

Synthetic studies on the glycosylation of the base residues of inosine and uridine

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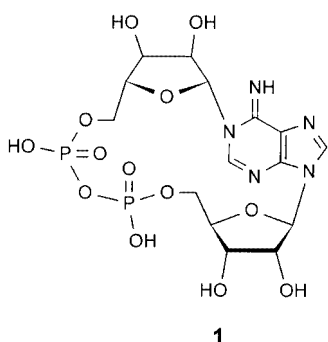
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Ribosylation and glucosylation of the base residues of inosine and uridine have been efficiently achieved using Mitsunobu reaction, leading to the N-1 and 6-O-glycosylinosine and N-3-glycosyluridine derivatives, all with β configuration at the glycosidic carbon. The unprecedented 5-amino-1-(β -D-ribofuranosyl)imidazole-4-[N-(β -D-glucopyranosyl)carboxamide] has also been synthesised.

Cyclic adenosine-5'-diphosphate ribose (cADPR, **1**) is a



recently discovered, naturally occurring metabolite¹ of nicotinamide adenine dinucleotide (NAD⁺), which has been shown to be a Ca²⁺-mobilising agent² in various cells and is even more active than inositol 1,4,5-triphosphate (IP₃).³ This important biological role, not yet fully understood, has largely stimulated studies on this molecule characterised by a very labile N-1 ribosyl bond which is rapidly, non-enzymatically hydrolysed, even in neutral aqueous solution,⁴ to give ADP-ribose.

In order to obtain more stable and still bioactive analogs of cADPR, a number of compounds structurally related to it have been synthesised to be used both as models to elucidate the structure-activity relationships in **1** and for their pharmacological properties. Ribosylation of purine nucleoside bases is one of the key steps in the synthesis of **1** and related derivatives, involving several problems associated with regio- and stereoselectivity requirements. Even if most of them are still unsolved, only a few studies have been addressed to the glycosylation of nucleoside bases. N-1 ribosylation of adenosine has been described using either enzymatic or chemical methods. Enzymatic methods,⁵ proposed for the intramolecular 1-ribosylation of the adenine base of NAD⁺ and leading to cADPR, provide an almost complete regio- and stereocontrolled reaction. However, the above procedures cannot be considered as general glycosylation strategies, being limited by the substrate specificity of the enzyme at the sole ribosylation. Alternatively the intramolecular ribosylation of NAD⁺ can be performed using a chemical strategy⁶ in which the cADPR was produced

in a stereoselective manner but in low yields. On the other hand, two chemical ribosylation approaches have been reported for the synthesis of 1,9-diribofuranosylpurines. The first one⁷ uses a ribosyl-1-imino ether derivative which, reacting with a precursor of purine nucleoside (5-amino-4-cyanoimidazonucleoside), leads to 1,9-diribofuranosyladenine. The second one, using 2,3,5-tri-*O*-benzoyl-1-bromoribose in a phase-transfer ribosylation of inosine, was reported to form both α and β stereoisomers of the N-1 and 6-O regioisomers.⁸

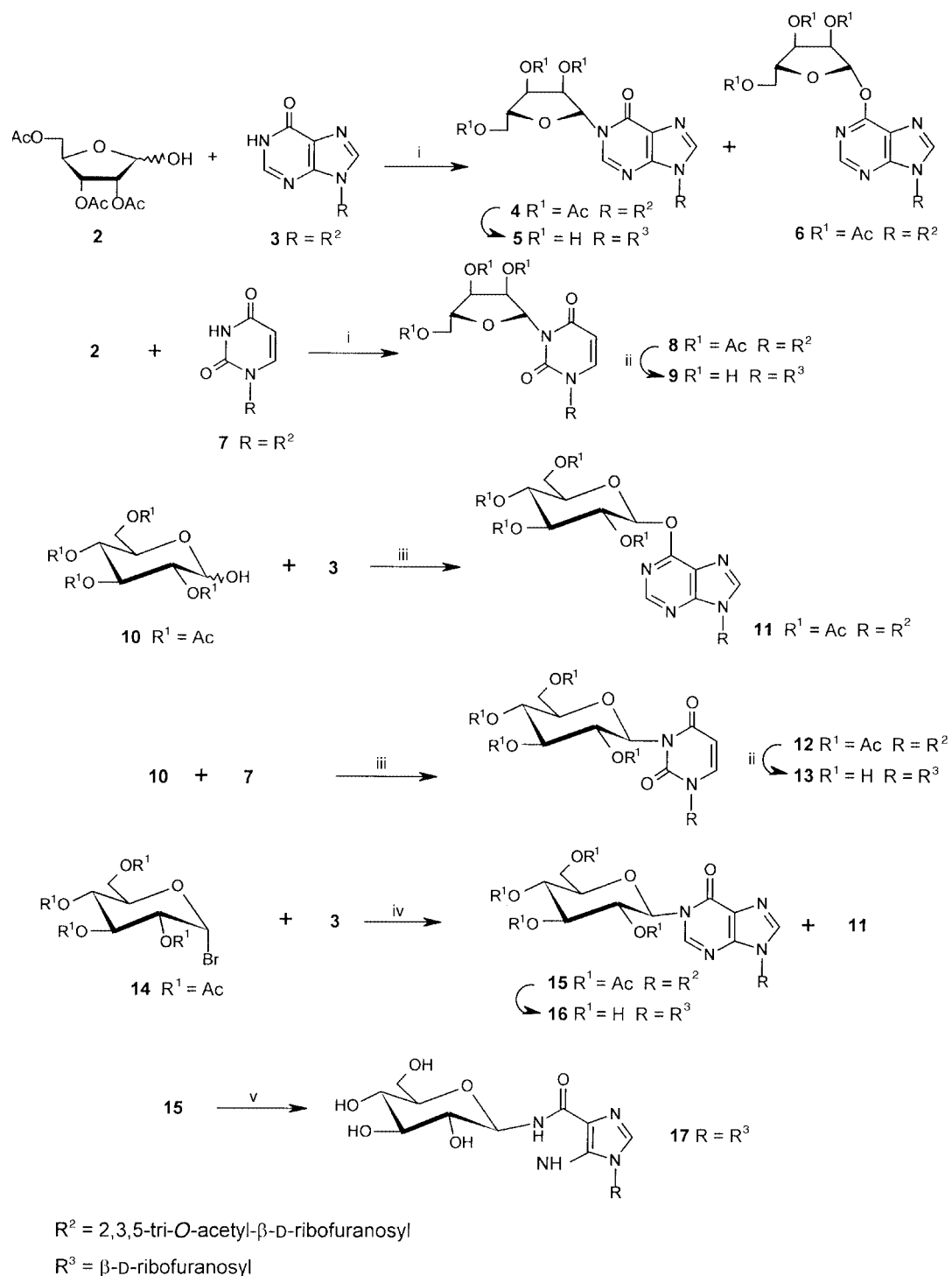
Chemical ribosylation of inosine derivatives is strongly limited, since upon the classical acid-catalyzed glycosylation methods⁹ the hypoxanthine ring of inosine reacts with the Lewis acid species thus becoming unreactive as a ribosyl acceptor. On the other hand, basic media, leading to deprotonation of inosine (pK_a 8.8), assure a good nucleophilicity to both the N-1 and 6-O positions, with reaction conditions and the nature of the electrophile strongly influencing the regioselectivity of the reaction.¹⁰ 1,3-Diribofuranosylpyrimidines, to our knowledge, have been reported only as undesired side products in the nucleoside synthesis performed by coupling the chosen heterocyclic bases with the sugar moieties.¹¹

Aiming at a mild and general procedure to obtain base-glycosylated nucleosides and producing useful intermediates of cyclic diglycosyl nucleotides structurally related to cADPR, we undertook a study on the ribosylation and glucosylation of inosine and uridine bases *via* Mitsunobu reaction. This reaction, largely exploited in the alkylation of nucleoside bases and also reported in some examples of N-glycosidic bond formation,¹² assures the introduction of a good activating group on the sugar anomeric carbon and the concomitant generation of a nucleophilic glycosyl acceptor by deprotonation of the amidic (or imidic) nitrogen of the base.

Results and discussions

In the proposed glycosylating strategy, 2,3,5-tri-*O*-acetyl-ribose (**2**, Scheme 1) and 2,3,4,6-tetra-*O*-acetylglucose **10** have been used as sugar substrates and the 2,3,5-tri-*O*-acetyl derivatives of inosine (**3**) and uridine (**7**) chosen as nucleoside starting materials.

In a typical reaction of ribosylation, 1.0 equiv. of acylated sugar **2**, obtained following literature procedures,¹³ was treated



Scheme 1 Reagents and conditions: i: tributylphosphine (2.5 equiv.), ADDP (2.5 equiv.), benzene, r.t., 10 h. ii: 0.01 M K_2CO_3 in H_2O -MeOH (1:1, v/v). iii: tributylphosphine (1.5 equiv.), ADDP (1.5 equiv.) benzene, r.t., 10 h. iv: DME, 50 °C, 5 h. v: conc. NH_4OH , 50 °C, 3 h.

with 1.2 equiv. of the sugar-protected nucleoside (**3** or **7**), in the presence of 2.5 equiv. of tri-*n*-butylphosphine and 2.5 equiv. of azodicarboxylic dipiperidine (ADDP) in anhydrous benzene for 10 h at r.t. In the case of glucosylation of **3** or **7** starting from compound **10**, high yields were obtained even when using a lower excess (1.5 equiv.) of both tri-*n*-butylphosphine and ADDP.

After silica gel chromatography, the isolated products were characterised by spectroscopic methods (1H , ^{13}C NMR and FAB MS spectrometry). In all cases, the configuration of the new glycosidic carbon has been assigned on the basis of NOESY and/or ROESY spectra, which show medium to strong NOE effects between H-1'' and H-4'' protons, in the case of ribose adducts, and between H-1'' and H-3'', H-5'' protons in

glucose derivatives, both in the sugar-protected or unprotected form. The ribosylation of inosine derivative **3** led, in all the tested conditions, to a mixture (1:5) of the N-1 and 6-O products (**4** and **6**, respectively) with an overall yield of 60%. On the other hand, reaction of **2** with uridine derivative **7** led to the sole N-3-ribosylated product **8** in 85% yields. When treated with **10**, substrate **3** gave exclusively the 6-O-glucosyl derivative **11** in almost quantitative yields; on the other hand, reaction of **10** with **7** yielded only N-3-glucosylated compound **12** in 85% yield. In all the studied cases only the products having β -configuration at the new glycosidic carbon have been isolated. As is well known, cyclic per-acetylated sugars, in acidic media, react with nucleophiles stereospecifically at the anomeric carbon due to the formation of the 1,2-acyloxonium ion. Upon

reaction under Mitsunobu conditions the 1-phosphonyl glycosyl derivative can be considered the very reactive precursor of the 1,2-acyloxonium ion.¹⁴

In the case of the pyrimidine ring of **7** we observed a total regioselectivity in the coupling (N-3 vs. 4-O position) even if thymine is reported to react with electrophiles in basic media at both positions.¹⁵ For the hypoxanthine ring of **3**, the ribosylation occurs at both the N-1 and 6-O positions, in accord with earlier observations.⁸ However, the glucosylation furnished only the 6-O regioisomer. This behaviour cannot be ascribed to a different reactivity of the glycosyl donors, since in principle they are very similar; anyway, the kind of ribose protecting group seems to play a key role in this reaction, as confirmed by the fact that when we used 2,3,5-tri-*O*-benzoylribose as sugar starting material only the 6-*O*- β -derivative was 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 reaction of protected inosine **3** with per-acetylated 1- α -bromoglucose **14** in the presence of a base. This reaction gave in all cases a mixture of the N-1 and 6-*O*-derivatives, both with the expected β -configuration, in ratios and overall yields strongly dependent on the solvent. The best results were obtained with 1,2-dimethoxyethane (DME) and K₂CO₃, which led to compounds **15** and **11** in a 1 : 1.2 ratio with 65% overall yield.

Different conditions were tested for the removal of the acetyl groups from the sugar moieties; as far as the synthesized *O*-derivatives are concerned, cleavage of the *O*-glycosidic bond occurred in all cases. *N*-derivatives were obtained in the sugar-protected form (**5**, **9**, **13** and **16**) in almost quantitative yields by treatment with 0.01 M K₂CO₃ in H₂O–MeOH (1 : 1, v/v). Remarkably, **15**, left in contact with conc. aq. ammonia, quantitatively converted into the unprecedented 5-amino-1- β -D-ribofuranosylimidazole-4-[*N*-(β -D-glucopyranosyl)carboxamide] (**17**, glucosylated AICAR), the synthesis of which is not trivial in other ways. The degradation of the purine six-membered ring leading to **17** can be attributed to the electron-withdrawing effect of the glucosyl moiety which, as reported for other appropriate N-1-substituted inosine derivatives,¹⁶ renders the 2-carbon electrophilic enough to react with nucleophiles producing a fast ring opening, followed by aminolysis of the formamidine intermediate.

Experimental

General methods

TLC plates (Merck, silica gel 60, F254) were developed in the following solvent systems: A [CHCl₃–MeOH (97 : 3, v/v)]; B [butan-1-ol–acetic acid–water (60 : 10 : 15, v/v)]. Column chromatography was performed on silica gel (Merck, Kieselgel 40, 0.063–0.200 mm). FAB mass spectra (positive) 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. Optical rotations were measured with a Perkin-Elmer 141 polarimeter at 25 °C and are quoted in units of 10⁻¹ deg cm² g⁻¹. Mps were determined on a Reichert Thermovar apparatus and are uncorrected.

Glycosylation by Mitsunobu reaction: general procedure; products **4**, **6**, **8**, **11** and **12**

To a solution of sugar **2** or **10** (1 equiv.) and per-acetylated nucleoside **3** or **7** (1.2 equiv.), dissolved in anhydrous benzene (2 cm³ per 0.1 mmol of sugar substrate), tri-*n*-butylphosphine and ADDP (1.5 equiv. in the reaction with **10**, 2.5 equiv. in the reaction with **2**) were sequentially added and the resulting mixture was left 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).

2',3',5'-Tri-*O*-acetyl-1-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)-inosine **4.** *R*_f 0.6 (system A); mp 52–54 °C; λ_{max} (CHCl₃)/nm 245 (ϵ /dm³ mol⁻¹ cm⁻¹ 9950) and 252 (9350); *m/z* (FAB) 652 (M⁺), 653 (MH⁺) [Found: HRMS (FAB⁺) *m/z* (M⁺), 652.1888. C₂₇H₃₂N₄O₁₅ requires *M*, 652.1864]; [α]_D –0.30 (*c* 2.4, CHCl₃); δ_{H} (CDCl₃) 8.23 (s, 1H, H-2), 7.91 (s, 1H, H-8), 6.74 (d, 1H, H-1'', *J* 4.8), 6.09 (d, 1H, H-1', *J* 5.0), 5.87 (apparent t, 1H, H-2'), 5.82 (apparent t, 1H, H-2''), 5.62 (apparent t, 1H, H-3'), 5.48 (apparent t, 1H, H-3''), 4.60 (m, 1H, H-4''), 4.44–4.16 (overlapped signals, 5H, H-4', H₂-5' and H₂-5''), 2.14–1.93 (several s, 18H, acetyl protons); δ_{C} (CDCl₃) 170.3, 169.5, 169.2, 168.5 (CH₃CO), 155.7 (C-6), 147.0 (C-4), 144.8 (C-2), 138.6 (C-8), 124.3 (C-5), 86.6 (C-1'), 83.9 (C-1''), 80.2 (C-4'), 79.9 (C-4''), 73.1 (C-3'), 71.0 (C-3''), 70.4 (C-2''), 69.9 (C-2'), 62.9 (overlapped, C-5' and C-5''), 20.7, 20.4, 19.8 (CH₃CO).

2',3',5'-Tri-*O*-acetyl-6-*O*-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)inosine **6.** *R*_f 0.75 (system A); mp 53–55 °C; λ_{max} (CHCl₃)/nm 247 (ϵ /dm³ mol⁻¹ cm⁻¹ 7600); *m/z* (FAB) 652 (M⁺) [Found: HRMS (FAB⁺) *m/z* (M⁺), 652.1879. C₂₇H₃₂N₄O₁₅ requires *M*, 652.1864]; [α]_D +1.17 (*c* 2.6, CHCl₃); δ_{H} (CDCl₃) 8.54 (s, 1H, H-2), 8.10 (s, 1H, H-8), 7.10 (d, 1H, H-1'', *J* 3.8), 6.19 (d, 1H, H-1', *J* 5.0), 5.92 (apparent t, 1H, H-2'), 5.63 (apparent t, 1H, H-3'), 5.32 (overlapped signals, 2H, H-2'' and H-3''), 4.57 (m, 1H, H-4''), 4.45–4.18 (overlapped signals, 5H, H-4', H₂-5' and H₂-5''), 2.18–2.01 (several s, 18H, acetyl protons); δ_{C} (CDCl₃) 170.5, 170.4, 170.2, 169.7, 169.5, 169.3 (CH₃CO), 159.3 (C-6), 152.4 and 152.1 (C-4 and C-2), 141.1 (C-8), 121.9 (C-5), 96.3 (C-1''), 86.4 (C-1'), 81.3 (C-4''), 80.3 (C-4'), 73.0 (C-3'), 70.5 and 70.4 (C-2'' and C-3''), 69.8 (C-2'), 63.2 and 62.9 (C-5' and C-5''), 20.7, 20.4, 20.3 (CH₃CO).

2',3',5'-Tri-*O*-acetyl-3-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)-uridine **8.** *R*_f 0.65 (system A); mp 50–53 °C; λ_{max} (CHCl₃)/nm 262 (ϵ /dm³ mol⁻¹ cm⁻¹ 7150); *m/z* (FAB) 629 (MH⁺) [Found: HRMS (FAB⁺) *m/z* (MH⁺), 629.1852. C₂₆H₃₃N₂O₁₆ requires *M*, 629.1830]; [α]_D +22.4 (*c* 0.84, CHCl₃); δ_{H} (CDCl₃) 7.37 (d, 1H, H-6, *J* 8.2), 6.40 (d, 1H, H-1'', *J* 2.1), 6.06 (d, 1H, H-1', *J* 5.2), 5.80 (d, 1H, H-5, *J* 8.2), 5.75 (dd, 1H, H-2''), 5.62 (dd, 1H, H-3', *J* 7.6 and 6.7), 5.30 (overlapped signals, 2H, H-2' and H-3'), 4.50–4.15 (overlapped signals, 6H, H-4', H-4'', H₂-5' and H₂-5''), 2.14–2.08 (several s, 18H, acetyl protons); δ_{C} (CDCl₃) 170.4, 169.8, 169.5, 169.4, 169.3, 169.1 (CH₃CO), 160.9 (C-4), 149.6 (C-2), 137.4 (C-6), 102.7 (C-5), 87.1 (C-1'), 86.1 (C-1''), 79.8 and 78.7 (C-4' and C-4''), 72.7 and 72.4 (C-3' and C-3''), 70.0 and 69.8 (C-2' and C-2''), 63.5 and 63.0 (C-5' and C-5''), 20.5, 20.3, 20.1 (CH₃CO).

2',3',5'-Tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl)inosine **11.** *R*_f 0.65 (system A); mp 116–120 °C; λ_{max} (CHCl₃)/nm 246 (ϵ /dm³ mol⁻¹ cm⁻¹ 8900); *m/z* (FAB) 725 (MH⁺) [Found: HRMS (FAB⁺) *m/z* (MH⁺), 725.2177. C₃₀H₃₇N₄O₁₇ requires *M*, 725.2154]; [α]_D –0.13 (*c* 0.10, CHCl₃); δ_{H} (CDCl₃) 8.57 (s, 1H, H-2), 8.11 (s, 1H, H-8), 6.50 (d, 1H, H-1'', *J* 8.1), 6.20 (d, 1H, H-1', *J* 5.4), 5.95 (apparent t, 1H, H-2'), 5.66 (apparent t, 1H, H-3'), 5.41–5.05 (overlapped signals, 3H, H-2'', H-3'' and H-4''), 4.45–3.92 (overlapped signals, 6H, H-4', H₂-5', H-5'' and H₂-6''), 2.15–1.98 (several s, 21H, acetyl protons); δ_{C} (CDCl₃) 170.4, 170.3, 169.3, 169.2 (CH₃CO), 158.8 (C-6), 152.9 (C-4), 152.0 (C-2), 141.6 (C-8), 122.3 (C-5), 94.1 (C-1''), 86.6 (C-1'), 81.2 (C-4''), 80.5 (C-4'), 73.1 (C-3'), 72.5 (C-3''), 70.8 and 70.7 (C-2' and C-2''), 68.0 (C-5''), 63.1 (C-5'), 61.6 (C-6''), 20.7, 20.6, 20.3 (CH₃CO).

2',3',5'-Tri-*O*-acetyl-3-(2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl)uridine **12.** *R*_f 0.75 (system A); mp 76–79 °C; λ_{max}

(CHCl₃)/nm 264 ($\epsilon/\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ 5980); m/z (FAB) 701 (MH⁺) [Found: HRMS (FAB⁺) m/z (MH⁺), 701.2078. C₂₁H₃₇N₂O₁₈ requires M , 701.2041]; [a]_D +4.22 (c 3.6, CHCl₃); δ_{H} (CDCl₃) 7.41 (d, 1H, H-6, J 8.1), 6.16 (d, 1H, H-1', J 5.8), 6.05 (d, 1H, H-1'', J 4.8), 5.75 (d, 1H, H-5, J 8.1), 5.34–5.27 (overlapped signals, 4H, H-2', H-3', H-2'' and H-3''), 5.17 (apparent t, 1H, H-4''), 4.41–4.14 (overlapped signals, 5H, H-4', H₂-5' and H₂-6''), 3.81 (m, 1H, H-5''), 2.11–1.97 (several s, 21H, acetyl protons); δ_{C} (CDCl₃) 170.5, 169.8, 169.6, 169.3, 169.2 (CH₃CO), 161.6 (C-4), 149.0 (C-2), 138.0 (C-6), 101.4 (C-5), 86.3 (C-1'), 79.9 (C-4'), 79.0 (C-1''), 74.6 (C-5''), 73.4 and 72.3 (C-3' and C-3''), 70.1 and 67.9 (C-2' and C-2''), 67.7 (C-4''), 63.2 (C-5'), 61.8 (C-6''), 20.6, 20.5, 20.4 (CH₃CO).

Synthesis of 15

100 mg (0.25 mmol) of 2,3,5-tri-*O*-acetylinosine were dissolved in 2 cm³ of anhydrous DME and left in contact with 70 mg (0.5 mmol) of K₂CO₃ at reflux for 1 h. Once cooled to 60 °C, the mixture was treated with 220 mg (0.5 mmol) of commercial 2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranosyl bromide **14**; after 6 h, the reaction mixture was evaporated to dryness and purified by silica gel column chromatography, which gave 54 mg of adduct **15** (0.075 mmol, 30%) and 64 mg of product **11** (0.088 mmol, 35%).

2',3',5'-Tri-*O*-acetyl-1-(2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl)inosine 15. R_f 0.55 (system A); mp 113–116 °C; λ_{max} (CHCl₃)/nm 245 ($\epsilon/\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ 7650) and 252 (7300); m/z (FAB) 724 (M⁺), 725 (MH⁺) [Found: HRMS (FAB⁺) m/z (MH⁺), 725.2182. C₃₀H₃₇N₄O₁₇ requires M , 725.2154]; [a]_D –20.8 (c 0.8, CHCl₃); δ_{H} (CDCl₃) 8.15 (s, 1H, H-2), 7.92 (s, 1H, H-8), 6.35 (d, 1H, H-1'', J 9.5), 6.11 (d, 1H, H-1', J 5.2), 5.82 (apparent t, 1H, H-2'), 5.60 (apparent t, 1H, H-3'), 5.50–5.16 (overlapped signals, 3H, H-2'', H-3'' and H-4''), 4.48–4.10 (overlapped signals, 5H, H-4', H₂-5' and H₂-6''), 4.02 (m, 1H, H-5''), 2.15–2.02 (several s, 21H, acetyl protons); δ_{C} (CDCl₃) 170.4, 170.2, 169.5, 169.2 (CH₃CO), 155.3 (C-6), 146.6 (C-4), 144.6 (C-2), 138.4 (C-8), 124.0 (C-5), 86.3 (C-1'), 80.3 (C-4'), 78.6 (C-1''), 75.3 (C-5''), 73.1 (C-3'), 72.6 (C-3''), 70.9 and 70.4 (C-2' and C-2''), 67.8 (C-4''), 62.8 (C-5'), 61.5 (C-6''), 20.6, 20.4, 20.2 (CH₃CO).

Deprotection of per-acetylated adducts; products 5, 9, 13, 16

Compounds **4**, **8**, **12** and **15** were treated with a 0.01 M K₂CO₃ solution in H₂O–MeOH (1:1, v/v; volumes of 5.0 cm³ of this solution were used per 0.1 mmol of the starting material) for 30 min at r.t. The reaction mixture was neutralised by addition of acetic acid, dried under reduced pressure and the crude product was then purified by preparative HPLC on an RP18 column (Bondapak C18, 7 μm , 19 \times 300 mm), eluting with H₂O–CH₃CN (95:5, v/v).

1-(β -D-Ribofuranosyl)inosine 5. R_f 0.5 (system B); mp 120–125 °C; λ_{max} (MeOH)/nm 245 ($\epsilon/\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ 7440) and 251 (7510); m/z (FAB) 401 (MH⁺) [Found: HRMS (FAB⁺) m/z (MH⁺), 401.3100. C₁₅H₂₁N₄O₉ requires M , 401.3085]; [a]_D –0.36 (c 0.76, MeOH); δ_{H} (D₂O) 8.55 (s, 1H, H-2), 8.40 (s, 1H, H-8), 6.60 (d, 1H, H-1'', J 4.0), 6.16 (d, 1H, H-1', J 5.6), 4.84 (apparent t, 1H, H-2'), 4.67 (apparent t, 1H, H-2''), 4.51–4.47 (overlapped signals, 3H, H-3', H-3'' and H-4''), 4.35 (m, 1H, H-4'), 4.03–3.86 (overlapped signals, 4H, H₂-5' and H₂-5''); δ_{C} (D₂O) 155.1 (C-6), 145.4 (C-4), 143.8 (C-2), 138.4 (C-8), 121.0 (C-5), 86.1 (C-1'), 84.5 (C-1''), 83.4 (C-4'), 80.9 (C-4''), 71.8 (overlapped signals C-3' and C-3''), 68.2 (overlapped signals C-2' and C-2''), 59.1 and 58.6 (C-5' and C-5'').

3-(β -D-Ribofuranosyl)uridine 9. R_f 0.4 (system B); mp 125–129 °C; λ_{max} (MeOH)/nm 266 ($\epsilon/\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ 7080); m/z (FAB) 377 (MH⁺) [Found: HRMS (FAB⁺) m/z (MH⁺), 377.1213. C₁₄H₂₁N₂O₁₀ requires M , 377.1196]; [a]_D +3.30 (c

0.93, MeOH); δ_{H} (D₂O) 7.86 (d, 1H, H-6, J 8.0), 6.27 (d, 1H, H-1'', J 3.5), 5.90 (d, 1H, H-5, J 8.0), 5.88 (d, 1H, H-1', J 4.4), 4.86 (m, 1H, H-2''), 4.43 (apparent t, 1H, H-3''), 4.32 (apparent t, 1H, H-2'), 4.20 (apparent t, 1H, H-3'), 4.13 (m, 1H, H-4'), 3.98–3.68 (overlapped signals, 5H, H-4'', H₂-5' and H₂-5''); δ_{C} (D₂O) 164.9 (C-4), 151.5 (C-2), 140.9 (C-6), 102.4 (C-5), 90.6 and 88.9 (C-1' and C-1''), 84.6 and 83.9 (C-4' and C-4''), 74.2 and 71.9 (C-3' and C-3''), 70.1 and 69.8 (C-2' and C-2''), 62.1 and 61.1 (C-5' and C-5'').

3-(β -D-Glucopyranosyl)uridine 13. R_f 0.3 (system B); mp 130–135 °C; λ_{max} (MeOH)/nm 266 ($\epsilon/\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ 6550); m/z (FAB) 407 (MH⁺) [Found: HRMS (FAB⁺) m/z (MH⁺), 407.1321. C₁₅H₂₃N₂O₁₁ requires M , 407.1302]; [a]_D +10.0 (c 0.68, MeOH); δ_{H} (D₂O) 7.99 (d, 1H, H-6, J 8.0), 6.08 (d, 1H, H-5, J 8.0), 5.98–5.91 (overlapped signals, 2H, H-1' and H-1''), 4.58 (apparent t, 1H, H-2''), 4.44 (m, 1H, H-2'), 4.32 (m, 1H, H-3'), 4.25 (m, 1H, H-4''), 4.06–3.86 (overlapped signals, 3H, H₂-5' and H-4''), 3.70–3.65 (overlapped signals, 4H, H-3'', H-5'' and H₂-6''); δ_{C} (DMSO-*d*₆; 90 °C; at r.t., two close signals per nucleus were observed, collapsing with increasing temperature, due to the presence of a couple of rotational isomers) 161.5 (C-4), 149.0 (C-2), 139.0 (C-6), 99.8 (C-5), 89.0 (C-1'), 84.2 (C-1''), 79.6 (C-4'), 77.7 (C-5''), 73.0 and 70.0 (C-3' and C-3''), 69.0 (C-2'), 67.8 (overlapped signals C-2'' and C-4''), 61.0 (C-5'), 60.3 (C-6'').

1-(β -D-Glucopyranosyl)inosine 16. R_f 0.1 (system B); mp 158–161 °C; λ_{max} (MeOH)/nm 251 ($\epsilon/\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ 2260); m/z (FAB) 431 (MH⁺) [Found: HRMS (FAB⁺) m/z (MH⁺), 431.1429. C₁₆H₂₃N₄O₁₀ requires M , 431.1414]; [a]_D –1.23 (c 1.2, MeOH); δ_{H} (D₂O) 8.62 (s, 1H, H-2), 8.45 (s, 1H, H-8), 6.20 (d, 1H, H-1', J 5.4), 6.15 (d, 1H, H-1'', J 9.3), 4.87 (apparent t, 1H, H-2'), 4.54 (apparent t, 1H, H-3'), 4.37 (m, 1H, H-4'), 4.05–3.73 (overlapped signals, 8H, H₂-5', H-2'', H-3'', H-4'', H-5'' and H₂-6''); δ_{C} (D₂O) 155.2 (C-6), 144.7 (C-4), 144.0 (C-2), 138.4 (C-8), 121.1 (C-5), 86.2 (C-1'), 83.3 (C-4'), 79.7 (C-1''), 77.0 (C-5''), 74.0 (C-3'), 72.0 (C-3''), 70.6 (C-2''), 68.1 (C-2'), 66.9 (C-4''), 59.1 (C-5'), 58.3 (C-6'').

Synthesis of 17

100 mg of **15** (0.14 mmol) were treated with 3 cm³ of conc. NH₄OH for 3 h at 50 °C. The solution was dried under reduced pressure and, after purification by preparative HPLC on an RP18 column (Bondapak C18, 7 μm , 19 \times 300 mm), eluting with H₂O–CH₃CN (95/5, v/v), 55 mg of pure **17** (95% yield) was obtained. The same results were obtained also when the reaction was carried out at r.t.

5-Amino-1-(β -D-ribofuranosyl)imidazole-4-[*N*-(β -D-glucopyranosyl)carboxamide] 17. R_f 0.12 (system B); mp >180 °C (decomp.); λ_{max} (MeOH)/nm 274 ($\epsilon/\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ 11 650); m/z (FAB) 421 (MH⁺) [Found: HRMS (FAB⁺) m/z (MH⁺), 421.1590. C₁₅H₂₅N₄O₁₀ requires m/z , 421.1571]; [a]_D –6.50 (c 0.6, MeOH); δ_{H} (D₂O) 7.44 (s, 1H, H-2), 5.64 (d, 1H, H-1', J 6.4), 5.07 (d, 1H, H-1'', J 8.2), 4.57 (apparent t, 1H, H-2'), 4.31 (apparent t, 1H, H-3'), 4.18 (m, 1H, H-4'), 3.92–3.43 (overlapped signals, 8H, H₂-5', H-2'', H-3'', H-4'', H-5'' and H₂-6''); δ_{C} (D₂O) 164.3 (CO), 142.3 (C-5), 128.6 (C-2), 109.7 (C-4), 85.5 (C-1'), 83.0 (C-4'), 77.0 (C-1''), 75.3 (C-5''), 74.3 (C-3'), 70.5 (C-3''), 69.7 (C-2''), 68.0 (C-2'), 67.2 (C-4''), 58.8 (C-5'), 58.4 (C-6'').

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