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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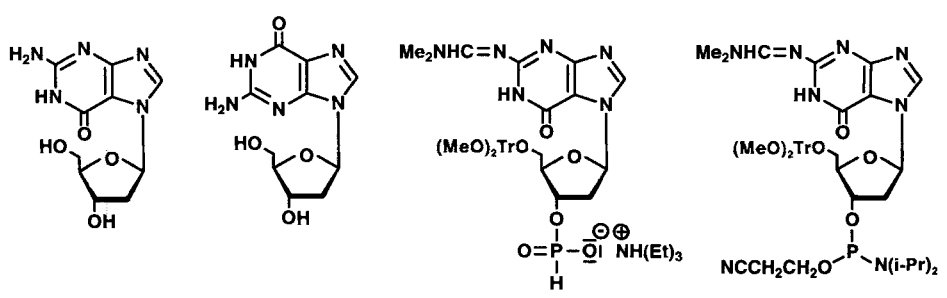
The synthesis of oligonucleotides containing N^7-(2-deoxy-β-D-*erythro*-pentofuranosyl)guanine (N^7G_d; **1**) is described. Compound **1** was prepared by nucleobase-anion glycosylation of 2-amino-6-methoxypurine (**5**) with 2-deoxy-3,5-di-*O*-(4-toluoyl)-α-D-*erythro*-pentofuranosyl chloride (**6**) followed by detoluoylation and displacement of the MeO group (**8**→**10**→**1**). Upon base protection with the (dimethylamino)methylidene residue (→**11**) the 4,4-dimethoxytrityl group was introduced at OH–C(5′) (→**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.* oligodeoxyxylonucleotides – 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

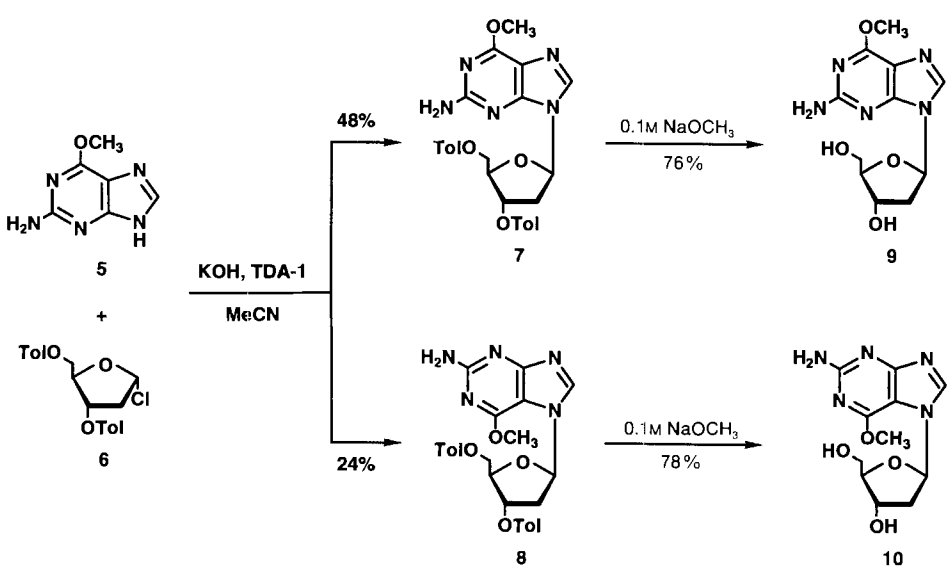
structure formed by an N^7-linked purine nucleoside. As in this case thymine is a constituent of the base pair, one may argue that the higher structural flexibility of the A-T pair – two oxo groups can act as proton acceptors – limits the duplex formation of N^7-linked purine bases to this particular 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 ($SnCl_4$/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 $CF_3SO_3SiMe_3$ 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 ^{1}H- and ^{13}C-NMR spectra. *Table 1* summarizes the ^{13}C-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, H_2O) is bathochromically shifted compared to 2′-deoxyguanosine (**2**; 254 nm, H_2O). 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 pK_a values of

Table 1. *^{13}C-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 (D_6)DMSO rel. to $SiMe_4$ at room temperature. [b]) From [^{1}H,^{13}C] 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	*m*	13	*m*	J(C(1′),H–C(1′))	171	169	169	165
J(C(5),H–C(1′))	4.4	*m*	4.6	*m*	J(C(3′),H–C(3′))	147	148	148	148
J(C(5),H–C(8))	4.4	*m*	4.2	*m*	J(C(4′),H–C(4′))	151	145	149	147
J(C(6),MeO)	*s*	*m*	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** ($t_{½}$ 35 min; 0.5N HCl, 25°; 280 nm, HPLC) is higher as compared to 2′-deoxyguanosine (**2**; $t_{½}$ 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 ^{1}H-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; →**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-

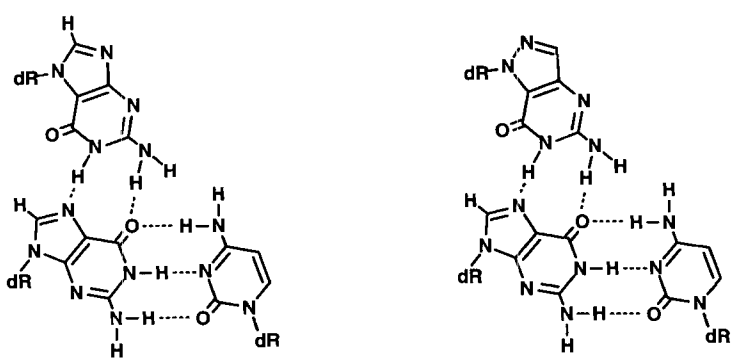
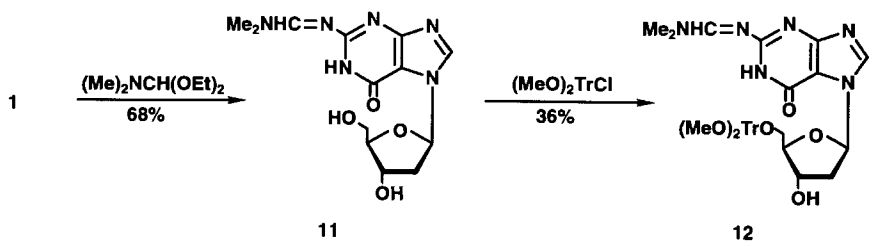
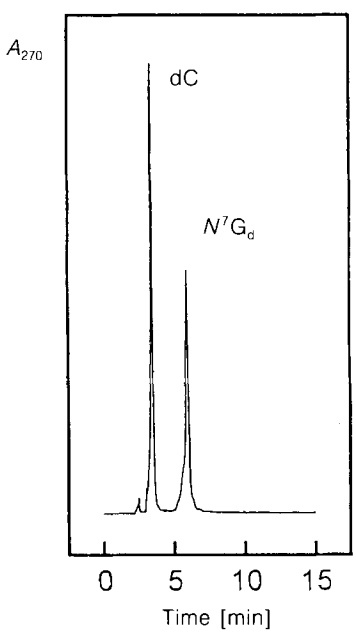


Fig. 1. N^7G_d-*Triplex pairing modes* [30] [31]. dR = 2′-deoxyribose.

Scheme 2



photometrically at 284 nm for **11** and at 280 nm for the corresponding dG derivative. Subsequently, 4,4′-dimethoxytritylation furnished the $(MeO)_2Tr$ 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 $d(N^7G{-}C)_3$ (**13**) and $d(N^7G{-}C)_6$ (**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*).



$d(N^7G{-}C)_3$ **13** $d(N^7G{-}C)_6$ **14**

$d(G{-}C)_3$ **15** $d(C{-}G)_3$ **16**

Fig. 2. *HPLC Profile of* **14**, *after enzymatic hydrolysis with snake-venom phosphodiesterase followed by alkaline phosphatase in 1* M 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 $d(N^7G{-}C)_3$ (**13**) was cooperative. However, the stability of its duplex was too low to determine the T_m value. Therefore, the experiments were

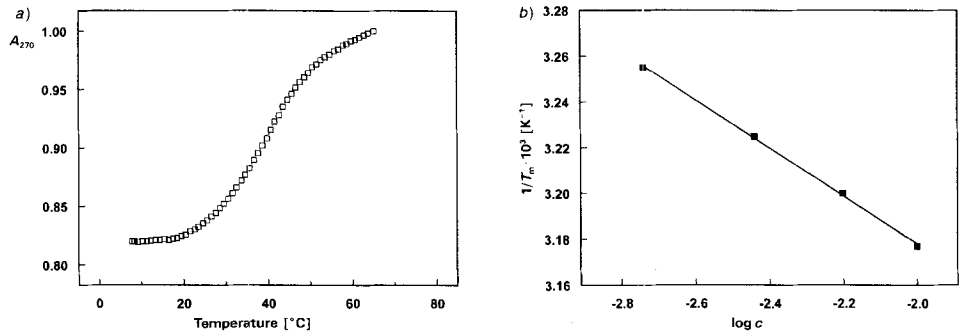


Fig. 3. a) *Normalized melting profile and* b) *graph of* $1/T_m$ vs. *log* c *of oligomer d(*N^7G–C)$_6$ (**14**). Measured at 270 nm in 1M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.0). The soln. contained 0.6 A_{260} units of the oligonucleotide.

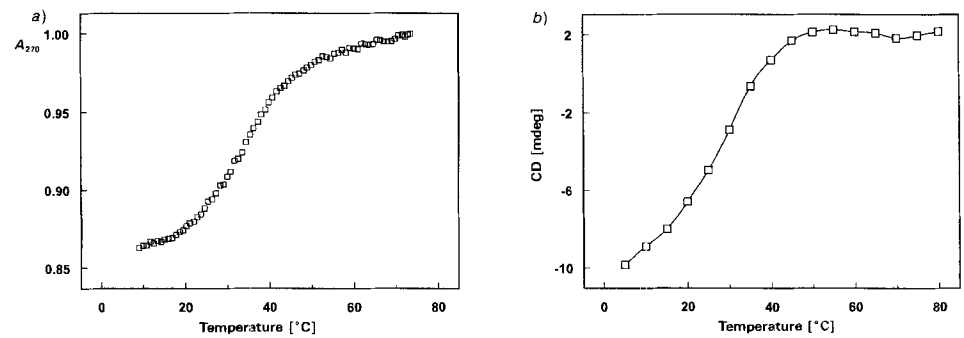


Fig. 4. a) *Normalized melting profile and* b) *temperature-dependent CD spectra of the* B_{2u} *transition of the oligomer d(*N^7G–C)$_6$ (**14**). Measured at 270 nm in 4M NaCl, 100 mM $MgCl_2$, and 10 mM Na-cacodylate (pH 7.0). The soln. contained 0.6 A_{260} units of the oligonucleotide.

untertaken 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 $MgCl_2$, 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 $MgCl_2$, 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/

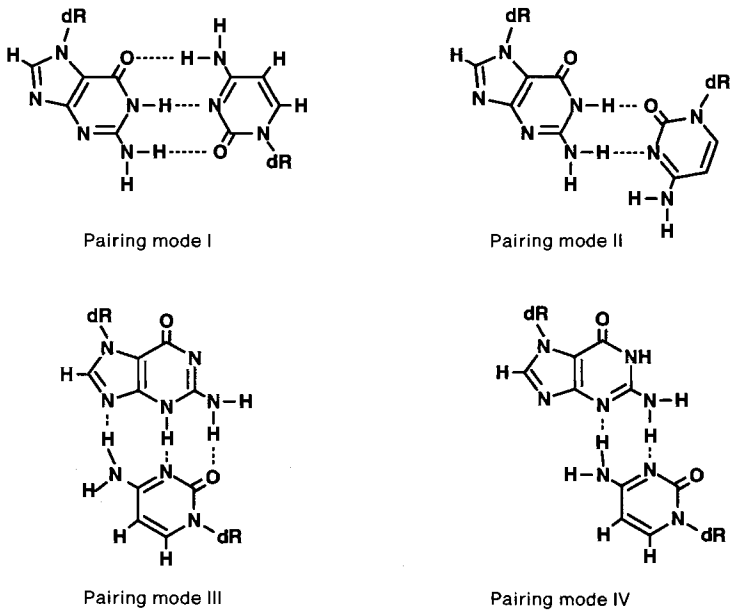


Fig. 5. *Possible* $N^7G_d \cdot dC$ *duplex-pairing modes.* dR = 2′-deoxyribose.

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.

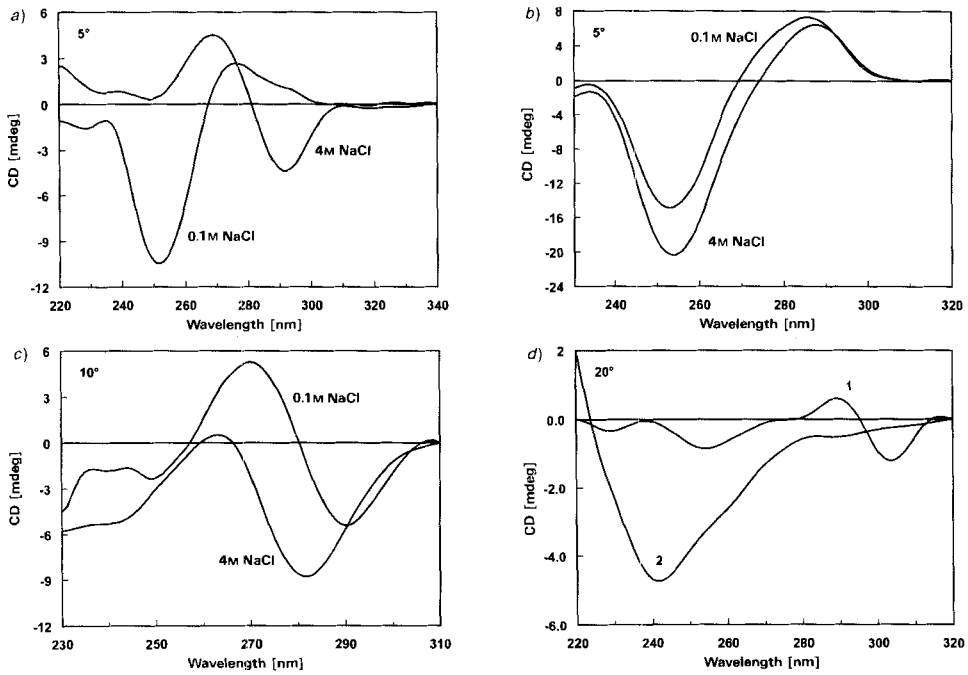


Fig. 6. *CD Spectra of* a) $d(C-G)_3$ (**16**), b) $d(G-C)_3$ (**15**), *and* c) $d(N^7G-C)_6$ (**14**) (in 0.1M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.0), or 4M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.0)) *and* d) *CD spectra of* N^7G_d (**1**) *and dG* (**2**; in 1M NaCl,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

Table 3. *^{1}H-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 (^{1}H) 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^7G_d = 2700, dG = 11700, dC ≈ 7300; ε_{280}: N^7G_d = 5800, G_d = 7500, dC = 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 anh. MeCN (60 ml). After 15 min, 2-amino-6-methoxypurine [18] (**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), than *B*) resulting in two main zones.

*2-Amino-9-[2-deoxy-3,5-di-*O*-(4-toluoyl)-β-*D*-erythro-pentofuranosyl]-6-methoxy-9*H*-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 (11400). ^{1}H-NMR ((D$_6$)DMSO): 2.36, 2.39 (2*s*, 2 Me); 2.68, 3.19 (2*m*, 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 $C_{27}H_{27}N_5O_6$ (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-7*H*-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}H-NMR ((D$_6$)DMSO): 2.36, 2.39 (2*s*, 2 Me); 2.73, 3.0 (2*m*, 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 $C_{27}H_{27}N_5O_6$ (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-7*H*-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}H-NMR ((D$_6$)DMSO): 2.3, 2.75 (2*m*, 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 $C_{11}H_{15}N_5O_4$ (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]-7*H*-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 eluated 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}H-NMR ((D$_6$)DMSO): 2.21, 2.26 (2*m*, 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 $C_{10}H_{13}N_5O_4$ (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}-7*H*-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 (11200). ^{1}H-NMR ((D$_6$)DMSO): 2.29 (2*m*, 2 H–C(2′)); 3.01, 3.14 (2*s*, 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*, –CH=); 8.60 (*s*, H–C(8)); 11.44 (*s*, NH). Anal. calc. for $C_{13}H_{18}N_6O_4$ (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}-7*H*-purin-6-one* (**12**). Compound **11** (300 mg, 0.93 mmol) was dried by repeated co-evaporation with anh. 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 (Na_2SO_4) 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). ^{1}H-NMR ((D_6)DMSO): 2.36 (2*m*, 2 H–C(2′)); 3.01, 3.17 (2*s*, Me_2N); 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 $C_{34}H_{36}N_6O_6$ (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 PCl_3 (214 µl, 2.5 mmol) and *N*-methylmorpholine (2.85 ml, 24.2 mmol) in CH_2Cl_2 (20 ml), 1,2,4-1*H*-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 CH_2Cl_2 (5 ml) was added dropwise, and the mixture was stirred for 20 min. The soln. was poured into 1M $(Et_3NH)HCO_3$ (pH 7.7, 15 ml), shaken, and separated. The aq. layer was extracted with CH_2Cl_2 (15 ml, twice), the combined org. layer dried (Na_2SO_4) and evaporated, and the residue chromatographed (silica gel, column 20 × 3 cm, *D* (250 ml), then *E*). The main zone was evaporated, dissolved in CH_2Cl_2 (10 ml), and extracted twice with 0.1M $(Et_3NH)HCO_3$ (10 ml). The org. layer was dried (Na_2SO_4) and evaporated. Colorless foam (390 mg, 55%). TLC (*D*): R_f 0.4. UV (MeOH): 235 (25 800), 286 (15 700). ^{1}H-NMR ((D_6)DMSO): 1.15 (*m*, Me); 2.41, 2.67 (2*m*, 2 H–C(2′)); 2.99 (*m*, 2 CH_2); 3.02, 3.14 (2*s*, Me_2N); 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* = 580, PH); 6.82–7.39 (2*m*, arom. H); 8.14 (*s*, –CH=); 8.69 (*s*, H–C(8)); 11.34 (*s*, NH). ^{31}P-NMR ((D_6)DMSO): 2.04; ^1J(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′-[(2-Cyanoethyl) N,N-Diisopropylphosphoramidite]* (**4**). To a soln. of **12** (40 mg, 0.66 mmol) and (i-Pr)$_2$EtN (90 µl, 0.51 mmol) in anh. CH_2Cl_2 (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 CH_2Cl_2 (10 ml) and quenched by adding 5% $NaHCO_3$ soln. (20 ml). Then the aq. layer was extracted with CH_2Cl_2 (3 × 20 ml), the combined org. layer dried (Na_2SO_4) and evaporated, and the colorless oil applied to FC (silica gel, column 9 × 3 cm, *H*): light yellow foam of **4** (20 mg, 38%). TLC (*H*): R_f 0.5, 0.4. ^{1}H-NMR ($CDCl_3$): 1.03–1.07 (*m*, Me_2CH); 2.32 (*t*, *J* = 6.5, CH_2CH_2CN); 2.28–2.41 (2*m*, 2 H–C(2′)); 2.95, 3.02 (2*s*, Me_2N); 3.25–3.28 (*m*, Me_2CH); 3.45 (*m*, 2 H–C(5′)); 3.50–3.53 (*t*, *J* = 6.6, OCH_2); 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)). ^{31}P-NMR ($CDCl_3$): 149.3.

Solid-Phase Synthesis of Oligodeoxyribonucleotides. Compounds **13** and **14** were synthesized on a 1-µmol scale using the 3′-phosphonate of [$(MeO)_2$Tr]bz^4C_d 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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