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A solid-phase approach to the synthesis of *N*-1-alkyl analogues of cyclic inosine-diphosphate-ribose (cIDPR)

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ABSTRACT

We report here an efficient solid-phase synthesis of *N*-1-alkyl-substituted analogues of cyclic inosine-diphosphate-ribose (cIDPR), a mimic of cyclic ADP-ribose (cADPR). Our synthetic strategy makes use of a polystyrene support to which inosine was bonded through a 2′,3′-acetal linkage. Insertion of a ω-hydroxy-polymethylene chain of variable length on N-1, followed by conversion into *N*-1-alkylinosine-bisphosphate derivatives and cyclization, allowed to obtain analogues of cIDPR of various ring size. The cyclization step was carried out both in solid-phase and in solution by pyrophosphate bond formation. The effect of the *N*-1-polymethylene chain length on the cyclization yields as well as the reaction conditions, which led to the solid-phase pyrophosphate bond formation, were thoroughly investigated.

1. Introduction

Cyclic ADP-ribose (cADPR, **1**, Fig. 1) is a metabolite involved in Ca^{2+} signalling in various cell types. It is biosynthesized from NAD^+ and regulates the calcium mobilization from intracellular stores in a wide variety of biological systems via ryanodine receptors.[1] New cADPR analogues, exhibiting both biological properties as agonists or antagonists and resistance to the enzymatic and chemical hydrolysis, are required to investigate cADPR-mediated Ca^{2+} signalling. In order to elucidate the structure–activity relationship, a number of analogues of cADPR have been synthesized using enzymatic, chemo-enzymatic and chemical approaches and pharmacologically tested.[2] 8-Br-cIDPR (**2**, Fig. 1), has been recently described as an agonist of cADPR/Ca^{2+} signalling system.[3] A series of *N*-1-substituted,[4] *N*-9-substituted[5] and C-8-substituted[3] cIDPR (**3**, Fig. 1) have been synthesized and some of them have been shown to retain an interesting Ca^{2+}-related biological activity. Particular attention has been devoted to the *N*-1-substitutions. In particular, the synthesis of *N*-1-carborybosyl,[6] *N*-1-glycosyl,[7] and *N*-1-ethoxymethyl[5a,7,8] analogues have been reported. In some cases, these modifications have also been combined with a C-8 substitution (i.e., 8-Cl, 8-Br, 8-N_3). Among the synthesized compounds, the *N*-1-ethoxymethyl-cIDPR (cIDPRE, **4**, Fig. 1), containing a structurally simplified *N*-1 molecular moiety, has shown a strong potency in inducing Ca^{2+} release in intact and permeabilized human Jurkat T lymphocytes.[4] The chemical synthesis proposed for the great part of these analogues, makes use of multistep sequences carried out in solution which require purification of the products after each reaction step. In an effort to facilitate the chemical synthesis of these substances as well as to enlarge the number of accessible structurally diverse analogues, we report here a new and general solid-phase approach to the synthesis of *N*-1-alkyl analogues of cIDPR (**18a–d**, Scheme 1).

2. Results and discussion

Our synthetic approach uses the inosine-binding solid support **7** as key intermediate. This was prepared in almost quantitative yield by reaction of the 4-(hydroxymethyl)-2′,3′-benzylidene-5′-TBDPS-inosine **6** with the commercially available polystyrenemonomethoxytrityl chloride resin (MMTCl, 1.3 mmol/g). In this way inosine was bound to the trityl resin by a linker having two acid labile functions (a benzyl–trityl ether and a 2′,3′-acetal linkage) that are selectively cleavable by treatment with anhydrous acids or aqueous acids, respectively. Carboxy-functionalized 2′,3′-benzylidene acetal linkers have previously been used to attach nucleosides to amino-loaded solid supports through amide bond formation.[9] Intermediate **6** was in turn prepared in 85% yield by reaction of 5′-protected inosine **5** with 4-(hydroxymethyl)-benzaldehyde dimethyl acetal in the presence of catalytic amounts of *p*-toluensulfonic acid (PTSA). This reaction proved highly diastereoselective giving the stereoisomer possessing the R configuration at the benzylidene acetal carbon (dr>96%) as ascertained by a 2D-

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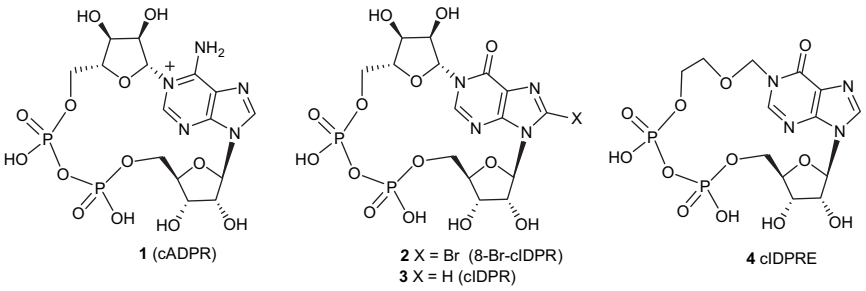
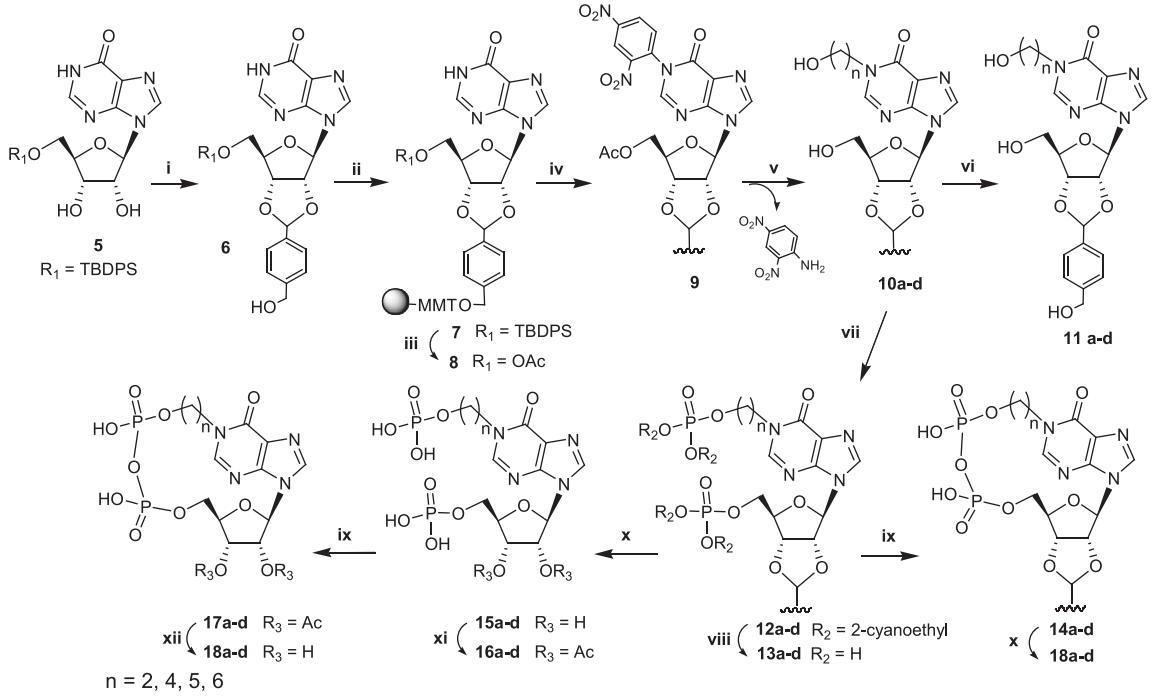


Figure 1. Structures of cADPR, cIDPR, 8-Br-cIDPR and cIDPRE.



Scheme 1. Reagents and conditions: (i) 4-(hydroxymethyl)-benzaldehyde dimethyl acetal (2.7 equiv), PTSA (0.3 equiv), DCM, 7 h, reflux; (ii) MMTCl resin, **6** (1.5 equiv), pyridine (1.5 mL/250 mg of resin), DMAP (0.2 equiv), 24 h, rt; (iii) (a) NH_4F (55 equiv), MeOH, reflux, 12 h, (b) Ac_2O in pyridine, 30 min, rt; (iv) DNCB (7.5 equiv), K_2CO_3 (7.5 equiv), DMF, 2 h, 80 °C.; (v) OH–$(CH_2)_{2,4-6}$–NH_2 (66 equiv), DMF, 8 h, 50 °C; (vi) TFA 2% in DCM, 8 min, rt; (vii) (a) $(CEO)_2PN(iPr)_2$ (24 equiv), tetrazole (48 equiv) in THF/DCM, 15 h, rt; (b) *tert*-butylhydroperoxide (69 equiv), decane/THF, 3 h, rt; (viii) conc. NH_4OH, 15 h, 55 °C; (ix) EDC (6.4 equiv for **13a–d** or 5.0 equiv for **16a–d**), DMF, 72 h, rt; (x) TFA/H_2O/DCM, 15 min, rt; (xi) Ac_2O in pyridine, 15 h, rt; (xii) conc. NH_4OH, 5 h, rt.

NOESY experiment that showed intense correlations between the acetal proton and the ribose H-2′ and H-3′ protons. In previous papers we demonstrated that *N*-1-(2,4-dinitrophenyl)-inosine can be easily converted into *N*-1-alkylated inosine by reaction with primary alkylamines, both in solution[10–12] or 5′-bound to a polymeric support.[13] In particular, reaction of the C-2 purine carbon with amino nucleophiles leads to the opening of the six-membered ring through the cleavage of the N1-C2 bond. The successive fast ring *re*-closure, favored by loss of 2,4-dinitroaniline, furnishes the *N*-1-alkylinosine derivatives in high yields. Based on these precedents, *N*-1-(2,4-dinitrophenyl)-inosine-containing support **9** was synthesized in high yields (94%) by reaction of **7** with 2,4-dinitrochlorobenzene (DNCB) in the presence of K_2CO_3. It is to be noted that a 5′ acetyl replaces the TBDMS protecting group in **8**. This protecting group modification (NH_4F treatment of **7** followed by acetylation) was suggested by the observation that the presence of a 5′-*O*-acetyl on the sugar furnishes higher yields of the dinitrophenylderivative **9** during the conversion of **8** to **9**. This transformation demonstrated that the above process works well on a solid support too, allowing the successful replacement of the time-consuming step required for the preparation of *N*-1-(2,4-dinitrophenyl)-inosine in solution before its loading onto the solid support.[13] Next, reaction of **9** with some ω-hydroxyalkylamines (HO–$(CH_2)_n$–NH_2, *n*=2,4–6, **a–d**, Table 1) furnished the *N*-1-

Table 1
Products and reaction yields

ω-hydroxyalkylamines	**11a–d** yield[a] (%)	**15a–d** yield[a] (%)	**18a–d** (from **13**) yield[b] (%)	**18a–d** (from **16**) yield[c];	**18a–d** yield[a] (%)
a OH–$(CH_2)_2$–NH_2	91	78	20	89	74
b OH–$(CH_2)_4$–NH_2	92	78	13	84	70
c OH–$(CH_2)_5$–NH_2	92	77	10	85	70
d OH–$(CH_2)_6$–NH_2	90	74	10	84	68

[a] Overall yield starting from support **7**.
[b] Calculated on the amount of **13** (0.063 mmol/g) converted in **18**.
[c] Calculated on the amount of **16** converted in **18**.

ω-hydroxyalkyl-inosine supports **10a–d** (90–92% yield) by the concomitant aminolysis of the 5′-*O*-acetyl group. It is to be noted that when 3-amino-propanol was used, the resin assumed an enduring gel-like consistency that prevented its further manipulation and therefore the synthesis of the expected *N*-1-(3-hydroxypropyl)-inosine. The reaction yield, as well as the structures of **10a–d**, were ascertained analyzing the HPLC purified nucleoside material **11a–d** released from a weighted amount of resin **10a–d** by treatment with a 2% TFA solution in dry CH_2Cl_2 (DCM) taking into account that the starting support **7** had a nucleoside functionalization of 0.72 mmol/g. Then, inosine derivatives **10a–d** were converted into the bis-protected diphosphate derivatives **12a–d** that, after phosphate deprotection, gave diphosphate derivatives **13a–d** suitable for cyclization by intramolecular pyrophosphate bond formation as detailed below.

The intramolecular cyclization is a crucial step in the chemical synthesis of cADPR, cIDPR and their derivatives, that requires the closure of a 18-membered cycle. The most employed method uses the condensation between a phosphomonoester and a *S*-phenyl phosphodiester group, promoted by $AgNO_3$ that converts the phosphorothioate into a reactive metaphosphate species.[6] However, this procedure, that minimizes the charge repulsion between the two phosphates, requires two distinct phosphorylation reactions and suitable protection/deprotection steps. In previous work, we demonstrated that in the 2′,3′-protected diphosphate **15c**, though characterized by a high conformational flexibility, the two charged phosphate functions could be efficiently condensed in dilute conditions in the presence of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC), generating a 19-membered ring.[11] Thus, following this approach, the reaction of **10a–d** with bis-cyanoethyl-*N*,*N*′-diisopropylphosphoramidite[14] $(CEO)_2PN(iPr)_2$ in THF, in the presence of tetrazole, furnished the bis-protected diphosphate derivatives **12a–d** (95–98% yield) after phosphorus oxidation with *tert*-butylhydroperoxide. The complete deprotection of both phosphates, achieved by treating **12a–d** with conc. aqueous ammonia, furnished supports **13a–d** in almost quantitative yields. The solid-phase phosphorylation yields, as well as the efficiency of ammonia deprotection of the phosphotriester groups, were evaluated analyzing the *N*-1-alkyl-inosine diphosphate derivatives **15a–d** (Table 1) released from **13a–d** by aqueous acid treatments (TFA/DCM/H_2O). The structures of **15a–d** were confirmed by spectroscopic data after HPLC purifications. Next, the intramolecular cyclization of supports **13a–d** by pyrophosphate bond formation was addressed. We observed that the previously employed conditions for the cyclization of **15c** in solution (EDC, 5.0 equiv, *N*-methylpyrrolidone, 60 h, rt) when applied to solid-supports **13a–d** led to very low yields of cyclic products **18a–d** (5–15%) even after prolonged reaction time (various EDC ratios, solvents and temperatures were tested). In particular, it was observed that high amounts (60–70%) of polymeric species were obtained, notwithstanding the pseudo-diluted conditions characterizing the solid-phase cyclization. The presence of these species, demonstrated by HPLC and MS analyses, can be explained by hypothesizing that the formation of intermolecular pyrophosphate bonds may occur due to the high functionalization of the support and the consequent low distance between the reactive inosine diphosphate moieties. On the other hand, only a modest increased yield of the cyclic products was obtained (12–22%) when the cyclization was carried out using an *ad hoc* synthesized support **7** characterized by a severe lowering of the support functionalization (up to 0.063 mmol/g). Therefore, we decided to perform the final cyclization step in solution. To this end, the diphosphate derivatives **15a–d** were converted quantitatively into the 2′,3′-di acetyl derivatives **16a–d** by reaction with acetic anhydride in pyridine solution. These compounds were subjected, without further purification, to the pyrophosphate bond formation by addition of EDC in DMF to give the cyclic diphosphate derivatives **17a–d** in high yields (Table 1). Finally removal of the acetyl groups, performed by aqueous conc. ammonia treatment, furnished the target products **18a–d** the structures of which were confirmed by ^1H, ^{31}P NMR and MS data. It is to be noted that the cyclization yields (84–89%, Table 1) are scarcely affected by the *N*-1-polymethylene chain lengths though the best yield was obtained with **18a** including the shortest, bis-methylene, chain. In addition, we also observed that almost the same cyclization yields were obtained when the reaction was carried out on the hydroxymethyl-acetylated 2′,3′-benzylidene derivatives of **15a–d** (not shown in Scheme 1) released from supports **13a–d** by anhydrous acidic treatment followed by standard acetylation of the *p*-CH_2OH group on the benzene ring. This second pathway demonstrated that a 2′,3′-bonded bulky group does not hamper the cyclization reaction and, furthermore, furnishes an alternative synthetic procedure, which uses an acid-labile 2′,3′-protecting group.

3. Conclusions

The herein reported solid-phase approach, besides the synthesis of several cIDPR analogues, furnishes a general and fast methodology for: (i) the synthesis of N-1 nucleoside analogues; (ii) the synthesis of their bis-phosphorylated derivatives; (iii) the intramolecular cyclization of bis-phosphate species by pyrophosphate bond formation. Particular attention has been devoted to the latter reaction that was carried out both in solid-phase and in solution. While the solid-phase cyclizations mostly lead to polymeric species, due to the formation of intermolecular pyrophosphate bonds, very high yields of the target cyclic products were obtained by performing the final cyclization step in solution.

4. Experimental section

4.1. General

4-Methoxytrityl chloride resin (MMT-Cl, 1% divinylbenzene, 200–400 mesh, 1.3 mmol g^{-1} substitution) was purchased from CBL Patras, Greece. Anhydrous solvents were used for 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 PO (bore of plug 2.5 mm), which were shaken on an orbital shaker, or in round bottomed flasks, when the process required high temperatures. The ^1H and ^{13}C NMR spectra were performed on a Varian Mercury Plus 400 MHz using $SiMe_4$ as an internal standard and CD_3OD and D_2O as solvents; chemical shifts are reported in parts per million (δ) relative to residual solvent signals: CD_2HOD 3.31, HDO 4.80 for ^1H NMR and CD_2HOD 49.0 for ^{13}C NMR. ^{31}P-NMR were performed on a Varian Unity Inova 500 MHz using H_3PO_4 85% as an external standard. RP-HPLC analyses and purifications were carried out on a Jasco UP-2075 Plus pump using a 4.8×150 mm C-18 reverse-phase column (particle size 5 μm) eluted with a linear gradient of CH_3CN in H_2O (from 0 to 100% in 60 min, flow 1.0 mL min^{-1}, system A) or with a linear gradient of CH_3CN in 0.1 M TEAB (pH 7.0, from 0 to 100% in 120 min, flow 1.0 mL min^{-1}, system B) equipped with a Jasco UV-2075 Plus UV detector. The UV spectra were recorded on a Jasco V-530 UV spectrophotometer. IR spectra were recorded on a Jasco FTIR 430 spectrometer. The High Resolution MS were recorded on a Bruker APEX II FT-ICR mass spectrometer using electron spray ionization (ESI) technique. Column chromatography was performed on silica gel (Merck, Kieselgel 60, 0.063–0.200 mm). Analytical TLC analyses were performed using F254 silica gel plates

(0.2 mm, Merck). TLC spots were detected under UV light (254 nm). Cationic exchange chromatographies were performed on Dowex 50 W X8 (Na^+ form, 50–100 mesh, Fluka).

*4.1.1. 5′-O-TBDPS-2′,3′-O-(4-hydroxymethyl-(1R)-benzyliden)-inosine **6**.* A mixture of **5** (1.0 g, 3.7 mmol), PTSA (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 refluxed for 7 h. The reaction was monitored by TLC (AcOEt/MeOH=95:5). After cooling, the solvent was removed under reduced pressure and the residue was applied on a silica gel column eluted with increasing amounts of MeOH in AcOEt (from 0 to 5%) to afford pure **6** (1.6 g, 70%) as an amorphous white solid; IR ν_{max} (neat) 3500 (broad, OH), 1690 (carbonyl) cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ_H 8.20, 7.80 (1H each, s's, H-8 and H-2), 7.61–7.24 (14H, complex signals, aromatic of TBDPS), 6.35 (1H, d, J=1.7 Hz, H-1′), 6.01 (1H, s, C*H*-benzylidene), 5.52 (1H, dd, J=6.6, 2.0 Hz, H-2′), 5.13 (1H, dd, J=6.7, 3.0 Hz, H-3′), 4.65 (2H, s, CH_2-benzylidene), 4.54 (1H, m, H-4′), 3.87 (1H, dd, J=11.2, 5.1 Hz, H_a-5′), 3.81 (1H, dd, J=11.2, 5.1 Hz, H_b-5′), 0.99 (9H, s, *t*-But); ^{13}C NMR (100 MHz, CD_3OD, assignments by a HSQC experiment) δ_C 20.0 (C-Si), 27.3 (3×CH_3, *t*-But), 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 carbons), 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); m/z (HRESIMS) 625.2497 ([M+H]$^+$, $C_{34}H_{37}N_4O_6Si$, requires, 625.2482).

4.2. General procedure for the scaffold synthesis

*4.2.1. Loading of **6** on MMT-Cl resin. Solid support **7**.* A mixture of **6** (1.0 g, 1.6 mmol) and DMAP (0.23 mmol, 0.028 g) co-evaporated with dry pyridine (3×1.5 mL) and dissolved in dry pyridine (6 mL) were added to the MMT-Cl resin (0.9 g, 1.1 mmol) and the whole was gently shaken for 24 h at rt. The obtained support **7** (inosine loading 0.72 mmol/g) was filtered and washed with DCM (3×5 mL), DCM/MeOH (1:1, v/v, 3×5 mL) and MeOH (3×5 mL), and finally dried under reduce pressure. The reaction yield (96%) was calculated both measuring the weight increment of the support and analyzing and quantizing the nucleoside material detached from the solid support by treatment with 2% TFA in DCM (v/v, 8 min, rt).

*4.2.2. Solid support **8**.* To the solid support **7** (1.0 g, 0.72 mmol), suspended in dry MeOH (20 mL), was added NH_4F (40 mmol, 1.5 g) and the mixture was refluxed for 12 h. The resin was filtered and washed with MeOH (3×5 mL), DCM/MeOH (1:1, v/v, 3×5 mL) and DCM (3×5 mL) and finally dried in vacuo. The 5′-hydroxy-inosine support was then acetylated by treatment with a solution of acetic anhydride in pyridine (2:8, v/v, 3 mL, 30 min, rt). After washing with pyridine (3×5 mL) and DCM (3×5 mL), support **8** was dried under reduced pressure.

*4.2.3. Solid support **9**.* To the solid support **8** (1.0 g, 0.87 mmol), suspended in dry DMF (20 mL), K_2CO_3 (0.9 g, 6.5 mmol) and DNCB (1.9 g, 6.5 mmol) were added and the mixture kept at 80 °C for 2 h. After filtration, the support was washed with DMF (3×5 mL), DMF/H_2O (1:1, v/v, 3×5 mL), H_2O (3×5 mL), H_2O/MeOH (1:1, v/v, 3×5 mL) and MeOH (3×5 mL) and then dried under reduced pressure to give support **9** (0.76 mmol/g). The reaction yield (94%) was evaluated by detaching 1-(2,4-dinitrophenyl)inosine[13] under acid hydrolytic conditions (TFA/H_2O/DCM (3:2:95, v/v/v, 15 min, rt)).

*4.2.4. Treatment of solid support **9** with ω-hydroxyalkylamines **a–d**; (supports **10a–d**).* Solid support **9** (0.10 g, 0.076 mmol), swollen in DMF, was left in contact with the appropriate ω-hydroxyalkylamine (**a–d**, 5.0 mmol, Table 1) in DMF (1.5 mL) under shaking for 8 h at 50 °C. After filtration and washings with DMF (3×5 mL), DMF/MeOH (1:1, v/v, 3×5 mL) and MeOH (3×5 mL), supports **10a–d** were dried under reduced pressure. The yields of *N*-1-alkyl-inosines **11a–d** (90–92%, from **9**) were calculated by quantization of the products obtained after HPLC purification (system A, see General) of the crude nucleoside material released from a weighed amount of resin by treatment with 2% (v/v) TFA in dry DCM for 8 min at rt followed by DMF washings (collected). ^1H NMR spectra confirmed the purity of the products.

*4.2.5. Solid-phase phosphorylation of supports **10a–d**; (supports **12a–d** and **13a–d**).* In a typical experiment, solid support **10a** (0.10 g, 0.070 mmol), swollen in dry THF, was treated with $(CEO)_2PN(iPr)_2$ (0.46 g, 1.7 mmol) and tetrazole (0.24 g, 3.4 mmol) in dry THF/DCM (9:1, v/v, 1.5 mL) and the whole was shaken for 15 h at rt. The obtained solid support was filtered and washed with THF (3×5 mL), THF/MeOH (1:1, v/v, 3×5 mL) and MeOH (3×5 mL) and finally dried in vacuo. Treatment with *tert*-butylhydroperoxide (5–6 M in decane, 0.85 mL, 4.8 mmol) in THF (1.5 mL) for 3 h at rt, followed by washings with THF (3×5 mL), THF/MeOH (1:1, v/v, 3×5 mL), and MeOH (3×5 mL), gave **12a**. Successively, support **12a** (0.10 g) was left in to contact with conc. NH_4OH for 15 h at 55 °C. After filtration and washings with H_2O (3×5 mL), H_2O/MeOH (1:1, v/v, 3×5 mL) and MeOH (3×5 mL), the resulting support **13a** was dried under reduced pressure. The solid-phase phosphorylation yields, as well as the efficiency of ammonia deprotection, were evaluated by analyzing the nucleotide material released from weighed amounts of **13a–d** by treatment with TFA/H_2O/DCM (3:2:95, v/v/v, 15 min, rt). HPLC purifications (system B, see General) furnished the *N*-1-alkyl-inosine-diphosphate derivatives **15a–d** the structures of which were confirmed by spectroscopic data (overall yields from **7** in Table 1). ^1H NMR and MS data, performed after cationic exchange and lyophilization (see General), confirmed the purity and the structure of the products.

*4.2.6. Pyrophosphate bond formation on supports **13a–d**.* In a typical experiment, support **13a** (0.050 g, 0.031 mmol), swollen in dry DMF, was treated with EDC (0.039 g, 0.20 mmol) and the mixture was shaken for 72 h at rt. After filtration and washings with DMF (3×5 mL), DMF/MeOH (1:1, v/v, 3×5 mL) and MeOH (3×5 mL), support **14a** was dried under reduced pressure. The yield of cyclic compound **18a** (22%, from **13a**, Table 1) was calculated by UV quantification of the product obtained after HPLC purification (system B, see General) of the crude nucleotide material released from a weighed amount of resin **14a** by treatment with TFA/H_2O/DCM (3:2:95, v/v/v, 15 min) at rt. ^1H NMR and MS data, performed after cationic exchange and lyophilization (see General), confirmed the purity and the structure of the product **18a**.

*4.2.7. Synthesis of compounds **16a–d**.* *N*-1-alkyl-inosine diphosphate **15a–d** (25 mg), were acetylated by reaction with a solution of Ac_2O in pyridine (2:8, v/v, 1 mL, 15 h) to give pure **16a–d** in almost quantitative yield. ^1H NMR spectra, performed after cationic exchange and lyophilization (see General) confirmed the structure of products.

*4.2.8. Pyrophosphate bond formation in solution; cyclic compounds **18a–d**.* In a typical experiment, to compound **16a** (10 mg, 0.018 mmol), dissolved in dry DMF (1.5 mL), EDC (17.2 mg, 0.090 mmol) was added and the solution was shaken for 72 at rt. The DMF was evaporated under reduced pressure and the crude mixture, dissolved in water, was purified by HPLC (system B, see General) to give **17a** (8.6 mg, 89%). The product was treated with

conc. NH_4OH for 5 h at rt and then dried under reduced pressure to give **18a** in almost quantitative yield.

The structure of **18a**, converted in sodium salt by cationic exchange chromatography (see General), was confirmed by ^1H and ^{31}P NMR spectroscopy and HRESIMS data.

4.2.9. 1-N-(2-Hydroxyethyl)-2′,3′-O-(4-hydroxymethyl-(1R)-benzyliden)inosine ***11a***. Colorless oil; IR ν_{max} (neat) 3600–2600 (broad, OH's), 1670 (strong, carbonyl) cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ_H 8.26, 8.23 (1H each, s's, H-8 and H-2), 7.40 (2H, d, J=8.0 Hz, aromatic), 7.29 (2H, d, J=8.0, aromatic), 6.23 (1H, d, J=2.9 Hz, H-1′), 6.13 (1H, s, C*H*Ph), 5.31 (1H, dd, J=6.4, 2.9 Hz, H-2′), 5.07 (1H, dd, J=6.4, 4.0 Hz, H-3′), 4.53 (2H, s, CH_2Ph), 4.31 (1H, m, H-4′), 4.11 (2H, t, J=4.8 Hz, CH_2N), 3.75–3.72 (4H, m, CH_2O, $H_{a,b}$-5′), *m*/*z* (HRESIMS) 431.1555 ($[M+H]^+$, $C_{20}H_{23}N_4O_7$, requires, 431.1567).

4.2.10. 1-N-(4-Hydroxybutyl)-2′,3′-O-(4-hydroxymethyl-(1R)-benzyliden)inosine ***11b***. Colorless oil; IR ν_{max} (neat) 3600–2600 (broad, OH's), 1672 (strong, carbonyl) cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ_H 8.26 (2H, br s, H-8 and H-2), 7.47 (2H, d, J=8.1 Hz, aromatic), 7.33 (2H, d, J=8.1 Hz, aromatic), 6.24 (1H, d, J=2.9 Hz, H-1′), 5.93 (1H, s, C*H*Ph), 5.31 (1H, dd, J=6.2, 2.8 Hz, H-2′), 5.02 (1H, dd, J=6.5, 2.3 Hz, H-3′), 4.55 (2H, s, CH_2Ph), 4.42 (1H, m, H-4′), 4.04 (2H, t, J=7.3 Hz, CH_2N), 3.67 (m, ABX system, J=10.4, 4.2 Hz, 2H, $H_{a,b}$-5′), 3.49 (2H, t, J=6.4 Hz, CH_2O), 1.75 (2H, m, CH_2), 1.48 (2H, m, CH_2); *m*/*z* (HRESIMS) 459.1842 ($[M+H]^+$, $C_{22}H_{27}N_4O_7$, requires, 459.1880).

4.2.11. 1-N-(5-Hydroxypentyl)-2′,3′-O-(4-hydroxymethyl-(1R)-benzyliden)inosine ***11c***. Amorphous solid; IR ν_{max} (neat) 3600–2600 (broad, OH's), 1670 (strong, carbonyl) cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ_H 8.35, 8.34 (s's, 1H each, H-8 and H-2), 7.57 (2H, d, J=8.1 Hz, aromatic), 7.43 (2H, d, J=8.1 Hz, aromatic), 6.33 (1H, d, J=2.93 Hz, H-1′), 6.03 (1H, s, C*H*Ph), 5.41 (1H, dd, J=6.4, 2.9 Hz, H-2′), 5.12 (1H, dd, J=6.4, 2.5 Hz, H-3′), 4.65 (2H, s, CH_2Ph), 4.51 (1H, m, H-4′), 4.11 (2H, t, J=7.2 Hz, CH_2N), 3.77 (m, ABX system, J=10.4, 4.1 Hz, 2H, $H_{a,b}$-5′), 3.55 (2H, t, J=6.3 Hz, CH_2O), 1.80 (2H, m, CH_2), 1.59 (2H, m, CH_2), 1.43 (2H, m, CH_2); *m*/*z* (HRESIMS) 473.2054 ($[M+H]^+$, $C_{23}H_{29}N_4O_7$, requires, 473.2036).

4.2.12. 1-N-(6-Hydroxyhexyl)-2′,3′-O-(4-hydroxymethyl-(1R)-benzyliden)inosine ***11d***. Amorphous solid; IR ν_{max} (neat) 3600–2600 (broad, OH's), 1670 (strong, carbonyl) cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ_H 8.26 (br s, 2H each, H-8 and H-2), 7.42 (2H, d, J=8.0 Hz, aromatic), 7.31 (2H, d, J=8.0 Hz, aromatic), 6.24 (1H, d, J=2.8 Hz, H-1′), 6.14 (1H, s, C*H*Ph), 5.33 (1H, m, H-2′), 5.08 (1H, dd, J=6.4, 4.1 Hz, H-3′), 4.55 (2H, s, CH_2Ph), 4.33 (1H, m, H-4′), 4.03 (2H, t, J=7.5 Hz, CH_2N), 3.77–3.65 (2H, m, $H_{a,b}$-5′), 3.46 (2H, t, J=6.4 Hz, CH_2O), 1.71 (2H, m, CH_2), 1.46 (2H, m, CH_2), 1.34 (4H, m, 2×CH_2); *m*/*z* (HRESIMS) 487.2234 ($[M+H]^+$, $C_{24}H_{31}N_4O_7$, requires, 487.2193).

4.2.13. 1-N-(2-O-Phosphorylethyl)-5′-O-phosphorylinosine ***15a***. Amorphous solid, sodium salt; IR ν_{max} (neat) 3600–2600 (broad, OH's), 1678 (strong, carbonyl), 1056, 915 (both strong, phosphates) cm^{-1}; ^1H NMR (400 MHz, D_2O) δ_H 8.55, 8.46, (s's, 1H each, H-8 and H-2), 6.16 (1H, d, J=4.8 Hz, H-1′), 4.78 (1H, covered by solvent signal, m, H-2′), 4.45 (1H, m, H-3′), 4.32 (3H, m, CH_2N and H-4′), 4.04 (4H, m, CH_2O and $H_{a,b}$-5′); ^{31}P-NMR (202 MHz, D_2O) δ_P 0.40, −0.11 (s's); *m*/*z* (HRESIMS) 471.0359 ($[M-H]^-$, $C_{12}H_{17}N_4O_{12}P_2$, requires 471.0318).

4.2.14. 1-N-(4-O-Phosphorylbutyl)-5′-O-phosphorylinosine ***15b***. Amorphous solid, sodium salt; IR ν_{max} (neat) 3600–2600 (broad, OH's), 1677 (strong, carbonyl), 1056, 915 (both strong, phosphates) cm^{-1}; ^1H NMR (400 MHz, D_2O) δ_H 8.44, 8.40 (1H each, s's, H-8 and H-2), 6.10 (1H, d, J=5.1 Hz, H-1′), 4.74 (1H, m, H-2′), 4.47 (1H, m, H-3′), 4.35 (1H, m, H-4′), 4.16 (2H, t, J=6.3 Hz, CH_2N), 4.08, 3.87 (2H each, m's, CH_2O and $H_{a,b}$-5′), 1.87 (2H, m, CH_2), 1.67 (2H, m, CH_2); ^{31}P-NMR (202 MHz, D_2O) δ_P 0.51, −0.20 (s's); *m*/*z* (HRESIMS) 499.0642 ($[M-H]^-$, $C_{14}H_{21}N_4O_{12}P_2$, requires 499.0631).

4.2.15. 1-N-(5-O-Phosphorylpentyl)-5′-O-phosphorylinosine ***15c***. Amorphous solid, sodium salt; IR ν_{max} (neat) 3600–2600 (broad, OH's), 1678 (strong, carbonyl), 1056, 915 (both strong, phosphates) cm^{-1}; ^1H NMR (400 MHz, D_2O) δ_H 8.59, 8.42 (1H each, s's, H-8 and H-2), 6.14 (1H, d, J=5.2 Hz, H-1′), 4.76 (1H, m, H-2′), 4.52 (1H, m, H-3′), 4.38 (1H, m, H-4′), 4.16 (2H, m, CH_2N); 4.04, 3.81 (2H each, m's, CH_2O and $H_{a,b}$-5′), 1.81 (2H, m, CH_2), 1.59 (2H, m, CH_2), 1.44 (2H, m, CH_2); ^{31}P-NMR (202 MHz, D_2O) δ_P 0.42, −0.16 (s's); *m*/*z* (HRESIMS) 513.0829 ($[M-H]^-$, $C_{15}H_{23}N_4O_{12}P_2$, requires 513.0788).

4.2.16. 1-N-(6-O-Phosphorylhexyl)-5′-O-phosphorylinosine ***15d***. Amorphous solid, sodium salt; IR ν_{max} (neat) 3600–2600 (broad, OH's), 1677 (strong, carbonyl), 1056, 915 (both strong, phosphates) cm^{-1}; ^1H NMR (400 MHz, D_2O) δ_H 8.52, 8.40 (1H each, s's, H-8 and H-2), 6.10 (1H, d, J=5.2 Hz, H-1′), 4.73 (1H, m, H-2′), 4.52 (1H, m, H-3′), 4.39 (1H, m, H-4′), 4.19 (2H, t, J=6.2 Hz, CH_2N), 4.06, 3.82 (m's, 2H each, CH_2O and $H_{a,b}$-5′), 1.82 (2H, m, CH_2), 1.62 (2H, m, CH_2), 1.41 (4H, m's, 2×CH_2); ^{31}P-NMR (202 MHz, D_2O) δ_P 0.46, −0.17 (s's); *m*/*z* (HRESIMS) 527.0932 ($[M-H]^-$, $C_{16}H_{25}N_4O_{12}P_2$, requires 527.0944).

4.2.17. 1-N-(2-O-Phosphorylethyl)-2′,3′-di-O-acetyl-5′-O-phosphorylinosine ***16a***. Amorphous solid, sodium salt; IR ν_{max} (neat) 3600–2600 (broad), 1742 (strong, carbonyl acetate), 1687 (strong, carbonyl), 1215 (acetate), 1056, 915 (both strong, phosphates) cm^{-1}; ^1H NMR (400 MHz, D_2O) δ_H 8.52, 8.41, (s's, 1H each, H-8 and H-2), 6.31 (1H, d, J=5.2 Hz, H-1′), 5.77 (1H, m, H-2′), 5.65 (1H, m, H-3′), 4.60 (1H, m, H-4′), 4.26 (2H, m, CH_2N), 4.12, 3.93 (2H each, m's, CH_2O and $H_{a,b}$-5′), 2.20 (3H, s, CH_3), 2.11 (3H, s, CH_3); ^{31}P-NMR (202 MHz, D_2O) δ_P 0.46, −0.15 (s's); *m*/*z* (HRESIMS) 555.0567 ($[M-H]^-$, $C_{16}H_{21}N_4O_{14}P_2$, requires 555.0529).

4.2.18. 1-N-(4-O-Phosphorylbutyl)-2′,3′-di-O-acetyl-5′-O-phosphorylinosine ***16b***. Amorphous solid, sodium salt; IR ν_{max} (neat) 3600–2600 (broad), 1740 (strong, carbonyl acetate), 1687 (strong, carbonyl), 1215 (acetate), 1056, 915 (both strong, phosphates) cm^{-1}; ^1H NMR (400 MHz, D_2O) δ_H 8.45, 8.41 (1H each, s's, H-8 and H-2), 6.36 (1H, d, J=5.6 Hz, H-1′), 5.79 (1H, m, H-2′), 5.65 (1H, m, H-3′), 4.61 (1H, m, H-4′), 4.27 (2H, m, CH_2N), 4.15, 3.98 (2H each, m's, CH_2O and $H_{a,b}$-5′), 2.18 (3H, s, CH_3), 2.09 (3H, s, CH_3), 1.87 (2H, m, CH_2), 1.68 (2H, m, CH_2); ^{31}P-NMR (202 MHz, D_2O) δ_P 0.48, −0.16 (s's); *m*/*z* (HRESIMS) 583.0883 ($[M-H]^-$, $C_{18}H_{25}N_4O_{14}P_2$, requires 583.0842).

4.2.19. 1-N-(5-O-Phosphorylpentyl)-2′,3′-di-O-acetyl-5′-O-phosphorylinosine ***16c***. Amorphous solid, sodium salt; IR ν_{max} (neat) 3600–2600 (broad), 1740 (strong, carbonyl acetate), 1687 (strong, carbonyl), 1215 (acetate), 1056, 915 (both strong, phosphates) cm^{-1}; ^1H NMR (400 MHz, D_2O) δ_H 8.55, 8.41 (1H each, s's, H-8 and H-2), 6.33 (1H, d, J=5.2 Hz, H-1′), 5.76 (1H, m, H-2′), 5.63 (1H, m, H-3′), 4.60 (1H, m, H-4′), 4.28 (2H, m, CH_2N), 4.16, 4.00 (2H each, m's, CH_2O and $H_{a,b}$-5′), 2.16 (3H, s, CH_3), 2.08 (3H, s, CH_3), 1.86 (2H, m, CH_2), 1.61 (2H, m, CH_2), 1.44 (2H, m, CH_2); ^{31}P-NMR (202 MHz, D_2O) δ_P 0.39, −0.18 (s's); *m*/*z* (HRESIMS) 597.1024 ($[M-H]^-$, $C_{19}H_{27}N_4O_{14}P_2$, requires 597.0999).

4.2.20. 1-N-(6-O-Phosphorylhexyl)-2′,3′-di-O-acetyl-5′-O-phosphorylinosine ***16d***. Amorphous solid, sodium salt; IR ν_{max} (neat) 3600–2600 (broad), 1742 (strong, carbonyl acetate), 1687 (strong, carbonyl), 1215 (acetate), 1056, 915 (both strong, phosphates) cm^{-1}; ^1H NMR (400 MHz, D_2O) δ_H 8.54, 8.41(1H each s's, H-8 and H-2), 6.29 (1H, d, J=5.4 Hz, H-1′), 5.80 (1H, m, H-2′), 5.63 (1H, m, H-3′), 4.59 (1H, m, H-4′), 4.26 (2H, m, CH_2N), 4.16, 3.98 (2H each, m's, CH_2O and

$H_{a,b}$-5′), 2.16 (3H, s, CH_3), 2.06 (3H, s, CH_3), 1.86 (2H, m, CH_2), 1.65 (2H, m, CH_2), 1.36 (4H, m, 2×CH_2); ^{31}P-NMR (202 MHz, D_2O) δ_P 0.45, −0.21 (s's); *m*/*z* (HRESIMS) 611.1116 ([M−H]$^-$, $C_{20}H_{29}N_4O_{14}P_2$, requires 611.1155).

4.2.21. 1-N-Ethyl-cyclic inosine-diphosphate ***18a***. Amorphous solid, sodium salt; IR ν_{max} (neat) 3600–2600 (broad, OH's), 1678 (strong, carbonyl), 1227, 1120, 1052, 935 (all strong, pyrophosphate) cm^{-1}; ^{1}H NMR (400 MHz, D_2O) δ_H 8.50, 8.40 (1H each, s's, H-8 and H-2), 6.13 (1H, d, *J*=5.1 Hz, H-1′), 5.34 (1H, m, H-2′), 4.42 (1H, m, H_a-5′), 4.78 (1H, m, H-3′), 4.30 (1H, m, H-4′), 4.27–4.13 (2H, m, CH_2N), 4.05 (1H, m, H_b-5′), 3.66 (2H, m, CH_2O); ^{31}P NMR (202 MHz, D_2O) δ_P −9.4, −10.5; *m*/*z* (HRESIMS) 453.0225 ([M−H]$^-$, $C_{12}H_{15}N_4O_{11}P_2$, requires 453.0213).

4.2.22. 1-N-Butyl-cyclic inosine–diphosphate ***18b***. Amorphous solid, sodium salt; IR ν_{max} (neat) 3600–2600 (broad, OH's), 1680 (strong, carbonyl), 1227, 1120, 1052, 935 (all strong, pyrophosphate) cm^{-1}; ^{1}H NMR (400 MHz, D_2O) δ_H 8.43, 8.19 (1H each, s's, H-8 and H-2), 6.03 (1H, d, *J*=3.4 Hz, H-1′), 5.41 (1H, m, H-2′), 4.75, (1H, m, H-3′), 4.39 (1H, m, H_a-5′), 4.32 (1H, m, H-4′), 4.24–4.10 (2H, m, CH_2N), 4.03 (1H, m, H_b-5′), 3.62 (2H, m, CH_2O), 1.80, 1.50 (2H each, m's, 2×CH_2); ^{31}P NMR (202 MHz, D_2O) δ_P −9.7, −10.4; *m*/*z* (HRESIMS) 481.0521 ([M−H]$^-$, $C_{14}H_{19}N_4O_{11}P_2$, requires 481.0526).

4.2.23. 1-N-Pentyl-cyclic inosine-diphosphate ***18c***. Amorphous solid, sodium salt; IR ν_{max} (neat) 3600–2600 (broad, OH's), 1680 (strong, carbonyl), 1227, 1120, 1052, 935 (all strong, pyrophosphate) cm^{-1}; ^{1}H NMR (400 MHz, D_2O) δ_H 8.42, 8.22 (1H each, s's, H-8 and H-2), 6.05 (1H, d, *J*=3.5 Hz, H-1′), 5.40 (1H, m, H-2′), 4.76 (1H, m, H-3′), 4.50 (1H, m, H_a-5′), 4.38 (1H, m, H-4′), 4.24 (2H, m, CH_2N), 4.03 (1H, m, H_b-5′), 3.82 (2H, m, CH_2O), 1.84, 1.58, 1.30 (2H each, m's, 3×CH_2). ^{31}P NMR (202 MHz, D_2O) δ_P −9.5, −10.5; *m*/*z* (HRESIMS) 495.0654 ([M−H]$^-$, $C_{15}H_{21}N_4O_{11}P_2$, requires 495.0682).

4.2.24. 1-N-Hexyl-cyclic inosine-diphosphate ***18d***. Amorphous solid, sodium salt; IR ν_{max} (neat) 3600–2600 (broad, OH's), 1679 (strong, carbonyl), 1227, 1120, 1052, 935 (all strong, pyrophosphate) cm^{-1}; ^{1}H NMR (400 MHz, D_2O) δ_H 8.55, 8.45 (1H each, s's H-2 and H-8), 6.16 (1H, d, *J*=5.1 Hz, H-1′), 5.43 (1H, m, H-2′), 4.74 (1H, m, H-3′), 4.53 (1H, m, H_a-5′), 4.41 (1H, m, H-4′), 4.20 (2H, m, CH_2N), 4.05 (1H, m, H_b-5′), 3.83 (2H, m, CH_2O), 1.80, 1.71, (2H each, m's, 2×CH_2), 1.41 (4H, m, 2×CH_2); ^{31}P NMR (202 MHz, D_2O) δ_P −9.6, −10.4; *m*/*z* (HRESIMS) 509.0860 ([M−H]$^-$, $C_{16}H_{23}N_4O_{11}P_2$, requires 509.0839).

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