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Synthesis and anti-viral activity of a series of D- and L-2′-deoxy-2′-fluororibonucleosides in the subgenomic HCV replicon system

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Abstract—Based on the discovery of (2′*R*)-D-2′-deoxy-2′-fluorocytidine as a potent anti-hepatitis C virus (HCV) agent, a series of D- and L-2′-deoxy-2′-fluororibonucleosides with modifications at 5- and/or 4-positions were synthesized and evaluated for their in vitro activity against HCV and bovine viral diarrhea virus (BVDV). The key step in the synthesis, the introduction of 2′-fluoro group, was achieved by either fluorination of 2,2′-anhydronucleosides with hydrogen fluoride–pyridine or potassium fluoride, or a fluorination of arabinonucleosides with DAST. Among the 27 analogues synthesized, only the 5-fluoro compound, namely (2′*R*)-D-2′-deoxy-2′,5-difluorocytidine (**13**), demonstrated potent anti-HCV activity and toxicity to ribosomal RNA. The replacement of the 4-amino group with a thiol group resulted in the loss of activity, while the 4-methylthio substituted analogue (**25**) exhibited inhibition of ribosomal RNA. As N^4-hydroxycytidine (NHC) had previously shown potent anti-HCV activity, we combined the two functionalities of the N^4-hydroxyl and the 2′-fluoro into one molecule, resulting (2′*R*)-D-2′-deoxy-2′-fluoro-N^4-hydroxycytidine (**23**). However, this nucleoside showed neither anti-HCV activity nor toxicity. All the L-forms of the analogues were devoid of anti-HCV activity. None of the compounds showed anti-BVDV activity, suggesting that the BVDV system cannot always predict anti-HCV activity.

1. Introduction

Hepatitis C virus (HCV) is an important pathogen affecting nearly 170 million people worldwide.[1] HCV infections become chronic in about 50% of cases,[2] and about 20% of these chronic patients develop liver cirrhosis that can lead to hepatocellular carcinoma.[3] The current therapy, based on interferon-alpha (IFN-α), alone or combination with ribavirin, is only moderately effective.[4] Therefore, there is a need for more effective anti-HCV agents. Recently, a ribonucleoside analogue, NM283, has shown potent anti-HCV activity,[5] and is in Phase II clinical trials by Idenix Pharmaceuticals. Earlier, we discovered that a sugar-fluorinated nucleoside, (2′*R*)-D-2′-deoxy-2′-fluorocytidine (**1**), had potent anti-HCV activity.[6] Based on the activity of this compound, we synthesized a series of D- and L-analogues, and evaluated them against bovine viral diarrhea virus (BVDV) and HCV in the replicon system. In addition, our discovery of a base-modified nucleoside, D-N^4-hydroxycytidine (NHC),[7] possessing anti-HCV activity prompted us to combine these two features in one molecule. Studying the structure–activity relationship of this class of nucleosides would increase our knowledge of the structural requirements for anti-viral agents for HCV, and would aid in the search for better anti-HCV agents. Herein we report the synthesis and the biological evaluation of D- and L-2′-deoxy-2′-fluororibonucleosides.

Keywords: HCV; BVDV; 2′-Deoxy-2′-fluororibonucleosides; 2′-Fluororibosides; Fluorination.
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2. Results and discussion

(2′*R*)-D-2′-Deoxy-2′-fluorouridine, the first 2′-deoxy-2′-fluororibonucleoside, was described by Cordington et al. four decades ago.[8] Since then, several 2′-deoxy-2′-fluororibonucleosides have been prepared.[9] The synthesis of 2′-deoxy-2′-fluororibonucleosides can be achieved primarily by three strategies: (i) fluorination of an appropriate nucleoside; (ii) condensation of an appropriate 2-fluorosugar and a nucleobase; and (iii) transglycosylation of a 2′-deoxy-2′-fluororibonucleoside with a nucleobase. Currently, there are two main approaches for the fluorination of nucleosides: one is through the substitution of anhydronucleosides with hydrogen fluoride-based reagents, and the other is by an S_N2 substitution of arabinonucleosides either directly with diethylaminosulfur trifluoride (DAST) or with tetrabutylammonium fluoride (TBAF) via a sulfonate intermediate. The first approach, the fluorination of anhydronucleosides, is a simple, short, and direct method to prepare carbohydrate-modified nucleosides, and the second approach, fluorination through an arabinonucleoside, is a relatively lengthy route. For the fluorination of anhydronucleosides, earlier efforts were focused on the use of the dangerous anhydrous hydrogen fluoride. More recently, the use of hydrogen fluoride–pyridine has been widely adopted. In our synthesis, the 2′-fluorine atom of L-2′-deoxy-2′-fluororibonucleosides was introduced by the fluorination of arabinonucleosides with DAST, while various fluorination approaches were used for the synthesis of D-analogues, based mainly on the accessibility.

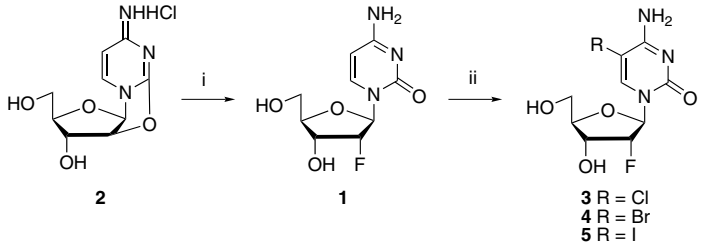
Originally, (2′*R*)-D-2′-deoxy-2′-fluorocytidine (**1**) was prepared from (2′*R*)-D-2′-deoxy-2′-fluorouridine via amination.[10] To the best of our knowledge, none of the (2′*R*)-2′-deoxy-2′-fluorocytidine analogues were prepared by direct substitution of the 2,2′-anhydrocytidine with hydrogen fluoride-based reagents. However, there was a report on the preparation of (2′*R*)-D-2′-deoxy-2′-fluorocytidine by the heating of the 2,2′-anhydrocytidine hydrofluoride salt.[11] The direct conversion of D-2,2′-anhydrocytidine to (2′*R*)-D-2′-deoxy-2′-fluorocytidine was achieved by Mengel and Guschlbauer utilizing potassium fluoride and crown ether.[12] This represents a simple and direct method for the preparation of (2′*R*)-2′-deoxy-2′-fluorocytidine nucleosides. We performed this reaction for the preparation of D-2′-deoxy-2′-fluorocytidine (**1**) and achieved a similar result (about 40% yield). From this compound, several other 5-halogenated cytidine analogues (**3**–**5**) were prepared by halogenation (Scheme 1). However, this fluorination did not work on D-2,2′-anhydro-5-fluorocytidine and D-2,2′-anhydro-5-methylcytidine. The replacement of potassium fluoride/crown ether with hydrogen fluoride–pyridine was also unsuccessful.

For the synthesis of (2′*R*)-D-2′-deoxy-2′,5-difluorouridine (**11**), a DAST fluorination approach was utilized. Thus, D-5-fluorouridine (**6**) was cyclized, protected, and hydrolyzed to the arabinose **9**. The fluorination and deprotection of **9** yielded (2′*R*)-D-2′-deoxy-2′,5-difluorouridine (**11**) in excellent yield, and the latter afforded (2′*R*)-D-2′-deoxy-2′,5-difluorocytidine (**13**) after acetylation and amination (Scheme 2).

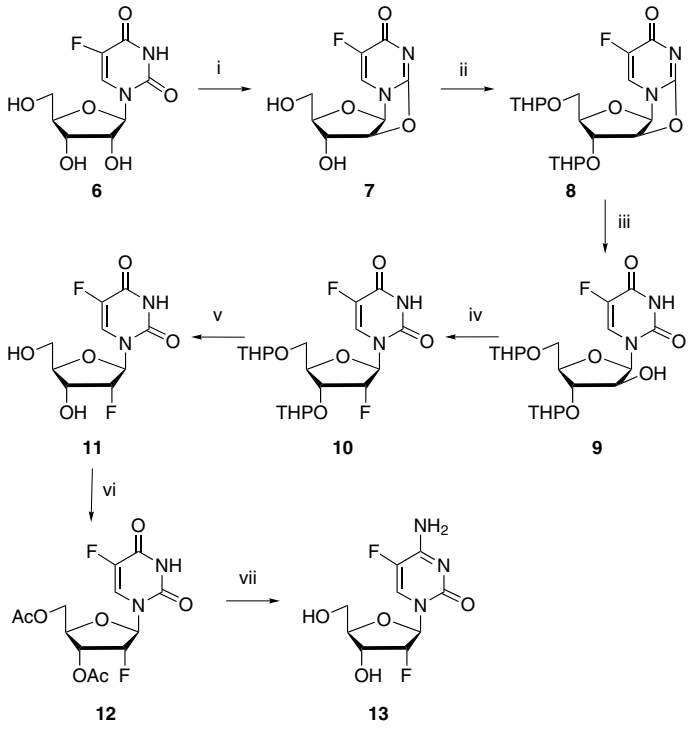
(2′*R*)-D-2′-Deoxy-2′-fluorouridine (**18**) was prepared from D-2,2′-anhydrouridine by fluorination with hydrogen fluoride–pyridine, as described by Kawasaki et al.[13] In a similar manner, (2′*R*)-D-2′-fluorothymidine (**19**) was also prepared in 31% yield. Acetylation followed by amination converted **19** to the 5-methylcytidine analogue **22** (Scheme 3). Following a literature process[10] 4-thio analogue **24** and 4-methylthio analogue **25** were synthesized, and (2′*R*)-D-2′-deoxy-2′-fluoroadenosine (**26**) was prepared according to the published procedures.[14] Unfortunately, the fluorination of the 5-fluoro-substituted anhydronucleoside with hydrogen fluoride–pyridine failed.

As both D-N^4-hydroxycytidine (NHC) and (2′*R*)-D-2′-deoxy-2′-fluorocytidine (**1**) have shown potent anti-HCV activity, it was of interest to combine the two functionalities into one molecule to improve the anti-viral potency and reduce cytotoxicity. Therefore, (2′*R*)-D-2′-deoxy-2′-fluoro-N^4-hydroxycytidine (**23**) was synthesized from (2′*R*)-D-2′-deoxy-2′-fluorouridine (**18**), by acetylation, sulfonation, hydroxyamination, and deprotection, as depicted in Scheme 3.

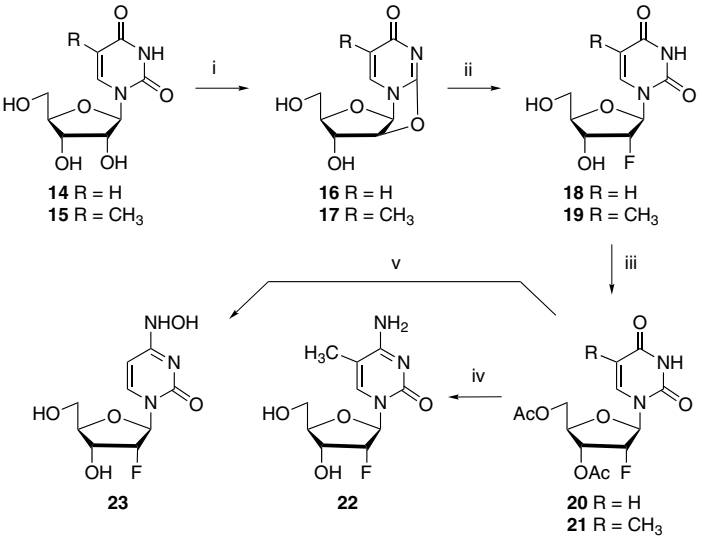
For the synthesis of the L-series of 2′-deoxy-2′-fluororibonucleosides, the fluorination of arabinonucleosides with DAST reagent was adopted, and the corresponding arabinonucleosides were prepared either by Holy's method[15] or Vorbrüggen sugar-base condensation.[16] Thus, L-2,2′-anhydrouridine (**27**) was protected, and hydrolyzed to give arabino-nucleoside **29**. Through a



Scheme 1. Reagents and conditions: (i) KF, dibenzo[18]crown-6, DMF, 120 °C, 4 h; (ii) BzCl, *m*-CPBA, DMF, rt, 30 min; or pyridine, Br_2, CCl_4, rt, 25 min; or MeOH, ICl, 50 °C, 4 h.



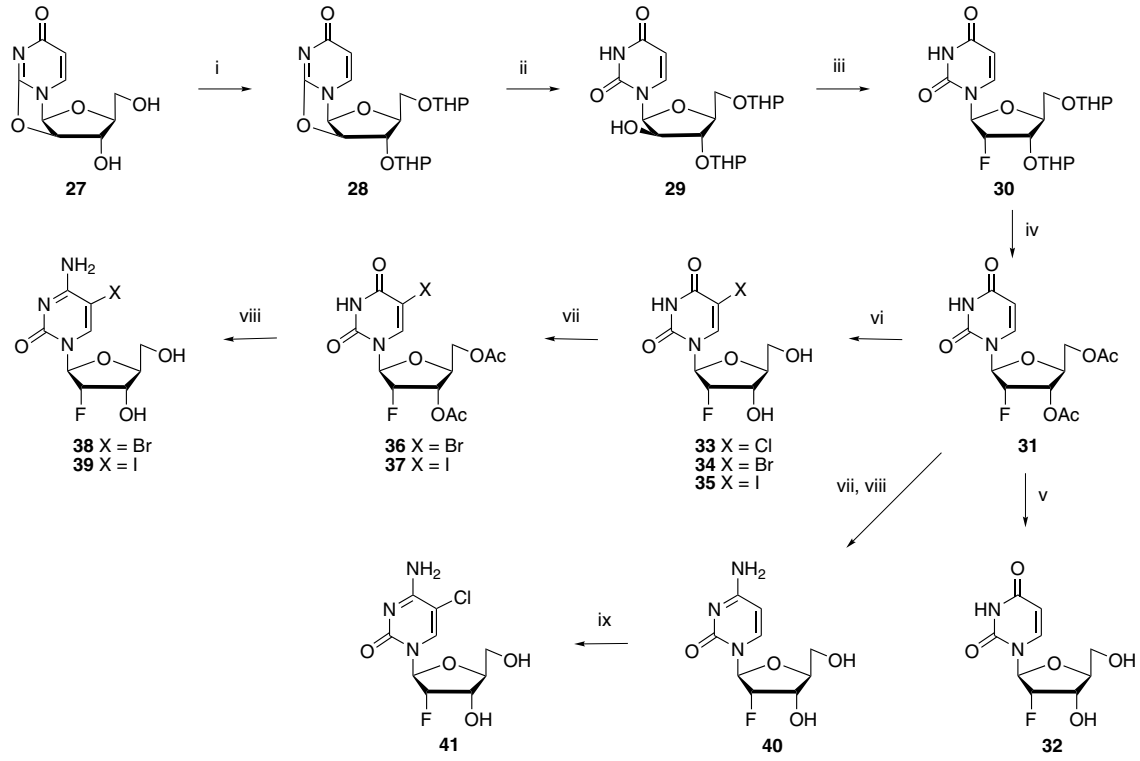
Scheme 2. Reagents and conditions: (i) HMPA, Ph_2CO, $NaHCO_3$, 135 °C, 40 min; (ii) DMF, 3,4-dihydro-2*H*-pyran, *p*-TsOH, 0 °C, 4 h; (iii) NaOH, MeOH, H_2O, rt, 2 h; (iv) DAST, CH_2Cl_2, pyridine, −70 to reflux, 4 h; (v) *p*-TsOH, MeOH, rt, 3 h; (vi) pyridine, Ac_2O, rt, overnight; (vii) (a) CH_3CN, Et_3N, TIPCl, DMAP, rt, 1 d; (b) NH_4OH, rt, 1 d.



Scheme 3. Reagents and conditions: (i) HMPA, Ph_2CO, $NaHCO_3$, 150 °C, 30 min; (ii) HF–pyridine, dioxane, 120–125 °C, 18 h; (iii) Ac_2O, pyridine; (iv) (a) CH_3CN, Et_3N, TIPCl, DMAP, rt, 1 d; (b) NH_4OH, rt, 1 d; (v) (a) CH_3CN, Et_3N, TIPCl, DMAP, rt, 1 d; (b) NH_2OH-HCl, rt, 1 d; (c) NH_3/MeOH, rt, 14 h.

fluorination with DAST, followed by deprotection and acetylation, protected (2′*S*)-L-2′-deoxy-2′-fluorouridine **31** was prepared in 41% yield. Deprotection of **31** afforded (2′*S*)-L-2′-deoxy-2′-fluorouridine (**32**), and amination of **31** yielded (2′*S*)-L-2′-deoxy-2′-fluorocytidine (**40**). Halogenation of **32** followed by deprotection produced 5-halogenated analogues **33**–**35**. 5-Bromo and 5-iodo analogues **34**–**35** were converted to their cytidine analogues **38**–**39** by acetylation and amination, whereas the chlorination of (2′*S*)-L-2′-deoxy-2′-fluorocytidine (**40**) yielded the 5-chlorocytidine analogue **41** (Scheme 4).

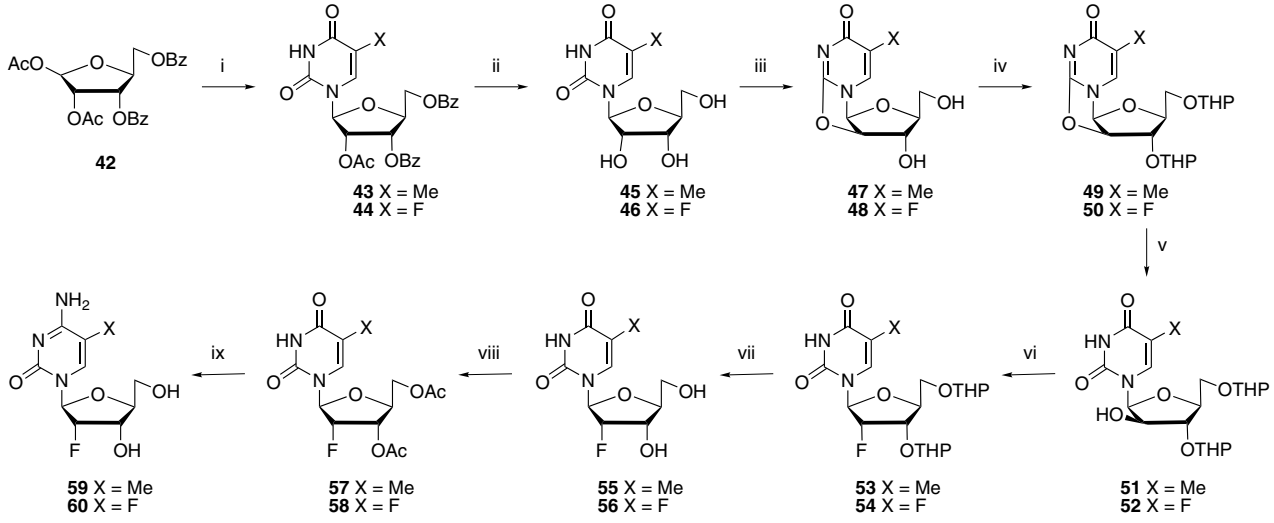
Because of the difficulty of the direct introduction of 5-methyl and 5-fluoro groups to the pyrimidine nucleosides, these nucleosides had to be prepared using a



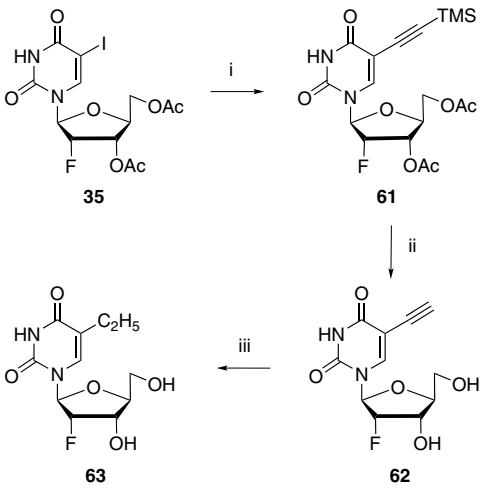
Scheme 4. Reagents and conditions: (i) DMF, 3,4-dihydro-2*H*-pyran, *p*-TsOH, 0 °C, 4 h; (ii) NaOH, MeOH, H_2O, rt, 2 h; (iii) DAST, CH_2Cl_2, pyridine, −70 °C to reflux, 4 h; (iv) (a) *p*-TsOH, MeOH, rt, 3 h; (b) pyridine, Ac_2O, rt, overnight; (v) NH_3/MeOH, rt, overnight; (vi) NCS, HOAc, 80–90 °C, 3 h; or HOAc, Ac_2O, Br_2, rt, overnight; or CH_2Cl_2, ICl, reflux, 5 h; (vii) Ac_2O, pyridine, rt, overnight; (viii) (a) 1,2,4-triazole, pyridine, $POCl_3$, rt, 17 h; (b) NH_4OH, rt, 4 h; (c) NH_3/MeOH, rt, 12 h; (ix) DMF, HCl, *m*-CPBA, rt, 2 h.

Vorbrüggen condensation of L-riboside and a silylated nucleobase. The condensation products **43** and **44** were deprotected, and cyclized to anhydronucleosides **47** and **48**. Protection by tetrahedronpyranyl group followed by hydrolysis gave the arabinonucleosides **51** and **52**, and fluorination with DAST followed by deprotection afforded (2′*S*)-L-2′-fluorothymidine (**55**) and (2′*S*)-L-2′-deoxy-2′, 5-fluorouridine (**56**). Through an acetylation and an amination, the uridine analogues were converted to the cytidine analogues **59** and **60** (Scheme 5).

5-Ethynyl, ethyl, and bromovinyl groups were converted from 5-iodouridine nucleosides via a palladium-



Scheme 5. Reagents and conditions: (i) (a) HMDS, nucleobase, reflux; (b) CH_3CN, TMSOTf; (ii) NaOMe, MeOH, rt, overnight; (iii) DMF, Ph_2CO, $NaHCO_3$, 150 °C, 1 h; (iv) DMF, 3,4-dihydro-2*H*-pyran, *p*-TsOH, 0 °C, 4 h; (v) NaOH, MeOH, H_2O, rt, 2 h; (vi) DAST, CH_2Cl_2, pyridine, −70 °C to reflux, 4 h; (vii) *p*-TsOH, MeOH, rt, 3 h; (viii) pyridine, Ac_2O, rt, overnight; (ix) (a) 1,2,4-triazole, pyridine, $POCl_3$, rt, 17 h; (b) NH_4OH, rt, 4 h; (c) NH_3/MeOH, rt, 12 h.



Scheme 6. Reagents and conditions: (i) Et_3N, CuI, $(Ph_3P)_2PdCl_2$, TMSCCH, 50–60 °C, 7 h; (ii) NaOMe, MeOH, rt, 15 h; (iii) EtOH, 10% Pd–C, H_2, 1 atm, 1 h.

mediated reaction.[17] From acetylated 5-iodo analogue **35**, after ethynylation followed by deprotection, 5-ethynyl analogue **62** was produced. Hydrogenation of **62** yielded 5-ethyl analogue **63** (Scheme 6). (2′*S*)-L-5-Bromovinyl-2′-deoxy-2′-fluorouridine (**64**) was prepared from 5-iodouridine analogue **35**, by a published procedure.[18]

The above synthesized 2′-deoxy-2′-fluororibonucleosides were evaluated in BVDV and HCV subgenomic replicon RNA-containing Huh7 cells, as described previously,[7,19] and the results of the selected nucleosides are shown in Table 1. For the BVDV assay, all the tested compounds showed no inhibitory activity, including the lead compound (2′*R*)-D-2′-deoxy-2′-fluorocytidine (**1**). For in vitro anti-HCV activity, in the D-series, among the 5-substituted cytidine analogues, only (2′*R*)-D-2′-deoxy-2′,5-difluorocytidine (**13**) exhibited potent anti-HCV activity and inhibition of ribosomal RNA. The 5-chloro, 5-bromo, 5-iodo, and 5-methyl substituted 2′-deoxy-2′-fluorocytidine analogues showed no anti-HCV activity. Similarly, the uridine analogues, (2′*R*)-D-2′-deoxy-2′-fluorouridine, (2′*R*)-D-2′-deoxy-2′,5-difluorouridine, and (2′*R*)-D-2′-fluorothymidine were not active against HCV. The replacement of the amino with a thiol group at the 4-position also resulted in an inactive compound **24**. However, the 4-methylthio analogue **25** demonstrated inhibition of ribosomal RNA. Unfortunately, the N^4-hydroxylamino analogue (**23**) showed neither activity against HCV nor inhibitory activity to ribosomal RNA.

In the L-series, none of the analogues, including (2′*S*)-L-2′-deoxy-2′-fluorocytidine (**40**), had any anti-HCV activity or anti-BVDV activity.

2′-Deoxy-2′-fluororibonucleoside analogues are known to be active against some RNA viruses. Several analogues of this class showed high activity against influenza A and B, and parainfluenza 1.[20] Also, (2′*R*)-D-2′-deoxy-2′-fluorocytidine has demonstrated inhibitory activity against herpes simplex virus type 1 and 2, pseudorabies and equine abortion virus.[21] It is surprising that our findings indicate that 2′-deoxy-2′-fluororibonucleosides possess anti-HCV activity, as some earlier works on HCV polymerase concluded that in order to be recognized by HCV RNA dependent RNA polymerase, a ribonucleoside was needed.[22] The discovery that 2′-deoxy-2′-fluorocytidine possesses potent anti-HCV activity suggests that the 2′-fluoro instead of 2′-hydroxyl group is recognized by the HCV RNA polymerase. In terms of Van der Vaar radii, the fluorine atom is closer to a hydrogen atom than a hydroxyl group. However, the fluorine atom may mimic the hydroxyl group, in terms of electronegativity and hydrogen bonding. The conformational study of 2′-fluorinated nucleosides also suggests that 2′-deoxy-2′-fluororibonucleosides are more like ribonucleosides than 2′-deoxyribonucleosides, since it has been confirmed that both 2′-deoxy-2′-fluororibonucleosides and ribonucleosides adopt a 3′-endo conformation.[23,24] However, the fact that the anti-HCV activity of 2′-deoxy-2′-fluorocytidine can be abolished by the addition of 2′-deoxycytidine, not by cytidine,[6] demonstrates that 2′-deoxy-2′-fluororibonucleosides are recognized as 2′-deoxynucleosides in at least one step in the catabolic pathway in HCV replicon cells.

It seems that the anti-HCV activity resides with the D-nucleosides. To the best of our knowledge, until now, no L-enantiomer has been reported to possess any specific anti-HCV activity in vitro. As more L-nucleosides are evaluated against HCV in vitro, this hypothesis will be further tested.

The fact that all the tested compounds showed no anti-BVDV activity may be due to the inadequate phosphorylation of these nucleosides in MBDK cells, as it has been shown that gemcitabine, a potent anti-cancer agent with nanomolar inhibition in most human cells, did not

Table 1. In vitro anti-BVDV and anti-HCV activity of selected 2′-deoxy-2′-fluororibonucleosides

Compound	Configuration	Base	4-Substitution	5-Substitution	EC_{90} (μM) BVDV	EC_{90} (μM) HCV	CC_{50} (μM) rRNA
1	D	C			>100	5.6	>100
13	D	C		F	ND	9.4	<1
23	D	C	NHOH		ND	>100	>100
25	D	U	SCH_3		>100	>100	8.7
NHC					5.4	5.0	>100
Ribavirin					1.5	⩾100	14.4

All the other nucleosides had values of EC_{90} and CC_{50} over 100 μM; ND: not determined; EC_{90}: effective concentration required for reducing the HCV RNA or BVDV levels by 90% in 96 h or 72 h.

CC_{50}: cytotoxic concentration required for reducing the rRNA levels by 50% in 96 h.

exhibit an inhibitory affect in this cell line.[7] This may suggest that the MDBK-cpBVDV assay is not always a reliable model for predicting anti-HCV activity in a replicon system.

3. Conclusions

A series of D- and L-2′-deoxy-2′-fluororibonucleosides were synthesized and evaluated for in vitro anti-HCV and anti-BVDV activity, as well as their inhibition of ribosomal RNA. The study revealed that (2′*R*)-D-2′-deoxy-2′,5-difluorocytidine (**13**) showed potent anti-HCV and inhibitory activity to ribosomal RNA. All of the other 5-modified D-nucleosides were not active against HCV, and all the L-series compounds were devoid of anti-HCV activity. The 4-methylthio analogue **25** showed inhibitory activity to ribosomal RNA. None of the tested compounds demonstrated anti-BVDV activity. Furthermore, (2′*R*)-D-2′-deoxy-2′-fluoro-N^4-hydroxylcytidine (**23**) was neither active nor toxic to liver cells.

4. Experimental

4.1. Materials

All reagents were used as received unless stated otherwise. Anhydrous solvents were purchased from Aldrich Chemical Company, Inc. (Milwaukee). Melting points (mp) were determined on an electrothermal digit melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were taken on a Varian Unity Plus 400 spectrometer at room temperature and reported in parts per million downfield from internal tetramethylsilane. Mass spectra were recorded at the Emory University Mass Spectrometry Center (Atlanta, GA). Polarimetries were measured on a Jasco DIP-370 digital polarimeter. Elemental analyses were performed by Atlantic Microlab Inc. (Norcross, GA). Analytic TLC was performed on Whatman LK6F silica gel plates. HPLC identification and purification were conducted with a Varian Prostar system. (2′*R*)-D-2′-Deoxy-2′-fluorocytidine (**1**),[12] D-2,2′-anhydrouridine (**16**),[25] (2′*R*)-D-2′-deoxy-2′-fluorouridine (**18**),[13] (2′*R*)-D-2′-deoxy-2′-fluoro-4-thiouridine (**24**),[10] (2′*R*)-D-2′-deoxy-2′-fluoro-4-methylthiouridine (**25**),[10] (2′*R*)-D-2′-deoxy-2′-fluoroadenosine (**26**),[14] and (2′*S*)-L-5-bromovinyl-2′-deoxy-2′-fluorouridine (**64**)[18] were prepared according to the published procedures.

4.1.1. (2′*R*)-β-D-5-Chloro-2′-deoxy-2′-fluorocytidine (3). To a solution of **1** (123 mg, 0.5 mmol) in DMF (1.5 mL) at room temperature was added BzCl (63 μL), followed by a solution of *m*-CPBA (125 mg) in DMF (0.5 mL). The solution was stirred at room temperature for 30 min, and then poured into cold water (10 mL). The resulting precipitate was filtered off and washed with water. The combined filtrates were evaporated in vacuo to dryness, and the residue was purified by flash chromatography on silica gel, eluting with CH_2Cl_2/MeOH (4:1), to give 33 mg (24%) of product as a white solid. Mp 176–178 °C (dec); ^{1}H NMR (DMSO-d_6) δ 8.35 (s, 1H, H-6), 7.93, 7.28 (2s, 2H, NH_2), 5.84 (d, J = 16.4 Hz, 1H, H-1′), 5.56 (d, J = 6.4 Hz, 1H, OH-3′), 5.37 (t, J = 4.8 Hz, 1H, OH-5′), 4.90 (dd, J = 4.0 and 52.8 Hz, 1H, H-2′), 4.21–4.09 (m, 1H, H-3′), 3.88 (d, J = 8.4 Hz, 1H, H-4′), 3.80, 3.60 (2m, 2H, H-5′). MS (FAB$^+$) *m/e* 286 [(M+Li)$^+$]; HRMS (FAB$^+$) calcd for ($C_9H_{11}ClFN_3O_4$+Li): 286.0582, found: 286.0577. Anal. Calcd for $C_9H_{11}ClFN_3O_4$+0.5H_2O: C 37.51, H 4.02, N 14.58. Found: C 37.78, H 3.99, N 13.94.

4.1.2. (2′*R*)-β-D-5-Bromo-2′-deoxy-2′-fluorocytidine (4). To a solution of **1** (72 mg, 0.29 mmol) in anhydrous pyridine (10 mL) at room temperature was added a solution of bromine (75 mg. 0.47 mmol) in CCl_4 (360 μL). The resulting orange colored solution was stirred at room temperature for 25 min, and then the solvent was evaporated to dryness. The residue was purified by flash chromatography on silica gel, eluting with CH_2Cl_2/MeOH (4:1), to give 80 mg (84%) of product as a pale yellow powder. Mp 165–167 °C (dec); ^{1}H NMR (DMSO-d_6) δ 8.42 (s, 1H, H-6), 7.93, 7.06 (2s, 2H, NH_2), 5.84 (d, J = 17.2 Hz, 1H, H-1′), 5.56 (d, J = 6.4 Hz, 1H, OH-3′), 5.37 (t, J = 4.4 Hz, 1H, OH-5′), 4.90 (dd, J = 4.0 and 53.2 Hz, 1H, H-2′), 4.21–4.09 (m, 1H, H-3′), 3.88 (d, J = 8.4 Hz, 1H, H-4′), 3.80, 3.60 (2m, 2H, H-5′). MS (FAB$^+$) *m/e* 330 [(M+Li)$^+$]; HRMS (FAB$^+$) calcd for ($C_9H_{11}BrFN_3O_4$+Li): 330.0077, found: 330.0071.

4.1.3. (2′*R*)-β-D-2′-Deoxy-2′-fluoro-5-iodocytidine (5). To a solution of **1** (123 mg, 0.5 mmol) in anhydrous MeOH (4 mL) was added ICl (163 mg, 1 mmol), and the solution was stirred at 50 °C under an argon atmosphere for 4 h. The solvent was evaporated to dryness and the residue was purified by flash chromatography on silica gel, eluting with CH_2Cl_2/MeOH (4:1), to give, after crystallization from EtOH/$CHCl_3$/hexane, 73 mg (39%) of product as a pale yellow crystalline solid. Mp 180–182 °C; ^{1}H NMR (DMSO-d_6) δ 8.45 (s, 1H, H-6), 7.90, 6.68 (2s, 2H, NH_2), 5.84 (d, J = 17.2 Hz, 1H, H-1′), 5.55 (d, J = 6.4 Hz, 1H, OH-3′), 5.36 (t, J = 4.8 Hz, 1H, OH-5′), 4.88 (dd, J = 4.4 and 53.2 Hz, 1H, H-2′), 4.21–4.09 (m, 1H, H-3′), 3.87 (d, J = 8.4 Hz, 1H, H-4′), 3.80, 3.58 (2m, 2H, H-5′). MS (FAB$^+$) *m/e* 378 [(M+Li)$^+$]. Anal. Calcd for $C_9H_{11}FIN_3O_4$: C 29.13, H 2.99, N 11.32. Found: C 29.07, H 3.02, N 11.15.

4.1.4. β-D-2,2′-Anhydro-5-fluorouridine (7). To a solution of **6** (1.60 g, 6.10 mmol) and diphenyl carbonate (1.74 g) in anhydrous HMPA (6 mL) was added $NaHCO_3$ (40 mg, mmol), and the mixture was stirred at 135 °C for 40 min. The resulting solution was cooled to room temperature, and poured into cold water (80 mL). The mixture was washed with $CHCl_3$ (3 × 40 mL), and concentrated in vacuo. The residue was crystallized from MeOH to give 575 mg (39%) of product as a white solid. Mp 222–224 °C; ^{1}H NMR (DMSO-d_6) δ 8.28 (d, J = 3.6 Hz, 1H, H-6), 6.31 (d, J = 5.6 Hz, 1H, H-1′), 5.91 (d, J = 3.2 Hz, 1H, H-2′), 5.25 (d, J = 6.4 Hz, OH-3′), 5.00 (t, J = 4.8 Hz, 1H, OH-5′), 4.40 (s, 1H, H-3′), 4.12 (m, 1H, H-4′), 3.31, 3.25 (2m, 2H, H-5′).

4.1.5. β-D-2,2′-Anhydro-5-fluoro-3′,5′-di-*O*-tetrahydropyranyluridine (8). To a solution of **7** (560 mg, 2.31 mmol) and *p*-TsOH (431 mg) in anhydrous DMF (10 mL) was added 3,4-dihydro-2*H*-pyran (5.6 mL, mmol), and the solution was stirred at 0 °C for 4 h. The solution was neutralized by the addition of Et_3N, and then the volatile was evaporated to dryness. The residue was dissolved in EtOAc (50 mL), washed with saturated aqueous $NaHCO_3$, dried over Na_2SO_4, and evaporated. The residue was triturated with hexane to give 640 mg (76%) of product as a white solid. This crude product was used for the next reaction without further purification.

4.1.6. β-D-3′,5′-Di-*O*-tetrahydropyranyl-5-fluoro-arabinouridine (9). A suspension of **8** (640 mg, 1.77 mmol) in MeOH (9 mL) and NaOH aqueous solution (1 N, 3 mL) was stirred at room temperature for 2 h. The solution was neutralized by 1 N HOAc. The mixture was evaporated and co-evaporated with EtOH. The residue was purified by flash chromatography on silica gel, eluting with CH_2Cl_2/MeOH (95:5), to give 680 mg (quant.) of product as a pale yellow solid. This crude product was used for the next reaction without further purification.

4.1.7. (2′*R*)-β-D-2′-Deoxy-2′,5-difluoro-3′,5′-di-*O*-tetrahydropyranyl-uridine (10). To a stirred mixture of **10** (680 mg, 1.77 mmol) in anhydrous CH_2Cl_2 (10 mL) and anhydrous pyridine (1.7 mL) at −70 °C was added DAST (887 mg, 5.5 mmol) and the mixture was allowed to warm up to room temperature. The mixture was heated at reflux for 4 h. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$ (2 mL) and ice water (10 mL). The mixture was extracted with CH_2Cl_2 (3 × 20 mL). The combined extracts were washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4), and concentrated to give 735 mg (96%) of product as brown oil. This crude product was used for the next reaction without further purification.

4.1.8. (2′*R*)-β-D-2′-Deoxy-2′,5-difluorouridine (11).[26] To a solution of **10** (735 mg, 1.70 mmol) in anhydrous MeOH (15 mL) was added *p*-TsOH (500 mg, mmol), and the solution was stirred at room temperature for 3 h. After the removal of the solvent by evaporation, the residue was co-evaporated with pyridine three times to give 427 mg (95%) of product as a brown solid. From this crude product, a sample of pure compound was obtained by recrystallization from MeOH/CH_2Cl_2/hexane, while the crude product was used for the next reaction without further purification. The NMR data was in agreement with that which was previously reported.[26] mp 135–137 °C; ^{1}H NMR (DMSO-d_6) δ 11.90 (s, 1H, NH), 8.34 (d, J = 7.2 Hz, 1H, H-6), 5.86 (d, J = 16.4 Hz, 1H, H-1′), 5.63 (d, J = 8.0 Hz, 1H, OH-3′), 5.41 (t, 1H, OH-5′), 5.02 (dd, J = 53.0 Hz, 1H, H-2′), 4.17 (m, 1H, H-3′), 3.90 (m, 1H, H-4′), 3.72 (m, 2H, H-5′).

4.1.9. (2′*R*)-β-D-2′-Deoxy-3′,5′-di-*O*-acetyl-2′,5-difluorouridine (12). To a solution of **11** (404 mg, 1.53 mmol) in anhydrous pyridine (5 mL) at 0 °C was added Ac_2O (1 mL, mmol), and the solution was stirred at room temperature overnight. After the removal of the solvent by evaporation, the residue was partitioned between water and $CHCl_3$. The organic layer was separated, dried (Na_2SO_4), evaporated, and co-evaporated with toluene to give 497 mg (93%) of product as a brown solid. This crude product was used for the next reaction without further purification.

4.1.10. (2′*R*)-β-D-2′-Deoxy-2′,5-difluorocytidine (13). To a solution of **12** (497 mg, 1.43 mmol) in anhydrous CH_3CN (20 mL) and Et_3N (0.80 mL, 5.71 mmol) at 0 °C was added TIPSCl (892 mg, 2.85 mmol), followed by DMAP (174 mg, 1.43 mmol). The solution was stirred at room temperature for 24 h, and then concentrated NH_4OH (50 mL) was added. After stirring at room temp for 24 h, the solvent was evaporated, and the residue was purified by flash chromatography on silica gel, eluting with CH_2Cl_2/MeOH (4:1), to give 101 mg (27%) of product as a pale yellow solid. Mp 164–166 °C; ^{1}H NMR (DMSO-d_6) δ 8.24 (d, J = 3.6 Hz, 1H, H-6), 7.84, 7.58 (2s, 2H, NH_2), 5.83 (d, J = 17.2 Hz, 1H, H-1′), 5.57 (d, J = 6.4 Hz, 1H, OH-3′), 5.35 (t, J = 4.8 Hz, 1H, OH-5′), 4.87 (dd, J = 4.4 and 53.2 Hz, 1H, H-2′), 4.20–4.08 (m, 1H, H-3′), 3.87 (d, J = 8.0 Hz, 1H, H-4′), 3.80, 3.60 (2m, 2H, H-5′); MS (FAB$^+$) *m/e* 270 [(M+Li)$^+$]; HRMS (FAB$^+$) calcd for ($C_9H_{11}F_2N_3O_4$+Li): 270.0878, found: 270.0869. Anal. Calcd for $C_9H_{11}F_2N_3O_4$+H_2O: C 38.44, H 4.66, N 14.94. Found: C 38.82, H 4.61, N 14.78.

4.1.11. β-D-2,2′-Anhydro-5-methyluridine (17). To a mixture of **15** (5.16 g, 20 mmol) and diphenyl carbonate (5.72 g) in anhydrous HMPA (35 mL) was added $NaHCO_3$ (130 mg, mmol), and the mixture was stirred at 150 °C for 30 min. The resulting solution was cooled to room temperature, and poured into cold water (120 mL). The mixture was washed with $CHCl_3$ (3 × 50 mL), and concentrated in vacuo. The residue was crystallized from MeOH to give 3.07 g (64%) of product as a white solid. ^{1}H NMR (DMSO-d_6) δ 7.75 (s, 1H, H-6), 6.29 (d, J = 6.0 Hz, 1H, H-1′), 5.88 (s, 1H, H-2′), 5.17 (d, J = 6.0 Hz, OH-3′), 4.97 (t, J = 5.2 Hz, 1H, OH-5′), 4.37 (s, 1H, H-3′), 4.06 (m, 1H, H-4′), 3.24, 3.17 (2m, 2H, H-5′), 1.79 (s, 3H, CH_3).

4.1.12. (2′*R*)-β-D-2′-Fluorothymidine (19).[14] A solution of **17** (3.0 g, 12.5 mmol) in anhydrous 1,4-dioxane (150 mL) and HF–pyridine (50 mL) was sealed in a tight stainless bomb and heated at 120–125 °C for 18 h. After cooling to room temperature, the solution was neutralized with solid $CaCO_3$, and stirred at room temperature overnight. The solid was filtered and washed with MeOH (50 mL). The combined filtrates were evaporated and the residue was purified by flash chromatography on silica gel, eluting with CH_2Cl_2/MeOH (4:1), to give 1.02 g (31%) of product as a white solid. Mp 185–187 °C; ^{1}H NMR (DMSO-d_6) δ 11.41 (s, 1H, NH), 7.80 (s, 1H, H-6), 5.91 (d, J = 6.0 Hz, 1H, H-1′), 5.62 (d, J = 6.0 Hz, OH-3′), 5.26 (t, J = 5.2 Hz, 1H, OH-5′), 5.02 (d, J = 52.8 Hz, 1H, H-2′), 4.21–4.11 (m, 1H, H-3′), 3.85 (d, J = 6.8 Hz, 1H, H-4′), 3.76, 3.59 (2m, 2H, H-5′), 1.75 (s, 3H, CH_3).

4.1.13. (2′*R*)-β-D-2′-Deoxy-3′,5′-di-*O*-acetyl-2′-fluorouridine (20). In an analogous manner to the preparation of **12**, the title compound **20** was prepared in the yield of 88%. This crude product was used for the next reaction without further purification.

4.1.14. (2′*R*)-β-D-3′,5′-Di-*O*-acetyl-2′-fluorothymidine (21). In an analogous manner to the preparation of **12**, the title compound **21** was prepared in a quantitative yield. This crude product was used for the next reaction without further purification.

4.1.15. (2′*R*)-β-D-2′-Deoxy-2′-fluoro-5-methylcytidine (22). In an analogous manner to the preparation of **13**, the title compound **22** was prepared in the yield of 57%. Mp 185–187 °C (dec); ^{1}H NMR (DMSO-d_6) δ 7.75 (s, 1H, H-6), 7.39, 6.87 (2s, 2H, NH_2), 5.88 (dd, J = 1.6 and 18.4 Hz, 1H, H-1′), 5.53 (d, J = 6.8 Hz, 1H, OH-3′), 5.20 (t, J = 5.2 Hz, 1H, OH-5′), 4.93, 4.79 (2m, 1H, H-2′), 4.20–4.05 (m, 1H, H-3′), 3.84 (m, 1H, H-4′), 3.77, 3.60 (2m, 2H, H-5′), 1.81 (s, 3H, CH_3). MS (FAB$^+$) *m/e* 266 [(M+Li)$^+$]; HRMS (FAB$^+$) calcd for ($C_{10}H_{14}FN_3O_4$+Li): 266.1128, found: 266.1117. Anal. Calcd for $C_{10}H_{14}FN_3O_4$+0.5H_2O: C 44.86, H 5.46, N 15.69. Found: C 45.87, H 5.39, N 15.81.

4.1.16. (2′*R*)-β-D-2′-Deoxy-2′-fluoro-N^4-hydroxycytidine (23). To a solution of **20** (430 mg, 1.3 mmol) in anhydrous CH_3CN (25 mL) and Et_3N (0.72 mL, 5.2 mmol) at 0 °C was added TIPSCl (813 mg, 2.6 mmol), followed by DMAP (159 mg, 1.3 mmol). The solution was stirred at room temperature for 24 h, and then NH_2OH–HCl (185 mg, 2.6 mmol) was added. After stirring at room temperature for 24 h, the solvent was evaporated, and the residue was passed through a silica gel column, eluting with CH_2Cl_2/MeOH (95:5). The eluent was concentrated to give white foam, to which NH_3/MeOH (2.0 M solution, 25 mL) was added. The mixture was stirred in a stoppered flask at room temperature for 14 h. After the removal of the solvent by evaporation, the residue was purified by flash chromatography on silica gel, eluting with CH_2Cl_2/MeOH (5:1), to give 133 mg (39%) of product as a white solid. Mp 201–203 °C (dec); ^{1}H NMR (DMSO-d_6) δ 10.02 (s, 1H, N*H*OH), 9.65 (s, 1H, NHO*H*), 7.06 (d, J = 8.0 Hz, 1H, H-6), 5.86 (d, J = 3.6 Hz, 1H, H-1′), 5.59 (d, J = 8.0 Hz, 1H, H-5), 5.57 (d, J = 6.4 Hz, 1H, OH-3′), 5.11 (t, J = 5.2 Hz, 1H, OH-5′), 5.04, 4.91 (2m, 1H, H-2′), 4.17–4.07 (m, 1H, H-3′), 3.81 (m, 1H, H-4′), 3.67, 3.55 (2m, 2H, H-5′). MS (FAB$^+$) *m/e* 268 [(M+Li)$^+$]. Anal. Calcd for $C_9H_{12}FN_3O_5$: C 41.38, H 4.63, N 16.09. Found: C 41.62, H 4.66, N 16.05.

4.1.17. (2′*S*)-β-L-3′,5′-Di-*O*-acetyl-2′-deoxy-2′-fluorouridine (31). To a suspension of β-L-2,2′-anhydrouridine (**27**) (17.0 g, 75.0 mmol) in DMF (300 mL) and 3,4-dihydropyran (180 mL) at 0 °C was added *p*-toluenesulfonic acid (14.0 g) and the mixture was stirred at 0 °C for 4 h when a clear solution was obtained. It was neutralized with Et_3N (30 mL) and then evaporated to dryness. The residue was redissolved in EtOAc, washed with saturated $NaHCO_3$, and dried ($MgSO_4$). Removal of solvent gave a residue, which was triturated with hexanes and filtered. The filter cake was washed with hexanes and dried to give (2′*S*)-β-L-2,2′-anhydro-3′,5′-di-*O*-tetrahydropyranyluridine (**28**) as a white solid (27.5 g, 93.0%), which was pure enough for the next reaction.

A suspension of **28** (23.0 g, 58.5 mmol) in MeOH (300 mL) and 1 N NaOH (100 mL) was stirred at room temperature for 2 h, then neutralized with dilute acetic acid. The mixture was evaporated to dryness and the residue was loaded to a silica gel pad and eluted with EtOAc to give (2′*S*)-β-L-3′,5′-di-*O*-tetrahydropyranyl-arabinouridine (**29**) as a white solid (22.6 g, 94.0%).

To a stirred mixture of **29** (20.6 g. 50.0 mmol) in CH_2Cl_2 (300 mL) and pyridine (50 mL) at −60 °C was added DAST (25.0 g, 155 mmol) under N_2. It was slowly warmed up to room temperature and then refluxed for 4 h. The reaction was quenched by saturated $NaHCO_3$ and ice-water, then extracted with CH_2Cl_2 (100 mL × 3), washed with saturated $NaHCO_3$, and dried ($MgSO_4$). Removal of solvent gave a dark-brown syrup (18.9 g), which was redissolved in MeOH (300 mL). To this solution was added *p*-toluenesulfonic acid (6 g) and the mixture was stirred at room temperature for 3 h. It was neutralized with pyridine (50 mL), co-evaporated with pyridine (50 mL × 2), and then redissolved in pyridine (100 mL). To this solution was added Ac_2O (20 mL) and the mixture was stirred at room temperature for 20 h. The removal of the solvent and recrystallization from EtOH gave **31** as a white solid (6.8 g, 41% from **29**): ^{1}H NMR ($CDCl_3$) δ 8.62 (s, 1H, NH, D_2O exchangeable), 7.33 (d, IH, H-6, J = 8.1 Hz). 5.72 (dd, 1H, H-1′, $J_{1',2'}$ = 2.0, $J_{1',F}$ = 25.0 Hz 5.70 (d, 1H, H-5, J = 8.1 Hz), 5.30 (ddd, 1H, H-2′, $J_{2',F}$ = 52.2 Hz 5.08 (ddd, 1H, H-3′, $J_{3'F}$ = 17.9 Hz), 4.31 (m, 3H, H-4′, H-5′a,b), 2.07, 2.03 (2s, 6H, Ac).

4.1.18. (2′*S*)-β-L-2′-Deoxy-2′-fluorouridine (32). Compound **31** was treated with satd NH_3/MeOH at room temperature for 20 h to give, after crystallization from water, **32** as a white solid (83%): mp 150–152 °C; $[\alpha]_D^{25}$ −50.26 (*c* 0.19, MeOH); ^{1}H NMR (DMSO-d_6) δ 11.39 (s, 1H NH, D_2O exchangeable), 7.91 (d, 1H H-6, J = 8.1 Hz), 5.87 (br d, 1H, H-1′, $J_{1'F}$ = 17.5 Hz), 5.62 (d, 1H 3′-OH, D_2O exchangeable), 5.61 (d. 1H H-5, J = 8.1 Hz), 5.20 (br s, 1H 5′-OH, D_2O exchangeable), 5.00 (dt, 1H H-2′, $J_{2'F}$ = 53.1 Hz), 4.13 (dm, 1H H-3′, $J_{3'F}$ = 20.8 Hz), 3.86 (m, 1H H-4′), 3.66 (m, 2H, H-5′a,b). Anal. Calcd for $C_9H_{11}FN_2O_5$+0.2H_2O: C 43.24, H 4.48, N 11.21. Found: C 43.18, H 4.60, N 11.06.

4.1.19. (2′*S*)-β-L-5-Chloro-2′-deoxy-2′-fluorouridine (33). A mixture of **31** (1.0 g, 3.0 mmol) and *N*-chlorosuccinimide (0.8 g, 6.0 mmol) in AcOH (30 mL) was stirred at 80–90 °C for 3 h. It was then poured into ice-water, extracted with $CHCl_3$ (50 mL × 3), washed with saturated $NaHCO_3$ and dried $MgSO_4$. After the removal of the solvent, the resulting white foam was treated with 0.2 N $NaOCH_3$/CH_3OH at room temperature overnight. After co-evaporation with EtOH, product **33** was obtained as a white powder (75%): mp 159–161 °C; $[\alpha]_D^{25}$: −17.60 (*c* 0.29, MeOH); ^{1}H NMR (DMSO-d_6) δ 8.47 (s, 1H, H-6), 5.86 (br d, 1H, H-1′,

$J_{1'F}$ = 15.6 Hz) 5.64 (d, 1H, 3′-OH, D_2O exchangeable), 5.44 (t, 1H, 5′-OH, D_2O exchangeable), 5.04 (dd, 1H, H-2′, $J_{2'F}$ = 52.9 Hz) 4.19 (dm, 1H, H-3′, $J_{3'F}$ = 23.8 Hz), 3.91 (m, 1H, H-4′), 3.73 (m, 2H, H-5′a,b). Anal. Calcd for $C_9H_{10}ClFN_2O_5$+0.8H_2O: C 36.61, H 3.93, N 9.49. Found: C 16.96, H 4.02, N 9.17.

4.1.20. (2′*S*)-β-L-5-Bromo-2′-deoxy-2′-fluorouridine (34). To a stirred mixture of **31** (1.0 g, 3.0 mmol) in Ac_2O (20 mL) and AcOH (10 mL) was added Br_2 (1 mL) in AcOH (3.0 mL), and the mixture was sealed and stirred at room temperature overnight. To this was added EtOH slowly, and the mixture was evaporated to dryness and collaborated with EtOH until no more fume formed. The residue was then purified by silica gel column chromatography (9:1 $CHCl_3$–CH_3OH) to give a white solid (0.89 g, 92%): mp 164–166 °C; $[\alpha]_D^{25}$ −8.66 (*c* 0.47, MeOH); ^{1}H NMR (DMSO-d_6) δ 11.89 (s, 1H, NH, D_2O exchangeable), 8.50 (s, 1H, H-6), 5.86 (br d, 1H, H-1′, $J_{1',F'}$ = 16.4 Hz), 5.63 (br s, 1H, 3′-OH, D_2O exchangeable), 5.44 (br s, 1H, 5′-OH, D_2O exchangeable), 4.99 (dd, 1H, H-2′, $J_{2',F}$ = 53.1 Hz), 4.18 (dm, 1H, H-3′, $J_{3',F}$ = 23.7 Hz), 3.90 (m, 1H, H-4′), 3.73 (m, 2H, H-5′a,b). Anal. Calcd for $C_9H_{10}BrFN_2O_5$+0.6H_2O: C 32.15, H 3.33, N 8.34. Found: C 32.03, H 3.27, N 8.34.

4.1.21. (2′*S*)-β-L-2′-Deoxy-2′-fluoro-5-iodouridine (35). A mixture of **31** (3.3 g, 10 mmol) and ICl (2.4 g, 15 mmol) in CH_2C1_2 (100 mL) was stirred at reflux for 5 h. It was then diluted with CH_2C1_2 (150 mL), washed successively with $NaHSO_3$ (100 mL × 3), saturated $NaHCO_3$ (50 mL × 2) and dried ($MgSO_4$). After removal of the solvent, the residue was treated with $NaOCH_3$/CH_3OH. Trituration in ether followed by recrystallization from water gave **35** as a white solid (78%): mp 217–218 °C; $[\alpha]_D^{25}$ +9.41 (*c* 0.36, MeOH); ^{1}H NMR (DMSO-d_6) δ 11.75 (s, 1H, NH, D_2O exchangeable), 8.54 (s, 1H, H-6), 5.86 (br d, 1H, H-1′, $J_{1',F}$ = 16.8 Hz), 5.62 (d, 1H, 3′-OH, D_2O exchangeable), 5.41 (t, 1H, 5′-OH, D_2O exchangeable), 5.04 (dd, 1H, H-2′, $J_{2',F}$ = 53.1 Hz), 4.18 (dm, 1H, H-3′, $J_{3',F}$ = 23.4 Hz), 3.90 (m, 1H, H-4′), 3.72 (m, 2H, H-5′a,b). Anal. Calcd for $C_9H_{10}FIN_2O_5$: C 29.05, H 2.71, N 7.53. Found: C 29.11, H 2.85, N 7.33.

4.1.22. (2′*S*)-β-L-5-Bromo-2′-deoxy-2′-fluorocytidine (38). Compound **34** was treated with Ac_2O in pyridine to give **36**, which was further treated in a similar manner as described for the synthesis of **40**. Trituration of the crude product with acetone gave **38** as a white solid (38%): mp >145 °C (dec); $[\alpha]_D^{25}$ −30.06 (*c* 0.54, MeOH); ^{1}H NMR (DMSO-d_6) δ 8.43 (s, 1H, H-6), 7.94, 7.08 (2s, 2H, NH_2, D_2O exchangeable), 5.85 (d, 1H, H-1′, $J_{1',F}$ = 17.1 Hz), 5.56 (d, 1H, 3′-OH, D_2O exchangeable), 5.38 (br s, 1H, 5′-OH, D_2O exchangeable), 4.91 (dd, 1H, H-2′, $J_{2',F}$ = 53.1 Hz), 4.16 (dm, 1H, H-3′, $J_{3',F}$ = 24.3 Hz), 3.86 (m, 1H, H-4′), 3.72 (m, 2H, H-5′a,b). Anal. Calcd for $C_9H_{11}BrFN_3O_4$+ 0.5C_2H_5OH: C 34.57, H 4.03, N 12.09. Found: C 34.36, H 3.78, N 11.78.

4.1.23. (2′*S*)-β-L-2′-Deoxy-2′-fluoro-5-iodocytidine (39). Compound **39** was prepared from **35** in 40% yield as described for **40**. Trituration of the crude product in acetone gave a yellow solid: mp 182–184 °C; $[\alpha]_D^{25}$ −6.66 (*c* 0.79, MeOH); ^{1}H NMR (DMSO-d_6) δ 8.46 (s, 1H, H-6), 7.91, 6.70 (2s, 2H, NH_2, D_2O exchangeable), 5.84 (d, 1H, H-1′, $J_{1',F}$ = 17.0 Hz), 5.56 (d, 1H, 3′-OH, D_2O exchangeable), 5.36 (t, 1H, 5′-OH, D_2O exchangeable), 4.90 (dd, 1H, H-2′, $J_{2',F}$ = 53.2 Hz), 4.15 (dm, 1H, H-3′, $J_{3',F}$ = 24.2 Hz), 3.88 (m, 1H, H-4′), 3.70 (m, 2H, H-5′a,b). Anal. Calcd for $C_9H_{11}FIN_3O_4$: C 29.13, H 2.99, N 11.32. Found: C 29.02, H 3.00, N 11.20.

4.1.24. (2′*S*)-β-L-2′-Deoxy-2′-fluorocytidine (40). To a stirred mixture of **31** (1.32 g, 4.0 mmol) and 1,2,4-triazole (2.20 g, 32.0 mmol) in pyridine (20 mL) was added $POCl_3$ (0.75 mL, 8.0 mmol), and the mixture was stirred at room temperature for 17 h. The solvent was evaporated and the residue was treated with NH_4OH–dioxane at room temperature for 4 h followed by satd NH_3/CH_3OH at room temperature for 12 h. The removal of the solvent gave a residue, which was recrystallized from cold water to give **40** as a white solid (750 mg, 77%): mp 164–167 °C; $[\alpha]_D^{25}$ −68.85 (*c* 0.35, MeOH); ^{1}H NMR (DMSO-d_6) δ 7.90 (d, 1H, H-6, *J* = 7.3 Hz), 7.24 (br d, 2H, NH_2, D_2O exchangeable), 5.99 (d, 1H, H-1′, $J_{1'F}$ = 17.8 Hz), 5.88 (d, 1H, H-5, *J* = 7.3 Hz), 5.73 (d, 1H, 3′-OH, D_2O exchangeable), 5.16 (t, 1H 5′-OH, D_2O exchangeable), 4.89 (dd, 1H H-2′, $J_{2'F}$ = 53.3 Hz), 4.11 (dm, IH, H-3′, $J_{3'F}$ = 22.4 Hz), 3.86 (m, 1H, H-4′), 3.68 (m, 2H, H-5′a,b). Anal. Calcd for $C_9H_{12}FN_3O_4$+2H_2O: C 38.41, H 5.69, N 14.94. Found: C 38.49, H 5.72, N 14.87.

4.1.25. (2′*S*)-β-L-5-Chloro-2′-deoxy-2′-fluorocytidine (41). To a solution of **40** (200 mg, 0.8 mmol) in DMF (5 mL) was added a solution of HCl/DMF (2 M, 0.6 mL) followed by a solution of *m*-CPBA (350 mg, 1.6 mmol) in DMF (1 mL). The mixture was stirred at room temperature for 2 h, then evaporated to dryness. The residue was suspended in H_2O and MeOH, filtered, and washed with H_2O. The combined filtrate was treated with concentrated NH_4OH and then evaporated to dryness. The residue was purified by silica gel column chromatography (10:1 $CHCl_3$/CH_2OH) and collaborated with Et_2O to give **41** as a white solid (65 mg, 28.5%): mp >105 °C (dec); $[\alpha]_D^{25}$ −47.74 (*c* 0.19, MeOH); ^{1}H NMR (DMSO-d_6) δ 8.54 (s, 1H, H-6), 8.54, 8.26 (2s, 2H, NH_2, D_2O exchangeable), 5.82 (d, 1H, H-1′, $J_{1',F}$ = 16.4 Hz), 4.94 (dd, 1H, H-2′. $J_{2',F}$ = 53.1 Hz), 4.14 (dm, 1H, H-3′, $J_{3',F}$ = 25.42 Hz), 3.91 (m, 1H, H-4′), 3.72 (dm, 2H, H-5′a,b). Anal. Calcd for $C_9H_{11}ClFN_3O_4$+HCl+0.5H_2O: C 33.21, H 4.00, N 12.92. Found: C 32.98, H 3.75, N 12.86.

4.1.26. β-L-1,2-Di-*O*-acetyl-3,5-di-*O*-benzoylribofuranose (42). 1,2-Di-*O*-isopropylidene-3,5-di-*O*-benzoyl-α-L-ribofuranose[27] (50 g, 125 mmol) was stirred in AcOH (500 mL), Ac_2O (1.50 mL), and concentrated H_2SO_4 (10 mL) at room temperature for 16 h. It was then poured into ice-water, extracted with $CHCl_3$, washed with satd $NaHCO_3$, dried ($MgSO_4$). The evaporation of the solvent gave a syrup, from which, a white crystal was obtained (35 g, 63%), as well as a syrup of 20 g (36%): ^{1}H NMR ($CDCl_3$) δ 8.10, 7.41 (m, 10H, Bz), 6.29 (br s, 1H, H-l), 5.80 (t, 1H, H-2), 5.58 (d, 1H,

H-3), 4.77, 4.50 (m, 3H, H-4, H-5a,b), 2.07, 2.00 (2s, 6H, Ac).

4.1.27. β-L-2,2′-Anhydro-5-methyluridine (47). A suspension of thymine (2.52 g, 20.0 mmol) in 1,1,1,3,3,3-hexamethyldisilazane (30 mL) was stirred at reflux under argon for 3 h to give a clear solution, which was then evaporated to dryness in vacuo to give an oil. To this was added **42** (4.42 g, 10.0 mmol) under argon followed by CH_3CN (40 mL), and the mixture was stirred at 0 °C, while TMSOTf (3.88 mL, 20.0 mmol) was added through a syringe, and the mixture was stirred at room temperature overnight. It was then poured into ice-water, extracted with $CHCl_3$ and washed with saturated $NaHCO_3$ and dried ($MgSO_4$). The removal of the solvent followed by recrystallization in MeOH gave **43** as a white solid (3.7 g, 73%).

Compound **43** (3.5 g, 6.9 mmol) was treated with NaOMe (1.5 g) in MeOH (50 mL) at room temperature overnight. It was then neutralized with Dowex 50w × 8 (H^+) resin and then filtered and washed with MeOH. The combined filtrate was evaporated to dryness and the residue was redissolved in water (50 mL), washed with Et_2O (50 mL × 2) and then evaporated to dryness. The residue was collaborated with EtOH to give **45** as a white foam (1.7 g, 96%).

To a stirred solution of **45** (1.6 g, 6.0 mmol) in DMF (10 mL) was added diphenylcarbonate (2.0 g, 11 mmol) and $NaHCO_3$ powder (100 mg). The mixture was stirred at 150 °C for 1 h and then cooled down to room temperature and poured into Et_2O (50 mL). After 30 min, the precipitate formed was filtered and washed with Et_2O (10 mL × 2). The filter cake was recrystallized twice from EtOH to give **47** as a white solid (1.35 g, 91.0%): mp 222–225 °C.

4.1.28. β-L-2,2′-Anhydro-5-fluorouridine (48). The title compound was prepared from 5-fluorouracil and **42** in a similar way as described for the synthesis of **47**; 81% yield for **44**, 94% yield for **46**, and 62% yield for **48**: mp 193–196 °C.

4.1.29. (2′*S*)-β-L-2′-Fluorothymidine (55). The title compound was prepared from **47** by using a similar procedure as described for the synthesis of **32**; **55**, white solid from water (83%): mp 185–188 °C; $[\alpha]_D^{25}$ −31.88 (*c* 0.26, MeOH); ^{1}H NMR (DMSO-d_6) δ 11.41 (s, 1H, NH, D_2O exchangeable), 7.80 (s, 1H, H-6), 5.92 (dd, 1H, H-1′, $J_{1',F}$ = 17.6 Hz), 5.62 (d, 1H, 3′-OH, D_2O exchangeable), 5.26 (t, 1H, 5′-OH, D_2O exchangeable), 5.02 (ddd, 1H, H-2′, $J_{2',F}$ = 53.2 Hz), 4.18 (dm, 1H, H-3′, $J_{3',F}$ = 20.1 Hz), 3.87 (m, 1H, H-4′), 3.68 (m, 2H, H-5′a,b), 1.77 (s, 3H, CH_3). Anal. Calcd for $C_{10}H_{13}FN_2O_5$: C 46.16, H 5.04, N 10.77. Found: C 45.99, H 5.09, N 10.78.

4.1.30. (2′*S*)-β-L-2′-Deoxy-2′,5-difluorouridine (56). The title compound was prepared from **48** by using a similar procedure as described for the synthesis of **32**; **56**, white powder (81%) after silica gel column chromatography (15:1/8:1 $CHCl_3/CH_3OH$): mp 134–136 °C; $[\alpha]_D^{25}$ −60.35 (*c* 0.14, MeOH); ^{1}H NMR (DMSO-d_6) δ 11.91 (s, 1H, NH, D_2O exchangeable), 8.34 (d, 1H, H-6, *J* = 7.1 Hz), 5.86 (d, 1H, H-1′, $J_{1',F}$ = 16.4 Hz), 5.63 (d, 1H, 3′-OH, D_2O exchangeable), 5.41 (t, 1H, 5′-OH, D_2O exchangeable), 5.02 (dd, 1H, H-2′, $J_{2',F}$ = 53.0 Hz), 4.16 (dm, 1H, H-3′, $J_{3',F}$ = 22.5 Hz), 3.90 (m, 1H, H-4′), 3.72 (m, 2H, H-5′a,b). Anal. Calcd for $C_9H_{10}F_2N_2O_5$+-H_2O: C 38.27, H 4.25, N 9.92. Found: C 38.42, H 4.33, N 9.90.

4.1.31. (2′*S*)-β-L-2′-Deoxy-2′-fluoro-5-methylcytidine (59). The title compound was prepared from **57** by using a similar procedure as described for the synthesis of **38**; **59**, 32% yield after crystallization from acetone: mp >143 °C (dec); $[\alpha]_D^{25}$ −49.68 (*c* 0.25, MeOH); ^{1}H NMR (DMSO-d_6) δ 7.76 (s, 1H, H-6), 7.42, 6.91 (2s, 2H, NH_2, D_2O exchangeable), 5.89 (dd, 1H, H-l′, $J_{1',F}$ = 18.1 Hz), 5.54 (d, 1H, 3′-OH, D_2O exchangeable), 5.22 (t, 1H, 5′-OH, D_2O exchangeable), 4.87 (dd, 1H, H-2′, $J_{2',F}$ = 53.5 Hz), 4.14 (dm, 1H, H-3′, $J_{3',F}$ = 22.4 Hz), 3.85 (m, 1H, H-4′), 3.68 (m, 2H, H-5′a,b), 1.77 (s, 3H, CH_3). Anal. Calcd for $C_{10}H_{14}FN_3O_4$+1.5H_2O: C 41.92, H 5.94, N 14.68. Found: C 41.50, H 5.37, N 15.10.

4.1.32. (2′*S*)-β-L-2′-Deoxy-2′,5-difluorocytidine (60). The title compound was prepared from **58** by using a similar procedure as described for the synthesis of **38**; **60**, 69% after silica gel column chromatography (10:1/5:1 $CHCl_3/CH_3OH$): hygroscopic solid; $[\alpha]_D^{25}$ −90.68 (*c* 0.16, MeOH); ^{1}H NMR (DMSO-d_6) δ 8.25 (d, 1H, H-6, *J* = 7.4 Hz), 7.85, 7.59 (2s, 2H, NH_2, D_2O exchangeable), 5.84 (d, 1H, H-1′, $J_{1',F}$ = 17.0 Hz), 5.58 (d, 1H, 3′-OH, D_2O exchangeable), 5.36 (t, 1H, 5′-OH, D_2O exchangeable), 4.89 (dd, 1H, H-2′, $J_{2',F}$ = 53.2 Hz), 4.14 (dm, 1H, H-3′, $J_{3',F}$ = 23.6 Hz), 3.88 (m, 1H, H-4′), 3.72 (m, 2H, H-5′a,b). Anal. Calcd for $C_9H_{11}F_2N_3O_4$+0.8H_2O: C 38.90, H 4.54, N 15.13. Found: C 38.99, H 4.50, N 15.02.

4.1.33. (2′*S*)-β-L-2′-Deoxy-2′-fluoro-5-ethynyluridine (62). Argon was bubbled through Et_3N (100 mL) for 0.5 h. To this was added compound **35** (1.22 g, 2.00 mmol), followed by CuI (50 mg), $(Ph_3P)_2PdCl_2$ (50 mg), and trimethylsilyl acetylene (0.71 mL, 5.0 mmol). The mixture was stirred at 50–60 °C for 7 h and then evaporated to dryness. The residue was redissolved in $CHCl_3$ (100 mL), washed with 5% EDTA solution (50 mL × 3) and satd $NaHCO_3$, dried ($MgSO_4$). Evaporation of solvent and purification on silica gel column (100:1 $CHCl_3/CH_3OH$) followed by crystallization from CH_3OH gave a white solid (530 mg). To the solid (480 mg) was added 0.2 N $NaOCH_3/CH_3OH$ and the solution was stirred at room temperature for 15 h. It was then neutralized with Dowex 50w × 8 (H+) resin and filtered, washed with MeOH. The combined filtrate was evaporated to dryness, triturated with Et_2O to give **62** as a pale yellow solid (205 mg, 42%): mp >189 °C (dec); $[\alpha]_D^{25}$ −8.15 (*c* 0.32, MeOH); ^{1}H NMR (DMSO-d_6) δ 11.71 (s, 1H, NH, D_2O exchangeable), 8.46 (s, 1H, H-6), 5.86 (br d, 1H, H-l′, $J_{1',F}$ = 16.7 Hz), 5.62 (br d, 1H, 3′-OH, D_2O exchangeable), 5.39 (br s, 1H, 5′-OH, D_2O exchangeable), 5.04 (dd, 1H, H-2′,

$J_{2',F}$ = 53.0 Hz), 4.18 (dm, 1H, H-3′, $J_{3',F}$ = 23.5 Hz), 4.10 (s, 1H, CCH), 3.86 (m, 1H, H-4′), 3.50 (m, 2H, H-5′a,b). Anal. Calcd for $C_{11}H_{11}FN_2O_5$: C 48.89, H 4.10, N 10.37. Found: C 48.63, H 4.15, N 10.37.

4.1.34. (2′*S*)-β-L-2′-Deoxy-2′-fluoro-5-ethyluridine (63). Compound **62** (108 mg, 0.40 mmol) was stirred in EtOH (10 mL) with 10% Pd–C (50 mg) under H_2 at 1 atm for 1 h, then filtered and washed with EtOH. The combined filtrate was evaporated to dryness and co-evaporated with ether to give **63** as a white solid (110 mg, 100%): mp 158–160 °C; $[\alpha]_D^{25}$ −21.79 (*c* 0.46, MeOH); ^{1}H NMR (DMSO-d_6) δ 11.35 (s, 1H, NH, D_2O exchangeable), 7.78 (s, 1H, H-6), 5.90 (br d, 1H, H-1′, $J_{1',F}$ = 17.6 Hz), 5.71 (br s, 1H, 3′-OH, D_2O exchangeable), 5.34 (br s, 1H, 5′-OH, D_2O exchangeable), 5.01 (dd, 1H, H-2′, $J_{2',F}$ = 53.2 Hz), 4.18 (dm, 1H, H-3′, $J_{3',F}$ = 20.9 Hz), 3.85 (m, 1H, H-4′), 3.76 (m, 2H, H-5′a,b), 2.18 (q, 2H, CH_2CH_3), 1.06 (t, 3H, CH_2CH_3). Anal. Calcd for $C_{11}H_{15}FN_2O_5$: C 48.18, H 5.51, N 10.21. Found: C 48.24, H 5.47, N 10.14.

4.2. Biological evaluations

4.2.1. Anti-BVDV quantitative real time PCR assay. Anti-viral assays were performed as described.[7,19] Madin-Darby bovine kidney cells (MDBK, ATCC-CCL22) were grown in DMEM/F12 (Gibco/BRL, Gaithersburg, MD) supplemented with 10% heat-inactivated horse serum (Gibco/BRL). Cells were seeded in 96-well plates at 5×10^3 cells/well and incubated for 1 h (for the experiments on exponentially growing cells) or for 72 h (for experiments on confluent cells). The monolayer was infected with cpBVDV at a multiplicity of infection (MOI) of 0.02 pfu/cell. After 45 min of infection, the viral inoculum was removed and the cells were washed twice with culture medium. Media or media containing test compound was added to those cells, followed by a 24 h incubation (exponentially growing cells) or 72 h (confluent cell monolayer). Cell culture medium was collected, clarified by centrifugation (2 min, 3000*g*, room temperature) and viral RNA was prepared (QIAamp viral RNA mini Kit, Qiagen, Valencia, CA). Viral RNA was detected using quantitative real-time PCR (Q-rt-PCR).[19] Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide; Schering-Plough, Raritan, NJ) was used as a positive control in these experiments.

4.2.2. HCV replicon assay. HCV subgenomic replicon RNA-containing Huh7 cells (Clone A cells; Apath, LLC, St. Louis, MO) were kept in exponential growth in DMEM media (high glucose, no pyruvate) containing 10% fetal bovine serum, 1X non-essential amino acids, penicillin–streptomycin–glutamine (100 units/L, 100 μg/L, and 2.92 mg/L, respectively), and G418 (500–1000 μg/mL). Anti-viral assays were performed in the same media without G418. Cells were seeded in a 96-well plate at 1000 cells per well and test compounds were added immediately after seeding. Incubation times differed according to the type of experiment. At the end of the incubation, total cellular RNA was isolated (Rneasy 96 kit, Qiagen, CA). Replicon RNA and an internal control (TaqMan Ribosomal RNA control Reagents, Applied Biosystems, CA) were amplified in a single-step multiplex RT-PCR protocol, as described.[19] Recombinant interferon alfa-2a (INF-α-2a; Roferon-A, Hoffman La Roche Inc, NJ) served as a positive control.

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