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8-AZA-7-DEAZAADENINE AND 7-DEAZAGUANINE: SYNTHESIS AND PROPERTIES OF NUCLEOSIDES AND OLIGONUCLEOTIDES WITH NUCLEOBASES LINKED AT POSITION-8

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### NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 20(4-7), 577-585 (2001)

# 8-AZA-7-DEAZAADENINE AND 7-DEAZAGUANINE: SYNTHESIS AND PROPERTIES OF NUCLEOSIDES AND OLIGONUCLEOTIDES WITH NUCLEOBASES LINKED AT POSITION-8

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# ABSTRACT

The 8-aza-7-deazaadenine (pyrazolo[3,4-d]pyrimidin-4-amine)  $N^{8}$ -(2'-deoxyribonucleoside) (2) and the 7-deazaguanine (pyrrolo[3,4-d]pyrimidine-2amin-(3*H*)-4-one) C<sup>8</sup>-(2'-deoxyribonucleoside) (4) were synthesized and incorporated in oligonucleotides employing phosphoramidite chemistry. Oligonucleotides carrying compound 2 are able to form base pairs with the four canonical DNA constituents without significant structural discrimination. The nucleoside 4 was obtained from the corresponding ribonucleoside by deoxygenation. Oligonucleotides containing compound 4 showed similar base pairing properties as those with 2'-deoxyisoguanosine.

# **INTRODUCTION**

The shape of the base pairs is of central importance for the structure of DNA. This is underlined by the isomorphous character of the bidentate dA-dT base pair and the tridentate base pair of dG-dC. Base pairs containing modified nucleobases form stable duplexes when the bases retain the shape of the natural molecule as well as their donor and acceptor sites. Aglycons which mimic the shape of the

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natural bases but are lacking donor and acceptor sites can stabilize DNA by vertical stacking. In the case of modified DNA it was shown that base pairing does not only depend on the shape of the bases and their donor-acceptor sites but that it is also the polymeric backbone which controls base pair recognition and stability. The altered properties of DNA backbone analogs such as PNA, hexose-DNA or locked-DNA (LNA) are examples for such a behaviour (1–3).

Up to now a significant number of modified purine and pyrimidine nucleosides being isosteric to the natural nucleic acids constituents have been incorporated into oligonucleotides. Among them, the pyrazolo[3,4-d] pyrimidine and the pyrrolo [3,4-d] pyrimidine nucleosides are ideal mimics of the purine compounds. The nucleobases have an almost identical shape as the purines, and their Watson-Crick recognition sites are not altered (4–7). This manuscript reports on the synthesis of nucleosides and oligonucleotides containing 8-aza-7-deazaadenine or 7-deazaguanine with a sugar moiety attached to the position-8 and not to nitrogen-9 which is the usual glycosylation site (purine numbering is used throughout this manuscript). For this purpose the nucleosides 2 and 4 were synthesized (Scheme 1), converted into their corresponding phosphoramidites, and oligonucleotides were prepared by solid-phase synthesis. Furthermore, the base pairing properties of compounds 2 and 4 were investigated in duplexes with antiparallel-strand orientation.

# **RESULTS AND DISCUSSION**

#### 1. Monomers

Synthesis of Nucleoside 2 and Its Phosphoramidite

The glycosylation of 8-aza-7-deaza-6-methoxypurine (5) with the  $\alpha$ -halogenose 6 yields the toluoylated N-9 and N-8 isomers which are normally separated on this stage (8,9). Now the crude reaction mixture was deprotected first



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#### 8-AZA-7-DEAZAADENINE AND 7-DEAZAGUANINE



Scheme 2. i) KOH, TDA-1, acetonitrile, r.t., 30 min. ii) 0.1 M NaOMe, MeOH, r.t., 5 h.

(0.1 M NaOMe/MeOH) furnishing the methoxy nucleosides 7 and 8 (Scheme 2). They were separated by silica gel flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>-MeOH 9:1) to give compound 7 (26%) and compound 8 (60%). Compound 7 was treated with methanol, saturated with ammonia, in an autoclave (100°C, 12 h) to give the nucleoside 2 (8).

The glycosylic bond stability (1 M HCl,  $25^{\circ}$ C) of compound **2** is significantly higher than that of dA (10). The sugar conformation of the nucleosides **1** and **2** was determined from the vicinal <sup>3</sup>J(H,H) coupling constants of the <sup>1</sup>H-NMR spectra measured in deuterium oxide by using the *PSEUROT* program (Version 6.2) (11,12). The nucleosides **2** shows populations of sugar conformers of 44% N and 56% S while those of the regularly linked nucleoside **1** are 37% N and 73% S (13). For comparison, 2'-deoxyadenosine shows a ratio of 28% N and 72% S (14).

Several protecting groups such as benzoyl, dimethylaminomethylidene, isobutyryl and acetyl residues were tested. As the isobutyryl derivative **9a** showed



Scheme 3. i) a) (i-Bu)<sub>2</sub>O, pyridine, r.t., 3 h. b) Ac<sub>2</sub>O, pyridine, r.t., 3 h. ii) DMTrCl, pyridine, r.t., 3 h. iii) (i-Pr)<sub>2</sub>NP(Cl)O(CH<sub>2</sub>)<sub>2</sub>CN, CH<sub>2</sub>Cl<sub>2</sub>, r.t., 20 min.

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a half-life of 6 min (25% aqueous ammonia, 40°C) it was used for further manipulation (Scheme 3). Then, compound **9a** was converted into its 4,4'-dimethoxytrityl derivative **10** (76%), and was treated with chloro(2-cyanoethoxy)- N,N-diisopropyl-amino-phosphine to give the phosphoramidite **11** in 80% yield (15).

Synthesis of the Nucleoside 4 and Its Phosphoramidite

Glycosylation of the unprotected 7-deazaguanine base with 1-*O*-acetyl-2,3,5tri-*O*-benzoyl- $\beta$ -D-ribofuranose in the presence of tin(IV) chloride yields the benzoyl protected C<sup>8</sup>-ribonucleoside in 56% yield (16). This compound was deprotected to give the 7-deazaguanine C<sup>8</sup>-ribofuranoside (**12a**) (16). The synthesis of the 2'-deoxyribonucleoside **4** from **12a** made use of the Barton deoxygenation procedure (Scheme 4).

Prior to this reaction, the amino group was protected with an isobutyryl residue employing the protocol of transient protection (17). As this group had to be removed



Scheme 4. i)  $(i-Pr)_2$ ClSiOSi $(i-Pr)_2$ Cl, pyridine, r.t., 12 h. ii) PhOC(S)Cl, acetonitrile, r.t., 12 h. iii)  $(n-Bu)_3$ SnH, AIBN, toluene, 60°C, 4 h. iv) 0.1 N TBAF/THF, r.t., 3 h. v) DMTrCl, pyridine, r.t., 3 h. vi)  $(i-Pr)_2$ NP(Cl)O(CH<sub>2</sub>)<sub>2</sub>CN, CH<sub>2</sub>Cl<sub>2</sub>, r.t., 20 min.



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later from the oligonucleotide the half-life value ( $\tau = 110 \text{ min}$ ) of the deprotection was determined in 25% aqueous ammonia (40°C). Prior to the derivatization of the 2'-hydroxyl group, compound **12b** was treated with Markiewicz's reagent to give the silyl derivative **13** (58%) (18). Treatment of **13** with phenoxythiocarbonyl chloride in acetonitrile furnished the 2'-O-phenoxythiocarbonyl derivative **14a** (48%) together with its 2'-O-N<sup>7</sup>-phenoxythiocarbonyl derivative **14b** (10%) as minor product. Reductive cleavage of **14a** with tributyltin(**IV**) hydride in toluene in the presence of  $\alpha$ , $\alpha'$ -azoisobutyronitrile (AIBN) yielded the 2'-deoxy derivative **15** (90%). The desilylation with 0.1 M tetra-n-butylammonium fluoride in anhydrous THF gave compound **16** (82%). Then, the 4,4'-dimethoxytriphenylmethyl group was introduced for the 5'-protection (74%). The phosphoramidite **17** was prepared by standard protocols from the 5'-protected derivative upon phosphitylation (78%).

# 2. Oligonucleotides

#### General

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Oligonucleotides containing compounds 2 or 4 were prepared by solid-phase synthesis using the standard protocol of an automated DNA synthesizer (ABI-392, Applied Biosystems) (19). The coupling efficiency of the phosphoramidites **11** and **17** was always higher than 98%. Deprotection was performed with 25% aqueous ammonia (16 h, 60°C), and the oligonucleotides were purified by reverse-phase HPLC (13). The homogenity of the oligonucleotides was proved by reverse phase HPLC. MALDI-TOF mass spectra were measured and are in agreement with the calculated data. The nucleoside composition was determined after digestion with snake-venom phosphodiesterase followed by alkaline phosphatase (20). For the hybridization experiments the duplex 5'-d(T-A-G-G-T-C-A-A-T-A-C-T)  $\cdot$  3'-d(A-T-C-C-A-G-T-T-A-T-G-A) (18  $\cdot$  19) was used as a reference to study the influence of base-modified nucleosides on the duplex stability. The melting curves were used to determine the thermodynamic data which were obtained by curve shape analysis using the program Meltwin 3.0 (21).

#### Oligonucleotides Containing Compound 2

In the cases of the duplexes carrying compound **2** only moderate  $T_m$ -decreases (4–6°C) were observed with regard to the reference duplex **18** · **19** (Table 1). Within the series of modified duplexes the  $T_m$ -difference was small ( $\Delta T_m = 2^\circ C$ ). The CD-spectra of the various duplexes showed the typical shape of a B-DNA (15). When the three canonical nucleosides dA, dG, and dC were positioned opposite to 2'-deoxyadenosine the  $T_m$ -values were decreased by 2–12°C (Table 1) while the dA analogue **2**, opposite to all of the four canonical nucleosides, results in a  $T_m$ -decrease of 4–6°C. From these experiments it is concluded that compound **2** 



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*Table 1.*  $T_m$ -Values of Oligonucleotides Containing One N<sup>8</sup>-8-aza-7-deaza-2'-deoxyadenosine (2) Residue Opposite to Each of the Four Canonical Nucleosides dA, dC, dG, and dT

Duplex		$T_m$ [°C] <sup>a</sup> )	∆H° [kcal/mol]	$\triangle S^{\circ}$ [cal/mol $\cdot$ K ]	$\Delta G_{298}^{\circ}$ [kcal/mol]
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)	18 19	50	-90	-252	-12.0
5'-d(T-A-G-G-T-C-A-A-T-A-C-T) 3'-d(A-T-C-C- <b>2</b> -G-T-T-A-T-G-A)	18 20	46	-76	-212	-9.9
5'-d(T-A-G-G-C-C-A-A-T-A-C-T) 3'-d(A-T-C-C- <b>2</b> -G-T-T-A-T-G-A)	21 20	46	-80	-225	-9.9
5'-d(T-A-G-G-A-C-A-A-T-A-C-T) 3'-d(A-T-C-C- <b>2-</b> G-T-T-A-T-G-A)	22 20	45	-77	-217	-9.7
5'-d(T-A-G-G-G-C-A-A-T-A-C-T) 3'-d(A-T-C-C- <b>2-</b> G-T-T-A-T-G-A)	23 20	44	-73	-205	-9.4
5'-d(T-A-G-G-C-C-A-A-T-A-C-T) 3'-d(A-T-C-C-A-G-T-T-A-T-G-A)	21 19	38	-68	-193	-8.0
5'-d(T-A-G-G-A-C-A-A-T-A-C-T) 3'-d(A-T-C-C-A-G-T-T-A-T-G-A)	22 19	40	-62	-172	-8.4
5'-d(T-A-G-G-G-C-A-A-T-A-C-T) 3'-d(A-T-C-C-A-G-T-T-A-T-G-A)	23 19	48	-80	-225	-10.5
5'-d(T-A-G-G- <b>3</b> -C-A-A-T-A-C-T) 3'-d(A-T-C-C- <b>A</b> -G-T-T-A-T-G-A)	24 19	48	-76	-211	-10.3

<sup>a</sup>) Measured at 260 nm in 1 M NaCl, 100 mM MgCl<sub>2</sub>, and 60 mM Na-cacodylate (pH 7.0) with 5  $\mu$ M single strand concentration.

is a universal nucleoside as it base pairs almost equally well with all of the four canonical DNA constituents.

#### Oligonucleotides Containing Compound 4

Oligonucleotides containing compound 4 were hybridized with an either complimentary strand having isoC<sub>d</sub>, and dC located opposite to 4. As a reference, the duplex 25  $\cdot$  19 is used which has a similar T<sub>m</sub>-value as the unmodified duplex 18  $\cdot$  19. As the Watson-Crick recognition site of compound 4 can act in a similar way as the recognition site of isoG<sub>d</sub> and not of dG, the oligonucleotide 26 was hybridized with 27. If the 4-residues are dispersed (29  $\cdot$  30) the T<sub>m</sub>-decrease is 0.5°C per residue. The corresponding duplexes with dC opposite to 4 (26  $\cdot$  19 and 18  $\cdot$  30) are rather labile ( $\sim$ 7°C/residue). This indicates that compound 4 acts as an analogue of 2'-deoxyisoguanosine, although it contains a 7-deazaguanine base. Compared to duplexes containing 4 in which only one of the strands is modified, the duplex 31  $\cdot$  32 contains modifications in both strands, and the hybrid is further destabilized with a T<sub>m</sub>-decrease of 3°C per 4-isoC<sub>d</sub> base pair.



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*Table 2.*  $T_m$ -Values and Thermodynamic Data of Oligonucleotide Duplexes Containing C<sup>8</sup>-linked 7-deaza-2'-deoxyguanosines (4) as well as  $c^7G_d$  (3) in Opposite to  $m^5iC_d$  and  $dC^a$ )

Duplex		$T_m$ [°C]	∆H° [kcal/mol]	$\triangle S^{\circ}$ [cal/mol $\cdot$ K ]	$\Delta { m G}^{\circ}_{298}$ [kcal/mol]
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)	18 19	50 (47)	-84 (-87)	-234 (-245)	-11.5 (-11.0)
5'-d(T-A- <b>3-3</b> -T-C-A-A-T-A-C-T) 3'-d(A-T-C-C-A-G-T-T-A-T-G-A)	25 19	(46)	(-98)	(-283)	(-10.5)
5'-d(T-A- <b>4-4</b> -T-C-A-A-T-A-C-T) 3'-d(A-T- <b>i</b> C- <b>i</b> C-A-G-T-T-A-T-G-A)	26 27	46 (42)	-82 (-73)	-230 (205)	-10.2 (-8.9)
5'-d(T-A-G-G-T-C-A-A-T-A-C-T) 3'-d(A-T-C-C-A- <b>3</b> -T-T-A-T- <b>3</b> -A)	18 28	(46)	(-84)	(-239)	(-10.1)
5'-d(T-A-G-G-T-iC-A-A-T-A-iC-T) 3'-d(A-T-C-C-A-4-T-T-A-T-4-A)	29 30	49 (45)	-76 (-65)	-210 (-180)	-10.7 (-9.4)
5'-d(T-A- <b>4-4</b> -T-C-A-A-T-A-C-T) 3'-d(A-T-C-C-A-G-T-T-A-T-G-A)	26 19	36 (32)	-52 (-35)	-143 (-93)	-7.9 (-6.7)
5'-d(T-A-G-G-T-C-A-A-T-A-C-T) 3'-d(A-T-C-C-A-4-T-T-A-T-4-A)	18 30	33 (32)	-39 (-46)	-102 (-127)	-7.3 (-7.1)
5'-d(T-iC-A-T-A-A-iC-T-4-A-T) 3'-d(A-4-T-A-T-T-4-A-iC-iC-T-A)	31 32	35 (31)	-45 (-45)	-121 (-124)	-7.6 (-6.9)

<sup>a</sup>) Measured at 260 nm in 1 M NaCl, 100 mM MgCl<sub>2</sub>, and 60 mM Na-cacodylate (pH 7.0) with 5  $\mu$ M single strand concentration. Data in parentheses are measured in 100 mM NaCl, 10 mM MgCl<sub>2</sub>, and 10 mM Na-cacodylate (pH 7.0). iC<sub>d</sub> = m<sup>5</sup>iC<sub>d</sub>.

#### 3. Proposed Base Pair Motifs

The new base pairs were constructed under the following assumptions: i) "Watson-Crick" and "Hoogsteen" base pairs are allowed as well as those with the reversed modes. ii) The nucleoside **2** can act as acceptor in a new Hoogsteen-like mode via nitrogen-9 and nitrogen-3. iii) The distances between the anomeric carbons of the universal nucleoside **2** and its natural counterparts should approximate those of the Watson-Crick base pairs (11 Å) (22). The length of the H-bonds was arbitrarily set to 2Å. When these factors as well as the duplex stabilities of oligonucleotides containing compound **2** are taken into account, the base pair motifs I–IV are suggested for the base pairing of the nucleoside **2** with the **4** canonical bases (Scheme 5) (15).

From the  $T_m$ -values and the thermodynamic data of Table 2 base pairs of compound 4 with  $iC_d$  and dC are constructed. It is obvious that the nucleoside 4 can form a strong base pair with 5-methyl-2'-deoxyisocytidine (motif VII) (Scheme 6). The motif VII represents a tridentate base pair and shows similarities to that of  $isoG_d$ - $iC_d$  (motif V). The base pair of 2 with dC is much weaker (motif VIII) also

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in comparison to the dG-dC pair (motif VIa or VIb for  $c^7G_d$ ). This shows that compound **4** is an analogue of  $isoG_d$  and not of dG.

# CONCLUSION

The pyrazolo[3,4-d]pyrimidine nucleoside **2** and the pyrrolo[3,4-d]pyrimidine nucleoside **4** represent a new class of nucleosides which form stable base pairs in oligonucleotide duplexes. Compound **2** hybridizes almost equally well with the four canonical DNA-constituents dA, dT, dG, and dC. Compound **4** can be considered as a 2'-deoxyisoguanosine analogue which forms a strong base pair with 2'-deoxy-5-methylisocytidine.

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