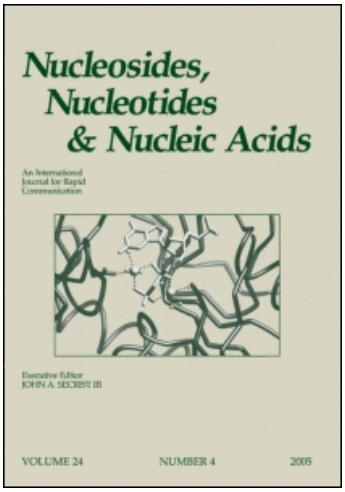


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Fluorescence Properties and Base Pair Stability of Oligonucleotides Containing 8-Aza-7-deaza-2'-deoxyisoinosine or 2'-Deoxyisoinosine

Frank Seela[a]; Georg Becher[a]; Yaoming Chen[a]
[a] Laboratorium für Organische und Bioorganische Chemie, Institut für Chemie, Universität Osnabrück, Osnabrück, Germany

To cite this Article Seela, Frank , Becher, Georg and Chen, Yaoming(2000) 'Fluorescence Properties and Base Pair Stability of Oligonucleotides Containing 8-Aza-7-deaza-2'-deoxyisoinosine or 2'-Deoxyisoinosine', Nucleosides, Nucleotides and Nucleic Acids, 19: 10, 1581 — 1598
To link to this Article: DOI: 10.1080/15257770008045448
URL: http://dx.doi.org/10.1080/15257770008045448

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 19(10–12), 1581–1598 (2000)

FLUORESCENCE PROPERTIES AND BASE PAIR STABILITY OF OLIGONUCLEOTIDES CONTAINING 8-AZA-7-DEAZA-2'-DEOXYISOINOSINE OR 2'-DEOXYISOINOSINE

Frank Seela*, Georg Becher and Yaoming Chen

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

Phone: +49(541)969-2791, Fax: +49(541)969-2370,

email: Fraseela@rz.uni-osnabrueck.de

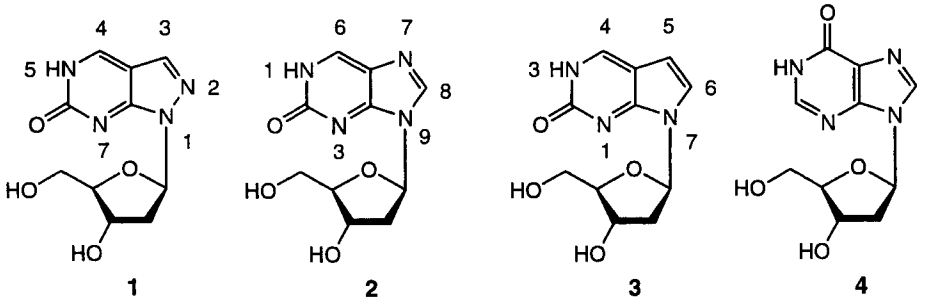
ABSTRACT: The fluorescence and the base pairing properties of 8-aza-7-deaza-2'-deoxyisoinosine (**1**) are described and compared with those of 2'-deoxyisoinosine (**2**). The corresponding phosphoramidites (**11**, **12**) are synthesized using the diphenylcarbamoyl (DPC) residue for the 2-oxo group protection. The nucleosides **1** and **2** base pair with 2'-deoxy-5-methylisocytidine in DNA duplexes with antiparallel chain orientation and with 2'-deoxycytidine in a parallel DNA. These base pairs are less stable than the canonical dA-dT pair and that of 2'-deoxyinosine (**4**) with 2'-deoxycytidine. The fluorescence of the nucleosides **1** and **2** is quenched (~95%) in duplex DNA. The residual fluorescence is used to determine the T_m-values, which are found to be the same as determined UV-spectrophotometrically.

Introduction

8-Aza-7-deaza-2'-deoxyisoinosine (**1**) represents the pyrazolo[3,4-d]pyrimidine analogue of 2'-deoxyisoinosine (**2**). The syntheses of **2** as well as of its pyrrolo[2,3-d] pyrimidine derivative **3** has been described recently.[1] An enzymatic procedure for the preparation of compound **2** was also reported.[2] Moreover, the conversion of **2** and its derivative 2',3'-didehydro-2',3'-dideoxyisoinosine into xanthosine derivatives by treatment with xanthine oxidase was investigated.[3,4] The diphenylcarbamoyl (DPC) residue was used as the 2-oxo protecting group during phosphoramidite synthesis.[5,6]

Oligonucleotides containing the nucleoside **2** have been described and the base pairing between 5-methylisocytosine and the four canonical bases were studied.[7-9]

The synthesis of the nucleoside **1** ($c^7z^8iI_d$) was performed as it was assumed that pyrazolo[3,4-d]pyrimidine nucleosides show a number of favorable properties compared to those of purine nucleosides, such as a more stable glycosylic bond and an increased duplex stability.[10] As nothing is known about the base pairing and fluorescence properties of **1**, oligonucleotides containing this nucleoside were prepared and their duplex stability was studied by temperature-dependent UV- and fluorescence spectra. These data were compared with those of oligonucleotides containing 2'-deoxyisoinosine (**2**) and with 2'-deoxyinosine (**4**).



Scheme 1

Results and Discussion

Monomers

The synthesis of 8-aza-7-deaza-2'-deoxyisoinosine (**1**) was performed by deamination of the corresponding 2-amino-8-aza-7-deazapurine 2'-deoxyribonucleoside [11] with $NaNO_2$ in acetic acid. Compound **1** is more stable in acidic solution than the parent nucleoside **2**. It is not hydrolyzed in 0.1 N HCl (30°C) within 6 h while the nucleoside **2** shows a half-life of only 24 min (30°C). Hydrolysis of **1** is observed in 1 N HCl (τ = 130 min). The hydrolysis was followed by HPLC on a RP-18 column. Similar to 7-deaza-2'-deoxyisoinosine (**3**), 8-aza-7-deaza-2'-deoxyisoinosine (**1**) is not oxidized by xanthine oxidase, while oxidation of 2'-deoxyisoinosine (**2**) leads to 2'-deoxyxanthosine.[3,4]

Like the purine nucleoside **2**, the pyrazolo[3,4-d]pyrimidine derivative **1** is fluorescent. It shows an emission maximum at 410 nm upon excitation at 316 nm. The maxima of the related nucleosides are: **2**, λ_{em} = 377 nm; λ_{ex} = 311 nm and **3**, λ_{em} = 440 nm; λ_{ex} = 328 nm. The spectra are shown in Figure 1. The Stokes shift is increased from the purine nucleoside **2** ($\Delta\lambda$ = 66 nm) via the pyrazolo[3,4-d]pyrimidine nucleoside **1** ($\Delta\lambda$ = 94 nm) to the pyrrolo[2,3-d]pyrimidine nucleoside **3** ($\Delta\lambda$ = 112 nm). The quantum yields of compounds **2** and **3** were already determined (Φ = 0.23).[3] These values served as a standard for the calculation of the quantum yield of **1**. The samples were excitated at a wavelength which was obtained from the UV curve intersection. The quantum yield was determined by comparing the integrated areas of the fluorescence of **2** and **3**. This leads to a quantum yield of Φ = 0.28 for compound **1**. Compared to the corresponding 2-amino nucleosides[13] the quantum yields of the 2-oxo compounds **1-3** are significantly decreased.

Next, compounds **1** and **2** were converted into the diphenylcarbamoyl (DPC) derivatives **5** and **8** [6,8] to protect the 2-oxo function. The stability of the DPC group was determined UV-spectrophotometrically by hydrolysis in 25% aq. NH_3 solution at 40°C. The half-life of deprotection was found to be 20 min for **8** and 19.5 min for **5**. Furthermore, the protecting group is stable in 5% dichloroacetic acid /CH_2Cl_2 (data not shown). As usual, the 4,4'-dimethoxytriphenylmethyl (DMT) group was introduced to protect the 5'-hydroxyl function.[12] In the case of **2** the overall yield of the last two reaction steps was only 29%. This yield was increased when the DMT group was introduced first, and **7** was treated with diphenylcarbamoyl chloride afterwards (**2** → **9** via **7**, 75%).

Compound **5** was converted to the DMT-protected nucleoside **6** in 75% yield. Intermediate **7** was transformed into the H-phosphonate **10** (PCl_3/N-methylmorpholine/ 1H-1,2,4-triazole, 88% yield). The phosphoramidites **11** and **12** were obtained from the derivatives **6** and **9** under standard conditions (Scheme 3).[13]

The UV-maxima of the derivatives **5** and **8** (λ_{max} = 268 and 267 nm in MeOH) are hypsochromically shifted (~ 50 nm) over that of the parent nucleosides **1** (321 nm) and **2** (322 nm). According to the UV-maximum of the related 2-methoxy-9-β-D-

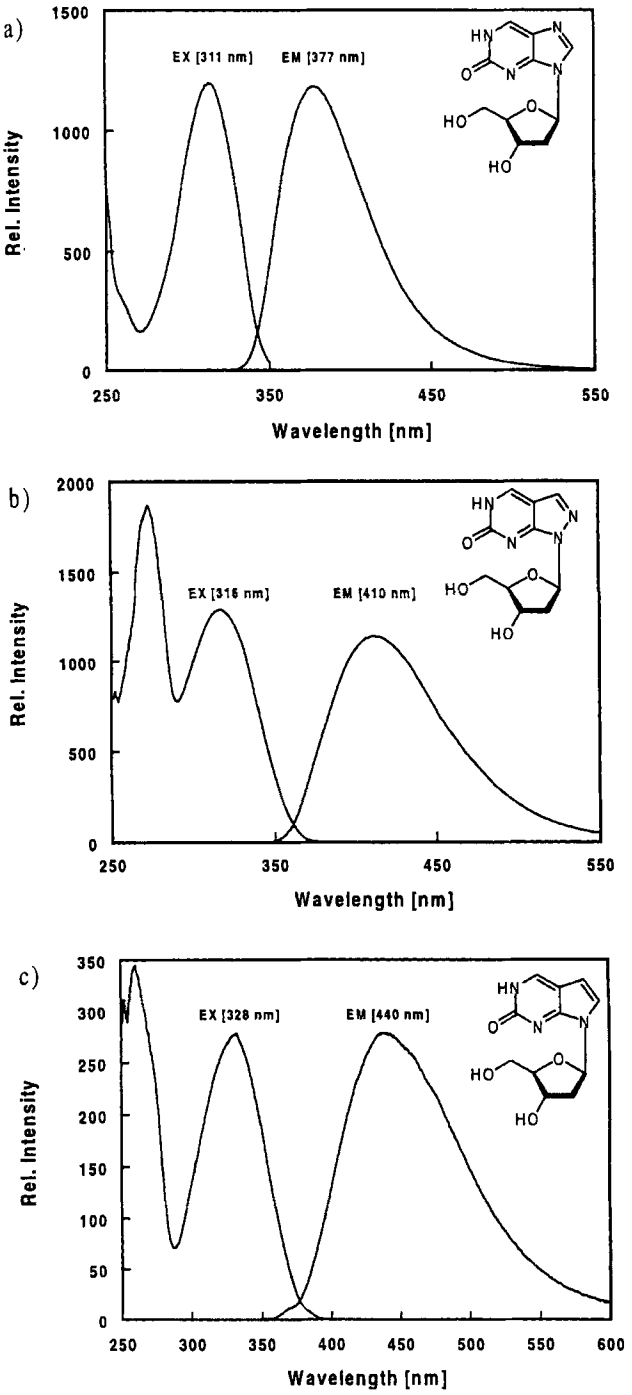
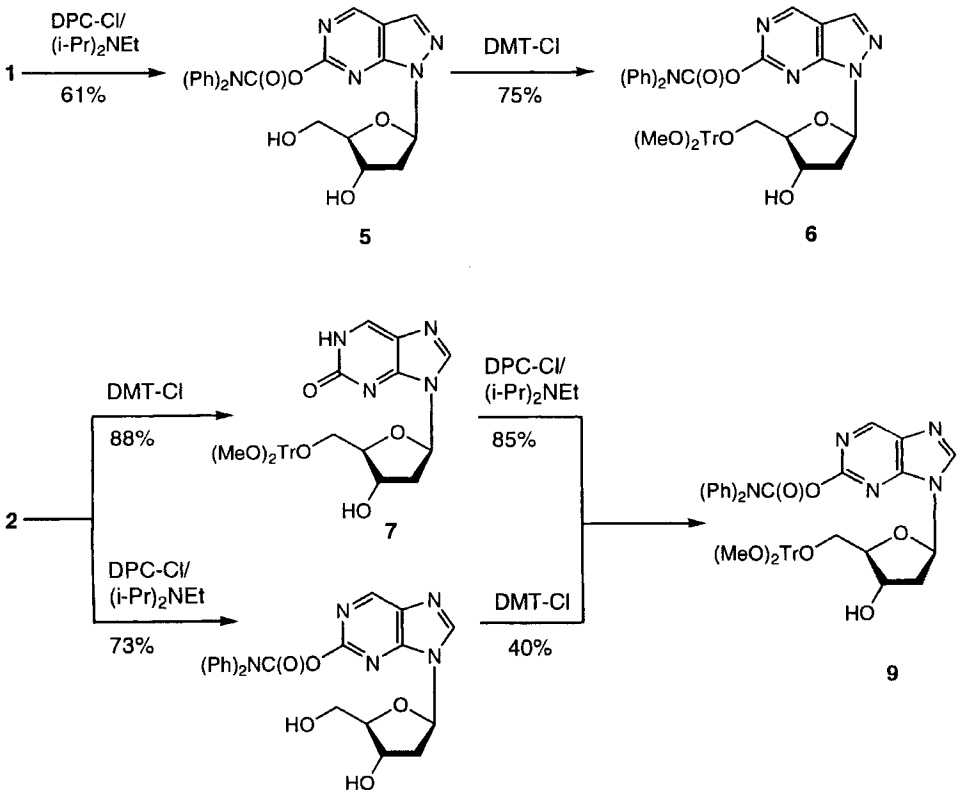
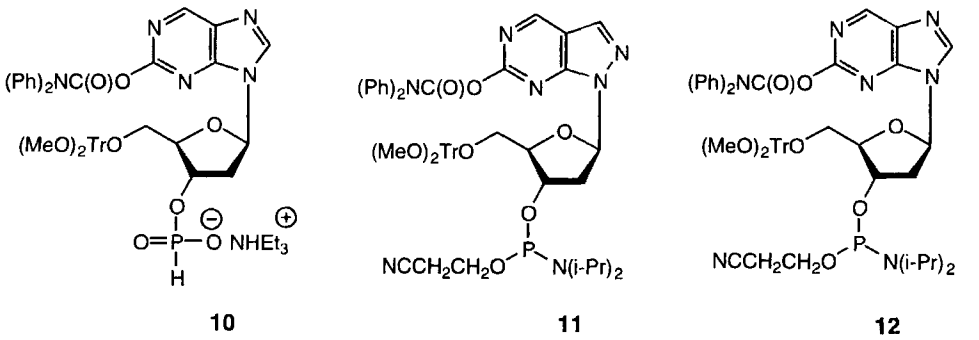


Fig.1. Fluorescence spectra of a) 2'-deoxyisoinosine (**2**), b) 8-aza-7-deaza-2'-deoxyisoinosine (**1**) and c) 7-deaza-2'-deoxyisoinosine (**3**), measured in water at 10^{-5} M concentration.



Scheme 2



Scheme 3

Table 1. ^{13}C-NMR Chemical Shifts of 2'-Deoxyisoinosine and Derivatives [a])

	C(2)[b] C(5)[c]	C(4) C(7a)	C(5) C(3a)	C(6) C(4)	C(8)	C(3)	C(1')	C(2')	C(3')	C(4')	C(5')
1	156.6[f]	159.2[f]	105.9	147.8		137.6	82.9	38.4	70.9	87.3	62.3
2	156.1	158.8	123.6	139.4	145.5		82.9	[e]	70.9	87.9	61.7
5[d]	154.9	154.8	114.1	151.7		141.4	86.5	40.0	73.1	89.1	63.9
6[d]	153.6	154.7	114.0	151.8		142.2	86.7	38.9	73.4	86.7	64.7
7	155.9	159.0	123.6	139.5	145.7		82.4	[e]	70.5	85.5	64.2
8	155.5	152.7	132.9	149.9	146.2		83.6	[e]	70.6	88.2	61.6
9	155.6	152.7	132.9	149.7	146.1		83.3	[e]	70.4	85.5	64.1
9[d]	156.3	152.7	129.8	150.1	144.0		84.0	40.9	72.2	86.2	64.2
10	155.6	152.8	133.0	150.0	145.9		83.7	[e]	72.7	85.6	63.9

a) Measured in (D_6)DMSO at 303 K. b) Purine numbering. c) Systematic numbering. d) Measured in $CDCl_3$. e) Superimposed by (D_6)DMSO. f) Tentative.

ribofuranosylpurine (λ_{max} = 281 nm) [14] the hypsochromic shift of the UV absorption demonstrates that the DPC group is attached to the 2-oxo position. Furthermore, compounds **5** and **8** were characterized by a 10-ppm downfield shift at C-6 and C-5 as well as ~6-ppm upfield shift at C-4 compared to nucleosides **1** and **2**. This was proven by ^{13}C-NMR as well as gated-decoupled ^{13}C-NMR spectra (Table 1).

Oligonucleotides

Synthesis and Characterization. Oligonucleotide synthesis was performed on solid-phase following the standard protocol.[7] The coupling efficiency of **11** and **12** was always higher than 95% (conductivity monitoring). The oligonucleotides were detritylated and purified on OPC cartridges[16] as well as by HPLC with a reversed phase RP-18 column (protocol for purification see **Experimental Part**). The homogeneity was proven by ion-exchange chromatography on a 4 X 50 mm NucleoPac PA-100 column (DIONEX, P/N 043018, USA). The composition of the oligonucleotides was analyzed by tandem hydrolysis with snake venom phosphodiesterase and alkaline phosphatase as described [5]

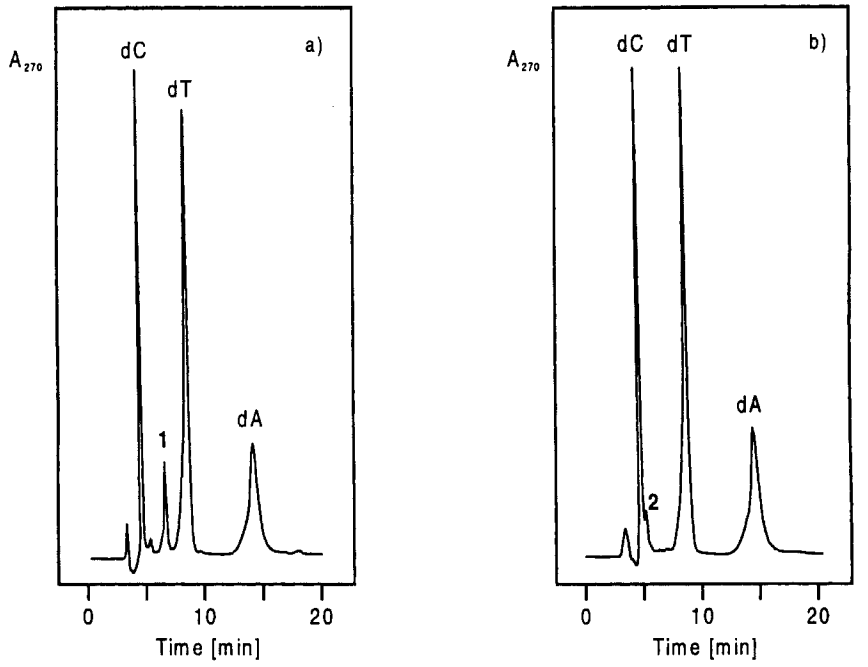


Fig. 2. HPLC Profile of the reaction products obtained after enzymatic hydrolysis of the oligomers **18** (a) and **19** (b) with snake-venom phosphodiesterase and alkaline phosphatase in 1M Tris-HCl buffer (pH 8.0) at 37°C. Column : RP-18 (200 x 10 mm) ; gradient : 0 – 30 min in 0.1 M (Et_3NH)OAc (pH 7.0) - MeCN; 95 :5.

(See **Fig.2,** the peak of compound **2** was identified by UV absorption). From the modified oligonucleotides containing **1** or **2** MALDI-TOF spectra were taken (see **Experimental Part**).

Base Pair Stability. First, compounds **1** and **2** were introduced into non self-complementary duplexes with an anti-parallel chain orientation. The base pair stabilities of me^5iC_d-(**1**) and me^5iC-(**2**) (motif I and II, Scheme 4) were determined and compared with those of the dT-dA as well as dC-dI base pair. The duplexes 5'-d(TAG GTT AAT ATT) • 3'-d(ATC CAA TTA TAA) (**13**)•(**14**) (T_m = 38°C) and 5'-d(TAG GTT AAT ATT) • 3'-d(ATC CA**4** TTA T**4**A) (**17**)•(**18**) (T_m = 35°C) served as standards. The replacement of a dT-dA by dC-dI base pair results in a small decrease of the duplex stability, while the incorporation of **1** or **2** opposite to me^5iC_d leads to a significant decrease of the T_m-value. It is found that the average stability of the duplex is reduced by

4.5°C (0.85 kcal/mol) per me^{5}iC$_d$-iI$_d$ substitution and 3.5°C (0.65 kcal/mol) per me^{5}iC$_d$-c^{7}z^{8}iI$_d$ modification. This observation is in contrast to the earlier observation describing an isoenergetic base pair of me^{5}iC$_d$ -iI$_d$ and of dA-dT.[8] Hybridization of **1** and **2** opposite to dT or dC results in a T_m-decrease of about 15°C (see Table 2), while mismatches are formed with dT or dC. The base pair me^{5}iC$_d$-(**1**) (Scheme 4, motif I) is always more stable than the me^{5}iC$_d$ – (**2**) base pair (Scheme 4, motif II). It should be mentioned that the me^{5}iC$_d$-iG$_d$ [17] base pair is more stable than that of dC-dG. Contrary to that, the me^{5}iC$_d$-(**2**) base pair is less stable than the dC-dI pair. So, it is shown that the base pair stability decreases in the order me^{5}iC$_d$-iG$_d$ > dC-dG >> dT-dA > dC-dI >> me^{5}iC$_d$-c^{7}z^{8}iI$_d$ > me^{5}iC$_d$-iI$_d$. The same tendency as observed for duplexes with two separated base pairs is found for those arranged in a consecutive manner, so that the nearest neighbor influence is minimized in these cases.

The duplex stability of non self-complementary duplexes with a parallel chain orientation was also investigated. In the parallel duplexes 5'-d(TAiGiGT**T**AATA**T**T) • 5'-d(ATCCAA**T**TATA**A**) and 5'-d(AiGTATTiGA**T**T**T**A) • 5'-d(TCATAACT**A**A**A**T) the dT-dA base pair (bold marked) was substituted by dC-(**1**), dC-(**2**) pairs (motif III and IV, Scheme 4) as well with dT- (**1**) and dT-(**2**) pairs. The stability of the parent duplexes (**33•34** and **35•36**) is reduced by 8°C when two dC-iI$_d$ were incorporated (**29•39**; **31•40**), while for dC-c^{7}z^{8}iI$_d$ the T_m is reduced by 7°C (**29•37**; **31•38**). No melting is observed in the case of dT- c^{7}z^{8}iI$_d$ or dT-iI$_d$ replacement (see Table 3). Thus, it can be seen that c^{7}z^{8}iI$_d$ or iI$_d$ form a stable base pair with dC in the parallel stranded duplex as supposed by Scheme 4.

Fluorescence Properties. Finally, the fluorescence properties of the oligomers **20** and **21** were analyzed. In a first experiment, the temperature-dependent spectra of the single strands were measured (**Fig. 3**). It should be mentioned, that the fluorescence intensity in the single strand or in the duplex is quenched by more than 95% compared to free nucleosides.[11] A temperature-dependent decrease of the fluorescence is observed, which is nearly linear. This phenomenon is due to a higher collision quenching by increasing the temperature. In a second set of experiments the oligomers **20**, **21** were hybridized with a double higher concentration of the oligomer **19** to ensure the complete hybridization.[20] When increasing the temperature, a sigmoidal melting profile was observed, leading to

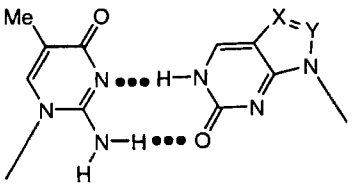
Table 2. T_m-Values and Thermodynamic Data of Duplexes with aps-Orientation [a, b, c])

Oligonucleotides	T_m	ΔG_{298}	Oligonucleotides	T_m	ΔG_{298}
5'-d(TAGGT**T**AATA**T**T) (**13**) 3'-d(ATCCA**A**TTATA**A**) (**14**)	38	-8.4	5'-d(TAGGT**G**AATA**G**T) (**15**) 3'-d(ATCCA**C**TTAT**C**A) (**16**)	46	-10.3
5'-d(TAGGT**C**AATA**C**T) (**17**) 3'-d(ATCCA**4**TTAT**4**A) (**18**)	35[15]	-7.8			
5'-d(TAGGT**i**CAATA**i**CT) (**19**) 3'-d(ATCCA **1**TTAT**1**A) (**20**)	31	-7.1	5'-d(TAGGT**i**CAATA**i**CT) (**19**) 3'-d(ATCCA**2**TTAT**2**A) (**21**)	29	-6.7
5'-d(TAGGT**T**AATA**T**T) (**13**) 3'-d(ATCCA**1**TTAT**1**A) (**20**)	26	-6.2	5'-d(TAGGT**T**AATA**T**T) (**13**) 3'-d(ATCCA**2**TTAT**2**A) (**21**)	24	-5.7
5'-d(TAGGT**C**AATA**C**T) (**17**) 3'-d(ATCCA**1**TTAT**1**A) (**20**)	22	-5.2	5'-d(TAGGT**C**AATA**C**T) (**17**) 3'-d(ATCCA**2**TTAT**2**A) (**21**)	19	-4.8
5'-d(TA**AA**CTAATACT) (**22**) 3'-d(AT**TT**GATTATGA) (**23**)	39	-8.5			
5'-d(TA**44**CTAATACT) (**24**) 3'-d(AT**CC**GATTATGA) (**25**)	36[15]	-7.6			
5'-d(TA**11**CTAATACT) (**26**) 3'-d(AT**i**C**i**CGATTATGA) (**27**)	31	-7.1	5'-d(TA**22**CTAATACT) (**28**) 3'-d(AT**i**C**i**CGATTATGA) (**27**)	30	-6.8
5'-d(TA**11**CTAATACT) (**26**) 3'-d(AT**TT**GATTATGA) (**23**)	24	-5.6	5'-d(TA**22**CTAATACT) (**28**) 3'-d(AT**TT**GATTATGA) (**23**)	22	-5.3
5'-d(TA**11**CTAATACT) (**26**) 3'-d(AT**CC**GATTATGA) (**25**)	23	-5.3	5'-d(TA**22**CTAATACT) (**28**) 3'-d(AT**CC**GATTATGA) (**25**)	22	-5.1

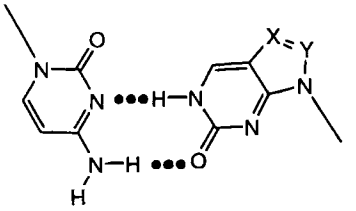
a) Measured in 0.1 M NaCl, 10 mM $MgCl_2$, 10 mM Na-cacodylate buffer, pH 7.0 at 260 nm with an oligomer concentration of 5 + 5μM. b) d(iC) = me^5iC_d = 2'-deoxy-5-methylisocytidine. c) Determination of ΔG_{298} was performed with Meltwin 3.0.[18]

the T_m-values of the duplexes (**19•20**) and (**19•21**) which are almost identical to those determined by UV-measurements (**Fig. 4**).

The results obtained from the base pairing studies and the fluorescence properties of oligonucleotides containing **1** and **2** show that the latter are useful probes to study the



aps- Watson-Crick base pair
motif I: X = C, Y = N
motif II: X = N, Y = C



ps-reverse Watson-Crick base pair
motif III: X = C, Y = N
motif IV: X = N, Y = C

Scheme 4

Table 3. T_m-Values and Thermodynamic Data of Duplexes with ps-Chain Orientation[a,b])

Oligonucleotides	T_m	ΔG_{298}	Oligonucleotides	T_m	ΔG_{298}
5'-d(TAiGiGT**C**AATA**C**T) (**29**) 5'-d(ATCCA**i**G**G**TTAT**i**G**A**) (**30**)	39[19]	-8.8	5'-d(AiGTATTiGA**CC**TA) (**31**) 5'-d(TCATAACT**i**G**i**GAT) (**32**)	44[19]	-10.3
5'-d(TAiGiGT**T**AATA**T**T) (**33**) 5'-d(ATCCAA**T**TATA**A**) (**34**)	28	-5.8	5'-d(AiGTATTiGA**TT**TA) (**35**) 5'-d(TCATAACTA**A**AT) (**36**)	28	-5.9
5'-d(TAiGiGT**C**AATA**C**T) (**29**) 5'-d(ATCCT**1**TTAT**1**A) (**37**)	21	-4.8	5'-d(AiGTATTiGA**CC**TA) (**31**) 5'-d(TCATAACT**11**AT) (**38**)	20	-4.8
5'-d(TAiGiGT**C**AATA**C**T) (**29**) 5'-d(ATCCT**2**TTAT**2**A) (**39**)	22	-4.8	5'-d(AiGTATTiGA**CC**TA) (**31**) 5'-d(TCATAACT**22**AT) (**40**)	21	-4.8
5'-d(TAiGiGT**T**AATA**T**T) (**33**) 5'-d(ATCCT**1**TTAT**1**A) (**37**)	[c])		5'-d(AiGTATTiGA**TT**TA) (**35**) 5'-d(TCATAACT**11**AT) (**38**)	[c])	
5'-d(TAiGiGT**T**AATA**T**T) (**33**) 5'-d(ATCCT**2**TTAT**2**A) (**39**)	[c])		5'-d(AiGTATTiGA**TT**TA) (**35**) 5'-d(TCATAACT**22**AT) (**40**)	[c])	

a) Measured in 1 M NaCl, 10mM $MgCl_2$, 10 mM Na-cacodylate buffer, pH 7.0 at 260 nm with an oligomer concentration of 5 + 5μM. b) d(iC) = me^5iC_d = 2'-deoxy-5-methylisocytidine. c) No melting.

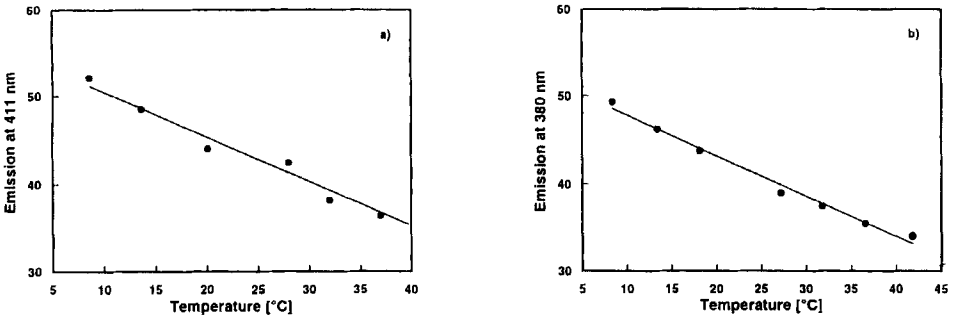


Fig. 3. Temperature-dependent fluorescence of the single strand **20** (a) and **21** (b) measured at the wavelength as indicated in 0.1 M NaCl, 10 mM $MgCl_2$ and 10 mM Na-cacodylate at pH 7.0 with (0.4 A_{260} units in 1 ml).

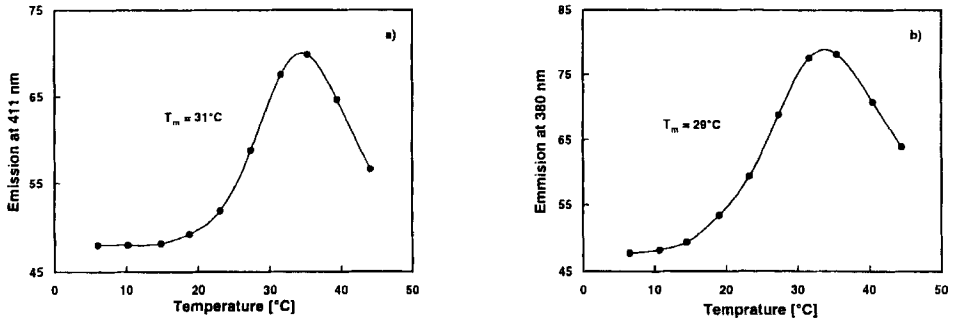


Fig. 4. Temperature-dependent fluorescence of the duplex **19** • **20** (a) and **19** • **21** (b) measured at the wavelength as indicated in 0.1 M NaCl, 10 mM $MgCl_2$ and 10 mM Na-cacodylate at pH 7.0; the concentration of the oligomer **19** (0.8 A_{260} units) was twice as high as that of **20** or **21**.

physical properties of single-stranded and double-stranded DNA. As the fluorescence of the monomers is strongly quenched when they are present in oligonucleotides, the release of the fluorescent components, e.g. by exonucleases, can be utilized to detect small quantities of nucleic acids or to determine kinetic parameters of enzymes.

EXPERIMENTAL PART

General: TLC: Aluminum sheets coated with 0.2-mm layer of silica gel *60* F_{254} (*Merck*, Germany). Flash chromatography (FC) was carried out at 0.5 bar (silica gel *60 H*

(*Merk*, Germany). A *Uvicord S* (*LKB instruments*, Sweden) UV recorder was used for detection. Solvent systems for TLC and FC: CH_2Cl_2/MeOH 95:5 (A), CH_2Cl_2/MeOH 9:1 (B), CH_2Cl_2/MeOH 3:1 (C), CH_2Cl_2/MeOH 4:1 (D), iPrOH/ H_2O/NH_3 7:2:1 (E), CH_2Cl_2/ acetone 98:2 (F), CH_2Cl_2/acetone 9:1 (G). UV Spectra: *Hitachi-150-20* spectrometer (*Hitachi*, Japan). M.p.: *Büchi-SMP-20* apparatus (*Büchi*, Switzerland). NMR Spectra: *Avance-DPX-250*, *Bruker-AC-250* and *AMX-500* spectrometer; δ values in ppm rel. to Me_4Si as internal standard (^1H- and ^{13}C-) or to external phosphoric acid (^{31}P). Fluorescence spectroscopy: F-4500 (*Hitachi*, Japan). Elemental analyses were performed by *Mikroanalytisches Laboratorium Beller*, Göttingen, Germany. MALDI-TOF spectra on a Bifex III, Scout MTP Instrument were provided by *Dr. T. Wenzel*, Bruker.

Synthesis and Purification of the Oligonucleotides 13 – 40. The synthesis was carried out on an automated DNA synthesizer (*Applied Biosystems*, Germany, model *ABI 392-08* for phosphoramidite chemistry) in a 1μmol scale with 3'-phosphoramidites of $[(MeO)_2Tr]ib^2G_d$, $[(MeO)_2Tr]bz^6A_d$, $[(MeO)_2Tr]bz^4C_d$ and $[(MeO)_2Tr]T_d$, together with the DPC-protected phosphoramidites of the compounds **11** and **12**. The synthesis of **13 – 40** followed the regular protocol of the DNA synthesizer for phosphoramidites.[13] After cleavage from the solid-support, the oligonucleotides were deprotected in 25% aq. NH_3 (12 – 15 h at 60°C). The purification of the 5'-(dimethoxytrityl)-oligomers were performed by OPC cartridges[16] as well as by reversed-phase HPLC (*RP-18*). The following solvent gradient was used (*A*, 0.1 M (Et_3NH)OAc (pH 7.0)/ MeCN 95:5; *B*, MeCN): 3 min 20% B in A, 12 min 15 – 40% B in A with a flow rate of 1.0 ml/min. To remove the 4,4'-dimethoxytrityl residues they were treated with 2.5% $CHCl_2COOH/CH_2Cl_2$ for 5 min at r.t. The detritylated oligomers were purified by reversed-phase HPLC with the gradient: 20 min 0 – 20% B in A with a flow rate of 1 ml/min. The oligomers were desalted on a short column (RP-18, silica gel) using H_2O for elution of the salt, while the oligomers were eluted with MeOH/H_2O 3:2. The oligomers were lyophilized on a *Speed-Vac* evaporator to yield colorless solids and stored frozen at –24°C.

Nucleoside Composition Analysis. The analyses were performed as described.[4] Extinction coefficients of the nucleoside constituents: ε_{260}: dA 15400, dT 8800, dG 11700, dC 7600, iG_d 7400, m^5iC_d 6300. Snake-venom phosphodiesterase (EC 3.1.4.1.,

Table 4. Molecular Masses M^+ of Oligonucleotides Determined by MALDI-TOF Mass Spectroscopy

Oligomer	M^+ (calc.)	M^+ (found)
5'-d(A**1**TATT**1**ACCTA) (**20**)	3613	3613
5'-d(A**2**TATT**2**ACCTA) (**21**)	3613	3613
5'-d(TA**11**CTAATACT) (**26**)	3613	3614
5'-d(TA**22**CTAATACT) (**28**)	3613	3613
5'-d(ATCCT**1**TTAT**1**A) (**37**)	3613	3613
5'-d(ATCCT**2**TTAT**2**A) (**39**)	3613	3614
5'-d(TCATAACT**11**AT) (**38**)	3613	3613
5'-d(TCATAACT**22**AT) (**40**)	3613	3614

Crotallus durissus) and alkaline phosphatase (EC 3.1.3.1., *E. coli*) were generous gifts from *Roche Diagnostics GmbH*, Germany.

Determination of T_m-Values and Thermodynamic Data. Absorbance *vs.* temperature profiles were measured on a *Cary-1/1E* UV/VIS spectrometer (*Varian*, Australia) equipped with a Cary thermoelectrical controller. The T_m-values were determined in the reference cell with a Pt-*100* resistor, and the thermodynamic data were calculated using the Meltwin 3.0 program[18].

1-(2-Deoxy-ß-D-erythro-pentofuranosyl)-1,5-dihydro-6H-pyrazolo[3,4-d]pyrimidin-6-one, (8-aza-7-deaza-2'-deoxyisoinosine) (1). To a solution of 6-amino-(2-deoxy-β-D-erythro-pentofuranosyl)-1H-pyrazolo[3,4-d]pyrimidine [11] (100 mg, 0.4 mmol) and $NaNO_2$ (125 mg, 1.81 mmol) in H_2O, AcOH (183 µl) was added dropwise at 50°C under stirring. Stirring was continued for 5 min, then the pH was adjusted to 8.0 (25% aq. NH_3). The solution was applied to a *Serdolit AD-4* column (4 x 20 cm, resin 0.1 - 0.2 mm; *Serva*, Germany). The column was washed with H_2O (300 ml), and the reaction product was eluted with H_2O/i-PrOH (9:1), the solvent was evaporated to give a colorless amorphous powder (70 mg, 69%). TLC (E): R_f 0.5. UV (MeOH): 242 (4700),

275 (4200), 322 (3900). ^{1}H-NMR (D_6-DMSO): 2.20 (m, 1H-C(2')); 2.69 (m, 1H-C(2')); 3.38 - 3.49 (m, 2H-C(5')); 3.78 (m, 1H-C(4')); 4.37 (m, 1H-C(3')); 4.92 (t, J = 5.6 Hz, OH-(5')) 5.37 (d, J = 4.5 Hz, OH-C(3')); 6.35 (t, J = 6.5, H-C(1')); 8.06 (s, 1H-C(7)); 8.72 (s, 1H-C(6)). Anal. calc. for $C_{10}H_{12}N_4O_4$ (252.2): C 47.62, H 4.80, N 22.21; found: C 47.48, H 4.78, N 22.01.

6-(N,N-Diphenylcarbamoyl)oxy-1-(2-deoxy-ß-D-erythro-pentofuranosyl)-1H-pyrazolo[3,4-d]pyrimidine (5). Compound **1** (100 mg, 0.4 mmol) was dried by coevaporation with dry pyridine (3 x 10 ml) and was suspended in dry pyridine (2 ml). Then, i-Pr_2EtN (100 µl) was added under stirring, and the mixture was cooled in an ice bath. Diphenylcarbamoyl chloride (140 mg, 0.6 mmol) was added, and stirring was continued for 3 h. The dark reaction mixture was poured into 5% aq. $NaHCO_3$ (10 ml) and extracted with CH_2Cl_2 (3 x 20 ml). The combined organic extracts were dried (anhydrous Na_2SO_4), filtered and concentrated to dryness. The residue was submitted to FC (silica gel, column 2 x 15 cm, eluant F followed by G), and the fractions of the main zone were collected and evaporated to give a colorless foam (110 mg, 61 %). TLC (B): R_f 0.3. UV (MeOH): 224 (16500); 268 (7900). ^{1}H-NMR ($CDCl_3$): 2.51 (m, 1H-C(2')); 2.91 (m, 1H-C(2')); 3.78 - 3.86 (m, 2H-C(5')); 4.16 (m, 1H-C(4')); 4.78 (m, H-C(3')); 4.93 (t, J = 5.5 Hz, OH-(5')); 5.31(d, J = 4.2 Hz, OH-(3')); 6.87 (t, J = 7.4 Hz, H-C(1')); 7.24-7.50 (m, aromatic H); 8.18 (s, H-C(7)); 9.10 (s, H-C(6)). Anal. calc. for $C_{23}H_{21}N_5O_5$ (447.4): C 61.74, H 4.73, N 15.65; found: C 61.79, H 4.78, N 15.62.

2-(N,N-Diphenylcarbamoyl)oxy-9-(2-deoxy-ß-D-erythro-pentofuranosyl)-9H-purine (8). As described for compound **5**, with compound **2**[1] (100 mg, 0.4 mmol), diphenylcarbamoyl chloride (140 mg, 0.6 mmol) and i-Pr_2EtN (100 µl) in anh. pyridine (2 ml). The mixture was stirred for 3 h at r.t, and after FC (silica gel, 2 x 15 cm, eluant A) a colorless foam (130 mg, 73%) was isolated. TLC (B): R_f 0.4. UV (MeOH): 224 (16300); 267 (7900). ^{1}H-NMR (D_6-DMSO): 2.37, 2.71 (2m, 2H-C(2')); 3.50 – 3.67 (m, 2H-C(5')); 3.88 (m, H-C(4')); 4.43 (m, H-C(3')); 4.95 (t, J = 5.43 Hz, OH-(5')); 5.36(d, J = 4.19 Hz, OH-(3')); 6.41 (t, J = 6.57 Hz, H-C(1')); 7.3-7.5 (m, aromatic H); 8.82 (s, H-C(8)); 9.11 (s, H-C(6)). Anal. calc. for $C_{23}H_{21}N_5O_5$ (447.4): C 61.74, H 4.73, N 15.65; found: C 61.89, H 4.88, N 15.50.

6-(N,N-Diphenylcarbamoyl)oxy-1-[2-deoxy-5-O-(4,4'-dimethoxytrityl)-ß-D-erythro-pentofuranosyl]-1H-pyrazolo[3,4-d]pyrimidine (6). Compound **5** (110 mg, 0.25 mmol) was dried by co-evaporation with dry pyridine (3 x 10 ml), suspended in dry pyridine (0.5 ml) and treated with $(MeO)_2TrCl$ (92 mg, 0.27 mmol) under stirring for 1.5 h at r.t. After addition of MeOH (2 ml), the reaction mixture was treated with 5% aq. $NaHCO_3$ (20 ml) and extracted with CH_2Cl_2 (3 x 20 ml) and then washed with water. The combined organic layers were dried (Na_2SO_4), filtered and evaporated. The residue was dissolved in CH_2Cl_2 and submitted to FC (silica gel column 2.5 x 10 cm, eluant F). The content of the main zone was isolated after evaporation, yielding a colorless foam (140 mg, 75%). TLC (F): R_f = 0.4. UV (MeOH): 237 (27500), 264 (10800), 272 (10800). ^{1}H-NMR ($CDCl_3$): 2.49 (m, 1H-C(2')); 2.99 (m, 1H-C(2')); 3.25 – 3.32 (m, 2H-C(5')); 3.78 (s, $2CH_3O$); 4.07 (m, H-C(4')); 4.87 (m, H-C(3')); 5.46 (d, J = 4.1 Hz, OH-(3')); 6.79 (t, J = 6.1 Hz, H-C(1')); 7.20-7.46 (m, aromatic H); 8.06 (s, H-C(7)); 9.05 (s, H-C(6)). Anal. calc. for $C_{44}H_{39}N_5O_7$ (749.81): C 70.48, H 5.24, N 9.34; found: C 70.59, H 5.29, N 9.38.

2-(N,N-Diphenylcarbamoyl)oxy-9-[2-deoxy-5-O-(4,4'-dimethoxytrityl)-ß-D-erythro-pentofuranosyl]-9H-purine (9). Method A: As described for **6**, with compound **8** (100 mg, 0.22 mmol), $(MeO)_2TrCl$ (90 mg, 0.27 mmol) in pyridine (3 ml). The solution was stirred overnight at r.t. and then diluted with 2 ml MeOH. After FC (silica gel, 2 x 8 cm, eluant A containing traces of Et_3N) from the main zone a colorless foam (66 mg, 40%) was isolated.

Method B: A solution of **7** [5] (300 mg, 0.54 mmol) and i-Pr_2EtN (150 µl) in dry pyridine (5 ml) was cooled in an ice bath. Diphenylcarbamoyl chloride (153 mg, 0.66 mmol) was added, and the solution was stirred for 1 h. The reaction mixture which became dark was poured into 5% aq. $NaHCO_3$ (20 ml), extracted with CH_2Cl_2 (3 x 20 ml), and the extract was washed with water. The combined organic layers were dried (Na_2SO_4) filtered and evaporated. The residue was submitted to FC (silica gel column 3 x 9 cm, eluant A containing traces of Et_3N). From the main zone a colorless foam (345 mg, 85 %) was obtained. The analytical data of this sample are identical with those of compound **7** prepared from compound **6**. TLC (B): R_f = 0.6. ^{1}H-NMR (D_6-DMSO): 2.38, 2.82 (2m,

2H-C(2')); 3.15 (m, 2H-C(5')); 3.67 (s, $2CH_3O$); 3.98 (m, H-C(4')); 4.47 (m, H-C(3')); 5.43 (d, J = 4.6 Hz, OH-(3')); 6.43 (t, J = 6.1 Hz, H-C(1')); 6.7-7.4 (m, aromatic H); 9.70 (s, H-C(8)); 9.12 (s, H-C(6)). Anal. calc. for $C_{44}H_{39}N_5O_7$ (749.81): C 70.48, H 5.24, N 9.34; found: C 70.64, H 5.33, N 9.30.

2-(N,N-Diphenylcarbamoyl)oxy-9-[2-deoxy-5-O-(4,4'-dimethoxytrityl)-ß-D-erythro-pentofuranosyl]-9H-purine 3'-H-phosphonate triethylammonium salt (10). To a solution of PCl_3 (350 µl, 4.0 mmol) and N-methylmorpholine (4.46 ml, 47 mmol) in CH_2Cl_2 (25 ml), 1,2,4-triazole (930 mg, 13 mmol) was added at r.t. under stirring. After 30 min the mixture was cooled to 0°C and a solution of compound **7** (620 mg, 0.83 mmol) in CH_2Cl_2 (25 ml) was slowly added. After stirring had continued for 30 min at r.t., the reaction mixture was poured into 1 M $(Et_3N)HCO_3$ (50 ml), shaken and separated. The aqueous layer was extracted with CH_2Cl_2 (3 x 50 ml). The combined organic extracts were dried (Na_2SO_4), filtered and evaporated. The residue was dissolved in CH_2Cl_2 and submitted to FC (silica gel column 3 x 9 cm, CH_2Cl_2/MeOH/Et_3N 88:10:2), the fraction of the main zone was collected and evaporated to dryness. The residue was dissolved in CH_2Cl_2 (100 ml) and washed with 0.1 M $(Et_3NH)HCO_3$ (5 x 20 ml). The organic layer was dried (Na_2SO_4), filtered and evaporated to give a colorless foam (670 mg, 88%). TLC (silica gel, CH_2Cl_2/MeOH/Et_3N 88:10:2): R_f = 0.3. ^{1}H-NMR (D_6-DMSO): 1.12 (t, J = 7.23 Hz, $3CH_3$); 2.55, (m, H-C(2')); 2.98 (q, J = 7.26 Hz, $3CH_2$); 3.18 (m, 2H-C(5')); 3.68 (s, $2CH_3O$); 4.18 (m, H-C(4')); 4.84 (m, H-C(3')); 6.67 (d, J (P,H) = 588 Hz, PH); 6.43 (t, J = 6.50 Hz H-C(1')); 6.7-7.5 (m, aromatic H); 8.70 (s, H-C(8)); 9.13 (s, H-C(6)). ^{31}P-NMR (D_6-DMSO): 1.25 (^{1}J (P,H) = 589 Hz, ^{3}J (P,H-C(3')) = 8.98 Hz). Anal. Calc. for $C_{50}H_{55}N_6O_9P$ (915.01): C 65.63, H 6.06, N 9.18; found: C 65.56, H 6.13, N 9.31.

6-(N,N-Diphenylcarbamoyl)oxy-1-[2-deoxy-5-O-(4,4'-dimethoxytrityl)-ß-D-erythro-pentofuranosyl]-1H-pyrazolo[3,4-d]pyrimidine 3'-[(2-Cyanoethyl)-N,N-diisopropylphosphoramidite] (11). To a soln. of compound **6** (130 mg, 0.17 mmol) and i-Pr_2EtN (85 µl, 0.61 mmol) in anh. THF (1 ml), chloro(2-cyanoethoxy)-(diisopropylamino)-phosphane (46 µl, 0.21 mmol) was added at r.t. After stirring for 30

min, the mixture was diluted with CH_2Cl_2/MeOH (10 ml) and quenched by adding 5% aq. $NaHCO_3$ soln. (5 ml). The aq. layer was extracted with CH_2Cl_2 (3 x 20 ml), the combined organic extracts were dried (anhydrous Na_2SO_4), filtered and evaporated to dryness. The residue was applied to FC (silica gel 2.5 x 10 cm, CH_2Cl_2/EtOAc 85:15). From the main zone a colorless foam (110 mg, 68%) was obtained. TLC (CH_2Cl_2/EtOAc 85:15) R_f 0.9. ^{31}P-NMR ($CDCl_3$): 149.76, 149.92.

2-(N,N-Diphenylcarbamoyl)oxy-9-[2-deoxy-5-O-(4,4'-dimethoxytrityl)- ß-D-erythro-pentofuranosyl]-9H-purine 3'-[(2-Cyanoethyl)-N,N-diisopropyl-phosphoramidite] (12). As described for **11**, with **9** (175 mg, 0.23 mmol), i-Pr_2EtN (160 µl, 0.92 mmol), chloro(2-cyanoethoxy)(diisopropyl-amino)phosphane (110 µl, 0.49 mmol) in anh. CH_2Cl_2 (3 ml) at r.t. After stirring for 30 min, the mixture was diluted with CH_2Cl_2 (10 ml). After FC (silica gel, 10 x 2.5 cm, CH_2Cl_2/EtOAc/Et_3N 47.5:47.5:5) a colorless foam (100 mg, 46%) was isolated. TLC (silica gel, CH_2Cl_2/EtOAc) R_f 0.9. ^{31}P-NMR ((D_6)DMSO): 149.75, 149.71.

Acknowledgements

We thank Dr. H. Rosemeyer and Mr. Y. He for measurement of the NMR spectra and Dr. T. Wenzel for the MALDI-TOF spectra. Financial support by the *Deutsche Forschungs-gemeinschaft* is gratefully acknowledged.

REFERENCES

1. Seela, F.; Chen, Y.; Bindig, U.; Kazimierczuk, Z. *Helv. Chim. Acta* **1994**, *77*, 194-202.
2. Beaussire, J. - J.; Pochet, S. *Nucleosides Nucleotides* **1995**, *14*, 805-808.
3. Seela, F.; Chen, Y.; Sauer, M. *Nucleosides Nucleotides* **1998**, *17*, 39-52.
4. Seela, F.; Chen, Y. *Nucleosides Nucleotides* **1995**, *14*, 863-866.
5. Seela, F.; Chen, Y.; Melenewski, A.; Rosemeyer, H.; Wei, C. *Acta Biochimica Polonica* **1996**, *43*, 45-52.
6. Kamimura, T.; Tsuchiya, M.; Koura, K.; Sekine, M.; Hata, T. *Tetrahedron Lett.* **1983**, *24*, 2775-2278.
7. Seela, F.; Chen, Y. *Nucleic Acids Res.* **1995**, *23*, 2499-2505.
8. Beaussire, J. - J.; Pochet, S., *Tetrahedron* **1998**, *54*, 13547-13556.
9. Beaussire, J. - J.; Pochet, S., *Nucleosides Nucleotides* **1999**, *18*, 403-410.
10. Seela, F.; Steker, H. *J. Chem. Soc. Perkin Trans. I* **1985**, 2573-2576.

11. Seela, F.; Becher, G. *Helv. Chim. Acta* **2000**, *83*, 928-942..
12. Schaller, H.; Weimann, G.; Lerch, B.; Khorana, H. G. *J. Am. Chem. Soc.* **1963**, *85*, 3821-3827.
13 Beaucage, S. L.; Caruthers, M. *Tetrahedron Lett.* **1981**, *22*, 1859-1862.
14. Schaeffer, H.J.; Thomas, H. J. *J. Am. Chem Soc.* **1958**, *80*, 4896-4899.
15. Seela, F.; Mittelbach, C. *Nucleosides Nucleotides* **1999**, *18*, 525-441.
16. Applied Biosystems, Oligonucleotide Purification Cartridges.
17. Horn, T.; Chang, C. - A.; Collins, M. L. *Tetrahedron Lett.* **1995**, *36*, 2033-2036.
18. McDowell, J. A.; Turner, D. H. *Biochemisty* **1996**, *35*, 14077-14089.
19. Seela, F.; Wei, C.; Becher, G.; Zulauf, M.; Leonard, P. *Bioorg. & Med. Chem. Lett.* **2000**, *10*, 289-292.
20. Ha, T.; Enderle, T.; Ogletree, D. F.; Chemila, D. S.; Selvin, P. R.; Weiss, S. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 6264-6269.