# 96. 2′-Deoxy-β-D-ribofuranosides of $N^6$-Methylated 7-Deazaadenine and 8-Aza-7-deazaadenine: Solid-Phase Synthesis of Oligodeoxyribonucleotides and Properties of Self-Complementary Duplexes

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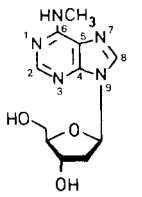
The syntheses of 7-deaza-$N^6$-methyladenine $N^9$-(2′-deoxy-β-D-ribofuranoside) (**2**) as well as of 8-aza-7-deaza-$N^6$-methyladenine $N^8$- and $N^9$-(2′-deoxyribofuranosides) (**3** and **4**, resp.) are described. A 4,4′-dimethoxytritylation followed by phosphitylation yielded the methyl phosphoramidites **12**–**14**. They were employed together with the phosphoramidite of 2′-deoxy-$N^6$-methyladenosine (**15**) in automated solid-phase oligonucleotide synthesis. Alternating or palindromic oligonucleotides derived from d(A-T)$_6$ or d(A-T-G-C-A-G-A*-T-C-T-G-C-A) but containing one methylated pyrrolo[2,3-*d*]pyrimidine or pyrazolo[3,4-*d*]pyrimidine moiety in place of a $N^6$-methylaminopurine (A*) were synthesized. Melting experiments showed that duplex destabilization induced by a $N^6$-Me group of 2′-deoxy-$N^6$-methyladenosine is reversed by incorporation of 8-aza-7-deaza-2′-deoxy-$N^6$-methyladenosine, whereas 7-deaza-2′-deoxy-$N^6$-methyladenosine decreased the $T_m$ value further. Regiospecific phosphodiester hydrolysis of d(A-T-G-C-A-G-m$^6$A-T-C-T-G-C-A) by the endodeoxyribonuclease Dpn I, yielding d(A-T-G-C-A-G-m$^6$A) and d(pT-C-T-G-C-A), was prevented when the residue c$^7$m$^6$A$_d$ (**2**), c$^7$m$^6$z$^8$A$_d$ (**3**), or c$^7$m$^6$z$^8$A$_d$′ (**4**) replaced m$^6$A$_d$ (**1**) indicating that N(7) of $N^6$-methyladenine is a proton-acceptor site for the endodeoxyribonuclease.

**1. Introduction.** – The methylation of the adenine 6-$NH_2$ group and of the 5-position of cytosine is the most common naturally occurring modification of the DNA molecule. With those methylated bases, gene expression is controlled [1] and enzymatic phosphodiester hydrolysis of DNA by endodeoxyribonucleases can be prevented [2]. As a consequence, it has been suggested that the Me group attached to 2′-deoxyadenosine alters DNA's tertiary structure [3].

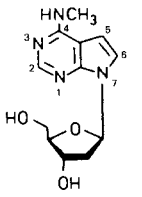
Up to now, 2′-deoxy-$N^6$-methyladenosine (m$^6$A$_d$, **1**) has been incorporated into DNA fragments employing chemical [4] [5] or enzymatic methods [3]. As the $T_m$ value of those oligonucleotides is strongly decreased and only little information exists about the interaction of endodeoxyribonucleases recognizing such methylated purines or pyrimidines [6], we became interested in $N^6$-methylated 2′-deoxyadenosine derivatives. They should lack potential enzyme binding positions (N(7) of purine) and form more stable duplexes by increased base stacking or stronger base pairing. In the following, we report on the syntheses of 7-deaza-2′-deoxy-$N^6$-methyladenosine[1]) (c$^7$m$^6$A$_d$, **2**) as well as of 8-aza-7-deaza-$N^6$-methyl-2′-deoxyadenosine (**3**) and its $N^8$-(2′-deoxy-β-D-ribofuranoside) **4** and on their conversion into the corresponding phosphoramidites **12**–**14**. These monomeric building blocks are then used together with regular phosphoramidites in automated oligonucleotide synthesis. The resulting oligomers are studied with respect to duplex stability or regiospecific phosphodiester hydrolysis by the endodeoxyribonuclease Dpn I.

[1]) Purine nomenclature is used throughout *Chapt. 1–4.*

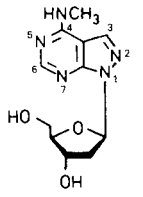
**2. Synthesis of 7-Deaza-$N^6$-methyladenine $N^9$-(2′-Deoxy-β-D-ribofuranoside) (2) as well as of 8-Aza-7-deaza-$N^6$-methyladenine $N^8$- and $N^9$-(2′-Deoxy-β-D-ribofuranosides) (3 and 4, respectively).** – For the preparation of **2**, three methods were considered: *i*) methylation of **9e** at N(1) followed by *Dimroth* rearrangement, as reported for the corresponding ribonucleoside [7], *ii*) nucleophilic displacement of a reactive 6-substituent by $MeNH_2$, and *iii*) glycosylation of 7-deaza-$N^6$-methyladenine (**5a**) with 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-*erythro*-pentofuranosyl chloride (**6**). As our laboratory has developed a method of regio- and stereoselective glycosylation of purine analogues with the



**1** (purine numbering)



**2** (systematic numbering)[1])



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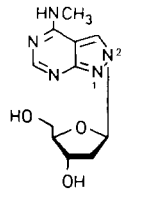
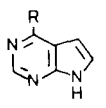
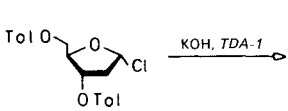


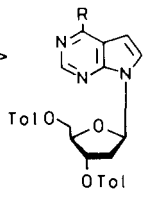
**4** (systematic numbering)[1])



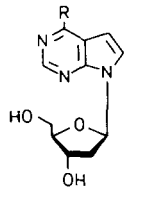
**5a** R = $CH_3NH$
**b** R = $CH_3S$
**c** R = Cl



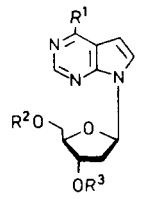
**6**



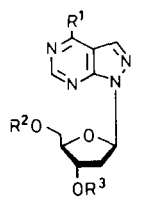
**7a** R = $CH_3NH$
**b** R = $CH_3S$
**c** R = Cl



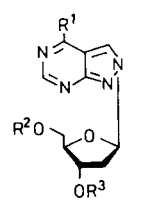
**8a** R = $CH_3S$
**b** R = Cl
**c** R = $C_2H_5S$
**d/d′** R = $C_2H_5SO$
**e** R = $C_2H_5SO_2$



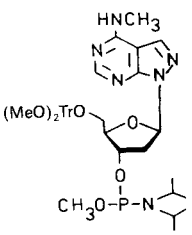
**9a** $R^1$ = $CH_3NH$, $R^2$ = H, $R^3$ = Tol
**b** $R^1$ = $CH_3NH$, $R^2$ = Tol, $R^3$ = H
**c** $R^1$ = $CH_3NH$, $R^2$ = $(MeO)_2Tr$, $R^3$ = Tol
**d** $R^1$ = $CH_3SO_2$, $R^2$ = $R^3$ = Tol
**e** $R^1$ = $NH_2$, $R^2$ = $R^3$ = H



**10a** $R^1$ = $CH_3O$, $R^2$ = $R^3$ = Tol
**b** $R^1$ = $CH_3NH$, $R^2$ = $(MeO)_2Tr$, $R^3$ = H
**c** $R^1$ = $NH_2$, $R^2$ = $R^3$ = H



**11a** $R^1$ = $CH_3O$, $R^2$ = $R^3$ = Tol
**b** $R^1$ = $CH_3NH$, $R^2$ = $(MeO)_2Tr$, $R^3$ = H
**c** $R^1$ = $NH_2$, $R^2$ = $R^3$ = H



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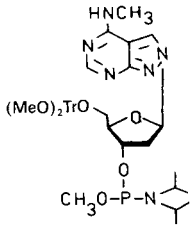
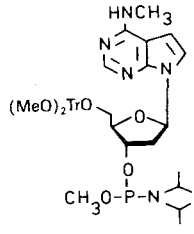
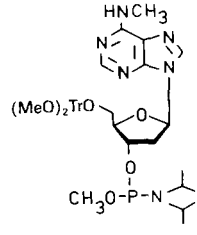


**13**



**14**



**15**

halogenose **6** [8] [9], we have employed solid-liquid phase-transfer glycosylation on compound **5a**. The reaction was performed in MeCN in the presence of powdered KOH and the cryptand *TDA-1* at room temperature. Within 15 min, **7a** was formed in 66% yield together with a minor amount of partially deprotected **9a** and **9b**. In contrast to glycosylations performed on other pyrrolo[2,3-*d*]pyrimidines, this reaction was particularly sensitive towards sugar deprotection. It is not clear whether this is due to a catalytic action of the MeNH group. However, detoluoylation of **7a**, **9a**, or **9b** in conc. aq. $NH_3$/MeOH yielded **2** which was isolated after chromatographic purification.

The glycosylation site as well as the anomeric configuration of **2** were determined by $^1$H-NMR NOE difference spectroscopy [10]. *Table 1* shows NOE's for H–C(4′) and $H_x$–C(2′) upon irradiation of H–C(1′) which indicate spatial proximity of those protons and confirm the β-D-configuration. The NOE of H–C(6) confirms the glycosylation position. The structure of the partially deprotected compounds **9a** and **9b** was assigned by the $^{13}$C-NMR data of the sugar moiety *(Table 2)*. Deprotection at the C(3′) position of **7a** (→ **9b**) shifts C(3′) upfield and C(2′) as well as C(1′) downfield (**9b**). Deprotection of the 5′-position (→ **9a**) results in an upfield shift of C(5′) and smaller downfield shift of the other C-atoms [11].

Table 1. *NOE Data* [%] *of* **2**, **8a**, *and* **11c** *upon Irradiation of H–C(1′) and of* **4** *upon Irradiation of H–C(3)* ($(D_6)Me_2SO$, 23°)

| | H–C(1′) | $H_x$–C(2′) | H–C(4′) | H–C(5′) | H–C(6)[a]) | H–C(3)[b]) |
|---|---|---|---|---|---|---|
| **2** | – | 6.1 | 2.3 | – | 4.8 | – |
| **8a** | – | 10.0 | 3.5 | – | 9.5 | – |
| **4** | 9.5 | 1.3 | 1.6 | 2.7 | – | – |
| **11c** | – | 4.8 | 2.0 | – | – | 8.3 |

[a]) Pyrrolo[2,3-*d*]pyrimidine numbering.
[b]) Pyrazolo[3,4-*d*]pyrimidine numbering.

Alternatively, compound **2** is accessible from the sugar-protected 6-chloro compound **7c** [11] by halogen displacement with $MeNH_2$ under simultaneous sugar deprotection in 65% yield. The reaction conditions required elevated temperature and a steel bomb, thus making this procedure more laborious. The reaction proceeded much faster and occurred already at 60° in a pressure bottle, when the sugar-protected sulfone **9d** was used, yielding **2** in 83%. For the synthesis of the sulfone **9d**, 4-(methylthio)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (**5b**) was glycosylated under the same conditions as already described for compound **5a**. The glycosylation product **7b** (80% yield) was deprotected to give **8a** which was oxidized with 3-chloroperbenzoic acid to **9d** as the only product. Under the same conditions, the oxidation of the deprotected 6-ethylthio compound **8c** prepared from **8b** with ethanethiol gave a mixture of the sulfone **8e** and the diastereoisomeric sulfoxides **8d**/**8d′**. The $^{13}$C-NMR chemical shifts of **5b**, **7b**, **8a**, and **9d** were assigned on the basis of gated-decoupled $^{13}$C-NMR spectra.

Preparation of **2** by methylation of **9e** was not considered as this route afforded too many steps. Comparing the total yields of the different synthetic routes (43% of **2** *via* **5c**, **5a**, **7a**; 53% of **2** *via* **5c**, **7c**; 54% of **2** *via* **5b**, **7b**, **9d**) shows that introduction of the MeNH group on the nucleoside level gave the best results. Although an additional step was necessary for the route *via* the sulfone, a reaction in a steel bomb was not required.

Table 2. *$^{13}$C-NMR Chemical Shifts* ($(D_6)Me_2SO$)[a]) *of Purine, Pyrrolo[2,3-d]pyrimidine, and Pyrazolo[3,4-d]pyrimidine 2′-Deoxyribofuranosides*[b])

| | C(2) (C(2)) [C(6)] | C(6) (C(4)) [C(4)] | C(5) (C(4a)) [C(3a)] | – (C(5)) [C(3)] | C(8) (C(6)) – | C(4) (C(7a)) [C(7a)] | $CH_2$ | $CH_3$ |
|---|---|---|---|---|---|---|---|---|
| **1** | 152.5 | 155.1 | 119.9 | – | 139.4 | 147.9 | – | 27.1 |
| **2** | 151.6 | 156.7 | 103.2 | 99.3 | 121.6 | 149.0 | – | 27.2 |
| **3** | 156.1 | 156.8 | 101.1 | 132.7 | – | 153.2 | – | 27.0 |
| **4** | 156.6 | 158.2 | 101.9 | 123.6 | – | 159.2 | – | 27.0 |
| **5a** | 151.5 | 156.7 | 102.6 | 98.6 | 120.8 | 150.5 | – | 27.2 |
| **5b** | 150.3 | 159.8 | 115.1 | 98.1 | 125.4 | 148.8 | – | 10.9 |
| **7a** | 151.8 | 156.2 | 103.4 | 100.1 | 121.1 | 149.4 | – | 27.2 |
| **7b** | 150.5 | 160.9 | 116.1 | 99.8 | 125.9 | 148.1 | – | 11.4 |
| **8a** | 150.5 | 160.6 | 115.9 | 99.3 | 125.9 | 147.8 | – | 11.4 |
| **8c** | 150.4 | 159.8 | 116.0 | 99.2 | 125.6 | 147.9 | 22.6 | 15.0 |
| **8d** | 151.52[c]) | 163.87[c]) | 117.38 | 100.01 | 129.08 | 149.97[c]) | 46.71 | 5.47 |
| **8d′** | 151.50[c]) | 163.87[c]) | 117.38 | 100.01 | 129.04 | 149.97[c]) | 46.68 | 5.41 |
| **8e** | 152.9[c]) | 153.9[c]) | 115.3 | 100.3 | 131.4 | 150.3[c]) | 46.1 | 6.8 |
| **9a** | 151.6 | 156.8 | 103.5 | 99.6 | 121.8 | 149.0 | – | 27.2 |
| **9b** | 151.9 | 156.7 | 103.2 | 99.6 | 121.0 | 149.3 | – | 27.2 |
| **9c** | 151.8 | 156.7 | 103.2 | 99.6 | 120.9 | 149.3 | – | 27.2/55.1 |
| **9d** | 153.2 | 155.6 | 114.3 | 100.6 | 131.4 | 150.4 | – | – |
| **10b** | 156.1 | 156.8 | 101.1 | 132.5 | – | 153.3 | – | 26.9/55.0 |
| **11b** | 156.5 | 158.3 | 102.2 | 124.1 | – | 159.3 | – | 27.0/55.0 |

| | C(1′) | C(2′) | C(3′) | C(4′) | C(5′) |
|---|---|---|---|---|---|
| **1** | 84.1 | –[d]) | 71.1 | 88.1 | 62.0 |
| **2** | 83.4 | –[d]) | 71.3 | 87.4 | 62.2 |
| **3** | 84.1 | 38.1 | 71.2 | 87.7 | 62.6 |
| **4** | 90.6 | 39.5 | 70.8 | 88.5 | 62.2 |
| **7a** | 81.1 | 36.2 | 75.2 | 83.2 | 64.4 |
| **7b** | 81.3 | 36.1 | 75.1 | 83.6 | 64.3 |
| **8a** | 83.1 | –[d]) | 71.1 | 87.5 | 62.0 |
| **8c** | 83.0 | 40.1 | 71.0 | 87.5 | 61.9 |
| **8d** | 83.00 | –[d]) | 71.00 | 87.67 | 61.86 |
| **8d′** | 82.94 | –[d]) | 71.00 | 87.67 | 61.86 |
| **8e** | 83.3 | –[d]) | 70.9 | 87.8 | 61.8 |
| **9a** | 83.9 | 37.0 | 76.2 | 84.7 | 62.0 |
| **9b** | 82.6 | –[d]) | 70.8 | 83.5 | 64.6 |
| **9c** | 82.5 | 38.0 | 70.9 | 85.5 | 64.3 |
| **9d** | 81.6 | 36.2 | 74.9 | 83.9 | 64.2 |
| **10b** | 83.5 | 38.3 | 70.9 | 85.2 | 64.5 |
| **11b** | 89.9 | 39.5 | 70.7 | 85.4 | 64.2 |

[a]) $\delta$ Values given in ppm relative to $Me_4Si$ as internal standard.
[b]) Pyrrolo[2,3-*d*]pyrimidine numbering in parentheses, pyrazolo[3,4-*d*]pyrimidine numbering in brackets.
[c]) Tentative.
[d]) Superimposed by $Me_2SO$.

For the synthesis of 8-aza-7-deaza-$N^6$-methyladenine $N^8$- and $N^9$-(2′-deoxy-$\beta$-D-ribofuranosides) (**3** and **4**, resp.), the already described 4-methoxy-1*H*-pyrazolo[3,4-*d*]-pyrimidine 2′-deoxy-$\beta$-D-ribofuranosides **10a** and **11a** [12a,b] were used as starting materials. As their MeO groups are much more reactive than those of pyrrolo[2,3-*d*]-

pyrimidines and even of purines, displacement by $MeNH_2$ occurred at 60° in a pressure bottle affording compounds **3** and **4**, respectively, in 95% yield. Their structures were confirmed by $^1$H- and $^{13}$C-NMR spectroscopy (see *Exper. Part* and *Table 2*). The strong NOE of H–C(1′) of **4** upon irradiation of H–C(3) (H–C(7) in purines numbering) provided the unequivocal assignment of H–C(6) and H–C(3) and showed that H–C(3) resonates downfield from H–C(6).

This conclusion should be transferable to compound **11c** and requires a correction of an earlier $^1$H-NMR assignment [12a]. This correction was verified by an NOE-difference spectrum of **11c** with saturation of H–C(1′) *(Table 1)*. In connection with these NMR experiments, $^1$H,$^{13}$C correlation spectra of **1** and dA have been measured. They showed that H–C(2) signals are located upfield as compared to H–C(8), which is opposite to an assignment reported earlier [13].

**3. Solid-Phase Synthesis of Oligonucleotides Containing 1–4.** – Solid-phase synthesis [14] of the oligonucleotides **19–27** was performed using P(III) chemistry [15] which

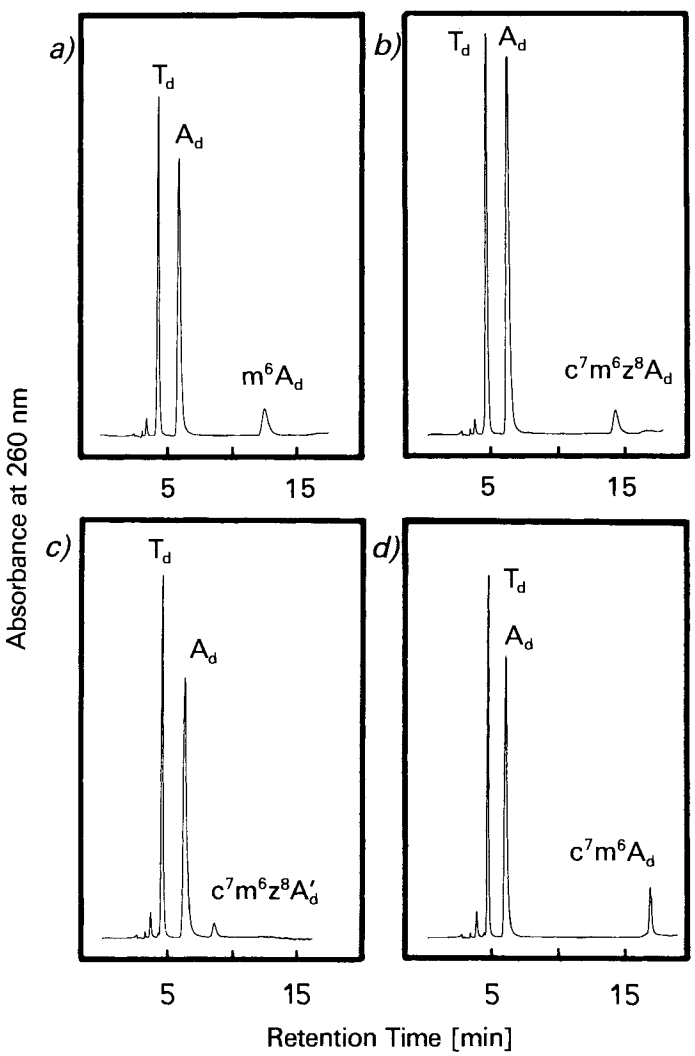



Fig. 1. *HPLC profiles after phosphodiester hydrolysis of* **19b–22b** *with snake-venom phosphodiesterase followed by alkaline phosphatase.* Gradient *IV*; conditions, see *Exper. Part. a*) From **19b**; *b*) from **20b**; *c*) from **21b**; *d*) from **22b**.

required the phosphoramidites **12–15** as monomeric building blocks. The synthesis of **15** has already been reported [4] [5]. For the synthesis of **12–14**, the 5′-OH group of **2–4** was protected with 4,4′-dimethoxytrityl chloride ($(MeO)_2TrCl$) to give **9c**, **10b**, and **11b**, respectively. The amorphous compounds were characterized by NMR spectroscopy and elemental analyses. The position of dimethoxytritylation was determined from the characteristic $^{13}C$-NMR upfield shift of the C(4′) signal and the downfield shift of C(5′) *(Table 2)*.

It has been shown earlier for 2′-deoxy-$N^6$-methyladenosine and related nucleosides that protection of the 6-methylamino group was not required during oligonucleotide synthesis [6]. Nucleobase protection was also not necessary in the case of **2–4**. The $AcNCH_3$ group, which can be formed upon the capping procedure of the oligonucleotide synthesis, will be cleaved together with the other base-protecting groups by $NH_3$ treatment. The phosphoramidites **12–14** were obtained from **9c**, **10b**, and **11b** by reaction with chloro(diisopropylamino)methoxyphosphine. They were purified by flash chromatography and characterized by $^{31}P$-NMR spectroscopy.

The synthesis of the oligonucleotides **19–27** was carried out on solid support in an automated DNA synthesizer using the regular phosphoramidites of dA, dT, dG, and dC

| d(A-T)$_6$ [13] | d(c$^7$z$^8$A-T)$_6$ [13] | d(c$^7$z$^8$A′-T)$_6$ [13] |
|---|---|---|
| **16** | **17** | **18** |
| d([(MeO)$_2$Tr]A-T-A-T-A-T-m$^6$A-T-A-T-A-T) | | d(A-T-A-T-A-T-m$^6$A-T-A-T-A-T) |
| **19a** | | **19b** |
| d([(MeO)$_2$Tr]A-T-A-T-A-T-c$^7$m$^6$z$^8$A-T-A-T-A-T) | | d(A-T-A-T-A-T-c$^7$m$^6$z$^8$A-T-A-T-A-T) |
| **20a** | | **20b** |
| d([(MeO)$_2$Tr]A-T-A-T-A-T-c$^7$m$^6$z$^8$A′-T-A-T-A-T) | | d(A-T-A-T-A-T-c$^7$m$^6$z$^8$A′-T-A-T-A-T) |
| **21a** | | **21b** |
| d([(MeO)$_2$Tr]A-T-A-T-A-T-c$^7$m$^6$A-T-A-T-A-T) | | d(A-T-A-T-A-T-c$^7$m$^6$A-T-A-T-A-T) |
| **22a** | | **22b** |
| d([(MeO)$_2$Tr]A-T-G-C-A-G-A-T-C-T-G-C-A) | | d(A-T-G-C-A-G-A-T-C-T-G-C-A) |
| **23a** | | **23b** |
| d([(MeO)$_2$Tr]A-T-G-C-A-G-m$^6$A-T-C-T-G-C-A) | | d(A-T-G-C-A-G-m$^6$A-T-C-T-G-C-A) |
| **24a** | | **24b** |
| d([(MeO)$_2$Tr]A-T-G-C-A-G-c$^7$m$^6$z$^8$A-T-C-T-G-C-A) | | d(A-T-G-C-A-G-c$^7$m$^6$z$^8$A-T-C-T-G-C-A) |
| **25a** | | **25b** |
| d([(MeO)$_2$Tr]A-T-G-C-A-G-c$^7$m$^6$z$^8$A′-T-C-T-G-C-A) | | d(A-T-G-C-A-G-c$^7$m$^6$z$^8$A′-T-C-T-G-C-A) |
| **26a** | | **26b** |
| d([(MeO)$_2$Tr]A-T-G-C-A-G-c$^7$m$^6$A-T-C-T-G-C-A) | | d(A-T-G-C-A-G-c$^7$m$^6$A-T-C-T-G-C-A) |
| **27a** | | **27b** |

c$^7$z$^8$A$_d$ = 8-aza-7-deaza-2′-deoxyadenosine; m$^6$A$_d$ = 2′-deoxy-$N^6$-methyladenosine; c$^7$m$^6$A$_d$ = 7-deaza-2′-deoxy-$N^6$-methyladenosine; c$^7$m$^6$z$^8$A$_d$ = 8-aza-7-deaza-2′-deoxy-$N^6$-methyladenosine. A′ is the symbol used for the 8-aza-7-deazaadenosine moiety with the unusual $N^8$- instead of the usual $N^9$-glycosylic linkage (purine numbering).

together with the modified compounds **12**–**15**. The syntheses followed a protocol of detritylation, coupling, capping, and oxidation according to [16]. The protected oligomers were demethylated with thiophenol and cleaved from the solid support by treatment with $NH_3$. After removal of the base-protecting groups by ammonolysis, purification of the $(MeO)_2Tr$-protected oligomers **19a**–**27a** by HPLC, and removal of the $(MeO)_2Tr$ residue with aq. AcOH, the oligomers **19b**–**27b** were recovered and again purified by HPLC to give colorless solids.

The nucleoside content of the oligonucleotides (see *Fig. 1*) was determined after tandem hydrolysis with snake-venom phosphodiesterase followed by alkaline phosphatase. UV Quantification of the HPLC pattern confirmed the molar amount of monomers.

**4. Physical Properties of the Oligomers 16–27b and Influence of 1–4 on the Phosphodiester Hydrolysis by Endodeoxyribonuclease Dpn I.** – It has been reported that $N^6$-methylation of dA within oligonucleotides decreases the $T_m$ value strongly [3] [4]. This is demonstrated by the $T_m$ values of $d(A\text{-}T)_6$ (**16**) and the modified dodecamer **19b**, showing a $\Delta T_m$ of 6° (*Table 3*). The destabilization by $N^6$-methylation has been attributed to a restricted rotation of the $N^6$-Me group, preferentially located in *cis* orientation with respect to N(1) [17]. This should interfere with *Watson-Crick* base pairing towards dT.

Table 3. *Melting Temperatures* ($T_m$ Values) *of the Oligonucleotides* **16**–**18**[a]), **19b**–**22b**[a]), *and* **23b**–**27b**[b])

| Oligomer | | $T_m$ [°] |
|---|---|---|
| $d(A\text{-}T)_6$ [12b] | (**16**) | 32 |
| $d(c^7z^8A\text{-}T)_6$ [12b] | (**17**) | 36 |
| $d(c^7z^8A'\text{-}T)_6$ [12b] | (**18**) | 48 |
| d(A-T-A-T-A-T-m$^6$A-T-A-T-A-T) | (**19b**) | 26 |
| d(A-T-A-T-A-T-c$^7$m$^6$z$^8$A-T-A-T-A-T) | (**20b**) | 28 |
| d(A-T-A-T-A-T-c$^7$m$^6$z$^8$A′-T-A-T-A-T) | (**21b**) | 31 |
| d(A-T-A-T-A-T-c$^7$m$^6$A-T-A-T-A-T) | (**22b**) | 24 |
| d(A-T-G-C-A-G-A-T-C-T-G-C-A) | (**23b**) | 59 |
| d(A-T-G-C-A-G-A-T-C-T-G-C-A) | (**23b**) | 59 |
| d(A-T-G-C-A-G-m$^6$A-T-C-T-G-C-A) | (**24b**) | 53 |
| d(A-T-G-C-A-G-c$^7$m$^6$z$^8$A-T-C-T-G-C-A) | (**25b**) | 54 |
| d(A-T-G-C-A-G-c$^7$m$^6$z$^8$A′-T-C-T-G-C-A) | (**26b**) | 55 |
| d(A-T-G-C-A-G-c$^7$m$^6$A-T-C-T-G-C-A) | (**27b**) | 51 |

[a]) In 1M NaCl, 0.1M $MgCl_2$, and 60 mM cacodylic acid, pH 7.0 at 4 μM single-strand concentration.
[b]) In 150 mM NaCl, 6 mM $MgCl_2$, 7 mM 2-mercaptoethanol, and bovine scrum albumine (100 μg/ml) at 4 μM single-strand concentration.

However, the situation seems to be much more complex. From the $+I$ effect of the Me group, the MeNH group of **1** is expected to be a weaker proton donor than an $NH_2$ group, thus destabilizing the H-bond to the carbonyl group of dT for electronic as well as for sterical reasons. Furthermore, the bathochromic shift (10 nm) in the UV of **1** as compared to dA indicates that $N^6$-methylation affects the electronic state of the nucleobase and thus stacking interactions. Finally, the $H_2O$ spine located within the major groove of an oligonucleotide duplex [18] may be affected by introducing a $N^6$-Me group. As the $H_2O$-accepting N(7) may not be accessible in case of **1** and is not present in compound **2**, a destabilization of the duplex may occur. The residues **3** or **4**, however, having an N-atom within the major groove (N(8)) may again allow the formation of a hydrate.

We have already reported on increased $T_m$ values of d(A-T)$_6$ (**16**) which contains 8-aza-7-deaza-2′-deoxyadenosine instead of dA residues [12b] and have explained this finding by a better base overlap. A stabilization is also observed in the case of the oligomer **20b** as compared to **19b**. However, the $T_m$ increase is smaller due to the fact that in this case, only one $m^6A_d$ is replaced by **3**. In contrast, the pyrrolo[2,3-*d*]pyrimidine nucleoside **2** destabilizes the duplex structure as demonstrated for the oligomer **22b** *(Table 3)*.

A strong stabilization of d(A-T)$_6$ (**16**) occurs also by replacing every dA residue with the 8-aza-7-deazaadenine $N^8$-(2′-deoxy-β-D-ribofuranoside) ( = $c^7z^8A'_d$; **11c**; see $T_m$ of **16** and **18** in *Table 3*) [12b]. The same phenomenon is observed in the case of **21b** when compared to **19b**. Because of the strong $T_m$ increase for the oligomer **18** as compared to **16**, a special tertiary structure of this duplex is assumed. As DNA tertiary structures can be visualized by the CD spectrum [19], we have measured the CD spectra of **16**–**18** and

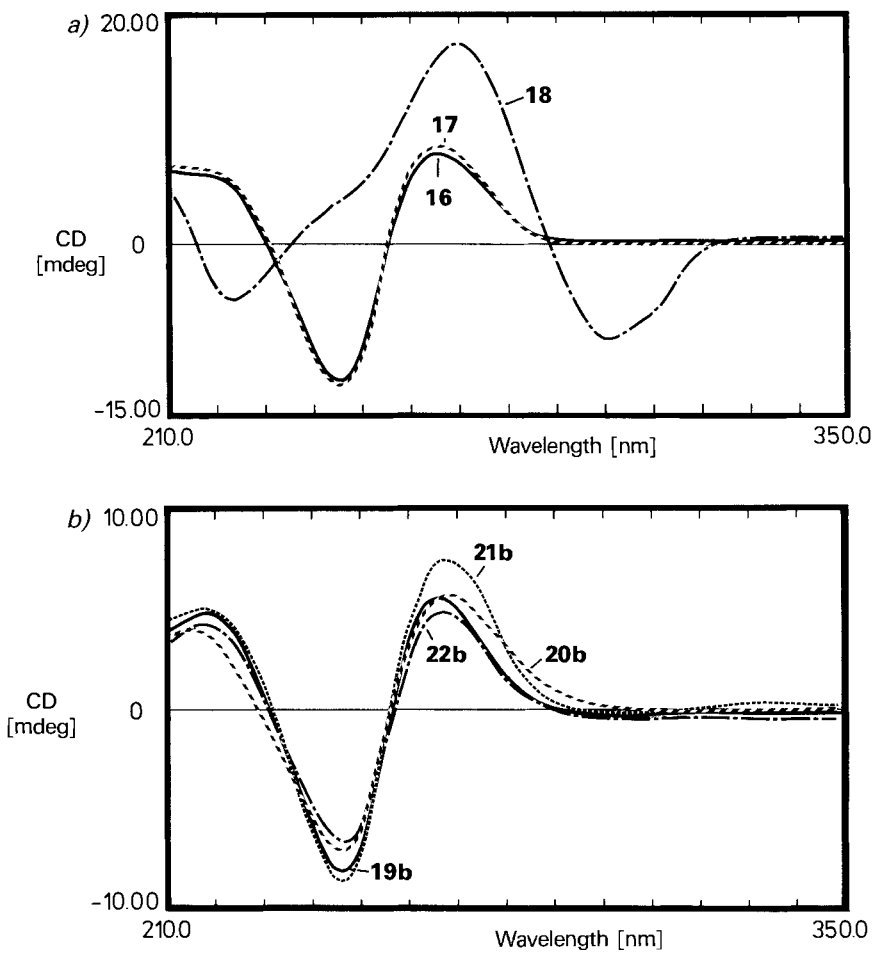



Fig. 2. *CD Spectra of the dodecamers* a) **16** (——), **17** (– – –), *and* **18** (— — —) *and* b) **19b** (——), **20b** (– – –), **21b** (-----), *and* **22b** (— — —). In 1M NaCl, 0.1M $MgCl_2$, and 60 mM sodium cacodylate at 10 μM single-strand concentration.

**19b–22b** (*Fig. 2*). The CD of **17** and **19b–22b** are very similar to that of d(A-T)$_6$ (**16**) indicating that those oligomers form a right-handed B-DNA-resembling structure as found for poly[d(A-T)]·poly[d(A-T)] at low salt concentration [20]. However, the CD of **18** is different (*Fig. 2a*). The existence of a negative *Cotton* effect at 300 nm and a positive band at 270 nm points to a left-handed helix as found for Z-DNA [21]. This left-handed DNA is characteristic for poly[d(G-C)]·poly[d(G-C)] at high salt concentration [22] and has also been observed for poly [d(A-T)]·poly[d(A-T)] at 5M NaCl in the presence of 95 mM $NiCl_2$ [20]. Characteristic features of Z-DNA are repeating elements of a C(3′)-*endo*/*syn* and C(2′)-*endo*/*anti* sugar conformation with the purine bases in *syn* orientation [23].

In the case of the **11c** residue a reversed *Watson-Crick* base pair between the modified adenine and thymine should be preferred. Computer modelling of a $c^7z^8A_d'$-$T_d$ base pair indicates that a left-handed Z-DNA can be formed if a reversed *Watson-Crick* base pair is formed. As the special CD spectrum is only found for **18**, (*Fig. 2a*) in which every dA residue is replaced by **11c**, but not for **21b** one can conclude that one $m^6A_d$ replacement by **4** is not sufficient to alter the structure of the whole duplex.

Apart from the alternating oligomers, we have determined the $T_m$ values of the 13-mers **23b–27b**. Compound **24b** contains the recognition sequence d(G-m$^6$A-T-C) of the endodeoxyribonuclease Dpn I (*Diplococcus* Pneumonia) [24–26]. This restriction enzyme is one of the rare examples where $N^6$-methylation of the adenine moiety within the recognition site is required for regiospecific phosphodiester hydrolysis [27]. In most other cases, $N^6$-methylation of adenine protects DNA from phosphodiester hydrolysis by restriction enzymes [2]. As it is unknown if N(7), located in the major groove of DNA, is necessary as a proton acceptor for enzym binding, we have incorporated compounds **2–4** instead of $m^6A_d$ into the 13-mer d(A-T-G-C-A-G-m$^6$A-T-C-T-G-C-A) (**24b**). The oligomers **23b–27b** were then subjected to hydrolysis by the endodeoxyribonuclease Dpn I. Enzymatic hydrolysis was followed by HPLC. As *Fig. 3* shows, the 13-mer **24b** is cleaved into the heptamer d(A-T-G-C-A-G-m$^6$A) and the hexamer d(pT-C-T-G-C-A) within 20 h at 22°. Complete hydrolysis of the oligomer by snake-venom phosphodiesterase followed by alkaline phosphatase resulted in a HPLC pattern (*Fig. 4*) from which the nucleoside

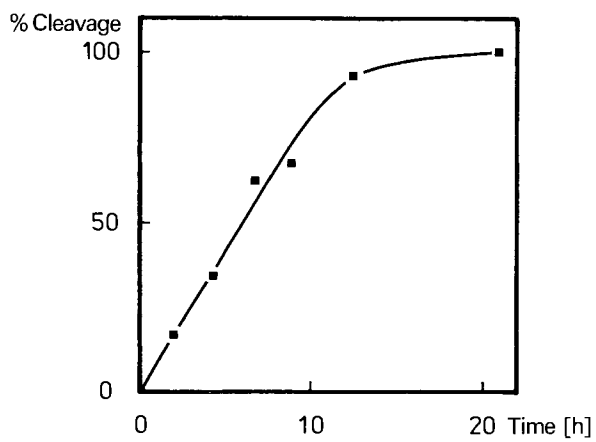



Fig. 3. *Time course of phosphodiester hydrolysis of the oligomer d(A-T-G-C-A-G-m$^6$A-T-C-T-G-C-A)* (**24b**) *by treatment with the endodeoxyribonuclease Dpn I.* Conditions, see *Exper. Part.*

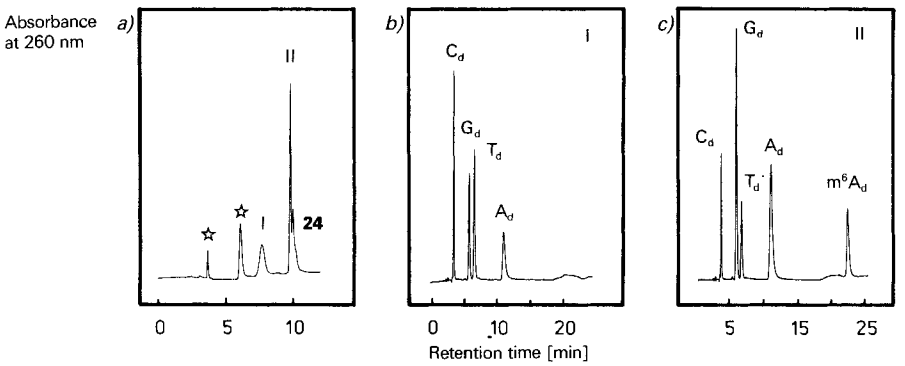



Fig. 4. *HPLC profiles of the products* a) *of phosphodiester hydrolysis of d(A-T-G-C-A-G-m⁶A-T-C-T-G-C-A)* (**24b**) *by the endodeoxyribonuclease Dpn I* (conditions, see *Exper. Part*; gradient *II*) *and b), c) after tandem hydrolysis of d(pT-C-T-G-C-A)* (peak I of *a*) *and d(A-T-G-C-A-G-m⁶A)* (peak II of *a*), *respectively, with snake-venom phosphodiesterase followed by alkaline phosphatase* (gradient *III*). ☆: buffer peaks.

content was calculated on the basis of the extinction coefficients. As expected, the non-methylated **23b** was not hydrolysed at all by the endodeoxyribonuclease Dpn I, a finding which confirms results on the oligonucleotide level already observed on native DNA [24]. None of the oligomers **25b–27b** containing the $m^6A_d$ derivatives **2–4** were hydrolyzed by the endodeoxyribonuclease Dpn I which shows that N(7) is required as a proton-acceptor position for regiospecific phosphodiester hydrolysis.

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## Experimental Part

*General.* The phosphoramidites were prepared from the 5′-tritylated and appropriately base-protected nucleosides $[(MeO)_2Tr]bz^6A_d$, $[(MeO)_2Tr]ib^2G_d$, $[(MeO)_2Tr]bz^4C_d$, and $[(MeO)_2Tr]T_d$ (*Biosyntech*, Hamburg, FRG). *Fractosil 500* polymeric support was purchased from *Biosyntech* (Hamburg, FRG). Snake-venom phosphodiesterase (EC 3.1.4.1., *Crotallus durissus*), alkaline phosphatase (EC 3.1.3.1., *E.coli*), and endodeoxyribonuclease DpnI were purchased from *Boehringer Mannheim* (FRG). Oligonucleotide synthesis was carried out on a automated DNA synthesizer, model *380B*, of *Applied Biosystems* (Weiterstadt, FRG) on a 1-μmol scale. TLC: siliga gel *SIL G-25 UV₂₅₄* plates (*Macherey-Nagel*, FRG). Flash chromatography: silica gel *60H* (*Merck*, FRG), at 0.9 bar; Solvent systems: $CH_2Cl_2$/MeOH 9:1 (*A*), $CH_2Cl_2$/MeOH 8:2 (*B*), $CH_2Cl_2$/acetone 7:3 (*C*), $CH_2Cl_2$/MeOH 95:5 (*D*), $CH_2Cl_2$/AcOEt/$Et_3N$ 45:45:10 (*E*), $CHCl_3$ (*F*), $CHCl_3$/MeOH 95:5 (*G*), $CHCl_3$/MeOH 9:1 (*H*), $CHCl_3$/MeOH 8:2 (*I*), cyclohexane/AcOEt 6:4 (*K*). UV spectra: *Hitachi-150-20* spectrophotometer; (*Hitachi*, Japan); $\lambda_{max}$ (ε) in nm. CD-spectra: *Jasco-J-600* spectropolarimeter. NMR spectra: *Bruker AC 250* spectrometer; δ in ppm relative to tetramethylsilane ( = 0 ppm) as internal standard ($^1H$ and $^{13}C$) or to external 85% phosphoric acid ($^{31}P$; = 0 ppm).

*Melting Experiments.* The melting experiments were carried out in a thermostatically controlled cell holder with a *Shimadzu-210-A* UV spectrophotometer connected with a *Kipp and Zonen BD 90* recorder. The increase of absorbance at the appropriate wavelength as a function of time was recorded, while the temp. of the soln. was increased linearly with time at a rate of 20°/h using a *Lauda PM-350* programmer and a *Lauda RCS 6* bath equipped with a *R 22* unit (*MWG Lauda*, FRG). The actual temp. was measured in the sample cell with a Pt resistor.

*HPLC Separation.* HPLC was carried out on a 4 × 250 and 4 × 25 mm (10 μm) *RP-18 LiChrosorb* column (*Merck*) using a *Merck-Hitachi* HPLC apparatus with one pump (model *655A-12*) connected with a proportion-

ing valve, a variable wavelength monitor (model *655A*), and a controller (model *L-5000*), connected with an integrator (model *D-2000*). The solvent gradients consisting of 0.1M $Et_3NHOAc$ (pH 7) MeCN 95:5 (*A*), and MeCN (*B*) were used in the following order: gradient *I*, 15 min 20–35% *B* in *A*; *II*, 10 min 5–20% *B* in *A*; *III*, 12 min 100% *A*, 13 min 0–10% *B* in *A*; *IV*, 8 min 100% *B*, 4 min 0–16% *B* in *A*.

*Phase-Transfer Glycosylation of 4-(Methylamino)-7*H*-pyrrolo[2,3-*d*]pyrimidine* (**5a**) *with 2-Deoxy-3,5-di-*O*-(*p*-toluoyl)-α-*D*-erythro-pentofuranosyl Chloride* (**6**). Powdered KOH (700 mg, 12.5 mmol), **5a** [28] (700 mg, 4.72 mmol), and tris[2-(2-methoxyethoxy)ethyl]amine (*TDA-1*; 100 µl, 0.31 mmol) were stirred at r.t. in anh. MeCN (50 ml). After 5 min, **6** [29] (1.85 g, 4.77 mmol) was added. Stirring was continued for 10 min, insoluble material removed by filtration and washed with MeCN, the filtrate evaporated, and the residue chromatographed on silica gel *60* (10 × 5 cm column): 3 zones were separated, containing **7a**, **9a**, and **9b**, respectively.

*7-[2′-Deoxy-3′,5′-di-*O*-(p-toluoyl)-β-*D*-erythro-pentofuranosyl]-4-(methylamino)-7*H*-pyrrolo[2,3-*d*]pyrimidine* (**7a**). From the fast migrating zone (eluant *G*), **7a** was isolated as colorless foam (1.56 g, 66%). TLC (silica gel, *H*): $R_f$ 0.5. UV (MeOH): 240 (35500), 271 (18000). $^1$H-NMR ($(D_6)$DMSO): 2.38, 2.40 (2*s*, 2 $CH_3$); 2.65 (*m*, $H_\beta$–C(2′)); 2.96 (*d*, *J* = 4.6, $CH_3N$); 3.07 (*m*, $H_\alpha$–C(2′)); 4.58 (*m*, H–C(4′), 2 H–C(5′)); 5.72 (*m*, H–C(3′)); 6.61 (*d*, *J* = 3.6, H–C(5)); 6.67 (*m*, H–C(1′)); 7.32, 7.93 (*m*, 8 arom. H, H–C(6)); 7.55 (*q*, *J* = 4.6, NH); 8.17 (*s*, H–C(2)). Anal. calc. for $C_{28}H_{28}N_4O_5$: C 67.19, H 5.63, N 11.19; found: C 67.00, H 5.67, N 11.01.

*7-[2′-Deoxy-3′-*O*-(p-toluoyl)-β-*D*-erythro-pentofuranosyl]-4-(methylamino)-7*H*-pyrrolo[2,3-*d*]pyrimidine* (**9a**). From the 2nd zone (eluant *H*), a colorless foam was obtained (110 mg, 6%). TLC (silica gel, *H*): $R_f$ 0.4. UV (MeOH): 239 (19100), 273 (15200). $^1$H-NMR ($(D_6)$DMSO): 2.41 (*s*, $CH_3$); 2.89 (*m*, $H_\alpha$–C(2′); $H_\beta$–C(2′) superimposed by DMSO); 2.96 (*d*, *J* = 4.6, $CH_3N$); 3.68 (*m*, 2 H–C(5′)); 4.19 (*m*, H–C(4′)); 5.51 (*t*, *J* = 5.7, OH–C(5′)); 5.58 (*m*, H–C(3′)); 6.60 (*m*, H–C(1′), H–C(1′), H–C(5)); 7.37, 7.95 (*m*, 4 arom. H); 7.44 (*d*, *J* = 3.7, H–C(6)); 7.58 (*q*, *J* = 4.6, NH); 8.16 (*s*, H–C(2)). Anal. calc. for $C_{20}H_{22}N_4O_4$: C 62.81, H 5.80, N 14.65; found: C 62.54, H 5.77, N 14.28.

*7-[2′-Deoxy-5′-*O*-(p-toluoyl)-β-*D*-erythro-pentofuranosyl]-4-(methylamino)-7*H*-pyrrolo[2,3-*d*]pyrimidine* (**9b**). The slow migrating zone (eluant *H*) afforded a colorless foam (100 mg, 6%). TLC (silica gel, *I*): $R_f$ 0.8. UV (MeOH): 236 (17900), 274 (15100). $^1$H-NMR ($(D_6$DMSO): 2.29 (*m*, $H_\beta$–C(2′)); 2.38 (*s*, $CH_3$); 2.64 (*m*, $H_\alpha$–C(2′)); 2.95 (*d*, *J* = 3.5, $CH_3N$); 4.10 (*m*, H–C(4′)); 4.35, 4.49 (*m*, 2 H–C(5′), H–C(3′)); 5.53 (*d*, *J* = 4.3, OH–C(3′)); 6.56 (*m*, H–C(1′), H–C(5)); 7.27 (*d*, *J* = 3.7, H–C(6)); 7.33, 7.86 (*m*, 4 arom. H); 7.52 (*q*, *J* = 3.7, NH); 8.16 (*s*, H–C(2)). Anal. calc. for $C_{20}H_{22}N_4O_4$: C 62.81, H 5.80, N 14.64; found: C 62.54, H 5.70, N 14.40.

*7-(2′-Deoxy-β-*D*-erythro-pentofuranosyl)-4-(methylamino)-7*H*-pyrrolo[2,3-*d*]pyrimidine* (**2**). *From* **7a**: A soln. of **7a** (100 mg, 0.2 mmol) in MeOH (25 ml, saturated with $NH_3$ at 0°) was stirred for 6 h at r.t. and evaporated. The residue was adsorbed on silica gel *60H* (2.0 g) and chromatographed on a silica-gel *60H* column (10 × 2 cm, *H*): **2** (40 mg, 76%), colorless foam.

*From* **7c**: A soln. of 4-chloro-7-[2′-deoxy-3′,5′-di-*O*-(*p*-toluoyl)-β-*D*-*erythro*-pentofuranosyl]-7*H*-pyrrolo-[2,3-*d*]pyrimidine [11] (**7c**; 1.0 g, 1.98 mmol) MeOH (100 ml) and 40% aq. $MeNH_2$ (10 ml) was treated in a steel bomb for 6 h at 150°. The cold mixture was evaporated, the residue dissolved in MeOH, adsorbed on silica gel (2.0 g), and chromatographed on a silica gel column (5 × 2 cm, *H*): the main zone yielded **2** as colorless foam (340 mg, 65%).

*From* **9d**: A soln. of **9d** (see below; 150 mg, 0.27 mmol) in 40% aq. $MeNH_2$/dioxane 1:1 (40 ml) was stored at 60° for 4 h in a pressure bottle. After evaporation of the solvent, the residue was chromatographed on silica gel (column: 4 × 3 cm, *G*). From the main zone, colorless amorphous **2** (60 mg, 83%) was isolated. TLC (silica gel, *I*): $R_f$ 0.4. UV (MeOH): 210 (30300), 274 (15600). $^1$H-NMR ($(D_6)$DMSO): 2.15 (*m*, $H_\beta$–C(2′)); 2.95 (*d*, *J* = 4.6, $CH_3N$); 3.54 (*m*, 2 H–C(5′)); 3.83 (*m*, H–C(4′)); 4.35 (*m*, H–C(3′)); 5.18 (*m*, OH–C(5′)); 5.27 (*m*, OH–C(3′)); 6.49 (*m*, H–C(1′)); 6.57 (*d*, *J* = 3.6, H–C(5)); 7.34 (*d*, *J* = 3.6, H–C(6)); 7.52 (*q*, *J* = 4.6, NH); 8.14 (*s*, H–C(2)). Anal. calc. for $C_{12}H_{16}N_4O_3$: C 54.54, H 6.10, N 21.20; found: C 54.61, H 6.11, N 20.95.

*7-(2′-Deoxy-β-*D*-erythro-pentofuranosyl)-4-(ethylthio)-7*H*-pyrrolo[2,3-*d*]pyrimidine* (**8c**). A mixture of **8b** (400 mg, 1.48 mmol) [11] [30], MeOH (5 ml), ethanethiol (6 ml), and 20% aq. NaOH soln. (2 ml) was stirred for 1 h at r.t. Then the soln. was neutralized with AcOH and evaporated. The residue was suspended in $CHCl_3$/MeOH 9:1, adsorbed on silica gel (2.0 g), and chromatographed on a silica-gel column (20 × 2 cm, *G*). The main zone gave a colorless foam upon evaporation which was crystallized from acetone: **8c** as colorless needles (410 mg, 94%). M.p. 140°. TLC (silica gel, *H*): $R_f$ 0.5. UV (MeOH): 222 (24000), 250 (7000), 293 (14000). $^1$H-NMR ($(D_6)$DMSO): 1.35 (*t*, *J* = 7.3, $CH_3CH_2S$); 2.23 (*m*, $H_\beta$–C(2′)); 2.52 (*m*, $H_\alpha$–C(2′)); 3.32 (*q*, *J* = 7.3, $CH_3CH_2S$); 3.55 (*m*, 2 H–C(5′)); 3.85 (*m*, H–C(4′)); 4.38 (*m*, H–C(3′)); 5.00 (*t*, *J* = 5.3, OH–C(5′)); 5.32 (*d*, *J* = 3.8, OH–C(3′)); 6.55 (*d*, *J* = 3.7, H–C(5)); 6.62 (*m*, H–C(1′)); 7.75 (*d*, *J* = 3.7, H–C(6)); 8.62 (*s*, H–C(2)). Anal. calc. for $C_{13}H_{17}N_3O_3S$: C 52.87, H 5.80, N 14.23, S 10.86; found: C 52.80, H 5.83, N 14.12, S 11.08.

*Oxidation of* **8c** *with 3-Chloroperbenzoic Acid.* To a soln. of **8c** (400 mg, 1.36 mmol) in EtOH (40 ml), 3-chloroperbenzoic acid (300 mg; technical grade, 80%) was added. After stirring for 30 min at r.t., the soln. was

evaporated and the residue chromatographed on silica gel *60H* (column: 10 × 5 cm, *G*). The diastereoisomeric mixture **8d**/**8d'** was isolated from the slower migrating main zone as a colorless foam (250 mg, 59%). TLC (silica gel, *H*): $R_f$ 0.3. The faster migrating zone yielded **8e** as a colorless foam (100 mg, 22%). TLC (silica gel, *H*): $R_f$ 0.3.

*7-(2'-Deoxy-β-*D-erythro-*pentofuranosyl)-4-(ethylsulfinyl)-7*H-*pyrrolo[2,3-*d*]*pyrimidine* (**8d**/**8d'**). UV (MeOH): 228 (21000), 286 (4000), 305 (4200). $^1$H-NMR ((D$_6$)DMSO): 1.10 (*t*, *J* = 7.3, C*H*$_3$CH$_2$); 2.29 (*m*, H$_\beta$–C(2')); 2.56 (*m*, H$_\alpha$–C(2')); 3.11, 3.31 (*m*, CH$_3$C*H*$_2$); 3.56 (*m*, 2 H–C(5')); 3.87 (*m*, H–C(4')); 4.40 (*m*, H–C(3')), 4.97 (*t*, *J* = 5.4, OH–C(5')); 5.36 (*d*, *J* = 4.1, OH–C(3')); 6.73 (*t*, *J* = 6.8, H–C(1')); 7.12 (*d*, *J* = 3.7, H–C(5)); 8.01 (*d*, *J* = 3.7, H–C(6)); 8.86 (*s*, H–C(2))[2]).

*7-(2'-Deoxy-β-*D-erythro-*pentofuranosyl)-4-(ethylsulfonyl)-7*H-*pyrrolo[2,3-*d*]*pyrimidine* (**8e**). UV (MeOH): 228 (26900), 285 (4200). $^1$H-NMR ((D$_6$)DMSO): 1.19 (*t*, *J* = 7.4, C*H*$_3$CH$_2$); 2.32 (*m*, H$_\beta$–C(2')); 2.58 (*m*, H$_\alpha$–C(2')); 3.57 (*m*, CH$_3$C*H*$_2$, 2 H–C(5')); 3.89 (*m*, H–C(4')); 4.42 (*m*, H–C(3')); 4.99 (*t*, *J* = 6.5, OH–C(5')); 5.37 (*d*, *J* = 4.2, OH–C(3')); 6.75 (*t*, *J* = 7.5, H–C(1')); 7.00 (*d*, *J* = 3.7, H–C(5)); 8.20 (*d*, *J* = 3.7, H–C(6)); 9.04 (*s*, H–C(2))[2]).

*7-[2'-Deoxy-3',5'-di-*O-(p-*toluoyl)-β-*D-erythro-*pentofuranosyl]-4-(methylthio)-7*H-*pyrrolo[2,3-*d*]*pyrimidine* (**7b**). Glycosylation was carried out as described for **7a** by using powdered KOH (650 mg, 11.6 mmol), *TDA-1* (100 µl, 0.31 mmol), 4-(methylthio)-7*H*-pyrrolo[2,3-*d*]pyrimidine [31] (**5b**; 500 mg, 3.03 mmol), and **6** (1200 mg, 3.10 mmol). After chromatographic purification (*F*), colorless crystals (1.26 g, 80%) were obtained from EtOH. M.p. 118°. TLC (silica gel, *K*): $R_f$ 0.7. UV (MeOH): 223 (38300), 240 (39200), 291 (14300). $^1$H-NMR ((D$_6$)DMSO): 2.37, 2.40 (2*s* CH$_3$); 2.65 (*s*, CH$_3$S); 2.73 (*m*, H$_\beta$–C(2')); 3.15 (*m*, H$_\alpha$–C(2')); 4.44 (*m*, H–C(4'), 2 H–C(5')); 5.75 (*m*, H–C(3')); 6.61 (*d*, *J* = 3.7, H–C(5)); 6.75 (*m*, H–C(1')); 7.34, 7.91 (*m*, 8 arom. H); 7.74 (*d*, *J* = 3.7, H–C(6)); 8.63 (*s*, H–C(2)). Anal. calc. for C$_{28}$H$_{27}$N$_3$O$_5$S: C 64.98, H 5.25, N 8.12, S 6.19; found: C 65.03, H 5.28, N 7.98, S 6.12.

*7-[2'-Deoxy-3',5'-di-*O-(p-*toluoyl)-β-*D-erythro-*pentofuranosyl]-4-(methylsulfonyl)-7*H-*pyrrolo[2,3-*d*]*pyrimidine* (**9d**). By warming, **7b** (1000 mg, 1.93 mmol) was dissolved in MeOH (50 ml) and 3-chloroperbenzoic acid (1.6 g; technical grade, 80%) was added and stirred for 1 h at r.t. Upon cooling, a precipitate was formed which was recrystallized from MeOH. Colorless crystals of **9d** (860 mg, 81%). M.p. 143°. TLC (silica gel, *K*): $R_f$ 0.5. UV (MeOH): 232 (49500). $^1$H-NMR ((D$_6$)DMSO): 2.38, 2.41 (2*s*, 2 CH$_3$); 2.80 (*m*, H$_\beta$–C(2')); 3.20 (*m*, H$_\alpha$–C(2')); 3.42 (*s*, CH$_3$SO$_2$); 4.61 (*m*, H–C(4'), 2 H–C(5')); 5.78 (*m*, H–C(3')); 6.87 (*m*, H–C(1')); 7.01 (*d*, *J* = 3.8, H–C(5)); 7.35, 7.92 (*m*, 8 arom. H); 8.19 (*d*, *J* = 3.8, H–C(6)); 9.02 (*s*, H–C(2)). Anal. calc. for C$_{28}$H$_{27}$N$_3$O$_7$S: C 61.19, H 4.95, N 7.65, S 5.83; found: C 61.25, H 5.00, N 7.58, S 5.82.

*7-(2'-Deoxy-β-*D-erythro-*pentofuranosyl)-4-(methylthio)-7*H-*pyrrolo[2,3-*d*]*pyrimidine* (**8a**). As described for **7a** (→**2**), **7b** (500 mg, 0.97 mmol) was deprotected. The soln. was evaporated, the residue adsorbed on silica gel *60* (2.0 g) and chromatographed on a silica-gel *60H* column (10 × 3 cm, *G*). The residue of the main zone was crystallized from acetone. Colorless crystals (250 mg, 92%). M.p. 148°. TLC (silica gel, *H*): $R_f$ 0.3. UV (MeOH): 222 (28000), 249 (8300), 291 (16000). $^1$H-NMR ((D$_6$)DMSO): 2.23 (*m*, H$_\beta$–C(2')); 2.54 (*m*, H$_\alpha$–C(2')); 2.65 (*s*, CH$_3$S); 3.56 (*m*, 2 H–C(5')); 3.85 (*m*, H–C(4')); 4.38 (*m*, H–C(3')); 5.00 (*t*, *J* = 5.5, OH–C(5')); 5.32 (*d*, *J* = 4.1, OH–C(3')); 6.58 (*d*, *J* = 3.8, H–C(5)); 6.62 (*m*, H–C(1')); 7.76 (*d*, *J* = 3.8, H–C(6)); 8.64 (*s*, H–C(2)). Anal. calc. for C$_{12}$H$_{15}$N$_3$O$_3$S: C 51.23, H 5.37, N 14.94, S 11.40; found: C 51.20, H 5.43, N 14.73, S 11.36.

*1-(2'-Deoxy-β-*D-erythro-*pentofuranosyl)-4-(methylamino)-1*H-*pyrazolo[3,4-*d*]*pyrimidine* (**3**). To a soln. of 1-[2'-deoxy-3,5-di-*O*-(*p*-toluoyl)-β-D-*erythro*-pentofuranosyl]-4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine [12a, b] (**10a**; 500 mg, 1 mmol) in dry MeOH (60 ml), 33% MeNH$_2$/EtOH (6 ml) was added and the mixture stored at 60° for 12 h. After evaporation, the residue was separated on a 15 × 4 cm column (silica gel *60H, A*) by flash chromatography. Isolation of the material of the main zone yielded colorless, amorphous **3** (250 mg, 95%). TLC (silica gel, *A*): $R_f$ 0.3. UV (MeOH): 281 (12800), 264 (sh) (9050). $^1$H-NMR ((D$_6$)DMSO): 8.33 (*q*, *J* = 4.5, NH); 8.29 (*s*, H–C(6)); 8.14 (*s*, H–C(3)); 6.55 (*t*, *J* = 6.4, H–C(1')); 5.27 (*d*, *J* = 4.5, OH–C(3')); 4.83 (*t*, *J* = 5.8, OH–C(5')); 4.44 (*m*, H–C(3')); 3.81 (*m*, H–C(4')); 3.42 (*m*, 2 H–C(5')); 2.98 (*d*, *J* = 4.4, CH$_3$N); 2.80 (*m*, H$_\alpha$–C(2')); 2.24 (*m*, H$_\beta$–C(2')). Anal. calc. for C$_{11}$H$_{15}$N$_5$O$_3$: C 49.81, H 5.70, N 26.40; found: C 49.69, H 5.69, N 26.49.

*2-(2'-Deoxy-β-*D-erythro-*pentofuranosyl)-4-(methylamino)-2*H-*pyrazolo[3,4-*d*]*pyrimidine* (**4**). As for **3**, using **11a** (1000 mg, 2 mmol), 33% MeNH$_2$/EtOH (10 ml), and dry MeOH (80 ml): colorless amorphous **4** (510 mg, 95%). TLC (silica gel, *B*): $R_f$ 0.4. UV (MeOH): 296 (13200). $^1$H-NMR ((D$_6$)DMSO): 8.48 (*s*, H–C(3)); 8.27 (*q*, *J* = 4.8, NH); 8.23 (*s*, H–C(6)); 6.31 (*t*, *J* = 5.9, H–C(1')); 5.33 (*d*, *J* = 4.3, OH–C(3')); 4.90 (*t*, *J* = 5.6, OH–C(5')); 4.39 (*m*, H–C(3')); 3.90 (*m*, H–C(4')); 3.52 (*m*, 2 H–C(5')); 2.96 (*d*, *J* = 4.6, CH$_3$N); 2.61 (*m*, H$_\alpha$–C(2')); 2.36 (*m*, H$_\beta$–C(2')). Anal. calc. for C$_{11}$H$_{15}$N$_5$O$_3$: C 49.80, H 5.70, N 26.40; found: C 49.56, H 5.72, N 26.37.

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[2]) No sufficient elemental analyses data were obtained due to decomposition of the materials on drying.

*1-[2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-β-*D*-erythro-pentofuranosyl]-4-(methylamino)-1*H*-pyrazolo[3,4-*d*]-pyrimidine* (**10b**). By co-evaporation with anh. pyridine (10 ml), **3** (400 mg, 1.5 mmol) was dried. Then, 4-(dimethylamino)pyridine (90 mg, 0.7 mmol) and 4,4'-dimethoxytrityl chloride (900 mg, 2.7 mmol) were added and stirred for 1 h under $N_2$ at r.t. (TLC control; silica gel, *C*). To the soln., 5% aq. $NaHCO_3$ soln. (50 ml) was added and the mixture extracted with $CH_2Cl_2$ (3 × 100 ml). The combined org. layers were dried ($Na_2SO_4$), filtered, and evaporated. The residue was flash chromatographed (9 × 3 cm column, silica gel, *C*). Isolation of the material of the main zone yielded colorless, amorphous **10b** (700 mg, 82%). TLC (silica gel, *A*): $R_f$ 0.66. UV (MeOH): 281 (13950), 231 (23650). $^1$H-NMR ((D$_6$)DMSO): 8.32 (*s*, H–C(6)); 8.29 (*m*, NH); 8.08 (*s*, H–C(3)); 6.59 (*m*, H–C(1')); 5.31 (*d*, *J* = 5, OH–C(3')); 4.57 (*m*, H–C(3')); 3.94 (*m*, H–C(4')); 3.69, 3.70 (2*s*, 2 $CH_3O$); 3.05 (*m*, 2 H–C(5')); 2.98 (*d*, *J* = 4.3, $CH_3N$); 2.79 (*m*, $H_\alpha$–C(2')); 2.31 (*m*, $H_\beta$–C(2')). Anal. calc. for $C_{32}H_{33}N_5O_5$: C 67.71, H 5.86, N 12.33; found: C 67.60, H 5.98, N 12.16.

*2-[2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-β-*D*-erythro-pentofuranosyl]-4-(methylamino)-1*H*-pyrazolo[3,4-*d*]-pyrimidine* (**11b**). As for **10b**, using **4** (500 mg, 1.9 mmol), 4,4'-dimethoxytrityl chloride (1.1 g, 3.3 mmol), and 4-(dimethylamino)pyridine (110 mg, 0.85 mmol): colorless amorphous **11b** (750 mg, 70%). TLC (silica gel, *D*): $R_f$ 0.3. UV (MeOH): 297 (12650), 234 (24000). $^1$H-NMR ((D$_6$)DMSO): 8.50 (*s*, H–C(3)); 8.27 (*m*, NH); 8.24 (*s*, H–C(6)); 6.38 (*m*, H–C(1')); 5.38 (*d*, *J* = 4.9, OH–C(3')); 4.48 (*m*, H–C(3')); 3.98 (*m*, H–C(4')); 3.70, 3.67 (2*s*, 2 $CH_3O$); 3.09 (*m*, 2 H–C(5')); 2.98 (*d*, *J* = 4.5, $CH_3N$); 2.71 (*m*, $H_\alpha$–C(2')); 2.36 (*m*, $H_\beta$–C(2')). Anal. calc. for $C_{32}H_{33}N_5O_5$: C 67.71, H 5.86, N 12.33; found: C 67.60, H 6.05, N 12.32.

*7-[2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-β-*D*-erythro-pentofuranosyl]-4-(methylamino)-1*H*-pyrrolo[2,3-*d*]-pyrimidine* (**9c**). As for **10b**, using **2** (310 mg, 1.2 mmol), 4,4'-dimethoxytrityl chloride (680 mg, 2 mmol), 4-(dimethylamino)pyridine (75 mg, 0.6 mmol), and anh. pyridine: **9c** (550 mg, 81%), colorless, amorphous material. TLC (silica gel, *A*): $R_f$ 0.6. UV (MeOH): 275 (17000). $^1$H-NMR ((D$_6$)DMSO): 8.14 (*s*, H–C(2)); 7.48 (*m*, NH); 6.53 (*m*, H–C(1')); 5.33 (*d*, *J* = 4.3, OH–C(3')); 4.35 (*m*, H–C(3')); 5.91 (*m*, H–C(4')); 3.72 (2*s*, 2 $CH_3O$); 3.14 (*m*, 2 H–C(5')); 2.95 (*d*, *J* = 4.6, $CH_3N$); 2.6 (*m*, $H_\alpha$–C(2')); 2.23 (*m*, $H_\beta$–C(2')). Anal. calc. for $C_{33}H_{34}N_3O_6$: C 69.95, H 6.05, N 9.88; found: C 70.05, H 6.12, N 9.90.

*General Procedure for the Preparation of the Phosphoramidites* **12–14**. The 5'-tritylated nuclcoside was dissolved in anh. MeCN (5 ml) in a flask preflushed with Ar. Chloro(diisopropylamino)methoxyphosphine and (i-Pr)$_2$EtN were added by syringe to the soln. kept under Ar at r.t. After 30 min, the soln. was added to 5% aq. $NaHCO_3$ (30 ml) and extracted 3 times with $CH_2Cl_2$. The org. layer was dried ($Na_2SO_4$) and evaporated and the foam purified by flash chromatography on silica gel *60H* (column 12 × 2.5 cm, *E*) affording the colorless, amorphous phosphoramidite.

*1-[2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-β-*D*-erythro-pentofuranosyl]-4-(methylamino)-1*H*-pyrazolo[3,4-*d*]-pyrimidine 3'-[Methyl* N,N-*Diisopropylphosphoramidite]* (**12**). From **10b** (400 mg, 0.7 mmol), chloro-(diisopropylamino)methoxyphosphine (0.14 ml, 0.7 mmol), and (i-Pr)$_2$EtN (0.36 ml, 2.1 mmol): colorless, amorphous **1** (350 mg, 69%). TLC (silica gel, *E*): $R_f$ 0.65. $^{31}$P-NMR ($CDCl_3$): 147.08.

*2-[2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-β-*D*-erythro-pentofuranosyl]-4-(methylamino)-1*H*-pyrazolo[3,4-*d*]-pyrimidine 3'-[Methyl* N,N-*Diisopropylphosphoramidite]* (**13**). As for **12**: **13** (370 mg, 71%) as colorless foam. TLC (silica gel, *E*): $R_f$ 0.35. $^{31}$P-NMR ($CDCl_3$): 148.4.

*7-[2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-β-*D*-erythro-pentofuranosyl]-4-(methylamino)-7*H*-pyrrolo[2,3-*d*]-pyrimidine 3'-[Methyl* N,N-*Diisopropylphosphoramidite]* (**14**). As described for **12**: colorless, amorphous **14** (310 mg, 61%). TLC (silica gel, *E*): $R_f$ 0.7. $^{31}$P-NMR ($CDCl_3$): 147.0, 147.2.

*Solid-Phase Synthesis of the Oligomers* **19–27**. The synthesis of the oligonucleotides was accomplished on a 1-μmol scale using the methyl phosphoramidites 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 the phosphoramidites **12–15**. The synthesis of **19–27** followed the regular protocol of the DNA synthesizer for methyl phosphoramidites [16]. Deprotection of $NH_2$ groups was carried out with 25% aq. $NH_3$ at 60° for 48 h. The 4,4'-dimethoxytrityl residues of **19a–27a** were removed by treatment with 80% aq. $CH_3CO_2H$, for 5 min at r.t. Purification was accomplished by HPLC on *RP-18* columns using solvent system *I* for the (MeO)$_2$Tr derivatives **19a–27a**, and *II* for the detritylated oligonucleotides **19a–27b**. The oligomers were desalted on a 25 × 4 HPLC cartridge (*RP-18* silica gel). Inorg. material was eluted with $H_2O$ (10 ml), while the oligomer was eluted with MeOH/$H_2O$ 3:2 (5 ml). The oligomers were lyophilized on a *Speed-Vac* evaporator to yield a colorless foam which was dissolved in $H_2O$ (1 ml) and stored frozen at −20°.

*Enzymatic Hydrolysis of the Oligomers* **19b–27b**. The oligomer (0.4 $A_{260}$ units) was dissolved in 0.1M *Tris*-HCl buffer (pH 8.5; 200 μl) and treated with snake-venom phosphodiesterase (3 μg) at 25° for 60 min and alkaline phosphatase (2 μg) for 15 min at 25°. The mixture was analyzed on reverse-phase HPLC (*RP-18*, solvent system *III* or *IV*). 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 ($\varepsilon_{260}$: $A_d$, 15400; $C_d$, 7300; $G_d$, 11700; $T_d$, 8800; $m^6A_d$, 11600; $m^6c^7A_d$, 9700; $c^7m^6z^8A_d$, 8400; $c^7m^6z^8A'_d$, 5300).

*Phosphodiester Hydrolysis of the Oligomers* **23b–27b** *with the Endodeoxyribonuclease Dpn I.* The oligomers **23b–27b** (16 μM) were dissolved in 6 mM *Tris*-HCl buffer (pH 7.7; 140 μl) containing 150 mM NaCl, 6 mM $MgCl_2$, 7 mM 2-mercaptoethanol, and bovine serum albumine (100 μg/ml) and treated with the endodeoxyribonuclease Dpn I (10 units) at 25°. Samples of 20 μl were taken at different intervals of time and analyzed on reverse-phase HPLC (*RP-18*, gradient *II*). The hydrolysis products were collected, lyophilized, and analyzed after hydrolysis with snake-venom phosphodiesterase and alkaline phosphatase, as described in the previous section.

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