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FLUORINATED 7-DEAZAPURINE 2′-DEOXYRIBONUCLEOSIDES: MODIFICATION AT THE NUCLEOBASE AND THE SUGAR MOIETY

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□ *7-Deaza-7-fluoro-purine 2′-deoxynucleosides as well as 2′-deoxy-2′-fluoroarabinofuranosyl nucleosides **1–8** were synthesized. The fluorine atom was introduced on the base level with Selectfluor. Nucleobase-anion glycosylation was then employed to form the nucleosides. Properties of the fluorine compounds were studied in solution and in solid state. Compound **4a** was incorporated into oligonucleotides where the stabilizing effect was observed.*

Keywords fluorination; pyrrolo[2,3-*d*]pyrimidine; glycosylation; sugar conformation; base pairing

INTRODUCTION

Replacement of hydrogen atoms or functional groups in an organic compound by fluorine atoms may cause a dramatic change in physical properties and biological activities, which is one of the reasons for the interest in the synthesis of fluoro compounds. Our group has expended effort to introduce the fluorine atom to nucleosides. We are interested in combining the favorable properties of pyrrolo[2,3-*d*]pyrimidine nucleosides with the well documented pharmaceutical behavior of fluorine substitution. Apart from the properties of monomeric nucleosides, 7-halogenated 7-deazapurine nucleosides can stabilize the DNA duplexes.[1–4] Herein, we report on the synthesis of nucleosides **1–3** and **6–8**, as well as the phosphoramidite **9** and **10**. The nucleosides **4a**, **b** and **5** were described recently[5] (Scheme 1). Oligonucleotides containing compound **4a** were prepared and their stability in DNA duplexes was determined.

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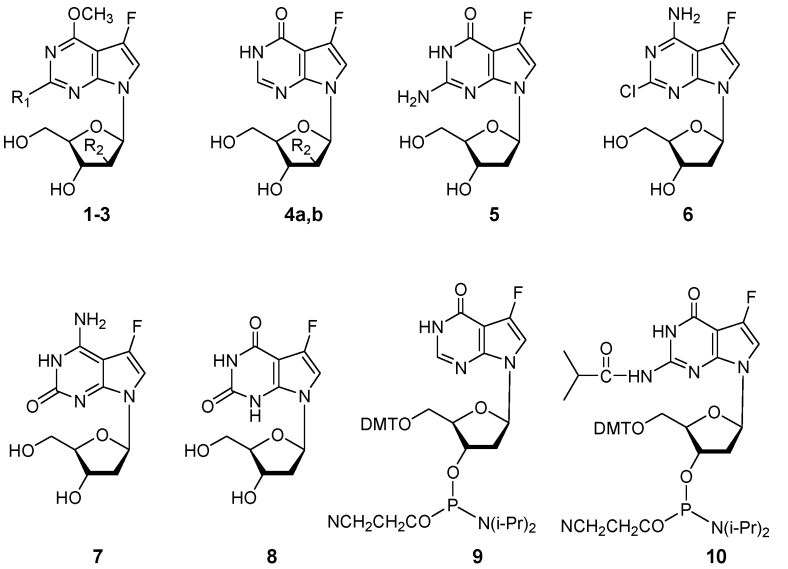


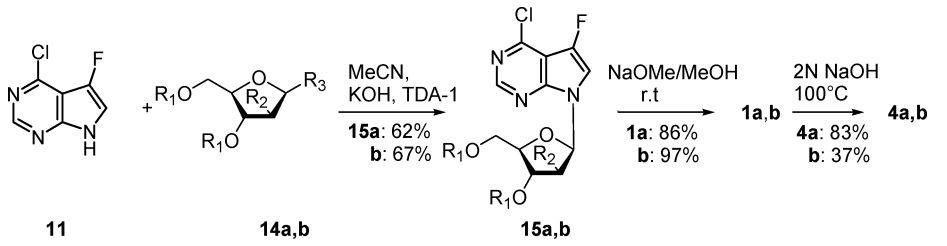
1a: R_1 = H, R_2 = H; **1b**: R_1 = H, R_2 = F; **2**: R_1 = NH_2, R_2 = H;
3: R_1 = OCH_3, R_2 = H; **4a**: R_2 = H; **4b**: R_2 = F.

SCHEME 1

RESULTS AND DISCUSSION

Earlier, a 7-fluoro residue was introduced in the nucleobase **11** employing Selectfluor.[6] The same strategy was used to synthesize the fluorinated pyrrolo[2,3-*d*]pyrimidine derivatives **12** and **13** by utilizing lower temperature conditions than used for **11**. The nucleobase-anion glycosylation reaction[7,8] of the base (**11-13**) afforded the 7-fluorinated inosine derivatives **4a**,**b**, as well as the 7-deaza-7-fluoro-2′-deoxyguanosine (**5**), 7-deaza-7-fluoro-2′-deoxyisoguanosine (**7**), and 7-deaza-7-fluoro-2′-deoxyxanthosine (**8**). Glycosylation of **11** with the halogenose **14a**,**b** gave the nucleosides **15a,b**, which were deprotected in $NaOCH_3$ to afford the 6-methoxy derivatives **1a,b**. Compounds **4a**,**b** were obtained by heating **1a,b** in aqueous NaOH (Scheme 2). Conformational analysis of the nucleosides **4a,b** showed that the fluorine substitution in the 2′-arabino position biased the conformation toward *N* (from 30% *N* for **4a** to 40% *N* for **4b**).[5]

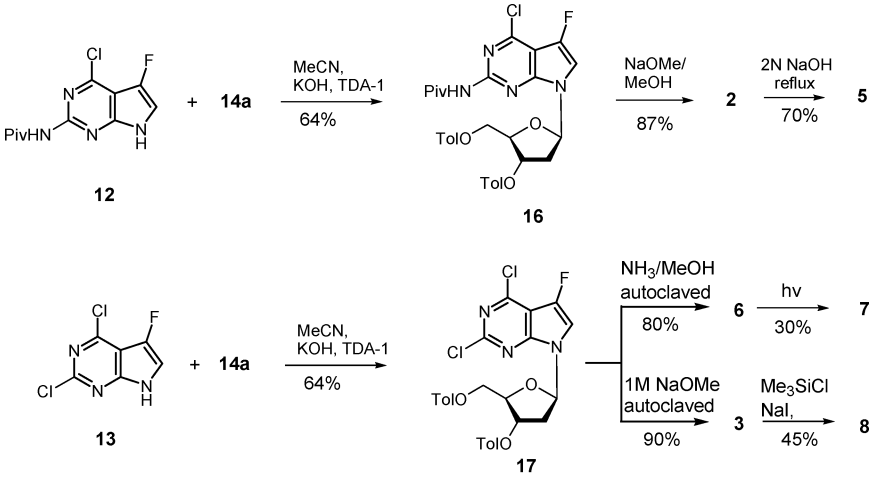
In a similar way, compounds **12** and **13** were glycosylated with **14a** to form the nucleosides **16** and **17**, which were deprotected in NaOMe/MeOH to give the intermediate methoxy derivatives **2** and **3**. Consequently, **2** and **3** were converted to 7-deaza-7-fluoro-2′-deoxyguanosine (**5**) and 7-deaza-7-fluoro-2′-deoxyxanthosine (**8**) in aqueous NaOH or with Me_3SiCl/NaI in MeCN. The isodG derivative **7** was obtained from **6** by photochemically induced displacement reaction performed in water (30% yield) (Scheme 3).



a: R_1 = Tol; R_2 = H; R_3 = Cl. **b**: R_1 = Bz; R_2 = F; R_3 = Br.

SCHEME 2

The 7-deazapurine 2′-deoxyribonucleosides **4a** and **5** were converted into the corresponding phosphoramidites **9** and **10** by standard procedures. Oligonucleotides containing compound **4a** were synthesized employing compound **9** in solid-phase oligonucleotide synthesis. As supposed to be an analog of the universal nucleoside 7-deaza-2′-deoxyinosine, compound **4a** was incorporated in the duplexes opposite to the canonical 2′-deoxynucleosides dA, dG, dC, and dT. According to Table 1, compound **4a** shows base pairing with duplex stabilities similar to 7-deaza-2′-deoxyinosine (**c**^{7}**Id**).[9] Thus, the 7-fluoro substituent has only a minor influence when only one 7-deaza-2′-deoxyinosine residue was replaced by the fluoro analog **4a**. However, a strong stabilizing effect was observed when twelve **4a** residues were incorporated in a self-complementary alternating 12-mer duplex **2323** (Table 1).



SCHEME 3

TABLE 1 T_m values and thermodynamic data of oligonucleotides containing **4a** and 7-deaza-2′-deoxyinosine (**c^{7}I**)[a,b]

Duplex	T_m	Duplex	T_m
5′-d(GGAAAA**4a**AAAAGG)-3′ (**18**) 3′-d(CCTTTT A TTTTCC)-5′ (**19**)	43	5′-d(GGAAAA**c^{7}I**AAAAGG)-3′ 3′-d(CCTTTT A TTTTCC)-5′	43[9]
5′-d(GGAAAA**4a**AAAAGG)-3′ (**18**) 3′-d(CCTTTT G TTTTCC)-5′ (**20**)	35	5′-d(GGAAAA**c^{7}I**AAAAGG)-3 3′-d(CCTTTT G TTTTCC)-5′	37[9]
5′-d(GGAAAA**4a**AAAAGG)-3′ (**18**) 3′-d(CCTTTT C TTTTCC)-5′ (**21**)	46	5′-d(GGAAAA**c^{7}I**AAAAGG)-3′ 3′-d(CCTTTT C TTTTCC)-5′	45[9]
5′-d(GGAAAA**4a**AAAAGG)-3′ (**18**) 3′-d(CCTTTT T TTTTCC)-5′ (**22**)	39	5′-d(GGAAAA**c^{7}I**AAAAGG)-3′ 3′-d(CCTTTT T TTTTCC)-5′	40[9]
5′-d(**4a**C**4a**C**4a**C**4a**C**4a**C**4a**C)-3′ (**23**) 3′-d(C**4a**C**4a**C**4a**C**4a**C**4a**C**4a**)-5′ (**23**)	29[c]	5′-d(**c^{7}I**C**c^{7}I**C**c^{7}I**C**c^{7}I**C**c^{7}I**C**c^{7}I**C)-3′ (**24**) 3′-d(C**c^{7}I**C**c^{7}I**C**c^{7}I**C**c^{7}I**C**c^{7}I**C**c^{7}I**)-5′ (**24**)	13[c]

[a]Thermodynamic parameters are derived from the fitting of melting curves measured at 260 nm in 1 M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate buffer, pH 7.0, with 5 μM + 5 μM single-strand concentration. [b] T_m values are given in °C. [c]Measured in 100 mM NaCl, 10 mM $MgCl_2$, and 10 mM Na-cacodylate buffer, pH 7.0, with 10 μM single-strand concentration.

CONCLUSION

7-deaza-7-fluoro-2′-deoxynucleosides were synthesized by nucleobase-anion glycosylation stereoselectively. A fluorine atom introduced in the 2′-arabino position biases the conformation of the sugar toward *N*. oligonucleotides containing 7-fluoro nucleosides increase the T_m value of duplex melting compared to the non-functionalized compounds (see duplex **23.23** versus **24.24**).

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