DOI: 10.1002/ejoc.201301913

Synthesis of Purine Nucleosides from D-Glucuronic Acid Derivatives and Evaluation of Their Cholinesterase-Inhibitory Activities

Nuno M. Xavier,*[a] Stefan Schwarz,[a,b] Pedro D. Vaz,[a] René Csuk,[b] and Amélia P. Rauter[a]

Keywords: Nucleosides / Nucleobases / Carbohydrates / Glycosylation / Biological activity / Uronic acids

Glucuronolactones were used as precursors for N^9 and N^7 purine nucleosides containing glucuronic acid derivatives in their structures. Acetylated *N*-benzylglucofuran- and glucopyranuronamides were synthesized in a few steps from glucofuranurono-6,3-lactone. They were converted into the corresponding furanosyl and pyranosyl uronamide-based nucleosides by *N*-glycosylation with silylated 2-acetamido-6-chloropurine in the presence of trimethylsilyl triflate. The triacetylated bicyclic lactone was coupled itself with the nucleobase to give bicyclic N^9,N^7 nucleosides. Tri-*O*-acetyl-glucopyranurono-6,1-lactone was used for the first time as a glycosyl donor for *N*-glycosylation, and led to β-configured N^9- and N^7-linked purinylglucuronides under reaction conditions similar to those used with the 1-*O*-acetyl-substituted glycosyl donors. The cholinesterase inhibitory profiles of the synthetic nucleosides bearing glucuronic acid derivatives as glycons were evaluated, and they showed moderate selective acetylcholinesterase inhibitory activities (K_i = 14.78–50.53 μM). The best inhibition was shown by the furanosyl N^9-linked uronamide-based purine nucleoside.

Introduction

Naturally-occurring nucleosides with uronic acid moieties or their derivatives in their structures are reported to have a variety of biological properties, namely antibacterial, antiviral, or antitumor activities.[1] Most of these compounds are pyrimidine nucleosides. Examples include the cytosine nucleosides Gougerotin and Blasticidin S, which contain, respectively, a 4-amido-glucuronamide motif and a 2,3-unsaturated 4-amido-uronic acid moiety.[2] Both of these molecules have broad-spectrum antibacterial activities, as well as antiviral and antitumor effects. Blasticidin S is also a fungicide, and it has been used against *Pyricularia oryzae*, which causes the serious rice blast disease in Asia.[3] The total synthesis of these natural compounds has been reported.[4] Among the few known natural purine nucleosides containing uronic acid moieties are the antibiotics amipurimycin and the miharamycins,[5,6] which also effectively inhibit the growth of *Pyricularia oryzae*. The distinguishing feature of these compounds is the presence of higher and branched-chain uronic acids in their structures. Although the total synthesis of these complex nucleosides has not yet been accomplished, syntheses of their core structures or of synthetic analogues have been reported.[7,8] Synthetic nucleosides bearing uronic acid moieties or their derivatives are molecules of potential pharmacological interest, bearing in mind the biological profiles of their naturally-occurring counterparts. Wolfrom and McWain[9] reported the first synthesis of nucleosides containing D-glucuronic acid derivatives as glycons, namely the N^9 adenine nucleoside of methyl tri-*O*-acetyl-D-glucuronate. They achieved the synthesis by coupling methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate bromide with 6-acetamido-9-chloromercuripurine. Treatment of the uronate-based nucleoside with methanolic ammonia gave the corresponding glucuronamide-containing nucleoside. Uronic acid, uronate, or uronamide-based nucleosides bearing pyrimidine,[10] uracil,[11] cytosine,[11] and purine,[12–15] moieties have been synthesized, and some of these compounds were found to have significant biological activities. Methods for their synthesis involve the *N*-glycosylation of silylated nucleobases with glucuronyl donors such as glycosyl halides, 1-*O*-acetyl donors,[11b,12] or methyl glycosides,[15] and the oxidation of the C-5′ hydroxy group of nucleosides.[11c,16] Among the reported biologically active compounds, 2-acetamido-6-chloropurine nucleosides containing a bicyclic uronate moiety were found to be cholinesterase (ChE) inhibitors, with high degrees of inhibition and high selectivities towards butyrylcholinesterase (BChE).[15] Inhibition of ChEs, which hydrolyse the neurotransmitter acetylcholine, is one of the major rational pharmacological strategies for the management of Alzheimer's disease.[17] These results encouraged us to explore the synthesis of a variety of new purine nucleo-

[a] Centro de Química e Bioquímica/Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Edificio C8, 5° Piso,
Campo Grande, 1749-016 Lisboa, Portugal
E-mail: nmxavier@fc.ul.pt
http://webpages.fc.ul.pt/~nmxavier/
[b] Bereich Organische Chemie, Martin-Luther-Universität Halle-Wittenberg,
Kurt-Mothes-Str. 2, 06120 Halle (Saale), Germany
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201301913.

sides with a uronic-acid-derived backbone with the aim of studying the potential of this family of compounds as anticholinesterase agents further.

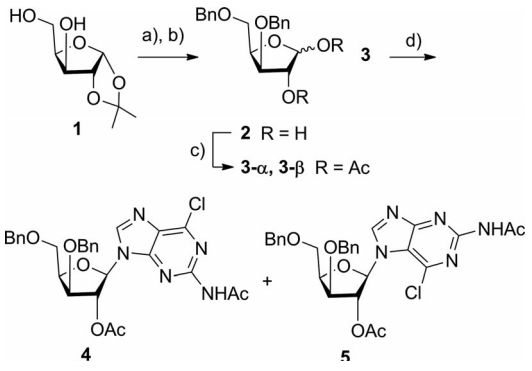
In this paper, we report the synthesis of N^9 and N^7 purine nucleosides containing glucofuranurono-6,3-lactone, *N*-benzylglucuronoamide, and glucuronic acid moieties, and the assessment of their cholinesterase (ChE) inhibitory activities.

1-*O*-Acetylglucuronyl derivatives, including tetra-*O*-acetylated furanosyl- and pyranosyl-glucuronoamides derived from glucofuranurono-6,3-lactone, and triacetylated glucofuranurono-6,3-lactone itself, were used as glycosyl donors for the microwave-assisted Lewis-acid-promoted coupling to the silylated nucleobase. Direct *N*-glucuronidation of glucopyranurono-6,1-lactone, leading to glucopyranuronic-acid-based purine nucleosides, is described for the first time.

Results and Discussion

Chemistry

The synthesis of a dibenzylated 1-*O*-acetyl-xylofuranosyl donor **3** (Scheme 1) was first undertaken. This would allow us to test the *N*-glycosylation conditions, but also the nucleosides derived from this donor would be included in the panel of compounds for assessment against ChE, along with those derived from glucuronic acid. The presence of a C-2 participating group to make the coupling of the nucleobase to the sugar stereoselective was also desired. β-Configured nucleosides typically show greater biological activities than their α-anomers.[18]



Scheme 1. *Reagents and conditions:* a) BnBr, NaH, DMF, room temp., 3 h; b) AcOH (70% aq.), reflux, 2 h, 80% over two steps; c) Ac_2O/py, room temp., 16 h, quant.; d) silylated 2-NHAc-6-Cl-purine, CH_3CN, 65 °C, MW, max. 150 W, 30 min, 52% (**4**) and 12% (**5**).

Hence, benzylation of 1,2-*O*-isopropylidene-α-D-xylofuranose (**1**) was followed by removal of the acetonide functionality to give known compound **2**.[19] Upon acetylation, this compound provided α- and β-configured diacetylated derivatives **3α** and **3β** in 20 and 80% yields, respectively, which could be separated by column chromatography.

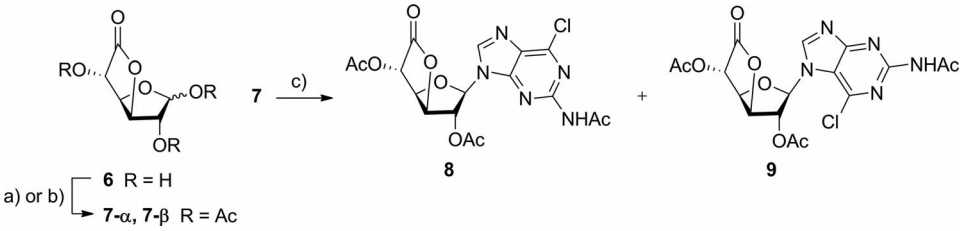
To further evaluate and compare the biological activities of both N^9 and N^7 nucleosides, an *N*-glycosylation method with 1-*O*-acetyl donors that would allow the formation of N^7 nucleosides, which are products of kinetic control, in a reasonable amount relative to the thermodynamic N^9 regioisomers, was also envisaged. Moreover, N^7 nucleosides were reported to have higher ChE inhibitory activities than their N^9 counterparts.[15]

Coupling of **3** with silylated 2-acetamido-6-chloropurine was carried out in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) in acetonitrile at 65 °C, conditions that are reported to favour the formation of N^7 purine nucleosides,[7c] and under microwave irradiation (MW) at 150 W maximum power for 30 min (Scheme 1). Thus, β-linked N^9 purine nucleoside **4** and its N^7 regioisomer **5** were obtained in 52 and 12% yields, respectively. HMBC experiments allowed the unambiguous assignment of **4** and **5**, providing conclusive evidence about the regiochemistry of the nucleosidic linkage. In the N^9 regioisomer (i.e., **4**), the anomeric proton (H-1′) correlates with C-4 of the purine, while in the N^7 nucleoside (i.e., **5**), H-1′ correlates with C-5 of the nucleobase. Among the distinguishing features of the ^1H and ^{13}C NMR spectra of **4** and **5** are the significant chemical shift differences of H-1′, H-8, C-4 and C-8, which are deshielded in the N^7 isomer, and of C-5, which is deshielded in the N^9-substituted purine.

HMBC experiments were also used to definitively assign the structures of further new N^9,N^7 nucleosides whose synthesis is described below.

Glucofuranurono-6,3-lactone (**6**; Scheme 2) was used as the precursor for purine nucleosides containing *N*-benzylglucuronamide moieties, and its triacetylated derivative was converted into bicyclic purine nucleosides.

Acetylation of **6** with acetic anhydride and pyridine at room temperature provided tri-*O*-acetylglucofuranurono-6,3-lactone in 92% yield as an anomeric mixture (**7α/7β** ratio, 1:0.39). Alternatively, treatment of **6** with acetic anhydride and iodine at 115 °C under MW irradiation



Scheme 2. *Reagents and conditions:* a) Ac_2O/py, room temp., 16 h, 92%; b) Ac_2O/I_2, 115 °C, MW, max. 300 W, 40 min, 62% (**7β**); c) silylated 2-NHAc-6-Cl-purine, CH_3CN, 65 °C, MW, max. 150 W, 30 min, 61% (**8**) and 9% (**9**).

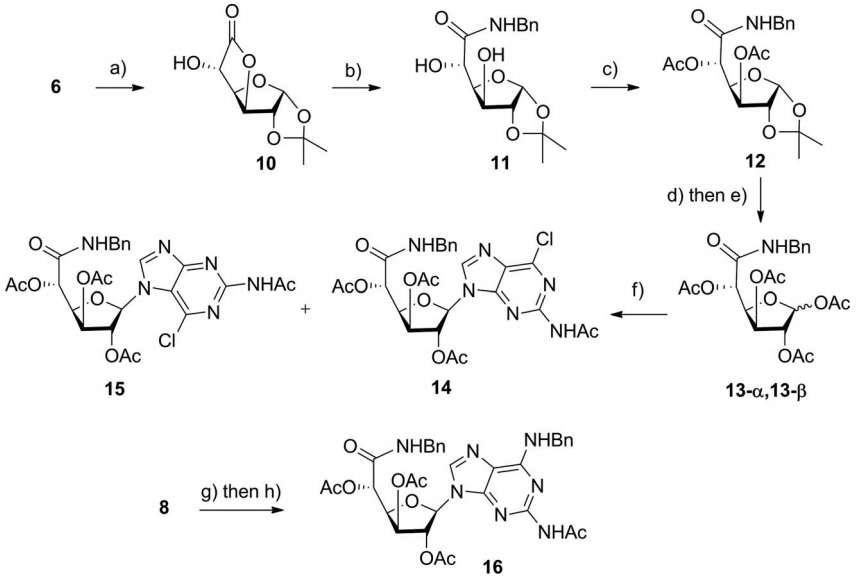
(300 W) for 40 min gave the β-anomer (i.e., **7β**) in 62% yield. *N*-Glycosylation of bis-silylated 2-acetamido-6-chloropurine with **7** under reaction conditions similar to those used with **3** gave glucofuranurono-6,3-lactone-based N^9 purine nucleoside **8** and N^7 compound **9** in 70% total yield, and with an N^9/N^7 ratio of 6.8:1. Although pure **8** could be obtained by subjecting the regioisomeric mixture to flash column chromatography, its regioisomer **9** was inseparably contaminated with compound **8** and could not be isolated, despite repeated separation attempts.

For the synthesis of glucuronamide-based nucleosides (Scheme 3), acetonide-protected glucofuranurono-6,3-lactone **10** was subjected to lactone ring opening with *N*-benzylamine to give *N*-benzylglucofuranuronamide derivative **11** in quantitative yield. Acetylation of **11** (Ac_2O/pyridine) gave 3,5-di-*O*-acetyl derivative **12**, which was treated with TFA (72% aq.) at room temperature to cleave the 1,2-*O*-isopropylidene group. Subsequent acetylation (Ac_2O/pyridine) provided *N*-benzyltetra-*O*-acetyl-glucofuranuronamide **13α,β** in 80% yield as a 1:0.75 mixture of α and β anomers. This anomeric mixture was subjected to MW-assisted TMSOTf-promoted *N*-glycosylation with silylated 2-acetamido-6-chloropurine to give furanuronamide-based N^9 purine nucleoside **14** and its N^7 counterpart **15** in 60% total yield, with a 2.8:1 N^9/N^7 ratio. As for the bicyclic-lactone-based nucleosides, which only had a very slight difference between their R_f values, only N^9 nucleoside **14** was isolable in pure form by flash chromatography.

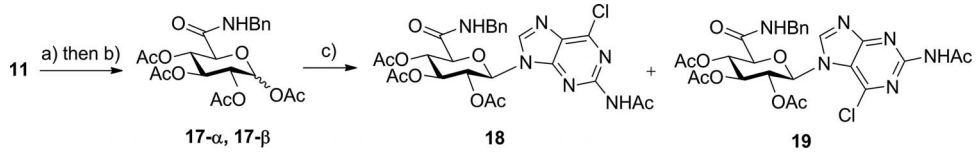
The synthesis of a 6-*N*-benzyladenine nucleoside analogue of **14** was also carried out in order to further evaluate and compare the ChE inhibitory activities of two compounds whose structures differ only in the purine C-6 substituent. Treatment of bicyclic N^9 purine nucleoside **8** with *N*-benzylamine at room temperature for 48 h effected both lactone ring opening and nucleophilic displacement at C-6, and subsequent acetylation gave **16**, albeit in modest yield (25%).

The replacement of the chlorine atom by a benzylamino group at C-6 of the nucleoside was confirmed by the significant changes in the chemical shifts of the signals of the purine moiety in the ^{13}C NMR spectrum. In particular, C-4, C-5, and C-8 of nucleoside **16** are shielded relative to those of **14**; they appeared at δ = 149.6, 116.3, and 137.4 ppm, respectively.

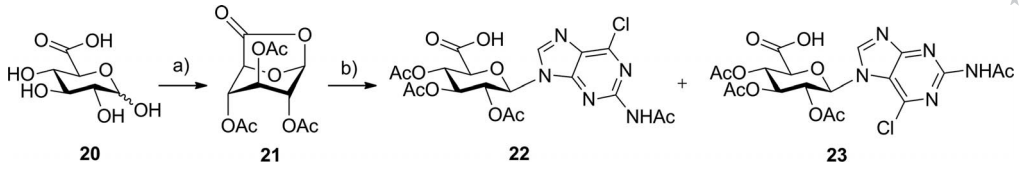
Pyranosyl counterparts of nucleosides **14** and **15** were then synthesized (Scheme 4). Peracetylated *N*-benzylglucopyranuronamide was accessed as an anomeric mixture (**17α/17β**, ratio 1:0.3) by acidic removal of the acetonide moiety of *N*-benzylfuranuronamide **11**, which resulted in ring expansion, followed by acetylation. Subsequent MW-assisted



Scheme 3. *Reagents and conditions:* a) H_2SO_4 (0.5%), acetone, quant.; b) $BnNH_2$, CH_2Cl_2, room temp., 16 h, quant; c) Ac_2O/py, room temp., 16 h, 95%; d) TFA (72% aq.), room temp., 3 h; e) Ac_2O/py, room temp., 20 min, 80% over two steps; f) silylated 2-NHAc-6-Cl-purine, CH_3CN, 65 °C, MW, max. 150 W, 40 min, 44% (**14**) and 16% (**15**); g) $BnNH_2$, CH_2Cl_2, room temp., 48 h; h) Ac_2O/py, room temp., 45 min, 25% over two steps.



Scheme 4. *Reagents and conditions:* a) TFA (60% aq.), room temp., 2.5 h; b) Ac_2O/py, room temp., 10 min, 71% over two steps; c) silylated 2-NHAc-6-Cl-purine, CH_3CN, 65 °C, MW, max. 150 W, 30 min, 42% (**18**) and 29% (**19**).



Scheme 5. *Reagents and conditions:* a) Ac_2O/I_2, 300 W, 115 °C, 20 min, 75%;[20] b) silylated 2-NHAc-6-Cl-purine, CH_3CN, 65 °C, MW, max. 150 W, 50 min, 52% (**22**) and 13% (**23**).

coupling of **17** with the silylated nucleobase gave N^9 purine nucleoside **18** and its N^7 regioisomer **19** in 71% yield, with a 1.5:1 N^9/N^7 ratio.

The generation of related nucleosides incorporating a glucuronic acid moiety was subsequently attempted. For this purpose, 2,3,4-tri-*O*-acetyl glucopyranurono-6,1-lactone (**21**; Scheme 5), easily synthesized by acetylation of glucuronic acid in the presence of iodine under microwave (MW) irradiation,[20] was used as glycosyl donor in the TMSOTf-promoted *N*-glycosylation of silylated 2-acetamido-6-chloropurine. Using coupling conditions similar to those used previously, N^9-linked purinylglucuronide **22** and N^7-linked compound **23** were obtained in 52 and 13% yields, respectively. No α-linked nucleosides were detected, showing that under these conditions, the presence of the 2-*O*-acetyl group in **21** is more important than the fused lactone for the reaction outcome. Although the lactone ring promotes an S_N2 pathway, it acts as an anomeric leaving group releasing the uronic acid functionality, while participation of the group at C-2 with oxocarbenium ring formation drives the β-stereoselectivity of the *N*-glycosylation reaction. The presence of the carboxylic acid in nucleosides **22** and **23** was proved by HRMS, which showed prominent peaks for the corresponding $[M - H + 2Na]^+$ molecular ions.

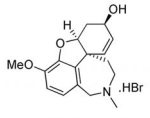
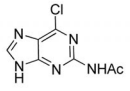
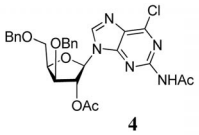
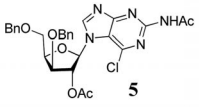
Although *O*- and *C*-glycosylation reactions using anomeric 6,1-lactones, in particular glucopyranurono-6,1-lactone, have been reported,[20–22] their use as glycosyl donors for nucleoside synthesis is, to the best of our knowledge, unprecedented.

Cholinesterase Inhibition Assessment

The in vitro inhibition of ChEs by the newly synthesized nucleosides was evaluated by the method of Ellman[23] using commercially available acetylcholinesterase (AChE) from *Electrophorus electricus*, and butyrylcholinesterase (BChE) from equine serum. Although AChE has been regarded as the most promising therapeutic target for the treatment of Alzheimer's disease (AD),[17a] BChE inhibition may provide additional benefits. Its activity increases over the course of the disease, while AChE activity progressively declines.[24] Consequently, as the severity of dementia progresses, ACh regulation may become increasingly dependent on BChE, so a dual inhibitory action may offer more sustained efficacy in advanced AD.

The synthetic nucleosides, along with 2-acetamido-6-chloropurine and galanthamine hydrobromide, a standard drug commonly used in the treatment AD, were evaluated as inhibitors of ChEs. Their inhibition constants K_i and the type of inhibition are given in Tables 1 and 2.

Table 1. Cholinesterase inibitory activities of purine nucleosides of di-*O*-benzylxylofuranose.

	K_i (AChE, M) [K_i' (AChE, M)]	K_i (BChE, M)	Type of inhibition
 Galantamine	0.54 ± 0.01	9.37 ± 0.67	competitive
	26.12 ± 2.80 (85.75 ± 10.67)	> 100	mixed-type
 **4**	> 20	> 20	n.d. (supposed: competitive)
 **5**	> 20	> 20	n.d. (supposed: competitive)

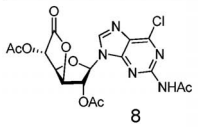
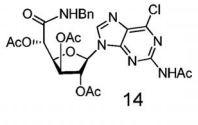
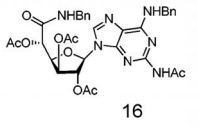
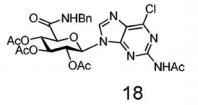
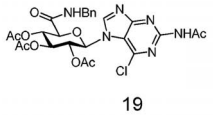
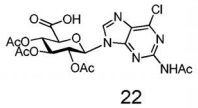
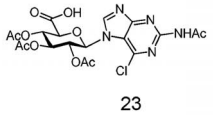
The K_i values of di-*O*-benzylxylofuranosyl-purine derivatives **4** and **5** (Table 1) could not be determined, due to the low solubility of these compounds at concentrations above 20 μM; no inhibitory effect from either compound was detected at lower concentrations.

All the glucuronic-acid-derived nucleosides showed moderate inhibitory activity and selectivity towards AChE, but they showed lower AChE inhibition than galanthamine hydrobromide by two orders of magnitude. None of them significantly inhibited BChE, i.e., at concentrations below 100 μM (Table 2). Most of the compounds showed competitive inhibition of AChE, with the exception of purinylglucuronides **22** and **23**, whose inhibition type was not determined.

The bicyclic glucofuranuronolactone-based nucleoside (i.e., **8**) had a K_i value similar to that of unsubstituted 2-acetamido-6-chloropurine.

Among the uronamide-based nucleosides, pyranosyl N^7 nucleoside **19** showed better AChE inhibition (K_i =

Table 2. Cholinesterase inhibitory activities of purine nucleosides containing glucuronic acid derivatives as glycons.

	K_i (AChE, M)	K_i (BChE, M)	Type of inhibition
 8	23.67 ± 7.58	> 100	competitive
 14	14.78 ± 0.62	> 100	competitive
 16	50.53 ± 6.75	> 100	competitive
 18	30.98 ± 5.39	> 100	competitive
 19	18.72 ± 2.87	> 100	competitive
 22	26.87 ± 8.03	> 100	n.d. (supposed: competitive)
 23	37.87 ± 4.46	> 100	n.d. (supposed: competitive)

18.72 μM) than N^9 compound **18** (K_i = 30.98 μM). Comparing glucuronamide N^9-linked nucleosides **14** and **18**, the inhibitory effect of the furanosyl derivative (i.e., **14**; K_i = 14.78 μM) was about twice as high as that of its pyranosyl isomer (i.e., **19**), and it was the best AChE inhibitor of the compounds tested. An *N*-benzylamino group at C-6 of the purine moiety of **16**, led to a threefold decrease of the enzymatic inhibition (K_i = 50.53 μM) relative to that of the 6-chloro derivative (i.e., **14**).

In terms of the glucuronic acid-based nucleosides, N^9 nucleoside **22** showed a lower K_i value than its N^7 regioisomer **23**. The inhibitory effect of **22** was not significantly different from that of its glucuronamide-based counterpart (i.e., **18**). The N^7-purinylglucuronic acid derivative (i.e., **23**) was, however, less active than the corresponding amide (i.e., **19**) by a factor of two.

Conclusions

Purine nucleosides containing D-glucuronic acid derivatives as sugar moieties were synthesized from glucuronolactone precursors. 1-*O*-Acetylglucofuranosyl or glucopyranosyl-uronamide donors as well as triacetylated glucofuranuronolactone were coupled with persilylated 2-acetamido-6-chloropurine in the presence of TMSOTf to give N^9 and N^7 nucleosides. Exclusive β-nucleoside formation was observed in all cases due to neighbouring-group participation. The regioisomeric N^9/N^7 ratios obtained in the *N*-glycosylation were shown to be dependent on the glycosyl donor structure. In the synthesis of furanosyl nucleosides, higher N^9/N^7 ratios were observed when bicyclic lactone **7** was coupled with the nucleobase; in this reaction, the N^9 product was favoured over the N^7 compound by a factor of about seven. The β-*N*-glycosylation achieved with **7** is driven by the participation of the 2-*O*-acetyl group. However, the sterically crowded nature of the β face, along with the strong conformational rigidity of **7** due to its fused ring system, probably result in a higher control of the regioselectivity in favour of the N^9 linkage, leading to the nucleoside in which the purine substituents are more distant. Purine β-*N*-glycosylation with furanuronamide **13**, whose structure is considerably less strained than **7**, with only a moderately bulky substituent (i.e., an *N*-benzyl group) on the furanose β face, gave the lowest N^9/N^7 ratio (2.8:1) of those obtained with glycofuranosyl donors. The coupling of the silylated purine with acetylated pyranuronamide **17**, whose structure is more flexible than that of **13** due to its six-membered ring, gave an increased amount of N^7 nucleoside and a N^9/N^7 ratio of 1.5:1.

A straightforward method for the stereoselective synthesis of glucuronic acid-containing nucleosides was explored, using acetylated glucopyranurono-6,1-lactone as a glycosyl donor for purine N-glycosylation. The fused lactone can be seen as a tethered anomeric acetate, with the uronic acid group acting as intramolecular leaving group. The C-2 acetate promoted the stereoselective formation of β-linked nucleosides.

The nucleosides containing glucuronic acid derivatives as glycons showed moderate and selective inhibition of acetylcholinesterase. The lowest K_i value for AChE inhibition (K_i = 14.78 μM) of the compounds tested was obtained with N^9-linked purinyl glucofuranuronamide **14**. This interesting result motivates future structural optimization and the synthesis of analogues to obtain improved biological activities.

Experimental Section

General Methods: All reactions were monitored by TLC on Merck 60 F_{254} silica gel aluminium plates with detection under UV light (254 nm) and/or by spraying with a solution of H_2SO_4 (10% in EtOH) or with a solution of cerium(IV) sulfate (0.2% w/v) and ammonium molybdate (5% w/v) in H_2SO_4 (6% aq.). Flash column chromatography was carried out on silica gel 60G (0.040–0.063 mm, E. Merck). Microwave-assisted synthesis was carried out with a CEM Discover system. NMR spectra were recorded with a Bruker Avance 400 spectrometer operating at 400.13 MHz for ^1H and 100.62 MHz for ^{13}C spectra. Chemical shifts are expressed in parts per million relative to tetramethylsilane. Spectra were calibrated using internal tetramethylsilane in the case of $CDCl_3$, or using the residual solvent peak as a reference. HRMS spectra were

acquired with an Apex Ultra FTICR Mass Spectrometer equipped with an Apollo II Dual ESI/MALDI ion source from Bruker Daltonics, and a 7T actively shielded magnet from Magnex Scientific. Optical rotations were measured with a Perkin–Elmer 343 polarimeter at 20 °C (589 nm, sodium D line). Melting points were determined with a Stuart SMP 30 apparatus.

1,2-Di-*O*-acetyl-3,5-di-*O*-benzyl-α-D-xylofuranose (3α) and 1,2-Di-*O*-acetyl-3,5-di-*O*-benzyl-β-D-xylofuranose (3β): Acetic anhydride (4 mL) was added to a solution of 3,5-di-*O*-benzyl-α,β-D-xylofuranose (100 mg, 0.30 mmol) in pyridine (5 mL), and the solution was stirred overnight at room temperature. The solvents were coevaporated with toluene, and the crude product was purified by flash column chromatography (petroleum ether/EtOAc, from 6:1 to 5:1) to give **3α** (25 mg, 20%) and **3β** (100 mg, 80%) as colourless oils.

Data for **3α**: R_f = 0.14 (EtOAc/petroleum ether, 1:7). $[\alpha]_D^{20}$ = +43 (c = 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.36–7.25 (m, 10 H, Ph), 6.40 (d, $^3J_{1,2}$ = 4.6 Hz, 1 H, 1-H), 5.15 (dd, $^3J_{1,2}$ = 4.6, $^3J_{2,3}$ = 6.2 Hz, 1 H, 2-H), 4.63 (d, part A of AB system, $^2J_{a,b}$ = 12.0 Hz, 1 H, a-H, CH_2Ph), 4.57–4.41 (m, 3 H, Bn), 4.32 (dt, 1 H, 4-H), 4.14 (dd, $^3J_{2,3}$ = 6.2, $^3J_{3,4}$ = 4.2 Hz, 1 H, 3-H), 3.51 (dd, part A of ABX system, $^3J_{4,5a}$ = 3.5, $^2J_{5a,5b}$ = 10.5 Hz, 1 H, 5a-H), 3.40 (dd, part B of ABX system, $^3J_{4,5b}$ = 3.6, $^2J_{5a,5b}$ = 10.5 Hz, 1 H, 5b-H), 2.13, 2.12 (2 s, 6 H, 2 CH_3, OAc) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.2, 170.2 (2 *C*O, OAc), 137.8, 137.8 (2 Cq, Ph), 128.6, 128.5, 128.0, 127.9, 127.9, 127.8 (*C*H, Ph), 94.7 (C-1), 83.5 (C-4), 75.5 (C-3), 73.6, 73.2 (2 CH_2Ph), 71.4 (C-2), 69.2 (C-5), 21.3, 20.7 (2 *C*H$_3$, 2 OAc) ppm. HRMS: calcd. for $C_{23}H_{26}O_7$ [M + Na]$^+$ 437.1571; found 437.1560; calcd. for [M + K]$^+$ 453.1310; found 453.1299.

Data for **3β**: R_f = 0.23 (EtOAc/petroleum ether, 1:7). $[\alpha]_D^{20}$ = +40 (c = 1.6, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.36–7.24 (m, 10 H, Ph), 6.12 (br. s, 1 H, 1-H), 5.30 (d, $^3J_{2,3}$ = 4.3 Hz, 1 H, 2-H), 4.62 (d, part A of AB system, $^3J_{a,b}$ = 11.6 Hz, 1 H, a-H, CH_2Ph), 4.53 (d, part A of AB system, $^2J_{a,b}$ = 12.2 Hz, 1 H, a-H, CH_2Ph), 4.49–4.42 (m, 4 H, 2 b-H, 2 CH_2Ph), 4.30 (dd, $^3J_{2,3}$ = 4.3, $^3J_{3,4}$ = 7.7 Hz, 1 H, 3-H), 4.22 (ddd, $^3J_{3,4}$ = 7.7, $^3J_{4,5a}$ = 2.9, $^3J_{4,5b}$ = 4.0 Hz, 1 H, 4-H), 3.68 (dd, part A of ABX system, $^3J_{4,5a}$ = 2.9, $^2J_{5a,5b}$ = 11.0 Hz, 1 H, 5a-H), 3.54 (dd, part B of ABX system, $^3J_{4,5b}$ = 4.0, $^2J_{5a,5b}$ = 11.0 Hz, 1 H, 5b-H), 2.12, 1.91 (2 s, 6 H, 2 CH_3, OAc) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.9, 169.2 (2 *C*O, OAc), 138.1, 137.3 (2 Cq, Ph), 128.5, 128.3, 128.1, 128.0, 127.6, 127.5 (C*H*, Ph), 98.5 (C-1), 81.4 (C-4), 76.4 (C-3), 73.6 (C-2), 73.2 (2 CH_2Ph), 69.0 (C-5), 20.9, 20.8 (2 *C*H$_3$, 2 OAc) ppm. HRMS: calcd. for $C_{23}H_{26}O_7$ [M + Na]$^+$ 437.1571; found 437.1563; calcd. for [M + K]$^+$ 453.1310; found 453.1301.

General Procedure for *N*-Glycosylation with 1-*O*-Acetylglycosyl Donors: 2-Acetamido-6-chloropurine (123 mg, 0.58 mmol) was suspended in anhydrous acetonitrile (2.5 mL) under N_2, and *N*,*O*-bis(trimethylsilyl)acetamide (0.28 mL, 1.16 mmol) was added. The mixture was stirred at room temp. for 20 min. Then, a solution of 1-*O*-acetylglycosyl donor (0.39 mmol) in anhydrous acetonitrile (2.5 mL) was added, followed by dropwise addition of trimethylsilyl triflate (0.45 mL, 2.51 mmol). The reaction mixture was exposed to microwave irradiation at 150 W (P_{max} = 250 Psi), and it was continuously stirred at 65 °C for 30–40 min. The mixture was then diluted with dichloromethane, and washed with satd. aq. $NaHCO_3$ solution. The aqueous phase was extracted with dichloromethane (3×), and the combined organic phases were dried with $MgSO_4$. After filtration and evaporation of the solvent, the residue was purified by column chromatography on silica gel.

2-Acetamido-6-chloro-9-(2-*O*-acetyl-3,5-di-*O*-benzyl-β-D-xylofuranosyl)purine (4) and 2-Acetamido-6-chloro-7-(2-*O*-acetyl-3,5-di-*O*-benzyl-β-D-xylofuranosyl)purine (5): According to the general procedure, and starting from 1,2-di-*O*-acetyl-3,5-di-*O*-benzyl-D-xylofuranose (**3**; 160 mg, 0.39 mmol) and 2-acetamido-6-chloropurine (123 mg, 0.58 mmol), the *N*-glycosylation of the corresponding silylated purine with **3** in the presence of trimethylsilyl triflate (0.45 mL, 2.51 mmol), was complete within 30 min. Purification by flash column chromatography on silica gel (EtOAc/petroleum ether, from 2:3 to 2:1) gave N^9 nucleoside **4** (114 mg, 52%) and its N^7 regioisomer **5** (25 mg, 12%) as colourless oils.

Data for **4**: R_f = 0.61 (EtOAc/petroleum ether, 7:3). $[\alpha]_D^{20}$ = +57 (c = 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.57 (br. s, 1 H, NH), 8.35 (s, 1 H, 8-H), 7.38–7.24 (m, 10 H, Ph), 6.16 (d, $^3J_{1',2'}$ = 3.3 Hz, 1 H, 1′-H), 5.70 (dd, $^3J_{1',2'}$ = 3.6, $^3J_{2',3'}$ = 4.7 Hz, 1 H, 2′-H), 4.69–4.44 (m, $^3J_{2',3'}$ = 4.7, $^3J_{3',4'}$ = 5.4, $^2J_{a,b}$ = 11.4, $^2J_{a,b}$ = 11.8 Hz, 5 H, 3′-H, 2 CH_2Ph) 4.34–4.29 (ddd, $^3J_{3',4'}$ = 5.4, $^3J_{4,5'a}$ = 2.1, $^3J_{4,5'b}$ = 3.5 Hz, 1 H, 4′-H), 3.82 (dd, part A of ABX system, $^3J_{4,5'a}$ = 2.1, $^2J_{5'a,5'b}$ = 10.8 Hz, 1 H, 5′a-H), 3.59 (dd, part B of ABX system, $^3J_{4,5'b}$ = 3.5, $^2J_{5'a,5'b}$ = 10.8 Hz, 1 H, 5′b-H), 2.49 (s, 3 H, CH_3, NHAc), 2.13 (s, 3 H, CH_3, OAc) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.0 (*C*O, NHAc, *C*O, Ac), 152.2 (C-4 or C-2), 152.1 (C-2 or C-4), 151.4 (C-6), 143.4 (C-8), 137.3, 137.3 (2 Cq, Ph), 128.7, 128.6 (*C*H, Ph), 128.5 (C-5), 128.3, 128.2, 128.1, 128.0 (*C*H, Ph), 87.7 (C-1′), 82.4 (C-4′), 76.1 (C-3′), 74.8 (C-2′), 73.6, 73.5 (2 CH_2Ph), 68.6 (C-5′), 25.3 (*C*H$_3$, NHAc), 20.8, (*C*H$_3$, OAc) ppm. HRMS: calcd. for $C_{28}H_{28}ClN_5O_6$ [M + H]$^+$ 566.1801; found 566.1787; calcd. for [M + Na]$^+$ 588.1620; found 588.1601.

Data for **5**: R_f = 0.25 (EtOAc/petroleum ether, 7:3). $[\alpha]_D^{20}$ = +40 (c = 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.92 (s, 1 H, 8-H), 8.18 (br. s, 1 H, NH), 7.42–7.20 (m, 10 H, Ph), 6.58 (d, $^3J_{1',2'}$ = 2.5 Hz, 1 H, 1′-H), 5.58 (dd, $^3J_{1',2'}$ = 2.5, $^3J_{2',3'}$ = 4.2 Hz, 1 H, 2′-H), 4.64–4.26 (m, $^3J_{2',3'}$ = 4.2, $^3J_{3',4'}$ = 6.5, $^2J_{a,b}$ = 11.9, $^2J_{a,b}$ = 11.1 Hz, 6 H, 3′-H, 4′-H, 2 CH_2Ph) 3.91 (br. d, part A of ABX system, $^2J_{5'a,5'b}$ = 10.8 Hz, 1 H, 5′a-H), 3.55 (br. d, part B of ABX system, $^2J_{5'a,5'b}$ = 10.8 Hz, 1 H, 5′b-H), 2.60 (s, 3 H, CH_3, NHAc), 2.18 (s, 3 H, CH_3, OAc) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.6 (*C*O, NHAc, *C*O, Ac), 163.9 (C-4), 152.7 (C-2 or C-6), 147.8 (C-8), 143.0 (C-2 or C-6), 137.0, 137.0 (2 Cq, Ph), 128.9, 128.7, 128.5 128.4, 128.3, 128.2 (*C*H, Ph), 118.2 (C-5), 89.2 (C-1′), 82.3 (C-4′), 75.6 (C-2′), 75.0 (C-3′), 73.7, 73.3 (2 CH_2Ph), 67.3 (C-5′), 25.4 (*C*H$_3$, NHAc), 20.8, (*C*H$_3$, OAc) ppm. HRMS: calcd. for $C_{28}H_{28}ClN_5O_6$ [M + H]$^+$ 566.1801; found 566.1782; calcd. for [M + Na]$^+$ 588.1620; found 588.1603.

1,2,5-Tri-*O*-acetyl-α,β-D-glucofuranurono-6,3-lactone (7α,7β)

Method 1: A mixture of glucofuranurono-6,3-lactone (250 mg, 1.42 mmol), pyridine (7 mL), and acetic anhydride (5 mL) was stirred overnight at room temp. After coevaporation with toluene, the residue was purified by flash column chromatography (EtOAc/petroleum ether, 1:4) to give triacetylated glucuronolactone **7α**,**7β** (393 mg, 92%; α/β ratio = 1:0.39) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.55 (d, $^3J_{1,2(\alpha)}$ = 4.8 Hz, 0.72 H, 1α-H), 6.17 (br. s, 0.28 H, 1β-H), 5.53 (d, $^3J_{4,5(\alpha)}$ = 5.2 Hz, 0.72 H, 5α-H), 5.36–5.21 (m, 1.56 H, 2α-H, 2β-H, 4β-H, 5β-H), 5.16–5.03 (m, 1.72 H, 3α-H, 4α-H, 3β-H), 2.22 (s, 2.16 H, CH_3, Ac), 2.16, 2.14, 2.13 (3 s, 3.84 H, 2 β-CH_3, β-Ac, α-CH_3, α-Ac), 2.09 (s, 2.16 H, CH_3, α-Ac), 2.06 (s, 0.84 H, CH_3, β-Ac) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.4, 169.7, 169.6, 169.3, 169.2, 169.2, 168.7, 168.6 (*C*O, α, β), 98.5 (C-1β), 94.8 (C-1α), 82.6 (C-3α), 81.7 (C-3β), 77.9 (C-2β), 76.8 (C-2α), 76.2 (C-4β), 75.4 (C-4α), 68.5 (C-5α), 68.0 (C-5β), 20.6, 20.6, 20.4, 20.1, 20.1, 19.8 (*C*H$_3$, Ac, α, β) ppm.

Method 2: Alternatively, acetylation of glucofuranurono-6,3-lactone with Ac_2O/I_2 under MW irradiation (300 W) gave only the β-anomer. I_2 (0.2 g) was added to a mixture of glucofuranurono-6,3-lactone (0.5 g, 2.84 mmol) and acetic anhydride (4 mL). The mixture was exposed to microwave irradiation at 300 W (P_{max} = 200 Psi) with continuous stirring at 115 °C for 40 min. The mixture was then diluted with CH_2Cl_2, and it was successively washed with satd. aq. $Na_2S_2O_3$, satd. aq. $NaHCO_3$, and brine. The organic phase was dried with $MgSO_4$. After filtration and concentration under vacuum, the residue was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether, 1:4) to give 1,2,5-tri-*O*-acetyl-β-D-glucofuranurono-6,3-lactone (532 mg, 62%) as white crystals.

Data for 1,2,5-Tri-*O*-acetyl-β-D-glucofuranurono-6,3-lactone (**7β**): M.p. 193.4–194.7 °C. $[\alpha]_D^{20}$ = +70 (*c* = 0.7, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.17 (br. s, 1-H), 5.31 (br. s, 2-H), 5.28–5.20 (m, $^3J_{3,4}$ = 4.6, $^3J_{4,5}$ = 7.3 Hz, 2 H, 4-H, 5-H), 5.09 (d, $^3J_{3,4}$ = 4.6 Hz, 1 H, 3-H), 2.17, 2.15, 2.08 (s, 3 CH_3, Ac) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.2, 169.5, 169.2, 168.9 (4 *C*O), 98.7 (C-1), 81.8 (C-3), 78.2 (C-2), 76.3 (C-4), 68.2 (C-5), 20.9, 20.7, 20.1 (3 *C*H_3, Ac) ppm. * HRMS: calcd. for $C_{12}H_{14}O_9$ [M + Na]$^+$ 325.0530; found 325.0525; calcd. for [M + K]$^+$ 341.0269; found 341.0265. *Data were in accordance with the published data.[25]

1-(2-Acetamido-6-chloropurin-9-yl)-2,5-di-*O*-acetyl-β-D-glucofuranurono-6,3-lactone (8) and 1-(2-Acetamido-6-chloropurin-7-yl)-2,5-di-*O*-acetyl-β-D-glucofuranurono-6,3-lactone (9): According to the general procedure, and starting from 1,2,5-tri-*O*-acetyl-α,β-D-glucofuranurono-6,3-lactone (**7**; 170 mg, 0.56 mmol) and 2-acetamido-6-chloropurine (150 mg, 0.71 mmol), the *N*-glycosylation of the corresponding silylated purine with **7** in the presence of trimethylsilyl triflate (0.55 mL, 3.06 mmol), was complete within 30 min. Flash column chromatography on silica gel (EtOAc/petroleum ether, from 5:1 to 6:1) gave **8** and **9** (176 mg, 70% total yield) in a ratio of 6.8:1. Pure N^9 nucleoside **8** (72 mg) was obtained as a white solid, while the N^7 compound (i.e., **9**) could not be isolated and was obtained in a mixture containing **8** (105 mg; ratio **8/9**, 3.6:1).

Data for **8**: R_f = 0.26 (EtOAc), m.p. 185.5–187.2 °C. $[\alpha]_D^{20}[\alpha]$ = +130 (*c* = 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.61 (br. s, 1 H, NH), 8.08 (s, 1 H, 8-H), 6.17 (d, $^3J_{1',2'}$ = 2.7 Hz, 1 H, 1′-H), 5.82 (d, $^3J_{1',2'}$ = 2.7 Hz, 1 H, 2′-H), 5.63 (d, $^3J_{4',5'}$ = 4.8 Hz, 1 H, 5′-H), 5.28–5.21 (m, $^3J_{3',4'}$ = 3.7, $^3J_{4',5'}$ = 4.8 Hz, 2 H, 3′-H, 4′-H), 2.49 (s, 3 H, CH_3, NHAc), 2.20, 2.17 (2 s, 6 H, CH_3, OAc) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.2 (*C*O, NHAc), 169.7, 169.6 (*C*O), 152.4 (C-2 or C-6), 151.9 (C-2 or C-6), 151.8 (C-4), 142.8 (C-8), 128.5 (C-5), 91.4 (C-1′), 82.4 (C-3′), 80.1 (C-2′), 77.9 (C-4′), 69.5 (C-5′), 25.3 (*C*H_3, NHAc), 20.6, 20.4 (2 *C*H_3, OAc) ppm. HMBC: 1′-H correlates with 151.8 (C-4) and 142.8 (C-8); 8-H correlates with 128.5 (C-5). HRMS: calcd. for $C_{17}H_{16}ClN_5O_8$ [M + H]$^+$ 454.0760; found 454.0748; calcd. for [M + Na]$^+$ 476.0580; found 476.0567.

Data for **9**: R_f = 0.17 (EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$)*: δ = 8.61 (br. s, 1 H, NH), 8.41 (s, 1 H, 8-H), 6.67 (d, $^3J_{1',2'}$ = 2.1 Hz, 1 H, 1′-H), 5.74–5.70 (m, $^3J_{1',2'}$ = 2.1, $^3J_{4',5'}$ = 4.6 Hz, 2 H, 2′-H, 5′-H), 5.35–5.31 (m, $^3J_{3',4'}$ = 3.3, $^3J_{4',5'}$ = 4.6 Hz, 1 H, 4′-H), 5.20 (d, $^3J_{3',4'}$ = 3.3 Hz, 1 H, 3′-H), 2.54 (s, 3 H, CH_3, NHAc), 2.22, 2.21 (2 s, 6 H, CH_3, OAc) ppm. ^{13}C NMR (100 MHz, $CDCl_3$)*: δ = 169.5, 169.2, 169.0 (*C*O), 163.4 (C-4), 152.9 (C-2 or C-6), 146.8 (C-8), 143.1 (C-2 or C-6), 118.4 (C-5), 91.8 (C-1′), 81.9 (C-3′), 79.9 (C-2′), 79.0 (C-4′), 69.6 (C-5′), 25.2 (*C*H_3, NHAc), 20.5, 20.3 (2 *C*H_3, OAc) ppm. *Data extracted from the spectrum of the **8/9** regioisomeric mixture.

***N*-Benzyl-1,2-*O*-isopropylidene-α-D-glucofuranuronamide (11):** 1,2-*O*-Isopropylidene-α-D-glucofuranurono-6,3-lactone (**10**; 150 mg, 0.69 mmol) was dissolved in anhydrous dichloromethane (5 mL) under nitrogen, and benzylamine (0.08 mL, 0.73 mmol) was added. The solution was stirred overnight at room temp. The solvent was removed under vacuum, and the residue was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether, 9:1) to give compound **11** (224 mg, quant.) as a white solid. R_f = 0.42 (EtOAc/petroleum ether, 9:1), m.p. 132.9–134.8 °C. $[\alpha]_D^{20}$ = –9 (*c* = 1.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37–7.23 (m, 5 H, Ph), 7.10 (t, $^3J_{NH,a\text{-}H}$ = $^3J_{NH,b\text{-}H}$ = 5.9 Hz, 1 H, N*H*), 5.96 (d, $^3J_{1,2}$ = 3.4 Hz, 1 H, 1-H), 4.60–4.45 (m, $^3J_{1,2}$ = 3.4, $^2J_{a,b}$ = 15.0, $^3J_{NH,a\text{-}H}$ = $^3J_{NH,b\text{-}H}$ = 5.9 Hz, 3 H, 2-H, CH_2Ph), 4.40 (d, $^3J_{3,4}$ = 2.4 Hz, 1 H, 3-H), 4.36 (d, $^3J_{4,5}$ = 7.1 Hz, 1 H, 5-H), 4.28 (dd, $^3J_{3,4}$ = 2.4, $^3J_{4,5}$ = 7.1 Hz, 1 H, 4-H), 1.47, 1.31 (2 s, 6 H, 2 CH_3, *i*Pr) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.1 (*C*O), 137.7 (Cq, Ph), 128.9, 128.7, 128.5 (*C*H, Ph), 112.4 (Cq, *i*Pr), 105.6 (C-1), 85.1 (C-2), 81.4 (C-4), 75.5 (C-3), 69.2 (C-5), 43.7 (*C*H_2Ph), 27.1, 26.3 (2 *C*H_3, *i*Pr) ppm. HRMS: calcd. for $C_{16}H_{21}NO_6$ [M + H]$^+$ 324.1442; found 324.1436; calcd. for [M + Na]$^+$ 346.1261; found 346.1255.

***N*-Benzyl-2,5-di-*O*-acetyl-1,2-*O*-isopropylidene-α-D-glucofuranuronamide (12):** *N*-Benzyl-1,2-*O*-isopropylidene-α-D-glucofuranuronamide (**11**; 100 mg, 0.31 mmol) was dissolved in pyridine (4 mL), and acetic anhydride (3 mL) was added. The reaction mixture was stirred overnight at room temp. The solvents were then coevaporated with toluene, and the residue was purified by flash column chromatography (ethyl acetate/petroleum ether, 3:1) to give compound **12** (120 mg, 95%) as a white solid. R_f = 0.71 (EtOAc/petroleum ether, 9:1), m.p. 129.2–131.0 °C. $[\alpha]_D^{20}$ = +11 (*c* = 0.9, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.38–7.22 (m, 5 H, Ph), 7.53 (t, $^3J_{NH,a\text{-}H}$ = $^3J_{NH,b\text{-}H}$ = 5.9 Hz, 1 H, N*H*), 5.96 (d, $^3J_{1,2}$ = 3.6 Hz, 1 H, 1-H), 5.41 (d, $^3J_{3,4}$ = 2.7 Hz, 1 H, 3-H), 5.16 (d, $^3J_{4,5}$ = 9.7 Hz, 1 H, 5-H), 4.63 (dd, $^3J_{3,4}$ = 2.7, $^3J_{4,5}$ = 9.7 Hz, 1 H, 4-H), 4.59–4.42 (m, $^3J_{1,2}$ = 3.6, $J_{a,b}$ = 15.0, $^3J_{NH,a\text{-}H}$ = $^3J_{NH,b\text{-}H}$ = 5.9 Hz, 3 H, 2-H, CH_2Ph), 2.12, 2.04 (2 s, 6 H, CH_3, OAc), 1.52, 1.32 (2 s, 6 H, 2 CH_3, *i*Pr) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.8, 169.4, 167.2 (3 *C*O), 137.9 (Cq, Ph), 128.8, 127.7, 127.6 (*C*H, Ph), 113.1 (Cq, *i*Pr), 105.6 (C-1), 83.0 (C-2), 77.5 (C-4), 75.3 (C-3), 69.2 (C-5), 43.7 (*C*H_2Ph), 26.9, 26.4 (2 *C*H_3, *i*Pr), 20.8, 20.7 (2 *C*H_3, OAc) ppm. HRMS: calcd. for $C_{20}H_{25}NO_8$ [M + H]$^+$ 408.1653; found 408.1646; calcd. for [M + Na]$^+$ 430.1472; found 430.1466.

***N*-Benzyl-1,2,3,5-tetra-*O*-acetyl-α,β-D-glucofuranuronamide (13α,13β):** A solution of *N*-benzyl-2,5-di-*O*-acetyl-α-D-glucofuranuronamide (70 mg, 0.17 mmol) in aq. trifluoroacetic acid (72%; 3.6 mL) was stirred at room temp. for 3 h. After coevaporation with toluene, the residue was stirred in pyridine (1.5 mL) and acetic anhydride (1 mL) at room temp. for 20 min. The solvents were then coevaporated with toluene, and the residue was purified by flash column chromatography (ethyl acetate/petroleum ether, 1:1) to give **13α**,**13β** (62 mg, 80% over two steps; anomeric mixture, α/β ratio, 1:0.75) as a colourless oil. R_f = 0.62 (EtOAc/petroleum ether, 4:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.38–7.22 (m, 5 H, Ph), 6.51–6.40 (m, $^3J_{1,2(\alpha)}$ = 4.6 Hz, 1.57 H, 1α-H, N*H* α, N*H* β), 6.15 (br. s, 0.43 H, 1β-H), 5.63 (dd, ^3J = 3.9, ^3J = 5.2 Hz, 0.57 H, 3α-H), 5.47 (dd, $^3J_{2,3(\beta)}$ = 1.1, $^3J_{3,4(\beta)}$ = 4.7 Hz, 0.43 H, 3β-H), 5.33–5.22 (m, 1.57 H, 2α-H, 5α-H, 5β-H), 5.17 (br. s, 0.43 H, 2β-H), 4.82–4.74 (m, 1 H, 4α-H, 4β-H), 4.58–4.49 (m, 1 H, aα-H, aβ-H, Bn), 4.47–4.37 (m, 1 H, bα-H, bβ-H, Bn), 2.13, 2.13, 2.13, 2.10, 2.08, 2.08, 2.07, 2.04 (each s, 12 H, CH_3, OAc, α,β) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.7, 169.6, 169.6, 169.5, 169.3, 169.2, 169.1, 169.1, 166.8, 166.6 (*C*O, α,β), 137.6, 137.5 (Cq, Ph α,β), 128.9, 128.9, 127.7, 127.7, 127.7 (*C*H, Ph, α,β), 99.4 (C-1β), 93.5

(C-1α), 80.0 (C-4β), 79.1 (C-2β), 77.1 (C-4α), 75.8 (C-2α), 73.9 (C-3α), 73.2 (C-3β), 70.3 (C-5α), 70.2 (C-5β), 43.8, 43.6 (*C*H_2Ph, α,β), 20.9, 20.7, 20.7, 20.7, 20.6, 20.6, 20.5 (*C*H_3, Ac, α,β) ppm. HRMS: calcd. for $C_{21}H_{25}NO_{10}$ $[M + H]^+$ 452.1551; found 452.1540; calcd. for $[M + Na]^+$ 474.1371; found 474.1359.

***N*-Benzyl-1-(2-acetamido-6-chloropurin-9-yl)-2,3,5-tri-*O*-acetyl-β-D-glucofuranuronamide (14) and *N*-Benzyl-1-(2-acetamido-6-chloropurin-7-yl)-2,3,5-tri-*O*-acetyl-β-D-glucofuranuronamide (15):** According to the general procedure, and starting from *N*-benzyl-1,2,3,5-tetra-*O*-acetyl-α,β-D-glucofuranuronamide (**13**; 48 mg, 0.11 mmol) and 2-acetamido-6-chloropurine (34 mg, 0.16 mmol), the *N*-glycosylation of the corresponding silylated purine with **13** in the presence of trimethylsilyl triflate (0.13 mL, 0.69 mmol), was complete within 40 min. Flash column chromatography on silica gel (EtOAc/petroleum ether, from 4:1 to 5:1) gave **14** and **15** (38 mg, 60% total yield) in a ratio of 2.8:1. Pure N^9 nucleoside **14** (10 mg) was obtained as a white solid, while the N^7 compound (i.e., **15**) could not be isolated and was obtained in a mixture containing **14** (28 mg, ratio **14/15**, 1.8:1).

Data for **14**: R_f = 0.41 (EtOAc), m.p. 198.8–200.0 °C. $[\alpha]_D^{20}$= +9 (*c* = 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.22 (s, 1 H, 8-H), 8.08 (s, 1 H, N*H*), 7.26–7.10 (m, 5 H, Ph), 6.67 (br.t, 1 H, N*H*), 6.14 (d, $^3J_{1',2'}$ = 2.1 Hz, 1 H, 1′-H), 5.67 (dd, $^3J_{2',3'}$ = 1.7, $^3J_{3',4'}$ = 4.0 Hz, 1 H, 3′-H), 5.53–5.44 (m, 1 H, 2′-H, 5′-H), 4.86 (dd, $^3J_{3',4'}$ = 4.0, $^3J_{4',5'}$ = 8.5 Hz, $^3J_{a,b}$ = 14.9, $^3J_{NH,a\text{-}H}$ = 5.8 Hz, 1 H, 4′-H), 4.47 (dd, part A of ABX system, 1 H, a-H from , $^3J_{NH,b\text{-}H}$ = 5.5, $^3J_{a,b}$ = 14.9 HzCH_2Ph), 4.39 (dd, part B of ABX system, 1 H, b-H from CH_2Ph), 2.47 (s, 3 H, CH_3, NHAc), 2.17, 2.14, 2.07 (3 s, 9 H, 3 CH_3, OAc) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.7, 169.3, 168.7, 166.3 (*C*O), 152.1 (C-4), 151.9 (C-2, C-6), 142.6 (C-8), 137.3 (Cq, Ph), 128.8 (*C*H, Ph), 127.8, 127.8, 127.8 (*C*H, Ph, C-5), 88.2 (C-1′), 79.7 (C-4′), 79.3 (C-2′), 73.6 (C-3′), 69.7 (C-5′), 43.8 (*C*H_2Ph), 25.3 (*C*H_3, NHAc), 20.7, 20.7, 20.3 (*C*H_3, OAc) ppm. HRMS: calcd. for $C_{26}H_{27}ClN_6O_9$ $[M + H]^+$ 603.1601; found 603.1605; calcd. for $[M + Na]^+$ 625.1420; found 625.1422.

Data for **15**: R_f = 0.31 (EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$)*: δ = 8.65 (s, 1 H, H-8), 8.29 (s, 1 H, N*H*), 7.26–7.10 (m, 5 H, Ph), 6.71 (br. s, 1 H, N*H*), 6.58 (br. s, 1 H, 1′-H), 5.59 (br. d, $^3J_{3',4'}$ = 3.2 Hz, 1 H, 3′-H), 5.45 (d, $^3J_{4',5'}$ = 8.6 Hz, 1 H, 5′-H), 5.33 (br. s, 1 H, 2′-H), 4.89 (dd, 1 H, 4′-H, $^3J_{3',4'}$ = 3.2, $J_{4',5'}$ = 8.6 Hz), 4.48 (d, *J* = 5.6 Hz, 2 H, CH_2Ph), 2.59 (s, 3 H, CH_3, NHAc), 2.22, 2.13, 2.01 (3 s, 9 H, 3 CH_3, OAc) ppm. ^{13}C NMR (100 MHz, $CDCl_3$)*: δ = 147.5 (C-8), 90.4 (C-1′), 80.9 (C-4′), 80.2 (C-2′), 73.5 (C-3′), 69.4 (C-5′) ppm. * Data extracted from the spectrum of the **14/15** regioisomeric mixture.

***N*-Benzyl-1-(2-acetamido-6-*N*-benzyladenin-9-yl)-2,3,5-tri-*O*-acetyl-β-D-glucofuranuronamide (16):** 2-Acetamido-6-chloro-9-(2′,5′-tri-*O*-acetyl-β-D-glucofuranosylurono-6′,3′-lactone)purine (**8**, 19 mg, 42 μmol) was dissolved in anhydrous dichloromethane (2.5 mL), and benzylamine (50 μL, 0.46 mmol) was added. The reaction mixture was stirred at room temp. for 2 d. The solvent was then evaporated, the residue was dissolved in pyridine (1.6 mL) and acetic anhydride (1 mL), and the solution was stirred at room temp. for 45 min. After coevaporation with toluene, the residue was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether, 5:1) to give compound **16** (7 mg, 25% over two steps) as a white solid. R_f = 0.48 (EtOAc/MeOH, 19:1), m.p. 202.4–204.3 °C. $[\alpha]_D^{20}$ = +3 (*c* = 0.6, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.85 (br. s, 2 H, N*H*Ac, 8-H), 7.40–7.11 (m, 10 H, Ph), 6.67 (br. s, 1 H, CON*H*Bn), 6.43 (br. s, 1 H, N*H*Bn), 6.17 (d, $^3J_{1',2'}$ = 1.7 Hz, 1 H, 1′-H), 5.62 (dd, $^3J_{2',3'}$ = 1.6, $^3J_{3',4'}$ = 4.0 Hz, 1 H, 3′-H), 5.58 (br. t, 1 H, 2′-H), 5.43 (d, $^3J_{4',5'}$ = 8.8 Hz, 1 H, 5′-H), 4.84–4.69 (m, $J_{3',4'}$ = 4.0, $^3J_{4',5'}$ = 8.8 Hz, 3 H, 4′-H, CH_2Ph), 4.49–4.36 (m, $^2J_{a,b}$ = 14.9, $^3J_{NH,a\text{-}H}$ = 5.6, $^3J_{NH,b\text{-}H}$ = 5.4 Hz, 2 H, CH_2Ph, amide), 2.46 (s, 3 H, CH_3, NHAc), 2.15, 2.12, 2.05 (3 s, 9 H, 3 CH_3, OAc) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.8, 169.1, 168.8 (*C*O), 166.5 (*C*O, amide), 149.6 (C-4)*, 137.4, 137.2 (C-8, Cq, Ph), 128.9, 128.8 127.7, 127.7 (*C*H, Ph), 116.3 (C-5)*, 87.9 (C-1′), 79.2 (C-4′), 79.1 (C-2′), 73.8 (C-3′), 69.7 (C-5′), 44.9 (NH*C*H_2Ph)**, 43.7 (*C*H_2Ph, amide), 25.3 (*C*H_3, NHAc), 20.7, 20.7 (*C*H_3, OAc). *inferred from HMBC, ** inferred from HMQC. HRMS: calcd. for $C_{33}H_{35}N_7O_9$ $[M + H]^+$ 674.2569; found 674.2554; calcd. for $[M + Na]^+$ 696.2389; found 696.2369.

***N*-Benzyl-1,2,3,4-tetra-*O*-acetyl-α,β-D-glucopyranuronamide (17α,17β):** A solution of *N*-benzyl-1,2-*O*-isopropylidene-α-D-glucofuranuronamide (**11**; 110 mg, 0.34 mmol) in aq. trifluoroacetic acid (60%; 2.3 mL) was stirred at room temp. for 2.5 h. After coevaporation with toluene, the residue was stirred in pyridine (2 mL) and acetic anhydride (1.2 mL) at room temp. for 10 min. The solvents were then coevaporated with toluene, and the residue was purified by flash column chromatography (ethyl acetate/petroleum ether, 1:1) to give **17α,17β** (109 mg, 71% over two steps; anomeric mixture, α/β ratio, 1:0.65) as a white solid. R_f = 0.35 (EtOAc/petroleum ether, 1:1). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.41–7.22 (m, 5 H, Ph), 6.68 (t, $^3J_{NH,a\alpha\text{-}H}$ = $^3J_{NH,b\alpha\text{-}H}$ = 5.7 Hz, 0.61 H, N*H* α), 6.61 (t, $^3J_{NH,a\beta\text{-}H}$ = $^3J_{NH,b\beta\text{-}H}$ = 5.5 Hz, 0.39 H, β-N*H*), 6.34 (d, $^3J_{1,2(\alpha)}$ = 3.7 Hz, 0.61 H, 1α-H), 5.75 (d, $^3J_{1,2(\beta)}$ = 8.3 Hz, 0.39 H, 1β-H), 5.54 (t, $^3J_{2,3(\alpha)}$ = $^3J_{3,4(\alpha)}$ = 10.1 Hz, 0.61 H, 3α-H), 5.36–5.18 (m, $^3J_{2,3(\beta)}$ = $^3J_{3,4(\beta)}$ = 9.3, $^3J_{3,4(\alpha)}$ = $^3J_{4,5(\alpha)}$ = 10.1, $J_{4,5(\beta)}$ = 9.6 Hz, 1.39 H, 3β-H, 4α-H, 4β-H), 5.15–5.01 (m, $^3J_{1,2(\alpha)}$ = 3.7, $^3J_{2,3(\alpha)}$ = 10.1, $J_{1,2(\beta)}$ = 8.3, $^3J_{2,3(\beta)}$ = 9.3 Hz, 1 H, 2α-H, 2β-H), 4.54–4.43 (m, $^2J_{a,b}$ = 14.7, $^3J_{NH,a\alpha\text{-}H}$ = 5.7, $^3J_{NH,a\beta\text{-}H}$ = 5.5 Hz, 1 H, aα-H, aβ-H, Bn), 4.39–4.30 (m, $^2J_{a,b}$ = 14.7, $^3J_{NH,b\alpha\text{-}H}$ = 5.7, $^3J_{NH,b\beta\text{-}H}$ = 5.5, $^3J_{4,5(\alpha)}$ = 10.1 Hz, 1.61 H, bα-H, bβ-H, 5α-H), 4.13 (d, $J_{4,5(\beta)}$ = 9.6 Hz, 0.39 H, 5β-H), 2.19 (s, 1.83 H, CH_3, OAc, α), 2.11, 2.09, 2.09, 2.04, 2.04, 2.02 (each s, 10.17 H, CH_3, OAc, α,β) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.0, 170.0, 169.9, 169.9, 168.9, 168.9, 167.9, 166.5, 166.0 (*C*O, α,β), 137.5 (Cq, Ph, β), 137.4 (Cq, Ph, α), 128.9, 128.9, 128.1, 128.0, 127.9, 127.8 (*C*H, Ph, α,β), 91.4 (C-1β), 88.4 (C-1α), 73.1 (C-5β), 72.1 (C-3β), 70.5 (C-5α), 70.3 (C-2β), 69.3 (C-4α), 69.1 (C-2α, C-4β), 69.0 (C-3α), 43.2, 43.2 (*C*H_2Ph, α,β), 20.9, 20.8, 20.7, 20.7, 20.6 (*C*H_3, Ac, α,β) ppm. HRMS: calcd. for $C_{21}H_{25}NO_{10}$ $[M + H]^+$ 452.1551; found 452.1542; calcd. for $[M + Na]^+$ 474.1371; found 474.1360.

***N*-Benzyl-1-(2-acetamido-6-chloropurin-9-yl)-2,3,4-tri-*O*-acetyl-β-D-glucopyranuronamide (18) and *N*-Benzyl-1-(2-acetamido-6-chloropurin-7-yl)-2,3,4-tri-*O*-acetyl-β-D-glucopyranuronamide (19):** According to the general procedure, and starting from *N*-benzyl-1,2,3,4-tetra-*O*-acetyl-α,β-D-glucopyranuronamide (**17**; 63 mg, 0.14 mmol) and 2-acetamido-6-chloropurine (43 mg, 0.2 mmol), the *N*-glycosylation of the corresponding silylated purine with **17** in the presence of trimethylsilyl triflate (0.16 mL, 0.88 mmol), was complete within 40 min. Purification by flash column chromatography on silica gel (EtOAc/petroleum ether, from 2.5:1 to 4:1) gave N^9 nucleoside **18** (35 mg, 42%) and its N^7 regioisomer **19** (24 mg, 29%) as white solids.

Data for **18**: R_f = 0.3 (EtOAc/petroleum ether, 5:1), m.p. 134.1–136.0 °C. $[\alpha]_D^{20}$ = –3 (*c* = 0.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.35 (br. t, 1 H, NH), 8.10 (s, 1 H, 8-H), 7.34–7.15 (m, 5 H, Ph), 7.02 (t, 1 H, N*H*), 5.86 (d, $^3J_{1',2'}$ = 9.3 Hz, 1 H, 1′-H), 5.74 (t, $^3J_{2',3'}$ = $^3J_{1',2'}$ = 9.3 Hz, 1 H, 2′-H), 5.52 (t, $^3J_{2',3'}$ = $^3J_{3',4'}$ = 9.3 Hz, 1 H, 3′-H), 5.44 (t, $^3J_{3',4'}$ = $^3J_{4',5'}$ = 9.3 Hz, 1 H, 4′-H), 4.47–4.31 (m, $^3J_{NH,a\text{-}H}$ = $^3J_{NH,b\text{-}H}$ = 5.8, $^2J_{a,b}$ = 15.2, $^3J_{4',5'}$ =

9.3 Hz, 3 H, 2 H, CH_2Ph, 5′-H), 2.42 (s, 3 H, CH_3, NHAc), 2.11, 2.05, 1.85 (3 s, 9 H, 3 CH_3, OAc) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.9, 169.7, 169.1, 165.5 (*C*O), 152.7 (C-4), 152.5, 152.0 (C-2, C-6), 142.3 (C-8), 137.7 (Cq, Ph), 128.8 (*C*H, Ph), 128.2 (C-5), 128.0, 127.7 (*C*H, Ph), 81.1 (C-1′), 75.5 (C-5′), 72.2 (C-3′), 69.4 (C-2′), 69.2 (C-4′), 43.3 (*C*H_2Ph), 25.3 (*C*H_3, NHAc), 20.8, 20.6, 20.3 (3 *C*H_3, OAc) ppm. HRMS: calcd. for $C_{26}H_{27}ClN_6O_9$ $[M + H]^+$ 603.1601; found 603.1583; calcd. for $[M + Na]^+$ 625.1420; found 625.1401.

Data for **19**: R_f = 0.3 (EtOAc), m.p. 137.6–139.3 °C. $[a]_D^{20}$ = –2 (*c* = 0.8, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.38 (s, 1 H, 8-H), 8.32 (br. s, 1 H, NH), 7.32–7.13 (m, 5 H, Ph), 7.00 (br.t, 1 H, N*H*), 6.16 (br. d, 1 H, 1′-H), 5.71 (br.t, 1 H, 2′-H), 5.55 (t, $^3J_{2',3'}$ = $^3J_{3',4'}$ = 9.4 Hz, 1 H, 3′-H), 5.48 (t, $^3J_{3',4'} \approx {}^3J_{4',5'} \approx$ 9.4 Hz, 1 H, 4′-H), 4.48 (dd, part A of ABX system, $^2J_{a,b}$ = 14.7, $^3J_{NH,H\text{-}a}$ = 6.4 Hz, 1 H, H-a from CH_2Ph), 4.39 (d, $^3J_{4',5'}$ = 9.6 Hz, 1 H, 5′-H), 4.31 (dd, part B of ABX system, $^2J_{a,b}$ = 14.7, $J_{NH,b\text{-}H}$ = 5.3 Hz, 1 H, b-H from CH_2Ph), 2.50 (s, 3 H, CH_3, NHAc), 2.12, 2.07, 1.90 (3 s, 9 H, 3 CH_3, OAc) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.9, 169.8 (*C*O), 165.3 (*C*O, amide), 153.0, 148.5 (C-8), 137.4 (Cq, Ph), 128.8 (*C*H, Ph), 128.0, 127.8 (*C*H, Ph), 75.2 (C-5′), 72.5 (C-3′),* 69.4 (C-2′),** 68.9 (C-4′), 43.2 (*C*H_2Ph), 25.3 (*C*H_3, NHAc), 20.8, 20.6, 20.4 (3 *C*H_3, OAc). * inferred from HMBC, ** inferred from HMQC. HRMS: calcd. for $C_{26}H_{27}ClN_6O_9$ $[M + H]^+$ 603.1601; found 603.1582; calcd. for $[M + Na]^+$ 625.1420; found 625.1400.

1-(2-Acetamido-6-chloropurin-9-yl)-2,3,4-tri-*O*-acetyl-β-D-glucopyranuronic Acid (22) and 1-(2-Acetamido-6-chloropurin-7-yl)-2,3,4-tri-*O*-acetyl-β-D-glucopyranuronic Acid (23): 2-Acetamido-6-chloropurine (121 mg, 0.57 mmol) was suspended in anhydrous acetonitrile (2.5 mL) under N_2, and *N*,*O*-bis(trimethylsilyl)acetamide (0.28 mL, 1.14 mmol) was added. The mixture was stirred at room temp. for 20 min. Then, a solution of 2,3,4-tri-*O*-acetyl-β-D-glucopyranurono-6,1-lactone (115 mg, 0.38 mmol) in anhydrous acetonitrile (2.5 mL) was added, followed by dropwise addition of trimethylsilyl triflate (0.45 mL, 2.47 mmol). The reaction mixture was exposed to microwave irradiation at 150 W (P_{max} = 250 Psi), and it was continuously stirred at 65 °C for 50 min. The mixture was then treated with triethylamine and concentrated. The residue was purified by flash column chromatography on silica gel (using ethyl acetate to ethyl acetate/methanol, 4:1 to 1:1) to give N^9 nucleoside **22** (102 mg, 52%) and its N^7 regioisomer **23** (25 mg, 13%) as colourless oils.

Data for **22**: R_f = 0.41 (EtOAc/MeOH, 1:1). $[a]_D^{20}$ = +8 (*c* = 0.8, MeOH). ^{1}H NMR (400 MHz, [D$_4$]methanol): δ = 8.81 (s, 1 H, 8-H), 6.29 (d, $^3J_{1',2'}$ = 9.1 Hz, 1 H, 1′-H), 5.69 (t, $^3J_{1',2'} = {}^3J_{2',3'}$ = 9.1 Hz, 1 H, 2′-H), 5.55 (br. t, $^3J_{2',3'}$ = 9.1, $J_{3',4'}$ = 9.8 Hz, 1 H, 3′-H), 5.40 (t, $^3J_{3',4'} = {}^3J_{4',5'}$ = 9.8 Hz, 1 H, 4′-H), 4.24 (d, $^3J_{4',5'}$ = 9.8 Hz, 1 H, 5′-H), 2.33 (s, 3 H, CH_3, NHAc), 2.05, 2.01, 1.73 (3 s, 9 H, 3 CH_3, OAc) ppm. ^{13}C NMR (100 MHz, [D$_4$]methanol): δ = 173.2 (*C*OOH), 171.4, 171.2, 170.8 (*C*O), 153.9 (C-4), 151.8 (C-2, C-6), 146.0 (C-8), 128.5 (C-5), 81.6 (C-1′), 78.3 (C-5′), 74.1 (C-3′), 72.2 (C-2′), 71.3 (C-4′), 24.7 (*C*H_3, NHAc), 20.7, 20.5, 20.0 (*C*H_3, OAc) ppm. HRMS: calcd. for $C_{19}H_{20}ClN_5O_{10}$ $[M + Na]^+$ 536.0791; found 536.0780; calcd. for $[M – H + 2Na]^+$ 558.0610; found 558.0598.

Data for **23**: R_f = 0.22 (EtOAc/MeOH, 1:1). $[a]_D^{20}$ = +30 (*c* = 1.1, MeOH). ^{1}H NMR (400 MHz, [D$_4$]methanol): δ = 9.08 (s, 1 H, 8-H), 6.38 (d, $^3J_{1',2'}$ = 8.8 Hz, 1 H, 1′-H), 5.67 (br. t, $^3J_{1',2'}$ = 8.8, $^3J_{2',3'}$ = 9.4 Hz, 1 H, 2′-H), 5.59 (t, $^3J_{2',3'} = {}^3J_{3',4'}$ = 9.4 Hz, 1 H, 3′-H), 5.41 (t, $^3J_{3',4'}$ = 9.4, $^3J_{4',5'}$ = 9.9 Hz, 1 H, 4′-H), 4.27 (d, $^3J_{4',5'}$ = 9.9 Hz, 1 H, 5′-H), 2.30 (s, 3 H, CH_3, NHAc), 2.05, 2.01, 1.80 (3 s, 9 H, 3 CH_3, OAc) ppm. ^{13}C NMR (100 MHz, [D$_4$]methanol): δ = 171.4, 171.1, 170.7 (*C*O), 154.1, 150.6 (C-8), 120.0 (C-5), 81.7 (C-1′), 78.4 (C-5′), 74.1 (C-3′), 73.0 (C-2′), 71.2 (C-4′), 24.6 (*C*H_3, NHAc), 20.7, 20.5, 20.0 (*C*H_3, OAc) ppm. HRMS: calcd. for $C_{19}H_{20}ClN_5O_{10}$ $[M + Na]^+$ 536.0791; found 536.0779; calcd. for $[M – H + 2Na]^+$ 558.0610; found 558.0595.

Enzymatic Studies

Spectrophometer and Chemicals: A TECAN SpectraFluorPlus working in kinetic mode and measuring the absorbance at 415 nm was used for the enzymatic studies. Acetylcholinesterase (from *Electrophorus electricus*), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), and acetylthiocholine iodide (ATChI) were purchased from Fluka. Butyrylcholinesterase (from equine serum) was purchased from Sigma, and butyrylthiocholine iodide (BTChI) was bought from Aldrich.

Preparation of Solutions: Preparation of Tris-HCl buffer (50 mM; pH 8.0): Tris(hydroxymethyl)aminomethane (606 mg) was dissolved in double-distilled water (100 mL) and the pH was adjusted to 8.0 ± 0.1 by the addition HCl. Buffer was freshly prepared and stored in the refrigerator. AChE solution (2.005 U/mL): the enzyme (271 U/mg; 0.037 mg) was dissolved in freshly prepared buffer pH 8.0 (5 mL) containing NaN_3 (0.98 mg). BChE solution (2.040 U/mL): the enzyme (7.54 U/mg, 1.353 mg) was dissolved in freshly prepared buffer pH 8.0 (5 mL) containing NaN_3 (0.98 mg). DTNB solution (3 mM): DTNB (23.8 mg) was dissolved in freshly prepared buffer pH 8.0 (20 mL) containing NaCl (116.8 mg) and $MgCl_2$ (38.0 mg). ATChI solution (15 mM): ATChI (43.4 mg) was dissolved in double-distilled water (10 mL). BTChI solution (15 mM): BTChI (47.6 mg) was dissolved in double-distilled water (10 mL). All solutions were stored in eppendorf vials in the refrigerator or freezer, if necessary. The pure compounds were initially dissolved in DMSO. Galantamine hydrobromide as standard was dissolved in double-distilled water. The final concentrations for the enzymatic assay were obtained by diluting the stock solution with double-distilled water. No inhibition by residual DMSO was detected (< 0.5%).

Enzyme Assays: A mixture of the DTNB solution (125 μL), enzyme (25 μL), and solutions of test compounds (25 μL, three different concentrations and once blank water) was prepared, and then incubated at 30 °C for 20 min. The substrate (25 μL, four different concentrations) was added to start the enzymatic reaction. The absorbance data (415 nm) was recorded at a controlled temperature of 30 °C for 30 min at 1 min intervals. All measurements were performed in triplicate. The final concentrations in the test were as follows: [AChE] = 2.005 U/mL, [BChE] = 2.040 U/mL, [DTNB] = 3 mM, [ATChI] = [BTChI] = 0.9375 mM, 0.625 mM, 0.325 mM, 0.1875 mM. The mode of inhibition as well as K_i and K_i' were determined using Lineweaver–Burk plot,[26] Dixon plot,[27] and Cornish–Bowden plot.[28]

Supporting Information (see footnote on the first page of this article): Copies of the ^{1}H and ^{13}C NMR spectra of new compounds.

Acknowledgments

The authors thank Fundação para a Ciência e Tecnologia (FCT) for financial support through the PEst-OE/QUI/UI0612/2013 project, and for the postdoctoral research grants to N. M. X. and S. S. (grants SFRH/BPD/91226/2012 and SFRH/BPD/81065/2011, respectively).

[1] V. A. Timoshchuk, *Pharm. Chem. J.* **1995**, *29*, 51–59.
[2] a) J. J. Fox, Y. Kuwada, K. A. Watanabe, *Tetrahedron Lett.* **1968**, *9*, 6029–6032; b) N. Ōtake, S. Takeuchi, T. Endō, H. Yonehara, *Tetrahedron Lett.* **1965**, *6*, 1411–1419.
[3] M. Kimura, I. Yamaguchi, *Pestic. Biochem. Physiol.* **1996**, *56*, 243–248.
[4] a) K. A. Watanabe, E. A. Falco, J. J. Fox, *J. Am. Chem. Soc.* **1972**, *94*, 3272–3274; b) Y. Ichikawa, K. Hirata, M. Ohbayashi, M. Isobe, *Eur. J. Inorg. Chem.* **2004**, *10*, 3241–3251.
[5] a) S. Harada, T. Kishi, *J. Antibiot.* **1977**, *30*, 11–16; b) T. Goto, Y. Toya, T. Ohgi, T. Kondo, *Tetrahedron Lett.* **1982**, *23*, 1271–1274.
[6] a) T. Noguchi, Y. Yasuda, T. Niida, T. Shomura, *Ann. Phytopath. Soc. Jpn.* **1968**, *34*, 323–327; b) H. Seto, M. Koyama, H. Ogino, T. Tsuruoka, S. Inouye, N. Otake, *Tetrahedron Lett.* **1983**, *24*, 1805–1808.
[7] a) A. P. Rauter, A. C. Fernandes, S. Czernecki, J.-M. Valery, *J. Org. Chem.* **1996**, *61*, 3594–3598; b) J. Xue, Z. Guo, *J. Carbohydr. Chem.* **2008**, *27*, 51–69; c) F. Marcelo, J. Jiménez-Barbero, J. Marrot, A. P. Rauter, P. Sinay, Y. Blériot, *Chem. Eur. J.* **2008**, *14*, 10066–10073.
[8] C. S. Stauffer, A. Datta, *J. Org. Chem.* **2008**, *73*, 4166–4174.
[9] M. L. Wolfrom, P. McWain, *J. Org. Chem.* **1965**, *30*, 1099–1101.
[10] T. Kishikawa, T. Yamazaki, H. Yiki, *Chem. Pharm. Bull.* **1966**, *14*, 1354–1360.
[11] a) T. Kishikawa, H. Yuki, *Chem. Pharm. Bull.* **1964**, *12*, 1259–1261; b) F. W. Lichtenthaler, A. Heerd, K. Strobel, *Chem. Lett.* **1974**, 449–452; c) R. F. Schinazi, M. S. Chen, W. H. Prusoff, *J. Med. Chem.* **1978**, *21*, 1141–1146.
[12] V. A. Timoshchuk, L. N. Kulinkovich, T. I. Olimpieva, E. I. Boreko, G. V. Vladyko, *Pharm. Chem. J.* **1988**, *22*, 41–49.
[13] P. Fischer, G. R. Lösch, R. R. Schmidt, *Chem. Ber.* **1981**, *114*, 2947–2955.
[14] P. G. Baraldi, F. Fruttarolo, M. A. Tabrizi, R. Romagnoli, D. Preti, A. Bovero, M. J. P. de Las Infantas, A. Moorman, K. Varani, P. Andrea Borea, *J. Med. Chem.* **2004**, *47*, 5535–5540.
[15] F. Marcelo, F. V. Silva, M. M. Goulart, J. Justino, P. Sinaÿ, Y. Blériot, A. P. Rauter, *Bioorg. Med. Chem.* **2009**, *17*, 5106–5166.
[16] R. N. Prasad, A. Fung, K. Tietje, H. H. Stein, H. D. Brondyk, *J. Med. Chem.* **1976**, *19*, 1180–1186.
[17] a) M. Singh, M. Kaur, H. Kukreja, R. Chugh, O. Silakari, D. Singh, *Eur. J. Med. Chem.* **2013**, *70*, 165–188; b) P. Anand, B. Singh, *Arch. Pharmacal Res.* **2013**, *36*, 375–399.
[18] L. J. Wilson, M. W. Hager, Y. A. El-Kattan, D. C. Liotta, *Synthesis* **1995**, 1465–1479.
[19] S. D. Lucas, H. Iding, A. Alker, H. P. Wessel, A. P. Rauter, *J. Carbohydr. Chem.* **2006**, *25*, 187–196.
[20] S. Rat, D. Mathiron, P. Michaud, J. Kovensky, A. Wadouachi, *Tetrahedron* **2007**, *63*, 12424–12428.
[21] M. Poláková, N. Pitt, M. Tosin, P. V. Murphy, *Angew. Chem. Int. Ed.* **2004**, *43*, 2518–2521; *Angew. Chem.* **2004**, *116*, 2572–2575.
[22] O. Gaertzen, A. M. Misske, P. Wolbers, H. M. R. Hoffmann, *Tetrahedron Lett.* **1999**, *40*, 6359–6363.
[23] G. L. Ellman, K. D. Courtney, V. Andres Jr, R. M. Featherstone, *Biochem. Pharmacol.* **1961**, *7*, 88–95.
[24] R. M. Lane, S. G. Potkin, A. Enz, *Int. J. Neuropsychopharmacol.* **2006**, *9*, 101–124.
[25] B. Liberek, D. Tuwalska, I. do Santos-Zounon, A. Konitz, A. Sikorski, Z. Smiatacz, *Carbohydr. Res.* **2006**, *341*, 2275–2285.
[26] H. Lineweaver, D. Burk, *J. Am. Chem. Soc.* **1934**, *56*, 658–666.
[27] M. Dixon, *Biochem. J.* **1953**, *55*, 170–171.
[28] A. Cornish-Bowden, *Biochem. J.* **1974**, *137*, 143–144.

Received: December 23, 2013
Published Online: March 6, 2014

