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Synthesis of 5,5-difluoro-5-phosphono-pent-2-en-1-yl nucleosides as potential antiviral agents†

F. Chevrier,[a] Z. Chamas,[a] T. Lequeux,[b] E. Pfund,[b] G. Andrei,[c] R. Snoeck,[c] V. Roy[a] and L. A. Agrofoglio *[a]

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A series of hitherto unknown acyclic 5,5-difluoro-5-phosphono-pent-2-en-1-yl-pyrimidines (**9a**, **b**, **13a**, **b**), -purines (**16a**, **b**) and -(1,2,4)-triazolo-3-carboxamide (**19**) were successfully synthesized from (*E*)-1-bromo-5-diethoxyphosphoryl-5,5-difluoro-pent-2-ene in a stereoselective manner. All the synthetized compounds were assayed for antiviral activity against various viruses, but were found to be neither active nor toxic.

Introduction

Viruses are infectious agents that can replicate their genome within host cells. Many antiviral drugs are nucleoside or nucleotide analogs. Acyclic nucleoside phosphonates (ANPs)[1] are a class of nucleotide analogs, originally developed by A. Holy's group,[2] which exhibit a broad spectrum of antiviral activities. ANPs possess a common structure, a nucleobase attached to an aliphatic side chain containing a phosphonate moiety (C–P), and have an increased metabolic stability and resistance to chemical and biological degradation.[3] Their activities are reliant on their diphosphorylation by NDP and NTP kinases, and further incorporation into the viral DNA where they can act as chain terminators. Three ANPs are in current clinical use for the treatment of serious viral infections, adefovir (PMEA), tenofovir [(*R*)-PMPA] and cidofovir [(*S*)-CDV] against hepatits B virus (HBV), human immunodeficiency virus (HIV) and cytomegalovirus (CMV), respectively, (Fig. 1).

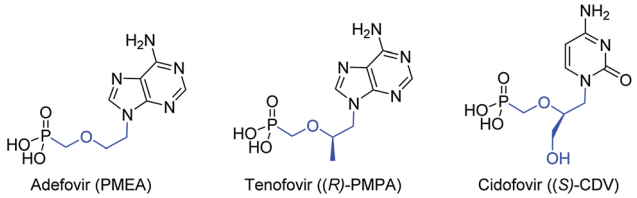


Fig. 1 Structure of approved antiviral acyclic nucleoside phosphonates.

Due to drug-resistant viruses and emerging viruses, in an effort to identify new nucleoside inhibitors of viral enzymes, new generations of ANPs, including fluorinated ANPs, were prepared and evaluated for their antiviral activity.[4,5] Over the last decade, our laboratory has developed a new family of ANPs based on the (*E*)-but-2-enyl linker between the phosphonate moiety and the nucleobase. Several of them exhibited antiviral activity against DNA and RNA viruses in submicromolar concentrations.[6] During our investigations, we have demonstrated that the N^1-[(*E*)-4-phosphono-but-2-en-1-yl]-thymine is a substrate of human TMPK and that the (*E*)-but-2-enyl moiety mimics the conformation of the C1′–O4′–C4′–C5′ atoms from the natural substrate, the thymidine 5′-monophosphate, (Fig. 2).[6] However, unlike natural nucleotide, our molecule missed the oxygen of the phosphate group (*e.g.*, –O–P). Thus, we turn our attention to the introduction of a *gem*-difluoromethylphosphonate moiety (*e.g.*, $-CF_2-P$), which is isopolar and isosteric to the phosphate group.[7]

In fact, due to specific properties of fluorine (high electronegativity, small steric size, hydrogen bond acceptor, …), its introduction into biologically active molecules could lead to major changes in their biological properties, such as reported by Halazy *et al.*[8] for the 9-(5,5-difluoro-5-phosphonopentyl)-

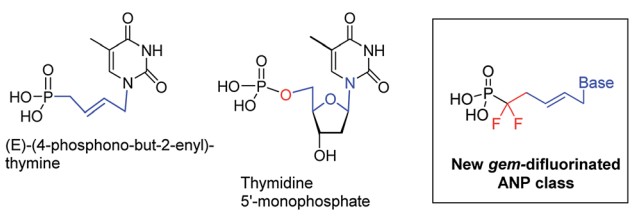


Fig. 2 Structure of newly synthesized 5,5-difluoro-5-phosphono-pent-2-en-1-yl nucleosides as potent mimics of dNMP.

[a]*Université d'Orléans et CNRS, ICOA, UMR 7311, F-45067, Orléans, France. E-mail: luigi.agrofoglio@univ-orleans.fr*

[b]*Laboratoire de Chimie Moléculaire et Thio-organique, ENSICAEN, UNICAEN, UMR, CNRS 6507, FR 3038, Caen, France*

[c]*REGA Institute for Medical Research, KU Leuven, Leuven, Belgium*

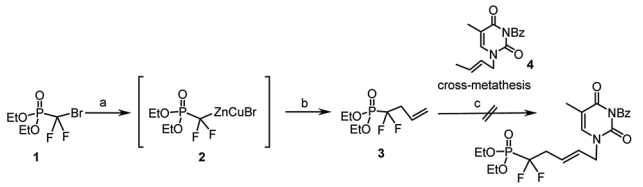
† Electronic supplementary information (ESI) available: ^1H, ^{13}C, ^{31}P and ^{19}F NMR data of selected compounds. See DOI: 10.1039/c7ra05153k

guanine, an inhibitor of purine nucleoside phosphorylase (PNP), a key enzyme in the purine metabolism.[9] Therefore, based on these findings, it was interesting to design and synthesize a new type of acyclic nucleoside phosphonate, the 5,5-difluoro-5-phosphono-pent-2-en-1-yl-pyrimidines, purines and -triazole, and to evaluate their inhibitory activity against several viruses.

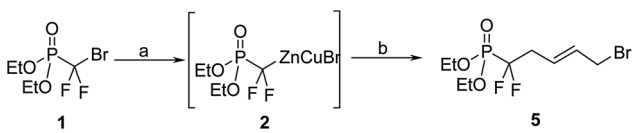
Results and discussion

Based on our previous work on the preparation of unsaturated acyclic nucleoside phosphonate using olefin cross-metathesis as key step,[10–12] we decided to utilize this reaction between the unsaturated *gem*-difluorophosphonate **3** and N^1-crotylated N^3-protected thymine.[10] The key intermediate **3** was synthesized from (diethoxyphosphinyl)difluoromethyl zinc bromide (**2**), allylic iodine, under CuBr catalysis, following the procedure introduced by Burton *et al.*[13,14] Compound **3** was then engaged in the reaction of cross metathesis in presence of N^3-benzoyl-N^1-crotylthymine **4** with Grubbs–Nolan catalyst[15] in dichloromethane. If cross-metathesis reactions were reported with fluorinated substrates,[16] despite all our attempts and contrary to our results with non-fluorinated phosphonate derivatives, we never obtained the desired product (Scheme 1); this could be due to the strong electron-withdrawing effect of the 2-*gem*-difluoro group and to the low reactivity of both cross partners with the metal alkylidene complex.

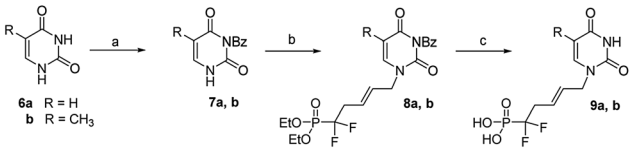
Alternatively we decided to introduce the nucleobase moiety through direct *N*-alkylation of protected and unprotected nucleobases with the corresponding (*E*)-1-bromo-5-diethoxyphosphoryl-5,5-difluoro-pent-2-ene (**5**). Starting from bromodifluoromethylphophonate (**1**), the previously described organo-zinc intermediate **2** was reacted with (*E*)-1,4-dibromo-but-2-ene[14] at 0 °C, to yield the desired compound **5** in 80% with no observed isomerization of the double bond (Scheme 2).



Scheme 1 Reagents and conditions: (a) Zn_{act}, 1,2-dibromoethane, TMSCl, THF_{anh}, 40 °C, 12 h; (b) CuBr, $LiCl_{act}$, allyl iodide, rt, 24 h, 35%; (c) **4** (1.5 eq.), Nolan–Grubb's II catalyst (10 mol%), CH_2Cl_{2anh}, reflux, 24 h.



Scheme 2 Reagents and conditions: (a) Zn_{act}, 1,2-dibromoethane, TMSCl, THF_{anh}, 40 °C, 12 h; (b) CuBr, $LiCl_{act}$, *trans*-1,4-dibromobutene, 0 °C, 4 h, 80%.

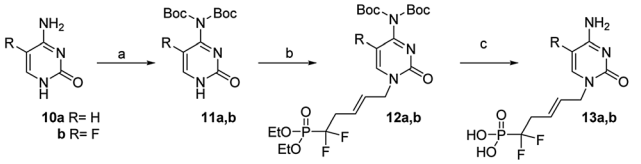


Scheme 3 Reagents and conditions: (a) (i) BzCl, CH_3CN/pyridine, rt (ii) K_2CO_3 (0.5 M), dioxane, 70 °C, 90% (for R = H) and 96% (for R = CH_3); (b) (*E*)-1-bromo-5-diethoxyphosphoryl-5,5-difluoro-pent-2-ene (**5**), Cs_2CO_3, dry DMF, 85% (for R = H) and 81% (for R = CH_3); (c) TMSBr, CH_2Cl_2, rt, 72 h, 90% (for R = H) and 96% (for R = CH_3).

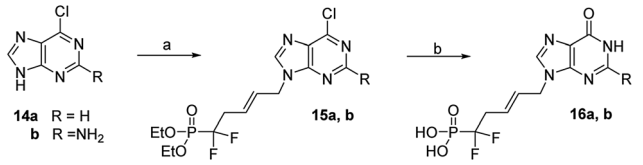
Then uracil **6a** was converted to its N^3-benzoyl derivative **7a** through a two steps procedure involving first the formation of N^1,N^3-dibenzoyl derivative in presence of an excess of benzoyl chloride in CH_3CN/pyridine mixture, then its selective N^1-deprotection by treatment with potassium carbonate in 1,4-dioxane, (Scheme 3).[17]

Similarly, thymine **6b** was converted to its N^3-protected derivative bromide their **7b**. Finally, the successful N^1-alkylation of **7a** and **7b** on **5** in the presence of cesium carbonate in DMF proceeded in good yields and excellent regioselectivities and afforded **8a** and **8b**, in 86% and 75% yields, respectively. Simultaneous deprotection of the N^3-benzoyl group and phosphonic esters with TMSBr/CH_2Cl_2 afforded analogues **9a** and **9b**, in good yields, respectively. These coupling conditions were extended to other nucleic bases. Cytosine **10a** and its fluorinated analogue **10b** were converted to N^4-bis(Boc)-cytosine derivatives **11a** and **11b**, respectively, in good yileds, through N^1,N^3,N^4-tris-Boc forms followed by regioselective N^1 deprotection with saturated solution of $NaHCO_3$ in methanol.[18] Alkylation at N^1 position of **11a** and **11b** in presence of derivative **5**, according to the same previous conditions using Cs_2CO_3, afford **12a** and **12b**, in 85% and 77% yield, respectively.[19] Deprotection with TMSBr afforded the expected free phosphonates **13a** and **13b**, respectively, in quantitative yield (Scheme 4).

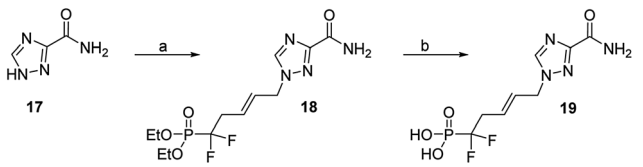
Direct coupling of the purines **14a** and **14b** with difluorophosphonate derivative **5** in DMF with Cs_2CO_3 during 20 h at rt provided the desired N^9-alkylated purine nucleotides **15a** and **15b** in 50 and 59% yields, respectively.[20] Then, 6-chloropurine derivative **15a** was converted to its hypoxanthine analogue **16a** by treatment with TMSBr to release phosphonic acid followed by refluxing an aqueous solution of hydrochloric acid in 54% overall yield, (Scheme 5).[21] Similarly, the 2-amino-6-chloropurine derivatives **15b** gave the guanine **16b** in 60% overall yields.



Scheme 4 Reagents and conditions: (a) (i) Boc_2O, DMAP, dry THF (ii) saturated $NaHCO_3$, MeOH 50 °C, 62%; (b) (*E*)-1-bromo-5-diethoxyphosphoryl-5,5-difluoro-pent-2-ene (**5**), Cs_2CO_3, dry DMF, 85% (for R = H) and 77% (for R = F); (c) TMSBr, CH_2Cl_2, rt, 72 h, quantitative for R = H and for R = F.



Scheme 5 Reagents and conditions: (a) (*E*)-1-bromo-5-diethoxyphosphoryl-5,5-difluoro-pent-2-ene (**5**), Cs_2CO_3, dry DMF, rt, 24 h, 50% (for R = H) and 59% (for R = NH_2); (b) (i) TMSBr, CH_2Cl_2, rt then (ii) HCl (1 M), reflux, 54% (for R = H) and 60% (for R = NH_2).



Scheme 6 Reagents and conditions: (a) (*E*)-1-bromo-5-diethoxyphosphoryl-5,5-difluoro-pent-2-ene (**5**), Cs_2CO_3, dry DMF, 42%; (b) TMSBr, CH_2Cl_2, rt, 72 h, 96%.

Finally, with respect to the broad-spectrum antiviral drug ribavirin, which possess a 1,2,4-triazole-3-carboxamide nucleobase, its acyclic difluorinated phosphonate **19** was synthesized from **17**, in a similar pathway, (Scheme 6).

All the synthesized 5,5-difluoro-5-phosphono-pent-2-en-1-yl nucleosides, **9a**, **b**, **13a**, **b**, **16a**, **b** and **19**, were evaluated against a wide variety of viruses, to determine their antiviral activity (EC_{50}) in HEL, MDCK, Vero and HeLa cell lines, as the effective concentration required to reduce virus-induced cytopathicity or plaque formation by 50%. Compounds were evaluated against vaccinia virus (VV), herpes simplex virus 1 (HSV-1) (KOS strain), herpes simplex virus 2 (HSV-2) (G strain), thymidine kinase deficient (TK-) HSV-1, vesicular stomatis virus (VSV), varicella-zoster virus (VZV) (TK^+ and TK^- strains), human cytomegalovirus (HCMV) (AD-169 and Davis strains) in HEL, vesicular stomatitis virus (VSV), Coxsackie B4, respiratory syncytial virus in HeLa cell cultures, parainfluenza-3, reovirus-1, Sindbis virus and Coxsackie B4 in Vero cells and influenza virus in MDCK cells. All of the synthesized compounds did not exhibit promising antiviral activity.

Conclusions

In summary, a series of hitherto unknown acyclic 5,5-difluoro-5-phosphono-pent-2-en-1-yl-pyrimidines (**9a**, **b**, **13a**, **b**), -purines (**16a**, **b**) and -(1,2,4)-triazolo-3-carboxamide (**19**) were successfully synthesized from (*E*)-1-bromo-5-diethoxyphosphoryl-5,5-difluoro-pent-2-ene (**5**) in a convergent stereoselective manner. Surprisingly, it was discovered that cross-metathesis, in our hand, cannot afford the desired difluorinated phosphonate compounds. However, the final nucleosides were obtained, in good yields, by *N*-alkylation of various nucleobases with (*E*)-diethyl-5-bromo-1,1-difluoropent-3-enylphosphonate.

However, none of the synthesized compounds showed significant antiviral activities. One plausible explanation could be due to a poor penetration to the cell and to the lack of next phosphorylation steps which could be dependent to the length of the acyclic chain.

Experimental section

General methods

Commercially available chemicals were of reagent grade and used as received. The reactions were monitored by thin layer chromatography (TLC) analysis using silica gel plates (Kieselgel 60F254, E. Merck). Column chromatography was performed on Silica Gel 60 M (0.040–0.063 mm, E. Merck). The ^1H and ^{13}C NMR spectra were recorded on a Varian InovaUnity 400 spectrometer (400 MHz) in (d_4) methanol, $CDCl_3$, shift values in parts per million relative to $SiMe_4$ as internal reference. High resolution mass spectra were performed on a Bruker maXis mass spectrometer by the "Federation de Recherche" ICOA/CBM (FR2708) platform. The following products are known products or previously reported by our group: N^3-benzoyluracil (**7a**), CAS registration 2775-87-3; N^3-benzoylthymine (**7b**), CAS registration 4330-20-5; N^4,N^4-bis(Boc)-cytosine (**11a**) CAS registration 1108637-28-0; 5-fluoro-N^4,N^4-bis(Boc)-cytosine (**11b**) CAS registration: 1450880-36-0.

(*E*)-1-Bromo-5-diethoxyphosphoryl-5,5-difluoro-pent-2-ene (5)

A suspension of zinc powder (441.3 mg, 6.75 mmol, 99.99% purity) in THF (0.4 mL) was added to a solution of 1,2-dibromoethane (0.96 mL, 1.13 mmol) in THF (5 mL) at room temperature, and was then warmed up to 65 °C. After 1 min, chlorotrimethylsilane (0.12 mL, 0.8 mmol) was added and the resulting mixture was stirred at 25 °C. After 15 min, the suspension was added dropwise to a solution of diethyl (bromodifluoro)methylphosphonate (0.8 mL, 4.5 mmol) in THF (2 mL), then the reaction mixture was stirred 12 h at 45 °C. CuBr (1.16 g, 8.1 mmol), activated LiCl (344.0 mg 8.1 mmol) and THF (4 mL) was added to the yellow solution at 0 °C under nitrogen, and then the resulting blue solution was stirred at 0 °C. After 10 min, *trans*-1,4-dibromo-2-butene (1.44 g, 6.75 mmol) was added and the reaction was stirred during 4 h at 0 °C. The mixture was filtered through celite and the filtrate was extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The residue was purified by silica gel column chromatography with petroleum ether–EtOAc (4 : 1) to give 2 (1.15 g, 80%) as a colourless oil. ^1H NMR (400 MHz, $CDCl_3$) δ 5.88–5.76 (m, 1H), 5.29–5.22 (m, 1H), 4.27 (q, J = 7.3 Hz, 2H), 4.25 (q, J = 7.3 Hz, 2H), 2.81 (m, 2H), 1.36 (t, J = 7.3 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 125.2, 127.1, 127.0, 126.9, 121.4, 64.6, 64.5, 39.1, 38.9, 38.7, 38.5, 16.5, 16.4; ^{19}F NMR (376 MHz, $CDCl_3$) δ −111.1, −111.4. ^{31}P NMR (162 MHz, $CDCl_3$) δ 6.9; HRMS (ESI): m/z $[M + H]^+$ calcd for $C_9H_{17}BrF_2O_3P$: 321.006487, found: 321.006126.

General procedure A: alkylation with nucleobases

A solution of nucleobase (1.3 equiv.) in dry DMF (3 mL), Cs_2CO_3 (1.3 equiv.) and (*E*)-diethyl-(5-bromo-1,1-difluoro)pent-3-enylphosphonate (1 equiv.) was stirred at room temperature

under argon for 16 h. After removal of DMF under vacuum, the residue was purified by silica gel column chromatography with CH_2Cl_2–MeOH (99 : 1 to 96 : 4) to the desired product.

***N*³-Benzoyl-1-[(*E*)-5-diethoxyphosphoryl-5,5-difluoro-pent-2-en-1-yl]-uracil (8a).** The title compound was prepared from N^3-benzoyluracil **7a** following procedure A to give **8a** (86%) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (dd, J = 8.0, 1.2 Hz, 2H), 7.62 (dd, J = 8.0, 1.2 Hz, 1H), 7.47 (t, J = 8.0 Hz, 2H), 7.32 (d, J = 8.0 Hz, 1H), 5.78 (m, 2H), 5.77 (d, J = 8.0 Hz, 1H), 4.35 (d, J = 6.0 Hz, 2H), 4.26 (q, J = 7.2 Hz, 2H), 4.24 (q, J = 7.2 Hz, 2H), 2.84 (m, 2H), 1.35 (t, J = 7.2 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.9, 162.5, 149.8, 143.7, 135.2, 131.5, 130.5, 129.6, 129.3, 125.7, 102.4, 64.8, 49.5, 37.7, 37.5, 37.4, 37.1, 16.5; ^{19}F NMR (376 MHz, $CDCl_3$) δ −111.1, −111.4; ^{31}P NMR (162 MHz, $CDCl_3$) δ 6.3; HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{20}H_{24}F_2N_2O_6P$: 457.134078, found: 457.133456.

***N*³-Benzoyl-1-[(*E*)-5-diethoxyphosphoryl-5,5-difluoro-pent-2-en-1-yl]-thymine (8b).** The title compound was prepared from N^3-benzoylthymine with typical procedure A to give **8b** (81%) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (dd, J = 8.0, 1.2 Hz, 2H), 7.63 (dd, J = 8.0, 1.2 Hz, 1H), 7.48 (t, J = 8.0 Hz, 2H), 7.17 (s, 1H), 5.79 (m, 2H), 4.35 (d, J = 6.0 Hz, 2H), 4.27 (q, J = 7.2 Hz, 2H), 4.25 (q, J = 7.2 Hz, 2H), 2.87 (m, 2H), 1.95 (s, 3H), 1.37 (t, J = 7.2 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.2, 163.2, 149.9, 139.5, 135.1, 131.7, 130.5, 130.0, 129.2, 125.7, 125.6, 125.5, 125.5, 111.2, 64.7, 49.2, 37.8, 37.6, 37.4, 37.2, 29.8, 16.5, 12.4; ^{19}F NMR (376 MHz, $CDCl_3$) δ −110.7 (t, J = 18.8 Hz), −111.0 (t, J = 18.8 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 6.4; HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{21}H_{26}F_2N_2O_6P$: 471.148990, found: 471.149106.

General procedure B: deprotection of diethylphosphonate nucleosides

A septum-sealed microwave tube charged with diethyl phosphonate derivative and trimethylsilylbromide (10.0 equiv.) in CH_3CN (0.1 M) was irradiated at 70 °C under microwave irradiation during 30 min. The progress of the reaction was monitored by TLC analysis. The reaction mixture was quenched with CH_3OH and concentrated under vacuum, then deionized H_2O (ELGA® water, 10 mL) was added and the aqueous layer was washed with CH_2Cl_2 (3 × 5 mL) and lyophilised to yield the expected phosphonic acid derivative.

***N*¹-[(*E*)-5,5-Difluoro-5-phosphono-pent-2-en-1-yl]-uracil (9a).** The title compound was prepared from phosphonate **8a** following procedure B to give (**9a**) (75%) as a colourless oil. ^{1}H NMR (400 MHz, MeOD) δ 7.57 (d, J = 8.0 Hz, 1H), 5.79 (m, 2H), 5.67 (d, J = 8.0 Hz, 1H), 2.85 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 166.9, 152.7, 147.0, 131.1, 126.2, 126.1, 102.55, 38.6, 38.34, 38.2, 38.0, 20.4; ^{19}F NMR (376 MHz, $CDCl_3$) δ −113.7, −114.0. ^{31}P NMR (162 MHz, $CDCl_3$) δ 5.3; HRMS (ESI): m/z [M + H]$^+$ calcd for $C_9H_{12}F_2N_2O_5P$: 297.044718, found: 297.044641.

***N*¹-[(*E*)-5,5-Difluoro-5-phosphono-pent-2-en-1-yl]-thymine (9b).** The title compound was prepared from phosphonate **8b** following procedure B to give (**9b**) (53%) as a colourless oil. ^{1}H NMR (400 MHz MeOD) δ 7.40 (s, 1H), 5.79 (m, 2H), 4.34 (d, J = 3.9 Hz, 4H), 2.85 (m, 2H), 1.87 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 167.0, 152.9, 142.7, 131.3, 126.0, 111.6, 38.5, 38.4, 38.3, 38.2, 21.1, 12.3; ^{19}F NMR (376 MHz, $CDCl_3$) δ −113.7, −114.0; ^{31}P NMR (162 MHz, $CDCl_3$) δ 5.3; HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{10}H_{14}F_2N_2O_5P$: 311.060279, found: 311.060291.

***N*⁴,*N*⁴-Bis(Boc)-1-[(*E*)-5-diethoxyphosphoryl-5,5-difluoro-pent-2-en-1-yl]-cytosine (12a).** To a solution of N^4,N^4-bis(Boc)cytosine **11a** (186 mg, 0.59 mmol, 1.0 equiv.) in dry DMF (2 mL) was added Cs_2CO_3 (195 mg, 0.65 mmol, 1.1 equiv.) and the *gem* difluorinated phosphonate **5** (211 mg, 0.65 mmol, 1.1 equiv.) at room temperature and stirred under an argon atmosphere for 3 h. The resulting mixture was then diluted with EtOAc (2 × 20 mL), quenched with water and extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The residue was purified by silica gel column chromatography with petroleum ether/EtOAc (98 : 2 to 1 : 2) to give product **12a** (279 mg, 85%) as a colourless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (d, J = 7.4 Hz, 1H), 6.99 (d, J = 7.4 Hz, 1H), 5.79 (m, 2H), 4.48 (d, J = 4.8 Hz, 2H), 4.27 (q, J = 7.3 Hz, 2H), 4.25 (q, J = 7.3 Hz, 2H), 2.83 (m, 2H), 1.37 (t, J = 7.1 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.3, 154.9, 149.6, 147.1, 130.2, 124.9, 96.5, 84.8, 64.5, 51.1, 37.4 (td, J = 21.5, 15.4 Hz), 27.7, 16.4; ^{19}F NMR (376 MHz, $CDCl_3$) δ −110.8 (t, J = 19.0 Hz), −111.1 (t, J = 19.0 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 7.09 (s), 6.44 (s), 5.78 (s); HRMS (ESI) m/z [M + H]$^+$ calcd for $C_{23}H_{37}F_2N_3O_8P$ 552.2281, found 552.2284.

***N*⁴,*N*⁴-Bis(Boc)-1-[(*E*)-5-diethoxyphosphoryl-5,5-difluoro-pent-2-en-1-yl]-5-fluorocytosine (12b).** To a solution of N^4,N^4-bis(Boc)-5-fluorocytosine **11b** (113.5 mg, 0.34 mmol, 1.0 equiv.) in dry DMF (1.5 mL) was added Cs_2CO_3 (123 mg, 0.38 mmol, 1.1 equiv.) and the *gem* difluorinated phosphonate **5** (121 mg, 0.38 mmol, 1.1 equiv.) at room temperature and stirred under an argon atmosphere for 3 h. The resulting mixture was then diluted with EtOAc (2 × 15 mL), quenched with water and extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The residue was purified by silica gel column chromatography with petroleum ether/EtOAc (98 : 2 to 1 : 2) to give product **12a** (149 mg, 77%) as a yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, J = 4.5 Hz, 1H), 5.90–5.76 (m, 2H), 4.53 (d, J = 5.4 Hz, 2H), 4.27 (q, J = 7.1 Hz, 2H), 4.25 (q, J = 7.1 Hz, 2H), 2.87 (m, 2H), 1.47 (s, 18H), 1.37 (t, J = 7.1 Hz, 6H).; ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.95 (d, J = 14.3 Hz), 153.84, 149.02, 142.02, 139.56, 133.07 (d, J = 34.73 Hz), 129.21, 126.94 (m), 84.89, 64.80 (d, J = 8.5 Hz), 51.57, 37.60 (m), 29.82, 27.86, 16.53 (d, J = 5.5 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ −110.8, −111.1, −156.23; ^{31}P NMR (162 MHz, $CDCl_3$) δ 6.23 (t, J = 105.8 Hz) HRMS (ESI) m/z [M + H]$^+$ calcd for $C_{23}H_{35}F_3N_3O_8P$ 570.2189, found 570.2187.

***N*¹-[(*E*)-5,5-Difluoro-5-phosphono-pent-2-en-1-yl]-cytosine (13a).** The title compound was prepared from phosphonate **12a** following procedure B to give **13a** (>98%) as a colorless oil; ^{1}H NMR (250 MHz, MeOD) δ 7.95 (d, J = 5.8 Hz, 1H), 6.14 (d, J = 5.8 Hz, 1H), 5.87 (m, 2H), 4.44 (d, J = 8.50 Hz, 2H), 2.87 (m, 2H); ^{13}C NMR (101 MHz, MeOD) δ 153.7, 153.4, 146.6, 136.0, 134.0, 133.7, 120.6, 36.9 (td, J = 22.1, 15.6 Hz); ^{19}F NMR (376 MHz, MeOD) δ −113.19, −113.46, −170.84; ^{31}P NMR (162 MHz, MeOD) δ 4.79 (t, J = 106.19 Hz); HRMS (ESI) m/z [M + H]$^+$ calcd for $C_9H_{11}F_3N_3O_4P$ 312.03665 found 312.03645.

***N*¹-[(*E*)-5,5-Difluoro-5-phosphono-pent-2-en-1-yl]-5-fluorocytosine (13b).** The title compound was prepared from phosphonate following procedure B to give **13b** (>98%) as a colorless oil; ^{1}H NMR (250 MHz, MeOD) δ 7.95 (d, J = 7.7 Hz, 1H), 6.17–6.08 (m, 1H), 5.88 (d, J = 4.3 Hz, 1H), 4.50 (d, J = 5.0 Hz, 2H), 3.02–2.77 (m, 2H); ^{13}C NMR (101 MHz, MeOD) δ 153.7, 153.4, 146.6, 136.0, 134.0, 133.7, 120.6, 36.9 (td, J = 22.1, 15.6 Hz); ^{19}F NMR (376 MHz, MeOD) δ −113.3 (t, J = 19.0 Hz), −113.6 (t, J = 18.9 Hz); ^{31}P NMR (162 MHz, MeOD) δ 5.68 (s), 5.04 (s), 4.40 (s); HRMS (ESI) m/z [M + H]$^+$ calcd for $C_9H_{13}F_2N_3O_4P$ 295.0606 found 295.0607.

***N*⁹-[(*E*)-5-Diethoxyphosphoryl-5,5-difluoro-pent-2-en-1-yl]-(6-chloro)purine (15a).** The title compound was prepared from 6-chloropurine **14a** following procedure A to give **15a** (50%) as a colourless oil. ^{1}H NMR (400 MHz $CDCl_3$) δ 8.55 (s, 1H), 7.96 (s, 1H), 5.80 (m, 2H), 5.80 (dt, J = 15.6, 6.8 Hz, 1H), 4.87 (d, J = 6.4 Hz, 2H), 4.27 (q, J = 7.2 Hz, 2H), 4.25 (q, J = 7.2 Hz, 2H), 2.86 (m, 2H), 1.36 (t, J = 7.2 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.2, 152.1, 151.7, 151.1, 145.1, 131.6, 130.1, 129.2, 125.8, 64.7, 45.5, 37.6, 37.4, 37.2, 37.0, 16.5; ^{19}F NMR (376 MHz, $CDCl_3$) δ −114.1, −113.7; ^{31}P NMR (162 MHz, $CDCl_3$) δ 6.3; HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{14}H_{19}ClF_2N_4O_3P$: 395.084844, found: 395.084588.

***N*⁹-[(*E*)-5-Diethoxyphosphoryl-5,5-difluoro-pent-2-en-1-yl]-(2-amino-6-chloro)purine (15b).** The title compound was prepared from 6-chloro-2-aminopurine (**14b**) following procedure A to give **15b** (59%) as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (s, 1H), 5.90–5.75 (m, 2H), 5.21 (s, 2H), 4.68 (d, J = 5.7 Hz, 2H), 4.26 (q, J = 7.1 Hz, 2H), 4.24 (q, J = 7.1 Hz, 2H), 2.85 (m, 2H), 1.35 (t, J = 7.1 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.1, 153.6, 151.3, 142.0, 129.7, 125.2, 124.8 (dd, J = 11.0, 5.6 Hz), 64.6 (d, J = 6.9 Hz), 44.9, 37.2 (td, J = 21.6, 15.6 Hz), 16.4 (d, J = 5.4 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ −110.9 (t, J = 18.9 Hz), −111.2 (t, J = 18.9 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 7.10 (dt, J = 14.8, 7.3 Hz), 6.44 (dt, J = 14.6, 7.1 Hz), 5.78 (dt, J = 15.0, 7.3 Hz); HRMS (ESI) m/z [M + H]$^+$ calcd for $C_{14}H_{20}ClF_2N_5O_3P$ 410.0955 found 410.0958.

***N*⁹-[(*E*)-5,5-Difluoro-5-phosphono-pent-2-en-1-yl]-hypoxanthine (16a).** The title compound was prepared from the 6-chlorouracil phosphonate analogue **15a** by treatment reflux acid (HCl, 1 M) to give **16a** (54%) as a colorless oil; ^{1}H NMR (400 MHz, DMSO) δ 12.31 (s, 1H), 8.08 (t, J = 10.6 Hz, 2H), 7.29–6.90 (m, 1H), 5.95–5.88 (m, 1H), 5.73–5.65 (m, 1H), 4.78 (d, J = 5.9 Hz, 2H), 2.76 (t, J = 19.7 Hz, 2H); ^{13}C NMR (101 MHz, DMSO) δ 157.0, 148.6, 146.1, 140.4, 130.5, 124.7 (d, J = 5.0 Hz), 124.1, 45.1, 37.2, 37.0–36.5 (m); ^{19}F NMR (376 MHz, DMSO) δ −111.8 (t, J = 19.8 Hz), −112.1 (t, J = 19.8 Hz); ^{31}P NMR (162 MHz, DMSO) δ 4.12 (t, J = 4.0 Hz), 3.51 (t, J = 4.6 Hz), 2.91 (t, J = 4.5 Hz); HRMS (ESI) m/z [M − H]$^-$ calcd for $C_{10}H_{10}F_2N_4O_4P$ 319.0413, found 319.0417.

***N*⁹-[(*E*)-5,5-Difluoro-5-phosphono-pent-2-en-1-yl]-guanine (16b).** The title compound was prepared from phosphonate **15b** by treatment reflux acid (HCl, 1 M) to give **16b** (60%) as a colorless oil; ^{1}H NMR (400 MHz, MeOD) δ 9.08 (s, 2H), 8.11 (s, 1H), 6.02 (s, 4H), 4.88 (d, J = 3.9 Hz, 4H), 2.92 (t, J = 18.7 Hz, 4H); ^{13}C NMR (101 MHz, MeOD) δ 162.0, 155.9, 153.7, 150.1, 136.6, 127.9–127.4 (m), 107.4, 46.1, 36.9 (d, J = 15.5 Hz); ^{19}F NMR (376 MHz, MeOD) δ −113.1 (t, J = 18.8 Hz), −113.4 (t, J = 18.8 Hz); ^{31}P NMR (162 MHz, MeOD) δ 5.68 (s), 5.30 (d, J = 103.5 Hz), 4.35 (s); HRMS (ESI) m/z [M − H]$^-$ calcd for $C_{10}H_{11}F_2N_5O_4P$ 334.0522, found 334.0529.

***N*¹-[(*E*)-5-Diethoxyphosphoryl-5,5-difluoro-pent-2-en-1-yl]-(3-carbamoyl)-1,2,4-triazol (18).** The title compound was prepared from 1*H*-1,2,4-triazole-3-carboxamide (**17**) following procedure A to give **18** (42%) as a colorless oil; ^{1}H NMR (400 MHz, MeOD) δ 8.49 (s, 1H), 6.04–5.96 (m, 1H), 5.85–5.71 (m, 1H), 4.93 (d, J = 6.1 Hz, 2H), 4.34–4.22 (m, 4H), 2.99–2.80 (m, 2H), 1.36 (t, J = 7.1 Hz, 6H); ^{13}C NMR (101 MHz, MeOD) δ 159.4, 149.6, 149.5–149.1 (m), 145.9, 130.7, 123.2 (dd, J = 10.9, 6.0 Hz), 120.4, 118.2, 64.8 (d, J = 7.0 Hz), 51.6, 45.7, 36.9 (dd, J = 21.6, 6.3 Hz), 15.4; ^{19}F NMR (376 MHz, MeOD) δ −111.8 (t, J = 19.2 Hz), −112.1 (t, J = 19.2 Hz); ^{31}P NMR (162 MHz, MeOD) δ 7.21 (dt, J = 15.7, 7.9 Hz), 6.54 (dt, J = 15.8, 7.9 Hz), 5.87 (dt, J = 15.9, 7.9 Hz); HRMS (ESI) m/z [M + H]$^+$ calcd for $C_{12}H_{20}F_2N_4O_4P$ 354.1185, found 354.1187.

***N*¹-[(*E*)-5,5-Difluoro-5-phosphono-pent-2-en-1-yl]-(3-carbamoyl)-1,2,4-triazole (19).** The title compound was prepared from phosphonate following procedure B from **18** to give **19** (>98%) as an oil; ^{1}H NMR (400 MHz, MeOD) δ 8.07 (s, 1H), 6.02–5.73 (m, 2H), 5.29 (d, J = 5.8 Hz, 2H), 2.97–2.72 (m, 2H); ^{13}C NMR (101 MHz, MeOD) δ 159.5, 149.5, 145.9, 129.8, 127.3, 124.4 (d, J = 5.8 Hz), 51.8, 36.9–36.4 (m); ^{19}F NMR (376 MHz, MeOD) δ −114.3 (t, J = 19.3 Hz), −114.6 (t, J = 19.3 Hz); ^{31}P NMR (162 MHz, MeOD) δ 5.68 (s), 5.30 (d, J = 103.5 Hz), 4.75 (s); HRMS (ESI) m/z [M + H]$^+$ calcd for $C_8H_{10}F_2N_4O_4P$ 295.0413 found 295.0414.

Antiviral activity assays

The antiviral assays were based on inhibition of virus-induced cytopathicity or plaque formation in HEL 299 (ATCC® CCL-137™) cell culture against herpes simplex virus 1 (HSV-1) (KOS), HSV-2 (G), vaccinia virus, vesicular stomatitis virus, cytomegalovirus (HCMV), and varicella-zoster virus (VZV). Moreover, the Vero (ATCC® CCL-81™) cell culture was utilized to test such compounds against parainfluenza-3, reovirus-1, Sindbis virus and Coxsackie B4. Furthermore, the novel compounds were evaluated in HeLa cell culture against vesicular stomatitis virus, Coxsackie virus B4, and respiratory syncytial virus or MDCK (ATCC® CCL-34™) [influenza A (H1N1; H3N2) and influenza B]. Confluent cell cultures (or nearly confluent for MDCK cells) in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus (1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures) or with 20 plaque forming units (PFU). After 1–2 h virus adsorption period, residual virus was removed, and the cell cultures were incubated in the presence of varying concentrations (200, 40, 8, 1.6, 0.32 μM) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. Antiviral activity was expressed as the EC_{50} or concentration required reducing virus-induced cytopathogenicity or viral plaque (VZV) plaque formation by 50%. The minimal cytotoxic concentration (MCC) of the compounds was defined as the compound concentration that caused a microscopically visible alteration of cell morphology. Alternatively, cytotoxicity of the test compounds was measured

based on inhibition of cell growth. HEL cells were seeded at a rate of 5×10^3 cells per well into 96-well microtiter plates and allowed to proliferate for 24 h. Then, medium containing different concentrations of the test compounds was added. After 3 days of incubation at 37 °C, the cell number was determined with a Coulter counter.

The cytostatic concentration was calculated as the CC_{50}, or the compound concentration required reducing cell proliferation by 50% relative to the number of cells in the untreated controls.

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