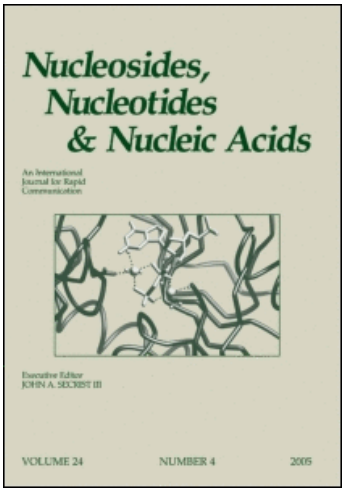

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Glycosylations of Inosine and Uridine Nucleoside Bases and Synthesis of the New 1-(β-D-Glucopyranosyl)-Inosine-5', 6''-diphosphate

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NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 19(8), 1289–1299 (2000)

GLYCOSYLATIONS OF INOSINE AND URIDINE NUCLEOSIDE BASES AND SYNTHESIS OF THE NEW 1-(β-D-GLUCOPYRANOSYL)-INOSINE-5',6''-DIPHOSPHATE

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ABSTRACT. Gluco- and ribosylation of the bases of sugar protected inosine and uridine were investigated, obtaining only adducts with β-configuration at the new glycosidic carbon; stereospecific insertion of a sugar moiety at the 1-N of inosine was achieved either using a Mitsunobu approach (for ribosylation) or by direct coupling of 1-α-bromoglucose **13** with 2',3',5'-tri-O-acetylinosine for glucosylation. 1-(β-D-glucosyl)-inosine, chosen as starting substrate for glucosylated analogs of cyclic IDP-ribose, was phosphorylated at the primary hydroxyls and tested in intramolecular pyrophosphate bond formation.

Nonenzymatic formation of stable sugar-nucleic acids adducts has been detected in patients with diabetes mellitus and, more generally, chemical modifications of DNA and RNA by sugars have been hypothesized to be responsible of nucleic acids strand breaks and to significatively contribute to the biochemical aging processes[1]. Severin and coworkers, investigating the reaction of guanosine and 2'-deoxyguanosine with glucose and ribose in heated aqueous solution as model study of DNA-sugars interactions, isolated, among various adducts, several base glycosylated derivatives[2]. Interestingly, modified nucleoside 5-(β-D-glucopyranosyl)-2'-deoxyuridine has been found in the variant surface glycoprotein gene of *Trypanosoma brucei*, a unicellular parasitic eukariote trasmitted by tsetse flies[3-6]. More recently, this modified thymidine linking a glucose unit

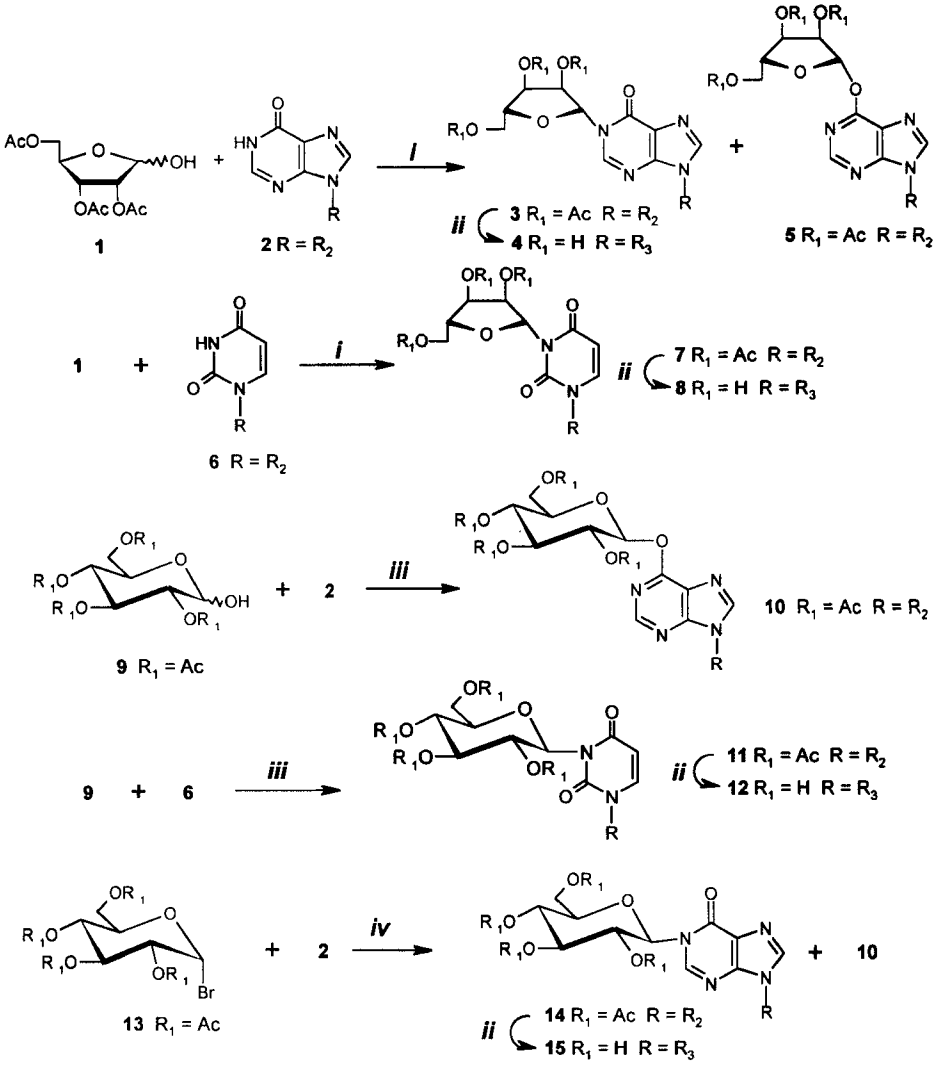
at the 5-position of the pyrimidine base and the corresponding 2-amino glucosylated product have been synthesized and, after appropriate functionalization, incorporated in oligonucleotide fragments to study the interactions of the sugar residue with double stranded DNA[7,8]. Presumably, glucose moieties, accomodated within the major groove, displace part of the hydration shell thus inducing an enhanced thermal stability in the resulting DNA duplex[7].

Another related topic is the preparation of useful intermediates of more stable and bioactive analogs of cyclic ADP-ribose (cADPR), a recently discovered naturally occurring metabolite of NAD^+ shown to be a potent Ca^{2+} mobilizing agent even more active than inositol 1,4,5-triphosphate[9]. This molecule, characterized by a very labile N-1 ribosyl bond[10], is rapidly nonenzymatically hydrolysed even in neutral aqueous solution, to give ADP-ribose.

Notwithstanding the relevance of the biological aspects connected with DNA or nucleosides glycosylations, to our knowledge very few studies have been addressed to the insertion of sugars onto the base of preformed nucleosides. This prompted us to undertake a synthetic study aimed at setting a general, efficient protocol to obtain base-glycosylated nucleosides to be studied for their pharmacological properties as such and successively phosphorylated to obtain analogs of cyclic IDP-ribose (cIDPR). We recently reported[11] preliminary results concerning the gluco- and ribosylation of inosine and uridine bases carried out exploiting the Mitsunobu reaction. In the proposed procedure, 2,3,5-tri-O-acetylribose (**1**) and 2,3,4,6-tetra-O-acetylglucose (**9**) have been used as sugar substrates and 2',3',5'-tri-O-acetylinosine (**2**) and 2',3',5'-tri-O-acetyluridine (**6**) chosen as nucleoside starting materials (scheme 1). The ribosylation of inosine derivative **2** led, in all the tested conditions, to a mixture (1:5) of the N-1 and O^6 adducts (**3** and **5**, respectively) with an overall yield of 60 %. On the other hand, reaction of **1** with uridine derivative **6** led to sole N-3 ribosylated product **7** in 85 % yields. When reacted with **9**, substrate **2** gave exclusively the O^6-glucosyl derivative **10** in almost quantitative yields; on the contrary, reaction of **9** with **6** yielded only N-3 glucosylated compound **11** in 85 % yields. In all the studied cases only the products having β-configuration at the new glycosidic carbon have been isolated.

Since the N-1 glucosyl derivative of inosine had never been isolated under the studied Mitsunobu conditions, we then tried the classical glycosylation route based on the

SCHEME 1



R_2 = 2,3,5-tri-O-acetyl-β-D-ribofuranosyl

R_3 = β-D-ribofuranosyl

Conditions: **i**: tributylphosphine (2.5 eq), ADDP (2.5 eq), benzene, r.t. 10 h: **ii**: 0.01 M K_2CO_3 in H_2O/MeOH (1:1,v/v); **iii**: tributylphosphine (1.5 eq), ADDP (1.5 eq) benzene, r.t. 10 h; **iv**: DME, 50 °C, 5 h; **v**: conc. NH_4OH, 50 °C, 3 h.

reaction of protected inosine **2** with peracetylated 1-α-bromoglucose **13** in the presence of a base. This reaction gave in all cases a mixture of the N-1 and O^6-derivatives, both with β-configuration, in ratios and overall yields strongly dependent on the solvent. The best results were obtained with DME and K_2CO_3, which led to compounds **14** and **10** in 1:1.2 ratio with 65 % overall yield. N-derivatives were obtained in the sugar-deprotected form (**4**, **8**, **12** and **15**) by treatment with 0.01 M K_2CO_3 in H_2O/MeOH (1:1, v/v). On the contrary the O-derivatives, under all the tested deacetylating conditions, resulted unstable being degraded to the nucleoside starting materials.

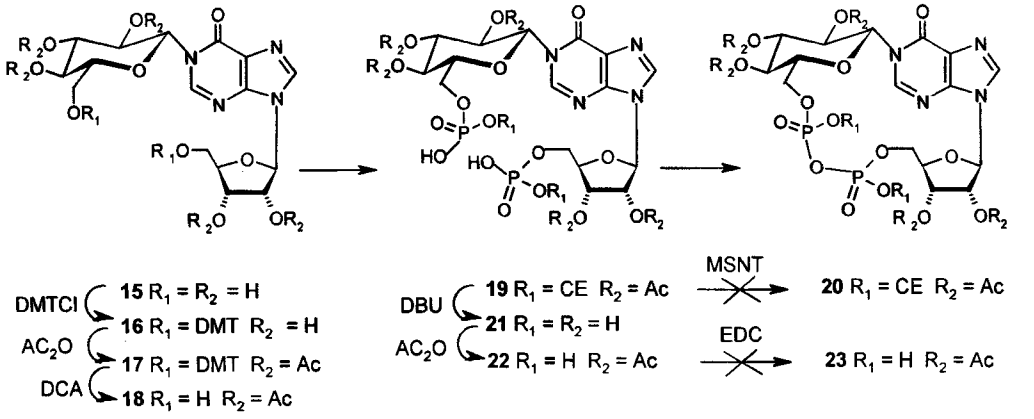
Aiming at new analogs of cADPR and cIDPR we decided to investigate the role of the glycosyl moiety linked at the N-1 position of the purine base in the biological activity of these metabolites, replacing the N-1 ribose with a 1-β-glucopyranosyl unit. We here report our results concerning the bis-phosphorylation of glucose derivative **15** and the successive attempts of cyclizing it through the formation of an intramolecular pyrophosphate linkage.

All the efforts to phosphorylate the 5'- and the 6''-hydroxyl groups of **15** using essentially the method described by Yoshikawa and coworkers[12] ($PO(OEt)_3/POCl_3$, 3 eq., 0 °C) failed. Therefore we decided to use as a nucleoside substrate for the bis-phosphorylation a glucosyl inosine derivative having the secondary hydroxy functions appropriately protected (**18**, Scheme 2).

For this purpose, **15** was converted into the 5',6''-bis-dimethoxytrityl derivative **16** by reaction with DMTCl/DMAP in DMF/Et_3N in 85 % yields. **16** was then treated with acetic anhydride in pyridine giving penta-acetylated derivative **17** in almost quantitative yields. Finally removal of DMT groups, achieved by treatment with DCA (10 %, v/v in CH_2Cl_2), led to desired compound **18** in 90 % yield. Compounds **16**, **17** and **18** have been purified by silica gel chromatography and their purity and identity ascertained by spectroscopic methods (^1H and ^{13}C NMR, UV and FAB MS).

For the phosphorylation step we used classical phosphoramidite reagents, basically adopting the synthetic procedure used by Matsuda and coworkers[4d] on similar substrates. Reaction of **18** with 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite and DIEA in anhydrous CH_3CN afforded the corresponding 5',6''-bisphosphoramidite compound. Without purification, this was then converted, by treatment with I_2/tetrazole, in the related bis-phosphodiester **19**, which was purified on a short RP18 column (80 % yields) and

SCHEME 2



DMTCl = 4,4'-dimethoxytritylchloride; DCA = dichloroacetic acid; DBU = 1,8-diazabicyclo[5.4.0.]undec-7-ene
MSNT = 1-(2-mesitylenesulfonyl)-3-nitro-1H-1,2,4-triazole; EDC =N-(dimethylaminopropyl)-N'-ethylcarbodiimide
CE = 2-cyanoethyl

characterized by ^{1}H, ^{31}P and ^{13}C NMR, UV and FAB MS. Addition of MSNT to **19** did not furnish the desired condensation of the 5' and 6''-phosphodiester functions to give product **20**. Therefore **19** was reacted with anhydrous DBU to remove the 2-cyanoethyl phosphate protecting groups in order to test the cyclization on the bis(phosphomonoester)derivative. Treatment with DBU led to the undesired, concomitant removal of the acetyl protecting groups, giving compound **21** in 95 % yields; this was successively peracetylated by reaction with acetic anhydride in pyridine obtaining **22** in almost quantitative yields. The intramolecular pyrophosphate formation between the two phosphoryl moieties of **22** was attempted using as condensing agent 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDC). Even if different reaction conditions and solvents were tested, cyclic compound **23** could not be isolated.

These negative results could be attributed, as hypothesized by Matsuda and coworkers[4d] for similar substrates, to the unfavourable anti conformation adopted by the N-1 glucosylated inosine nucleotide. Efforts to obtain the 8-bromo derivative of the synthesized N-1 glucosylated compounds, which presumably adopts a more favourable conformation for the cyclization reaction, are actually underway in our laboratories.

Further studies will be also addressed to the insertion in ODN chains of compounds **4**, **8**, **12** and **15** to investigate the binding properties of the resulting oligonucleotides with complementary single or double stranded DNA or RNA fragments.

Experimental Section.

General Methods.

Thin Layer Chromatography was carried out on Merck coated plates (silica gel 60, F254). Column chromatography was performed on silica gel (Merck, Kieselgel 40, 0.063-0.200 mm). FAB mass spectra were determined on a ZAB 2SE spectrometer. NMR spectra were recorded on Bruker WM-400 and on Varian- Gemini 200 spectrometers. All chemical shifts are expressed in ppm with respect to the residual solvent signal. *J* values are given in Hz. UV measurements were performed on a Perkin Elmer Lambda 7 spectrophotometer.

General procedure of glycosylation by Mitsunobu reaction; synthesis of compounds 3, 5, 7, 10 and 11.

Sugar **1** or **9** (1 eq) and peracylated nucleoside **2** or **6** (1.2 eq), dissolved in anhydrous benzene (2 mL per 0.1 mmol of sugar substrate), were treated with tri-*n*-butylphosphine and ADDP (1.5 eq in the reaction with **9**, 2.5 eq when reacting **1**) and the resulting mixture was left under stirring at room temperature. After 10 h the reaction mixture was concentrated under reduced pressure and then purified on a silica gel column eluted with benzene/ethyl acetate (65/35, v/v). Following this procedure compound **3** could be isolated in 10 %, **5** in 50 %, **7** in 85 %, **10** in 97 % and **11** in 85 % yields. For the characterization of the synthesized compounds see ref. 11.

Synthesis of 1-(2",3",4",6"-tetra-O-acetyl-β-D-glucopyranosyl)-2',3',5'-tri-O-acetylinosine (14).

To 100 mg (0.25 mmol) of 2',3',5'-tri-O-acetylinosine (**2**), dissolved in 2 mL of anhydrous DME, 70 mg (0.5 mmol) of K_2CO_3 were added and the resulting mixture was left at reflux for 1 h. Successively 220 mg (0.5 mmol) of 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (**31**) were added to the suspension, previously cooled to 60 °C;

after 6 h, the reaction mixture was taken to dryness and purified on a silica gel column eluted with a gradient of CH_3OH in $CHCl_3$. 54 mg of compound **14**[11] (0.075 mmol, 30 % yield) and 64 mg of **10**[11] (0.088 mmol, 35 % yield) were obtained.

Deprotection of peracetylated compounds; synthesis of 4, 8, 12 and 15.

Compounds **3**, **7**, **11** and **14** were treated with a 0.01 M solution of K_2CO_3 in H_2O/CH_3OH (1:1, v/v; for 0.1 mmol of substrate 5.0 mL of the cited solution were used) for 30 min at r.t. The reaction mixture, neutralized by addition of acetic acid, was concentrated under reduced pressure and the crude purified by HPLC on an RP18 column (μBondapak C18, Waters, 7 μm, 3.9x300 mm) eluted with H_2O/CH_3CN (95/5, v/v). After purification, **4** was recovered in 75 % yields while **8**, **12** and **15** could be isolated in 80 % yields. For the characterization of compounds **4**, **8**, **12** and **15** see ref. 11.

1-[6"-O-(4,4'-dimethoxytrityl)-β-D-glucopyranosyl]-5'-O-(4,4'-dimethoxytrityl)-inosine (16).

100 mg (0.23 mmol) of **15,** coevaporated several times with anhydrous pyridine and then dissolved in 2.5 mL of DMF/triethylamine (2:1, v/v), were treated with 195 mg (0.57 mmol) of 4,4'-dimethoxytritylchloride. The reaction, kept at 80 °C for 2-4 h, was quenched by addition of water and the mixture concentrated under reduced pressure. After purification on a silica gel column, eluted with a gradient of CH_3OH in $CHCl_3$/pyridine (1: 0.1, v/v, from 0 % to 10 %), 202 mg of ditritylated compound **16** were obtained (85 % yield). Rf 0.75 (eluent $CHCl_3/CH_3OH$ 8:2, v/v); m/z (FAB, positive ions) 1035 $(M + H)^+$, 733 $(M - DMT + 2 H^+)$, 303 $(DMT)^+$. δ_H (CD_3OD) 8.25 (s, 1H, H-2), 8.18 (s, 1H, H-8), 7.47-6.72 (complex signals, 26 H, aromatic protons of DMT groups), 6.05 (d, 1H, H-1'), 6.01 (d, 1H, H-1"), 4.43 (apparent t, 1H, H-3'), 4.25 (m, 1H, H-4'), 3.77-3.35 (overlapped signals, 8H, H-5', H-2", H-3'', H-4", H-5" and H-6"), 3.70 (s, 12 H, 4 OCH_3 of DMT groups); H-2' resonance is submerged by the residual solvent signal. δ_C (CD_3OD) 158.4 (C-6), 147.1 e 146.7 (C-4 and C-2), 139.7 (C-8), 123.5 (C-5), 160.6, 150.0, 137.9, 131.0, 129.9, 129,3, 129,2, 128.2, 114.7, 114.5 (aromatic carbons of DMT groups), 88.3 (C-1'), 86.1 (C-4'), 83.6 (quaternary C of DMT groups), 82.1 (C-1''), 79.3 (C-5''), 75.2 (C-3'), 75.1 (C-3''), 72.7 (C-2''), 72.1 (C-2'), 71.5 (C-4''), 65.3 (C-5'), 62.9 (C-6''), 56.2 (OCH_3 of DMT groups).

1-[6"-O-(4,4'-dimethoxytrityl)-2'',3'',4''-tri-O-acetyl-β-D-glucopyranosyl]-5'-O-(4,4'-dimethoxytrityl)-2',3'-di-O-acetylinosine (17).

To 100 mg (0.097 mmol) of **16**, dissolved in 2.0 mL of pyridine, 0.6 mL of acetic anhydride were added and the resulting mixture left at r.t. for 2 h. The solution was successively dried and coevaporated several times with *n*-eptane and three times with benzene. 120 mg of **17**, pure by TLC (Rf 0.7, eluent $CHCl_3/CH_3OH$, 98:2, v:v), and by ^1H NMR, were obtained (99 % yields). m/z (FAB, positive ions) 1245 $(M + H)^+$, 943 (M - DMT + 2 H^+), 641 (M - 2DMT + 3 H^+), 303 $(DMT)^+$. δ_H ($CDCl_3$) 8.16 (s, 1H, H-2), 7.92 (s, 1H, H-8), 7.47-6.75 (complex signals, 26 H, aromatic protons of DMT groups), 6.33 (d, 1H, H-1''), 6.24 (d, 1H, H-1'), 6.00 (m, 1H, H-2'), 5.66 (m, 1H, H-3'), 5.41-5.20 (overlapped signals, 3H, H-2'', H-3'' and H-4''), 4.33 (m, 1H, H-4'), 3.87 (m, 1H, H-5"), 3.76 (s, 12 H, 4 OCH_3 of DMT groups), 3.46 (m, 2H, H-5'), 3.23 (m, 2H, H-6''). δ_C ($CDCl_3$) 169.6 and 169.2 ($CH_3\underline{C}O$), 158.4 (C-6), 144.5 and 144.0 (C-4 and C-2), 138.0 (C-8), 121.2 (C-5), 158.6, 135.0, 130.0, 128.1, 127.9, 127.7, 127.0, 113.3, 113.0 (aromatic carbons of DMT groups), 87.1 (C-1'), 86.2 (C-4'), 84.3 (quaternary C of DMT groups), 82.7 (C-1''), 78.7 (C-5''), 73.1 (C-3'), 73.0 (C-3''), 71.8 (C-2''), 72.5 (C-2'), 68.2 (C-4''), 62.9 (C-5'), 61.3 (C-6''), 55.1 (OCH_3 of DMT groups).

1-(2",3",4"-tri-O-acetyl-β-D-glucopyranosyl)-2',3'-di-O-acetylinosine (18).

100 mg (0.080 mmol) of **17**, dissolved in 2 mL of CH_2Cl_2, were treated with 4 mL of a 10 % dichloroacetic solution in CH_2Cl_2. The reaction, complete in 20-30 min at r.t., was quenched by addition of pyridine. The resulting mixture was transferred into a separatory funnel, diluted with CH_2Cl_2 and washed with water. The organic phase, taken to dryness, was purified on silica gel plates, eluted with $CHCl_3/CH_3OH$ 95:5 (v:v). The bands at Rf 0.25, eluted with $CHCl_3/CH_3OH$ (7:3, v/v), gave 46 mg (0.072 mmol) of **18**, pure by TLC and ^1H NMR (90 % yield). m/z (FAB, positive ions) 641 $(M + H)^+$, 640 $(M)^+$, 425 $(M - 5\ CH_3CO)^+$. δ_H ($CDCl_3$) 8.28 (s, 1H, H-2), 7.86 (s, 1H, H-8), 6.24 (d, 1H, H-1''), 6.01 (d, 1H, H-1'), 5.87 (t, 1H, H-2'), 5.60 (overlapped signals, 2H, H-3' and H-2''), 5.17 (overlapped signals, 2H, H-3'' and H-4''), 4.35 (m, 1H, H-4'), 3.84 (overlapped signals, 5H, H-5', H-5" and H-6"), 2.17, 2.10, 2.05, 2.02, 1.86 (5 s's, 3H each, $C\underline{H}_3CO$). δ_C ($CDCl_3$) 170.2, 169.7 and 169.4 ($CH_3\underline{C}O$), 155.0 (C-6), 145.9 (C-4), 145.3 (C-2), 140.0

(C-8), 125.0 (C-5), 88.1 (C-1'), 86.1 (C-4'), 78.8 (C-1''), 77.9 (C-5"), 73.1 (C-3'), 72.4 (C-2' and C-3''), 71.9 (C-2"), 68.3 (C-4''), 62.4 (C-5'), 61.1 (C-6''), 20.7, 20.6 and 20.3 ($\underline{C}H_3CO$).

1-[6"-(2-cyanoethylphosphoryl)-2",3",4"-tri-O-acetyl-β-D-glucopyranosyl]-2',3'-di-O-acetylinosine-5'-(2-cyanoethylphosphate) (19).

To 30 mg (0.047 mmol) of **18**, dissolved in 1.5 mL of anhydrous acetonitrile, 0.3 mL of DIEA and 0.045 mL (0.19 mmol) of 2-cyanoethyl, N,N-diisopropylchlorophosphoramidite were added. After 30 min at r.t., the reaction mixture was diluted with $CHCl_3$, transferred into a separatory funnel, washed with water and the organic phase was taken to dryness. The residue was then treated with 3 mL of a 0.45 M 1-H-tetrazole solution in CH_3CN/H_2O (95:5; v/v) and the mixture left at r.t.. After 1 h 3 mL of a 0.1 M iodine solution in tetrahydrofurane/pyridine/H_2O (1.1, v/v) were added and, after 20 min at r.t., the reaction mixture was concentrated under reduced pressure. The residue, dissolved in water, was chromatographed on a short RP18 column eluted with a gradient of CH_3OH in H_2O (from 0 to 50 %). 48 mg (0.041 mmol) of **19** (as ethyldiisopropylammonium salt), pure by TLC and ^1H NMR, were obtained (87 % yields). Rf 0.40 [eluent: isopropanol/NH_4OH/H_2O, 55:35:10 (v:v)]; m/z (FAB, negative ions) 905 $(M - H)^-$. δ_H (D_2O) 8.71 (s, 1H, H-2), 8.46 (s, 1H, H-8), 6.41 (overlapped signals, 2H, H-1' and H-1''), 5.81 (t, 1H, H-2'), 5.72 (t, 1H, H-3'), 5.62 (overlapped signals, 2H, H-2'' and H-4''), 5.42 (t, 1H, H-3''), 4.67 (m, 1H, H-4'), 4.30-4.01 (overlapped signals, 9H, H-5', H-5", H-6" and 2 $OC\underline{H}_2CH_2CN$ groups), 2.79 (m, 4H, 2 $OCH_2C\underline{H}_2CN$ groups), 2.24, 2.18, 2.12, 2.11, 1.93 (5 s's, 3H each, $C\underline{H}_3CO$). δ_P (D_2O) 1.41. δ_C (D_2O) 171.6, 171.5, 171.4, 171.0, 170.9 ($CH_3\underline{C}O$), 155.8 (C-6), 148.0 (C-4), 145.3 (C-2), 140.1 (C-8), 126.2 (C-5), 118,1 (CN), 84.8 (C-1'), 80.9 (C-4'), 79.0 (C-1''), 74.0 (C-5"), 73.0 (C-3'), 71.9 (C-2'), 70.2 (C-2"), 70.3 (C-3''), 62.8 (C-4"), 61.9 (C-5'), 61.1 (C-6"), 59.4 ($O\underline{C}H_2CH_2CN$), 20.3 ($OCH_2\underline{C}H_2CN$), 20.2, 20.2, 20.1, 20.1, 20.0 ($\underline{C}H_3CO$) .

1-(6"-phosphoryl-β-D-glucopyranosyl)-inosine-5'-phosphate (21).

20 mg (0.017 mmol) of **19** (as ethyldiisopropylammonium salt) were treated with 50 μL of anhydrous DBU anidro at r.t. for 18 h. The reaction mixture, concentrated under reduced pressure, was dissolved in water and chromatographed on a short RP18 column

eluted with a gradient of CH_3OH in H_2O (from 0 to 50 %). Fractions eluted with H_2O/CH_3OH (9:1, v:v), collected and taken to dryness, gave 12 mg of **21** (0.014 mmol, as $DBUH^+$ salt), pure by TLC and ^1H NMR (95 % yields): Rf 0.10 [eluent: isopropanol/NH_4OH/H_2O, 55:35:10 (v:v)]; δ_H (D_2O) 8.60 (s, 1H, H-2), 8.53 (s, 1H, H-8), 6.16 (d, 1H, H-1', J = 5.4), 6.07 (d, 1H, H-1'', J = 9.4), 4.53 (t, 1H, H-3'), 4.38 (m, 1H, H-4'), 4.04 (m, 2H, H-5'), 3.60-3.42 (overlapped signals, 6H, H-2'', H-3", H-4'', H-5'' and H-6"); H-2' resonance is submerged by the residual solvent signal.

1-(6"-phosphoryl-2",3",4"-tri-O-acetyl-β-D-glucopyranosyl]-2',3'-di-O-acetylinosine-5'-phosphate (22).

10 mg of **21** (0.012 mmol, as $DBUH^+$ salt) were treated with acetic anhydride in pyridine (1mL, 1:1, v:v) and the resulting mixture left at r.t. for 48 h. The solution was successively dried and coevaporated several times with *n*-eptane and three times with benzene. The crude was redissolved in water and purified by HPLC on a RP18 column (μBondapak C18, Waters, 7 μm, 3.9x300 mm) eluted with a gradient of CH_3CN in 0.1 M TEAB (from 0 to 100 % in 40 min, flow 0.7 mL/min). Peak at 14.2 min, collected and taken to dryness, gave 10 mg of **22** (0.0092 mmol, as $DBUH^+$ salt). Rf 0.5 [eluent: $CHCl_3/CH_3OH/H_2O$, 14:6:1 (v:v)]; δ_H (D_2O) 8.69 (s, 1H, H-2), 8.60 (s, 1H, H-8), 6.41 (d, 2H, H-1' and H-1''), 5.76 (t, 1H, H-3'), 5.65 (m, 2H, H-2''and H-3"), 5.48 (m, 1H, H-4''), 5.35 (t, 1H, H-2'), 4.31 (m, 1H, H-4'), 4.14-3.95 (overlapped signals, 5H, H-5', H-5'' and H-6"), 2.24, 2.16, 2.09, 2.08 and 1.90 (s's, 3H each, acetyl protons).

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