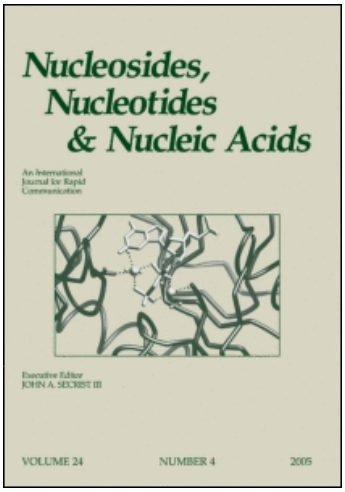

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7-Deaza-2'-deoxyinosine: A Stable Nucleoside with the Ambiguous Base Pairing Properties of 2'-Deoxyinosine

Frank Seela[a]; Cathrin Mittelbach[a]
[a] Laboratorium für Organische und Bioorganische Chemie, Institut für Chemie, Universität Osnabrück, Osnabrück, Germany

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7-DEAZA-2'-DEOXYINOSINE: A STABLE NUCLEOSIDE WITH THE AMBIGUOUS BASE PAIRING PROPERTIES OF 2'-DEOXYINOSINE

Frank Seela* and Cathrin Mittelbach

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

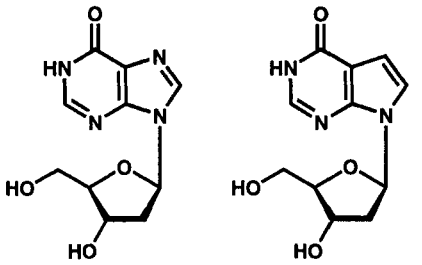
ABSTRACT: The base pairing ambiguity of 7-deaza-2'-deoxyinosine (c^7I_d, **2**) was studied and was found to be the same as that of 2'-deoxyinosine. The duplex stability decreases in the order [d(c^7I-C) > d(c^7I-A) > d(c^7I-T) > d(c^7I-G)]. Modified nucleosides were used to probe the various base pair motifs which were the same for dI and c^7I_d. The 7-deazapurine nucleoside (**2**) is extremely stable against acid or base. As oligonucleotides can be prepared using phosphoramidite chemistry and DNA is accessible by enzymic polymerisation of the triphosphate of **2**, the latter can be used as an universal nucleoside for the sequencing of DNA by chemical degradation and is otherwise a facile substitute of 2'-deoxyinosine when stability in acidic or alkaline solution is required.

INTRODUCTION

The naturally-occurring ribonucleoside inosine is known to form wobble base pairs at the ambiguous positions of the anticodon of tRNAs.[1,2] The corresponding 2'-deoxyinosine (dI, **1**) is the classical universal nucleoside which shows ambiguous base pairing with the four natural components of DNA.[1] Universal nucleosides, such as **1** are useful for primers or for probes in DNA technology.[3-6] In the mean time several other nucleosides with the capability of ambiguous base pairing have been synthesised and their pairing properties were investigated.[7-16]

Recently, it was shown that 7-deazapurine nucleosides including 7-deaza-2'-deoxyinosine (**2**) can protect DNA from formamide degradation at positions where the modified base is incorporated.[17] This is the result of the stability of compound **2** towards such nucleophiles as well as towards acid or base[18]; neither the N-glycosylic bond nor the five-membered ring are cleaved even under rigorous conditions.

According to the fact that purine nucleosides are degraded while 7-deazapurine nucleosides are stable, the latter can be used for chemical footprinting experiments or for chemical sequencing.[17] As nothing is known about the base pairing properties of 7-deaza-2'-deoxyinosine (**2**) this work will focus on the topic whether 7-deaza-2'-deoxyinosine (**2**) can replace 2'-deoxyinosine with regard to ambiguous base pairing. A comparative study of base pair stability will be presented using oligonucleotides which contain either compound **1** or **2** and the four common nucleosides opposite to them. Finally, the base pair motifs which were anticipated for 2'-deoxyinosine[4,19-25] will be proven with this analogue which is not able to form Hoogsteen base pairs. According to the favourable properties of compound **2** it will be useful to substitute 2'-deoxyinosine (**1**) in reactions which degrade the parent purine nucleoside.



1 **2**

RESULTS AND DISCUSSION

*1. Chemical and physical properties of the nucleosides **1** and **2**.*

The nucleoside **2** has been synthesised earlier using liquid - liquid transfer glycosylation.[18] As the glycosylation yield was rather low (40%) the nucleobase anion glycosylation[26,27] of 4-methoxy-2-methylthio-7H-pyrrolo[2,3-d]pyrimidine[28] with 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-*erythro*-pentofuranosyl chloride[29] in MeCN, in the presence of KOH and tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1) was performed. This increased the glycosylation yield to 71%. The displacement of the methoxy group and the removal of the methylthio residue was carried out as described.[18] Compound **2** was protected with a DMT residue at the 5' position[13] and converted into the cyanoethylphosphoramidite using standard methods.[15,30]

A significant difference between the nucleosides **1** and **2** regarding their N-glycosylic bond stability is observed. Compared to 2'-deoxyinosine (**1**) (t1/2 = 27 min in 0.1 N HCl at 30°C)[31] the N-glycosylic bond of 7-deaza-2'-deoxyinosine (**2**) is extremely

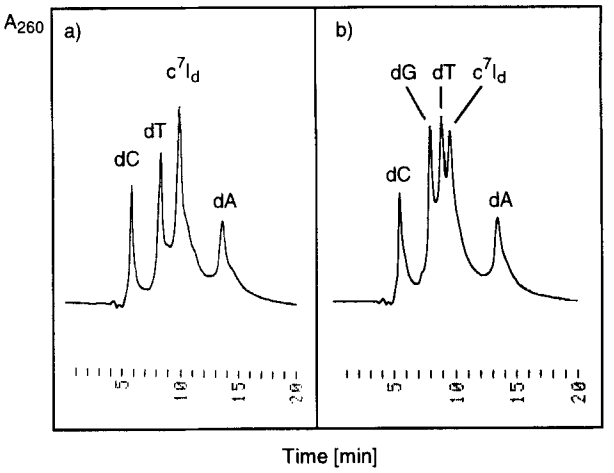


FIG. 1 *HPLC-* Profiles of the oligonucleotides **11** (a) and **16** (b) after enzymic hydrolysis with snake-venom phosphodiesterase followed by alkaline phosphatase in 1M Tris-HCl buffer (pH 8.3). The HPLC profiles were measured at 260 nm using 20 min 100% 0.1M (Et_3NH)OAc (pH 7.0)/MeCN, 95:5 with a flow rate of 0.6 ml/min.

stable (t1/2 = 51 h in 2 N HCl ; 35 h in 6 N HCl, both measured at 30°C). Also the 5-membered ring of compound **2** is not opened even under harsh treatment with base.[18] Furthermore, compound **2** is not able to form Hoogsteen base pairs. The Watson-Crick base pairing ability, however, is expected to be the same for compound **1** and **2**. The pK_a-value of deprotonation of compound **2** is 9.8 compared to 9.2 for 2'-deoxyinosine (**1**). Compound **2** is protonated with a pK_a = 1.3, while **1** shows a pK_a-value of 1.5. On RP-18 HPLC compound **2** shows a higher retention time (higher lipophilicity), due to the replacement of nitrogen-7 by a C-H residue. The extinction coefficient (ε_{260}) of compound **2** is 10000; for compound **1** 7700.

2. Oligonucleotide synthesis

Oligonucleotide synthesis was performed on solid-phase using the phosphoramidites of **2**, **27**[30], **28**[32] and **29**[33] and their composition was confirmed by tandem hydrolysis with snake venom phosphodiesterase and alkaline phosphatase, followed by HPLC analysis of the digest. Representative examples of the composition pattern are shown in Figure 1. Also the MALDI TOF-spectra were taken in several cases (see Table 6).

3. Stability of the duplexes 5'-d(T-A-X-X-T-C-A-A-T-A-C-T) • 3'-d(A-T-Y-Y-A-G-T-T-A-T-G-A) with X = c^7I_d or dI and Y = dC, dA, dT and dG.

The pairing properties of 2'-deoxyinosine (**1**) with the four common bases were investigated earlier in a number of manuscripts.[3,4,19-25] For this study we have selected the duplex 5'-d(T-A-G-G-T-C-A-A-T-A-C-T) (**3**) • 3'-d(A-T-C-C-A-G-T-T-A-T-G-A) (**4**) which is routinely used in our laboratory to study the influence of modified bases on the duplex stability. This duplex shows an almost random distribution of the four DNA bases. Here, the replacement of two consecutive dG-residues by compound **1** and **2** is investigated.

It is known that dI behaves like a 2'-deoxyguanosine analogue.[24] Due to the lack of the nitrogen 7 in compound **2** the analogous compound is the 7-deaza-2'-deoxyguanosine (c^7G_d, **29**). Table 1 starts with the reference duplexes **3•4** and **5•4** followed by those containing the 2'-deoxyderivatives **1** and **2**. By incorporation of **1** instead of dG the T_m value of the duplexes with dC decreases from 48°C (**3•4**, Table 1) to 36°C found for the duplex **6•4** (ΔT_m=12°C). In the case of oligomers containing compound **2** and its analogue c^7G_d (**29**) the T_m value differences (ΔT_m) between the duplexes d(c^7G-C) (**5•4**, Table 1) and d(c^7I-C) (**10•4**, Table 1) amount to 10°C.

Most of the duplexes contain the base pair replacement in a consecutive manner. The location in either one or the other chain has no influence on the T_m value (see duplexes **6•4** and **18•7** or **10•4** and **18•13**, Table 1). For comparison duplexes are also presented in which the modified base pairs are separated. This rearrangement has no effects in the case of the duplex containing two c^7I_d-dC base pairs. For both duplexes (**18•13** and **3•11**) the same T_m values were observed but differences were found in the thermodynamic data. In contrast to this, the insertion of two separated c^7I_d-dG base pairs (**12•4**) leads to a T_m decrease of around 5°C compared to the oligomer duplex containing the consecutive base pairs (**3•13**).

Reference studies were made in which the modified base pairs are embedded in the same environment to exclude nearest neighbours effects. For these investigations we used the oligomer duplexes described by Ohtsuka et al.[3] The modified nucleosides (c^7I_d, dI) are located at position X in the middle of a purine-rich strand whereas the four common nucleosides are located in position Y in an oligo(pyrimidine) region. The following duplexes of oligodeoxynucleotides 5'-d(G-G-A-A-A-A-X-A-A-A-A-G-G) • 5'-d(C-C-T-T-T-T-Y-T-T-T-T-C-C) in which X is dI (**21**) or c^7I_d (**22**) and Y is dC (**23**), dA (**24**), dT (**25**) or dG (**26**) were synthesised (Table 2).

TABLE 1 T_m values and thermodynamic data of duplex formation of oligodeoxynucleotides containing compounds **1** or **2** opposite to the 4 common nucleosides [a]).

Oligomer	T_m [°C]	ΔH [kcal/mol]	ΔS [cal/K mol]	ΔG^{298} [kcal/mol]
5'-d(T-A-G-G-T-C-A-A-T-A-C-T) (**3**) 3'-d(A-T-C-C-A-G-T-T-A-T-G-A) (**4**)	48	-89	-253	-10.9
5'-d(T-A-c^{7}G-c^{7}G-T-C-A-A-T-A-C-T) (**5**) 3'-d(A-T- C- C- A-G-T-T-A-T-G-A) (**4**)	45	-90	-250	-10.0
5'-d(T-A-**1**- **1**-T-C-A-A-T-A-C-T) (**6**) 3'-d(A-T-**C**-**C**-A-G-T-T-A-T-G-A) (**4**)	36	-79	-231	-7.60
5'-d(T-A-**C**-**C**-T-C-A-A-T-A-C-T) (**18**) 3'-d(A-T-**1**-**1**- A-G-T-T-A-T-G-A) (**7**)	36	-85	-250	-7.63
5'-d(T-A-**2**- **2**-T-C-A-A-T-A-C-T) (**10**) 3'-d(A-T-**C**-**C**-A-G-T-T-A-T-G-A) (**4**)	35	-75	-220	-7.29
5'-d(T-A-**C**-**C**-T-C-A-A-T-A-C-T) (**18**) 3'-d(A-T-**2**-**2**-A-G-T-T-A-T-G-A) (**13**)	35	-84	-247	-7.34
5'-d(T-A-**A**-**A**-T-C-A-A-T-A-C-T) (**19**) 3'-d(A-T-**1**-**1**- A-G-T-T-A-T-G-A) (**7**)	31	-77	-229	-6.13
5'-d(T-A-**A**-**A**-T-C-A-A-T-A-C-T) (**19**) 3'-d(A-T-**2**-**2**-A-G-T-T-A-T-G-A) (**13**)	33	-73	-214	-6.89
5'-d(T-A-**T**-**T**-T-C-A-A-T-A-C-T) (**20**) 3'-d(A-T-**1**-**1**-A-G-T-T-A-T-G-A) (**7**)	21	-53	-153	-5.23
5'-d(T-A-**T**-**T**-T-C-A-A-T-A-C-T) (**20**) 3'-d(A-T-**2**-**2**-A-G-T-T-A-T-G-A) (**13**)	24	-58	-170	-5.27
5'-d(T-A-**G**-**G**-T-C-A-A-T-A-C-T) (**3**) 3'-d(A-T-**1**- **1**- A-G-T-T-A-T-G-A) (**7**)	22	-60	-178	-4.93
5'-d(T-A-**G**-**G**-T-C-A-A-T-A-C-T) (**3**) 3'-d(A-T-**2**- **2**-A-G-T-T-A-T-G-A) (**13**)	20	-71	-216	-4.08
5'-d(T-A-G-G-T-**2**-A-A-T-A- **2**-T) (**12**) 3'-d(A-T-C-C-A-**G**-T-T-A-T-**G**-A) (**4**)	15	---	---	---
5'-d(T-A-G-G-T-**C**-A-A-T-A-**C**-T) (**3**) 3'-d(A-T-C-C-A-**2**-T-T-A-T- **2**-A) (**11**)	35	-75	-217	-7.5
5'-d(T-A-**c^{7}G**-**c^{7}G**-T-C-A-A-T-A-C-T) (**5**) 3'-d(A-T- **1** - **1**- A-G-T-T-A-T-G-A) (**7**)	no melting			
5'-d(T-A-**c^{7}G**-**c^{7}G**-T-C-A-A-T-A-C-T) (**5**) 3'-d(A-T- **2** - **2**- A-G-T-T-A-T-G-A) (**13**)	no melting			

[a]) Measured at 260nm in 0.1M NaCl, 0.01M Na-cacodylate and 0.01M $MgCl_2$ (pH 7.0) at 5 µmol single strand concentration.

TABLE 2 T_m values and thermodynamic data of duplex formation of 5'-(G-G-A-A-A-A-X-A-A-A-A-G-G) • 3'-d(C-C-T-T-T-T-Y-T-T-T-T-C-C) [a].

dX/dY	T_m [°C]	ΔH [kcal/mol]	ΔS [cal/K mol]	ΔG^{298} [kcal/mol]
I/C(**21•23**)	47	-104	-299	-11.5
I/A(**21•24**)	44	-92	-266	-10.0
I/T(**21•25**)	38	-105	-311	-8.4
I/G(**21•26**)	37	-99	-292	-8.1
c^7I/C(**22•23**)	45	-82	-234	-9.8
c^7I/A(**22•24**)	43	-94	-270	-10.4
c^7I/T(**22•25**)	40	-101	-298	-9.0
c^7I/G(**22•26**)	37	-93	-274	-7.9

[a]) Measured at 260 nm in 0.1 M NaCl, 10 mM Na-cacodylate, 10 mM $MgCl_2$, pH 7 at 5 μM single strand concentration.

From the experiments of Tables 1 and 2 performed with oligonucleotides containing either compound **1** or **2** it is apparent that the compound **2** behaves very similar to the parent compound **1** regarding base pair stability and base pair ambiguity. The T_m values of the duplexes of both tables decrease in the order [d(c^7I-C) > d(c^7I-A) > d(c^7I-T) > d(c^7I-G)] as those observed earlier[3] for the duplexes containing dI shown in Table 2.

When dG was replaced by c^7G_d (see Table 1) in the base pair with compound **1** or **2** (**5•7** or **5•13**, Table 1) duplex melting was not observed while duplexes containing dI/c^7I_d-dG base pairs (**3•7** and **3•13**, Table 1) showed a melting profile. Consequence regarding base pairing will be discussed later. The effects of **2** and **1** are very similar in all cases, so it is concluded that compound **2** can fully replace compound **1** as ambiguous base.

4. Stability of the duplexes 5'-d(T-A-G-G-T-C-A-A-T-A-C-T)• 3'-d(A-T-C-C-A-G-T-T-A-T-G-A) with two dI or c^7I_d residues in place of two dA or dT.

The investigations performed in section 3 used the standard duplex **3•4** and studied the replacement of dG-dC pairs by those of compound **1** or **2** with the four common nucleosides located opposite to them. In these cases base pairs with 3 hydrogen bonds were replaced by those which can form only two H-bridges. Now, (Table 3) two dA-dT base pairs are substituted by dA-dI/c^7I_d as well as by dT-dI/c^7I_d pairs.

TABLE 3 T_m values and thermodynamic data of duplex formation of oligodeoxynucleotides containing compounds **1** or **2** in non-consecutive positions.

Oligomer	T_m [°C]	ΔH [kcal/mol]	ΔS [cal/K mol]	ΔG^{298} [kcal/mol]
5'-d(T-A-G-G-T-C-A-A-T-A-C-T) (**3**) 3'-d(A-T-C-C-A-G-T-T-A-T-G-A) (**4**)	48	-89	-253	-10.9
5'-d(T-A-G-G-**2**-C-A-A-**2**-A-C-T) (**16**) 3'-d(A-T-C-C-**A**-G-T-T-**A**-T-G-A) (**4**)	44	-84	-240	-9.8
5'-d(T-**A**-G-G-T-C-**A**-A-T-A-C-T) (**3**) 3'-d(A-**2**-C-C-A-G- **2**-T-A-T-G-A) (**17**)	39	-82	-238	-8.6
5'-d(T-**A**-G-G-T-C-**A**-A-T-A-C-T) (**3**) 3'-d(A-**1**-C-C-A-G- **1**-T-A-T-G-A) (**9**)	38	-73	-208	-8.4
5'-d(T-**2**-G-G-T-C-**2**-A-T-A-C-T) (**14**) 3'-d(A-**T**-C-C-A-G-**T**-T-A-T-G-A) (**4**)	38	-75	-216	-8.1
5'-d(T-**1**-G-G-T-C-**1**-A-T-A-C-T) (**8**) 3'-d(A-**T**-C-C-A-G-**T**-T-A-T-G-A) (**4**)	35	-74	-214	-7.5
5'-d(T-A-G-G-**T**-C-A-A-**T**-A-C-T) (**3**) 3'-d(A-T-C-C-**2**-G-T-T-**2**-T-G-A) (**15**)	35	-73	-213	-7.3

[a]) measured at 260 nm in 10 mM Na-cacodylate, 10 mM $MgCl_2$, 100 mM NaCl (pH 7) at 5 μM single strand concentration.

This represents a replacement of a base pair with 2 H-bonds by another one also containing two H-bonds. From this it was expected that the T_m decrease is much lower compared to that described above (Table 1). Indeed, this is found in all cases (Table 3). Nevertheless, the destabilisation is greater in the case of the dI-dT pair compared to that of dI-dA. Also nearest neighbour influences can be seen. A stabilisation is observed when c^7I_d (**2**) is replacing dI (**1**) in the base pairing with dT. The difference between the T_m values (ΔT_m) of these duplexes (**14•4** and **8•4**, Table 3) amounts to 3°C.

To underline the results shown in the Tables 1 and 3 four instead of two c^7I_d - base pairs were incorporated into the duplex **3•4**. In one group the pyrimidine bases were replaced by compound **2**, in the other group the purine bases (Table 4).

The substitution of dT residues by c^7I_d (**16•17**, Table 4) within the dA-dT pair shows a smaller destabilisation than in the case of the replacement of dA (**14•15**, Table 4)

TABLE 4 T_m values and thermodynamic data of duplex formation of oligonucleotides containing compound **2** and the four modified bases.

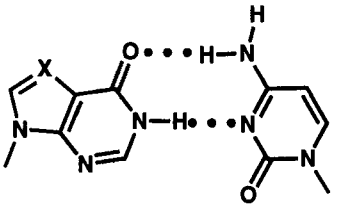
Oligomer	T_m [°C]	ΔH [kcal/mol]	ΔS [cal/K mol]	ΔG^{298} [kcal/mol]
5'-d(T-A-G-G-T-C-A-A-T-A-C-T) (**3**) 3'-d(A-T-C-C-A-G-T-T-A-T-G-A) (**4**)		48 -89	-253	-10.9
5'-d(T-**A**-G-G-**2**-C-**A**-A-**2**-A-C-T) (**16**) 3'-d(A-**2**-C-C-**A**-G-**2**-T-**A**-T-G-A) (**17**)		32 -62	-178	-7.0
5'-d(T-**2**-G-G-**T**-C-**2**-A-**T**-A-C-T) (**14**) 3'-d(A-**T**-C-C-**2**-G-**T**-T-**2**-T-G-A) (**15**)		21 -62	-187	-4.7
5'-d(T-A-**2**- **2**-T-**C**-A-A-T-A-**C**-T) (**10**) 3'-d(A-T-**C**-**C**-A-**2**-T-T-A-T- **2**-A) (**11**)		25 -77	-233	-4.4
5'-d(T-A-**G**-**G**-T-**2**-A-A-T-A- **2**-T) (**12**) 3'-d(A-**T**-**2**- **2**-A-**G**-T-T-A-T-**G**-A) (**13**)		<10		

[a]) measured at 260 nm in 10 mM Na-cacodylate, 10 mM $MgCl_2$, 100 mM NaCl (pH 7) at 5 μM of single strand concentration.

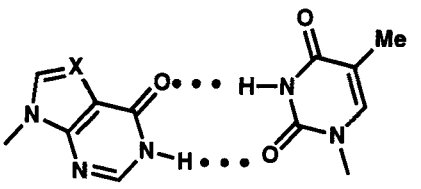
which leads to a severe destabilisation. The opposite is true for the replacement of a dG-dC base pair. In this case the strong destabilisation occurs when compound **2** is replacing dC (**12**•**13**, Table 4). In both cases sequence influences have also to be considered.

5. Comparison of the proposed dI-base pair motifs and those derived from hybridisation studies with dI or c^7I_d opposite to c^7A_d, c^1A_d and c^7G_d.

The finding that the various base pairs of compound **1** and **2** with the four common DNA-constituents shows almost the same stability prompted us to compare the base pair motifs already proposed for compound **1**.[4,19-24] The proposed structures for the dI base pairs with X = N on the basis of X-ray analyses or nmr spectroscopy[20-24] are depicted in Figure 2. When compound **2** is used instead of **1** the motifs with X = CH are expected. At first, the pyrimidine-purine base pairs are discussed. The corresponding oligonucleotides of Table 1 indicate no significant differences of the T_m values in the cases when compound **1** or **2** are located opposite to dC. In the case of dT opposite to **1** or **2** the T_m values of the 7-deazapurine-containing oligonucleotides



dI/c^{7}I$_d$ - dC
X = N(Ref 4), CH
Watson-Crick
motif I



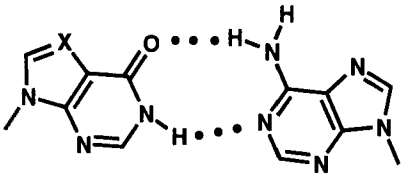
dI/c^{7}I$_d$ - dT
X = N(Ref 21), CH
Watson-Crick
motif II

FIG. 2a Base pair motifs of compounds **1** and **2** with complementary pyrimidine nucleosides.

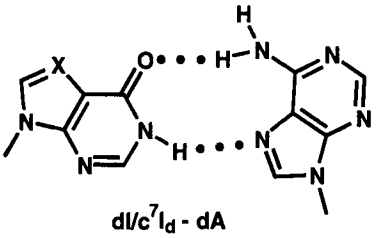
are only slightly enhanced over those having purine nucleosides. From this observation it is concluded that the base pairing modes for compound **2** are the same as for compound **1** (motif I and II, Figure 2a). Moreover, these findings support the 2'-deoxyinosine-dC as well as dT pairing modes which have been suggested earlier.

In the case of the purine-purine motifs (Figure 2b) the situation is more complicated. Base pairs between the two purine rings can occur under the participation of two pyrimidine moieties as well as of a pyrimidine and an imidazole ring (Hoogsteen pairs). In the case of the base pair of dI with dA the motifs III, IV and V have been reported. In contrast to the results determined by ^{1}H nmr spectroscopy suggesting motif III with dI and dA in anti conformation[22] a base pair of the Hoogsteen type (motif IV, Figure 2b) with dI in the anti and dA in the syn conformation was determined by single crystal X-ray analysis.[24] Also a third structure was reported which was found to be stable below pH 6.5. In this case the dA base is in the anti conformation and is protonated; the dI-residue is in the syn-conformation and nitrogen-7 of this residue is participating in the base pair (Hoogsteen motif V, Figure 2b).[20]

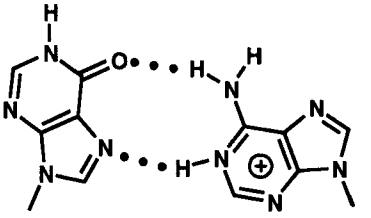
From the studies reported earlier it seems likely that the dI-dA base pairs motif shows a certain variability. Oligonucleotide duplexes might prefer one or the other motif depending on the sequence of the oligonucleotide and/or certain neighbours located next to the modified base pair. As the T_m value of the duplex containing c^{7}I$_d$ opposite to dA (**19•13**, Table 1) is slightly higher than the duplex dI-dA (**19•7**, Table 1) the participation of the 7-nitrogen of dI can be excluded which will exclude the base



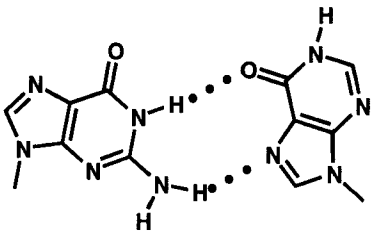
dI/c^{7}I$_d$ - dA
X = N(Ref 22), CH
Watson-Crick
motif III



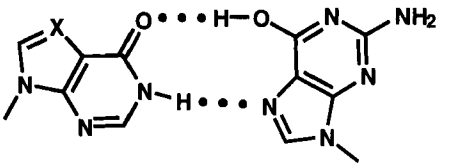
dI/c^{7}I$_d$ - dA
X = N(Ref 24), CH
Hoogsteen
motif IV



dI - dA
(Ref 20)
Hoogsteen
motif V



dI - dG
(Ref 23)
Hoogsteen
motif VI



dI/c^{7}I$_d$ - dG
X = N ,CH
Hoogsteen
motif VII

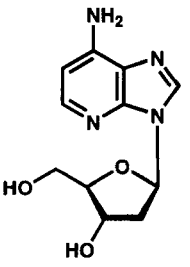
FIG. 2b Base pair motifs of compounds **1** and **2** with complementary purine nucleosides.

pair motif V at least under neutral conditions. The remaining motifs - the Watson-Crick motif III vs the Hoogsteen motifs IV (both Figure 2b) - were studied by substituting the dA-residues by 1-deaza-2'-deoxyadenosine (**27**) or 7-deaza-2'-deoxyadenosine (**28**) which were positioned opposite to the dI or c^{7}I$_d$ residues (duplexes **32•9**, **32•17**, **16•33**, **30•9**, **30•17**, **16•31**, Table 5).

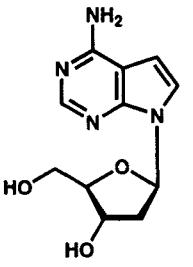
TABLE 5 T_m values and thermodynamic data of duplex formation of oligonucleotides containing c^7I_d and dI opposite dA, c^7A_d and c^1A_d [a]).

Oligomer	T_m [°C]	ΔH [kcal/mol]	ΔS [cal/K mol]	ΔG^{298} [kcal/mol]
5'-d(T-**A**-G-G-T-C-**A**-A-T-A-C-T) (**3**) 3'-d(A-**1**-C-C-A-G- **1**-T-A-T-G-A) (**9**)	38	-73	-208	-8.4
5'-d(T-**c⁷A**-G-G-T-C-**c⁷A**-A-T-A-C-T) (**30**) 3'-d(A- **1** -C-C-A-G- **1** -T-A-T-G-A) (**9**)	38	-70	-201	-8.2
5'-d(T-**c¹A**-G-G-T-C-**c¹A**-A-T-A-C-T) (**32**) 3'-d(A- **1** -C-C-A-G- **1** -T-A-T-G-A) (**9**)	32	-64	-185	-6.8
5'-d(T-**A**-G-G-T-C-**A**-A-T-A-C-T) (**3**) 3'-d(A-**2**-C-C-A-G- **2**-T-A-T-G-A) (**17**)	39	-82	-238	-8.6
5'-d(T-**c⁷A**-G-G-T-C-**c⁷A**-A-T-A-C-T) (**30**) 3'-d(A- **2** -C-C-A-G- **2** -T-A-T-G-A) (**17**)	35	-74	-214	-7.9
5'-d(T-**c¹A**-G-G-T-C-**c¹A**-A-T-A-C-T) (**32**) 3'-d(A- **2** -C-C-A-G- **2** -T-A-T-G-A) (**17**)	32	-69	-200	-6.7
5'-d(T-A-G-G-**2**-C-A-A-**2**-A-C-T) (**16**) 3'-d(A-T-C-C-**A**-G-T-T-**A**-T-G-A) (**4**)	44	-84	-240	-9.8
5'-d(T-A-G-G- **2** -C-A-A- **2** -A-C-T) (**16**) 3'-d(A-T-C-C-**c⁷A**-G-T-T-**c⁷A**-T-G-A) (**31**)	39	-70	-200	-8.4
5'-d(T-A-G-G- **2** -C-A-A- **2** -A-C-T) (**16**) 3'-d(A-T-C-C-**c¹A**-G-T-T-**c¹A**-T-G-A) (**33**)	32	-79	-212	-6.7

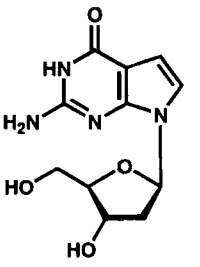
[a]) measured at 260 nm in 10 mM Na-cacodylate, 10 mM $MgCl_2$, 100 mM NaCl (pH 7) at 5 μM of single strand concentration.



27



28



29

In the case of the incorporation of 7-deaza-2'-deoxyadenosine (c^7A_d, **28**) instead of dA the base pairing is limited to the Watson-Crick motif III (Figure 2b). According to Table 5 the T_m values of the oligonucleotide duplex with dI opposite to c^7A_d and dA (**30•9** and **3•9**, Table 5) are the same and also the thermodynamic data for the dI-dA pairs are very similar. The situation of the base pair of c^7I_d (**2**) opposite to dA or c^7A_d (**28**) are somewhat different. The duplex **30•17** shows a T_m value which is 4°C lower than that of **3•17**. The same is observed for the duplex **16•31** compared to **16•4**. A possible explanation would be the formation of the duplex with the motif V with dA in the non-protonated state under conditions when motif III cannot be formed.

Contrary to these findings the incorporation of 1-deaza-2'-deoxyadenosine (c^1A_d, **27**) opposite to compound **1** or **2** shows the same effect and results in a T_m decrease of 6°C; a very similar decrease of the enthalpies of duplex formation can be seen (see duplexes **32•9** and **32•17**). This T_m decrease becomes larger (12°C) when the c^1A_d residues are located in another position in the other strand (see duplexes **16•33** vs. **16•4**). According to that, the base pair motif IV is extremely unlike to occur in solution as it should not be affected as strongly as in the absence of nitrogen-1 in the dA-residue. Therefore, the motif III (Figure 2b) which was suggested by Ohtsuka et al.[22] is preferred to occur in our case. However, these experiments do not exclude the switching from one base pair motif to another when such hydrogen bonding is possible between various donor and acceptor sites. The CD spectra of the duplex **16•31** containing c^7A_d at position X shows similarities to that of the parent duplex **16•4**. The spectra of the duplex **16•33** with X = c^1A_d is rather different which might be due to the presence of two Hoogsteen base pairs.

Finally, the base pair motifs of dI or c^7I_d opposite dG have to be discussed. The proposed motif VI for the dI-dG pair[23] is not in agreement with our findings observed with modified bases. The oligonucleotide duplexes **3•7** and **3•13** (Table 1) indicate that the 7-deazapurine compound **2** leads to nearly the same T_m value as the parent 2'-deoxyinosine (**1**). This excludes the Hoogsteen motif VI which requires the participation of nitrogen-7 of the hypoxanthine moiety. It is not easy to envisage another base pair motif. A possible pairing mode would be the motif VII with one of the keto groups in the enol form and under participation of nitrogen-7 of the guanine moiety (Figure 2b). This is underlined by the finding that the incorporation of 7-deaza-2'-deoxyguanosine (**29**) opposite to compound **1** or **2** does not lead to a sigmoidal melting profiles (Table 1).

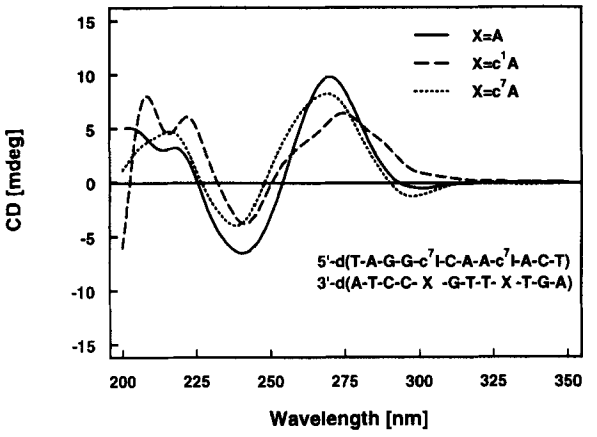


FIG. 3 CD Spectra of the oligonucleotide duplexes of 5'-d(T-A-G-G-**2**-C-A-A-**2**-A-C-T)•3'-d(A-T-C-C-**X**-G-T-T-**X**-T-G-A) in which X is dA, c^7A_d or c^1A_d. The spectra were taken at 25°C in 0.1M NaCl, 10 mM $MgCl_2$, 10 mM Na-cacodylate at pH 7.0. The single strand concentration was 5 μM.

CONCLUSIONS

From the T_m measurements and thermodynamic data of oligonucleotide duplexes containing compounds **1** or **2** opposite to the 4 common nucleosides it is apparent that 7-deaza-2'-deoxyinosine (**2**) is an efficient substitute of 2'-deoxyinosine (**1**). Only minor changes are observed in the duplex stability of oligonucleotides containing dI or c^7I_d. The stability order of base pairs decreases in the order [d(c^7I-C) > d(c^7I-A) > d(c^7I-T) > d(c^7I-G)] which is the same as found for dI. Also, the base pair motifs suggested for the nucleoside **1** with dC, dT and dA in opposite position were confirmed by the use of compound **2** as well as by base-modified nucleosides.[12] In the case of the dI-dG base pair the suggested motif is not in accordance with our observations. As compound **2** is extremely stable against treatment with acid or base it is an efficient substitute for 2'-deoxyinosine. The successful incorporation of the triphosphate of compound **2** by DNA-polymerases[34] and the availability of a phosphoramidite suggests its use as universal nucleoside in chemical sequencing procedures[17] as well as for the synthesis of primers and probes in the cases when 2'-deoxyinosine is too labile.

MATERIALS AND METHODS

General. Reverse phase HPLC was carried out on a 4x250 mm RP-18 (10 μm) LiChrosorb column (Merck) with a Merck-Hitachi HPLC pump (model 655 A-12)

connected with a variable-wavelength monitor (model 655-A), a controller (model L-5000), and an integrator (model D-2000). Snake-venom phosphodiesterase (EC 3.1.4.1, *crotallus durissus*) and alkaline phosphatase (EC 3.1.3.1, *E.coli*) were generous gifts of Roche Diagnostics, Germany.

7-(2'-Deoxy-β-D-erythro-pentofuranosyl)-3,7-dihydro-4H-pyrrolo [2,3-d] pyrimidin-4-one (**2**).

To a suspension of 4-methoxy-2-methylthio-7H-pyrrolo[2,3-d]pyrimidine[28] (500 mg, 2.56 mmol) in anh. MeCN (40 ml), powdered KOH (600 mg, 8.91 mmol) and TDA-1 (= tris[2-(2-methoxyethoxy)ethyl]amine, 100 µl, 0.31 mmol) were added under stirring at r.t. The stirring was continued for 10 min, 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-*erythro*-pentofuranosyl chloride (1.16g, 2.98 mmol)[29] was introduced and the reaction was continued for another 15 min. Insoluble material was filtered off, the filtrate was evaporated and the residue was stirred with 0.5M NaOMe in MeOH (40 ml) for 12 h. The solution was adsorbed on silica gel and applied to flash chromatography (column 9x4, CH_2Cl_2/MeOH 9:1). The content of the main zone was isolated and crystallized from H_2O. Colourless needles (570 mg, 71%). The 6-oxo group was generated thereafter and the 2-methylthio residue was removed following reference 18.

7-[2'-Deoxy-5-O-(4,4'-dimethoxytrityl)-β-D-erythro-pentofuranosyl]-3,7-dihydro-4H-pyrrolo [2,3-d] pyrimidin-4-one 3'-(2-Cyanoethyl-N,N-Diisopropylphosphoramidite).

The nucleoside **2** was protected at the 5'-OH group with the 4,4'-dimethoxytrityl chloride using standard conditions.[13] To a soln. of the latter (230 mg, 0.41 mmol) in anh. CH_2Cl_2 (2 ml) under Ar, $(i\text{-}Pr)_2EtN$ (226 µl, 1.3 mmol) and chloro(2-cyano-ethoxy)(diisopropylamino)-phosphine (290 µl, 1.3 mmol) were added at r.t. After stirring for 30 min, the soln. was diluted with CH_2Cl_2 (10 ml), and 5% aq. $NaHCO_3$ soln. (5 ml) was added. The mixture was extracted with CH_2Cl_2 (3x20 ml), the combined org. layers were dried (Na_2SO_4), filtered and evaporated. The residue was applied to flash chromatography (silica gel, column 8x2cm, CH_2Cl_2/EtOAc 95:5). Colourless foam (222 mg, 72 %). ^{31}P NMR ($CDCl_3$) δ 150.0, 150.2 ppm.

Oligonucleotides. The solid-phase synthesis were carried out on a DNA synthesizer, model 392 (*Applied Biosystems,* Weiterstadt, Germany) using the corresponding phosphoramidites in a 1-µmol scale. The oligonucleotides were removed from the solid

TABLE 6 Molecular weights determined by MALDI-TOF spectra

oligomer	M^+(calc) [Da]	m/z [Da]
5'-d(T-A-**2**-**2**-T-C-A-A-T-A-C-T)	3612	3612
5'-d(A-**2**-T-A-T-T-**2**-A-C-C-T-A)	3612	3612
5'-d(T-A-G-G-T-**2**-A-A-T-A-**2**-T)	3692	3691
5'-d(A-G-T-A-T-T-G-A-**2**-**2**-T-A)	3692	3693
5'-d(T-**2**-G-G-T-C-**2**-A-T-A-C-T)	3644	3646
5'-d(A-G-T-**2**-T-T-G-**2**-C-C-T-A)	3644	3644
5'-d(T-A-G-G-**2**-C-A-A-**2**-A-C-T)	3662	3666
5'-d(A-G-T-A-T-**2**-G-A-C-C-**2**-A)	3662	3664

support and were deprotected under standard conditions.[35] The purification of the oligonucleotides was performed using oligonucleotide cartridges (*OPC, Applied Biosystems*).[36] The MALDI-TOF-spectra were measured by Mrs. Julia Gross, Institut für Medizinische Physik und Biophysik (head: Prof. Dr. F. Hillenkamp), Westfälische Wilhelms - Universität, Münster, Germany.

Melting experiments. The thermal dissociation of the duplexes was measured by temperature-dependent UV-melting profiles using a *Cary-1-E UV/VIS* spectrophotometer (*Varian*, Australia) equipped with a *Cary* thermoelectrical controller; the actual temp. was measured in the reference cell with a Pt-*100* resistor. The Tables 1-5 summarise the T_m values as well as the thermodynamic data (MeltWin[37]) of the duplex formations.

CD spectra. The CD spectra were measured in 1 cm cuvettes using a *Jasco* 600 spectropolarimeter (Jasco, Tokio, Japan) connected with a temperature controller (*Lauda RCS* 6, Germany) and a bath (*Lauda RK* 20, Germany).

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