

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

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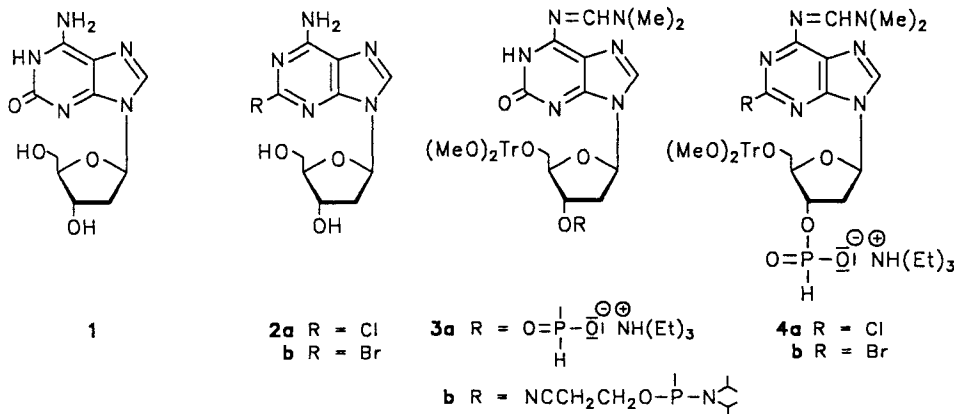
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Dedicated to Prof. Dr. F. Eckstein on the occasion of his 60th birthday

(27. VII. 92)

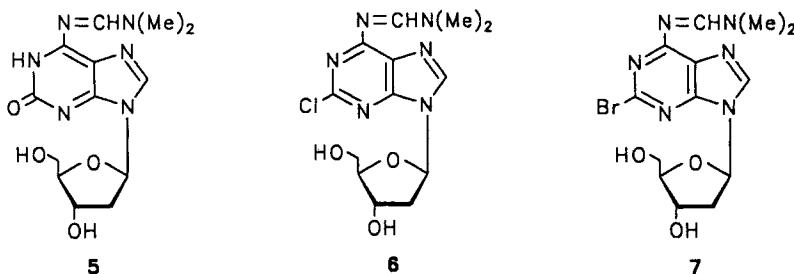
Oligonucleotides containing 2'-deoxyisoguanosine (**1**) or 2-chloro-2'-deoxyadenosine (**2a**) have been prepared by solid-phase synthesis. Suitably protected phosphonates **3a**, **4a**, and **4b** as well as the phosphoramidite of **1** have been obtained from the nucleosides **1**, **2a**, or **2b** 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 isoC₄ 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 ¹H- and ¹³C-NMR spectra (*Table 1* and *Exper. Part*). ¹³C-NMR Chemical shifts were assigned from the gated-decoupled spectra (*Table 2*). According to the ¹H-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 ¹H-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.



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. NH₃ 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 ¹³C-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	°)	72.5	85.5	63.9	55.0
4a	83.9	°)	71.5	85.5	64.0	55.0
4b	83.9	°)	71.5	85.5	64.0	55.0
5 ^{b)}	83.5	°)	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	°)	70.6	85.5	64.3	55.1
9	83.6	°)	70.6	86.2	64.2	55.0
10	83.6	°)	70.6	86.1	64.2	55.0

a) Analogously to the amidine protected nucleoside.

b) From gated-decoupled spectra.

c) Superimposed by DMSO.

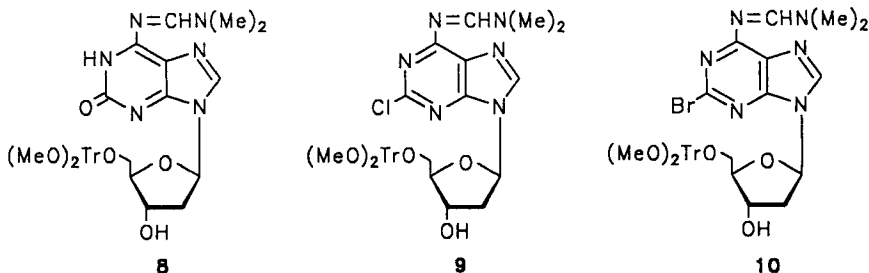
Table 2. $J(\text{C},\text{H})$ Values [Hz] of (Dimethylamino)methylidene Nucleosides^{a)}

$J(\text{C}(8),\text{H}-\text{C}(8))$	214.1	214.3	214.4
$J(\text{C}(8),\text{H}-\text{C}(1'))$	4.9	4.0	4.1
$J(\text{C}(6),\text{N}=\text{CHNMe}_2)$	6.1	7.2	7.0
$J(\text{C}(5),\text{H}-\text{C}(8))$	11.8	11.6	11.6
$J(\text{C}(4),\text{H}-\text{C}(8)), \text{H}-\text{C}(1')$	<i>m</i>	<i>m</i>	<i>m</i>
$J(\text{C}(=\text{N}-\text{C}),\text{N}=\text{CHNMe}_2)$	161.5	184.5	177.9
$J(\text{C}(1'),\text{H}-\text{C}(1'))$	164.6	166.7	166.7
$J(\text{C}(3'),\text{H}-\text{C}(3'))$	152.3	148.8	150.2
$J(\text{C}(4'),\text{H}-\text{C}(4'))$	148.4	146.6	146.6
$J(\text{C}(5'),\text{H}-\text{C}(5'))$	140.9	139.0	139.0

a) From spectra measured in (D_6)DMSO.

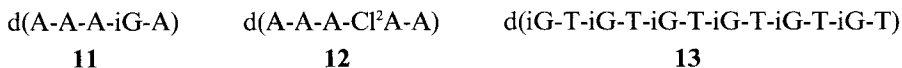
tives [19]. Tritylation of compound **5** was more difficult as in the case of **6** or **7**, and some starting material was recovered.

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

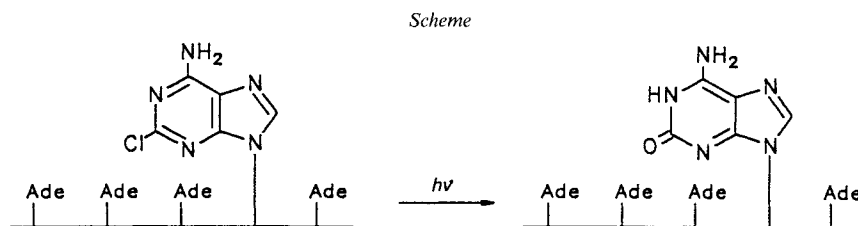


amounts of modified building blocks is more easy in the case of phosphonates than of phosphoramidites. Reaction of **8–10** with PCl_3 /*N*-methylmorpholine/1,2,4-triazole in CH_2Cl_2 afforded the 3'-phosphonates **3a** and **4a, b**. They were purified chromatographically and isolated as triethylammonium 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 $(\text{MeO})_2\text{Tr}$ -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:



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



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 $\text{d}(\text{A-T})_6$ was 30°.

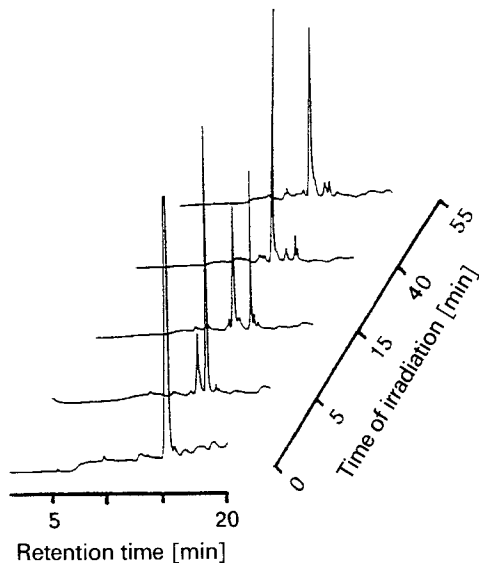


Fig. 1. HPLC Profile obtained from the photochemical conversion of **12** into **11** by irradiation at 254 nm with a 4-W Hg lamp. Samples were taken within interval of time and injected into the HPLC. Gradient III. Flow rate was 1.0 ml/min.

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

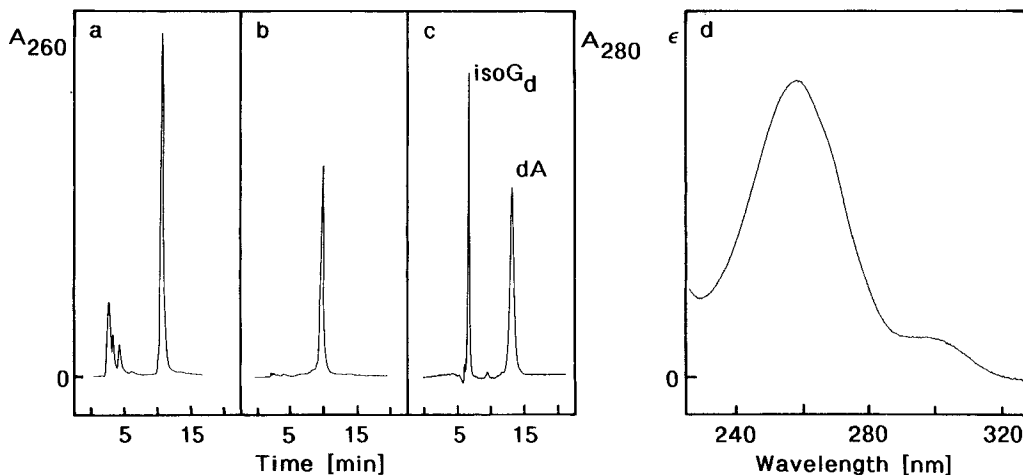


Fig. 2. HPLC Profiles and UV spectra of the oligonucleotide **11**. a) Crude DMT-protected, gradient I; b) unprotected, gradient II; c) enzymatic digest, gradient III. a) and b) at 260 nm, flow rate 1.0 ml/min; c) at 280 nm, 0.6 ml/min; d) UV spectrum of **11** in H₂O.

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).

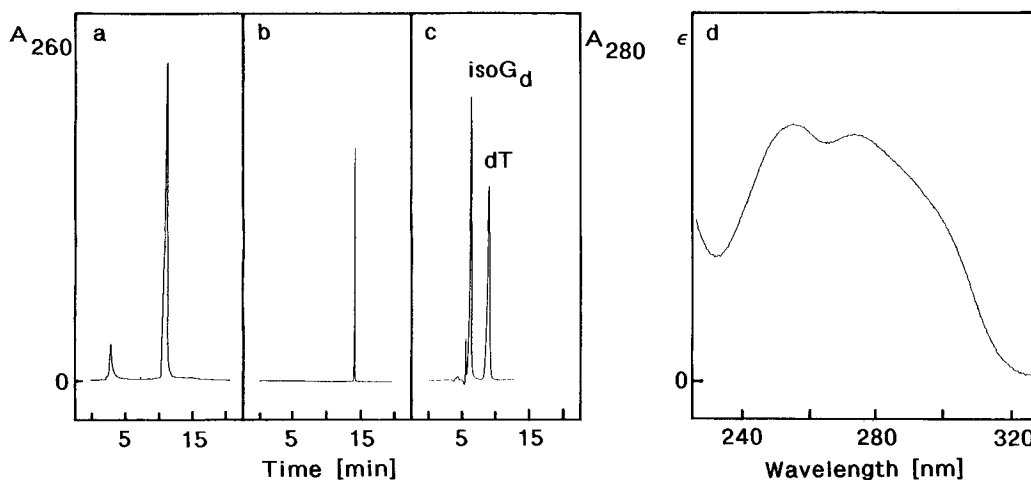


Fig. 3. HPLC Profiles and UV spectra of the oligonucleotide **13**. a) DMT-protected, gradient I; b) unprotected, gradient II; c) enzymatic digest, gradient III. a) and b) at 260 nm, flow rate, 1.0 ml/min; c) at 280 nm, 0.6 ml/min; d) UV-spectrum of **13** in H₂O.

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.

We thank Mrs. A. Heckel for the preparation of **3a**, **b**, and **8**, Mr. T. Wenzel for the solid-phase synthesis, and Mrs. I. Strodholz for the purification of the oligomers. Financial support by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie is gratefully acknowledged.

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 H₂O (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*) H₂O (500 ml), *ii*) H₂O/*i*-PrOH 95:5 (500 ml). Compound **1** was eluted with H₂O/*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 Et₃NHOAc (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]-9H-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 (CH₂Cl₂/MeOH 8:2): *R*_f 0.62. UV (MeOH): 338 (9400), 259 (6100), 223 (9100). ¹H-NMR ((D₆)DMSO): 2.18, 2.58 (2*m*, H-C(2')); 3.10, 3.20 (2*s*, 2 CH₃N); 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 (1'*t*', *J* = 6.3, H-C(1')); 8.05 (*s*, H-C(8)); 9.17 (*s*, N=CHNMe₂); 11.3 (NH). Anal. calc. for C₁₃H₁₈N₆O₄ (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-9H-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 CH₂Cl₂/MeOH 9:1: colorless foam (295 mg, 82%). TLC (CH₂Cl₂/MeOH 9:1): *R*_f 0.38. UV (MeOH): 318 (23600), 266 (7200), 235 (12200). ¹H-NMR ((D₆)DMSO): 2.32, 2.65 (2*m*, H-C(2')); 3.14, 3.22 (2*s*, 2 CH₃N); 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 (1'*t*', *J* = 6.3, H-C(1')); 8.46 (*s*, H-C(8)); 8.80 (*s*, N=CHNMe₂). Anal. calc. for C₁₃H₁₇ClN₆O₃ (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-9H-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 (CH₂Cl₂/MeOH 9:1): *R*_f 0.33. UV (MeOH): 318 (28600), 238 (13000). ¹H-NMR ((D₆)DMSO): 2.32, 2.67 (2*m*, H-C(2')); 3.14, 3.23 (2*s*, 2 CH₃N); 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 (1'*t*', *J* = 6.3, H-C(1')); 8.44 (*s*, H-C(8)); 8.85 (*s*, N=CHNMe₂). Anal. calc. for C₁₃H₁₇BrN₆O₃ (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]-9H-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 CH₂Cl₂, and submitted to FC (column 20 × 4 cm). The column was washed with CH₂Cl₂, and compound **8** was eluted with CH₂Cl₂/MeOH 9:1, containing traces of Et₃N. 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 (CH₂Cl₂/MeOH/Et₃N 80:10:5): *R*_f 0.60. ¹H-NMR ((D₆)DMSO): 2.26, 2.59 (2*m*, H-C(2')); 3.08, 3.18 (2*s*, 2 CH₃N); 3.70, 3.71 (2*s*, CH₃O); 3.92 (*m*, H-C(4')); 4.37 (*m*, H-C(3')); 5.43 (*m*, HO-C(3')); 6.15 (1'*t*', *J* = 6.8, H-C(1')); 6.8–7.4 (*m*, arom. H); 7.93 (*s*, H-C(8)); 9.15 (*s*, N=CHNMe₂); 11.09 (NH). Anal. calc. for C₃₄H₃₆N₆O₆ (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 $\times$ 5 cm column with CH₂Cl₂/MeOH 9:1: colorless solid (390 mg, 61%). TLC (CH₂Cl₂/MeOH 9:1): *R_f* 0.41. ¹H-NMR ((D₆)DMSO): 2.37, 2.82 (2*m*, H-C(2')); 3.14, 3.23 (2*s*, 2 CH₃N); 3.69, 3.71 (2*s*, CH₃O); 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=CHNMe₂). Anal. calc. for C₃₄H₃₅ClN₆O₅ (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 (CH₂Cl₂/MeOH 9:1): *R_f* 0.37. ¹H-NMR ((D₆)DMSO): 2.23, 2.68 (2*m*, H-C(2')); 3.01, 3.09 (2*s*, 2 CH₃N); 3.57, 3.58 (2*s*, CH₃O); 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=CHNMe₂). Anal. calc. for C₃₄H₃₅BrN₆O₅ (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 PCl₃ (270 μ l, 3.1 mmol) and *N*-methylmorpholine (3.5 ml) in CH₂Cl₂ (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 CH₂Cl₂ (25 ml), was added slowly. After stirring for 30 min at r.t., the mixture was poured into 1*M* (Et₃NH)HCO₃ (35 ml), shaken, and separated. The aq. layer was extracted with CH₂Cl₂ (3 $\times$ 30 ml). The combined org. extracts were dried, and the colorless foam was submitted to four prep. silica-gel plates (20 $\times$ 20 cm) and developed in CH₂Cl₂/MeOH/Et₃N 80:10:5. The residue of the main zone gave a colorless foam (320 mg, 63%). TLC (CH₂Cl₂/MeOH/Et₃N 80:10:5): *R_f* 0.40. ¹H-NMR ((D₆)DMSO): 1.1–1.2 (*m*, CH₃CH₂); 2.25, 2.65 (2*m*, H-C(2')); 2.7–2.8 (*m*, CH₃CH₂); 3.16, 3.25 (2*s*, 2 CH₃N); 3.25 (*s*, H-C(5')); 3.77, 3.78 (2*s*, 2 CH₃O); 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=CHNMe₂). ³¹P-NMR ((D₆)DMSO): 2.23 (¹*J*(P,H) = 585, ³*J*(P,H) = 8.1). Anal. calc. for C₄₀H₅₂N₇O₈P (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 (CH₂Cl₂/AcOEt/MeOH/Et₃N 45:40:5:10): *R_f* 0.45, 0.50. ³¹P-NMR ((D₆)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 CH₂Cl₂ (10 ml) were introduced in a soln. of PCl₃ (135 μ l, 1.55 mmol), *N*-methylmorpholine (1.7 ml, 15.2 mmol) and 1,2,4-triazole (358 mg, 5 mmol) in CH₂Cl₂ (10 ml). Purification was performed by FC on a 20 $\times$ 5 cm column with CH₂Cl₂/Et₃N and then with CH₂Cl₂/MeOH/Et₃N 88:10:2, yielding a colorless foam (190 mg, 75%). TLC (CH₂Cl₂/MeOH/Et₃N 88:10:2): *R_f* 0.35. ¹H-NMR ((D₆)DMSO): 0.9–1.2 (*m*, CH₃CH₂); 2.37, 2.82 (2*m*, H-C(2')); 2.60–2.64 (*m*, CH₃CH₂); 3.14, 3.22 (2*s*, 2 CH₃N); 3.69, 3.71 (2*s*, 2 CH₃O); 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=CHNMe₂). ³¹P-NMR ((D₆)DMSO): 1.09 (¹*J*(P,H) = 585, ³*J*(P,H) = 9.1). Anal. calc. for C₄₀H₅₁ClN₇O₇P (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 (CH₂Cl₂/MeOH/Et₃N 88:10:2): *R_f* 0.35. ¹H-NMR ((D₆)DMSO): 1.0–1.2 (*m*, CH₃CH₂); 2.23, 2.82 (2*m*, H-C(2')); 2.7–2.9 (*m*, CH₃CH₂); 3.14, 3.23 (2*s*, 2 CH₃N); 3.70, 3.71 (2*s*, 2 CH₃O); 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=CHNMe₂). ³¹P-NMR ((D₆)DMSO): 0.90 (¹*J*(P,H) = 585, ³*J*(P,H) = 9.1).

Solid-Phase Synthesis of the Oligonucleotides 11–13. The synthesis was carried out on a 1- μ m scale using the phosphonates of [(MeO)₂Tr]T_A, [(MeO)₂Tr]bz^oA_o, 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 NH₂-protecting groups was carried out with 25% NH₃/H₂O at 60° for 48 h. The DMT-oligomers were purified by HPLC on a 25 $\times$ 4 mm *RP-18* column (gradient *I*), isolated, and the 4,4'-dimethoxytrityl residues were removed by treatment with 80% AcOEt/H₂O 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 $\times$ 25-mm HPLC cartridge (*RP-18*, silica gel) using H₂O (10 ml) for the elution of the salt, while the oligomer was eluted with

MeOH/H₂O 3:2 (5 ml). The nucleotides were lyophilized on a *Speed-Vac* evaporator. Colorless solids, which were dissolved in H₂O (100 µl) and stored frozen at –18°. The yields are between 3 and 14 A₂₆₀ units.

Photochemical Conversion of the Oligonucleotide 12 to 11. A soln. of **12** (2 A₂₆₀ units) in H₂O (100 µl) was irradiated with 4-W Hg resonance lamp in quartz cuvettes. Samples were taken after intervals of time (*Fig. 1*), and the reaction was followed by HPLC. Then, conc. aq. NH₃ (100 µl) was added and the solvent evaporated. The oligonucleotide was identical with **11** obtained from **1**.

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