Synthesis and antiviral activities of fluorinated acyclic nucleoside phosphonates

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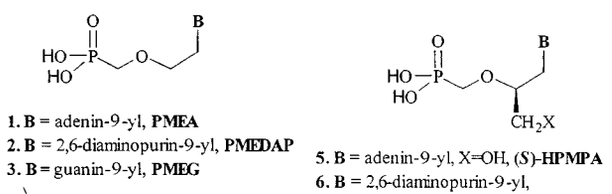
Novel α-fluoro derivatives of PME and HPMP were synthesized by electrophilic fluorination of 1-*tert*-butyldimethylsiloxy-2-[(diethoxyphosphoryl)methoxy]ethane **15** and 3-*O*-benzyl-2-*O*-[(diethoxyphosphoryl)methyl]-1-*O*-(*tert*-butyldimethylsiloxy)glycerol **22**, respectively. The first series of acyclic nucleoside phosphonates possessing the α-fluoro(phosphoryl)methoxy group were prepared by coupling of F-PME or F-HPMP derivatives **18**, **26**, or **27** with the corresponding purine or pyrimidine nucleic bases under either modified Mitsunobu conditions or base-catalyzed alkylation conditions. Treatment of the diesters of F-PMEA **25a**–**c**, F-PMEG **25f** and F-PMEC **25g** with concentrated aqueous ammonia led to the formation of the corresponding monoammonium salts of monoethyl phosphonate **30a**, **30d**, **30f** and **30g**. The synthesized fluorinated acyclic nucleoside phosphonates were tested against herpes viruses, respiratory viruses, hepatitis B virus and HIV. The monoammonium salt of the monoethyl ester of F-PMEA **30a** was found to be active against human cytomegalovirus (HCMV), Epstein–Barr virus and measles with EC_{50} values of 5.6, 1.6 and 32 μg mL^{-1}, respectively.

Introduction

Analogues of acyclic nucleoside phosphonates, in which the furanose ring of a nucleotide is replaced with an acyclic side chain and the $POCH_2$ unit of the monophosphate is replaced with a bioisostere, PCH_2O, have attracted considerable attention recently for their broad-spectrum antiviral activity against several DNA and RNA viruses.[1] For example, 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA **1**) (Scheme 1) exhibits *in vitro*



Scheme 1

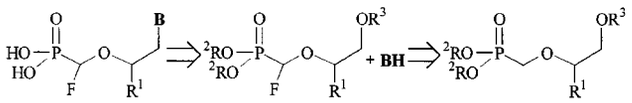
and *in vivo* activities against human immunodeficiency virus (HIV),[2] herpes simplex virus (HSV),[3] murine cytomegalovirus (MCMV),[4] hepatitis B virus (HBV),[5] as well as other DNA viruses and retroviruses.[6a,b] PMEA is undergoing clinical trials against HBV infection,[7] and the bis(pivaloyloxymethyl) ester prodrug of PMEA, bis(POM)-PMEA **4**, is now in phase II/III clinical trials for the treatment of HIV infection.[8a,b] PMEDAP **2** displays antiviral activities against HIV, CMV and Murine Sarcoma Virus (MSV).[9a,b] PMEG **3** has remarkable activity against papillomaviruses.[10] (*S*)-HPMPA **5**, (*S*)-HPMPDAP **6** and (*S*)-HPMPG **7** also show potent activities against retroviruses.[11a–c] HPMPC **8** (Vistide) has been approved by the FDA for the treatment of CMV retinitis in AIDS patients.[8b] The *R*-enantiomers of the PMP derivatives (*R*)-PMPA and (*R*)-PMPDAP possess potent antiviral effects against retroviruses.[12a,b] The fluoromethyl derivative FPMPA **11** was found to be active both *in vitro* and *in vivo* against retroviruses.[6c] A series of analogues modified in the PME and HPMP skeleton as well as in the heterocyclic portion has been synthesized and studied for structure–activity relationships.[1,13a,b]

In our efforts to search for new antiviral agents with improved activity and selectivity, we were interested in investigating the α-fluoromethoxy analogues of acyclic nucleoside phosphonates. Blackburn[14] and Chambers[15] and their co-workers have demonstrated that α-fluoroalkylphosphonates are more effective analogues of phosphates compared with the non-fluorinated phosphonates because the CFH or CF_2 group can both sterically and electronically mimic an oxygen,[16] enabling the second dissociation constant, pK_a2, to more closely mirror those of the phosphates due to the electron-withdrawing effect of fluorine. In addition, fluorine can act as a possible electron pair donor and retain the capacity of being a hydrogen bond acceptor, thus providing a possible additional binding site for enzymes. It is anticipated that replacement of the α-CH_2 group by CHF or CF_2 functionality in the PME- and HPMP-based acyclic nucleoside phosphonates would lead to enhanced therapeutic efficacy and improved pharmacological properties.

During the course of our study, we have developed a method for the preparation of the key intermediates diethyl (2-hydroxyethoxy)fluoromethylphosphonate **18** and 3-*O*-benzyl-2-*O*-[(diethoxyphosphoryl)fluoromethyl]glycerol **24**, and, for the first time, successfully synthesized a series of novel fluorinated analogues of the acyclic nucleoside phosphonates. These synthesized compounds were screened for their antiviral activities. The preliminary results on the synthesis of **18** were reported previously.[17] Herein we report our results concerning the synthesis and the *in vitro* biological activities of these fluorine-containing acyclic nucleoside phosphonates.

Results and discussion

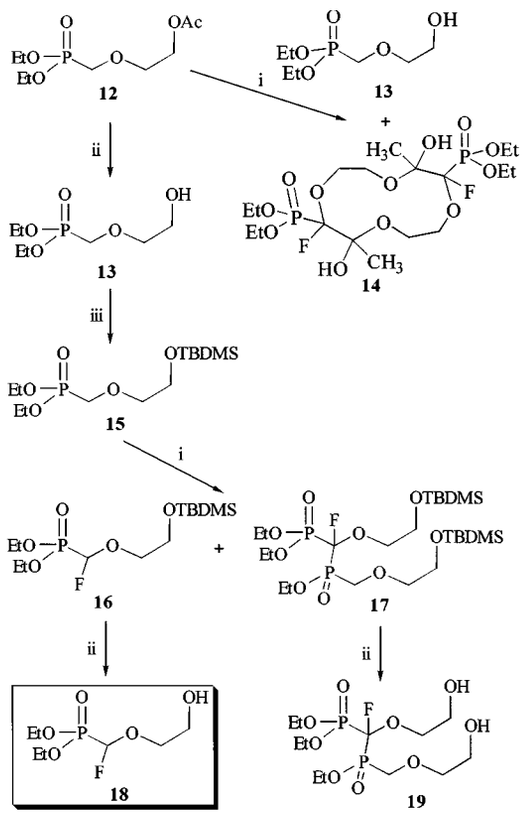
Retrosynthetic analysis of the target compounds revealed that the fluorine-containing PMEA, PMEC and HPMPC analogues can be constructed by the coupling of fluorine-containing PME and HPMP side chains with the corresponding purine and



Scheme 2

pyrimidine heterocyclic bases (Scheme 2). The α-fluorophosphono ether functionality can be introduced by electrophilic fluorination of the alkylphosphonate carbanion formed by deprotonation of a phosphorylmethoxy ether using a base.

Thus, the PME side chain **12** was prepared according to a literature procedure (Scheme 3).[18,19] Electrophilic fluorination

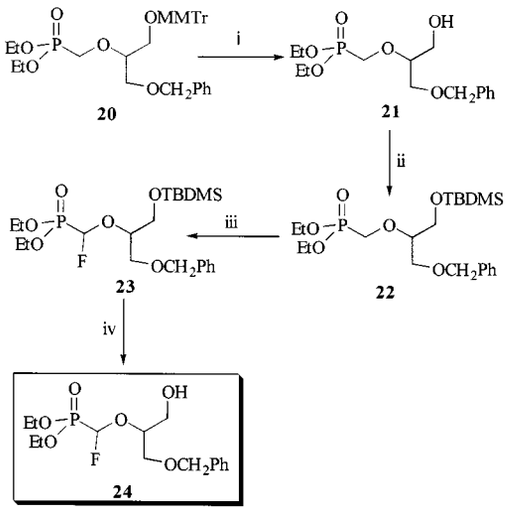


Scheme 3 *Reagents*: i, *sec*-BuLi, $(PhSO_2)_2NF$; ii, Dowex (H^+) resin; iii, TBDMS-Cl, DMAP, Et_3N.

of diethyl (2-acetoxyethoxy)methylphosphonate **12** was initially attempted using *N*-fluoro-*N*-(phenylsulfonyl)benzenesulfonamide $[(PhSO_2)_2NF]$[20] in the presence of NaH as a base. Instead of the desired product, a hydrolyzed alcohol **13** and a dimeric fluorine-substituted cyclic hemiketal **14** were obtained. A mechanism for the formation of the hemiketal was proposed previously,[17] which involves migration of the acyl group from O to C, followed by the electrophilic fluorination and dimerization. Changing the base from NaH to LDA, LHMDS, KHMDS or *sec*-BuLi did not affect the course of the reaction.

Compound **12** was then converted into *tert*-butyldimethylsilyl (TBDMS)-protected compound **15** in a two-step process (Scheme 3). Fluorination of **15** was carried out at −78 to 0 °C by using $(PhSO_2)_2NF$ as the fluorinating agent and *sec*-BuLi as the base.[17] The key intermediate 1-(*tert*-butyldimethylsiloxy)-2-[(diethoxyphosphoryl)fluoromethoxy]ethane **16** was formed in moderate yield (27%), along with a small amount of dimer **17** (~5% yield). Treatment of **16** with Dowex (H^+) ion exchange resin at ambient temperature yielded diethyl (2-hydroxyethoxy)-fluoromethylphosphonate **18** in 57% yield after purification by column chromatography on silica gel. Dimer **17** was also desilylated under similar conditions to yield compound **19** in 53% yield.

The fluorine-containing HPMP side chain **24** was synthesized by electrophilic fluorination of the protected HPMP

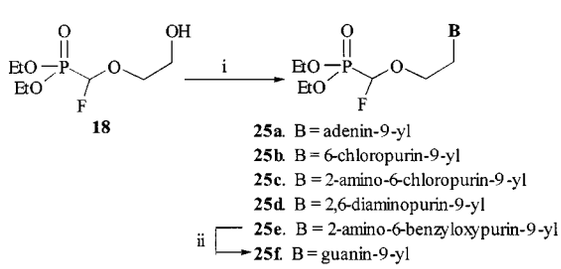


Scheme 4 *Reagents*: i, Amberlyst (H^+) ion-exchange resin, MeOH; ii, TBDMS-Cl, DMAP, CH_2Cl_2; iii, *n*-BuLi, $(PhSO_2)_2NF$; iv, Dowex (H^+) resin, EtOH.

side chain intermediate **22** (Scheme 4). The HPMP intermediate **20** was prepared according to a literature procedure,[21] and initially attempted for electrophilic fluorination. However, only starting compound **20** was recovered. The monomethoxytrityl (MMTr) protecting group was then removed by treatment of **20** with Amberlyst-15 (H^+) ion-exchange resin in MeOH at room temperature to provide the alcohol **21** in 60% yield. Treatment of **21** with *tert*-butyldimethylsilyl chloride in the presence of triethylamine and DMAP furnished the TBDMS-protected side chain **22** in 93% yield.

Fluorination of compound **22** at −78 °C by using $(PhSO_2)_2$-NF as the fluorinating agent and *sec*-BuLi as the base produced the desired compound, 1-*O*-(*tert*-butyldimethylsiloxy)-3-*O*-benzyl-2-*O*-[(diethoxyphosphoryl)fluoromethyl]glycerol **23** in only 5% yield. The unfavorable steric interactions between the OTBDMS-protected HPMP side chain and *sec*-BuLi may account for the low yield. Thus, *sec*-BuLi was replaced by *n*-BuLi and the fluorination was carried out under similar conditions to afford compound **23** in 29% yield (Scheme 4). Investigation of this reaction using other bases has also been conducted. However, replacement of *n*-BuLi with NaH, KH, LDA or KHMDS did not lead to the formation of the desired product. Only starting compound **22** was recovered from the reactions. Removal of the TBDMS group of **23** was accomplished by treatment with Dowex (H^+) ion exchange resin to afford compound **24** in 76% yield after column chromatographic purification.

In our previous work, we have identified the Mitsunobu reaction to be an efficient method for direct coupling of adenine to a variety of free alcohol side chains.[19] Modified Mitsunobu conditions were thus applied to coupling the fluorine-containing free alcohol, diethyl (2-hydroxyethoxy)fluoromethylphosphonate **18**, with purine bases (Scheme 5). It appeared that the outcome of the Mitsunobu coupling reaction depended strongly on the reaction temperature. While N^9-

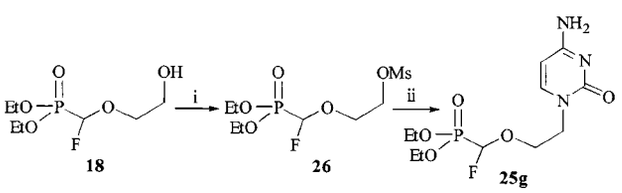


Scheme 5 *Reagents*: i, Nucleic base, DEAD, Ph_3P, DMF; ii, H_2, Pd/C, EtOH.

ethyladenine was isolated as the major product in 42% yield when the reaction was run at 0 °C, the desired 9-{2-[(diethoxyphosphoryl)fluoromethoxy]ethyl}adenine **25a** was obtained in 46% yield when **18** was coupled with adenine at −10 °C. The same procedure was applied to the coupling of 6-chloropurine with **18** to afford compound **25b** in 32% isolated yield. The corresponding 2-amino-6-chloropurine derivative **25c** was also prepared at −30 °C in 45% yield. However, under various reaction conditions, 2,6-diaminopurine led to the formation of N^9-ethyl-2,6-diaminopurine as the sole product, without any detection of the desired product **25d**. It is noteworthy that no other regioisomers have been detected from these Mitsunobu reactions.

Attempted coupling of guanine with F-PME side chain **18** under Mitsunobu conditions failed to afford the expected product. The extremely low solubility of guanine in the reaction medium unfavorably influences the process of its alkylation. To circumvent the problem of low solubility, 2-amino-6-benzyloxypurine was then chosen as a precursor of guanine, which was synthesized according to literature reports.[22a,b] Coupling of 2-amino-6-benzyloxypurine with **18** under Mitsunobu conditions at −40 °C afforded the corresponding 2-amino-6-benzyloxypurine derivative **25e** in 24% yield (Scheme 5). Removal of the benzyl protecting group was accomplished by hydrogenolysis using palladium on activated carbon as the catalyst[23] to provide the diethyl ester of F-PMEG (**25f**) in 37% yield. It should be noted that attempts to couple the fluorinated dimeric side chain **19** (Scheme 3) with adenine under Mitsunobu conditions failed to yield the corresponding dimeric alkylated adenine-based product.

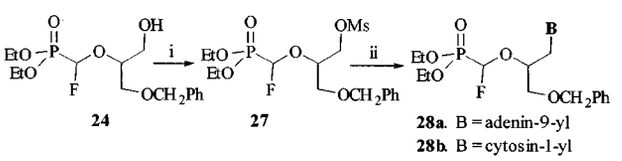
Base-catalyzed alkylation conditions were used for coupling cytosine with the mesylate of the F-PME side chain (**26**) since the fluorinated side chain **18** did not couple with cytosine under Mitsunobu conditions (Scheme 6). Thus, upon treatment with



Scheme 6 *Reagents*: i, MsCl, Et_3N, CH_2Cl_2; ii, cytosine, $CsCO_3$, DMF.

mesyl chloride in the presence of triethylamine, compound **18** was converted into the corresponding mesylate **26**. Coupling of **26** with cytosine in the presence of Cs_2CO_3[13b] successfully formed the desired N^1-derivative, the diethyl ester of F-PMEC **25g**, in 26% yield.

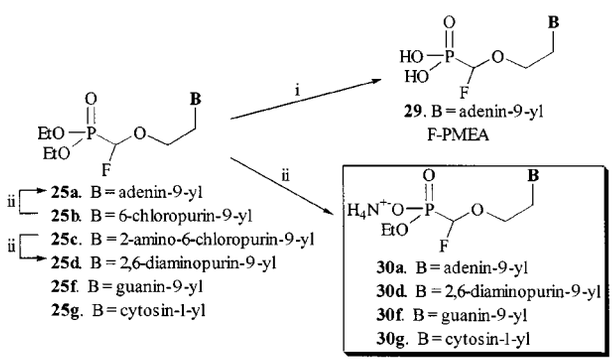
The fluorine-containing derivatives of HPMP nucleoside phosphonates were also synthesized, including 1-{2-[(diethoxyphosphoryl)fluoromethoxy]-3-benzyloxypropyl}cytosine **28a** and 1-{2-[(diethoxyphosphoryl)fluoromethoxy]-3-benzyloxypropyl}adenine **28b** (Scheme 7). The initial attempts to couple



Scheme 7 *Reagents*: i, MsCl, Et_3N, CH_2Cl_2; ii, nucleic base, $CsCO_3$, DMF.

the F-HPMP side chain **24** with cytosine or adenine under Mitsunobu conditions failed to afford the expected products. A small amount of N^9-ethyladenine (6% yield) was separated from the adenine reaction. Fluorine-containing alcohol **24** was then converted to mesylate **27** by treatment with mesyl chloride and triethylamine. The coupling of mesylate fluorine-containing side chain **27** with cytosine or adenine was achieved using Cs_2CO_3 as a base at elevated temperature to produce the corresponding acyclic nucleoside phosphonates **28a** and **28b**.

Bromotrimethylsilane (TMSBr) has been frequently used as an efficient reagent for dealkylation of phosphonate dialkyl ester to generate the corresponding phosphonic acid.[24a–c] Hydrolysis of the nonfluorinated PMEA diethyl ester using TMSBr proceeded smoothly to afford PMEA in 49% yield.[17,19] However, removal of the ethyl groups from 9-{2-[(diethoxyphosphoryl)fluoromethoxy]ethyl}adenine **25a** using the typical procedure (room temperature) did not afford the desired product. Instead, cleavage at the ether bond was observed, which led to the formation of the N^9-(2-hydroxyethyl)adenine. The decomposition was possibly caused by the hydrogen bromide released during the reaction and the sensitivity of the resulting α-fluoro phosphonomethoxy ether moiety. Nevertheless, direct attack of the ether oxygen by TMSBr could not be ruled out. When the reaction was carried out at a lower temperature (−10 °C), a mixture of 9-[2-(phosphonofluoromethoxy)ethyl]adenine **29** and 9-(2-hydroxyethyl)adenine was obtained (Scheme 8). Compound **29** was stable upon storage,



Scheme 8 *Reagents*: i, TMSBr, CH_2Cl_2; ii, conc. aqueous NH_3.

but it gradually degraded in a week to N^9-hydroxyethyladenine in aqueous solution when stored in a refrigerator below 0 °C.

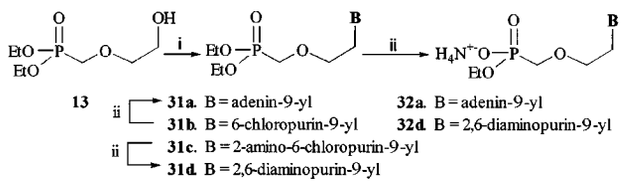
Alternatively, the hydrolysis of the diesters of F-PME and F-HPMP nucleoside phosphonates under mild alkaline conditions was then investigated. Treatment of the diesters of F-PMEA **25a**, F-PMEG **25f** and F-PMEC **25g** with concentrated aqueous ammonia led to the formation of the corresponding monoammonium salts of monoethyl phosphonate **30a**, **30f** and **30g**, respectively (Scheme 8). The same treatment of fluorine-containing 6-chloropurine and 2-amino-6-chloropurine derivatives **25b** and **25c** with concentrated aqueous ammonia resulted in the corresponding monoesters **30a** and **30d**, with concomitant replacement of the chlorine by an amino group. This replacement under such mild conditions was surprising because the chlorine attached to the 6-position of the purine ring is quite stable and its replacement by an amino group usually has to be performed with methanolic NH_3 at elevated temperature in a sealed vessel.

The monoesters **30a** and **30d** were remarkably stable and resistant to further hydrolysis under both basic and enzymatic conditions. Attempts to remove the second ethyl group from compound **30a** were carried out according to a literature procedure[25] using phosphodiesterases (such as *C. atrox* phosphodiesterase and phosphodiesterase I from *crotalus ademantens veron*) in 0.1 M triethylammonium bicarbonate buffer (TEAB, pH 7.4–8.5). However, no reaction occurred over a two day period. It should be noted that further treatment of the monoester with TMSBr led to degradation, resulting in the formation of N^9-(2-hydroxyethyl)adenine.

For purposes of comparison, the non-fluorinated diesters of **31a–d** were also prepared by coupling the non-fluorinated side chain **13** with the corresponding purine nucleic bases under Mitsunobu conditions (Scheme 9).[19] The diesters **31a–d** were then treated with concentrated aqueous ammonia. The reac-

Table 1 ^{19}F NMR Spectra of diesters and monoesters of fluorine-containing PME and HPMP nucleoside phosphonate analogues

Compounds	δ_1 (ppm)	$^2J_{P,F}$ (Hz)	$^2J_{H,F}$ (Hz)	δ_2 (ppm)	$^2J_{P,F}$ (Hz)	$^2J_{P,F}$ (Hz)
diester						
25a	−143.0	97.4	58.7	—	—	—
25b	−143.5	95.3	58.2	—	—	—
25c	−143.3	97.5	57.4	—	—	—
25f	−142.8	99.8	57.2	—	—	—
25g	−141.9	96.4	59.2	—	—	—
28a	−139.9	100.9	58.0	−137.4	100.7	57.5
28b	−141.0	97.6	57.8	−138.0	101.0	57.5
monoester						
30a	−140.7	80.7	57.7	—	—	—
30d	−143.5	86.4	59.1	—	—	—
30f	−143.6	84.2	58.0	—	—	—
30g	−142.2	82.2	58.8	—	—	—



Scheme 9 *Reagents*: i, Purine base, DEAD, Ph_3P, DMF; ii, conc. aqueous NH_3.

tions of **31a** and **31c** appeared much slower than those of the corresponding fluorinated analogues **25a** and **25c**. Approximately 50% of **31a** was converted to **32a** in 10 days. For **31b** and **31c**, the major products were **31a** and **31d**, with small amounts of **32a** and **32d** being detected. It is not surprising that the rates of hydrolysis of the non-fluorinated derivatives decrease in comparison with the fluorinated analogues. The strong electron-withdrawing effect of the fluorine atom enhances the rate of removal of ethoxide ion. It has also been reported that introduction of fluorine into the cyclic phosphates increased the rate of their hydrolysis.[26]

All the diesters and monoesters of the fluorine-containing PME nucleotide analogues **25a**,**b**,**c**,**f**,**g** and **30a,d,f,g** exhibit doublet of doublet peaks at about −140 ppm in their ^{19}F NMR spectra, with coupling constants being $^2J_{P,F}$ = 97–100 Hz and $^2J_{H,F}$ = 57–58 Hz (Table 1). For derivatives of F-HPMP nucleoside phosphonates **28a** and **28b**, two sets of doublet of doublets are observed in their ^{19}F NMR spectra, indicating different chemical shifts for the diastereomers. The coupling constants between fluorine and phosphorus in the monoesters **30a,d,f,g** are smaller ($^2J_{P,F}$ = 80–86 Hz) than those of the diesters ($^2J_{P,F}$ = 95–100 Hz). The ^{13}C NMR chemical shift of the heterocyclic carbons of the synthesized fluorine-containing and the nonfluorinated PME nucleoside phosphonate analogues are summarized in Table 2.

Table 2 ^{13}C NMR shifts for the heteroaromatic ring carbons of diesters and monoesters of PME purine nucleotide phosphonate analogues

Compound	δ_2 (ppm)	δ_4 (ppm)	δ_5 (ppm)	δ_6 (ppm)	δ_8 (ppm)
diester					
25a	153.1	149.9	119.5	155.7	141.3
25b	146.1	133.5	129.0	152.1	131.7
25c	154.3	149.5	123.3	160.0	143.3
31a	152.9	149.5	119.5	155.7	141.4
31b	146.3	132.1	128.5	151.8	131.4
31c	153.8	151.2	125.0	159.2	143.3
31d	156.1	151.9	114.0	160.0	138.8
monoester					
30a	152.0	149.3	118.5	155.7	141.5
30d	154.5	152.5	113.0	160.0	137.0

Biological evaluation and discussion

9-[2-(Phosphonofluoromethoxy)ethyl]adenine **29** and its diester **25a** were screened in the *in vitro* XTT assay for (XTT = 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide) for anti-HIV activity. It was found that these two compounds were inactive against HIV-1. As a PMEA analogue, the diester **25a** must be hydrolyzed and phosphorylated into the corresponding mono- and di-phosphonate in order to confer anti-HIV activity. The fact that **25a** is inactive implies that it might not be hydrolyzed and phosphorylated in the course of the assay. The inactivity of the acid **29** may be accounted for by its instability in aqueous solution. The monoesters **30a**, **30d**, **30f** and **30g** were evaluated for their antiviral activities against HIV. It was observed that these monoammonium salts were not active against HIV in the *in vitro* assay. Ethyl ammonium [2-(adenin-9-yl)ethoxy]fluoromethylphosphonate **30a** was also evaluated for its antiviral activities against hepatitis B virus (HBV), Herpes viruses (HSV-1, HSV-2, CMV, VZV and EBV) and respiratory viruses (adenovirus type 1, measles, parainfluenza type 3, influenza A, influenza B and respiratory syncytial virus). The results for compound **30a** are summarized in Table 3. Compound **30a** was not active against HIV and hepatitis B virus (HBV) in the *in vitro* assays, suggesting that either the monoester of F-PMEA may not be phosphorylated in these cells or its diphosphate, if formed, may have low binding affinity to the reverse transcriptase of HIV and HBV. However, despite poor activity against HSV-1 and HSV-2, compound **30a** exhibited potent activity against both human cytomegalovirus (HCMV) and Epstein–Barr virus (EBV), but somewhat less potent activity against measles.

The fact that the corresponding non-fluorinated monoethyl ester has been reported to be antivirally inactive[27] implies that introduction of fluorine into the α-position of the phosphonate plays a key role in maintaining the biological activity of **30a**, which may be due to the increased acidity of the phosphonic acid or altered mechanism of action. These results appear to suggest a significant level of phosphorylation of **30a**, either directly or *via* the hydrolyzed product of **30a** by host enzymes; however, its diphosphate may be a poor inhibitor of herpes simplex virus (HSV-1 and HSV-2) DNA polymerase, but an effective inhibitor of the HCMV DNA polymerase. Whether **30a** acts as a prodrug of **29** or exerts an antiviral effect without cleavage of the ester group will be a subject of future studies.

Experimental

Melting points were obtained with a Laboratory Devices Mel-Temp apparatus and were corrected. TLC analyses were performed on analytical thin layer plates coated with silica gel 60 F_{254} (Merck) and components were visualized under UV light and/or stained with iodine. Column chromatography was

Table 3 Antiviral activity of ethyl ammonium [2-(adenin-9-yl)ethoxy]fluoromethylphosphonate **30a**[a]

Virus cell strain	Compound **30a**			Positive Drug Control
	EC_{50}/μg mL^{-1}	IC_{50}/μg mL^{-1}	SI (IC_{50}/EC_{50})	EC_{50}/μg mL^{-1}
HIV-1/CEM-SS/RF	inactive[b]	non-toxic[b]		
HBV/2.2.15 cells	inactive[b]	non-toxic[b]		
HSV-1/HFF/E-377	>100	>100		0.08 (Acyclovir)
HSV-2/HFF/MS	>100	>100		0.5 (Acyclovir)
HCMV/HFF/AD-169	5.6	94.8	16.9	0.05 (Ganciclovir)
Adenovirus type 1/A549/adenoid	>100	>100		10 (HPMPA)
Epstein–Barr virus/DAUDI/P3HR-1	1.6	>50	>31	1.2 (Acyclovir)
Measles/CV-1/CC	32	>100	>3	1 (Ribavirin)
Parainfluenza type 3/MA-104/C243	>100	>100		10 (Ribavirin)
Respiratory syncytial virus/MA-104/Utah 89	>100	>100		8 (Ribavirin)

[a] EC_{50} value is the concentration required to inhibit viral cytopathogenicity by 50%; IC_{50} value is the concentration required to inhibit cell proliferation by 50%. [b] The value was >100 μM.

performed using silica gel 60 (70–230 mesh from EM Science). ^{1}H, ^{13}C, ^{19}F, and ^{31}P NMR spectra were recorded on a Varian VX-300 NMR spectrometer or a Varian Gemini 2300 NMR spectrometer. Chemical shifts (δ) are expressed in ppm and J values are given in Hz. Me_4Si was used as an internal standard for ^{1}H NMR and ^{13}C NMR, while trifluoroacetic acid (TFA) and phosphoric acid (H_3PO_4) were used as external standards for ^{19}F NMR and ^{31}P NMR, respectively. The infrared spectra were recorded on a Midac M series FT-IR. Mass spectra were recorded on a Finnegan MAT 90 mass spectrometer or a Finnegan MAT LCQ-MS spectrometer. UV spectra were measured in MeOH solution on a Hitachi U-2000 UV–VIS spectrophotometer. Elemental analyses were carried out at Midwest Microlab, Indianapolis, Indiana. Chemical reagents and anhydrous solvents were purchased from Aldrich Chemical Co. unless otherwise stated.

1-(*tert*-Butyldimethylsiloxy)-2-[(diethoxyphosphoryl)methoxy]-ethane 15

To a 100 mL single-necked round bottom flask equipped with a magnetic stirrer were added 2-[(diethoxyphosphoryl)methoxy]-ethanol **13**[21] (5.0 g, 23.6 mmol), anhydrous CH_2Cl_2 (40 mL), *tert*-butyldimethylsilyl chloride (3.9 g, 25.9 mmol), triethylamine (3.6 g, 35.4 mmol) and 4-dimethylaminopyridine (0.1 g, 0.8 mmol). The mixture was stirred under nitrogen at room temperature for 16 h, after which time it was diluted with CH_2Cl_2 (70 mL) and washed successively with 10% aqueous K_2CO_3 (40 mL) and brine (40 mL). The organic layer was separated and dried over anhydrous Na_2SO_4. After concentration under vacuum, 8 g of pale yellow oil was obtained. Purification by column chromatography on silica gel (EtOAc) provided the desired product **15** (6.4 g, 83% yield) as a colorless oil (Found: C, 47.87; H, 9.29. Calc. for $C_{13}H_{31}O_5PSi$: C, 47.83; H, 9.57%); ν_{max}/cm^{-1} 3479, 2932, 2959, 1472, 1254, 1030, 963; δ_H(300 MHz; $CDCl_3$) 4.11 (4H, quintet, J 7.1, $2CH_2OP$), 3.81 (2H, d, J 8.2, OCH_2P), 3.72 (2H, t, J 4.8, CH_2O), 3.59 (2H, t, J 5.0, CH_2O), 1.28 (6H, t, J 7.1, $2CH_3$), 0.83 [9H, s, $(CH_3)_3CSi$], 0.00 [6H, s, $(CH_3)_2Si$]; δ_C(75 MHz; $CDCl_3$) 74.68 (d, $^2J_{C,P}$ 11.3, CH_2OP), 65.47 (d, $^1J_{C,P}$ 165.2, CH_2P), 62.58 (CH_2OSi), 62.30 (d, $^3J_{C,P}$ 6.6, CH_2O), 25.80 [$(CH_3)_3CSi$], 18.30 [$SiC(CH_3)_3$], 16.40 (d, $^3J_{C,P}$ 5.7, CH_3), −5.42 [$(CH_3)_2Si$]; m/z (EI) 311($M^+ - CH_3$), 269 ($M^+ - C_4H_9$).

1-(*tert*-Butyldimethylsiloxy)-2-[(diethoxyphosphoryl)fluoro-methoxy]ethane 16

Anhydrous THF (20 mL) in a three-necked round bottom flask equipped with a thermometer, a magnetic stirrer, and an addition funnel was cooled to −78 °C under nitrogen, to which a solution of *sec*-BuLi (1.3 M in hexanes, 4.7 mL, 6.07 mmol) was added. After the temperature had equilibrated to −78 °C, a solution of the protected PME alcohol **15** (1.65 g, 5.06 mmol) in anhydrous THF (6 mL) was added dropwise to the light yellow solution. The reaction mixture was stirred at −78 °C for 0.5 h, followed by dropwise addition of *N*-fluoro-*N*-(phenylsulfonyl)benzenesulfonamide (3.0 g, 9.52 mmol) in anhydrous THF (20 mL). The resulting mixture was stirred at −78 °C for 2 h, gradually warmed to 0 °C, and then diluted with Et_2O (100 mL). Saturated aqueous NH_4Cl (30 mL) was added and the organic layer was separated, washed successively with H_2O (30 mL), brine (30 mL), and dried over anhydrous Na_2SO_4. The crude product (3 g), obtained after concentration under vacuum, was purified by column chromatography on silica gel (EtOAc) to afford the desired product **16** (480 mg, 27% yield) as a pale yellow oil (Found: C, 44.93; H, 8.54. Calc. for $C_{13}H_{30}FO_5PSi$: C, 45.33; H, 8.78%); ν_{max}/cm^{-1} 3430, 2934, 2859, 1651, 1474, 1393, 1254, 1163, 1026, 835, 777; δ_H(300 MHz; $CDCl_3$) 5.57 (1H, dd, $^1J_{F,H}$ 58.6, $^2J_{P,H}$ 14.0, CHFP), 4.26 (4H, quintet, J 7.0, $2CH_2O$), 3.84 (4H, m, $2CH_2O$), 1.36 (6H, t, J 7.0, $2CH_3$), 0.89 [9H, s, $(CH_3)_3CSi$], 0.06 [6H, s, $(CH_3)_2Si$]; δ_C(75 MHz; $CDCl_3$) 107.92 (dd, $^1J_{C,P}$ 230.8, $^1J_{C,F}$ 216.9, CHFP), 73.25 (d, $^2J_{C,P}$ 15.1, CH_2OP), 63.77 (dd, $^3J_{C,F}$ 12.6 , $^3J_{C,P}$ 6.2, CH_2O), 62.12 (CH_2OSi), 25.78 (Me_3CSi), 18.22 ($SiCMe_3$), 16.40 (d, $^3J_{C,P}$ 5.5, CH_3), −5.49 (Me_2Si); δ_F(280 MHz; $CDCl_3$; TFA) −141.76 (dd, $^2J_{F,P}$ 99.2, $^2J_{F,H}$ 58.6); m/z (CI) 345 ($M^+ + 1$).

Compound **17** was separated and characterized as the monofluoro dimer by-product. The yield of the dimer varied from 5 to 30% depending on the reaction conditions. The dimer **17** was more polar than **16**. Compound **17** was obtained as a pale yellow oil (Found: C, 46.60; H, 8.90; F, 3.13. Calc. for $C_{24}H_{55}FO_9P_2Si_2$: C, 46.14; H, 8.87; F, 3.04%); ν_{max}/cm^{-1} 3499, 2932, 2859, 1640, 1474, 1258, 1101, 1026, 959, 835, 777; δ_H(300 MHz; $CDCl_3$) 4.30–4.13 (10 H, m, CH_2PO, $3CH_2OP$, CH_2OCF), 3.80 (6H, m, $3CH_2O$), 1.36 (9H, m, $3CH_3$), 0.88 (18H, 2s, $2Me_3CSi$), 0.05 (12 H, 2s, $2Me_2Si$); δ_C(75 MHz; $CDCl_3$) 115.85–97.66 (m, CFP), 75.11 (d, $^2J_{C,P}$ 7.6, CH_2OP), 69.14 (m, CH_2O), 66.44 (d, $^1J_{C,P}$ 110.8, CH_2P), 64.92 (d, $^3J_{C,P}$ 6.7, CH_2O), 64.30 (d, $^2J_{C,P}$ 7.1, CH_2OP), 63.51 (d, $^2J_{C,P}$ 7.3, CH_2OP), 62.46 (CH_2OSi), 61.96 (CH_2OSi), 25.86 (Me_3CSi), 25.76 (Me_3CSi), 16.40 (d, $^3J_{C,P}$ 6.0, CH_3), −5.37 (Me_2Si), −5.45 (Me_2Si); δ_F(280 MHz; $CDCl_3$; TFA) −135.59 (dd, $^2J_{F,P}$ 106.1, $^2J_{F,P}$ 85.3); δ_P(146 MHz; $CDCl_3$; H_3PO_4) 34.21 (dd, $J_{F,P}$ 85.3, $J_{P,P}$ 61.2), 8.91 (dt, $^2J_{P,F}$ 99.9, $^2J_{P,P}$ 60.8); m/z (CI) 625($M^+ + 1$).

Diethyl (2-hydroxyethoxy)fluoromethylphosphonate 18

Into a 50 mL single-necked round bottom flask equipped with a magnetic stirrer were placed the protected F-PME alcohol **16** (1.23 g, 3.58 mmol), ethanol (20 mL) and Dowex 50WX8 (H^+-form) ion exchange resin (3 g), which had been prewashed thoroughly with ethanol. The mixture was stirred at room temperature overnight and completion of the reaction was determined by TLC analysis. The mixture was filtered, the resin

washed with ethanol (3 × 30 mL), and the combined filtrates were concentrated under vacuum. The residue was purified by column chromatography on silica gel (CH_2Cl_2–MeOH, 9:1) to afford the desired product **18** (0.35 g, 43% yield) as a colorless oil (Found: C, 37.87; H, 7.42. Calc. for $C_7H_{16}FO_5P$: C, 36.53; H, 7.01%); ν_{max}/cm^{-1} 3422, 2986, 2935, 1447, 1395, 1248, 1163, 1024; δ_H(300 MHz; $CDCl_3$) 5.59 (1H, dd, $^2J_{F,H}$ 57.0, $^2J_{P,H}$ 14.0, CHF), 4.26 (4H, m, $2CH_2OP$), 3.89 (1H, s, OH), 3.83 (4H, m, CH_2OH and CH_2O), 1.36 (6H, m, $2CH_3$); δ_C(75 MHz; $CDCl_3$) 108.0 (dd $^1J_{C,P}$ 229.7, $^1J_{C,F}$ 213.4, CHF), 74.5 (d, $^2J_{C,P}$ 14.1, CH_2OP), 63.7 (dd, $^3J_{C,F}$ 37.3, $^3J_{C,P}$ 6.2, CH_2O), 61.4 (CH_2OH), 16.4 (d, $^3J_{C,P}$ 5.0, CH_3); δ_F(280 MHz; $CDCl_3$; TFA) −140.5 (dd, $^2J_{F,P}$ 99.8, $^2J_{F,H}$ 57.5); *m*/*z* (CI) 231 ($M^+ + 1$).

Diethyl α-[ethoxy(2-hydroxyethoxymethyl)phosphoryl]-α-(2-hydroxyethoxy)fluoromethylphosphonate 19

The dimer OH side chain **19** was prepared from the TBDMS protected F-PME dimer **17** under conditions similar to those used for the preparation of the fluorinated F-PME alcohol **18**. After purification by column chromatography on silica gel (CH_2Cl_2–MeOH, 9:1), compound **19** (53% yield) was obtained as a colorless oil (Found: C, 36.74; H, 6.97; P, 14.46. Calc. for $C_{12}H_{27}FO_9P_2$: C, 36.37; H, 6.87; P, 15.63%); ν_{max}/cm^{-1} 3414, 2986, 1651, 1445, 1370, 1252, 1163, 1024, 982; δ_H(300 MHz; $CDCl_3$) 4.32 (8H, m, $2CH_2O$, CH_2OP, and CH_2P), 3.76 (8H, 2t, $2CH_2OP$ and $2CH_2OH$), 1.40 (9H, 2t, *J* 4.4, $3CH_3$); δ_C(75 MHz; $CDCl_3$) 111.79 (ddd, $^1J_{C,P}$ 251.5, $^1J_{C,P}$ 251.3, $^1J_{C,F}$ 112.7, CPFP), 75.45 (d, $^2J_{C,P}$ 9.5, CH_2OP), 69.65 (m, CH_2OP), 66.10 (d, $^1J_{C,P}$ 113.4, CH_2P), 65.20 (d, $^3J_{C,P}$ 6.6, CH_2O), 64.58 (d, $^3J_{C,P}$ 6.8, CH_2O), 63.68 (d, $^3J_{C,P}$ 7.5, CH_2O), 61.32 (CH_2OH), 61.16 (CH_2OH), 16.46 (CH_3), 16.38 (CH_3); δ_F(280 MHz; $CDCl_3$; TFA) −136.11 (dd, $^2J_{F,P}$ 100.8, $^2J_{F,P}$ 85.5; δ_P(146 MHz; $CDCl_3$; H_3PO_4) 34.0 (dd, $^2J_{F,P}$ 85.5, $^2J_{P,P}$ 62.3), 8.3 (dd, $^2J_{F,P}$ 100.0, $^2J_{P,P}$ 63.0); *m*/*z* (CI) 397 ($M^+ + 1$).

1-*O*-(*tert*-Butyldimethylsiloxy)-2-*O*-[(diethoxyphosphoryl)-methyl]-3-*O*-benzylglycerol 22

To a 250 mL single-necked round bottom flask equipped with a magnetic stirrer were added 2-*O*-[(diethoxyphosphoryl)methyl]-3-*O*-benzylglycerol **21**[21] (4.0 g, 12.1 mmol), anhydrous CH_2Cl_2 (60 mL), *tert*-butyldimethylsilyl chloride (2.0 g, 12.3 mmol), triethylamine (1.8 g, 18.1 mmol) and 4-dimethylaminopyridine (0.1 g, 0.8 mmol). The mixture was stirred under nitrogen at room temperature for 16 h, after which it was diluted with CH_2Cl_2 (100 mL) and washed successively with 10% aqueous K_2CO_3 (80 mL) and brine (80 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum to yield 8 g of a pale yellow oil. Purification by column chromatography on silica gel (EtOAc) provided the desired product **22** (5.0 g, 93% yield) as a pale yellow oil (Found: C, 56.33; H, 8.67. Calc. for $C_{21}H_{39}O_6PSi$: 56.48; H, 8.80%); ν_{max}/cm^{-1} 3474, 3032, 2955, 2930, 2859, 1497, 1472, 1391, 1254, 1100, 1028, 996, 837, 779, 738.8, 698; δ_H(300 MHz; $CDCl_3$) 7.32–7.34 (5H, m, C_6H_5), 4.54 (2H, s, OCH_2Ph), 4.15–4.18 (4H, m, $POCH_2$), 4.02 (2H, d, *J* 8.7, OCH_2P), 3.56–3.71 (5H, m, 1-H, 2-H, and 3-H), 1.29–1.35 (6H, m, CH_3), 0.88 [9H, s, $(CH_3)_3Si$], 0.05 (6H, s, $2CH_3Si$); δ_C(75 MHz; $CDCl_3$) 138.27, 128.41, and 127.66 (aromatic C), 81.83 (d, $^3J_{C,P}$ 10.9, C-2), 73.37 (OCH_2Ph), 70.09 (C-3), 64.56 (d, $^1J_{C,P}$ 166.0, OCH_2P), 62.89 (C-1), 62.23(d, $^2J_{C,P}$ 6.3, CH_2OP), 25.71[$(CH_3)_3Si$], 18.07 (CSi), 16.32 (d, $^3J_{C,P}$ 5.1, CH_3), −5.65 (CH_3Si); *m*/*z* (ESI) 447.5 ($M^+ + 1$).

1-*O*-(*tert*-Butyldimethylsiloxy)-2-*O*-[(diethoxyphosphoryl)-fluoromethyl]-3-*O*-benzylglycerol 23

To a 500 mL three-necked round bottom flask equipped with a thermometer, a magnetic stirrer and an addition funnel were added the TBDMS-protected glycerol derivative **22** (3.9 g, 8.74 mmol) and 250 mL of anhydrous THF under nitrogen. The solution was cooled to −78 °C and a solution of *n*-BuLi (5.6 mL, 2.5 M in hexane, 14.0 mmol) was then added. The reaction mixture was stirred at −78 °C for 1.5 h and *N*-fluoro-*N*-(phenylsulfonyl)benzenesulfonamide (4.7 g, 14.9 mmol) in anhydrous THF (50 mL) was added dropwise. The resulting mixture was stirred at −78 °C for 2.5 h and then gradually warmed to −10 °C. The reaction mixture was then diluted with Et_2O (100 mL). Saturated aqueous NH_4Cl (2 × 80 mL) was added and the organic layer was separated, washed with brine (80 mL), and dried over anhydrous Na_2SO_4. The crude product (6 g), obtained after concentration under vacuum, was purified by column chromatography on silica gel (hexane–EtOAc, 7:3, 1 L; hexane–EtOAc, 1:1, 1 L; EtOAc 500 mL) to afford the desired product **23** (1.2 g, 29% yield) as a pale yellow oil (Found: C, 53.61; H, 8.22. Calc. for $C_{21}H_{38}FO_6PSi$: 54.30; H, 8.24%); ν_{max}/cm^{-1} 2955, 2932, 2859, 1472, 1391, 1258, 1165, 1100, 1026, 980, 837, 779; δ_H(300 MHz; $CDCl_3$) 7.35–7.29 (5H, m, C_6H_5), 5.78 (1H, dd, $^2J_{F,H}$ 58.5, $^2J_{P,H}$ 14.1, PCHF), 4.55 (2H, s, OCH_2Ph), 4.20–4.26 (4H, m, $POCH_2$), 4.03 (1H, m, 2-H), 3.82–3.62 (4H, m, 1-H and 3-H), 1.30–1.37 (6H, m, CH_3), 0.88 [9H, s, $(CH_3)_3Si$], 0.06 (6H, s, $2CH_3Si$); δ_C(75 MHz; $CDCl_3$) 138.10, 138.03, 128.46, 128.44, and 127.71 (aromatic), 109.04 (dd, $^1J_{C,P}$ 227.9, $^1J_{C,F}$ 217.0, CHFP), 106.0 (dd, $^2J_{C,P}$ 231.3, $^2J_{C,F}$ 219.0, CHFP), 81.94 (d, $^3J_{C,F}$ 14.0, C-2), 81.73 (d, $^3J_{C,F}$ 14.0, C-2), 73.50 and 73.47 (2s, OCH_2Ph), 69.51 (C-3), 63.75 (C-1), 63.18 (CH_2OP), 62.76 (CH_2OP), 25.68 [$(CH_3)_3Si$], 18.05 (CSi), 16.29 (d, *J* 5.1, CH_3), −5.70 (CH_3Si); δ_F(280 MHz; $CDCl_3$; TFA) −138.50 (dd, $^2J_{F,P}$ 100.7, $^2J_{F,H}$ 58.7), −138.86 (dd, $^2J_{F,P}$ 100.7, $^2J_{F,H}$ 58.7); *m*/*z* (ESI) 482.4 ($M^+ + 1$).

2-*O*-[(Diethoxyphosphoryl)fluoromethyl]-3-*O*-benzylglycerol 24

To a 50 mL single-necked round bottom flask equipped with a magnetic stirrer were added 1-*O*-(*tert*-butyldimethylsiloxy)-2-*O*-[(diethoxyphosphoryl)fluoromethyl]-3-*O*-benzylglycerol **23** (1.1 g, 2.37 mmol), ethanol (20 mL), and Dowex 50WX8 (H^+-form) ion exchange resin (3 g). The mixture was stirred at room temperature overnight and completion of the reaction was determined by TLC analysis. The mixture was filtered, the resin washed with ethanol (3 × 30 mL), and the combined filtrates were concentrated under vacuum. The residue was purified by column chromatography on silica gel (CH_2Cl_2–MeOH, 93:7) to provide the desired product **24** (0.6 g, 76% yield) as a colorless oil (Found: C, 50.91; H, 7.12. Calc. for $C_{15}H_{24}FO_6P$, 51.43; H, 6.91%); ν_{max}/cm^{-1} 3414, 2984, 2942, 1454, 1370, 1250, 1163, 1100, 1026, 982, 741, 700; δ_H (300 MHz; $CDCl_3$) 7.31–7.33 (5H, m, C_6H_5), 5.75 (1/2H, dd, $^2J_{F,H}$ 57.9, $^2J_{P,H}$ 13.2, PCHF), 5.72 (1/2H, dd, $^2J_{F,H}$ 55.4, $^2J_{P,H}$ 13.8, PCHF), 4.55 (2H, s, OCH_2Ph), 4.21–4.28 (4H, m, $POCH_2$), 4.05 (1H, m, H-2), 3.74–3.66 (4H, m, 1-H and 3-H), 2.56 (2H, br s, OH), 1.30–1.39 (6H, m, $2CH_3$); δ_C(75 MHz; $CDCl_3$) 137.84, 137.68, 128.53, 128.49, 127.92, 127.85, 127.73, and 127.70 (aromatic H), 107.66 (2dd, $^1J_{C,P}$ 254.5, $^1J_{F,C}$ 218.5, CHFP), 84.67 (d, $^3J_{C,F}$13.1, C-2), 82.40 (d, $^3J_{C,F}$ 13.7, C-2), 73.60 and 73.51 (2s, OCH_2Ph), 70.09 and 69.66 (C-3), 63.86 (C-1), 63.04 (CH_2OP), 62.61 (CH_2OP), 16.30 (d, $^3J_{C,P}$ 5.1, CH_3); δ_F(280 MHz; $CDCl_3$; TFA) −136.44 (dd, $^2J_{F,P}$ 103.9, $^2J_{F,H}$ 55.7), −137.86 (dd, $^2J_{F,P}$ 101.7, $^2J_{F,H}$ 59.4); *m*/*z* (ESI) 351.3 ($M^+ + 1$).

1-*O*-(Methylsulfonyl)- 2-*O*-[(diethoxyphosphoryl)fluoromethyl]-3-*O*-benzylglycerol 27

To a solution of 2-*O*-[(diethoxyphosphoryl)fluoromethyl]-3-*O*-benzylglycerol **24** (130 mg, 0.37 mmol) and methanesulfonyl chloride (50 mg, 0.43 mmol) in 5 mL of anhydrous CH_2Cl_2 was added triethylamine (75 mg, 0.74 mmol) dropwise at 0 °C under a nitrogen atmosphere. The resulting solution was stirred at 0 °C for 1 h, then gradually warmed to room temperature and stirred overnight. Ice-water (10 mL) was added to the solution and the aqueous layer was separated and extracted with CH_2Cl_2 (2 × 10 mL). The combined CH_2Cl_2 extracts were dried over

anhydrous sodium sulfate and filtered, and solvent was removed under vacuum to afford 150 mg (94% yield) of desired compound **27** as a pale yellow oil, which was used in the next reaction without further purification; ν_{max}/cm^{-1} 2986, 2933, 2870, 1725, 1454, 1358, 1258, 1177, 1024, 974, 833, 745, 700; δ_H(300 MHz; $CDCl_3$) 7.30–7.35 (5H, m, C_6H_5), 5.68 and 5.61 (1H, 2dd, $^2J_{F,H}$ 57.8, $^2J_{P,H}$ 12.8, CHFP), 4.55 (2H, s, OCH_2Ph), 4.32–4.50 (2H, m, 1-H), 4.16–4.28 (4H, m, CH_2OP), 3.76–3.61 (3H, m, 2-H and 3-H), 3.06 (3H, s, OCH_3), 1.39–1.31 (6H, m, $2CH_3$); *m*/*z* (ESI) 429.3 ($M^+ + 1$).

Procedure A. General procedure for the coupling of fluorinated alcohol side chains with purine bases under Mitsunobu conditions—preparation of compounds 25a, 25b, 25c and 25e

A mixture of a purine base (1 mol), diethyl (2-hydroxyethoxy)-fluoromethylphosphonate **18** (1 mol) and triphenylphosphine (1.5 mol) in anhydrous DMF was stirred at room temperature for 30 min. The reaction mixture was then cooled to −5 to −40 °C and a solution of diethyl azodicarboxylate (DEAD) (2 mol) in anhydrous DMF was added dropwise at such a rate as to maintain the reaction temperature at −5 to −40 °C. The reaction mixture was stirred for 3 h and then allowed to warm to room temperature and stirred overnight. Any unreacted purine base was removed by filtration and solvent was removed under vacuum. The crude product was purified by column chromatography on silica gel (CH_2Cl_2–MeOH) to afford the desired products.

Procedure B. General procedure for the coupling of fluorinated side chains with purine and pyrimidine bases under base-catalyzed conditions—preparation of compounds 25g, 28a and 28b

A mixture of 1-*O*-(methylsulfonyl)-2-[diethoxyphosphoryl)-fluoromethoxy]ethane **26** or 1-*O*-(methylsulfonyl)-2-*O*-[(diethoxyphosphoryl)fluoromethyl]-3-*O*-benzylglycerol **27** (1 mol), purine or pyrimidine base (1 mol), caesium carbonate (1.5 mol) and anhydrous DMF was stirred at 60–80 °C under a nitrogen atmosphere for 5–7 h. The solvent was removed under reduced pressure and the residue purified by column chromatography on silica gel to afford the desired compounds.[13b]

9-{2-[(Diethoxyphosphoryl)fluoromethoxy]ethyl}adenine 25a. Compound **25a** was prepared from adenine and the alcohol **18** according to procedure A (−5 °C) in 39% yield after purification by column chromatography on silica gel (CH_2Cl_2–MeOH, 9:1). An analytical sample was obtained by recrystallizing twice from EtOAc to afford **25a** as an off-white solid, mp 148–150 °C (Found: C, 41.88; H, 5.52; N, 19.75. Calc. for $C_{12}H_{19}FN_5O_4P$: C, 41.51; H, 5.51; N, 20.14%); λ_{max}(MeOH)/nm 260.0 (ε/dm^3 mol^{-1} cm^{-1} 10,300); ν_{max}(KBr)/cm^{-1} 3356, 3283, 3129, 2978, 1715, 1678, 1605, 1422, 1366, 1256, 1024; δ_H(300 MHz; $CDCl_3$) 8.35 (1H, s, 2-H), 7.94 (1H, s, 8-H), 5.80 (2H, s, NH_2), 5.41 (1H, dd, $^2J_{F,H}$ 58.2, $^2J_{P,H}$ 13.0, CHFP), 4.48 (2H, m, CH_2N), 4.15 (6H, m, CH_2O and $2CH_2OP$), 1.30 (6H, 2t, $2CH_3$); δ_C(75 MHz; $CDCl_3$) 155.7 (C-6), 153.1 (C-2), 149.9 (C-4), 141.3 (C-8), 119.5 (C-5), 107.5 (dd, $^1J_{C,P}$ 234.4, $^1J_{C,F}$ 216.2, CHFP), 70.0 (d, $^2J_{C,P}$ 15.5, $POCH_2$), 64.0 (d, $^3J_{C,P}$ 6.3, CH_2O), 43.3 (CH_2N), 15.5 (d, $^3J_{C,P}$ 3.4, CH_3); δ_F(280 MHz; $CDCl_3$; TFA) −143.0 (dd, $^2J_{F,P}$ 97.4, $^2J_{F,H}$ 58.7); *m*/*z* (CI) 348 ($M^+ + 1$), 328 ($M^+ - HF$).

6-Chloro-9-{2-[(diethoxyphosphoryl)fluoromethoxy]ethyl}-purine 25b. The title compound was synthesized from 6-chloropurine and the alcohol **18** according to procedure A (−10 °C) in 32% yield. The desired product **25b** was obtained as a thick oil after purification by column chromatography on silica gel (CH_2Cl_2–MeOH, 95:5), which solidified upon standing at room temperature for a couple of days. An analytical sample was obtained by triturating with hexane and diethyl ether, mp 53–55 °C (Found: C, 39.01; H, 4.62; N, 14.90. Calc. for $C_{12}H_{17}ClFN_4O_4P$: C, 39.30; H, 4.67; N, 15.28%); λ_{max}(MeOH)/nm 264.5 (ε/dm^3 mol^{-1} cm^{-1} 9,000); ν_{max}(KBr)/cm^{-1} 3451, 2986, 2932, 1595, 1501, 1404, 1335, 1254, 1148, 1024, 934; δ_H(300 MHz; $CDCl_3$) 8.76 (1H, s, 2-H), 8.28 (1H, s, 8-H), 5.42 (1H, dd, $^2J_{F,H}$ 57.3, $^2J_{P,H}$ 12.9, CHFP), 4.60 (2H, m, CH_2N), 4.19 (6H, m, CH_2O and $2CH_2OP$), 1.33 (6H, 2t, $2CH_3$); δ_C(75 MHz; $CDCl_3$) 152.1 (C-6), 146.1 (C-2), 133.5 (C-4), 131.7 (C-8), 129 (C-5), 107.4 (dd, $^1J_{C,P}$ 234.9, $^1J_{C,F}$ 216.4, CHFP), 69.4 (d, $^2J_{C,P}$ 14.9, CH_2OP), 64.2 (d, $^3J_{C,P}$ 6.9, CH_2O), 43.8 (CH_2N), 16.3 (d, $^3J_{C,P}$ 5.7, CH_3); δ_F(280 MHz; $CDCl_3$; TFA) −143.5 (dd, $^2J_{F,P}$ 95.3, $^2J_{F,H}$ 58.2); *m*/*z* (CI) 367 ($M^+ + 1$), 347 ($M^+ - HF$).

2-Amino-6-chloro-9-{2-[(diethoxyphosphoryl)fluoromethoxy]-ethyl}purine 25c. The title compound was obtained from 2-amino-6-chloropurine and the alcohol **18** according to procedure A (−30 °C) in 45% yield after purification by column chromatography on silica gel (CH_2Cl_2–MeOH, 9:1). An analytical sample was obtained by recrystallization from CH_2Cl_2–hexane as a white solid, mp 103–105 °C (Found: C, 37.92; H, 4.80; N, 18.32. Calc. for $C_{12}H_{18}ClFN_5O_4P$: C, 37.76; H, 4.75; N, 18.35%); λ_{max}(MeOH)/nm 309.0 (ε/dm^3 mol^{-1} cm^{-1} 8,250) and 246.5 (7,000); ν_{max}(KBr)/cm^{-1} 3399, 3341, 3225, 2996, 1651, 1615, 1566, 1532, 1474, 1418, 1354, 1242, 1157, 1051, 1015, 907; δ_H(300 MHz; DMSO-d_6) 8.07 (1H, s, 8-H), 6.92 (2H, s, NH_2), 5.81 (1H, dd, $^2J_{F,H}$ 57.1, $^2J_{P,H}$ 13.7, CHFP), 4.33 (2H, m, CH_2N), 4.20 (2H, m, CH_2O), 4.01 (4H, m, CH_2OP), 1.16 (6H, 2t, ^1J 6.9, $2CH_3$); δ_C(75 MHz; DMSO-d_6) 160 (C-6), 154.3 (C-2), 149.5 (C-4), 143.4 (C-8), 123.3 (C-5), 107.3 (dd, $^1J_{C,P}$ 227.5, $^1J_{C,F}$ 211.7, CHFP), 69.1 (d, $^3J_{C,P}$ 5.4, CH_2O), 63.2 (d, $^2J_{C,P}$ 6.9, CH_2OP), 42.6 (CH_2N), 16.0 (d, $^3J_{C,P}$ 5.1, CH_3); δ_F(280 MHz; DMSO-d_6; TFA) −143.2 (dd, $^2J_{F,P}$ 97.5, $^2J_{F,H}$ 57.4); *m*/*z* (CI) 382 ($M^+ + 1$).

2-Amino-6-benzyloxy-9-{2-[(diethoxyphosphoryl)fluoro-methoxy]ethyl}purine 25e. The title compound was obtained from 2-amino-6-benzyloxypurine[22a,b] and the alcohol **18** according to procedure A at −40 °C. The crude product was purified by column chromatography on silica gel (CH_2Cl_2–MeOH, 93:7) to afford the desired product **25e** as a thick oil in 24% yield, which semi-solidified upon standing at room temperature for a couple of days. An analytical sample was obtained by triturating with hexane–EtOAc. λ_{max}(MeOH)/nm 283.5 (ε/dm^3 mol^{-1} cm^{-1} 11,370) and 247.0 (10,830); ν_{max}(KBr)/cm^{-1} 3327, 3212, 2994, 1582, 1458, 1412, 1356, 1248, 1028, 982, 791, 700; δ_H(300 MHz; $CDCl_3$) 7.70 (1H, s, 8-H), 7.49–7.33 (5H, m, C_6H_5), 5.57 (1H, s, OCH_2Ph), 5.41 (1H, dd, $^2J_{F,H}$ 57.8, $^2J_{P,H}$ 13.1, CHFP), 4.83 (2H, br s, NH_2), 4.35–4.09 (8H, m, CH_2N, CH_2O, and $2CH_2OP$), 1.33–1.29 (6H, m, $2CH_3$); δ_C(75 MHz; $CDCl_3$) 161.21, 159.31, 154.09, 140.50, 136.55, 129.01, 128.44, 128.33, 128.04, 128.02, 115.57, 107.58 (dd, $^1J_{C,P}$ 233.9, $^1J_{C,F}$ 216.1, CHF), 100.19, 97.42, 69.9 (d, $^2J_{C,P}$ 15.5, CH_2OP), 64.0 (d, $^3J_{C,P}$ 6.9, CH_2O), 43.26 (CH_2N), 16.3 (d, $^3J_{C,P}$ 5.7, CH_3); *m*/*z* (ESI) 454.5 ($M^+ + 1$).

9-{2-[(Diethoxyphosphoryl)fluoromethoxy]ethyl}guanine 25f. A suspension of 2-amino-6-benzyloxy-9-{2-[(diethoxyphosphoryl)fluoromethoxy]ethyl}purine **25e** (200 mg, 0.442 mmol) and palladium on activated carbon (10%, 20 mg) in 20 mL of absolute ethanol was stirred overnight under a hydrogen atmosphere at room temperature. The catalyst was removed by filtration and the filtrate concentrated under vacuum to afford 120 mg of crude product as a sticky residue, which was purified by column chromatography on silica gel (CH_2Cl_2–MeOH, 8:2) to afford the desired compound **25f** (50 mg, 37% yield) as a white solid, mp 120 °C (decomp.); λ_{max}(MeOH)/nm 270 (ε/dm^3 mol^{-1} cm^{-1} 7,480) and 253.0 (10,880); ν_{max}(KBr)/cm^{-1} 3331, 3135, 2980, 2764, 1688, 1611, 1539, 1481, 1375, 1256, 1161, 1024, 980, 781, 693; δ_H(300 MHz; CD_3OD) 7.74 (1H, s, 8-H), 5.64 (1H, dd, $^2J_{F,H}$ 57.5, $^2J_{P,H}$ 13.7, CHFP), 4.36–4.25 (3H, m,

CH_2N and 1/2CH_2O), 4.19–4.07 (5H, m, 1/2CH_2O and 2CH_2OP), 1.30–1.23 (6H, m, 2CH_3); δ_C(75 MHz; CD_3OD) 164.69, 155.53, 154.09, 140.25, 140.09, 108.70 (dd, $^1J_{C,P}$ 231.6, $^1J_{C,F}$ 216.7, CHF), 70.98 (d, $^2J_{C,P}$ 16.1, CH_2OP), 64.0 (d, $^3J_{C,P}$ 6.3, CH_2O), 44.09 (CH_2N), 16.62 (d, $^3J_{C,P}$ 5.7, CH_3); δ_F(280 MHz; $CDCl_3$; TFA) −142.83 (dd, $^2J_{F,P}$ 99.8, $^2J_{F,H}$ 57.2); *m*/*z* (ESI) 364.3 ($M^+ + 1$).

1-{2-[(Diethoxyphosphoryl)fluoromethoxy]ethyl}cytosine 25g. The title compound was obtained as an off-white solid from cytosine and 1-*O*-(methylsulfonyl)-2-(diethoxyphosphoryl-fluoromethoxy)ethane **26** according to procedure B in 26% yield after purification by column chromatography on silica gel (CH_2Cl_2–MeOH, 4:1). An analytical sample was obtained by recrystallization from MeOH–CH_2Cl_2 (95:5), mp 164–166 °C (decomp.) (Found: C, 40.55; H, 5.97; N, 12.87. Calc. for $C_{11}H_{19}FN_3O_5P$: C, 40.87; H, 5.92; N, 13.00%); λ_{max}(MeOH)/nm 272.5 (ε/dm^3 mol^{-1} cm^{-1} 6,700) and 252.2 (4,800); ν_{max}(KBr)/cm^{-1} 3343, 3153, 2990, 1659, 1618, 1510, 1487, 1391, 1250, 1163, 1028, 980, 791, 689; δ_H(300 MHz; $CDCl_3$) 7.30 (1H, d, *J* 7.2, 5-H), 5.62 (1H, d, *J* 7.2, 6-H), 5.34 (1H, dd, $^2J_{F,H}$ 58.1, $^2J_{P,H}$ 13.4, CHFP), 4.13 (4H, m, CH_2O and CH_2OP), 4.04 (2H, m, CH_2N), 1.27 (6H, 2t, 2CH_3); δ_C(75 MHz; $CDCl_3$) 166.14 (C-4), 156.44 (C-2), 147.01 (C-6), 107.73 (dd, $^1J_{C,P}$ 233.6, $^1J_{C,F}$ 217.0, CHFP), 93.86 (C-5), 70.32 (d, $^2J_{C,P}$ 15.5, CH_2O), 64.02 (d, $^3J_{C,P}$ 6.3, CH_2O), 49.84 (CH_2N), 16.35 (d, $^3J_{C,P}$ 5.1, CH_3); δ_F(280 MHz; $CDCl_3$; TFA) −141.9 (dd, $^2J_{F,P}$ 96.2, $^2J_{H,F}$ 59.2; *m*/*z* (CI) 323 ($M^+ + 1$).

1-*O*-(Methylsulfonyl)-2-[(diethoxyphosphoryl)fluoromethoxy]-ethanol 26. To a solution of the fluorinated alcohol **18** (550 mg, 2.18 mmol) and methanesulfonyl chloride (265 mg, 2.3 mmol) in anhydrous CH_2Cl_2 (30 mL) was added dropwise triethylamine (380 mg, 4.35 mmol) at 0 °C under a nitrogen atmosphere. The resulting solution was stirred at 0 °C for 1 h, then gradually warmed to room temperature and stirred for an additional 3 h. Ice-water (30 mL) was added to the solution and the aqueous layer was separated and extracted with CH_2Cl_2 (2 × 50 mL). The combined CH_2Cl_2 extracts were dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by column chromatography on silica gel (CH_2Cl_2–MeOH, 95:5) to provide the title compound **26** as a pale yellow oil in quantitative yield (Found: C, 32.38; H, 6.12. Calc. for $C_8H_{18}FO_7PS$: C, 31.17; H, 5.89%); ν_{max}/cm^{-1} 2988, 2940, 1354, 1254, 1175, 1024, 976, 926, 797; δ_H(300 MHz; $CDCl_3$) 5.49 (1H, dd, $^2J_{F,H}$ 57.6, $^2J_{P,H}$ 12.9, CHFP), 4.45 (2H, t, *J* 5.1, CH_2N), 4.25 (4H, m, CH_2O and CH_2OP), 3.09 (3H, s, CH_3SO_2), 1.37 (6H, t, *J* 6.6, CH_3); δ_C(75 MHz; $CDCl_3$) 107.6 (dd, $^1J_{C,P}$ 234.7, $^1J_{C,F}$ 216.4, CHFP), 69.5 (d, $^2J_{C,P}$ 16.1, CH_2OP), 67.9 (s, CH_2O), 64.2 (d, $^3J_{C,P}$ 6.9, CH_2O), 37.7 (CH_3SO_2), 16.3 (d, $^3J_{C,P}$ 5.7, CH_3); δ_F(280 MHz; $CDCl_3$; TFA) −143.03 (dd, $^2J_{F,P}$ 96.0, $^2J_{F,C}$ 57.7, CHFP); *m*/*z* (CI) 309 ($M^+ + 1$), 289 ($M^+ - HF$).

9-{3-Benzyloxy-2-[(diethoxyphosphoryl)fluoromethoxy]-propyl}adenine 28a. The title compound was prepared from adenine and 2-(diethoxyphosphorylfluoromethoxy)-1-*O*-(methylsulfonyl)ethanol **27** according to procedure B. The reaction mixture was stirred at 70 °C under a nitrogen atmosphere for 7 h. After purification by column chromatography on silica gel (CH_2Cl_2–MeOH, 9:1), compound **28a** (28% yield) was obtained as a semi-solid; λ_{max}(MeOH)/nm 259.5 (ε/dm^3 mol^{-1} cm^{-1} 17,040); ν_{max}(KBr)/cm^{-1} 3331, 3198, 3003, 2868, 1651, 1599, 1478, 1418, 1300, 1250, 1154, 1024, 984, 799, 743, 700, 650; δ_H(300 MHz; $CDCl_3$) 8.36 and 8.35 (1H, 2s, 2-H), 7.93 and 7.92 (1H, 2s, 8-H), 7.36–7.27 (5H, m, C_6H_5), 5.66 (1/2H, dd, $^2J_{F,H}$ 57.5, $^2J_{P,H}$ 13.1, PCHF), 5.62 (2H, br s, NH_2), 5.39 (1/2H, dd, $^2J_{F,H}$ 57.3, $^2J_{P,H}$ 12.8, PCHF), 4.52 (2H, s, OCH_2Ph), 4.42 (2H, m, H-1′), 4.14 (4H, m, $POCH_2$), 3.71–3.64 (2H, m, H-3′), 3.55–3.52 (1H, m, H-1′), 1.35–1.28 (6 H, m, 2CH_3); δ_C(75 MHz; $CDCl_3$) 155.5, 153.2, 153.1, 142.0, 141.8, 137.5, 137.4, 128.6, 128.1, 128.1, 127.9, 107.21 (2dd, $^1J_{C,P}$ 251.4, $^1J_{C,F}$ 218.7, CHFP), 80.4 (d, $^2J_{C,P}$ 14.9, $POCH_2$), 78.8 (d, $^3J_{C,P}$ 14.3, C-2′), 73.8 (s, OCH_2Ph), 69.7 (C-3′), 44.7 (C-1′), 16.3 (CH_3); δ_F(280 MHz; $CDCl_3$; TFA) −138.04 (dd, $^2J_{F,P}$ 101.0, $^2J_{F,H}$ 57.5, CHFP), −141.04 (dd, $^2J_{F,P}$ 97.6, $^2J_{F,H}$ 57.8, CHFP); *m*/*z* (ESI) 468.3 ($M^+ + 1$).

1-{3-Benzyloxy-2-[(diethoxyphosphoryl)fluoromethoxy]-propyl}cytosine 28b. The title compound was prepared from cytosine and 1-*O*-(methylsulfonyl)-2-*O*-[(diethoxyphosphoryl)-fluoromethyl]-3-*O*-benzylglycerol **27** according to procedure B. The reaction mixture was stirred at 70 °C under a nitrogen atmosphere for 7 h. After purification by column chromatography on silica gel (CH_2Cl_2–MeOH, 9:1), compound **28b** (41% yield) was obtained as an off-white solid, mp 50–55 °C (soften), 130 °C (decomp.) (Found: C, 50.92; H, 6.34; N, 9.27. Calc. for $C_{19}H_{27}FN_3O_6P$: 51.47; H, 6.14; N, 9.48%); λ_{max}-(MeOH)/nm 272 (ε/dm^3 mol^{-1} cm^{-1} 3,800) and 253.0 (2,800); ν_{max}(KBr)/cm^{-1} 3349, 2920, 1653, 1491.1, 1389, 1248, 1157, 1024, 984, 789, 700; δ_H(300 MHz; $CDCl_3$) 7.67 (1H, 2d, *J* 7.2, 6-H), 7.36–7.34 (5H, m, C_6H_5), 7.01 (2H, br s, NH_2), 5.84 (1H, 2dd, $^2J_{F,H}$ 57.6, $^2J_{P,H}$ 13.2, PCHF), 5.64 (1H, 2d, *J* 7.2, 5-H), 4.52 (2H, s, OCH_2Ph), 4.31 (1H, m, 2′-H), 4.14 (4H, m, $POCH_2$), 3.76–3.51 (4H, m, 1′-H and 3′-H), 1.26–1.18 (6H, m, 2CH_3); δ_C(75 MHz; $CDCl_3$) 166.1, 156.6, 156.4, 137.7, 137.6, 128.5, 128.0, 127.9, 127.8, 105.4 (dd, $^1J_{C,P}$ 234.7, $^1J_{C,F}$ 219.9, CHFP), 105.4 (dd, $^1J_{C,P}$ 232.6, $^1J_{C,F}$ 219.2, CHFP), 93.6, 93.5, 80.5 (d, $^2J_{C,P}$ 14.9, CH_2OP), 79.5 (d, $^2J_{C,P}$ 14.9, CH_2OP), 73.6 (OCH_2Ph), 70.3 (C-3), 63.6 (C-2), 51.5 and 51.0 (CH_2N), 16.30 (d, $^3J_{C,P}$ 5.1, CH_3); δ_F(280 MHz; $CDCl_3$; TFA) −137.41 (dd, $^2J_{F,P}$ 100.7, $^2J_{F,H}$ 57.5, CHFP), −139.9 (dd, $^2J_{F,P}$ 100.9, $^2J_{F,H}$ 58.0, CHFP); *m*/*z* (ESI) 444.3 ($M^+ + 1$).

9-[2-(Phosphonofluoromethoxy)ethyl]adenine 29

9-{2-[(Diethoxyphosphoryl)fluoromethoxy]ethyl}adenine **25a** (100 mg, 0.29 mmol) was dissolved in anhydrous CH_2Cl_2 (4 mL) and cooled to −10 °C in an ice-water bath, to which was added bromotrimethylsilane (0.4 mL, 2.9 mmol) dropwise while stirring. The resulting mixture was stirred at −10 °C for 4.5 h and then gradually warmed to ambient temperature and stirred overnight. Solvent was removed under vacuum to afford a white foam solid, which was dissolved in H_2O (1 mL). Acetone (2 mL) was added to the aqueous solution and the mixture was stirred at ambient temperature for 15 min. A white precipitate formed and was collected by filtration to afford 36 mg (43% yield) of compound **1**, mp 210 °C (decomp.); δ_H(300 MHz; DMSO-d_6) 8.21 (1H, s, 2-H), 8.18 (1H, s, 8-H), 7.70 (2H, s, NH_2), 5.51 (1H, dd, $^2J_{F,H}$ 57.6, $^2J_{P,H}$ 12.0, CHFP), 4.39 (2H, m, CH_2N), 3.95 (2H, m, CH_2O); δ_F(280 MHz; DMSO-d_6; TFA) −143.75 (dd, $^2J_{F,P}$ 92.1, $^2J_{F,H}$ 58.2, CHFP).

Procedure C. General procedure for the hydrolysis of diesters of the fluorinated acyclic nucleoside phosphonates under basic conditions—preparation of compounds 30a, 30d, 30f, 30g, 32a and 32d

A suspension of a diester of fluorinated acyclic nucleoside phosphonate in concentrated aqueous ammonia was stirred at room temperature for 1–10 days. Solvent was removed under vacuum at 30 °C to leave a crude product, which was further purified to afford the desired product.

Ethyl ammonium [2-(adenin-9-yl)ethoxy]fluoromethylphosphonate 30a. Compound **30a** was prepared from 9-{2-[(diethoxyphosphoryl)fluoromethoxy]ethyl}adenine **25a** according to procedure C with a reaction time of 2 days. The crude product was recrystallized from MeOH–CH_2Cl_2 to afford the desired product **30a** (55% yield) as a white solid, mp 180 °C (decomp.) (Found: C, 35.15; H, 5.26; N, 23.43. Calc. for $C_{10}H_{18}FN_6O_4P$: C, 35.72; H, 5.40; N, 24.99%); λ_{max}(MeOH)/nm 259.5

(ε/dm^3 mol^{-1} cm^{-1} 11,700); ν_{max}(KBr)/cm^{-1} 3351, 3187, 2988, 2890, 1669, 1615, 1499, 1419.7, 1333, 1202, 1146, 1050, 976, 790, 652; δ_H(300 MHz; DMSO-d_6) 8.22 (1H, s, 2-H), 8.13 (1H, s, 8-H), 7.27 (2H, s, NH_2), 5.17 (1H, dd, $^2J_{FH}$ 57.9, $^2J_{P,H}$ 9.6, CHFP), 4.34 (2H, t, J 5.0, CH_2N), 4.03 (2H, m, CH_2O), 3.67 (2H, m, CH_2OP), 1.00 (3H, t, J 7.1, CH_3); δ_C(75 MHz; DMSO-d_6) 155.7(C-6), 152.0 (C-2), 149.3 (C-4), 141.5 (C-8), 118.5 (C-5), 110.6 (dd, $^1J_{C,P}$ 228.6, $^1J_{C,F}$ 191.3, CHF), 68.2 (d, $^2J_{C,P}$ 12.6, CH_2OP), 60.1 (d, $^3J_{C,P}$ 4.1, CH_2O), 42.7 (CH_2N), 16.9 (CH_3); δ_F(280 MHz; DMSO-d_6; TFA) −140.7 (dd, $^2J_{F,P}$ 80.7, $^2J_{F,H}$ 57.7; *m*/*z* (+ve FAB) 320 (M^- − NH_2); *m*/*z* (−ve FAB) 318 (M − NH_4^+).

Ethyl ammonium [2-(2,6-diaminopurine-9-yl)ethoxy]fluoromethylphosphonate 30d. The title compound **30d** was prepared from 2-amino-6-chloro-9-{2-[(diethoxyphosphoryl)fluoromethoxy]ethyl}purine **25d** according to procedure C with a reaction time of 3 days. The crude product was triturated with CH_2Cl_2 twice to afford compound **30d** (87% yield) as an off-white solid. An analytical sample was obtained by recrystallization from MeOH, mp 230 °C (decomp.) (Found: C, 33.40; H, 4.96; N, 23.29. Calc. for $C_{10}H_{19}FN_7O_4P$: C, 34.19; H, 5.45; N, 27.92%); λ_{max}(MeOH)/nm 280.5 (ε/dm^3 mol^{-1} cm^{-1} 10,500) and 247.5 (9,600); ν_{max}(KBr)/cm^{-1} 3644, 3366, 3138, 1671, 1586, 1535, 1483, 1410, 1331, 1200, 1073, 965, 768; δ_H(300 MHz; acetic acid-d_4) 8.11 (1H, s, 8-H), 5.72 (1H, dd, $^2J_{F,H}$ 59.1, $^2J_{P,H}$ 11.7, CHFP), 4.30 (4H, m, CH_2N and CH_2O), 4.13 (2H, app. quartet, J 6.9, CH_2OP), 1.27 (3H, t, J 6.9, CH_3); δ_C(75 MHz; acetic acid-d_4) 160.0 (C-6), 154.5 (C-2), 152.5 (C-4), 137.0 (C-8), 116.0 (dd, $^1J_{C,P}$ 282.8, $^1J_{C,F}$ 188.6, CHFP), 113.0 (C-5), 69.9 (CH_2OP), 63.6 (CH_2O), 46.2 (CH_2N), 16.9 (CH_3); δ_F(280 MHz; acetic acid-d_4; TFA) −143.5 (dd, $^2J_{F,P}$ 86.4, $^2J_{F,H}$ 59.1, CHFP); *m*/*z* (+ve FAB) 335 (M^+ − 18); *m*/*z* (−ve FAB) 333 (M^- − NH_2).

Ethyl ammonium [2-(guanin-9-yl)ethoxy]fluoromethylphosphonate 30f. The title compound **30f** was prepared from 9-{2-[(diethoxyphosphoryl)fluoromethoxy]ethyl}guanine **25f** according to procedure C with a reaction time of 2 days. The crude product was purified by recrystallization from CH_2Cl_2–MeOH to afford compound **30f** (52% yield) as a white solid, mp 190 °C (decomp.); λ_{max}(MeOH)/nm 275 (ε/dm^3 mol^{-1} cm^{-1} 4,990) and 253.5 (7,990); ν_{max}(KBr)/cm^{-1} 3381, 3138, 2998, 2360, 1684, 1586, 1476, 1366, 1220, 1046, 953; δ_H(300 MHz; CD_3OD) 7.85 (1H, s, 8-H), 5.33 (1H, dd, $^2J_{F,H}$ 58.4, $^2J_{P,H}$ 11.0, CHFP), 4.33–4.30 (2H, m, CH_2N), 4.08–4.03 (2H, m, CH_2O), 3.93–3.86 (2H, m, CH_2OP), 1.19–1.14 (3H, t, J 7.1, CH_3); δ_C(75 MHz; CD_3OD) 159.54, 155.46, 153.28, 140.67, 121.0, 115.18 (dd, $^1J_{C,P}$ 231.3, $^1J_{C,F}$ 199.2, CHFP), 73.87 (d, $^2J_{C,P}$ 13.7, CH_2OP), 66.48 (d, $^3J_{C,P}$ 5.7, CH_2O), 48.11 (CH_2N), 20.75 (d, $^3J_{C,P}$ 5.7, CH_3); δ_F(280 MHz; CD_3OD; TFA) −143.62 (dd, $^2J_{F,P}$ 84.2, $^2J_{F,H}$ 58.0, CHFP).

Ethyl ammonium [2-(cytosin-1-yl)ethoxy]fluoromethylphosphonate 30g. The title compound **30g** was prepared from 1-{2-[(diethoxyphosphoryl)fluoromethoxy]ethyl}cytosine **25g** according to procedure C with a reaction time of 1 day. The crude product was triturated with CH_2Cl_2 twice to afford compound **30g** (77% yield) as an off-white solid, mp 120 °C (decomp.) (Found: C, 34.68; H, 5.81; N, 14.89. Calc. for $C_9H_{18}FN_4O_5P$: C, 34.62; H, 5.81; N, 17.94%); λ_{max}(MeOH)/nm 277.5 (ε/dm^3 mol^{-1} cm^{-1} 10,700) and 249.5 (5,100); ν_{max}(KBr)/cm^{-1} 3352, 3187, 3086, 2984, 1719, 1655, 1495, 1366, 1283, 1223, 1160, 1049, 955, 785; δ_H(300 MHz; CD_3OD) 7.67 (1H, d, J 7.2, 5-H), 5.79 (1H, d, J 7.2, 6-H), 5.25 (1H, dd, $^2J_{F,H}$ 58.4, $^2J_{P,H}$ 10.4, CHFP), 3.89 (4H, m, CH_2O and CH_2N), 3.80 (2H, apparent quartet, J 7.2, CH_2OP), 1.10 (3H, t, J 7.2, CH_3); δ_C(75 MHz; CD_3OD) 168.19 (C-4), 153.5 (C-2), 148.03 (C-6), 110.60 (dd, $^1J_{C,P}$ 229.6, $^1J_{C,F}$ 191.8, CHF), 92.96 (C-5), 67.91 (d, $^2J_{C,P}$ 12.5, CH_2OP), 60.24 (d, $^3J_{C,P}$ 5.7, CH_2O), 48.53 (CH_2N), 16.86 (d, $^3J_{C,P}$ 5.7, CH_3); δ_F(280 MHz; CD_3OD; TFA) −142.2 (dd, $^2J_{F,P}$ 82.2, $^2J_{H,F}$ 58.8, CHFP); *m*/*z* (+ve FAB) 294 (M^+ − NH_2); *m*/*z* (−ve FAB) 296 (M^- − NH_4^+).

Ethyl ammonium [2-(adenin-9-yl)ethoxy]methylphosphonate 32a. The title compound **32a** was prepared from 9-[2-(diethoxyphosphorylmethoxy)ethyl]adenine[19] according to procedure C with a reaction time of 9 days. The crude product was purified by trituration with CH_2Cl_2 to afford **32a** (24% yield) as a white solid, mp 220 °C (decomp.); λ_{max}(MeOH)/nm 260.0 (ε/dm^3 mol^{-1} cm^{-1} 13,980); ν_{max}(KBr)/cm^{-1} 3285, 3152, 2978, 2798, 2214, 1935, 1682, 1609, 1481, 1422, 1312, 1253, 1119, 1065, 935.5, 799, 714, 648; δ_H(300 MHz; DMSO-d_6) 8.20 (1H, s, 2-H), 8.12 (1H, s, 8-H), 7.16 (2H, s, NH_2), 4.29 (2H, t, J 5.0, CH_2N), 3.82 (2H, t, J 4.8, CH_2O), 3.66 (2H, app. quintet, J 7.1, CH_2OP), 3.42 (2H, d, $^2J_{P,H}$ 8.1, CH_2P), 1.02 (3H, t, J 7.2, CH_3).

Ethyl ammonium [2-(2,6-diaminopurin-9-yl)ethoxy]methylphosphonate 32d. The title compound **32d** was prepared from 2-amino-6-chloro-9-[2-(diethoxyphosphorylmethoxy)ethyl]-purine[21] according to procedure C with a reaction time of 10 days. The crude product was triturated with CH_2Cl_2 twice to afford **32d** (25% yield) as an off-white solid, mp 190 °C (decomp.); λ_{max}(MeOH)/nm 281.0 (ε/dm^3 mol^{-1} cm^{-1} 3,630) and 254 (3,180); δ_H(300 MHz; DMSO-d_6) 7.80 (1H, s, H-8), 7.01 (2H, br s, NH_2), 6.14 (2H, br s, NH_2), 4.14 (2H, t, J 4.8, CH_2N), 3.76 (4H, m, CH_2P and CH_2O), 1.08 (3H, t, J 6.9, CH_3).

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