DOI: 10.1002/ejoc.200901271

Facile Solid-Phase Synthesis of AICAR 5′-Monophosphate (ZMP) and Its 4-*N*-Alkyl Derivatives

Giorgia Oliviero,[a] Stefano D'Errico,[a] Nicola Borbone,[a] Jussara Amato,[a] Vincenzo Piccialli,[b] Gennaro Piccialli,*[a] and Luciano Mayol[a]

Keywords: ZMP / Nucleotides / Solid-phase synthesis / Phosphorylation

We report herein a facile, solid-phase synthesis of 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide-5′-monophosphate (ZMP), a biosynthetic precursor of purine nucleotides, as well as a small collection of its 4-*N*-alkyl derivatives. The very difficult, direct, chemical phosphorylation of 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide (AICAR) was circumvented by installing a suitable, fully protected, phosphate group on the 5′-position of *N*-1-(2,4-dinitrophenyl)-inosine, connected to the solid support through the 2′,3′-positions, prior to the purine degradation, which led to the 5-amino-imidazole-4-carboxamide moiety. A plausible reaction mechanism for the formation of the ZMP imidazole was also reported.

Introduction

Nucleosides and nucleotides exhibit a broad spectrum of biological activities including antiviral, anticancer, antibacterial and antiparasitic activity, which generally result from their ability to inhibit specific enzymes. Both naturally occurring and synthetic nucleosides and nucleotides are of value in antiviral chemotherapy, with nucleoside mimics significantly contributing to the arsenal of agents for the treatment of diseases ranging from chickenpox to acquired immunodeficiency syndrome (AIDS). Imidazole nucleosides and nucleotides closely related to their purine counterparts have, to a large extent, been neglected, possibly because they are not constituents of nucleic acids. However, it is well established that imidazole nucleotides are important intermediates in de novo purine nucleotide biosynthesis.[1] In addition, they are also produced during histidine biosynthesis and are associated with histamine metabolism.[2]

In recent years, particular attention has been paid to 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide (AICAR, Figure 1) and its phosphorylated derivative, 5-amino-imidazole-4-carboxamide ribotide (ZMP, Figure 1), a central intermediate in the de novo biosynthesis of purine nucleotides.[3] AICAR is phosphorylated in cells by adenosine kinase to ZMP, which mimics 5′-adenosine monophosphate (AMP) and activates 5′-adenosine monophosphate-activated protein kinase (AMPK)[4] without altering the cellular levels of ATP, ADP or AMP.[5] The activation of AMPK has been reported to be associated with a variety of beneficial effects on the cardiovascular system, including an increase of the regional blood flow and local adenosine concentration, inhibition of neutrophil activation, suppression of platelet activation, prevention of intracoronary thrombosis, prevention of oxidant-induced injury and potentiation of the protective mechanisms induced by myocardial preconditioning.[3] Furthermore, recent studies have suggested that AMPK is a mediator of glucose transport in skeletal muscles[6] and that its activation strongly inhibits basal and insulin-stimulated glucose uptake, lipogenesis, glucose oxidation as well as lactate production in fat cells.[7] In this context, ZMP is expected to possess an activity higher than that of AICAR toward AMPK activation, and both have been indicated as promising prodrugs. Consequently, the production of ZMP and new ZMP derivatives is an appealing objective in the field of medicinal chemistry.

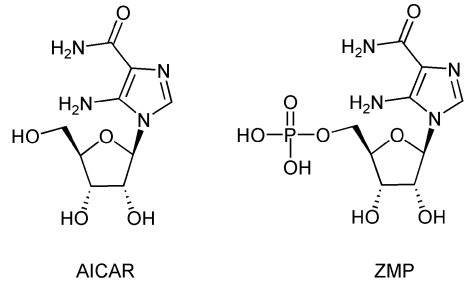


Figure 1. AICAR and ZMP (AICA ribotide).

As experienced by others, we found the direct chemical preparation of ZMP from AICAR to be very difficult. It has been reported[8] that only adenosine kinase efficiently

[a] Dipartimento di Chimica delle Sostanze Naturali, Università degli Studi di Napoli "Federico II",
Via D. Montesano 49, 80131 Napoli, Italy
Fax: +39-081-678552
E-mail: picciall@unina.it
[b] Dipartimento di Chimica Organica e Biochimica, Università degli Studi di Napoli "Federico II",
Via Cynthia 4, 80126, Napoli, Italy

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.200901271.

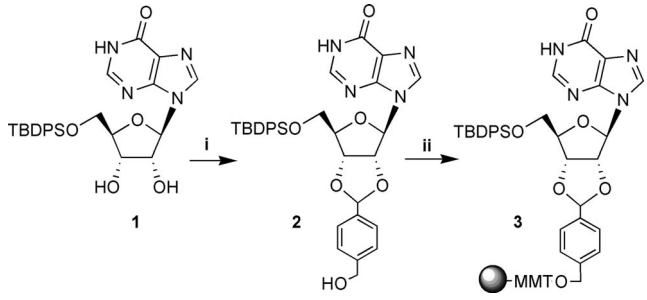
catalyses the phosphorylation of AICAR to ZMP. The first chemical synthesis of ZMP appeared in a paper published in 1961.[9] In this work 1-methoxymethyl-2′,3′-isopropylideninosine 5′-di-*p*-nitrophenyl phosphate was converted into ZMP by the degradation of the purine with sodium hydroxide and the enzymatic removal of the *p*-nitrophenyl groups with a yield of 11–16%. In an alternative synthetic procedure, which used as the starting material the methyl 5-aminoimidazole-4-carboxylate ribonucleoside, a 40–50% yield of ZMP was obtained.[10] In addition, to the best of our knowledge, only two 4-*N*-alkyl AICA-ribotides have been synthesised so far, both showing inhibition of inosine-monophosphate cyclohydrolase, an enzyme involved in the de novo purine biosynthetic pathway.[11] These 4-*N*-alkyl ribotides were obtained in low to moderate yields by the 5′-phosphorylation of the corresponding 4-*N*-alkyl ribosides. We report herein a fast and high-yielding, solid-phase approach for the preparation of ZMP and 4-*N*-alkyl AICA-ribotides as well as evidence on the mechanism of conversion of the purine of inosine into the imidazole ring of ZMP.

Results and Discussion

In previous studies we had shown that AICAR and its 4-*N*-alkyl derivatives (Scheme 1) can be easily obtained starting from *N*-1-(2,4-dinitrophenyl)inosine and *N*-1-alkylinosines, respectively.[12] In particular, the introduction of the strongly electron–withdrawing, 2,4-dinitrophenyl group on *N*-1 of inosine increases the electrophilicity of the purine C-2, allowing its reaction with amino nucleophiles. Therefore, the treatment of the activated inosine with ethylenediamine (EDA) quantitatively gave AICAR, whereas its reaction with alkylamines furnished *N*-1-alkylinosines, which could, in turn, be converted into 4-*N*-alkyl AICARs by NaOH-mediated, purine degradation.[13]

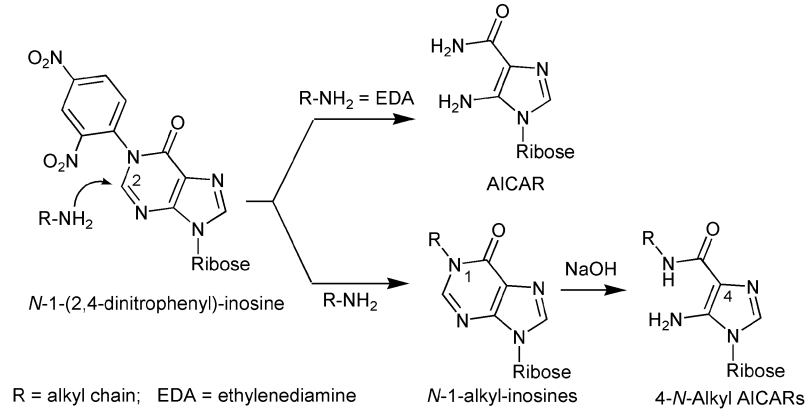
These precedents provided the basis for this paper, wherein we describe the synthesis of ZMP and a collection of its 4-*N*-alkyl derivatives by the introduction of a suitable phosphorylation step into the synthetic routes described above.

Our synthetic approach used the inosine-binding solid support **3** (0.72 mmol/g, Scheme 2) as a key intermediate. This was prepared, in almost quantitative yield, by the reaction of the 4-(hydroxymethyl)-2′,3′-benzylidene-5′-TBDPS-inosine (**2**, Scheme 2) with the commercially available polystyrene monomethoxytrityl chloride resin (MMTCl resin, 1.3 mmol/g). In this way, inosine was bound to the trityl resin by a linker having two acid-labile groups (a benzyl trityl ether and a 2′,3′-acetal), which are selectively cleavable by treatment with anhydrous and aqueous acid, respectively. Intermediate **2** was, in turn, prepared in 85% yield by the reaction of 5′-protected inosine (**1**, Scheme 2) with 4-(hydroxymethyl)benzaldehyde dimethyl acetal in the presence of catalytic amounts of TsOH. This reaction proved highly diastereoselective giving the stereoisomer possessing the (*R*) configuration at the benzylidene acetal carbon (*de* > 96%), as ascertained by a 2D-NOESY experiment, which showed intense correlations between the acetal proton and the ribose 2′-H and 3′-H protons.



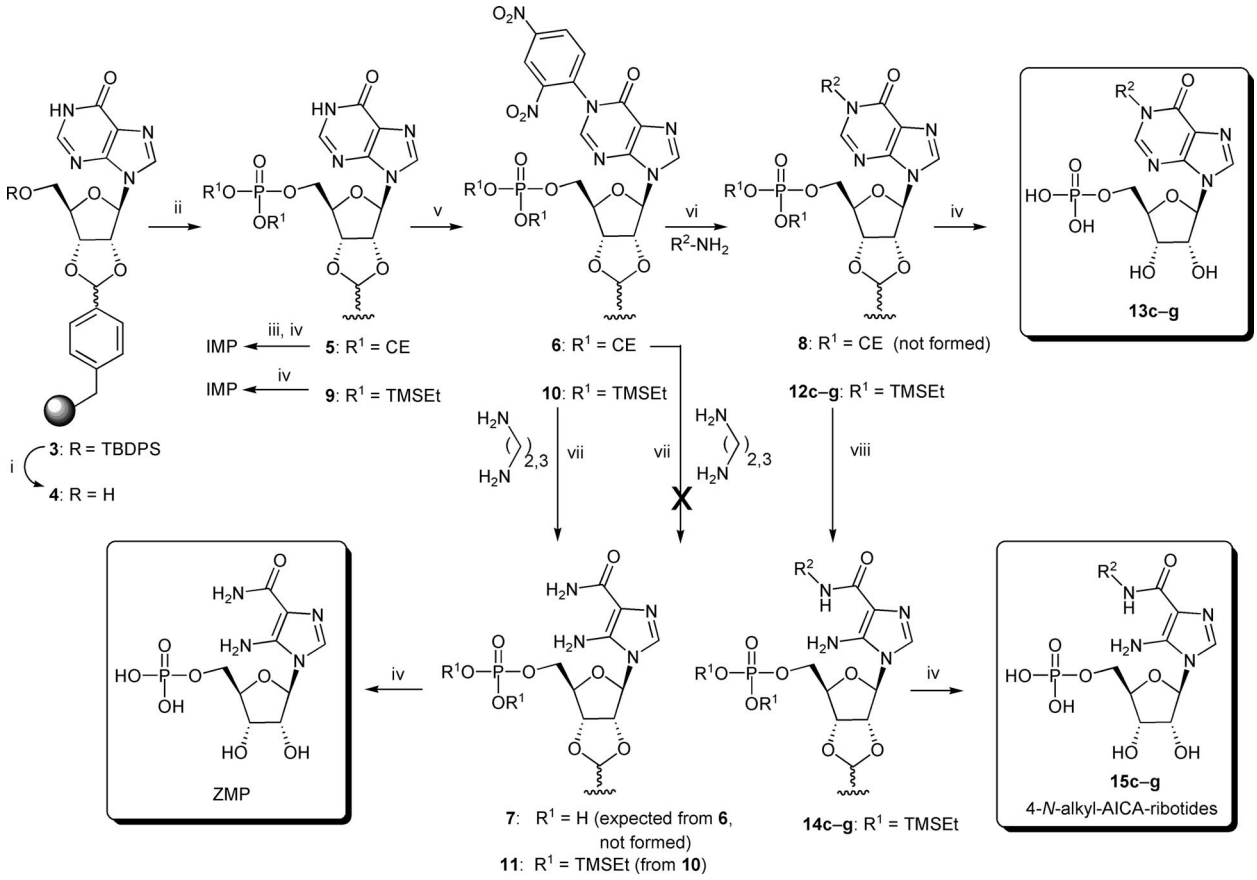
Scheme 2. Reagents and conditions: (i) 4-(hydroxymethyl)benzaldehyde dimethyl acetal, *p*-toluenesulfonic acid (TsOH), DCM, 7 h, reflux; (ii) MMTCl resin, pyridine, DMAP, 24 h, room temp.

The key step of the whole synthetic pathway (Scheme 3) proved to be the phosphorylation of the 5′-OH group. In particular, the choice of suitable protecting groups on the phosphate moiety was found to be crucial in order to obtain ZMP and its 4-*N*-alkyl derivatives in high yields and purity. Thus, the desilylation of support **3** in the presence of NH_4F gave support **4**, which furnished the fully 5′-protected inosine phosphate **5** upon treatment with bis-cyanoethyl-*N*,*N*′-



Scheme 1. Synthetic pathways leading to AICAR, *N*-1-alkylinosines and 4-*N*-alkyl AICARs.

diisopropylphosphoramidite [$(i\text{Pr})_2\text{NP(OCE)}_2$], in the presence of tetrazole, followed by phosphate oxidation with *tert*-butyl hydroperoxide (*t*BuOOH). The yield (95% from **4**) of the phosphorylation step was evaluated by detaching inosine 5′-monophosphate (IMP) by treatment of support **5** with concentrated aqueous ammonia followed by acid. Compound **5**, suspended in dry DMF, was then treated with 2,4-dinitrochlorobenzene (DNCB) in the presence of K_2CO_3 to give support **6**. In an attempt to obtain ZMP through a one-pot procedure, support **6** was treated with EDA, a reagent that could both degrade the purine to AICA by acting as a nucleophile on C-2 as well as deprotect the phosphate by acting as a base capable of promoting the removal of the 2-cyanoethyl protecting groups by β-elimination, to give, in principle, resin **7**. Disappointingly, the acidic treatment of a weighed amount of the resulting resin gave a complex mixture of products, from which ZMP was isolated in less than 10% yield. Likewise, the reaction of **6** with alkylamines failed to give the expected *N*-1-alkylinosine supports **8**. We hypothesise that a partial or complete deprotection of the phosphate took place by a reaction with either the diamine or the alkylamine, which caused the process to take a different course. These results prompted us to employ an alternative phosphorylating reagent having base-stable protecting groups. We focused our attention on the commercially available bis-trimethylsilylethoxy-*N*,*N*′-diisopropylphosphoramidite[14] [$(i\text{Pr})_2\text{NP(OTMSEt)}_2$], in which the TMSEt groups can be removed by fluoride or trifluoroacetic acid treatment. This phosphorylating reagent has largely been employed to phosphorylate sugars and peptides[15] but, to the best of our knowledge, it has never been used with nucleosides. According to the new strategy, the reaction of **4** with $(i\text{Pr})_2\text{NP(OTMSEt)}_2$ in THF, in the presence of tetrazole, afforded support **9** in high yield after the usual phosphate oxidation with *t*BuOOH (released IMP, 95% yield). Support **9** was then converted into support **10** as described above for support **5**. Pleasingly, the treatment of **10** with EDA in DMF, followed by the acidic cleavage of the nucleotidic material from **11**, gave ZMP in an overall 86% yield (from **3**). ^{1}H and ^{31}P NMR and UV data were in full agreement with those obtained from the commercially available, authentic sample. We note that the treatment of support **11** with TFA, followed by washing with H_2O, allowed for the release of the nucleotidic material from the resin as well as the deprotection of the phosphate group.



Scheme 3. Reagents and conditions: (i) NH_4F, MeOH, reflux, 15 h; (ii) a) $(i\text{Pr})_2\text{NP(OCE)}_2$ or $(i\text{Pr})_2\text{NP(OTMSEt)}_2$, tetrazole, room temp., 15 h; b) *t*BuOOH, THF, room temp., 2.5 h; (iii) concentrated NH_4OH, 15 h, 55 °C; (iv) TFA (2% in DCM), room temp., 20 min, followed by washing with H_2O; (v) DNCB, K_2CO_3, DMF, 80 °C, 3 h; (vi) R_2-NH_2, DMF, 50 °C, 8 h; (vii) EDA or 1,3-diaminopropane, DMF, 50 °C, 8 h; (viii) NaOH (2 M in EtOH), reflux, 5 h.

The stability of TMSEt groups under basic conditions opened the way to the synthesis of 4-*N*-alkyl AICA-ribotide derivatives **15c**–**g**. In particular, we explored the possibility of alkylating the *N*-1 position of the inosine followed by opening of the purine in the presence of the fully protected phosphate group. Thus, the reaction of **10** with some alkylamines (R^2-NH_2, **c**–**g**, Table 1) furnished the *N*-1-alkylinosine supports **12c**–**g**. Next, supports **12c**–**g** were incubated in a ethanolic NaOH to give supports **14c**–**g**. The TFA treatment of **14c**–**g** furnished 4-*N*-alkyl AICA-ribotide derivatives **15c**–**g** in 74–77% yield (Table 1), the structures of which were confirmed by spectroscopic data. On the other hand, the acid treatment of supports **12c**–**g** provided, in high yields (Table 1), *N*-1-alkylinosine-5′-phosphates **13c**–**g** as well. The use of 5-hydroxypentylamine (**f**) and butylamine (**g**) allowed us to enlarge the chemical diversity attainable through the above synthetic routes and prove the compatibility of the chemistry with a hydroxyl-bearing side chain on *N*-1.

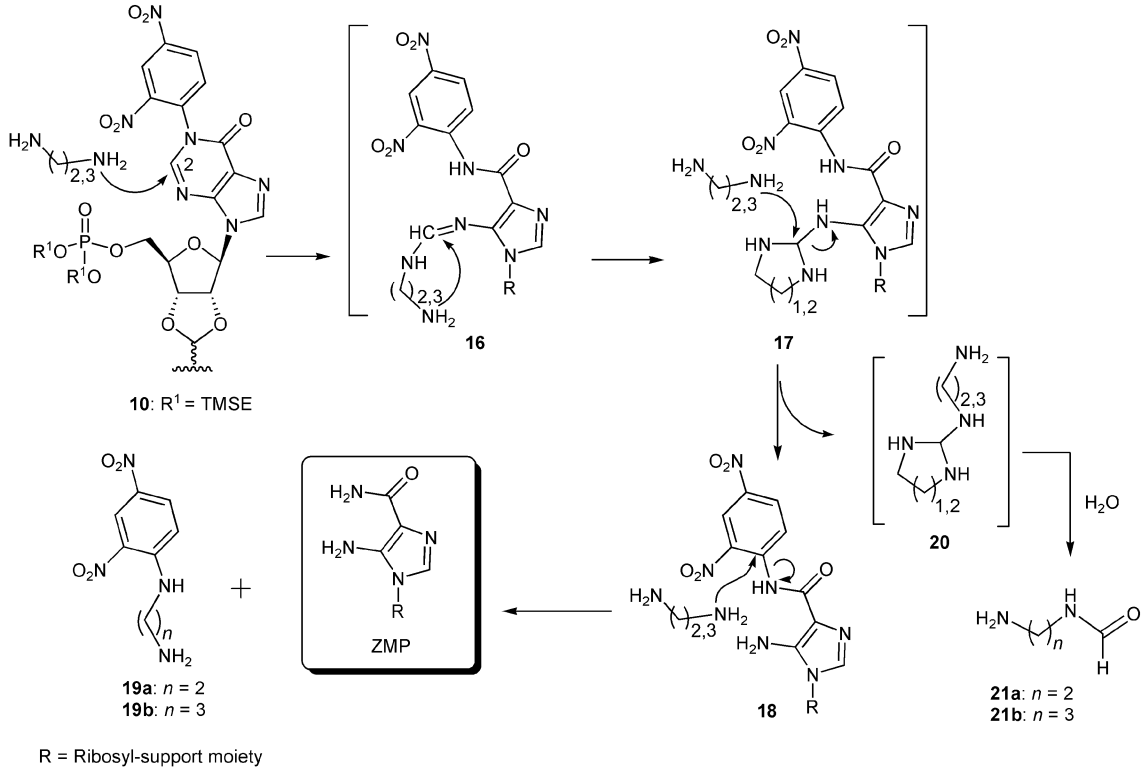
Finally, we studied the reaction of *N*-1-(2,4-dinitrophenylinosine) support **10** with some linear α,ω-diaminoalkanes. In particular, we wanted to shed light on the diverse reactivity of **10**, which leads to *N*-1-alkylinosines or ZMP when reacted with alkylamines or EDA, respectively.

In our previous studies on the opening-reclosure of the purine of inosine,[16] we demonstrated that a formamidine intermediate (**16**, Scheme 4) was formed upon the attack of amino nucleophiles on C-2. We hypothesised that a similar intermediate was formed upon the attack of EDA on **10** and that the free, terminal, amino group could be responsible for the purine degradation by an intramolecular attack on the formamidine carbon. This hypothesis prompted us to test the reaction of **10** with a set of linear α,ω-diaminoalkanes of different chain length [NH_2-$(CH_2)_n$-NH_2, n = 2–6]. Interestingly, we found that if the diamine was comprised of two or three methylene groups, ZMP was formed (Scheme 4). When longer diamines (four to six methylene groups) were used, purine reclosure was observed with the formation of *N*-1-ω-aminoalkylinosines (**12c**–**e**, Scheme 5). These findings suggested that if the diamine is short, the terminal amino group in **16** can attack the formamidine carbon forming the cyclic orthoamide **17**, which then undergoes degradation by the attack of a second diamine molecule at the orthoamide carbon to afford AICAR derivative **18** and orthoamides **20**. ZMP was eventually obtained through the attack of an additional diamine on the dinitrophenyl *ipso* carbon with the concomitant formation of N-substituted 2,4-dinitroanilines **19**. On the other hand, a

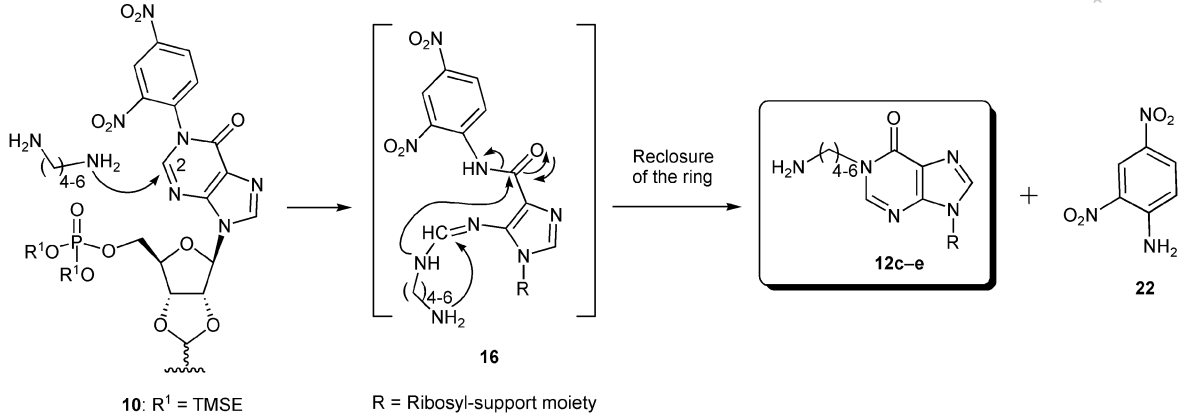
Table 1. Products and reaction yields.

Entry	R^2–NH_2	Yield of ZMP [%][a]	Yield of **13** [%][a]	Yield of **15** [%][a]
1	H_2N–$(CH_2)_2$–NH_2	86	n.f.	n.f.
2	H_2N–$(CH_2)_3$–NH_2	85	n.f.	n.f.
3	H_2N–$(CH_2)_4$–NH_2	17	69	75
4	H_2N–$(CH_2)_5$–NH_2	8	77	74
5	H_2N–$(CH_2)_6$–NH_2	n.f.	83	76
6	OH–$(CH_2)_5$–NH_2	n.f.	83	77
7	H_3C–$(CH_2)_3$–NH_2	n.f.	84	77

[a] Overall yield starting from support **3**; n.f.: not formed.



Scheme 4. A mechanistic hypothesis that explains the formation of ZMP by the reaction of **10** with shorter α,ω-diaminoalkanes.



Scheme 5. A mechanistic hypothesis for the formation of *N*-1-(ω-aminoalkyl)inosines by the reaction of **10** with longer α,ω-diaminoalkanes.

longer diamine possesses increased conformational freedom, and the alternative route (Scheme 5), leading to **12c–e**, can operate, wherein the N linked to the amidine carbon in **16** participates in the reclosure of the purine by attacking the carbonyl group and causing dinitroaniline displacement. In summary, with a too-long diamine (n = 4–6), the formation of cyclic orthoamides **17** is disfavoured because an unfavourable seven- to nine-membered ring would be involved. On the contrary, this step can easily take place when the formation of a five-/six-membered ring (n = 2,3) is involved, leading to AICAR through the purine system degradation. These hypothesised mechanisms were supported by the isolation of formamides **21** from the hydrolysis of cyclic orthoamides **20** and 2,4-dinitroaniline **22** or *N*-substituted 2,4-dinitroanilines **19** from **16**, depending on the length of the diamine employed (see Schemes 4 and 5).

Conclusions

In conclusion, we have described an efficient, solid-phase synthesis of ZMP, 4-*N*-alkyl AICA-ribotides and 5′-phosphate *N*-1-alkylinosines. The main goal of the synthesis, the installation of the phosphate at the 5′ position of AICAR, has been successfully achieved by postponing the degradation of the purine until after the phosphorylation step. The latter has been performed using $(i\text{Pr})_2\text{NP(OTMSEt)}_2$, where the TMSEt protecting groups are stable under basic conditions and removable by mild acidic treatment. In addition, a plausible hypothesis for the formation of the 5-aminoimidazole-4-carboxamide ring and *N*-1-alkylinosines has been given by studying the reaction of *N*-1-(2,4-dinitrophenyl)inosine 5′-phosphate with a set of linear α,ω-diaminoalkanes.

Experimental Section

General Methods: 4-Methoxytrityl chloride resin (MMTCl resin, 1% divinylbenzene, 200–400 mesh, 1.3 mmol/g loading) was purchased from CBL Patras, Greece. Anhydrous solvents were used for all reactions. All the other reagents were obtained from commercial sources and were used without further purification. The reactions on solid phase were performed using glass columns [10 mm diameter, 100 mm length, with fused-in sintered glass-disc of porosity 3 (bore of plug: 2.5 mm)], which were shaken in an orbital shaker. High-temperature reactions were performed in round-bottomed flasks. Column chromatography was performed on Merck silica gel (Kieselgel 60, 0.063–0.200 mm); preparative thin-layer chromatography was performed on TLC plates (Merck, silica gel 60, F_{254}, 0.5 mm thick). ^{1}H and ^{13}C NMR spectra were acquired with a Varian Mercury Plus 400 MHz or a Varian Unity Inova 500 MHz instrument using D_2O, CD_3OD or [D_6]acetone as the solvent. Chemical shifts were reported in parts per million (δ) relative to the residual solvent signal [^{1}H: 4.80 (HDO), 3.31 (CD_2HOD) and 2.09 ([D_5H]acetone) ppm; ^{13}C: 49.0 (CD_3OD) ppm]. ^{31}P NMR were performed with a Varian Unity Inova 500 MHz instrument using 85% H_3PO_4 as an external standard (δ = 0 ppm). IR spectra were recorded with a Jasco FT-IR 430 spectrometer. UV spectra were recorded with a Jasco V-530 UV spectrophotometer. Mass spectra were recorded with an Applied Biosystems API 2000 mass spectrometer using electron spray ionisation (ESI) in negative mode. RP-HPLC analyses and purifications were carried out with a Jasco UP-2075 Plus pump, equipped with a Jasco UV-2075 Plus UV detector and a C-18, reverse-phase column (5 μm, 4.8 × 150 mm), eluted with a linear gradient of CH_3CN in 0.1 M triethylammonium hydrogen carbonate buffer (TEAB, pH = 7.0). System A: from 0 to 20% CH_3CN over 90 min, flow rate: 1.6 mL/min; System B: from 0 to 100% CH_3CN over 90 min, flow rate: 1.6 mL/min).

5′-*O*-TBDPS-2′,3′-*O*-[4-Hydroxymethyl-(1*R*)-benzyliden]inosine (2): A mixture of **1** (1.0 g, 3.7 mmol), TsOH (0.19 g, 0.99 mmol) and 4-(hydroxymethyl)benzaldehyde dimethyl acetal (9.9 mmol, 1.8 g) was suspended in dry DCM (15 mL), and the solution was refluxed for 7 h. The reaction was monitored by TLC (AcOEt/MeOH = 95:5). After the mixture was cooled, the solvent was removed under reduced pressure, and the residue was applied to a silica gel column and eluted with increasing amounts of MeOH in AcOEt (from 0 to 5%) to afford pure **2** (1.6 g, 70%) as an amorphous, white solid. IR (neat): $\tilde{\nu}$ = 3500 (broad, OH), 1690 (carbonyl) cm^{-1}. ^{1}H NMR (400 MHz, CD_3OD): δ = 8.20 (s, 1 H, 8-H or 2-H), 7.80 (s, 1 H, 8-H or 2-H), 7.61–7.24 (complex signals, 14 H, aromatic of TBDPS), 6.35 (d, J = 1.7 Hz, 1 H, 1′-H), 6.01 (s, 1 H, C*H*-benzylidene), 5.52 (dd, J = 6.6, 2.0 Hz, 1 H, 2′-H), 5.13 (dd, J = 6.7, 3.0 Hz, 1 H, 3′-H), 4.65 (s, 2 H, CH_2-benzylidene), 4.54 (m, 1 H, 4′-H), 3.87 (dd, J = 11.2, 5.1 Hz, 1 H, 5′-H$_a$), 3.81 (dd, J =

11.2, 5.1 Hz, 1 H, 5′-H_b), 0.99 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (100 MHz, CD_3OD, assignments were made by an HSQC experiment): δ = 20.0 (C-Si) 27.3 (3 CH_3, *t*Bu), 64.8 (CH_2Ph), 65.5 (C-5′), 83.8 (C-4′), 86.4 (C-2′), 89.1 (C-3′), 91.9 (C-1′), 108.8 (CH acetal), 126.2 (C-5), 127.9 (C-2 and C-6 benzylidene), 128.2 (C-3 and C-5 benzylidene), 128.7, 128.8, 130.9, 131.0, 134.0, 134.3, 135.7 (aromatic), 136.7 (C-1 benzylidene), 136.7 (aromatic), 141.3 (C-8), 144.7 (C-4 benzylidene), 146.5 (C-2), 149.2 (C-4), 158.8 (C-6) ppm. ESI-MS: *m*/*z* = 625 $[M + H]^+$.

Loading of 2 onto MMTCl Resin–Solid Support 3: A mixture of **2** (1.0 g, 1.6 mmol) and DMAP (0.23 mmol, 0.028 g), coevaporated with dry pyridine (3 × 1.5 mL) and dissolved in dry pyridine (6 mL), was added to the MMTCl resin (0.9 g, 1.1 mmol), and the mixture was gently shaken for 24 h at room temp. The obtained support **3** (inosine loading of 0.72 mmol/g) was filtered and washed with DCM (3 × 5 mL), DCM/MeOH (1:1, 3 × 5 mL) and MeOH (3 × 5 mL) and finally dried under reduced pressure. The reaction yield (96%) was calculated both by measuring the weight increase of the support and analysing and quantitating the nucleoside material detached from the solid support by treatment with 2% TFA in DCM (8 min, room temp.).

ZMP: Solid support **3** (0.50 g, 0.36 mmol) was desilylated by treatment with NH_4F according to a reported procedure.[17] Support **4** (0.22 g, 0.37 mmol), previously swollen in dry THF, was treated with $(i\text{Pr})_2\text{NP(OTMSEt)}_2$ (1.6 g, 4.4 mmol) and tetrazole (0.48 g, 6.6 mmol) in dry THF/DCM (9:1, 4.5 mL) and shaken for 15 h at room temp. After the resin was filtered, the solid support was washed with THF (3 × 5 mL), THF/MeOH (1:1, 3 × 5 mL) and MeOH (3 × 5 mL) and finally dried in vacuo. The resin was treated with *t*BuOOH (5.5 M in decane, 2.2 mL, 12.4 mmol) in THF (4.5 mL) for 2.5 h at room temp. and then washed with THF (3 × 5 mL), THF/MeOH (1:1, 3 × 5 mL) and MeOH (3 × 5 mL) to give support **9** with a 0.67 mmol/g inosine-5′-monophosphate loading. The reaction yield (95%) was evaluated by detaching inosine-5′-monophosphate by treating the resin with 2% TFA in DCM for 20 min at room temp., washing it with H_2O and collecting the filtrates.

To the solid support **9** (0.30 g, 0.2 mmol), suspended in dry DMF (6 mL), K_2CO_3 (0.14 g, 1.0 mmol) and DNCB (0.30 g, 1.0 mmol) were added, and the reaction was maintained at 80 °C for 3 h. After the resin was filtered, the support was washed with DMF (3 × 5 mL), DMF/H_2O (1:1, 3 × 5 mL), H_2O (3 × 5 mL), H_2O/MeOH (1:1, 3 × 5 mL) and MeOH (3 × 5 mL) and then dried under reduced pressure to give support **10**.

Support **10** (0.10 g, 0.059 mmol) was incubated with EDA (0.30 g, 5.0 mmol, **a**, Table 1) or 1,3-diaminopropane (0.37 g, 5.0 mmol, **b**, Table 1) in DMF (4.5 mL) under shaking for 8 h at 50 °C. After the resin was filtered and washed with DMF (3 × 5 mL), DMF/MeOH (1:1, 3 × 5 mL) and MeOH (3 × 5 mL), support **11** was dried under reduced pressure. Cleavage of the nucleotidic material was performed by treating the resin with 2% TFA in DCM for 20 min at room temp., washing it with H_2O and collecting the filtrates. The crude material, purified according to the procedure reported in the General Methods (System A), furnished 18 mg (86% from **3**) of pure ZMP, which showed chromatographic behaviour and spectroscopic data identical to those exhibited by an authentic specimen. The washings of resin **11** from the reaction of support **10** with EDA or 1,3-diaminopropane, collected as described above, were dried under reduced pressure. The crude material was purified on TLC plates using $CHCl_3$/MeOH (7:3) as the solvent system. The bands were detected either by direct exposure to UV light (254 nm) or by treatment of a narrow strip of the plates with ninhydrin. The relevant portions of the plate were then scratched from the plates and eluted with $CHCl_3$/MeOH (6:4) to give **19a** and **21a** or **19b** and **21b**.

***N*-(2,4-Dinitrophenyl)-1,2-diaminoethane (19a):** Amorphous yellow solid. ^{1}H NMR (400 MHz, [D_6]acetone): δ = 9.03 (d, *J* = 2.7 Hz, 1 H, 3-H phenyl), 8.34 (dd, *J* = 9.6, 2.7 Hz, 1 H, 5-H phenyl), 7.38 (d, *J* = 9.6 Hz, 1 H, 6-H phenyl), 3.81 (m, 2 H, CH_2NH), 3.62 (t, *J* = 5.9 Hz, 2 H, CH_2NH_2) ppm. ESI-MS: *m*/*z* = 227 $[M + H]^+$.

***N*-(2,4-Dinitrophenyl)-1,3-diaminopropane (19b):** Amorphous yellow solid. ^{1}H NMR (400 MHz, [D_6]acetone): δ = 9.02 (d, *J* = 2.7 Hz, 1 H, 3-H phenyl), 8.33 (dd, *J* = 9.5, 2.7 Hz, 1 H, 5-H phenyl), 7.31 (d, *J* = 9.5 Hz, 1 H, 6-H phenyl), 3.72 (m, 2 H, CH_2NH), 3.41 (t, *J* = 5.7 Hz, 2 H, CH_2NH_2) ppm. 2.09 (CH_2, obscured by the residual solvent signal); ESI-MS: *m*/*z* = 241 $[M + H]^+$.

***N*-(2-Aminoethyl)formamide (21a):** Oil. ^{1}H NMR (500 MHz, CD_3OD): δ = 8.13 (s, 1 H, formamide), 3.41 (t, *J* = 6.1 Hz, 2 H, CH_2NH), 2.91 (t, *J* = 6.1 Hz, 2 H, CH_2NH_2) ppm. ESI-MS: *m*/*z* = 89 $[M + H]^+$.

***N*-(2-Aminopropyl)formamide (21b):** Oil. ^{1}H NMR (400 MHz, CD_3OD): δ = 8.05 (s, 1 H, formamide), 3.31 (CH_2NH, obscured by the residual solvent signal), 2.69 (t, *J* = 6.1 Hz, 2 H, CH_2NH_2), 1.68 (m, 2 H, CH_2) ppm. ESI-MS: *m*/*z* = 103 $[M + H]^+$.

13c–g: Support **10** (0.10 g, 0.059 mmol), obtained as described above for the synthesis of ZMP, swollen in DMF, was incubated with the appropriate amine (**c**–**g**, 5.0 mmol, Table 1) in DMF (1.5 mL) under shaking for 8 h at 50 °C. After the resin was filtered and washed with DMF (3 × 5 mL), DMF/MeOH (1:1, 3 × 5 mL) and MeOH (3 × 5 mL), supports **12c**–**g** were dried under reduced pressure. The yields of *N*-1-alkylinosine 5′-monophosphates **13c**–**g** (69–84% from **3**, Table 1) were calculated by UV quantitation of the products obtained after HPLC purification (System B, see the General Methods) of the crude nucleotidic material released from a weighed amount of resin **12c**–**g** by treating it with 2% TFA in DCM for 20 min at room temp., washing it with H_2O and collecting the filtrates. In particular, 19 mg of **13c**, 21 mg of **13d**, 23 mg of **13e**, 23 mg of **13f** and 22 mg of **13g** were obtained. ^{1}H and ^{31}P NMR, UV, IR and MS data confirmed the purity and the structure of the products (see below).

15c–g: Supports **12c**–**g** (0.10 g, 0.060–0.062 mmol) were suspended in ethanolic NaOH (2.0 M in EtOH, 2.0 mL) for 5 h at reflux. After the resin was cooled, it was filtered, washed with EtOH (3 × 5 mL), EtOH/H_2O (1:1, 3 × 5 mL), H_2O (3 × 5 mL) and MeOH (3 × 5 mL) and finally dried under reduced pressure, giving supports **14c**–**g**. The yields of 4-*N*-alkyl AICA ribotides **15c**–**g** (74–77%, from **3**) were calculated by UV quantitation of the products obtained after HPLC purification (System B, see the General Methods) of the crude nucleotidic materials released from a weighed amount of resin **14c**–**g** by treating it with 2% TFA in DCM for 20 min at room temp., washing it with H_2O and collecting the filtrate. In particular, 21 mg of **15c**, 21 mg of **15d**, 23 mg of **15e**, 22 mg of **15f** and 21 mg of **15g** were obtained. ^{31}P NMR, UV, IR and MS data confirmed the purity and the structure of the products (see below).

1-(4-Aminobutyl)inosine 5′-Monophosphate (13c): Amorphous solid. IR (neat): $\tilde{\nu}$ = 3600–2600 (broad, OHs and NH_2), 1681 (strong, C=O), 1069, 973 (both strong, phosphate) cm^{-1}. ^{1}H NMR (500 MHz, D_2O): δ = 8.51 (s, 1 H, 8-H or 2-H), 8.39 (s, 1 H, 8-H or 2-H), 6.10 (d, *J* = 5.8 Hz, 1 H, 1′-H), 4.80 (2′-H, obscured by the residual solvent signal), 4.52 (m, 1 H, 3′-H), 4.40 (m, 1 H, 4′-H), 4.19 (t, *J* = 6.9 Hz, 2 H, CH_2N), 4.05 (m, 2 H, 5′$_{a,b}$-H), 3.04 (t, *J* = 7.4 Hz, 2 H, CH_2NH_2), 1.89 (m, 2 H, CH_2), 1.72 (m, 2 H,

CH_2) ppm. ^{31}P NMR (202 MHz, D_2O): δ = 3.81 (s) ppm. UV (H_2O): λ_{max} = 250 nm; ESI-MS: *m*/*z* = 418 [M – H]$^-$.

1-(5-Aminopentyl)inosine 5′-Monophosphate (13d): Amorphous solid. IR (neat): $\tilde{\nu}$ = 3600–2600 (broad, OHs and NH_2), 1682 (strong, C=O), 1058 and 941 (both strong, phosphate) cm^{-1}. ^1H NMR (500 MHz, D_2O): δ = 8.45 (s, 1 H, 8-H or 2-H), 8.37 (s, 1 H, 8-H or 2-H), 6.10 (d, *J* = 5.8 Hz, 1 H, 1′-H), 4.80 (2′-H, obscured by the residual solvent signal), 4.50 (m, 1 H, 3′-H), 4.37 (m, 1 H, 4′-H), 4.15 (t, *J* = 6.9 Hz, 2 H, CH_2N), 4.13–4.06 (m, 2 H, 5′$_{a,b}$-H), 2.98 (t, *J* = 7.5 Hz, 2 H, C*H*$_2$NH$_2$), 1.83 (m, 2 H, CH_2), 1.70 (m, 2 H, CH_2), 1.42 (m, 2 H, CH_2) ppm. ^{31}P NMR (202 MHz, D_2O): δ = 2.15 (s) ppm. UV (H_2O): λ_{max} = 250 nm; ESI-MS: *m*/*z* = 432 [M – H]$^-$.

1-(6-Aminohexyl)inosine 5′-Monophosphate (13e): Amorphous solid. IR (neat): $\tilde{\nu}$ = 3600–2600 (broad, OHs and NH_2), 1671 (strong, C=O), 1175 and 1126 (both strong, phosphate) cm^{-1}. ^1H NMR (400 MHz, D_2O): δ = 8.42 (s, 1 H, 8-H or 2-H), 8.37 (s, 1 H, 8-H or 2-H), 6.10 (d, *J* = 5.9 Hz, 1 H, 1′-H), 4.80 (2′-H, obscured by the residual solvent signal), 4.49 (m, 1 H, 3′-H), 4.37 (m, 1 H, 4′-H), 4.16–4.09 (complex signal, 4 H, CH_2N, 5′$_{a,b}$-H), 3.18 (q, *J* = 7.3 Hz, CH_2 triethylammonium), 2.96 (t, *J* = 7.7 Hz, 2 H, C*H*$_2$NH$_2$), 1.79 (m, 2 H, CH_2), 1.64 (m, 2 H, CH_2), 1.39 (m, 4 H, 2 CH_2), 1.27 (t, *J* = 7.3 Hz, CH_3 triethylammonium) ppm. ^{31}P NMR (202 MHz, D_2O): δ = 1.26 (s) ppm. UV (H_2O): λ_{max} = 250 nm; ESI-MS: *m*/*z* = 446 [M – H]$^-$.

1-(5-Hydroxypentyl)inosine 5′-Monophosphate (13f): Amorphous solid. IR (neat): $\tilde{\nu}$ = 3600–2600 (broad, OHs), 1678 (strong, C=O), 1201 and 956 (both strong, phosphate) cm^{-1}. ^1H NMR (400 MHz, D_2O): δ = 8.44 (s, 1 H, 8-H or 2-H), 8.39 (s, 1 H, 8-H or 2-H), 6.12 (d, *J* = 5.8 Hz, 1 H, 1′-H), 4.75 (m, 1 H, 2′-H), 4.49 (m, 1 H, 3′-H), 4.38 (m, 1 H, 4′-H), 4.17–4.09 (complex signal, 4 H, CH_2N and 5′$_{a,b}$-H), 3.58 (t, *J* = 6.5 Hz, 3 H, CH_2O), 3.19 (q, *J* = 7.3 Hz, CH_2 triethylammonium), 1.81 (m, 2 H, CH_2), 1.58 (m, 2 H, CH_2), 1.39 (m, 2 H, CH_2), 1.27 (t, *J* = 7.3 Hz, CH_3 triethylammonium) ppm. ^{31}P NMR (202 MHz, D_2O): δ = 1.25 (s) ppm. UV (H_2O): λ_{max} = 250 nm; ESI-MS: *m*/*z* = 433 [M – H]$^-$.

1-Butylinosine 5′-Monophosphate (13g): Amorphous solid. IR (neat): $\tilde{\nu}$ = 3600–2600 (broad, OHs), 1683 (strong, C=O), 1057 and 945 (both strong, phosphate) cm^{-1}. ^1H NMR (400 MHz, D_2O): δ = 8.38 (s, 1 H, 8-H or 2-H), 8.43 (s, 1 H, 8-H or 2-H), 6.11 (d, *J* = 5.9 Hz, 1 H, 1′-H), 4.75 (m, 1 H, 2′-H), 4.49 (m, 1 H, 3′-H), 4.37 (m, 1 H, 4′-H), 4.15–4.08 (complex signal, 4 H, CH_2N and 5′$_{a,b}$-H), 3.18 (q, *J* = 7.3 Hz, CH_2 triethylammonium), 1.75 (m, 2 H, CH_2), 1.34 (m, 2 H, CH_2), 1.26 (t, *J* = 7.3 Hz, CH_3 triethylammonium), 0.91 (t, *J* = 7.4 Hz, 3 H, CH_3) ppm. ^{31}P NMR (202 MHz, D_2O): δ = 1.33 (s) ppm. UV (H_2O): λ_{max} = 250 nm; ESI-MS: *m*/*z* = 403 [M – H]$^-$.

5-Amino-1-(β-D-ribofuranosyl)imidazole-4-[*N*-(4-aminobutyl)]carboxamide 5′-Monophosphate (15c): Amorphous solid. IR (neat): $\tilde{\nu}$ = 3600–2600 (broad, OHs and NH_2s), 1626 (strong, C=O), 1558 (strong, amide II band), 1082 and 975 (both strong, phosphate) cm^{-1}. ^1H NMR (500 MHz, D_2O): δ = 7.52 (s, 1 H, 2-H), 5.65 (d, *J* = 6.8 Hz, 1 H, 1′-H), 4.66 (m, 1 H, 2′-H), 4.45–4.39 (m, 1 H, 3′-H), 4.31 (m, 1 H, 4′-H), 4.10–3.99 (m, 2 H, 5′$_{a,b}$-H), 3.38 (m, 2 H, CH_2N), 3.03 (br. t, *J* = 7.6 Hz, 2 H, C*H*$_2$NH$_2$), 1.77–1.61 (m, 4 H, 2 CH_2) ppm. ^{31}P NMR (202 MHz, D_2O): δ = 4.38 (s) ppm. UV (H_2O): λ_{max} = 268 nm; ESI-MS: *m*/*z* = 408 [M – H]$^-$.

5-Amino-1-(β-D-ribofuranosyl)imidazole-4-[*N*-(5-aminopentyl)]carboxamide 5′-Monophosphate (15d): Amorphous solid. IR (neat): $\tilde{\nu}$ = 3600–2600 (broad, OHs and NH_2s), 1626 (strong, C=O), 1563 (strong, amide II band), 1082 and 974 (both strong, phosphate) cm^{-1}. ^1H NMR (400 MHz, D_2O): δ = 7.51 (s, 1 H, 2-H), 5.64 (d, *J* = 7.0 Hz, 1 H, 1′-H), 4.66 (m, 1 H, 2′-H), 4.44–4.39 (m, 1 H, 3′-H), 4.30 (m, 1 H, 4′-H), 4.06–3.97 (m, 2 H, 5′$_{a,b}$-H), 3.34 (t, *J* = 6.1 Hz, 2 H, CH_2N), 2.99 (t, *J* = 7.3 Hz, 2 H, C*H*$_2$NH$_2$), 1.69 (m, 2 H, CH_2), 1.61 (m, 2 H, CH_2), 1.42 (m, 2 H, CH_2) ppm. ^{31}P NMR (202 MHz, D_2O): δ = 2.81 (s) ppm. UV (H_2O): λ_{max} = 267 nm; ESI-MS: *m*/*z* = 422 [M – H]$^-$.

5-Amino-1-(β-D-ribofuranosyl)imidazole-4-[*N*-(6-aminohexyl)]carboxamide 5′-Monophosphate (15e): Amorphous solid. IR (neat): $\tilde{\nu}$ = 3600–2600 (broad, OHs and NH_2s), 1681 (strong, C=O), 1627 (strong, amide II band), 1132 and 991 (both strong, phosphate) cm^{-1}. ^1H NMR (400 MHz, D_2O): δ = 7.52 (s, 1 H, 2-H), 5.65 (d, *J* = 6.8 Hz, 1 H, 1′-H), 4.63 (m, 1 H, 2′-H), 4.43–4.39 (m, 1 H, 3′-H), 4.31 (m, 1 H, 4′-H), 4.09–4.04 (m, 2 H, 5′$_{a,b}$-H), 3.34 (br. t, 2 H, CH_2N), 3.19 (q, *J* = 7.3 Hz, CH_2 triethylammonium), 2.98 (t, *J* = 7.2 Hz, 2 H, C*H*$_2$NH$_2$), 1.70–1.54 (m, 4 H, 2 CH_2), 1.46–1.35 (m, 4 H, 2 CH_2), 1.27 (t, *J* = 7.3 Hz, CH_3 triethylammonium) ppm. ^{31}P NMR (202 MHz, D_2O): δ = 2.93 (s) ppm. UV (H_2O): λ_{max} = 268 nm; ESI-MS: *m*/*z* = 436 [M – H]$^-$.

5-Amino-1-(β-D-ribofuranosyl)imidazole-4-[*N*-(5-hydroxypentyl)]-carboxamide 5′-Monophosphate (15f): Amorphous solid. IR (neat): $\tilde{\nu}$ = 3600–2600 (broad, OHs and NH_2), 1683 (strong, C=O), 1635 (strong, amide II band), 1133 and 991 (both strong, phosphate) cm^{-1}. ^1H NMR (500 MHz, D_2O): δ = 7.52 (s, 1 H, 2-H), 5.66 (d, *J* = 7.01 Hz, 1 H, 1′-H), 4.65 (m, 1 H, 2′-H), 4.42 (m, 1 H, 3′-H), 4.32 (m, 1 H, 4′-H), 4.06 (m, 2 H, 5′$_{a,b}$-H), 3.61 (t, *J* = 6.5 Hz, 2 H, CH_2O), 3.34 (t, *J* = 6.4 Hz, 2 H, CH_2N), 3.20 (q, *J* = 7.3 Hz, CH_2 triethylammonium), 1.59 (m, 4 H, 2 CH_2), 1.41 (m, 2 H, CH_2), 1.28 (t, *J* = 7.3 Hz, CH_3 triethylammonium) ppm. ^{31}P NMR (202 MHz, D_2O): δ = 3.54 (s) ppm. UV (H_2O): λ_{max} = 266 nm; ESI-MS: *m*/*z* = 423 [M – H]$^-$.

5-Amino-1-(β-D-ribofuranosyl)imidazole-4-(*N*-butyl)carboxamide 5′-Monophosphate (15g): Amorphous solid. IR (neat): $\tilde{\nu}$ = 3600–2600 (broad, OHs and NH_2), 1624 (strong, C=O), 1559 (strong, amide II band), 1081 and 982 (both strong, phosphate) cm^{-1}. ^1H NMR (400 MHz, D_2O): δ = 7.51 (s, 1 H, 2-H), 5.65 (d, *J* = 6.8 Hz, 1 H, 1′-H), 4.63 (m, 1 H, 2′-H), 4.41 (m, 1 H, 3′-H), 4.30 (m, 1 H, 4′-H), 4.05 (m, 2 H, 5′$_{a,b}$-H), 3.32 (br. t, 2 H, CH_2N), 3.19 (q, *J* = 7.3 Hz, CH_2 triethylammonium), 1.54 (m, 2 H, CH_2), 1.35 (m, 2 H, CH_2), 1.27 (t, *J* = 7.3 Hz, CH_3 triethylammonium), 0.90 (t, *J* = 7.2 Hz, 3 H, CH_3) ppm. ^{31}P NMR (202 MHz, D_2O): δ = 2.78 (s) ppm. UV (H_2O): λ_{max} = 266 nm; ESI-MS: *m*/*z* = 393 [M – H]$^-$.

Supporting Information (see also the footnote on the first page of this article): ^1H and ^{13}C NMR spectra of **2**, ^1H and ^{31}P NMR spectra of **13c**–**g** and **15c**–**g**, and ^1H NMR spectra of **19a**,**b** and **21a**,**b**.

Acknowledgments

We are grateful to the Italian Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST) (PRIN 2007), for financial support in favour of this investigation. We are also grateful to the Centro di Servizio Interdipartimentale di Analisi Strumentale of the University of Napoli Federico II for NMR facilities. The authors are thankful to Dr Luisa Cuorvo for technical assistance.

[1] C. Hartman, J. M. Buchanan, *Ann. Rev. Biochem.* **1959**, *28*, 365–410.

[2] a) G. Shaw, in: *Chemistry of Nucleosides and Nucleotides* (Ed.: L. B. Townsend), Plenum Press, New York, **1994**, vol. III, chapter 4; b) L. B. Townsend, *Chem. Rev.* **1967**, *67*, 533–563.

[3] N. Nath, S. Giri, R. Prasad, M. L. Salem, A. K. Singh, I. Singh, *J. Immunol.* **2005**, *175*, 566–574.
[4] G. A. Rutter, G. da Silva Xavier, I. Lecler, *Biochem. J.* **2003**, *375*, 1–16.
[5] S. L. Longnus, R. B. Wambolt, H. L. Parson, R. W. Brownsey, M. F. Allard, *AJP-Regul. Integr. Comp. Physiol.* **2003**, *284*, 936–944.
[6] I. P. Salt, J. M. C. Connell, G. W. Gould, *Diabetes* **2000**, *49*, 1649–1656.
[7] M. P. Gaidhu, S. Fediuc, R. B. Ceddia, *J. Biol. Chem.* **2006**, *281*, 25956–25964.
[8] R. A. Mehl, T. P. Begley, J. Label, *J. Labelled Compd. Radiopharm.* **2002**, *45*, 1097–1102.
[9] E. Shaw, *J. Am. Chem. Soc.* **1961**, *83*, 4770–4772.
[10] G. Shaw, D. V. Wilson, C. P. Green, *J. Chem. Soc.* **1964**, 2650–2657.
[11] a) I. E. Burrows, G. Shaw, *J. Chem. Soc. Perkin Trans. 1* **1967**, *11*, 1088–1093; b) M. Wall, J. Hoon Shim, S. J. Benkovic, *Biochemistry* **2000**, *39*, 11303–11311.
[12] a) L. De Napoli, A. Messere, D. Montesarchio, G. Piccialli, M. Varra, *J. Chem. Soc. Perkin Trans. 1* **1997**, 2079–2082; b) G. Oliviero, J. Amato, N. Borbone, S. D′Errico, G. Piccialli, L. Mayol, *Tetrahedron Lett.* **2007**, *48*, 397–400.
[13] a) E. Casanova, A. I. Hernàndez, E. M. Priego, S. Liekens, M. J. Camarasa, J. Balzarini, M. J. Pérez-Pérez, *J. Med. Chem.* **2006**, *49*, 5562–5570; b) G. Oliviero, J. Amato, N. Borbone, S. D'Errico, E. Bucci, V. Piccialli, M. Varra, G. Piccialli, L. Mayol, *Tetrahedron* **2008**, *64*, 6475–6481.
[14] K. C. Ross, D. L. Rathbone, W. Thomson, S. Freeman, *J. Chem. Soc. Perkin Trans. 1* **1995**, *4*, 421–426.
[15] a) H.-G. Chao, M. S. Bernatowicz, C. E. Klimas, G. R. Matsueda, *Tetrahedron Lett.* **1993**, *34*, 3377–3380; b) T. Müller, R. R. Schmidt, *Liebigs Ann./Recueil* **1997**, 1907–1914; c) S. L. Mellor, D. A. Wellings, J.-A. Fehrentz, M. Paris, J. Martinez, N. J. Ede, A. M. Bray, D. J. Evans, G. B. Bloomberg, in: *Fmoc Solid Phase Synthesis. A practical approach* (Eds: W. C. Chan, P. D. White), Oxford University Press, Oxford, **2000**, p. 177; d) K. Barlos, D. Gatos, S. Koutsogianni, *J. Pept. Res.* **1998**, *51*, 194–200; e) K. Barlos, D. Gatos, S. Kapolos, C. Poulos, W. Schafer, Y. Wenqing, *J. Pept. Res.* **1991**, *38*, 555–561; f) K. Barlos, D. Gatos, S. Koutsogianni, W. Schafer, G. Stavropoulos, Y. Wenqing, *Tetrahedron Lett.* **1991**, *32*, 471–474.
[16] L. De Napoli, A. Messere, D. Montesarchio, G. Piccialli, *J. Org. Chem.* **1995**, *60*, 2251–2253.
[17] W. Zheng, M. J. Robins, *Tetrahedron Lett.* **1992**, *33*, 1177–1180.

Received: November 5, 2009
Published Online: January 28, 2010

