173. Synthesis of Phosphonates and Oligodeoxyribonucleotides Derived from 2′-Deoxyisoguanosine and 2′-Deoxy-2-haloadenosines

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Dedicated to Prof. Dr. *F. Eckstein* on the occasion of his 60th birthday

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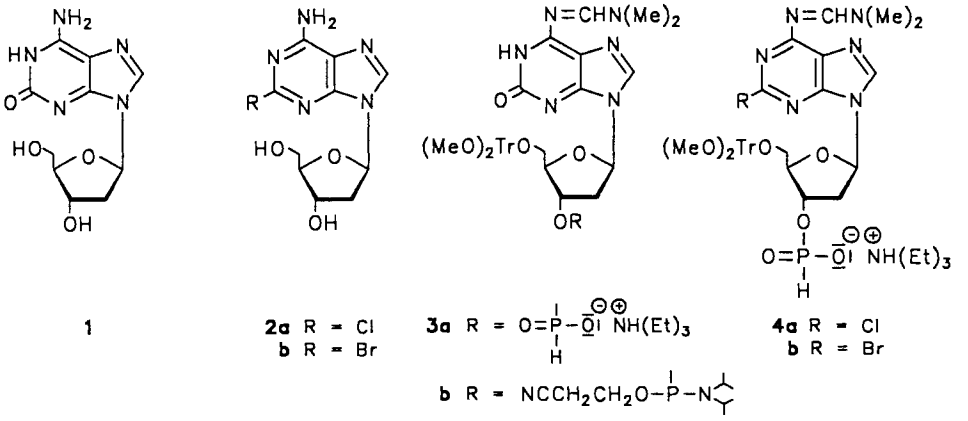
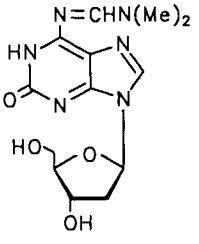
Oligonucleotides containing 2′-deoxyisoguanosine (**1**) or 2-chloro-2′-deoxyadenosine (**2a**) have been prepared by solid-phase synthesis. Suitably protected phosphonates **3a**, **4a**, and **4b** as well as the phosphoramidite of **1** have been obtained from the nucleosides **1**, **2a**, or **2b** *via* the (dimethylamino)methylidene derivatives **5**–**7**. 4,4′-Dimethoxytrityl groups were introduced to yield the base-protected derivatives **8**–**10**. Alternatively to the direct incorporation of **1** into oligonucleotides, they were also obtained by the photochemical conversion of a **2a** residue within the oligonucleotide chain.

Introduction. – The recognition of DNA single strands during duplex or triplex formation depends on the nitrogen pattern of the nucleobase [1], the type and position of exocyclic substituents [2], and the positioning of bases on a particular sugar-phosphate backbone [3] [4]. All three structural elements can be altered leading to new DNA structures with uncommon recognition pattern. Recently, the 5′-triphosphate of 2′-deoxyisoguanosine (**1**) has been incorporated enzymatically into DNA fragments. It was found opposite to $isoC_d$ when DNA polymerase I was used on a modified template [5].



Polyribonucleotides of isoguanosine have also been prepared using polynucleotides phosphorylase [6] or T7 RNA polymerase [5]. Recently, the photochemical synthesis of **1** [7] from the antileukemic 2-chloro-2′-deoxyadenosine (**2a**) [8] has been reported. Here, we report on the synthesis of suitably protected phosphonates and phosphoramidites **3a**, **b** and **4a**, **b** for oligonucleotide synthesis. Two different strategies will be followed: *i*) direct incorporation of **1** using the building block **3a** or *ii*) incorporation of the precursor **2a** *via* **4a** followed by photochemical conversion into a **1** residue within an oligonucleotide.

Results and Discussion. – Starting materials for the synthesis of the phosphonates **3a** or **4a**, **b** were the nucleosides **1**, **2a** [9] [10], or **2b** [11]. Reaction of **1**, **2a**, or **2b** with *N*,*N*-dimethylformamide diethyl acetal afforded the amidines **5**–**7**. Their structure was confirmed by ^{1}H- and ^{13}C-NMR spectra (*Table 1* and *Exper. Part*). ^{13}C-NMR Chemical shifts were assigned from the gated-decoupled spectra (*Table 2*). According to the ^{1}H-NMR spectrum of **5**, the 5′-OH group is shifted downfield compared to the compounds **6** or **7**, implying H-bonding. An H-bridge between HO–C(5′), and the 2-oxo group or H–N(3) of the base can be considered leading to a preferred '*syn*'-conformation. This was substantiated by ^{1}H-NMR-NOE measurements. Upon irradiation of H–C(8) of compound **5** an NOE of 6.4% was observed for H–C(1′). Using a graphical method for the determination of '*syn*'/'*anti*'-populations around the *N*-glycosylic bond [12], a '*syn*'-population of 60% was found.



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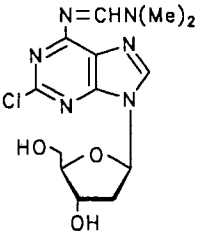

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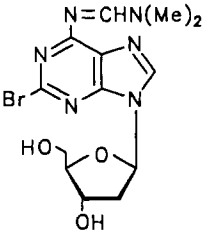

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The introduction of the (dimethylamino)methylidene group [13] circumvents the transient protection of the sugar OH groups, stabilizes the *N*-glycosylic bond, and it can be removed under much milder conditions as acyl-protecting groups [14] [15]. Furthermore, it has been shown that amidine-protected residues of common or modified nucleosides can be used effectively during phosphoramidite [16] or phosphonate synthesis [17–21] of oligonucleotides.

To test the utility of the amidine protecting group for oligonucleotide synthesis, compounds **5**–**7** were hydrolyzed in 25% aq. NH_3 at 40°. The reaction was followed by UV spectrophotometry at 335 nm for **5**, at 264 nm for **6**, and 316 nm for **7**. A half-life time of 5.4 min was observed for **5** and of 1.4 min for **6** and **7**. This made the amidine-protected derivatives suitable for oligonucleotide synthesis and compatible to regular DNA constituents. Next, the 4,4′-dimethoxytrityl (DMT) residues were introduced. Standard conditions were chosen [19] affording **8**–**10**. The position of tritylation was confirmed by the ^{13}C-NMR spectra exhibiting upfield shifts of C(4′) and the downfield shifts of C(5′) compared to the parent 2′-deoxynucleosides and similar to other DMT-protected deriva-

Table 1. *^{13}C-NMR Chemical Shifts of Purine 2′-Deoxyribonucleosides in (D_6)DMSO at 23°*

	C(2)	C(4)	C(5)	C(6)	C(8)	NCH_3
3a[a])	156.5	157.4	113.5	154.4	139.2	34.3[a])
4a[a])	152.5	152.3	125.0	160.2	141.6	34.9/41.0
4b[a])	143.7	152.2	125.4	160.0	141.8	34.9/41.0
5[b])	156.5	157.0	113.4	154.6	140.1	34.3/41.1
6[b])	152.4	152.2	124.7	160.2	141.6	34.9/41.0
7[b])	143.6	152.2	125.1	160.1	141.4	34.9/41.0
8[a])	156.6	157.6	113.4	154.5	139.6	34.4/41.2
9[a])	152.5	152.2	125.0	160.2	141.8	34.9/41.0
10[a])	143.7	152.1	125.4	160.0	141.6	34.9/41.0
	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	OCH_3
3a	84.5	[c])	72.5	85.5	63.9	55.0
4a	83.9	[c])	71.5	85.5	64.0	55.0
4b	83.9	[c])	71.5	85.5	64.0	55.0
5[b])	83.5	[c])	70.9	87.9	61.1	
6[b])	83.6	39.2	70.7	88.0	61.6	
7[b])	83.6	39.2	70.7	88.0	61.6	
8	82.3	[c])	70.6	85.5	64.3	55.1
9	83.6	[c])	70.6	86.2	64.2	55.0
10	83.6	[c])	70.6	86.1	64.2	55.0

[a]) Analogously to the amidine protected nucleoside.
[b]) From gated-decoupled spectra.
[c]) Superimposed by DMSO.

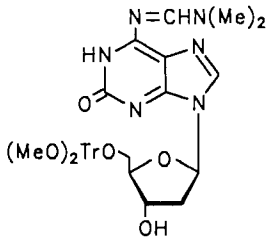
Table 2. *J(C,H) Values* [Hz] *of (Dimethylamino)methylidene Nucleosides*[a])

J(C(8),H–C(8))	214.1	214.3	214.4
J(C(8),H–C(1′))	4.9	4.0	4.1
J(C(6),N=C*H*NMe_2)	6.1	7.2	7.0
J(C(5),H–C(8))	11.8	11.6	11.6
J(C(4),H–C(8)), H–C(1′)	*m*	*m*	*m*
J(C(=N–C),N=C*H*NMe_2)	161.5	184.5	177.9
J(C(1′),H–C(1′))	164.6	166.7	166.7
J(C(3′),H–C(3′))	152.3	148.8	150.2
J(C(4′),H–C(4′))	148.4	146.6	146.6
J(C(5′),H–C(5′))	140.9	139.0	139.0

[a]) From spectra measured in (D_6)DMSO.

tives [19]. Tritylation of compound **5** was more difficult as in the case of **6** or **7**, and some starting material was recovered.

Two approaches are commonly used for solid-phase oligonucleotide synthesis: phosphoramidite or phosphonate chemistry. Although the phosphoramidite protocol offers advantages during the synthesis of long-chain oligonucleotides, preparation of modified oligonucleotides or large-scale synthesis of oligonucleotides are better to perform with phosphonates. Monomeric phosphonates are extremely stable against oxidation, allowing their recovery after oligonucleotide synthesis, and purification of small



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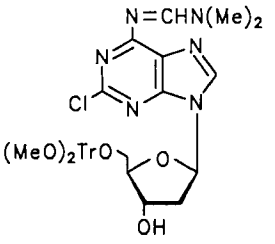

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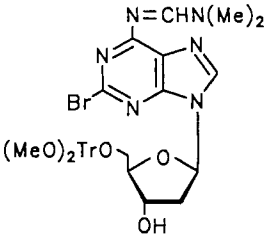

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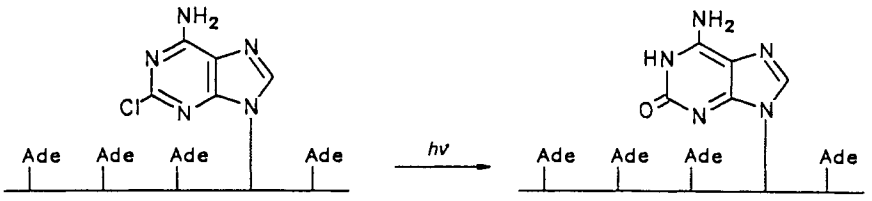
amounts of modified building blocks is more easy in the case of phosphonates than of phosphoramidites. Reaction of **8**–**10** with PCl_3/*N*-methylmorpholine/1,2,4-triazole in CH_2Cl_2 afforded the 3′-phosphonates **3a** and **4a**, **b**. They were purified chromatographically and isolated as triethylammonium salts. The phosphoramidite **3b** was prepared for comparison purposes.

The phosphonates **3a** and **4a** were then employed in solid-phase synthesis, together with the 3′-phosphonates of regular 2′-deoxyribonucleosides. Oligonucleotide synthesis was performed in an automated synthesizer. The protocol of detritylation, activation, coupling, and capping followed the user bulletin of *Applied Biosystems* [22]. Oxidation with I_2 was carried out on the oligomeric level. The $(MeO)_2Tr$-protected oligonucleotides were removed from the support with NH_3 and then purified by reverse-phase *RP-18* HPLC. Detritylation in aq. AcOH was followed by neutralization with Et_3N, and the products were again submitted to *RP-18* HPLC, desalted, and lyophilized. The following oligonucleotides were prepared:

d(A-A-A-iG-A)	d(A-A-A-Cl^{2}A-A)	d(iG-T-iG-T-iG-T-iG-T-iG-T-iG-T)
11	**12**	**13**

The oligomer **11** was prepared by two different routes: direct solid-phase synthesis using the phosponate **3a**, or synthesis of the precursor oligomer **12** with **4a**. The **2a**-containing oligomer **12** was then converted photochemically into **11** (*Scheme*). This was

Scheme



accomplished by irradiation with a Hg lamp, followed by HPLC purification. *Fig. 1* shows the HPLC profile of samples, taken from the conversion of **12** into **11** at different intervals of time. The oligomer obtained by the direct synthesis showed identical properties as that obtained photochemically. Apart from that, the oligonucleotide **13** was synthesized by the direct route. Compound **13** formed a duplex in 60 mM Na-cacodylate, 100 mM $MgCl_2$, 1M NaCl with a T_m value 27°; the T_m of the corresponding $d(A\text{-}T)_6$ was 30°.

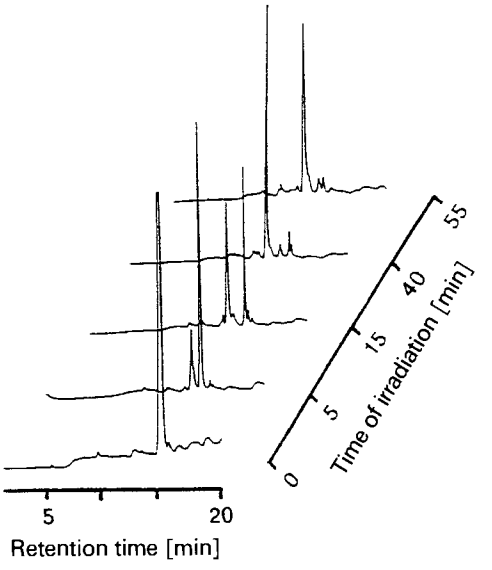


Fig. 1. *HPLC Profile obtained from the photochemical conversion of* **12** *into* **11** *by irradiation at 254 nm with a 4-W Hg lamp.* Samples were taken within interval of time and injected into the HPLC. Gradient *III*. Flow rate was 1.0 ml/min.

The nucleoside content was determined by two different methods. As compound **1** has UV maxima at 247 (9100) and 292 nm (10100) [7], the long-wavelength maximum is separated from dA (*Fig. 2, d*). Accordingly, the content of **1** incorporated into an oligonucleotide can be directly calculated from the UV spectrum of the oligomer. Furthermore, the oligonucleotide content was determined after tandem hydrolysis with

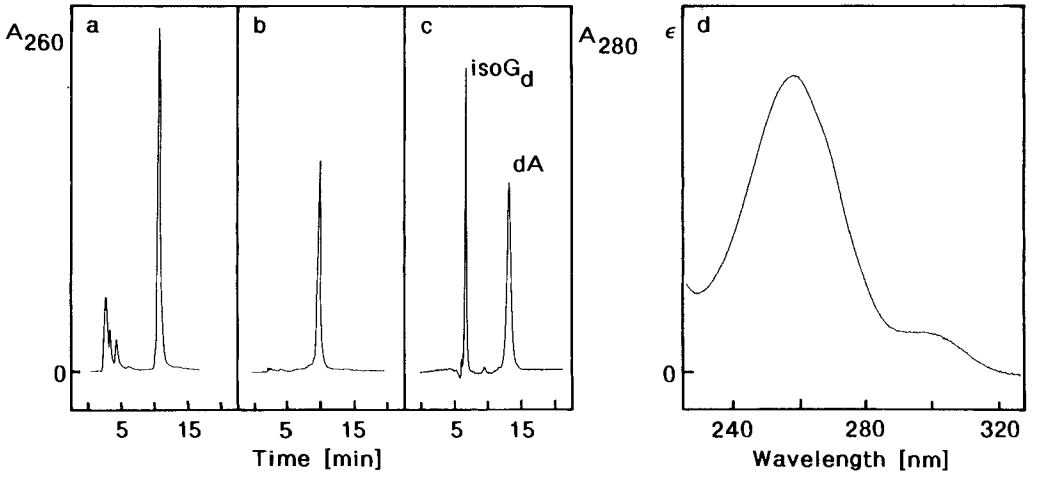


Fig. 2. *HPLC Profiles and UV spectra of the oligonucleotide* **11**. *a*) Crude DMT-protected, gradient *I*; *b*) unprotected, gradient *II*); *c*) enzymatic digest, gradient *III*. *a*) and *b*) at 260 nm, flow rate 1.0 ml/min; *c*) at 280 nm, 0.6 ml/min; *d*) UV spectrum of **11** in H_2O.

snake-venom phosphodiesterase followed by alkaline phosphatase [19]. Reverse-phase HPLC of the hydrolysis mixture was used for the separation of the enzymatic digest. Due to the differences of UV maxima of the regular nucleosides and **1**, the HPLC analysis of the digest was performed at 280 nm; in all other cases at 260 nm. *Fig. 2* shows HPLC profiles and the UV spectrum from the oligomer **11**: the crude DMT-protected compound (*a*), the oligonucleotide (*b*), the enzymatic digest of the oligonucleotide (*c*), and the UV spectrum of the oligonucleotide (*d*). *Fig. 3* shows the same for the oligonucleotide **13**. From these data, a ratio of **1** *vs.* dA was determined for **11**: (1:4.3 by HPLC; 1:3.9 by UV). In the case of the oligomer **13**, there is an overlap of the UV spectrum of **1** with that of dT (*Fig. 3, d*). In this case, the ratio of **1** *vs.* dT was determined from the HPLC pattern of the enzymatic digest and found to be 1:1.15 (**1**/dT).

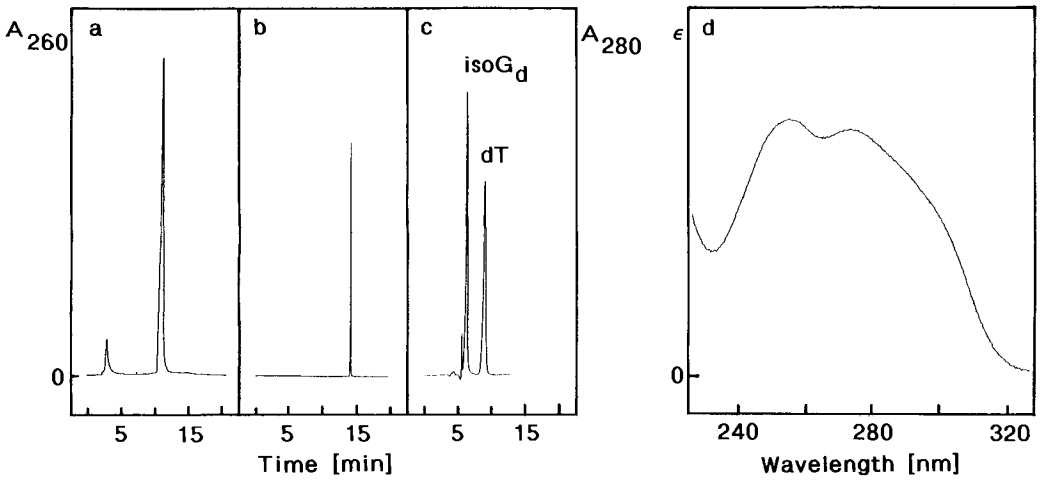


Fig. 3. *HPLC Profiles and UV spectra of the oligonucleotide* **13**. *a*) DMT-protected, gradient *I*; *b*) unprotected, gradient *II*; *c*) enzymatic digest, gradient *III*. *a*) and *b*) at 260 nm, flow rate, 1.0 ml/min; *c*) at 280 nm, 0.6 ml/min; *d*) UV-spectrum of **13** in H_2O.

Although compound **1** can now be incorporated at any position of an oligonucleotide, the formation of 1*H*- and 3*H*-tautomers may lead to mispairing in duplex DNA. Base-pairing of **1** with $isoC_d$ but also with dT [5] [23] has been already observed. Detailed studies on the mutagenicity of **1** as well as on the base-pairing, duplex structure, and nuclease resistance of oligonucleotides containing **1** are under current investigation.

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Experimental Part

General. See [24]. The phosphonates of the regular building blocks were purchased from *Sigma*, St. Louis, and the *Fractosil*-linked 2′-deoxyribonucleosides from *Milligen*, Eschborn, Germany. Snake-venom phosphodiesterase (*EC 3.1.15.1*, *Crotallus durissus*) and alkaline phosphatase (*EC 3.1.3.1*, *E. coli*) are products of *Boehringer*, Mannheim, Germany. Oligonucleotide synthesis was carried out on an automated DNA synthesizer, model *380B*, *Applied Biosystems* (Weiterstadt, Germany). Prep.-scale synthesis of **1** was carried out under conditions already described in [7] but using a 500-ml volumetric cylinder fitted with a *TNN 15/32* low-pressure 15-W resonance Hg lamp (*Heräus*, Germany). Compound **2a** (100 mg) was dissolved in H_2O (400 ml) and irradiated for 70–75 min. The pH should always be kept above 7. For chromatographic purification a 0.1–0.2-mm *Serdolit AD-4* ion-exchange resin (*Serva*, Germany, column 4 × 22 cm) was used. The soln. obtained after irradiation was applied on the column, and the resin was washed with *i*) H_2O (500 ml), *ii*) H_2O/i-PrOH 95:5 (500 ml). Compound **1** was eluted with H_2O/i-PrOH 9:1 (200–300 ml) and was obtained from i-PrOH/acetone 1:1 as colorless powder. For enzymatic hydrolysis of the oligomers, see [19].

HPLC Separation of Oligonucleotides. HPLC was carried out on a 4 × 250 mm (10 μm) *RP-18 LiChrosorb* column (*Merck*, Germany) using a *Merck-Hitachi* HPLC apparatus. The solvents gradients consisting of 0.1M Et_3NHOAc (pH 7.0), MeCN 95:5 (*A*), and MeCN (*B*) were used in the following order: gradient *I* (3 min 15% *B* in *A*, 12 min 15–40% *B* in *A*, 5 min 40–15% *B* in *A*); gradient *II* (20 min 20% *B* in *A*); gradient *III* (20 min 100% *A*).

*9-(2′-Deoxy-β-*D-erythro-*pentofuranosyl)-6-[(dimethylamino)methylidene]-9*H-*isoguanine* (**5**). A soln. of compound **1** (200 mg, 0.74 mmol) [7] in abs. DMF (10 ml) is stirred in the presence of *N,N*-dimethylformamide diethyl acetal (2 ml, 11.7 mmol) at r.t. for 24 h. The solvent was evaporated and co-evaporated with toluene and then with acetone. FC on silica gel (column: 25 × 4.5 cm) afforded a main zone. It was evaporated and the residue crystallized from MeOH. Pale yellow crystals (150 mg, 63%). M.p. of 178° (dec.). TLC (CH_2Cl_2/MeOH 8:2): R_f 0.62. UV (MeOH): 338 (9400), 259 (6100), 223 (9100). ^{1}H-NMR ((D$_6$)DMSO): 2.18, 2.58 (2*m*, H–C(2′)); 3.10, 3.20 (2*s*, 2 CH_3N); 3.50 (2*m*, H–C(5′)); 3.86 (*m*, H–C(4′)); 4.37 (*m*, H–C(3′)); 5.24 (*d*, *J* = 4, HO–C(3′)); 5.34 (*m*, HO–C(3′)); 6.15 (‘*t*’, *J* = 6.3, H–C(1′)); 8.05 (*s*, H–C(8)); 9.17 (*s*, N=C*H*NMe$_2$); 11.3 (NH). Anal. calc. for $C_{13}H_{18}N_6O_4$ (322.3): C 48.44, H 5.63, N 26.07; found: C 48.25, H 5.69, N 25.87.

*2-Chloro-9-(2′-deoxy-β-*D-erythro-*pentofuranosyl)-6-{[(dimethylamino)methylidene]amino}-9*H-*purine* (**6**). Compound **6** was prepared as described for **5** except that the following amounts were used: **2a** (300 mg, 1.05 mmol) [9] [10], DMF (3 ml), *N,N*-dimethylformamide diethyl acetal (0.9 ml, 5.2 mmol). FC on a 15 × 5 cm column with CH_2Cl_2/MeOH 9:1: colorless foam (295 mg, 82%). TLC (CH_2Cl_2/MeOH 9:1): R_f 0.38. UV (MeOH): 318 (23600), 266 (7200), 235 (12200). ^{1}H-NMR ((D$_6$)DMSO): 2.32, 2.65 (2*m*, H–C(2′)); 3.14, 3.22 (2*s*, 2 CH_3N); 2.54 (*m*, H–C(5′)); 3.87 (*m*, H–C(4′)); 4.40 (*m*, H–C(3′)); 4.96 (*t*, *J* = 5.5, HO–C(5′)); 5.30 (*d*, *J* = 3.8, HO–C(3′)); 6.31 (‘*t*’, *J* = 6.3, H–C(1′)); 8.46 (*s*, H–C(8)); 8.80 (*s*, N=C*H*NMe$_2$). Anal. calc. for $C_{13}H_{17}ClN_6O_3$ (340.77): C 45.82, H 5.03, N 24.66; found: C 45.97, H 5.10, N 24.50.

*2-Bromo-9-(2′-deoxy-β-*D-erythro-*pentofuranosyl)-6-{[(dimethylamino)methylidene]amino}-9*H-*purine* (**7**). Compound **7** was prepared as described for **5** except that the following amounts were used: **2b** (330 mg, 1.0 mmol) [11], DMF (3 ml) *N,N*-dimethylformamide diethyl acetal (0.85 ml, 5 mmol). FC as described for **6**: colorless amorphous solid (306 mg, 80%). TLC (CH_2Cl_2/MeOH 9:1): R_f 0.33. UV (MeOH): 318 (28600), 238 (13000). ^{1}H-NMR ((D$_6$)DMSO): 2.32, 2.67 (2*m*, H–C(2′)); 3.14, 3.23 (2*s*, 2 CH_3N); 3.56 (*m*, H–C(5′)); 3.86 (*m*, H–C(4′)); 4.39 (*m*, H–C(3′)); 4.95 (*t*, *J* = 5.5, HO–C(5′)); 5.33 (*d*, *J* = 4.3, HO–C(3′)); 6.31 (‘*t*’, *J* = 6.3, H–C(1′)); 8.44 (*s*, H–C(8)); 8.85 (*s*, N=C*H*NMe$_2$). Anal. calc. for $C_{13}H_{17}BrN_6O_3$ (385.22): C 40.53, H 4.45, N 21.82; found: C 40.66, H 4.50, N 21.79.

*9-[2′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-β-*D-erythro-*pentofuranosyl]-6-[(dimethylamino)methylidene]-9*H-*isoguanine* (**8**). Compound **5** (750 mg, 2.33 mmol) was dried by repeated co-evaporation from anh. pyridine and then dissolved in anh. pyridine (15 ml). At r.t., 4,4′-dimethoxytrityl chloride (DMT-Cl; 1.1 g, 3.25 mmol) was added and the soln. stirred for 12 h. Then, the solvent was evaporated, the residue co-evaporated with toluene, dissolved in CH_2Cl_2, and submitted to FC (column 20 × 4 cm). The column was washed with CH_2Cl_2, and compound **8** was eluted with CH_2Cl_2/MeOH 9:1, containing traces of Et_3N. The fractions of the main zone were pooled, and the solvent was evaporated: colorless powder (560 mg, 39%). From a second zone, **5** (230 mg) was recovered. TLC (CH_2Cl_2/MeOH/Et_3N 80:10:5): R_f 0.60. ^{1}H-NMR ((D$_6$)DMSO): 2.26, 2.59 (2*m*, H–C(2′)); 3.08, 3.18 (2*s*, 2 CH_3N); 3.70, 3.71 (2*s*, CH_3O); 3.92 (*m*, H–C(4′)); 4.37 (*m*, H–C(3′)); 5.43 (*m*, HO–C(3′)); 6.15 (‘*t*’, *J* = 6.8, H–C(1′)); 6.8–7.4 (*m*, arom. H); 7.93 (*s*, H–C(8)); 9.15 (*s*, N=C*H*NMe$_2$); 11.09 (NH). Anal. calc. for $C_{34}H_{36}N_6O_6$ (624.71): C 65.37, H 5.81, N 13.45; found: C 65.30, 5.99, N 13.44.

*2-Chloro-9-[2'-deoxy-5'-O-(4,4'-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-6-{[(dimethylamino)methylidene]amino}-9H-purine* (**9**). Compound **9** was prepared analogously to **8**. The following amounts were used: **6** (340 mg, 1.0 mmol), pyridine (2 ml), 4,4'-dimethoxytrityl chloride (440 mg, 1.3 mmol). FC on a 20 × 5 cm column with CH_2Cl_2/MeOH 9:1: colorless solid (390 mg, 61%). TLC (CH_2Cl_2/MeOH 9:1): R_f 0.41. ^{1}H-NMR ((D$_6$)DMSO): 2.37, 2.82 (2*m*, H–C(2')); 3.14, 3.23 (2*s*, 2 CH_3N); 3.69, 3.71 (2*s*, CH_3O); 3.98 (*m*, H–C(4')); 4.46 (*m*, H–C(3')); 5.39 (*d*, *J* = 4.5, HO–C(3')); 6.35 ('*t*', *J* = 6.2, H–C(1')); 6.7–7.3 (*m*, 13 arom. H); 8.37 (*s*, H–C(8)); 8.84 (*s*, N=CHNMe$_2$). Anal. calc. for $C_{34}H_{35}ClN_6O_5$ (643.14): C 63.49, H 5.49, N 13.06; found: C 63.30, H 5.68, N 12.88.

*2-Bromo-9-[2'-deoxy-5'-O-(4,4'-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-6-{[(dimethylamino)methylidene]amino}-9H-purine* (**10**). Compound **10** was prepared as described for **8**. The following amounts were used: **7** (250 mg, 0.65 mmol), pyridine (2 ml), 4,4'-dimethoxytrityl chloride (330 mg, 0.98 mmol). FC see **9**: colorless solid (260 mg, 58%). TLC (CH_2Cl_2/MeOH 9:1): R_f 0.37. ^{1}H-NMR ((D$_6$)DMSO): 2.23, 2.68 (2*m*, H–C(2')); 3.01, 3.09 (2*s* 2 CH_3N); 3.57, 3.58 (2*s*, CH_3O); 3.84 (*m*, H–C(4')); 4.33 (*m*, H–C(3')); 5.24 (*d*, *J* = 4.5, HO–C(3')); 6.22 ('*t*', *J* = 5.8, H–C(1')); 6.6–7.2 (*m*, arom. H); 8.21 (*s*, H–C(8)); 8.69 (*s*, N=CHNMe$_2$). Anal. calc. for $C_{34}H_{35}BrN_6O_5$ (687.59): C 59.39, H 5.13, N 12.22; found: C 59.46, H 5.40, N 12.09.

*9-[2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-6-[(dimethylamino)methylidene]-9H-isoguanine 3'-Triethylammonium Phosphonate* (**3a**). To a soln. of PCl_3 (270 µl, 3.1 mmol) and *N*-methylmorpholine (3.5 ml) in CH_2Cl_2 (25 ml), 1,2,4-triazole (716 mg, 10 mmol) was added. After stirring for 30 min, the soln. was cooled to 0°, and **8** (413 mg, 0.64 mmol), dissolved in CH_2Cl_2 (25 ml), was added slowly. After stirring for 30 min at r.t., the mixture was poured into 1M $(Et_3NH)HCO_3$ (35 ml), shaken, and separated. The aq. layer was extracted with CH_2Cl_2 (3 × 30 ml). The combined org. extracts were dried, and the colorless foam was submitted to four prep. silica-gel plates (20 × 20 cm) and developed in CH_2Cl_2/MeOH/Et_3N 80:10:5. The residue of the main zone gave a colorless foam (320 mg, 63%). TLC (CH_2Cl_2/MeOH/Et_3N 80:10:5): R_f 0.40. ^{1}H-NMR ((D$_6$)DMSO): 1.1–1.2 (*m*, CH_3CH_2); 2.25, 2.65 (2*m*, H–C(2')); 2.7–2.8 (*m*, CH_3CH_2); 3.16, 3.25 (2*s*, 2 CH_3N); 3.25 (*s*, H–C(5')); 3.77, 3.78 (2*s*, 2 CH_3O); 4.16 (*m*, H–C(4')); 4.79 (*m*, H–C(3')); 6.20 ('*t*', *J* = 6, HO–C(5')); 6.8–7.4 (*m*, arom. H); 7.97 (*s*, H–C(8)); 9.15 (*s*, N=CHNMe$_2$). ^{31}P-NMR ((D$_6$)DMSO): 2.23 (^1J(P,H) = 585, ^3J(P,H) = 8.1). Anal. calc. for $C_{40}H_{52}N_7O_8P$ (789.87): C 60.82, H 6.63, N 12.41; found: C 60.80, H 6.79, N 12.41.

*9-[2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-6-[(dimethylamino)methylidene]-9H-isoguanine 3'-[(2-Cyanoethyl) N,N-Diisopropylphosphoramidite]* (**3b**). In an anal.-scale experiment, **8** was treated and worked up according to [25]: colorless solid (37%). TLC (CH_2Cl_2/AcOEt/MeOH/Et_3N 45:40:5:10): R_f 0.45, 0.50. ^{31}P-NMR ((D$_6$)DMSO): 148.95; 148.27.

*2-Chloro-9-[2'-deoxy-5'-O-(4,4'-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-6-{[(dimethylamino)methylidene]amino}-9H-purine 3'-Triethylammonium Phosphonate* (**4a**). Compound **4a** was prepared as described for **3a**, except that the following amounts were used: **9** (200 mg, 31 mmol) dissolved in CH_2Cl_2 (10 ml) were introduced in a soln. of PCl_3 (135 µl, 1.55 mmol), *N*-methylmorpholine (1.7 ml, 15.2 mmol) and 1,2,4-triazole (358 mg, 5 mmol) in CH_2Cl_2 (10 ml). Purification was performed by FC on a 20 × 5 cm column with CH_2Cl_2/Et_3N and then with CH_2Cl_2/MeOH/Et_3N 88:10:2, yielding a colorless foam (190 mg, 75%). TLC (CH_2Cl_2/MeOH/Et_3N 88:10:2): R_f 0.35. ^{1}H-NMR ((D$_6$)DMSO): 0.9–1.2 (*m*, CH_3CH_2); 2.37, 2.82 (2*m*, H–C(2')); 2.60–2.64 (*m*, CH_3CH_2); 3.14, 3.22 (2*s*, 2 CH_3N); 3.69, 3.71 (2*s*, 2 CH_3O); 4.16 (*m*, H–C(4')); 4.77 (*m*, H–C(3')); 6.34 ('*t*', *J* = 6.2, H–C(1')); 6.63 (*d*, *J* = 585, PH); 6.7–7.3 (*m*, arom. H); 8.34 (*s*, H–C(8)); 8.85 (*s*, N=CHNMe$_2$). ^{31}P-NMR ((D$_6$)DMSO): 1.09 (^1J(P,H) = 585, ^3J(P,H) = 9.1). Anal. calc. for $C_{40}H_{51}ClN_7O_7P$ (808.31): C 59.43, H 6.36, N 12.13; found: C 59.26, H 6.50, N 11.93.

*2-Bromo-9-[2'-deoxy-5'-O-(4,4'-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-6-{[(dimethylamino)methylidene]amino}-9H-purine 3'-Triethylammonium Phosphonate* (**4b**). Analogously to **4a**, **4b** was prepared. Colorless foam (128 mg, 48%). TLC (CH_2Cl_2/MeOH/Et_3N 88:10:2): R_f 0.35. ^{1}H-NMR ((D$_6$)DMSO): 1.0–1.2 (*m*, CH_3CH_2); 2.23, 2.82 (2*m*, H–C(2')); 2.7–2.9 (*m*, CH_3CH_2); 3.14, 3.23 (2*s*, 2 CH_3N); 3.70, 3.71 (2*s*, 2 CH_3O); 4.14 (*m*, H–C(4')); 4.72 (*m*, H–C(3')); 6.31 ('*t*', *J* = 6.2, H–C(1')); 6.63 (*d*, *J* = 585, PH); 6.7–7.3 (*m*, arom. H); 8.31 (*s*, H–C(8)); 8.82 (*s*, N=CHNMe$_2$). ^{31}P-NMR ((D$_6$)DMSO): 0.90 (^1J(P,H) = 585, ^3J(P,H) = 9.1).

Solid-Phase Synthesis of the Oligonucleotides **11–13**. The synthesis was carried out on a 1-µM scale using the phosphonates of [(MeO)$_2$Tr]T$_d$, [(MeO)$_2$Tr]bz^{6}A$_d$, and **3a** or **4a** and the solid supports CPG-dT or CPG-dA. The synthesis of **11–13** followed the regular protocol of the DNA synthesizer for phosphonates [22]. Deprotection from the support and of the NH_2-protecting groups was carried out with 25% NH_3/H_2O at 60° for 48 h. The DMT-oligomers were purified by HPLC on a 25 × 4 mm *RP-18* column (gradient *I*), isolated, and the 4,4'-dimethoxytrityl residues were removed by treatment with 80% AcOEt/H_2O for 5 min at r.t. HPLC purification was carried out as indicated above but using gradient *II*. The oligomers were desalted on a 4 × 25-mm HPLC cartridge (*RP-18*, silica gel) using H_2O (10 ml) for the elution of the salt, while the oligomer was eluted with

MeOH/H_2O 3:2 (5 ml). The nucleotides were lyophilized on a *Speed-Vac* evaporator. Colorless solids, which were dissolved in H_2O (100 µl) and stored frozen at −18°. The yields are between 3 and 14 A_{260} units.

Photochemical Conversion of the Oligonucleotide **12** *to* **11**. A soln. of **12** (2 A_{260} units) in H_2O (100 µl) was irradiated with 4-W Hg resonance lamp in quarz cuvettes. Samples were taken after intervals of time (*Fig. 1*), and the reaction was followed by HPLC. Then, conc. aq. NH_3 (100 µl) was added and the solvent evaporated. The oligonucleotide was identical with **11** obtained from **1**.

REFERENCES

[1] F. Seela, Q.-H. Tran-Thi, D. Franzen, *Biochemistry* **1982,** *21,* 4338.
[2] P. W. R. Corfield, W. N. Hunter, T. Brown, P. Robinson, O. Kennard, *Nucleic Acids Res.* **1987,** *15,* 7935.
[3] A. Eschenmoser, M. Dobler, *Helv. Chim. Acta* **1992,** *75,* 218.
[4] H. Rosemeyer, F. Seela, *Helv. Chim. Acta* **1991,** *74,* 748.
[5] C. Switzer, S. E. Moroney, S. A. Benner, *J. Am. Chem. Soc.* **1989,** *111,* 8322.
[6] T. Golas, M. Fikus, Z. Kazimierczuk, D. Shugar, *Eur. J. Biochem.* **1976,** *65,* 183.
[7] Z. Kazimierczuk, R. Mertens, W. Kawczynski, F. Seela, *Helv. Chim. Acta* **1991,** *74,* 1742.
[8] Z. Kazimierczuk, J. A. Vilpo, F. Seela, *Helv. Chim. Acta* **1992,** *75,* 2289.
[9] L. F. Christensen, A. D. Broom, M. J. Robins, A. Bloch, *J. Med. Chem.* **1972,** *15,* 735.
[10] Z. Kazimierczuk, H. B. Cottam, G. R. Revankar, R. K. Robins, *J. Am. Chem. Soc.* **1984,** *106,* 6379.
[11] Z. Kazimierczuk, J. Vilpo, C. Hildebrand, G. Wright, *J. Med. Chem.* **1990,** *33,* 1683.
[12] H. Rosemeyer, G. Toth, B. Golankiewicz, Z. Kazimierczuk, W. Bourgeois, U. Kretschmer, H.-P. Muth, F. Seela, *J. Org. Chem.* **1990,** *55,* 5784.
[13] J. Zemlicka, A. Holy, *Collect. Czech. Chem. Commun.* **1967,** *32,* 3159.
[14] L. J. McBride, R. Kierzek, S. L. Beaucage, M. H. Caruthers, *J. Am. Chem. Soc.* **1986,** *108,* 2040.
[15] M. Krecmerova, F. Seela, *Nucleos. Nucleot.* **1992,** *11,* 1393.
[16] Applied Biosystems, 'Users Bulletin for DNA Synthesizer', 1990, number 57.
[17] B. C. Froehler, M. D. Matteucci, *Tetrahedron Lett.* **1986,** *27,* 469.
[18] P. J. Garegg, I. Lindh, T. Regberg, J. Stawinski, R. Strömberg, *Tetrahedron Lett.* **1986,** *27,* 4051.
[19] F. Seela, S. Lampe, *Helv. Chim. Acta* **1991,** *74,* 1790.
[20] H. Rosemeyer, M. Krecmerova, F. Seela, *Helv. Chim. Acta* **1991,** *74,* 2054.
[21] F. Seela, K. Mersmann, *Heterocycles* **1992,** *34,* 229.
[22] Applied Biosystems, 'Model 381B DNA Synthesizer Users Manual', Version 1.23, 1987.
[23] F. Seela, A. Heckel, unpublished data.
[24] Z. Kazimierczuk, F. Seela, *Helv. Chim. Acta* **1990,** *73,* 316.
[25] F. Seela, T. Wenzel, *Helv. Chim. Acta* **1992,** *75,* 1111.