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Preparation of C5-substituted O^6,5′-cyclouridine

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ABSTRACT

The synthesis of *hitherto unknown* C5-substituted O^6,5′-cyclouridines is described. The 2′,3′-isopropylidene-uridine was treated with *N*-halogenosuccinimides forming appropriate bridged 5-halogeno derivatives. Using lithiation method, bromine substituent at C5 position was exchanged into various alkyl and alkenyl derivatives.

1. Introduction

Modified nucleosides and nucleotides exhibit broad spectrum of biological activities.[1–5] Cyclonucleosides, compounds with great structural similarity to classical nucleosides, but bearing additional linkage between the heterocyclic ring and the sugar moiety, are known from early fifties. The first cyclic purine nucleoside was synthesized by Todd et al.[6] in 1951 meanwhile the first pyrimidine cyclonucleoside possessing a O^6,5′-anhydro linkage, the 5,5-diiodo-5,6-dihydro-2′-deoxy-O^6,5′-cyclouridine (**1**), was obtained by Chang et al.[7] in 1963 as a minor product during the investigation of 2′-deoxycytidine iodination. The bridged structure was correctly assigned two years later and further transformations of novel compound leading to new bridged derivatives **2a**,**b** were described.[8] Since then, the synthesis and properties of compounds with O^6,5′-cyclopyrimidines have been reported.[9] Those compounds are mainly use as intermediates for the synthesis of various nucleosides such as the anti-HIV stavudine.[10] More recently, Schinazi et al. reported[11] a novel nucleoside-based *N*-cyclic compound, which exhibited a potent in vitro anti-HCV activity, meanwhile Len et al. reviewed the synthesis of C–C bridge cyclonucleosides.[12] Additionally, multibridged nucleosides, e.g., **3**, possessing O^6,2′- or O^6,3′-bridge together with O^6,5′-bridge were also synthesized.[13]

Thus, based on our on-going research program, we decided to report herein the synthesis of *hitherto unknown* cyclonucleoside derivatives type **4**, bearing a O^6,5′-anhydro bridge for potential antiviral activities (Fig. 1).

2. Results and discussion

Early investigations[10] showed that the O^6,5′-bridge is reversibly formed in basic solutions of 2′,3′-*O*-*iso*popylideneuridine, during the halogenation of nucleosides with *N*-halogenosuccinimides,[14] after treatment of 5-iodonucleosides with alkoxides,[15] or during treatment of protected pyrimidine nucleosides with lead tetraacetate.[16] Sekine et al.[17] observed the formation of O^6,5′-bridge during the deoxygenation of thymidine derivatives, while Hirota et al.[18] obtained the O^6,5′-cyclourididine derivatives during the oxidative coupling of 2′,3′-isopropylideneuridine with methyl acrylate.

Thus, starting from halogenation of the 2′,3′-isopropylideneuridine (**5**), with excess of *N*-halogenosuccinimide,[19] the 5,5-dihalogeno nucleosides **6**–**8** were obtained in moderate yields, respectively (Scheme 1). Compounds **7** and **8** were directly treated with 1 M NaOH to the corresponding 5-halogeno derivatives **10** and **11**, respectively.

In contrast, the dichloro intermediate **6** turns out to be stable in basic conditions, at rt, and cannot be directly transformed into its 5′-monochloroderivative **9**. Dichlorinated analogue **6** has been

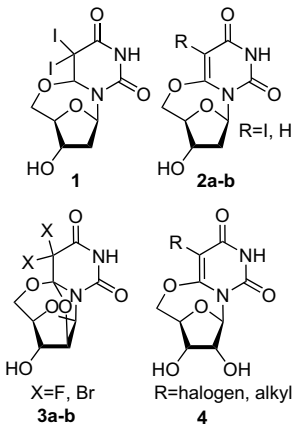

Figure 1.

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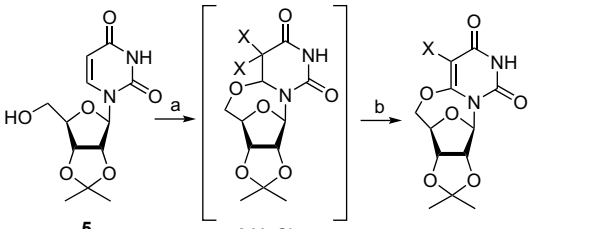


Scheme 1. *Reagents and conditions*: (a) 3 equiv of NXS, DMF, rt, overnight; (b) 1 M NaOH, 30 min, then 1 M HCl to pH=7.

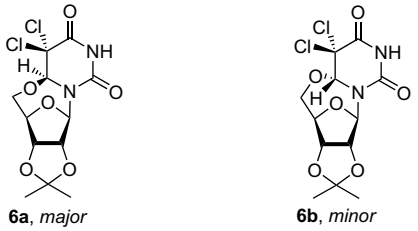


Figure 2.

isolated as a mixture of two diastereoisomers, a major one **6a** and a minor isomer **6b** in a ratio 84:16 (Fig. 2).

These compounds were separated by column chromatography with a total yield of 51%. Interestingly the two compounds crystallized differently out of a $CDCl_3$ solution: compound **6a** yields fine agglomerated needles whereas compound **6b** afforded thin parallelograms. A detailed NMR analysis allowed us to determine the stereochemistry of **6a** and **6b**, respectively. 2D-NOESY spectra (relaxation delay 1 s, mixing time 800 ms) allowed the unequivocally characterization **6a** and **6b**.

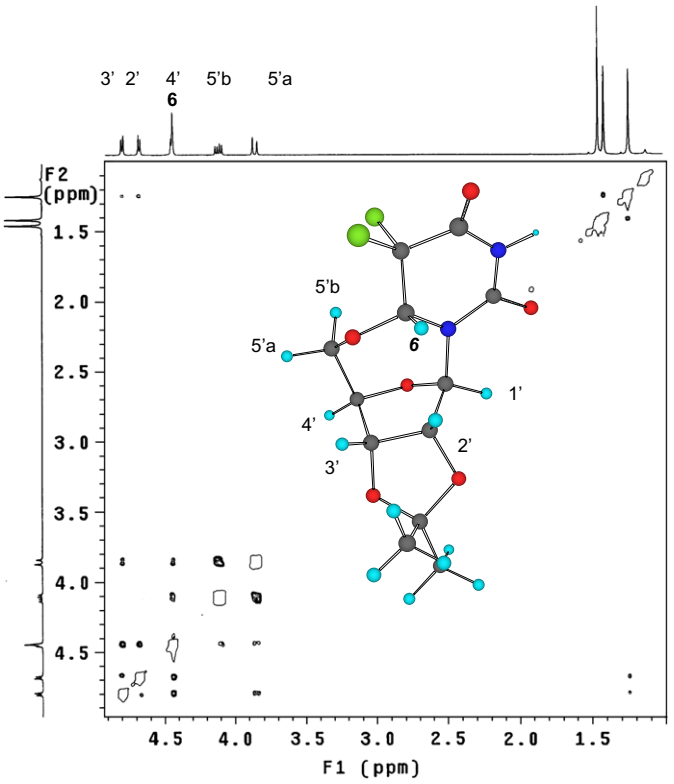


Figure 3. 2D-NOESY of **6b** with 3D view of the molecule.

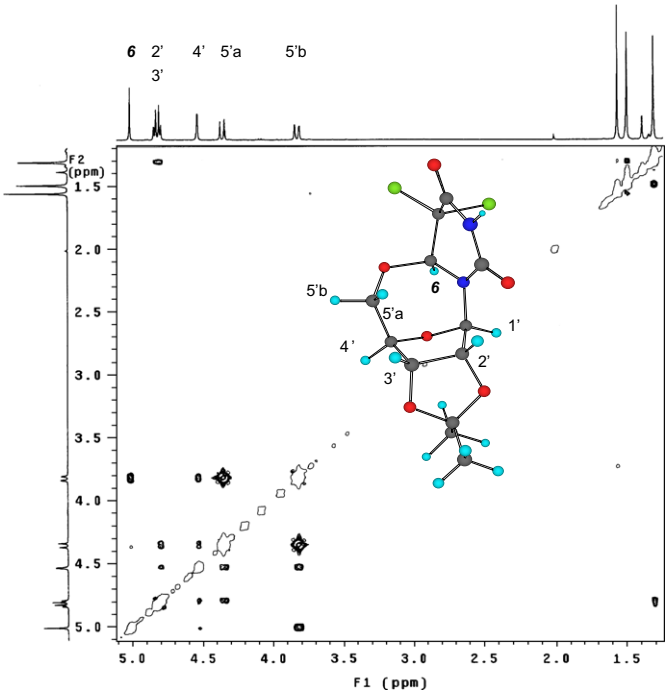


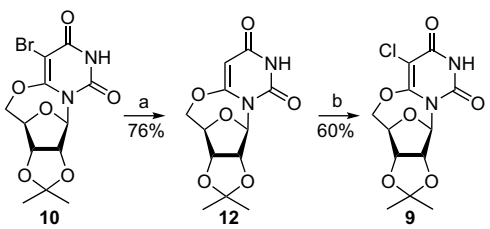
Figure 4. 2D-NOESY of **6a** with 3D view of the molecule.

In the NOESY spectrum of **6b** (Fig. 3) NOE interactions can be clearly seen involving H6 proton (s at 4.44 ppm partly overlapping the signal of H4′) and both H2′ (d at 4.80, 5.6 Hz) and H3′ protons (d at 4.68, 5.6 Hz) as it might be expected if one considers the constrained seven-membered ring involving cyclouridine and nucleoside. Protons H2′ and H3′ also display some weak interactions with one of the methyl groups of the protecting ketal group. In addition, weaker correlations can be also seen between the H4′ proton and the protons of the bridging methylene group (H5′a at 3.86 ppm, d, 12.7 Hz and H5′b at 4.11 ppm, dd, 12.4 and 6 Hz) and between H3′ and H5′a. The latter weak cross peak allows quite precisely to distinguish between H2′ and H3′ and also to individually attribute the signals of protons H5′a and H5′b of the methylene bridge.

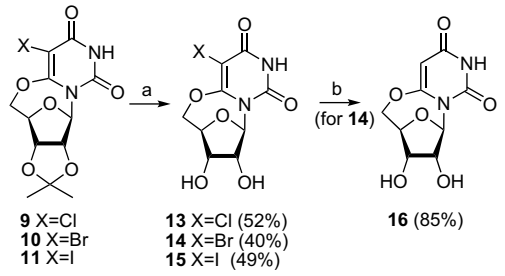
The assignment of ^1H and ^{13}C NMR signals was confirmed based on ^1H, ^{13}C, ^1H–^1H COSY, ^1H, ^{13}C COSY (gHMQC) and ^1H, ^{13}C-long range correlation spectra (gHMBC).

In the NOESY spectrum of **6a** (Fig. 4) the NOE contacts involving H6 and H2′ and H3′ found in the spectrum of **6b** are clearly missing. Proton H6 strongly interacts with one proton (H5′b) of the bridging methylene group at 3.83 ppm (dd, 12.8 and 1.9 Hz) and, at a lesser extend, with protons H4′ (d, 1.8 Hz) and H1′ (s, 6.33 ppm, not displayed on the figure). To supplement this, protons H2′ and H3′ clearly show correlation peaks with proton H5′a and one of the methyl group of the ketal protecting group.

To obtain the desired 5-chloro-2′,3′-isopropylidene-O^6,5′-cyclouridine (**9**), we have firstly hydrogenated the bromoderivative **10** to the 2′,3′-isopropylidene-O^6,5′-cyclouridine (**12**),[8] which by treatment with sulfuryl chloride[20] (or NCS)[13c] gave **9** in 60% yield (Scheme 2).



Scheme 2. *Reagents and conditions*: (a) H_2/Pd/C, THF, pyridine; (b) SO_2Cl_2, CH_2Cl_2, rt, overnight.



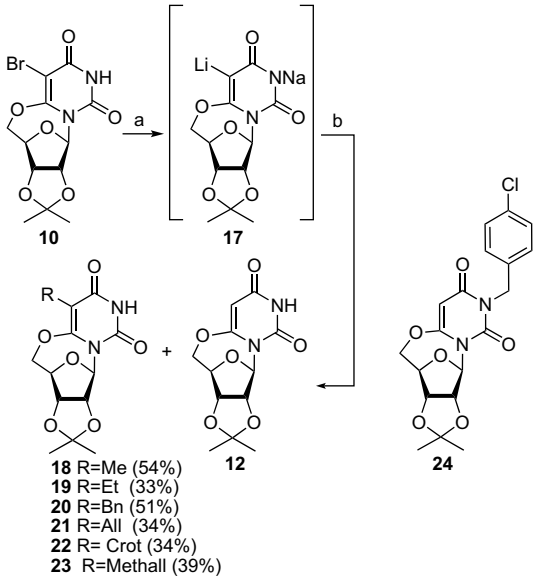
Scheme 3. *Reagents and conditions*: (a) TFA/H_2O 1:1; (b) H_2/Pd/C, TEA, MeOH.

The obtained halogenated 2′,3′-isopropylidene-O^6,5′-cyclouridine derivatives **6**, **9**–**11** were then deprotected with 50% TFA (Scheme 3) to **13**–**16**, respectively, in yields ranging from 40% to 52%. The O^6,5′-cyclouridine (**16**) cannot be obtained from **12** as, under acidic conditions, it is converted to the barbituric nucleoside.[15a,b] Thus, to obtain the O^6,5′-cyclouridine (**16**), the bromoderivative **14** was hydrogenated.[8]

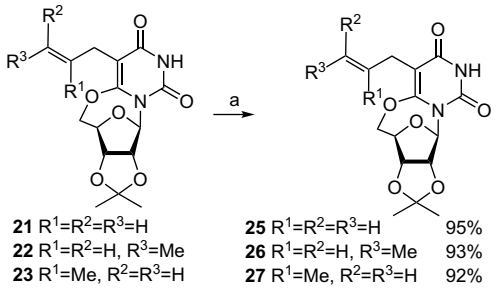
Having halogenated O^6,5′-cyclouridine derivatives, we decided to synthesize some 5-alkyl- and 5-alkenyl-derivatives of O^6,5′-cyclouridine through a lithiation/alkylation step.[21] For this purpose, the 5′-bromoderivative **10** was treated with 1 equiv NaH and 2 equiv of BuLi followed by addition of 5 equiv of appropriate nucleophile (Scheme 4). 5-Substitued derivatives **18**–**23** were obtained with moderate yields together with various amount of dehalogenated nucleoside **12**. When using propyl iodide, no reaction was observed except the dehalogenation of **10** to **12**. When 4-chlorobenzyl bromide was applied, only the *N*-alkylated product **24** was isolated.

The introduction of alkenyl substituent at C5 position is of interest as it can make possible further transformations of the bridged molecule such as addition to the double bound. Because the lithiation method allowed only the synthesis of methyl, ethyl, and benzyl but not the propyl derivative, other 5-alkyl homologues were obtained through the hydrogenation of alkenyl derivatives such as **21**–**23**. Compounds **25**–**27** were then obtained (Scheme 5).

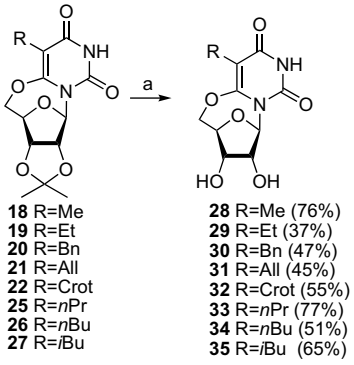
All obtained alkyl and allyl derivatives **18**–**22** and **25**–**27** were successfully deprotected with 10% TFA solution to final products **28**–**35** with moderate to high yield (Scheme 6).



Scheme 4. *Reagents and conditions*: (a) 1 equiv NaH, 10 min, rt, THF then 2 equiv BuLi, −78 °C; (b) 5 equiv nucleophile RI.



Scheme 5. *Reagents and conditions*: (a) Pd/C/H_2, THF.



Scheme 6. *Reagents and conditions*: (a) 10% TFA in H_2O, 1 h, 70 °C.

For all cyclonucleosides, characteristic AB systems were observed by ^{1}H NMR data. For protected compounds, two AB systems appeared, e.g., for C5′ protons and for C2′/C3′ protons. For deprotected compounds, only AB system for C5′ protons still existed, while C2′/C3′ protons usually had identical shifts giving one multiplet.

3. Conclusion and perspectives

Finally, some O^6,5′-cyclouridine derivatives bearing halogeno-, alkyl- and allyl-substituents at C5 position were synthesized. Applied method allowed synthesizing compounds with moderate yield. No activity against HCV was found. Future modifications of 5-halo-O^6,5′-cyclouridines under Pd(0) conditions will be reported elsewhere.

4. Experimental

4.1. General

Commercially available chemicals were used as received. Reactions were monitored by thin-layer chromatography (TLC) analysis using silica gel plates (Kieselgel 60 F_{254}). Compounds were visualized by UV irradiation and/or spraying with ethanol solution (2.5%) in phosphomolybdic acid, followed by charring at 150 °C. Column chromatography was performed on silica gel 60 M (0.040–0.063 mm).

The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE DPX 250 (^{1}H: 249.88 MHz, ^{13}C: 62.84 MHz) and Varian Inova Unity 400 spectrometer (^{1}H 399.91 MHz, ^{13}C: 100.54 MHz) in DMSO-d_6 or mixture of $CDCl_3$/DMSO-d_6. The NMR spectra were recorded at rt. The chemical shifts are given in parts per million relative to the residual signal of the solvent, TMS being used as calibration standard with different deuterated solvents.

Signals are reported as s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), q_i (quintuplet), and m (multiplet) with the relevant coupling constants *J* in hertz. For protected compounds to differentiate signals from aliphatic chain and isopropylidene group: *i* signals derived from isopropylidene group, *c* signals from aliphatic chain in C5 position. Polarity index was measured on Perkin–Elmer Model 341 polarimeter. Evidence of purity has been done from a proton-decoupled ^{13}C NMR spectrum with a signal-to-noise ratio sufficient to permit seeing peak with 5% of the intensity of the strongest peak.

4.2. Procedure for synthesis of dichloroderivative (6a, 6b)

5 g of 2′,3′-isopropylideneuridine was dissolved in 150 ml of dry DMF, then 3 equiv of NCS was added. Reaction mixture was stirred overnight at rt then the solvent was evaporated and the residue purified by liquid column chromatography on silica gel yielding 2.70 g of **6a** and 0.53 g of **6b**, respectively.

*4.2.1. 5,5-Dichloro-5,6-dihydro-2′,3′-isopropylidene-O^6,5′-clouridine (**6a**, major)*

$[\alpha]_D$ −61 (*c* 1.0, CH_2Cl_2). ^1H NMR (400 MHz, DMSO-d_6): 1.27 (s, 3H, CH_{3i}), 1.42 (s, 3H, CH_{3i}), 3.79 (dd, 1H, J_1=1.6, 12.6 Hz, H5′), 4.40 (d, 1H, *J*=12.6 Hz, H5′), 4.54 (s, 1H, H4′), 4.68 (d, 1H, *J*=5.8 Hz, H3′), 4.89 (d, 1H, *J*=5.8 Hz, H2′), 5.62 (s, 1H, H6), 6.11 (1H, s, H1′), 11.53 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 24.4 (CH_{3i}), 26.2 (CH_{3i}), 76.1 (C5′), 81.2 (C5), 82.1 (C2′), 84.6 (C4′), 86.0 (C3′), 89.3 (C6), 90.5 (C1′), 111.5 ($C^{IV}{}_i$), 148.8 (C2), 160.8 (C4); HRMS (ESI): *m*/*z* calcd for $C_{12}H_{14}Cl_2N_2O_6Na$ (M^++Na): 376.1475, found: 376.1472.

*4.2.2. 5,5-Dichloro-5,6-dihydro-2′,3′-isopropylidene-O^6,5′-clouridine (**6b**, minor)*

$[\alpha]_D$ +29 (*c* 1.0, DMF). ^1H NMR (400 MHz, DMSO-d_6): 1.20 (s, 3H, CH_{3i}), 1.42 (s, 3H, CH_{3i}), 3.94 (d, 1H, J_2=12.4 Hz, H5′), 4.06 (dd, 1H, J_1=6.2, 12.4 Hz, H5′), 4.47 (d, 1H, H4′), 4.83 (d, 1H, *J*=5.7 Hz, H2′), 5.20 (d, 1H, *J*=5.7 Hz, H3′), 5.22 (s, 1H, H6), 5.96 (1H, s, H1′), 11.81 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 24.1 (CH_{3i}), 25.6 (CH_{3i}), 70.0 (C5′), 77.7 (C5), 82.4 (C2′), 84.0 (C4′), 85.7, 89.9 (C3′, C6), 89.2 (C1′), 111.3 ($C^{IV}{}_i$), 151.0 (C2), 160.9 (C4); HRMS (ESI): *m*/*z* calcd for $C_{12}H_{14}Cl_2N_2O_6Na$ (M^++Na): 376.1475, found: 376.1474.

4.3. General procedure for bromination or iodination of uridine

1 equiv of 2′,3′-isopropylideneuridine (**5**) was dissolved in DMF (50 mL), then 3 equiv of appropriate *N*-halogenosuccinimide (either NBS or NIS) was added. The reaction mixture was stirred overnight at rt, then the solvents were co-evaporated with toluene (3×20 mL). The 5,5-dihaloderivatives (**7** or **8**) were purified by precipitation with water. NMR showed that further purification is not required. The 5,5-dibromoderivative **7** and the 5,5-diiododerivative **8** were not isolated, but directly treated with large excess of 1 M NaOH. The solution was neutralized with 1 M HCl and the 5-bromo derivative **10** and 5-iododerivative **11** precipitated, respectively. After filtration, compounds were dried under reduced pressure and used in the next steps without any further purification.

*4.3.1. 5-Bromo-2′,3′-isopropylidene-O^6,5′-clouridine (**10**)*

$[\alpha]_D$ +80 (*c* 1.0, CH_2Cl_2). ^1H NMR (250 MHz, DMSO-d_6): 1.28 (s, 3H, CH_{3i}), 1.42 (s, 3H, CH_{3i}), 4.12 (d, 1H, *J*=12.3 Hz, H5′), 4.64 (s, 1H, H4′), 4.71 (d, 1H, *J*=12.3 Hz, H5′), 4.94 (d, 1H, *J*=5.6 Hz, H3′), 5.00 (d, 1H, *J*=5.6 Hz, H2′), 6.26 (1H, s, H1′), 11.85 (s, 1H, NH); ^{13}C NMR (62 MHz, DMSO-d_6): 24.3 (CH_{3i}), 25.9 (CH_{3i}), 77.3 (C5′), 81.6 (C2′), 83.5 (C4′), 84.3 (C3′), 85.6 (C5), 89.7 (C1′), 111.5 ($C^{IV}{}_i$), 148.7 (C2), 157.9 (C6), 159.5 (C4); HRMS (ESI): *m*/*z* calcd for $C_{12}H_{13}BrN_2O_6Na$ (M^++Na): 384.1382, found: 384.1379.

*4.3.2. 5-Iodo-2′,3′-isopropylidene-O^6,5′-cyclouridine (**11**)*

$[\alpha]_D$ +72 (*c* 1.0, CH_2Cl_2). ^1H NMR (250 MHz, DMSO-d_6): 1.29 (s, 3H, CH_{3i}), 1.42 (s, 3H, CH_{3i}), 4.02 (d, 1H, *J*=12.2 Hz, H5′), 4.51–4.53 (m, 2H, H4′, H5′), 4.91 (d, 1H, *J*=5.3 Hz, H3′), 5.00 (d, 1H, *J*=5.3 Hz, H2′), 6.25 (1H, s, H1′), 11.71 (s, 1H, NH); ^{13}C NMR (62 MHz, DMSO-d_6): 24.6 (CH_{3i}), 25.9 (CH_{3i}), 60.9 (C5), 76.8 (C5′), 81.7 (C2′), 83.4 (C4′), 84.3 (C3′), 89.4 (C1′), 111.5 ($C^{IV}{}_i$), 149.4 (C2), 160.0 (C6), 161.1 (C4); HRMS (ESI): *m*/*z* calcd for $C_{12}H_{13}IN_2O_6Na$ (M^++Na): 431.1387, found: 431.1384.

*4.3.3. 5-Chloro-2′,3′-isopropylidene-O^6,5′-cyclouridine (**9**)*

3 g of 5-bromo-O^6,5′-cyclo-2′,3′-*O*-isopropylidene uridine (**10**) (8.32 mmol, 1 equiv) was dissolved in 50 ml of THF, then 3.47 ml (24.96 mmol, 3 equiv) of TEA was added followed by 100 mg of of Pd/C. Hydrogen was connected and reaction mixture was stirred overnight at rt. Solvent were evaporated and the residue purified by liquid chromatography on silica gel (petroleum ether/EtOAc, 4:6) to give 1.78 g of **12** as a white solid.

*Physico-chemical data of **12***: $[\alpha]_D$ +77 (*c* 1.0, CH_2Cl_2). ^1H NMR (400 MHz, DMSO-d_6): 1.28 (s, 3H, CH_{3i}), 1.42 (s, 3H, CH_{3i}), 3.98 (dd, 1H, J_1=0.9 Hz, J_2=12.8 Hz, H5′), 4.56–4.63 (m, 2H, H4′, H5′), 4.89 (d, H, *J*=5.6 Hz, H3′), 4.95 (d, H, *J*=5.6 Hz, H2′), 5.27 (1H, s, H5), 6.25 (1H, s, H1′), 11.39 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 24.3 (CH_{3i}), 25.9 (CH_{3i}), 77.2 (C5′), 81.6 (C2′), 83.3 (C4′), 84.4 (C3′), 88.8 (C1′), 89.7 (C5), 111.5 ($C^{IV}{}_i$), 149.9 (C2), 161.0 (C6), 163.1 (C4); HRMS (ESI): *m*/*z* calcd for $C_{12}H_{14}N_2O_6Na$ (M^++Na): 305.2421, found: 305.2418.

1.7 g (6.0 mmol, 1 equiv) of **12** was dissolved in CH_2Cl_2, then 0.54 (6.6 mmol, 1.1 equiv) of sulfuryl chloride was added. The reaction mixture was stirred overnight in rt. Next day solvent was evaporated and product was purified by chromatography using 1% MeOH in CH_2Cl_2 to yield 1.13 g of **9** (60%).

*Physico-chemical data of **9***: $[\alpha]_D$ +80 (*c* 1.0, CH_2Cl_2). ^1H NMR (250 MHz, DMSO-d_6): 1.29 (s, 3H, CH_{3i}), 1.42 (s, 3H, CH_{3i}), 4.16 (d, 1H, *J*=12.3 Hz, H5′), 4.64 (s, 1H, H4′), 4.73 (d, 1H, *J*=12.3 Hz, H5′), 4.94 (d, 1H, *J*=5.6 Hz, H3′), 5.00 (d, 1H, *J*=5.6 Hz, H2′), 6.25 (1H, s, H1′), 11.88 (s, 1H, NH); ^{13}C NMR (62 MHz, DMSO-d_6): 24.3 (CH_{3i}), 25.9 (CH_{3i}), 77.6 (C5′), 81.6 (C2′), 83.5 (C4′), 84.3 (C3′), 89.7 (C1′), 96.2 (C5), 111.5 ($C^{IV}{}_i$), 148.3 (C2), 156.9 (C6), 159.3 (C4); HRMS (ESI): *m*/*z* calcd for $C_{12}H_{13}ClN_2O_6Na$ (M^++Na): 339.6869, found: 339.6867.

4.4. General procedure for deprotection of halogenated derivatives (6, 9–11)

Isopropylidene derivatives were, respectively, treated with 1 M HCl (for **6**) or with 50% TFA in H_2O (for **9**–**11**). The reaction mixture was stirred at rt, then solvent was evaporated. Because of poor solubility of final products, the residues were washed several times with methanol, dried under the vacuum pump. Products **13**–**16** were obtained in a pure form, respectively.

*4.4.1. 5-Chloro-O^6,5′-clouridine (**13**)*

$[\alpha]_D$ +101 (*c* 1.0, DMF). ^1H NMR (250 MHz, DMSO-d_6): 4.08 (d, 1H, *J*=12.4 Hz, H5′), 4.28–4.38 (m, 2H, H2′, H3′), 4.41 (s, 1H, H4′), 4.69 (d, 1H, *J*=12.4 Hz, H5′), 4.93 (br s, 2′OH, 3′OH), 6.18 (s, 1H, H1′), 11.84 (s, 1H, NH); ^{13}C NMR (62 MHz, DMSO-d_6): 71.9 (C3′), 76.4 (C2′), 77.7 (C5′), 86.5 (C4′), 92.3 (C1′), 96.3 (C5), 148.2 (C2), 157.3 (C6), 159.3 (C4); HRMS (ESI): *m*/*z* calcd for $C_9H_9ClN_2O_6Na$ (M^++Na): 299.6223, found: 299.6219.

*4.4.2. 5-Bromo-O^6,5′-clouridine (**14**)*

$[\alpha]_D$ +93 (*c* 1.0, DMF). ^1H NMR (400 MHz, DMSO-d_6): 4.05 (dd, 1H, J_1=0.8, 12.4 Hz, H5′), 4.24–4.38 (m, 2H, H2′, H3′), 4.41 (s, 1H,

H4′), 4.67 (dd, 1H, J_1=0.9, 12.4 Hz, H5′), 5.24 (d, 1H, J_1=4.1 Hz, 3′OH), 5.44 (d, 1H, J_1=6.8 Hz, 2′OH), 6.19 (s, 1H, H1′), 11.80 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 71.9 (C3′), 76.4 (C2′), 77.7 (C5′), 86.5 (C4′), 85.7 (C5), 92.3 (C1′), 148.8 (C2), 158.3 (C6), 159.5 (C4); HRMS (ESI): m/z calcd for $C_9H_9BrN_2O_6Na$ (M^++Na): 344.0736, found: 344.0735.

*4.4.3. 5-Iodo-O^6,5′-cyclouridine (**15**)*

$[\alpha]_D$ +53 (c 1.0, DMF). ^{1}H NMR (250 MHz, DMSO-d_6): 3.94 (d, H, J=12.3 Hz, H5′), 4.32 (s, 2H, H2′, H3′), 4.62 (d, H, J=12.3 Hz, H5′), 4.41 (s, 1H, H4′), 6.18 (s, 1H, H1′), 11.68 (s, 1H, NH); ^{13}C NMR (62 MHz, DMSO-d_6): 61.9 (C5), 73.0 (C3′), 77.4 (C2′), 77.9 (C5′), 87.4 (C4′), 93.0 (C1′), 150.4 (C2), 161.4, 161.9 (C6, C4); HRMS (ESI): m/z calcd for $C_9H_9IN_2O_6Na$ (M^++Na): 391.0741, found: 391.0738.

4.5. Synthesis of O^6,5′-cyclouridine (16)

2 g of 5-bromo-O^6,5′-cyclouridine (**14**) was dispersed in 40 ml mixture of MeOH/TEA 1:1, then Pd/C was added and hydrogen was connected through septum. Reaction mixture was stirred overnight at rt. After filtration, solvents were removed, and the residue was washed again on funnel with DMF. Solution after washing with DMF was collected, evaporated, and co-evaporated with toluene. Obtained O^6,5′-cyclouridine (**16**) did not require further purification. Yield: 1.28 g (85%). $[\alpha]_D$ +84 (c 1.0, DMF). ^{1}H NMR (400 MHz, DMSO-d_6): 3.93 (dd, 1H, J_1=0.8, 12.6 Hz, H5′), 4.23–4.34 (m, 2H, H2′, H3′), 4.40 (s, 1H, H4′), 4.54 (dd, 1H, J_1=1.2 Hz, J_2=12.6 Hz, H5′), 5.19 (br s, 3′OH), 5.26 (s, 1H, H5), 5.45 (d, 1H, J=6.1 Hz, 2′OH), 6.17 (s, 1H, H1′), 11.34 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 72.0 (C3′), 76.6 (C2′), 77.5 (C5′), 86.3 (C4′), 89.8 (C5), 91.3 (C1′), 150.0 (C2), 161.5 (C6), 163.1 (C4); HRMS (ESI): m/z calcd for $C_9H_{10}N_2O_6Na$ (M^++Na): 265.1775, found: 265.1773.

4.6. General procedure of nucleophilic addition of alkyl and allyl halides

1 equiv of 5-bromo-2′,3′-isopropylidene-O^6,5′-cyclouridine (**10**) was dissolved in dry THF and 1 equiv of sodium hydride was added followed by 3 equiv of HMPA (in case of alkenyl derivatives). The reaction mixture was cooled down under nitrogen to −78 °C, then 2 equiv of n-BuLi was added. After stirring for a 30 min, 5 equiv of appropriate nucleophile was added. The reaction mixture was stirred at −78 °C overnight, then warmed up to rt. Satd NH_4Cl was added, organic phase was separated, and aqueous phase was extracted three times with CH_2Cl_2. Organic phases were evaporated and the residue was purified by liquid chromatography on silica gel (petroleum ether/EtOAc, 7:3 then 6:4).

*4.6.1. 5-Methyl-2′,3′-isopropylidene-O^6,5′-cyclouridine (**18**)*

$[\alpha]_D$ +74 (c 1.0, CH_2Cl_2). ^{1}H NMR (250 MHz, $CDCl_3$): 1.36 (s, 3H, CH_{3i}), 1.54 (s, 3H, CH_{3i}), 1.85 (s, 3H, CH_{3c}), 3.94 (dd, 1H, J_1=0.9 Hz, J_2=12.4 Hz, H5′), 4.51 (dd, 1H, J_1=1.3 Hz, J_2=12.4 Hz, H5′), 4.60 (s, 1H, H4′), 4.84 (d, 1H, J=5.6 Hz, H3′), 4.94 (d, 1H, J=5.6 Hz, H2′), 6.59 (1H, s, H1′), 9.24 (s, 1H, NH); ^{13}C NMR (62 MHz, $CDCl_3$): 7.6 (CH_{3c}), 24.6 (CH_{3i}), 26.2 (CH_{3i}), 76.7 (C5′), 82.4 (C2′), 84.0 (C4′), 85.6 (C3′), 89.9 (C1′), 98.9 (C5), 113.0 (C^{IV}_i), 148.9 (C2), 156.9 (C6), 164.1 (C4); HRMS (ESI): m/z calcd for $C_{12}H_{16}N_2O_6Na$ (M^++Na): 307.2579, found: 307.2577.

*4.6.2. 5-Ethyl-2′,3′-isopropylidene-O^6,5′-cyclouridine (**19**)*

$[\alpha]_D$ +71 (c 1.0, CH_2Cl_2). ^{1}H NMR (250 MHz, $CDCl_3$): 1.04 (t, 3H, J=7.4 Hz, CH_{3c}), 1.37 (s, 3H, CH_{3i}), 1.54 (s, 3H, CH_{3i}), 2.35 (dq, 2H, J_1=2.8 Hz, J_2=7.5 Hz, CH_{2c}), 3.93 (dd, 1H, J_1=0.7 Hz, J_2=12.4 Hz, H5′), 4.53 (dd, 1H, J_1=1.2 Hz, J_2=12.4 Hz, H5′), 4.60 (s, 1H, H4′), 4.84 (d, 1H, J=5.6 Hz, H3′), 4.95 (d, 1H, J=5.6 Hz, H2′), 6.60 (1H, s, H1′), 9.24 (s, 1H, NH); ^{13}C NMR (62 MHz, $CDCl_3$): 13.9 (CH_{3c}), 16.1 (CH_{2c}), 24.6 (CH_{3i}), 26.2 (CH_{3i}), 77.1 (C5′), 82.4 (C2′), 84.0 (C4′), 85.6 (C3′), 89.9 (C1′), 104.9 (C5), 113.0 (C^{IV}_i), 149.0 (C2), 157.0 (C6), 163.6 (C4); HRMS (ESI): m/z calcd for $C_{14}H_{18}N_2O_6Na$ (M^++Na): 333.2957, found: 333.2956.

*4.6.3. 5-Benzyl-2′,3′-isopropylidene-O^6,5′-cyclouridine (**20**)*

$[\alpha]_D$ +115 (c 1.0, CH_2Cl_2). ^{1}H NMR (250 MHz, $CDCl_3$): 1.32 (s, 3H, CH_{3i}), 1.50 (s, 3H, CH_{3i}), 3.48–3.52 (m, 2H, CH_{2c}), 3.93 (d, 1H, J=14.5 Hz, H5′), 4.27 (dd, 1H, J_1=1.1 Hz, J_2=12.5 Hz, H5′), 4.60 (s, 1H, H4′), 4.90 (d, 1H, J=5.6 Hz, H3′), 4.91 (d, 1H, J=5.6 Hz, H2′), 6.58 (1H, s, H1′), 7.13–7.31 (m, 5H, 5CH_{Ar}), 8.96 (s, 1H, NH); ^{13}C NMR (62 MHz, $CDCl_3$): 24.7 (CH_{3i}), 26.2 (CH_{3i}), 28.6 (CH_{2c}), 76.9 (C5′), 82.4 (C2′), 84.0 (C4′), 85.6 (C3′), 89.9 (C1′), 103.3 (C5), 113.1 (C^{IV}_i), 126.3 (CH_{Ar}), 128.3 (CH_{Ar}), 128.5 (CH_{Ar}), 140.0 (C^{IV}_{Ar}), 148.8 (C2), 157.9 (C6), 163.4 (C4); HRMS (ESI): m/z calcd for $C_{19}H_{20}N_2O_6Na$ (M^++Na): 395.3665, found: 395.3662.

*4.6.4. 5-Allyl-2′,3′-isopropylidene-O^6,5′-cyclouridine (**21**)*

$[\alpha]_D$ +96 (c 1.0, CH_2Cl_2). ^{1}H NMR (250 MHz, $CDCl_3$): 1.36 (s, 3H, CH_{3i}), 1.54 (s, 3H, CH_{3i}), 2.97–3.21 (m, 2H, CH_{2c}), 3.93 (dd, 1H, J_1=0.7 Hz, J_2=12.4 Hz, H5′), 4.47 (dd, 1H, J_1=1.2 Hz, J_2=12.5 Hz, H5′), 4.59 (s, 1H, H4′), 4.84 (d, 1H, J=5.6 Hz, H2′), 4.94 (d, 1H, J=5.6 Hz, H2′), 4.96–5.11 (2H, m, CH_2=), 5.72–9.94 (1H, m, CH=), 6.60 (1H, s, H1′), 9.44 (s, 1H, NH); ^{13}C NMR (62 MHz, $CDCl_3$): 24.6 (CH_{3i}), 26.2 (CH_{3i}), 28.6 (CH_{2c}), 77.1 (C5′), 82.4 (C2′), 84.0 (C4′), 85.5 (C3′), 89.9 (C1′), 101.3 (C5), 113.0 (C^{IV}_i), 115.3 (CH_2=), 135.3 (CH=), 149.0 (C2), 157.8 (C6), 163.4 (C4); HRMS (ESI): m/z calcd for $C_{15}H_{18}N_2O_6Na$ (M^++Na): 345.3067, found: 345.3065.

*4.6.5. 5-Crotyl-2′,3′-isopropylidene-O^6,5′-cyclouridine (**22**) (as a E/Z mixture)*

^{1}H NMR (400 MHz, $CDCl_3$): 1.36 (s, 3H, CH_{3i}), 1.54 (s, 3H, CH_{3i}), 1.63 (d, J=5.3 Hz, CH_{3c}), 1.71 (d, J=6.8 Hz, CH_{3c}), 2.93–3.16 (m, 2H, CH_{2c}), 3.88–3.97 (m, 1H, H5′), 4.47 (dd, 1H, J_1=0.5 Hz, J_2=12.4 Hz, H5′), 4.58 (s, 1H, H4′), 4.84 (d, H, J=5.6 Hz, H3′), 4.94 (d, H, J=5.6 Hz, H2′), 5.28–5.53 (m, 2H, CH=), 6.59 (1H, s, H1′), 9.30, 9.35 (2×s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): 12.7, 17.7 (CH_{3c}), 20.3, 25.5 (CH_{2c}), 24.6, 26.2 (CH_{3i}), 77.0, 77.1 (C5′), 82.4 (C2′), 84.0 (C4′), 85.5 (C3′), 89.9 (C1′), 102.3, 102.7 (C5), 113.1 (C^{IV}_i), 124.9, 126.1, 127.0, 127.6 (C^{III}=), 149.0 (C2), 157.4 (C6), 163.4, 163.5 (C4); HRMS (ESI): m/z calcd for $C_{16}H_{20}N_2O_6Na$ (M^++Na): 359.3335, found: 359.3330.

*4.6.6. 5-Methallyl-2′,3′-isopropylidene-O^6,5′-cyclouridine (**23**)*

$[\alpha]_D$ +85 (c 1.0, CH_2Cl_2). ^{1}H NMR (250 MHz, $CDCl_3$): 1.35 (s, 3H CH_{3i}), 1.54 (s, 3H CH_{3i}), 1.75 (s, 3H, CH_{3i}), 2.88–3.14 (m, 2H, CH_{2c}), 3.92 (d, 1H, J=12.5 Hz, H5′), 4.47 (dd, 1H, J_1=0.8 Hz, J_2=12.4 Hz, H5′), 4.60 (d, 2H, J=11.6 Hz, CH_2=), 4.74 (s, 1H, H4′), 4.83 (d, 1H, J=5.6 Hz, H3′), 4.94 (d, 1H, J=5.6 Hz, H2′), 6.60 (1H, s, H1′), 9.61 (s, 1H, NH); ^{13}C NMR (62 MHz, $CDCl_3$): 22.5 (CH_{3c}), 24.6 (CH_{3i}), 26.1 (CH_{3i}), 30.2 (CH_{2c}), 77.0 (C5′), 82.3 (C2′), 84.0 (C4′), 85.5 (C3′), 89.8 (C1′), 101.3 (C5), 110.4 (CH_2=), 113.0 (C^{IV}_i), 143.4 (C^{IV}_c), 149.0 (C2), 157.9 (C6), 163.7 (C4); HRMS (ESI): m/z calcd for $C_{16}H_{20}N_2O_6Na$ (M^++Na): 359.3335, found: 359.3332.

*4.6.7. N^3-(4-Chlorobenzyl)-2′,3′-isopropylidene-O^6,5′-cyclouridine (**24**)*

$[\alpha]_D$ +71 (c 1.0, CH_2Cl_2). ^{1}H NMR (250 MHz, $CDCl_3$): 1.33 (s, 3H, CH_{3i}), 1.52 (s, 3H, CH_{3i}), 3.98 (dd, 1H, J=0.8, 12.5 Hz, H5′), 4.45 (dd, 1H, J_1=1.3, 12.5 Hz, H5′), 4.79 (d, 1H, J=5.6 Hz, H3′), 4.89 (d, 1H, J=5.6 Hz, H2′), 5.03 (d, 1H, J=2.4 Hz, CH_{2c}), 5.45 (1H, s, H5), 6.61 (1H, s, H1′), 7.26 (d, 2H, J=8.5 Hz, CH_{Ar}), 7.43 (d, 2H, J=8.5 Hz, CH_{Ar}); ^{13}C NMR (62 MHz, $CDCl_3$): 24.7 (CH_{3i}), 26.2 (CH_{3i}), 44.1 (CH_{2c}), 77.4 (C5′), 82.2 (C2′), 83.9 (C4′), 85.6 (C3′), 90.5, 90.8 (C1′, C5), 113.1 (C^{IV}_i), 128.4 (CH_{Ar}), 130.8 (CH_{Ar}), 133.7 (C^{IV}_{Ar}), 134.9 (C^{IV}_{Ar}), 150.2 (C2), 159.8 (C6), 162.6 (C4); HRMS (ESI): m/z calcd for $C_{19}H_{19}ClN_2O_6Na$ (M^++Na): 429.8113, found: 429.8110.

4.7. Hydrogenation of alkenyl derivatives 21–23

Alkenyl derivatives dissolved in THF (30 ml), palladium on carbon, and hydrogen were stirred overnight at rt, respectively. Catalyst was filtrated on Celite then solvent were evaporated and the residue was chromatographed using petroleum ether/EtOAc, 7:3.

*4.7.1. 5-Propyl-2′,3′-isopropylidene-O^6,5′-cyclouridine (**25**)*

$[\alpha]_D$ +61 (*c* 1.0, CH_2Cl_2). ^{1}H NMR (250 MHz, $CDCl_3$): 0.91 (t, 3H, *J*=7.4 Hz, CH_{3c}), 1.36 (s, 3H, CH_{3i}), 1.54 (s, 3H, CH_{3i}), 1.45 (q, *J*=7.4 Hz, 2H, CH_2), 2.20–2.40 (m, 2H, CH_{2c}), 3.93 (d, 1H, *J*=12.3 Hz, H5′), 4.53 (dd, 1H, J_1=1.0, 12.4 Hz, H5′), 4.60 (s, 1H, H4′), 4.83 (d, 1H, *J*=5.6 Hz, H3′), 4.95 (d, 1H, *J*=5.6 Hz, H2′), 6.60 (1H, s, H1′), 9.49 (s, 1H, NH); ^{13}C NMR (62 MHz, $CDCl_3$): 13.84 (CH_{3c}), 22.1 (CH_{2c}), 24.5 (CH_{2c}), 24.6 (CH_{3i}), 26.2 (CH_{3i}), 77.0 (C5′), 82.4 (C2′), 83.9 (C4′), 85.5 (C3′), 89.8 (C1′), 103.3 (C5), 113.0 (C^{IV}_i), 149.1 (C2), 157.1 (C6), 163.9 (C4); HRMS (ESI): *m*/*z* calcd for $C_{15}H_{20}N_2O_6Na$ (M^++Na): 347.3225, found: 347.3223.

*4.7.2. 5-n-Butyl-2′,3′-isopropylidene-O^6,5′-cyclouridine (**26**)*

$[\alpha]_D$ +66 (*c* 1.0, CH_2Cl_2). ^{1}H NMR (250 MHz, $CDCl_3$): 0.91 (t, 3H, *J*=7.4 Hz, CH_{3c}), 1.21–1.48 (m, 7H, CH_{3i}, $2CH_{2c}$), 1.54 (s, 3H, CH_{3i}), 2.15–2.43 (m, 2H, CH_{2c}), 3.93 (d, 1H, J_2=12.3 Hz, H5′), 4.51 (dd, 1H, J_1=1.1 Hz, J_2=12.4 Hz, H5′), 4.59 (s, 1H, H4′), 4.83 (d, 1H, *J*=5.6 Hz, H3′), 4.94 (d, 1H, *J*=5.6 Hz, H2′), 6.60 (1H, s, H1′), 8.97 (s, 1H, NH); ^{13}C NMR (62 MHz, $CDCl_3$): 13.9 (CH_{3c}), 22.4 (CH_{2c}), 22.5 (CH_{2c}), 24.7 (CH_{3i}), 26.2 (CH_{3i}), 31.2 (CH_{2c}), 77.1 (C5′), 82.4 (C2′), 84.0 (C4′), 85.6 (C3′), 89.9 (C1′), 103.6 (C5), 113.1 (C^{IV}_i), 148.9 (C2), 157.1 (C6), 163.6 (C4); HRMS (ESI): *m*/*z* calcd for $C_{16}H_{22}N_2O_6Na$ (M^++Na): 361.3493, found: 361.3488.

*4.7.3. 5-iso-Butyl-2′,3′-isopropylidene-O^6,5′-cyclouridine (**27**)*

^{1}H NMR (250 MHz, $CDCl_3$): 0.87 (d, 3H, *J*=2.8 Hz, CH_{3c}), 0.90 (d, 3H, *J*=2.8 Hz, CH_{3c}), 1.36 (s, 3H, CH_{3i}), 1.54 (s, 3H, CH_{3i}), 1.72–1.88 (m, 1H, CH_c), 2.15–2.26 (m, 2H, CH_{2c}), 3.93 (d, 1H, *J*=12.4 Hz, H5′), 4.49 (dd, 1H, J_1=1.1, 12.4 Hz, H5′), 4.59 (s, 1H, H4′), 4.81 (d, 1H, *J*=5.6 Hz, H3′), 4.94 (d, 1H, *J*=5.6 Hz, H2′), 6.60 (1H, s, H1′), 9.31 (s, 1H, NH); ^{13}C NMR (62 MHz, $CDCl_3$): 22.2 (CH_{3c}), 22.6 (CH_{3c}), 24.7 (CH_{3i}), 26.2 (CH_{3i}), 27.5 (CH_c), 31.5 (CH_{2c}), 77.0 (C5′), 82.3 (C2′), 83.9 (C4′), 85.6 (C3′), 89.8 (C1′), 102.4 (C5), 113.0 (C^{IV}_i), 149.1 (C2), 157.3 (C6), 163.9 (C4); HRMS (ESI): *m*/*z* calcd for $C_{16}H_{22}N_2O_6Na$ (M^++Na): 361.3493, found: 361.3489.

4.8. General deprotection of 5-alkyl and allyl derivatives

Isopropylidene derivatives were treated with 10% TFA in H_2O for 1 h in 70 °C. Solvent was evaporated and co-evaporated with toluene. The residue was dissolved was purified by liquid chromatography on silica gel (petroleum/EtOAc, from 5:5 to 7:3).

*4.8.1. 5-Methyl-O^6,5′-cyclouridine (**28**)*

$[\alpha]_D$ +70 (*c* 1.0, DMF). ^{1}H NMR (250 MHz, DMSO-d_6): 1.69 (s, 3H, CH_3), 3.88 (d, 1H, *J*=12.4 Hz, H5′), 4.25–4.34 (m, 2H, H3′, H4′), 4.37 (s, 1H, H4′), 4.57 (d, 1H, *J*=12.4 Hz, H5′), 5.19 (d, 1H, *J*=3.7 Hz, 3′OH), 5.45 (d, 1H, J_1=6.3 Hz, 2′OH), 6.18 (s, 1H, H1′), 11.34 (s, 1H, NH); ^{13}C NMR (62 MHz, DMSO-d_6): 7.5 (CH_3), 72.2 (C3′), 76.5 (C2′), 76.7 (C5′), 86.5 (C4′), 91.3 (C1′), 97.0 (C5), 149.2 (C2), 156.9 (C6), 164.0 (C4); HRMS (ESI): *m*/*z* calcd for $C_{10}H_{12}N_2O_6Na$ (M^++Na): 279.2043, found: 279.2039.

*4.8.2. 5-Ethyl-O^6,5′-cyclouridine (**29**)*

$[\alpha]_D$ +76 (*c* 1.0, DMF). ^{1}H NMR (250 MHz, DMSO-d_6): 0.94 (t, 3H, *J*=7.3 Hz, CH_3), 2.20 (q, 2H, *J*=7.3 Hz, CH_2), 3.86 (d, 1H, *J*=12.4 Hz, H5′), 4.25–4.42 (m, 2H, H3′, H4′), 4.36 (s, 1H, H4′), 4.61 (d, 1H, *J*=12.4 Hz, H5′), 5.17 (br s, 1H, 3′OH), 5.44 (d, 1H, *J*=5.2 Hz, 2′OH), 6.19 (s, 1H, H1′), 11.34 (s, 1H, NH); ^{13}C NMR (62 MHz, DMSO-d_6): 13.8 (CH_3), 15.6 (CH_2), 72.2 (C3′), 76.5 (C2′), 77.2 (C5′), 86.4 (C4′), 91.3 (C1′), 103.1 (C5), 149.2 (C2), 157.0 (C6), 163.5 (C4); HRMS (ESI): *m*/*z* calcd for $C_{11}H_{14}N_2O_6Na$ (M^++Na): 293.2311, found: 293.2307.

*4.8.3. 5-Benzyl-O^6,5′-cyclouridine (**30**)*

$[\alpha]_D$ +98 (*c* 1.0, DMF). ^{1}H NMR (250 MHz, DMSO-d_6): 3.43–3.72 (m, 3H, CH_2, H5′), 4.23–4.40 (m, 2H, H3′, H4′), 4.34 (s, 1H, H4′), 4.52 (d, 1H, *J*=12.6 Hz, H5′), 5.18 (br s, 1H, 3′OH), 5.44 (d, 1H, J_1=6.6 Hz, 2′OH), 6.20 (s, 1H, H1′), 7.10–7.33 (m, 5H, H_{Ar}), 11.45 (s, 1H, NH); ^{13}C NMR (62 MHz, DMSO-d_6): 27.7 (CH_2), 72.1 (C3′), 76.6 (C2′), 77.0 (C5′), 86.3 (C4′), 91.4 (C1′), 100.9 (C5), 125.8 (CH_{Ar}), 127.9 (CH_{Ar}), 128.2 (CH_{Ar}), 140.2 (C^{IV}_{Ar}), 149.2 (C2), 157.9 (C6), 163.5 (C4); HRMS (ESI): *m*/*z* calcd for $C_{16}H_{16}N_2O_6Na$ (M^++Na): 355.3019, found: 355.3016.

*4.8.4. 5-Allyl-O^6,5′-cyclouridine (**31**)*

$[\alpha]_D$ +92 (*c* 1.0, DMF). ^{1}H NMR (250 MHz, DMSO-d_6): 2.93 (d, 2H, *J*=5.9 Hz, CH_2), 3.82 (d, 1H, *J*=12.6 Hz, H5′), 4.23–4.33 (m, 2H, H3′, H4′), 4.36 (s, 1H, H4′), 4.57 (d, 1H, *J*=12.6 Hz, H5′), 4.87–5.05 (m, 2H, CH_2=), 5.18 (d, 1H, J_1=3.9 Hz, 3′OH), 5.44 (d, 1H, J_1=6.4 Hz, 2′OH), 5.66–5.87 (m, H, CH=), 6.19 (s, 1H, H1′), 11.39 (s, 1H, NH); ^{13}C NMR (62 MHz, DMSO-d_6): 26.2 (CH_2), 72.2 (C3′), 76.6 (C2′), 77.2 (C5′), 86.4 (C4′), 91.4 (C1′), 99.4 (C5), 114 (CH_2=), 135.9 (CH=), 149.3 (C2), 157.8 (C6), 163.3 (C4); HRMS (ESI): *m*/*z* calcd for $C_{12}H_{14}N_2O_6Na$ (M^++Na): 305.2421, found: 305.2417.

*4.8.5. 5-Crotyl-O^6,5′-cyclouridine (**32**) (as a Z/E mixture)*

^{1}H NMR (400 MHz, DMSO-d_6): 1.53–1.68 (m, 3H, CH_3), 2.79–3.03 (m, 2H, CH_2), 3.81 (m, 1H, H5′), 4.24–4.34 (m, 2H, H3′, H4′), 4.36 (s, 1H, H4′), 4.57 (dd, 1H, J_1=4.2 Hz, J_2=12.4 Hz, H5′), 5.17 (d, 1H, *J*=3.2 Hz, 3′OH), 5.23–4.43 (m, 2H, 2CH=), 5.45 (d, 1H, J_1=6.1 Hz, 2′OH), 6.18 (s, 1H, H1′), 11.38 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 12.6, 17.5 (CH_3), 20.0, 25.0 (CH_2), 72.2 (C3′), 76.6 (C2′), 77.1 (C5′), 86.5 (C4′), 91.3 (C1′), 100.4, 100.9 (C5), 123.9, 124.9, 127.9, 128.6 (CH=), 149.2, 149.3 (C2), 157.5 (C6), 163.4, 163.5 (C4); HRMS (ESI): *m*/*z* calcd for $C_{13}H_{16}N_2O_6Na$ (M^++Na): 319.2689, found: 319.2687.

*4.8.6. 5-n-Propyl-O^6,5′-cyclouridine (**33**)*

$[\alpha]_D$ +66 (*c* 1.0, DMF). ^{1}H NMR (250 MHz, DMSO-d_6): 0.83 (t, 3H, *J*=7.3 Hz, CH_3), 1.34 (q, *J*=7.4 Hz, 2H, CH_2), 2.16 (t, 2H, *J*=7.4 Hz, CH_2), 3.84 (d, 1H, *J*=12.3 Hz, H5′), 4.30 (s, 2H, H2′, H3′), 4.36 (s, 1H, H4′), 4.59 (d, 1H, *J*=12.3 Hz, H5′), 5.4 (br s, 2H, 3′OH, 2′OH), 6.19 (s, 1H, H1′), 11.4 (br s, 1H, NH); ^{13}C NMR (62 MHz, DMSO-d_6): 13.7 (CH_3), 21.7 (CH_2), 24.2 (CH_2), 72.2 (C3′), 76.6 (C2′), 77.2 (C5′), 86.4 (C4′), 91.3 (C1′), 101.3 (C5), 149.3 (C2), 157.3 (C6), 163.7 (C4); HRMS (ESI): *m*/*z* calcd for $C_{12}H_{16}N_2O_6Na$ (M^++Na): 307.2579, found: 307.2576.

*4.8.7. 5-n-Butyl-O^6,5′-cyclouridine (**34**)*

$[\alpha]_D$ +67 (*c* 1.0, DMF). ^{1}H NMR (250 MHz, DMSO-d_6): 0.86 (t, 3H, *J*=6.9 Hz, CH_3), 1.15–1.41 (m, 4H, 2×CH_2), 2.19 (t, 2H, *J*=6.4 Hz, CH_2), 3.84 (d, 1H, *J*=12.3 Hz, H5′), 4.25–4.33 (m, 2H, H2′, H3′), 4.36 (s, 1H, H4′), 4.59 (d, 1H, *J*=12.3 Hz, H5′), 5.17 (d, 1H, *J*=3.2 Hz, 3′OH), 5.44 (d, 1H, J_1=6.3 Hz, 2′OH), 6.19 (s, 1H, H1′), 11.33 (s, 1H, NH); ^{13}C NMR (62 MHz, DMSO-d_6): 13.4 (CH_3), 21.5 (CH_2), 30.4 (CH_2) (*n*-butyl), 71.8 (C3′), 76.2 (C2′), 76.8 (C5′), 86.0 (C4′), 90.9 (C1′), 101.2 (C5), 148.9 (C2), 156.8 (C6), 163.3 (C4); HRMS (ESI): *m*/*z* calcd for $C_{13}H_{18}N_2O_6Na$ (M^++Na): 321.2847, found: 321.2845.

*4.8.8. 5-iso-Butyl-O^6,5′-cyclouridine (**35**)*

$[\alpha]_D$ +79 (*c* 1.0, DMF). ^{1}H NMR (250 MHz, CD_3OD): 0.90 (t, 6H, *J*=6.3 Hz, $2CH_3$), 1.82 (m, *J*=6.3 Hz, 1H, CH), 2.10–2.33 (m, 2H, CH_2),

3.96 (dd, 1H, J=1.1, 12.5 Hz, H5′), 4.45 (br s, 2H, H2′, H3′), 4.47 (s, 1H, H4′), 4.60 (dd, 1H, J_1=1.3 Hz, J_2=12.5 Hz, H5′), 6.41 (s, 1H, H1′), 8.00 (s, 1H, NH); ^{13}C NMR (62 MHz, CD_3OD): 22.6 (CH_3), 23.0 (CH_3), 29.0 (CH), 32.5 (CH_2), 74.2 (C3′), 78.4 (C2′), 78.6 (C5′), 88.3 (C4′), 93.4 (C1′), 103.0 (C5), 151.2 (C2), 159.8 (C6), 166.6 (C4); HRMS (ESI): m/z calcd for $C_{13}H_{18}N_2O_6Na$ (M^++Na): 321.2847, found: 321.2844.

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