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Graphical Abstract

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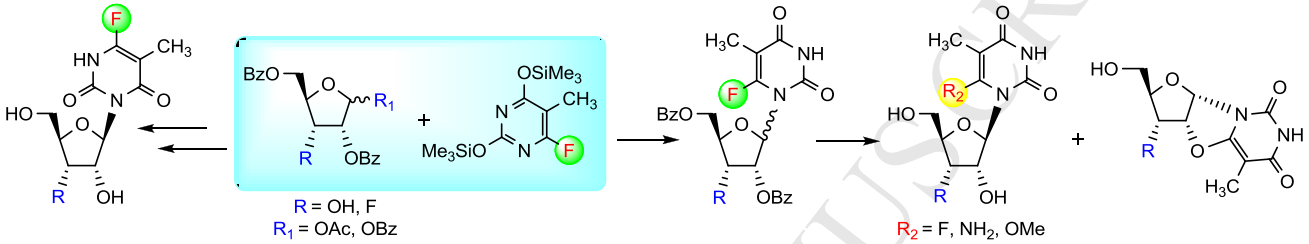

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Synthesis of novel 6-substituted thymine ribonucleosides and their 3′-fluorinated analogues

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ABSTRACT

Nine novel 6-fluorothymine nucleoside analogues of both N(1)-*α*/*β* and N(3)-*β*-ribo series were prepared by the Vorbrüggen method starting from persilylated 6-fluorothymine and 1-O-acetyl-2,3,5-tri-O-benzoyl-*β*-D-ribofuranose, 1-O-acetyl-2,5-di-O-benzoyl-3-deoxy-3-fluoro-*α*,*β*-D-ribofuranose or 1,2,3,5-tetra-O-benzoyl-*β*-D-ribofuranose and its *α*-anomer. Protected N(3)-*β*-D-ribofuranosides were prepared as sole products in high yields at room temperature. A mixture of benzoylated N(1)-*β*- and *α*-anomeric ribonucleosides was obtained at lower temperatures. Yields of *β*-anomers and stereoselectivities (*β*:*α* = 2.2/4.5:1) of the condensation reactions depended on reaction conditions and the structure of the glycosylating agent. Debenzoylation of 6-fluorothymine N(1)- or N(3)-*β*-D-ribofuranosides and their 3′-fluorodeoxy analogues by LiOH monohydrate in MeCN/H_2O resulted in the corresponding fluorinated nucleosides in good yields, whereas the deprotection of N(1)-*α*-ribofuranosides under the same conditions unexpectedly yielded 6,2′-O-*α*-D-anhydronucleosides. 6-Substituted (OMe, NH_2) thymine *β*-ribonucleosides were prepared by the treatment of protected N(1)-*β*-D-ribosides with nucleophilic agents.

1. Introduction

Fluorinated nucleosides have been shown to possess interesting physicochemical and biological properties.[1-3] Bioisosteric replacement of a hydroxyl group or hydrogen atom by fluorine atom(s) is a classic approach in medicinal chemistry to improve the pharmacological properties of a biologically active molecule. As the most electronegative element, fluorine can serve as an isopolar and isosteric mimic of a hydroxyl group because the C-F bond length is close to the C-O bond length. Moreover, fluorine is the second smallest atom, similar in size to hydrogen and substitution of the latter by fluorine in small molecule can cause significant electronic consequences without much distortion of the geometry.[4] Introduction of a fluorine atom to a nucleoside molecule may change substrate metabolism due to the higher strength of the C-F bond compared to that of the C-H bond resulting in higher biological activity and chemical stability of fluorinated derivatives.

All these factors along with the clinical importance of nucleoside-based antiviral and anticancer drugs have determined the historic interest to medicinal chemistry of fluorinated nucleosides. C(5)-Fluoro substitution of the pyrimidine nucleobase led to development of 5-fluorouracil, ftorafur (tegafur***)*** and nowadays capecitabine which are clinically used as anticancer drugs for the treatment of solid or hematological malignancies.[2,5,6] Unlike 5-fluoropyrimidines, 6-fluorothymine nucleosides are little studied.

There are only a few papers published on the synthesis and antiviral activity of glycosides of this hard-to-reach fluorinated pyrimidine or their analogues obtained by the substitution of the C(6)-fluorine atom with different nucleophilic agents (Fig. 1).[7,8]

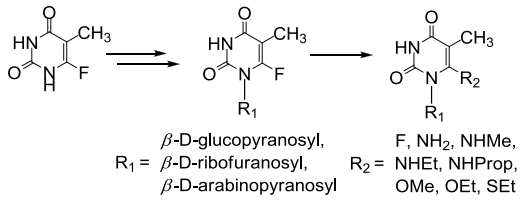

Fig. 1. Glycosides of 6-fluorothymine and their analogues.

Synthesis of 6-fluoro-5-methyluridine was carried out by the coupling of silylated 6-fluorothymine with peracetylated D-ribose in the presence of $SnCl_4$ in 1,2-dichloroethane followed by deprotection of the intermediate nucleoside under acidic treatment or with zinc acetate.[8] Studentsov *et al.* described the synthesis of 6-fluorothymine arabinonucleosides.[9] It should be noted that the 6-fluoro analogue of natural thymine arabinoside

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(spongothymidine) inhibits development of viral infections and displays antitumor activity. Langen and co-workers found that the reaction of silylated 6-fluorothymine with α-acetobromoglucose in the presence of $SnCl_4$ gives only the acetylated N(1)-β-D-nucleoside in 75% yield.[7,8] Deprotection of the latter by HCl treatment in methanol resulted in 60% yield of the target nucleoside.[8]

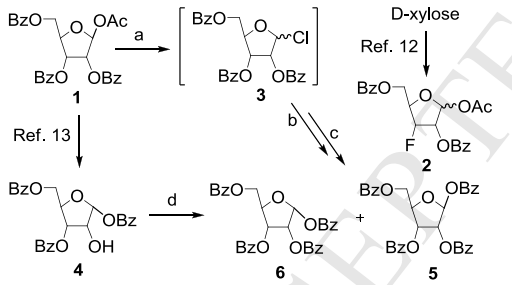
It is noteworthy that deacetylation of protected 6-fluorothymine β-D-glucopyranoside or -ribofuranoside with methanolic ammonia or sodium methoxide led to fluorine atom replacement at the C(6)-position to afford the corresponding 6-amino- and -methoxy-substituted thymine nucleosides.[8] Treatment of the deacetylated 6-fluorothymine ribonucleoside by different nucleophilic agents resulted in 6-substituted thymine nucleosides.[7] Some C(6)-modified pyrimidine nucleoside analogues showed antiviral and anticancer activities.[8-10]

Within our research program on the study of fluorinated nucleosides we have investigated new aspects of the synthesis of 1-(β-D-ribofuranosyl)-6-fluorothymine and synthesized its novel analogues with a fluorine atom at the C(3′)-position of the ribose moiety starting from available carbohydrate precursors for investigation of biological properties.

2. Results and discussion

2.1. Preparation of acylated D-ribose derivatives

Acylated derivatives of D-ribofuranose **1**, **2**, **5** and **6** (Scheme 1) were used as glycosylating agents in the coupling reactions with 6-fluorothymine.[9] 1-O-Acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (**1**) and 1-O-acetyl-2,5-di-O-benzoyl-3-deoxy-3-fluoro-α,β-D-ribofuranose (**2**) were obtained from D-ribose[11] and D-xylose[12] according to the known procedures. Perbenzoylated D-ribofuranose derivatives **5** and **6** were prepared from readily available 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (**1**) in 50% and 24% yields, respectively.

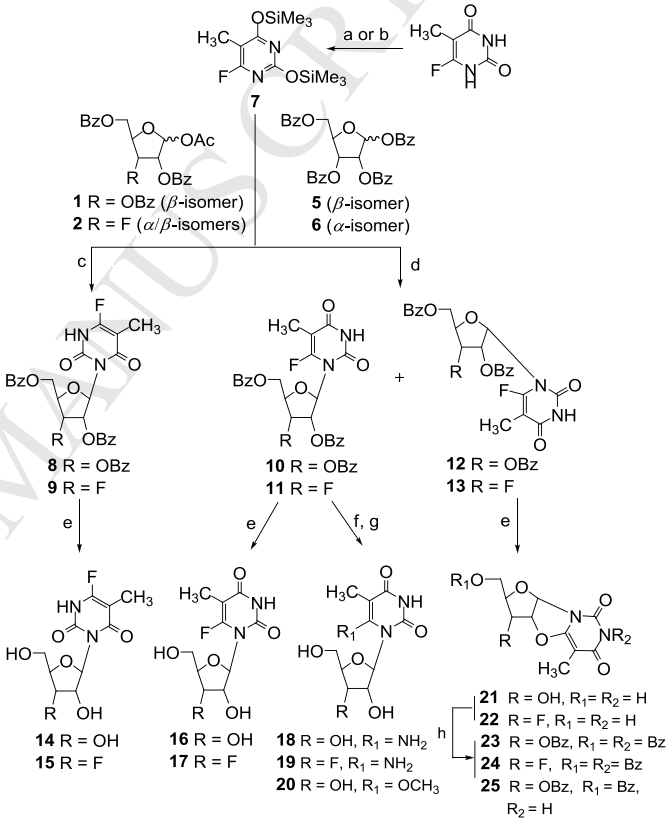


Scheme 1. *Reagents and conditions:* a) HCl, CH_2Cl_2, 0 °C, 1.5 h; b) CH_2Cl_2, MeOH, H_2O, rt, 1 h; c) BzCl, Py/CH_2Cl_2, rt, 20 h; d) BzCl, Py, rt, 20 h.

Chlorination of **1** with gaseous HCl in methylene chloride followed by hydrolysis of intermediate **3** and benzoylation of the forming 2,3,5-tri-O-benzoyl-D-ribofuranose gave rise to 1,2,3,5-tetra-O-benzoyl-β-D-ribofuranose (**5**) and its α-anomer **6** (Scheme 1). In addition, the latter was also prepared via 1,3,5-tri-O-benzoyl-α-D-ribofuranose[13] from acetate **1**.

2.2. Glycosylation reactions: regio- and stereo-selectivities of D-ribose couplings under Vorbrüggen conditions

Condensation reactions of persilylated 6-fluorothymine (**7**) with D-ribose derivatives **1**, **2**, **5** and **6** in the presence of trimethylsilyl triflate (TMSOTf) under Vorbrüggen method[14] conditions followed by deprotection of intermediate ribonucleosides were investigated for the synthesis of fluorinated nucleosides (Scheme 2).



Scheme 2. *Reagents and conditions*: a) HMDS, TMSCl, reflux, 8 h; b) BSTFA, CH_3CN, rt, 2 h; c) TMSOTf, MeCN, rt, 4 h; d) TMSOTf, MeCN, 0 °C→ -15 °C, 4 h (Table 1, entries 1-9); e) LiOH·H_2O, MeCN, H_2O, rt, 5 h; f) NH_3/MeOH, rt, 24 h; g) MeONa/MeOH, rt, 2 h; h) BzCl, Py, rt, 20 h.

Table 1. Preparation of benzoylated 6-fluorothymine ribonucleosides from acylated D-ribose derivatives **1**, **2**, **5** and **6**

Entry	Sugar	Silylation conditions of 6-fluorothymine	Condensation conditions[a]	Products	β/α Ratio[b]	Yield of β (%)[c]
1	**1**	HMDS, TMSCl, reflux; 8 h	TMSOTf, MeCN, rt, 4 h	N(3)-β (**8**)	-	85
2	**1**	HMDS, TMSCl, reflux; 8 h	TMSOTf, MeCN, 0 °C→ -15 °C, 4 h	N(1)-β/α (**10**/**12**)	3.0:1	42
3	**1**	BSTFA, CH_3CN, rt, 2 h	TMSOTf, MeCN, 0 °C→ -15 °C, 4 h	N(1)-β/α (**10**/**12**)	2.2:1	53
4	**1**	BSTFA, CH_3CN, rt, 2 h	TMSOTf, MeCN/$(CH_2Cl)_2$, 0 °C→ -15 °C, 4 h	N(1)-β/α (**10**/**12**)	3.2:1	55
5	**2**	HMDS, TMSCl, reflux; 8 h	TMSOTf, MeCN, rt, 4 h	N(3)-β (**9**)	-	78
6	**2**	HMDS, TMSCl, reflux; 8 h	TMSOTf, MeCN, 0 °C→ -15 °C, 4 h	N(1)-β/α (**11**/**13**)	3.7:1	45
7	**5**	BSTFA, CH_3CN, rt, 2 h	TMSOTf, MeCN, 0 °C→ -15 °C, 4 h	N(1)-β/α (**10**/**12**)	4.3:1	60
8	**5**	BSTFA, CH_3CN, rt, 2 h	TMSOTf, MeCN/$(CH_2Cl)_2$, 0 °C→ -15 °C, 4 h	N(1)-β/α (**10**/**12**)	4.5:1	53
9	**6**	BSTFA, CH_3CN, rt, 2 h	TMSOTf, MeCN, 0 °C→ -15 °C, 4 h	N(1)-β/α (**10**/**12**)	2.4:1	49

[a] Molar ratio sugar : pyrimidine base : TMSOTf – 1:1.2:1.1.
[b] Ratio was determined by ^{1}H NMR spectroscopic analysis of anomeric mixture in $CDCl_3$ after isolation by column chromatography.
[c] Isolated yield of benzoylated β-ribonucleoside by column chromatography.

Firstly, we undertook a search for stereoselective reaction conditions for the N-glycosylation of 2,4-bis-O-(trimethylsilyl)-6-fluorothymine (**7**). This was prepared by treatment of 6-fluorothymine with hexamethyldisilazane (HMDS) / trimethylsilyl chloride (TMSCl) under reflux or with N,O-bis-trimethylsilyl trifluoroacetamide[15] (BSTFA) in CH_3CN at room temperature, in order to synthesise benzoylated 6-fluorothymine *β*-nucleosides. Results of the investigation on condensation reactions of **7** with four sugars under different conditions are summarized in Table 1. Silyl derivative **7**, prepared using HMDS/TMSCl, was condensed with sugars **1** or **2** in anhydrous MeCN in the presence of TMSOTf at room temperature to give the unexpected protected N(3)-*β*-isomers **8** (85%) or **9** (78%), respectively, as the only reaction products (Table 1, entries 1 and 5).

Similar coupling reactions of the silylated base **7** with acyl derivatives **1** or **2** at low temperature (from 0 °C to -15 °C) in anhydrous MeCN gave protected N(1)-*β*-ribonucleosides **10** (42%) or **11** (45%) as the main products, along with N(1)-*α*-nucleosides **12** (14%) and **13** (11%). These isomeric products were isolated by column chromatography on silica gel. Formation of N(3)-isomeric derivatives **8** and **9** was not observed under the reaction conditions stated (entries 2 and 6). These findings on the glycosylation reactions of the silylated base **7** in MeCN indicate that the regio- and stereo-selectivity of N-glycosylation depends on temperature. The undesired exclusive formation of a N(3)-glycoside bond took place at room temperature. Felczak et al. noted also that the condensation of uracil derivatives containing an electrophilic trifluoromethyl substituent at C(6) with 1-O-acetyl-2,3,5-tri-O-benzoyl-*β*-D-ribofuranose (**1**) led to formation of only the N(3)-ribosides despite the use of various conditions.[10] Probably, the size and character of the C(6)-substituent in the pyrimidine heterocycle is critical for its N-glycosylation. It was reported that condensation of a silylated 6-chlorouracil with 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride under heating gave only the N(3)-*β*-D-riboside in low yield.[16] The coupling reaction of silylated 6-methylmercaptouracil with 1-bromo-2,3,5-tri-O-benzoyl-D-ribofuranose in MeCN also gave the corresponding N(3)-*β*-D-riboside of 6-substituted uracil.[17]

However, at lower temperatures the desired N(1)-*β*-anomers **10** and **11** were prepared as the main classical condensation products along with *α*-anomers of riboside **12** and fluorodeoxy nucleoside **13** (ratios *β*:*α*–3.0/3.7:1). Further, the coupling reactions of the silylated base **7** prepared using BSTFA were carried out with acyl derivate **1** in anhydrous MeCN or a mixture of MeCN/$(CH_2Cl)_2$ in the presence of TMSOTf (Table 1, entries 3 and 4). The protected N(1)-*β*-ribonucleoside **10** was prepared in 53-55% yield after chromatographic isolation on silica gel. In a continuation of this investigation, we also studied available 1,2,3,5-tetra-O-benzoyl-*β*-D-ribofuranose (**5**) and its *α*-anomer **6** in the condensation reactions with **7** (entries 7-9) and, as a result, the N(1)-*β*-ribonucleoside **10** was obtained in 49-60% yield. It should be noted that the best yield of the intermediate protected nucleoside and good stereoselectivities (ratios *β*:*α* – 4.3/4.5:1) of the condensation reactions were achieved at lower temperatures using benzoate **5** as glycosylating agent (entries 7-8). These results differ from the known approach,[8] leading to exclusively N(1)-*β*-glycoside of 6-fluorothymine with application of 1,2,3,5-tetra-O-acetyl-*β*-D-ribofuranose and the silylated base **7** at room temperature in dichloroethane in the presence of $SnCl_4$. According to previous communications,[18,19] the formation of *α*-anomers of ribonucleosides as by-products was observed during the condensation of the D-ribofuranose derivative with persilylated bases in few cases, e.g. the glycosylation of 5-fluorouracil in dichloroethane in the presence of $SnCl_4$ or without. On the whole, these findings, as well as the literature data,[7,8] provide evidence that the outcome of the glycosylation reactions of **7** with different acyl D-ribose derivatives strongly depends on the reaction conditions viz. Lewis acid, temperature, the method used for preparing the silylated base, and the structure and anomeric stereochemistry of the glycosylating agent.

2.3. Deprotection of 6-fluorothymine *β*-ribosides and intramolecular cyclization of *α*-ribosides

Secondly, deprotection of the benzoylated 6-fluorothymine D-ribofuranosides **8-13** as in the case of acetylated 6-fluorothymine glycosides,[7] represented another task to be solved for the efficient synthesis of target nucleosides. Standard deprotection of individually protected nucleosides **10** and **11** with methanolic ammonia resulted in the substitution of the fluorine atom at the C(6)-position of the heterocycle by the amino group with the formation of 6-aminothymine substituted nucleosides **18** and **19** in high yields (72-75%). The 6-methoxythymine ribonucleoside **20** was isolated after treatment of **10** with NaOMe in methanol at room temperature in 68% yield after chromatographic purification on silica gel.

We have found that the treatment of individually N(1)-*β*-nucleosides **10** and **11** with LiOH monohydrate in a mixture of acetonitrile-water (3:1.3, v/v) led to target nucleosides **16** and **17** in high yields (75-79%) compared to the previously described methods for removal of the acetyl groups of 6-fluorothymine nucleosides using zinc acetate or HCl in methanol.[7,8] In a similar way, the removal of the benzoyl groups in N(3)-*β*-ribonucleosides **8** and **9** gave nucleoside analogues **14** and **15** with 57% and 63 % yield, respectively.

Deprotection of individually protected *α*-nucleosides of 6-fluorothymine **12** and **13** with LiOH monohydrate in a mixture of acetonitrile-water and subsequent chromatographic purification unexpectedly gave pure 6,2′-O-*α*-D-anhydronucleosides **21** and **22** in 81% and 85% yields, respectively. One can assume that the formation of anhydronucleosides **21** and **22** resulted from an intramolecular substitution of the fluorine atom at the C(6)-position of the heterocycle by the activated hydroxyl group at the C(2′)-atom of the intermediate deprotected nucleoside during removal of the protective groups of nucleosides **12** and **13** under the basic conditions. The benzoylation of the prepared *α*-cyclonucleosides **21** and **22** was carried out with benzoyl chloride in pyridine. This led to tribenzoate **23** and dibenzoate **24** isolated by column chromatography on silica gel in 75% and 81% yields, respectively. In the case of compound **21**, dibenzoate **25** (20%) was also isolated from the reaction mixture by column chromatography.

2.4. Structural analysis

The structures of all synthesized nucleosides were proved by various spectroscopic methods. The assignment of ^1H and ^{13}C NMR spectra was conducted with support from 2D NMR spectroscopy (^1H-^1H COSY, NOESY and ^1H-^{13}C HMBC).

2.4.1. Regio- and stereoisomeric assignment. Comparison of the UV absorption spectra of the isomeric pairs of 6-fluorothymine nucleosides **14**, **15** and **16**, **17** in acidic, neutral and alkaline aqueous solutions revealed a significant bathochromic shift of the absorption maxima to 281 nm (lg ε 4.0 and 3.96) for N(3)-nucleosides **14** and **15** not only at pH 13, which is typical for N(3)-nucleosides,[20] but also at the neutral pH (see experimental part). It should be noted that similar spectral behavior have been described for 3-(*β*-D-ribofuranosyl)-6-trifluoromethyluracil at neutral and alkaline pH as compared to the spectrum at pH 1.[10]

Table 2. Selected ^{13}C, ^{19}F, ^{1}H NMR data for 6-fluorohymine ribonucleosides and their analogues [some $J_{C,F}$, $J_{H',H'}$, $J_{H',F}$ values are given in brackets][a]

Com-pound	Chemical shift δ (ppm), in brackets J (Hz)											
	C(2) [$J_{C2,F6}$]	C(4) [$J_{C4,F6}$]	C(6) [$J_{C6,F6}$]	C(1′) [$J_{C1',F6}$]	C(2′) [$J_{C2',F6}$]	C(4′) [$J_{C4',F6}$]	F-C(6)	F-C(3′)	H(1′) [$J_{1',2'}$]	H(2′) [$J_{2',3'}$]	H(3′) [$J_{3',4'}$]	H(4′)
8	150.0	163.7	157.1	87.2	74.1	79.2	-96.43	-	6.60	6.16-6.13		4.72-4.65 m
	d [9.1]	d [17.5]	d [267.5]	br s	s	s	s		br s	m		(H-4′, H-5′′)
9	150.2	163.7	157.1	86.1	73.0	79.5	-95.82	-205.39	6.59	6.04	5.72	4.61-4.55 m
	d [8.9]	d [17.1]	d [270.3]	br s	d	d	s	m	br s	br s	br d	(H-4′, H-5′′)
10	147.7	163.2	157.7	87.9	74.0	79.4	-98.94	-	6.26	6.03	5.92	4.69-4.66
	d [4.5]	d [18.3]	d [271.3]	d [4.8]	d [3.5]	s	s		br s	dd [6.4]	t	m
11	147.7	163.4	157.4	86.5	72.8	79.9	-99.36	-204.27	6.24	5.97	5.59	4.62-4.55 m
	d [3.7]	d [18.2]	d [271.4]	d [4.9]	d	d	s	m	br s	dd [6.3]	dt	(H-4′, H-5′′)
12	147.9	163.0	158.4	85.8	70.6	79.7	-94.51	-	7.04	6.09	5.79	4.98-4.95
	d [4.7]	d [17.6]	d [272.7]	s	s	d [5.4]	s		d [5.7]	t	t	m
13	148.0	163.1	158.5	84.8	70.5	80.7	-94.12	-202.96	6.97	5.84	5.41	4.95-4.90
	d [4.8]	d [17.4]	d [271.1]	s	d	dd [3.8]	s	m	d [6.7]	dt	ddd [4.2]	dm
14	156.8	169.0	167.4	90.0	72.8	85.7	-75.43	-	6.40	4.72	4.39	3.92-3.89
	d [31.5]	d [17.5]	d [242.8]	s	s	s	s		d [4.1]	dd [6.2]	t	m
15	158.6	169.6	168.9	88.7	71.2	84.3	-74.23	-202.29	6.45	5.05	5.06	4.21-4.14
	d [29.7]	d [17.6]	d [245.9]	s	d	d	s	m	d [6.1]	dt [5.2]	ddd [3.0]	dm
16	148.3	163.3	157.9	88.5	71.4	84.4	-97.89	-	5.87	4.32	3.95-3.92	3.71-3.68
	d [3.9]	d [17.9]	d [269.7]	s	d [3.9]	s	s		d [4.3]	t	m	m
17	148.4	163.2	157.8	86.8	69.5	82.5	-98.24	-199.82	5.91	4.64	4.96	4.10-4.03
	d [4.1]	d [18.1]	d [270.5]	s	dd [5.6]	d	s	m	d [6.9]	dt [4.6]	ddd [2.3]	dm
21	146.5	165.3	159.0	85.8	80.2	84.3	-	-	6.14	5.19	4.01	3.56
	s	s	s	s	s	s			d [4.8]	t [5.1]	dd [9.3]	ddd
22	146.5	165.4	159.1	86.5	81.6	79.1	-	-212.65	6.21	5.49	5.17	4.03
	s	s	s	d	d	d		dd	d [5.1]	dt [5.2]	ddd [7.3]	ddd

[a] **8-13** – $CDCl_3$; **14**, **15** – CD_3OD; **16**, **17**, **21** and **22** – DMSO-d6

^{13}C NMR data presented in Table 2 provides additional confirmation for the assigned structures of N(1)-*β*-, N(3)-*β*-ribonucleoside derivatives **14-17**. In the ^{13}C NMR spectra of each pair of isomeric nucleosides, downfield shift of the C(6)-atoms of the N(3)-nucleosides **14** and **15** to 9-11 ppm was observed as compared with N(1)-nucleosides **16** and **17**.The magnitude of the constant $J_{C6,F6}$ for N(3)-isomers in comparison with their N(1)-counterparts was reduced by 26 Hz and 24 Hz, respectively. It is interesting to note a significant change in the vicinal coupling constant between the C(2)-atom and fluorine atom at the C(6)-position: the magnitude of $^{3}J_{C2,F6}$ is equal to 3.9 Hz for N(1)-isomer **16**, but for N(3)-nucleoside **14** the one comprises 31.5 Hz, whereas the magnitudes of $^{3}J_{C4,F6}$ vicinal couplings are similar for the both regioisomers.

Configurations at the anomeric centers of prepared 6-fluorothymine nucleosides **10-13** were determined on the basis of NMR data. Long-range $^{3}J_{C,F}$ couplings of C(1′) to F(6) were observed in the ^{13}C NMR spectra of *β*-nucleosides **10** and **11** (4.8 Hz and 4.9 Hz, respectively) (Table 2). Six-bonds couplings between carbon C(4′) and F(6) were observed in spectra of the *α*-isomers **12** and **13** (5.4 Hz and 3.8 Hz, respectively). These confirm the accession of the pentofuranose to the N(1)-atom of 6-fluorothymine in both condensation products and their *α*-anomeric configurations. The latter couplings ($^{6}J_{C4',F6}$) generally indicate spatial proximities of the nuclei involved and favorable non-bonded interactions between the fluorine of the heterocyclic base and carbon atom C(4′) in ”through-space” C-F and C-H couplings.[21] Decrease of the ”through-space” C-F coupling magnitude ($^{6}J_{C4',F6}$ = 3.8 Hz) for 3′-fluororiboside **13** likely reflects molecule conformational features caused by repulsion taking place between the fluorine atoms of the heterobase and carbohydrate moiety. Such repulsion leads to an increase in the distance between the C(4′)- and F(6)-atoms. This observation also confirms a major contributor to the carbon couplings to be the direct ”through-space” carbon-fluorine couplings in contrast to its ”through-bond” counterpart.[21]

The signal of the H(1′)-proton of N(3)-*β*-riboside **8** displayed in the ^{1}H NMR spectrum as a broad singlet at 6.60 ppm was shifted to a lower field of 0.3 ppm compared to N(1)-*β*-riboside **10**. The most informative feature in the ^{1}H NMR spectra of *α*-anomer **12** is the downfield shift of H(1′) and H(4′) resonances[12] in comparison with the those of the corresponding *β*-nucleoside **10** (Table 2).

It should be noted that the described ^{1}H NMR spectra features observed for a number of 6-fluorothymine analogues **8**, **10** and **12** are the same for 3′-deoxy-3′-fluoro-D-ribosides of 6-fluorothymine **9**, **11** and **13** and the corresponding deprotected nucleosides **14-17** (Table 2). The signals of the 6-amino groups as well as the 6-methoxy group are displayed as singlets at 6.51, 6.59 ppm, and 3.88 ppm in the ^{1}H NMR spectra of 6-substituted thymine nucleosides **18-20**, respectively.

The configuration at the anomeric center of *β*-nucleoside **10** and its *α*-anomer **12** was also confirmed by 2D NMR spectroscopy (Fig. 2). It is noteworthy that in the case of nucleoside **10** an obvious cross-peak between H(4′) and H(1′) observed in the NOESY spectrum provides evidence in favor of its *β*-anomeric configuration. This cross-peak is the most intensive and twice more stronger than the interaction between H(1′) and H(2′). The NOE-effect involving protons of CH_3 and C(5′)-O-benzoyl (meta-proton, 7.42 ppm) groups found in the spectrum of **10** are clearly missing in that of **12.**

In the NOESY spectrum of the benzoylated *α*-riboside **12**, a weak cross-peak between H(4′) and H(1′) (10% of the intensity of the H(1′)-H(2′) cross-peak and 30% of that of H(1′)-H(3′) was found. The presence of such interaction can be explained by a conformational flexibility of the molecule. The structure is not rigid and, at a certain moment, the protons are getting closer to raise a signal. In addition, a NOE-effect was also observed between H(5′) and H(3′)-protons (Fig. 2). Slight interactions could be also seen between the protons of the CH_3 group (d at 1.75 ppm, $J_{CH3,F6}$ = 2.3 Hz) and the ortho- (7.85 ppm) and meta- (7.38 ppm) protons of the C(2′)-O-benzoyl group.

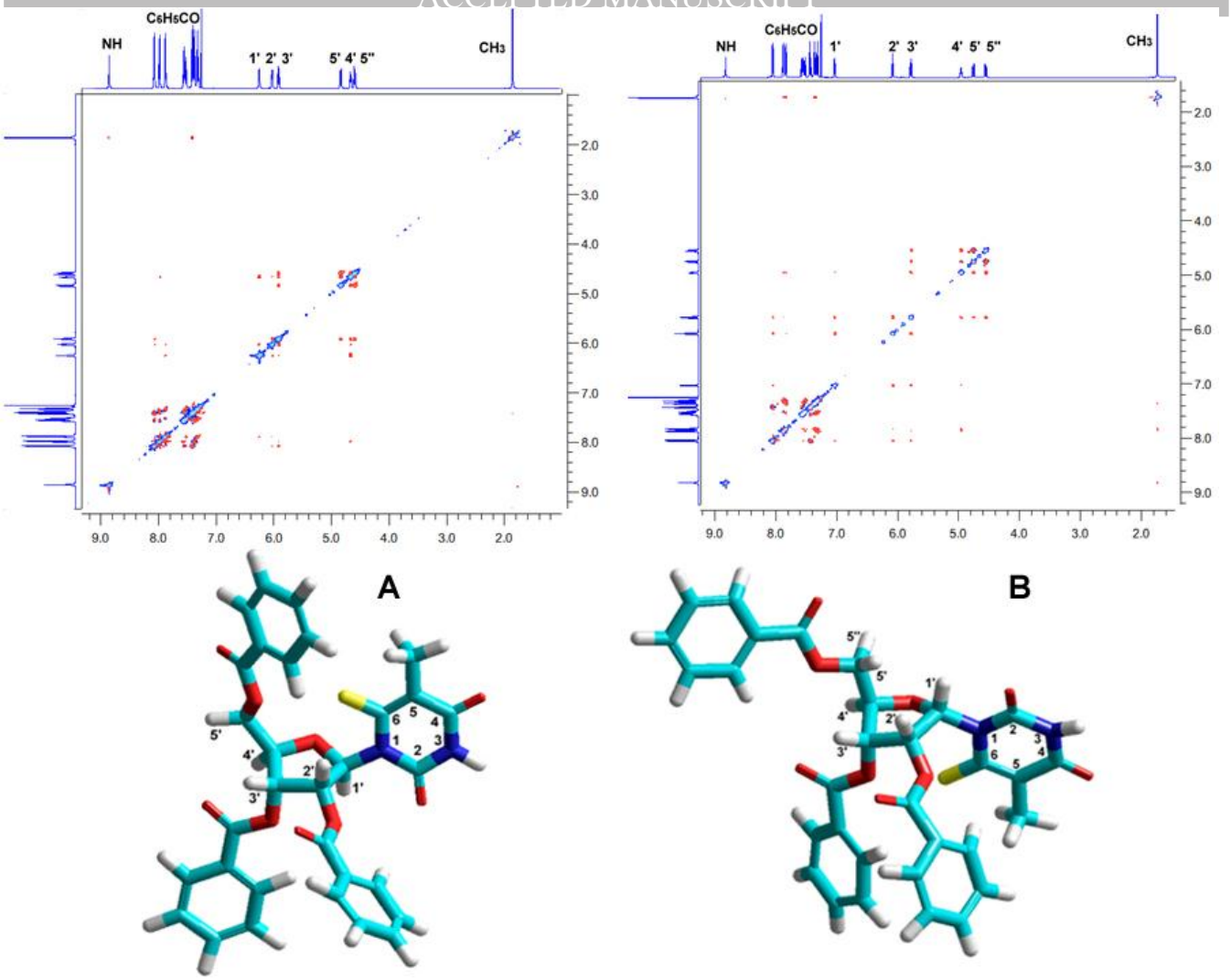


Fig. 2. 2D NOESY of *β*-riboside **10** (A) and *α*-riboside **12** (B) with 3D view of the molecules obtained using HyperChem 8.0.

The ^{19}F NMR spectra of compounds **8-13**, **16** and **17** exhibit the characteristic peaks near -96.69 ppm assigned to the F(6)-atom. The signal of the fluorine atom for deprotected N(3)-nucleosides **14** and **15** is shifted upfield on 22-24 ppm compared to N(1)-isomers **16** and **17** and observed at -75 ppm, which is obviously due to the location of the D-ribofuranose.

2.4.2. Cyclization. The surprising structures of 6,2′-O-*α*-D-anhydronucleosides **21** and **22** have been confirmed by NMR spectroscopy, including 2D techniques, and by comparison with the spectral data obtained for their benzoyl derivatives **23-25**. Analysis of the ^{1}H NMR spectra of benzoylated *α*-nucleoside derivatives **12** and **25** showed that chemical shifts of the H(1′), H(2′), H(3′) and H(4′) protons of the *α*-cyclonucleoside **25** shifted into the strong field (Δ = 0.5 ppm for H(1′) and H(3′); Δ = 0.4 ppm for H(2′) and H(4′). Chemical shifts of the H(5′) and H(5″) signals are not changed.

The most informative NMR spectra features of *α*-cyclonucleosides are (i) shifts of the H(2′) resonance signal at 0.85-0.87 ppm to a lower field for compound **21** and fluoride **22** compared to *β*-ribosides **16** and **17** and (ii) absence of signals of the 2′-OH groups, indicating the presence of 6,2′-anhydro linkage. Furthermore, the fluorine at the C(6)-atom of the heterocycle is absent in the ^{19}F NMR spectra of compounds **21** and **22**, and there is only a signal of fluorine at the C(3′)-atom (dd, -212.65 ppm) for 3′-fluoro-cyclonucleoside **22**.

It should be emphasized that synthesis of 6,2′-O-*α*-D-anhydronucleosides has not been earlier described. However, several processes was reported for the preparation of pyrimidine 6,2′-O-*β*-D-anhydronucleosides.[22-25] Folkers *et al.* have synthesized a series of C(5)-substituted 6,2′-anhydrouridines[22] by the treatment of *β*-ribofuranosyl barbiturate acid derivatives with thiocarbonyldiimidazole under refluxing in toluene, among them 6,2′-anhydro-1-(*β*-D-arabinofuranosyl)-6-hydroxythymine (**27**) (Fig. 3), an isomeric analogue of **21**, which was prepared in 60% yield.[22] 6,2′-Anhydro-1-(*β*-D-arabinofuranosyl)-6-hydroxyuridine (**28**) was also prepared from the alkali treatment of 1-*β*-D-arabinofuranosyl-5-halogenuracils with sodium methoxide in methanol or sodium hydroxide.[23,24] The structures of the 6,2′-anhydrouridines were established by the ^{1}H NMR and UV absorption spectra.

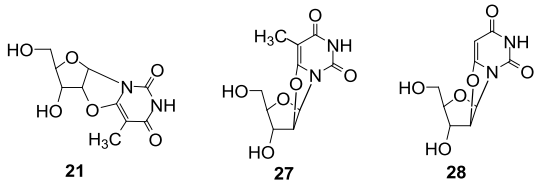


Fig. 3. Structures of 6,2′-O-D-anhydrouridines.

It is worth noting that the change of anomeric configuration from 6,2′-anhydro-1-(*β*-D-arabinofuranosyl)-6-hydroxythymine

(**27**)[22] to 6,2′-anhydro-1-(α-D-ribofuranosyl)-6-hydroxythymine (**21**) is accompanied by a shift of the signal H(3′) (dd at 4.01 ppm) to a higher-field (Δ = 0.38 ppm) versus H(3′) (s at 4.39 ppm)[22] for β-nucleoside. However, differences in ^{1}H NMR chemical shifts of the H(1′) (Δ = 0.11 ppm) and H(2′) (Δ = 0.04 ppm) protons of the isomeric nucleosides are slight, and the $J_{1',2'}$ magnitudes (4.8 and 5.0 Hz) are very close for the both nucleosides with 1,2-*cis* arrangements of H(1′)- and H(2′)-protons. The coupling constant (J = 9.3 Hz) between H(3′)- and H(4′)-protons is found in the ^{1}H NMR spectrum of **21** while clearly missing[22] in the spectrum of the known β-6,2′-anhydronucleoside **27**. The value of $J_{3',4'}$ (2.0 Hz)[23] observed for β-6,2′-anhydrouridine **28**, the structural analogue of **27**, is much below that of α-cyclonucleoside **21**. These data demonstrate the extremely tense conformation of prepared novel α-cyclonucleosides. Conformationally constrained cyclic pyrimidine nucleosides of this family are likely to be of interest for preparation of modified oligonucleotides with interesting antisense properties.[26]

It should be noted that in the CD-spectrum of 6,2′-O-α-anhydronucleosides **21** and **22** we observed strongly pronounced positive Cotton effects at 255 nm (bands B_{2u}) with large amplitudes (θ = 27×10^3 and θ = 20×10^3, respectively) as compared to CD-spectral data prepared for 6-fluorothymine β-ribofuranosides **14** and **16** under the same conditions (Fig. 4). This phenomenon could likely be explained by the rigid conformation of the obtained anhydronucleosides in comparison with 6-fluorothymine β-ribonucleosides **14** and **16**.

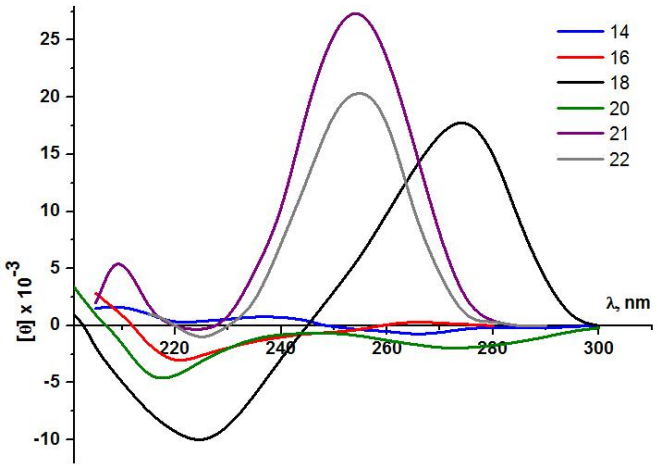


Fig. 4. CD-spectra of compounds **14**, **16**, **18**, **20-22** in water.

From the CD-spectra of N(1)-ribosides **16**, **18** and **20** bearing different substituents at the C(6)-atom of the heterocycle and N(3)-isomer **14**, which has two carbonyl groups close to the anomeric center, one can assume that the size of the substituent as well its electronegativity causing change in the electronic structure of the modified pyrimidine bases exerts significant influence on the shape of the CD-spectra bands and amplitude of Cotton effect.

3. Conclusion

New 6-substituted thymine ribonucleosides and their 3′-fluorinated analogues have been synthesized. It is shown that the regio- and stereoselectivity of N-glycosylation of persilylated 6-fluorothymine with acyl derivatives of D-ribose in MeCN in the presence of TMSOTf depends on temperature and leads to the formation of N(1)-β- and N(1)-α-ribonucleosides or only N(3)-β--isomers. Yields (42-60%) of target benzoylated 6-fluorothymine N(1)-β-ribonucleosides and stereoselectivities of the condensation reactions (ratios β:α – 2.2/4.5:1) at lower temperatures depends on the silylation procedure of the heterocyclic base and the structure of glycosylating agent. The use of BSTFA instead of HMDS/TMSCl for the silylation of 6-fluorothymine resulted in better total yields of protected 6-fluorothymine N(1)-β- and -α-ribonucleosides in the condensation reactions from acetate **1**. Among four D-ribose derivatives, good yields of the β-nucleoside and the best stereoselectivities (ratios β:α – 4.3/4.5:1) of the coupling reactions were achieved from β-benzoate **5**. It was found that mild deprotection of the benzoyl groups of intermediate 6-fluorothymine β-nucleosides using LiOH monohydrate in a mixture of acetonitrile-water resulted in the target nucleosides in high yields. An effective method for the preparation of 6-fluorothymine N(1)-β-ribonucleoside was devised in 47% overall yield from 1,2,3,5-tetra-O-benzoyl-β-D-ribofuranose. A new approach to the synthesis of 6,2′-O-α-D-anhydronucleosides has been developed as a result of the intramolecular substitution reaction of the fluorine atom at the C(6)-position of the heterocycle by the C(2′)-hydroxyl group of an intermediate deprotected nucleoside during removal of the protective groups of 6-fluorothymine N(1)-α-ribonucleosides under the basic reaction conditions. The anomeric configuration of the glycosidic bonds was determined by 2D NMR spectroscopy. It is noteworthy that the couplings $^6J_{C4',F6}$ caused by spatial proximities of the nuclei were detected for protected 6-fluorothymine α-nucleosides and this fact possibly indicates the preferable direct "through-space" interaction between fluorine atom of heterocyclic base and C(4′) carbon atom in contrast to its "through-bond" counterpart.

4. Experimental

4.1. General

Organic solvents were purified and dried by standard methods before use. UV absorption spectra were measured on a Varian Cary 100 spectrometer. NMR spectra were registered on a Bruker Avance 500 MHz spectrometer at 500 MHz (^{1}H), 125 MHz (^{13}C) and 470 MHz (^{19}F). Chemical shifts (δ) are given in ppm related to internal $SiMe_4$ and coupling constants (J) in Hz. IR spectra were measured on a Perkin Elmer Spectrum 100 FT-IR spectrometer. High-resolution mass spectra (HRMS) were recorded on an Agilent 6550 Q-ToF instrument using ESI (electrospray ionization). Melting points were determined on an electrically heated melting point apparatus and were uncorrected. CD spectra were obtained on a Jasco J-20 spectropolarimeter. Preparative column chromatography was performed on silica gel Merck 60 (70-230 mesh). TLC analysis was carried out using TLC Silica gel 60 F_{254} (aluminium-backed sheets, Merck). The following solvent systems were used: hexane-EtOAc, 3:2, v/v (A), $CHCl_3$- MeOH, 4:1, v/v (B).

4.2. Synthesis of perbenzoylated D-ribofuranose derivatives

*4.2.1. 1,2,3,5-Tetra-O-benzoyl-β-D-ribofuranose (**5**) and its α-isomer **6**.* Hydrogen chloride was bubbled into an ice-cold solution of **1** (0.80 g, 1.58 mmol) in methylene chloride (15 mL) for 1.5 h. After being kept at 2-8 °C for 5 h, the solution was concentrated under vacuum. To the obtained crude 1-chloro derivative **3** was added a mixture of CH_2Cl_2 (7 mL), MeOH (7 mL) and H_2O (1.5 mL). The mixture was stirred for 1 h at ambient temperature and diluted with $CHCl_3$ (150 mL). The organic phase was washed with 5% $NaHCO_3$ (50 mL), dried over Na_2SO_4 and evaporated to dryness. The obtained residue was applied to a chromatographic column (silica gel, 80 cm^3) and

eluted with a linear ethyl acetate gradient (20→30%, v/v, 400 mL). The fractions containing the product were collected and evaporated to yield 0.61 g of colorless oil. To this ice-cooled residue in a mixture of anhydrous pyridine (0.3 mL) and CH_2Cl_2 (10 mL) was added benzoyl chloride (300 mL, 2.58 mmol), the reaction mixture was stirred for 20 h at room temperature. After standard workup the obtained residue was applied to a chromatographic column (silica gel, 100 cm^3) and eluted with $CHCl_3$/hexane (5:1, v/v, 500 mL). The fractions containing the products **5** and **6** were collected and evaporation to dryness under vacuum. **5**: Yield 50%; white solid; m.p. 120-121 °C (from EtOH), (lit.[27] mp 120-121 °C); ^{1}H NMR ($CDCl_3$): δ 8.04-7.21 (20H, m, Bz), 6.68 (1H, s, H1′), 6.02-5.98 (2H, m, H2′, H3′), 4.89-4.86 (1H, m, H4′), 4.75 (1H, dd, J = 3.9, J = 12.2, H5′), 4.57 (1H, dd, J = 4.5, H5″); ^{13}C NMR ($CDCl_3$): δ 166.1, 165.4, 165.0 and 164.7 (4×C_6H_5C=O), 133.7-128.3 (24×C_{ar}), 99.1 (C1′), 79.9 (C4′), 75.1 (C2′ or C3′), 71.4 (C2′ or C3′), 63.7 (C5′); IR (KBr,): ν 1725, 1601, 1585, 1451, 1316, 1268, 1178, 1115, 1067, 1026, 953, 710 cm^{-1}; HRMS $(M+Na)^+$: calcd. for $C_{33}H_{26}NaO_9$: 589.1475; found 589.1471.

6: Yield 24%; colorless oil, ^{1}H NMR ($CDCl_3$): δ 8.13-7.28 (20H, m, Bz), 6.95 (1H, d, J = 4.4, H1′), 5.92 (1H, dd, J = 2.1, J = 6.5, H3′), 5.75-5.72 (1H, m, H2′), 4.93-4.91 (1H, m, H4′), 4.78 (1H, dd, J = 3.0, J = 12.1, H5′), 4.68 (1H, dd, J = 3.5, H5″); ^{13}C NMR ($CDCl_3$): δ 166.1, 165.7, 165.1 and 165.0 (4×C_6H_5C=O), 133.6-128.4 (24×C_{ar}), 95.0 (C1′), 82.9 (C4′), 71.5 (C2′ or C3′), 70.8 (C2′ or C3′), 64.0 (C5′); IR (KBr,): ν 1725, 1601, 1585, 1451, 1316, 1268, 1178, 1136, 1112, 1067, 1023, 956, 708 cm^{-1}; HRMS $(M+Na)^+$: calcd. for $C_{33}H_{26}NaO_9$: 589.1475; found 589.1473.

*4.2.2. 1,2,3,5-Tetra-O-benzoyl-α-D-ribofuranose (**6**).* To a ice-cooled solution of 1,3,5-tri-O-benzoyl-α-D-ribofuranose (**4**) (0.50 g, 1.08 mmol) in anhydrous pyridine (10 mL) was added benzoyl chloride (0.25 mL, 2.15 mmol) and the mixture was stirred for 20 h at room temperature. After standard workup the obtained residue was applied to a chromatographic column (silica gel, 60 cm^3) and eluted with a linear ethyl acetate gradient (20→30% v/v, 300 mL) in hexane. The fractions containing the product were collected and evaporation to yield 0.58 g (95%) of **6** as colorless oil. **6**: similarly 4.2.1.

4.3. General procedure for the glycosylation of 6-fluorothymine with acetates 1 and 2

Method A: A suspension of 6-fluorothymine (0.35 g, 2.43 mmol) in mixture of hexamethyldisilazane (7 mL) and trimethylchlorosilane (0.02 mL, 0.16 mmol) was refluxed under nitrogen for 8 h. The obtained homogeneous solution was evaporated to dryness under vacuum, then the residue was codistilled with anhydrous toluene (2 × 15 mL). To the obtained 2,4-bis-O-(trimethylsilyl)-6-fluourothymine (**7**), was added a solution of acetates **1** or **2** (2.11 mmol) in anhydrous MeCN (6.5 mL). To this ice-cooled mixture TMSOTf (0.42 mL, 2.32 mmol) was added, and the resulting solution was stirred for 5 min at +5 °C. Then the reaction mixture was brought to room temperature and stirred for 4 h, and diluted with CH_2Cl_2 (150 mL). The organic phase was washed with 5% $NaHCO_3$ (2 × 50 mL), dried over Na_2SO_4 and evaporated to dryness. The obtained residue was applied to a chromatographic column (silica gel, 100 cm^3) and eluted with a linear ethyl acetate gradient (15→50%, v/v, 1200 mL) in hexane. The fractions containing the individual products **8** or **9** were collected and evaporated to dryness under vacuum.

*4.3.1. 3-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)-6-fluorothymine (**8**).* Yield 85%; white solid, m.p. 180-182 °C (from Et_2O/hexane); R_f (A) 0.21; ^{1}H NMR ($CDCl_3$): δ 8.08-7.30 (15H, m, Bz), 6.60 (1H, br s, H1′), 6.16-6.13 (2H, m, H2′, H3′), 4.78 (1H, dd, J = 2.8, J = 11.3, H5′), 4.72-4.65 (2H, m, H4′, H5″), 1.84 (3H, d, J = 1.5, CH_3); ^{13}C NMR ($CDCl_3$): δ 166.5, 165.7 and 165.4 (3×C_6H_5C=O), 163.7 (d, J = 17.5, C4), 157.1 (d, J = 267.5, C6), 150.0 (d, J = 9.1, C2), 133.6-128.4 (18×C_{ar}), 90.9 (d, J = 13.0, C5), 87.2 (br s, C1′), 79.2 (C4′), 74.1 (C2′), 71.2 (C3′), 63.7 (C5′), 6.7 (CH_3); ^{19}F NMR ($CDCl_3$): δ -96.43 (s, FC6); IR (KBr,): ν 1725, 1664, 1602, 1452, 1315, 1271, 1177, 1123, 1071, 1027, 711 cm^{-1}; HRMS $[M+NH_4]^+$ calcd for $C_{31}H_{29}FN_3O_9$: 606.1888, found 606.1887.

*4.3.2. 3-(2,5-Di-O-benzoyl-3-deoxy-3-fluoro-β-D-ribofuranosyl)-6-fluorothymine (**9**).* Yield 78%; white solid, m.p. 167-169 °C (from Et_2O/hexane); R_f (A) 0.20; ^{1}H NMR ($CDCl_3$): δ 8.08-7.39 (10H, m, Bz), 6.59 (1H, br s, H1′), 6.04 (1H, br s, H2′), 5.72 (1H, br d, J = 54.6, H3′), 4.70 (1H, d, J = 11.2, H5′), 4.61-4.55 (2H, m, H4′, H5″), 1.80 (3H, d, J = 1.1, CH_3); ^{13}C NMR ($CDCl_3$): δ 166.3 and 165.7 (2×C_6H_5C=O), 163.7 (d, J = 17.1, C4), 157.1 (d, J = 270.3, C6), 150.2 (d, J = 8.9, C2), 133.7-128.3 (12×C_{ar}), 90.7 (d, J = 15.8, C5), 88.0 (d, J = 193.9, C3′), 86.1 (br s, C1′), 79.5 (d, J = 25.7, C4′), 73.0 (d, J = 7.5, C2′), 63.1 (C5′), 6.5 (CH_3); ^{19}F NMR ($CDCl_3$): δ -95.82 (s, FC6), -205.39 (m, FC′3); IR (KBr,): ν 1725, 1664, 1602, 1452, 1315, 1271, 1177, 1110, 1071, 1027, 711 cm^{-1}; HRMS $[M+Na]^+$ calcd for $C_{24}H_{20}F_2N_2O_7Na$: 509.1136, found 509.1129.

Method B: To 2,4-bis-O-(trimethylsilyl)-6-fluourothymine (**7**), prepared as in method A, a solution of acetate **1** or **2** (2.11 mmol) in anhydrous MeCN (6.5 mL) was added. To this ice-cooled mixture TMSOTf (0.42 mL, 2.32 mmol) was added under stirring. Then the reaction mixture was stirred for 4 h from 0 °C to -15 °C. After the standard workup, the obtained residue was applied to a chromatographic column (silica gel, 150 cm^3) and eluted with a linear ethyl acetate gradient (15→35% v/v, 1600 ml) in hexane. The fractions containing the individual products **10** and **12** (or **11** and **13**) were collected and evaporated to dryness under vacuum.

*4.3.3. 1-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)-6-fluorothymine (**10**).* Yield 42%; white solid, m.p. 93-95 °C (from Et_2O/hexane); R_f (A) 0.54; ^{1}H NMR ($CDCl_3$): δ 8.81 (1H, s, NH), 8.08-7.31 (15H, m, Bz), 6.26 (1H, br s, H1′), 6.03 (1H, dd, J = 2.6, J = 6.4, H2′), 5.92 (1H, t, H3′), 4.84 (1H, dd, J = 3.1, J = 12.0, H5′), 4.69-4.66 (1H, m, H4′), 4.61 (1H, dd, J = 5.1, H5″), 1.86 (3H, d, J = 2.3, CH_3); ^{13}C NMR ($CDCl_3$): δ 166.3, 165.7 and 165.4 (3×C_6H_5C=O), 163.2 (d, J = 18.3, C4), 157.7 (d, J = 271.3, C6), 147.7 (d, J = 4.5, C2), 133.9-128.5 (18×C_{ar}), 92.8 (d, J = 16.7, C5), 87.9 (d, J = 4.8, C1′), 79.4 (C4′), 74.0 (d, J = 3.5, C2′), 70.5 (C3′), 63.3 (C5′), 6.6 (CH_3); ^{19}F NMR ($CDCl_3$): δ -98.94 (s, FC6); IR (KBr,): ν 1730, 1684, 1602, 1469, 1452, 1316, 1271, 1178, 1123, 1097, 1071, 1027, 712 cm^{-1}; HRMS $[M+NH_4]^+$ calcd for $C_{31}H_{29}FN_3O_9$: 606.1888, found 606.1883.

*4.3.4. 1-(2,5-Di-O-benzoyl-3-deoxy-3-fluoro-β-D-ribofuranosyl)-6-fluorothymine (**11**).* Yield 45%; white solid, m.p. 82-84 °C (from Et_2O/hexane); R_f (A) 0.53; ^{1}H NMR ($CDCl_3$): δ 8.39 (s, 1H, NH), 8.09-7.44 (10H, m, Bz), 6.24 (1H, br s, H1′), 5.97 (1H, dd, J = 6.3, J = 10.4, H2′), 5.59 (1H, dt, J = 53.1, H3′), 4.75 (1H, dt, J = 3.7, J = 11.8, H5′), 4.62-4.55 (2H, m, J = 5.0, H4′, H5″), 1.85 (3H, d, J = 2.4, CH_3); ^{13}C NMR ($CDCl_3$): δ 166.1 and 165.5 (2×C_6H_5C=O), 163.4 (d, J = 18.2, C4), 157.4 (d, J = 271.4, C6), 147.7 (d, J = 3.7, C2), 133.9-128.5 (12×C_{ar}), 92.7 (d, J = 16.5, C5), 87.7 (d, J = 194.4, C3′), 86.5 (d, J = 4.9, C1′), 79.9 (d, J = 25.4, C4′), 72.8 (d, J = 12.5, C2′), 62.8 (d, J = 3.0, C5′), 6.4 (CH_3); ^{19}F NMR ($CDCl_3$): δ -99.36 (s, FC6), -204.27 (m, FC′3); IR (KBr, cm^{-1}): ν 1730, 1684, 1602, 1469, 1452, 1316, 1271,

1178, 1123, 1097, 1071, 1027, 712; HRMS $[M+Na]^+$ calcd for $C_{24}H_{20}F_2N_2O_7Na$: 509.1136, found 509.1130.

*4.3.5. 1-(2,3,5-Tri-O-benzoyl-α-D-ribofuranosyl)-6-fluorothymine (**12**).* Yield 14%; white solid, m.p. 90-92 °C (from Et_2O/hexane); R_f (A) 0.46; ^1H NMR ($CDCl_3$): δ 8.61 (1H, s, NH), 8.07-7.31 (15H, m, Bz), 7.04 (1H, d, J = 5.7, H1′), 6.09 (1H, t, H2′), 5.79 (1H, t, H3′), 4.98-4.95 (1H, m, H4′), 4.77 (1H, dd, J = 3.1, J = 12.2, H5′), 4.56 (1H, dd, J = 4.0, H5″), 1.74 (3H, d, J = 2.3, CH_3); ^{13}C NMR ($CDCl_3$): δ 166.2, 165.2 and 164.6 (3×C_6H_5C=O), 163.0 (d, J = 17.6, C4), 158.4 (d, J = 272.7, C6), 147.9 (d, J = 4.7, C2), 134.1-128.3 (18×C_{ar}), 92.5 (d, J = 16.6, C5), 85.8 (C1′), 79.7 (d, J = 5.4, C4′), 71.4 (C3′), 70.6 (C2′), 63.6 (C5′), 6.2 (CH_3); ^{19}F NMR ($CDCl_3$): δ -94.51 (s, FC6); IR (KBr): ν 1730, 1679, 1602, 1475, 1452, 1417, 1316, 1270, 1178, 1135, 1096, 1069, 1026, 712 cm^{-1}; HRMS $[M+Na]^+$ calcd for $C_{31}H_{25}FN_2O_9Na$: 611.1442, found 611.1449.

*4.3.6. 1-(2,5-Di-O-benzoyl-3-deoxy-3-fluoro-α-D-ribofuranosyl)-6-fluorothymine (**13**).* Yield 11%; white solid, m.p. 78-80 °C (from Et_2O/hexane); R_f (A) 0.43; ^1H NMR ($CDCl_3$): δ 8.18 (1H, s, NH), 8.06-7.43 (10H, m, Bz), 6.97 (1H, d, J = 6.7, H1′), 5.84 (1H, dt, J = 12.8, H2′), 5.41 (1H, ddd, J = 4.2, J = 5.9, J = 52.7, H3′), 4.95-4.90 (1H, dm, J = 18.5, H4′), 4.65 (1H, dd, J = 3.2, J = 12.3, H5′), 4.53 (1H, dd, J = 3.5, H5″), 1.85 (3H, d, J = 2.4, CH_3); ^{13}C NMR ($CDCl_3$): δ 166.0 and 164.6 (2×C_6H_5C=O), 163.1 (d, J = 17.4, C4), 158.5 (d, J = 271.1, C6), 148.0 (d, J = 4.8, C2), 134.1-128.6 (12×C_{ar}), 92.5 (d, J = 16.8, C5), 88.8 (d, J = 198.7, C3′), 84.8 (C1′), 80.7 (dd, J = 3.8, J = 25.4, C4′), 70.5 (d, J = 14.3, C2′), 63.6 (d, J = 5.7, C5′), 6.2 (CH_3); ^{19}F NMR ($CDCl_3$): δ -94.12 (s, FC6), -202.96 (m, FC3′); IR (KBr): ν 1730, 1679, 1602, 1475, 1452, 1417, 1316, 1270, 1178, 1133, 1095, 1069, 1026, 712 cm^{-1}; HRMS $[M+Na]^+$ calcd for $C_{24}H_{20}F_2N_2O_7Na$: 509.1136; found 509.1132.

4.4. General procedure for the glycosylation of 6-fluorothymine with benzoates 5 and 6

To a suspension of 6-fluorothymine (0.10 g, 0.69 mmol) in anhydrous MeCN (5.5 mL) was added BSTFA (0.55 mL, 2.07 mmol), the mixture was stirred for 2 h at room temperature and the obtained homogeneous solution was evaporated to dryness under vacuum. To the obtained 2,4-bis-O-(trimethylsilyl)-6-fluourothymine (**7**) a solution of benzoate **5** or **6** (0.31 g, 0.55 mmol) in anhydrous MeCN (4.5 mL) was added. To this ice-cooled mixture TMSOTf (1.1 mL, 0.61 mmol) was added under stirring, then the reaction mixture was stirred for 4 h from 0 °C to -15 °C. After the standard workup, the obtained residue was applied to a chromatographic column (silica gel, 60 cm^3) and eluted with a linear ethyl acetate gradient (15→35% v/v, 600 mL) in hexane. The fractions containing the products **10** and **12** were collected and evaporated to yield *β*-anomer 0.19 g (60%), *α*:*β* - 4.3:1 from **5** and 0.16 g (51%), *α*:*β* - 2.4:1 from **6**.

4.5. General procedure for the debenzoylation of 6-fluorothymine ribonucleosides 8-13 with LiOH monohydrate

To a solution of benzoylated 6-fluorothymine nucleoside **8-13** (0.21 mmol) in MeCN (31 mL) and H_2O (13 mL) was added LiOH·H_2O (0.76 mmol for **8**, **10**, **12** and 0.52 mmol for **9**, **11**, **13**). The reaction mixture was stirred for 5 h at room temperature, neutralized by the addition of Amberlite IRG-5 (H^+-form) ion-exchange resin, then filtered, washed with methanol and evaporated to dryness under vacuum. The obtained residue was purified by column chromatography (silica gel, 50 cm^3) using a linear MeOH gradient (0→15% v/v, 600 mL) in $CHCl_3$. The fractions containing the individual products **14-17** and **21**, **22** were collected and evaporated to dryness under vacuum.

*4.5.1. 3-(β-D-Ribofuranosyl)-6-fluorothymine (**14**).* Yield 57%; white solid, m.p. 179-181 °C (from Et_2O/hexane); R_f (B) 0.21; UV λ, nm (lg ε): pH 1, λ_{max} 257 (3.83), λ_{min} 232 (3.43); pH 7, λ_{max} 281 (4.0), λ_{min} 247 (2.59); pH 13, λ_{max} 281 (4.0), λ_{min} 247 (3.18); ^1H NMR (CD_3OD): δ 6.40 (1H, d, J = 4.1, H1′), 4.72 (1H, dd, J = 6.2, H2′), 4.39 (1H, t, H3′), 3.92-3.89 (1H, m, H4′), 3.81 (1H, dd, J = 2.6, J = 11.9, H5′), 3.68 (1H, dd, J = 4.8, H5″), 1.77 (3H, d, J <1, CH_3); ^{13}C NMR (CD_3OD): δ 169.0 (d, J = 17.5, C4), 167.4 (d, J = 242.8, C6), 156.8 (d, J = 31.5, C2), 90.0 (C1′), 87.3 (d, J = 25.9, C5), 85.7 (C4′), 72.8 (C2′), 71.6 (C3′), 63.7 (C5′), 6.9 (CH_3); ^{19}F NMR (CD_3OD): δ -75.43 (s, FC6); IR (KBr): ν 1724, 1657, 1636, 1477, 1439, 1382, 1257, 1143, 1104, 1050, 859 cm^{-1}; HRMS $[M+Na]^+$ calcd for $C_{10}H_{13}FN_2O_6Na$: 299.0655, found 299.0650.

*4.5.2. 3-(3-Deoxy-3-fluoro-β-D-ribofuranosyl)-6-fluorothymine (**15**).* Yield 63%; colorless oil; UV λ, nm (lg ε): pH 1, λ_{max} 258 (3.82), λ_{min} 233 (3.42); pH 7, λ_{max} 282 (3.96), λ_{min} 248 (2.57); pH 13, λ_{max} 282 (3.96), λ_{min} 248 (3.05); NMR (CD_3OD): δ 6.45 (1H, d, J = 6.1, H1′), 5.06 (1H, ddd, J = 3.0, J = 5.2, J = 56.1, H3′), 5.05 (1H, dt, J = 15.2, H2′), 4.21-4.14 (1H, dm, J = 25.0, H4′), 3.74 (1H, dd, J = 3.2, J = 12.1, H5′), 3.66 (1H, dd, J = 3.8, H5″), 1.74 (3H, d, J <1, CH_3); ^{13}C NMR (CD_3OD): δ 169.6 (d, J = 17.6, C4), 168.9 (d, J = 245.9, C6), 158.6 (d, J = 29.7, C2), 93.5 (d, J = 182.9, C3′), 88.7 (C1′), 87.4 (d, J = 26.5, C5), 84.3 (d, J = 23.4, C4′), 71.2 (d, J = 15.3, C2′), 63.3 (d, J = 8.3, C5′), 7.1 (CH_3); ^{19}F NMR (CD_3OD): δ -74.23 (s, FC6), -202.29 (m, FC3′); HRMS $[M+Na]^+$ calcd for $C_{10}H_{12}F_2N_2O_5Na$: 301.0612, found 301.0607.

*4.5.3. 1-(β-D-Ribofuranosyl)-6-fluorothymine (**16**).* Yield 79%; white solid, m.p. 135-136 °C (from Et_2O); R_f (B) 0.67; UV λ, nm (lg ε): pH 1, λ_{max} 255 (4.03), λ_{min} 228 (3.53); pH 7, λ_{max} 255 (4.03), λ_{min} 228 (3.53); pH 13, λ_{max} 258 (3.86), λ_{min} 241 (3.68); ^1H NMR (DMSO-d6): δ 11.63 (1H, br s, NH), 5.87 (1H, d, J = 4.3, H1′), 5.33 (1H, d, J = 5.2, 2′OH), 5.07 (1H, d, J = 6.0, 3′OH), 4.76 (1H, t, J = 5.7, 5′OH), 4.32 (1H, t, H2′), 3.95-3.92 (1H, m, H3′), 3.71-3.68 (1H, m, H4′), 3.55 (1H, dd, J = 3.7, J = 11.8, H5′), 3.45-3.40 (1H, m, J = 5.7, H5″), 1.73 (3H, d, J = 2.4, CH_3); ^{13}C NMR (DMSO-d_6): δ 163.3 (d, J = 17.9, C4), 157.9 (d, J = 269.7, C6), 148.3 (d, J = 3.9, C2), 90.9 (d, J = 15.6, C5), 88.5 (C1′), 84.4 (C4′), 71.4 (d, J = 3.9, C2′), 69.2 (C3′), 61.4 (C5′), 6.1 (CH_3); ^{19}F NMR (DMSO-d6): δ -97.89 (s, FC6); IR (KBr): ν 1724, 1685, 1669, 1475, 1427, 1387, 1232, 1121, 1098, 1064, 1036, 857 cm^{-1}; HRMS $[M+Na]^+$ calcd for $C_{10}H_{13}FN_2O_6Na$: 299.065, found 299.0651.

*4.5.4. 1-(3-Deoxy-3-fluoro-β-D-ribofuranosyl)-6-fluorothymine (**17**).* Yield 75%; colorless oil; R_f (B) 0.72; UV λ, nm (lg ε): pH 1, λ_{max} 255 (3.95), λ_{min} 228 (3.61); pH 7, λ_{max} 255 (3.95), λ_{min} 228 (3.61); pH 13, λ_{max} 258 (3.84), λ_{min} 241 (3.69); ^1H NMR (DMSO-d_6): δ 11.72 (1H, br s, NH), 5.91 (1H, d, J = 6.9, H1′), 5.87 (1H, d, J = 5.8, 2′OH), 4.98 (1H, t, J = 5.4, 5′OH), 4.96 (1H, ddd, J = 2.3, J = 4.6, J = 54.4, H3′), 4.64 (1H, dt, J = 19.1, H2′), 4.10-4.03 (1H, dm, J = 25.2, H4′), 3.52-3.50 (2H, m, H5′, H5″), 1.73 (3H, d, J = 2.3, CH_3); ^{13}C NMR (DMSO-d_6): δ 163.2 (d, J = 18.1, C4), 157.8 (d, J = 270.5, C6), 148.4 (d, J = 4.1, C2), 91.2 (d, J = 15.5, C5), 91.0 (d, J = 183.3, C3′), 86.8 (C1′), 82.5 (d, J = 22.1, C4′), 69.5 (dd, J = 5.6, J = 15.8, C2′), 60.4 (d, J = 8.3, C5′), 6.1 (CH_3); ^{19}F NMR (DMSO-d_6): δ -98.24 (s, FC6), -199.82 (m, FC3′). HRMS $[M+Na]^+$ calcd for $C_{10}H_{12}F_2N_2O_5Na$: 301.0612, found 301.0604.

*4.5.5. 6,2′-Anhydro-1-(α-D-ribofuranosyl)-6-hydroxythymine (**21**).* Yield 81%; white solid, m.p. 234-236 °C (from Et_2O); R_f

(B) 0.53; UV λ, nm (lg ε): pH 1, λ_{max} 260 (4.08); pH 7, λ_{max} 260 (4.14); pH 13, λ_{max} 262 (3.97); ^{1}H NMR (DMSO-d_6): δ 11.04 (1H, br s, NH), 6.14 (1H, d, J = 4.8, H1′), 5.72 (1H, d, J = 5.7, 3′OH), 5.19 (1H, t, H2′), 4.86 (1H, t, J = 5.1, 5′OH), 4.01 (1H, dd, J = 5.1, J = 9.3, H3′), 3.68 (1H, d, H5′), 3.56 (1H, ddd, J = 1.6, J = 4.2, J = 9.3, H4′) 3.43 (1H, dd, J = 12.4, H5″), 1.68 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6): δ 165.3 (C4), 159.0 (C6), 146.5 (C2), 85.8 (C1′), 84.3 (C4′), 81.3 (C5), 80.2 (C2′), 69.6 (C3′), 59.2 (C5′), 6.7 (CH_3); IR (KBr): ν 1750, 1682, 1665, 1650, 1489, 1228, 1049, 1034, 1003, 1021, 772 cm^{-1}; HRMS [M+H]$^+$ calcd for $C_{10}H_{13}N_2O_6$: 257.0774, found 257.0767.

*4.5.6. 6,2′-Anhydro-1-(3-deoxy-3-fluoro-α-D-ribofuranosyl)-6-hydroxythymine (**22**).* Yield 85%; white solid, m.p. 221-223 °C (from Et_2O); R_f (B) 0.63; UV λ, nm (lg ε): pH 1, λ_{max} 259 (4.06); pH 7, λ_{max} 259 (4.11); pH 13, λ_{max} 261 (3.93); ^{1}H NMR (DMSO-d_6): δ 11.13 (1H, s, NH), 6.21 (1H, d, J = 5.1, H1′), 5.49 (1H, dt, J = 1.7, H2′), 5.22 (1H, br s, 5′OH), 5.17 (1H, ddd, J = 5.2, J = 7.3, J = 50.5, H3′), 4.03 (1H, ddd, J = 7.3, J = 14.2, H4′), 3.66 (1H, dd, J = 2.6, J = 12.6, H5′), 3.54 (1H, dd, J = 3.6, H5″), 1.67 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6): δ 165.4 (C4), 159.1 (C6), 146.5 (C2), 88.2 (d, J = 192.8, C3′), 86.5 (d, J = 4.8, C1′), 81.8 (C5), 81.6 (d, J = 12.7, C2′), 79.1 (d, J = 22.8, C4′), 58.9 (C5′), 6.7 (CH_3); ^{19}F NMR (DMSO-d_6): δ -212.65 (dd, FC3′); IR (KBr): ν 1734, 1673, 1662, 1619, 1489, 1226, 1105, 1053, 1034, 767 cm^{-1}; HRMS [M+H]$^+$ calcd for $C_{10}H_{12}FN_2O_5$: 259.0730, found 259.0726.

4.6. General procedure for the debenzoylation of 6-fluorothymine ribonucleosides 10 and 11 with methanolic ammonia

Compound **10** or **11** (0.50 mmol) was dissolved in MeOH (15 mL) saturated with NH_3 at 0 °C and stirred for 24 h at room temperature. The reaction mixture was evaporated to dryness under vacuum and the obtained residue was purified by column chromatography (silica gel, 60 cm^3) using a linear MeOH gradient (0→30% v/v, 500 mL) in $CHCl_3$. The fractions containing the individual product **18** or **19** were collected and evaporated to dryness under vacuum.

*4.6.1. 1-(β-D-Ribofuranosyl)-6-aminothymine (**18**).* Yield 72%; white solid, m.p. 124-126 °C (from EtOH); R_f (B) 0.23; UV λ, nm (lg ε): pH 1, λ_{max} 280 (4.26); pH 7, λ_{max} 280 (4.26); pH 13, λ_{max} 281 (4.11); ^{1}H NMR (DMSO-d_6): δ 10.57 (1H, s, NH), 6.51 (2H, s, NH_2), 6.24 (1H, d, J = 7.3, H1′), 5.48 (1H, t, J = 4.6, 5′OH), 5.24 (1H, d, J = 6.4, 3′OH), 5.04 (1H, d, J = 4.9, 2′OH), 4.41-4.37 (1H, m, H2′), 4.05-4.02 (1H, m, H3′), 3.81 (1H, br s, H4′), 3.64-3.57 (2H, m, J = 11.8, H5′, H5″), 1.65 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6): δ 162.2 (C4), 151.7 (C2), 150.5 (C6), 87.9 (C1′), 84.7 (C4′), 82.17 (C5), 69.2 (C2′), 69.1 (C3′), 60.0 (C5′), 8.1 (CH_3); IR (KBr): ν 1715, 1693, 1627, 1572, 1505, 1293, 1191, 1117, 1072, 1044 cm^{-1}; HRMS [M+Na]$^+$ calcd for $C_{10}H_{15}N_3O_6Na$: 296.0859, found 509.0845.

*4.6.2. 1-(3-Deoxy-3-fluoro-β-D-ribofuranosyl)-6-aminothymine (**19**).* Yield 75%; white solid; R_f (B) 0.42; UV λ, nm (lg ε): pH 1, λ_{max} 280 (4.23); pH 7, λ_{max} 280 (4.23); pH 13, λ_{max} 281 (4.03); ^{1}H NMR (DMSO-d_6): δ 10.64 (1H, s, NH), 6.59 (2H, s, NH_2), 6.33 (1H, d, J = 8.9, H1′), 5.80 (1H, d, J = 6.3, 2′OH), 5.70 (1H, br s, 5′OH), 4.96 (1H, dd, J = 55.6, H3′), 4.65 (1H, dm, J = 22.1, H2′), 4.21 (1H, d, J = 30.4, H4′), 3.75 (1H, dd, J = 4.3, J = 11.8, H5′), 3.57 (1H, d, H5″), 1.66 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6): δ 162.2 (C4), 151.4 (C6), 150.6 (C2), 91.9 (d, J = 182.2, C3′), 86.2 (C1′), 82.6 (d, J = 24.1, C4′), 82.1 (C5), 67.9 (d, J = 15.2, C2′), 60.0 (d, J = 11.0, C5′), 8.2 (CH_3); ^{19}F NMR (DMSO-d_6): δ -195.54 (m, FC3′); HRMS [M+Na]$^+$ calcd for $C_{10}H_{14}FN_3O_5Na$: 298.0815, found 298.0810.

4.7. Procedure for the debenzoylation of 6-fluorothymine ribonucleosides 10 with NaOMe in methanol

Compound **10** (0.43 mmol) was dissolved in anhydrous MeOH (20 mL). To this was added a methanolic solution of 1.25 M NaOMe (2 mL). The reaction mixture was stirred for 2 h at room temperature and neutralized by glacial AcOH. The mixture was evaporated to dryness under vacuum and the residue was purified with column chromatography (silica gel, 40 cm^3) using a linear MeOH gradient (0→30% v/v, 400 mL) in $CHCl_3$. The fractions containing the product **20** were collected and evaporated to dryness.

*4.7.1. 1-(β-D-Ribofuranosyl)-6-methoxythymine (**20**).* Yield 68%; white solid, m.p. 172-174 °C (from Et_2O); R_f (B) 0.64; UV λ, nm (lg ε): pH 1, λ_{max} 265 (4.02); pH 7, λ_{max} 265 (4.02); pH 13, λ_{max} 266 (3.89); ^{1}H NMR (DMSO-d_6): δ 11.35 (1H, br s, NH), 5.71 (1H, br s, H1′), 5.21 (1H, br s, 2′OH), 4.95 (1H, br s, 3′OH), 4.72 (1H, t, 5′OH), 4.41 (1H, d, H2′), 3.95 (1H, t, H3′), 3.88 (3H, s, OCH_3), 3.67-3.64 (1H, m, H4′), 3.60 (1H, dd, J = 3.5, J = 11.8, H5′), 3.46 (1H, dd, J = 6.1, H5″), 1.77 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6): δ 164.1 (C4), 159.1 (C6), 149.4 (C2), 96.7 (C1′), 89.0 (C4′), 83.9 (C5), 70.9 (C2′), 69.3 (C3′), 62.6 (OCH_3), 61.5 (C5′), 7.9 (CH_3); IR (KBr): ν 1707, 1698, 1670, 1623, 1476, 1246, 1133, 1100, 1044, 1021 cm^{-1}; HRMS [M+Na]$^+$ calcd for $C_{11}H_{16}N_2O_7Na$: 311.0855, found 311.0849.

4.8. General procedure for the benzoylation of anhydronucleosides 21 and 22

To a ice-cooled solution of 6,2′-anhydronucleosides **21** or **22** (0.07 mmol) in anhydrous pyridine (1 mL) was added benzoyl chloride (0.42 mmol for **21** and 0.32 mmol for **22**) and the mixture was stirred for 20 h at room temperature. After standard workup the obtained residue was applied to a chromatographic column (silica gel, 30 cm^3) and eluted with a linear ethyl acetate gradient (0→30% v/v, 300 mL) in hexane. The fractions containing the individual product **23**, **24** or **25** were collected and evaporated to dryness under vacuum.

*4.8.1. 6,2′-Anhydro-1-(3,5-di-O-benzoyl-α-D-ribofuranosyl)-N-3-benzoyl-6-hydroxythymine (**23**).* Yield 75%; colorless oil; ^{1}H NMR ($CDCl_3$): δ 8.05-7.38 (15H, m, Bz), 6.54 (1H, d, J = 4.9, H1′), 5.72 (1H, t, H2′), 5.31 (1H, dd, J = 5.3, J = 8.9, H3′), 4.74 (1H, dd, J = 3.5, J = 12.2, H5′), 4.64-4.57 (2H, m, H5″, H4′), 1.75 (3H, s, CH_3); HRMS [M+Na]$^+$ calcd for $C_{31}H_{24}N_2O_9Na$: 591.1380, found 591.1377.

*4.8.2. 6,2′-Anhydro-1-(5-O-benzoyl-3-deoxy-3-fluoro-α-D-ribofuranosyl)-N-3-benzoyl-6-hydroxythymine (**24**).* Yield 81%; colorless oil; ^{1}H NMR ($CDCl_3$): δ 8.05-7.45 (10H, m, Bz), 6.45 (1H, d, J = 4.9, H1′), 5.41 (1H, t, H2′), 5.13 (1H, ddd, J = 5.2, J = 8.2, J = 50.1, H3′), 4.71 (1H, dd, J = 3.4, J = 12.5, H5′), 4.61 (1H, dd, J = 4.0, H5″), 4.49-4.44 (1H, m, H4′), 1.91 (3H, s, CH_3); HRMS [M+Na]$^+$ calcd for $C_{24}H_{19}FN_2O_7Na$: 489.1074, found 489.1076.

*4.8.3. 6,2′-Anhydro-1-(3,5-di-O-benzoyl-α-D-ribofuranosyl)-6-hydroxythymine (**25**).* Yield 20%; colorless oil; ^{1}H NMR ($CDCl_3$): δ 8.52 (s, 1H, NH), 8.03-7.37 (10H, m, Bz), 6.51 (1H, d, J = 5.0, H1′,), 5.68 (1H, t, H2’), 5.28 (1H, dd, J = 5.4, J = 9.1, H3’), 4.73 (1H, dd, J = 3.7, J = 12.4, H-5′), 4.60 (1H, dd, J = 4.2, H5″), 4.53-4.50 (1H, m, H4′), 1.73 (3H, s, CH_3); HRMS [M+Na]$^+$ calcd for $C_{24}H_{20}N_2O_8Na$: 487.1117, found 487.1125.

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Supplementary material

Supplementary data Supplementary data related to this article can be found in the online version.

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