



HHS Public Access

Author manuscript

ChemMedChem. Author manuscript; available in PMC 2023 January 05.

Published in final edited form as:

ChemMedChem. 2022 January 05; 17(1): e202100568. doi:10.1002/cmdc.202100568.

Halogen Dance-Based Synthesis of Phosphonomethoxyethyl (PME) Substituted 2-Aminothiazoles as Potent Inhibitors of Bacterial Adenylate Cyclases

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Abstract

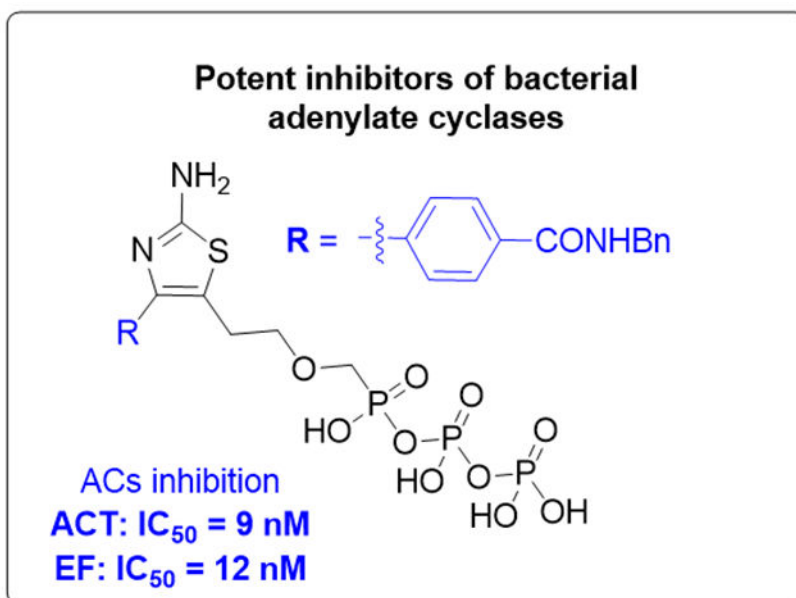
A series of acyclic nucleoside phosphonates (ANPs) was designed as inhibitors of bacterial adenylate cyclases (ACs), where adenine was replaced with 2-amino-4-arylthiazoles. The target compounds were prepared using the Halogen Dance reaction. Final ACs inhibitors were evaluated in cell-based assays (prodrugs) and cell-free assays (phosphono diphosphates). Novel ANPs were potent inhibitors of adenylate cyclase toxin (ACT) from *Bordetella pertussis* and edema factor (EF) from *Bacillus anthracis*, with substantial selectivity over mammalian enzymes AC1, AC2, and AC5. Six of the new ANPs were more potent or equipotent ACT inhibitors ($IC_{50} = 9\text{--}18$ nM), and one of them was more potent EF inhibitor ($IC_{50} = 12$ nM), compared to adefovir diphosphate (PMEApp) with $IC_{50} = 18$ nM for ACT and $IC_{50} = 36$ nM for EF. Thus, these compounds represent the most potent ACT/EF inhibitors based on ANPs reported to date. The potency of the phosphorodiamidates to inhibit ACT activity in J774A.1 macrophage cells was somewhat weaker, where the most potent derivative had $IC_{50} = 490$ nM compared to $IC_{50} = 150$ nM of the analogous adefovir phosphorodiamidate. The results suggest that more efficient type of phosphonate prodrugs would be desirable to increase concentrations of the ANP-based active species in the cells in order to proceed with the development of ANPs as potential antitoxin therapeutics.

Graphical Abstract

Novel acyclic nucleoside phosphonates (ANPs) derived from substituted 2-aminothiazole represent the most potent inhibitors (in the form of phosphono diphosphates in enzymatic assays) of bacterial adenylate cyclases (adenylate cyclase toxin and edema factor) based on ANPs. The target ANPs were synthesized using Halogen Dance reaction.

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The authors declare no competing financial interest.



Keywords

Acyclic nucleoside phosphonates; Adenylate cyclase; *Bordetella pertussis*; Inhibitors; Prodrugs

Introduction

Adenylate cyclases (ACs) form a large and diverse family of enzymes, which are broadly expressed in different kingdoms of life.^[1] ACs catalyze an intramolecular conversion of ATP to cyclic adenosine 3',5'-monophosphate (cAMP), a universal second messenger involved in passing on signals, that reach the cellular membrane, to intracellular targets.^[2] cAMP is involved in a wide range of key physiological processes, from virulence mechanisms in various bacteria to regulation of metabolism and gene transcription in mammals.^[3,4] The cytosolic levels of cAMP are strictly regulated mostly by its synthesis by diverse ACs and by cAMP degradation by phosphodiesterases (PDEs).^[1,2] Substantial alteration in the intracellular cAMP concentration has a profound effect on essential cellular processes and can be exploited to facilitate the invasion and survival of various pathogens in the host, thus contributing to pathogenesis of infectious diseases.^[5-7] It has been speculated, that inhibition of microbial AC-based toxins could be exploited as potential prophylactic or post-exposure treatment strategy to combat infectious diseases.^[7-9]

It has been reported that adefovir diphosphate (PMEApp, Fig. 1), the active metabolite of prodrug bis(POM)PMEA (Fig. 1), was active against both adenylate cyclase toxin (ACT) from *Bordetella pertussis*^[10] and adenylate cyclase-based edema factor (EF) from *Bacillus anthracis*.^[11] Furthermore PMEApp and its mimics were shown to inhibit ACs derived from rat brain.^[12]

Subsequently, an extensive structure-activity relationship study was performed with structurally modified adefovir (PMEA) analogues as inhibitors of bacterial adenylate cyclase

toxins: the SAR study covered adefovir derivatives modified either at the nucleobase^[13,14] or at the aliphatic moiety.^[15,16] Some of the compounds also exhibited an ability to selectively modulate mammalian ACs (especially AC1), with an anticipated potential for development of non-opioid alternatives for the treatment of inflammatory and neuropathic pain.^[14]

It has been recently reported,^[17] that the adenine moiety in adefovir could be replaced by another heterocycle that is able to mimic the adenine nucleobase, namely 2-aminothiazole, leading to compounds **II** (Fig. 2). The design of such compounds was based on compound MB05032 (Fig. 2),^[18-21] a potent and selective inhibitor of fructose 1,6-bisphosphatase (FBPase). It was shown that 2-aminothiazoles **II** bearing an aryl substituent in C5 position and a phosphonomethoxyethyl (PME) moiety in C4 position were potent inhibitors of bacterial AC toxins both as phosphorodiamidate prodrugs in J744A.1 murine macrophages or as phosphono diphosphates (nucleoside triphosphate analogues) in a cell-free assay.^[17]

The goal of the current study was to synthesize and evaluate a series of regioisomers derived from the previously reported 5-aryl-4-PME-2-aminothiazoles **II**, i.e. 4-aryl-5-PME-2-aminothiazoles **III** (Fig. 2).

In order to get an access to target compounds **III** (Fig. 2), synthetic methodology was developed and optimized starting from commercially available Boc-protected 2-aminothiazole. The final compounds were prepared in the form of their phosphorodiamidate prodrugs^[22-24] for cell-based assays and as their phosphono diphosphates for enzymatic assays. The cell-permeable phosphorodiamidate prodrugs bearing non-toxic L-phenylalanine isopropyl ester moieties were selected based on the previous observation,^[25] that PME A prodrug **I** (Fig. 1) was significantly less cytotoxic and showed better plasma stability profiles compared to bis(POM)PMEA (Fig. 1).

Results and Discussion

Synthesis

Starting Boc-protected 2-aminothiazole **1**^[26] was converted into the key intermediate, 2-amino-5-bromothiazole derivative **2** (Scheme 1), in a 74% yield using *N*-bromosuccinimide (NBS). 5-Bromothiazole derivative **2** was then subjected to the Halogen Dance (HD) reaction,^[27,28] using LDA and iodine as an electrophile, but desired 4-bromo-5-iodothiazole **3** was obtained only in a 37% yield. The next step was the transformation of 5-iodothiazole **3** into 5-vinylthiazole **4** using Suzuki-Miyaura cross-coupling and this step required an optimization of the reaction conditions: the reaction conversion was low with 1 equiv. of vinyltrifluoroborate, while large excess (5 equiv.) of the reagent led to the increased formation of undesired 4,5-divinyl-2-aminothiazole product (MW: 252.3, observed by UPLC-MS); eventually, the use of 3 equiv. of vinyltrifluoroborate afforded desired 5-vinylthiazole **4** in a 65% yield (Scheme 1). Nevertheless, the low yield (37%) of the HD reaction, overall high number of synthetic steps, and the necessity to optimize the subsequent transformation of 5-vinyl group into the 2-hydroxyethyl moiety compelled us to rethink the whole strategy at this point.

We decided to introduce the 2-hydroxyethyl moiety directly using the HD reaction with oxirane as an electrophile. The HD reaction of 5-bromothiazole **2** with LDA followed by treatment with oxirane afforded the desired product as a lithium salt, which after hydrolysis gave compound **5** (Scheme 1) in a 53% yield. However, we decided to directly exploit the lithium salt of compound **5** for the subsequent *in situ* alkylation of the 2-hydroxyethyl moiety.

A sequential *in situ* treatment of 5-bromothiazole **2** with LDA, oxirane, and subsequently with TfOCH₂P(O)(OiPr)₂ afforded the desired phosphonate intermediate **6** (Scheme 2) in an overall 62% yield. It should be mentioned, that this satisfactory yield was obtained after a thorough optimization process and that several crucial factors should be taken into consideration for this one pot synthesis. First, each reaction step demanded a specific temperature: optimal temperature for the HD reaction was –20 °C, for the oxirane opening +10 °C, and for the final alkylation –40 °C. The second key factor was the presence of 3 equiv. of each of the reagents, i.e. LDA, oxirane, and the alkylating agent. The use of 1 or 2 equiv. of LDA dramatically decreased the HD reaction conversion, which is in accordance with the literature.^[29] Similarly, significantly lower yields of compound **6** were obtained when only 1–2 equiv. of oxirane or of the alkylating agent were employed. Thirdly, the reaction time was crucial too: the reaction was ideally stopped 2 h after the addition of the alkylating agent despite the incomplete conversion (UPLC-MS), as after 3 h, the amount of bis-alkylated by-product **7** started to increase substantially (UPLC-MS).

Key 4-bromothiazole intermediate **6** was used in the Suzuki-Miyaura coupling for the synthesis of a series of 4-aryl substituted 2-aminothiazole-based ANPs **8a–8i** (Scheme 2) in 28–77% yields (Table 1). Phosphonate diesters **8a–8i** were subsequently converted into final phosphorodiamidate prodrugs **9a–9i** (29–61%) by their transformation into the corresponding silyl esters first, followed by the treatment with L-phenylalanine isopropyl ester under the standard reaction conditions (Aldriethiol-2, pyridine, Et₃N, 70 °C) previously reported by our group.^[30] Finally, compounds **8a–8i** were used for the preparation of corresponding phosphono diphosphates **10a–10i** (4–13%), the active triphosphate analogues (Table 1): transformation of **8a–8i** into the corresponding silyl esters, followed by the treatment with carbonyldiimidazole and tributylammonium pyrophosphate.^[31] The low yields of compounds **10a–10i** were primarily caused by the low conversion, by relative instability of the phosphono diphosphates, and by their laborious purification process.

It should be noted, that the assignment of the regiochemistry of compounds **8a–8i** was based on 2D NMR spectroscopy measurements, namely H,C-HMBC experiments, which correlate hydrogen and carbon signals usually *via* two or three covalent bonds. The chemical shifts of carbon atoms in positions C4 and C5 of the thiazole ring can be easily distinguished because C5 (neighboring the sulfur atom) is found in the region of 115–120 ppm while C4 (next to the nitrogen) can be found close to 145 ppm. The aromatic hydrogen atoms in *ortho* position to the thiazole ring have a clear three-bond correlation with carbon C4 in the HMBC spectra, while the aliphatic CH₂ hydrogen atoms next to the thiazole ring have both the two-bond correlation to C5 and three-bond correlation to C4. The hydrogen atoms of the second next CH₂ group have only three-bond correlations with carbon C5. The structure of the thiazole derivatives prepared earlier^[17] was also unequivocally determined by HMBC experiments.

Inhibition of ACT in cell-based assays—The general putative cleavage of phosphorodiamidates^[32] releasing the parent ANPs is similar to the metabolic activation of phosphoramidates (ProTides),^[33] which is well-described.^[34] The released phosphonates need to be further phosphorylated to the active species, phosphono diphosphates (nucleoside triphosphate analogues), as it was previously shown,^[17] that parent ANPs are not active in the enzymatic assays. Thus, all prepared phosphorodiamidates **9a–9i** were tested for their ability to inhibit *B. pertussis* adenylate cyclase toxin (ACT) activity in J774A.1 macrophage cells (Table 2). Murine J774A.1 cells were incubated with various concentrations of tested compounds and subsequently exposed to ACT. The cells were lysed and the amount of cAMP was determined. Two of the prepared compounds (**9b** and **9f**) exhibited a single digit μM activity. The best ACT inhibitor from the series proved to be compound **9d** with $\text{IC}_{50} = 490 \text{ nM}$, which was only about three times less potent than phosphorodiamidate prodrug of adefovir **I** ($\text{IC}_{50} = 147 \text{ nM}$, Table 2).^[14] In general, the novel 4-aryl-5-PME-2-aminothiazole analogues **9a–9i** exhibited somewhat lower potency to inhibit ACT in the cell-based assay compared to previously published regioisomeric 5-aryl-4-PME-2-aminothiazoles (Table 2, values in red color),^[17] with the exception of compound **9d**, (4-isopropoxyphenyl)thiazole derivative ($\text{IC}_{50} = 490 \text{ nM}$), which is slightly (1.4 fold) more potent compared to its corresponding regioisomer **II** ($\text{IC}_{50} = 680 \text{ nM}$). On the other hand, the most potent inhibitor from the previous series was 5-[3-(benzyloxy)phenyl]thiazole derivative ($\text{IC}_{50} = 260 \text{ nM}$, Table 2),^[17] while the 4-[3-(benzyloxy)phenyl]thiazole regioisomer **9c** from the current study was inactive ($\text{IC}_{50} > 10 \mu\text{M}$, Table 2). Thus, no clear correlation as for the ACT inhibition in the cell-based assay can be made between the two regioisomeric series of compounds.

Inhibition of mammalian ACs

All prepared phosphorodiamidates **9a–9i** were tested against human transmembrane AC isoforms at a single concentration of $30 \mu\text{M}$ (Table 2). Human AC1, AC2, and AC5, representing the three major mAC subfamilies, were overexpressed in HEK293 cells, which exhibit drastically reduced forskolin (FSK)-stimulated cAMP formation in the absence of recombinant AC.^[35] Most of the compounds failed to markedly inhibit any of the mACs tested, thus revealing promising selectivity for the bacterial ACs. From the whole series, compound **9a** was the most potent AC1 inhibitor (61% of remaining AC activity) with a good to moderate selectivity over AC2 (99%) and AC5 (79%). Treatment of AC2 with **9d** resulted in a moderate increase in activity, reaching 150% activity compared to FSK-stimulated control, a phenomenon that has previously been noted with some AC inhibitors.^[14,36] Only two compounds inhibited AC5 activity more than 20%: **9a** and **9f** inhibited this isoform to 79% and 73% activity, respectively. Previously described isoform selective inhibitors of AC1 and AC2 were also included as controls and performed as expected:^[36,37] the AC1-specific ST034307 reduced activity by over 99%, and SKF83566, an AC2-selective inhibitor, resulted in 85% inhibition of AC2.

Direct inhibition of ACT and EF activity

In order to observe direct enzymatic effect of the studied compounds (without the influence of the cell-penetration and prodrug biotransformation), triphosphate analogues **10a–10i** were

tested in a cell-free assay, along with PMEApp as a reference compound, for their inhibitory activity against two commercially available bacterial adenylate cyclases, adenylate cyclase toxin (ACT from *B. pertussis*) expressed recombinantly in *E. coli*, and edema factor (EF) from *Bacillus anthracis*. Compounds **10a–10i** (Table 3) proved to be very potent (mostly submicromolar) inhibitors of both ACT and EF, and (with the exception of **10h**) more potent compared to the previously reported regioisomers **II** (Fig. 2).^[17] In general, ACT seems to be more susceptible to inhibition by 2-aminothiazole-based ANPpp **10a–10i** than EF, which is also in agreement with previously reported regioisomeric analogues **II**.^[17] Six of the compounds (**10b**, **10c**, **10d**, **10f**, **10g**, and **10i**) are more potent or equipotent ACT inhibitors ($IC_{50} = 9.0\text{--}18.4$ nM) compared to PMEApp ($IC_{50} = 17.8$ nM) and, thus, they represent the most potent ANP-based inhibitors of bacterial ACs known up to date. From the whole series, derivative **10i** is the most potent ACT ($IC_{50} = 9$ nM) and EF ($IC_{50} = 12$ nM) inhibitor, twice and three times more potent against ACT and EF, respectively, than PMEApp (Table 3).

Conclusion

Nine novel acyclic nucleoside phosphonates (ANPs) containing the 4-aryl-2-aminothiazole moiety as a replacement for the nucleobase (adenine) were designed and prepared. The compounds were prepared as phosphonate diesters (**8a–8i**), in the form of phosphorodiamidate prodrugs (**9a–9i**), and as phosphono diphosphate analogues (**10a–10i**). Several prodrugs (namely **9b**, **9d**, and **9f**) were found to be potent (single digit μM or submicromolar) inhibitors of adenylate cyclase toxin (ACT) from *Bordetella pertussis* in the macrophage cell-based assay, they were non-cytotoxic, and did not markedly inhibit any of the tested mammalian ACs (mACs). The observed selectivity for ACT over mACs qualifies the 2-aminothiazole-based ANPs for potential therapeutic applications at concentrations that effectively neutralize bacterial toxins without deleterious side-effects through interference with the host ACs. The most potent compound of the series, 2-amino-4-(4-isopropoxyphenyl)thiazole derivative **9d**, inhibits ACT with $IC_{50} = 490$ nM, while it only slightly (by 29%) decreased AC1 activity and moderately (by 50%) increased AC2 activity. On the other hand, compound **9a** proved to be the most efficacious (61% of remaining AC activity) AC1 inhibitor, with some observed selectivity over AC2 and AC5 (with 99% and 79% remaining AC activity, respectively). In general, the phosphono diphosphate analogues **10a–10i** proved to be very potent inhibitors of bacterial adenylate cyclase toxins, where ACT from *B. pertussis* was more sensitive than EF from *B. anthracis*. Phosphono diphosphate **10i** was the most potent inhibitor of both ACT ($IC_{50} = 9$ nM) and EF ($IC_{50} = 11.6$ nM) in the series and represents the most potent ANP-based inhibitor of bacterial adenylate cyclases (ACT and EF) known to date. The noticeable discrepancy between the potency of studied analogues in cell-based (prodrugs) vs. enzymatic (phosphono diphosphates) assays could be explained by somewhat varying ability of the studied compounds to cross cell membranes and/or to undergo the biotransformation of the phosphorodiamidates into the enzymatically active triphosphate analogues. At the moment, development of more efficient prodrug strategy is in progress in order to evaluate the most potent analogues in the mouse models of pertussis and/or anthrax.

Experimental Section

Chemistry – general methods

Unless otherwise stated, solvents were evaporated at 40 °C/2 kPa and the compounds were dried over P₂O₅ 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, Fluorochem Ltd.). Solvents were dried by standard procedures; pyridine was stored over molecular sieves (4 Å); tetrahydrofuran (THF) was freshly distilled from LiAlH₄ pellets under Ar. TLC was performed on plates of Kieselgel 60 F254 (Merck). Preparative TLC was performed on plates of UNIPLATE, SILICA GEL GF, 2000 µm, 20 × 20 cm (Analtech). 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 crude mixtures using gradient MeOH/H₂O 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. NMR spectra were recorded on Bruker Avance III 500 (¹H at 500 MHz, ¹³C at 125.8 MHz and ³¹P at 202.4 MHz) and/or Bruker Avance III 400 (¹H at 401 MHz, ¹³C at 100.8 MHz) spectrometers 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). The purity of target compounds was determined by HPLC (H₂O-CH₃CN, linear gradient) and was higher than 95%.

Method A. General procedure for Suzuki-Miyaura cross-coupling reactions of 2-amino-4-bromo-thiazole derivatives

—A solution of **6** (0.50 g, 1.0 mmol) in THF (2 mL) under Ar was added to a mixture of precatalyst Xphos Pd G2 (31 mg, 0.04 mmol) and corresponding boronic acid (1.5 mmol) in degassed aqueous THF (21 mL, THF:H₂O, 4:1) under Ar, followed by a degassed solution of K₃PO₄ (640 mg, 3.0 mmol) in H₂O (2 mL). The resulting mixture was stirred at 50 °C overnight and then cooled down. Aqueous NH₄Cl solution (50%, 10 mL) was added and the mixture was extracted with EtOAc (3 × 25 mL). Combined organic layers were dried (Na₂SO₄), volatiles were removed *in vacuo* and the residue was dissolved in conc. TFA (5 mL). After 5 min at RT, the volatiles were removed *in vacuo* and the residue was codistilled with water (3 × 10 mL). The crude product was adsorbed onto the C18 silica gel and purified by reverse phase flash chromatography (0–100% H₂O in CH₃CN with 0.1 % HCOOH) to give the desired product.

Method B. General procedure for synthesis of bis-(L-phenylalanine ethyl ester) prodrugs of phosphonates^[30]

—TMSBr (1 mL) was added to the corresponding phosphonate diester **8** (1.0 mmol) dissolved in dry pyridine (10 mL) and the reaction mixture was stirred at 20 °C overnight. Volatiles were removed *in vacuo*, while the moisture sensitive intermediate 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 Et₃N (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 CHCl₃) followed by C18 reversed phase column chromatography (0–100% CH₃CN in water). The UV absorbing fractions were collected and evaporated *in vacuo*. The product was dissolved in dioxane (2 mL) and lyophilized to give a white amorphous solid.

Method C. General procedure for synthesis of phosphono diphosphates^[31]—

TMSBr (1 mL) was added to the corresponding phosphonate diester **8** (1.0 mmol) dissolved in dry pyridine (10 mL) under Ar. The reaction mixture was stirred at 20 °C overnight and evaporated. Aqueous TEAB solution (2 M, 2 mL), 1.0 M aqueous tetrabutylammonium hydroxide solution (2.5 mL, 2.5 mmol), and dioxane (5 mL) were added and volatiles were evaporated. The residue was dissolved in water (10 mL) and applied on C18 column (0–100% CH₃CN in water). Collected fractions with product were evaporated, the residue was codistilled with dioxane (3 × 5 mL) and dissolved in DMSO (10 mL). Carbonyldiimidazole (1.62 g, 10.0 mmol) was added and the mixture was stirred at RT for 2 h. MeOH (0.40 mL, 10.0 mmol) was added and the mixture was stirred at RT for another 30 min. Tributylammonium pyrophosphate (5.47 g, 10.0 mmol) was added and the mixture was stirred at RT overnight. Reaction mixture was diluted with water (5 mL) and applied on the RPC18 column (0–100% CH₃CN in water). UV absorbing fractions with the product were evaporated and purified on POROS 50 HQ column (TEAB 0.05–1 M) and applied on Dowex 50 in Na⁺ cycle. The product was lyophilized to give a white amorphous solid.

Preparation of fresh LDA solution—2.7 M BuLi in hexane (0.48 mL, 1.3 mmol) was added dropwise to a solution of *N,N*-diisopropylamine (0.2 mL, 1.4 mmol) in THF (3.5 mL) cooled to –78 °C under Ar. The resulting solution was directly used in the next reaction step.

Compounds synthesis and characterization

5-Bromo-2-(*tert*-butoxycarbonylamino)thiazole (2): A suspension of 2-(*tert*-butoxycarbonylamino)thiazole (6.3 g, 31.5 mmol) and *N*-bromosuccinimide (6.2 g, 34.6 mmol) in THF (100 mL) was stirred at room temperature for 12 h. The reaction mixture was filtered and purified by flash chromatography (linear gradient from hexane to 10% MeOH/EtOAc) to give **2** (6.5 g, 74%) as a white solid. ESI-MS, *m/z* (%): 301 [M+Na⁺] (100). ¹H NMR (DMSO-*d*₆): δ 11.73 (brs, 1H, NH); 7.43 (s, 1H, CH-4); 1.48 (s, 9H, CH₃). ¹³C NMR (DMSO-*d*₆): δ 160.16 (C-2); 152.97 (OCON); 139.00 (C-4); 100.59 (C-5); 81.72 (C-CH₃); 27.84 (CH₃). HR-MS (ESI+): *m/z* [M + Na]⁺ calculated for: C₈H₁₁O₂N₂BrNaS, 300.9617, found: 300.9620.

4-Bromo-5-iodo-2-(*tert*-butoxycarbonylamino)thiazole (3): A solution of **2** (1.0 g, 3.6 mmol) in dry THF (4 mL) was added dropwise to a freshly prepared THF solution of LDA (11.9 mmol) under Ar at 0 °C. The resulting solution was stirred at 0 °C for further 15 min. The reaction mixture was allowed to warm up to RT and a solution of iodine (3.0 g, 11.9 mmol) in THF (5 mL) was added dropwise. The reaction mixture was stirred at RT for 1 h and then diluted with EtOAc (150 mL) and with 50 % sat. NH₄Cl/H₂O solution (40 mL). The organic layer was washed with water (100 mL) and brine (100 mL), and

dried (Na_2SO_4). The solvent was evaporated and the crude product was purified by flash chromatography (10% EtOAc/cyclohexane) to give **3** (0.5 g, 37%) as a white solid. ESI-MS, m/z (%): 404.9 $[\text{M}+\text{H}^+]$ (60). ^1H NMR (CDCl_3): δ 9.06 (brs, 1H, NH); 1.56 (s, 9H, CH_3). ^{13}C NMR (CDCl_3): δ 164.62 (C-2); 151.80 (OCON); 129.86 (C-4); 83.88 ($\underline{\text{C}}\text{-CH}_3$); 65.07 (C-5); 28.28 (CH_3). HR-MS (ESI+): m/z $[\text{M} + \text{Na}]^+$ calculated for: $\text{C}_8\text{H}_{10}\text{O}_2\text{N}_2\text{BrINaS}$, 426.8583, found: 426.8585.

4-Bromo-5-vinyl-2-(tert-butoxycarbonylamino)thiazole (4): *N,N*-Diisopropylethylamine (0.04 mL, 0.25 mmol) was added to the mixture of **3** (0.10 g, 0.25 mmol), $\text{Pd}(\text{dppf})\text{Cl}_2 \cdot \text{CH}_2\text{Cl}_2$ (0.02 g, 10 mol%) and potassium vinyltrifluoroborate (0.10 g, 0.74 mmol) in degassed 96% aq. EtOH (5 mL) under Ar. The reaction mixture was heated to 100 °C for 4 h and then allowed to cool down to RT. Volatiles were removed *in vacuo*, the residue was dissolved in Et_2O (10 mL) and washed with 20% aq. NH_4Cl solution (2×10 mL) and brine (10 mL). Organic layer was dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by preparative TLC (10% cyclohexane/EtOAc) to give **4** (75 mg, 65%) as a white solid. ESI-MS, m/z (%): 327.0 $[\text{M}+\text{Na}^+]$ (100). ^1H NMR ($\text{DMSO}-d_6$): δ 11.88 (brs, 1H, NH); 6.65 (dd, $J = 17.4$, $J = 11.0$ Hz, 1H, H-1'); 5.49 (d, $J = 17.3$ Hz, 1H, H-2'a); 5.28 (d, $J = 11.3$ Hz, 1H, H-2'b); 1.48 (s, 9H, CH_3). ^{13}C NMR ($\text{DMSO}-d_6$): δ 164.32 (C-2); 152.65 (OCON); 126.53 (C-1'); 124.24 (C-5); 121.14 (C-4); 115.87 (C-2'); 82.12 ($\underline{\text{C}}\text{-CH}_3$); 27.80 (CH_3). HR-MS (ESI+): m/z $[\text{M} + \text{Na}]^+$ calculated for: $\text{C}_{10}\text{H}_{13}\text{O}_2\text{N}_2\text{BrNaS}$, 326.9773, found: 326.9776.

Bromo-5-(ethanol-2-yl)-2-(tert-butoxycarbonylamino)thiazole (5): A solution of **2** (0.50 g, 1.8 mmol) in dry THF (10 mL) was added dropwise to a freshly prepared THF solution of LDA (5.9 mmol) under Ar at 0 °C. The resulting solution was stirred at 0 °C for further 15 min and 3.0 M THF solution of oxirane (1.97 mL, 5.9 mmol) was added dropwise. The temperature was kept at 0 °C for further 30 min and then the reaction mixture was allowed to warm up to 10 °C and stirred for additional 2 h. The resulting slurry was diluted with water (10 mL) and volatiles were evaporated *in vacuo*. The residue was purified by flash chromatography (0.5–3% MeOH/toluene, checked at $\lambda_{\text{max}} = 270$ nm) to give **5** (0.31 g, 53%) as a white solid. ESI-MS, m/z (%): 345.0 $[\text{M}+\text{Na}^+]$ (100). ^1H NMR ($\text{DMSO}-d_6$): δ 11.55 (brs, 1H, NH); 4.93 (m, 1H, OH); 3.56 (m, 2H, O- CH_2); 2.77 (t, $J(\text{CH}_2, \text{CH}_2) = 6.3$ Hz, 2H, 5- CH_2); 1.48 (s, 9H, CH_3). ^{13}C NMR ($\text{DMSO}-d_6$): δ 158.55 (C-2); 152.70 (OCON); 122.99 (C-5); 118.70 (C-4); 81.53 ($\underline{\text{C}}\text{-CH}_3$); 60.25 (O- CH_2); 30.57 (5- CH_2); 27.84 (CH_3). HR-MS (ESI+): m/z $[\text{M} + \text{Na}]^+$ calculated for: $\text{C}_{10}\text{H}_{15}\text{O}_3\text{N}_2\text{BrNaS}$, 344.9879, found: 344.9882.

4-Bromo-2-(tert-butoxycarbonylamino)-5-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}-thiazole (6): A solution of **2** (1.0 g, 3.6 mmol) in dry THF (20 mL) was added dropwise to a freshly prepared THF solution of LDA (11.9 mmol) under AR at -20 °C and after 15 min, THF solution of oxirane (3 M, 4 mL, 11.9 mmol) was added dropwise at -20 °C. The reaction mixture was allowed to warm up to 10 °C (1 h), when the brown solution changed into a brownish slurry. The reaction mixture was cooled down to -40 °C and a solution of diisopropylphosphonemethyl triflate (3.9 g, 11.9 mmol) in THF (30 mL) was added dropwise. The reaction mixture was stirred at -40 °C for

2 h. Then, 50 % sat. NH₄Cl/H₂O solution (40 mL) was added and the mixture was washed with EtOAc (150 mL). The organic layer was washed by water (100 mL) and brine (100 mL) and dried (Na₂SO₄). The solvent was evaporated and the crude product was purified by flash chromatography (linear gradient from cyclohexane to 10% MeOH/EtOAc) to give **6** (1.15 g, 64%) as a white solid. ESI-MS, *m/z* (%): 523.0 [M+Na⁺] (100). ¹H NMR (DMSO-*d*₆): δ 11.58 (s, 1H, NH); 4.70–4.48 (m, 2H, CH-*iPr*); 3.76 (d, *J*(CH₂,P) = 8.3 Hz, 2H, P-CH₂); 3.68 (t, *J*(CH₂,CH₂) = 5.9 Hz, 2H, O-CH₂); 2.88 (t, *J*(CH₂,CH₂) = 6.1 Hz, 2H, 5-CH₂); 1.47 (s, 9H, CH₃); 1.24–1.19 (m, 12H, 2 x CH₃). ¹³C NMR (DMSO-*d*₆): δ 158.75 (C-2); 152.70 (OCN); 122.44 (C-5); 119.12 (C-4); 81.58 (C-CH₃); 71.41 (d, *J* = 11.8 Hz, OCH₂); 70.24 (d, *J* = 6.4 Hz, CH-*iPr*); 64.81 (d, *J* = 164.1 Hz, P-CH₂); 27.83 (C-CH₃); 27.36 (C-1'); 23.82, 23.79, 23.71, 23.67 (4 x CH₃). HR-MS (ESI+): *m/z* [M + Na]⁺ calculated for: C₁₇H₃₀O₆N₂BrNaPS, 523.0638, found: 523.0643.

2-Amino-5-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}-4-phenylthiazole

(8a): Treatment of **6** (0.40 g, 0.8 mmol) by Method A afforded **8a** (0.24 g, 74%) as a yellowish oil. ESI-MS, *m/z* (%): 399 [M+H⁺] (100). ¹H NMR (DMSO-*d*₆): δ 7.58–7.46 (m, 2H, H-2'); 7.35–7.40 (m, 2H, H-3'); 7.32–7.23 (m, 1H, H-4'); 6.84 (bs, 2H, NH₂); 4.57 (m, 2H, CH-*iPr*); 3.74 (d, *J*(CH₂, P) = 8.1 Hz, 2H, P-CH₂); 3.69 (t, *J*(CH₂,CH₂) = 6.5 Hz, 2H, O-CH₂); 2.92 (t, *J*(CH₂,CH₂) = 6.5 Hz, 2H, 5-CH₂); 1.22 and 1.21 2 x (d, *J*(CH₃,CH) = 7.7 Hz, 6H, CH₃). ¹³C NMR (DMSO-*d*₆): δ 165.46 (C-2); 146.23 (C-4); 135.40 (C-1'); 128.23 and 128.07 (C-2', 3'); 127.02 (C-4'); 116.82 (C-5); 72.98 d, *J*(C-O-C-P) = 11.5 Hz, (O-CH₂); 71.15 (d, *J*(C-O-P) = 6.4 Hz, CH-*iPr*); 64.81 (d, *J*(C-P) = 163.9 Hz, CH₂-P); 27.16 (5-CH₂); 23.84, 23.82, 23.80 and 23.71 (CH₃-*iPr*). HR-MS (ESI+): *m/z* [M + H]⁺ calculated for: C₁₈H₂₈N₂O₄PS, 399.1502, found: 399.1502.

2-Amino-5-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}-4-(3-butoxyphenyl)thiazole

(8b): Treatment of **6** (0.40 g, 0.8 mmol) by Method A afforded **8b** (0.19 g, 51%) as a yellowish oil. ESI-MS, *m/z* (%): 471 [M+H⁺] (100). ¹H NMR (DMSO-*d*₆): δ 7.24–7.29 (m, 1H, H-5'); 7.12–7.00 (m, 2H, H-2', 6'); 6.84–6.86 (m, 3H, C-4', NH₂); 4.51–4.63 (m, 2H, CH-*iPr*); 3.97 (t, *J*(CH₂,CH₂) = 6.5 Hz, 2H, 3'-O-CH₂); 3.74 (d, *J*(CH₂,P) = 8.1 Hz, 2H, P-CH₂); 3.69 (t, *J*(CH₂,CH₂) = 6.5 Hz, 2H, O-CH₂); 2.94 (t, *J*(CH₂,CH₂) = 6.5 Hz, 2H, 5-CH₂); 1.79–1.63 (m, 2H, -O-CH₂-CH₂); 1.58–1.35 (m, 2H, CH₂-CH₃); 1.23 (d, *J*(CH₃,CH) = 8.0 Hz, 6H, CH₃); 1.21 (d, *J*(CH₃,CH) = 8.0 Hz, 6H, CH₃); 0.94 (t, *J*(CH₃,CH₂) = 7.4 Hz, 3H, CH₂CH₃). ¹³C NMR (DMSO-*d*₆): δ 165.38 (C-2); 158.48 (C-3'); 146.15 (C-4); 136.78 (C-1'); 128.97 (C-5'); 120.42 (C-6'); 117.00 (C-5); 114.01 (C-2'); 113.48 (C-4'); 72.96 d, *J*(C-O-C-P) = 11.3 Hz, (O-CH₂); 71.16 (d, *J*(C-O-P) = 6.3 Hz, CH-*iPr*); 67.04 (3'-O-CH₂); 64.85 d, *J*(C-P) = 164.1 Hz, (CH₂-P); 30.80 (3''-O-CH₂-CH₂); 27.19 (5-CH₂); 23.84, 23.81, 23.71 and 23.66 (CH₃-*iPr*); 18.77 (CH₂-CH₃); 13.72 (CH₂-CH₃). HR-MS (ESI+): *m/z* [M + H]⁺ calculated for: C₂₂H₃₆N₂O₅PS, 471.2077, found: 471.2075.

2-Amino-5-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}-4-[3-(benzyloxy)phenyl]thiazole (8c): Treatment of **6** (0.40 g, 0.8 mmol)

by Method A afforded **8c** (0.18 g, 55%) as a colourless oil. ESI-MS, *m/z* (%): 505 [M+H⁺] (100). ¹H NMR (DMSO-*d*₆): δ 7.45 (m, 2H, H-2''); 7.38 (m, 2H, H-3''); 7.07–7.33 (m, 5H, H-2', 4', 5', 6', 4''); 6.84 (bs, 2H, NH₂); 5.13 (s, 2H, 1''-CH₂); 4.63–4.51 (m, 2H, CH-

iPr); 3.74 (d, $J(\text{CH}_2, \text{P}) = 8.1$ Hz, 2H, P-CH₂); 3.67 (t, $J(\text{CH}_2, \text{CH}_2) = 6.4$ Hz, 2H, O-CH₂); 2.90 (t, $J(\text{CH}_2, \text{CH}_2) = 6.5$ Hz, 2H, 5-CH₂); 1.23 (d, $J(\text{CH}_3, \text{CH}) = 8.3$ Hz, 6H, CH₃); 1.21 (d, $J(\text{CH}_3, \text{CH}) = 8.3$ Hz, 6H, CH₃). ¹³C NMR (DMSO-*d*₆): δ 165.45 (C-2); 158.12 (C-3'); 146.03 (C-4); 137.19 (C-1''); 136.80 (C-1'); 129.89 (C-5'); 128.46 (C-3''); 127.79 (C-4''); 127.62 (C-2''); 120.89 (C-6'); 117.23 (C-5); 114.43 (C-2'); 113.84 (C-4'); 72.98 d, $J(\text{C-O-C-P}) = 11.5$ Hz, (O-CH₂); 70.24 (d, $J(\text{C-O-P}) = 6.3$ Hz, CH-*iPr*); 69.12 (3'-O-CH₂); 64.88 d, $J(\text{C-P}) = 164.0$ Hz, (CH₂-P); 27.19 (5-CH₂); 23.86, 23.83, 23.73 and 23.68 (CH₃-*iPr*). HR-MS (ESI+): *m/z* [M + H]⁺ calculated for: C₂₅H₃₄N₂O₅PS, 505.1921, found: 505.1919.

2-Amino-5-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}-4-(4-isopropoxyphenyl)thiazole (8d): Treatment of

6 (0.50 g, 1.0 mmol) by Method A afforded **8d** (0.29 g, 63%) as a yellowish oil. ESI-MS, *m/z* (%): 457 [M+H⁺] (100). ¹H NMR (DMSO-*d*₆): δ 7.48–7.37 (m, 2H, H-2'); 7.02–6.76 (m, 2H, H-3'); 4.62–4.58 (m, 3H, 4'-O-CH, CH-*iPr*); 3.75 (d, $J(\text{CH}_2, \text{P}) = 8.1$ Hz, 2H, P-CH₂); 3.69 (t, $J(\text{CH}_2, \text{CH}_2) = 6.4$ Hz, 2H, O-CH₂); 2.89 (t, $J(\text{CH}_2, \text{CH}_2) = 6.4$ Hz, 2H, 5-CH₂); 1.26 (d, 6H, $J(\text{CH}_3, \text{CH}) = 6.0$ Hz, 4'-O-CH-CH₃); 1.23 (m, 6H, CH₃); 1.21 (m, 6H, CH₃). ¹³C NMR (DMSO-*d*₆): δ 166.07 (C-2); 156.99 (C-4'); 146.26 (C-4); 129.73 (C-2', C-6'); 127.91 (C-1'); 115.47 (C-5); 115.17 (C-3', 5'); 72.59 d, $J(\text{C-O-C-P}) = 11.6$ Hz, (O-CH₂); 70.15 (d, $J(\text{C-O-P}) = 6.4$ Hz, CH-*iPr*); 69.16 (4'-O-CH); 64.80 d, $J(\text{C-P}) = 164.0$ Hz, (CH₂-P); 26.96 (5-CH₂); 23.84, 23.80, 23.71 and 23.66 (CH₃-*iPr*); 21.82 (4''-O-CH-CH₃). HR-MS (ESI+): *m/z* [M + H]⁺ calculated for: C₂₁H₃₄N₂O₅PS, 457.1921, found: 457.1919.

2-Amino-5-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}-4-(2-fluorophenyl)thiazole (8e): Treatment of **6** (0.50 g, 1.0 mmol) by Method A afforded **8e** (0.29 g, 70%) as a

brownish oil. ESI-MS, *m/z* (%): 417 [M+H⁺] (100). ¹H NMR (DMSO-*d*₆): δ 7.35–7.47 (m, 2H, H-4'', 6''); 7.19–7.28 (m, 2H, H-3'', 5''); 6.86 (bs, 2H, NH₂); 4.45–4.65 (m, 2H, CH-*iPr*); 3.70 (d, $J(\text{P,CH}_2) = 8.2$ Hz, 2H, P-CH₂); 3.60 (t, $J(\text{CH}_2, \text{CH}_2) = 6.6$ Hz, 2H, O-CH₂); 2.71 (t, $J(\text{CH}_2, \text{CH}_2) = 6.4$ Hz, 2H, 5-CH₂); 1.22 (d, $J(\text{CH}_3, \text{CH}) = 6.2$ Hz, 6H, CH₃); 1.20 (d, $J(\text{CH}_3, \text{CH}) = 6.2$ Hz, 6H, CH₃). ¹³C NMR (DMSO-*d*₆): δ 166.00 (C-2); 159.08 (d, $J(2''\text{-F}) = 245.8$ Hz, C-2''); 140.61 (C-4); 131.80 (d, $J(6''\text{-F}) = 3.4$ Hz, C-6''); 129.65 (d, $J(4''\text{-F}) = 8.2$ Hz, C-4''); 124.15 (d, $J(5''\text{-F}) = 3.3$ Hz, C-5''); 123.22 (d, $J(1''\text{-F}) = 15.0$ Hz, C-1''); 118.75 (C-5); 115.72 (d, $J(3''\text{-F}) = 22.4$ Hz, C-3''); 72.67 d, $J(\text{C-O-C-P}) = 11.5$ Hz, (O-CH₂); 70.15 $J(\text{C-O-P}) = 6.4$ Hz, (CH-*iPr*); 64.75 d, $J(\text{C-P}) = 164.1$ Hz, (CH₂-P); 27.14 (5-CH₂); 23.84, 23.80, 23.70 and 23.65 (CH₃-*iPr*). HR-MS (ESI+): *m/z* [M + H]⁺ calculated for: C₁₈H₂₇N₂O₄FPS, 417.1408, found: 417.1408.

2-Amino-5-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}-4-[3-(fluoro)phenyl]thiazole (8f): Treatment of **6** (0.50 g, 1.0 mmol) by Method A afforded **8f** (0.27 g, 65%) as a

brownish oil. ESI-MS, *m/z* (%): 417 [M+H⁺] (80). ¹H NMR (DMSO-*d*₆): δ 7.39–7.47 (m, 2H, H-5', 6'); 7.29–7.36 (m, 1H, H-2'); 7.09–7.17 (m, 1H, H-4'); 6.89 (bs, 2H, NH₂); 4.57 (sept, $J(\text{CH}, \text{CH}_3) = 6.2$ Hz, 2H, CH-*iPr*); 3.75 (d, $J(\text{P,CH}_2) = 8.2$ Hz, 2H, P-CH₂); 3.70 (t, $J(\text{CH}_2, \text{CH}_2) = 6.4$ Hz, 2H, O-CH₂); 2.95 (t, $J(\text{CH}_2, \text{CH}_2) = 6.4$ Hz, 2H, 5-CH₂); 1.22 (d, $J(\text{CH}_3, \text{CH}) = 6.2$ Hz, 6H, CH₃); 1.20 (d, $J(\text{CH}_3, \text{CH}) = 6.2$ Hz, 6H, CH₃). ¹³C NMR (DMSO-*d*₆): δ 166.02 (C-2); 162.46 (d, $J(2'\text{-F}) = 242.7$ Hz, C-2'); 145.35 (d, $J(4, \text{F}) = 2.4$ Hz, C-4); 138.25 (d, $J(1'\text{-F}) = 8.2$ Hz, C-1'); 130.48 (d, $J(5'\text{-F}) = 8.5$ Hz, C-5'); 124.69

(d, $J(6',F) = 2.5$ Hz, C-6'); 118.66 (C-5); 115.19 (d, $J(2',F) = 22.1$ Hz, C-2'); 114.23 (d, $J(4',F) = 20.9$ Hz, C-4'); 73.35 d, $J(C-O-C-P) = 11.6$ Hz, (O-CH₂); 70.63 $J(C-O-P) = 6.3$ Hz, (CH-*iPr*); 65.32 d, $J(C-P) = 164.0$ Hz, (CH₂-P); 27.59 (5-CH₂); 24.29, 24.25, 24.15 and 24.11 (CH₃-*iPr*). HR-MS (ESI+): m/z [M + H]⁺ calculated for: C₁₈H₂₇N₂O₄FPS, 417.1408, found: 417.1408.

2-Amino-5-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}-4-[3-(N-methylsulfamoyl)phenyl]thiazole (8g): Treatment of

6 (0.50 g, 1.0 mmol) by Method A afforded **8g** (0.38 g, 77%) as a brownish oil. ESI-MS, m/z (%): 492 [M+H⁺] (100). ¹H NMR (DMSO-*d*₆): δ 9.78 (bs, 1H, SO₂NH); 7.46–6.93 (m, 6H, H-2', 4' 5', 6', NH₂); 4.57 (sept., $J(CH,CH_3) = 6.2$ Hz, 2H, CH-*iPr*); 3.75 (d, $J(P,CH_2) = 8.2$ Hz, 2H, P-CH₂); 3.69 (d, $J(CH_2,CH_2) = 6.4$ Hz, 2H, O-CH₂); 2.97 (s, 3H, CH₃NH); 2.92 (t, $J(CH_2,CH_2) = 6.4$ Hz, 2H, 5-CH₂); 1.23 (d, $J(CH_3,CH) = 6.2$ Hz, 6H, CH₃); 1.21 (d, $J(CH_3,CH) = 6.2$ Hz, 6H, CH₃). ¹³C NMR (DMSO-*d*₆): δ 165.82 (C-2); 144.68 (C-4); 138.34 and 138.39 (C-3', C-1'); 129.30 (C-5'); 123.83 (C-6'); 119.76 (C-2'); 118.60 (C-4'); 117.30 (C-5); 72.76 d, $J(C-O-C-P) = 11.5$ Hz, (O-CH₂); 70.21 (d, $J(C-O-P) = 6.4$ Hz, CH-*iPr*); 64.79 d, $J(C-P) = 164.1$ Hz, (CH₂-P); 39.31 (NH-CH₃); 27.06 (5-CH₂); 23.85, 23.82, 23.72 and 23.68 (CH₃-*iPr*). HR-MS (ESI+): m/z [M + H]⁺ calculated for: C₁₉H₃₁N₃O₆PS₂, 492.1386, found: 492.1386.

2-Amino-5-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}-4-[4-(methylcarbamoyl)phenyl]thiazole (8h): Treatment of **6** (0.50 g, 1.0 mmol) by Method

A afforded **8h** (0.22 g, 49%) as a brownish oil. ESI-MS, m/z (%): 456 [M+H⁺] (50); 478 [M+Na⁺]. ¹H NMR (DMSO-*d*₆): δ 8.43 (q, 1H, $J(NH,CH_3) = 4.2$ Hz, NHCH₃); 7.80–7.85 (m, 2H, H-2', 6'); 7.58–7.63 (m, 2H, H-3', 5'); 6.89 (bs, 2H, NH₂); 4.57 (sept., $J(CH,CH_3) = 6.2$ Hz, 2H, CH-*iPr*); 3.74 (d, $J(P,CH_2) = 8.1$ Hz, 2H, P-CH₂); 3.70 (t, $J(CH_2,CH_2) = 6.4$ Hz, 2H, O-CH₂); 2.95 (t, $J(CH_2,CH_2) = 6.4$ Hz, 2H, 5-CH₂); 2.79 (d, $J(CH_3,NH) = 4.5$ Hz, 3H, NH-CH₃); 1.22 (d, $J(CH_3,CH) = 6.2$ Hz, 6H, CH₃); 1.20 (d, $J(CH_3,CH) = 6.2$ Hz, 6H, CH₃). ¹³C NMR (DMSO-*d*₆): δ 166.34 (CON); 165.58 (C-2); 145.40 (C-4); 137.87 (C-4'); 132.84 (C-1'); 127.95 (C-2'); 126.93 (C-3'); 118.25 (C-5); 72.88 d, $J(C-O-C-P) = 11.7$ Hz, (O-CH₂); 70.17 $J(C-O-P) = 6.4$ Hz, (CH-*iPr*); 64.82 d, $J(C-P) = 164.0$ Hz, (CH₂-P); 27.19 (5-CH₂); 26.26 (NH-CH₃); 23.85, 23.81, 23.71 and 23.67 (CH₃-*iPr*). HR-MS (ESI+): m/z [M + H]⁺ calculated for: C₂₀H₃₁N₃O₅PS, 456.1717, found: 456.1717.

2-Amino-5-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}-4-[4-(benzylcarbamoyl)phenyl]thiazole (8i): Treatment of **6** (0.50 g, 1.0 mmol)

by Method A afforded **8i** (0.30 g, 56%) as a brownish oil. ESI-MS, m/z (%): 554 [M+Na⁺] (100). ¹H NMR (DMSO-*d*₆): δ 9.05 (t, $J(NH,CH_2) = 6.1$ Hz, CO-NH); 7.87–7.94 (m, 2H, H-3'); 7.60–7.66 (m, 2H, H-2'); 7.29–7.36 (m, 4H, H-2'', 3''); 7.21–7.28 (m, 1H, H-4''); 6.88 (bs, 2H, NH₂); 4.57 (sept., $J(CH,CH_3) = 6.2$ Hz, 2H, CH-*iPr*); 4.49 (d, $J(CH_2,NH) = 6.0$ Hz, 2H, NH-CH₂); 3.74 (d, $J(P,CH_2) = 8.2$ Hz, 2H, P-CH₂); 3.70 (t, $J(CH_2,CH_2) = 6.4$ Hz, 2H, O-CH₂); 2.96 (t, $J(CH_2,CH_2) = 6.4$ Hz, 2H, 5-CH₂); 1.22 (d, $J(CH_3,CH) = 6.2$ Hz, 6H, CH₃); 1.20 (d, $J(CH_3,CH) = 6.2$ Hz, 6H, CH₃). ¹³C NMR (DMSO-*d*₆): δ 165.92 (CO-NH); 165.58 (C-2); 145.48 (C-4); 139.75 (C-1''); 138.15 (C-1'); 132.63 (C-4'); 128.27 (C-3''); 127.98 (C-2'); 127.19 (C-3'); 127.15 (C-2''); 126.72 (C-4''); 118.35 (C-5);

72.88 d, $J(\text{C-O-C-P}) = 11.4$ Hz, (O-CH₂); 70.17 $J(\text{C-O-P}) = 6.3$ Hz, (CH-*iPr*); 64.82 d, $J(\text{C-P}) = 163.9$ Hz, (CH₂-P); 27.19 (5-CH₂); 23.85, 23.81, 23.71 and 23.67 (CH₃-*iPr*). HR-MS (ESI+): m/z [M + H]⁺ calculated for: C₂₆H₃₄N₃O₅PSNa, 554.1849, found: 554.1847.

Bis(L-phenylalanine isopropyl ester) prodrug of 2-amino-5-[2-

(phosphorylmethoxy)ethyl]-4-(phenyl)thiazole (9a).: Treatment of **8a** (0.14 g, 0.35 mmol) by Method B afforded **9a** (0.13 g, 55%) as a colourless foam. ESI-MS, m/z (%): 693 [M+H⁺] (100). ¹H NMR (DMSO-*d*₆): δ 7.50 (m, 2H, H-2''); 7.37 (m, 2H, H-3''); 7.11–7.31 (m, 11H, H-2', 3', 4', 4''); 6.85 (bs, 2H, NH₂); 4.81 and 4.77 2 × (sept., $J(\text{CH}, \text{CH}_3) = 6.3$ Hz, 1H, CH-*iPr*); 4.42 and 4.09 2 × (t, $J(\text{NH}, \text{CH}) = J(\text{NH}, \text{P}) = 11.3$ Hz, 1H, NH); 3.84–3.97 (m, 2H, NH-CH); 3.50 (t, $J(\text{CH}_2, \text{CH}_2) = 7.1$ Hz, 2H, O-CH₂); 3.20–3.30 (m, 2H, P-CH₂); 2.87 (t, $J = 7.1$ Hz, 2H, 5-CH₂); 2.74–2.91 (m, 4H, CH₂Ph); 1.15, 1.11, 1.06 and 1.01, 4 × (d, $J(\text{CH}_3, \text{CH}) = 6.3$ Hz, 3H, CH₃). ¹³C NMR (DMSO-*d*₆): δ 172.50 (d $J(\text{C-C-N-P}) = 2.9$, COO); 172.34 (d $J(\text{C-C-N-P}) = 5.1$, COO); 165.59 (C-2); 146.44 (C-4); 137.19 and 137.34 (C-1'); 135.64 (C-1''); 129.71 (C-2'); 128.38 (C-3'); 128.31, 128.28 (C-2'', 3''); 127.25 (C-4''); 126.71 and 126.66 (C-4'); 116.54 (C-5); 73.04 d, $J(\text{C-O-C-P}) = 11.0$ Hz, (O-CH₂); 68.17 and 68.04 (CH-*iPr*); 67.75 d, $J(\text{C-P}) = 134.9$ Hz, (CH₂-P); 54.18 and 54.14 (NH-CH); 39.90 (CH₂Ph); 27.36 (5-CH₂); 21.72, 21.66, 21.60 and 21.54 (CH₃-*iPr*); HR-MS (ESI+): m/z [M + H]⁺ calculated for: C₃₆H₄₆N₄O₆PS, 693.2870, found: 693.2879.

Bis(L-phenylalanine isopropyl ester) prodrug of 2-amino-5-[2-

(phosphorylmethoxy)ethyl]-4-(3-butoxyphenyl)thiazole (9b).: Treatment of **8b** (0.17 g, 0.35 mmol) by Method B afforded **9b** (0.12 g, 43%) as a colourless solid. ESI-MS, m/z (%): 765 [M+H⁺] (100). ¹H NMR (DMSO-*d*₆): 7.11–7.27 (m, 11H, H-2', 3', 4', 5''); 7.02–7.06 (m, 2H, H-2'', 6''); 6.84–6.86 (m, 3H, C-4'', NH₂); 4.81 and 4.77 2 × (sept., $J(\text{CH}, \text{CH}_3) = 6.3$ Hz, 1H, CH-*iPr*); 4.41 and 4.06 2 × (t, $J(\text{NH}, \text{CH}) = J(\text{NH}, \text{P}) = 11.3$ Hz, 1H, NH); 3.95 (t, 2H, $J(\text{CH}_2, \text{CH}_2) = 6.5$ Hz, 3''-O-CH₂); 3.83–3.97 (m, 2H, NH-CH); 3.49 (t, $J(\text{CH}_2, \text{CH}_2) = 7.2$ Hz, 2H, O-CH₂); 3.19–3.28 (m, 2H, P-CH₂); 2.73–2.90 (m, 6H, 5-CH₂, CH₂Ph); 1.68 (m, 2H, 3''-O-CH₂-CH₂); 1.42 (m, 2H, CH₂-CH₃); 1.15, 1.11, 1.06 and 1.01, 4 × (d, $J(\text{CH}_3, \text{CH}) = 6.3$ Hz, 3H, CH₃). ¹³C NMR (DMSO-*d*₆): δ 172.49 (d $J(\text{C-C-N-P}) = 3.0$, COO); 172.33 (d $J(\text{C-C-N-P}) = 5.1$, COO); 165.54 (C-2); 158.72 (C-3''); 146.24 (C-4); 137.34 and 137.20 (C-1'); 136.97 (C-1''); 129.72 (C-2'); 129.38 (C-5''); 128.30 and 128.27 (C-3'); 126.70 and 126.65 (C-4'); 120.54 (C-6''); 116.73 (C-5); 114.18 (C-2''); 113.67 (C-4''); 72.98 d, $J(\text{C-O-C-P}) = 10.7$ Hz, (O-CH₂); 68.17 and 68.04 (CH-*iPr*); 67.71 d, $J(\text{C-P}) = 134.7$ Hz, (CH₂-P); 67.25 (3''-O-CH₂); 54.18 and 54.14 (NH-CH); 39.90 (CH₂Ph); 31.02 (3''-O-CH₂-CH₂); 27.40 (5-CH₂); 21.72, 21.66, 21.60 and 21.54 (CH₃-*iPr*); 19.01 (CH₂-CH₃); 13.97 (CH₂-CH₃). HR-MS (ESI+): m/z [M + H]⁺ calculated for: C₄₀H₅₄N₄O₇PS, 765.3445, found: 765.3435.

Bis(L-phenylalanine isopropyl ester) prodrug of 2-amino-5-[2-

(phosphorylmethoxy)ethyl]-4-[3-(benzyloxy)phenyl]thiazole (9c).: Treatment of **8c** (0.18 g, 0.35 mmol) by Method B afforded **9c** (0.12 g, 43%) as an amorphous yellowish solid. ESI-MS, m/z (%): 799 [M+H⁺] (100). ¹H NMR (DMSO-*d*₆): δ 7.45 (m, 2H, H-2'''); 7.38 (m, 2H, H-3'''); 7.07–7.33 (m, 14H, H-2', 3', 4', 2'', 5'', 6'', 4'''); 6.85 (bs, 2H, NH₂); 5.11 (s, 2H, 1'''-CH₂); 4.80 and 4.76 2 × (sept., $J(\text{CH}, \text{CH}_3) = 6.3$ Hz, 1H, CH-*iPr*); 4.41 and

4.07 2 × (t, $J(\text{NH},\text{CH}) = J(\text{NH},\text{P}) = 11.3$ Hz, 1H, NH); 3.83–3.97 (m, 2H, NH-CH); 3.48 (t, $J(\text{CH}_2,\text{CH}_2) = 7.1$ Hz, 2H, O-CH₂); 3.20–3.28 (m, 2H, P-CH₂); 2.73–2.90 (m, 6H, 5-CH₂, CH₂Ph); 1.15, 1.10, 1.06 and 1.01, 4 × (d, $J(\text{CH}_3,\text{CH}) = 6.3$ Hz, 3H, CH₃). ¹³C NMR (DMSO-*d*₆): δ 172.49 (d $J(\text{C}-\text{C}-\text{N}-\text{P}) = 3.0$, COO); 172.33 (d $J(\text{C}-\text{C}-\text{N}-\text{P}) = 5.1$, COO); 165.56 (C-2); 158.36 (C-3''); 146.15 (C-4); 137.34 and 137.33 (C-1', 1'''); 137.19 (C-1'); 137.03 (C-1''); 129.71 (C-2'); 129.42 (C-5''); 128.66 (C-3''') 128.30 and 128.27 (C-3'); 128.01 (C-4'''); 127.81 (C-2'''); 126.70 and 126.65 (C-4'); 120.96 (C-6''); 116.88 (C-5); 114.60 (C-2''); 113.96 (C-4''); 72.98 d, $J(\text{C}-\text{O}-\text{C}-\text{P}) = 10.7$ Hz, (O-CH₂); 69.30 (1'''-CH₂); 68.16 and 68.03 (CH-*iPr*); 67.76 d, $J(\text{C}-\text{P}) = 134.9$ Hz, (CH₂-P); 54.17 and 54.13 (NH-CH); 39.90 (CH₂Ph); 27.36 (5-CH₂); 21.71, 21.65, 21.59 and 21.53 (CH₃-*iPr*). HR-MS (ESI+): *m/z* [M + H]⁺ calculated for: C₄₃H₅₂N₄O₇PS, 799.3289, found: 799.3277.

Bis(L-phenylalanine isopropyl ester) prodrug of 2-amino-5-[[2-(phosphoryl-methoxy)ethyl]-4-(4-isopropoxyphenyl)thiazole (9d): Treatment of **8d** (0.16 g, 0.35 mmol) by Method B afforded **9d** (0.12 g, 46%) as an amorphous white solid. ESI-MS, *m/z* (%): 751 [M+H⁺] (100). ¹H NMR (DMSO-*d*₆): δ 7.41 (m, 2H, H-2''); 7.11–7.26 (m, 10H, H-2', 3', 4'); 6.89 (m, 2H, H-3''); 6.80 (bs, 2H, NH₂); 4.81 and 4.77 2 × (sept., $J(\text{CH},\text{CH}_3) = 6.3$ Hz, 1H, CH-*iPr*); 4.58 (sept., 2H, $J(\text{CH}_3,\text{CH}) = 6.0$ Hz, 4''-O-CH); 4.43 and 4.09 2 × (t, $J(\text{NH},\text{CH}) = J(\text{NH},\text{P}) = 11.3$ Hz, 1H, NH); 3.84–3.97 (m, 2H, NH-CH); 3.49 (m, 2H, O-CH₂); 3.21–3.30 (m, 2H, P-CH₂); 2.75–2.91 (m, 6H, 5-CH₂, CH₂Ph); 1.26 (d, 6H, $J(\text{CH}_3,\text{CH}) = 6.0$ Hz, 4''-O-CH-CH₃); 1.15, 1.11, 1.06 and 1.01, 4 × (d, $J(\text{CH}_3,\text{CH}) = 6.3$ Hz, 3H, CH₃). ¹³C NMR (DMSO-*d*₆): δ 172.49 (d $J(\text{C}-\text{C}-\text{N}-\text{P}) = 3.0$, COO); 172.32 (d $J(\text{C}-\text{C}-\text{N}-\text{P}) = 5.1$, COO); 165.33 (C-2); 156.66 (C-4''); 146.26 (C-4); 137.34 and 137.18 (C-1'); 129.71 (C-2'); 129.62 (C-2''); 128.30 and 128.27 (C-3'); 127.91 (C-1''); 126.69 and 126.65 (C-4'); 115.25 (C-3''); 114.98 (C-5); 73.10 d, $J(\text{C}-\text{O}-\text{C}-\text{P}) = 10.9$ Hz, (O-CH₂); 69.24 (4''-O-CH); 68.16 and 68.03 (CH-*iPr*); 67.76 d, $J(\text{C}-\text{P}) = 134.9$ Hz, (CH₂-P); 54.17 and 54.15 (NH-CH); 39.90 (CH₂Ph); 27.39 (5-CH₂); 22.08 (4''-O-CH-CH₃); 21.71, 21.59, 21.65 and 21.53 (CH₃-*iPr*). HR-MS (ESI+): *m/z* [M + H]⁺ calculated for: C₃₉H₅₂N₄O₇PS, 751.3289, found: 751.3280.

Bis(L-phenylalanine isopropyl ester) prodrug of 2-amino-5-[2-(phosphoryl-methoxy)ethyl]-4-(2-fluorophenyl)thiazole (9e): Treatment of **8e** (0.15 g, 0.35 mmol) by Method B afforded **9e** (0.12 g, 47%) as an amorphous white solid. ESI-MS, *m/z* (%): 711 [M+H⁺] (100). ¹H NMR (DMSO-*d*₆): δ 7.37–7.44 (m, 2H, H-4'', 6''); 7.10–7.26 (m, 12H, H-2', 3', 4', 3'', 5''); 6.87 (bs, 2H, NH₂); 4.80 and 4.76 2 × (sept., $J(\text{CH},\text{CH}_3) = 6.3$ Hz, 1H, CH-*iPr*); 4.39 and 4.06 2x (t, $J(\text{NH},\text{CH}) = J(\text{NH},\text{P}) = 11.3$ Hz, 1H, NH); 3.82–3.95 (m, 2H, NH-CH); 3.40 (t, $J(\text{CH}_2,\text{CH}_2) = 7.2$ Hz, 2H, O-CH₂); 3.16–3.24 (m, 2H, P-CH₂); 2.73–2.90 (m, 4H, CH₂Ph); 2.63 (t, $J(\text{CH}_2,\text{CH}_2) = 7.1$ Hz, 2H, 5-CH₂); 1.15, 1.10, 1.06 and 1.01, 4 × (d, $J(\text{CH}_3,\text{CH}) = 6.3$ Hz, 3H, CH₃). ¹³C NMR (DMSO-*d*₆): δ 172.47 (d $J(\text{C}-\text{C}-\text{N}-\text{P}) = 3.1$, COO); 172.34 (d $J(\text{C}-\text{C}-\text{N}-\text{P}) = 5.2$, COO); 166.13 (C-2); 159.25 (d, $J(2'',\text{F}) = 245.6$ Hz, C-2''); 140.78 (C-4); 137.33 and 137.20 (C-1'); 131.98 (d, $J(6'',\text{F}) = 3.2$ Hz, C-6''); 129.91 (d, $J(4'',\text{F}) = 8.2$ Hz, C-4''); 129.71 (C-2'); 128.29 and 128.25 (C-3'); 126.69 and 126.65 (C-4'); 124.45 (d, $J(5'',\text{F}) = 3.2$ Hz, C-5''); 123.40 (d, $J(1'',\text{F}) = 15.1$ Hz, C-1''); 118.47 (C-5); 115.96 (d, $J(3'',\text{F}) = 22.4$ Hz, C-3''); 72.76 d, $J(\text{C}-\text{O}-\text{C}-\text{P}) = 11.2$ Hz, (O-CH₂); 68.16 and 68.02 (CH-*iPr*); 67.66 d, $J(\text{C}-\text{P}) = 135.1$

Hz, (CH₂-P); 54.17 and 54.12 (NH-CH); 39.90 (CH₂Ph); 27.28 (d, *J* = 3.1 Hz, 5-CH₂); 21.71, 21.65, 21.59 and 21.53 (CH₃-*iPr*). HR-MS (ESI+): *m/z* [M + H]⁺ calculated for: C₃₆H₄₅N₄O₆FPS, 711.2776, found: 711.2768.

Bis(L-phenylalanine isopropyl ester) prodrug of 2-amino-5-[2-

(phosphoryl-methoxy)ethyl]-4-(3-fluorophenyl)thiazole (9f): Treatment of **8f** (0.15 g, 0.35 mmol) by Method B afforded **9f** (0.09 g, 35%) as an amorphous solid. ESI-MS, *m/z* (%): 733 [M+Na⁺] (100). ¹H NMR (DMSO-*d*₆): δ 7.41 (m, 1H, H-5''); 7.36 (dt, *J*(6'',5'') = 7.8 Hz, *J*(6'',2'') = 1.3 Hz, 1H, H-6''); 7.29 (ddd, *J*(2'',F) = 10.6 Hz, *J*(2'',4'') = 2.7 Hz, *J*(2'',6'') = 1.5 Hz, 1H, H-2''); 7.10–7.26 (m, 11H, H-2', 3', 4', 4''); 6.91 (bs, 2H, NH₂); 4.80 and 4.77 2 × (sept., *J*(CH,CH₃) = 6.3 Hz, 1H, CH-*iPr*); 4.41 and 4.08 2x (t, *J*(NH,CH) = *J*(NH,P) = 11.3 Hz, 1H, NH); 3.84–3.97 (m, 2H, NH-CH); 3.51 (t, *J*(CH₂,CH₂) = 7.0 Hz, 2H, O-CH₂); 3.22–3.30 (m, 2H, P-CH₂); 2.74–2.90 (m, 6H, CH₂Ph, 5-CH₂); 1.15, 1.10, 1.06 and 1.01, 4 × (d, *J*(CH₃,CH) = 6.3 Hz, 3H, CH₃). ¹³C NMR (DMSO-*d*₆): δ 172.49 (d *J*(C-C-N-P) = 2.9, COO); 172.33 (d *J*(C-C-N-P) = 5.1, COO); 165.71 (C-2); 162.24 (d, *J*(3'',F) = 242.7 Hz, C-3''); 144.95 (d, *J*(4,F) = 2.5 Hz, C-4); 137.95 (d, *J*(1'',F) = 8.0 Hz, C-1''); 137.33 and 137.19 (C-1'); 130.34 (d, *J*(5''-F) = 8.5 Hz, C-5''); 129.71 and 129.70 (C-2'); 128.30 and 128.26 (C-3'); 126.69 and 126.65 (C-4'); 124.36 (d, *J*(6'',F) = 2.6 Hz, C-6''); 117.90 (C-5); 114.91 (d, *J*(2'',F) = 22.1 Hz, C-2''); 114.03 (d, *J*(4'',F) = 20.9 Hz, C-4''); 72.86 d, *J*(C-O-C-P) = 10.8 Hz, (O-CH₂); 68.16 and 68.03 (CH-*iPr*); 67.76 d, *J*(C-P) = 134.6 Hz, (CH₂-P); 54.17 and 54.13 (NH-CH); 39.90 (CH₂Ph); 27.33 (5-CH₂); 21.71, 21.65, 21.59 and 21.53 (CH₃-*iPr*). HR-MS (ESI+): *m/z* [M + H]⁺ calculated for: C₃₆H₄₅N₄O₆FPS, 711.2776, found: 711.2767.

Bis(L-phenylalanine isopropyl ester) prodrug of 2-amino-5-[2-

(phosphoryl-methoxy)ethyl]-4-[3-(N-methylsulfamoyl)phenyl]thiazole (9g): Treatment of **8g** (0.17 g, 0.35 mmol) by Method B afforded **9g** (0.11 g, 40%) as a yellowish amorphous solid. ESI-MS, *m/z* (%): 808 [M+Na⁺] (100). ¹H NMR (DMSO-*d*₆): δ 9.85 (bs, 1H, SO₂NH); 7.43 (t, *J*(2'',4'') = *J*(2'',6'') = 2.0 Hz, 2H, H-2''); 7.32 (t, *J*(5'',4'') = *J*(5'',6'') = 7.9 Hz, 2H, H-5''); 7.10–7.26 (m, 12H, H-2', 3', 4', 4'',6''); 6.88 (bs, 2H, NH₂); 4.80 and 4.77 2 × (sept., *J*(CH,CH₃) = 6.3 Hz, 1H, CH-*iPr*); 4.45 (t, *J*(NH,CH) = *J*(NH,P) = 11.4 Hz, 1H, NH); 4.12 (dd, *J*(NH,CH) = *J*(NH,P) = 12.2 Hz, 1H, NH); 3.83–3.99 (m, 2H, NH-CH); 3.51 (t, *J*(CH₂,CH₂) = 7.1 Hz, 2H, O-CH₂); 3.21–3.32 (m, 2H, P-CH₂); 2.74–2.91 (m, 6H, 5-CH₂, CH₂Ph); 1.15, 1.10, 1.06 and 1.01, 4 × (d, *J*(CH₃,CH) = 6.3 Hz, 3H, CH₃). ¹³C NMR (DMSO-*d*₆): δ 172.49 (d *J*(C-C-N-P) = 3.0, COO); 172.31 (d *J*(C-C-N-P) = 5.0, COO); 165.64 (C-2); 145.98 (C-4); 138.58 (C-1'') and 131.61 (C-3''); 137.33 and 137.16 (C-1'); 129.72 and 129.70 (C-2'); 129.26 (C-5''); 128.31 and 128.27 (C-3'); 126.71 and 126.66 (C-4'); 123.83 (C-6''); 119.68 (C-2''); 118.46 (C-4''); 117.05 (C-5); 72.97 d, *J*(C-O-C-P) = 10.6 Hz, (O-CH₂); 68.18 and 68.05 (CH-*iPr*); 67.72 d, *J*(C-P) = 134.9 Hz, (CH₂-P); 54.14 (NH-CH); 39.90 (CH₂Ph); 39.30 (NH-CH₃); 27.38 (5-CH₂); 21.71, 21.65, 21.59 and 21.53 (CH₃-*iPr*). HR-MS (ESI+): *m/z* [M + H]⁺ calculated for: C₃₇H₄₉N₅O₈PS₂, 786.2755, found: 786.2743.

Bis(L-phenylalanine isopropyl ester) prodrug of 2-amino-5-[2-

(phosphoryl-methoxy)ethyl]-4-[4-(methylcarbamoyl)phenyl]thiazole (9h): Treatment of

8h (0.16 g, 0.35 mmol) by Method B afforded **9h** (0.16 g, 61%) as an amorphous white solid. ESI-MS, m/z (%): 772 [M+Na⁺] (100). ¹H NMR (DMSO-*d*₆): δ 8.45 (q, J (NH,CH₃) = 4.5 Hz, 1H, CONH); 7.85 (m, 2H, H-3''); 7.59 (m, 2H, H-2''); 7.10–7.26 (m, 10H, H-2', 3', 4'); 6.90 (bs, 2H, NH₂); 4.81 and 4.77 2 × (sept., J (CH,CH₃) = 6.3 Hz, 1H, CH-*iPr*); 4.42 (t, J (NH,CH) = J (NH,P) = 11.3 Hz, 1H, NH); 4.09 (dd, J (NH,CH) = 10.6 Hz, J (NH,P) = 12.0 Hz, 1H, NH); 3.83–3.97 (m, 2H, NH-CH); 3.51 (t, J (CH₂,CH₂) = 7.1 Hz, 2H, O-CH₂); 3.21–3.30 (m, 2H, P-CH₂); 2.74–2.91 (m, 6H, 5-CH₂, CH₂Ph); 2.78 (d, J (CH₃-NH) = 4.6 Hz, 3H, NH-CH₃); 1.15, 1.10, 1.06 and 1.01, 4 × (d, J (CH₃,CH) = 6.3 Hz, 3H, CH₃). ¹³C NMR (DMSO-*d*₆): δ 172.50 (d J (C-C-N-P) = 2.8, COO); 172.34 (d J (C-C-N-P) = 5.1, COO); 166.54 (CON); 165.70 (C-2); 145.56 (C-4); 138.10 (C-1'') 137.33 and 137.19 (C-1'); 133.05 (C-4''); 129.71 (C-2'); 128.30 and 128.27 (C-3'); 128.10 (C-2''); 127.24 (C-3''); 126.70 and 126.65 (C-4'); 117.95 (C-5); 72.88 d, J (C-O-C-P) = 10.8 Hz, (O-CH₂); 68.17 and 68.04 (CH-*iPr*); 67.75 d, J (C-P) = 134.9 Hz, (CH₂-P); 54.17 and 54.12 (NH-CH); 39.90 (CH₂Ph); 27.39 (5-CH₂); 26.50 (NH-CH₃); 21.71, 21.65, 21.59 and 21.53 (CH₃-*iPr*). HR-MS (ESI+): m/z [M + H]⁺ calculated for: C₃₈H₄₉N₅O₇PS, 750.3085, found: 750.3092.

Bis(L-phenylalanine isopropyl ester) prodrug of 2-amino-5-[2-

(phosphoryl-methoxy)ethyl]-4-[4-(benzylcarbamoyl)phenyl]thiazole (9i): Treatment of **8i** (0.19 g, 0.35 mmol) by Method B afforded **9i** (0.13 g, 44%) as an amorphous white solid. ESI-MS, m/z (%): 848 [M+Na⁺] (100). ¹H NMR (DMSO-*d*₆): δ 9.07 (t, J (NH,CH₂) = 6.0 Hz, CO-NH); 7.92 (m, 2H, H-3''); 7.61 (m, 2H, H-2''); 7.30–7.34 (m, 4H, H-2''', 3'''); 7.10–7.26 (m, 11H, H-2', 3', 4', 4'''); 6.92 (bs, 2H, NH₂); 4.81 and 4.76 2 × (sept., J (CH,CH₃) = 6.3 Hz, 1H, CH-*iPr*); 4.49 (d, J (CH₂,NH) = 6.0 Hz, 2H, NH-CH₂); 4.41 and 4.09 2x (t, J (NH,CH) = J (NH,P) = 11.3 Hz, 1H, NH); 3.83–3.98 (m, 2H, NH-CH); 3.51 (t, J (CH₂,CH₂) = 7.2 Hz, 2H, O-CH₂); 3.22–3.30 (m, 2H, P-CH₂); 2.74–2.92 (m, 6H, CH₂Ph, 5-CH₂); 1.14, 1.10, 1.06 and 1.01, 4 × (d, J (CH₃,CH) = 6.3 Hz, 3H, CH₃). ¹³C NMR (DMSO-*d*₆): δ 172.50 (d J (C-C-N-P) = 2.9, COO); 172.34 (d J (C-C-N-P) = 5.1, COO); 166.11 (CO-NH); 165.73 (C-2); 145.57 (C-4); 139.96 (C-1'''); 138.32 (C-1''); 137.32 and 137.18 (C-1'); 132.86 (C-4''); 129.71 and 129.70 (C-2'); 128.49 (C-3'''); 128.30 and 128.26 (C-3'); 128.14 (C-2''); 127.46 (C-3''); 127.40 (C-2'''); 126.94 (C-4'''); 126.69 and 126.65 (C-4'); 118.04 (C-5); 72.88 d, J (C-O-C-P) = 10.7 Hz, (O-CH₂); 68.17 and 68.04 (CH-*iPr*); 67.75 d, J (C-P) = 134.9 Hz, (CH₂-P); 54.17 and 54.12 (NH-CH); 42.80 (NH-CH₂); 39.90 (CH₂Ph); 27.39 (5-CH₂); 21.71, 21.65, 21.59 and 21.52 (CH₃-*iPr*). HR-MS (ESI+): m/z [M + H]⁺ calculated for: C₄₄H₅₃N₅O₇PS, 826.3398, found: 826.3386.

[2-(2-Amino-4-phenylthiazol-5-yl)ethoxy]methyl]phosphonic acid diphosphate

(10a): Treatment of **8a** (0.10 g, 0.25 mmol) by Method C afforded **10a** (0.015 g, 13%) as an amorphous white solid. ESI-MS, m/z (%): 473 [M-H] (100). ¹H NMR (D₂O): δ 7.43–7.52 (m, 5H, H-2', 3', 4'); 3.78–3.81 (m, 4H, CH₂-O, P-CH₂); 3.02 (t, J (CH₂,CH₂) = 6.2 Hz, 2H, 5-CH₂). ¹³C NMR (D₂O): δ 169.00 (C-2); 144.91 (C-4); 134.17 (C-1'); 129.40 (C-2') and 129.37 (C-3'); 128.97 (C-4'); 120.34 (C-5); 73.44 d, J (C-O-C-P) = 10.4 Hz, (O-CH₂); 67.24 d, J (C-P) = 163.0 Hz, (CH₂-P); 27.15 (5-CH₂). ³¹P NMR (121.4 MHz, D₂O): 9.38 d J (P-O-P) = 26.2 Hz; -8.38 d J (P-O-P) = 19.9 Hz; -21.92 dd J (P-O-P) = 26.4 and 19.7 Hz. HR-MS (ESI⁻): m/z [M - H] calculated for: C₁₂H₁₆N₂O₁₀P₃S: 472.9744, found: 472.9742.

{[2-(2-Amino-4-(3-butoxyphenyl)thiazol-5-yl)ethoxy]methyl}phosphonic acid

diphosphate (10b).: Treatment of **8b** (0.10 g, 0.21 mmol) by Method C afforded **10b** (11 mg, 9%) as an amorphous white solid. ESI-MS, m/z (%): 545 [M-H] (100). $^1\text{H NMR}$ (D_2O): δ 7.42 (t, 2H, $J(5',4') = J(5',6') = 7.9$ Hz, H-5'); 7.12 (m, 1H, H-6'); 7.01–7.06 (m, 2H, H-2', 4'); 4.10 (t, $J(\text{CH}_2, \text{CH}_2) = 8.7$ Hz, 2H, $\text{CH}_3(\text{CH}_2)_2\text{CH}_2$); 3.77–3.81 (m, 4H, P- CH_2 , O- CH_2); 3.01 (t, $J(\text{CH}_2, \text{CH}_2) = 6.1$ Hz, 2H, 5- CH_2); 1.75 (m, 2H, $\text{CH}_3\text{CH}_2\text{CH}_2$); 1.46 (m, 2H, CH_3CH_2); 0.94 (t, $J(\text{CH}_2, \text{CH}_2) = 7.4$ Hz, 2H, CH_3). $^{13}\text{C NMR}$ (D_2O): δ 169.03 (C-2); 158.90 (C-3'); 143.78 (C-4); 135.33 (C-1'); 130.68 (C-5'); 122.33 (C-6'); 120.52 (C-5); 115.58 and 115.60 (C-2' and C-4'); 73.31 d, $J(\text{C-O-C-P}) = 10.6$ Hz, (O- CH_2); 67.23 d, $J(\text{C-P}) = 163.4$ Hz, ($\text{CH}_2\text{-P}$); 63.90 ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2$); 31.19 ($\text{CH}_3\text{CH}_2\text{CH}_2$); 27.21 (5- CH_2); 19.30 (CH_3CH_2); 13.78 (CH_3). $^{31}\text{P NMR}$ (D_2O) 9.41 d $J(\text{P-O-P}) = 26.5$ Hz; -8.88 m; -22.04 m. HR-MS (ESI⁻): m/z [M - H] calculated for: $\text{C}_{16}\text{H}_{24}\text{N}_2\text{O}_{11}\text{P}_3\text{S}$: 545.0319, found: 545.0318.

{[2-(2-Amino-4-(3-(benzyloxy)phenyl)thiazol-5-yl)ethoxy]methyl}phosphonic acid

diphosphate (10c).: Treatment of **8c** (0.10 g, 0.20 mmol) by Method C afforded **10c** (12 mg, 9%) as an amorphous white solid. ESI-MS, m/z (%): 579 [M-H] (100). $^1\text{H NMR}$ (D_2O): δ 7.37–7.45 (m, 6H, H-5', 2'', 3'', 4''); 7.06–7.09 (m, 2H, H-4', 6'); 6.94 (m, 1H, H-2'); 5.11 (s, 2H, 1''- CH_2); 3.76 (d, 2H, $J(\text{H-C-P}) = 8.7$ Hz, P- CH_2); 3.63 (t, $J(\text{CH}_2, \text{CH}_2) = 5.8$ Hz, 2H, $\text{CH}_2\text{-O}$); 2.80 (t, $J(\text{CH}_2, \text{CH}_2) = 5.8$ Hz, 2H, 5- CH_2). $^{13}\text{C NMR}$ (D_2O): δ 169.31 (C-2); 158.42 (C-3'); 139.38 (C-4); 137.09 (C-1''); 132.94 (C-1'); 130.94 (C-5'); 129.56 (C-3''); 129.10 (C-4''); 128.58 (C-2''); 122.43 (C-6'); 119.81 (C-5); 116.68 (C-4'); 115.82 (C-2'); 72.70 d, $J(\text{C-O-C-P}) = 11.6$ Hz, (O- CH_2); 70.92 (1''- CH_2); 67.14 d, $J(\text{C-P}) = 163.8$ Hz, ($\text{CH}_2\text{-P}$); 27.02 (5- CH_2). $^{31}\text{P NMR}$ (D_2O): 9.34 d $J(\text{P-O-P}) = 26.2$ Hz; -9.67 d $J(\text{P-O-P}) = 19.7$ Hz; -22.28 dd $J(\text{P-O-P}) = 22.6$ and 19.4 Hz. HR-MS (ESI⁻): m/z [M - H] calculated for: $\text{C}_{19}\text{H}_{22}\text{N}_2\text{O}_{11}\text{P}_3\text{S}$: 579.0163, found: 579.0161.

{[2-(2-Amino-4-(4-isopropoxyphenyl)thiazol-5-yl)ethoxy]methyl}phosphonic acid

diphosphate (10d).: Treatment of **8d** (0.107 g, 0.23 mmol) by Method C afforded **10d** (13 mg, 9%) as an amorphous white solid. ESI-MS, m/z (%): 531 [M-H] (100). $^1\text{H NMR}$ (D_2O): δ 7.42 (m, 2H, H-2'); 7.05 (m, 2H, H-3'); 4.72 (sept, 1H, $\text{CH-}i\text{Pr}$); 3.81 (d, $J(\text{C-H-P}) = 8.5$ Hz, 2H, P- CH_2); 3.77 (t, $J(\text{CH}_2, \text{CH}_2) = 6.1$ Hz, 2H, O- CH_2); 2.97 (t, $J(\text{CH}_2, \text{CH}_2) = 6.1$ Hz, 2H, 5- CH_2); 1.34 (d, $J(\text{CH}_3, \text{CH}) = 6.1$ Hz, 6H, CH_3). $^{13}\text{C NMR}$ (D_2O): δ 168.84 (C-2); 157.37 (C-4'); 143.26 (C-4); 130.76 (C-2'); 126.68 (C-1'); 119.19 (C-5); 116.95 (C-3'); 73.27 d, $J(\text{C-O-C-P}) = 10.5$ Hz, (O- CH_2); 72.31 ($\text{CH-}i\text{Pr}$); 67.19 d, $J(\text{C,P}) = 163.4$ Hz, ($\text{CH}_2\text{-P}$); 27.10 (5- CH_2) 21.72 ($\text{CH}_3\text{-}i\text{Pr}$). $^{31}\text{P NMR}$ (D_2O): 9.41 d $J(\text{P-O-P}) = 26.0$ Hz; -8.3 d $J(\text{P-O-P}) = 18.3$ Hz; -21.82 m. HR-MS (ESI⁻): m/z [M - H] calculated for: $\text{C}_{15}\text{H}_{22}\text{N}_2\text{O}_{11}\text{P}_3\text{S}$: 531.0163, found: 531.0160.

{[2-(2-Amino-4-(2-fluorophenyl)thiazol-5-yl)ethoxy]methyl}phosphonic acid

diphosphate (10e).: Treatment of **8e** (0.104 g, 0.25 mmol) by Method C afforded **10e** (0.010 g, 7%) as amorphous solid. ESI-MS, m/z (%): 491 [M-H] (100). $^1\text{H NMR}$ (D_2O): δ 7.46 (m, 1H, H-4'); 7.43 (td, $J(6',5') = J(6',\text{F}) = 7.6$ Hz, $J(6',4') = 1.8$ Hz, 1H, H-6'); 7.29 (td, $J(5',6') = J(5',4') = 7.5$ Hz, $J(5',3') = 1.1$ Hz, 1H, H-5'); 7.25 (ddd, $J(3',\text{F}) = 10.5$ Hz, $J(3',4') = 8.3$ Hz, $J(3',5') = 1.2$ Hz, 1H, H-3'); 3.78 (d, $J(\text{H-C-P}) = 8.5$ Hz, 2H, P- CH_2);

3.75 (t, $J(\text{CH}_2, \text{CH}_2) = 6.3$ Hz, 2H, O-CH₂); 2.87 (t, $J(\text{CH}_2, \text{CH}_2) = 6.3$ Hz, 2H, 5-CH₂). ¹³C NMR (D₂O): δ 169.20 (C-2); 160.44 (d, $J(2', \text{F}) = 245.0$ Hz, (C-2'); 140.45 (C-4); 132.38 (C-6'); 131.21 (d, $J(4', \text{F}) = 8.3$ Hz, (C-4'); 125.12 (d, $J(5', \text{F}) = 3.4$ Hz, (C-5'); 122.81 (C-5); 116.54 (d, $J(3', \text{F}) = 22.1$ Hz, (C-3'); 73.31 d, $J(\text{C-O-C-P}) = 10.2$ Hz, (O-CH₂); 67.36 d, $J(\text{C-P}) = 162.6$ Hz, (CH₂-P); 27.13 (5-CH₂). ³¹P NMR (D₂O): 9.16 d $J(\text{P-O-P}) = 25.8$ Hz; -5.79 m; -21.18 m. HR-MS (ESI⁻): m/z [M - H] calculated for: C₁₂H₁₅N₂O₁₀FP₃S: 490.9650, found: 490.9648.

{[2-(2-Amino-4-(3-fluorophenyl)thiazol-5-yl)ethoxy]methyl}phosphonic acid

diphosphate (10f): Treatment of **8f** (91 mg, 0.22 mmol) by Method C afforded **10f** (8 mg, 7%) as an amorphous white solid. ESI-MS, m/z (%): 491 [M-H] (100). ¹H NMR (D₂O): δ 7.47 (ddd, $J(5', 4') = 8.5$ Hz, $J(5', 6') = 7.7$ Hz, $J(5', \text{F}) = 6.1$ Hz, 1H, H-5'); 7.32 (ddd, $J(6', 5') = 7.7$ Hz, $J(6', 2') = 1.5$ Hz, $J(6', 4') = 0.9$ Hz, 1H, H-6'); 7.25 (ddd, $J(2', \text{F}) = 10.1$ Hz, $J(2', 4') = 2.6$ Hz, $J(2', 6') = 1.5$ Hz, 1H, H-2'); 7.16 (m, 1H, H-4'); 3.78–3.81 (m, 4H, O-CH₂, P-CH₂); 3.03 (t, $J(\text{CH}_2, \text{CH}_2) = 6.3$ Hz, 2H, 5-CH₂). ¹³C NMR (D₂O): δ 168.88 (C-2); 163.08 (d, $J(3', \text{F}) = 243.7$ Hz, (C-3'); 144.78 (C-4); 136.75 (d, $J(1', \text{F}) = 8.2$ Hz, (C-1'); 130.93 (d, $J(5', \text{F}) = 8.7$ Hz, (C-5'); 125.20 (d, $J(6', \text{F}) = 2.8$ Hz, (C-6'); 121.24 (C-5); 115.98 (d, $J(2', \text{F}) = 22.4$ Hz, (C-2'); 115.40 (d, $J(4', \text{F}) = 21.0$ Hz, (C-4'); 73.37 d, $J(\text{C-O-C-P}) = 10.3$ Hz, (O-CH₂); 67.24 d, $J(\text{C-P}) = 162.9$ Hz, (CH₂-P); 27.10 (5-CH₂). ³¹P NMR (D₂O): 9.30 d $J(\text{P-O-P}) = 26.1$ Hz; -7.37 m; -21.62 m. HR-MS (ESI⁻): m/z [M - H] calculated for: C₁₂H₁₅N₂O₁₀FP₃S: 490.9650, found: 490.9646.

{[2-(2-Amino-4-(3-(N-methylsulfamoyl)phenyl)thiazol-5-yl)ethoxy]methyl}phosphonic acid diphosphate (10g):

Treatment of **8g** (0.10 g, 0.20 mmol) by Method C afforded **10g** (10 mg, 8%) as an amorphous white solid. ESI-MS, m/z (%): 565 [M-H] (100). ¹H NMR (D₂O): δ 7.81 (m, 2H, H-3'); 7.62 (m, 2H, H-2'); 3.78–3.82 (m, 4H, O-CH₂, P-CH₂); 3.04 (t, $J(\text{CH}_2, \text{CH}_2) = 6.2$ Hz, 2H, 5-CH₂); 2.94 (s, 3H, CH₃). ¹³C NMR (D₂O): δ 171.12 (CON); 169.06 (C-2); 144.74 (C-4); 138.00 (C-1'); 133.61 (C-4'); 129.59 (C-2'); 127.92 (C-3'); 121.81 (C-5); 73.41 d, $J(\text{C-O-C-P}) = 10.4$ Hz, (O-CH₂); 67.21 d, $J(\text{C-P}) = 163.0$ Hz, (CH₂-P); 27.23 (5-CH₂); 27.09 (CH₃). ³¹P NMR (D₂O): 9.40 d $J(\text{P-O-P}) = 26.3$ Hz; -8.65 m; -21.99 m. HR-MS (ESI⁻): m/z [M - H] calculated for: C₁₃H₁₉N₃O₁₂P₃S₂: 565.9629, found: 565.9626.

{[2-(2-Amino-4-(4-(methylcarbamoyl)phenyl)thiazol-5-yl)ethoxy]methyl}phosphonic acid diphosphate (10h):

Treatment of **8h** (0.100 g, 0.22 mmol) by Method C afforded **10h** (5 mg, 4%) as an amorphous white solid. ESI-MS, m/z (%): 530 [M-H] (100). ¹H NMR (D₂O): δ 7.81 (m, 2H, H-3'); 7.62 (m, 2H, H-2'); 3.78–3.82 (m, 4H, O-CH₂, P-CH₂); 3.04 (t, $J(\text{CH}_2, \text{CH}_2) = 6.2$ Hz, 2H, 5-CH₂); 2.94 (s, 3H, CH₃). ¹³C NMR (D₂O): δ 171.12 (CON); 169.06 (C-2); 144.74 (C-4); 138.00 (C-1'); 133.61 (C-4'); 129.59 (C-2'); 127.92 (C-3'); 121.81 (C-5); 73.41 d, $J(\text{C-O-C-P}) = 10.4$ Hz, (O-CH₂); 67.21 d, $J(\text{C-P}) = 163.0$ Hz, (CH₂-P); 27.23 (5-CH₂); 27.09 (CH₃). ³¹P NMR (D₂O): 9.40 d $J(\text{P-O-P}) = 26.3$ Hz; -8.65 m; -21.99 m. HR-MS (ESI⁻): m/z [M - H] calculated for: C₁₄H₁₉N₃O₁₁P₃S: 529.9959, found: 529.9955.

{[2-(2-Amino-4-(4-(benzylcarbamoyl)phenyl)thiazol-5-yl)ethoxy]methyl}phosphonic acid diphosphate (10i). Treatment of **8i** (0.103 g, 0.19 mmol) by Method C afforded **10i** (12 mg, 9%) as an amorphous white solid. ESI-MS, m/z (%): 606 [M-H] (100). ^1H NMR (D_2O): δ 7.83 (m, 2H, H-3'); 7.60 (m, 2H, H-2'); 7.44–7.39 (m, 4H, H-2'', 3''); 7.35 (m, 1H, H-4''); 4.59 (s, 2H, 1''-CH₂); 3.80–3.82 (m, 4H, O-CH₂, P-CH₂); 3.04 (t, $J(\text{CH}_2, \text{CH}_2) = 6.3$ Hz, 2H, 5-CH₂). ^{13}C NMR (D_2O): δ 171.02 (CON); 168.90 (C-2); 144.97 (C-4); 138.70 (C-1''); 138.28 (C-1'); 133.33 (C-4'); 129.47 and 129.45 (C-2' and C-3''); 128.08 (C-4''); 127.97 and 127.86 (C-2'' and C-3'); 121.84 (C-5); 73.40 d, $J(\text{C-O-C-P}) = 10.3$ Hz, (O-CH₂); 67.30 d, $J(\text{C-P}) = 162.7$ Hz, (CH₂-P); 44.10 (1''-CH₂); 27.23 (5-CH₂). ^{31}P NMR (D_2O): 9.30 d $J(\text{P-O-P}) = 26.1$ Hz; -6.85 bd $J(\text{P-O-P}) = 18.6$ Hz; -21.40 m. HR-MS (ESI⁻): m/z [M - H] calculated for: C₂₀H₂₃N₃O₁₁P₃S: 606.0272, found: 606.0268.

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 CaCl₂, 1.2 mM MgCl₂, 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 $\mu\text{mol}/\text{min}/\text{mg}$) 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 mACs—Materials: Forskolin (FSK) and 3-isobutyl-1-methylxanthine (IBMX) were purchased from Sigma–Aldrich (St. Louis, MO, USA). OptiMEM, DMEM, and antibiotic-antimycotic were purchased from Life Technologies (Grand Island, NY, USA). FCI and BCS were purchased from Hyclone (Logan, UT, USA). Geneticin was purchased from InvivoGen (San Diego, CA, USA). **Methods:** HEK293 cells with CRISPR-Cas9 knockout of endogenous AC3 and AC6 (HEK 3/6) stably expressing recombinant AC1, AC2, and AC5 were grown, cryopreserved, and assayed with the prepared compounds at 30 μM concentration as described previously.^[35] Briefly, frozen cells were rapidly thawed in a 37 °C water bath, added to 9 ml of 37 °C OptiMEM, pelleted at 500 $\times g$, resuspended in fresh OptiMEM to achieve a cell density of 1.75×10^5 cells/ml (AC1 and AC5) or 7×10^5 (AC2), and 10 μL cells were added to each well. Cells were allowed to recover for 1

hour at 37 °C, 5% CO₂ prior to the addition of 5 µL of 4X concentrated test compound or vehicle control in OptiMEM for 30 minutes at 37 °C, 5% CO₂. Accumulation of cAMP was stimulated with 4X forskolin (5 µM final) or vehicle control in OptiMEM and plate was incubated for 1 hour at ambient temperature, after which cAMP accumulation was terminated by addition of HTRF reagents (Cisbio, Bedford, MA) as previously described.^[35] After 1 hour at ambient temperature, HTRF signal was measured using a Synergy Neo2 (Biotek, Winooski, VT), and cAMP was quantified as previously described, according to manufacturer directions.^[35] Data were normalized to cAMP formation in the absence of forskolin as 0% activity, and in the presence of 5 µM forskolin in the absence of inhibitor to 100%. Data represent the mean of N = 3 experiments, each performed with duplicate wells.

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 MnCl₂, 1 mM EDTA, 1 µM CaCl₂, 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 [³H]ATP to [³H]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.

Acknowledgements

This research was funded by the Institute of Organic Chemistry and Biochemistry (RVO 61388963), the Ministry of Education, Youth and Sports (MŠMT in Czech) in the program INTER-EXCELLENCE (project LTAUSA18086), and the National Institutes of Health (MH101673).

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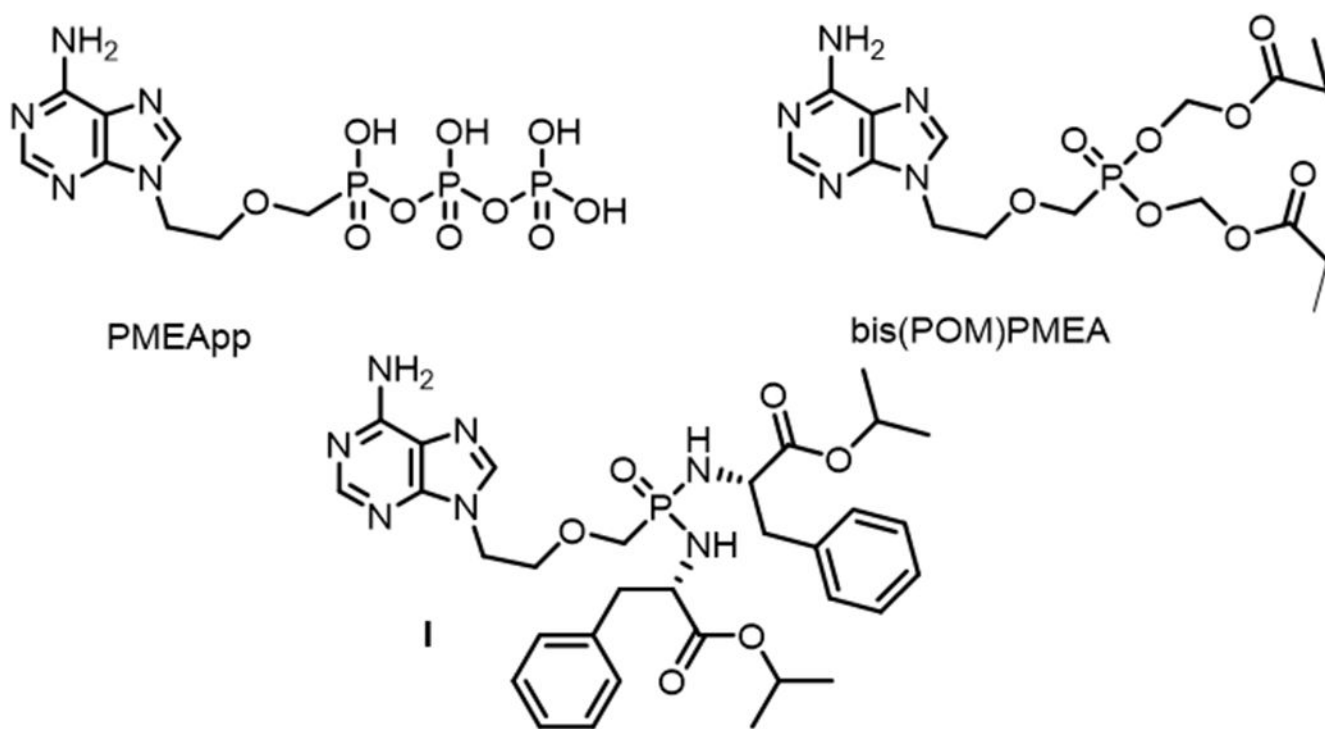


Figure 1. Structures of metabolically active adefovir disphosphate (PMEApp) and PMEa prodrugs.

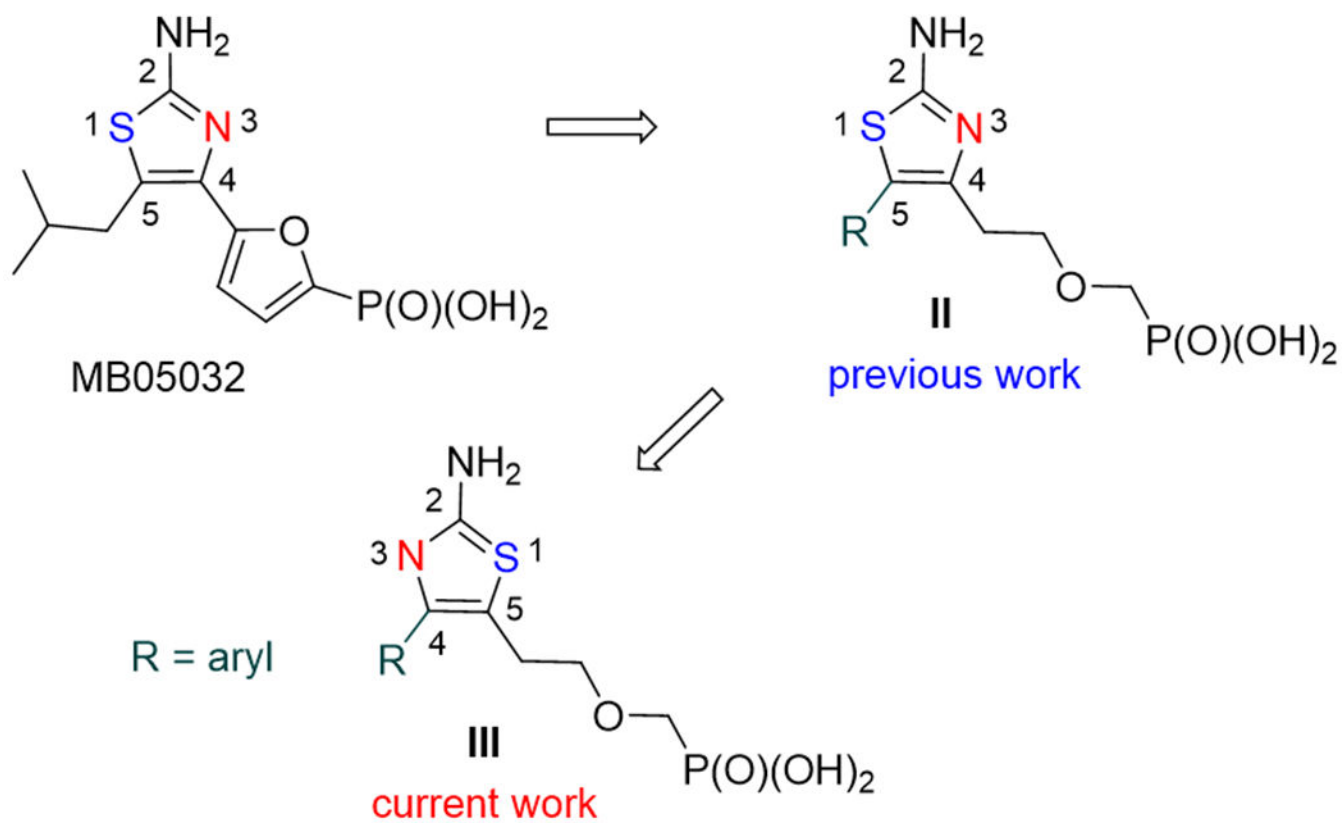
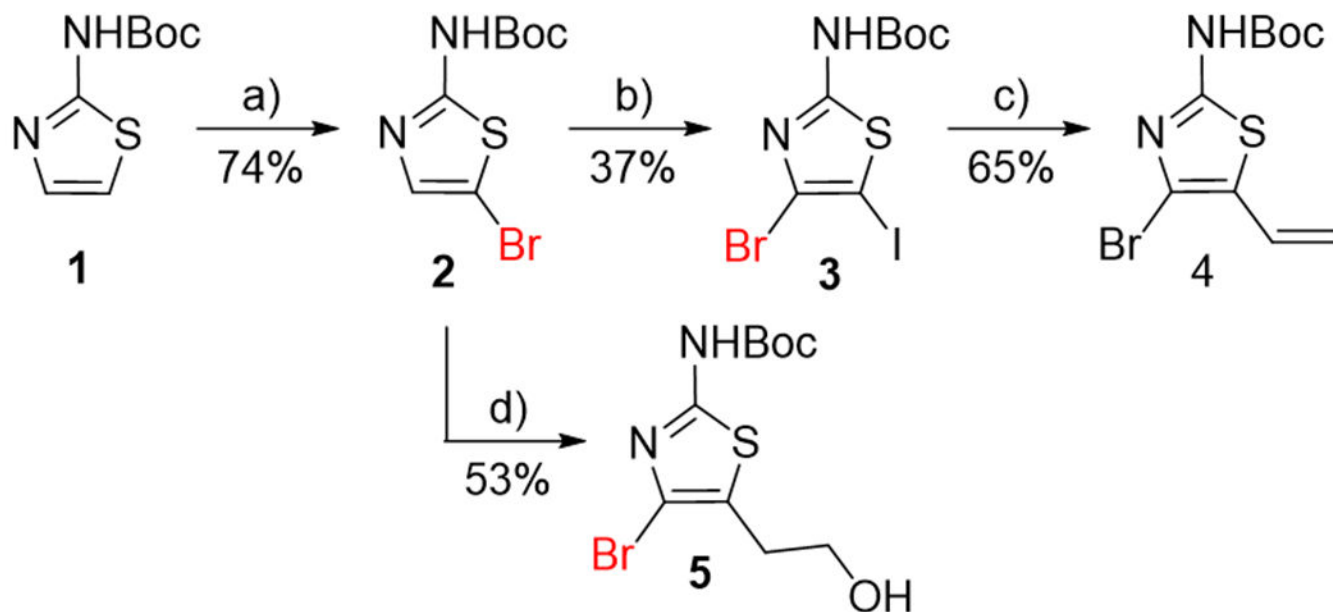
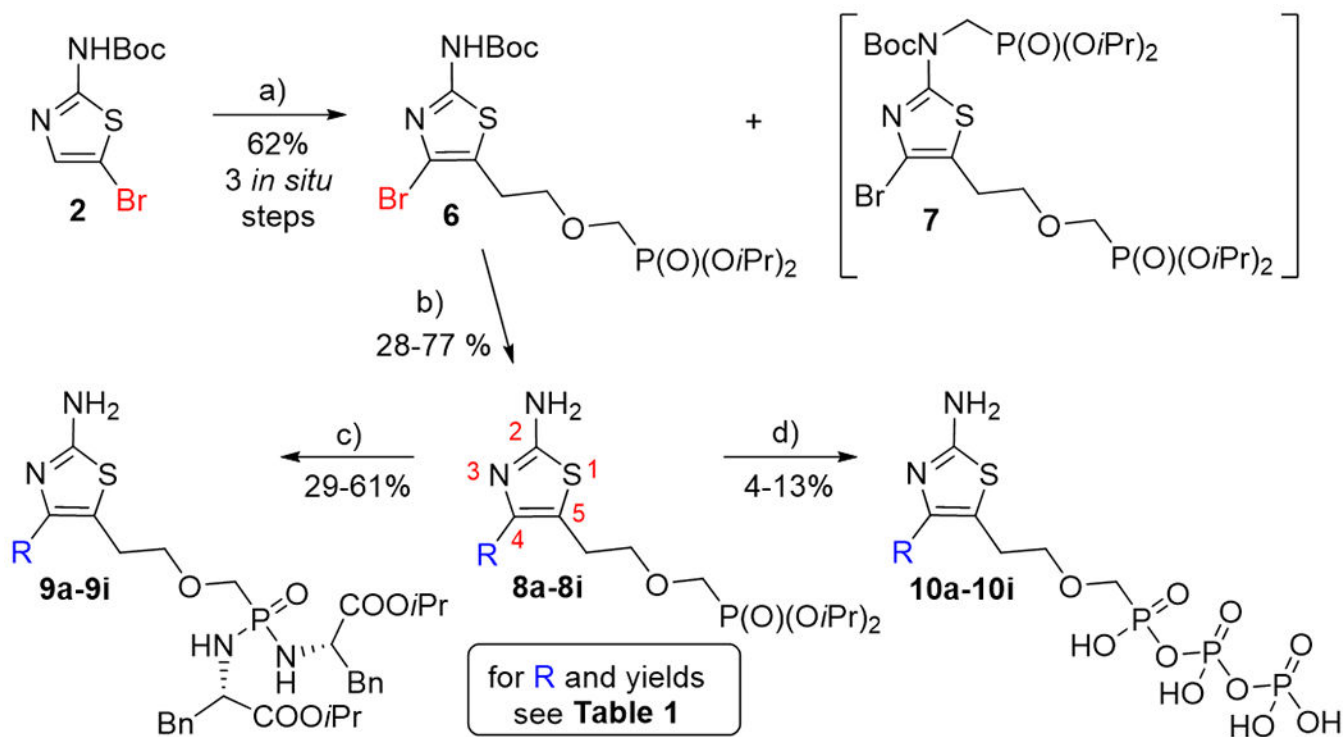


Figure 2. Structures of MB 05032, recently reported 5-aryl-4-PME-2-aminothiazoles **II**, and their 4-aryl-5-PME-2-aminothiazole regioisomers **III** prepared in this work.

**Scheme 1.**

Synthesis of 2-(thiazol-5-yl)ethan-1-ol derivative **5**. Reagents and conditions: a) NBS, THF, RT; b) LDA, THF, 0 °C, then I₂, THF, RT; c) potassium vinyltrifluoroborate, *i*Pr₂NEt, Pd(dppf)Cl₂·CH₂Cl₂, EtOH, 100 °C; d) LDA, THF, 0 °C, then oxirane, THF, 0–10 °C, then H₂O.

**Scheme 2.**

Synthesis of ANPs **8**, the corresponding phosphorodiamidate prodrugs **9**, and phosphono disphosphates **10**. Reagents and conditions: a) LDA, THF, $-20\text{ }^{\circ}\text{C}$, then oxirane, THF, $10\text{ }^{\circ}\text{C}$, then $\text{TfOCH}_2\text{P}(\text{O})(\text{O}i\text{Pr})_2$, $-40\text{ }^{\circ}\text{C}$; b) XPhos Pd G2, R-B(OH)₂, K₃PO₄, THF/H₂O (4:1), $40\text{ }^{\circ}\text{C}$, then TFA, RT, 5 min; c) TMSBr, pyridine, RT, then (L)-NH₂CH(Bn)COO*i*Pr·HCl, Aldrithiol™, PPh₃, Et₃N, pyridine, $70\text{ }^{\circ}\text{C}$; d) (i) TMSBr, pyridine, RT, (ii) 2M aq. TEAB and TBAH, (iii) CDI, (Bu₃N)₂P₂O₇, DMSO.

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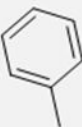
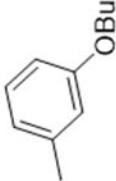
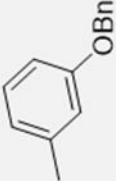
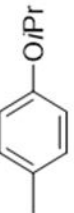
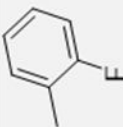
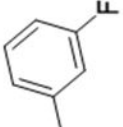
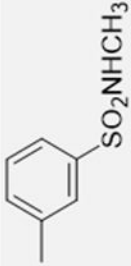
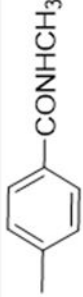
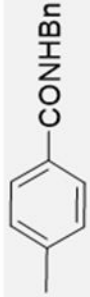
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Table 1:

Yields of Suzuki coupling products **8a–8i**, phosphorodiamidate prodrugs **9a–9i**, and phosphono diphosphates **10a–10i** (Scheme 2)

R =	Comp 8	Yield [%] ^[a]	Comp 9	Yield [%] ^[a]	Comp 10	Yield [%] ^[a]
	8a	74	9a	55	10a	13
	8b	51	9b	43	10b	9
	8c	55	9c	43	10c	9
	8d	63	9d	46	10d	9
	8e	70	9e	47	10e	7
	8f	65	9f	35	10f	7
	8g	77	9g	40	10g	8
	8h	49	9h	61	10h	4
	8i	56	9i	44	10i	9

Isolated yields.

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ACT inhibition (J774A.1 cells), cytotoxic effects (J774A.1 cells), and mammalian AC1, AC2 and AC5 inhibition (HEK293 cells) of phosphorodiamidate prodrugs **9a–9i**. The ACT inhibition of prodrugs **9a–9i** is compared with the potency of their previously reported^[17] regioisomers **II** (Fig. 2).

Table 2.

Comp	ACT IC ₅₀ [μM] ^[d]	ACT for II ^[17] IC ₅₀ [μM] ^[d]	Viability [%] ^[b]	% FSK (5 μM) Stimulated Activity ^[d]	AC1	AC2	AC5
I ^[c]	0.15 ± 0.04	--	93		64 ± 9	200 ± 45	99 ± 8
9a	>10	1.82 ± 0.58	112		61 ± 12	99 ± 26	79 ± 16
9b	8.72 ± 1.33	1.62 ± 0.36	106		90 ± 15	137 ± 30	81 ± 15
9c	>10	0.26 ± 0.05	103		88 ± 14	126 ± 33	91 ± 9
9d	0.49 ± 0.10	0.68 ± 0.08	103		71 ± 18	150 ± 28	87 ± 10
9e	>10	ND	101		74 ± 13	98 ± 16	81 ± 17
9f	5.19 ± 0.76	0.45 ± 0.17	110		79 ± 22	139 ± 60	73 ± 7
9g	>10	4.30 ± 0.29	101		86 ± 41	115 ± 32	81 ± 9
9h	>10	0.66 ± 0.18	101		119 ± 15	112 ± 28	84 ± 5
9i	13.1 ± 0.80	ND	108		102 ± 21	115 ± 33	81 ± 12
-FSK	ND	ND	ND		0 ± 0	0 ± 0	0 ± 0
+FSK	ND	ND	ND		100 ± 0	100 ± 0	100 ± 0
ST034307	ND	ND	ND		0 ± 0	ND	ND
SKF83566	ND	ND	ND		ND	15 ± 12	ND

^[a]Data represent the mean ± SD of at least three independent experiments; IC₅₀ = 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]Previously published data for compound **I**, ref. [14].

^[d]Data represent the mean ± SD of at least three independent experiments at a fixed prodrug concentration (30 μM).

FSK; forskolin. ND: not determined.

Table 3.

Direct inhibition of bacterial ACs (ACT from *B. pertussis* and EF from *B. anthracis*) by prepared ANPpp **10a–10i** and their comparison with the previously reported^[17] regioisomers **II** (Fig. 2), where available.

Compound	IC ₅₀ [nM] ^[a]		Regioisomers II ^b - IC ₅₀ [nM] ^[a]	
	ACT	EF	ACT	EF
10a	25.2 ± 5.7	192.7 ± 16.4	--	--
10b	18.4 ± 6.6	174.9 ± 35.7	--	--
10c	16.7 ± 0.4	67.3 ± 5.4	192 ± 13	235 ± 37
10d	17.1 ± 7.1	263.8 ± 44	185 ± 31	428 ± 11
10e	311.9 ± 7.3	7,992 ± 1,222	--	--
10f	12 ± 0.2	120.9 ± 37.3	313 ± 55	1,335 ± 375
10g	15.4 ± 4.5	5,792 ± 685	--	--
10h	115.7 ± 22.4	162.3 ± 54.3	37.4 ± 7.6	704 ± 50
10i	8.96 ± 0.07	11.6 ± 1.7	--	--
PMIEApp	17.8 ± 6.9	36.0 ± 2.1	--	--

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

^[b]Data taken from ref. [17].