

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

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The synthesis of oligonucleotides containing 7-(2-deoxy- $\beta$ -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 (dimethyl-amino)methylidene residue was introduced ( $\rightarrow$ **9**). Amidine **9** was blocked at OH-C(5') with the dimethoxytrityl residue ((MeO)<sub>2</sub>Tr), 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[( $N^7A$ )<sub>11</sub>-A] (**11**) formed a duplex with d(T)<sub>12</sub> (**13**). Antiparallel chain polarity and reverse *Watson-Crick* base pairing was deduced from duplex formation of the self-complementary d[( $N^7A$ )<sub>8</sub>-T]<sub>8</sub> (**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  $\alpha$ -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- $\beta$ -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).

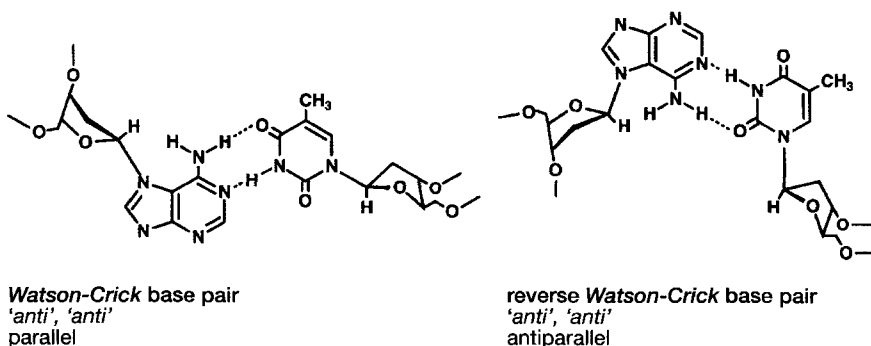
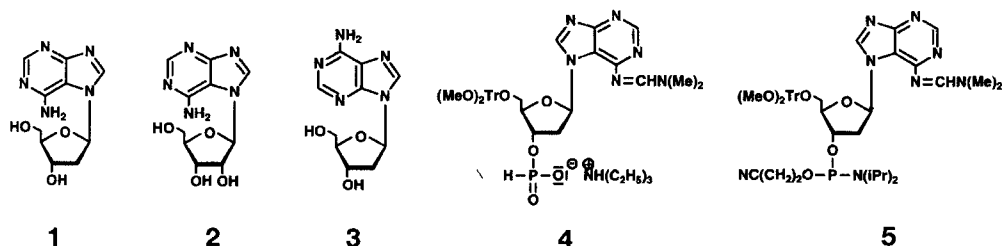


Fig. 1. Base-pairing pattern of  $N^7A_d$  (**1**) and  $T_d$ . According to the structure of **1**, Hoogsteen base pairing is excluded. For steric reasons, 'syn'-conformation of  $N^7A_d$  is not possible.

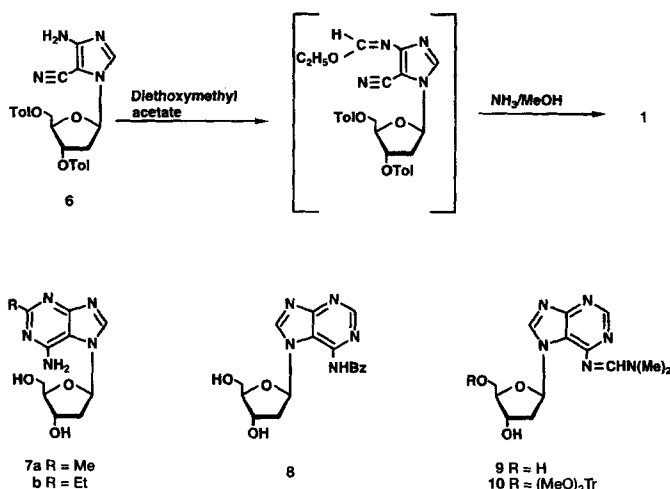
The first example of a naturally occurring purine nucleoside which contains the ribose moiety at another position as  $N^9$  was 7-( $\alpha$ -D-ribofuranosyl)adenine. It was isolated from pseudovitamin B<sub>12</sub> [6]. Its structure was elucidated by *Montgomery* and *Thomas* [7]. The synthesis of the corresponding  $\beta$ -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<sub>d</sub>; **3**).



**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)- $\alpha$ -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  $\text{NH}_3/\text{MeOH}$  furnishing nucleoside **1** after detoluoylation. The latter was crystallized from  $\text{H}_2\text{O}$  or  $\text{MeOH}$  and exhibits an UV maximum at 272 nm compared to 260 nm of 2'-deoxyadenosine (**3**). On TLC (silica gel,  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  4:1), **1** migrates slower than **3**. The reaction of **6** with triethyl orthoacetate gave the 2-methyl derivative **7a**; the corresponding  $\alpha$ -D-ribofuranoside was earlier isolated from the naturally occurring vitamin B<sub>12m</sub> [15]. The 2-ethyl derivative **7b** was also prepared. The position of sugar attachment ( $N^7$ ) and the anomeric configuration ( $\beta$ -D) of **1** and **7a, b** were confirmed by  $^1\text{H-NMR}$  NOE difference spectroscopy [16]. In the case of **1**, NOE's on the  $\text{NH}_2$  group (2%) and on  $\text{H-C}(4')$  (3.2%) were observed upon irradiation of  $\text{H-C}(1')$ .

It is established that acid-catalyzed depurination of A<sub>d</sub> (**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<sub>3</sub>Si 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<sup>6</sup>A<sub>d</sub> (0.1N HCl, 25°) [17]. Additionally, compound **8** is difficult to debenzoylate. While bz<sup>6</sup>A<sub>d</sub> has a half-life value of 170 min (25% aq. NH<sub>3</sub> at 40°) [20], compound **8** requires 540 min, even at higher temperature (60°). The destabilization of the N-glycosylic bond upon benzylation 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<sub>3</sub> 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<sup>6</sup>-[(dimethylamino)methylidene]adenine, the slower one the starting material **9**. A half-life value of 119 min (0.01N HCl) was found (A<sub>d</sub>: 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 studied in conc. NH<sub>3</sub> at 40°; *t*<sub>1/2</sub> was 16.8 min, compared to 9 min for (mfa)<sup>6</sup>A<sub>d</sub> [20].

Table 1 summarizes the <sup>13</sup>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 <sup>3</sup>J coupling of C(8) with the anomeric proton is observed apart from the <sup>1</sup>J couplings. Also C(6) shows a <sup>3</sup>J coupling (11 Hz) with H–C(2) which is

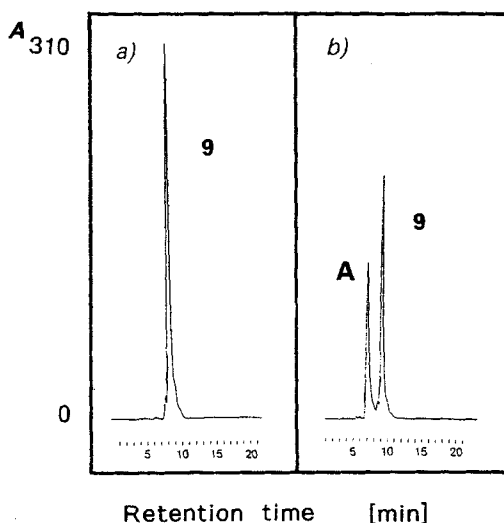


Fig. 2. HPLC Pattern (310 nm) of the hydrolysis mixture of the amidine **9** in 0.01N HCl at 25°. *N*<sup>6</sup>-[(Dimethylamino)methylidene]adenine (**A**). a) 0 min; b) 60 min; conditions, see *Exper. Part*.

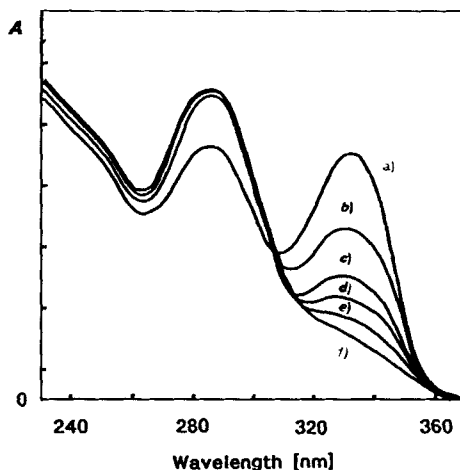


Fig. 3. UV Spectra (25°) of compound **8** in a) MeOH, b) MeOH/H<sub>2</sub>O 4:1, c) MeOH/H<sub>2</sub>O 3:2, d) MeOH/H<sub>2</sub>O 2:3, e) MeOH/H<sub>2</sub>O 1:4, and f) H<sub>2</sub>O

not observed in the case of 2'-deoxyadenosine. The <sup>13</sup>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.  $^{13}\text{C}$ -NMR Chemical Shifts of Purine 2'-Deoxyribonucleosides<sup>a)b)</sup>

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

<sup>a)</sup> Spectra measured in (D)<sub>6</sub>DMSO rel. to TMS. <sup>b)</sup> From  $^1\text{H}$ ,  $^{13}\text{C}$  gated-decoupled spectra. <sup>c)</sup> Not detected.

very marked change on changing the solvent from MeOH to H<sub>2</sub>O. 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<sub>2</sub>O results in a stepwise shift of the UV maxima (Fig. 3). The UV spectrum of **8** measured in H<sub>2</sub>O shows similar maxima as bz<sup>6</sup>A<sub>4</sub> which is believed to carry the proton at the exocyclic NH<sub>2</sub> 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<sub>3</sub>/*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<sub>3</sub> solution and purified as 5'-(MeO)<sub>2</sub>Tr-protected derivatives by reversed-phase HPLC (for an example, see Fig. 4a). Then the (MeO)<sub>2</sub>Tr residues were removed with 60% aqueous AcOH and the mixtures neutralized with Et<sub>3</sub>N, 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 <sup>7</sup> A) <sub>11</sub> -A] | d(A <sub>12</sub> ) | d(T <sub>12</sub> ) | d[(N <sup>7</sup> A) <sub>8</sub> -T <sub>8</sub> ] | d[(N <sup>7</sup> A)-T <sub>6</sub> ] | d(A-T <sub>6</sub> ) |
| <b>11</b>                              | <b>12</b>           | <b>13</b>           | <b>14</b>                                           | <b>15</b>                             | <b>16</b>            |

*Properties of N<sup>7</sup>A<sub>x</sub>-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<sub>d</sub> residues is protonated. Hoogsteen base pairing keeps both strands together, and the strands show parallel chain orientation [27] [28]. Homooligomers containing ddGlc(A<sub>6</sub>) show reverse Hoogsteen base pairing already under neutral conditions [1]. Within the oligomer d[(N<sup>7</sup>A)<sub>11</sub>-A] (**11**), the situation is different. As the N(7) atom is the glycosyla-

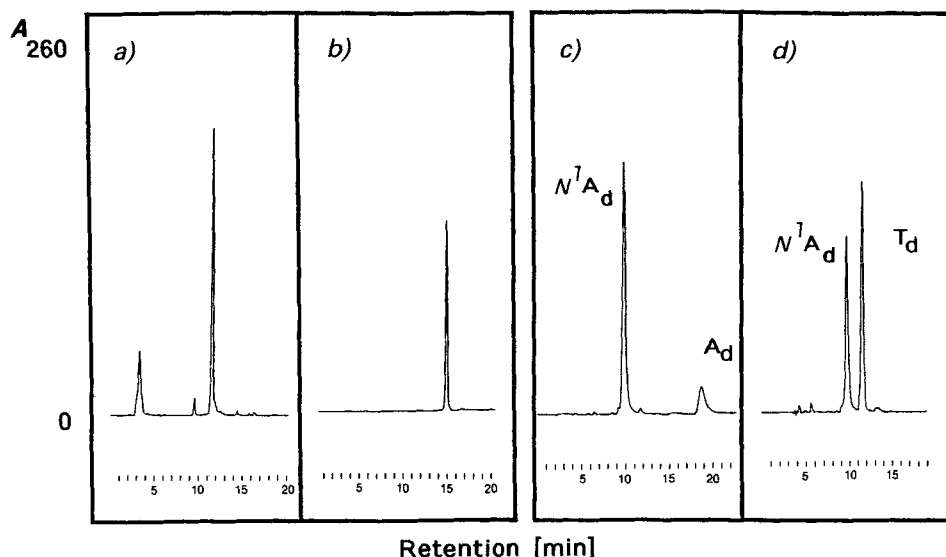


Fig. 4. HPLC Profiles a) of the crude 5'-(MeO)<sub>2</sub>Tr-protected oligomer of **11**, b) of **11**, and c) d) of the mixture obtained by enzymatic hydrolysis of **11** (c) or **14** (d) with snake-venom phosphodiesterase followed by alkaline phosphatase. Conditions, see *Exper. Part*.

tion site, such duplexes should not be formed. This was confirmed by melting experiments. At pH 5.0, a sigmoidal melting profile (10–80°) was not detected for **11**, neither at 260 nm 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

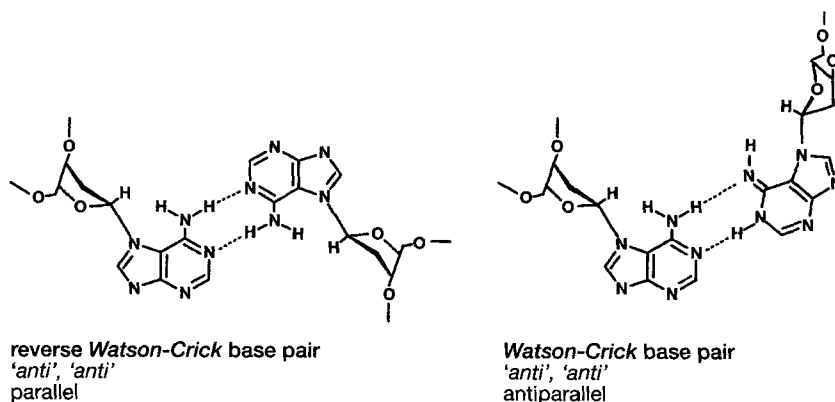


Fig. 5. Possible self-pairing modes of oligonucleotides containing compound **1**. According to the structure of **1**, Hoogsteen base pairing is excluded. For steric reasons, 'syn'-conformation of  $N^7A_d$  is not possible.

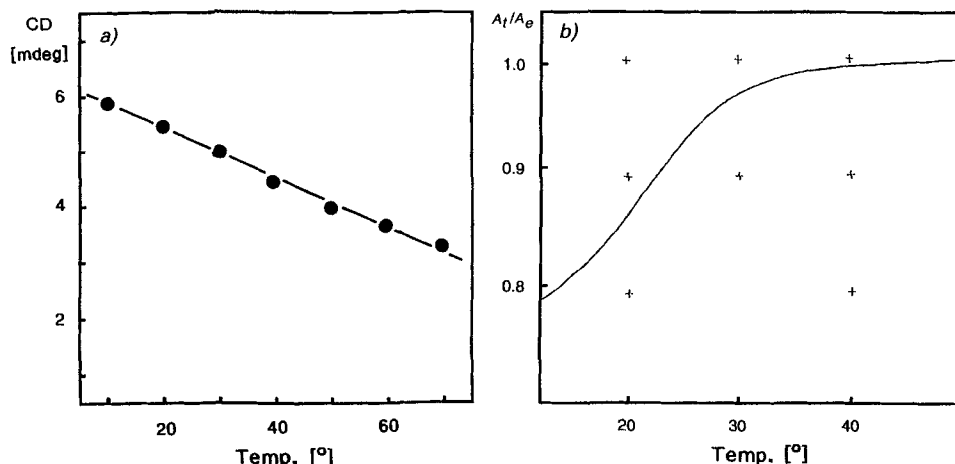


Fig. 6. a) Temperature-dependent  $B_{2u}$  transition of the CD spectra of  $d[(N^7A)_{11}-A]$  (11). b) Normalized melting profile of  $d[(N^7A)_{11}-A] \cdot d(T)_{12}$  (11 · 13). Spectra were measured in 60 mM Na-cacodylate buffer (pH 7.0, 1M NaCl, 100 mM  $MgCl_2$ ) at 6.5  $\mu M$  oligonucleotide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

Next, the duplex formation of  $d[(N^7A)_{11}-A]$  with  $d(T)_{12}$  [29] ( $\rightarrow$  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  $\pi-\pi^*$  transitions ( $B_{1u}$ ,  $B_{2u}$ ) were measured as a function of temperature

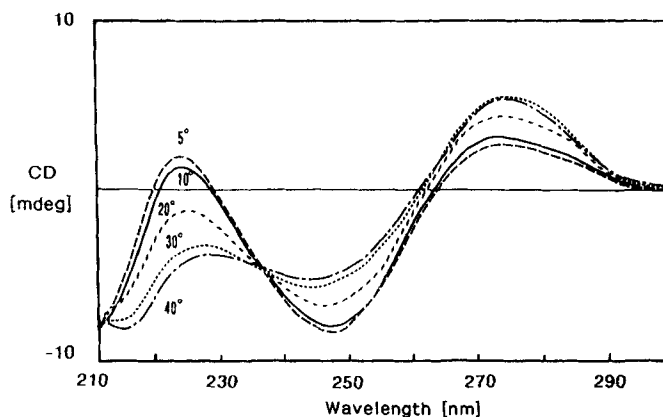


Fig. 7. Temperature-dependent CD spectra of  $d[(N^7A)_{11}-A] \cdot d(T)_{12}$  (11 · 13) at 5, 10, 20, 30, and 40°. Conditions, see Fig. 6b.

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  $\mu$ mol), we observed a single phase cooperative melting with a  $T_m$  value of 35° (Fig. 8a).

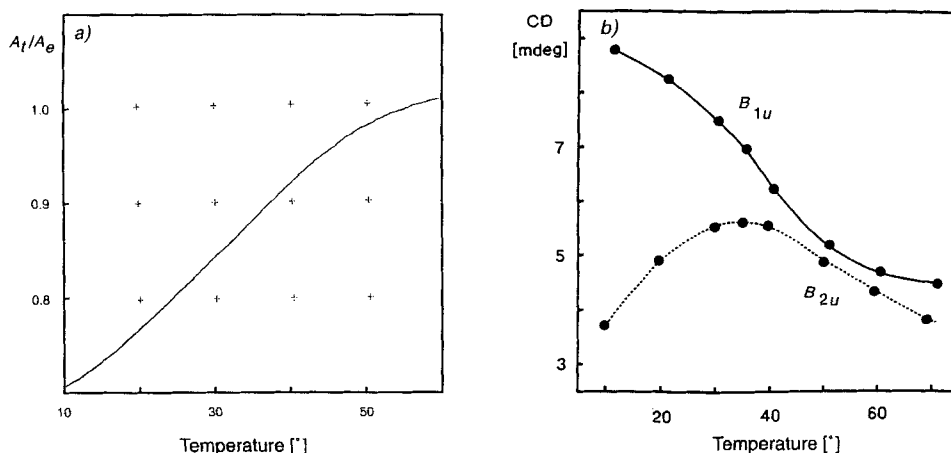


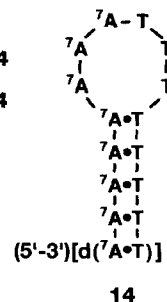
Fig. 8. a) Normalized melting profile of  $d[(N^7A)_8T_8]$  (**14**) and b) melting profiles obtained from the temperature-dependent  $B_{1u}$  and  $B_{2u}$  transitions of the CD spectra of  $d[(N^7A)_8T_8]$  (**14**). Spectra were measured in 60 mM Na-cacodylate buffer (pH 7.0, 1M NaCl, 100 mM  $MgCl_2$ ) at 4.5  $\mu$ M 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  $\mu$ M to 2.1  $\mu$ M), 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(7'A-7A-7A-7A-7A-7A-7A-T-T-T-T-T-T-T-T)] 14  
 (3'-5')[d(T-T-T-T-T-T-T-T-7A-7A-7A-7A-7A-7A-7A)] 14

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

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



7A=N<sup>7</sup>A<sub>d</sub>

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<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1, *B* = CH<sub>2</sub>Cl<sub>2</sub>/MeOH 4:1, *C* = CH<sub>2</sub>Cl<sub>2</sub>/MeOH/Et<sub>3</sub>N 83:15:2, *D* = CH<sub>2</sub>Cl<sub>2</sub>/MeOH/Et<sub>3</sub>N 88:10:2, *E* = CH<sub>2</sub>Cl<sub>2</sub>/Et<sub>3</sub>N 97:3, *F* = AcOEt/CH<sub>2</sub>Cl<sub>2</sub>/Et<sub>3</sub>N 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<sub>3</sub>N (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<sub>3</sub>NH)OAc (pH 7.0)/MeCN 95:5; *B* = MeCN. Gradient: 10 min 10–20% *B* in *A*; flow rate 1.0 ml/min. ( $\epsilon_{260}$ : A<sub>d</sub> 15400, T<sub>d</sub> 8800, N<sup>7</sup>A<sub>d</sub> 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<sub>3</sub>NH)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<sub>f</sub>* 0.28. UV (MeOH): 272 (8400). <sup>1</sup>H-NMR ((D<sub>6</sub>)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<sub>2</sub>); 8.23 (*s*, H-C(8)); 8.54 (*s*, H-C(2)). Anal. calc. for C<sub>10</sub>H<sub>13</sub>N<sub>5</sub>O<sub>3</sub> (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<sub>f</sub>* 0.35. UV (MeOH): 272 (8300). <sup>1</sup>H-NMR ((D<sub>6</sub>)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<sub>2</sub>); 8.44 (*s*, H-C(8)). Anal. calc. for C<sub>11</sub>H<sub>15</sub>N<sub>5</sub>O<sub>3</sub> (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). <sup>1</sup>H-NMR ((D<sub>6</sub>)DMSO): 1.22 (*t*, MeCH<sub>2</sub>); 2.30–2.45 (2*m*, 2 H–C(2')); 2.65 (*q*, MeCH<sub>2</sub>); 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<sub>2</sub>); 8.44 (*s*, H–C(8)). Anal. calc. for C<sub>12</sub>H<sub>17</sub>N<sub>3</sub>O<sub>3</sub> (279.3): C 51.60, H 6.13, N 25.07; found: C 51.74, H 6.27, N 25.05.

**N<sup>6</sup>-Benzoyl-7-(2-deoxy-β-D-erythro-pentofuranosyl)adenine (8)**. Compound **1** (200 mg, 0.80 mmol) was dried by co-evaporation with anhyd. pyridine and then suspended in pyridine (10 ml). Me<sub>3</sub>SiCl (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<sub>2</sub>O (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). <sup>1</sup>H-NMR (D<sub>2</sub>O): 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<sub>17</sub>H<sub>17</sub>N<sub>5</sub>O<sub>4</sub> (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<sup>6</sup>-[(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). <sup>1</sup>H-NMR ((D<sub>6</sub>)DMSO): 2.30 (2*m*, 2 H–C(2')); 3.11, 3.20 (2*s*, Me<sub>2</sub>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<sub>13</sub>H<sub>18</sub>N<sub>6</sub>O<sub>3</sub> (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<sup>6</sup>-[(dimethylamino)methylidene]adenine (10)**. Compound **9** (130 mg, 0.42 mmol) was dried by co-evaporation (twice) with dry pyridine and suspended in anhyd. 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<sub>3</sub> soln. (20 ml), the mixture was extracted 3 times with CH<sub>2</sub>Cl<sub>2</sub> (50 ml, each). The combined org. layer was dried (Na<sub>2</sub>SO<sub>4</sub>), 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). <sup>1</sup>H-NMR ((D<sub>6</sub>)DMSO): 2.50, 2.70 (2*m*, 2 H–C(2')); 3.11, 3.20 (2*s*, Me<sub>2</sub>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<sub>34</sub>H<sub>36</sub>N<sub>6</sub>O<sub>5</sub> (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<sup>6</sup>-[(dimethylamino)methylidene]adenine 3'-(Triethylammonium Phosphonate) (4)**. To a soln. of PCl<sub>3</sub> (75 μl, 0.86 mmol) and *N*-methylmorpholine (0.95 ml, 8.52 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (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<sub>2</sub>Cl<sub>2</sub> (10 ml) was added dropwise and the mixture stirred for 10 min. Thereupon, the mixture was poured into 1*M* (Et<sub>3</sub>NH)HCO<sub>3</sub> (TBK, pH 7.7, 10 ml), shaken, and separated. The aq. layer was extracted twice with CH<sub>2</sub>Cl<sub>2</sub>, the combined org. extract dried (Na<sub>2</sub>SO<sub>4</sub>) 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<sub>2</sub>Cl<sub>2</sub> (20 ml) and extracted twice with 0.1*M* (Et<sub>3</sub>NH)HCO<sub>3</sub> (5 ml). The org. layer was dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated: **4** as colorless foam (90 mg, 71%). TLC (*D*):  $R_f$  0.5. UV (MeOH): 321 (28800). <sup>1</sup>H-NMR ((D<sub>6</sub>)DMSO): 1.20 (*m*, Me); 2.50 (*m*, 2 H–C(2')); 2.93–3.00 (*m*, 2 CH<sub>2</sub>); 3.12, 3.20 (2*s*, Me<sub>2</sub>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=). <sup>31</sup>P-NMR ((D<sub>6</sub>)DMSO): 0.95; <sup>1</sup>*J*(P,H) = 583.

**7-[2-Deoxy-5-O-(4,4'-dimethoxytriphenylmethyl)-β-D-erythro-pentofuranosyl]-N<sup>6</sup>-[(dimethylamino)methylidene]adenine 3'-[(2-Cyanoethyl) *N,N*-Diisopropylphosphoramidite] (5)**. To a soln. of **10** (40 mg, 0.07 mmol) and (i-Pr)<sub>2</sub>EtN (110 μl, 0.63 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (2 ml), chloro(2-cyanoethoxy)(diisopropylamino)phosphane (90 μl, 0.39 mmol) was added within 2 min under N<sub>2</sub>. After stirring for 60 min, the reaction was quenched by adding 5% aq. NaHCO<sub>3</sub> soln. (4 ml), the mixture extracted twice with CH<sub>2</sub>Cl<sub>2</sub> (10 ml, each), and the org. layer dried (Na<sub>2</sub>SO<sub>4</sub>) 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. <sup>31</sup>P-NMR ((D<sub>6</sub>)DMSO): 148.3.

Table 2. Retention Times and Hypochromicities of Oligomers

| Oligomer                                                      | Retention time [min] <sup>a)</sup> | Hypochromicity [%] <sup>b)</sup> |
|---------------------------------------------------------------|------------------------------------|----------------------------------|
| d[(N <sup>7</sup> A) <sub>11</sub> -A] <b>11</b>              | 10.9 (I), 13.8 (II)                | 22.4                             |
| d[(N <sup>7</sup> A) <sub>8</sub> -T <sub>8</sub> ] <b>14</b> | 10.7 (I), 15.3 (II)                | 21.0                             |
| d[(N <sup>7</sup> A)-T <sub>6</sub> ] <b>15</b>               | 11.1 (I), 14.3 (II)                | 16.5                             |

<sup>a)</sup> The first values (gradient I) refer to the (MeO)<sub>2</sub>Tr derivatives; gradient II was used for the deprotected oligomers; flow rate 3.5 ml/min. <sup>b)</sup> Enzymatic hypochromicity at 260 nm.

*Solid-Phase Synthesis of the Oligonucleotides 11, 14, and 15.* The synthesis of **11**, **14**, and **15** was carried out on a 1-μmol scale using the 3'-phosphonates of [(MeO)<sub>2</sub>Tr]bz<sup>6</sup>A<sub>d</sub>, [(MeO)<sub>2</sub>Tr]T<sub>d</sub>, and [(MeO)<sub>2</sub>Tr]fam<sup>6</sup>N<sup>7</sup>A<sub>d</sub> (**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)<sub>2</sub>Tr derivatives and hypochromicities of **11**, **14**, and **15** were determined (Table 2).

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