SYNTHESIS OF 2′-*C*-METHYLCYTIDINE AND 2′-*C*-METHYLURIDINE DERIVATIVES MODIFIED IN THE 3′-POSITION AS POTENTIAL ANTIVIRAL AGENTS

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Dedicated to Professor Antonín Holý on the occasion of his 70th birthday in recognition of his outstanding contributions to the area of nucleic acid chemistry.

As part of our anti-hepatitis C program, we recently discovered 2′-*C*-methylcytidine (**1**) and 2′-*C*-methyluridine (**2**), which are potent inhibitors in cell culture of several viruses (bovine viral diarrhea virus (BVDV), yellow fever virus (YFV)) closely related to HCV. In order to characterize structure–activity relationships, we introduced some structural and functional modifications into the 3′-position of 2′-*C*-methylcytidine and 2′-*C*-methyluridine. All these hitherto unknown compounds thus synthesized were tested for the activity against a wide range of viruses and found to be inactive.

Keywords: Pyrimidines; Antivirals; Hepatitis C virus; 2′-*C*-Methyl branched ribonucleosides; Deoxygenation; Fluorination; RNA viruses; Nucleosides.

Hepatitis C virus (HCV) is the pathogen responsible for one of the most common chronic blood-born infections. Indeed, the prevalence of HCV infection is now thought to be around five-fold greater than HIV infection[1] with an estimated 170 million people infected worldwide[2–4]. While often asymptomatic, HCV can progress to chronic hepatitis, leading to liver cirrhosis and sometimes to hepatocellular carcinoma[1,2]. Current therapy for chronic hepatitis C [1,5,6] consists of pegylated (or non-pegylated) interferon α combined with the nucleoside analog ribavirin, neither of which acts directly on the virus, and whose antiviral effects seem to be mediated by mul-

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tiple indirect mechanisms, involving immunologic routes. However, those compounds are both poorly tolerated and have limited efficacy, with less than 50% response rates among patients infected with the most prevalent HCV genotype (1b)[7,8]. There is therefore an urgent need for more effective and better tolerated anti-HCV agents[9].

As part of our ongoing effort to develop new chemotherapeutic agents against HCV, we identified and reported a series of branched-sugar ribonucleosides that could be useful for that purpose[10,11]. Among them, 2′-*C*-methylcytidine **1** (Fig. 1), and its somewhat less active uridine counterpart 2′-*C*-methyluridine **2 (**Fig. 1), were found to be selective inhibitors in cell culture of a number of *Flaviviridae* including the pestivirus BVDV (bovine viral diarrhea virus, a surrogate model for HCV), and the flavivirus YFV (yellow fever virus)[10,11]. Encouraged by these results, we performed a structure–activity relationship (SAR) study focused on evaluation of modifications at the 3′-position of the sugar moiety. We report herein the synthesis of several hitherto unknown 3′-modified 2′-*C*-methylribonucleosides, in both the uracil and cytosine series (Fig. 1).

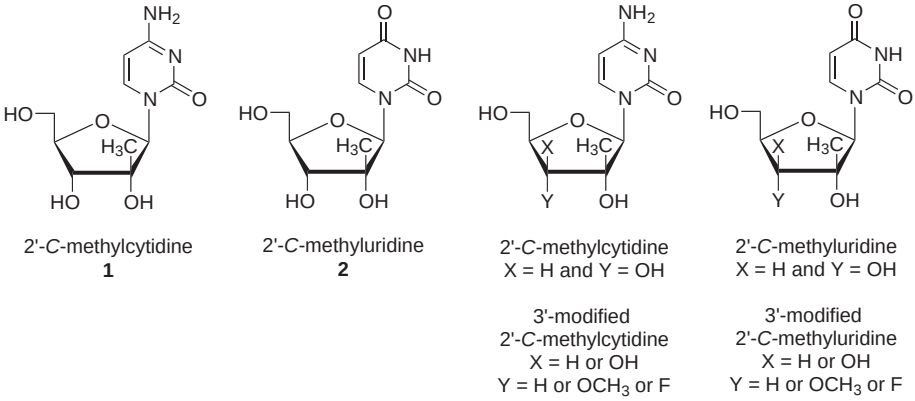


FIG. 1
Structures of 2′-*C*-methylcytidine (**1**), 2′-*C*-methyluridine (**2**) and of their hitherto unknown derivatives modified at the 3′-position

As a number of triphosphate derivatives of nucleosides lacking the 3′-hydroxy group have been identified as potent inhibitors of the HCV NS5B-polymerase[12], first we decided to synthesize the respective 3′-deoxy derivatives of 2′-*C*-methylcytidine and 2′-*C*-methyluridine as well as their xylofuranosyl derivatives.

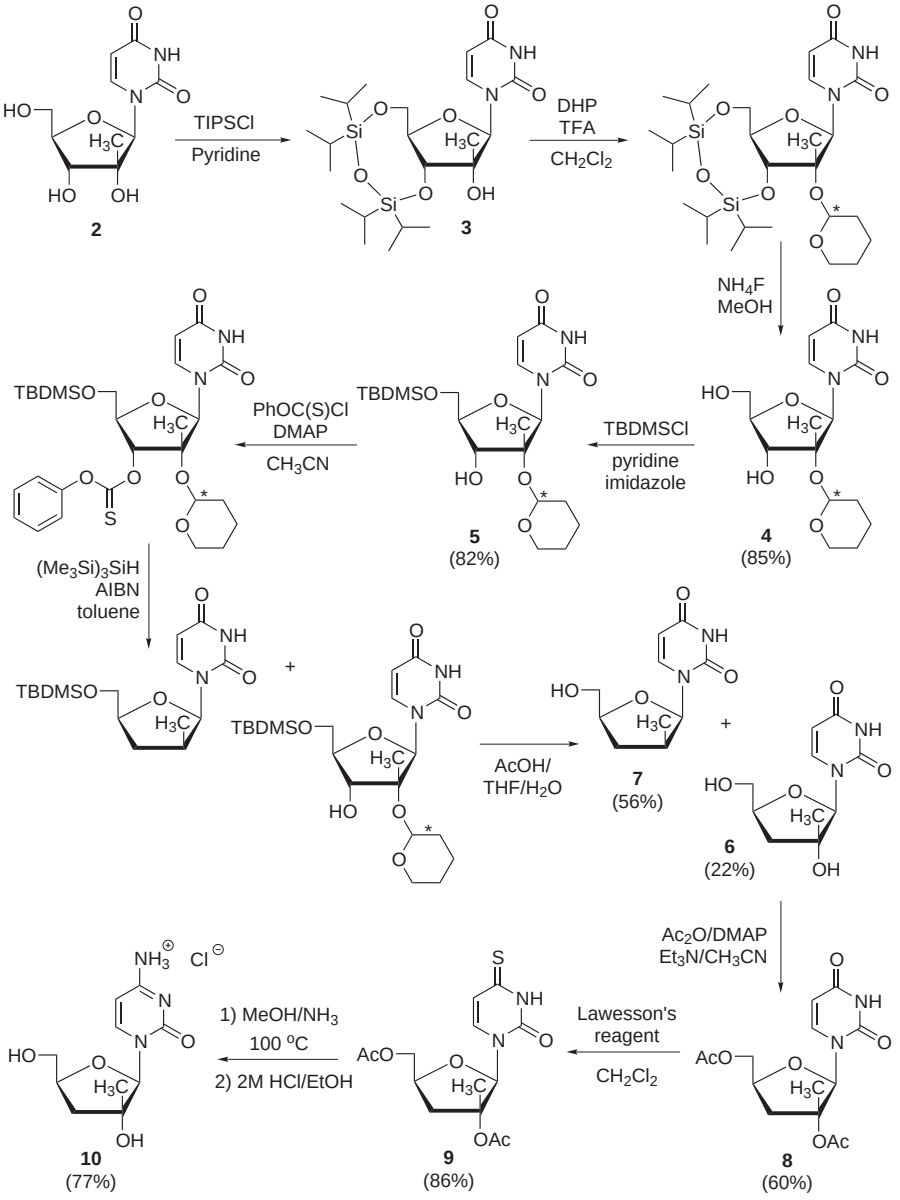
Then, we considered the effect of either *O*-methylation or fluorination of the 3′-hydroxy group. The first modification was suggested due to the fact

that, although this modification introduced in the 2′-position of 2′-*C*-methyladenosine has led to an inactive compound[12], 2′-*O*-methylcytidine and 2′-*O*-methylguanosine are potent inhibitors of HCV replication that specifically target the NS5B polymerase[12–14]. Finally, and based on the potent HCV inhibition properties of 2′-deoxy-2′-fluorocytidine[15] and 2′-deoxy-2′-fluoro-2′-*C*-methylcytidine[16], we decided to study the effect of fluorination on the 3′-position of 2′-*C*-methylcytidine.

RESULTS AND DISCUSSION

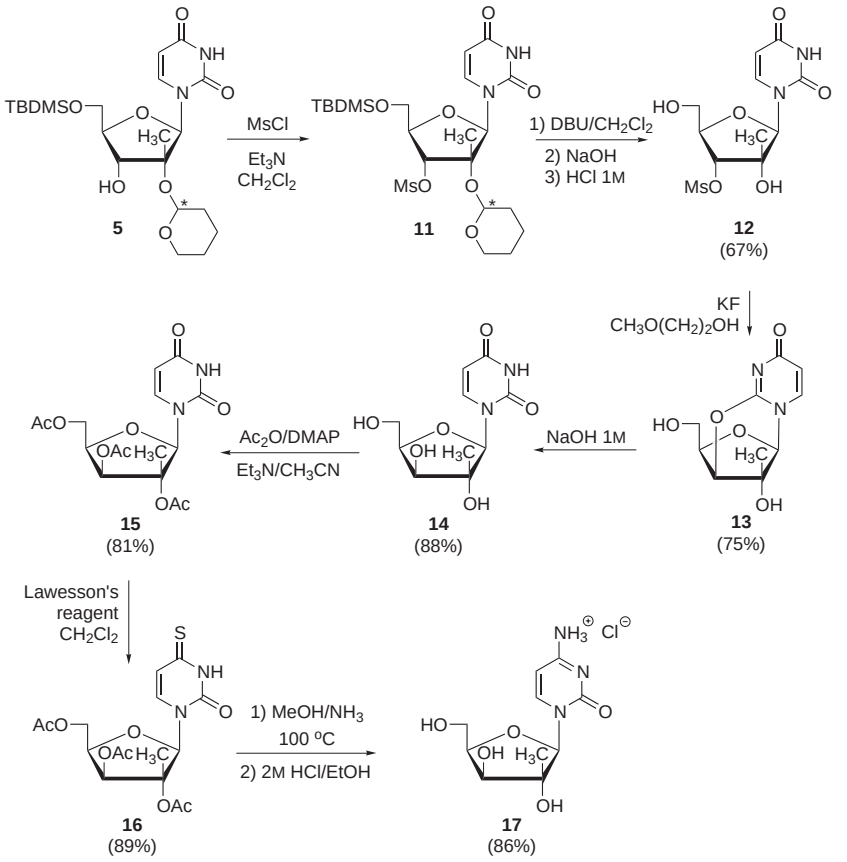
3′-Deoxy-2′-*C*-methyluridine (**6**) and 3′-deoxy-2′-*C*-methylcytidine hydrochloride (**10**) were synthesized starting from 2′-*C*-methyluridine (**2**)[17,18] (Scheme 1). First, the 3′- and 5′-hydroxy groups of **2** were protected with the bifunctional reagent 1,1,3,3-tetraisopropyldisiloxane (TIPDS), followed by tetrahydropyranylation of the 2′-hydroxy group in the presence of dihydropyran and trifluoroacetic acid[19,20]. Removal of the silyl group with excess ammonium fluoride (NH_4F) in methanol[21], an economical alternative to tetrabutylammonium fluoride in tetrahydrofuran (TBAF/THF), followed by selective silylation of the 5′ primary hydroxy group using a reported method[22] afforded the protected key intermediate **5** in 70% overall yield. Barton deoxygenation of compound **5** led to the desired 3′-deoxynucleoside **6** contaminated with a large amount of the corresponding 2′,3′-dideoxy derivative **7**. This undesired side reaction resulted from an unexpected 2′-hydroxy group deprotection which occurred during the deoxygenation step and can be explained by the presence of hydrochloric acid during reaction with chlorothioformate reagent. After removal of both silyl and THP groups using 4:2:1 acetic acid–tetrahydrofuran–water at room temperature[23], compounds **6** and **7** were isolated in 22 and 56% yields, respectively. 3′-Deoxy-2′-*C*-methyluridine **6** was acetylated, and aminated to its cytidine analog by sequential treatment with Lawesson's reagent[24] followed by methanol saturated with ammonia at 100 °C to give 3′-deoxy-2′-*C*-methylcytidine which was later quantitatively transformed into its hydrochloride **10** (77% yield).

Key intermediate **5** was also used as the starting material for the synthesis of 1-(2-*C*-methyl-β-D-xylofuranosyl)uracil and -cytosine derivatives (Scheme 2). Thus, mesylation of the secondary hydroxy group of **5**, using mesyl chloride and triethylamine in anhydrous methylene chloride, gave 3′-*O*-mesyl derivative **11** in quantitative yield. An attempt to invert the configuration via anhydro bond formation, using DBU followed by treatment with sodium hydroxide failed and led, after quenching with hydro-



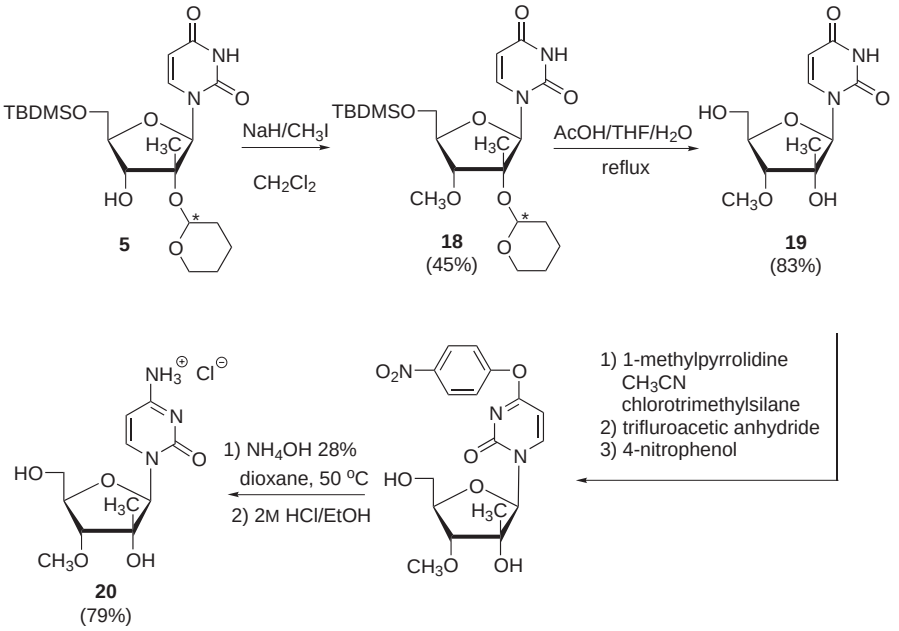
SCHEME 1
Synthesis of 3′-deoxy-2′-*C*-methyluridine (**6**) and 3′-deoxy-2′-*C*-methylcytidine hydrochloride (**10**)

chloric acid, to the unprotected mesyl derivative **12** in 67% yield. Surprisingly, treatment of **12** with potassium fluoride in 2-methoxyethanol led to the formation of 2,3′-anhydro-2′-*C*-methyluridine (**13**) in 75% yield. Finally, opening of the anhydro bond was effected quantitatively with sodium hydroxide and gave the desired 1-(2-*C*-methyl-β-D-xylofuranosyl)-uracil (**14**) in 88% yield. Here again, compound **14** was acetylated to give **15**, which was treated with Lawesson's reagent to give the thiouridine derivative **16**. Finally, treatment of **16** with ammonia at 100 °C led to desired 1-(2-*C*-methyl-β-D-xylofuranosyl)cytidine (**17**) which was later transformed into its hydrochloride.



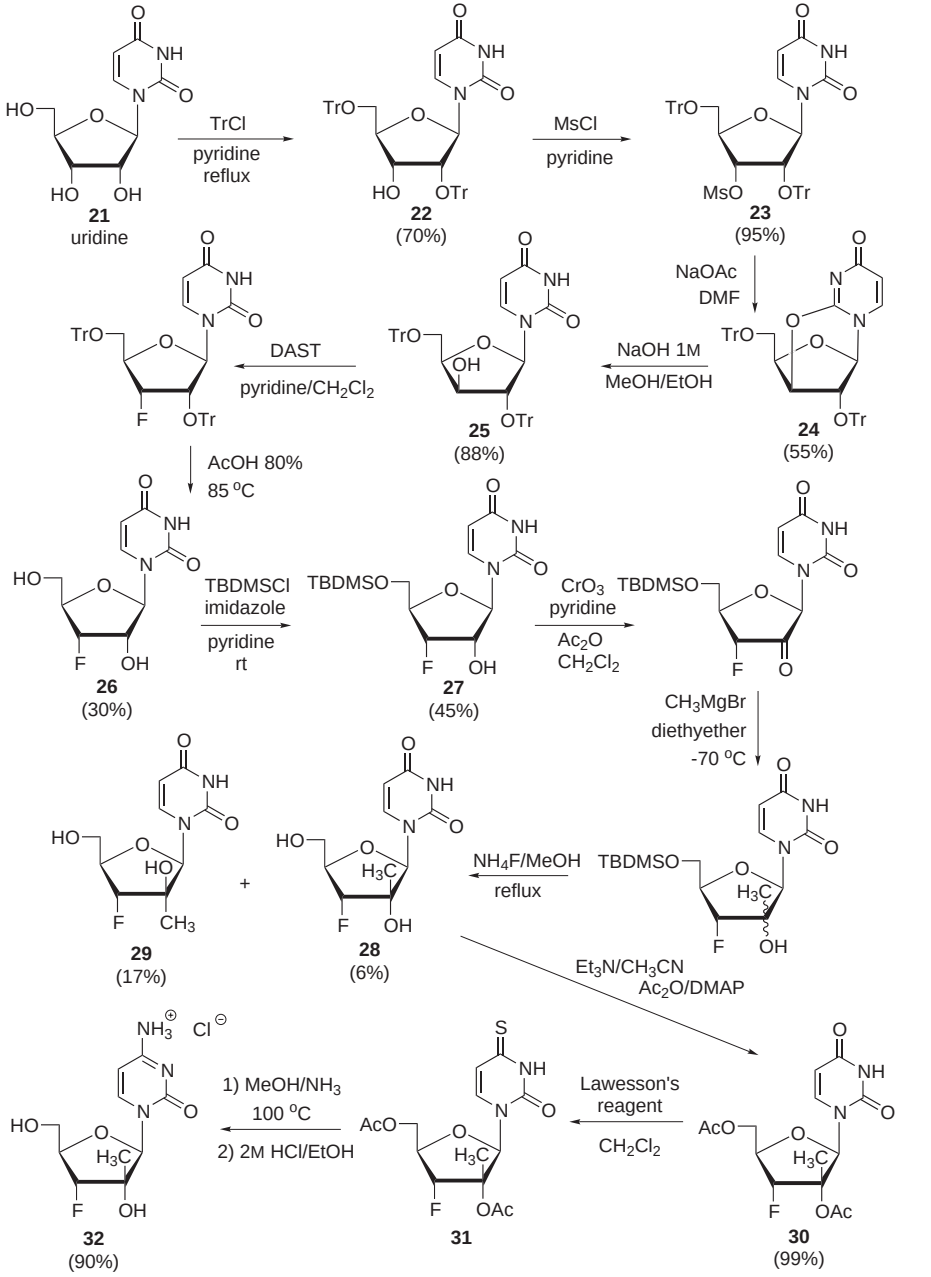
SCHEME 2
Synthesis of 1-(2-*C*-methyl-β-D-xylofuranosyl)uracil (**14**) and 1-(2-*C*-methyl-β-D-xylofuranosyl)-cytosine hydrochloride (**17**)

Key intermediate **5** was also used for the synthesis of 2′-*C*,3′-*O*-dimethyluridine and -cytidine derivatives (Scheme 3). 3′-*O*-Alkylation of **5** was performed by reaction with methyl iodide in anhydrous methylene chloride in the presence of sodium hydride[25]. 2′-*C*,3′-*O*-Dimethyluridine (**19**) was isolated in 83% yield after removal of the protecting groups, and converted to the corresponding cytidine derivative by 4-nitrophenylation[26]. Thus, following trimethylsilylation of **19** in acetonitrile, the resulting protected nucleoside was reacted successively with trifluoroacetic anhydride and 1-methylpyrrolidine, and finally with 4-nitrophenol. The 4-*O*-(4-nitrophenyl) derivative was treated directly with ammonia in aqueous dioxane to give 2′-*C*,3′-*O*-dimethylcytidine which was isolated in 79% overall yield, and transformed into its hydrochloride **20**.



SCHEME 3
Synthesis of 2′-*C*,3′-*O*-dimethyluridine (**19**) and 2′-*C*,3′-*O*-dimethylcytidine hydrochloride (**20**)

Several attempts to introduce a fluorine atom into the 3′-position starting from a suitably protected 2,3′-anhydro-2′-*C*-methyluridine or 1-(2-*C*-methyl-β-D-xylofuranosyl)uracil were unsuccessful. So, we developed another strategy for the preparation of 3′-deoxy-3′-fluoro-2′-*C*-methyluridine and -cytidine derivatives. This involved preliminary 3′-fluorination of protected uridine before introducing the 2′-*C*-methyl branching (Scheme 4).



SCHEME 4
Synthesis of 3′-deoxy-3′-fluoro-2′-*C*-methylcytidine hydrochloride (**32**)

Selective 2′,5′-di-*O*-tritylation of uridine led to the intermediate **22** in 70% yield which was then quantitatively mesylated at the 3′-position, and treated with sodium acetate at 100 °C [27] to afford the anhydro derivative **24** in 55% yield. After breaking off the 2,3′-anhydro bond in basic conditions, fluorination was achieved with diethylaminosulfur trifluoride (DAST)[28] to give, after detritylation, 3′-deoxy-3′-fluorouridine (**26**) in 30% yield. This compound was selectively silylated in the 5′-position and subsequently oxidized under Jones' conditions[12] to give a 2′-keto intermediate, which was reacted with methylmagnesium bromide[12] and deprotected to afford the two diastereoisomers **28** and **29** (isolated in 6 and 17% yields, respectively). Finally, 3′-deoxy-3′-fluoro-2′-*C*-methyluridine (**28**) was transformed in three steps into its cytosine counterpart, according to the strategy described for compounds **10** and **17**.

All the synthesized 3′-modified 2′-*C*-methylnucleosides **6**, **10**, **14**, **17**, **19**, **20**, and **32** were evaluated for their antiviral activity in cell-based assays against a large range of RNA viruses including bovine viral diarrhea virus (BVDV), yellow fever virus (YFV), dengue virus type 2 (DENV-2), West Nile virus (WNV), respiratory syncytial virus (RSV), and the retrovirus human immunodeficiency virus type-1 (HIV-1). However, none of them showed any significant antiviral activity or toxicity up to concentrations of 100 μmol/l.

In conclusion, several hitherto unkown 3′-modified 2′-*C*-methylribonucleosides were synthesized from 2′-*C*-methyluridine or uridine, and tested against a wide range of RNA viruses. Unfortunately, the introduction of modifications at the 3′-position of the biologically active compounds 2′-*C*-methylcytidine (**1**) and 2′-*C*-methyluridine (**2**) resulted in loss of antiviral activity.

EXPERIMENTAL

General Procedure

^{1}H NMR spectra were recorded at ambient temperature on a Bruker AC 200 MHz, 250 MHz, 300 MHz or 400 MHz spectrometer. Chemical shifts (δ-scale) are quoted in ppm referenced to the residual solvent peak (DMSO-d_6) set at 2.49 ppm; coupling constants (*J*) are given in Hz. FAB mass spectra were recorded in the positive- or negative-ion mode on a JEOL DX 300 mass spectrometer operating with a JMA-DA 5000 mass data system and using a mixture of glycerol (G)/thioglycerol (T) (1:1, v/v) as the matrix. Melting points were determined in open capillary tubes with a Büchi B-545 apparatus and are uncorrected. UV spectra were recorded on a Uvikon XS spectrophotometer. Elemental analyses were carried out by the Service de Microanalyses du CNRS, Division de Vernaison (France). Thin-layer chromatography (TLC) was performed on precoated aluminium sheets of silica gel 60 F_{254} (Merck, Art. 5554), visualization of products being accomplished by UV absorbance and by charring

with 10% ethanolic sulfuric acid on heating or with a 0.2% ethanolic ninhydrin solution for compounds bearing an amine function.

Column chromatography was carried out on silica gel 60 (Merck, Art. 9385). Evaporation of solvents was carried out in a rotary evaporator under reduced pressure. All moisture-sensitive reactions were carried out under rigorously anhydrous conditions under argon atmosphere using oven-dried glassware. Solvents were dried and distilled prior to use and solids were dried over P_2O_5 under reduced pressure. Analytical high-performance liquid chromatography (HPLC) studies were carried out on a Waters Associates unit (multisolvent delivery system, 717 autosampler injector, 996 photodiode array detector and a Millenium data workstation) using a reverse-phase analytical column (Nova-Pak® Silica 60°A 4 μm, C18, 150 × 3.9 mm). The compound to be analyzed was eluted using a linear gradient of acetonitrile in 20 mM triethylammonium acetate buffer (TEAC, pH 7) programmed over a 10, 15 or 30-min period with a flow rate of 1 ml/min.

2′-*C*-Methyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)uridine (**3**)

To a solution of 2′-*C*-methyluridine (**2;** 5.25 g, 20.3 mmol) in dry pyridine (60 ml) was slowly added at 0 °C 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane ($TIPSCl_2$; 7.6 ml, 24.4 mmol). The solution was stirred at room temperature for 3 h, and the reaction was quenched by addition of water. The residue was diluted with methylene chloride, and washed successively with saturated solution of sodium hydrogencarbonate and water. The combined organic phases were dried over anhydrous sodium sulfate, evaporated under reduced pressure, and coevaporated with toluene. Silica gel column chromatography of the residue, using a stepwise gradient of diethyl ether (50–70%) in petroleum ether, afforded the title compound **3** (10.7 g, quantitative yield). FAB positive, *m/z* (GT): 501 $(M + H)^+$, 389 $(M - B)^+$, 261 $(TIPSO + H)^+$, 113 $(B + 2 H)^+$; FAB negative, *m/z* (GT): 499 $(M - H)^-$, 111 $(B)^-$. ^{1}H NMR (250 MHz, DMSO-d_6): 11.32 s, 1 H, D_2O exchangeable (NH); 7.69 d, 1 H, *J*(6,5) = 8.0 (H-6); 5.77 s, 1 H (H-1′); 5.59 d, 1 H, *J*(5,6) = 8.0 (H-5); 5.48 s, 1 H, D_2O exchangeable (OH-2′); 4.3–3.7 m, 4 H (H-5′a, H-5′b, H-4′ and H-3′); 1.1 m, 31 H (isopropyl and CH_3).

2′-*C*-Methyl-2′-*O*-(tetrahydropyran-2-yl)uridine (**4**)

To a stirred solution of compound **3** (1.00 g, 2.00 mmol) and dihydropyran (1.8 ml, 20.0 mmol) in dry methylene chloride (10 ml), was added trifluoroacetic acid (TFA, 0.23 ml, 3.00 mmol), and the mixture was stirred at room temperature for 8 h. The reaction profile was followed by TLC and the reaction mixture was recharged twice with dihydropyran (2 × 1.8 ml, 2 × 20.0 mmol) and once with TFA (0.23 ml, 3.00 mmol). The solution was then neutralized by addition of 0.2 M solution of sodium methanolate in methanol (32 ml, 6.40 mmol), diluted with methylene chloride, washed successively with aqueous $NaHCO_3$ solution and water, and evaporated. The residue was purified by silica gel column chromatography (eluent: diethyl ether, 40–50% in petroleum ether). Appropriate fractions were collected, evaporated to dryness and taken up in dry methanol (40 ml). To that solution was added ammonium fluoride (1.00 g, 26.0 mmol) and the reaction mixture was refluxed for 1 h. After evaporation of the solvent, the crude mixture was purified by silica gel column chromatography (eluent: methanol, 0–6% in methylene chloride) to afford the title compound **4** as a diastereoisomeric mixture (0.58 g, 85%). FAB positive, *m/z* (GT): 685 $(2 M + H)^+$, 343 $(M + H)^+$, 113 $(B + 2 H)^+$; FAB negative, *m/z* (GT): 683 $(2 M - H)^-$, 341

$(M - H)^-$, 111 $(B)^-$. ^{1}H NMR (200 MHz, DMSO-d_6): 11.40 s, 1 H, D_2O exchangeable (NH); 8.08 d, 1 H, *J*(6,5) = 8.1 (H-6); 6.18 and 5.82 2s, 1 H (H-1′); 5.62 2d, 1 H, *J*(5,6) = 8.1 (H-5); 5.3–4.9 2m, 3 H, 2 signals D_2O exchangeable (OH-3′, OH-5′, CH); 3.9–3.5 m, 6 H (H-5′a, H-5′b, H-4′, H-3′, CH_2); 1.5 m, 6 H (3 CH_2); 1.13 s, 3 H (CH_3).

5′-*O*-(*tert*-Butyldimethylsilyl)-2′-*C*-methyl-2′-*O*-(tetrahydropyran-2-yl)uridine (**5**)

To a stirred solution of compound **4** (4.65 g, 13.6 mmol) and *tert*-butyldimethylsilyl chloride (2.20 g, 15.0 mmol) in dry pyridine (100 ml) was added imidazole (1.40 g, 20.4 mmol), and the mixture was stirred at room temperature for 2 h. The reaction profile was followed by TLC and the reaction mixture was recharged with *tert*-butyldimethylsilyl chloride (0.82 g, 4.80 mmol and 1.13 g, 6.80 mmol) and imidazole (0.55 g, 8.16 mmol). After 2 h, the reaction was still incomplete. It was recharged with additional *tert*-butyldimethylsilyl chloride (1.13 g, 6.80 mmol) and imidazole (0.70 g, 10.2 mmol). An aqueous solution of $NaHCO_3$ was then slowly added and the resulting mixture was extracted with ethyl acetate. The organic phase was washed with water, dried over anhydrous sodium sulfate, and evaporated under pressure. The crude mixture was purified by silica gel column chromatography (eluent: diethyl ether, 50–70% in petroleum ether) to afford the title compound **5** as a diastereoisomeric mixture (5.06 g, 82%). FAB positive, *m/z* (GT): 913 $(2\ M + H)^+$, 549 $(M + G + H)^+$, 457 $(M + H)^+$, 373 $(M - THP + 2\ H)^+$, 345 $(M - B)^+$, 113 $(B + 2\ H)^+$, 85 $(THP)^+$; FAB negative, *m/z* (GT): 911 $(2\ M - H)^-$, 547 $(M + G - H)^-$, 455 $(M - H)^-$, 111 $(B)^-$. ^{1}H NMR (250 MHz, DMSO-d_6): 11.43 s, 1 H, D_2O exchangeable (NH); 7.91 d, 1 H, *J*(6,5) = 8.1 (H-6); 6.16 and 5.80 2s, 1 H (H-1′); 5.49 2d, 1 H, *J*(5,6) = 8.1 (H-5); 5.24 and 5.04 2d, 1 H, D_2O exchangeable (OH-3′), 5.1 m, 1 H *(*CH); 4.1–3.5 m, 6 H (H-5′a, H-5′b, H-4′, H-3′, CH_2); 1.9–1.3 m, 6 H (3 CH_2); 1.07 2s, 3 H (CH_3); 0.88 s, 9 H ($(CH_3)_3C$); 0.08 s, 6 H ($Si(CH_3)_2$).

3′-Deoxy-2′-*C*-methyluridine (**6**) and 2′,3′-Dideoxy-2′-*C*-methyluridine (**7**)

To a solution of intermediate **5** (1.20 g, 2.63 mmol) in dry acetonitrile (24 ml), were successively added *O*-phenyl chlorothioformate (0.49 ml, 3.55 mmol) and 4-(dimethylamino)pyridine (800 mg, 6.57 mmol), and the reaction mixture was stirred at room temperature overnight. After evaporation of the solvent, the residue was diluted with ethyl acetate and washed with water. The organic phase was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was dissolved in anhydrous toluene (30 ml) and refluxed with 2,2′-azoisobutyronitrile (130 mg, 0.79 mmol) and tris(trimethylsilyl)silane (1.2 ml, 3.95 mmol). After removal of the solvent, the crude mixture was taken up in a mixture of acetic acid–tetrahydrofuran–water (4:2:1, 160 ml) and stirred at room temperature for 2 days. After evaporation of the solvents and coevaporation with toluene, the crude mixture was purified by silica gel column chromatography (eluent: methanol, 0–6% in methylene chloride) to isolate 3′-deoxy-2′-*C*-methyluridine **6** (141 mg, 22%) along with a less polar side product 2′,3′-dideoxy-2′-*C*-methyluridine **7** (116 mg, 56%). The desired product **6** was crystallized from methanol–chloroform, m.p. 197–199 °C. For $C_{10}H_{14}N_2O_5 \cdot 0.4H_2O$ (249.4) calculated: 48.15% C, 5.98% H, 11.23% N; found: 48.28% C, 5.69% H, 11.06% N. FAB positive, *m/z* (GT): 485 $(2\ M + H)^+$, 335 $(M + G + H)^+$, 243 $(M + H)^+$, 131 $(M - B)^+$, 113 $(B + 2\ H)^+$; FAB negative, *m/z* (GT): 483 $(2\ M - H)^-$, 333 $(M + G - H)^-$, 241 $(M - H)^-$, 111 $(B)^-$. ^{1}H NMR (250 MHz, DMSO-d_6): 11.32 s, 1 H, D_2O exchangeable (NH); 8.13 d, 1 H, *J*(6,5) = 8.1 (H-6); 5.77 s, 1 H (H-1′); 5.59 d, 1 H, *J*(5,6) = 8.1 (H-5); 5.44 s, 1 H, D_2O exchangeable (OH-2′);

5.18 t, 1 H, *J*(OH,5′) = 4.7, D_2O exchangeable (OH-5′); 4.3 m, 1 H (H-4′); 3.9–3.5 m, 2 H (H-5′a, H-5′b); 1.9–1.7 m, 2 H (H-3′a, H-3′b); 1.09 s, 3 H (CH_3). ^{13}C NMR (400 MHz, DMSO-d_6): 163.03 (C-4); 150.75 (C-2); 140.22 (C-6); 101.15 (C-5); 92.04 (C-1′); 80.58 (C-4′); 80.31 (C-2′); 60.49 (C-5′); 39.46 (C-3′); 21.96 (CH_3). HPLC R_t = 3.89 min (gradient from 0 to 80% CH_3CN in 20 mM triethylammonium acetate buffer programmed over a 30-min period, flow rate of 1 ml/min), λ_{max} = 264 nm. UV (H_2O): λ_{max} = 262.2 nm (ε 11000), λ_{min} = 229.9 nm (ε 2100).

Compound **7**: FAB positive, *m/z* (GT): 453 (2 M + H)$^+$, 319 (M + G + H)$^+$, 227 (M + H)$^+$, 115 (M – B)$^+$, 113 (B + 2 H)$^+$; FAB negative, *m/z* (GT): 451 (2 M – H)$^-$, 225 (M – H)$^-$, 111 (B)$^-$. ^1H NMR (250 MHz, DMSO-d_6): 11.28 s, 1 H, D_2O exchangeable (NH); 8.10 d, 1 H, *J*(6,5) = 8.0 (H-6); 6.03 d, 1 H, *J*(1′,2′) = 7.1 (H-1′); 5.57 d, 1 H, *J*(5,6) = 8.0 (H-5); 5.1 m, 1 H, D_2O exchangeable (OH-5′); 4.0 m, 1 H (H-4′); 3.8–3.5 m, 2 H (H-5′a, H-5′b); 2.7 m, 1 H (H-2′); 1.9 m, 1 H (H-3′a); 1.5 m, 1 H (H-3′b); 0.81 d, 3 H, *J*(CH_3,2′) = 6.8 (CH_3). HPLC R_t = 4.67 min (gradient from 0 to 50% CH_3CN in 20 mM triethylammonium acetate buffer programmed over a 15-min period, flow rate of 1 ml/min), λ_{max} = 266.4 nm. UV (H_2O): λ_{max} = 262.5 nm (ε 10200), λ_{min} = 229.9 nm (ε 1900).

2′,5′-Di-*O*-acetyl-3′-deoxy-2′-*C*-methyluridine (**8**)

To a solution of compound **6** (80 mg, 0.33 mmol) and DMAP (7 mg, 0.05 mmol) in a mixture of dry acetonitrile (3.7 ml) and triethylamine (0.12 ml), was added acetic anhydride (0.15 ml, 1.58 mmol). The reaction mixture was stirred at room temperature overnight. The solvents were evaporated to dryness and the crude mixture was purified by silica gel column chromatography to give **8** (65 mg, 60%). ^1H NMR (200 MHz, DMSO-d_6): 11.48 s, 1 H, D_2O exchangeable (NH); 7.62 d, 1 H, *J*(6,5) = 8.1 (H-6); 6.18 s, 1 H (H-1′); 5.88 d, 1 H, *J*(5,6) = 8.1 (H-5); 4.5–4.2 m, 3 H (H-4′, H-5′a, H-5′b); 2.7 m, 1 H (H-3′a), 2.1 2 s, 6 H (2 CH_3), 2.0 m, 1 H (H-3′b); 1.40 s, 3 H (CH_3).

3′-Deoxy-2′-*C*-methylcytidine Hydrochloride (**10**)

A mixture of compound **8** (95 mg, 0.29 mmol) and Lawesson's reagent (166 mg, 0.40 mmol) was refluxed in anhydrous methylene chloride (4.4 ml) overnight. The solvent was evaporated to dryness and the crude mixture purified by silica gel column chromatography to give 2′,5′-di-*O*-acetyl-3′-deoxy-2′-*C*-methyl-4-thiouridine **9** (86 mg, 86%). This intermediate was then treated with methanol saturated with ammonia at 100 °C for 3 h. After removal of methanol under reduced pressure, the crude mixture was purified by silica gel column chromatography (eluent: methanol, 10–20% in methylene chloride) to give 3′-deoxy-2′-*C*-methylcytidine (47 mg, 77%) which was transformed into its hydrochloride **10** after three coevaporations with 1 M hydrochloric acid (0.230 ml) and ethanol (1.4 ml), and precipitated. FAB positive, *m/z* (GT): 483 (2 M + H)$^+$, 242 (M + H)$^+$, 131 (M – B)$^+$, 112 (B + 2 H)$^+$; FAB negative, *m/z* (GT): 276 (M + Cl)$^-$, 110 (B)$^-$. ^1H NMR (200 MHz, DMSO-d_6): 9.63 s and 8.54 2s, 3 H, D_2O exchangeable (NH_3^+); 8.52 d, 1 H, *J*(6,5) = 7.6 (H-6); 6.10 d, 1 H, *J*(5,6) = 7.6 (H-5); 5.77 s, 1 H (H-1′); 5.6 and 5.2 2m, 2 H, D_2O exchangeable (OH-2′ and OH-5′); 4.2 m, 1 H (H-4′); 3.9–3.5 m, 2 H (H-5′a, H-5′b); 1.9–1.7 m, 2 H (H-3′a, H-3′b); 1.13 s, 3 H (CH_3). ^{13}C NMR (400 MHz, DMSO-d_6): 159.82 (C-4); 148.34 (C-2); 144.30 (C-6); 94.20 (C-5); 93.28 (C-1′); 81.70 (C-4′); 81.05 (C-2′); 60.85 (C-5′); 38.67 (C-3′); 22.32 (CH_3). HPLC R_t = 3.40 min (gradient from 0 to 80% CH_3CN in 20 mM triethylammonium acetate buffer programmed over a 15-min period, flow rate of 1 ml/min), λ_{max} = 273.5 nm.

5′-*O*-(*tert*-Butyldimethylsilyl)-3′-*O*-mesyl-2′-*C*-methyl-2′-*O*-(tetrahydropyran-2-yl)-uridine (**11**)

Compound **5** (2.17 g, 4.75 mmol) dissolved in dry methylene chloride (150 ml) was reacted with mesyl chloride (0.9 ml, 10.9 mmol) at 0 °C in the presence of triethylamine (64 ml). The reaction mixture was stirred at 0 °C for 2 h and recharged with additional mesyl chloride (2 × 0.9 ml, 2 × 10.9 mmol). The reaction was allowed to warm to room temperature and stirred for one more hour. The solvents were evaporated under reduced pressure and the crude mixture purified by silica gel column chromatography (eluent: 80% diethyl ether in petroleum ether) to afford compound **11** as a diastereoisomeric mixture (2.66 g, quantitative yield). FAB positive, *m/z* (GT): 627 $(M + G + H)^+$, 535 $(M + H)^+$, 423 $(M - B)^+$, 113 $(B + 2\ H)^+$; FAB negative, *m/z* (GT): 1067 $(2\ M - H)^-$, 625 $(M + G - H)^-$, 533 $(M - H)^-$, 111 $(B)^-$. ^{1}H NMR (250 MHz, DMSO-d_6): 11.46 s, 1 H, D_2O exchangeable (NH); 7.76 2d, 1 H, *J*(6,5) = 8.1 (H-6); 6.21 and 5.86 2s, 1 H (H-1′); 5.54 2d, 1 H, *J*(5,6) = 8.10 (H-5); 5.1–5.0 2m, 1 H (CH); 4.7 m, 1 H (H-3′); 4.1 m, 1 H (H-4′); 4.0–3.7 m, 3 H (H-5′a, H-5′b, CH_2); 3.5 m, 1 H (CH_2); 3.26 s, 3 H (SCH_3); 1.8–1.4 m, 6 H (3 CH_2); 1.2 m, 3 H (CH_3); 0.85 s, 9 H ($(CH_3)_3C$); 0.04 s, 6 H ($Si(CH_3)_2$).

3′-*O*-Mesyl-2′-*C*-methyluridine (**12**)

To a solution of compound **11** (2.33 g, 4.37 mmol) in dry methylene chloride (130 ml) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 3.2 ml, 21.8 mmol). The reaction mixture was refluxed for 3 days and recharged with additional DBU (3.2 ml, 21.8 mmol). The reaction was quenched by addition of 0.5 M of hydrochloric acid. The organic phase was separated, washed with water, dried over anhydrous sodium sulfate and evaporated to dryness. The crude mixture was filtered through a silica gel column (eluent: 2% methanol in methylene chloride) and treated with 1 M sodium hydroxide (80 ml) at room temperature overnight. The reaction mixture was quenched by addition of a 1 M hydrochloric acid and partitioned between water and methylene chloride. The aqueous layer was evaporated under reduced pressure and purified by silica gel column chromatography (eluent: 10% methanol in methylene chloride) to give the title compound **12** (985 mg, 67%). FAB positive, *m/z* (GT): 429 $(M + G + H)^+$, 337 $(M + H)^+$, 113 $(B + 2\ H)^+$; FAB negative, *m/z* (GT): 335 $(M - H)^-$, 111 $(B)^-$. ^{1}H NMR (400 MHz, DMSO-d_6): 11.40 s, 1 H, D_2O exchangeable (NH); 7.99 d, 1 H, *J*(6,5) = 8.1 (H-6); 5.93 s, 1 H, D_2O exchangeable (OH-2′); 5.85 s, 1 H (H-1′); 5.68 d, 1 H, *J*(5,6) = 8.10 (H-5); 5.42 s, 1 H, D_2O exchangeable (OH-5′); 4.72 d, 1 H, *J*(3′,4′) = 8.9 (H-3′); 4.1 m, 1 H (H-4′); 3.9–3.7 m, 2 H (H-5′a, H-5′b); 3.3 m, 3 H (SCH_3); 1.14 s, 3 H (CH_3).

2,3′-Anhydro-2′-*C*-methyluridine (**13**)

To a solution of compound **12** (827 mg, 2.46 mmol) in 2-methoxyethanol (8.4 ml) was added potassium fluoride (KF; 715 mg, 12.3 mmol). The reaction mixture was stirred at room temperature for 5 h. After evaporation of the solvent, the crude mixture was purified by silica gel column chromatography (eluent: methanol, 10–15% in methylene chloride) to give the anhydro derivative **13** (520 mg, 75%). FAB positive, *m/z* (GT): 481 $(2\ M + H)^+$, 333 $(M + G + H)^+$, 241 $(M + H)^+$; FAB negative, *m/z* (GT): 479 $(2\ M - H)^-$, 331 $(M + G - H)^-$, 239 $(M - H)^-$. ^{1}H NMR (250 MHz, DMSO-d_6): 7.77 d, 1 H, *J*(6,5) = 8.1 (H-6); 5.98 s, 1 H, D_2O exchangeable (OH-2′); 5.9 m, 2 H (H-1′, H-5); 4.93 t, 1 H, *J*(OH,5′) = 5.3, D_2O exchangeable (OH-5′); 4.1 m, 1 H (H-3′); 3.9 q, 1 H, *J*(4′,5′) = 4.7 (H-4′); 3.4–3.3 m, 2 H (H-5′a, H-5′b);

1.49 s, 3 H (CH_3). HPLC R_t = 2.84 min (gradient from 0 to 50% CH_3CN in 20 mM triethylammonium acetate buffer programmed over a 15-min period, flow rate of 1 ml/min), λ_{max} = 249.9 nm. UV (H_2O): λ_{max} = 248.0 nm (ε 8600), λ_{min} = 232.5 nm (ε 6700).

1-(2-*C*-Methyl-β-D-xylofuranosyl)uracil (**14**)

Compound **13** (483 mg, 2.0 mmol) was treated with a 1 M solution of sodium hydroxide (22 ml) at room temperature for 1 h, then the solvent was evaporated under reduced pressure. The crude mixture was purified by silica gel column chromatography (eluent: 10% methanol in methylene chloride) to afford title compound **14** (455 mg, 88%). For $C_{10}H_{14}N_2O_6$ (258.2) calculated: 46.51% C, 5.46% H, 10.85% N; found: 46.62% C, 5.68% H, 10.46% N. FAB positive, *m/z* (GT): 259 $(M + H)^+$, 131 $(M - B)^+$, 113 $(B + 2 H)^+$; FAB negative, *m/z* (GT): 257 $(M - H)^-$, 111 $(B)^-$. ^{1}H NMR (200 MHz, DMSO-d_6): 11.26 s, 1 H, D_2O exchangeable (NH); 7.67 d, 1 H, *J*(6,5) = 8.1 (H-6); 5.79 s, 1 H (H-1′); 5.53 d, 1 H, *J*(5,6) = 8.1 (H-5); 5.49 d, 1 H, D_2O exchangeable (OH-3′); 5.27 s, 1 H, D_2O exchangeable (OH-2′); 5.16 t, 1 H, *J*(OH,5′) = 5.3, D_2O exchangeable (OH-5′); 3.8–3.6 m, 4 H (H-3′, H-4′, H-5′a, H-5′b); 1.16 s, 3 H (CH_3). HPLC R_t = 3.63 min (gradient from 0 to 50 % CH_3CN in 20 mM triethylammonium acetate buffer programmed over a 15-min period, flow rate of 1 ml/min), λ_{max} = 262.9 nm. UV (H_2O): λ_{max} = 261.3 nm (ε 9600), λ_{min} = 229.0 nm (ε 2000).

1-(2,3,5-Tri-*O*-acetyl-2-*C*-methyl-β-D-xylofuranosyl)uracil (**15**)

To a solution of compound **14** (363 mg, 1.40 mmol) and DMAP (12 mg, 0.10 mmol) in a mixture of dry acetonitrile (15 ml) and triethylamine (0.77 ml) was added acetic anhydride (0.48 ml, 5.04 mmol). The reaction mixture was stirred at room temperature for 5 h, then recharged with additional DMAP (2 × 12 mg, 2 × 0.10 mmol) and acetic anhydride (2 × 0.16 ml, 2 × 1.7 mmol). The reaction was complete after 20 h and the solvents were evaporated to dryness. Column chromatography on silica gel using methanol (0–2%) in methylene chloride as eluent gave **15** (436 mg, 81%). FAB positive, *m/z* (GT): 385 $(M + H)^+$, 273 $(M - B)^+$, 113 $(B + 2 H)^+$, 43 $(Ac)^+$; FAB negative, *m/z* (GT): 383 $(M - H)^-$, 111 $(B)^-$, 59 $(AcO)^-$. ^{1}H NMR (250 MHz, DMSO-d_6): 11.48 s, 1 H, D_2O exchangeable (NH); 7.71 d, 1 H, *J*(6,5) = 8.2 (H-6); 6.02 s, 1 H (H-1′); 5.68 d, 1 H, *J*(5,6) = 8.2 (H-5); 5.44 m, 1 H (H-3′); 4.4–4.0 m, 3 H (H-4′, H-5′a, H-5′b); 2.12 s, 3 H (CH_3); 2.05 s, 3 H (CH_3); 1.97 s, 3 H (CH_3); 1.53 s, 3 H (CH_3).

1-(2,3,5-Tri-*O*-acetyl-2-*C*-methyl-β-D-xylofuranosyl)-4-thiouracil (**16**)

A mixture of compound **15** (430 mg, 1.12 mmol) and Lawesson's reagent (1.27 g, 3.14 mmol) was refluxed in anhydrous methylene chloride (17 ml) for 48 h. The solvent was evaporated to dryness and the crude mixture purified by silica gel column chromatography to give compound **16** (397 mg, 89%). FAB positive, *m/z* (GT): 801 $(2 M + H)^+$, 401 $(M + H)^+$, 273 $(M - B)^+$, 129 $(B + 2 H)^+$, 43 $(Ac)^+$; FAB negative, *m/z* (GT): 399 $(M - H)^-$, 127 $(B)^-$, 59 $(AcO)^-$. ^{1}H NMR (250 MHz, DMSO-d_6): 12.91 s, 1 H, D_2O exchangeable (NH); 7.65 d, 1 H, *J*(6,5) = 7.6 (H-6); 6.40 d, 1 H, *J*(5,6) = 7.6 (H-5); 6.05 s, 1 H (H-1′); 5.46 d, 1 H, *J*(3′,4′) = 4.7 (H-3′); 4.5–4.1 m, 3 H (H-4′, H-5′a, H-5′b); 2.16 s, 3 H (CH_3); 2.08 s, 3 H (CH_3); 2.01 s, 3 H (CH_3); 1.58 s, 3 H (CH_3).

1-(2-*C*-Methyl-β-D-xylofuranosyl)cytosine Hydrochloride (**17**)

Compound **16** (390 mg, 1.02 mmol) was treated with methanol saturated with ammonia (25 ml) at 100 °C for 3 h. After removal of methanol under reduced pressure, the crude mixture was purified by silica gel column chromatography (eluent: methanol, 10–20% in methylene chloride) to give 1-(2-*C*-methyl-β-D-xylofuranosyl)cytosine (225 mg, 86%) which was transformed into its hydrochloride **17** after three coevaporations with a mixture of 1 M hydrochloric acid (0.10 ml) and ethanol (0.60 ml), and precipitated. For $C_{10}H_{16}ClN_3O_5 \cdot 0.15HCl$ (299.2) calculated: 40.15% C, 5.44% H, 13.63% Cl, 14.05% N; found: 39.77% C, 5.48% H, 13.48% Cl, 13.78% N. FAB positive, *m/z* (GT): 258 $(M - Cl)^+$, 112 $(B + 2\ H)^+$; FAB negative, *m/z* (GT): 292 $(M - H)^-$. ^{1}H NMR (250 MHz, DMSO-d_6): 9.71 s and 8.64 2s, 3 H, D_2O exchangeable (NH_3^+); 8.01 d, 1 H, *J*(6,5) = 7.9 (H-6); 6.14 d, 1 H, *J*(5,6) = 7.9 (H-5); 5.83 s, 1 H (H-1′); 5.6 and 5.3 2m, 2 H, D_2O exchangeable (OH-2′ and OH-5′); 3.8–3.6 m, 4 H (H-3′, H-4′, H-5′a, H-5′b); 1.21 s, 3 H (CH_3). ^{13}C NMR (400 MHz, DMSO-d_6): 159.32 (C-4); 147.25 (C-2); 146.37 (C-6); 92.63 (C-5); 88.94 (C-1′); 85.39 (C-4′); 78.59 (C-2′); 76.86 (C-3′); 60.93 (C-5′); 19.47 (CH_3). HPLC R_t = 3.06 min (gradient from 0 to 50% CH_3CN in 20 mM triethylammonium acetate buffer programmed over a 15-min period, flow rate of 1 ml/min), λ_{max} = 272.3 nm. UV (H_2O): λ_{max} = 271.0 nm (ε 9400), λ_{min} = 250.0 nm (ε 6000), λ_s = 233.0 nm (ε 8000).

5′-*O*-*tert*-Butyldimethylsilyl-2′-*C*,3′-*O*-dimethyl-2′-*O*-(tetrahydropyran-2-yl)uridine (**18**)

To a solution of compound **5** (2.24 g, 4.90 mmol) in dry methylene chloride (49 ml) was added at 0 °C sodium hydride 60% (386 mg, 9.80 mmol) and methyl iodide (2.25 ml, 36.3 mmol). The reaction mixture was heated at 40 °C for 24 h, recharged with additional sodium hydride (98 mg, 2.48 mmol) and methyl iodide (0.61 ml, 9.83 mmol) and stirred one more day at 40 °C. The reaction mixture was diluted with methylene chloride and washed successively with 0.5 M hydrochloric acid and water. The combined organic layers were evaporated under reduced pressure and the crude mixture was purified by silica gel column chromatography to give compound **18** (1.05 g, 45%) in a low yield due to the presence of starting material. FAB positive, *m/z* (GT): 471 $(M + H)^+$, 387 $(M - THP + 2\ H)^+$, 275 $(M - B - THP + H)^+$, 113 $(B + 2\ H)^+$, 85 $(THP)^+$; FAB negative, *m/z* (GT): 469 $(M - H)^-$, 385 $(M - THP)^-$, 111 $(B)^-$. ^{1}H NMR (400 MHz, DMSO-d_6): 11.40 s, 1 H, D_2O exchangeable (NH); 7.77 2d, 1 H, *J*(6,5) = 8.1 (H-6); 6.66 s, 1 H (H-1′); 5.41 2d, 1 H, *J*(5,6) = 8.1 (H-5); 5.1–5.0 2m, 1 H (CH); 4.0–3.7 m, 5 H (H-4, H-5′a, H-5′b,CH_2); 3.39 d, 1 H, *J*(3′,4′) = 9.8 (CH_2); 3.34 s, 3 H (OCH_3); 1.8–1.1 m, 6 H (3 CH_2); 1.03 s, 3 H (CH_3); 0.82 s, 9 H ($(CH_3)_3C$); 0.01 2s, 6 H ($Si(CH_3)_2$).

2′-*C*,3′-*O*-Dimethyluridine (**19**)

A solution of compound **18** (1.04 g, 2.20 mmol) in a mixture of acetic acid–tetrahydrofuran–water (4:2:1, 67 ml) was refluxed for 42 h, and the solvents were evaporated to dryness. The crude mixture was coevaporated with toluene and purified by silica gel column chromatography (eluent: methanol, 0–8% in methylene chloride) to afford the title compound **19** (502 mg, 83%) which was recrystallized from ethyl acetate, m.p. 176–177 °C. For $C_{11}H_{16}N_2O_6$ (272.3) calculated: 48.53% C, 5.92% H, 10.29% N; found: 48.37% C, 5.93% H, 10.36% N. FAB positive, *m/z* (GT): 273 $(M + H)^+$, 161 $(M - B)^+$, 113 $(B + 2\ H)^+$; FAB negative, *m/z* (GT): 379 $(M + T - H)^-$, 271 $(M - H)^-$, 111 $(B)^-$. ^{1}H NMR (200 MHz, DMSO-d_6): 11.39 s, 1 H, D_2O exchangeable (NH); 8.04 d, 1 H, *J*(5,6) = 8.1 (H-6); 5.76 s, 1 H (H-1′); 5.64 d, 1 H,

J(6,5) = 8.1 (H-5); 5.32 t, 1 H, D_2O exchangeable (OH-5′); 5.22 s, 1 H, D_2O exchangeable (OH-2′); 4.0–3.5 m, 4 H (H-3′, H-4′, H-5′a, H-5′b); 3.35 s, 3 H (OCH_3); 1.12 s, 3 H (CH_3). ^{13}C NMR (400 MHz, DMSO-d_6): 162.99 (C-4); 150.67 (C-2); 140.32 (C-6); 101.42 (C-5); 91.09 (C-1′); 81.07 and 81.01 (C-4′ and C-3′); 78.12 (C-2′); 59.14 (C-5′); 58.98 (OCH_3); 21.01 (CH_3). HPLC R_t = 6.01 min (gradient from 0 to 50% CH_3CN in 20 mM triethylammonium acetate buffer programmed over a 15-min period, flow rate of 1 ml/min), λ_{max} = 264.0 nm. UV (H_2O): λ_{max} = 262.1 nm (ε 10 600), λ_{min} = 229.6 nm (ε 1900).

2′-*C*,3′-*O*-Dimethylcytidine Hydrochloride (**20**)

A solution of **19** (286 mg, 1.05 mmol), 1-methylpyrrolidine (1.0 ml), and chlorotrimethylsilane (0.40 ml, 3.15 mmol) in anhydrous acetonitrile (10.5 ml) was stirred at room temperature for 1 h. The solution was cooled to 0 °C, treated with trifluoroacetic anhydride (0.44 ml, 3.15 mmol) and stirred at the same temperature for 30 min. 4-Nitrophenol (438 mg, 3.15 mmol) was added and the solution was further stirred at 0 °C for 3 h. The reaction was quenched by adding water, and the solvents were evaporated under reduced pressure. The residue was taken up in dichloromethane, washed with saturated aqueous $NaHCO_3$ solution, and water. The organic layer was evaporated under reduced pressure, and the crude residue was purified by silica gel column chromatography (eluent: methanol, 0–5% in methylene chloride) to afford the 4-nitrophenyl intermediate which was further taken up in dioxane (26 ml) and treated with a 28% aqueous NH_4OH (5.2 ml). The solution was heated to 50 °C overnight. Evaporation of the solvents under reduced pressure, and chromatographic separation using a gradient of methanol (0–20%) in dichloromethane lead to 2′-*C*,3′-*O*-dimethylcytidine (225 mg, 79%). The compound was transformed into its hydrochloride **20** after three coevaporations with a mixture of 1 M hydrochloric acid (0.665 ml) and ethanol (12.5 ml), and crystallized from ethanol, m.p. 219–222 °C. For $C_{11}H_{18}ClN_3O_5$ (307.7) calculated: 42.93% C, 5.90% H, 11.52% Cl, 13.65% N; found: 42.90% C, 5.82% H, 11.57% Cl, 13.70% N. FAB positive, *m/z* (GT): 543 (2 M + H)$^+$, 364 (M + G + H)$^+$, 272 (M + H)$^+$, 161 (M – B)$^+$, 112 (B + 2 H)$^+$; FAB negative, *m/z* (GT): 306 (M + Cl)$^-$, 270 (M – H)$^-$, 110 (B)$^-$. ^1H NMR (200 MHz, DMSO-d_6): 9.86 and 8.68 2s, 3 H, D_2O exchangeable (NH_3^+); 8.43 d, 1 H, *J*(6,5) = 7.8 (H-6); 6.16 d, 1 H, *J*(5,6) = 7.8 (H-5); 5.77 s, 1 H (H-1′); 5.2–4.9 m, 2 H, D_2O exchangeable (OH-2′ and OH-5′); 3.8–3.6 m, 4 H (H-3′, H-4′, H-5′a, H-5′b); 1.16 s, 3 H (CH_3). ^{13}C NMR (400 MHz, DMSO-d_6): 159.27 (C-4); 147.29 (C-2); 143.94 (C-6); 93.95 (C-5); 91.76 (C-1′); 81.54 (C-4′); 80.55 (C-3′); 78.38 (C-2′); 59.08 (C-5′ and OCH_3); 20.70 (CH_3). HPLC R_t = 5.28 min (gradient from 0 to 50% CH_3CN in 20 mM triethylammonium acetate buffer programmed over a 15-min period, flow rate of 1 ml/min), λ_{max} = 273.5 nm. UV (H_2O) λ_{max} = 271.2 nm (ε 9800), λ_{min} = 248.8 nm (ε 6400).

2′,5′-Di-*O*-trityluridine (**22**)

To a solution of uridine **21** (40.0 g, 164 mmol) in anhydrous pyridine (820 ml) was added portionwise triphenylmethyl chloride (170 g, 615 mmol). The reaction mixture was refluxed for 2 h, then heated at 60 °C overnight. The solvent was evaporated under reduced pressure and the residue partitioned between a saturated aqueous $NaHCO_3$ solution and water. The organic layer was washed successively with 0.2 M hydrochloric solution and water, and evaporated to dryness. The crude mixture was purified by silica gel column chromatography (eluent: 30% ethyl acetate in hexane) to give compound **22** (83.6 g, 70%). FAB positive, *m/z* (GT): 729 (M + H)$^+$, 469 (M – TrO)$^+$, 243 (Tr)$^+$; FAB negative, *m/z* (GT): 727 (M – H)$^-$, 485

$(M - Tr)^-$, 110 $(B)^-$. ^{1}H NMR (400 MHz, DMSO-d_6): 11.5 s, 1 H, D_2O exchangeable (NH); 7.6–7.2 m, 31 H (trityl and H-6); 6.21 d, 1 H, J(1′,2′) = 7.2 (H-1′); 5.22 d, 1 H, J(5,6) = 8.0 (H-5); 4.88 d, 1 H, J(OH-3′) = 5.3, D_2O exchangeable (OH-3′); 4.4 m, 1 H (H-2′); 3.9 m, 1 H (H-4′); 3.1–2.9 m, 3 H (H-3′, H-5′a, H-5′b).

3′-*O*-Mesyl-2′,5′-di-*O*-trityluridine (**23**)

Compound **22** (60.0 g, 82.4 mmol) dissolved in dry pyridine (410 ml) was reacted with mesyl chloride (13.0 ml, 165 mmol) and stirred for 3 h. The reaction mixture was diluted with methylene chloride and washed successively with saturated aqueous $NaHCO_3$ solution, 0.2 M hydrochloric solution, and water. The combined organic layers were dried over anhydrous sodium sulfate, filtered, evaporated under reduced pressure and coevaporated with toluene. Precipitation in a mixture of ethyl acetate/diethyl ether afforded compound **23** (63.0 g, 95%). FAB positive, *m/z* (GT): 807 $(M + H)^+$, 711 $(M - MsO)^+$, 243 $(Tr)^+$; FAB negative, *m/z* (GT): 805 $(M - H)^-$, 727 $(M - Ms)^-$, 563 $(M - Tr)^-$, 111 $(B)^-$, 95 $(MsO)^-$. ^{1}H NMR (250 MHz, DMSO-d_6): 11.51 s, 1 H, D_2O exchangeable (NH); 7.4–7.1 m, 31 H (trityl, H-6); 6.13 d, 1 H, J(1′,2′) = 7.75 (H-1′); 5.28 d, 1 H, J(5,6) = 8.0 (H-5); 4.6 m, 1 H (H-2′); 4.3 m, 1 H (H-3′); 3.88 d, 1 H, J(4′,5′) = 4.67 (H-4′); 3.12 s, 3 H (SCH_3); 3.0 m, 2 H (H-5′a, H-5′b).

2,3′-Anhydro-2′,5′-di-*O*-trityluridine (**24**)

A mixture of compound **23** (63.0 g, 78.0 mmol) and sodium acetate (32.0 g, 390 mmol) in anhydrous dimethylformamide (390 ml) was stirred at 100 °C until TLC showed the absence of starting material. The reaction mixture was cooled to room temperature and evaporated to dryness. The residue was dissolved in methylene chloride and washed successively with saturated aqueous $NaHCO_3$ solution and water. The combined organic layers were evaporated under reduced pressure. Precipitation in a mixture of methylene chloride/diethyl ether afforded 12.1 g of pure compound **24**. The filtrate was evaporated to dryness and purified by silica gel column chromatography (eluent: 30% ethyl acetate in hexane) to afford 18.1 g of compound **24** (55% total yield). FAB positive, *m/z* (GT): 711 $(M + H)^+$, 469 $(M - Tr + 2\ H)^+$, 243 $(Tr)^+$. ^{1}H NMR (250 MHz, DMSO-d_6): 7.5–7.1 m, 31 H (trityl, H-6); 5.65 d, 1 H, J(5,6) = 7.4 (H-5); 4.88 s, 1 H (H-1′); 4.5 m, 2 H (H-2′, H-3′); 4.4 m, 1 H (H-4′); 3.2–3.0 m, 2 H (H-5′a, H-5′b).

1-(2,5-Di-*O*-trityl-β-D-xylofuranosyl)uracil (**25**)

A solution of compound **24** (1.05 g, 1.48 mmol) in a mixture of methanol (5.0 ml) and ethanol (5.0 ml) was refluxed with a 1 M solution of sodium hydroxide (48 ml) at room temperature for 4 days. The reaction was quenched by addition of acetic acid and the solvent were evaporated under reduced pressure. The residue was dissolved in methylene chloride, washed with brine and evaporated to dryness. The crude mixture was purified by silica gel column chromatography (eluent: 40% ethyl acetate in hexane) to afford the title compound (946 mg, 88%). FAB positive, *m/z* (GT): 729 $(M + H)^+$, 243 $(Tr)^+$; FAB negative, *m/z* (GT): 485 $(M - TR)^-$. ^{1}H NMR (200 MHz, DMSO-d_6): 11.42 s, 1 H, D_2O exchangeable (NH); 7.5–7.2 m, 31 H (trityl, H-6); 6.12 s, 1 H (H-1′); 5.57 d, 1 H, J(5,6) = 8.1 (H-5); 5.0 s, 1 H, D_2O exchangeable (OH-3′); 4.0–3.9 m, 2 H (H-2′, H-4′); 3.3–3.0 m, 2 H (H-5′a, H-5′b); 2.9 m, 1 H (H-3′).

3′-Deoxy-3′-fluorouridine (**26**)

Compound **25** (830 mg, 1.14 mmol) was dissolved in a mixture of anhydrous methylene chloride (10 ml) and pyridine (0.30 ml). To that solution was added, at 0 °C, diethylaminosulfur trifluoride (DAST; 0.30 ml, 2.28 mmol) and the reaction mixture was stirred at room temperature for 3 h. The reaction was then recharged with additional DAST (2 × 0.07 ml, 2 × 0.57 mmol) and stopped after 24 h. The reaction mixture was diluted with methylene chloride and washed successively with saturated aqueous $NaHCO_3$, brine and water. The combined organic layers were evaporated to dryness and the residue was partly purified by silica gel column chromatography (eluent: 30% ethyl acetate in hexane). The collected fractions were evaporated and treated with 80% aqueous acetic acid (30 ml). The reaction mixture was heated at 85 °C for 3 h, allowed to cool to room temperature, and extracted with ethyl acetate and water. The aqueous layer was evaporated to dryness and coevaporated with absolute ethanol. The residue was purified by silica gel column chromatography (eluent: methanol, 5–10% in methylene chloride) to afford compound **26** (80.0 mg, 30%). FAB positive, *m/z* (GT): 339 $(M + G + H)^+$, 247 $(M + H)^+$; FAB negative, *m/z* (GT): 353 $(M + T - H)^-$, 245 $(M - H)^-$; 111 $(B)^-$. ^{1}H NMR (200 MHz, DMSO-d_6): 11.5 s, 1 H, D_2O exchangeable (NH); 7.84 d, 1 H, *J*(6,5) = 8.2 (H-6); 5.88 m, 2 H, 1 signal D_2O exchangeable (OH-2′, H-1′); 5.82 d, 1 H, *J*(5,6) = 8.2 (H-5); 5.30 t, 1 H, *J*(OH-5′,5′) = 4.9, D_2O exchangeable (OH-5′); 4.9 dd, 1 H, *J*(3′,4′) = 4.4, *J*(3′,F) = 54.7 (H-3′); 4.2–4.0 m, 2 H (H-2′, H4′); 4.1 m, 2 H (H-5′a, H-5′b). HPLC R_t = 3.83 min (gradient from 0 to 80% CH_3CN in 20 mM triethylammonium acetate buffer programmed over a 30-min period, flow rate 1 ml/min), λ_{max} = 260.5 nm.

5′-*O*-(*tert*-Butyldimethylsilyl)-3′-deoxy-3′-fluorouridine (**27**)

To a solution of compound **26** (1.30 g, 5.28 mmol) in dry pyridine (20 ml) were added imidazole (360 mg, 5.28 mmol) and *tert*-butyldimethylsilyl chloride (717 mg, 4.75 mmol). The reaction mixture was stirred at room temperature for 2 h and recharged with additional *tert*-butyldimethylsilyl chloride (560 mg, 3.71 mmol) until the reaction was complete. Then solution of aqueous $NaHCO_3$ was slowly added and the resulting mixture was extracted with ethyl acetate and washed successively with saturated aqueous $NaHCO_3$, 0.5 M HCl, and brine. The combined organic layers were evaporated under pressure and coevaporated with toluene. The crude mixture was purified by silica gel column chromatography (eluent: 40% ethyl acetate in hexane) to afford the title compound (850 mg, 45%). The low yield was due to the concomitant formation of 2′,5′-di-*O*-(*tert*-butyldimethylsilyl)-3′-deoxy-3′-fluorouridine (1.20 g, 48%). FAB positive, *m/z* (GT): 721 $(2\ M + H)^+$, 361 $(M + H)^+$, 249 $(M - B)^+$, 113 $(B + 2\ H)^+$; FAB negative, *m/z* (GT): 719 $(2\ M - H)^-$, 359 $(M - H)^-$, 111 $(B)^-$. ^{1}H NMR (300 MHz, DMSO-d_6): 11.35 s, 1 H, D_2O exchangeable (NH); 7.61 d, 1 H, *J*(6,5) = 8.1 (H-6); 5.8 m, 2 H, 1 signal D_2O exchangeable (H-1′, OH-2′); 5.60 d, 1 H, *J*(5,6) = 8.1 (H-5); 4.83 dd, 1 H, *J*(3′,4′) = 3.7, *J*(3′,F) = 54.1 (H-3′); 4.29 m, 1 H (H-4′); 3.7 m, 2 H (H-5′a, H-5′b); 0.8 m, 9 H $((CH_3)_3C)$; –0.03 s, 6 H $(Si(CH_3)_2)$.

3′-Deoxy-3′-fluoro-2′-*C*-methyluridine (**28**)

Anhydrous pyridine (2.9 ml) and acetic anhydride (2.2 ml) were added successively to an ice-cold suspension of chromium(VI) oxide (2.22 g, 22.2 mmol) in anhydrous methylene chloride (90 ml), while stirring at room temperature under argon atmosphere was continued until a homogeneous solution was obtained. Compound **27** (3.2 g, 8.83 mmol) was added,

and stirring was continued for 2 h. The reaction mixture was poured into cold ethyl acetate and the resulting precipitate was filtered off. The filtrate was concentrated and coevaporated with toluene. The crude product was used in the next step without purification. To its solution in anhydrous diethyl ether (45 ml) was slowly added, at –78 °C, a 3 M methylmagnesium bromide solution in diethyl ether (5.3 ml, 15.9 mmol). The reaction mixture was stirred at –78 °C for 1 h and then allowed to come to room temperature. The reaction was stopped after 3 h and the mixture poured into saturated aqueous ammonium chloride. The organic phase was washed with brine, dried over anhydrous sodium sulfate and evaporated to dryness. The residue was dissolved in anhydrous methanol (80 ml) and treated with ammonium fluoride (3.2 g, 88.9 mmol). The reaction mixture was refluxed for 3 h, then the solvent was evaporated under reduced pressure. The crude mixture was purified by silica gel column chromatography (eluent: methanol, 0–5% in methylene chloride) to provide the desired compound **28** (128 mg, 6%) with its epimer at C-2′ **29** (400 mg, 17%).

Compound **28**: FAB positive, *m/z* (GT): 521 $(2\ M + H)^+$, 369 $(M + T + H)^+$; 353 $(M + G + H)^+$; 261 $(M + H)^+$, 149 $(M - B)^+$, 113 $(B + 2\ H)^+$; FAB negative, *m/z* (GT): 367 $(M + T - H)^-$, 259 $(M - H)^-$, 111 $(B)^-$. ^{1}H NMR (400 MHz, DMSO-d_6): 11.41 s, 1 H, D_2O exchangeable (NH); 7.90 d, 1 H, *J*(6,5) = 8.1 (H-6); 5.8 m, 2 H, 1 signal D_2O exchangeable (H-1′, OH-2′); 5.64 d, 1 H, *J*(5,6) = 8.1 (H-5); 5.37 t, 1 H, *J*(OH-5′) = 5.0, D_2O exchangeable (OH-5′); 4.72 dd, 1 H, *J*(3′,4′) = 8.3, *J*(3′,F) = 44.9 (H-3′); 4.1 m, 1 H (H-4′); 3.9–3.6 m, 2 H (H-5′a, H-5′b); 1.13 s, 3 H (CH_3).

1-(3-Deoxy-3-fluoro-2-*C*-methyl-β-D-arabinofuranosyl)uracil **29**: FAB positive, *m/z* (GT): 521 $(2\ M + H)^+$, 353 $(M + G + H)^+$; 261 $(M + H)^+$, 149 $(M - B)^+$, 113 $(B + 2\ H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6): 11.35 s, 1 H, D_2O exchangeable (NH); 7.58 d, 1 H, *J*(6,5) = 8.2 (H-6); 5.9 m, 1 H (H-1′); 5.81 s, 1 H, D_2O exchangeable (OH-2′); 5.61 d, 1 H, *J*(5,6) = 8.2 (H-5); 5.25 t, 1 H, *J*(OH-5′) = 5.4, D_2O exchangeable (OH-5′); 4.72 dd, 1 H, *J*(3′,4′) = 1.9, *J*(3′,F) = 52.7 (H-3′); 4.1 m, 1 H (H-4′); 3.7 m, 2 H (H-5′a, H-5′b); 1.25 s, 3 H (CH_3).

2′,5′-Di-*O*-acetyl-3′-deoxy-3′-fluoro-2′-*C*-methyluridine (**30**)

To a solution of compound **28** (128 mg, 0.49 mmol) and DMAP (4 mg, 0.03 mmol) in a mixture of dry acetonitrile (4.0 ml) and triethylamine (0.20 ml) was added acetic anhydride (0.12 ml, 1.22 mmol). The reaction mixture was stirred at room temperature overnight, then recharged with additional DMAP (4 mg, 0.03 mmol) and acetic anhydride (0.12 ml, 1.22 mmol). The reaction was stirred one more day and the solvents were evaporated to dryness. Column chromatography on silica gel using 0–5% of methanol in methylene chloride as eluent gave **30** (168 mg, 99%). FAB positive, *m/z* (GT): 345 $(M + H)^+$, 233 $(M - B)^+$, 113 $(B + 2\ H)^+$, 43 $(Ac)^+$; FAB negative, *m/z* (GT): 343 $(M - H)^-$, 111 $(B)^-$, 59 $(AcO)^-$. ^{1}H NMR (200 MHz, DMSO-d_6): 7.63 d, 1 H, *J*(6,5) = 8.1 (H-6); 6.06 s, 1 H (H-1′); 5.68 d, 1 H, *J*(5,6) = 8.1 (H-5); 5.3 m, 1 H, *J*(3′,4′) = 4.8, *J*(3′,F) = 51.9 (H-3′); 4.4–4.2 m, 3 H (H-4′, H-5′a, H-5′b); 2.06 s, 3 H (OCH_3); 1.97 s, 3 H (OCH_3); 1.58 2s, 3 H (CH_3).

3′-Deoxy-3′-fluoro-2′-*C*-methylcytidine Hydrochloride (**32**)

A mixture of compound **30** (160 mg, 0.46 mmol) and Lawesson's reagent (200 mg, 0.48 mmol) was refluxed in anhydrous methylene chloride (12 ml). The solvent was evaporated to dryness and the crude mixture purified by silica gel column chromatography (eluent: methanol, 0–5% in methylene chloride) to give compound **31** (268 mg, quantitative yield) which was then treated with methanol saturated with ammonia (12 ml) at 100 °C for 3 h. After re-

moval of methanol under reduced pressure, the crude mixture was purified by silica gel column chromatography (eluent: methanol, 0–15% in methylene chloride) to give title compound **32** (108 mg, 90%). This compound was transformed into its hydrochloride after three coevaporations with a mixture of 2 M hydrochloric acid (0.34 ml) and ethanol (0.69 ml), and precipitated. For $C_{10}H_{15}ClFN_3O_4$ (295.7) calculated: 40.62% C, 5.11% H, 11.99% Cl, 14.21% N; found: 40.57% C, 5.23% H, 11.72% Cl, 14.10% N. FAB positive, *m/z* (GT): 519 $(2\ M + H)^+$; 352 $(M + G + H)^+$, 260 $(M + H)^+$, 112 $(B + 2\ H)^+$; FAB negative, *m/z* (GT): 517 $(2\ M - H)^-$; 258 $(M - H)^-$. ^{1}H NMR (300 MHz, DMSO-d_6): 9.71 8.62 2s, 3 H, D_2O exchangeable (NH_3^+); 8.26 d, 1 H, *J*(6,5) = 7.8 (H-6); 6.13 d, 1 H, *J*(5,6) = 7.8 (H-5); 5.82 s, 1 H (H-1′); 4.74 dd, 1 H, *J*(3′,4′) = 8.2, *J*(3′,F) = 47.1 (H-3′); 4.2 m, 1 H (H-4′); 3.9–3.6 m, 2 H (H-5′a, H-5′b); 1.15 s, 3 H (CH_3). ^{13}C NMR (400 MHz, DMSO-d_6): 160.02 (C-4); 148.35 (C-2); 144.13 (C-6); 94.65 (C-5); 91.16 d, *J*(F,C) = 1.9 (C-3′); 91.82 (C-1′); 80.38 d, *J*(F,C) = 0.2 (C-4′); 77.71 d, *J*(F,C) = 0.1 (C-2′); 59.03 (C-5′); 19.85 (CH_3). HPLC R_t = 3.66 min (gradient from 0 to 40% CH_3CN in 20 mM triethylammonium acetate buffer programmed over a 10-min period, flow rate of 1 ml/min), λ_{max} = 271.1 nm. UV (H_2O): λ_{max} = 270.1 nm (ε 8300), λ_{min} = 249.0 nm (ε 5800).

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