

Pyrazolo[3,4-*d*][1,2,3]triazine DNA: Synthesis and Base Pairing of 7-Deaza-2,8-diaza-2'-deoxyadenosine

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Received March 16, 2004

7-Deaza-2,8-diaza-2'-deoxyadenosine (**4**) was synthesized from 8-aza-7-deaza-2'-deoxyadenosine (**1**) via the 1,*N*⁶-etheno derivative **5**. Ring opening with sodium hydroxide followed by ring closure in the presence of sodium nitrite formed the tricyclic intermediate **5** from which the transiently introduced "etheno" moiety was removed with NBS. Compound **4** was converted to the phosphoramidite **11**, which was employed in solid-phase oligonucleotide synthesis. Base pairing studies on **4**, incorporated in a 12-mer duplex, showed that this adenine nucleoside analogue forms a strong base pair with dG but not with dT. This novel base pair is as stable as that of the canonical dA-dT pair. As a result of the absence of nitrogen-7 compound **4** is expected to form a face to face base pair with dG.

Introduction

The aza and deaza derivatives of purine nucleosides attract attention because of their biological activities as antifungal, antiviral, and anticancer agents.^{1–3} They are also valuable tools in bioorganic chemistry, molecular biology, and nanotechnology.^{4–9} Meanwhile, a variety of such base-modified nucleosides are available either by chemical synthesis or from natural origin. Among them are the nucleosides modified in the imidazole moiety such as the 7-deazapurine nucleosides,^{10,11} the 8-aza-7-deazapurine nucleosides and the 8-azapurine nucleosides.^{12–18} Compounds such as **1** (Figure 1), which can

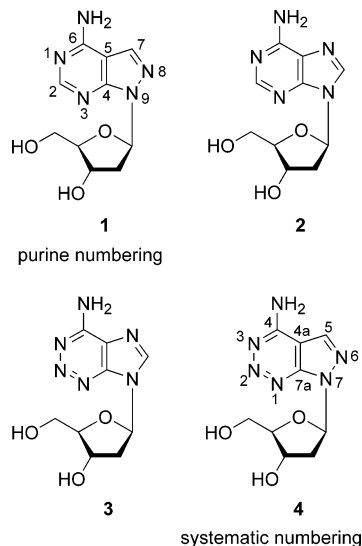


FIGURE 1.

be considered to be isosteric to 2'-deoxyadenosine (**2**), have been already incorporated in duplex DNA.¹⁹

The 2-azapurines, such as 2-azaadenine and 2-azahypoxanthine, have also long been known to inhibit the growth of both microbial and mammalian cells.^{20–29} Although the physical properties of these nucleosides have been studied,³⁰ very few were incorporated in oligonucleotides.³¹ Recently, 2-aza-2'-deoxyadenosine (**3**) was synthesized by our laboratory, and its behavior within oligonucleotide duplexes was studied.^{32,33} In con-

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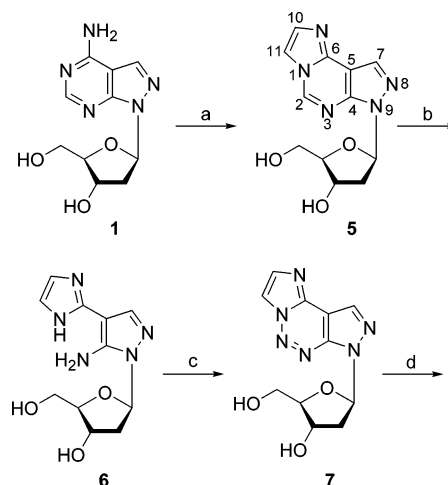
tinuation of the work on 2-azapurine nucleosides and oligonucleotides and as the result of the unusual base pairing properties of **3**, the related 7-deaza-2,8-diazapurine (7*H*-pyrazolo[3,4-*d*][1,2,3]triazine) system is now investigated (purine numbering is used throughout the general part). This manuscript reports on the synthesis of 7-deaza-2,8-diaza-2'-deoxyadenosine (**4**), its conversion into a building block for oligonucleotide synthesis, and its base pairing properties within 12-mer duplexes.

Results and Discussion

1. Synthesis of the Monomers. Up to now, several procedures have been developed for the synthesis of 2-azapurine nucleosides, such as ring annulation on imidazole nucleosides^{21,28,34–43} and the glycosylation of the 2-azapurine base.^{32,44,45} The ribonucleoside of 7-deaza-2,8-diazapurine has already been prepared by Montgomery et al. in 1972.^{21,28} However, the synthesis of the 2'-deoxyribonucleoside **4** has not been reported so far. Herein the synthesis of **4** is performed using the nucleoside **1**³⁴ as starting material, thereby forming its 1,*N*⁶-etheno derivative **5** as central intermediate (Scheme 1). This synthetic route has been elaborated earlier for other compounds^{34–37} and was recently successfully used for the synthesis of **3** by our group.³³

Reaction of **1** with chloroacetaldehyde at pH 4.5–5 gave 1,*N*⁶-etheno-8-aza-7-deaza-2'-deoxyadenosine (**5**) in 82% yield, which was treated with 1 M sodium hydroxide at room temperature to give the derivative **6** (83% yield). Subsequent treatment of **6** with sodium nitrite in 80%

SCHEME 1^a



^a Reagents and conditions: (a) ClCH₂CHO, 1 M aq NaOAc buffer, pH 4.5–5, 82%; (b) 1 N NaOH, 83%; (c) NaNO₂, 80% aq HOAc, 72%; (d) NBS, 1 M aq NaOAc buffer, pH 4–4.5, 66%.

TABLE 1. ¹³C NMR Data of Nucleosides^a

	C(2) ^b	C(4) ^b	C(5) ^b	C(6) ^b	C(7) ^b	C(10) ^b	C(11) ^b
1 ³⁴	155.7	153.6	100.4	157.3	132.8		
5	140.3	145.0	103.3	139.4	131.8	132.4 ^c	112.9 ^c
6 ^e		146.6 ^c	96.3 ^c	142.5 ^c	137.5	122.4 ^c	122.4 ^c
7		142.7	104.3	131.1	131.3	132.9 ^c	115.3 ^c
4		149.2	96.0	153.2	132.9		
8		147.9	99.4	150.3	136.8		
9		147.6	99.9	150.3	136.8		
10		147.9	99.4	150.2	136.6		

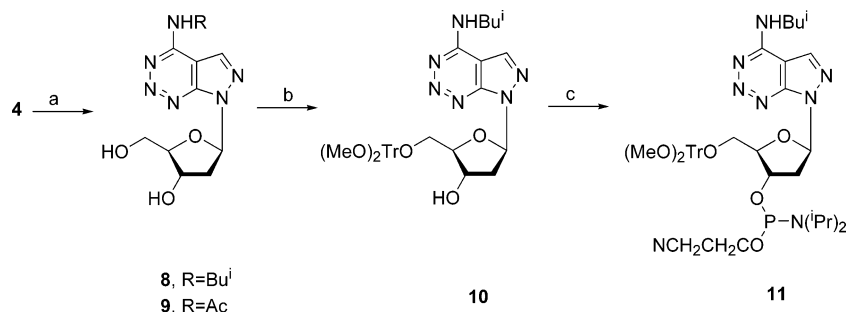
	C(1') ^b	C(2') ^b	C(3') ^b	C(4') ^b	C(5') ^b	O-CH ₃	C=O	CH	CH ₃
1 ³⁴	84.1	38.0	71.0	87.5	62.3				
5	84.3	38.0	71.1	87.9	62.4				
6 ^e	86.0	<i>d</i>	72.2	88.0	63.2				
7	85.7	<i>d</i>	70.8	88.2	62.1				
4	84.9	<i>d</i>	71.0	87.9	62.3				
8	84.8	<i>d</i>	70.7	87.9	62.0		177.4	34.6	19.0
9	84.9	<i>d</i>	70.7	87.9	62.0		170.6		23.9
10	84.8	<i>d</i>	70.4	85.7	64.0	54.7	177.4	34.6	19.0

^a Measured in DMSO-*d*₆ at 303 K. ^b Purine numbering. ^c Tentative. ^d Superimposed by the signal of DMSO-*d*₆. ^e DMSO-*d*₆–0.4 aq NH₄OAc.

aqueous acetic acid afforded 1,*N*⁶-etheno-7-deaza-2,8-diaza-2'-deoxyadenosine (**7**) in 72%. Afterward compound **7** was treated with *N*-bromosuccinimide at pH 4–4.5 to result in **4** (66% yield). The overall yield amounts to 32% for the four steps. The structures of all compounds were confirmed by ¹H and ¹³C NMR spectra and by elemental analyses (see Table 1 and Experimental Section). The ¹³C NMR resonances were assigned with the help of gated-decoupled spectra (Supporting Information).

Next, the stability of the *N*-glycosylic bond of **4** was measured in aqueous hydrochloric acid (0.1 M, rt). The reaction was monitored by UV spectroscopy at 291 nm. The stability of **4** was found to be similar (τ = 41 min) to that of dA (**2**) (τ = 45 min) but lower than that of z²A_d (**3**) (τ = 63 min). The UV maximum of **4** (λ_{max} = 310 nm) shows a strong red shift compared to compound **1** (λ_{max} = 260 nm). In acidic medium, the maximum is blue shifted from λ_{max} = 310 nm to λ_{max} = 290 nm. Next, the *pK* value of protonation of compound **4** was measured

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SCHEME 2^a

^a Reagents and conditions: (a) (i) $(\text{CH}_3)_3\text{SiCl}$, Py, (ii) $(\text{Bu}^t)_2\text{O}$ for **8** or Ac_2O for **9**, then (iii) 10% NH_3 , 70% for **8**, 60% for **9**; (b) $(\text{MeO})_2\text{TrCl}$, Py, 80%; (c) $^i\text{Pr}_2\text{NP}(\text{Cl})\text{OCH}_2\text{CH}_2\text{CN}$, $^i\text{Pr}_2\text{EtN}$, CH_2Cl_2 , 72%.

and compared with that of **3**.⁴⁶ Both compounds show very similar values ($\text{pK}_a = 1.7$ for **4** and 1.9 for **3**), which are quite different from those of **1** ($\text{pK}_a = 4.0$) and **2** ($\text{pK}_a = 3.8$).⁴⁷ We assume that the site of protonation is the same for compound **4** (N-1) as it is for dA.

For the synthesis of oligonucleotides the phosphoramidite **11** was prepared. It involves at first the protection of the amino group of **4**. Previous studies have shown that the benzoyl group is inappropriate in this case because of its long half-life.³³ To look for a more appropriate group, the isobutyryl and acetyl groups were selected for protection. Thus, compound **4** was treated with trimethylsilyl chloride and isobutyric anhydride or acetic anhydride as described by Jones et al.⁴⁸ to give compound **8** and **9** in 70% and 60% yield, respectively (Scheme 2). Then, the stability of these protecting groups was studied. The τ values of hydrolysis were determined in concentrated aqueous ammonia (40 °C) and monitored by UV spectroscopy at 235 nm showing that both the isobutyryl and the acetyl group have appropriate half-lives of 51 min for **8** and of 31 min for **9**. Because of the higher yield of **8**, it was used for further manipulations. The introduction of the 4,4'-dimethoxytrityl group followed the standard protocol, giving compound **10** in 80% yield.⁴⁹ Subsequent phosphitylation with chloro-(2-cyanoethoxy)-*N,N*-(diisopropylamino)phosphine gave the phosphoramidite **11** (72% yield).⁵⁰ All new compounds were characterized by ^1H and ^{13}C NMR spectra and by elemental analysis (see Table 1 and Experimental Section).

2. Synthesis and Base Pairing Properties of Oligonucleotides. The oligonucleotide synthesis was performed applying the phosphoramidite **11** in solid-phase oligonucleotide chemistry in an automatized DNA synthesizer on controlled pore glass (CPG) 500 serving as solid phase.⁵¹ The oligomers were deprotected by incubation with a 25% aqueous NH_3 solution (60 °C, 20 h) and purified by RP-18 HPLC. The oligomers synthesized are

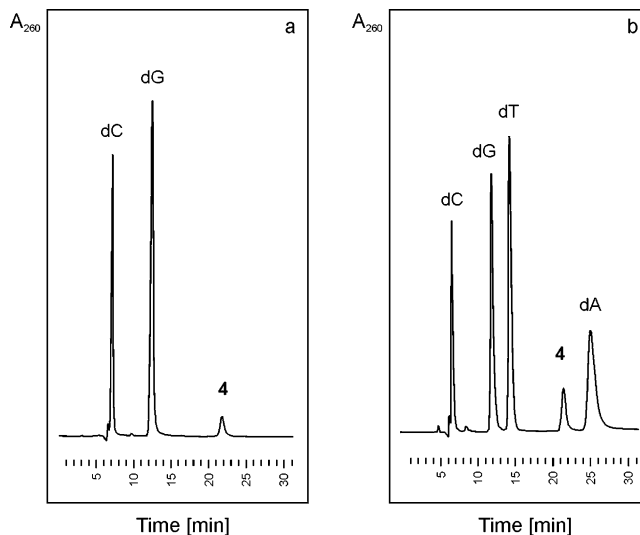


FIGURE 2. RP-18 HPLC-elution profile of enzymatic digests of the oligonucleotides 5'-d(**4** CGC GCG) (**16**) (a) and 5'-d(AGT **4**TT G4C CTA) (**15**) (b).

shown in the following tables. For the investigation of the base pairing selectivity and base pair stability of compound **4**, two oligonucleotides, 5'-d(TAG GTC AAT ACT) (**12**) and 3'-d(ATC CAG TTA TGA) (**13**), were synthesized to form the already known reference duplex **12**·**13**.¹⁸ For characterization, the oligonucleotides **14**, **15**, and **16** were hydrolyzed with snake venom phosphodiesterase, followed by alkaline phosphatase¹⁹ to yield the nucleosides, which were separated by RP-18 HPLC (Figure 2 and Supporting Information). The oligonucleotides were also characterized by MALDI-TOF spectra (Supporting Information). The masses were in good agreement with the calculated values (Supporting Information).

Initially, compound **4**, as an analogue of dA, was incorporated opposite to dT. As can be seen from Table 2, the incorporation of one **4**-dT base pair reduces the T_m of the duplex **17**·**13** as well as of **12**·**18** by 5 and 4 °C, respectively. Incorporation of two modified base pairs shows a decrease of the T_m value within the range of 8–13 °C (**19**·**13**, **12**·**15**, **20**·**13**). In these cases the position of incorporation results in slightly different stabilities due to nearest neighbor influences. Consecutive incorporation of **4**-dT base pairs led to a decrease stronger than of that of separated base pairs. The duplex **20**·**13** possessing a terminal modification has a minor effect on the T_m value

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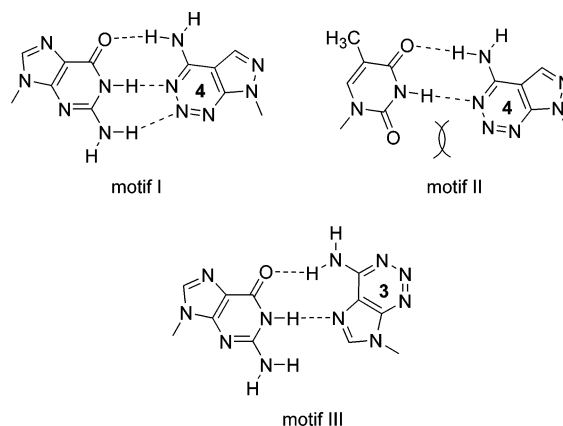
TABLE 2. T_m Values and Thermodynamic Data of Oligonucleotide Duplexes Containing Regular and Base-Modified Nucleoside Residues^{a,b}

duplex	no.	T_m [°C]	ΔT_m^c	ΔG^{310} [kcal/mol] ^d
5'-d(TAG GTC AAT ACT)	(12)	47	0	-10.4
3'-d(ATC CAG TTA TGA)	(13)			
5'-d(TAG GTC A4T ACT)	(17)	42	-5	-8.8
3'-d(ATC CAG TTA TGA)	(13)			
5'-d(TAG GTC AAT ACT)	(12)	43	-4	-9.1
3'-d(ATC C4G TTA TGA)	(18)			
5'-d(TAG GTC 4 4 T ACT)	(19)	34	-6.5	-7.0
3'-d(ATC CAG TTA TGA)	(13)			
5'-d(TAG GTC AAT ACT)	(12)	37	-5	-7.6
3'-d(ATC C4G T T4 TGA)	(15)			
5'-d(T 4G GTC AAT 4CT)	(20)	39	-4	-8.0
3'-d(ATC CAG TTA TGA)	(13)			
5'-d(TAG GTC AAT ACT) ⁵²	(12)	44	0	-9.2
3'-d(ATC CAG TGA TGA)	(21)			
5'-d(TAG GGC AAT ACT) ⁵²	(22)	48	0	-10.5
3'-d(ATC CAG TTA TGA)	(13)			
5'-d(TAG GTC A4T ACT)	(17)	46	+2	-9.6
3'-d(ATC CAG TGA TGA)	(21)			
5'-d(TAG GGC AAT ACT)	(22)	48	0	-10.6
3'-d(ATC C4G TTA TGA)	(18)			
5'-d(TAG GTC 4 4T ACT)	(19)	46	+1	-9.2
3'-d(ATC CAG GGA TGA)	(23)			
5'-d(TAG GGC AAG ACT)	(24)	46	+1	-9.4
3'-d(ATC C 4G T T 4 TGA)	(15)			
5'-d(T 4G GTC AAT 4 CT)	(20)	48	+1	-10.0
3'-d(AGC CAG TTA GGA)	(25)			
5'-d(TAG GTC A4T ACT)	(17)	34	-13	-7.0
3'-d(ATC CAG TAA TGA)	(26)			
5'-d(TAG GAC AAT ACT)	(27)	37	-10	-7.8
3'-d(ATC C4G TTA TGA)	(18)			
5'-d(TAG GTC 4 4T ACT)	(19)	21	-13	-5.7
3'-d(ATC CAG AAA TGA)	(30)			
5'-d(TAG GAC AAA ACT)	(31)	21	-13	-5.3
3'-d(ATC C 4G T T 4 TGA)	(15)			
5'-d(TAG GTC A4T ACT)	(17)	32	-15	-6.8
3'-d(ATC CAG TCA TGA)	(28)			
5'-d(TAG GCC AAT ACT)	(29)	36	-11	-7.4
3'-d(ATC C 4G TTA TGA)	(18)			
5'-d(TAG GTC 4 4T ACT)	(19)	20	-13.5	-5.2
3'-d(ATC CAG CCA TGA)	(32)			
5'-d(TAG GCC AAC ACT)	(33)	20	-13.5	-5.3
3'-d(ATC C 4G T T 4 TGA)	(15)			

^a **4** = $z^2c^7z^8A_d$ = 7-deaza-2,8-diaza-2'-deoxyadenosine. ^b Determined in 10 mM sodium cacodylate buffer, pH 7.0, containing 100 mM NaCl and 10 mM MgCl₂. ^c Per modified base pair. ^d 1 cal = 4.184 J.

as a modification in a central position (**12**·**15**). These observations demonstrate that the **4**-dT base pair is significantly less stable than that of dA-dT.

Next, compound **4** was incorporated into a duplex position opposite to dG. In these cases the duplexes of **12**·**21** and **22**·**13** are used as reference compounds. Replacement of one dA-dG base pair by one **4**-dG pair (**17**·**21**; **22**·**18**) resulted in no significant change of the T_m value ($\Delta T_m = 1-2$ °C). Incorporation of two **4**-dG pairs shows also no significant changes in the stability ($\Delta T_m = 1-2$ °C). All consecutive central **4**-dG pairs (**19**·**23**), separated central **4**-dG pairs (**24**·**15**), and peripheral **4**-dG base pairs (**20**·**25**) exhibit similar stabilities with $\Delta T_m = 1-2$ °C. Moreover, no matter if one or two **4**-dG were introduced, the duplexes have the same stability as that of the standard duplexes (**12**·**13**, **12**·**21**, and **22**·**13**) with a ΔT_m range of 1–2 °C. Thus, the nucleoside **4** forms a

SCHEME 3^a

^a Face to face base pair motifs of (I) **4**-dG and (II) **4**-dT; (III) alternative Hoogsteen motif for **3**-dG base pair.

TABLE 3. Comparison of T_m Values and Thermodynamic Data of Duplex Containing $z^2c^7z^8A_d$ **4** and z^2A_d **3^{a,b}**

duplex	no.	T_m [°C]	ΔT_m^c	ΔG^{310} [kcal/mol] ^d
5'-d(TAG GTC 4 4T ACT)	(19)	46	+1	-9.2
3'-d(ATC CAG GGA TGA)	(23)			
5'-d(TAG GTC 3 3T ACT)	(34)	46	+1	-10.1
3'-d(ATC CAG GGA TGA)	(23)			
5'-d(TAG GGC AAG ACT)	(24)	46	+1	-9.4
3'-d(ATC C 4G T T 4 TGA)	(15)			
5'-d(TAG GGC AAG ACT)	(24)	45	+0.5	-11.5
3'-d(ATC C 3G T T 3 TGA)	(35)			
5'-d(TAG GTC 4 4T ACT)	(19)	34	-6.5	-7.0
3'-d(ATC CAG TTA TGA)	(13)			
5'-d(TAG GTC 3 3T ACT)	(34)	37	-5	-7.7
3'-d(ATC CAG TTA TGA)	(13)			
5'-d(TAG GTC AAT ACT)	(12)	37	-5	-7.6
3'-d(ATC C 4G TT 4 TGA)	(15)			
5'-d(TAG GTC AAT ACT)	(12)	37	-5	-7.6
3'-d(ATC C 3G TT 3 TGA)	(35)			

^a **4** = $z^2c^7z^8A_d$ = 7-deaza-2,8-diaza-2'-deoxyadenosine; **3** = z^2A_d = 2-aza-2'-deoxyadenosine. ^b Determined in 10 mM sodium cacodylate buffer, pH 7.0, containing 100 mM NaCl and 10 mM MgCl₂. ^c Per modified base pair. ^d 1 cal = 4.184 J.

strong base pair with dG comparable to that of a dA-dG pair (**12**·**21** and **22**·**13**) or that of dA-dT (**12**·**13**).

In the following experiments the nucleoside **4** was introduced opposite to dA or dC residues. This results in a dramatic decrease of the T_m values. One incorporation opposite to dA (**17**·**26** and **27**·**18**) reduces the T_m by -13 and -10 °C, respectively, and opposite to dC (**17**·**28** and **29**·**18**) by -15 and -11 °C. In both cases, two incorporations (**19**·**30**, **31**·**15**, **19**·**32**, and **33**·**15**) show a decrease of the T_m values in the range of 26–27 °C. Regarding these results, the base of compound **4** forms a strong base pair with guanine but a much weaker one with thymine, adenine, and cytosine with an order of relative stability of dG > dT > dA \approx dC, which is similar to **3**.³³

It seems interesting to compare the duplex stability of compounds **3** and **4**. Because of the absence of nitrogen-7 in compound **4** this molecule cannot form a Hoogsteen base pair, which makes the formation of a face to face base pair as shown in motif (I) very likely (Scheme 3). As seen from Table 3 the incorporation of the nucleosides **3** or **4** opposite to dG causes duplexes of almost identical

TABLE 4. T_m Values and Thermodynamic Data of Oligonucleotides with Dangling Nucleotides^{a,b}

duplex	no.	T_m [°C]	ΔT_m	ΔG^{310} [kcal/mol] ^c
5'-d(CGC GCG) ³³	(36)	46	0	-8.2
3'-d(GCG CGC)	(36)			
5'-d(4 CGC GCG) ^d	(16)	57	+11	-10.1
3'-d(GCG CGC 4)	(16)			
5'-d(3 CGC GCG) ³³	(38)	53	+7	-9.7
3'-d(GCG CGC 3)	(38)			
5'-d(A CGC GCG) ³³	(39)	55	+9	-10.1
3'-d(GCG CGC A)	(39)			
5'-d(T CGC GCG) ³³	(40)	53	+7	-9.6
3'-d(GCG CGC T)	(40)			
5'-d(c ⁷ A CGC GCG) ³³	(37)	57	+11	-10.0
3'-d(GCG CGC c ⁷ A)	(37)			

^a 4 = z²c⁷z⁸A_d = 7-deaza-2,8-diaza-2'-deoxyadenosine; 3 = z²A_d = 2-aza-2'-deoxyadenosine; c⁷A_d = 7-deaza-2'-deoxyadenosine.

^b Determined in 0.01 M Na₂HPO₄, 1 M NaCl, pH 7.0. ^c 1 cal = 4.184 J. ^d Determined in 10 mM sodium cacodylate buffer, pH 7.0, containing 100 mM NaCl and 10 mM MgCl₂.

stability (see 19·23, 15·24 and 34·23, 24·35). Moreover, these duplexes exhibit T_m values nearly identical to those of the standard duplex 12·13. Also the introduction of 3 or 4 opposite to thymine yields duplexes with a similar reduced stability (19·13, 12·15 and 34·13, 12·35) and a T_m decrease ($\Delta T_m = -5$ °C) in comparison to the duplex with canonical base pairs as in the duplex 12·13. Thus, the interchange of a nitrogen between the positions 7 and 8 has rather little influence on the duplex stability. It is worth noting that all T_m values of duplex formation are in good correspondence to the ΔG values. We have also studied the influence of the nucleoside 4 forming a dangling end at the terminus of d(G-C)₃. This should reflect the stacking ability in the absence of hydrogen bonding. For this purpose the oligonucleotide duplexes with overhanging ends were synthesized and compared to related compounds from which the influence of the dangling end was already reported (see duplexes of Table 4). According to this Table the stacking of compound 4 is at least as strong as those of other "purine" nucleosides.

Conclusion

Compound 4 has the same capability to form strong base pairs with dG and much weaker ones with dT as was reported for other 2-azapurine nucleosides.³³ The strength of the 4-dG base pair is similar to that of the dG-dA pair or that of dA-dT. The most likely motifs for the base pairs are (I) for 4-dG and (II) for 4-dT (Scheme 3). The two 2-aza nucleosides show pK_a values of 1.7 (4) and 1.9 (3). In the absence of the nitrogen at the 2-position the pK_a value is 3.8 for dA (2)⁴⁷ and 4.0 for compound 1. These differences reflect the weaker proton acceptor capabilities of the 2-azapurines. Consequently, they are also weaker proton acceptors in the 3-dT or 4-dT pairs compared to those of compound 1 or 2 with dT. On the other hand, the base pairs between 3 or 4 with dG are very stable thus reaching the stability of a dA-dT pair. According to the inability of compound 4 to form a Hoogsteen pair as is possible in the case of compound 3 (motif (III)), the motif (I) is suggested for the 4-dG pair and motif (II) for the pairing mode of 4 with dT. Compound 3 is expected to form the same type of a base pair motif. The lower stability of the 4-dT pair compared

to the 4-dG pair might result from a steric clash of the 2-oxo group of dT with nitrogen-2 of 4 as well as an electronic repulsion of these residues. In contrary, attracting forces (H-bonding) are expected to stabilize the base pair of 3 or 4 with dG (see motif (I)). As the motif (I) is formed by two "purine" bases one might argue that this will destabilize the sugar phosphate backbone and distort the double helix. However, this unfavorable situation might be compensated by the stronger stacking of the odd "purine-purine" base pairs as has been reported for the face to face pair of dG-dA.⁵³

Experimental Section

General. Solvents were technical grade and distilled before use. Solvent systems for TLC and chromatography: (A) CH₂Cl₂/CH₃OH 95:5 v/v; (B) CH₂Cl₂/CH₃OH 9:1 v/v; (C) CH₂Cl₂/CH₃OH 85:15 v/v; (D) CH₂Cl₂/CH₃OH 8:2 v/v; (E) H₂O/PrOH 9:1 v/v; (F) CH₂Cl₂/CH₃COCH₃ 9:1 v/v; (G) CH₂Cl₂/CH₃COCH₃ 85:15 v/v; (H) CH₂Cl₂/CH₃COCH₃ 8:2 v/v. Flash chromatography: 0.4 bar, silica gel 60H. TLC: aluminum sheet, silica gel 60 F₂₅₄ (0.2 mm). NMR spectra: measured at 250.13 MHz for ¹H and 125.13 MHz for ¹³C, δ values in ppm relative to internal SiMe₄ (¹H, ¹³C) or external H₃PO₄.

7-(2-Deoxy- β -D-erythro-pentofuranosyl)-imidazo[1,2-*c*]-7H-pyrazolo[4,3-*e*]pyrimidine (5). To a solution of compound 1³⁴ (3.0 g, 12.00 mmol) in aqueous sodium acetate buffer (1 M, pH 4.5–5.0, 70 mL) at 40–50 °C was added chloroacetaldehyde (50% aqueous solution, 15 mL). The solution was stirred at room temperature for 70 h. The reaction mixture was evaporated, chromatographed (silica gel, column 15 cm \times 3 cm, solvent system A \rightarrow C), and the product was crystallized from MeOH/EtOAc (1:1, v/v). Compound 5 (2.71 g, 82%) was isolated as colorless needles, mp 197–200 °C. TLC (silica gel, solvent system B): R_f 0.4. UV (MeOH): λ_{max} 278 nm (ϵ 4500), 257 nm (ϵ 8100), 232 nm (ϵ 41000). ¹H NMR (DMSO-*d*₆): δ 2.34, 2.88 (2m, 2H, H₂-C(2')), 3.54 (m, 2H, H₂-C(5')), 3.86 (m, 1H, H-C(4')), 4.49 (m, 1H, H-C(3')), 4.78 (dd, 1H, OH-C(5')), 5.35 (d, J = 3.4 Hz, 1H, OH-C(3')), 6.72 ("t", J = 6.3 Hz, 1H, H-C(1')), 7.52 (d, J = 1.6 Hz, 1H, H-C(11)), 8.06 (d, J = 1.6 Hz, 1H, H-C(10)), 8.40 (s, 1H, H-C(7)), 9.32 (s, 1H, H-C(2)). Anal. Calcd for C₁₂H₁₃N₅O₃ (275.26): C, 52.36; H, 4.76; N, 25.44. Found: C, 52.61; H, 4.90; N, 25.15.

5-Amino-1-(2-deoxy- β -D-erythro-pentofuranosyl)-4-(imidazol-2-yl)pyrazole (6). Compound 5 (1.2 g, 4.36 mmol) was stirred with aqueous NaOH (1 N, 20 mL) overnight at room temperature. The reaction solution was acidified to pH 7.0 with hydrochloric acid (2 N), concentrated, and purified by FC (silica gel, column 10 cm \times 3 cm, solvent system C) to yield the title compound 6 as a yellowish foam (960 mg, 83%). TLC (silica gel, solvent system C): R_f 0.3. UV (MeOH): λ_{max} 254 nm (ϵ 10500). ¹H NMR (DMSO-*d*₆): δ 2.11, 2.71 (2m, 2H, H₂-C(2')), 3.45 (m, 2H, H₂-C(5')), 3.80 (m, 1H, H-C(4')), 4.37 (m, 1H, H-C(3')), 4.99 (dd, 1H, OH-C(5')), 5.21 (d, J = 3.8 Hz, 1H, OH-C(3')), 6.12 ("t", J = 6.4 Hz, 1H, H-C(1')), 6.37 (s, 2H, H-C(7), H-C(8)), 6.95 (s, 2H, NH₂), 7.65 (s, 1H, H-C(3)), 11.94 (s, 1H, NH). Anal. Calcd for C₁₁H₁₅N₅O₃ (265.27): C, 49.81; H, 5.70; N, 26.40. Found: C, 49.83; H, 5.78; N, 26.36.

7-(2-Deoxy- β -D-erythro-pentofuranosyl)-imidazo[1,2-*c*]-7H-pyrazolo[4,3-*e*][1,2,3]triazine (7). A solution of compound 6 (240 mg, 0.91 mmol) in 80% aqueous HOAc (20 mL) was treated with sodium nitrite (70 mg, 1 mmol) during 1 h in an ice bath. The reaction solution was evaporated, dissolved in water, and evaporated repeatedly to remove HOAc. The residue was then chromatographed on silica gel (column 15 cm \times 3 cm, solvent system A \rightarrow C) to give 7 as a yellow foam (180 mg, 72%). TLC (silica gel, solvent system B): R_f 0.3. UV

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(MeOH): λ_{\max} 295 nm (ϵ 4300), 233 nm (ϵ 34800). ^1H NMR (DMSO- d_6): δ 2.47, 2.97 (2m, 2H, $\text{H}_2\text{-C}(2')$), 3.55 (m, 2H, $\text{H}_2\text{-C}(5')$), 3.91 (m, 1H, $\text{H-C}(4')$), 4.54 (m, 1H, $\text{H-C}(3')$), 4.74 ("t", $J = 5.7$ Hz, 1H, $\text{OH-C}(5')$), 5.43 (d, $J = 4.7$ Hz, 1H, $\text{OH-C}(3')$), 6.98 ("t", $J = 6.1$ Hz, 1H, $\text{H-C}(1')$), 7.79 (d, $J = 1.6$ Hz, 1H, $\text{H-C}(10)$), 8.70 (s, 1H, $\text{H-C}(7)$), 8.77 (d, $J = 1.6$ Hz, 1H, $\text{H-C}(11)$). Anal. Calcd for $\text{C}_{11}\text{H}_{12}\text{N}_6\text{O}_3$ (276.25): C, 47.83; H, 4.38; N, 30.42. Found: C, 48.00; H, 4.28; N, 30.05.

4-Amino-7-(2-deoxy- β -D-erythro-pentofuranosyl)-7H-pyrazolo[3,4-*d*][1,2,3]triazine (7-deaza-2,8-diaza-2'-deoxy-adenosine) (4). To a solution of compound **7** (276 mg, 0.97 mmol) in aqueous sodium acetate buffer (1 M, pH 4.0–4.5, 60 mL) at 40–50 °C was added *N*-bromosuccinimide (1.4 g, 7.87 mmol), the reaction mixture was stirred at room temperature overnight. The solution was then evaporated. The residue was dissolved in water and desalted with a Serdolit column (column 10 cm \times 2 cm, solvent system $\text{H}_2\text{O} \rightarrow \text{E}$), purified by chromatography (silica gel, column 6 cm \times 3 cm, solvent system B), and recrystallized from MeOH/ H_2O (1:1, v/v) to yield **4** as colorless crystals (161 mg, 66%), mp 178–181 °C. TLC (silica gel, solvent system C): R_f 0.4. UV (MeOH): λ_{\max} 309 nm (ϵ 6900), 260 nm (ϵ 4100). ^1H NMR (DMSO- d_6): δ 2.36, 2.88 (2m, 2H, $\text{H}_2\text{-C}(2')$), 3.53 (m, 2H, $\text{H}_2\text{-C}(5')$), 3.85 (m, 1H, $\text{H-C}(4')$), 4.48 (m, 1H, $\text{H-C}(3')$), 4.76 ("t", $J = 5.7$ Hz, 1H, $\text{OH-C}(5')$), 5.35 (d, $J = 4.4$ Hz, 1H, $\text{OH-C}(3')$), 6.78 ("t", $J = 6.3$ Hz, 1H, $\text{H-C}(1')$), 8.82 (s, 3H, NH_2 and $\text{H-C}(7)$). Anal. Calcd for $\text{C}_9\text{H}_{12}\text{N}_6\text{O}_3$ (252.23): C, 42.86; H, 4.80; N, 33.32. Found: C, 42.78; H, 4.83; N, 33.18.

7-(2-Deoxy- β -D-erythro-pentofuranosyl)-4-isobutyl-rylamino-7H-pyrazolo[3,4-*d*][1,2,3]triazine (8). After being coevaporated twice with anhydrous pyridine, compound **4** (100 mg, 0.4 mmol) was suspended in anhydrous pyridine (1.6 mL) and treated with trimethylsilyl chloride (260 μL , 2.05 mmol) at room temperature during 15 min. To the reaction solution was added isobutyl anhydride (110 μL , 0.66 mmol), and the mixture was stirred at room temperature for 3 h. The reaction mixture was cooled in an ice–water bath and treated with H_2O (400 μL) for 15 min and then with aqueous NH_3 (25% aq, 400 μL) for further 15 min. The mixture was then evaporated to dryness and coevaporated with toluene. The residue was purified over a silica gel column (10 cm \times 1 cm, solvent system B) to yield **8** as colorless foam (90 mg, 70%). TLC (silica gel, solvent system B): R_f 0.3. UV (MeOH): λ_{\max} 306 nm (ϵ 4700), 266 nm (ϵ 7200). ^1H NMR (DMSO- d_6): δ 1.18 (d, 6H, 2 CH_3), 2.43 (m, 1H, $\text{H}_\alpha\text{-C}(2')$), 2.92 (m, 1H, CH), 2.97 (m, 1H, $\text{H}_\beta\text{-C}(2')$), 3.53 (m, 2H, $\text{H}_2\text{-C}(5')$), 3.87 (m, 1H, $\text{H-C}(4')$), 4.51 (m, 1H, $\text{H-C}(3')$), 4.77 ("t", $J = 5.4$ Hz, 1H, $\text{OH-C}(5')$), 5.39 (d, $J = 4.7$ Hz, 1H, $\text{OH-C}(3')$), 6.94 ("t", $J = 6.1$ Hz, 1H, $\text{H-C}(1')$), 8.67 (s, 1H, $\text{H-C}(7)$), 11.92 (s, 1H, NH). Anal. Calcd for $\text{C}_{13}\text{H}_{18}\text{N}_6\text{O}_4$ (322.32): C, 48.44; H, 5.63; N, 26.07. Found: C, 48.62; H, 5.55; N, 25.86.

4-Acetylamino-7-(2-deoxy- β -D-erythro-pentofuranosyl)-7H-pyrazolo[3,4-*d*][1,2,3]triazine (9). The procedure described for **8** was used but with acetic anhydride (110 μL , 1.17 mmol) instead of isobutyl anhydride to yield a colorless foam (70 mg, 60%). TLC (silica gel, solvent system B): R_f 0.3. UV (MeOH): λ_{\max} 303 nm (ϵ 3900), 265 nm (ϵ 5600). ^1H NMR (DMSO- d_6): δ 2.30 (s, 3H, CH_3), 2.42, 2.92 (2m, 2H, $\text{H}_2\text{-C}(2')$), 3.45 (m, 2H, $\text{H}_2\text{-C}(5')$), 3.88 (m, 1H, $\text{H-C}(4')$), 4.51 (m, 1H, $\text{H-C}(3')$), 4.77 (m, 1H, $\text{OH-C}(5')$), 5.39 (m, 1H, $\text{OH-C}(3')$), 6.93 ("t", $J = 5.8$ Hz, 1H, $\text{H-C}(1')$), 8.62 (s, 1H, $\text{H-C}(7)$), 11.92 (s, 1H, NH). Anal. Calcd for $\text{C}_{11}\text{H}_{14}\text{N}_6\text{O}_4$ (294.27): C, 44.90; H, 4.80; N, 28.56. Found: C, 45.05; H, 4.99; N, 28.42.

7-[2-Deoxy-5-*O*-(4,4'-dimethoxytriphenyl)methyl- β -D-erythro-pentofuranosyl]-4-isobutyl-rylamino-7H-pyrazolo[3,4-*d*][1,2,3]triazine (10). Compound **8** (400 mg, 1.24 mmol) was coevaporated twice with anhydrous pyridine and then dissolved in anhydrous pyridine (15 mL). This solution was treated with 4,4'-dimethoxytriphenylmethyl chloride (605 mg, 1.79 mmol) at room temperature for 4 h. Thereupon, MeOH (3 mL) was added, and the stirring was continued for 10 min. The solution was concentrated to half of the volume, and $\text{CH}_2\text{-Cl}_2$ (70 mL) was added. This was washed twice with an aqueous NaHCO_3 (5%, 2×40 mL), twice with water (2×40 mL), and once with a saturated NaCl solution (40 mL). The organic phase was dried over Na_2SO_4 , filtered, and evaporated, and the residue was chromatographed on silica gel (column 10 cm \times 3 cm, solvent system F \rightarrow H) to yield the nucleoside **10** as a yellow foam (620 mg, 80%). TLC (silica gel, solvent system H): R_f 0.3. UV (MeOH): λ_{\max} 313 nm (ϵ 8300), 267 nm (ϵ 12600), 236 nm (ϵ 33600). ^1H NMR (DMSO- d_6): δ 1.18 (d, 6H, 2 CH_3), 2.47 (m, 1H, $\text{H}_\alpha\text{-C}(2')$), 2.98 (m, 1H, CH), 3.06 (m, 1H, $\text{H}_\beta\text{-C}(2')$), 3.68, 3.70 (2s, 6H, 2 OCH_3), 3.73 (m, 1H, $\text{H-C}(5')$), 4.01 (m, 1H, $\text{H-C}(4')$), 4.62 (m, 1H, $\text{H-C}(3')$), 5.44 (d, $J = 5.0$ Hz, 1H, $\text{OH-C}(3')$), 6.70 (m, 4H, phenyl-H), 6.99 (m, 1H, $\text{H-C}(1')$), 7.12, 7.24 (2m, 9H, phenyl-H), 8.61 (s, 1H, $\text{H-C}(7)$), 11.94 (s, 1H, NH). Anal. Calcd for $\text{C}_{34}\text{H}_{36}\text{N}_6\text{O}_6$ (624.69): C, 65.37; H, 5.81; N, 13.43. Found: C, 65.67; H, 5.92; N, 13.37.

7-[2-Deoxy-5-*O*-(4,4'-dimethoxytriphenyl)methyl- β -D-erythro-pentofuranosyl]-4-isobutyl-rylamino-7H-pyrazolo[3,4-*d*][1,2,3]triazine-3'-[(2-cyanoethyl)-*N,N*-diisopropylphosphoramidite] (11). Compound **10** (400 mg, 0.64 mmol) was coevaporated twice with anhydrous pyridine and then dissolved in CH_2Cl_2 (20 mL). *N,N*-Diisopropylethylamine (210 μL , 1.21 mmol) and chloro-(2-cyanoethoxy)-*N,N*-diisopropylaminophosphine (210 μL , 0.94 mmol) were added under argon atmosphere. After 20 min of stirring at room temperature, CH_2Cl_2 (40 mL) was added. The organic phase was washed twice with an aqueous solution of NaHCO_3 (5%, 2×20 mL) and once with a saturated solution of NaCl (20 mL). The organic layer was dried over Na_2SO_4 , filtered, and evaporated. The residue was purified by FC (silica gel, column 10 cm \times 3 cm, solvent system G) to furnish **11** as colorless foam (382 mg, 72%). TLC (silica gel, solvent system G): R_f 0.4. ^{31}P NMR (CDCl_3): δ 149.9, 149.74. Anal. Calcd for $\text{C}_{43}\text{H}_{53}\text{N}_8\text{O}_7\text{P}$ (824.90): C, 62.61; H, 6.48; N, 13.58. Found: C, 62.93; H, 6.61; N, 13.37.

Acknowledgment. We thank Dr. Helmut Rosemeyer and Dr. Yang He for measuring the NMR spectra and Mrs. E. Feiling for the oligonucleotide syntheses. We also thank Mrs. E.-M. Becker for the pK measurements and Mrs. M. Dubiel for her help during the preparation of the manuscript. Financial support by the European Community (Grant QLRT-2001-00506, "Flavitherapeutics") is gratefully acknowledged.

Supporting Information Available: General experimental methods for oligonucleotides, $J(\text{H,C})$ coupling constant (Hz) of nucleosides, coupling yields of oligo-2'-deoxyribonucleotides, MALDI-TOF spectrum of the oligonucleotide 5'-d[TAG GTC AAT ACT] (**17**), and composition of the oligonucleotides **14**, **15**, and **16**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

JO0401501