Organic & Biomolecular Chemistry

PAPER

View Article Online
View Journal | View Issue

Cite this: *Org. Biomol. Chem.*, 2014, **12**, 2446

Microwave-assisted synthesis of novel purine nucleosides as selective cholinesterase inhibitors†

S. Schwarz,[a,b] R. Csuk[b] and A. P. Rauter*[a]

Received 19th January 2014,
Accepted 7th February 2014

DOI: 10.1039/c4ob00142g

www.rsc.org/obc

Alzheimer's disease (AD), the most common form of senile dementia, is characterized by high butyrylcholinesterase (BChE) levels in the brain in later AD stages, for which no treatment is available. Pursuing our studies on selective BChE inhibitors, that may contribute to understand the role of this enzyme in disease progression, we present now microwave-assisted synthesis and anticholinesterase activity of a new nucleoside series embodying 6-chloropurine or 2-acetamido-6-chloropurine linked to D-glucosyl, D-galactosyl and D-mannosyl residues. It was designed to assess the contribution of sugar stereochemistry, purine structure and linkage to the sugar for cholinesterase inhibition efficiency and selectivity. Compounds were subjected to Ellman's assay and their inhibition constants determined. The α-anomers were the most active compounds, while selectivity for BChE or acetylcholinesterase (AChE) inhibition could be tuned by the purine base, by the glycosyl moiety and by N^7-ligation. Some of the nucleosides were far more potent than the drug galantamine, and the most promising competitive and selective BChE inhibitor, the N^7-linked 2-acetamido-α-D-mannosylpurine, showed a K_i of 50 nM and a selectivity factor of 340 fold for BChE over AChE.

Introduction

More than 100 years after Alois Alzheimer first described a new neurodegenerative disease,[1] that now bears his name, there is still no cure available for it. Alzheimer's disease (AD) is a multifactorial disease characterized by the presence of β-amyloid plaques and neurofibrillar tangles in the brain and also a decrease of the level of the neurotransmitter acetylcholine (ACh), that causes a dysfunction of the cholinergic activity, which results in a decline in memory and recognition.[2–4] The level of acetylcholine in the brain is controlled by the hydrolase acetylcholinesterase (AChE, E.C. 3.1.1.7). Also butyrylcholinesterase (BChE, E.C. 3.1.1.8) is able to hydrolyze ACh, although it does not have the same affinity as AChE.[5] It is known that about 80% of the cholinergic activity inside the human brain is caused by AChE.[6] However AChE levels decrease in AD, while those of BChE are highly increased. The ratio of BChE/AChE changes from 0.5 in the normal brain to 11 in the AD brain[7] but the reduced AChE activity may be upregulated by BChE,[8,9] whose activity is doubled in advanced AD.[10,11] Current treatment of AD is based on the AChE inhibitors galantamine[12] and donepezil[13,14] and the dual cholinesterase inhibitor rivastigmine[15] that raise the ACh level and improve cognitive abilities.[16,17] However these drugs are only effective in the early stages of disease.

Histochemical analysis of Alzheimer's disease (AD) brain tissues indicates that BChE is also present in β-amyloid (Aβ) plaques and recent studies in a transgenic mouse model of AD have suggested that it may play a role in AD plaque maturation.[18] Currently under investigation,[19] the influence of BChE on AD pathology is still unclear.

Selective BChE inhibitors will facilitate a better understanding of the role of this enzyme on AD progression. One of the first successful attempts to obtain selective BChE inhibitors was accomplished by Greig *et al.* investigating derivatives of cymserin,[7] followed by Takahashi describing norcymserin derivatives.[20] Other groups succeeded by dealing with derivatives of polyphenol[21] and quinazolinimine.[22] The use of purine nucleotides appeared to be promising and cycloSal pronucleotides showed selectivity towards inhibition of BChE.[23,24] More recently Marcelo *et al.* synthesized a series of highly active purine nucleosides.[25] Those compounds, bearing a benzyl protected bicyclic sugar moiety N^7 and β-linked to 2-acetamido-6-chloropurine, exhibited BChE selectivity, and one of them showed nM inhibition of the same order of magnitude as that of rivastigmine. These results encouraged us to launch our investigation into the impact of the sugar and purine structures on this new family of selective BChE inhibitors. Therefore, a series of benzylated glycosyl donors with D-*gluco*, D-*manno* and D-*galacto* configuration were linked either

[a]*Centro de Química e Bioquímica/Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, Ed. C8, Piso 5, 1749-016 Lisboa, Portugal. E-mail: aprauter@fc.ul.pt*
[b]*Bereich Organische Chemie, Martin-Luther-Universität Halle-Wittenberg, Kurt-Mothes-Str. 2, D-06120 Halle (Saale), Germany*
†Electronic supplementary information (ESI) available. See DOI: 10.1039/c4ob00142g

to 6-chloropurine (CP) or 2-acetamido-6-chloropurine (ACP). The reaction conditions of Marcelo *et al.*[25] were optimized for this family of compounds and microwave-assisted reactions were carried out for the glycosylation reaction. The inhibitory activities of all compounds were determined by Ellman's assays using AChE and BChE, aiming to find efficient and selective BChE inhibitors of a simplified structure when compared to the parent nucleosides previously described by our group.[25]

Results and discussion

Chemistry

6-Chloropurine nucleoside synthesis has been described starting from 1-*O*-acetyl glycosyl donors and mediated by stannic chloride in acetonitrile to give N^9-regioselectivity with the ribofuranosyl donor[26] and N^7-regioselectivity with the glucosyl donor.[26] Alternatively, TMSOTf mediated reaction gave the product resulting from thermodynamic control, the N^9-regioisomer.[27,28] Furthermore, the direct coupling of a persilylated purine with a methyl furanoside using Lewis acid activation of the anomeric centre by stannic chloride[30] or by TMSOTf[31] has been accomplished. Also a furanosyl halide has been activated by stannic chloride to give a purine nucleoside.[29] Other leaving groups have also been used, namely the phenylsulfanyl group activated by *N*-iodosuccimide/triflic acid to yield the N^9-regioisomer.[27] Marcelo *et al.* applied TMSOTf in acetonitrile for the first time to couple methyl bicyclic hexosides to a purine scaffold with β-stereoselectivity and N^7-regioselectivity in 58–65% yield.[25] Hence, we started from the perbenzylated methyl glycoside precursors and investigated their conjugation with persilylated CP as well as ACP. The influence of the concentration of activating TMSOTf as well as that of microwave irradiation onto yield and reaction stereo- and regioselectivity were studied. Four products were formed that differed in their anomeric configuration and linkage of the glycosyl group to purine either in the N^7- or N^9-position (Table 1). For CP glucosylation the ratio α/β is in favor of the β-anomer for both regioisomers, but the β-selectivity for the N^7-isomer increased with the concentration of the activator. In ACP glucosylation the same trends were observed but N^9-selectivity detected for CP glucosylation did not occur.

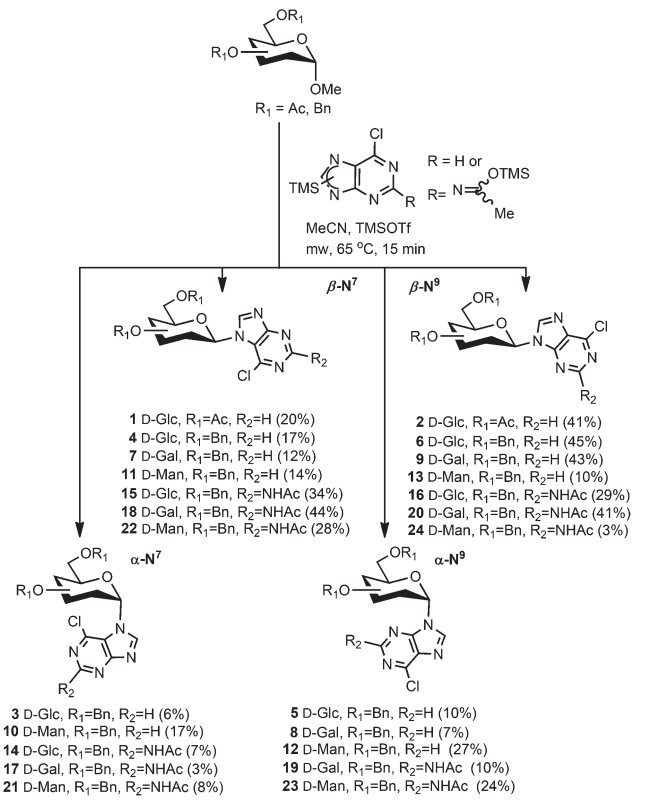
When using microwave irradiation instead of conventional heating, the time taken for glucosylation completion of CP and ACP dropped significantly from 2 h to 15 min but reaction regioselectivity and stereoselectivity were not significantly altered. Consequently, all compounds were further synthesized by microwave-assisted synthesis (Scheme 1).

The anomeric configuration as well as the substitution at N^7 or N^9 of the compounds was determined from their ^{1}H- and ^{13}C NMR spectra, COSY, HMQC, HMBC and gHSQC experiments. The chemical shift of purine carbon 5 can be used to distinguish between N^7- and N^9-substitution. It was found to be about δ = 122 ppm for N^7 CP and about δ = 118 ppm for N^7 ACP, while substitution at N^9 resulted in chemical shifts of about δ = 131 ppm for CP and about δ = 128 ppm for ACP derivatives. The anomeric configuration was unambiguously assigned from the coupling constant $^3J_{1,2}$ and the chemical shift of H-1 of the glycosyl group. For D-*gluco* or D-*galacto* configurated products the coupling constants $^3J_{1,2}$ = 5 Hz (for glucosyl group) and $^3J_{1,2}$ = 3 Hz (for galactosyl group) confirm the α-configuration with H-1 chemical shifts observed between δ = 6.1 and δ = 6.4 ppm. The corresponding β-anomers presented H-1 chemical shifts between δ = 5.4 and δ = 5.7 ppm and coupling constants $^3J_{1,2}$ ranging from 7 to 9 Hz, characteristic of the *trans*-diaxial position of H-1 and

Table 1 Conditions for the reaction of methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucoside with the purines CP or ACP[a]

Purine	TMSOTf (eq.)	Time	N^7/N^9	N^7 α/β	N^9 α/β	Total yield
CP	2	2 h	1/1.4	1/1.7	1/6.0	56%
CP	4	2 h	1/3.8	1/2.6	1/5.8	75%
CP	8	2 h	1/3.4	1/3.6	1/5.9	78%
CP	8	15 min (mw)	1/2.5	1/2.2	1/4.6	81%
ACP	2	2 h	1/1.4	1.1/1	1/4.0	2%
ACP	4	2 h	1.3/1	1/2.2	1/8.5	80%
ACP	8	2 h	1/1.2	1/6.2	1/8.2	71%
ACP	8	15 min (mw)	1.3/1	1/5.0	1/9.3	74%

[a] Yields and product ratios were determined by ^{1}H NMR experiments; 1 equivalent of the monosaccharide and 1.5 equivalents of the silylated purine in dry acetonitrile were used under conventional heating or microwave-assisted heating (mw) at 65 °C. No product was formed with both purines in the presence of 1 equivalent of TMSOTf.



Scheme 1 Reagents and conditions: silylated CP or ACP, TMSOTf, acetonitrile, microwave irradiation (150 W), 65 °C, 15 min.

H-2. For D-*manno* configurated compounds, coupling constants $^3J_{1,2}$ of approx. J = 9 Hz for the α-anomers and 1 Hz for the β-anomers were obtained.

In the ^{13}C NMR spectra, chemical shifts of δ = 79 to δ = 81 ppm for the α-anomers and of δ = 83 to δ = 85 ppm for the β-anomers were found in excellent agreement with those previously reported for related nucleosides.[25]

The highest β/α ratio (9 : 1), determined by ^{1}H NMR, was found in the glucosylation of 2-acetamido-6-chloropurine, while galactosylation of 6-chloropurine afforded the highest β/α ratio (8 : 1). N^9-regioselectivity was observed for the chloropurine nucleosides bearing galactosyl or glucosyl residues while introduction of the substituent 2-acetamido favours N^7-linked nucleosides leading to a N^9/N^7 ratio *ca.* 1.0. However, the N^7-linked D-*manno* nucleosides bearing the 2-acetamido-6-chloropurine exhibit a β/α ratio (3.5 : 1) while for the 6-chloropurine no selectivity occurs. With both the purine bases, α-selectivity is found for the N^9-linked nucleosides.

Biology

All compounds were subjected to Ellman's assay[32] varying substrate and compound concentration to evaluate the inhibition constants K_i (dissociation constant of enzyme–inhibitor complex in competitive inhibition) and K_i' (dissociation constant of enzyme–substrate–inhibitor complex in non-competitive inhibition) and the type of inhibition caused by the 24 synthesized nucleosides. The data for the compounds, for their parent purines and for galantamine hydrobromide – a common drug used for treating AD – are compiled in Table 2. Since the glycosyl residues carry four benzyl groups, with the exception of compounds **1** and **2**, the solubility was poor under the assay conditions. Thus, for some of the compounds, specific data could not be obtained. For these compounds the maximum compound concentration was too low to cause a detectable inhibition, although the cut-off of our assay (100 μM) was not reached. Hence, it was not possible to receive enough data for a definite statement of structure–activity relationship studies for all the compounds tested. Nevertheless, some structural principles as well as tendencies could be determined. First of all, most of the compounds showed a competitive inhibition on both cholinesterases as galantamine hydrobromide did. Galantamine hydrobromide has shown a higher impact on AChE than on BChE. The selectivity factor of galantamine, given by the quotient of K_i (AChE) and K_i (BChE), is 0.06. The unsubstituted purines CP and ACP showed no inhibition on BChE at all, but a linear mixed type inhibition on AChE. This allowed the determination of their selectivity for AChE inhibition. The D-*gluco* and D-*galacto* chloropurine nucleosides were selective for AChE inhibition, with the exclusion of both the perbenzylated N^7-β-nucleosides that could not be evaluated due to their poor solubility under the assay conditions. In addition, N^7- or N^9-ligation does not seem to be crucial for AChE selective inhibition of chloropurine nucleosides, also including those of the D-*manno* series.

The introduction of the perbenzylated mannosyl substituent increased the inhibitory activity considerably. With the exception of both N^9-β-diastereomers, inhibitory constants ranging from 50 nM to 30 μM were determined. It is noteworthy that low inhibition constants were shown by both anomers N^7-linked to ACP when compared to their N^7-linked CP counterparts. The α-anomers were in general the most active compounds for the inhibition of both cholinesterases. However, selectivity for BChE inhibition was mostly shown by the 2-acetamidochloropurine nucleosides, particularly those which exhibit N^7-ligation to the α-D-glucosyl or the α-D-mannosyl group, with the exception of compound **11**, the single chloropurine nucleoside that is a BChE selective inhibitor.

Within all 24 compounds studied, compound **21**, bearing a 2-acetamido-6-chloropurine base N^7-linked to a α-D-mannosyl group, was the most potent BChE inhibitor possessing a K_i of 50 nM. Furthermore, this nucleoside showed an extraordinary selectivity as demonstrated by a selectivity factor of 340.

Table 2 Inhibitory constant K_i for nucleosides **1–24** as determined by Ellman's assay with BChE and AChE in comparison to galantamine hydrobromide[a]

	Purine	N^7/N^9	α/β	Sacch	K_i (μM) (BChE)	K_i (μM) (AChE)
Galantamine hydrobromide					**9.4 ± 0.7**	**0.5 ± 0.0**
6-Chloropurine (CP)					>100	55.5 ± 3.9[c]
2-Acetamido-6-chloropurine (ACP)					>100	26.1 ± 2.8[d]
1	CP	N^7	β	Glc	>100	28.9 ± 2.1
2	CP	N^9	β	Glc	>100	17.5 ± 2.6[e]
3	CP	N^7	α	Glc	>20	9.6 ± 2.3
4	CP	N^7	β	Glc	>2	>2
5	CP	N^9	α	Glc	>100	17.0 ± 2.0
6	CP	N^9	β	Glc	>100	16.0 ± 2.7[f]
7	CP	N^7	β	Gal	>20	>20
8	**CP**	**N^9**	**α**	**Gal**	**>10**	**5.6 ± 0.8**
9	CP	N^9	β	Gal	>100	25.6 ± 2.1
10	CP	N^7	α	Man	30.9 ± 2.2	23.4 ± 3.6
11	**CP**	**N^7**	**β**	**Man**	**9.6 ± 0.7**	**28.5 ± 6.5**
12	**CP**	**N^9**	**α**	**Man**	**2.8 ± 0.3**	**2.4 ± 0.3**
13	CP	N^9	β	Man	>20	>20
14	**ACP**	**N^7**	**α**	**Glc**	**2.5 ± 0.3**	**>10**
15	ACP	N^7	β	Glc	>100	23.0 ± 1.9
16	ACP	N^9	β	Glc	>2	>2
17	ACP	N^7	α	Gal	50.0 ± 7.0	42.0 ± 9.2
18	ACP	N^7	β	Gal	>20	>20
19	ACP	N^9	α	Gal	>2	>2
20	ACP	N^9	β	Gal	>2	>2
21	**ACP**	**N^7**	**α**	**Man**	**0.05 ± 0.01**[b]	**18.1 ± 4.8**
22	**ACP**	**N^7**	**β**	**Man**	**1.4 ± 0.1**	**3.0 ± 0.3**
23	ACP	N^9	α	Man	10.3 ± 2.6	17.1 ± 2.5
24	ACP	N^9	β	Man	>10	>10

[a] Four different substrate concentrations and 4 different inhibitor concentrations were used. Each experiment was performed in triplicate. Poor compound solubility limited the evaluation of K_i when lying above the given concentration. [b] K_i' = 0.7 ± 0.1 μM. [c] K_i' = 115.4 ± 20.5 μM. [d] K_i' = 85.8 ± 10.7 μM. [e] K_i' = 74.3 ± 7.1 μM. [f] K_i' = 44.6 ± 14.3 μM.

Experimental

General experimental procedures

Reagents were purchased from commercial suppliers without any further purification. Microwave assisted synthesis was performed with a CEM Discover and Explorer SP. Melting points

were measured with a Melting Point Apparatus, SMP3, Stuart Scientific, Bibby and were not corrected. NMR spectra were recorded on a BRUKER Avance 400 spectrometer at 298 K with trimethylsilane as an internal standard, δ are given in ppm and J in Hz. Mass spectra were taken on a FINNIGAN MAT TSQ 7000 (electrospray, voltage 4.5 kV, sheath gas nitrogen) instrument. Elemental analyses were carried out on a Foss-Heraeus Vario EL unit. Optical rotations were determined on a Perkin-Elmer 341 polarimeter. TLC was performed on silica gel (Merck 5554). Solvents were dried before use according to usual procedures. The purity of the compounds was checked by HPLC-DAD and found to be >95% for each compound.

N-glycosylation: general procedure

N,*O*-Bis(trimethylsilyl)acetamide (BSA) (1.5 eq. for CP and 3.0 eq. for ACP) was added to a mixture of the respective purine (1.5 eq.) in dry acetonitrile. The mixture was stirred at room temperature for 40 min.

Then, the corresponding methyl glycoside (1 eq.), dissolved in dry acetonitrile, and trimethylsilyl trifluoromethanesulfonate (TMSOTf) were added. The reaction was performed under microwave irradiation (150 W, 65 °C, 15 min). The mixture was poured into dichloromethane, washed with a saturated solution of Na_2CO_3 and extracted with dichloromethane (3 × 15 mL). The combined organic layers were washed with brine, dried ($MgSO_4$) and concentrated and compounds isolated and purified by column chromatography.

6-Chloro-7-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)purine (1) and 6-chloro-9-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)purine (2). The compounds obtained by the reaction of methyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (70 mg, 0.18 mmol) and CP (44 mg, 0.27 mmol) according to the general procedure. Purification by column chromatography (EtOAc–cyclohexane 1 : 1) yielded **1** (17.5 mg; 20%) and **2** (35.8 mg; 41%).

*Data for compound **1**.* Colourless oil; R_f = 0.35 (EtOAc–hexane, 3 : 1). $[\alpha]_D^{20}$ −12° (*c* 1.03, $CHCl_3$). ESI-MS *m*/*z* (%): 485.0 ($[M + H]^+$, 100), 507.3 ($[M + Na]^+$, 22), 969.9 ($[2M + H]^{2+}$, 10), 991.0 ($[2M + Na]^+$, 16). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.94 (s, 1H, H-2), 8.50 (s, 1H, H-8), 6.18 (d, J = 8.1 Hz, 1H, H-1′), 5.69 (dd, J = 8.9 Hz, J = 8.1 Hz, 1H, H-2′), 5.48 (dd, J = 9.3 Hz, J = 8.8 Hz, 1H, H-3′), 5.28 (dd, J = 9.6 Hz, J = 9.3 Hz, 1H, H-4′), 4.28 (dd, J = 12.5 Hz, J = 4.7 Hz, 1H, H-6′a), 4.18 (d, J = 12.5 Hz, 1H, H-6′b), 4.10 (m, 1H, H-5′), 2.08 (s, 6H, 2 × Ac-CH_3), 2.05 (s, 3H, Ac-CH_3), 2.04 (s, 3H, Ac-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4 (Ac-COO), 169.9 (Ac-COO), 169.2 (Ac-COO), 169.0 (Ac-COO), 162.2 (C-4), 153.1 (C-2), 146.3 (C-8), 142.9 (C-6), 122.2 (C-5), 82.6 (C-1′), 75.0 (C-5′), 72.9 (C-3′), 69.8 (C-2′), 67.5 (C-4′), 61.4 (C-6′), 20.6 (Ac-CH_3), 20.5 (Ac-CH_3), 20.5 (Ac-CH_3), 20.2 (Ac-CH_3). Elemental anal. calcd for $C_{19}H_{21}ClN_4O_9$: C, 47.07; H, 4.37; N, 11.56; found: C, 46.86; H, 4.50; N, 11.31.

*Data for compound **2**.* Colourless crystals; mp 175–179 °C; R_f = 0.69 (EtOAc–hexane, 3 : 1). $[\alpha]_D^{20}$ −15° (*c* 1.08, $CHCl_3$). ESI-MS *m*/*z* (%): 485.1 ($[M + H]^+$, 100), 507.4 ($[M + Na]^+$, 30), 969.8 ($[2M + H]^{2+}$, 8), 991.3 ($[2M + Na]^+$, 18). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.79 (s, 1H, H-2), 8.32 (s, 1H, H-8), 5.95 (d, J = 9.4 Hz, 1H, H-1′), 5.67 (dd, J = 9.4 Hz, J = 9.4 Hz, 1H, H-2′), 5.48 (dd, J = 9.4 Hz, J = 9.4 Hz, 1H, H-3′), 5.31 (dd, J = 9.6 Hz, J = 9.4 Hz, 1H, H-4′), 4.30 (dd, J = 12.7 Hz, J = 4.7 Hz, 1H, H-6′a), 4.16 (d, J = 12.7 Hz, 1H, H-6′b), 4.05 (m, 1H, H-5′), 2.08 (s, 3H, Ac-CH_3), 2.07 (s, 3H, Ac-CH_3), 2.04 (s, 3H, Ac-CH_3), 1.78 (s, 3H, Ac-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4 (Ac-COO), 169.9 (Ac-COO), 169.3 (Ac-COO), 168.9 (Ac-COO), 152.5 (C-2), 151.7 (C-4), 151.6 (C-6), 142.9 (C-8), 131.5 (C-5), 80.8 (C-1′), 75.2 (C-5′), 72.7 (C-3′), 70.1 (C-2′), 67.6 (C-4′), 61.4 (C-6′), 20.7 (Ac-CH_3), 20.5 (Ac-CH_3), 20.5 (Ac-CH_3), 20.1 (Ac-CH_3). Elemental anal. calcd for $C_{19}H_{21}ClN_4O_9$: C, 47.07; H, 4.37; N, 11.56; found: C, 46.93; H, 4.47; N, 11.47.

6-Chloro-7-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)purine (3), 6-chloro-7-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)purine (4), 6-chloro-9-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)purine (5) and 6-chloro-9-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-purine (6). The compounds were obtained by the reaction of methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside (100 mg, 0.18 mmol) and CP (44 mg, 0.27 mmol) according to the general procedure. Purification by column chromatography (EtOAc–cyclohexane 1 : 1) yielded **3** (7.3 mg; 6%), **4** (20.7 mg; 17%), **5** (12.2 mg; 10%) and **6** (54.8 mg; 45%).

*Data for compound **3**.* Colourless oil; R_f = 0.27 (EtOAc–hexane 1 : 2). $[\alpha]_D^{20}$ −13° (*c* 0.88, $CHCl_3$). ESI-MS *m*/*z* (%): 676.9 ($[M + H]^+$, 100), 699.1 ($[M + Na]^+$, 20), 1353.7 ($[2M + H]^+$, 22), 1374.9 ($[2M + Na]^+$, 12). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.85 (s, 1H, H-2), 8.70 (s, 1H, H-8), 7.43–7.02 (m, 18H, Bn H), 6.90 (m, 2H, Bn H), 6.35 (d, J = 4.8 Hz, 1H, H-1′), 4.85 (d, J = 11.3 Hz, 1H, Bn-C*H*H), 4.80 (d, J = 11.3 Hz, 1H, Bn-CH*H*), 4.75 (d, J = 11.1 Hz, 1H, Bn-C*H*H), 4.70 (d, J = 12.1 Hz, 1H, Bn-C*H*H), 4.57 (d, J = 11.1 Hz, 1H, Bn-CH*H*), 4.56 (d, J = 12.2 Hz, 1H, Bn-C*H*H), 4.48 (d, J = 12.2 Hz, 1H, Bn-CH*H*), 4.32 (d, J = 12.1 Hz, 1H, Bn-CH*H*), 4.12 (dd, J = 6.8 Hz, J = 4.8 Hz, 1H, H-2′), 4.06 (dd, J = 6.8 Hz, J = 6.6 Hz, 1H, H-3′), 3.86 (dd, J = 9.1 Hz, J = 6.6 Hz, 1H, H-4′), 3.68–3.64 (m, 2H, H-6′a and H-5′), 3.56 (m, 1H, H-6′b). ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.7 (C-4), 152.4 (C-2), 148.7 (C-8), 143.0 (C-6), 137.6 (Bn-Cq), 137.5 (Bn-Cq), 137.4 (Bn-Cq), 136.1 (Bn-Cq), 128.6 (Bn), 128.6 (Bn), 128.5 (Bn), 128.5 (Bn), 128.4 (Bn), 128.4 (Bn), 128.4 (Bn), 128.4 (Bn), 128.4 (Bn), 128.2 (Bn), 128.1 (Bn), 128.1 (Bn), 128.1 (Bn), 128.1 (Bn), 128.0 (Bn), 127.8 (Bn), 127.8 (Bn), 127.8 (Bn), 127.7 (Bn), 127.7 (Bn), 121.7 (C-5), 81.0 (C-1′), 80.6 (C-3′), 77.2 (C-2′), 76.5 (C-4′), 74.4 (Bn-CH_2), 74.2 (Bn-CH_2), 73.9 (Bn-CH_2), 73.5 (Bn-CH_2), 73.2 (C-5′), 67.9 (C-6′). Elemental anal. calcd for $C_{39}H_{37}ClN_4O_5$: C, 69.17; H, 5.51; N, 8.27; found: C, 68.86; H, 5.72; N, 8.12.

*Data for compound **4**.* Colourless oil; R_f = 0.10 (EtOAc–hexane 1 : 2). $[\alpha]_D^{20}$ −9° (*c* 1.01, $CHCl_3$). ESI-MS *m*/*z* (%): 587.1 ($[M - Bn + H]^+$, 90), 609.3 ($[M - Bn + Na]^+$, 48), 677.0 ($[M + H]^+$, 100). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.84 (s, 1H, H-2), 8.23 (s, 1H, H-8), 7.40–7.17 (m, 15H, BnH), 7.07–6.95 (m, 3H, BnH), 6.74 (m, 2H, BnH), 5.72 (br, 1H, H-1′), 5.01 (d, J = 10.9 Hz, 1H, Bn-C*H*H), 4.95 (d, J = 10.9 Hz, 1H, Bn-CH*H*), 4.89 (d, J = 10.7 Hz, 1H, Bn-C*H*H), 4.64 (d, J = 10.7 Hz, 1H, Bn-CH*H*), 4.63 (d, J = 11.5 Hz, 1H, Bn-C*H*H), 4.54 (d, J = 12.1 Hz, 1H, Bn-C*H*H), 4.48 (d, J = 12.1 Hz, 1H, Bn-CH*H*), 4.23 (d, J = 11.5 Hz, 1H, Bn-CH*H*), 3.97 (m, 1H, H-2′), 3.95–3.86 (m, 2H, H-3′ and H-5′), 3.78–3.70 (m, 3H, H-4′ and H-6′a and H-6′b). ^{13}C NMR

(100 MHz, $CDCl_3$): δ 161.4 (C-4), 152.1 (C-2), 147.0 (C-8), 143.1 (C-6), 137.8 (Bn-Cq), 137.5 (Bn-Cq), 137.5 (Bn-Cq), 135.8 (Bn-Cq), 128.6 (Bn), 128.6 (Bn), 128.5 (Bn), 128.5 (Bn), 128.4 (Bn), 128.4 (Bn), 128.2 (Bn), 128.2 (Bn), 128.1 (Bn), 128.0 (Bn), 128.0 (Bn), 128.0 (Bn), 128.0 (Bn), 127.9 (Bn), 127.9 (Bn), 127.8 (Bn), 127.8 (Bn), 127.8 (Bn), 127.7 (Bn), 127.7 (Bn), 122.1 (C-5), 86.0 (C-3′), 85.2 (C-1′), 80.1 (C-2′), 78.1 (C-4′), 77.2 (C-5′), 75.9 (Bn-CH_2), 75.3 (Bn-CH_2), 74.7 (Bn-CH_2), 73.5 (Bn-CH_2), 68.2 (C-6′). Elemental anal. calcd for $C_{39}H_{37}ClN_4O_5$: C, 69.17; H, 5.51; N, 8.27; found: C, 68.98; H, 5.42; N, 8.02.

*Data for compound **5**.* Colourless oil; R_f = 0.55 (EtOAc–hexane 1 : 2). $[\alpha]_D^{20}$ +28° (*c* 1.07, $CHCl_3$). ESI-MS *m*/*z* (%): 677.1 ([M + H]$^+$, 100), 699.3 ([M + Na]$^+$, 16), 1354.8 ([2M + H]$^+$, 18). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.67 (s, 1H, H-2), 8.40 (s, 1H, H-8), 7.40–7.08 (m, 18H, BnH), 6.92 (m, 2H, BnH), 6.26 (d, *J* = 4.9 Hz, 1H, H-1′), 4.86 (d, *J* = 11.4 Hz, 1H, Bn-C*H*H), 4.80 (d, *J* = 11.4 Hz, 1H, Bn-CH*H*), 4.74 (d, *J* = 11.0 Hz, 1H, Bn-C*H*H), 4.60 (d, *J* = 11.8 Hz, 1H, Bn-C*H*H), 4.54 (d, *J* = 11.0 Hz, 1H, Bn-CH*H*), 4.54 (d, *J* = 12.0 Hz, 1H, Bn-C*H*H), 4.45 (d, *J* = 11.8 Hz, 1H, Bn-CH*H*), 4.32 (d, *J* = 12.0 Hz, 1H, Bn-CH*H*), 4.16 (dd, *J* = 7.2 Hz, *J* = 6.0 Hz, 1H, H-3′), 4.12 (dd, *J* = 7.2 Hz, *J* = 4.9 Hz, 1H, H-2′), 3.85 (dd, *J* = 9.4 Hz, *J* = 5.9 Hz, 1H, H-4′), 3.81 (m, 1H, H-5′), 3.70 (dd, *J* = 10.8 Hz, *J* = 3.8 Hz, 1H, H-6′a), 3.59 (dd, *J* = 10.8 Hz, *J* = 2.1 Hz, 1H, H-6′b). ^{13}C NMR (100 MHz, $CDCl_3$): δ 152.0 (C-2), 151.6 (C-4), 150.9 (C-6), 144.9 (C-8), 138.1 (Bn-Cq), 137.7 (Bn-Cq), 137.7 (Bn-Cq), 136.3 (Bn-Cq), 131.3 (C-5), 128.5 (Bn), 128.5 (Bn), 128.4 (Bn), 128.4 (Bn), 128.4 (Bn), 128.4 (Bn), 128.3 (Bn), 128.3 (Bn), 128.2 (Bn), 128.0 (Bn), 128.0 (Bn), 128.0 (Bn), 127.9 (Bn), 127.9 (Bn), 127.9 (Bn), 127.9 (Bn), 127.9 (Bn), 127.7 (Bn), 127.7 (Bn), 127.7 (Bn), 79.8 (C-3′), 79.4 (C-1′), 76.3 (C-2′), 76.1 (C-4′), 73.6 (C-5′), 74.1 (Bn-CH_2), 74.0 (Bn-CH_2), 73.5 (Bn-CH_2), 73.2 (Bn-CH_2), 67.9 (C-6′). Elemental anal. calcd for $C_{39}H_{37}ClN_4O_5$: C, 69.17; H, 5.51; N, 8.27; found: C, 68.84; H, 5.70; N, 8.03.

*Data for compound **6**.* Colourless crystals; mp 146–149 °C; R_f = 0.35 (EtOAc–hexane 1 : 2). $[\alpha]_D^{20}$ −24° (*c* 1.03, $CHCl_3$). ESI-MS *m*/*z* (%): 677.1 ([M + H]$^+$, 100), 699.3 ([M + Na]$^+$, 29), 1354.7 ([2M + H]$^+$, 12). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.68 (s, 1H, H-2), 8.03 (s, 1H, H-8), 7.39–7.27 (m, 13H, BnH), 7.23–7.18 (m, 2H, BnH), 7.12 (m, 1H, BnH), 6.99 (m, 2H, BnH), 5.59 (d, *J* = 9.0 Hz, 1H, H-1′), 4.99 (d, *J* = 11.0 Hz, 1H, Bn-C*H*H), 4.94 (d, *J* = 11.0 Hz, 1H, Bn-CH*H*), 4.88 (d, *J* = 10.7 Hz, 1H, Bn-C*H*H), 4.65 (d, *J* = 10.7 Hz, 1H, Bn-CH*H*), 4.61 (d, *J* = 11.7 Hz, 1H, Bn-C*H*H), 4.55 (d, *J* = 12.2 Hz, 1H, Bn-C*H*H), 4.48 (d, *J* = 12.2 Hz, 1H, Bn-CH*H*), 4.17 (d, *J* = 11.7 Hz, 1H, Bn-CH*H*), 4.07 (dd, *J* = 9.0 Hz, *J* = 9.0 Hz, 1H, H-2′), 3.91 (dd, *J* = 9.0 Hz, *J* = 8.3 Hz, 1H, H-3′), 3.87 (dd, *J* = 9.6 Hz, *J* = 9.1 Hz, 1H, H-4′), 3.77–3.68 (m, 3H, H-6′a and H-5′ and H-6′b). ^{13}C NMR (100 MHz, $CDCl_3$): δ 152.0 (C-2), 151.4 (C-4), 151.0 (C-6), 143.2 (C-8), 137.9 (Bn-Cq), 137.7 (Bn-Cq), 137.6 (Bn-Cq), 136.2 (Bn-Cq), 131.5 (C-5), 128.5 (Bn), 128.5 (Bn), 128.5 (Bn), 128.5 (Bn), 128.4 (Bn), 128.4 (Bn), 128.1 (Bn), 128.1 (Bn), 128.1 (Bn), 128.0 (Bn), 128.0 (Bn), 127.9 (Bn), 127.8 (Bn), 127.8 (Bn), 127.8 (Bn), 127.7 (Bn), 127.7 (Bn), 127.7 (Bn), 127.7 (Bn), 127.7 (Bn), 85.9 (C-3′), 83.3 (C-1′), 79.9 (C-2′), 78.3 (C-5′), 77.2 (C-4′), 75.9 (Bn-CH_2), 75.3 (Bn-CH_2), 74.9 (Bn-CH_2), 73.5 (Bn-CH_2), 68.2 (C-6′). Elemental anal. calcd for $C_{39}H_{37}ClN_4O_5$: C, 69.17; H, 5.51; N, 8.27; found: C, 69.02; H, 5.38; N, 8.11.

6-Chloro-7-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)purine (7), 6-chloro-9-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-purine (8) and 6-chloro-9-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)purine (9). The compounds were obtained by the reaction of methyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranoside (120 mg, 0.21 mmol) and CP (49 mg, 0.32 mmol) according to the general procedure. Purification by column chromatography (EtOAc–cyclohexane 1 : 1) yielded **7** (17 mg; 12%), **8** (9.9 mg; 7%) and **9** (60.9 mg; 43%).

*Data for compound **7**.* Colourless oil; R_f = 0.30 (EtOAc–hexane 1 : 1). $[\alpha]_D^{20}$ −22° (*c* 1.00, $CHCl_3$). ESI-MS *m*/*z* (%): 587.0 ([M − Bn + H]$^+$, 100), 609.3 ([M − Bn + Na]$^+$, 70), 676.9 ([M + H]$^+$, 7). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.83 (s, 1H, H-2), 8.26 (s, 1H, H-8), 7.44–7.23 (m, 15H, BnH), 7.11–6.96 (m, 3H, BnH), 6.75 (m, 2H, BnH), 5.78 (d, *J* = 8.1 Hz, 1H, H-1′), 5.01 (d, *J* = 11.2 Hz, 1H, Bn-C*H*H), 4.85 (d, *J* = 11.6 Hz, 1H, Bn-C*H*H), 4.78 (d, *J* = 11.6 Hz, 1H, Bn-CH*H*), 4.70 (d, *J* = 11.4 Hz, 1H, Bn-C*H*H), 4.36 (d, *J* = 11.2 Hz, 1H, Bn-CH*H*), 4.48 (d, *J* = 11.8 Hz, 1H, Bn-C*H*H), 4.43 (d, *J* = 11.8 Hz, 1H, Bn-CH*H*), 4.28 (d, *J* = 11.4 Hz, 1H, Bn-CH*H*), 4.29 (m, 1H, H-2′), 4.10 (dd, *J* = 1.9 Hz, *J* = 1.9 Hz, 1H, H-4′), 3.86 (dd, *J* = 6.3 Hz, *J* = 6.3 Hz, 1H, H-5′), 3.81 (dd, *J* = 9.4 Hz, *J* = 2.5 Hz, 1H, H-3′), 3.66–3.58 (m, 2H, H-6′a and H-6′b). ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.6 (C-4), 152.1 (C-2), 147.2 (C-8), 143.2 (C-6), 138.2 (Bn-Cq), 137.5 (Bn-Cq), 137.4 (Bn-Cq), 136.2 (Bn-Cq), 128.6 (Bn), 128.6 (Bn), 128.5 (Bn), 128.5 (Bn), 128.4 (Bn), 128.4 (Bn), 128.2 (Bn), 128.2 (Bn), 128.0 (Bn), 128.0 (Bn), 128.0 (Bn), 128.0 (Bn), 128.0 (Bn), 127.9 (Bn), 127.9 (Bn), 127.8 (Bn), 127.8 (Bn), 127.8 (Bn), 127.6 (Bn), 127.6 (Bn), 122.3 (C-5), 85.2 (C-1′), 83.4 (C-3′), 77.2 (C-2′), 76.6 (C-5′), 75.0 (Bn-CH_2), 74.9 (Bn-CH_2), 73.6 (Bn-CH_2), 73.1 (C-4), 72.6 (Bn-CH_2), 68.2 (C-6′). Elemental anal. calcd for $C_{39}H_{37}ClN_4O_5$: C, 69.17; H, 5.51; N, 8.27; found: C, 68.97; H, 5.69; N, 7.99.

*Data for compound **8**.* Colourless oil; R_f = 0.43 (EtOAc–hexane 1 : 2). $[\alpha]_D^{20}$ −14° (*c* 1.00, $CHCl_3$). ESI-MS *m*/*z* (%): 587.0 ([M − Bn + H]$^+$, 72), 609.3 ([M − Bn + Na]$^+$, 62), 677.0 ([M + H]$^+$, 100), 1354.8 ([2M + H]$^+$, 29). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.65 (s, 1H, H-2), 8.38 (s, 1H, H-8), 7.44–7.28 (m, 14H, BnH), 7.18–7.09 (m, 4H, BnH), 6.85 (m, 2H, BnH), 6.29 (d, *J* = 3.0 Hz, 1H, H-1′), 4.82 (d, *J* = 12.1 Hz, 1H, Bn-C*H*H), 4.71 (d, *J* = 11.7 Hz, 1H, Bn-C*H*H), 4.69 (d, *J* = 11.7 Hz, 1H, Bn-CH*H*), 4.64 (d, *J* = 12.1 Hz, 1H, Bn-CH*H*), 4.53 (d, *J* = 14.4 Hz, 1H, Bn-C*H*H), 4.51 (d, *J* = 14.4 Hz, 1H, Bn-CH*H*), 4.41 (d, *J* = 11.9 Hz, 1H, Bn-C*H*H), 4.39 (m, 1H, H-5′), 4.21 (dd, *J* = 4.9 Hz, *J* = 2.9 Hz, 1H, H-4′), 4.13 (d, *J* = 11.9 Hz, 1H, Bn-CH*H*), 4.09–4.06 (m, 2H, H-6′a and H-3′), 3.93 (dd, *J* = 5.5 Hz, *J* = 3.1 Hz, 1H, H-2′), 3.82 (dd, *J* = 11.3 Hz, *J* = 3.8 Hz, 1H, H-6′b). ^{13}C NMR (100 MHz, $CDCl_3$): δ 151.6 (C-2), 150.8 (C-4), 150.5 (C-6), 145.2 (C-8), 137.8 (Bn-Cq), 137.8 (Bn-Cq), 137.6 (Bn-Cq), 136.0 (Bn-Cq), 131.1 (C-5), 128.5 (Bn), 128.5 (Bn), 128.5 (Bn), 128.5 (Bn), 128.3 (Bn), 128.3 (Bn), 128.2 (Bn), 128.2 (Bn), 128.2 (Bn), 128.1 (Bn), 128.1 (Bn), 128.0 (Bn), 127.9 (Bn), 127.9 (Bn), 127.9 (Bn), 127.8 (Bn), 127.8 (Bn), 127.7 (Bn), 127.7 (Bn), 127.6 (Bn), 77.2 (C-1′), 75.5 (C-5′), 74.4 (C-2′), 74.4 (C-3′), 73.4 (Bn-CH_2), 73.3 (Bn-CH_2), 73.2 (Bn-CH_2), 72.8

(Bn-CH_2), 72.6 (C-4′), 65.8 (C-6′). Elemental anal. calcd for $C_{39}H_{37}ClN_4O_5$: C, 69.17; H, 5.51; N, 8.27; found: C, 69.13; H, 5.59; N, 8.13.

Data for compound ***9***. Colourless oil; R_f = 0.30 (EtOAc–hexane 1 : 2). $[\alpha]_D^{20}$ −16° (*c* 0.92, $CHCl_3$). ESI-MS *m*/*z* (%): 497.1 ($[M - 2Bn + H]^+$, 8), 519.3 ($[M - 2Bn + Na]^+$, 12), 587.1 ($[M - Bn + H]^+$, 23), 609.4 ($[M - Bn + Na]^+$, 24), 677.1 ($[M + H]^+$, 100), 699.3 ($[M + Na]^+$, 49). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.72 (s, 1H, H-2), 8.07 (s, 1H, H-8), 7.44–7.24 (m, 15H, BnH), 7.16 (m, 1H, BnH), 7.03 (m, 2H, BnH), 6.64 (m, 2H, BnH), 5.68 (d, *J* = 9.0 Hz, 1H, H-1′), 5.05 (d, *J* = 11.5 Hz, 1H, Bn-C*H*H), 4.84 (d, *J* = 11.7 Hz, 1H, Bn-C*H*H), 4.79 (d, *J* = 11.7 Hz, 1H, Bn-CH*H*), 4.70 (d, *J* = 11.5 Hz, 1H, Bn-CH*H*), 4.69 (d, *J* = 11.6 Hz, 1H, Bn-C*H*H), 4.48 (d, *J* = 11.9 Hz, 1H, Bn-C*H*H), 4.43 (d, *J* = 11.9 Hz, 1H, Bn-CH*H*), 4.32 (dd, *J* = 9.2 Hz, *J* = 9.0 Hz, 1H, H-2′), 4.24 (d, *J* = 14.4 Hz, 1H, Bn-CH*H*), 4.09 (m, 1H, H-4′), 3.86 (m, 2H, H-5′ and H-3′), 3.61–3.59 (m, 2H, H-6′a and H-6′b). ^{13}C NMR (100 MHz, $CDCl_3$): δ 152.0 (C-2), 151.6 (C-4), 150.8 (C-6), 143.0 (C-8), 138.3 (Bn-Cq), 137.7 (Bn-Cq), 137.4 (Bn-Cq), 136.4 (Bn-Cq), 131.1 (C-5), 128.6 (Bn), 128.6 (Bn), 128.4 (Bn), 128.4 (Bn), 128.3 (Bn), 128.3 (Bn), 128.1 (Bn), 128.1 (Bn), 128.0 (Bn), 127.9 (Bn), 127.9 (Bn), 127.9 (Bn), 127.9 (Bn), 127.9 (Bn), 127.9 (Bn), 127.8 (Bn), 127.8 (Bn), 127.8 (Bn), 127.6 (Bn), 127.6 (Bn), 83.4 (C-3′), 82.8 (C-1′), 77.7 (C-2′), 76.6 (C-5′), 75.1 (Bn-CH_2), 74.7 (Bn-CH_2), 73.6 (Bn-CH_2), 73.2 (C-4′), 72.8 (Bn-CH_2), 68.2 (C-6′). Elemental anal. calcd for $C_{39}H_{37}ClN_4O_5$: C, 69.17; H, 5.51; N, 8.27; found: C, 69.00; H, 5.77; N, 8.17.

6-Chloro-7-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)purine (10), 6-chloro-7-(2,3,4,6-tetra-*O*-benzyl-β-D-mannopyranosyl)-purine (11), 6-chloro-9-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)purine (12) and 6-chloro-9-(2,3,4,6-tetra-*O*-benzyl-β-D-mannopyranosyl)purine (13). The compounds were obtained by the reaction of methyl 2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranoside (250 mg, 0.45 mmol) and CP (104 mg, 0.67 mmol) according to the general procedure. Purification by column chromatography (EtOAc–cyclohexane 1 : 1) yielded **10** (52.2 mg; 17%), **11** (42.8 mg; 14%), **12** (82.6 mg; 27%) and **13** (30.6 mg; 10%)

Data for compound ***10***. Colourless oil; R_f = 0.46 (EtOAc–cyclohexane 1 : 1). $[\alpha]_D^{20}$ +82° (*c* 0.86, $CHCl_3$). ESI-MS *m*/*z* (%): 676.9 ($[M + H]^+$, 100), 699.3 ($[M + Na]^+$, 6). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.87 (s, 1H, H-2), 8.28 (s, 1H, H-8), 7.41–7.12 (m, 18H, BnH), 6.82 (m, 2H, BnH), 6.49 (d, *J* = 9.1 Hz, 1H, H-1′), 4.69 (d, *J* = 12.0 Hz, 1H, Bn-C*H*H), 4.62 (d, *J* = 4.3 Hz, 1H, Bn-C*H*H), 4.59 (d, *J* = 4.3 Hz, 1H, Bn-CH*H*), 4.50 (s, 2H, Bn-CH_2), 4.44 (d, *J* = 12.0 Hz, 1H, Bn-CH*H*), 4.39 (dd, *J* = 6.6 Hz, *J* = 6.6 Hz, 1H, H-5′), 4.28 (d, *J* = 11.9 Hz, 1H, Bn-C*H*H), 4.12 (dd, *J* = 9.1 Hz, *J* = 2.8 Hz, 1H, H-2′), 4.06 (dd, *J* = 2.8 Hz, *J* = 2.8 Hz, 1H, H-3′), 4.02 (d, *J* = 11.9 Hz, 1H, Bn-CH*H*), 3.94 (dd, *J* = 10.3 Hz, *J* = 7.6 Hz, 1H, H-6′a), 3.83 (dd, *J* = 3.5 Hz, *J* = 1.3 Hz, 1H, H-4′), 3.71 (dd, *J* = 10.3 Hz, *J* = 5.9 Hz, 1H, H-6′b). ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.6 (C-4), 152.2 (C-2), 146.9 (C-8), 143.4 (C-6), 137.6 (Bn-Cq), 137.4 (Bn-Cq), 137.3 (Bn-Cq), 136.0 (Bn-Cq), 128.6 (Bn), 128.6 (Bn), 128.5 (Bn), 128.5 (Bn), 128.5 (Bn), 128.5 (Bn), 128.4 (Bn), 128.4 (Bn), 128.3 (Bn), 128.2 (Bn), 128.1 (Bn), 127.8 (Bn), 127.8 (Bn), 127.8 (Bn), 127.8 (Bn), 127.8 (Bn), 127.8 (Bn), 127.8 (Bn), 127.7 (Bn), 127.7 (Bn), 122.6 (C-5), 78.6 (C-1′), 74.1 (C-3′), 77.1 (C-5′), 75.4 (C-2′), 73.3 (Bn-CH_2), 73.3 (Bn-CH_2), 71.9 (Bn-CH_2), 73.5 (C-4′), 71.3 (Bn-CH_2), 67.5 (C-6′). Elemental anal. calcd for $C_{39}H_{37}ClN_4O_5$: C, 69.17; H, 5.51; N, 8.27; found: C, 68.96; H, 5.63; N, 8.11.

Data for compound ***11***. Colourless oil; R_f = 0.31 (EtOAc–hexane 1 : 1). $[\alpha]_D^{20}$ +62° (*c* 1.02, $CHCl_3$). ESI-MS *m*/*z* (%): 587.1 ($[M - Bn + H]^+$, 100), 609.3 ($[M - Bn + Na]^+$, 29), 677.0 ($[M + H]^+$, 4). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.77 (s, 1H, H-2), 8.51 (s, 1H, H-8), 7.46–7.23 (m, 15H, BnH), 6.92–6.86 (m, 3H, BnH), 6.73 (m, 2H, BnH), 6.90 (d, *J* = 1.0 Hz, 1H, H-1′), 4.98 (d, *J* = 10.8 Hz, 1H, Bn-C*H*H), 4.90 (d, *J* = 11.7 Hz, 1H, Bn-C*H*H), 4.80 (d, *J* = 11.7 Hz, 1H, Bn-CH*H*), 4.68 (d, *J* = 11.8 Hz, 1H, Bn-C*H*H), 4.66 (d, *J* = 10.8 Hz, 1H, Bn-CH*H*), 4.64 (d, *J* = 12.4 Hz, 1H, Bn-C*H*H), 4.56 (d, *J* = 12.4 Hz, 1H, Bn-CH*H*), 4.07 (dd, *J* = 9.5 Hz, *J* = 9.5 Hz, 1H, H-4′), 4.02 (dd, *J* = 2.5 Hz, *J* = 1.0 Hz, 1H, H-2′), 3.84 (dd, *J* = 9.5 Hz, *J* = 2.5 Hz, 1H, H-3′), 3.78 (m, 2H, H-6′a and H-6′b), 3.71 (ddd, *J* = 9.5 Hz, *J* = 3.6 Hz, *J* = 3.6 Hz, 1H, H-5′), 4.34 (d, *J* = 11.8 Hz, 1H, Bn-CH*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.8 (C-4), 151.8 (C-2), 147.9 (C-8), 141.1 (C-6), 137.8 (Bn-Cq), 137.7 (Bn-Cq), 137.4 (Bn-Cq), 135.6 (Bn-Cq), 128.8 (Bn), 128.8 (Bn), 128.5 (Bn), 128.5 (Bn), 128.5 (Bn), 128.5 (Bn), 128.4 (Bn), 128.4 (Bn), 128.3 (Bn), 128.1 (Bn), 128.1 (Bn), 128.0 (Bn), 128.0 (Bn), 128.0 (Bn), 128.0 (Bn), 127.9 (Bn), 127.8 (Bn), 127.7 (Bn), 127.7 (Bn), 127.7 (Bn), 120.4 (C-5), 83.9 (C-1′), 82.3 (C-3′), 75.4 (Bn-CH_2), 74.1 (Bn-CH_2), 74.0 (Bn-CH_2), 73.6 (C-5′), 73.5 (Bn-CH_2), 72.8 (C-2′), 73.5 (C-4′), 67.8 (C-6′). Elemental anal. calcd for $C_{39}H_{37}ClN_4O_5$: C, 69.17; H, 5.51; N, 8.27; found: C, 69.04; H, 5.62; N, 8.17.

Data for compound ***12***. Colourless oil; R_f = 0.27 (EtOAc–hexane 1 : 2). $[\alpha]_D^{20}$ +64° (*c* 1.58, $CHCl_3$). ESI-MS *m*/*z* (%): 677.0 ($[M + H]^+$, 100), 699.1 ($[M + Na]^+$, 26), 1354.8 ($[2M + 2H]^+$, 23), 1374.9 ($[2M + Na]^+$, 18). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.60 (s, 1H, H-2), 8.11 (s, 1H, H-8), 7.39–7.20 (m, 15H, BnH), 7.16 (m, 1H, BnH), 7.09 (m, 2H, BnH), 6.88 (m, 2H, BnH), 6.03 (d, *J* = 8.1 Hz, 1H, H-1′), 4.76 (dd, *J* = 8.1 Hz, *J* = 2.6 Hz, 1H, H-2′), 4.72 (d, *J* = 12.3 Hz, 1H, Bn-C*H*H), 4.65 (d, *J* = 12.3 Hz, 1H, Bn-CH*H*), 4.52 (d, *J* = 12.2 Hz, 1H, Bn-C*H*H), 4.50–4.42 (m, 5H, Bn-CH_2 and H-5′), 4.16 (d, *J* = 12.2 Hz, 1H, Bn-CH*H*), 3.99 (dd, *J* = 2.8 Hz, *J* = 2.8 Hz, 1H, H-3′), 3.86 (dd, *J* = 5.8 Hz, *J* = 3.2 Hz, 1H, H-4′), 3.75 (dd, *J* = 10.8 Hz, *J* = 6.0 Hz, 1H, H-6′a), 3.65 (dd, *J* = 10.8 Hz, *J* = 3.9 Hz, 1H, H-6′b). ^{13}C NMR (100 MHz, $CDCl_3$): δ 151.7 (C-2), 151.4 (C-4), 150.9 (C-6), 144.9 (C-8), 137.8 (Bn-Cq), 137.6 (Bn-Cq), 137.5 (Bn-Cq), 136.4 (Bn-Cq), 132.0 (C-5), 128.5 (Bn), 128.5 (Bn), 128.5 (Bn), 128.5 (Bn), 128.5 (Bn), 128.3 (Bn), 128.3 (Bn), 128.2 (Bn), 128.2 (Bn), 128.1 (Bn), 128.1 (Bn), 128.0 (Bn), 128.0 (Bn), 128.0 (Bn), 127.9 (Bn), 127.9 (Bn), 127.9 (Bn), 127.7 (Bn), 127.7 (Bn), 127.7 (Bn), 80.9 (C-1′), 75.8 (C-5′), 74.9 (C-4′), 74.1 (C-3′), 73.2 (Bn-CH_2), 72.8 (Bn-CH_2), 72.7 (Bn-CH_2), 72.1 (C-2′), 71.6 (Bn-CH_2), 68.3 (C-6′). Elemental anal. calcd for $C_{39}H_{37}ClN_4O_5$: C, 69.17; H, 5.51; N, 8.27; found: C, 69.00; H, 5.64; N, 7.97.

Data for compound ***13***. Colourless oil; R_f = 0.24 (EtOAc–hexane 1 : 2). $[\alpha]_D^{20}$ +56° (*c* 0.91, $CHCl_3$). ESI-MS *m*/*z* (%): 677.1 ($[M + H]^+$, 100), 699.3 ($[M + Na]^+$, 38). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.55 (s, 1H, H-2), 8.38 (s, 1H, H-8), 7.43–7.20 (m,

15H, BnH), 7.06–6.98 (m, 3H, BnH), 6.86 (m, 2H, BnH), 5.81 (d, J = 1.1 Hz, 1H, H-1′), 4.95 (d, J = 10.8 Hz, 1H, Bn-C*H*H), 4.84 (m, 2H, Bn-CH_2), 4.76 (d, J = 11.6 Hz, 1H, Bn-C*H*H), 4.65 (d, J = 10.8 Hz, 1H, Bn-CH*H*), 4.62 (d, J = 12.3 Hz, 1H, Bn-C*H*H), 4.56 (d, J = 12.3 Hz, 1H, Bn-CH*H*), 4.33 (d, J = 11.6 Hz, 1H, Bn-CH*H*), 4.09 (m, 2H, H-2′ and H-4′), 3.91 (dd, J = 9.4 Hz, J = 2.6 Hz, 1H, H-3′), 3.77–3.72 (m, 3H, H-6′a and H-6′b and H-5′). ^{13}C NMR (100 MHz, $CDCl_3$): δ 151.3 (C-2), 150.6 (C-6), 149.8 (C-4), 144.6 (C-8), 137.8 (Bn-Cq), 137.8 (Bn-Cq), 137.6 (Bn-Cq), 136.0 (Bn-Cq), 130.7 (C-5), 128.6 (Bn), 128.6 (Bn), 128.5 (Bn), 128.5 (Bn), 128.5 (Bn), 128.5 (Bn), 128.5 (Bn), 128.4 (Bn), 128.4 (Bn), 128.1 (Bn), 128.1 (Bn), 128.0 (Bn), 128.0 (Bn), 128.0 (Bn), 127.9 (Bn), 127.8 (Bn), 127.7 (Bn), 127.7 (Bn), 127.6 (Bn), 127.6 (Bn), 83.1 (C-3′), 82.1 (C-1′), 78.8 (C-5′), 75.5 (Bn-CH_2), 74.1 (C-4′), 74.4 (Bn-CH_2), 73.5 (Bn-CH_2), 73.2 (Bn-CH_2), 72.6 (C-2′), 68.8 (C-6′). Elemental anal. calcd for $C_{39}H_{37}ClN_4O_5$: C, 69.17; H, 5.51; N, 8.27; found: C, 69.04; H, 5.76; N, 8.04.

2-(Acetamido)-6-chloro-7-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)purine (14), 2-(acetamido)-6-chloro-7-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)purine (15) and 2-(acetamido)-6-chloro-9-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)purine (16). The compounds were obtained by the reaction of methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside (39 mg, 0.07 mmol) and ACP (23 mg, 0.11 mmol) according to the general procedure. Purification by column chromatography (EtOAc–cyclohexane 1 : 1) yielded **14** (3.6 mg; 7%), **15** (17.5 mg; 34%) and **16** (14.9 mg; 29%).

*Data for compound **14**.* Yellow crystals; mp 81–84 °C; R_f = 0.23 (EtOAc–hexane 1 : 1). $[\alpha]_D^{20}$ +33° (c 0.88, $CHCl_3$). ESI-MS m/z (%): 734.2 ($[M + H]^+$, 100), 756.3 ($[M + Na]^+$, 54), 1467.1 ($[2M + H]^+$, 34). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.61 (s, 1H, H-8), 8.06 (s, 1H, NH), 7.42–7.24 (m, 12H, BnH), 7.21–7.08 (m, 6H, BnH), 6.96 (m, 2H, BnH), 6.24 (d, J = 4.8 Hz, 1H, H-1′), 4.86 (d, J = 11.2 Hz, 1H, Bn-C*H*H), 4.81 (d, J = 11.2 Hz, 1H, Bn-CH*H*), 4.75 (d, J = 11.1 Hz, 1H, Bn-C*H*H), 4.71 (d, J = 12.1 Hz, 1H, Bn-C*H*H), 4.56 (d, J = 11.1 Hz, 1H, Bn-CH*H*), 4.55 (d, J = 12.2 Hz, 1H, Bn-C*H*H), 4.48 (d, J = 12.2 Hz, 1H, Bn-CH*H*), 4.36 (d, J = 12.1 Hz, 1H, Bn-CH*H*), 4.09 (dd, J = 6.7 Hz, J = 4.8 Hz, 1H, H-2′), 4.04 (dd, J = 7.4 Hz, J = 6.7 Hz, 1H, H-3′), 3.84 (dd, J = 9.5 Hz, J = 6.7 Hz, 1H, H-4′), 3.66 (dd, J = 10.6 Hz, J = 3.5 Hz, 1H, H-6′a), 3.56 (m, 2H, H-5′ and H-6′b), 2.63 (s, 3H, Ac-Me). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.1 (Ac-COO), 162.8 (C-4), 152.4 (C-2), 149.3 (C-8), 143.6 (C-6), 137.5 (Bn-Cq), 137.5 (Bn-Cq), 137.5 (Bn-Cq), 136.2 (Bn-Cq), 128.6 (Bn), 128.6 (Bn), 128.5 (Bn), 128.5 (Bn), 128.5 (Bn), 128.5 (Bn), 128.4 (Bn), 128.4 (Bn), 128.4 (Bn), 128.2 (Bn), 128.2 (Bn), 128.1 (Bn), 128.1 (Bn), 128.0 (Bn), 127.9 (Bn), 127.9 (Bn), 127.9 (Bn), 127.8 (Bn), 127.7 (Bn), 127.7 (Bn), 118.2 (C-5), 81.0 (C-1′), 80.8 (C-3′), 76.9 (C-2′), 76.6 (C-4′), 74.6 (Bn-CH_2), 74.3 (Bn-CH_2), 74.0 (Bn-CH_2), 73.5 (Bn-CH_2), 73.1 (C-5′), 68.0 (C-6′), 25.2 (Ac-Me). Elemental anal. calcd for $C_{41}H_{40}ClN_5O_6$: C, 67.07; H, 5.49; N, 9.54; found: C, 66.87; H, 5.63; N, 9.32.

*Data for compound **15**.* Yellow crystals; mp 71–74 °C; R_f = 0.09 (EtOAc–hexane 1 : 1). $[\alpha]_D^{20}$ −13° (c 0.99, $CHCl_3$). ESI-MS m/z (%): 734.13 ($[M + H]^+$, 100), 756.2 ($[M + Na]^+$, 78). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15 (s, 1H, H-8), 8.06 (s, 1H, NH), 7.39–7.17 (m, 15H, BnH), 7.10–6.99 (m, 3H, BnH), 6.80 (m, 2H, BnH), 5.61 (br, 1H, H-1′), 5.00 (d, J = 10.9 Hz, 1H, Bn-C*H*H), 4.94 (d, J = 10.9 Hz, 1H, Bn-CH*H*), 4.88 (d, J = 10.7 Hz, 1H, Bn-C*H*H), 4.64 (d, J = 11.5 Hz, 1H, Bn-C*H*H), 4.63 (d, J = 10.7 Hz, 1H, Bn-CH*H*), 4.54 (d, J = 12.1 Hz, 1H, Bn-C*H*H), 4.48 (d, J = 12.1 Hz, 1H, Bn-CH*H*), 4.24 (d, J = 11.5 Hz, 1H, Bn-CH*H*), 3.94–3.84 (m, 3H, H-2′ and H-3′ and H-5′), 3.77–3.67 (m, 3H, H-4′ and H-6′a and H-6′b), 2.64 (s, 3H, Ac-Me). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.6 (Ac-COO), 162.8 (C-4), 152.1 (C-2), 149.3 (C-8), 143.6 (C-6), 137.7 (Bn-Cq), 137.5 (Bn-Cq), 136.6 (Bn-Cq), 136.0 (Bn-Cq), 128.6 (Bn), 128.6 (Bn), 128.5 (Bn), 128.5 (Bn), 128.4 (Bn), 128.4 (Bn), 128.3 (Bn), 128.3 (Bn), 128.1 (Bn), 128.1 (Bn), 128.0 (Bn), 128.0 (Bn), 128.0 (Bn), 127.9 (Bn), 127.9 (Bn), 127.8 (Bn), 127.8 (Bn), 127.8 (Bn), 127.7 (Bn), 127.7 (Bn), 117.9 (C-5), 85.9 (C-1′), 85.9 (C-3′), 78.1 (C-4′), 77.2 (C-2′), 77.2 (C-5′), 75.9 (Bn-CH_2), 75.3 (Bn-CH_2), 74.7 (Bn-CH_2), 73.5 (Bn-CH_2), 68.3 (C-6′), 25.2 (Ac-Me). Elemental anal. calcd for $C_{41}H_{40}ClN_5O_6$: C, 67.07; H, 5.49; N, 9.54; found: C, 66.81; H, 5.52; N, 9.33.

*Data for compound **16**.* Colourless oil; R_f = 0.53 (EtOAc–hexane 1 : 1). $[\alpha]_D^{20}$ −24° (c 1.01, $CHCl_3$). ESI-MS m/z (%): 734.2 ($[M + H]^+$, 88), 756.3 ($[M + Na]^+$, 100), 1468.9 ($[2M + H]^+$, 28), 1489.7 ($[2M + Na]^+$, 10). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05 (s, 1H, NH), 8.92 (s, 1H, H-8), 7.39–7.18 (m, 15H, BnH), 7.12–6.98 (m, 3H, BnH), 6.71 (m, 2H, BnH), 5.38 (d, J = 9.1 Hz, 1H, H-1′), 5.00 (d, J = 11.0 Hz, 1H, Bn-C*H*H), 4.94 (d, J = 11.0 Hz, 1H, Bn-CH*H*), 4.88 (d, J = 10.7 Hz, 1H, Bn-C*H*H), 4.64 (d, J = 10.7 Hz, 1H, Bn-CH*H*), 4.61 (d, J = 11.6 Hz, 1H, Bn-C*H*H), 4.52 (d, J = 12.1 Hz, 1H, Bn-C*H*H), 4.45 (d, J = 12.1 Hz, 1H, Bn-CH*H*), 4.24 (d, J = 11.6 Hz, 1H, Bn-CH*H*), 4.11 (dd, J = 9.0 Hz, J = 9.0 Hz, 1H, H-2′), 3.87 (dd, J = 9.0 Hz, J = 9.0 Hz, 1H, H-3′), 3.80 (dd, J = 9.0 Hz, J = 9.0 Hz, 1H, H-4′), 3.75–3.68 (m, 3H, H-6′a and H-6′b and H-5′), 2.50 (s, 3H, Ac-Me). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.1 (Ac-COO), 151.9 (C-2), 151.8 (C-4), 151.2 (C-6), 143.0 (C-8), 137.9 (Bn-Cq), 137.5 (Bn-Cq), 137.5 (Bn-Cq), 136.3 (Bn-Cq), 128.5 (Bn), 128.5 (Bn), 128.5 (Bn), 128.5 (Bn), 128.4 (Bn), 128.4 (Bn), 128.0 (Bn), 128.0 (Bn), 128.0 (Bn), 128.0 (Bn), 128.0 (Bn), 128.0 (Bn), 127.9 (Bn), 127.9 (Bn), 127.9 (Bn), 127.8 (Bn), 127.8 (Bn), 127.8 (Bn), 127.8 (C-5), 127.7 (Bn), 127.7 (Bn), 85.9 (C-3′), 83.8 (C-1′), 75.1 (C-2′), 77.9 (C-5′), 77.2 (C-4′), 75.8 (Bn-CH_2), 75.2 (Bn-CH_2), 74.5 (Bn-CH_2), 73.5 (Bn-CH_2), 68.2 (C-6′), 25.2 (Ac-Me). Elemental anal. calcd for $C_{41}H_{40}ClN_5O_6$: C, 67.07; H, 5.49; N, 9.54; found: C, 66.81; H, 5.70; N, 9.42.

2-(Acetamido)-6-chloro-7-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)purine (17), 2-(acetamido)-6-chloro-7-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)purine (18), 2-(acetamido)-6-chloro-9-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)purine (19) and 2-(acetamido)-6-chloro-9-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)purine (20). The compounds were obtained by the reaction of methyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranoside (600 mg, 1.08 mmol) and ACP (340 mg, 1.62 mmol) according to the general procedure. Purification by column chromatography (EtOAc–cyclohexane, 1 : 1) yielded **17** (25.2 mg; 3%), **18** (346.5 mg; 44%), **19** (78.8 mg; 10%) and **20** (322.9 mg; 41%).

*Data for compound **17**.* Yellow oil; R_f = 0.11 (EtOAc–heptane 3 : 2). $[\alpha]_D^{20}$ +3° (*c* 1.00, $CHCl_3$). ESI-MS *m*/*z* (%): 734.1 ($[M + H]^+$, 100), 756.3 ($[M + Na]^+$, 28), 1467.8 ($[2M + H]^+$, 58), 1491.1 ($[2M + Na]^+$, 28). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.56 (s, 1H, H-8), 8.28 (s, 1H, NH), 7.47–7.22 (m, 15H, BnH), 7.19–7.08 (m, 3H, BnH), 6.86 (m, 2H, BnH), 6.36 (d, *J* = 2.4 Hz, 1H, H-1′), 4.84 (d, *J* = 11.8 Hz, 1H, Bn-C*H*H), 4.75 (d, *J* = 11.6 Hz, 1H, Bn-C*H*H), 4.70 (d, *J* = 11.8 Hz, 1H, Bn-CH*H*), 4.67 (d, *J* = 11.6 Hz, 1H, Bn-CH*H*), 4.55 (d, *J* = 11.6 Hz, 1H, Bn-C*H*H), 4.50 (d, *J* = 11.6 Hz, 1H, Bn-CH*H*), 4.48 (d, *J* = 11.9 Hz, 1H, Bn-C*H*H), 4.28–4.20 (m, 2H, H-5′ and H-4′), 4.14 (d, *J* = 11.9 Hz, 1H, Bn-CH*H*), 4.01–3.95 (m, 3H, H-3′ and H-6′a and H-2′), 3.81 (dd, *J* = 11.4 Hz, *J* = 3.5 Hz, 1H, H-6′b), 2.67 (s, 3H, Ac-Me). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.6 (Ac-COO), 162.8 (C-4), 152.1 (C-2), 149.2 (C-8), 142.6 (C-6), 137.7 (Bn-Cq), 137.6 (Bn-Cq), 137.5 (Bn-Cq), 135.9 (Bn-Cq), 128.6 (Bn), 128.6 (Bn), 128.5 (Bn), 128.5 (Bn), 128.5 (Bn), 128.4 (Bn), 128.3 (Bn), 128.3 (Bn), 128.3 (Bn), 128.3 (Bn), 128.2 (Bn), 128.1 (Bn), 128.0 (Bn), 127.9 (Bn), 127.9 (Bn), 127.8 (Bn), 127.8 (Bn), 127.7 (Bn), 127.7 (Bn), 127.7 (Bn), 117.7 (C-5), 78.5 (C-1′), 75.1 (C-5′), 74.7 (C-3′), 74.4 (C-2′), 73.4 (Bn-CH_2), 73.3 (Bn-CH_2), 73.1 (Bn-CH_2), 73.0 (Bn-CH_2), 72.5 (C-4′), 65.5 (C-6′), 25.2 (Ac-Me). Elemental anal. calcd for $C_{41}H_{40}ClN_5O_6$: C, 67.07; H, 5.49; N, 9.54; found: C, 66.77; H, 5.53; N, 9.39.

*Data for compound **18**.* Yellow oil; R_f = 0.26 (EtOAc–cyclohexane 1 : 1). $[\alpha]_D^{20}$ −4° (*c* 1.26, $CHCl_3$). ESI-MS *m*/*z* (%): 734.2 ($[M + H]^+$, 82), 756.4 ($[M + Na]^+$, 22), 1468.9 ($[2M + H]^+$, 100), 1491.2 ($[2M + Na]^+$, 76). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.16 (s, 1H, H-8), 8.00 (s, 1H, NH), 7.43–7.23 (m, 15H, BnH), 7.12–7.01 (m, 3H, BnH), 6.81 (m, 2H, BnH), 5.65 (d, *J* = 7.3 Hz, 1H, H-1′), 5.00 (d, *J* = 11.2 Hz, 1H, Bn-C*H*H), 4.84 (d, *J* = 11.6 Hz, 1H, Bn-C*H*H), 4.77 (d, *J* = 11.6 Hz, 1H, Bn-CH*H*), 4.71 (d, *J* = 11.4 Hz, 1H, Bn-C*H*H), 4.62 (d, *J* = 11.2 Hz, 1H, Bn-CH*H*), 4.48 (d, *J* = 11.8 Hz, 1H, Bn-C*H*H), 4.43 (d, *J* = 11.8 Hz, 1H, Bn-CH*H*), 4.29 (d, *J* = 11.4 Hz, 1H, Bn-CH*H*), 4.28 (m, 1H, H-2′), 4.09 (d, *J* = 2.5 Hz, 1H, H-4′), 3.84 (m, 1H, H-5′), 3.79 (dd, *J* = 9.3 Hz, *J* = 2.5 Hz, 1H, H-3′), 3.66–3.57 (m, 2H, H-6′a and H-6′b), 2.63 (s, 3H, Ac-Me). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.4 (Ac-COO), 162.9 (C-4), 152.0 (C-2), 147.9 (C-8), 143.7 (C-6), 138.1 (Bn-Cq), 137.5 (Bn-Cq), 137.3 (Bn-Cq), 136.0 (Bn-Cq), 128.6 (Bn), 128.6 (Bn), 128.5 (Bn), 128.5 (Bn), 128.4 (Bn), 128.4 (Bn), 128.2 (Bn), 128.2 (Bn), 128.1 (Bn), 128.1 (Bn), 128.0 (Bn), 128.0 (Bn), 127.9 (Bn), 127.9 (Bn), 127.9 (Bn), 127.9 (Bn), 127.9 (Bn), 127.6 (Bn), 127.6 (Bn), 118.7 (C-5), 85.2 (C-1′), 83.6 (C-3′), 77.2 (C-2′), 76.5 (C-5′), 75.1 (Bn-CH_2), 75.0 (Bn-CH_2), 73.6 (Bn-CH_2), 73.2 (C-4′), 72.6 (Bn-CH_2), 68.2 (C-6′), 25.2 (Ac-Me). Elemental anal. calcd for $C_{41}H_{40}ClN_5O_6$: C, 67.07; H, 5.49; N, 9.54; found: C, 67.00; H, 5.67; N, 9.40.

*Data for compound **19**.* Yellow oil; R_f = 0.56 (EtOAc–heptane 3 : 2). $[\alpha]_D^{20}$ +5° (*c* 0.99, $CHCl_3$). ESI-MS *m*/*z* (%): 734.1 ($[M + H]^+$, 100), 756.4 ($[M + Na]^+$, 54), 1467.5 ($[2M + H]^+$, 38), 1492.1 ($[2M + Na]^+$, 22). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.25 (s, 1H, H-8), 8.04 (s, 1H, NH), 7.47–7.12 (m, 18H, BnH), 6.89 (m, 2H, BnH), 6.08 (d, *J* = 3.0 Hz, 1H, H-1′), 4.86 (d, *J* = 12.0 Hz, 1H, Bn-C*H*H), 4.77 (d, *J* = 11.8 Hz, 1H, Bn-C*H*H), 4.71 (d, *J* = 12.0 Hz, 1H, Bn-CH*H*), 4.68 (d, *J* = 11.8 Hz, 1H, Bn-CH*H*), 4.53 (m, 2H, Bn-CH_2), 4.52 (d, *J* = 12.0 Hz, 1H, Bn-C*H*H), 4.44–4.28 (m, 2H, H-5′ and H-4′), 4.20 (d, *J* = 12.0 Hz, 1H, Bn-CH*H*), 4.15 (m, 1H, H-3′), 4.03 (dd, *J* = 11.2 Hz, *J* = 8.3 Hz, 1H, H-6′a), 3.97 (m, 1H, H-2′), 3.81 (dd, *J* = 11.2 Hz, *J* = 3.4 Hz, 1H, H-6′b), 2.44 (s, 3H, Ac-Me). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.5 (Ac-COO), 152.2 (C-2), 151.5 (C-4), 150.9 (C-6), 144.3 (C-8), 137.8 (Bn-Cq), 137.7 (Bn-Cq), 137.7 (Bn-Cq), 136.2 (Bn-Cq), 128.5 (Bn), 128.5 (Bn), 128.5 (Bn), 128.5 (Bn), 128.3 (Bn), 128.3 (Bn), 128.2 (Bn), 128.2 (Bn), 128.2 (Bn), 128.0 (Bn), 128.0 (Bn), 127.9 (Bn), 127.9 (Bn), 127.8 (Bn), 127.8 (Bn), 127.7 (Bn), 127.7 (Bn), 127.7 (Bn), 127.8 (C-5), 127.7 (Bn), 127.6 (Bn), 77.1 (C-1′), 75.4 (C-5′), 74.7 (C-3′), 74.1 (C-2′), 73.3 (Bn-CH_2), 73.2 (Bn-CH_2), 73.1 (Bn-CH_2), 73.0 (Bn-CH_2), 72.9 (C-4′), 65.9 (C-6′), 25.1 (Ac-Me). Elemental anal. calcd for $C_{41}H_{40}ClN_5O_6$: C, 67.07; H, 5.49; N, 9.54; found: C, 69.80; H, 5.69; N, 9.41.

*Data for compound **20**.* A colourless oil; R_f = 0.57 (EtOAc–hexane 1 : 1). $[\alpha]_D^{20}$ −14° (*c* 1.02, $CHCl_3$). ESI-MS *m*/*z* (%): 734.3 ($[M + H]^+$, 100), 756.5 ($[M + Na]^+$, 56), 1467.9 ($[2M + H]^+$, 22). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93 (s, 1H, NH), 7.92 (s, 1H, H-8), 7.43–7.22 (m, 15H, BnH), 7.10 (m, 1H, BnH), 7.01 (m, 2H, BnH), 6.73 (m, 2H, BnH), 5.35 (d, *J* = 9.0 Hz, 1H, H-1′), 4.96 (d, *J* = 11.4 Hz, 1H, Bn-C*H*H), 4.81 (d, *J* = 11.7 Hz, 1H, Bn-C*H*H), 4.75 (d, *J* = 11.7 Hz, 1H, Bn-CH*H*), 4.68 (d, *J* = 11.8 Hz, 1H, Bn-C*H*H), 4.64 (d, *J* = 11.8 Hz, 1H, Bn-CH*H*), 4.45 (d, *J* = 11.9 Hz, 1H, Bn-C*H*H), 4.43 (m, 1H, H-2′), 4.40 (d, *J* = 11.9 Hz, 1H, Bn-CH*H*), 4.29 (d, *J* = 11.4 Hz, 1H, Bn-CH*H*), 4.06 (m, 1H, H-4′), 3.79 (dd, *J* = 6.3 Hz, *J* = 6.3 Hz, 1H, H-5′), 3.76 (dd, *J* = 9.6 Hz, *J* = 2.6 Hz, 1H, H-3′), 3.55 (m, 2H, H-6′a and H-6′b), 2.42 (s, 3H, Ac-Me). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.3 (Ac-COO), 152.1 (C-2), 151.9 (C-4), 151.1 (C-6), 143.1 (C-8), 138.2 (Bn-Cq), 137.6 (Bn-Cq), 137.4 (Bn-Cq), 136.6 (Bn-Cq), 128.6 (Bn), 128.6 (Bn), 128.4 (Bn), 128.4 (Bn), 128.4 (Bn), 128.4 (Bn), 128.1 (Bn), 128.1 (Bn), 128.1 (Bn), 128.0 (Bn), 128.0 (Bn), 128.0 (Bn), 128.0 (Bn), 128.0 (Bn), 128.0 (Bn), 128.0 (Bn), 128.0 (Bn), 127.8 (C-5), 127.6 (Bn), 127.6 (Bn), 83.9 (C-1′), 83.6 (C-3′), 76.5 (C-5′), 75.7 (C-2′), 74.8 (Bn-CH_2), 74.8 (Bn-CH_2), 73.6 (Bn-CH_2), 73.0 (C-4′), 72.6 (Bn-CH_2), 68.2 (C-6′), 25.2 (Ac-Me). Elemental anal. calcd for $C_{41}H_{40}ClN_5O_6$: C, 67.07; H, 5.49; N, 9.54; found: C, 66.92; H, 5.61; N, 9.47.

2-(Acetamido)-6-chloro-7-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)purine (21), 2-(acetamido)-6-chloro-7-(2,3,4,6-tetra-*O*-benzyl-β-D-mannopyranosyl)purine (22), 2-(acetamido)-6-chloro-9-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)purine (23) and 2-(acetamido)-6-chloro-9-(2,3,4,6-tetra-*O*-benzyl-β-D-mannopyranosyl)purine (24). The compounds were obtained by the reaction of methyl 2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranoside (250 mg, 0.45 mmol) and ACP (112 mg, 0.54 mmol) according to the general procedure. Purification by column chromatography (EtOAc–cyclohexane 1 : 1) yielded **21** (27.1 mg; 8%), **22** (92.5 mg; 28%), **23** (81.3 mg; 24%) and **24** (10.2 mg, 3%).

*Data for compound **21**.* Yellow oil; R_f = 0.47 (EtOAc–hexane 4 : 1). $[\alpha]_D^{20}$ +38° (*c* 1.03, $CHCl_3$). ESI-MS *m*/*z* (%): 734.1 ($[M + H]^+$, 100), 756.3 ($[M + Na]^+$, 58), 1467.9 ($[2M + H]^+$, 20), 1488.7 ($[2M + Na]^+$, 8). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.16 (s, 1H, H-8), 8.02 (s, 1H, NH), 7.41–7.14 (m, 18H, BnH), 6.88 (m, 2H, BnH), 6.36 (d, *J* = 8.9 Hz, 1H, H-1′), 4.68 (d, *J* = 12.0 Hz, 1H, Bn-C*H*H),

4.60 (d, J = 12.0 Hz, 1H, Bn-CH*H*), 4.59 (d, J = 11.6 Hz, 1H, Bn-C*H*H), 4.50 (s, 2H, Bn-CH_2), 4.44 (d, J = 11.6 Hz, 1H, Bn-CH*H*), 4.37 (m, 1H, H-5′), 4.30 (d, J = 11.9 Hz, 1H, Bn-C*H*H), 4.11 (dd, J = 8.9 Hz, J = 2.5 Hz, 1H, H-2′), 4.05 (m, 1H, H-3′), 4.04 (d, J = 11.9 Hz, 1H, Bn-CH*H*), 3.92 (dd, J = 10.2 Hz, J = 7.5 Hz, 1H, H-6′a), 3.82 (dd, J = 3.3 Hz, J = 1.2 Hz, 1H, H-4′), 3.69 (dd, J = 10.2 Hz, J = 5.9 Hz, 1H, H-6′b), 2.63 (s, 3H, Ac-Me). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.5 (Ac-COO), 162.9 (C-4), 152.1 (C-2), 147.6 (C-8), 143.8 (C-6), 137.6 (Bn-Cq), 137.4 (Bn-Cq), 137.3 (Bn-Cq), 136.1 (Bn-Cq), 128.6 (Bn), 128.6 (Bn), 128.5 (Bn), 128.5 (Bn), 128.5 (Bn), 128.5 (Bn), 128.4 (Bn), 128.4 (Bn), 128.2 (Bn), 128.1 (Bn), 128.1 (Bn), 127.9 (Bn), 127.9 (Bn), 127.9 (Bn), 127.9 (Bn), 127.8 (Bn), 127.8 (Bn), 127.8 (Bn), 127.7 (Bn), 127.7 (Bn), 119.1 (C-5), 78.6 (C-1′), 77.2 (C-5′), 75.1 (C-2′), 74.1 (C-4′), 73.5 (C-3′), 73.3 (Bn-CH_2), 73.3 (Bn-CH_2), 71.9 (Bn-CH_2), 71.3 (Bn-CH_2), 67.4 (C-6′), 25.2 (Ac-Me). Elemental anal. calcd for $C_{41}H_{40}ClN_5O_6$: C, 67.07; H, 5.49; N, 9.54; found: C, 66.82; H, 5.54; N, 9.31.

Data for compound ***22****.* Yellow crystals; mp 80–82 °C; R_f = 0.36 (EtOAc–hexane 4 : 1). $[\alpha]_D^{20}$ +74° (c 0.98, $CHCl_3$). ESI-MS m/z (%): 734.5 ($[M + H]^+$, 70), 756.5 ($[M + Na]^+$, 20), 1469.2 ($[2M + H]^+$, 100), 1491.3 ($[2M + Na]^+$, 44). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.34 (s, 1H, H-8), 8.09 (s, 1H, NH), 7.38–7.16 (m, 15H, BnH), 6.89 (m, 3H, BnH), 6.71 (m, 2H, BnH), 5.75 (d, J = 1.0 Hz, 1H, H-1′), 4.90 (d, J = 10.9 Hz, 1H, Bn-C*H*H), 4.83 (d, J = 11.8 Hz, 1H, Bn-C*H*H), 4.72 (d, J = 11.8 Hz, 1H, Bn-CH*H*), 4.61 (d, J = 11.8 Hz, 1H, Bn-C*H*H), 4.59 (d, J = 10.9 Hz, 1H, Bn-CH*H*), 4.55 (d, J = 12.4 Hz, 1H, Bn-C*H*H), 4.49 (d, J = 12.4 Hz, 1H, Bn-CH*H*), 4.26 (d, J = 11.8 Hz, 1H, Bn-CH*H*), 3.98 (dd, J = 9.4 Hz, J = 9.4 Hz, 1H, H-4′), 3.91 (m, 1H, H-2′), 3.75 (dd, J = 9.4 Hz, J = 2.5 Hz, 1H, H-3′), 3.71 (m, 3H, H-6′a and H-6′b and H-5′), 2.56 (s, 3H, Ac-Me). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.2 (Ac-COO), 162.9 (C-4), 152.9 (C-2), 148.5 (C-8), 141.5 (C-6), 137.7 (Bn-Cq), 137.7 (Bn-Cq), 137.4 (Bn-Cq), 135.8 (Bn-Cq), 128.8 (Bn), 128.8 (Bn), 128.6 (Bn), 128.6 (Bn), 128.5 (Bn), 128.5 (Bn), 128.4 (Bn), 128.4 (Bn), 128.4 (Bn), 128.2 (Bn), 128.2 (Bn), 128.1 (Bn), 128.1 (Bn), 128.0 (Bn), 128.0 (Bn), 128.0 (Bn), 127.8 (Bn), 127.8 (Bn), 127.8 (Bn), 127.6 (Bn), 116.9 (C-5), 83.9 (C-1′), 82.3 (C-3′), 78.9 (C-5′), 74.0 (C-4′), 75.2 (Bn-CH_2), 74.2 (Bn-CH_2), 73.7 (Bn-CH_2), 73.4 (Bn-CH_2), 72.9 (C-2′), 68.7 (C-6′), 25.2 (Ac-Me). Elemental anal. calcd for $C_{41}H_{40}ClN_5O_6$: C, 67.07; H, 5.49; N, 9.54; found: C, 66.87; H, 5.39; N, 9.47.

Data for compound ***23****.* Yellow oil; R_f = 0.73 (EtOAc–hexane 1 : 1). $[\alpha]_D^{20}$ +50° (c 1.22, $CHCl_3$). ESI-MS m/z (%): 734.5 ($[M + H]^+$, 100), 756.5 ($[M + Na]^+$, 47), 1467.1 ($[2M + H]^+$, 24), 1491.1 ($[2M + Na]^+$, 34). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.94 (s, 1H, H-8), 7.94 (s, 1H, NH), 7.37–7.00 (m, 18H, BnH), 6.82 (m, 2H, BnH), 5.85 (d, J = 8.6 Hz, 1H, H-1′), 4.68 (d, J = 12.1 Hz, 1H, Bn-C*H*H), 4.65 (m, 1H, H-2′), 4.62 (d, J = 12.1 Hz, 1H, Bn-CH*H*), 4.48 (d, J = 12.1 Hz, 1H, Bn-C*H*H), 4.43 (d, J = 12.6 Hz, 1H, Bn-C*H*H), 4.42 (br, 2H, Bn-CH_2), 4.40 (m, 1H, H-5′), 4.40 (d, J = 12.6 Hz, 1H, Bn-CH*H*), 4.10 (d, J = 12.1 Hz, 1H, Bn-CH*H*), 3.96 (dd, J = 2.7 Hz, J = 2.7 Hz, 1H, H-3′), 3.70 (dd, J = 10.7 Hz, J = 6.3 Hz, 1H, H-6′a), 3.80 (dd, J = 5.2 Hz, J = 2.9 Hz, 1H, H-4′), 3.59 (dd, J = 10.6 Hz, J = 4.2 Hz, 1H, H-6′b), 2.34 (s, 3H, Ac-Me). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5 (Ac-COO), 152.1 (C-2), 151.5 (C-4), 151.2 (C-6), 144.5 (C-8), 137.8 (Bn-Cq), 137.5 (Bn-Cq), 137.3 (Bn-Cq), 136.5 (Bn-Cq), 128.6 (Bn), 128.6 (Bn), 128.5 (Bn), 128.5 (Bn), 128.5 (Bn), 128.4 (Bn), 128.4 (Bn), 128.3 (Bn), 128.2 (Bn), 128.2 (Bn), 128.2 (C-5), 128.1 (Bn), 128.1 (Bn), 128.0 (Bn), 128.0 (Bn), 128.0 (Bn), 127.9 (Bn), 127.9 (Bn), 127.7 (Bn), 127.6 (Bn), 127.6 (Bn), 80.7 (C-1′), 76.0 (C-5′), 74.7 (C-4′), 73.6 (C-3′), 73.1 (C-2′), 72.7 (Bn-CH_2), 72.1 (Bn-CH_2), 71.9 (Bn-CH_2), 71.2 (Bn-CH_2), 68.1 (C-6′), 25.1 (Ac-Me). Elemental anal. calcd for $C_{41}H_{40}ClN_5O_6$: C, 67.07; H, 5.49; N, 9.54; found: C, 66.91; H, 5.52; N, 9.41.

Data for compound ***24****.* Yellow oil; R_f = 0.61 (EtOAc–hexane 1 : 1). $[\alpha]_D^{20}$ +77° (c 1.00, $CHCl_3$). ESI-MS m/z (%): 734.5 ($[M + H]^+$, 100), 756.6 ($[M + Na]^+$, 52). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.21 (s, 1H, H-8), 8.19 (s, 1H, NH), 7.38–7.16 (m, 15H, BnH), 7.03–6.96 (m, 3H, BnH), 6.83 (m, 2H, BnH), 5.62 (br, 1H, H-1′), 4.89 (d, J = 10.8 Hz, 1H, Bn-C*H*H), 4.79 (m, 2H, Bn-CH_2), 4.72 (d, J = 11.6 Hz, 1H, Bn-C*H*H), 4.59 (d, J = 10.8 Hz, 1H, Bn-CH*H*), 4.56 (d, J = 12.2 Hz, 1H, Bn-C*H*H), 4.49 (d, J = 12.2 Hz, 1H, Bn-CH*H*), 4.32 (d, J = 11.6 Hz, 1H, Bn-CH*H*), 4.05 (m, 1H, H-2′), 4.02 (dd, J = 9.5 Hz, J = 9.5 Hz, 1H, H-4′), 3.84 (dd, J = 9.4 Hz, J = 2.5 Hz, 1H, H-3′), 3.72–3.64 (m, 3H, H-6′a and H-6′b and H-5′), 2.38 (s, 3H, Ac-Me). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4 (Ac-COO), 151.3 (C-4), 150.8 (C-2), 150.6 (C-6), 143.9 (C-8), 137.8 (Bn-Cq), 137.8 (Bn-Cq), 137.6 (Bn-Cq), 136.2 (Bn-Cq), 128.6 (Bn), 128.6 (Bn), 128.5 (Bn), 128.5 (Bn), 128.4 (Bn), 128.4 (Bn), 128.4 (Bn), 128.4 (Bn), 128.3 (C-5), 128.0 (Bn), 128.0 (Bn), 128.0 (Bn), 127.9 (Bn), 127.9 (Bn), 127.9 (Bn), 127.8 (Bn), 127.7 (Bn), 127.7 (Bn), 127.7 (Bn), 127.7 (Bn), 127.7 (Bn), 82.8 (C-3′), 82.1 (C-1′), 78.7 (C-5′), 75.3 (Bn-CH_2), 74.3 (Bn-CH_2), 74.1 (C-4′), 73.4 (Bn-CH_2), 73.1 (Bn-CH_2), 72.5 (C-2′), 68.7 (C-6′), 25.0 (Ac-Me). Elemental anal. calcd for $C_{41}H_{40}ClN_5O_6$: C, 67.07; H, 5.49; N, 9.54; found: C, 66.98; H, 5.54; N, 9.37.

Enzymatic studies

Spectrophotometer and chemicals. A TECAN SpectraFluor-Plus working on the 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 were purchased from Fluka. Butyrylcholinesterase (from equine serum) was purchased from Sigma and butyrylthiocholine iodide was bought from Aldrich.

Solutions preparation. Preparation of 50 mM Tris-HCl buffer pH 8.0: tris(hydroxymethyl)aminomethane (606 mg) was dissolved in bidistilled water (100 mL) and adjusted with HCl to a pH of 8.0 ± 0.1. Buffer was freshly prepared and stored in the refrigerator. AChE solution 2.005 U mL^{-1}: the enzyme (271 U mg^{-1}, 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^{-1}: the enzyme (7.54 U mg^{-1}, 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 bidistilled water (10 mL). BTChI solution 15 mM: BTChI (47.6 mg) was dissolved in

bidistilled water (10 mL). All solutions were stored in eppendorf caps in the refrigerator or freezer, if necessary. The pure compounds were initially dissolved in DMSO, galantamine hydrobromide as standard was dissolved in bidistilled water. The final concentrations for the enzymatic assay were yielded by diluting the stock solution with bidistilled water. No inhibition was detected by residual DMSO (<0.5%).

Enzyme assay. A mixture of the DTNB solution (125 μL), enzyme (25 μL) and compounds solution (25 μL, 3 different concentrations and once blank water) was prepared and incubated at 30 °C for 20 min. The substrate (25 μL, 4 different concentrations) was added to start the enzymatic reaction. The absorbance data (415 nm) were recorded under a controlled temperature of 30 °C for 30 min at 1 min intervals. All measurements were performed as triplicates. The final concentrations in the test were as follows: [AChE] = 2.005 U mL^{-1}, [BChE] = 2.040 U mL^{-1}, [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 the Lineweaver–Burk plot,[33] the Dixon plot[34] and the Cornish-Bowden plot.[35]

Conclusions

A series of 24 new nucleosides were synthesized and tested, leading to new insights into the structural features required for BChE or AChE selective inhibition. Selectivity for both these enzymes can be tuned by the purine base, the anomeric configuration and the glycosyl moiety. The 6-chloropurine nucleosides are mainly AChE selective inhibitors, with the exception of their mannosyl nucleosides, which also inhibit BChE. The most potent and selective BChE inhibitors embody the 2-acetamido-6-chloropurine base N^7-linked to the mannosyl and the glucosyl moieties, and the α-anomers are the most promising isomers, exhibiting activities ranging from K_i 50 nM to 2.46 μM. When compared to the purine bicyclic nucleosides previously designed by us, this new series of compounds is much more easy to synthesize, and includes the 2-acetamido-N^7-α-D-mannosylpurine **21**, a competitive and selective 50 nM BChE inhibitor. This new lead and its BChE selective inhibitor analogs represent new molecular entities that may be useful for the understanding of BChE's role in AD with the potential to further contribute to new therapeutic approaches to AD treatment.

Acknowledgements

The authors wish to thank Dr Nuno M. Xavier and the Erasmus student Laure Mathon-Claudon for support in the compounds' synthesis. Fundação para a Ciência e a Tecnologia is gratefully acknowledged for Stefan Schwarz Postdoc research grant SFRH/BPD/81065/2011 and the support of the project Pest-OE/QUI/UI0612/2013. The ESI-MS spectra were generously measured by Dr Ralph Kluge.

References

1 A. Alzheimer, *Allg. Zschr. Psychiatr. Psych. gerichtl. Med.*, 1907, **64**, 146.
2 P. J. Whitehouse, D. L. Price, A. W. Clark, J. T. Coyle and M. R. DeLong, *Ann. Neurol.*, 1981, **10**, 122.
3 R. T. Bartus, R. L. I. Dean, B. Beer and A. S. Lippa, *Science*, 1982, **217**, 408.
4 J. T. Coyle, D. L. Price and M. R. DeLong, *Science*, 1983, **219**, 1184.
5 N. H. Greig, T. Utsuki, Q.-S. Yu, X. Zhu, H. W. Holloway, T. Perry, B. Lee, D. K. Ingram and D. K. Lahiri, *Curr. Med. Res. Opin.*, 2001, **17**, 159.
6 E. Giacobini, in *Morphological and Biochemical Correlates of Neural Activity*, ed. M. M. Cohen and R. S. Snyder, Harper and Row, New York, 1964, pp. 15–38.
7 N. H. Greig, T. Utsuki, D. K. Ingram, Y. Wang, G. Pepeu, C. Scali, Q.-S. Yu, J. Mamczarz, H. W. Holloway, T. Giordano, D. Chen, K. Furukawa, K. Sambamurti, A. Brossi and D. K. Lahiri, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 17213.
8 E. Giacobini, in *Cholinesterases and Cholinesterase Inhibitors*, ed. E. Giacobini, Martin Dunitz, London, 2000, pp. 181–226.
9 M.-M. Mesulam, A. Guillozet, P. Shaw, A. Levey, E. G. Duysen and O. Lockridge, *Neuroscience*, 2002, **110**, 627.
10 E. K. Perry, B. E. Tomlinson, G. Blessed, K. Bergmann, P. H. Gibson and R. H. Perry, *Br. Med. J.*, 1978, **2**, 1457.
11 E. Giacobini, *Int. J. Geriatr. Psychiatry*, 2003, **18**, 1.
12 V. Zarotsky, J. J. Sramek and N. R. Cutler, *Am. J. Health Syst. Pharm.*, 2003, **60**, 446.
13 H. Sugimoto, *Pure Appl. Chem.*, 1999, **71**, 2031.
14 H. Sugimoto, Y. Yamanishi, Y. Iimura and Y. Kawakami, *Curr. Med. Chem.*, 2000, **7**, 303.
15 M. W. Jann, *Pharmacotherapy*, 2000, **20**, 1.
16 T. Darreh-Shori, S. Vijayaraghavan, S. Aeinehband, F. Piehl, R. P. F. Lindblom, B. Nilsson, K. N. Ekdahl, B. Långström, O. Almkvist and A. Nordberg, *Neurobiol. Aging*, 2013, **34**, 2465.
17 R. León, A. G. Garcia and J. Marco-Contelles, *Med. Res. Rev.*, 2013, **33**, 139.
18 S. Darvesh, M. K. Cash, G. A. Reid, E. Martin, A. Mitnitski and C. Geula, *J. Neuropathol. Exp. Neurol.*, 2012, **71**, 2.
19 R. M. Lane and Y. He, *Alzheimers Dement.*, 2013, **9**, 1.
20 J. Takahashi, I. Hijikuro, T. Kihara, M. G. Murugesh, S. Fuse, R. Kunimoto, Y. Tsumura, A. Akaike, T. Niidome, Y. Okuno, T. Takahashi and H. Sugimoto, *Bioorg. Med. Chem.*, 2010, **20**, 1718.
21 Y. J. Woo, B. H. Lee, G. H. Yeun, H. J. Kim, M.-H. Won, S. H. Kim, B. H. Lee and J. H. Park, *Bull. Korean Chem. Soc.*, 2011, **32**, 2593.
22 X. Chen, I. G. Tikhonova and M. Decker, *Bioorg. Med. Chem.*, 2011, **19**, 1222.
23 C. Meier, C. Ducho, U. Görbig, R. Esnouf and J. Balzarini, *J. Med. Chem.*, 2004, **47**, 2839.

24 C. Ducho, U. Görbig, S. Jessel, N. Gisch, J. Balzarini and C. Meier, *J. Med. Chem.*, 2007, **50**, 1335.
25 F. Marcelo, F. V. M. Silva, M. Goulart, J. Justino, P. Sinaÿ, Y. Blériot and A. P. Rauter, *Bioorg. Med. Chem.*, 2009, **17**, 5106.
26 R. N. Shah, J. Baptista, G. R. Perdomo, J. P. Carver and J. J. Krepinsky, *J. Carbohydr. Chem.*, 1987, **6**, 645.
27 P. Garner, J. U. Yoo and R. Sarabu, *Tetrahedron*, 1992, **48**, 4259.
28 P. Garner and S. Ramakanth, *J. Org. Chem.*, 1988, **53**, 1294.
29 P. I. Pradeepkumar, P. Cheruku, O. Plashkevych, P. Acharya, S. Gohil and J. Chattopadhyaya, *J. Am. Chem. Soc.*, 2004, **126**, 11484.
30 M. J. Robins, Z. Guo and S. F. Wnuk, *J. Am. Chem. Soc.*, 1997, **119**, 3637.
31 Z.-G. Gao, S.-K. Kim, T. Biadatti, W. Chen, K. Lee, D. Barak, S. G. Kim, C. R. Johnson and K. A. Jacobson, *J. Med. Chem.*, 2002, **45**, 4471.
32 S. Di Giovanni, A. Borloz, A. Urbain, A. Marston, K. Hostettmann, P. A. Carrupt and M. Reist, *Eur. J. Pharm. Sci.*, 2008, **33**, 109.
33 H. Lineweaver and D. Burk, *J. Am. Chem. Soc.*, 1934, **56**, 658.
34 M. Dixon, *Biochem. J.*, 1953, **55**, 170.
35 A. Cornish-Bowden, *Biochem. J.*, 1974, **137**, 143.