Highly Efficient Method for C-5 Halogenation of Pyrimidine-Based Nucleosides in Ionic Liquids

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Abstract: A novel, highly efficient, convenient, and benign methodology for C-5 halogenation of pyrimidine-based nucleosides has been developed using *N*-halosuccinimides as halogenating reagents without using any catalyst in ionic liquid medium. The ionic liquids were successfully recovered and reused for all the reactions.

Key words: ionic liquids, nucleosides, halogenation

The rational variation at C-5 position of pyrimidine-based nucleosides can enhance their properties in terms of oral bioavailability, metabolic stability, pharmacokinetics, etc. 5-Halopyrimidine nucleosides are of great pharmaceutical interest and have been extensively investigated due to their antineoplastic and antiviral properties.[1–3] For example, idoxuridine, also known as 5-iodo-2′-deoxyuridine marketed as Herples and Stoxil® is one of the drugs used for conjunctival and corneal disease associated with feline herpes virus. Also, 5-chlorouridines have shown selective anti-HIV activity.[4] Since cytidine can be incorporated into PNA and DNA more efficiently than uridine,[5,6] several antiviral studies have been carried out using 5-halocytidines.[7,8] Also, 5-halopyrimidine nucleosides have been used extensively as intermediates for the synthesis of a wide range of modified nucleosides showing activity, mainly against HSV-1 and HSV-2 (herpes simplex virus type 1 and 2) and VZV (varicella-zoster virus).[9–12] Radiolabeled halogenated nucleosides are used for cellular biochemistry for purposes of understanding nucleoside transporter affinities, specificity of enzyme-substrate complexes and their overall interactions and as mechanistic probes for DNA metabolism studies.[13,14] Therefore, new methods for the synthesis of 5-halopyrimidine nucleosides are of great interest.

One approach to synthesize 5-halopyrimidine nucleosides is the coupling of a protected chlorosugar and halogenated base, but this method requires multiple steps and often results in a mixture of α- and β-anomers.[15,16] The other approach is the direct halogenation of protected or unprotected nucleosides.[17] For the first time, synthesis of 5-bromouridine from uridine was reported using bromine water through an bromohydroxy intermediate, which on heating in acidic ethanol gave the desired product.[18] Later, bromination of uridine and cytidine has been carried out using *N*-bromosuccinimide in DMF.[19] In similar attempts, *N*-chlorosuccinimide has been used with excess of pyridine[4] or with glacial acetic acid to obtain 5-chloropyrimidine nucleosides in prolonged reaction time.[20] Chlorination of pyrimidine nucleosides has also been carried out using acyl chlorides/DMF/MCPBA system in moderate yields.[21] Bromination and chlorination of deoxycytidine has been achieved using Br_2/CCl_4 and Cl_2/CCl_4, respectively, in acetic acid/pyridine under anhydrous conditions in low to moderate yields.[22] The first report for C-5 iodination of uridines was using I_2/HNO_3 or KI/H_2SO_4.[14] Later, *N*-iodosuccinimide or iodine monochloride (ICl) were used in DMSO or *N*-ethylacetamide for iodination of pyrimidine nucleosides using different catalysts.[23] Iodination of pyrimidine nucleosides has also been reported using $I_2/HIO_3/CCl_4$ in acetic acid in low to moderate yields.[24] Iodination of uridines via 5-mercuro intermediates was also achieved in aqueous alcohol.[25] Recently, uridine and cytidine were reacted with *N*-iodosuccinimide using DMF as a solvent under microwave conditions to give the corresponding iodo derivatives.[26] Asakura and Robins reported a C-5 halogenation of uridines with iodine and lithium halides in the presence of ceric ammonium nitrate (CAN) using acetic acid–acetonitrile as solvent.[27,28] Kumar et al. reported halogenation of uridines using *N*-halosuccinimides and ICl in the presence of excess sodium azide (NaN_3), which required elongated reaction time up to 48 hours.[29] All these reported methods for nucleoside halogenation are associated with several disadvantages, namely harsh reaction conditions, longer reaction time, use of toxic oxidizing agents (e.g., HIO_3, HNO_3, CAN) or catalysts (e.g., NaN_3), toxic and difficult to handle halogenating agents (e.g., Cl_2, Br_2, and ICl), inert reaction conditions, highly toxic and high boiling solvents (e.g., AcOH, DMF, CCl_4), which are difficult to remove and often get contaminated with products, etc. Furthermore, all these reactions involves multiple workup steps such as neutralization, extraction, purification, etc., which are time-consuming and lower down the product yield.

Over the last decade, ionic liquids (ILs) have emerged as effective alternatives to conventional organic solvents due to their attractive properties, including their negligible vapor pressure, recyclability, high thermal stability and their ability to dissolve wide range of compounds.[30,31] Our efforts of finding suitable solvents for nucleoside chemistry led to designing new ILs, which give high solubility of nucleosides. These solvents are found to be efficient reaction

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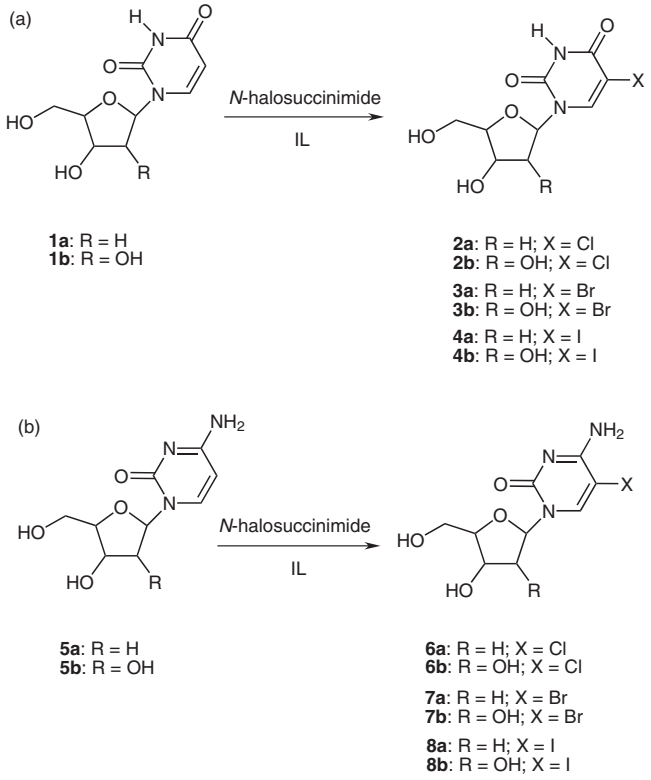
media for selective modifications of nucleosides giving high yields under mild conditions.[32,33] Recently, we reported the utility of ionic liquids towards the synthesis of nucleoside-based antiviral drugs.[34]

In the current report, a new method for C-5 halogenation of uridines and cytidines (Scheme 1) is described using ionic liquids as reaction medium and *N*-halosuccinimides as halogenating agents without using any catalyst. We found that ionic liquids with oxygenated anions give high solubility of nucleosides,[32,33] therefore the ionic liquids selected in this study are 1-methoxyethyl-3-methylimidazolium methanesulfonate ([MoeMIm][Ms]), 1-methoxyethyl-3-methylimidazolium trifluoroacetate ([MoeMIm][TFA]), 1-butyl-3-methylimidazolium methanesulfonate ([BMIm][Ms]), and 1-butyl-3-methylimidazolium trifluoroacetate ([BMIm][TFA]) (Figure 1).

Chlorination of 2′-deoxyuridine (**1a**) and uridine (**1b**) was first carried out at room temperature (25 °C) in IL [MoeMIm][Ms] using *N*-chlorosuccinimide (NCS) (1.5 equiv) as halogenating agent. The reactions were slow and took 20 hours for completion to give the corresponding chlorinated products **2a**[29] and **2b**[29] in 92 and 90% yield, respectively (Table 1, entries 1 and 2). When the same reactions were carried out at 50 °C, the reaction time dropped significantly to just 20 minutes for complete conversion with high isolated yields (Table 1, entries 3 and 4). It is well documented in the literature that changing the cation or anion of an IL can change the course of a reaction, including the kinetics. To investigate this effect we carried out the chlorination of **1a** and **1b** in ILs [MoeMIm][TFA], [BMIm][TFA], and [BMIm][Ms]. We observed that the reactions in ILs having trifluoroacetate as anion (Table 1, entries 5 to 8) were slower as compared to those with methanesulfonate as anion. It is worth mentioning that the IL [BMIm][Ms] is solid at room temperature and its reactions were carried out at its melting point (60 °C), which resulted in further decrease of reaction time to 10 minutes with high product yield (Table 1, entries 9 and 10). When similar reaction conditions were applied for the chlorination of 2′-deoxycytidine (**5a**) and cytidine (**5b**) using ILs [MoeMIm][Ms] (Table 1, entries 11 and 12) and [BMIm][Ms] (Table 1, entries 13 and 14), the corresponding chlorinated derivatives **6a**[21] and **6b**[35] were obtained in high yields, although the reaction time required for completion of these reactions were longer as compared with the chlorination of **1a** and **1b**.



Scheme 1 IL-mediated C-5 halogenation of: (a) uridines and (b) cytidines

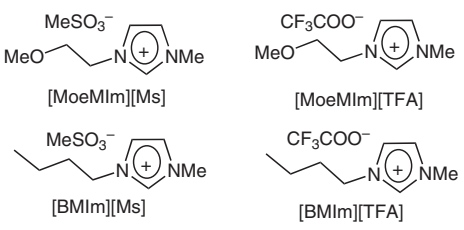


Figure 1 Ionic liquids used in this study

Table 1 Chlorination of Uridines and Cytidines with *N*-Chlorosuccinimide in ILs

Entry	IL	Substrate/ Product	Time (min)/ Temp (°C)	Yield (%) in fresh IL (in recovered IL)
1	[MoeMIm][Ms]	**1a/2a**	20 h/25	92
2	[MoeMIm][Ms]	**1b/2b**	20 h/25	90
3	[MoeMIm][Ms]	**1a/2a**	20/50	92 (93)
4	[MoeMIm][Ms]	**1b/2b**	20/50	92 (91)
5	[MoeMIm][TFA]	**1a/2a**	45/50	87 (85)
6	[MoeMIm][TFA]	**1b/2b**	45/50	94 (90)
7	[BMIm][TFA]	**1a/2a**	45/50	89 (86)
8	[BMIm][TFA]	**1b/2b**	45/50	86 (85)
9	[BMIm][Ms]	**1a/2a**	10/60	89 (85)
10	[BMIm][Ms]	**1b/2b**	10/60	87 (87)
11	[MoeMIm][Ms]	**5a/6a**	60/50	84 (85)
12	[MoeMIm][Ms]	**5b/6b**	60/50	86 (85)
13	[BMIm][Ms]	**5a/6a**	45/60	80 (80)
14	[BMIm][Ms]	**5b/6b**	45/60	82 (81)

Bromination of nucleosides **1a**, **1b**, **5a**, and **5b** was carried out using *N*-bromosuccinimide (NBS) (1.5 equiv) in all four ionic liquids. It took only 5 minutes to complete the reaction of **1a** and **1b** at 25 °C in ILs [MoeMIm][Ms], [BMIm][TFA], and [MoeMIm][TFA] and at 60 °C in IL

[BMIm][Ms] to give the corresponding brominated derivatives **3a**[29] and **3b**[29] (Table 2, entries 1–8). The reaction time for bromination of **5a** and **5b** was 30 minutes at 25 °C in ILs [MoeMIm][Ms], [BMIm][TFA], and [MoeMIm][TFA], while it took only 20 minutes at 60 °C in IL [BMIm][Ms] to give the brominated derivatives **7a**[22] and **7b**[36] (Table 2, entries 9–16). These are the shortest reaction times ever reported for nucleoside reactions studied here, and also giving high yields without using any catalyst or oxidizing agent.

Interestingly, when iodination of uridine (**1b**) was carried out with *N*-iodosuccinimide (NIS) (2.5 equiv) in [MoeMIm][Ms] and [BMIm][Ms], no reaction was observed at 25 °C or even at elevated temperature. However, the reaction was complete in 24 hours to give 5-iodouridine (**4b**) in 75% isolated yield, when carried out in [MoeMIm][TFA] at 25 °C (Table 3, entry 1). The reaction time was decreased to four hours for iodination of both **1a** and **1b** when the reactions were carried out at 60 °C in [MoeMIm][TFA] and [BMIm][TFA] to give the corresponding iodinated derivatives **4a**[29] and **4b**[29] (Table 3, entries 2–5) in good yields. Similarly, no reaction was observed when iodination of **5a** and **5b** was carried out in [MoeMIm][Ms] and [BMIm][Ms] having methanesulfonate as anion. However, the reactions were complete in four hours at 60 °C to give corresponding products **8a**[37] and **8b**[38] when reactions were carried out in ILs [MoeMIm][TFA] and [BMIm][TFA] having trifluoroacetate as anion (Table 3, entries 6–9).

Table 2 Bromination of Uridines and Cytidines with *N*-Bromosuccinimide in ILs

Entry	IL	Substrate/Product	Time (min)/Temp (°C)	Yield (%) in fresh IL (in recovered IL)
1	[MoeMIm][Ms]	**1a/3a**	5/25	90 (90)
2	[MoeMIm][Ms]	**1b/3b**	5/25	82 (89)
3	[BMIm][TFA]	**1a/3a**	5/25	82 (81)
4	[BMIm][TFA]	**1b/3b**	5/25	85 (84)
5	[MoeMIm][TFA]	**1a/3a**	5/25	98 (95)
6	[MoeMIm][TFA]	**1b/3b**	5/25	85 (82)
7	[BMIm][Ms]	**1a/3a**	5/60	84 (84)
8	[BMIm][Ms]	**1b/3b**	5/60	82 (83)
9	[MoeMIm][Ms]	**5a/7a**	30/25	82 (80)
10	[MoeMIm][Ms]	**5b/7b**	30/25	87 (85)
11	[BMIm][TFA]	**5a/7a**	30/25	83 (82)
12	[BMIm][TFA]	**5b/7b**	30/25	80 (81)
13	[MoeMIm][TFA]	**5a/7a**	30/25	85 (80)
14	[MoeMIm][TFA]	**5b/7b**	30/25	86 (85)
15	[BMIm][Ms]	**5a/7a**	20/60	82 (81)
16	[BMIm][Ms]	**5b/7b**	20/60	85 (85)

Table 3 Iodination of Uridines and Cytidines with *N*-Iodosuccinimide in ILs

Entry	IL	Substrate/Product	Time (min)/Temp (°C)	Yield (%) in fresh IL (in recovered IL)
1	[MoeMIm][TFA]	**1b/4b**	24/25	75
2	[MoeMIm][TFA]	**1b/4b**	4/60	88 (86)
3	[MoeMIm][TFA]	**1a/4a**	4/60	89 (85)
4	[BMIm][TFA]	**1b/4b**	4/60	77 (75)
5	[BMIm][TFA]	**1a/4a**	4/60	74 (70)
6	[MoeMIm][TFA]	**5b/8b**	4/60	64 (62)
7	[MoeMIm][TFA]	**5a/8a**	4/60	61 (60)
8	[BMIm][TFA]	**5b/8b**	4/60	58 (59)
9	[BMIm][TFA]	**5a/8a**	4/60	60 (60)

It is important to note that 10–15 mL of highly polar organic solvents (e.g., pyridine, DMF, etc.) are required to dissolve 1 mmol of nucleosides studied here. On the other hand due to high solubility in ILs, our methodology requires only 1–1.5 mL of ILs to dissolve these nucleosides. Therefore, the solvent consumption is decreased by 10-fold, which make the reaction easy to handle and workup. After completion, the reaction mixture was diluted with methanol and loaded on the silica gel column, which was eluted by MeOH–CH_2Cl_2 in increasing order of polarity to isolate the pure halogenated nucleosides. Once the product was isolated, the column was eluted with 80% MeOH–CH_2Cl_2 to recover the IL. The recovered IL was dried overnight in vacuum oven at 60 °C and reused giving products in similar yields as reported in Tables 1, 2, and 3. It is important to note that, there was no inert atmosphere or special reaction assembly needed for these reactions.

All the products were characterized by ^{1}H, ^{13}C, COSY, and HSQC NMR, and high resolution LC-MS experiments. It is important to mention that NH_2 protons of halogenated cytidines **6a**, **6b**, **7a**, **7b**, **8a**, and **8b** appeared separately in the ^{1}H NMR spectra due to restricted rotation of C–N bond caused by introduction of bulky halogens at C- 5 position. When the spectra of these compounds were taken at 80 °C, both protons of NH_2 group appeared together and in doing so an upfield shift of 0.20–0.4 ppm for the OH protons was also observed. As representative example, the ^{1}H NMR spectra of **6a** and **6b** at 25 °C and 80 °C are given in Figure 2.

In summary, ionic liquids proved to be highly efficient, convenient, and benign reaction medium for C-5 halogenation of pyrimidine-based nucleosides. Several highlights of this new methodology are: easy to handle reagents, reuse of reaction media, easy workup and purification, high product yields, and very short reaction time without using any catalyst. Thus, the reactions reported here make it a perfect protocol for the C-5 halogenation of pyrimidine-based nucleosides.

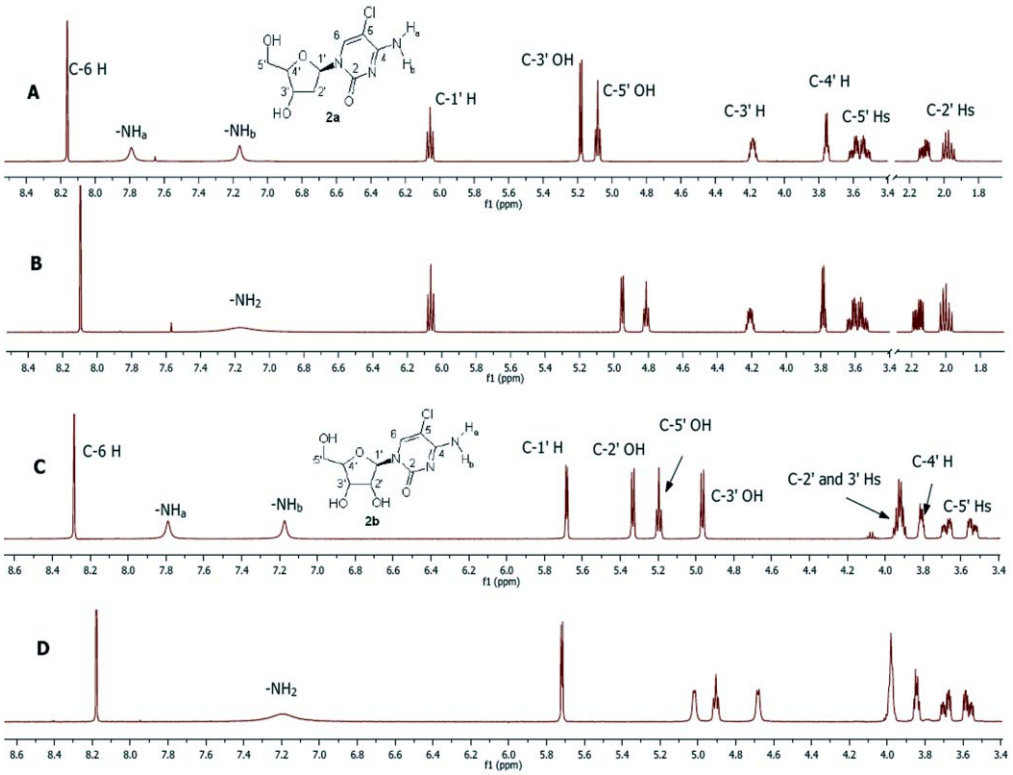


Figure 2 ^{1}H NMR spectra of (**A**) **6a** at 25 °C; (**B**) **6a** at 80 °C; (**C**) **6b** at 25 °C; (**D**) **6b** at 80 °C

TLCs were run on precoated Merck silica gel 60F_{254} plates and observed by charring with 5% H_2SO_4/EtOH and with UV light. The products were isolated and purified using Teledyne ISCO Rf flash chromatography system with MeOH and CH_2Cl_2 as eluents. For verification of the product, the LC-MS was taken on an Agilent 1200 series system with an Agilent 6210 Time-of-Flight mass detector. The ^{1}H (400 MHz), ^{13}C (101 MHz), COSY, and HSQC NMR spectra were taken on a Varian 400MR spectrophotometer with TMS as the internal standard. All the chemicals were purchased from Sigma-Aldrich Co. and used as obtained. Ionic liquid 1-butyl-3-methylimidazolium methanesulfonate ([BMIm][Ms]) was purchased from Aldrich Chemicals Co. Ionic liquids 1-methoxyethyl-3-methylimidazolium methanesulfonate ([MoeMIm][Ms]), 1-methoxyethyl-3-methylimidazolium trifluoroacetate ([MoeMIm][TFA]), and 1-butyl-3-methylimidazolium trifluoroacetate ([BMIm][TFA]) were prepared following the literature protocol.[33]

Halogenation of the Nucleosides in Ionic Liquids; General Procedure

The nucleoside **1a**, **1b**, **5a**, or **5b** (1 mmol) was dissolved in an ionic liquid (1.5 mL) in a vial followed by the addition of *N*-halosuccinimide (1.5 mmol for *N*-chlorosuccinimide and *N*-bromosuccinimide, 2.5 mmol for *N*-iodosuccinimide). The reaction mixture was then stirred at appropriate temperature (Tables 1– 3) and the progress of the reaction was monitored by TLC (small aliquot of the reaction mixture diluted with MeOH was used for spotting). After completion of the reaction, the mixture was diluted with MeOH (3 mL) and loaded on silica gel column, which was eluted with MeOH–CH_2Cl_2 in increasing order of polarity. The combined fractions containing the product were concentrated and dried under vacuum to isolate the halogenated derivatives as white powder. Once the product was recovered from the column, it was eluted with 80% MeOH–CH_2Cl_2 to recover the ionic liquids. The combined fractions containing the IL were concentrated under vacuum, dried in a vacuum oven at 60 °C overnight, and reused for the same reaction. The reaction time, temperature, isolated yields in fresh and recovered ILs are mentioned in Tables 1, 2, and 3.

5-Chloro-2′-deoxyuridine (2a)[29]

White solid; mp 174–178 °C (dec.) [Lit.[29] mp 173–176 °C (dec.)].

^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.15–2.03 (m, 2 H), 3.57 (dd, *J* = 11.8, 25.1 Hz, 2 H), 3.77 (q, *J* = 3.2 Hz, 1 H), 4.21 (s, 1 H), 5.14 (s, 1 H), 5.22 (d, *J* = 3.8 Hz, 1 H), 6.08 (t, *J* = 6.5 Hz, 1 H), 8.28 (s, 1 H), 11.80 (s, 1 H).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 40.09, 60.89, 70.02, 84.73, 87.45, 107.27, 137.39, 150.40, 160.09.

LC-MS (ESI-TOF): *m*/*z* [M + H]$^+$ calcd for $C_9H_{12}ClN_2O_6$: 263.04293; found: 263.05489l.

5-Chlorouridine (2b)[29]

White solid; mp 210–216 °C (dec.) [Lit.[29] mp 215–220 °C (dec.)].

^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.64–3.54 (m, 1 H), 3.74–3.64 (m, 1 H), 3.87 (dt, *J* = 2.7, 5.0 Hz, 1 H), 3.99 (dd, *J* = 4.9, 9.8 Hz, 1 H), 4.05 (dd, *J* = 4.8, 9.6 Hz, 1 H), 5.08 (d, *J* = 5.4 Hz, 1 H), 5.28 (t, *J* = 4.7 Hz, 1 H), 5.43 (d, *J* = 5.3 Hz, 1 H), 5.73 (d, *J* = 4.5 Hz, 1 H), 8.41 (s, 1 H), 11.85 (s, 1 H).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 60.14, 69.24, 73.93, 84.68, 88.50, 107.12, 137.89, 149.74, 158.99.

LC-MS (ESI-TOF): *m*/*z* [M + H]$^+$ calcd for $C_9H_{12}ClN_2O_6$: 279.03784; found: 279.04976.

5-Bromo-2′-deoxyuridine (3a)[29]

White solid; mp 174–176 °C (dec.) [Lit.[29] mp 175–179 °C (dec.)].

^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.19–2.01 (m, 2 H), 3.57 (dd, *J* = 11.7, 25.3 Hz, 2 H), 3.77 (q, *J* = 3.2 Hz, 1 H), 4.21 (d, *J* = 3.3 Hz, 1 H), 5.14 (s, 1 H), 5.22 (s, 1 H), 6.07 (t, *J* = 6.5 Hz, 1 H), 8.36 (s, 1 H), 11.76 (s, 1 H).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 40.13, 60.79, 69.96, 84.86, 87.57, 95.66, 140.27, 149.74, 159.17.

LC-MS (ESI-TOF): *m*/*z* [M + H]$^+$ calcd for $C_9H_{12}BrN_2O_5$: 307.0063; found: 1306.99241.

5-Bromouridine (3b)[29]
White solid; mp 190–195 °C (dec.) [Lit.[29] mp 190–198 °C (dec.)].

^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.59–3.50 (m, 1 H), 3.67 (dd, *J* = 6.7, 9.8 Hz, 1 H), 3.84 (dt, *J* = 2.7, 4.9 Hz, 1 H), 3.96 (d, *J* = 4.2 Hz, 1 H), 4.01 (dd, *J* = 4.7, 9.4 Hz, 1 H), 5.04 (d, *J* = 4.6 Hz, 1 H), 5.25 (t, *J* = 4.7 Hz, 1 H), 5.40 (d, *J* = 5.2 Hz, 1 H), 5.70 (d, *J* = 4.5 Hz, 1 H), 8.45 (s, 1 H), 11.78 (s, 1 H).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 60.11, 69.25, 73.94, 84.67, 88.49, 95.69, 140.35, 149.97, 159.14.

LC-MS (ESI-TOF): *m*/*z* [M + H]$^+$ calcd for $C_9H_{12}BrN_2O_6$: 322.98733; found: 323.0019.

5-Iodo-2′-deoxyuridine (4a)[29]
White solid; mp 172–180 °C (dec.) [Lit.[29] mp 170–180 °C (dec.)].

^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.12 (dd, *J* = 4.6, 8.5 Hz, 2 H), 3.67–3.51 (m, 2 H), 3.79 (q, *J* = 3.2 Hz, 1 H), 4.24 (dt, *J* = 4.3, 8.6 Hz, 1 H), 5.14 (t, *J* = 4.8 Hz, 1 H), 5.24 (d, *J* = 4.2 Hz, 1 H), 6.10 (t, *J* = 6.5 Hz, 1 H), 8.40 (s, 1 H), 11.66 (s, 1 H).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 40.18, 60.80, 69.25, 69.99, 84.63, 87.50, 145.03, 150.09, 160.48.

LC-MS (ESI-TOF): *m*/*z* [M + H]$^+$ calcd for $C_9H_{12}IN_2O_5$: 354.97854; found: 354.995241.

5-Iodouridine (4b)[29]
White solid; mp 205–210 °C (dec.) [Lit.[29] mp 207–209 °C (dec.)].

^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.62–3.53 (m, 1 H), 3.73–3.64 (m, 1 H), 3.87 (dt, *J* = 2.6, 4.9 Hz, 1 H), 4.01 (dq, *J* = 4.6, 21.6 Hz, 2 H), 5.07 (d, *J* = 5.0 Hz, 1 H), 5.26 (t, *J* = 4.5 Hz, 1 H), 5.41 (d, *J* = 5.0 Hz, 1 H), 5.72 (d, *J* = 4.6 Hz, 1 H), 8.48 (s, 1 H), 11.68 (s, 1 H).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 60.18, 69.31, 73.95, 84.73, 88.27, 96.17, 145.11, 150.35, 160.48.

LC-MS (ESI-TOF): *m*/*z* [M + H]$^+$ calcd for $C_9H_{12}IN_2O_6$: 370.97346; found: 370.99221.

5-Chloro-2′-deoxycytidine (6a)[21]
Highly viscous transparent material, which turned into a white semi-solid over time.

^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): δ = 2.03–1.92 (m, 1 H), 2.11 (ddd, *J* = 3.8, 6.1, 13.2 Hz, 1 H), 3.56–3.50 (m, 1 H), 8.16 (s, 1 H), 3.63–3.57 (m, 1 H), 3.76 (q, *J* = 3.4 Hz, 1 H), 4.18 (td, *J* = 3.8, 7.6 Hz, 1 H), 5.08 (t, *J* = 5.0 Hz, 1 H), 5.18 (d, *J* = 4.3 Hz, 1 H), 6.06 (t, *J* = 6.4 Hz, 1 H), 7.16 (s, 1 H), 7.79 (s, 1 H).

^{1}H NMR (400 MHz, DMSO-d_6, 80 °C): δ = 2.05–1.95 (m, 1 H), 2.20–2.11 (m, 1 H), 3.59–3.52 (m, 1 H), 3.65–3.59 (m, 1 H), 3.79 (q, *J* = 3.6 Hz, 1 H), 4.21 (td, *J* = 3.9, 7.7 Hz, 1 H), 4.81 (t, *J* = 5.1 Hz, 1 H), 4.95 (d, *J* = 4.4 Hz, 1 H), 6.06 (t, *J* = 6.5 Hz, 1 H), 7.33–6.97 (m, 2 H), 8.10 (s, 1 H).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 41.41, 61.54, 70.62, 86.05, 88.06, 99.52, 139.87, 154.22, 162.01.

LC-MS (ESI-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_9H_{12}ClN_3O_4$ + Na: 284.04140; found: 284.04074

5-Chlorocytidine (6b)[35]
White solid; mp 212–215 °C (Lit.[35] mp 211–214 °C (dec.)].

^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): δ = 3.54 (ddd, *J* = 2.7, 4.9, 12.1 Hz, 1 H), 3.67 (ddd, *J* = 2.8, 4.8, 12.1 Hz, 1 H), 3.81 (dt, *J* = 2.6, 5.3 Hz, 1 H), 3.98–3.88 (m, 2 H), 4.97 (d, *J* = 5.5 Hz, 1 H), 5.20 (s, 1 H), 5.33 (d, *J* = 5.0 Hz, 1 H), 5.68 (d, *J* = 3.4 Hz, 1 H), 7.17 (s, 1 H), 7.79 (s, 1 H), 8.29 (s, 1 H).

^{1}H NMR (400 MHz, DMSO-d_6, 80 °C): δ = 3.57 (ddd, *J* = 3.2, 4.9, 12.1 Hz, 1 H), 3.69 (ddd, *J* = 3.0, 4.8, 12.1 Hz, 1 H), 3.84 (dt, *J* = 3.0, 5.0 Hz, 1 H), 4.02–3.94 (m, 2 H), 4.68 (d, *J* = 4.4 Hz, 1 H), 4.90 (t, *J* = 5.0 Hz, 1 H), 5.02 (d, *J* = 3.8 Hz, 1 H), 5.75–5.69 (m, 1 H), 7.19 (s, 2 H), 8.18 (s, 1 H).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 60.56, 69.46, 74.97, 84.68, 90.12, 140.21, 154.45, 162.00.

LC-MS (ESI-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_9H_{12}ClN_3O_5$ + Na: 300.03632; found: 300.03475.

5-Bromo-2′-deoxycytidine (7a)[22]
Highly viscous transparent material, which turned into a white semi-solid over time.

^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): δ = 2.02–1.93 (m, 1 H), 2.11 (ddd, *J* = 3.8, 6.1, 13.2 Hz, 1 H), 3.56–3.48 (m, 1 H), 3.60 (ddd, *J* = 3.4, 5.0, 11.8 Hz, 1 H), 3.75 (q, *J* = 3.4 Hz, 1 H), 4.18 (td, *J* = 3.9, 7.7 Hz, 1 H), 5.08 (t, *J* = 5.0 Hz, 1 H), 5.18 (d, *J* = 4.3 Hz, 1 H), 6.05 (t, *J* = 6.4 Hz, 1 H), 6.94 (s, 1 H), 7.80 (s, 1 H), 8.25–8.21 (m, 1 H).

^{1}H NMR (400 MHz, DMSO-d_6, 80 °C): δ = 2.01 (dd, *J* = 6.6, 13.3 Hz, 1 H), 2.16 (ddd, *J* = 3.8, 6.1, 13.3 Hz, 1 H), 3.55 (ddd, *J* = 3.8, 5.0, 11.8 Hz, 1 H), 3.62 (ddd, *J* = 3.5, 5.0, 11.8 Hz, 1 H), 3.79 (q, *J* = 3.6 Hz, 1 H), 4.21 (td, *J* = 3.9, 7.8 Hz, 1 H), 4.81 (t, *J* = 6.5 Hz, 1 H), 4.95 (d, *J* = 4.4 Hz, 1 H), 7.07 (s, 2 H), 8.17 (s, 1 H).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 40.79, 48.60, 60.82, 69.91, 85.40, 86.18, 87.40, 141.93, 153.64, 161.82.

LC-MS (ESI-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_9H_{12}BrN_3O_4$ + Na: 327.99089; found: 327.98891.

5-Bromocytidine (7b)[36]
White solid; mp 182–185 °C [Lit.[36] mp 183–185 °C (dec.)].

^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): δ = 3.54 (ddd, *J* = 2.6, 4.8, 12.1 Hz, 1 H), 3.67 (ddd, *J* = 2.8, 4.8, 12.1 Hz, 1 H), 3.85–3.78 (m, 1 H), 3.98–3.88 (m, 1 H), 4.98 (d, *J* = 5.4 Hz, 1 H), 5.20 (t, *J* = 4.8 Hz, 1 H), 5.34 (d, *J* = 5.0 Hz, 1 H), 5.68 (d, *J* = 3.4 Hz, 1 H), 6.96 (s, 1 H), 7.79 (s, 1 H), 8.36 (s, 1 H).

^{1}H NMR (400 MHz, DMSO-d_6, 80 °C): δ = 3.57 (ddd, *J* = 3.2, 4.6, 12.1 Hz, 1 H), 3.69 (ddd, *J* = 3.1, 4.4, 12.0 Hz, 1 H), 3.89–3.82 (m, 1 H), 3.98 (s, 1 H), 4.69 (d, *J* = 2.8 Hz, 1 H), 4.91 (t, *J* = 4.6 Hz, 1 H), 5.02 (d, *J* = 1.9 Hz, 1 H), 5.72 (d, *J* = 3.5 Hz, 1 H), 7.10 (s, 2 H), 8.25 (s, 1 H).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 60.52, 69.45, 74.98, 84.70, 86.88, 90.11, 142.92, 154.56, 162.49.

LC-MS (ESI-TOF): *m*/*z* [M + H]$^+$ calcd for $C_9H_{13}BrN_3O_5$: 322.003859; found: 322.00262.

5-Iodo-2′-deoxycytidine (8a)[37]
White solid; mp 185–188 °C.

^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): δ = 2.02–1.91 (m, 1 H), 2.10 (ddd, *J* = 3.8, 6.1, 13.2 Hz, 1 H), 3.51 (ddd, *J* = 3.5, 4.9, 11.8 Hz, 1 H), 3.59 (ddd, *J* = 3.4, 5.0, 11.8 Hz, 1 H), 3.75 (q, *J* = 3.4 Hz, 1 H), 4.20–4.14 (m, 1 H), 5.07 (t, *J* = 5.0 Hz, 1 H), 5.17 (d, *J* = 4.3 Hz, 1 H), 6.04 (t, *J* = 6.4 Hz, 1 H), 6.56 (s, 1 H), 7.76 (s, 1 H), 8.25 (s, 1 H).

^{1}H NMR (400 MHz, DMSO-d_6, 80 °C): δ = 1.99 (dt, *J* = 6.5, 13.2 Hz, 1 H), 2.20–2.11 (m, 1 H), 3.58–3.50 (m, 1 H), 3.61 (ddd, *J* = 3.7, 4.7, 11.7 Hz, 1 H), 3.78 (q, *J* = 3.6 Hz, 1 H), 4.20 (td, *J* = 3.8, 7.6 Hz, 1 H), 4.80 (t, *J* = 5.0 Hz, 1 H), 4.94 (d, *J* = 4.4 Hz, 1 H), 6.05 (t, *J* = 6.5 Hz, 1 H), 6.88 (s, 2 H), 8.21 (s, 1 H).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 41.48, 57.20, 61.49, 70.58, 85.91, 147.93, 154.56, 164.35.

LC-MS (ESI-TOF): *m/z* [M + H]$^+$ calcd for $C_9H_{13}IN_3O_4$: 353.99507; found: 353.99299.

5-Iodocytidine (8b)[38]

Highly viscous transparent material, which turned white semi-solid with time.

^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): δ = 3.53 (ddd, *J* = 2.7, 4.9, 12.0 Hz, 1,H), 3.67 (ddd, *J* = 2.8, 4.8, 12.0 Hz, 1,H), 3.84–3.78 (m, 1,H), 3.96–3.87 (m, 2 H), 4.96 (d, *J* = 5.4 Hz, 1 H), 5.19 (t, *J* = 4.8 Hz, 1 H), 5.32 (d, *J* = 5.1 Hz, 1 H), 5.68 (d, *J* = 3.5 Hz, 1 H), 6.58 (s, 1 H), 7.76 (s, 1 H), 8.38 (s, 1 H).

^{1}H NMR (400 MHz, DMSO-d_6, 80 °C): δ = 3.61–3.52 (m, 1 H), 3.73–3.64 (m, 1 H), 3.88–3.82 (m, 1 H), 3.97 (s, 2 H), 4.67 (d, *J* = 2.4 Hz, 1 H), 4.89 (t, *J* = 4.4 Hz, 1 H), 5.01 (d, *J* = 2.2 Hz, 1 H), 5.71 (d, *J* = 3.5 Hz, 1 H), 6.88 (s, 2 H), 8.28 (s, 1 H).

^{13}C NMR (101 MHz, DMSO-d_6): δ = 57.21, 60.53, 69.48, 74.96, 84.67, 90.04, 148.28, 154.79, 164.35.

LC-MS (ESI-TOF): *m/z* [M + Na]$^+$ calcd for $C_9H_{13}IN_3O_5$ + Na: 369.98999; found: 369.98867.

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