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Synthesis of new mannosyl, galactosyl and glucosyl theophylline nucleosides with potential activity as antagonists of adenosine receptors. DEMA-induced cyclization of glycosylideneiminouracils

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Abstract—The synthesis of D-mannosyl, D-galactosyl and D-glucosyl theophylline nucleosides by diethoxymethyl acetate (DEMA)-induced cyclization of 4-amino-5-glycosylideneimino-1,3-dimethyluracil is reported. 8-Methyltheophylline derivatives of the same sugars were also prepared by Ac_2O/H^+-induced cyclization of their imine precursors. This approach has allowed β-D-mannopyranosyl-, α-D-galactofuranosyl- and β-D-glucofuranosyltheophylline nucleosides to be synthesized for the first time. The inhibition of specific binding at A_1, A_{2A}, A_{2B} and A_3 adenosine receptors in the mannose derivatives is also reported.

Keywords: Iminouracil; Mannosyl, galactosyl and glucosyl nucleosides; Theophylline

1. Introduction

Nucleoside analogues are amongst the most complex and promising anticancer drugs on account of the growing number of anticancer nucleoside analogues available, the abundance of molecular targets for them and the wide variety of effective drug resistance mechanisms.[1] Nucleosides additionally exhibit antiviral activity (e.g., against the human immunodeficiency virus or hepatitis B virus);[2] also, they act as antagonists of various adenosine receptors (particularly those substituted at position 8 in the purine ring).[3] New efforts should therefore be made with a view to fully exploit their synthetic potential.

7-Glycopyranosyl theophylline nucleosides have been prepared in three different ways involving direct coupling of the base and sugar moiety, namely: (i) from theophylline and glycopyranose pentaacetate (fusion method);[4] (ii) from theophylline silver[5] and mercury salts[6] and tetra-*O*-acetyl glycopyranosyl bromide (heavy-metal method); and (iii) from a silylated theophylline and glycopyranose pentaacetate (Vorbrüggen method).[7] Only the β-anomer has thus been obtained for gluco- and galactopyranosyl nucleosides, and the α-anomer for mannopyranosyl nucleosides using these condensation procedures. These results can be explained in the light of the generally accepted reaction mechanism, where glycosidation is produced by an attack of the base to an acetoxonium ion intermediate at the site opposite the 2-acetoxy group to give 1′,2′-*trans*-nucleosides alone (Scheme 1).[8]

The stereochemistry of the process is governed by the axial arrangement of the 2-acetoxy group in mannopyranose pentaacetate, which leads to the α-nucleoside (Scheme 1). To the best of our knowledge, the synthesis of a β-anomer of mannosyl theophylline nucleosides by sugar–nucleobase condensation methods

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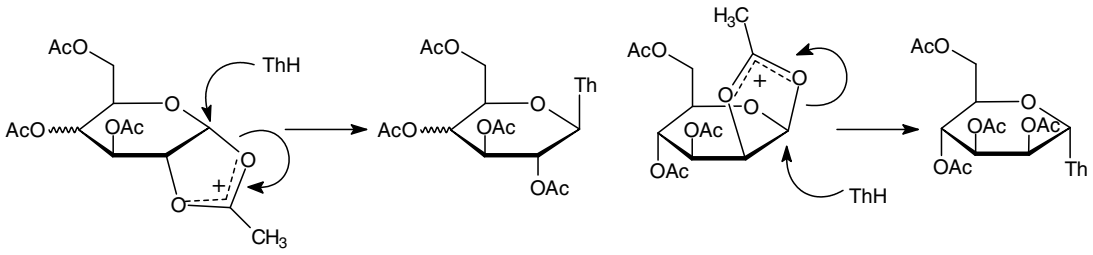


Scheme 1. Mechanism for glucosyl and galactosyl (left) and mannosyl (right) nucleosides.

has previously never been reported. Lichtenthaler and Nakagawa, however, have reported a theophylline β-nucleoside of 3-amino-3-deoxy-β-D-mannopyranose (and derivatives) formed by periodate oxidation and ring expansion via nitromethane reaction with 7-(β-D-ribofuranosyl)theophylline.[9]

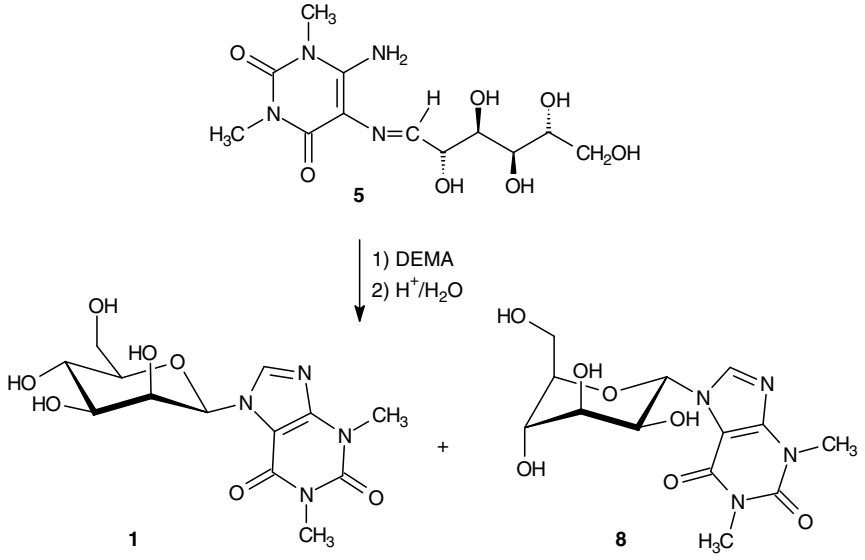
In previous work, we accomplished the synthesis of theophylline nucleosides with D-ribose, D-glucose and L-arabinose by building the imidazole and sugar rings from acyclic imine precursors such as 4-amino-1,3-dimethyl-5-*N*-glycosylideniminouracil as an alternative to direct glycosidation.[10,11] In this work, we developed the first synthesis for β-mannosides 7-(β-D-mannopyranosyl)theophylline (**1**) and 7-(β-D-mannopyranosyl)-8-methyltheophylline (**2**). Also, we further explored this synthetic approach and extended it to mannosyl, glucosyl and galactosyl nucleosides by preparing new nucleoside derivatives for α-D-galactofuranose (**3**) and β-D-glucofuranose (**4**). The starting imines (**5**–**7**) were obtained by the condensation of 4,5-diamino-1,3-dimethyluracil with D-mannose, D-galactose and D-glucose, respectively, in methanol, following the previously reported procedures.[12]

2. Results and discussion

2.1. DEMA-induced cyclization

2.1.1. Mannose derivatives. Treating the imine of mannose **5** with diethoxymethyl acetate (DEMA)[11] at 120 °C for 1 h and workup, provided a crude product whose ^{1}H NMR spectrum in D_2O showed the presence of a mixture containing two main products. After separation by column chromatography and purification by crystallization from ethanol, the major compound was identified as 7-(β-D-mannopyranosyl)theophylline (**1**) (yield 62%) and the minor one 7-(α-D-mannopyranosyl)theophylline (**8**) (yield 20%) (see Scheme 2).

The ^{1}H NMR spectrum for **1** in D_2O exhibits a singlet at 8.05 ppm corresponding to H-8 in theophylline. The anomeric proton gives a broad doublet at 5.92 ppm, whose coupling constant (J <1 Hz) is consistent with an axial–equatorial arrangement with H-2′. This, together with the triplet at 3.53 ppm for H-4′ (J 9.7 Hz), which reflects a *trans*-diaxial arrangement with H-3′ and H-5′, is consistent with the presence of the β-anomer in a $^4C_1$ conformation.

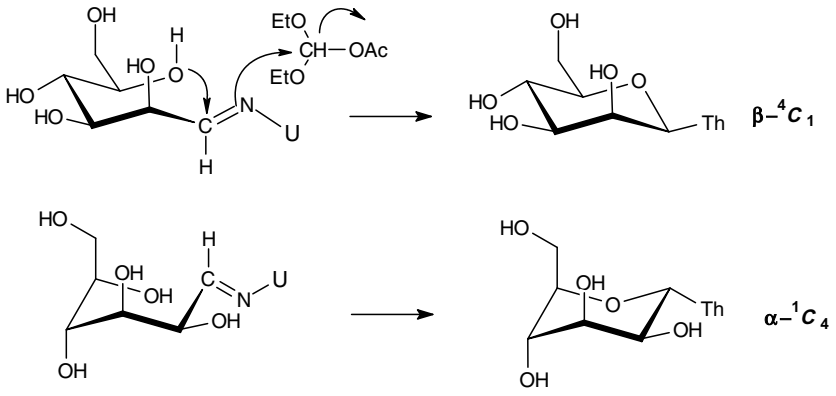


Scheme 2.

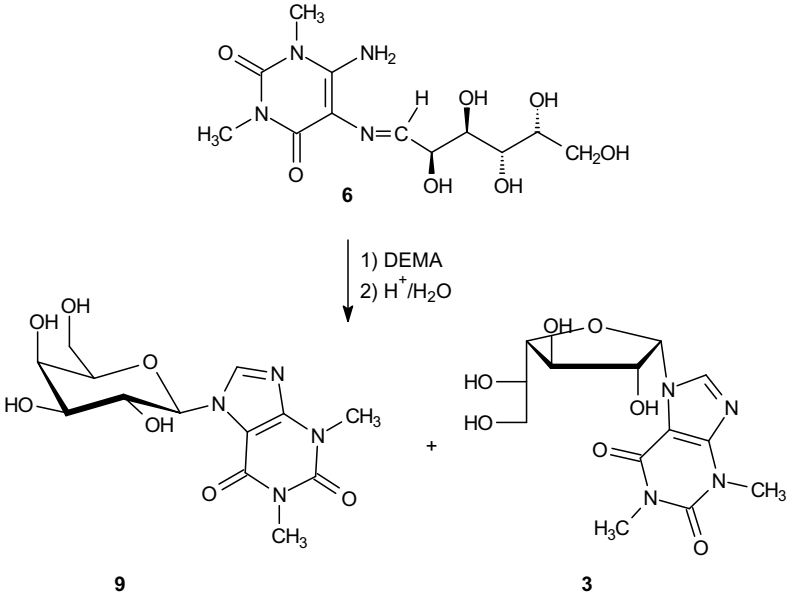
Compound **8** was identified by comparison with the previously reported data.[4,7a] Its ^{1}H NMR spectrum in DMSO-d_6 exhibits a J value between H-1′ and H-2′ of 9.0 Hz and that in D_2O one of 6.3 Hz.[4] This has been ascribed to a preferential $^1C_4$ conformation in α-anomer **8** in DMSO-d_6 solution (Scheme 2) and to a rapid equilibrium with $^4C_1$ in D_2O.[13] The $^1C_4$ conformation, with the aglycon in an equatorial arrangement, is favoured in DMSO-d_6, even in the presence of three axial substituents. This is consistent with the reported data which suggest a preferred equatorial arrangement for amino groups in cyclic sugars.[4] By contrast, β-anomer **1** only occurs in a $^4C_1$ conformation since the aglycon is equatorially arranged and the mannopyranose ring bears only one axial substituent.

In contrast with other synthetic methods for 7-(α-D-mannopyranosyl)theophylline nucleosides, the stereoselectivity on the β-anomer arises in the first step of a two-step reaction. First, DEMA attacks the nitrogen atom in the imino group, and simultaneously the oxygen in the hydroxyl group on C-5′ attacks C-1′ to form the pyranose ring; second, cyclization of 4,5-diaminouracil produces to the imidazole ring and hence theophylline. The attack of the oxygen atom of the hydroxyl group on C-5′ over C-1′ to form the pyranose ring in the first step can take place on both sides of the plane, thus leading to both α- and β-anomers (Scheme 3).

2.1.2. Galactose derivatives. 4-Amino-1,3-dimethyl-5-*N*-galactosylideneiminouracil (**6**) was treated with DEMA under the same conditions as for **5** (Scheme 4). Crude reaction in D_2O showed the product consisting of a 55:45 mixture of 7-(β-D-galactopyranosyl)theophylline (**9**) and 7-(α-D-galactofuranosyl)theophylline (**3**), which were resolved by chromatography.



Scheme 3. DEMA-induced cyclization of mannosylideneimine **5**.



Scheme 4.

The ^{1}H NMR spectrum for **9** in D_2O exhibits a singlet at 8.19 ppm corresponding to theophylline H-8. The anomeric proton in the sugar moiety appears as a doublet at 5.65 ppm (J 9.4 Hz), H-2′ as a triplet at 4.05 ppm (J 9.4 Hz), and H-3′ as a doublet of doublets at 3.72 ppm ($J_{3',2'}$ 9.4 Hz and $J_{3',4'}$ 3.5 Hz), all of which are consistent with the proposed structure.

In the ^{1}H NMR spectrum for compound **3** in D_2O, the anomeric proton of the sugar moiety appears as a doublet at 5.90 ppm (J 5.2 Hz), H-2′ and H-3′ appear as two triplets at 4.50 ppm (J 5.2 and 4.17 Hz, respectively), and H-4′ gives a doublet of doublets at 4.25 ppm (J 5.2 and 3.6 Hz). The ^{13}C NMR spectrum exhibits the signal corresponding to C-4′ at 84.8 ppm (HSQC experiments), that for C-2′ at 80.7 ppm, and that for C-1′ at 91.8 ppm, which is consistent with a furanose ring. A NOESY experiment exposed NOEs among H-1′, H-4′ and H-2′. All these results are consistent with the proposed structure.

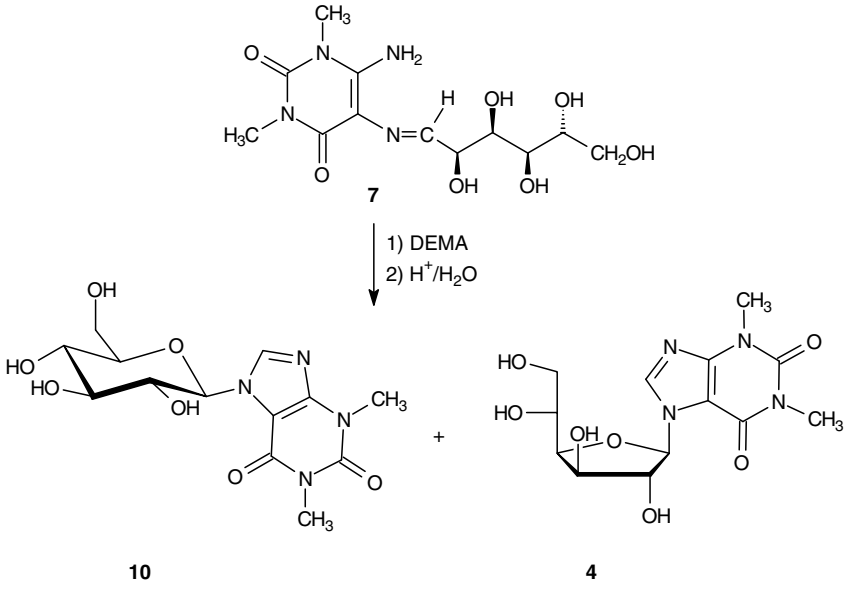
2.1.3. Glucose derivatives. We previously found the reaction of 4-amino-1,3-dimethyl-5-*N*-glucosylideneiminouracil (**7**) with DEMA at room temperature for 20 h afforded 7-(β-D-glucopyranosyl)theophylline (**10**) in 60% yield.[11] However, the presence of nucleoside **3,** with the sugar in galactofuranose form, amongst the products led us to revisit the process by applying the conditions used for **5** (i.e., a shorter reaction time and a higher temperature) to the imine derivative of glucose **7**. The ^{1}H NMR spectrum for the crude reaction in D_2O revealed the presence of a 69:20 mixture of two products in addition to two minor ones (~10%). The most abundant product crystallized as a white solid from the reaction mixture and was identified as **10** (Scheme 5) by comparison with the reported data.[11,13] The second major product was isolated by column chromatography and identified as 7-(β-D-glucofuranosyl)theophylline (**4**) from its spectroscopic data.

The ^{1}H NMR spectrum for **4** in D_2O exhibits a singlet at 5.95 ppm corresponding to the anomeric proton, which is consistent with a glucofuranose structure. The signals assigned to C-1′, C-4′ and C-2′ in the ^{13}C NMR spectrum for the compound in D_2O appear at 94.0, 84.4 and 82.3 ppm (HSQC experiment), respectively, which confirms the presence of a furanose ring. A NOESY experiment exposed NOEs amongst H-1′, H-3′ and H-4′. All these results are consistent with a glucofuranose ring with the base in a β-configuration.

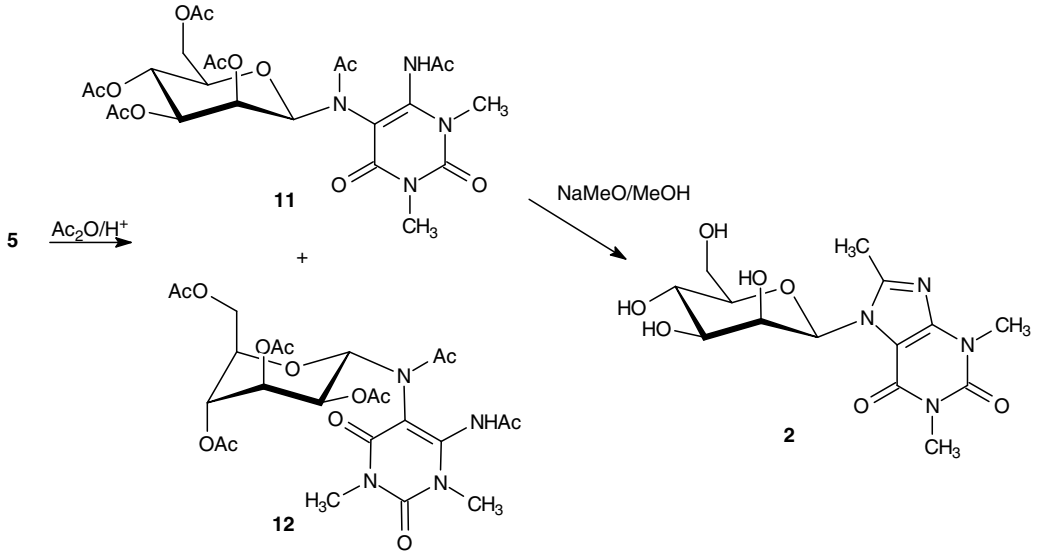
2.2. Ac_2O/H^+-induced cyclization

The cyclization procedure for the imine of mannose **5** was also conducted with Ac_2O/H^+ as the cyclization agent to obtain the C-8 methyl-substituted derivative. The 8-methyltheophylline nucleoside of mannose was thus obtained in two steps involving (i) cyclization of the sugar to the mannopyranosylaminouracil derivatives **11** and **12**, and (ii) cyclization to purine. The reaction of imine **5** with acetic anhydride and sulfuric acid as catalysts at 30 °C for 4 h afforded **11** and **12** in 65% and 8% yield, respectively (Scheme 6).

The ^{1}H NMR spectrum for **5** in $CDCl_3$ exhibits a broad singlet at 6.20 ppm that can be assigned to the H-1′ proton, which is consistent with an axial–equatorial arrangement with H-2′, and a triplet at 5.14 ppm (J 10.0 Hz) corresponding to H-4′ which is consistent with a *trans*-diaxial arrangement of this proton with H-3′ and H-5′. Both the results testify to the presence



Scheme 5.



Scheme 6.

of the β-anomer in a $^4C_1$ conformation. Judging from its $J_{1',2'}$ value, 9.3 Hz, compound **6** is the α-anomer of **5** in a $^1C_4$ conformation.

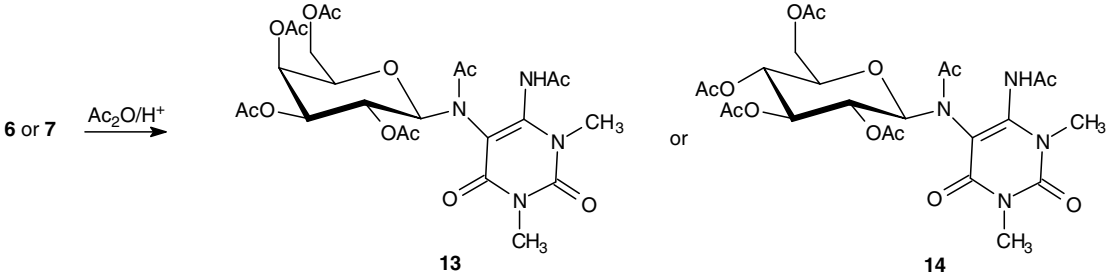
β-Anomer **11** is virtually the sole isomer obtained in this reaction. This is another example where the formation of an amino glycoside with the aglycon in an equatorial arrangement is favoured. These results can be justified in the light of a mechanism similar to that described above.

The second step was carried out by refluxing a solution of **11** with NaOMe in MeOH, which gave 7-(β-D-mannopyranosyl)-8-methyltheophylline (**2**) in 82% yield (Scheme 6). The presence of a singlet at 2.49 ppm (3H) corresponding to the C-8 methyl group in the ^{1}H NMR spectrum confirms the formation of the purine ring. Also, the broad singlet at 6.11 ppm is consistent with an axial–equatorial arrangement for H-1′ and H-2′, and the triplet at 3.51 ppm (J 9.7 Hz), which corresponds to H-4′ in a *trans*-diaxial arrangement with H-3′ and H-5′, confirms a β-$^4C_1$ conformation for **2**.

Treating imines **6** and **7**[14] with acetic anhydride in an acid medium under the same conditions as for **5** provided 4-acetylamino-5-*N*-acetyl(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl) amino-1,3-dimethyluracil (**13**) and 4-acetylamino-5-*N*-acetyl(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl) amino-1,3-dimethyluracil (**14**), respectively, in ~80% yield (Scheme 7). Whereas the mannose derivative was accompanied by 8% of α-anomer **6**, no other isomers of these compounds were detected. N-Glycosides **13** and **14** gave the corresponding 8-methyltheophylline nucleosides of galactose and glucose, both in good yields.[15]

The ^{1}H NMR spectrum for **13** in $CDCl_3$ exhibits a doublet at 6.20 ppm (J 9.6 Hz) corresponding to the anomeric proton, which is consistent with a *trans*-diaxial arrangement of H-1′ and H-2′, in addition to a double doublet at 5.06 ppm ($J_{3',2'}$ 9.6 Hz and $J_{3',4'}$ 3.2 Hz) that can be assigned to H-3′. These results are consistent with a *trans*-diaxial arrangement of H-2′ with respect to H-3′, and an axial–equatorial arrangement of H-3′ with respect to H-4′; also, they agree with the structure of the β-anomer in a $^4C_1$ conformation for compound **13**.

Based on the proposed mechanism for the DEMA- or Ac_2O-induced cyclization of the imine precursors, the reaction should yield four nucleosides, namely, two anomers with the sugar moiety in the pyranose form



Scheme 7.

and another two with the moiety in furanose form (Scheme 3). The outcome depends on the attack side of the C-4′ or C-5′ hydroxyl group over the $C_{1'}$=N imino group during the cyclization step.

In all the cases, the reaction with DEMA yielded two major nucleosides and two minor ones as established by ^{1}H NMR spectroscopy. Also, the major isomer with a β-glycopyranose structure and having the theophylline group in an equatorial arrangement was the most abundant.

Nucleosides with galactose (**3**) or glucose (**4**) with a furanose structure were obtained in 20% and 42% yield, respectively (Table 1). However, only the α-anomer for the galactose nucleosides and the β-anomer for the glucose nucleosides were isolated. This can be explained by assuming the aglycon and the dihydroxyethyl group in the sugar (C-5′ and C-6′) to be on the same side of the furanose ring during the cyclization, probably as a result of hydrogen bonding. Under these conditions, the disparate configuration of C-4′ in galactose and glucose drives the cyclization to the α- and β-anomer, respectively (Fig. 1).

No furanoside isomer was detected in appreciable amounts for the mannose nucleosides, the yield of isolated α and β products being around 80%. One plausible explanation for this result is that all bulky groups of the sugar moiety in the intermediate of the cyclization to the β-furanosyl derivative are on the same side of the furanose ring, so cyclization is hindered. In addition, the α-pyranosyl derivative was obtained in 20% yield. The stability of the $^1C_4$ conformation with the aglycon in an equatorial position (widely reported) is one likely reason.

Interestingly, no furanose derivatives were obtained in the Ac_2O/H^+-induced cyclization reactions either. This can be ascribed to high steric hindrance between the acetyl groups in the furanose form of the reaction intermediate (Fig. 1).

2.3. Binding assays

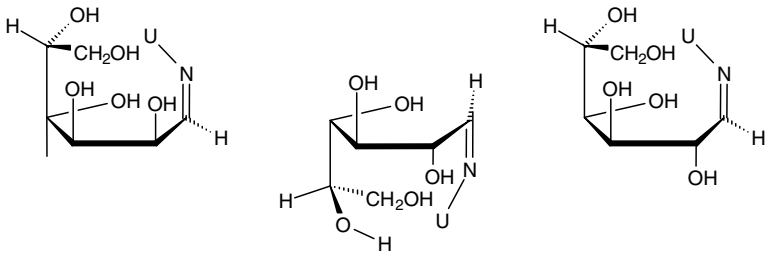
As noted in Section 1, nucleosides are known to act as antagonists of adenosine receptors (particularly those substituted at position 8 in the purine ring).[3] We report here a previous study on radioligand binding at adenosine receptors in some of the synthesized mannosyl nucleosides. Compounds **1**, **8** and **12** were assayed with human recombinant A_1, A_{2A}, A_{2B} and A_3 receptors as described elsewhere.[16] Radioligand assays were performed in vitro with such receptors by measuring the percent inhibition of specific binding at a single concentration (10 μM). The results are summarized in Table 2.

The three compounds exhibited percent inhibition values below 50% at 10 μM. We may therefore assume that these compounds have a low affinity for the receptors. However, based on the data obtained in radioligand binding assays involving recombinant human A_1, A_{2A}, A_{2B} and A_3 receptors (Table 2), we can hypothesize that the modification of compounds **1**, **8** and **12** may afford compounds with an increased affinity for adenosine receptors.

Table 1. Cyclization conditions for the imines of mannose (**5**), galactose (**6**) and glucose (**7**)

Imine	Reaction Cond.[a] reagent|T|t	Nucleoside #/yield (%)	H-1′	H-2′	H-3′	H-4′	C-1′	C-4′
			δ (ppm)/J (Hz)				δ (ppm)	
5	DEMA|120 °C|1 h	**1** (62)	5.92/*br s*	4.01/*3.0*	3.71/*9.0–3.0*	3.53/*9.7*	83.8	80.3
		8 (20)	6.10/*6.3*	4.42/*6.4–3.4*	With H-6′	3.73/*5.2*	80.7	78.9
5	Ac_2O/H^+|30 °C|4 h	**2** (53)	6.11/*br s*	3.91/*3.3*	With H-6′	3.51/*9.7*	86.7	80.8
6	DEMA|120 °C|1 h	**9** (50)	5.65/*9.4*	4.05/*9.4*	3.72/*9.4–3.5*	With H-5′	85.9	78.7
		3 (35)	5.90/*5.2*	4.50/*5.2*	4.17/*5.2*	4.25/*5.3–3.6*	91.8	84.8
7	DEMA|120 °C|1 h	**10** (61)	—	—	—	—	—	—
		4 (15)	5.95/*s*	4.46/*s*	4.16/*2.7*	4.20/*8.8–2.7*	94	84.4

[a] DEMA-induced reactions were followed by acid treatment, and Ac_2O/H^+-induced reactions by treatment with NaOMe in refluxing methanol.



Imine of mannose (**5**) Imine of galactose (**6**) Imine of glucose (**7**)

Figure 1. Cyclization conformation of the three glycosylimino derivatives.

Table 2. Percent displacement ± eem of specific binding at A_1, A_{2A}, A_{2B} and A_3 receptors exhibited by the mannose nucleosides (10 μM)

Compound	A_1	A_{2A}	A_{2B}	A_3
1	27 ± 1	1 ± 3	17 ± 3	10 ± 3
8	15 ± 1	23 ± 1	2 ± 3	27 ± 1
12	17 ± 2	12 ± 1	47 ± 2	34 ± 2

3. Conclusions

The DEMA- or Ac_2O/H^+-induced cyclization of acyclic precursors such as glycosylideneiminouracils provides a powerful, simple method for the synthesis of nucleosides in good yields. While synthesizing other theophylline nucleosides by direct glycosydation requires the prior preparation of various starting materials such as the acetylated sugar, acetylated glycosyl halides and, occasionally, the theophylline derivatives as well, the proposed method only requires the glycosylideniminouracil as a starting material, and this can be easily obtained in an almost quantitative manner by the condensation of the sugar with 4,5-diamino-1,3-dimethyluracil.[12] Also, the cyclization mechanism depends on the particular configuration of the sugar[17] and provides various routes for obtaining nucleosides which cannot be prepared in other ways. The stereoselectivity of the process can be ascribed to the most energetically favored conformation of the acyclic intermediate of the sugar during cyclization. In this work, we prepared four new nucleosides (**1**–**4**) using this methodology. With the mannose precursor, the method affords the β-anomer of mannopyranosyl theophylline in a $^4C_1$ conformation (**1**); by contrast, existing methods only afford preparation of the α-anomer. In addition, the β-anomer of the 8-methyltheophylline derivative (**2**) is obtained when Ac_2O/H^+ is used. The sugars in the new galactose (**3**) and glucose (**4**) nucleosides are in the furanose form.

We are currently in the process of optimizing the DEMA cyclization conditions with a view to extending the reaction to other sugars, and also of using the anhydride/H^+ cyclization of glycosylimines to the prepare 8-substituted nucleosides active as adenosine receptors. Especially interesting are those with oligoethyleneglycol chains at the C-8 position of the purine ring, preparation of which from acyclic precursors is currently under way.

4. Experimental

4.1. General

Melting points were determined with a Gallenkamp instrument and are reported uncorrected. Optical rotations were measured with a Perkin–Elmer Model 241 polarimeter and are given in 10^{-1} deg cm^2 g^{-1} units. UV spectra were recorded on a Hewlett–Packard 8452A spectrophotometer, and IR spectra on Beckman Aculab IV and Perkin–Elmer 883 spectrophotometers. Mass spectrometry was done with a Thermo Finnigan instrument, using the direct injection and electron-impact (EI) modes. ^{1}H NMR and ^{13}C NMR spectra were recorded on 200 MHz AC 200 and 400 MHz ARX 400 Bruker spectrometers, using the residual solvent peak in $CDCl_3$ (δ_H 7.24 for ^{1}H and δ_C 77.0 for ^{13}C), deuterium oxide (with CD_3OD as the internal standard, δ_H 4.8 for ^{1}H and δ_C 49.1 for ^{13}C) or CD_3SOCD_3 (δ_H 2.50 for ^{1}H and δ_C 39.5 for ^{13}C). Chemical shifts are given in δ units. TLC analyses were performed on E. Merck Silica Gel 60 F_{254} plates, and column chromatography on Silica Gel 60 (0.040–0.063 mm). DEMA was purchased from Aldrich Chemical Co. and used as received.

4.2. DEMA-induced cyclization

4.2.1. 7-(β-D-Mannopyranosyl)theophylline (1) and 7-(α-D-mannopyranosyl)theophylline (8). Imine **5** (1.0 g, 3.0 mmol) was treated with DEMA (10 mL) at 120 °C for 1 h. Following the concentration of the reaction mixture at low pressure and the addition of water (10 mL), the resulting residue was evaporated to dryness. The crude reaction was treated with an 8:2 AcOH–water mixture (15 mL) at 20 °C. After stirring for 18 h, the solvent was removed in vacuo, and the residue was treated with NaOMe in MeOH (0.05 M, 20 mL) for 4 h and neutralized with Amberlite IR 120 (H^+) resin. After solvent evaporation, the residue was chromatographed in 8:1:1 acetonitrile–TBME–water to obtain **1** and **8**. The products were recrystallized from ethanol.

Compound **1**: yield, 639 mg (62%), white needles, mp 250–251 °C, $[\alpha]_D^{23}$ +110 (*c* 1.0, H_2O). ^{1}H NMR (400 MHz, D_2O): δ 3.11 (s, 3H, NMe), 3.31 (s, 3H, NMe), 3.49–3.55 (m, 2H, H-4′, H-5′), 3.65 (dd, 1H, *J* 12.4, 5.8 Hz, H-6′), 3.71 (dd, 1H, *J* 9.0, 3.0 Hz, H-3′), 3.80 (br d, 1H, *J* 12.4 Hz, H-6″), 4.01 (d, 1H, *J* 3.0 Hz, H-2′), 5.92 (br d, 1H, $J < 1$ Hz, H-1′), 8.05 (s, 1H, H-8). ^{13}C NMR (100 MHz, DMSO-d_6): δ 27.8 (NMe), 29.7 (NMe), 60.8, 65.8, 69.9, 73.1, 80.3, 83.8 (C-1′), 104.8 (C-5), 141.6 (C-8), 148.3 (C-4), 151.0 (C-6), 154.5 (C-2). EIMS: *m*/*z* (%) 342 (6, M^+), 180 (100), 95 (20). Anal. Calcd for $C_{13}H_{18}N_4O_7{\cdot}0.5H_2O$: C, 44.45; H, 5.45; N, 15.95. Found: C, 44.27; H, 5.20; N, 15.76.

Compound **8**: yield, 206 mg (20%), white solid, mp 198–200 °C, $[\alpha]_D^{22}$ +86 (*c* 1.0, H_2O) [lit.:[4] mp 199–200 °C, $[\alpha]_D$ +86]. ^{1}H NMR (400 MHz, D_2O): δ 3.15 (s, 3H, NMe), 3.51 (s, 3H, NMe), 3.56 (dd, 1H, *J* 12.8, 3.2 Hz, H-6″), 3.63 (m, 1H, H-5′), 3.73 (pt, 1H, *J* 5.2 Hz, H-4′), 3.89–3.91 (m, 2H, H-3′ and H-6″), 4.42 (dd, 1H, *J* 6.4, 3.4 Hz, H-2′), 6.10 (d, 1H, *J* 6.3 Hz, H-1′), 8.13 (s, 1H, H-8). ^{13}C NMR (100 MHz, D_2O): δ 28.0, 29.8, 59.1, 67.0, 67.8, 70.7, 78.9, 80.7, 107.0, 141.5, 148.6, 152.4, 155.6. EIMS: *m*/*z* (%): 342 (12, M^+), 180 (100). Anal. Calcd for $C_{13}H_{18}N_4O_7{\cdot}H_2O$: C, 44.33; H, 5.59; N, 15.55. Found: C, 44.47; H, 5.71; N, 15.32.

4.2.2. 7-(β-D-Galactopyranosyl)theophylline (9) and 7-(α-D-galactofuranosyl)theophylline (3). Imine **6** (1.0 g, 3.0 mmol) was treated with DEMA, using the same procedure as for **5**. After evaporation of the solvent, the residue was chromatographed in 8:1:0.5 acetonitrile–TBME–water to obtain **9** and **3**.

Compound **9**: yield, 515 mg (50%), white solid, mp 250 °C, $[\alpha]_D^{24}$ +25 [lit.:[18] mp 248 °C, $[\alpha]_D$ +23]. ^{1}H NMR (400 MHz, D_2O): δ 3.19 (s, 3H, NMe), 3.38 (s, 3H, NMe), 3.66 (m, 2H, H-6′ and H-6″), 3.72 (dd, 1H, $J_{3',2'}$ 9.4, $J_{3',4'}$ 3.5 Hz, H-3′), 3.84 (m, 2H, H-4′ and H-5′), 4.05 (pt, 1H, J 9.4 Hz, H-2′), 5.65 (d, 1H, J 9.4 Hz, H-1′), 8.19 (s, 1H, H-8). ^{13}C NMR (100 MHz, D_2O): δ 28.9 and 30.7 (Me−N^1 and Me−N^3), 61.4 (C-6′), 69.5, 70.8, 73.7, 78.7, 85.9 (C-1′), 110.3 (C-5), 142.8 (C-8), 149.7 (C-4), 153.2 (C-2), 156.4 (C-6). EIMS: m/z (%): 342 (3, M^+), 180 (100). Anal. Calcd for $C_{13}H_{18}N_4O_7$: C 45.60, H 5.30, N 16.37. Found C 45.68, H 5.36, N 16.17.

Compound **3**: yield, 360 mg (35%), white solid, mp 267 °C, $[\alpha]_D^{24}$ −12. ^{1}H NMR (400 MHz, D_2O): δ 3.15 (s, 3H, NMe), 3.33 (s, 3H, NMe), 3.70 (m, 1H, H-5′), 4.17 (pt, 1H, J 5.2 Hz, H-3′), 4.25 (dd, 1H, $J_{4',3'}$ 5.2 Hz, $J_{4',5'}$ 3.6 Hz, H-4′), 4.49 (m, 2H, H-6′ and H-6″), 4.50 (pt, 1H, J 5.2 Hz, H-2′), 5.90 (d, 1H, J 5.2 Hz, H-1′), 8.01 (s, 1H, H-9). ^{13}C NMR (100 MHz, D_2O): δ 29.2, 31.0 (Me−N^1 and Me−N^3), 63.5 (C-6′), 71.5 (C-5′), 75.7 (C-3′), 80.7 (C-2′), 84.8 (C-4′), 91.8 (C-1′), 107.5 (C-5), 143.5 (C-8), 150.8 (C-4), 153.6 (C-2), 156.7 (C-6). EIMS: m/z (%): 342 (6, M^+), 180 (100). Anal. Calcd for $C_{13}H_{18}N_4O_7$: C, 45.60; H, 5.30; N, 16.37. Found: C, 45.48; H, 5.50; N, 16.17.

4.2.3. 7-(β-D-Glucopyranosyl)theophylline (10) and 7-(β-D-glucofuranosyl)theophylline (4). Compound **7** (1.0 g, 3.0 mmol) was treated with DEMA, using the same procedure as for **5**. A crystallized white solid was obtained from the reaction mixture that was identified as **10**. Then, the methanolic solution was concentrated to dryness, and the solid residue was separated by chromatography in 9:1 CH_2Cl_2–MeOH to obtain **10** and **4**.

Compound **10**: yield, 628 mg (61%), white solid, mp 273–274 °C, $[\alpha]_D^{24}$ −44 (*c* 1.0, water) [lit.:[11,18] mp 272–277 °C, $[\alpha]_D$ −45].

Compound **4**: yield, 154 mg (15%), white solid, mp 185 °C, $[\alpha]_D^{22}$ +35 (*c* 1, H_2O). ^{1}H NMR (400 MHz, D_2O): δ 3.13 (s, 3H, NMe), 3.30 (s, 3H, NMe), 3.64 (dd, 1H, $J_{6'',5'}$ 5.6 Hz, $J_{6',6''}$ 12.4 Hz, H-6″), 3.76 (dd, 1H, $J_{6',5'}$ 2.8 Hz, $J_{6',6''}$ 12.4 Hz, H-6′), 4.02 (m, 1H, H-5′), 4.16 (d, 1H, $J_{3',4'}$ 2.7 Hz, H-3′), 4.20 (dd, 1H, $J_{4',5'}$ 8.8 Hz, $J_{4',3'}$ 2.7 Hz, H-4′), 4.26 (s, 1H, H-2′), 5.95 (s, 1H, H-1′), 7.95 (s, 1H, H-9). ^{13}C NMR (100 MHz, D_2O): δ 29.4, 31.2 (Me−N^1 and Me−N^3), 64.7 (C-6′), 69.8 (C-3′), 75.9 (C-5′), 82.3 (C-2′), 84.4 (C-4′), 94.0 (C-1′), 107.6 (C-5), 141.3 (C-8), 150.3 (C-4), 153.8 (C-2), 157.0 (C-6). EIMS: m/z (%): 342 (2, M^+), 180 (100), 95 (19). Anal. Calcd for $C_{13}H_{18}N_4O_7$: C, 45.60; H, 5.30; N, 16.37. Found: C, 45.70; H, 5.41; N, 16.52.

4.3. Ac_2O/H^+-induced cyclization

4.3.1. 4-Acetylamino-5-*N*-acetyl(2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl)amino-1,3-dimethyluracil (11), and 4-acetylamino-5-*N*-acetyl(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)amino-1,3-dimethyluracil (12). Imine **5** (0.44 g, 1.3 mmol) was treated with a mixture of Ac_2O (20 mL) and H_2SO_4 (0.04 mL) at 30 °C for 4 h. The reaction mixture was concentrated to dryness at low pressure, and the residue was dissolved in MeOH. The solution was kept at 20 °C for 30 min. The MeOH was then evaporated to dryness, and the residue was dissolved in chloroform, washed with aq $NaHCO_3$ and water, and dried over anhyd Na_2SO_4. The chloroform was eliminated to obtain a solid foam, and recrystallization from MeOH gave compound **11** in pure form. The mother liquor consisted of a mixture of compounds **11** and **12**, which were separated by column chromatophy in 10:1 ether–acetone.

Compound **11**: yield, 503 mg (65%), white solid, mp 134–139 °C, $[\alpha]_D^{22}$ −93 (*c* 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.84–2.17 (6 × s, 3H each, COMe), 3.32 (s, 3H, NMe), 3.41 (s, 3H, NMe), 3.92 (m, 1H, H-5′), 4.08 (dd, 1H, J 12.6, 5.7 Hz, H-6″), 4.38 (dd, 1H, J 12.7, 1.7 Hz, H-6′), 5.11 (m, 2H, H-3′), 5.14 (d, 1H, H-4′), 5.49 (d, 1H, J 2.1 Hz, H-2′), 6.20 (s, 1H, H-1′), 8.60 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.2, 20.5, 20.7, 20.8, 21.2, 23.2, 28.7, 32.8, 62.4, 65.0, 69.3, 71.7, 75.8, 80.4, 107.2, 147.7, 150.5, 160.2, 168.3, 169.5 169.6, 169.7, 170.4, 172.3. EIMS: m/z (%): 584 (1, M^+), 542 (7), 223 (26), 169 (100), 422 (30), 254 (19), 109 (55). Anal. Calcd for $C_{24}H_{32}N_4O_{13}$: C, 49.31; H, 5.52; N, 9.58. Found: C, 49.22; H, 5.35; N, 9.30.

Compound **12**: yield, 54 mg (7%), oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.84–2.10 (6 × s, 3H each, 6 × COMe), 3.30 (s, 3H, NMe), 3.36 (s, 3H, NMe), 4.20 (dd, 1H, J 9.6, 3.5 Hz, H-4′), 4.24 (dd, 1H, J 12.6, 2.2 Hz, H-6′), 4.45 (dd, 1H, J 12.6, 2.7 Hz, H-6″), 4.70 (br s, 1H, NH), 4.80 (dd, 1H, J 9.3, 2.2 Hz, H-2′), 5.15 (m, 1H, H-5′), 5.47 (pt, 1H, J 3.8 Hz, H-3′), 6.65 (d, 1H, J 9.3 Hz, H-1′). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.4, 20.6, 20.8, 20.9, 22.0, 23.2, 28.9, 33.0, 61.5, 68.0, 69.2, 69.9, 74.8, 85.2, 104.4, 149.4, 150.5, 160.4, 169.4, 169.5 169.7, 169.9, 170.8, 173.3. EIMS: m/z (%): 584 (2, M^+), 331 (59), 212 (31), 169 (100), 109 (38). Anal. Calcd for $C_{24}H_{32}N_4O_{13}$: C, 49.31; H, 5.52; N, 9.58. Found: C, 49.02; H, 5.43; N, 9.50.

4.3.2. 7-(β-D-Mannopyranosyl)-8-methyltheophylline (2). Compound **11** (400 mg, 0.68 mmol) was treated with NaOMe in refluxing MeOH (15 mL, 0.05 M) for 4 h. After cooling, the reaction mixture was neutralized with Amberlite IR 120 (H^+) resin. The solution was concen-

trated at low pressure. The residue was crystallized from EtOH to obtain 200 mg (82%) of **2** as colourless needles: mp 230–235 °C, $[\alpha]_D^{23}$ +115 (*c* 0.95, H_2O). IR (KBr) *v* cm^{-1}: 3600–3000, 2943, 1700, 1654, 1095. UV (MeOH) λ_{max} nm (logε): 210 (3.80), 276 (3.71). ^1H NMR (400 MHz, D_2O): δ 2.49 (s, 3H, Me–C-8), 3.09 (s, 3H, NMe), 3.26 (s, 3H, NMe), 3.42 (m, 3H, H-5′), 3.51 (t, 1H, *J* 9.7 Hz, H-4′), 3.67 (m, 2H, H-3′, H-6′), 3.82 (dd, 1H, *J* 12.4, 1.5 Hz, H-6″), 3.91 (d, 1H, *J* 3.3 Hz, H-2′), 6.11 (br s, 1H, H-1′). ^{13}C NMR (100 MHz, D_2O): δ 17.3, 29.1, 30.9, 62.1, 67.3, 72.5, 73.5, 80.8, 86.7, 107.0, 148.8, 153.4, 155.8, 156.3. EIMS: *m*/*z* (%): 356 (2, M^+), 194 (100), 109 (20), 68 (28). Anal. Calcd for $C_{14}H_{20}N_4O_7{\cdot}1H_2O$: C, 44.92; H, 5.92; N, 14.97. Found: C, 44.82; H, 5.45; N, 14.89.

4.3.3. 4-Acetylamino-5-*N*-acetyl(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)amino-1,3-dimethyluracil (13). Imine **6** (1.0 g, 3.0 mmol) was treated with a mixture of Ac_2O (35 mL) and H_2SO_4 (0.08 mL) at 30 °C for 4 h. The reaction mixture was concentrated to dryness at low pressure, and the residue dissolved in MeOH. The solution was kept at 20 °C for 1 h. The MeOH was then removed to obtain a solid foam, and the residue was dissolved in chloroform, washed with aq $NaHCO_3$ and water, and dried over anhyd Na_2SO_4. The solid residue was crystallized from EtOH to obtain **13**,[14] yield 1.4 g (80%), mp 133 °C [lit.:[14a] mp 132–133 °C], $[\alpha]_D^{24}$ −50 (*c* 1.0, $CHCl_3$). IR (KBr) *v* cm^{-1}: 3355, 3250, 1745, 1700. ^1H NMR (400 MHz, $CDCl_3$): δ 1.85–2.35 (6 × s, 3H each, 6 acetates), 3.30 (s, 3H, NMe), 3.38 (s, 3H, NMe), 4.03 (dd, 1H, $J_{6'',6'}$ 11.0 Hz, $J_{6'',5'}$ 6.8 Hz, H-6″), 5.17 (m, 1H, H-5′), 4.24 (dd, 1H, $J_{6',6''}$ 11.0 Hz, $J_{6',5'}$ 5.6 Hz, H-6′), 5.00 (pt, 1H, $J_{2',1'}$ and $J_{2',3'}$ 9.6 Hz, H-2′), 5.06 (dd, 1H, $J_{3',2'}$ 9.6 Hz, $J_{3',4'}$ 3.2 Hz, H-3′), 5.41 (pd, 1H, *J* 3.2 Hz, H-4′), 6.20 (d, 1H, $J_{1',2'}$ 9.6 Hz, H-1′), 9.18 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.4, 20.6, 20.7, 21.1, 21.6, 23.4, 29.2, 33.2, 61.4, 64.6, 67.1, 72.3, 73.3, 83.3, 104.5, 123.6, 150.4, 160.2, 169.4, 169.6, 169.8, 170.1, 170.2, 173.0. EIMS: *m*/*z* (%): 584 (M^+, 1), 182 (80), 170 (100). Anal. Calcd for $C_{24}H_{32}N_4O_{13}$: C, 49.31; H, 5.52; N, 9.58. Found: C, 49.29; H, 5.64; N, 9.42.

4.3.4. 4-Acetylamino-5-*N*-acetyl(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)amino-1,3-dimethyluracil (14). Following the same procedure as for **13** (Section 4.3.3), from imine **7** (1.0 g, 3.0 mmol) compound **14** was obtained: yield 1.4 g (80%), mp 229 °C, $[\alpha]_D^{24}$ −99.5 (*c* 1.0, $CHCl_3$).[14] IR (KBr) *v* cm^{-1}: 3355, 3250, 1745, 1700. ^1H NMR (400 MHz, $CDCl_3$): δ 1.86–2.08 (6 × s, 3H each, 6 acetates), 3.27 (s, 3H, NMe), 3.37 (s, 3H, NMe), 3.92 (m, 1H, H-5′), 4.24 (m, 2H, H-6′ and H-6″), 4.83 (pt, 1H, $J_{4',5'}$ 10.0 Hz, H-4′), 5.02 (pt, 1H, $J_{3',4'}$ 10.0 Hz, H-3′), 5.21 (pt, 1H, $J_{3',2'}$ 10.0 Hz, H-2′), 6.22 (d, 1H, $J_{1',2'}$ 10.0 Hz, H-1′), 9.02 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.4, 20.6, 20.7, 21.1, 21.6, 23.4, 29.2, 33.2, 61.4, 64.6, 67.1, 72.3, 73.3, 83.3, 104.6, 149.2, 150.2, 160.2, 169.2, 169.3, 169.8, 170.1, 170.3, 173.0. EIMS: *m*/*z* (%): 584 (1), 182 (80), 170 (100). Anal. Calcd for $C_{24}H_{32}N_4O_{13}$: C, 49.31; H, 5.52; N, 9.58. Found: C, 49.29; H, 5.64; N, 9.42.

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