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2′-Derivatisation of 3′-*C*-Methyl Pyrimidine Nucleosides

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The stereoselective synthesis of some 3′-deoxy-3′-*C*-methyl pyrimidine nucleosides is reported. The studied modifications concern the inversion of the configuration in 2′-position as well as the introduction of fluoro and azido substituents. The corresponding arabinonucleoside and 2′-substituted ribonucleoside analogs of uracil and cytosine have been obtained and fully characterized. Attempts to introduce fluoro and azido substituents with inversion at C-2′ are also presented.

Introduction

The current pandemic associated with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its health, economic, environmental, and social consequences highlight the need for therapeutic treatments in parallel to the development of prophylactic strategies. In the search for efficient antiviral small-molecules against this disease, drug repurposing (also called repositioning, redirecting, or reprofiling) was first extensively studied.[1] This last has some advantages over new drug discovery since chemical synthesis steps, manufacturing processes, reliable safety, and pharmacological properties in early clinical development phases are already available. Nucleoside and nucleotide analogs, which have a long and rich history in the field of medicinal chemistry, are in consequence well represented in this race to arsenal COVID-19 therapeutics.

During the last decades, extensive modifications of the nucleoside/tide endogenous scaffolds have been reported.[2] Among them, the introduction of a methyl group on the osidic residue has led to new molecules endowed with a wide range of biological activities. As an example, (2′*S*)-2′-deoxy-2′-*C*-methylcytidine[3] (SMDC, Figure 1), as well as 3′-C-methyladenosine[4] (Figure 1), exhibited potent activities against various human cancer cell lines. In addition, the 2′-*C*-methyl series is known to include potent inhibitors of hepatitis C virus (HCV) replication and Sofosbuvir (Figure 1) was approved by the United States Food and Drug Administration (FDA) for the treatment of this disease.[5]

As part of our work on this topic, we previously reported preliminary studies in the 3′-*C*-methyl nucleoside series[6] with the synthesis of 3′-deoxy and 2′,3′-dideoxy-3′-*C*-methyl nucleosides bearing naturally occurring nucleobases and various aglycones of pharmacological interest (Figure 2).

We focused our investigations in this series by designing various modifications in the 2′-position. Modifications such as inversion of configuration, introduction of fluoro, azido and amino substituents were envisaged. Thus, the stereoselective synthesis of the resulting 3′-deoxy-3′-*C*-methyl nucleosides bearing uracil and cytosine is reported herein (Figure 2).

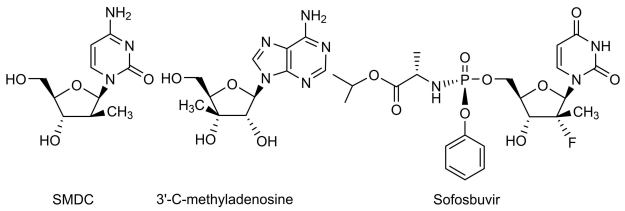

Figure 1. Examples of *C*-methyl nucleoside/tide analogs with biological activities.

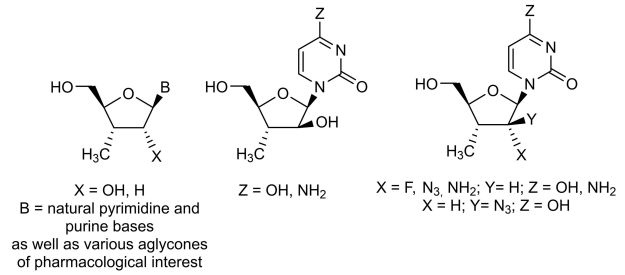

Figure 2. Previously reported 3′-*C*-nucleosides and additional structural analogs investigated in this study.

Results and Discussion

3′-Deoxy-3′-C-methyl arabinonucleoside analogs. Anhydronucleosides are useful starting materials for the stereochemically-controlled introduction of functional groups into the secondary positions of the sugar moiety.[7] In this respect, the anhydronucleoside **2** (Scheme 1) appeared as a suitable candidate for various modifications with 2′-inversion of configuration. The latter was obtained from the benzoylated nucleoside **1**[6a] under Mitsunobu conditions. Treatment of **2** with an aqueous sodium hydroxide solution gave the arabino derivative of uracile **3**. This nucleoside was previously published according to a non-regiospecific reaction involving the opening of a 2′,3′-epoxide.[8] Conversion of the uracil aglycone of **3** into the cytosine

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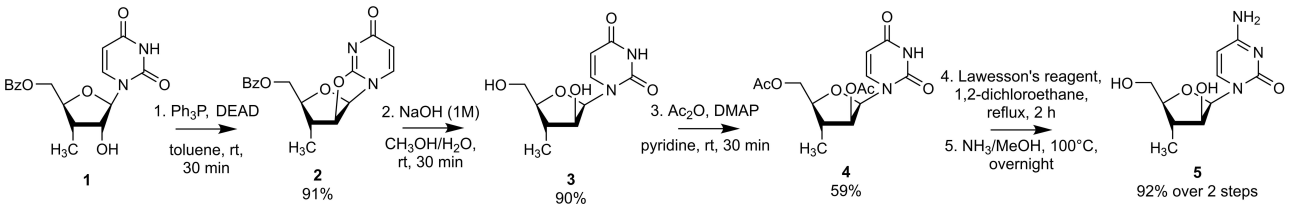


Scheme 1. Synthesis of 3′-deoxy-3′-*C*-methyl arabinonucleoside analogs **3** and **5**.

counterpart required acetylation to obtain the fully protected nucleoside **4**. Then, the desired arabino derivative of cytosine **5** was obtained in two steps from **4**, *via* the reaction with Lawesson's reagent,[9] followed by the treatment of the 4-thioamide intermediate with methanolic ammonia at 100 °C in a stainless-steel bomb.

The proposed structures of these arabinonucleosides were based on UV, ^{1}H, and ^{13}C NMR and mass spectral data. The ^{1}H spectrum shows H-1′ as a doublet with coupling constants ($J_{1'-2'} = 6.1$ and 5.8 Hz for **3** and **5**, respectively) in agreement with the arabinose configuration of the 2′-hydroxyl function.[10] In addition, the signal of the 3′-methyl substituent ($\delta \simeq 1.0$ ppm) appeared as doublet and the CH_3-$H_{3'}$ coupling constants were 6.6 and 6.7 Hz for **3** and **5** respectively, similar to those observed for the 3′-*C*-methyl-β-D-ribo- and 2′-deoxy-β-D-ribonucleoside analogs.[6]

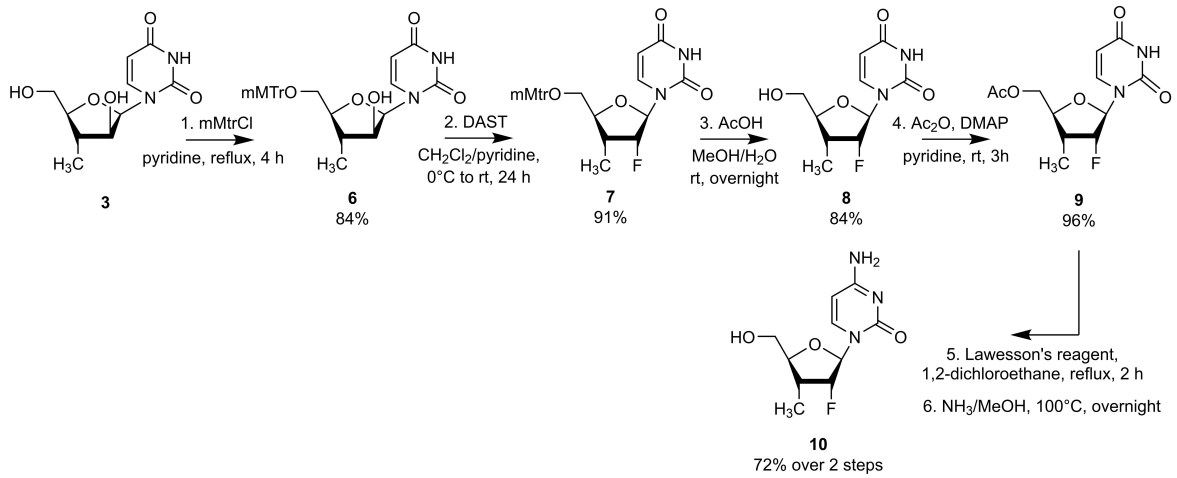
2′-Fluoro-3′-deoxy-3′-C-methyl ribonucleoside analogs. Several routes have been previously reported for the synthesis of 2′-deoxy-2′-fluoro-β-D-ribonucleosides, including the opening of 2,2′-anhydronucleosides with a fluorinating agent (HF/dioxane or KF/crown ether),[11] the coupling of a suitably blocked 2-deoxy-2-fluororibofuranoside with appropriate heterocyclic bases,[12] an enzyme-catalyzed transglycosylation,[13] the nucleophilic displacement of 2′-*O*-trifluoromethanesulfonyl arabinonucleosides by tetra-*n*-butylammonium fluoride (TBAF),[14] and the direct introduction of fluorine atom by using diethylaminosulfur trifluoride (DAST) and an arabinonucleoside.[15] We selected this last methodology for the preparation of the fluoro derivative **8** (Scheme 2). Thus, the previously obtained arabinonucleoside **3** reacted with 4-monomethoxytrityl chloride to afford the corresponding 5′-*O*-protected nucleoside **6**, which was treated with DAST to give rise to intermediate **7**. Then, acidic treatment of **7** led to the desired fluorinated ribonucleoside **8** which was crystallized in methanol. The uracil derivative **8** was converted to **10** using the same methodology as for compound **5** (Scheme 1).

The structures of fluoronucleosides **8** and **10** were confirmed by UV, ^{1}H, ^{13}C, ^{19}F NMR, and mass spectra analysis. ^{1}H NMR spectra of these derivatives exhibited a downfield shift of the H-2′ due to the electron-withdrawing effect of fluorine.

Sarah Couturier graduated in organic chemistry from the Engineer School ENSSPICAM (Aix-Marseille) and obtained her Ph.D. (2004) at the University of Montpellier. Starting in industry in 2005, she contributed to the success of innovative projects of the API Services and Chemical Development Department of a CRO in Northern Ireland. She joined SERATEC (Courville sur Eure, France) in 2007, a Drug Substance CDMO dedicated to low volume and complex APIs in a context of quality excellence. She is currently R&D Director, developing synthetic route for API for the pharmaceutical industry and acceptable to the health authorities.

Suzanne Peyrottes graduated in chemistry from Montpellier University and completed her postdoctoral study at the MRC (Cambridge, UK). She is currently Research Director at CNRS, working on the design of potential therapeutic agents to treat infections and cancers while developing new synthetic methodologies related to nucleic acid components. She heads the team "Nucleosides & Phosphorylated Effectors" belonging to the Institute of Biomolecules Max Mousseron (IBMM, Montpellier, France).

Christian Périgaud obtained his Ph.D. (1991) at the University of Montpellier with G. Gosselin in the laboratory of Pr. J.-L. Imbach working on nucleoside chemistry. Back to Montpellier after a postdoctoral work with Pr. J.-P. Sommadossi, he was appointed as associate professor (1993), then professor (2001). His scientific interests focused on the development of anti-infective agents, prodrug concepts as well as tools for the understanding of biological processes. He has co-authored more than 130 publications. He has also served for his University as vice-president for research (2008-2012) and the French Ministry of Higher Education and Research (2013-2020).

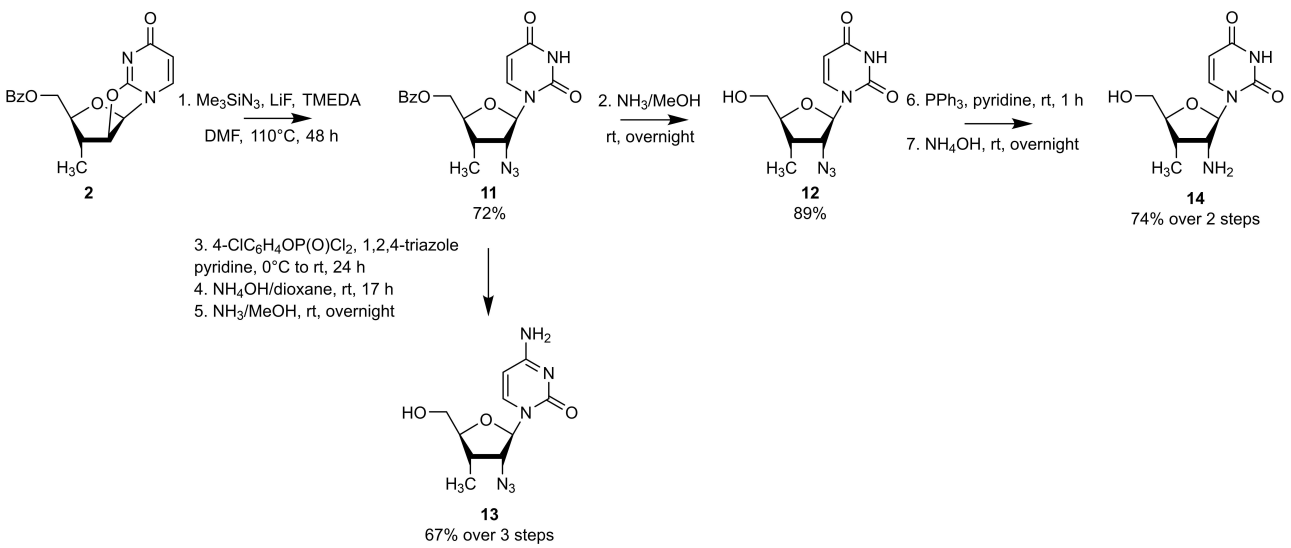


Scheme 2. Synthesis of 2′-fluoro-3′-deoxy-3′-*C*-methyl ribonucleoside analogs **8** and **10**.

Illustrated by NMR data observed for **8**, the assignments of H-1′ (δ = 5.79 ppm), H-2′ (δ = 5.00 ppm), and H-3′ (δ = 2.12 ppm) are consistent with the vicinal and geminal H–F coupling values found: 17.5, 52.3, and 35.9 Hz respectively.[16] The large proton-fluorine coupling constants allow ready assignments of the protons but the spectrum was insufficiently resolved in the used solvent to determine the magnitude of $J_{1'-2'}$ (known to be particularly low for 1′,2′-*trans* stereoisomers)[17] and $J_{3'-4'}$. The (2′*R*) configuration of the fluorinated nucleosides **8** and **10** was also assigned from ^{13}C NMR spectra analysis. For the two compounds, the measured $J_{C1'-F}$ (37 Hz) is similar to that observed for other 2′-deoxy-2′-fluoronucleoside analogs characterized by a 1′,2′-*trans* stereoisomerism.[18] In addition, we measured a coupling constant between the 3′-methyl group (δ ≃ 7.5 ppm, d) and the fluorine atom (J_{CH3-F} = 7.6 and 8.3 Hz for **8** and **10**, respectively) which is only observed when these two substituents are in a *cis* position.[19]

2′-Azido- and 2′-amino-3′-deoxy-3′-C-methyl ribonucleoside analogs. The synthesis of the 2′-azido derivative **11** (Scheme 3) was carried out according to a published procedure involving the *in situ* generation of lithium azide from lithium fluoride and azidotrimethylsilane in the presence of N,N,N′,N′-tetramethylethylenediamine (TMEDA) in DMF.[20] In these conditions, the introduction of the azido group on the 2′-position was obtained with satisfactory yield from the previously described anhydronucleoside **2**. Thus, treatment of the intermediate **11** with methanolic ammonia afforded the desired 2′-azido-ribonucleoside **12**. The corresponding cytosine analog **13** cannot be obtained by a thiation procedure using Lawesson's reagent,[9b] and we selected Sung's methodology for its preparation.[21]

Thus, the reaction of the protected nucleoside **11** with 4-chlorophenyl phosphorodichloridate and 1,2,4-triazole in pyridine at room temperature led to the corresponding 4-triazolylpyrimidinone derivative (not shown) which was purified



Scheme 3. Synthesis of 2′-azido- and 2′-amino-3′-deoxy-3′-*C*-methyl ribonucleoside analogs **12**–**14.**

on silica gel column chromatography. This intermediate was treated with aqueous ammonia in dioxane, followed by methanolic ammonia, to give rise to the desired nucleoside **13**.

Various synthetic protocols have been described for converting azides to amines,[22] among them the Staudinger procedure[23] appeared to be the most promising in the nucleoside field. Thus, 2′-azido nucleoside **12** was converted in presence of triphenylphosphine into a phosphinimine intermediate, the latter being hydrolyzed with aqueous ammonia to afford the 2′-amino-ribonucleoside analog **14**.

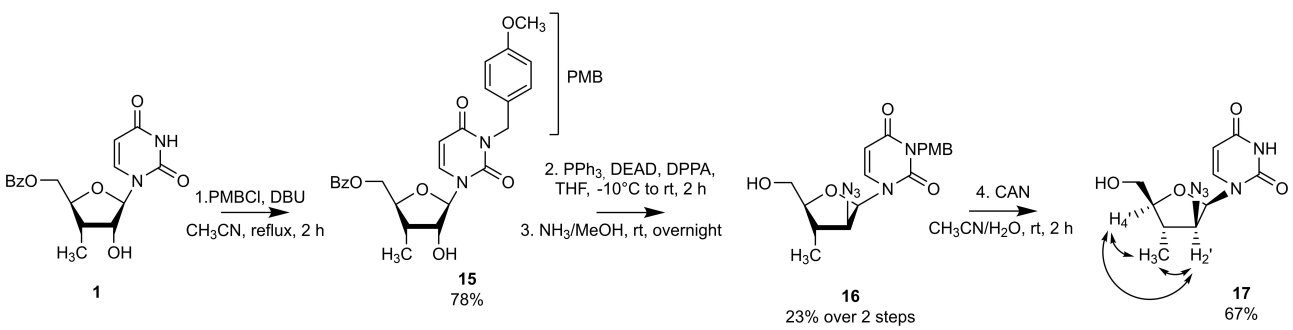
The structures of nucleosides **12**–**14** were in accordance with UV, IR, ^{1}H, ^{13}C NMR, and mass spectral data. The infrared spectra of compounds **11**–**13** exhibited a strong absorption band at 2110 cm^{-1}, characteristic of the azido function. Compared to ^{1}H NMR spectra of fluorinated nucleosides **8** and **10**, the chemical shifts and coupling constants measured for derivatives **12**–**14** reflect the relationship between the electronegativity of the 2′-substituents and the conformation of the sugar residue. The assignments of H-1′/H-2′ for **8** ($\delta = 5.83/5.12$ ppm), **12** ($\delta = 5.73/4.42$ ppm), and **14** ($\delta = 5.50/3.30$ ppm) are consistent with the electronegativity of fluorine, azido, and amino functions. It is well known that coupling constants $J_{1'-2'}$ is primarily conformation-dependent. Sugar puckering of natural ribo- and deoxyribonucleosides exist in dynamic equilibria between two major conformers: the North (N) and the South (S).[24] NMR studies of 2′-substituted ribonucleoside analogs demonstrated that the contribution of the N form (2′-*exo*-3′-*exo*) increases with the electronegativity of the 2′-substituent.[25] In addition, it seems reasonable that the bulky methyl group in 3′-position adopts an equatorial position of the sugar plane increasing the N conformer population. Thus, as observed for 2′-fluoro derivatives, the anomeric protons of 2′-azido-ribonucleosides **12** and **13** appeared as a singlet in accordance with the Karplus equation.

In the case of the 2′-amino derivative **14**, the measured $J_{1'-2}$ (2.5 Hz) seems to indicate that the equilibrium of the canonical conformers would be less in favor of the N form. This result was already observed for other 2′-amino pyrimidine and purine nucleosides.[25,26] In addition, the (2′R) configuration of the 2′-azido nucleoside **12** was corroborated by the NOE effects observed between H-6, H-2′, and H-3′ protons. Finally, the ^{13}C NMR analysis showed a downfield C2′ chemical shifts correlated to the electronegativity of the 2′-substituent for **14** ($\delta = 59.8$ ppm), **12** ($\delta = 69.8$ ppm) and **10** ($\delta = 98.5$ ppm).

Attempts to obtain 2′-modified-3′-deoxy-3′-C-methyl arabinonucleoside analogs. Fluorination. The introduction of a substituent at the C-2′-β (arabino) position in pyrimidine nucleosides by nucleophilic reactions cannot be achieved due to the neighboring-group participation of the carbonyl function of the aglycon.[7] It is well known that the *intra*-molecular attack of the 2-carbonyl group of the nucleobase on C-2′ of the sugar moiety supersedes the *inter*-molecular nucleophilic substitution. To circumvent this competition and to limit direct S_N2 reaction at the 2′-position of uridine derivatives, the protection of N^3-imide function has attracted attention and various protecting groups have been reported.[27] Among them, we selected *p*-methoxybenzyl (PMB).[28] The PBM protecting group is often used in nucleoside chemistry due to its compatibility with a wide range of reaction conditions and its stability in acid and alkaline media.[29]

Thus, the 5′-*O*-benzoylated precursor **1** was treated with 4-methoxylbenzyl chloride in presence of DBU to afford the N^3-protected nucleoside **15** in 78% yield (Scheme 4). This compound submitted to usual fluorination conditions led to the unexpected 2′-fluoro-ribonucleoside analog (see supporting information). Unfortunately, the retention of the configuration of C-2′ sugar residue demonstrates that this strategy cannot circumvent the neighboring group participation by the respective O^2-atom in fluorination conditions. Using N^3-benzyl uridine analogs, similar results have just been reported during the writing of this article.[30]

Azidation. The N^3-protected nucleoside **15** treated under the conditions used for the preparation of nucleoside **11** (Scheme 3) gave rise to a complex mixture of at least three compounds (see supporting information). A chromatography separation allows separating one of them, which turns out to be the unexpected 2′-azido-ribonucleoside analog presenting retention of the configuration. The other mixture fractions were collected, treated with methanolic ammonia to provide after purification the 2′,3′-unsaturated nucleoside analog and the target nucleoside **16** in low yield (23%). Finally, the PBM group is removed from the purified intermediate **16** using the mild oxidizing reagent ceric ammonium nitrate (CAN) to afford the desired 2′-azido-3′-deoxy-3′-*C*-methyl arabinonucleoside **17**.



Scheme 4. Synthesis of the 1-(2′-azido-2′,3′-dideoxy-3′-*C*-methyl-β-D-arabinofuranosyl)uracil **17**

The (2′*S*) configuration of the azidonucleoside **17** was assigned in comparison to its stereoisomer **12** on the basis of NMR spectra and NOE experiments. In the ^{1}H NMR spectrum of **17** the signal for the anomeric proton appeared at a lower field resonance ($\delta = 6.13$ ppm) than that of the isomer **12** ($\delta =$ 5.73 ppm). The larger coupling constant (6.5 Hz) for the H-1′ proton of **17** while the anomeric proton of **12** appeared as a singlet providing additional support to the 1′,2-*cis* configuration of **17**. These typical ^{1}H NMR patterns are in agreement with the abundant literature in the field, especially with the empirical rules used for the determination of the anomeric configuration. Finally, irradiations of the 3′-methyl group of the nucleoside **17** gave large NOE effects (5.0%) on H-2′ and H-4′ protons (Scheme 4). A similar effect was also observed between the same protons.

Conclusion

In summary, the stereoselective synthesis of 3′-deoxy-3′-*C*-methyl nucleoside analogs of uracil and cytosine bearing several modifications at the 2′-position of the sugar residue was undertaken with the hope of discovering new compounds endowed with potential activities. The corresponding arabino-nucleoside and 2′-substituted ribonucleoside analogs were relatively straightforward to obtain, whereas attempts to introduce a substituent at C-2′-β (arabino) position of uracil nucleosides by nucleophilic reactions failed (fluorination) or led to complex mixtures (azidation). These results are due to the neighboring group participation of the carbonyl function of the aglycon despite the protection of N^3-imide function. The biological evaluations of these compounds are currently in progress.

Experimental Section

General Information: Melting points (m.p.) were determined in open capillary tubes on a Gallenkamp MFB-595-010M apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 300 MHz on a Bruker 300 Advance, ^{19}F spectra at 250 MHz on Bruker 250. Chemical shifts are quoted in parts per million (ppm) using residual non-deuterated solvents as internal references. Deuterium exchange, decoupling, and COSY experiments were performed in order to confirm proton assignments. Coupling constants, *J*, are reported in Hertz. 2D ^{1}H-^{13}C heteronuclear COSY spectra were recorded for the attribution of ^{13}C signals. UV spectra were recorded on an Uvikon 931 (Kontron). IR spectra were recorded on a Perkin Elmer Paragon 1000 FT-IR. FAB mass spectra were recorded in the positive-ion or negative-ion mode on a JEOL JMS DX 300 using thioglycerol/glycerol (1:1, v/v, G–T) as matrix. ESI HRMS were recorded in the positive mode on a Micromass Q-TOF Waters. TLC was performed on pre-coated aluminium sheets of silica gel 60 F_{254} (Merck, Art. 9385), visualization of products being accomplished by UV absorbance followed by charring with 5% ethanolic sulphuric acid with heating. Solvents were reagent grade or purified by distillation prior to use, and solids were dried over P_2O_5 under reduced pressure at rt. Moisture-sensitive reactions were performed under an argon atmosphere using oven-dried glassware. All aqueous solutions were saturated with the specified salt unless otherwise indicated. The nucleoside analog **1** was obtained following a previously published procedure.[6a]

2,2′-Anhydro-1-(5-*O*-benzoyl-3-deoxy-3-*C*-methyl-β-D-*erythro*-pento-furanosyl)uracil (2): To a solution of the protected nucleoside **1** (1.81 g, 5.23 mmol) in anhydrous toluene (93 mL) were added triphenylphosphine (2.73 g, 10.46 mmol) and diethyl azodicarboxylate (DEAD, 1.61 mL, 10.46 mmol). After stirring 30 min at room temperature, the reaction mixture was evaporated under reduced pressure and the resulting residue was subjected to silica gel column chromatography using as eluent a stepwise gradient of methanol (0–5%) in dichloromethane to afford the title compound **2** (1.55 g, 91% yield) as white solid. ^{1}H NMR ($CDCl_3$) $\delta = 7.87$–7.34 (m, 5H, C_6H_5CO), 7.29 (d, $J = 7.5$, 1H, H-6), 6.11 (d, $J = 5.6$, 1H, H-1′), 5.98 (d, $J = 7.5$, 1H, H-5), 5.06 (dd, $J = 5.6$, $J = 2.7$, 1H, H-2′), 4.31 (m, 1H, H-5′), 4.20 (m, 2H, H-4′, H-5″), 2.67 (m, 1H, H-3′), 1.27 (d, $J = 7.3$, 3H, CH_3); ^{13}C NMR ($CDCl_3$) $\delta = 171.8$ (CO), 165.9 (C-4), 159.6 (C-2), 134.9 (C-6), 133.5-128.6 (Ph), 110.4 (C-5), 90.3 (C-1′), 89.2 (C-2′), 85.7 (C-4′), 64.0 (C-5′), 41.5 (C-3′), 16.4 (CH_3); UV (EtOH 95) $\lambda_{max} = 259$ nm ($\varepsilon = 10\,700$); MS: *m/z* (FAB > 0) 657 $(2M+H)^+$, 329 $(M+H)^+$; *m/z* (FAB < 0) 327 $(M-H)^-$.

1-(3-Deoxy-3-*C*-methyl-β-D-arabinofuranosyl)uracil (3): The 2,2′-*O*-anhydronucleoside **2** (0.74 g, 2.25 mmol) was dissolved in methanol/water (22.5 mL, 1/1 : v/v) and treated with 1 M NaOH (22.5 mL) for 30 min, then neutralized with glacial AcOH and evaporated to dryness. The crude material was purified by silica gel column chromatography using a stepwise gradient of methanol (0–10%) in dichloromethane to afford the title compound **3** (0.49 g, 90% yield) as white solid, which was crystallised from MeOH. m.p. 213–214 °C [litt.: 211–213 °C,[8c] 208–210 °C (MeOH/toluene/petroleum ether);[8b] ^{1}H NMR (DMSO-*d6*) $\delta = 11.20$ (s, 1H, NH), 7.88 (d, $J = 8.1$, 1H, H-6), 5.98 (d, $J = 6.1$, 1H, H-1′), 5.54 (d, $J = 8.1$, 1H, H-5), 5.1 (bs, 1H, OH-5′), 3.98 (pt, 1H, H-2′), 3.68 (m, 1H, H-5′), 3.57 (m, 2H, H-5″, H-4′), 3.38 (bs, 1H, OH-2′), 1.93 (1H, m, H-3′), 1.00 (d, $J = 6.6$, 3H, CH_3). ^{13}C NMR (DMSO-*d6*) $\delta = 163.3$ (C-4), 150.6 (C-2), 142.1 (C-6), 100.0 (C-5), 83.5 (C-1′), 83.4 (C-4′), 76.6 (C-2′), 60.0 (C-5′), 38.6 (C-3′), 14.0 (CH_3); UV (EtOH 95) $\lambda_{max} = 262$ nm ($\varepsilon = 9500$); MS: *m/z* (FAB > 0) 243 $(M+H)^+$; HRMS (ESI^+): *m/z* calcd. for $C_{10}H_{15}N_2O_5$ $(M+H)^+$: 243.0981; found: 243.0978; elemental analysis calcd (%) for $C_{10}H_{14}N_2O_5$: C, 49.58; H, 5.83; N, 11.56; found: C, 49.89; H, 5.76; N, 11.52.

1-(2,5-Di-*O*-acetyl-3-deoxy-3-*C*-methyl-β-D-arabinofuranosyl) uracil (4): To a solution of the arabinonucleoside **3** (94.2 mg, 0.39 mmol) in anhydrous pyridine (2.3 mL) were added acetic anhydride (0.54 mL) and a catalytic amount of 4-dimethylaminopyridine (DMAP). The solution was stirred 30 min at room temperature and then, neutralised with solid $NaHCO_3$. The reaction mixture was extracted with diethyl ether and the organic layer was evaporated to dryness. The crude material was purified by silica gel column chromatography using a stepwise gradient of methanol (0–2%) in dichloromethane to afford the title compound **4** (75.0 mg, 59% yield) as white solid.^{1}H NMR ($CDCl_3$) $\delta = 8.50$ (s, 1H, NH), 7.50 (d, $J = 8.2$, 1H, H-6), 6.11 (d, $J = 5.1$, 1H, H-1′), 5.66 (dd, $J = 8.1$, $J = 2.2$, 1H, H-5), 5.11 (pt, 1H, H-2′), 4.28 (dd, $J = 5.5$, $J = 12.3$, 1H, H-5″), 4.23 (dd, $J = 3.7$, $J = 12.3$, 1H, H-5′), 3.81 (m, 1H, H-4′), 2.13 (m, 1H, H-3′), 2.07 (s, 3H, CH_3CO), 1.94 (s, 3H, CH_3CO), 1.15 (d, $J = 7.0$, 3H, CH_3); ^{13}C NMR ($CDCl_3$) $\delta = 170.5$ (CO), 169.3 (CO), 162.7 (C-4), 149.7 (C-2), 140.6 (C-6), 101.4 (C-5), 83.9 (C-1′), 81.5 (C-4′), 77.5 (C-2′), 63.7 (C-5′), 39.9 (C-3′), 20.8 (CH_3CO), 20.6 (CH_3CO), 15.3 (CH_3); UV (EtOH 95) $\lambda_{max} = 259$ nm ($\varepsilon = 9900$); MS: *m/z* (FAB > 0) 653 $(2M+H)^+$, 327 $(M+H)^+$, 215 $(S)^+$, 113 $(BH_2)^+$; *m/z* (FAB < 0) 651 $(2M-H)^-$, 325 $(M-H)^-$, 111 $(B)^-$.

1-(3-Deoxy-3-*C*-methyl-β-D-arabinofuranosyl)cytosine (5): Lawesson's reagent (64.8 mg, 0.16 mmol) was added to a solution of the protected nucleoside **4** (75.0 mg, 0.23 mmol) in anhydrous 1,2-dichloroethane (7.3 mL) and the reaction mixture was refluxed for

2 h. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography using a stepwise gradient of methanol (0–1%) in dichloromethane affording the corresponding 4-thioamide intermediate as orange foam. The latter was dissolved in methanolic ammonia (7 mL, saturated beforehand at −10 °C and stoppered tightly) and heated overnight at 100 °C in a stainless-steel bomb. After cooling to room temperature, the solution was evaporated under reduced pressure and the residue was subjected to silica gel column chromatography using as eluent a stepwise gradient of methanol (0–15%) in dichloromethane to afford the title compound **5** (51.0 mg, 92% yield) as white solid. ^{1}H NMR (DMSO-*d6*) δ = 7.76 (d, *J* = 7.4, 1H, H-6), 7.01 (pd, 2H, NH_2), 6.02 (d, *J* = 5.8, 1H, H-1′), 5.65 (d, *J* = 7.4, 1H, H-5), 5.29 (d, *J* = 5.4, 1H, OH-2′), 5.02 (pt, 1H, OH-5′), 3.92 (m, 1H, H-2′), 3.65 (m, 1H, H-5′), 3.56 (m, 1H, H-5″), 3.49 (m, 1H, H-4′), 1.92 (m, 1H, H-3′), 1.01 (d, *J* = 6.7, 3H, CH_3); ^{13}C NMR (DMSO-*d6*) δ = 165.4 (C-4), 155.5 (C-2), 142.7 (C-6), 92.5 (C-5), 84.1 (C-1′), 83.2 (C-4′), 76.5 (C-2′), 60.7 (C-5′), 39.5 (C-3′), 14.6 (CH_3); UV (EtOH 95) λ_{max} = 272 nm (ε = 8000); MS: *m/z* (FAB > 0) 242 $(M+H)^+$, 112 $(BH_2)^+$; *m/z* (FAB < 0) 240 $(M-H)^-$; HRMS (ESI^+): *m/z* calcd. for $C_{10}H_{16}N_3O_4$ $(M+H)^+$: 242.1141; found: 242.1138.

1-(3-Deoxy-3-*C*-methyl-5-*O*-monomethoxytrityl-β-D-arabino-furanosyl)uracil (6): 4-Monomethoxytrityl chloride (372.0 mg, 1.21 mmol) was added to a solution of the protected nucleoside **3** (265.3 mg, 1.10 mmol) in anhydrous pyridine (4.9 mL) and the reaction mixture was refluxed for 4 h. After cooling to room temperature, methanol (4 mL) was added and the reaction mixture was evaporated under reduced pressure. The resulting residue was subjected to silica gel column chromatography using as eluent a stepwise gradient of methanol (0–5%) in dichloromethane to afford the title compound **6** (472.2 mg, 84% yield) as foam. ^{1}H NMR ($CDCl_3$) δ = 9.76 (bs, 1H, NH), 8.21 (d, *J* = 8.1, 1H, H-6), 7.37-7.09 (m, 12H, Ph), 6.78 (m, 2H, Ph), 6.00 (d, *J* = 5.8, 1H, H-1′), 5.31 (dd, *J* = 8.1, *J* = 1.3, 1H, H-5), 4.37 (bs, 1H, OH-2′), 4.12 (m, 1H, H-2′), 3.73 (s, 3H, CH_3O), 3.57 (m, 1H, H-4′), 3.51 (dd, *J* = 1.7, *J* = 11.2, 1H, H-5′), 3.27 (dd, *J* = 2.4, *J* = 11.2, 1H, H-5″), 2.26 (m, 1H, H-3′), 0.97 (d, *J* = 6.5, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ = 164.4 (C-4), 158.8 (Ph), 151.6 (C-2), 143.9-125.3 (Ph), 141.8 (C-6), 113.3 (Ph), 101.5 (C-5), 87.2 (CPh_3), 85.4 (C-1′), 83.3 (C-4′), 78.1 (C-2′), 61.3 (C-5′), 55.3 (OCH_3), 37.3 (C-3′), 13.6 (CH_3); UV (EtOH 95) λ_{max} = 264 nm (ε = 10600), 230 nm (ε = 15900); MS: *m/z* (FAB > 0) 515 $(M+H)^+$, 113 $(BH_2)^+$; *m/z* (FAB < 0) 1027 $(2M-H)^-$, 513 $(M-H)^-$, 241(M-mMTr)$^-$,111 $(B)^-$.

1-(2,3-Dideoxy-2-fluoro-3-*C*-methyl-5-*O*-monomethoxytrityl-β-D-ribofuranosyl)uracil (7): To a solution of the protected arabinonucleoside **6** (435.0 mg, 0.85 mmol) in a mixture of dry dichloromethane and pyridine (5.1 mL, 92/8 : v/v) at 0 °C was added DAST (166 μL, 1.35 mmol). The reaction mixture was stirred at room temperature one day, neutralized with saturated $NaHCO_3$ and diluted with dichloromethane (30 mL). The organic layer was washed with water, dried over sodium sulfate and evaporated to dryness. The residue was purified by silica gel column chromatography using as eluent a stepwise gradient of methanol (0–2%) in dichloromethane to afford the title compound **7** (398.7 mg, 91% yield) as white foam. ^{1}H NMR ($CDCl_3$) δ = 10.55 (bs, 1H, NH), 8.16 (d, *J* = 8.1, 1H, H-6), 7.46–7.22 (m, 12H, Ph), 6.87 (m, 2H, Ph), 6.05 (d, *J* = 16.3, 1H, H-1′), 5.40 (d, *J* = 8.1, 1H, H-5), 5.00 (dd, *J* = 3.7, *J* = 51.4, 1H, H-2′), 4.02 (m, 1H, H-4′), 3.78 (s, 3H, CH_3O), 3.73 (dd, *J* = 1.6, *J* = 11.5, 1H, H-5′), 3.36 (dd, *J* = 2.3, *J* = 11.5, 1H, H-5″), 2.56 (dm, *J* = 34.1, 1H, H-3′), 1.01 (d, *J* = 6.7, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ = 164.1 (C-4), 158.8 (Ph), 150.5 (C-2), 143.8-123.9 (Ph), 140.0 (C-6), 113.7 (Ph), 102.0 (C-5), 98.2 (d, *J* = 185.7, C-2′), 89.3 (d, *J* = 38.0, C-1′), 87.2 (CPh_3), 85.0 (C-4′), 60.6 (C-5′), 55.2 (OCH_3), 35.6 (d, *J* = 19.6, C-3′), 8.0 (d, *J* = 7.6, CH_3); ^{19}F NMR ($CDCl_3$) δ = −196,5 (m, *J* = 16.3, *J* = 51.4, *J* = 34.1, F-2′); UV (EtOH 95) λ_{max} = 260 nm (ε = 11300), 231 nm (ε = 17500); MS: *m/z* (FAB > 0) 517 $(M+H)^+$, 113 $(BH_2)^+$; *m/z* (FAB < 0) 1031 $(2M-H)^-$, 515 $(M-H)^-$, 243 (M-mMTr)$^-$, 111 $(B)^-$.

1-(2,3-Dideoxy-2-fluoro-3-*C*-methyl-β-D-ribofuranosyl)uracil (8): A solution of the protected nucleoside **7** (454.9 mg, 0.88 mmol) in a mixture of AcOH, methanol and water (26 mL, 8/1/1 : v/v/v) was stirred overnight at room temperature. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography using a stepwise gradient of methanol (0–10%) in dichloromethane to afford the title compound **8** (180.1 mg, 84% yield), which was crystallised from MeOH. m.p. 214–215 °C; ^{1}H NMR (DMSO-*d6*) δ = 11.4 (bs, 1H, NH), 8.06 (d, *J* = 8.1, 1H, H-6), 5.83 (d, *J* = 17.5, 1H, H-1′), 5.58 (d, *J* = 8.1, 1H, H-5), 5.26 (pt, 1H, OH-5′), 5.12 (dd, *J* = 4.0, *J* = 52.3, 1H, H-2′), 3.85 (m, 1H, H-5′), 3.82 (m, 1H, H-4′), 3.60 (m, 1H, H-5″), 2.29 (dm, *J* = 35.9, 1H, H-3′), 1.00 (d, *J* = 6.7, 3H, CH_3); ^{13}C NMR (DMSO-*d6*) δ = 163.3 (C-4), 150.2 (C-2), 139.9 (C-6), 100.8 (C-5), 98.5 (d, *J* = 181.1, C-2′), 88.5 (d, *J* = 37.0, C-1′), 85.8 (C-4′), 58.8 (C-5′), 34.4 (d, *J* = 19.6, C-3′), 7.9 (d, *J* = 7.6, CH_3); ^{19}F NMR (DMSO-*d6*) δ = −195,6 (m, *J* = 17.5, *J* = 52.3, *J* = 35.9, F-2′); UV (EtOH 95) λ_{max} = 259 nm (ε = 10300); MS: *m/z* (FAB > 0) 489 $(2M+H)^+$, 245 $(M+H)^+$, 113 $(BH_2)^+$; *m/z* (FAB < 0) 487 $(2M-H)^-$, 243 $(M-H)^-$, 111 $(B)^-$; HRMS (ESI^+): *m/z* calcd. for $C_{10}H_{14}FN_2O_4$ $(M+H)^+$: 245.0938; found: 245.0938.

1-(5-*O*-Acetyl-2,3-dideoxy-2-fluoro-3-*C*-methyl-β-D-ribofuranosyl)-uracil (9): To a solution of the nucleoside **8** (94.6 mg, 0.39 mmol) in anhydrous pyridine (3.9 mL) were added acetic anhydride (0.467 mL, 5.42 mmol) and a catalytic amount of DMAP. After stirring 3 h at room temperature, EtOH 95 was added and the reaction mixture was evaporated to dryness. The crude material was purified by silica gel column chromatography using a stepwise gradient of methanol (0–5%) in dichloromethane to afford the title compound **9** (107.4 mg, 96% yield) as white foam. ^{1}H NMR ($CDCl_3$) δ = 8.84 (bs, 1H, NH), 7.60 (d, *J* = 8.2, 1H, H-6), 5.79 (d, *J* = 17.3, 1H, H-1′), 5.65 (d, *J* = 8.2, 1H, H-5), 5.00 (dd, *J* = 4.2, *J* = 51.5, 1H, H-2′), 4.33 (m, 2H, H-5′, H-5″), 4.10 (m, 1H, H-4′), 2.12 (m, 1H, H-3′), 2.06 (s, 3H, CH_3O), 1.08 (dd, *J* = 6.8, *J* = 0.8, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ = 170.3 (CO), 162.9 (C-4), 149.8 (C-2), 139.6 (C-6), 101.9 (C-5), 97.5 (d, *J* = 186.4, C-2′), 90.9 (d, *J* = 37.7, C-1′), 83.5 (C-4′), 62.4 (C-5′), 36.5 (d, *J* = 20.4, C-3′), 20.7 (CH_3CO), 8.1 (d, *J* = 8.3, CH_3); ^{19}F NMR ($CDCl_3$) δ = −195,4 (m, *J* = 17.3, *J* = 51.5, *J* = 33.9, *J* = 0.8, F-2′); UV (EtOH 95) λ_{max} = 258 nm (ε = 9700); MS: *m/z* (FAB > 0) 573 $(2M+H)^+$, 287 $(M+H)^+$, 175 $(S)^+$, 113 $(BH_2)^+$; *m/z* (FAB < 0) 571 $(2M-H)^-$, 285 $(M-H)^-$, 111 $(B)^-$.

1-(2,3-Dideoxy-2-fluoro-3-*C*-methyl-β-D-ribofuranosyl)cytosine (10): Lawesson's reagent (80.5 mg, 0.20 mmol) was added to a solution of the protected nucleoside **9** (81.4 mg, 0.28 mmol) in anhydrous 1,2-dichloroethane (9 mL) and the reaction mixture was refluxed for 2 h. The solvent was evaporated under reduced pressure and the residue was purified using silica gel column chromatography using a stepwise gradient of methanol (0–1%) in dichloromethane to give the corresponding 4-thioamide intermediate as a yellow foam. The foam was dissolved in methanolic ammonia (10 mL, saturated beforehand at −10 °C and stoppered tightly) and heated overnight at 100 °C in a stainless-steel bomb. After cooling to room temperature, the solution was evaporated under reduced pressure and the residue was subjected to silica gel column chromatography using as eluent a stepwise gradient of methanol (0–15%) in dichloromethane to afford the title compound **10** (50.0 mg, 72% yield) as white solid. ^{1}H NMR (D_2O) δ = 7.84 (d, *J* = 7.5, 1H, H-6), 5.89 (d, *J* = 7.5, 1H, H-5), 5.86 (d, *J* = 17.7, 1H, H-1′), 4.96 (dd, *J* = 4.0, *J* = 51.8, 1H, H-2′), 3.97 (m, 1H, H-4), 3.92 (m, 1H, H-5′), 3.69 (dd, *J* = 4.0, *J* = 13.0, 1H, H-5″), 2.13 (dm, *J* = 35.2, 1H, H-3′), 0.98 (d, *J* = 6.8, 3H, CH_3); ^{13}C NMR (D_2O) δ = 171.0 (C-4), 166.3 (C-2), 141.1 (C-6), 98.7 (d, *J* = 182.6, C-2′), 95.5 (C-5), 90.0 (d, *J* = 37.7, C-1′), 86.0 (C-4′), 60.0 (C-5′), 35.4 (d, *J* = 19.6, C-3′), 7.2 (d, *J* = 8.3, CH_3); ^{19}F NMR (D_2O) δ = −196.1 (m, *J* = 17.7, *J* = 51.8, *J* = 33.9,

F-2′); UV (EtOH 95) λ_{max} = 272 nm (ε = 8700); MS *m/z* (FAB > 0) 244 $(M+H)^+$, 112 $(BH_2)^+$; HRMS (ESI^+): *m/z* calcd. for $C_{10}H_{15}FN_3O_3$ $(M+H)^+$: 244.1097; found: 244.1098.

1-(2-Azido-5-*O*-benzoyl-2,3-dideoxy-3-*C*-methyl-β-D-ribofuranosyl)-uracil (11): Lithium fluoride (10.96 mg, 0.42 mmol) was suspended in anhydrous DMF (0.26 mL) at room temperature. To the stirred suspension was added N,N,N′,N′-tetramethylethylenediamine (TMEDA, 0.23 mL, 1.54 mmol) followed by azidotrimethylsilane (65 μL, 0.49 mmol). The reaction mixture was heated 3 h at 110 °C and the 2,2′-*O*-anhydronucleoside **2** (84.4 mg, 0.26 mmol) was added. After stirring 48 h at 110 °C, the solvent was evaporated under reduced pressure and the residue was subjected to silica gel column chromatography using as eluent a stepwise gradient of methanol (0–2 %) in dichloromethane to afford the title compound **11** (95.4 mg, 72 % yield) which was crystallised from EtOH 100. m.p. 153–154 °C; ^{1}H NMR ($CDCl_3$) δ = 9.25 (bs, 1H, NH), 7.94 (m, 2H, Ph), 7.71 (d, *J* = 8.2, 1H, H-6), 7.58–7.39 (m, 3H, Ph), 5.76 (s, 1H, H-1′), 5.39 (d, *J* = 8.2, 1H, H-5), 4.62 (dd, *J* = 2.4, *J* = 13.0, 1H, H-5′), 4.57 (dd, *J* = 3.1, *J* = 13.0, 1H, H-5″), 4.15 (d, *J* = 5.5, 1H, H-2′), 4.11 (m, 1H, H-4′), 2.17 (m, 1H, H-3′), 1.10 (d, *J* = 6.7, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ = 166.0 (CO), 162.9 (C-4), 149.9 (C-2), 138.7 (C-6), 133.8–128.7 (Ph), 101.7 (C-5), 90.4 (C-1′), 84.2 (C-4′), 70.1 (C-2′), 62.0 (C-5′), 35.4 (C-3′), 9.3 (CH_3); UV (EtOH 95) λ_{max} = 261 nm (ε = 10400), 228 nm (ε = 15500); IR υ = 2112 (s) cm^{-1} (N_3); MS: *m/z* (FAB > 0) 743 $(2M+H)^+$, 372 $(M+H)^+$, 260 $(S)^+$, 113 $(BH_2)^+$; *m/z* (FAB > 0) 370 $(M-H)^-$, 111 $(B)^-$.

1-(2-Azido-2,3-dideoxy-3-*C*-methyl-β-D-ribofuranosyl)uracil (12): The protected nucleoside **11** (604.8 mg, 1.63 mmol) was dissolved in methanolic ammonia (40.7 mL, saturated beforehand at −10 °C and stoppered tightly) and the resulting solution was stirred overnight at room temperature. The solution was evaporated under reduced pressure and the residue was subjected to silica gel column chromatography using as eluent a stepwise gradient of methanol (0–5 %) in dichloromethane to afford the title compound **12** (385 mg, 89 % yield) which was crystallised from EtOH 100. m.p. 167–168 °C; ^{1}H NMR (DMSO-*d6*) δ = 11.4 (bs, 1H, NH), 8.10 (d, *J* = 8.1, 1H, H-6), 5.73 (s, 1H, H-1′), 5.58 (dd, *J* = 8.1, *J* = 1.9, 1H, H-5), 5.23 (pt, 1H, OH-5′), 4.42 (d, *J* = 5.6, 1H, H-2′), 3.80 (ddd, *J* = 2.2, *J* = 5.5, *J* = 12.7, 1H, H-5′), 3.69 (m, 1H, H-4′), 3.55 (ddd, *J* = 2.4, *J* = 4.8, *J* = 12.7, 1H, H-5″), 2.30 (m, 1H, H-3′), 0.98 (d, *J* = 6.7, 3H, CH_3); ^{13}C NMR (DMSO-*d6*) δ = 163.3 (C-4), 150.3 (C-2), 139.6 (C-6), 100.6 (C-5), 88.7 (C-1′), 86.3 (C-4′), 69.8 (C-2′), 58.8 (C-5′), 33.7 (C-3′), 9.4 (CH_3); UV (EtOH 95) λ_{max} = 261 nm (ε = 10400); IR υ = 2110 (s) cm^{-1} (N_3); MS: *m/z* (FAB > 0) 268 $(M+H)^+$, 113 $(BH_2)^+$; HRMS (ESI^+): calcd. for $C_{10}H_{14}N_5O_4$ $(M+H)^+$: 268.1046; found: 268.1050.

1-(2-Azido-2,3-dideoxy-3-*C*-methyl-β-D-ribofuranosyl)cytosine (13): To a solution of the protected nucleoside **11** (49.3 mg, 0.13 mmol) in dry pyridine (820 μL) was added 1,2,4-triazole (120.6 mg, 1.75 mmol) followed by the dropwise addition at 0 °C of 4-chlorophenylphosphorodichloridate (95 μL, 0.59 mmol). The reaction mixture was stirred one day at room temperature, and the solvent was evaporated to dryness. The residue was dissolved in dichloromethane (8.7 mL) and washed with water. After decantation, the organic layer was dried (Na_2SO_4), concentrated to dryness and the residue was purified by silica gel column chromatography using as eluent a stepwise gradient of methanol (0–3 %) in dichloromethane to afford the 4-triazolyl intermediate. This last was then dissolved in NH_4OH (28 %)/dioxane (1.85 mL, 1/3, v/v) and the reaction mixture was stirred overnight at room temperature. After evaporation to dryness, the resulting residue was subjected to silica gel column chromatography using as eluent a stepwise gradient of methanol (0–10 %) in dichloromethane to afford the title compound **13** (23.7 mg, 67 % yield) as white solid. ^{1}H NMR (DMSO-*d6*) δ = 8.07 (d, *J* = 7.5, 1H, H-6), 7.15 (bs, 2H, NH_2) 5.71 (s, 1H, H-1′), 5.69 (d, *J* = 7.5, 1H, H-5), 5.19 (pt, 1H, OH-5′), 4.24 (d, *J* = 5.5, 1H, H-2′), 3.80 (m, 1H, H-5′), 3.69 (m, 1H, H-4′), 3.55 (m, 1H, H-5″), 2.20 (m, 1H, H-3′), 0.96 (d, *J* = 6.7, 3H, CH_3); ^{13}C NMR (DMSO-*d6*) δ = 166.9 (C-4), 155.0 (C-2), 140.2 (C-6), 93.0 (C-5), 89.2 (C-1′), 86.1 (C-4′), 70.1 (C-2′), 58.9 (C-5′), 33.4(C-3′), 9.3 (CH_3); UV (EtOH 95) λ_{max} = 272 nm (ε = 8900); IR υ = 2110 (s) cm^{-1} (N_3); MS: *m/z* (FAB > 0) 533 $(2M+H)^+$, 267 $(M+H)^+$, 112 $(BH_2)^+$; *m/z* (FAB < 0) 531 $(2M-H)^-$, 265 $(M-H)^-$; HRMS (ESI^+): calcd. for $C_{10}H_{15}N_6O_3$ $(M+H)^+$: 267.1206; found: 267.1206.

1-(2-Amino-2,3-dideoxy-3-*C*-methyl-β-D-ribofuranosyl)uracil (14): A solution of the nucleoside analog **12** (213.1 mg, 0.80 mmol) and triphenylphosphine (315.7 mg, 1.20 mmol) in anhydrous pyridine (10 mL) was stirred 1 h at room temperature, then NH_4OH (20 %, 6.2 mL) was added. After overnight stirring at room temperature, the volatiles were evaporated under reduced pressure and the residue was subjected to silica gel column chromatography using as eluent a stepwise gradient of methanol (0–15 %) in dichloromethane to afford the title compound **14** (142.0 mg, 74 % yield) which was lyophilised in water. ^{1}H NMR (DMSO-*d6*) δ = 11.3 (bs, 1H, NH), 8.06 (d, *J* = 8.1, 1H, H-6), 5.55 (d, *J* = 8.1, 1H, H-5), 5.50 (d, *J* = 2.5, 1H, H-1′), 5.07 (pt, 1H, OH-5′), 3.80 (m, 1H, H-4′), 3.73 (ddd, *J* = 2.0, *J* = 4.8, *J* = 12.3, 1H, H-5′), 3.52 (ddd, *J* = 3.6, *J* = 7.4, *J* = 12.3, 1H, H-5″), 3.30 (dd, *J* = 2.5, *J* = 6.4, 1H, H-2′), 2.10 (m, 1H, H-3′), 0.98 (d, *J* = 6.7, 3H, CH_3); ^{13}C NMR (DMSO-*d6*) δ = 163.3 (C-4), 150.6 (C-2), 140.5 (C-6), 100.5 (C-5), 91.3 (C-1′), 85.7 (C-4′), 60.4 (C-5′), 59.8 (C-2′), 34.7 (C-3′), 10.1 (CH_3); UV (EtOH 95) λ_{max} = 262 nm (ε = 10200); MS: *m/z* (FAB > 0) 242 $(M+H)^+$, 130 $(S)^+$, 113 $(BH_2)^+$; *m/z* (FAB < 0) 240 $(M-H)^-$, 111 $(B)^-$; HRMS (ESI^+): calcd. for $C_{10}H_{16}N_3O_4$ $(M+H)^+$: 242.1141; found: 242.1141.

1-(5-*O*-Benzoyl-3-deoxy-3-*C*-methyl-β-D-ribofuranosyl)-3-(*p*-methoxybenzyl)uracil (15): To a suspension of nucleoside analog **1** (780 mg, 2.25 mmol) in anhydrous acetonitrile (22.5 mL) were added *p*-methoxybenzyl chloride (550 μL, 4.05 mmol) and DBU (673 μL, 4.05 mmol). The reaction mixture was refluxed for 2 h then, cooled to 0 °C, hydrolyzed with a $KHSO_4$ (5 %) solution and diluted with dichloromethane. After decantation, the organic layer was dried (Na_2SO_4), concentrated to dryness and the residue was purified by silica gel column chromatography using as eluent a stepwise gradient of methanol (0–2 %) in dichloromethane to afford the title compound **15** (819 mg, 78 % yield) as white foam.^{1}H NMR ($CDCl_3$) δ = 7.94 (m, 2H, Ph), 7.66 (d, *J* = 8.1, 1H, H-6), 7.57–7.33 (m, 5H, Ph), 6.75 (m, 2H, Ph), 5.58 (s, 1H, H-1′), 5.47 (d, *J* = 8.1, 1H, H-5), 4.95 (d, *J* = 13.6, 2H, CH_2Ph), 4.63 (dd, *J* = 2.4, *J* = 12.9, 1H, H-5′), 4.55 (dd, *J* = 3.3, *J* = 12.9, 1H, H-5″), 4.25 (m, 1H, H-4′), 4.15 (d, *J* = 5.1, 1H, H-2′), 3.70 (s, 3H, CH_3O), 2,85 (bs, 1H, OH-2′), 2.04 (m, 1H, H-3′), 1.07 (d, *J* = 6.8, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ = 167.6 (CO), 164.0 (C-4), 160.6 (CH_2Ph), 152.6 (C-2), 138.2 (C-6), 135.1-130.1 (Ph), 115.2 (CH_2Ph), 102.7 (C-5), 95.1 (C-1′), 85.5 (C-4′), 80.2 (C-2′), 64.2 (C-5′), 56.7 (OCH_3), 44.9 (CH_2Ph), 37.7 (C-3′), 10.0 (CH_3); UV (EtOH 95) λ_{max} = 264 nm (ε = 11500), 225 nm (ε = 27900); MS: *m/z* (FAB > 0) 933 $(2M+H)^+$, 467 $(M+H)^+$; *m/z* (FAB < 0) 465 $(M-H)^-$, 231 $(B)^-$.

1-(2-Azido-2,3-dideoxy-3-*C*-methyl-β-D-arabinofuranosyl)-3-(*p*-methoxybenzyl)-uracil (16): To a solution of the protected nucleoside **15** (0.64 g, 1.37 mmol) in dry THF (13.5 mL) was added triphenylphosphine (1.07 g, 4.10 mmol). The reaction mixture was cooled at −10 °C and a solution of diethyl azodicarboxylate (DEAD, 0.64 mL, 4.105 mmol) and diphenylphosphorylazide (DPPA, 0.89 mL, 4.10 mmol) in dry THF was added dropwise. After stirring 10 min at −10 °C then 2 h at room temperature, the solvent was evaporated under reduced pressure and the residue was subjected to silica gel column chromatography using as eluent a stepwise gradient of methanol (0–1 %) in dichloromethane. The collected fractions containing products with R_f = 0.3 (diethyl ether/petroleum ether, 8/2 : v/v) were evaporated and the residue was subjected to silica gel column chromatography using an isocratic elution of diethyl ether/petroleum ether (1/1 : v/v). The appropriate fractions

were evaporated under reduced pressure and the residue (0.42 g, 0.90 mmol) was dissolved in methanolic ammonia (30 mL, saturated beforehand at −10 °C and stoppered tightly). The resulting solution was stirred overnight at room temperature then, evaporated under reduced pressure. The residue was subjected to reverse phase column chromatography using as eluent a stepwise gradient of acetonitrile (15–20 %) in water to afford the title compound **16** (121.8 mg, 23 % yield) which was crystallised from EtOH 100. m.p. 156–157 °C; ^{1}H NMR ($CDCl_3$) δ = 7.73 (d, *J* = 8.1 Hz, 1H, H-6), 7.40-7.33 (m, 2H, Ph), 6.74 (m, 2H, Ph), 6.16 (d, *J* = 6.1, 1H, H-1′), 5.68 (d, *J* = 8.1, 1H, H-5), 4.99 (m, 2H, CH_2Ph), 3.91 (m, 2H, H-5′, H-2′), 3.70 (s, 3H, CH_3O), 3,67 (s, 1H, H-5″), 3.60 (m, 1H, H-4′), 2.13 (m, 1H, H-3′), 1.10 (d, *J* = 6.7, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ = 162.8 (CO), 159.0 (C-4), 151.1 (C-2), 138.3 (C-6), 133.2-130.3 (Ph), 113.7 (Ph), 101.5 (C-5), 84.6 (C-4′), 84.5 (C-1′), 69.5 (C-2′), 60.8 (C-5′), 55.2 (CH_3O), 43.6 (CH_2Ph), 37.5 (C-3′), 14.6 (CH_3); UV (EtOH 95) λ_{max} = 263 nm (ε = 10 500), 221 nm (ε = 15 200); IR υ = 2112 (s) cm^{-1} (N_3); MS: *m/z* (FAB > 0) 388 $(M+H)^+$; *m/z* (FAB < 0) 231 $(B)^-$.

1-(2-Azido-2,3-dideoxy-3-*C*-methyl-1-β-D-arabinofuranosyl)uracil (17): To a solution of the nucleoside **16** (134.6 mg, 0.35 mmol) in a mixture of acetonitrile and water (11 mL, 8/2:v/v) was added ceric ammonium nitrate (CAN, 1.14 g, 2.09 mmol). The reaction mixture was stirred 2 h at room temperature, then evaporated under reduced pressure. The crude residue was dissolved in dichloromethane (10 mL) and washed with water. After decantation, the organic layer was dried (Na_2SO_4), concentrated to dryness and the residue was purified by silica gel column chromatography using as eluent a stepwise gradient of methanol (0–5 %) in dichloromethane to afford the title compound **17** (62.3 mg, 67 % yield) which was lyophilised in water. ^{1}H NMR (DMSO-*d6*) δ = 11.40 (bs, 1H, NH), 8.04 (d, *J* = 8.1, 1H, H-6), 6.13 (d, *J* = 6.5, 1H, H-1′), 5.61 (d, *J* = 8.1, 1H, H-5), 5.24 (pt, 1H, OH-5′), 4.33 (dd, *J* = 6.5, *J* = 10.2, 1H, H-2′), 3.75 (m, 1H, H-5′), 3.61 (m, 2H, H-4′, H-5″), 2.03 (m, 1H, H-3′), 1.07 (d, *J* = 6.4, 3H, CH_3); ^{13}C NMR (DMSO-*d6*) δ = 163.0 (C-4), 150.5 (C-2), 140.5 (C-6), 100.9 (C-5), 84.4 (C-4′), 82.8 (C-1′), 68.4 (C-2′), 58.9 (C-5′), 36.0 (C-3′), 13.2 (CH_3); IR υ = 2112 (s) cm^{-1} (N_3); UV (EtOH 95) λ_{max} = 261 nm (E = 10 000); MS: *m/z* (FAB > 0) 535 $(2M+H)^+$, 268 $(M+H)^+$, 113 $(BH_2)^+$; *m/z* (FAB < 0) 533 $(2M-H)^-$, 266 $(M-H)^-$, 111 $(B)^-$; HRMS (ESI$^+$): calcd. for $C_{10}H_{14}N_5O_4$ $(M+H)^+$: 268.1046; found: 268.1046.

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Conflict of Interest

The authors declare no conflict of interest.

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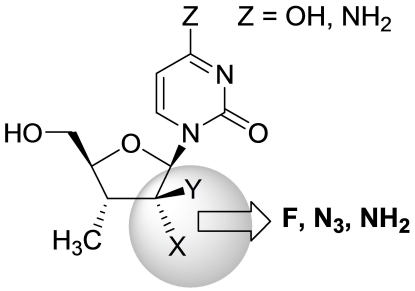
FULL PAPERS

The study of nucleoside and nucleotide analogs has been the subject of extensive development during these last decades. In this field, additions or substitutions on the sugar residue had led to potent drugs in antiviral and anticancer chemotherapies. Herein, we reported the stereoselective synthesis of a new series of pyrimidine nucleosides bearing a double modification in the 2′ and 3′ positions of the sugar scaffold.



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