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3-Bromopyrazolo[3,4-*d*]pyrimidine 2′-Deoxy-2′-fluoro-β-D-arabinonucleosides: Modified DNA Constituents with an Unusually Rigid Sugar *N*-Conformation

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Received February 13, 2003

The nucleobase anion glycosylation of 3-bromo-4-isopropoxy-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (**6**) with 3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranosyl bromide (**5**) furnished the protected N^1-β-D-nucleosides **7** (60%) and **8** (ca. 2%) along with the N^2-β-D-regioisomer **9** (9%). Debenzoylation of compounds **7** and **9** yielded the nucleosides **10** (81%) and **11** (76%). Compound **10** was transformed to the 2′-deoxyguanosine derivative **1** [6-amino-3-bromo-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one] (85% yield) and the purine-2,6-diamine analogue **2** [3-bromo-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4, 6-diamine] (78%). Both nucleosides form more than 98% *N*-conformer population (P_N ca. 358° and ψ_m ca. 37°) in aqueous solution. Single-crystal X-ray analysis of **1** showed that the sugar moiety displays also the *N*-conformation [$P = 347.3°$ and $\psi_m = 34.4°$] in the solid state. The remarkable rigid *N*-conformation of the pyrazolo[3,4-*d*]pyrimidine 2′-deoxy-2′-fluoro-β-D-arabinonucleosides **1** and **2** observed in solution is different from that of the parent purine 2′-deoxy-2′-fluoro-β-D-arabinonucleosides **3** and **4**, which are in equilibrium showing almost equal distribution of the *N*/*S*-conformers.

Introduction

It is well-known that the introduction of a fluorine atom into a natural nucleoside strongly effects the chemical, physical, and biological properties of the molecule.[1] Most of such analogues of the natural nucleosides manifest high pharmacological activity;[2−4] some of them are very important chemotherapeutic drugs for the treatment of various viral and cancer diseases, being in use or in clinical evaluation.[5] Among the fluoro-containing analogues of natural nucleosides the 2′-deoxy-2′-fluoro-β-D-arabinofuranosyl nucleosides and their β-L-enantiomers attract particular attention.[6−9] The fluoroarabino analogue of the natural nucleoside antibiotic sangivamycin develops a rather interesting anti-(human cytomegalovirus) activity.[7] Recently, we have shown that 2′-deoxy-2′-fluoro-β-D-arabinofuranosyl pyrazolo[3,4-*d*]pyrimidine nucleosides display significant activity against HBV.[9]

The introduction of a fluorine atom instead of hydrogen into the sugar moiety of nucleosides causes only a minor change in the size of the molecule but strongly influences the *S*/*N*-conformational equilibrium of the pentofuranose ring in solution. Because of the anomeric effect,[10,11] the equilibrium is shifted to one side or another depending on the position and stereochemistry of a fluorine substituent.[12−16] Pyrazolo[3,4-*d*]pyrimidine nucleobases, as mimics of purines, also affect the sugar conformation of nu-

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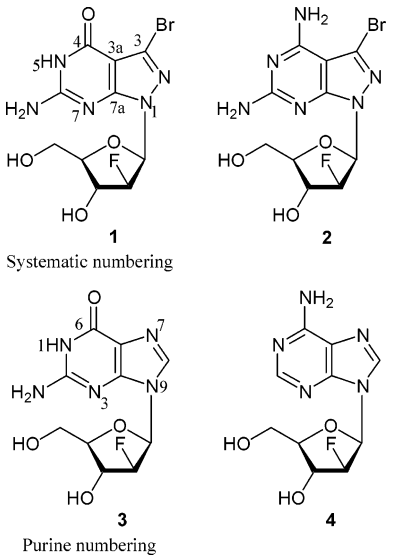
10.1021/jo030051p CCC: $25.00
Published on Web 06/07/2003

SCHEME 1



cleosides. This type of a nucleobase, as found in the case of 8-aza-7-deaza-guanosine and related analogues, also impairs quartet formation when incorporated into oligonucleotide chains.[17] Continuing our studies on the conformation of the pyrazolo[3,4-*d*]pyrimidine nucleosides,[18−20] the present work describes the synthesis and the conformational properties of the 2′-deoxy-2′-fluoro-β-D-arabinofuranosyl nucleosides **1** and **2**. Their properties will be compared with the structurally related 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)guanine ($^{2'F}$Guo, **3**) and -adenine ($^{2'F}$Ado, **4**) (Scheme 1).[21]

Results and Discussion

1. Synthesis. Different routes have been developed to synthesize 2′-deoxy-2′-fluoro-β-D-arabinofuranosyl nucleosides of the natural purines and their analogues (see recent reviews[22,23]). Most of these studies have dealt with the condensation of purine bases[24−27] and related analogues[7,9] with 3,5-di-*O*-acyl-2-deoxy-2-fluoro-D-arabinofuranosyl bromide (convergent approach). However, these glycosylation reactions suffer from low efficiency of the glycosylation step with regard to yield and stereoselectivity. An alternative synthetic approach comprises the nucleophilic displacement of an activated 2′-hydroxyl group of a β-D-ribofuranosyl nucleoside by the fluorine anion proceeding with Walden inversion.[23] However, the lengthy preparation of blocked ribonucleosides and the dependence of the reaction course on the nucleobase structure are the main drawbacks of this approach.

Previous studies[9] on the glycosylation of 4-methoxypyrazolo[3,4-*d*]pyrimidine with 3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranosyl bromide (**5**) used sodium hydride [28] or DBU [29] for the generation of the nucleobase anion. In the first case, the condensation gave rather low overall yields and an unfavorable distribution of the isomers. In the second case, the formation of a mixture of the α-D- and β-D-anomers of N^1- and N^2-glycosides (94%, overall yield) was observed, in which the desired N^1-β-D-anomer was abundant (45%). The unsatisfactory stereochemical outcome of these reactions prompted us to employ another inorganic base for the nucleobase anion glycosylation.[30] Thus, the condensation of 3-bromo-4-isopropoxy-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (**6**)[31] was performed with the halogenose **5**[32] in MeCN in the presence of powdered KOH and TDA-1 for 20 min at rt (Scheme 2). This reaction resulted in the formation of the desired N^1-β-D-nucleoside **7** (60%) along with the N^2-β-D-regioisomer **9** (ca. 9%) and a trace amount of the 3′-debenzoylated derivative **8** (ca. 2%). Note that the formation of an N^2-isomeric glycoside was not accompanied by the dehalogenation, which is different from the closely related glycosylation of **6**.[31] In one experiment, conducted for 40 min at rt, the formation of the partially debenzoylated nucleoside **8** (21%) together with the aforementioned nucleosides **7** (25%) and **9** (4%) was observed, and the compounds were separated by flash-chromatography.

The stereoselective formation of the β-D-nucleosides described above corresponds to a protocol developed for the synthesis of purine or modified purine β-D-2′-deoxyribonucleosides developed in our laboratory in 1983.[30] In this case, a stereochemically pure 2-deoxy-α-D-ribofuranosyl chloride reacts with a highly reactive nucleobase anion to yield a 2′-deoxy-β-D-ribonucleoside, exclusively.[30] As the α-D-configuration of the deoxyfluoro sugar bromide **5** was already established in 1988,[32] it is likely that the stereochemical control of 2′-fluoro nucleoside formation follows the same mechanism as that of the 2′-deoxyribonucleoside synthesis. Although this reaction proceeds under Walden inversion, the formation of an oxonium ion has to be considered as an intermediate that

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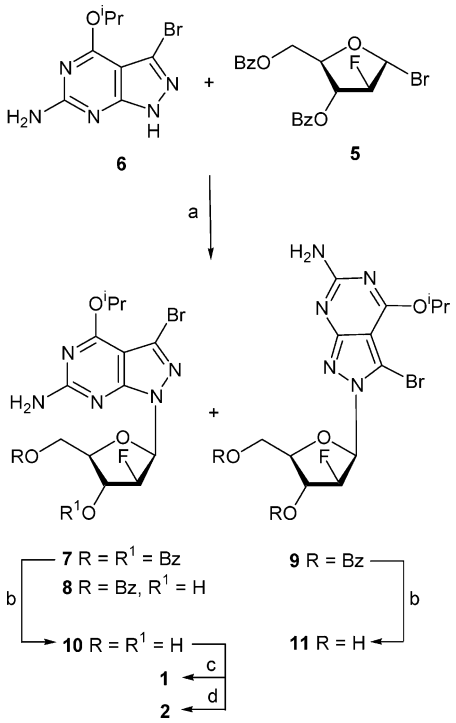
SCHEME 2[a]



[a] Reagents and conditions: (a) **6** + KOH, MeCN, rt, 15 min, + TDA-1, rt, 15 min, + **5**/MeCN, rt, (1) 20 min, **7**, 60%; **8**, 2%; **9**, 9%; (2) 40 min, **7**, 25%; **8**, 21%; **9**, 4.2%; (b) 0.1 M *i*-PrONa/*i*-PrOH, rt, 30 min (**10**, 81%; **11**, 76%); (c) 2 N aq NaOH, 50−70 °C, 3 h (**1**, 85%); (d) aq 25% ammonia, 60 °C, 2 weeks (**2**, 78%).

is preferentially attacked by the nitrogen nucleophile from the β-D face (kinetic control). The protected nucleosides **7** and **9** were debenzoylated with 0.1 N *i*-PrONa in *i*-PrOH to afford the corresponding nucleosides **10** and **11**. Compound **10** was then converted to the guanine analogue **1** by the treatment with 2 N NaOH or to the purin-2,6-diamine analogue **2** by ammonolysis in 25% aqueous ammonia solution.

The structure of all synthesized compounds was proved by ^{1}H and ^{13}C NMR spectroscopy (Tables 1 and 2). An assignment of the carbon resonances of the bases in nucleosides **1**, **2**, and **7**−**11** was made according to a previous publication.[31] We were surprised to find out that the fluorine resonances in the ^{19}F NMR spectra of the aforementioned compounds are split into a doublet of a doublet (geminal H2′/F and vicinal H3′/F couplings) and not into a doublet of two doublets as a result of an additional vicinal H1′/F coupling. It means that there is only one vicinal H/F coupling as distinct from the closely related nucleosides **3** and **4**. It implies that the conformation of the pentofuranose ring of pyrazolo[3,4-*d*]pyrimidine nucleosides differs substantially from that of **3** and **4**.[21] Moreover, rather essential differences have also been observed in the ^{13}C NMR spectra with regard to the $^3J_{C4',F}$ coupling constants of nucleosides under investigation vs the same couplings previously measured for **3** and **4**.[21] The differences in the pentofuranose ring coupling constants ($^3J_{H,H}$, $^3J_{H,F}$, and $^3J_{C4',F}$) prompted us to study the structural and stereochemical peculiarities in more detail.

2. Conformation in the Solid State. The single-crystal X-ray analysis of compound **1** confirms the N^1-β-D-configuration of the molecule[33] (Figure 1). Furthermore, the crystal structure is characterized by the anti orientation about the glycosylic bond with the O4′−C1′-N1−C7a torsion angle of $\chi = 108.4°$ and the *N* conformation of the pentofuranose ring [the phase angle of pseudorotation: $P = 347.3°$ ($E_2 \rightleftarrows {}^3T_2$) and the puckering amplitude: $\psi_m = 34.4°$]. Taking into account the similarity of the $^3J_{H,H}$ and $^3J_{H,F}$, and the $^3J_{C4',F}$ couplings and the identity of the protocol one can suggest that compounds **1**, **2**, and **7**, **8**, **10** are N^1-β-D-isomers and **9** and **11** are the N^2-β-D-regioisomers. It is noteworthy that the pentofuranose ring of 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine was found to be also in the *N* conformation in the crystal [$P = 10.0°$ ($^3T_2 \rightleftarrows {}^3E$) and $\psi_m = 38.2°$].[12] On the contrary, the sugar moiety of 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-fluorouracil in the solid-state occupies the *E* (east) part of the pseudorotation cycle [$P = 101.6°$ ($^0T_1$); $\psi_m = 43.2°$],[34] whereas that of 9-(2-deoxy-2-fluoro-β-L-arabinofuranosyl)purine is between the $_2T^1$ and E_2 state ($P = 330.5°$; $\psi_m = 40.2°$).[35] These data demonstrate a rather big diversity of the 2-deoxy-2-fluoro-β-D-arabinofuranosyl ring conformations formed in the crystalline state. Obviously, the strong crystal packing forces do not allow the molecule to adopt the most favored sugar conformation which is formed in solution.

3. Conformation in Solution. The conformational analysis of the furanose puckering of nucleosides **1**, **2**, **10**, and **11** was then performed in solution using the PSEUROT program (version 6.3).[36] This program calculates the best fits of three $^3J_{H,H}$ and two $^3J_{H,F}$ experimental coupling constants to the five conformational parameters (*P* and ψ_m for both the *N*- and *S*-type conformers and the corresponding mole fractions). Chattopadhyaya and co-workers have recently developed a new seven-parameter Karplus-type relation between vicinal proton-fluorine coupling constants and the corresponding H−C−C−F torsion angles.[14] This relation correlates $^3J_{H,F}$ with the H−C−C−F torsion angle employing the correction terms for both the substituents electronegativity and for the H−C−C and F−C−C bond angles. The values of $a_{FC2'C1'} = 112.8°$, $a_{FC2'C3'} = 108.9°$, $a_{C2'C1'H} = 113.6°$, and $a_{C2'C3'H} = 109.9°$ have been taken from[14] for 1-(2,3-dideoxy-2-fluoro-β-D-arabinofuranosyl)uracil and used in all calculations included in Table 3.

It has been previously shown that compounds **3** and **4** are in the $N \leftrightarrows S$ pseudorotational equilibrium in solution showing a ratio of ca. 40:60 (Table 3).[21] A priori, one might expect that the nucleosides **1**, **2**, **10**, and **11** will also exist in solution in a mixture of the *N* and *S* pseudorotamers. Contrary to this expectation, we found that the compounds described above are almost exclusively in the *N*-conformation (98−100%, Table 3). Previously, it has been noticed that the $N \leftrightarrows S$ pseudorotational equilibrium of the sugar moieties of nucleosides

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TABLE 1. ^{1}H NMR Data for Nucleosides 1, 2, 7−11[a]

	chemical shifts, δ_{TMS}, ppm					coupling constants, Hz ^3J(H,H)			^3J(H,F)		
compd	H-1′	H-2′	H-3′	H-4′	H-5′/ H-5″	1′,2′	2′,3′	3′,4′	1′,F	3′,F	others
1	6.25 d	5.33 dt	4.56 m	3.74 m	3.63 m	6.30	7.5	7.2	<1.0	18.4	10.85 (s, 1H, N*H*), 6.90 (br.s, 2H, NH_2), 5.86 (d, 1H, ^3J = 5.58 Hz; 3′-O*H*), 4.83 (“t“, 1H, ^3J = 5.48 Hz; 5′-O*H*)
2	6.31 d	5.31 dt	4.58 m	3.73 m	3.63 m	5.98	8.0	7.5	<1.0	18.50	6.83 (br signal, 1H, N*H*), 6.45 (s, 3H, XNH_2 and Y-N*H*), 5.84 (d, 1H, ^3J = 5.50 Hz, 3′-O*H*), 4.83 (“t“, 1H, ^3J = 5.61 Hz, 5′-O*H*)
7	6.58 d	6.02 dt	6.29 m	4.61 m	4.54 m	6.60	≈7.0	≈7.0	<1.0	16.96	7.14 (s, 2H, NH_2), 5.45 (m, 1H, ^3J = 6.15 Hz, Me_2C*H*), 1.36 [d, 6H, (CH_3)$_2$CH]
8	6.42 d	5.46 dt	4.88 m	4.57 m	4.44 m	6.68	≈7.3	n.d.	<1.0	17.60	7.08 (s, 2H, NH_2), 6.07 (d, 1H, ^3J = 5.37 Hz, 3′-O*H*), 5.44 (m, 1H, ^3J = 6.18 Hz; Me_2C*H*), 1.35 [d, 6H, (CH_3)$_2$CH]
9	6.64 d	6.03 dt	6.43 dt	4.70 m	4.63 m	6.57	7.0	7.5	<1.0	16.47	6.75 (s, 2H, NH_2), 5.46 (m, 1H, ^3J = 6.19 Hz, Me_2C*H*), 1.37 [d, 6H, (CH_3)$_2$CH]
10	6.35 d	5.34 dt	4.58 m	3.74 m	3.63 m	6.28	7.53	7.0	<1.0	18.36	7.06 (s, 2H, NH_2), 5.85 (d, 1H, ^3J = 5.30 Hz, 3′-O*H*), 5.44 (m, 1H, ^3J = 6.19 Hz; Me_2C*H*), 4.81 (“t”, 1H, ^3J = 5.38 Hz, 5′-O*H*), 1.35 [d, 6H, (CH_3)$_2$CH]
11	6.40 d	5.39 dt	4.73 m	3.83 m	3.76 br.t	6.60	7.5	7.0	<1.0	18.83	6.66 (s, 2H, NH_2), 5.92 (d, 1H, ^3J = 5.65 Hz, 3′-O*H*), 5.42 (m, 1H, ^3J = 6.23 Hz; Me_2C*H*), 4.81 (“t”, 1H, ^3J = 5.50 Hz; 5′-O*H*), 1.36 [d, 6H, (CH_3)$_2$CH]

[a] The spectra have been measured in DMSO-d_6.

TABLE 2. ^{13}C NMR Data of Modified Nucleosides

	chemical shifts, δ_{TMS}, ppm											
	base[a,b]					sugar					others	
compd	C-2 (C-6)	C-4 (C-7a)	C-6 (C-4)	C-5 (C-3a)	C-8 (C-3)	C-1′ ($^2J_{F,C}$)	C-2′ ($^1J_{F,C}$)	C-3′ ($^2J_{F,C}$)	C4′ ($^3J_{F,C}$)	C5′	*C*H (CH_3)$_2$	CH (*C*H_3)$_2$
1	156.33	157.61	157.86	98.91	123.52	80.75 (17.86)	95.55 (196.63)	72.82 (19.94)	83.14 (10.76)	63.43		
2	157.38	158.30	162.81	93.93	119.83	79.52 (18.30)	94.71 (196.00)	72.04 (19.88)	82.04 (10.38)	62.53		
7	162.73	159.36	162.60	95.74	120.96	79.73 (17.67)	91.73 (198.89)	74.55 (21.70)	76.23 (9.75)	64.15	69.41	21.61
8	162.59	159.20	162.52	95.64	120.37	79.46 (18.05)	≈92 (≈200)	71.37 (19.69)	77.9 (≈10)	64.07	69.29	21.62
9	163.75	162.87	161.88	98.93	109.01	83.65 (17.99)	92.14 (201.09)	74.37 (22.33)	77.37 (9.50)	64.64	69.38	21.57
10	162.55	159.14	162.50	95.63	119.95	79.84 (17.93)	94.71 (196.00)	72.03 (19.81)	82.15 (10.69)	62.52	69.28	21.62
11	163.67	162.45	161.79	98.56	108.30	83.23 (18.23)	95.22 (197.17)	70.92 (20.19)	83.80 (11.20)	61.96	69.32	21.60

[a] Purine numbering and, in parentheses, systematic numbering of the base. [b] The assignments of the carbon resonances of pyrazolo[3,4-*d*]pyrimidine bases are made according to ref 31; tentative assignments are displayed in italic.

is driven by the various stereoelectronic gauche and anomeric effects. The anomeric effect depends on the electronic nature of the heterocyclic base and drives the $N \leftrightarrows S$ pseudorotational equilibrium of the sugar moieties of β-D-nucleosides toward the *N*-type conformers.[10,11,37−41] Furthermore, it was observed that the higher electron-attracting properties of the base, the more the $N \leftrightarrows S$ pseudorotational equilibrium is biased toward the *N*-conformation.[19,20] The $N \leftrightarrows S$ equilibrium in the case of pyrazolo[3,4-*d*]pyrimidine 2′-deoxynucleoside analogues of dA and dG shows an *N*-conformer population of 37−36% while those of 2′-deoxyadenosine (2′dAdo) and -guanosine (2′dGuo) is ca. 30%.[19,20] It was suggested that these differences are connected with the higher strength of the anomeric effect of pyrazolo[3,4-*d*]pyrimidine bases compared to that of purines or the pyrrolo[2,3-*d*]pyrimidines (7-deazapurines). An introduction of a fluorine atom at C2′ of 2′dAdo and 2′dGuo in the *ara* “up” configuration results in a bias toward the *N* conformation to 57−58% (Table 3).[21] This effect has been discussed in terms of the gauche orientation of the electronegative substituents within the pentofuranose ring, and the gauche effect of the ring oxygen and a fluorine atom at C2′ was considered as the predominant factor that governs the overall sugar conformation.[13,14,21] Nevertheless, the contribution of the electronic structure of the heterocyclic base[18−20,37] together with electronegative substituents of the sugar

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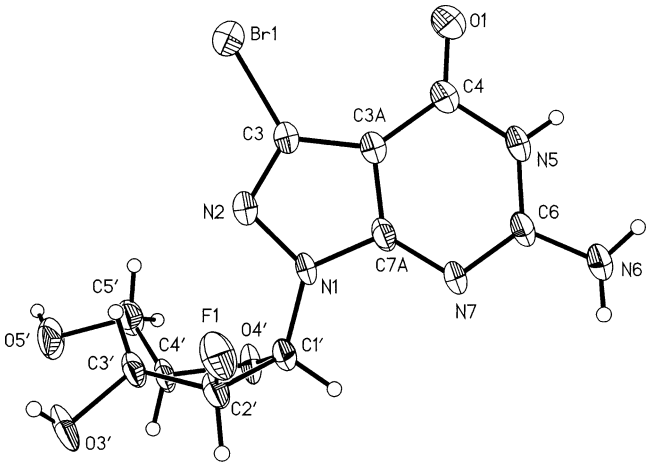

FIGURE 1. Crystal structure of compound **1**: the anti conformation of the base about the glycosyl bond (torsion angle χ (O4′−C1′−N1−C7a) is 108.0°], the N conformation of the pentofuranose ring (P = 347.3°, ψ_{max} = 34.4°). The complete data of the structure will be published elsewhere.[33]

TABLE 3. Pseudorotational Parameters of Nucleosides 1−4, 10, and 11[a]

compd	P_N	$\psi_{m(N)}$	P_S	$\psi_{m(S)}$	rms	$\lvert\Delta J_{max}\rvert$	% N
1 $^3T_2$	−2.1	36.7	108.0[b]	42.0[b]	0.554	2.18	98
2	−2.1	38.0	126.0[b]	42.0[b]	0.790	3.06	100
3[c] $_4E \leftrightarrows {}_4T^0$	65.8	43[b]	172.8	43[b]	0.160	0.48	57
4[c]	70.3	43[b]	167.7	43[b]	0.181	0.89	58
10	−4.6	36.3	108.0[b]	42.0[b]	0.579	2.38	98
11	−2.8	35.3	108.0[b]	42.0[b]	0.493	2.05	10

[a] The coupling constants of the pentofuranose ring of compounds **1**, **2**, **10**, and **11**, $^3J_{H,H}$ and $^3J_{H,F}$, were not simulated; the bond angles values are: $a_{FC2'C1'}$ = 112.8, $a_{FC2'C3'}$ = 108.9, $a_{C2'C1'H}$ = 113.6, and $a_{C2'C3'H}$ = 109.9 which have been taken from[14] for 1-(2,3-dideoxy-2-fluoro-β-D-arabinofuranosyl)uracil and used in all calculations. [b] The values indicated were fixed during the final calculations. [c] The data for the 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenine ($^{2'F}$Ado; **4**) and -guanine ($^{2'F}$Guo; **3**) are calculated using the aforementioned values of the *a* angles and are included for comparison.

moiety [37] attract attention. The stereochemical peculiarities of nucleosides described in the present communication manifest the crucial importance of the electronic structure of the pyrazolo[3,4-*d*]pyrimidine base within an interplay of the anomeric and gauche interactions giving rise to the "rigid" pentofuranose structure. Such "rigid" sugar N-conformations have been also realized with bicyclic sugar ring systems in LNA[42] and oxetane-constrained nucleosides and oligonucleotides.[43]

The values of $^3J_{F,C4'}$ spin-couplings supply an additional information on the dominating *N*-type furanose ring conformation of compounds **1**, **2**, **10**, and **11**. Indeed, the trans anti-periplanar arrangement of a fluorine atom and the C4′ of the furanose ring is realized in the *N* conformation and the $^3J_{F,C4'}$ of 10.38−11.20 Hz unambiguously reflects such a conformation (Table 1). The most striking finding on the nucleosides **1**, **2**, **10**, and **11** is the existence of an exclusive *N*-conformation ($^3T_2$; C3′-*endo*-C2′-*exo*) of the furanose rings in solution. The calculated P_N value of compound **1** is 358° identical to the data obtained from the X-ray analysis, which is not always the case. Nevertheless, the furanose ring of compound **1** has a very similar *N* conformation in the solid state and in solution. Taking these considerations into account, it can be concluded that not only the fluorine substituent of the sugar moiety, but also the nucleobase plays an important role directing the sugar moiety of the nucleoside **1** into the *N* conformation in solution. Preliminary molecular modeling studies on compound **1** using the HyperChem package (version 7.0) support this observation (see the Supporting Information).

Conclusion

It was shown that the nucleobase anion glycosylation[30] of 3-halogeno-4-isopropoxy-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine with the fluoro sugar bromide **5** proceeds in a stereoselective way with almost exclusive formation of the β-D-anomers leading to the fluoro purine nucleosides **1** and **2**. Regarding the canonical DNA constituents dA and dG, a fluoro substituent in the 2′-up position drives the sugar conformation toward a higher N-population (98−100% for 1 and 2; 57−58% in the case of **3** and **4)**. The combination of the C3-halogenated 1*H*-pyrazolo[3,4-*d*]pyrimidine with the 2′-fluorine "up" substituent as in nucleosides **1** and **2** leads to a "locked" sugar *N*-conformation (almost 100% N). This "locked" N-conformation mimics the sugar backbone units of RNA. In these cases the formation of a "DNA" /RNA heteroduplex will be favored. Currently, we are studying the biochemical and biophysical consequences of the incorporation of the nucleosides **1** and **2** into nucleic acids.

Experimental Section

General Methods. 1,3,5-Tri-*O*-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranose was a commercial product. The solvents were distilled from technical grade for use. Pyridine was refluxed with CaH_2 for 6 h before distillation. TLC was performed on aluminum sheets coated with silica gel 60 F_{254}, (0.2 mm, Merck, Germany) and flash chromatography (FC) was performed on silica gel 60 H with 0.4 bar. For NMR spectra, δ values are in parts per million downfield from the chemical shift of internal $SiMe_4$ (^{1}H, ^{13}C) or external $CFCl_3$ (^{19}F). They are measured in DMSO-d_6 at 250.13 MHz for ^{1}H, 62.89 MHz for ^{13}C, and 235.36 MHz for ^{19}F. *J* values are reported in Hz. Microanalyses were performed by Mikroanalytisches Labor Beller (Göttingen, Germany).

1-Bromo-2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-α-D-arabinofuranose (5).[32] 1,3,5-Tri-*O*-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranose (1.0 g, 2.15 mmol) was dissolved in CH_2Cl_2 (5 mL), 30% solution of HBr in acetic acid (1.2 mL) was added, and the reaction mixture was stirred at rt for 16 h and evaporated to dryness. The oily residue was redissolved in CH_2Cl_2 (20 mL), washed with water (5 mL) and then with an aqueous saturated $NaHCO_3$ solution (5 mL), dried, and concentrated to a viscous syrup and further dried under high vacuum for 18 h at rt. The colorless syrup **5** was used in the next step without purification.

Glycosylation of 3-Bromo-4-isopropoxy-1*H*-pyrazolo-[3,4-*d*]pyrimidin-6-amine (6) with the Bromide 5. A. Compound **6** (0.53 g, 1.95 mmol) and KOH (0.54 g, 9.6 mmol) were suspended in MeCN (40 mL) and stirred for 15 min at rt. Then, the phase-transfer catalyst TDA-1 (0.15 mL) was added, and stirring was continued for another 15 min. The

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solution of bromide **5** in MeCN (5 mL) was added in five portions to the reaction mixture during 10 min, and it was stirred for another 10 min and filtered. The filtrate was evaporated to dryness, and the residue was applied to FC [silica gel, column 4 × 15 cm, elution with petroleum ether/EtOAc stepwise gradient, 20:1 (100 mL), 15:1 (200 mL), 10:1 (200 mL) and 5:1 (200 mL)]. The fractions containing individual compounds were combined and evaporated to give three compounds in the order of their elution.

3-Bromo-1-(2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-β-D-arabinofuranosyl)-4-isopropoxy-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (7). Colorless solid (0.72 g, 60%). TLC (silica gel, petroleum ether/ethyl acetate, 2:1): R_f 0.78. UV (MeOH): λ_{max} 275 (10 100); 230 (55 300). ^{19}F NMR: −204.24 (dd, $^2J_{F,H2'}$ = 54.1, $^3J_{F,H3'}$ = 16.5). Anal. Calcd for $C_{27}H_{25}BrFN_5O_6$ (614.4): C, 52.78; H, 4.10; N, 11.40. Found: C, 53.11; H, 4.20; N, 11.39.

3-Bromo-2-(2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-β-D-arabinofuranosyl)-4-isopropoxy-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (9). Colorless solid (107 mg, 9%). TLC (silica gel, petroleum ether/ethyl acetate, 2:1): R_f 0.44. ^{19}F NMR: −205.87 (dd, $^2J_{F,H2'}$ = 51.8, $^3J_{F,H3'}$ = 16.5). Anal. Calcd for $C_{27}H_{25}BrFN_5O_6$ (614.4): C, 52.77; H, 4.07; N, 11.40. Found: C, 53.01; H, 4.03; N, 11.52.

3-Bromo-1-(5-*O*-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-4-isopropoxy-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (8). Colorless solid (15 mg, 2%). TLC (silica gel, petroleum ether/ethyl acetate, 2:1): R_f 0.38. UV (MeOH): λ_{max} 275 (10 700); 230 (52 000). ^{19}F NMR: −205.72 (dd, $^2J_{F,H2'}$ = 53.0, $^3J_{F,H3'}$ = 17.6). Anal. Calcd for $C_{20}H_{21}BrFN_5O_5$ (510.3): C, 47.07; H, 4.15; N, 13.72. Found: C, 47.31; H, 4.05; N, 13.27.

B. Compound **6** (1.2 g, 4.4 mmol) and KOH (1.2 g, 21.4 mmol) were suspended in MeCN (100 mL) and stirred for 15 min. Then, the phase-transfer catalyst TDA-1 (0.2 mL) was added, and stirring was continued for another 15 min. The solution of bromide **5** [prepared from 1,3,5-tri-*O*-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranose (2.0 g, 4.31 mmol) as described above] in MeCN (5 mL) was added in five portions to the reaction mixture during 10 min, and the mixture was stirred for another 30 min. Workup and chromatographic purification as described in **A** gave compounds **7** (0.65 g, 25%), **9** (0.11 g, 4%) and **8** (0.46 g, 21%).

3-Bromo-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-4-isopropoxy-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (10). Compound **7** (300 mg, 0.49 mmol) was suspended in *i*-PrONa/*i*-PrOH (0.1 M, 20 mL) and stirred at rt for 30 min. The reaction mixture was neutralized with acetic acid, silica gel (10 g) was added to the solution, and the mixture was evaporated to dryness. The products, adsorbed to silica gel, were applied on the top of a column [silica gel, column 3.2 × 8 cm; elution with CH_2Cl_2/MeOH, 20:1 (200 mL), 15:1 (300 mL) and 10:1 (200 mL)], the fractions, containing the desired material, were collected and evaporated to dryness to give compound **10** as a colorless solid (160 mg, 81%). TLC (silica gel, CH_2Cl_2/MeOH, 9:1): R_f 0.42. UV (MeOH): λ_{max} 231 (33 700); 258 (10 000); 277 (10 400). ^{19}F NMR: −204.60 (dd, $^2J_{F,H2'}$ = 54.1, $^3J_{F,H3'}$ = 18.8). Anal. Calcd for $C_{13}H_{17}BrFN_5O_4$ (406.2): C, 38.44; H, 4.22; N, 17.24. Found: C, 38.42; H, 4.31; N, 16.79.

3-Bromo-2-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-4-isopropoxy-2*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (11). Compound **9** (80 mg, 0.13 mmol) was debenzoylated as described above to give nucleoside **11** (40 mg, 76%) as a colorless solid, which was pure according to TLC (silica gel, CH_2Cl_2/MeOH 9:1): R_f = 0.21. UV (MeOH): λ_{max} 226 (28 900); 271 (10 000); 303 (6000), and 340 (1560). ^{19}F NMR: −205.95 (dd, $^2J_{F,H2'}$ = 52.95, $^3J_{F,H3'}$ = 20.0). Anal. Calcd for $C_{13}H_{17}BrFN_5O_4$ (406.2): C, 38.44; H, 4.22. Found: C, 38.39; H, 4.29.

6-Amino-3-bromo-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (1). Compound **10** (0.1 g, 0.246 mmol) was dissolved in MeOH (3 mL), aq NaOH solution (2 N, 20 mL) was added, and the reaction mixture was heated at 50−70 °C for 3 h. Progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was neutralized with acetic acid, silica gel (10 g) was added to the solution, and the suspension was evaporated to dryness. The products adsorbed to silica gel were applied on the top of a column [silica gel, column 3.2 × 5 cm; elution with CH_2Cl_2/MeOH, 20:1 (200 mL), 15:1 (400 mL) and 10:1 (400 mL)], the fractions containing the desired material were collected and evaporated to dryness to give compound **1** as a colorless solid (76 mg, 85%), which was pure according to TLC (silica gel, CH_2Cl_2/MeOH, 9:1): R_f 0.25. UV (MeOH): λ_{max} 256 (13300); 12500 at 260; ^{19}F NMR: −205.23 (dd, $^2J_{F,H2'}$ = 54.1, $^3J_{F,H3'}$ = 18.8). MS: calcd 364.0199, found 364.0. Anal. Calcd for $C_{10}H_{11}BrFN_5O_4$ (364.1), C, 32.98; H, 3.04; N, 19.23. Found: C, 33.02; H, 3.00; N, 19.50.

3-Bromo-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diamine (2). Compound **10** (0.5 g, 1.2 mmol) was heated at 60 °C for 2 weeks in a 25% aq ammonia solution (50 mL) in a tightly sealed bottle. The reaction mixture was evaporated to dryness, and the residue was crystallized from MeOH to afford compound **2** (0.35 g, 78%) as hexagonal transparent crystals. TLC (silica gel, CH_2Cl_2/MeOH, 9:1): R_f 0.29. UV (MeOH): λ_{max} 229 (27 300); 261 (9300); 277 (8700); λ_{max} 260 nm (9200). ^{19}F NMR: −204.81 (dd, $^2J_{F,H2'}$ = 54.1, $^3J_{F,H3'}$ = 18.8). Anal. Calcd for $C_{10}H_{12}BrFN_6O_3$ (363.1): C, 33.07; H, 3.33; N, 23.14. Found: C, 33.15; H, 3.25; N, 23.13.

Acknowledgment. We are indebted to Dr. K. A. Watanabe for helpful discussions and Mr. Y. He and Dr. H. Rosemeyer for the NMR measurements. We also thank Mr. H. Eickmeyer for performing the X-ray analysis.

Supporting Information Available: The Hyperchem calculation for compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO030051P