

133. Hoogsteen-Duplex DNA: Synthesis and Base Pairing of Oligonucleotides Containing 1-Deaza-2'-deoxyadenosine

by Frank Seela* and Thomas Wenzel

Laboratorium für Organische und Bioorganische Chemie, Institut für Chemie, Universität Osnabrück,
Barbarastr. 7, D-49069 Osnabrück

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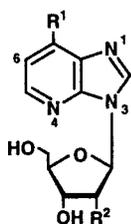
Oligodeoxyribonucleotides containing 1-deaza-2'-deoxyadenosine (= 7-amino-3-(2-deoxy- β -D-erythro-pentofuranosyl)-3H-imidazo[4,5-b]pyridine; **1b**) form *Hoogsteen* duplexes. *Watson-Crick* base pairs cannot be built up due to the absence of N(1). For these studies, oligonucleotide building blocks – the phosphonate **3a** and the phosphoramidite **3b** – were prepared from **1b** via **4a** and **5**, as well as the *Fractosil*-linked **6b**, and used in solid-phase synthesis. The applicability of various *N*-protecting groups (see **4a–c**) was also studied. The *Hoogsteen* duplex d[(c¹A)₂₀·d(T₂₀)] (**11·13**; T_m 15°) is less stable than d(A₂₀·d(T₂₀)] (**12·13**; T_m 60°). The block oligomers d[(c¹A)₁₀-T₁₀] (**14**) and d[T₁₀-(c¹A)₁₀] (**15**) containing purine and pyrimidine bases in the same strand are also able to form duplexes with each other. The chain polarity was found to be parallel.

1. Introduction. – The formation of DNA duplex or triplex structures is controlled by the N-atom pattern of the nucleobase, their exocyclic substituents, and the composition and conformation of the sugar-phosphate backbone. Triplex-stranded DNA structures recently attracted renewed interest due to their possible occurrence *in vivo* [1]. The third strand can also block DNA recognition, which is the basis of new potential therapeutic agents [2]. These selective binding properties were also used to introduce cuts at specific sites of DNA by attaching DNA-cleaving reagents [3]. In all these systems, the pyrimidine oligomer is positioned parallel to the purine strand of the *Watson-Crick* duplex.

In the case of 7-deazapurine-containing oligonucleotides, it was demonstrated that base pairing can be restricted to the *Watson-Crick* mode, if the purine N(7) is absent [4] [5]; triplexes are not formed. Likewise, exclusive *Hoogsteen* base pairing should occur, if the purine N(1) moiety is replaced by a CH group (1-deazapurines). As a result, oligonucleotides containing 1-deaza-2'-deoxyadenosine (purine numbering is used throughout *Sect.* 2) should form non-*Watson-Crick* duplex structures, selectively, either by *Hoogsteen* or by reverse-*Hoogsteen* base pairing.

The 1-deaza-2'-deoxyadenosine (c¹A_d; **1b**) is structurally related to 2'-deoxyadenosine (dA; **2a**) but shows a high population of the 'syn'-conformer (data not shown) [6]. Its synthesis was reported [7–9]. Also the corresponding ribonucleoside was prepared [7]. The 1-deazapurine nucleosides are inhibitors of adenosine deaminase [10] [11]. The incorporation of **1c** in dinucleoside monophosphates [12] as well as in homopolynucleotides was described [13] [14].

There are several reports on non-*Watson-Crick* duplex structures. All of them contain studies with homopolyribonucleotides of non-defined chain length. The polyribonucleotides were prepared from nucleoside diphosphates with polynucleotide phosphorylase. Non-*Watson-Crick* base pairing was detected between poly(c¹A) and poly(U) [14]. Also

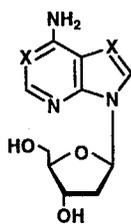


1a R¹ = NO₂, R² = H

b R¹ = NH₂, R² = H

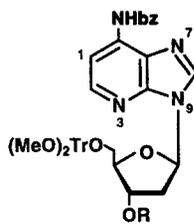
c R¹ = NH₂, R² = OH

(systematic numbering)



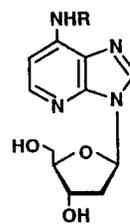
2a X = N

b X = CH



3a R = -PH(O)O[⊖]Et₃NH[⊕]

b R = -P[(i-Pr)₂N][O(CH₂)₂CN]
(purine numbering)



4a R = bz

b R = mac

c R = piv

polyribonucleotide A·U duplexes with bulky C(2) groups at the purine moiety formed such base pairs [15]. Recently, a parallel-stranded *Hoogsteen* duplex was described using oligonucleotides of a particular sequence [16]: the purine strand contained adenine and guanine moieties, whereas the other was constructed from thymidine and 2'-deoxycytidine. The presence of G·C base pairs restricted *Hoogsteen*-duplex formation to the acidic medium. Above pH 6.0, an imperfect *Watson-Crick* duplex was formed [16].

In the following, we report for the first time on a *Hoogsteen* duplex DNA which is stable under neutral conditions. This duplex is constructed from two oligonucleotide strands: one contains 1-deaza-2'-deoxyadenosine (**1b**) and the other (= 2'-deoxyribosylthymine = dT). For the synthetic part of this work, the oligonucleotide building blocks **3a** and **3b** were prepared allowing the incorporation of **1b** into any position of an oligonucleotide chain.

2. Results and Discussion. – *Monomers.* Earlier, syntheses of compound **1b** started from the ribonucleoside [7] or used the microbial transglycosylation of imidazo[4,5-*b*]pyridine bases [8]. We prepared compound **1b** from the nitronucleoside **1a** [17]. Compound **1a** was reduced with *Raney*-Ni catalyst/hydrazine monohydrate in 87% yield [9]. Various protecting groups (benzoyl (bz), methoxyacetyl (mac), pivaloyl (piv)) were studied to block the amino group of **1b**. Reaction of **1b** with benzoyl chloride (transient protection [18]) afforded the benzoyl derivative **4a** (77% yield). Similarly, the pivaloyl-protected **4c** was prepared (82%). The methoxyacetyl derivative **4b** (78%) was obtained *via* peracylation of compound **1b** with MeOCH₂COCl followed by selective deprotection of the sugar-protecting groups [19].

Next, the stability of the protecting groups was studied, as they had to be compatible to those of the regular nucleosides. The time course of deprotection (25% aqueous NH₃ solution, 50°) was followed by HPLC analysis at 260 nm (*Fig. 1*). Compound **1b** was always faster-migrating than **4a–c**. From these experiments, the following half-life values were determined: *t*_{1/2} 2070 min for **4c**, 125 min for **4a**, and 20 min for **4b**. As the benzoyl-protected **4a** showed a similar stability as bz⁶A_d (*t*_{1/2} 71 min) [20], we continued the synthesis with this nucleoside derivative. However, the more labile MeOCH₂CO group will be the group of choice in oligoribonucleotide synthesis [21]. Surprisingly, the benzoyl derivative **4a** was more stable than **1b** against depurination, which is opposite to bz⁶A_d vs. dA [22].

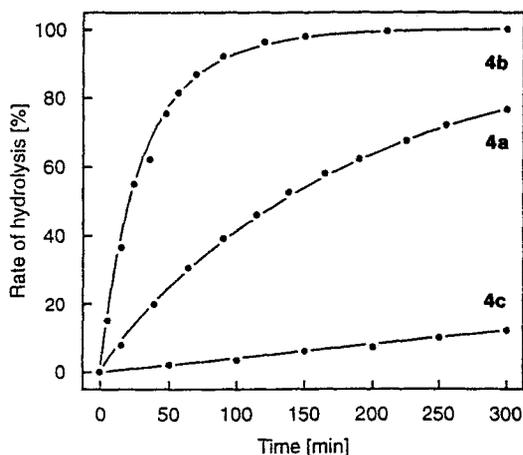
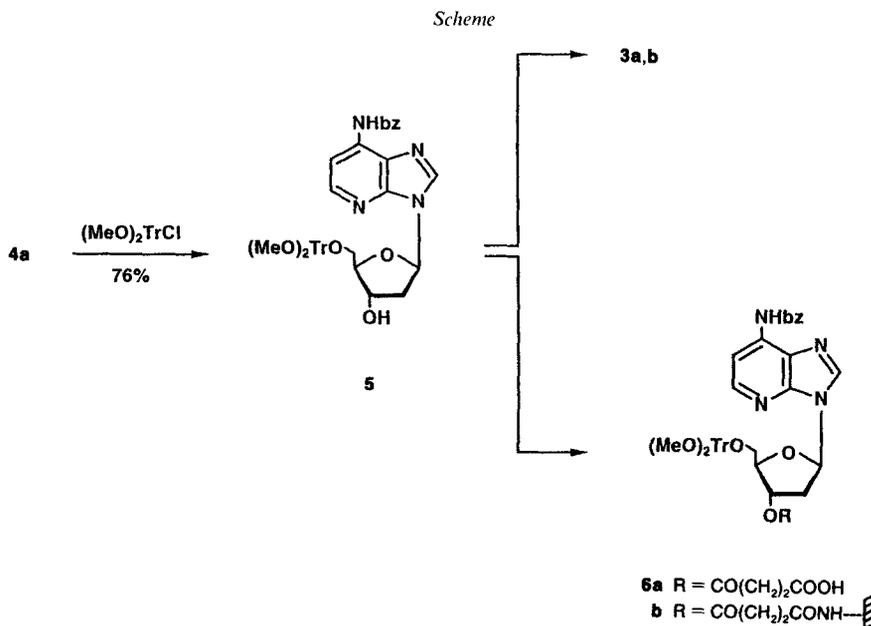


Fig. 1. Time course of the deprotection of **4a–c** in 25% aqueous NH_3 solution at 50° . From a stock solution of the nucleoside, a sample (200 μl) was diluted with 25% NH_3 solution (2 ml) and stored at 50° . Samples (100 μl) were taken after a certain interval of time and neutralized with 0.1N HCl. An aliquot (100 μl) was examined by HPLC (solvent: *A* = 0.1M $(\text{Et}_3\text{NH})\text{OAc}$ (pH 7.0)/MeCN 95:5 and *B* = MeCN; gradient: 20 min 20% *B* in *A*, flow rate 0.8 ml/min). The UV absorbance was measured at 260 nm and quantification made on the basis of peak areas and extinction coefficients.

The 4,4'-dimethoxytritylation was performed under standard conditions furnishing compound **5** in 76% yield (*Scheme*). The reaction of **5** with tris(1*H*-1,2,4-triazol-1-yl)phosphane [23] gave the phosphonate **3a**. This triethylammonium salt was purified chromatographically (84% yield). Phosphonylation of **5** with chloro(2-cyanoethoxy)(diisopropylamino)phosphane [24] furnished the phosphoramidite **3b** (85% yield; diastereoisomer mixture). Succinylation of **5** [25] gave acid **6a** (81%) which was activated *via* the 4-nitrophenyl ester and linked to amino-functionalized *Fractosil* yielding **6b** [26]. The ligand concentration was 66 $\mu\text{mol/g}$ solid support.



We made a complete assignment of the ^{13}C -NMR chemical shifts (*Table 1*) of the 1-deazapurine derivatives on the basis of $J(\text{C},\text{H})$ coupling constants. ^1H - and ^{31}P -NMR Spectra as well as ^1H -NMR NOE difference spectroscopy (*Table 2*) were used for further characterization.

Table 1. ^{13}C -NMR Chemical Shifts of 2-Deoxyribofuranosyl Derivatives of Imidazo[4,5-*b*]pyridine in (D_6)DMSO at 23 $^\circ$)^{a)}

	C(2)	C(3a)	C(5)	C(6)	C(7)	C(7a)
1a	147.9	143.7	144.8	112.1	150.0	127.2
b	139.4	146.4	144.2	102.3	147.2	123.6
3a	142.0	147.0	144.8	109.1	137.1	126.6
4a	142.2	147.0	144.8	109.1	137.2	126.8
b	142.3	146.8	145.2	106.9	136.2	125.4
c	142.2	146.7	145.0	107.9	137.1	126.1
5	142.2	147.1	144.9	109.0	137.1	126.7
6a	142.5	147.1	145.1	109.3	137.3	126.8
	C(1')	C(2')	C(3')	C(4')	C(5')	C=O
1a	84.1	39.4	70.5	88.1	61.5	–
b	84.5	39.3	71.4	88.1	62.2	–
3a	83.8	37.8	72.9	84.9	63.8	166.1
4a	84.0	39.5	70.9	88.0	61.9	166.2
b	84.1	39.4	71.0	88.1	61.9	169.3
c	84.1	39.4	71.0	88.0	61.9	177.2
5	83.6	39.2	70.8	85.8	64.1	166.2
6a	84.1	35.4	74.9	83.5	64.0	166.3, 166.5, 172.3
	Alkyl	CH ₂	MeO	MeCH ₂	MeCH ₂	
3a	–	–	54.9	8.5	45.2	
4a	–	–	–	–	–	
b	–	71.4	58.9	–	–	
c	27.0, 27.1	–	–	–	–	
5	–	–	54.9	–	–	
6a	–	29.6, 30.0	–	–	–	

a) δ Values in ppm rel. to Me₄Si as internal standard; systematic numbering.

b) From ^1H , ^{13}C -gated-decoupled spectra.

Table 2. NOE Data [%] of Compounds **1a** and **1b**)^{a)}

	Irradiation	NOE [%] at					
		H–C(1')	H _z –C(2')	H _{β} –C(2')	H–C(3')	H–C(4')	H–C(2)
1a	H–C(1')	–	6.9	–	–	1.8	4.6
	H–C(2)	4.4	–	3.4	1.4	–	–
1b	H–C(2)	^{c)}	–	2.4	0.9	–	–

a) In (D_6)DMSO at 23 $^\circ$.

b) Systematic numbering.

c) NOE superimposed by H–C(6) and NH₂.

The ^{13}C -NMR signal of C(7a) (systematic numbering) exhibits a *dd* multiplicity due to the coupling to H-C(6) and H-C(2), whereas C(3a) shows a *td* multiplicity resulting from the coupling to H-C(2), H-C(5), and H-C(1'). The chemical shifts of C(2), C(5), and C(6), and those of the sugar moiety were assigned by 2D ^1H , ^{13}C -correlation spectra.

The assignment of the anomeric configuration was established on compound **1a**: ^1H -NMR NOE's at $\text{H}_2\text{-C}(2')$, H-C(4'), and H-C(2) upon irradiation on H-C(1') confirmed the β -D-configuration and N(3) as glycosylation site. Furthermore, it was observed that the NO_2 compound **1a** adopted *ca.* 62% '*anti*'-conformation, whereas the '*syn*'-conformation (67%) was preferred in the case of **1b**. Both values were determined by NOE experiments using a graph published earlier [28]. A preferred '*syn*'-conformation was also reported for the ribonucleoside [29]. The predominance of the '*syn*'-conformation of **1b** was supported by a deshielding of the OH-C(5') resonance (**1b**: 5.82 ppm; **1a**: 5.02 ppm) pointing to an intramolecular H-bond between the 5'-OH group and N(4).

Oligonucleotides. The efficiency of the phosphonate **3a** in solid-phase oligonucleotide synthesis was tested in a competition experiment. Approximately equimolar amounts of the phosphonate building blocks of c^1A_d (see **3a**) and of dA were employed as mixture during the oligonucleotide synthesis using standard conditions [23] [30]. The yield of each coupling step [31] was better than 99%. After treatment with NH_3 , HPLC analysis of the synthesized 5'-protected oligomers $\text{d}\{[(\text{MeO})_2\text{Tr}]\text{T-T-A-T-T}\}$ (**7a**) and $\text{d}\{[(\text{MeO})_2\text{Tr}]\text{T-T-c}^1\text{A-T-T}\}$ (**7b**) showed that different chromatographical mobilities result, even if only one dA residue is replaced by c^1A_d . The oligomers **7a** and **7b** were separated by HPLC, detritylated, and again submitted to HPLC (Figs. 3, a, b). The composition of **7c** and **7d** was then determined by hydrolysis with snake-venom phosphodiesterase followed by alkaline phosphatase yielding the monomeric nucleosides (Figs. 3c, d). A significant bathochromic shift (6 nm) was observed in the UV spectrum of **7d** (λ_{max} 270 nm) compared to **7c** (λ_{max} 264 nm). The corresponding monomers showed the following maxima: dA at 259 nm (15400) and c^1A_d at 264 (12800) and 280 nm (sh, 10400).

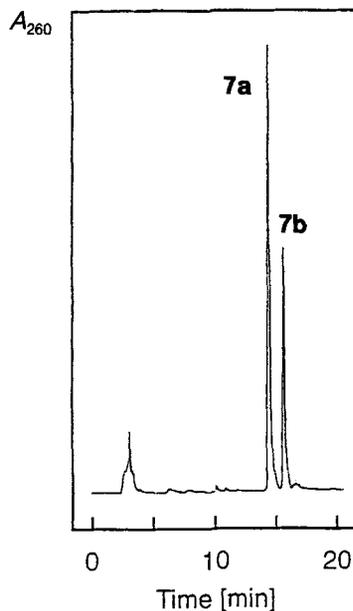


Fig. 2. HPLC Profile of the crude 5'-protected $\text{d}\{[(\text{MeO})_2\text{Tr}]\text{T-T-A-T-T}\}$ (**7a**) and $\text{d}\{[(\text{MeO})_2\text{Tr}]\text{T-T-c}^1\text{A-T-T}\}$ (**7b**). Gradient I; for details, see *Exper. Part*.

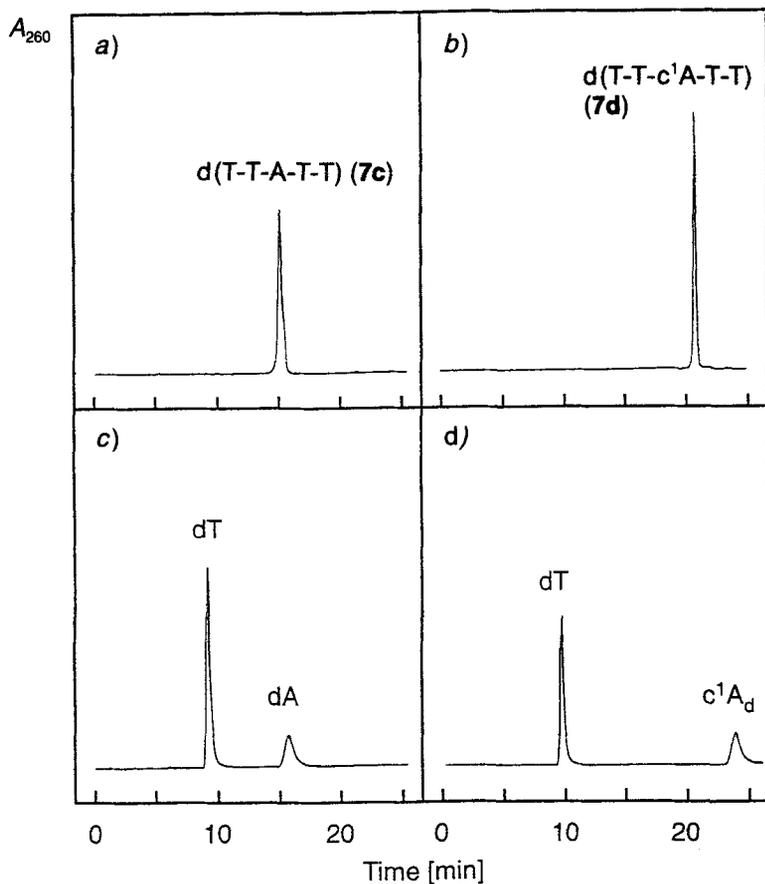


Fig. 3. HPLC Profiles of a) $d(T-T-A-T-T)$ (7c), (gradient II) b) $d(T-T-c^1A-T-T)$ (7d) (gradient II), and c) d) the nucleoside mixtures obtained after hydrolysis with snake-venom phosphodiesterase followed by alkaline phosphatase (gradient III). For details, see *Exper. Part*.

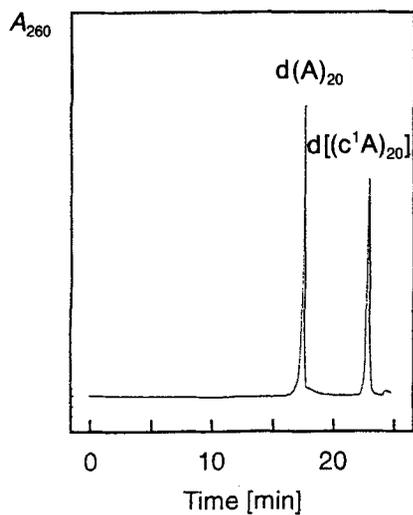
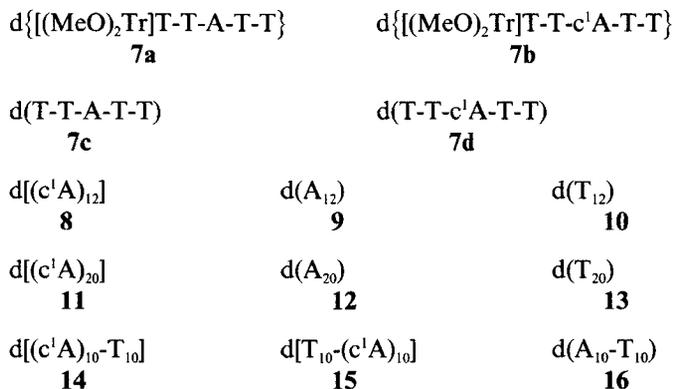


Fig. 4. HPLC Profiles of a synthetic mixture of $d(A)_{20}$ (12) and $d[(c^1A)_{20}]$ (11). Gradient II; for details, see *Exper. Part*.

For further studies regarding the base-pairing of c¹A_d-containing oligonucleotides, the oligomers **8–16** were synthesized by the standard protocol of phosphonate synthesis [30] [32]. The increased acid sensitivity of the *N*-glycosyl bond of c¹A_d compared to dA (data not shown) required the detritylation of the debenzoylated oligomers (2.5% CHCl₂CO₂H) at decreased temperature (0° instead of room temperature). All other workup conditions were identical to those described earlier [20]. *Fig. 4* shows the HPLC profile (*RP-18* silica-gel column) of a synthetic mixture of d(A₂₀) and d[(c¹A)₂₀] indicating the lipophilicity of the modified oligonucleotide.



Duplex Formation and Base Pairing of Oligonucleotides. It was reported that oligonucleotides containing 1,7-dideaza-2'-deoxyadenosine cannot form duplexes with dT, neither by the *Watson-Crick* nor by the *Hoogsteen* mode [20]. In the case of 7-deazapurine-containing oligonucleotides in which only N(7) is absent, the base pairing is restricted to the *Watson-Crick* mode [4] [5]. Consequently, it was expected that *Hoogsteen* duplexes are formed selectively, when oligonucleotides are utilized containing c¹A_d (**1b**) instead of dA. It was assumed that these duplexes are less stable than those formed by *Watson-Crick* base pairs. This information can be drawn from pyrimidine/purine/pyrimidine triplex structures. In this case, the third strand bound to the *Watson-Crick* duplex dissociates from the major groove before the *Watson-Crick* strands are separated [33].

At first, the self-pairing of oligodeoxyribonucleotides containing c¹A_d was studied. Self-pairing was expected as poly(c¹A) prepared enzymatically from 1-deazaadenosine 5'-diphosphate with polynucleotide phosphorylase can form such duplexes. The latter showed cooperative melting under acidic conditions (pH 5.7, *T_m* 40°) [14], and a similar duplex structure was suggested as reported for poly(A) [34]. It was also reported that the p*K_a* of poly(c¹A) was *ca.* 1.5 units higher than that of the monomer c¹A; in the case of poly(A) *vs.* A, the difference was even higher (2.4 units). As this was very unlikely, the p*K_a* values of the deoxyribonucleoside c¹A_d (**1b**) as well as of the oligodeoxyribonucleotide d[(c¹A)₂₀] (**11**) were determined (see *Exper. Part*). A p*K_a* value of 4.6 was found for **1b** which is similar to that of the ribonucleoside c¹A (p*K_a* = 4.7 [14]) but different from that of dA (**2a**: p*K_a* = 3.8). The graph of the pH titration of **11** (*Fig. 5*) resulted in a p*K_a* value of 5.2, a value higher by 0.6 units than that of the monomer **1b**. Next, melting experiments of d[(c¹A)₂₀] (**11**) were carried out in acidic solutions (pH 5.0: 0.1M NaCl, 0.05M NaOAc; pH 5.7: 0.1M NaCl, 0.05M NaOAc) as well as under neutral conditions (pH 7.0: 60 mM

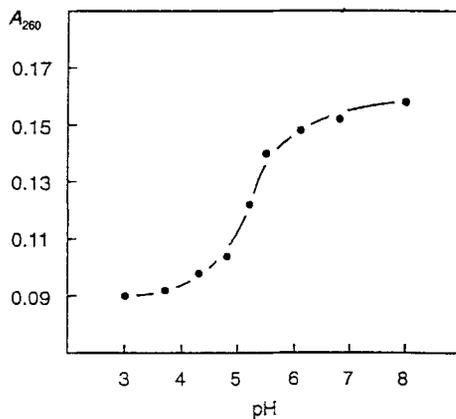


Fig. 5. Titration plot of $d[(c^1A)_{20}]$ (11; pH vs. UV absorption) in buffer solution. For details, see *Exper. Part* and *Sect. 2*.

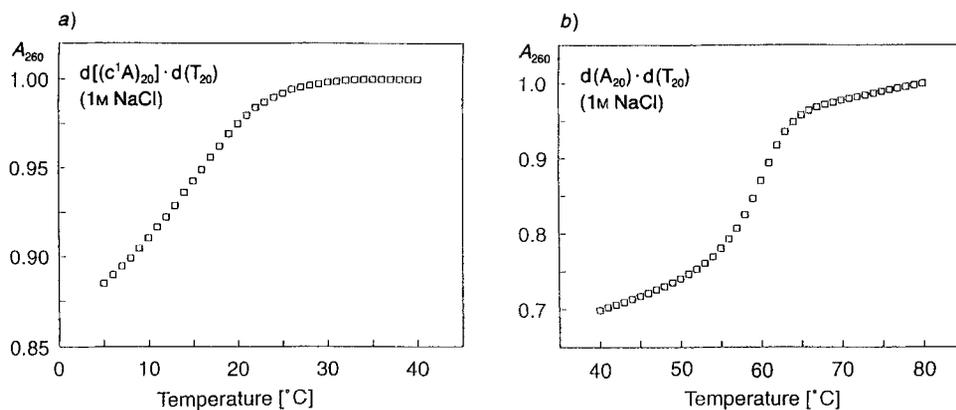


Fig. 6. Normalized melting profiles of a) $d[(c^1A)_{20}] \cdot d(T_{20})$ (11 · 13) and b) $d(A_{20}) \cdot d(T_{20})$ (12 · 13). The melting curves were measured at 260 nm in 60 mM Na-cacodylate, 1M NaCl, and 100 mM $MgCl_2$ at pH 7.0; the oligomer concentration was 5.0 μM of single strands.

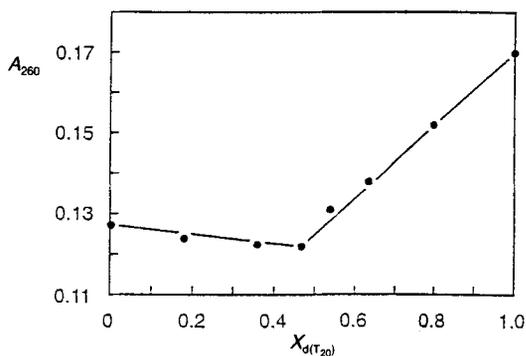


Fig. 7. Mixing experiments of $d[(c^1A)_{20}] \cdot d(T_{20})$ (11 · 13). For details, see *Exper. Part*.

Na-cacodylate, 1M NaCl, 0.1M MgCl₂). In all cases, no sigmoidal melting profile was observed, neither at 260 nor at 280 nm, and the hypochromicity change was smaller than 5%. These results demonstrated that self-pairing of d[(c¹A)₂₀] (**11**) did not take place. Similarly, self-pairing of short regular oligomers such as d(A₂₀) (**12**) did also not occur under acidic conditions, although the hypochromicity change was larger (14%) in this case. Adenine/adenine self-pairing occurred even on short oligonucleotides when the phosphodiester backbone was altered, e.g. in the case of ddGlc(A)₆ [35]. The observation that poly(c¹A) formed duplexes by self-pairing [14] similar to poly(A) [34] indicates that a certain chain length is required to observe this phenomenon. Different from poly(c¹A) (*h* = 27%) [14], the hypochromicity of d[(c¹A)₂₀] (**11**) was only 3% (thermal melting, neutral solution).

Next, the melting of d[(c¹A)₁₂] (**8**) and d(T₁₂) (**10**) was studied. The separate strands as well as the **8**·**10** mixture exhibited only a small increase of the absorbance (data not shown). The related *Watson-Crick* duplex of d(A₁₂)·d(T₁₂) (**9**·**10**) showed already a *T_m* of 43° [36]. This *T_m* value was 17° higher when the chain lengths were increased from the 12-mers to 20-mers: d(A₂₀)·d(T₂₀) (**12**·**13**) measured under identical conditions had a *T_m* value of 60° (*Fig. 6*).

A complete UV melting curve was obtained for the 20-mers **11**·**13** from which a *T_m* value of 15° was determined (*Fig. 6*). The thermal hypochromicity (260 nm) measured between 5° and 80° was smaller for **11**·**13** (12%) than for **12**·**13** (40%). These results demonstrate that a non-*Watson-Crick* duplex was formed between **11** and **13**. The duplex formation was also established from the mixing profile [4], measured in 0.1M NaCl, 50 mM Na-cacodylate, and 1 mM MgCl₂ at pH 7.0 and 8° (see *Exper. Part*), which gave a straight line with an intersection at 50% d[(c¹A)₂₀] demonstrating the 1:1 stoichiometry of the complex (*Fig. 7*).

In the case of duplex **11**·**13**, parallel as well as antiparallel strand orientation had to be considered. Thus, we focussed our interest on the oligomers **14** and **15**. These oligomers contain the same tracts of c¹A_n and dT residues within one strand, but they differ regarding their 3'- and 5'-termini, the latter being a c¹A_n cluster in **14** and a dT segment in **15**. The type of experiment performed with **14** and **15** (see below) can be used in duplex DNA to discriminate between parallel and antiparallel strand orientation. In this regard, it is important to note that in Pyr·Pur·Pyr triplex structures, the strands must always contain only one type of bases, either purines or pyrimidines to form perfect triple helices. To expand the recognition mode to all four base pairs, the nonnatural deoxyribonucleoside 1-(2-deoxy-β-D-erythro-pentofuranosyl)-4-[3-(benzamido)phenyl]-1*H*-imidazole was synthesized which forms triplexes with d(T·A), d(C·G), d(A·T), and d(G·C) [37]. The situation is different in *Hoogsteen*- or reverse-*Hoogsteen*-duplex DNA. Similar to *Watson-Crick* DNA, each purine and each pyrimidine base has a free binding site for base pairing. The only difference is the participation of purine N(7) instead of the purine N(1) during duplex formation. However, the presence of purine and pyrimidine bases in the same strand may cause geometrical problems due to glycosylic torsion angles and repulsions in the sugar-phosphate backbone.

In an earlier experiment (see above), self-pairing of d[(c¹A)₂₀] was excluded and c¹A_n·dT base pairing was established from homooligomers. As a consequence, only the antiparallel duplexes **14**·**14** or **15**·**15** as well as the parallel duplex of **14**·**15** had to be considered.

We already stated that various arrangements have to be considered for the non-*Watson-Crick* duplex formed by $d[(c^1A)_{20}]$ (**11**) with $d(T_{20})$ (**13**): Parallel as well as antiparallel strand orientation, *Hoogsteen* as well as reverse-*Hoogsteen* base pairing (Fig. 9). Nevertheless, the melting experiments carried out on the block oligomers **14** and **15** make a parallel strand orientation also very likely for the duplex **11**·**13**. But even in the case of parallel chain orientation, *Hoogsteen* or reverse-*Hoogsteen* base pairs may be formed.

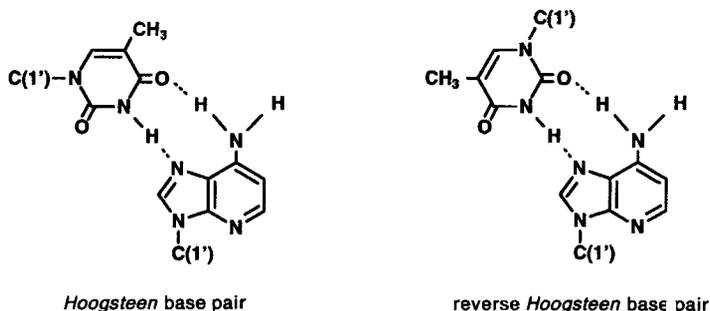


Fig. 9. Base-pairing pattern

To differentiate between *Hoogsteen* and reverse-*Hoogsteen* base pairing, we extracted structural information from Pyr·Pur·Pyr triplex structures and built up models which follow these instructions. Unfortunately, only very little precise structural information is available for triplex structures. *Arnott* and coworkers provided an A-DNA model for $d(T_n) \cdot d(A_n) \cdot d(T_n)$ based on fiber-diffraction data and molecular modelling [38] [39]. This model was used for structural interpretation of DNA triplex structures. It was recently modified by *Ragunathan et al.*, favoring a B-DNA structure for the partial *Watson-Crick* structure which was proved by the fact that distamycin A forms complexes only with B- and not with A-DNA [40] [41]. This new model has a dyad symmetry between the two identical antiparallel dT strands. The base pairing of the third strand is of the *Hoogsteen* mode, the sugar pucker is C(2')-*endo* which is in line with NMR data [42], and the glycosylic torsion angles are 'anti'. If these data are used to build up a duplex DNA from $d[(c^1A)_{20}]$ (**11**) and $d(T_{20})$ (**13**), a regular helix is formed with pyrimidine bases in the 'anti'-conformation and the 1-deazapurine bases in the high-'syn'-conformation. The base pairing is very similar to that of the triplex structure (*Hoogsteen* mode). We also constructed a *Hoogsteen* helix from $d[(c^1A)_{10}-T_{10}] \cdot d[T_{10}-(c^1A)_{10}]$ (**14**·**15**). The first ten base pairs were constructed in the same manner as described for the duplex **11**·**13**. When the sequence of one strand changes from purine to pyrimidine bases, the glycosylic torsion angles also change to 'anti'-conformation for the purine bases and high-'syn'-conformation for the pyrimidine residues. Furthermore, the sugar-phosphate backbone shows a change of the helicity. The duplex **14**·**15** was also constructed from reverse-*Hoogsteen* base pairs. In this case, the first ten base pairs are arranged with the purine nucleosides in high-'anti'- and the pyrimidine residues in 'anti'-conformation. At the purine-pyrimidine junction, the conformation is changed to 'syn' for the purine bases and to high-'syn' for the thymidine residues. In this regard, a conclusion on the base-pairing

pattern of the duplex **14·15** (*Hoogsteen* or reverse-*Hoogsteen*) cannot be given. However, in the light of the large number of data available for homopyrimidine·homopurine·homopyrimidine triplexes [33] [38–41], the duplex $d[(c^1A)_{20}] \cdot d(T_{20})$ (**11·13**) is expected to show *Hoogsteen* base pairing.

With regard to polymeric drugs, *Hoogsteen* base pairing was reported to block the flow of information from *Watson-Crick* duplex DNA to mRNA via triplex formation. Now, *Hoogsteen* base pairing can also be considered to complex single-stranded nucleic acids, such as mRNA. This would expand the antisense concepts [43] [44] from antiparallel to parallel oligonucleotide duplex structures.

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Experimental Part

General. See [45]. 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*), $CH_2Cl_2/AcOEt/Et_3N$ 45:45:10 (*F*), $MeCN/H_2O$ 9:1 (*G*).

HPLC Separation. HPLC was carried out according to [45]. 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*, 5 min 20% *B* in *A*, flow rate 1 ml/min; system *III*, 30 min 100% *A*, flow rate 0.6 ml/min.

pK_a Values. The pK_a values were determined UV-spectrophotometrically at 20° in a buffer according to [46] for **1b** and in *Sørensen*-phosphate buffer [47] for **11**. The absorbance of identical amounts was measured within the pH range 3.0–8.0. The mixtures were equilibrated for 10 min in each experiment, and the absorbance at 260 nm was plotted against the pH.

Melting Experiments. Melting curves were measured with a *Cary-1/3* UV/VIS spectrophotometer (*Varian*, Australia) using a temp. increase of 30°/h. The actual temp. was measured in the reference cell with a Pt-100 resistor. The *T_m* values were calculated using the software package '2hDNA' (Dr. *Apel*, *Varian*, Darmstadt, Germany).

Oligonucleotide UV-Mixing Experiments. Experiments using $d[(c^1A)_{20}]$ and $d(T_{20})$ were performed according to [4] at 260 nm in 0.05M aq. Na-cacodylate (pH 7.0) containing 0.1M NaCl and 1 mM $MgCl_2$. Before measurement, the mixtures were allowed to equilibrate for 15 min at 8°.

7-Amino-3-(2-deoxy-β-D-erythro-pentofuranosyl)-3H-imidazo[4,5-b]pyridine (1b). A soln. of **1a** [17] (800 mg, 2.85 mmol) in $MeOH$ (200 ml) containing hydrazine-hydrate (15 ml) was hydrogenated in the presence of *Raney-Ni* catalyst (5 g) at r.t. After the mixture became colourless (60 min), the catalyst was filtered off, the filtrate evaporated, and the residue adsorbed on silica gel and applied to FC (silica gel, column 20 × 3 cm, *C*): **1b** (620 mg, 87%). Colorless crystals ($MeOH$). M.p. 217°. TLC (*C*): *R_f* 0.7. UV ($MeOH$): 264 (12800), 280 (10400). ¹H-NMR ((D₆)DMSO): 2.23 (*m*, H_α-C(2')); 2.81 (*m*, H_β-C(2')); 3.71 (*m*, 2 H-C(5')); 3.94 (*m*, H-C(4')); 4.44 (*m*, H-C(3')); 5.31 (*d*, *J* = 3.5, OH-C(3')); 5.82 (*m*, OH-C(5')); 6.40 (*m*, H-C(1')); 6.40 (*d*, *J* = 5.2, H-C(6)); 6.47 (*s*, NH₂); 7.81 (*d*, *J* = 5.5, H-C(5)); 8.28 (*s*, H-C(2)). Anal. calc. for C₁₁H₁₄N₄O₃ (250.26): C 52.79, H 5.64, N 22.39; found: C 52.96, H 5.74, N 22.22.

7-(Benzoylamino)-3-(2-deoxy-β-D-erythro-pentofuranosyl)-3H-imidazo[4,5-b]pyridine (4a). Compound **1b** (600 mg, 2.40 mmol) was dried by co-evaporation with anhyd. pyridine and then dissolved in pyridine (20 ml). Me_3SiCl (1.54 ml, 12.0 mmol) was added under Ar. The soln. was stirred for 15 min, treated with benzoyl chloride (1.39 ml, 12.0 mmol), and maintained at r.t. for 2 h. The mixture was cooled to 0° and hydrolyzed with H₂O (2.5 ml). After 5 min, the mixture was treated with 25% aq. NH₃ soln. (5 ml) and stirred for another 30 min. The solvent was evaporated and the oily residue co-evaporated with toluene and acetone. The residue, dissolved in $MeOH$, was adsorbed on silica gel and chromatographed (silica gel, column 20 × 3 cm, *B*): Colorless crystals (650 mg, 77%). M.p. 127–128° (acetone). TLC (*B*): *R_f* 0.3. UV ($MeOH$): 231 (sh, 11900), 282 (24300). ¹H-NMR ((D₆)DMSO): 2.32 (*m*, H_α-C(2')); 2.81 (*m*, H_β-C(2')); 3.58 (*m*, 2 H-C(5')); 3.91 (*m*, H-C(4')); 4.45 (*m*, H-C(3')); 5.21 (*t*, *J* = 5.4,

OH–C(5''); 5.36 (*d*, *J* = 3.7, OH–C(3'')); 6.51 (*t'*, *J* = 6.7, H–C(1'')); 7.52–8.05 (*m*, H–C(6), Ph); 8.31 (*d*, *J* = 5.4, H–C(5)); 8.64 (*s*, H–C(2)); 10.42 (*s*, NH–C(7)). Anal. calc. for C₁₈H₁₈N₄O₄ (354.37): C 61.01, H 5.12, N 15.81; found: C 61.23, H 5.27, N 15.51.

3-(2-Deoxy-β-D-erythro-pentofuranosyl)-7-(methoxyacetylamino)-3H-imidazo[4,5-b]pyridine (**4b**). Compound **1b** (100 mg, 0.4 mmol) was dried by two evaporations with anhyd. pyridine (5 ml), dissolved in dry pyridine, and treated with methoxyacetyl chloride (220 μl, 2.4 mmol). After 2 h of stirring at r.t., H₂O (500 μl) was added to destroy the acyl chloride. After another 30 min, the soln. was evaporated and the residue dissolved in CH₂Cl₂ (30 ml) and extracted 3 times with 5% aq. NaHCO₃ soln. followed by H₂O (20 ml each). The org. layer was dried (Na₂SO₄) and evaporated. The yellow oil was stirred for 30 min with Et₃N/pyridine/H₂O 1:1:3 (15 ml) at r.t., the soln. evaporated, the oily residue co-evaporated twice with toluene (10 ml each) and acetone (10 ml each), and the residue applied to FC (silica gel, column 15 × 2 cm, *B*): colorless foam (100 mg, 78%). TLC (silica gel, *B*): R_f 0.3. UV (MeOH): 271 (19800), 279 (sh, 16700). ¹H-NMR ((D₆)DMSO): 2.32 (*m*, H_α–C(2'')); 2.78 (*m*, H_β–C(2'')); 3.43 (*s*, MeO); 3.54 (*m*, 1 H–C(5'')); 3.61 (*m*, 1 H–C(5'')); 3.89 (*m*, H–C(4'')); 4.17 (*s*, CH₂); 4.43 (*m*, H–C(3'')); 5.17 (*m*, OH–C(5'')); 5.33 (*d*, *J* = 2.9, OH–C(3'')); 6.48 (*t'*, *J* = 5.9, H–C(1'')); 8.08 (*d*, *J* = 5.0, H–C(6)); 8.26 (*d*, *J* = 5.0, H–C(5)); 8.60 (*s*, H–C(2)); 9.82 (*s*, NH–C(7)). Anal. calc. for C₁₄H₁₈N₄O₅ (322.30): C 52.17, H 5.63, N 17.38; found: C 52.25, H 5.65, N 17.24.

3-(2-Deoxy-β-D-erythro-pentofuranosyl)-7-(pivaloylamino)-3H-imidazo[4,5-b]pyridine (**4c**). Compound **1b** (100 mg, 0.4 mmol) was dried by co-evaporation with dry pyridine, dissolved in anhyd. pyridine (5 ml), and treated with Me₃SiCl (410 μl, 6.2 mmol). After 30 min of stirring at r.t., pivaloyl chloride (196 μl, 2.0 mmol) was added and the soln. stirred for 1 h. The mixture was cooled to 0° and hydrolyzed with H₂O (400 μl, 5 min) and 25% aq. NH₃ soln. (800 μl, 30 min). The solvent was evaporated, the oily residue dissolved in CH₂Cl₂ (50 ml) and extracted 3 times with 5% aq. NaHCO₃ soln. followed by H₂O (20 ml each), the org. layer dried (Na₂SO₄) and co-evaporated with toluene (2 × 10 ml) and acetone (2 × 10 ml), and the residue dissolved in *B* and applied to FC (silica gel, column 15 × 2 cm, *B*): colorless foam (110 mg, 82%). TLC (silica gel, *B*): R_f 0.4. UV (MeOH): 271 (20800), 281 (sh, 17600). ¹H-NMR ((D₆)DMSO): 1.29 (*m*, *t*-Bu), 2.25 (*m*, H_α–C(2'')); 2.76 (*m*, H_β–C(2'')); 3.53 (*m*, 1 H–C(5'')); 3.60 (*m*, 1 H–C(5'')); 4.01 (*m*, H–C(4'')); 4.22 (*m*, H–C(3'')); 5.18 (*m*, OH–C(5'')); 5.32 (*m*, OH–C(3'')); 6.47 (*t'*, *J* = 6.8, H–C(1'')); 7.95 (*d*, *J* = 5.4, H–C(6)); 8.24 (*d*, *J* = 5.5, H–C(5)); 8.59 (*s*, H–C(2)); 9.18 (*s*, NH–C(7)). Anal. calc. for C₁₆H₂₂N₄O₄ (334.40): C 57.47, H 6.63, N 16.76; found: C 57.26, H 6.69, N 16.67.

7-(Benzoylamino)-3-[2-deoxy-5-O-(4,4'-dimethoxytriphenylmethyl)-β-D-erythro-pentofuranosyl]-3H-imidazo[4,5-b]pyridine (**5**). Compound **4a** (550 mg, 1.55 mmol) in anhyd. pyridine (20 ml) was stirred under Ar for 2 h in the presence of 4,4'-dimethoxytriphenylmethyl chloride (1.0 g, 2.95 mmol) at r.t. After addition of 5% aq. NaHCO₃ soln. (30 ml), the mixture was extracted with CH₂Cl₂ (5 × 30 ml), the combined org. layer dried (Na₂SO₄) and evaporated, and the residue dissolved in *B* and applied to FC (silica gel, column 30 × 3 cm, *A*). The main zone was evaporated and the oily residue dissolved in CH₂Cl₂ (10 ml) and poured into cold hexane/Et₂O 1:1 (50 ml). Compound **5** precipitated in colorless needles (770 mg, 76%). M.p. 130°. TLC (*A*): R_f 0.3. UV (MeOH): 234 (32000), 282 (26800). ¹H-NMR ((D₆)DMSO): 2.40 (*m*, H_α–C(2'')); 2.97 (*m*, H_β–C(2'')); 3.21 (*m*, 2 H–C(5'')); 3.69 (*s*, MeO); 3.71 (*s*, MeO); 4.04 (*m*, H–C(4'')); 4.54 (*m*, H–C(3'')); 5.42 (*d*, *J* = 4.4, OH–C(3'')); 6.54 (*t'*, *J* = 6.4, H–C(1'')); 6.76–8.04 (*m*, H–C(6), Bz, (MeO)₂Tr); 8.27 (*d*, *J* = 5.5, H–C(5)); 8.54 (*s*, H–C(2)); 10.41 (*s*, NH–C(7)). Anal. calc. for C₃₉H₃₆N₄O₆ (656.75): C 71.33, H 5.53, N 8.53; found: C 71.29, H 5.67, N 8.51.

7-(Benzoylamino)-3-[2-deoxy-5-O-(4,4'-dimethoxytriphenylmethyl)-β-D-erythro-pentofuranosyl]-3H-imidazo[4,5-b]pyridine 3'-(Triethylammonium Phosphonate) (**3a**). To a soln. of PCl₃ (290 μl, 3.4 mmol) and *N*-methylmorpholine (3.8 ml, 34.0 mmol) in anhyd. CH₂Cl₂ (30 ml), 1*H*-1,2,4-triazole (770 mg, 11.1 mmol) was added under Ar. After stirring for 30 min, the soln. was cooled to 0°, and **5** (400 mg, 0.61 mmol) in anhyd. pyridine (20 ml) was added dropwise within 10 min. After stirring for 20 min at r.t., the mixture was poured into 1M aq. (Et₃NH)HCO₃ (TBK; pH 7.5; 35 ml), shaken, and separated. The aq. layer was extracted with CH₂Cl₂ (3 × 30 ml), the combined org. extract dried (Na₂SO₄), filtered, and evaporated, and the colorless foam applied to FC (silica gel, column 15 × 3 cm, *D* (0.5 l), then *E*). The residue of the main zone was dissolved in CH₂Cl₂ (50 ml) and extracted with 0.1M aq. (Et₃NH)HCO₃ (5 × 50 ml). The org. layer was dried (Na₂SO₄) and evaporated: colorless foam (420 mg, 84%). TLC (*E*): R_f 0.4. UV (MeOH): 233 (31200), 282 (25900). ¹H-NMR ((D₆)DMSO): 1.15 (*m*, MeCH₂NH); 2.55 (*m*, H_α–C(2'')); 2.97 (*m*, MeCH₂NH); 2.99 (*m*, H_β–C(2'')); 3.07 (*m*, 2 H–C(5'')); 3.70 (*s*, MeO); 3.71 (*s*, MeO); 4.22 (*m*, H–C(4'')); 4.90 (*m*, H–C(3'')); 6.53 (*t'*, *J* = 6.8, H–C(1'')); 6.69 (*d*, *J* = 587.2, PH); 7.31–8.02 (*m*, Bz, (MeO)₂Tr); 8.04 (*d*, *J* = 5.2, H–C(6)); 8.25 (*d*, *J* = 5.4, H–C(5)); 8.52 (*s*, H–C(2)); 10.40 (*s*, NH–C(7)). ³¹P-NMR ((D₆)DMSO): 2.56 (¹J(P,H) = 587.2, ³J(P,H–C(4')) = 8.3). Anal. calc. for C₄₅H₅₂N₅O₈P (821.90): C 65.76, H 6.38, N 8.52; found: C 65.73, H 6.39, N 8.58.

7-(Benzoylamino)-3-[2-deoxy-5-O-(4,4'-dimethoxytriphenylmethyl)-β-D-erythro-pentofuranosyl]-3H-imidazo[4,5-b]pyridine 3'-(2-Cyanoethyl) N,N-Diisopropylphosphoramidite (**3b**). A soln. of **5** (300 mg, 0.46 mmol) in

anh. CH_2Cl_2 (10 ml) was preflushed with Ar. Then (i-Pr) $_2$ EtN (240 μl , 1.38 mmol) and chloro(2-cyanoethoxy)(diisopropylamino)phosphane (300 μl , 1.36 mmol) were added, and the mixture was kept under Ar at r.t. After stirring for 1 h, 5% aq. NaHCO_3 soln. (10 ml) was added and the soln. extracted several times with CH_2Cl_2 . The org. layer was dried (Na_2SO_4), filtered, and evaporated. The residue dissolved in *F* was applied to FC (silica gel, *F*): colorless foam (330 mg, 85%). TLC (silica gel, *F*): R_f 0.6, 0.7. $^1\text{H-NMR}$ ((D_6) DMSO): 1.01–1.14 (*m*, Me_2CH); 2.52 (*m*, $\text{H}_\alpha\text{-C}(2'')$); 2.67 (*t*, $J = 6.5$, $\text{CH}_2\text{CH}_2\text{CN}$); 2.77 (*t*, $J = 6.5$, $\text{CH}_2\text{CH}_2\text{CN}$); 3.05 (*m*, $\text{H}_\beta\text{-C}(2'')$); 3.20 (*m*, 1 $\text{H-C}(5')$); 3.54 (*m*, 1 $\text{H-C}(5')$); 3.69 (*m*, 2 MeO); 4.15 (*m*, $\text{H-C}(4')$); 5.82 (*m*, $\text{H-C}(3')$); 6.50 (*m*, $\text{H-C}(1')$); 6.77–8.04 (*m*, Bz, $(\text{MeO})_2\text{Tr}$, $\text{H-C}(6)$); 8.22 (*d*, $J = 5.4$, $\text{H-C}(5)$); 8.55 (*s*, $\text{H-C}(2)$); 10.40 (*s*, $\text{NH-C}(7)$). $^{31}\text{P-NMR}$ ((D_6) DMSO): faster migrating zone: 148.2 (*dd*, $J(\text{P,H}) = 6.2$, and 11.3); slower migrating zone: 148.8 (*dd*, $J(\text{P,H}) = 5.7$, 11.0).

7-(Benzoylamino)-3-[2-deoxy-5-O-(4,4'-dimethoxytriphenylmethyl)- β -D-erythro-pentofuranosyl]-3H-imidazo[4,5-b]pyridine 3'-(3-Carboxypropanoate) (**6a**). To a soln. of dry **5** (200 mg, 0.31 mmol) in anh. 1,2-dichloroethane (610 μl), 4-(dimethylamino)pyridine (18.7 mg, 0.15 mmol), succinic anhydride (45.9 mg, 0.46 mmol), and Et_3N (42.3 μl , 0.31 mmol) were added. The mixture was stirred for 30 min at 50°. Then the soln. was diluted with 1,2-dichloroethane (10 ml), washed with ice-cold 10% aq. citric acid soln. (3 \times 8 ml) and H_2O (3 \times 8 ml). The org. layer was dried (Na_2SO_4) and evaporated and the residue submitted to FC (silica gel, column 10 \times 3 cm, *G*). After evaporation of the main zone, the residue was dissolved in CH_2Cl_2 (5 ml) and precipitated from Et_2O /hexane 1:1 (20 ml): colorless powder (190 mg, 81%). TLC (*G*): R_f 0.7. UV (MeOH): 233 (26700), 282 (22300). $^1\text{H-NMR}$ ((D_6) DMSO): 2.49 (*m*, $\text{H}_\alpha\text{-C}(2'')$); 2.94 (*m*, $\text{H}_\beta\text{-C}(2'')$); 3.28 (*m*, 2 $\text{H-C}(5')$); 3.50 (*m*, 2 CH_2 , $\text{H-C}(4')$); 3.69 (*s*, 2 MeO); 4.19 (*m*, $\text{H-C}(3')$); 5.41 (*s*, COOH); 6.52 (*t'*, $J = 6.5$, $\text{H-C}(1')$); 6.75–8.09 (*m*, $\text{H-C}(6)$, Bz, $(\text{MeO})_2\text{Tr}$); 8.20 (*d*, $J = 5.3$, $\text{H-C}(5)$); 8.55 (*s*, $\text{H-C}(2)$); 10.44 (*s*, $\text{NH-C}(7)$). Anal. calc. for $\text{C}_{43}\text{H}_{40}\text{N}_4\text{O}_9$ (756.80): C 68.24, H 5.33, N 7.40; found: C 68.01, H 5.46, N 7.41.

7-(Benzoylamino)-3-[2-deoxy-5-O-(4,4'-dimethoxytriphenylmethyl)- β -D-erythro-pentofuranosyl]-3H-imidazo[4,5-b]pyridine 3'-[3-(N-Fructosilcarbamoyl)propanoate] (**6b**). To a soln. of **6a** (100 mg, 0.13 mmol) in 1,4-dioxane/pyridine 95:5 (1 ml), 4-nitrophenol (33 mg, 0.24 mmol) and *N,N*-dicyclohexylcarbodiimide (50 mg, 0.25 mmol) were added under stirring at r.t. After 2 h, dicyclohexylurea was removed by filtration. To the filtrate, Fractosil 200 (200 mg, 450 μequiv . NH_2/g) and DMF (1 ml) were added. Then, Et_3N (200 μl) was introduced and the suspension shaken for 4 h at r.t. Ac_2O (60 μl) was added and shaking continued for another 30 min. The Fractosil derivative was filtered off, washed with DMF, EtOH, and Et_2O , and dried *in vacuo*. The amount of silica-gel-bound nucleoside was determined by treatment of **6b** (5 mg) with 0.1M TsOH (10 ml) in MeCN. From the absorbance at 498 nm of the supernatant, 66 μmol of linked **6a/g** Fractosil was calculated ($(\text{MeO})_2\text{Tr} = 70000$).

Solid-Phase Synthesis of the Oligonucleotides 7–16. The synthesis of the oligonucleotides was performed on a 1- μmol scale using the 3'-phosphonates of $[(\text{MeO})_2\text{Tr}]A_d$ and $[(\text{MeO})_2\text{Tr}]T_d$ as well as **3a** and following the protocol of the DNA synthesizer for 3'-phosphonates [30] [48]. Results in Table 3.

Table 3. Retention Times and Yields of Oligonucleotides

Oligomer	7c	7d	8	9	10	11	12	13	14	15	16
Retention time [min] ^{a)}	15.2	20.8	21.6	15.5	13.7	23.1	16.9	14.6	22.1	22.0	17.1
Yield [%] ^{b)}	28	15	6	7	15	5	8	16	9	7	13

a) The retention times refer to gradient II.

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

Enzymatic Hydrolysis of the Oligomers 7–16 and Determination of the Hypochromicity. A soln. of the oligonucleotide (0.5 A_{260} units) in 0.1M Tris \cdot HCl buffer (pH 8.3, 200 μl) was treated with snake-venom phosphodiesterase (3 μg) at 37° for 120 min and alkaline phosphatase (3 μg) for 90 min at 37°. The mixture was analyzed on reversed-phase HPLC (RP-18, solvent 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; $c'A_d$ 11500; T_d 8800).

The hypochromicity *H* (%) was determined by enzymatic digestion to 0.5 A_{260} units of the corresponding oligonucleotides as described above.

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