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Synthesis of a novel *N*-1 carbocyclic, *N*-9 butyl analogue of cyclic ADP ribose (cADPR)

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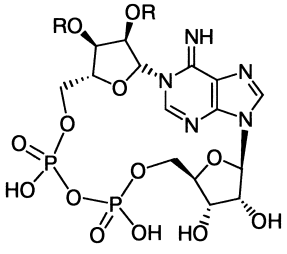
Abstract—A new analogue **1** of cADPR was prepared through a synthetic pathway starting from 6-chloropurine **2** which underwent two sequential alkylations at N-9 and N-1, with formation of the intermediate **8**. The successive bis-phosphorylation of hydroxyalkyl functions, followed by deprotection and reprotection steps, afforded the derivative **13**, the substrate for the cyclization reaction. This was carried out according to the Matsuda procedure and led to the intramolecular pyrophosphate bond formation, thus affording **14**. The final deprotection of **14**, in alkaline conditions, gave the target compound **1** in good yield.

1. Introduction

Cyclic-ADP ribose (cADPR, Fig. 1), a naturally occurring compound related to NAD^+, is a Ca^{2+} messenger, important in regulating various cellular functions in a wide range of species including humans.[1] This metabolite is capable of mobilizing calcium ions more actively than inositol 1,4,5-triphosphate (IP_3),[2] through a completely independent mechanism of action from IP_3.[3,4] Several enzymes involved in the metabolism of cADPR have been described, among which the ubiquitous ADP-ribosyl cyclase, first discovered in sea urchin eggs and particularly abundant in *Aplysia california*. The *Aplysia* cyclase, whose sequence is homologous to antigens CD38 on human lymphocytes and BST1/BP3 on bone marrow cells, shares with all members of the cyclase family a significant catalytic activity in the cyclization of NAD^+ and $NADP^+$ to cADPR.[5–9] This property has been exploited to enzymatically produce more stable cADPR analogues as tools to investigate the biological role of cADPR. In fact, such studies are severely prevented by the high lability of this calcium-releasing metabolite (the *N*-1 ribosyl bond is rapidly hydrolyzed in neutral aqueous solution, even in the absence of enzymes) coupled to its extremely low concentration in cells. On the other hand, the analogues, which can be obtained by enzymatic cyclization of modified substrates are limited due to the enzyme specificity. Thus, chemical methods are needed to prepare new and more stable cADPR analogues.

Several laboratories, in order to obtain molecules having more stable linkages, have prepared *N*-1 or *N*-9 carbocyclic analogues of cADPR. To this end, Matsuda and co-workers have reported the first total synthesis of a stable analogue of cADPR having a carbocyclic ribose mimic unit linked at the N-1 position of the inosine base[10] (cIDPR). It is well established that structural modifications of naturally occurring nucleosides may produce new derivatives capable of exerting interesting and useful biological properties.[11,12] This



cADPR

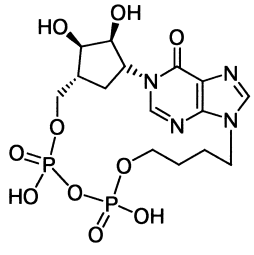


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Figure 1.

Keywords: cADPR; pyrophosphate bond; cyclization.

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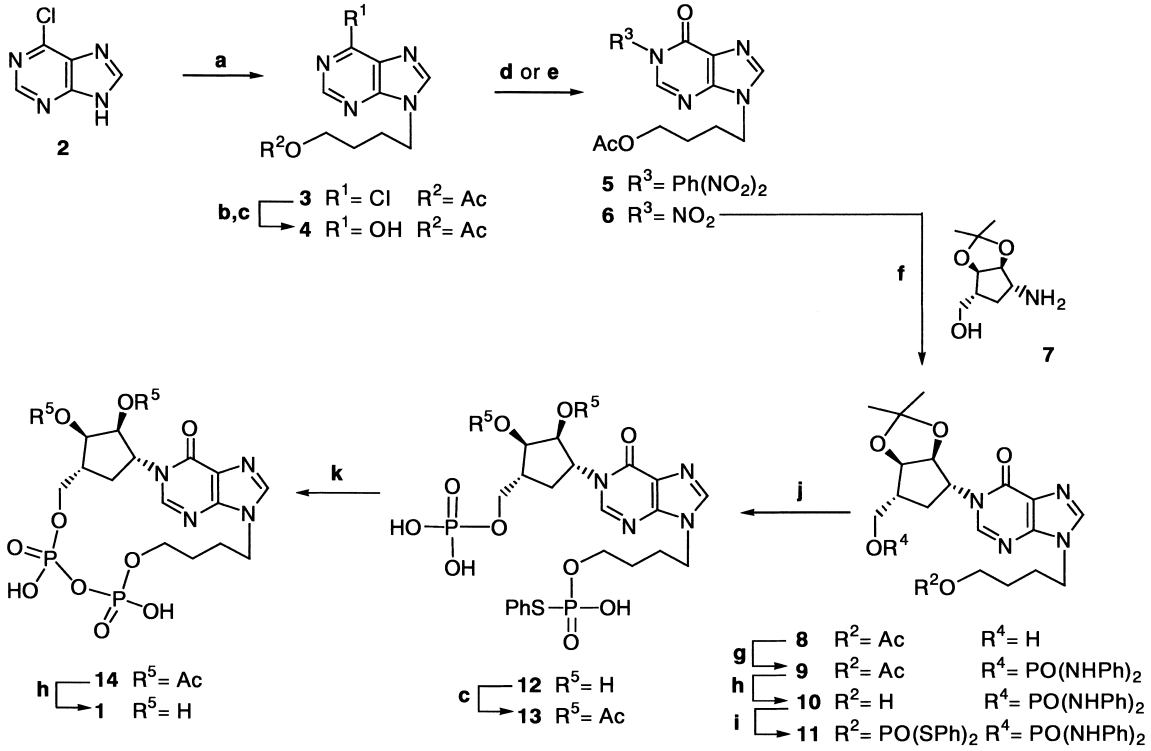
prompted us to undertake a synthetic study aimed at obtaining further stable analogues of cADPR. In this paper we describe the chemical synthesis of the cIDPR analogue **1**, characterized by the presence of carbaribosyl and butyl moieties at N-1 and N-9 of hypoxanthine, respectively. This compound is, to the best of our knowledge, the first example of a new class of molecules characterized by a linear chain at N-9. In fact, despite the biological activity of acyclonucleotides,[12–16] only one attempt to obtain such a derivative with a methoxyethyl chain at N-9 appeared in the literature, which failed at the level of enzymatic cyclization of the pertinent NAD^+ analogue precursor.[11] As for similar compounds, **1** is expected to be resistant to both enzymatic and chemical hydrolysis since the adenine base is replaced by its congener hypoxanthine and the N-1 ribosyl unit by an alkyl moiety. Biological tests performed on this analogue might be valuable in supplying useful information about the role of the *N*-9 ribosyl moiety of cADPR in the mechanism of Ca^{2+} modulation.

2. Results and discussion

The synthetic strategy adopted for **1** is shown in Scheme 1. A key reaction is the cyclization of products **13** with the formation of an intramolecular pyrophosphate linkage. This step has been reported as the most critical one in a number of works having cIDPR as targets. We adopted the procedure recently described by Matsuda,[10] exploiting the condensation of a phenylthiophosphate and a phosphomonoester functions in the presence of I_2 as a promoter.

Starting from 6-chloropurine **2**, alkylation at the N-9 position was first attempted by Mitsunobu procedure,[17] using 4-benzyloxy-1-butanol, in the presence of triphenylphosphine/diethylazodicarboxylate (PPh_3/DEAD) as an activator, in several reaction conditions. In any case, only poor yields of the desired N-9 alkylated product were observed. However, high yields of product **3** (85%) could be achieved by reacting **2** with 4-acetoxybutylchloride in DMF in the presence of 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU). Hydrolysis of **3** with aqueous 1 M HCl at 90°C, followed by reacetylation of the primary hydroxyl function with Ac_2O, gave the hypoxanthine derivative **4** in 70% yield. To attach the carbaribosyl structure at N-1, we tried the same procedure used to prepare *N*-1 alkylinosine from *N*-1-(2,4-dinitrophenyl)inosine and an alkylamine.[18] Consequently, we first synthesized the *N*-1-(2,4-dinitrophenyl) derivative **5** by reaction of **4** with 2,4-dinitrochlorobenzene and K_2CO_3 in DMF. However, successive treatment of **5** with the carbocyclic amine **7**[19] did not afford the expected N-1 alkylated product **8**. In fact, even performing the reaction at high temperature (up to 85°C) for a long time, only the *N*-(2,4-dinitrophenyl)amine derivative was observed.

This result could be explained as follows. In the case of *N*-9 ribosyl analogues of **5**, the formation of a N-alkylated derivative proceeds through a rearrangement of the pyrimidine ring, induced by a nucleophilic attack of an amine at C-2, whose electrophilicity must be enhanced by the presence of an electron-withdrawing group at N-1.[18] Particularly, the amine **7** was shown to readily react with 2,4-dinitrophenylinosine, affording the corresponding N-1 substituted derivative, thus indicating that steric effects should not hamper the above reaction.[10] Therefore, we



Scheme 1. *Reagents*: (a) DBU, $AcOCH_2(CH_2)_2CH_2Cl$, DMF, 80°C, 85%; (b) HCl, 1N, 90°C, 70%; (c) Ac_2O, Py, rt, quant.; (d) $ClPh(NO_2)_2$, DMF, 80°C, 90%; (e) NH_4NO_3,TFAA, CH_2Cl_2, 0°C, 90%; (f) DMF, 80°C, 75%; (g) $ClPO(NHPh)_2$, Py, 80°C, 90%; (h) K_2CO_3,CH_3OH, rt, 97%; (i) $C_6H_{11}NH_3OPO(SPh)_2$,Py, rt 80%; (j) (i) isoamylnitrite, Ac_2O, AcOH, Py, rt; (ii) H_3PO_2, Et_3N, Py, rt, total yield 80%; (k) I_2, MS 3 Å, Py, rt, 80%.

concluded that the failure of the reaction of **5** with **7** was to ascribe to the reduced electrophilicity at C-2 of the latter compound, when compared to that of *N*-1-(2,4-dinitrophenyl)inosine, due to the electron–donor effect of the alkyl group at N-9. To confirm this hypothesis, we decided to introduce a more effective electron-withdrawing group at N-1, such as the nitro group. As expected, the desired intermediate **8** could be obtained in 75% yield by reaction of **7** with the *N*-1-nitrohypoxanthine derivative **6**, prepared according to the procedure proposed by Vilarrasa and co-workers,[20] by treatment of **4** with ammonium nitrate and trifluoroacetic anhydride (TFAA) in dichloromethane. Successively, the di(anilino)phosphoryl group was introduced by reacting **8** with $(PhNH)_2POCl$[21] to give **9**. After removal of the acetyl protecting group of **9** with K_2CO_3 in MeOH, the bis(phenylthio)phosphoryl group was introduced on the primary hydroxyl of the *N*-9 alkyl chain by treatment of the resulting compound **10** with *S*,*S*-diphenylphosphorodithioate (cyclohexylammonium salt) in the presence of 2,4,6-triisopropylbenzenesulphonylchloride (TPSCl) and tetrazole in pyridine,[22] to give the protected bisphosphate derivative **11**. Sequential treatment of **11** with isoamyl nitrite in pyridine/AcOH/Ac_2O (2:1:1) and H_3PO_2[23] resulted in the concomitant removal of protecting groups of both phosphates and carbocyclic hydroxyls, thus affording **12** (triethylammonium salts, 80% overall yield). Acetylation of **12** with Ac_2O in pyridine gave, in almost quantitative yield, the substrate for the intramolecular condensation **13** as a pyridinium salt whose proton decoupled ^{31}P NMR spectrum showed two singlets at 20.84 and 4.49 ppm. The cyclization reaction was performed by slowly adding **13**, dissolved in pyridine, to a solution of I_2 in the same solvent, in the presence of activated molecular sieves. The ^{31}P NMR spectrum of the crude product did not display the aforementioned signals of the phosphate groups of compound **13**, thus indicating its complete consumption. On the other hand, resonances typical of pyrophosphate species were present. Successive HPLC fractionation resulted in the pure di-acetylated derivative **14** as the major product and a mixture of the two mono-acetylated products, resistant to any further purification. Final deacetylation of both di- and mono-acetylated intermediates was carried out by treatment with K_2CO_3 in MeOH to give after purification on HPLC, (Fig. 2) the target compound **1** in 90% yield (from **13**). The

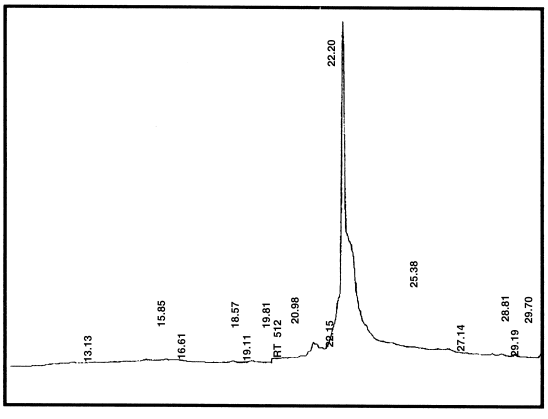


Figure 2. HPLC profile of **1**. Column Nucleosil 300-7 C-18 (10×250 mm^2, 7 μm) eluted with linear gradient of CH_3CN in TEAA 0.1 M, pH=7.00 from 0 to 38% in 30 min, flow 2 mL min^{-1}.

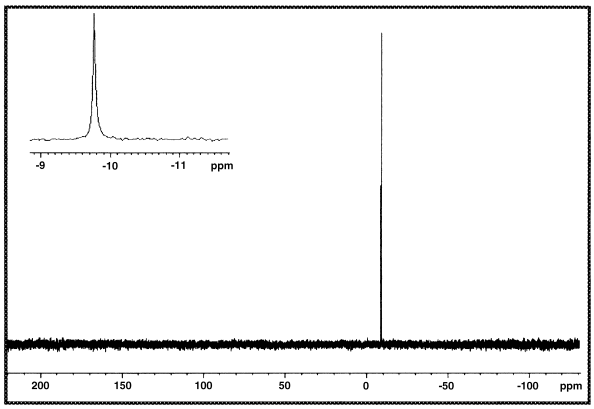


Figure 3. ^{31}P NMR spectrum (D_2O, 162 MHz ^1H decoupled) of **1**.

structure of **1** was confirmed by spectroscopic data (^{31}P NMR spectrum in Fig. 3) and HRMS (FAB).

3. Conclusions

In this paper we have described the efficient synthesis of a **1**, a new deribosylated analogue of cADPR, carrying a carbaribosyl and a butyl moieties at N-1 and N-9, respectively. In particular, a carbocyclic ribose unit was successfully introduced at N-1 by reacting amine **7** with the *N*-1 nitrohypoxanthine derivative **6**. The latter compound might prove to be a valuable intermediate for N-1 alkylation of *N*-9 alkyl hypoxanthines, thanks to the enhanced reactivity at C-2, on comparison with that of the corresponding *N*-1-(2,4-dinitrophenyl) derivative **5**.After bis-phosphorylation of **8**, which led to compound **11**, the successive phosphate deprotection caused the removal of isopropylidene protecting group, also. This allowed us to reprotect carbocyclic hydroxyls by acetylation and, after the cyclization step, regenerate the two alcoholic functions in more favorable conditions than those required for removal of isopropylidene, which generally causes a drop of yields of the target compound.[10]

4. Experimental

4.1. General methods

^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker AMX 500 spectrometer. Residual proton and carbon signals of the solvent ($CDCl_3$ δ=7.26 and 77.5, respectively) were used as internal references. ^{31}P NMR were recorded on a Bruker WM 400 spectrometer (85% H_3PO_4 as a external standard). NMR signals were assigned to the pertinent nuclei through two-dimensional ^1H–^1H COSY experiments. Fast ion bombardment mass spectra (FABMS) were recorded in a glycerin matrix on a VG PROSPEC instrument. General reagents and solvents were purchased from Sigma–Aldrich–Fluka.

4.2. Synthesis

4.2.1. 6-Chloro-9-(4-acetoxybutyl)purine (3). A mixture of **2** (1.0 g, 6.50 mmol) and 4-chlorobutyl acetate (1.95 g, 13.00 mmol) in DMF (15.0 mL), in the presence of DBU

(1.18 g, 7.86 mmol), was stirred at 80°C for 6 h, under N_2 atmosphere. The mixture was dried in vacuo and the product purified on a silica gel column eluted with increasing amount of CH_3OH in $CHCl_3$. The fractions eluted with 5% of CH_3OH furnished 1.48 g of **3** (85% yield) as yellow oil. IR ν_{max} ($CHCl_3$) 3004, 2961, 1734, 1594, 1562, 1245 cm^{-1}; ^1H NMR δ_H (500 MHz, DMSO-d_6) δ 8.73 (1H, s, H-2 or H-8), 8.12 (1H, s, H-8 or H-2), 4.33 (2H, t, J=7.2 Hz, CH_2N), 4.09 (2H, t, J=6.3 Hz, CH_2O), 2.05 (3H, s, CH_3), 2.00 and 1.67 (2H each, m's, alkyl chain methylene protons). ^{13}C NMR δ_c (125 MHz, DMSO-d_6) 170.3, 155.2, 152.5, 148.8, 147.2, 130.3, 62.2, 47.0, 29.5, 27.5, 21.2. HRMS (FAB^+): MH^+, found 269.0814. $C_{11}H_{14}ClN_4O_2$ requires 269.0805.

4.2.2. 9-(4-Acetoxybutyl)hypoxanthine (4). Compound **3** (1.40 g, 5.22 mmol) was treated with 15.0 mL of aqueous 1 M HCl at 90°C. After 6 h, the mixture was dried under reduced pressure and treated with 10.0 mL of Ac_2O/pyridine solution (2:3 v/v). After 6 h at room temperature, the dried mixture was purified by a silica gel column eluted with increasing amounts of CH_3OH in $CHCl_3$. The fractions eluted with 20% of CH_3OH furnished 913 mg (70% yield) of **4** as white amorphous solid. IR nmax ($CHCl_3$) 3022, 2959, 1733, 1687, 1368, 1245 cm^{-1}; ^1H NMR δ_H (500 MHz, $CDCl_3$) 8.15 (1H, s, H-2 or H-8), 7.80 (1H, s, H-8 or H-2), 4.25 (2H, t, J=7.2 Hz, CH_2N), 4.09 (2H, t, J=6.3 Hz, CH_2O), 2.03 (3H, s, CH_3), 1.90 and 1.65 (2H each, m's, alkyl chain methylene protons); ^{13}C NMR δ_C (125 MHz, $CDCl_3$) 170.2, 151.4, 150.8, 145.1, 141.4, 131.3, 62.6, 44.3, 26.5, 25.1, 21.2; HRMS (FAB^+): MH^+, found 251.1155. $C_{11}H_{15}N_4O_3$ requires 251.1144.

4.2.3. 1-(2,4-Dinitrophenyl)-9-(4-acetoxybutyl)hypoxanthine (5). A mixture of **4** (100 mg, 0.40 mmol), K_2CO_3 (110 mg, 0.80 mmol), and 2,4-dinitrochlorobenzene (161 mg, 0.80 mmol) was stirred in DMF (10.0 mL) at 80°C for 2.5 h. The mixture was dried and purified on a silica gel column and eluted with increasing amount of CH_3OH in $CHCl_3$.The fractions eluted with 3% of CH_3OH furnished 150 mg of **5** (90% yield) as yellow foam. IR ν_{max} ($CHCl_3$) 2961, 2054, 1708, 1541, 1350 cm^{-1}; ^1H NMR δ_H (500 MHz, $CDCl_3$) 9.01 (1H, d, J=2.5 Hz, $(NO_2)_2Ph$), 8.65 (1H, dd, J=2.6, 8.6, $(NO_2)_2Ph$), 8.01 (1H, s, H-2 or H-8), 7.83 (1H, s, H-8 or H-2), 7.71 (1H, d, J=8.6 Hz, $(NO_2)_2Ph$), 4.23 (2H, m, CH_2N), 4.12 (2H, m, CH_2O), 2.05 (3H, s, CH_3), 1.99 and 1.71 (2H each, m's, alkyl chain methylene protons); ^{13}C NMR δ_C (125 MHz, $CDCl_3$) 171.6, 165.0, 148.0, 147.8, 147.5, 144.5, 140.8, 135.8, 132.0, 128.3, 124.5, 121.5, 63.0, 43.5, 27.3, 26.2, 21.3; HRMS (FAB^+): MH^+, found 417.1143. $C_{17}H_{17}N_6O_7$ requires 417.1159.

4.2.4. 1-Nitro-9-(4-acetoxybutyl)hypoxanthine (6). TFAA (6.05 g, 28.80 mmol) was added to a suspension of finely powdered NH_4NO_3 (921 mg, 11.52 mmol) in anhydrous CH_2Cl_2 (30.0 mL) at 0°C. The mixture was vigorously stirred at room temperature until the solid was dissolved (45 min) and cooled to 0°C. Compound **4** (900 mg, 3.60 mmol) in CH_2Cl_2 (15.5 mL) was added, and the resulting solution stirred for 1 h. The reaction was quenched adding 5 mL of CH_2Cl_2 and phosphate buffer (pH=7.0, 2×25.0 mL). The organic layer was dried under reduced pressure and the product purified on a silica gel column, eluted with increasing amount of CH_3OH in $CHCl_3$. The fractions eluted with 20% of CH_3OH furnished **6**, 956 mg (90% yield) as yellow oil. IR ν_{max} ($CHCl_3$) 3029, 2958, 1747, 1628, 1577, 1367, 1233 cm^{-1}; ^1H NMR δ_H (500 MHz, $CDCl_3$) 8.73 (1H, s, H-2 or H-8), 7.83 (1H, s, H-8 or H-2), 4.09 (2H, t, J=7.2 Hz, CH_2N), 4.35 (2H, t, J=6.3 Hz, CH_2O), 2.03 (3H, s, CH_3), 1.92 and 1.78 (2H each, m's, alkyl chain methylene protons); ^{13}C NMR δ_C (125 MHz, $CDCl_3$) 171.2, 150.2, 146.3, 141.4, 140.7, 124.6, 63.4, 44.3, 27.3, 25.9, 21.2; HRMS (FAB^+): MH^+, found 296.1002. $C_{11}H_{14}N_5O_5$ requires 296.0995.

4.2.5. 1-[(1*R*,2*S*,3*R*,4*R*)-2,3-(Isopropylidenedioxy)-4-hydroxymethyl-cyclopentyl]-9-(4-acetoxybutyl)hypoxanthine (8). A mixture of **6** (800 mg, 2.71 mmol) and amine **7** (608 mg, 3.25 mmol) in DMF (8.0 mL) was stirred at 80°C for 4 h. The resulting mixture was evaporated under reduced pressure and the residue purified on a silica gel column, eluted with increasing amount of CH_3OH in $CHCl_3$.The fractions eluted with 5% of CH_3OH furnished 854 mg (75% yield) of **8** as yellow oil. IR ν_{max} ($CHCl_3$) 3025, 2948, 1733, 1699, 1653, 1542, 1375 cm^{-1}; ^1H NMR δ_H (500 MHz, $CDCl_3$) 8.03 (1H, s, H-2 or H-8), 7.78 (1H, s, H-8 or H-2), 5.19 (1H, m, H-2′), 4.75 (1H, m, H-3′), 4.63 (1H, m, H-1′), 4.19 (2H, t, J=7.2 Hz, CH_2N), 4.09 (2H, t, J=6.3 Hz, CH_2O), 3.80 (2H, br. d, H-6′a, H-6′b), 2.40–2.12 (3H, m, H-4′, H-5′a, H-5′b), 2.05 (3H, s, CH_3), 1.89 and 1.60 (2H each, m's, alkyl chain methylene protons), 1.50 and 1.21 (3H each, s's, isopropylidene methyl protons); ^{13}C NMR δ_C (125 MHz, $CDCl_3$) 171.6, 156.9, 147.8, 146.9, 140.6, 124.8, 113.3, 83.7, 82.6, 66.1, 64.0, 62.0, 47.0, 44.2, 33.0, 28.6, 28.0, 27.3, 25.6, 21.3; HRMS (FAB^+): MH^+, found 421.2070. $C_{20}H_{29}N_4O_6$ requires 421.2087.

4.2.6. 1-[(1*R*,2*S*,3*R*,4*R*)-2,3-(Isopropylidenedioxy)-4-[[(dianilinophosphoryl)oxy]methyl]cyclopentyl]-9-(4-acetoxybutyl)hypoxanthine (9). To a solution of **8** (900 mg, 2.14 mmol) in dry pyridine (10.0 mL), $(PhNH)_2POCl$ (2.28 g, 8.57 mmol) was added and the mixture stirred at room temperature overnight. The mixture was dried under reduced pressure and chromatographed on a silica gel column eluted with increasing amount of CH_3OH in $CHCl_3$. The fractions eluted with 15% of CH_3OH in $CHCL_3$ furnished 1.25 g of **9** (90% yield) as yellow oil. IR ν_{max} ($CHCl_3$) 3017, 1732, 1698, 1604, 1500, 1385 cm^{-1}; ^1H NMR δ_H (500 MHz, $CDCl_3$) 7.90 (1H, s, H-2 or H-8), 7.75 (1H, s, H-8 or H-2), 7.20–6.88 (10H, m, PhNH), 6.38, 6.15 (1H each, each d, J=10.2 Hz, PhNH), 5.10 (1H, m, H-2′), 4.81 (1H, m, H-3′), 4.50 (1H, m, H-1′), 4.33, 4.28 (1H each, each m, H-6′a, H-6′b) 4.18 (2H, t, J=7.2 Hz, CH_2N), 4.08 (2H, t, J=6.3 Hz, CH_2O), 2.51–2.49 (2H, m, H-5′a, H-4′), 2.27 (1H, m, H-5′b), 2.01 (3H, s, CH_3), 1.90 and 1.60 (2H each, m's, alkyl chain methylene protons), 1.48 and 1.21 (3H each, s's, isopropylidene methyl protons); ^{13}C NMR δ_C (125 MHz, $CDCl_3$) 171.2, 156.5, 147.5, 146.9, 140.0, 135.8, 130.1, 126.2, 122.5, 117.5, 115.0, 84.9, 82.0, 66.9, 66.2, 65.0, 47.3, 44.3, 31.8, 29.6, 27.5, 27.0, 22.6, 21.3; HRMS (FAB^+): MH^+, found 651.2716. $C_{32}H_{40}N_6O_7P$ requires 651.2696.

4.2.7. 1-[(1*R*,2*S*,3*R*,4*R*)-2,3-(Isopropylidenedioxy)-4-[[(dianilinophosphoryl)oxy]methyl]cyclopentyl]-9-(4-hydroxybutyl)hypoxanthine (10). To a solution of **9** (1.0 g,

1.54 mmol) in CH_3OH (15.0 mL), finely powdered K_2CO_3 (844 mg, 6.04 mmol) was added and the mixture stirred at room temperature for 20 min. The reaction was quenched by addition of aqueous 1 M $KHSO_4$ (7.0 mL) and dried under reduced pressure. The residue was purified on a silica gel column eluted with increasing amount of CH_3OH in $CHCl_3$.The fractions eluted with 30% of CH_3OH furnished 907 mg of **10** (97% yield) as yellow oil. IR ν_{max} ($CHCl_3$) 3019, 1682, 1607, 1498, 1394 cm^{-1}; ^1H NMR δ_H (500 MHz, CD_3OD) 8.32 (1H, s, H-2 or H-8), 8.21 (1H, s, H-8 or H-2), 7.20–6.88 (10H, m, PhNH), 5.09 (1H, m, H-2′), 4.93 (1H, m, H-3′), 4.71 (1H, m, H-1′), 4.33 (2H, t, J=7.2 Hz, CH_2N), 4.25 (2H, m, H-6′a, H-6′b), 3.60 (2H, t, J=6.1 Hz, CH_2O), 2.56 (1H, m, H-4′), 2.31 (2H, m, H-5′a, H-5′b), 2.07 and 1.55 (2H each, m's, alkyl chain methylene protons), 1.52 and 1.30 (3H each, s's, isopropylidene methyl protons); ^{13}C NMR δ_C (125 MHz, CD^3OD) 157.5, 147.5, 147.0, 140.3, 136.1, 130.0, 126.4, 122.5, 117.6, 115.2, 85.0, 82.3, 67.0, 65.1, 62.5, 47.5, 44.3, 31.8, 29.8, 27.6, 27.1, 23.5; HRMS (FAB^+): MH^+, found 609.2573. $C_{30}H_{38}N_6O_6P$ requires 609.2590.

4.2.8. 1-[(1*R*,2*S*,3*R*,4*R*)-2,3-(Isopropylidenedioxy)-4-[[(dianilinophosphoryl)oxy]methyl]cyclopentyl]-9-[4-*O*-[bis(phenylthio)phosphoryl]butyl]hypoxanthine (11). To a solution of **10** (900 mg, 1.48 mmol) in dry pyridine (9.0 mL) *S*,*S*-diphenylphosphorodithioate (674 mg, 1.77 mmol) (cyclohexylammonium salt) and TPSCl (1.21 g, 4.00 mmol) were added and the reaction stirred for 8 h at room temperature under N_2 atmosphere. The mixture was dried under reduced pressure and the residue purified on a silica gel column eluted with increasing amount of CH_3OH in $CHCl_3$. The fractions eluted with 5% of CH_3OH furnished 1.03 g of **11** (80% yield) as yellow oil. IR ν_{max} ($CHCl_3$) 2989, 1685, 1602, 1498, 1217 cm^{-1}; 1H δ_H (500 MHz, $CDCl_3$) 7.90 (1H, s, H-2 or H-8), 7.78 (1H, s, H-8 or H-2), 7.51–7.30 (10H, m, PhS), 7.13–6.80 (10H, m, PhNH), 6.55, 6.40 (1H each, each d, J=10.2 Hz, NH), 5.09 (1H, m, H-2′), 4.81 (1H, m, H-3′), 4.52 (1H, m, H-1′), 4.28 (2H, m, H-6′a, H-6′b), 4.23 (2H, m, CH_2O), 4.06 (2H, t, J=7.2 Hz, CH_2N), 2.56 (2H, m, H-5′a, H-4′), 2.50 (1H, m, H-5′b), 1.85 and 1.65 (2H each, m's, alkyl chain methylene protons), 1.49 and 1.22 (3H each, s's, isopropylidene methyl protons); ^{13}C NMR δ_C (125 MHz, $CDCl_3$) 156.9, 147.9, 147.0, 140.5, 140.0, 135.5, 129.9, 129.7, 129.4, 126.5, 126.4, 121.8, 118.3, 113.4, 83.6, 82.2, 67.2, 66.5, 66.2, 45.8, 43.6, 32.6, 28.0, 27.3, 26.6, 25.5; HRMS (FAB^+): MH^+, found 873.2436. $C_{42}H_{47}N_6O_7P_2S_2$ requires 873.2423.

4.2.9. 1-[(1*R*,2*S*,3*R*,4*R*)-2,3-(Dihydroxy)-4-[[(dianilinophosphoryl)oxy]methyl]cyclopentyl]-9-[4-*O*-[bis(phenylthio)phosphoryl]butyl]hypoxanthine (12). A mixture of **11** (262 mg, 0.30 mmol) and isoamyl nitrite (606 μL, 4.50 mmol) in pyridine/AcOH/Ac_2O (2:1:1, v/v 9.0 mL) was stirred at room temperature for 8 h. The reaction mixture was evaporated (at <50°C) and, successively, dissolved in a mixture of H_3PO_2 (306 μL, 6.00 mmol), Et_3N (417 μL, 3.00 mmol) and pyridine (7.5 mL) and the resulting solution was stirred at room temperature for 11 h. After the mixture was evaporated (at <50° C) and the residue dissolved in TEAA buffer (0.1 M, pH 7.0). The solution was purified by a C_{18} reversed phase HPLC column using a linear gradient from 0 to 30% in 45 min of CH_3CN in TEAA buffer (0.1 M, pH 7.0). Appropriate fractions were evaporated and excess of TEAA removed by C_{18} reversed phase HPLC column using a linear gradient from 0 to 30% in 35 min of CH_3CN aqueous to give 166 mg of **12** (80% yield) as a triethylammonium salt. IR ν_{max} (liquid film) 3020, 1670, 1598, 1506, 1202 cm^{-1}; ^1H NMR δ_H (500 MHz, D_2O) 8.31 (1H, s, H-2 or H-8), 7.98 (1H, s, H-8 or H-2), 7.33–6.95 (5H, m, PhS), 4.99 (1H, m, H-1′), 4.45 (1H, m, H-2′), 4.10 (1H, m, H-3′), 4.08 (2H, t, J=7.2 Hz, CH_2N), 3.91–3.81 (4H, m, H-6′a, H-6′b, CH_2O), 2.30 (2H, m, H-4′, H-5′a), 1.70 (1H, m, H-5′b), 1.62 and 1.48 (2H each, m's, alkyl chain methylene protons); ^{13}C NMR δ_C (125 MHz, CD_3OD) 158.0, 148.2, 147.5, 142.5, 134.2, 133.0, 130.3, 127.5, 125.0, 76.2, 74.8, 67.5, 66.4, 62.0, 45.6, 44.7, 28.8, 28.6, 28.0; HRMS (FAB^-): $[M-H]^-$, found 589.0948. $C_{21}H_{27}N_4O_{10}P_2S$ requires 589,0923.

4.2.10. 1-[(1*R*,2*S*,3*R*,4*R*)-2,3-(Diacetoxy)-4-[[(dianilinophosphoryl)oxy]methyl]cyclopentyl]-9-[4-*O*-[bis(phenylthio)phosphoryl]butyl]hypoxanthine (13). Compound **12** (28 mg, 0.40 mmol) was treated with 1.0 mL of Ac_2O/pyridine solution 2:3 (v/v). After 6 h at room temperature, the dried mixture was lyophilized thus giving 30 mg of **13** (95% yield) as a triethylammonium salt. IR ν_{max} (liquid film) 3034, 1710, 1620, 1518, 1240, 1105 cm^{-1}; ^1H NMR δ_H (500 MHz, CD_3OD), 8.40 (1H, s, H-2), 8.08 (1H, s, H-8), 7.60–7.13 (5H, m, PhS), 5.71 (1H, br. t, H-2′), 5.45 (1H, br. t, H-3′), 5.18 (1H, m, H-1′), 4.22–4.12 (4H, m, CH_2N, H-6′a, H-6′b), 4.02 (2H, m, CH_2O), 2.60, (1H, m, H-4′), 2.40 (1H, m, H-5′a), 2.18 (1H, m, H-5′b), 2.15, 2.01 (3H each, each s, CH_3), 1.88 and 1.65 (2H each, m's, alkyl chain methylene protons); ^{13}C NMR δ_C (125 MHz, CD_3OD) 172.4, 172.5, 158.0, 148.9, 147.9, 142.5, 133.5, 133.1, 129.8, 127.7, 124.3, 76.0, 74.3, 67.5, 66.1, 62.3, 45.0, 43.1, 29.5, 28.2, 28.0, 20.2, 20.1; ^{31}P NMR δ_P (162 MHz, D_2O, decoupled with 1H) 20.84, 4.49; HRMS (FAB^-): $[M-H]^-$, found 673.1112. $C_{25}H_{31}N_4O_{12}P_2S$ requires 673.1134.

4.2.11. Compound 14. A solution of **13** (22 mg, 28.4 μmol) in pyridine (24.0 mL) was slowly added over 15 h, using a syringe pump, to a mixture of I_2 (150 mg, 591 mmol) and dried 3 Å molecular sieves (1.5 g) in pyridine (24.0 mL) at room temperature in the dark. After filtering and washing with H_2O, the combined filtrate and washings were evaporated to dryness. The residue was dissolved in TEAA buffer (1 M, pH 7.0, 3.0 mL), and purified by a C_{18} reversed phase HPLC column using a linear gradient from 0 to 30% in 45 min of CH_3CN in TEAA buffer (0.1 M, pH 7.0). Appropriate fractions were evaporated and excess of TEAA removed by C_{18} reversed phase HPLC column using a linear gradient from 0 to 30% in 35 min of CH_3CN aqueous to give **14** (triethylammonium salt), 15 mg (80% yield). IR ν_{max} (liquid film) 3015, 1734, 1698, 1508, 1245, 1100, 1085 cm^{-1}; ^1H NMR δ_H (500 MHz, D_2O), 8.53 (1H, s, H-2 or H-8), 8.24 (1H, s, H-8 or H-2), 5.67 (1H, m, H-2′), 5.45 (1H, m, H-3′), 5.23 (1H, m, H-1′), 4.37 (2H, t, J=7.2 Hz, CH_2N), 4.30 (2H, m, H-6′a, H-6′b) 4.12 (2H, t, CH_2O, J=6.3 Hz), 2.62 (1H, m, H-5′a), 2.45 (1H, m, H-4′), 2.19 (1H, m, H-5′b), 2.07 and 1.65 (2H each, m's, alkyl chain methylene protons), 2.10 and 1.98 (3H each, s's,

acetyl methyl protons); ^{13}C NMR δ_C (125 MHz, D_2O), 173.3, 173.2, 158.1, 147.2, 147.1, 142.0, 122.9, 75.1, 72.8, 65.5, 64.9, 61.8, 45.5, 41.5, 36.1, 26.0, 26.9, 20.1, 19.8; ^{31}P NMR δ_P (162 MHz, D_2O, decoupled with 1H) −11.06; HRMS (FAB$^-$): $[M-H]^-$, found 563.0923. $C_{19}H_{25}N_4O_{12}P_2$ requires 563.0944.

4.2.12. Compound 1. To a solution of **14** (10 mg, 0.015 mmol) in CH_3OH (1.0 mL) K_2CO_3 (9 mg, 0.060 mmol) was added and the mixture stirred at room temperature for 20 min. The reaction was quenched by addition of 70.0 μL of 1 M aqueous solution $KHSO_4$. After drying under reduced pressure, the residue was dissolved in TEAA buffer (1 M, pH 7.0, 1.0 mL) and purified by a C_{18} reversed phase HPLC column using a linear gradient from 0 to 38% in 30 min of CH_3CN in TEAA buffer (0.1 M, pH 7.0), (flow 2 mL min^{-1}, retention time: 22.20 min). Appropriate fractions were evaporated and excess of TEAA removed by C_{18} reversed phase HPLC column using a linear gradient from 0 to 30% in 35 min of CH_3CN aqueous to give **1** as triethylammonium salt, 8 mg (90% yield). IR ν_{max} (liquid film) 3020, 1670, 1509, 1265, 1180, 1085 cm^{-1}; ^1H NMR δ_H (500 MHz, D_2O) 8.35 (1H, s, H-2 or H-8), 8.06 (1H, s, H-8 or H-2), 5.02 (1H, m, H-1′), 4.51 (1H, m, H-2′), 4.18 (2H, t, J=7.2 Hz, CH_2N), 4.13 (1H, m, H-3′), 3.94 (2H, m, H-6′a, H-6′b), 3.74 (2H, m, CH_2O), 2.42 (1H, m, H-5′a), 2.26 (1H, m, H-4′), 1.77 and 1.61 (2H each, m's, alkyl chain methylene protons), 1.72 (1H, m, H-5′b); ^{13}C NMR δ_C (125 MHz, D_2O), 159.6, 146.8, 143.6, 142.5, 124.6, 74.2, 72.0, 66.0, 63.0, 62.0, 44.7, 43.8, 28.2, 26.5, 26.0. ^{31}P NMR δ_P (162 MHz, D_2O, decoupled with 1H) −9.80; HRMS (FAB$^-$): $[M-H]^-$, found 479.0737. $C_{15}H_{21}N_4O_{10}P_2$ requires 479.0733.

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