65. Oligodeoxyribonucleotides Containing 4-Aminobenzimidazole in Place of Adenine: Solid-Phase Synthesis and Base Pairing

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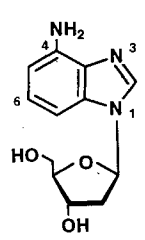
(14.II.95)

Oligonucleotides containing 4-aminobenzimidazole 2′-deoxyribofuranoside (1,3-dideaza-2′-deoxyadenosine; $c^1c^3A_d$, **1**) were synthesized. For this purpose, various NH_2-protecting groups were investigated, and the [(9*H*-fluoren-9-yl)methoxy]carbonyl group was selected for phosphoramidite protection (→ **4c**). Apart from the phosphoramidite **3**, the phosphonate **2** was prepared. Compound **1** was incorporated in a homooligonucleotide as well as in oligomers containing naturally occurring nucleosides. The T_m values and the thermodynamic data of various duplexes (**11**·**10**, **17**·**10**, **18**·**10**) containing 4-aminobenzimidazole were determined. Although $d[(c^1c^3A)_{20}]$ (**11**) does not form a *Hoogsteen* duplex with $d(T_{20})$ (**10**) as observed with $d[(c^1A)_{20}]$, it destabilizes the *Watson-Crick* duplexes to a much smaller extent than it was expected from a bulged loop structure. Apparently, 4-aminobenzimidazole interacts with regular nucleoside residues within a *Watson-Crick* duplex structure, most likely by vertical stacking. According to the low basicity of the amino group, only weak H-bonding is expected.

Introduction. – The synthesis of oligonucleotides containing 4-aminobenzimidazole residues was encouraged by the discovery that *Hoogsteen* duplexes were exclusively formed when a 1-deazapurine replaced adenine in homooligonucleotides [1]. According to this, a selective *Hoogsteen* base pairing was also expected for $d[(c^1c^3A)_{20}]\cdot d(T_{20})$. The [2-^{14}C]benzimidazole was already incorporated into ribonucleic acids by *E. coli* cells [2]. It was reported that benzimidazole as well as a number of derivatives can cause mutagenic events which might be due to mispairing in naturally occurring DNA or RNA. From the structural point of view, the benzimidazole moiety may act as universal base as it was reported for hypoxanthine [3], pyrrole [4], and indole 2′-deoxyribofuranosides [5].

The 5,6-dimethyl-1-(α-D-ribofuranosyl)benzimidazole (for a review, see [6]) is a constituent of vitamin B_{12} [7]. Benzimidazole 2′,5′- and 3′,5′-linked dinucleotides and tri-



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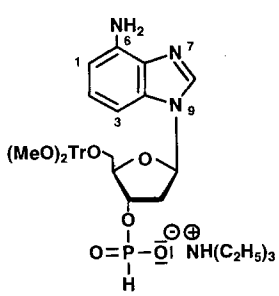

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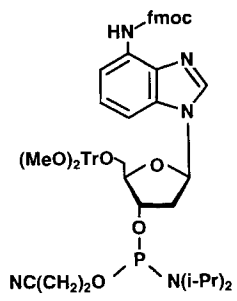

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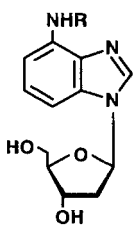
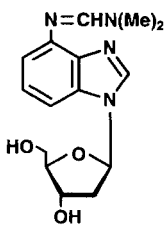
nucleotides were already chemically synthesized [8] [9]. However, to the best of our knowledge, no solid-phase oligonucleotide synthesis of benzimidazole oligonucleotides has been described so far. In the following, we report on the synthesis of the oligonucleotide building blocks **2** and **3** derived from 1,3-dideaza-2′-deoxyadenosine (4-aminobenzimidazole 2′-deoxyribofuranoside; $c^1c^3A_d$, **1**) and their incorporation into oligonucleotides. Furthermore, the base pairing of these oligonucleotides will be studied.

Results and Discussion. – *Monomers.* Conventional glycosylation techniques were used for the synthesis of benzimidazole ribonucleosides such as 1,3-dideazaadenosine [10] [11]. Benzimidazole 2′-deoxyribonucleosides were synthesized in a non-stereoselective manner [12] as well as stereospecifically [13]. Recently, 1,3-dideaza-2′-deoxyadenosine (**1**) was obtained in our laboratory in a stereochemically controlled fashion [14] starting from 4-nitrobenzimidazole and 1-chloro-2-deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-*erythro*-pentofuranose.

For the NH_2-protection of **1**, benzoyl (bz), acetyl (ac), and [(9*H*-fluoren-9-yl)methoxy]carbonyl (fmoc) groups were introduced using the transient-protection protocol [15] (→ **4a**, **4b** [16], and **4c**). The phenoxyacetyl (pac) and the methoxyacetyl (mac) derivatives – **4d** [16] and **4e** – were obtained *via* peracylation followed by selective deprotection of the sugar moiety [17]. The (dimethylamino)methylidene ($m_2$fa) group which circumvents the intermediate sugar protection (compound **5** [18]) is not stable in protic solvents [19].



4a R = bz
b R = ac
c R = fmoc
d R = pac
e R = mac



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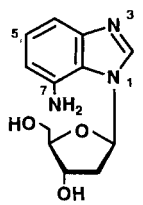

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Various reports on ^{13}C-NMR data of benzimidazoles were already published [20–23]. However, only a few data are available for nucleosides. According to the complex ^{1}H,^{13}C gated-decoupled spectra, the chemical shifts were also assigned by 2D-^{1}H,^{13}C-COLOC NMR spectra (*Table 1*). In this connection, the assignment of the ^{13}C-NMR data of compound **1** and of its regioisomer **6** had to be revised [14], although the chemical shifts could be fully confirmed. *Table 2* summarizes the coupling constants showing that C(4) and C(7a) exhibit a *d* multiplicity resulting from the couplings to H–C(5) and H–C(7), respectively; C(3a) shows a complex coupling pattern. Nevertheless, the significant assignment of the bridgehead C-atoms and C(4) resulted from 2D-^{1}H,^{13}C-COLOC NMR spectra (excitations of the ^3J couplings). In this case, C(4) shows a cross-peak to H–C(6), whereas C(7a) exhibits cross-peaks to H–C(6) and H–C(2). The assignment of the bridgehead C(3a) was made on the basis of the cross-peaks to H–C(5) and H–C(2) which could be obtained from the 2D-^{1}H,^{13}C-COLOC NMR spectra.

To test the suitability of the various protecting groups for oligonucleotide synthesis, their half-life values ($t_{1/2}$) were measured in 25% aqueous NH_3 solution. The data were

Table 1. *^{13}C-NMR Chemical Shifts* ((D$_6$)DMSO) *of Benzimidazole 2′-Deoxyribofuranosides*[a])[b])

	C(2)[c]) C(8)[d])	C(3a)[c]) C(5)[d])	C(4)[c]) C(6)[d])	C(5)[c]) C(1)[d])	C(6)[c]) C(2)[d])	C(7)[c]) C(3)[d])	C(7a)[c]) C(4)[d])
1	139.1	132.4	140.4	104.8	123.8	98.7	133.7
2	138.9	132.4	140.5	105.0	123.9	99.1	133.7
4a	141.2	136.2	129.4	113.9	122.8	107.6	134.4
b	140.9	134.8	130.3	112.3	123.1	106.4	133.3
c	141.1	135.4	129.8	112.1	123.2	106.5	133.5
d	141.4	134.5	129.3	111.3	123.3	107.2	133.2
e	141.3	134.3	128.9	110.7	123.3	107.0	133.1
5	140.3	136.9	143.1	115.4	123.4	104.1	134.3
6	141.4	145.9	108.7	122.9	109.2	135.2	123.0
7	140.7	135.5	129.9	111.9	123.1	106.6	133.5
8a	140.9	135.3	129.9	113.1	123.1	106.8	133.2
20	138.2	122.4	135.9	109.4	126.9	100.8	131.6

	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	C=O or CH=	CH_3O
1	84.1	–	70.6	87.4	61.7	–	–
2	84.6	38.6	72.5	84.4	63.6	–	55.0, 55.1
4a	84.6	–	70.4	87.5	61.4	165.0	–
b	84.6	–	70.5	87.6	61.6	169.0	–
c	84.7	38.9	70.6	87.7	61.6	153.8	–
d	84.7	–	70.5	87.7	61.5	166.7	–
e	84.8	–	70.5	87.7	61.5	167.7	58.9
5	84.4	–	70.5	87.5	61.6	156.0	–
6	84.9	–	70.0	87.5	61.6	–	–
7	84.6	39.0	70.5	85.6	63.9	153.8	55.0
8a	84.8	36.5	74.3	85.8	66.2	153.6, 158.0, 171.9	–
20	86.5	–	69.8	88.5	60.9	–	–

[a]) δ Values in ppm rel. to Me_4Si as internal standard. [b]) From ^{1}H,^{13}C gated-decoupled spectra. [c]) Systematic numbering. [d]) Purine numbering.

Table 2. *J(C,H) Values* [Hz] *of Benzimidazole 2′-Deoxyribofuranosides*[a])[b])

	1	**4c**	**4e**	**5**	**20**
J(C(2),H–C(2))	208.6	210.0	210.2	208.8	218.6
J(C(2),H–C(1′))	4.0	2.5	3.5	3.9	3.0
J(C(4),H–C(5))	*m*	8.8	10.1	7.5	9.4
J(C(5),H–C(5))	150.5	164.8	164.6	156.3	160.6
J(C(5),H–C(6))	5.8	–	8.2	5.3	7.1
J(C(6),H–C(6))	156.9	159.7	159.5	159.4	160.4
J(C(6),H–C(5))	–	–	–	–	–
J(C(7),H–C(7))	165.1	164.8	165.3	164.9	169.8
J(C(7),H–C(6))	8.3	8.8	8.2	8.5	7.9
J(C(3a),H–C(2))	*m*	*m*	*m*	*m*	*m*
J(C(7a),H–C(7))	*m*	11.3	11.2	11.1	11.1
J(C(1′),H–C(1′))	162.7	164.8	164.0	164.9	171.9
J(C(2′),H–C(2′))	–	–	–	–	–
J(C(3′),H–C(3′))	148.0	149.0	148.8	148.7	147.7
J(C(4′),H–C(4′))	148.0	149.0	148.8	148.7	147.4
J(C(5′),H–C(5′))	138.7	139.9	139.8	138.9	139.9

[a]) Data taken from measurements in (D$_6$)DMSO at 23°; systematic numbering. [b]) Multiplets (*m*) are unresolved.

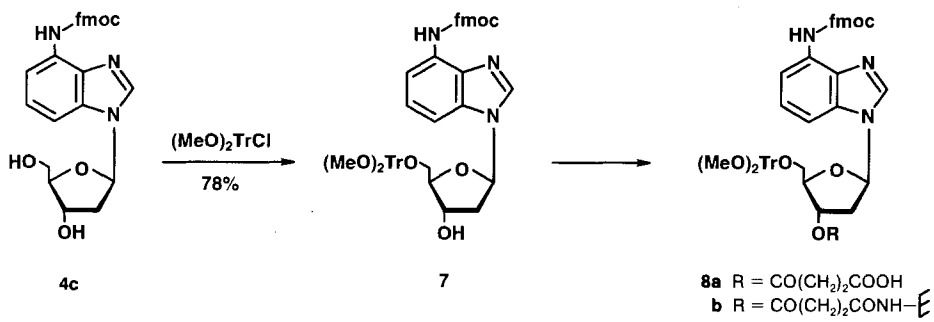
drawn from HPLC analysis or UV-spectrophotometrically at the wavelength and temperature indicated in *Table 3*. It can be seen that only the formamidine-protected **5** and the fmoc-protected **4c** were suitable for solid-phase oligonucleotide synthesis. The other protecting groups were much too stable. As the fmoc group is also exceptionally stable under neutral and acid conditions [24] which is not true for the amidine residue [18] [19], compound **4c** was selected for further experiments.

Table 3. *Half-Life Values ($t_{1/2}$) of Deprotection of Benzimidazole 2'-Deoxyribonucleosides in 25% Aqueous NH_3 Solution*[a])

		λ [nm]	$t_{1/2}$ [min]
$bz^6c^1c^3A_d$	(**4a**)	270	> 5000[b])
$ac^6c^1c^3A_d$	(**4b**)	292	> 5000[b])
$fmoc^6c^1c^3A_d$	(**4c**)	245	9[c])
$pac^6c^1c^3A_d$	(**4d**)	296	390[b])
$mac^6c^1c^3A_d$	(**4e**)	260	270[b])
$(m_2fa)^6c^1c^3A_d$	(**5**)	299	47[d])

[a]) At 10 μM nucleoside concentration. [b]) Hydrolyzed at 60° and determined by HPLC analysis at 60°. [c]) Determined UV-spectrophotometrically at 30°. [d]) See *Footnote c*, at 50°.

Scheme



Compound **4c** was converted into the 4,4'-dimethoxytrityl ($(MeO)_2Tr$) derivative **7** under standard conditions which was isolated in 79% yield (*Scheme*). It was expected that reaction of **7** with PCl_3/*N*-methylmorpholine/1*H*-1,2,4-triazole would give the fully protected phosphonate. However, the fmoc protecting group was lost during workup. As the sensitivity of the fmoc group against bases is well documented in the case of amino acids [24], the use of this residue in the oligonucleotide synthesis seems to be limited. The purification of the phosphonate required Et_3N as a base. As a consequence, the fmoc residue was already split off, and the partially deprotected **2** was isolated as the triethylammonium salt. These results were confirmed by the fact that the cleavage of the fmoc residue of **4c** occurred already in 1M aqueous $(Et_3NH)HCO_3$ (pH 7.5) at 30° ($t_{1/2}$ 75 min) and also with Et_3N/MeCN 1:1 ($t_{1/2}$ 30 min). This observation is also in line with other fmoc-protected nucleosides which lost the protecting group already under weak alkaline conditions (Et_3N/pyridine mixtures) [25] [26].

On the other hand, the fmoc-protected phosphoramidite **3** was accessible. Phosphonylation of **7** with chloro(2-cyanoethoxy)(diisopropylamino)phosphane furnished the diastereoisomer mixture **3** in 70% yield in an analytically pure form. Apart from the phosphoramidite, the silica-linked **8b** was prepared. Succinylation of **7** [27] gave **8a** (83%), which was activated *via* the 4-nitrophenyl ester and linked to amino-functionalized *Fractosil* yielding **8b** [28]. The ligand concentration was 72 μmol/g of solid support.

Oligonucleotides. The phosphonate **2** as well as the phosphoramidite **3** were used in automated solid-phase synthesis of oligonucleotides. The latter were prepared according to a protocol published recently [29–31]. The oligonucleotides were removed from the support by the action of conc. aqueous NH_3 solution (60°) and then purified as 5′-*O*-$(MeO)_2$Tr derivatives by *RP-18* HPLC. After detritylation with 2.5% $CHCl_2CO_2H$ in CH_2Cl_2, the oligomers were again submitted to *RP-18* HPLC, desalted, and lyophilized. The composition of the oligonucleotides **9**–**18** was determined by tandem hydrolysis with snake-venom phosphodiesterase and alkaline phosphatase followed by HPLC. Compound **1** was found to be much more stable towards cleavage at the N-glycosylic bond than dA (**1**: 49 min, 1N HCl, 40° [14]; dA: 3.5 min, 1N HCl, 25° [32]). As a consequence, 'depurination', a problem caused by dA residues, was not occurring in oligonucleotides containing **1**.

$d(A_{20})$ **9** $d(T_{20})$ **10** $d[(c^1c^3A)_{20}]$ **11**

d(G-T-A-G-A-A-T-T-C-T-A-C) [40] **12** d(G-T-A-G-c^{1}c^{3}A-c^{1}c^{3}A-T-T-C-T-A-C) **13**

d(G-T-A-G-c^{1}c^{7}A-c^{1}c^{7}A-T-T-C-T-A-C) [40] **14** d(G-T-A-G-c^{1}A-A-T-T-C-T-A-C) **15**

d(G-T-A-G-c^{1}A-c^{1}A-T-T-C-T-A-C) **16** $d[A_9\text{-}(c^1c^3A)_2\text{-}A_9]$ **17** $d[A_8\text{-}(c^1c^3A)_4\text{-}A_8]$ **18**

Recently, *Jones et al.* reported that H-phosphonate oligonucleotide synthesis can be carried out without amino-protecting groups [33]. To establish whether or not the non-protected phosphonate **2** can be used in solid-phase synthesis, the oligonucleotide **13** was prepared. For comparison, the same oligonucleotide was synthesized using the phosphoramidite **3**. *Figs. 1a* and *1b* show that **13** was formed in both cases effectively. The correct composition of monomers was determined as described before; thus, amino protection is not necessary for phosphonate **1** in solid-phase synthesis.

Properties of Homooligonucleotides. Recently, we showed that oligonucleotides containing 1-deaza-2′-deoxyadenosine can form *Hoogsteen* duplexes [1] [34]. In this connection, it was of interest to establish if this is also possible with oligonucleotides containing 1,3-dideaza-2′-deoxyadenosine. Another point of interest was the behaviour of **1** within oligonucleotides which contain only a few of the 4-amino-benzimidazoles residue within a regular *Watson-Crick* (*WC*) duplex.

In the first experiment, the hybridization of the oligomer $d[(c^1c^3A)_{20}]$ (**11**) with $d(T_{20})$ (**10**) was studied. Previous investigations showed that the *WC* duplex $d(A_{20})\cdot d(T_{20})$ (**9**·**10**)

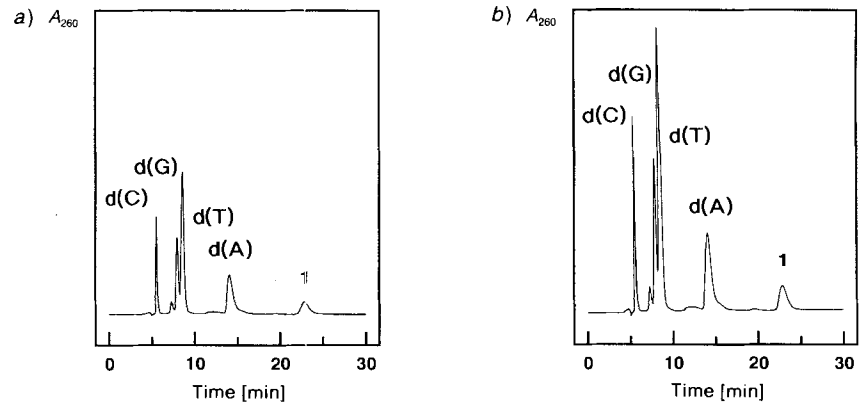
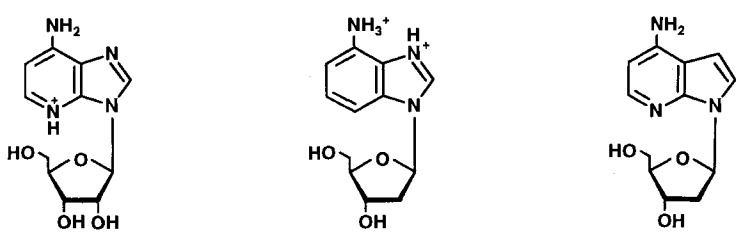


Fig. 1. *HPLC Profiles of d(G-T-A-G-c^{1}c^{3}A-c^{1}c^{3}A-T-T-C-T-A-C)* (**13**) a) *synthesized from the phosphonate* **2** *and* b) *from the phosphoramidite* **3**, *after enzymatic tandem hydrolysis in 0.1* M Tris-*HCl buffer* (pH 8.3) *with snake-venom phosphodiesterase followed by alkaline phosphatase.* For details, see *Exper. Part.*

has a T_m value of 60° [1], whereas the T_m value of the *Hoogsteen* duplex $d(c^1A_{20})\cdot d(T_{20})$ is around 15°. In the case of $d[(c^1c^3A)_{20}]$ (**11**) and $d(T_{20})$ (**10**), base pairing was not detected. Only a linear increase of the UV_{260} absorbance ($h = 3\%$) was observed. This result was unexpected as the structurally related $d(c^1A_{20})$ formed a *Hoogsteen* base pair with $d(T_{20})$. To explain this behaviour, it was neccessary to compare the donor/acceptor properties of 1,3-dideaza-2′-deoxyadenosine (**1**) with those of 1-deazaadenosine (**19**). Some information can be drawn from the protonation patterns of these nucleosides.

Earlier UV measurements established the first protonation site of **1** to be N(7) ($pK_a = 4.5$; purine numbering); also a second protonation was observed on the amino group ($pK_a = 0.7$) [14]. This was supported by ^{13}C-NMR experiments measured in neutral as well as acidic DMSO solution (*Table 1*). The assignment was made on the basis of ^{1}H,^{13}C gated-decoupled spectra of **20**, *i.e.*, of doubly protonated **1** (*Table 2*). The situation was different in the case of 1-deazaadenosine. Here, the site of the first protonation is N(3) (**19**·H$^+$). This was also deduced from the ^{13}C-NMR spectra measured under neutral and acidic conditions in DMSO solution [35]. The comparison of half-lives of the amino-protecting groups supported the strong basicity of the amino groups of **1**. In the series of compounds **1**, 1-deaza-2′-deoxyadenosine (c^1A_d), and 2′-deoxyadenosine, the stability of the N^6-benzoyl groups decreased from $bz^6c^1c^3A_d$ (> 5000 min) to $bz^6c^1A_d$ (125 min), and bz^6A_d (71 min). This immediately indicated that the amino group of **1** is much



19·H$^+$ **20** **21**

more basic than that of c^1A_d and is, therefore, only a very weak proton donor during base pairing. As the T_m value of the *Hoogsteen* duplex of $d[(c^1A)_{20}] \cdot d(T_{20})$ is already low (15°), a *Hoogsteen* duplex formation of $d[(c^1c^3A)_{20}] \cdot d(T_{20})$ is expected even less stable, which is in agreement with the experimental findings.

Hairpin-Structures in Self-complementary Oligonucleotides. It was already reported that duplex formation is favoured in the case of the dodecamer **12** at high oligomer concentration, whereas in the low-concentration range hairpins are formed [36]. It was also shown that hairpins become the major species in self-complementary oligonucleotides when the central base pairs are destabilized [37]. As the melting of hairpins is independent of the concentration, and a destabilization of the innermost base pairs is expected for **13**, the T_m values of oligomer **13** were measured within an oligonucleotide concentration range of 1–24 μM (see *Fig. 2b*): the T_m value of **13** (T_m *43°*) was independent of the oligonucleotide concentration indicating exclusive hairpin formation.

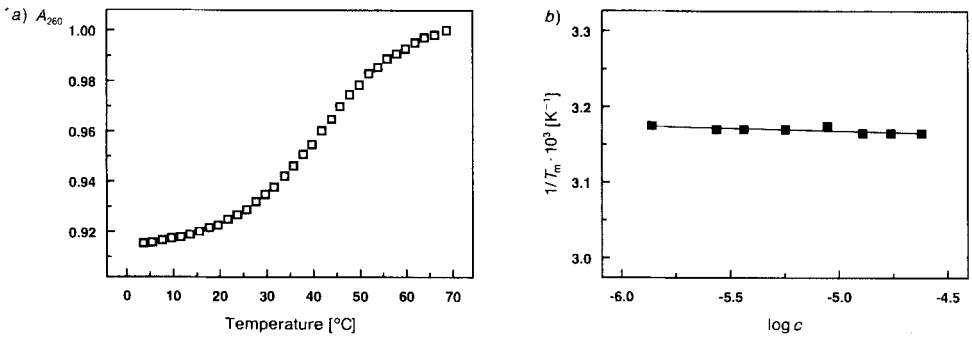


Fig. 2. a) *Normalized melting profile of d(G-T-A-G-c^{1}c^{3}A-c^{1}c^{3}A-T-T-C-T-A-C)* (**13**) *and* b) $1/T_m$ vs. *log* c *of* **13**. Measured at 260 nm in 60 mM Na-cacodylate, 1M NaCl, and 100 mM $MgCl_2$ at pH 7.0; oligomer concentration in *Fig. 2a*, 5.0 μM of single strands.

Next, the thermodynamic parameters (ΔH and ΔS) of **13** were calculated [38] using a two-state model for helix-coil transition (*Table 4*). The low enthalpy ($\Delta H = -38.2$ kcal/mol) and entropy values ($\Delta S = -121$ cal/K·mol) which are in line with calculated parameters for hairpin formation [39] confirmed the hairpin structure of **13**. Hairpin formation was also observed earlier, when the innermost dA segment of **12** was replaced by

Table 4. T_m *Values and Thermodynamic Data of 1,3-Dideazaadenine-Containing Oligodeoxyribonucleotides*[a])

	T_m [°C]	h_{mel} [%]	ΔH [kcal/mol]	ΔS [cal/mol·K]
$d(A_{20}) \cdot d(T_{20})$ (**9·10**)	60.0	40	–190.1	–571
$d[A_9\text{-}(c^1c^3A)_2\text{-}A_9] \cdot d(T_{20})$ (**17·10**)	54.0	22	–179.2	–549
$d[A_8\text{-}(c^1c^3A)_4\text{-}A_8] \cdot d(T_{20})$ (**18·10**)	51.0	22	–169.8	–524
$d[(c^1c^3A)_{20}] \cdot d(T_{20})$ (**11·10**)	–[b])	3	–	–
d(G-T-A-G-A-A-T-T-C-T-A-C) (**12**) [40]	48.0	27	–82.0	–254
d(G-T-A-G-c^{1}c^{3}A-c^{1}c^{3}A-T-T-C-T-A-C) (**13**)	43.0	8	–38.2	–121
d(G-T-A-G-c^{1}c^{7}A-c^{1}c^{7}A-T-T-C-T-A-C) (**14**) [40]	43.0	15	–35.0	–112

[a]) Measured in 1M NaCl containing 100 mM $MgCl_2$ and 60 mM Na-cacodylate, pH 7.0 at 260 nm; single-strand concentration, 5 μM. [b]) No cooperative melting.

1,7-dideaza-2′-deoxyadenosine [40]. The T_m value as well as the thermodynamic data of the oligonucleotide **14** were almost identical with those of **13** (T_m 43°, $\Delta H = -35.0$ kcal/mol, $\Delta S = -112$ cal/K·mol).

With regard to hairpin formation, the oligonucleotides **15** or **16** containing one or two 1-deaza-2′-deoxyadenosine residues showed a different behaviour. The oligomer **15** exhibited a T_m of 30° which was decreased in going to the parent oligonucleotide **12** (48°). The T_m value was further decreased in the case of **16** ($T_m < 20°$). Sigmoidal melting profiles and concentration-dependent T_m values (see *Fig. 3*) confirmed that a duplex was formed. These results clearly showed that in the case of self-complementary oligomers such as **12**, only c^1A_d can be involved in a one-H-bond *Watson-Crick* base pairing. Neither $c^1c^7A_d$ nor $c^1c^3A_d$ residues do form such base pairs. Apparently, the 4-NH_2 group of the benzimidazole nucleoside is too basic to act as proton donor not only in *Hoogsteen* (see homooligonucleotides) but also in *Watson-Crick* base pairing.

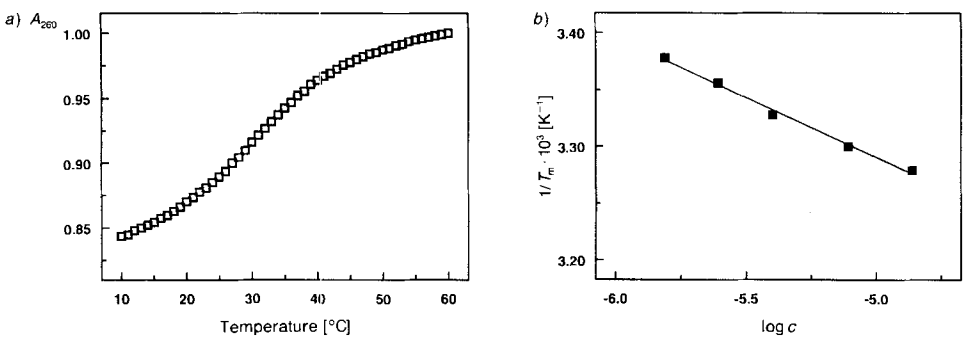
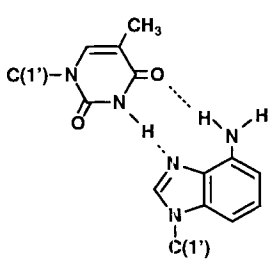
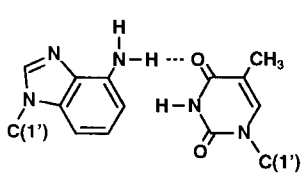


Fig. 3. *a) Normalized melting profile of d(G-T-A-G-c^1A-A-T-T-C-T-A-C)* (**15**; conditions, see *Fig. 2*; oligomer concentration, 8.0 μM of single strands) *and* b) $1/T_m$ vs. *log* c *of compound* **15** (same buffer as in *Fig. 2*)

Base Pairing of Oligonucleotides with dA and $c^1c^3A_d$ Residues. According to the points discussed above, it can be expected that in adenine/1,3-dideazaadenine-containing oligonucleotides the 1,3-dideazaadenine moiety forms only one very weak H-bond in a *Watson-Crick* base pair. However, *Hoogsteen* base pairing with two weak H-bonds in the base pair can be considered in oligonucleotides stabilized by a number of regular *WC*



Possible *Hoogsteen* base pair of $c^1c^3A_d$ and dT



Possible *Watson-Crick* base pair of $c^1c^3A_d$ and dT

base pairs. For this purpose, the oligomers **17**·**10** and **18**·**10** were synthesized. Cooperative monophasic melting profiles were found in 1M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (see *e.g.*, *Fig. 4*). Compared with the parent duplex **9**·**10** (T_m 60°), the T_m value of **17**·**10** was 54° and that of **18**·**10** 51°. The T_m values of the duplexes **17**·**10** and **18**·**10** were confirmed by CD spectra. Both π–π^* transitions (B_{1u}, B_{2u}) were measured as a function of temperature between 10 and 80°. In both cases, sigmoidal monophasic melting profiles were obtained (see *e.g.*, *Fig. 5*).

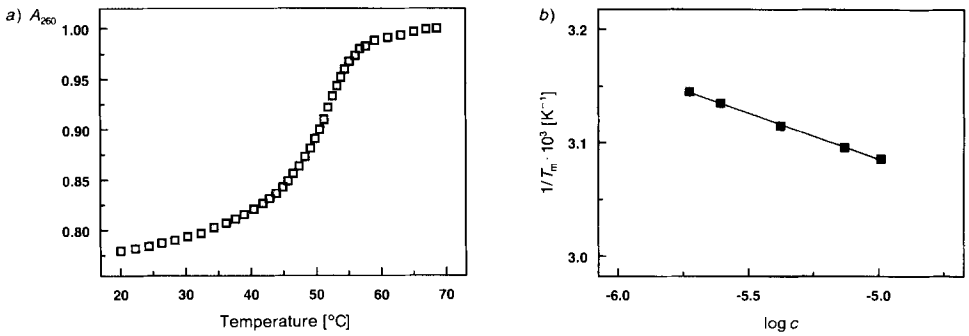


Fig. 4. a) *Normalized melting profile of* $d[A_8\text{-}(c^1c^3A)_4\text{-}A_8]\cdot d(T_{20})$ (**18**·**10**) (same buffer as in *Fig. 2*; oligomer concentration, 5.0 μM of single strands) *and* b) $1/T_m$ vs. *log* c *of compound* **18**·**10** (conditions, see *Fig. 2*)

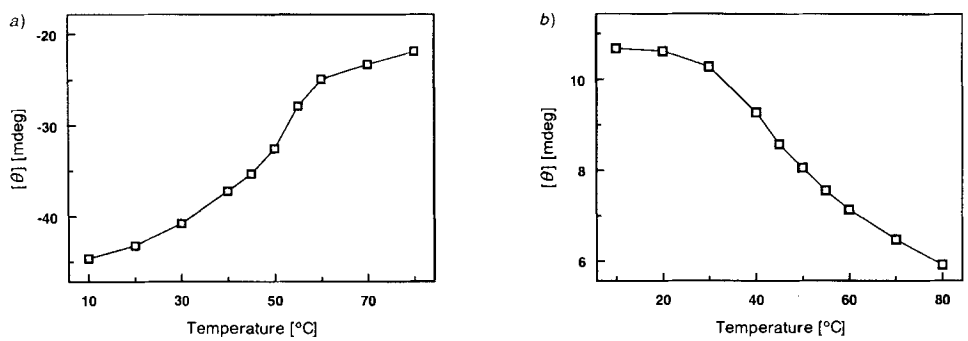


Fig. 5. *Temperature-dependent CD spectra of* a) *the* B_{1u} *and* b) *the* B_{2u} *transitions of* $d[A_8\text{-}(c^1c^3A)_4\text{-}A_8]\cdot d(T_{20})$ (**18**·**10**). Buffer as in *Fig. 2*; oligomer concentration, 5.0 μM of single strands.

When the oligonucleotide concentration of **17**·**10** or **18**·**10** was reduced from 10 μM to 2.5 μM, the T_m decreased by *ca.* 5° in both cases indicating duplex melting (see *Fig. 4b*). As the modified duplexes **17**·**10** as well as **18**·**10** showed only a moderate decrease of the T_m values compared with the parent duplex **9**·**10** (ΔT_m −6.0° for **17**·**10**, and −9.0° for **18**·**10**), it was considered that 4-aminobenzimidazole can take part in base interaction. Therefore, the thermodynamic data were determined according to [38] (*Table 4*). The experimentally determined ΔH values of the duplexes **17**·**10** and **18**·**10** were reduced compared to the parent **9**·**10**, from $\Delta H = -190.1$ kcal/mol to −179.2 kcal/mol and −169.8 kcal/mol, respectively ($\Delta\Delta H = 10.9$ kcal/mol for **17**·**10** and $\Delta\Delta H = 20.3$ kcal/mol for **18**·**10**). *Breslauer et al.* [41] reported that the ΔH values of oligonucleotides can be calculated

from the sum of ΔH increments of A–T base pairs, in our case −9.5 kcal/mol for each A–T base pair. If the 4-aminobenzimidazole nucleosides do not take part in the base pairing and a bulged loop is formed, ΔH values of −171 kcal/mol for **17**·**10** and −152 kcal/mol for **18**·**10** are calculated. Thus, in comparison with the experimentally determined $\Delta\Delta H$'s the calculated values are almost twice as high. This indicates that in **17**·**10** or **18**·**10**, the nucleoside **1** takes part in the base interaction within the duplex structure.

The stabilization found in the case of 4-aminobenzimidazole residues may be attributed to the following features: *i*) The base is turned around forming a *Hoogsteen* or a reverse *Hoogsteen*-like base pair under participation of only one H-bond between the 1,3-dideazaadenine N(7) as acceptor and the H–N(3) of dT as donor. Related two-H-bond *Hoogsteen* base pairs were already observed for hypoxanthine residues opposite to 2′-deoxyadenosine [3] [42] [43] or 2′-deoxyguanosine [3] [44] in an otherwise regular *WC*-duplex structure. *ii*) The 4-aminobenzimidazole residue replacing the adenine moiety in the regular *WC* duplex does not form H-bonds with dT. The location of the 4-aminobenzimidazole moiety is similar to that found for the parent adenine. The observed stabilization results mainly from vertical stacking interaction, whereby the benzimidazole acts similarly as an intercalator. This type of interaction was already observed in oligonucleotides containing 3-nitropyrrole residues [4]. According to the points discussed above, the 4-aminobenzimidazole residue may act as an 'ambiguous base' which will be the subject of further investigations. Exclusive *Hoogsteen* base pairing with parallel chains[1]), as found between the c^1A_d (see **19**) and dT moiety [1], is not observed.

We thank Dr. *A. Helfen* and Mr. *S. Freimund* for providing us with some baseprotected intermediates and Mr. *H. Debelak* for supporting us in the preparation of the computer drawings. Financial support by the *Deutsche Forschungsgemeinschaft* is gratefully acknowledged.

Experimental Part

General. See [1]. The phosphonates and (2-cyanoethyl) phosphoramidites were purchased from *Sigma*, St. Louis, and CPG (30–50 μmol of immobilized protected 2′-deoxynucleoside/g of solid support) from *Milligene*, Eschborn, Germany. Oligonucleotide synthesis was carried out on a DNA synthesizer, model *380 B*, *Applied Biosystems*, Weiterstadt, Germany. Melting experiments were carried out as described [45] (linear temp. increase from 10 to 70°). The enzymatic hydrolysis of the oligomers and the hydrolysis was carried out as described [1]. 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 (λ_{260}: A_d 15400, C_d 7300, G_d 11700, T_d 8800, and $c^1c^3A_d$ 7150). Hypochromicity values were determined by enzymatic digestion of 0.3 A_{260} units of the corresponding oligodeoxyribonucleotides as described in [1]. HPLC: see [1]; gradients consisting of 0.1M $(Et_3NH)OAc$ (pH 7.0)/MeCN 95:5 (*A*) and MeCN (*B*) were used; 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 1.0 ml/min; gradient *II*, 20 min 0–20% *B* in *A*, flow rate 1.0 ml/min; gradient *III*, 30 min 100% *B* in *A*, flow rate 0.6 ml/min. Solvent systems for flash chromatography (FC) and TLC: CH_2Cl_2/MeOH 95:5 (*A*), CH_2Cl_2/MeOH 9:1 (*B*), CH_2Cl_2/MeOH 8:2 (*C*), CH_2Cl_2/Et_3N 98:2 (*D*), CH_2Cl_2/MeOH/Et_3N 88:10:2 (*E*), CH_2Cl_2/AcOEt/Et_3N 91.5:8:0.5 (*F*), MeCN/H_2O 9:1 (*G*).

*4-(Benzoylamino)-1-(2-deoxy-β-*D*-*erythro*-pentofuranosyl)benzimidazole* (**4a**). Compound **1** (100 mg, 0.4 mmol) was dissolved in anh. pyridine (2 ml). Me_3SiCl (520 μl, 4.1 mmol) was added, and the soln. was stirred for 15 min, treated with benzoyl chloride (57 μl, 0.49 mmol), and maintained at r.t. for 2 h. The mixture was cooled to 0°

[1]) A parallel strand orientation was established from a complex formed between (5′–3′)[d($^1A_{10}$-T_{10})] with (5′–3′)[d(T_{10}-$^1A_{10}$)] as discussed in [1] (p. 1494). Unfortunately, the formulae of this complex (**14**·**15**) contain an error. The chain polarity of **15** within the duplex (**14**·**15**) is (5′–3′) and not (3′–5′).

and hydrolyzed with H_2O (1 ml). After 5 min, 25% aq. NH_3 soln. (1 ml) was added and the soln. stirred for another 30 min. The solvent was evaporated and the oily residue co-evaporated twice with toluene and applied to FC (silica gel, column 20 × 3 cm, *B*). Subsequent crystallization from AcOEt yielded colourless crystals (100 mg, 71%). M.p. 154°. TLC (*B*): R_f 0.4. UV (MeOH): 282 (15500). ^{1}H-NMR ((D)$_6$DMSO): 2.36 (*m*, H_α–C(2′)); 2.60 (*m*, H_β–C(2′)); 3.58 (*m*, 2 H–C(5′)); 3.88 (*m*, H–C(4′)); 4.41 (*m*, H–C(3′)); 5.00 (*t*, *J* = 5.0, OH–C(5′)); 5.38 (*d*, *J* = 4.0, OH–C(3′)); 6.39 ('*t*', *J* = 6.5, H–C(1′)); 7.28 (*t*, *J* = 8.5, H–C(6)); 7.51–7.63 (*m*, arom. H); 7.66 (*d*, *J* = 7.8, H–C(7)); 8.02 (*d*, *J* = 7.5, H–C(5)); 8.50 (*s*, H–C(2)); 9.90 (*s*, NH–C(4)). Anal. calc. for $C_{19}H_{19}N_3O_4$ (353.38): C 64.58, H 5.42, N 11.89; found: C 64.67, H 5.54, N 11.87.

*4-(Acetylamino)-1-(2-deoxy-β-*D-erythro-*pentofuranosyl)benzimidazole* (**4b**). As described for **4a**, with **1** (100 mg, 0.4 mmol), pyridine (2 ml), Me_3SiCl (520 µl, 4.1 mmol), and Ac_2O (200 µl, 2.1 mmol). Hydrolysis at 0° with H_2O (700 µl, 10 min) followed by 25% aq. NH_3 soln. (1 ml, 10 min). FC (silica gel, column 20 × 3 cm, *C*) gave **4b** (80 mg, 68%). Colourless crystals (Et_2O-diffusion method). M.p. 170°. TLC (*C*): R_f 0.8. UV (MeOH): 271 (14200). ^{1}H-NMR ((D)$_6$DMSO): 2.17 (*s*, Me); 2.33 (*m*, H_α–C(2′)); 2.49 (*m*, H_β–C(2′)); 3.56 (*m*, 2 H–C(5′)); 3.86 (*m*, H–C(4′)); 4.39 (*m*, H–C(3′)); 5.00 (*t*, *J* = 4.8, OH–C(5′)); 5.38 (*d*, *J* = 4.1, OH–C(3′)); 6.34 ('*t*', *J* = 6.6, H–C(1′)); 7.17 (*t*, *J* = 8.3, H–C(6)); 7.39 (*d*, *J* = 8.0, H–C(7)); 7.95 (*d*, *J* = 7.4, H–C(5)); 8.45 (*s*, H–C(2)); 9.03 (*s*, NH–C(4)). Anal. calc. for $C_{14}H_{17}N_3O_4$ (291.36): C 57.72, H 5.88, N 14.42; found: C 57.42, H 5.79, N 14.30.

*1-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-4-{[(9H-fluoren-9-yl)methoxy]carbonylamino}benzimidazole* (**4c**). As described for **4a**, with **1** (600 mg, 2.4 mmol), pyridine (20 ml), Me_3SiCl (1.5 ml, 11.7 mmol; 30 min), and [(9*H*-fluoren-9-yl)methoxy]carbonyl chloride (780 mg, 2.9 mmol; 90 min). Hydrolysis at 0° with H_2O (10 ml; 1 h at r.t.). The residue was dissolved in MeOH, adsorbed on silica gel, and applied to FC (silica gel, column 20 × 3 cm, *B*). The colourless foam was crystallized from MeCN: colourless needles (860 mg, 76%). M.p. 146–147°. TLC (*B*): R_f 0.4. UV (MeOH): 265 (35200), 288 (9800), 299 (6300). ^{1}H-NMR ((D)$_6$DMSO): 2.31 (*m*, H_α–C(2′)); 2.62 (*m*, H_β–C(2′)); 3.58 (*m*, 2 H–C(5′)); 3.89 (*m*, H–C(4′)); 4.31 (*m*, CH(9) of fmoc); 4.43 (*m*, H–C(3′), CH_2O); 4.98 (*t*, *J* = 5.1, OH–C(5′)); 5.36 (*d*, *J* = 3.9, OH–C(3′)); 6.35 ('*t*', *J* = 6.6, H–C(1′)); 7.18 (*t*, *J* = 8.0, H–C(6)); 7.30 (*d*, *J* = 8.0, H–C(7)); 7.60 (*d*, *J* = 7.5, H–C(5)); 7.79–7.90 (*m*, arom. H); 8.47 (*s*, H–C(2)); 9.39 (*s*, NH–C(4)). Anal. calc. for $C_{27}H_{25}N_3O_5$ (471.52): C 68.78, H 5.34, N 8.91; found: C 68.58, H 5.50, N 8.92.

*1-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-4-[(phenoxyacetyl)amino]benzimidazole* (**4d**). Compound **1** (200 mg, 0.8 mmol) was dissolved in anh. pyridine (8 ml), and phenoxyacetic anhydride (1.36 g, 4.8 mmol) was added. After stirring for 90 min, H_2O (80 µl) was added, and the soln. was evaporated after further 30 min. The residue was dissolved in CH_2Cl_2 (15 ml) and extracted 3× with 5% aq. $NaHCO_3$ soln. followed by 15 ml of H_2O. The org. layer was dried (Na_2SO_4) and evaporated. The oily residue was stirred for 1 h with Et_3N/pyridine/H_2O 1:1:3 (6 ml) at r.t. The soln. was evaporated and the residue co-evaporated twice with toluene (10 ml, each) and then applied to FC (silica gel, column 15 × 3 cm, *B*). Subsequent crystallization from AcOEt afforded **4d** (220 mg, 72%). Colourless needles. M.p. 186°. TLC (*B*): R_f 0.2. UV (MeOH): 269 (15500). ^{1}H-NMR ((D)$_6$DMSO): 2.34 (*m*, H_α–C(2′)); 2.58 (*m*, H_β–C(2′)); 3.56 (*m*, 2 H–C(5′)); 3.89 (*m*, H–C(4′)); 4.41 (*m*, H–C(3′)); 4.84 (*s*, CH_2O); 4.98 (*t*, *J* = 5.2, OH–C(5′)); 5.37 (*d*, *J* = 4.1, OH–C(3′)); 6.37 ('*t*', *J* = 6.4, H–C(1′)); 6.97–7.38 (*m*, H–C(6), arom. H); 7.46 (*d*, *J* = 8.1, H–C(7)); 8.10 (*d*, *J* = 7.8, H–C(5)); 8.48 (*s*, H–C(2)); 9.81 (*s*, NH–C(4)). Anal. calc. for $C_{20}H_{21}N_3O_5$ (383.41): C 62.65, H 5.52, N 10.96; found: C 62.77, H 5.69, N 10.78.

*1-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-4-[(methoxyacetyl)amino]benzimidazole* (**4e**). As described for **4d**, with **1** (400 mg, 1.6 mmol), pyridine (20 ml), methoxyacetyl chloride (880 µl, 9.7 mmol; 2 h), and H_2O (2 ml; 30 min). Workup with CH_2Cl_2 (50 ml), 5% aq. $NaHCO_3$ (3 × 30 ml), and H_2O (30 ml, each), then Et_3N/pyridine/H_2O 1:1:3 (50 ml; 30 min). FC (silica gel, column 20 × 3 cm, *B*) gave colourless foam (380 mg, 74%). TLC (*B*): R_f 0.3. UV (MeOH): 271 (16100). ^{1}H-NMR ((D)$_6$DMSO): 2.32 (*m*, H_α–C(2′)); 2.59 (*m*, H_β–C(2′)); 3.47 (*s*, MeO); 3.56 (*m*, 2 H–C(5′)); 3.88 (*m*, H–C(4′)); 4.10 (*s*, CH_2O); 4.40 (*m*, H–C(3′)); 4.93 (*t*, *J* = 5.3, OH–C(5′)); 5.32 (*d*, *J* = 4.2, OH–C(3′)); 6.35 ('*t*', *J* = 6.7, H–C(1′)); 7.22 (*t*, *J* = 8.0, H–C(6)); 7.43 (*d*, *J* = 8.2, H–C(7)); 8.05 (*d*, *J* = 7.9, H–C(5)); 8.45 (*s*, H–C(2)); 9.41 (*s*, NH–C(4)). Anal. calc. for $C_{15}H_{19}N_3O_5$ (321.335): C 56.07, H 5.96, N 13.08; found: C 56.21, H 5.99, N 13.10.

*1-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-4-{[(dimethylamino)methylidene]amino}benzimidazole* (**5**). A soln. of **1** (720 mg, 2.9 mmol) in anh. DMF (15 ml) was stirred in the presence of freshly distilled *N*,*N*-dimethylformamide diethyl acetal (18 ml, 105 mmol) for 48 h at 60°. The solvent was evaporated and co-evaporated twice with toluene, anh. acetone, and anh. MeOH (10 ml, each). The yellow solid was crystallized from dry acetone/dry MeOH 10:1: slightly yellow crystals (720 mg, 82%). M.p. 173°. TLC (*B*): R_f 0.1. UV (MeOH): 286 (13800). ^{1}H-NMR ((D)$_6$DMSO): 2.30 (*m*, H_α–C(2′)); 2.59 (*m*, H_β–C(2′)); 2.99 (*s*, Me_2N); 3.54 (*m*, 2 H–C(5′)); 3.85 (*m*, H–C(4′)); 4.37 (*m*, H–C(3′)); 4.94 (*t*, *J* = 5.0, OH–C(5′)); 5.33 (*d*, *J* = 3.7, OH–C(3′)); 6.29 ('*t*', *J* = 6.6, H–C(1′)); 6.69 (*d*, *J* = 8.0, H–C(5)); 7.06 (*t*, *J* = 7.7, H–C(6)); 7.15 (*d*, *J* = 8.0, H–C(7)); 8.31 (*s*, H–C(2)); 8.57 (*s*, N=CH). Anal. calc. for $C_{15}H_{20}N_4O_3$ (304.35): C 59.20, H 6.62, N 18.41; found: C 59.13, H 6.69, N 18.36.

*1-[2-Deoxy-5-O-(4,4'-dimethoxytriphenylmethyl)-β-*D*-erythro-pentofuranosyl]-4-{[(9H-fluoren-9-yl)-methoxy]carbonylamino}benzimidazole* (7). Compound **4c** (700 mg, 1.5 mmol) in anh. pyridine (20 ml) was stirred for 90 min in the presence of 4,4'-dimethoxytriphenylmethyl chloride (1.0 g, 2.95 mmol). The mixture was poured into 5% aq. $NaHCO_3$ soln. (50 ml) and extracted with CH_2Cl_2 (3 × 50 ml). The combined org. layer was dried (Na_2SO_4) and evaporated and the residue applied to FC (silica gel, column 20 × 3 cm, *A*). The main zone was concentrated (2 ml) and poured into petroleum ether at r.t. Compound 7 precipitated as colourless, amorphous powder (910 mg, 78%). TLC (*A*): R_f 0.4. UV (MeOH): 235 (sh, 22900), 265 (31300), 286 (sh, 9500), 299 (5500). ^{1}H-NMR ((D)$_6$DMSO): 2.45 (*m*, H_α–C(2')); 2.74 (*m*, H_β–C(2')); 3.14 (*m*, 2 H–C(5')); 3.68 (*s*, MeO); 3.70 (*s*, MeO); 4.01 (*m*, H–C(4')); 4.31 (*m*, CH(9) of fmoc); 4.42 (*m*, H–C(3'), CH_2O); 5.42 (*d*, *J* = 4.1, OH–C(3')); 6.38 ('*t*', *J* = 6.3, H–C(1')); 6.76 (*m*, arom. H); 7.11 (*t*, *J* = 8.0, H–C(6)); 7.16–7.44 (*m*, H–C(7), arom. H); 7.61 (*d*, *J* = 7.6, H–C(5)); 7.80–7.91 (*m*, arom. H); 8.37 (*s*, H–C(2)); 9.39 (*s*, NH–C(4)). Anal. calc. for $C_{48}H_{43}N_3O_7$ (773.89): C 74.50, H 5.60, N 5.43; found: C 74.44, H 5.64, N 5.46.

*4-Amino-1-[2-deoxy-5-O-(4,4'-dimethoxytriphenylmethyl)-β-*D*-erythro-pentofuranosyl]benzimidazole 3'-(Triethylammonium Phosphonate)* (**2**). To a soln. of PCl_3 (227 µl, 2.7 mmol) and *N*-methylmorpholine (3.0 ml, 26.8 mmol) in dry CH_2Cl_2 (25 ml), 1*H*-1,2,4-triazole (600 mg, 8.6 mmol) was added. After 30 min stirring at r.t., the soln. was cooled to 0° and a soln. of 7 (400 mg, 0.52 mmol) in dry CH_2Cl_2 (15 ml) was introduced dropwise within 10 min. The mixture was stirred for another 20 min at r.t. and then hydrolyzed with 1M $(Et_3NH)HCO_3$ buffer (30 ml, pH 7.5). The aq. layer was extracted with CH_2Cl_2 (3 × 20 ml) and the org. layer dried (Na_2SO_4) and evaporated. FC (silica gel, column 10 × 3 cm, *D* (200 ml), then *E*) yielded a colourless foam (150 mg, 40%), after washing with 0.1M $(Et_3NH)HCO_3$ buffer (5 × 10 ml), drying (Na_2SO_4), and co-evaporation with CH_2Cl_2. TLC (*E*): R_f 0.4. UV (MeOH): 235 (23300), 256 (7600), 291 (5400). ^{1}H-NMR ((D)$_6$DMSO): 1.14 (*m*, (*Me*CH_2)$_3$N); 2.55 (*m*, H_α–C(2')); 2.78 (*m*, H_β–C(2')); 2.98 (*m*, (MeC*H*$_2$)$_3$N); 3.16 (*m*, 2 H–C(5')); 3.69 (*s*, MeO); 3.71 (*s*, MeO); 4.13 (*m*, H–C(4')); 4.83 (*m*, H–C(3')); 5.75 (*s*, NH_2–C(4)); 6.25 ('*t*', *J* = 6.4, H–C(1')); 6.39 (*d*, *J* = 7.0, H–C(5)); 6.64 (*d*, *J* = 594, PH); 6.76–7.20 (*m*, H–C(7), arom. H); 7.32 (*t*, *J* = 7.8, H–C(6)); 8.13 (*s*, H–C(2)). Anal. calc. for $C_{39}H_{49}N_4O_7P$ (716.82): C 65.35, H 6.89, N 7.82; found: C 65.21, H 6.95, N 7.88.

*1-[2-Deoxy-5-O-(4,4'-dimethoxytriphenylmethyl)-β-*D*-erythro-pentofuranosyl]-4-{[(9H-fluoren-9-yl)-methoxy]carbonylamino}benzimidazole 3'-[(2-Cyanoethyl) N,N-Diisopropylphosphoramidite]* (**3**). To a soln. of 7 (600 mg, 0.8 mmol) and (i-Pr)$_2$EtN (405 µl, 2.3 mmol) in anh. CH_2Cl_2 (10 ml), chloro(2-cyanoethoxy)(diisopropylamino)phosphane (510 µl, 2.3 mmol) was added at r.t. After stirring for 30 min, the mixture was diluted with CH_2Cl_2 (40 ml) and quenched by adding 5% aq. $NaHCO_3$ soln. (30 ml). Then the aq. layer was extracted with CH_2Cl_2 (3 × 20 ml), the combined org. layer dried (Na_2SO_4) and evaporated, and the colourless oil applied to FC (silica gel, column 10 × 3 cm, *F*): colourless amorphous **3** (530 mg, 68%), diastereoisomer mixture. TLC (*F*): R_f 0.4, 0.3. ^{1}H-NMR ($CDCl_3$): 1.08–1.20 (*m*, Me_2CH); 2.45 (*t*, *J* = 6.4, CH_2CH_2CN); 2.61 (*t*, *J* = 6.1, CH_2CH_2CN); 2.68–2.74 (*m*, H–C(2')); 3.63 (*m*, 2 H–C(5')); 3.68 (*s*, MeO); 3.70 (*s*, MeO); 4.11 (*m*, H–C(4')); 4.30 (*m*, CH(9) of fmoc); 4.52 (*t*, *J* = 6.8, CH_2O); 4.71 (*m*, H–C(3')); 6.28 ('*t*', *J* = 6.6, H–C(1')); 6.73–6.78 (*m*, arom. H); 7.14 (*t*, *J* = 8.7, H–C(6)); 7.21–7.42 (*m*, H–C(7), arom. H); 7.63 (*d*, *J* = 7.4, H–C(5)); 7.68–7.99 (*m*, arom. H); 8.35 (*s*, H–C(2)); 9.44 (*s*, NH–C(4)). Anal. calc. for $C_{57}H_{60}N_5O_8P$ (974.11): C 70.28, H 6.21, N 7.19; found: C 70.44, H 6.22, N 7.18.

*1-[2-Deoxy-5-O-(4,4'-dimethoxytriphenylmethyl)-β-*D*-erythro-pentofuranosyl]-4-{[(9H-fluoren-9-yl)-methoxy]carbonylamino}benzimidazole 3'-(3-Carboxypropanoate)* (**8a**). To a soln. of dry 7 (300 mg, 0.39 mmol) in anh. 1,2-dichloroethane (750 µl), 4-(dimethylamino)pyridine (24 mg, 0.23 mmol), succinic anhydride (58 mg, 0.69 mmol), and Et_3N (53 µl, 0.47 mmol) were added. The mixture was stirred for 20 min at 50°. Then the soln. was diluted with 1,2-dichloroethane (10 ml), washed with ice-cold 10% aq. citric acid soln. (3 × 10 ml) followed by H_2O (10 ml), dried (Na_2SO_4) and evaporated. The residue was applied to FC (silica gel, column 15 × 3 cm, *G*): colourless amorphous solid (280 mg, 82%). TLC (*G*): R_f 0.8. UV (MeOH): 235 (sh, 26300), 265 (36600), 285 (sh, 11700), 299 (6900). ^{1}H-NMR ((D)$_6$DMSO): 2.54 (*m*, H_α–C(2')); 2.95 (*m*, H_β–C(2')); 3.23 (*m*, 2 H–C(5')); 3.30 (*m*, Me); 3.70 (*s*, CH_2, 2 MeO); 4.20 (*m*, H–C(4')); 4.31 (*m*, CH(9) of fmoc); 4.43 (*t*, *J* = 6.2, CH_2O); 5.42 (*m*, H–C(3')); 6.40 ('*t*', *J* = 6.5, H–(1')); 6.80 (*m*, arom. H); 7.03 (*t*, *J* = 8.0, H–C(6)); 7.20–7.42 (*m*, H–C(7), arom. H); 7.56 (*d*, *J* = 7.2, H–C(5)); 7.81–7.90 (*m*, arom. H); 8.38 (*s*, H–C(2)); 9.34 (*s*, NH–C(4)). Anal. calc. for $C_{52}H_{47}N_3O_{10}$ (873.96): C 71.46, H 5.42, N 4.81; found: C 71.33, H 5.48, N 4.84.

*1-[2-Deoxy-5-O-(4,4'-dimethoxytriphenylmethyl)-β-*D*-erythro-pentofuranosyl]-4-{[(9H-fluoren-9-yl)-methoxy]carbonylamino}benzimidazole 3'-[3-(*N*-Fractosil*carbamoyl*)propanoate* (**8b**). A soln. of **8a** (200 mg, 0.23 mmol) in 1,4-dioxane/pyridine 95:5 (2 ml) was treated with 4-nitrophenol (48 mg, 0.34 mmol) and dicyclohexylcarbodiimide (94 mg, 0.46 mmol). The mixture was stirred for 2 h at r.t. and dicyclohexylurea filtered off. The filtrate was added to a suspension of amino-linked silica gel (*Fractosil 200*, 450 µmol NH_2/g; *Merck*) in dry DMF (2 ml). Then Et_3N (400 µl) was introduced and the mixture shaken for 4 h at r.t. Ac_2O (120 µl) was added and shaking

continued for another 30 min. Silica gel was filtered off, washed with DMF, EtOH, and Et_2O and dried *in vacuo*. The amount of covalently linked **1** was determined after the release of dimethoxytrityl cation from the support (5 mg). Upon treatment with 0.1M TsOH in MeCN (1 ml), the loading was found to be 72 μmol/g modified *Fractosil*.

Solid-Phase Synthesis of the Oligodeoxyribonucleotides **9–18**. The oligomers **9**, **10**, and **13** were synthesized on a 1-μmol scale using the 3′-phosphonates of $[(MeO)_2Tr]bz^6A_d$, $[(MeO)_2Tr]ib^2G_d$, $[(MeO)_2Tr]bz^4C_d$, and $[(MeO)_2Tr]T_d$ as well as compound **2**, whereas the oligomers **12**, and **14–16** were synthesized according to [3] [34]. The syntheses of the oligodeoxyribonucleotides **11**, **13**, **17**, and **18** were carried out on a 1-μmol scale using the commercial cyanoethyl phosphoramidites as well as compound **3**. The syntheses and deprotection of the oligonucleotides **9**, **10**, and **13** followed a slightly modified protocol of the DNA synthesizer for 3′-phosphonates [29] [30], whereas the oligomers **11**, **13**, **17**, and **18** were synthesized employing 2-cyanoethyl phosphoramidite chemistry [31]. Yields, hypochromicities, and retention times (*Trityl-Off*, gradient *II*) of the oligomers **11**, and **13–18** are shown in *Table 5*.

Table 5. *Retention Times and Yields of Oligonucleotides*

	11	**13**	**14**	**15**	**17**	**18**
Retention time [min][a])	21.8	18.1	19.3	19.7	18.6	18.8
Hypochromicity [%]	6	8	19	16	27	21
Yield [%][b])	5	6	9	7	17	14

[a]) The retention times refer to gradient *II*.

[b]) The yields were calculated on the basis of silica-gel-bound nucleosides.

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