Organic & Biomolecular Chemistry

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Cite this: *Org. Biomol. Chem.*, 2023, **21**, 2809

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

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Received 1st February 2023,
Accepted 9th March 2023

DOI: 10.1039/d3ob00161j

rsc.li/obc

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 CF_3 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 (m^5C) and 5-hydroxymethylcytidine (hm^5C), and products of their oxidation, 5-formylcytidine (f^5C) and 5-carboxycytidine (ca^5C), 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 m^5C-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-CF_3-substituted pyrimidine nucleosides are widely used drugs with antitumor and antiviral activities.[14] Notably, the 5-CF_3-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-CF_3U, **2a**) or 5-trifluoromethylcytidine (5-CF_3C, **2b**) precursors. The employed strategy does not require any protecting groups for regioselective C5-trifluoromethylation of pyrimidine ribonucleosides and the subsequent 5-CF_3 conversion.

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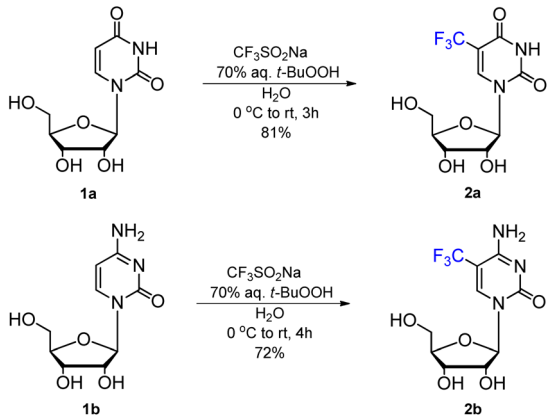
† Electronic supplementary information (ESI) available: Spectral characterization of nucleosides **2a–7a** and **2b–6b**. See DOI: https://doi.org/10.1039/d3ob00161j



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 trifluoromethanesulfinate (CF_3SO_2Na, known as the Langlois reagent) in combination with *tert*-butylhydroperoxide (*t*-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 CF_3SO_2Na and *t*-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 studies[24] 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 ($Fe^{2+}/H_2O_2/H_2SO_4$; Y = 11–53%)[26] or photoinduced reaction with *e.g.* trifluoromethyl sulfones or the Langlois reagent as the source of CF_3 radicals (Y = 38–42%).[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 CF_3SO_2Na (3 equiv.) and 70% aqueous (aq.) solution of *t*-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

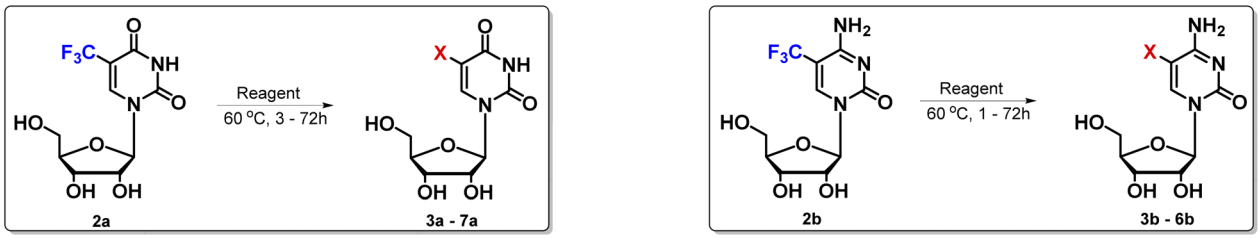
In general, the CF_3 group attached to the aromatic ring is stable and chemically inert under neutral conditions. The reactivity of the aromatic CF_3 group rises rapidly in alkaline solutions at elevated temperature. The first conversions of the 5-CF_3-2′-deoxyuridine nucleoside into 5-carboxy- or 5-cyano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

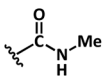
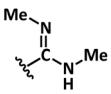
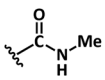
Very recently, Ito *et al.* demonstrated the synthetic scope of 5-CF_3dU and 5-CF_3dC conversions at the oligonucleotide level.[29] Since CF_3-modified nucleosides in the *ribo* series have never been considered as precursors to introduce various C5-functional groups, we evaluated the reactivity of 5-CF_3U (**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-CF_3U/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^{5}U, **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^{5}C) is a naturally existing modification identified in mRNA sequences as a product of m^{5}C or hm^{5}C epigenetic nucleoside oxidation.[10] The biological function of ca^{5}C 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 (–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-CF_3U and 5-CF_3C 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^2CO^3/MeOH (2) Dowex H	72	**4a**	90
4	**2b**		(1) 50 mM K_2CO_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 CF_3dU (2 weeks, 37 °C) in a methanolic solution of K_2CO_3 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 K_2CO_3 in dry MeOH heated to 60 °C afforded the *ortho* ester intermediate 5-(trimethoxymethyl)uridine (5-$(MeO)_3$C-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-CF_3-cytidine (**2b**) conversion. In this case, the reaction of **2b** with 50 mM K_2CO_3 methanolic solution (72 h, 60 °C) resulted in the formation of the 5-$(MeO)_3$C-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 ca^{5}C-phosphoramidite and then the ca^{5}C-modified RNA fragment.[19]

Next, we tested the reactivity of 5-CF_3U (**2a**) and 5-CF_3C (**2b**) with ammonia. According to the literature, the ammonolysis of CF_3-aromatic systems provided exclusively cyano derivatives; none of the primary amides was formed.[28c,29,33] For instance, the incubation of 5-CF_3dU with conc. NH_3(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-CF_3U 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. NH_3 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-CF_3-cytidine **2b** with 30% aq. NH_3–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-CF_3U aminolysis with methylamine as a nucleophilic reagent. Ito *et al.* previously reported the reactivity of 5-CF_3dU- and 5-CF_3dC-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. $MeNH_2$ solutions were tested. Incubation of **2a** with 4% aq. $MeNH_2$ at 60 °C for 20 h (Table 1, entry 7) resulted in the full conversion of the CF_3 group to *N*-methylcarbamoyl C(O)NHMe (product **6a**) in 65% yield. The use of a 10-fold higher $MeNH_2$ 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-CF_3-cytidine (**2b**) with 4% aq. $MeNH_2$ (4 h, 60 °C) or 40% aq. $MeNH_2$ (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-CF_3dU and 5-CF_3-dC with $MeNH_2$.[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-CF_3U, **2a** and 5-CF_3C, **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 60 (230–400 mesh, Fluka). NMR spectra were recorded on a Bruker Avance II Plus 700 spectrometer at 700 (for ^{1}H) and 176 (for ^{13}C) MHz or on a JEOL 400 spectrometer at 376 (for ^{19}F) MHz. Chemical shifts (δ) are reported in ppm relative to TMS (internal standard) for ^{1}H and ^{13}C. 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 H_2O (6.2 mL), and CF_3SO_2Na (0.96 g, 6.15 mmol) was added. After cooling the reaction mixture to 0 °C, *t*-BuOOH (70% in H_2O, 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 $CHCl_3$) to give 5-trifluoromethyluridine **2a** (0.52 g, 81%). TLC: R_f = 0.57 ($CHCl_3$/MeOH, 8 : 2, v/v); **^{1}H NMR** (700 MHz, D_2O) 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); **^{13}C NMR** (176 MHz, D_2O) 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, CF_3), 142.90 (q, J = 5.28 Hz, C6), 150.68 (C2), 161.54 (C4); **^{19}F NMR** (376 MHz, D_2O) δ: −63.26 ; HRMS (ESI) calcd for $C_{10}H_{10}N_2O_6F_3$ $[M - H]^-$ 311.0490, found 311.0496 (ESI, Fig. S1–S3†).

Synthesis of 5-trifluoromethylcytidine (2b)

Cytidine **1b** (1 g, 4.11 mmol) was dissolved in H_2O (12 mL); then CF_3SO_2Na (1.86 g, 12.33 mmol) was added and the solution was stirred at 0 °C. After 10 min, *t*-BuOOH (70% in H_2O, 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 $CHCl_3$) to give compound **2b** as a yellow solid (0.92 g, 72%). TLC: R_f = 0.34 ($CHCl_3$/MeOH, 8 : 2, v/v); **^{1}H NMR** (700 MHz, D_2O) 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); **^{13}C NMR** (176 MHz, D_2O) 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, CF_3),

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 $C_{10}H_{11}N_3O_5F_3$ $[M - H]^-$ 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); **^{1}H NMR** (700 MHz, D_2O) δ (ppm): 3.89 (dd, 1H, J = 3.5 Hz, J = 12.6 Hz, H5″), 4.05 (dd, 1H, J = 2.8 Hz, J = 13.3 Hz, H5′), 4.21–4.23 (m, 1H, H4′), 4.29 (dd, 1H, J = 5.1 Hz, J = 7.0 Hz, H3′), 4.41 (dd, 1H, J = 2.8 Hz, J = 5.1 Hz, H2′), 5.96 (d, 1H, J = 2.8 Hz, H1′), 9.08 (s, 1H, H6); **^{13}C NMR** (176 MHz, D_2O) δ (ppm): 59.66 (C5′), 68.47 (C3′), 74.36 (C2′), 83.95 (C4′), 90.65 (C1′), 103.22 (C5), 149.20 (C6), 150.43 (C2), 164.33 (C4), 166.08 (COOH); HRMS (ESI) calcd for $C_{10}H_{11}N_2O_8$ $[M - H]^-$ 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) $CHCl_3$/MeOH, 9 : 1, v/v, (2) $EtOAc/Me_2CO/AcOH/H_2O$, 5 : 3 : 1 : 1, v/v/v/v; **^{1}H NMR** (700 MHz, D_2O) δ (ppm): 3.89 (dd, 1H, J = 3.5 Hz, J = 13.3 Hz, H5″), 4.07 (dd, 1H, J = 2.8 Hz, J = 13.3 Hz, H5′), 4.22–4.24 (m, 1H, H4′), 4.28 (dd, 1H, J = 4.9 Hz, J = 7.7 Hz, H3′), 4.40 (dd, 1H, J = 2.8 Hz, J = 4.9 Hz, H2′), 5.95 (d, 1H, J = 2.8 Hz, H1′), 9.24 (s, 1H, H6); **^{13}C NMR** (176 MHz, D_2O) δ (ppm): 62.33 (C5′), 71.11 (C3′), 76.92 (C2′), 86.63 (C4′), 93.44 (C1′), 102.72 (C5), 151.45 (C6), 151.65 (C2), 161.65 (C4), 169.82 (COOH) ; HRMS (ESI) calcd for $C_{10}H_{12}N_3O_7$ $[M - H]^-$ 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 K_2CO_3/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 ($BuOH/AcOH/H_2O$, 5 : 1 : 4, v/v/v); **^{1}H NMR** (700 MHz, D_2O) δ (ppm): 3.21 (s, 9H, 3 × O–CH_3), 3.86 (m, 1H, J = 3.5 Hz, J = 12.6 Hz, H5″), 4.01 (dd, 1H, J = 2.8 Hz, J = 12.6 Hz, H5′), 4.16–4.19 (m, 1H, H4′), 4.26 (dd, 1H, J = 4.9 Hz, J = 7.0 Hz, H3′), 4.37 (dd, 1H, J = 2.8 Hz, J = 4.9 Hz, H2′), 5.94 (d, 1H, J = 2.8 Hz, H1′), 8.50 (s, 1H, H6) (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 ($CHCl_3$/MeOH, 8 : 2, v/v), R_f = 0.2 ($BuOH/AcOH/H_2O$, 5 : 1 : 4, v/v/v); **^{1}H NMR** (700 MHz, D_2O) δ (ppm): 3.88–3.91 (m, 4H, O–CH_3, H5″), 4.07 (dd, 1H, J = 2.8 Hz, J = 13.0 Hz, H5′), 4.20–4.22 (m, 1H, H4′), 4.29–4.31 (m, 1H, H3′), 4.39–4.40 (m, 1H, H2′), 5.96 (d, 1H, J = 2.5 Hz, H1′), 9.10 (s, 1H, H6); **^{13}C NMR** (176 MHz, D_2O) δ (ppm): 52.43 (CH_3–O), 59.48 (C5′), 68.35 (C3′), 74.39 (C2′), 83.78 (C4′), 90.58 (C1′), 104.17 (C5), 149.13 (C6), 150.61 (C2), 162.15 (C4), 164.73 (O–C═O); HRMS (ESI) calcd for $C_{11}H_{13}N_2O_8$ $[M - H]^-$ 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 K_2CO_3/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 ($CHCl_3$/MeOH, 8 : 2, v/v); **^{1}H NMR** (700 MHz, D_2O) δ (ppm): 3.23 (s, 9H, 3 × O–CH_3), 3.84–3.88 (m, 1H, H5″), 4.04 (dd, 1H, J = 2.8 Hz, J = 12.6 Hz, H5′), 4.16–4.19 (m, 1H, H4′), 4.24 (dd, 1H, J = 4.9 Hz, J = 7.7 Hz, H3′), 4.31 (dd, 1H, J = 2.8 Hz, J = 4.9 Hz, H2′), 5.92 (d, 1H, J = 2.8 Hz, H1′), 8.51 (s, 1H, H6) (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 ($CHCl_3$/MeOH, 8 : 2, v/v); **^{1}H NMR** (700 MHz, D_2O) δ (ppm): 3.88–3.91 (m, 4H, O–CH_3, H5″), 4.08–4.10 (m, 1H, H5′), 4.18–4.20 (m, 1H, H4′), 4.24–4.27 (m, 1H, H3′), 4.31–4.33 (m, 1H, H2′), 5.91 (m, 1H, H1′), 9.21 (s, 1H, H6); **^{13}C NMR** (176 MHz, D_2O) δ (ppm): 52.52 (CH_3–O), 59.03 (C5′), 67.79 (C3′), 74.53 (C2′), 83.24 (C4′), 91.25 (C1′), 96.90 (C5), 149.32 (C6), 154.33 (C2), 162.53 (C4), 165.77 (O–C═O); HRMS (ESI) calcd for $C_{11}H_{14}N_3O_7$ $[M - H]^-$ 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. NH_3–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 (H_2O/EtOH/Me_2CO/AcOEt, 0.5 : 1 : 1 : 6, v/v/v/v); **^{1}H NMR** (700 MHz, D_2O) δ (ppm): 3.88 (dd, 1H, J = 3.5 Hz, J = 12.6, H5″), 4.03 (dd, 1H, J = 2.1 Hz, J = 12.6 Hz, H5′), 4.19–4.21 (m, 1H, H4′), 4.24 (dd, 1H, J = 5.6 Hz, J = 7.0 Hz, H3′), 4.37 (dd, 1H, J = 2.8 Hz, J = 4.9 Hz, H2′), 5.89 (d, 1H, J = 2.8 Hz, H1′), 8.83 (s, 1H, H6); **^{13}C NMR** (176 MHz, D_2O) δ (ppm): 59.90 (C5′), 68.48 (C3′), 74.27 (C2′), 84.08 (C4′), 89.01 (C5), 90.77 (C1′), 113.91 (CN), 150.09 (C2), 150.37 (C6), 162.40 (C4); HRMS (ESI) calcd for $C_{10}H_{10}N_3O_6$ $[M - H]^-$ 268.0570, found 268.0579; IR (ATR) cm^{-1}: 2241 (C≡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 (H_2O/EtOH/Me_2CO/AcOEt, 0.5 : 1 : 1 : 6, v/v/v/v); **^{1}H NMR** (700 MHz, D_2O) 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); **^{13}C NMR** (176 MHz, D_2O) 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 $C_{10}H_{11}N_4O_5$ [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. $MeNH_2$ 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 $CHCl_3$) to give product **6a** in 65% yield. TLC: R_f = 0.56 (BuOH/AcOH/H_2O, 5 : 1 : 4, v/v/v); **^{1}H NMR** (700 MHz, D_2O) δ: 2.93 (s, 3H, NH–C$\underline{H}_3$), 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); **^{13}C NMR** (176 MHz, D_2O) δ (ppm): 25.86 (NH–CH_3), 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 $C_{11}H_{14}N_3O_7$ [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 $CHCl_3$) in 60% yield. TLC: R_f = 0.36 (BuOH/AcOH/H_2O, 5 : 1 : 4, v/v/v); **^{1}H NMR** (700 MHz, D_2O) δ (ppm): 2.87 (s, 3H, C$\underline{H}_3$–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); **^{13}C NMR** (176 MHz, D_2O) δ (ppm): 26.04 (CH_3–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 $C_{11}H_{15}N_4O_6$ [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*-methylcarbamoylcytidine (6b)

Nucleoside **2b** (0.15 g, 0.48 mmol) was dissolved in 40% aq. $MeNH_2$ 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 $CHCl_3$) 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. $MeNH_2$ 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, $CHCl_3$/MeOH/H_2O, 65 : 25 : 4 v/v/v) to give product **7a** in 82% yield. TLC: R_f = 0.13 (BuOH/AcOH/H_2O, 5 : 1 : 4, v/v/v); **^{1}H NMR** (700 MHz, D_2O) δ: 2.99 (s, 3H, NH–C$\underline{H}_3$), 3.02 (s, 3H, ═N–CH_3), 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); **^{13}C NMR** (176 MHz, D_2O) δ: 28.63 (NH–CH_3), 30.91 (═N–CH_3), 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 $C_{12}H_{17}N_4O_6$ [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.

Acknowledgements

This work was supported by a grant UMO-2021/43/B/ST4/01570 from the National Science Centre to G. L. This paper was completed while the first author (K. P.) was a doctoral candidate in the Interdisciplinary Doctoral School at the Lodz University of Technology, Poland.

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