131. 8-Aza-7-deaza-2′-deoxyguanosine: Phosphoramidite Synthesis and Properties of Octanucleotides

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Base-modified octanucleotides derived from d(G1–G2–A–A–T–T–C–C) (**11**) but containing 8-aza-7-deaza-2′-deoxyguanosine (**2**) instead of 2′-deoxyguanosine (**1**) have been prepared by solid-phase synthesis employing P(III) chemistry. Isobutyrylation of **2**, followed by 4,4′-dimethoxytritylation and subsequent phosphitylation yielded the methyl or the cyanoethyl phosphoramidites **6a** or **6b**, respectively. They were used as building blocks in automated DNA synthesis. The resulting octanucleotides **12**–**14** containing **2** showed increased T_m values compared to the parent oligomer **11**. The oligomers **11**–**14** were employed as sequence-specific probes in endodeoxyribonuclease Eco RI oligonucleotide recognition. Whereas displacement of dG-2 (enzymic cleavage site of **11**) abolished phosphodiester hydrolysis, replacement of dG-1 enhanced the cleavage rate compared to **11**.

Introduction. – Recently, 8-aza-7-deaza-2′-deoxyguanosine ($z^8c^7G_d$; **2**) has been synthesized by phase-transfer glycosylation [1] [2] and was later converted into the 2′,3′-dideoxyribofuranoside [2][1]). As compound **2** represents one of the most closest structural analoga of the DNA constituent 2′-deoxyguanosine (dG; **1**), being still capable to form *Watson-Crick* base pairs, we decided to synthesize phosphoramidites of **2**, which are applicable in automated DNA synthesis machines.

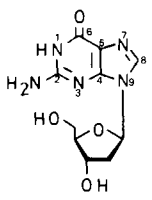
Due to the altered electronic state of **2** compared to that of 2′-deoxyguanosine (**1**), changes in the reactivities of nucleobase-protecting groups have to be considered. Kinetic data of base-catalyzed hydrolysis of acyl- or amidine-protected **2** were determined in order to select the most appropriate protecting group. Then, protection of the 5′-OH of the sugar moiety allowed phosphoramidite synthesis by established procedures [4] [5]. Combining the resulting phosphoramidites **6a** or **6b** with regular phosphoramidites, oligonucleotides are accessable with compound **2** at any position of incorporation.

Here we present the synthesis of octanucleotides derived from d(G1–G2–A–A–T–T–C–C)[1]) (**11**) containing the recognition sequence of the endodeoxyribonuclease Eco RI [6]. We have chosen these oligomers as **11** forms duplexes under appropriate salt condition and is regioselectively hydrolyzed by the endonuclease [7]. As the phosphodiester bond is cleaved between dG and dA, it is of interest whether the enzyme accepts compound **2** as a substitute of dG-1 or dG-2 within an oligomer. Earlier studies have considered the N^7 and O^6 atoms of dG-2 within **11** as the most probable

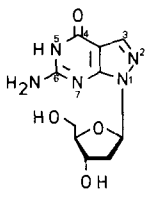
[1]) For the symbols of protecting groups, nucleosides and nucleotides, see IUPAC-IUB conventions [3]. In the symbolism for **2** ($z^8c^7G_d$) and related nucleosides, the numbering of **1** is used which differs from the systematic numbering given in *Formula* **2**.

proton-acceptor sites for arginine-200 (guanidino residue) of the enzyme [8]. Replacement of dG by **2** should proof the regiochemical demands of the enzyme with regard to proton-acceptor positions.

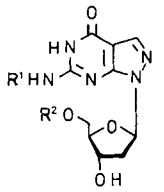
Results and Discussion. – For the synthesis of oligonucleotides containing 8-aza-7-deaza-2′-deoxyguanosine (**2**), P(III) chemistry on solid support was chosen which required the preparation of the phosphoramidites **6a** and **6b**. For this purpose, the 6-NH_2 group of **2** had to be protected. The isobutyryl residue (ib)[1]) was chosen to this end as this group has already successfully been employed in the case of 2′-deoxyguanosine (**1**) [9].



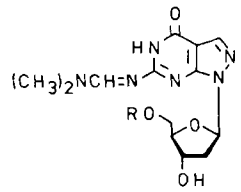
1



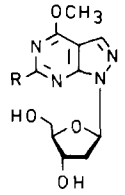
2



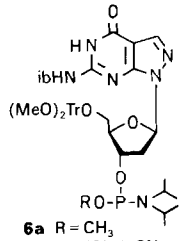
3a R^1 = ib; R^2 = H
b R^1 = ib; R^2 = $(MeO)_2Tr$



4a R = H
b R = $(MeO)_2Tr$



5a R = NH_2
b R = N=CHN$(CH_3)_2$



6a R = CH_3
b R = $(CH_2)_2CN$

For isobutyrylation, the protocol of transient protection was used [10]. Crystalline **3a** was isolated in 64% yield. Alternatively, we converted **2** into the formamidine **4a** using the protocol described for regular nucleosides [11] [12]. This route circumvented intermediate protection of the sugar OH groups and yielded crystalline **4a** in 85% yield. The structure of the acyl- as well as of amidine-protected nucleosides **4a** and **5b** was confirmed by elemental analysis and ^{1}H-NMR spectroscopy. *Table 1* shows the ^{13}C-NMR data of these compounds which were unambiguously assigned by gated decoupled ^{13}C-NMR spectra *(Table 2)*.

As the N^6 protecting group of **2** has to be stable during oligonucleotide synthesis but removable during deprotection with 25% aq. NH_3 soln., we carried out hydrolysis experiments at 40°. The kinetics was followed by UV spectrophotometry. *Table 3* shows that **3a** was hydrolyzed faster than the N^2-isobutyrylated dG [9] or the pyrrolo[2,3-*d*]-pyrimidine congener $ib^2c^7G_d$ [13]. The formamidine **4a** was even less stable as acyl-protected **3a**. This is in line with the results on the corresponding formamidine of dG [11] and similar to that of the formamidine of c^7G_d [14]. However, on alteration of the electronic structure of the nucleobase, *e.g.* by methylation of O^4 of **2** (→**5a**), NH_2-protecting groups became much more stable. As a result, the formamidine **5b** was the one with the slowest hydrolysis rate of this series *(Table 3)*.

Table 1. *^{13}C-NMR Chemical Shifts of Compound* **2** *and Related Nucleosides in (D_6)DMSO*[a])

	C(3)	C(3a)	C(4)	C(6)	C(7a)	C=O	CH_3O
2	134.9	99.7	157.4	154.6	155.2	–	–
3a	135.7	102.9	155.9	150.2	152.9	180.6	–
3b	135.7	103.0	155.9	150.1	152.8	180.7	55.0
4a	135.3	102.3	159.2	158.6	154.7	–	–
4b	135.2	102.3	159.1	158.6	154.6	–	–
5a	132.0	96.1	163.4	161.9	157.6	–	53.2
5b	132.2	98.7	163.1	165.0	157.6	–	53.6

	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	CH	CH_3
2	83.1	37.9	71.0	87.3	62.4	–	–
3a	83.6	37.9	71.0	87.8	62.5	34.9	18.9, 18.6
3b	83.7	38.2	70.8	85.6	64.4	34.9	18.9, 18.8
4a	83.3	38.1	71.3	87.7	62.6	158.6	34.9
4b	83.0	38.4	71.0	85.3	64.6	158.7	34.9
5a	83.3	37.8	71.0	87.3	62.4	–	–
5b	83.8	38.0	71.2	87.6	62.6	159.0	34.7

[a]) TMS as internal standard.

Table 2. *J(C, H) Values* [Hz] *of the Nucleosides* **3a**, **4a**, *and* **5b**[a])

J(C, H)	**3a**	**4a**	**5b**
C(3), *H*–C(3)	193.1	190.9	193.8
C(3a), *H*–C(3)	10.3	10.1	10.7
C(4), *CH*$_3$O	–	–	3.9
C(6), *CH*=N	–	6.8	7.0
*C*H, C*H*	131.4	179.7	178.0
C(7a), *H*–C(3)	3.6	3.8	3.7
C(7a), *H*–C(1′)	–	1.2	1.1
C(1′), *H*–C(1′)	164.5	169.1	164.7
C(3′), *H*–C(3′)	148.8	147.5	148.6
C(4′), *H*–C(4′)	148.7	148.2	146.9
C(5′), *H*–C(5′)	139.8	140.0	140.1

[a]) Measured in (D_6)DMSO.

Table 3. *$\tau_{1/2}$ Values of NH_2-Protected 2′-Deoxyguanosine Derivatives*[a]) *in 25% aq. NH_3 Solution at 40°*

Compound[1])		$\tau_{1/2}$ [min][b])
ib^2G_d	[9]	112
$ib^2c^7G_d$	[13]	109
$ib^2z^8c^7G_d$	**3a**	37
$m_2fa^2G_d$	[11]	19
$m_2fa^2c^7G_d$	[14]	22
$m_2fa^2z^8c^7G_d$	**4a**	16
$m_2fa^2m^6z^8c^7G_d$	**5b**	65

[a]) 30 μM nucleoside concentration.
[b]) Measured by UV spectrophotometry at 300 nm.

The ^{1}H-NMR spectra of the formamidines **4a** and **5b** show 2 separate *s* for the Me resonances at r.t. in (D_6)DMSO. This is caused by the restricted rotation around the C–NMe bond demonstrating the partial double-bond character. At elevated temperature, however, the Me signals of **5b** coincided (100°) but, depending on the nucleobase structure, coalescence was not observed in other cases due to the height of the rotation barrier and the limitation of the temperature. Compound **5b** exhibiting the lowest rotation barrier was the most stable against base-catalyzed hydrolysis.

Next, the 5′-OH groups of **3a** and **4a** were protected with the 4,4′-dimethoxytriphenylmethyl ($(MeO)_2Tr$[1]) residue. The reaction was carried out similarly as described for the parent 2′-deoxyguanosine [15]. The 5′-*O*-protection (→**3b** and **4b**, resp.) was confirmed by ^{13}C-NMR spectra which showed downfield shifts for C(5′) and upfield shifts for C(4′), both indicating 5′-*O*-alkylation *(Table 1)*.

We have chosen compound **3b** for further experiments as the formamidine protecting group of **4b** was too labile to be used in oligonucleotide synthesis. The conversion of **3b** into the phosphoramidite **6a** followed a protocol which was originally developed by *Caruthers* for regular methyl phosphoramidites [4]. The cyanoethyl amidite **6b** was prepared according to the method of *Köster* and coworkers [5]. Both phosphoramidites were purified by flash chromatography and characterized by ^{31}P-NMR spectroscopy. The phosphoramidites **6a** and **6b** together with those of dA, dG, dC, and dT were then employed in automated DNA synthesis on solid support [16] using a purchasable DNA synthesizer. The synthesis of **7**–**14** followed a protocol of detritylation, coupling, and oxidation according to [17]. The octamers were purified by HPLC and desalted as described in the *Exper. Part.*

d([(MeO)$_2$Tr]G–G–A–A–T–T–C–C)	(**7**)	d(G–G–A–A–T–T–C–C)	(**11**)
d([(MeO)$_2$Tr]G–z^{8}c^{7}G–A–A–T–T–C–C)	(**8**)	d(z^{8}c^{7}G–z^{8}c^{7}G–A–A–T–T–C–C)	(**12**)
d([(MeO)$_2$Tr]z^{8}c^{7}G–z^{8}c^{7}G–A–A–T–T–C–C)	(**9**)	d(G–z^{8}c^{7}G–A–A–T–T–C–C)	(**13**)
d([(MeO)$_2$Tr]z^{8}c^{7}G–G–A–A–T–T–C–C)	(**10**)	d(z^{8}c^{7}G–G–A–A–T–T–C–C)	(**14**)

The nucleoside content of the purified octamers **11**–**14** was determined after hydrolysis with snake-venom phosphodiesterase followed by digestion with alkaline phosphatase. Expected amounts of **2** as well as of dA, dC, dG, and dT were detected by UV spectrophotometry after HPLC separation *(e.g. Fig. 1c)*. This indicates that irreversible side reactions at the lactam moiety of **2** did not take place, and a protection of this group was unnecessary for oligonucleotide synthesis.

As the endodeoxyribonuclease Eco RI, the enzyme which was used later for phosphodiester-hydrolysis experiments, requires duplexes for regioselective phosphodiester hydrolysis of the oligonucleotide chain, melting profiles under appropriate reaction conditions were carried out. *Table 4* shows that with an increasing number of incorporated **2**, higher T_m values are observed. Similar results have been observed on oligonucleotides containing 8-aza-7-deaza-2′-deoxyadenosine [18].

According to the melting curves of **11**–**14**, a temperature of 20° was sufficient for enzymic cleavage as under this condition duplex formation was complete. The relative rates of hydrolysis of the oligomers **11**–**14** by the endodeoxyribonuclease Eco RI was determined by HPLC analysis. At identical oligomer concentration, the time course of released dimers was measured at certain intervals of time *(Fig. 2)*. *Fig. 1a* shows a typical HPLC pattern of the hydrolysis products of the oligomer **14**, after cleavage with the endodeoxyribonuclease Eco RI.

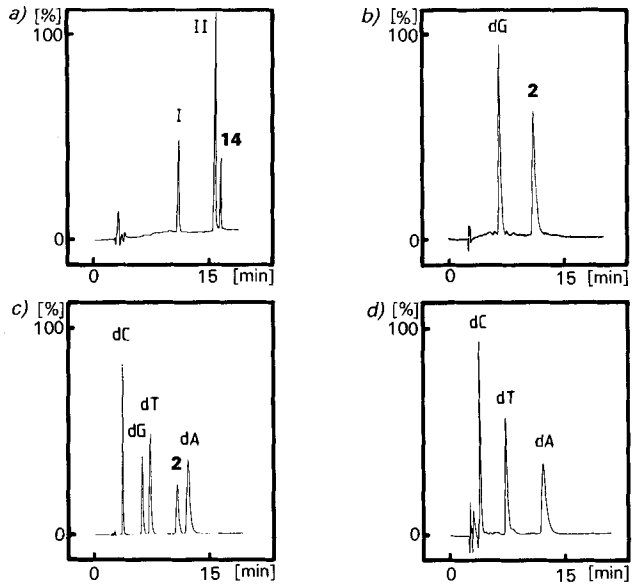


Fig. 1. *HPLC profile of the products* a) *of phosphodiester hydrolysis of* $d(z^8c^7G-G-A-A-T-T-C-C)$ (**14**) *by the endodeoxyribonuclease Eco RI* (conditions, see *Exper. Part;* solvent III) *and* b–d) *after tandem hydrolysis of* $d(z^8c^7G-G)$ (peak I of *a)*), **14**, *and* $d(pA-A-T-T-C-C)$ (peak II of *a)*), *respectively, with snake-venom phosphodiesterase followed by alkaline phosphatase* (solvent V). Absorbance [%] at 260 nm *vs.* retention time [min].

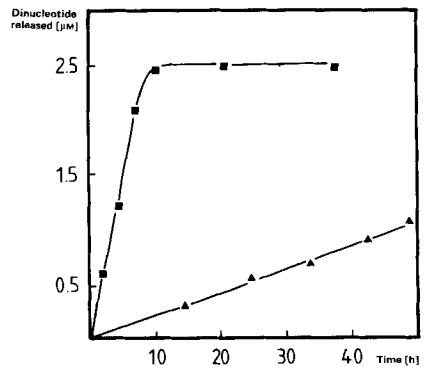


Fig. 2. *Time courses of phosphodiester hydrolysis of the oligomers* $d(G-G-A-A-T-T-C-C)$ (**11**; ▲) *and* $d(z^8c^7G-G-A-A-T-T-C-C)$ (**14**; ■) *by the endodeoxyribonuclease Eco RI.* Oligomer concentration was 2.5 μM. Samples (1 ml, H_2O) containing 10 mM *Tris*-HCl, pH 7.5, 20 mM $MgCl_2$, and 80 mM NaCl were incubated at 20° with the endodeoxyribonuclease Eco RI. Aliquots were analyzed by reverse phase HPLC (solvent III).

Table 4. *Melting Temperatures* (T_m Values) *of the Oligomers* **11**–**14** *Observed at 260 nm*[a])

Oligomer		T_m [°C]
d(G–G–A–A–T–T–C–C)	(**11**)	30
$d(z^8c^7G-z^8c^7G-A-A-T-T-C-C)$	(**12**)	37
$d(G-z^8c^7G-A-A-T-T-C-C)$	(**13**)	33
$d(z^8c^7G-G-A-A-T-T-C-C)$	(**14**)	33

[a]) T_m Values were determined in 10 mM *Tris*-HCl buffer containing 80 mM NaCl, 20 mM $MgCl_2$, and an oligomer concentration of 3 μM (single strands).

In order to confirm the peak contents, the cleavage products d(z^8c^7G–G) and d(pA–A–T–T–C–C) were isolated, and, like **14** itself, hydrolyzed by snake-venom phosphodiesterase and, subsequently, by alkaline phosphatase: the nucleoside mixtures obtained were analyzed by HPLC (see *Fig. 1b, 1c,* and *1d*). For quantification, the integrals of peak areas and the extinction coefficients of the nucleosides were used, thus confirming the expected ratio.

The results of endodeoxyribonuclease Eco RI cleavage of **11** and **14** are depicted in *Fig. 2*. Neither **12** nor **13** was cleaved within 48 h. The hydrolysis of the octamer **11** was *ca.* 42% at that time but was complete upon prolongated treatment. Surprisingly, a significant increase of the hydrolysis rate was observed when compound **2** replaced dG-1 outside of the recognition sequence (see *Fig. 2,* **14**). It needs further investigation to determine wether compound **2** alters the duplex structure allowing the enzyme to bind more efficiently or if the k_{off} rate of the hydrolysis product d(z^8c^7G–G) is increased compared to that of d(G–G).

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Experimental Part

General. See [19]. The protected phosphoramidites were synthesized according to the procedure of *Baron et al.* [4] and *Sinha et al.* [5]. CPG (70 μmol of immobilized protected 2′-deoxynucleoside/g of solide support) was purchased from *Biosyntech* (Hamburg, FRG). Snake-venom phosphodiesterase (EC 3.1.16.1, from *Crotallus durissus*), alkaline phosphatase (EC 3.1.3.1, from *E. coli*), and endodeoxyribonuclease Eco RI (EC 3.1.23.13, from *E. coli BS5*) were products of *Boehringer Mannheim GmbH* (FRG). Solvent systems: CH_2Cl_2/MeOH 9:1 (*A*), CH_2Cl_2/acetone 9:1 (*B*), CH_2Cl_2/acetone 4:1 (*C*), CH_2Cl_2/AcOEt/Et_3N 45:45:10 (*D*). HPLC: gradients containing 0.1M AcONHEt$_3$, pH 7.0/MeCN (95:5) (*A*), MeCN (*B*), H_2O (*C*), and MeOH/H_2O 3:2 (*D*) were used: I, 15 min (15–60% *B*) in *A*; II, 15 min (0–25% *B*) in *A*; III, 30 min (0–20% *B*) in *A*; IV, 15 min *C* – 10 min *D*; V, 100% *A*.

*1-(2′-Deoxy-β-*D*-*erythro*-pentofuranosyl)-6-[(2-methylpropionyl)amino]-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one* (**3a**). For 3 h, **2** (179 mg, 0.67 mmol) suspended in CH_3CN (3 ml) was stirred in the presence of hexamethyldisilazane (2 ml). The solvent was evaporated (40°/h.v.) and the residue dissolved in pyridine (3 ml), and isobutyric anhydride (0.4 ml) and 4-(dimethylamino)pyridine (8.8 mg, 0.07 mmol) were subsequently added. After stirring for 2 h at r.t., the mixture was hydrolyzed upon addition of MeOH (30 ml) within 48 h. After addition of H_2O (5 ml), the soln. was evaporated. The residue was dissolved in MeOH (4 ml) from which it crystallized within 12 h at 4°: 145 mg (64%) of colorless needles. M.p. 211°. TLC (silica gel, *A*): R_f 0.4. UV (MeOH): 263 (sh, 13900), 269 (14800). ^{1}H-NMR ((D$_6$)DMSO): 1.12 (*d*, *J* = 6.8, CH_3); 2.23, 2.74 (2*m*, CH, 2 H–C(2′)); 3.49 (*m*, CH_2(5′)); 3.78 (*m*, H–C(4′)); 4.42 (*m*, H–C(3′)); 4.73 (*t*, *J* = 5.5, OH–C(5′)); 5.27 (*d*, *J* = 4.5, OH–C(3′)); 6.40 (*t*′, *J* = 6.3, H–C(1′)); 8.08 (*s*, H–C(3)). Anal. calc. for $C_{14}H_{19}N_5O_5$: C 49.85, H 5.68, N 20.76; found: C 49.92, H 5.77, N 20.78.

*1-[2′-Deoxy-5′-*O*-(dimethoxytrityl)-β-*D*-erythro-pentofuranosyl]-6-[(2-methylpropionyl)amino]-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one* (**3b**). By coevaporation with anh. pyridine (5 ml), **3a** (500 mg, 1.5 mmol) was dried. The residue was dissolved in pyridine (15 ml) and stirred with 4,4′-dimethoxytrityl chloride (600 mg, 1.8 mmol) in the presence of (i-Pr)$_2$EtN (0.3 ml, 1.8 mmol) for 1.5 h at r.t. The soln. was poured in 5% aq. $NaHCO_3$ soln. (100 ml), extracted with CH_2Cl_2 (2 × 100 ml), the combined org. extract dried (Na_2SO_4), and the solvent evaporated. Silica-gel chromatography (column 5 × 15 cm, *C*) furnished a colorless foam (810 mg, 86%). TLC (silica gel, *C*): R_f 0.4. UV (CH_2Cl_2): 275 (14000). ^{1}H-NMR ((D$_6$)DMSO): 1.12 (*d*, *J* = 6.7, 2 CH_3); 2.30, 2.79 (2*m*, CH, 2 H–C(2′)); 3.00 (*m*, CH_2(5′)); 3.70, 3.71 (2*s*, 2 CH_3O); 3.93 (*m*, H–C(4′)); 4.51 (*m*, H–C(3′)); 5.31 (*d*, *J* = 4.5, OH–C(3′)); 6.42 (*dd*, *J* = 5.3, 4.5, H–C(1′)); 6.77, 7.20 (2*m*, 13 arom. H); 8.03 (*s*, H–C(3)). Anal. calc. for $C_{35}H_{37}N_5O_7$: C 65.71, H 5.83, N 10.94; found: C 65.52, H 5.96, N 10.74.

*1-(2′-Deoxy-β-*D*-*erythro*-pentofuranosyl)-6-[(dimethylamino)methylidenamino]-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one* (**4a**). To **2** (500 mg, 1.9 mmol) in anh. DMF (5 ml), *N,N*-dimethylformamide diethyl acetal (3 ml)

was added under stirring and the reaction continued at 50° for 2 h. The solvent was evaporated and the resultant oil repeatedly evaporated with toluene and acetone to yield a colorless foam. Crystallization from H_2O afforded colorless needles (510 mg, 85%). M.p. 227°. TLC (silica gel, *A*): R_f 0.6. UV (MeOH): 241 (20200), 300 (25200). ^{1}H-NMR ((D$_6$)DMSO): 2.19, 2.73 (2*m*, 2 H–C(2′)); 3.06, 3.19 (2*s*, 2 CH_3); 3.38, 3.50 (2*m*, CH_2(5′)); 3.80 (*m*, H–C(4′)); 4.41 (*m*, H–C(3′)); 4.75 (*t*, *J* = 5.8, OH–C(5′)); 5.26 (*d*, *J* = 4.1, OH–C(3′)); 6.48 (*t*′, *J* = 6.4, H–C(1′)); 7.91 (*s*, H–C(3)); 8.69 (*s*, CH); 11.29 (*s*, NH). Anal. calc. for $C_{13}H_{18}N_6O_4$: C 48.44, H 5.63, N 26.07; found: C 48.56, H 5.60, N 26.15.

*1-(2′-Deoxy-β-*D-erythro-*pentofuranosyl)-6-[(dimethylamino)methylidenamino]-4-methoxy-1*H-*pyrazolo[3,4-*d*]pyrimidine* (**5b**) was prepared as described for **4a**, except that **5a** (200 mg, 0.7 mmol) was used. Crystallization from MeOH afforded colorless needles (221 mg, 92%). M.p. 213°. TLC (silica gel, *A*): R_f 0.5. UV (MeOH): 247 (15400), 300 (28100). ^{1}H-NMR ((D$_6$)DMSO): 2.25, 2.77 (2*m*, 2 H–C(2′)); 3.05, 3.17 (2*s*, 2 CH_3); 3.39, 3.50 (2*m*, CH_2(5′)); 3.80 (*m*, H–C(4′)); 4.04 (1*s*, CH_3O); 4.42 (*m*, H–C(3′)); 4.77 (*t*, *J* = 5.7, OH–C(5′)); 5.26 (*d*, *J* = 4.4, OH–C(3′)); 6.56 (*t*′, *J* = 6.6, H–C(1′)); 8.03 (*s*, H–C(3)); 8.75 (*s*, CH). Anal. calc. for $C_{14}H_{20}N_6O_4$: C 49.99, H 5.99, N 24.99; found: C 49.81, H 5.94, N 25.00.

*1-[2′-Deoxy-5′-*O*-(dimethoxytrityl)-β-*D-erythro-*pentofuranosyl]-6-[(dimethylamino)methylidenamino]-1*H-*pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one* (**4b**). To a soln. of **4a** (500 mg, 1.6 mmol) in anh. pyridine (5 ml), 4,4′-dimethoxytrityl chloride (600 mg, 1.7 mmol) and (i-Pr)$_2$EtN (400 µl, 2.3 mmol) were added. The mixture was stirred for 3 h at r.t., poured into 5% $NaHCO_3$ soln. (50 ml), extracted with CH_2Cl_2 (2 × 50 ml), and the combined org. extract dried (Na_2SO_4) and evaporated. The oily residue was redissolved in CH_2Cl_2 and chromatographed on silica gel (column 20 × 2.5 cm, *B*). From the main zone, colorless amorphous **4b** (710 mg, 73%) was obtained upon evaporation. TLC (silica gel, *B*): R_f 0.2. UV (MeOH): 236 (35700), 300 (23500). ^{1}H-NMR ((D$_6$)DMSO): 2.25, 2.70 (2*m*, 2 H–C(2′)); 3.06, 3.18 (2*s*, CH_3, CH_2(5′)); 3.71, 3.72 (2*s*, 2 CH_3O); 3.92 (*m*, H–C(4′)); 4.52 (*m*, H–C(3′)); 5.31 (*d*, *J* = 2.8, OH–C(3′)); 6.51 (*m*, H–C(1′)); 6.78, 7.19, 7.33 (*m*, 13 arom. H); 7.84 (*s*, H–C(3)); 8.72 (*s*, 1 CH); 11.32 (*s*, NH). Anal. calc. for $C_{34}H_{36}N_6O_6$: C 65.37, H 5.81, N 13.45; found: C 65.09, H 5.84, N 13.26.

*1-[2′-Deoxy-5′-*O*-(dimethoxytrityl)-β-*D-erythro-*pentofuranosyl]-6-[(2-methylpropionyl)amino]-1*H-*pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one 3′-[Methyl* N,N-*Diisopropylphosphoramidite]* (**6a**). A soln. of **3b** (500 mg, 0.8 mmol) in anh. CH_2Cl_2 was stirred with bis(diisopropylamino)methoxyphosphine (262 mg, 1.0 mmol) and diisopropylammonium tetrazolide (171 mg, 1.0 mmol) for 1 h at r.t. under Ar. The soln. was diluted with CH_2Cl_2 (20 ml) and washed with 5% aq. $NaHCO_3$ soln. (20 ml) and the org. layer dried (Na_2SO_4) and evaporated. Chromatography on silica gel *60 H* (column 2 × 15 cm, *D*) furnished a main zone from which **6a** (556 mg, 89%) was isolated as colorless foam. TLC (silica gel, *D*): R_f 0.9. ^{31}P-NMR ($CDCl_3$): 147.14.

*1-[2′-Deoxy-5′-*O*-(dimethoxytrityl)-β-*D-erythro-*pentofuranosyl]-6-[(2-methylpropionyl)amino]-1*H-*pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one 3′-[(2-Cyanoethyl)* N,N-*Diisopropylphosphoramidite]* (**6b**). A soln. of **3b** (250 mg, 0.39 mmol) in dry THF was stirred with (i-Pr)$_2$EtN (200 µl, 1.2 mmol) at r.t. Chloro(2-cyanoethoxy)(diisopropylamino)phosphine (142 mg, 0.6 mmol) was added within 2 min by syringe under Ar. The salt was filtered off (Ar), the filtrate evaporated, its residue dissolved in AcOEt, and the soln. washed with sat. aq. $NaHCO_3$ soln., dried (Na_2SO_4), filtered, and chromatographed on silica gel (column: 10 × 2 cm, *C*). Coevaporation of the main zone with acetone afforded a colorless foam (268 mg, 82%). TLC (silica gel, *D*): R_f 0.9. ^{31}P-NMR ($CDCl_3$): 146.4.

Oligomers 7–**14**. The oligomers **7**–**14** were synthesized on an *Applied Biosystems* DNA synthesizer *(Model 680 B)* employing methyl phosphoramidite [4] (for **6a**) or (2-cyanoethyl) phosphoramidite [5] chemistry (for **6b**).

Table 5. *Purification of Oligonucleotides* **7**–**14** *by HPLC*

		Retention times [min]	Solvent
d([(MeO)$_2$Tr]G–G–A–A–T–T–C–C)	(**7**)	10.8	I
d([(MeO)$_2$Tr]G–z^{8}c^{7}G–A–A–T–T–C–C)	(**8**)	10.9	I
d([(MeO)$_2$Tr]z^{8}c^{7}G–z^{8}c^{7}G–A–A–T–T–C–C)	(**9**)	10.5	I
d([(MeO)$_2$Tr]z^{8}c^{7}G–G–A–A–T–T–C–C)	(**10**)	10.6	I
d(G–G–A–A–T–T–C–C)	(**11**)	13.0	II
d(z^{8}c^{7}G–z^{8}c^{7}G–A–A–T–T–C–C)	(**12**)	12.8	II
d(G–z^{8}c^{7}G–A–A–T–T–C–C)	(**13**)	13.1	II
d(z^{8}c^{7}G–G–A–A–T–T–C–C)	(**14**)	12.6	II

Synthesis was carried out on solid support (CPG) on a 1-μmol scale. The reaction cycle of detritylation, coupling, oxidation, and capping followed the user manual [17]. Cleavage of the Me group was accomplished by the action of thiophenol. Removal of the oligonucleotide from the solid support was carried out with 25% NH_3 soln. at r.t. on the stage of the 5'-dimethoxytritylated oligomers. This procedure also removed the cyanoethyl protecting group. Further incubation hydrolyzed the nucleobase protecting groups. The 5'-O-protected oligomers **7**–**10** were purified by HPLC (solvent I; see *Table 5*); detritylation was performed by the action of 80% AcOH/H_2O for 5 min. After evaporation of the acid, the oligomer was dissolved in H_2O (5 ml) and extracted with Et_2O. The oligomers **11**–**14** were then purified by reverse-phase HPLC with solvent II (see *Table 5*). Samples of the main zone were lyophilized and desalted by reverse-phase HPLC with solvent IV. After lyophilization, the oligomers (0.20–0.35 μmol; 20–35%) were dissolved in H_2O (100 μl) and stored frozen at −20°.

Enzymatic Hydrolysis of **11**–**14**. The oligomer (*ca.* 0.3 A_{260} units) in 0.1M *Tris*-HCl buffer of pH 8.5 (500 μl) was digested with snake-venom phosphodiesterase (2 μg) for 30 min at 37°. Further incubation with alkaline phosphatase (1 μg, 25°, 10 min) yielded a mixture of the nucleosides. After separation by HPLC (solvent V), quantification was made on the basis of the peak areas and the extinction coefficients of the nucleosides (dC 7300, dG 11400, dA 15400, dT 8800, **2** 11400).

Phosphodiester Hydrolysis of **11**–**14** *with the Endodeoxyribonuclease Eco RI*. The oligonucleotide (2.5 μM single strand concentration) in H_2O (1 ml) containing 10 mM *Tris*-HCl, 20 mM $MgCl_2$, and 80 mM NaCl at pH 7.5 was digested with the endodeoxyribonuclease Eco RI (100 units, each). The mixture was incubated at 20°. Aliquots were analyzed at different intervals of time by HPLC (solvent III).

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