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Two-step conversion of uridine and cytidine to variously C5-C functionalized analogs†

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C5-substituted pyrimidine nucleosides are an important class of molecules that have practical use as biological probes and pharmaceuticals. Herein we report an operationally simple protocol for C5-functionalization of uridine and cytidine via transformation of underexploited 5-trifluoromethyluridine or 5-trifluoromethylcytidine, respectively. The unique reactivity of the CF₃ group in the aromatic ring allowed the direct incorporation of several distinct C5-C “carbon substituents”: carboxyl, nitrile, ester, amide, and amidine.

Introduction

Modified ribonucleosides constitute an exceptionally important class of biomolecules that are present in all three domains of life.¹ To date, more than 150 modifications have been identified in RNA. About 100 of them have been identified in tRNAs, including C5-substituted cytidines and uridines. Most of them are located at the 34 position of the tRNA anticodon (known as the wobble position) and play a crucial role in the accuracy and efficiency of protein biosynthesis. Wobble nucleosides facilitate the third codon letter recognition, particularly in non-cognate codons by stabilizing the codon–anticodon interactions and modelling the ribosome-acceptable anticodon loop architecture.^{2–4} Recently, the regulatory and signalling role of RNA ribonucleosides has been discovered,^{4,5} e.g. two epigenetic modifications, 5-methylcytidine (m⁵C) and 5-hydroxymethylcytidine (hm⁵C), and products of their oxidation, 5-formylcytidine (f⁵C) and 5-carboxycytidine (ca⁵C), were supposed to regulate the translation process.^{5–8} Notably, their shortage was associated with intellectual disabilities and cancer.^{6,9,10}

Due to their ubiquity, naturally modified nucleosides have found widespread applications as biological probes in biochemistry and medicine. For instance, fluorescently labelled m⁵C-oxidized products were investigated to find a more sensitive method for their identification.⁷ In addition, 5-substituted uridines were used as models to assess the structure–function relationships.¹¹

Unnatural modified nucleosides are frequently evaluated as potential drugs in the treatment of existing and emerging viral and bacterial diseases and cancer.^{12–14} Incorporation of the C5-substituent in pyrimidine bases was found to increase drug bioavailability, biological activity, and/or stability under cellular conditions. Among others, 5-fluorine- and 5-CF₃-substituted pyrimidine nucleosides are widely used drugs with anti-tumor and antiviral activities.¹⁴ Notably, the 5-CF₃-dU nucleoside is an antiviral drug commercially known as trifluridine, approved by the Food and Drug Administration (FDA) for the treatment of epithelial keratitis caused by Herpes Simplex virus 1 and 2 (HSV-1 and HCV-2).

In the light of the foregoing discussion, it is important to develop straightforward and effective methods for the synthesis of natural and artificial modified nucleosides.

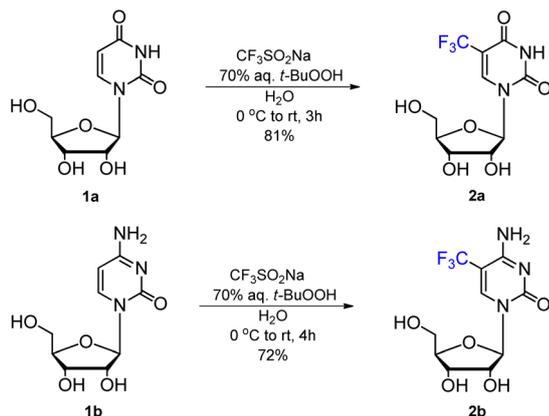
According to literature data, the incorporation of a “carbon substituent” at the C5 position of uridine or cytidine involved hydroxymethylation,¹⁵ aminomethylation by the Mannich reaction,¹⁶ Pd-catalyzed reaction of 5-iodo-nucleosides to introduce alkyl, alkenyl, alkynyl or aryl groups,^{13,17} reaction of a C5-lithiated nucleoside with an appropriate electrophile,^{18,19} substitution of 5-halo derivatives with the generated carboanion^{19,20} and radical malonylation induced by Mn(III) or Ce(IV) compounds.²¹ All these strategies require, however, the additional ribose protection and final deprotection steps, which are costly and time-consuming.

Herein, we present a facile, two-step approach to introduce several chemically diverse “carbon substituents” (carboxyl, nitrile, ester, amide, or amidine) at the C5 position of uridine (**1a**, Scheme 1) and cytidine (**1b**) nucleosides using underexploited 5-trifluoromethyluridine (5-CF₃U, **2a**) or 5-trifluoromethylcytidine (5-CF₃C, **2b**) precursors. The employed strategy does not require any protecting groups for regioselective C5-trifluoromethylation of pyrimidine ribonucleosides and the subsequent 5-CF₃ conversion.

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Scheme 1 Synthesis of 5-trifluoromethyluridine (5- CF_3U , **2a**) and 5-trifluoromethylcytidine (5- CF_3C , **2b**).

Results and discussion

Synthesis of 5-trifluoromethyluridine (5- CF_3U) and 5-trifluoromethylcytidine (5- CF_3C)

Radical trifluoromethylation is a valuable method for the preparation of CF_3 -tagged aromatic heterocycles, in which the hydrogen atom bonded to the aromatic system is replaced with a trifluoromethyl group without prior substrate prefunctionalization. Pivotal progress in radical trifluoromethylation was made by Baran *et al.*, who developed a transition metal-free approach based on two easy-to-handle, commercially available reactants: sodium trifluoromethanesulfonate ($\text{CF}_3\text{SO}_2\text{Na}$, known as the Langlois reagent) in combination with *tert*-butylhydroperoxide ($t\text{-BuOOH}$) as the radical source.²² This method was successfully applied to monotrifluoromethylation of 2'-deoxyuridine (dU) providing 5- CF_3dU as the only regiomer in 57% yield.²²

Using the $\text{CF}_3\text{SO}_2\text{Na}$ and $t\text{-BuOOH}$ reagent system, several other CF_3 -containing nucleosides (mostly 2'-deoxy derivatives) have been successfully synthesized and evaluated as potential antiviral or antitumor drugs,²³ biochemical probes for ^{19}F NMR studies²⁴ or substrates in the preparation of CF_3 -functionalized oligonucleotides.²⁵ In case of the 5- CF_3U and 5- CF_3C ribonucleosides, two general methods based on the peroxide-generated CF_3 radicals have been reported in the literature: trifluoromethylation of U/C with gaseous CF_3I in the presence of the Fenton oxidation reagent ($\text{Fe}^{2+}/\text{H}_2\text{O}_2/\text{H}_2\text{SO}_4$; $Y = 11\text{--}53\%$)²⁶ or photoinduced reaction with *e.g.* trifluoromethyl sulfones or the Langlois reagent as the source of CF_3 radicals ($Y = 38\text{--}42\%$).²⁷

In our studies, 5- CF_3 -uridine (**2a**, Scheme 1) and 5- CF_3 -cytidine (**2b**) were synthesized under metal-free conditions, using an excess of $\text{CF}_3\text{SO}_2\text{Na}$ (3 equiv.) and 70% aqueous (aq.) solution of $t\text{-BuOOH}$ (5 equiv.). The reaction mixture was left for 3–4 h at room temperature. Products **2a** and **2b** were isolated by column chromatography with 81% and 72% yields, respectively, and characterized by NMR and MS spectroscopy (ESI, Fig. S1–S6†). Since the starting material remained unreacted, the reaction conditions were optimized by extending the reaction time and/or increasing the excess of the reagents;

however, no improvement in the substrate consumption was observed. Notably, when the trifluoromethylation of U/C was carried out using a 4-fold higher amount of the starting material (2 vs. 0.5 g) using the same reactant ratios and conditions as for the small-scale reaction, an *ca.* 10% decrease in the yields of CF_3 -nucleosides was observed, with similar recovery yields of the starting nucleosides.

Reactivity of 5- CF_3U and 5- CF_3C under alkaline conditions

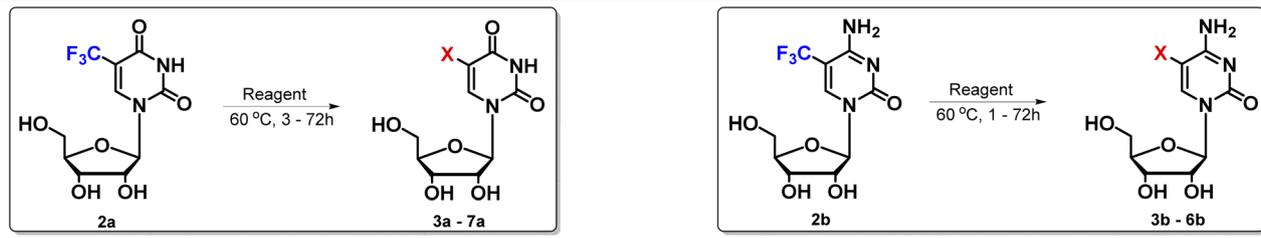
In general, the CF_3 group attached to the aromatic ring is stable and chemically inert under neutral conditions. The reactivity of the aromatic CF_3 group rises rapidly in alkaline solutions at elevated temperature. The first conversions of the 5- CF_3 -2'-deoxyuridine nucleoside into 5-carboxy- or 5-cyano derivatives in heated aqueous sodium hydroxide or ammonia, respectively, were presented three decades ago.²⁸

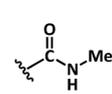
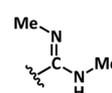
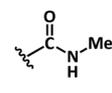
Very recently, Ito *et al.* demonstrated the synthetic scope of 5- CF_3dU and 5- CF_3dC conversions at the oligonucleotide level.²⁹ Since CF_3 -modified nucleosides in the *ribo* series have never been considered as precursors to introduce various C5-functional groups, we evaluated the reactivity of 5- CF_3U (**2a**) and 5- CF_3C (**2b**) with several nucleophiles, involving hydroxide and alkoxide ions, ammonia, and methylamine (Table 1). To select the most suitable conversion conditions, small-scale preliminary experiments were performed with CF_3 -uridine (**2a**) (*ca.* 150 μmol of the starting material). The developed reaction conditions were then used for 5- CF_3C (**2b**) and optimized. To assess whether the method is reproducible and scalable, some of the 5- $\text{CF}_3\text{U/C}$ conversions were performed on a larger scale (1 g of **2a/2b** vs. 0.15 g).

First, we investigated the alkaline hydrolysis of 5- CF_3U (**2a**) into 5-carboxyuridine (ca^5U , **3a**, Table 1, entry 1). NaOH aq. solution at 60 °C was used since hydroxide anions were reported as the most effective reactants to convert CF_3 -containing aromatic heterocycles to COOH -functionalized derivatives.^{28–30} To optimize the reaction conditions, we tested the reactivity of 5- CF_3U at various NaOH concentrations. When 20 mM aq. NaOH was used, we observed incomplete 5- CF_3U conversion even after 20 h. A 5-fold increase in NaOH concentration improved the reaction yield (65% after 12 h), although an unconsumed substrate was still observed. With further increase in aq. NaOH concentration to 0.5 M, 90% yield was achieved and the reaction time was reduced to 4 h (Table 1, entry 1). We found these conditions of **2a** hydrolysis to be optimal and used them for 5- CF_3 -cytidine (**2b**) conversion (Table 1, entry 2). In this case, the reaction took only 1 h, affording 5-carboxycytidine (**3b**) in 85% yield. It is worth noting that 5-carboxycytidine (ca^5C) is a naturally existing modification identified in mRNA sequences as a product of m^5C or hm^5C epigenetic nucleoside oxidation.¹⁰ The biological function of ca^5C is still elusive, although its regulatory role in the translation process has been speculated.^{7,10}

5- CF_3U was then exploited for conversion of the CF_3 group to the methyl ester residue ($-\text{COOMe}$). Analysis of the literature revealed that sodium methoxide in methanol is a commonly used reagent for CF_3 -compound methanolysis.^{31,32} More recently,



Table 1 The conditions for the conversion of 5-CF₃U and 5-CF₃C to C5-substituted derivatives


| Entry | Substrate | -X | Reagent | Time (h) | Product | Yield (%) |
|-------|-----------|---|---|----------|---------|-----------------|
| 1 | 2a | | 0.5 M aq. NaOH | 4 | 3a | 90 |
| 2 | 2b |  | 0.5 M aq. NaOH | 1 | 3b | 85 |
| 3 | 2a |  | (1) 50 mM K ₂ CO ₃ /MeOH (2) Dowex H | 72 | 4a | 90 |
| 4 | 2b |  | (1) 50 mM K ₂ CO ₃ /MeOH (2) 0.5 M aq. HCl | 72 | 4b | 72 |
| 5 | 2a | | 30% aq. NH ₃ /EtOH (3/1 v/v) | 4 | 5a | 70 |
| 6 | 2b |  | 30% aq. NH ₃ /EtOH (3/1 v/v) | 3 | 5b | 70 |
| 7 | 2a |  | 4% aq. MeNH ₂ | 20 | 6a | 65 |
| 8 | 2a |  | 40% aq. MeNH ₂ | 3 | 7a | 82 ^a |
| 9 | 2b | | 4% aq. MeNH ₂ | 4 | 6b | 60 |
| 10 | 2b |  | 40% aq. MeNH ₂ | 2 | 6b | 65 ^a |

^a Small-scale reaction yield (ca. 150 μmol of 2a/2b).

Markley *et al.* found that prolonged incubation of CF₃dU (2 weeks, 37 °C) in a methanolic solution of K₂CO₃ leads to the 5-methoxycarbonyl derivative in a quantitative yield.³³ We tested both conditions reported in the literature. The reaction of 2a with 50 mM solution of K₂CO₃ in dry MeOH heated to 60 °C afforded the *ortho* ester intermediate 5-(trimethoxymethyl)uridine (5-(MeO)₃C-uridine) after 72 h (ESI, Fig. S10[†]). The reaction mixture was diluted with methanol and acidified with Dowex-H⁺, furnishing 5-methoxycarbonyluridine (4a) in 90% yield (Table 1, entry 3). In turn, the use of 75% MeONa methanolic solution at 60 °C turned out to be less effective, affording 5-methoxycarbonyluridine (4a) in 70% yield after 24 h.

The former conditions of methanolysis were established as optimal and used for 5-CF₃-cytidine (2b) conversion. In this case, the reaction of 2b with 50 mM K₂CO₃ methanolic solution (72 h, 60 °C) resulted in the formation of the 5-(MeO)₃C-cytidine orthoester derivative, stable under Dowex-

H⁺ acidification conditions (ESI, Fig. S26[†]). Thus, in the next experiment, the *ortho* ester was *in situ* readily hydrolyzed with mild aq. acid (0.5 M aq. HCl, 12 h, rt), affording 5-methoxycarbonylcytidine (4b) in 72% yield (Table 1, entry 4). Notably, 5-methoxycarbonylcytidine (4b) can be considered as a COOH-protected synthon for the preparation of ca⁵C-phosphoramidite and then the ca⁵C-modified RNA fragment.¹⁹

Next, we tested the reactivity of 5-CF₃U (2a) and 5-CF₃C (2b) with ammonia. According to the literature, the ammonolysis of CF₃-aromatic systems provided exclusively cyano derivatives; none of the primary amides was formed.^{28c,29,33} For instance, the incubation of 5-CF₃dU with conc. NH₃(aq.) for 16 h at 55 °C furnished 5-cyano-dU in 95% yield.³³ The high conversion efficiency encouraged us to apply these conditions to 5-CF₃U ammonolysis. The reaction was conducted in a sealed vial tube for 5 h at 60 °C, affording 5-cyanouridine (5a) in 70% yield. In the next experiment, the mixture of 30% aq. NH₃ and



EtOH (3 : 1, v/v) was used at 60 °C (Table 1, entry 5). Full conversion of **2a** was observed after 4 h (TLC analysis), affording product **5a** in 70% yield. Since the latter conditions are commonly used during alkaline deprotection of RNA oligomers, this variant of ammonolysis was employed for the synthesis of 5-CN-cytidine (**5b**). The reaction of 5-CF₃-cytidine **2b** with 30% aq. NH₃-EtOH (3 : 1, v/v) proceeded for 3 h at 60 °C to afford **5b** in 70% yield (Table 1, entry 6).

Finally, we performed several trials of 5-CF₃U aminolysis with methylamine as a nucleophilic reagent. Ito *et al.* previously reported the reactivity of 5-CF₃dU- and 5-CF₃dC-DNA with 2–4% aq. methylamine or butylamine, demonstrating the formation of primary amides or symmetrical amidine compounds.²⁹ It is beneficial that the applied methodology of amide bond formation does not require any coupling reagent. In our studies, 4% and 40% aq. MeNH₂ solutions were tested. Incubation of **2a** with 4% aq. MeNH₂ at 60 °C for 20 h (Table 1, entry 7) resulted in the full conversion of the CF₃ group to *N*-methylcarbamoyl C(O)NHMe (product **6a**) in 65% yield. The use of a 10-fold higher MeNH₂ concentration for 3 h led to the formation of **7a** containing the *N,N*-dimethylamidinyl group in 82% yield (Table 1, entry 8). The TLC-controlled analysis of the reaction clearly showed that the amidinyl residue is formed *via* the amide intermediate. Interestingly, the reaction of 5-CF₃-cytidine (**2b**) with 4% aq. MeNH₂ (4 h, 60 °C) or 40% aq. MeNH₂ (2 h, 60 °C) afforded exclusively the amide-type compound **6b** in ca. 60% yields (Table 1, entries 9 and 10). The extension of the reaction time to 20 h did not change the chemical status of the amide product. In the past, Ito *et al.* reported a similar divergence between the reactivity of 5-CF₃dU and 5-CF₃dC with MeNH₂.²⁹ Based on the DFT calculations, the authors showed that the imine carbon in the amidinyl residue of cytosine is more sensitive to water attack leading to amide group formation than in the case of amidinyl-uridine.

Conclusions

Compared to the multi-step and complex procedures reported in the literature for C5-C functionalization of uridine and cytidine,^{15–21} the approach described herein involves only two reactions and stable, easy to handle commercially available reactants. The regioselectively obtained 5-trifluoromethylated uridines and cytidines (5-CF₃U, **2a** and 5-CF₃C, **2b**) proved to be useful substrates for a rapid, effective (*Y* = 60–90%) and scalable process of the C5-functionality with the carboxyl, nitrile, ester, amide, or amidine group.

Experimental section

General methods

All solid compounds were dried under a high vacuum prior to use. The reaction progress was monitored by analytical thin layer chromatography (TLC) on silica gel-coated plates (60F254). Column chromatography was performed on silica gel

(230–400 mesh, Fluka). NMR spectra were recorded on a Bruker Avance II Plus 700 spectrometer at 700 (for ¹H) and 176 (for ¹³C) MHz or on a JEOL 400 spectrometer at 376 (for ¹⁹F) MHz. Chemical shifts (δ) are reported in ppm relative to TMS (internal standard) for ¹H and ¹³C. Multiplicities are described as s (singlet), d (doublet), dd (doublet of doublets), dt (doublet of triplets), q (quartet) and m (multiplet). Coupling constants (*J*) are reported in hertz. IR spectra were recorded using an FTIR ALPHA instrument (Bruker) equipped with a Platinum ATR QuickSnap module in the range of 4000–400 cm⁻¹. High-resolution mass spectrometry (HRMS) measurements were performed using a Synapt G2Si mass spectrometer (Waters) equipped with an ESI source and a quadrupole-time-of-flight mass analyzer. The measurement was performed in the negative ion mode. The results of the measurements were processed using the MassLynx 4.1 software (Waters).

Synthesis of 5-trifluoromethyluridine (**2a**)

Uridine **1a** (0.50 g, 2.05 mmol) was dissolved in H₂O (6.2 mL), and CF₃SO₂Na (0.96 g, 6.15 mmol) was added. After cooling the reaction mixture to 0 °C, *t*-BuOOH (70% in H₂O, 1.34 mL, 10.25 mmol) was added dropwise; then the reaction mixture was warmed to rt and stirred for 3 h. The reaction mixture was concentrated under reduced pressure, and co-evaporated with anhydrous toluene (2 × 5 mL). The crude product was purified by column chromatography (silica gel, 0–12% MeOH in CHCl₃) to give 5-trifluoromethyluridine **2a** (0.52 g, 81%). TLC: *R*_f = 0.57 (CHCl₃/MeOH, 8 : 2, v/v); ¹H NMR (700 MHz, D₂O) δ (ppm): 3.89 (dd, 1H, *J* = 2.7 Hz, *J* = 13.0 Hz, H5''), 4.05 (dd, 1H, *J* = 2.5 Hz, *J* = 13.0 Hz, H5'), 4.18–4.22 (m, 1H, H4'), 4.29 (dd, 1H, *J* = 5.0 Hz, *J* = 7.2 Hz, H3'), 4.38 (dd, 1H, *J* = 2.5 Hz, *J* = 5.0 Hz, H2'), 5.93 (d, 1H, *J* = 2.5 Hz, H1'), 8.77 (s, 1H, H6); ¹³C NMR (176 MHz, D₂O) δ (ppm): 59.35 (C5'), 68.26 (C3'), 74.34 (C2'), 83.76 (C4'), 90.39 (C1'), 104.36 (q, *J* = 33.26 Hz, C5), 122.15 (q, *J* = 268.75 Hz, CF₃), 142.90 (q, *J* = 5.28 Hz, C6), 150.68 (C2), 161.54 (C4); ¹⁹F NMR (376 MHz, D₂O) δ : -63.26; HRMS (ESI) calcd for C₁₀H₁₀N₂O₆F₃ [M - H]⁻ 311.0490, found 311.0496 (ESI, Fig. S1–S3†).

Synthesis of 5-trifluoromethylcytidine (**2b**)

Cytidine **1b** (1 g, 4.11 mmol) was dissolved in H₂O (12 mL); then CF₃SO₂Na (1.86 g, 12.33 mmol) was added and the solution was stirred at 0 °C. After 10 min, *t*-BuOOH (70% in H₂O, 2.82 mL, 20.55 mmol) was added dropwise, and the reaction mixture was warmed to rt and stirred for 4 h. Then the reaction mixture was evaporated under reduced pressure and co-evaporated with anhydrous toluene (3 × 10 mL). The crude product was purified by column chromatography (silica gel, 0–18% MeOH in CHCl₃) to give compound **2b** as a yellow solid (0.92 g, 72%). TLC: *R*_f = 0.34 (CHCl₃/MeOH, 8 : 2, v/v); ¹H NMR (700 MHz, D₂O) δ (ppm): 3.89 (dd, 1H, *J* = 2.8 Hz, *J* = 13.3 Hz, H5''), 4.07 (dd, 1H, *J* = 2.8 Hz, *J* = 13.3 Hz, H5'), 4.19 (dt, 1H, *J* = 2.8 Hz, *J* = 8.0 Hz, H4'), 4.25 (dd, 1H, *J* = 4.9 Hz, *J* = 8.0 Hz, H3'), 4.32 (dd, 1H, *J* = 2.1 Hz, *J* = 4.9 Hz, H2'), 5.90 (d, 1H, *J* = 2.1 Hz, H1'), 8.76 (s, 1H, H6); ¹³C NMR (176 MHz, D₂O) δ (ppm): 59.18 (C5'), 67.90 (C3'), 74.54 (C2'), 83.27 (C4'), 91.08 (C1'), 97.69 (q, *J* = 35.02 Hz, C5), 122.84 (q, *J* = 269.45 Hz, CF₃),



143.43 (q, $J = 6.16$ Hz, C6), 156.27 (C2), 161.30 (C4); ^{19}F NMR (376 MHz, D_2O) δ : -62.70 ; HRMS (ESI) calcd for $\text{C}_{10}\text{H}_{11}\text{N}_3\text{O}_5\text{F}_3$ $[\text{M} - \text{H}]^-$ 310.0651, found 310.0660 (ESI, Fig. S4–S6 †).

Synthesis of 5-carboxyuridine (3a) and 5-carboxycytidine (3b)

Nucleoside **2a/2b** (1 g, 3.2 mmol) was treated with 0.5 M aq. NaOH solution (100 mL) and incubated at 60 °C for 4 h (compound **2a**) or 1 h (compound **2b**). The reaction mixture was diluted with water and passed through cation exchange resin (Dowex 50WX2-100, H^+ form). The fraction containing the nucleoside was concentrated under reduced pressure and lyophilized to give compound **3a** in 90% yield. TLC: $R_f = 0.16$ (BuOH/ H_2O , 85 : 15, v/v); ^1H NMR (700 MHz, D_2O) δ (ppm): 3.89 (dd, 1H, $J = 3.5$ Hz, $J = 12.6$ Hz, $\text{H}5''$), 4.05 (dd, 1H, $J = 2.8$ Hz, $J = 13.3$ Hz, $\text{H}5'$), 4.21–4.23 (m, 1H, $\text{H}4'$), 4.29 (dd, 1H, $J = 5.1$ Hz, $J = 7.0$ Hz, $\text{H}3'$), 4.41 (dd, 1H, $J = 2.8$ Hz, $J = 5.1$ Hz, $\text{H}2'$), 5.96 (d, 1H, $J = 2.8$ Hz, $\text{H}1'$), 9.08 (s, 1H, $\text{H}6$); ^{13}C NMR (176 MHz, D_2O) δ (ppm): 59.66 ($\text{C}5'$), 68.47 ($\text{C}3'$), 74.36 ($\text{C}2'$), 83.95 ($\text{C}4'$), 90.65 ($\text{C}1'$), 103.22 (C5), 149.20 (C6), 150.43 (C2), 164.33 (C4), 166.08 (COOH); HRMS (ESI) calcd for $\text{C}_{10}\text{H}_{11}\text{N}_2\text{O}_8$ $[\text{M} - \text{H}]^-$ 287.0515, found 287.0522; IR (ATR) cm^{-1} : 3354 (O–H), 1712 (C=O) (ESI, Fig. S7–S9 †).

Compound **3b** was obtained in 85% yield. TLC: $R_f = 0.38$ (1) $\text{CHCl}_3/\text{MeOH}$, 9 : 1, v/v, (2) $\text{EtOAc}/\text{Me}_2\text{CO}/\text{AcOH}/\text{H}_2\text{O}$, 5 : 3 : 1 : 1, v/v/v/v; ^1H NMR (700 MHz, D_2O) δ (ppm): 3.89 (dd, 1H, $J = 3.5$ Hz, $J = 13.3$ Hz, $\text{H}5''$), 4.07 (dd, 1H, $J = 2.8$ Hz, $J = 13.3$ Hz, $\text{H}5'$), 4.22–4.24 (m, 1H, $\text{H}4'$), 4.28 (dd, 1H, $J = 4.9$ Hz, $J = 7.7$ Hz, $\text{H}3'$), 4.40 (dd, 1H, $J = 2.8$ Hz, $J = 4.9$ Hz, $\text{H}2'$), 5.95 (d, 1H, $J = 2.8$ Hz, $\text{H}1'$), 9.24 (s, 1H, $\text{H}6$); ^{13}C NMR (176 MHz, D_2O) δ (ppm): 62.33 ($\text{C}5'$), 71.11 ($\text{C}3'$), 76.92 ($\text{C}2'$), 86.63 ($\text{C}4'$), 93.44 ($\text{C}1'$), 102.72 (C5), 151.45 (C6), 151.65 (C2), 161.65 (C4), 169.82 (COOH); HRMS (ESI) calcd for $\text{C}_{10}\text{H}_{12}\text{N}_3\text{O}_7$ $[\text{M} - \text{H}]^-$ 286.0675, found 286.0684; IR (ATR) cm^{-1} : 3226 (O–H), 1726 (C=O) (ESI, Fig. S23–S25 †).

Synthesis of 5-methoxycarbonyluridine (4a)

Nucleoside **2a** (1 g, 3.2 mmol) was treated with 50 mM $\text{K}_2\text{CO}_3/\text{MeOH}$ (140 mL) and the mixture was stirred for 72 h at 60 °C. The amount sufficient for spectral analysis was evaporated under reduced pressure and purified by column chromatography (silica gel, 0–15% MeOH in CHCl_3) affording 5-(trimethoxymethyl)uridine. TLC: $R_f = 0.33$ (BuOH/ $\text{AcOH}/\text{H}_2\text{O}$, 5 : 1 : 4, v/v/v); ^1H NMR (700 MHz, D_2O) δ (ppm): 3.21 (s, 9H, 3 \times O– CH_3), 3.86 (m, 1H, $J = 3.5$ Hz, $J = 12.6$ Hz, $\text{H}5''$), 4.01 (dd, 1H, $J = 2.8$ Hz, $J = 12.6$ Hz, $\text{H}5'$), 4.16–4.19 (m, 1H, $\text{H}4'$), 4.26 (dd, 1H, $J = 4.9$ Hz, $J = 7.0$ Hz, $\text{H}3'$), 4.37 (dd, 1H, $J = 2.8$ Hz, $J = 4.9$ Hz, $\text{H}2'$), 5.94 (d, 1H, $J = 2.8$ Hz, $\text{H}1'$), 8.50 (s, 1H, $\text{H}6$) (ESI, Fig. S10 †). The residual part of the reaction was diluted with anhydrous methanol and acidified with cation exchange resin (Dowex 50WX2-100, H^+ form). After resin filtration, the mixture was evaporated under reduced pressure and purified by column chromatography (silica gel, 0–15% MeOH in CHCl_3) affording compound **4a** in 90% yield. TLC: $R_f = 0.41$ ($\text{CHCl}_3/\text{MeOH}$, 8 : 2, v/v), $R_f = 0.2$ (BuOH/ $\text{AcOH}/\text{H}_2\text{O}$, 5 : 1 : 4, v/v/v); ^1H NMR (700 MHz, D_2O) δ (ppm): 3.88–3.91 (m, 4H, O– CH_3 , $\text{H}5''$), 4.07 (dd, 1H, $J = 2.8$ Hz, $J = 13.0$ Hz, $\text{H}5'$), 4.20–4.22 (m, 1H,

$\text{H}4'$), 4.29–4.31 (m, 1H, $\text{H}3'$), 4.39–4.40 (m, 1H, $\text{H}2'$), 5.96 (d, 1H, $J = 2.5$ Hz, $\text{H}1'$), 9.10 (s, 1H, $\text{H}6$); ^{13}C NMR (176 MHz, D_2O) δ (ppm): 52.43 ($\text{CH}_3\text{--O}$), 59.48 ($\text{C}5'$), 68.35 ($\text{C}3'$), 74.39 ($\text{C}2'$), 83.78 ($\text{C}4'$), 90.58 ($\text{C}1'$), 104.17 (C5), 149.13 (C6), 150.61 (C2), 162.15 (C4), 164.73 (O–C=O); HRMS (ESI) calcd for $\text{C}_{11}\text{H}_{13}\text{N}_2\text{O}_8$ $[\text{M} - \text{H}]^-$ 301.0672, found 301.0680; IR (ATR) cm^{-1} : 2946 (C–H), 1681 (C=O), 1087 (C–O–C) (see ESI, Fig. S11–S13 †).

Synthesis of 5-methoxycarbonylcytidine (4b)

Nucleoside **2b** (1 g, 3.2 mmol) was treated with 50 mM $\text{K}_2\text{CO}_3/\text{MeOH}$ (140 mL) and the mixture was stirred for 72 h at 60 °C. The amount sufficient for spectral analysis was evaporated under reduced pressure and purified by column chromatography (silica gel, 0–20% MeOH in CHCl_3), affording 5-(trimethoxymethyl)cytidine. TLC: $R_f = 0.43$ ($\text{CHCl}_3/\text{MeOH}$, 8 : 2, v/v); ^1H NMR (700 MHz, D_2O) δ (ppm): 3.23 (s, 9H, 3 \times O– CH_3), 3.84–3.88 (m, 1H, $\text{H}5''$), 4.04 (dd, 1H, $J = 2.8$ Hz, $J = 12.6$ Hz, $\text{H}5'$), 4.16–4.19 (m, 1H, $\text{H}4'$), 4.24 (dd, 1H, $J = 4.9$ Hz, $J = 7.7$ Hz, $\text{H}3'$), 4.31 (dd, 1H, $J = 2.8$ Hz, $J = 4.9$ Hz, $\text{H}2'$), 5.92 (d, 1H, $J = 2.8$ Hz, $\text{H}1'$), 8.51 (s, 1H, $\text{H}6$) (see ESI, Fig. S26 †). To the residual part of the reaction, 0.5 M aq. HCl was added dropwise. The mixture was stirred at rt for 12 h, concentrated under reduced pressure and purified by column chromatography (silica gel, 0–20% MeOH in CHCl_3) to give compound **4b** in 72% yield. TLC: $R_f = 0.38$ ($\text{CHCl}_3/\text{MeOH}$, 8 : 2, v/v); ^1H NMR (700 MHz, D_2O) δ (ppm): 3.88–3.91 (m, 4H, O– CH_3 , $\text{H}5''$), 4.08–4.10 (m, 1H, $\text{H}5'$), 4.18–4.20 (m, 1H, $\text{H}4'$), 4.24–4.27 (m, 1H, $\text{H}3'$), 4.31–4.33 (m, 1H, $\text{H}2'$), 5.91 (m, 1H, $\text{H}1'$), 9.21 (s, 1H, $\text{H}6$); ^{13}C NMR (176 MHz, D_2O) δ (ppm): 52.52 ($\text{CH}_3\text{--O}$), 59.03 ($\text{C}5'$), 67.79 ($\text{C}3'$), 74.53 ($\text{C}2'$), 83.24 ($\text{C}4'$), 91.25 ($\text{C}1'$), 96.90 (C5), 149.32 (C6), 154.33 (C2), 162.53 (C4), 165.77 (O–C=O); HRMS (ESI) calcd for $\text{C}_{11}\text{H}_{14}\text{N}_3\text{O}_7$ $[\text{M} - \text{H}]^-$ 300.0832, found 300.0842; IR (ATR) cm^{-1} : 2940 (C–H), 1710 (C=O), 1098 (C–O–C) (see ESI, Fig. S27–S29 †).

Synthesis of 5-cyanouridine (5a) and 5-cyanocytidine (5b)

Nucleoside **2a/2b** (1 g, 3.2 mmol) was treated with 30% aq. $\text{NH}_3\text{--EtOH}$ (140 mL, 3 : 1, v/v). The reaction mixture was stirred in a sealed vial tube at 60 °C for 4 h (compound **2a**) or 3 h (compound **2b**). Then the mixture was cooled to rt and evaporated under reduced pressure. Compound **5a** was purified by column chromatography (silica gel, 0–10% MeOH in CHCl_3) to obtain a white foam in 70% yield. TLC: $R_f = 0.78$ ($\text{H}_2\text{O}/\text{EtOH}/\text{Me}_2\text{CO}/\text{AcOEt}$, 0.5 : 1 : 1 : 6, v/v/v/v); ^1H NMR (700 MHz, D_2O) δ (ppm): 3.88 (dd, 1H, $J = 3.5$ Hz, $J = 12.6$ Hz, $\text{H}5''$), 4.03 (dd, 1H, $J = 2.1$ Hz, $J = 12.6$ Hz, $\text{H}5'$), 4.19–4.21 (m, 1H, $\text{H}4'$), 4.24 (dd, 1H, $J = 5.6$ Hz, $J = 7.0$ Hz, $\text{H}3'$), 4.37 (dd, 1H, $J = 2.8$ Hz, $J = 4.9$ Hz, $\text{H}2'$), 5.89 (d, 1H, $J = 2.8$ Hz, $\text{H}1'$), 8.83 (s, 1H, $\text{H}6$); ^{13}C NMR (176 MHz, D_2O) δ (ppm): 59.90 ($\text{C}5'$), 68.48 ($\text{C}3'$), 74.27 ($\text{C}2'$), 84.08 ($\text{C}4'$), 89.01 (C5), 90.77 ($\text{C}1'$), 113.91 (CN), 150.09 (C2), 150.37 (C6), 162.40 (C4); HRMS (ESI) calcd for $\text{C}_{10}\text{H}_{10}\text{N}_3\text{O}_6$ $[\text{M} - \text{H}]^-$ 268.0570, found 268.0579; IR (ATR) cm^{-1} : 2241 (C \equiv N) (see ESI, Fig. S14–S16 †).

Compound **5b** was purified by column chromatography (silica gel, 0–12% MeOH in CHCl_3) to obtain a white foam in



70% yield. TLC: $R_f = 0.47$ (H₂O/EtOH/Me₂CO/AcOEt, 0.5 : 1 : 1 : 6, v/v/v/v); ¹H NMR (700 MHz, D₂O) δ (ppm): 3.89 (dd, 1H, $J = 3.5$ Hz, $J = 13.3$ Hz, H5''), 4.06 (dd, 1H, $J = 2.1$ Hz, $J = 13.3$ Hz, H5'), 4.20–4.21 (m, 2H, H3', H4'), 4.31–4.32 (m, 1H, H2'), 5.86 (d, 1H, $J = 2.1$ Hz, H1'), 8.78 (s, 1H, H6); ¹³C NMR (176 MHz, D₂O) δ (ppm): 59.84 (C5'), 68.26 (C3'), 74.47 (C2'), 81.17 (C5), 83.61 (C4'), 91.45 (C1'), 114.26 (CN), 150.66 (C6), 155.24 (C2), 163.31 (C4); HRMS (ESI) calcd for C₁₀H₁₁N₄O₅ [M – H][–] 267.0729, found 267.0724; IR (ATR) cm^{–1}: 2225 (C≡N) (see ESI, Fig. S30–S32†).

Synthesis of 5-*N*-methylcarbamoyluridine (6a) and 5-*N*-methylcarbamoylcytidine (6b)

Nucleoside **2a/2b** (1 g, 3.2 mmol) was treated with 4% aq. MeNH₂ solution (140 mL) and incubated in a sealed vial tube for 20 h at 60 °C. The reaction mixture was cooled to rt and evaporated under reduced pressure. The obtained residue was purified by column chromatography (silica gel, 0–30% MeOH in CHCl₃) to give product **6a** in 65% yield. TLC: $R_f = 0.56$ (BuOH/AcOH/H₂O, 5 : 1 : 4, v/v/v); ¹H NMR (700 MHz, D₂O) δ : 2.93 (s, 3H, NH–CH₃), 3.87 (dd, 1H, $J = 4.2$ Hz, $J = 12.6$ Hz, H5''), 4.01 (dd, 1H, $J = 2.8$ Hz, $J = 12.6$ Hz, H5'), 4.19–4.21 (m, 1H, H4'), 4.27–4.29 (m, 1H, H3'), 4.39 (dd, 1H, $J = 3.5$ Hz, $J = 5.2$ Hz, H2'), 5.97 (d, 1H, $J = 3.5$ Hz, H1'), 8.81 (s, 1H, H6); ¹³C NMR (176 MHz, D₂O) δ (ppm): 25.86 (NH–CH₃), 60.29 (C5'), 69.05 (C3'), 74.25 (C2'), 84.19 (C4'), 90.17 (C1'), 106.11 (C5), 146.24 (C6), 150.88 (C2), 163.89 (C4), 164.53 (CO–NH); HRMS (ESI) calcd for C₁₁H₁₄N₃O₇ [M – H][–] 300.0832, found 300.0839; IR (ATR) cm^{–1}: 3313 (N–H), 2936 (C–H), 1680 (C=O) (see ESI, Fig. S17–S19†).

Compound **6b** was purified by column chromatography (silica gel, 0–30% MeOH in CHCl₃) in 60% yield. TLC: $R_f = 0.36$ (BuOH/AcOH/H₂O, 5 : 1 : 4, v/v/v); ¹H NMR (700 MHz, D₂O) δ (ppm): 2.87 (s, 3H, CH₃–NH), 3.89 (dd, 1H, $J = 3.5$ Hz, $J = 12.6$ Hz, H5''), 4.06 (dd, 1H, $J = 2.8$ Hz, $J = 12.6$ Hz, H5'), 4.18–4.20 (m, 1H, H4'), 4.27 (dd, 1H, $J = 4.9$ Hz, $J = 7.7$ Hz, H3'), 4.33 (dd, 1H, $J = 2.8$ Hz, $J = 5.6$ Hz, H2'), 5.88 (d, 1H, $J = 2.1$ Hz, H1'), 8.60 (s, 1H, H6); ¹³C NMR (176 MHz, D₂O) δ (ppm): 26.04 (CH₃–NH), 59.76 (C5'), 68.19 (C3'), 74.37 (C2'), 83.48 (C4'), 90.95 (C1'), 101.44 (C5), 143.70 (C6), 156.10 (C2), 163.55 (C4), 167.14 (CONH); HRMS (ESI) calcd for C₁₁H₁₅N₄O₆ [M – H][–] 299.0992, found 299.0998; IR (ATR) cm^{–1}: 3302 (N–H), 2925 (C–H), 1646 (C=O) (see ESI, Fig. S33–S35†).

Synthesis of 5-*N,N'*-dimethylamidinyluridine (7a)

Nucleoside **2b** (0.15 g, 0.48 mmol) was dissolved in 40% aq. MeNH₂ solution (10 mL) and incubated in a sealed vial tube for 2 h at 60 °C. The reaction mixture was concentrated under reduced pressure. The crude residue was purified by column chromatography (silica gel, 0–30% MeOH in CHCl₃) to give product **6b** in 65% yield. Spectral data were identical to those described above for **6b**.

Synthesis of 5-(*N,N'*-dimethylamidinyl)uridine (7a)

Nucleoside **2a** (0.15 g, 0.48 mmol) was treated with 40% aq. MeNH₂ solution (21 mL) and incubated in a sealed vial tube

for 3 h at 60 °C. The reaction mixture was cooled to rt and evaporated under reduced pressure. The obtained residue was purified by column chromatography (silica gel, CHCl₃/MeOH/H₂O, 65 : 25 : 4 v/v/v) to give product **7a** in 82% yield. TLC: $R_f = 0.13$ (BuOH/AcOH/H₂O, 5 : 1 : 4, v/v/v); ¹H NMR (700 MHz, D₂O) δ : 2.99 (s, 3H, NH–CH₃), 3.02 (s, 3H, =N–CH₃), 3.85 (dd, 1H, $J = 2.8$ Hz, $J = 13.0$ Hz, H5''), 4.00 (dd, 1H, $J = 2.8$ Hz, $J = 13.3$ Hz, H5'), 4.16–4.20 (m, 1H, H4'), 4.25–4.27 (m, 1H, H3'), 4.36 (dd, 1H, $J = 2.8$ Hz, $J = 4.9$ Hz, H2'), 5.92 (d, 1H, $J = 2.8$ Hz, H1'), 8.46 (s, 1H, H6); ¹³C NMR (176 MHz, D₂O) δ : 28.63 (NH–CH₃), 30.91 (=N–CH₃), 59.91 (C5'), 68.56 (C3'), 74.29 (C2'), 83.80 (C4'), 90.49 (C1'), 103.74 (C5), 144.22 (C6), 148.29 (C2), 155.60 (C4), 160.18 (NH–C=N); HRMS (ESI) calcd for C₁₂H₁₇N₄O₆ [M – H][–] 313.1148, found 313.1153; IR (ATR) cm^{–1}: 3201 (N–H), 2925 (C–H), 1644 (C=N) (see ESI, Fig. S20–S22†).

Author contributions

G. L. designed and supervised the study; K. P. performed the experiments and characterization of novel compounds; A. K. and S. D. assisted with chemical work; K. P. prepared the initial draft including data presentation; and G. L. wrote the manuscript with input from all the authors.

Conflicts of interest

There are no conflicts to declare.

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