EFFICIENT SYNTHESIS AND BIOLOGICAL PROPERTIES OF THE 2′-TRIFLUOROMETHYL ANALOGUES OF ACYCLIC NUCLEOSIDES AND ACYCLIC NUCLEOSIDE PHOSPHONATES

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Dedicated to the 75th anniversary of Professor Antonín Holý birthday and 25th anniversary of the discovery of antiviral nucleoside phosphonates.

Efficient and optimized procedure for the preparation of several acyclic nucleosides and acyclic nucleoside phosphonates substituted at the C-2′ position of the aliphatic part by the trifluoromethyl group is described. Trifluoromethyloxirane was found to be an excellent reagent for the introduction of the 1,1,1-trifluoropropan-2-ol moiety. Surprisingly, the next reaction of these 1,1,1-trifluoropropan-2-ols with the reagent for the introduction of the methylphosphonic residue afforded the desired phosphonates in very high yields and finally a novel simple and scalable procedure for the isolation of free phosphonic acids, after the reaction of dialkyl phosphonates with bromotrimethylsilane, was developed. Prepared compounds were evaluated for their biological properties, but none of the prepared phosphonic acids or acyclic nucleosides exhibits any antiviral, antiproliferative or anti-toxin activities.
Keywords: Nucleosides; Nucleotides; Phosphorus; Fluorine; Biological activity; Antibiotics.

The nucleoside analogues rank among the most used therapeutics in the antiviral therapy[1]. In the last two decades, research focused also on the acyclic analogues of nucleotides, which led to the discovery of the new antiviral class of drugs – acyclic nucleoside phosphonates (ANPs) with improved features such as higher barrier to resistance, higher chemical and enzymatic stability and better oral bio-availability[2]. For further increase of the catabolic stability, the fluorine atom is often introduced into the biologically active molecules. The high stability of the C–F bond and small van der Waals radius makes the fluorine atom a good and enzymatically stable mi-

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metic of the C–H bond. Fluorinated compounds based on the naturally occurring molecules are known to exert biological activity[3]. The fluorinated derivatives of nucleosides were studied as well and interesting biological activity was demonstrated in many cases, e.g. antineoplastic effect of 5-fluorouracil[4] or 2′-fluoro nucleosides[5].

In terms of trifluoromethylated nucleoside analogues, there are several ways how to introduce the CF_3- functional group into the molecule. The most profound reagent in that matter is (trifluoromethyl)trimethylsilane (Ruppert's reagent) in combination with Lewis acid[6]. The introduction of CF_3- group into various positions of the purine moiety of the nucleoside analogues was achieved using this methodology and led to several biologically active compounds (e.g. 6-trifuoromethylpurine riboside showed moderate cytotoxic activity)[7]. The derivate of 6-trifluoromethylpurine was also used for the intercalation into DNA and as the consequence for the inhibition of RNA-editing adenosine deaminase[8]. Another important nucleoside analogue bearing the CF_3- group is trifluridine (5-trifluoromethyluridine, antiviral-ophthalmic drug). The fact, that molecules bearing the CF_3- group are indeed established among biologically active compounds is supported by the fact that Merck index features 142 compounds bearing CF_3- group. Among the commercially most successful drugs are celecoxib (anti-inflammatory drug – selective COX-2 inhibitor), efavirenz (anti-HIV drug, non-nucleoside reverse transcriptase inhibitor) and pleconaril (anti-*Plecornaviridae* drug – prevents the virus attachment to the cells and the uncoating of the viral RNA). There are few examples of purine nucleoside analogues bearing CF_3- group on the aliphatic part of the molecule. Various ribonucleosides bearing CF_3- group in the 2′-position of the ribose were synthesized[9] and evaluated for their antiviral and cytotoxic activity[10], but with no remarkable effect. Adenine and cytosine nucleosides bearing 3′-CF_3- substituted ribose were also examined and their dideoxy- and didehydro- analogues showed moderate anti-HBV activity[11]. The purine derivate bearing CF_3- on the aliphatic chain in the same three carbonyl distance was proven to treat *Trypanosoma brucei* infections[12]. The 4′-CF_3- derivative of deoxynepatocin was also synthetized[13], but the report lacks the analysis of biological activities.

In this paper we report the effective synthesis of the CF_3- analogues of tenofovir, the established anti-HIV drug and its precursors. Although some of the mentioned compounds were already synthesized, the formerly used approach offered the desired compounds in very low yields[14]. Our synthetic pathway, which is based on the use of the trifluoromethyloxirane, afforded

the CF_3- derivatives of the ANPs in good yields. This approach was already used for the preparation of the corresponding pyrimidine derivatives[15]. The goal of our work was to determinate the anti-HIV, anti-RSV and anti-HCV activities of these compounds, which were not yet known. Simultaneously, we evaluated the potential of these compounds to inhibit adenylate cyclase toxins from pathogenic bacteria such as *Bordetella pertussis* or *Bacillus anthracis*. This was inspired by the fact that adefovir was previously described to be an excellent inhibitor[16] of the *B. anthracis* toxin. According to our docking studies it was predicted that the CF_3- modification could further increase the inhibitory activity. The unique potential of the anti-toxin therapy to combat the Anthrax threat was discussed and reviewed elsewhere[17].

RESULTS AND DISCUSSION

The X-ray structure of the complex of adefovir with the adenylate cyclase toxin from *B. anthracis*[16] was used for the rational drug design. X-ray data were obtained from PDB database (PDB ID: 1PKO)[18]. Visualization and docking studies were performed with the ArgusLab software[19]. The hydrogen atom in the C-2′ position of adefovir was replaced by CF_3- group using the implemented function in the ArgusLab software. Without any further optimization of the structure new inhibitor bearing the CF_3- group nicely fits into the cavity in the active site of the adenylate cyclase toxin. It was found that both 2′-CF_3- enantiomers can bind into the active site of the toxin. Figure 1 displays the overlay of (*S*)-2′-CF_3- and (*R*)-2′-CF_3- derivatives

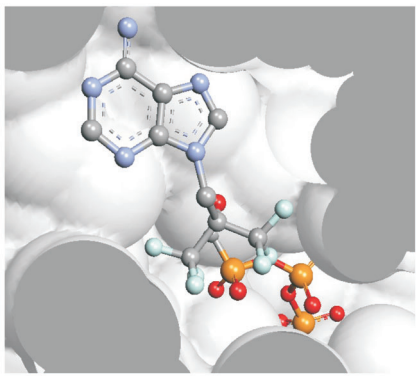
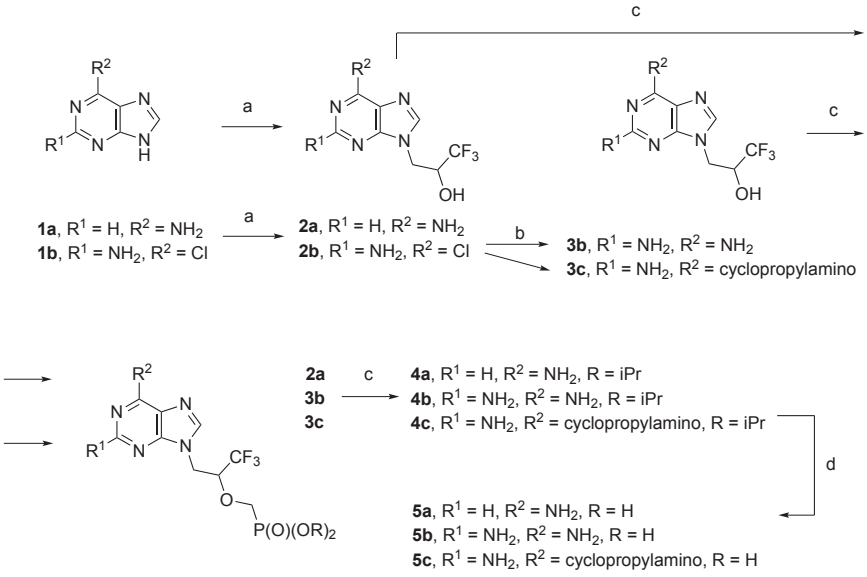


FIG. 1
The overlay of (*S*)-2′-CF_3- and (*R*)-2′-CF_3- derivatives of adefovir in the complex with adenylate cyclase toxin from *Bacillus anthracis*. Fluorine atoms are displayed in cyan

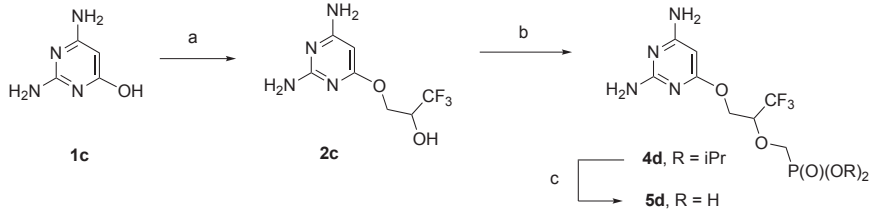
of adefovir in the complex with adenylate cyclase toxin from *B. anthracis*. Therefore, various purine and purine mimicking (((1,1,1-trifluoropropan-2-yl)oxy)methyl)phosphonic acids were prepared and their ability to inhibit the bacterial toxin was tested in cell-based *in vitro* model.

At first, the alkylation of adenine (**1a**), 2-amino-6-chloropurine (**1b**) and 2,4-diamino-6-hydroxypyrimidine (**1c**) with trifluoromethyloxirane afforded the corresponding 1,1,1-trifluoropropan-2-ol intermediates **2** (Schemes 1 and 2). Trifluoromethyloxirane was found to be an excellent re-



SCHEME 1

(a) Trifluoromethyloxirane, Cs_2CO_3/DMF; (b) NH_3/EtOH or cyclopropylamine/MeCN; (c) $BrCH_2P(O)(OiPr)_2$, NaH/DMF; (d) Me_3SiBr/MeCN



SCHEME 2

(a) Trifluoromethyloxirane, Cs_2CO_3/DMF; (b) $BrCH_2P(O)(OiPr)_2$, NaH/DMF; (c) Me_3SiBr/MeCN

agent for the introduction of the 1,1,1-trifluoropropan-2-ol residue. It is commercially available (as a racemic mixture or as a pure enantiomer) and it is reactive enough. The only disadvantage is the high volatility of this reagent. For this reason, the reaction has to be performed in close-vessel mode. The effect of the catalyzing base for the generation of the salt of the purine derivatives was also scrutinized and it was found that cesium carbonate is the best base for this reaction, which allows the reaction to be performed at 50 °C and leads to the full conversion after 20 h. Sodium or potassium carbonate could also be used but then the reaction requires higher reaction temperatures and prolonged times compared to cesium carbonate. The pure N^9 isomer (**2a** and **2b**) or O isomer (**2c**) was then isolated by column chromatography in good yields (65–85%). The amount of corresponding N^7 isomer for purines and various N isomers for pyrimidine was very low and it was not possible to isolate these pure isomers by one single chromatography. The treatment of the intermediate **2b** with high excess of ammonia or cyclopropylamine afforded compounds **3b** and **3c** in high yields (Scheme 1), where the pure products were isolated by filtration through the silica gel.

In order to obtain the phosphonate derivatives **4**, the compounds **2a**, **2b**, **3b** and **3c** were reacted with diisopropyl bromomethylphosphonate[20] (Schemes 1 and 2). Surprisingly, in contrast to previously reported cases[21], this reaction afforded the desired products in very high yields (81–89%) without any optimization and even more surprisingly no alkylation of unprotected amino groups was observed. This finding could be explained by the stabilization of the secondary alkoxide anion by the presence of the three fluorine atoms in the β-position.

Finally, hydrolysis of diisopropyl esters **4** under the standard conditions[21,22] using Me_3SiBr/MeCN afforded required free phosphonic acids **5** (Schemes 1 and 2). Isolation of free phosphonic acids is usually very laborious, where two ion exchange chromatographies together with HPLC or crystallization are used for the final purification. In our approach the free phosphonic acids **5** were isolated by novel procedure. After the evaporation of the reaction mixture in vacuo and codistillation with acetonitrile, the residue was sonicated in aqueous ethanol (50%) at room temperature for 10 min. During this time the free phosphonic acid quantitatively precipitated off. Formed trimethylsilanol was evaporated together with water and ethanol. The residue was sonicated again in aqueous ethanol (50%) at room temperature for 10 min. The solid precipitated free phosphonic acids were filtered off (whereas all impurities and salts stay in the solution).

Recrystallization from the mixture of water and ethanol afforded the desired products in high purity as white crystals.

All prepared compounds were screened for their antiviral and antiproliferative activities. Surprisingly, none of the targeted phosphonic acids **5** exhibits any anti-HIV, anti-RSV, anti-HCV and antiproliferative activities.

The anti-toxin activity towards bacterial adenylate cyclase was assayed in mouse J774A.1 macrophages. The exponentially growing cells were preincubated with the compounds for 5 h after which they were exposed to the recombinant adenylate cyclase from *B. pertussis* for 30 min. cAMP accumulation in the cells (a marker of adenylate cyclase activity) was monitored via indirect competitive ELISA method. Adefovir and its prodrug adefovir dipivoxil were employed as reference inhibitors. As expected, adenylate cyclase toxin induced a marked, approximately 10-fold increase in cellular cAMP. Adefovir largely prevented this effect and adefovir dipivoxil was even more efficient resulting in nearly complete inhibition of adenylate cyclase toxin. On the other hand, no activity was observed with the newly prepared CF_3- nucleoside phosphonate analogs (Table I).

With respect to the fact that adefovir was shown to inhibit adenylate cyclase in its diphosphorylated form[16] we assume that the lack of activity of the hereby presented compounds can possibly be explained by their insufficient intracellular phosphorylation. Therefore, diphosphates of here reported (((1,1,1-trifluoropropan-2-yl)oxy)methyl)phosphonic acids (triphosphate analogs) will be prepared and their ability to inhibit the

TABLE I
The effect of selected compounds on adenylate cyclase toxin (ACT)-induced cAMP accumulation in J774A.1 cells. Data are expressed as means of 3 replicates ± S.D.

	cAMP level, %	Relative inhibition efficiency vs ACT, %
Untreated control	100	
ACT	958 ± 137	
Adefovir	294 ± 31	77
Adefovir dipivoxil	143 ± 18	95
2a	1026 ± 187	n.i.[a]
5a	1088 ± 118	n.i.[a]

[a] No inhibition.

adenylate cyclase toxin will be tested in direct *in vitro* enzymatic assay. If these diphosphates prove to be active, enzymatically stable analogs and their prodrugs will be prepared and their activity will be tested again in the cell-based assay.

In conclusion, the efficient procedure for the preparation of acyclic nucleosides and acyclic nucleoside phosphonates bearing the trifluoromethyl group in the C-2′ position, using trifluoromethyloxirane as a key reagent, is reported. None of the prepared compounds exhibited any promising biological properties in the tested assays.

EXPERIMENTAL

Unless otherwise stated, the solvents were evaporated at 40 °C/2 kPa and the compounds were dried in vacuo over P_2O_5. The NMR spectra were recorded on a Bruker Avance 500 (^{1}H at 500 MHz, ^{13}C at 125.8 MHz). The chemical shifts (in ppm, δ-scale) were referenced to the signal of DMSO (δ 2.50 and 39.7) or to dioxane (δ 3.75 and 67.19). The coupling constants (*J*) are given in Hz. The assignment of the carbons was based on C,H-HSQC and C,H-HMBC experiments. The melting points were determined on a Kofler block and are uncorrected. The mass spectra were measured on a LCQ Fleet spectrometer (Thermo Fisher Scientific) using ESI ionization. The high-resolution mass spectra were measured on a LTQ Orbitrap XL spectrometer (Thermo Fisher Scientific) using ESI ionization. The microwave syntheses were carried out using the CEM Discover instrument.

Reaction with Trifluoromethyloxirane. General Procedure

A mixture of purine or pyrimidine heterocyclic base (10 mmol), 4-(dimethylamino)pyridine (12 mg, 0.1 mmol), trifluoromethyloxirane (1.12 g, 10 mmol) and cesium carbonate (1.63 g, 5 mmol) in dimethylformamide (100 ml) was heated in sealed flask at 50 °C for 20 h to achieve full conversion, according to the TLC (silica gel, chloroform/methanol 90:10). The mixture was concentrated in vacuo and the desired product was purified by the column chromatography (silica gel, chloroform/methanol gradient elution 100:0–90:10) and crystallization (ethyl acetate/hexane 1:1, hexane added after disolution in ethyl acetate).

3-(6-Amino-9H-purin-9-yl)-1,1,1-trifluoropropan-2-ol (**2a**). Yield 2.10 g (85%) of white crystals, m.p. 175–176 °C. ^{1}H NMR (DMSO-d_6): 8.16 s, 1 H (H-2); 8.13 s, 1 H (H-8); 7.30 bs, 2 H (NH_2); 6.79 d, 1 H, *J*(OH,2′) = 6.6 (OH); 4.50 m, 1 H (H-2′); 4.44 dd, 1 H, J_{gem} = 14.2, *J*(1′a,2′) = 3.4 (H-1′a); 4.30 dd, 1 H, J_{gem} = 14.2, *J*(1′b,2′) = 9.1 (H-1′b). ^{13}C NMR (DMSO-d_6): 156.25 (C-6); 152.82 (C-2); 149.80 (C-4); 141.79 (C-8); 125.11 q, *J*(3′,F) = 283.3 (C-3′); 118.81 (C-5); 67.12 q, *J*(2′,F) = 29.8 (C-2′); 43.30 (C-1′). ESI MS, *m/z* (%): 246.1 (100) [M$^-$]. HR ESI MS: for $C_8H_7F_3N_5O$ calculated 246.0603; found 246.0601. For $C_8H_8F_3N_5O$ (247.18) calculated: 38.87% C, 3.26% H, 28.33% N; found: 38.68% C, 3.34% H, 28.19% N.

3-(2-Amino-6-chloro-9H-purin-9-yl)-1,1,1-trifluoropropan-2-ol (**2b**). Yield 4.10 g, starting from 20 mmol (73%) of white crystals, m.p. 151–152 °C. ^{1}H NMR (DMSO-d_6): 8.12 s, 1 H (H-8); 7.00 bs, 2 H (NH_2); 6.79 bs, 1 H (OH); 4.50 m, 1 H (H-2′); 4.33 dd, 1 H, J_{gem} = 14.2, *J*(1′a,2′) = 3.4 (H-1′a); 4.21 dd, 1 H, J_{gem} = 14.2, *J*(1′b,2′) = 9.5 (H-1′b). ^{13}C NMR (DMSO-d_6): 160.11 (C-2); 154.48 (C-4); 149.68 (C-6); 144.04 (C-8); 125.05 q, *J*(3′,F) = 283.4 (C-3′); 123.41 (C-5); 66.85 q, *J*(2′,F) = 29.7 (C-2′); 43.21 (C-1′). ESI MS, *m/z* (%): 280.1 (100) [M$^-$]. HR ESI

MS: for $C_8H_6ClF_3N_5O$ calculated 280.0213; found 280.0214. For $C_8H_7ClF_3N_5O$ (281.62) calculated: 34.12% C, 2.51% H, 24.87% N; found: 34.37% C, 2.69% H, 24.62% N.

3-((2,6-Diaminopyrimidin-4-yl)oxy)-1,1,1-trifluoropropan-2-ol (**2c**). Yield 1.54 g (65%) of white crystals, m.p. 189–190 °C. ^{1}H NMR (DMSO-d_6): 6.60 bs, 1 H, J(OH,2′) = 6.7 (OH); 6.09 bs, 2 H and 5.98 bs, 2 H (NH_2); 5.06 s, 1 H (H-5); 4.27 m, 2 H (H-1′a, H-2′); 4.18 dd, 1 H, J_{gem} = 11.8, J(1′b,2′) = 7.4 (H-1′b). ^{13}C NMR (DMSO-d_6): 169.58 (C-4); 166.32 (C-2); 163.05 (C-6); 125.36 q, J(3′,F) = 283.4 (C-3′); 76.52 (C-5); 67.66 q, J(2′,F) = 29.2 (C-2′); 63.30 (C-1′). ESI MS, *m/z* (%): 237.1 (100) [M$^-$]. HR ESI MS: for $C_7H_8F_3N_4O_2$ calculated 237.0599; found 237.0597. For $C_7H_9F_3N_4O_2$ (238.17) calculated: 35.30% C, 3.81% H, 23.52% N; found: 35.53% C, 3.90% H, 23.47% N.

Reaction with Amines. General Procedure

A mixture of compound **2b** (1.971 g, 7 mmol) with 2 M ethanolic ammonia (30 ml, 60 mmol) or cyclopropylamine (3.420 g, 60 mmol) in 30 ml of ethanol was portioned into 10 ml microwave vials which were irradiated in microwave reactor at 120 °C for 10 min to achieve total conversion, according to the TLC (silica gel, chloroform/methanol 90:10). The reaction mixtures were evaporated together and the solid residue was partially dissolved in 40 ml of ethyl acetate and this mixture was filtered through the layer of silica gel (5 cm). The filtrate was evaporated in vacuo and the solid precipitate was recrystallized (ethyl acetate/hexane 1:1, hexane added after disolution in ethyl acetate).

3-(2,6-Diamino-9H-purin-9-yl)-1,1,1-trifluoropropan-2-ol (**3b**). Yield 1.63 mg (89%) of white crystals, m.p. 196–197 °C. ^{1}H NMR (DMSO-d_6): 7.70 s, 1 H (H-8); 6.80 d, 1 H, J(OH,2′) = 6.1 (OH); 6.75 s, 2 H (NH_2-6); 5.88 s, 2 H (NH_2-2); 4.45 m, 1 H (H-2′); 4.24 dd, 1 H, J_{gem} = 14.2, J(1′b,2′) = 3.4 (H-1′b); 4.10 dd, 1 H, J_{gem} = 14.2, J(1′a,2′) = 9.4 (H-1′a). ^{13}C NMR (DMSO-d_6): 160.54 (C-2); 156.41 (C-6); 151.99 (C-4); 138.43 (C-8); 125.19 q, J(3′,F) = 283.O (C-3′); 113.20 (C-5); 67.08 q, J(2′,F) = 29.5 (C-2′); 42.90 (C-1′). ESI MS, *m/z* (%): 261.2 (100) [M$^-$]. HR ESI MS: for $C_8H_8F_3N_6O$ calculated 261.0712; found 261.0710. For $C_8H_9F_3N_6O$ (262.19) calculated: 36.65% C, 3.46% H, 32.05% N; found: 36.47% C, 3.66% H, 31.83% N.

3-(2-Amino-6-(cyclopropylamino)-9H-purin-9-yl)-1,1,1-trifluoropropan-2-ol (**3c**). Yield 1.78 g (84%) of white crystals, m.p. 144–145 °C. ^{1}H NMR (DMSO-d_6): 7.69 s, 1 H (H-8); 7.35 s, 1 H (NH-6); 6.79 s, 1 H (OH); 5.93 s, 2 H (NH_2-2); 4.45 m, 1 H (H-2′); 4.25 dd, J_{gem} = 14.2, J(1′b,2′) = 3.4 (H-1′b); 4.11 dd, J_{gem} = 14.2, J(1′a,2′) = 9.3 (H-1′a); 3.01 bs, 1 H (CHcypr.); 0.66 m, 2 H and 0.57 m, 2 H (2 × CH_2cypr.). ^{13}C NMR (DMSO-d_6): 160.45 (C-2); 156.17 (C-6); 151.47 (C-4); 138.15 (C-8); 125.18 q, J(3′,F) = 283.0 (C-3′); 113.45 (C-5); 67.07 q, J(3′,F) = 29.2 (C-2′); 42.85 (C-1′); 23.91 (CHcypr.); 6.70 (CH_2cypr.). ESI MS, *m/z* (%): 301.2 (100) [M$^-$]. HR ESI MS: for $C_{11}H_{12}F_3N_6O$ calculated 301.1025; found 301.1028. For $C_{11}H_{13}F_3N_6O$ (302.26) calculated: 43.71% C, 4.34% H, 27.80% N; found: 43.95% C, 4.57% H, 27.62% N.

Reaction with Diisopropyl Bromomethylphosphonate. General Procedure

A mixture containing substituted 1,1,1-trifluoropropan-2-ol (5 mmol) and 60% sodium hydride dispersion (260 mg, 6.5 mmol) in dry DMF (150ml) was stirred under argon atmosphere at room temperature for 10 min. Then, diisopropyl bromomethylphosphonate (1.36 g, 5.25 mmol) was added and the mixture was stirred at room temperature overnight. The solvent was evaporated in vacuo and the brown residue was dissolved in chloroform and fil-

tered through Celite. The filtrate was concentrated in vacuo and purified on a silica gel column (chloroform/methanol gradient elution 100:0–90:10).

Diisopropyl (((3-(6-amino-9H-purin-9-yl)-1,1,1-trifluoropropan-2-yl)oxy)methyl)phosphonate (**4a**). Yield 1.89 g (89%) of colorless oil. ^{1}H NMR (DMSO-d_6): 8.16 s, 1 H (H-2); 8.11 s, 1 H (H-8); 7.30 s, 2 H (NH_2); 4.69 m, 1 H (H-2′); 4.58–4.46 m, 4 H (H-1′, CHiPr); 4.00 dd, 1 H, J_{gem} = 13.7, J(PCH$_2$b,P) = 9.2 (PCH$_2$b); 3.92 dd, J_{gem} = 13.7, J(PCH$_2$a,P) = 10.0 (PCH$_2$a); 1.21 d, 3 H, 1.19 d, 6 H and 1.12 d, 3 H, J(CH$_3$,CH) = 6.2 (CH$_3$). ^{13}C NMR (DMSO-d_6): 156.24 (C-6); 152.88 (C-2); 146.86 (C-4); 141.42 (C-8); 124.34 q, J(3′,F) = 283.9 (C-3′); 118.55 (C-5); 76.39 qd, J(2′,F) = 29.5, J(2′,P) = 14.0 (C-2′); 70.88 d and 70.83 d, 2 C, J(CH,P) = 6.3 (CHiPr); 66.05 d, J(CH,P) = 165.7 (PCH$_2$); 40.81 (C-1′); 23.95 d and 23.90 d, 2 C, J(CH$_3$,P) = 3.8, 23.77 d and 23.69 d, 2 C, J(CH$_3$,P) = 4.6 (CH$_3$). ESI MS, *m/z* (%): 424.1 (100) [M$^-$]. HR ESI MS: for $C_{15}H_{22}F_3N_5O_4P$ calculated 424.1362; found 424.1360. For $C_{15}H_{23}F_3N_5O_4P$ (425.34) calculated: 42.36% C, 5.45% H, 16.47% N; found: 42.49% C, 5.68% H, 16.30% N.

Diisopropyl (((3-(2,6-diamino-9H-purin-9-yl)-1,1,1-trifluoropropan-2-yl)oxy)methyl)phosphonate (**4b**). Yield 1.78 g (81%) of colorless oil. ^{1}H NMR (DMSO-d_6): 7.71 s, 1 H (H-8); 6.72 bs, 2 H (NH_2-6); 5.83 bs, 2 H (NH_2-2); 4.64 m, 1 H (H-2′); 4.49–4.57 m, 2 H (CHiPr); 4.34 dd, 1 H, J_{gem} = 14.8, J(1′a,2′) = 3.7 (H-1′a); 4.27 dd, J_{gem} = 14.8, J(1′b,2′) = 7.7 (H-1′b); 3.95 dd, J_{gem} = 13.5, J(H,C,P) = 9.5 (PCH$_2$a); 3.84 dd, 1 H, J_{gem} = 13.5, J(H,C,P) = 10.0 (PCH$_2$b); 1.22 d, 3 H, 1.20 d, 3 H, 1.19 d, 3 H and 1.15 d, 3 H, J(CH$_3$,CH) = 6.2 (CH$_3$). ^{13}C NMR (DMSO-d_6): 160.61 (C-2); 156.36 (C-6); 152.05 (C-4); 137.86 (C-8); 124.39 q, J(3′,F) = 284.3 (C-3′); 112.92 (C-5); 76.48 qd, J(2′,F) = 28.9, J(2′,P) = 14.5 (C-2′); 70.85 m (CHiPr); 66.18 d, J(C,P) = 165.5 (PCH$_2$); 40.51 (C-1′); 23.69–23.97 m (CH$_3$). ESI MS, *m/z* (%): 441.2 (100) [MH$^+$], 463.1 (20) [MNa$^+$]. HR ESI MS: for $C_{15}H_{25}F_3N_6O_4P$ calculated 441.1627; found 441.1624. For $C_{15}H_{24}F_3N_6O_4P$ (440.36) calculated: 40.91% C, 5.49% H, 19.08% N; found: 40.78% C, 5.41% H, 18.95% N.

Diisopropyl (((3-(2-amino-6-(cyclopropylamino)-9H-purin-9-yl)-1,1,1-trifluoropropan-2-yl)oxy)-methyl)phosphonate (**4c**). Yield 2.10 g (87%) of colorless oil. ^{1}H NMR (DMSO-d_6): 7.70 s, 1 H (H-8); 7.35 bs, 1 H (NH); 5.89 bs, 2 H (NH_2); 4.64 m, 1 H (H-2′); 4.48–4.57 m, 2 H (CHiPr); 4.35 dd, 1 H, J_{gem} = 14.8, J(1′a,2′) = 3.8 (H-1′a); 4.28 dd, J_{gem} = 14.8, J(1′b,2′) = 7.8 (H-1′b); 3.95 dd, J_{gem} = 13.6, J(H,C,P) = 9.4 (PCH$_2$a); 3.83 dd, 1 H, J_{gem} = 13.6, J(H,C,P) = 10.0 (PCH$_2$b); 3.01 bs, 1 H (CHcypr.); 1.22 d, 3 H, 1.19 d, 3 H, 1.19 d, 3 H and 1.14 d, 3 H, J(CH$_3$,CH) = 6.2 (CH$_3$); 0.65 m, 2 H and 0.57 m, 2 H (CH$_2$cypr.). ^{13}C NMR (DMSO-d_6): 160.52 (C-2); 156.12 (C-6); 151.6 (C-4); 137.61 (C-8); 124.39 q, J(3′,F) = 284.1 (C-3′); 113.18 (C-5); 76.48 qd, J(2′,F) = 29.1, J(2′,P) = 14.1 (C-2′); 70.84 m (CHiPr); 66.20 d, J(C,P) = 165.6 (PCH$_2$); 40.49 (C-1′); 23.68–23.96 m (CH$_3$); 6.62 (CH$_2$cypr.). ESI MS, *m/z* (%): 481.2 (100) [MH$^+$]. HR ESI MS: for $C_{18}H_{29}F_3N_6O_4P$ calculated 481.1940; found 481.1940. For $C_{18}H_{28}F_3N_6O_4P$ (480.42) calculated: 45.00% C, 5.87% H, 17.49% N; found: 45.16% C, 5.64% H, 17.24% N.

Diisopropyl (((3-((2,6-diaminopyrimidin-4-yl)oxy)-1,1,1-trifluoropropan-2-yl)oxy)methyl)phosphonate (**4d**). Yield 1.75 g (84%) of colorless oil. ^{1}H NMR (DMSO-d_6): 6.11 s, 2 H (NH_2-2); 5.98 s, 2 H (NH_2-6); 5.05 s, 1 H (H-5); 4.60 d sept and 4.59 d sept, 1 H and 1 H, J(CH,P) = 7.7, J(CH,CH$_3$) = 6.2 (CHiPr); 4.51 m, 1 H (H-2′); 4.46 dd, 1 H, J_{gem} = 11.9, J(1′b,2′) = 6.0 (H-1′b); 4.29 dd, 1 H, J_{gem} = 11.9, J(1′a,2′) = 4.2 (H-1′a); 4.10 dd, 1 H, J_{gem} = 13.7, J(PCH$_2$b,P) = 9.4 (PCH$_2$b); 4.04 dd, 1 H, J_{gem} = 13.7, J(PCH$_2$a,P) = 9.2 (PCH$_2$a); 1.24 d, 3 H, 1.24 d, 3 H, 1.22, d, 3 H and 1.22 d, 3 H, J(CH$_3$,CH) = 6.2 (CH$_3$). ^{13}C NMR (DMSO-d_6): 169.28 (C-4); 166.37 (C-2); 163.04 (C-6); 124.48 q, J(3′,F) = 284.0 (C-3′); 76.64 qd, J(2′,F) = 29.7, J(2′,P) = 14.0 (C-2′); 76.42 (C-5); 70.90 d and 70.87 d, J(CH,P) = 6.2 (CHiPr); 65.84 d,

$J(CH_2,P)$ = 163.9 (PCH_2); 61.15 (C-1′); 24.03 d and 24.02 d, $J(CH_3,P)$ = 3.7, $J(CH_3,P)$ = 3.8 (CH_3); 23.80 d, $J(CH_3,P)$ = 4.5 (CH_3). ESI MS, *m/z* (%): 415.1 (100) [M^-]. HR ESI MS: for $C_{14}H_{23}F_3N_4O_5P$ calculated 415.1358; found 415.1359. For $C_{14}H_{24}F_3N_4O_5P$ (416.33) calculated: 40.39% C, 5.81% H, 13.46% N; found: 40.24% C, 5.98% H, 13.27% N.

Reaction with Bromotrimethylsilane. General Procedure

A mixture of diisopropyl ester (2 mmol), acetonitrile (40 ml), and $BrSiMe_3$ (4 ml) was stirred overnight at room temperature. After evaporation in vacuo and codistillation with acetonitrile, the residue was sonicated in aqueous ethanol (50 ml, 50%) for 10 min. This mixture was well evaporated in vacuo (45 °C, 2 mbar) and the residue was sonicated again in aqueous ethanol (50 ml, 50%) for 10 min. The formed solid precipitate was filtered of and recrystallized from water–ethanol mixture 1:1 (ethanol added after disolution in water) to afford the desired product as white crystals.

(((3-(6-Amino-9H-purin-9-yl)-1,1,1-trifluoropropan-2-yl)oxy)methyl)phosphonic acid (**5a**). Yield 0.48 g (71%) of white crystals, m.p. > 250 °C. ^{1}H NMR (D_2O): 8.28 s, 1 H (H-8); 8.12 s, 1 H (H-2); 4.63 dd, 1 H, J_{gem} = 15.2, J(1′a,2′) = 3.9 (H-1′a); 4.52 dd, 1 H, J_{gem} = 15.2, J(1′b,2′) = 6.6 (H-1′b); 4.40 m, 1 H (H-2′); 3.83 dd, 1 H, J_{gem} = 12.4, J(H,C,P) = 9.5 (PCH_2a); 3.56 dd, 1 H, J_{gem} = 12.5, J(H,C,P) = 9.4 (PCH_2b). ^{13}C NMR (D_2O): 155.86 (C-6); 152.83 (C-2); 149.29 (C-4); 143.93 (C-8); 124.64 q, J(3′,F) = 284.0 (C-3′); 118.47 (C-5); 77.27 qd, J(2′,F) = 30.0, J(2′,P) = 12.8 (C-2′); 70.59 d, J(C,P) = 152.7 (PCH_2); 42.46 (C-1′). ESI MS, *m/z* (%): 340.1 (100) [M^-]. HR ESI MS: for $C_9H_{10}F_3N_5O_4P$ calculated 340.0422; found 340.0423. For $C_9H_{11}F_3N_5O_4P{\cdot}0.8H_2O$ (355.60) calculated: 30.40% C, 3.57% H, 16.03% F, 19.69% N; found: 30.68% C, 3.65% H, 15.75% F, 19.50% N.

(((3-(2,6-Diamino-9H-purin-9-yl)-1,1,1-trifluoropropan-2-yl)oxy)methyl)phosphonic acid (**5b**). Yield 0.59 mg (83%) of white crystals, m.p. > 250 °C. ^{1}H NMR (D_2O): 7.99 s, 1 H (H-8); 4.48 m, 1 H (H-1′a); 4.34–4.40 m, 2 H (H-1′b, H-2′); 3.78 dd, 1 H, J_{gem} = 12.2, J(H,C,P) = 9.6, 3.56 dd, 1 H, J_{gem} = 12.2, J(H,C,P) = 9.3 (PCH_2). ^{13}C NMR (D_2O): 160.36 (C-2); 156.42 (C-6); 151.58 (C-4); 141.61 (C-8); 124.76 q, J(C,F) = 283.9 (C-3′); 113.03 (C-5); 77.26 qd, J(2′,F) = 29.5, J(2′,P) = 12.4 (C-2′); 71.13 d, J(C,P) = 151.0 (PCH_2); 42.16 (C-1′). ESI MS, *m/z* (%): 357.2 (100) [MH^+], 379.2 (70) [MNa^+]. HR ESI MS: for $C_9H_{13}F_3N_6O_4P$ calculated 357.0688; found 357.0687. For $C_9H_{12}F_3N_6O_4P{\cdot}0.9H_2O$ (372.41) calculated: 29.03% C, 3.73% H, 15.30% F, 22.57% N; found: 29.27% C, 3.85% H, 15.11% F, 22.40% N.

(((3-(2-Amino-6-(cyclopropylamino)-9H-purin-9-yl)-1,1,1-trifluoropropan-2-yl)oxy)methyl)-phosphonic acid (**5c**). Yield 0.58 mg (73%) of white crystals, m.p. > 250 °C. ^{1}H NMR (D_2O): 7.98 s, 1 H (H-8); 4.48 m, 1 H (H-1′a); 4.30–4.41 m, 2 H (H-1′b, H-2′); 3.72 dd, 1 H, J_{gem} = 11.8, J(H,C,P) = 9.8 (PCH_2a); 3.49 dd, 1 H, J_{gem} = 12.0, J(H,C,P) = 9.5 (PCH_2b); 2.82 bs, 1 H (CHcypr.); 0.86 m, 2 H and 0.65 m, 2 H (CH_2cypr.). ^{13}C NMR (D_2O): 160.83 (C-2); 156.92 (C-6); 149.82 (C-4); 141.16 (C-8); 124.84 q, J(C,F) = 284.5 (C-3′); 113.44 (C-5); 77.24 qd, J(2′,F) = 29.1, J(2′,P) = 13.0 (C-2′); 71.71 d, J(C,P) = 149.0 (PCH_2); 42.15 (C-1′); 24.24 (CHcypr.); 7.31 (CH_2cypr.). ESI MS, *m/z* (%): 397.2 (100) [MH^+], 419.2 (82) [MNa^+]. HR ESI MS: for $C_{12}H_{17}F_3N_6O_4P$ calculated 397.1001; found 397.1003. For $C_{12}H_{16}F_3N_6O_4P{\cdot}0.5H_2O$ (405.27) calculated: 36.37% C, 4.07% H, 14.38% F, 21.21% N; found: 36.58% C, 4.24% H, 14.26% F, 21.11% N.

(((3-((2,6-Diaminopyrimidin-4-yl)oxy)-1,1,1-trifluoropropan-2-yl)oxy)methyl)phosphonic acid (**5d**). Yield 0.64 mg (81%) of white crystals, m.p. > 250 °C. ^{1}H NMR (D_2O + NaOD): 5.45 s, 1 H (H-5); 4.48 m, 1 H (H-1′b); 4.39 m, 1 H (H-1′a); 4.36 m, 1 H (H-2′); 3.81 dd, 1 H, J_{gem} = 12.0,

$J(CH_2,P)$ = 9.3 (PCH_2b); 3.75 dd, 1 H, J_{gem} = 12.0, $J(CH_2,P)$ = 9.5 (PCH_2a). ^{13}C NMR (D_2O + NaOD): 170.91 (C-4); 167.30 (C-2); 163.29 (C-6); 124.95 q, J(3′,F) = 284.0 (C-3′); 77.42 (C-5); 77.20 qd, J(2′,F) = 29.4, J(2′,P) = 11.7 (C-2′); 71.0 d, $J(CH_2,P)$ = 148.6 (PCH_2); 64.09 (C-1′). ESI MS, *m/z* (%): 333.1 (100) [MH^+], 355.1 (56) [MNa^+]. HR ESI MS: for $C_8H_{13}F_3N_4O_5P$ calculated 333.0576; found 333.0573. For $C_8H_{12}F_3N_4O_5P$ (332.17) calculated: 28.93% C, 3.64% H, 16.87% N; found: 28.75% C, 3.88% H, 16.60% N.

Cell Culture and cAMP Measurement

J774A.1 mouse macrophages (ATCC) were cultured in DMEM medium supplemented with 10% serum, 1% penicillin/streptomycin and 2 mM GlutaMax™ at 37 °C and 5% CO_2. Cells were seeded in a 96-well plate at a concentration of 50 000 cells per well and left to attach overnight. Prior to the experiment, cells were washed with HBSS and 10 μM compounds dissolved at the same vehicle were added to the wells. Following a 5 h preincubation, cells were exposed to 0.4 μg/ml of adenylate cyclase toxin from *B. pertussis* (Enzo Life Sciences) for 30 min. Reaction was completed by the addition of lysis buffer (50 μl/well) and the cellular content was extracted by shaking the plate at 250 rpm for 10 min. The plate was then centrifuged to remove cell debris and the supernatant was assayed for cAMP content using the CatchPoint™ cAMP immunoassay kit (Molecular Devices) according to the manufacturer's instructions. Data were analyzed using GraphPad Prism (GraphPad software).

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