

Influence of 4'-Substitution on the Activity of Gemcitabine and Its ProTide Against VZV and SARS-CoV-2

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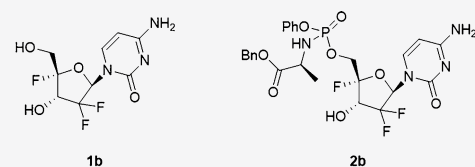
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ABSTRACT: In addition to its therapeutic value as a chemotherapy drug, gemcitabine is of ongoing interest to the scientific community for its broad-spectrum antiviral activity. Herein the synthesis of 4'-methoxy- and 4'-fluoro-substituted gemcitabine analogues along with their phosphoramidate prodrugs is described. Among these derivatives, 4'-fluorogemcitabine proved to be active against varicella zoster virus (VZV, TK+ strain) with an EC_{50} of 0.042 μM and produced significant cytotoxicity ($CC_{50} = 0.11 \mu\text{M}$). Upon derivatization of this trifluoro nucleoside as its prodrug, decreased anti-VZV activity was observed, but with a concomitantly improved selectivity index (SI = 36). When this prodrug was tested against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), its antiviral activity ($EC_{50} = 0.73 \mu\text{M}$) was comparable to or slightly lower than its cytotoxic concentration in measurements of cell growth and cell morphology, respectively.

KEYWORDS: Gemcitabine analogues, phosphoramidate prodrug, varicella zoster virus, severe acute respiratory syndrome coronavirus 2, antiviral activity



	Virus	EC_{50} (μM)	CC_{50} (μM)	SI
1b	VZV (TK ⁺)	0.042	0.11	2
	SARS-CoV-2	0.096	0.26	2
2b	VZV (TK ⁺)	2.32	84.35	36
	SARS-CoV-2	0.73	1.44	<2

Gemcitabine (2'-deoxy-2',2'-difluorocytidine, dFdC; [Figure 1](#)) is a pyrimidine nucleoside analogue that is in

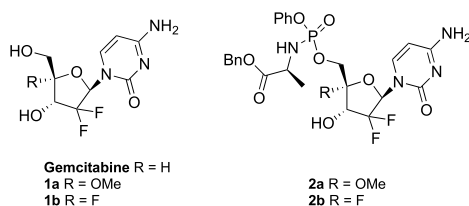


Figure 1. Gemcitabine and its 4'-substituted derivatives investigated in this study.

clinical use for the treatment of various solid tumors, including nonsmall cell lung, pancreatic, bladder, and breast cancer.¹ By acting as a potent antimetabolite, it inhibits two cellular processes that are both required for DNA biosynthesis, i.e., nucleotide reduction and replicative DNA chain elongation. Upon phosphorylation in cells, it is converted to its biologically active metabolites, namely, gemcitabine diphosphate (dFdCDP) and triphosphate (dFdCTP).² While dFdCDP acts as an inhibitor of ribonucleotide reductase (RNR), thus preventing the biosynthesis of deoxynucleotide building blocks that are required for DNA synthesis, dFdCTP is competitively incorporated into DNA in place of natural 2'-deoxycytosine-5'-triphosphate (dCTP).³

Furthermore, gemcitabine was also demonstrated to exert broad-spectrum in vitro activity against a range of RNA viruses. In particular, its antiviral inhibitory effect was assessed against Zika virus (ZIKV) ($EC_{50} = 0.01 \mu\text{M}$),⁴ hepatitis C virus (HCV) ($EC_{50} = 12 \text{ nM}$),⁵ human immunodeficiency virus 1 (HIV-1) ($EC_{50} = 16.3 \text{ nM}$),⁶ and poliovirus ($IC_{50} = 0.3 \mu\text{M}$)⁷ as well as respiratory viruses such as influenza A virus (IAV) ($EC_{50} = 0.068 \mu\text{M}$),⁸ human rhinovirus (HRV) ($EC_{50} = 0.81\text{--}1.92 \mu\text{M}$),⁹ Middle East respiratory syndrome coronavirus (MERS-CoV) ($EC_{50} = 1.22 \mu\text{M}$),¹⁰ and severe acute respiratory syndrome coronavirus (SARS-CoV) ($EC_{50} = 4.95 \mu\text{M}$).¹⁰ In addition, a recent report described the activity of this modified nucleoside against the emerging coronavirus SARS-CoV-2 ($EC_{50} = 1.24 \mu\text{M}$) that is responsible for the current worldwide viral pneumonia outbreak.¹¹

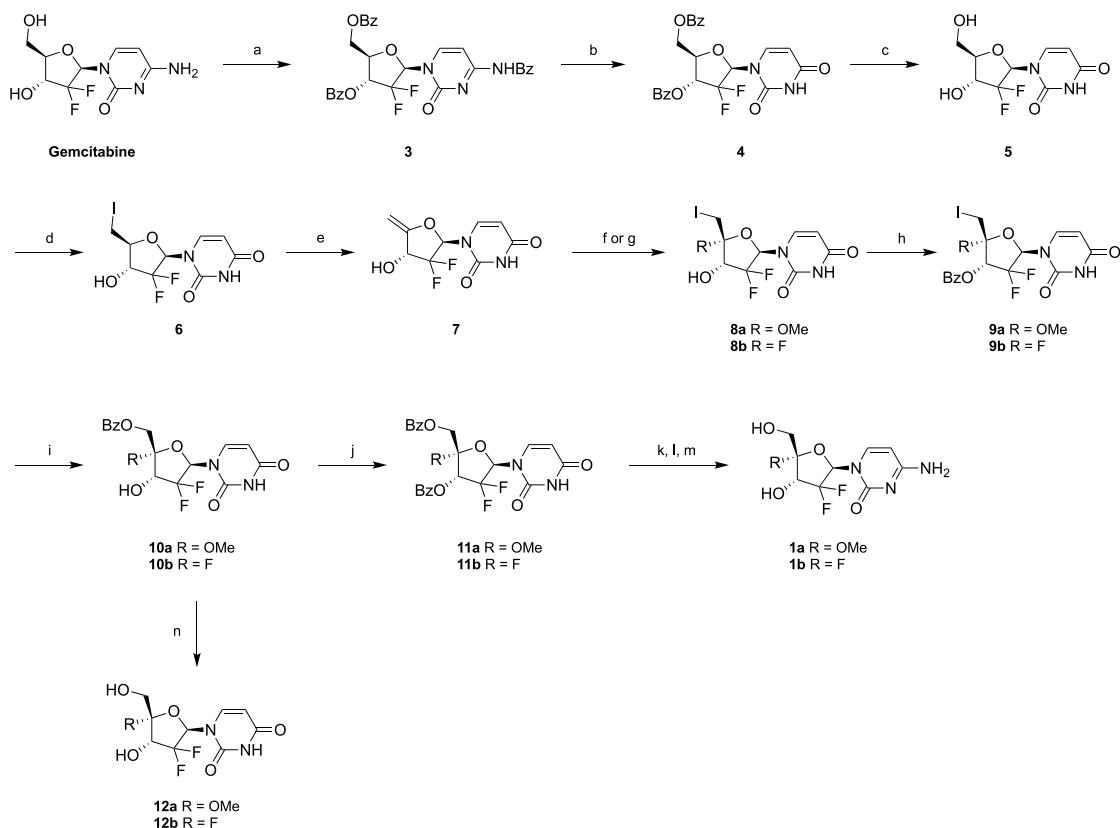
The modification of gemcitabine as a means to convert a life-saving antitumoral drug into an active compound for the treatment of severe viral infections represents an attractive research endeavor. In this context, a variety of gemcitabine analogues have been synthesized to increase its selectivity as

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Scheme 1. Synthetic Routes for the Preparation of 4'-Substituted Gemcitabine Analogues^a

^aReagents and conditions: (a) BzCl, pyr, 93%; (b) 80% AcOH, reflux, 90%; (c) 7 M NH₃ in MeOH, 85%; (d) imidazole, PPh₃, I₂, THF, 84%; (e) DBU, THF, 80 °C, 65%; (f) I₂, PbCO₃, anhydrous MeOH for **8a**, 61%; (g) I₂, AgF, anhydrous THF for **8b**, 35%; (h) BzCl, anhydrous pyr, 0 °C, 94 and 87% for **9a** and **9b**, respectively; (i) *m*-CPBA, CH₂Cl₂/H₂O, 40 °C, 72 and 59% for **10a** and **10b**, respectively; (j) BzCl, triethylamine, DMAP, anhydrous THF, 65 and 75% for **11a** and **11b**, respectively; (k) POCl₃, 1,2,4-triazole, anhydrous MeCN; (l) 25% NH₄OH(aq), MeCN; (m) 7 M NH₃ in MeOH, 38 and 56% for **1a** and **1b**, respectively, over three steps; (n) 7 M NH₃ in MeOH, 68 and 51% for **12a** and **12b**, respectively.

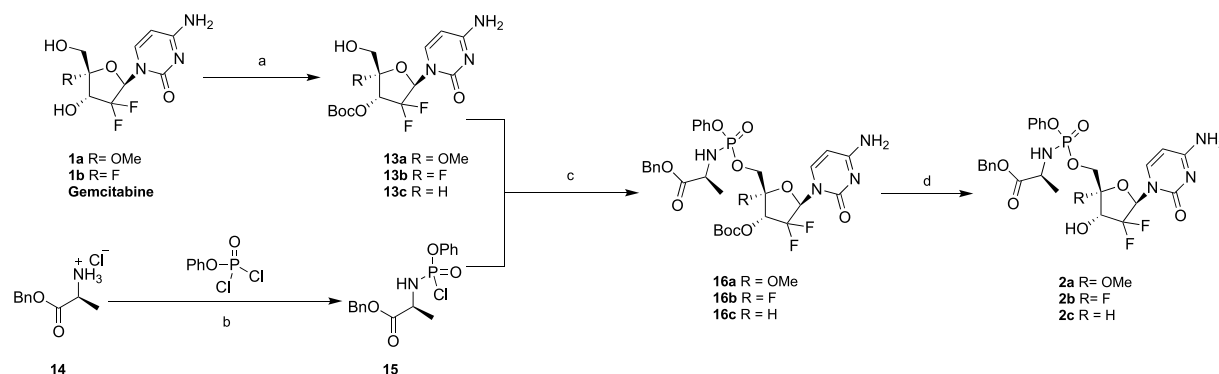
antiviral agent.^{12,13} The rationale behind this strategy aims at reducing the inhibitory activity of dFdCDP and dFdCTP against the cellular RNR and polymerase, respectively, while maintaining the viral polymerase inhibitory capacity of the dFdCTP metabolite.

It is well-known that subtle structural modifications of nucleosides can have a profound impact on their biological profiles. In this study, we selected a methoxy group as an electron-withdrawing substituent via the inductive effect and a fluorine atom as a strongly inductive electron-withdrawing substituent to modify the 4'-position of gemcitabine in order to identify compounds that could lead to reduced cell toxicity while retaining activity against selected viruses. Sterically, both substituents are small, as larger groups might be detrimental for an effective interaction with the target enzymes. In addition, the presence of substituents with different electronic properties might influence the reactivity of the primary hydroxyl group, which needs to undergo intracellular phosphorylation to deliver the active metabolite. The synthesis of 4'-methoxy- and 4'-fluorogemcitabine analogues (**1a** and **1b**, respectively; Figure 1) was complemented by that of the corresponding prodrugs (**2a** and **2b**; Figure 1). A comparison of the antiviral activity of a nucleoside itself (which still needs to undergo three consecutive phosphorylations) and its prodrug (bypassing the first phosphorylation to the nucleoside

monophosphate) was expected to provide useful information for the design of new gemcitabine analogues.

Herein, varicella zoster virus (VZV) and human cytomegalovirus (HCMV) were chosen as the two most important herpes viruses. Although there are anti-VZV and anti-HCMV drugs on the market, toxicity is an issue for some of these drugs, and the emergence of drug resistance has been described for all of them among immunocompromised patients. Novel nontoxic antiviral chemotherapeutics that are more potent and effective than the currently available drugs are therefore required for the treatment of these viral infections in at-risk populations. At the same time, SARS-CoV-2 was privileged among RNA viruses because of the extremely urgent need to develop new antiviral treatments for this infection. As shown in Scheme 1, commercially available gemcitabine served as a convenient starting point for the introduction of fluoro and methoxy substituents at the 4'-position.

First, uridine congener **5** was obtained in an overall yield of 71% via a three-step sequence including complete functional group protection, nucleobase deamination, and O-debenzoylation using standard literature procedures.¹⁴ Next, the primary hydroxyl group of compound **5** was substituted with an iodine atom upon treatment with iodine and triphenylphosphine to yield **6** in good yield (84%). When **6** was subjected to an elimination reaction under strongly basic conditions, methylene derivative **7** was obtained in 65% yield and served as a key

Scheme 2. Conversion of Gemcitabine and Its 4'-Substituted Analogues to Their Phosphoramidate Prodrugs^a

^aReagents and conditions: (a) DBDC, Na₂CO₃, dioxane/H₂O, 35% for **13a**, 23% for **13b**, and 59% for **13c**; (b) phenyl dichlorophosphate, triethylamine, CH₂Cl₂, -78 °C, quantitative yield; (c) *tert*-butylmagnesium chloride, anhydrous THF; (d) trifluoroacetic acid/CH₂Cl₂, 0 °C, 49% for **16a**, 54% for **16b**, and 32% for **16c**.

Table 1. Antiviral Activities and Cytotoxicities of 4'-Substituted Gemcitabine Analogues and Their Prodrugs against VZV and HCMV

compound	antiviral activity, EC ₅₀ (μM) ^a				HEL cytotoxicity (μM)		VZV SI ^e	
	VZV		HCMV		MCC ^b	CC ₅₀ ^c	TK ⁺ strain	TK ⁻ strain
	TK ⁺ strain	TK ⁻ strain	AD-169 strain	Davis strain				
gemcitabine	0.028 ± 0.020	0.054 ± 0.012	0.074 ± 0.004	0.053 ± 0.049	20	0.0036 ± 0.0003	<1	<1
1a	>100	>100	>100	>100	>100	ND ^d	–	–
1b	0.042 ± 0.014	0.166 ± 0.13	0.815 ± 0.64	0.93 ± 0.75	20	0.11	2	<1
12a	>100	>100	>100	>100	>100	ND ^d	–	–
12b	>100	>100	>100	>100	>100	ND ^d	–	–
2a	>100	>100	>100	>100	>100	ND ^d	–	–
2b	2.32 ± 0.43	2.84 ± 0.021	15.47 ± 6.40	4.00 ± 0.00	>100	84.35 ± 22.1	36	29
2c	0.074 ± 0.006	0.089 ± 0.011	0.066 ± 0.007	0.032 ± 0.00	>100	0.34 ± 0.25	4	3
acyclovir	1.6	22.15 ± 11.6	ND ^d	ND ^d	>440	424 ± 21.7	265	19
brivudin	0.036 ± 0.004	6.04	ND ^d	ND ^d	>300	>300	>8333	>49
ganciclovir	ND ^d	ND ^d	2.77 ± 0.53	1.67 ± 0.48	350	231.22 ± 49.2	–	–
cidofovir	ND ^d	ND ^d	0.65 ± 0.38	0.34 ± 0.056	300	150.02 ± 29.1	–	–

^aEffective concentration required to reduce virus plaque formation by 50%. Virus input was 100 plaque forming units (PFU). ^bMinimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology. ^cCytotoxic concentration required to reduce the cell growth by 50%. ^dNot determined. ^eSI = CC₅₀/EC₅₀.

synthon for the further introduction of a methoxy or fluorine substituent at the 4'-position to afford intermediate compounds **8a** and **8b** in 61 and 35% yield, respectively. The stereoselectivity of this step was most likely due to the participation of the C2 carbonyl of the uracil nucleobase via formation of a 4'-anhydro intermediate, as previously postulated.^{14–16} Subsequent protection of the 3'-hydroxy group of **8a** and **8b** as a benzoyl moiety furnished compounds **9a** and **9b** in 94 and 87% yield, respectively; these were further converted to **10a** and **10b** by transfer of the 3'-benzoyl group to the 5'-position upon treatment with *m*-CPBA in a solvent mixture (CH₂Cl₂/H₂O, 4/1). Then the benzoyl group of **10a** and **10b** was deprotected to afford 4'-methoxy- and 4'-fluorouridine analogues **12a** and **12b** in 68 and 51% yield, respectively. Alternatively, the 3'-hydroxy group of compounds **10a** and **10b** was reprotected using benzoyl chloride in pyridine followed by nucleobase conversion via a triazole intermediate and final deprotection under basic conditions to afford compounds **1a** and **1b** in 38 and 56% yield, respectively, over three steps.^{15,17}

The aryloxy triester phosphoramidate prodrug or ProTide technology is a strategy that has proven to be very effective in

bypassing the first rate-limiting phosphorylation step in the nucleoside activation cascade to their 5'-triphosphate forms and facilitate intracellular delivery.¹⁸ For instance, the application of this approach to gemcitabine led to enhanced biological activities and decreased drug resistance.¹⁹ Herein, phosphoramidate prodrugs of compounds **1a** and **1b** along with that of gemcitabine were synthesized as shown in Scheme 2. First, **1a**, **1b**, and gemcitabine were selectively protected at the 3'-position by treatment with di-*tert*-butyl dicarbonate (DBDC) and sodium carbonate in a 4:1 mixture of dioxane and water as the solvent to afford compounds **13a–c**, respectively. In parallel, L-alanine benzyl ester hydrochloride (**14**) was converted to its phenyl aminoacyl phosphorochloridate **15** upon reaction with phenyl phosphorochloridate. Then compounds **13a–c** were reacted with compound **15** to furnish compounds **16a–c** in moderate yields. Finally, the Boc protecting group was cleaved under acidic conditions to afford the desired compounds **2a–c**.

All of the novel compounds (**1a**, **1b**, and **2a–c**) were evaluated for their antiviral activity (expressed as EC₅₀) against VZV (strains TK⁺, thymidine kinase-competent, and TK⁻, thymidine kinase-deficient) and HCMV (strains AD-169 and

Table 2. Antiviral Activities and Cytotoxicities of 4'-Substituted Gemcitabine Analogues and Their Prodrugs against SARS-CoV-2 in Vero Cells

compound	antiviral activity, EC ₅₀ (μM) ^a		cytotoxicity (μM)	
	UC-1074 strain	UC-1075 strain	MCC ^b	CC ₅₀ ^c
gemcitabine	>0.0032 ± 0	>0.0016 ± 0	0.008	0.0043 ± 0.0008
1a	ND ^d	≥86.2 ± 20.4	>100	>100 ± 0
1b	0.36	0.096 ± 0.034	0.16	0.26 ± 0.13
2a	>100	≥55.1 ± 42.8	≥100	≥93.6 ± 11.1
2b	≥2.4 ± 2.3	0.73 ± 0.15	4 ± 0	1.44 ± 0.62
2c	>0.032 ± 0	>0.0128 ± 0	0.048 ± 0.018	0.020 ± 0.009
remdesivir	5.8 ± 3.1	1.52 ± 1.60	>40	>40 ± 0
hydroxychloroquine	8.1 ± 2.4	1.74 ± 0.68	100	36.9 ± 7.5

^aEffective concentration required to reduce virus plaque formation by 50%. Virus input was 100 PFU. ^bMinimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology. ^cCytotoxic concentration required to reduce cell growth by 50%. ^dNot determined.

Davis), with concomitant determination of cytotoxicity and cytostatic effects (CC₅₀) (Table 1). Gemcitabine was included as a reference compound, and acyclovir, brivudine, ganciclovir, and cidofovir were included as positive controls.

The potent cytostatic activity of gemcitabine in human embryonic lung (HEL) fibroblasts (CC₅₀ = 0.0036 μM) leads to a complete absence of selective antiviral activity. The phosphoramidate prodrug of gemcitabine (compound **2c**) was 100-fold less inhibitory toward HEL cell growth (CC₅₀ = 0.34 μM), which is in agreement with what was previously observed for other ProTides of gemcitabine. The introduction of a fluorine at the 4'-position of gemcitabine yielded compound **1b**, which was in general less active against VZV and HCMV. Remarkably, an EC₅₀ of 0.042 μM was observed against the TK⁺ strain of VZV, making it only 2-fold less active than gemcitabine. Moreover, **1b** displayed a 30-fold lower cytostatic effect than gemcitabine (CC₅₀ = 0.11 μM vs 0.0036 μM for gemcitabine).

Interestingly, phosphoramidate prodrug **2b** is less active than the parent nucleoside **1b**, showing moderate activity against the TK⁺ and TK⁻ strains of VZV with EC₅₀ values in the 2–3 μM range. However, a 700-fold lower cytostatic activity was observed for ProTide **2b** in comparison with the parent compound **1b**. Consequently, compound **2b** showed a selectivity index (SI) of 36, which was higher than those of **1b** (SI = 2) and **2c** (SI = 4). On the other hand, the 4'-methoxy gemcitabine analogue **1a** and the 4'-substituted uridine analogues **12a** and **12b** were completely devoid of activity against VZV and HCMV.

In a second screening assay, the cytosine-containing compounds were tested against two different clinical isolates of SARS-CoV-2 in Vero cells (Table 2). Remdesivir and hydroxychloroquine were included as positive controls. As expected, the high cytotoxicity of gemcitabine in Vero cells precluded selective antiviral activity. The application of the ProTide technology to gemcitabine (affording compound **2c**) led to decreased cytotoxicity but also lack of activity against SARS-CoV-2. The introduction of a 4'-methoxy group afforded compound **1a** and the corresponding prodrug **2a**, both of which were devoid of antiviral activity and cytotoxicity. The presence of a 4'-fluorine substituent (compound **1b**) reduced the cytotoxicity of gemcitabine, although no selective antiviral activity was observed. The 4'-fluorophosphoramidate prodrug **2b** was less cytostatic (CC₅₀ = 1.44 μM) than the parent nucleoside **1b** and displayed minimal antiviral activity.

Interestingly, in this cellular test system, compounds **1b** and **2b** were 20 and 10 times more active than remdesivir, respectively.

In summary, the synthesis of 4'-methoxy- and 4'-fluorogemcitabine analogues and their phosphoramidate prodrugs and an evaluation of their antiviral activities against VZV, HCMV, and SARS-CoV-2 have been described. The introduction of a fluorine atom at the 4'-position led to a trifluorinated gemcitabine analogue that exhibited potent activity but no selectivity against these three viruses. A phosphoramidate prodrug of the 4'-fluoro congener displayed reduced activity against VZV but less pronounced cytotoxicity with an enhanced selectivity index. A similar although somewhat reduced effect was observed against HCMV. Unfortunately, this effect was not determined upon testing against SARS-CoV-2.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsmchemlett.0c00485>.

Experimental details and characterization data for the reported compounds, NMR spectra, and biological assays (PDF)

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

HCMV, human cytomegalovirus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; VZV, varicella zoster virus

REFERENCES

- (1) Hertel, L. W.; Boder, G. B.; Kroin, J. S.; Rinzel, S. M.; Poore, G. A.; Todd, G. C.; Grindey, G. B. Evaluation of the Antitumor-Activity of Gemcitabine (2',2'-Difluoro-2'-deoxycytidine). *Cancer Res.* **1990**, *50*, 4417–4422.
- (2) van der Donk, W. A.; Yu, G. X.; Perez, L.; Sanchez, R. J.; Stubbe, J.; Samano, V.; Robins, M. J. Detection of a New Substrate-Derived Radical During Inactivation of Ribonucleotide Reductase from *Escherichia coli* by Gemcitabine 5'-Diphosphate. *Biochemistry* **1998**, *37*, 6419–6426.
- (3) Wang, J.; Lohman, G. J. S.; Stubbe, J. Enhanced Subunit Interactions with Gemcitabine-5'-Diphosphate Inhibit Ribonucleotide Reductases. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 14324–14329.
- (4) Kuivanen, S.; Bespalov, M. M.; Nandania, J.; Ianevski, A.; Velagapudi, V.; De Brabander, J. K.; Kainov, D. E.; Vapalahti, O. Obatoclox, Saliphenylamide and Gemcitabine Inhibit Zika Virus Infection in Vitro and Differentially Affect Cellular Signaling, Transcription and Metabolism. *Antiviral Res.* **2017**, *139*, 117–128.
- (5) Beran, R. K. F.; Sharma, R.; Corsa, A. C.; Tian, Y.; Golde, J.; Lundgaard, G.; Delaney, W. E.; Zhong, W. D.; Greenstein, A. E. Cellular Growth Kinetics Distinguish a Cyclophilin Inhibitor from an HSP90 Inhibitor as a Selective Inhibitor of Hepatitis C Virus. *PLoS One* **2012**, *7*, e30286.
- (6) Clouser, C. L.; Holtz, C. M.; Mullett, M.; Crankshaw, D. L.; Briggs, J. E.; O'Sullivan, M. G.; Patterson, S. E.; Mansky, L. M. Activity of a Novel Combined Antiretroviral Therapy of Gemcitabine and Decitabine in a Mouse Model for HIV-1. *Antimicrob. Agents Chemother.* **2012**, *56*, 1942–1948.
- (7) Zhang, Z. R.; Yang, E. Z.; Hu, C. M.; Cheng, H.; Chen, C. Y.; Huang, D.; Wang, R.; Zhao, Y.; Rong, L. J.; Vignuzzi, M.; Shen, H. B.; Shen, L.; Chen, Z. W. Cell-Based High-Throughput Screening Assay Identifies 2',2'-Difluoro-2'-deoxycytidine Gemcitabine as a Potential Antipoliiovirus Agent. *ACS Infect. Dis.* **2017**, *3*, 45–53.
- (8) Denisova, O. V.; Kakkola, L.; Feng, L.; Stenman, J.; Nagaraj, A.; Lampe, J.; Yadav, B.; Aittokallio, T.; Kaukinen, P.; Ahola, T.; Kuivanen, S.; Vapalahti, O.; Kantele, A.; Tynell, J.; Julkunen, I.; Kallio-Kokko, H.; Paavilainen, H.; Hukkanen, V.; Elliott, R. M.; De Brabander, J. K.; Saelens, X.; Kainov, D. E. Obatoclox, Saliphenylamide, and Gemcitabine Inhibit Influenza A Virus Infection. *J. Biol. Chem.* **2012**, *287*, 35324–35332.
- (9) Song, J. H.; Kim, S. R.; Heo, E. Y.; Lee, J. Y.; Kim, D. E.; Cho, S.; Chang, S. Y.; Yoon, B. I.; Seong, J.; Ko, H. J. Antiviral Activity of Gemcitabine Against Human Rhinovirus in Vitro and in Vivo. *Antiviral Res.* **2017**, *145*, 6–13.
- (10) Dyal, J.; Coleman, C. M.; Hart, B. J.; Venkataraman, T.; Holbrook, M. R.; Kindrachuk, J.; Johnson, R. F.; Olinger, G. G.; Jahrling, P. B.; Laidlaw, M.; Johansen, L. M.; Lear-Rooney, C. M.; Glass, P. J.; Hensley, L. E.; Frieman, M. B. Repurposing of Clinically Developed Drugs for Treatment of Middle East Respiratory

Syndrome Coronavirus Infection. *Antimicrob. Agents Chemother.* **2014**, *58*, 4885–4893.

(11) Zhang, Y.-N.; Zhang, Q.-Y.; Li, X.-D.; Xiong, J.; Xiao, S.-Q.; Wang, Z.; Zhang, Z.-R.; Deng, C.-L.; Yang, X.-L.; Wei, H.-P.; Yuan, Z.-M.; Ye, H.-Q.; Zhang, B. Gemcitabine, Lycorine and Oxysophoridine Inhibit Novel Coronavirus (SARS-CoV-2) in Cell Culture. *Emerging Microbes Infect.* **2020**, *9*, 1170–1173.

(12) Patent application WO 2014209979 A1.

(13) Patent application WO 2015054465 A1.

(14) Wang, G. Y.; Deval, J.; Hong, J.; Dyatkina, N.; Prhac, M.; Taylor, J.; Fung, A.; Jin, Z. N.; Stevens, S. K.; Serebryany, V.; Liu, J. W.; Zhang, Q. L.; Tam, Y. E.; Chanda, S. M.; Smith, D. B.; Symons, J. A.; Blatt, L. M.; Beigelman, L. Discovery of 4'-Chloromethyl-2'-deoxy-3',5'-di-O-isobutyryl-2'-fluorocytidine (ALS-8176), A First-in-Class RSV Polymerase Inhibitor for Treatment of Human Respiratory Syncytial Virus Infection. *J. Med. Chem.* **2015**, *58*, 1862–1878.

(15) Martinez-Montero, S.; Deleavey, G. F.; Kulkarni, A.; Martin-Pintado, N.; Lindovska, P.; Thomson, M.; Gonzalez, C.; Gotte, M.; Damha, M. J. Rigid 2',4'-Difluororibonucleosides: Synthesis, Conformational Analysis, and Incorporation into Nascent RNA by HCV Polymerase. *J. Org. Chem.* **2014**, *79*, 5627–5635.

(16) Martinez-Montero, S.; Deleavey, G. F.; Dierker-Viik, A.; Lindovska, P.; Iliina, T.; Portella, G.; Orozco, M.; Parniak, M. A.; Gonzalez, C.; Damha, M. J. Synthesis and Properties of 2'-Deoxy-2',4'-difluoroarabino-Modified Nucleic Acids. *J. Org. Chem.* **2015**, *80*, 3083–3091.

(17) Divakar, K. J.; Reese, C. B. 4-(1,2,4-Triazol-1-yl) and 4-(3-Nitro-1,2,4-triazol-1-yl)-1-(β -D-2,3,5-tri-O-acetyl arabinofuranosyl)-pyrimidin-2(1H)-ones—Valuable Intermediates in the Synthesis of Derivatives of 1-(β -D-Arabinofuranosyl)Cytosine (Ara-C). *J. Chem. Soc., Perkin Trans. 1* **1982**, 1171–1176.

(18) Mehellou, Y.; Balzarini, J.; McGuigan, C. Aryloxy Phosphoramidate Triesters: A Technology for Delivering Monophosphorylated Nucleosides and Sugars into Cells. *ChemMedChem* **2009**, *4*, 1779–1791.

(19) Slusarczyk, M.; Lopez, M. H.; Balzarini, J.; Mason, M.; Jiang, W. G.; Blagden, S.; Thompson, E.; Ghazaly, E.; McGuigan, C. Application of ProTide Technology to Gemcitabine: A Successful Approach to Overcome the Key Cancer Resistance Mechanisms Leads to a New Agent (NUC-1031) in Clinical Development. *J. Med. Chem.* **2014**, *57*, 1531–1542.