8. Duplex Stabilization of DNA: Oligonucleotides Containing 7-Substituted 7-Deazaadenines

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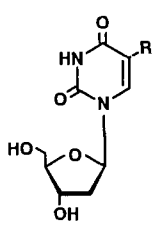
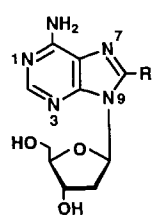
The oligonucleotide building blocks **4b–d** derived from 7-bromo-, 7-chloro-, and 7-methyl-substituted 7-deaza-2′-deoxyadenosines **3b–d** were prepared. They were employed in the solid-phase synthesis of the oligonucleotides **7–25**. The dA residues of the homomer d(A_{12}), the alternating d[(A-T)$_6$], and the palindromic d(G-T-A-G-A-A-T-T-C-T-A-C) were replaced by **3b–d** as well as by the parent 7-deaza-2′-deoxyadenosine (**3a**). The melting profiles and CD spectra of oligonucleotide duplexes, showing this major groove modification, were measured, and the T_m values as well as the thermodynamic data were determined. It was found that small substituents such as Br, Cl, or Me introduced in the 7-position of a 7-deazaadenine residue increase the duplex stability compared to oligonucleotides containing adenine.

Introduction. – The 5-methyl group of thymidine (T_d; **1c**) stabilizes the structure of oligodeoxyribonucleotide duplexes containing d[(A-T)$_n$] base pairs compared to those built up from d[(A-U)$_n$] [1]. A Br substituent or other not too bulky substituents introduced in the 5-position (**1b**) of 2′-deoxyuridine (**1a**) increases the duplex stability further [2] [3]. The situation is opposite for 8-substituted purines **2b**, **c**. In this case, Me or Br substituents also located in the major groove decrease the stability of a duplex [4].

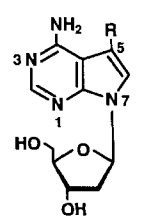


1a R = H
b R = Br
c R = Me



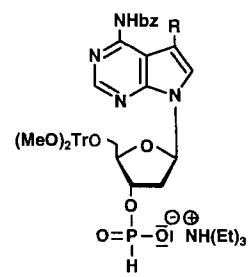
purine numbering

2a R = H
b R = Br
c R = Me



systematic numbering

3a R = H
b R = Br
c R = Cl
d R = Me



4a R = H
b R = Br
c R = Cl
d R = Me

The inspection of the B-DNA duplex structure shows that 5-substituents of pyrimidine residues which are located in the major groove of B-DNA have steric freedom. A similar situation may occur for 7-substituted purines. However, the N(7) atom of the purine system limits the functionalization by substituents, *e.g.* Me groups, as the molecule

becomes charged after formation of such a derivative. As a consequence, another heterocyclic system has to be chosen, when a DNA should to be functionalized in that position. Pyrrolo[2,3-*d*]pyrimidine nucleosides [5] containing a CH group instead of the N(7) atom are ideal molecules for this type of functionalization.

Various 7-substituted 7-deazapurine ribonucleosides, such as toyocamycin, sangivamycin, queuosine, or archaeosine [6–15] were isolated from natural origin, either as monomeric nucleosides or as constituents of nucleic acids. A 7-substituted nucleoside with a 5′-deoxyribose sugar moiety, namely 7-deaza-5′-deoxy-7-iodoadenosine (= 5′-deoxy-5-iodotubercidin), was isolated from marine red algae [16] and shown to be an inhibitor of adenosine kinase [14]. Moreover, 7-deazapurine oligoribonucleotides as well as oligodeoxyribonucleotides were prepared either enzymatically or by total synthesis [17] [18].

In a previous publication, the synthesis of various 7-substituted 7-deaza-2′-deoxyadenosine derivatives, *i.e.*, **3b–d**, was described [19]. These nucleosides are now used to study the role of substituents located in the major groove of B-DNA. The experiments with such oligonucleotides will show similarities to those with oligomers substituted at C(5) of the pyrimidine moiety. In the following, the synthesis of oligonucleotide building blocks derived from 7-Br- (**3b**), 7-Cl- (**3c**), and 7-Me-substituted (**3d**) 7-deaza-2′-deoxyadenosines will be reported which are then used in solid-phase synthesis of oligonucleotides. The base pairing with complementary dT residues will be studied in homooligonucleotides, alternating oligonucleotides, and palindromic structures.

Results and Discussion. – *Building Blocks* **4b–d**. The 7-deaza-2′-deoxyadenosine derivatives **3b–d** were prepared as described earlier [19]. The benzoyl (bz) residue was chosen to protect the NH_2 groups. Compounds **5b–d** were prepared using the protocol of transient protection [20] and were isolated crystalline (60–70% yield; *Scheme*). To compare the stabilities of the protecting groups of **5b–d**, the latter were hydrolyzed in 25% aq. NH_3 solution. The half-life values were determined UV-spectrophotometrically at the maximum difference of wavelength between educts and products. According to *Table 1*, compounds **5a**, **c**, **d** are more difficult to deprotect than bz^6A_d. Only **5b** has a half-life value similar to that of bz^6A_d. Since oligodeoxyribonucleotides are not sensitive towards harsh ammonia treatment, the synthesis was continued with these N^6-benzoyl derivatives.

Scheme

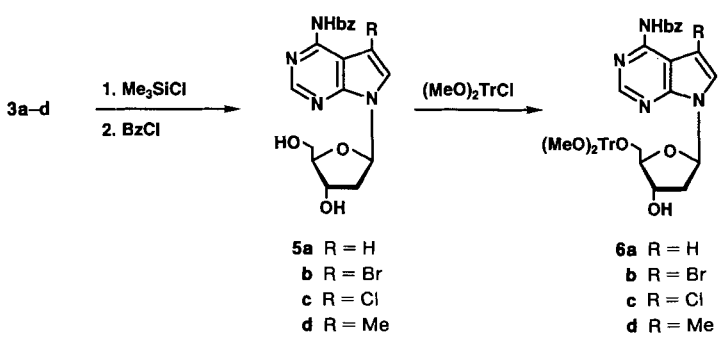


Table 1. *Half-Life Values ($t_{1/2}$) of Debenzoylation of Pyrrolo[2,3-d]pyrimidine 2′-Deoxyribonucleosides in 25% aq. NH_3 Solution*[a])

	λ [nm]	$t_{1/2}$ [min]		λ [nm]	$t_{1/2}$ [nm]
bz^6A_d [21]	295	36	$Me^7bz^6c^7A_d$ (**5d**)	305	60
$bz^6c^7A_d$ (**5a**) [21]	304	65	$fma^6c^7A_d$ [21]	312	10
$Br^7bz^6c^7A_d$ (**5b**)	305	40	$Br^7fma^6c^7A_d$	317	40[b])
$Cl^7bz^6c^7A_d$ (**5c**)	305	60			

[a]) At 60°. [b]) At 50°.

Nevertheless, a benzoyl-protected 7-deazaadenine moiety should not be used during oligoribonucleotide synthesis [22]. Subsequently, the 4,4′-dimethoxytriphenylmethyl ($(MeO)_2Tr$) group was introduced under standard conditions to block the 5′-OH group [23]. Compounds **6b–d** were isolated in yields of 75–80%. From those, the phosphonates **4b–d** were prepared using PCl_3/*N*-methylmorpholine/1*H*-1,2,4-triazole [23].

Table 2. *^{13}C-NMR Chemical Shifts of Pyrrolo[2,3-d]pyrimidine Nucleosides*[a])

	C(2)[b]) C(2)[c])	C(6)[b]) C(4)[c])	C(5)[b]) C(4a)[c])	C(7)[b]) C(5)[c])	C(8)[b]) C(6)[c])	C(4)[b]) C(7a)[c])	Me	C=O	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)
3a	151.3	157.3	102.8	99.4	121.4	149.5		–	85.8	[d])	70.8	87.8	61.7
b	152.5	157.0	101.0	86.8	121.5	149.3		–	83.0	[d])	71.0	87.5	62.0
c	152.7	156.8	99.9	102.8	119.0	148.8		–	83.0	[d])	71.0	87.5	62.0
d	151.4	157.9	102.7	109.8	119.0	150.3	11.9	–	82.6	[d])	71.1	87.1	62.2
bz^6A_d	151.5	150.3	125.9	–	143.0	151.9		165.7	83.7	[d])	70.7	88.0	61.6
5a	150.0	151.0	109.4	103.1	124.3	152.0		165.7	82.9	[d])	70.9	87.3	61.9
b	[e])	151.4	110.9	88.3	126.3	150.7		168.3	83.0	[d])	70.8	87.6	61.7
c	151.3	151.0	110.0[f])	104.0[f])	123.7	150.3		167.5	83.0	[d])	70.8	87.6	61.7
d	150.4	151.2[f])	110.8[f])	113.9[f])	124.0	152.3[f])	11.2	167.2	82.5	[d])	71.1	87.4	62.1
6a	150.9	152.0	109.3	103.0	123.9	149.9			82.7	[d])	70.6	85.2	63.9
b	151.9[g])	151.9[g])	110.8	88.3	126.6	151.9[g])			83.0	[d])	70.6	85.5	64.1
c	151.4	151.6	110.1	104.1	124.0	150.7			83.1	[d])	70.7	85.8	64.2
d	[e])	[e])	110.8[f])	113.9[f])	124.0	[e])	11.1		82.5	[d])	70.9	85.5	64.2
4a	150.5	151.4	109.6	103.6	124.3	152.4			83.0	[d])	73.0	84.5	64.0
b	151.7[g])	151.0[g])	113.3	88.9	126.2	151.0[g])			83.3	[d])	72.9	84.9	64.0
c	151.4	[e])	[e])	104.0	124.0	[e])			83.2	[d])	72.9	85.7	64.0
d	150.6[g])	151.6[g])	110.8[f])	113.0[f])	123.6	152.0[g])	11.1		82.7	[d])	72.9	84.4	64.0

[a]) Measured in (D_6)DMSO at 25°. [b]) Purine numbering. [c]) Systematic numbering. [d]) Superimposed by DMSO. [e]) Not detectable. [f]) Tentative. [g]) Very broad signals.

All compounds were characterized by ^1H- and ^{13}C-NMR spectra (*Table 2*). The assignment of the ^{13}C-signals of the 7-substituted 7-deaza-2′-deoxyadenosine derivatives **3a–d** and **5a–d** resulted from the splitting patterns of the gated-decoupled ^{13}C-NMR spectra (*Table 3*). It can be seen that the C(7) signal of the 7-deaza-2′-deoxyadenosine (**3a**) is shifted upfield upon bromination (**3b**) and downfield when a Cl or Me group is introduced (**3c**, **d**). In the case of the halo compounds, the signal of the bridgehead atom C(5) is also shifted. Benzoylation affects all signals of the base moiety. A few signals appear as broad peaks. This behavior might be explained by tautomeric equilibria.

Table 3. *J(C,H) Values* [Hz] *of Pyrrolo[2,3-d]pyrimidine 2'-Deoxyribonucleosides*[a])

	3a	3b	3c	3d		3a	3b	3c	3d
J(C(2),H–C(2))	197	199	199	197	J(C(5),H–C(6))	7.9	–	–	–
J(C(2),H–C(4))	10.8	11	11.0	11.1	$J(CH_3,C(6))$	–	–	–	5.2
J(C(5),H–C(5))	168	–	–	–	J(C(6),H–C(4a))	7.6	5.7	5.3	6.6
J(C(6),H–C(5))	7.0	3.3	2.9	6.3	$J(NH_2,C(4a))$	4.0	–	1.1	3.1
$J(CH_3,C(5))$	–	–	–	6.5	J(C(2),H–C(7a))	10.6	9.0	11.2	11.2
J(C(6),H–C(6))	188	194	194	186	J(C(6),H–C(7a))	2.4	2.9	1.4	2.5
J(C(1'),H–C(6))	4.4	4.7	4.8	5.4	J(C(1'),H–C(7a))	7.0	7.0	7.4	7.9

[a]) Data taken from measurements in (D_6)DMSO at 25°. Systematic numbering.

The UV spectra of the benzoylated compounds show peculiarities which point to various tautomers formed in solvents of different polarity (*Fig. 1*). While bz^6A_d exhibits an UV maximum at 275 nm (H_2O), the maximum of compound **5a** is shifted bathochromically to 305 nm. The parent nucleosides show a much smaller difference (dA 260 nm, **3a** 270 nm). On the other hand, the bromo (**5b**) as well as the methyl compound **5d** in H_2O exhibit two maxima around 275 and 310 nm, respectively. These maxima show different intensities in H_2O and in dioxane. Also the long-wavelength maximum of **5d** is shifted bathochromically in dioxane (*Fig. 1*). Neither bz^6A_d nor $bz^6c^7A_d$ show these changes. It was suggested that benzoylated adenine residues form different tautomeric species [24] in aprotic and protic solvents, with preference of the lactam form in the protic and the lactim form in the aprotic solvent. This may be also considered for compounds **5b** and **5d**.

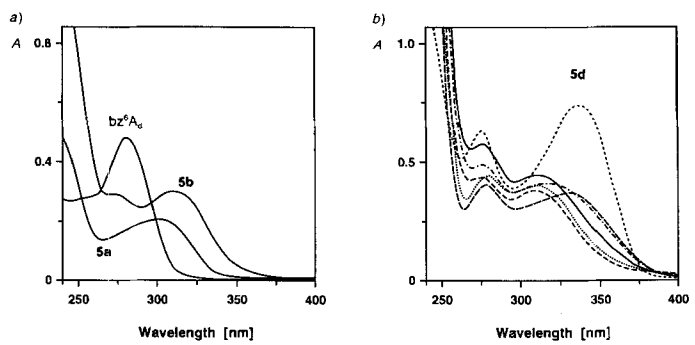


Fig. 1. *UV Spectra of compounds* **5a**, **b**, **d** *and* bz^6A_d: a) bz^6A_d, **5a**, *and* **5b** *in* H_2O *and* b) **5d** *in dioxane* (------), *dioxane*/H_2O *8:2* (————), *dioxane*/H_2O *6:4* (–·–·–), *dioxane*/H_2O *4:6* (·····), *dioxane*/H_2O *2:8* (– – –), *and* H_2O (———)

Oligonucleotide Synthesis. The phosphonates **4a–d** as well as those of the regular DNA constituents were employed in automated oligonucleotide synthesis. The synthesis followed a protocol of phosphonate chemistry which was published recently [25]. The oligonucleotides were recovered from the polymer support, deprotected, and purified as described [26]. Thus, the oligonucleotides **7–25** were synthesized.

d(A_{12}) (**7**)
d[(c^7A)$_{11}$-A] (**8**)
d[(Br^7c^7A)$_{11}$-A] (**9**)
d[(Me^7c^7A)$_{11}$-A] (**10**)
d(T_{12}) (**11**)
d[(A-T)$_6$] (**12**)
d[(c^7A-T)$_6$] (**13**)
d[(Br^7c^7A-T)$_6$] (**14**)
d[(Cl^7c^7A-T)$_6$] (**15**)
d[(Me^7c^7A-T)$_6$] (**16**)

d(G-T-A-G-A-A-T-T-C-T-A-C) (**17**)
d(G-T-A-G-Br^7c^7A-A-T-T-C-T-A-C) (**18**)
d(G-T-A-G-A-Br^7c^7A-T-T-C-T-A-C) (**19**)
d(G-T-A-G-Br^7c^7A-Br^7c^7A-T-T-C-T-A-C) (**20**)
d(G-T-Br^7c^7A-G-A-A-T-T-C-T-A-C) (**21**)
d(G-T-A-G-A-A-T-T-C-T-Br^7c^7A-C) (**22**)
d(G-T-A-G-Me^7c^7A-A-T-T-C-T-A-C) (**23**)
d(G-T-A-G-A-Me^7c^7A-T-T-C-T-A-C) (**24**)
d(G-T-A-G-Me^7c^7A-Me^7c^7A-T-T-C-T-A-C) (**25**)

Their nucleoside composition was established from HPLC profiles obtained after hydrolysis with snake-venom phosphodiesterase followed by alkaline phosphatase. The hydrophobic behavior of 7-deazapurine nucleosides **3a–d** as well as of the 'homooligomers' **8–10** resulted in an increased retention time compared to dA and d(A_{12}) (**7**), respectively, as seen in *Fig. 2*.

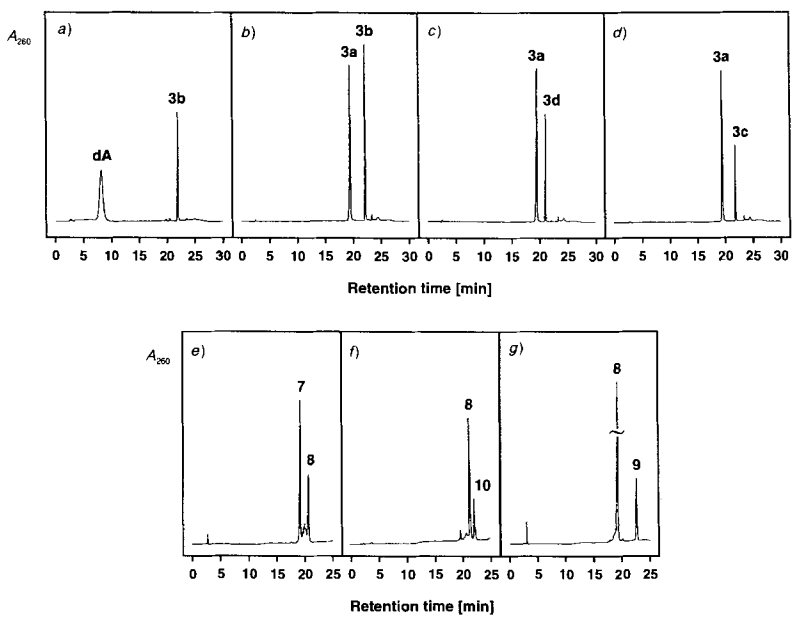


Fig. 2. *HPLC Profiles of the mixtures of the nucleosides* (gradient III) a) *dA*/**3b**, b) **3a**/**3b**, c) **3a**/**3d**, *and* d) **3a**/**3c**, *and of the mixtures of the 'homooligonucleotides'* (gradient II), e) **7**/**8**, f) **8**/**10**, *and* g) **8**/**9**

Duplex Stability of Oligonucleotides. Studies of synthetic oligonucleotides contributed to a large extent to our current understanding of the properties of DNA. Oligo- and polynucleotides containing (A-T)$_n$ base pairs received special attention. The structure of poly(dA)·poly(dT) differs from that of random B-DNA. Experiments showed that there is a bent in the helix axis and a certain stiffness of the A tract [27] [28]. A cross-strand

bifurcated H-bond between the NH_2 group of adenine and the C(4)=O of thymine was suggested [29]. These bonds cannot be formed in alternating structures. The alternating poly[d(A-T)]·poly[d(A-T)] is much more flexible and has an alternating structure with a small helical twist of the d(A-T) steps and a larger one at the d(T-A) steps [30] [31]. This results in a polymorphic structure which can adopt more than one conformation depending on the environment.

Small pyrimidine 5-substituents stabilize both, the duplexes of homopolynucleotides as well as those of alternating polynucleotides. The T_m value of poly(dU)·poly(dA) was found to be 55° (1M NaCl). It was increased by the introduction of a 5-Me group (poly(dT)·poly(dA), T_m 89°) [32] [33]. Smaller effects were found in the case of alternating polynucleotides. The T_m value of poly[d(A-T)] (60°, 0.1M NaCl) was only four degrees higher than that of poly[d(A-U)] (56°, 0.1M NaCl) [16] [17]. Replacement of the 5-Me group by a Br-substituent stabilized the duplex further (poly[d(A-Br^{5}U)]·poly-[d(A-Br^{5}U)], T_m 70° (0.1M NaCl)).

The inspection of a B-DNA duplex structure shows that small pyrimidine 5-substituents have a considerable steric freedom in the major groove of B-DNA. However, if their size is increased, *e.g.* by the introduction of i-Pr residues, a structural change and a decrease of the T_m value was observed [34]. It was proposed on the basis of computational studies of dA tracts that the proximity of the thymine Me groups in poly(dA)·poly(dT) facilitates hydrophobic and/or stacking interactions between the bases. These interactions are reduced if the substituents alternate between the strands as in poly[d(A-T)]·poly[d(A-T)]. Consequently, the stability of poly(dA)·poly(dT) is more affected by the absence of Me groups as poly[d(A-T)]. As already mentioned, a similar situation should exist in 7-substituted 7-deazapurines (pyrrolo[2,3-*d*]pyrimidines).

It was reported that poly(c^{7}A)·poly(U) (T_m 43°) has a lower duplex stability than poly(A)·poly(U) (T_m 61°) [35]. A similar observation was made by comparing the melting profiles of the duplexes d[(c^{7}A)$_{11}$-A]·d(T$_{12}$) (**8**·**11**) with d(A$_{12}$)·d(T$_{12}$) (**7**·**11**) (*Fig. 3*). From the melting curves of the oligonucleotides, the T_m values were determined and the thermodynamic parameters ΔH, ΔS, and ΔG^0 calculated using the two-state model for helix-coil transition [36–38] (*Table 4*). As it can be seen, the ΔH value of **8**·**11** (−93 kcal/mol) is similar to that of **7**·**11** (−89 kcal/mol). The destabilization of the duplex structure of the 7-deazapurine-containing oligonucleotide is mainly driven by the unfavorable reaction entropy.

In principle, the reaction entropy of short double-helix formations from single strands is unfavorable, as two single strands form only one double strand. During this process, the rotation as well as the translation energy of two single strands are reduced. This unfavorable situation is compensated by a favorable entropy term which has its origin in the release of H_2O from the polar groups of the two single strands upon duplex formation. If the base moieties of a single stranded d(A$_{12}$) (**7**) are inspected, H_2O molecules can be associated with the NH_2 groups as well as with N(1) and N(7). It is apparent that the H_2O which is located in positions used for base pairing has to be removed during duplex formation. It can also be assumed that the H_2O molecules bound to N(7) are expelled from the major groove, at least in part. The situation is different in the case of d[(c^{7}A)$_{11}$-A] (**8**). Here, less H_2O is bound to the single strand as N(7) is absent and the molecule has lost one acceptor site. This will result in a less favorable gain of entropy during duplex formation of oligonucleotides containing 7-deazapurines com-

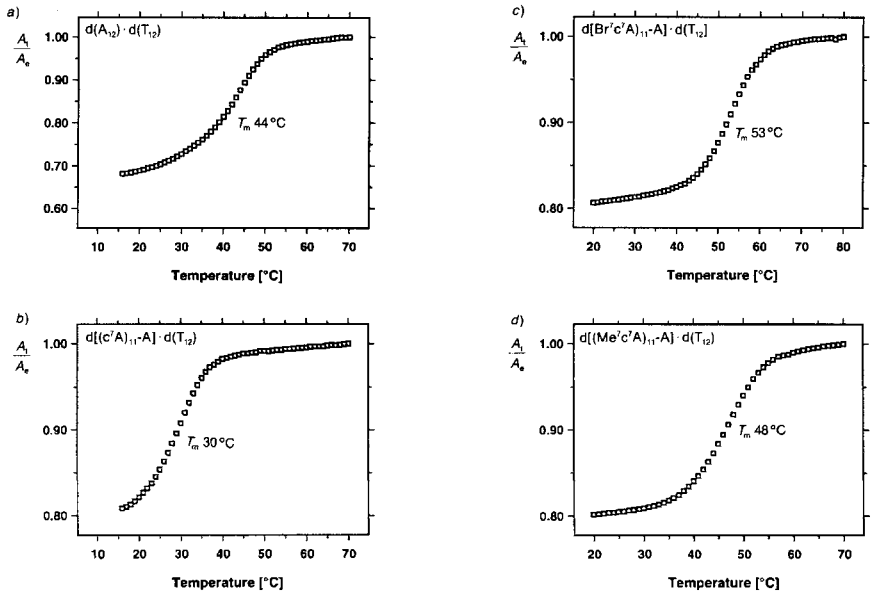


Fig. 3. *Normalized melting profiles of the homooligonucleotide duplexes* a) **7·11**, b) **8·11**, c) **9·11**, *and* d) **10·11**. Measured ar 260 nm in 1M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.1) at 7.5 μM single-strand concentration.

Table 4. T_m *Values and Thermodynamic Parameters of Oligonucleotide Melting*[a]) *of the 'Homooligomers'* **7·11**, **8·11**, **9·11**, *and* **10·11**

Oligonucleotide		T_m [°C][b])	ΔH [kcal/mol]	ΔS [cal/mol·K]
$d(A_{12})\cdot d(T_{12})$	**7·11**	44	– 89	– 281
$d[(c^7A)_{11}\text{-}A]\cdot d(T_{12})$	**8·11**	30	– 93	– 309
$d[(Br^7c^7A)_{11}\text{-}A]\cdot d(T_{12})$	**9·11**	53	– 114	– 349
$d[(Me^7c^7A)_{11}\text{-}A]\cdot d(T_{12})$	**10·11**	48	– 105	– 331

[a]) Oligomer concentration, 7.5 μM of single strands.
[b]) Measured in 1M NaCl containing 60 mM Na-cacodylate, 100 mM $MgCl_2$, pH 7.1.

pared to purines resulting in a more unfavorable total entropy change, which is actually the case (*Table 4*). This situation becomes even more pronounced when the 7-deaza-purine moiety carries hydrophobic Me or Br substituents at C(7) (*Table 4*).

According to *Table 4*, the stabilization of the duplexes $d[(Br^7c^7A)_{11}\text{-}A]\cdot d(T_{12})$ (**9·11**) or $d[(Me^7c^7A)_{11}\text{-}A]\cdot d(T_{12})$ (**10·11**) is due to the large reaction enthalpy. This change results from the formation of H-bonds as well as of stacking interactions. In the case of $d[(Me^7c^7A)_{11}\text{-}A]\cdot d(T_{12})$, both strands carry a spine of Me groups within the major groove. Both spines can contribute to the stacking of the duplex. As a consequence, the duplex $d[(Me^7c^7A)_{11}\text{-}A]\cdot d(T_{12})$ (T_m 48°) is much more stable than that of $d[(c^7A)_{11}\text{-}A]\cdot d(T_{12})$ showing a T_m value of only 30°. The stabilizing influence of the Me groups can compensate the unfavorable entropy change which is caused by the 7-deazaadenine base in comparison to parent adenine. The T_m value is further increased if a 7-Br substituent is

replacing the Me group. In the case of d[(Br^{7}c^{7}A)$_{11}$-A]·d(T$_{12}$), the T_m value of the duplex is already 9° higher than that of d(A$_{12}$)·d(T$_{12}$) (*Table 4*).

The situation with regard to the reaction entropy of the alternating oligonucleotide d[(c^{7}A-T)$_6$]·d[(c^{7}A-T)$_6$] (**13·13**) is similar to that of the 'homooligomers'. Nevertheless, a favorable reaction enthalpy increases the T_m value of the 7-deazaadenine duplex already above the parent d[(A-T)$_6$]·d[(A-T)$_6$] (**12·12**; *Fig. 4*, *Table 5*). These data are supported

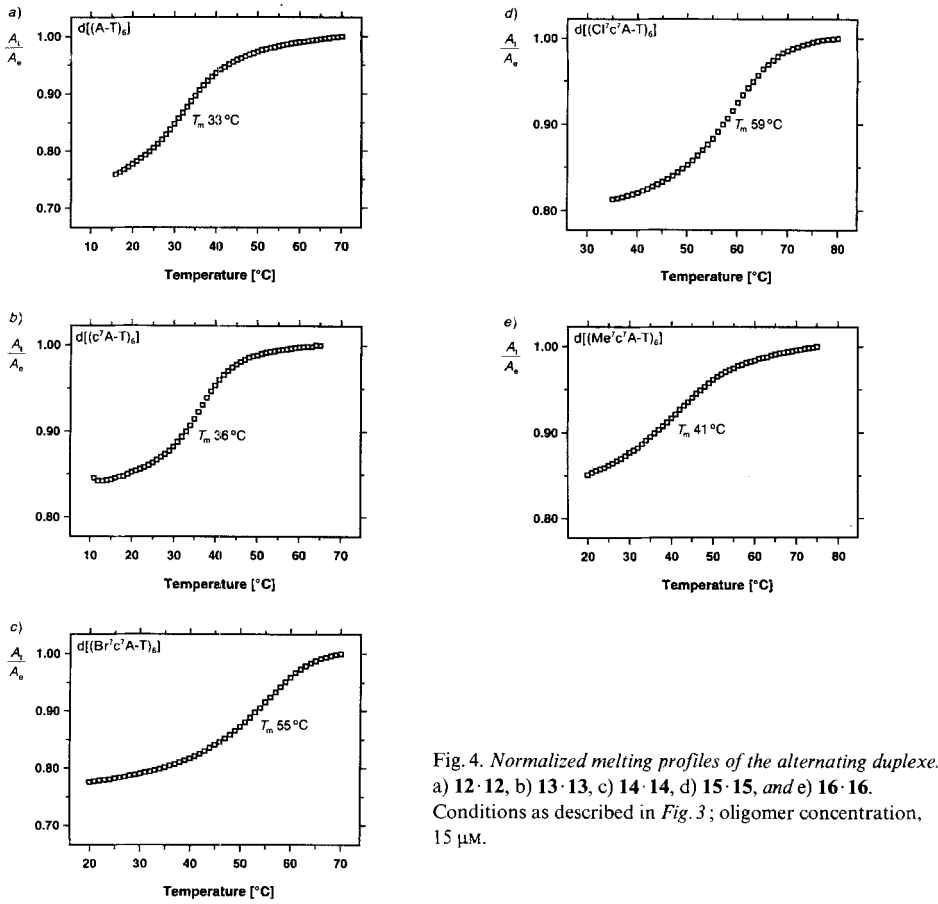


Fig. 4. *Normalized melting profiles of the alternating duplexes* a) **12·12**, b) **13·13**, c) **14·14**, d) **15·15**, *and* e) **16·16**. Conditions as described in *Fig. 3*; oligomer concentration, 15 μM.

Table 5. T$_m$ *Values and Thermodynamic Data of the Alternating Oligonucleotide Duplexes of* **12–16**[a])

Oligonucleotide		T_m [°C][b])	ΔH [kcal/mol]	ΔS [cal/mol·K]
d[(A-T)$_6$]·d[(A-T)$_6$]	**12·12**	33	−67	−219
d[(c^{7}A-T)$_6$]·d[(c^{7}A-T)$_6$]	**13·13**	36	−84	−271
d[(Br^{7}c^{7}A-T)$_6$]·d[(Br^{7}c^{7}A-T)$_6$]	**14·14**	55	−60	−184
d[(Cl^{7}c^{7}A-T)$_6$]·d[(Cl^{7}c^{7}A-T)$_6$]	**15·15**	59	−72	−218
d[(Me^{7}c^{7}A-T)$_6$]·d[(Me^{7}c^{7}A-T)$_6$]	**16·16**	41	−45	−142

[a]) Oligomer concentration, 15 μM of single strands.
[b]) Measured in 1M NaCl containing 60 mM Na-cacodylate and 100 mM $MgCl_2$, pH 7.1.

by the very similar values of poly[d(c^{7}A-T)]·poly[d(c^{7}A-T)] compared to poly[d(A-T)]·poly[d(A-T)] [17]. As discussed earlier, the T_m differences vary with different salt concentrations [39]. The T_m values of the alternating oligomers containing the 7-substituted 7-deazaadenosines (**14**·**14**, **15**·**15**, **16**·**16**) are further increased. However, the thermodynamic situation is now quite different. In these cases, the duplex stabilization is driven by the favorable reaction entropies. The enthalpic therms are in disfavor of duplex formation. As discussed above, the alternating d[(A-T)$_n$] has an extraordinary structure being more flexible than that of the homooligomers with a small helical twist of the d(A-T) steps and a large one of the d(T-A) units [30] [31].

The CD spectra of the duplexes of the oligomers **7**–**10** with d(T$_{12}$) (**11**) are quite different from those of the monomers, showing only small negative *Cotton* effects. The duplexes show characteristics of a B-DNA with a positive B_{2u} transition as well as a negative B_{1u} transition. The CD spectra were measured as a function of temperature between 10° and 70° (*Fig. 5*). The T_m values obtained from temperature-dependent ellipticities of the B_{1u} transitions (*Fig. 6*) were identical with the T_m values determined from the UV spectra (*Fig. 3*). Inspection of the temperature-dependent CD spectra exhibit some peculiarities: In the case of d(A$_{12}$)·d(T$_{12}$) (**7**·**11**), an isoelliptic point (262 nm) is observed over the whole temperature range. The positive *Cotton* effect at 257 nm which appears at low temperature is reduced upon heating. All other 'homooligomer' duplexes show different behavior. Upon heating up to the melting temperature, the CD spectra display a continuous shift with no isoelliptic point in the long-wavelength region (> 260 nm). Above the melting temperature, the CD spectra of the 'homooligonucleotides' display isoelliptic points between 260 and 290 nm, indicating a simple single-strand stack/unstack two-state equilibrium. Interestingly, d[(c^{7}A)$_{11}$-A]·d(T$_{12}$) (**8**·**11**) shows an inversion of the

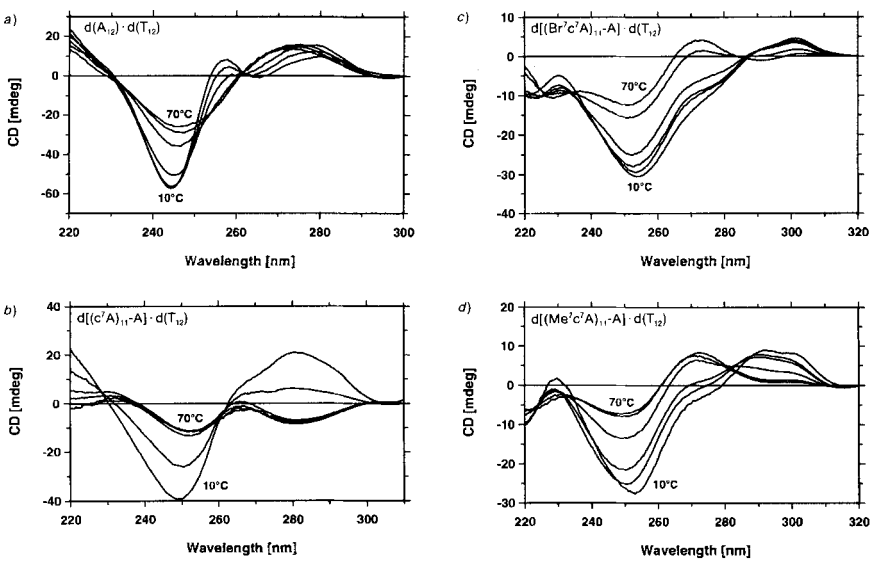


Fig. 5. *Temperature-dependent CD spectra of the duplexes* a) *d(A$_{12}$)·d(T$_{12}$)* (**7**·**11**), b) *d[(c^{7}A)$_{11}$-A]·d(T$_{12}$)* (**8**·**11**), c) *d[(Br^{7}c^{7}A)$_{11}$-A]·d(T$_{12}$)* (**9**·**11**), *and* d) *d[(Me^{7}c^{7}A)$_{11}$-A]·d(T$_{12}$)* (**10**·**11**). Measured at 10° and 30–70° at 7.5 μM single-strand concentration. Conditions as in *Fig. 3*.

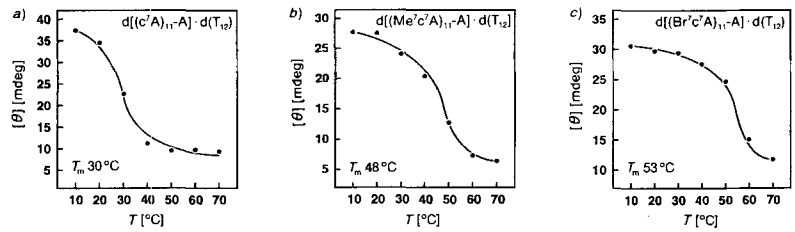


Fig. 6. *Temperature-dependent ellipticities* [Θ] *of the* B_{1u} *transitions of the 'homooligonucleotide' duplexes* a) *d[(c⁷A)₁₁-A]·d(T₁₂)* (**7·11**), b) *d[(Me⁷c⁷A)₁₁-A]·d(T₁₂)* (**10·11**), *and* c) *d[(Br⁷c⁷A)₁₁-A]·d(T₁₂)* (**9·11**). Oligomer single-strand concentration, 7.5 μM°, buffer, see *Fig. 3*.

positive B_{2u} transition (282 nm) to a negative *Cotton* effect upon heating. Moreover, the strong negative B_{1u} transition (249 nm) is reduced to *ca.* 30% of its intensity.

Apart from the complete replacement of adenine residues by 7-deazaadenine derivatives, the substitution of one or two adenine residues by 7-bromo-7-deazaadenine or 7-methyl-7-deazaadenine within the sequence d(G-T-A-G-A-A-T-T-C-T-A-C) was also studied. We have selected this oligonucleotide as it is self-complementary and contains the recognition site of the endodeoxyribonuclease Eco RI [40]. As it can be seen from *Table 6*, these replacements increase the T_m value already slightly above the dA-containing duplex **17·17**. This is different to the oligomer **17** modified by c^7A_d residues. The corresponding modified duplex is slightly less stable than the unmodified one [40]. The

Table 6. T_m *Values and Thermodynamic Data of Palindromic Oligonucleotides* **17–25**[a])

Oligonucleotide[b])		T_m [°C][c])	ΔH [kcal/mol]	ΔS [cal/mol·K]
[d(G-T-A-G-A-A-T-T-C-T-A-C)]$_2$	**17·17**	51	– 94	– 291
[d(G-T-A-G-Br⁷c⁷A-A-T-T-C-T-A-C)]$_2$	**18·18**	51	– 110	– 340
[d(G-T-A-G-A-Br⁷c⁷A-T-T-C-T-A-C)]$_2$	**19·19**	55	– 112	– 343
[d(G-T-A-G-Br⁷c⁷A-Br⁷c⁷A-T-T-C-T-A-C)]$_2$	**20·20**	53	– 109	– 333
[d(G-T-Br⁷c⁷A-G-A-A-T-T-C-T-A-C)]$_2$	**21·21**	50	– 121	– 373
[d(G-T-A-G-A-A-T-T-C-T-Br⁷c⁷A-C)]$_2$	**22·22**	54	– 104	– 315
[d(G-T-A-G-Me⁷c⁷A-A-T-T-C-T-A-C)]$_2$	**23·23**	50	– 103	– 320
[d(G-T-A-G-A-Me⁷c⁷A-T-T-C-T-A-C)]$_2$	**24·24**	53	– 116	– 357
[d(G-T-A-G-Me⁷c⁷A-Me⁷c⁷A-T-T-C-T-A-C)]$_2$	**25·25**	50	– 114	– 354

[a]) Oligomer concentration, 13 μM of single strands.
[b]) For convenience, the dots for association of the chains are omitted.
[c]) Measured in 1M NaCl, 100 mM $MgCl_2$, 60 mM Na-cacodylate buffer, pH 7.1.

thermodynamic data of the duplexes of the palindromic oligonucleotides **17–25** show similarities to those of the homooligonucleotide duplexes. The reaction entropy is unfavorable in all oligonucleotides containing 7-substituted 7-deazaadenines. But this situation is compensated by the more favorable enthalpies.

In conclusion, it was shown for the first time that Me groups as well as Br or Cl substituents introduced at the 7-position of 7-deazapurine-containing oligonucleotides stabilize the oligonucleotide duplex structure; the unsubstituted 7-deazapurines destabi-

lize the duplex compared to the parent oligomers containing purines. In alternating d[(A-T)$_n$] duplexes, the destabilization by c^7A_d is small and can lead even to stabilization under high salt concentration conditions. The duplex stabilization of 7-deazapurine residues carrying not too bulky substituents at the 7-position leads to duplex structures which are even much more stable than the parent purine-containing DNA fragments.

In general, the Me or halo substituents introduced at the 7-position of 7-deazapurines cause changes similar to those of 5-substituted pyrimidines. Also, other not too bulky 7-substituents including linking moieties to reporter groups (fluorescence labels, intercalators, or chemically or enzymatically acting residues *etc.*; data not shown) stabilize the DNA duplex structure.

Experimental Part

General. See [26]. Oligonucleotide synthesis is carried out on a DNA synthesizer, model *381A* (*Applied Biosystems*, Weiterstadt, Germany), on a 1-μmol scale using the phosphonate cycle given in *Table 7*.

Table 7. *Oligonucleotide Synthesis*

Step	Reagents and solvents	Time [s]
A. *Coupling Procedure*		
1. Detritylation	2.5% $Cl_2CHCOOH$ in CH_2Cl_2	5 × 20
2. Wash	MeCN	2 × 20
	MeCN/pyridine 1:1	1 × 15
	MeCN	1 × 20
3. Condensation	0.04M phosphonate and 0.2M pivaloyl chloride both in	
	MeCN/pyridine 1:1	1 × 20
	wait	1 × 40
4. Wash	MeCN/pyridine 1:1	2 × 15
	MeCN	1 × 20
5. Capping	0.1M triisopropyl phosphite in MeCN/pyridine 1:1	1 × 20
	wait	1 × 30
6. Wash	MeCN/pyridine 1:1	1 × 15
	MeCN	1 × 20
B. *Manual Oxidation*		
1.	Oxidizer I: a 1:1 mixture (2 ml)[a]) of 0.2M I_2 in THF and *N*-methylmorpholine/H_2O/THF 1:1:8	10 × 5
	wait	1 × 150
2.	Oxidizer II: a 1:1 mixture (2 ml)[a]) of 0.2M I_2 in THF and Et_3N/H_2O/THF 1:1:8	10 × 5
	wait	1 × 150
3. Wash	2% Et_3N in MeCN (2 ml)	10 × 5

[a]) Mixed before use.

Chromatography. Flash chromatography (FC) and TLC: petroleum ether/AcOEt 4:1 (*A*), CH_2Cl_2/MeOH 9:1 (*B*), CH_2Cl_2/MeOH/Et_3N 88:10:2 (*C*), CH_2Cl_2/Et_3N 98:2 (*D*). Prep. HPLC: 250 × 4 mm *RP-18* column; *Merck-Hitachi* HPLC; gradients of 0.1M (Et_3NH)OAc (pH 7.0)/MeCN 95:5 (*A*) and MeCN (*B*); gradient *I*: 3 min 15% *B* in *A*, 7 min 15–40% *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*: 12 min 100% *A*, 8 min 0–40% *B* in *A*, 5 min 40–0% *B* in *A*, 5 min 100% *A*, flow rate 1 ml/min.

Enzymatic Hydrolysis of the Oligomers and Determination of the Hypochromicity. Hydrolysis was carried out as described [26]. The mixture was analyzed on reversed-phase HPLC (*RP-18*, gradient *III*). Quantification of the constituents was made on the basis of the peak areas, which were divided by the extinction coefficients of the nucleoside (ε_{260} values: A_d 15400, C_d 7300, G_d 11400, T_d 8800, $Br^7c^7A_d$ 5300, $Me^7c^7A_d$ 4900, and $Cl^7c^7A_d$ 6300). Hypochromicity values were determined by enzymatic digestion of 0.2 A_{260} units of the corresponding oligonucleotides as described in [26].

Melting Experiments. Experiments were carried out with a *Cary 1E* UV-VIS spectrophotometer (*Varian*, Melbourne, Australia) according to [41]. The temperature was increased by 60°/h. The evaluation of the melting curves was made as follows: data pairs were transformed into an ASCII code and displayed graphically using the graphic program *Fig.P* (version 1.2a, 1993, *Fig.P Software Corp.*, Durham, NC, USA).

*4-(Benzoylamino)-5-bromo-7-(2-deoxy-β-*D-erythro-*pentofuranosyl)-7H-pyrrolo[2,3-*d*]pyrimidine* (**5b**). The 5-bromo-2′-deoxytubercidin (**3b**) [19] (1.31 g, 4.0 mmol) was dried by co-evaporation with anh. pyridine, dissolved in pyridine (5 ml), and treated with Me_3SiCl (5.2 ml, 40.6 mmol) under stirring at r.t. After 30 min, benzoyl chloride (520 µl, 4.1 mmol) was added and the mixture kept at r.t. for 2 h. The mixture was cooled to 0°, diluted with H_2O (5 ml), and after 10 min treated with 25% aq. NH_3 soln. (8 ml, 30 min, r.t.). The soln. was evaporated and the residue dissolved in H_2O (20 ml), extracted with AcOEt, and applied to a silica gel *60* column (20 × 5 cm, solvent *B*): pale yellow crystals (1.2 g, 69%). M.p. 198° (from MeOH/H_2O). TLC (silica gel, *B*): R_f 0.4. ^{1}H-NMR ((D$_6$)DMSO): 2.27 (*m*, H_α–C(2′)); 2.50 (*m*, H_β–C(2′)); 3.56 (*m*, 2 H–C(5′)); 3.86 (*m*, H–C(4′)); 4.38 (*m*, H–C(3′)); 5.01 (*t*, OH–C(5′)); 5.34 (*d*, OH–C(3′)); 6.69 (*dd*, *J* = 6.7, H–C(1′)); 7.58 (*m*, 3 arom. H); 8.04 (*d*, 2 arom. H); 8.04 (*s*, H–C(6)); 8.72 (*s*, H–C(2)); 11.0 (br., NH–C(4)). Anal. calc. for $C_{18}H_{17}BrN_4O_4$ (433.27): C 49.90, H 3.95, N 12.93; found: C 50.05, H 4.10, N 13.05.

*4-(Benzoylamino)-5-chloro-7-(2-deoxy-β-*D-erythro-*pentofuranosyl)-7H-pyrrolo[2,3-*d*]pyrimidine* (**5c**). The 5-chloro-2′-deoxytubercidin (**3c**) [19] (1.14 g, 4.0 mmol) was benzoylated analogously to **3b**. After FC, **5c** was isolated from the slower migrating zone as a light-yellow solid (0.93 g, 60%). M.p. 190° (from MeOH/H_2O). TLC (silica gel, *B*): R_f 0.4. ^{1}H-NMR ((D$_6$)DMSO): 2.31 (*m*, H_α–C(2′)); 2.57 (*m*, H_β–C(2′)); 3.58 (*m*, 2 H–C(5′)); 3.89 (*m*, H–C(4′)); 4.41 (*m*, H–C(3′)); 5.00 (*t*, OH–C(5′)); 5.33 (*d*, OH–C(3′)); 6.72 (*dd*, *J* = 6.7, H–C(1′)); 7.44–7.65 (*m*, 3 arom. H); 8.00 (*s*, H–C(6)); 8.05 (*d*, 2 arom. H); 8.72 (*s*, H–C(2)); 11.2 (br., NH–C(4)). Anal. calc. for $C_{18}H_{17}ClN_4O_4$ (388.8): C 55.61, H 4.41, N 14.41; found: C 55.71, H 4.54, N 14.3.

*4-(Benzoylamino)-7-(2-deoxy-β-*D-erythro-*pentofuranosyl)-5-methyl-7H-pyrrolo[2,3-*d*]pyrimidine* (**5d**). The 5-methyl-2′-deoxytubercidin (**3d**) [19] (1.06 g, 4.0 mmol) was benzoylated analogously to **3b**. After FC, **5d** was obtained from the slow migrating main zone as a light-yellow solid (1.07 g, 73%). M.p. 196° (from MeOH/H_2O). TLC (silica gel, *B*): R_f 0.3. ^{1}H-NMR ((D$_6$)DMSO): 2.09 (*m*, H_α–C(2′)); 2.21 (*s*, Me–C(5)); 2.50 (*m*, H_β–C(2′)); 3.53 (*m*, 2 H–C(5′)); 3.83 (*m*, H–C(4′)); 4.36 (*m*, H–C(3′)); 4.97 (*t*, OH–C(5′)); 5.32 (*d*, OH–C(3′)); 6.65 (*dd*, *J* = 6.7, H–C(1′)); 7.53 (*s*, H–C(6)); 7.57 (*m*, 3 arom. H); 8.05 (*d*, 2 arom. H); 8.60 (*s*, H–C(2)); 10.95 (br., NH–C(4)). Anal. calc. for $C_{19}H_{20}N_4O_4$ (368.40): C 61.95, H 5.47, N 15.21; found: C 62.08, H 5.65, N 15.00.

*4-(Benzoylamino)-5-bromo-7-[2-deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D-erythro-*pentofuranosyl]-7H-pyrrolo[2,3-*d*]pyrimidine* (**6b**). A mixture of **5b** (500 mg, 1.15 mmol) in anh. pyridine (20 ml) was stirred under Ar in the presence of 4,4′-dimethoxytriphenylmethyl chloride ($(MeO)_2TrCl$; 585 mg, 1.73 mmol) at r.t. After addition of 5% aq. $NaHCO_3$ soln. (10 ml), the mixture was extracted with CH_2Cl_2 (3 × 20 ml), the combined org. phase dried (Na_2SO_4) and evaporated, and the residue applied to FC (silica gel, column 20 × 5 cm, *B*): **6b** as yellow amorphous foam. It was dissolved in CH_2Cl_2 (5 ml) and poured into hexane (500 ml): yellow amorphous foam (620 mg, 73%). TLC (silica gel, *B*): R_f 0.6. ^{1}H-NMR ((D$_6$)DMSO): 2.30 (*m*, H_α–C(2′)); 2.50 (*m*, H_β–C(2′)); 3.15 (*m*, 2 H–C(5′)); 3.73 (*s*, 2 MeO); 3.98 (*m*, H–C(4′)); 4.42 (*m*, H–C(3′)); 5.40 (*d*, OH–C(3′)); 6.69 (*dd*, *J* = 6.7, H–C(1′)); 6.84 (*m*, 4 H of $(MeO)_2Tr$); 7.2–7.8 (*m*, 12 arom. H); 7.87 (*s*, H–C(6)); 8.06 (*m*, 2 arom. H); 8.70 (*s*, H–C(2)); 11.0 (br., NH–C(4)). Anal. calc. for $C_{39}H_{35}BrN_4O_6$ (735.64): C 63.68, H 4.80, N 7.62; found: C 63.85, H 4.67, N 7.52.

*4-(Benzoylamino)-5-chloro-7-[2-deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D-erythro-*pentofuranosyl]-7H-pyrrolo[2,3-*d*]pyrimidine* (**6c**). As described for **5b**, **5c** (500 mg, 1.29 mmol) was treated with $(MeO)_2TrCl$ (660 mg, 1.95 mmol). FC yielded **6c** (680 mg, 76%). Yellow amorphous solid. TLC (silica gel, *B*): R_f 0.5. ^{1}H-NMR ((D$_6$)DMSO): 2.30 (*m*, H_α–C(2′)); 2.50 (*m*, H_β–C(2′)); 3.15 (*m*, 2 H–C(5′)); 3.73 (*s*, 2 MeO); 3.98 (*m*, H–C(4′)); 4.42 (*m*, H–C(3′)); 5.40 (*d*, OH–C(3′)); 6.69 (*dd*, *J* = 6.7, H–C(1′)); 6.84 (*m*, 4 H of $(MeO)_2Tr$); 7.2–7.8 (*m*, 12 arom. H); 7.87 (*s*, H–C(6)); 8.06 (*m*, 2 arom. H); 8.70 (*s*, H–C(2)); 11.0 (br., NH–C(4)). Anal. calc. for $C_{39}H_{35}ClN_4O_6$ (691.19): C 67.77, H 5.10, N 8.11; found: C 67.70, H 5.05, N 8.19.

*4-(Benzoylamino)-7-[2-deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D-erythro-*pentofuranosyl]-5-methyl-7H-pyrrolo[2,3-*d*]pyrimidine* (**6d**). As described for **5b**, **5d** (500 mg, 1.36 mmol) was tritylated with $(MeO)_2TrCl$ (690 mg, 2.05 mmol). FC yielded **6d** (700 mg, 77%). Yellow amorphous solid. TLC (silica gel, *B*): R_f 0.5. ^{1}H-NMR ((D$_6$)DMSO): 2.08 (*s*, Me–C(5)); 2.30 (*m*, H_α–C(2′)); 2.50 (*m*, H_β–C(2′)); 3.19 (*m*, 2 H–C(5′)); 3.73 (*s*, 2 MeO);

3.97 (*m*, H–C(4′)); 4.44 (*m*, H–C(3′)); 5.39 (*d*, OH–C(3′)); 6.67 (*dd*, *J* = 6.7, H–C(1′)); 6.85 (*m*, 4 H of $(MeO)_2Tr$); 7.58 (*s*, H–C(6)); 7.2–7.8 (*m*, 12 arom. H); 8.06 (*m*, 2 arom. H); 8.60 (*s*, H–C(2)); 10.95 (br., NH–C(4)). Anal. calc. for $C_{40}H_{38}N_4O_6$ (670.77): C 71.63, H 5.71, N 8.35; found: C 71.48, H 5.71, N 8.31.

*4-(Benzoylamino)-5-bromo-7-[2-deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D*-*erythro*-pentofuranosyl]-7*H*-pyrrolo[2,3-d]pyrimidine 3′-(Triethylammonium Phosphonate)* (**4b**). To a soln. of PCl_3 (290 µl, 3.3 mmol) and *N*-methylmorpholine (3.44 g, 34.0 mmol) in anh. CH_2Cl_2 (30 ml), 1*H*-1,2,4-triazole (775 mg, 11.2 mmol) was added under Ar. After stirring for 30 min at r.t., the soln. was cooled to 0°, and **6b** (500 mg, 0.68 mmol) in anh. CH_2Cl_2 (10 ml) was added dropwise within 5 min. After stirring for 10 min at r.t., the mixture was poured into 1M aq. $(Et_3NH)HCO_3$ (TBK, pH 7.5, 30 ml), shaken, and separated. The aq. layer was extracted 3 times with CH_2Cl_2 (10 ml each), the combined org. phase dried (Na_2SO_4) and evaporated, and the colorless foam applied to FC (silica gel, column 20 × 5 cm, 1 l *D*, then *C*). The residue of the main zone was dissolved in CH_2Cl_2 (30 ml) and extracted with 1M aq. $(Et_3NH)HCO_3$ (5 × 20 ml). The org. layer was dried (Na_2SO_4) and evaporated: colorless foam (410 mg, 67%). TLC (silica gel, *C*): R_f 0.7. ^{1}H-NMR ((D_6)DMSO): 1.16 (*t*, 3 *Me*CH_2); 2.50 (*m*, H_α–C(2′)); 2.78 (*m*, H_β–C(2′)); 3.00 (*q*, 3 MeCH_2); 3.22 (*m*, 2 H–C(5′)); 3.73 (*s*, 2 MeO); 4.17 (*m*, H–C(4′)); 4.82 (*m*, H–C(3′)); 6.69 (*dd*, *J* = 6.7, H–C(1′)); 6.68 (*d*, *J* = 588.5, PH); 6.90 (*m*, 4 H of $(MeO)_2Tr$); 7.2–7.7 (*m*, 12 arom. H); 7.86 (*s*, H–C(6)); 8.07 (*m*, 2 arom. H); 8.70 (*s*, H–C(2)); 11.05 (br., NH–C(4)). ^{31}P-NMR ((D_6)DMSO): 1.16 (^1J(P,H) = 588.3, ^3J(P,H) = 8.6).

*4-(Benzylamino)-5-chloro-7-[2-deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D*-*erythro*-pentofuranosyl]-7*H*-pyrrolo[2,3-d]pyrimidine 3′-(Triethylammonium Phosphonate)* (**4c**). As described for **4b**, with **6c** (500 mg, 0.74 mmol), PCl_3 (315 µl, 3.5 mmol), *N*-methylmorpholine (3.75 g, 37.0 mmol), and 1*H*-1,2,4-triazole (0.84 g, 12.3 mmol): colorless foam (445 mg, 72%). TLC (silica gel, *C*): R_f 0.6. ^{1}H-NMR ((D_6)DMSO): 1.16 (*t*, 3 *Me*CH_2); 2.50 (*m*, H_α–C(2′)); 2.74 (*m*, H_β–C(2′)); 3.00 (*q*, 3 MeCH_2); 3.33 (*m*, 2 H–C(5′)); 3.72 (*s*, 2 MeO); 4.15 (*m*, H–C(4′)); 4.78 (*m*, H–C(3′)); 6.66 (*d*, *J* = 585.8, PH); 6.69 (*dd*, *J* = 7.8, H–C(1′)); 6.84 (*m*, 4 H of $(MeO)_2Tr$); 7.2–7.7 (*m*, 12 arom. H); 7.79 (*s*, H–C(6)); 8.04 (*m*, 2 arom. H); 8.69 (*s*, H–C(2)); 10.6 (br., NH–C(4)). ^{31}P-NMR ((D_6)DMSO): 1.16 (^1J(P,H) = 588.3, ^3J(P,H) = 8.6).

*4-(Benzoylamino)-7-[2-deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D*-*erythro*-pentofuranosyl]-5-methyl-7*H*-pyrrolo[2,3-d]pyrimidine 3′-(Triethylammonium Phosphonate)* (**4d**). As described for **4b**, with **6d** (500 mg, 0.75 mmol), PCl_3 (320 µl, 3.6 mmol), *N*-methylmorpholine (3.8 g, 37.5 mmol), and 1*H*-1,2,4-triazole (0.85 g,

Table 8. *Data of Oligonucleotides* **7**–**25**

	A_{260} units	t_R [min][a])	Composition							
			A_d	G_d	T_d	C_d	c^7A_d	$Cl^7c^7A_d$	$Me^7c^7A_d$	$Br^7c^7A_d$
7	8.3	17.7	–	–	–	–	–	–	–	–
8	5.0	19.2	1.0	–	–	–	11.5	–	–	–
9	4.0	22.6	1.0	–	–	–	–	–	–	11.7
10	4.0	20.0	1.0	–	–	–	–	–	11.4	–
11	7.7	13.7	–	–	–	–	–	–	–	–
12	9.5	14.8	5.6	–	6.0	–	–	–	–	–
13	8.2	20.2	–	–	6.0	–	5.8	–	–	–
14	7.4	18.0	–	–	6.0	–	–	–	–	6.1
15	6.7	19.9	–	–	6.0	–	–	6.2	–	–
16	5.2	17.9	–	–	6.0	–	–	–	5.7	–
17	10.3	16.8	3.7	2.0	3.9	2.2	–	–	–	–
18	7.4	19.2	2.7	2.0	4.0	2.1	–	–	–	1.2
19	7.5	19.7	2.8	2.0	3.8	2.2	–	–	–	1.1
20	11.2	17.9	1.8	2.0	3.9	2.3	–	–	–	2.2
21	14.4	17.0	2.7	2.0	3.8	2.2	–	–	–	0.8
22	6.8	18.7	2.9	1.8	3.8	2.0	–	–	–	0.9
23	6.8	17.9	2.6	2.0	3.7	2.2	–	–	0.9	–
24	7.3	17.8	2.7	2.0	3.8	2.2	–	–	1.1	–
25	22.0	16.5	1.7	2.0	3.9	2.1	–	–	1.8	–

[a]) Gradient *II*.

12.5 mmol): colorless foam (440 mg, 70%). TLC (silica gel, *C*): R_f 0.7. ^{1}H-NMR ((D$_6$)DMSO): 1.16 (*t*, 3 *Me*CH$_2$); 2.09 (*s*, Me–C(5)); 2.24 (*m*, H$_\alpha$–C(2′)); 2.67 (*m*, H$_\beta$–C(2′)); 3.00 (*q*, 3 MeC*H*$_2$); 3.20 (*m*, 2 H–C(5′)); 3.73 (*s*, 2 MeO); 4.13 (*m*, H–C(4′)); 4.83 (*m*, H–C(3′)); 6.65 (*dd*, *J* = 6.7, H–C(1′)); 6.68 (*d*, *J* = 588.5, PH); 6.85 (*m*, 4 H of (MeO)$_2$*Tr*); 7.58 (*s*, H–C(6)); 7.2–7.6 (*m*, 12 arom. H); 8.05 (*m*, 2 arom. H); 8.60 (*s*, H–C(2)); 10.98 (br., NH–C(4)). ^{31}P-NMR ((D$_6$)DMSO): 1.08 (^1J(P,H) = 576.6 ^3J(P,H) = 8.9).

Solid-Phase Synthesis of the Oligonucleotides **7–25**. The oligonucleotides were synthesized in a 1-μmol scale with an automated DNA synthesizer employing the phosphonate-synthesis protocol (see *Table 7*). The oligonucleotides **7–25** were recovered from the synthesizer as the 5′-*O*-(MeO)$_2$Tr derivatives. Deprotection of NH_2 groups was carried out with 25% NH_3/H_2O at 60° for 48 h. The 5′-*O*-(MeO)$_2$Tr-protected oligonucleotides were purified by HPLC (*RP-18* columns, 250 × 4 mm, 7 μm, gradient *I*). The (MeO)$_2$Tr residues were removed with 80% AcOH/H_2O (800 μl) for 30 min at r.t. The oligomers **7–25** were purified by HPLC (gradient *II*). A 4 × 25 mm cartridge (*RP-18* silica gel) was used for desalting using H_2O for the elution of salt, while the oligomers were eluted with MeOH/H_2O 3:2. The oligonucleotides were lyophilized on a *Speed-Vac* evaporator and the colorless residues dissolved in H_2O (100 μl) and stored frozen at –20°. Data: *Table 8*.

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