173. 3-Deazaguanine N^7- and N^9-(2′-Deoxy-β-D-ribofuranosides): Building Blocks for Solid-Phase Synthesis and Incorporation into Oligodeoxyribonucleotides

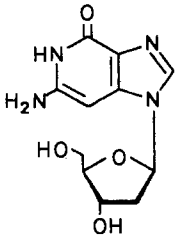
by **Frank Seela*** and **Sigrid Lampe**

Laboratorium für Organische und Bioorganische Chemie, Fachbereich Biologie/Chemie, Universität Osnabrück, Barbarastr. 7, D–4500 Osnabrück

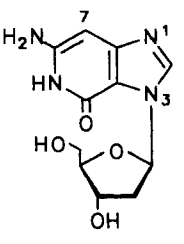
(24.IX.91)

Oligonucleotides containing 3-deaza-2′-deoxyguanosine (**1**) or its N^7-regioisomer **2** were prepared by solid-phase synthesis using P^{III} chemistry. Protection of **1** or **2** with *N*,*N*-dimethylformamide diethyl acetal followed by 4,4′-dimethoxytritylation afforded imidazo[4,5-*c*]pyridines **10b** and **11b**, respectively. The latter were converted into the 3′-phosphonates **10c** or **11c**, respectively; the cyanoethyl *N*,*N*-diisopropylphosphoramidite **10d** was also prepared. The oligonucleotide building blocks were employed in automated solid-phase synthesis. The self-complementary oligomers **13**, **15**, and **17** were prepared and characterized by enzymatic hydrolysis with snake-venom phosphodiesterase followed by alkaline phosphatase. There CD spectra exhibited the general structure of a B-DNA.

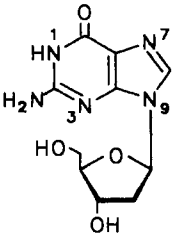
Introduction. – The 3-deazaguanine as well as its 2′-deoxy-β-D-ribofuranoside exhibit a broad spectrum of antiviral and antibacterial activity [1]. Hitherto unknown are building blocks for solid-phase synthesis as well as oligonucleotides containing 3-deaza-



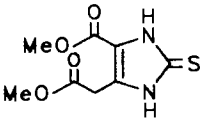
1



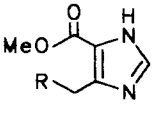
2 (systematic numbering)



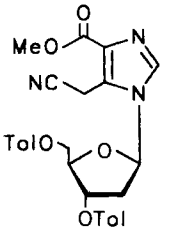
3 (purine numbering)



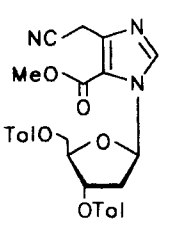
4



5a R = COOMe
b R = $CONH_2$
c R = CN



6



7

2′-deoxyguanosine (**1**) or its N^7-regioisomer **2**. The latter are valuable probes for the study of protein-nucleic acid interactions. The replacement of 2′-deoxyguanosine (G_d; **3**) by **1** within oligonucleotides results in a minor groove modification of the duplex. As N(3) can be considered as binding site for metals, proteins, or antibiotics [2], our laboratory [3] as well as others [4] [5] have focused their interest on the synthesis of such DNA fragments. In the following, we report on the synthesis of appropriately protected building blocks of 3-deaza-2′-deoxyguanosine (**1**) as well as of its N^7-regioisomer **2** and their use in automated solid-phase oligonucleotide synthesis.

Results and Discussion. – The 3-deaza-2′-deoxyguanosine (c^3G_d; **1**) and its N^7-regioisomer **2** ($c^3G'_d$; purine numbering is used throughout the *General Part*) were synthesized earlier [6] [7] employing 3,5-di-*O*-(4-toluoyl)-*β*-D-*erythro*-pentofuranosyl chloride [8] and the nucleobase anions of methyl 5(4)-(cyanomethyl)-1*H*-imidazole-4(5)-carboxylate (**5c**); compound **5c** was prepared *via* compounds **4**, **5a**, and **5b** [9] [10]. The ratio of glycosylation products **6** and **7** was reported to be 1:1 [7]. In our hands, a 3:5 ratio was formed. The N^7-glycosylated compounds can be obtained exclusively using the 2-chloro or 2-(methylthio) derivative of **5c** [11]. Regarding the glycosylation, we observed that an excess of inorganic base decreased the reaction yield. This could be due to C-anion formation at the exocyclic methylene group which can interfere with N-anion formation. The imidazole derivative **6** which was converted earlier into 3-deaza-2′-deoxyguanosine (**1**) by a two-step procedure was now directly transformed into **1** in 65% yield by treatment of **6** with NH_3/MeOH in a pressure bottle. Analogously, compound **7** was converted into the N^7-regioisomer **2**.

^{1}H-NOE Measurements [12] on compound **1** with saturation of H–C(1′) confirmed both N^9-glycosylation and *β*-D-configuration (data see *Exper. Part*), in agreement with the earlier assignment based on UV data [7] [13]. Compounds **1** and **2** were also characterized by ^{13}C-NMR spectra (*Table 1*). The pronounced chemical-shift differences of the bridgehead C(3a) (**2**: 10 ppm upfield compared to **1**) and C(7a) (**2**: 10 ppm downfield compared to **1**) of **1** and **2** is in agreement with the assignment of the regioisomers, but the differences do not follow the trend observed for methylated 3-deazapurines [14]. Nucleosides **1** and **2** exhibit significantly different stabilities in 1N HCl (*Table 2*), the

Table 1. *^{13}C-NMR Chemical Shifts* ($(D_6)Me_2SO$; in ppm) *of 2′-Deoxyribofuranosyl Derivatives of Imidazo[4,5-c]pyridines*[a]). Systematic numbering.

	C(2)	C(3a)	C(4)	C(6)	C(7)	C(7a)
1	136.7	123.0	156.7	147.8	70.7	142.4
2	142.5	112.9	153.7[b])	146.6[b])	77.9	152.9
8a	138.5	127.7[b])	155.7	n.d.[b])	80.6	139.9
b	138.9[b])	127.7	155.7	139.6[b])	80.4	138.3
c	138.3	126.6	155.7	140.1	80.5	138.5
d	139.8	127.5	155.8	138.5[b])	80.1	138.8
e	138.9[b])	127.6	156.2	139.6[b])	80.1	138.9
9	138.0[b])	128.7	155.6	140.0[b])	80.2	138.8
10a	137.7	127.0	157.4	151.0	77.1	141.0
b	137.4	127.2	157.5	151.1	77.4	141.3
c	137.2	127.1	157.3	151.1	77.1	141.1
11a	142.3	116.3	154.8	151.3	84.2	150.1
c	141.7	116.4	154.7	151.2	85.6	150.2

Table 1 (cont.)

	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)
1	84.1	39.4	70.6	87.5	61.7
2	85.0	41.5	70.4	87.7	61.6
8a	84.6	39.2	73.8	81.7	63.4
b	84.6	38.9	70.5	87.8	61.6
c	84.3	38.4	70.1	85.8	63.6
d	84.9	39.2	73.9	81.5	63.4
e	84.6	38.8	70.6	87.8	61.6
9	84.7	39.2	72.2	85.0[f])	63.4
10a	84.4	38.9	70.6	87.7	61.6
b	84.1	38.6	70.4	85.8	64.1
c	84.3	39.4	72.3[e])	84.8[d])	63.7
11a	84.9	39.3	72.2	87.6	61.7
c	84.1	39.4	72.4[e])	84.7[f])	63.8

	CH=N, C=O(ib), C=O(ac)	CH(ib)	Me(ib), Me(ac), MeN	$(MeO)_2Tr$	$(CH_3CH_2)_3NH^+$, $(CH_3CH_2)_3NH^+$
8a	176.8, 175.9, 175.8	33.1, 33.0, 35.3	19.1, 18.8, 18.7		
b	176.9	35.3	19.2		
c	176.8	35.4	19.1	55.0	
d	170.1		23.9, 20.8, 20.4		
e	170.3		24.0		
9	177.1	35.3	19.2	55.0	45.5, 8.6
10a	155.1		34.2		
b	155.0		34.2	55.1	
c	154.9		34.0	54.9	45.3, 8.6
11a	154.5[b])		34.8		
c	154.5[b])		34.0	58.4	45.5, 8.4

[a]) Measured at 23°. [b]) Tentative. [c]) $^2J(P,C(3')) = 4.3$ Hz. [d]) $^3J(P,C(4')) = 4.5$ Hz. [e]) $^2J(P,C(3')) = 4.9$ Hz. [f]) According to P coupling.

Table 2. *Half-Life Values* ($\tau/2$) *of Deprotection or N-Glycosylic Bond Hydrolyses of 3-Deazaguanine and Guanine 2′-Deoxyribofuranosides*

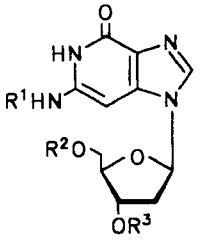
	$\tau/2$ [min]							
	1	**2**	**8b**	**8e**	**10a**	**11a**	ib^2G_d [21]	$m_2fa^2G_d$ [21][b])
1N HCl[a])	73	39	–	285	440	–	–	–
25% aq. NH_3 soln.[a])	–	–	> 500	27	28	12	112	19

[a]) Measured at 40° at the wavelength of maximal difference in UV absorbance. [b]) fa = Me_2NCH=.

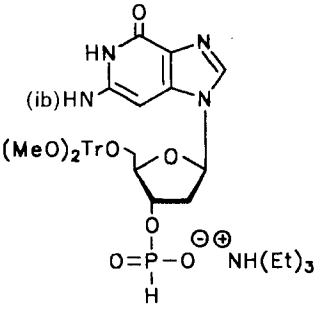
N^7-isomer **2** being hydrolysed two times faster than the N^9-compound **1**. This can be traced back to a destabilisation of the N-glycosylic bond due to a steric repulsion between the C(6)=O group of the nucleobase and the sugar moiety. Similar results were reported for the regioisomeric 3-deazaadenine 2′-deoxy-β-D-ribofuranosides [15] as well as for purine nucleosides [16] [17].

Next, protection of the exocyclic NH_2 group was performed. In the case of **1**, three different protecting groups, isobutyryl (ib), acetyl (ac), and formamidine (Me_2NCH=),

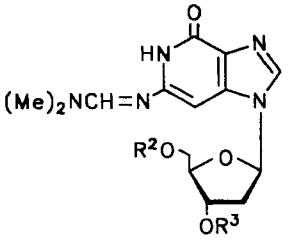
were studied. Peracylation of **1** with the corresponding acyl anhydrides afforded compounds **8a** and **8d**, respectively. *O*-Acyl groups were selectively removed by either 2N NaOH or 25% NH_3/H_2O yielding the monoacylated compounds **8b** and **8e**, respectively. As shown later, alkaline hydrolysis of the exocyclic amino-protecting groups was more difficult to achieve in the case of 3-deazaguanine nucleosides compared to the parent purine nucleosides. This enhanced stability increased the selectivity for the removal of the sugar- *vs.* the base-protecting groups. As a consequence, weak bases like ammonia can be used for selective sugar deacylation, a reagent which cannot be applied in the case of derivatives of dG (**3**). Additionally, compound **1** was reacted with *N*,*N*-dimethylformamide diethyl acetal [18] to yield the (dimethylamino)methylidene derivative **10a**; the N^7-isomer **2** was protected similarly (→**11a**).



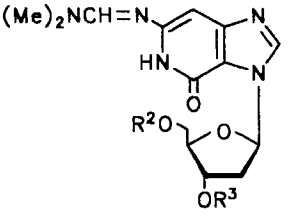
8a $R^1 = R^2 = R^3 = ib = Me_2CHCO$
b $R^1 = ib$, $R^2 = R^3 = H$
c $R^1 = ib$, $R^2 = (MeO)_2Tr$, $R^3 = H$
d $R^1 = R^2 = R^3 = ac$
e $R^1 = ac$, $R^2 = R^3 = H$



9



10a $R^2 = R^3 = H$
b $R^2 = (MeO)_2Tr$, $R^3 = H$
c $R^2 = (MeO)_2Tr$, $R^3 = PH(O)O^-(Et)_3NH^+$
d $R^2 = (MeO)_2Tr$, $R^3 = P[(i\text{-}Pr)_2N](OCH_2CH_2CN)$



11a $R^2 = R^3 = H$
b $R^2 = (MeO)_2Tr$, $R^3 = H$
c $R^2 = (MeO)_2Tr$, $R^3 = PH(O)O^-(Et)_3NH^+$

Acylation of the sugar moiety of **1** or **2** results in a 2–3-ppm downfield shift of C(3′) and C(5′) and a 6-ppm upfield shift of C(4′) (see **8a** and **8d** *Table 1*). The *N*-acyl compounds **8b** and **8e** show similar chemical shifts for the sugar moiety as the parent **1**. Phosphonate formation, as in the case of **10c** or **11c**, affects the C(3′), C(4′), and C(5′) signals.

Compounds **8b**, **8e**, and **10a** were hydrolysed with 25% NH_3/H_2O at 40° and the corresponding half-life values determined. The hydrolyses were followed by UV at the appropriate wavelength of maximal absorbance difference between educt and product. Both the acetyl and the (dimethylamino)methylidene residue could be cleaved off at rates

suitable for oligonucleotide synthesis (see *Table 2*: $\tau/2 = 27$ min for **8e** and 28 min for **10a**), while complete hydrolysis of **8b** (isobutyryl residue) needed more than 3 days (TLC monitoring). Hydrolysis of **11a** with 25% NH_3/H_2O (40°) proceeded at a significantly faster rate ($\tau/2 = 12$ min) compared to the N^9-isomer **10a** ($\tau/2 = 28$ min). It is known that a (dimethylamino)methylidene residue stabilizes the N-glycosylic bond [19]. Indeed, the hydrolysis of compounds **8e** and **10a** in 1N HCl at 40° gave $\tau/2$ values of 285 and 440 min, respectively.

Next, compounds **8b** and **10a** were converted into the 4,4′-dimethoxytrityl ($(MeO)_2Tr$) derivatives **8c** and **10b**, respectively; protection of OH–C(5′) was proved by a downfield shift ($\Delta\delta(C) = 2$–3 ppm) of the C(5′) signals compared to those of the starting compounds (*Table 1*). Reaction of **8c** and **10b** with PCl_3/*N*-methylmorpholine/1,2,4-triazole in CH_2Cl_2 [20] afforded the corresponding 3′-phosphonates as triethylammonium salts **9** and **10c**, respectively. They were characterized by ^1H-, ^{13}C-, and ^{31}P-NMR spectra. Moreover, the N^7-regioisomer **11a** was converted into its 3′-phosphonate **11c** *via* the 5′-dimethoxytritylated precursor **11b**. As an alternative to phosphonate **10c**, phosphoramidite **10d** (mixture of diastereoisomers) was prepared, from **10b** and chloro[(2-cyanoethyl)diisopropylamino]phosphane [21].

To prove the utility of 3′-phosphonates **10c** and **11c** in automated solid-phase oligonucleotides synthesis, they were employed together with the 3′-phosphonates of regular 2′-deoxynucleosides. Thus, the oligonucleotides **13**, **15**, and **17** were synthesized and compared with the similarly prepared parent compounds **12**, **14**, and **16**. Oligonucleotide **13** represents a structure containing the sequence of an Eco RI recognition site, and **15** and **17** are also self-complementary structures containing only dG and dC. Automated oligonucleotide synthesis was performed on solid support employing phosphonate chemistry [20]. The protocol of detritylation, activation, coupling, and capping followed the user bulletin [22]. Oxidation with I_2 in pyridine/H_2O/THF was carried out on the oligomeric level. The yield of each coupling step (data not shown) was measured quantitatively (95–98%) by monitoring the liberation of the $(MeO)_2Tr$ cation spectrophotometrically (λ_{max} 498 nm ($\varepsilon = 70000$)) according to [22]. The $(MeO)_2Tr$-protected oligonucleotides were removed from the support with ammonia and then purified by reversed-phase *RP-18* HPLC. Detritylation (80% AcOH/H_2O) was followed by neutralization with Et_3N, and the products were again submitted to *RP-18* HPLC, desalted, and lyophilized.

The incorporation of c^3G_d (**1**) into oligonucleotide **13** was proved by enzymatic tandem hydrolysis of **13** using snake-venom phosphodiesterase followed by alkaline

d(G-T-A-G-A-A-T-T-C-T-A-C)
12

d(G-T-A-c^3G-A-A-T-T-C-T-A-C)
13

d(C-G-C-G-C-G)
14

d(C-G-C-c^3G-C-G)
15

d(G-C-G-C-G-C)
16

d(G-C-c^3G′-C-G-C)
17

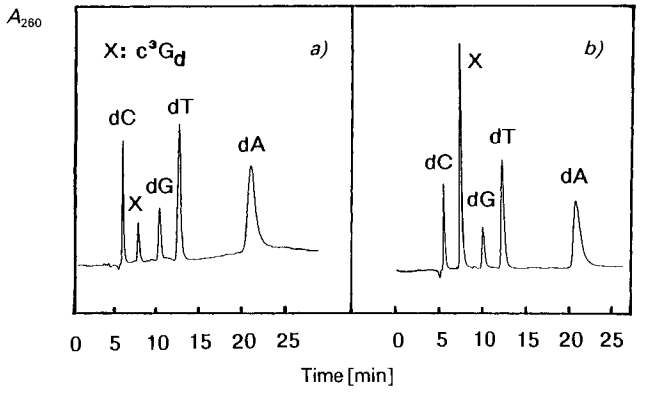


Fig. 1. a) *HPLC profile* (system *III*) *obtained from hydrolysis of d(G–T–A–*c^3*G–A–A–T–T–C–T–A–C)* (**13**; 3 μM of single strand) *with snake-venom phosphodiesterase and alkaline phosphatase, after a total incubation time of 75 min* (37°). b) *Same experiment, but with addition of synthetic* **1**. Conditions, see *Exper. Part.* t_R (**1**) 8 min.

phosphatase (*Fig. 1*). The identity of compound **1** was proved by addition of synthetic **1**. Same protocols were carried out in the case of **15** and **17** (*Figs. 2* and *3*). In all cases, integration of the peaks of HPLC analyses demonstrated the correct gross composition of the oligonucleotides.

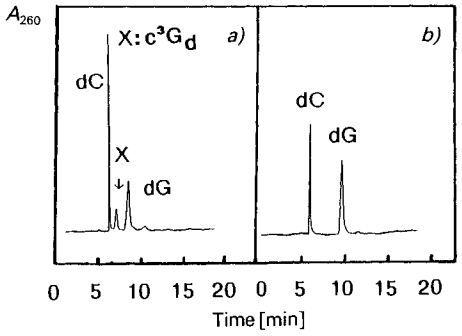


Fig. 2. *HPLC profiles* (system *III*) *of the enzymatic digest of* a) **14** *and* b) **15**. Conditions, see *Fig. 1*, except that the flow rate was 1.0 ml/min in case of **15**.

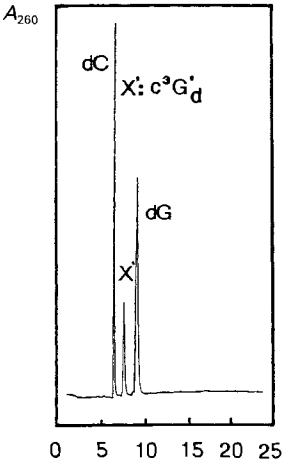


Fig. 3. *HPLC profile* (system *III*) *of the enzymatic digest of* **17**. Flow rate 1.0 ml/min.

Fig. 4 displays the CD spectra of **13** as well as that of the parent oligomer **12**. Both exhibit the characteristics of a general B-DNA structure, but in case of **13**, a distinct shoulder at 260 nm of the B_{1u} transition points to a slightly different fine structure.

Fig. 5 shows the time course of phosphodiester hydrolysis of **13** by either snake-venom or calf-spleen phosphodiesterase. As can be seen, the oligonucleotide 3′-nucleotidohydrolase (calf spleen) hydrolyses the oligomer **13** to an extent of only one half (hypochromicity = $h = 9\%$) of that of the oligonucleotide 5′-nucleotidohydrolase (snake-venom;

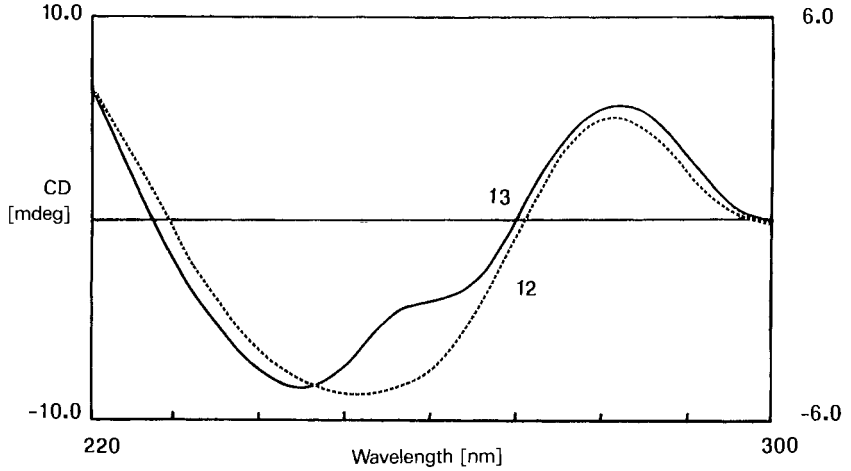


Fig. 4. *CD-Spectra of the oligomers* **12** (right-hand scale) *and* **13** (left-hand scale). 3.1 μM of single strand in 60 mM Na-cacodylate buffer, pH 7.0, 1M NaCl, 100 mM $MgCl_2$.

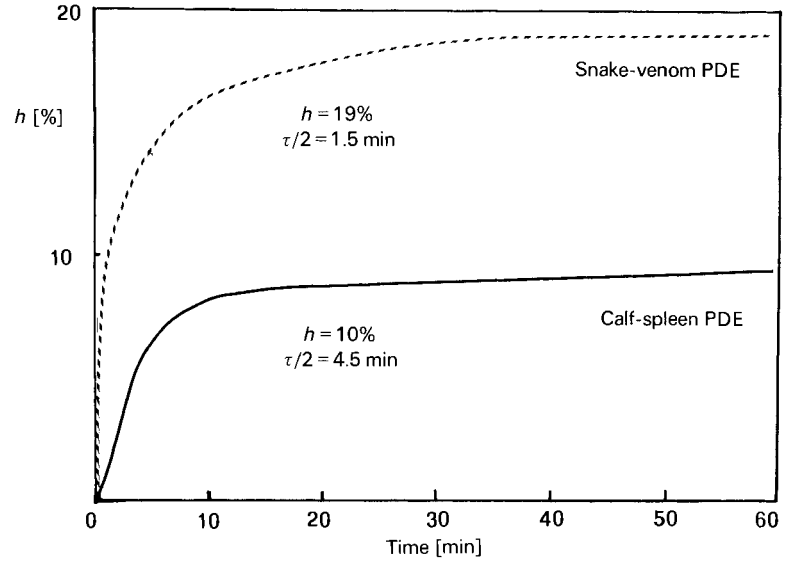


Fig. 5. *Time course of phosphodiester hydrolysis of* **13** *by either snake-venom phosphodiesterase or calf-spleen phosphodiesterase at r.t.* Conditions, see *Exper. Part*; h = hypochromicity.

h = 19%). This low cleavage hypochromicity implies that calf-spleen phosphodiesterase is only able to catalyse the liberation of a few nucleoside 3′-monophosphates (starting at the 3′-terminus) from the oligomer. Moreover, the half-life values of enzymatic hydrolyses are significantly higher compared to the hydrolysis of the unmodified parent oligomer **12** ($\tau/2$ (snake-venom PDE) 0.4 min; $\tau/2$ (calf-spleen PDE) 2.7 min [23]). These results demonstrate a certain protection of an oligonucleotide against the action of nonspecific exonucleases even by a single point modification.

The successful incorporation of **1** and of its regioisomer **2** into oligonucleotides opens a wide range of experiments to elucidate changes in secondary and tertiary structure caused by the lipophilisation (CH *vs.* N) within the DNA double helix as well with respect to the interaction with DNA-processing enzymes or other proteins.

We thank Dr. *H. Rosemeyer* for helpful discussion. Financial support by the *Deutsche Forschungsgemeinschaft* and the *Fonds der Chemischen Industrie* is gratefully acknowledged.

Experimental Part

General: See [24]. FC = flash chromatography. The phosphonates of regular 2′-deoxyribunocleosides were purchased from *Sigma*, St. Louis, and the *Fractosil*-linked 2′-deoxyribonucleosides from *Milligene*, Eschborn, Germany. Snake-venom phosphodiesterase (EC 3.1.15.1, *Crotallus durissus*), calf-spleen phosphodiesterase (EC 3.1.16.1), and alkaline phosphatase (EC 3.1.3.1, *E. coli*) are products of *Boehringer*, Mannheim, Germany. Oligonucleotide synthesis was carried out on an automated DNA synthesizer, model *380 B*, *Applied Biosystems*, Weiterstadt, Germany. CD Spectra: *Jasco 600* spectropolarimeter.

*Methyl 2,3-Dihydro-5-[(methoxycarbonyl)methyl]-2-thioxo-1*H*-imidazole-4-carboxylate* (**4**) was prepared from dimethyl 3-oxopentanedioate (128.0 g, 546 mmol) according to [10], except the workup procedure. The reaction mixture was extracted with AcOEt (10 times, 5 l, total). After drying (Na_2SO_4), the soln. was evaporated and the product (15.0 g, 11.9%) used without further purification. Recrystallisation from H_2O gave yellowish needles. TLC (CH_2Cl_2/acetone 97:3): R_f 0.4. M.p. 220° ([10]: m.p. 220–222°). ^{1}H-NMR ((D_6)DMSO): 12.7 (br. *s*, 2 NH); 3.84 (*s*, CH_2); 3.75, 3.66 (2*s*, 2 Me). ^{13}C-NMR ((D_6)DMSO): 168.7, 162.5, 158.5 (2 *C*OOCH$_3$, C(2)); 130.7, 117.0 (C(4), C(5)); 52.3 (CH_3O); 51.7 (CH_3O); 30.1 (CH_2–C(5)).

*Methyl 4-[(Methoxycarbonyl)methyl]-1*H*-imidazole-5-carboxylate* (**5a**) was prepared from **4** according to [10]. Recrystallisation from H_2O gave yellowish crystals. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.4. M.p. 172° ([10]: m.p. 163–178°). ^{1}H-NMR ((D_6)DMSO): 12.87 (br. *s*, NH); 7.75 (*s*, H–C(2)); 3.94 (*s*, CH_2); 3.76 (*s*, Me); 3.63 (*s*, Me).

*Methyl 4-(Carbamoylmethyl)-1*H*-imidazole-5-carboxylate* (**5b**) was prepared from **5a** (3.0 g, 15.1 mmol) according to [10]: 2.0 g (72%). Recrystallisation from H_2O gave colourless needles. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.14. M.p. 230° ([6]: 242–244°). ^{1}H-NMR ((D_6)DMSO): 12.65 (br. *s*, NH); 7.69 (*s*, H–C(2)); 7.22 (br. *d*, NH_2); 3.75 (*s*, CH_2, CH_3).

*6-Amino-1-(2′-deoxy-β-*D-erythro-*pentofuranosyl)-1*H*-imidazo[4,5-c]pyridin-4(5*H*)-one* (**1**). A soln. of **6** (1.4 g, 2.7 mmol) in NH_3/MeOH (saturated at 0°) was heated to 50° for 72 h in a pressure bottle. After evaporation, the residue was submitted to FC (silica gel *60H*, column 4 × 20 cm, AcOEt/H_2O/i-PrOH 4:2:1, upper layer, 0.5 bar): 470 mg (65%) of **1** [7]. TLC (AcOEt/H_2O/i-PrOH 4:2:1, upper layer): R_f 0.15. ^{1}H-NMR: irr. of H–C(1′)→NOE for H–C(2) (4.2%), H–C(7) (5.9%), H–C(3′) (1.3%), H–C(4′) (2.9%), H_α–C(2′) (6.4%).

*1-[2′-Deoxy-3′,5′-bis-*O*-(2-methylpropionyl)-β-*D-erythro-*pentofuranosyl]-6-[(2-methylpropionyl)amino]-1*H*-imidazo[4,5-c]pyridin-4(5*H*)-one* (**8a**). Compound **1** (100 mg, 0.38 mmol) was dried by repeated coevaporation from anh. pyridine. After addition of pyridine/isobutyric anhydride 1:1 (8 ml), the mixture was refluxed for 3 h and then evaporated. The residue was submitted to FC (silica gel *60H*, column 20 × 4 cm, 0.5 bar, CH_2Cl_2/MeOH 9:1): **8a** (130 mg, 73%). Yellowish foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.5. UV (MeOH): 270 (13100), 305 (12600). ^{1}H-NMR ((D_6)DMSO): 11.29 (br. *s*, NH); 10.30 (br. *s*, NH); 8.16 (*s*, H–C(2′)); 6.60 (*s*, H–C(7)); 6.18 ('*t*', *J* = 5.2, H–C(1′)); 5.33 (*m*, H–C(3′)); 4.26–4.15 (*m*, H–C(4′), CH_2(5′)); 2.83, 2.60 (2*m*, CH_2(2′)); 1.17, 1.14 (2*s*, *J* = 2.7, Me_2CHCO); 1.07 ('*t*', *J* = 5.0, Me_2CHCO). Anal. calc. for $C_{23}H_{32}N_4O_7$ (476.5): C 57.97, H 6.77, N 11.76; found: C 58.17, H 6.90, N 11.56.

*1-(2′-Deoxy-β-*D-erythro-*pentofuranosyl)-6-[(2-methylpropionyl)amino]-1*H*-imidazo[4,5-c]pyridin-4(5*H*)-one* (**8b**). To a soln. of **8a** (200 mg, 0.42 mmol) in MeOH at 0°, cold 2M NaOH was added until pH 12.5 was reached. After 20 min, the reaction was quenched by addition of ion-exchange resin (*Dowex WX-8*, pyridinium form). The neutral soln. was filtered and the resin washed with MeOH. The combined filtrates were evaporated: **8b** (102 mg, 72%). Crystallisation from H_2O gave colourless needles. TLC (CH_2Cl_2/MeOH 95:5): R_f 0.3. M.p. 235°. UV (MeOH): 271 (15400), 304 (14600). ^{1}H-NMR ((D_6)DMSO): 11.24 (br. *s*, NH); 10.30 (br. *s*, NH); 8.16 (*s*, H–C(2)); 6.55 (*s*, H–C(7)); 6.10 ('*t*', *J* = 6.6, H–C(1′)); 5.36 (*d*, *J* = 4.2, OH–C(3′)); 4.93 ('*t*', *J* = 5.4, OH–C(5′)); 4.35 (*m*, H–C(3′)); 3.84 (*m*, H–C(4′)); 3.52 (*m*, CH_2(5′)); 2.5 (*m*, H_β–C(2′)); 2.32 (*m*, H_α–C(2′)); 1.14, 1.15 (*d*, *J* = 6.7, 2 Me). Anal. calc. for $C_{15}H_{20}N_4O_5$ (336.3): C 53.57, H 5.99, N 16.66; found: C 53.61, H 6.04, N 16.63.

*1-[2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-β-*D*-erythro-pentofuranosyl]-6-[(2-methylpropionyl)amino]-1H-imidazo[4,5-c]pyridin-4(5H)-one* (**8c**). Compound **8b** (100 mg, 0.3 mmol) was dried by coevaporation with anh. pyridine and then dissolved in pyridine (3.0 ml). After addition of 4-(dimethylamino)pyridine (16 mg, 0.13 mmol) and 4,4'-dimethoxytrityl chloride (150 mg, 0.4 mmol), the mixture was stirred for 3 h at r.t. Then, the soln. was poured into 5% aq. $NaHCO_3$ soln. (15 ml) and extracted twice with CH_2Cl_2 (15 ml). The combined org. layers were dried (Na_2SO_4), filtered, and evaporated. FC (silica gel *60H*, column 20 × 2.5 cm, 0.5 bar, CH_2Cl_2/MeOH 9:1) afforded **8c** (91 mg, 47.5%). Colourless foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.35. UV (MeOH): 273 (14800), 304 (16600). ^{1}H-NMR ((D$_6$)DMSO): 11.30 (br. *s*, NH); 10.47 (br. *s*, NH); 8.12 (*s*, H–C(2)); 7.23–7.11 (arom. H); 6.79–6.69 (arom. H, H–C(7)); 6.18 ('*t*', *J* = 5.6, H–C(1')); 5.45 (*d*, *J* = 4.8, OH–C(3')); 4.36 (*m*, H–C(3')); 4.08 (*m*, H–C(4')); 3.70 (*s*, 2 MeO); 3.06 (*m*, CH_2(5')); 2.71 (*m*, H_β–C(2')); 2.5 (*m*, H_α–C(2')); 1.14–1.10 (*m*, Me_2CHCO). Anal. calc. for $C_{36}H_{38}N_4O_7$ (638.7): C 67.69, H 6.00, N 8.77; found: C 67.64, H 6.13, N 8.77.

*1-[2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-β-*D*-erythro-pentofuranosyl]-6-[(2-methylpropionyl)amino]-1H-imidazo[4,5-c]pyridin-4(5H)-one 3'-(Triethylammonium Phosphonate)* (**9**). To a soln. of PCl_3 (75 µl, 0.08 mmol) and *N*-methylmorpholine (1.0 ml, 0.83 mmol) in CH_2Cl_2 (10 ml) was added 1,2,4-triazole (0.2 g, 2.8 mmol). After stirring for 30 min, the soln. was cooled to 0°, and **8c** (80 mg, 0.13 mmol) which had been dried by evaporation from anh. MeCN and dissolved in CH_2Cl_2 (4 ml) was added slowly. After stirring for 10 min at r.t., the mixture was poured into 1 M aq. (Et_3NH)HCO_3 (*TBK*; pH 8.0; 30 ml [25]), shaken, and separated. The aq. layer was extracted with CH_2Cl_2 (30 ml), the combined org. extract dried (Na_2SO_4) and evaporated, and the colourless foam submitted to FC (silica gel *60H* column 4 × 15 cm, 0.5 bar, CH_2Cl_2/MeOH/Et_2N 88:10:2). The residue of the main zone was dissolved in CH_2Cl_2 (10 ml) and extracted with 1M aq. (Et_3NH)HCO_3 (20 ml; pH 8.0). The org. layer was dried (Na_2SO_4) and evaporated: **9** (40 mg, 39.7%). Colourless foam. TLC (CH_2Cl_2/MeOH/Et_3N 88:10:2): R_f 0.3. UV (MeOH): 271 (17400), 305 (14500). ^{1}H-NMR ((D$_6$)DMSO): 11.4 (br. *s*, NH); 10.85 (*s*, NH); 8.02 (*s*, H–C(2)); 6.63 (*d*, ^1J(H,P) = 587, PH); 7.23–7.10, 6.67–6.67 (2*m*, arom. H, H–C(7)); 6.16 (*m*, H–C(1')); 4.74 (*m*, H–C(3')); 4.15 (*m*, H–C(4')); 3.69 (*s*, 2 MeO); 2.99 (*m*, CH_2(5'), CH_3CH_2); 2.5 (*m*, CH_2(2')); 1.14 (*m*, Me_2CHCO, CH_3CH_2). ^{31}P-NMR ((D$_6$)DMSO): 1.25 (^1J(P,H) = 587, ^3J(P,*H*–C(3')) = 8.3).

*6-(Acetylamino)-1-(3',5'-di-O-acetyl-2'-deoxy-β-*D*-erythro-pentofuranosyl)-1H-imidazo[4,5-c]pyridin-4(5H)-one* (**8d**). Compound **1** (100 mg, 0.38 mmol) was dried by repeated coevaporation with anh. pyridine and dissolved in pyridine (1.5 ml). After addition of Ac_2O (3 ml), the mixture was stirred at r.t. overnight and then evaporated. The residue was submitted to FC (silica gel *60H*, column 20 × 4 cm, 0.5 bar, CH_2Cl_2/MeOH 95:5): **8d** (116 mg, 79%). Colourless foam. TLC (CH_2Cl_2/MeOH 95:5): R_f 0.34. UV (MeOH): 269 (15800), 303 (14700). ^{1}H-NMR ((D$_6$)DMSO): 11.47 (br. *s*, NH); 10.96 (br. *s*, NH); 8.18 (*s*, H–C(2)); 6.61 (*s*, H–C(7)); 6.17 ('*t*', *J* = 6.3, H–C(1')); 5.28 (*m*, H–C(3')); 4.26–4.13 (2*m*, H–C(4'), H–C(5')); 2.86 (*m*, H_β–C(2')); 2.58 (*m*, H_α–C(2')); 2.10, 1.91 (2*s*, 3 Me). Anal. calc. for $C_{17}H_{20}N_4O_7$ (392.4): C 52.04, H 5.14, N 14.28; found: C 52.38, H 5.22, N 14.14.

*6-(Acetylamino)-1-(2'-deoxy-β-*D*-erythro-pentofuranosyl)-1H-imidazo[4,5-c]pyridin-4(5H)-one* (**8e**). A soln. of **8d** (160 mg, 0.41 mmol) in 25% NH_3/H_2O (10 ml) was stirred for 30 min at r.t. After evaporation, the residue was submitted to FC (silica gel *60H*, column 15 × 4 cm, 0.5 bar, CH_2Cl_2/MeOH 7:3) and the product recrystallised from MeOH: **8e** (79 mg, 63%). Colourless needles. TLC (CH_2Cl_2/MeOH 7:3): R_f 0.53. M.p. > 250°. UV (MeOH): 269 (16800), 303 (15200). ^{1}H-NMR ((D$_6$)DMSO): 11.47 (br. *s*, NH); 10.85 (br. *s*, NH); 8.17 (*s*, H–C(2)); 6.54 (*s*, H–C(7)); 6.12 ('*t*', *J* = 6.6, H–C(1')); 5.40 (*m*, OH–C(3')); 4.96 (*m*, OH–C(5')); 4.35 (*m*, H–C(3')); 3.85 (*m*, H–C(4')); 3.53 (*m*, CH_2(5')); 2.5 (*m*, H_β–C(2')); 2.32 (*m*, H_α–C(2')); 2.12 (*s*, Me). Anal. calc. for $C_{13}H_{16}N_4O_5$ (308.3): C 50.65, H 5.23, N 18.17; found: C 50.73, H 5.40, N 18.02.

*1-(2'-Deoxy-β-*D*-erythro-pentofuranosyl)-6-{[(dimethylamino)methylidene]amino}-1H-imidazo[4,5-c]pyridin-4(5H)-one* (**10a**). To a soln. of **1** (200 mg, 0.75 mmol) in anh. amine-free DMF (5 ml), *N*,*N*-dimethylformamide diethyl acetal (3 ml) was added. After stirring for 24 h at r.t., the soln. was evaporated and the residue repeatedly coevaporated from toluene. FC (silica gel *60H*, column 20 × 4 cm, 0.5 bar, CH_2Cl_2/MeOH 8:2) gave **10a** (196 mg, 81%). Colourless foam. TLC (CH_2Cl_2/MeOH 8:2): R_f 0.2. UV (MeOH): 316 (18700). ^{1}H-NMR ((D$_6$)DMSO): 10.64 (br. *s*, NH); 8.06, 8.02 (2*s*, H–C(2), H–C=N); 6.10 (*m*, *s*, H–C(1'), H–C(7)); 5.36 (*d*, *J* = 3.6, OH–C(3')); 5.03 ('*t*', *J* = 5, OH–C(5')); 4.37 (*m*, H–C(3')); 3.85 (*m*, H–C(4')); 3.55 (*m*, CH_2(5')); 3.06, 2.95 (2*s*, 2 Me); 2.26 (*m*, H_α–C(2')). Anal. calc. for $C_{14}H_{19}N_5O_4$ (321.3): C 52.33, H 5.96, N 21.79; found: C 52.59, H 6.05, N 21.49.

*1-[2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-β-*D*-erythro-pentofuranosyl]-6-{[(dimethylamino)methylidene]-amino}-1H-imidazo[4,5-c]pyridin-4(5H)-one* (**10b**). Compound **10a** (100 mg, 0.31 mmol) was dried by repeated coevaporation from anh. pyridine and then dissolved in anh. pyridine (4 ml). At r.t. 4-(dimethylamino)pyridine (16 mg, 0.13 mmol) and 4,4'-dimethoxytrityl chloride (168 mg, 0.49 mmol) were added and stirred for 3 h. Then the soln. was poured into 5% aq. $NaHCO_3$ soln. (40 ml) and extracted twice with CH_2Cl_2 (30 ml). The combined org. layers were dried (Na_2SO_4), filtered, and evaporated. The residue was submitted to FC (silica gel *60H*, column 15 × 4 cm, 0.5 bar, CH_2Cl_2/MeOH 9:1): **10b** (137 mg, 70.8%). Colourless foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.5.

UV (MeOH): 283 (14800), 316 (18000). ^{1}H-NMR ((D$_6$)DMSO): 10.68 (br. *s*, NH); 7.95, 7.93 (2*s*, H–C(2), H–C=N); 7.38–7.18, 6.82–6.77 (*m*, arom. H); 6.13 (*'t'*, *J* = 6.5, H–C(1')); 6.07 (*s*, H–C(7)); 5.45 (*d*, *J* = 4.4, OH–C(3')); 4.35 (*m*, H–C(3')); 3.96 (*m*, H–C(4')); 3.71 (*s*, 2 MeO); 3.11 (*m*, CH$_2$(5')); 2.98, 2.91 (2*s*, Me); 2.64 (*m*, H$_\beta$–C(2')); 2.37 (*m*, H$_\alpha$–C(2')). Anal. calc. for $C_{35}H_{37}N_5O_6$ (623.7): C 67.40, H 5.98, N 11.22; found: C 67.32, H 6.11, N 11.26.

*1-[2'-Deoxy-5'-*O*-(4,4'-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-6-{[(dimethylamino)methylidene]-amino}-1*H*-imidazo[4,5-c]pyridin-4(5*H*)-one 3'-(Triethylammonium Phosphonate)* (**10c**). As described for **9**, from **10b** (300 mg, 0.48 mmol): colourless foam (283 mg, 74.6%). TLC (CH$_2$Cl$_2$/MeOH/Et$_3$N 88:10:2): R_f 0.25. UV (MeOH): 283 (15200), 318 (16400). ^{1}H-NMR ((D$_6$)DMSO): 10.64 (br. *s*, NH); 7.96, 7.91 (2*s*, H–C(2), H–C=N); 6.60 (*d*, ^1J(P,H) = 580, PH); 7.34–7.18, 6.61–6.79 (2*m*, arom. H); 6.13 (*m*, H–C(1')); 6.06 (*s*, H–C(7)); 4.73 (*m*, H–C(3')); 4.13 (*m*, H–C(4')); 3.72 (*s*, 2 MeO); 3.13 (*m*, CH$_2$(5')); 2.96, 2.91 (2*s*, 2 Me); 2.83 (*m*, CH$_3$C*H*$_2$); 2.7 (*m*, H$_\beta$–C(2')); 1.08 (*t*, C*H*$_3$CH$_2$). ^{31}P-NMR ((D$_6$)DMSO): 1.24 (*dd*, ^1J(P,H) = 580, ^3J(P,*H*–C(3')) = 9).

*1-[2'-Deoxy-5'-*O*-(4,4'-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-6-{[(dimethylamino)methylidene]-amino}-1*H*-imidazo[4,5-c]pyridin-4(5*H*)-one 3'-[(2-Cyanoethyl) N,N-Diisopropylphosphoramidite]* (**10d**). To a soln. of **10b** (50 mg, 0.08 mmol) in CH$_2$Cl$_2$ (1 ml), (i-Pr)$_2$EtN (56 µl, 0.286 mmol) and chloro(2-cyanoethoxy)(diisopropylamino)phosphane (115 µl, 0.511 mmol) were added. The soln. was stirred for 2 h at r.t. under Ar. After addition of 5% aq. NaHCO$_3$ soln. (3 ml), the mixture was extracted twice with CH$_2$Cl$_2$ (30 ml), the extract dried (Na$_2$SO$_4$) and evaporated, and the residue submitted to FC (silica gel *60H*, column 10 × 2 cm, 0.5 bar, CH$_2$Cl$_2$/AcOEt/Et$_3$N 45:45:10): two partially overlapping zones of diastereoisomers of **10d** as colourless foam (31 mg, 46.4%). TLC (CH$_2$Cl$_2$/AcOEt/Et$_3$N 45:45:10): R_f 0.2. ^{31}P-NMR ((D$_6$)DMSO): 149.2, 148.4.

*3-(2'-Deoxy-β-*D*-erythro-pentofuranosyl)-6-{[(dimethylamino)methylidene]amino}-3*H*-imidazo[4,5-c]pyridin-4(5*H*)-one* (**11a**). As described for **10a**, from **2** (200 mg, 0.75 mmol): 195 mg (81%) of yellowish foam. Crystallisation from MeOH gave colourless crystals. TLC (CH$_2$Cl$_2$/MeOH 9:1): R_f 0.3. M.p. 205°. UV (MeOH): 317 (19000). ^{1}H-NMR ((D$_6$)DMSO): 10.87 (br. *s*, NH); 8.38 (*s*, H–C(2)); 7.99 (*s*, H–C=N); 6.76 (*'t'*, *J*(H–C(1'),H–C(2')) = 6.4, H–C(1')); 5.99 (*s*, H–C(7)); 5.34 (*d*, *J* = 3.9, OH–C(3')); 5.08 (*t*, *J* = 5.3, OH–C(5')); 4.35 (*m*, H–C(3')); 4.13 (*m*, H–C(4')); 3.84 (*m*, CH$_2$(5')); 2.94, 3.05 (2*s*, 2 Me); 2.26–2.47 (2*m*, CH$_2$(2')). Anal. calc. for $C_{14}H_{19}N_5O_4$ (321.3): C 52.33, H 5.96, N 21.79; found: C 52.19, H 6.14, N 21.45.

*3-[2'-Deoxy-5'-*O*-(4,4'-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-6-{[(dimethylamino)methylidene]-amino}-3*H*-imidazo[4,5-c]pyridine-5(4*H*)-one 3'-(Triethylammonium Phosphonate)* (**11c**). As described for **10b**, **11a** (200 mg, 0.62 mmol) gave **11b** as colourless foam (158 mg, 40.7%; TLC (CH$_2$Cl$_2$/MeOH 9:1): R_f 0.4) which was converted without further purification.

From **11b** (200 mg, 0.32 mmol), **11c** was obtained as described for **10c**: colourless foam (83 mg, 32.8%). TLC (CH$_2$Cl$_2$/MeOH/Et$_3$N 88:10:2): R_f 0.4. UV (MeOH): 283 (15100), 317 (16400). ^{1}H-NMR ((D$_6$)DMSO): 10.71 (br. *s*, NH); 8.00 (*s*, H–C(2)); 7.81 (*s*, H–C=N); 6.46 (*d*, ^1J(P,H) = 585, PH); 7.23–7.07, 6.70–6.68 (*m*, arom. H, H–C(1')); 5.81 (*s*, H–C(7)); 4.72 (*m*, H–C(3')); 3.96 (*m*, H–C(4')); 3.57 (*s*, 2 MeO); 2.84–2.61 (*m*, Me, CH$_3$C*H*$_2$); 2.43 (*m*, H$_\beta$–C(2')); 1.02 (*t*, C*H*$_3$CH$_2$). ^{31}P-NMR ((D$_6$)DMSO): 1.12 (*dd*, ^1J(P,H) = 586, ^3J(P,H–C(3') = 8.5).

Solid-Phase Synthesis of the Oligonucleotides **12**–**17**. The syntheses were carried out on a 1-µmol scale using the 3'-hydrogen phosphonates of [(MeO)$_2$Tr]bz^{6}A$_d$, [(MeO)$_2$Tr]ib^{2}G$_d$, [(MeO)$_2$Tr]bz^{4}C$_d$, and [(MeO)$_2$Tr]T$_d$ as well as **10c** and **11c**. The synthesis of **12**–**17** followed the regular protocol of the DNA synthesizer for 3'-hydrogen phosphonates [22]. Deprotection of NH$_2$ groups was carried out with 25% NH$_3$/H$_2$O at 60° for 48 h. The 4,4'-dimethoxytrityl residues of the oligomers were removed by treatment with 80% AcOH/H$_2$O for 5 min at r.t. HPLC purification was carried out on *RP-18* columns using solvent system *I* for the (MeO)$_2$Tr derivatives, and *II* for the detritylated oligomers (see below). The oligomers were desalted on a 4 × 25 mm HPLC cartridge (*RP-18* silica gel) using H$_2$O (10 ml) for elution of the salt, while the oligomer was eluted with MeOH/H$_2$O 3:2 (5 ml). The nucleotides **12**–**17** were lyophilised on a *Speed-Vac* evaporator: colourless foams which were dissolved in H$_2$O (100 µl) and stored frozen at –18°.

Enzymatic Hydrolysis of the Oligomers **12**–**17** *and Determination of the Hypochromicity of* **12** *and* **13**. The oligonucleotide (0.2 A_{260} units) was dissolved in 0.1M *Tris*-HCl buffer (pH 8.3, 200 µl) and treated with either snake-venom phosphodiesterase (6 µg) at 37° for 45 min and alkaline phosphatase (2 µg) for 30 min at 37° or with calf-spleen phosphodiesterase (12 µg) and alkaline phosphatase. The mixture was analysed on HPLC (*RP-18*, solvent system *III*, followed by solvent system *II*; see below). 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}: A$_d$, 15400; C$_d$, 7300; G$_d$, 11700; T$_d$ 8800; c^{3}G$_d$ (**1**) 7400; c^{3}G'$_d$ (**2**) 8100).

Hypochromicity values of **12** and **13** were determined by enzymatic digestion of 0.5 A_{260} units of the corresponding oligonucleotide as described above. The hypochromicity values were calculated using the equation: $h = [(\varepsilon_{monomer} - \varepsilon_{oligomer}) \cdot (\varepsilon_{monomer})^{-1}] \cdot 100\%$. The extinction coefficient of the oligonucleotide was taken as the

sum of the extinction coefficients of the constituent monomeric deoxynucleosides. Time courses of phosphodiester hydrolysis of oligonucleotides were measured as follows: The oligonucleotide was dissolved in 0.1M *Tris*-HCl buffer (pH 8.3), and alkaline phosphatase (2 μg) was added. Reaction was started by addition of either snake-venom phosphodiesterase (6 μg) or calf-spleen phosphodiesterase (12 μg). The increase of A_{260} was continuously measured in thermostatted quartz cuvettes (1-cm path length) at 23°; the final absorbance was normalised to 100% of hydrolysis.

HPLC Purification of **12**–**17** *and Analyses of* **13**–**15** *and* **17**. HPLC analyses were carried out according to [23]. Solvent systems: 0.1M (Et_3NH)OAc (pH 7)/MeCN 95:5 (*A*) and MeCN (*B*) were used in the following order: system *I*, 3 min 15% *B* in *A*, 7 min 15–40% *B* in *A*, 5 min 40% *B* in *A*, 5 min 40–15% *B* in *A*, flow rate 1 ml/min; *II*, 20 min 0–20% *B* in *A*, flow rate 1 ml/min; *III*, 15 min 100% *A*, flow rate 0.6 ml/min.

REFERENCES

[1] Y. Mizuno, 'The Organic Chemistry of Nucleic Acids', in 'Studies in Organic Chemistry', Elsevier, Amsterdam, 1986, Vol. 24, pp. 33.
[2] B. F. Baker, P. B. Dervan, *J. Am. Chem. Soc.* **1989,** *111,* 2700.
[3] F. Seela, W. Bourgeois, H. Rosemeyer, T. Grein, *Collect. Czech. Chem. Commun.* **1990,** *55,* 37.
[4] P. C. Newman, V. U. Nwosu, D. M. Williams, R. Cosstick, F. Seela, B. A. Connolly, *Biochemistry* **1990,** *29,* 9891.
[5] P. C. Newman, D. M. Williams, R. Cosstick, F. Seela, B. A. Connolly, *Biochemistry* **1990,** *29,* 9902.
[6] A. M. Mian, T. A. Khwaja, *J. Med. Chem.* **1983,** *26,* 286.
[7] G. R. Revankar, P. K. Gupta, A. D. Adams, N. K. Dalley, P. A. McKernan, P. D. Cook, P. G. Canonico, R. K. Robins, *J. Med. Chem.* **1984,** *27,* 1389.
[8] M. Hoffer, *Chem. Ber.* **1960,** *93,* 3041.
[9] P. D. Cook, R. J. Rousseau, A. M. Miau, P. Dea, R. B. Meyer, Jr., R. K. Robins, *J. Am. Chem. Soc.* **1976,** *98,* 1492.
[10] R. K. Robins, J. H. Horner, C. V. Greco, C. W. Noell, C. G. Beames, Jr., *J. Org. Chem.* **1963,** *28,* 3041.
[11] P. K. Gupta, R. K. Robins, G. R. Revankar, *Nucleic Acids Res.* **1985,** *13,* 5341.
[12] H. Rosemeyer, G. Toth, F. Seela, *Nucleos. Nucleot.* **1989,** *8,* 587.
[13] D. G. Bartholomew, P. D. Dea, R. K. Robins, G. R. Revankar, *J. Org. Chem.* **1975,** *40,* 3708.
[14] G. B. Barlin, M. D. Fenn, *Aust. J. Chem.* **1981,** *34,* 1341.
[15] F. Seela, H. Rosemeyer, S. Fischer, *Helv. Chim. Acta* **1990,** *73,* 1602.
[16] R. P. Panzica, R. J. Rousseau, R. K. Robins, L. B. Townsend, *J. Am. Chem. Soc.* **1972,** *94,* 4708.
[17] Z. Kazimierczuk, R. Mertens, W. Kawczynski, F. Seela, *Helv. Chim. Acta* **1991,** *74,* 1742.
[18] J. Zemlicka, A. Holy, *Collect. Czech. Chem. Commun.* **1967,** *32,* 3159.
[19] L. J. McBride, M. H. Caruthers, *Tetrahedron Lett.* **1983,** *24,* 2953.
[20] B. C. Froehler, P. G. Ng, M. D. Matteucci, *Nucleic Acids Res.* **1986,** *14,* 5399.
[21] F. Seela, H. Driller, *Helv. Chim. Acta* **1988,** *71,* 1191.
[22] Applied Biosystems, 'Users Manual of the DNA Synthesizer' 380B.
[23] H. Rosemeyer, F. Seela, *Helv. Chim. Acta* **1991,** *74,* 748.
[24] F. Seela, K. Kaiser, U. Bindig, *Helv. Chim. Acta* **1989,** *72,* 868.
[25] H. Rosemeyer, F. Seela, *Carbohydr. Res.* **1978,** *62,* 155.