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Synthesis of fluorinated acyclic nucleoside phosphonates with 5-azacytosine base moiety

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ABSTRACT

With respect to the strong antiviral activity of (*S*)-1-[3-hydroxy-2-(phosphonomethoxy)propyl]-5-azacytosine various types of its side chain fluorinated analogues were prepared. The title compound, (*S*)-1-[3-fluoro-2-(phosphonomethoxy)propyl]-5-azacytosine (FPMP-5-azaC) was synthesised by the condensation reaction of (*S*)-2-[(diisopropoxyphosphoryl)methoxy)-3-fluoropropyl *p*-toluenesulfonate with a sodium salt of 5-azacytosine followed by separation of appropriate N^1 and O^2 regioisomers and ester hydrolysis. Transformations of FPMP-5-azaC to its 5,6-dihydro-5-azacytosine counterpart, amino acid phosphoramidate prodrugs and systems with an annelated five-membered imidazole ring, i.e. imidazo [1,2-a][1,3,5]triazine derivatives were also carried out. 1-(2-Phosphonomethoxy-3,3,3-trifluoropropyl)-5-azacytosine was prepared from 5-azacytosine and trifluoromethyloxirane to form 1-(3,3,3-trifluoro-2-hydroxypropyl)-5-azacytosine which was treated with diisopropyl bromomethanephosphonate followed by deprotection of esters. Antiviral activity of all newly prepared compounds was studied. FPMP-5-azaC diisopropyl ester inhibited the replication of herpes viruses with EC_{50} values that were about three times higher than that of the reference anti-HCMV drug ganciclovir without displaying cytotoxicity.

1. Introduction

The chemistry of acyclic nucleoside phosphonates (ANPs) is a key research topic of the Institute of Organic Chemistry and Biochemistry in Prague. The importance of these compounds lies in their biological activities, especially antiviral [1–4]. Three compounds of this class have already became commercially available drugs: cidofovir for the treatment of human cytomegalovirus (CMV) retinitis in AIDS patients; adefovir in its prodrug form adefovir dipivoxil for the treatment of hepatitis B (HBV); and tenofovir, either as tenofovir disoproxil fumarate, or more recently (since 2015) also as a new prodrug form tenofovir alafenamide (TAF), for the treatment of HIV and HBV infections [5]. The introduction of tenofovir to the market had a crucial influence on the transformation of HIV/AIDS from a mortal threat to a manageable chronic disease [6]. Besides, long-term systematic investigation of ANPs has enabled the development of many other therapeutically promising structures, which have been synthesised but have never advanced to the stage of preclinical/clinical investigations. Among others, this concerns fluorinated purine 3-fluoro-2-[(phosphonomethoxy)propyl] derivatives [7] and acyclic nucleoside phosphonates with 5-azacytosine base moiety [8–10].

5-Azacytosine ANPs were originally synthesised with the aim to search for new epigenetic (hypomethylating) agents; contrary to 5-azacytosine nucleosides (azacitidine, decitabine), however, this activity has not been found. It is evident that the presence of the sugar moiety is essential for this effect [11]. On the other hand, we found a new class of antiviral agents selective against DNA viruses. The lead compound of this group is 5-azacytosine analogue of cidofovir, 1-(*S*)-[3-hydroxy-2-(phosphonomethoxy)propyl]-5-azacytosine, HPMP-5-azaC (Fig. 1). In comparison with cidofovir, its antiviral activity data are similar, but the toxicity profile is more favourable, with the selectivity index (the ratio of the 50% cytotoxic concentration (CC_{50}) to the 50% effective concentration (EC_{50})) being 2- to 16-fold higher than that of cidofovir [8]. The antiviral activity of HPMP-5-azaC has been increased many times by the transformation to ester prodrugs derived from its cyclic form [9].

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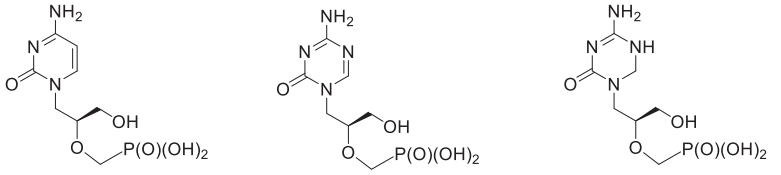


Fig. 1. The structures of anti-DNA virus agent cidofovir and its 5-azacytosine analogue, HPMP-5-azaC.

The only disadvantage of HPMP-5-azaC is its rather complicated metabolic profile caused by its chemical (and also enzymatic) instability [12,13]. Therefore, the compound was finally not advanced to the stage of clinical investigations. Other 5-azacytosine ANPs have better stability but they are either inactive or their antiviral activity is only marginal. Interestingly, some of them, e.g. 2-(phosphonomethoxy)ethyl derivative exhibited antiviral activity when converted into appropriate prodrugs [10].

In this study, we investigate a new type of 5-azacytosine ANPs modified with fluorine at the acyclic part. Fluorinated derivatives, especially nucleoside and nucleotide analogues are compounds of great interest [14]. An introduction of the fluorine atom into biologically active molecules often leads to remarkable changes in their physical, biological, and pharmacokinetic properties due to its electronegativity, size, omniphobicity or lipophilicity and electrostatic interactions [15]. In clinical practice, fluorinated nucleosides are utilised as effective cytostatics (e.g. gemcitabine) or antivirals (trifluridine, sofosbuvir) [16,17]. Within the group of ANPs, 3-fluoro-2-(phosphonomethoxy)propyl (FPMP) derivatives have been identified as potent and selective inhibitors of retroviruses. The adenine derivative, (*S*)-9-[3-fluoro-2-(phosphonomethoxy)propyl] adenine, ((*S*)-FPMPA) exhibits even better *in vivo* anti-HIV and anti-HBV parameters than adefovir. The activity against HIV is also known for both enantiomers of 9-[3-fluoro-2-(phosphonomethoxy)propyl]-2,6-diaminopurine (FPMPDAP) and its guanine counterpart [7,18]. Structures of these compounds are depicted in Fig. 2.

This paper describes the synthesis and biological activities of side-chain fluorinated analogues of HPMP-5-azaC and their derivatives with further modifications on the base moiety. These modifications have been designed with the aim to stabilise the 5-azacytosine ring.

Moreover, it must be taken into account that ANPs as compounds bearing (a) free phosphonate group(s) have a polar character responsible for their limited intracellular delivery and low oral bioavailability. The usual way to overcome this problem is the transformation of such compounds to appropriate prodrugs. The most frequently used ANP prodrugs are acyloxyalkyl (pivaloyloxymethyl, POM), alkyloxyalkyl, S-acylthioethyl (SATE), aryl, cyclic 1-aryl-1,3-propanyl and cyclosaligenyl phophonate esters, phosphoramidates bearing amino acid esters and proTides [19]. Despite promising *in vitro* or *in vivo* studies, only a few of them have been proceeded into clinical trials. This can be attributed to the difficulty of the achievement of the right prodrug property balance, in particular suitable chemical stability, rate of conversion, safety with respect to by-products, the avoidance of stereoisomers, adequate physical properties (the ability to crystallise, solubility), and the ability to synthesize substantial quantities. The synthesis of ANP prodrugs and the investigation of their pharmacological properties have been the subject of many reviews [20,21].

Regarding the above-mentioned facts, the transformation of our new structures to prodrug forms is an integral part of this work.

2. Chemistry

The most logical method of the preparation of FPMP-5-azaC reveals direct condensation of the sodium salt of 5-azacytosine with an appropriate fluorinated precursor. Such a reactive precursor can be (*S*)-2-[(diisopropoxyphosphoryl)methoxy)-3-fluoropropyl *p*-toluenesulphonate (FPMP synthon, **1**) or its methanesulphonyl analogue. The synthon approach has already been reported for the preparation of purine FPMP derivatives by Jindrich et al. [22] Their synthesis started from protected 5′-O-ribonolactone and included as many as nine reaction steps. Another, more efficient synthesis of FPMP synthons utilises enantiomeric fluorohydrines accessible from commercially available O-tritylated glycidols by reaction with potassium hydrogen difluoride [23]. In our present work, we have investigated the fluorination of (*R*)-3-benzyloxy-2-[(diisopropylphosphoryl)methoxy)]propanol (**3**), a compound utilised in our laboratory as a common starting material for the preparation of 3-hydroxy-2-(phosphonomethoxy) propyl derivatives. This synthon is accessible on a large scale by a three-step synthesis from (*S*)-2-(trityloxymethyl)oxirane (**2**) (Scheme 1) [24].

Our first experiments with the fluorination of **3** were carried out *via* previous activation of the hydroxyl group, e.g. by the reaction with *p*-toluenesulphonyl chloride [25]. Although the reaction of tosylate **4**, formed in this way, with tetrabutylammonium fluoride proceeded satisfactorily, the total preparative yield of the fluoro derivative **5** was not high because of the complicated and laborious isolation and purification of the tosylate intermediate.

An alternative way is the direct fluorination of the hydroxy derivative **3**. This approach has never been explored in the area of ANP synthons whose synthesis is usually performed as a large-scale multi-step process without the isolation of intermediates. The fluorination reagents generally used for the substitution of hydroxy groups are DAST [26], Olah's reagent [27], and fluoroalkylamine reagents [28].

We have recently described perfluorobutane-1-sulphonyl fluoride as a nucleophilic fluorination reagent for the preparation of thymidine phosphorylase inhibitors (*S*) and (*R*)-1-[3-fluoro-2-(phosphonomethoxy)propyl]thymines (FPMPT) [29–31]. Unlike various strongly corrosive reagents, e.g. DAST or deoxofluor, perfluorobutane-1-sulphonyl fluoride is stable, only little moisture-sensitive and easy to handle. Its use for the direct fluorination of ANPs is known only from for several examples. In the above mentioned FPMPT synthesis, the appropriate starting material was the 3-hydroxy-2-[(phosphonomethoxy)propyl] derivative of 4-O-methoxy-5-methylpyrimidinone (not thymine). The reason is that these fluorinations are sensitive to the presence of acidic hydrogen atoms in the starting molecule. For example, the performance of the same reaction with the diisopropyl ester of HPMPT (containing NH at the position 3 of the thymine moiety), results in elimination under basic conditions resulting in a six-membered ring closure through the side-chain hydroxy group [29]. Similarly, this fluorination method also fails with cytosine derivatives (HPMPC).

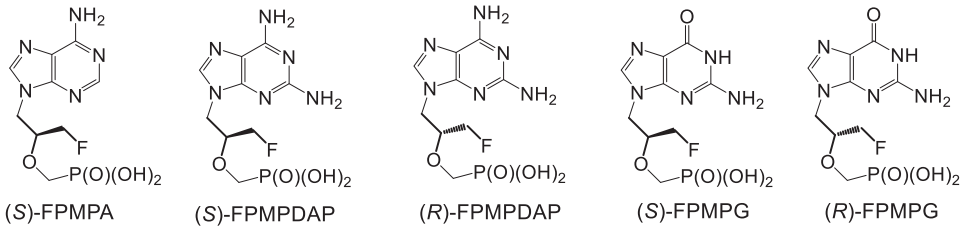
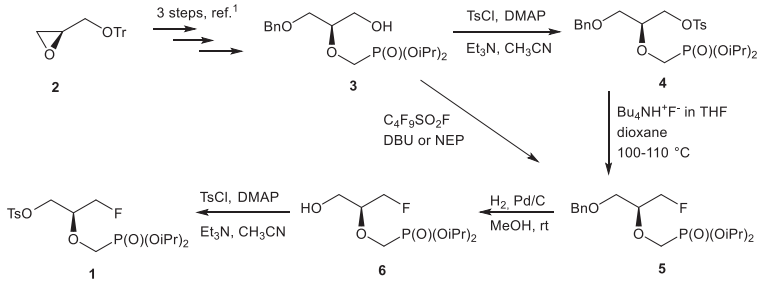


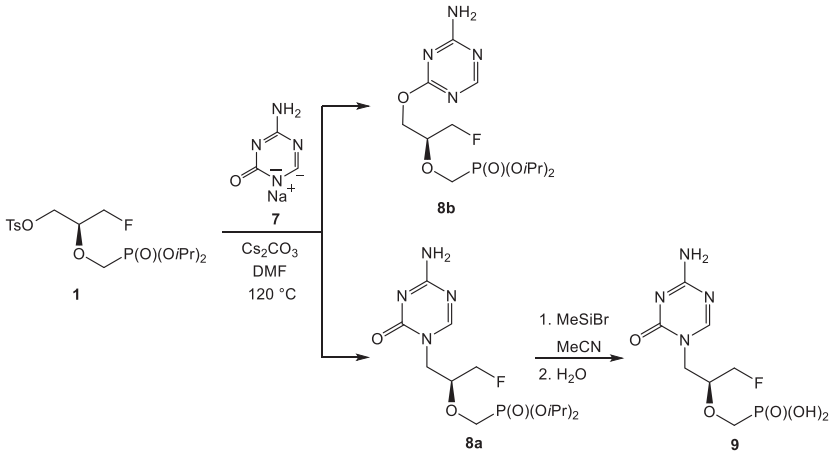
Fig. 2. The structures of the antiretroviral 3-fluoro-2-(phosphonomethoxy)propyl derivatives of purine bases.



Scheme 1. The **s**ynthesis of (*S*)-2-[(diisopropoxyphosphoryl)methoxy)-3-fluoropropyl *p*-toluenesulphonate (FPMP synthon, **1**).

An important aspect of syntheses with perfluorobutane-1-sulphonyl fluoride is the selection of reaction conditions: base and solvent [32]. The base should be a good proton acceptor with low nucleophilicity and moderate basicity to avoid elimination. It the preparation of fluoro derivative **5** from (*R*)-3-benzyloxy-2-[(diisopropylphosphoryl)methoxy)]propanol (**3**), we chose between *N*-ethylpiperidine (NEP) and 1,8-diazabicyclo [5.4.0]undec-7-ene (DBU). The reaction with NEP had been reported to provide better results in the syntheses of fluorinated nucleosides [32], but the preparation of our FPMP precursor proceeded satisfactory using DBU. The reaction was carried out in toluene, initially at room temperature. Although the yields of **5** were 42–50% the reaction rate was slow. A significant decrease of reaction time was achieved by heating to 90 °C. Full conversion was observed when DBU was added for a few hours (3–4 h). The course of fluorination reaction with NEP was worse and required excess of perfluorobutanesulphonyl fluoride (90% conversion after 9 h). The final FPMP synthon, (*S*)-2-[(diisopropoxyphosphoryl)methoxy)-3-fluoropropyl *p*-toluenesulphonate (**1**), was obtained from **5** by the removal of a benzyl group *via* catalytic hydrogenation to give intermediate **6** which was further subjected to tosylation (Scheme 1).

The condensation of (*S*)-2-[(diisopropoxyphosphoryl) methoxy)-3-fluoropropyl *p*-toluenesulphonate (**1**) with a sodium salt of 5-azacytosine (**7**) was performed by heating in *N,N*-dimethylformamide with catalytic amount of caesium carbonate. Reaction afforded a mixture of N^1 and O^2 regioisomers **8a** and **8b**, whose ratio depended on the reaction time (Scheme 2). The highest regioselectivity was observed when 5-azacytosine sodium salt was heated with phosphonate **1** at 120 °C for 11 h. In this case, only N^1-isomer **8a** was detected but its preparative yield was low (28%). Further extension of the reaction time resulted in increased formation of O^2-isomer **8b**. After 24 h at 120 °C, the ratio of regioisomers **8a**:**8b** was 7:4 while their preparative yields were 28% (**8a**) and 16% (**8b**). Regarding these observations, it is obvious that the alkylation is a balanced process and the desired N^1-isomer is formed as a product possessing kinetic stability. The final product, free phosphonic acid **9** (FPMP-5-azaC) was obtained from diisopropyl ester **8a** by the standard procedure of deprotection by



Scheme 2. The condensation of (*S*)-2-[(diisopropoxyphosphoryl)methoxy)-3-fluoropropyl p-toluenesulphonate (FPMP synthon, **1**) with a sodium salt of 5-azacytosine (**7**).

bromotrimethylsilane followed by hydrolysis.

In order to have also its prodrug form available for antiviral screening we selected its phosphoramidate prodrug **11**. The synthesis was performed by the modified procedure originally developed by Jansa [33] based on the reaction of diisopropyl ester **8a** with bromotrimethylsilane, first giving the intermediary bis(-trimethylsilyl) ester **10**. This intermediate can be converted without isolation to the desired bis(amidate) **11** by a reaction with an appropriate amino acid ester activated by 2,2′-dithiopyridine (Aldrithiol™) and triphenylphosphine (Scheme 3). A direct reaction of **9** with ethyl L-alanine, Aldrithiol™ and triphenylphosphine was not successful, giving the desired bis(amidate) **11** in low yields only, together with a number of by-products.

A significant problem of 5-azacytosine compounds is their hydrolytic instability which substantially compromise their pharmaceutical potential [11–13]. This instability is caused by the electron deficiency in position 6 of the triazine ring, where the imine-resembling carbon could be easily attacked by a nucleophile, *e.g.* an hydroxyl ion, accompanied by the cleavage of the ring [11,12]. On the other hand, the conversion of 5-azacytosine compounds into their 5,6-dihydro-5-azacytosine counterparts with sp^3-hybridisied carbon (CH_2) at the position 6, leads to the compounds which are hydrolytically stable. Nucleosides modified in this way, e.g. 5,6-dihydro-5-azacytidine and 2′-deoxy-5,6-dihydro-5-azacytidine, still preserve their antitumor and hypomethylating activities. They are lower in comparison with the original 5-azacytosine derivatives but the compounds are less toxic [11,34]. In the field of ANPs, we previously reported the 5,6-dihydro derivative of HPMP-5-azaC (Fig. 1). The compound has preserved its anti-DNA virus activity (albeit lower than HPMP-5-azaC); nevertheless in contrast to HPMP-5-azaC, it also exhibits inhibitory activity against HCV replication, and is hydrolytically stable [8].

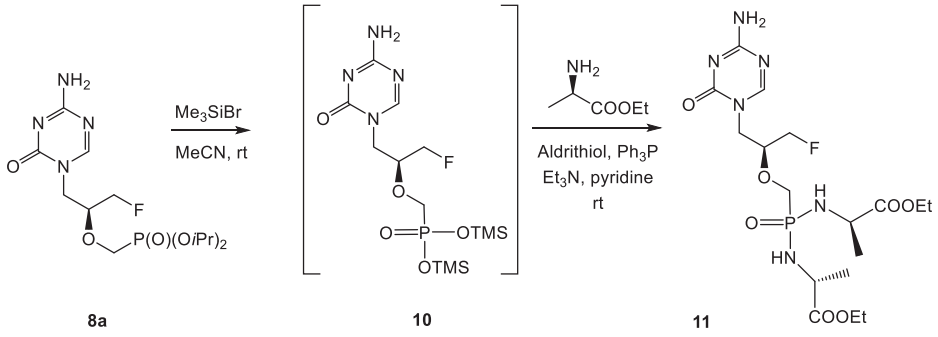
The transformation of FPMP-5-azaC into the corresponding 5,6-dihydro derivative **13** was carried out on the level of its diisopropyl ester **8a** *via* catalytic hydrogenation. This step was followed by the deprotection of ester groups in the intermediate **12** using bromotrimethylsilane (Scheme 4).

The guanidine grouping of the 5,6-dihydro-5-azacytosine moiety provides a unique opportunity for the preparation of imidazo [1,2-a][1,3,5]triazine derivatives – compounds with four hydrogen-bonding sites bearing an annelated 5-membered imidazole ring. These analogues can be formed by reaction of 5,6-dihydro-5-azacytosine derivatives with appropriate carbonyl compounds. We have described the coupling of 5,6-dihydro-5-azacytosine phosphonate **12** with various 2-bromoacetophenones **14a-c**. (Scheme 5). Although the desired annelated products **15a-c** were formed in all cases, the imidazole imino group was found sensitive towards further alkylation with some 2-bromoacetophenones. When the compound **12** was treated with 2-bromoacetophenones **14b** and **14c** alkylated by-products **16b** and **16c** were isolated together with products **15b**, and **15c**. On the other hand, 2-bromoacetophenone **14a** did not afford any further alkylated product.

Surprisingly enough, a serious problem occurred in the deprotection of diisopropyl ester groups to the final phosphonic acids **17a-c**. The usual way using bromotrimethylsilane completely failed, leading to a mixture of decomposition products. Another possibility was a microwave-assisted acid hydrolysis. This method of the transformation of phosphonate esters to free ANPs has been originally developed at the IOCB by Jansa and Holý. This method is usually very clean and the isolation of products is easy [35,36]. In our experiments, we hydrolysed **15a-c** with two equivalents of HCl at 150 °C for 5–15 min under micro-wave irradiation. Unfortunately, the ring of the triazine moiety opened and the C-6 methylene group was hydrolytically split off. This indicated the destabilisation of the 5,6-dihydro-5-azacytosine ring as a consequence of imidazole-triazine annelation. On the other hand, we had observed earlier that 5-azacytosine ANPs can have better stability in the cases when their phosphonic function is masked by an appropriate prodrug group [9,11,37]. Taking into account this finding, we selected another synthetic strategy starting from bis(amidate) prodrug of FPMP-5-azaC **11**. Compound **11** can be easily reduced to the corresponding 5,6-dihydro form **18** which is then subjected to condensation reaction with 2-bromoacetophenones to form the desired annelated structures **19a-c** in their prodrug forms (Scheme 6). The other way, Jansa's method [33] for the transformation of phosphonate ester **12** into phosphoramidate **18** doesn't take place. This may have been caused by the presence of acetic acid in the structure of **12**. Condensation reactions using 2,2′-dipyridyldisulphide (Aldrithiol) and triphenylphosphine are known to proceed *via* a cyclic intermediate whose formation requires a proton coming from a nucleophile [38] (e.g. amino acid ester). It is possible that acetic acid preferentially protonates a pyridine unit of 2-thiopyridine which then cannot activate a nucleophile and phosphonate units.

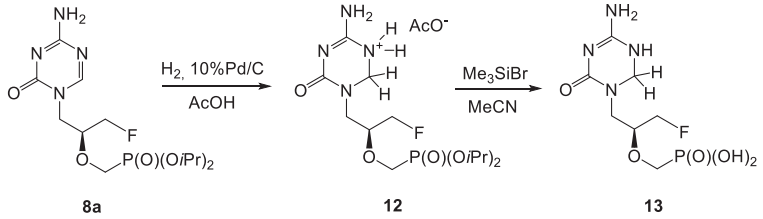
Besides FPMP derivatives, we also worked on the syntheses of 5-azacytosine ANPs bearing *gem*-difluorinated arrangement by the method developed in our laboratory for purine *gem*-difluorinated ANPs [36]. Unfortunately, our effort failed because of the instability of the triazine ring during the deprotection of phosphonate ester groups (with bromotrimethylsilane as well as under microwave conditions).

On the other hand, ANPs with trifluoromethyl arrangement are known to be stable towards Lewis acids. Therefore, 5-azacytosine phosphonate **25** could be prepared quite easily (Scheme 7). To a first approximation, the compounds were prepared as racemates using cheap and easily accessible trifluoromethyloxirane. This approach is sufficient for the evaluation of the true biological potential of the compounds. If any biological activity were sought,

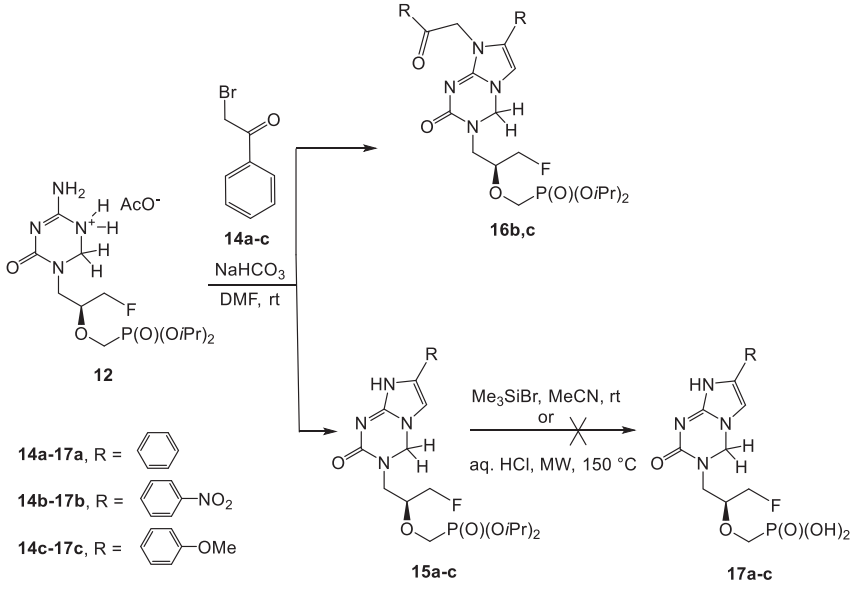


8a 10 11

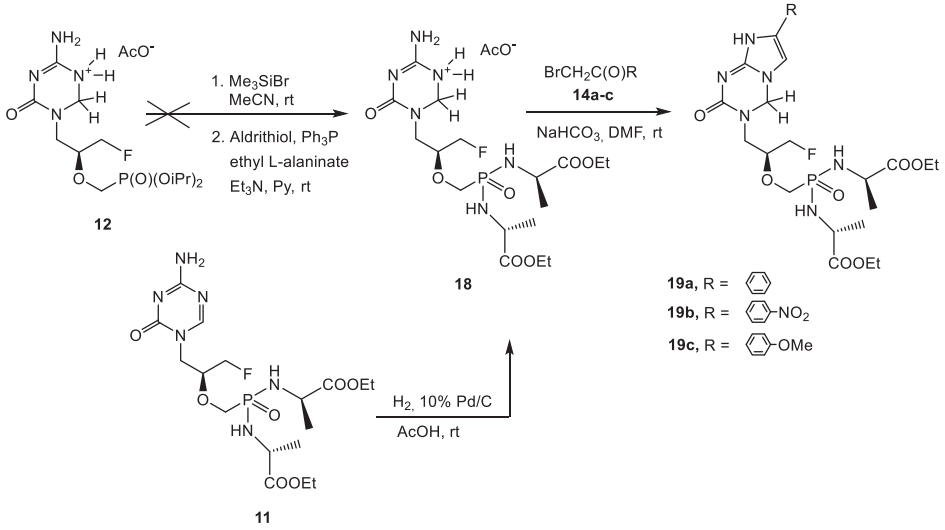
Scheme 3. The synthesis of the bis(amide) prodrug of 1-(*S*)-[3-fluoro-2-(phosphonomethoxy)propyl]-5-azacytosine.



Scheme 4. The synthesis of 1-(*S*)-5,6-dihydro-[3-fluoro-2-(phosphonomethoxy)propyl]-5-azacytosine (**13**).



Scheme 5. The transformation of 5,6-dihydro-5-azacytosine ANPs to their imidazo [1,2-a][1,3,5]triazine derivatives **15a-c**.

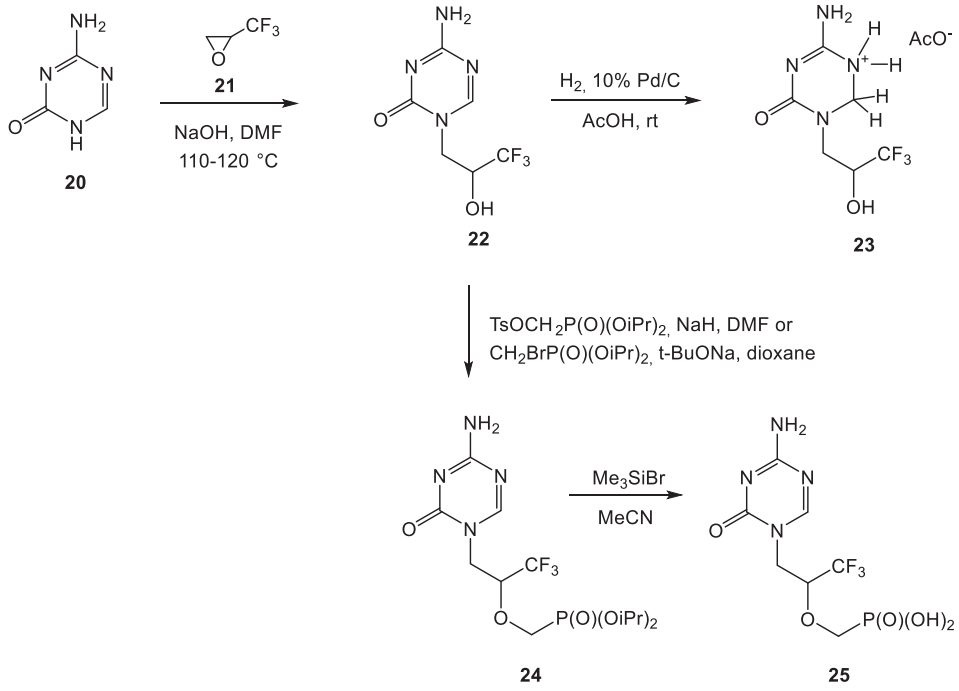


Scheme 6. The synthesis of the bis(amidate) prodrug of 5,6-dihydro-FPMP-5azaC (**18**) and its imidazo [1,2-a][1,3,5]triazine analogues (**19a-c**).

pure enantiomers would have to be subsequently synthesised.

The synthesis is based on the treatment of 5-azacytosine (**20**) with trifluoromethyloxirane (**21**) followed by the reaction of the formed hydroxy derivative **22** with appropriate phosphonate synthon to give the desired phosphonate in the form of ester **24.**

For this purpose, two phosphonate synthons were investigated. While the reaction with diisopropyl tosyloxymethanephosphonate and sodium hydride afforded only low yields of **24**, the conversion increased substantially when more reactive diisopropyl bromomethanephosphonate and sodium *tert*-butoxide were used. In



Scheme 7. The synthesis of 5-azacytosine ANPs with trifluoromethyl arrangement.

agreement with our previous observations, the presence of fluorine atom or trifluoromethyl arrangement in the intermediate (e.g. **22**) may significantly decrease the nucleophilicity of the secondary alcohol group compared to corresponding non-fluorinated analogues [36]. The final deprotection of ester groups with bromotrimethylsilane afforded free phosphonic acid **25**. Additionally, the catalytic hydrogenation of 5-azacytosine derivative **22** in the presence of acetic acid afforded 5,6-dihydro derivative **23** in the form of an acetate salt.

3. Biological activity

The synthesised side-chain fluorinated analogues of HPMP-5-azaC were evaluated for their inhibitory activity against a wide range of DNA and RNA viruses: in human embryonic lung (HEL) cells [herpes simplex virus-1 (KOS strain), herpes simplex virus-2 (G strain), thymidine kinase deficient (acyclovir resistant) herpes simplex virus-1 (TK− KOS ACVr), vaccinia virus, vesicular stomatitis virus, human cytomegalovirus (HCMV) (AD-169 strain and Davis strains), varicella-zoster virus (VZV) (TK + VZV strain and TK− VZV strains))], in HeLa cell cultures [vesicular stomatitis virus, Coxsackie virus B4 and respiratory syncytial virus], in Vero cell cultures [parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4, Punta Toro virus, yellow fever virus], in CRFK cell cultures [feline corona virus (FIPV)], and in MDCK cell cultures [influenza A virus (H1N1 and H3N2 subtypes) and influenza B virus]. Ganciclovir, cidofovir, acyclovir, brivudin, zanamivir, amantadine, rimantadine, ribavirin, dextran sulphate (molecular weight 10000, DS-10000), mycophenolic acid, Hippeastrum hybrid agglutinin (HHA), and Urtica dioica agglutinin (UDA) were used as the reference compounds. The antiviral activity was expressed as EC_{50}, i.e. the compound concentration required to decrease virus plaque formation (VZV) or virus-induced cytopathogenicity (other viruses) by 50%. While none of the compounds showed any activity against RNA viruses, compounds **8a** and **22** were able to inhibit, albeit weakly, the replication of herpesviruses (Table 1). The FPMP-5-azaC diisopropyl ester (**8a**) inhibited the replication of HCMV with EC_{50} values that were about three times higher than those of the reference anti-HCMV drug ganciclovir without displaying cytotoxicity for HEL cells up to the highest concentration tested (100 μM). The compound **8a** proved significantly less active against wild-type strains of HSV-1, HSV-2 and VZV than the reference drug acyclovir but was in contrast to acyclovir remained active against TK-deficient viruses. This derivative proved inactive against other DNA viruses such as vaccinia virus (poxvirus) and adenoviruses. Compound **22** (3,3,3-trifluoro-2-hydroxypropyl-5-azaC) showed marginal activity against herpesviruses and adenovirus 2 with EC_{50} values in the range of 40–270 μM.

4. Conclusions

Several synthetic strategies for side-chain fluorinated acyclic nucleotide analogues bearing 5-azacytosine and imidazo [1,2-a][1,3,5]triazine base moieties have been developed. Highly polar free phosphonic acids were transformed into amidate prodrugs. All new compounds were subjected to the investigation of biological (antiviral) activities. Except for the FPMP 5-azaC diisopropyl ester (**8a**), which had some activity against human cytomegalovirus and herpesviruses and for 1-(3,3,3-trifluoro-2-hydroxypropyl)-5-azacytosine (**22**) with negligible activity against herpesviruses and adenovirus, none of the newly synthesised acyclic nucleoside analogues (ANPs) exhibited antiviral activity.

5. Experimental

5.1. General

Unless stated otherwise, solvents were evaporated at 40 °C/2 kPa and compounds were dried at 13 Pa. Column chromatography was performed on silica gel 60 μm (Fluka). Analytical TLC was performed on silica gel 60 F_{254} plates (Merck) in the solvent systems: chloroform-methanol 7:1 (S1), chloroform-methanol 7:2 (S2) and 10:1 (S3), ethyl acetate-acetone-ethanol-water 18:3:2:2 (S4) or 16:3:3:2 (S5), 2-propanol-25% aq. ammonia-water 7:1:2 (S6).

Table 1
The *in vitro* antiviral activity of fluorinated 5-azacytosine derivatives against DNA viruses compared to reference antiviral drugs acyclovir (ACV), brivudine (BVDU), ganciclovir (GCV) and cidofovir (CDV).

No.	Antiviral activity - EC_{50} (μM)[a]									Cytotoxicity (μM)	
	HCMV		VZV		HSV-1 Kos (TK^+)	HSV-2 G (TK^+)	HSV-1 ACV-R (TK^-)	VACV	Adeno-2	MCC[b]	CC_{50}[c]
	AD-169	Davis	Oka (TK^+)	07/1 (TK^-)							
8a	24.5 ± 13.6	7.9 ± 5.5	11.7 ± 4.0	8.8 ± 0.2	39 ± 19	17.5 ± 10.6	32.5 ± 12.5	>100	>100	>100	>100
9	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	ND[d]
11	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	ND
13	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	ND
18	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	ND
19a	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	ND
19b	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	ND
19c	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	ND
22	231 ± 12.9	200 ± 17.8	40.6 ± 11.7	126 ± 13.8	230 ± 29	230 ± 29	>446	>446	268 ± 178	>446	>446
23	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	ND
25	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	ND
ACV	ND	ND	1.44 ± 0.79	43.9 ± 19.8	0.48 ± 0.27	0.21 ± 0.12	48.9 ± 57.0	>250	ND	>440	>440
BVDU	ND	ND	0.023 ± 0.021	26.7 ± 19.0	0.03 ± 0.02	123 ± 26	1.6 ± 0.6	11.3 ± 6.4	ND	>300	>300
GCV	6.7 ± 2.9	3.0 ± 2.7	ND	ND	0.03 ± 0.01	0.03 ± 0	1.1 ± 0.7	>100	ND	>350	>350
CDV	0.93 ± 0.34	0.73 ± 0.31	ND	ND	1.9 ± 1.2	1.3 ± 0.4	1.3 ± 0.3	26 ± 12	8.3 ± 2.1	>300	>300

[a] The effective concentration required to reduce virus plaque formation (VZV) or virus-induced cytopathic effect (HCMV, HSV-1, HSV-2, vaccinia virus (VACV) and adenovirus-2) by 50%. The virus input was (1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures) or 20 plaque forming units (PFU) in the case of VZV.
[b] The minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology.
[c] The cytostatic concentration required to reduce cell growth by 50%.
[d] Not determined.

Preparative TLC was carried out on 45 × 18 × 0.4 cm loose-layer silica gel containing UV indicator (S7). Compound **13** was purified on a column (3.3 × 1.7 cm) of spherical reverse phase silica gel C18 (45–75 μm), 70 Å (Versa Flash, Supelco), (S8). Microwave-assisted hydrolysis was performed on a CEM Discover reactor (USA) under the following conditions: the power of 50–150 W, the maximum pressure of 1.7 MPa (17 Bar), the temperature profile of 0→150 °C (1 min), followed by 150 °C (25 min). The reactions were performed in a high-pressure test tube (10 mL), equipped with a septum and a magnetic stirrer. Reaction time was modified according to the rate of conversion of the starting esters. IR spectra were recorded on a FTIR Spectrometer Bruker IFS 55 (Equinox) in KBr, CCl_4 and $CHCl_3$. ^1H and ^{13}C NMR spectra were acquired on Bruker Avance 600 III HD and Bruker Avance 500 III HD spectrometers operating at 600.1 and 500.0 MHz for ^1H and 150.9 and 125.7 MHz for ^{13}C, respectively. $^{31}P\{^1H\}$ NMR spectra were recorded on Bruker Avance 500 III HD or Bruker Avance 400 III HD operating at 202.3 or 162.0 MHz, respectively. ^{19}F NMR spectra were recorded on Bruker Avance 500 III HD operating at 470.3 MHz. NMR samples were dissolved in $CDCl_3$, DMSO-d_6, CD_3OD or D_2O and NMR spectra were referenced to the residual solvent signal ($CDCl_3$: $\delta(^1H) = 7.26$ ppm, $\delta(^{13}C) = 77.0$ ppm; DMSO-d_6: $\delta(^1H) = 2.50$ ppm, $\delta(^{13}C) = 39.7$ ppm; CD_3OD-d_6: $\delta(^1H) = 3.31$ ppm, $\delta(^{13}C) = 49.0$ ppm). Aqueous samples were referenced to signals of 1,4-dioxane as an external standard ($\delta(^1H) = 3.75$ ppm, $\delta(^{13}C) = 69.3$ ppm). The complete assignment of NMR signals was based on a combination of 1D and 2D correlation experiments (H,H–COSY, H,C-HSQC, H,C-HMBC). The general numbering scheme for the assignment of NMR signals is outlined in Fig. 3. Mass spectra were measured on an LTQ Orbitrap XL (Thermo Fisher Scientific) operated in the ESI mode. Optical rotations were measured on a Autopol IV polarimeter (Rudolph Research Analytical, U.S.A.) at 20 °C, $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. The concentrations for specific rotation measuements are in the units of g/100 mL.

5.2. Materials and chemicals

Standard chemicals and ion-exchange resins were purchased from Sigma-Aldrich (Czech Republic). The synthetic precursor **3** was prepared from tritylglycidyl ether (**2**) according to Ref. [24] The sodium salt of 5-azacytosine **7** was prepared by the treatment of 5-azacytosine with sodium methoxide according to Ref. [24] Diisopropyl bromomethylphosphonate was purchased from Alfa Aesar. Trifluoromethyloxirane was purchased from Fluorochem (Wesley Street, Old Glossop). Anhydrous solvents were purchased from Sigma-Aldrich and Penta (Czech Republic).

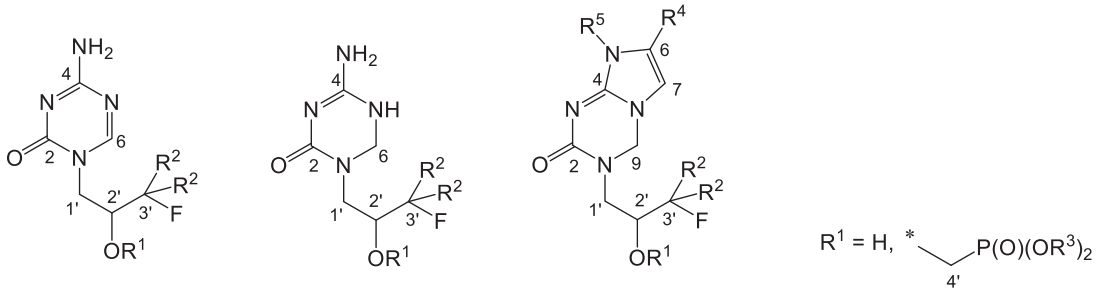


Fig. 3. The general numbering scheme for assignment of NMR signals.

5.3. (S)-3-(Benzyloxy)-2-[(diisopropoxyphosphoryl)methoxy] propyl 4-methylbenzenesulphonate (4)

A solution of **3** (63 g, 138 mmol) in acetonitrile (400 mL) was cooled to 0 °C and triethylamine (159 mL, 1.1 mol), TsCl (40 g, 166 mmol) and DMAP (50 mg) were added. The mixture was stirred at room temperature for 54 h (until full conversion), then concentrated *in vacuo* and the residue was partitioned between ether and water (600 mL/100 mL). An organic layer was dried over magnesium sulphate and evaporated. The obtained material was used for the next step without purification. Yield: 51 g (58%) of **4** as yellow oil. ^{1}H, and ^{13}C NMR data were identical to those reported in Ref. [39].

5.4. Diisopropyl (S)-{[(1-(benzyloxy)-3-fluoropropan-2-yl)oxy] methyl}phosphonate (5)

5.4.1. Method 1

A mixture of **4** (51 g, 99.1 mmol) and 1 M TBAF solution in THF (147 mL) was refluxed vigorously in dioxane (500 mL) at 100–110 °C for 6.5 h. The mixture was concentrated *in vacuo* and the residue was partitioned between ether and water (500 mL/60 mL). An aqueous layer was extracted with ether (2 × 200 mL). Combined organic extracts were dried over $MgSO_4$ and evaporated. The residue was used without further purification. Yield: 31 g (86%) of **5** as yellow oil; $[\alpha]_D$ +13.6 (c 0.295, $CHCl_3$). IR: (CCl_4) ν_{max} 3090, 3066, 3033, 2981, 2934, 1635, 1604, 1586, 1495, 1453, 1467, 1386, 1376, 1315, 1267, 1191, 1178, 1142, 1071, 1106, 1030, 1010, 991, 889, 710, 611 cm^{-1}. ESIMS *m/z* 363.1 $(M + 1)^+$ (48). HRMS *m/z* (ESI) calcd. for $C_{17}H_{28}O_5FNaP$ $(M + 1)^+$ 385.1551 found 385.1551.^{1}H NMR (500.0 MHz, $CDCl_3$): 1.308, 1.314, 1.321, 1.328 (4 × d, 4 × 3H, J_{vic} = 6.2, (C**H$_3$**)$_2$CH); 3.58 (ddd, 1H, J_{gem} = 10.2, $J_{1'b,2'}$ = 5.5, $J_{H,F}$ = 1.2, H-1′b); 3.62 (ddd, 1H, J_{gem} = 10.2, $J_{1'a,2'}$ = 5.4, $J_{H,F}$ = 1.9, H-1′a); 3.89 (m, 1H, H-2′); 3.93 (d, 2H, $J_{H,P}$ = 8.7, H-4′); 4.51 (ddd, 1H, $J_{H,F}$ = 47.3, J_{gem} = 10.1, $J_{3'b,2'}$ = 5.6, H-3′b); 4.53 (s, 2H, CH_2Ph); 4.57 (ddd, 1H, $J_{H,F}$ = 47.3, J_{gem} = 10.1, $J_{3'a,2'}$ = 3.6, H-3′a); 4.68–4.80 (m, 2H, C**H**$(CH_3)_2$); 7.25–7.37 (m, 5H, H-*o,m,p*-Ph). ^{13}C NMR (125.7 MHz, $CDCl_3$): 23.9 (d, $J_{C,P}$ = 4.6, (**C**H_3)$_2$CH); 24.1 (d, $J_{C,P}$ = 3.8, (**C**H_3)$_2$CH); 65.2 (d, $J_{C,P}$ = 167.9, CH_2-4′); 68.5 (d, $J_{C,P}$ = 7.8, CH_2-1′); 71.2, 71.2 (2 × d, $J_{C,P}$ = 6.5, **C**H$(CH_3)_2$); 73.5 (CH_2Ph); 79.3 (dd, $J_{C,F}$ = 18.8, $J_{C,P}$ = 10.7, CH-2′); 83.2 (d, $J_{C,F}$ = 171.2, CH_2-3′); 127.6 (CH-*o*-Ph); 127.8 (CH-*p*-Ph); 128.4 (CH-*m*-Ph); 137.7 (C-*i*-Ph). ^{31}P{^{1}H} NMR (202.3 MHz, $CDCl_3$): 19.41.^{19}F NMR (470.4 MHz, $CDCl_3$): −226.78 (td, J = 47.3, 19.1).

5.4.2. Method 2

A mixture of DBU (250 μL, 1.7 mmol) or *N*-ethylpyrrolidine (86 μL,0.6 mmol) and 92% perfluorobutane-1-sulphonyl fluoride (410 mg, 1.2 mmol) was stirred in toluene (1 ml) at room temperature for 10 min. A solution of **3** (300 mg, 0.8 mmol) in toluene (3 ml) was added and the resulting mixture was heated at 90 °C for 4 h (TLC control in ethyl acetate). The mixture was evaporated and the residue was chromatographed on a silica gel column in a gradient of hexane (100%) to hexane – ethyl acetate (2:9). The yield of **5**: 150 mg (50%, reaction with DBU) or 126 mg (42%, reaction with NEP), yellow oil. ^{1}H, and ^{13}C NMR data were identical to those described in Method 1.

5.5. Diisopropyl (S) {[(1-fluoro-3-hydroxypropan-2-yl)oxy]methyl} phosphonate (6)

A solution of **5** (31 g, 85.6 mmol) in methanol (300 mL) was hydrogenated in a high pressure autoclave (10 atm) over 10% palladium on charcoal (20 g, suspended in water (20 mL)) for 48 h. The mixture was filtered through a Celite pad and the filtrate was concentrated *in vacuo*. The residue was chromatographed on silica gel in ethyl acetate. Yield: 13.3 g (57%) of **6** as yellowish oil. ESIMS *m/z* 273.1 $(M + 1)^+$ (22). HRMS *m/z* (ESI) calcd. for $C_{10}H_{23}O_5FP$ $(M + 1)^+$ 273.1261, found 273.1262.^{1}H NMR (500.0 MHz, $CDCl_3$): 1.33, 1.346, 1.349 (3 × d, 12H, J_{vic} = 6.2, (C**H$_3$**)$_2$CH); 3.10 (bs, 1H, OH); 3.64 (m, 1H, H-1′b); 3.74–3.81 (m, 2H, H-1′a,2′); 3.81 (dd, 1H, J_{gem} = 14.1, $J_{H,P}$ = 8.4, H-4′b); 4.05 (dd, 1H, J_{gem} = 14.1, $J_{H,P}$ = 7.5, H-4′a); 4.40–4.56 (m, 2H, H-3′); 4.69–4.83 (m, 2H, C**H**$(CH_3)_2$). ^{13}C NMR (125.7 MHz, $CDCl_3$): 23.9 (d, $J_{C,P}$ = 4.6, (**C**H_3)$_2$CH); 24.0 (d, $J_{C,P}$ = 4.2, (**C**H_3)$_2$CH); 24.1 (d, $J_{C,P}$ = 3.8, (**C**H_3)$_2$CH); 61.0 (d, $J_{C,P}$ = 7.9, CH_2-1′); 65.4 (d, $J_{C,P}$ = 169.0, CH_2-4′); 71.4 (d, $J_{C,P}$ = 6.9, **C**H$(CH_3)_2$); 71.8 (d, $J_{C,P}$ = 6.6, **C**H$(CH_3)_2$); 82.2 (dd, $J_{C,F}$ = 18.7, $J_{C,P}$ = 7.9, CH-2′); 82.9 (d, $J_{C,F}$ = 170.8, CH_2-3′). ^{31}P{^{1}H} NMR (202.3 MHz, $CDCl_3$): 20.63.^{19}F NMR (470.4 MHz, $CDCl_3$): −226.42 (td, J = 47.2, 17.0).

5.6. (S)-2-[(Diisopropoxyphosphoryl)methoxy]-3-fluoropropyl 4-methylbenzenesulphonate (1)

Triethylamine (18 mL, 129 mmol), tosyl chloride (7.31 g, 38.3 mmol) and dimethylaminopyridine (20 mg, catalytic amount) were added to a 0 °C cold solution of **6** (5 g, 20.0 mmol) in acetonitrile (250 mL). The mixture was stirred at room temperature for 48 h, then evaporated, and the residue was extracted with a mixture of ether and brine (200 mL/50 mL). The organic layer was dried ($MgSO_4$) and evaporated and the residue chromatographed on silica gel in ethyl acetate. Yield 5.1 g (65%) of **1** as yellow oil. R_f (EtOAc) 0.50; $[\alpha]_D$ +11.8 (c 0.651, $CHCl_3$). IR: (CCl_4) ν_{max} 3068, 3034, 2981, 2934, 2875, 1599, 1496, 1467, 1453, 1403, 1385, 1376, 1375, 1307, 1289, 1260, 1212, 1191, 1180, 1142, 1119, 1106, 1113, 1099, 1042, 889, 833, 705, 666, 571, 555 cm^{-1}. ESIMS *m/z* 427.1 $(M + 1)^+$ (42). HRMS *m/z* (ESI) calcd. for $C_{17}H_{29}O_7FPS$ $(M + 1)^+$ 427.1350, found 427.1351. Anal. Calcd. for $C_{17}H_{28}FO_7PS$: C, 47.9; H, 6.6; P, 7.3; S, 7.5; F, 4.5. Found: C, 48.2; H, 6.6; P, 7.5; S, 7.2; F, 4.3. NMR data are in agreement with the literature [22,23].

5.7. The synthesis of 5-azacytosine FPMP-derivatives 8a and 8b

A mixture of **1** (1.5 g, 3.5 mmol), the sodium salt of 5-azacytosine [24] (**7**) (456 mg, 3.4 mmol) and caesium carbonate (10 mg, 0.03 mmol) in dimethylformamide (11 mL) was heated at 120 °C for 24 h. The mixture was concentrated *in vacuo*, the residue was coevaporated with toluene (2 × 2 mL) and chromatographed on a preparative TLC plate (S7) in dichloromethane-methanol (7:1).

5.7.1. Diisopropyl (S)-{[(1-(4-amino-2-oxo-1,3,5-triazin-1(2H)-yl)-3-fluoropropan-2-yl)oxy]methyl}phosphonate (8a)

Yield: 359 mg (29%) of a white amorphous solid. R_f = 0.40 (S1). $[\alpha]_D$ −55.8 (c 0.276, $CHCl_3$). IR: (CCl_4) ν_{max} 3540, 3487, 3422, 3305, 3305, 3185, 3120, 2985, 2937, 1652, 1634, 1691, 1599, 1570, 1507, 1463, 1440, 1388, 1376, 1244, 1178, 1142, 1104, 1127, 1015, 997, 890 cm^{-1}. ESIMS *m/z* 389.1 $(M + 1)^+$ (100). HRMS *m/z* (ESI) calcd. for $C_{13}H_{24}O_5$ N_4FNaP $(M + Na)^+$ 389.1361, found 389.1466.^{1}H NMR (500.0 MHz, $CDCl_3$): 1.30, 1.31, 1.33 (3 × d, 12H, J_{vic} = 6.0, (C**H$_3$**)$_2$CH); 3.68 (dd, 1H, J_{gem} = 14.0, $J_{1'b,2'}$ = 8.1, H-1′b); 3.74 (dd, 1H, J_{gem} = 13.7, $J_{H,P}$ = 9.3, H-4′b); 3.93 (dd, 1H, J_{gem} = 13.7, $J_{H,P}$ = 8.5, H-4′a); 4.00 (ddddd, 1H, $J_{H,F}$ = 22.8, $J_{2',1'}$ = 8.1, 3.1, $J_{2',3'}$ = 4.5, 2.7, H-2′); 4.21 (dd, 1H, J_{gem} = 14.0, $J_{1'a,2'}$ = 3.1, H-1′a); 4.46 (ddd, 1H, $J_{H,F}$ = 47.1, J_{gem} = 10.7, $J_{3'b,2'}$ = 4.5, H-3′b); 4.69 (ddd, 1H, $J_{H,F}$ = 47.1, J_{gem} = 10.7, $J_{3'a,2'}$ = 2.7, H-3′a); 4.69–4.78 (m, 2H, C**H**$(CH_3)_2$); 5.67 (bs, 1H, NH_a**H$_b$**); 6.42 (bs, 1H, N**H$_a$**H$_b$); 8.04 (s, 1H, H-6). ^{13}C NMR (125.7 MHz, $CDCl_3$): 23.9 (d, $J_{C,P}$ = 4.6, (**C**H_3)$_2$CH); 24.0 (d, $J_{C,P}$ = 3.9, (**C**H_3)$_2$CH); 24.0 (d, $J_{C,P}$ = 4.0, (**C**H_3)$_2$CH); 47.7 (d, $J_{C,F}$ = 9.1, CH_2-1′); 65.2 (d, $J_{C,P}$ = 168.5, CH_2-4′); 71.3 (d, $J_{C,P}$ = 6.6, **C**H$(CH_3)_2$); 71.4 (d, $J_{C,P}$ = 6.7, **C**H$(CH_3)_2$); 77.7 (dd, $J_{C,F}$ = 18.5, $J_{C,P}$ = 10.4, CH-2′); 82.0 (d, $J_{C,F}$ = 173.6, CH_2-3′); 154.4 (C-2); 159.5 (CH-6); 166.5 (C-4). ^{31}P{^{1}H}

NMR (202.3 MHz, $CDCl_3$): 18.80.^{19}F NMR (470.4 MHz, $CDCl_3$): −229.09 (td, J = 47.1, 22.8).

5.7.2. Diisopropyl (S)- ({1-[(4-amino-1,3,5-triazin-2-yl)oxy]-3-fluoropropan-2-yl}oxymethyl)phosphonate (8b)

Yield: 201 mg (16%) of yellowish oil. R_f = 0.63 (S1). IR: (CCl_4) ν_{max} 3552, 3501, 3434, 3328, 3328, 3201, 2981, 2937, 1658, 1636, 1611, 1587, 1577, 1542, 1464, 1455, 1428, 1386, 1376, 1337, 1312, 1245, 1179, 1142, 1106, 1011, 993, 890, 823 cm^{-1}. ESIMS *m/z* 367.2 $(M + 1)^+$ (73). HRMS *m/z* (ESI) calcd. for $C_{13}H_{25}O_5\ N_4FP\ (M + 1)^+$ 367.1541, found 367.1542.^{1}H NMR (500.0 MHz, $CDCl_3$): 1.310, 1.313, 1.322, 1.323 (4 × d, 4 × 3H, J_{vic} = 6.1, (CH_3)$_2$CH); 3.94 (dd, 1H, J_{gem} = 13.6, $J_{H,P}$ = 8.6, H-4′b); 3.98 (dd, 1H, J_{gem} = 13.6, $J_{H,P}$ = 8.8, H-4′a); 4.06 (m, 1H, H-2′); 4.42−4.50 (m, 2H, H-1′); 4.58 (ddd, 1H, $J_{H,F}$ = 47.1, J_{gem} = 10.2, $J_{3'b,2'}$ = 5.2, H-3′b); 4.69 (ddd, 1H, $J_{H,F}$ = 47.1, J_{gem} = 10.2, $J_{3'a,2'}$ = 3.8, H-3′a); 4.75 (m, 2H, C**H**(CH_3)$_2$); 5.79 (bs, 2H, NH_2); 8.35 (s, 1H, H-6). ^{13}C NMR (125.7 MHz, $CDCl_3$): 23.9, 23.9 (2 × d, $J_{C,P}$ = 4.7, (**C**H_3)$_2$CH); 24.1, 24.1 (2 × d, $J_{C,P}$ = 3.7, (**C**H_3)$_2$CH); 64.9 (d, $J_{C,F}$ = 7.8, CH_2-1′); 65.31 (d, $J_{C,P}$ = 168.3, CH_2-4′); 71.3, 71.36 (2 × d, $J_{C,P}$ = 6.6, **C**H(CH_3)$_2$); 78.0 (dd, $J_{C,F}$ = 19.4, $J_{C,P}$ = 10.4, CH-2′); 82.3 (d, $J_{C,F}$ = 172.5, CH_2-3′); 167.9 (C-4); 168.10 (CH-6); 170.1 (C-2); ^{31}P{^{1}H} NMR (202.3 MHz, $CDCl_3$): 18.97.^{19}F NMR (470.4 MHz, $CDCl_3$): −227.58 (td, J = 47.1, 19.1).

5.8. (S)-{[1-(4-Amino-2-oxo-1,3,5-triazin-1(2H)-yl)-3-fluoropropan-2-yl]oxymethyl}phosphonic acid (9)

Bromotrimethylsilane (0.9 ml, 6.8 mmol) was added to a solution of **8a** (228 mg, 0.6 mmol) in acetonitrile (15 ml). The mixture was stirred at room temperature overnight, evaporated and the residue coevaporated with water (2 × 2 ml). The residue was dissolved in water (3 ml) and applied onto a column of Dowex 1 (acetate form). The ion exchanger was washed with water, than with a gradient 0−1 M acetic acid and finally with 1 M formic acid. Appropriate UV absorbing fractions were evaporated, the residue co-evaporated with methanol (2 × 5 mL) and toluene (2 × 5 mL), and the product was finally lyophilised from water. Yield: 158 mg (74%) of **9** as a white foam. $[\alpha]_D$ −53.3 (c 0.377, H_2O). IR: (KBr) ν_{max} 3385, 3107, 1681, 1616, 1524, 1308, 1203, 1176, 1003, 991 cm^{-1}. ESIMS *m/z* 283.0 $(M + 1)^+$ (47). HRMS *m/z* (ESI) calcd. for $C_7H_{13}O_5\ N_4FP\ (M + 1)^+$ 283.0602 found 283.0603. Anal. Calcd. for $C_7H_{12}FO_5\ N_4P$. 3.3H_2O: C, 24.6; H, 5.5; N, 16.4; P, 9.1; F, 5.6. Found: C, 24.9; H, 5.2; N, 16.2; P, 8.9; F, 5.3.^{1}H NMR (500.0 MHz, D_2O): 3.69 (dd, 1H, J_{gem} = 13.7, $J_{H,P}$ = 9.0, H-4′b); 3.85−4.07 (m, 4H, H-1′,2′,4′a); 4.59 (ddd, 1H, $J_{H,F}$ = 46.3, J_{gem} = 10.8, $J_{3'b,2'}$ = 3.1, H-3′b); 4.77 (ddd, 1H, $J_{H,F}$ = 47.1, J_{gem} = 10.8, $J_{3'a,2'}$ = 2.8, H-3′a); 8.47 (s, 1H, H-6). ^{13}C NMR (125.7 MHz, D_2O): 50.2 (d, $J_{C,F}$ = 8.2, CH_2-1′); 68.0 (d, $J_{C,P}$ = 158.4, CH_2-4′); 79.4 (dd, $J_{C,F}$ = 18.6, $J_{C,P}$ = 9.8, CH-2′); 84.5 (d, $J_{C,F}$ = 168.6, CH_2-3′); 150.6 (C-2); 162.6 (C-4); 165.0 (CH-6). ^{31}P{^{1}H} NMR (202.3 MHz, D_2O): 16.56.^{19}F NMR (470.4 MHz, $CDCl_3$): −228.54 (ddd, J = 47.1, 46.3, 22.9).

5.9. Bis(isopropyl L-phenylalanine) amidate prodrug of (S)-1-[3-fluoro-2-(phosphonomethoxy)propyl]-5-azacytosine (11)

A mixture of **8a** (150 mg, 0.4 mmol), acetonitrile (10 ml) and bromotrimethylsilane (544 μL, 4.1 mmol) was stirred at room temperature overnight, evaporated (under argon), and the residue was co-evaporated with toluene (2 × 2 ml). The residue and ethyl L-alaninate (252 mg, 1.6 mmol) were dissolved in pyridine (3.3 mL) and triethylamine (0.8 mL). The solution was warmed to 50 °C and another solution consisting of Aldrithiol-2 (541 mg, 2.5 mmol), triphenylphosphine and pyridine (4.1 mL) was added. The resulting mixture was heated at 50 °C for 4 h and further stirred at room temperature overnight. The mixture was evaporated, and the residue was chromatographed on silica gel in a gradient of 0−15% methanol in chloroform. Yield: 128 mg (64%) of **11** as white amorphous solid. $[\alpha]_D$ −55.8 (c 0.251, $CHCl_3$). IR: ($CHCl_3$) ν_{max} 3538, 3487, 3421, 3394, 3199, 2988, 2940, 2877, 1733, 1690, 1463, 1635, 1599, 1507, 1478, 1391, 1377, 1368, 1108, 1062, 1020, 991 cm^{-1}. ESIMS *m/z* 503.0 $(M + Na)^+$ (100). HRMS *m/z* (ESI) calcd. for $C_{17}H_{30}O_7\ N_6FNaP\ (M + Na)^+$ 503.1790, found 503.1790. Anal. Calcd for $C_{17}H_{30}FO_7\ N_6FP$. 1.5H_2O: C, 40.2; H, 6.6; N, 16.6; P, 6.0; F, 3.7. Found: C, 40.5; H, 6.3; N, 16.3; P, 6.0; F, 3.7.^{1}H NMR (500.0 MHz, CD_3OD): 1.26, 1.29 (2 × t, 2 × 3H, J_{vic} = 7.1, C**H$_3$**CH_2O); 1.39 (dd, 3H, J_{vic} = 7.2, $J_{H,P}$ = 1.0, H-3-Ala); 1.40 (d, 3H, J_{vic} = 7.2, H-3-Ala); 3.69 (dd, 1H, J_{gem} = 13.0, $J_{H,P}$ = 11.5, H-4′b); 3.79 (dd, 1H, J_{gem} = 14.2, $J_{1'b,2'}$ = 8.2, H-1′b); 3.89−3.99 (m, 3H, H-2′, H-2-Ala); 4.00 (dd, 1H, J_{gem} = 13.0, $J_{H,P}$ = 7.0, H-4′a); 4.09−4.25 (m, 5H, H-1′a, CH_3C**H$_2$**O); 4.51 (ddd, 1H, $J_{H,F}$ = 47.1, J_{gem} = 10.7, $J_{3'b,2'}$ = 4.4, H-3′b); 4.70 (ddd, 1H, $J_{H,F}$ = 47.6, J_{gem} = 10.7, $J_{3'a,2'}$ = 3.0, H-3′a); 8.26 (s, 1H, H-6). ^{13}C NMR (125.7 MHz, CD_3OD): 14.5, 14.5 (**C**H_3CH_2O); 20.8 (d, $J_{C,P}$ = 6.9, CH_3-3-Ala); 21.1 (d, $J_{C,P}$ = 5.3, CH_3-3-Ala); 48.8 (CH_2-1′); 49.5, 50.3 (CH-2-Ala); 62.4, 62.4 (CH_3**C**H_2O); 67.3 (d, $J_{C,P}$ = 136.6, CH_2-4′); 79.5 (dd, $J_{C,F}$ = 18.5, $J_{C,P}$ = 12.8, CH-2′); 83.3 (d, $J_{C,F}$ = 171.1, CH_2-3′); 157.28 (C-2); 161.4 (CH-6); 168.3 (C-4); 175.7 (d, $J_{C,P}$ = 2.2, C-1-Ala); 175.8 (d, $J_{C,P}$ = 4.5, C-1-Ala). ^{31}P{^{1}H} NMR (202.3 MHz, CD_3OD): 24.66.^{19}F NMR (470.4 MHz, CD_3OD): −229.85 (ddd, J = 47.6, 47.1, 22.4).

5.10. (S)-6-Amino-3-{2-[(diisopropoxyphosphoryl)methoxy]-3-fluoropropyl}-4-oxo-1,2,3,4-tetrahydro-1,3,5-triazin-1-ium acetate (12)

Compound **8a** (351 mg, 0.9 mmol) was hydrogenated in acetic acid (10 mL) on 10% palladium on charcoal (109 mg) at atmospheric pressure (TLC control in S1) for 24 h. The mixture was filtered through a Celite pad and the filtrate was evaporated. The residue was co-evaporated with toluene (2 × 5 mL), methanol (5 mL) and hexane (5 mL). Yield: 351 mg (90%) of **12** as colourless oil. $[\alpha]_D$ −7.7 (c 0.350, $CHCl_3$). IR: ($CHCl_3$) ν_{max} 3320, 3189, 2985, 2748, 2502, 1716, 1654, 1609, 1557, 1467, 1409, 1235, 1179, 1142, 1002, 891, 653, 601 cm^{-1}. ESIMS *m/z* 367.2 $(M - 1)^-$ (100). HRMS *m/z* (ESI) calcd. for $C_{13}H_{25}O_5\ N_4FP\ (M - 1)^-$ 367.1552, found 367.1547.^{1}H NMR (500.0 MHz, $CDCl_3$): 1.31, 1.33, 1.34 (3 × d, 12H, J_{vic} = 6.0, (CH_3)$_2$CH); 1.99 (bs, 3H, CH_3COO); 3.31 (dd, 1H, J_{gem} = 14.7, $J_{1'b,2'}$ = 7.4, H-1′b); 3.63 (dd, 1H, J_{gem} = 14.7, $J_{1'a,2'}$ = 3.8, H-1′a); 3.81 (dd, 1H, J_{gem} = 13.6, $J_{H,P}$ = 9.6, H-4′b); 3.93 (m, 1H, H-2′); 3.94 (dd, 1H, J_{gem} = 13.6, $J_{H,P}$ = 9.0, H-4′a); 4.43 (ddd, 1H, $J_{H,F}$ = 47.2, J_{gem} = 10.6, $J_{3'b,2'}$ = 5.0, H-3′b); 4.57 (ddd, 1H, $J_{H,F}$ = 47.2, J_{gem} = 10.6, $J_{3'a,2'}$ = 2.9, H-3′a); 4.61 (d, 1H, J_{gem} = 9.6, H-6b); 4.67−4.95 (m, 2H, C**H**(CH_3)$_2$); 4.79 (d, 1H, J_{gem} = 9.6, H-6a); 8.88 (bs, 4H, NH_2 + NH_2^+). ^{13}C NMR (125.7 MHz, $CDCl_3$): 22.9 (**C**H_3COO); 23.9, 24.0 (2 × d, $J_{C,P}$ = 4.6, (**C**H_3)$_2$CH); 24.0, 24.0 (2 × d, $J_{C,P}$ = 3.9, (**C**H_3)$_2$CH); 46.2 (d, $J_{C,F}$ = 8.7, CH_2-1′); 57.4 (CH_2-6); 64.9 (d, $J_{C,P}$ = 169.9, CH_2-4′); 71.7, 72.0 (2 × d, $J_{C,P}$ = 6.8, **C**H(CH_3)$_2$); 79.3 (dd, $J_{C,F}$ = 18.3, $J_{C,P}$ = 11.8, CH-2′); 82.5 (d, $J_{C,F}$ = 172.9, CH_2-3′); 153.6 (C-2); 156.5 (C-4); 177.8 (CH_3**C**OO). ^{31}P{^{1}H} NMR (202.3 MHz, $CDCl_3$): 19.07.^{19}F NMR (470.4 MHz, $CDCl_3$): −227.48 (td, J = 47.2, 20.9).

5.11. (S)-1-[3-Fluoro-2-(phosphonomethoxy)propyl]-5,6-dihydro-5-azacytosine (13)

A mixture of **12** (332 mg, 0.8 mmol), acetonitrile (19 ml) and bromotrimethylsilane (1.0 ml, 7.8 mmol) was stirred at room temperature overnight, than evaporated and the residue was co-evaporated with acetonitrile (2 × 3 mL) and water (2 × 3 ml). The residue was extracted with ether/water (5 mL/30 mL), ethyl acetate/water (5mL/30 mL) and ether/water (5 mL/30 mL). The aqueous layer was evaporated, and the residue was co-evaporated

with methanol (5 mL), toluene (2 × 5 mL) and ethyl acetate (5 mL). The final purification was performed on a reverse phase C18 column (S8) in water, and the product was lyophilised from water. Yield: 180 mg (75%) of **11** as a yellowish foam. $[\alpha]_D$ −13.8 (c 0.276, D_2O). IR: (KBr) ν_{max} 3409, 2810, 2732, 2700, 2330, 1700, 1623, 1172, 1110, 1063, 1005 cm^{-1}. ESIMS *m/z* 285.1 $(M + 1)^+$ (92). HRMS *m/z* (ESI) calcd. for $C_7H_{15}O_5N_4FP$ $(M + 1)^+$ 285.0759, found 285.0760. Anal. Calcd for $C_7H_{14}FO_5N_4FP.9H_2O$: C, 18.8; H, 7.1; N, 12.6; P, 6.9; F, 4.3. Found: C, 18.7; H, 7.0; N, 12.9; P, 6.9; F, 4.0.^{1}H NMR (500.0 MHz, D_2O): 3.53 (dd, 1H, J_{gem} = 15.1, $J_{1'b,2'}$ = 7.8, H-1′b); 3.72 (dd, 1H, J_{gem} = 15.1, $J_{1'a,2'}$ = 3.8, H-1′a); 3.77 (dd, 1H, J_{gem} = 13.3, $J_{H,P}$ = 9.8, H-4′b); 3.92 (dd, 1H, J_{gem} = 13.3, $J_{H,P}$ = 9.0, H-4′a); 3.96 (m, 1H, H-2′); 4.53 (ddd, 1H, $J_{H,F}$ = 46.6, J_{gem} = 10.8, $J_{3'b,2'}$ = 4.0, H-3′b); 4.69 (ddd, 1H, $J_{H,F}$ = 47.3, J_{gem} = 10.8, $J_{3'a,2'}$ = 3.2, H-3′a); 4.80 (d, 1H, J_{gem} = 10.5, H-6b); 4.89 (d, 1H, J_{gem} = 10.5, H-6a). ^{13}C NMR (125.7 MHz, D_2O): 48.5 (d, $J_{C,F}$ = 8.0, CH_2-1′); 59.0 (CH_2-6); 68.2 (d, $J_{C,P}$ = 159.4, CH_2-4′); 81.3 (dd, $J_{C,F}$ = 18.3, $J_{C,P}$ = 11.5, CH-2′); 85.1 (d, $J_{C,F}$ = 167.7, CH_2-3′); 154.3 (C-2); 156.6 (C-4). $^{31}P\{^1H\}$ NMR (202.3 MHz, D_2O): 17.74.^{19}F NMR (470.4 MHz, D_2O): −229.37 (ddd, J = 47.3, 46.6, 23.8).

5.12. The reaction of compound 12 with 1-aryl-2-bromoacetophenones (14a-c)

A mixture of **12** (0.51 mmol), bromoacetophenone **14a-c** (0.5 mmol) and sodium bicarbonate (43 mg, 0.5 mmol) in DMF (10 mL) was stirred at room temperature for 24 h. The mixture was evaporated and the residue was chromatographed on a preparative TLC plate (S7) in the system mentioned below.

5.12.1. Diisopropyl (S)-{[(1-fluoro-3-(2-oxo-7-phenyl-1,2,8,8a-tetrahydroimidazo[1,2-a][1,3,5]triazin-3(4H)-yl)propan-2-yl)oxy]methyl}phosphonate (15a)

Yield: 95 mg (40%) of **15a** as yellowish oil. Chromatography in the chloroform-methanol (15:1) system, R_f = 0.42 (S3). IR: (CCl_4) ν_{max} 3447, 3198, 3156, 3059, 3035, 2981, 2935, 2876, 1692, 1611, 1602, 1561, 1531, 1468, 1468, 1452, 1452, 1386, 1376, 1316, 1282, 1250, 1179, 1142, 1105, 1009, 993, 890, 710 cm^{-1}. ESIMS *m/z* 469.3 $(M + 1)^+$ (92). HRMS *m/z* (ESI) calcd. for $C_{21}H_{31}O_5N_4FP$ $(M + 1)^+$ 469.1938, found 469.1943.^{1}H NMR (500.0 MHz, $CDCl_3$): 1.28, 1.30, 1.31 (3 × d, 12H, J_{vic} = 6.2, (C**H$_3$**)$_2$CH); 3.42 (dd, 1H, J_{gem} = 14.7, $J_{1'b,2'}$ = 7.5, H-1′b); 3.78 (dd, 1H, J_{gem} = 13.5, $J_{H,P}$ = 9.7, H-4′b); 3.87 (dd, 1H, J_{gem} = 14.7, $J_{1'a,2'}$ = 3.4, H-1′a); 4.01 (dd, 1H, J_{gem} = 13.5, $J_{H,P}$ = 8.7, H-4′a); 4.05 (m, 1H, H-2′); 4.48 (ddd, 1H, $J_{H,F}$ = 47.4, J_{gem} = 10.6, $J_{3'b,2'}$ = 5.2, H-3′b); 4.66 (ddd, 1H, $J_{H,F}$ = 47.4, J_{gem} = 10.6, $J_{3'a,2'}$ = 2.8, H-3′a); 4.67–4.77 (m, 2H, $(CH_3)_2$C**H**); 5.32, 5.61 (2 × d, 2 × 1H, J_{gem} = 8.9, H-9); 6.94 (s, 1H, H-7); 7.23 (m, 1H, H-*p*-Ph); 7.33–7.37 (m, 2H, H-*m*-Ph); 7.67–7.71 (m, 2H, H-*o*-Ph). ^{13}C NMR (125.7 MHz, $CDCl_3$): 23.9, 24.0 (2 × d, $J_{C,P}$ = 4.6, (**C**H_3)$_2$CH); 24.0, 24.1 (2 × d, $J_{C,P}$ = 3.9, (**C**H_3)$_2$CH); 46.8 (d, $J_{C,F}$ = 9.3, CH_2-1′); 60.4 (CH_2-9); 65.3 (d, $J_{C,P}$ = 169.4, CH_2-4′); 71.2, 71.5 (2 × d, $J_{C,P}$ = 6.7, **C**H$(CH_3)_2$); 80.1 (dd, $J_{C,F}$ = 18.1, $J_{C,P}$ = 11.5, CH-2′); 82.8 (d, $J_{C,F}$ = 172.8, CH_2-3′); 107.6 (CH-7); 124.7 (CH-*o*-Ph); 127.0 (CH-*m*-Ph); 128.6 (CH-*o*-Ph); 133.3 (C-*i*-Ph); 140.1 (C-6); 141.7 (C-4); 151.6 (C-2). $^{31}P\{^1H\}$ NMR (202.3 MHz, $CDCl_3$): 19.19. ^{19}F NMR (470.4 MHz, $CDCl_3$): −227.83 (td, J = 47.4, 21.1).

5.12.2. Diisopropyl (S)-{[(1-fluoro-3-(7-(4-nitrophenyl)-2-oxo-1,2,8,8a-tetrahydroimidazo[1,2-a][1,3,5]triazin-3(4H)-yl)propan-2-yl)oxy]methyl}phosphonate (15b)

Yield: 141 mg (54%) of **15b** as a dark yellow solid. Chromatography in the chloroform-methanol (20:1) system, R_f = 0.32. IR: ($CHCl_3$) ν_{max} 3431, 2984, 2877, 1697, 1608, 1561, 1530, 1485, 1467, 1427, 1388, 1377, 1342, 1283, 1249, 1180, 1142, 1110, 1102, 1015, 998, 950, 861, 854, 646, 509 cm^{-1}. ESIMS *m/z* 514.2 $(M + 1)^+$ (38). HRMS *m/z* (ESI) calcd. for $C_{21}H_{30}O_7N_5FP$ $(M + 1)^+$ 514.1861, found 514.1863.^{1}H NMR (500.0 MHz, $CDCl_3$): 1.28, 1.30, 1.32, 1.33 (4 × d, 4 × 3H, J_{vic} = 6.2, (C**H$_3$**)$_2$CH); 3.42 (dd, 1H, J_{gem} = 14.8, $J_{1'b,2'}$ = 7.6, H-1′b); 3.78 (dd, 1H, J_{gem} = 13.4, $J_{H,P}$ = 9.9, H-4′b); 3.92 (dd, 1H, J_{gem} = 14.8, $J_{1'a,2'}$ = 3.1, H-1′b); 4.02 (dd, 1H, J_{gem} = 13.4, $J_{H,P}$ = 8.9, H-4′a); 4.02 (m, 1H, H-2′); 4.49 (ddd, 1H, $J_{H,F}$ = 47.3, J_{gem} = 10.6, $J_{3'b,2'}$ = 5.1, H-3′b); 4.64 (ddd, 1H, $J_{H,F}$ = 47.3, J_{gem} = 10.6, $J_{3'a,2'}$ = 2.8, H-3′a); 4.66–4.78 (m, 2H, C**H**$(CH_3)_2$); 5.37, 5.71 (2 × d, 2 × 1H, J_{gem} = 9.1, H-9); 7.15 (s, 1H, H-7); 7.84 (m, 2H, H-*o*-$C_6H_4NO_2$); 8.20 (m, 2H, H-*m*-$C_6H_4NO_2$); 9.13 (bs, 1H, NH). ^{13}C NMR (125.7 MHz, $CDCl_3$): 23.9, 24.0 (2 × d, $J_{C,P}$ = 4.5, (**C**H_3)$_2$CH); 24.0, 24.1 (2 × d, $J_{C,P}$ = 3.9, (**C**H_3)$_2$CH); 46.9 (d, $J_{C,F}$ = 9.3, CH_2-1′); 60.3 (CH_2-9); 65.3 (d, $J_{C,P}$ = 169.9, CH_2-4′); 71.2 (d, $J_{C,P}$ = 6.7, **C**H$(CH_3)_2$); 71.6 (d, $J_{C,P}$ = 6.6, **C**H$(CH_3)_2$); 80.2 (dd, $J_{C,F}$ = 18.2, $J_{C,P}$ = 11.9, CH-2′); 82.7 (d, $J_{C,F}$ = 173.2, CH_2-3′); 110.5 (CH-7); 124.2 (CH-*m*-$C_6H_4NO_2$); 125.0 (CH-*o*-$C_6H_4NO_2$); 138.0 (C-6); 139.7 (C-*i*-$C_6H_4NO_2$); 142.7 (C-4); 146.4 (C-*p*-$C_6H_4NO_2$); 151.5 (C-2).$^{31}P\{^1H\}$ NMR (202.3 MHz, $CDCl_3$): 19.07.^{19}F NMR (470.4 MHz, $CDCl_3$): −227.83 (td, J = 47.3, 21.1).

5.12.3. Diisopropyl (S)-{[(1-fluoro-3-(7-(4-nitrophenyl)-8-(2-(4-nitrophenyl)-2-oxoethyl)-2-oxo-1,2,8,8a-tetrahydroimidazo[1,2-a][1,3,5]triazin-3(4H)-yl)propan-2-yl)oxy]methyl}phosphonate (16b)

Yield: 31 mg (9%) of **16b** as a yellow amorphous solid. Chromatography in the chloroform-methanol (20:1) system, R_f = 0.40. ESIMS *m/z* 699.2 $(M + Na)^+$ (100). HRMS *m/z* (ESI) calcd. for $C_{29}H_{34}O_{10}N_6FNaP$ $(M + Na)^+$ 699.1950, found 699.1952.^{1}H NMR (500.0 MHz, $CDCl_3$): 1.27, 1.29, 1.30 (3 × d, 12H, J_{vic} = 6.2, (C**H$_3$**)$_2$CH); 3.51 (dd, 1H, J_{gem} = 14.9, $J_{1'b,2'}$ = 7.3, H-1′b); 3.80 (dd, 1H, J_{gem} = 13.2, $J_{H,P}$ = 9.9, H-4′b); 3.89–4.02 (m, 3H, H-1′a,2′,4′a); 4.51 (ddd, 1H, $J_{H,F}$ = 47.3, J_{gem} = 10.5, $J_{3'b,2'}$ = 5.3, H-3′b); 4.61 (ddd, 1H, $J_{H,F}$ = 47.3, J_{gem} = 10.5, $J_{3'a,2'}$ = 2.9, H-3′a); 4.62–4.72 (m, 2H, C**H**$(CH_3)_2$); 5.01, 5.11 (2 × d, 2 × 1H, J_{gem} = 9.5, H-9); 5.22 (s, 2H, CH_2CO); 7.56 (s, 1H, H-7); 7.78–7.82 (m, 2H, H-*o*-$C_6H_4NO_2$-6); 8.15–8.19 (m, 2H, H-*m*-$C_6H_4NO_2$-6); 8.19–8.22 (m, 2H, H-*o*-$C_6H_4NO_2$); 8.36–8.39 (m, 2H, H-*o*-$C_6H_4NO_2$). ^{13}C NMR (125.7 MHz, $CDCl_3$): 23.9 (d, $J_{C,P}$ = 4.5, (**C**H_3)$_2$CH); 24.0 (d, $J_{C,P}$ = 4.3, (**C**H_3)$_2$CH); 24.0 (d, $J_{C,P}$ = 4.0, (**C**H_3)$_2$CH); 46.1 (d, $J_{C,F}$ = 9.0, CH_2-1′); 53.8 (**C**H_2CO); 63.8 (CH_2-9); 65.4 (d, $J_{C,P}$ = 170.3, CH_2-4′); 71.2 (d, $J_{C,P}$ = 6.9, **C**H$(CH_3)_2$); 71.7 (d, $J_{C,P}$ = 6.7, **C**H$(CH_3)_2$); 80.2 (dd, $J_{C,F}$ = 18.5, $J_{C,P}$ = 12.6, CH-2′); 82.6 (d, $J_{C,F}$ = 173.5, CH_2-3′); 109.3 (CH-7); 124.0 (CH-*m*-$C_6H_4NO_2$-6); 124.1 (CH-*m*-$C_6H_4NO_2$); 125.1 (CH-*o*-$C_6H_4NO_2$-6); 129.3 (CH-*o*-$C_6H_4NO_2$); 138.4 (C-6); 139.3 (C-*i*-$C_6H_4NO_2$-6); 139.5 (C-*i*-$C_6H_4NO_2$); 146.6 (C-*p*-$C_6H_4NO_2$-6); 147.9 (C-2); 149.7 (C-4); 150.7 (C-*p*-$C_6H_4NO_2$); 193.5 (CO). $^{31}P\{^1H\}$ NMR (202.3 MHz, $CDCl_3$): 18.04.

5.12.4. Diisopropyl (S)-{[(1-fluoro-3-(7-(4-methoxyphenyl)-2-oxo-1,2,8,8a-tetrahydroimidazo[1,2-a][1,3,5]triazin-3(4H)-yl)propan-2-yl)oxy]methyl}phosphonate (15c)

Yield: 102 mg (40%) of **15c** as a yellow amorphous solid. Chromatography in the chloroform-methanol (20:1) system, R_f = 0.40. IR: ($CHCl_3$) ν_{max} 3432, 3147, 2984, 2937, 2911, 2839, 1693, 1632, 1600, 1512, 1500, 1564, 1466, 1420, 1387, 1377, 1248, 1174, 1143, 1104, 1033, 1020, 1011, 997, 891, 835, 508 cm^{-1}. ESIMS *m/z* 499.2 $(M + 1)^+$ (32). HRMS *m/z* (ESI) calcd. for $C_{22}H_{33}O_6N_4FP$ $(M + 1)^+$ 499.2116, found 499.2117.^{1}H NMR (500.1 MHz, $CDCl_3$): 1.28, 1.298, 1.302, 1.31 (4 × d, 4 × 3H, J_{vic} = 6.2, (C**H$_3$**)$_2$CH); 3.42 (dd, 1H, J_{gem} = 14.8, $J_{1'b,2'}$ = 7.4, H-1′b); 3.78 (dd, 1H, J_{gem} = 13.4, $J_{H,P}$ = 9.7, H-4′b); 3.81 (s, 3H, CH_3O); 3.85 (dd, 1H, J_{gem} = 14.8, $J_{1'a,2'}$ = 2.3, H-1′a); 4.00 (dd, 1H, J_{gem} = 13.4, $J_{H,P}$ = 8.7, H-4′a); 4.03 (m, 1H, H-2′); 4.48 (ddd, 1H, $J_{H,F}$ = 47.3, J_{gem} = 10.6, $J_{3'b,2'}$ = 5.2, H-3′b); 4.65 (ddd, 1H, $J_{H,F}$ = 47.3, J_{gem} = 10.6, $J_{3'a,2'}$ = 2.7, H-3′a); 4.67–4.76 (m, 2H, C**H**$(CH_3)_2$); 5.30, 5.57 (2 × d, 2 × 1H, J_{gem} = 8.8, H-9); 6.81 (s, 1H, H-7); 6.86–6.90 (m, 2H, H-*m*-C_6H_4OMe); 7.59–7.63 (m, 2H, H-*o*-C_6H_4OMe). ^{13}C NMR (125.7 MHz, $CDCl_3$): 23.9, 24.0 (2 × d, $J_{C,P}$ = 4.5, (**C**H_3)$_2$CH); 24.0, 24.1 (2 × d, $J_{C,P}$ = 3.9, (**C**H_3)$_2$CH); 46.8 (d, $J_{C,F}$ = 9.3, CH_2-1′); 55.3

(CH_3O); 60.4 (CH_2-9); 65.3 (d, $J_{C,P} = 169.4$, CH_2-4′); 71.2, 71.5 (2 × d, $J_{C,P} = 6.6$, **C**H(CH_3)$_2$); 80.1 (dd, $J_{C,F} = 18.1$, $J_{C,P} = 11.6$, CH-2′); 82.9 (d, $J_{C,F} = 172.8$, CH_2-3′); 106.4 (CH-7); 114.0 (CH-*m*-C_6H_4OMe); 126.0 (C-*i*-C_6H_4OMe); 126.1 (CH-*o*-C_6H_4OMe); 139.7 (C-6); 141.9 (C-4); 151.6 (C-2); 158.8 (C-*p*-C_6H_4OMe). $^{31}P\{^1H\}$ NMR (202.3 MHz, $CDCl_3$): 18.58.^{19}F NMR (470.4 MHz, $CDCl_3$): −227.58 (td, $J = 47.3$, 21.2).

5.12.5. Diisopropyl (S)-{[(1-fluoro-3-(7-(4-methoxyphenyl)-8-(2-(4-methoxyphenyl)-2-oxoethyl)-2-oxo-1,2,8,8a-tetrahydroimidazo[1,2-a][1,3,5]triazin-3(4H)-yl)propan-2-yl)oxy]methyl}phosphonate (16c)

Yield: 39 mg (10%) of **16c** as a yellow amorphous solid. Chromatography in the system chloroform-methanol (20:1) system, $R_f = 0.46$. IR: ($CHCl_3$) ν_{max} 2984, 2938, 2903, 2841, 1711, 1639, 1623, 1603, 1576, 1512, 1505, 1465, 1443, 1423, 1387, 1376, 1247, 1239, 1172, 1143, 1115, 1104, 1032, 1020, 1011, 997, 890, 836, 632, 508 cm^{-1}. ESIMS *m/z* 647.3 $(M + 1)^+$ (100). HRMS *m/z* (ESI) calcd. for $C_{31}H_{41}O_8N_4FP$ $(M + 1)^+$ 647.2641, found 647.2643.^{1}H NMR (500.0 MHz, $CDCl_3$): 1.27, 1.28, 1.29 (3 × d, 12H, $J_{vic} = 6.2$, (C**H$_3$**)$_2$CH); 3.55 (dd, 1H, $J_{gem} = 14.7$, $J_{1'b,2'} = 6,9$, H-1′b); 3.78−3.83 (m, 4H, H-1′a, CH_3O−C_6H_4OMe-6); 3.83 (dd, 1H, $J_{gem} = 13.5$, $J_{H,P} = 9.3$, H-4′b); 3.88 (s, 3H, CH_3O−C_6H_4OMe); 3.96 (dd, 1H, $J_{gem} = 13.5$, $J_{H,P} = 8.9$, H-4′a); 3.99 (m, 1H, H-2′); 4.51 (ddd, 1H, $J_{H,F} = 47.3$, $J_{gem} = 10.5$, $J_{3'b,2'} = 5.2$, H-3′b); 4.64 (ddd, 1H, $J_{H,F} = 47.3$, $J_{gem} = 10.5$, $J_{3'a,2'} = 3.0$, H-3′a); 4.64−4.73 (m, 2H, C**H**(CH_3)$_2$); 4.85, 4.97 (2 × d, 2 × 1H, $J_{gem} = 9.5$, H-6); 4.99, 5.09 (2 × bd, 2 × 1H, $J_{gem} = 17.8$, CH_2CO); 6.84−6.88 (m, 2H, H-*m*-C_6H_4OMe-6); 6.94−6.98 (m, 2H, H-*m*-C_6H_4OMe); 7.26 (s, 1H, H-7); 7.60−7.63 (m, 2H, H-*o*-C_6H_4OMe-6); 7.97−8.00 (m, 2H, H-*o*-C_6H_4OMe-6). ^{13}C NMR (125.7 MHz, $CDCl_3$): 23.9, 23.9 (2 × d, $J_{C,P} = 4.6$, (**C**H_3)$_2$CH); 24.0, 24.0 (2 × d, $J_{C,P} = 3.8$, (**C**H_3)$_2$CH); 46.4 (d, $J_{C,F} = 8.8$, CH_2-1′); 52.8 (**C**H_2CO); 55.2 (CH_3O−-C_6H_4OMe-6); 55.5 (CH_3O−C_6H_4OMe); 64.0 (CH_2-9); 65.3 (d, $J_{C,P} = 169.0$, CH_2-4′); 71.1, 71.4 (2 × d, $J_{C,P} = 6.7$, **C**H(CH_3)$_2$); 79.7 (dd, $J_{C,F} = 18.4$, $J_{C,P} = 11.1$, CH-2′); 82.8 (d, $J_{C,F} = 172.8$, CH_2-3′); 105.1 (CH-7); 113.8 (CH-*m*-C_6H_4OMe-6); 114.0 (CH-*m*-C_6H_4OMe); 126.0 (C-*i*-C_6H_4OMe-6); 126.4 (CH-*o*-C_6H_4OMe-6); 127.9 (C-*i*-C_6H_4OMe); 130.4 (CH-*o*-C_6H_4OMe); 140.1 (C-6); 148.4 (C-2); 149.5 (C-4); 159.0 (C-*p*-C_6H_4OMe-6); 164.0 (C-*p*-C_6H_4OMe); 192.7 (CO). $^{31}P\{^1H\}$ NMR (202.3 MHz, $CDCl_3$): 18.44.

5.13. Bis(isopropyl L-phenylalanine) amidate prodrug of (S)-1-[3-fluoro-2-(phosphonomethoxy)propyl]-5,6-dihydro-5-azacytosine (18)

A solution of **11** (154 mg, 0.3 mmol) in acetic acid (4 mL) was hydrogenated at elevated pressure (10 bar) on 10% palladium on charcoal (120 mg) for 24 h. The suspension was filtered through a Celite pad and the filtrate evaporated. The residue was chromatographed on preparative TLC plate (S7) in chloroform-methanol (6:1). Yield: 66 mg (39%) of a white amorphous solid. $[\alpha]_D$ −39.7 (c 0.297, $CHCl_3$). IR: (KBr) ν_{max} 3332, 3196, 2886−2563, 1736, 1700, 1613, 1590, 1559, 1429, 1408, 1377, 1152, 1099, 1062, 663, 602 cm^{-1}. ESIMS *m/z* 483.1 $(M + 1)^+$ (100). HRMS *m/z* (ESI) calcd. for $C_{17}H_{33}O_7N_6FP$ $(M + 1)^+$ 483.2127, found 483.2127.^{1}H NMR (500.0 MHz, CD_3OD): 1.27, 1.28 (2 × t, 2 × 3H, $J_{vic} = 7.1$, C**H$_3$**CH_2O); 1.397, 1.399 (2 × d, 2 × 3H, $J_{vic} = 7.2$, H-3-Ala); 1.93 (bs, 3H, CH_3COO); 3.46 (dd, 1H, $J_{gem} = 15.0$, $J_{1'b,2'} = 6.7$, H-1′b); 3.61 (dd, 1H, $J_{gem} = 15.0$, $J_{1'a,2'} = 4.3$, H-1′a); 3.81 (dd, 1H, $J_{gem} = 13.2$, $J_{H,P} = 10.0$, H-4′b); 3.87−4.04 (m, 4H, H-2′,4′a, H-2-Ala); 4.11−4.25 (m, 4H, CH_3C**H$_2$**O); 4.46 (ddd, 1H, $J_{H,F} = 47.5$, $J_{gem} = 10.6$, $J_{3'b,2'} = 5.0$, H-3′b); 4.61 (ddd, 1H, $J_{H,F} = 47.5$, $J_{gem} = 10.6$, $J_{3'a,2'} = 3.0$, H-3′a); 4.68, 4.79 (2 × d, 2 × 1H, $J_{gem} = 9.8$, H-6). ^{13}C NMR (125.7 MHz, CD_3OD): 14.5 (**C**H_3CH_2O); 21.1 (d, $J_{C,P} = 5.9$, CH_3-3-Ala); 21.2 (d, $J_{C,P} = 4.9$, CH_3-3-Ala); 23.6 (**C**H_3COO); 46.9 (d, $J_{C,F} = 8.5$, CH_2-1′); 49.9, 50.1 (CH-2-Ala); 59.7 (CH_2-6); 62.4, 62.4 (CH_3**C**H_2O); 67.8 (d, $J_{C,P} = 136.8$, CH_2-4′); 80.9 (dd, $J_{C,F} = 18.3$, $J_{C,P} = 12.1$, CH-2′); 83.9 (d, $J_{C,F} = 170.5$, CH_2-3′); 156.9 (C-2); 157.7 (C-4); 175.8 (d, $J_{C,P} = 4.8$, C-1-Ala); 175.8 (d, $J_{C,P} = 3.4$, C-1-Ala); 180.4 (CH_3**C**OO). $^{31}P\{^1H\}$ NMR (202.3 MHz, CD_3OD): 24.58.^{19}F NMR (470.4 MHz, CD_3OD): −228.48 (td, $J = 47.5$, 21.2).

5.14. The condensation of 1-aryl-2-bromoacetophenones (14a-c) with phosphoramidate prodrug 18

A mixture of **18** (0.5 mmol), bromoacetophenone **14a-c** (0.5 mmol) and sodium bicarbonate (39 mg, 0.5 mmol) in DMF (13 mL) was stirred at room temperature for 6 h. The mixture was evaporated and the residue was chromatographed on a preparative TLC plate (S7) in the system described below.

5.14.1. Diethyl 2,2'-(((((1-fluoro-3-(2-oxo-7-phenyl-2,8-dihydroimidazo[1,2-a][1,3,5]triazin-3(4H)-yl)propan-2-yl)oxy)methyl)phosphoryl)bis(azanediyl))-dipropionate (19a)

Yield: 43 mg (16%) of a yellow amorphous solid. Chromatography in system S3, $R_f = 0.50$. IR: (KBr) ν_{max} 3434, 1736, 1686, 1609, 1392, 1376, 1151, 1098, 1063, 1022, 993, 760, 695 cm^{-1}. ESIMS *m/z* 605.2 $(M + Na)^+$ (100). HRMS *m/z* (ESI) calcd. for $C_{25}H_{36}O_7N_6FPNa$ $(M + Na)^+$ 605.2259, found 605.2263.^{1}H NMR (500.0 MHz, CD_3OD): 1.21, 1.26 (2 × t, 2 × 3H, $J_{vic} = 7.1$, C**H$_3$**CH_2O); 1.33 (dd, 3H, $J_{vic} = 7.2$, $J_{H,P} = 0.2$, H-3-Ala); 1.39 (d, 3H, $J_{vic} = 7.2$, H-3-Ala); 3.55 (dd, 1H, $J_{gem} = 14.9$, $J_{1'b,2'} = 7.5$, H-1′b); 3.788 (dd, 1H, $J_{gem} = 13.1$, $J_{H,P} = 11.1$, H-4′b); 3.790 (ddd, 1H, $J_{gem} = 14.9$, $J_{vic} = 3.4$, $J_{H,F} = 0.7$, H-1′a); 3.92−4.00 (m, 2H, H-2-Ala); 4.00−4.07 (m, 2H, H-2′,4′a); 4.07−4.22 (m, 4H, CH_3C**H$_2$**O); 4.52 (ddd, 1H, $J_{H,F} = 47.5$, $J_{gem} = 10.6$, $J_{3'b,2'} = 4.8$, H-3′b); 4.69 (ddd, 1H, $J_{H,F} = 47.5$, $J_{gem} = 10.6$, $J_{3'a,2'} = 3.0$, H-3′a); 5.45, 5.61 (2 × d, 2 × 1H, $J_{gem} = 8.9$, H-9); 7.212 (s, 1H, H-7); 7.214 (m, 1H, H-*p*-Ph); 7.31−7.35 (m, 2H, H-*m*-Ph); 7.63−7.66 (m, 2H, H-*o*-Ph). ^{13}C NMR (125.7 MHz, CD_3OD): 14.5, 14.5 (**C**H_3CH_2O); 21.0 (d, $J_{C,P} = 6.2$, CH_3-3-Ala); 21.1 (d, $J_{C,P} = 5.1$, CH_3-3-Ala); 47.8 (d, $J_{C,F} = 8.8$, CH_2-1′); 49.7, 50.2 (CH-2-Ala); 61.6 (CH_2-9); 62.4, 62.4 (CH_3**C**H_2O); 67.6 (d, $J_{C,P} = 136.9$, CH_2-4′); 81.3 (dd, $J_{C,F} = 18.2$, $J_{C,P} = 12.5$, CH-2′); 83.9 (d, $J_{C,F} = 170.6$, CH_2-3′); 109.8 (CH-7); 125.7 (CH-*o*-Ph); 127.9 (CH-*p*-Ph); 129.6 (CH-*m*-Ph); 135.0 (C-*i*-Ph); 140.5 (C-6); 143.8 (C-4); 153.9 (C-2); 175.8 (d, $J_{C,P} = 3.1$, C-1-Ala); 175.9 (d, $J_{C,P} = 4.8$, C-1-Ala). $^{31}P\{^1H\}$ NMR (162.0 MHz, CD_3OD): 25.81.

5.14.2. Diethyl 2,2'-(((((1-fluoro-3-(7-(4-nitrophenyl)-2-oxo-2,8-dihydroimidazo[1,2-a][1,3,5]triazin-3(4H)-yl)propan-2-yl)oxy)methyl)phosphoryl)bis(azanediyl))-dipropionate (19b)

Yield: 80 mg (28%) of a brown amorphous solid. Chromatography in system S3, $R_f = 0.44$. IR: (KBr) ν_{max} 3428, 3395, 1732, 1696, 1530, 1392, 1378, 1342, 1146, 1110, 1097, 1608, 1562, 1516, 1422, 1312, 1289, 1063, 1020, 992, 861, 854 cm^{-1}. ESIMS *m/z* 650.1 $(M + Na)^+$ (100). HRMS *m/z* (ESI) calcd. for $C_{25}H_{35}O_9N_7FPNa$ $(M + Na)^+$ 650.2110, found 650.2111.^{1}H NMR (500.0 MHz, CD_3OD): 1.21, 1.26 (2 × t, 2 × 3H, $J_{vic} = 7.1$, C**H$_3$**CH_2O); 1.34, 1.38 (2 × d, 2 × 3H, $J_{vic} = 7.2$, H-3-Ala); 3.55 (dd, 1H, $J_{gem} = 14.9$, $J_{1'b,2'} = 7.5$, H-1′b); 3.79 (dd, 1H, $J_{gem} = 13.2$, $J_{H,P} = 11.0$, H-4′b); 3.80 (dd, 1H, $J_{gem} = 14.9$, $J_{1'a,2'} = 3.5$, H-1′a); 3.92−4.07 (m, 4H, H-2′,4′a, H-2-Ala); 4.07−4.23 (m, 4H, CH_3C**H$_2$**O); 4.52 (ddd, 1H, $J_{H,F} = 47.5$, $J_{gem} = 10.6$, $J_{3'b,2'} = 4.8$, H-3′b); 4.69 (ddd, 1H, $J_{H,F} = 47.5$, $J_{gem} = 10.6$, $J_{3'a,2'} = 3.0$, H-3′a); 5.48, 5.65 (2 × d, 2 × 1H, $J_{gem} = 9.1$, H-9); 7.50 (s, 1H, H-7); 7.86−7.90 (m, 2H, H-*o*-$C_6H_4NO_2$); 8.20−8.23 (m, 2H, H-*m*-$C_6H_4NO_2$). ^{13}C NMR (125.7 MHz, CD_3OD): 14.5, 14.5 (**C**H_3CH_2O); 21.0 (d, $J_{C,P} = 6.1$, CH_3-3-Ala); 21.1 (d, $J_{C,P} = 5.1$, CH_3-3-Ala); 47.8 (d,

$J_{C,F}$ = 8.8, CH_2-1′); 49.7, 50.1 (CH-2-Ala); 61.6 (CH_2-9); 62.4, 62.4 (CH_3**C**H_2O); 67.6 (d, $J_{C,P}$ = 136.9, CH_2-4′); 81.3 (dd, $J_{C,F}$ = 18.1, $J_{C,P}$ = 12.4, CH-2′); 83.8 (d, $J_{C,F}$ = 170.6, CH_2-3′); 113.0 (CH-7); 125.0 (CH-*m*-$C_6H_4NO_2$); 125.8 (CH-*o*-$C_6H_4NO_2$); 138.4 (C-6); 141.8 (C-*i*-$C_6H_4NO_2$); 144.7 (C-4); 147.5 (C-*p*-$C_6H_4NO_2$); 153.9 (C-2); 175.8 (d, $J_{C,P}$ = 3.3, C-1-Ala); 175.9 (d, $J_{C,P}$ = 4.9, C-1-Ala). $^{31}P\{^1H\}$ NMR (202.3 MHz, CD_3OD): 24.68.^{19}F NMR (470.4 MHz, CD_3OD): −228.91 (td, J = 47.5, 21.7).

5.14.3. Diethyl 2,2'-(((((1-fluoro-3-(7-(4-methoxyphenyl)-2-oxo-2,8-dihydroimidazo[1,2-a][1,3,5]triazin-3(4H)-yl)propan-2-yl)oxy)methyl)phosphoryl)bis(azanediyl))-dipropionate (19c)

Yield: 48 mg (17%) of a brown amorphous solid. Chromatography in the chloroform-methanol (15:1) system, R_f = 0.33. IR: (KBr) ν_{max} 3431, 3391, 2839, 1733, 1693, 1599, 1585, 1500, 1440, 1420, 1392, 1377, 1301, 1247, 1173, 1147, 1097, 1063, 1031, 1022, 993, 835 cm^{-1}. ESIMS *m/z* 635.1 $(M + Na)^+$ (100). HRMS *m/z* (ESI) calcd. for $C_{26}H_{38}O_8N_6FPNa$ $(M + Na)^+$ 635.2365, found 635.2366.^{1}H NMR (500.0 MHz, CD_3OD): 1.21, 1.26 (2 × t, 2 × 3H, J_{vic} = 7.1, C**H**$_3$$CH_2O$); 1.33 (dd, 3H, J_{vic} = 7.2, $J_{H,P}$ = 0.2, H-3-Ala); 1.38 (d, 3H, J_{vic} = 7.2, H-3-Ala); 3.54 (dd, 1H, J_{gem} = 14.9, $J_{1'b,2'}$ = 7.5, H-1′b); 3.76−3.82 (m, 2H, H-1′a,4′b); 3.80 (s, 3H, CH_3O); 3.92−4.07 (m, 4H, H-2′,4′a, H-2-Ala); 4.07−4.23 (m, 4H, CH_3C**H**$_2$O); 4.52 (ddd, 1H, $J_{H,F}$ = 47.5, J_{gem} = 10.6, $J_{3'b,2'}$ = 4.8, H-3′b); 4.68 (ddd, 1H, $J_{H,F}$ = 47.5, J_{gem} = 10.6, $J_{3'a,2'}$ = 3.0, H-3′a); 5.42, 5.59 (2 × d, 2 × 1H, J_{gem} = 8.9, H-9); 6.88−6.93 (m, 2H, H-*m*-C_6H_4OMe); 7.08 (s, 1H, H-7); 7.54−7.59 (m, 2H, H-*o*-C_6H_4OMe). ^{13}C NMR (125.7 MHz, CD_3OD): 14.5, 14.5 (**C**H_3CH_2O); 21.0 (d, $J_{C,P}$ = 6.2, CH_3-3-Ala); 21.1 (d, $J_{C,P}$ = 5.0, CH_3-3-Ala); 47.7 (d, $J_{C,F}$ = 8.8, CH_2-1′); 49.7, 50.2 (CH-2-Ala); 61.5 (CH_2-9); 62.4, 62.4 (CH_3**C**H_2O); 67.6 (d, $J_{C,P}$ = 136.9, CH_2-4′); 81.3 (dd, $J_{C,F}$ = 18.2, $J_{C,P}$ = 12.4, CH-2′); 83.9 (d, $J_{C,F}$ = 170.6, CH_2-3′); 108.6 (CH-7); 115.0 (CH-*m*-C_6H_4OMe); 127.0 (CH-*o*-C_6H_4OMe); 127.7 (C-*i*-C_6H_4OMe); 140.5 (C-6); 143.6 (C-4); 154.0 (C-2); 160.3 (C-*p*-C_6H_4OMe); 175.8 (d, $J_{C,P}$ = 3.2, C-1-Ala); 175.89 (d, $J_{C,P}$ = 4.9, C-1-Ala). $^{31}P\{^1H\}$ NMR (202.3 MHz, CD_3OD): 24.69. ^{19}F NMR (470.4 MHz, CD_3OD): −228.88 (td, J = 47.5, 21.6).

5.15. 4-Amino-1-(3,3,3-trifluoro-2-hydroxypropyl)-1,3,5-triazin-2(1H)-one (22)

Sodium hydroxide (0.65 g, 16.3 mmol) was added to a 105 °C hot mixture of 5-azacytosine (1.7 g, 13.4 mmol) and trifluoromethyloxirane (**21**) (1.5 g, 13.4 mmol) in DMF (80 mL). The mixture was heated to 110−120 °C for 18 h, then evaporated, and the residue was chromatographed on silica gel in S4. Yield: 2.2 g (73%) of a white amorphous solid. IR: (KBr) ν_{max} 6422, 3210, 1694, 1640, 1512, 1481, 1049, 1132 cm^{-1}. ESIMS *m/z* 225.1 $(M + 1)^+$ (100). HRMS *m/z* (ESI) calcd. for $C_6H_8O_2N_4F_3$ $(M + 1)^+$ 225.0599, found 225.0594.^{1}H NMR (500.0 MHz, DMSO-d_6): 3.61 (dd, 1H, J_{gem} = 13.6, $J_{1'b,2'}$ = 9.5, H-1′b); 4.08 (dd, 1H, J_{gem} = 13.6, $J_{1'a,2'}$ = 2.9, H-1′a); 4.24 (m, 1H, H-2′); 6.74 (d, 1H, $J_{OH,2'}$ = 6.6, OH); 7.50, 7.51 (2 × bd, 2 × 1H, J_{gem} = 2.3, NH_2); 8.19 (s, 1H, H-6). ^{13}C NMR (125.7 MHz, DMSO-d_6): 46.7 (CH_2-1′; 65.98 (q, $J_{C,F}$ = 29.7, CH-2′); 125.0 (q, $J_{C,F}$ = 283.1, C-3′); 154.0 (C-2); 160.0 (CH-6); 166.7 (C-4).

5.16. 6-Amino-4-oxo-3-(3,3,3-trifluoro-2-hydroxypropyl)-1,2,3,4-tetrahydro-1,3,5-triazin-1-ium acetate (23)

A solution of **22** (200 mg, 0.9 mmol) in acetic acid (10 mL) was hydrogenated on 10% palladium on charcoal (66 mg) for 48 h. The suspension was filtered through a Celite pad and the filtrate was concentrated *in vacuo*. The residue was crystallised from methanol. Yield:118 mg (46%) of white solid; mp 185−186 °C. IR: (KBr) ν_{max} 3402, 3225, 3127, 3015, 1738, 1699, 1662, 1512, 1407, 1267, 1125, 1036, 696, 569, 545 cm^{-1}. ESIMS *m/z* 227.1 $(M + 1)^+$ (100). HRMS *m/z* (ESI) calcd. for $C_6H_{10}O_2N_4F_3$ $(M + 1)^+$ 227.0750, found 227.0751. Anal. Calcd for $C_8H_{13}F_3O_4N_4$: C, 33.6; H, 4.6; N, 19.6; F, 19.9. Found: C, 33.3; H, 4.4; N, 19.4; F, 19.4.^{1}H NMR (600.1 MHz, DMSO-d_6): 1.88 (s, 3H, CH_3CO); 3.11 (dd, 1H, J_{gem} = 14.2, $J_{1'b,2'}$ = 8.8, H-1′b); 3.62 (dd, 1H, J_{gem} = 14.2, $J_{1'a,2'}$ = 3.5, H-1′a); 4.18 (dqd, 1H, $J_{2',1'}$ = 8.8, 3.5, $J_{H,F}$ = 7.4, H-2′); 4.49, 4.59 (2 × d, 2 × 1H, J_{gem} = 10.1, H-6). ^{13}C NMR (150.9 MHz, DMSO-d_6): 21.7 (**C**H_3CO); 45.1 (CH_2-1′); 61.2 (CH_2-6); 67.5 (q, $J_{C,F}$ = 28.5, CH-2′); 125.3 (q, $J_{C,F}$ = 283.7, C-3′); 153.3 (C-4); 155.0 (C-2); 172.8 (CH_3**C**O). ^{19}F NMR (470.4 MHZ, DMSO-d_6): −73.77 (d, $J_{F,H}$ = 7.4).

5.17. Diisopropyl {[(3-(4-amino-2-oxo-1,3,5-triazin-1(2H)-yl)-1,1,1-trifluoropropan-2-yloxy]methyl}phosphonate (24)

Sodium *tert*-butoxide (97%, 199 mg, 2.0 mmol) was added to a solution of **22** (300 mg, 1.3 mmol) in DMF (10 mL) at −20 °C and the mixture was stirred for 15 min. Diisopropyl bromomethanephosphonate (0.7 mL, 2.7 mmol) was added, the mixture was warmed up to room temperature and stirred for 4 days. Additional portions of sodium *tert*-butoxide and diisopropyl bromomethanephosphonate (1 eq. each) were added and the stirring continued for 24 h (TLC control in S2). The mixture was evaporated and the residue was chromatographed on a preparative TLC plate (S7) in the dichloromethane-methanol (7:2) system. Yield: 304 mg (57%) of white amorphous solid. ESIMS *m/z* 425.1 $(M + 1)^+$ (100). HRMS *m/z* (ESI) calcd. for $C_{13}H_{22}O_5N_4F_3NaP$ $(M + 1)^+$ 425.1172, found 425.1173.^{1}H NMR (500.0 MHz, CD_3OD): 1.31, 1.330, 1.334, 1.34 (4 × d, 4 × 3H, J_{vic} = 6.2, (C**H**$_3$)$_2$CH); 3.86 (dd, 1H, J_{gem} = 14.3, $J_{1'b,2'}$ = 8.9, H-1′b); 3.95 (dd, 1H, J_{gem} = 13.9, $J_{H,P}$ = 10.0, H-4′b); 4.13 (dd, 1H, J_{gem} = 13.9, $J_{H,P}$ = 8.5, H-4′a); 4.30 (dd, 1H, J_{gem} = 14.3, $J_{1'a,2'}$ = 3.0, H-1′a); 4.41 (dqd, 1H, $J_{2',1'}$ = 8.9, 3.0, $J_{H,F}$ = 6.3, H-2′); 4.63−4.75 (m, 2H, $(CH_3)_2$C**H**); 8.18 (s, 1H, H-6). ^{13}C NMR (125.7 MHz, CD_3OD): 24.1−24.3 ((**C**H_3)$_2$CH); 47.0 (CH_2-1′); 67.7 (d, $J_{C,P}$ = 168.6, CH_2-4′); 73.4 (d, $J_{C,P}$ = 6.5, $(CH_3)_2$**C**H); 73.7 (d, $J_{C,P}$ = 6.7, $(CH_3)_2$**C**H); 77.5 (qd, $J_{C,F}$ = 29.8, $J_{C,P}$ = 12.3, CH-2′); 125.6 (q, $J_{C,F}$ = 284.1, C-3′); 156.8 (C-2); 161.1 (CH-6); 168.3 (C-4). $^{31}P\{^1H\}$ NMR (202.3 MHz, CD_3OD): 18.04.^{19}F NMR (470.4 MHZ, D_2O): −73.26 (d, $J_{F,H}$ = 6.3).

5.18. {[(3-(4-Amino-2-oxo-1,3,5-triazin-1(2H)-yl)-1,1,1-trifluoropropan-2-yl)oxy]methyl}phosphonic acid (25)

Bromotrimethylsilane (0.9 ml, 6.8 mmol) was added to a solution of **24** (272 mg, 0.7 mmol) in acetonitrile (15 ml). The mixture was stirred at room temperature overnight, evaporated and the residue was co-distilled with water (2 × 2 ml) and toluene (2 × 5 mL). The crude product was applied onto a Dowex 1 (AcO^- form) column. An anion exchanger was washed with water, followed by a gradient of 0−1 M acetic acid. Product containing fractions were evaporated, and the residue was co-evaporated with methanol (2 × 5 mL) and toluene (2 × 5 mL). The product was lyophilised from water. Yield: 45 mg (19%) of white amorphous solid. IR: (KBr) ν_{max} 3435, 3060, 2680, 2365, 1772, 1666, 1621, 1561, 1527, 1371, 1273, 1187, 1148, 1130, 1187, 1111, 988, 778 cm^{-1}. ESIMS *m/z* 317.0 $(M + 1)^+$ (100). HRMS *m/z* (ESI) calcd. for $C_7H_9O_5N_4F_3P$ $(M + 1)^+$ 317.0268, found 317.0266. Anal. Calcd for $C_7H_{10}F_3O_5N_4P$ .2H_2O: C, 23.6; H, 3.4; N, 15.7; P, 8.7; F, 16.00. Found: C, 23.4; H, 3.7; N, 15.4; P, 8.04, F, 15.8.^{1}H NMR (500.0 MHz, D_2O): 3.83 (dd, 1H, J_{gem} = 14.0, $J_{H,P}$ = 7.6, H-4′b); 4.06 (dd, 1H, J_{gem} = 14.0, $J_{H,P}$ = 8.5, H-4′a); 4.22 (dd, 1H, J_{gem} = 15.5, $J_{1'b,2'}$ = 9.7, H-1′b); 4.47 (dd, 1H, J_{gem} = 15.5, $J_{1'a,2'}$ = 3.0, H-1′a); 4.58 (dqd, 1H, $J_{2',1'}$ = 9.7, 3.0, $J_{H,F}$ = 6.1,

H-2′); 8.36 (s, 1H, H-6). ^{13}C NMR (125.7 MHz, D_2O): 45.7 (CH_2-1′); 71.0 (d, $J_{C,P} = 156.1$, CH_2-4′); 78.6 (qd, $J_{C,F} = 30.3$, $J_{C,P} = 7.8$, CH-2′); 126.5 (q, $J_{C,F} = 285.2$, C-3′); 150.1 (C-2); 160.8 (CH-6); 163.4 (C-4). $^{31}P\{^1H\}$ NMR (202.3 MHz, D_2O): 15.45. ^{19}F NMR (470.4 MHZ, D_2O): −76.79 (bs).

Biological assays

The compounds were evaluated against different herpesviruses, including herpes simplex virus type 1 (HSV-1) strain Kos, thymidine kinase-deficient (TK^-) HSV-1 Kos strain resistant to ACV (ACV^r), herpes simplex virus type 2 (HSV-2) strain G, varicella-zoster virus (VZV) strain Oka (TK^+), TK^- VZV strain 07-1, human cytomegalovirus (HCMV) strains AD-169 and Davis as well as vaccinia virus, adenovirus-2 adeno-2), vesicular stomatitis virus, parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4, Punta Toro virus, respiratory syncytial virus (RSV), feline coronovirus (FIPV) and influenza A virus subtypes H1N1 (A/PR/8), H3N2 (A/HK/7/87) and influenza B virus (B/HK/5/72). The antiviral assays were based on the inhibition of virus-induced cytopathicity or plaque formation in human embryonic lung (HEL) fibroblasts, African green monkey kidney cells (Vero), human epithelial cervix carcinoma cells (HeLa), Crandell-Rees feline kidney cells (CRFK), and Madin Darby canine kidney cells (MDCK). Confluent cell cultures 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) and the cell cultures were incubated in the presence of varying concentrations of the test compounds. Viral cytopathicity or plaque formation (VZV) was recorded as soon as it reached completion in the control virus-infected cell cultures that had not been treated with the test compounds. Antiviral activity was expressed as the EC_{50} or compound concentration required to reduce virus-induced cytopathicity or viral plaque formation by 50%.

The cytotoxicity of the tested compounds was expressed as the minimum cytotoxic concentration (MCC) or the compound concentration that caused a microscopically detectable alteration of cell morphology. Alternatively, the cytostatic activity of the test compounds was measured based on inhibition of cell growth. HEL cells were seeded at a rate of 5×10^3 cells/well into 96-well microtiter plates and allowed to proliferate for 24 h. Subsequently, a medium containing different concentrations of the test compounds was added. After three 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 to reduce cell proliferation by 50% relative to the number of cells in the untreated controls.

Conflicts of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

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