87. 1,7-Dideaza-2′-deoxyadenosine: Building Blocks for Solid-Phase Synthesis and Secondary Structure of Base-Modified Oligodeoxyribonucleotides

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Dedicated to Prof. *W. Pfleiderer* on the occasion of his 65th birthday

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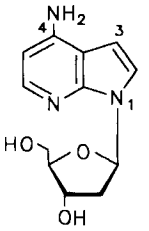
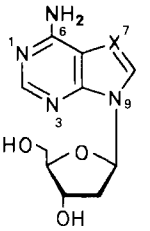
The 1,7-dideaza-2′-deoxyadenosine ($c^1c^7A_d$; **1**) was converted into building blocks **3a,b** for solid-phase oligodeoxyribonucleotide synthesis. Testing various *N*-protecting groups – benzoyl, phenoxyacetyl, [(fluoren-9-yl)methoxy]carbonyl, and (dimethylamino)methylidene – only the latter two were found to be suitable (**1** → **4b, d**). Ensuing 4,4′-dimethoxytritylation of **4d** and phosphitylation afforded the 3′-phosphonate **3a** or the 3′-[(2-cyanoethyl)diisopropylphosphoramidite] **3b**. Self-complementary oligonucleotides with alternating dA or $c^1c^7A_d$ and dT residues (7 and **8**) as well as palindromic oligomers such as d(C-G-C-G-c^1c^7A-c^1c^7A-T-T-C-G-C-G) (**10**) and d(G-T-A-G-c^1c^7A-c^1c^7A-T-T-C-T-A-C) (**12**) were synthesized. Duplex stability was decreased because **1** cannot form *Watson-Crick* or *Hoogsteen* base pairs if incorporated into oligonucleotides. On the other hand, the structural modifications in **10** and **12** forced these palindromic oligomers to form hairpin structures.

Introduction. – Base-modified nucleosides are useful tools to study the structural properties of DNA and RNA fragments as well as their interaction with metals, antibiotics, or proteins. Pyrrolo[2,3-*d*]pyrimidine 2′-deoxyribonucleosides, *e.g.* **2b**, were used for this purpose. The absence of the purine N-atom N(7) located in the major groove of duplex DNA prevents the hydrolytic action of endodeoxyribonucleases [1] [2]. Moreover, secondary interactions of dG residues, causing band compression during electrophoresis of oligonucleotides, can be avoided upon *Sanger* dideoxy sequencing [3].

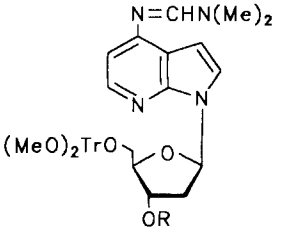
Pyrrolo[2,3-*b*]pyridine (1,7-dideazapurine, 7-azaindole) nucleosides are isosteric to the parent purine compounds, but they neither contain the purine N-atoms N(1) nor N(7) [4–6]. According to that, *Watson-Crick* and/or *Hoogsteen* base pairing are expected not to occur, if such a nucleoside is incorporated into a DNA fragment. On the other hand, oligonucleotide regions containing such a modified nucleoside within a larger piece of a



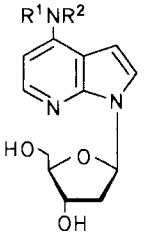
1 (systematic numbering)



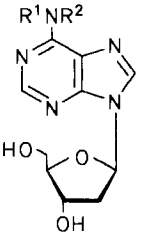
2a X = N (purine numbering)
b X = CH



3a R = PH(O)O⁻Et_3NH^+
b R = P[(i-Pr)$_2$N](OCH$_2$CH$_2$CN)



4a $R^1 = H$, $R^2 = bz$
b $R^1 = H$, $R^2 = fmoc$
c $R^1 = H$, $R^2 = pac$
d R^1, $R^2 = m_2fa$



5a $R^1 = H$, $R^2 = bz$
b $R^1 = H$, $R^2 = fmoc$
c $R^1 = H$, $R^2 = pac$
d R^1, $R^2 = m_2fa$

regular DNA can only be stabilized by vertical base-stacking. Thus, different secondary structures such as bulge loops or hairpins should be favored. In the following, we describe the synthesis of a suitably protected phosphonate **3a** or phosphoramidite **3b** of 1,7-dideaza-2′-deoxyadenosine (**1**) as building block for automated solid-phase synthesis. Moreover, the secondary structure of self-complementary oligonucleotides containing **1** is investigated.

Results and Discussion. – Recently, 1,7-dideaza-2′-deoxyadenosine (**1**) was synthesized in our laboratory in a stereochemically controlled reaction [7]. The 2′,3′-dideoxyribonucleoside was also prepared [8]. The ribonucleoside was described earlier by others [9]. Compound **1** is extraordinarily stable at the N-glycosylic bond [7] which is the result of the sugar attachment to the pyrrole moiety. As a consequence, 'depurination' which is a severe problem in the case of oligodeoxyribonucleotide synthesis with benzoylated dA is not expected to occur. On the other hand, compound **1** is easier protonated than the parent dA. A pK_a value of 6.1 was determined spectrophotometrically at 270 and 295 nm for **1** (see *Exper. Part*). Compared to 3,7-dideaza-2′-deoxyadenosine (pK_a 8.6) [10], the nucleoside is less basic but easier to protonate than 7-deaza-2′-deoxyadenosine (**2b**: pK_a 5.3) [10] or even 2′-deoxyadenosine (**2a**: pK_a 3.8). A pK_a value of **1** published recently [7] is too low and has to be revised. As this pK_a value was measured below 210 nm, UV measurement interfered with the absorption of the buffer solution.

To synthesize an oligonucleotide building block being compatible with those of regular DNA constituents, various protecting groups were studied. For 4-NH_2 protection of **1**, the benzoyl (bz), [(fluoren-9-yl)methoxy]carbonyl (fmoc), phenoxyacetyl (pac), and (dimethylamino)methylidene (m_2fa) groups were chosen. Reaction of **1** with benzoyl chloride under temporarily protection of the sugar OH groups by Me_3Si residues (transient protection) [11] afforded compound **4a** in 74% yield, after chromatography. Surprisingly, the use of phenoxyacetyl anhydride for 4-NH_2 protection [12] of **1** was difficult, and formation of **4c** was only accomplished under vigorous conditions in low yield (20%). Similarly to benzoyl derivative **4a**, **4b** was obtained by reaction of **1** with [(fluoren-9-yl)methoxy]carbonyl (fmoc) chloride [13] [14] in 77% yield. The preparation of compound **4d** [15] circumvented the intermediate protection of the sugar OH groups; it was isolated in 76% yield. Compounds **4a–d** were characterized by elemental analysis and ^1H- and ^{13}C-NMR spectra (*Table 1*). The assignment of the quarternary aglycone signals was made on the basis of J(C,H) coupling constants. The chemical shifts of C(2), C(3), C(5),

Table 1. *^{13}C-NMR Chemical Shifts of 2′-Deoxyribofuranosyl Derivatives of Pyrrolo[2,3-b]pyridines in (D_6)DMSO at 23°*[a])

	C(2)	C(3)	C(3a)	C(4)	C(5)	C(6)	C(7a)	MeO	N=CH
1 [7]	122.0	98.6	108.4	148.6	100.2	143.4	148.1	–	–
3a	122.6	99.6	115.3	152.2	106.5	143.7	148.9	55.0	155.0
4a	124.5	100.1	112.7	138.8	107.9	143.2	148.7	–	–
b	124.1	99.2	111.1	138.9	104.9	143.7	148.2	–	–
c	124.6	99.8	111.4	137.8	106.4	143.4	148.3	–	–
d	123.7	99.2	115.7	152.4	106.3	143.2	148.5	–	154.9
6	122.8	99.5	115.3	152.2	106.4	143.6	148.9	55.0	154.9

	Me_2N	C=O	CH_3CH_2	CH_3CH_2	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)
1	–	–	–	–	84.4	[b])	71.5	87.4	62.5
3a	34.0	–	45.2	8.5	82.5	[b])	72.9	83.9	64.0
4a	–	166.7	–	–	83.2	39.6	71.3	87.3	62.2
b	–	153.5	–	–	83.2	[b])	71.1	87.2	62.1
c	–	167.6	–	–	83.1	39.7	71.1	87.2	62.1
d	34.0	–	–	-	83.6	39.7	71.2	87.1	62.2
6	30.9/34.0	–	–	-	82.4	39.7	71.0	84.9	64.0

[a]) δ Values given in ppm relative to Me_4Si as internal standard.
[b]) Superimposed by DMSO.

and those of the sugar moiety were assigned by 2D ^{1}H,^{13}C-correlation spectra. The sugar signals of the various base-protected compounds **4a–d** correspond to those of the unprotected nucleoside **1** [7] which already were assigned.

As the 4-NH_2 protecting groups of **1** had to be stable during the oligonucleotide synthesis but removable under mild alkaline conditions, we carried out hydrolysis experiments in concentrated aqueous NH_3 solution. TLC monitoring confirmed that deprotection gave back nucleoside **1** as the only reaction product. Quantitative kinetic measurements were carried out spectrophotometrically at a wavelength of maximal UV-absorption difference [16]. In contrast to bz^6A_d (**5a**) **4a** was not hydrolyzed in conc. NH_3 at 50° (*Table 2*). The fmoc derivative **4b** was much more labile, with a half-life time of deprotection of 40 min which seems to be perfect for oligonucleotide synthesis. The amidine **4d** as well as the phenoxyacetylated **4c** exhibited much longer half-life times, but their deprotection times were still in the range which allows their use in solid-phase synthesis. A

Table 2. *Half-Life Values ($t_{1/2}$) of Deprotection of Pyrrolo[2,3-b]pyridine 2′-Deoxyribonucleosides in 25% aq. NH_3 Solution*[a])

Compound	$t_{1/2}$ [min]	Compound	$t_{1/2}$ [min]
$bz^6c^1c^7A_d$ (**4a**)	∞[b])	bz^6A_d (**5a**)	71[c])
$fmoc^6c^1c^7A_d$ (**4b**)	40[c])	–	–
$pac^6c^1c^7A_d$ (**4c**)	188[d])	pac^6A_d (**5c**)	1[b])
$(m_2fa)^6c^1c^7A_d$ (**4d**)	243[d])	$(m_2fa)^6A_d$ (**5d**)	4[f])

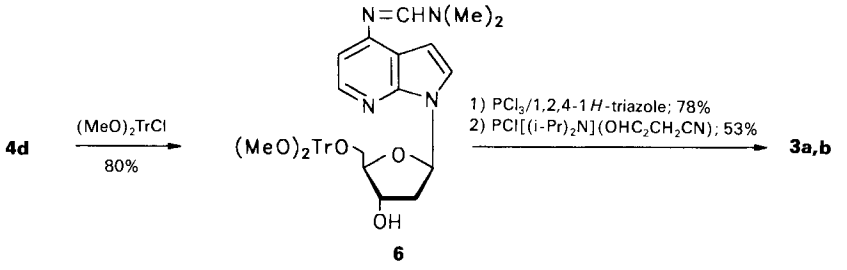
[a]) At 20 μM nucleoside concentration and 50°. [b]) Measured UV-spectrophotometrically at 295 nm. [c]) See *Footnote b*, at 255 nm. [d]) See *Footnote b*, at 308 nm. [e]) See *Footnote b*, at 310 nm. [f]) See *Footnote b*, at 311 nm.

comparison of compounds **4a**, **4c**, and **4d** with the corresponding dA derivatives **5a**, **5c**, and **5d** showed similar trends for the modified and parent compounds (*Table 2*). The higher stability of the base-protecting groups in the case of **4a,c,d** results from the increase of the basicity of the 1,7-dideazaadenosine 4-amino group compared with that of dA (**2a**); supplementary N-atoms in the dA system have the same effect as $-M$ substituents, which decrease the basicity.

From the data described above, the fmoc group would be the most suitable to be used further. Although this group was already employed for NH_2 protection in oligonucleotide synthesis in solution [14], it was noticed that the yields of fmoc-protected dA **5b** varied from experiment to experiment [17]. Later it was shown that apart from the mono-protected derivative, also the bis-protected compound was formed which is not easy to convert into the mono-protected derivative [14]. This was not observed in the case of **4b** (77% yield). However, in this work, the (dimethylamino)methylidene derivative **4d** was preferred. This group is now widely used for the protection of dA in solid-phase oligonucleotide synthesis [18a].

Reaction of **4d**, with an excess of 4,4′-dimethoxytriphenylmethyl chloride ($(MeO)_2TrCl$) in the presence of 4-(dimethylamino)pyridine and purification by flash chromatography yielded compound **6** (80%; *Scheme*). The downfield ^{13}C-NMR shift of C(5′) and an upfield location of C(4′) confirmed 5′-protection (*Table 1*). The reaction of the protected deoxynucleoside **6** with tris(1,2,4-triazolyl)phosphite [19] followed by hydrolysis with aqueous $(Et_3NH)HCO_3$ (*TBK*, 1M, pH 7.5) gave **3a** in 78% yield ($J(P,C(3')) = 18$ and $J(P,C(4')) = 20$ Hz). Phosphitylation of **6** with chloro(2-cyanoethoxy) (diisopropylamino)phosphane [20] afforded the phosphoramidite **3b**, isolated as a diastereoisomer mixture (^{31}P-NMR: 148.1, 148.7 ppm) in 53% yield.

Scheme



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The phosphonate **3a** was then employed in solid-phase oligonucleotide synthesis [19] using an *ABI-380B* synthesizer. The protocol of detritylation, activation (adamantanoyl chloride), coupling, and capping followed the *ABI* user bulletin [18b]. Oxidation with I_2 in pyridine/H_2O/THF was carried out on the oligomeric level. The $(MeO)_2Tr$-protected oligonucleotides were split off from the polymer support by concentrated NH_3 solution and purified by reversed-phase *RP-18* HPLC. Detritylation (80% AcOH/H_2O) was followed by neutralization with Et_3N. The oligomers were again submitted to *RP-18* HPLC, desalted, and lyophilized. Thus, the oligonucleotides **7**, **8**, **10**, and **12** as well as the known sequences **9**, **11**, and **13** were synthesized.

Their nucleoside content was determined after tandem hydrolysis with snake-venom phosphodiesterase followed by alkaline phosphatase [16]. Reversed-phase RP-18 HPLC

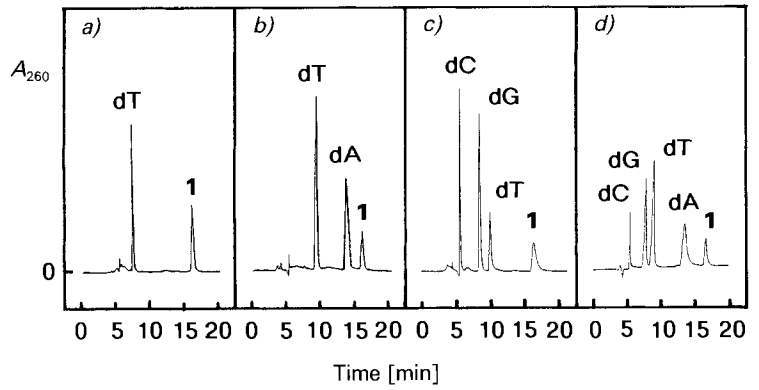

Fig. 1. *HPLC Profiles after tandem hydrolysis of* a) *d(c*^{1}*c*^{7}*A-T)*$_6$ (**8**), b) *d(A-T-A-T-c*^{1}*c*^{7}*A-T-c*^{1}*c*^{7}*A-T-A-T-A-T)* (**7**), c) *d(C-G-C-G-c*^{1}*c*^{7}*A-c*^{1}*c*^{7}*A-T-T-C-G-C-G)* (**10**), *and* d) *d(G-T-A-G-c*^{1}*c*^{7}*A-c*^{1}*c*^{7}*A-T-T-C-T-A-C)* (**12**) *with snake-venom phosphodiesterase followed by alkaline phosphatase in 0.1*M Tris-*HCl buffer* (pH 8.3). Incubation time, 75 min (37°).

of the hydrolysis mixture separated the nucleosides (see *Fig. 1*, for **7**, **8**, **10**, and **12**). Quantitative UV spectrophotometry using the extinction coefficients of the monomers confirmed the molar amount of monomeric constituents.

d(A-T-A-T-c^{1}c^{7}A-T-c^{1}c^{7}A-T-A-T-A-T) **7**

d(c^{1}c^{7}A-T)$_6$ **8**

d(A-T)$_6$ **9**

d(C-G-C-G-c^{1}c^{7}A-c^{1}c^{7}A-T-T-C-G-C-G) **10**

d(C-G-C-G-A-A-T-T-C-G-C-G) **11**

d(G-T-A-G-c^{1}c^{7}A-c^{1}c^{7}A-T-T-C-T-A-C) **12**

d(G-T-A-G-A-A-T-T-C-T-A-C) **13**

Next the T_m values of the alternating dodecamers **7**–**9** were determined at 260 nm at pH 7.0 in 1M NaCl (*Table 3*). The accuracy of the measurement was ±1°. Except for compound **8**, cooperative mono-phasic melting profiles were observed. The alternating oligomer **7** containing two modified dA residues **1** exhibited a lower T_m value as the

Table 3. T_m *Values and Thermal Hypochromicities of the Oligonucleotides* **7**–**13**

Oligonucleotide		t_m [°][a])[b])	h_{therm} [%][b])[c])
d(A-T-A-T-c^{1}c^{7}A-T-c^{1}c^{7}A-T-A-T-A-T)	(**7**)	24	12
d(c^{1}c^{7}A-T)$_6$	(**8**)	–[d])	1
d(A-T)$_6$	(**9**)	30	23
d(C-G-C-G-c^{1}c^{7}A-c^{1}c^{7}A-T-T-C-G-C-G)	(**10**)	63	9
d(C-G-C-G-A-A-T-T-C-G-C-G)	(**11**)	49	22
d(G-T-A-G-c^{1}c^{7}A-c^{1}c^{7}A-T-T-C-T-A-C)	(**12**)	43	15
d(G-T-A-G-A-A-T-T-C-T-A-C)	(**13**)	48	27

[a]) 5 μM single-strand concentration. [b]) Measured in 60 mM cacodylate buffer, pH 7.0, 1M NaCl, 100 mM $MgCl_2$. [c]) h_{therm}: 10–80°; 260 nm. [d]) No cooperative melting.

parent oligomer **9** [16]. As expected, a total replacement of dA by **1** as in **8** resulted in the complete loss of base-pairing, due to the absence of N(1) and N(7).

As it is unknown whether the 1,7-dideazaadenine moieties within a single-stranded oligomer such as **8** show nearest-neighbor interactions with dT, the hypochromicity (*h*, determined at 260 nm) was measured [21]. The hypochromicity of oligomer **8** was determined by the melting curves within the temperature range 10–80° and found to be almost zero at 260 nm (*Table 3*). The value of *h* decreased to about one half, if only two of six dA residues of **9** were replaced (see **7**). These differences are also found in the CD spectra of **8** and **9** (*Fig. 2*). The oligomer **9** exhibits a B_{2u} transition at 268 nm with a positive *Cotton* effect and a B_{1u} transition at 246 nm with a negative sign. The CD spectra of **8** show a small positive *Cotton* effect at 243 nm and a negative one below 220 nm. These differences demonstrate that $d(A\text{-}T)_6$ is a B-type duplex, whereas **8** is only a single strand. To prove whether the oligomer **8** has still some ordered secondary structure, we measured the CD spectra of synthetic 1:1 mixtures dT/**1** at a concentration corresponding to that

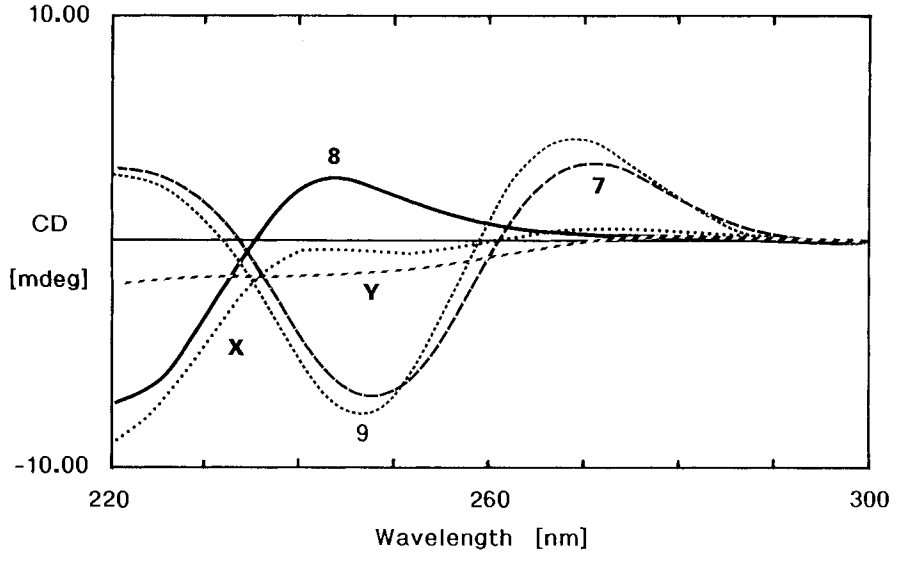

Fig. 2. *CD spectra of* **7**, **8**, *d(A-T)*$_6$ (**9**), *a 1:1 mixture* **1**/*dT* (**X**) *and a 1:1 mixture dA/dT* (**Y**). Measured in 60 mM Na cacodylate buffer (pH 7.0, 1M NaCl, 100 mM $MgCl_2$), at 8°; the oligomer concentration was *ca.* 5.0 μM and the monomeric mixtures contained 25 μM dT and 25 μM dA or compound **1**.

of **8**. From *Fig. 2*, it can be seen that the spectra of the oligomer **8** and the mixture of its monomeric constituents **1** and dT are different. This demonstrates that the single-stranded oligomer **8** has still a secondary structure, most probably caused by stacking interaction. The CD spectrum of the partially modified oligomer **7** shows only minor changes compared to **9**. According to that, it is still a duplex without interaction in the innermost segment which can be described as bulge loop duplex.

To get more information about the secondary structure of the modified oligomers, the thermodynamic parameters (ΔH and ΔS) were calculated from each melting profile at various concentrations (1–20 μM) [22]. Each truncated melting curve was fitted to a

Table 4. *Thermodynamic Parameters for Helix Formation of the Dodecamers* **7**–**13**[a])

	ΔH_{calc} [kcal/mol]	ΔH_{exp} [kcal/mol]	ΔS [cal/mol·K]	ΔG[b]) [kcal/mol]	$\Delta\Delta G$ [kcal/mol]
7	–42	–40	–133	+0	–1
8	–	–	–	–	–
9 [16]	–66	–59	–195	–1	–
10	–47	–52	–144	–9	+2
11 [25] [31]	–125	–102	–317	–7	–
12	–30	–35	–112	–2	–4
13	–95	–82	–254	–6	–

[a]) Measured in 60 mM cacodylate buffer, pH 7.0, 1M NaCl, 100 mM $MgCl_2$. [b]) Calculated at 25°

two-state model using a least-square fitting program [23]. The $\Delta\Delta G$ value of oligomer **7** (*Table 4*) together with the reduced T_m value reflect the lower stability of the bulge loop duplex compared to the parent oligomer **9**. As expected from the reduced number of *Watson-Crick* base pairs within the duplex of **7** (8 instead of 12 base pairs), the ΔH of **7** is 19 kcal/mol lower as that of **9**. If one assumes that the duplex of **7** contains two base-paired segments d(A-T-A-T)·d(T-A-T-A), the ΔH can be calculated from the sum of ΔH increments of d(A-T) base pairs (–6 kcal/mol of each A·T base pair), reduced by the nucleation energy as reported [24]. According to that, a value of –42 kcal/mol was calculated for **7** compared to –40 kcal/mol determined experimentally. As the duplex regions of **7** contain only 4 base pairs at each site of the modified segment, calculations as described above have to be interpreted with care. However, it appears, that the d(A-T) segment containing modified dA residues does not contribute much to the duplex stabilization of **7**.

As can be seen from *Fig. 3b*, the heat of helix formation is almost independent on the temperature in the case of **9** but varies in the case of **7**. This implies that the region of d(c^1c^7A-T) within the duplex of **7** has some secondary structure – at least at low tempera-

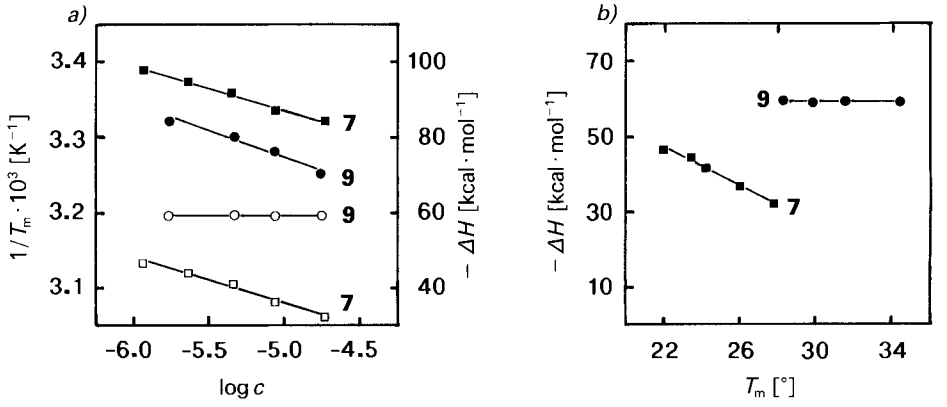

Fig. 3. a) $1/T_m$ vs. *log* c *and* –ΔH vs. *log* c (● and ■, $1/T_m$ scale; ○ and □, $-\Delta H$ scale) *and* b) –ΔH vs. T_m *of compounds* **7** *and* **9**. Same buffer as in *Fig. 2*.

ture – which is melted out at higher values. This results in a decrease of the heat of helix formation as demonstrated. This structural change is also evident from the dependence of $1/T_m$ on log c (*Fig. 3a*).

It was already reported for the *Dickerson* dodecamer **11** that depending on the NaCl concentration, hairpins and duplexes can be present simultaneously at low salt concentrations, whereas duplex formation is favored at high salt concentrations [25] [26]. Fortunately, hairpins and duplexes can be detected separately by UV in the case of **11**. The shoulder at 280 nm in the UV spectrum of dG, which is absent in dA, allows the detection of the d(G-C) melting without detecting d(A-T) melting [26]. As the number of d(G-C) base pairs is not changed upon melting of duplex **11** into 2 hairpins, the T_m observed at 280 nm represents melting of the hairpin. The small difference of the T_m values (1M NaCl, 5 μM oligonucleotide concentration) of **11** at 260 nm (49°) and 280 nm (50°) indicates that oligomer **11** forms a duplex at 1M NaCl almost exclusively. However, at lower salt concentration, hairpins are favored [27].

Compound **10** exhibits a much higher T_m value than **11** (ΔT_m = 14°) and a much lower hypochromicity (*Table 3*). This points to different secondary structures of **10** and **11**; most probably to a hairpin in the case of **10**. As the melting of a hairpin does not depend on the concentration T_m values were measured within an oligonucleotide concentration range of 1–20 μM. *Fig. 4a* shows the dependence of T_m of the oligomer **11** (●) on the concentration, whereas T_m of **10** (■) is independent. The ΔH value of **10** is also almost

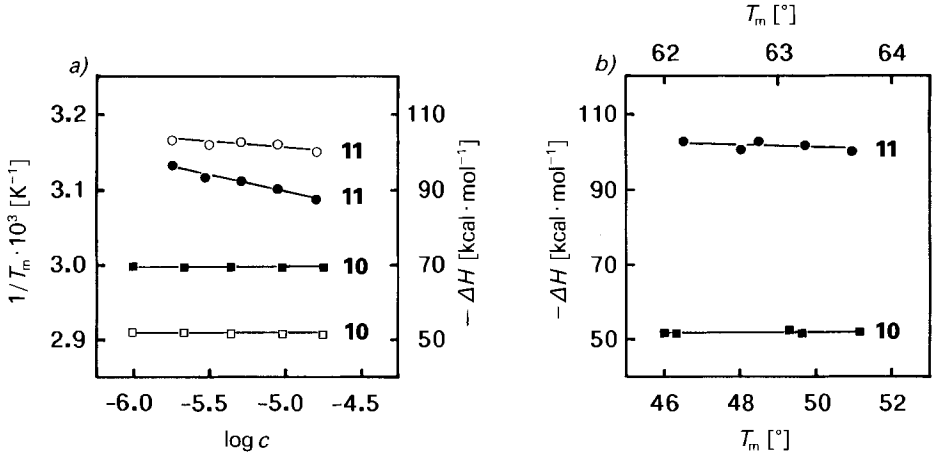

Fig. 4. a) $1/T_m$ vs. *log* c *and* $-\Delta H$ vs. *log* c (scale according *Fig. 3*) *and* b) $-\Delta H$ vs. T_m *of compounds* **10** *and* **11** (full circles, lower scale; full squares, upper scale). Buffer according to *Fig. 3*.

independent on T_m (*Fig. 4b*), whereas **11** shows a slight dependence. According to that, oligonucleotide **10** is a hairpin and **11** is a duplex.

As discussed above, hairpin formation of **11** is facilitated by decreasing *Watson-Crick* base-pairing interactions within the innermost part of an oligonucleotide. This was already shown by the replacement of dA residues by propane-1,3-diol moieties or 2′-deoxyxylofuranosyladenine (xA_d) residues [27] [28]. Consequently, duplex formation

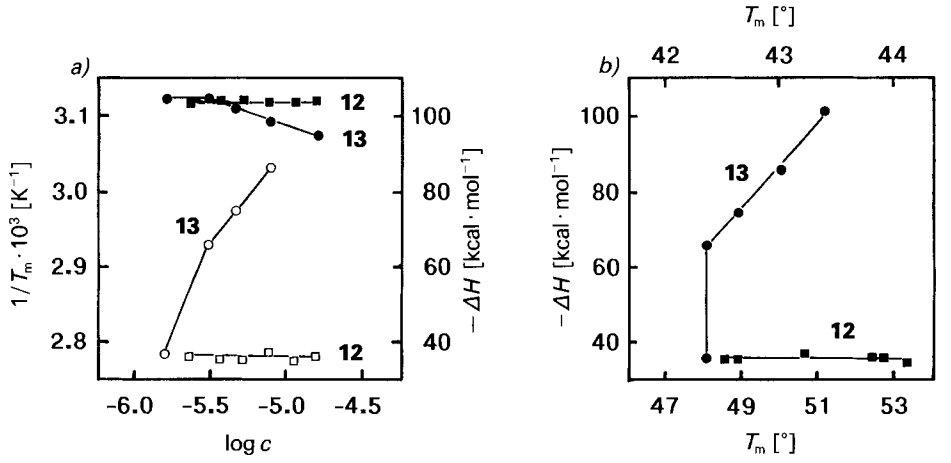

Fig. 5. a) $1/T_m$ vs. *log* c *and* $-\Delta H$ vs. *log* c (scale according *Fig. 3a*) *and* b) $-\Delta H$ vs. T_m *of compounds* **12** *and* **13** (scale according *Fig. 4b*). Buffer according to *Fig. 3*.

should be favored if *Watson-Crick* base pairing is decreased in the flanking regions of an oligonucleotide. This is the case with compound **13**. According to the bend observed in the graphs of $1/T_m$ *vs.* log *c* or $-\Delta H$ *vs.* log *c* (*Fig. 5a*) as well as $-\Delta H$ *vs.* T_m (*Fig. 5b*), oligomer **13** forms duplexes and hairpins within the same concentration range [28]. But again, exclusive hairpin formation is observed, if the innermost dA residues are replaced by modified nucleosides (*e.g.* **1**) such as in compound **12** (*Fig. 5a*).

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Experimental Part

General. See [29]. The phosphonates of regular 2′-deoxynucleosides were purchased from *Sigma*, St. Louis, and the *Fractosil*-linked 2′-deoxynucleosides from *Milligene*, Eschborn, Germany. Snake-venom phosphodiesterase (EC 3.1.15.1, *Crotallus durissus*) and alkaline phosphatase (EC 3.1.3.1, *E. coli*) are products of *Boehringer*, Mannheim, Germany. Oligonucleotide syntheses were carried out on an automated DNA synthesizer, model *380 B*, of *Applied Biosystems*, Weiterstadt, Germany. Flash chromatography (FC) and TLC: solvent systems: CH_2Cl_2/MeOH 9:1 (*A*), $CHCl_3$/MeOH 9:1 (*B*), CH_2Cl_2/Et_3N 98:2 (*C*), CH_2Cl_2/MeOH/Et_3N 88:10:2 (*D*), CH_2Cl_2/AcOEt/Et_3N 45:45:10 (*E*). The pK_a value was determined according to [30] at 295 and 270 nm in a buffer of the ionic strength of 0.01 at 20°. The hypochromicity ($h = [(\varepsilon_{monomer} - \varepsilon_{polymer}) \cdot (\varepsilon_{monomer})^{-1}] \cdot 100\,\%$) of **7–13** (*Table 3*) was determined from the melting curves. The extinction coefficient of the oligonucleotide was calculated from the sum of the extinction coefficients of the monomeric 2′-deoxyribonucleosides divided by the hyperchromicity. The analysis of changes in the melting curves of oligonucleotides as a function of temp. were calculated according to a two-state stacked-unstacked equilibrium according to $\ln K = \ln [(E^s - E)/(E - E^u)] = \Delta S/R - \Delta H/RT$ (E = absorbancy at a particular wavelength, s = stacked state, and u = unstacked state). The program included an interactive least-squares fitting of both, the melting curve ($E = f(T)$) and the corresponding *van t'Hoff* plot, as well as the plotting of both graphs [27]. CD Spectra: *Jasco-600* spectropolarimeter; thermostatically controlled 1-cm cuvettes connected with a *Lauda RCS 6* bath. Microanalyses were performed by 'Mikroanalytisches Labor Beller', Göttingen, Germany.

HPLC Separation. Prep. HPLC was carried out on a 250 × 4 mm *RP-18* precolumn by a *Merck-Hitachi* HPLC with one pump (model *655-A-12*) connected with a proportioning valve, a variable-wavelength monitor

(modcl *655 A*), a controller (model *L-5000*), and an integrator (model *D-2000*). The solvent gradients consisting of 0.1M $(Et_3NH)OAc$ (pH 7.0)/MeCN 95:5 (*A*) and MeCN (*B*) were used in the following order: gradient *I*, 3 min 15% *B* in *A*, 7 min 15–40% *B* in *A*, 5 min 40% *B* in *A*, 5 min 40–15% *B* in *A*, flow rate 1 ml/min; gradient *II*, 20 min 0–20% *B* in *A*, flow rate 1 ml/min; gradient *III*, 20 min 100% *A*, flow rate 0.6 ml/min.

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 *R22* unit (*MWG Lauda*, Germany). The actual temp. was measured in the reference cell with at Pt resistor. Melting hypochromicity values were calculated from the initial and final absorbances as described above.

*4-(Benzoylamino)-1-(2'-deoxy-β-*D-erythro-*pentofuranosyl)-1*H-*pyrrolo[2,3-*b*]pyridine* (**4a**). Compound **1** [7] (200 mg, 0.80 mmol) was dried by co-evaporation with anh. pyridine and then suspended in pyridine (10 ml). Me_3SiCl (512 µl, 4.0 mmol) was added under Ar and the soln. stirred for 15 min, treated with benzoyl chloride (464 µl, 4.0 mmol), and maintained at r.t. for 2 h. The mixture was cooled to 0° and hydrolyzed with H_2O (800 µl). After 5 min, the resultant was treated with 25% aq. NH_3 soln. (1.6 ml) and stirred for another 30 min. The solvent was evaporated and the oily residue co-evaporated twice with toluene. It was redissolved in solvent *A* and applied to FC (0.1 bar, silica gel, column 20 × 5 cm, solvent *A*): colorless amorphous **4a** (210 mg, 74%). TLC (silica gel, solvent *A*): R_f 0.4. UV (MeOH): 302 (10900). ^{1}H-NMR ((D_6)DMSO): 2.21 (*m*, H_α–C(2')); 2.60 (*m*, H_β–C(2')); 3.57 (*m*, 2 H–C(5')); 3.85 (*m*, H–C(4')); 4.38 (*m*, H–C(3')); 5.11 (*t*, *J* = 5.7, OH–C(5')); 5.29 (*d*, *J* = 4.0, OH–C(3')); 6.71 (*dd*, *J* = 6.1, 8.3, H–C(1')); 6.90 (*d*, *J* = 3.7, H–C(3)); 7.54–8.00 (*m*, 5 arom. H, bz); 7.66 (*d*, *J* = 3.8, H–C(2)); 7.76 (*d*, *J* = 5.4, H–C(5)); 8.20 (*d*, *J* = 5.4, H–C(6)); 10.48 (*s*, NH–C(4)). Anal. calc. for $C_{19}H_{19}N_3O_4$ (353.4): C 64.58, H 5.42, N 11.89; found: C 64.57, H 5.762, N 11.76.

*1-(2'-Deoxy-β-*D-erythro-*pentofuranosyl)-4-{[(fluoren-9-yl)methoxy]carbonylamino}-1*H-*pyrrolo[2,3-*b*]pyridine* (**4b**). To a suspension of **1** (200 mg, 0.80 mmol) in anh. pyridine (10 ml), Me_3SiCl (1.0 ml, 7.9 mmol) was added under Ar. After stirring for 3 h at r.t., the soln. was treated with [(fluoren-9-yl)methoxy]carbonyl chloride (268 mg, 1.0 mmol), stirred for another 3 h and cooled to 0°. Upon dilution with H_2O (5 ml), the mixture was stirred again overnight at r.t. and the volume reduced. The conc. soln. was diluted with H_2O/AcOEt 2:1 (30 ml). After separation of the org. layer, the aq. layer was extracted with AcOEt (5 times, 10 ml each), the combined org. extract dried (Na_2SO_4) and evaporated, and the pale yellow foam applied to FC (silica gel, column 20 × 5 cm, solvent *A*): colourless amorphous **4b** (290 mg, 77%). TLC (silica gel, solvent *A*): R_f 0.5. UV (MeOH): 265 (26000), 289 (17700), 298 (16200). ^{1}H-NMR ((D_6)DMSO): 2.21 (*m*, H_α–C(2')); 2.60 (*m*, H_β–C(2')); 3.57 (*m*, 2 H–C(5')); 3.86 (*m*, H–C(4')); 4.40 (*m*, H–C(3')); 4.58 (*d*, *J* = 6.8, CH_2, fmoc); 5.13 (*t*, *J* = 5.6, OH–C(5')); 5.29 (*d*, *J* = 4.0, OH–C(3')); 6.69 (*dd*, *J* = 6.0, 8.2, H–C(1')); 6.99 (*d*, *J* = 3.7, H–C(3)); 7.35–7.49 (*m*, 4 arom. H, fmoc); 7.56 (*d*, *J* = 5.3, H–C(5)); 7.63 (*d*, *J* = 3.8, H–C(2)); 7.81–7.95 (*m*, 4 arom. H, fmoc); 8.09 (*d*, *J* = 5.5, H–C(6)); 10.16 (*s*, NH–C(4)). Anal. calc. for $C_{27}H_{25}N_3O_5$ (471.5): C 68.78, H 5.34, N 8.91; found: C 68.76, H 5.36, N 8.80.

*1-(2'-Deoxy-β-*D-erythro-*pentofuranosyl)-4-[(phenoxyacetyl)amino]-1*H-*pyrrolo[2,3-*b*]pyridine* (**4c**). Compound **1** (100 mg, 0.40 mmol) was dried by co-evaporation with dry pyridine (10 ml), then suspended in anh. pyridine (100 ml), and treated with phenoxyacetic anhydride (1030 mg, 3.6 mmol). After heating for 48 h under reflux, the mixture was allowed to cool to r.t. H_2O (10 ml) was added and the mixture evaporated. The residue was dissolved in $CHCl_3$ (50 ml) and extracted (5 times) with 5% $NaHCO_3$ soln. (50 ml, each) followed by 50 ml of H_2O. The org. layer was dried (Na_2SO_4) and evaporated. The yelow oil was stirred for 15 min with Et_3N/pyridine/H_2O 1:1:3 (10 ml) at r.t. The soln. was evaporated and the oily residue co-evaporated twice with toluene (10 ml, each) and acetone (10 ml, each). The residue was applied to FC (silica gel, column 20 × 5 cm, solvent *B*): **4c** (30 mg, 20%). Colorless needles. M.p. 176–177° (MeOH). TLC (silica gel, solvent *B*): R_f 0.2. UV (MeOH): 292 (14500). ^{1}H-NMR ((D_6)DMSO): 2.21 (*m*, H_α–C(2')); 2.58 (*m*, H_β–C(2')); 3.56 (*m*, 2 H–C(5')); 3.84 (*m*, H–C(4')); 4.36 (*m*, H–C(3')); 4.89 (*s*, CH_2, pac); 5.09 (*t*, *J* = 5.7, OH–C(5')); 5.27 (*d*, *J* = 4.0, OH–C(3')); 6.68 (*dd*, *J* = 6.0, 8.1, H–C(1')); 6.83 (*d*, *J* = 3.7, H–C(3)); 6.94–7.35 (*m*, 5 arom. H, pac); 7.67 (*d*, *J* = 3.8, H–C(2)); 7.81 (*d*, *J* = 5.4, H–C(5)); 8.14 (*d*, *J* = 5.4, H–C(6)); 10.27 (*s*, NH–C(4)). Anal. calc. for $C_{20}H_{21}N_3O_5$ (383.4): C 62.65, H 5.52, N 10.96; found: C 62.52, H 5.60, N 10.80.

*1-(2'-Deoxy-β-*D-erythro-*pentofuranosyl)-4-{[(dimethylamino)methylidene]amino}-1*H-*pyrrolo[2,3-*b*]pyridine* (**4d**). A soln. of **1** (300 mg, 1.20 mmol) in DMF (30 ml) containing *N*,*N*-dimethylformamide diethyl acetal (3 ml, 17.5 mmol) was stirred for 6 h at 40°. The solvent was evaporated and the oily residue coevaporated twice with toluene (10 ml, each) and acetone (10 ml, each). The residue was dissolved in CH_2Cl_2 and applied to FC (silica gel, column 20 × 5 cm, solvent *A*): **4d** (280 mg, 76%). Colorless leafs from acetone. M.p. 158–159°. TLC (silica gel, solvent *A*): R_f 0.3. UV (MeOH): 308 (17800). ^{1}H-NMR ((D_6)DMSO): 2.17 (*m*, H_α–C(2')); 2.62 (*m*, H_β–C(2'));

3.05, 3.10 (2*s*, Me_2N); 3.57 (*m*, 2 H–C(5′)); 3.86 (*m*, H–C(4′)); 4.39 (*m*, H–C(3′)); 5.15 (*t*, *J* = 5.8, OH–C(5′)); 5.28 (*d*, *J* = 3.7, OH–C(3′)); 6.52 (*d*, *J* = 3.6, H–C(3)); 6.63 (*d*, *J* = 5.3, H–C(5)); 6.64 (*m*, H–C(1′)); 7.51 (*d*, *J* = 3.7, H–C(2)); 7.99 (*d*, *J* = 5.3, H–C(6)); 8.06 (*s*, N=CH). Anal. calc. for $C_{15}H_{20}N_4O_3$ (304.3): C 59.20, H 6.62, N 18.41; found: C 59.21, H 6.55, N 18.33.

*1-[2′-Deoxy-5′-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D*-*erythro*-pentofuranosyl]-4-{[(dimethylamino)methylidene]amino}-1*H*-pyrrolo[2,3-b]pyridine* (**6**). Compound **4d** (200 mg, 0.66 mmol) in anh. pyridine (10 ml) was stirred under Ar for 3 h in the presence of 4-(dimethylamino)pyridine (40 mg, 0.33 mmol) and 4,4′-dimethoxytriphenylmethyl chloride (445 mg, 1.31 mmol) at r.t. (TLC monitoring (silica gel, solvent *A*)). After addition of 5% aq. $NaHCO_3$ soln. (20 ml), the mixture was extracted 5 times with CH_2Cl_2 (20 ml, each), the combined org. layers were dried (Na_2SO_4) and evaporated, and the residue applied to FC (silica gel, column 25 × 5 cm, solvent *A*). The main zone was concentrated (2 ml) and poured into hexane/Et_2O 1:1 at 0°. Compound **6** precipitated in yellow crystals (320 mg, 80%). M.p. 106°. TLC (silica gel, solvent *A*): R_f 0.6. UV (MeOH): 286 (13500), 306 (16100). ^{1}H-NMR ((D_6)DMSO): 2.24 (*m*, H_α–C(2′)); 2.59 (*m*, H_β–C(2′)); 3.02, 3.08 (2*s*, Me_2N); 3.40 (*m*, 2 H–C(5′)); 3.74 (*m*, 2 MeO); 3.94 (*m*, H–C(4′)); 4.36 (*m*, H–C(3′)); 5.32 (*d*, *J* = 4.2, OH–C(3′)); 6.49 (*d*, *J* = 3.2, H–C(3)); 6.61 (*d*, *J* = 5.3, H–C(5)); 6.70 (‘*t*’, *J* = 6.9, H–C(1′)); 6.82–7.40 (*m*, 13 arom. H, $(MeO)_2Tr$); 7.34 (*d*, *J* = 3.0, H–C(2)); 7.99 (*d*, *J* = 5.0, H–C(6)); 8.03 (*s*, N=CH). Anal. calc. for $C_{36}H_{38}N_4O_5$ (606.7): C 71.27, H 6.31, N 9.23; found: C 71.09, H 6.38, N 9.04.

*1-[2′-Deoxy-5′-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D*-*erythro*-pentofuranosyl]-4-{[(dimethylamino)methylidene]amino}-1*H*-pyrrolo[2,3-b]pyridine 3′-(Triethylammonium Phosphonate)* (**3a**). To a soln. of PCl_3 (290 µl, 3.3 mmol) and *N*-methylmorpholine (3.8 ml, 34.4 mol) in anh. CH_2Cl_2 (30 ml), 1,2,4-1*H*-triazole (768 mg, 11.1 mmol) was added under Ar. After stirring for 30 min at r.t., the soln. was cooled to 0°, and **6** (400 mg, 0.66 mmol) in anh. CH_2Cl_2 (20 ml) was added dropwise within 10 min. After stirring for 10 min at r.t., the mixture was poured into 1M aq. $(Et_3NH)HCO_3$ (*TBK*, pH 7.5, 36 ml), shaken, and separated. The aq. layer was extracted 3 times with CH_2Cl_2 (30 ml), the combined org. extract dried (Na_2SO_4), filtered, and evaporated, and the colourless foam applied to FC (silica gel, column 30 × 5 cm, solvent *C* (1 l), then *D*). The residue of the main zone was dissolved in CH_2Cl_2 (50 ml) and extracted 10 times with aq. $(Et_3NH)HCO_3$ (0.1M, 25 ml). The org. layer was dried (Na_2SO_4) and evaporated: **3a** (395 mg, 78%). Colorless foam. TLC (silica gel, solvent *D*): R_f 0.6. UV (MeOH): 285 (12800), 305 (14600). ^{1}H-NMR ((D_6)DMSO): 1.15 (*t*, *J* = 7.3, CH_3CH_2NH); 2.38 (*m*, H_α–C(2′)); 2.61 (*m*, H_β–C(2′)); 2.98 (*q*, CH_3CH_2NH); 3.08 (*s*, Me_2N); 3.18 (*d*, *J* = 4.5, 2 H–C(5′)); 3.73 (*m*, 2 MeO); 4.13 (*m*, H–C(4′)); 4.76 (*m*, H–C(3′)); 6.50 (*d*, *J* = 3.6, H–C(3)); 6.62 (*d*, *J* = 5.3, H–C(1′)); 6.66 (*d*, *J* = 584.6, PH); 6.70 (*dd*, *J* = 5.8, 8.3, H–C(1′)); 6.80–7.41 (*m*, 13 arom. H, $(MeO)_2Tr$); 7.35 (*d*, *J* = 3.6, H–C(2)); 7.99 (*d*, *J* = 5.3, H–C(6)); 8.04 (*s*, N=CH). ^{31}P-NMR ((D_6)DMSO): 1.13 (^1J(P,H) = 584.6, ^3J(P,H) = 8.5). Anal. calc. for $C_{42}H_{54}N_5O_7P$ (771.9): C 65.35, H 7.05, N 9.07; found: C 65.40, H 7.23, N 8.99.

*1-[2′-Deoxy-5′-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D*-*erythro*-pentofuranosyl]-4-{[(dimethylamino)methylidene]amino}-1*H*-pyrrolo[2,3-b]pyridine 3′-[(2-Cyanoethyl)* N,N*-Diisopropylphosporamidite]* (**3b**). Compound **6** (50 mg, 0.082 mmol) in anh. CH_2Cl_2 (1.0 ml) was preflushed with Ar. (i-Pr)$_2$EtN (50 µl, 0.287 mmol) and chloro(2-cyanoethoxy)(diisopropylamino)phosphane (60 mg, 0.252 mmol) were added, and the mixture was kept under Ar at r.t. After stirring for 1 h, 5% aq. $NaHCO_3$ soln. (3.0 ml) was added and the soln. extracted several times with CH_2Cl_2. The org. layer was dried (Na_2SO_4), filtered, and evaporated and the residue applied to FC (silica gel, column 10 × 2 cm, solvent *E*): colorless amorphous **3b** (35 mg, 53%), diastereoisomer mixture. TLC (silica gel, solvent *E*): R_f 0.7, 0.6. ^{31}P-NMR ((D_6)DMSO): 148.1, 148.7.

Solid-Phase Synthesis of the Oligomers **7**–**13**. The synthesis of the oligonucleotides was accomplished on a 1-µmol scale using the 3′-phosphonates of [$(MeO)_2$Tr]bz^6A_d, [$(MeO)_2$Tr]ib^2G_d, [$(MeO)_2$Tr]bz^4C_d and [$(MeO)_2$Tr]T_d as well as compound **3a**. The synthesis of **7**–**13** followed the regular protocol of the DNA synthesizer for 3′-phosphonates [18b]. The oligonucleotides **7**–**13** were recovered from the synthesizer as the 5′-dimethoxytritylated derivatives. After treatment with 25% aq. NH_3 soln. for 60 h at 60° (cleavage of the nucleobase protecting groups), the 5′-dimethoxytritylated oligomers were purified by HPLC (see below; *RP-18* columns, 250 × 4 mm, 7 µm, solvent *I*). Detritylation was performed by 80% AcOH/H_2O for 2 min at r.t. After removal of the acid, the oligomers were purified agian by HPLC (*RP-18* column, 250 × 4 mm, 7 µm, solvent *II*). The oligomers were desalted on a 25 × 4 mm HPLC cartridge (*RP-18*, silica gel). Inorg. material was eluted with MeOH/H_2O 3:2 (6 ml). After lyophilization on a *Speed-Vac* evaporator, the oligonucleotides were dissolved in H_2O (100 µl) and stored frozen at –20°. The yields were calculated on the basis of silica-gel-bound nucleosides: **7** (43%), **8** (22%), **10** (16%), and **12** (23%). Retention times (t_R in min; solvent *II*): **7** (16.41), **8** (16.27), **10** (14.49), and **12** (14.72).

Enzymatic Hydrolysis of the Oligomers. The oligonucleotides (0.2 A_{260} units) were dissolved in 0.1M *Tris*-HCl buffer (pH 8.3; 200 µl) and treated with snake-venom phosphodiesterase (3 µg) at 37° for 45 min and alkaline

phosphatase (3 μg) for 30 min at 37°. The mixture was analyzed on reversed-phase HPLC (*RP-18*, solvent system *III*; see below). Quantification of the material was made on the basis of the peak areas, which were divided by the extinction coefficients of the nucleoside constituents (ε_{260}: A_d 15400; $c^1c^7A_d$ 8000; C_d 7300; G_d 11700; T_d 8800).

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