

43. N^7 -DNA: Synthesis and Base Pairing of Oligonucleotides Containing N^7 -(2-Deoxy- β -D-erythro-pentofuranosyl)guanine (N^7G_d)

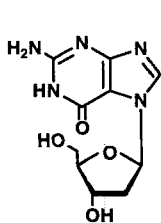
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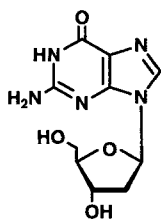
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The synthesis of oligonucleotides containing N^7 -(2-deoxy- β -D-erythro-pentofuranosyl)guanine (N^7G_d ; **1**) is described. Compound **1** was prepared by nucleobase-anion glycosylation of 2-amino-6-methoxypurine (**5**) with 2-deoxy-3,5-di-*O*-(4-toluoyl)- α -D-erythro-pentofuranosyl chloride (**6**) followed by detoluoylation and displacement of the MeO group (**8** $\rightarrow$ **10** $\rightarrow$ **1**). Upon base protection with the (dimethylamino)methylidene residue ($\rightarrow$ **11**) the 4,4-dimethoxytrityl group was introduced at OH-C(5') ($\rightarrow$ **12**). The phosphonate **3** and the phosphoramidite **4** were prepared and used in solid-phase oligonucleotide synthesis. The self-complementary dodecamer d(N^7G-C)₆ shows sigmoidal melting. The T_m of the duplex is 40°. This demonstrates that guanine residues linked *via* N(7) of purine to the phosphodiester backbone are able to undergo base pairing with cytosine.

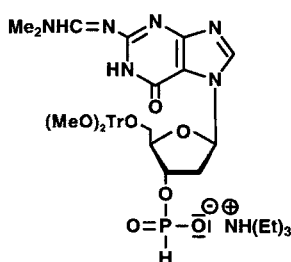
The base pairing of nucleic acids is controlled by the donor/acceptor pattern between purine and pyrimidine bases as well as by the structural, configurational, and conformational characteristics of the nucleic-acid backbone [1–4]. Peptidyl nucleic acids (PNA's) [5] or those with modified bases [6] can form duplex structures following the common modes of nucleic-acid base pairing. Nevertheless, oligonucleotides with a configurationally altered sugar-phosphate backbone – *e.g.* oligodeoxyxynucleotides – have been shown to possess an altered sequence-specific base-pair stability compared to those of regular DNA [7]. In hexopyranose DNA, the guanine-guanine base pair was found to be more stable than that of guanine-cytosine [8]. It was also demonstrated that modification of the nucleobases have a significant influence on the complex stability. As part of this work, nucleosides with unusually linked bases such as N^7 -(2-deoxy- β -D-erythro-pentofuranosyl) adenine have been incorporated in oligonucleotides. They form parallel duplexes with oligomeric dT [9]. This was the first example of a *Watson-Crick* duplex



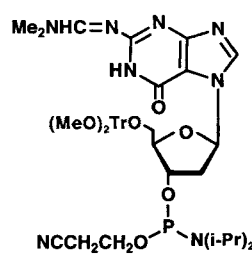
1



2



3



4

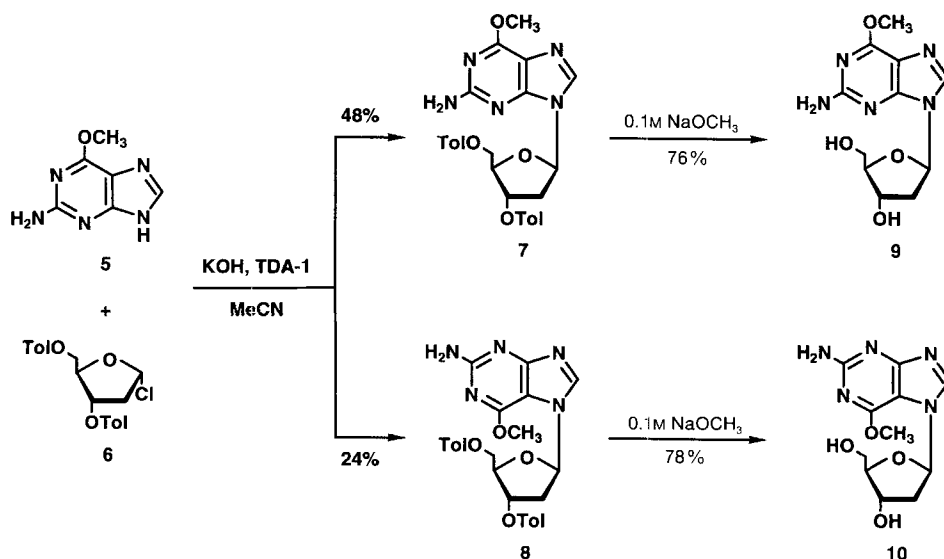
structure formed by an N^7 -linked purine nucleoside. As in this case thymine is a constituent of the base pair, one may argue that the higher structural flexibility of the A-T pair – two oxo groups can act as proton acceptors – limits the duplex formation of N^7 -linked purine bases to this particular base pair.

In this communication, we report on the synthesis and base pairing of oligonucleotides containing N^7 -(2-deoxy- β -D-erythro-pentofuranosyl)guanine (N^7G_d ; **1**), a regioisomer of 2'-deoxyguanosine (**2**). Compound **1** was protected and converted into the phosphonate **3** and the phosphoramidite **4**. The oligonucleotide building blocks **3** and **4** were employed in solid-phase synthesis. It will be shown that N^7G_d (**1**) can form base pairs with dC in self-complementary oligonucleotide duplexes.

Results and Discussion. – *Monomers.* Usually, the N^7 -purine nucleosides are the by-products formed during glycosylation of purines under the common reaction conditions. Also the nucleobase-anion glycosylation [10] of purines with 2-deoxy-3,5-di-*O*-(4-toluoyl)- α -D-erythro-pentofuranosyl chloride (**6**) [11] furnished the N^7 -(β -D-2-deoxyribofuranoside) as minor components [12]. In the case of 2-amino-6-chloropurine, only a 9–15% yield of the N^7 -isomer was obtained [13] [15]. The glycosylation product was converted into 2'-deoxy- N^7 -guanosine (**1**) [14]. So far it was not possible to increase the amount of the N^7 -isomers significantly by variation of the reaction conditions. This is different from the glycosylation performed on the silylated bases under *Vorbrüggen* conditions ($\text{SnCl}_4/\text{MeCN}$). The N^2 -acetylguanine reacted with 1,2,3,5-tetra-*O*-acetyl- β -D-ribofuranose or 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl- β -D-ribofuranose at ambient temperature giving the N^7 -ribonucleoside almost exclusively; the N^9 -isomer was formed under thermodynamic control at elevated temperature in CH_2Cl_2 with $\text{CF}_3\text{SO}_3\text{SiMe}_3$ as catalyst [16].

It has been found that the N^7/N^9 ratio can be changed during nucleobase-anion glycosylation towards the N^7 -isomer when 6-methoxypurine was used instead of 6-

Scheme 1



chloropurine [17]. As a similar result was expected for other 6-methoxy derivatives, the anion of compound **5** [18] was glycosylated under the same conditions: in MeCN with halogenose **6** in the presence of powdered KOH and TDA-1 [10] [19], the *N*⁷-isomer **8** was obtained in 24% yield together with the *N*⁹-compound **7** in 48% (Scheme 1). The configuration of the glycosylation products is β -D in all cases (see NOE data). The compounds **7** and **8** were separated chromatographically and deblocked with NaOMe/MeOH. The methoxy nucleosides **9** and **10** were isolated as colorless needles (76 and 78%). The UV maximum of the *N*⁷-isomer **10** (294 nm) shows a bathochromic shift compared to the *N*⁹-isomer (281 nm), an observation which is typical for regioisomeric purine nucleosides [20] [21].

Next, compound **10** was transformed into 7-(2-deoxy- β -D-erythro-pentofuranosyl)-guanine (**1**) in aqueous 2N NaOH at elevated temperature (50°). The strong alkaline conditions did not lead to imidazole-ring opening by attack of OH ions at C(8). Apparently, the guanine anion protects the molecule from nucleophilic attack. The overall yield of this three-step synthesis is 15% based on 2-amino-6-methoxypurine (**5**). An alternative synthetic route which used the *N*⁷-glycosylation of isobutyrylated guanine [16] with the *ribo*-sugar derivative followed by deoxygenation of *N*⁷-(ribofuranosyl)guanine [22] gave a 12% overall yield. However, this method is laborious as it requires 7 reaction steps [23].

The position of glycosylation (*N*⁷) and the anomeric configuration (β -D) of **1** and **10** were assigned unambiguously from ¹H- and ¹³C-NMR spectra. Table 1 summarizes the ¹³C-NMR chemical shifts of the *N*⁷-glycosylated purine nucleosides as well as of their *N*⁹-isomers assigned by gated-decoupled spectra (Table 2). The change of the glycosylation position from *N*⁹ to *N*⁷ results in a significant downfield shift of C(4) and an upfield shift of C(5) being in accordance with observations on ribonucleosides [24]. Furthermore, the NOE's of compound **10** (MeO group 1.5%, OH-C(3') 1.3%, and H-C(4') 2.4% upon irradiation of H-C(1')) confirmed β -D-configuration. The long-wavelength UV maximum of compound **1** (282 nm, H₂O) is bathochromically shifted compared to 2'-deoxyguanosine (**2**; 254 nm, H₂O). The hypsochromic shift occurring on compound **1** in 0.1N HCl (248 nm) is diagnostic for *N*⁷-substituted guanine nucleosides [25]; 2'-deoxyguanosine shows almost no change under these circumstances. The p*K*_a values of

Table 1. ¹³C-NMR Chemical Shifts of Purine 2'-Deoxyribonucleosides^{a)}b)

	C(2)	C(4)	C(5)	C(6)	C(8)	MeO	Me	C(1')	C(2')	C(3')	C(4')	C(5')	C=N
1	153.9 ^{d)}	160.4	107.5	155.5 ^{d)}	141.0			85.5	41.2	70.3	87.8	61.5	
2	153.8 ^{d)}	151.0	117.0	156.7 ^{d)}	135.4			82.7	40.0	70.6	87.7	61.6	
3	157.0 ^{d)}	159.2	110.3	156.0 ^{d)}	141.0	55.0	34.5	85.5	40.2	72.2	85.3	64.3	155.0
4	158.3 ^{d)}	160.0	110.5	156.1 ^{d)}	140.6	55.2		86.6 ^{d)}	41.2 ^{c)}	72.9 ^{c)}	86.5 ^{c)}	63.2	154.8
7	159.9 ^{d)}	153.9	114.2	160.8 ^{d)}	137.8	53.3		84.3	35.5	75.2	81.5	64.2	
8	159.9 ^{d)}	164.4	104.6	156.8 ^{d)}	143.9	55.6		85.9	37.2	74.7	81.4	64.2	
9	159.7	153.7	114.0	160.6	137.7	53.1		82.8	40.1	70.7	87.6	61.5	
10	159.8	164.0	105.2	156.7	143.2	53.5		85.9	40.7	70.4	87.8	61.5	
11	156.7 ^{d)}	159.3	110.2	155.2 ^{d)}	141.6			85.6	41.3	70.4	87.9	61.5	157.8
12	157.7 ^{d)}	159.2	110.3	156.6 ^{d)}	141.0	55.0	34.0	85.5	40.5	70.3	85.8	64.1	158.0

^{a)} Spectra measured in (D₆)DMSO rel. to SiMe₄ at room temperature. ^{b)} From [¹H, ¹³C] gated-decoupled spectra.

^{c)} Splitting pattern is due to C,P coupling and/or to the differences of the chemical shifts of the diastereoisomers.

^{d)} Tentative.

Table 2. $J(C,H)$ Coupling Constants [Hz] of Purine 2'-Deoxyribonucleosides^{a)}^{b)}

	1	9	10	11		1	9	10	11
$J(C(4),H-C(8))$	13	<i>m</i>	13	<i>m</i>	$J(C(1'),H-C(1'))$	171	169	169	165
$J(C(5),H-C(1'))$	4.4	<i>m</i>	4.6	<i>m</i>	$J(C(3'),H-C(3'))$	147	148	148	148
$J(C(5),H-C(8))$	4.4	<i>m</i>	4.2	<i>m</i>	$J(C(4'),H-C(4'))$	151	145	149	147
$J(C(6),MeO)$	<i>s</i>	<i>m</i>	3.1	–	$J(C(5'),H-C(5'))$	143	140	140	140
$J(C(8),H-C(8))$	210	211	211	213	$J(MeO)$	–	147	147	–
$J(C(8),H-C(1'))$	4.8	4.0	4.0	4.0	$J(=N-C-H)$	–	–	–	160
					$J(=N-C,Me)$	–	–	–	12

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

deprotonation [26] is 9.5 for compound **1** and also for dG. Compound **1** (pK_a 3.1) is easier to protonate than dG (pK_a 1.6). The glycosylic-bond stability of the N^7 -nucleoside **1** ($t_{1/2}$ 35 min; 0.5N HCl, 25°; 280 nm, HPLC) is higher as compared to 2'-deoxyguanosine (**2**; $t_{1/2}$ 11 min; same conditions). The same tendency has already been observed for the N^7 - and the N^9 -isomer of the pair of isoguanine nucleosides [27] [28]. The situation is different, however, in the case of adenine 2'-deoxyribofuranosides. Here, the N^9 -nucleoside is hydrolytically more stable than the N^7 -isomer [17] [29].

Oligonucleotides. The N^7 -(2-deoxy- β -D-erythro-pentofuranosyl)guanine (N^7G_d ; **1**) as well as a related pyrazolo[4,3-*d*]pyrimidine derivative (P1) have been already incorporated in regular oligonucleotides. They were used to study triplex stability [30] [31]. It was observed that the incorporation of one N^7G_d residue in place of dC gave a more stable triplex than that of the parent oligomer. On the basis of 1H -NMR NOE data, the base-pairing modes in Scheme 2 were proposed.

We have prepared the phosphonate **3** as well as the phosphoramidite **4** both containing the (dimethylamino)methylidene residue as amino-protecting group [32]. This group was introduced into **1** under standard conditions (room temperature, overnight; $\rightarrow$ **11**) [33] [34]. The half-life value for deprotection was 6 min for compound **11** compared to 19 min for dG (25% aqueous NH_3 solution at 40°). The reaction was followed UV-spectro-

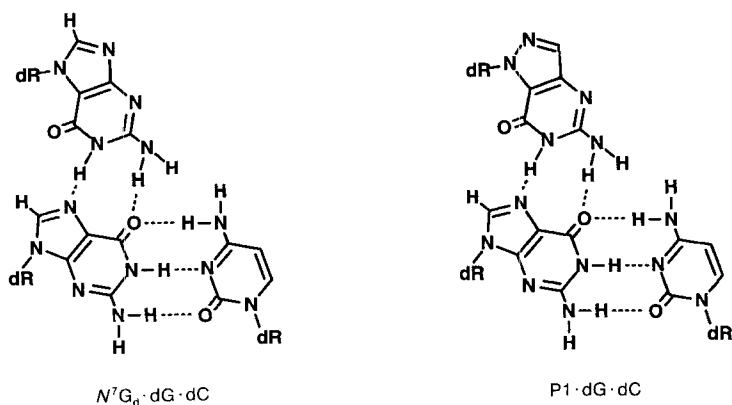
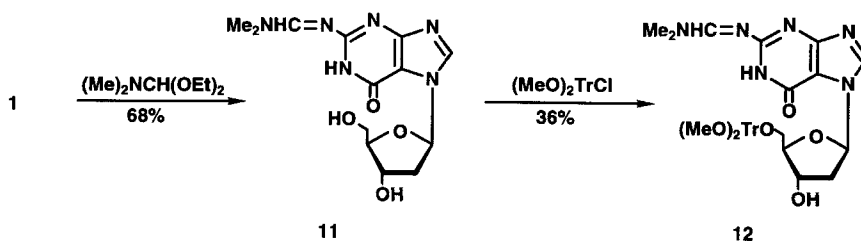


Fig. 1. N^7G_d Triplex pairing modes [30] [31]. dR = 2'-deoxyribose.

Scheme 2



photometrically at 284 nm for **11** and at 280 nm for the corresponding dG derivative. Subsequently, 4,4'-dimethoxytritylation furnished the $(\text{MeO})_2\text{Tr}$ derivative **12**. The phosphonate **3** and the phosphoramidite **4** were prepared under standard conditions and were characterized by ^1H -, ^{13}C -, and ^{31}P -NMR spectroscopy. These building blocks were used in automated solid-phase synthesis furnishing the self-complementary oligonucleotides $\text{d}(\text{N}^7\text{G}-\text{C})_3$ (**13**) and $\text{d}(\text{N}^7\text{G}-\text{C})_6$ (**14**). The synthetic protocol followed methods published earlier [35] [36]. The oligonucleotides were removed from the solid support with conc. aqueous ammonia (60°) and purified on an *OPC*-cartridge [37]. The composition of the oligonucleotides **13** and **14** was determined by tandem hydrolysis with snake-venom phosphodiesterase and alkaline phosphatase followed by HPLC (see Fig. 2).

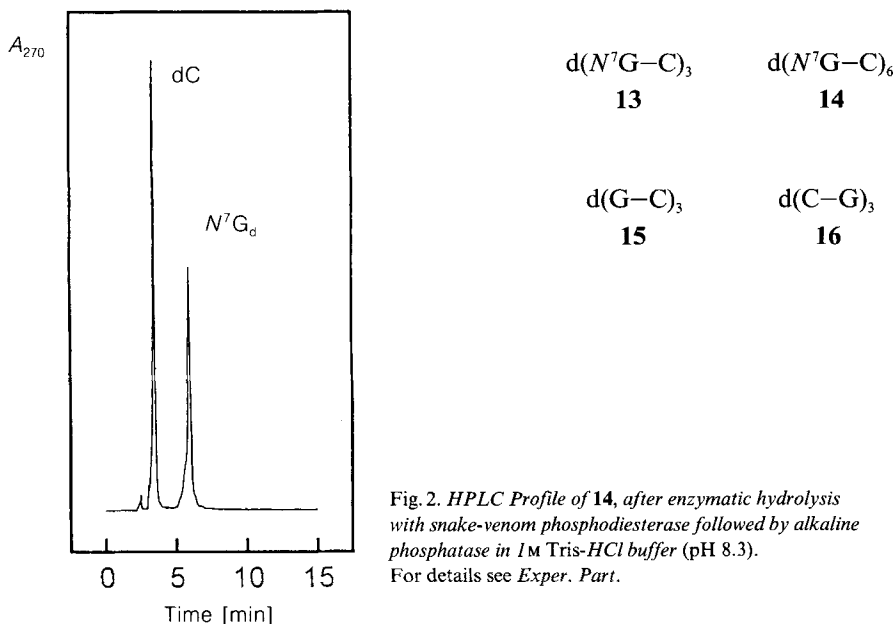


Fig. 2. HPLC Profile of **14**, after enzymatic hydrolysis with snake-venom phosphodiesterase followed by alkaline phosphatase in 1M Tris-HCl buffer (pH 8.3). For details see *Exper. Part*.

As the N^7 -nucleoside **1** contains an unchanged pyrimidine substituent pattern which is necessary to undergo base pairing with dC, formation of the oligonucleotide **13** was expected. The melting curve of $\text{d}(\text{N}^7\text{G}-\text{C})_3$ (**13**) was cooperative. However, the stability of its duplex was too low to determine the T_m value. Therefore, the experiments were

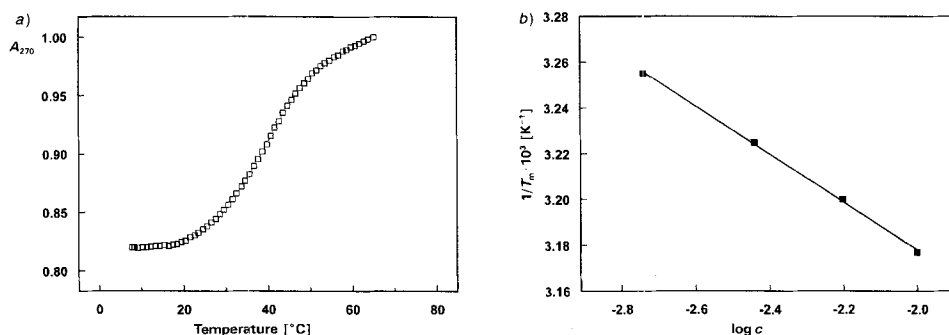


Fig. 3. a) Normalized melting profile and b) graph of $1/T_m$ vs. $\log c$ of oligomer $d(N^7G-C)_6$ (14). Measured at 270 nm in 1M NaCl, 100 mM MgCl₂, and 60 mM Na-cacodylate (pH 7.0). The soln. contained 0.6 A_{260} units of the oligonucleotide.

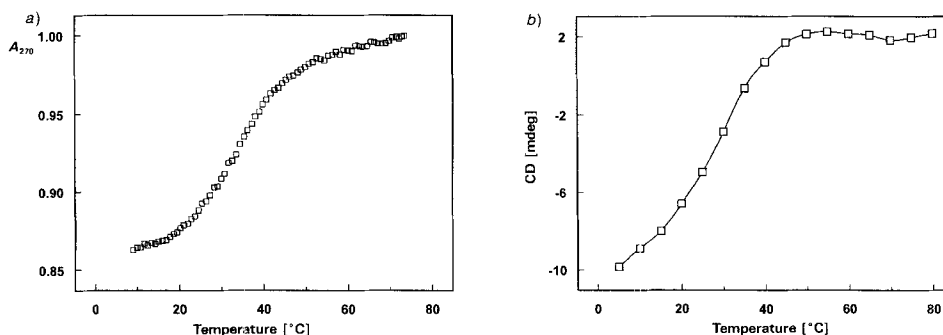


Fig. 4. a) Normalized melting profile and b) temperature-dependent CD spectra of the B_{2u} transition of the oligomer $d(N^7G-C)_6$ (14). Measured at 270 nm in 4M NaCl, 100 mM MgCl₂, and 10 mM Na-cacodylate (pH 7.0). The soln. contained 0.6 A_{260} units of the oligonucleotide.

undertaken on 12-mer $d(N^7G-C)_6$ (14). This oligomers gave a complete melting curve with a T_m 40° (1M NaCl, 100 mM MgCl₂, 60 mM Na-cacodylate, see Fig. 3, a). The duplex formation was confirmed by concentration-dependent measurement of the T_m values (Fig. 3, b). The T_m value showed almost no change between 0.1M NaCl and 1M NaCl. A decrease from 40 to 34° was observed at higher salt concentration (4M NaCl, Fig. 4, a). This was similar to the parent $d(G-C)_3$ (15) [38]. Nevertheless, $d(N^7G-C)_6$ is significantly less stable than the parent oligomers containing 2'-deoxyguanosine residues. A chain length as in dodecamer $d(N^7G-C)_6$ (14; T_m 40°) was necessary to reach about the stability of the hexamer $d(G-C)_3$ (T_m 46°).

As it was not clear whether this destabilization is the result of enthalpic or entropic changes, the thermodynamic data were determined from the melting profiles using a two-state model [39]. All measurements were carried out in 1M NaCl, 100 mM MgCl₂, and 60 mM Na-cacodylate buffer. The modified dodecamer $d(N^7G-C)_6$ (14; T_m 40°) gave the following values: $\Delta H = -63.0$ kcal/mol, $\Delta S = -204$ cal/mol·K. Similar values were already observed for the hexamer $d(G-C)_3$ (15; $\Delta H = -72$ kcal/mol, $\Delta S = -226$ cal/

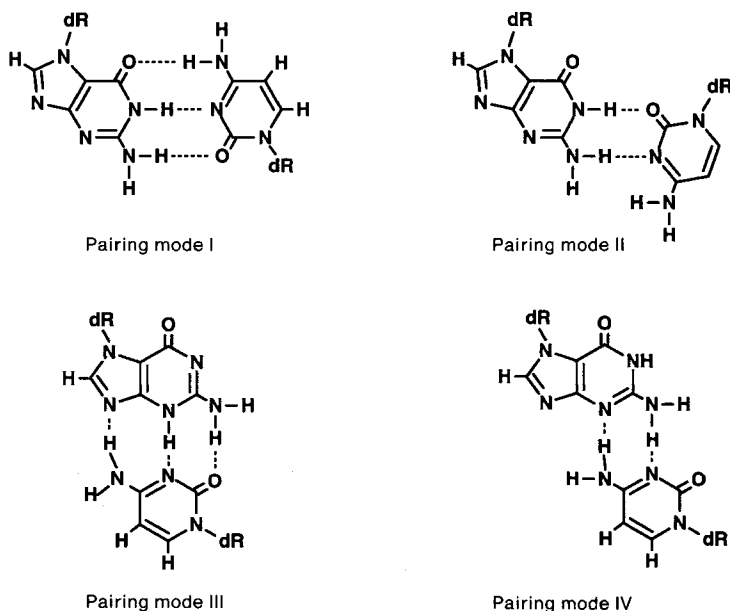


Fig. 5. Possible N^7G_d -dC duplex-pairing modes. dR = 2'-deoxyribose.

mol·K) with a T_m of 46° [40]. The enthalpy change per base pair is -24 kcal/mol for dG-dC and only -10.5 kcal/mol for N^7G_d -dC. On the other hand, the entropy change is -37.6 cal/mol·K for dG-dC and -34 cal/mol·K for N^7G_d -dC. These values are calculated for antiparallel duplexes with a full set of base pairs. The enthalpic destabilization of $d(N^7G-C)_6$ (**14**) raises the question about the structure of the base pair. Apart from the 'normal' base pairing (mode I), other modes (II-IV) have to be considered (see Fig. 5). Also a parallel arrangement of the oligonucleotide chains cannot be excluded which would decrease the number of base pairs from 12 to 10.

The CD spectra of $d(N^7G-C)_6$ (**14**; see Fig. 6, c) show an inverse Cotton effect compared to the parent oligonucleotide d(G-C)₃ (**15**; Fig. 6, b) with a positive transition at 270 nm and a strong negative Cotton effect at 290 nm. The spectra are temperature-dependent and were used to confirm the melting profiles (Fig. 4, b). As known from d(C-G)₃ (**16**), a B-Z-DNA transition takes place between low and high salt concentration (1M → 4M NaCl) [38]. This change was not observed in the case of $d(N^7G-C)_6$ (**14**). However, the CD spectrum of $d(N^7G-C)_3$ (**13**) shows some similarity with that of the Z-form of d(C-G)₃ (**15**; see Fig. 6, a). Nevertheless, the sugar attachment at N(7) changes already the electronic properties of the base in relation to chiral centres of the sugar moiety. As a result, the CD of compounds **1** and **2** show already different CD spectra (see Fig. 6, d). Surprisingly, the 'syn'/'anti' population of conformers determined by NOE measurements [41] (see Table 3) were similar in the case of the N^7 -nucleoside **1** (70% anti) and 2'-deoxyguanosine (**2**; 70% anti). Up to now, it is difficult to extract more detailed information on the duplex structure of $d(N^7G-C)_6$ from these data. Further NMR experiments or X-ray data are required to establish the structure of this new base pair.

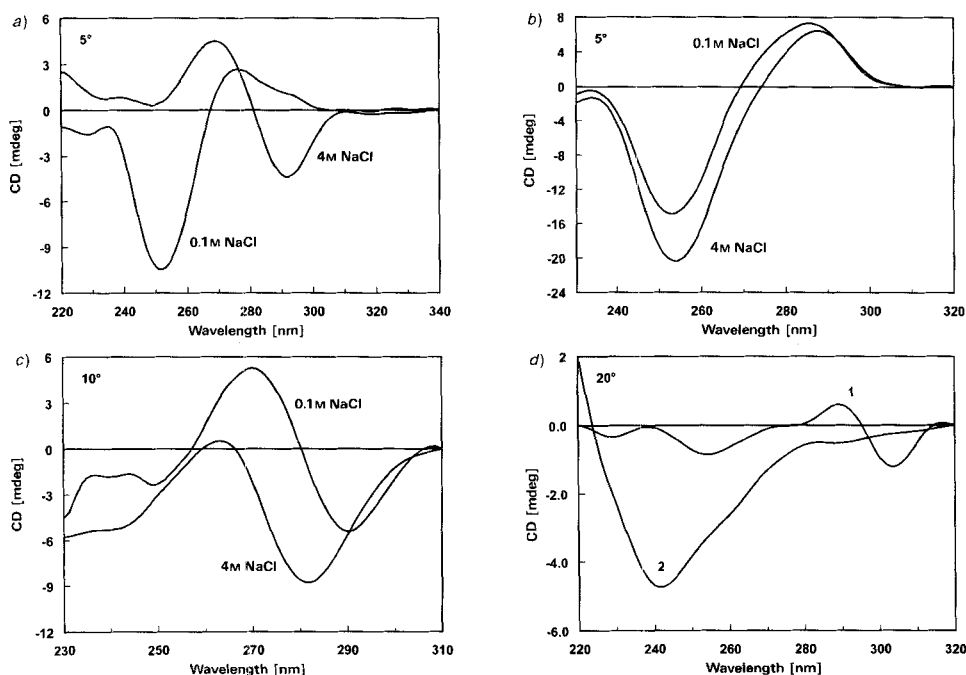


Fig. 6. CD Spectra of a) $d(C-G)_3$ (**16**), b) $d(G-C)_3$ (**15**), and c) $d(N^7G-C)_6$ (**14**) (in 0.1 M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.0), or 4 M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.0)) and d) CD spectra of N^7G_4 (**1**) and dG (**2**; in 1 M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.0)). The oligonucleotide concentration was 3 μM .

Table 3. 1H -NMR NOE Data of Purine 2'-Deoxyribofuranosides^{a)}

	Irradiation	NOE [%]
1	H-C(8)	H-C(1') (3.4), H-C(3') (1.1)
2	H-C(8)	H-C(1') (3.1), H-C(2') (3.7), H-C(3') (1.1)
	H-C(1')	H-C(8) (3.0), H-C(2') (1.8), H-C(4') (1.8)
10	H-C(8)	H-C(1') (3.8), H-C(3') (1.5), H-C(2') (5.5), OH-C(5') (1.5)
	H-C(1')	H-C(8) (3.9), H-C(4') (2.4), H-C(2') (7.2), MeO (1.5), OH-C(3') (1.3)
	MeO	H-C(8) (0.7), H-C(1') (2.9), OH-C(3') (1.8), OH-C(5') (2.1), NH_2 (1.0)

^{a)} In (D_6)DMSO measured at 23°.

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Experimental Part

General. See [42]. MeCN was distilled from CaH_2 . The phosphonate of dC was purchased from *Sigma*, St. Louis, and CPG (controlled pore glass; 30–50 μmol of immobilized protected 2'-deoxynucleoside/g of solid support) from *Milligene*, Eschborn, Germany. TLC scanning: CS-930 TLC Scanner (*Shimadzu*, Japan). Column flash chromatography (FC) at 0.8 bar; silica gel 60 *H* (*Merck*, FRG). Solvent systems for FC and TLC: $CH_2Cl_2/MeOH$ 98:2 (*A*), $CH_2Cl_2/MeOH$ 9:1 (*B*), $CH_2Cl_2/MeOH$ 4:1 (*C*), $CH_2Cl_2/MeOH/Et_3N$ 88:10:2 (*D*), $CH_2Cl_2/MeOH/Et_3N$ 78:20:2 (*E*), *i*-PrOH/(25% aq. NH_3 soln./ H_2O 3:1:1 (*F*), H_2O/i -PrOH 9:1 (*G*), $AcOEt/CH_2Cl_2/Et_3N$ 45:45:10 (*H*). *Uvicord S* detector and *Ultra Rac II* fractions collector (*LKB Instruments*, Sweden).

M.p.: Büchi-SMP-20 apparatus (Büchi, Switzerland). UV Spectra: 150-20 spectrometer (Hitachi, Japan). NMR Spectra: AC-250 Bruker spectrometer; operational frequencies 250.134 (^1H) and 62.898 (^{13}C) MHz; δ values rel. to Me_4Si as internal standard. Microanalyses were performed by Mikroanalytisches Laboratorium Beller, Göttingen, Germany. Oligonucleotide synthesis was carried out on a DNA synthesizer, model 380 B, Applied Biosystems, Weiterstadt, Germany. Melting curves were measured with a 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. The enzymatic hydrolysis of the oligomers was carried out as described in [43].

N-Glycosylic-Bond Hydrolysis. As described in [9], with a stock soln. of **1** (1.5 mg, 1.5 ml). ϵ_{260} : $N^7\text{G}_d = 2700$, $d\text{G} = 11\,700$, $d\text{C} = 7300$; ϵ_{280} : $N^7\text{G}_d = 5800$, $\text{G}_d = 7500$, $d\text{C} = 6900$. HPLC Separation. See [9].

Glycosylation of 2-Amino-6-methoxypurine (5) with 2-Deoxy-3,5-di-O-(4-toluoyl)- α -D-erythro-pentofuranosyl Chloride (6). Powdered KOH (650 mg, 11.6 mmol) and TDA-1 (tris[2-(2-methoxyethoxy)ethyl]amine; 60 μl ; 0.18 mmol) were suspended in anhyd. MeCN (60 ml). After 15 min, 2-amino-6-methoxypurine [**5**] (500 mg, 3.0 mmol) was added and stirring continued for another 15 min. Then the halogenose **6** [**11**] (1.2 g, 3.1 mmol) was introduced in portions. After 15 min, the insoluble material was filtered off and the solvent evaporated. The residue was applied to FC (silica gel, column 20×4 cm, A (500 ml), then B) resulting in two main zones.

2-Amino-9-[2-deoxy-3,5-di-O-(4-toluoyl)- β -D-erythro-pentofuranosyl]-6-methoxy-9H-purine (7). From the faster migrating zone, a pale yellow foam was isolated. Crystallization from EtOH afforded colorless needles (750 mg, 48%). M.p. 185°. TLC (B): R_f 0.6. UV (MeOH): 281 (11 400). $^1\text{H-NMR}$ ((D_6) DMSO): 2.36, 2.39 (2s, 2 Me); 2.68, 3.19 (2m, 2 H-C(2')); 3.95 (s, MeO); 4.50–4.62 (m, H-C(4'), 2 H-C(5')); 5.73 (m, H-C(3')); 6.38 ('t', $J = 7.2$, H-C(1')); 6.50 (s, NH_2); 7.3–7.9 (arom. H); 8.07 (s, H-C(8)). Anal. calc. for $\text{C}_{27}\text{H}_{27}\text{N}_5\text{O}_6$ (517.54): C 62.66, H 5.26, N 13.53; found: C 62.64, H 5.28, N 13.50.

2-Amino-7-[2-deoxy-3,5-di-O-(4-toluoyl)- β -D-erythro-pentofuranosyl]-6-methoxy-7H-purine (8). From the slow migrating zone, **8** was isolated as a foam (370 mg, 24%). TLC (B): R_f 0.4. UV (MeOH): 294 (8200). $^1\text{H-NMR}$ ((D_6) DMSO): 2.36, 2.39 (2s, 2 Me); 2.73, 3.0 (2m, 2 H-C(2')); 4.0 (s, MeO); 4.46–4.60 (m, H-C(4'), 2 H-C(5')); 5.66 (m, H-C(3')); 6.24 (s, NH_2); 6.47 ('t', $J = 6.5$, H-C(1')); 7.3–7.9 (arom. H); 8.40 (s, H-C(8)). Anal. calc. for $\text{C}_{27}\text{H}_{27}\text{N}_5\text{O}_6$ (517.54): C 62.66, H 5.26, N 13.53; found: C 62.54, H 5.19, N 13.58.

2-Amino-7-(2-deoxy- β -D-erythro-pentofuranosyl)-6-methoxy-7H-purine (10). A soln. of **8** (500 mg, 1.0 mmol) was stirred with 0.1M NaOMe in MeOH (50 ml) for 1 h. The mixture was neutralized with AcOH, evaporated, and applied to FC (silica gel, column 15×4 cm, B (300 ml), then C). Colorless needles (H_2O ; 236 mg, 84%). M.p. 155°. TLC (C): R_f 0.4. UV (MeOH): 246 (7500), 294 (8800). $^1\text{H-NMR}$ ((D_6) DMSO): 2.3, 2.75 (2m, 2 H-C(2')); 3.45–3.52 (m, 2 H-C(5')); 3.62 (m, H-C(4')); 3.97 (s, MeO); 4.29 (m, H-C(3')); 4.93 (t, $J = 5.0$, OH-C(5')); 5.29 (d, $J = 3.5$, OH-C(3')); 6.16 (s, NH_2); 6.30 ('t', $J = 6.4$, H-C(1')); 8.39 (s, H-C(8)). Anal. calc. for $\text{C}_{11}\text{H}_{15}\text{N}_5\text{O}_4$ (281.3): C 46.97, H 5.38, N 24.90; found: C 46.93, H 5.44, N 24.71.

2-Amino-7-[2-deoxy- β -D-erythro-pentofuranosyl]-7H-purin-6-one (= 2'-Deoxy- N^7 -guanosine; **1).** Compound **10** (390 mg, 1.39 mmol) was heated in 2N aq. NaOH (40 ml) at 50° for 24 h. The soln. was cooled, neutralized with AcOH, diluted with H_2O (300 ml), and applied to Amberlite XAD-4 (column 20×4 cm, G). The column was washed with H_2O (500 ml) and the compound eluted with i-PrOH/ H_2O 9:1. Upon evaporation, a pale yellow solid (300 mg, 81%) was obtained. TLC (F): R_f 0.7. UV (MeOH): 243 (4800), 283 (5700). $^1\text{H-NMR}$ ((D_6) DMSO): 2.21, 2.26 (2m, 2 H-C(2')); 3.51–3.55 (m, 2 H-C(5')); 3.80 (m, H-C(4')); 4.29 (m, H-C(3')); 4.93 (t, $J = 4.9$, OH-C(5')); 5.24 (d, $J = 3.7$, OH-C(3')); 6.17 (s, NH_2); 6.42 ('t', $J = 6.4$, H-C(1')); 8.24 (s, H-C(8)); 10.68 (s, NH). Anal. calc. for $\text{C}_{10}\text{H}_{13}\text{N}_5\text{O}_4$ (267.2): C 44.94, H 4.90, N 26.21; found: C 45.13, H 5.00, N 26.06.

7-(2-Deoxy- β -D-erythro-pentofuranosyl)- N^2 -{[(dimethylamino)methylidene]amino}-7H-purin-6-one (11). Compound **1** (270 mg, 1.01 mmol) was dissolved in dry DMF (20 ml) and stirred with N,N -dimethylformamide diethyl acetal (3 ml, 17.5 mmol) for 12 h at r.t. The soln. was evaporated and the oily residue co-evaporated with toluene (10 ml, twice) and applied to FC (silica gel, column 20×3 cm, C). Colorless crystals from MeOH (220 mg, 68%). M.p. 186°. TLC (C): R_f 0.6. UV (MeOH): 236 (8600), 284 (11 200). $^1\text{H-NMR}$ ((D_6) DMSO): 2.29 (2m, 2 H-C(2')); 3.01, 3.14 (2s, Me_2N); 3.55 (m, 2 H-C(5')); 3.83 (m, H-C(4')); 4.30 (m, H-C(3')); 4.95 ('t', $J = 5.4$, OH-C(5')); 5.26 (d, $J = 3.9$, OH-C(3')); 6.50 ('t', $J = 6.6$, H-C(1')); 8.34 (s, $-\text{CH}=\text{N}-$); 8.60 (s, H-C(8)); 11.44 (s, NH). Anal. calc. for $\text{C}_{13}\text{H}_{18}\text{N}_6\text{O}_4$ (322.3): C 48.44, H 5.63, N 26.07; found: C 48.60, H 5.70, N 25.99.

7-[2-Deoxy-5-O-bis(4-methoxyphenyl)phenylmethyl]- β -D-erythro-pentofuranosyl]- N^2 -{[(dimethylamino)methylidene]amino}-7H-purin-6-one (12). Compound **11** (300 mg, 0.93 mmol) was dried by repeated co-evaporation with anhyd. pyridine and suspended in dry pyridine (2 ml). The soln. was stirred under Ar in the presence of 4-(dimethylamino)pyridine (10 mg, 0.08 mmol) and bis(4-methoxyphenyl)phenylmethyl chloride (383 mg, 1.12 mmol) for 12 h. The mixture was diluted with 5% aq. NaHCO_3 soln. (20 ml) and extracted with CH_2Cl_2

(3 × 50 ml). The combined org. layer was dried (Na₂SO₄) and evaporated and the residue chromatographed (silica gel, column 20 × 3 cm, *D*). The main zone afforded a colorless foam (210 mg, 36%). TLC (*D*): *R*_f 0.6. UV (MeOH): 285 (11 200). ¹H-NMR ((D₆)DMSO): 2.36 (2*m*, 2 H–C(2′)); 3.01, 3.17 (2*s*, Me₂N); 3.72 (*s*, 2 MeO); 3.94 (*m*, H–C(4′)); 4.30 (*m*, H–C(3′)); 5.32 (*d*, *J* = 4.3, OH–C(3′)); 6.56 (‘*t*’, *J* = 5.8, H–C(1′)); 6.81–7.35 (2*m*, arom. H); 8.15 (*s*, –CH=); 8.60 (*s*, H–C(8)); 11.37 (*s*, NH). Anal. calc. for C₃₄H₃₆N₆O₆ (624.7): C 65.37, H 5.81, N 13.45; found: C 65.45, H 5.84, N 13.47.

7-*{2-Deoxy-5-O-[bis(4-methoxyphenyl)phenylmethyl]-β-D-erythro-pentofuranosyl}-N²-{[(dimethylamino)methylidene]amino}-7H-purin-6-one 3′-(Triethylammonium Phosphonate)* (**3**). To a soln. of PCl₃ (214 μl, 2.5 mmol) and *N*-methylmorpholine (2.85 ml, 24.2 mmol) in CH₂Cl₂ (20 ml), 1,2,4-*1H*-triazole (560 mg, 8.26 mmol) was added and the mixture stirred for 30 min at r.t. After cooling (0°), a soln. of **11** (320 mg, 0.50 mmol) in CH₂Cl₂ (5 ml) was added dropwise, and the mixture was stirred for 20 min. The soln. was poured into 1*M* (Et₃NH)HCO₃ (pH 7.7, 15 ml), shaken, and separated. The aq. layer was extracted with CH₂Cl₂ (15 ml, twice), the combined org. layer dried (Na₂SO₄) and evaporated, and the residue chromatographed (silica gel, column 20 × 3 cm, *D* (250 ml), then *E*). The main zone was evaporated, dissolved in CH₂Cl₂ (10 ml), and extracted twice with 0.1*M* (Et₃NH)HCO₃ (10 ml). The org. layer was dried (Na₂SO₄) and evaporated. Colorless foam (390 mg, 55%). TLC (*D*): *R*_f 0.4. UV (MeOH): 235 (25 800), 286 (15 700). ¹H-NMR ((D₆)DMSO): 1.15 (*m*, Me); 2.41, 2.67 (2*m*, 2 H–C(2′)); 2.99 (*m*, 2 CH₂); 3.02, 3.14 (2*s*, Me₂N); 3.41 (*m*, 2 H–C(5′)); 3.73 (*s*, 2 MeO); 4.10 (*m*, H–C(4′)); 4.70 (*m*, H–C(3′)); 6.56 (‘*t*’, *J* = 6.6, H–C(1′)); 7.27 (*d*, *J* = 5.80, PH); 6.82–7.39 (2*m*, arom. H); 8.14 (*s*, –CH=); 8.69 (*s*, H–C(8)); 11.34 (*s*, NH). ³¹P-NMR ((D₆)DMSO): 2.04; ¹*J*(P,H) = 580.

7-*{2-Deoxy-5-O-[bis(4-methoxyphenyl)phenylmethyl]-β-D-erythro-pentofuranosyl}-N²-{[(dimethylamino)methylidene]amino}-7H-purin-6-one 3′-f-(2-Cyanoethyl) N,N-Diisopropylphosphoramidite* (**4**). To a soln. of **12** (40 mg, 0.66 mmol) and (i-Pr)₂EtN (90 μl, 0.51 mmol) in anhyd. CH₂Cl₂ (2 ml), chloro(2-cyanoethoxy)(diisopropylamino)phosphane (113 μl, 0.51 mmol) was added at r.t. After stirring for 30 min, the mixture was diluted with CH₂Cl₂ (10 ml) and quenched by adding 5% NaHCO₃ soln. (20 ml). Then the aq. layer was extracted with CH₂Cl₂ (3 × 20 ml), the combined org. layer dried (Na₂SO₄) and evaporated, and the colorless oil applied to FC (silica gel, column 9 × 3 cm, *H*): light yellow foam of **4** (20 mg, 38%). TLC (*H*): *R*_f 0.5, 0.4. ¹H-NMR (CDCl₃): 1.03–1.07 (*m*, Me₂CH); 2.32 (*t*, *J* = 6.5, CH₂CH₂CN); 2.28–2.41 (2*m*, 2 H–C(2′)); 2.95, 3.02 (2*s*, Me₂N); 3.25–3.28 (*m*, Me₂CH); 3.45 (*m*, 2 H–C(5′)); 3.50–3.53 (*t*, *J* = 6.6, OCH₂); 3.66 (*s*, 2 MeO); 4.15 (*m*, H–C(4′)); 4.48 (*m*, H–C(3′)); 6.51 (‘*t*’, *J* = 6.2, H–C(1′)); 6.69–6.70 (*m*, arom. H); 7.14–7.31 (*m*, arom. H); 7.93 (*s*, –CH=); 8.67 (*s*, H–C(8)). ³¹P-NMR (CDCl₃): 149.3.

Solid-Phase Synthesis of Oligodeoxyribonucleotides. Compounds **13** and **14** were synthesized on a 1-μmol scale using the 3′-phosphonate of [(MeO)₂Tr]bz⁴C₄ as well as compound **3** (Table 4). The synthesis and deprotection of the oligonucleotides **13** and **14** followed a preparation protocol for 3′-phosphonates [36].

Table 4. Data of Oligonucleotides **13–15**

	13	14	15 [44]
Retention time [min] ^{a)}	12.6	17.5	17.8
Thermal hypochromicity [%]	17 ^{b)}	22 ^{b)}	30 ^{c)}
Yield [%] ^{b)}	^{d)}	20	36

^{a)} The retention times refer to gradient *II* (see [9]). ^{b)} At 270 nm. ^{c)} At 260 nm. ^{d)} Not determined.

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