



Synthesis of *N*-methyl-*D*-ribofuranuronamide nucleosides

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ABSTRACT

The synthesis of *N*-methyl-*D*-ribofuranuronamide nucleosides is described. The key route is the rearrangement of a 1,2-*O*-isopropylidene protected furanose sugar with a carboxamide function in the 4-position to a ribopyranuronamide ring. The Lewis acid catalyzed condensation of adenine and thymine nucleobases with the per-*O*-acetylated *N*-methyl-*D*-ribofuranuronamide sugar is used to give the target nucleosides as a mixture of the α and β anomers. The mixture was separated and the final compounds were obtained by deacetylation in basic conditions.

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1. Introduction

Modification of the sugar moiety of nucleosides has given a plethora of biological active compounds.¹ Many modified nucleosides exhibiting remarkable antiviral and anticancer properties have been discovered in this way.² The biological activity of these nucleoside analogues is highly related to their substrate specificity for cellular and viral kinase and, as triphosphate, for cellular and viral polymerases.^{3,4}

In the series of azasugar nucleosides, *D*- and *L*-nucleoside analogues with a pyrrolidine,⁵ pyridine,⁶ piperidine^{7,8} and five-, six-, seven-membered lactam^{9–12} ring have been synthesized and biologically evaluated. These nucleoside analogues are characterized by the presence of one nitrogen atom in the sugar ring moiety. Replacement of the oxygen atom of a furanose nucleoside by an amide function would deliver us ribopyranuronamide nucleosides. The amide resonance would give the nucleosides with a restricted conformational flexibility as is seen with cyclohexenyl nucleosides.¹³

Few attempts have been described before to synthesize pyranuronamide nucleosides.^{14,15} In 1987, Timoshchuk¹⁴ described the synthesis of the *N*-unsubstituted- α -*D*-xylopyranuronamide sugar moiety by the rearrangement of the α -*D*-xylopyranuronamide in $\text{BF}_3 \cdot \text{Et}_2\text{O}$ and acetic anhydride. However, they failed to introduce the base moiety using fluorouracil as example. In 2005, Van Rompaey et al.¹⁵ isolated the acetyl-protected 4-azido 6-oxopiperidinyl nucleoside with the 6-chloropurine base, as unexpected compound during a sugar–base condensation reaction.

In this paper, we describe the synthesis of this class of nucleoside analogues (**1a**, **1b** and **2a**, **2b**) with an adenine and thymine base and in which the sugar moiety of the nucleoside is replaced by an *N*-methyl-*D*-ribofuranuronamide ring (Fig. 1). These compounds have been evaluated for their potential antiviral activity and have been found to be inactive.

2. Results and discussion

The four nucleosides **1a**, **1b** and **2a**, **2b** were synthesized from 3-*O*-acetyl-1,2:5,6-di-*O*-isopropylidene- α -*D*-allofuranose (**3**) (Scheme 1).

The synthesis of the starting material **3** has been described previously.^{16,17} We followed the literature procedure and obtained compound **3** starting from 1,2:5,6-di-*O*-isopropylidene- α -*D*-glucofuranose by inversion of configuration in the 3-position and

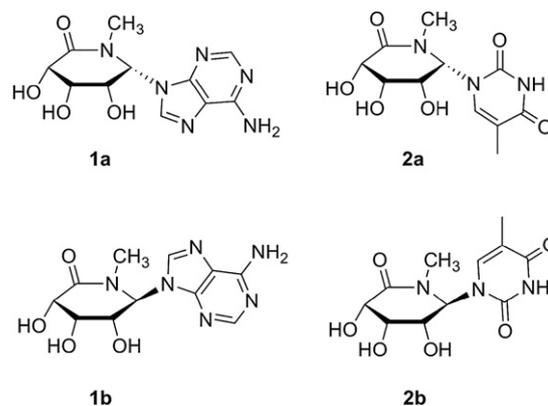
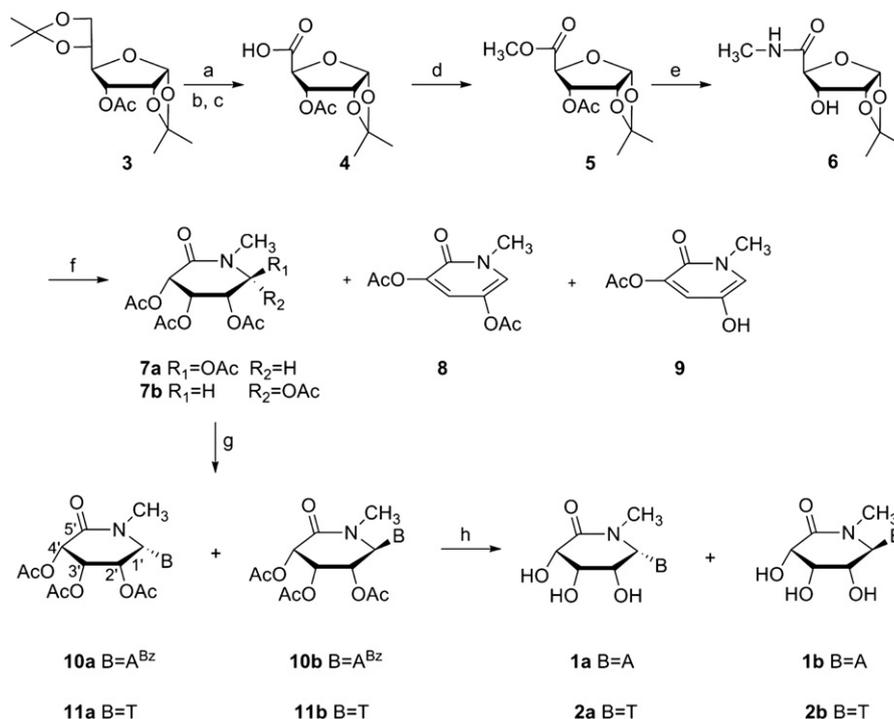


Figure 1. Structures of **1a**, **1b** and **2a**, **2b**.

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Scheme 1. Reagents: (a) 75% AcOH; (b) KIO₄, CH₃OH/H₂O; (c) KMnO₄, 50% AcOH; (d) EDAC, DMAP, CH₃OH; (e) CH₃NH₂, THF; (f) BF₃·Et₂O, acetic anhydride; (g) silylated thymine or N⁶-benzoyladenine, SnCl₄, CH₃CN; (h) saturated NH₃ in CH₃OH.

followed by acetylation.^{18–20} Removal of the 5,6-*O*-isopropylidene protection group was carried out under mild acidic conditions (75% acetic acid). Without purification, the obtained diol was oxidized to an aldehyde intermediate and further to the carboxylic acid **4**.²¹ The methylamide group of **6** was introduced by esterification of the carboxylic acid **4** to yield compound **5** followed by reaction with methylamine under pressure.^{22,23}

The critical reaction in this synthetic scheme is the rearrangement of the furanose ring to the *N*-methyl-*D*-ribofuranuronamide ring starting from *N*-methyl-1,2-*O*-isopropylidene- α -*D*-ribofuranuronamide (**6**) in a reproducible way.¹⁴ This rearrangement was accomplished using BF₃·Et₂O followed by acetylation using acetic anhydride to obtain *N*-methyl-1,2,3,4-tetra-*O*-acetyl-*D*-ribofuranuronamides (**7a** and **7b**). However, these compounds easily undergo elimination reaction in the presence of Lewis acid. Thin layer chromatographic analysis of the reaction indicated that four products are formed. The two more mobile compounds were separated by column chromatography, and shown by ¹H NMR to be the desired products, **7a** and **7b**. The anomeric configuration of the compounds **7a** and **7b** was deduced from the ¹H NMR coupling constants and confirmed where appropriate by Noediff experiments. In this way, compound **7b** was shown to be the α -anomer mainly on the basis of the small long-range coupling of 1.3 Hz between H-1' and H-3', pointing to a *W*-coupling path between these two protons. This can happen only when both protons are equatorial and thus *cis*-oriented, and therefore only possible in the α -anomer. The rather high coupling constant $J_{2',3'}=7.9$ Hz in the other isomer **7a**, which has thus the β -configuration, can be rationalized by the adoption of a more boat-like conformation of this six-membered lactam ring. This also explains the absence of a large $J_{1',2'}$ coupling between these two *trans*-oriented protons.

The less mobile material consisted of two aromatic compounds **8** and **9**. They were separated by column chromatography. Both compounds are more likely originated by the elimination reaction of **7a** and **7b**. Compound **8** is the less stable compound and is deacetylated easily to **9**. Compound **9** is the less mobile and more stable component of the mixture.

Having the key *N*-methyl-*D*-ribofuranuronamide sugar moiety in hand, final conversion to the target nucleosides was attempted using silylated thymine and N⁶-benzoyladenine in the presence of SnCl₄.^{24–26} The nucleosidation reactions led, in both cases, to moderate yield of the adenine and thymine nucleoside analogues. The reaction did not proceed exclusively via the neighboring group effect of the 2'-*O*-Ac group to give preponderant β -anomers.²⁶ The desired nucleosides were obtained as a mixture of diastereoisomers (**10a**, **10b** and **11a**, **11b**). The final compounds **1a**, **1b** and **2a**, **2b** were obtained by deprotection using saturated ammonia in methanol.²⁴ Preparative TLC or column chromatographic purification followed by HPLC was used to purify the acetyl-protected nucleoside precursors and the final products.

The structure of compounds **1a**, **1b** was unambiguously assigned using a combination of NMR techniques (H,H-COSY, H,C-HSQC, and H,C-HMBC). Compound **1b** shows a large ¹H NMR coupling constant ($J=8.0$ Hz), which is typical for a *trans* di-axial relationship between H-1' and H-2'. This implies a β -configuration. Compound **1a** is then the α -anomer having a slightly distorted half-chair conformation and resulting in an increased coupling constant between H-2' and H-3' ($J_{2',3'}=5.5$ Hz), and the disappearance of the long-range H-1'/H-3' *W*-coupling. Noediff at H-1' gave a significant signal increase only for H-2', and thus confirms the C-1' conformation and at the same time the α -configuration of **1a**. In the same way, we characterized compounds **2a** and **2b** and also the protected derivatives **10a**, **10b**, and **11a**, **11b**, since they all showed very similar ¹H-couplings as in **1a** and **1b**. Compound **1a** is the more mobile one on TLC of the two diastereoisomers. Normally we had expected that the presence of the 2'-*O*-acetyl group would direct sugar–base condensation reaction in the direction of the β -anomer via intermediate **12** (Fig. 2). Apparently, this is not the sole mechanism as a considerable amount of the α -anomer is formed. The *N*-Me group may participate in the reaction mechanism analogous to the condensation reaction of *N*-methyloxycarbonyl protected pyrrolidine and nucleobase via the formation of an iminium ion (**13**).²⁶ The infrared absorption of compound **7a** (1694 cm⁻¹) is situated at somewhat higher wavelength than that of *N*-methyl- α -piperidone

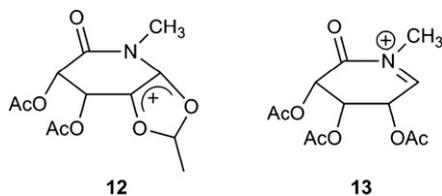


Figure 2. Possible intermediates of condensation reaction.

(1669 cm^{-1}), which may indicate lesser amide resonance.^{27–29} This suggests that the lone pair electrons on the nitrogen atom of the cyclic amide (**7a** and **7b**) can be involved in the mechanism of sugar–base condensation reaction.

In order to overcome the formation of α -nucleoside during the condensation reaction, we tried different condensation conditions. By using SnCl_2^{30} as the catalyst, also a mixture of α and β anomers was formed. The condensation reaction was not successful using TMS–OTf as catalyst (no nucleosides were formed).

Additionally, we also attempted to introduce the base moiety to the *N*-unsubstituted-*D*-ribofuranuronamide sugar moiety, which was obtained from compound **5** by ammonolysis and further rearrangement.¹⁴ By using silylated thymine catalyzed with SnCl_4 , we found that the *N*-unsubstituted sugar moiety was unstable, and the corresponding nucleoside could not be obtained.

We have also attempted to synthesize the 3'-OMe analogue starting from 3-*O*-methylated 1,2:5,6-di-*O*-isopropylidene-*D*-allofuranose and following a similar reaction sequence.³¹ The yield of the key rearrangement, however, was only about 10%. More side product **8** and **9** were obtained. Methylation of the 3-OH group gives rise to an easier elimination reaction in contrast to what was expected.

No antiviral activity has been found when the compounds **1a**, **1b** and **2a**, **2b** were evaluated against Parainfluenza-3-virus, Reovirus-1, Sindbis virus, Coxsackie virus B4, Punta Toro virus, Feline Corona virus, Feline Herpes virus, Herpes simplex virus-1, Herpes simplex virus-2, Vaccinia virus, Vesicular stomatitis virus, Respiratory syncytial virus, Influenza virus A, Influenza virus B. No cytotoxicity was detected in MDCK cells (Madin Darby Canine Kidney cells), HeLa cells, CRFK cells (Crandell-Rees Feline Kidney cells), and Vero cells.

3. Conclusions

We reported the synthesis of *N*-methyl-*D*-ribofuranuronamide nucleosides **1a**, **1b** and **2a**, **2b** by a rearrangement of a furanose sugar with a carboxamide function in the 4-position to a ribopyranuronamide ring followed by condensation with adenine and thymine nucleobases, catalyzed by SnCl_4 . The compound without the *N*-methyl group on the ribopyranuronamide sugar moiety proved to be less stable during the condensation reaction. No antiviral activity and toxicity was found for the four deprotected nucleosides **1a**, **1b** and **2a**, **2b**.

4. Experimental

4.1. General

For all reactions, analytical grade solvents were used. All moisture-sensitive reactions were carried out in oven-dried glassware (135 °C). Anhydrous CH_3CN was refluxed over calcium hydride and distilled. A Bruker Avance II 500 MHz spectrometer and a Bruker Avance 300 MHz apparatus were used for ^1H NMR and ^{13}C NMR. Accurate mass spectra were recorded on a Fourier transform ion-cyclotron resonance (FTICR) mass spectrometer, apex-Qe (Bruker Daltonics, Bremen, Germany) with a passively shielded 9.4 T superconducting magnet equipped with an Apollo 2 CombiSource

(Bruker Daltonics, Bremen, Germany). Tuning in positive electro-spray mode to resolution of 95,000 (at m/z 400) and calibration (from m/z 100 to 1500) were performed using poly-DL-alanine (Sigma–Aldrich, St. Louis, MO). Spray voltage was set to 4000 V, capillary temperature 240 °C. Samples were infused in a water/acetonitrile (1:1) mixture with a flow rate of 180 $\mu\text{L}/\text{h}$; 32 scans of 512k datapoints were acquired and averaged. For the acquisition and processing, software ApexControl 1.0 and DataAnalysis 3.4 (Bruker Daltonics, Bremen, Germany) were used, respectively. Precoated aluminum sheets (MN ALUGRAM SIL G/UV₂₅₄ 20×20 cm) were used for TLC; the spots were examined with UV light. Preparative TLC was performed on 20×40 cm plate coated with MN-Silica Gel P/UV 254. Column chromatography was performed on ICN silica gel 63–200, 60 Å. Analytical HPLC was performed on waters 600E-2487 system using Alltima HP C18 column (4.6×250 mm) at the flow rate of 1 mL/min by a gradient elution of acetonitrile and water. Preparative HPLC was performed on waters 1525-2487 system using Prep C18 5 μm column 19×150 mm at the flow rate of 16 mL/min by a gradient elution of acetonitrile and water. Elemental analyses were performed at the Department of Chemistry, K.U. Leuven. For sake of clarity of the NMR signal assignment, sugar protons and carbons are numbered with a prime, and signals of base protons and carbons are given without prime.

4.2. 3-*O*-Acetyl-1,2-*O*-isopropylidene- α -*D*-5-ribofuranonic acid (**4**)

Compound **3** (4.43 g, 14.65 mmol) was dissolved in 75% acetic acid (37 mL) at room temperature and stirred for 2 days. Solvents were removed in vacuo. The residue obtained was dissolved in methanol (30 mL) and water (45 mL) was added. Then KIO_4 (3.69 g, 16.04 mmol) was added portionwise to the solution, while cooling by ice bath and vigorously stirring. After 5 h, the reaction mixture was diluted with methanol (50 mL), and then the inorganic salts were filtered and washed with ethanol. The filtrate was evaporated. The syrup obtained was dissolved in 50% acetic acid (70 mL) and the solution was cooled to 10 °C. KMnO_4 (4.74 g, 29.99 mmol) was added in small portions while stirring. After 6 h, sodium sulfite was added to the solution until MnO_2 disappears, and the pH of the solution was adjusted to 2 with concentrated hydrochloric acid. The solution was extracted with CH_2Cl_2 (80 mL×4), the extracts were washed with water, dried over Na_2SO_4 , filtered, and evaporated to afford compound **4** (3.08 g, 85.4%) as a syrup. ^1H NMR (300 MHz, MeOD): δ 5.92 (d, 1H, $J=3.6$ Hz, H-1'), 5.01 (dd, 1H, $J_1=4.8$ Hz, $J_2=8.7$ Hz, H-3'), 4.84 (m, 1H, H-2'), 4.50 (d, 1H, $J=8.8$ Hz, H-4'), 2.11 (s, 3H, Me), 1.53 (s, 3H, Me), 1.34 (s, 3H, Me); ^{13}C NMR (75 MHz, MeOD): δ 172.61 (COOH), 171.43 (Me–CO), 114.74 (isopropylidene–C), 106.39 (C-1'), 79.13 (C-4'), 77.33 (C-2'), 75.49 (C-3'), 27.01 (Me), 26.86 (Me), 20.36 (Me); HRMS for $\text{C}_{10}\text{H}_{14}\text{O}_7$ ($\text{M}+\text{Na}$)⁺ calcd: 269.0637, found: 269.0630.

4.3. Methyl 3-*O*-acetyl-1,2-*O*-isopropylidene- α -*D*-ribofuranuronate (**5**)

N-Ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDAC, 722 mg, 3.77 mmol) and DMAP (18 mg, 0.15 mmol) were added to a solution of compound **4** (370 mg, 1.50 mmol) in MeOH (11 mL), and the reaction mixture was stirred for 24 h at room temperature. After the solvent was removed by rotary evaporation, the residue was dissolved in dichloromethane (70 mL), washed with water (60 mL) and brine (60 mL), dried over anhydrous Na_2SO_4 , filtered, and concentrated to dryness. The residue was purified by column chromatography (*n*-hexane/EtOAc, 3:1) to yield compound **5** (300 mg, 76.9%) as a colorless syrup. ^1H NMR (300 MHz, CDCl_3): δ 5.79 (d, 1H, $J=3.6$ Hz, H-1'), 4.85 (dd, 1H, $J_1=4.7$ Hz, $J_2=8.7$ Hz, H-3'), 4.68 (dd, 1H, $J_1=3.7$ Hz, $J_2=4.7$ Hz, H-2'), 4.40 (d, 1H, $J=8.8$ Hz, H-4'), 3.62 (s, 3H, Me), 1.99 (s, 3H, Me), 1.40 (s,

3H, Me), 1.20 (s, 3H, Me); ^{13}C NMR (75 MHz, CDCl_3): δ 169.74 (–COOMe), 169.31 (Me–CO), 113.60 (isopropylidene–C), 104.69 (C-1'), 77.50 (C-4'), 75.90 (C-2'), 73.89 (C-3'), 52.44 (COO–Me), 26.57 (Me), 26.52 (Me), 20.36 (Me); HRMS for $\text{C}_{11}\text{H}_{16}\text{O}_7$ ($\text{M}+\text{Na}$) $^+$ calcd: 283.0794, found: 283.0788.

4.4. N-Methyl-1,2-O-isopropylidene- α -D-ribofuranuronamide (6)

A solution of compound **5** (310 mg, 1.19 mmol) and 2 M methylamine in THF (7 mL) was heated for 24 h at 55 °C in a sealed tube. The reaction mixture was concentrated to dryness, and the residue was purified by column chromatography (*n*-hexane/EtOAc/MeOH, 5:5:1) to yield **6** (250 mg, 96.6%). ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 7.97 (q, 1H, $J=4.6$ Hz, NH), 5.74 (d, 1H, $J=3.4$ Hz, H-1'), 5.34 (d, 1H, $J=6.7$ Hz, 3'-OH, exchanged with D_2O), 4.48 (t, 1H, $J=3.9$ Hz, H-2'), 4.03 (d, 1H, $J=8.9$ Hz, H-4'), 3.92 (m, 1H, H-3'), 2.59 (d, 3H, $J=4.7$ Hz, NH–Me), 1.45 (s, 3H, Me), 1.27 (s, 3H, Me); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$ and one drop D_2O): δ 169.38 (CO), 111.96 (isopropylidene–C), 103.95 (C-1'), 79.51 (C-2'), 78.61 (C-3'), 73.93 (C-4'), 26.83 (NH–Me), 26.51 (Me), 25.34 (Me); HRMS for $\text{C}_9\text{H}_{15}\text{NO}_5$ ($\text{M}+\text{Na}$) $^+$ calcd: 240.0848, found: 240.0840. Elemental analysis found, %: C 49.37; H 7.08; N 6.30. Calculated, %: C 49.76; H 6.96; N 6.45.

4.5. N-Methyl-1,2,3,4-tetra-O-acetyl-D-ribofuranuronamide (7)

To a solution of compound **6** (840 mg, 3.87 mmol) in acetic anhydride (15 mL) was added 0.4 mL of $\text{BF}_3 \cdot \text{Et}_2\text{O}$. The reaction mixture was stirred for 19 h and another 0.2 mL of $\text{BF}_3 \cdot \text{Et}_2\text{O}$ was added. The reaction was continued to stir for 4 h and then poured into iced water and extracted with CH_2Cl_2 (60 mL \times 3). The combined extracts were washed with saturated NaHCO_3 solution and water, dried over anhydrous Na_2SO_4 , filtered, and concentrated to dryness. The residue was purified by column chromatography (*n*-hexane/EtOAc, 2:1) to yield compounds **7a** and **7b** (430 mg, 32.2%) as a syrup. A second column chromatographic purification was needed to obtain analytical pure compounds. The compound with a large $J_{2',3'}=7.9$ Hz could be assigned as **7a**, while the one with a small $J_{1',3'}=1.3$ Hz, $J_{1',2'}=4.4$ Hz could be assigned as **7b**. Data for **7a** (β -anomer): ^1H NMR (300 MHz, CDCl_3) δ 6.27 (d, 1H, $J=4.0$ Hz, H-1'), 5.79 (d, 1H, $J=4.8$ Hz, H-4'), 5.54 (dd, 1H, $J_1=4.8$ Hz, $J_2=7.9$ Hz, H-3'), 5.39 (dd, 1H, $J_1=4.0$ Hz, $J_2=7.9$ Hz, H-2'), 2.99 (s, 3H, N–Me), 2.14 (s, 6H, 2Me), 2.06 (s, 3H, Me), 2.05 (s, 3H, Me); ^{13}C NMR (75 MHz, CDCl_3) δ 170.13, 169.76, 169.64, 169.55, 165.33, 79.05 (C-1'), 67.46 (C-4'), 67.21 (C-2'), 66.90 (C-3'), 33.74 (N–Me), 20.81 (Me), 20.67 (Me), 20.62 (2Me); IR (film on KBr tablet, cm^{-1}): 2937 (m, $\nu_{\text{C-H}}$, CH_3), 1748 (s, $\nu_{\text{C=O}}$, OAc), 1694 (s, $\nu_{\text{C=O}}$, CONCH₃), 1372 (s, $\nu_{\text{O-C}}$, OAc), 1215 (s, $\nu_{\text{C-O}}$), 1047 (s, $\nu_{\text{C-N}}$); HRMS for $\text{C}_{14}\text{H}_{19}\text{NO}_9$ ($\text{M}+\text{H}$) $^+$ calcd: 346.1132, found: 346.1133. Data for **7b** (α -anomer): ^1H NMR (300 MHz, CDCl_3) δ 6.32 (dd, 1H, $J_{1',2'}=4.4$ Hz, $J_{1',3'}=1.3$ Hz, H-1'), 5.70 (ddd, 1H, $J_{3',4'}=3.7$ Hz, $J_{3',2'}=2.3$ Hz, $J_{3',1'}=1.3$ Hz, H-3'), 5.56 (d, 1H, $J=3.7$ Hz, H-4'), 5.43 (dd, 1H, $J_{2',1'}=4.4$ Hz, $J_{2',3'}=2.3$ Hz, H-2'), 2.94 (s, 3H, N–Me), 2.15 (s, 3H, Me), 2.14 (s, 3H, Me), 2.12 (s, 3H, Me), 2.03 (s, 3H, Me); ^{13}C NMR (75 MHz, CDCl_3) δ 170.29, 169.99, 169.56, 169.27, 165.54, 79.65 (C-1'), 68.60 (C-3'), 66.73 (C-4'), 64.56 (C-2'), 33.29 (N–Me), 20.83 (Me), 20.68 (Me), 20.54 (Me), 20.41 (Me); HRMS for $\text{C}_{14}\text{H}_{19}\text{NO}_9$ ($\text{M}+\text{H}$) $^+$ calcd: 346.1132, found: 346.1138.

4.6. 1-Methyl-2-oxo-1,2-dihydropyridine-3,5-diyl diacetate (8) and 5-hydroxy-1-methyl-2-oxo-1,2-dihydropyridin-3-yl acetate (9)

After compounds **7a** and **7b** were obtained, the column chromatography was further eluted with *n*-hexane/EtOAc (1:1 to 2:3) to yield compound **8** (320 mg, 36.7%) as a yellowish solid and

compound **9** (100 mg, 14.1%) as a white solid. Data for **8**: ^1H NMR (300 MHz, CDCl_3) δ 7.16 (d, 1H, $J=2.9$ Hz, H-6), 7.01 (d, 1H, $J=2.9$ Hz, H-4), 3.46 (s, 3H, N–Me), 2.21 (s, 3H, Me), 2.15 (s, 3H, Me); ^{13}C NMR (75 MHz, CDCl_3) δ 169.11, 168.27, 156.50 (C-2), 140.92 (C-3), 131.22 (C-5), 127.49 (C-6), 126.14 (C-4), 38.04 (N–Me), 20.83 (Me), 20.70 (Me); HRMS for $\text{C}_{10}\text{H}_{11}\text{NO}_5$ ($\text{M}+\text{H}$) $^+$ calcd: 226.0710, found: 226.0708. Data for **9**: ^1H NMR (300 MHz, CDCl_3) δ 7.04 (br, 1H, 5-OH), 6.83 (d, 1H, $J=2.7$ Hz, H-6), 6.68 (d, 1H, $J=2.7$ Hz, H-4), 3.59 (s, 3H, N–Me), 2.25 (s, 3H, Me); ^{13}C NMR (75 MHz, CDCl_3) δ 169.36, 157.7 (C-2), 146.40 (C-3), 133.77 (C-5), 119.72 (C-6), 111.00 (C-4), 37.68 (N–Me), 20.94 (Me); HRMS for $\text{C}_8\text{H}_9\text{NO}_4$ ($\text{M}+\text{H}$) $^+$ calcd: 184.0604, found: 184.0603. Elemental analysis found, %: C 52.04; H 5.20; N 6.92. Calculated, %: C 52.46; H 4.95; N 7.65.

4.7. (2S,3S,4S)-N-Methyl-1-(N⁶-benzoyladenine-9-yl)-2,3,4-tri-O-acetyl-D-ribofuranuronamides (10a and 10b)

N^6 -Benzoyladenine (0.29 g, 1.21 mmol), ammonia sulfate (10 mg, 0.08 mmol) and 15 mL of HMDS were added to a dried flask. The mixture was refluxed overnight under nitrogen. HMDS was removed in vacuo with exclusion of moisture. To the flask with the residue was added a solution of compounds **7a** and **7b** (410 mg, 1.19 mmol) in 12 mL of dry CH_3CN and the solution was cooled to 0 °C and followed by dropwise addition of SnCl_4 (280 μL , 2.39 mmol), under N_2 . The reaction mixture was stirred overnight at room temperature and then poured into ice-cold saturated aqueous NaHCO_3 solution (20 mL). It was extracted with CH_2Cl_2 (60 mL \times 3) and then NaHCO_3 solution layer was concentrated to a small volume. The residue was taken up in H_2O (30 mL) and CH_2Cl_2 (60 mL). The combined CH_2Cl_2 layer was washed with water, dried over anhydrous Na_2SO_4 , filtered, and concentrated in vacuo. To obtain an analytical sample, the brown crude solid product (390 mg, 62.5%) was further purified by preparative TLC ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 12:1) and then by preparative HPLC with 25% CH_3CN in water to yield **10a** and **10b**. Data for (1S,2S,3S,4S)-isomer (α -anomer) **10a**: ^1H NMR (300 MHz, CDCl_3) δ 8.90 (s, 1H, NH), 8.81 (s, 1H, H-2), 8.06 (s, 1H, H-8), 8.03 (d, 2H, $J=7.2$ Hz, benzoyl), 7.64 (t, 1H, $J=7.2$ Hz, benzoyl), 7.55 (t, 2H, $J=7.3$ Hz, benzoyl), 6.42 (d, 1H, $J=4.7$ Hz, H-1'), 5.97 (d, 1H, $J=3.6$ Hz, H-4'), 5.73 (dd, 1H, $J_1=3.6$ Hz, $J_2=6.6$ Hz, H-3'), 5.63 (dd, 1H, $J_1=4.6$ Hz, $J_2=6.6$ Hz, H-2'), 2.82 (s, 3H, N–Me), 2.21 (s, 3H, Me), 2.18 (s, 3H, Me), 2.02 (s, 3H, Me); ^{13}C NMR (75 MHz, CDCl_3) δ 169.79 (CO–acetyl), 169.06 (CO–acetyl), 168.62 (CO–acetyl), 165.56 (CO–N–Me), 164.58 (CO–NH–benzoyl), 153.43 (C-2), 151.98 (C-4), 150.05 (C-6), 141.22 (C-8), 133.37 (C–benzoyl), 133.22 (C–benzoyl), 129.15 (C–benzoyl), 128.03 (C–benzoyl), 122.95 (C-5), 67.73 (C-1'), 67.09 (C-3'), 66.77 (C-4'), 66.04 (C-2'), 32.27 (N–Me), 20.82 (Me), 20.70 (Me), 20.64 (Me); HRMS for $\text{C}_{24}\text{H}_{24}\text{N}_6\text{O}_8$ ($\text{M}+\text{H}$) $^+$ calcd: 525.1728, found: 525.1727. Data for (1R,2S,3S,4S)-isomer (β -anomer) **10b**: ^1H NMR (500 MHz, CDCl_3) δ 8.94 (s, 1H, NH), 8.82 (s, 1H, H-2), 8.13 (s, 1H, H-8), 8.03 (d, 2H, $J=7.5$ Hz, *o*-benzoyl), 7.64 (t, 1H, $J=7.5$ Hz, benzoyl), 7.55 (t, 2H, $J=7.6$ Hz, *m*-benzoyl), 6.04 (dd, 1H, $J_1=2.0$ Hz, $J_2=8.0$ Hz, H-2'), 6.01 (d, 1H, $J=2.4$ Hz, H-4'), 5.88 (overl. m, 1H, H-3'), 5.87 (d, 1H, $J=8.0$ Hz, H-1'), 2.73 (s, 3H, N–Me), 2.23 (s, 3H, Me), 2.22 (s, 3H, Me), 1.92 (s, 3H, Me); ^{13}C NMR (75 MHz, CDCl_3) δ 169.96 (CO–acetyl), 169.70 (CO–acetyl), 168.95 (CO–acetyl), 164.76 (CO–N–Me), 164.64 (CO–NH–benzoyl), 153.52 (C-2), 151.85 (C-4), 150.16 (C-6), 141.38 (C-8), 133.20 (Cp–benzoyl), 133.18 (Ci–benzoyl), 129.10 (Co–benzoyl), 128.04 (Cm–benzoyl), 124.82 (C-5), 70.32 (C-1'), 68.69 (C-3'), 68.42 (C-2'), 67.12 (C-4'), 31.03 (N–Me), 20.97 (Me), 20.69 (Me), 20.43 (Me); HRMS for $\text{C}_{24}\text{H}_{24}\text{N}_6\text{O}_8$ ($\text{M}+\text{H}$) $^+$ calcd: 525.1728, found: 525.1715.

4.8. (2S,3S,4S)-N-Methyl-1-(thymine-1-yl)-2,3,4-tri-O-acetyl-D-ribofuranuronamides (11a and 11b)

Thymine (0.18 g, 1.43 mmol), ammonia sulfate (20 mg, 0.15 mmol) and 4 mL of HMDS were added to a dried flask. The

mixture was refluxed overnight under nitrogen. HMDS was removed in vacuo with exclusion of moisture. To the flask with the residue was added a solution of compounds **7a** and **7b** (400 mg, 1.16 mmol) in 10 mL of dry CH₃CN and the solution was cooled to 0 °C and followed by dropwise addition of SnCl₄ (300 μL, 2.56 mmol), under N₂. The reaction mixture was stirred overnight at room temperature and then poured into ice-cold saturated aqueous NaHCO₃ solution (30 mL). It was extracted with CH₂Cl₂ (60 mL×3) and then the NaHCO₃ solution layer was concentrated to a small volume. The residue was divided between H₂O (30 mL) and CH₂Cl₂ (60 mL). The combined CH₂Cl₂ layer was washed with water, dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo. To obtain an analytical sample, the brown crude solid product (280 mg, 58.7%) was further purified by preparative TLC (CH₂Cl₂/MeOH, 12:1) and then by preparative HPLC with 15% CH₃CN in water to yield **11a** and **11b**. Data for (1*R*,2*S*,3*S*,4*S*)-isomer (α-anomer) (**11a**): ¹H NMR (300 MHz, CDCl₃) δ 8.05 (br, 1H, NH), 7.05 (s, 1H, H-6), 5.93 (d, 1H, *J*=3.2 Hz, H-1'), 5.76 (d, 1H, *J*=3.5 Hz, H-4'), 5.46 (dd, 1H, *J*₁=3.7 Hz, *J*₂=7.0 Hz, H-3'), 5.37 (dd, 1H, *J*₁=3.1 Hz, *J*₂=7.0 Hz, H-2'), 2.91 (s, 3H, N-Me), 2.20 (s, 3H, Me), 2.16 (s, 3H, Me), 2.01 (s, 3H, Me), 1.97 (s, 3H, Me); ¹³C NMR (75 MHz, CDCl₃) δ 170.01, 169.24, 169.00, 168.57, 165.22, 150.89, 134.08, 112.15, 71.53 (C-1'), 67.53 (C-2'), 67.02 (C-3'), 66.77 (C-4'), 33.80 (N-Me), 20.76 (Me), 20.68 (Me), 20.62 (Me), 13.00 (5-Me); HRMS for C₁₇H₂₁N₃O₉ (M+Na)⁺ calcd: 434.1176, found: 434.1167. Data for (1*S*,2*S*,3*S*,4*S*)-isomer (β-anomer) (**11b**): ¹H NMR (300 MHz, DMSO-*d*₆) δ 11.49 (br, 1H, NH), 7.82 (s, 1H, H-6), 6.01 (br, 1H, H-1'), 5.83 (br, 1H), 5.65 (br, 2H), 2.68 (s, 3H, N-Me), 2.15 (s, 3H, Me), 2.07 (s, Me), 1.97 (s, 3H, Me), 1.78 (s, 3H, Me); ¹³C NMR (75 MHz, DMSO-*d*₆) δ 170.62, 169.72, 169.51, 165.33, 164.22, 151.42, 136.63, 109.84, 69.00 (C-1'), 67.49 (C-4'), 67.22 (C-2' and C-3'), 30.34 (N-Me), 20.97 (Me), 20.77 (2Me), 12.59 (5-Me); HRMS for C₁₇H₂₁N₃O₉ (M+Na)⁺ calcd: 434.1176, found: 434.1166.

4.9. (2*S*,3*S*,4*S*)-*N*-Methyl-1-(adenin-9-yl)-*D*-ribofuranuronamides (**1a** and **1b**)

A solution of the crude product obtained in the previous reaction (compounds **10a** and **10b**) was dissolved in MeOH saturated with ammonia (35 mL) and stirred at room temperature for 1 day. The reaction mixture was concentrated, and the residue was purified by chromatography (*n*-hexane/EtOAc/MeOH, 5:5:0.5 to 5:5:1) to yield compound **1a** (45 mg, 12.9% starting from **7a** and **7b**) and compound **1b** (100 mg, 28.6% starting from **7a** and **7b**). An analytical sample was obtained by purification with preparative HPLC with 1% CH₃CN in water. Data for (1*S*,2*S*,3*S*,4*S*)-isomer (α-anomer) (**1a**): ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.15 (s, 1H, H-2), 8.11 (s, 1H, H-8), 7.30 (s, 2H, NH₂), 6.05 (d, 1H, *J*=4.2 Hz, H'-1), 5.99 (br, 1H, 2'-OH), 5.66 (br, 1H, 3'-OH), 5.21 (br, 1H, 4'-OH), 4.39 (d, 1H, *J*=2.9 Hz, H-4'), 4.08 (dd, 1H, *J*₁=2.9 Hz, *J*₂=5.5 Hz, H-3'), 4.03 (dd, 1H, *J*₁=4.2 Hz, *J*₂=5.4 Hz, H-2'), 2.50 (s, 3H, N-Me); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 171.99 (CO), 156.05 (C-6), 152.72 (C-2), 149.50 (C-4), 140.82 (C-8), 118.26 (C-5), 70.94 (C-3'), 68.80 (C-1'), 67.51 (C-2'), 67.37 (C-4'), 30.67 (N-Me); HRMS for C₁₁H₁₄N₆O₄ (M+H)⁺ calcd: 295.1149, found: 295.1147. Data for (1*R*,2*S*,3*S*,4*S*)-isomer (β-anomer) (**1b**): ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.38 (s, 1H, H-8), 8.13 (s, 1H, H-2), 7.31 (s, 2H, NH₂), 5.61 (d, 1H, *J*=8.0 Hz, H-1'), 5.61 (br, 1H, 2'-OH), 5.56 (br, 1H, 3'-OH), 5.11 (d, 1H, *J*=4.4 Hz, 4'-OH), 4.52 (dd, 1H, *J*₁=2.1 Hz, *J*₂=8.0 Hz, H-2'), 4.28 (d, 1H, *J*=2.4 Hz, H-4'), 4.05 (t, 1H, *J*=2.3 Hz, H-3'), 2.45 (s, 3H, N-Me); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 170.84 (CO), 156.21 (C-6), 152.76 (C-2), 149.67 (C-4), 140.80 (C-8), 119.30 (C-5), 72.42 (C-1'), 71.80 (C-3'), 69.45 (C-4'), 68.60 (C-2'), 29.76 (N-Me); HRMS for C₁₁H₁₄N₆O₄ (M+H)⁺ calcd: 295.1149, found: 295.1146.

*Coupling constants derived from ¹H NMR (300 MHz, MeOD).

4.10. (2*S*,3*S*,4*S*)-*N*-Methyl-1-(thymine-1-yl)-*D*-ribofuranuronamides (**2a** and **2b**)

A solution of the crude product obtained in the previous reaction (compounds **11a** and **11b**) was dissolved in MeOH saturated with ammonia (45 mL) and stirred at room temperature overnight. The reaction mixture was concentrated, and the residue was purified by chromatography (*n*-hexane/EtOAc/MeOH, 5:5:0.5 to 5:5:1) to yield compound **2a** (50 mg, 15.1% starting from **7a** and **7b**) and compound **2b** (100 mg, 30.2% starting from **7a** and **7b**). An analytical sample was obtained by purification with preparative HPLC with 1% CH₃CN in water. Data for (1*R*,2*S*,3*S*,4*S*)-isomer (α-anomer) (**2a**): ¹H NMR (500 MHz, DMSO-*d*₆, at 60 °C): δ 11.12 (br, 1H, NH-3), 7.22 (q, 1H, *J*=1.0 Hz, H-6), 5.70 (br, 1H, 2'-OH), 5.61 (d, 1H, *J*=3.9 Hz, H-1'), 5.45 (br, 1H, 4'-OH), 5.17 (br, 1H, 3'-OH), 4.13 (d, 1H, *J*=3.1 Hz, H-4'), 3.94 (dd, 1H, *J*₁=3.9 Hz, *J*₂=6.7 Hz, H-2'), 3.80 (dd, 1H, *J*₁=3.2 Hz, *J*₂=6.9 Hz, H-3'), 2.66 (s, 3H, N-Me), 1.77 (d, 3H, *J*=1.0 Hz, Me); ¹³C NMR (125 MHz, DMSO-*d*₆): δ 170.86 (CO), 163.84 (C-4), 151.13 (C-2), 136.08 (C-6), 109.28 (C-5), 73.59 (C-1'), 69.94 (C-3'), 69.21 (C-2'), 68.96 (C-4'), 31.24 (N-Me), 12.55 (5-Me); HRMS for C₁₁H₁₅N₃O₆ (M+Na)⁺ calcd: 308.0859, found: 308.0851. Data for (1*S*,2*S*,3*S*,4*S*)-isomer (β-anomer) (**2b**): ¹H NMR (500 MHz, DMSO-*d*₆) δ 11.34 (br, 1H, NH-3), 7.62 (q, 1H, *J*=1.1 Hz, H-6), 5.68 (br, 1H, H-1'), 5.60 (d, 1H, *J*=5.9 Hz, 2'-OH), 5.44 (d, 1H, *J*=3.5 Hz, 3'-OH), 4.99 (d, 1H, *J*=3.2 Hz, 4'-OH), 4.22 (br, 1H, H-4'), 3.98 (m, 1H, H-3'), 3.94 (br, 1H, H-2'), 2.58 (s, 3H, N-Me), 1.79 (s, 3H, Me); ¹³C NMR (75 MHz, DMSO-*d*₆) δ 171.83 (CO), 163.80 (C-4), 151.45 (C-2), 136.15 (C-6), 110.72 (C-5), 71.66 (C-3'), 70.83 (C-1'), 69.06 (C-4'), 68.18 (C-2'), 29.49 (N-Me), 12.05 (5-Me); HRMS for C₁₁H₁₅N₃O₆ (M+Na)⁺ calcd: 308.0859, found: 308.0853.

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