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Synthesis of 5-haloethynyl- and 5-(1,2-dihalo)vinyluracil nucleosides: Antiviral activity and cellular toxicity

Vanessa Escuret,[b] Vincent Aucagne,[a] Nicolas Joubert,[a] David Durantel,[b] Kimberly L. Rapp,[c] Raymond F. Schinazi,[c] Fabien Zoulim[b] and Luigi A. Agrofoglio[a,*]

[a]*Institut de Chimie Organique et Analytique, ICOA UMR 6005, UFR Sciences, BP 6759, 45067 Orléans Cedex 2, France*
[b]*Inserm U271, 151 Cours Albert Thomas, 69003 Lyon, France*
[c]*Veterans Affairs Medical Center and Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, Atlanta, GA 30033, USA*

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Abstract—In this article, we report the synthesis of hitherto unknown 5-haloethynyl and 5-(1,2-dihalo)vinyluracil nucleosides in the 2′-deoxy, 3′-deoxy- and ribosyl series, and we discuss their in vitro anti-HIV and anti-HCV activities and cellular toxicitites. As a result, on the basis of their selectivity index (SI) obtained with the HCV replicon system, but also on their cytotoxicity on peripheral blood mononuclear, CEM and VERO cell lines, the best compounds were the 5-bromoethynyluridine (SI = 3.2) and the 5-(1-chloro-2-iodo)vinyluridine (SI > 2.8).

1. Introduction

HCV infection remains a main public health problem with 175 million people infected in the world. Currently, the best treatment available consists in the combination of pegylated interferon alpha 2b (IFNα-2b) and ribavirin [**1**, 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide, Fig. 1]. Twenty percent of patients infected with HCV genotype 2 or 3 and 50% of patients infected with HCV genotype 1 are non-responders to this treatment.[1–3] Moreover, IFN and ribavirin are responsible for numerous adverse effects. Therefore, a new treatment regimen based on more potent and specific inhibitors of HCV replication is required to improve the current antiviral strategies against chronic HCV infection. The inhibition of NS5B bearing the RNA-dependent RNA polymerase activity has been intensively studied[4] because of the central role of this protein in viral replication. Thus, specific inhibitors of this domain may represent potent antiviral agents.[5,6] The pharmaceutical importance of nucleoside analogues against the HIV, HBV or HSV DNA polymerases has prompted the design and the synthesis of nucleoside analogues against viral RNA polymerase.

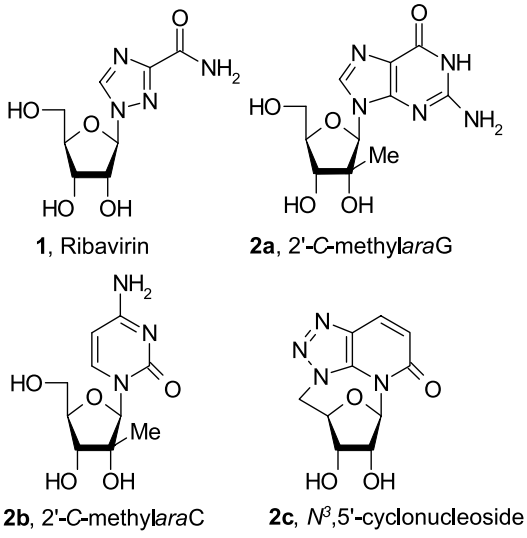

Figure 1. Some nucleoside inhibitors of NS5B.

Keywords: Nucleosides; HCV replicon; 5-Haloethynyl nucleosides; 5-(1,2-Dihalo)ethynyl nucleosides.
* Corresponding author. Tel.: +33 2 3849 4582; fax: +33 2 3841 7281; e-mail: luigi.agrofoglio@univ-orleans.fr

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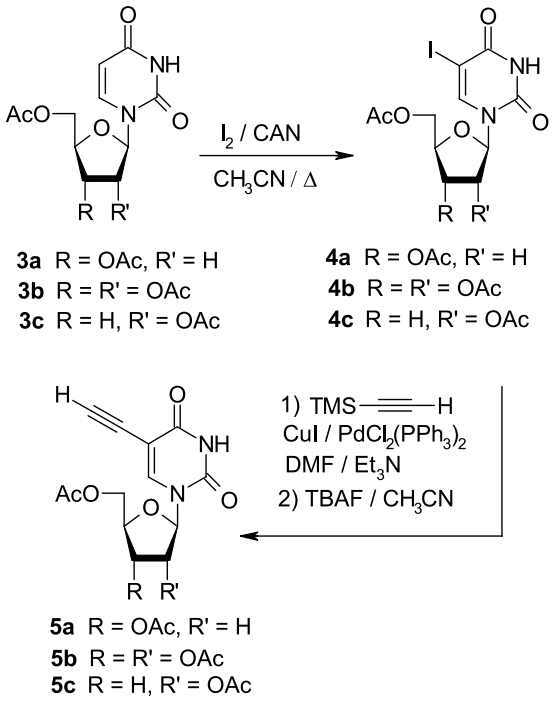
While numerous non-nucleoside inhibitors of NS5B-mediated HCV RNA replication have been reported,[7] only a few nucleoside inhibitors have been identified so far,[8] (Fig. 1). Among nucleoside inhibitors,[9b] the 2′-C-methylarabinoguanosine (**2a**) and the cytosine analogue (**2b**)[10a] are phosphorylated in cultured uninfected cells and are orally bioavailable in primates; meanwhile, the triazolo analogue (**2c**) exhibited an effective concentration (EC_{50}) of 19.7 μM on HCV replicon without any toxicity on peripheral blood mononuclear (PBM) and VERO cells.[10b] The use of these compounds effectively eliminates wild-type viruses and those with mutations in the polymerase gene survive. These selected mutants are therefore resistant to the drugs.[9,10] Therefore, the discovery of new inhibitors of HCV is mandatory in the perspective of the development of combination therapy.

Thus, as a part of our drug discovery programme, we report here the synthesis and evaluation of 18, hitherto unknown, 5-haloethynyl- and 5-(1,2-dihalo)vinyluracil-(2′-deoxy-, ribo- and 3′-deoxy)-nucleosides. The anti-HCV activity and the cellular toxicity were evaluated for these new 5-halosubstituted uridine derivatives using a cell line harbouring a genotype-1b HCV subgenomic replicon,[11] and for some compounds, against the bovine viral diarrhoea virus (BVDV) strain NADL. The molecules were also tested on HIV-1 using different cell lines and their cytotoxicities evaluated in lymphocytic CEM, African Green monkey (VERO) and activated human PBM cells.

2. Chemistry

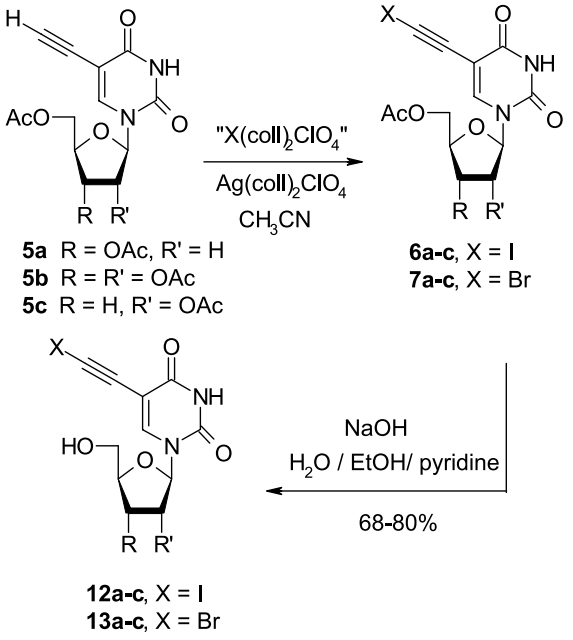
Halogenated pyrimidines **4a–c** can be prepared by direct reaction with halogens following the procedure recently reported by Asakura and Robins[12] via a cerium(IV)-mediated halogenation at the C-5 of uracil derivatives (Scheme 1). Thus, the treatment of known acetylated nucleosides **3a–c** with elemental iodine and ceric ammonium nitrate (CAN) at 80 °C gave the corresponding protected 5-iodouracil nucleosides **4a–c** with excellent yields. This CAN-mediated halogenation has advantages over other available methods as mild reaction conditions and short reaction times are employed. The introduction of a C-5-alkynyl group was performed by a Pd(0)-mediated reaction, using the Sonogashira conditions.[13] Thus, reaction of the resulting pure acetylated 5-iodouracil **4a–c** with trimethylsilylacetylene (TMS) in the presence of Et_3N (base), CuI (cocatalyst) and $PdCl_2(PPh_3)_2$ (catalyst) in anhydrous dimethylsufoxide (DMSO) at room temperature, followed by the removal of the trimethylsilyl group with *n*-Bu_4NF yielded the corresponding 5-ethynyluracil nucleosides **5a–c** with a high efficiency (80–99%). In most cases, it has been observed that slight elevations in the reaction temperature led to an increased rate of coupling. In all cases, a by-product, furanopyrimidine derivatives,[14] has been either isolated with a <7% yield or just detected by thin-layer chromatography (TLC).



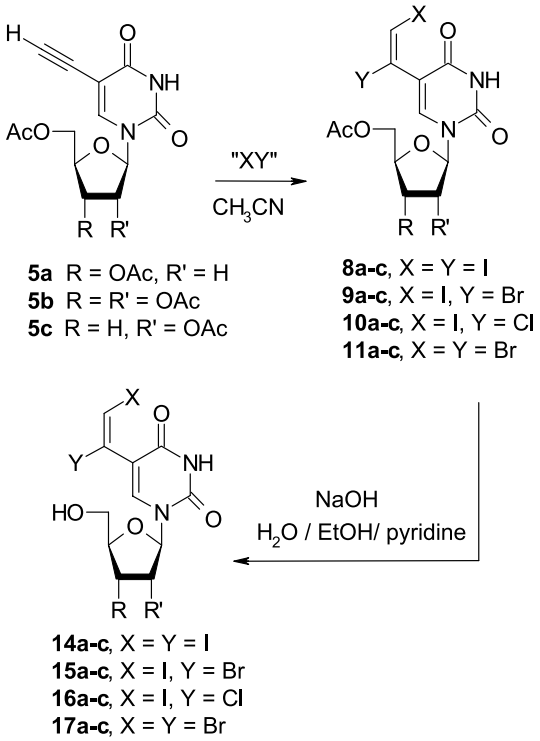
Scheme 1. Synthesis of 5-ethynyluracil acetylated nucleosides **5a–c**.

We then turned our attention to the synthesis of 5-haloethynyluracil nucleosides **12a–c** and **13a–c**, for which only the 5-bromoethynyl-2′-deoxyuridine has been already reported by Eger et al.[15] to exhibit a certain selectivity [similar to the *E*-5-(bromovinyl)-2′-deoxyuridine] towards HSV-1. The halogenation of alkynes **5a–c** was realized using either the iodonium di-*syn*-collidine perchlorate (IDCP, for iodination) or bromonium di-*syn*-collidine perchlorate ($Br(coll)_2ClO_4$, for bromination) in anhydrous CH_3CN (Scheme 2). Both reagents are known to act as very reactive electrophile (I^+ and Br^+, respectively). The 5-iodoethynyluracil nucleosides **6a–c** were obtained with a good yield ranging from 68 to 93%, whereas the bromination yielded the desired 5-bromoethynyluracil derivatives **7a–c** in lower yields (from 21 to 58%). The final deacylation of nucleosides **6a–c** and **7a–c** was performed under smooth basic conditions (1 M NaOH in a mixture of H_2O/EtOH/pyridine), and the 5-haloethynyluracil nucleosides **12a–c** (for X = I) and **13a–c** (for X = Br) were obtained, respectively, in good-to-moderate yields.

Finally, we worked on the dihalogenation of 5-ethynyl nucleosides to reach the hitherto unknown 5-(1,2-dihalogenated)vinyluracil nucleosides **14a–c** to **17a–c** (Scheme 3). Several halogenating reagents (i.e., I_2, IBr, ICl and Br_2) were used to obtain the corresponding 1,2-dihaloderivatives. In all cases of halo-iodination, the formation of vicinal halo-iodoalkenes (**8a–c** to **10a–c**) occurred with high anti-stereospecificity, implicating the intermediary of an iodonium ion in the reaction sequence, and a high and unique regioselectivity, implicating a predominantly Markovnikov addition. The formation of **11a–c** using Br_2 occurs also with an *E*-stereochemistry.



Scheme 2. Synthesis of 5-haloethynyluracil nucleosides **12a**–**c**, **13a**–**c**.



Scheme 3. Synthesis of 5-(1,2-dihalo)vinyluracil nucleosides from **14a**–**c** to **17a**–**c**.

3. Biological assay

All synthesized compounds, the 5-haloethynyluracil nucleosides (**12a**–**c** and **13a**–**c**) and the 5-(1,2-dihalo)vinyluracil nucleosides (**14a**–**c** to **17a**–**c**) along with the known antiviral compounds (ribavirin and IFNα-2b for HCV, and AZT for HIV), were tested for their antiviral activities in vitro, and the results are given in Table 1. Human PBM cells were used to assay their ability to inhibit HIV-1.[16] Among the 2′-deoxyribosyl nucleosides, **12a**–**17a** exhibited moderate anti-HIV activity with a median EC_{50} ranging from 0.9 to 73.3 μM; the best anti-HIV activity was exhibited by the 5-(1-bromo-2-iodo)vinyl-2′-deoxyuridine (**15a**) (EC_{50} = 0.9 μM, SI = 14.8). Nevertheless, all those compounds also showed toxicity against PBM, CEM or VERO cells, probably by inhibiting cellular DNA synthesis. Among the C-5-substituted uridines, **12b**–**17b**, the best (but moderate) anti-HIV activity was achieved for compound **12b** (5-iodoethynyluridine) with an EC_{50} of 20.8 μM (SI > 4.8); this nucleoside analogue did not exhibited any toxicity in PBM, CEM or VERO cells. In the 3′-deoxyribosyl series, no or very weak anti-HIV activity was noted for compounds **12c**–**17c** that were also not toxic.

The HCV subgenomic replicon system was used to assess the inhibitory activity of the synthesized compounds on the HCV replication. Among the 2′-deoxyuridine analogues (**12a**–**17a**), the 5-iodoethynyl-2′-deoxyuridine (**12a**) and the 5-(1-chloro-2-iodo)-2′-deoxyuridine (**16a**) have the best selectivity index (SI) on HCV replication, respectively, of 3.2 and 2.7. It is worth noting that ribavirin in the same system had an SI < 1, although its mechanism of action on HCV replication remains debated. However, in this series, all iodinated analogues were also the most toxic ones in Huh7 cells and also in PBM, CEM and VERO cells, whereas the bromo derivative compounds (**13a** and **17a**) were less toxic, but also unable to inhibit HCV significantly. The important cell toxicity exhibited by the 2′-deoxyuridine derivatives is also in favour of a non-specific inhibition of viral genomic replication by those compounds. Indeed, in the replicon system, it was shown that HCV replication is dependent on cell cycle progression. Drugs that have a cytostatic effect may therefore exhibit HCV replication by an indirect mechanism. Efficient compounds for inhibiting HCV replication were also found among the uridine analogues. The 5-bromoethynyluridine (**13b**), the 5-(1,2-diiodo)vinyluridine (**14b**) and the 5-(1-chloro,2-iodo)vinyluridine (**16b**) exhibited an EC_{50} of 58, 162 and 75 μM, respectively, against HCV replicon, with a CC_{50} > 200 μM for **14b** and **16b**. The selectivity index against HCV, for **13b**, **14b** and **16b**, was found to be equal to 3.2, 1.5 and 2.8, respectively. Finally, the 3′-deoxyribosyl nucleosides (**12c**–**17c**) did not show any antiviral activity against neither HCV or BVDV (Table 2).

Ribavirin, which shows activity in vivo in chronically infected patients together with IFNα, is inactive against HCV replication in the replicon system, but inhibits viral replication in the BVDV system in combination with IFNα.[17] It is well known that ribavirin when used alone has no anti-HCV activity in humans infected with HCV. On the basis of observation of possibly toxic compounds (**12b**, **14a** and **15a**) that exhibited only modest antiviral activity (SI < 1), this may therefore indicate for **13b** and **16b** a specific anti-HCV effect, as these compounds were not active against the replication of BVDV, a closely related virus member of the Flaviviridae family

Table 1. Evaluation of the antiviral activity and cell viability (obtained with neutral red test) against HCV replication using cell line harbouring HCV subgenomic replicon, against HIV-1 in PBM cells and of the cytotoxicity against PBM, CEM and VERO cells in vitro

	Compound	Anti-HCV activity and cytotoxicity on replicon system				Anti-HIV activity			Cytotoxicity (CC_{50})		
		CC_{50}	EC_{50}	EC_{90}	SI	EC_{50}	EC_{90}	SI	PBM	CEM	VERO
	IFNα-2b	**>1000**	**19**	**760**	**52**						
	Ribavirin	**110**	**170**	**>512**	**<1**						
	MPA	**7**	**40**		**<1**						
	AZT					**0.016**		**>6250**	**>100**	**14.0**	**29.0**
2′-Deoxyribosyl moiety	**12a**	**>182**	**57**	**>239**	**>3.2**	**9.2**	**34.8**	**<1**	**7.5**	**15.8**	**>100**
	13a	>200	>200	>200	⩽1	73.3	>100	>1.4	>100	>100	>100
	14a	77	>161	>263	<1	10.3	39.1	1.4	14.7	19.5	19.5
	15a	43	>100	>100	<1	0.9	6.9	14.8	13.3	0.13	6.6
	16a	**213**	**80**	**>207**	**2.7**	**2.6**	**20.3**	**1.2**	**3.2**	**14.8**	**1.4**
	17a	>200	<200	>200	⩽1	43.2	86.9	>2.3	>100	43.9	>100
Ribosyl moiety	**12b**	148	>200	>200	<1	20.8	>100	>4.8	>100	>100	>100
	13b	**188**	**58**	**>200**	**3.2**	**95.2**	**>100**	**1**	**>100**	**>100**	**>100**
	14b	**>250**	**>162**	**>250**	**>1.5**	**>100**	**>100**	**⩽1**	**100**	**41.7**	**49.5**
	15b	>100	>100	>100	1	62.4	>100	>1.6	>100	93.6	>100
	16b	**>200**	**75**	**>250**	**>2.8**	**90.8**	**>100**	**1.1**	**>100**	**>100**	**100**
	17b	>200	>200	>200	⩽ 1	60.1	>100	>1.7	>100	>100	59.8
3′-Deoxyribosyl moiety	**12c**	>200	>200	>200	⩽ 1	88.7	>100	>1.1	>100	>100	3.2
	13c	>300	>300	>300	⩽ 1	>100	>100	⩽ 1	>100	>100	6.4
	14c	>200	>200	>200	⩽ 1	49.4	>100	1.8	87.2	>100	42.6
	15c	>200	>200	>200	⩽ 1	44.5	90.7	1.6	71.5	79.3	>100
	16c	>200	>200	>200	⩽ 1	47.8	>100	2	94.4	66.6	15.3
	17c	>200	=200	>200	⩽ 1	81.1	>100	>1.2	>100	79.3	7.8

Results are expressed in μM, except for IFN which is expressed as international units/ml. Selectivity index (SI) is the ratio between CC_{50} and EC_{50}.

Table 2. Neutral red testing and antiviral assay on BVDV strain NADL expressed in μM

Compound	CC_{50} (Neutral red)	EC_{50} CPE inhibition[a] (3rd day pi)[b]	SI
Ribavirin	>100	36	>2.8
12a–c, **13b**, **14a–c**, **15c**, **16a–c**	>100	>100	<1

[a] Cytopathic effect.
[b] Post inoculation.

(Table 2). This may suggest that the MDBK-cpBVDV assay is not always a reliable model for predicting anti-HCV activity. Therefore, the active and non-toxic **13b** and **16b** warrant further evaluation in combination with IFNα both in in vitro and in animal models.[22]

In summary, the substituted 2′-deoxyuridines **12a** and **16a**, and the substituted uridines **13b** and **16b** exhibited anti-HCV activity in the HCV replicon system. Nevertheless, the best compounds, based on their SI obtained with the HCV replicon system, but also on their cytotoxicity on PBM, CEM and VERO cell lines, are bearing the 5-(2-bromoethynyl)-uridine (**13b**) and the (*E*)-5-(1-chloro-2-iodovinyl)uridine (**16b**). Finally, of significance was the finding that all active compounds have a 3′-hydroxyl group (either in the ribosyl or 2′-deoxyribosyl series) while all 3′-deoxyuridine analogues did not possess any antiviral activity neither against HCV nor against HIV-1. As all of these active compounds possess a 3′-hydroxyl group on the ribose, our results confirm the crucial role of the 3′-hydroxyl group for inhibition of the HCV polymerase.[18] Thus, based on the anti-HCV activity and cytotoxicity, the order of potency for these anti-HCV nucleoside analogues was found to be: ribosyl- > 2′-deoxyribosyl- > 3′-deoxyribosyl substituted nucleoside analogues. Our results are also in accordance with other observations of the general incapacity of 3′-deoxy purine and pyrimidine ribonucleosides to inhibit the HCV RNA replication in cell assays, despite their efficient inhibition in in vitro NS5B assays.[8d]

It will be interesting to compare their anti-HCV activity to that of IFNα and to study whether their combination with IFNα may lead to a synergistic antiviral effect.

4. Experimental

4.1. Chemistry

4.1.1. General methods. Commercially available chemicals and solvents were of reagent grade and used as received. Dry tetrahydrofuran, pyridine and dichloromethane were obtained from distillation over CaH_2 or Na, *N*,*N*-dimethylformamide over BaO. The reactions were monitored by TLC analysis using silica gel plates (Kieselgel 60 F_{254}; E. Merck). Compounds were visualized by UV irradiation and/or spraying with 20% H_2SO_4 in EtOH, followed by charring at 150 °C. Column chromatography was performed on Silica Gel 60 M (0.040–0.063 mm; E. Merck). Melting points were recorded on a Büchi (Dr. Tottoli) and were uncorrected. Optical rotations were measured at 20–25 °C with a Perkin-Elmer Model 141 polarimeter. ^{1}H and ^{13}C NMR

spectra were recorded on a Bruker AVANCE DPX 250 Fourier Transform spectrometer at 250 MHz for ^{1}H and 62.9 MHz for ^{13}C, respectively, using tetramethylsilane as the internal standard; signals are reported as s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Mass spectra were recorded on a Perkin-Elmer SCIEX API-300 (heated nebullizer) spectrometer. High-resolution mass analyses (HRMS) were performed by the CRMPO, University of Rennes 1–Fr using the fast atom bombardment or electron spray ionization mode. The nomenclature of the obtained compounds is in accordance with the IUPAC rules and was checked with Autonome. The numbering and assignment of the chemical shifts for all described compounds are related to the corresponding ribose derivatives. 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.

4.1.2. General procedure for iodination of acetylated nucleosides. A solution of acetylated nucleoside **3a–c** (15 mmol) in dry CH_3CN (150 mL), CAN (4.94 g, 0.6 equiv) and I_2 (2.28 g, 0.6 equiv) was refluxed until completion (typically 1 h, checked by TLC). After cooling to rt solvents were evaporated under reduced pressure, and the dark oily residue was dissolved in AcOEt (300 mL) and H_2O (50 mL). The biphasic mixture was cooled in an ice bath, and a saturated $Na_2S_2O_3$ solution was smoothly added until complete decolouration. The organic layer was washed with water (2× 50 mL) and brine (50 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The white foam was triturated with pentane (50 mL), filtered and dried under reduced pressure to afford pure iodinated compounds **4a–c**, respectively.

4.1.3. 3′,5′-Di-*O*-acetyl-2′-deoxy-5-iodouridine (4a). The physicochemical data of this compound are in accordance with those previously published.[12,19]

4.1.4. 2′,3′,5′-Tri-*O*-acetyl-5-iodouridine (4b). The physicochemical data of this compound are in accordance with those previously published.[12,19]

4.1.5. 2′,5′-Di-*O*-acetyl-3′-deoxy-5-iodouridine (4c). 89% yield; ^{1}H NMR ($CDCl_3$) δ 9.53 (br s, 1H, NH), 7.93 (s, 1H, H-6), 5.82 (d, $J = 1.6$ Hz, 1H, H-1′), 5.30 (m, 1H, H-2′), 4.55 (m, 1H, H-4′), 4.41 (dd, $J = 12.8$, 2.8 Hz, 1H, H-5′α), 4.33 (dd, $J = 12.8$, 3.8 Hz, 1H, H-5′β), 2.31–1.95 (m, 2H, H-3′), 2.23 (s, 3H, OAc), 2.13 (s, 3H, OAc).

4.1.6. General procedure for Sonogashira cross-coupling and subsequent desilylation. Iodinated nucleoside **4a–c** (5 mmol) was dissolved in a mixture of dry DMF (15 mL), dry Et_3N (2.06 mL, 3 equiv) and TMS (2.06 mL, 3 equiv). CuI (190 mg, 0.2 equiv) and $PdCl_2(PPh_3)_2$ (350 mg, 0.1 equiv) were then added and the reaction mixture was stirred at rt until completion (typically 5–20 h, checked by TLC). Solvents were evaporated under reduced pressure. The oily residue was dissolved in AcOEt (250 mL) and then washed with water (5× 40 mL) and brine (40 mL). The organic layer was dried over $MgSO_4$ and the solvents were evaporated under reduced pressure to a dark oil. A first purification using a short path flash chromatography (eluent:CH_2Cl_2, then MeOH/CH_2Cl_2 95:5) afforded the desired compound contaminated with coloured reaction coproducts. Pure silylated alkynes (2 mmol), obtained after a second flash chromatography (eluent: hexanes/AcOEt, 7:3 then 1:1), were dissolved in dry CH_3CN (20 mL). TBAF monohydrate (583 mg, 1.05 equiv) was added and the resulting solution was stirred at rt until completion (typically 30 min to 2 h, checked by TLC). Solvents were evaporated under reduced pressure at rt and the oily residue was submitted to a flash column chromatography (eluent: hexanes/AcOEt 1:1 then AcOEt then MeOH/AcOEt 98:2) to afford pure 5-ethynyl nucleosides **5a–c**.

4.1.7. 3′,5′-Di-*O*-acetyl-2′-deoxy-5-ethynyluridine (5a). The physicochemical data of this compound are fully related with those previously published.[20]

4.1.8. 2′,3′,5′-Tri-*O*-acetyl-5-ethynyluridine (5b). The physicochemical data of this compound are fully related with those previously published.[21]

4.1.9. 2′,5′-Di-*O*-acetyl-3′-deoxy-5-ethynyluridine (5c). 75% yield; $[\alpha]_D^{20}$ −42 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 9.8 (br s, 1H, NH), 7.88 (s, 1H, H-6), 5.78 (s, 1H, H-1′), 5.31 (d, $J = 7.5$ Hz, 1H, H-2′), 4.48 (m, 1H, H-4′), 4.35 (dd, $J = 12.5$, 1.8 Hz, 1H, H-5′α), 4.25 (dd, $J = 12.5$, 3.4 Hz, 1H, H-5′β), 3.1 (s, 1H, H-Csp), 2.31–1.95 (m, 2H, H-3′), 2.12 (s, 3H, OAc), 2.05 (s, 3H, OAc). HRMS *m*/*z* calcd for $C_{15}H_{16}N_2O_7Na$, 360.2968; found, 360.2971.

4.1.10. General procedure for monohalogenation of 5-ethynyl nucleosides. 5-Ethynyl nucleoside **5a–c** (0.5 mmol) was dissolved in 5 mL dry CH_3CN, and the mixture was cooled in an ice bath. 0.7 mmol (1.4 equiv) of the halogenating reagent (i.e., IDCP or $Br(coll)_2ClO_4$) and 11 mg $Ag(coll)_2ClO4$ (0.05 equiv) were added and the mixture was stirred in the dark at rt (typically 2–20 h, checked by TLC). The reaction mixture was cooled to 0 °C then quenched by a saturated $Na_2S_2O_3$ solution (2 mL) and extracted with AcOEt (4× 10 mL). The organic layer was washed with water (10 mL), a 1 M HCl solution (3× 5 mL), water (2× 5 mL) and brine (10 mL) and then dried over $MgSO_4$ and concentrated in vacuo. The solid residue was purified by flash chromatography (eluent: hexanes/AcOEt, 1:1, v/v) to leave the desired monohalogenated compound (**6a–c** and **7a–c**).

4.1.11. 3′,5′-Di-*O*-acetyl-5-(2-iodoethynyl)-2′-deoxyuridine (6a). 93% yield; mp 175–177 (dec) °C; $[\alpha]_D^{20}$ −32 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 11.68 (br s, 1H, NH), 8.02 (s, 1H, H-6), 6.12 (t, $J = 7.1$ Hz, 1H, H-1′), 5.20 (m, 1H, H-3′), 4.33–4.15 (m, 3H, H-4′,5′), 2.55 (m, 1H, H-2′α), 2.31 (m, 1H, H-2′β), 2.07 (s, 3H, OAc), 2.05 (s, 3H, OAc). HRMS *m*/*z* calcd for $C_{15}H_{15}IN_2O_7Na$, 486.1982; found, 486.1986.

4.1.12. 2′,3′,5′-Tri-*O*-acetyl-5-(2-iodoethynyl)-uridine (6b). 68% yield; mp 100–102 °C; $[\alpha]_D^{20}$ −71 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 9.41 (br s, 1H, NH), 7.91 (s, 1H, H-6), 6.07 (d, J = 4.6 Hz, 1H, H-1′), 5.35 (m, 2H, H-2′,3′), 4.38 (m, 3H, H-4′,5′), 2.25 (s, 3H, OAc), 2.12 (s, 2 × 3H, 2 × OAc). HRMS *m*/*z* calcd for $C_{17}H_{17}IN_2O_9Na$, 544.2352; found 544.2349.

4.1.13. 2′,5′-Di-*O*-acetyl-5-(2-iodoethynyl)-3′-deoxyuridine (6c). 76% yield as an oil; $[\alpha]_D^{20}$ −49 (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 11.68 (br s, 1H, NH), 7.98 (s, 1H, H-6), 5.74 (s, 1H, H-1′), 5.30 (d, J = 7.5 Hz, 1H, H-2′), 4.43 (m, 1H, H-4′), 4.28 (br s, 2H, H-5′), 2.31–1.95 (m, 2H, H-3′), 2.09 (s, 3H, OAc), 2.05 (s, 3H, OAc). HRMS *m*/*z* calcd for $C_{15}H_{15}IN_2O_7Na$, 486.1982; found, 486.1986.

4.1.14. 3′,5′-Di-*O*-acetyl-5-(2-bromoethynyl)-2′-deoxyuridine (7a). 21% yield as an oil; $[\alpha]_D^{20}$ −68 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.88 (br s, 1H, NH), 7.95 (s, 1H, H-6), 6.31 (dd, J = 7.3, 6.4 Hz, 1H, H-1′), 5.25 (m, 1H, H-3′), 4.38–4.30 (m, 3H, H-4′,5′), 2.55 (m, 1H, H-2′α), 2.31 (m, 1H, H-2′β), 2.18 (s, 3H, OAc), 2.12 (s, 3H, OAc). HRMS *m*/*z* calcd for $C_{15}H_{15}BrN_2O_7Na$, 439.1978; found, 439.1981.

4.1.15. 2′,3′,5′-Tri-*O*-acetyl-5-(2-bromoethynyl)-uridine (7b). 58% yield as a colourless oil; $[\alpha]_D^{20}$ −59 (*c* 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 9.75 (br s, 1H, NH), 7.92 (s, 1H, H-6), 6.07 (d, J = 4.4 Hz, 1H, H-1′), 5.35 (m, 2H, H-2′,3′), 4.37 (m, 3H, H-4′,5′), 2.22 (s, 3H, OAc), 2.12 (s, 2 × 3H, 2 × OAc). HRMS *m*/*z* calcd for $C_{17}H_{17}BrN_2O_9Na$, 497.2348; found 497.2351.

4.1.16. 2′,5′-Di-*O*-acetyl-5-(2-bromoethynyl)-3′-deoxyuridine (7c). 25% yield as an oil; $[\alpha]_D^{20}$ −52 (*c* 0.4, $CHCl_3$); ^1H NMR (DMSO-d_6) δ 11.75 (br s, 1H, NH), 8.07 (s, 1H, H-6), 5.85 (s, 1H, H-1′), 5.31 (d, J = 7.5 Hz, 1H, H-2′), 4.43 (m, 1H, H-4′), 4.28 (br s, 2H, H-5′), 2.31-1.95 (m, 2H, H-3′), 2.08 (s, 3H, OAc), 2.06 (s, 3H, OAc). HRMS *m*/*z* calcd for $C_{15}H_{15}BrN_2O_7Na$, 439.1978; found 439.1975.

4.1.17. General procedure for dihalogenation of 5-ethynyl nucleosides. 5-Ethynyl nucleoside **5a**–**c** (0.5 mmol) was dissolved in 5 mL dry CH_3CN, and the mixture was cooled in an ice bath. 0.6 mmol (1.2 equiv) of a solution of the halogenating reagent (i.e., I_2, IBr, ICl or Br_2) in 1 mL dry CH_3CN was added dropwise and the mixture was stirred at 0 °C until completion (typically 15 min to 2 h, checked by TLC). The reaction mixture was quenched at 0 °C by a saturated $Na_2S_2O_3$ solution (2 mL) and then extracted with AcOEt (3× 10 mL). The organic layer was washed with water (2× 5 mL) and brine (10 mL) then dried over $MgSO_4$ and concentrated in vacuo to let the desired dihalogenated compound (**8a**–**c** to **11a**–**c**). Pure analytical samples were obtained using flash chromatography (eluent: hexanes/AcOEt, 1:1).

4.1.18. (*E*)-3′,5′-Di-*O*-acetyl-5-(1,2-diiodovinyl)-2′-deoxyuridine (8a). 94% yield; mp 76–78 °C; $[\alpha]_D^{20}$ −19 (*c* 0.3, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 9.72 (br s, 1H, NH), 7.67 (s, 1H, H-6), 7.45 (s, 1H, =CHI), 6.36 (dd, J = 8.1, 5.6 Hz, 1H, H-1′), 5.25 (m, 1H, H-3′), 4.50–4.28 (m, 3H, H-4′,5′), 2.60 (m, 1H, H-2′α), 2.30 (m, 1H, H-2′β), 2.17 (s, 3H, OAc), 2.08 (s, 3H, OAc). HRMS *m*/*z* calcd for $C_{15}H_{16}I_2N_2O_7Na$, 614.1106; found, 614.1110.

4.1.19. (*E*)-2′,3′,5′-Tri-*O*-acetyl-5-(1,2-diiodovinyl)uridine (8b). 98% yield; mp 83–85 °C; $[\alpha]_D^{20}$ −41 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 9.56 (br s, 1H, NH), 7.58 (s, 1H, H-6), 7.40 (s, 1H, =CHI), 6.15 (d, J = 4.6 Hz, 1H, H-1′), 5.36 (m, 2H, H-2′,3′), 4.38 (br s, 3H, H-4′,5′), 2.19 (s, 3H, OAc), 2.14 (s, 3H, OAc), 2.11 (s, 3H, OAc). HRMS *m*/*z* calcd for $C_{17}H_{18}I_2N_2O_9Na$, 672.1476; found, 672.1480.

4.1.20. (*E*)-2′,5′-Di-*O*-acetyl-5-(1,2-diiodovinyl)-3′-deoxyuridine (8c). 87% yield; mp 75–77 °C; $[\alpha]_D^{20}$ −23 (*c* 0.3, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 9.38 (br s, 1H, NH), 7.64 (s, 1H, H-6), 7.39 (s, 1H, =CHI), 5.92 (d, J = 1.6 Hz, 1H, H-1′), 5.38 (m, 1H, H-4′), 4.58 (m, 1H, H-2′), 4.44 (dd, J = 12.5, 4.4 Hz, 1H, H-5′α), 4.33 (dd, 1H, H-5′β), 2.31–1.95 (m, 2H, H-3′), 2.14 (s, 3H, OAc), 2.12 (s, 3H, OAc). HRMS *m*/*z* calcd for $C_{15}H_{16}I_2N_2O_7Na$, 614.1106; found, *m*/*z* 614.1101.

4.1.21. (*E*)-3′,5′-Di-*O*-acetyl-5-(1-bromo-2-iodovinyl)-2′-deoxyuridine (9a). 96% yield; mp 82–84 °C; $[\alpha]_D^{20}$ −14 (*c* 4.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 9.78 (br s, 1H, NH), 7.71 (s, 1H, H-6), 7.08 (s, 1H, =CHI), 6.25 (dd, J = 8.1, 5.6 Hz, 1H, H-1′), 5.28 (m, 1H, H-3′), 4.50–4.05 (m, 3H, H4′, 5′), 2.58 (m, 1H, H-2′α), 2.20 (m, 1H, H-2′β), 2.12 (s, 3H, OAc), 1.96 (s, 3H, OAc). HRMS *m*/*z* calcd for $C_{15}H_{16}BrIN_2O_7Na$, 567.1102; found, 567.1107.

4.1.22. (*E*)-2′,3′,5′-Tri-*O*-acetyl-5-(1-bromo-2-iodovinyl)-uridine (9b). 92% yield; mp 86–88 °C; $[\alpha]_D^{20}$ −39 (*c* 2.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 9.57 (br s, 1H, NH), 7.66 (s, 1H, H-6), 7.15 (s, 1H, =CHI), 6.15 (d, J = 4.7 Hz, 1H, H-1′), 5.38 (m, 2H, H-2′,3′), 4.38 (m, 3H, H-4′,5′), 2.18 (s, 3H, OAc), 2.15 (s, 3H, OAc), 2.13 (s, 3H, OAc). HRMS *m*/*z* calcd for $C_{17}H_{18}BrIN_2O_9Na$, 625.1469; found, *m*/*z* 625.1473.

4.1.23. (*E*)-2′,5′-Di-*O*-acetyl-5-(1-bromo-2-iodovinyl)-3′-deoxyuridine (9c). 89% yield as an oil; $[\alpha]_D^{20}$ −19 (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 9.63 (br s, 1H, NH), 7.61 (s, 1H, H-6), 7.13 (s, 1H, =CHI), 5.92 (s, 1H, H-1′), 5.38 (m, 1H, H-4′), 4.58 (m, 1H, H-2′), 4.44 (dd, J = 12.4, 2.6 Hz, 1H, H-5′α), 4.33 (dd, J = 12.4, 4.4 Hz, 1H, H-5′β), 2.31–1.95 (m, 2H, H-3′), 2.16 (s, 3H, OAc), 2.14 (s, 3H, OAc). HRMS *m*/*z* calcd for $C_{15}H_{16}BrIN_2O_7Na$, 567.1102; found, *m*/*z* 567.1105.

4.1.24. (*E*)-3′,5′-Di-*O*-acetyl-5-(1-chloro-2-iodovinyl)-2′-deoxyuridine (10a). 91% yield; mp 81–83 °C; $[\alpha]_D^{20}$ −14 (*c* 0.7, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 9.28 (br s, 1H, NH), 7.75 (s, 1H, H-6), 6.91 (s, 1H, =CHI), 6.32 (m, 1H, H-1′), 5.24 (m, 1H, H-3′), 4.50–4.30 (m, 3H, H-4′,5′), 2.75 (m, 1H, H-2′α), 2.18 (m, 1H, H-2′β), 2.13 (s, 2 × 3H, 2 × OAc). HRMS *m*/*z* calcd for $C_{15}H_{16}ClIN_2O_7Na$, 522.6592; found, 522.6597.

4.1.25. (*E*)-2′,3′,5′-Tri-*O*-acetyl-5-(1-chloro-2-iodovinyl)uridine (10b). 97% yield; mp 85–87 °C; $[\alpha]_D^{20}$ −27 (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 9.43 (br s, 1H, NH), 7.68 (s, 1H, H-6), 6.92 (s, 1H, =CHI), 6.15 (br s, 1H, H-1′), 5.36 (m, 2H, H-2′,3′), 4.38 (m, 3H, H-4′,5′), 2.17 (s, 3H, OAc), 2.14 (s, 3H, OAc), 2.12 (s, 3H, OAc). HRMS *m/z* calcd for $C_{17}H_{18}ClIN_2O_9Na$, 580.6962; found, 580.6958.

4.1.26. (*E*)-2′,5′-Di-*O*-acetyl-5-(1-chloro-2-iodovinyl)-3′-deoxyuridine (10c). 83% yield; mp 87–89 °C; $[\alpha]_D^{20}$ −13 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 9.75 (br s, 1H, NH), 7.74 (s, 1H, H-6), 6.90 (s, 1H, =CHI), 5.91 (d, $J = 1.7$ Hz, 1H, H-1′), 5.38 (m, 1H, H-4′), 4.58 (m, 1H, H-2′), 4.43 (dd, $J = 12.5$, 2.8 Hz, 1H, H-5′α), 4.33 (dd, $J = 12.5$, 4.1 Hz, 1H, H-5′β), 2.35-1.95 (m, 2H, H-3′), 2.14 (s, 2 × 3H, 2 × OAc). HRMS *m/z* calcd for $C_{15}H_{16}ClIN_2O_7Na$, 522.6592; found, 522.6593.

4.1.27. 3′,5′-Di-*O*-acetyl-5-(1,2-dibromovinyl)-2′-deoxyuridine (11a). 97% yield; mp 79–81 °C; $[\alpha]_D^{20}$ −15 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 9.85 (br s, 1H, NH), 7.75 (s, 1H, H-6), 6.89 (s, 1H, =CHBr), 6.33 (dd, $J = 8.1$, 5.6 Hz, 1H, H-1′), 5.24 (m, 1H, H-3′), 4.45–4.25 (m, 3H, H-4′,5′), 2.65 (m, 1H, H-2′α), 2.18 (m, 1H, H-2′β), 2.14 (s, 2 × 3H, 2 × OAc). HRMS *m/z* calcd for $C_{15}H_{16}Br_2N_2O_7Na$, 520.1098; found, 520.1099.

4.1.28. (*E*)-2′,3′,5′-Tri-*O*-acetyl-5-(1,2-dibromo-vinyl)uridine (11b). 92% yield; mp 83–85 °C; $[\alpha]_D^{20}$ −43 (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 9.38 (br s, 1H, NH), 7.66 (s, 1H, H-6), 6.90 (s, 1H, =CHBr), 6.12 (d, $J = 4.8$ Hz, 1H, H-1′), 5.36 (m, 2H, H-2′,3′), 4.38 (m, 3H, H-4′,5′), 2.17 (s, 3H, OAc), 2.14 (s, 3H, OAc), 2.12 (s, 3H, OAc). HRMS *m/z* calcd for $C_{17}H_{18}Br_2N_2O_9Na$, 578.1468; found, 578.1473.

4.1.29. 2′,5′-Di-*O*-acetyl-5-(1,2-dibromovinyl)-3′-deoxyuridine (11c). 82% yield; mp 78–80 °C; $[\alpha]_D^{20}$ −16 (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 9.61 (br s, 1H, NH), 7.72 (s, 1H, H-6), 6.88 (s, 1H, =CHI), 5.88 (d, $J = 1.5$ Hz, 1H, H-1′), 5.38 (m, 1H, H-4′), 4.58 (m, 1H, H-2′), 4.43 (dd, $J = 12.5$, 2.8 Hz, 1H, H-5′α), 4.33 (dd, $J = 12.5$, 4.1 Hz, 1H, H-5′β), 2.35–1.95 (m, 2H, H-3′), 2.15 (s, 3H, OAc), 2.14 (s, 3H, OAc). HRMS *m/z* calcd for $C_{15}H_{16}Br_2N_2O_7Na$, 520.1098; found, 520.1103.

4.1.30. General procedure for deacetylation. Acetylated nucleoside analogue (**6a**–**c** to **11a**–**c**) (1 mmol) was dissolved in 10 mL pyridine and 5 mL EtOH. The reaction mixture was cooled to −10 °C and 5 mL of a 1 M NaOH aqueous solution was added. The resulting solution was stirred at this temperature until completion (typically 1–4 h, checked by TLC). The reaction mixture was neutralized with Dowex 50X2-200 then filtered through a fritted glass funnel. Solvents were evaporated in vacuo, and the oily residue was submitted to a flash column chromatography using an appropriate eluent (typically hexanes/EtOAc 25:75 then EtOAc then MeOH/AcOEt 99:1) to let pure **12a**–**c** to **17a**–**c**.

4.1.31. 5-(2-Iodoethynyl)-2′-deoxyuridine (12a). 68% yield; mp 135–137 °C; $[\alpha]_D^{20}$ +14 (*c* 0.8, MeOH); ^1H NMR (CD_3OD) δ 8.32 (s, 1H, H-6), 6.22 (t, $J = 6.6$ Hz, 1H, H-1′), 4.41 (m, 1H, H-3′), 3.95 (m, 1H, H-4′), 3.83 (dd, $J = 12.1$, 3.0 Hz, 1H, H-5′α), 3.73 (dd, $J = 12.1$, 3.4 Hz, 1H, H-5′β), 2.40–2.12 (m, 2H, H-2′). HRMS *m/z* calcd for $C_{11}H_{11}IN_2O_5Na$, 402.1229; found, 402.1231.

4.1.32. 5-(2-Iodoethynyl)-uridine (12b). 70% yield as an oil; $[\alpha]_D^{20}$ −12 (*c* 0.2, MeOH); ^1H NMR (DMSO-d_6) δ 11.65 (br s, 1H, NH), 8.29 (s, 1H, H-6), 5.72 (m, 1H, H-1′), 5.05–5.12 (3 × br s, 3 × OH), 4.05–3.95 (m, 2H, H-2′,3′), 3.85 (m, 1H, H-4′), 3.70–355 (m, 2H, H-5′). HRMS *m/z* calcd for $C_{11}H_{11}IN_2O_6Na$, 418.1223; found, 418.1219.

4.1.33. 5-(2-Iodoethynyl)-3′-deoxyuridine (12c). 66% yield as an oil; $[\alpha]_D^{20}$ +6 (*c* 1.0, MeOH); ^1H NMR (DMSO-d_6) δ 11.65(s, 1H, NH), 8.48 (s, 1H, H-6), 5.59 (s, 1H, H-1′), 5.54 (d, $J = 4.1$ Hz, 1H, OH), 5.23 (t, $J = 5.1$ Hz, 1H, OH), 4.32 (m, 1H, H-4′), 4.21 (m, 1H, H-2′), 3.75 (m, 1H, H-5′α), 3.52 (m, 1H, H-5′β), 1.98 (m, 1H, H-3′α), 1.76 (m, 1H, H-3′β). HRMS *m/z* calcd for $C_{11}H_{11}IN_2O_5Na$, 402.1229; found, 402.1233.

4.1.34. 5-(2-Bromoethynyl)-2′-deoxyuridine (13a). 80% yield as an oil; $[\alpha]_D^{20}$ +3 (*c* 0.3, MeOH); ^1H NMR (CD_3OD) δ 8.30 (s, 1H, H-6), 6.05 (t, $J = 6.3$ Hz, 1H, H-1′), 4.21 (m, 1H, H-3′), 3.78 (m, 1H, H-4′), 3.65–3.50 (m, 2H, H-5′), 2.15 (dd, $J = 5.6$, 5.0 Hz, 2H, H-2′). HRMS *m/z* calcd for $C_{11}H_{11}BrN_2O_5Na$, 355.1225; found, 355.1227.

4.1.35. 5-(2-Bromoethynyl)-uridine (13b). 74% yield as an oil; $[\alpha]_D^{20}$ −23 (*c* 0.5, MeOH); ^1H NMR (DMSO-d_6) δ 11.69 (br s, 1H, NH), 8.39 (s, 1H, H-6), 5.73 (d, $J = 4.7$ Hz, 1H, H-1′), 5.41 (d, $J = 5.3$ Hz, OH), 5.24 (t, $J = 4.7$ Hz, OH), 5.08 (d, $J = 5.3$ Hz, OH), 4.10–3.91 (m, 2H, H-2′,3′), 3.85 (m, 1H, H-4′), 3.75–350 (m, 2H, H-5′). HRMS *m/z* calcd for $C_{11}H_{11}BrN_2O_6Na$, 371.1219; found, 371.1224.

4.1.36. 5-(2-Bromoethynyl)-3′-deoxyuridine (13c). 72% yield as an oil; $[\alpha]_D^{20}$ −29 (*c* 1.0, MeOH); ^1H NMR (DMSO-d_6) δ 11.63(s, 1H, NH), 8.53 (s, 1H, H-6), 5.59 (s, 1H, H-1′), 5.56 (d, $J = 4.1$ Hz, 1H, OH), 5.26 (t, $J = 5.1$ Hz, 1H, OH), 4.32 (m, 1H, H-4′), 4.25 (m, 1H, H-2′), 3.81 (m, 1H, H-5′α), 3.61 (m, 1H, H-5′β), 1.95 (m, 1H, H-3′α), 1.72 (m, 1H, H-3′β). HRMS *m/z* calcd for $C_{11}H_{11}BrN_2O_5Na$, 355.1225; found, 355.1223.

4.1.37. (*E*)-5-(1,2-Diiodovinyl)-2′-deoxyuridine (14a). 72% yield; mp 103–105 °C; $[\alpha]_D^{20}$ +28 (*c* 0.3, MeOH); ^1H NMR (CD_3OD) δ 8.27 (s, 1H, H-6), 7.52 (s, 1H, =CHI), 6.28 (t, $J = 6.3$ Hz, 1H, H-1′), 4.43 (m, 1H, H-3′), 3.97 (m, 1H, H-4′), 3.83 (dd, $J = 11.9$, 3.2 Hz, 1H, H-5′α), 3.73 (m, $J = 11.9$, 3.4 Hz, 1H, H-5′β), 2.43–2.12 (m, 2H, H-2′). HRMS *m/z* calcd for $C_{11}H_{12}I_2N_2O_5$-Na, 530.0353; found, 530.0558.

4.1.38. (*E*)-5-(1,2-Diiodovinyl)uridine (14b). 82% yield; mp 107–109 °C; $[\alpha]_D^{20}$ +5 (*c* 1.0, MeOH); ^1H NMR

(DMSO-d_6) δ 11.65 (br s, 1H, NH), 8.05 (s, 1H, H-6), 7.05 (s, IH, =CHI), 5.80 (d, J = 4.7 Hz, 1H, H-1′), 5.50–5.10 (3 × br s, 3 × OH), 4.12–3.95 (m, 2H, H-2′,3′), 3.87 (m, 1H, H-4′), 3.75–355 (m, 2H, H-5′). HRMS m/z calcd for $C_{11}H_{12}I_2N_2O_6Na$, 546.0347; found, 546.0351.

4.1.39. (*E*)-5-(1,2-Diiodovinyl)-3′-deoxyuridine (14c). 76% yield as an oil; $[\alpha]_D^{20}$ +1.0 (c 0.8, MeOH); ^{1}H NMR (DMSO-d_6 + D_2O) δ 8.21 (s, 1H, H-6), 7.55 (s, 1H, =CHI), 5.61 (s, 1H, H-1′), 4.32 (m, 1H, H-4′), 4.25 (m, 1H, H-2′), 3.81 (m, 1H, H-5′α), 3.52 (m, 1H, H-5′β), 1.97 (m, 1H, H-3′α), 1.74 (m, 1H, H-3′β). HRMS m/z calcd for $C_{11}H_{12}I_2N_2O_5Na$, 530.0553; found, 530.0554.

4.1.40. (*E*)-5-(1-Bromo-2-iodovinyl)-2′-deoxyuridine (15a). 78% yield as an oil; $[\alpha]_D^{20}$ +26 (c 2.0, MeOH); ^{1}H NMR (CD_3OD) δ 8.35 (s, 1H, H-6), 7.39 (s, 1H, =CHI), 6.25 (t, J = 6.5 Hz, 1H, H-1′), 4.45 (m, 1H, H-3′), 3.94 (m, 1H, H-4′), 3.86 (dd, J = 11.9, 3.2 Hz, 1H, H-5′α), 3.72 (dd, J = 11.9, 3.4 Hz, 1H, H-5′β), 2.45–2.15 (m, 2H, H-2′). HRMS m/z calcd for $C_{11}H_{12}BrIN_2O_5Na$, 483.0349; found, 483.0352.

4.1.41. (*E*)-5-(1-Bromo-2-iodovinyl)uridine (15b). 76% yield as an oil; $[\alpha]_D^{20}$ −1 (c 0.3, MeOH); ^{1}H NMR (D_2O) δ 8.27 (s, 1H, H-6), 7.45 (s, IH, =CHI), 5.79 (d, J = 4.7 Hz, 1H, H-1′), 4.15–3.92 (m, 2H, H-2′,3′), 3.88 (m, 1H, H-4′), 3.67 (dd, J = 11.8, 2.5 Hz, 1H, H-5′α), 3.56 (dd, 1H, H-5′β). HRMS m/z calcd for $C_{11}H_{12}BrIN_2O_6Na$, 499.0343; found, m/z 499.0348.

4.1.42. (*E*)-5-(1-Bromo-2-iodovinyl)-3′-deoxyuridine (15c). 68% yield as an oil; $[\alpha]_D^{20}$ +3 (c 1.0, MeOH); ^{1}H NMR (DMSO-d_6 + D_2O) δ 8.35 (s, 1H, H-6), 7.39 (s, 1H, =CHI), 5.61 (d, J = 1.3 Hz, 1H, H-1′), 4.32 (m, 1H, H-4′), 4.25 (m, 1H, H-2′), 3.76 (dd, J = 12.2, 2.4 Hz, 1H, H-5′α), 3.52 (dd, J = 12.2, 2.8 Hz, 1H, H-5′β), 1.97 (m, 1H, H-3′α), 1.75 (m, 1H, H-3′β). HRMS m/z calcd for $C_{11}H_{12}BrIN_2O_5Na$, 483.0349; found, m/z 483.0344.

4.1.43. (*E*)-5-(1-Chloro-2-iodovinyl)-2′-deoxyuridine (16a). 71% yield; mp 109–111 °C; $[\alpha]_D^{20}$ +34 (c 0.5, MeOH); ^{1}H NMR (CD_3OD) δ 8.38 (s, 1H, H-6), 7.06 (s, 1H, =CHI), 6.30 (t, J = 6.5 Hz, 1H, H-1′), 4.43 (ddd, J = 6.5, 3.8, 3.6 Hz, 1H, H-3′), 3.96 (ddd, J = 3.6, 3.4, 3.0 Hz, 1H, H-4′), 3.83 (dd, J = 11.9, 3.1 Hz, 1H, H-5′α), 3.73 (dd, J = 11.9, 3.4 Hz, 1H, H-5′β), 2.45–2.10 (m, 2H, H-2′). HRMS m/z calcd for $C_{11}H_{12}ClIN_2O_5Na$, 438.5839; found, m/z 438.5843.

4.1.44. (*E*)-5-(1-Chloro-2-iodovinyl)uridine (16b). 78% yield; mp 109–111 °C; $[\alpha]_D^{20}$ +5 (c 0.4, MeOH); ^{1}H NMR (D_2O) δ 8.45 (s, 1H, H-6), 7.05 (s, IH, =CHI), 5.89 (br s, 1H, H-1′), 4.30 (br s, 2H, H-2′,3′), 4.10 (m, 1H, H-4′), 3.85 (dd, J = 11.5, 2.2 Hz, 1H, H-5′α), 3.75 (dd, 1H, H-5′β). HRMS m/z calcd for $C_{11}H_{12}ICIN_2O_6Na$, 454.5833; found, m/z 454.5835.

4.1.45. (*E*)-5-(1-Chloro-2-iodovinyl)-3′-deoxyuridine (16c). 70% yield as an oil; $[\alpha]_D^{20}$ +2 (c 2.0, MeOH); ^{1}H NMR (DMSO-d_6 + D_2O) δ 8.38 (s, 1H, H-6), 7.18 (s, 1H, =CHI), 5.65 (d, J = 1.2 Hz, 1H, H-1′), 4.32 (m, 1H, H-4′), 4.25 (m, 1H, H-2′), 3.76 (dd, J = 12.3, 2.6 Hz, 1H, H-5′α), 3.52 (dd, J = 12.3, 2.8 Hz, 1H, H-5′β), 2.01 (m, 1H, H-3′α), 1.75 (m, 1H, H-3′β). HRMS m/z calcd for $C_{11}H_{12}ClIN_2O_5Na$, 438.5839; found, m/z 438.5837.

4.1.46. (*E*)-5-(1,2-Dibromovinyl)-2′-deoxyuridine (17a). 85% yield as an oil; $[\alpha]_D^{20}$ +26 (c 0.5, MeOH); ^{1}H NMR (CD_3OD) δ 8.39 (s, 1H, H-6), 7.06 (s, 1H, =CHBr), 6.28 (t, J = 6.5 Hz, 1H, H-1′), 4.41 (m, 1H, H-3′), 3.95 (m, 1H, H-4′), 3.82 (dd, J = 11.9, 2.9 Hz, 1H, H-5′α), 3.73 (dd, J = 11.9, 3.4 Hz, 1H, H-5′β), 2.45–2.10 (m, 2H, H-2′). HRMS m/z calcd for $C_{11}H_{12}Br_2N_2O_5Na$, 436.0345; found, m/z 436.0348.

4.1.47. (*E*)-5-(1,2-Dibromovinyl)uridine (17b). 72% yield as an oil; $[\alpha]_D^{20}$ −14 (c 0.5, MeOH); ^{1}H NMR (D_2O) δ 8.45 (s, 1H, H-6), 7.07 (s, IH, =CHBr), 5.93 (d, J = 5.2 Hz, 1H, H-1′), 4.18 (m, 2H, H-2′,3′), 4.05 (m, 1H, H-4′), 3.88 (dd, J = 12.5, 2.5 Hz, 1H, H-5′α), 3.74 (dd, J = 12.5, 2.8 Hz, 1H, H-5′β). HRMS m/z calcd for $C_{11}H_{12}Br_2N_2O_6Na$, 452.0339; found, 452.0342.

4.1.48. (*E*)-5-(1,2-Dibromovinyl)-3′-deoxyuridine (17c). 74% yield as an oil; $[\alpha]_D^{20}$ +5 (c 1.0, MeOH); ^{1}H NMR (DMSO-d_6 + D_2O) δ 8.42 (s, 1H, H-6), 7.25 (s, 1H, =CHBr), 5.64 (br s, 1H, H-1′), 4.35 (m, 1H, H-4′), 4.25 (m, 1H, H-2′), 3.78 (m, H-5′α), 3.54 (m, H-5′β), 1.95 (m, 1H, H-3′α), 1.75 (m, 1H, H-3′β). HRMS m/z calcd for $C_{11}H_{12}Br_2N_2O_5Na$, 436.0345; found, 436.0340.

4.2. Biological evaluation

4.2.1. Antiviral and Cytotoxicity assays for HCV. Huh7 cells harbouring the subgenomic HCV replicon BM4-5, kindly provided by Seeger,[11] were used in this study. Cells were maintained in Dulbecco's modified Eagle's medium high glucose 4.5 g/L (LifeTechnologies) supplemented with 10% fetal bovine serum, 1% L-glutamine, 1% penicillin and streptomycin, 1% L-pyruvate and 500 μg/mL of geneticin (G418; Invitrogen). Geneticin was used to select cells permitting the HCV RNA replication. Cells were passaged every 4 days with a 1:4 ratio.

Cells were seeded in 6-well plates at a density of 2.5×10^5 cells per well, 16 h before the beginning of treatment. Cells were treated with the molecules administered at different concentrations in complete medium that did not contain geneticin. The administration of each drug was renewed every day for three consecutive days. Ribavirin (ICN Pharmaceuticals), mycophenolic acid (Sigma) and IFNα-2b(IntronA®) were used in the same conditions as positive controls. The concentrations used for these drugs were, 0, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256, and 512 μM for ribavirin: 0, 2.5, 5, 10, 20, and 40 μM for mycophenolic acid, and 0, 0.1, 1, 10, 100 and 1000 UI/mL for IFNα-2b. Total RNA was extracted at the end of treatment (24 h after the last day of treatment) with the 'Extract all' reagent (Eurobio), which is a mix of phenol and guanidinium thiocyanate. Northern blot analysis was then performed using

the NorthernMax™-Gly kit (Ambion), following manufacturer's instructions. Five micrograms of total RNA were denaturated in glyoxal buffer at 50 °C for 30 min, separated by 1.1% agarose gel electrophoresis and then transferred for 12 h onto a charged nylon membrane (HybondN+; Amersham). Hybridization was carried out with three different [^{32}P]CTP-labelled riboprobes obtained by in vitro transcription (Riboprobe in vitro transcription system; Promega). Two probes were complementary to the NS5A region of the HCV genome of negative polarity and positive polarity. A third probe was complementary to the beta-actin mRNA and obtained by in vitro transcription from a specific plasmid (pTRI beta actin human, reference 7424; Ambion). First, the blot was hybridized with the riboprobes directed against the negative strand of HCV RNA and beta-actin mRNA. After one night of hybridization at 68 °C, the membrane was washed, and then exposed to X-ray film and a phosphor screen (phosphorimager). This screen was then scanned and quantitative analysis was achieved using ImageQuant software. The amount of beta-actin mRNA was used as an internal loading control to standardize the amount of HCV RNA detected. The same membrane was subsequently hybridized with the negative sense riboprobe to determine the level of positive strand HCV RNA, using the same approach.

For cell viability assays, cells were seeded in 96-well plates at a density of 12,500 cells per well. They were treated by the different molecules with the same concentrations and conditions than those used for the antiviral assays. Then, cell viability was measured by neutral red assay. Neutral red which specifically colours lysosomes and its accumulation depends on cellular membrane integrity. The yield of neutral red incorporated in cells is proportional to the number of living cells. At the end of treatment, culture medium was removed; cells were washed by PBS and then coloured with neutral red at 0.005% for 3 h at 37 °C. Cells were then fixed 1 min by formol calcium and lysed by a treatment with a v/v mixture of acetic acid and ethanol. After 15 min incubation, absorbance was read at 490 nm.

4.2.2. Antiviral and cytotoxicity assays for BVDV. Non-cytopathic-BVDV-free MDBK cells (European Collection of Animal Cell Cultures, Porton Down, UK) were kindly provided by Dr. N. Zitzmann (Oxford University). Cells were propagated in DMEM F12 (Eurobio) supplemented with 10% horse serum (Life Technologies), 1% L-glutamin (Gibco), 1% penicillin and streptomycin (Gibco). The NADL cytopathic (CP) BVDV strain was obtained from ATCC.

MDBK cells were seeded in microwell plates (96 wells) at a density of 1×10^5 cells per well, and then infected with BVDV (strain NADL) [at a dilution inducing 100% cytopathic effect (CPE) 3 days postinoculation, that is, approximately 10 plaque forming units (pfu) per well at 3 days postinfection] for 1 h, at 37 °C. After a wash with DMEM, infected cells were incubated for 3 days in the presence or absence of drug; each concentration of drug was added in 12 consecutive wells. The appearance of CPE was visually checked the third day postinoculation to evaluate the EC_{50} on BVDV (strain NADL), according to the different conditions of treatment. By definition, the EC_{50} is the concentration of drug that inhibits 50% of the CPE in comparison to cells inoculated but untreated presenting 100% of CPE. Uninfected MDBK cells were grown in the absence or presence of varying concentrations of drug tested. After 3 days, toxicity was evaluated by neutral red colouration as described previously.

4.2.3. Cell culture assays for HIV-1. Antiviral and cytotoxicity assays were conducted as described recently by Stuyver et al.[16] HIV-1 antiviral assays were performed in activated primary human PBM.

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