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Nucleobase Modified Adefovir (PMEA) Analogues as Potent and Selective Inhibitors of Adenylate Cyclases from *Bordetella pertussis* and *Bacillus anthracis*

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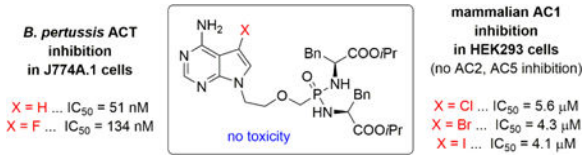
Abstract

A series of 13 acyclic nucleoside phosphonates (ANPs) as bisamidate prodrugs was prepared. Five compounds were found to be non-cytotoxic and selective inhibitors of *Bordetella pertussis* adenylate cyclase toxin (ACT) in J774A.1 macrophage cell-based assays. The 8-aza-7-deazapurine derivative of adefovir (PMEA) was the most potent ACT inhibitor in the series (IC_{50} = 16 nM) with substantial selectivity over mammalian adenylate cyclases (mACs). AC inhibitory properties of the most potent analogues were confirmed by direct evaluation of the corresponding phosphonodiphosphates in cell-free assays and were found to be potent inhibitors of both ACT and edema factor (EF) from *Bacillus anthracis* (IC_{50} values ranging from 0.5 to 21 nM). Moreover, 7-halo-7-deazapurine analogues of PMEA were discovered to be potent and selective mammalian AC1 inhibitors (no inhibition of AC2 and AC5) with IC_{50} values ranging from 4.1–5.6 μM in HEK293 cell-based assays.

Graphical Abstract



A novel series of acyclic nucleoside phosphonates derived from adefovir (PMEA) with modified purine nucleobases was prepared where some compounds (e.g. 8-aza-7-deazapurine analogue) are potent and selective inhibitors of bacterial adenylate cyclases (adenylate cyclase toxin from *B. pertussis* and edema factor from *B. anthracis*) and some analogues (7-halo-7-deazapurine analogues) are selective inhibitors of mammalian AC1 over AC2 and AC5.

Keywords

adenylate cyclase; inhibitor; *Bordetella pertussis*; *Bacillus anthracis*; adefovir

Introduction

Bordetella pertussis, a strictly human pathogen, causes acute or chronic respiratory infections in the tracheobronchial tree, called whooping cough.[1] *B. pertussis* is easily transmitted from infected to susceptible people through droplets. It is estimated that there were 24,1 million pertussis cases and 160 700 deaths from pertussis in children younger than 5 years in 2014,[2] whereas the most endangered group are children aged less than 12 months.[3,4]

B. pertussis is usually treated with antibiotics.[5,6] Despite the fact that *B. pertussis* resistance to antibiotics has been reported sporadically,[7] the level of bacterial resistance keeps rising.[8] *Bordetella pertussis* produces several virulent factors,[9–11] which help it to evade the host organism during the infection.[10] The calmodulin-dependent adenylate cyclase toxin (ACT) is considered to be an essential virulence factor,[11] since it has been demonstrated that the virulence of *B. pertussis* ACT-deficient mutant was significantly reduced.[12–14] Thus, the development of potential antitoxin therapies represents a viable approach for whooping cough treatment.

ACT binds to the surface receptors (integrin CD11b/CD18) on myeloid phagocytic cells and transports its AC domain into the cytosol in a two-step process.[15] The pore-forming activity[16,17] leads to an increased intracellular calcium concentration and calmodulin-mediated activation of ACT which is responsible for the conversion of intracellular ATP into the second messenger cAMP.[10,13,18–20] This massive and non-physiological increases of intracellular cAMP levels completely disrupt cellular signalling pathways. ACT has been shown to impair a number of key metabolic functions of human immune effector cells,[21–26] facilitating an effective invasion and colonization of the toxin-producing bacteria in the host organism. Moreover, the suppression of the immune response can make the host organism more vulnerable to additional infections.

Acyclic nucleoside phosphonates (ANPs)[27] possess a broad spectrum of biological activities. The most pronounced is their antiviral effect,[28] however ANPs exhibit also cytostatic,[29,30,] antiparasitic,[31–35] antibacterial,[36] and immuno-modulatory[37–40] properties. It was also found that the approved anti-HBV drug adefovir dipivoxil (bis(POM)PMEA, **I**, Fig. 1), upon its intracellular conversion into the active metabolite adefovir diphosphate (PMEApp, **II**, Fig. 1), inhibited *Bordetella pertussis* ACT.[41] Bis(POM)PMEA (**I**) was also active against adenylate cyclase-based edema factor (EF) from *Bacillus anthracis* in CHO and BMMΦ cells.[42] Recently, various bisamidate prodrugs of PMEA, e.g. bis(L-phenylalanine isopropyl ester) PMEA (**III**, Fig. 1), with enhanced plasma stability profile and low toxicity were evaluated as potentially more suitable drug candidates for antitoxin therapy despite their somewhat lower efficacy to inhibit ACT compared with bis(POM)PMEA (**I**).[43] Based on these results,[43] and taking into account various aspects of the prodrug strategy (stability, cytotoxicity, cell membrane permeability, ease of synthesis and handling), L-phenylalanine isopropyl ester moiety (as in compound **III**, Fig. 1) has been selected as a representative type of bisamidate prodrug for the evaluation of any other novel nucleotide analogues.

The present work is a part of a broad structure-activity relationship (SAR) study mapping the influence of each structural part of the acyclic nucleotide analogues, i.e. an aliphatic moiety and a nucleobase, on the inhibition of bacterial adenylate cyclases, namely ACT and EF (Fig. 2). In recent years, the molecule of adefovir (PMEA) was structurally modified and evaluated in this regard, but no significant improvement of the ACT inhibition was observed with ANPs bearing variously modified aliphatic chains (chain length, oxygen position and/or branching, unpublished results). Thus, the presence of the unmodified 2-(phosphonomethoxy)ethyl (PME) moiety turned out to be crucial for the preservation of the ACT inhibition.

Next, we tried to modify the adenine moiety by means of various substitutions. A modification of the 6-amino group by its alkylation led to a decrease of activity, while a substitution of the C-8 position (e.g. amino, oxo, thio, methylthio derivatives, Fig. 2) resulted in a complete loss of the antitoxin activity (unpublished results). On the other hand, PMEA bisamidates substituted with various functional groups in the C-2 position of the purine moiety showed activity against ACT in a cell-based assay, however, they were weaker inhibitors than the corresponding base-unsubstituted PMEA derivative **III** (Fig. 1).[44]

Further structural changes of the heterocyclic scaffold, namely aza- and deaza-modifications, supported by the docking into the crystal structure of the ACT – PMEApp complex,[41,45] represent the next logical step in the design of future ACT inhibitors. Herein, we prepared and evaluated a series of ANPs derived from adefovir (PMEA) derivative **III** with modified nucleobases that are more or less able to mimic the adenine scaffold (Fig. 2). The common feature of such nucleobases is the bicyclic heterocycle (usually 6 + 5 atoms) and the presence of an unmodified “6-aminopurine” group for potential hydrogen bond interactions with the target enzymes (ACT and EF).

Results and Discussion

Synthesis.

First, we aimed to prepare all four possible mono-deazaadenine analogues of PMEA (adefovir), namely 1-deaza-PMEA, 3-deaza-PMEA, 7-deaza-PMEA, and 9-deaza-PMEA, in the form of their isopropyl ester bis(L-phenylalanine) bisamidate prodrugs. Phosphonates **1a** and **1b** (Scheme 1), prepared according to previously reported procedures,[46, 47] served as the starting material for the synthesis of 1-deaza- and 3-deazaadenine analogues, compounds **2a** and **2b**, respectively. Silyl esters, preformed from isopropyl esters **1a** and **1b** (using TMSBr in pyridine at room temperature) were treated with L-phenylalanine isopropyl ester under standard reaction conditions (Aldrithiol-2, pyridine, Et_3N, 70 °C), developed previously in our laboratory,[48] to give desired bisamidates **2a** and **2b** in 60% and 16% yields, respectively.

The synthesis of 7-deazaadenine analogue **6a** and its 7-halogenated versions **6b**–**6e** (Scheme 2) started from commercially available 7-deazapurine (7*H*-pyrrolo[2,3-d]pyrimidine) derivatives **3a**–**3e**. Although phosphonate diester **5a** (Scheme 2) has been reported by Holý et al.,[49] here we developed a more efficient method of its synthesis. Alkylation of 6-chloro-7-deazapurine (**3a**) with diisopropyl [(2-chloroethoxy)-methyl]phosphonate[49] in the presence of Cs_2CO_3 in DMSO at 80 °C, followed by ammonolysis with ethanolic ammonia

at 100 °C afforded compound **5a** in a 39% overall yield (compared to an overall 23% yield of **5a**,[49] when starting from 2-methylsulfanyl-7-deazaadenine). Compounds **3b**–**3e** were analogously converted into 7-halo-7-deazaadenine derivatives **5b**–**5e** (Scheme 2) in two steps and with good yields. Corresponding bisamidates **6a**–**6e** (Scheme 2) were then prepared from phosphonate diesters **5a**–**5e** by the standard procedure.[48]

To the best of our knowledge, synthesis of 9-deaza-PMEA (**14**, Scheme 3) has not been reported so far. This might have been due to its demanding synthesis and/or inaccessibility of suitable C-C cross-coupling methods at times, when the other aza/deaza analogues were prepared.[46,47,49] In order to synthesize 9-deaza-PMEA and its derivatives for biological evaluation, commercially available 6-chloro-9-deazapurine (4-chloro-5*H*-pyrrolo[3,2-d]pyrimidine, **7**, Scheme 3) was first subjected to iodination with *N*-iodosuccinimide in THF to give 6-chloro-9-iodo-9-deazapurine (**8**) in a 85% yield.[50] After the introduction of SEM protecting group using (2-chloromethoxyethyl)trimethylsilane (SEMCl) and NaH in DMF, SEM derivative **9** was subjected to the Stille cross-coupling reaction with vinyltributyltin under catalysis of $Pd(t\text{-}Bu_3P)_2$ in THF to afford 9-vinyl-9-deazapurine derivative **10** (Scheme 3) in a 69% yield. These are the optimized reaction conditions as various protecting groups at the N-7 position and several other conditions were evaluated as well. For example, the use of other palladium catalysts during the Stille cross-coupling reaction of compound **9** with vinyltributyltin led to complex reaction mixtures containing various ratios of the desired product **10**, the homocoupling product, starting compound **9**, as well as the dehalogenated starting compound. Utilization of the bromo analogue of compound **9**, i.e. 9-bromo-6-chloro-9-deazapurine, as starting compound also significantly reduced yields of the desired intermediate **10**.

Desired 9-(2-hydroxyethyl)-9-deazapurine derivative **11** (Scheme 3) was obtained in a 79% yield by hydroboration[51] of compound **10**, followed by *in situ* oxidation with aqueous sodium perborate. The subsequent alkylation of compound **11** to give phosphonate **12** was accomplished by modified alkylation conditions[52] using $CF_3SO_2OCH_2P(O)(OiPr)_2$ in the presence of *n*-BuLi at low temperature. Obtained phosphonate **12** was treated with ethanolic ammonia to give protected 9-deazaadenine intermediate **13** in a good yield. The simultaneous removal of SEM and isopropyl ester groups from **13** using microwave-assisted hydrolysis with aqueous HCl[53] yielded free phosphonic acid **14** in a 56% yield, while treatment of **13** by the standard procedure[48] (with an addition of TMSI to remove SEM protecting group) afforded desired bisamidate prodrug **15** (Scheme 3) in a 44% yield.

Secondly, we focused on the synthesis of the 8-aza-7-deazapurine and 8-azapurine analogues, compounds **18a** and **18b** (Scheme 4), respectively. Diisopropyl ester **17a** was synthesized from 8-aza-7-deazaadenine (**16a**) in a 53% yield using an analogous procedure as above (heating of diisopropyl [(2-chloroethoxy)methyl]phosphonate[49] with preformed sodium salt of the nucleobase in DMF at 100 °C) reported previously for the preparation of the corresponding phosphonate diethyl ester.[54] Similarly, alkylation of 8-azaadenine (**16b**) with the same alkylating agent in DMF using DBU as a base afforded desired product **17b** in a 32% yield (together with 32% of the corresponding 8-regioisomer).[55] Finally, phosphonate

diesters **17a** and **17b** were converted to bisamidates **18a** and **18b** by the standard procedure[48] (Scheme 4) in moderate yields.

We have decided to extend our structure-activity relationship study for ANPs bearing other non-purine bases that are able to mimic the adenine moiety and preserve the exocyclic "6-aminopurine" group. Thus, thieno[3,2-*d*]pyrimidine, quinazoline, and pyrrolo[2,1-*f*][1,2,4]triazine were selected as suitable starting materials. The common feature of these heterocycles is their 9-deaza character. Thus, the above reported methodology for the preparation of 9-deaza-PMEA prodrug **15** (Scheme 3) was employed for the synthesis of target compounds.

Treatment of commercially available 7-bromo-4-chloro-thieno[3,2-*d*]pyrimidine (**19**, Scheme 5) with vinyltributyltin under the above developed Stille cross-coupling reaction conditions (catalysis with Pd(*t*-$Bu_3P)_2$ in THF) afforded only traces of the desired vinyl derivative **20**, but an addition of CuI increased the yield of **20** to 50%. Tandem hydroboration and oxidation of vinyl compound **20** gave 2-hydroxyethyl derivative **21** in a good yield, as well as the subsequent alkylation of the hydroxyl group with $CF_3SO_2OCH_2P(O)(OiPr)_2$ in the presence of BuLi at −78 °C to yield phosphonate **22** (Scheme 5). Bisamidate prodrug **24** was obtained in good yield by ammonolysis of chloro derivative **22** (to give **23** in a 66% yield), followed by treatment of phosphonate **23** by the standard procedure.[48]

Starting 8-bromoquinazolin-4(3H)-one (**25**, Scheme 6) was first treated with 2,4-dimethoxybenzylamine, BOP reagent, and DBU at elevated temperature to afford 2,4-dimethoxybenzyl (DMB)-protected 8-bromo-4-aminochinazoline **26**. Compound **26** was then converted step by step into vinyl derivative **27** (Stille cross-coupling with vinyltributyltin, Pd(*t*-$Bu_3P)_2$ and CuI in *N*-methylpyrrolidone), into 2-hydroxyethyl compound **28** (hydroboration[51] and *in-situ* oxidation), and into phosphonate **29** (alkylation with $CF_3SO_2OCH_2P(O)(OiPr)_2$). DMB deprotection with TFA in DCM (to give **30** in a 49% yield) followed by standard bisamidate formation[48] yielded final analogue **31** (Scheme 6) in a good overall yield.

Commercially available 4-chloropyrrolo[2,1-*f*][1,2,4]triazine (**32**, Scheme 7) was transformed, using microwave-assisted treatment with 2,4-dimethoxybenzylamine and Et_3N in absolute ethanol, into DMB-protected derivative **33**, which was then iodinated with NIS to give compound **34** in a high yield. 4-Aminopyrrolo[2,1-*f*][1,2,4]triazine derivative **39** (Scheme 7) was prepared in a good overall yield starting from the iodo derivative **34** in analogy to the step by step synthetic methodology described for compound **31** (Scheme 6).

Finally, for enzymatic assays, triphosphate analogues **40a**, **40b**, and **41** (Scheme 8) were prepared from compounds **5a**, **17a**, and **14**, respectively, employing the standard morpholidate methodology reported by Holý and Rosenberg.[56]

Inhibition of ACT in the cell-based assay.

All prepared bisamidates **2a**, **2b**, **6a-6e**, **15**, **18a-18b**, **24**, **31**, and **39** were tested for their ability to inhibit ACT activity in J774A.1 macrophage cells (Table 1). For comparison,

bis(POM)PMEA (**I**) and PMEA bisamidate **III** (Fig. 1) were used as reference compounds. Murine macrophage cells J774A.1 were preincubated with various concentrations of tested prodrugs and subsequently exposed to *B. pertussis* ACT. The cells were lysed and the cAMP content was determined. Compounds that exhibited inhibitory activity in the low micromolar and sub-micromolar range (Table 1) were also evaluated for their effects on the viability of J774A.1 cells under the same conditions of the cAMP assay, in order to exclude false positives due to potential cytotoxic effects of the compounds.

All prepared bisamidates, except derivatives **2a**, **2b**, **15**, and **31**, inhibited ACT in low micromolar to submicromolar range. Three bisamidates (**6a**, **6b**, and **18a**) were more potent ACT inhibitors than the parent PMEA derivative **III** (Table 1). The most potent ACT inhibitor within the bisamidate prodrug series in the macrophage cell-based assay was compound **18a**, the 8-aza-7-deazapurine derivative of PMEA, with a IC_{50} value of 16 nM (Table 1). Compound **18a** is about an order of magnitude more potent ACT inhibitor than parent PMEA derivative **III** and, thus, as a promising ACT inhibitor, warrants further biological and pharmacological evaluation.

Deaza-modifications of the pyrimidine part of the purine moiety, as seen in compounds **2a** and **2b**, as well as the replacement of the adenine moiety with 4-aminoquinazoline base in derivative **31** led to a substantial loss of inhibitory activity. On the other hand, modification of the imidazole part of the purine scaffold, as seen in compounds **6a**, **6b**, and **18a**, seemed to be well-tolerated by the bacterial AC enzyme and led to an increase in potency compared to parent PMEA derivative **III**. Interestingly, novel 9-deaza-PMEA derivative **15** did not exhibit any ACT inhibitory effects (IC_{50} value >10 μM, Table 1). As the key structural difference between compound **15** and parent PMEA analogue **III** is the presence of the acidic hydrogen atom at the N-7 position, compound **15** (in the form of diphosphate **41**) was selected for further evaluation at the enzymatic level (see cell-free assay results).

Finally, all bisamidates evaluated in the cell-based assay exhibited improved cytotoxicity profiles compared to adefovir dipivoxil (bis(POM)PMEA, **I**, Fig. 1, Table 1) with most of them being non-toxic under the cAMP assay conditions.

Inhibition of ACT activity in a cell-free assay.

Selected compounds, i.e. two most active derivatives **6a** and **18a** (Table 1), as well as novel 9-deazapurine analogue **15**, were prepared in the form of corresponding phosphonodiphosphates **40a**, **40b** and **41**, respectively, representing the metabolically active species. Their direct anti-AC activity was assessed and compared with that of parent PMEApp (**II**, Fig. 1). The compounds were tested on two commercially available *B. pertussis* ACTs (from ENZO and Sigma), recombinantly expressed in *E. coli*, and on EF from *Bacillus anthracis* (Table 2). Compounds **40a** and **40b** were equally or more potent inhibitors of all enzymes tested compared to PMEApp (**II**) with IC_{50} values ranging from 0.5 to 21 nM. In the case of the ACT enzyme from Sigma, the 7-deaza derivative of PMEApp, i.e. compound **40a**, was found to be about 30 times more potent than PMEApp (**II**). Similarly, the novel 9-deazapurine derivative of PMEApp (**II**), compound **41**, was shown to be nearly equipotent compared to compounds **40a** and **40b**, as well as to parent

PMEApp (**II**, Table 2). Thus, the apparent lack of inhibitory effect of bisamidate **15** (analogue of **41**) in the cell-based assay (Table 1) is speculated to be due to the inefficient phosphorylation (by cellular kinases) of intermediate phosphonate **14** (released from bisamidate **15** in the cell) to the biologically active species **41**.

Molecular modelling of the most potent inhibitor for all studied bacterial adenylate cyclases, 7-deaza-PMEApp (**40a**), was performed using the recently reinterpreted[57] crystal structure of adenylate cyclase domain (ACD) from *B. pertussis* ACT with calmodulin (CaM) and PMEApp (PDB ID:1ZOT).[41] The docking revealed almost identical binding mode for both PMEApp (**II**) and compound **40a** (Fig. 3). The improved binding potential of **40a** is speculated to be due to the higher electron density of the pyrrol moiety when compared to the imidazole ring in PMEApp and, thus, stronger C-H – **π** interaction between Asn304 and the pyrrol moiety. Moreover, the hydrogen at position C-7 of **40a** is placed nearby (2.45 Å) the carbonyl group of Gly299 suggesting that their direct interaction may also be important (Fig. 3).

Inhibition of mammalian ACs.

All prepared ANPs in the bisamidate form were also evaluated as potential inhibitors of mammalian adenylate cyclases (mACs). Specifically, the enzyme isoforms AC1, AC2, and AC5, representing the three major mAC subfamilies, were tested to explore the potential selectivity of studied compounds for bacterial ACs over mACs (Table 3). Most of the compounds failed to markedly inhibit any of the mACs tested, revealing promising selectivity for the bacterial ACs. Several compounds even seemed to potentiate AC2 (e.g. **I**, **6a**, **6c**, **6d**, **18a**, **18b**, and **24**) and/or AC5 (e.g. **I**, **6d**, **6e**, and **31**) at 30 μM.

A number of compounds appeared to selectively inhibit AC1 (e.g. **6b**, **6c**, **6d**, **6e**, **31**, and **39**, Table 3). Interestingly, 7-halo-7-deazapurine analogues **6c**, **6d**, and **6e** were found to be the most efficacious AC1 inhibitors from the whole series, therefore, IC_{50} values were also determined for these compounds (Fig. 4, Table 4). Moreover, the potentiation of these compounds at AC2 was only evident at the highest concentration tested.

All three 7-halo-7-deazapurine analogues **6c**, **6d**, and **6e** were selective AC1 inhibitors (no AC2 and AC5 inhibition was observed) with potency in low μM range (IC_{50} values of 4.1–5.6 μM, Table 4). AC1 belongs to a group of mACs stimulated by calcium in a calmodulin-dependent manner, and AC1 inhibitors have a great potential for treating neuropathic and inflammatory pain.[58,59] Thus, compounds **6c**, **6d**, and **6e** warrant further study with AC1 knockout mice and their development as potent and selective AC1 inhibitors. Furthermore, the inhibitory activity of these 7-halo-7-deazapurine analogues on both the bacterial ACs and mammalian AC1 may suggest a shared binding domain, as both the bacterial ACs and the mammalian AC1 isoforms are also regulated by calcium/calmodulin.

Conclusions

A series of 13 acyclic nucleoside phosphonates (ANPs) bearing 2-(phosphonomethoxy)ethyl (PME) moiety in the form of bisamidate prodrug was synthesized as potential inhibitors of adenylate cyclases (ACs) of *Bordetella pertussis* (ACT) and *Bacillus anthracis* (EF). The

prepared compounds are characterized by the replacement of the purine moiety of adefovir (PMEA) with various bicyclic heterocycles, namely 7*H*-pyrrolo[2,3-*d*]pyrimidine (7-deazapurine), 1*H*-pyrazolo[3,4-*d*]pyrimidine (8-aza-7-deazapurine), 3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidine (8-azaapurine), 5*H*-pyrrolo[3,2-*d*]pyrimidine (9-deazapurine), thieno[3,2-d]pyrimidine, quinazoline, and pyrrolo[2,1-f][1,2,4]triazine (4-aza-7,9-dideazapurine). Bisamidate prodrugs with L-phenylalanine isopropyl ester were used based on their significantly improved stability in plasma and decreased cytotoxicity compared to the original adefovir dipivoxil (bis(POM)PMEA).[43]

Prepared compounds, with the exception of four derivatives (**2a**, **2b**, **15**, and **31**), are potent inhibitors of adenylate cyclase toxin from *Bordetella pertussis* (ACT) in the J774A.1 macrophage cell-based assay. The SAR study suggested that structural modification of the pyrimidine part of the molecule is not tolerated but aza-, deaza-, and even 7-thia-modifications in the imidazole part of the molecule seem to preserve or even improve potency. An additional substitution of the C-7 position of 7-deaza derivative **6a** by halogen atom led to decrease of inhibitory activity. Compound **18a**, 8-aza-7-deazapurine derivative of PMEA, was identified as the most potent ACT inhibitor in the series with 16 nM inhibitory potency towards ACT, substantial selectivity over mammalian ACs, and no observed cytotoxicity. As such, the compound represents a promising lead structure for further pharmacological evaluation in the mouse model of pertussis or anthrax.

The two most active ANP bisamidates identified in the macrophage cell-based assay, 7-deaza-PMEA derivative **6a** and 8-aza-7-deaza-PMEA derivative **18a**, were also prepared as ANP-diphosphates **40a** and **40b**, respectively, for the evaluation of their direct interaction with the proteins of interest in a cell-free assay. Compounds **40a** and **40b** were equally or more potent inhibitors of bacterial adenylate cyclases tested (ACT Enzo, ACT Sigma, and EF) compared to parent PMEApp (**II**). In fact, compound **40a**, was up to 30 times more potent than PMEApp on ACT from *B.pertussis*.

Synthesis of novel 9-deaza-PMEA (**14**) was designed and executed. Although its bisamidate analogue **15** was not a particularly efficient ACT inhibitor in the murine macrophage cell-based assay, 9-deaza-PMEApp (**41**) exhibited similar potency to PMEApp (**II**) on all three bacterial ACs tested *in vitro*. The lack of the potency of compound **15** in the cellular assay is speculated to be due to its inefficient intracellular transformation into the active nucleoside triphosphate analogue **41**.

Finally, several of the prepared compounds were discovered to inhibit mammalian AC1 with a considerable efficacy and a noticeable selectivity over AC2 and AC5. The 7-halo-7-deazapurine derivatives **6c**, **6d**, and **6e** were selective AC1 inhibitors with IC_{50} values in the range 4.1–5.6 μM. These compounds may represent promising lead structures for further optimization of their structure and for potential development of new agents for the treatment of human neurological and/or inflammatory diseases.

Experimental Section

Chemistry:

Unless otherwise stated, solvents were evaporated at 40 °C/2 kPa and the compounds were dried over P_2O_5 at 2 kPa. The microwave-assisted reactions were carried out in CEM Discover (Explorer) microwave apparatus. Chemicals and reagents were obtained from commercial sources (Sigma–Aldrich and Fluorochem Ltd.), bis(POM)PMEA was obtained from SANTIAGO company. Solvents were dried by standard procedures. Pyridine was stored over molecular sieves (4 Å). Tetrahydrofuran (THF) was freshly distilled from $LiAlH_4$ pellets under Ar. TLC was performed on plates of Kieselgel 60 F254 (Merck). NMR spectra were recorded on Bruker Avance 500 (^1H at 500 MHz, ^{13}C at 125.8 MHz) spectrometer with TMS as internal standard or referenced to the residual solvent signal. Mass spectra were measured on UPLC-MS (Waters SQD-2), and HR-MS were taken on a LTQ Orbitrap XL spectrometer using electrospray ionization (ESI). Preparative HPLC purifications were performed on columns packed with 10 μm C18 reversed phase resin (Phenomenex Gemini 10 μm 21 × 250 mm) on Waters Delta 600 chromatography system in ca. 200 mg batches of mixtures using gradient MeOH/H_2O as eluent. Flash chromatography on normal phase and on reversed phase was performed on Reveleris Flash Chromatography System. The deionization was performed on column Redisep®Rf GOLD C18 Teledyne ISCO. Preparative HPLC purification of triphosphate analogues was performed on a column packed with POROS® HQ 50 mm (50mL) with use of a gradient of TEAB in water (0.05–0.5 M). The purity of target compounds was determined by HPLC (H_2O-CH_3CN, linear gradient) and was higher than 95%.

Method A. General procedure for synthesis of bis-(L-phenylalanine ethyl ester) prodrugs of phosphonates:[48]

TMSBr (1 mL) was added to the corresponding phosphonate diester (1.0 mmol) dissolved in dry pyridine (10 mL). The reaction mixture was stirred at RT overnight. Volatiles were removed, and the moisture sensitive product was permanently kept under Ar. Solid isopropyl ester L-phenylalanine hydrochloride (0.97 g, 4.0 mmol) was added to the intermediate, followed by dry pyridine (8 mL) and dry Et_3N (2 mL) under Ar. The mixture was preheated to 70 °C and freshly prepared solution of Aldrithiol-2 (1.37 g, 6.2 mmol) and triphenylphosphine (1.64 g, 6.2 mmol) in pyridine (10 mL) was added. The resulting mixture was stirred at 70 °C for 72 h. Reaction mixture was evaporated *in vacuo* and the residue was purified by column chromatography (0–100% MeOH in a mixture of Hexane:EtOAc, 6:4) followed by C18 reversed phase column chromatography (0–100% MeOH in water).

Method B. General procedure for alkylation of modified nucleobases:

Diisopropyl [(2-chloroethoxy)methyl]phosphonate[49] (2.0 g, 7.9 mmol) was added to the solution of halogenated nucleobase (5.9 mmol) in DMSO (12 mL) under argon at RT. The reaction mixture was warmed up to 80 °C, Cs_2CO_3 (1.25 g, 3.9 mmol) was added, and the resulting mixture was stirred at 80 °C. The mixture was cooled down, dissolved in Et_2O (100 mL) and extracted with 80% aqueous solution of sat. NH_4Cl (3 × 30 mL). Organic

phase was dried over Na_2SO_4. Volatiles were removed *in vacuo* and the residue was purified by flash column chromatography (MeOH:$CHCl_3$, 0–10% gradient).

Method C. General procedure for hydroboration of vinyl compounds:[51]

9-BBN (0.5 M in THF) (16 mL, 8.0 mmol) was added dropwise to the solution of vinyl compound (4.0 mmol) in THF (10 mL) at 0 °C under argon within 15 min. The reaction mixture was allowed to warm up to RT and stirred at RT for 3 h. $NaBO_3.4\ H_2O$ (2.4 g) in water (50 mL) was added and the mixture was stirred for additional 3 h. The reaction mixture was filtered, $CHCl_3$ (50 mL) was added, the organic phase was washed with 10% aqueous NaCl (2 × 25 mL) and brine (25 mL), and then dried over Na_2SO_4. Volatiles were removed *in vacuo* and the residue was purified by column chromatography.

Method D. General procedure for alkylation of 2-hydroxyethyl derivatives with $CF_3SO_2CH_2P(O)(O\mathit{i}Pr)_2$:

A solution of BuLi (2.5 M in hexane, 1.42 mL, 3.55 mmol) was added dropwise to the corresponding 2-hydroxyethyl compound (2.8 mmol) in THF (23 mL) under argon at −78 °C and the mixture was stirred at −78 °C for 10 min. $CF_3SO_2CH_2P(O)(O\mathit{i}Pr)_2$ (2.24 g, 6.8 mmol) in THF (5 mL) was added and the reaction mixture was stirred at −78 °C for 15 min. The resulting mixture was allowed to warm up to −40 °C (in approx. 3 h) and a mixture of sat. NH_4Cl in H_2O (1:1, 25 mL) was added, followed by EtOAc (150 mL). The mixture was washed with H_2O (50 mL) and brine (50 mL) and then dried over Na_2SO_4. Volatiles were removed and the residue was purified by C18 reverse-phase chromatography (H_2O:MeOH, 0–100%).

Method E. General procedure for preparation of phosphonate diphosphates:[56]

A solution of dicyclohexylcarbodiimide (0.44 g, 2.2 mmol) in *t*-BuOH (4 mL) was added dropwise (2 h) to the refluxing mixture of corresponding phosphonic acid (0.5 mmol) and morpholine (0.19 mL) in 50% aqueous *t*-BuOH (16 mL). The reaction mixture was refluxed overnight, cooled down, and concentrated to half-volume *in vacuo* (bath max. 35 °C). The mixture was diluted with H_2O (10 mL) and extracted with Et_2O (3 × 10mL). The aqueous layer was evaporated *in vacuo* and codistilled with EtOH and toluene. The residue was dissolved in DMF (4.5 mL) and Bu_3N (0.35 mL, 1.5 mmol) and tributylammoniumpyrophosphate (0.33 g, 1.5 mmol) were added. The solution was stirred at RT overnight. The reaction mixture was pour into Et_2O. The solid was filtered off, washed with Et_2O and dissolved in 1 M aqueous TEAB (5 mL). Volatiles were removed *in vacuo* (bath 30 °C), the residue was dissolved in 0.05 M aqueous TEAB (5 mL) and applied on column POROS 50 HQ and eluted by TEAB (0.05–1 M gradient). The corresponding eluate was evaporated *in vacuo*, codistilled with H_2O (3 × 5 mL) and applied on column of Dowex (50 × 8) (Na^+ cycle). The UV absorbing fraction was lyophilized.

9-{2-[(Diisopropoxyphosphoryl)methoxy]ethyl}−1-deazaadenine (1a):

Compound **1a** was synthesized from 1-deazaadenine according to the reported procedure.[46,47]

9-{2-[(Diisopropoxyphosphoryl)methoxy]ethyl}−3-deazaadenine (1b):

Diisopropyl ((2-chloroethoxy)methyl)phosphonate (1.93 g, 7.5 mmol) was added to the reaction mixture (preheated at 80 °C for 1 h) of NaH (60 % susp. in mineral oil, 0.3 g, 7.5 mmol) and 3-deazaadenine (7.5 mmol) in DMF (150 mL). The resulting mixture was stirred at 100 °C overnight. The clear solution was cooled down and volatiles were removed *in vacuo*. The residue was codistilled with toluene and purified by column chromatography (MeOH:$CHCl_3$, 0–10%) to give **1b** (1.64 g, 62%) as yellowish oil. ESI-MS, *m*/*z* (%): 357 [M+H^+] (100). ^{1}H NMR (DMSO-d_6): δ 1.12 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 1.16 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 3.76 (d, *J*(CH_2, P) = 8.3 Hz, 2H, P-CH_2); 3.84 (m, 2H, H-2'); 4.34 (m, 2H, H-1'); 4.47 (m, 2H, CH*i*Pr); 6.09 (brs, 2H, NH_2); 6.81 (d, *J*(3, 2) = 5.8 Hz, 1H, H-3); 7.65 (d, *J*(2, 3) = 5.8 Hz, 1H, H-2); 8.01 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): δ 23.79 (d, *J*(C-C-O-P) = 4.5 Hz, CH_3*i*Pr); 23.92 (d, *J*(C-C-O-P) = 3.8 Hz, CH_3*i*Pr); 44.20 (C-1'); 64.87 (d, *J*(C-P) = 164.1 Hz, CH_2-P); 70.32 (d, *J*(C-O-P) = 6.4 Hz, CH*i*Pr); 70.91 (d, *J*(2'-P) = 11.6 Hz, C-2'); 96.80 (C-3); 126.75 (C-5); 138.55 (C-4); 140.30 (C-2); 141.93 (C-8); 152.49 (C-6). HR-MS (ESI+): m/z [M + H]$^+$ calculated for: $C_{15}H_{26}N_4O_4P$, 357.1686, found: 357.1687.

Bis(L-phenylalanine isopropyl ester) prodrug of 9-[2-(phosphorylmethoxy)ethyl]-1-deazaadenine (2a):

Treatment of **1a** (0.17 g, 0.60 mmol) by Method A afforded **2a** (0.21 g, 60%) as a colourless foam. ESI-MS, *m*/*z* (%): 651 [M+H^+] (100). ^{1}H NMR (DMSO-d_6): δ 1.01, 1.07, 1.11 and 1.16 (d, *J*(CH_3, CH) = 6.3 Hz, 12H, CH_3); 2.71–2.89 (m, 4H, -CH_2Ph); 3.21 (dd, *J*(CH_2a, P) = 8.4 Hz, *J*(gem) = 13.2 Hz, 1H, P-CH_2a); 3.30 (dd, *J*(CH_2b, P) = 7.8 Hz, *J*(gem) = 13.2 Hz, 1H, P-CH_2b); 3.68 (t, *J*(2', 1') = 5.3 Hz, 2H, H-2'); 3.83–4.01 m, 2H, NH-CH); 4.13 (t, *J*(NH, CH) = *J*(NH, P) = 11.3 Hz, 1H, CH-NH); 4.25 (t, *J*(1', 2') = 5.3 Hz, 2H, H-1'); 4.45 (t, *J*(NH, CH) = *J*(NH, P) = 11.5 Hz, 1H, CH-NH); 4.78 a 4.82 (2x sept, 1H, *J*(CH_3, CH) = 6.3 Hz, CH*i*Pr); 6.28 (brs, 2H, NH_2); 7.08 (m, 10H, Ph-ortho, meta, para); 6.34 (d, *J*(2, 1) = 5.5 Hz, 1H, H-2); 7.82 (d, *J*(2, 1) = 5.5 Hz, 1H, H-2); 8.03 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): δ 21.47, 21.54, 21.60 a 21.67 (CH_3*i*Pr); 39.90 (CH_2Bn); 42.42 (C-1'); 54.02 a 54.24 (NH-CH); 67.48 (d, *J*(C-P) = 135.3 Hz, CH_2-P); 67.98 a 68.10 (CH*i*Pr); 70.61 (d, *J*(2'-P) = 11.4 Hz, C-2'); 102.12 (C-1); 122.88 (C-5); 126.58 a 126.64 (Ph-para); 128.22 a 128.26 (Ph-meta); 129.65 (Ph-ortho); 137.22 a 136.29 (ipso); 140.85 (C-8); 144.66 (C-2); 146.95 (C-6); 147.44 (C-4); 172.34 and 172.47 m (COO). HR-MS (ESI+): m/z [M + H]$^+$ calculated for: $C_{33}H_{44}N_6O_6P$, 651.3055, found: 651.3055.

Bis(L-phenylalanine isopropyl ester) prodrug of 9-[2-(phosphorylmethoxy)ethyl]-3-deazaadenine (2b):

Treatment of **1** (0.35 g, 1.0 mmol) by Method A afforded **2b** (0.11 g, 16%) as a colourless foam. ESI-MS, *m*/*z* (%): 651 [M+H^+] (100). ^{1}H NMR (DMSO-d_6): δ 1.00–1.17, (m, 12H, CH_3); 2.67–2.88 (m, 4H, -CH_2Ph); 3.16–3.43 (m, 2H, P-CH_2); 3.64 (m, 2H, H-2'); 3.83–4.00 m, 2H, NH-CH); 4.10 (m, 1H, CH-NH); 4.25 (m, 2H, H-1'); 4.41 (m, 1H, CH-NH); 4.73–4.86 (m, 2H, CH*i*Pr); 6.08 (brs, 2H, NH_2); 6.79 (d, *J*(3, 2) = 5.8 Hz, 1H, H-3); 7.05–7.26 (m, 10H, Ph-ortho, meta, para); 7.65 (d, *J*(2, 3) = 5.8 Hz, 1H, H-2); 8.03 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): δ 21.47, 21.54, 21.61 a 21.67 (CH_3*i*Pr); 39.90 (CH_2Bn); 44.31

(C-1'); 54.01 and 54.22 (NH-CH); 67.57 (d, *J*(C-P) = 135.0 Hz, CH_2-P); 67.98 and 68.12 (CH*i*Pr); 70.86 (d, *J*(2'-P) = 11.0 Hz, C-2'); 96.72 (C-3); 126.57 and 126.65 (Ph-para); 126.72 (C-5); 128.20 and 128.26 (Ph-meta); 129.62 (Ph-ortho); 137.17 and 137.27 (ipso); 138.58 (C-4); 140.37 (C-2); 142.00 (C-8); 152.50 (C-6); 172.31–172.52 m (COO). HR-MS (ESI+): m/z $[M + H]^+$ calculated for: $C_{33}H_{44}N_6O_6P$, 651.3055, found: 651.3055.

9-{2-[(Diisopropoxyphosphoryl)methoxy]ethyl}−6-chloro-7-deazapurine (4a):

For the synthesis of **4a** look at the preparation of compound **5a**.

9-{2-[(Diisopropoxyphosphoryl)methoxy]ethyl}−7-fluoro-6-chloro-7-deazapurine (4b):

Treatment of **3b** (2.0 g, 11.7 mmol) by Method B (4 h at 80 °C) and chromatography (MeOH:$CHCl_3$, 5:95) afforded **4b** (2.77 g, 60%) as a yellowish oil. ESI-MS, *m*/*z* (%): 394 $[M+H^+]$ (100). ^{1}H NMR ($CDCl_3$): δ 1.25 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 1.29 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 3.70 (d, *J*(H-C-P) = 8.4 Hz, 2H, P-CH_2); 3.89 (t, *J*(1', 2') = 4.9 Hz, 2H, H-1'); 4.44 (t, *J*(2', 1') = 4.9 Hz, 2H, H-2'); 4.61–4.75 (m, 2H, CH*i*Pr); 7.24 (d, *J*(8, F) = 2.6 Hz, 1H, H-8); 8.58 (s, 1H, H-2). ^{13}C NMR ($CDCl_3$): δ 23.89 (d *J*(C-C-O-P) = 4.6 Hz, CH_3*i*Pr); 23.97 (d *J*(C-C-O-P) = 4.0 Hz, CH_3*i*Pr); 44.13 (C-1'); 65.91 (d, *J*(C-P) = 168.2 Hz, CH_2-P); 71.15 (d, *J*(C-O-P) = 6.8 Hz, CH*i*Pr); 71.64 (d, *J*(2'-P) = 10.7 Hz, C-2'); 106.69 (d, *J*(5, F) = 13.7 Hz, C-5); 113.34 (d, *J*(8, F) = 26.2 Hz, C-8); 140.62 (d, *J*(C-F) 251.6 Hz, C-7); 146.22 (C-4); 150.40 (C-6); 151.12 (C-2). HR-MS (ESI+): m/z $[M + H]^+$ calculated for: $C_{15}H_{23}ClFN_3O_4P$, 394.1093, found: 394.1090.

6,7-Dichloro-9-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}−7-deazapurine (4c):

Treatment of **3c** (1.0 g, 5.3 mmol) by Method B (5 h at 80 °C) and chromatography (MeOH:$CHCl_3$, 5:95) afforded **4c** (1.83 g, 84%) as a yellowish oil. ESI-MS, *m*/*z* (%): 410 $[M+H^+]$ (100). ^{1}H NMR (DMSO-d_6): δ 1.08 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 1.14 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 3.75 (d, *J*(H-C-P) = 8.4 Hz, 2H, P-CH_2); 3.90 (t, *J*(1', 2') = 5.0 Hz, 2H, H-1'); 4.39–4.46 (m, 2H, CH*i*Pr); 4.45–4.47 (m, 2H, H-2'); 7.95 (s, 1H, H-8); 8.67 (s, 1H, H-2). ^{13}C NMR (DMSO-d_6): δ 23.38 (d *J*(C-C-O-P) = 4.5 Hz, CH_3*i*Pr); 23.56 (d, *J*(C-C-O-P) = 3.8 Hz, CH_3*i*Pr); 43.96 (C-1'); 64.36 (d, *J*(C-P) = 164.1 Hz, CH_2-P); 69.95 (d, *J*(C-O-P) = 6.4 Hz, CH*i*Pr); 70.21 (d, *J*(2'-P) = 11.7 Hz, C-2'); 100.97 (C-5); 112.59 (C-7); 128.89 (C-8); 149.45 and 149.84 (C-4, 6); 150.83 (C-2). HR-MS (ESI+): m/z $[M + H]^+$ calculated for: $C_{15}H_{23}Cl_2N_3O_4P$, 410.0799, found: 410.0798.

7-Bromo-9-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}−6-chloro-7-deazapurine (4d):

Treatment of **3d** (1.0 g, 4.3 mmol) by Method B (4 h at 80 °C) and chromatography (MeOH:$CHCl_3$, 6:94) afforded **4d** (1.67 g, 85%) as a colourless oil. ESI-MS, *m*/*z* (%): 454 $[M+H^+]$ (100). ^{1}H NMR (DMSO-d_6): δ 1.08 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 1.14 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 3.76 (d, *J*(H-C-P) = 8.4 Hz, 2H, P-CH_2); 3.90 (t, *J*(1', 2') = 5.0 Hz, 2H, H-1'); 4.38–4.46 (m, 2H, CH*i*Pr); 4.45–4.48 (m, 2H, H-2'); 7.98 (s, 1H, H-8); 8.67 (s, 1H, H-2). ^{13}C NMR (DMSO-d_6): δ 23.40 (d, *J*(C-C-O-P) = 4.5 Hz, CH_3*i*Pr); 23.57 (d, *J*(C-C-O-P) = 3.8 Hz, CH_3*i*Pr); 44.05 (C-1'); 64.37 (d, *J*(C-P) = 163.99 Hz, CH_2-P); 69.96 (d, *J*(C-O-P) = 6.4 Hz, CH*i*Pr); 70.22 (d, *J*(2'-P) = 11.8 Hz, C-2'); 85.23 (C-7); 113.78

(C-5); 131.38 (C-8); 150.92 and 151.15 (C-4 and C-6); 155.96 (C-2). HR-MS (ESI+): m/z $[M + H]^+$ calculated for: $C_{15}H_{23}BrClN_3O_4P$, 454.0293, found: 454.0293.

9-{2-[(Diisopropoxyphosphoryl)methoxy]ethyl}−6-chloro-7-iodo-7-deazapurine (4e):

Treatment of **3e** (1.5 g, 5.3 mmol) by Method B (5 h at 80 °C) and chromatography (MeOH:$CHCl_3$, 5:95) afforded **4e** (1.92 g, 72%) as a yellowish oil. ESI-MS, *m*/*z* (%): 502 $[M+H^+]$ (10); 524 $[M+Na^+]$ (100). ^{1}H NMR (DMSO-d_6): δ 1.08 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 1.14 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 3.80 (d, *J*(H-C-P) = 8.3 Hz, 2H, P-CH_2); 3.72–3.77 (m, 2H, H-1'); 4.40–4.49 (m, 2H, H-2'); 4.56–4.64 (m, 2H, CH*i*Pr); 7.97 (s, 1H, H-8); 8.64 (s, 1H, H-2). ^{13}C NMR (DMSO-d_6): δ 24.22 (d, *J*(C-C-O-P) = 4.6 Hz, CH_3*i*Pr); 24.34 (d, *J*(C-C-O-P) = 3.7 Hz, CH_3*i*Pr); 43.68 (C-1'); 65.08 (d, *J*(C-P) = 164.4 Hz, CH_2-P); 70.70 (d, *J*(C-O-P) = 6.4 Hz, CH*i*Pr); 72.74 (d, *J*(2'-P) = 11.9 Hz, C-2'); 51.80 (C-7); 116.61 (C-5); 137.18 (C-8); 150.80 (C-2); 151.08 and 151.34 (C-4 and C-6). HR-MS (ESI+): m/z $[M + Na]^+$ calculated for: $C_{15}H_{22}ClIN_3O_4PNa$, 523.9973, found: 523.9974.

9-{2-[(Diisopropoxyphosphoryl)methoxy]ethyl}−7-deazaadenine (5a):

Diisopropyl ((2-chloroethoxy)methyl) phosphonate[49] (13.6 g, 52.0 mmol) was added to the preheated (80 °C) reaction mixture of **3a** (6.0 g, 39.0 mmol) and Cs_2CO_3 (8.2 g, 25.4 mmol) in DMSO (90 mL). The resulting mixture was stirred at 80 °C for 5 h and then cooled down to RT. Aqueous solution of NH_4Cl (20%, 300 mL) was added and the mixture was washed with Et_2O (3 × 100 mL). The combined organic layers were concentrated and codistilled with toluene (3 × 25 mL) to give crude **4a**. A mixture of crude **4a** in ethanolic ammonia solution (40 mL) was heated at 100 °C for 24 h. The solvent was removed *in vacuo* and the residue was applied on Dowex (50 × 8) and washed with 30% aqueous MeOH solution successively. The column was then washed with 2.5% aqueous ammonia solution, the UV absorbing fraction was evaporated *in vacuo* and repurified on C18 column (H_2O:MeOH, 0–100%) to give **5a** (5.48 g, 39%) as a yellowish oil. The analytical data are in agreement with published data.[49]

9-{2-[(Diisopropoxyphosphoryl)methoxy]ethyl}−7-fluoro-7-deazaadenine (5b):

Compound **4b** (1.92 g, 4.87 mmol) was dissolved in EtOH/NH_3 (30 mL) and stirred at 100 °C for 16 h. The volatiles were removed and the residue was purified by C18 reversed phase chromatography (H_2O:MeOH 0–100%) to give **5b** (0.89 g, 49%) as a yellowish oil. ESI-MS, *m*/*z* (%): 375 $[M+H^+]$ (100). ^{1}H NMR ($CDCl_3$): δ 1.27 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 1.30 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 3.71 (d, *J*(H-C-P) = 8.5 Hz, 2H, P-CH_2); 3.88 (t, *J*(1', 2') = 5.1 Hz, 2H, H-1'); 4.33 (t, *J*(2', 1') = 5.1 Hz, 2H, H-2'); 4.63–4.75 (m, 2H, CH*i*Pr); 5.41 (brs, 2H, NH_2); 6.88 (d, *J*(8, F) = 2.5 Hz, 1H, H-8); 8.23 (s, 1H, H-2). ^{13}C NMR ($CDCl_3$): δ 23.91 (d, *J*(C-C-O-P) = 4.7 Hz, CH_3*i*Pr); 24.01 (d, *J*(C-C-O-P) = 3.9 Hz, CH_3*i*Pr); 43.74 (C-1'); 65.97 (d, *J*(C-P) = 167.9 Hz, CH_2-P); 71.11 (d, *J*(C-O-P) = 6.7 Hz, CH*i*Pr); 72.11 (d, *J*(2'-P) = 11.2 Hz, C-2'); 93.19 (d, *J*(5, F) = 14.9 Hz, C-5); 107.98 (d, *J*(8, F) = 26.4 Hz, C-8); 142.52 (d, *J*(C, F) = 244.1 Hz, C-7); 145.65 (d, *J*(4, F) = 2.5 Hz, C-4); 152.66 (C-2); 155.43 (d, *J*(6, F) = 2.9 Hz, C-6). HR-MS (ESI+): m/z $[M + H]^+$ calculated for: $C_{15}H_{25}N_4O_4FP$, 375.1592, found: 375.1591.

9-{2-[(Diisopropoxyphosphoryl)methoxy]ethyl}−7-chloro-7-deazaadenine (5c):

Compound **4c** (1.6 g, 3.9 mmol) was dissolved in EtOH/NH_3 (50 mL) and stirred at 100 °C for 16 h. The volatiles were removed and the residue was purified by C18 reversed phase chromatography (H_2O:MeOH 0–100%) to give **5c** (1.00 g, 66%) as a colourless oil. ESI-MS, *m*/*z* (%): 391 [M+H^+] (100). ^{1}H NMR (DMSO-d_6): δ 1.13 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 1.17 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 3.75 (d, *J*(H-C-P) = 8.5 Hz, 2H, P-CH_2); 3.83 (m, 2H, H-2'); 4.27 (m, 2H, H-1');); 4.83 (d of septets, *J*(H-C-O-P) = 7.7 Hz, *J*(CH, CH_3) = 6.2 Hz, 2H, CH*i*Pr); 6.81 (brs, 2H, NH_2); 7.34 (s, 1H, H-8); 8.08 (s, 1H, H-2). ^{13}C NMR (DMSO-d_6): δ 23.76 (d, *J*(C-C-O-P) = 4.5 Hz, (CH_3*i*Pr); 23.92 (d, *J*(C-C-O-P) = 3.8 Hz, CH_3*i*Pr); 43.37 (C-1'); 64.65 (d, *J*(C-P) = 163.9 Hz, CH_2-P); 70.34 (d, *J*(C-O-P) = 6.4 Hz, CH*i*Pr); 70.94 (d, *J*(2'-P) = 12.1 Hz, C-2'); 99.65 (C-5); 101.11 (C-7); 122.17 (C-8); 148.73 (C-4); 152.48 (C-2); 156.81 (C-6). HR-MS (ESI+): m/z [M + H]$^+$ calculated for: $C_{15}H_{25}N_4O_4ClP$, 391.1297, found: 391.1298.

9-{2-[(Diisopropoxyphosphoryl)methoxy]ethyl}−7-bromo-7-deazaadenine (5d):

Compound **4d** (1.5 g, 3.3 mmol), was dissolved in EtOH/NH_3 (40 mL) and stirred at 100 °C for 16 h. The volatiles were removed and the residue was purified by C18 reversed phase chromatography (H_2O:MeOH 0–100%) to give **5d** (1.17 g, 81%) as a colourless oil. ESI-MS, *m*/*z* (%): 435 [M+H^+] (100). ^{1}H NMR (DMSO-d_6): δ 1.13 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 1.17 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 3.75 (d, *J*(H-C-P) = 8.5 Hz, 2H, P-CH_2); 3.83 (m, 2H, H-2'); 4.27 (m, 2H, H-1'); 4.47 (d of septets, *J*(H-C-O-P) = 7.7 Hz, *J*(CH, CH_3) = 6.2 Hz, 2H, CH*i*Pr); 6.71 (brs, 2H, NH_2); 7.39 (s, 1H, H-8); 8.09 (s, 1H, H-2). ^{13}C NMR (DMSO-d_6): δ 23.77 (d *J*(C-C-O-P) = 4.5 Hz, CH_3*i*Pr); 23.93 (d, *J*(C-C-O-P) = 3.8 Hz, CH_3*i*Pr); 43.47 (C-1'); 64.66 (d, *J*(C-P) = 164.0 Hz, CH_2-P); 70.34 (d, *J*(C-O-P) = 6.4 Hz, CH*i*Pr); 70.95 (d, *J*(2'-P) = 12.0 Hz, C-2'); 85.00 (C-7); 100.85 (C-5); 124.66 (C-8); 149.24 (C-4); 152.40 (C-2); 157.06 (C-6). HR-MS (ESI+): m/z [M + H]$^+$ calculated for: $C_{15}H_{25}N_4O_4BrP$, 435.0791, found: 435.0792.

9-{2-[(Diisopropoxyphosphoryl)methoxy]ethyl}−7-iodo-7-deazaadenine (5e):

Compound **4e** (1.92 g, 3.8 mmol), was dissolved in EtOH/NH_3 (30 mL) and stirred at 100 °C for 16 h. The solvent was removed and the residue was purified by C18 reversed phase chromatography (H_2O:MeOH 0–100%) to give **5e** (1.70 g, 92%) as a yellowish oil. ESI-MS, *m*/*z* (%): 483 [M+H^+] (67); 505 [M+Na^+] (100). ^{1}H NMR (DMSO-d_6): δ 1.13 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 1.18 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 3.75 (d, *J*(H-C-P) = 8.5 Hz, 2H, P-CH_2); 3.83 (t, *J*(1', 2') = 5.2 Hz, 2H, H-1'); 4.29 (t, *J*(2', 1') = 5.2 Hz, 2H, H-2'); 4.48 (d of septets, *J*(H-C-O-P) = 7.7 Hz, *J*(CH, CH_3) = 6.2 Hz, 2H, CH*i*Pr); 6.59 (brs, 2H, NH_2); 7.42 (s, 1H, H-8); 8.09 (s, 1H, H-2). ^{13}C NMR (DMSO-d_6): δ 23.52 (d, *J*(C-C-O-P) = 4.5 Hz, CH_3*i*Pr); 23.67 (d, *J*(C-C-O-P) = 3.7 Hz, CH_3*i*Pr); 43.25 (C-1'); 49.47 (C-7); 64.39 (d, *J*(C-P) = 163.9 Hz, CH_2-P); 70.04 (d, *J*(C-O-P) = 6.4 Hz, CH*i*Pr); 70.70 (d, *J*(2'-P) = 12.1 Hz, C-2'); 102.76 (C-5); 129.65 (C-8); 149.56 (C-4); 151.61 (C-2); 156.98 (C-6). HR-MS (ESI+): m/z [M + H]$^+$ calculated for: $C_{15}H_{25}N_4O_4IP$, 483.0653, found: 483.0654.

Bis(L-phenylalanine isopropyl ester) prodrug of 9-[2-(phosphorylmethoxy)ethyl]-7-deazaadenine (6a):

Treatment of **5a** (0.35 g, 0.98 mmol) by Method A afforded **6a** (0.38 g, 60%) as a whitish amorphous solid. ESI-MS, *m*/*z* (%): 651 [M+H$^+$] (100). ^{1}H NMR (DMSO-d_6): δ 1.15, 1.19, 1.20 and 1.24 (4 x d, *J*(CH_3, CH) = 6.3 Hz, 12H, CH_3); 2.76 (dd, *J*(CH_2, CH) = 6.9 Hz, *J*(gem) = 13.7 Hz, 1H, CH_2aPh); 2.81 (dd, *J*(NH, CH) = 11.1 Hz, *J*(NH, P) = 12.6 Hz, 1H, P-NH); 2.82 (dd, *J*(CH_2a, Ph) = 7.7 Hz, *J*(gem) = 13.7 Hz, 1H, CH_2aPh); 2.93 (dd, *J*(CH_2, CH) = 5.7 Hz, *J*(gem) = 13.7 Hz, 1H, CH_2bPh); 3.00 (dd, *J*(CH_2, CH) = 5.7 Hz, *J*(gem) = 13.6 Hz, 1H, CH_2bPh); 3.14 (bt, *J*(NH, CH) = *J*(NH, P) = 10.7 Hz, 1H, NH); 3.17 (dd, *J*(CH_2a, P) = 9.2 Hz, *J*(gem) = 12.9 Hz, 1H, P-CH_2a); 3.26 (dd, *J*(CH_2b, P) = 8.5 Hz, *J*(gem) = 12.9 Hz, 1H, P-CH_2b); 3.65 (m, 2H, H-2'); 4.04 (dddd, *J*(CH, CH_2b) = 5.7 Hz, *J*(CH, CH_2a) = 6.9 Hz, *J*(CH, P) = 9.0 Hz, *J*(CH, NH) = 11.0 Hz, 1H, CH-NH); 4.16 (dddd, *J*(CH, CH_2b) = 5.7 Hz, *J*(CH, CH_2a) = 7.7 Hz, *J*(CH, P) = 9.5 Hz, *J*(CH, NH) = 11.0 Hz, 1H, CH-NH); 4.29 (m, 2H, H-1'); 4.94 and 4.98 (2x sept, 1H, CH*i*Pr); 5.24 (brs, 2H, NH_2); 6.27 (d, *J*(6, 5) = 3.6 Hz, 1H, H-7); 6.99 (d, *J*(8, 7) = 3.6 Hz, 1H, H-8); 7.05 and 7.11 (2x m, 4H, *o*-Bn); 7.16–7.27 (m, 6H, *m,p*-Bn); 8.30 (s, 1H, H-2). ^{13}C NMR (DMSO-d_6): δ 21.62, 21.70, 21.71 and 21.79 (CH_3*i*Pr); 40.61 (d, *J*(C-P) = 4.0 Hz, CH_2Bn); 40.80 (d, *J*(C-P) = 5.3 Hz, CH_2Bn); 44.26 (C-1'); 53.62 and 54.02 (NH-CH); 67.95 (d, *J*(C-P) = 136.6 Hz, CH_2-P); 69.07 and 69.12 (CH*i*Pr); 71.96 (d, *J*(2'-P) = 13.4 Hz, C-2'); 97.40 (C-7); 103.14 (C-5); 125.99 (C-8); 126.84 and 126.88 (Ph-para); 128.32 and 128.37 (Ph-meta); 129.62 and 129.66 (Ph-ortho); 136.29 and 136.52 (ipso); 150.13 (C-4); 151.68 (C-2); 156.63 (C-6); 172.37 and 172.54 (m, COO). HR-MS (ESI+): m/z [M + H]$^+$ calculated for: $C_{33}H_{44}N_6O_6P$, 651.3055, found: 651.3054.

Bis(L-phenylalanine isopropyl ester) prodrug of 9-[2-(phosphorylmethoxy)ethyl]-7-fluoro-7-deazaadenine (6b):

Treatment of **5b** (0.35 g, 0.93 mmol) by Method A afforded **6b** (0.32 g, 51%) as a whitish amorphous solid. ESI-MS, *m*/*z* (%): 669 [M+H$^+$] (50); 691 [M+Na$^+$] (100). ^{1}H NMR (DMSO-d_6): δ 1.01, 1.06, 1.11 and 1.16 (4 x d, *J*(CH_3, CH) = 6.3 Hz, 12H, CH_3); 2.73 (dd, *J*(gem) = 13.4 Hz, *J*(CH_2, CH) = 7.1 Hz, 2H, $PhCH_2$); 2.77–2.87 (m, 4H, $PhCH_2$); 3.22 (dd, *J*(CH_2a, P) = 8.3 Hz, *J*(gem) = 13.1 Hz, 1H, P-CH_2a); 3.26 (dd, *J*(CH_2b, P) = 8.0 Hz, *J*(gem) = 13.1 Hz, 1H, P-CH_2b); 3.62 (brd, *J*(2', 1') = 5.1 Hz, 2H, H-2'); 3.87 (ddt, *J*(CH, NH) = 10.5 Hz, *J*(CH, P) = 9.0 Hz, *J*(CH, CH_2) = 7.0 Hz, 1H, CH-NH); 3.93 (ddt, *J*(CH, NH) = 10.8 Hz, *J*(CH, P) = 9.3 Hz, *J*(CH, CH_2) = 7.0 Hz, 1H, CH-NH); 4.12 (dd, *J*(NH, P) = 12.0 Hz, *J*(NH, CH) = 10.6 Hz, 1H, P-NH); 4.17 (brtd, *J*(1', 2'a) = *J*(1', 2'b) = 5.0 Hz, $J_{l.r.}$ = 3.7 Hz, 2H, H-1'); 4.41 (dd, *J*(NH, P) = 11.9 Hz, *J*(NH, CH) = 10.8 Hz, 1H, P-NH); 4.77 (sept, *J*(CH, CH_3) = 6.3 Hz, 2H, CH*i*Pr); 4.82 (sept, *J*(CH, CH_3) = 6.3 Hz, 2H, CH*i*Pr); 6.91 (brs, 2H, NH_2); 7.10 (m, 2H, Ph-ortho); 7.14 (d, *J*(8, F) = 2.3 Hz, 1H, H-8); 7.15 (m, 2H, Ph-ortho); 7.17–7.27 (m, 6H, Ph-meta, para); 8.05 (s, 1H, H-2). ^{13}C NMR (DMSO-d_6): δ 21.47, 21.54, 21.59 and 21.67 (CH_3*i*Pr); 40.20 (CH_2Ph); 43.02 (C-1'); 54.05 and 54.19 (NH-CH); 67.52 (d, *J*(C-P) = 135.1 Hz, CH_2-P); 67.99 and 68.12 (CH*i*Pr); 71.11 (d, *J*(2'-P) = 11.9 Hz, C-2'); 92.02 (d, *J*(2'-P) = 15.0 Hz, C-5); 107.51 (d, *J*(C, F) = 26.0 Hz, C-8); 126.61 and 126.65 (Ph-para); 128.22 and 128.25 (Ph-meta); 129.64 and 129.65 (Ph-ortho); 137.20 and 137.30 (ipso); 141.95 (d, *J*(C, F) = 243.5 Hz, C-7); 145.43 (d, *J*(4, F) = 2.9 Hz, C-4); 152.73 (C-2);

155.96 (C-6); 172.33 and 172.46 *J*(C-C-N-P) = 5.1 Hz and *J*(C-C-N-P) = 2.9 Hz, COO). HR-MS (ESI+): m/z $[M + H]^+$ calculated for: $C_{33}H_{42}FN_6O_6P$, 669.2960, found: 669.2958.

Bis(L-phenylalanine isopropyl ester) prodrug of 9-[2-(phosphorylmethoxy)ethyl]-7-chloro-7-deazaadenine (6c):

Treatment of **5c** (0.50 g, 1.3 mmol) by Method A afforded **6c** (0.49 g, 56%) as a whitish amorphous solid. ESI-MS, *m*/*z* (%): 685 $[M+H^+]$ (100). ^{1}H NMR (DMSO-d_6): δ 1.00, 1.06, 1.11 and 1.16 (4 x d, *J*(CH_3, CH) = 6.3 Hz, 12H, CH_3); 2.71–2.88 (m, 4H, $PhCH_2$); 3.19–3.29 (m, 2H, P-CH_2); 3.63 (m, 2H, H-2'); 3.83–3.96 (m, 2H, CH-NH); 4.11 (m, 1H, NH); 4.21 (m. 2H, H-1'); 4.41 (m, 1H, NH); 4.77 (sept, *J*(CH, CH_3) = 6.3 Hz, 2H, CH*i*Pr); 4.82 (sept, *J*(CH, CH_3) = 6.3 Hz, 2H, CH*i*Pr); 6.78 (brs, 2H, NH_2); 7.07 (m, 10 H, Ph-ortho, meta, para); 7.36 (s, 1H, H-8); 8.09 (s, 1H, H-2). ^{13}C NMR (DMSO-d_6): δ 21.47, 21.54, 21.60 and 21.67 (CH_3*i*Pr); 40.20 (CH_2Ph); 43.46 (C-1'); 54.05 and 54.20 (NH-CH); 67.48 (d, *J*(C-P) = 135.2 Hz, CH_2-P); 67.98 and 68.12 (CH*i*Pr); 70.93 (d, *J*(2'-P) = 11.5 Hz, C-2'); 99.68 (C-5); 101.17 (C-7); 122.26 (C-8); 126.60 and 126.65 (Ph-para); 128.21 and 128.25 (Ph-meta); 129.63 and 129.64 (Ph-ortho); 137.20 and 137.30 (ipso); 148.70 (C-4); 152.66 (C-2); 156.92 (C-6); 172.32–172.47 m (COO). HR-MS (ESI+): m/z $[M + H]^+$ calculated for: $C_{33}H_{43}N_6O_6ClP$, 685.2665, found: 685.2667.

Bis(L-phenylalanine isopropyl ester) prodrug of 9-[2-(phosphorylmethoxy)ethyl]-7-bromo-7-deazaadenine (6d):

Treatment of **5d** (0.50 g, 1.2 mmol) by Method A afforded **6d** (0.31 g, 37%) as a whitish amorphous solid. ESI-MS, *m*/*z* (%): 729 $[M+H^+]$ (100). ^{1}H NMR (DMSO-d_6): δ 1.00, 1.06, 1.11 and 1.16 (4 x d, *J*(CH_3, CH) = 6.2 Hz, 12H, CH_3); 2.71–2.88 (m, 4H, $PhCH_2$); 3.18–3.29 (m, 2H, P-CH_2); 3.64 (m, 2H, H-2'); 3.83–3.96 (m, 2H, CH-NH); 4.10 (m, 1H, P-NH); 4.22 (m, 2H, H-1'); 4.41 (m, 1H, P-NH); 4.77 (sept, *J*(CH, CH_3) = 6.3 Hz, 2H, CH*i*Pr); 4.82 (sept, *J*(CH, CH_3) = 6.3 Hz, 2H, CH*i*Pr); 6.71 (brs, 2H, NH_2); 7.08–7.26 (m, 10H, Ph-ortho, meta, para); 7.42 (s, 1H, H-8); 8.10 (s, 1H, H-2). ^{13}C NMR (DMSO-d_6): δ 21.47, 21.55, 21.61 and 21.68 (CH_3*i*Pr); 40.21 (CH_2Ph); 43.58 (C-1'); 54.05 and 54.21 (NH-CH); 67.47 (d, *J*(C-P) = 135.3 Hz, CH_2-P); 67.98 and 68.12 (CH*i*Pr); 70.91 (d, *J*(2'-P) = 11.4 Hz, C-2'); 85.12 (C-7); 100.87 (C-5); 124.77 (C-8); 126.60 and 126.65 (Ph-para); 128.21 and 128.25 (Ph-meta); 129.25 and 129.64 (Ph-ortho); 137.20 and 137.30 (ipso); 149.19 (C-4); 152.47 (C-2); 157.09 (C-6); 172.32–172.47 m (COO). HR-MS (ESI+): m/z $[M + H]^+$ calculated for: $C_{33}H_{43}BrN_6O_6P$, 729.2160, found: 729.2163.

Bis(L-phenylalanine isopropyl ester) prodrug of 9-[2-(phosphorylmethoxy)ethyl]-7-iodo-7-deazaadenine (6e):

Treatment of **5e** (0.83 g, 1.7 mmol) by Method A afforded **6e** (0.48 g, 62%) as a whitish amorphous solid. ESI-MS, *m*/*z* (%): 777 $[M+H^+]$ (100). ^{1}H NMR (DMSO-d_6): δ 1.01, 1.06, 1.11 and 1.16 (d, *J*(CH_3, CH) = 6.3 Hz, 12H, CH_3); 2.71–2.73 (m, 4H, $PhCH_2$); 3.20 (dd, *J*(CH_2b, P) = 8.2 Hz, *J*(gem) = 13.2 Hz, 1H, P-CH_2b); 3.27 (dd, *J*(CH_2a, P) = 7.9 Hz, *J*(gem) = 13.2 Hz, 1H, P-CH_2a); 3.64 (m, 2H, H-2'); 3.83–3.96 (m, 2H, CH-NH); 4.08 (m, 1H, P-NH); 4.22 (m, 2H, H-1'); 4.40 (m, 1H, P-NH); 4.78 (sept, *J*(CH, CH_3) = 6.2 Hz, 2H, CH*i*Pr); 4.82 (sept, *J*(CH, CH_3) = 6.2 Hz, 2H, CH*i*Pr); 6.59 (brs, 2H, NH_2); 7.07–7.25 (m, 10H, Ph-

ortho, meta, para); 7.44 (s, 1H, H-8); 8.10 (s, 1H, H-2). ^{13}C NMR (DMSO-d_6): δ 21.49, 21.56, 21.62 and 21.69 (CH_3*i*Pr); 40.21 (CH_2Ph); 43.65 (C-1'); 49.92 (C-7); 54.05 and 54.21 (NH-CH); 67.46 (d, *J*(C-P) = 135.4 Hz, CH_2-P); 67.98 and 68.12 (CH*i*Pr); 70.95 (d, *J*(2'-P) = 11.4 Hz, C-2'); 103.08 (C-5); 126.59 and 126.64 (Ph-para); 128.21 and 128.25 (Ph-meta); 129.65 (Ph-ortho); 130.00 (C-8); 137.19 and 137.29 (ipso); 149.83 (C-4); 151.98 (C-2); 157.32 (C-6); 172.32–172.47 m (COO). HR-MS (ESI+): m/z $[M + H]^+$ calculated for: $C_{33}H_{43}IN_6O_6P$, 777.2021, found: 777.2022.

6-Chloro-9-iodo-9-deazapurine (8):[50]

N-Iodosuccinimide (8.06 g, 35.8 mmol) was added to a solution of 6-chloro-9-deazapurine **7** (5.0 g, 32.6 mmol) in dry THF (70 mL) under Ar at RT. The reaction mixture was strirred at RT for 1 h. Volatiles were removed and the residue was dissolved in $CHCl_3$ (250 mL) and washed with H_2O (100 mL), 10% $Na_2S_2O_3$ (100 mL), brine (100 mL) and then dried over Na_2SO_4. The solvent was removed *in vacuo* to give **8** (7.70 g, 85%) as white solid, which was used in the next step without further purification The analytical data are identical with reported data.[50,60]

6-Chloro-9-iodo-7-((2-(trimethylsilyl)ethoxy)methyl)-9-deazapurine (9):

NaH (60% susp. in mineral oil, 1.01 g, 27.5 mmol) was added to a solution of **8** (5.64 g, 20.2 mmol) in DMF (75 mL) under Ar at RT. After 40 min, SEMCl (5.36 mL, 30.2 mmol) was added to the mixture at RT. The resulting mixture was stirred at RT overnight, then sat. NH_4Cl (5 mL) was added. Solvents were removed, the residue was dissolved in EtOAc (300 mL), extracted with H_2O (2 × 50 mL) and brine (50 mL) and then dried over Na_2SO_4. Volatiles were removed and the residue crystalized from hexan/EtOAc to give **9** (7.8 g, 94%) as a white amorphous solid. ESI-MS, *m*/*z* (%): 410 $[M+H^+]$ (100). ^{1}H NMR (DMSO-d_6): −0.12 (s, 9H, CH_3); 0.79 (t, *J*(CH_2, CH_2) = 7.8 Hz, 2H, Si-CH_2); 3.50 (t, *J*(CH_2, CH_2) = 7.8 Hz, 2H, Si-CH_2-CH_2); 5.76 (s, 2H, N-CH_2); 8.42 (s, 1H, H-8); 8.75 (s, 1H, H-2). ^{13}C NMR (DMSO-d_6): δ −1.57 (CH_3); 16.90 (Si-CH_2); 58.90 (C-9); 65.27 (Si-CH_2-CH_2); 76.72 (N-CH_2); 123.30 (C-5); 141.89 (C-6); 142.29 (C-8); 150.24 (C-2); 152.67 (C-4). HR-MS (ESI +): m/z $[M + H]^+$ calculated for: $C_{12}H_{18}ClIN_3OSi$, 409.9947, found: 409.9945.

6-Chloro-9-vinyl-7-((2-(trimethylsilyl)ethoxy)methyl)-9-deazapurine (10):

Tributyl(vinyl)tin (1.94 mL, 6.6 mmol) was added to a solution of **9** (2.3 g, 5.6 mmol) and Pd(*t*-$Bu_3P)_2$ (0.1 g, 0.2 mmol) in dioxane (20 mL) under Ar. The resulting mixture was stirred at RT for 24 h, cooled to RT and sat. NH_4Cl (5 mL) and EDTA (10 mL) were added. After 15 min, EtOAc (250 mL) was added to the stirred slurry. The resulting mixture was filtered over Cellite. The organic layer was separated and washed with sat. EDTA solution (50 mL), H_2O (50 mL), brine (50 mL) and then dried over Na_2SO_4. Volatiles were removed *in vacuo*, and the residue was purified by flash chromatography (Hex:EtOAc 0–70%) to give **10** (1.2 g, 69%) as a yellowish oil. ESI-MS, *m*/*z* (%): 310 $[M+H^+]$ (100). ^{1}H NMR (DMSO-d_6): δ −0.12 (s, 9H, CH_3); 0.80 (t, *J*(CH_2, CH_2) = 7.8 Hz, 2H, Si-CH_2); 3.50 (t, *J*(CH_2, CH_2) = 7.8 Hz, 2H, Si-CH_2-CH_2); 5.34 (dd, *J*(H-C=C-H) = 11.3 Hz, *J*(gem) = 2.2 Hz 1H, CH_2=CH); 5.76 (s, 2H, N-CH_2); 6.30 (dd, *J*(H-C=C-H) = 17.7 Hz, *J*(gem) = 2.2 Hz, 1H, CH_2=CH); 6.83 (dd, *J*(H-C=C-H) = 17.7 Hz, *J*(H-C=C-H) = 11.4 Hz, 1H, CH_2=CH); 8.28

(s, 1H, H-8); 8.74 (s, 1H, H-2). ^{13}C NMR (DMSO-d_6): δ −1.25 (CH_3); 17.22 (Si-CH_2); 65.49 (Si-CH_2-$\underline{CH_2}$); 76.88 (N-CH_2); 114.11 (C-9); 115.18 ($\underline{CH_2}$=CH); 123.98 (C-5); 126.51 (CH_2=$\underline{CH}$); 137.53 (C-8); 142.18 (C-6); 150.30 (C-2); 150.49 (C-4). HR-MS (ESI+): m/z $[M + H]^+$ calculated for: $C_{14}H_{21}ClN_3OSi$, 310.1137, found: 310.1134.

6-Chloro-7-((2-(trimethylsilyl)ethoxy)methyl)-9-(2-hydroxyethyl)-9-deazapurine (11):

Treatment of **10** (1.2 g, 3.9 mmol) by Method C afforded **11** (1.0 g, 79%) as yellowish oil. ESI-MS, *m*/*z* (%): 350 $[M+Na^+]$ (100). ^{1}H NMR (DMSO-d_6): δ −0.12 (s, 9H, CH_3); 0.80 (t, *J*(CH_2, CH_2) = 7.8 Hz, 2H, Si-CH_2); 2.88 (t, *J*(CH_2, CH_2) = 7.0 Hz, 2H, H-1'); 3.47 (t, *J*(CH_2, CH_2) = 7.8 Hz, 2H, Si-CH_2-$\underline{CH_2}$); 3.68 (dt, *J*(CH_2, CH_2) = 7.0 Hz, *J* = 5.4 Hz, H-2'); 5.74 (s, 2H, N-CH_2); 8.00 (s, 1H, H-8); 8.66 (s, 1H, H-2). ^{13}C NMR (DMSO-d_6): δ −1.56 (CH_3); 16.98 (Si-CH_2); 26.87 (C-1'); 60.50 (C-2'); 65.00 (Si-CH_2-$\underline{CH_2}$); 76.21 (N-CH_2); 113.07 (C-9); 122.93 (C-5); 137.12 (C-8); 141.32 (C-6); 149.13 (C-2); 151.76 (C-4). HR-MS (ESI+): m/z $[M + H]^+$ calculated for: $C_{14}H_{22}ClN_3O_2Si$, 350.1062, found: 350.1066.

9-{2-[(Diisopropoxyphosphoryl)methoxy]ethyl}−6-chloro-7-((2-(trimethylsilyl)ethoxy)methyl)-9-deazapurine (12):

Treatment of **11** (3.0 g, 9.2 mmol) by Method D afforded recovered starting material **11** (1.40 g, 46%) and **12** (2.38 g, 51%) as yellowish oils. ESI-MS, *m*/*z* (%): 506 $[M+H^+]$ (75); 528 $[M+Na^+]$ (100). ^{1}H NMR (DMSO-d_6): δ −0.11 (s, 9H, CH_3); 0.80 (m, 2H, Si-CH_2); 1.16 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 1.20 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 2.98 (td, *J*(1', 2') = 6.8 Hz, *J*(1', 8) = 0.8 Hz, 2H, H-1'); 3.49 (m, 2H, Si-CH_2-$\underline{CH_2}$); 3.75 (d, *J*(H-C-P) = 8.3 Hz, 2H, P-CH_2); 3.82 (t, *J*(2', 1') = 6.8 Hz, 2H, H-2'); 4.54 (d septet, *J*(H-C-O-P) = 7.7 Hz, *J*(CH, CH_3) = 6.2 Hz, 2H, CH*i*Pr); 5.73 (s, 2H, N-CH_2); 8.02 (t, *J*(8, 1') = 0.8 Hz, 1H, H-8); 8.67 (s, 1H, H-2). ^{13}C NMR (DMSO-d_6): δ −1.28 (Si-CH_3); 17.26 (Si-CH_2); 23.57 (C-1'); 23.78 (d, *J*(C-C-O-P) = 4.5 Hz, CH_3*i*Pr); 23.94 (d, *J*(C-C-O-P) = 3.8 Hz, CH_3*i*Pr); 64.79 (d, *J*(C-P) = 164.7 Hz, CH_2-P); 65.34 (Si-CH_2-$\underline{CH_2}$); 70.24 (d, *J*(C-O-P) = 6.4 Hz, CH*i*Pr); 71.85 (d, *J*(2'-P) = 11.8 Hz, C-2'); 76.55 (N-CH_2); 112.61 (C-9); 123.22 (C-5); 137.39 (C-8); 141.64 (C-6); 149.51 (C-2); 151.92 (C-4). HR-MS (ESI+): m/z $[M + H]^+$ calculated for: $C_{21}H_{38}ClN_3O_5PSi$, 506.2001, found: 506.2004.

9-{2-[(Diisopropoxyphosphoryl)methoxy]ethyl}−7-((2-(trimethylsilyl)-ethoxy)methyl)-9-deazaadenine (13):

Compound **12** (1.0 g, 2.0 mmol), was dissolved ina EtOH/NH_3 mixture (40 mL) and stirred at 100 °C for 16 h. Volatiles were removed and the residue was purified by C18 reversed phase chromatography (H_2O:MeOH 0–100%) to give **13** (0.71 g, 74%) as a white amorphous solid. ESI-MS, *m*/*z* (%): 487 $[M+H^+]$ (100). ^{1}H NMR (DMSO-d_6): δ −0.06 (s, 9H, CH_3); 0.85 (m, 2H, Si-CH_2); 1.19 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 1.22 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 2.86 (td, *J*(1', 2') = 7.0 Hz, *J*(1', 8) = 0.8 Hz, 2H, H-1'); 3.49 (m, 2H, Si-CH_2-$\underline{CH_2}$); 3.74 (d, *J*(H-C-P) = 8.3 Hz, 2H, P-CH_2); 3.76 (t, *J*(2', 1') = 7.1 Hz, 2H, H-2'); 4.56 (d septet, *J*(H-C-O-P) = 7.7 Hz, *J*(CH, CH_3) = 6.2 Hz, 2H, CH*i*Pr); 5.54 (s, 2H, N-CH_2); 6.58 (brs, 2H, NH_2); 7.46 (t, *J*(8, 1') = 0.8 Hz, 1H, H-8); 8.67 (s, 1H, H-2). ^{13}C NMR (DMSO-d_6): δ −1.27 (Si-CH_3); 17.27 (Si-CH_2); 23.82 (C-1'); 23.82 (d *J*(C-C-O-P) = 4.3 Hz, CH_3*i*Pr); 23.96 (d, *J*(C-C-O-P) = 3.8 Hz, CH_3*i*Pr); 64.79 (d, *J*(C-P) = 164.6 Hz,

CH_2-P); 64.89 (Si-CH_2-$\underline{CH_2}$); 70.23 (d, *J*(C-O-P) = 6.4 Hz, CH*i*Pr); 72.32 (d, *J*(2'-P) = 11.8 Hz, C-2'); 76.90 (N-CH_2); 111.10 (C-9); 114.21 (C-5); 131.45 (C-8); 148.57 (C-4); 150.40 (C-2); 150.97 (C-6). HR-MS (ESI+): m/z $[M + H]^+$ calculated for: $C_{21}H_{40}N_4O_5PSi$, 487.2500, found: 487.2495.

9-[2-(Phosphonomethoxy)ethyl]-9-deazaadenine (14):

Compound **13** (0.5 g, 1 mmol) was suspended in H_2O (20 mL) and conc. HCl (0.46 mL, 5.15 mmol). The reaction mixture was heated to 130 °C for 40 min in microwave reactor. Volatiles were removed and the residue was codistilled with H_2O (2 × 5 mL) and dissolved in 3% aq. NH_3 (1 mL). The solution was applied on Dowex 1 × 2 (acetate form) column and eluted with H_2O and grad. 0.05 M-1M aq. acetic acid. The product containing UV-absorbing fractions were collected, evaporated and codistilled with water (2 × 5 mL). Crystallization from H_2O gave **14** (0.16 g, 56%) as white crystals, m.p. >250 °C (dec.). ESI-MS, *m*/*z* (%): 273 $[M+H^+]$ (81); 295 $[M+Na^+]$ (100). ^{1}H NMR (DMSO-d_6): δ 2.94 (td, *J*(1', 2') = 6.9 Hz, *J*(1', 8) = 0.8 Hz, 2H, H-1'); 3.53 (d, *J*(H-C-P) = 8.6 Hz, 2H, P-CH_2); 3.82 (t, *J*(2', 1') = 7.1 Hz, 2H, H-2'); 7.39 (t, *J*(8, 1') = 0.8 Hz, 1H, H-8); 8.05 (s, 1H, H-2). ^{13}C NMR (DMSO-d_6): δ 24.11 (C-1'); 69.36 (d, *J*(C-P) = 150.2 Hz, CH_2-P); 72.86 (d, *J*(2'-P) = 10.1 Hz, C-2'); 111.95 (C-9); 114.83 (C-5); 129.87 (C-8); 145.46 (C-4); 149.77 (C-2); 150.99 (C-6). HR-MS (ESI+): m/z $[M + H]^+$ calculated for: $C_9H_{14}N_4O_4P$, 273.0747, found: 273.0744.

Bis(L-phenylalanine isopropyl ester) prodrug of 9-[2-(phosphorylmethoxy)ethyl]-9-deazaadenine (15):

Bromotrimethyl-silane (1.0 mL) was added to **13** (0.5 g, 1 mmol) in dry pyridine (10 mL) and the resulting mixture was stirred at RT overnight. Iodotrimethylsilane (1.0 mL) was added and the reaction mixture was stirred for additional 2 h. Subsequent treatment by Method A afforded **15** (0.29 g, 44%) as a whitish amorphous solid. ESI-MS, *m*/*z* (%): 651 $[M+H^+]$ (100). ^{1}H NMR (DMSO-d_6): δ 1.03, 1.08, 1.12 and 1.17 (d, *J*(CH_3, CH) = 6.2 Hz, 12H, CH_3); 2.75–2.91 (m, 6H, CH_2Ph, H-1'); 3.24–3.33 (m, 2H, P-CH_2); 3.59 (m, 2H, H-2'); 3.89 and 3.96 (2x m, 2H, NH-$\underline{CH}$); 4.08 (dd, *J*(NH-P) = 12.1 Hz, *J*(NH-CH) = 10.5 Hz, P-NH); 4.40 (dd, *J*(NH-P) = 11.8 Hz, *J*(NH-CH) = 10.7 Hz, P-NH); 4.80 (sept, *J*(CH, CH_3) = 6.3 Hz, 2H, CH*i*Pr); 4.83 (sept, *J*(CH, CH_3) = 6.3 Hz, 2H, CH*i*Pr); 6.62 (brs, 2H, NH_2); 7.12–7.27 (m, 10H, Ph); 7.32 (dt, *J*(8, 7) = 2.8 Hz, *J*(8, 1') = 0.8 Hz, 1H, H-8); 8.09 (s, 1H, H-2); 10.65 (brs, 1H, H-7). ^{13}C NMR (DMSO-d_6): δ 21.48, 21.55, 21.61 and 21.67 (CH_3*i*Pr); 24.14 (C-1'); 39.90 (CH_2Ph); 54.10 and 54.11 (NH-CH); 67.57 (d, *J*(C-P) = 134.5 Hz, CH_2-P); 67.98 and 68.11 (CH*i*Pr); 72.61 (d, *J*(2'-P) = 11.9 Hz, C-2'); 111.34 (C-9); 113.38 (C-5); 125.98 (C-8); 126.63 (Ph-para); 128.24 (Ph-meta); 129.65 (Ph-ortho); 137.15 and 137.32 (ipso); 146.04 (C-4); 150.05 (C-2); 150.46 (C-6); 172.27–172.49 m, (COO). HR-MS (ESI+): m/z $[M + H]^+$ calculated for: $C_{33}H_{44}N_6O_6P$, 651.3055, found: 651.3053.

9-{2-[(Diisopropoxyphosphoryl)methoxy]ethyl}−7-deaza-8-azaadenine (17a):

Treatment of 6-amino-7-deaza-8-azapurine (**16a**, 1.0 g, 7.4 mmol) according to the lit.[54] afforded **17a** (1.4 g, 53%) as a yellowish oil. ESI-MS, *m*/*z* (%): 358 $[M+H^+]$ (100). ^{1}H NMR (DMSO-d_6): δ 1.20 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 1.25 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 3.73 (d, *J*(CH_2, P) = 8.5 Hz, 2H, P-CH_2); 4.07 (t, *J*(1', 2') = 5.6 Hz, 2H, H-1');

4.59 (t, *J*(2', 1') = 5.6 Hz, 2H, H-2'); 4.68–4.56 (m, 2H, CH*i*Pr); 6.07 (brs, 2H, NH_2); 7.97 (s, 1H, H-8); 8.35 (s, 1H, H-2). ^{13}C NMR (DMSO-d_6): δ 23.81, 23.86, 23.98 and 24.01 (CH_3*i*Pr); 46.48 (C-1'); 65.74 (d, *J*(C-P) = 167.3 Hz, CH_2-P); 70.89 (d, *J*(2'-P) = 11.3 Hz, C-2'); 71.16 (d, *J*(C-O-P) = 6.6 Hz, CH*i*Pr); 100.65 (C-5); 131.11 (C-7); 153.92 (C-4); 155.87 (C-2); 157.61 (C-6). HR-MS (ESI+): m/z [M + H]$^+$ calculated for: $C_{14}H_{25}N_5O_4P$, 358.1639, found: 358.1639.

9-{2-[(Diisopropoxyphosphoryl)methoxy]ethyl}−8-azaadenine (17b):

Diisopropyl ((2-chloroethoxy)methyl)phosphonate[55] (2.65 g, 10.3 mmol) dissolved in DMF (4 mL) was added to a preheated (80 °C) reaction mixture of 8-azaadenine (**16a**, 1.0 g, 7.3 mmol) and DBU (2.63 mL, 17.2 mmol) in DMF (8 mL). The resulting mixture was heated to 100 °C overnight. The clear solution was cooled down and volatiles were removed *in vacuo*. The residue was codistilled with toluene (2 × 5 mL) and purified by column chromatography EtOH:$CHCl_3$ (0–10%) to give **17b** (0.85 g, 32%) and the corresponding 8-regioisomer **(**0.85 g, 32%) as yellowish oils. Compound **17b**: ESI-MS, *m*/*z* (%): 359 [M+H$^+$] (11); 381 [M+Na$^+$] (100). ^{1}H NMR (DMSO-d_6): δ 1.04 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 1.11 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 3.74 (d, *J*(H-C-P) = 8.4 Hz, 2H, P-CH_2); 4.06 (m, 2H, H-2'); 4.40 (d septet, *J*(CH, CH_3) = 6.2 Hz, *J*(H-C-O-P) = 7.7 Hz, 2H, CH*i*Pr); 4.71 (m, 2H, H-1'); 8.28 (s, 1H, H-2); 8.06 and 8.41 (brs, 2H, NH_2). ^{13}C NMR (DMSO-d_6): δ 23.66 (d *J*(C-C-O-P) = 4.6 Hz, CH_3*i*Pr); 23.87 (d *J*(C-C-O-P) = 3.8 Hz, CH_3*i*Pr); 46.03 (C-1'); 64.66 (d, *J*(C-P) = 163.7 Hz, CH_2-P); 69.87 (d, *J*(2'-P) = 12.1 Hz, C-2'); 70.33 (d, *J*(C-O-P) = 6.4 Hz, C-2'); 123.98 (C-5); 149.33 (C-4); 156.40 (C-6); 156.83 (C-2). HR-MS (ESI+): m/z [M + H]$^+$ calculated for: $C_{13}H_{24}N_6O_4P$, 359.1591, found: 359.1592.

Bis(L-phenylalanine isopropyl ester) prodrug of 9-[2-(phosphorylmethoxy)ethyl]-7-deaza-8-azaadenine (18a):

Treatment of **17a** (0.70 g, 2.0 mmol) by Method A afforded **18a** (0.51 g, 40%) as a whitish amorphous solid. ESI-MS, *m*/*z* (%): 652 [M+H$^+$] (48); 674 [M+Na$^+$] (100). ^{1}H NMR (DMSO-d_6): δ 1.00, 1.05, 1.11 and 1.15 (4 x d, *J*(CH_3, CH) = 6.3 Hz, 12H, CH_3); 2.71 (dd, *J*(gem) = 13.4 Hz, *J*(CH_2-CH) = 8.2 Hz, 1H, $PhCH_2$); 2.74–2.86 (m, 3H, $PhCH_2$); 3.23 (dd, *J*(CH_2b, P) = 8.0 Hz, *J*(gem) = 13.3 Hz, 1H, P-CH_2b); 3.27 (dd, *J*(CH_2a, P) = 8.3 Hz, *J*(gem) = 13.3 Hz, 1H, P-CH_2a); 3.77 (m, 2H, H-2'); 3.83 (m, 1H, CH-NH); 3.91 (m, 1H, CH-NH); 4.04 (dd, *J*(NH, P) = 12.0 Hz, *J*(NH, CH) = 10.7 Hz, 1H, P-NH); 4.30–4.45 (m, 3H, H-1', P-NH); 4.77 (sept, *J*(CH, CH_3) = 6.3 Hz, 2H, CH*i*Pr); 4.80 (sept, *J*(CH, CH_3) = 6.3 Hz, 2H, CH*i*Pr); 7.08 and 7.15 (2x m, 2H, Ph-ortho); 7.16–7.28 (m, 6H, Ph-meta, para); 7.70 (brm, 2H, NH_2); 8.09 (s, 1H, H-8); 8.16 (s, 1H, H-2). ^{13}C NMR (DMSO-d_6): δ 21.47, 21.54, 21.61 and 21.67 (CH_3*i*Pr); 40.10 and 40.20 (CH_2Ph); 45.79 (C-1'); 54.09 and 54.14 (NH-CH); 67.30 (d, *J*(C-P) = 135.0 Hz, CH_2-P); 67.98 and 68.09 (CH*i*Pr); 70.24 (d, *J*(2'-P) = 11.4 Hz, C-2'); 100.25 (C-5); 126.62 and 126.65 (Ph-para); 128.24 and 128.25 (Ph-meta); 129.65 (Ph-ortho); 132.28 (C-7); 137.15 and 137.30 (ipso); 153.49 (C-4); 156.12 (C-2); 158.28 (C-6); 172.25 (d, *J*(C-C-N-P) = 5.1 Hz, COO); 172.43 (d, *J*(C-C-N-P) = 3.0 Hz, COO). HR-MS (ESI+): m/z [M + H]$^+$ calculated for: $C_{32}H_{43}N_7O_6P$, 652.3007, found: 652.3007.

Bis(L-phenylalanine isopropyl ester) prodrug of 9-[2-(phosphorylmethoxy)ethyl]-8-azaadenine (18b):

Treatment of **17b** (0.46 g, 1.3 mmol) by Method A afforded **18b** (0.38 g, 45%) as a whitish amorphous solid. ESI-MS, *m*/*z* (%): 653 [M+H$^+$] (10); 675 [M+Na$^+$] (100). ^{1}H NMR (DMSO-d_6): δ 1.00, 1.05, 1.10 and 1.14 (4 x d, *J*(CH$_3$, CH) = 6.3 Hz, 12H, CH$_3$); 2.68–2.85 (m, 4H, PhCH$_2$); 3.24–3.33 (m, 2H, P-CH$_2$); 3.79–3.96 (m, 4H, H-2', CH-NH); 4.02 (m, 1H, P-NH); 4.38 (m, 1H, P-NH); 3.65 (m, 2H, H-2'); 4.72–4.81 (m, 2H, CH*i*Pr); 7.06–7.26 (m, 10H, Ph-ortho, meta, para); 8.26 (s, 1H, H-2); 8.07 and 8.40 (2x brs, 1H, NH$_2$). ^{13}C NMR (DMSO-d_6): δ 21.45, 21.52, 21.59 and 21.65 (CH$_3$*i*Pr); 40.16 (CH$_2$Ph); 45.98 (C-1'); 54.05 and 54.09 (NH-CH); 67.35 (d, *J*(C-P) = 135.6 Hz, CH$_2$-P); 67.97 and 68.08 (CH*i*Pr); 69.85 (d, *J*(2'-P) = 10.9 Hz, C-2'); 124.01 (C-5); 126.59 and 126.64 (Ph-para); 128.21 and 128.24 (Ph-meta); 129.62 (Ph-ortho); 137.10 and 137.27 (ipso); 149.29 (C-4); 156.43 (C-6); 156.93 (C-2); 172.21 (d, *J*(C-C-N-P) = 5.2 Hz, COO); 172.40 (d, *J*(C-C-N-P) = 2.9 Hz, COO). HR-MS (ESI+): m/z [M + H]$^+$ calculated for: $C_{31}H_{42}N_8O_6P$, 653.2959, found: 653.6962.

4-Chloro-7-vinylthieno[3,2-*d*]pyrimidine (20):

Tributyl(vinyl)tin (7.7 mL, 26.3 mmol) was added to a solution of 7-bromo-4-chlorothieno[3,2-d]pyrimidine (**19**, 4.19 g, 16.9 mmol), Pd(*t*-Bu$_3$P)$_2$ (0.37 g, 0.72 mmol) and CuI (0.48 g, 2.5 mmol) in dioxane (54 mL) under Ar. The resulting mixture was heated to 120 °C for 24 h, cooled to RT and aq. solution of KF (4.0 g in 30 mL) was added. After 15 min, EtOAc (100 mL) was added to the resulting stirred slurry. The resulting mixture was filtered over Cellite after additional 15 min. The organic layer was separated and washed with sat. EDTA solution (50 mL), H$_2$O (50 mL), brine (30 mL) and then dried over Na$_2$SO$_4$. Volatiles were removed *in vacuo*, and the residue was purified by flash chromatography (grad. Hex:CHCl$_3$ 0–100% and subsequent grad. MeOH v CHCl$_3$ 0–10%) to give **20** (2.33 g, 50%) as a yellow oil. ESI-MS, *m*/*z* (%): 195 [M$^+$] (100). ^{1}H NMR (DMSO-d_6): δ ppm 5.55 (dd, *J*(vic) = 11.3 Hz, *J*(gem) = 1.7 Hz, 1H, CH$_2$b); 6.48 (dd, *J*(vic) = 17.8 Hz, *J*(gem) = 1.7 Hz, 1H, CH$_2$a); 7.04 (ddm, *J*(vic) = 17.8 Hz, *J*(vic) = 11.3 Hz, 1H, CH=); 8.62 (s, 1H, H-8); 9.11 (s, 1H, H-2). ^{13}C NMR (DMSO-d_6): δ 118.47 (CH$_2$=CH-); 127.80 (CH$_2$=CH-); 131.02 (C-5); 133.95 (C-9); 135.42 (C-8); 154.19 (C-6); 154.53 (C-2); 159.34 (C-4). HR-MS (EI+): m/z [M]$^+$ calculated for: $C_8H_5N_2SCl$, 195.9862, found: 195.9861.

4-Chloro-7-(2-hydroxyethyl)thieno[3,2-*d*]pyrimidine (21):

Treatment of **20** (1.3 g, 6.7 mmol) by Method C (additional 9-BBN (26.8 mL, 13.4 mmol) was added after 3 h at RT and the reaction mixture was stirred overnight; column chromatography in grad. MeOH:CHCl$_3$ 0–10%) afforded **21** (0.91 g, 64%) as a yellowish oil. ESI-MS, *m*/*z* (%): 215 [M+H$^+$] (100). ^{1}H NMR (DMSO-d_6): δ 3.04 (td, *J*(1', 2') = 6.7 Hz, *J*(1', 8) = 1.0 Hz, 2H, H-1'); 3.74 (t, *J*(2', 1') = 6.7 Hz, 2H, H-2'); 4.80 (brs, 1H, OH); 8.27 (t, *J*(8, 1') = 1.0 Hz, 1H, H-8); 9.05 (s, 1H, H-2). ^{13}C NMR (DMSO-d_6): δ 31.01 (C-1'); 60.04 (C-2'); 130.46 (C-5); 135.13 (C-8); 135.47 (C-9); 154.03 (C-6); 154.21 (C-2); 160.98 (C-4). HR-MS (ESI+): m/z [M + H]$^+$ calculated for: $C_8H_8N_2OClS$, 215.0040, found: 215.0040.

4-Chloro-7-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}thieno[3,2-*d*]pyrimidine (22):

Treatment of **21** (0.60 g, 2.8 mmol) by Method D afforded **22** (0.58 g, 53%) as yellowish oil, which was used in the next reaction step without further purification and characterization.

4-Amino-7-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}thieno[3,2-*d*]pyrimidine (23):

Compound **22** (0.86 g, 2.2 mmol) was dissolved in EtOH/NH_3 (50 mL) and stirred at 100 °C for 24 h. The solvent was removed and the residue was purified by C18 reversed phase chromatography (H_2O:MeOH 0–100%) to give **23** (0.54 g, 66%) as a yellowish oil. ESI-MS, *m*/*z* (%): 374 [M+H^+] (36); 396 [M+Na^+] (100). ^{1}H NMR (DMSO-d_6): δ 1.17 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 1.20 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 3.02 (td, *J*(1', 2') = 6.7 Hz, *J*(1', 8) = 1.0 Hz, 2H, H-1'); 3.75 (d, *J*(H-C-P) = 8.4 Hz, 2H, P-CH_2); 3.82 (t, *J*(2', 1') = 6.7 Hz, 2H, H-2'); 4.54 (d septet, *J*(H-C-O-P) = 7.7 Hz, *J*(CH, CH_3) = 6.2 Hz, 2H, CH*i*Pr); 7.38 (brs, 2H, NH_2); 7.82 (t, *J*(8, 1') = 1.0 Hz, 1H, H-8); 8.38 (s, 1H, H-2). ^{13}C NMR (DMSO-d_6): δ 23.84 (d, *J*(C-C-O-P) = 4.5 Hz, CH_3*i*Pr); 23.98 (d, *J*(C-C-O-P) = 3.7 Hz, (CH_3*i*Pr); 27.46 (C-1'); 64.73 (d, *J*(C-P) = 164.4 Hz, CH_2-P); 70.28 (d, *J*(C-O-P) = 6.3 Hz, CH*i*Pr); 71.46 (d, *J*(C-O-C-P) = 12.3 Hz, C-2'); 114.19 (C-5); 128.96 (C-8); 133.83 (C-9); 154.69 (C-2); 158.56 (C-4); 158.66 (C-6). HR-MS (ESI+): m/z [M + H]$^+$ calculated for: $C_{15}H_{25}N_3O_4PS$, 374.1298, found: 374.1298.

Bis(L-phenylalanine isopropyl ester) prodrug of (4-aminothieno[3,2-*d*]pyrimidin-7-yl)ethoxy)methyl)phosphonic acid (24):

Treatment of **23** (0.27 g, 0.7 mmol) by Method A afforded **24** (0.25 g, 51%) as a whitish amorphous solid. ESI-MS, *m*/*z* (%): 668 [M+H^+] (35); 690 [M+Na^+] (100). ^{1}H NMR (DMSO-d_6): δ 1.02, 1.07, 1.12 and 1.16 (4 x d, *J*(CH_3, CH) = 6.3 Hz, 12H, CH_3); 2.74–2.90 (m, 4H, PhCH_2); 2.97 (td, *J*(CH_2-CH_2) = 6.7 Hz, *J*(1', 8) = 0.9 Hz, 2H, H-1'); 3.23–3.32 (m, 2H, P-CH_2); 3.64 (t, *J*(2', 1') = 7.0 Hz, 2H, H-2'); 3.85–4.00 (m, 2H, NH-CH); 4.10 (dd, *J*(NH-P) = 11.9 Hz, *J*(NH-CH) = 10.5 Hz, 1H, P-NH); 4.42 (m, 1H, P-NH); 4.79 (sept, *J*(CH, CH_3) = 6.3 Hz, 2H, CH*i*Pr); 4.82 (sept, *J*(CH, CH_3) = 6.3 Hz, 2H, CH*i*Pr); 7.10–7.26 (m, 10H, Ph-ortho, meta, para); 7.38 (brs, 2H, NH_2); 7.81 (t, *J*(8, CH_2) = 1.0 Hz, 1H, H-8); 8.39 (s, 1H, H-2). ^{13}C NMR (DMSO-d_6): δ 21.50, 21.56, 21.63 and 21.69 (CH_3*i*Pr); 27.43 (C-1'); 39.53 (CH_2Ph); 54.11 and 54.13 (NH-CH); 67.55 (d, *J*(C-P) = 135.3 Hz, CH_2-P); 68.01 and 68.14 (CH*i*Pr); 71.31 (d, *J*(2'-P) = 12.2 Hz, C-2'); 114.17 (C-5); 126.63 and 126.65 (Ph-para); 128.23 and 128.25 (Ph-meta); 128.96 (C-8); 129.66 and 129.67 (Ph-ortho); 133.90 (C-9); 137.17 and 137.32 (ipso); 154.71 (C-2); 158.56 and 158.66 (C-4 and C-6); 172.32 (d, *J*(C-C-N-P) = 5.3 Hz, COO); 172.50 (d, *J*(C-C-N-P) = 3.0 Hz, COO). HR-MS (ESI+): m/z [M + H]$^+$ calculated for: $C_{33}H_{43}N_5O_6PS$, 668.2666, found: 668.2667.

8-Bromo-4-(2,4-dimethoxybenzyl)aminoquinazoline (26):

DBU (3.4 mL, 22.7 mmol) was added to a solution of 8-bromo-4-oxochinazoline **25** (2.0 g, 8.9 mmol) and BOP (6.0 g, 13.6 mmol) in CH_3CN (60 mL) under Ar. The mixture was stirred at RT for 10 min and then preheated to 80 °C. 2,4-Dimethoxybenzylamin (4 mL, 26.4 mmol) was added and the mixture was stirred at 80 °C overnight. Volatiles were removed after cooling down. The residue was dissolved in EtOAc (300 mL) and washed with brine (60 mL) and dried by Na_2SO_4. The residue was purified by flash chromatography (grad.

MeOH-$CHCl_3$ 0–10%) to give **26** (1.73 g, yield 55%) as a white amorphous solid. ESI-MS, *m*/*z* (%): 374 [M+H$^+$] (100). ^{1}H NMR (DMSO-d_6): δ 3,72 (s, 3H, 4'-OCH_3); 3.82 (s, 3H, 2'-OCH_3); 4.67 (bd, *J*(CH_2-NH) = 5.6 Hz, 2H, $PhCH_2$); 6.44 (dd, *J*(5', 6') = 8.4 Hz, *J*(5', 3') = 2.4 Hz, 1H, H-5'); 6.58 (d, *J*(3', 5') = 2.4 Hz, 1H, H-3'); 7.08 (d, *J*(6', 5') = 8.4 Hz, 1H, H-6'); 7.42 (dd, *J*(6, 5) = 8.3 Hz, *J*(6, 7) = 7.6 Hz, 1H, H-6); 8.12 (dd, *J*(7, 6) = 7.6 Hz, *J*(7, 5) = 1.1 Hz, 1H, H-7); 8.36 (dd, *J*(5, 6) = 8.4 Hz, *J*(5, 7) = 1.1 Hz, 1H, H-5); 8.51 (s, 1H, H-2); 8.76 (brt, *J*(NH-CH_2) = 5.7 Hz, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 39.14 ($PhCH_2$); 55.37 (4'-OCH_3); 55.62 (2'-OCH_3); 98.46 (C-3'); 104.47 (C-5'); 116.50 (C-4a); 118.46 (C-1'); 122.87 (C-8); 122.90 (C-5); 126.44 (C-6); 128.48 (C-6'); 136.21 (C-7); 146.74 (C-8a); 156.20 (C-2); 157.97 (C-2'); 159.80 and 159.88 (C-4' and C-4). HR-MS (ESI+): m/z [M + H]$^+$ calculated for: $C_{17}H_{17}N_3O_2Br$, 374.0499, found: 374.0499.

4-(2,4-Dimethoxybenzyl)amino-8-vinylquinazoline (27):

Tributyl(vinyl)tin (1.9 mL, 6.6 mmol) was added to the solution of **26** (2.0 g, 5.3 mmol), CuI (0.31 g, 1.6 mmol) and Pd(*t*-$Bu_3P)_2$ (0.1 g, 0.2 mmol) in NMP (40 mL) under Ar. The resulting mixture was heated at 120 °C for 24 h, then cooled to RT and an aqueous solution of KF (2.0 g in 15 mL) was added. After 15 min, EtOAc (120 mL) was added to the resulting slurry. After 15 min, the resulting mixture was filtered over Cellite. The organic layer was separated and washed with a sat. EDTA solution (50 mL), H_2O (50 mL), brine (50 mL) and then dried over Na_2SO_4. Volatiles were removed *in vacuo*, and the residue was purified by flash chromatography (grad. Hex:$CHCl_3$ 0–100% and grad. MeOH in $CHCl_3$ 0–10%) to give **27** (0.76 g, 44%) as a white oil. ESI-MS, *m*/*z* (%): 322 [M+H$^+$] (100). ^{1}H NMR (DMSO-d_6): δ 3.72 (s, 3H, 4'-OCH_3); 3.82 (s, 3H, 2'-OCH_3); 4.66 (bd, *J*(CH_2-NH) = 5.7 Hz, 2H, $PhCH_2$); 5.41 (dd, *J*(vic) = 11.2 Hz, *J*(gem) = 1.5 Hz, 1H, CH_2b); 6.00 (dd, *J*(vic) = 18.0 Hz, *J*(gem) = 1.5 Hz, 1H, CH_2a); 6.43 (dd, *J*(5', 6') = 8.4 Hz, *J*(5', 3') = 2.4 Hz, 1H, H-5'); 6.57 (d, *J*(3', 5') = 2.4 Hz, 1H, H-3'); 7.07 (dm, *J*(6', 5') = 8.4 Hz, 1H, H-6'); 7.50 (ddd, *J*(6, 5) = 8.3 Hz, *J*(6, 7) = 7.4 Hz, *J*(6, 8-CH) = 0.5 Hz, 1H, H-6); 7.76 (ddm, *J*(vic) = 18.0 Hz, *J*(vic) = 11.2 Hz, 1H, CH=); 8.05 (ddd, *J*(7, 6) = 7.4 Hz, *J*(7, 5) = 1.3 Hz, *J*(7,8-CH) = 0.5 Hz, 1H, H-7); 8.27 (dd, *J*(5, 6) = 8.4 Hz, *J*(5, 7) = 1.3 Hz, 1H, H-5); 8.46 (s, 1H, H-2); 8.58 (brt, *J*(NH-CH_2) = 5.8 Hz, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 38.85 ($PhCH_2$); 55.37 (4'-OCH_3); 55.62 (2'-OCH_3); 98.44 (C-3'); 104.44 (C-5'); 115.26 (C-4a); 115.84 ($\underline{CH_2}$=CH-); 118.80 (C-1'); 122.55 (C-5); 125.40 (C-6); 128.05 (C-7); 128.32 (C-6'); 132.28 (CH_2=$\underline{CH}$-); 134.07 (C-8); 146.56 (C-8a); 154.78 (C-2); 157.93 (C-2'); 159.81 and 159.84 (C-4' a C-4). HR-MS (ESI+): m/z [M + H]$^+$ calculated for: $C_{19}H_{20}N_3O_2$, 322.1550, found: 322.1550.

4-(2,4-Dimethoxybenzyl)amino-8-(2-hydroxyethyl)quinazoline (28):

Treatment of **27** (0.7 g, 2.2 mmol) by Method C (column chromatography in gradient MeOH:$CHCl_3$ 0–10%) afforded **28** (0.27 g, 44%) as a yellowish oil. ESI-MS, *m*/*z* (%): 340 [M+H$^+$] (100). ^{1}H NMR (DMSO-d_6): δ 3.19 (t, *J*(1', 2') = 7.1 Hz, 2H, H-1'); 3.67 (t, *J*(2', 1') = 7.1 Hz, 2H, H-2'); 3.72 (s, 3H, 4"-OCH_3); 3.82 (s, 3H, 2"-OCH_3); 4.65 (bd, *J*(CH_2-NH) = 5.8 Hz, 2H, $PhCH_2$); 4.74 (brs, 1H, OH); 6.43 (dd, *J*(5", 6") = 8.4 Hz, *J*(5", 3") = 2.4 Hz, 1H, H-5"); 6.57 (d, *J*(3", 5") = 2.4 Hz, 1H, H-3"); 7.05 (dm, *J*(6", 5") = 8.4 Hz, 1H, H-6"); 7.41 (dd, *J*(6, 5) = 8.3 Hz, *J*(6, 7) = 7.2 Hz, 1H, H-6); 7.64 (dm, *J*(7, 6) = 7.2 Hz, 1H, H-7); 8.18 (dd, *J*(5, 6) = 8.5 Hz, *J*(5, 7) = 1.4 Hz, 1H, H-5); 8.45 (s, 1H, H-2); 8.52 (brt, *J*(NH-CH_2) =

5.8 Hz, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 34.94 (C-1'); 38.79 ($PhCH_2$); 55.36 (4"-OCH_3); 55.61 (2"-OCH_3); 61.72 (C-2'); 98.42 (C-3"); 104.43 (C-5"); 114.95 (C-4a); 118.93 (C-1"); 120.86 (C-5); 125.23 (C-6); 128.19 (C-6"); 133.08 (C-7); 136.94 (C-8); 147.91 (C-8a); 154.53 (C-2); 157.89 (C-2"); 159.76 (C-4"); 160.04 (C-4). HR-MS (ESI+): m/z $[M + H]^+$ calculated for: $C_{19}H_{22}N_3O_3$, 340.1656, found: 340.1656.

4-(2,4-Dimethoxybenzyl)amino-8-{2-[(diisopropoxyphosphoryl)-methoxy]ethyl}quinazoline (29):

Treatment of **28** (0.27 g, 0.8 mmol) by Method D afforded **29** (0.17 g, 42%) as a yellowish oil. ESI-MS, *m*/*z* (%): 518 $[M+H^+]$ (100). ^{1}H NMR (DMSO-d_6): δ 1.16 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 1.19 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 3.29 (t, *J*(1', 2') = 6.7 Hz, 2H, H-1'); 3.72 (s, 3H, 4"-OCH_3); 3.73 (d, *J*(H-C-P) = 8.5 Hz, 2H, P-CH_2); 3.80 (t, *J*(CH_2, CH_2) = 6.7 Hz, 2H, H-2'); 3.82 (s, 3H, 2"-OCH_3); 4.52 (d septet, *J*(H-C-O-P) = 7.6 Hz, *J*(CH, CH_3) = 6.2 Hz, 2H, CH*i*Pr); 4.66 (m, 2H, $PhCH_2$); 6.42 (dd, *J*(5", 6") = 8.4 Hz, *J*(5", 3") = 2.4 Hz, 1H, H-5"); 6.57 (d, *J*(3", 5") = 2.4 Hz, 1H, H-3"); 7.05 (d, *J*(6", 5") = 8.4 Hz, 1H, H-6"); 7.42 (dd, *J*(6, 5) = 8.2 Hz, *J*(6, 7) = 7.3 Hz, 1H, H-6); 7.67 (dd, *J*(7, 6) = 7.1 Hz, *J*(7, 5) = 0.5 Hz, 1H, H-7); 8.19 (dd, *J*(5, 6) = 8.3 Hz, *J*(5, 7) = 1.0 Hz, 1H, H-5); 8.45 (s, 1H, H-2); 8.52 (brt, *J*(NH-CH_2) = 5.7 Hz, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 23.55 (d *J*(C-C-O-P) = 4.5 Hz, CH_3*i*Pr); 23.68 (d *J*(C-C-O-P) = 3.7 Hz, CH_3*i*Pr); 30.65 (C-1'); 38.49 ($PhCH_2$); 55.06 (4"-OCH_3); 55.31 (2"-OCH_3); 64.48 (d, *J*(C-P) = 164.5 Hz, CH_2-P); 69.93 (d, *J*(C-O-P) = 6.4 Hz, CH*i*Pr); 72.43 (d, *J*(C-O-C-P) = 12.3 Hz, C-2'); 98.13 (C-3"); 104.12 (C-5"); 114.66 (C-4a); 118.62 (C-1"); 120.80 (C-5); 124.81 (C-6); 127.89 (C-6"); 132.67 (C-7); 135.65 (C-8); 147.57 (C-8a); 154.29 (C-2); 157.87 (C-2"); 159.46 and 159.70 (C-4"a C-4). HR-MS (ESI+): m/z $[M + H]^+$ calculated for: $C_{26}H_{37}N_6O_3P$, 518.2415, found: 518.2414.

4-Amino-8-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}quinazoline (30):

Compound **29** (0.4 g, 0.8 mmol) was dissolved in a mixture of CH_2Cl_2 (1 mL) and CF_3COOH (1 mL). The reaction mixture was stirred at RT overnight, then aq. NH_3 (1 mL) and $CHCl_3$ (150 mL) were added. The organic layer was washed with H_2O (50ml) and brine (50 mL). Volatiles were removed and the residue was purified by flash chromatography (gradient MeOH:$CHCl_3$ 0–10%) to give **30** (0.14 g, (49%) as a yellowish solid ESI-MS, *m*/*z* (%): 368 $[M+H^+]$ (100). ^{1}H NMR (DMSO-d_6): δ 1.16 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 1.19 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 3.27 (t, *J*(1', 2') = 6.8 Hz, 2H, H-1'); 3.73 (d, *J*(H-C-P) = 8.4 Hz, 2H, P-CH_2); 3.80 (t, *J*(2', 1') = 6.8 Hz, 2H, H-2'); 4.52 (d septet, *J*(H-C-O-P) = 7.7 Hz, *J*(CH, CH_3) = 6.2 Hz, 2H, CH*i*Pr); 7.38 (dd, *J*(6, 5) = 8.3 Hz, *J*(6, 7) = 7.2 Hz, 1H, H-6); 7.65 (dm, *J*(7, 6) = 7.2 Hz, 1H, H-7); 7.71 (brs, 2H, NH_2); 8.06 (dd, *J*(5, 6) = 8.3 Hz, *J*(5, 7) = 1.4 Hz, 1H, H-5); 8.41 (s, 1H, H-2). ^{13}C NMR (DMSO-d_6): δ 23.85 (d, *J*(C-C-O-P) = 4.6 Hz, CH_3*i*Pr); 23.99 (d, *J*(C-C-O-P) = 3.7 Hz, CH_3*i*Pr); 30.88 (C-1'); 64.76 (d, *J*(C-P) = 164.4 Hz, CH_2-P); 70.25 (d, *J*(C-O-P) = 6.4 Hz, CH*i*Pr); 72.70 (d, *J*(2'-P) = 12.3 Hz, C-2'); 114.36 (C-4a); 121.93 (C-5); 124.89 (C-6); 133.19 (C-7); 135.73 (C-8); 148.36 (C-8a); 154.92 (C-2); 162.29 (C-4). HR-MS (ESI+): m/z $[M + H]^+$ calculated for: $C_{17}H_{27}N_3O_4P$, 368.1734, found: 368.1734.

Bis(L-phenylalanine isopropyl ester) prodrug of (4-aminoquinazoline-8-yl)ethoxy)methyl)phosphonic acid (31):

Treatment of **30** (0.14 g, 0.38 mmol) by Method A afforded **31** (0.12 g, 46%) as a whitish amorphous solid. ESI-MS, *m*/*z* (%): 662 [M+H$^+$] (100). ^{1}H NMR (DMSO-d_6): δ 1.03, 1.07, 1.12 and 1.16 (4 x d, *J*(CH$_3$, CH) = 6.2 Hz, 12H, CH$_3$); 2.75–2.90 (m, 4H, PhCH$_2$); 3.18–3.33 (m, 4H, P-CH$_2$, H-1'); 3.59–3.66 (m, 2H, H-2'); 3.82–3.98 (m, 2H, NH-CH); 4.05 (m, 1H, P-NH); 4.37 (m, 1H, P-NH); 4.79 (sept, *J*(CH, CH$_3$) = 6.3 Hz, 2H, CH*i*Pr); 4.82 (sept, *J*(CH, CH$_3$) = 6.3 Hz, 2H, CH*i*Pr); 7.10–7.25 (m, 10H, Ph-ortho, meta, para); 7.34 (dd, *J*(6, 5) = 8.3 Hz, *J*(6, 7) = 7.2 Hz, 1H, H-6); 7.61 (dd, *J*(7, 6) = 7.2 Hz, *J*(7, 5) = 1.4 Hz, 1H, H-7); 7.71 (brs, 2H, NH$_2$); 8.06 (dd, *J*(5, 6) = 8.4 Hz, *J*(5, 7) = 1.4 Hz, 1H, H-5); 8.42 (s, 1H, H-2). ^{13}C NMR (DMSO-d_6): δ 21.50, 21.56, 21.62 and 21.68 (CH$_3$*i*Pr); 30.92 (C-1'); 40.22 (CH$_2$Ph); 54.04 and 54.13 (NH-CH); 67.66 (d, *J*(C-P) = 135.6 Hz, CH$_2$-P); 68.01 and 68.14 (CH*i*Pr); 72.59 (d, *J*(C-O-C-P) = 12.3 Hz, C-2'); 114.38 (C-4a); 121.93 (C-5); 124.91 (C-6); 126.62 and 126.63 (Ph-para); 128.22 and 128.23 (Ph-meta); 129.65 and 129.67 (Ph-ortho); 133.08 (C-7); 135.73 (C-8); 137.13 and 137.33 (ipso); 148.35 (C-8a); 154.94 (C-2); 162.30 (C-4); 172.26 (d, *J*(C-C-N-P) = 5.4 Hz, COO); 172.47 (d, *J*(C-C-N-P) = 3.0 Hz, COO). HR-MS (ESI+): m/z [M + H]$^+$ calculated for: $C_{32}H_{47}N_4O_9P$, 662.3075, found: 662.3081.

4-(2,4-Dimethoxybenzyl)aminopyrrolo[2,1-*f*][1,2,4]triazine (33):

2,4-Dimethoxybenzylamine (4.62 mL, 18.5 mmol) was added to a solution of 4-chloro-aminopyrrolo[2,1-f][1,2,4]triazine (**32**, 1.75 g, 11.4 mmol) in abs. EtOH (21 mL) and Et$_3$N (3.5 mL). The resulting mixture was heated at 120 °C for 1 h in microwave reactor. Volatiles were removed and the residue was purified on C18 RP in (H$_2$O:MeOH 0–100%) to give **33** (2.76 g, 85%) as an amorphous solid. ESI-MS, *m*/*z* (%): 285 [M+H$^+$] (45); 307 [M+Na$^+$] (100). ^{1}H NMR (DMSO-d_6): δ 3.73 (s, 3H, 4'-OCH$_3$); 3.81 (s, 3H, 2'-OCH$_3$); 4.60 (d, *J*(NH-CH$_2$) = 5.7 Hz, 2H, PhCH$_2$); 6.47 (dd, *J*(5', 6') = 8.4 Hz, *J*(5', 3') = 2.4 Hz, 1H, H-5'); 6.58 (d, *J*(3', 5') = 2.4 Hz, 1H, H-3'); 6.60 (dd, *J*(6, 5) = 4.3 Hz, *J*(6, 7) = 2.6 Hz, 1H, H-6); 6.96 (dd, *J*(5, 6) = 4.3 Hz, *J*(5, 7) = 1.6 Hz, 1H, H-5); 7.11 (d, *J*(6', 5') = 8.3 Hz, 2H, H-6'); 7.60 (dd, *J*(7, 6) = 2.6 Hz, *J*(7, 5) = 1.6 Hz, 1H, H-7); 7.84 (s, 1H, H-2); 8.48 (brt, *J*(NH-CH$_2$) = 5.7 Hz, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 37.69 (PhCH$_2$); 55.10 (4'-OCH$_3$); 55.34 (2'-OCH$_3$); 98.18 (C-3'); 100.73 (C-5); 104.26 (C-5'); 109.88 (C-6); 114.50 (C-4a); 117.97 (C-7); 118.37 (C-1'); 128.55 (C-6'); 147.46 (C-2); 153.78 (C-4); 157.63 (C-2'); 159.68 (C-4'). HR-MS (ESI+): m/z [M + H]$^+$ calculated for: $C_{15}H_{17}N_4O_2$, 285.1346, found: 285.1347.

4-(2,4-Dimethoxybenzyl)amino-7-iodo-pyrrolo[2,1-*f*][1,2,4]triazine (34):

N-Iodosuccinimide (3.1 g, 13.8 mmol) was added to the solution of **33** (3.6 g, 12.6 mmol) in dry CH$_3$CN (40 mL) cooled to 0 °C under Ar. The reaction mixture was stirred at 0 °C for 1 h. Volatiles were removed and the residue was dissolved in CHCl$_3$ (250 mL) and washed with H$_2$O (100 mL), 10% Na$_2$S$_2$O$_3$ (100 mL), brine (100mL) and then dried over Na$_2$SO$_4$. The solvent was removed *in vacuo* and the residue was purified by flash chromatography (grad. MeOH:CHCl$_3$ 0–10%) to give **34** (4.0 g, 77%) as a yellowish oil. ESI-MS, *m*/*z* (%): 411 [M+H$^+$] (65); 433 [M+Na$^+$] (100). ^{1}H NMR (DMSO-d_6): δ 3.73 (s, 3H, 4'-OCH$_3$); 3.80 (s, 3H, 2'-OCH$_3$); 4.60 (d, *J*(NH-CH$_2$) = 5.7 Hz, 2H, PhCH$_2$); 6.46 (dd, *J*(5', 6') = 8.4 Hz,

J(5', 3') = 2.4 Hz, 1H, H-5'); 6.57 (d, *J*(3', 5') = 2.4 Hz, 1H, H-3'); 6.83 (d, *J*(6, 5) = 4.4 Hz, 1H, H-6); 7.09–7.11 (m, 2H, H-5 and 6'); 7.97 (s, 1H, H-2); 8.57 (brt, *J*(NH-CH_2) = 5.7 Hz, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 38.20 ($PhCH_2$); 55.41 (4'-OCH_3); 55.65 (2'-OCH_3); 71.71 (C-7); 98.49 (C-3'); 103.74 (C-5); 104.57 (C-5'); 118.04 (C-4a); 118.44 (C-1'); 118.47 (C-6); 128.94 (C-6'); 148.46 (C-2); 153.76 (C-4); 157.96 (C-2'); 160.04 (C-4'). HR-MS (ESI +): m/z [M + H]$^+$ calculated for: $C_{15}H_{16}N_4O_2I$, 411.0313, found: 411.0315.

4-(2,4-Dimethoxybenzyl)amino-7-vinyl-pyrrolo[2,1-*f*][1,2,4]triazine (35):

Tributyl(vinyl)tin (1.4 mL, 4.8 mmol) was added to the solution of **34** (1.58 g, 5.6 mmol), CuI (87 mg, 0.45 mmol) and Pd(*t*-$Bu_3P)_2$ (0.1 g, 0.2 mmol) in NMP (30 mL) under Ar. The resulting mixture was heated at 120 °C for 5 h, then cooled to RT and an aqueous solution of KF (1.3 g in 10 mL) was added. After 15 min, EtOAc (120 mL) was added and resulting slurry was stirred for 15 min. The mixture was filtered over Cellite, the organic layer was separated and washed with a sat. EDTA solution (50 mL), H_2O (50 mL), brine (50 mL) and then dried over Na_2SO_4. Volatiles were removed *in vacuo*, and the residue was purified by flash chromatography ((Hex:$CHCl_3$(1:1) together with grad MeOH 1–10%) to give **35** (1.08 g, 90%) as a yellowish oil. ESI-MS, *m*/*z* (%): 311 [M+H$^+$] (65); 333 [M+Na$^+$] (100). ^{1}H NMR (DMSO-d_6): δ 3.73 (s, 3H, 4'-OCH_3); 3.81 (s, 3H, 2'-OCH_3); 4.60 (bd, *J*(CH_2-NH) = 5.7 Hz, 2H, $PhCH_2$); 5.27 (dd, *J*(vic) = 11.5 Hz, *J*(gem) = 1.7 Hz, 1H, CH_2b); 5.93 (dd, *J*(vic) = 17.8 Hz, *J*(gem) = 1.7 Hz, 1H, CH_2a); 6.47 (dd, *J*(5', 6') = 8.4 Hz, *J*(5', 3') = 2.4 Hz, 1H, H-5'); 6.58 (d, *J*(3', 5') = 2.4 Hz, 1H, H-3'); 7.10 (d, *J*(6', 5') = 8.4 Hz, 1H, H-6'); 6.87–7.02 (m, 2H, H-5 and H-6); 7.93 (s, 1H, H-2); 8.49 (brt, *J*(NH-CH_2) = 5.7 Hz, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 37.74 ($PhCH_2$); 55.10 (4'-OCH_3); 55.34 (2'-OCH_3); 98.18 (C-3'); 101.49 (C-5); 104.27 (C-5'); 108.26 (C-6); 113.18 (CH=$\underline{CH_2}$); 115.07 (C-4a); 118.31 (C-1'); 123.95 ($\underline{CH}$=CH_2); 128.18 (C-7); 128.58 (C-6'); 147.64 (C-2); 153.76 (C-4); 157.64 (C-2'); 159.70 (C-4'). HR-MS (ESI+): m/z [M + H]$^+$ calculated for: $C_{17}H_{19}N_4O_2$, 311.1503, found: 311.1503.

4-(2,4-Dimethoxybenzyl)amino-7-(2-hydroxyethyl)-pyrrolo[2,1-*f*][1,2,4]triazine (36):

Treatment of **35** (1.0 g, 3.2 mmol) by Method C (column chromatography in grad. MeOH:$CHCl_3$ 0–10%) afforded **36** (0.56 g, 53%) as a yellowish oil. ESI-MS, *m*/*z* (%): 329 [M+H$^+$] (30); 351 [M+Na$^+$] (100). ^{1}H NMR (DMSO-d_6): δ 3.00 (t, *J*(1', 2') = 7.1 Hz, 2H, H-1'); 3.68 (m, 2H, H-2'); 3.73 (s, 3H, 4"-OCH_3); 3.81 (s, 3H, 2"-OCH_3); 4.59 (d, *J*(NH-CH_2) = 5.8 Hz, 2H, $PhCH_2$); 4.73 (brt, *J*(OH, CH_2) = 5.4 Hz, 1H, OH); 6.45 (d, *J*(6, 5) = 4.3 Hz, 1H, H-6); 6.46 (dd, *J*(5", 6") = 8.3 Hz, *J*(5", 3") = 2.4 Hz, 1H, H-5"); 6.57 (d, *J*(3", 5") = 2.4 Hz, 1H, H-3"); 6.60 (d, *J*(5, 6) = 4.3 Hz, 1H, H-5); 7.08 (d, *J*(6", 5") = 8.3 Hz, 2H, H-6"); 7.85 (s, 1H, H-2); 8.36 (brt, *J*(NH-CH_2) = 5.8 Hz, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 29.02 (C-1'); 37.88 ($PhCH_2$); 55.40 (4"-OCH_3); 55.63 (2"-OCH_3); 59.44 (C-2'); 98.44 (C-3"); 100.37 (C-5); 104.54 (C-5"); 109.45 (C-6); 114.25 (C-4a); 118.86 (C-1"); 128.05 (C-7); 128.68 (C-6"); 147.34 (C-2); 154.11 (C-4); 157.78 (C-2"); 159.92 (C-4"). HR-MS (ESI+): m/z [M + H]$^+$ calculated for: $C_{17}H_{21}N_4O_3$, 329.1608, found: 329.1608.

4-(2,4-Dimethoxybenzyl)amino-7-{2-[(diisopropoxyphosphoryl)-methoxy]ethyl}pyrrolo[2,1-*f*][1,2,4]triazine (37):

Treatment of **36** (0.53 g, 1.7 mmol) by Method D afforded **37** (0.85 g, 98%) as a yellowish oil. ESI-MS, *m*/*z* (%): 507 [M+H$^+$] (100). ^{1}H NMR (DMSO-d_6): δ 1.18 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 1.21 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 3.10 (t, *J*(1', 2') = 6.7 Hz, 2H, H-1'); 3.73 (s, 3H, 4"-OCH_3); 3.75 (d, *J*(H-C-P) = 8.4 Hz, 2H, P-CH_2); 3.80 (t, *J*(CH_2, CH_2) = 6.7 Hz, 2H, H-2'); 3.82 (s, 3H, 2"-OCH_3); 4.54 (d septet, *J*(H-C-O-P) = 7.7 Hz, *J*(CH, CH_3) = 6.2 Hz, 2H, CH*i*Pr); 4.59 (m, 2H, PhCH_2); 6.45 (dd, *J*(5", 6") = 8.4 Hz, *J*(5", 3") = 2.4 Hz, 1H, H-5"); 6.49 (d, *J*(6, 5) = 4.3 Hz, 1H, H-6); 6.57 (d, *J*(3", 5") = 2.4 Hz, 1H, H-3"); 6.90 (d, *J*(5, 6) = 4.3 Hz, 1H, H-5); 7.08 (dt, *J*(6", 5") = 8.4 Hz, *J*(6", CH_2) = 0.8 Hz, 2H, H-6"); 7.86 (s, 1H, H-2); 8.37 (brt, *J*(NH-CH_2) = 5.9 Hz, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 23.85 (d, *J*(C-C-O-P) = 4.5 Hz, CH_3*i*Pr); 23.99 (d *J*(C-C-O-P) = 3.7 Hz, CH_3*i*Pr); 25.37 (C-1'); 37.88 (PhCH_2); 55.38 (4"-OCH_3); 55.62 (2"-OCH_3); 64.78 (d, *J*(C-P) = 164.4 Hz, CH_2-P); 70.28 (d, *J*(C-O-P) = 6.4 Hz, CH*i*Pr); 70.54 (d, *J*(C-O-C-P) = 12.1 Hz, C-2'); 98.43 (C-3"); 100.40 (C-5); 104.51 (C-5"); 109.55 (C-6);114.36 (C-4a); 118.80 (C-1"); 127.18 (C-7); 128.67 (C-6"); 147.37 (C-2); 154.06 (C-4); 157.87 (C-2"); 159.92 (C-4"). HR-MS (ESI+): m/z [M + H]$^+$ calculated for: $C_{24}H_{36}N_4O_6P$, 507.2367, found: 507.2367.

4-Amino-7-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}pyrrolo[2,1-*f*][1,2,4]triazine (38):

Compound **37** (0.7 g, 1.4 mmol) was dissolved in a mixture of CH_2Cl_2 (10 mL) and CF_3COOH (10 mL). The reaction mixture was stirred at RT for 3 h, cooled with ice and aq. NH_3 (10 mL) and $CHCl_3$ (150 mL) were added. The organic layer was washed with H_2O (50ml) and brine (50 mL). Volatiles were removed and the residue was purified by flash chromatography (grad. MeOH:$CHCl_3$ 0–10%) to give **38** (0.3 g, 61%) as a white amorphous solid. ESI-MS, *m*/*z* (%): 357 [M+H$^+$] (100). ^{1}H NMR (DMSO-d_6): δ 1.18 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 1.21 (d, *J*(CH_3, CH) = 6.2 Hz, 6H, CH_3); 3.10 (t, *J*(1', 2') = 6.7 Hz, 2H, H-1'); 3.75 (d, *J*(H-C-P) = 8.3 Hz, 2H, P-CH_2); 3.80 (t, *J*(2', 1') = 6.7 Hz, 2H, H-2'); 4.54 (d septet, *J*(H-C-O-P) = 7.7 Hz, *J*(CH, CH_3) = 6.2 Hz, 2H, CH*i*Pr); 6.49 (d, *J*(6, 5) = 4.3 Hz, 1H, H-6); 6.80 (d, *J*(5, 6) = 4.3 Hz, 1H, H-5); 7.58 (brs, 2H, NH_2); 7.81 (s, 1H, H-2). ^{13}C NMR (DMSO-d_6): δ 23.85 (d *J*(C-C-O-P) = 4.5 Hz, CH_3*i*Pr); 24.00 (d, *J*(C-C-O-P) = 3.7 Hz, CH_3*i*Pr); 25.39 (C-1'); 64.79 (d, *J*(C-P) = 164.5 Hz, CH_2-P); 70.30 (d, *J*(C-O-P) = 6.4 Hz, CH*i*Pr); 70.50 (d, *J*(C-O-C-P) = 12.2 Hz, C-2'); 100.85 (C-5); 109.69 (C-6);114.13 (C-4a); 127.23 (C-7); 147.84 (C-2); 155.68 (C-4). HR-MS (ESI+): m/z [M + H]$^+$ calculated for: $C_{15}H_{26}N_4O_4P$, 357.1686, found: 357.1687.

Bis(L-phenylalanine isopropyl ester) prodrug of 2-(4-aminopyrrolo[2,1-*f*][1,2,4]triazin-7-yl)ethoxy)methyl)phosphonic acid (39):

Treatment of **38** (0.29 g, 0.8 mmol) by Method A afforded **39** (0.27 g, 50%) as a whitish amorphous solid. ESI-MS, *m*/*z* (%): 651 [M+H$^+$] (100). ^{1}H NMR (DMSO-d_6): δ 1.03, 1.07, 1.13 and 1.16 (d, *J*(CH_3, CH) = 6.2 Hz, 12H, CH_3); 2.74–2.90 (m, 4H, CH_2Ph); 3.01–3.08 (m, 2H, H-1'); 3.22–3.34 (m, 2H, P-CH_2); 3.59–3.66 (m, 2H, H-2'); 3.85–3.98 (2x m, 2H, NH-CH); 4.06 (m, 1H, P-NH); 4.38 (m, 1H, P-NH); 4.79 (sept, *J*(CH, CH_3) = 6.3 Hz, 1H, CH*i*Pr); 4.82 (sept, *J*(CH, CH_3) = 6.3 Hz, 1H, CH*i*Pr); 6.47 (d, *J*(6, 5) = 4.3 Hz, 1H, H-6); 6.81 (d, *J*(5, 6) = 4.3 Hz, 1H, H-5); 7.10–7.26 (m, 10H, Ph-ortho, meta, para); 7.59 (s, 2H,

NH_2); 7.81 (s, 1H, H-2). ^{13}C NMR (DMSO-d_6): δ 21.49, 21.55, 21.62 and 21.68 (CH_3*i*Pr); 25.42 (C-1'); 40.10 (CH_2Ph); 54.09 and 54.13 (NH-CH); 67.61 (d, *J*(C-P) = 135.8 Hz, CH_2-P); 68.00 and 68.13 (CH*i*Pr); 70.33 (d, *J*(2'-P) = 12.1 Hz, C-2'); 100.93 (C-5); 109.62 (C-6); 114.13 (C-4a); 126.63 and 126.64 (Ph-para); 127.20 (C-7); 128.22 and 128.24 (Ph-meta); 129.66 and 129.66 (Ph-ortho); 137.16 and 137.32 (ipso); 147.86 (C-2); 155.68 (C-4); 172.28 (d, *J*(C-C-N-P) = 5.3 Hz, COO); 172.46 (d, *J*(C-C-N-P) = 3.0 Hz, COO). HR-MS (ESI+): m/z $[M + H]^+$ calculated for: $C_{33}H_{44}N_6O_6P$, 651.3055, found: 651.3050.

(7-Deazaadenine-9-yl)ethoxy)methyl)phosphonic acid diphosphate (40a):

Compound **5a** (0.204 g, 0.57 mmol) was converted into the free phosphonate (0.15 g, 0.55 mmol) according to the reported procedure,[49] and subsequent treatment by Method E afforded **40a** (90 mg, 38%) as an amorphous solid after lyophilization. ESI-MS, *m*/*z* (%): 431 $[M^-]$ (100). ^1H NMR (D_2O): δ 3.85 (d, *J*(H-C-P) = 8.7 Hz, 2H, P-CH_2); 3.98 (t, *J*(2', 1') = 5.1 Hz, 2H, H-2'); 4.36 (t, *J*(1', 2') = 5.1 Hz, 2H, H-1'); 6.55 (d, *J*(7, 8) = 3.6 Hz, 1H, H-7); 7.38 (d, *J*(8, 7) = 3.6 Hz, 1H, H-8); 8.07 (s, 1H, H-2). ^{13}C NMR (D_2O): δ 44.90 (C-1'); 67.03 (d, *J*(C-P) = 164.5 Hz, CH_2-P); 71.57 (d, *J*(2'-P) = 10.9 Hz, C-2'); 100.63 (C-7); 102.44 (C-5); 128.73 (C-8); 145.10 (C-2); 147.31 (C-4); 153.10 (C-6). ^{31}P NMR (D_2O) −22.41 (m, P_β); −9.30 (m, P_α); 8.80 (d, P_γ).

(8-Aza-7-deazaadenine-9-yl)ethoxy)methyl)phosphonic acid diphosphate (40b):

Compound **17a** (0.28 g, 0.8 mmol) was converted into the free phosphonate (0.16 g, 0.58 mmol) according to the reported procedure,[54] and subsequent treatment by Method E afforded **40b** (110 mg, 45%) as an amorphous solid after lyophilization. ESI-MS, *m*/*z* (%): 432 $[M^-]$ (100). ^1H NMR (D_2O): δ 3.76 (d, *J*(H-C-P) = 8.0 Hz, 2H, P-CH_2); 4.07 (t, *J*(2', 1') = 5.3 Hz, 2H, H-2'); 4.56 (t, *J*(1', 2') = 5.3 Hz, 2H, H-1'); 8.12 (s, 1H, H-7); 8.21 (s, 1H, H-2). ^{13}C NMR (D_2O): δ 47.36 (C-1'); 67.34 (d, *J*(C-P) = 162.5 Hz, CH_2-P); 71.18 (d, *J*(2'-P) = 9.1 Hz, C-2'); 101.08 (C-5); 133.72 (C-7); 152.95 (C-4); 156.32 (C-2); 158.96 (C-6). ^{31}P NMR (D_2O) −22.03 (m, P_β); −8.89 (m, P_α); 9.37 (d, *J*(P-O-P) = 26.2 Hz, P_γ).

(9-Deazaadenine-9-yl)ethoxy)methyl)phosphonic acid diphosphate (41):

Treatment of **14** (0.12 g, 0.44 mmol) by Method E afforded **41** (61 mg, 32%) as a whitish amorphous solid after lyophilization. ESI-MS, *m*/*z* (%): 431 $[M^-]$ (100). ^1H NMR (D_2O): δ 2.95 (t, *J*(1', 2') = 5.9 Hz, 2H, H-1'); 3.83 (t, *J*(2', 1') = 6.0 Hz, 2H, H-2'); 3.88 (d, *J*(H-C-P) = 8.6 Hz, 2H, P-CH_2); 7.51 (s, 1H, H-8); 8.31 (s, 1H, H-2). ^{13}C NMR (D_2O): δ 24.61 (C-1'); 67.11 (d, *J*(C-P) = 162.5 Hz, CH_2-P); 73.54 (d, *J*(2'-P) = 11.8 Hz, C-2'); 110.04 (C-9); 113.19 (C-5); 131.67 (C-8); 135.41 (C-4); 145.50 (C-2); 151.92 (C-6). ^{31}P NMR (D_2O) −21.82 (dd, *J*(P-O-P) = 25.4 Hz, *J*(P-O-P) = 19.8 Hz P_β); −7.84 (d, *J*(P-O-P) = 19.8 Hz P_α); 9.81 (d, *J*(P-O-P) = 25.4 Hz, P_γ).

Inhibition of ACT – cell free assay:

AC enzymatic activity was evaluated by determining the conversion of $[^3H]$ ATP to $[^3H]$cAMP. Each assay mixture contained 3 μM BSA, 20 mM HEPES (pH 7.4), 10 mM $MnCl_2$, 1 mM EDTA, 1 μM $CaCl_2$, 0.1 mM cold ATP, 20 μCi [2,8-^3H]ATP (ARC, St. Louis, MO, USA; specific activity 20 Ci/mmol), 1.2 μM calmodulin and tested compound at a

concentration of 0 – 100 μM. Inhibition of AC activity was evaluated towards three different commercially available bacterial adenylate cyclases: ACT (Sigma), specific activity 65 μmol/min/mg, ACT (Enzo), specific activity 115 μmol/min/mg and finally, EF-A (LBL), specific activity 830 μmol/min/mg, with the final enzyme concentration of 1.1 nM , 0.67 nM and 0.12 nM, respectively. The incubation was carried out for 30 min at 30 °C in a reaction volume of 50 μL. A 2 μL aliquot of the assay mixture was spotted onto a polyethyleneimine-cellulose TLC plate, and developed in 4M LiCl:1 M acetic acid (1:4). After developing the TLC plate, the spots containing ATP and cAMP were quantified using Radio-TLC scanner RITA (RAYTEST, Germany) equipped with the evaluation software GINA STAR TLC. Inhibition rates were calculated from the percentage of [^{3}H]ATP to [^{3}H]cAMP conversion. K_i values were calculated using the Graphpad Prism 5 software (San Diego, CA, USA). All assays were performed in duplicate with three independent experiments, and results are presented as mean values ± SD.

Inhibition of ACT – cell based assay:

J774A.1 cells were seeded in a 96-well plate at 5×10^4 cells per well, and left to attach overnight. Prior to the experiment, cells were washed with HBSS (135 mM NaCl, 5.9 mM KCl, 1.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 25 mM glucose, 10 mM HEPES [pH 7.4]) and pre-incubated with the tested compounds at concentrations of 0.001–30 μM for 5 h. After that, cells were exposed to ACT (2 nM) from *B.pertussis* (Enzo Life Sciences, Palo Alto, CA; SA=115 μmol/min/mg) for 30 min. Finally, the cAMP content was determined by using the CatchPointcAMP immunoassay kit (Molecular Devices, Wokingham, UK). Briefly, the reaction was terminated by the addition of lysis buffer to the treated cells (50 μL per well), the cellular content was extracted by shaking the plate at 250 rpm for 10 min. The plate was then centrifuged to remove cell debris, the supernatant was transferred to the immunoassay plate, and immunoassays were carried out according to the manufacturer's instructions. Fluorescence signal was acquired using an Infinite M1000 plate reader (Tecan Systems Inc., San Jose, CA, USA).

Effect on the viability of J774A.1 cells:

J774A.1 cells were plated onto white 96-well assay plates at 5×10^4 cells per well and allowed to attach overnight. Cells were then washed with HBSS and treated with 10 μM compounds for 5 h. Cell viability was then assessed with the Cell Titer-Glo Luminescent Cell Viability assay (Promega, Madison, WI, USA) according to the manufacturer's instructions. Measurement of luminescence signal was performed by use of a GENios micro plate reader (Tecan Systems). Data are expressed as a percentage of the viability of control (untreated) cells.

Assays with mAC. Materials:

3-Isobutyl-1-methylxanthine (IBMX), and A23187 were purchased from Sigma-Aldrich (St. Louis, MO). Phorbol 12-myristate 13-acetate (PMA), and forskolin (FSK) were purchased from Tocris Bioscience (Ellisville, MO). Opti-MEM, antibiotic-antimycotic 100x solution, and Dulbecco's modified Eagle's medium (DMEM) were purchased from Life technologies (Grand Island, NY). FetalClone I serum and bovine calf serum, were purchased from Hyclone (Logan, UT). G418 was purchased from InvivoGen (San Diego, CA). The

homogenous time-resolved fluorescence (HTRF) cAMP kits were purchased from Cisbio Bioassays (Bedford, MA). The tested compounds were prepared as 50 mM stocks in DMSO, and stored at −20 °C.

Compound Screening (30 μM) and Concentration Response Curves:

Cryopreserved HEK293 cells stably expressing human AC1, human AC2, or human AC5 were thawed rapidly at 37 °C, and resuspended in 10mL of Opti-MEM. Cells were centrifuged at 500 g for 5 min. The supernatant was aspirated, and the cell pellet was resuspended in 10 mL of Opti-MEM for a second centrifugation step. The cells were plated in a 384-well plate, and incubated for 1 h in a humidified incubator at 37 °C and 5% CO_2. Cells were then incubated with each corresponding compound at 30 μM or a concentration response curve as indicated for 30 min, followed by the addition of each cell line's corresponding selective stimulant diluted in Opti-MEM and 500 μM IBMX (AC1: 3 μM A23187, AC2: 100 nM PMA and AC5: 1 μM FSK). The stimulation was terminated by the addition of equal parts of the Cisbio HTRF kit reagents, cAMP-d2 and the anti-cAMP cryptate conjugate. After 1 h of incubation, the homogenous time-resolved fluorescence energy transfer (HTR-FRET) was measured using a Synergy4 (BioTek) fluorescence plate reader (excitation filter: 330/80 nm and emission filters: 620/10 nm and 665/8 nm). The resulting cAMP concentrations were calculated in GraphPad Prism by interpolating the 620/665 nm fluorescence ratio values from a cAMP standard curve in parallel. Compound concentration response curves were also analyzed using GraphPad Prism.

Molecular Modelling:

Recently reinterpreted[57] crystal structure of adenylate cyclase domain (ACD) from *Bordetella pertussis* adenylate cyclase toxin (ACT) with calmodulin (CaM) and bound PMEApp (PDB ID:1ZOT, resolution 2.2 Å)[41] was used for molecular modelling study. The pre-processed protein was prepared with MOE Structure Preparation and Protonate 3D tools with the default setup. 7-Deaza-PMEApp (compound **40a**) was properly protonated and minimized to RMS gradient of 0.001 kcal/mol. For docking study, the Template docking protocol was selected with substructure setup where all atoms of the acyclic moiety (purine base was excluded) of the PMEApp were selected as the Query in the setup. Bond rotation of ligands was allowed and default placement and refinement methods were used with 50 retained structures after the placement and 30 retained structures after the refinement. For all calculations Amber12:EHT mixed force-field was used with R-Field solvent model.

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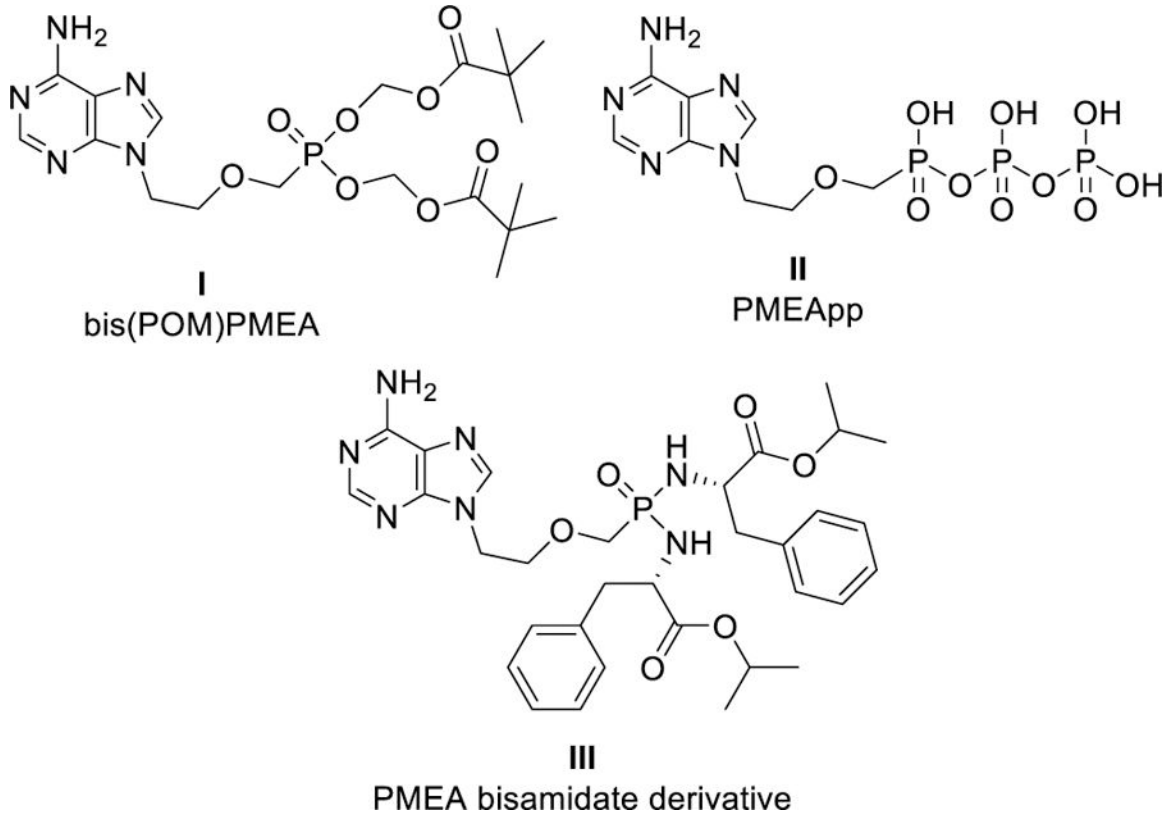


Figure 1.
PMEA prodrugs **I** and **III** and metabolically active PMEA diphosphate **II**.

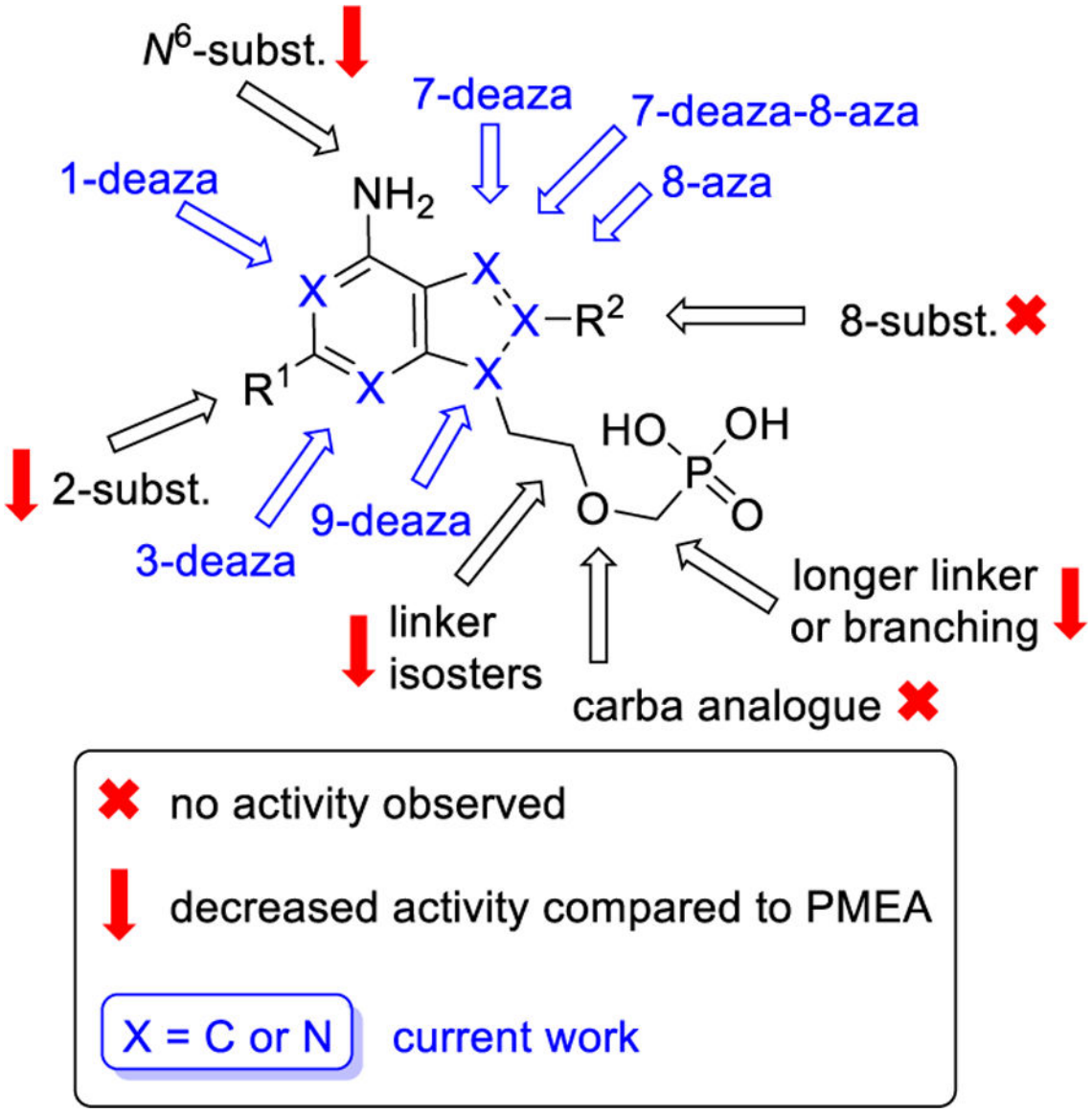


Figure 2.

Overview of the structure activity relationship studies of adefovir (PMEA) analogues as ACT inhibitors. Blue colour depicts the positions on the purine scaffold modified in this work, namely positions C-1, C-3, C-7, C-8, and C-9.

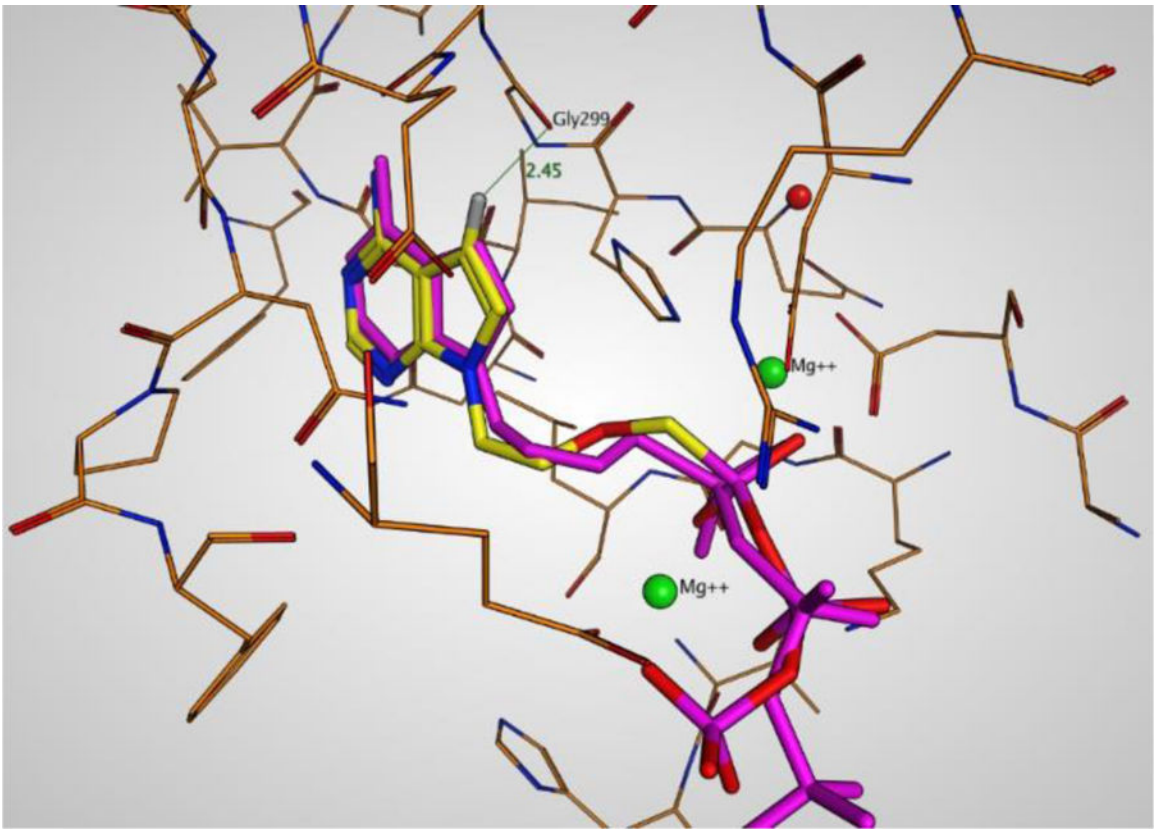


Figure 3.

Docking study of 7-deaza-PMEApp (compound **40a**) to adenylate cyclase domain of ACT in comparison with PMEApp. Picture description: Purple structure – optimized position of parent PMEApp (**II**). Yellow structure - docking results for compound **40a**. Red dot - Mg902 changed to a water molecule; Green dots - Mg901 and Mg903 ions.

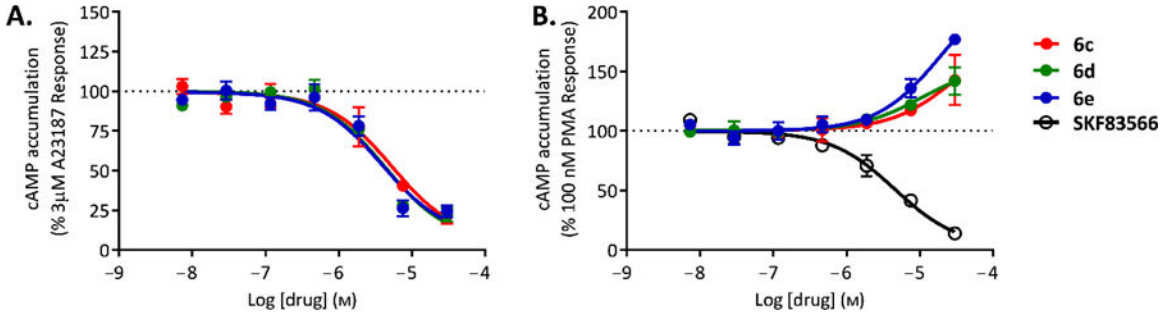
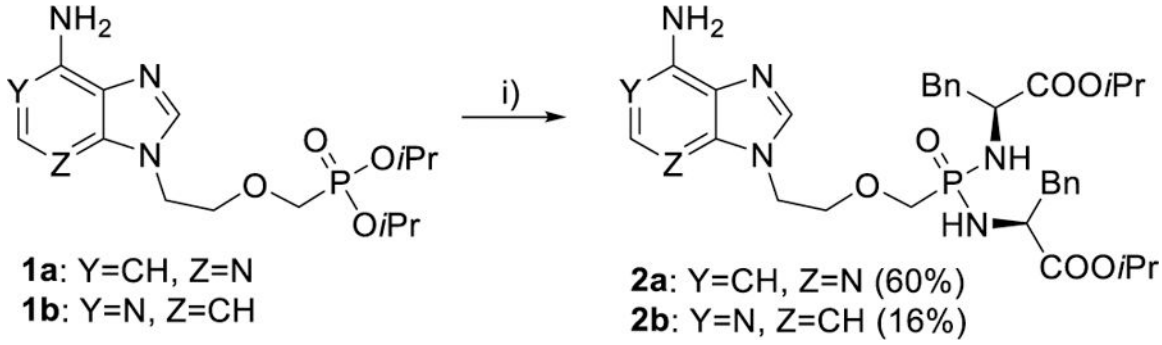


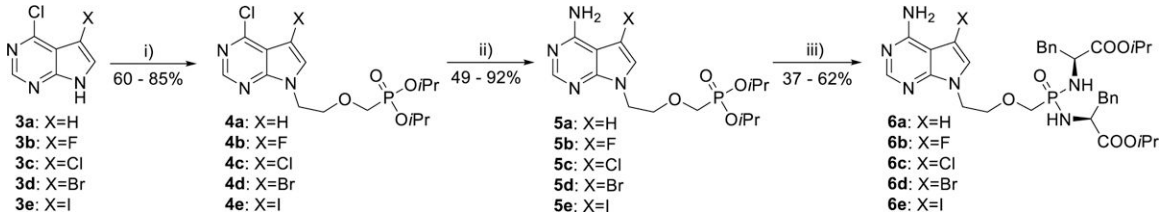
Figure 4.

Dose response curves of compounds **6c**, **6d**, and **6e** at AC1 and AC2. Increasing concentrations of the compounds were added to HEK293 cells expressing AC1 (A) or AC2 (B) followed by selective AC stimulation using the calcium ionophore A23187 or the phorbol ester PMA as indicated. SKF83566 is a selective inhibitor of AC2. Data shown represent the mean and SEM of three independent experiments conducted in duplicate.



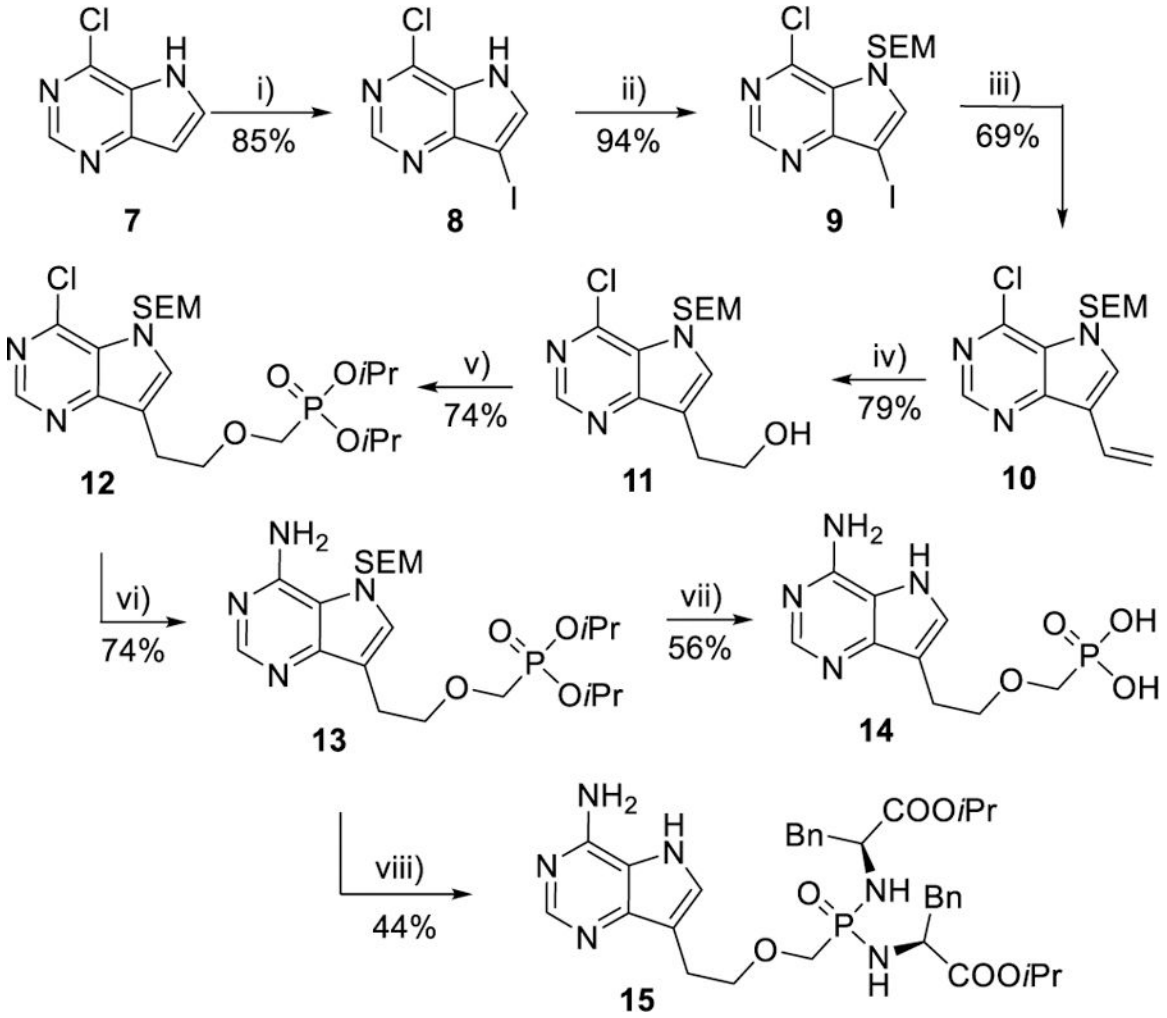
Scheme 1.

Synthesis of 1-deaza- and 3-deazaadenine derivatives **2a** and **2b**. Reagents and conditions: i) TMSBr, pyridine, rt; then (L)-$NH_2CH(Bn)COOiPr \cdot HCl$, PPh_3, Aldrithiol-2, pyridine, Et_3N, 70 °C.



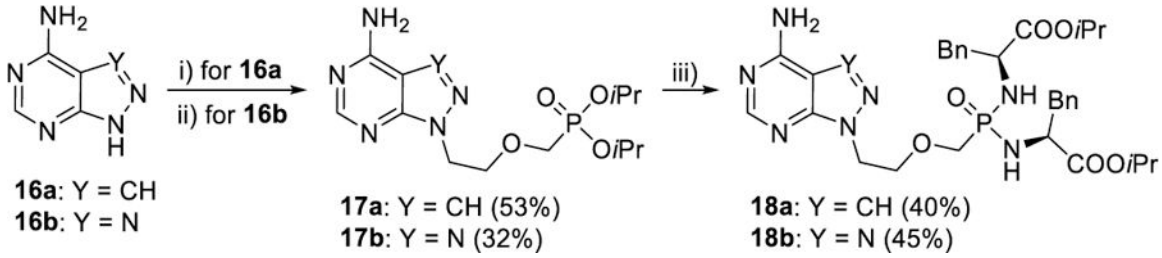
Scheme 2.

Synthesis of 7-deazadenine derivatives **6a**–**6e**. Reagents and conditions: i) $Cl(CH_2)_2OCH_2P(O)(OiPr)_2$, Cs_2CO_3, DMSO, 80 °C; ii) $EtOH/NH_3$, 100 °C; iii) TMSBr, pyridine, rt; then (L)-$NH_2CH(Bn)COOiPr$·HCl, PPh_3, Aldrithiol-2, pyridine, Et_3N, 70 °C.



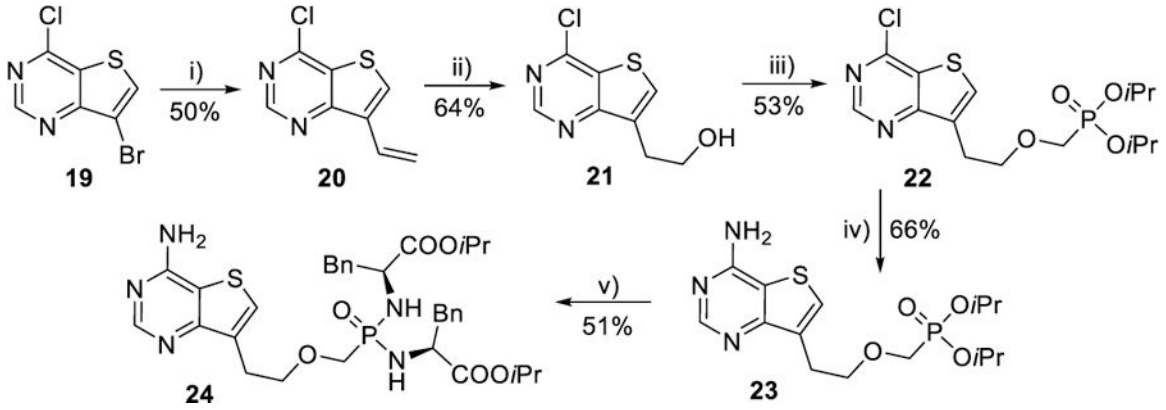
Scheme 3.

Synthesis of 9-deazapurine derivatives **14** and **15**. Reagents and conditions: i) NIS, THF, rt; ii) $(CH_3)_3Si(CH_2)_2OCH_2Cl$, NaH, DMF, rt; iii) CH_2=$CHSnBu_3$, Pd(*t*-$Bu_3P)_2$, THF, rt; iv) 9-BBN, THF, 0 °C to rt; then aq. $NaBO_3$; v) *n*-BuLi, $CF_3SO_2OCH_2P(O)(OiPr)_2$, THF, −78 °C; vi) EtOH/$NH_3$, 100 °C; vii) HCl 2 eq, H_2O,130 °C; viii) TMSBr/TMSI, pyridine, rt; then (L)-$NH_2CH(Bn)COOiPr$·HCl, PPh_3, Aldrithiol-2, pyridine, Et_3N, 70 °C.



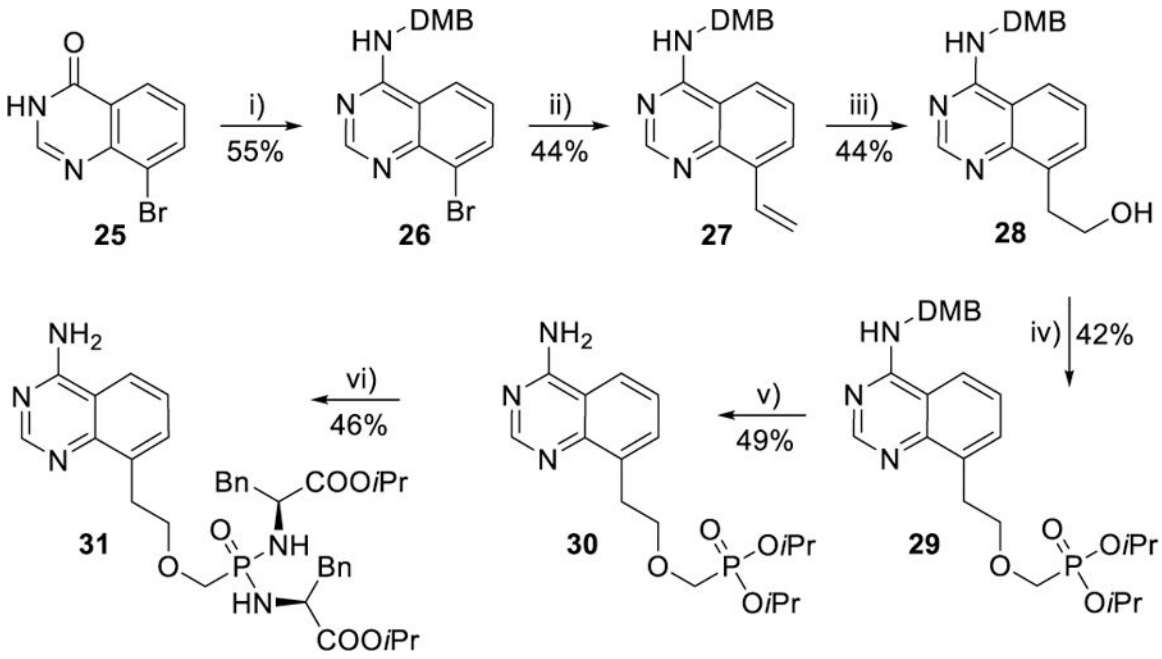
Scheme 4.

Synthesis of compounds **18a** and **18b**. Reagents and conditions: i) NaH, DMF, 80 °C, then $Cl(CH_2)_2OCH_2P(O)(OiPr)_2$, DMF, 100 °C; ii) $Cl(CH_2)_2OCH_2P(O)(OiPr)_2$, DBU, DMF, 100 °C; iii) TMSBr, pyridine, rt; then (L)-$NH_2CH(Bn)COOiPr{\cdot}HCl$, PPh_3, Aldrithiol-2, pyridine, Et_3N, 70 °C.



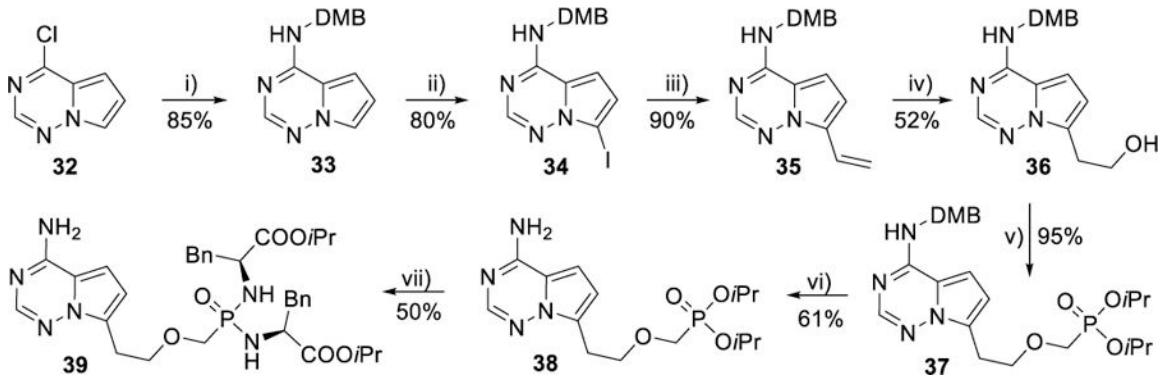
Scheme 5.

Synthesis of thieno[3,2-*d*]pyrimidine analogue **24**. Reagents and conditions: i) CH_2=$CHSnBu_3$, Pd(*t*-$Bu_3P)_2$, CuI, dioxane, 120 °C; ii) 1) 9-BBN, THF, 0 °C to rt, 2) aq. $NaBO_3$; iii) *n*-BuLi, $CF_3SO_2OCH_2P(O)(OiPr)_2$, THF, −78 °C; iv) EtOH/$NH_3$, 100 °C; v) TMSBr, pyridine, rt; then (L)-$NH_2CH(Bn)COOiPr$·HCl, PPh_3, Aldrithiol-2, pyridine, Et_3N, 70 °C.



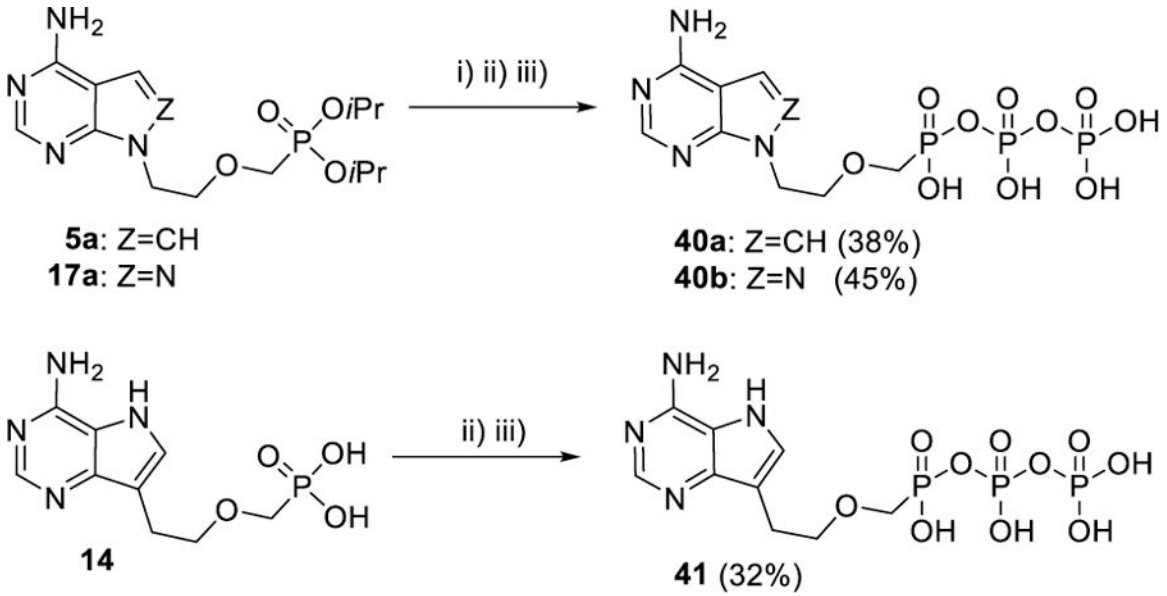
Scheme 6.

Synthesis of 4-aminoquinazoline analogue **31**. i) BOP reagent, DBU, DMF, CH_3CN, 2,4-dimethoxybenzylamine, 80 °C; ii) CH_2=$CHSnBu_3$, Pd(*t*-$Bu_3P)_2$, CuI, dioxane, 120 °C; iii) 9-BBN, 0 °C to RT; then aq. $NaBO_3$; iv) *n*-BuLi, $CF_3SO_2OCH_2P(O)(OiPr)_2$, THF, −78 °C; v) CF_3COOH, CH_2Cl_2, rt; vi) TMSBr, pyridine, rt; then (L)-$NH_2CH(Bn)COOiPr$·HCl, PPh_3, Aldrithiol-2, pyridine, Et_3N, 70 °C.



Scheme 7.

Synthesis of 4-aminopyrrolo[2,1-*f*][1,2,4]triazine derivative **39**. i) Et_3N, EtOH, 2,4-dimethoxybenzylamine, 120 °C MW; ii) NIS, CH_3CN, RT; iii) CH_2=$CHSnBu_3$, Pd(*t*-$Bu_3P)_2$, CuI, NMP, 120 °C; iv) 9-BBN, THF, 0 °C to RT; then aq. $NaBO_3$; v) *n*-BuLi, $CF_3SO_2OCH_2P(O)(OiPr)_2$, THF, −78 °C; vi) CF_3COOH, CH_2Cl_2, rt; vii) TMSBr, pyridine, rt then (L)-$NH_2CH(Bn)COOiPr$·HCl, PPh_3, Aldrithiol-2, pyridine, Et_3N, 70 °C.



Scheme 8.

Synthesis of phosphonodiphosphates **40a**, **40b**, and **41**. Reagents and conditions: i) TMSBr/CH_3CN; ii) DCC, morpholine, *t*BuOH, H_2O, reflux; iii) $(Bu_3N)_2P_2O_7$, Bu_3N, DMF.

Table 1.

ACT inhibition and cytotoxic effects of the base-modified PMEA derivatives measured in J774A.1 cells.

Compound	IC_{50} (μM)[a]	Viability (%)[b]
I	0.006 ± 0.001	59
III	0.147 ± 0.034	93
2a	>10	ND[c]
2b	>10	ND
6a	0.051 ± 0.008	120
6b	0.134 ± 0.019	90
6c	1.268 ± 0.222	89
6d	3.780 ± 0.360	105
6e	2.185 ± 0.454	106
15	>10	ND
18a	0.016 ± 0.004	109
18b	0.208 ± 0.088	101
24	0.196 ± 0.037	84
31	>10	ND
39	1.235 ± 0.603	96

[a] Data represent the mean ± SD of at least three independent experiments. IC_{50} = concentration of a compound causing a 50% decrease in ACT-induced cAMP accumulation.

[b] Data represent the percentage of cell viability at a fixed prodrug concentration (10 μM) versus untreated control.

[c] ND: not determined.

Table 2.

IC_{50} values of selected ANP-diphosphates for ACT and EF.

Compound	IC_{50} (μM)[a]		
	ACT Enzo	ACT Sigma	EF
II	13.6 ± 4.7	16.0 ± 0.1	11.5 ± 0.6
40a	4.08 ± 0.75	0.51 ± 0.12	2.54 ± 0.65
40b	20.8 ± 0.1	12.7 ± 0.3	5.28 ± 1.33
41	13.3 ± 2.3	9.32 ± 2.61	20.9 ± 1.8

[a]Data represent the mean ± SD of at least three independent experiments.

Table 3.

Mammalian AC1, AC2 and AC5 inhibition with base-modified ANPs at 30 μM measured in HEK293 cells.

Compound	Response of Control (%)[a]		
	AC1	AC2	AC5
I	177 ± 9	391 ± 30	345 ± 24
III	64 ± 9	200 ± 45	99 ± 8
2a	88 ± 6	149 ± 15	98 ± 4
2b	88 ± 15	107 ± 26	77 ± 5
6a	66 ± 15	207 ± 39	139 ± 14
6b	48 ± 12	176 ± 16	108 ± 8
6c	23 ± 4	236 ± 39	182 ± 10
6d	24 ± 5	238 ± 49	215 ± 17
6e	22 ± 6	182 ± 50	229 ± 33
15	62 ± 9	189 ± 27	134 ± 15
18a	71 ± 11	223 ± 37	147 ± 2
18b	79 ± 18	308 ± 12	176 ± 17
24	63 ± 5	262 ± 46	178 ± 30
31	56 ± 6	197 ± 12	220 ± 13
39	56 ± 7	174 ± 3	172 ± 12
SQ22536[b]	71 ± 6	52 ± 1	67 ± 12
NKY80[c]	74 ± 10	123 ± 27	54 ± 5
SKF83566[d]	82 ± 9	4 ± 4	103 ± 14

[a]Data are the mean ± SEM relative to the control response (100%) of at least two independent experiments. Values greater than 100% represent a potentiated response.

[b],[c]SQ22536 and NKY80 are non-selective P-site inhibitors.

[d]SKF83566 is a selective inhibitor of AC2.

Table 4.

IC_{50} values and % activity on AC1 and AC2 in HEK293 cells.

Compound	AC1		AC2	
	% Response[a]	IC_{50} (μM)[b]	% Response	IC_{50} (μM)
6c	22 ±5	5.6 ±1.7	143 ±21	ND[c]
6d	22 ±4	4.3 ±0.5	142 ±11	ND
6e	25 ±4	4.1 ±1.1	177 ±3	ND
SKF83566	ND	ND	14 ±5	5.0 ±1.6

[a]Percentages shown correspond to % mAC activity at 30 μM inhibitor normalized to the control response (no inhibitor). Values greater than 100% represent a potentiated response.

[b]Values are shown as mean and SEM of three independent experiments.

[c]ND, not determined.