5.5). Anal. calc. for $C_{55}H_{63}N_6O_{10}P$ (999.11): C 66.12, H 6.36, N 8.41; found: C 66.02, H 6.48, N 8.50.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D-erythro-*pentofuranosyl]-4-[(2-methylpropanoyl)amino]-7*H-*pyrrolo[2,3-*d*]-pyrimidin-2-yl Diphenylcarbamate 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**6**). As described for **4**, with **19** (320 mg, 0.38 mmol), CH_2Cl_2 (11 ml), 2-cyanoethyl diisopropylphosphoramidochloridite (0.34 ml, 1.52 mmol), and ^{i}Pr$_2$EtN (0.26 ml, 1.49 mmol). After stirring at r.t. for 2.5 h and workup with 5% aq.

$NaHCO_3$ soln. (11 ml) and CH_2Cl_2 (3×8 ml), the combined org. extract was dried (Na_2SO_4) for 1.5 h and evaporated and the oil submitted to FC (silica gel, column 2×10 cm, CH_2Cl_2/Me_2CO 93 : 7 containing a few drops of Et_3N): **6** (337 mg, 85%). Colourless foam. TLC (CH_2Cl_2/Me_2CO 9 : 1): R_f 0.80. ^{31}P-NMR ($CDCl_3$): 149.7, 149.9.

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