126. 3-Deaza-2′-deoxyadenosine: Synthesis *via* 4-(Methylthio)-1*H*-imidazo[4,5-*c*]pyridine 2′-Deoxyribonucleosides and Properties of Oligonucleotides

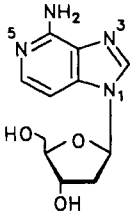
by **Frank Seela***, **Thomas Grein**, and **Samuel Samnick**

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

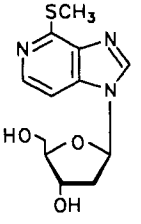
(25.V.92)

The synthesis of 4-(methylthio)-1*H*-imidazo[4,5-*c*]pyridine 2′-deoxy-β-D-ribonucleosides **2** and **9** and the conversion of the N^1-isomer **2** into the 2′,3′-didehydro-2′,3′-dideoxyribonucleoside **3a** or (*via* **7**) 3-deaza-2′-deoxyadenosine (**1**) is described. Phosphonate building blocks of **1** were employed in solid-phase synthesis of self-complementary base-modified oligonucleotides. Their properties were studied with regard to duplex stability and hydrolysis by the restriction enzyme *Eco*RI.

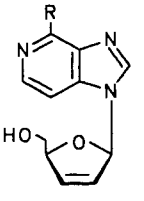
Introduction. – The 3-deaza-2′-deoxyadenosine [1] (**1**) is a structural analogue of the naturally occurring DNA constituent 2′-deoxyadenosine (dA) in which N(3) is replaced by CH. As N(3) is located within the minor groove of double-stranded DNA, it acts as acceptor for DNA ligands. The replacement of dA by compound **1** can alter the properties of this binding domain. Compound **1** was synthesized stereoselectively from 4-chloro-1*H*-imidazo[4,5-*c*]pyridine or 4,6-dichloro-1*H*-imidazo[4,5-*c*]pyridine anions and the halogenose **6** [2–5]. The incorporation of compound **1** into oligonucleotides was accomplished first in 1987 [6] using phosphotriester chemistry and was carried out later by solid-phase methods employing phosphoramidites [5] or phosphonates [7]. Apart from **1**, the 2′,3′-dideoxyribonucleoside and the 2′,3′-didehydro-2′,3′-dideoxynucleoside **3b** of 3-deazaadenine were prepared as potential antiviral agents [8].



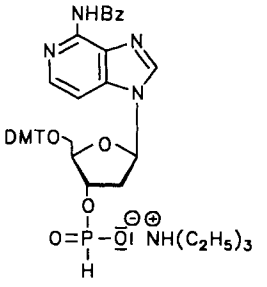
1 systematic numbering



2



3a R = SCH_3
b R = NH_2



4

In the course of this work, it became apparent that the use of the 4-chloro compounds as nucleobase precursors exhibit drawbacks: *i*) the glycosylation is not regioselective; N^1- and N^3-(2′-deoxyribofuranosides) are formed [2] and *ii*) the 4-chloro substituent is difficult to displace with nucleophiles, such as ammonia. Therefore, the glycosylation of methylthio compound **5** was now considered which should allow the transformation into a sulfone, the latter being easier accessible to nucleophilic displacement reactions. Compound **2**, obtained *via* intermediate methylthio compound **7**, was also converted into the 2′,3′-didehydro-2′,3′-dideoxynucleoside **3a**. Furthermore, oligonucleotides representing the recognition sequence of the endodeoxyribonuclease *Eco* RI containing **1** were synthesized from phosphonate **4**, and their regioselective hydrolysis by the restriction enzyme was studied.

Results and Discussion. – *3-Deazanucleosides.* Starting material was the methylthio compound **5** [9] which was prepared from 4-chloroimidazo[4,5-*c*]pyridine [10]. For this purpose, the latter was converted into the imidazo[4,5-*c*]pyridine-4-thione by sodium hydrosulfide [11]. Alkylation with MeI afforded compound **5** [9]. It was crystallized from aqueous, slightly alkaline solution (ammonia) as we had shown that 3-deazapurines are much more basic than purines and can easily form salts, even under neutral condition [2].

Glycosylation of the anion of **5** with the anomerically pure halogenose **6** [12] in MeCN in the presence of powdered KOH and tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1) [13–15] afforded the two regioisomers **7** and **8** in a ratio of 2.4:1 (*Scheme 1*). The ratio of regioisomers of the corresponding reaction products using 4-chloro-1*H*-imidazo[4,5-*c*]-pyridine was 2.5:1 [2]. Although the ratio of regioisomers was not changed, the total yield of the glycosylation products was improved. The regioisomers **7** and **8** were deprotected (NH_3/MeOH) yielding the nucleosides **2** and **9**, respectively. The glycosylation position and the anomeric configuration of **2** and **9** were unambiguously assigned by ^{1}H-NMR

Scheme 1

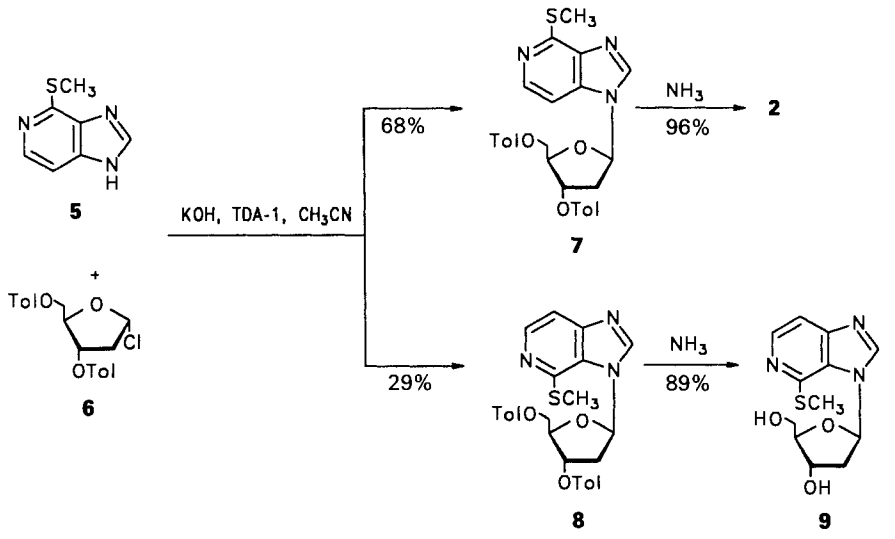


Table 1. *NOE Data [%] of Imidazo[4,5-c]pyridine Nucleosides in (D_6)DMSO at 23°.* Systematic numbering.

	Irradiated proton	Observed NOE [%]
2	H–C(1′)	H–C(2) (6.0), H–C(7) (5.0), H_α–C(2′) (6.9), H–C(4′) (1.8)
	H–C(2)	H–C(2) (0.0), H–C(6) (13.5), H–C(1′) (5.3), H_α–C(2′) (0.0), H–C(3′) (0.0)
9	H–C(1′)	H–C(2) (0.0), H–C(7) (0.0), H_α–C(2′) (7.1), H–C(4′) (0.0)
	H–C(2)	H–C(2) (0.0), H–C(6) (0.0), H–C(1′) (0.0), H_α–C(2′) (7.4), H–C(3′) (2.3)

Table 2. *^{13}C-NMR Chemical Shifts of Imidazo[4,5-c]pyridine Nucleosides in ($(D_6)Me_2SO$).* Systematic numbering.

	C(2)	C(3a)	C(4)	C(6)	C(7)	C(7a)
1 [2]	139.7	126.8	152.4	140.6	97.2	137.5
2	142.2[a])	137.7[b])	150.7[a])	141.4[a])	103.7[a])	136.1[b])
3a	142.8	137.7	150.5	141.6	103.5	136.5
5	142.4	137.6	149.3	141.0	104.9	136.2
8	144.3	128.4	142.6	141.3	111.4	148.0
9	144.2[a])	128.3[d])	142.4	141.0[a])	111.3[a])	147.9[d])
10	142.0	137.9	150.9	141.3	103.9	136.2
11	142.0	137.6	150.9	141.5	103.8	136.1
12	145.8[a])	136.7[c])	148.3	140.1[a])	111.3[a])	136.7[c])
13	145.5	136.7[c])	148.0	140.1	111.8	136.7[c])

	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	MeS	$MeSO_2$	C=O	
1 [2]	84.6	39.9	70.5	87.6	61.5				
2	84.9[a])	40.1	70.3	87.8[a])	61.3	11.2			
3a	88.2	125.4	135.0	89.8	62.8	11.0			
5						11.3			
8	85.6	DMSO	74.5	81.6	64.0	12.6		21.5, 21.2	165.2, 165.4
9	86.0[b])	41.7	70.0	87.9[b])	61.1	12.5			
10	84.7	40.3	69.8	87.0	64.0	11.3		16.9, 26.7	
11	84.2[c])	40.2	79.6	84.5[c])	63.0	11.3		18.7, 26.6	
12	85.7	DMSO	74.8	82.2	64.2		41.7	21.3, 21.4	165.5, 165.7
13	85.4	DMSO	70.4	88.2	61.3		41.6		

[a]) From ^{1}H,^{13}C-correlation spectra. [b]) From INAPT spectra. [c]) Tentative. [d]) From gated-decoupled spectra.

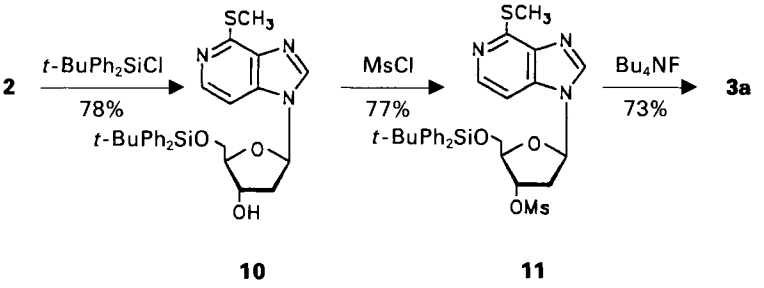
NOE difference spectroscopy [16] (*Table 1*). Further evidence of the structure of the regioisomers was obtained from the ^{13}C-NMR data (*Table 2*). The chemical shifts of the regioisomers were similar to those of the 4-chloro compounds [2].

A NOE at H–C(2) and H–C(7) upon saturation of H–C(1′) proves N(1) as glycosylation site in the case of **7**. Unfortunately, the regioisomer **9** did not show the expected NOE at H–C(2) when H–C(1′) was irradiated. This is due to the '*anti*'-orientation of the base, induced by the bulky MeS group. In an analogous experiment, H–C(2) was irradiated giving similar results (*Table 1*). The N^3-substituted compound **9** showed a downfield shift of the C(7a) NMR signal by *ca.* 12 ppm in comparison to the N^1-isomer **2**. On the other hand, the C(3a) signal of **9** is shifted upfield compared to **2** (9.4 ppm). The base **5** shows almost the same chemical shifts as the base moiety of compound **2** indicating that the proton is located at N(1). ^{13}C-NMR assignments of N^1- and N^3-methylated imidazo[4,5-*c*]pyridine taken from measurements in H_2O differ from our data [17].

Recently, 3-deaza-2′,3′-didehydro-2′,3′-dideoxyadenosine (**3b**) was prepared from its ribonucleoside *via* 2′-bromo-3′-acetates [8]. Compound **3b** was then converted into 2′,3′-dideoxyribonucleoside $O^{5'}$-(dialkyl phosphates) which showed moderate anti-HIV activ-

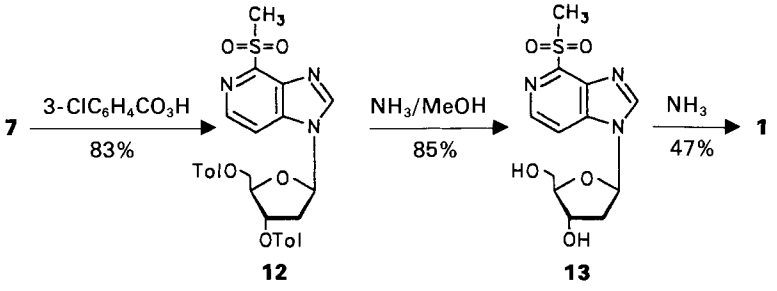
ity [8]. We employed an alternative route for the synthesis of the related 3-deazapurine 2′,3′-didehydro-2′,3′-dideoxyribonucleoside **3a** which can be used as precursor for various 2′,3′-didehydro-2′,3′-dideoxyribonucleosides. Thus, 2′-deoxyribonucleoside **2** was protected with (*t*-Bu)Ph_2SiCl to give the silyl derivative **10** regioselectively (^{13}C-NMR downfield shift of C(5′), 2.7 ppm (*Table 2*); *Scheme 2*). Treatment of **10** with methanesulfonyl chloride (MsCl) afforded mesylate **11** which was converted with Bu_4NF to crystalline **3a**, under removal of the silyl protecting group and simultaneous elimination of the MsO group.

Scheme 2



Next, the conversion of the 4-MeS group into an amino group was studied. The reactivity of the MeS group of compound **2** against ammonia is low compared to that of the Cl substituent. It was not displaced in a pressure bottle at 50°. However, the reactivity was increased by oxidation to the corresponding sulfone. Thus, the protected nucleoside **7** was oxidized with 3-chloroperbenzoic acid and the resulting sulfone **12** deprotected either with 0.1N NaOH/dioxane or with NH_3/MeOH yielding the highly fluorescent compound **13** (*Scheme 3*). Conversion of compound **13** into **1** occurred upon treatment with aq. ammonia at 50° in a pressure bottle. The corresponding 6-chloro derivative did not react under these conditions.

Scheme 3



It was already reported that 3-deaza-2′-deoxyadenosine is hydrolytically less labile than dA by a factor of 10 [2]. The sensitivity of the N-glycosylic bond against acid is increased in the case of the N^3-glycosylated isomers, *e.g.* **9**, compared to the N^1-com-

Table 3. *Half-life Values ($t_{1/2}$) for Proton-Catalyzed Hydrolysis of 3-Deazapurine Nucleosides, Measured at 25°*

	$t_{1/2}$ [min] in aq. HCl solution		
	1.0N	0.1N	0.01N
dA	1.6		
1 [2]	17		
2	11		
13		46	
3a		6	
$c^3A_{d'}$[a]) [2]			1.9
9			1.3

[a]) $c^3A_{d'}$ = 3-deazaadenine N^3-(2′-deoxy-β-D-ribofuranoside).

pound **2** (*Table 3*). Electron-withdrawing substituent at C(4) destabilize the N-glycosylic bond (see **13**). As expected, the most labile compound within the series of N^1-nucleosides was the 2′,3′-dideoxy-2′,3′-didehydro-nucleoside **3a**.

Oligonucleotides Containing **1**. Oligonucleotides containing **1** were already prepared [5–7]. Homooligoribonucleotides were obtained enzymatically from its 5′-diphosphate [18]. According to the fact that compound **1** can be used to modify the minor groove of a DNA duplex, it was employed as structural probe for bended DNA [7]. Replacement of dA residues within $d(A)_6$ tracts reduced bending if the replacement occurred at the 3′-site, whereas at the 5′-end, bending was almost the same as observed in the case of the parent oligonucleotide.

It was reported that compound **1** incorporated into oligonucleotides did not change the T_m value of a duplex [5]. However, we showed that duplexes containing $d(c^3A)$ tracts had strongly decreased T_m values. This work was now extended to self-complementary oligomers with an alternating d(A-T) sequence or palindromic compounds, recognized by restriction enzymes.

Oligonucleotides **14–18** were obtained by solid-phase oligonucleotide synthesis using the phosphonate **4** [7] together with the phosphonates of regular DNA constituents in an automated DNA synthesizer. The synthesis of the single-stranded oligomers followed a protocol of detritylation, activation, coupling, and capping described in [19]. After removal of the base-protecting groups by ammonolysis, the 5′-protected oligomers were purified by reversed-phase HPLC, detritylated, again submitted to reversed-phase chromatography, and desaltet (see *Exper. Part*). The composition of the oligonucleotides was proved by determination of the nucleoside content after tandem-hydrolysis with snake-venom phosphodiesterase and alkaline phosphatase followed by *RP-18* HPLC [20] (see *Fig. 1*). Integration of the peaks demonstrated the correct composition of the oligonucleotides and showed that no side-reaction had occurred during oligonucleotide synthesis.

$d(c^3A\text{-}T)_6$ **14**

$d(A\text{-}T)_6$ **15**

d(G-T-A-G-c^3A-c^3A-T-T-C-T-A-C) **16**

d(G-T-c^3A-G-A-A-T-T-C-T-c^3A-C) **17**

d(G-T-A-G-A-A-T-T-C-T-A-C) **18**

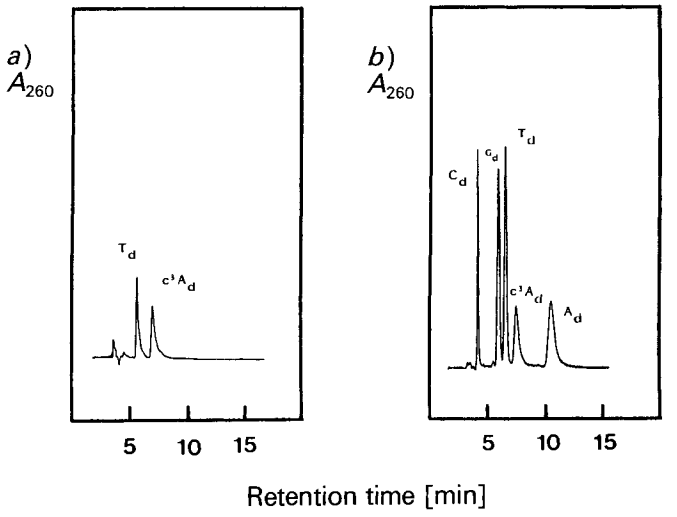


Fig. 1. *HPLC profiles of the enzymatic hydrolysis products of* a) *d(c³A-T)₆* (**14**) *and* b) *d(G-T-A-G-c³A-c³A-T-T-C-T-A-C)* (**16**) *with snake-venom phosphodiesterase followed by alkaline phosphatase.* Conditions, see *Exper. Part.*

Next, T_m values of the oligomers **14**–**18** were measured. The T_m value of **15**, determined UV-spectrophotometrically at 260 nm, was found to be 30° (5 μM single-strand concentration; *Table 4*). The temperature-dependent CD spectra of **15**, however, measured within the range of 5–80° resulted in different T_m values for the B_{1u} (31°) and the B_{2u} (48°) transitions (*Table 4* and *Fig. 2*). This indicated that different species were present in solution, a finding which is in agreement with earlier observation [22–26]. However, a

Table 4. T_m *Values, Thermodynamic Data, and Thermal/Enzymatic Hypochromicities of the Self-Complementary Oligonucleotides* **14**–**18**

	T_m [°C]			ΔH^b) [kcal/mol]	ΔS^b) [kcal/mol·K]	Hypochromicity [%]	
	UV[a]) (260 nm)	CD[a])				UV[c]) (260 nm)	UV[d]) (260 nm)
		B_{1u}	B_{2u}				
14	< 25		18				22
15	30	31	48	–29	–96	21	26
15 [30]	30			–30[e])	–98[e])	23	
16	43	50	49	–15	–48	20	29
17	34	33	42	–40	–130	22	26
18	49	50	50	–49	–153	27	35
18 [30]	48			–41[e])	–127[e])	27	

[a]) Measured at 3–5 μM single-strand concentration in 60 mM Na-cacodylate buffer (pH 7.0), 1M NaCl, and 100 mM $MgCl_2$.
[b]) Calculated on the basis of single strands.
[c]) Thermal hypochromicity.
[d]) Enzymatic hypochromicity.
[e]) Average values. Conditions, see *Exper. Part.*

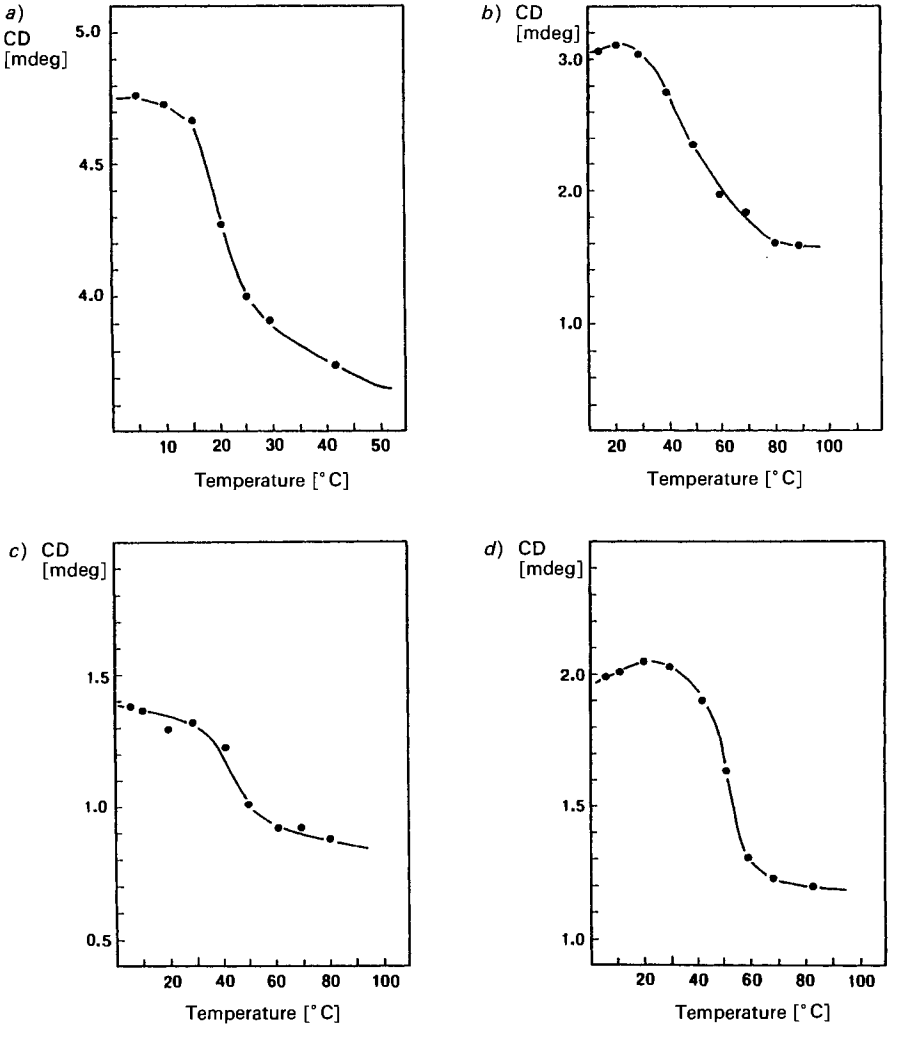


Fig. 2. *Temperature-dependent CD spectra* (CD *vs.* temp.; B_{2u} transition) *of* a) *d(c³A-T)₆* (**14**), b) *d(A-T)₆* (**15**), c) *d(G-T-A-G-c³A-c³A-T-T-C-T-A-C)* (**16**), *and* d) *d(G-T-A-G-A-A-T-T-C-T-A-C)* (**18**). In 60 mM Na cacodylate buffer (pH 7.0), 1M NaCl, and 100 mM $MgCl_2$ (3.0 μM single-strand concentration).

preference of duplexes at high salt concentration was found. In the case of oligomer **14**, only the B_{2u} transition of the temperature-dependent CD measurement allowed the determination of a transition which was lower than in the case of **15**. The melting and the enzymatic hypochromicities of the modified oligomers were slightly decreased (see *Table 4*). From the UV and CD data, it was apparent that c^3A_d residues strongly destabilized base-pairing within *Watson-Crick* duplex structures (*Table 4*). Analogous experiments were performed with the oligomers **16**–**18**. The T_m values of **16** and **17** containing two modified bases in the centre or near the ends of the chain differred strongly and were both

lower than that of the parent oligomer **18**. According to recent findings, hairpins or duplexes can be formed at this high salt buffer around a concentration of 3 μM oligomer [21].

According to the ΔH values and the identical T_m values (*Table 4*) observed at the B_{1u} and B_{2u} transition, oligomer **18** should be a duplex. Depending on the position of the modification, self-complementary oligomers such as **16** and **17** can be forced either to form hairpins or duplexes. This is due to the stability of the base pairs of the modified structure compared to the parent one. In our case, base-pairing was decreased, if compound **1** was replacing dA, most probably by competition of solvent protonation of the base moiety of **1** with base-pairing. This was supported by the finding that compound **1** was protonated already at neutral conditions ($pK_{BH^+} = 7.3$ [2]), whereas protonation of dA occurred at $pK_{BH^+} = 3.8$.

The very low ΔH and ΔS values of oligomer **16** (*Table 4*), which were calculated according to a program developed recently [27], showed that the incorporation of **1** affected the stability of an oligomer in many ways. This was also shown by the strong difference between the B_{1u} and B_{2u} transition found for compound **17** (*Table 4*).

Earlier, nucleoside **1** was incorporated into the recognition sequence d(G-A-T-A-T-C) of the restriction enzyme *Eco*RV [5]. It was observed that incorporation of c^3A_d into either the central or outer dA–dT base-pair resulted in a substantial reduction in the rate of phosphodiester hydrolysis. We now have made some preliminary experiments with the oligomers **16** and **17** containing the recognition site of *Eco*RI. In a previous paper, we showed that the replacement of only one dA residue by another deazanucleoside (c^7A_d) within the sequence d(G-A-A-T-T-C) reduced the hydrolysis by *Eco*RI, whereas replacement of both dA's protected the duplex from hydrolysis completely [20]. This was later

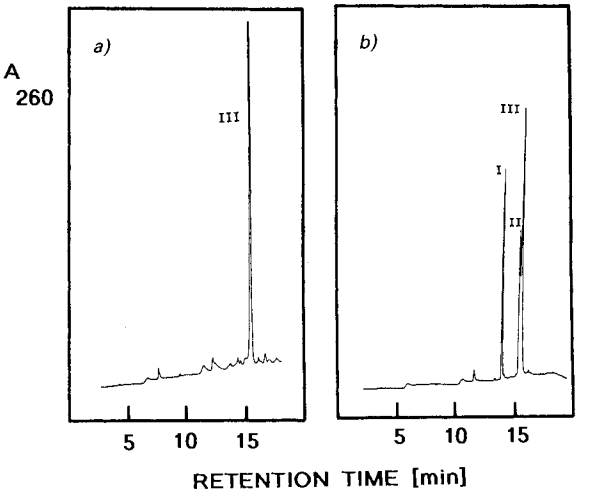


Fig. 3. *HPLC profiles of the reaction mixture from the cleavage of d(G-T-A-G-c³A-c³A-T-T-C-T-A-C)* (**16**) *by the endodeoxyribonuclease* Eco*RI* (conditions, see *Exper. Part*; solvent system *IV*) a) *at the beginning and* b) *after 101 min.* **I**, d(G-T-A-G); **II**, d(pc³A-c³A-T-T-C-T-A-C); **III**, dodecanucleotide **16**.

confirmed on high-molecular DNA prepared by PCR amplification [28]. To study the influence of c^3A_d/dA replacement on the dodecanucleotides **16**–**18**, we incubated these compounds with *Eco* RI and followed the hydrolysis by HPLC. The starting oligomer **18** or **16** was separated from the tetramer d(G-T-A-G) and the octamer d(pA-A-T-T-C-T-A-C) or d(pc^{3}A-c^{3}A-T-T-C-T-A-C), respectively (*Fig. 3*; conditions, see *Exper. Part*). It was observed that, differently from c^7A_d, the replacement of both dA residues by c^3A_d did not protect the duplex from hydrolysis, although hydrolysis was slow ($t_{1/2}$ (**16**), 90 min; $t_{1/2}$ (**18**), 80 min).

Surprisingly, incubation of the oligomer d(G-T-c^{3}A-G-A-A-T-T-C-T-c^{3}A-C) (**17**) which is modified in the flanking regions showed no measurable cleavage within 27 h. As we have shown above that self-complementary oligomers form different species depending on the position of modification, this finding may be explained with the special properties of short self-complementary structures but has to be evaluated further.

We thank Dr. *H. Rosemeyer* for discussion of the NMR experiments. Financial support by the *Deutsche Forschungsgemeinschaft* is gratefully acknowledged.

Experimental Part

General. See [29]. The phosphonates of regular 2′-deoxynucleosides were purchased from *Sigma* (St. Louis, USA) and CPG-immobilized protected 2′-deoxynucleosides (30 μmol/g of solid support) from *Milligene* (Eschborn, Germany). Snake-venom phosphodiesterase (EC 3.1.4.1, *Crotalus durissus*), alkaline phosphatase (EC 3.1.3.1, calf intestine), and the endodeoxyribonuclease *Eco* RI, high enzyme concentration (EC 3.1.23.13), were products of *Boehringer Mannheim* (Germany). Lyophilization was performed with a *Speed-Vac* concentrator (*Savant Instruments*, Farmingdale, N.Y.). TLC: silica gel *G-25 UV254* plates, *Macherey-Nagel* (Düren, Germany). Flash chromatography (FC): 0.5 bar, silica gel *60* (*Merck*, Germany); solvent systems: CH_2Cl_2/MeOH 95:5 (*A*), CH_2Cl_2/MeOH 9:1 (*B*), CH_2Cl_2/acetone 95:5 (*C*), CH_2Cl_2/acetone 93:7 (*D*), CH_2Cl_2/MeOH 8:2 (*E*), CH_2Cl_2/MeOH 95:2 (*F*), CH_2Cl_2/MeOH 97:3 (*G*), and CH_2Cl_2/MeOH 99:1 (*H*). CD Spectra: *Jasco 600* spectropolarimeter; thermostatically controlled 1-cm cuvettes connected with a *Lauda RCS 6* bath (*Lauda-Königshofen*, Germany). The melting hypochromicity ($h = [(\varepsilon_{simplex} - \varepsilon_{duplex}) \cdot (\varepsilon_{simplex})^{-1}] \cdot 100\%$) of **14**–**18** (*Table 4*) was determined from the melting curves. The extinction coefficient of the oligonucleotide was calculated from the sum of the extinction coefficients of the monomeric 2′-deoxyribonucleosides divided by the hypochromicity.

*4-(Methylthio)-1*H*-imidazo[4,5-*c*]pyridine* (**5**) was prepared from imidazo[4,5-*c*]pyridin-4-thione [11] as described [9]. The product was isolated upon acidification with 10% aq. HCl soln. and crystallization from H_2O/MeOH at pH 8.0 (ammonia). TLC (*B*): R_f 0.55. UV (MeOH): 283 (11000), 216 (17800). ^{1}H-NMR ((D$_6$)DMSO): 8.30 (*s*, H–C(2)); 8.18 (*d*, *J* = 5.6, H–C(6)); 7.32 (*d*, *J* = 5.6, H–C(7)); 2.60 (*s*, MeS).

*1-[2′-Deoxy-3′,5′-di-*O*-(4-toluoyl)-β-*D*-erythro-pentofuranosyl]-4-(methylthio)-1*H*-imidazo[4,5-*c*]pyridine* (**7**) *and 3-[2′-Deoxy-3′,5′-di-*O*-(4-toluoyl)-β-*D*-erythro-pentofuranosyl]-4-(methylthio)-1*H*-imidazo[4,5-*c*]pyridine* (**8**). Powdered KOH (650 mg, 11.6 mmol) and tris[2-(2-methoxyethoxy)ethyl]amin (TDA-1; 50 μl, 0.15 mmol) were added to a soln. of **5** (370 mg, 2.24 mmol) in anh. MeCN (50 ml). The mixture was stirred at r.t. for 5 min. Then 2-deoxy-3,5-di-*O*-(4-toluoyl)-β-*D*-*erythro*-pentofuranosyl chloride (**6**; 1.0 g, 2.57 mmol) [11] was added within 5 min, and stirring was continued at r.t. for another 15 min. The mixture was filtered over *Celite*, the solvent evaporated, and the resultant oil chromatographed (silica gel *60* H, column 25 × 6 cm, solvent *C*).

From the zone with R_f 0.55, **7** (786 mg, 68%) was obtained. Colourless crystals. M.p. 120–122° (EtOH). UV (MeOH): 282 (15300), 240 (33000). ^{1}H-NMR ((D$_6$)DMSO): 8.56 (*s*, H–C(2)); 8.13 (*d*, *J* = 5.7, H–C(6)); 8.13–7.82 (*m*, arom. H); 7.53 (*d*, *J* = 5.7, H–C(7)); 7.42–7.31 (*m*, arom. H); 6.61 (*m*, H–C(1′)); 5.75 (*m*, H–C(3′)); 4.55–4.67 (*m*, H–C(4′), 2 H–C(5′)); 3.01 (*m*, H_α–C(2′)); 2.81 (*m*, H_β–C(2′)); 2.60 (*s*, MeS); 2.43, 2.40 (2*s*, Me). Anal. calc. for $C_{28}H_{27}N_3O_5S$: C 64.98, H 5.26, N 8.12, S 6.19; found: C 64.99, H 5.34, N 8.12, S 6.15.

The zone with R_f 0.47 afforded **8** (334 mg, 29%). Colourless crystals. M.p. 116–117° (EtOH). UV (MeOH): 296 (6300), 240 (29300). ^{1}H-NMR ((D$_6$)DMSO): 8.77 (*s*, H–C(2)); 8.30 (*d*, *J* = 5.6, H–C(6)); 8.01–7.71 (*m*, arom.

H); 7.49 (*d*, $J = 5.6$, H–C(7)); 7.54–7.52 (*m*, arom. H); 7.0 ('*t*', H–C(1')); 5.76 (*m*, H–C(3')); 4.59–4.66 (*m*, H–C(4'), 2 H–C(5')); 3.13 (*m*, H_α–C(2')); 2.93 (*m*, Hβ–C(2')); 2.69 (*s*, MeS); 2.41, 2.37 (2*s*, Me). Anal. calc. for $C_{28}H_{27}N_3O_5S$: C 64.98, H 5.26, N 8.12; found: C 64.88, H 5.38, N 8.00.

*1-(2'-Deoxy-β-*D-erythro-*pentofuranosyl)-4-(methylthio)-1*H-*imidazo[4,5-*c*]pyridine* (**2**). At r.t., **7** (460 mg, 0.89 mmol) was stirred in NH_3/MeOH (saturated at 0°) for 24 h. The solvent was evaporated and the residue applied to FC (column 10 × 4 cm, solvent *B*): colourless crystals (239 mg, 96%). TLC (*B*): R_f 0.41. M.p. 175–178° (acetone). UV (MeOH): 283 (13700), 218 (18000). ^{1}H-NMR ((D$_6$)DMSO): 8.58 (*s*, H–C(2)); 8.24 (*d*, $J = 5.7$, H–C(6)); 7.56 (*d*, $J = 5.7$, H–C(7)); 6.38 ('*t*', H–C(1')); 5.39 (*d*, OH–C(3')); 5.03 (*t*, OH–C(5')); 4.41 (*m*, H–C(3')); 3.9 (*m*, H–C(4')); 3.60 (*m*, 2 H–C(5')); 2.60 (*s*, MeS); 2.52 (*m*, H_α–C(2')); 2.34 (*m*, H_β–C(2')). Anal. calc. for $C_{12}H_{15}N_3O_3S$: C 51.23, H 5.37, N 14.94; found: C 51.10, H 5.39, N 14.74.

*3-(2'-Deoxy-β-*D-erythro-*pentofuranosyl)-4-(methylthio)-1*H-*imidazo[4,5-*c*]pyridine* (**9**). As described for **2**, with **8** (600 mg, 1.16 mmol): colourless crystals (291 mg, 89%). TLC (*B*): R_f 0.44. M.p. 158–161°. UV (MeOH): 296 (10500), 249 (sh, 5900), 217 (19000). ^{1}H-NMR ((D$_6$)DMSO): 8.79 (*s*, H–C(2)); 8.26 (*d*, $J = 5.6$, H–C(6)); 7.46 (*d*, $J = 5.6$, H–C(7)); 6.85 (*t*, H–C(1')); 5.42 (*m*, OH–C(3')); 5.09 (*t*, OH–C(5')); 4.42 (*m*, H–C(3')); 3.95 (*m*, H–C(4')); 3.62 (*m*, 2 H–C(5')); 2.61 (*s*, MeS); 2.57 (*m*, H_α–C(2')); 2.41 (*m*, H_β–C(2')). Anal. calc. for $C_{12}H_{15}N_3O_3S$: C 51.23, H 5.37, N 14.94; found: C 51.34, H 5.49, N 14.79.

*1-[2'-Deoxy-3',5'-di-*O*-(4-toluoyl)-β-*D-erythro-*pentofuranosyl]-4-(methylsulfonyl)-1*H-*imidazo[4,5-*c*]pyridine* (**12**). A soln. of **7** (250 mg, 0.48 mmol) in MeOH (10 ml) was treated with 3-chloroperbenzoic acid (70%; 600 mg, 3.51 mmol) at r.t. for 30 min. The solvent was evaporated and the residue applied to FC (column 10 × 4 cm, solvent *D*). From the main zone, a colourless powder was obtained (220 mg, 83%). TLC (*D*): R_f 0.42. UV (MeOH): 273 (8300), 266 (sh, 7800), 241 (30200). ^{1}H-NMR ((D$_6$)DMSO): 8.90 (*s*, H–C(2)); 8.39 (*d*, $J = 5.5$, H–C(6)); 8.16 (*d*, $J = 5.5$, H–C(7)); 7.99–7.30 (4*m*, arom. H); 6.72 ('*t*', H–C(1')); 5.76 (*m*, H–C(3')); 4.65–4.56 (*m*, H–C(4'), 2 H–C(5')); 3.49 (*s*, $MeSO_2$); 3.11 (*m*, H_α–C(2')); 2.88 (*m*, H_β–C(2')); 2.42, 2.37 (2*s*, Me). Anal. calc. for $C_{28}H_{27}N_3O_7S$: C 61.19, H 4.95, N 7.65; found: C 61.20, H 5.00, N 7.55.

*1-(2'-Deoxy-β-*D-erythro-*pentofuranosyl)-4-(methylsulfonyl)-1*H-*imidazo[4,5-*c*]pyridine* (**13**). *Method A:* At r.t., **12** (380 mg, 0.69 mmol) was stirred for 1 h in 1,4-dioxane/0.1N aq. NaOH 1:1 (20 ml). A precipitate was formed and separated by filtration. The soln. was neutralized with 0.01N HCl, the solvent evaporated, and the residue applied to FC (column 15 × 3 cm, solvent *B*): colourless foam (169 mg, 78%).

Method B: At r.t., **12** (380 mg, 0.69 mmol) was stirred for 20 h in NH_3/MeOH (saturated at 0°). The solvent was evaporated and the residue applied to FC (column 10 × 4 cm, solvent *B*): colourless foam (183 mg, 85%). TLC (*B*): R_f 0.69. UV (MeOH): 276 (6800). ^{1}H-NMR ((D$_6$)DMSO): 8.87 (*s*, H–C(2)); 8.48 (*d*, $J = 5.5$, H–C(6)); 8.23 (*d*, $J = 5.5$, H–C(7)); 6.49 ('*t*', H–C(1')); 5.40 (*d*, OH–C(3')); 5.05 (*t*, OH–C(5')); 4.44 (*m*, H–C(3')); 3.92 (*m*, H–C(4')); 3.58 (*m*, 2 H–C(5')); 3.51 (*s*, $MeSO_2$); 2.62 (*m*, H_α–C(2')); 2.42 (*m*, H_β–C(2')). Anal. calc. for $C_{12}H_{15}N_3O_5S$: C 46.00, H 4.83, N 13.41; found: C 46.20, H 4.84, N 13.49.

*1-{2'-Deoxy-5'-*O*-[(*tert*-butyl)diphenylsilyl]-β-*D-erythro-*pentofuranosyl}-4-(methylthio)-1*H-*imidazo[4,5-*c*]pyridine* (**10**). Compound **2** (430 mg, 1.53 mmol) was co-evaporated with anh. pyridine and then dissolved in anh. pyridine (10 ml). The soln. was cooled to 0° and (*t*-Bu)Ph_2SiCl (0.4 ml, 1.56 mmol) added dropwise under Ar while stirring. Stirring was continued for 24 h at r.t. The solvent was evaporated, the residue co-evaporated with toluene (2 × 10 ml), and the residue applied to FC (column 15 × 4 cm, solvent *G*): colourless crystals (620 mg, 78%). TLC (*A*): R_f 0.34. M.p. 68–71°(MeOH). ^{1}H-NMR ((D$_6$)DMSO): 8.47 (*s*, H–C(2)); 8.11 (*d*, $J = 6.6$, H–C(6)); 7.60–7.31 (*m*, arom. H, H–C(7)); 6.40 ('*t*', H–C(1')); 5.50 (*d*, $J = 4.4$, OH–C(3')); 4.52 (*m*, H–C(3')); 4.14–3.71 (*m*, H–C(4'), 2 H–C(5')); 2.60 (*s*, MeS); 2.38–2.52 (*m*, 2 H–C(2')); 0.97 (*s*, *t*-Bu). Anal. calc. for $C_{28}H_{33}N_3O_3SSi$: C 64.71, H 6.40, N 8.08; found: C 64.64, H 6.54, N 8.01.

*1-{2'-Deoxy-5'-*O*-[(*tert*-butyl)diphenylsilyl]-3'-*O*-(methylsulfonyl)-β-*D-erythro-*pentofuranosyl}-4-(methylthio)-1*H-*imidazo[4,5-*c*]pyridine* (**11**). A soln. of **10** (375 mg, 0.72 mmol) in CH_2Cl_2 (10 ml) containing pyridine (3 ml) was cooled to 0° and MsCl (0.55 ml, 7.2 mmol) added while stirring. Stirring was continued for 4 h at r.t., then MeOH (2.5 ml) was added. After 15 min, the soln. was diluted with $CHCl_3$ (50 ml) and washed with 0.1N HCl and H_2O (50 ml, each). The org. layer was dried (Na_2SO_4), filtered, and evaporated. The residue was applied to FC (column 10 × 3 cm, solvent *E*): colourless foam (330 mg, 77%). TLC (*G*): R_f 0.51. ^{1}H-NMR ((D$_6$)DMSO): 8.49 (*s*, H–C(2)); 8.07 (*d*, $J = 5.70$, H–C(6)); 7.31–7.59 (*m*, arom. H); 7.5 (*d*, $J = 5.7$, H–C(7)); 6.48 (*t*, H–C(1')); 5.50 (*m*, H–C(3')); 4.33 (*m*, H–C(4')); 3.86 (*m*, 2 H–C(5')); 3.17 (*s*, $MeSO_2$); 2.98 (*m*, H_α–C(2')); 2.83 (*m*, H_β–C(2')); 2.59 (*s*, MeS); 0.97 (*s*, *t*-Bu). Anal. calc. for $C_{29}H_{35}N_3O_5S_2Si$: C 58.26, H 5.90, N 7.03; found: C 58.03, H 5.95, N 7.01.

*1-(2',3'-Dideoxy-β-*D-glycero-*pent-2'-enofuranosyl)-4-(methylthio)-1*H-*imidazo[4,5-*c*]pyridine* (**3a**). To a soln. of **11** (600 mg, 1.0 mmol) in anh. THF (15 ml), 1M Bu_4NF in THF (7 ml) was added. The mixture was stirred at 50° for 2.5 h and then evaporated. The residue was applied to FC (column 4 × 10 cm, solvent *A*): colourless crystals (193 mg, 73%). M.p. 137–140° (MeOH). TLC (*A*): R_f 0.35. UV (MeOH): 283 (13300), 219 (16700).

^{1}H-NMR ((D$_6$)DMSO): 8.36 (*s*, H–C(2)); 8.24 (*d*, *J* = 5.7, H–C(6)); 7.53 (*d*, *J* = 5.7, H–C(7)); 7.04 (*m*, H–C(1′)); 6.54 (*d*, *J* = 5.7, H–C(3′)); 6.24 (*d*, *J* = 5.6, 2 H–C(2′)); 4.93 (*m*, H–C(4′), OH–C(5′)); 3.54 (*t*, 2 H–C(5′)); 2.60 (*s*, MeS). Anal. calc. for $C_{12}H_{13}N_3O_2S$: C 54.74, H 4.98, N 15.96; found: C 54.84, H 5.02, N 15.93.

*4-Amino-1-(2′-deoxy-β-*D*-*erythro*-pentofuranosyl)-1*H*-imidazo[4,5-c]pyridine* (**1**). A soln. of **13** (500 mg, 1.6 mmol) in 25% aq. NH_3 soln. (60 ml) and 30% H_2O_2 soln. (3 ml) was stirred in a closed vessel at 60° for 24 h. After cooling, the solvent was evaporated and the residue applied to FC (column 15 × 3 cm, solvent *F*). From the main zone, colourless crystals (187 mg, 47%) were isolated. M.p. 206–209° (H_2O; [2]: 209–211°).

Solid-Phase Synthesis of the Oligomers **14**–**18**. The syntheses were performed on a 1-μmol scale using the 3′-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 compound **4**. The synthesis of **14**–**18** followed the regular protocol of the DNA synthesizer for 3′-phosphonates [19]. The oligomers were recovered from the synthesizer as 5′-dimethoxytritylated derivatives. Deprotection of NH_2 groups was carried out by 25% NH_3/H_2O soln.: at 60° within 20 h for **15** and **18** and within 48 h for **14**, **16**, and **17**. The dimethoxytrityl residues of the oligomers were removed by treatment with 80% AcOH/H_2O for 10 min at r.t. Purification was accomplished by HPLC (see below) on *RP-18* columns using solvent system *I* for the (MeO)$_2$Tr derivatives of the oligonucleotides and solvent system *II* for the detritylated oligomers. The oligonucleotides were desalted on a 4 × 25 mm HPLC cartridge (*RP-18* silica gel) using H_2O (15 ml) for elution of the salt, while the oligomer was eluted with MeOH/H_2O 3:2 (5 ml). Each oligomer **14**–**18** was lyophilized and the colourless residue dissolved in H_2O (100 μl) and stored frozen at −25°. The yield was around 5 A_{260} units.

Enzymatic Hydrolysis of the Oligomers and Determination of the Cleavage Hypochromicity of **14**–**18**. The oligonucleotides (0.2 A_{260} units) were dissolved in 0.1M *Tris*-HCl buffer (pH 8.3; 200 μl) and treated with snake-venom phosphodiesterase (6 μg) at 37° for 45 min and with alkaline phosphatase (2 μg) for 30 min at 37°. The mixture was analyzed on reversed-phase HPLC (*RP-18*, solvent system *IV*; 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}: dA, 15400; dC, 7300; dG, 11700; dT, 8800; c^{3}A$_d$ (**1**), 10200). Values obtained from **14**–**18** were in agreement with calculated data. Hypochromicity values were determined by enzymatic digestion of 0.2 A_{260} units of the corresponding oligonucleotides as described above. The hypochromicity values were calculated using the equation: $h = [(\varepsilon_{simplex} - \varepsilon_{duplex}) \cdot (\varepsilon_{simplex})^{-1}] \cdot 100\%$. The extinction coefficient of the oligonucleotide was taken as the sum of the extinction coefficients of the constituent monomeric deoxynucleosides.

HPLC Separation. HPLC equipment: see [29]. Solvent systems: *A′*, 0.1M (Et_3NH)OAc/MeCN 95:5 (pH 8.0); *B′*, MeCN; *C′*, H_2O; *D′*, MeOH/H_2O 3:2. System *I*, 15 min 15–40% *B′* in *A′*; system *II*, 20 min 0–20% *B′* in *A′*; system *III*, 15 min *C′*, then 10 min *D′*; system *IV*, 100% *A′*; system *V*, 20 min 0–40% *B′* in *A′*; system *VI*, 15 min 0–20% *B′* in *A′*.

Oligomer Hydrolysis by the Endodeoxyribonuclease Eco*RI*. Experiments were carried out in 50 mM *Tris*-HCl buffer (100 μl, pH 7.5), containing 100 mM NaCl, 10 mM $MgCl_2$, and 1 mM dithioerythrol. The oligomer (0.4 A_{260} units) was incubated with endodeoxyribonuclease *Eco* RI (high concentration; 3 μl = 270 units). With respect to the T_m value (compd. **17**, 34°) of the modified oligomer, the mixtures were held at 23°. The reaction was monitored by HPLC with solvent system *VI* (see HPLC separation).

REFERENCES

[1] T. Itoh, T. Yamaguchi, Y. Mizuno, *J. Carbohydr. Nucleos. Nucleot.* **1981,** *8,* 119.
[2] F. Seela, H. Rosemeyer, S. Fischer, *Helv. Chim. Acta* **1990,** *73,* 1602.
[3] P. Serafinowski, *Synthesis* **1990,** 757.
[4] Z. Kazimierczuk, H. B. Cottam, G. R. Revankar, R. K. Robins, *J. Am. Chem. Soc.* **1984,** *106,* 6379.
[5] R. Cosstick, X. Li, D. K. Tuli, D. M. Williams, B. A. Connolly, P. C. Newman, *Nucleic Acids Res.* **1990,** *18,* 4771.
[6] A. Ono, T. Ueda, *Nucleic Acids Res.* **1987,** *15,* 3059.
[7] F. Seela, T. Grein, *Nucleic Acids Res.* **1992,** *20,* 2297.
[8] P. Franchetti, L. Cappellacci, G. Cristalli, M. Grifantini, A. Pani, P. La Colla, G. Nocentini, *Nucleos. Nucleot.* **1991,** *10,* 1551.
[9] K. B. De Roos, C. A. Salemink, *Recl. Trav. Chim. Pays-Bas* **1971,** *90,* 1166.
[10] Y. Mizuno, T. Itoh, K. Saito, *Chem. Pharm. Bull.* **1964,** *12,* 866.
[11] R. J. Rousseau, R. K. Robins, *J. Heterocycl. Chem.* **1965,** *2,* 196.
[12] M. Hoffer, *Chem. Ber.* **1960,** *93,* 2777.
[13] H. D. Winkeler, F. Seela, *J. Org. Chem.* **1983,** *48,* 3119.

[14] F. Seela, B. Westermann, U. Bindig, *J. Chem. Soc., Perkin Trans. 1* **1988,** 697.
[15] G. J. Soula, *J. Org. Chem.* **1985,** *50,* 3717.
[16] H. Rosemeyer, G. Tóth, F. Seela, *Nucleos. Nucleot.* **1989,** *8*(4), 587.
[17] G. B. Barlin, M. D. Fenn, *Aust. J. Chem.* **1981,** *34,* 1341.
[18] L. Hagenberg, H. G. Gassen, H. Matthaei, *Biochem. Biophys. Res. Commun.* **1973,** *50,* 1104.
[19] *Applied Biosystems,* 'User Bulletin', Weiterstadt, Germany, 1987, p. 44.
[20] F. Seela, A. Kehne, *Biochemistry* **1987,** *26,* 2232.
[21] H. Rosemeyer, M. Krečmerova, F. Seela, *Helv. Chim. Acta* **1991,** *74,* 2054.
[22] I. E. Scheffler, E. L. Elson, R. L. Baldwin, *J. Mol. Biol.* **1968,** *36,* 291.
[23] I. E. Scheffler, E. L. Elson, R. L. Baldwin, *J. Mol. Biol.* **1970,** *48,* 145.
[24] R. L. Baldwin, *Acc. Chem. Res.* **1971,** *4,* 265.
[25] D. B. Haniford, D. E. Pulleyblank, *Nucleic Acids Res.* **1985,** *13,* 4343.
[26] J. A. McClellan, E. Palecek, D. M. J. Lilley, *Nucleic Acids Res.* **1986,** *14,* 9291.
[27] J. Kehrhahn, University of Osnabrück.
[28] F. Seela, A. Röling, *Nucleic Acids Res.* **1992,** *20,* 55.
[29] F. Seela, K. Kaiser, U. Bindig, *Helv. Chim. Acta* **1989,** *72,* 868.
[30] F. Seela, T. Wenzel, *Helv. Chim. Acta* **1992,** *75,* 1111.