European Journal of Medicinal Chemistry 90 (2015) 595–602

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

New antitumor 6-chloropurine nucleosides inducing apoptosis and G2/M cell cycle arrest

Stefan Schwarz [a, b], Bianka Siewert [b], René Csuk [b], Amélia P. Rauter [a, *]

[a] Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, Ed. C8, Piso 5, 1749-016 Lisboa, Portugal
[b] Bereich Organische Chemie, Martin-Luther-Universität Halle-Wittenberg, Kurt-Mothes-Str. 2, D-06120 Halle (Saale), Germany

ARTICLE INFO

Article history:
Received 21 July 2014
Received in revised form
29 October 2014
Accepted 9 November 2014
Available online 11 November 2014

Keywords:
6-Chloropurine
N-glycosylation
Antitumor
SRB assay
Cell cycle analysis
Acridine orange

ABSTRACT

Treating cancer has been challenging for decades, following countless approaches and attempts. Nucleosides, alone or as part of nucleotides, are vital elements of living systems and have shown pharmacological effects, *e.g.* as antibiotic or antiviral agents. We investigated the antitumor potential on human melanoma, lung and ovarian carcinomas, and on colon adenocarcinoma of a new series of purine nucleosides based on a 6-chloropurine or a 2-acetamido-6-chloropurine scaffold linked to perbenzylated hexosyl (glucosyl, galactosyl and mannosyl) residues. All compounds were tested in a sulforhodamine B (SRB) assay for their cytotoxicity and provided micromolar GI_{50} values with order of magnitude comparable to structurally similar chemotherapeutics, namely 2-chloro-2′-deoxyadenosine (cladribine). Furthermore, the induction of apoptosis was established and cell cycle analysis was accomplished demonstrating a G2/M cell cycle arrest.

1. Introduction

Purine nucleosides and nucleotides have been major targets of anticancer research for several decades. One of the first investigations was accomplished by the group of Noell in 1962 synthesizing a series of thioguanines and 2-amino-6-alkylthiopurine derivatives [1]. Studies dealing with compounds containing purine moieties, *e.g.* in gold (III) complexes [2] or adenine derivatives [3–5] have also been reported and effective camptothecin purine derivatives possessing GI_{50} values in micromolar range were synthesized by Li et al. [6]. Caba et al. [7] reported on an antiproliferative agent against the MCF-7 adenocarcinoma with micromolar GI_{50} value embodying tetrahydrobenzoxazepine N^9-linked to a 6-chloropurine while a coumarin N^9-linked to a 2-amino-6-chloropurine showed moderate activity (25–35 μM) against the HeLa, HepG2 and SW620 cell lines [9]. Voller et al. accomplished substitutions at different positions of a 6-aminopurine scaffold and have shown that some of the ribosides exhibited micromolar anticancer activity while their N^7- or N^9-linked glucoside analogues were not active [8]. In addition, a 9-norbornyl-6-chloropurine was recently reported as a novel antileukemic compound [10]. The previously reported anticancer potential of 6-chloropurine derived compounds encouraged us to investigate, for the first time, a series of purines embodying a 6-chloro substitution (CP) or both 6-chloro- and 2-acetamido groups (ACP), linked at N^7 or N^9 to perbenzylated d-*glucosyl*, D-*mannosyl* and D-*galactosyl* residues. The reaction conditions described by Marcelo et al. [11] using a silylated base was optimized for this type of nucleosides. The anticancer activity was determined using a sulforhodamine B (SRB) assay to yield GI_{50} values for human melanoma, lung and ovarian carcinoma, and colon adenocarcinoma cancer cell lines. Furthermore, the substances were tested on murine embryonic fibroblasts (NiH 3T3) to investigate their tumor cell-to-control-specifity. In addition, acridine orange/propidium iodide assays, DNA laddering experiments and cell cycle analyses were performed for the most active compound to gain some information about the mode of action of this family of new anticancer molecular entities.

2. Results

2.1. Chemistry

Nucleoside synthesis can be performed by a two steps procedure, starting with the acetylation [12] or halogenation [13,14] of

* Corresponding author. Faculdade de Ciências, Universidade de Lisboa, Centro de Química e Bioquímica/Departamento de Química e Bioquímica, Campo Grande, Ed. C8, Piso 5, 1749-016 Lisboa, Portugal.
E-mail address: aprauter@fc.ul.pt (A.P. Rauter).

http://dx.doi.org/10.1016/j.ejmech.2014.11.019
0223-5234/

Table 1
Experimental conditions for the reaction of methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glycoside with the purine (CP or ACP). 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, conventional heating at 65 °C was performed.

Entry	Purine	Eq. TMSOTf	Time	β-N^7/N^9	Overall yield
1	CP	1	24 h		n.d.[a]
2	CP	2	2 h	1/1.9	43%
3	CP	4	2 h	1/4.5	62%
4	CP	8	2 h	1/3.8	65%
5	ACP	1	24 h		n.d.
6	ACP	2	2 h	1/2.4	1%
7	ACP	4	2 h	1/1	62%
8	ACP	8	2 h	1/1.3	63%

[a] n.d: no product detected.

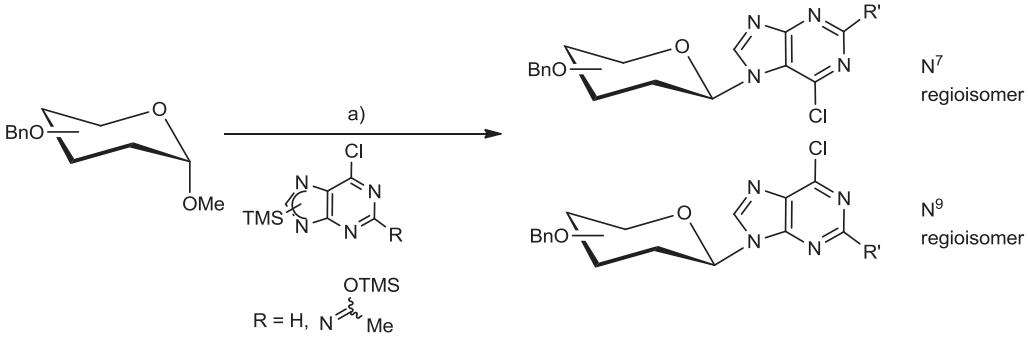
suitable precursors, followed by reaction with the heterocyclic base. A direct access to nucleosides can be gained by Lewis acid activation with tin chloride [15,16] or TMSOTf [17] of methyl glycosides in a reaction employing a persilylated purine. Marcelo et al. applied TMSOTf in acetonitrile to link regioselectively bicyclic pyranosides to purine scaffolds at their N^7 position with β-stereoselectivity [11]. These conditions were investigated in this study for this nucleoside series. Therefore, monosaccharidyl donors were prepared according to established procedures [18]. In the next step, the molarity of TMSOTf was investigated regarding its impact on the reaction yield and N^7/N^9 ratio. The proportions of the reaction products formed, determined by ^{1}H NMR, are compiled in Table 1, showing that the use of eight equivalents of TMSOTf worked best regarding the overall yields, while the N^7/N^9 ratio did not change significantly by increasing the concentration of TMSOTf. However, the expected N^7 regioselectivity obtained by Marcelo et al. [11] in the presence of TMSOTf in acetonitrile occurred only for the β-mannosylation and the β-galactosylation of 2-acetamido-6-chloropurine, while the thermodynamically controlled N^9 nucleoside was the major β-anomer resulting from the N-glucosylation and the N-galactosylation of 6-chloropurine. All four isomers (α/β, N^7/N^9) could be detected using these reaction conditions [19]. Nevertheless, only the β-anomers (Scheme 1) were subjected to biological testings, in as much as they were the predominant species when introducing the glycosyl or galactosyl moieties and the α-anomers appeared in very low amount. Therefore, also the minor β-mannosyl nucleosides were isolated and tested in order to receive comparable biological data to correlate structure with bioactivity in this family of compounds. The hydroxy groups of the glycosyl moities remained benzylated, since the presence of benzyl groups enhances the cytotoxicity of glycosylated compounds [20,21]. Moreover, benzyl groups represent a suitable metabolic protection.

The configuration of the anomeric center as well as the purine substitution pattern could be confirmed by the observed chemical shifts in the ^{1}H and ^{13}C NMR spectra and the $^3J_{H\text{-}1,H\text{-}2}$ coupling constant of the anomeric proton. Chemical shifts of $\delta = 5.4$ ppm to $\delta = 5.7$ ppm and coupling constants of $J = 7$ to $J = 9$ Hz were obtained for D-*gluco* and D-*galacto* configurated derivatives proving an axial position of the anomeric proton, while coupling constants of approx. $J = 1$ Hz were determined for D-*manno* configurated compounds. In the ^{13}C NMR spectra, the chemical shifts of $\delta = 83$ to $\delta = 85$ ppm for the anomeric carbon are in full agreement with data given in the literature for the anomeric carbon of other β-hexopyranosyl nucleosides [22–24]. The distinction between N^7 or N^9 substitution at the purine scaffold can be achieved by the chemical shift of purine carbon 5. In CP and ACP nucleosides the resonance of C-5 ranges from $\delta = 131$ ppm to $\delta = 128$ ppm indicating N^9 substitution while N^7 substitution was characterized by C-5 chemical shifts of about $\delta = 122$ ppm for CP and about $\delta = 118$ ppm for ACP nucleosides. These assignments were also confirmed with NMR HMBC experiments.

2.2. Biology

The cytotoxic activities of all synthesized compounds are represented by their GI$_{50}$ values given in Table 2. The values were determined in photometric SRB assays, using four different human tumor cell lines as well as murine embryonic fibroblasts (NiH 3T3). Some general tendencies could be observed within the bounds of our study. While the purines CP and ACP did not show any activity below 30 μM (cut-off), their N-glycosylation, increased considerably their cytotoxic activity. In general, the ACP nucleosides showed higher activities than the corresponding CP analogues. Furthermore, N^7 derivatives showed lower GI$_{50}$ values when compared to their N^9 regioisomers.

The influence of the glycosyl moiety was, however, less significant. The introduction of glucosyl and galactosyl groups showed similar results, *e.g.* GI$_{50}$ values from 18 to above 30 μM were determined for compounds **2** and **4**, both N^9 CP regioisomers,



1 R' = H, Glc-N^7-CP (19%) **7** R' = NHAc, Glc-N^7-ACP (25%)
2 R' = H, Glc-N^9-CP (30%) **8** R' = NHAc, Glc-N^9-ACP (35%)
3 R' = H, Gal-N^7-CP (15%) **9** R' = NHAc, Gal-N^7-ACP (47%)
4 R' = H, Gal-N^9-CP (44%) **10** R' = NHAc, Gal-N^9-ACP (18%)
5 R' = H, Man-N^7-CP (16%) **11** R' = NHAc, Man-N^7-ACP (26%)
6 R' = H, Man-N^9-CP (13%) **12** R' = NHAc, Man-N^9-ACP (3%)

Scheme 1. Synthesis of the nucleosides. CP: 6-chloropurine; ACP: 2-acetamido-6-chloropurine. Reagents and conditions: (a) TMSOTf, CH_3CN, 65 °C, 2 h.

exhibiting the lowest activities amongst the CP nucleosides tested. In contrast, those nucleosides bearing a mannosyl group showed the highest cytotoxic potential. The only exception was compound **5**, a N^7 CP nucleoside, possessing GI_{50} values between 9 and 14 μM. The corresponding ACP derivative **11** exhibited GI_{50} values of 1.4–2.2 μM and is the most potent compound of this investigation. This compound exhibited a higher cytotoxicity than betulinic acid (GI_{50} from 11.0 to 14.9 μM [25,26]) or tamoxifen (GI_{50} from 7.6 to 9.7 μM [27]), two common antitumor drugs. However, no selectivity was detected when these substances were tested on murine embryonic fibroblasts NiH 3T3, a non-malignant cell line.

The ability to trigger apoptosis is a fundamental quality of potent anticancer drugs. Consequently, the most active compound **11** of this study was subjected to further screening, applying a dye exclusion assay (acridine orange/propidium iodide, AO/PI) [28,29], DNA laddering assay [25] and cell-cycle analysis [30]. The appearance of green fluorescent cells with sections of diverse intensity indicated that **11** is able to trigger apoptosis in HT-29 cells (Fig. 1). This circumstance was confirmed by the results of the DNA laddering test (Fig. 2) showing the characteristic DNA fragmentation.

As a result of the AO/PI assay, the induction of a programmed cell death (PCD) [31] by **11** could be demonstrated. Membrane integrity is a typical hallmark of PCDs, like *e.g.* apoptosis. For an accidental cell death (necrosis) a membrane disruption is described, instead. Necrotic cells are stained in this assay red, due to intercalation of the cell membrane impermeable dye (propidium iodide, PI) into the DNA double strand [32].

Additionally, investigation of the cell morphology suggested a PCD mediated by apoptosis, due to the occurrence of typical membrane blabbing. Also, the occurrence of many mitotic cells indicates a potential interruption in the last cell cycle phase (mitosis); some of the cells seem to be arrested in the telophase.

To establish the induction of apoptosis, the DNA enriched fraction of the death-cells was submitted to a gel-electrophoresis based fragmentation assay (DNA-ladder assay). As depicted in Fig 1 by the occurrence of well-defined 178 kBp DNA fragments and multiples thereof, treatment of the cells with **11** led to DNA fragmentation and thus, to the activation of the caspase-activated DNAse (CAD) [33]. The latter is another typical hallmark of the caspase mediated apoptosis.

Triggering of apoptosis is known to be possible *via* several caspase dependent routes, besides an intrinsic and an extrinsic way, an inhibition of the cell cycle regulating cyclin-dependent kinases (cdks) is known to effect the cell cycle distribution and hence to trigger apoptosis [34]. 2,6,9-Trisubstituted purine derivatives are able to inhibit CDKs and some of them led to a specific G2 phase arrest, promoting apoptosis in MT-2 cells [35], while several other CDK inhibiting purine analogues were shown to induce a significant G1 arrest or to trigger apoptosis independently from the trapping of the cell cycle [36]. To gain a deeper insight into the modulation of the cell cycle, cells were treated with **11** and submitted to a cell cycle distribution assay. As shown in Fig. 3, a significant G2/M phase arrest was induced by the compound after an incubation period of 24 h.

3. Conclusions

A new family of anticancer nucleosides embodying a 6-chloropurine or a 2-acetamido-6-chloropurine linked to a hexosyl moiety is here disclosed. The structural features common to the most active compounds are the 2-acetamido group and the N^7 glycosyl linkage, in contrast to the N^9-linkage present in the antitumor purine derivatives described in the literature. The mannosyl group was present in the most active nucleoside, but the analogue galactosyl nucleoside also showed GI_{50} values of the same order of magnitude. They were active on human melanoma, lung and ovarian carcinomas, and colon adenocarcinoma in low micromolar range, and the most active compounds provided data of the same order of magnitude (*e.g.* compound **11** with GI_{50} 1.5 μM) as that of the structurally similar chemotherapeutic 2-chloro-2′-deoxyadenosine (cladribine, GI_{50} 2.43 μM on U266 leukemia [35]).

A membrane-stability analysis (AO/PI assay), as well as a DNA-fragmentation assay with the most potent compound **11** confirmed the cytocidal effect leading to apoptosis. In addition, cell cycle analyses suggest an induced mode-of-action *via* modulation of DNA-synthesis. The process is a known hallmark of several clinical-approved and structurally related deoxyadenosine analogues (*e.g.* fludarabine, pentostatin or cladribine [5,38,39]). Treatment with these drugs offered several benefits, for example promising overall survival rates at 12 years of 75–87% for the treatment of hairy cell leukemia with 2-chlorodeoxyadenosine [40]. However, resistance of the tumor cells *via* a down regulation of drug-activating enzymes (dCK and dGK [41]) urges the necessity of further improvements [37,42]. Our findings confirm the potential of hexopyranosyl glycon based purines as a rewarding starting point for further pharmacological investigations.

Table 2
Cytotoxicity (GI_{50} in μM) of compounds **1**–**12** measured in SRB-assays with 4 different human cancer cell lines and non-malignant murine embryonic fibroblasts (NiH 3T3) in comparison to their parent purines CP and ACP[a] as well as to betulinic acid [25,26] and tamoxifen [27].

	Purine, purine nucleoside[b]	N^7/N^9	518A2 (melanoma)	A2780 (lung carcinoma)	A549 (ovarian carcinoma)	HT-29 (colon adenocarcinoma)	NiH 3T3
	CP		>30	>30	>30	>30	>30
	ACP		>30	>30	>30	>30	>30
1	GlcCP	N^7	4.7 ± 0.1	6.3 ± 0.1	4.2 ± 0.2	6.8 ± 0.4	6.9 ± 0.1
2	GlcCP	N^9	>30	>30	17.9 ± 8.7	n.d.	29.2 ± 13.9
3	GalCP	N^7	9.0 ± 0.3	4.0 ± 1.0	8.1 ± 0.7	19.7 ± 1.1	7.9 ± 2.9
4	GalCP	N^9	>30	>30	20.2 ± 16.4	n.d.	18.6 ± 6.1
5	ManCP	N^7	13.5 ± 0.6	10.9 ± 0.1	9.4 ± 0.2	11.0 ± 1.1	9.8 ± 2.4
6	ManCP	N^9	13.7 ± 3.4	27.1 ± 1.9	29.5 ± 6.4	25.3 ± 1.5	11.2 ± 5.2
7	GlcACP	N^7	3.4 ± 0.1	3.5 ± 0.1	4.6 ± 0.4	3.9 ± 0.1	3.6 ± 0.1
8	GlcACP	N^9	7.6 ± 0.1	15.6 ± 0.8	23.2 ± 0.6	>30	8.8 ± 0.3
9	GalACP	N^7	3.8 ± 0.1	5.5 ± 0.3	4.1 ± 0.4	4.1 ± 0.6	4.2 ± 0.8
10	GalACP	N^9	8.9 ± 4.4	11.0 ± 3.2	17.5 ± 6.8	15.7 ± 8.4	7.3 ± 3.0
11	ManACP	N^7	2.0 ± 0.1	2.2 ± 0.1	1.6 ± 0.2	1.5 ± 0.1	1.4 ± 0.1
12	ManACP	N^9	11.5 ± 0.3	15.4 ± 0.2	13.0 ± 0.1	13.2 ± 0.2	8.7 ± 0.2
–	Betulinic acid	–	11.9 ± 0.6	11.0 ± 0.6	14.9 ± 0.7	16.1 ± 0.8	10.0 ± 0.5
–	Tamoxifen	–	7.6 ± 0.5	7.8 ± 0.5	9.7 ± 0.7	–	7.3 ± 0.5

[a] GI_{50} values represent mean values of 3 independent measurements and were calculated applying the two-parametric Hill slope equation.
[b] Betulinic acid and tamoxifen are standard anticancer drugs.

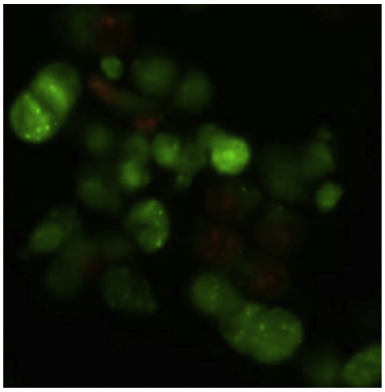


Fig. 1. Results of AO/PI assay using HT-29 cells treated with compound **11** (5 μM) for 24 h, floated cells collected.

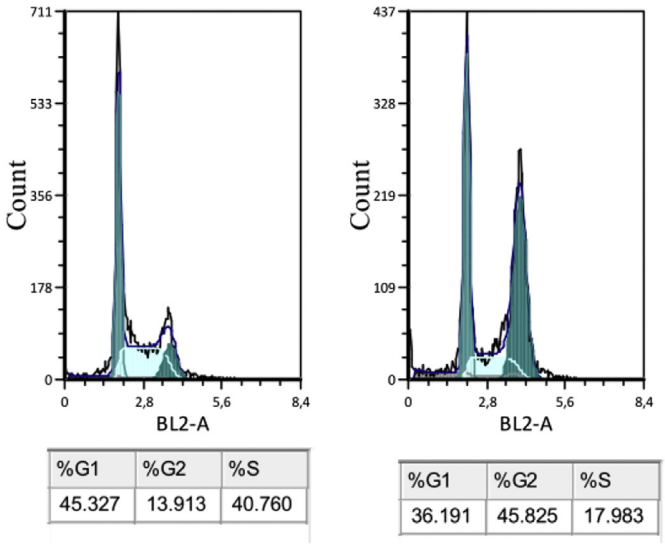


Fig. 3. Results of the cell cycle analysis in a size-number-diagram using HT-29 cells, left – control, right – compound **11** (5 μM); incubation time was 24 h.

4. Experimental section

4.1. Synthesis and analysis

Reagents were bought from commercial suppliers without any further purification. Melting points were measured with a Melting Point Apparatus, SMP3, Stuart Scientific, Bibby and were not corrected. NMR spectra were recorded on 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 measured 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.

4.2. General procedure for the N-glycosylation

N,*O*-Bis(trimethylsilyl)acetamide (BSA) (1.5 eq. for 6-chloropurine and 3.0 eq. for 2-acetamido-6-chloropurine) was added to a mixture of the respective purine (1.5 eq.) in dry acetonitrile (10 mL). The mixture was stirred at room temperature for 40 min. Then, the methyl glycoside (1 eq.), dissolved in dry acetonitrile (2 mL), and trimethylsilyl trifluoromethanesulfonate (TMSOTf, 8 eq.) were added. After continuous stirring at 65 °C for 2 h, the solution was poured into dichloromethane (10 mL), 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. Compounds were isolated by column chromatography.

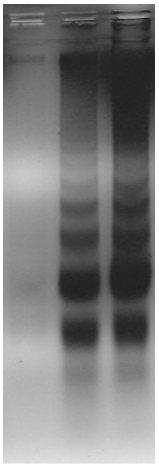


Fig. 2. DNA fragmentation scatter of the DNA laddering assay using HT-29 cells tested with compound **11** (5 μM) for 24 h.

4.2.1. 6-Chloro-7-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl) purine (**1**) and 6-chloro-9-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)purine (**2**)

The compounds were obtained by the reaction of methyl 2,3,4,6-tetra-*O*-benzyl α-D-glucopyranoside (280 mg, 0.50 mmol) and 6-chloropurine (122 mg, 0.75 mmol) according to the general procedure. Purification by column chromatography (ethyl acetate/cyclohexane 1:1) afforded **1** (65 mg, 19%) and **2** (103 mg, 30%). Data for **1**: colorless oil; $R_f = 0.10$ (ethyl acetate/hexane 1:2); $[\alpha] = -9°$ (c 1.01, $CHCl_3$); MS (ESI): m/z (%) = 587.1 ($[M\text{-}Bn+H]^+$, 90), 609.3 ($[M\text{-}Bn+Na]^+$, 48), 677.0 ($[M+H]^+$, 100); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.84$ (s, 1H, H-2), 8.23 (s, 1H, H-8), 7.40–7.17 (m, 15H, Bn-*arom.*), 7.07–6.95 (m, 3H, Bn-*arom.*), 6.74 (m, 2H, Bn-*arom.*), 5.72 (br, 1H, H-1′), 5.01 (part A of AB system, 1H, Bn-C*H*H′, $J = 10.9$), 4.95 (part B of AB system, 1H, Bn-CH*H*′, $J = 10.9$), 4.89 (part A of AB system, 1H, Bn-C*H*H′, $J = 10.7$), 4.64 (part B of AB system, 1H, Bn-CH*H*′, $J = 10.7$), 4.63 (part A of AB system, 1H, Bn-C*H*H′, $J = 11.5$), 4.54 (part A of AB system, 1H, Bn-C*H*H′, $J = 12.1$), 4.48 (part B of AB system, 1H, Bn-CH*H*′, $J = 12.1$), 4.23 (part B of AB system, 1H, Bn-CH*H*′, $J = 11.5$), 3.97–3.86 (br, 3H, H-2′ and H-3′ and H-5′), 3.78–3.70 (br, 3H, H-4′ and H-6′a and H-6′b) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 161.4$ (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-*arom.*), 128.6 (Bn-*arom.*), 128.5 (Bn-*arom.*), 128.5 (Bn-*arom.*), 128.4 (Bn-*arom.*), 128.4 (Bn-*arom.*), 128.2 (Bn-*arom.*), 128.2 (Bn-*arom.*), 128.1 (Bn-*arom.*), 128.0 (Bn-*arom.*), 128.0 (Bn-*arom.*), 128.0 (Bn-*arom.*), 128.0 (Bn-*arom.*), 127.9 (Bn-*arom.*), 127.9 (Bn-*arom.*), 127.8 (Bn-*arom.*), 127.8 (Bn-*arom.*), 127.8 (Bn-*arom.*), 127.7 (Bn-*arom.*), 127.7 (Bn-*arom.*), 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′) ppm. Elemental analysis calculated for $C_{39}H_{37}ClN_4O_5$ (677.2): C, 69.17; H, 5.51; N, 8.27; found: C, 68.98; H, 5.42; N, 8.02.

Data for **2**: colorless crystals; mp 146–149 °C; R_F = 0.35 (ethyl acetate/hexane 1:2); [α] = −24° (*c* 1.03, $CHCl_3$); MS (ESI): *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, Bn-*arom.*), 7.23–7.18 (m, 2H, Bn-*arom.*), 7.12 (m, 1H, Bn-*arom.*), 6.99 (m, 2H, Bn-*arom.*), 5.59 (d, 1H, H-1′, *J* = 9.0), 4.99 (part A of AB system, 1H, Bn-C*H*H′, *J* = 11.0), 4.94 (part B of AB system, 1H, Bn-CH*H*′, *J* = 11.0), 4.88 (part A of AB system, 1H, Bn-C*H*H′, *J* = 10.7), 4.65 (part B of AB system, 1H, Bn-CH*H*′, *J* = 10.7), 4.61 (part A of AB system, 1H, Bn-C*H*H′, *J* = 11.7), 4.55 (part A of AB system, 1H, Bn-C*H*H′, *J* = 12.2), 4.48 (part B of AB system, 1H, Bn-CH*H*′, *J* = 12.2), 4.17 (part B of AB system, 1H, Bn-CH*H*′, *J* = 11.7), 4.07 (t, 1H, H-2′, *J* = 9.0), 3.91 (dd, 1H, H-3′, *J* = 9.0, 8.3), 3.87 (dd, 1H, H-4′, *J* = 9.6, 9.1), 3.76–3.69 (br, 3H, H-6′a and H-5′ and H-6′b) ppm; ^{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-*arom.*), 128.5 (Bn-*arom.*), 128.5 (Bn-*arom.*), 128.5 (Bn-*arom.*), 128.4 (Bn-*arom.*), 128.4 (Bn-*arom.*), 128.1 (Bn-*arom.*), 128.1 (Bn-*arom.*), 128.1 (Bn-*arom.*), 128.0 (Bn-*arom.*), 128.0 (Bn-*arom.*), 127.9 (Bn-*arom.*), 127.8 (Bn-*arom.*), 127.8 (Bn-*arom.*), 127.8 (Bn-*arom.*), 127.7 (Bn-*arom.*), 127.7 (Bn-*arom.*), 127.7 (Bn-*arom.*), 127.7 (Bn-*arom.*), 127.7 (Bn-*arom.*), 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′) ppm. Elemental analysis calculated for $C_{39}H_{37}ClN_4O_5$ (677.2): C, 69.17; H, 5.51; N, 8.27; found: C, 69.02; H, 5.38; N, 8.11.

4.2.2. 6-Chloro-7-(2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl) purine (**3**) and 6-chloro-9-(2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl)purine (**4**)

The compounds were obtained by the reaction of methyl 2,3,4,6-tetra-*O*-benzyl α-D-galactopyranoside (280 mg, 0.50 mmol) and 6-chloropurine (122 mg, 0.75 mmol) according to the general procedure. Purification by column chromatography (ethyl acetate/cyclohexane 1:1) afforded **3** (51 mg, 15%) and **4** (151 mg, 44%). Data for **3**: colorless oil; R_f = 0.30 (ethyl acetate/hexane 1:1); [α] = −22° (*c* 1.00, $CHCl_3$); MS (ESI): *m*/*z* (%) = 587.0 ($[M\text{-}Bn+H]^+$, 100), 609.3 ($[M\text{-}Bn+Na]^+$, 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, Bn-*arom.*), 7.11–6.96 (m, 3H, Bn-*arom.*), 6.75 (m, 2H, Bn-*arom.*), 5.78 (d, 1H, H-1′, *J* = 8.1), 5.01 (part A of AB system, 1H, Bn-C*H*H′, *J* = 11.2), 4.85 (part A of AB system, 1H, Bn-C*H*H′, *J* = 11.6), 4.78 (part B of AB system, 1H, Bn-CH*H*′, *J* = 11.6), 4.70 (part A of AB system, 1H, Bn-C*H*H′, *J* = 11.4), 4.36 (part B of AB system, 1H, Bn-CH*H*′, *J* = 11.2), 4.48 (part A of AB system, 1H, Bn-C*H*H′, *J* = 11.8), 4.43 (part B of AB system, 1H, Bn-CH*H*′, *J* = 11.8), 4.28 (part B of AB system, 1H, Bn-CH*H*′, *J* = 11.4), 4.29 (dd, 1H, H-2), 4.10 (t, 1H, H-4, *J* = 1.9), 3.86 (t, 1H, H-5, *J* = 6.3), 3.81 (dd, 1H, H-3, *J* = 9.4, 2.5), 3.66–3.58 (m, 2H, H-6′a and H-6′b) ppm; ^{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-*arom.*), 128.6 (Bn-*arom.*), 128.5 (Bn-*arom.*), 128.5 (Bn-*arom.*), 128.4 (Bn-*arom.*), 128.4 (Bn-*arom.*), 128.2 (Bn-*arom.*), 128.2 (Bn-*arom.*), 128.0 (Bn-*arom.*), 128.0 (Bn-*arom.*), 128.0 (Bn-*arom.*), 128.0 (Bn-*arom.*), 128.0 (Bn-*arom.*), 127.9 (Bn-*arom.*), 127.9 (Bn-*arom.*), 127.8 (Bn-*arom.*), 127.8 (Bn-*arom.*), 127.8 (Bn-*arom.*), 127.6 (Bn-*arom.*), 127.6 (Bn-*arom.*), 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′) ppm. Elemental analysis calculated for $C_{39}H_{37}ClN_4O_5$ (677.2): C, 69.17; H, 5.51; N, 8.27; found: C, 68.97; H, 5.69; N, 7.99.

Data for **4**: colorless oil; R_f = 0.30 (ethyl acetate/hexane 1:2); [α] = −16° (*c* 0.92, $CHCl_3$); MS (ESI): *m*/*z* (%) = 497.1 ($[M\text{-}2Bn+H]^+$, 8), 519.3 ($[M\text{-}2Bn+Na]^+$, 12), 587.1 ($[M\text{-}Bn+H]^+$, 23), 609.4 ($[M\text{-}Bn+Na]^+$, 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, Bn-*arom.*), 7.16 (m, 1H, Bn-*arom.*), 7.03 (m, 2H, Bn-*arom.*), 6.64 (m, 2H, Bn-*arom.*), 5.68 (d, 1H, H-1′, *J* = 9.0), 5.05 (part A of AB system, 1H, Bn-C*H*H′, *J* = 11.5), 4.84 (part A of AB system, 1H, Bn-C*H*H′, *J* = 11.7), 4.79 (part B of AB system, 1H, Bn-CH*H*′, *J* = 11.7), 4.70 (part B of AB system, 1H, Bn-CH*H*′, *J* = 11.5), 4.69 (part A of AB system, 1H, Bn-C*H*H′, *J* = 11.6), 4.48 (part A of AB system, 1H, Bn-C*H*H′, *J* = 11.9), 4.43 (part B of AB system, 1H, Bn-CH*H*′, *J* = 11.9), 4.32 (dd, 1H, H-2′, *J* = 9.2, 9.0), 4.24 (part B of AB system, 1H, Bn-CH*H*′, *J* = 11.6), 4.09 (br, 1H, H-4′), 3.87–3.84 (br, 2H, H-5′ and H-3′), 3.61–3.59 (br, 2H, H-6′a and H-6′b) ppm; ^{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-*arom.*), 128.6 (Bn-*arom.*), 128.4 (Bn-*arom.*), 128.4 (Bn-*arom.*), 128.3 (Bn-*arom.*), 128.3 (Bn-*arom.*), 128.1 (Bn-*arom.*), 128.1 (Bn-*arom.*), 128.0 (Bn-*arom.*), 127.9 (Bn-*arom.*), 127.9 (Bn-*arom.*), 127.9 (Bn-*arom.*), 127.9 (Bn-*arom.*), 127.9 (Bn-*arom.*), 127.9 (Bn-*arom.*), 127.8 (Bn-*arom.*), 127.8 (Bn-*arom.*), 127.8 (Bn-*arom.*), 127.6 (Bn-*arom.*), 127.6 (Bn-*arom.*), 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′) ppm. Elemental analysis calculated for $C_{39}H_{37}ClN_4O_5$ (677.2): C, 69.17; H, 5.51; N, 8.27; found: C, 69.00; H, 5.77; N, 8.17.

4.2.3. 6-Chloro-7-(2,3,4,6-tetra-O-benzyl-β-D-mannopyranosyl) purine (**5**) and 6-chloro-9-(2,3,4,6-tetra-O-benzyl-β-D-mannopyranosyl)purine (**6**)

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 6-chloropurine (104 mg, 0.67 mmol) according to the general procedure. Purification by column chromatography (ethyl acetate/cyclohexane 1:1) afforded **5** (49 mg, 16%) and **6** (40 mg, 13%). Data for **5**: colorless oil; R_f = 0.31 (ethyl acetate/hexane 1:1); [α] = +62° (*c* 1.02, $CHCl_3$); MS (ESI): *m*/*z* (%) = 587.1 ($[M\text{-}Bn+H]^+$, 100), 609.3 ($[M\text{-}Bn+Na]^+$, 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, Bn-*arom.*), 6.92–6.86 (m, 3H, Bn-*arom.*), 6.73 (m, 2H, Bn-*arom.*), 6.90 (d, 1H, H-1′, *J* = 1.0), 4.98 (part A of AB system, 1H, Bn-C*H*H′, *J* = 10.8), 4.90 (part A of AB system, 1H, Bn-C*H*H′, *J* = 11.7), 4.80 (part B of AB system, 1H, Bn-CH*H*′, *J* = 11.7), 4.68 (part A of AB system, 1H, Bn-C*H*H′, *J* = 11.8), 4.66 (part B of AB system, 1H, Bn-CH*H*′, *J* = 10.8), 4.64 (part A of AB system, 1H, Bn-C*H*H′, *J* = 12.4), 4.56 (part B of AB system, 1H, Bn-CH*H*′, *J* = 12.4), 4.07 (t, 1H, H-4′, *J* = 9.5), 4.02 (dd, 1H, H-2′, *J* = 2.5, 1.0), 3.84 (dd, 1H, H-3′, *J* = 9.5, 2.5), 3.79–3.77 (br, 2H, H-6′a and H-6′b), 3.71 (dt, 1H, H-5′, *J* = 9.5, 3.6), 4.34 (part B of AB system, 1H, Bn-CH*H*′, *J* = 11.8) ppm; ^{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-*arom.*), 128.8 (Bn-*arom.*), 128.5 (Bn-*arom.*), 128.5 (Bn-*arom.*), 128.5 (Bn-*arom.*), 128.5 (Bn-*arom.*), 128.4 (Bn-*arom.*), 128.4 (Bn-*arom.*), 128.3 (Bn-*arom.*), 128.1 (Bn-*arom.*), 128.1 (Bn-*arom.*), 128.0 (Bn-*arom.*), 128.0 (Bn-*arom.*), 128.0 (Bn-*arom.*), 128.0 (Bn-*arom.*), 127.9 (Bn-*arom.*), 127.8 (Bn-*arom.*), 127.7 (Bn-*arom.*), 127.7 (Bn-*arom.*), 127.7 (Bn-*arom.*), 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′) ppm. Elemental analysis calculated for $C_{39}H_{37}ClN_4O_5$ (677.2): C, 69.17; H, 5.51; N, 8.27; found: C, 69.04; H, 5.62; N, 8.17.

Data for **6**: colorless oil; R_f = 0.24 (ethyl acetate/hexane 1:2); [α] = +56° (*c* 0.91, $CHCl_3$); MS (ESI): *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, Bn-*arom.*), 7.06–6.98 (m, 3H, Bn-*arom.*), 6.86 (m, 2H, Bn-*arom.*), 5.81 (d, 1H, H-1′, *J* = 1.1), 4.95 (part A of AB system, 1H, Bn-C*H*H′, *J* = 10.8), 4.84 (m, 2H, Bn-CH_2), 4.76 (part A of AB system, 1H, Bn-C*H*H′, *J* = 11.6), 4.65 (part B of AB system, 1H, Bn-CH*H*′, *J* = 10.8), 4.62 (part A of AB system, 1H, Bn-C*H*H′, *J* = 12.3), 4.56 (part B of AB system, 1H, Bn-CH*H*′, *J* = 12.3), 4.33 (part B of AB system, 1H, Bn-CH*H*′, *J* = 11.6), 4.08–4.10 (br, 2H, H-2′ and H-4′), 3.91 (dd, 1H, H-3′, *J* = 9.4, 2.6), 3.77–3.71 (br, 3H, H-

6′a and H-6′b and H-5′) ppm; ^{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-*arom.*), 128.6 (Bn-*arom.*), 128.5 (Bn-*arom.*), 128.5 (Bn-*arom.*), 128.5 (Bn-*arom.*), 128.5 (Bn-*arom.*), 128.5 (Bn-*arom.*), 128.4 (Bn-*arom.*), 128.4 (Bn-*arom.*), 128.1 (Bn-*arom.*), 128.1 (Bn-*arom.*), 128.0 (Bn-*arom.*), 128.0 (Bn-*arom.*), 128.0 (Bn-*arom.*), 127.9 (Bn-*arom.*), 127.8 (Bn-*arom.*), 127.7 (Bn-*arom.*), 127.7 (Bn-*arom.*), 127.6 (Bn-*arom.*), 127.6 (Bn-*arom.*), 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′) ppm. Elemental analysis calculated for $C_{39}H_{37}ClN_4O_5$ (677.2): C, 69.17; H, 5.51; N, 8.27; found: C, 69.04; H, 5.76; N, 8.04.

4.2.4. 2-Acetamido-6-chloro-7-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)purine (**7**) and 2-acetamido-6-chloro-9-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)purine (**8**)

The compounds were obtained by reaction of methyl 2,3,4,6-tetra-*O*-benzyl α-D-glucopyranoside (280 mg, 0.50 mmol) and 2-acetamido-6-chloropurine (156 mg, 0.75 mmol) according to the general procedure. Purification by column chromatography (ethyl acetate/cyclohexane 1:1) afforded **7** (91 mg, 25%) and **8** (127 mg, 35%). Data for **7**: yellow crystals; mp 71–74 °C; R_f = 0.09 (ethyl acetate/hexane 1:1); [α] = −13° (*c* 0.99, $CHCl_3$); MS (ESI): *m*/*z* (%) = 734.1 ([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, Bn-*arom.*), 7.10–6.99 (m, 3H, Bn-*arom.*), 6.80 (m, 2H, Bn-*arom.*), 5.61 (br, 1H, H-1′), 5.00 (part A of AB system, 1H, Bn-C*H*H′, *J* = 10.9), 4.94 (part B of AB system, 1H, Bn-CH*H*′, *J* = 10.9), 4.88 (part A of AB system, 1H, Bn-C*H*H′, *J* = 10.7), 4.64 (part A of AB system, 1H, Bn-C*H*H′, *J* = 11.5), 4.63 (part B of AB system, 1H, Bn-CH*H*′, *J* = 10.7), 4.54 (part A of AB system, 1H, Bn-C*H*H′, *J* = 12.1), 4.48 (part B of AB system, 1H, Bn-CH*H*′, *J* = 12.1), 4.24 (part B of AB system, 1H, Bn-CH*H*′, *J* = 11.5), 3.94–3.84 (br, 3H, H-2′ and H-3′ and H-5′), 3.77–3.67 (br, 3H, H-4′ and H-6′ and H-6′b), 2.64 (s, 3H, Ac-Me) ppm; ^{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-*arom.*), 128.6 (Bn-*arom.*), 128.5 (Bn-*arom.*), 128.5 (Bn-*arom.*), 128.4 (Bn-*arom.*), 128.4 (Bn-*arom.*), 128.3 (Bn-*arom.*), 128.3 (Bn-*arom.*), 128.1 (Bn-*arom.*), 128.1 (Bn-*arom.*), 128.0 (Bn-*arom.*), 128.0 (Bn-*arom.*), 128.0 (Bn-*arom.*), 127.9 (Bn-*arom.*), 127.9 (Bn-*arom.*), 127.8 (Bn-*arom.*), 127.8 (Bn-*arom.*), 127.8 (Bn-*arom.*), 127.7 (Bn-*arom.*), 127.7 (Bn-*arom.*), 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-xCH_2), 73.5 (Bn-CH_2), 68.3 (C-6′), 25.2 (Ac-Me) ppm. Elemental analysis calculated for $C_{41}H_{40}ClN_5O_6$ (734.2): C, 67.07; H, 5.49; N, 9.54; found: C, 66.81; H, 5.52; N, 9.33.

Data for **8**: colorless oil; R_f = 0.53 (ethyl acetate/hexane 1:1); [α] = −24° (*c* 1.01, $CHCl_3$); MS (ESI): *m*/*z* (%) = 734.2 ([M+H]$^+$, 84), 756.3 ([M+Na]$^+$, 100), 1468.9 ([2M+H]$^+$, 22), 1489.7 ([2M+Na]$^+$, 8); ^{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, Bn-*arom.*), 7.12–6.98 (m, 3H, Bn-*arom.*), 6.71 (m, 2H, Bn-*arom.*), 5.38 (d, 1H, H-1′, *J* = 9.1), 5.00 (part A of AB system, 1H, Bn-C*H*H′, *J* = 11.0), 4.94 (part B of AB system, 1H, Bn-CH*H*′, *J* = 11.0), 4.88 (part A of AB system, 1H, Bn-C*H*H′, *J* = 10.7), 4.64 (part B of AB system, 1H, Bn-CH*H*′, *J* = 10.7), 4.61 (part A of AB system, 1H, Bn-C*H*H′, *J* = 11.6), 4.52 (part A of AB system, 1H, Bn-C*H*H′, *J* = 12.1), 4.45 (part B of AB system, 1H, Bn-CH*H*′, *J* = 12.1), 4.24 (part B of AB system, 1H, Bn-CH*H*′, *J* = 11.6), 4.11 (t, 1H, H-2′, *J* = 9.0), 3.87 (t, 1H, H-3′, *J* = 9.0), 3.80 (t, 1H, H-4′, *J* = 9.0), 3.75–3.68 (br, 3H, H-6′a and H-6′b and H-5′), 2.50 (s, 3H, Ac-Me) ppm; ^{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-*arom.*), 128.5 (Bn-*arom.*), 128.5 (Bn-*arom.*), 128.5 (Bn-*arom.*), 128.4 (Bn-*arom.*), 128.4 (Bn-*arom.*), 128.0 (Bn-*arom.*), 128.0 (Bn-*arom.*), 128.0 (Bn-*arom.*), 128.0 (Bn-*arom.*), 128.0 (Bn-*arom.*), 127.9 (Bn-*arom.*), 127.9 (Bn-*arom.*), 127.9 (Bn-*arom.*), 127.8 (Bn-*arom.*), 127.8 (Bn-*arom.*), 127.8 (Bn-*arom.*), 127.8 (C-5), 127.7 (Bn-*arom.*), 127.7 (Bn-*arom.*), 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) ppm. Elemental analysis calculated for $C_{41}H_{40}ClN_5O_6$ (734.2): C, 67.07; H, 5.49; N, 9.54; found: C, 66.81; H, 5.70; N, 9.42.

4.2.5. 2-Acetamido-6-chloro-7-(2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl)purine (**9**) and 2-acetamido-6-chloro-9-(2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl)purine (**10**)

The compounds were obtained by reaction of methyl 2,3,4,6-tetra-*O*-benzyl α-D-galactopyranoside (280 mg, 0.50 mmol) and ACP (156 mg, 0.75 mmol) according to the general procedure. Purification by column chromatography (ethyl acetate/cyclohexane, 1:1) gave **9** (172 mg, 47%) and 1**0** (66 mg, 18%). Data for **9**: yellow oil; R_f = 0.26 (ethyl acetate/cyclohexane 1:1); [α] = −4° (*c* 1.26, $CHCl_3$); MS (ESI): *m*/*z* (%) = 734.2 ([M+H]$^+$, 82), 756.4 ([M+Na]$^+$, 22), 1468.9 ([2M+H]$^+$, 100), 1491.2 ([2M+Na]$^+$, 72); ^{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, Bn-*arom.*), 7.12–7.01 (m, 3H, Bn-*arom.*), 6.81 (m, 2H, Bn-*arom.*), 5.65 (d, 1H, H-1′, *J* = 7.3), 5.00 (part A of AB system, 1H, Bn-C*H*H′, *J* = 11.2), 4.84 (part A of AB system, 1H, Bn-C*H*H′, *J* = 11.6), 4.77 (part B of AB system, 1H, Bn-CH*H*′, *J* = 11.6), 4.71 (part A of AB system, 1H, Bn-C*H*H′, *J* = 11.4), 4.62 (part B of AB system, 1H, Bn-CH*H*′, *J* = 11.2), 4.48 (part A of AB system, 1H, Bn-C*H*H′, *J* = 11.8), 4.43 (part B of AB system, 1H, Bn-CH*H*′, *J* = 11.8), 4.29 (part B of AB system, 1H, Bn-CH*H*′, *J* = 11.4), 4.28 (br, 1H, H-2′), 4.09 (t, 1H, H-4′, *J* = 2.5), 3.84 (br, 1H, H-5′), 3.79 (dd, 1H, H-3′, *J* = 9.3, 2.5), 3.66–3.57 (br, 2H, H-6′a and H-6′b), 2.63 (s, 3H, Ac-Me) ppm; ^{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-*arom.*), 128.6 (Bn-*arom.*), 128.5 (Bn-*arom.*), 128.5 (Bn-*arom.*), 128.4 (Bn-*arom.*), 128.4 (Bn-*arom.*), 128.2 (Bn-*arom.*), 128.2 (Bn-*arom.*), 128.1 (Bn-*arom.*), 128.1 (Bn-*arom.*), 128.0 (Bn-*arom.*), 128.0 (Bn-*arom.*), 127.9 (Bn-*arom.*), 127.9 (Bn-*arom.*), 127.9 (Bn-*arom.*), 127.9 (Bn-*arom.*), 127.9 (Bn-*arom.*), 127.9 (Bn-*arom.*), 127.6 (Bn-*arom.*), 127.6 (Bn-*arom.*), 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) ppm; analysis calculated for $C_{41}H_{40}ClN_5O_6$ (734.2): C, 67.07; H, 5.49; N, 9.54; found: C, 67.00; H, 5.67; N, 9.40.

Data for 1**0**: a colorless oil; R_f = 0.57 (ethyl acetate/hexane 1:1); [α] = −14° (*c* 1.02, $CHCl_3$); MS (ESI): *m*/*z* (%) = 734.3 ([M+H]$^+$, 100), 756.5 ([M+Na]$^+$, 56), 1467.9 ([2M+H]$^+$, 12); ^{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, Bn-*arom.*), 7.10 (m, 1H, Bn-*arom.*), 7.01 (m, 2H, Bn-*arom.*), 6.73 (m, 2H, Bn-*arom.*), 5.35 (d, 1H, H-1′, *J* = 9.0), 4.96 (part A of AB system, 1H, Bn-C*H*H′, *J* = 11.4), 4.81 (part A of AB system, 1H, Bn-C*H*H′, *J* = 11.7), 4.75 (part B of AB system, 1H, Bn-CH*H*′, *J* = 11.7), 4.68 (part A of AB system, 1H, Bn-C*H*H′, *J* = 11.8), 4.64 (part B of AB system, 1H, Bn-CH*H*′, *J* = 11.8), 4.45 (part A of AB system, 1H, Bn-C*H*H′, *J* = 11.9), 4.43 (m, 1H, H-2), 4.40 (part B of AB system, 1H, Bn-CH*H*′, *J* = 11.9), 4.29 (part B of AB system, 1H, Bn-CH*H*′, *J* = 11.4), 4.06 (d, 1H, H-4′, *J* = 1.9), 3.79 (t, 1H, H-5′, *J* = 6.3), 3.76 (dd, 1H, H-3′, *J* = 9.6, 2.6), 3.54–3.56 (br, 2H, H-6′a and H-6′b), 2.42 (s, 3H, Ac-Me) ppm; ^{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-*arom.*), 128.6 (Bn-*arom.*), 128.4 (Bn-*arom.*), 128.4 (Bn-*arom.*), 128.4 (Bn-*arom.*), 128.4 (Bn-*arom.*), 128.1 (Bn-*arom.*), 128.1 (Bn-*arom.*), 128.1 (Bn-*arom.*), 128.0 (Bn-*arom.*), 128.0 (Bn-*arom.*), 128.0 (Bn-*arom.*), 128.0 (Bn-*arom.*), 128.0 (Bn-*arom.*), 128.0 (Bn-*arom.*), 128.0 (Bn-*arom.*), 128.0 (Bn-*arom.*), 127.8 (C-5), 127.6 (Bn-*arom.*), 127.6 (Bn-*arom.*), 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)

ppm; analysis calculated for $C_{41}H_{40}ClN_5O_6$ (734.2): C, 67.07; H, 5.49; N, 9.54; found: C, 66.92; H, 5.61; N, 9.47.

4.2.6. 2-Acetamido-6-chloro-7-(2,3,4,6-tetra-O-benzyl-β-D-mannopyranosyl)purine (**11**) and 2-acetamido-6-chloro-9-(2,3,4,6-tetra-O-benzyl-β-D-mannopyranosyl)purine (**12**)

The compounds were obtained by the reaction of methyl 2,3,4,6-tetra-*O*-benzyl α-D-mannopyranoside (280 mg, 0.50 mmol) and ACP (156 mg, 0.75 mmol) according to the general procedure. Purification by column chromatography (ethyl acetate/cyclohexane 1:1) yielded **11** (95 mg, 26%) and **12** (11 mg, 3%). Data for **11**: yellow crystals; mp 80–82 °C; $R_f = 0.36$ (ethyl acetate/hexane 4:1); $[\alpha] = +74°$ (*c* 0.98, $CHCl_3$); MS (ESI): *m/z* (%) = 734.5 ($[M+H]^+$, 66), 756.5 ($[M+Na]^+$, 24), 1469.2 ($[2M+H]^+$, 100), 1491.3 ($[2M+Na]^+$, 40); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.34$ (s, 1H, H-8), 8.09 (s, 1H, NH), 7.38–7.16 (m, 15H, Bn-*arom.*), 6.89 (m, 3H, Bn-*arom.*), 6.71 (m, 2H, Bn-*arom.*), 5.75 (d, 1H, H-1′, $J = 1.0$), 4.90 (part A of AB system, 1H, Bn-C*H*H′, $J = 10.9$), 4.83 (part A of AB system, 1H, Bn-C*H*H′, $J = 11.8$), 4.72 (part B of AB system, 1H, Bn-CH*H*′, $J = 11.8$), 4.61 (part A of AB system, 1H, Bn-C*H*H′, $J = 11.8$), 4.59 (part B of AB system, 1H, Bn-CH*H*′, $J = 10.9$), 4.55 (part A of AB system, 1H, Bn-C*H*H′, $J = 12.4$), 4.49 (part B of AB system, 1H, Bn-CH*H*′, $J = 12.4$), 4.26 (part B of AB system, 1H, Bn-CH*H*′, $J = 11.8$), 3.98 (t, 1H, H-4′, $J = 9.4$), 3.91 (br, 1H, H-2′), 3.75 (dd, 1H, H-3′, $J = 9.4, 2.5$), 3.70 (m, 2H, H-6′a and H-6′b), 3.63 (m, 1H, H-5′), 2.56 (s, 3H, Ac-Me) ppm; ^{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-*arom.*), 128.8 (Bn-*arom.*), 128.6 (Bn-*arom.*), 128.6 (Bn-*arom.*), 128.5 (Bn-*arom.*), 128.5 (Bn-*arom.*), 128.4 (Bn-*arom.*), 128.4 (Bn-*arom.*), 128.4 (Bn-*arom.*), 128.2 (Bn-*arom.*), 128.2 (Bn-*arom.*), 128.1 (Bn-*arom.*), 128.1 (Bn-*arom.*), 128.0 (Bn-*arom.*), 128.0 (Bn-*arom.*), 128.0 (Bn-*arom.*), 127.8 (Bn-*arom.*), 127.8 (Bn-*arom.*), 127.8 (Bn-*arom.*), 127.6 (Bn-*arom.*), 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) ppm; analysis calculated for $C_{41}H_{40}ClN_5O_6$ (734.2): C, 67.07; H, 5.49; N, 9.54; found: C, 66.87; H, 5.39; N, 9.47.

Data for **12**: yellow oil; $R_f = 0.61$ (ethyl acetate/hexane 1:1); $[\alpha] = +77°$ (*c* 1.00, $CHCl_3$); MS (ESI): *m/z* (%) = 734.5 ($[M+H]^+$, 100), 756.6 ($[M+Na]^+$, 50), 1256.5 ($[2M+H]^+$, 12); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.21$ (s, 1H, H-8), 8.19 (s, 1H, NH), 7.38–7.16 (m, 15H, Bn-*arom.*), 7.03–6.96 (m, 3H, Bn-*arom.*), 6.83 (m, 2H, Bn-*arom.*), 5.62 (br, 1H, H-1′), 4.89 (part A of AB system, 1H, Bn-C*H*H′, $J = 10.8$), 4.79 (m, 2H, Bn-CH_2), 4.72 (part A of AB system, 1H, Bn-C*H*H′, $J = 11.6$), 4.59 (part B of AB system, 1H, Bn-CH*H*′, $J = 10.8$), 4.56 (part A of AB system, 1H, Bn-C*H*H′, $J = 12.2$), 4.49 (part B of AB system, 1H, Bn-CH*H*′, $J = 12.2$), 4.32 (part B of AB system, 1H, Bn-CH*H*′, $J = 11.6$), 4.05 (br, 1H, H-2′), 4.02 (t, 1H, H-4, $J = 9.5$), 3.84 (dd, 1H, H-3′, $J = 9.4, 2.5$), 3.71–3.65 (br, 3H, H-6′a and H-6′b and H-5′), 2.38 (s, 3H, Ac-Me) ppm; ^{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-*arom.*), 128.6 (Bn-*arom.*), 128.5 (Bn-*arom.*), 128.5 (Bn-*arom.*), 128.4 (Bn-*arom.*), 128.4 (Bn-*arom.*), 128.4 (Bn-*arom.*), 128.4 (Bn-*arom.*), 128.3 (C-5), 128.0 (Bn-*arom.*), 128.0 (Bn-*arom.*), 128.0 (Bn-*arom.*), 127.9 (Bn-*arom.*), 127.9 (Bn-*arom.*), 127.9 (Bn-*arom.*), 127.8 (Bn-*arom.*), 127.7 (Bn-*arom.*), 127.7 (Bn-*arom.*), 127.7 (Bn-*arom.*), 127.7 (Bn-*arom.*), 127.7 (Bn-*arom.*), 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) ppm; analysis calculated for $C_{41}H_{40}ClN_5O_6$ (734.2): C, 67.07; H, 5.49; N, 9.54; found: C, 66.98; H, 5.54; N, 9.37.

4.3. Cell lines and culture conditions

The human cell lines 518A2 (melanoma), A549 (lung carcinoma), A2780 (ovarian carcinoma), HT-29 (colon adenocarcinoma) and the NiH 3T3 mouse fibroblast cell line were included in this study. Cultures were maintained as monolayer in RPMI 1640 (PAA Laboratories, Pasching, Germany) supplemented with 10% heat inactivated fetal bovine serum (Biochrom AG, Berlin, Germany) and penicillin/streptomycin (PAA Laboratories) at 37 °C in a humidified atmosphere of 5% CO_2/95% air.

4.4. Cytotoxicity assay [43]

The cytotoxicity of the compounds was evaluated using the sulforhodamine-B (SRB) (Sigma Aldrich) microculture colorimetric assay. In short, exponentially growing cells were seeded into 96-well plates on day 0 at the appropriate cell densities to prevent confluence of the cells during the period of experiment. After 24 h, the cells were treated with serial dilutions of the compounds (0–100 μM) for 96 h. The final concentration of DMSO or DMF solvent never exceeded 0.5%, which was non-toxic to the cells. The percentages of surviving cells relative to untreated controls were determined 96 h after the beginning of drug exposure. After a 96 h treatment, the supernatant medium from the 96 well plates was discarded and the cells were fixed with 10% TCA. For a thorough fixation, the plates were allowed to rest at 4 °C. After fixation, the cells were washed in a strip washer. The washing was done four times with water using alternate dispensing and aspiration procedures. The plates were then dyed with 100 μl of 0.4% SRB (sulforhodamine B) for about 20 min. After dying, the plates were washed with 1% acetic acid to remove the excess of the dye and allowed to air dry overnight. 100 μl of 10 mM Tris base solution were added to each well, and absorbance was measured at $\lambda = 570$ nm (using a 96 well plate reader, Tecan Spectra, Crailsheim, Germany). The GI_{50} value was determined from three independent measurements applying the two-parametric Hill slope equation.

4.5. Acridine orange/propidium iodide dye exclusion assay (AO/PI)

Apoptotic cell death was analyzed by acridine orange/ethidium bromide dye using fluorescence microscopy on A549 cells. Therefore approx. 500.000 cells were seeded in cell culture flasks and were allowed to grow for 24 h. The medium was removed, and the substance loaded medium was added. After 24–48 h, the supernatant medium was collected and centrifuged; the pellet was suspended in phosphate-buffer saline (PBS) and centrifuged again. The liquid was removed and the pellet was suspended in PBS. After mixing the suspension with a solution of AO/PI, analysis was performed under a fluorescence microscope. While a green fluorescence showed apoptosis, a red colored nucleus indicated necrotic cells [28,29].

4.6. DNA laddering

The DNA fragmentation assay was performed as described previously [25].

4.7. Cell cycle analysis

Approximately $1*10^6$ cells (HT29, A2780 or NiH 3T3) were seeded in cell culture flasks (25 cm^2), and the cells were allowed to grow for 24 h. After removing of the used medium, the substance loaded medium was reloaded (or a blank fresh medium as a control). After 24 or 48 h, the living cells were harvested, washed with PBS (with Mg^{2+} and Ca^{2+}) twice and ethanol fixed (70%, 4 °C, 1 h). After removing of the fixation and permeabilization agent, the cells were washed with PBS buffer (with Mg^{2+} and Ca^{2+}, containing 1% BSA and 0.1% NaN_3, 3 × 1 mL, 1000 rpm) and adjusted to $1*10^5$ million cells. The pellet was gently suspended in staining buffer (PBS buffer containing BSA, RNAase, NaN_3 and PI analog

Darzynkiewicz et al. [30]) and incubated for 30 min at 37 °C. Analyses were performed using the Attune® FACS machine; collecting data from the BL-3A channel. Doublet cells were excluded from the measurements by plotting BL-3A against BL-3H. For each cell cycle distribution 20.000 events were collected. Distribution was calculated by the method of Dean et al. [44].

Acknowledgments

Fundação para a Ciência e a Tecnologia is gratefully acknowledged for the postdoc research grant of Stefan Schwarz (FCT, Project SFRH/BPD/81065/2011, Portugal) and for the support of the project Pest-OE/QUI/UI0612/2013. The European Commission is also gratefully acknowledged for the approval of INOVAFUNAGEING commitment. The authors also wish to thank the support of the "Gründerwerkstatt – Biowissenschaften" and Dr. Ralph Kluge for running compounds' elemental analysis. The cell lines were kindly provided by Dr. T. Müller (Dept. of Haematology/Oncology, Univ. Halle).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.11.019.

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