58. N^7-DNA: Synthesis and Base Pairing of Oligonucleotides Containing 7-(2-Deoxy-β-D-*erythro*-pentofuranosyl)adenine (N^7A_d)

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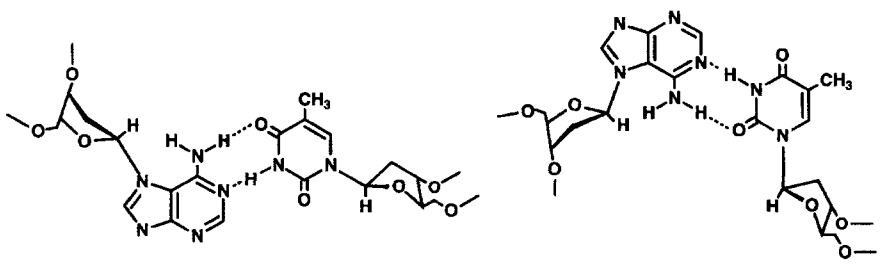
The synthesis of oligonucleotides containing 7-(2-deoxy-β-D-*erythro*-pentofuranosyl)adenine (N^7A_d; **1**) is described. Compound **1** was obtained from the precursor 4-amino-1*H*-imidazole-5-carbonitrile 2-deoxyribonucleoside **6** and was found to be much more labile than A_d. The N^6-benzoyl protecting group (see **8**) destabilized the N-glycosylic bond further and was difficult to remove by NH_3-catalyzed hydrolysis. Therefore, a (dimethylamino)methylidene residue was introduced (→**9**). Amidine **9** was blocked at OH–C(5′) with the dimethoxytrityl residue ($(MeO)_2Tr$), and phosphonate **4** as well as phosphoramidite **5** were prepared under standard conditions. Phosphonate **4** was employed in solid-phase oligonucleotide synthesis. Homooligonucleotides as well as self-complementary oligonucleotides were prepared. The oligomer d[$(N^7A)_{11}$-A] (**11**) formed a duplex with $d(T_{12})$ (**13**). Antiparallel chain polarity and reverse *Watson-Crick* base pairing was deduced from duplex formation of the self-complementary d[$(N^7A)_8$-T_8] (**14**).

Watson-Crick base pairing between purine and pyrimidine bases is not restricted to natural DNA or RNA molecules but can occur in other oligomeric structures. Oligo(2′-deoxyxylonucleotides) containing a modified sugar-phosphate backbone [1–3] or α-L-nucleosides are examples for such a behavior [4]. Base pairing of oligonucleotides containing unusually linked pyrazolo[3,4-*d*]pyrimidine nucleoside was reported [5]. From model studies, it is expected that oligonucleotides containing 7-(2-deoxy-β-D-*erythro*-pentofuranosyl)adenine (N^7A_d; **1**) can form base pairs with oligo(dT). *Watson-Crick* base pairing results in parallel strand orientation, whereas reverse *Watson-Crick* base pairing yields antiparallel strands (*Fig. 1*).

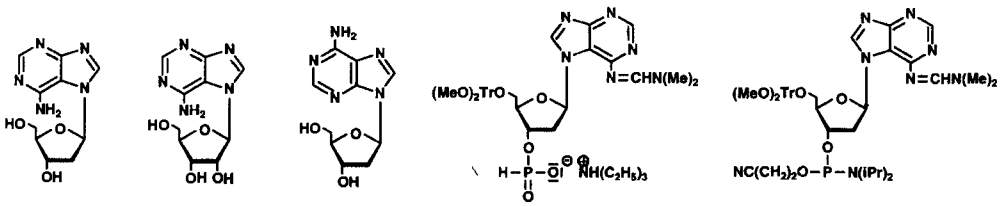


Watson-Crick **base pair**
'anti', 'anti'
parallel

reverse *Watson-Crick* base pair
'anti', 'anti'
antiparallel

Fig. 1. *Base-pairing pattern of* N^7A_d (**1**) *and* T_d. According to the structure of **1**, *Hoogsteen* base pairing is excluded. For steric reasons, *'syn'*-conformation of N^7A_d is not possible.

The first example of a naturally occurring purine nucleoside which contains the ribose moiety at another position as N^9 was 7-(α-D-ribofuranosyl)adenine. It was isolated from pseudovitamin B_{12} [6]. Its structure was elucidated by *Montgomery* and *Thomas* [7]. The synthesis of the corresponding β-D-anomer **2** was described earlier [8] [9]. In the following, the synthesis of compound **1** is reported using a 4-amino-1*H*-imidazole-5-carbonitrile 2′-deoxyribonucleoside as intermediate [10]. Phosphonate **4** and the phosphoramidite **5** were also prepared. Phosphonate **4** was used in solid-phase synthesis of various oligonucleotides containing **1**. These oligomers were studied with respect to duplex formation, complex stability, and strand orientation, and were compared to oligonucleotides containing 2′-deoxyadenosine (A_d; **3**).

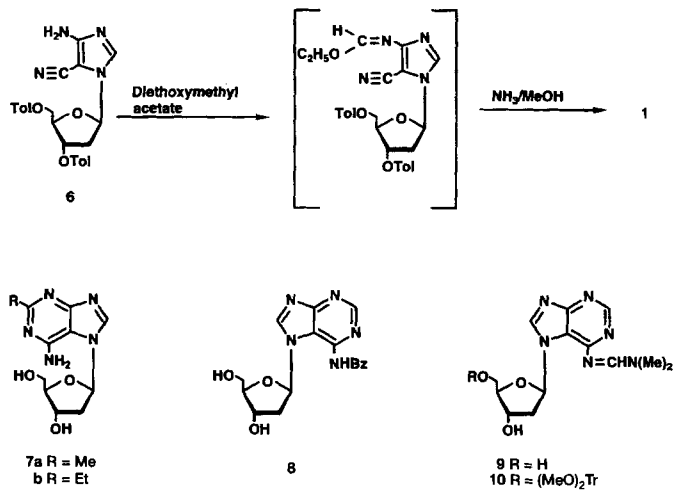


1 **2** **3** **4** **5**

Results and Discussion. – *Nucleoside* **1** *and Incorporation into Oligodeoxyribonucleotides.* Nucleobase-anion glycosylation of 6-substituted purines with 2′-deoxy-3′, 5′-di-*O*-(4-toluoyl)-α-D-*erythro*-pentofuranosyl chloride [11] yields the N^9-nucleosides as the main product and the N^7-regioisomers as the minor compounds [12] [13]. The situation changes if imidazole derivatives instead of purines are used as precursors. Glycosylation of 5-amino-1*H*-imidazole-4-carbonitrile [14] with the above mentioned halogenose gave compound **6** as main product (45%) and the N^3-regioisomer as minor component (21%) [10]. The imidazole nucleosides are pivotal intermediates for the synthesis of purine nucleosides. Condensation of **6** with diethoxymethyl acetate formed a 4-(ethoxymethylidene)amino intermediate which shows an increased mobility compared to the starting material (*Scheme*). It was not purified but directly treated with NH_3/MeOH furnishing nucleoside **1** after detoluoylation. The latter was crystallized from H_2O or MeOH and exhibits an UV maximum at 272 nm compared to 260 nm of 2′-deoxyadenosine (**3**). On TLC (silica gel, CH_2Cl_2/MeOH 4:1), **1** migrates slower than **3**. The reaction of **6** with triethyl orthoacetate gave the 2-methyl derivative **7a**; the corresponding α-D-ribofuranoside was earlier isolated from the naturally occurring vitamin B_{12m} [15]. The 2-ethyl derivative **7b** was also prepared. The position of sugar attachment (N^7) and the anomeric configuration (β-D) of **1** and **7a,b** were confirmed by ^{1}H-NMR NOE difference spectroscopy [16]. In the case of **1**, NOE's on the NH_2 group (2%) and on H–C(4′) (3.2%) were observed upon irradiation of H–C(1′).

It is established that acid-catalyzed depurination of A_d (**3**) is the most critical step during chain elongation (detritylation) in solid-phase oligonucleotide synthesis. N-Glycosylic-bond hydrolysis of **1** was studied UV-spectrophotometrically at 25° by the decrease of the 273-nm absorbance. A half-life of 4.7 (0.1N HCl) and 55 min (0.01N HCl) was observed for the N^7-nucleoside **1**. The N^9-nucleoside **3** is more stable (95 min) [17]. Ribonucleoside **2** shows a similar tendency of glycosylic-bond stability but is less labile [18].

Scheme



Reaction of **1** with benzoyl chloride under temporarily protection of the sugar OH groups by the Me_3Si residue (transient protection) [19] afforded compound **8** in only 38% yield. The low yield results from the instability of the N-glycosylic bond. This is verified by a half-life value of only 13 min (0.01N HCl, 25°) for **8** compared to 34 min for bz^6A_d (0.1N HCl, 25°) [17]. Additionally, compound **8** is difficult to debenzoylate. While bz^6A_d has a half-life value of 170 min (25% aq. NH_3 at 40°) [20], compound **8** requires 540 min, even at higher temperature (60°). The destabilization of the N-glycosylic bond upon benzoylation of **1** and the alkaline stability of the protecting group prompted us to use another residue. It was reported that the (dimethylamino)methylidene residue stabilize the N-glycosylic bond of 2′-deoxyadenosine [17] [21] and can easily be removed by NH_3 hydrolysis. Thus, amidine **9** was prepared by condensation of **1** with dimethylformamide dimethyl acetal and isolated crystalline in 75% yield. In the case of **9**, the stability of the N-glycosylic bond could not be determined UV-spectrophotometrically, as changes of the UV spectra appeared due to depurination and to deprotection of the formamidine residue. The half-life time was, therefore, determined by HPLC analysis. Hydrolysis of **9** was carried out in 0.01N HCl at 25°, and samples were taken at certain time intervals and neutralized, and aliquots were injected into the HPLC (310 nm). Two peaks were identified; the faster migrating was N^6-[(dimethylamino)methylidene]adenine, the slower one the starting material **9**. A half-life value of 119 min (0.01N HCl) was found (A_d: 3.2 h in 0.1N HCl). If the HPLC analysis was not monitored at 310 nm but at 260 nm, adenine was detected (data not shown). Therefore, the half-life value represents depurination together with deprotection of the base. The stability of amidine **9** was studies in conc. NH_3 at 40°; $t_{1/2}$ was 16.8 min, compared to 9 min for $(mfa)^6A_d$ [20].

Table 1 summarizes the ^{13}C-NMR chemical shifts of compound **1** and derivatives used during the synthesis. Gated-decoupled spectra were used for the assignment of the C-signals. In the case of **1**, a ^3J coupling of C(8) with the anomeric proton is observed apart from the ^1J couplings. Also C(6) shows a ^3J coupling (11 Hz) with H–C(2) which is

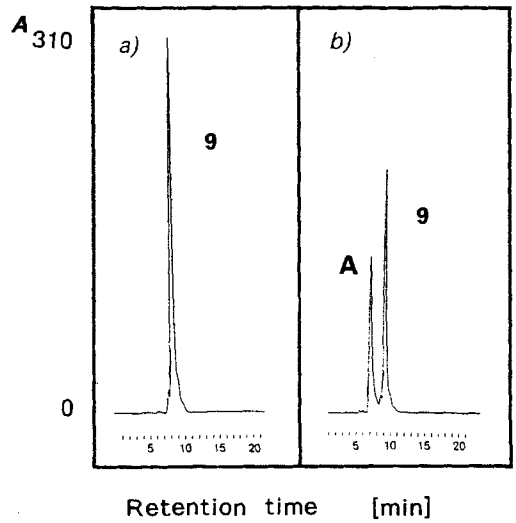


Fig. 2. *HPLC Pattern* (310 nm) *of the hydrolysis mixture of the amidine* **9** *in 0.01* N *HCl at 25°. N*^{6}-[(Dimethylamino)methylidene]adenine (**A**). *a*) 0 min; *b*) 60 min; conditions, see *Exper. Part.*

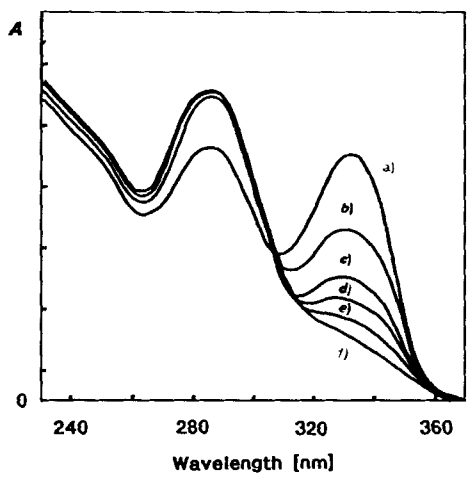


Fig. 3. *UV Spectra* (25°) *of compound* **8** *in* a) *MeOH*, b) *MeOH*/H_2O *4:1*, c) *MeOH*/H_2O *3:2*, d) *MeOH*/H_2O *2:3*, e) *MeOH*/H_2O *1:4, and* f) H_2O

not observed in the case of 2′-deoxyadenosine. The ^{13}C-NMR spectrum of compound **8** shows peculiarities. Only the signals of the sugar moiety and the benzoyl group can be detected, while the signals of the base do not appear. Moreover, the signal of C(1′) has a comparatively small intensity. This behaviour might be explained by an equilibrium of tautomeric forms with a proton located either at the amide group or at ring N-atoms.

The formation of different tautomeric forms of **8** can be deduced from the UV spectra measured in solvents of different polarity (*Fig. 3*). The absorption spectrum of the benzoyl compound **8** in MeOH exhibits two maxima at 330 and 285 nm. It undergoes a

Table 1. *^{13}C-NMR Chemical Shifts of Purine 2′-Deoxyribonucleosides*[a])[b])

	C(2)	C(4)	C(5)	C(6)	C(8)	CH_2	Me_3		C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	C=N
1	152.6	159.4	110.4	151.6	143.6			**1**	85.4	40.9	69.3	87.7	60.4	
2 [22]	152.8	160.7	110.2	151.7	144.6			**2** [22]	89.4	75.0	69.0	86.4	60.5	
3	152.5	148.9	119.4	156.2	139.7			**3**	84.1	DMSO	71.1	88.1	62.0	
4	152.2	160.9	116.3	154.7	143.1			**4**	85.7	DMSO	71.8	86.3	63.4	156.6
7a	161.0	161.6	108.6	151.6	143.6	25.4		**7a**	85.4	41.0	69.4	87.7	60.5	
b	165.3	161.6	108.8	151.5	143.6	31.1	13.1	**b**	85.4	40.9	69.4	87.7	60.5	
8	[c])	[c])	[c])	[c])	[c])			**8**	86.7	41.9	69.8	88.2	61.1	
9	152.0	160.9	116.2	154.5	143.6			**9**	86.5	40.7	69.9	88.0	61.2	156.6
10	152.1	160.9	116.3	154.4	143.3			**10**	85.8	40.5	70.0	85.7	63.9	156.6

[a]) Spectra measured in $(D)_6$DMSO rel. to TMS. [b]) From ^{1}H,^{13}C gated-decoupled spectra. [c]) Not detected.

very marked change on changing the solvent from MeOH to H_2O. In this latter case, only one maximum at 285 nm is observed. One maximum, but now at 335 nm, is observed in dioxane, too. Titration of a MeOH solution of **8** with H_2O results in a stepwise shift of the UV maxima (*Fig. 3*). The UV spectrum of **8** measured in H_2O shows similar maxima as bz^6A_d which is believed to carry the proton at the exocyclic NH_2 group. It is suggested that the UV change found for **8** represents the formation of tautomeric species in aprotic or protic solvents.

The reaction of **9** with an excess of 4,4′-dimethoxytriphenylmethyl chloride [23] in the presence of 4-(dimethylamino)pyridine and purification by flash chromatography furnished **10** (62% yield). The latter was reacted with PCl_3/*N*-methylmorpholine/1*H*-1,2,4-triazole to give the 3′-phosphonate **4**. Alternatively, **10** was converted into the phosphoramidite **5** using chloro(2-cyanoethoxy)(diisopropylamino)phosphane [24]. Phosphonate **4** and those of regular 2′-deoxynucleosides were used for the synthesis of the oligonucleotides **11**–**16**, employing pivaloyl chloride as coupling reagent. The synthesis protocol followed a repeated oxidation cycle to ensure complete oxidation of the phosphonate diester bonds [23] [25]. The oligonucleotides were removed from the polymer support with 25% aqueous NH_3 solution and purified as 5′-$(MeO)_2$Tr-protected derivatives by reversed-phase HPLC (for an example, see *Fig. 4a*). Then the $(MeO)_2$Tr residues were removed with 60% aqueous AcOH and the mixtures neutralized with Et_3N, again submitted to reversed-phase HPLC purification (see *Fig. 4b*), desalted, and lyophilized. The composition of the oligomers **11**–**16** was determined by tandem-hydrolysis with snake-venom phosphodiesterase and alkaline phosphatase. Reversed-phase HPLC of the hydrolysis mixtures separated the monomers (*Fig. 4c, d*). Quantification confirmed the calculated nucleoside composition.

d[(N^7A)$_{11}$-A]	d(A_{12})	d(T_{12})	d[(N^7A)$_8$-T_8]	d[(N^7A)-T_6]	d(A-T_6)
11	**12**	**13**	**14**	**15**	**16**

Properties of N^7A_d*-Containing Oligonucleotides.* Poly(A) forms a duplex under acidic conditions (pH 5.0) [26]. Within the duplex structure, the N(1) atom of one or both A_d residues is protonated. *Hoogsteen* base pairing keeps both strands together, and the strands show parallel chain orientation [27] [28]. Homooligomers containing ddGlc(A_6) show reverse *Hoogsteen* base pairing already under neutral conditions [1]. Within the oligomer d[(N^7A)$_{11}$-A] (**11**), the situation is different. As the N(7) atom is the glycosyla-

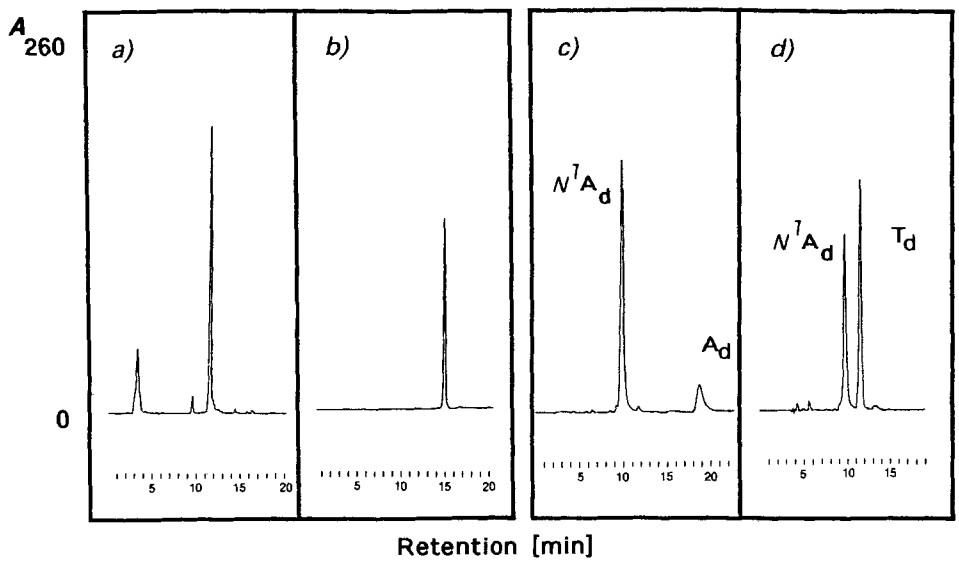
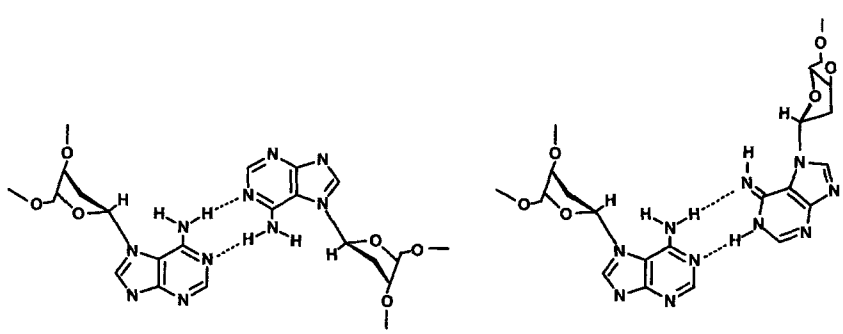


Fig. 4. *HPLC Profiles* a) *of the crude 5'-(MeO)$_2$Tr-protected oligomer of* **11**, b) *of* **11**, *and* c) d) *of the mixture obtained by enzymatic hydrolysis of* **11** (*c*)) *or* **14** (*d*)) *with snake-venom phosphodiesterase followed by alkaline phosphatase.* Conditions, see *Exper. Part.*

tion site, such duplexes should not be formed. This was confirmed by melting experiments. At pH 5.0, a sigmoidal melting profil (10–80°) was not detected for **11**, neither at 260 nor at 280 nm.

Possible antiparallel and parallel duplex structures as shown in *Fig. 5* may be furnished under neutral conditions for **11**. This self-aggregation would either be formed by a 'reverse' *Watson-Crick* (parallel) or by a *Watson-Crick* base pair (antiparallel, amino-imino tautomer). But only a linear decrease of the B_{2u} transition of the CD spectra was found between 10 and 70° under these conditions (*Fig. 6a*). UV-Spectrophotometric



reverse *Watson-Crick* base pair
'anti', 'anti'
parallel

Watson-Crick base pair
'anti', 'anti'
antiparallel

Fig. 5. *Possible self-pairing modes of oligonucleotides containing compound* **1**. According to the structure of **1**, *Hoogsteen* base pairing is excluded. For steric reasons, *'syn'*-conformation of N^7A_d is not possible.

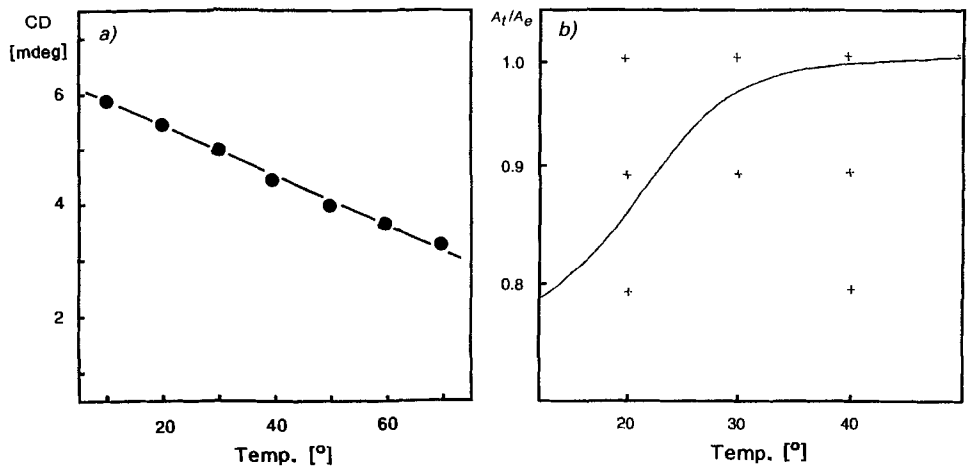


Fig. 6. a) *Temperature-dependent* B_{2u} *transition of the CD spectra of* $d[(N^7A)_{11}\text{-}A]$ (**11**). b) *Normalized melting profile of* $d[(N^7A)_{11}\text{-}A]\cdot d(T)_{12}$ (**11**·**13**). Spectra were measured in 60 mM Na-cacodylate buffer (pH 7.0, 1M NaCl, 100 mM $MgCl_2$) at 6.5 μM oligonucleotide concentration (singlestrands).

studies lead to a similar result. However, a hypochromicity change appeared and was determined to be 6% (260 nm) and 12% (280 nm). These values are similar to that of $d(A_{12})$ (13%, 260 nm [2]) and indicate a highly ordered structure of the adenine moieties in $d[(N^7A)_{11}\text{-}A]$.

Next, the duplex formation of $d[(N^7A)_{11}\text{-}A]$ with $d(T_{12})$ [29] (→**11**·**13**) was studied. A cooperative UV melting profile was observed with a T_m value of 22° (*Fig. 6b*). This value is much lower than that of $d(A)_{12}\cdot d(T)_{12}$ (**12**·**13**) measured under identical conditions (T_m 43°) [29]. The thermal hypochromicity at 260 nm (10–80°) was 21% for **11**·**13** and 31% for **12**·**13**. Duplex formation between **11** and **13** was also confirmed by CD spectroscopy (*Fig. 7*). Both $\pi\text{–}\pi^*$ transitions (B_{1u}, B_{2u}) were measured as a function of temperature

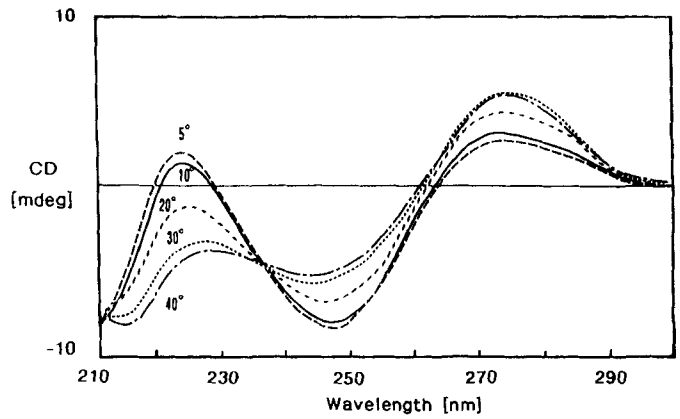


Fig. 7. *Temperature-dependent CD spectra of* $d[(N^7A)_{11}\text{-}A]\cdot d(T)_{12}$ (**11**·**13**) *at 5, 10, 20, 30, and 40°.* Conditions, see *Fig. 6b*.

between 10 and 70°. The T_m value obtained from the B_{1u} transition (22°) is nearly identical with the T_m value determined from the UV profile. The B_{2u} transition exhibits a maximum at 30°.

The antiparallel orientation of DNA strands is the preferred arrangement in the DNA duplex structure. However, oligonucleotides with appropriate sequences of A_d and T_d can form stable duplexes with a parallel strand orientation [30] [31]; this duplex is formed by reverse *Watson-Crick* base pairing [32]. According to *Fig. 1*, parallel and antiparallel strand orientations are possible in the case of homooligonucleotides containing N^7A_d.

Because of the still unknown polarity of the duplex **11**·**13**, we studied the duplex formation of oligomer **14**. According to the structure of this oligonucleotide, it can be used to discriminate between parallel and antiparallel strand orientation. Parallel strand orientation (p.s. **14**·**14**) would require N^7A_d-N^7A_d as well as T_d-T_d base pairing. The self-pairing between the adenine moiety can be excluded as it was already done for the homooligomer **11** (see above). In the case of the oligomer **14** (4.5 μmol), we observed a single phase cooperative melting with a T_m value of 35° (*Fig. 8a*).

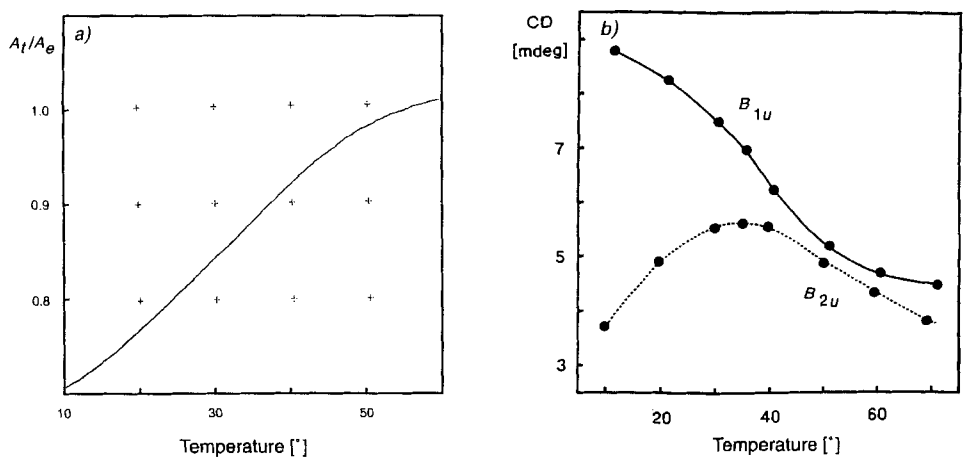


Fig. 8. a) *Normalized melting profile of d[(N^7A)$_8$-T_8]* (**14**) *and* b) *melting profiles obtained from the temperature-dependent* B_{1u} *and* B_{2u} *transitions of the CD spectra of d[(N^7A)$_8$-T_8]* (**14**). Spectra were measured in 60 mM Na-cacodylate buffer (pH 7.0, 1M NaCl, 100 mM $MgCl_2$) at 4.5 μM oligonucleotide concentration.

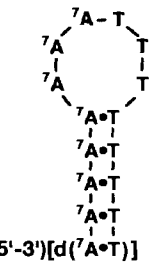
As the parallel duplex structure was already excluded, the observed sigmoid UV profile of **14** can be only explained by the melting of an antiparallel duplex (ap. s. **14**·**14** or a hairpin structure). This melting is also observed by temperature-dependent CD spectra (*Fig. 8b*). The transition at 245 nm is strongly temperature-dependent, and the temperature profile leads to the same T_m as already determined from the UV measurement. The B_{2u} transition at 269 nm shows a maximum at the T_m value (*Fig. 8b*). When the oligonucleotide concentration of **14** was reduced by a factor of *ca.* 2 (from 4.5 μM to 2.1 μM), the T_m decreased from 35° to 32°. This is expected for duplex melting. However, more detailed investigations have to be carried out to exclude hairpin structures. Nevertheless, base-pairing has to occur by the reverse *Watson-Crick* mode in both cases. These results clearly show that oligonucleotides containing N^7A_d (**1**) form base pairs with their T_d

ap.s. 14·14: (5'-3')[d(⁷A-⁷A-⁷A-⁷A-⁷A-⁷A-⁷A-T-T-T-T-T-T-T-T-T-)] **14**

(3'-5')[d (T-T-T-T-T-T-T-T-T-⁷A-⁷A-⁷A-⁷A-⁷A-⁷A-⁷A-⁷A)] **14**

p.s. 14·14: (5'-3')[d(⁷A-⁷A-⁷A-⁷A-⁷A-⁷A-⁷A-⁷A-T-T-T-T-T-T-T-T-T-T)] **14**

(5'-3')[d(⁷A-⁷A-⁷A-⁷A-⁷A-⁷A-⁷A-⁷A-T-T-T-T-T-T-T-T-T-T)] **14**



(5'-3')[d(⁷A•T)]

14

⁷A=N⁷A$_d$ **ap.s. Hairpin**

counterparts following an antiparallel strand orientation. Experiments on oligonucleotides containing 7-(2-deoxy-β-D-*erythro*-pentofuranosyl)guanine are under current investigation [33].

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Experimental Part

General. See [29]. Melting curves were measured with a *Cary-1/3* UV/VIS spectrophotometer (*Varian*, Australia) equipped with a *Cary* thermoelectrical controller. The actual temperature was measured in the reference cell with a Pt-100 resistor. The T_m values were calculated using the software package '2hDNA' (*Varian*, Darmstadt, Germany). Solvent systems for flash chromatography (FC): *A* = CH_2Cl_2/MeOH 9:1, *B* = CH_2Cl_2/MeOH 4:1, *C* = CH_2Cl_2/MeOH/Et_3N 83:15:2, *D* = CH_2Cl_2/MeOH/Et_3N 88:10:2, *E* = CH_2Cl_2/Et_3N 97:3, *F* = AcOEt/CH_2Cl_2/Et_3N 45:45:10.

N-Glycosylic-Bond Hydrolysis. HPLC: From an aq. nucleoside stock soln. of **9** (1.5 mg, 1.5 ml), 200 µl were diluted with 0.01N aq. HCl (1.5 ml). The mixture was stored at 25°, samples (200 µl) were taken and neutralized with Et_3N (20 µl). An aliquot (100 µl) was injected into the HPLC. Quantification was made on basis of peak areas. Solvent systems: *A* = 0.1M (Et_3NH)OAc (pH 7.0)/MeCN 95:5; *B* = MeCN. Gradient: 10 min 10–20% *B* in *A*; flow rate 1.0 ml/min. (ε_{260}: A_d 15400, T_d 8800, N^7A_d 6500).

HPLC Separation. HPLC was carried out on a 4 × 250 and 4 × 25 mm (7 µmol) *RP-18 LiChrosorb* column (*Merck*) using a *Merck-Hitachi* HPLC apparatus. The solvent systems were *A* = 0.1M (Et_3NH)OAc (pH 7.0)/MeCN 95:5 and *B* = MeCN and used in the following order: gradient *I*, 15 min 15–40% *B* in *A*; gradient *II*, 20 min 0–20% *B* in *A*.

*7-(2-Deoxy-β-*D-erythro-*pentofuranosyl)adenine* (**1**). A soln. of 4-amino-1-[2-deoxy-3,5-di-*O*-(4-toluoyl)-β-D-*erythro*-pentofuranosyl]-1*H*-imidazole-5-carbonitrile [10] (**6**; 400 mg, 0.87 mmol) in diethoxymethyl acetate was heated under reflux for 4 h. The soln. was evaporated, the resulting syrup dissolved in toluene, and again evaporated. Methanolic ammonia (60 ml, previously sat. at 0°) was added and the soln. stirred for 24 h at r.t. After evaporation, the residue was purified by FC (column 20 × 3 cm, *B*): **1** as colorless solid. After crystallization from MeOH, colorless crystals (150 mg, 69%). M.p. 187°. TLC (*B*): R_f 0.28. UV (MeOH): 272 (8400). ^{1}H-NMR ((D$_6$)DMSO): 2.33, 2.50 (2*m*, 2 H–C(2')); 3.57 (*m*, 2 H–C(5')); 3.92 (*m*, H–C(4')); 4.42 (*m*, H–C(3')); 5.17 (*t*, *J* = 4.8, OH–C(5')); 5.43 (*d*, *J* = 3.9, OH–C(3')); 6.33 ('*t*', *J* = 6.4, H–C(1')); 7.00 (br., NH_2); 8.23 (*s*, H–C(8)); 8.54 (*s*, H–C(2)). Anal. calc. for $C_{10}H_{13}N_5O_3$ (251.3): C 47.81, H 5.22, N 27.88; found: C 47.77, H 5.28, N 27.73.

*7-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-2-methyladenine* (**7a**). To a soln. of **6** (300 mg, 0.65 mmol) in triethyl orthoacetate (10 ml), AcOH (0.1 ml) was added. The soln. was stirred under reflux for 4 h, then evaporated and the residue treated and purified as described for **1**. The colorless solid **7a** (105 mg, 61%) was crystallized from MeOH to give colorless crystals. M.p. > 250°. TLC (*B*): R_f 0.35. UV (MeOH): 272 (8300). ^{1}H-NMR ((D$_6$)DMSO): 2.25, 2.45 (2*m*, 2 H–C(2')); 2.38 (*s*, Me); 3.56 (*m*, 2 H–C(5')); 3.89 (*m*, H–C(4')); 4.38 (*m*, H–C(3')); 5.14 (*t*, *J* = 4.9, OH–C(5')); 5.40 (*d*, *J* = 4.6, OH–C(3')); 6.27 ('*t*', *J* = 6.4, H–C(1')); 6.86 (br., NH_2); 8.44 (*s*, H–C(8)). Anal. calc. for $C_{11}H_{15}N_5O_3$ (265.2): C 49.81, H 5.70, N 26.40; found: C 49.64, H 5.78, N 26.28.

*7-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-2-ethyladenine* (**7b**). To a soln. of **6** (450 mg, 0.97 mmol) in triethyl orthopropionate (10 ml), AcOH (0.1 ml) was added. The soln. was stirred under reflux for 3 h and then evaporated. The resulting syrup was dissolved in toluene, the soln. again evaporated, and the residue dissolved in methanolic

ammonia (60 ml, previously sat. at 0°) and stirred at 60° for 24 h. Workup as described for **1**. Flash chromatography yielded 150 mg (56%) of **7b** and crystallization from MeOH colorless crystals. M.p. 171–173°. TLC (*B*): R_f 0.45. UV (MeOH): 274 (8900). ^{1}H-NMR ((D$_6$)DMSO): 1.22 (*t*, *Me*CH$_2$); 2.30–2.45 (2*m*, 2 H–C(2′)); 2.65 (*q*, MeC*H*$_2$); 3.54 (*m*, 2 H–C(5′)); 3.89 (*m*, H–C(4′)); 4.38 (*m*, H–C(3′)); 5.13 (*t*, *J* = 4.9, OH–C(5′)); 5.39 (*d*, *J* = 4.5, OH–C(3′)); 6.28 ('*t*', *J* = 6.3, H–C(1′)); 6.84 (br., NH$_2$); 8.44 (*s*, H–C(8)). Anal. calc. for $C_{12}H_{17}N_5O_3$ (279.3): C 51.60, H 6.13, N 25.07; found: C 51.74, H 6.27, N 25.05.

N^6-*Benzoyl-7-(2-deoxy-β-*D-erythro-*pentofuranosyl)adenine* (**8**). Compound **1** (200 mg, 0.80 mmol) was dried by co-evaporation with anh. pyridine and then suspended in pyridine (10 ml). Me_3SiCl (0.52 ml, 4 mmol) was added under Ar and the soln. stirred for 2 h (TLC control), treated with benzoyl chloride (0.46 ml, 3.90 mmol), and maintained at r.t. for 3 h. The mixture was cooled to 0° and hydrolyzed with H_2O (1 ml). After 5 min, the resultant was treated with 25% aq. ammonia (2 ml) and stirred for another 60 min. The solvent was evaporated and the residue co-evaporated twice with toluene. Chromatography (silica gel *60H*, column 20 × 3 cm, *D*) afforded **8** as colorless solid which was crystallized from MeOH: colorless crystals (110 mg, 38%). M.p. 204°. TLC (*D*): R_f 0.4. UV (MeOH): 286 (10900), 328 (10900). ^{1}H-NMR (D_2O): 2.60, 2.70 (2*m*, 2 H–C(2′)); 3.60, 3.70 (2*m*, 2 H–C(5′)); 4.09 (*m*, H–C(4′)); 4.33 (*m*, H–C(3′)); 6.67 ('*t*', *J* = 3.0, H–C(1′)); 7.46–8.05 (*m*, arom. H); 8.71 (*s*, H–C(2)); 8.75 (*s*, H–C(8)). Anal. calc. for $C_{17}H_{17}N_5O_4$ (355.3): C 57.47, H 4.82, N 19.71; found: C 57.56, H 4.86, N 19.71.

*7-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-*N^6*-[(dimethylamino)methylidene]adenine* (**9**). Compound **1** (250 mg, 1.0 mmol), dried by co-evaporation with dry DMF, was dissolved in dry DMF (20 ml). After treatment with *N,N*-dimethylformamide diethyl acetale (3 ml, 17.5 mmol) the soln. was stirred at 40° for 2 h and then evaporated. The oily residue was co-evaporated twice with toluene (10 ml, each) and chromatographed (silica gel *60H*, column 20 × 3 cm, *D*). The product was crystallized from acetone/MeOH (9:1): colorless crystals (230 mg, 75%). M.p. 183–184°. TLC (*A*): R_f 0.25. UV (MeOH): 320 (29000). ^{1}H-NMR ((D$_6$)DMSO): 2.30 (2*m*, 2 H–C(2′)); 3.11, 3.20 (2*s*, Me$_2$N); 3.60 (*m*, 2 H–C(5′)); 3.87 (*m*, H–C(4′)); 4.32 (*m*, H–C(3′)); 5.04 (*t*, *J* = 5.2, OH–C(5′)); 5.26 (*d*, *J* = 4.0, OH–C(3′)); 7.02 ('*t*', *J* = 6.2, H–C(1′)); 8.42 (*s*, H–C(2)); 8.71 (*s*, H–C(8)); 8.89 (*s*, –CH=). Anal. calc. for $C_{13}H_{18}N_6O_3$ (306.3): C 50.97, H 5.92, N 27.43; found: C 51.09, H 5.93, N 27.43.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D-erythro-*pentofuranosyl]-*N^6*-[(dimethylamino)methylidene]adenine* (**10**). Compound **9** (130 mg, 0.42 mmol) was dried by co-evaporation (twice) with dry pyridine and suspended in anh. pyridine (10 ml). The soln. was stirred under Ar in the presence of 4-(dimethylamino)pyridine (30 mg, 0.24 mmol) and 4,4′-dimethoxytriphenylmethyl chloride (300 mg, 0.9 mmol) for 3 h. After addition of 5% aq. $NaHCO_3$ soln. (20 ml), the mixture was extracted 3 times with CH_2Cl_2 (50 ml, each). The combined org. layer was dried (Na_2SO_4), filtered, and evaporated and the residue chromatographed (silica gel *60H*, column 20 × 3 cm, *D*): **10** as colorless foam (160 mg, 62%). TLC (*D*): R_f 0.7. UV (MeOH): 321 (27800). ^{1}H-NMR ((D$_6$)DMSO): 2.50, 2.70 (2*m*, 2 H–C(2′)); 3.11, 3.20 (2*s*, Me$_2$N); 3.41 (*m*, 2 H–C(5′)); 3.71 (*s*, 2 MeO); 4.00 (*m*, H–C(4′)); 4.33 (*m*, H–C(3′)); 5.37 (*d*, *J* = 4.0, OH–C(3′)); 5.82–6.85, 7.23–7.39 (2*m*, arom. H); 7.06 ('*t*', *J* = 5.9, H–C(1′)); 8.44, 8.47 (2*s*, H–C(2), H–C(8)); 8.90 (*s*, –CH=). Anal. calc. for $C_{34}H_{36}N_6O_5$ (608.3): C 67.09, H 5.96, N 13.81; found: C 66.85, H 6.05, N 13.60.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D-erythro-*pentofuranosyl]-*N^6*-[(dimethylamino)methylidene]adenine 3′-(Triethylammonium Phosphonate)* (**4**). To a soln. of PCl_3 (75 µl, 0.86 mmol) and *N*-methylmorpholine (0.95 ml, 8.52 mmol) in CH_2Cl_2 (20 ml), 1*H*-1,2,4-triazole (200 mg, 2.8 mmol) was added and the mixture stirred for 30 min at r.t. After cooling to 0°, a soln. of **10** (100 mg, 0.17 mmol) in CH_2Cl_2 (10 ml) was added dropwise and the mixture stirred for 10 min. Thereupon, the mixture was poured into 1M $(Et_3NH)HCO_3$ (TBK, pH 7.7, 10 ml), shaken, and separated. The aq. layer was extracted twice with CH_2Cl_2, the combined org. extract dried (Na_2SO_4) and evaporated, and the residue chromatographed (silica gel *60H*, column 20 × 3 cm, 600 ml *E*, then *D*). The content of the main zone was dissolved in CH_2Cl_2 (20 ml) and extracted twice with 0.1M $(Et_3NH)HCO_3$ (5 ml). The org. layer was dried (Na_2SO_4) and evaporated: **4** as colorless foam (90 mg, 71%). TLC (*D*): R_f 0.5. UV (MeOH): 321 (28800). ^{1}H-NMR ((D$_6$)DMSO): 1.20 (*m*, Me); 2.50 (*m*, 2 H–C(2′)); 2.93–3.00 (*m*, 2 CH$_2$); 3.12, 3.20 (2*s*, Me$_2$N); 3.25 (*m*, 2 H–C(5′)); 3.72 (*s*, 2 MeO); 4.13 (*m*, H–C(4′)); 4.70 (*m*, H–C(3′)); 6.82–6.91, 7.23–7.39 (2*m*, arom. H); 7.04 ('*t*', *J* = 6.9, H–C(1′)); 8.45, 8.46 (2*s*, H–C(2), H–C(8)); 8.90 (*s*, –CH=). ^{31}P-NMR ((D$_6$)DMSO): 0.95; ^1J(P,H) = 583.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D-erythro-*pentofuranosyl]-*N^6*-[(dimethylamino)methylidene]adenine 3′-[(2-Cyanoethyl)* N,N-*Diisopropylphosphoramidite]* (**5**). To a soln. of **10** (40 mg, 0.07 mmol) and (i-Pr)$_2$EtN (110 µl, 0.63 mmol) in dry CH_2Cl_2 (2 ml), chloro(2-cyanoethoxy)(diisopropylamino)phosphane (90 µl, 0.39 mmol) was added within 2 min under N_2. After stirring for 60 min, the reaction was quenched by adding 5% aq. $NaHCO_3$ soln. (4 ml), the mixture extracted twice with CH_2Cl_2 (10 ml, each), and the org. layer dried (Na_2SO_4) and evaporated. FC (column 9 × 3 cm, *F*) gave two partially overlapping zones of **5** (diastereoisomers; 30 mg, 56%). TLC (*F*): R_f 0.52, 0.60. ^{31}P-NMR ((D$_6$)DMSO): 148.3.

Table 2. *Retention Times and Hypochromicities of Oligomers*

Oligomer	Retention time [min][a]	Hypochromicity [%][b]
d[$(N^7A)_{11}$-A] **11**	10.9 (*I*), 13.8 (*II*)	22.4
d[$(N^7A)_8$-T_8] **14**	10.7 (*I*), 15.3 (*II*)	21.0
d[(N^7A)-T_6] **15**	11.1 (*I*), 14.3 (*II*)	16.5

[a]) The first values (gradient *I*) refer to the $(MeO)_2Tr$ derivatives; gradient *II* was used for the deprotected oligomers; flow rate 3.5 ml/min. [b]) Enzymatic hypochromicity at 260 nm.

Solid-Phase Synthesis of the Oligonucleotides **11**, **14**, *and* **15**. The synthesis of **11**, **14**, amd **15** was carried out on a 1-μmol scale using the 3′-phosphonates of $[(MeO)_2Tr]bz^6A_d$, $[(MeO)_2Tr]T_d$, and $[(MeO)_2Tr]fam^6N^7A_d$ (**4**) and following the regular protocol of the DNA synthesizer for 3′-hydrogen phosphonates, which was slightly modified [20] [25]. The 4,4′-dimethoxytrityl residues of the oligomers were removed by treatment with 60% AcOH for 20 min at r.t. Retention times of **11**, **14**, and **15** and of their 5′-$(MeO)_2Tr$ derivates and hypochromicities of **11**, **14**, and **15** were determined (*Table 2*).

REFERENCES

[1] J. Hunziker, H.-J. Roth, M. Böhringer, A. Giger, U. Diederichsen, M. Göbel, R. Krishnan, B. Jaun, C. Leumann, A. Eschenmoser, *Helv. Chim. Acta* **1993,** *76,* 259.
[2] H. Rosemeyer, F. Seela, *Helv. Chim. Acta* **1991,** *74,* 748.
[3] U. Urata, E. Ogura, K. Shinohara, Y. Ueda, M. Akagi, *Nucleic Acids Res.* **1992,** *20,* 3325.
[4] C. Gagnor, J. R. Bertrand, S. Thenet, M. Lemaitre, F. Morvan, B. Rayner, C. Malvy, B. Lebleu, J.-L. Imbach, C. Paoletti, *Nucleic Acids Res.* **1987,** *15,* 10419.
[5] F. Seela, K. Kaiser, *Helv. Chim. Acta* **1988,** *71,* 1813.
[6] W. Friedrich, K. Bernhauer, *Chem. Ber.* **1956,** *89,* 2507.
[7] J. A. Montgomery, H. J. Thomas, *J. Am. Chem. Soc.* **1963,** *85,* 2672.
[8] J. A. Montgomery, H. J. Thomas, *J. Am. Chem. Soc.* **1965,** *87,* 5442.
[9] R. J. Rousseau, R. K. Robins, L. B. Townsend, *J. Am. Chem. Soc.* **1968,** *90,* 2661.
[10] Z. Kazimierczuk, F. Seela, *Liebigs Ann. Chem.* **1991,** 695.
[11] M. Hoffer, *Chem. Ber.* **1960,** *93,* 2777.
[12] Z. Kazimierczuk, H. B. Cottam, G. R. Revankar, R. K. Robins, *J. Am. Chem. Soc.* **1984,** *106,* 6379.
[13] F. Seela, H. Winter, *Bioorg. Med. Chem. Lett.* **1993,** *3,* 273.
[14] J. P. Ferris, L. I. Orgel, *J. Am. Chem. Soc.* **1965,** *87,* 4976.
[15] W. Friedrich, K. Bernhauer, *Chem. Ber.* **1957,** *90,* 465.
[16] H. Rosemeyer, G. Toth, F. Seela, *Nucleos. Nucleot.* **1989,** *8,* 587; D. Neuhaus, M. Williamson, 'The Nuclear Overhauser Effect', in 'Structural and Conformational Analysis', Verlag Chemie, Weinheim, 1989.
[17] M. Krecmerova, F. Seela, *Nucleos. Nucleot.* **1992,** *11,* 1393.
[18] R. P. Panzica, R. J. Rousseau, R. K. Robins, L. B. Townsend, *J. Am. Chem. Soc.* **1972,** *94,* 4708.
[19] G. S. Ti, B. L. Gaffney, R. A. Jones, *J. Am. Chem. Soc.* **1982,** *104,* 1316.
[20] F. Seela, T. Grein, *Nucleic Acids Res.* **1992,** *20,* 2297.
[21] B. C. Froehler, M. D. Matteucci, *Nucleic Acids Res.* **1983,** *11,* 8031.
[22] M.-T. Chenon, R. J. Pugmire, D. M. Grant, R. P. Panzica, L. B. Townsend, *J. Am. Chem. Soc.* **1975,** *97,* 4627.
[23] B. C. Froehler, P. G. Ng, M. D. Matteucci, *Nucleic Acids Res.* **1986,** *14,* 5399.
[24] N. D. Sinha, J. Biernat, J. McManus, H. Köster, *Nucleic Acids Res.* **1984,** *12,* 1316.
[25] B. C. Froehler, in 'Methods in Molecular Biology', Ed. E. S. Agrawal, Humana Press, Totowa, N.J., 1993.
[26] J. R. Fresco, E. Klemperer, *Ann. N. Y. Acad. Sci.* **1959,** *81,* 730.
[27] A. Rich, D. R. Davies, F. H. C. Crick, J. D. Watson, *J. Mol. Biol.* **1961,** *3,* 71.
[28] D. B. Lerner, D. R. Kearns, *Biopolymers* **1981,** *20,* 803.
[29] H. Rosemeyer, F. Seela, *Helv. Chim. Acta* **1991,** *74,* 748.
[30] N. B. Ramsing, T. M. Jovin, *Nucleic Acids Res.* **1988,** *16,* 6659.
[31] M. W. Germann, B. W. Kalish, J. H. van de Sande, *Biochemistry* **1988,** *27,* 8302.
[32] N. Pattabiraman, *Biopolymers* **1986,** *25,* 1603.
[33] F. Seela, P. Leonard, manuscript in preparation.