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Synthesis and evaluation of NHC derivatives and 4′-fluorouridine prodrugs†

Li Xiang,[a,b] Tianwen Hu,[b,c] Haitao Xue,[b,c] Wenfang Pan,[b,c] Yuanchao Xie*[d] and Jingshan Shen *[a,b,c]

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β-D-N^4-Hydroxycytidine (NHC) derivatives with structural modifications at the C_4', O_4' or C_6 position and 4′-fluorouridine prodrugs were synthesized and evaluated for their antiviral activities against respiratory syncytial virus (RSV) or influenza virus (IFV) *in vitro*. The NHC derivatives were found inactive, but 4′-fluorouridine and its prodrugs had potent anti-RSV and anti-IFV activities. 4′-Fluorouridine was proved to be a nucleoside with poor stability, but the tri-ester prodrugs exhibited enhanced stability, especially tri-isobutyrate ester **1a**. This prodrug also showed excellent oral pharmacokinetic properties in rats, with potential to be an oral antiviral candidate.

Introduction

The emergence or re-emergence of epidemic viruses poses a serious threat to human health and economic security, exemplified by SARS-CoV-2 which has been circulating for nearly three years since the global outbreak in early 2020.[1] Using small-molecule antiviral agents is one of the most effective measures to cope with viral infections. As a well-known class of antiviral agents, nucleoside analogs that function by targeting the conserved active site of viral polymerase have been of research interest in the discovery of antiviral drugs. In recent years, two nucleoside drugs, remdesivir[2] and molnupiravir,[3] used for treating COVID-19 have been marketed, and their application may be further expanded due to their broad-spectrum antiviral properties.[4,5]

β-D-N^4-Hydroxycytidine (NHC, EIDD-1931), a cytidine analog, is the parent nucleoside of molnupiravir (EIDD-2801) (Fig. 1). During the catalysis of cellular kinases, it is converted to NHC triphosphate, which can substitute for cytidine triphosphate or uridine triphosphate by the SARS-CoV-2 RNA dependent RNA polymerase (RdRp) to embed in the growing RNA chain, thus resulting in a lethal mutation.[6] NHC also demonstrates antiviral activities against multiple other RNA viruses, including Ebola virus,[7] Venezuelan equine encephalitis virus (VEEV),[8] respiratory syncytial virus (RSV) and influenza A virus (IAV).[9] In BALB/cJ mice infected with RSV or IAV, oral administration of NHC significantly reduced the viral load in the lungs and alleviated disease biomarkers. This nucleoside showed favorable oral pharmacokinetic (PK) properties in mice and ferrets.[9]

From a chemical perspective, NHC bearing a hydroxylamine group at the base C_4 position is very similar to cytidine and uridine, and able to form hydrogen bonds with adenosine and guanosine, respectively. Because of the high structural similarity, viral polymerase is unable to discriminate NHC triphosphate from natural pyrimidine nucleoside triphosphates, and therefore accepts it as a substrate for viral RNA elongation. For the design of antiviral nucleoside analogs, we envisaged that large structural changes in natural nucleosides may have a negative impact on the antiviral activity. Accordingly, in this study, we designed and synthesized several NHC derivatives with minor structural modifications, including 4′-fluorination, replacement of the 4′-oxygen atom of ribose with a sulfur atom, and replacement of the C_6 carbon atom with a nitro atom in the base moiety of NHC. To our disappointment,

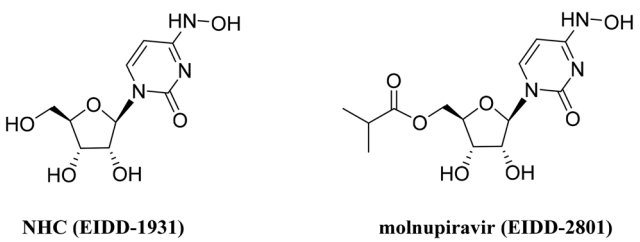

Fig. 1 The structures of NHC and molnupiravir.

[a]*School of Chinese Materia Medica, Nanjing University of Chinese Medicine, Nanjing 210023, P. R. China. E-mail: shenjingshan@simm.ac.cn*
[b]*State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, P. R. China*
[c]*University of Chinese Academy of Sciences, Beijing 100049, P. R. China*
[d]*Lingang Laboratory, Shanghai 200031, P. R. China. E-mail: xieyuanchao@lglab.ac.cn*

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these nucleoside derivatives did not show inhibitory effects against IFV or RSV. However, 4′-fluorouridine tri-isobutyrate which was used for the synthesis of 4′-fluoro-substituted NHC was found to be a potent replication inhibitor against IFV. We further synthesized several ester prodrugs of 4′-fluorouridine and evaluated their antiviral activities, chemical stability in buffer solution and PK properties, which provided tri-isobutyrate ester **1a** as a promising oral antiviral candidate.

Results and discussion

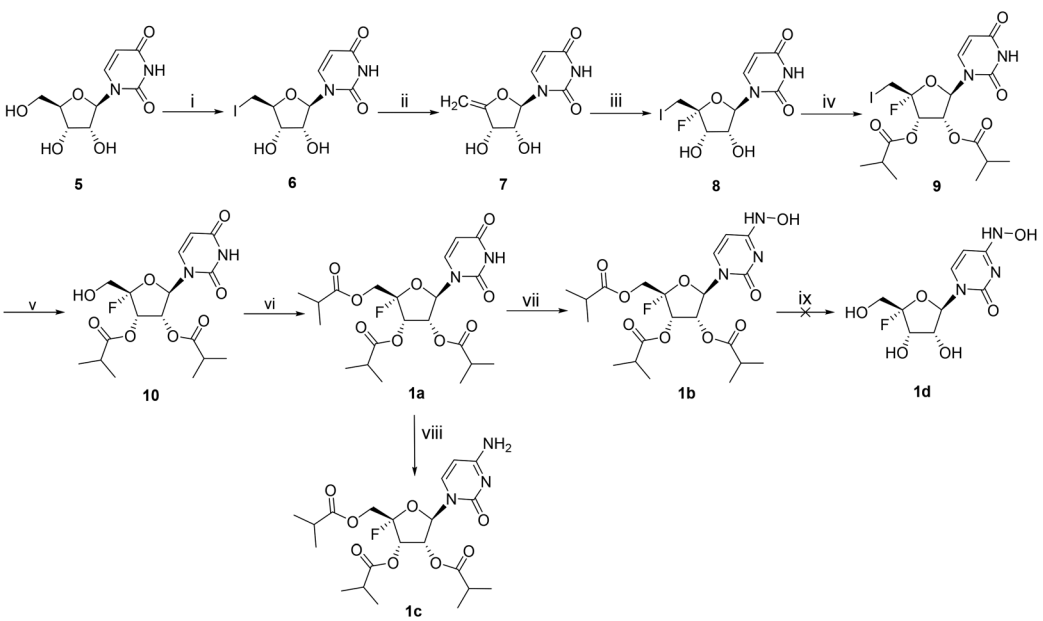
Synthesis of NHC derivatives and 4′-fluorouridine prodrugs

The synthetic approach towards compounds **1a–d** is shown in Scheme 1. The methods for introducing a fluorine atom into the C_4' position of ribonucleosides have been well documented.[10–12] Starting from uridine **5**, the 5′-hydroxyl group was readily converted into iodine through an Appel reaction, and the obtained intermediate **6** was subjected to elimination in the presence of sodium methoxide (CH_3ONa)/methanol to yield **7**. The synthesis of the key intermediate **10** involved three sequential steps: iodofluorination, isobutyrylation and oxidation. The isobutyrylation of the 5′-hydroxyl group of **10** afforded tri-isobutyrate ester **1a**, which subsequently underwent hydroxylamination at the C_4 position of the base moiety to provide **1b** in 18% yield using 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl), 4-dimethylaminopyridine (DMAP), triethylamine, and hydroxylamine hydrochloride. Compound **1c** was obtained in the same way. We tried to synthesize **1b** by direct hydroxylamination of **1c** by following a recently reported method,[13] but it was not achieved. The reaction gave complicated products which was mainly due to the low stability of the 4′-fluorine skeleton when heated. The removal of the isobutyryl groups of **1b** was performed with 0.2 equiv. of potassium carbonate in methanol, but we did not obtain the pure product **1d** because it was easily decomposed during the purification process.

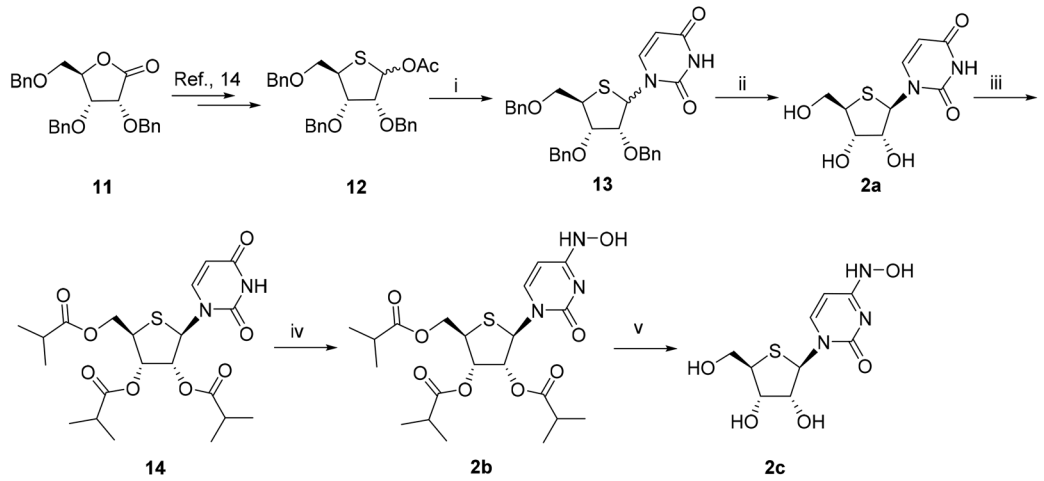
The synthesis of **2a–c** is shown in Scheme 2. Compound **12** was synthesized starting from 2,3,5-tri-*O*-benzyl-D-ribonolactone **11** according to reported methods.[14] The reaction of **12** with uracil under Vorbruggen glycosylation reaction conditions yielded an anomeric mixture **13** ($\alpha:\beta = 1:1$) in 85% overall yield. After debenzylation with BCl_3, the obtained crude was purified by silica gel column chromatography to give β-nucleoside **2a** in 76% yield. Compound **2a** was then esterified with isobutyric anhydride, followed by hydroxylamination using the aforementioned method to give **2b** in 54% yield over two steps. After the removal of the isobutyryl group, compound **2c** was obtained.

The 6-azauridine derivatives **3a–d** were synthesized as shown in Scheme 3. The reaction of commercial 6-azauracil **16** with peracylated sugar **15** yielded β-nucleoside **17** when using the general glycosylation method. Compound **17** was treated with phosphorus oxychloride/Et_3N to generate an active intermediate, which then reacted with hydroxylamine hydrochloride and ammonia to give **3a** and **3c**, respectively. The removal of the acetyl group of **3a** and **17** in ammonia methanol solution afforded nucleosides **3b** and **3d** that were solidified in methyl *tert*-butyl ether (MTBE).

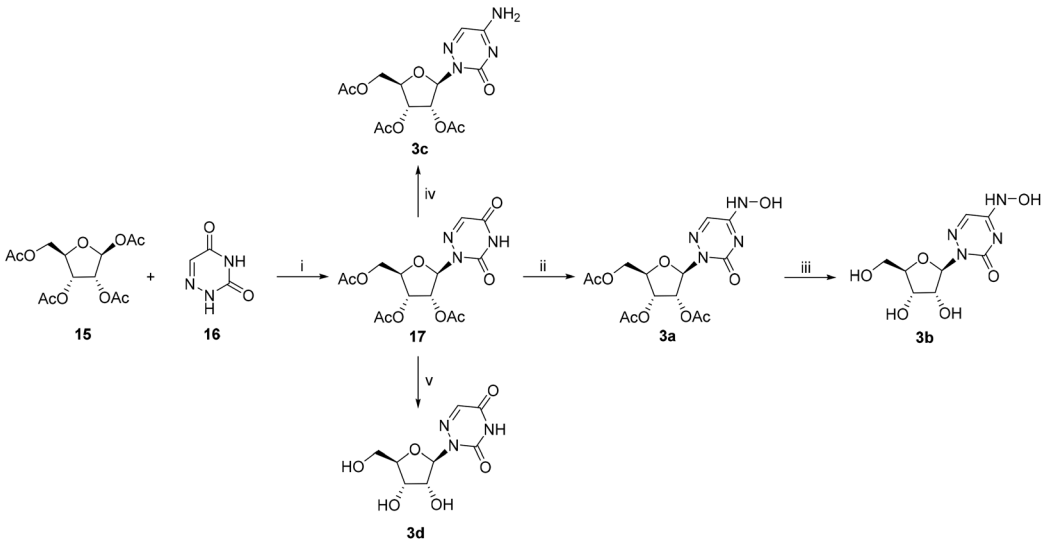
Five ester prodrugs (**4a–e**) of 4′-fluorouridine were synthesized, and the routes are shown in Scheme 4. The 2′ and 3′ hydroxyl groups of intermediate **8** were protected with benzyl-



Scheme 1 Reagents and conditions: (i) PPh_3, I_2, pyridine, 0 °C to RT, 67%; (ii) CH_3ONa, MeOH, 67 °C, 57%; (iii) NIS, $Et_3N{\cdot}3HF$, acetonitrile, 0 °C to RT, 77%; (iv) isobutyric anhydride, DMAP, Et_3N, CH_2Cl_2, RT, 47%; (v) TBAOH, TFA, *m*-CPBA, CH_2Cl_2, RT, 52%; (vi) isobutyric anhydride, DMAP, Et_3N, CH_2Cl_2, 0 °C to RT, 98%; (vii) TPSCl, DMAP, Et_3N, $NH_2OH{\cdot}HCl$, CH_2Cl_2, 0 °C to RT, 18%; (viii) TPSCl, DMAP, Et_3N, 25% $NH_3{\cdot}H_2O$, CH_2Cl_2, 0 °C to RT, 14%.



Scheme 2 Reagents and conditions: (i) (a) uracil, HMDS, (b) TMSOTf, acetonitrile, −10 °C to RT, 85%; (ii) BCl_3, CH_2Cl_2, −70 °C to −30 °C, 76%; (iii) isobutyric anhydride, DMAP, Et_3N, CH_2Cl_2, RT, 85%; (iv) (a). 2,4,6-Triisopropylbenzenesulfonyl chloride, DIPEA, DMAP, CH_2Cl_2, 0 °C to RT, (b). hydroxylamine hydrochloride, DIPEA, 64%; (v) NH_3/MeOH, RT, 80%.



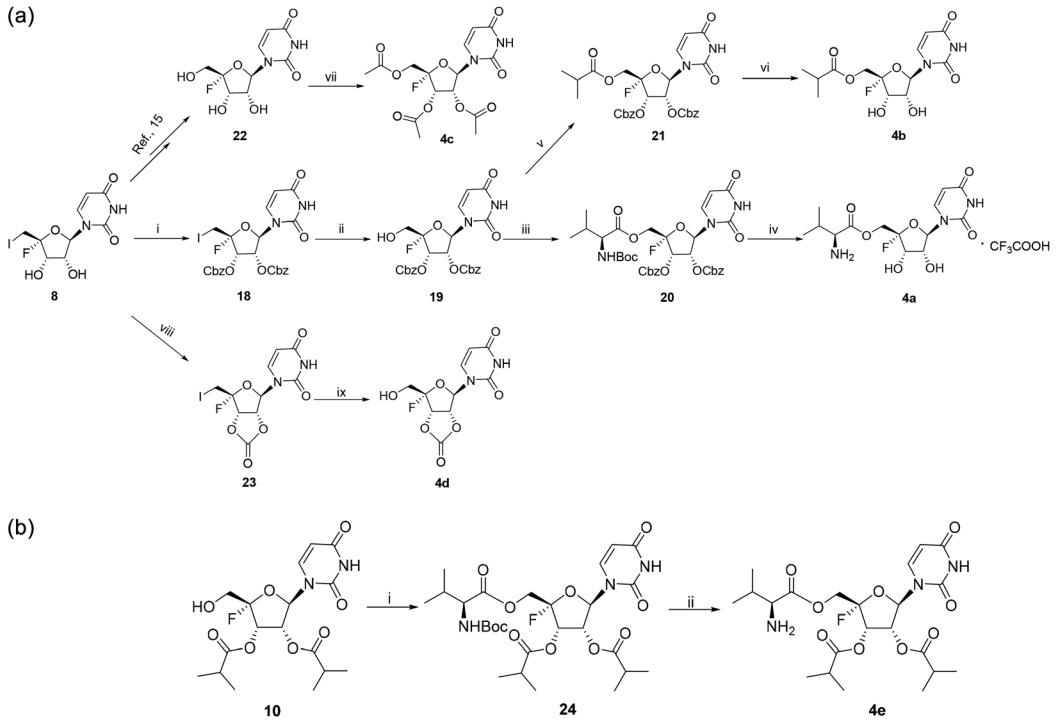
Scheme 3 Reagents and conditions: (i) (a) $(NH_4)_2SO_4$, HMDS, 120 °C, (b) $SnCl_4$, CH_2Cl_2, RT, 64.5%; (ii) $POCl_3$, Et_3N, hydroxylamine hydrochloride, acetonitrile, 0 °C to RT, 17.3%; (iii) NH_3/MeOH, RT, 60.2%; (iv) $POCl_3$, Et_3N, 25% $NH_3 \cdot H_2O$, acetonitrile, 0 °C to RT, 45%; (v) NH_3/MeOH, RT, 60.2%.

oxycarbonyl (Cbz) to give **18**, followed by oxidation to yield intermediate **19**. The condensation of **19** with *N*-Boc-L-valine and isobutyric anhydride under general reaction conditions gave **20** and **21**, respectively. After the removal of the protecting groups, compounds **4a** and **4b** were obtained. Notably, more than 50% of **4a** was found to be decomposed after being stored for 30 days at room temperature in a sealed tube. The 4′-fluorine substituted uridine **22** was prepared by following the reported procedure[15] and acetylation of **22** with acetic anhydride smoothly gave triacetate **4c**. The treatment of **8** with 1,1′-carbonyldiimidazole in THF afforded **23** in a good yield, and compound **4d** was obtained from **23** by the same reaction used for **19**. Compound **4e** was easily synthesized from intermediate **10** by condensation with *N*-Boc-L-valine and Boc-deprotection.

Antiviral activity assays

The antiviral activities of the synthesized compounds were evaluated by the cytopathic effect (CPE) assay against RSV (ATCC VR-26 strain) and IFV (H1N1 strain) in Hep-2 cells and MDCK cells, respectively. Data were expressed as the inhibition rate, EC_{50} (concentrations reducing viral replication by 50%) and CC_{50} (concentrations decreasing cell viability by 50%) values. The results are summarized in Table 1.

In the beginning, we designed and synthesized compounds **1b**, **2c** and **3b** as NHC derivatives all possessing a C_4 hydroxylamine group. Compound **1b** was a tri-isobutyrate ester of 4′-fluorine-substituted NHC; **2c** and **3b** were a 4′-thionucleoside and a 6-azanucleoside, respectively. In our study, NHC was



Scheme 4 **4a**: Reagents and conditions: (i) benzyl chloroformate, 1-methylimidazole, CH_2Cl_2, 0 °C to RT, 97%; (ii) TBAOH, TFA, CH_2Cl_2, 0 °C to RT, 32%; (iii) *N*-Boc-L-valine, HOBT, EDCI, DMAP, CH_2Cl_2, 0 °C to RT, 76%; (iv) (a) TFA, CH_2Cl_2, RT; (b) 10% Pd/C, H_2, ethanol, RT, 68% over two steps; (v) isobutyric anhydride, DMAP, Et_3N, CH_2Cl_2, RT, 70%; (vi) 10% Pd/C, H_2, ethanol, RT, 75%; (vii) acetic anhydride, DMAP, Et_3N, EA, 0 °C to RT, 89%; (viii) 1,1′-carbonyldiimidazole, THF, RT, 51%; (ix) TBAOH, TFA, *m*-CPBA, CH_2Cl_2, RT, 69%. **4b**: (i) *N*-Boc-L-valine, HOBT, EDCI, DMAP, CH_2Cl_2, 0 °C to RT, 85%; (ii) TFA, CH_2Cl_2, RT, 83%.

found to inhibit the replication of RSV and IFV with an EC_{50} of 4.6 μM and 0.77 μM, respectively. However, the three compounds didn't show any inhibitory activity against the two viruses, neither did ester prodrugs **2b** and **3a**. Previous studies have indicated that the steric conformation of nucleoside triphosphates was crucial for binding with the active site of viral polymerase and being incorporated into the growing chain.[12] We speculated that the absence of antiviral activities of these NHC derivatives was due to the minor structural modifications which likely altered the steric conformation of nucleosides compared with NHC, thus leading to disfavored binding with the viral polymerase. In addition, the loss of antiviral activities may also be related to reduced phosphorylation efficiency.

Compound **2a** is a thionucleoside and its phosphoramidate prodrugs were reported to exhibit anti-hepatitis C activity.[16] Recently, a patent disclosed **2a** as a potent antiviral agent against SARS-CoV-2 with EC_{50} values in a low micromolar range.[17] In our study, **2a** was found inactive against RSV and IFV, indicating that this nucleoside may have a narrow antiviral spectrum. 6-Azauridine and 6-azacytidine had been shown to have anti-cancer and antiviral activities.[18,19] However, we observed that the acetate ester prodrug **3c** was inactive against IFV and 6-azauridine **3d** was highly cytotoxic to MDCK cells, so these nucleosides were not considered for further evaluation.

The 4′-fluorine nucleoside tri-isobutyrate esters **1a** and **1c** were found to be highly potent against IFV at a concentration of 5.0 μM (with inhibition rates of 99.8% and 97.4%, respectively). Moreover, they did not show cytotoxic effects at this concentration. Because of the presence of the 4′-fluorine atom, the 4′-fluoro-nucleosides were likely susceptible to nucleophilic attack, as evidenced by poor stability in H_2O described in previous research.[20] In our synthetic study, we found that the stability of 4′-fluorouridine was better than that of the cytidine analog. Therefore, we further synthesized 4′-fluorouridine **22** and its several ester prodrugs with modifications on the hydroxyl groups. Compounds **1a** and **4c** were two tri-ester prodrugs; **4a** and **4b** were the 5′-L-valine ester and 5′-isobutyrate ester prodrug, respectively; **4d** had a carbonate group masking the 2′ and 3′ hydroxyl groups and **4e** contained a 5′-L-valine ester and two isobutyrate ester groups. Nucleoside **22** showed potent antiviral activities against RSV and IFV with an EC_{50} value of 2.32 μM and less than 0.12 μM, respectively. All these prodrugs exhibited similar activities with anti-RSV EC_{50} values ranging from 1.13 μM to 4.90 μM and anti-IFV EC_{50} values ranging from less than 0.12 μM to 0.96 μM. This result suggested that the prodrug moiety could be metabolized in cells to release the parent nucleoside.

Stability studies

4′-Fluorouridine had been shown to be very labile in water and it decomposed slower in phosphate buffer with uracil as the main degradation product.[21] The chemical stability of 4′-fluoro-

Table 1 Antiviral activity of the synthesized NHC derivatives and 4′-fluorouridine prodrugs against RSV (ATCC VR-26 strain) and IFV (H1N1 strain) viruses

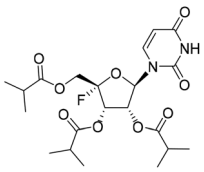
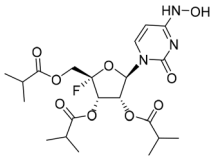
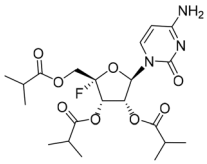
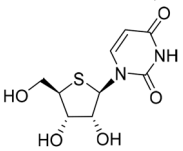
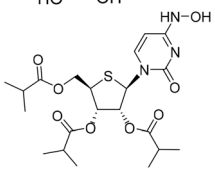
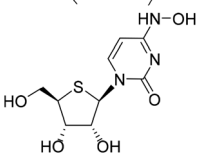
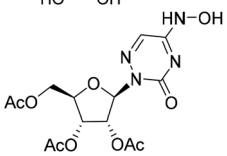
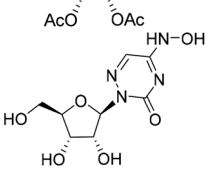
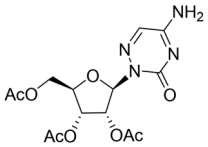
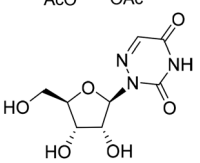
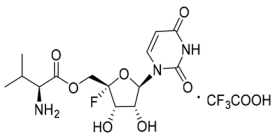
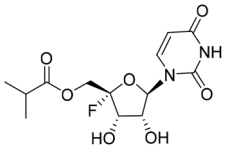
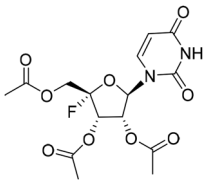
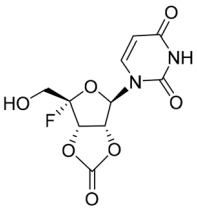
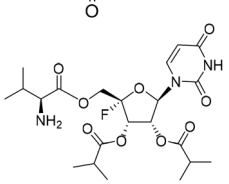
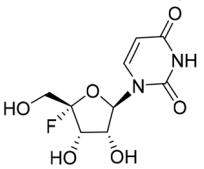
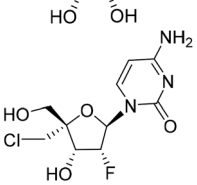
Compd.	Structure	RSV (Hep-2 cells)		IFV (MDCK cells)	
		EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)
1a		1.13	>10	0.34	>10
1b		−0.1% [a]	—	2.0% [a]	—
1c		—	—	97.4% [a]	—
2a		−1.6% [a]	—	−1.2% [a]	—
2b		—	—	6.1% [a]	—
2c		−8.9% [a]	—	6.5% [a]	—
3a		—	—	2.0% [a]	—
3b		2.0% [a]	—	7.5% [a]	—
3c		—	—	−7.4% [a]	—
3d		—	—	Cytotoxic[a]	—

Table 1 *(Contd.)*

Compd.	Structure	RSV (Hep-2 cells)		IFV (MDCK cells)	
		EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)
4a		4.90	>10	<0.12	>10
4b		1.46	>10	0.23	>10
4c		1.43	>10	0.96	>10
4d		3.25	>10	<0.12	>10
4e		1.86	>10	0.42	>10
22		2.32	>10	<0.12	>10
ALS-8112		0.48	>10	—	—

[a] Inhibition rate at 5 μM unless other specified. "—" means undetermined.

uridine **22** and four ester derivatives (**1a** and **4b–d**) was determined through the content change by using HPLC in pH 2.0 and 4.0 buffer at 30 °C after 7 days, and the result is shown in Table 2. It was found that compound **22** had poor stability with 45% and 73% decrease in pH 4.0 and pH 2.0 buffer, respectively. Among the four ester derivatives, compound **1a** exhibited the best stability profile with only a slight change of the content (0.24% and 0.46% decrease in pH 4.0 and pH 2.0 buffer, respectively). In another experiment, we observed that the level of uracil increased in a nearly linear manner within 7 days for 4′-fluorouridine (**22**) in pH 2.0 buffer (Fig. 2). Relatively, **4d** showed significantly enhanced stability with only

Table 2 The chemical stability of 4′-fluorouridine (**22**) and four ester derivatives in different buffers at 30 °C after 7 days

Compound	pH 2.0 Change of content (%)	pH 4.0 Change of content (%)
1a	−0.46	−0.24
4b	−7.70	−6.90
4c	−2.23	−0.35
4d	−5.80	−7.70
22	−73.0	−45.0

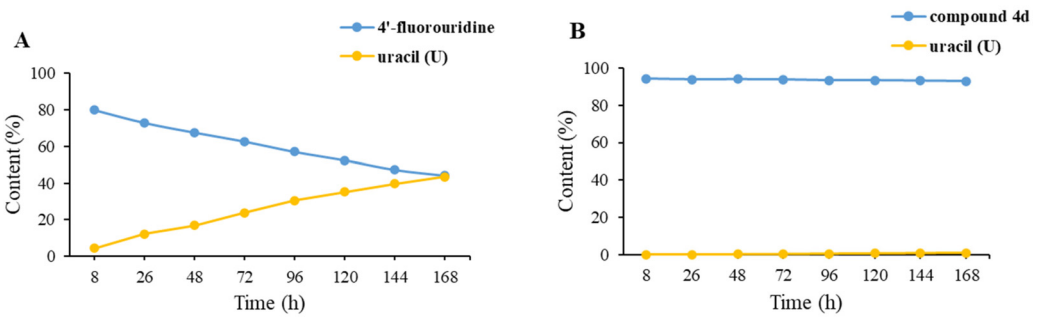


Fig. 2 The stability of (A) 4′-fluorouridine and (B) compound **4d** and formation of uracil (U) in pH = 2.0 buffer at 30 °C after 7 days.

Table 3 Pharmacokinetic (PK) parameters of compounds **22** and **1a** in rats

Compd.	Route	T_{max} (h)	C_{max} (ng mL^{-1})	$T_{1/2}$ (h)	AUC_{0-t} (ng h mL^{-1})	$AUC_{0-\infty}$ (ng h mL^{-1})	$MRT_{0-\infty}$ (%)	F (%)
22	p.o.[a]	2.00	2495	7.75	13 757	14 910	7.63	82.9
	i.v.[a]	—	2618	6.09	8294	8691	6.89	
22	p.o.[b]	0.67	2345	5.87	11 555	12 105	5.54	
1a	p.o.[b]	0.83	3234	8.22	15 615	16 936	7.26	

[a] The vehicle for administration is 5% DMSO + 5% ethanol + 40% PEG400 + 50% saline. [b] The vehicle for administration is 10% DMSO + 10% solutol + 80%(20%HP-β-CD).

a little amount of uracil being generated (1.09% at day 7, ESI†). Based on this result, it could be concluded that modifications of all three hydroxyl groups would greatly improve the stability of the 4′-fluorinated nucleoside.

Pharmacokinetic studies

Given the low chemical stability of **22**, we were curious about the pharmacokinetic (PK) properties of this compound. Therefore, we conducted a PK study of **22** and prodrug **1a** in SD rats. Compound **22** was administered at an intravenous dose of 5 mg kg^{-1} and an oral dose of 10 mg kg^{-1}. The oral bioavailability was 82.9% calculated based on the plasma exposure of the parent nucleoside, suggesting that this nucleoside was efficiently absorbed (Table 3). The half-life of the nucleoside after oral administration was 7.75 h which was a little longer than that after intravenous administration (6.09 h). For the oral administration group at 10 mg kg^{-1}, the maximum concentration (C_{max}) reached 2495 ng ml^{-1} (9.5 μM) which was much higher than the EC_{50} value against IFV and RSV. Generally, compound **22** showed excellent PK properties. In another experiment, we compared the PK parameters of compound **22** with those of **1a** at the same molar dose (10 mg kg^{-1} for **22** and 18 mg kg^{-1} for **1a**). It was pleasing to find that **1a** had better oral PK properties than compound **22** in terms of C_{max}, $T_{1/2}$ and AUC_{0-t}.

Conclusions

In summary, we synthesized several NHC derivatives and 4′-fluorouridine prodrugs. The NHC derivatives did not exhibit activities against IFV or RSV in our lab, and innovative work would be warranted for the discovery of novel nucleoside antiviral agents. Recent research has investigated the therapeutic potential of 4′-fluorouridine against RSV and SARS-CoV-2 infection in animal models.[15] However, 4′-fluorouridine has poor chemical stability which may significantly limit its application. Our study presented that after esterification, the chemical stability of the derived 4′-fluorouridine prodrugs was improved. Tri-isobutyrate ester **1a** with its excellent stability profile and PK properties has the potential to be a new oral antiviral candidate.

Experimental section

General methods

All reagents were directly used without further purification. All water-sensitive reactions were carried out under anhydrous conditions using raw materials dried in a vacuum drying chamber overnight; the glassware was kept in an oven at 100 °C for several hours and reagents were dried using molecular sieves. The reactions were monitored by TLC with 0.2 mm silica gel plates (HSGF 254). The purification procedure of some compounds was performed by silica gel column chromatography (200–300 mesh). ^{1}H-NMR spectra and ^{13}C-NMR spectra were determined on Bruker 400 MHz, 500 MHz or 600 MHz and Bruker 126 MHz, 201 MHz or 151 MHz instruments with TMS as an internal standard, respectively. All of the spectra were obtained with MestReNova software. LRMS was measured on a Finnigan LTQ and HRMS was measured on a 1290–6545 UHPLC-QTOF. The raw materials **5**, **15** and **16** were purchased from Shanghai

Haohong Scientific Co., Ltd, and intermediate **11** was obtained from Topharman Shanghai Co., Ltd.

Method A: General procedure for the synthesis of prodrugs with anhydrides (isobutyric anhydride and acetic anhydride)

To a solution of raw material in DCM (EA for the synthesis of **4c**), triethylamine, DMAP and anhydrides were sequentially added at 0 °C. After stirring at rt until the raw material was completely consumed, the mixture was diluted with DCM and washed successively with aqueous 1 M hydrochloric acid solution and saturated aqueous $NaHCO_3$ solution. The organic layer was dried (Na_2SO_4), filtered and concentrated *in vacuo*. The crude was purified by silica gel column chromatography to afford target compounds.

Method B: General procedure for the synthesis of prodrugs with *N*-Boc-L-valine

To a solution of raw material (1 eq.) in DCM *N*-Boc-L-valine (1.4 eq.), HOBT (1.5 eq.), EDCI (2.2 eq.) and DMAP (4 eq.) were sequentially added at 0 °C. After stirring at rt until the substrate was completely consumed as monitored by TLC, the mixture was diluted with DCM and washed successively with aqueous 1 M hydrochloric acid solution and saturated aqueous $NaHCO_3$ solution. The organic layer was dried (Na_2SO_4), filtered and concentrated *in vacuo*. The crude was purified by silica gel column chromatography (PE : EA = 1 : 1) to afford the intermediate. After removing the Boc group using trifluoroacetic acid, the target compounds were obtained.

1-((2*R*,3*R*,4*S*,5*S*)-3,4-Dihydroxy-5-(iodomethyl)tetrahydrofuran-2-yl)pyrimidine-2,4(1*H*,3*H*)-dione (6)

To a solution of triphenylphosphine (9.43 g, 36 mmol) in pyridine (80 mL) at 0 °C, iodine (9.25 g, 36 mmol) was added and the mixture was stirred for 10 min at 20 °C. Uridine **5** (7.32 g, 30 mmol) was added, the mixture was stirred at rt for 6 h, and then quenched with saturated aqueous $Na_2S_2O_3$ solution. The resulting solution was concentrated and the residue was partitioned between water and tetrahydrofuran. The organic portion was washed with saturated brine, dried with Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (DCM : MeOH = 100 : 1 → 15 : 1) to give compound **6** as a yellow solid (8.5 g, yield 67%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.39 (s, 1H), 7.68 (d, J = 8.1 Hz, 1H), 5.80 (d, J = 5.9 Hz, 1H), 5.68 (dd, J = 8.1, 2.1 Hz, 1H), 5.49 (d, J = 5.8 Hz, 1H), 5.37 (d, J = 5.1 Hz, 1H), 4.19 (q, J = 5.7 Hz, 1H), 3.91–3.81 (m, 2H), 3.55 (dd, J = 10.5, 5.5 Hz, 1H), 3.40 (dd, J = 10.5, 6.6 Hz, 1H).

1-((2*R*,3*R*,4*S*)-3,4-Dihydroxy-5-methylenetetrahydrofuran-2-yl) pyrimidine-2,4(1*H*,3*H*)-dione (7)

A fresh solution of sodium methoxide in MeOH was prepared by adding sodium (830 mg, 36 mmol) to methanol (30 mL) in an ice bath. To a solution of **6** (8.5 g, 24 mmol) in anhydrous MeOH (30 mL), the above prepared solution was added. The reaction mixture was stirred under reflux (67 °C) for 3 h, then adjusted to pH = 8–9 with aqueous 1 M hydrochloric acid solution and concentrated to dryness. Chromatography on a silica gel column (DCM : MeOH = 15 : 1) afforded **7** as a white solid (3.1 g, yield 57%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.44 (s, 1H), 7.60 (d, J = 8.1 Hz, 1H), 5.98 (d, J = 5.4 Hz, 1H), 5.65 (d, J = 8.0 Hz, 1H), 5.61 (s, 1H), 5.47 (d, J = 5.4 Hz, 1H), 4.41 (s, 1H), 4.35 (s, 1H), 4.26 (d, J = 5.0 Hz, 1H), 4.19 (s, 1H).

1-((2*R*,3*R*,4*S*,5*R*)-5-Fluoro-3,4-dihydroxy-5-(iodomethyl) tetrahydrofuran-2-yl)pyrimidine-2,4(1*H*,3*H*)-dione (8)

To a solution of **7** (3.1 g, 13.7 mmol) and triethylamine trihydrofluoride (2.7 g, 16.5 mmol) in anhydrous acetonitrile (60 mL), *N*-iodosuccinimide (3.7 g, 16.5 mmol) was added in portions at 0 °C. After stirring for 5 min, the mixture was filtered and washed with DCM to give **8** as a white solid (4.1 g, yield 77%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.50 (s, 1H), 7.67 (d, J = 8.1 Hz, 1H), 5.91 (d, J = 3.0 Hz, 1H), 5.76 (d, J = 5.3 Hz, 1H), 5.68 (dd, J = 8.0, 2.2 Hz, 1H), 5.42 (d, J = 8.9 Hz, 1H), 4.41–4.33 (m, 1H), 4.31–4.26 (m, 1H), 3.62–3.46 (m, 2H).

(2*R*,3*S*,4*R*,5*R*)-5-(2,4-Dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2-fluoro-2-(iodomethyl)tetrahydrofuran-3,4-diyl bis(2-methylpropanoate) (9)

Compound **9** was prepared by following the same procedure as described for method A. The reaction of **8** (2.0 g, 5.38 mmol) with triethylamine (4.35 g, 43.04 mmol), DMAP (0.33 g, 2.69 mmol) and isobutyric anhydride (1.78 g, 11.29 mmol) gave **9** as a white solid (1.3 g, yield 47%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.60 (s, 1H), 7.80 (d, J = 8.1 Hz, 1H), 6.02 (d, J = 2.4 Hz, 1H), 5.78 (dd, J = 19.0, 7.3 Hz, 1H), 5.73 (d, J = 8.0 Hz, 1H), 5.59 (dd, J = 7.3, 2.4 Hz, 1H), 3.65–3.49 (m, 2H), 2.65–2.58 (m, 2H), 1.16–1.10 (m, 12H).

(2*S*,3*S*,4*R*,5*R*)-5-(2,4-Dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2-fluoro-2-(hydroxymethyl)tetrahydrofuran-3,4-diyl bis(2-methylpropanoate) (10)

Tetrabutylammonium hydroxide (3.30 g, 40% aqueous solution, 12.7 mmol) was adjusted to pH ≈ 4 by adding TFA. The resulting solution was added to a solution of **9** (1.3 g, 2.54 mmol) in DCM (10 mL). *m*-Chloroperbenzonic (2.19 g, 12.7 mmol) was added in portions under vigorous stirring and the resulting reaction mixture was stirred at rt for 7 h. The reaction was quenched with saturated aqueous $Na_2S_2O_3$ solution and extracted with EA and THF. The combined organic portions were dried (Na_2SO_4) and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (PE : EA = 1 : 3) to give compound **10** as a white solid (520 mg, yield 52%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.54 (s, 1H), 7.79 (d, J = 8.1 Hz, 1H), 6.01 (d, J = 2.5 Hz, 1H), 5.74–5.65 (m, 2H), 5.54 (dd, J = 7.1, 2.5 Hz, 1H), 5.50 (t, J = 6.3 Hz, 1H), 2.63–2.53 (m, J = 6.8 Hz, 2H), 1.14–1.06 (m, 12H).

(2*S*,3*S*,4*R*,5*R*)-5-(2,4-Dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2-fluoro-2-((isobutyryloxy)methyl)tetrahydrofuran-3,4-diyl bis(2-methylpropanoate) (1a)

Compound **1a** was prepared by following the same procedure as described for method A. The reaction of **10** (520 mg,

1.33 mmol) with triethylamine (653 mg, 5.32 mmol), DMAP (67 mg, 0.66 mmol) and isobutyric anhydride (231 mg, 1.46 mmol) gave **1a** as a white solid (600 mg, yield 98%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.59 (s, 1H), 7.78 (d, J = 8.1 Hz, 1H), 6.03 (d, J = 2.2 Hz, 1H), 5.81 (dd, J = 20.0, 7.3 Hz, 1H), 5.70 (dd, J = 8.0, 2.2 Hz, 1H), 5.58 (dd, J = 7.2, 2.1 Hz, 1H), 4.40–4.24 (m, 2H), 2.64–2.52 (m, 3H), 1.13–1.07 (m, 18H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 175.27, 175.18, 174.65, 163.18, 150.06, 144.13, 114.58 (d, J = 233.6 Hz), 102.17, 94.02, 70.29, 69.02 (d, J = 19.5 Hz), 61.33 (d, J = 36.9 Hz), 33.08, 33.05, 33.01, 18.62, 18.60, 18.55, 18.55, 18.50, 18.37. HRMS (ESI$^+$) m/z: calcd for $C_{21}H_{29}FN_2O_9$ $[M + H]^+$, 473.1930; found, 473.1932.

(2*S*,3*S*,4*R*,5*R*)-2-Fluoro-5-(6-(hydroxyamino)-2-oxo-1l3,3-oxazin-3(2*H*)-yl)-2-((isobutyryloxy)methyl)tetrahydrofuran-3,4-diyl bis(2-methylpropanoate) (1b)

To a solution of **1a** (200.0 mg, 0.43 mmol) in DCM (15 mL), triethylamine (173.7 mg, 1.72 mmol), DMAP (24.4 mg, 0.22 mmol) and TPSCl (260.6 mg, 0.86 mmol) were sequentially added at 0 °C. After stirring at rt for 1 h, TLC was used to monitor that the substrate was completely consumed, then triethylamine (347.4 mg, 3.44 mmol) and hydroxyamine hydrochloride (298.9 mg, 4.30 mmol) were added. After stirring at rt for 4 h, the mixture was diluted with DCM and washed successively with aqueous 1 M hydrochloric acid solution and saturated aqueous $NaHCO_3$ solution. The organic layer was dried (Na_2SO_4), filtered and concentrated *in vacuo*. The crude was purified by silica gel column chromatography (PE : EA = 1 : 1) to afford **1b** as a white solid (40 mg, yield 18%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.15 (s, 1H), 9.90 (s, 1H), 7.00 (d, J = 8.2 Hz, 1H), 5.77 (dd, J = 19.5, 7.3 Hz, 1H), 5.60 (dd, J = 8.1, 1.8 Hz, 1H), 5.52 (dd, J = 7.3, 2.4 Hz, 1H), 4.41–4.21 (m, 2H), 2.66–2.52 (m, 3H), 1.13–1.07 (m, 18H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 175.30, 175.16, 174.67, 148.87, 142.98, 133.11, 114.21 (d, J = 233.3 Hz), 98.98, 93.34, 70.12, 69.10 (d, J = 19.7 Hz), 61.58 (d, J = 36.0 Hz), 33.01, 33.01, 32.96, 18.63, 18.57, 18.56, 18.55, 18.51, 18.39. HRMS (ESI$^-$) m/z: calcd for $C_{21}H_{30}FN_3O_9$ $[M - H]^-$, 486.1893; found, 486.1900.

(2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-2-oxo-1λ^3,3-oxazin-3(2*H*)-yl)-2-fluoro-2-((isobutyryloxy)methyl)tetrahydrofuran-3,4-diyl bis(2-methylpropanoate) (1c)

Compound **1c** was obtained by following the same procedure as described for **1b**. The reaction of **1a** (100 mg, 0.215 mmol) with triethylamine (86.9 mg, 0.86 mmol), DMAP (12.2 mg, 0.11 mmol) and 25% ammonium hydroxide (301 mg, 2.15 mmol) gave **1c** as a white solid (14 mg, yield 14%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.68 (d, J = 7.4 Hz, 1H), 7.43 (d, J = 9.0 Hz, 2H), 5.94–5.84 (m, 2H), 5.73 (d, J = 7.4 Hz, 1H), 5.56 (dd, J = 7.2, 1.9 Hz, 1H), 4.39–4.22 (m, 2H), 2.67–2.52 (m, 3H), 1.14–1.06 (m, 18H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 175.32, 175.29, 174.70, 166.33, 154.37, 145.28, 114.71 (d, J = 233.0 Hz), 95.75, 94.63, 70.61, 69.69 (d, J = 19.2 Hz), 61.70 (d, J = 36.4 Hz), 33.07, 33.07, 32.99, 18.61, 18.61, 18.58, 18.55, 18.55, 18.40. HRMS (ESI$^+$) m/z: calcd for $C_{21}H_{30}FN_3O_8$ $[M + H]^+$, 472.209; found, 472.2092.

1-*O*-Acetyl-2,3,5-tri-*O*-benzyl-4-thio-α,β-D-ribofuranose (12)

Compound **12** was synthesized according to the literature procedure (α : β = 6 : 1).[14] 12α: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.43–7.26 (m, 15H), 6.26 (d, J = 4.7 Hz, 1H), 4.78–4.45 (m, 6H), 4.17–4.13 (m, 1H), 4.07 (t, J = 4.4 Hz, 1H), 3.80–3.77 (m, 1H), 3.43 (dd, J = 10.0, 5.4 Hz, 1H), 3.40 (dd, J = 10.0, 7.7 Hz, 1H), 2.04 (s, 1H). 12β: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.40–7.24 (m, 15H), 6.01 (d, J = 1.9 Hz, 1H), 4.81–4.45 (m, 6H), 4.14 (dd, J = 3.3, 2.0 Hz, 1H), 4.01 (dd, J = 8.4, 3.5 Hz, 1H), 3.91–3.86 (m, 1H), 3.82 (dd, J = 9.8, 4.4 Hz, 1H), 3.62 (dd, J = 9.8, 7.0 Hz, 1H), 2.06 (s, 3H). LRMS (ESI$^+$) m/z: calcd for $C_{28}H_{30}O_5S$ $[M + H]^+$, 478.18; found, 479.6.

1-((3*R*,4*S*,5*R*)-3,4-Bis(benzyloxy)-5-((benzyloxy)methyl) tetrahydrothiophen-2-yl)pyrimidine-2,4(1*H*,3*H*)-dione (13)

Compound **12** (1.4 g, 3.0 mmol, a mixture of α and β) and silylated uracil (6.0 mmol) were dissolved in acetonitrile (20 mL) and then treated with TMSOTf (1.5 g, 6.6 mmol) at −10 °C. After stirring for 30 min, the temperature was raised to rt. When the starting materials were completely consumed, the reaction was quenched with saturated aqueous $NaHCO_3$ solution and then concentrated to remove acetonitrile. The residue was extracted with EA. The organic layer was washed with brine, dried using Na_2SO_4 and then filtered. After concentration, the residue was purified by flash chromatography (PE : EA = 10 : 1) to afford **13** (1.4 g, yield 85%, α : β = 1 : 1). 13α: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04 (d, J = 8.2 Hz, 1H), 7.39–7.19 (m, 15H), 6.33 (d, J = 3.4 Hz, 1H), 5.55 (dd, J = 8.2, 2.2 Hz, 1H), 4.75–4.44 (m, 6H), 4.00 (dd, J = 5.9, 3.6 Hz, 1H), 3.96 (t, J = 3.5 Hz, 1H), 3.76–3.73 (m, 1H), 3.69 (dd, J = 10.2, 3.0 Hz, 1H), 3.49 (dd, J = 9.9, 6.2 Hz, 1H). 13β: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.09 (d, J = 8.2 Hz, 1H), 7.39–7.19 (m, 15H), 6.04 (d, J = 5.9 Hz, 1H), 5.07 (dd, J = 8.2, 2.2 Hz, 1H), 4.75–4.44 (m, 6H), 4.24 (dd, J = 5.4, 3.6 Hz, 1H), 3.91 (t, J = 3.5 Hz, 1H), 3.89 (dd, J = 17.7, 4.0 Hz, 1H), 3.83 (dd, J = 10.2, 2.5 Hz, 1H), 3.56 (dd, J = 9.9, 5.6 Hz, 1H). LRMS (ESI$^+$) m/z: calcd for $C_{30}H_{30}N_2O_5S$ $[M + H]^+$, 530.19; found, 531.5.

1-((2*R*,3*R*,4*S*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl) tetrahydrothiophen-2-yl)pyrimidine-2,4(1*H*,3*H*)-dione (2a)

A solution of **13** (700 mg, 1.32 mmol, β anomer) in DCM (5 mL) was treated with 1.0 M BCl_3 solution in DCM (5.3 mL, 5.28 mmol) at −78 °C. After the addition, the temperature was slowly increased to −30 °C. When the raw materials were consumed, the reaction solution was quenched with methanol, then the pH of the reaction solution was adjusted to 7–8 with $NaHCO_3$ solution, concentrated and purified by reverse-phase silica gel column chromatography (H_2O : MeOH = 100 : 1 → 50 : 1) to obtain compound **2a** (261 mg, yield 76%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.32 (s, 1H), 7.99 (d, J = 8.1 Hz, 1H), 5.90 (d, J = 7.4 Hz, 1H), 5.68 (d, J = 8.1 Hz, 1H), 5.49 (d, J = 5.8 Hz, 1H), 5.28 (d, J = 4.3 Hz, 1H), 5.18 (t, J = 5.3 Hz, 1H), 4.16 (dt, J = 8.2, 4.0 Hz, 1H), 4.03 (q, J = 3.1 Hz, 1H), 3.68–3.52 (m, 2H), 3.22–3.18 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 162.97, 151.16, 141.52, 102.14, 76.33, 73.00, 63.11, 62.28, 52.98. HRMS (ESI$^+$) m/z: calcd for $C_9H_{12}N_2O_5S$ $[M + Na]^+$, 283.0359; found, 283.0355.

(2*R*,3*R*,4*S*,5*R*)-2-(2,4-Dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-5-((isobutyryloxy)methyl)tetrahydrothiophene-3,4-diyl bis(2-methylpropanoate) (14)

Compound **14** was synthesized according to method A. The reaction of **2a** (260 mg, 1.0 mmol) with triethylamine (1.0 g, 10.4 mmol), DMAP (43 mg, 0.35 mmol) and isobutyric anhydride (823 mg, 5.2 mmol) gave **14** as a white solid (400 mg, yield 85%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.46 (d, J = 1.8 Hz, 1H), 8.02 (d, J = 8.1 Hz, 1H), 6.19 (d, J = 7.8 Hz, 1H), 5.76 (dd, J = 8.1, 2.2 Hz, 1H), 5.68 (dd, J = 7.7, 4.0 Hz, 1H), 5.53–5.45 (m, 1H), 4.46 (dd, J = 11.6, 7.4 Hz, 1H), 4.34 (dd, J = 11.6, 5.9 Hz, 1H), 3.70–3.64 (m, 1H), 2.69–2.56 (m, 2H), 2.50–2.46 (m, 1H), 1.15 (dd, J = 14.0, 7.0 Hz, 12H), 1.03 (dd, J = 7.0, 4.1 Hz, 6H). LRMS (ESI$^+$) m/z: calcd for $C_{21}H_{30}N_2O_8S$ [M + H]$^+$, 470.17; found, 471.3.

(2*R*,3*R*,4*S*,5*R*)-2-(4-(Hydroxyamino)-2-oxopyrimidin-1(2*H*)-yl)-5-((isobutyryloxy)methyl)tetrahydrothiophene-3,4-diyl bis(2-methylpropanoate) (2b)

Compound **14** (150.0 mg, 0.32 mmol), DIPEA (207.0 mg, 1.6 mmol) and DMAP (4 mg, cat.) were sequentially added to DCM (10 mL), followed by TPSCl (194.0 mg, 0.64 mmol) at 0 °C for 10 min. After stirring at rt for 2 h, DIPEA (207.0 mg, 1.6 mmol) and hydroxyamine hydrochloride (111.0 mg, 1.6 mmol) were added at 0 °C for 10 min. After stirring at rt for 2 h, the mixture was diluted with DCM and washed successively with aqueous 1 M hydrochloric acid solution and saturated aqueous $NaHCO_3$ solution. The organic layer was dried (Na_2SO_4), filtered and concentrated *in vacuo*. The crude was purified by silica gel column chromatography (PE : EA = 1 : 1) to afford **2b** as a foam (100 mg, yield 64%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 10.09 (s, 1H), 9.72 (d, J = 2.2 Hz, 1H), 7.18 (d, J = 8.3 Hz, 1H), 6.20 (d, J = 8.3 Hz, 1H), 5.68 (dd, J = 8.2, 2.0 Hz, 1H), 5.58 (dd, J = 8.3, 4.0 Hz, 1H), 5.47 (dd, J = 4.0, 2.2 Hz, 1H), 4.42–4.28 (m, 2H), 3.65–3.60 (m, 1H), 2.69–2.54 (m, 2H), 2.49–2.45 (m, 1H), 1.15 (d, J = 7.0 Hz, 3H), 1.12 (dd, J = 6.9, 3.2 Hz, 9H), 1.02 (dd, J = 7.0, 2.8 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 175.70, 174.89, 174.80, 149.43, 142.89, 129.59, 99.74, 73.68, 72.22, 64.33, 60.19, 46.55, 33.17, 33.17, 33.08, 18.81, 18.66, 18.66, 18.49, 18.41, 18.32. HRMS (ESI$^+$) m/z: calcd for $C_{21}H_{31}N_3O_8S$ [M + H]$^+$, 486.1905; found, 486.1902.

1-((2*R*,3*R*,4*S*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl) tetrahydrothiophen-2-yl)-4-(hydroxyamino)pyrimidin-2(1*H*)-one (2c)

Compound **2b** (50 mg, 0.1 mmol) was added to ammonia solution in methanol (8 mL) and stirred at rt. After the raw material was completely consumed, the reaction solution was concentrated and slurried with methyl *tert*-butyl ether (MTBE) to obtain **2c** as an off-white solid (22 mg, yield 80%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.96 (s, 1H), 9.47 (s, 1H), 7.15 (d, J = 8.3 Hz, 1H), 5.90 (d, J = 8.0 Hz, 1H), 5.61 (d, J = 8.2 Hz, 1H), 5.33 (d, J = 6.3 Hz, 1H), 5.21 (d, J = 4.1 Hz, 1H), 5.14 (t, J = 5.4 Hz, 1H), 4.12–4.06 (m, 1H), 4.02 (td, J = 3.8, 2.1 Hz, 1H), 3.61–3.50 (m, 2H), 3.17–3.12 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 149.89, 143.45, 130.38, 98.86, 75.44, 73.10, 63.56, 61.69, 52.72. HRMS (ESI$^-$) m/z: calcd for $C_9H_{13}N_3O_5S$ [M − H]$^-$, 274.0503; found, 274.0502.

(2*R*,3*R*,4*R*,5*R*)-2-(Acetoxymethyl)-5-(3,5-dioxo-4,5-dihydro-1,2,4-triazin-2(3*H*)-yl)tetrahydrofuran-3,4-diyl diacetate (17)

6-Azauracil **16** (904 mg, 8 mmol) and $(NH_4)_2SO_4$ (52.8 mg, 0.4 mmol) were added to HMDS (20 mL) and stirred at 130 °C for 2 h. After the raw material was completely consumed, the reaction solution was concentrated. A solution of 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose **15** (1.27 g, 4 mmol) in DCM (30 mL) was treated with the concentrate, followed by the addition of $SnCl_4$ (2.5 g, 9.6 mmol) at 0 °C. The reaction was stirred at rt for 3 h and extracted with EA. The organic layers were washed with saturated aqueous $NaHCO_3$ solution and brine, then dried using Na_2SO_4. After concentration, the residue was purified by flash chromatography (PE : EA = 2 : 1 → 1 : 1) to afford **17** as a white foamy solid (950 mg, yield 64.5%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.36 (s, 1H), 7.67 (d, J = 7.2 Hz, 1H), 6.13 (d, J = 3.2 Hz, 1H), 5.54 (dd, J = 5.5, 3.3 Hz, 1H), 5.35 (t, J = 5.9 Hz, 1H), 4.31 (ddd, J = 27.5, 10.8, 4.1 Hz, 2H), 4.10–4.02 (m, 1H), 2.10 (s, 3H), 2.07 (s, 3H), 2.03 (s, 3H).

(2*R*,3*R*,4*R*,5*R*)-2-(Acetoxymethyl)-5-(5-(hydroxyamino)-3-oxo-1,2,4-triazin-2(3*H*)-yl)tetrahydrofuran-3,4-diyl diacetate (3a)

To a solution of **17** (100 mg, 0.27 mmol) and triethylamine (136 mg, 1.35 mmol) in acetonitrile (6 mL), $POCl_3$ (82.9 mg, 0.54 mmol) was added at 0 °C. After stirring at rt for 10 min, a solution of hydroxylamine hydrochloride (187.7 mg, 2.7 mmol) and triethylamine (273 mg, 2.7 mmol) in acetonitrile (1 mL) was added. The mixture was stirred at rt for 1 h, and treated with the same post-processing operation. The residue was purified by thin layer chromatography (PE : EA = 2 : 1), and then slurred in a mixed solution of petroleum ether and dichloromethane to obtain compound **3a** as a white solid (18 mg, yield 17.3%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.10 (s, 1H), 10.79 (s, 1H), 7.54 (d, J = 1.8 Hz, 1H), 6.03 (d, J = 3.7 Hz, 1H), 5.49 (dd, J = 5.6, 3.7 Hz, 1H), 5.28 (t, J = 5.8 Hz, 1H), 4.32 (dd, J = 12.0, 3.5 Hz, 1H), 4.21 (q, J = 5.3 Hz, 1H), 4.03 (dd, J = 12.0, 5.3 Hz, 1H), 2.07 (s, 3H), 2.06 (s, 3H), 2.02 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 170.05, 169.49, 169.46, 146.60, 139.05, 135.60, 86.91, 78.22, 71.75, 70.24, 62.87, 20.54, 20.34, 20.34. HRMS (ESI$^+$) m/z: calcd for $C_{14}H_{18}N_4O_9$ [M + H]$^+$, 387.1147; found, 387.1152.

2-((2*R*,3*R*,4*S*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl) tetrahydrofuran-2-yl)-5-(hydroxyamino)-1,2,4-triazin-3(2*H*)-one (3b)

Compound **3b** was prepared by following the same procedure as described for **2c**. **3a** (59 mg, 0.15 mmol) was treated with ammonia solution in methanol (2 mL) to yield **3b** as a white solid (24 mg, yield 60.2%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.95 (s, 1H), 10.62 (d, J = 2.0 Hz, 1H), 7.43 (d, J = 1.7 Hz, 1H), 5.80 (d, J = 4.2 Hz, 1H), 5.17 (d, J = 5.5 Hz, 1H), 4.97 (d, J = 5.8 Hz, 1H), 4.67 (t, J = 5.8 Hz, 1H), 4.21–4.13 (m, 1H), 3.92 (q, J = 5.5 Hz, 1H), 3.75–3.69 (m, 1H), 3.52–3.43 (m, 1H), 3.39–3.35

(m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 147.03, 139.28, 134.55, 88.62, 84.06, 71.61, 70.44, 62.25. HRMS (ESI$^-$) m/z: calcd for $C_8H_{12}N_4O_6$ [M − H]$^-$, 259.0684; found, 259.0684.

(2*R*,3*R*,4*R*,5*R*)-2-(Acetoxymethyl)-5-(5-amino-3-oxo-1,2,4-triazin-2(3*H*)-yl)tetrahydrofuran-3,4-diyl diacetate (3c)

Compound **3c** was obtained by following the same procedure as described for **3a**. The reaction of **17** (20 mg, 0.054 mmol) with triethylamine (27.3 mg, 0.27 mmol), $POCl_3$ (16.9 mg, 0.11 mmol) and 25% ammonium hydroxide (78 mg, 0.57 mmol) gave **3c** as a white solid (9 mg, yield 45%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.19 (s, 1H), 8.04 (s, 1H), 7.56 (s, 1H), 6.16 (d, J = 3.5 Hz, 1H), 5.51 (dd, J = 5.6, 3.4 Hz, 1H), 5.34 (t, J = 5.9 Hz, 1H), 4.32 (dd, J = 12.0, 3.5 Hz, 1H), 4.27–4.22 (m, 1H), 4.03 (dd, J = 12.0, 5.1 Hz, 1H), 2.09–2.05 (m, 6H), 2.01 (s, 3H). ^{13}C NMR (201 MHz, DMSO-d_6): δ 169.97, 169.48, 169.41, 158.84, 152.68, 128.54, 88.42, 78.53, 72.18, 70.34, 62.93, 20.48, 20.32, 20.30. HRMS (ESI$^+$) m/z: calcd for $C_{14}H_{18}N_4O_8$ [M + Na]$^+$, 393.1017; found, 393.1019.

2-((2*R*,3*R*,4*S*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl) tetrahydrofuran-2-yl)-1,2,4-triazine-3,5(2*H*,4*H*)-dione (3d)

Following the procedure for the synthesis of **2c**, **3d** (24 mg, yield 60.2%) was obtained as a white solid from **17** (59 mg, 0.15 mmol). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.24 (s, 1H), 7.53 (d, J = 4.1 Hz, 1H), 5.88 (d, J = 3.8 Hz, 1H), 5.26 (s, 1H), 5.04 (d, J = 5.2 Hz, 1H), 4.66 (s, 1H), 4.22 (s, 1H), 3.99 (d, J = 6.3 Hz, 1H), 3.78 (d, J = 5.3 Hz, 1H), 3.54–3.34 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 157.25, 148.97, 136.31, 89.37, 84.53, 72.25, 70.36, 62.02. HRMS (ESI$^-$) m/z: calcd for $C_8H_{11}N_3O_6$ [M − H]$^-$, 244.0575; found, 244.0573.

Dibenzyl ((2*R*,3*S*,4*R*,5*R*)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2-fluoro-2-(iodomethyl)tetrahydrofuran-3,4-diyl) bis (carbonate) (18)

Benzyl chloroformate (68.08 g, 399.11 mmol) and 1-methylimidazole (43.66 g, 532.14 mmol) were added to a solution of **8** (33.0 g, 88.69 mmol) in DCM (330 mL) at 0 °C. The reaction was stirred for 2 h at rt, after which the solution was washed successively with aqueous 1 M hydrochloric acid solution and saturated aqueous $NaHCO_3$ solution. The organic layer was dried (Na_2SO_4), filtered and concentrated *in vacuo*. The crude was slurried in propan-2-ol for 2 h to afford **18** as an off-white solid (55 g, yield 97%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.63 (d, J = 2.2 Hz, 1H), 7.79 (d, J = 8.1 Hz, 1H), 7.44–7.28 (m, 10H), 6.14 (d, J = 2.1 Hz, 1H), 5.81 (dd, J = 19.0, 7.2 Hz, 1H), 5.71 (dd, J = 8.0, 2.2 Hz, 1H), 5.63 (dd, J = 7.1, 2.1 Hz, 1H), 5.25–5.06 (m, 4H), 3.68 (dd, J = 11.9, 8.3 Hz, 1H), 3.53 (dd, J = 22.8, 11.9 Hz, 1H). LRMS (ESI$^-$) m/z: calcd for $C_{25}H_{22}FIN_2O_9$ [M − H]$^-$, 640.04; found, 639.06.

Dibenzyl ((2*S*,3*S*,4*R*,5*R*)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2-fluoro-2-(hydroxymethyl)tetrahydrofuran-3,4-diyl) bis (carbonate) (19)

Tetrabutylammonium hydroxide (60.77 g, 40% aqueous solution, 93.69 mmol) was treated with trifluoroacetic acid (10.68 g, 93.69 mmol) at 0 °C, and the mixture was stirred for 10 min. A solution of **18** (20 g, 31.23 mmol) in DCM (160 mL) was added and the reaction mixture was stirred overnight at rt. After completion, the reaction was quenched by the addition of saturated aqueous $Na_2S_2O_3$ solution (160 mL). The organic phase was washed twice with water, dried with Na_2SO_4 and filtered. Solvent was evaporated and the residue was purified by column chromatography (PE : acetone = 1 : 1) to give compound **19** as an off-white solid (5.3 g, yield 32%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.54 (s, 1H), 7.77 (d, J = 8.0 Hz, 1H), 7.38–7.34 (m, 10H), 6.12 (d, J = 2.3 Hz, 1H), 5.75 (dd, J = 19.2, 7.0 Hz, 1H), 5.68 (d, J = 8.0 Hz, 1H), 5.60 (dd, J = 7.0, 2.2 Hz, 1H), 5.48 (t, J = 6.3 Hz, 1H), 5.23–5.09 (m, 4H), 3.62 (t, J = 7.9 Hz, 2H).

((2*S*,3*S*,4*R*,5*R*)-3,4-Bis(((benzyloxy)carbonyl)oxy)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2-fluorotetrahydrofuran-2-yl) methyl (*tert*-butoxycarbonyl)-L-valinate (20)

Compound **20** (314 mg, yield 76%) was synthesized according to method B from **19** (300 mg, 0.57 mmol). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.61 (s, 1H), 7.77 (d, J = 8.0 Hz, 1H), 7.40–7.33 (m, 10H), 7.15 (d, J = 8.2 Hz, 1H), 6.19 (d, J = 2.1 Hz, 1H), 5.84 (dd, J = 19.4, 7.2 Hz, 1H), 5.70 (dd, J = 8.0, 2.2 Hz, 1H), 5.61 (dd, J = 7.2, 2.0 Hz, 1H), 5.24–5.10 (m, 4H), 4.39 (d, J = 12.4 Hz, 2H), 3.89 (dd, J = 8.2, 5.9 Hz, 1H), 2.06–1.99 (m, 1H), 1.37 (s, 9H), 0.82 (dd, J = 6.9, 5.1 Hz, 6H). LRMS (ESI$^-$) m/z: calcd for $C_{35}H_{40}FN_3O_{13}$ [M − H]$^-$, 729.25; found, 728.1.

((2*S*,3*S*,4*R*,5*R*)-5-(2,4-Dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2-fluoro-3,4-dihydroxytetrahydrofuran-2-yl) methyl L-valinate trifluoroacetate (4a)

To a stirred solution of **20** (270.3 mg, 0.37 mmol) in DCM (3 mL), trifluoroacetic acid (0.3 mL, 3.7 mmol) was added. The reaction mixture was stirred at rt for 3 h and then concentrated to obtain the crude product. Then the crude product (233.2 mg, 0.37 mmol, yield calculated for 100%) was dissolved in ethanol (10 mL) and treated with 10% Pd/C (78.7 mg, 0.07 mmol) under hydrogen at rt for 2 h. The reaction solution was filtered and concentrated. The residue was slurried in MTBE and DCM to give **4a** as a brown solid (120 mg, yield 68% over two steps). ^{1}H NMR (400 MHz, methanol-d_4): δ 7.61 (d, J = 8.0 Hz, 1H), 5.78 (d, J = 2.1 Hz, 1H), 5.70 (d, J = 8.0 Hz, 1H), 4.65–4.53 (m, 3H), 4.47 (dd, J = 7.1, 2.1 Hz, 1H), 4.01 (d, J = 4.4 Hz, 1H), 2.42–2.31 (m, 1H), 1.09 (s, 3H), 1.08 (s, 3H). LRMS (ESI$^+$) m/z: calcd for $C_{14}H_{20}FN_3O_7$ [M + H]$^+$, 361.13; found, 362.2.

((2*S*,3*S*,4*R*,5*R*)-3,4-Bis(((benzyloxy)carbonyl)oxy)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2-fluorotetrahydrofuran-2-yl) methyl isobutyrate (21)

21 was obtained by following method A. The reaction of **19** (800 mg, 1.51 mmol) with triethylamine (229.2 mg, 2.67 mmol), DMAP (36.9 mg, 0.30 mmol) and isobutyric anhydride (286.5 mg, 1.81 mmol) gave **21** as a white solid (623 mg, yield 70%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.61 (s, 1H), 7.77 (d, J = 8.1 Hz, 1H), 7.41–7.32 (m, 10H), 6.18 (d, J = 2.0 Hz, 1H),

5.86 (dd, J = 19.9, 7.1 Hz, 1H), 5.69 (dd, J = 8.0, 2.2 Hz, 1H), 5.62 (dd, J = 7.1, 1.9 Hz, 1H), 5.23–5.09 (m, 4H), 4.42–4.29 (m, 2H), 2.49–2.43 (m, 1H), 1.05–1.02 (m, 6H). LRMS (ESI^-) m/z: calcd for $C_{29}H_{29}FN_2O_{11}$ $[M + Cl]^-$, 600.18; found, 635.0.

((2*S*,3*S*,4*R*,5*R*)-5-(2,4-Dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2-fluoro-3,4-dihydroxytetrahydrofuran-2-yl) methyl isobutyrate (4b)

By following the procedure for the synthesis of **4a**, **4b** (250 mg, yield 75%) was obtained as a white solid from **21** (606.3 mg, 1.01 mmol). ^{1}H NMR (500 MHz, methanol-d_4): δ 7.59 (d, J = 8.0 Hz, 1H), 5.88 (d, J = 2.1 Hz, 1H), 5.70 (d, J = 8.0 Hz, 1H), 4.52 (dd, J = 19.2, 6.8 Hz, 1H), 4.42–4.38 (m, 1H), 4.38–4.24 (m, 2H), 2.70–2.58 (m, 1H), 1.19–1.16 (m, 6H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 175.46, 163.05, 150.22, 142.38, 115.45 (d, J = 231.1 Hz), 102.37, 93.46, 70.30, 70.00 (d, J = 21.0 Hz), 61.59 (d, J = 39.1 Hz), 33.11, 26.86, 18.71. HRMS (ESI^+) m/z: calcd for $C_{13}H_{17}FN_2O_7$ $[M + Na]^+$, 355.0912; found, 355.0912.

1-((2*R*,3*R*,4*S*,5*S*)-5-Fluoro-3,4-dihydroxy-5-(hydroxymethyl) tetrahydrofuran-2-yl)pyrimidine-2,4(1*H*,3*H*)-dione (22)

Compound **22** was synthesized starting from **8** by a reported method.[15] ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.14 (s, 1H), 7.69 (d, J = 8.1 Hz, 1H), 5.99 (d, J = 2.7 Hz, 1H), 5.67 (dd, J = 10.2, 6.5 Hz, 2H), 5.48 (d, J = 6.1 Hz, 1H), 5.18 (d, J = 8.7 Hz, 1H), 4.25 (dt, J = 17.1, 7.2 Hz, 1H), 4.14 (d, J = 4.3 Hz, 1H), 3.56 (t, J = 5.2 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 163.03, 150.34, 140.91, 117.46 (d, J = 230.8 Hz), 102.18, 91.28, 71.45, 68.76 (d, J = 20.1 Hz), 60.02 (d, J = 42.0 Hz). HRMS (ESI^+) m/z: calcd for $C_9H_{11}FN_2O_6$ $[M + H]^+$, 263.0674; found, 263.0676.

1-((2*R*,3*R*,4*S*,5*S*)-5-Fluoro-3,4-dihydroxy-5-(hydroxymethyl) tetrahydrofuran-2-yl)pyrimidine-2,4(1*H*,3*H*)-dione (4c)

Compound **4c** was obtained by following method A. To a solution of **22** (100 mg, 0.38 mmol) in EA (2 mL), triethylamine (192.3 mg, 1.90 mmol), DMAP (9.3 mg, 0.08 mmol) and acetic anhydride (136.3 mg, 1.34 mmol) were sequentially added at 0 °C. After stirring at rt for 2 h, the mixture was diluted with EA and washed successively with aqueous 1 M hydrochloric acid solution and saturated aqueous $NaHCO_3$ solution. The organic layer was dried (Na_2SO_4), filtered and concentrated *in vacuo*. The crude was slurried to afford **4c** as a white solid (132 mg, yield 89%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.60 (s, 1H), 7.77 (d, J = 8.0 Hz, 1H), 6.06 (s, 1H), 5.79 (dd, J = 19.9, 7.3 Hz, 1H), 5.71 (d, J = 8.0 Hz, 1H), 5.56 (d, J = 7.4 Hz, 1H), 4.43 (t, J = 11.7 Hz, 1H), 4.25 (t, J = 11.5 Hz, 1H), 2.10 (s, 6H), 2.04 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 169.64, 169.49, 168.99, 163.12, 150.00, 143.97, 114.41 (d, J = 233.9 Hz), 102.18, 93.79, 70.27, 68.98 (d, J = 19.5 Hz), 61.37 (d, J = 35.7 Hz), 20.30, 20.26, 20.04. HRMS (ESI^+) m/z: calcd for $C_{15}H_{17}FN_2O_9$ $[M + H]^+$, 389.0991; found, 389.0990.

1-((3^a*R*,4*R*,6*R*,6^a*S*)-6-Fluoro-6-(iodomethyl)-2-oxotetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)pyrimidine-2,4(1*H*,3*H*)-dione (23)

A solution of **8** (950 mg, 2.55 mmol) in THF (5 mL) was treated with 1,1′-carbonyldiimidazole (616 mg, 3.80 mmol) at rt for 30 min and then concentrated. The residue was purified by flash chromatography (PE : EA = 1 : 1 → 1 : 2) to obtain compound **23** as a white foamy solid (520 mg, yield 51%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.64 (s, 1H), 7.79 (d, J = 8.0 Hz, 1H), 6.31 (s, 1H), 5.77 (d, J = 7.3 Hz, 1H), 5.72 (d, 1H), 5.56 (dd, J = 11.4, 7.3 Hz, 1H), 3.62 (d, J = 18.2 Hz, 2H).

1-((3a*R*,4*R*,6*S*,6a*S*)-6-Fluoro-6-(hydroxymethyl)-2-oxotetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)pyrimidine-2,4(1*H*,3*H*)-dione (4d)

By following the procedure for the synthesis of **10**, **4d** (220 mg, yield 69%) was obtained as a white solid from **23** (440 mg, 1.1 mmol). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.59 (s, 1H), 7.78 (d, J = 8.0 Hz, 1H), 6.27 (s, 1H), 5.73 (d, J = 7.3 Hz, 1H), 5.70 (d, J = 8.0 Hz, 1H), 5.58 (t, J = 6.5 Hz, 1H), 5.53 (dd, J = 12.1, 7.3 Hz, 1H), 3.76–3.55 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 163.35, 153.65, 150.23, 144.13, 117.55 (d, J = 234.6 Hz), 102.08, 93.52, 81.28, 78.32 (d, J = 20.0 Hz), 61.55 (d, J = 32.0 Hz). HRMS (ESI^+) m/z: calcd for $C_{10}H_9FN_2O_7$ $[M + H]^+$, 289.0467; found, 289.0466.

(2*S*,3*S*,4*R*,5*R*)-2-((((*tert*-Butoxycarbonyl)-L-valyl)oxy)methyl)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2-fluorotetrahydrofuran-3,4-diyl bis(2-methylpropanoate) (24)

Compound **24** (254.6 mg, yield 85%) was synthesized according to method B from **10** (200.0 mg, 0.50 mmol). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.58 (s, 1H), 7.78 (d, J = 8.1 Hz, 1H), 7.16 (d, J = 8.2 Hz, 1H), 6.05 (d, J = 2.3 Hz, 1H), 5.76 (dd, J = 19.4, 7.3 Hz, 1H), 5.71 (dd, J = 8.1, 2.1 Hz, 1H), 5.56 (dd, J = 7.3, 2.4 Hz, 1H), 4.41–4.29 (m, 2H), 3.91 (dd, J = 8.2, 5.9 Hz, 1H), 2.65–2.56 (m, 2H), 2.08–2.01 (m, 1H), 1.37 (s, 9H), 1.14–1.05 (m, 12H), 0.85 (t, J = 7.4 Hz, 6H). LRMS (ESI^-) m/z: calcd for $C_{27}H_{40}FN_3O_{11}$ $[M + Cl]^-$, 601.26; found, 636.2.

(2*S*,3*S*,4*R*,5*R*)-2-(((L-Valyl)oxy)methyl)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2-fluorotetrahydrofuran-3,4-diyl bis (2-methylpropanoate) (4e)

To a stirred solution of **24** (50.0 mg, 0.08 mmol) in DCM (1 mL), trifluoroacetic acid (0.1 mL, 0.83 mmol) was added. The reaction mixture was stirred at rt for 30 min and then concentrated. The residue was purified by thin layer chromatography (DCM : MeOH = 20 : 1) to obtain **4e** as a white foamy solid (34.6 mg, yield 83%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.78 (d, J = 8.0 Hz, 1H), 6.03 (d, J = 2.2 Hz, 1H), 5.81 (dd, J = 19.7, 7.3 Hz, 1H), 5.70 (d, J = 8.0 Hz, 1H), 5.58 (dd, J = 7.3, 2.2 Hz, 1H), 4.41–4.27 (m, 2H), 3.14 (d, J = 5.2 Hz, 1H), 2.59 (pd, J = 7.0, 2.5 Hz, 2H), 1.92–1.81 (m, 1H), 1.15–1.08 (m, 12H), 0.86 (d, J = 6.9 Hz, 3H), 0.79 (d, J = 6.8 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 175.21, 174.85, 168.41, 163.23, 150.18, 144.02, 114.26 (d, J = 233.9 Hz), 102.31, 93.94, 70.41, 69.26 (d, J = 18.9 Hz), 62.77 (d, J = 36.4 Hz), 57.34, 33.07, 33.02, 29.30, 18.63, 18.59, 18.53, 18.40, 17.85, 17.56. HRMS (ESI^+) m/z: calcd for $C_{22}H_{32}FN_3O_9$ $[M + H]^+$, 502.2195; found, 502.2200.

Antiviral assays and cytotoxicity

Cytopathic effect (CPE) assay was used for testing the inhibitory activity of the compounds against respiratory syncytial virus (RSV) and influenza virus (IFV) replication.

Anti-RSV activity assay

Hep-2 cells were plated on 96-well plates (6×10^3 cells per well) and incubated with 5% CO_2 overnight at 37 °C. The following day, serial dilutions of the test compounds were added, with ALS-8112 as the positive control, followed by the addition of RSV/A Long (ATCC VR-26). After incubating for 5 days, the cell viability and cytotoxicity were evaluated using a spectrophotometric plate reader and calculated using GraphPad Prism software. The results are expressed as EC_{50} (concentrations reducing viral replication by 50%) and CC_{50} (concentrations decreasing cell viability by 50%) values.

Anti-IFV activity assay

Pre-seeded MDCK cells (1.5×10^4 cells per well) were treated with indicated concentrations of the test compounds, and then infected with IFV A (California/07/2009 (H1N1) pdm09 (ATCC, VR-1894)). After 5 days of incubation, CCK-8 was added, and the absorbance of each well was read on a spectrophotometric plate reader. The EC_{50} and CC_{50} values were calculated with GraphPad Prism software.

Stability assays

Ammonium dihydrogen phosphate (0.115 g) was dissolved in pure water (100 mL) to prepare the buffer solution (30 mL), and phosphoric acid was added to adjust the pH to 4.0. The pH 2.0 buffer solution was prepared in the same way. Compound **1a**, **4b–d** or **22** (0.5 mg) was added to a 1 : 1 (v/v) mixture of acetonitrile and buffer solutions (pH = 2.0/4.0) to prepare the incubation mixture (0.5 mg mL^{-1}), which was incubated at rt for 7 days. The samples (5 μL) were analyzed by HPLC. The determination of the purity of compounds **1a**, **4b–d** and **22** was performed on a YMC Pack Pro C_4 column (250 × 4.6 mm, 5 μM). The wavelength of the UV detector was 210 nm. Eluent A: H_2O; B: acetonitrile. Gradient: from 0 to 5 min: 95% of A; from 20 to 25 min: 10% of A. The flow rate is 1.0 mL min^{-1}.

Pharmacokinetic studies

The PK studies of compound **22** in SD rats were conducted at HQ Bioscience Co., Ltd. Before the experiment, SD rats ($N = 3$ for each group, male, 180–280 g) were fasted for 12 h, except the intravenous group, and allowed to drink water. Four hours after administration, the rats were fed. The compound dissolved in 5% DMSO + 5% ethanol + 40% PEG400 + 50% saline was administered orally at 10 mg kg^{-1} and intravenously at 5 mg kg^{-1}, respectively. 0.2 mL blood sample was collected from the jugular vein and placed in heparin sodium anticoagulation tubes at 0.25, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, and 24 h after administration, and centrifuged at 2000 *g* at 4 °C within 10 min. After centrifugation, the plasma samples (30 μL) were accurately aspirated into a plastic tube, which contained 600 μL methanol solution with the internal standard (tolbutamide, 100 ng mL^{-1}), and frozen in a refrigerator at an ultra-low temperature for testing. The concentration of **22** in plasma was determined by LC-MS/MS.

The tri-isobutyrate ester **1a** and compound **22** dissolved in 10% DMSO + 10% solutol + 80% (20% HP-β-CD) were orally administered at the same molar dose (10 mg kg^{-1} for **22**, and 18 mg kg^{-1} for **1a**). The procedure for PK studies in rats was the same as described above.

The analysis was performed on a system of LC-MS010 (5500) and data processing was performed using Analyst1.6.3 (Sciex). The analytes were separated using an Agela Venusil ASB-C_{18} column (150 Å, 3 μM, 4.6 × 50 mm) with an injection volume of 3.00 μL. Mobile system: (A) water with 0.5 mM ammonium acetate; B: methanol. Gradient: from 0 to 0.40 min: 35% of B; from 0.40 to 1.20 min: 35%–90% of B; from 1.20 to 2.40 min: 90% of B; from 2.40 to 2.50 min: 90%–35% of B; from 2.50 to 3.50 min: 35% of B. Flow rate: from 0 to 0.20 min: 0.5500 mL min^{-1}, from 0.20 to 3.5 min: 0.700 mL min^{-1} with the column oven temperature at 40 °C. General MS parameters were set as follows: ion source, ESI; injection voltage: −4500 V; Gas1: 50 psi; Gas2: 50 psi; ion source temperature: 500 °C; dwell time: 80 ms. Auto-MS negative mode was used.

Conflicts of interest

There are no conflicts to declare.

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