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QUARTERLY

Dedicated to Professor David Shugar on the occasion of his 80th birthday

Synthesis and application of novel nucleoside phosphonates and phosphoramidites modified at the base moiety*

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The H-phosphonates and phosphoramidites of 2′-deoxyisoguanosine, 2′-deoxyisoinosine, 5-aza-7-deaza-2′-deoxyguanosine, and N^1-methyl-2′-deoxyformycin A were prepared. The diphenylcarbamoyl group was chosen for the 2-O-protection of 2′-deoxyisoinosine and 2′-deoxyisoguanosine, and dimethylaminoalkylidene groups were used to block the amino function of the various monomers. The synthesis of isoguanine oligonucleotides was found to be much more efficient using the 2-O-protected building blocks compared to those without oxygen protection. Oligodeoxynucleotides containing 2′-deoxyisoguanosine and 2′-deoxycytidine form parallel duplex structures. The self-complementary duplex containing 5-aza-7-deaza-2′-deoxyguanosine and 2′-deoxycytidine forms a stable duplex in acidic solution (pH = 5.0) while it is destabilized under neutral conditions.

Antisense oligonucleotides targeted to certain segments within single-stranded regions of m-RNA have conquered the center of DNA research within the last few years [1, 2]. The exploration and synthesis of novel oligonucleotide building blocks with modified bases is important for the progression of this work. Modified bases can form unusual base pairs, may stabilize the DNA-duplex thermodynamically, and can lead to oligonucleotides being resistant to exo- and endonucleases [3, 4]. This paper reports on the synthesis of four different DNA building blocks derived from 2′-deoxyisoguanosine (**1**), 2′-deoxyisoinosine (**2**), N^1-methyl-2′-deoxyformycin A (**3**), and 5-aza-7-deaza-2′-deoxyguanosine (**4**). Two of them (**1** and **4**) form unusual base pairs. The compounds **2** and **3** are fluorescent. For formulae see Scheme 1.

RESULTS AND DISCUSSION

Base-modified building blocks for solid-phase oligonucleotide synthesis

Phosphonates and phosphoramidites of 2′-deoxyisoguanosine and 2′-deoxyisoinosine

The 2-oxo groups of 2′-deoxyisoguanosine (**1**) and 2′-deoxyisoinosine (**2**) are more reactive than the 6-oxo groups of 2′-deoxyguanosine and 2′-deoxyinosine ([5] and F. Seela & Y. Chen, unpublished results). Therefore, the coupling

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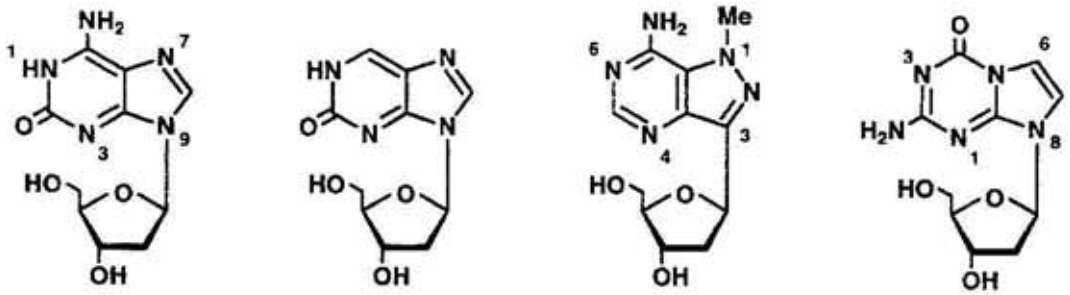
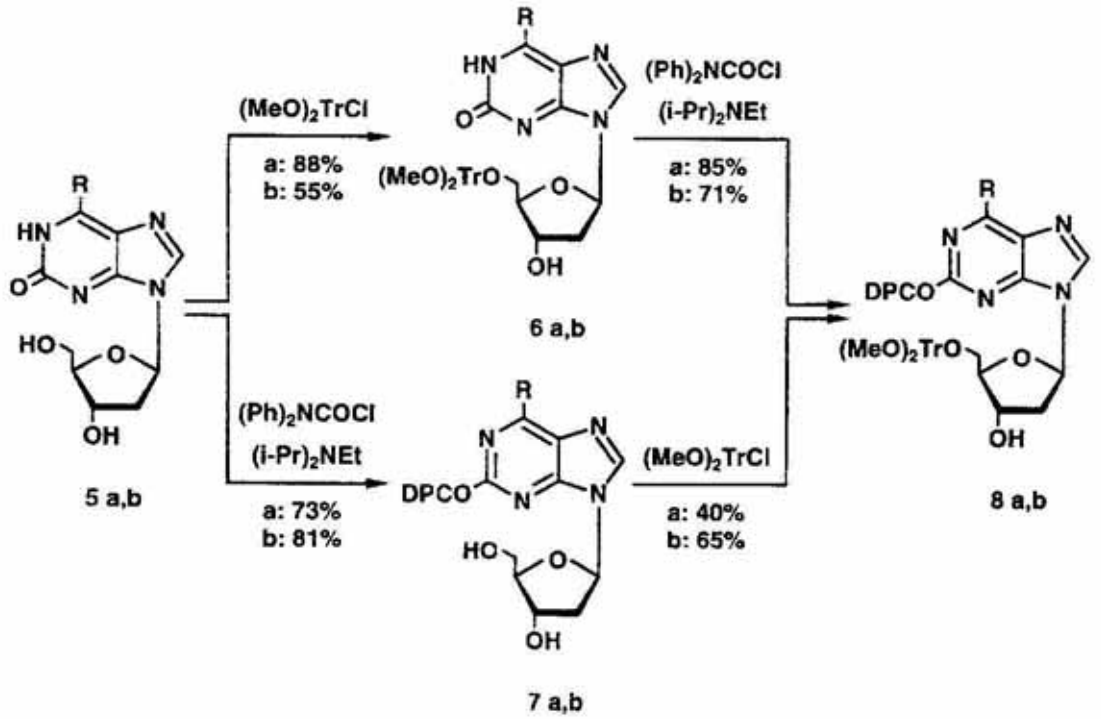
Abbreviations: DPC, diphenylcarbamoyl group; DPC-Cl, DPC chloride; DMF, dimethylformamide; DMT, dimethoxytriphenylmethyl; THF, tetrahydrofurane.

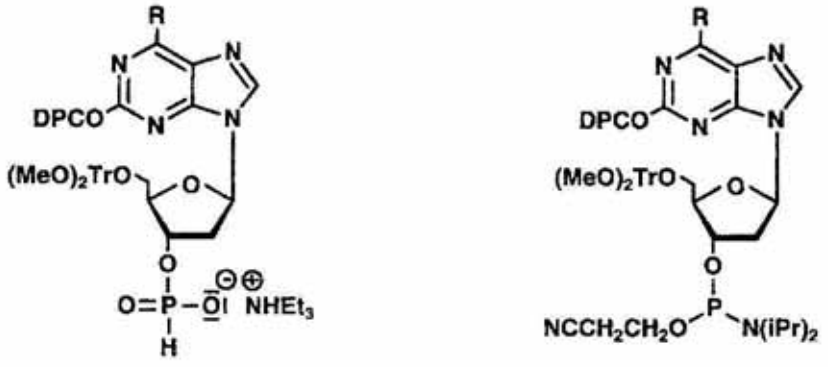


1 2 3 4

Scheme 1



5 a,b 6 a,b 7 a,b 8 a,b



9 a,b 10 a,b

a: R = H

b: R = N=C(Me)N(Me)2

DPC = C(=O)N(Ph)2

Scheme 2

efficiency is low when 2′-deoxyisoinosine- or 2′-deoxyisoguanosine phosphoramidites are used in oligonucleotide synthesis without protecting the 2-oxo group [6, 7]. The coupling yield of phosphonates is higher, but it is still difficult to incorporate consecutive residues into the growing oligonucleotide chain. In order to improve the coupling yield further the 4-nitrophenylethyl group was introduced as 2-oxo protecting group [8]. Nevertheless, protection and deprotection procedures are laborious in this case. As the diphenylcarbamoyl (DPC) group has already been used for the 6-oxo group protection of guanosine [9] it is now applied for the protection of compounds **1** and **2**. The DPC group has the advantage of great lipophilicity which facilitates chromatographic separation. The group is also sufficiently stable under acidic or basic conditions [9].

The reaction of 2′-deoxyisoguanosine (**1**) with *N,N*-dimethylacetamide dimethyl acetal in methanol [10] afforded the amidine derivative **5b** (90% yield). The latter as well as 2′-deoxyisoinosine (**2** = **5a**) were converted to the 5′-*O*-dimethoxytrityl-derivatives **6a,b** using standard conditions. For formulae see Scheme 2.

The diphenylcarbamoyl protecting group was introduced into compounds **6a,b** using diphenylcarbamoyl chloride (DPC-Cl)/diisopropylethyl amine in dry pyridine (**8a,b**). On an alternative route compounds **5a,b** were reacted directly with DPC-Cl yielding the derivatives **7a,b** regioselectively (**7a**: 73%; **7b**: 81%). The half-lives of deprotection were 20 min for **7a** and 54 min for **7b** (25% aq. NH_3, 40°C). Subsequent tritylation of **7a,b** with $(MeO)_2TrCl$ gave compounds **8a,b**. Next, the phosphonates **9a,b** and the phosphoramidites **10a,b** were prepared and purified by column chromatography. All compounds were characterized by UV-, ^1H-, ^{13}C-, and ^{31}P-NMR spectra as well as by elemental analyses. The ^{13}C-NMR data are summarized in Table 1.

Synthesis of N^1-methyl-2′-deoxyformycin A

Formycin A (**11**) — a cytotoxic analog of adenosine — shows strong fluorescence and phosphorescence [11]. The X-ray analysis [12] demonstrated that it exhibits a special glycosylic bond conformation (χ = –110°; "high-*anti*"). Poly(formycin A) [13] reveals inverted ORD-spectra relative to poly(A) from which a left-handed helical sense of the polynucleotide chain was deduced. More recently 2′-deoxyfor-

Table 1

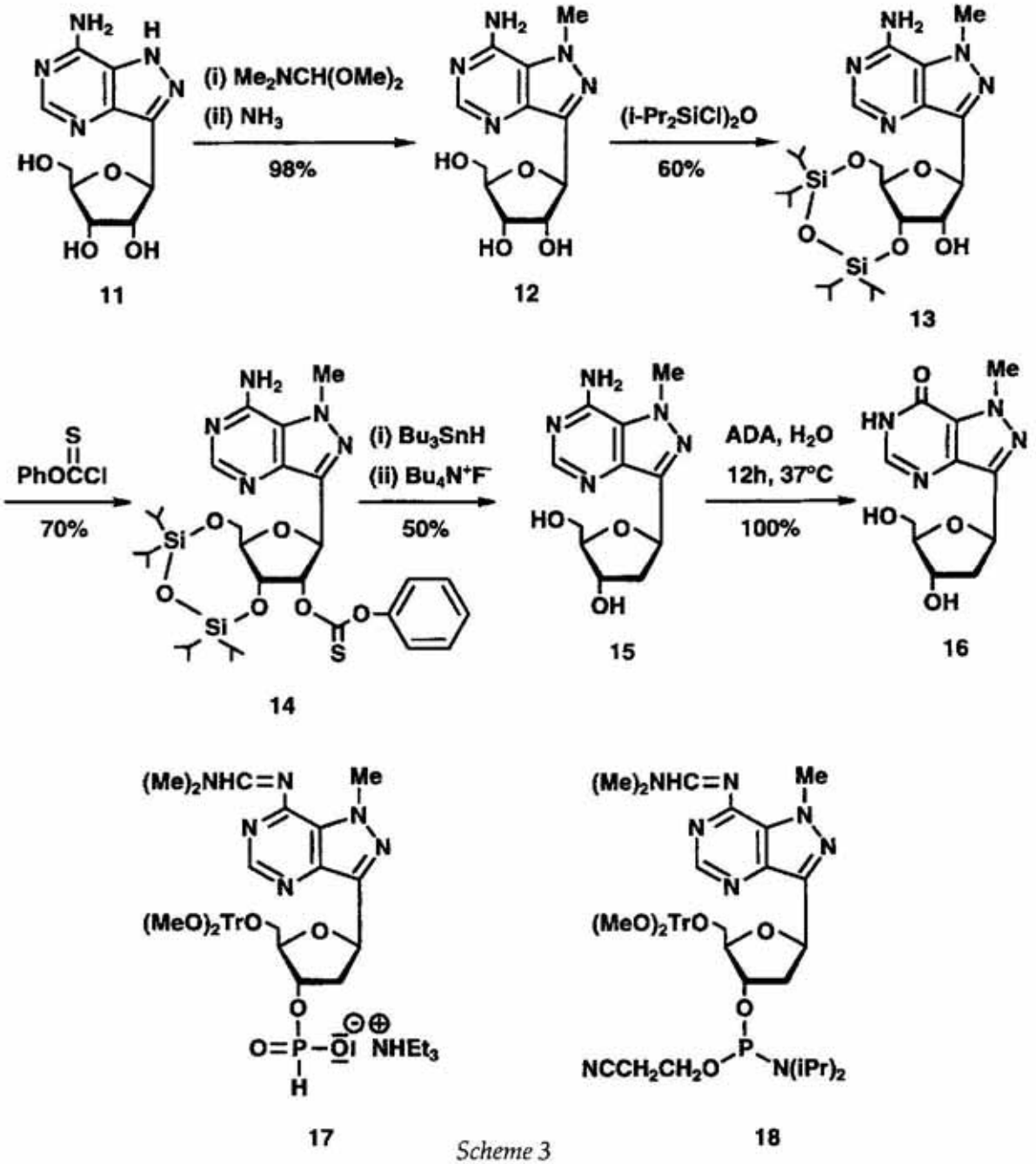
^{13}C-NMR chemical shifts of 2′-deoxyisoinosine and 2′-deoxyisoguanosine derivatives in d_6-DMSO at 303 K

Compd.[a]	C-2	C-4	C-5	C-6	C-8	C=O	N=C	C-1′	C-2′[d]	C-3′	C-4′	C-5′
5a	156.1	158.8	123.6	139.4	145.5	–	–	82.9	–	70.9	87.9	61.7
6a	155.9	159.0	123.6	139.5	145.7	–	–	82.4	–	70.5	85.5	64.2
7a[b]	155.5	152.7	132.9	149.9	146.2	151.5	–	83.6	–	70.6	88.2	61.6
8a	155.6	152.7	132.9	149.7	146.1	151.5	–	83.3	–	70.4	85.5	64.1
9a	155.6	152.8	133.0	150.0	145.9	151.4	–	83.7	–	72.7	85.6	63.9
5b[b]	156.4[c]	157.0	113.2	153.6[c]	140.1	–	163.9	83.6	–	71.0	88.0	62.1
6b	156.8[c]	157.4	113.2	153.6[c]	139.4	–	163.7	82.1	–	70.7	85.5	64.3
7b[b]	160.7[c]	141.9	123.9	155.3[c]	141.1	151.5	162.2	83.4	–	70.7	87.9	61.7
8b	160.9[c]	142.0	124.0	155.7[c]	140.9	151.7	162.3	82.9	–	70.6	85.5	64.2
9b	160.8[c]	141.9	123.9	155.5[c]	140.5	151.5	162.2	83.0	–	72.5	85.5	63.7

[a] Purine numbering. [b] From gated-decoupled spectra. [c] Tentative. [d] Superimposed by DMSO.

mycin A was incorporated into oligodeoxynucleotides [14]. Ambiguous base pairing [15] caused by the N(1)-H ⇔ N(2)-H tautomerism was reported [16] which can be circumvented using N^1-methyl 2′-deoxyformycin A (**15** = **3**).

The reaction of formycin A (**11**) with *N*,*N*-dimethylformamide dimethyl acetal (65°C, 14 h, DMF) followed by hydrolysis with conc. NH_3 aq. (room temp., 3 days) resulted in an almost quantitative formation of N^1-methylformycin A (**12**) [17]. The reaction follows a mechanism of *inter*molecular transmethylation [18]. Condensation of compound **12** with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in pyridine (20 h, room temp.) gave the 3′,5′-O-protected derivative **13** in 60% yield [19]. Subsequent treatment of **13** with phenoxythiocarbonyl chloride in the presence of *N*,*N*-dimethylaminopyridine (MeCN, Ar, 20 h, room temp.) gave the 2′-*O*-phenylthiocarbonyl derivative **14** in 70% yield. The latter was deoxygenated (Bu_3SnH, AIBN, toluene, Ar, 75°C, 4 h) and desilylated (Bu_4NF,



Scheme 3

1 M in THF, 75°C, 2 h) affording crystalline **15** (m.p. 220–222°C (EtOH)). **15**: ^{1}H-NMR (d_6-DMSO): δ, 8.11(s, H-5); 7.40 (br. s., NH_2); 5.70 (m, H-1'); 5.32 (t, 5'-OH); 5.04 (m, 3'-OH); 4.34 (m, H-3'); 4.19 (s, CH_3); 3.88 (m, H-4'); 3.58 and 3.48 (m, H_2-5'); ≈2.5 (m, H_β-2'); 2.02 (m, H_α-2').

The Michaelis-Menten constants for the enzymatic deamination of N^1-methyl-2'-deoxyformycin A (**15**) were measured (K_m = 800 μM; V_{max} = 50 mM/min·mg of enzyme = 0.25 mM/min·unit). Despite the fact that the enzymic deamination is slow [20], compound **15** (10 mg, H_2O, 12 h, 37°C, 100 units) was converted into crystalline N^1-methyl-2'-deoxyformycin B (**16**) on a preparative scale (m.p. 200–202°C (EtOH-H_2O, 1:1)). **16**: ^{1}H-NMR (d_6-DMSO): δ, 7.86 (s, H-5); 5.28 (dd, J = 10.4, 5.7 Hz, H-1'); 5.07 and 4.99 (2 x OH); 4.30 (m, H-3'); 4.15 (s, CH_3); 3.83 (m, H-4'); 3.52 (m, H_2-5'); ≈2.5 (m, H_β-2'); 2.03 (m, H_α-2'). Standard reaction conditions can be used for the conversion of N^1-methyl-2'-deoxyformycin A into the phosphonate **17** and the phosphoramidite **18** (Scheme 3).

All compounds were characterized by ^{1}H- and ^{13}C-NMR [21], as well as by mass spectra (for ^{13}C-NMR data, see Table 2). Upon introduction of the silyl clamp (**13**) only the ^{13}C-NMR signal of C-3' but not that of C-5' is affected. The 2'-deoxynucleoside **15** shows intensive fluorescence in water with a broad excitation maximum at 300 nm and an emission maximum at 335 nm (shoulders at 325 and 350 nm).

Phosphonates and phosphoramidites of 5-aza-7-deaza-2'-deoxyguanosine

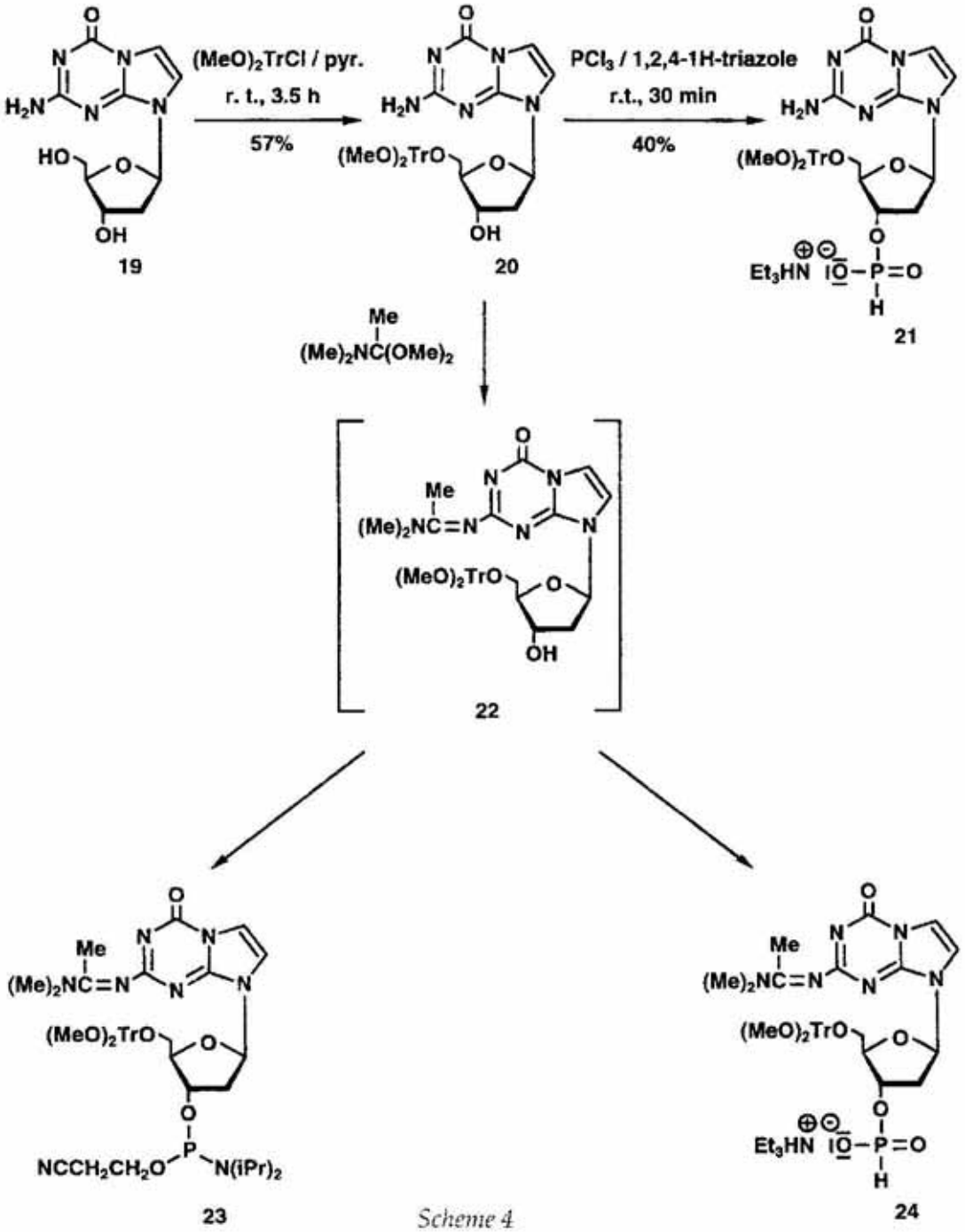
5-Aza-7-deaza-2'-deoxyguanosine ($c^7z^5G_d$, **19** = **4**, Scheme 4) has been synthesized in our laboratory in 1987 [22]. The normally stereoselective glycosylation leads to anomeric mixtures when the anion of 2-isobutyrylamino-8-H-imidazo[1,2-a]-s-triazin-4-one is glycosylated with 2-deoxy-di-*O*-(*p*-toluoyl)-α-D-*erythro*-pentofuranosyl chloride [22]. The nucleoside **19** shows a pK value of protonation (pK = 3.5) which is similar to that of deoxyguanosine (3.7) [23].

5-Aza-7-deaza-2'-deoxyguanosine (**19** = **4**) was reacted with 4,4'-dimethoxytriphenylmethyl chloride (pyridine, room temp., 3.5 h) to yield the 5'-O-DMT-derivative **20** (57%). Subsequent protection of **20** with *N*,*N*-dimethylacetaldehyde dimethyl acetal gave the dimethylaminoethylidine derivative **22** which was not isolated but converted directly into the phosphonate **24** (PCl_3/*N*-methylmorpholine/1,2,4-triazole) (Scheme 4). Also the phosphoramidite **23** can be prepared from **22**. Alternatively, the 5'-O-DMT-protected **20** was converted into the 3'-phosphonate without prior protection of the amino function (→**21**). All compounds were characterized by ^{1}H, ^{13}C-, and ^{31}P-NMR spectra. Table 3 summarizes the ^{13}C-NMR data.

Table 2
^{13}C-NMR data of N^1-methylformycin derivatives in d_6-DMSO at 303 K

Compd.[a]	C-3	C-3a	C-5	C-7	C-7a	C-1'	C-2'	C-3'	C-4'	C-5'	CH_3
11	143.2	138.3	151.4	151.6	123.4	78.2	75.3	72.6	86.1	62.6	–
12	142.0	140.2	151.0	151.2	122.0	78.1	75.0	72.3	86.0	62.6	40.1
13	141.2	140.6	151.3	151.0	121.7	72.6	74.0	78.9	80.3	62.0	38.6
14	140.3	139.4	151.6	151.0	121.8	85.7	75.7	71.6	80.7	61.2	38.9
15	142.8	140.1	151.0	151.2	122.1	73.9	[b]	73.1	88.3	62.8	40.0
16	143.9	136.9	143.2	153.6	126.5	73.1	[b]	72.8	88.1	62.7	38.9

[a]Systematic numbering. [b]Superimposed by DMSO.



Scheme 4

Table 3

^{13}C-NMR chemical shifts of 5-Aza-7-deaza-2'-deoxyguanosine in d_6-DMSO at 303 K

Compd.[a]	C-4	C-8a	C-2	C-7	C-6	C-1'	C-2'	C-3'	C-4'	C-5'	CH_3
19	165.4	150.2	150.1	114.2	108.4	87.7	38.8	70.6	82.8	61.6	–
20	164.9	150.2	149.7	114.1	108.3	85.6	38.6	70.1	82.6	63.9	–
21	165.3	150.4	149.9	113.7	108.4	85.6	37.6	72.3	82.7	63.7	–
24	162.0	150.2	149.9	115.1	108.1	85.6	37.9	72.1	83.1	63.6	16.0

[a] Systematic numbering.

Oligonucleotides

Oligonucleotides containing 2'-deoxyisoguanosine

The DPC-protected phosphonate **9b** as well as the phosphoramidite **10b** were used for solid-phase oligonucleotide synthesis. Oligonucleotides such as **26** or **27**, containing consecutive iG_d-residues, were only accessible by using building blocks with oxygen protection such as **9b** or **10b**.

5'- d(iGCiGCiGC)	5'- d(iGiGiGCCC)	5'- d(CCCiGiGiG)
25	**26**	**27**

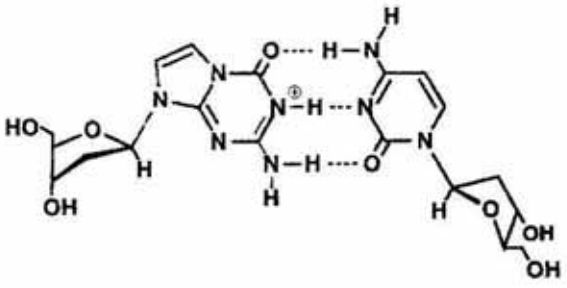
The self-complementary oligomer **25** shows cooperative melting with a t_m of 32°C (8.4 μM of single strands, 1 M NaCl, 0.1 M $MgCl_2$, 60 mM Na-cacodylate buffer, pH 7.0). When the oligomers **26** and **27** were hybridized a t_m value of 46°C was observed. As both individual oligonucleotides exhibit only low t_m values (**26**: 17°C; **27**: 16°C) it can be concluded that a parallel orientated duplex **26·27** is formed.

Oligonucleotides with 5-aza-7-deaza-2'-deoxyguanosine

As mentioned earlier unusual base pairing was expected for compound **4** with 2'-deoxycytidine. For this purpose the oligonucleotide **28** was synthesized and duplex formation with **30** was studied. For comparison the oligomer **29** was prepared.

5'- d(T_5-c^7z^5G-T_6)	5'- d(T_5GT_6)	5'- d(A_6CA_6)
28	**29**	**30**

The oligomers **28** and **29** were hybridized with an equimolar amount of the oligonucleotide **30** (5 μM single strand concentration). Next, the duplex stability was studied as a function of the pH using 10 mM Na-cacodylate, 10 mM $MgCl_2$, 100 mM NaCl at pH 5.0, 6.0, 7.0, 8.5, and 9.5 (not shown). In all cases cooperative melting profiles were observed. Figure 1A shows a graph of t_m values of the modified duplex **28·30** plotted against the pH value of the solution. Figure 1B displays the graph for the parent duplex **29·30**. The t_m values of the non-modified duplex are almost independent from the pH (5.0–9.5) while the t_m values of the modified duplex increased from 22°C to 35°C when the pH value was decreased (9.5 to 5.0) [24]. At low pH the t_m value of **28·30** is similar to that of the non-modified duplex **29·30**. The inflection point (7.7) of this graph represents the pK value of the modified base within the oligonucleotide chain (Scheme 5).



Scheme 5

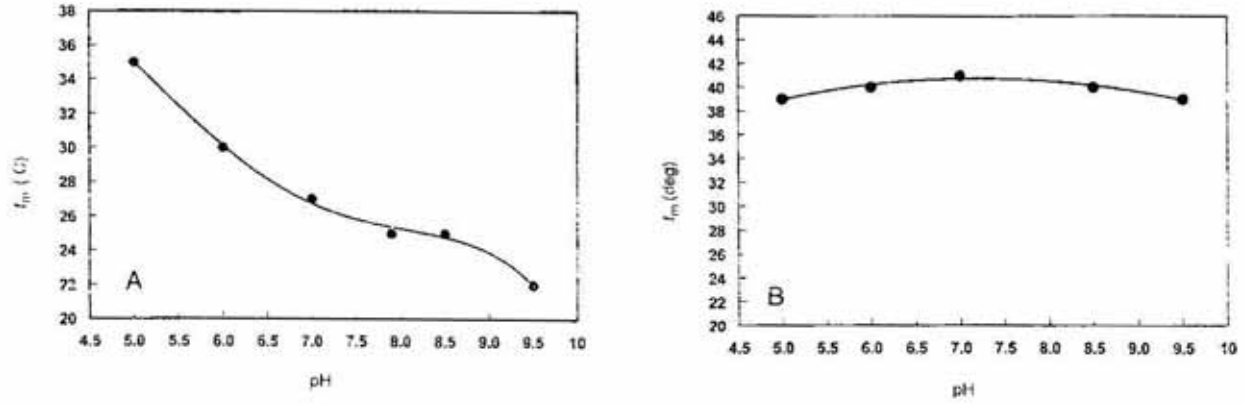


Fig. 1. t_m Values of the duplex **28·30** *(A) and of* **29·30** *(B) as a function of pH.*

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