6. Oligonucleotides Containing Consecutive 2′-Deoxyisoguanosine Residues: Synthesis, Duplexes with Parallel Chain Orientation, and Aggregation

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

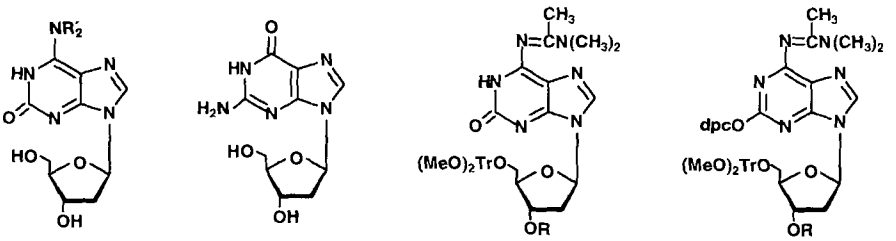
Laboratorium für Organische und Bioorganische Chemie, Institut für Chemie, Universität Osnabrück, Barbarastrasse 7, D–49069 Osnabrück

(26.VIII.96)

The 2′-deoxyisoguanosine phosphonates **3a** and **4a** and the phosphoramidites **3b** and **4b** were prepared as building blocks for solid-phase oligonucleotide synthesis. The diphenylcarbamoyl (dpc) residue was introduced as 2-oxo protecting group which stabilizes the *N*-glycosylic bond against hydrolysis and prevents the molecule from side reactions. The dpc-protected building blocks **4a,b** were employed in solid-phase synthesis and were found to be much more efficient than the unprotected compounds **3a,b**. Oligonucleotides with alternating (**11**) or consecutive isoguanine residues (**13**–**15**) were synthesized. They form duplexes with parallel chain orientation. The aggregate $d(T_4\text{-}iG_4\text{-}T_4)$ (**15**) containing four consecutive 2′-deoxyisoguanosine is shown to be a tetramer similar to that of $d(T_4\text{-}G_4\text{-}T_4)$.

Introduction. – The oxo group of 2′-deoxyisoguanosine ($isoG_d$ or iG_d; **1**) shows an obviously higher reactivity than that of 2′-deoxyguanosine (**2**) as it is readily acylated or tosylated. On the other hand, it is difficult to acylate its exocyclic NH_2 function without prior protection of the 2-oxo group [1]. Furthermore, nucleoside **1** is much more sensitive towards acid-catalyzed *N*-glycosylic-bond hydrolysis than 2′-deoxyguanosine or 2′-deoxyadenosine. These properties give rise to problems during oligonucleotide synthesis. It was found to be difficult to incorporate several consecutive isoguanine nucleoside residues into an oligonucleotide without protection of the 2-oxo group [2]. Nevertheless, oligonucleotides containing only one isolated or several non-consecutive 2′-deoxyisoguanosine residues can be prepared using phosphonate or phosphoramidite chemistry [2] [3]. Recently, the (4-nitrophenyl)ethyl residue [4] and also the allyl group [5] have been used as 2-oxo protecting groups for the isoguanine base. Homomeric isoguanine oligonucleotides have been synthesized in the case of pyranose-DNA or pRNA [6]. This paper reports on the synthesis of oligonucleotides with consecutive isoguanine residues employing the diphenylcarbamoyl (dpc; $C(O)NPh_2$) group for the protection of the 2-oxo function of 2′-deoxyisoguanosine. The protected phosphonates **3a** and **4a** and phosphoramidites **3b** and **4b** were prepared and employed in solid-phase synthesis [7].

Additionally, it will be shown that isoguanine forms a base pair with cytosine resulting in DNA duplexes with parallel chain orientation. This investigation goes back to an earlier report of our laboratory, which showed duplex formation of the self-complementary $d[(iG-C)_3]$ [8]. This base pair is different from that of isoC-isoG [4] [9] or m^5 isoC-isoG [10] resulting in duplexes with antiparallel chains. Finally, we report on the oligonucleotide $d(T_4\text{-}iG_4\text{-}T_4)$ forming a tetraplex similar to that of $d(T_4\text{-}G_4\text{-}T_4)$.



1 R′ = H
10 R'_2 = Me_2NCH=

2

3a,b

4a,b,c dpc = $C(O)NPh_2$

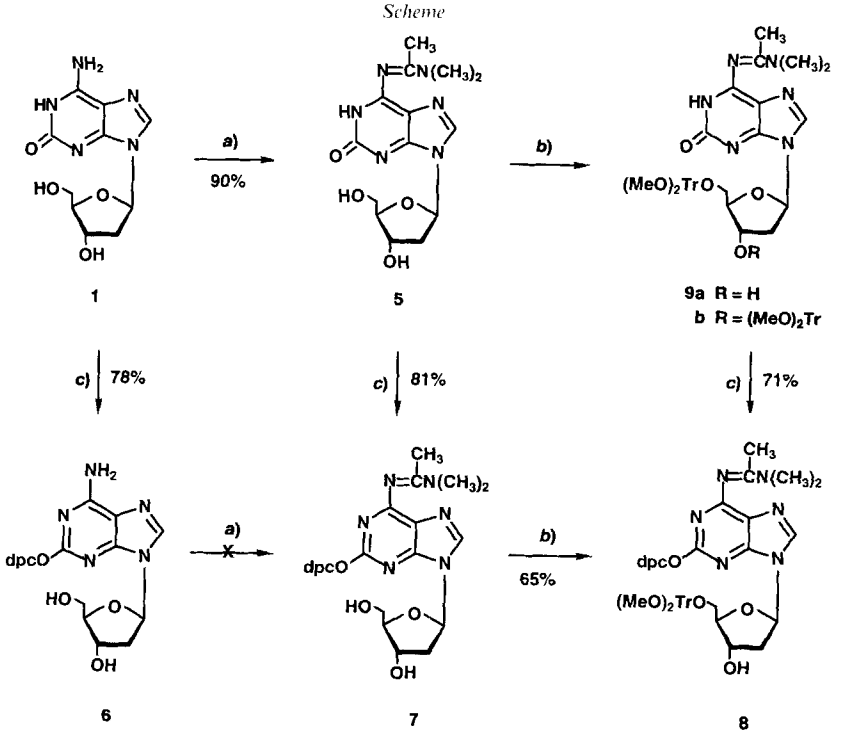
a R = $PH(=O)OH \cdot Et_3N$

b R = $P(OCH_2CH_2CN)[N(i\text{-}Pr)_2]$

c R = $C(O)(CH_2)_2C(O)NH$–⟨ ⟩

Results and Discussion. – *Synthesis of Nucleosides.* Earlier, the diphenylcarbamoyl (dpc) group has been used in nucleoside and oligonucleotide synthesis to protect the 6-oxo function of guanine or guanosine [11–17]. As the dpc group can be introduced at the 6-oxo group of 2′-deoxyguanosine (**2**) in a regioselective manner [11] [12], the same was expected for the 2-oxo group of 2′-deoxyisoguanosine (**1**). Thus, either the deoxyribonucleoside **1** [18] [19] or its amidine **5** was reacted with diphenylcarbamoyl chloride (dpc-Cl) in the presence of diisopropylethylamine (*Scheme*). Amidine **5** was obtained from **1** by

Scheme



a) $Me_2NC(OMe)_2Me$, MeOH, r.t., 3.75 h. *b*) $(MeO)_2TrCl$, pyridine, r.t.; **9a**, 40%; **9b**, 17%. *c*) dpc-Cl, $(i\text{-}Pr)_2EtN$, pyridine, r.t.

treatment with dimethylacetamide dimethyl acetal in MeOH [20]. Both dpc derivatives **6** and **7** were purified chromatographically and characterized spectroscopically. Colored impurities, which were formed during the reaction with dpc-Cl were removed chromatographically. In the case of **6**, the dpc group shifts the UV maximum of compound **1** hypsochromically from 292 to 260 nm. A similar hypsochromic shift is observed on diphenylcarbamoylation of amidine **5**, from 337 and 251 nm (**5**) to 309 and 233 nm (**7**).

Compounds **5** and **7** were converted into the 5′-*O*-(dimethoxytrityl)-protected ($(MeO)_2Tr$) derivatives **9a** and **8** under standard conditions. As side product, the 3′,5′-bis-*O*-protected compound **9b** was obtained. This results from the low reactivity of the 5′-OH group of **5** which may form a H-bond with the base moiety [3]. Compound **8** could also be prepared by reaction of **9a** with dpc-Cl. However, the total yield from **5** *via* **7** to **8** (53%) was higher than that from **5** *via* **9a** to **8** (28%). So, the first route was chosen for further experiments. The final reaction of **9a** or **8** with PCl_3/*N*-methylmorpholine/1*H*-1,2,4-triazole in CH_2Cl_2 furnished the 3′-phosphonates **3a** and **4a**, which were purified chromatographically and isolated as triethylammonium salts. The phosphoramidites **3b** and **4b** were prepared by treatment of **9a** or **8** with chloro(2-cyanoethoxy)(diisopropylamino)phosphine in the presence of diisopropylethylamine. Additionally, the polymer-linked 2′-deoxyisoguanosine **4c** was prepared for the synthesis of oligomer **14** using a standard protocol [22].

Protecting-Group Stability. To test the applicability of the protecting groups for oligonucleotide synthesis, their stability was first studied under basic conditions. The deprotection was followed UV-spectrophotometrically. Earlier, the (dimethylamino)methylidene residue was introduced as an amino-protecting group at NH_2–C(6) of compound **1**. The resulting 9-(2-deoxy-β-D-*erythro*-pentofuranosyl)-6-{[(dimethylamino)methylidene]amino}-1,9-dihydro-2*H*-purin-2-one (**10**) [3] showed a half-life (τ) of 6 min (conc. ammonia at 40°; *Table 1*). The half-life of deprotection was increased to 91 min in the case of acetamidine **5**. Under the same conditions, the dpc derivative **6** led to a value of 70 min. If the dpc protecting group was used in combination with the acetamidine group (compound **7**) the half life was 54 min. This last value shows that the combination of protecting groups is suitable for fast deprotection under alkaline conditions.

Table 1. *Half-Life Values* τ *of Deprotection of 2′-Deoxyisoguanosine Derivatives in 25% Aqueous Ammonia at 40°*

	UV λ [nm]	Half-life τ [min]		UV λ [nm]	Half-life τ [min]
5	310	91	**7**	310	54
6	284	70	**10**[a])	335	6

[a]) Data according to [3].

With regard to the stability under acidic conditions, it was reported that the glycosylic bond of 2′-deoxyisoguanosine (**1**) is very labile (see *Table 2*) and its hydrolysis is ten times faster than that of 2′-deoxyguanosine (**2**) [19]. The stability of **1** is in the range of 2′,3′-dideoxyguanosine [19]. As the low glycosylic-bond stability can cause depurination during acid-catalyzed detritylation in oligonucleotides synthesis it is of interest to study the glycosylic-bond stability of the dpc derivative **7** as well as of the precursor molecules **5** and **6**. All reactions were performed in 0.1N aqueous HCl at 25°. The kinetics was followed

UV-spectrophotometrically at the wavelength of maximal difference of UV absorbance. The hydrolyzed compounds were identified by HPLC; kinetic data are summarized in *Table 2*. As indicated by *Fig. 1,a*, hydrolysis of the dpc derivative **6** occurs exclusively at the *N*-glycosylic bond, and (diphenylcarbamoyl)isoguanine (dpc^{2}iGua) is the only reaction product formed after 3.5 h. This indicates that the dpc protecting group is sufficiently stable against acid. The half-life for glycosylic-bond hydrolysis of **6** is 125 min which is increased by about a factor of 10 over that of the 2-oxo-unprotected nucleoside **1** (14 min; *Table 2*). The values for 2′-deoxy-2-methoxyadenosine ((MeO)^{2}A$_d$) fall in the same range. From this it is apparent that the protection of the 2-oxo group of 2′-deoxyisoguanosine with the dpc group reduces depurination during the acid-catalyzed detritylation step in oligonucleotide synthesis which has to be considered as a limitation during oligonucleotide synthesis.

The 2′-deoxy-N^6-[(dimethylamino)ethylidene]isoguanosine (dae^{6}iG$_d$: **5**) has a similar stability as the parent nucleoside **1** (*Table 2*). According to the HPLC pattern (*Fig. 1,b*) N^6[(dimethylamino)ethylidene]isoguanine (dae^{6}iGua) is formed at the beginning of this reaction. According to the half-life values, the amidine protecting group does not stabilize the glycosylic bond of 2′-deoxyisoguanosine as it is found in the case of 2′-deoxyadenosine [21]. The acid-catalyzed hydrolysis of the bis-protected compound **7** (dae^6-dpc^{2}iG$_d$) furnishes 2-*O*-(diphenylcarbamoyl)-N^6-[(dimethylamino)ethylidene]isoguanine (dae^{6}dpc^{2}iGua) and starting material (*Fig. 1,c*). Again glycosylic-bond hydrolysis is observed. As found for the dpc protected compound **6**, the glycosylic bond is also stabilized in this case as compared to that of 2′-deoxyisoguanosine (**1**) or its amidine derivative **5** (*Table 2*). From these experiments, it can be concluded that the use of the dpc protecting group has the following advantages: *i*) The dpc group is easily introduced into iG$_d$ and can be removed under standard conditions of oligonucleotide chemistry; *ii*) The dpc group stabilizes the glycosylic bond of iG$_d$. As a result, problems which are attributed to depurination can be circumvented.

Often isoguanine nucleosides show only a few of the nucleobase ^{13}C-NMR signals if the spectra are measured in (D$_6$)DMSO. This problem can be overcome by performing the measurements in a mixture of (D$_6$)DMSO/0.4M aqueous NH$_4$OAc using long relaxation delays (10 s) during acquisition of NMR spectra [1]. However, this is not necessary when the base moiety carries a protecting group (see data in *Table 3*). The assignment of the ^{13}C-NMR signals of 2′-deoxy-2-*O*-(diphenylcarbamoyl)isoguanosine (**6**) follows that of 2′-deoxy-2-methoxyadenosine [1]. However, the assignment of C(2) and C(6) of **6** is still tentative as the gated-decoupled ^{13}C-NMR spectrum show a *singlet* in both cases. The [(dimethylamino)ethylidene]-nucleoside **5** exhibits similar chemical shifts for the signals of the base moiety as found for the (dimethylamino)methylidene derivative [3].

Table 2. *Half-Life Values τ of Isoguanine Nucleosides in 0.1* N *HCl at 25° Measured UV-Spectrophotometrically*

	τ[min]	UV λ[nm]
iG$_d$ (**1**)	14 (8; [19])	236
(MeO)^{2}A$_d$	160	248
dae^{6}iG$_d$[a]) (**5**)	13	235
dpc^{2}iG$_d$[a]) (**6**)	125	261
dae^{6}dpc^{2}iG$_d$[a]) (**7**)	284	285

[a]) dae = 1-(dimethylamino)ethylidene; dpc = diphenylcarbamoyl.

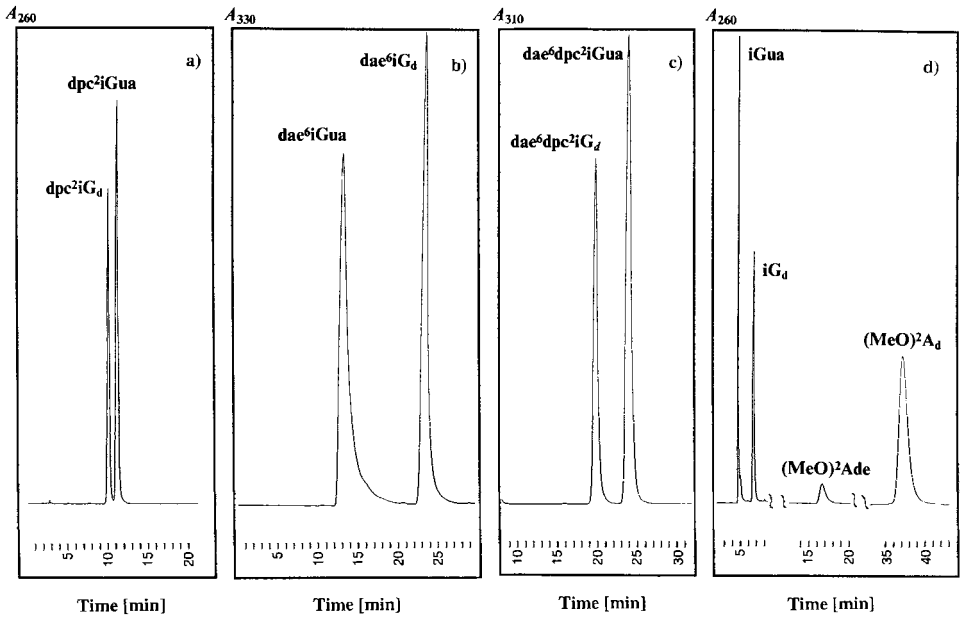


Fig. 1. *HPLC Profiles of the hydrolysis products of 2'-deoxyisoguanosine and its derivatives formed in 0.1* N *HCl*: a) *dpc²iG$_d$* (**6**; time: 3.5 h; HPLC conditions *II*); b) *dae⁶iG$_d$* (**5**; time: 10 min; HPLC conditions *I*); c) *dae⁶dpc²iG$_d$* (**7**; time: 2 h; HPLC conditions *II*); d) *mixture of iG$_d$* (**1**) *and 2'-deoxy-2-methoxyadenosine* ((MeO)²A$_d$) (time: 22 min; HPLC conditions *I*). HPLC Conditions: 5% MeCN in 0.1M (Et_3NH)OAc, pH 7.0 (*A*) and MeCN (*B*) were used; condition *I*: only *A*, flow rate 0.6 ml/min; condition *II*: 25% *B* in *A*, flow rate 1.0 ml/min.

Table 3. *^{13}C-NMR Chemical Shifts of Isoguanosine Derivatives in (D_6)DMSO at 25°*

	C(2)[a])	C(4)	C(5)	C(6)[a])	C(8)	C=O)	C=N
1[b])	156.7	152.5	109.3	154.1	137.5	–	–
5[d])	156.4	157.0	113.2	153.6	140.1	–	163.9
9a	156.8	157.4	113.2	153.6	139.4	–	163.7
b	156.5	158.0	113.2	153.5	138.7	–	163.6
3a	156.7	157.2	113.2	153.6	139.3	–	163.9
6[d])	157.1	150.1	117.6	155.7	139.6	151.5	–
7[d])	160.7	141.9	123.9	155.3	141.1	151.5	162.2
8	160.9	142.0	124.0	155.7	140.9	151.7	162.3
4a	160.8	141.9	123.9	155.5	140.5	151.5	162.2
	C(1')	C(2')	C(3')	C(4')	C(5')	MeO	Me
1[b])	83.7	[c])	71.1	88.1	62.1	–	–
5[d])	83.6	[c])	71.0	88.0	62.1	–	18.8
9a	82.1	[c])	70.7	85.5	64.3	55.1	18.6
b	82.4	38.5	74.6	85.5	64.4	54.9	18.5
3a	82.4	[c])	72.5	85.6	[e])	55.0	18.7
6[d])	83.6	[c])	70.8	87.9	61.8	–	–
7[d])	83.4	[c])	70.7	87.9	61.7	–	17.2
8	82.9	[c])	70.6	85.5	64.2	55.0	17.3
4a	83.0	[c])	72.5	85.5	63.7	54.9	17.2

[a]) Tentative. [b]) Data from [1]. [c]) Superimposed by DMSO. [d]) From gated-decoupled spectra. [e]) Not detected.

Oligonucleotides. As discussed above, the use of the 2-*O*-unprotected 2′-deoxyisoguanosine building blocks **3a,b** gives rise to difficulties in solid-phase oligonucleotide synthesis. The coupling yields are always lower compared to those of regular compounds. The problem is particularly severe when an isoguanine building block is added to the growing oligonucleotide chain having already a 2-oxo-unprotected isoguanine base at the 5′-end. The situation is improved when the sequence is alternating. In this case, the phosphonate **3a** gives suitable results, and self-complementary oligonucleotides containing several non-consecutive isoguanine residues were prepared by this route [22]. As a consequence, the dpc-protected phosphonate **4a** was used during the synthesis of the oligonucleotides **11** and **13**–**15**. In these cases, oligonucleotides with consecutive isoguanine residues were obtained in sufficient yield. The situation was further improved, and the coupling yield based on the liberation of 4′,4′-dimethoxytrityl residues reached standard values when the coupling time was increased from 40 to 100 s. Apparently, the bulkiness of the dpc group blocking the 2-oxo function decreases the coupling efficiency. After deprotection and purification, the nucleoside composition of oligonucleotides was determined by enzymatic hydrolysis using snake-venom phosphodiesterase followed by alkaline phosphatase and analyzed by reversed-phase HPLC (*Fig. 2*). They were also characterized by MALDI-TOF mass spectra. The following oligomers were prepared:

5′-d(iG-C-iG-C-iG-C) (**11**)
5′-d(G-C-G-C-G-C) (**12**)
5′-d(iG-iG-iG-C-C-C) (**13**)
5′-d(C-C-C-iG-iG-iG) (**14**)
5′-d(T-T-T-T-iG-iG-iG-iG-T-T-T-T) (**15**)

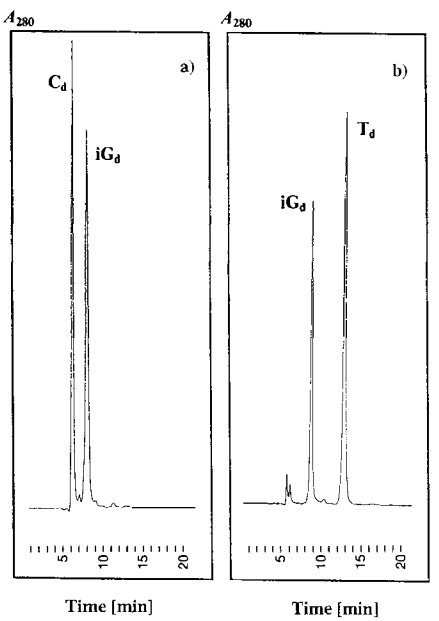
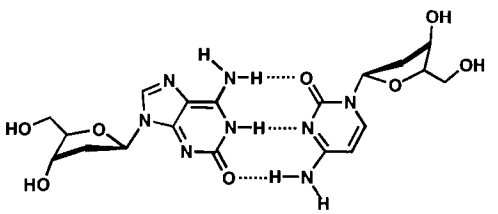


Fig. 2. *HPLC Profiles of the hydrolysis products of* a) *d[(iG-C)$_3$]* (**11**) *and* b) *d(T$_4$-iG$_4$-T$_4$)* (**15**) *formed by snake-venom phosphodiesterase followed by alkaline phosphatase.* Conditions, see *Exper. Part.*

An isoguanine·isocytosine base pair has been reported by *Benner* in 1989 [23]. However, other isoguanine base-pairing modes are conceivable in duplex DNA. The formation of a reversed *Watson-Crick* pair of 2′-deoxyisoguanosine with 2′-deoxycytidine has been observed in our laboratory in 1993 [8]. Mismatches can be also formed [10]. Base pairing between isoguanine and guanine was found by *Eschenmoser* and coworkers in pRNA [5] [6].

The self-complementary oligomer 5′-d[(iG-C)$_3$] (**11**) [8] shows cooperative melting (*Table 4*). According to the reversed *Watson-Crick* motive of the $iG_d \cdot C_d$ base pair **A**, it is expected that **11** forms parallel strands in duplex structures [7]. This is now confirmed by the following experiment: The self-complementary oligonucleotides 5′-d(iG$_3$-C$_3$) (**13**) as well as 5′-d(C$_3$-iG$_3$) (**14**) show T_m values below 17°. This points to an incomplete hybridization of only 3 $iG_d \cdot C_d$ base pairs in a duplex with parallel chain orientation. However, when 1 equiv. of 5′-d(iG$_3$-C$_3$) (**13**) is mixed with 5′-d(C$_3$-iG$_3$) (**14**) a hybrid is formed showing a T_m increase of 30° (*Table 4*). This duplex is considered to form now 6 $iG_d \cdot C_d$ base pairs. According to these observation, the chain orientation is parallel in these oligonucleotides.



A $iG_d \cdot C_d$

parallel strands

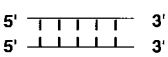


Table 4. T_m- *Values of Duplexes Formed By Oligonuleotides* **11**–**14**[a])

	Base pairs	Number of base pairs	T_m Values [°]	
			1M NaCl[b])	0.1M NaCl[c])
11·11	5′-d(iG-C-iG-C-iG-C) 5′-d(iG-C-iG-C-iG-C)	5	31	31
12·12	5′-d(G-C-G-C-G-C) 3′-d(C-G-C-G-C-G)	6	46	46
13·13	5′-d(iG-iG-iG-C-C-C) 5′-d(iG-iG-iG-C-C-C)	3	17	[d])
14·14	5′-d(C-C-C-iG-iG-iG) 5′-d(C-C-C-iG-iG-iG)	3	16	[d])
14·13	5′-d(C-C-C-iG-iG-iG) 5′-d(iG-iG-iG-C-C-C)	6	47	44

[a]) Oligonucleotide concentration was 10 μM. [b]) With 0.1M $MgCl_2$, 60 mM Na-cacodylate buffer, pH 7.0. [c]) With 0.01M $MgCl_2$, 60 mM Na-cacodylate buffer, pH 7.3. [d]) Not measured.

Isoguanine nucleosides and nucleotides form aggregates in aqueous solution. An iG-quartet structure has been proposed by *Shugar* and coworkers [24] and *Davis* and coworkers [25]. Nevertheless, a tetrameric species has not been detected. We have performed ion-exchange HPLC on a *Dionex-NucleoPac™-PA100* column (4 × 50 mm) with the oligonucleotide d(T_4-iG_4-T_4) (**15**) at 30° using a 1M NaCl solution as eluent. Two well separated peaks were observed (*Fig. 3,a*). The fast migrating peak belongs to the single

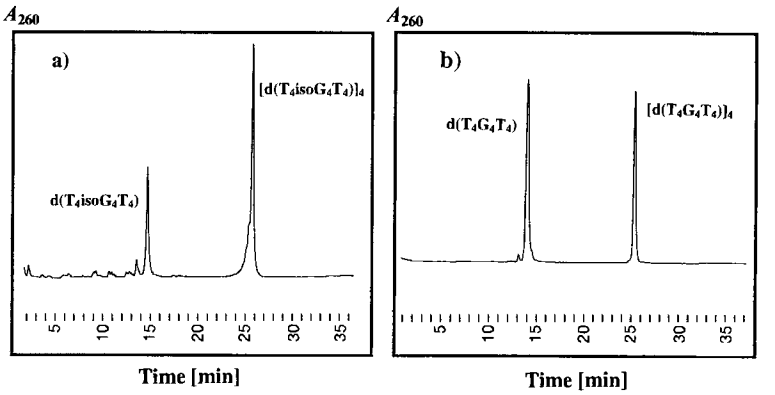


Fig. 3. *Ion-exchange HPLC profiles of* a) *5′-d(*T_4-iG_4-T_4*)* (**15**) *and* b) *5′-d(*T_4-G_4-T_4*) on a* Dionex *column at 30°.* Conditions, see *Exper. Part.*

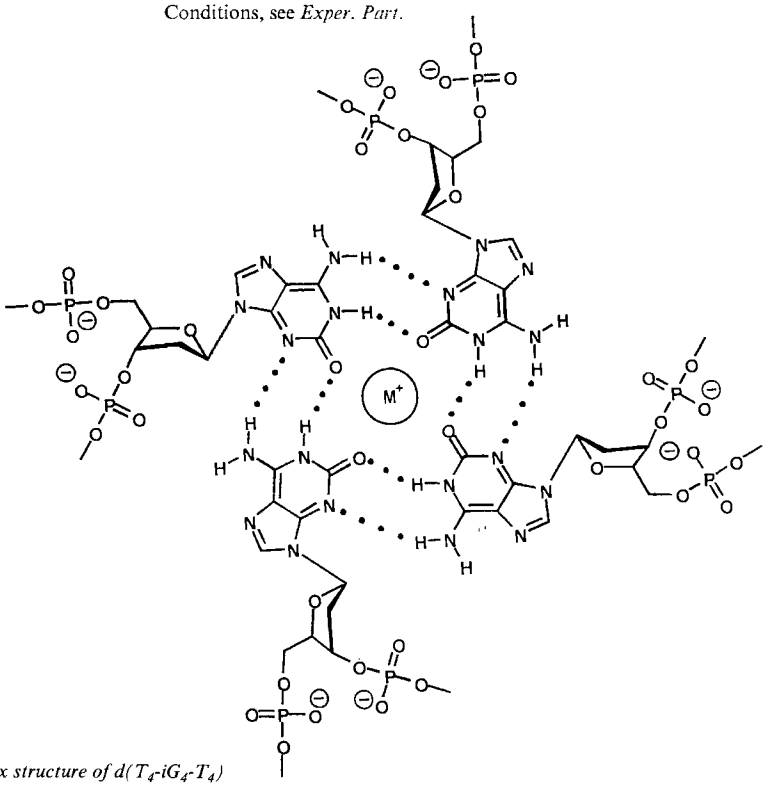


Fig. 4. *Possible tetraplex structure of d(*T_4-iG_4-T_4*)*

strand, while the slow-migrating one represents the aggregate. For comparison, we synthesized the oligomer d(T_4-G_4-T_4) which is known to form a tetrameric structure. Ion-exchange HPLC under identical conditions (*Fig. 3b*) showed two peaks with similar retention times as found for d(T_4-iG_4-T_4). As the ion-exchange column discriminates molecules by the number of negative charges of the sugar-phosphate backbone, this number has to be identical in the case of d(T_4-iG_4-T_4) and d(T_4-G_4-T_4). Therefore, the aggregates of G_d and iG_d have to have the same molecular weight and a tetrameric structure. This result is the first example demonstrating the formation of a defined tetraplex species in the case of an isoguanine-containing oligonucleotide. One possible structure for the tetraplex is shown in *Fig. 4*, which rests on measurements performed recently on monomeric isoguanosine [25]. However, other structures [6] [24] cannot be excluded. Further investigations on iG_d-rich oligonucleotides are currently undertaken in our laboratory which should give more information on isoguanine base pairing.

We thank Drs. *N. Ramzaeva* and *H. Rosemeyer* for the NMR spectra, Mrs. *E. Feiling* for excellent technical assistance, Mr. *A. Melenewski* for providing us with the ion-exchange HPLC and Mr. *Y. Chen* for helpful discussion. Financial support by the *Fonds der Chemischen Industrie* is gratefully acknowledged.

Experimental Part

General. See [26]. The solid-phase synthesis of oligonucleotides was carried out on an automated DNA synthesizer (*Applied Bioystems*, 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. Thin-layer chromatography (TLC): TLC aluminium sheets silica gel *60* F_{254} (0.2 mm, *Merck*, Germany). Reversed-phase HPLC: 4 × 250-mm *RP-18* (10 μm)-*LiChrosorb* column (*Merck*) with a *Merck-Hitachi* HPLC pump (model *655 A-12*) connected with a variable-wavelength monitor (model *655-A*), a controller (model *L-5000*), and an integrator (model *D-2000*). UV Spectra: *U-3200* spectrometer (*Hitachi*, Japan); λ_{max} in nm, ε in M^{-1} cm^{-1}, half-life values were measured using a temperature controller (*Lauda*, Germany). NMR Spectra: *AC-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 soln. (^{31}P).

Alkaline Hydrolysis of Nucleosides. The half-lives of nucleosides were measured in 25% aq. ammonia soln. at 40°. The reaction was followed UV-spectrophotometrically at the wavelength as indicated.

Acidic Hydrolysis of Nucleosides. a) *By UV Spectroscopy*: The reactions were performed in 0.1N aq. HCl at 25°. The hydrolysis was followed at 25° at the wavelength shown in *Table 2*. b) *By HPLC Analysis*: The nucleoside (2–3 mg) was dissolved in 0.1N aq. HCl (1.5 ml) and kept at 25°. After certain intervals of time (see *Fig. 1*), the mixture was quenched (pH 7) with Et_3N. A sample (50 μl) of the soln. was injected into the HPLC apparatus, and spectra were recorded at a wavelength as indicated in *Table 2*. For the retention times and gradients, see *Table 2* and *Fig. 1*.

Rp-18 *HPLC Separation of Oligonucleotides.* HPLC was carried out as reported [3]. The following solvent systems were used: 0.1M (Et_3NH)OAc (pH 7.0)/MeCN 95:5 (*A*) and MeCN (*B*). They were used in the following order: gradient *I*, 3 min 15% *B* in *A*, 12 min 15–40% *B* in *A*, 5 min 40–15% *B* in *A*; gradient *II*, 20 min 0–20% *B* in *A*; gradient *III*, 20 min 100% *A*.

Ion-exchange HPLC. Compound **15** (0.3 A_{260} units) was injected into a *Dionex-NucleoPac™-PA100* column (4 × 50 mm) at 30° connected with a HPLC apparatus. The column was eluted using the following solvent systems: 25 mM *Tris*·HCl containing 1 mM EDTA buffer (pH 8.0)/MeCN 90:10 (*A*) and 25 mM *Tris*·HCl, 1.0M NaCl containing 1 mM EDTA buffer (pH 8.0)/MeCN 90:10 (*B*). The following gradient was used: 30 min 20–80% *B* in *A*, 5 min 80% *B* in *A*, 5 min 80–20% *B* in *A*, with the flow rate of 0.75 ml/min. The HPLC profile was recorded at 260 nm.

Composition Analysis of Oligonucleotides. The oligonucleotide (0.2 A_{260} units) was dissolved in 0.1M *Tris* · HCl buffer (pH 8.3; 200 µl) and treated with snake-venom phosphodiesterase (3 µl) at 37° for 45 min and alkaline phosphatase (3 µl) at 37° for 30 min. The mixture was analyzed on reversed-phase HPLC (*RP-18*, gradient *II*, at 280 nm). Quantification of the material was made on the basis of the peak areas, which were divided by the extinction coefficients of the nucleoside constituents (ε_{260}: isoG$_d$ 4300, T$_d$ 8800, C$_d$ 7600; ε_{280}: isoG$_d$ 7300, T$_d$ 6337, C$_d$ 6900).

*6-Amino-9-(2-deoxy-β-*D-erythro-*pentofuranosyl)-9*H*-purin-2-yl Diphenylcarbamate* (**6**). Compound **1** (579 mg, 2.2 mmol) was dried by repeated co-evaporation from anh. pyridine and then suspended in anh. pyridine (10.0 ml). Diphenylcarbamoyl chloride (722 mg, 3.1 mmol) and (i-Pr)$_2$EtN (512 µl, 3 mmol) were added, and the mixture was stirred at r.t. for 5.5 h. The mixture was poured into 5% aq. NaHCO$_3$ soln. (16 ml) and extracted with CH_2Cl_2 (4 × 8 ml), the extract dried (Na_2SO_4) and evaporated, and the residue applied to FC (silica gel, 2.5 × 10 cm, CH_2Cl_2/MeOH 99:1 to 90:10): **6** as a foam (782 mg, 77%). The product was treated with H_2O, filtered, and washed with Et_2O to give a sample for analysis. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.36. UV (MeOH): 260 (18300), 238 (20500). ^{1}H-NMR ((D$_6$)DMSO): 2.25 (*m*, 1 H–C(2′)); 2.63 (*m*, 1 H–C(2′)); 3.57 (*m*, 2 H–C(5′)); 3.85 (*m*, H–C(4′)); 4.38 (*m*, H–C(3′)); 5.00 (*t*, *J* = 5.5, OH–C(5′)); 5.28 (*d*, *J* = 4.1, OH–C(3′)); 6.25 (*t*, *J* = 6.75, H–C(1′)); 7.28–7.42 (*m*, arom. H); 7.68 (*s*, NH$_2$); 8.32 (*s*, H–C(8)). Anal. calc. for $C_{23}H_2N_6O_5$ (462.46): C 59.73, H 4.79, N 18.17; found: C 59.91, H 4.86, N 18.06.

*9-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-6-{[1-(dimethylamino)ethylidene]amino}-1,9-dihydro-2*H*-purin-2-one* (**5**). To the suspension of **1** (505 mg, 1.9 mmol) in MeOH (25 ml), *N*,*N*-dimethylacetamide dimethyl acetal (0.75 ml, 5.0 mmol) was added. The mixture was stirred at r.t. for 4 h, then evaporated and co-evaporated with MeOH and the residue applied to FC (silica gel, 2.5 × 12 cm, CH_2Cl_2/MeOH 9:1→8:2): **5** (573 mg, 90%). Pale-yellow powder. TLC (CH_2Cl_2/MeOH 3:2): R_f 0.5. UV (MeOH): 337 (21100), 251 (9500). ^{1}H-NMR ((D$_6$)DMSO): 2.12 (*s*, *Me*C(NMe$_2$)); 2.17 (*m*, 1 H–C(2′)); 2.60 (*m*, 1 H–C(2′)); 3.08 (*s*, MeN); 3.15 (*s*, MeN); 3.54 (*m*, 2 H–C(5′)); 3.83 (*m*, H–C(4′)); 4.34 (*m*, H–C(3′)); 5.25 (*d*, *J* = 3.8, OH–C(3′)); 5.40 (*m*, OH–C(5′)); 6.10 (*t*, *J* = 6.4, H–C(1′)); 7.98 (*s*, H–C(8)); 10.92 (br. *s*, NH). Anal. calc. for $C_{14}H_{20}N_6O_4$ (336.35): C 49.99, H 5.99, N 24.99; found: C 49.85, H 6.10, N 24.89.

*9-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-6-{[1-(dimethylamino)ethylidene]amino}-9*H*-purin-2-yl Diphenylcarbamate* (**7**). To a soln. of **5** (247.3 mg, 0.74 mmol) in anh. pyridine (5.0 ml), diphenylcarbamoyl chloride (259.1 mg, 1.12 mmol) and (i-Pr)$_2$EtN (190 µl, 1.10 mmol) were added. The mixture was stirred at r.t. for 40 min and then poured into 5% aq. NaHCO$_3$ soln. (7 ml) and extracted with CH_2Cl_2 (3 × 5 ml). The extract was dried (Na_2SO_4) and evaporated and the residue applied to FC (silica gel, 2.5 × 8 cm, CH_2Cl_2/MeOH 99:1→95:5): **7** (318 mg, 81%). Pale-yellow solid. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.47. UV (MeOH): 309 (23800), 233 (28000). ^{1}H-NMR ((D$_6$)DMSO): 2.15 (*s*, *Me*C(NMe$_2$)); 2.34 (*m*, 1 H–C(2′)); 2.71 (*m*, 1 H–C(2′)); 3.18 (br. *s*, Me$_2$N); 3.58 (*m*, 1 H–C(5′)); 3.65 (*m*, 1 H–C(5′)); 3.92 (*m*, H–C(4′)); 4.46 (br. *s*, H–C(3′)); 5.04 (*t*, *J* = 5.5, OH–C(5′)); 5.35 (*d*, *J* = 4.15, OH–C(3′)); 6.36 (*t*, *J* = 6.95, H–C(1′)); 7.35–7.49 (*m*, arom. H); 8.45 (*s*, H–C(8)). Anal. calc. for $C_{27}H_{29}N_7O_5$ (531.56): C 61.01, H 5.50; found: C 60.97, H 5.79.

*9-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D-erythro-*pentofuranosyl]-6-{[1-(dimethylamino)ethylidene]amino}-1,9-dihydro-2*H*-purin-2-one* (**9a**) *and 9-[2-Deoxy-3,5-*O*-bis(4,4′-dimethoxytrityl)-β-*D-erythro-*pentofuranosyl]-6-{[1-(dimethylamino)ethylidene]amino}-1,9-dihydro-2*H*-purin-2-one* (**9b**). Compound **5** (491 mg, 1.46 mmol) was dried by repeated co-evaporation from anh. pyridine and then dissolved in anh. pyridine (10 ml). At r.t., 4,4′-dimethoxytrityl chloride ((MeO)$_2$TrCl; 700 mg, 2.07 mmol) was added under stirring, and stirring was continued for 17 h. After addition of MeOH (3 ml) the mixture was evaporated, the residue dissolved in CH_2Cl_2 (20 ml), the soln. washed with 5% aq. NaHCO$_3$ soln. (10 ml) and then with H_2O (10 ml), dried (Na_2SO_4), and evaporated, and the residue applied to FC (silica gel, 4 × 12 cm, washing with CH_2Cl_2/Me$_2$CO 8:2, then CH_2Cl_2/MeOH 9:1): more polar **9a** (377 mg, 40%) and less polar **9b** (234 mg, 17%).

9a: pale-yellow powder. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.37. UV (MeOH): 337 (18700), 222 (41200). ^{1}H-NMR ((D$_6$)DMSO): 2.07 (*s*, *Me*C(NMe$_2$)); 2.25 (*m*, 1 H–C(2′)); 2.63 (*m*, 1 H–C(2′)); 3.08 (*s*, MeN); 3.13 (*s*, MeN); 3.69 (*s*, MeO); 3.71 (*s*, MeO); 3.91 (*m*, H–C(4′)); 4.37 (*m*, H–C(3′)); 5.33 (*m*, OH–C(3′)); 6.12 (*t*, *J* = 6.3, H–C(1′)); 6.80–7.36 (*m*, arom. H); 7.87 (*s*, H–C(8)). Anal. calc. for $C_{35}H_{38}N_6O_6$ (638.72): C 65.82, H 6.00, N 13.16; found: C 65.85, H 6.07, N 12.99.

9b: colorless foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.45. UV (MeOH): 338 (19300), 225 (48400). ^{1}H-NMR ((D$_6$)DMSO): 1.72 (*m*, 1 H–C(2′)); 2.09 (*s*, *Me*C(NMe$_2$)); 2.18 (*m*, 1 H–C(2′)); 2.93 (*m*, 1 H–C(5′)); 2.99 (*m*, 1 H–C(5′)); 3.08 (*s*, MeN); 3.14 (*s*, MeN); 3.70–3.74 (*m*, 4 MeO); 3.98 (*s*, H–C(4′)); 4.20 (*d*, H–C(3′)); 6.11 (*t*, *J* = 6.5, H–C(1′)); 6.78–7.40 (*m*, arom. H); 7.69 (*s*, H–C(8)); 10.88 (*s*, NH). Anal. calc. for $C_{56}H_{56}N_6O_8$ (941.11): C 71.47, H 6.00, N 8.93; found: C 71.27, H 6.11, N 9.02.

*9-[2-Deoxy-5-O-(4,4'-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl]-6-{[1-(dimethylamino)ethylidene]-amino}-9H-purin-2-yl Diphenylcarbamate* (**8**). a) *From* **9a**. To a soln. of **9a** (319.2 mg, 0.5 mmol) in dry pyridine (5.0 ml), diphenylcarbamoyl chloride (207.8 mg, 0.897 mmol) and (i-Pr)$_2$EtN (130 µl, 0.76 mmol) were added. The mixture was stirred at r.t. for 30 min, poured in 5% aq. NaHCO$_3$ soln. (5.0 ml), and extracted with CH_2Cl_2 (3 × 6 ml). The extract was washed with H_2O (20 ml), dried (Na_2SO_4), and evaporated and the residue applied to FC (silica gel, 2 × 10 cm, CH_2Cl_2/Me_2CO 95:5→85:15): **8** (298 mg, 71%). Colorless solid. TLC (CH_2Cl_2/MeOH 95:5): R_f 0.31. UV (MeOH): 309 (21700), 234 (47000). ^{1}H-NMR ((D$_6$)DMSO): 2.08 (*s*, *Me*C(NMe$_2$)); 2.38 (*m*, 1 H–C(2')); 2.76 (*m*, 1 H–C(2')); 3.13 (*s*, Me$_2$N); 3.17 (*m*, 2 H–C(5')); 3.71 (*s*, 2 MeO); 3.97 (*s*, H–C(4')); 4.46 (*m*, H–C(3')); 5.36 (*d*, *J* = 4.63, OH–C(3')); 6.35 (*t*, *J* = 6.3, H–C(1')); 6.78–7.43 (*m*, arom. H); 8.28 (*s*, H–C(8)). Anal. calc. for $C_{48}H_{47}N_7O_7$ (833.94): C 69.09, H 5.68, N 11.76; found: C 68.98, H 5.70, N 11.62.

b) *From* **7**. Compound **7** (106.2 mg, 0.20 mmol) was dried by repeated co-evaporation from anh. pyridine and then dissolved in anh. pyridine (2.5 ml). (MeO)$_2$TrCl (81.3 mg, 0.24 mmol) was added at r.t. and the soln. stirred for 21.5 h. MeOH (2 ml) and 5% aq. NaHCO$_3$ soln. (5 ml) were added. The mixture was extracted with CH_2Cl_2 (3 × 5 ml), the org. layer dried (Na_2SO_4) and evaporated and the residue applied to FC (silica gel, 2 × 10 cm, washing with CH_2Cl_2, then CH_2Cl_2/Me_2CO 95:5→85:15): **8** (108 mg, 65%). Colorless foam.

*9-[2-Deoxy-5-O-(4,4'-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl]-6-{[1-(dimethylamino)ethylidene]-amino}-1,9-dihydro-2H-purin-2-one 3'-(Triethylammonium Phosphonate)* (**3a**). To the mixture of 1*H*-1,2,4-triazole (1020 mg, 14.8 mmol) and *N*-methylmorpholine (4.5 ml) in CH_2Cl_2 (25 ml) was introduced PCl_3 (420 µl, 4.68 mmol) under stirring. After 30 min, the soln. was cooled to 0° and **9a** (528.8 mg, 0.828 mmol) in CH_2Cl_2 (25 ml) added slowly. Stirring was continued at r.t. for 35 min and then the mixture poured into 1M (Et$_3$NH)HCO$_3$ (50 ml). After shaking, the aq. phase was extracted with CH_2Cl_2 (4 × 15 ml), the combined org. phase dried (Na_2SO_4) and evaporated, and the residue applied to FC (silica gel, 2.5 × 10 cm, washing with CH_2Cl_2/Et$_3$N 98:2, then CH_2Cl_2/MeOH/Et$_3$N 93:5:2→88:10:2) to give a colorless foam (537 mg). This product was dissolved in CH_2Cl_2 (10 ml) and washed with 0.1M (Et$_3$NH)HCO$_3$ buffer (5 × 20 ml). The aq. layers were extracted with CH_2Cl_2 (2 × 10 ml) and the combined CH_2Cl_2 layers dried (Na_2SO_4) and evaporated: **3a** (473 mg, 71%). Colorless foam. TLC (CH_2Cl_2/MeOH/Et$_3$N 88:10:2): R_f 0.42. UV (MeOH): 337 (20200). ^{1}H-NMR ((D$_6$)DMSO): 1.12–1.23 (*m*, 3 *Me*CH$_2$); 2.09 (*s*, *Me*C(NMe$_2$)); 2.76 (*m*, 1 H–C(2')); 2.99–3.14 (*m*, 3 MeC*H*$_2$); 3.36 (*s*, MeN); 3.38 (*s*, MeN); 3.71 (*s*, 2 MeO); 4.06 (*m*, H–C(4')); 4.75 (*m*, H–C(3')); 5.44, 7.77 (2*s*, P–H); 6.10 (*m*, H–C(1')); 6.60–7.33 (*m*, arom. H); 7.85 (*s*, H–C(8)). ^{31}P-NMR ((D$_6$)DMSO): 1.10 (^1J(P,H) = 585, ^3J(P,H) = 8.1). Anal. calc. for $C_{41}H_{54}N_7O_8P$ (803.89): C 61.26, H 6.77, N 12.20; found: C 61.11, H 6.77, N 12.02.

*9-[2-Deoxy-5-O-(4,4'-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl]-6-{[1-(dimethylamino)ethylidene]-amino}-1,9-dihydro-2H-purin-2-one 3'-(2-Cyanoethyl N,N-Diisopropylphosphoramidite)* (**3b**). A soln. of **9a** (239 mg, 0.37 mmol) in CH_2Cl_2 (8 ml) was flushed with Ar. Then chloro(2-cyanoethoxy)(diisopropylamino)-phosphane (0.25 ml, 1.12 mmol) together with (i-Pr)$_2$EtN (0.20 ml, 1.12 mmol) was added under Ar. After stirring for 2 h at r.t., a further portion of chloro(2-cyanoethoxy)(diisopropylamino)phosphane (0.25 ml, 1.12 mmol) and (i-Pr)$_2$EtN (0.20 ml, 1.12 mmol) were added. Stirring was continued for additional 2 h. Then 5% aq. NaHCO$_3$ soln. (8 ml) was added and the mixture extracted with CH_2Cl_2 (3 × 3 ml). The extract was dried (Na_2SO_4) and evaporated. The oily residue was co-evaporated with toluene and the residue applied to FC (silica gel, 2.5 × 10 cm, CH_2Cl_2/AcOEt/MeOH /Et$_3$N 45:40:10:5) to give an oil. This was dissolved in CH_2Cl_2 furnishing **3b** as a pale yellow solid (202 mg, 65%) upon precipitation in hexane. TLC (CH_2Cl_2/AcOEt/MeOH/Et$_3$N 45:45:5:10): R_f 0.54. ^{31}P-NMR ($CDCl_3$): 149.1, 149.2.

*9-[2-Deoxy-5-O-(4,4'-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl]-6-{[1-(dimethylamino)ethylidene]-amino}-9H-purin-2-yl Diphenylcarbamate 3'-(Triethylammonium Phosphonate)* (**4a**). As described for **3a**, with 1*H*-1,2,4-triazole (220 mg, 3.2 mmol), *N*-methylmorpholine (1.0 ml), CH_2Cl_2 (6.0 ml), PCl_3 (83 µl, 0.9 mmol), **8** (153 mg, 0.18 mmol), and CH_2Cl_2 (6.0 ml). Workup with 1M (Et$_3$NH)HCO$_3$ (11.0 ml) and CH_2Cl_2 (4 × 3 ml) and FC (silica gel, 2 × 8 cm, CH_2Cl_2/MeOH/Et$_3$N 94:4:2) gave a colorless foam (160 mg, 89%). Further purification as described for **3a** with CH_2Cl_2 (6.0 ml), 0.1M (Et$_3$NH)HCO$_3$ buffer (5 × 10 ml), and CH_2Cl_2 (2 × 8 ml) yielded **4a** (136 mg, 76%). Colorless foam. TLC (CH_2Cl_2/MeOH/Et$_3$N 88:10:2): R_f 0.53. UV (MeOH): 308 (21600), 234 (47400). ^{1}H-NMR ((D$_6$)DMSO): 1.07 (*m*, 3 *Me*CH$_2$); 2.07 (*s*, *Me*C(NMe$_2$)); 2.79 (*m*, 3 MeC*H*$_2$); 3.11 (br. *s*, Me$_2$N); 3.16 (*m*, 1 H–C(5')); 3.69 (*s*, 2 MeO); 4.12 (*m*, H–C(4')); 4.79 (*m*, H–C(3')); 5.47, 7.81 (2*s*, P–H); 6.31 (*t*, *J* = 6.3, H–C(1')); 6.76–7.42 (*m*, arom. H); 8.25 (*s*, H–C(8)). ^{31}P-NMR ((D$_6$)DMSO): 1.15 (^1J(P,H) = 585, ^3J(P,H) = 8.2). Anal. calc. for $C_{54}H_{63}N_8O_9P$ (999.11): C 64.92, H 6.36, N 11.22; found: C 65.06, H 6.38, N 11.35.

*9-[2-Deoxy-5-O-(4,4'-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl]-6-{[1-(dimethylamino)ethylidene]-amino}-9H-purin-2-yl Diphenylcarbamate 3'-(2-Cyanoethyl N,N-Diisopropylphosphoramidite)* (**4b**). A soln. of **8** (219 mg, 0.26 mmol) in CH_2Cl_2 (7.5 ml) was preflushed with Ar and kept under Ar. Then chloro(2-cyano-ethoxy)(diisopropylamino)phosphane (0.23 ml, 1.03 mmol) and (i-Pr)$_2$EtN (0.18 ml, 1.03 mmol) were added at r.t.,

and stirring was continued for 2.5 h. Then 5% aq. $NaHCO_3$ soln. (7.5 ml) was added, the aq. layer extracted with CH_2Cl_2 (3 × 3 ml), and the combined org. phase dried (Na_2SO_4, 1.5 h) and evaporated. The oily residue was dissolved in CH_2Cl_2, and a white powder was isolated from precipitation in hexane (200 ml): **4b** (252 mg, 94%). TLC (CH_2Cl_2/AcOEt/Et_3N 45:45:10): R_f 0.56. ^{31}P-NMR ($CDCl_3$): 149.4, 149.5.

Solid-Phase Synthesis of the Oligonucleotides **11**–**15**. a) *Phosphonate Protocol.* The synthesis was carried out on a 1-μmol scale using the 3′-phosphonates of $[(MeO)_2Tr]T_d$, $[(MeO)_2Tr]bz^4C_d$, together with **3a** or **4a** and *CPG-500*-T_d or *CPG-500*-C_d. The syntheses of **11**–**15** was performed using the cycle described in *Table 5*.

Table 5. *Oligonucleotide Synthesis*

Step[a])	Reagents and solvents	Time [s]
A. *Coupling procedure*		
1. Wash	MeCN	20
2. Detritylation	2.5% $Cl_2CHCOOH$ in CH_2Cl_2	5 × 20
3. Wash	MeCN	2 × 20
	MeCN/pyridine 1:1	20
	MeCN	20
4. Condensation	0.1M phosphonate and 0.2M pivaloyl chloride both in MeCN/pyridine 1:1	20
	wait	100
5. Wash	MeCN/pyridine 1:1	20
	MeCN	20
6. Capping	0.1M diisopropyl phosphite and 0.2M pivaloyl chloride both in MeCN/pyridine 1:1	20
	wait	90
7. Wash	MeCN/pyridine 1:1	20
	MeCN	20
B. *Automatic oxidation*		
1. Oxidizer I	1:1 mixture of 0.2M I_2 in THF and *N*-methylmorpholine/H_2O/THF 1:1:8	5 × 21
2. Oxidizer II	1:1 mixture of 0.2M I_2 in THF and Et_3N/H_2O/THF 1:1:8	5 × 21
3. Wash	MeCN	1 × 30, 1 × 20, 3 × 10
C. *Cleavage from the solid support*		
1. Hydrolysis	33% NH_3 soln.	1 × 18
	wait	1 × 900
2. Repeat step 1 two times		
3.	33% NH_3 soln.	1 × 15
4. Wash	MeCN	1 × 30

[a]) The syntheses were carried out on a DNA synthesizer, model *ABI 380 B* (*Applied Biosystems*) upgraded to the fast coupling cycle (version 2.01).

b) *Phosphoramidite Protocol.* The oligonucleotides **11** and **13** were synthesized also using the 3′-phosphoramidite of $[(MeO)_2Tr]bz^4C_d$, dpc-protected phosphoramidite **4b**, and the solid suppert *CPG-500*-C_d. The synthesis was carried out on a 1-μmol scale using standard conditions.

c) *Deprotection and Purification.* Deprotection of the oligonucleotides was performed in 25% aq. NH_3 at 60° for 18 h. The 5′-$[(MeO)_2Tr]$-oligomers were purified by HPLC (250 × 4 mm *RP-18* column, gradient *I*), isolated, and the $(MeO)_2Tr$ residues were removed by treatment with 2.5% CCl_2HCOOH/CH_2Cl_2 for 5 min at r.t. The detritylated oligomers were purified by HPLC (gradient *II*). The oligomers were desalted on a 4-cm 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 colorless solids which were dissolved in 100 μl of H_2O and stored frozen at −18°. HPLC (gradient *II*): t_R 18.2 (**11**), 20.1 (**13**), 19.6 min (**15**). MS (MALDI-TOF): $[M + H]^+$ at 1792.9 (**11**), 1792 (**13**).

REFERENCES

[1] F. Seela, C. Wei, Z. Kazimierczuk, *Helv. Chim. Acta* **1995,** *78,* 1843.
[2] T. Tuschl, M. M. P. Ng, W. Pieken, F. Benseler, F. Eckstein, *Biochemistry* **1993,** *32,* 11658.
[3] F. Seela, R. Mertens, Z. Kazimierczuk, *Helv. Chim. Acta* **1992,** *75,* 2298.
[4] C. Y. Switzer, S. E. Moroney, S. A. Benner, *Biochemistry* **1993,** *32,* 10489.
[5] S. Pitsch, R. Krishnamurthy, M. Bolli, S. Wendeborn, A. Holzner, M. Minton, C. Lesueur, I. Schlönvogt, B. Jaun, A. Eschenmoser, *Helv. Chim. Acta* **1995,** *78,* 1621.
[6] R. Krishnamurthy, S. Pitsch, M. Minton, C. Miculka, N. Windhab, A. Eschenmoser, *Angew. Chem.* **1996,** *108,* 1619.
[7] F. Seela, Y. Chen, A. Melenewski, H. Rosemeyer, C. Wei, *Acta Biochim. Polon.* **1996,** *43,* 45.
[8] F. Seela, B. Gabler, Z. Kazimierczuk, *Collect. Czech. Chem. Commun.* **1993,** *58,* 170.
[9] Y. Tor, P. B. Dervan, *J. Am. Chem. Soc.* **1993,** *115,* 4461.
[10] T. Horn, C.-A. Chang, M. L. Collins, *Tetrahedron Lett.* **1995,** *36,* 2033.
[11] T. Kamimura, M. Tsuchiya, K. Koura, M. Sekine, T. Hata, *Tetrahedron Lett.* **1983,** *24,* 2775.
[12] T. Kamimura, M. Tsuchiya, K. Urakami, K. Koura, M. Sekine, K. Shinozaki, K. Miura, T. Hata, *J. Am. Chem. Soc.* **1984,** *106,* 4552.
[13] R. Zou, M. J. Robins, *Can. J. Chem.* **1987,** *65,* 1436.
[14] S. Janardhanam, K. P. Nambiar, *J. Chem. Soc., Chem. Commun.* **1994,** 1009.
[15] M. Sekine, J. Heikkila, T. Hata, *Tetrahedron Lett.* **1987,** *28,* 5691.
[16] J. Nielsen, M. Taagaard, J. E. Marugg, J. H. van Boom, O. Dahl, *Nucleic Acids Res.* **1986,** *14,* 7391.
[17] M. Kamaike, Y. Hasegawa, Y. Ishido, *Nucleosides Nucleotides* **1988,** *7,* 37.
[18] Z. Kazimierczuk, R. Mertens, W. Kawczynski, F. Seela, *Helv. Chim. Acta* **1991,** *74,* 1742.
[19] F. Seela, B. Gabler, *Helv. Chim. Acta* **1994,** *77,* 622.
[20] L. J. McBride, R. Kierzek, S. L. Beaucage, M. H. Caruthers, *J. Am. Chem. Soc.* **1986,** *108,* 2040.
[21] M. Krecmerova, F. Seela, *Nucleosides Nucleotides* **1992,** *11,* 1393.
[22] F. Seela, T. Fröhlich, *Helv. Chim. Acta* **1994,** *77,* 399.
[23] C. Switzer, S. E. Moroney, S. A. Benner, *J. Am. Chem. Soc.* **1989,** *111,* 8322.
[24] T. Golas, M. Fikus, Z. Kazimierczuk, D. Shugar, *Eur. J. Biochem.* **1976,** *65,* 183.
[25] J. T. Davis, S. Tirumala, J. R. Jenssen, E. Radler, D. Fabris, *J. Org. Chem.* **1995,** *60,* 4167.
[26] W. Bourgeois, F. Seela, *J. Chem. Soc., Perkin Trans. 1* **1991,** 279.