

152. *N*⁶-(Carbamoylmethyl)-2'-deoxyadenosine, a Rare DNA Constituent: Phosphoramidite Synthesis and Properties of Palindromic Dodecanucleotides

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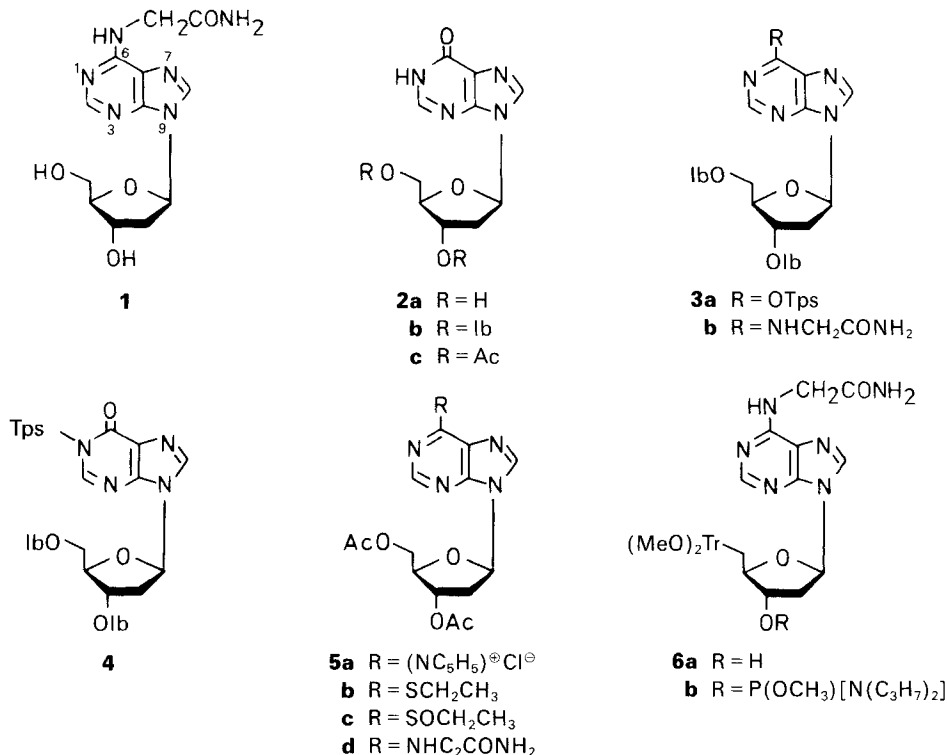
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*N*⁶-(Carbamoylmethyl)-2'-deoxyadenosine (**1**), a modified nucleoside occurring in bacteriophage Mu, was synthesized by two different routes. Glycinamide was introduced by nucleophilic displacement of (2,4,6-triisopropylphenyl)sulfonyloxy or ethylsulfinyl groups at C(6) of the purine moiety. Compound **1** was converted into the protected phosphoramidite **6b** and employed in solid-phase synthesis of the self-complementary oligonucleotides **7–14**. Replacement of 2'-deoxyadenosine by **1** led to a strong decrease of the *T*_m values of the oligomers d(A-T)₆ (**7**) and d(A-T-G-A-A-G-C-T-T-C-A-T) (**10**), respectively. As the oligomer **10** contains the recognition site d(A-A-G-C-T-T) of the endodeoxyribonuclease HindIII, it was subjected to sequence-specific hydrolysis experiments. Replacement of the first or second A_d by **1** prevented enzymatic phosphodiester hydrolysis (results with **11** and **12**). In contrast, slow hydrolysis was observed if the less bulky *N*⁶-methyl-2'-deoxyadenosine replaced the second A_d residue (results with **14**).

Introduction. – DNA of *Escherichia coli* bacteriophage Mu is stable to several endodeoxyribonucleases [1] [2]. This phenomenon requires at least two genes, namely the *mom*⁺ gene of the phage Mu and the host *E. coli* *dam*⁺ gene. From the *mom*⁺ gene a protein is encoded which modifies up to 15% of phage DNA adenine residues [3]. This modification occurs exclusively within the sequence d(C/G-A-G/C-N-Y) and is positively controlled by the *dam*⁺ methyltransferase [4]. The modified adenosine base (designated as xA) observed during mild acid hydrolysis was believed to contain a free carboxyl group. Later mass spectrometric analysis identified the structure as *N*⁶-(carbamoylmethyl)-2'-deoxyadenosine (**1**; x⁶A_d) [3].

In earlier experiments, we have employed short synthetic DNA fragments of 8 to 12 base pairs length to study the influence of rare or modified nucleosides on duplex stability and recognition by endodeoxyribonucleases, in particular that of EcoRI [5] [6]. Now, we focused our interest on the rare 2'-deoxyribonucleoside **1**, its incorporation into oligonucleotides, and the restriction of phosphodiester hydrolysis by the endodeoxyribonuclease HindIII. For this purpose, two new routes for the synthesis of **1** are described. One of these routes allowed large-scale preparation which was necessary to obtain the appropriately protected phosphoramidite **6b**. This compound was used in oligonucleotide synthesis to yield self-complementary dodecanucleotides. They were subject to physical studies with respect to duplex stability and to hydrolysis by the endodeoxyribonuclease HindIII.



Ib = Isobutyryl; Tps = (2,4,6-triisopropylphenyl)sulfonyl; (MeO)₂Tr = dimethoxytrityl

Synthesis of *N*⁶-(Carbamoylmethyl)-2'-deoxyadenosine (1). – A known route of synthesis for the modified nucleoside **1** uses 6-chloro-9-(β-D-2'-deoxyribofuranosyl)-9*H*-purine [3] as reactive intermediate, which can be obtained from 2'-deoxyinosine (**2a**) by the action of thionyl chloride in DMF [7] or by the action of *tert*-butyl nitrite in CCl₄ [8]. As the synthesis of the 6-chloro compound did not lead to reproducible yields, we looked for a different procedure using other reactive substituents at C(6) of purine, which should allow to introduce glycinamide by a nucleophilic displacement reaction.

Starting material for the synthesis of **1** was 2'-deoxyinosine (**2a**) which was obtained from 2'-deoxyadenosine in 87% yield by deamination with adenosine deaminase. After protection of the 3'- and 5'-OH groups with isobutyryl or acetyl residues, compounds **2b** and **2c** were obtained in yields of 92 and 85%, respectively. The glycinamide residue was now introduced on two different routes.

At first, 3',5'-isobutyrylated 2'-deoxyinosine **2b** was treated with 2,4,6-triisopropylphenylsulfonyl chloride (TpsCl) in the presence of base. The reaction was followed by TLC and was complete within 5 min. The products were separated by flash chromatography to give **3a** and **4** in a ratio of nearly 1:1. This was in contrast to the reaction with 2'-deoxyguanosine where only one reaction product was formed [9].

¹H- and ¹³C-NMR spectra of the products obtained from the slow and the fast migrating zone showed the typical signals of the 2'-deoxyribofuranosyl and the adenine moieties as well as those of the (2,4,6-triisopropyl-

Table 1. ^{13}C -NMR Chemical Shifts of Precursor Molecules of N^6 -(Carbamoylmethyl)-2'-deoxyadenosine (**1**) and Precursor Derivatives

	C(2)	C(4)	C(5)	C(6)	C(8)	C(1')	C(2')	C(3')	C(4')	C(5')	CH(aryl)	CH ₃ (aryl)
2a^b	145.8	147.8	124.4	156.6	138.5	83.5	39.5	70.6	87.9	61.6		
2b^b	145.4	148.6	125.3	158.9	138.0	82.9	38.1	74.1	84.8	63.5		
2c^a	145.9	148.0	124.6	156.5	138.6	81.7	35.7	74.2	83.6	63.5		
3a^b	151.5	153.7	123.7	154.3	142.6	83.1	37.8	74.0	85.1	63.4	34.2	23.2–24.5
4^b	142.9	146.4	125.2	155.3	138.4	83.1	38.0	74.0	84.9	63.4	29.4	23.3–24.3
5a^c	155.2	146.7	125.7	150.5	155.2	82.8	36.3	74.6	85.6	64.1		
5b^b	147.9	152.0	131.9	161.9	140.6	82.7	37.5	74.5	84.7	63.7		
5c^b	151.23	152.56	131.26	162.07	144.26	82.85	37.37	74.13	85.00	63.54		
		152.56	131.36	162.11	144.46	82.89	37.60	74.21	85.15	63.59		
	C=O	CH ₃ CO	CH ₂ S	CH (aliph.)	CH ₃ (aliph.)	CH (pyridinio)						
2b^b	176.2, 176.4			33.8, 33.9	18.7–18.9							
2c^a	170.0, 170.1	20.5, 20.8										
3a^b	176.1, 176.3			33.8, 33.8	18.7–18.8							
4^b	176.1, 176.3			33.8, 33.8	18.7–18.8							
5a^c	173.7, 173.8	20.5, 20.7				128.6, 143.5, 148.1						
5b^b	170.3, 170.4	20.8, 20.9	23.3		14.8							
5c^b	170.28 ^d	20.78	47.11		6.30							
	170.32 ^d	20.92	47.13		6.33							

^a) In (D₆)DMSO. ^b) In CDCl₃. ^c) In D₂O. ^d) Assignment to diastereoisomers tentatively.

phenyl) group (Table 1). It has been reported that in heterocycles [10], ^{13}C -NMR signals of C-atoms located in α -position to a NH function are shifted downfield if the NH proton tautomerizes to other positions except to C(α). Whereas the slowly migrating compound, as compared to the starting material **2b**, exhibited only minor shifts of the ^{13}C -NMR signals of the base moiety, the faster migrating compound showed a strong downfield shift of the C(2) signal (+ 5.9 ppm, Table 1). According to this, the faster migrating material was assigned structure **3a** and the slower one structure **4**.

Treatment of **3a** with a 10-fold excess of glycineamide yielded **3b** exclusively. The regioisomer **4** did not react with the amino-acid amide. ^1H - and ^{13}C -NMR spectra (Table 2), and elemental analysis identified the reaction product as compound **3b** having a glycineamide group at C(6). After removal of the isobutyryl residues with conc. NH_3 , compound **1** was obtained as colorless crystals exhibiting identical ^1H -NMR data as reported earlier [3]. Due to the nonregioselective sulfonylation, the total yield of this route based on **2a** as starting material was only 28%. As a result, we looked for a more efficient synthesis.

The 2-chlorophenyl phosphodichloridate has been widely employed as coupling reagent in the phosphotriester synthesis of oligonucleotides [11]. However, several side products have been observed in the case of nucleosides with lactam moieties [12]. *Adamiak et al.* have isolated pyridinium derivatives as intermediates [13]. These pyridinium salts were stable in the case of the pyrimidine and purine ribofuranosides as well as of the pyrimidine 2'-deoxyribofuranosides [13].

We now have shown that also a purine 2'-deoxyribofuranoside such as compound **2c** can be isolated as its pyridinium derivative **5a** according to the procedure of *Adamiak et al.* [13]. The product was purified by flash chromatography and its structure confirmed by ^1H - and ^{13}C -NMR spectroscopy (Table 1). As reported for the pyridinium salt resulting from inosine, the deoxynucleoside **5a** also showed strong fluorescence.

Compound **5a** was stable for several days at 4°. Attempts to exchange the pyridinium residue against glycnamide failed; due to the *Zincke* reaction [14] which was initiated by glycnamide 2'-deoxyadenosine was formed. To overcome this difficulty, a 6-ethylthio group was introduced as a temporary substituent yielding compound **5b** after a nucleophilic displacement reaction. Unfortunately, the two-step procedure **2c**→**5a**→**5b** with the isolation of **5a** resulted in a moderate overall yield. Therefore, **2c** was converted into **5b** without isolation of **5a**; this increased the total yield of **5b** to 83%.

Oxidation of **5b** with *m*-chloroperbenzoic acid in EtOH led to a uniform product which was purified by flash chromatography to give **5c** in 78% yield. Elemental analysis proved the conversion of **5b** into a sulfoxide, further oxidation to the sulfone as reported for methylthio derivatives [15] was not observed. In keeping with the monooxidation of **5b**, the sulfoxide **5c** was a diastereoisomeric mixture. As deduced from the ¹H- and ¹³C-NMR spectra (Table 1) which exhibited 2 signals for nearly each C- or H-atom in a 1:1 ratio, the oxidation was not stereoselective under these conditions.

The exchange of the ethylsulfinyl group of **5c** against glycnamide occurred in dry DMF at 80° within 1 h. Flash chromatography gave **5d** which crystallized from EtOH/Et₂O. ¹H- and ¹³C-NMR spectroscopy confirmed its structure. The OH-protecting groups

Table 2. ¹³C-NMR Chemical Shifts of N⁶-(Carbamoylmethyl)-2'-deoxyadenosine (**1**) and 2'-Deoxyadenosine Derivatives in CDCl₃

	C(2)	C(4)	C(5)	C(6)	C(8)	C(1')	C(2')	C(3')	C(4')	C(5')	CH ₂ N	CONH ₂
1 ^{a)}	152.0	148.6	119.7	154.5	139.4	83.9	39.6	70.8	87.9	61.8	43.2	171.0
dA ^{a)}	152.2	149.3	119.6	156.3	140.2	85.8	40.2	70.8	87.8	61.7		
3b	153.0	149.3	120.5	154.5	138.5	82.8	37.6	74.2	84.6	63.6	44.5	172.1
5d	153.1	149.2	120.5	154.4	138.7	82.5	37.4	74.6	84.6	63.8	44.5	172.2
6a	152.9	149.3	120.4	154.4	139.0	84.4	40.3	72.4	86.2	63.9	44.5	172.3
							39.7	73.9	86.1	63.7		
6b	152.9	149.4	120.5	154.5	139.0	84.7			85.9		44.6	172.3
							39.7	73.4	84.8	63.5		
	C=O		CH ₃ CO		CH ₃ O		C(aryl)		CH ₃ OP			
5d	170.4, 170.6		20.8, 21.0									
6a					55.2		126.9–130.1					
6b					55.2		126.8–135.6		50.3, 50.6			

^{a)} In (D₆)DMSO.

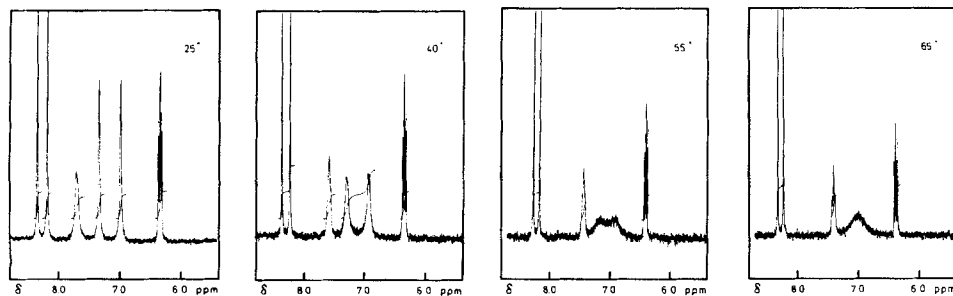


Fig. 1. Temperature-dependent ¹H-NMR spectra of **1** in (D₆)DMSO. The downfield part of the spectrum is shown, with the signals of H-C(8), H-C(2), H-C(1'), and the amide protons.

were split off by the action of conc. aq. ammonia yielding nucleoside **1** as colorless crystals in 82%. The overall yield of the conversion of **2c** into **1** was 40%.

The ^{13}C -NMR spectrum of **1** showed no significant shifts for the heterocyclic moiety in relation to 2'-deoxyadenosine and its N^6 -methyl derivative, but two additional signals for the CH_2N group (43.2 ppm) and the carboxamide function (171.0 ppm) (Table 2). In the ^1H -NMR spectrum, however, the amide protons showed 2 well separated signals at 25°. These signals were broadened at higher temperature and showed complete coalescence at 65° (Fig. 1).

Synthesis of the Self-Complementary Oligonucleotides 7–14 Employing the Phosphoramidite 6b. – To study the influence of compound **1** on duplex structure and recognition of the endodeoxyribonuclease HindIII [16], the self-complementary oligomers **7–12** were synthesized. For this purpose, the 5'-OH group of **1** was protected with 4,4'-dimethoxytrityl chloride to give **6a** in 83% yield. As shown by Ikehara and co-workers [17] for 2'-deoxy- N^6 -methyladenosine, the protection of the secondary 6-amino function was not necessary for oligonucleotide synthesis by the phosphotriester approach on solid support [15]. We also omitted the protection of the secondary methylamino group of **6a**. However, treatment of **6a** with chloro(diisopropylamino)methoxyphosphine [18] yielded several side products. As a consequence, phosphinylation of **6a** was carried out with the less reactive bis(diisopropylamino)methoxyphosphine and an equimolar amount of triethylammonium tetrazolide [19]; thus, the phosphoramidite **6b** was isolated in 71% after purification.

7	d(A-T) ₆	11	d(A-T-G- x^6 A-A-G-C-T-T-C-A-T)
8	d(A-T-A-T-A-T- x^6 A-T-A-T-A-T)	12	d(A-T-G-A- x^6 A-G-C-T-T-C-A-T)
9	d(x^6 A-T) ₆	13	d(A-T-G- m^6 A-A-G-C-T-T-C-A-T)
10	d(A-T-G-A-A-G-C-T-T-C-A-T)	14	d(A-T-G-A- m^6 A-A-G-C-T-T-C-A-T)

$x^6\text{A}_d$ = N^6 -(Carbamoylmethyl)-2'-deoxyadenosine (**1**)

$m^6\text{A}_d$ = 2'-Deoxy- N^6 -methyladenosine

The synthesis of the oligonucleotides **7–14** was carried out on an automated DNA synthesizer using the regular methyl phosphoramidites [18], the modified phosphoramidite **6b**, or the methylphosphoramidite of 2'-deoxy- N^6 -methyladenosine [17]. The fully protected oligomers were detritylated on solid support, treated with thiophenol, and split off from CPG by the action of conc. NH_3 . After hydrolysis of the base-protecting groups in the same medium at 60° for 16 h, the product was purified on reverse-phase HPLC and desalted yielding single-peak products. Analysis of the oligonucleotides was accomplished on the nucleoside composition after hydrolysis with snake-venom phosphodiesterase and alkaline phosphatase. The separation of the cleavage products was performed on reverse-phase HPLC and compared with the authentic 2'-deoxy-ribofuranosides.

The separation of 2'-deoxyadenosine and its N^6 -derivative **1** was difficult, since this modification resulted in nearly identical HPLC retention times on reverse-phase silica. This problem was solved by using the solvent system II which contained less MeCN (Fig. 2).

The analysis of the unmodified oligomers **7** and **10** and of the N^6 -methyladenine-modified compounds **13** and **14** agreed well with the calculated contents of nucleosides. However, the analysis of oligomers **8**, **9**, **11**, and **12** showed a reduced content of **1** in relation to the calculated values. A significant amount of a side product with a lowered

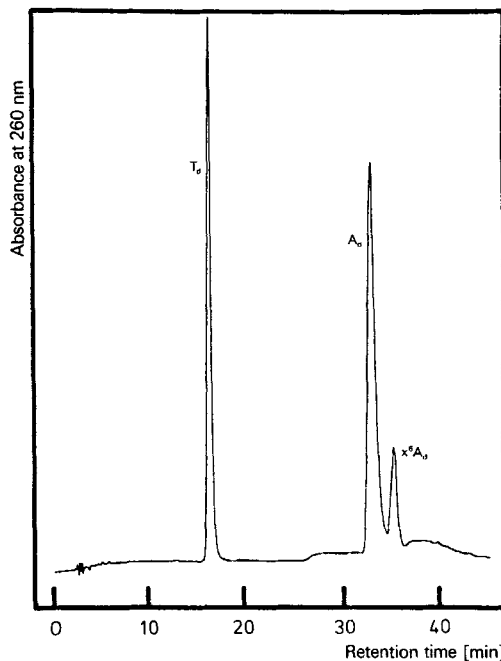


Fig. 2. HPLC profile of phosphodiester-hydrolysis products of the oligomer **8** after treatment with snake-venom phosphodiesterase and alkaline phosphatase. Conditions, see *Exper. Part*; $x^*A_0 = N^6$ -(carbamoylmethyl)-2'-deoxyadenosine (**1**).

retention time (43 min) was formed. The side reaction might be due to the formation of an imidazolidone ring system, generated by ring closure between the amide group and a carbonyl function of an N^6 -acylated compound, the latter being formed by the reaction with Ac_2O during the capping procedure. To overcome this side reaction, we omitted the capping procedure during synthesis of the oligonucleotides containing compound **1** and increased the coupling time for the phosphoramidites. As a result, no side reaction took place and HPLC analysis of the hydrolysed oligonucleotides gave the calculated nucleoside content.

Physical Properties of the Oligonucleotides 7–14 and Influence of 1 on Endodeoxyribonuclease HindIII Cleavage. – Studies of *Hattman et al.* [4] showed that high molecular weight DNA with a 15% modification of 2'-deoxyadenosine by compound **1** exhibited a decrease in the melting temperature T_m of *ca.* 5° compared to unmodified DNA. In order to study this phenomenon on short oligonucleotides, we compared the melting profiles of $d(A-T)_6$ (**7**) with that of **8** containing one modified nucleoside **1** instead of 2'-deoxyadenosine (17% A_0 modification). Under the conditions described in *Table 3*, a T_m value below 15° was observed for **8** compared to 26° for $d(A-T)_6$ (**7**) [20]. Replacement of all adenine bases ($\rightarrow$ **9**) resulted in single-stranded oligomers which did not form duplexes at all under those experimental conditions.

Similar experiments by *Ono and Ueda* [21] showed that replacement of one adenine base by a N^6 -methyladenine residue within a decamer also affected the T_m value very strongly, even on modification of only one of the strands ($d(C-C-A-G-A-T-C-T-C-$

Table 3. T_m Values of the Oligonucleotides 7–14

Compd.	Oligomer ^{a)}	T_m [°]
7 ^{b)}	d(A-T) ₆	26
8 ^{b)}	d(A-T-A-T-A-T-A-x ⁶ A-T-A-T-A-T)	< 15
9 ^{b)}	d(x ⁶ A-T) ₆	–
10 ^{c)}	d(A-T-G-A-A-G-C-T-T-C-A-T)	47
11 ^{c)}	d(A-T-G-x ⁶ A-A-G-C-T-T-C-A-T)	32
12 ^{c)}	d(A-T-G-A-x ⁶ A-G-C-T-T-C-A-T)	32
13 ^{c)}	d(A-T-G-m ⁶ A-A-G-C-T-T-C-A-T)	41
14 ^{c)}	d(A-T-G-A-m ⁶ A-G-C-T-T-C-A-T)	41

a) x⁶A_d = N⁶-(Carbamoylmethyl)-2'-deoxyadenosine (1); m⁶A_d = 2'-deoxy-N⁶-methyladenosine.

b) In 10 mM Tris-HCl, pH 7.5, 20 mM MgCl₂, 80 mM NaCl.

c) In 50 mM Tris-HCl, pH 8.0, containing 10 mM MgCl₂ and 50 mM NaCl. The oligomer concentration was ca. 3 μM on single strands.

C)·d(G-G-A-G-A-T-C-T-G-G), T_m 40°; d(C-C-A-G-m⁶T-C-T-C-C)·d(G-G-A-G-A-T-C-T-G-G), T_m 27°). This strong decrease of the T_m value may be explained by (i) an altered helix structure induced by the methylated adenine moiety [22]; (ii) a shift of the single-strand-duplex equilibrium towards the single-stranded form, but without significant change of the duplex structure [23].

Melting experiments with the oligonucleotides **10**, **13**, and **14** under cleavage conditions showed that the incorporation of 2'-deoxy-N⁶-methyladenosine decreased the T_m from 47° for **10** to 41° for **13** and **14** (Table 3). The T_m values for the corresponding oligomers **11** and **12** (containing **1**) were found to be 32°. The larger decrease of the T_m values of **11** and **12** (15°) compared with that of **13** and **14** (6°) may be due to the bulkiness of the amino-acid substituent. This can affect both the association kinetics of the single strands as well as the helix structure.

As the Me group of N⁶-methyladenine is located in the major groove of double-stranded DNA, potential binding sites for endonucleases are blocked [24]. The same was expected for DNA fragments containing compound **1**. Former studies of our laboratory on oligomers containing 8 to 12 nucleoside residues, which represented recognition sequences of endodeoxyribonucleases, showed that these oligomers were useful probes to study the interaction of an endodeoxyribonuclease with its cognate DNA fragment [5] [6].

We focused our interest on the oligomers **10–14** containing the HindIII recognition site d(A-A-G-C-T-T) [20]. For comparative cleavage experiments, the following dodecamers were studied: (i) the oligomer **10** containing the non-modified recognition site of HindIII, (ii) the oligomers **11** and **12** with x⁶A_d (**1**) instead of the first or second A_d residue within the recognition site, and (iii) the oligomers **13** and **14** having either the first or second A_d residue replaced by 2'-deoxy-N⁶-methyladenosine (m⁶A_d) within d(A-A-G-C-T-T). As the cleavage of oligonucleotides by restriction endonucleases like HindIII requires duplex structures, the cleavage experiments were carried out at 25° in order to ensure duplex formation under cleavage conditions. At 25°, the unmodified oligonucleotide **10** was cleaved within 16 h into fragments d(A-T-G-A) and d(pA-G-C-T-T-C-A-T) (Fig. 3). They were identified after hydrolysis with snake-venom phosphodiesterase and subsequently with alkaline phosphatase. Under the same conditions, both modified oligonucleotides **11** and **12** (containing **1**) were not cleaved by HindIII within 72 h. Under

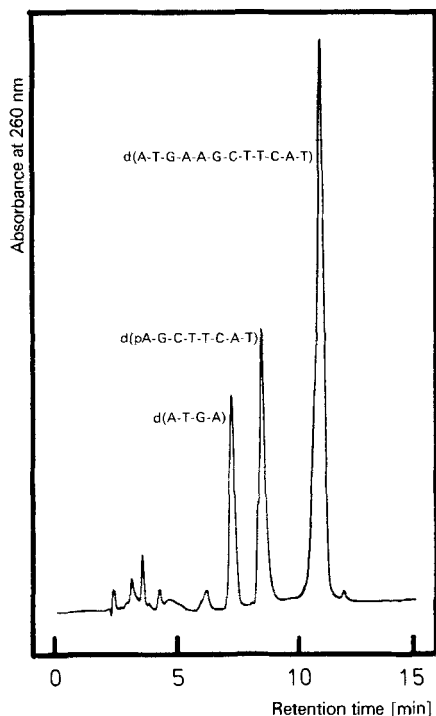


Fig. 3. HPLC profile of phosphodiester-hydrolysis products of the oligomer **10** after treatment with the endodeoxyribonuclease *Hind*III. For conditions, see *Exper. Part*.

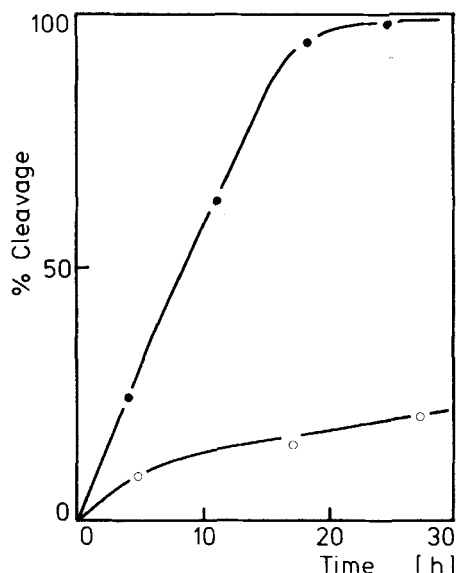


Fig. 4. Time course of phosphodiester hydrolysis of the oligomers *d*(A-T-G-A-A-G-C-T-T-C-A-T) (**10**); ● and *d*(A-T-G-A-*m*⁶A-G-C-T-T-C-A-T) (**14**); ○ on treatment with the endodeoxyribonuclease *Hind*III. For conditions, see *Exper. Part*.

the same conditions, **13** was not hydrolyzed by *Hind*III, whereas **14** was cleaved slowly by the enzyme (Fig. 4).

The fact that *d*(A-T-G-*x*⁶A-A-G-C-T-T-C-A-T) (**11**) was stable against *Hind*III cleavage was not surprising, as methylation at the amino group of the first adenine base ($\rightarrow$ **13**) prevents cleavage by the endonuclease *Hind*III [16]. The resistance of **12** with the modified nucleoside **1** replacing the second 2'-deoxyadenosine of the recognition site was rather unexpected, since methylation at this position ($\rightarrow$ **14**) only increased cleavage time. One explanation for this finding might be the bulkiness of the glycinamide moiety compared to that of the Me group. Due to this, the interaction of the endonuclease with **12** is more hindered than with **14**. Future studies have to explore the biosynthetic mechanism of the formation of compound **1** and other properties of this residue with respect to DNA structure and function.

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Experimental Part

General. CH_2Cl_2 and MeCN were distilled from CaH_2 and stored over 3-Å molecular sieves. Tetrazole and 4-(dimethylamino)pyridine were sublimated under reduced pressure. The phosphoramidites were prepared from the 5'-tritylated and appropriately base-protected nucleosides $[(\text{MeO})_2\text{Tr}]\text{bzAd}$, $[(\text{MeO})_2\text{Tr}]\text{ibGd}$, $[(\text{MeO})_2\text{Tr}]\text{bzCd}$, and $[(\text{MeO})_2\text{Tr}]\text{Td}$ (Biosyntech, Hamburg, FRG) according to the procedure of McBride and Caruthers [18]. The 2'-deoxyinosine (**2a**) was prepared enzymatically by the action of adenosine deaminase (EC 3.5.4.4, Boehringer, Mannheim, FRG) from 2'-deoxyadenosine (Biomol, Ilvesheim, FRG). Endodeoxyribonuclease HindIII (EC 3.1.23.20) was purchased from Boehringer (Mannheim, FRG). Glycinamide was prepared from glycynamide hydrochloride by ion-exchange chromatography on Dowex ion-exchange resin (OH^- form). Fractosil 500 polymeric support was purchased from Biosyntech (Hamburg, FRG). Bis(diisopropylamino)methoxyphosphine and triethylammonium tetrazolide were prepared as described [19] and stored at -18° . Oligonucleotide synthesis was carried out on an automated DNA synthesizer, model 380B, of Applied Biosystems (Weiterstadt, FRG). Column flash chromatography: 0.5 bar, silica gel 60-H (Merck, FRG); solvent proportions in v/v ; UV detector (254 nm). Reverse-phase HPLC: prepacked columns (Merck, FRG; LiChrosorb RP-18, 4×250 , 7 μm), connected with a RP-18 4×25 precolumn, Hitachi-Merck HPLC with one pump (model 655-12) connected with a proportioning valve, a variable wavelength monitor (model 655A), a controller (model L-5000), and an integrator (model D-2000); solvent systems consisting of 0.1M Et_3NHOAc of pH 7.0 (A), 0.1M Et_3NHOAc of pH 7.0 containing 5% MeCN (B), and MeCN (C) were used in the following order: system I: 10–25% C in B for 10 min; system II: 40% B in A for 20 min, 60% B in A for 10 min, 100% B for 15 min; flow rates for I and II 1 ml/min. M.p.: Linstrom apparatus (Wagner & Munz, München, FRG). Melting curves: Teflon-stoppered cuvettes with 10 mm light path length in a thermostatically controlled cell holder; Shimadzu-210-A recording spectrophotometer connected with an IBM-XT computer; the increase of absorbance at 260 nm was recorded while the temp. of the soln. was increased linearly with time at a rate of $20^\circ/\text{h}$ using a Lauda-PM-351 programmer and a Lauda RCS-6 bath equipped with a R-22 unit (MWG Lauda, Lauda-Königshofen, FRG); the actual temp. was measured in the probe cell with a Pt-100 resistor. UV: Uvicon-180 spectrophotometer (Kontron; Switzerland) and Hitachi-150-20 spectrophotometer (Hitachi, Japan). NMR: Bruker-AC-250 and Bruker-WM-250 spectrometers; δ in ppm rel. to 85% H_3PO_4 for ^{31}P and to Me_4Si for ^1H and ^{13}C , positive δ if downfield with respect to the standard. Elemental analyses: Mikroanalytisches Labor Beller (Göttingen, FRG).

9-(2'-Deoxy-3',5'-di-O-isobutyril- β -D-erythro-pentofuranosyl)-1H-purin-6(9H)-one (**2b**). To a soln. of 2'-deoxyinosine (**2a**; 2.0 g, 7.9 mmol) in anhyd. pyridine (50 ml), isobutyric anhydride (2.5 g, 25.5 mmol) was added. After stirring for 1 h at r.t., the soln. was poured into 10% aq. NaHCO_3 soln. (100 ml). The aq. layer was saturated with NaCl and extracted with CH_2Cl_2 (5×100 ml). The org. layers were dried (Na_2SO_4), evaporated, and coevaporated with toluene (2×20 ml). The residue was crystallized from $\text{EtOH}/\text{Et}_2\text{O}$: **2b** (2.85 g, 92%), colorless needles. M.p. 175° . TLC (silica gel, $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9:1): R_f 0.6. UV (MeOH): 244 (12300), 249 (12200). $^1\text{H-NMR}$ (CDCl_3): 1.17 (m, 4 CH_3); 2.33 (m, $\text{H}_\beta\text{-C}(2')$, 2 CH); 2.84 (m, $\text{H}_\alpha\text{-C}(2')$); 4.33 (m, $\text{H-C}(4')$, 2 $\text{H-C}(5')$); 5.38 (m, $\text{H-C}(3')$); 6.37 (dd, $J = 6.0$, 7.9, $\text{H-C}(1')$); 8.01 (s, $\text{H-C}(8)$); 8.18 (s, $\text{H-C}(2)$); 11.3 (s, NH). Anal. calc. for $\text{C}_{18}\text{H}_{24}\text{N}_4\text{O}_6$: C 55.10, H 6.16, N 14.28; found: C 55.14, H 6.25, N 14.40.

9-(2'-Deoxy-3',5'-di-O-isobutyril- β -D-erythro-pentofuranosyl)-6-O-[(2,4,6-triisopropylphenyl)sulfonyloxy]-9H-purine (**3a**) and 9-(2'-Deoxy-3',5'-di-O-isobutyril- β -D-erythro-pentofuranosyl)-1-[(2,4,6-triisopropylphenyl)sulfonyl]-1H-purine-6(9H)-one (**4**). Compound **2b** (2.00 g, 5.10 mmol) in anhyd. CH_2Cl_2 (20 ml) was treated with 2,4,6-triisopropylphenylsulfonyl chloride (3.10 g, 10.2 mmol) for 5 min in the presence of Et_3N (3 ml) and 4-(dimethylamino)pyridine (62 mg, 0.51 mmol). The mixture was diluted with toluene (10 ml) and evaporated. The oily residue was dissolved in CH_2Cl_2 and separated by flash chromatography (column 25×4 cm, $\text{CH}_2\text{Cl}_2/\text{acetone}$ 975:25). From the faster migrating zone, **3a** was obtained as a colorless foam (1.55 g, 46%). TLC (silica gel, $\text{CH}_2\text{Cl}_2/\text{acetone}$ 95:5): R_f 0.6. UV (CH_2Cl_2): 240 (17000), 255 (sh, 12300), 285 (sh, 2100). $^1\text{H-NMR}$ (CDCl_3): 1.18 (m, 10 CH_3); 2.58 (m, $\text{H}_\beta\text{-C}(2')$, 2 CH); 2.90 (m, $\text{H}_\alpha\text{-C}(2')$), (CH_3)₂CH-C(arom.); 4.32 (m, 2 (CH_3)₂CH-C(arom.), $\text{H-C}(4')$, 2 $\text{H-C}(5')$); 5.41 (m, $J = 6.0$, 7.9, $\text{H-C}(1')$); 7.20 (s, 2 arom. H); 8.22 (s, $\text{H-C}(8)$); 8.56 (s, $\text{H-C}(2)$). Anal. calc. for $\text{C}_{33}\text{H}_{46}\text{N}_4\text{O}_8$: C 60.17, H 7.04, N 8.50; found: C 60.54, H 7.20, N 8.47.

After evaporation of the second zone, **4** was obtained as a colorless foam (1.45 g, 43%). TLC (silica gel, $\text{CH}_2\text{Cl}_2/\text{acetone}$ 95:5): R_f 0.4. UV (CH_2Cl_2): 243 (22000), 285 (6700). $^1\text{H-NMR}$ (CDCl_3): 1.19 (m, 10 CH_3); 2.51–2.90 (m, 2 $\text{H-C}(2')$, 2 CH, (CH_3)₂CH-C(arom.)); 4.08 (sept., 2 (CH_3)₂CH-C(arom.)); 4.33 (m, $\text{H-C}(4')$, 2 $\text{H-C}(5')$); 5.10 (m, $\text{H-C}(3')$); 6.34 (dd, $J = 6.0$, 7.9, $\text{H-C}(1')$); 7.16 (s, 2 arom. H); 7.91 (s, $\text{H-C}(8)$); 8.84 (s, $\text{H-C}(2)$). Anal. calc. for $\text{C}_{33}\text{H}_{46}\text{N}_4\text{O}_8$: C 60.17, H 7.04, N 8.50; found: C 60.10, H 7.07, N 8.50.

N^6 -(Carbamoylmethyl)-2'-deoxy-3',5'-di-O-isobutyriladenosine (**3b**). Compound **3a** (2.95 g, 4.48 mmol) and glycynamide (3.00 g, 41 mmol) in dry DMF were stirred at 80° for 2 h. The solvent was removed, and after

co-evaporation with toluene (2×20 ml), the residue was purified by flash chromatography (column 25×3 cm, $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 96:4). The main zone was collected and evaporated. Compound **3b** crystallized from Et_2O in colorless needles (1.69 g, 84%). M.p. 115° . TLC (silica gel, $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9:1): R_f 0.4. UV (MeOH): 265 (16800). $^1\text{H-NMR}$ (CDCl_3): 1.14 (*dd*, 2 CH_3); 1.19 (*d*, 2 CH_3); 2.57 (*m*, $\text{H}_b\text{-C}(2')$, 2 CH); 2.90 (*m*, $\text{H}_a\text{-C}(2')$); 4.35 (*m*, $\text{H-C}(4')$, 2 $\text{H-C}(5')$, CH_2N); 5.39 (*m*, $\text{H-C}(3')$); 5.63 (*br. s*, NH); 6.31 (*br. s*, NH); 6.40 (*dd*, $J = 5.9, 8.1$, $\text{H-C}(1')$); 6.63 (*t*, $J = 5.8$, NHCH_2); 7.99 (*s*, $\text{H-C}(8)$); 8.39 (*s*, $\text{H-C}(2)$). Anal. calc. for $\text{C}_{20}\text{H}_{28}\text{N}_6\text{O}_6$: C 53.56, H 6.29, N 18.74; found: C 53.42, H 6.32, N 18.75.

9-(3',5'-Di-O-acetyl-2'-deoxy- β -D-erythro-pentofuranosyl)-1H-purine-6-(9H)-one (**2c**). A soln. of **2a** (3.00 g, 11.9 mmol) in anhyd. pyridine (200 ml) was treated with Ac_2O (8 ml, 83 mmol) at r.t. After 2 h, the solvent was evaporated and the residue crystallized from H_2O : **2c** as colorless needles (3.4 g, 85%). M.p. 191° . TLC (silica gel, $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9:1): R_f 0.50. UV (MeOH): 244 (11800), 249 (11900). $^1\text{H-NMR}$ (CDCl_3): 2.06, 2.13 (2*s*, 2 CH_3); 2.59 (*m*, $\text{H}_b\text{-C}(2')$); 2.89 (*m*, $\text{H}_a\text{-C}(2')$); 4.36 (*m*, $\text{H-C}(4')$, 2 $\text{H-C}(5')$); 5.39 (*m*, $\text{H-C}(3')$); 6.38 (*dd*, $J = 6.0, 7.7$, $\text{H-C}(1')$); 8.02 (*s*, $\text{H-C}(8)$); 8.32 (*s*, $\text{H-C}(2)$); 13.12 (*br.*, NH). Anal. calc. for $\text{C}_{14}\text{H}_{16}\text{N}_4\text{O}_6$: C 49.99, H 4.80, N 16.66; found: C 49.83, H 4.71, N 16.77.

9-(3',5'-Di-O-acetyl-2'-deoxy- β -D-erythro-pentofuranosyl)-6-(ethylthio)-9H-purine (**5b**). A soln. of **2c** (2.00 g, 6.0 mmol) in anhyd. pyridine (20 ml) was treated with 4-chlorophenyl phosphodichloridate (2 ml, 12.2 mmol) for 15 min at 5° and stirred for 24 h at r.t. Pyridine was evaporated and the oily residue (**5a**) treated with 30 ml of ice-cold H_2O for 10 min and shaken with charcoal (200 mg). After filtration, the solvent was removed and the residue¹⁾ dissolved in 1,4-dioxane (20 ml). Ethanthiol (3 ml, 70 mmol) was added and the mixture stirred for 24 h at r.t. and extracted with CH_2Cl_2 (3×20 ml). The org. layer was washed with H_2O , dried (Na_2SO_4), and purified by flash chromatography (500 ml of CH_2Cl_2 , then 500 ml of $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 95:5). The main zone was evaporated, heated in the presence of charcoal (100 mg), and filtered. Compound **5b** crystallized upon evaporation in colorless needles (1.87 g, 82%). M.p. 68° . TLC (silica gel, $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9:1): R_f 0.9. UV (MeOH): 284 (19500), 290 (19300). $^1\text{H-NMR}$ (CDCl_3): 1.43 (*t*, $J = 7.3$, CH_3); 2.06, 2.12 (2*s*, 2 CH_3); 2.61 (*m*, $\text{H}_b\text{-C}(2')$); 2.97 (*m*, $\text{H}_a\text{-C}(2')$); 3.37 (*q*, $J = 7.3$, CH_2S); 4.36 (*m*, $\text{H-C}(4')$, 2 $\text{H-C}(5')$); 5.42 (*m*, $\text{H-C}(3')$); 6.42 (*dd*, $J = 6.0, 7.9$, $\text{H-C}(1')$); 8.12 (*s*, $\text{H-C}(8)$); 8.68 (*s*, $\text{H-C}(2)$). Anal. calc. for $\text{C}_{16}\text{H}_{20}\text{N}_4\text{O}_5\text{S}$: C 50.51, H 5.31, N 14.73, S 8.43; found: C 50.45, H 5.17, N 14.81, S 8.42.

9-(3',5'-Di-O-acetyl-2'-deoxy- β -D-erythro-pentofuranosyl)-6-[(*RS*)-ethylsulfinyl]-9H-purine (**5c**). To a soln. of **5b** (1.2 g, 3.2 mmol) in EtOH (30 ml), *m*-chloroperbenzoic acid (2.16 g, 12.5 mmol) was given in portions within 2 h. The solvent was evaporated and the residue purified by flash chromatography (500 ml of $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 98:2, then 500 ml of $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 95:5). The main zone was evaporated and the residue crystallized from $\text{EtOH}/\text{Et}_2\text{O}$: **5c** as colorless crystals (1.02 g, 81%). M.p. 139° . TLC (silica gel, $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9:1): R_f 0.7. UV (MeOH): 278 (9500). $^1\text{H-NMR}$ (CDCl_3): 1.32, 1.33 (2*t*, $J = 7.4, 3\text{H}$); 2.08, 2.14 (2*s*, 2 CH_3); 2.69 (*m*, $\text{H}_b\text{-C}(2')$); 3.00 (*m*, $\text{H}_a\text{-C}(2')$); 3.37, 3.38 (2*q*, $J = 7.4$, CH_2SO); 4.39 (*m*, $\text{H-C}(4')$, 2 $\text{H-C}(5')$); 5.46 (*m*, $\text{H-C}(3')$); 6.53 (*dd*, $J = 6.0, 7.8$, $\text{H-C}(1')$); 8.36, 8.37 (2*s*, $\text{H-C}(8)$); 9.09 (*s*, $\text{H-C}(2)$). Anal. calc. for $\text{C}_{16}\text{H}_{20}\text{N}_4\text{O}_6\text{S}$: C 48.47, H 5.10, N 14.14, S 8.09; found: C 48.70, H 5.32, N 14.04, S 7.92.

3',5'-Di-O-acetyl-N⁶-(carbamoylmethyl)-2'-deoxyadenosine (**5d**). Compound **5c** (750 mg, 1.89 mmol) in anhyd. DMF was stirred with glycineamide (1.6 g, 21.6 mmol) for 1 h at 80° . The mixture was concentrated, coevaporated with toluene (2×10 ml), and purified by flash chromatography (column 10×2.5 cm; $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 95:5). The main zone was evaporated and crystallized from $\text{EtOH}/\text{Et}_2\text{O}$: **5d** as colorless crystals (607 mg, 82%). M.p. 105° . TLC (silica gel, $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9:1): R_f 0.42. UV (MeOH): 265 (16300). $^1\text{H-NMR}$ (CDCl_3): 2.07, 2.12 (2*s*, CH_3); 2.59 (*m*, $\text{H-C}(2')$); 2.96 (*m*, $\text{H}_a\text{-C}(2')$); 4.37 (*m*, $\text{H-C}(4')$, 2 $\text{H-C}(5')$, CH_2N); 5.42 (*m*, $\text{H-C}(3')$); 5.95 (*br. s*, NH); 6.41 (*dd*, $J = 6.0, 7.9$, $\text{H-C}(1')$); 6.49 (*br. s*, NH); 7.00 (*t*, $J = 5.8$, NHCH_2); 8.01 (*s*, $\text{H-C}(8)$); 8.38 (*s*, $\text{H-C}(2)$). Anal. calc. for $\text{C}_{16}\text{H}_{18}\text{N}_6\text{O}_6$: C 48.97, H 5.15, N 21.42; found: C 49.13, H 5.10, N 21.31.

N⁶-(Carbamoylmethyl)-2'-deoxyadenosine (**1**). Compounds **3b** (500 mg, 1.12 mmol) or **5d** (450 mg, 1.14 mmol) were stirred in a mixture of 1,4-dioxane/conc. aq. NH_3 1:1 (20 ml) at r.t. for 36 h. The solvent was evaporated and the residue crystallized from MeOH: **1** as colorless needles (30 mg, 87% from **3b**; 290 mg, 83% from **5d**). M.p. $215\text{--}216^\circ$ ($[3]: 211^\circ$). TLC (silica gel, $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 4:1): R_f 0.3. UV (MeOH): 265 (17600). $^1\text{H-NMR}$ ($(\text{D}_6)\text{DMSO}$): 2.26 (*m*, $\text{H}_b\text{-C}(2')$); 2.72 (*m*, $\text{H}_a\text{-C}(2')$); 3.59 (*m*, 2 $\text{H-C}(5')$); 3.89 (*m*, $\text{H-C}(4')$); 4.02 (*br.*

¹⁾ Anal. Data of 4-[9-(3',5'-Di-O-acetyl-2'-deoxy- β -D-erythro-pentofuranosyl)-9H-purin-6-yl]pyridinium Chloride (**5a**): TLC (silica gel, $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9:1): R_f 0.1. UV (H_2O): 295 (sh), 272. $^1\text{H-NMR}$ (D_2O): 2.07, 2.27 (2*s*, 2 CH_3); 2.90 (*m*, $\text{H}_b\text{-C}(2')$); 3.24 (*m*, $\text{H}_a\text{-C}(2')$); 4.42 (*m*, 2 $\text{H-C}(5')$); 4.58 (*m*, $\text{H-C}(4')$); 5.61 (*m*, $\text{H-C}(3')$); 6.77 (*t*, $J = 6.1$, $\text{H-C}(1')$); 8.47 (*t*, 2H, 2 $\text{H-C}(\beta)$ of pyridinium); 9.20 (*s*, $\text{H-C}(8)$); 10.08 (*d*, 2 $\text{H-C}(\alpha)$ of pyridinium); 10.10 (*s*, $\text{H-C}(2)$).

CH₂N); 4.42 (*m*, H–C(3'')); 5.20 (*t*, *J* = 5.1, OH–C(5')); 5.31 (*d*, *J* = 4.0, OH–C(3'')); 6.37 (*t'*, *J* = 6.3, 7.4, H–C(1'')); 7.01, 7.36 (*s*, NH₂); 7.73 (*br.*, NH); 8.21 (*s*, H–C(8)); 8.37 (*s*, H–C(2)). Anal. calc. for C₁₂H₁₆N₆O₄: C 46.75, H 5.23, N 27.26; found: C 46.91, N 5.38, N 27.41.

N⁶-(Carbamoylmethyl)-2'-deoxy-5'-O-(4,4'-dimethoxytriphenylmethyl)adenosine (**6a**). Compound **1** (600 mg, 1.95 mmol), coevaporated with dry pyridine (10 ml), was dissolved in pyridine (10 ml) and treated with 4,4'-dimethoxytriphenylmethyl chloride (680 mg, 1.00 mmol). After stirring for 2 h, the mixture was diluted with 5% aq. NaHCO₃ soln. (50 ml) and extracted with CH₂Cl₂ (3 × 60 ml). The org. layer was dried (Na₂SO₄), filtered, concentrated, and purified by flash chromatography (column 25 × 3 cm; 300 ml of CH₂Cl₂/acetone 1:1 then 300 ml of CH₂Cl₂/MeOH 9:1). Evaporation of the main zone and co-evaporation with acetone yielded **6a** as a colorless foam (990 mg, 83%). TLC (silica gel, CH₂Cl₂/MeOH 9:1): *R_f* 0.7. UV (CH₂Cl₂): 236 (19500), 266 (16100). ¹H-NMR (CDCl₃): 2.52 (*m*, H_b–C(2'), OH); 2.82 (*m*, H_a–C(2')); 3.39 (*m*, 2 H–C(5')); 3.75 (*s*, 2 CH₃O); 4.11 (*m*, H–C(3'')); 4.65 (*m*, H–C(4'')) 5.64 (*br.*, NH); 6.41 (*m*, H–C(1''), NH); 6.63 (*t*, *J* = 2.5, *NHCH*₂); 6.74–7.38 (*m*, arom. H); 7.93 (*s*, H–C(8)); 8.30 (*s*, H–C(2)). Anal. calc. for C₃₃H₃₄N₆O₆: C 64.91, H 5.61, N 13.76; found: C 64.80, H 5.72 N 13.67.

N⁶-(Carbamoylmethyl)-2'-deoxy-3'-O-[(diisopropylamino)methoxyphosphino]-5'-O-(4,4'-dimethoxytriphenylmethyl)adenosine (**6b**). Compound **6a** (618 mg, 1.01 mmol) in dry CH₂Cl₂ was stirred with bis(diisopropylamino)methoxyphosphine (194 mg, 1.10 mmol) and diisopropylammonium tetrazolide (188 mg, 1.10 mmol) for 1 h under Ar. The mixture was diluted with CH₂Cl₂ (15 ml) and washed with H₂O. The org. layer was dried (Na₂SO₄), filtered, and purified by flash chromatography (silica gel, column 10 × 3 cm, CH₂Cl₂/AcOEt/(Et)₃N 45:45:10): **6b** as colorless foam after evaporation and co-evaporation with acetone (51 mg, 70%). TLC (silica gel, CH₂Cl₂/AcOEt/(Et)₃N 45:45:10): *R_f* 0.8. UV (MeOH): 238 (20800), 266 (17600). ³¹P-NMR (CDCl₃): 150.2. Anal. calc. for C₄₀H₅₃N₇O₇P: C 61.99, H 6.91, N 12.65; found: C 62.40, H 6.53, N 12.66.

Solid-Phase Synthesis of the Oligomers 7–14. The synthesis of the oligonucleotides was accomplished in a 1-μmol scale using the methyl phosphoramidites of [(MeO)₂Tr]bzA_d, [(MeO)₂Tr]ibG_d, [(MeO)₂Tr]bzC_d, [(MeO)₂Tr]T_d [18], [(MeO)₂Tr]mA_d [17] as well as **6b**. When **6b** was used during the synthesis, oligomerization was carried out without capping, but with an increased coupling time (2 min) for the phosphoramidites. The synthesis of **7**, **10**, **13**, and **14** followed the regular protocol²⁾ of the DNA synthesizer for methyl phosphoramidites [21]. The 5'-dimethoxytrityl group was split off on solid support, the oligomers were treated with thiophenol and deprotected by the action of conc. NH₃ (24 h, 60°). Purification was accomplished by HPLC on *RP-18* columns using solvent system I. The oligomers were desalted on a 25 × 4 HPLC cartridge (*RP-18* silica gel). Inorg. material was deluted with H₂O (10 ml), while the oligomer was eluted with MeOH/H₂O 3:2 (5 ml). The oligomers were lyophilized on a *Speed-Vac* evaporator to yield a colorless foam which was dissolved in H₂O (1 ml) and stored frozen at –20°. Yields were between 0.27–0.36 μmol (27–36%) of isolated material.

Enzymatic Hydrolysis of the Oligomers 7–14. The Oligomer (0.3 *A*₂₆₀ units) was dissolved in 0.1M *Tris*-HCl buffer (pH 8.5; 100 μl) and treated with snake-venom phosphodiesterase (3 μg) at 25° for 45 min and alkaline phosphatase (2 μg) for 10 min at the same temp. The mixture was analyzed on reverse-phase HPLC (*RP-18*, solvent system II). Quantification of the material was made at 260 nm on the basis of the peak areas which were divided by the extinction coefficients of the nucleoside constituents (*ε*₂₆₀: A_d, 15400; m⁶A_d, 11600; **1**, 15800; C_d, 7300; G_d, 11300; T_d, 8800).

Phosphodiester Hydrolysis of the Oligomers 10–14 with the Endodeoxyribonuclease HindIII. The oligomers **10–14** (0.3 *A*₂₆₀ units, each) were dissolved in 50 mM *Tris*-HCl buffer (pH 8.0) containing 10 mM MgCl₂ and 50 mM NaCl (100 μl) and treated with the endodeoxyribonuclease HindIII (5 μl of enzyme suspension containing 12 units of enzyme per μl of buffer, 10 mM *Tris*-HCl, pH 7.4, 250 mM NaCl, 0.1 mM EDTA, 1 mM dithioerythrol, 50% (*v/v*) glycerol) at 25°. Samples of 10 μl were taken at different intervals of time and analyzed on reverse-phase HPLC (*RP-18*, solvent system I). The cleavage product were collected, lyophilized, and analyzed on their nucleoside content after cleavage with snake-venom phosphodiesterase and alkaline phosphatase, as described in the previous section.

²⁾ Users manual of the DNA synthesizer 380B, edition 1986, synthesis cycle abi002 and depr003.

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