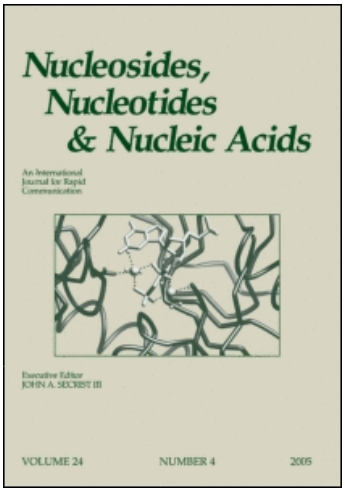

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Frank Seela[ab]; Xiaohua Peng[ab]; Kuiying Xu[ab]
[a] Laboratorium für Organische und Bioorganische Chemie, Universität Osnabrück, Osnabrück, Germany [b] Laboratory of Bioorganic Chemistry and Chemical Biology, Center for Nanotechnology, Münster, Germany

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MISMATCH DISCRIMINATION OF BASE-MODIFIED NUCLEIC ACIDS AND THEIR CONSTITUENTS: NON-WATSON-CRICK BASE PAIRING INDUCED BY TAUTOMERIZATION

Frank Seela, Xiaohua Peng, and Kuiying Xu □ *Laboratorium für Organische und Bioorganische Chemie, Universität Osnabrück, Osnabrück, Germany and Laboratory of Bioorganic Chemistry and Chemical Biology, Center for Nanotechnology, Münster, Germany*

□ *The effect of tautomerism on the mismatch discrimination was studied on 7-deazapurine and 8-aza-7-deazapurine analogues of isoguanosine. 7-Halogenated 7-deaza-2′-deoxyisoguanosines show better base pair discrimination than 2′-deoxyisoguanosine due to the more favored keto tautomer formation. 8-Aza-7-deazaisoguanosine and its 7-halogeno derivatives also show higher keto tautomer population than that of isoguanosine, but the 7-halogens do not bias the tautomeric equilibrium significantly as it is observed for the 7-deaza-2′-deoxyisoguanosine derivatives.*

Keywords Nucleosides; oligonucleotides; 7-deazapurines; 8-aza-7-deazapurines; base pair discrimination; tautomers

INTRODUCTION

It is well documented that canonical DNA constituents can form stable non-Watson-Crick base pairs. However, the degree of fidelity achieved by the association of Watson-Crick base pairs is not sufficient to maintain genetic integrity. DNA damages are caused by many factors, e.g., oxidation, irradiation and normal metabolic processes inside the cell. Furthermore, the tautomerism of the canonical nucleobases and the formation of wobble base pairs lead to the DNA mutation.[1] Diagnostic tools have been developed to detect mutation (single nucleotide polymorphisms, SNPs) by hybridization in solution or on polymer surfaces (biochips). Modified nucleosides carrying fluorescent dyes (e.g., 7-deazapurine nucleoside triphosphates) are used in these protocols.[2] Here, the effect of the tautomerism was studied with 7-deaza-2′-deoxyisoguanosine, 8-aza-7-deazaisoguanosine and their

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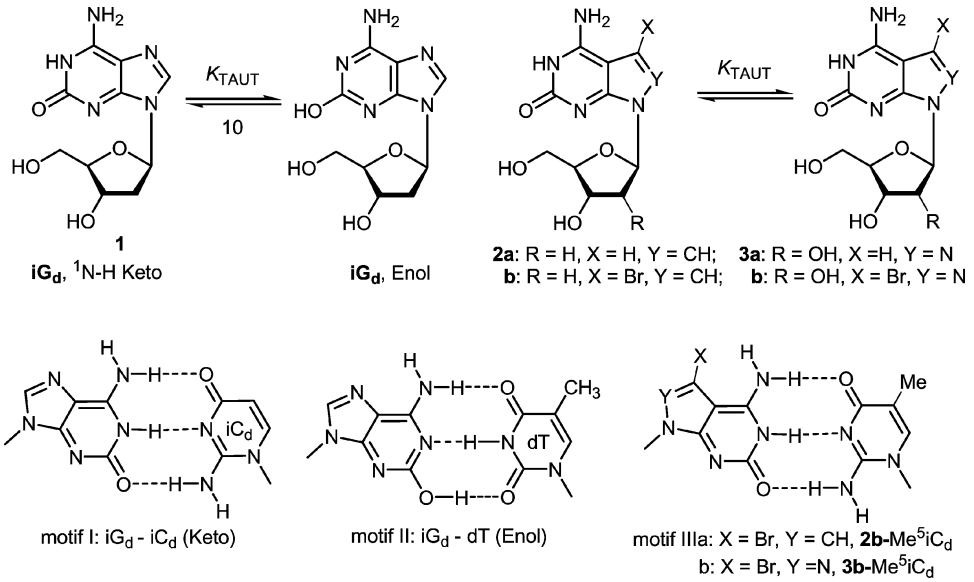
Address correspondence to Frank Seela, Laboratorium für Organische und Bioorganische Chemie, Universität Osnabrück, Barbarastraβe 7, 49069 Onsabrück, Germany. E-mail: Frank.Seela@uni-onsabruck.de

7-halogenated derivatives. Experiments regarding mismatch discrimination are performed.

RESULTS AND DISCUSSION

Base Recognition of 7-Deaza-2′-Deoxyisoguanosine, 8-Aza-7-Deazaisoguanosine, and Their 7-Halogenated Derivatives

2′-Deoxyisoguanosine (iG_d, **1**) is one of the most promiscuous nucleosides. The ambiguity of base pairing caused by the tautomerism of iG_d (motifs I and II; Scheme 1) limits its use as an additional "letter in the genetic alphabet."[3] This has been correlated to a particular property of the isoguanine base which adopts the form of an enol tautomer much more easily than the canonical DNA nucleobases (K_{TAUT} = [keto]/[enol] ≈ 10).[4] 7-Deaza-2′-deoxyisoguanosine (c^7iG_d, **2a**) shows a more favored keto form population (K_{TAUT} ≈10^3) than that of iG_d.[5] However, compound **2a** decreases the duplex stability and still shows base pair ambiguity as is observed for iG_d. We have found that the introduction of 7-halogen substituents greatly increases the keto content with K_{TAUT} ≈10,000 for 7-chloro-, or 7-bromo-7-deaza-2′-deoxyisoguanosine (**2b**) (Scheme 1).[6] As we want to combine the advantages of high duplex stability with better base discrimination, compounds **2a, b** were incorporated into oligonucleotides and their base recognition was investigated in both parallel (*ps*) and antiparallel duplex (*aps*) DNA.



SCHEME 1 Tautomerism of iG_d and **2, 3** and base pair motifs.

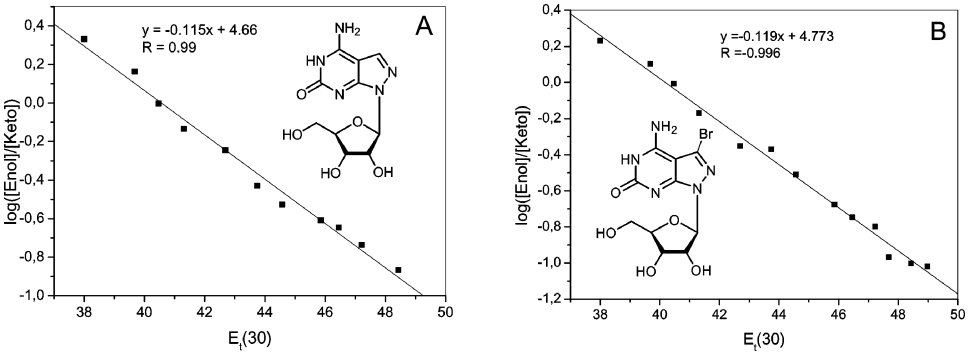
TABLE 1 T_m-values of duplexes with mismatches opposite to **iG**$_d$ and **2a,b**[a,b]

	3′-d(ATCCA**X**TTATGA)-5′ (*aps*) 5′-d(TAGGT**Y**AATACT)-3′					5′-d(TiCATAAiCT**X**XAT)-3′*c* (*ps*) 5′-d(AG TATT GA**Y**CTA)-3′				
X\Y[c]	iC	C	G	T	A	C	G	T	A	iC
iG$_d$	54	44	44	42 (−12)	32	44	36	33 (-11)	34	40
2a	54	43	42	42 (−12)	29	44	33	31 (-13)	32	37
2b	**57**	47	45	40 (−**17**)	27	**49**	36	31 (−**18**)	31	45

[a]Measured in 1.0 M NaCl, 0.1 M $MgCl_2$, and 60 mM Na-cacodylate buffer, pH 7.0, with 5 μM + 5 μM single-strand concentration.
[b]The data in parentheses are ΔT_m ($T_m^{\text{base mismatch}} - T_m^{\text{base match}}$).
[c]d(iC) = ^{Me}iC$_d$ = 5-Methyl-2′-deoxyisocytidine. [c]**X** stands for the bases in the row (**iG**$_d$**, 2a, 2b**), **Y** for the bases in the line (iC, C, G, T, A).

As shown in Table 1, the incorporation of the 7-halogenated derivatives **2b** results in an increase of the T_m-values both in *aps* and *ps* DNA duplexes base pairing with iC($\Delta T_m = +3.0 \sim 5.0°C$). Furthermore, compound **2b** shows an enhanced base discrimination (in particular against dT) both in *aps* and *ps* DNA indicated from higher $|\Delta T_m|$ values (**1** ≈ **2a** < **2b**). Apparently, a tridentate mispair cannot be formed between the halogenated compound **2b** with dT due to the low content of the enol form ($K_{TAUT} \approx 10^4$), while the base pair formed with 2′-deoxy-5-methyisocytidine (motif IIIa; *aps* DNA) or 2′-deoxycytidine (*ps* DNA) is predominant.

Next, the tautomeric equilibria of the 8-aza-7-deazaisoguanosines **3a, b** were determined as described.[5,6] Scheme 2 shows the linear relationship of log[K_{TAUT}] and the $E_T(30)$[9] in the range of 38-50. The K_{TAUT} was calculated by extrapolating this relationship to the $E_T(30)$ value of water (63.1).[7] The tautomeric equilibrium constants for compound **3a, b** were found as 400 for **3a**, 570 for **3b** (Table 2). These values are lower than those of the corresponding 7-deazapurine derivatives **2a, b** but higher



SCHEME 2 Plot of log([enol]/[keto]) versus $E_T(30)$ for compound **3a** (A) and **3b** (B) in mixtures of dioxane ($E_T(30) = 36.0$) and water ($E_T(30) = 63.1$).

TABLE 2 Tautomeric equilibrium constants for isoguanine derivatives[a]

Compound	K_{TAUT}	Compound	K_{TAUT}
2a	1880	**3a**	400
2b	19700	**3b**	570

[a] K_{TAUT} = [keto]/[enol], measured in dioxane-water mixtures.

than that of isoguanosine (10).[4] That means compared to **2a, b**, the nucleosides **3a, b** are more enolized; however, compared to isoguanosine, compound **3a, b** enol population is lower. The base pairing properties of 8-aza-7-deaza-2′-deoxyisoguanosine and 7-halogenated derivatives have already been reported.[8] Studies on the base pair discrimination within oligonucleotides are still in progress.

In summary, 7-halogenated 7-deaza-2′-deoxyisoguanosines, show better base discrimination than 2′-deoxyisoguanosine or 7-deaza-2′-deoxyisoguanosine due to the increased keto population (K_{TAUT} of 20,000 *vs.* 2000 or 10). In comparison, in 8-aza-7-deazaisoguanosines, 7-halogen substituents do not affect the tautomeric equilibrium significantly.

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