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Polynucleotides and Their Components in the Processes of Aromatic Nucleophilic Substitution: I. Chemistry and Dynamics of Nucleotide Arylation with Pentafluoropyridine; Obtaining of Synthons for Molecular Design of Nucleic Acid Analogues

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Abstract—Pentafluoropyridine reacts with thymidine, adenosine, and uridine hydroxy groups to give quantitative yields of the corresponding nucleoside di- and triaryl ethers. The nucleophilic substitution reactions proceed successively and in parallel, with the slowest step being the nucleophilic substitution of the nucleoside secondary hydroxyls. The resulting ethers contain tetrafluoropyridyl moieties, which could be smoothly modified by nucleophilic substitution of fluorine atoms. The ethers are useful intermediate synthons (both isolated and *in situ*) for molecular design of oligonucleotide analogues.

Key words: arylation of nucleosides, pentafluoropyridine, S_NAr

INTRODUCTION

The development of new approaches to modification of nucleosides, nucleotides, heterocyclic bases, and oligonucleotides is important for the solution of a number of fundamental and applied problems [1–6]. In most cases, the nucleosides are primarily functionalized by their interaction with electrophiles, whereas the subsequent transformations of the resulting electrophilic moieties are carried out by the reactions with nucleophiles. This strategy is used for the synthesis of compounds bearing reporter groups, conjugates with nucleic acids, and other hybrid molecules. However, the number of methods for introduction of electrophilic moieties into the molecules of, for example, oligonucleotides is rather limited [4].

It is obvious that biopolymers interacting with electrophiles can be regarded as specific polyfunctional reagents containing ensembles of nucleophilic targets (amino and hydroxy groups) that mutually affect each other. Such structural organization implies specific chemical properties of these compounds in comparison with the properties of monofunctional nucleophilic compounds. A comparative study of chemical properties of an isolated molecule and that in an ensemble with other molecules is among "a new set of great tasks", whose solution may be of fundamental significance [7].

One of the topical practical aspects of this problem is the development of new approaches to the selective modification of complex polyfunctional compounds of medicinal use in order to increase their efficiency.

We believe that the reaction of polyfunctional molecules with activated arenes may be a fruitful approach to their modification; the arenes could bear several nucleophilic atoms or groups, which could ensure further transformations of the resulting products in S_NAr processes. This work opens a cycle of publications, the goal of which is the study of general regularities of interaction of polyfunctional molecules (components of NAs) with activated aromatic compounds, clarification of the specificity of these processes in comparison with reactions of the corresponding mononucleophiles, and use of the results for the molecular design of more complicated molecules, e.g., constructs similar to synthetic analogues of nucleic acids. Some specific schemes of molecular design of such analogues will be presented in the next communication.

RESULTS AND DISCUSSION

We carried out the reaction of thymidine (**II**), adenosine (**IX**), and uridine (**X**) with pentafluoropyridine (**I**), which led to (**V**), (**XI**), and (**XII**) after the substitution of the first halogen atom of (**I**) (Schemes 1 and 2). Each of the products possesses at least three

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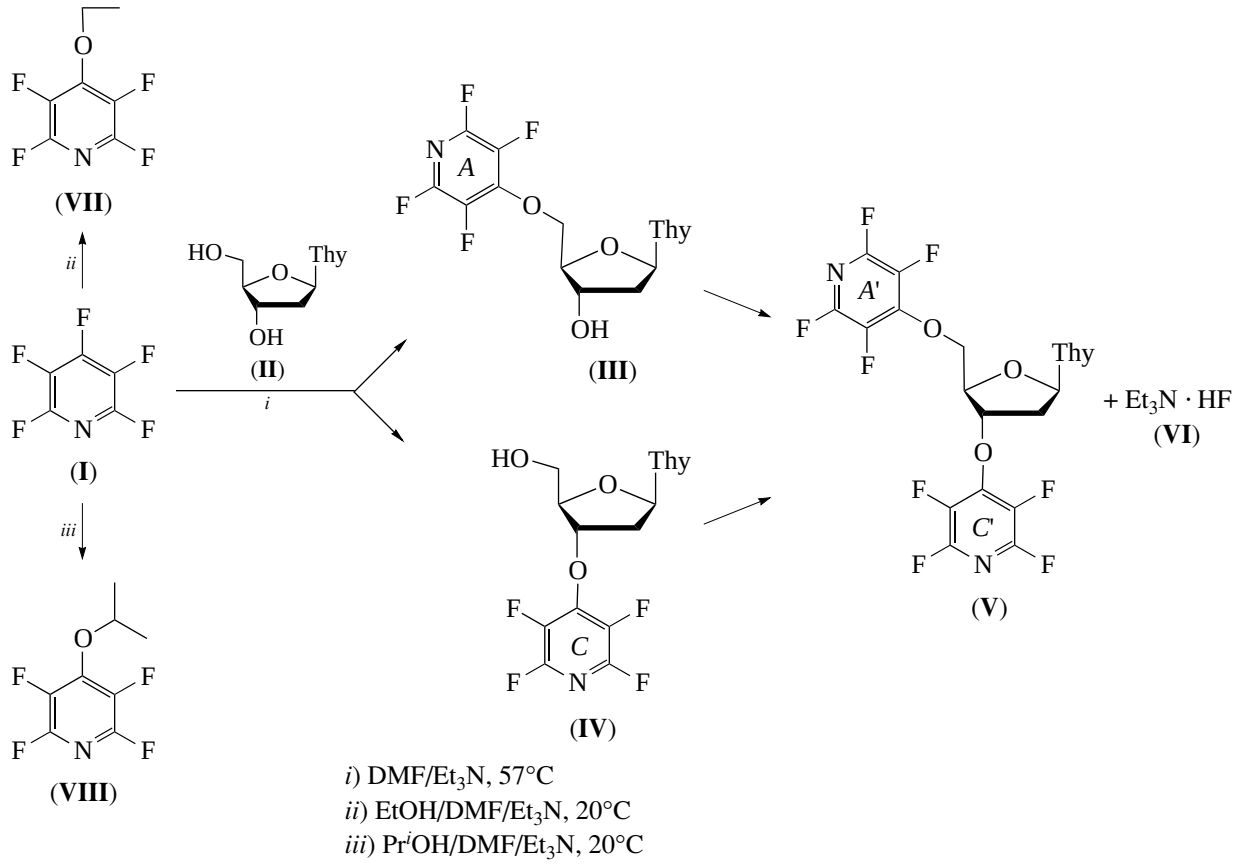
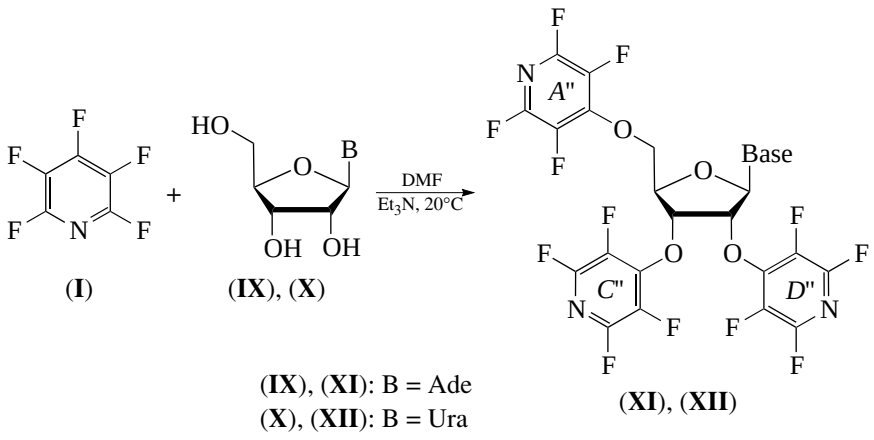


Scheme 1.



Scheme 2.

fluorine atoms capable of subsequent nucleophilic substitutions, which predetermines the possibilities of their further modifications. The dynamics of the processes shown in Scheme 1 was monitored by analyzing homogeneous reaction mixtures by ^{19}F NMR (Fig. 1).

We studied the spectra of ethers (**VII**) and (**VIII**) formed in the reactions of pentafluoropyridine with primary or secondary alcohols in order to assign the ^{19}F NMR resonances of (**III**), (**IV**), and (**V**) resulting from the arylation of primary or secondary hydroxy groups of thymidine (Scheme 1 and Fig. 2).

As follows from the fragments of spectra presented in Fig. 2, the multiplets of β,β'-fluorine atoms of (**VII**) are shifted upfield (3.86 ppm; here and further, chemical shifts are given for the centers of symmetrical multiplets) in comparison with those in isopropyl ether (**VIII**) (4.89 ppm). Similar shifts were observed for the centers of multiplet signals of the fluorine atoms in pyridine positions 2 and 6. On the basis of these data, one can assign with a high probability the low-field multiplets at 5.59 and 5.18 ppm to fluorine atoms in pyridine positions 3 and 5 of *C*' and *C* rings of (**V**) and (**IV**), respectively. Then, the higher field multiplets at 4.60 and 4.51 ppm should be assigned to fluorine atoms in positions 3 and 5 of rings *A* and *A*' attached to primary hydroxyls in (**III**) and (**V**). Another argument in favor of the proposed assignment can be the fact that the rates of formation of ether (**III**) [as well as the ethyl ether (**VII**)] were much higher than those of arylation of secondary hydroxy groups.

Therefore, the dynamics of the process can be monitored by means of direct measurement of integral intensities of the resonances of fluorine atoms in *C*' and *C* rings. The content of ether (**III**), the multiplet of which is partially overlaps with the resonances of fragment *A*' (Fig. 2), was determined by subtraction of the integral intensity of the fluorine atoms of the diether (**V**) ring *C*'.

The experimental data in Fig. 1 confirm the transformations presented in Scheme 1. As expected, the fluorine atom in position 4 of pentafluoropyridine is substituted in the course of reaction, and an exponential decrease in the intensity of resonance at 29.5 ppm (curve *1*) reflects this fact. A correlation between the decrease in the substrate amount and the accumulation of reaction products is observed at any moment of the reaction. The concentrations of ethers (**III**) and (**IV**) grow from zero to their maxima after three hours and then again fall to zero. The rate of formation of (**V**) is initially equal to zero. Then, it reaches its maximum at the highest total concentrations of ethers (**III**) and (**IV**), with the tangent of the angle of slope being 0.6, 1.2, 1.4, 2.8, 1.8, and 1.5 after 1.5, 2.15, 2.65, 3.1, 3.6, and 4.05 h, respectively. The *S*-like character of curve *4* also confirms the formation of ether (**V**) from its precursors (**III**) and (**IV**) in successive processes. The initial rate of formation of (**III**) is almost three times higher than that of (**IV**) (Fig. 1, curves *2* and *3*, respectively), which could result from both a higher ionization degree of the primary hydroxy group in the presence of triethylamine (the reaction did not proceed in the absence of the base) and lower sterical hindrances in comparison with secondary hydroxyl. On the basis of these data, one can presume that compounds of type (**III**) and (**IV**) can be obtained separately due to an increased difference between their formation rates, which could be achieved using substrates containing sterically hindered reaction centers (e.g., 3,5-dichloro-2,4,6-trifluoropyridine).

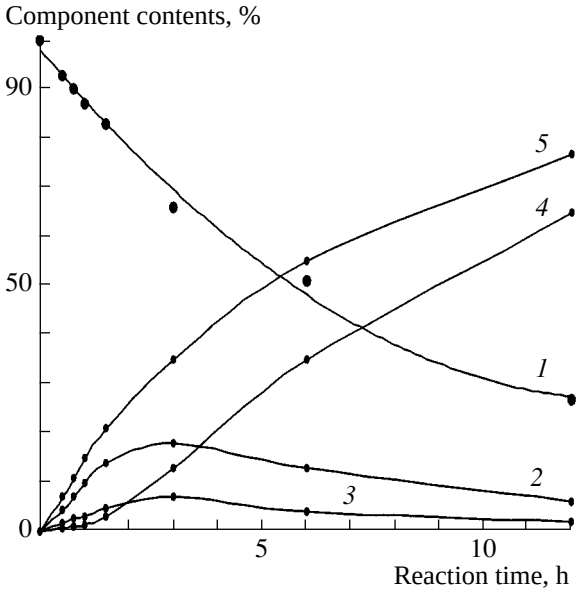


Fig. 1. The time dependences of relative contents $(I_i/I_0) \times 100$ of components in the process of thymidine arylation with pentafluoropyridine. I_i and I_0 are integral intensities of fluorine resonances of the reaction components and the initial pentafluoropyridine content, respectively. *1*, Dynamics of substrate (**I**) expenditure; *2* and *3*, accumulation and decrease of intermediates (**III**) and (**IV**), respectively; *4*, formation of diether (**V**); *5*, accumulation of triethylamine hydrofluoride (**VI**) and the total content of ethers (**III**)–(**V**).

Our data on the differences in arylaton rates of primary and secondary hydroxy groups of thymidine and mononucleophiles (ethanol and isopropanol) deserve a special discussion for elucidation of probable specific properties of polynucleophilic reagents (Scheme 1). The ratios of formation rates of ethers (**VII**) and (**VIII**) measured at the initial parts of kinetic curves and under the conditions identical to those used for the accumulation of thymidine ethers (**III**) and (**IV**) are as follows: (**III**)/(**IV**) = 3.1 (*1*), (**VII**)/(**VIII**) = 4.3 (*2*), (**III**)/(**VII**) = 2.2 (*3*), and (**IV**)/(**VIII**) = 2.5 (*4*). The first two ratios are reasonable and can be explained by a higher ionization degree of the primary hydroxy function in the presence of triethylamine and lesser steric hindrances for its arylation in comparison with the secondary hydroxyl. On the other hand, the next two ratios (*3*) and (*4*) contradict not only to the first two ratios, but also to the published data [8], according to which just a simple increase in the number of methylene groups in alcoholates should decrease the nucleophile reactivity (which is reflected in the *1/2* ratios) rather than increase the *3*/*4* ratio. Thus, the hydroxy groups of thymidine attached to a more bulky pentose backbone turn out to be more reactive than similar nucleophilic centers in simple isolated molecules. A possible explanation of the specificity of a polynucleophilic target can be the involvement of hydrogen atom of one of the hydroxy groups in the intramolecular salvation, which results in a stabilization of intermediate anionic Meisenheimer-like σ com-

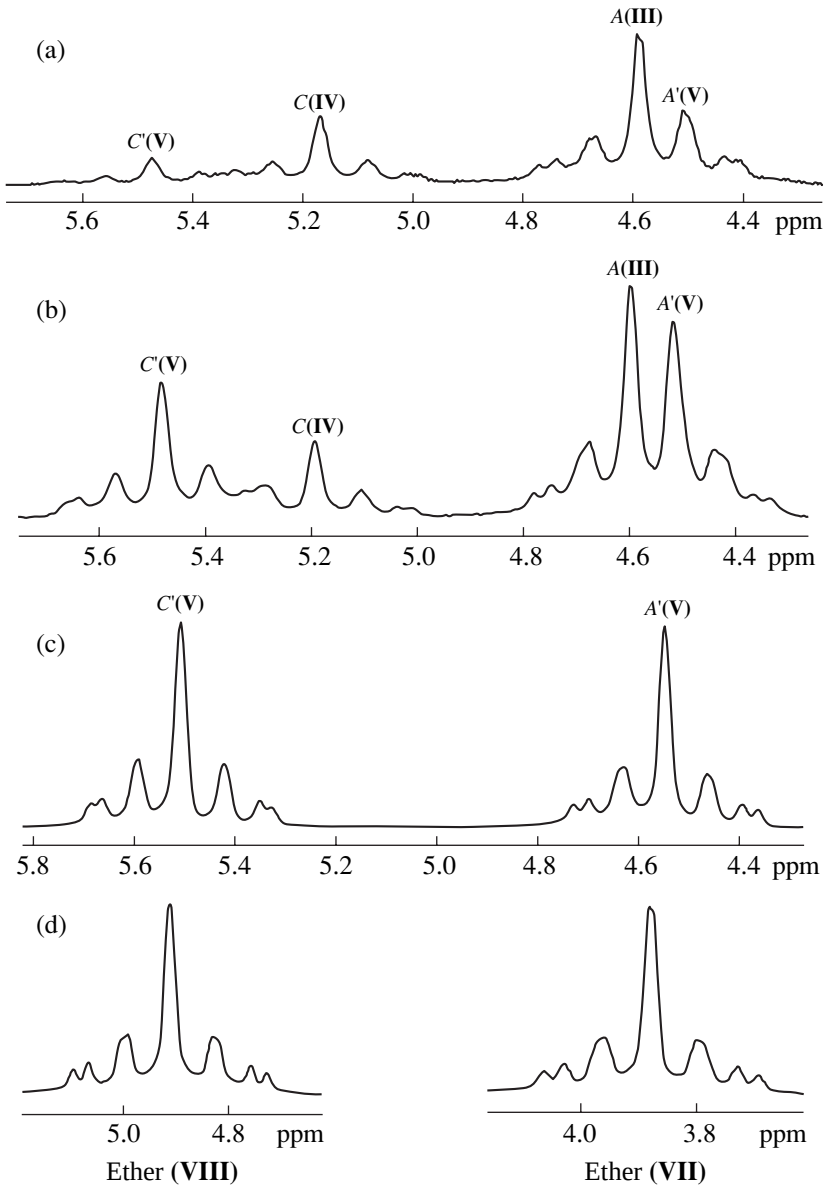


Fig. 2. Parts of ^{19}F NMR spectra of the reaction mixtures presented in Scheme 1 that correspond to β,β'-fluorine atoms of pyridyl rings *A*, *C*, *A*', and *C*' of (**III**), (**IV**), and (**V**). The spectra were registered after (a) 0.75, (b) 3, and (c, final) 16 h; (d), the parts of ^{19}F NMR spectra of the reaction mixtures corresponding to β,β"-fluorine atoms of pyridyl rings of ethers (**VII**) and (**VIII**)

plex. The formation of such an intermediate is probably a rate-limiting stage in the process under study, as it is commonly the case in the majority of reactions of activated arenes with anionic reagents. Another factor favoring the nucleoside transformation into more active reagent can be the formation of a spirocomplex with a successive participation of two hydroxyls, which is a more stable than the classical Meisenheimer complex [9].

Modification of the third hydroxy group in adenosine (**IX**) and uridine (**X**) under the same conditions (Scheme 2) requires a longer reaction time, which may result from greater steric hindrances along with the growing number of hydroxy groups and aryl residues in the pentose backbone. These hindrances can hamper both the formation of intermediates and the probable involvement of hydroxy groups in the intramolecular stabilization. As in the case of thymidine, primary hydroxyl reacts noticeably faster than the secondary hydroxyls, which clearly follows from the dynamics of ratios of integral intensities of multiplets (4.78 and 4.82 ppm) of fluorine atoms disposed in β-positions relative the ring *A*" nitrogen atom and the total intensity of overlapped downfield low-field resonances (6.72 and

6.18 ppm) of the corresponding diether fragments (*C*'' and *D*''). Like diether (**V**), triethers (**XI**) and (**XII**) were quantitatively accumulated in the reaction mixture.

According to ^{19}F NMR, diether (**V**) formed in quantitative yield, displays no visible changes for the next 54 h at 57°C (data not given), which predefines its possible use [as well the use of triethers (**XI**) and (**XII**)] as *in situ* substrates for further modifications in S_NAr-like processes. These compounds contain several mobile fluorine atoms in positions 2 and 6 of pyridine rings and can be regarded as intermediates for the molecular design of polyfunctional compounds, e.g., oligonucleotide analogues. We hope to describe such studies in future.

EXPERIMENTAL

^1H and ^{19}F NMR spectra were registered on a Bruker WP 200 SY spectrometer (Germany) at 295 K and working frequencies of 200.13 and 188.28 MHz, respectively. The values of chemical shifts (δ, ppm) were measured relative to internal standards $SiMe_4$ in $CDCl_3$ for ^1H and C_6F_6 in DMF for ^{19}F NMR. The coupling constants *J* are given in Hz. Melting points were determined on a Koefler S 30 A/G table (Germany).

Thymidine was from Yamasa Shoyu Co., Ltd (Japan), adenosine from Reanal (Hungary), and uridine (pure grade) from NPO Biokhimreaktiv (Russia). Pentafluoropyridine had no admixtures according to ^{19}F NMR spectrum. Silica gel with a particle size of 40–100 μm was used for column chromatography. Solvents were purified according to the known procedures [10] and stored over molecular sieves 4Å.

The completeness of reactions and homogeneity of the resulting products were monitored by TLC and HPLC. TLC was carried out on precoated TLC plates Kieselgel $60F_{254}$ (Merck, Germany) in (A) 10 : 1 and (B) 20 : 1 $CHCl_3$–MeOH mixtures. Analytical HPLC was carried out on a microcolumn liquid chromatograph Milikhrom-1 (Nauchpribor, Orel, Russia) using a CHROM system for collection and analysis of chromatographic data [11], a Nucleosil 100-5 C-18, 5 μm column (Macherey–Nagel, Germany) and elution for 20 min in a gradient of CH_3CN (70–90%) in 0.1% TFA at the flow rate of 100 μl/min.

3',5'-*O*-Bis(2,3,5,6-tetrafluoropyrid-4-yl)thymidine (V). Pentafluropyridine (166 mg, 0.98 mmol) and triethylamine (111 mg, 1.10 mmol) were added to a solution of thymidine (116 mg, 0.48 mmol) in DMF (0.5 ml), and the reaction mixture was kept for 16 h at 56–58°C. The completion of reaction was monitored by ^{19}F NMR according to the absence of pentafluropyridine. The reaction mixture was poured into ice water and neutralized with concentrated HCl. The resulting precipitate was filtered, washed with distilled water, and dried in air and then in vacuum of a water-jet pump over NaOH to give the product; yield 250 mg (96% from thymidine) with the purity of 91% (here and hereinafter, according to HPLC). The product with R_f 0.5 (A) was purified by column chromatography on silica gel (elution with $CHCl_3$). A portion of the product (100 mg) isolated by chromatography was recrystallized from 20% isopropanol in hexane to give (**V**) (36 mg) with purity exceeding 99%; mp 88–90°C; ^1H NMR: 1.86 (3 H, s, CH_3), 2.48–2.79 (2 H, m, H2'), 4.60 (1 H, br. s, H4'), 4.80 (2 H, br. s, H5'), 5.52 (1 H, br. s, H3'), 6.30 (1 H, apparent t, $J_{1',2'}$ 7.1, H1'), 7.17 (1 H, s, H6), and 9.59 (1 H, br. s, 3-NH); ^{19}F NMR: 4.58 [2 F, m, 5'-(β,β'-difluoropyrid-4-yl)], 5.55 [2 F, m, 3'-(β,β'-difluoropyrid-4-yl)], 70.69 [2 F, m, 5'-(α,α'-difluoropyrid-4-yl)], and 71.16 [2 F, m, 3'-(α,α'-difluoropyrid-4-yl)]. Found, %: C 44.12 and 45.31; H 2.29 and 2.42; N 10.43 and 10.25; F 28.36 and 28.59. $C_{20}H_{12}F_8N_4O_5$. Calc., %: C 44.46, H 2.24, N 10.37, F 28.13.

2',3',5'-*O*-Tris(2,3,5,6-tetrafluoropyrid-4-yl)adenosine (XI). Pentafluoropyridine (506 mg, 2.99 mmol) and triethylamine (331 mg, 3.28 mmol) were added to a solution of adenosine (250 mg, 0.94 mmol) in DMF (2 ml), and the reaction mixture was kept for 8 days at room temperature. The reaction was monitored and the product (**XI**) was isolated as described above for (**V**) with quantitative yield (810 mg) and 97% purity. After recrystallization from propanol the purity of (**XI**) exceeded 99%; mp 81–83°C; ^1H NMR: 4.78–5.00 (3 H, m, H5', H4'), 5.92 (2 H, br. s, 6-NH), 6.12 (1 H, apparent t, *J* 4.8, H3'), 6.34–6.40 (2 H, m, H1' and H2'), 7.91 (1 H, s, H8), and 8.18 (1 H, s, H2). ^{19}F NMR: 4.78 [2 F, m, 5'-(β,β'-difluoropyrid-4-yl)], 6.72 [4 F, m, 3'-(β,β'-difluoropyrid-4-yl)], 70.57 [2 F, m, 5'-(α,α'-difluoropyrid-4-yl)], and 71.51 [4 F, m, 3'-(α,α'-difluoropyrid-4-yl)]. Found, %: C 42.51 and 42.23; H 1.96 and 1.73; N 15.41; F 32.26 and 32.56. $C_{25}H_{10}F_{12}N_8O_4$. Calc., %: C 42.03, H 1.41, N 15.69, F 31.91.

2',3',5'-*O*-Tris(2,3,5,6-tetrafluoropyrid-4-yl)uridine (XII). Pentafluropyridine (554 mg, 3.28 mmol) and triethylamine (363 mg, 3.58 mmol) were added to a solution of uridine (250 mg, 1.02 mmol) in DMF (2 ml), and the reaction mixture was kept for 6 days at room temperature. The product was isolated in a yield 86% (709 mg, the purity of 86%) as described above. Purification by column chromatography on silica gel (elution with 49 : 1 $CHCl_3$–MeOH) yielded the product with R_f 0.5 (B), which was recrystallized from 7 : 3 propanol–water to give (**XII**) (315 mg) with the purity of 99%; mp 78–80°C. ^1H NMR: 4.76–5.00 (3 H, m, H5' and H4'), 5.61 (2 H, br. s, H2' and H3'), 5.77 (1 H, d, *J* 8.1, H5), 5.96 (1 H, s, H1'), 7.42 (1 H, d, *J* 8.1, H6), 9.52 (1 H, br. s, 3-NH). ^{19}F NMR: 4.82 [2 F, m, 5'-(β,β'-difluoropyrid-4-yl)], 6.18 [4 F, m, 3'-(β,β'-difluoropyrid-4-yl)], 70.64 [2 F, m, 5'-(α,α'-difluoropyrid-4-yl)], 71.52 [4 F, m, 3'-(α,α'-difluoropyrid-4-yl)]. Found, %: C 41.79 and 42.08; H 1.59 and 1.71, N 10.01, F 32.56 and 32.84. $C_{24}H_9F_{12}N_5O_6$. Calc., %: C 41.70, H 1.31, N 10.13, F 32.97.

The study of thymidine interaction with pentafluoropyridine by the ^{19}F NMR technique. Pentafluropyridine (147 mg, 0.87 mmol) and triethylamine (96 mg, 0.95 mmol) were added to a solution of thymidine (100 mg, 0.41 mmol) in DMF (0.4 ml), and the reaction mixture was kept at room temperature, while registering ^{19}F NMR spectra 0.5, 0.75, 1.0, 1.5, 3.0, 6.0, and 12 h after the beginning of the reaction. Relative contents of the products were determined according to integral intensities of resonances in the range of 1–6 ppm (Figs. 1, 2).

The study of ethanol and isopropanol interaction with pentafluoropyridine by the ^{19}F NMR technique. Triethylamine (97 mg, 0.95 mmol) and ethanol (44 mg, 0.95 mmol) or isopropanol (57 mg, 0.95 mmol) were added to a solution of pentafluropyridine (154 mg, 0.91 mmol) in DMF (0.4 ml), and the reaction mixtures were kept at room temperature, while registering ^{19}F NMR spectra 10, 20, 30, and 40 h after the beginning of the reactions. Relative contents of the products were determined according to integral intensities of resonances in the range of 1–6 ppm.

4-Ethoxy-2,3,5,6-tetrafluoropyridine (VII); ^{19}F NMR: 3.86 (2 F, m, F3 and F5), 70.41 (2 F, m, F2 and F6). The contents of (**VII**) at the above-mentioned time points were 66, 82, 87, and 91%, respectively.

4-Isopropyloxy-2,3,5,6-tetrafluoropyridine (VIII); ^{19}F NMR: 4.89 (2 F, m, F3 and F5), 70.67 (2 F, m, F2 and F6). The contents of (**VIII**) at the above-mentioned time points were 13, 24, 35, and 43%, respectively.

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