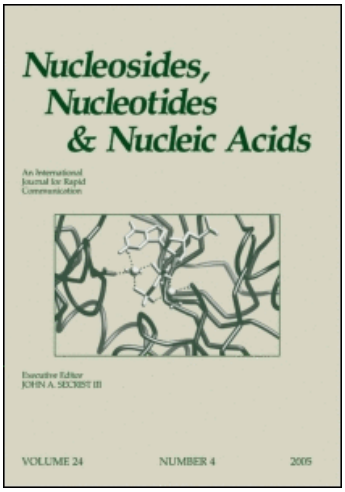

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Oligonucleotides With Purine Nitrogen-7 as Glycosylation Site

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OLIGONUCLEOTIDES WITH PURINE NITROGEN-7 AS GLYCOSYLATION SITE

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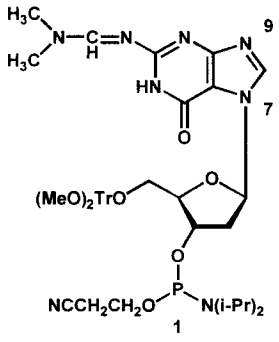
ABSTRACT: The synthesis of phosphoramidites (**2** and **3**) derived from hypoxanthine and isoguanine N^7-2'-deoxyribonucleosides is described. Solid-phase synthesis furnishes oligonucleotides containing N^7-glycosylated purines. New base pairs between purine N^7- and N^9-nucleosides are proposed.

The base pairing of nucleic acids is controlled by the donor/acceptor pattern between purine and pyrimidine bases as well as by the structural, configurational, and conformational characteristics of the nucleic acid backbone. The base pairing of N^7-(2-deoxy-β-D-*erythro*-pentofuranosyl)adenine (^7A_d) with dT was the first report on the duplex formation of an N^7-purine oligonucleotide.[1-3] Previously, N^7-(2-deoxy-β-D-*erythro*-pentofuranosyl)guanine (^7G_d) has shown to form a duplex of considerable stability in d(^{7}G-C)$_6$.[4] This work will now be extended to other purine N^7-nucleosides such as N^7-(2-deoxy-β-D-*erythro*-pentofuranosyl)hypoxanthine (^7I_d, **7**)[5] and N^7-(2-deoxy-β-D-*erythro*-pentofuranosyl)isoguanine (^7iG_d, **9**).[6] The phosphoramidite building blocks **1** and **4** have already been synthesized.[3,4] Studies with **5** and **6** are in progress. Now the phosphoramidites **2** and **3** are synthesized and oligonucleotides containing ^7iG_d or ^7I_d are prepared.



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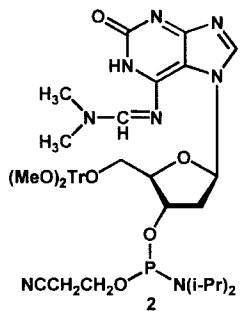

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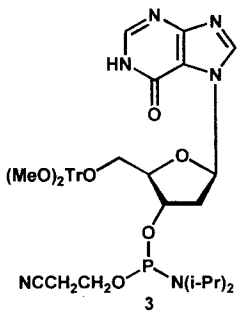

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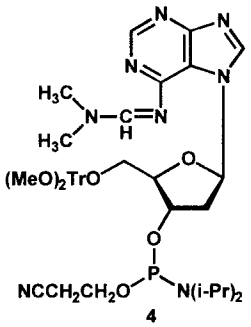

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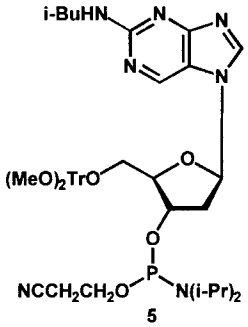

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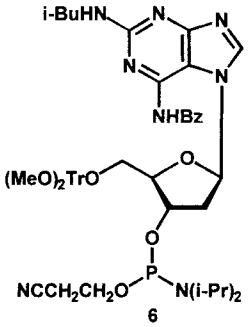

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The (dimethylamino)methylidene residue was used for protection of the amino group of **9**. Compound **10** as well as the nucleoside **7** were transformed into the 4,4'-dimethoxytrityl derivatives **8** and **11** under standard conditions. Also the phosphonates of the nucleosides **7** and **9** were prepared. They carry the same protecting groups as the corresponding phosphoramidites. Table 1 summarizes selected ^{13}C-NMR data which were used for structural characterization.

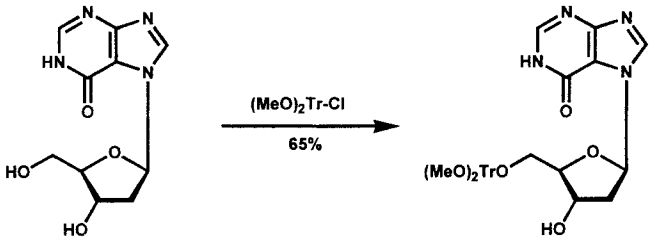

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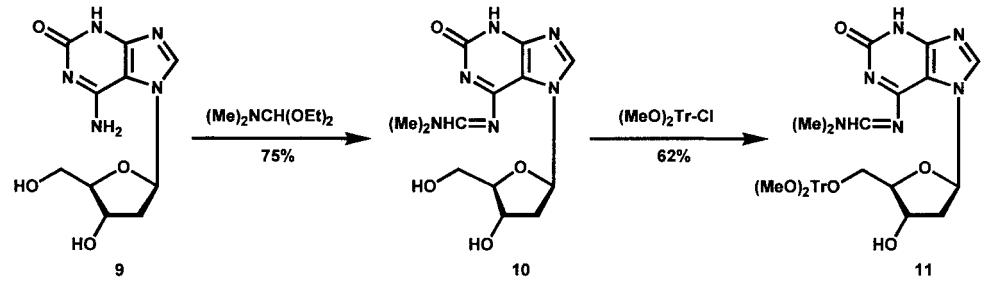
TABLE 1. ^{13}C-NMR Chemical Shifts of Purine N^7-2'-Deoxyribofuranosides [a] [b])

	C-2	C-4	C-5	C-6	C-8
7[5]	144.9	157.4	114.3	154.4	141.5
8	144.8	157.5	114.4	154.0	141.2
9[6]	154.0 [c])	[d])	102.8 [c])	156.6 [c])	141.6 [c])
11	156.8 [c])	[d])	108.5 [c])	157.0 [c])	140.0 [c])
	C1'	**C2'**	**C3'**	**C4'**	**C5'**
7[5]	85.9	39.4	70.3	88.0	61.2
8	85.5	40.7	70.2	86.0	64.0
9[6]	85.5	38.6	69.3	87.9	60.5
11	85.7	41.8	69.8	86.0	63.7

[a]) Spectra measured in (D_6)DMSO rel. to $SiMe_4$ at room temperature.
[b]) From [^{1}H,^{13}C] gated-decoupled spectra. [c]) Tentative. [d]) Not detected.



Two sets of oligonucleotides were synthesized containing either two ^7G_d- or two ^7iG_d-residues in the center of d(T_{12}) or replacing the dG-residues of d(TAGGTCAATACT) (Tables 2, 3). The solid-phase synthesis was performed on a ABI 392 synthesizer using the standard protocol. The coupling efficiency of the modified phosphoramidites was the same as found for the regular ones. The composition of the oligomers was confirmed by MALDI-TOF mass spectra and enzymatic composition analysis.

Next, the duplex stability of the oligonucleotides was analyzed by T_m-measurements (Tables 2 and 3). For this purpose oligonucleotides of the sequences d(T_5XXT_5) and

TABLE 2. T_m-Values and Thermodynamic Data of Duplex Melting of 5'-d(TTTTTXXTTTTT) 5'-d(AAAAAYYAAAAA)[a] containing ^7G_d and ^7iG_d.

XX YY	T_m [°C]	ΔH [kcal/mol]	ΔS [cal/mol K]	h [%]
TT AA	37	-89	-281	22
GG CC	39	-90	-294	n.d.
$^7G^7G$ CC	34	-84	-273	19
$^7G^7G$ GG	28	-80	-262	23
$^7G^7G$ c^7G c^7G	26	-82	-274	21
$^7G^7G$ AA	14	-76	-266	22
$^7G^7G$ TT	15	-72	-251	24
GG GG	<10	n.d.	n.d.	n.d.
$^7iG^7iG$ GG	30	-62	-204	20
$^7iG^7iG$ c^7G c^7G	27	-68	-228	23
$^7iG^7iG$ CC	20	-84	-287	20
$^7iG^7iG$ AA	20	-69	-234	24
$^7iG^7iG$ TT	<10	-88	-313	22

[a] Measured at 260 nm in 0.1 M NaCl containing 10 mM $MgCl_2$, and 10 mM Na-cacodylate (pH 7.0) at 5 μmol single strand concentration; n.d. : not detected.

TABLE 3. T_m-Values and Thermodynamic Data of Duplex Melting of 5'-d(TAXXTCAATACT) 5'-d(ATYYAGTTATGA) [a] containing ^7G_d and ^7I_d.

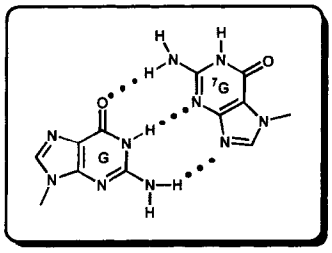
XX YY	T_m [°C]	ΔH [kcal/mol]	ΔS [cal/mol K]	h [%]
GG CC	47	-94	-292	26
GG $^7G^7G$	37	-83	-287	26
c^7Gc^7G $^7G^7G$	39	-84	-271	27
$^7G^7G$ $^7G^7G$	37	-61	-196	20
GG $^7I^7I$	30	-65	-218	23
c^7Gc^7G $^7I^7I$	29	-60	-215	25
$^7G^7G$ $^7I^7I$	28	-74	-246	22

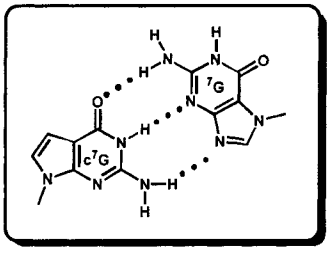
[a] Conditions see Table 2.

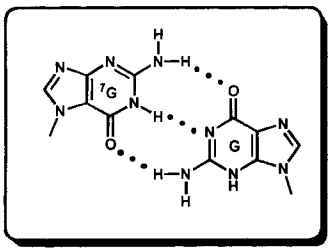
d(A_5YYA_5) were hybridized. In the case of d(T_5XXT_5) X is either ^7G_d, ^7iG_d while Y stands for dA, dG, dT, dC or 7-deaza-2'-deoxyguanosine (c^7G_d) in d(A_5YYA_5) (Table 2). In another experiment the N^7-nucleosides were incorporated into the sequence d(ATYYAGTTATGA) and were hybridized with d(TAXXTCAATACT)(Table 3). Here, Y represents ^7G_d or ^7I_d and X is dG, ^7G_d or c^7G_d. In all cases sigmoidal melting profiles were observed from which thermodynamic data were calculated.

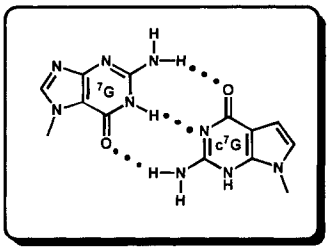
From the Tables 2 and 3 it is apparent that the T_m-values decrease only moderately when ^7G_d is located opposite to dG or c^7G_d. As expected, a relatively high T_m-value is found for duplexes in which ^7G_d is located opposite to dC. The oligonucleotides containing ^7iG_d seem to form base pairs with dG or c^7G_d (Table 2). However, the enthalpic data for the duplex formation are much lower when ^7iG_d is located opposite to dG compared to those containing ^7G_d. In all the cases where the ^7G_d is located opposite to dA or dT duplexes are less stable. A similar trend of duplex stability is found for ^7G_d in both sets of oligonucleotide duplexes. It is also apparent that duplexes containing ^7I_d are considerably less stable than those containing ^7G_d supporting base pairing between ^7G_d and dG.

According to the stabilities of the various oligonucleotide duplexes base pairing is proposed for ^7G_d • dG, ^7G_d • c^7G_d and ^7G_d • ^7G_d. Base pairs can also be considered for ^7iG_d • dG and ^7iG_d • c^7G_d. Base pairing between ^7A_d • dT and ^7G_d • dC has already been reported.[3,4] As it was of interest to establish structural motives for the various base pairing modes, models were constructed and inserted into the B-DNA duplex. Those

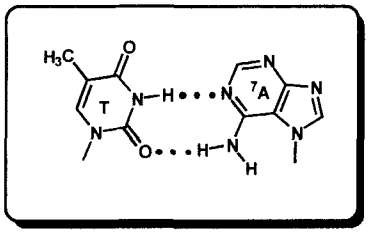


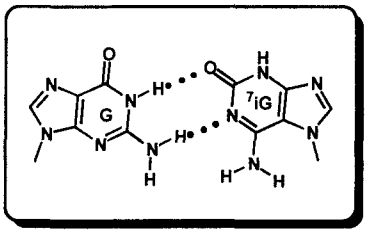






base pairs which show the best fitting are depicted below. Nevertheless, a final conclusion on the various motives can only be given after structural elucidation of such duplexes using NMR-spectroscopy or single crystal X-ray analysis.





Apart from the base pairing properties of the 7-glycosylated purines the nucleosides show strong fluorescence under alkaline conditions (NaOH, pH 12.0). Excitation of $^{7}G_{d}$ at 282 nm results in an emission at 363 nm. Among the various purine N^{7}-nucleosides the fluorescence of $^{7}iG_{d}$ was particularly strong. While excited at 295 nm the emission maximum appeared at 361 nm.

Acknowledgments

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REFERENCES

1. Seela, F.; Winter, H. *10th International Roundtable* **1992**, Park City, USA.
2. Seela, F.; Winter, H. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 273.
3. Seela, F.; Winter, H. *Helv. Chim. Acta* **1994**, *77*, 597.
4. Seela, F.; Leonard, P. *Helv. Chim. Acta* **1996**, *79*, 477.
5. Seela, F.; Winter, H. *Nucleosides & Nucleotides* **1995**, *14*, 129.
6. Kazimierczuk, Z.; Mertens, R.; Kawczynski, W.; Seela, F. *Helv. Chim. Acta* **1991**, *74*, 1742.