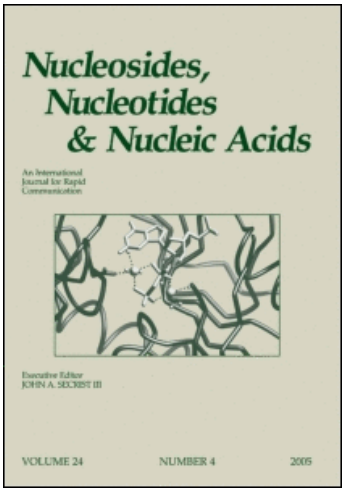

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6-AZAPYRIMIDINE AND 7-DEAZAPURINE 2′-DEOXY-2′-FLUOROARABINONUCLEOSIDES: SYNTHESIS, CONFORMATION AND PROPERTIES OF OLIGONUCLEOTIDES

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▫ *The synthesis of 2′-deoxy-2′-fluoro-β-D-arabinofuranosyl nucleosides (**1b, 2b,** and **3b**) were described and their conformation in solution as well as in the solid state was determined. In addition to this, building blocks **10a,b** and **13a,b** were prepared and employed in solid-phase oligonucleotide synthesis. For compounds **1a** and **1b** the lactime proton is protected to avoid unresolved degradation of its phosphoramidites **10a,b**. UV-melting studies have been carried out to assess the thermal stability of oligonucleotides containing compounds **1a,b,** and **3a,b**.*

Keywords Conformation, Imide Protection, Solid-Phase Synthesis

INTRODUCTION

The introduction of a fluorine substituent into a naturally occuring nucleoside strongly effects the chemical, physical, and biological properties of the molecule.[1] Among the various positions used for the fluorination, the 5-position of a pyrimidine moiety and/or the 2′-position of the sugar moiety are the most important modification sites. Recently, it was shown that the 2′-deoxy-2′-fluoro-β-D-arabinofuranosyl pyrazolo[3,4-*d*]pyrimidines display significant activity against HBV.[2] The 2′-fluorine atom strongly influences the *S/N* conformational equilibrium of the pentofuranose ring. To explore this, we synthesized the 2′-deoxy-2′-fluoro-β-D-arabinofuranosyl nucleosides **1b,2b,3b** as well as their 2′-deoxyribo nucleoside analogues **1a,2a,3a** and investigated their conformation in the solid state and in solution (Scheme 1).

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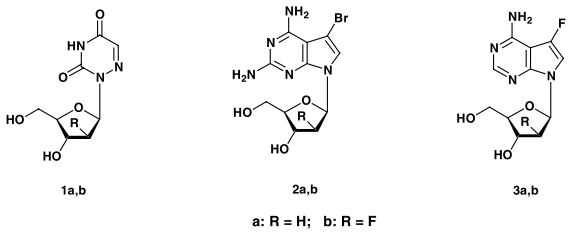


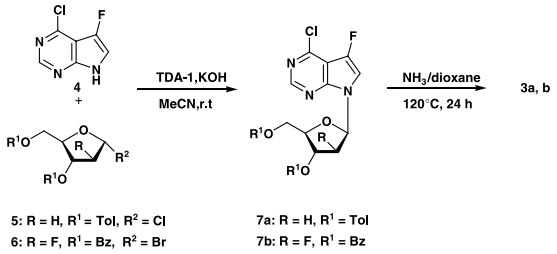
SCHEME 1

RESULTS AND DISCUSSION

The nucleosides **3a,b** were synthesized using the 5-fluoropyrrolo[2,3-d]pyrimidine base **4**[3] and the sugar components **5** or **6** by nucleobase anion glycosylation. The intermediates **7a,b** were deblocked to give pyrrolo[2,3-d]pyrimidine nucleosides **3a,b** (Scheme 2).

Single crystal X-ray analysis of compounds **1b**[4] and **3b** established an *N*-conformation. A conformational analysis of the furanose ring was performed also in solution from the ^3J(H,H) and ^3J(H,F) coupling constants of ^{1}H-NMR spectra measured in (D_2O) applying *PSEUROT* program (Version 6.3).[5] According to Table 1 the 2′-up fluorine atom shifts the sugar conformation from *S* to *N* (**2a**:29%N; **2b**:37%N[6] and **3a**:30%N; **3b**:33%N). When a nitrogen atom is located next to the glycosylation site, both the gauche effect of the 2′-fluoro substituent and the anomeric effect of the nucleobase drive the sugar conformation almost exclusively to *N*.[7] (100% N-conformation for **1b**).

In order to investigate properties of oligonucleotides containing the nucleoside residues **1a,b** and **3a,b**, the phosphoramidites **10a,b** and **13a,b** were prepared and employed in solid-phase synthesis. However, difficulties appeared upon the isolation of the phosphoramidites of **1a,b** without base protection. The presence of nitrogen at position-6 makes the lactim moiety acidic and degrades the phosphoramidite residue. To avoid this, protecting groups were introduced. Among them, the *o*-anisoyl chloride was found to be the best protecting group. Thus, compounds **8a,b** were prepared and converted via **9a,b** to **10a,b**. In a



SCHEME 2

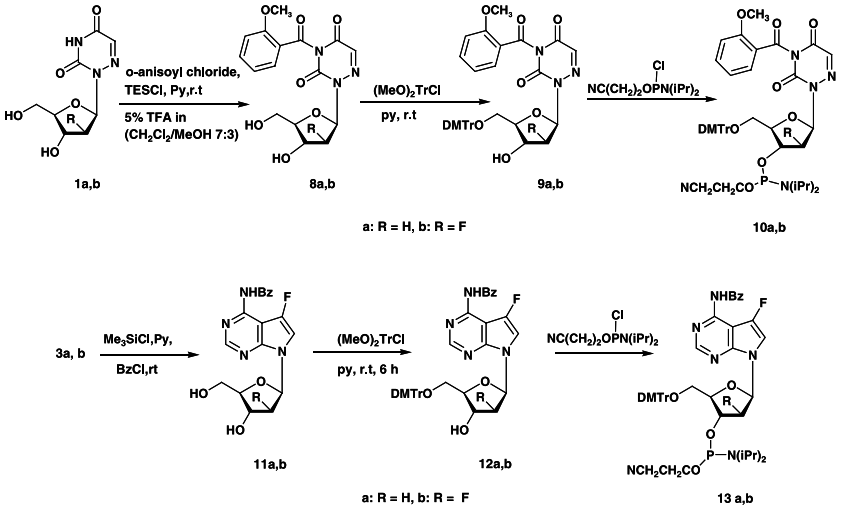
TABLE 1 Sugar Conformation of the Modified Nucleosides in Solution

	Conformation	
Compound	% N	% S
1a	58	42
1b	100	0
2a	29	71
2b	37	63
3a	30	70
3b	33	67

similar way the nucleosides **3a,b** were transformed to **13a,b** (Scheme 3). Both phosphoramidites gave high coupling yields (>99%) in oligonucleotide synthesis.

Oligonucleotides containing **1a,b** or **3a,b** were synthesized and hybridized to form the duplexes shown in Table 2. As only hybridization experiments have been performed in the DNA series it was expected that a modification with **1a,b** decreases the T_m values due to the presence of a nitrogen at the 6-position.[8] A fluorine substituent present in the 5-position as in compound **3a** stabilizes the duplex slightly, while two fluorine residues as in compound **3b** lead to the same T_m as found for the standard duplex. Studies with respect to DNA-RNA hybrids are currently performed.

The conformational analysis of a number of 2′-deoxy-2′-fluoro-β-D-arabinonucleosides in solution as well as in solid state was performed. The most striking observation is that some of the ortho-azanucleosides (**1b**) with a fluoro subtituent on the 2′-arabino position exclusively exists in an unusually rigid *N*-conformation in



SCHEME 3

TABLE 2 T_m Values, Thermodynamic Data of Oligonucleotide Duplexes Containing Regular and Base Modified Nucleosides **1a, 1b, 3a, 4b**

Duplex	T_m (°C)	ΔT_m[b]	$\Delta G^{310°}$ (kcal/mol)[c]
5′-d(AGTATTGACCTA)3′-d(TCATAACTGGAT)	50[a]	0	−12.0
5′-d(AG**1a**ATTGACC**1a**A)3′-d(TCATAACTGGAT)	42[a]	−4	−8.5
5′-d(TAGG**1a**CAA**1a**ACT)3′-d(ATCCAGTTATGA)	38[a]	−6	−7.0
5′d(AG**1b**A**1b1b**GACC**1b**A)3′-d(TCATAACTGGAT)	20[a]	−7.5	−3.7
5′-d(AGT**3a**TTG**3a**CCTA)3′-d(TCATAACTGGAT)	53[a]	1.5	−12.4
5′-d(AGT**3b**TTG**3b**CCTA)3′-d(TCATAACTGGAT)	50[a]	0	−10.9

[a]Data measured in 1 M NaCl, 100 mM MgCl2, and 60 mM Na-cacodylate buffer (pH 7.0).
[b]Per modified base pair.
[c]1 cal = 4.184 J.

solution due to the presence of a nitrogen atom located next to the glycosylation site. Both the gauche effect of the 2′-fluoro atom and the anomeric effect of the nucleobase drives the sugar moieties to adopt almost exclusively to the *N*-conformation. Additionally, the importance of the lactime protecting group (N-3) for the successful isolation of the phosphoramidites **10a,b** was described. The *o*-anisoylchloride was found to be the best protecting group.

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