Synthesis and Antiproliferative Evaluation of Novel D-Glucuronamide-based Nucleosides and (Triazolyl)methyl Amide-linked Pseudodisaccharide Nucleosides

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Abstract

The synthesis and antiproliferative evaluation of novel D-glucopyranuronamide-containing nucleosides is described. Based on our previously reported anticancer D-glucuronamide-based nucleosides, new analogues comprising *N/O*-dodecyl or *N*-propargyl substituents at the glucuronamide unit and anomerically N-linked 2-acetamido-6-chloropurine, 6-chloropurine or 4-(6-chloropurinyl)methyl triazole motifs were synthesized in 4-6 steps starting from acetonide-protected glucofuranurono-6,3-lactone. The methodologies were based on the access to *N*-substituted glycopyranuronamide precursors, namely 1,2-*O*-acetyl derivatives or glucuronoamidyl azides for further N-glycosidation with a nucleobase or 1,3-dipolar cycloaddition with N^9 and N^7-propargyl-6-chloropurines, respectively. *N*-Propargyl glucuronamide-based N^9-linked purine nucleosides were converted into (triazolyl)methyl amide-6,6-linked pseudodisaccharide nucleosides via cycloaddition with methyl 6-azido glucopyranoside. A CuI/Amberlyst A-21 catalytic system employed in the cycloaddition reactions also effected conversion into 6-dimethylamino purine nucleosides. Antiproliferative evaluation in chronic myeloid leukemia (K562) and breast cancer (MCF-7) cells revealed significant effects exhibited by the synthesized monododecylated purine-containing nucleosides. A *N*-propargyl 3-*O*-dodecyl glucuronamide derivative comprising a N^9-β-linked 6-chloropurine moiety was the most active compound against MCF-7 cells (GI_{50} = 11.9 μM) while a related α-(purinyl)methyltriazole nucleoside comprising a N^7-linked 6-chloropurine moiety exhibited the highest activity against K562 cells was (GI_{50} = 8.0 μM).
Flow cytometry analysis and immunoblotting analysis of apoptosis-related proteins in K562 cells treated with the *N*-propargyl 3-*O*-dodecyl glucuronamide-based N^9-linked 6-chloropurine nucleoside indicated that it acts via apoptosis induction.

1. Introduction

Nucleosides, nucleotides and their analogues are among the most relevant groups of compounds in bioorganic and medicinal chemistry. The importance of such molecules arises from their ability to mimic their physiological counterparts eliciting interference with nucleotide-dependent biological processes or enzymes, which are fundamental in health but also crucial for the development of various diseases. Hence, non-physiological nucleos(t)ides and analogues may target nucleic acid synthesis, affecting cell division, or within-host pathogen replication, as well as other events mediated by nucleotides and nucleotide-dependent enzymes involved in cell division, signalling or metabolism [1,2]. Various nucleos(t)ide analogues became approved drugs for cancer and viral infections [1-3], while the propensity of either natural or synthetic compounds of these groups to exhibit antimicrobial activity has been well reported [4-7].

Their importance for tackling current issues and challenges in medicinal chemistry is further demonstrated by the number of drugs of these groups that are included in the WHO Model Lists of Essential Medicines. More recently the nucleos(t)ide analogues remdesivir [8] and molnupiravir [9] were approved or authorized for emergency use, respectively, by the FDA for COVID-19 treatment, being remdesivir the first approved drug and molnupiravir the first oral drug against this disease.

Chemotherapeutic resistance is often a major drawback on the use of nucleos(t)ide analogues in clinics, along with low bioavailability [10], urging therefore the development of new molecules of these groups that display better cell-penetrating abilities and exhibit alternative mechanisms of action. Strategies for the design of nucleos(t)ide analogues include simple modifications at purine, pyrimidine or at ribose/2-deoxyribose moieties, the use of other nitrogenous heteroaromatic systems or other glycosyl units, the installation of phosphate group mimetic motifs or modification on the type or location of the bond connecting nucleobase and sugar (*e.g.* C-nucleosides or isonucleosides, respectively). In the last few years we have been engaged into the exploitation of the synthesis and therapeutic potential of novel nucleoside and nucleotide analogues based on *N*-substituted D-glucuronamide templates [11-14]. D-Glucuronamide is a rather unexploited glycosyl unit in nucleos(t)ide chemistry. Few examples of reported D-glucuronamide-based nucleosides comprise the natural compounds gougerotin [15] and its semisynthetic analogues [15,16], bagougeramines [17] and aspiculamycin [18], which display antimicrobial activities. Concerning synthetic derivatives, reports on the literature are scarce, although a *N*-methyl riburonamide-based 2-chloro-N^6-(3-iodobenzyl)adenine nucleoside CF102 achieved clinical trials for hepatocellular carcinoma, acting as A3 adenosine receptor antagonist [19,20]. The use of D-glucuronamide as glycosyl unit in the design of new potentially bioactive nucleosides is also encouraged by the significant biological properties described for D-glucuronamide-containing molecules, especially anticancer effects [21]. Moreover, the inclusion of the D-glucuronamide moiety offers opportunities for performing structural variations in a regioselective manner at C-6 on a gluco-configured template by varying the N-substitution, allowing the installation of

various kinds of moieties as a part of a rather stable amide functionality and enabling bioactivity tuning of the nucleos(t)ide analogue. We have previously reported the synthesis of *N*-dodecyl glucuronamide-based nucleosides, including pyranosyl and furanosyl derivatives, and their antiproliferative effects in chronic myeloid leukemia (K562) and breast adenocarcinoma cells (MCF-7), from which molecules exhibiting activities at single digit micromolar order of concentration, comparable or higher than that of a standard drug against MCF-7 cells, and with ability to induce apoptosis, were revealed (Figure. 1) [12,14].

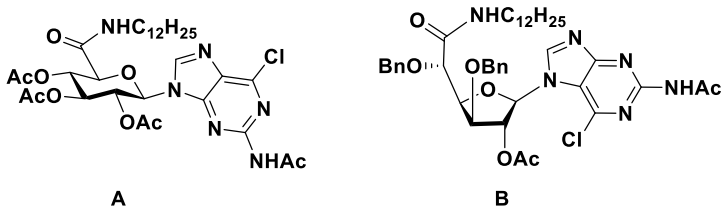


Figure. 1. *N*-Dodecyl glucuronamide-based nucleosides exhibiting significant antiproliferative activities in K562 and MCF cells and ability to induce apoptosis.

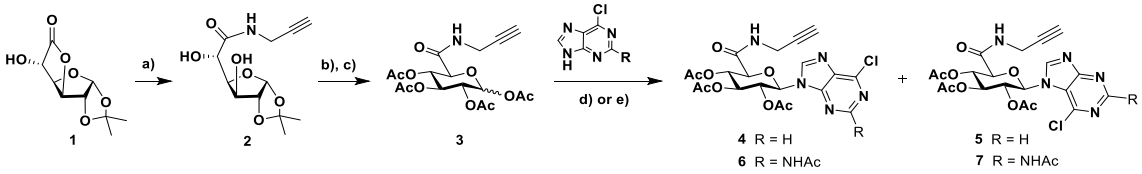
Motivated by the previous encouraging findings, we report herein a further exploitation on the synthesis of new structures of glucopyranuronamide-based nucleosides and evaluation of their anticancer potential. Based on the structure of lead compound **A** (Figure. 1), the synthesis of nucleoside analogues comprising *N*-propargyl or *N*-dodecyl glucuronamide-derived moieties and anomerically *N*-linked purine or (purinyl)methyl triazole units was performed. The 6-chloropurine moiety was especially used as nucleobase to evaluate its suitability to elicit antiproliferative activities when combined with D-glucuronamide-based systems as the previously exploited 2-acetamido-6-chloropurine system does. Per-*O*-acetylated *N*-propargyl glucuronamide-based nucleosides were also converted into corresponding (triazolyl)methyl amide-6,6-linked pseudodisaccharide nucleosides to further assess the anticancer potential of this type of structure, which was firstly proposed by us as potential mimetic of nucleoside diphosphate sugars in which the (triazolyl)methyl amide system is proposed a surrogate of the diphosphate moiety [11]. More recently, the synthesis and antiflaviviral potential of 1,5-linked pseudodisaccharide nucleoside analogues of such type of structures were reported [22].

6-Chloropurine *N*-propargyl glucuronamide-containing nucleoside analogues of compound **A** possessing a 3-*O*-dodecyl group, which may increase the cell-penetrating ability of the molecules, were accessed, aiming at evaluating the effect of moving the dodecyl moiety from the amide system to a hydroxyl group on the antiproliferative activity of the molecules. Moreover, analogues of **A** comprising a novel type of D-glucopyranuronamide-based nucleosidic framework having a methylene 1,2,3-triazolyl fragment between the 6-chloropurine system and the glucuronamide moiety, *i.e.* (purinyl)methyltriazole nucleosides were accessed.

2. Results and Discussion

2.1. Chemistry

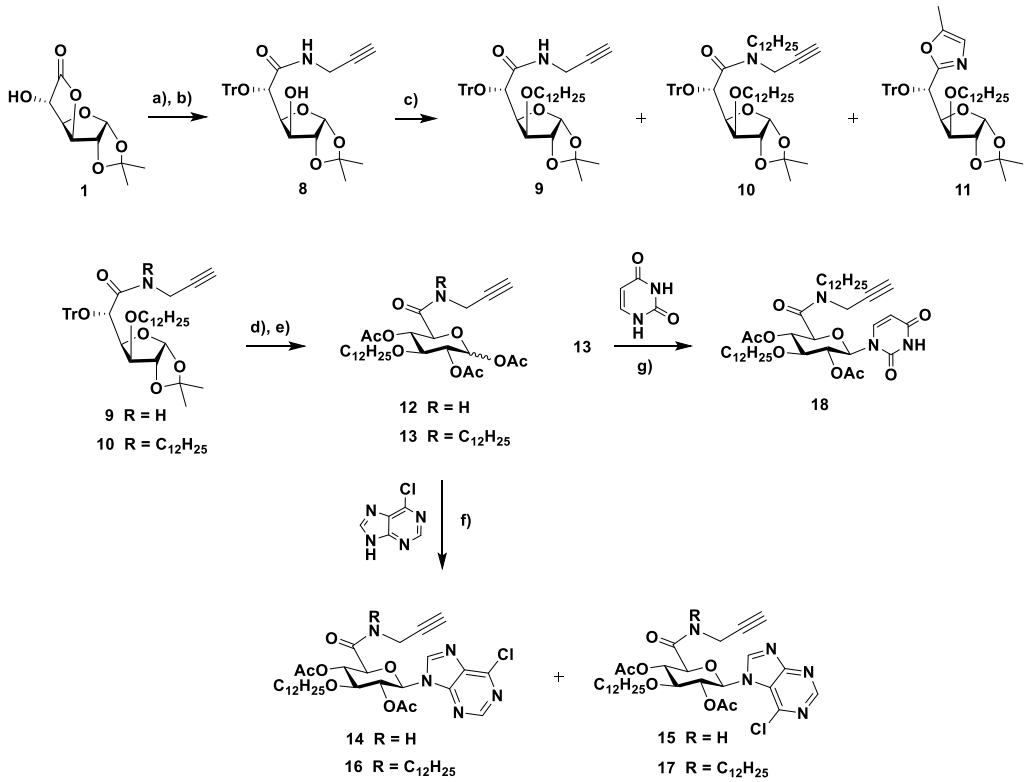
1,2-*O*-Isopropylidene glucofuranurono-6,3-lactone (**1**) was the starting material for the synthesis of glucopyranuronamide precursors for further nucleosidation, via opening of the lactone moiety with amines. Hence the tetra-*O*-acetylated *N*-propargyl glucopyranuronamidyl donor (**3**) was synthesized as previously reported by us, through amidation of **1** with propargylamine followed by 1,2-acetonide cleavage with aqueous trifluoroacetic acid (TFA, 70% aq. soln.) and subsequent acetylation in pyridine [11]. Treatment of **3** with 6-chloropurine and 2-acetamido-6-chloropurine, which were silylated with bis(trimethylsilyl)acetamide (BSA) [11], under microwave irradiation (Pmax 250 Psi, 150 W maximum power) in the presence of trimethylsilyl triflate (TMSOTf) as Lewis acid (Scheme 1) furnished the corresponding N^9- (**4**, **6**) and N^7-linked nucleosides (**5**, **7**) in 59% and 69% total yields and in virtually 1:1 regioisomeric ratios. Regioisomer assignments were unambiguously made based on HMBC correlations, namely those between the anomeric proton (H-1') and C-4 of the purine moiety (for the N9 nucleosides) or those between H-1' and purine C-5 for the N7 counterparts.



Scheme 1. Reactions and conditions: (a) propargylamine, CH_2Cl_2, r. t., 16 h, quant. **[**11], (b) TFA (70% aq.), 40 ºC, 30 min; c) Ac_2O, py, 30 min, 87%, α/β = 1:0.5, 2 steps [11]; (d) 6-Cl-purine, BSA, TMSOTf, CH_3CN, 65 °C, MW, max. 150 W, 1.5 h, 33% (**4**), 34% (**5**); (e) 2-NHAc-6-Cl-purine, BSA, TMSOTf, MeCN, 65 °C, MW, max. 150 W, 1.5 h, 30% (**6**) and 29% (**7**) [11].

For the synthesis of related *N*-propargyl glucuronamide-based nucleosides containing a 3-*O*-dodecyl group (Scheme 2), glucuronolactone **1** was subjected to tritylation using trityl chloride (TrCl) in the presence of dimethylaminopyridine (DMAP) and subsequent treatment with propargylamine, leading to amide **8** in 65% overall yield. Dodecylation of **8** via nucleophilic substitution with dodecyl bromide (2 equiv.) in the presence of sodium hydride afforded the 3-O-dodecyl derivative **9** in 18% along with the *N*,*O*-didodecylated product **10** (38%) and the 5-methyl-2-oxazole derivative **11** as minor product (4%). The ^{1}H NMR spectrum of the didodecyl derivative **10** showed notorious peak doubling, indicating the existence of *cis* and *trans*-rotamers in a 1:1 ratio in $CDCl_3$ solution, due to the hindered rotation about the amide C-N bond. Variable temperature (VT) ^{1}H-NMR

experiments of **10** in $CDCl_3$ solution showed no significant change on the rotameric ratio with heating up to 45 ºC. In CD_3CN solution at ambient temperature the rotameric ratio was changed to 1:2 and VT ^1H NMR experiments over the temperature range 20-60 ºC demonstrated a gradual coalescence of the two sets of signals. The structure of the oxazole **11** was elucidated by NMR and HRMS data. The ^1H NMR spectrum of **11** showed the methyl substituent protons resonating at 2.13 ppm and the signal of H-4" at 6.34 ppm, while its ^{13}C NMR spectrum with support from HSQC and/or HMBC experiments led to the assignments of signals for C-2", C-4" and C-5" at 161.0, 122.7 and 148.1 ppm, respectively. The formation of the **11** is not surprising since it is well reported that *N*-propargylamides are prompted to cyclize or isomerize into oxazoles under various reaction conditions [23]. Such cyclization has been particularly exploited using metal catalysis [24,25] or iodine-mediated activation of the triple bond [. Only a few base-mediated examples of *N*-propargylamide cyclization into oxazoles have been reported, namely for *N*-(1-arylpropargyl)amides indicating that allenic intermediates may be involved in such transformation [26-29]. In our case, since amide NH deprotonation by sodium hydride is possible, the resulting anion may rearrange into the corresponding oxazol-5(4*H*)-ylidenemethanide which upon protonation by traces of water contained in DMF and further basic-catalysed allylic rearrangement leads to the more stable oxazole system. Both mono *O*-dodecylated and di-*N*,*O*-dodecylated *N*-propargyl glucuronofuranuronamide derivatives **9** and **10** were subjected to acetonide hydrolysis (TFA, 70% aq. soln.) and further acetylation (Ac_2O/py) leading to 1,2-di-*O*-acetyl glucopyranuronamide derivatives **12** and **13.** N-Nucleosidation of **12** and **13** was performed with silylated 6-chloropurine under similar microwave-assisted conditions as previously mentioned leading to the corresponding N^9-linked nucleosides **14**, **16** in 77% and 17% yields, and to their N^7-linked regioisomers **15** and **17** in 3% and 10% yields, respectively. The glucopyranuronamidyl donor **13** was also subjected to *N*-glycosidation with uracil to afford solely the N^1-linked uracil nucleoside **18** in a moderate yield (52%), whose regiochemistry was unambiguously assigned through the observed HMBC correlations between H-1' of the glycosyl unit and C-2/C-6 of the uracil moiety.

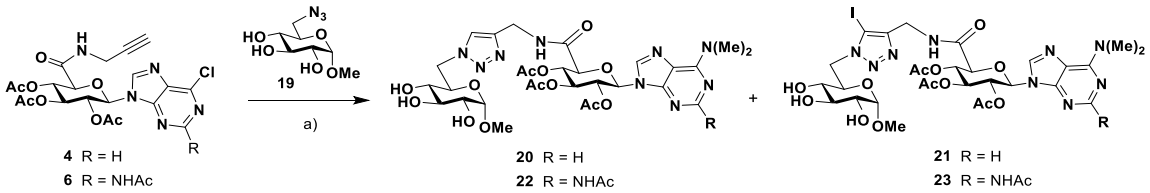


Scheme 2. Reactions and conditions: (a) TrCl, DMAP, CH_2Cl_2 /pyridine, 40 °C, 5 d; (b) propargylamine, CH_2Cl_2, r. t., 4 d, 65%, 2 steps; (c) $C_{12}H_{25}Br$, NaH, DMF, 17 h, r. t., 18% (**9**), 38% (**10**), 4% **(11**); (d) TFA (70% aq.), 40 ºC, 2 h (for **9**), r.t., 16 h (for **10**); (e) Ac_2O, py, 30 min, 75%, (**12**, α/β = 1:0.6), 70%, (**13**, α/β = 1:0.7), 2 steps; (f) 6-Cl-purine, BSA, TMSOTf, CH_3CN, 65 °C, MW, max. 150 W, 1.5 h, 77% (**14**), 3% (**15**), 27% (**16**), 10% (**17**); (g) uracil, BSA, TMSOTf, CH_3CN, 65 °C, MW, max. 150 W, 1.5 h, 52%.

The access to pseudodisaccharide nucleosides in which the glucuronamide-based nucleoside system is connected through its amide function to a methyl glucosidyl moiety at C-6 via a methylenetriazole fragment was based on our reported methodology involving a 1,3-dipolar cycloaddition between a *N*-propargyl nucleoside and a 6-azido glucopyranoside [11]. Hence the purine N^9-linked nucleosides **4**-**5** were coupled with methyl 6-azido glucopyranoside (**19**) in the presence of a catalytic system in which CuI is supported on Amberlyste A21 resin [30]. In both cases, dimethylaminopurine pseudodisaccharide nucleosides (**20**, **22**) were obtained in modest similar yields (27%−29%) along with their 5-iodotriazole derivatives as secondary products (**21**, **23**, 23% and 9% yields, respectively).

The ^{1}H NMR signals for H-2 (δ 8.23, 8.21 ppm) of the dimethylaminopurine purine moiety in the nucleosides **20** and **21** appear at significantly lower chemical shifts than that in the 6-chloropurine nucleoside **4** (δ 8.81 ppm) while in the ^{13}C NMR spectra signals for C-8 are shifted upfield in **20**-**23** (δ 139.1, 138.4) relatively to those in the 6-chloropurine/2-acetamido-6-chlotropurine nucleosides **4** and **5** (δ 143.1 and 142.4 ppm,

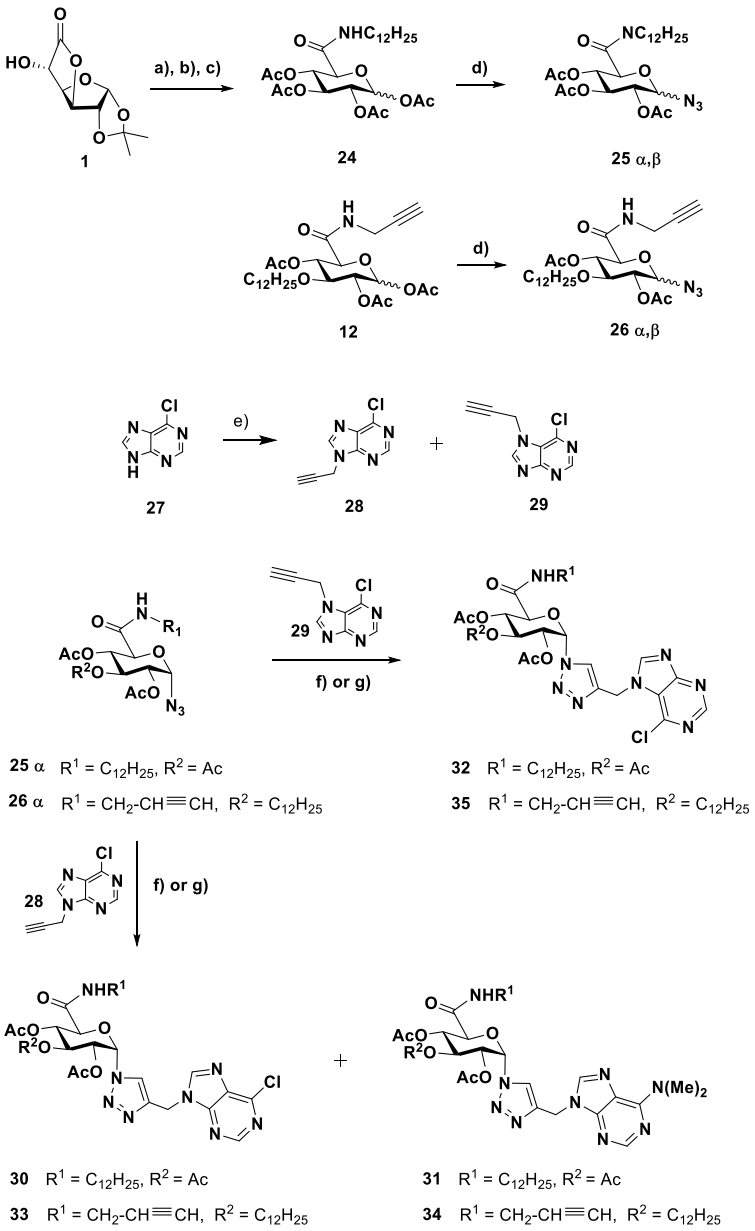
respectively). NMR Identification of the iodo nucleosides **21-23** relied on the absence of the signals for the triazole proton (H-9') while their triazole quaternary carbon signals (C-8′) appeared at δ 83.1 and 83.6 ppm. The presence of dimethylamino groups in the Amberlyst A-21 resin of the catalytic system herein employed elicited both chloride replacement by the dimethylamino group in the purine moieties as well as 5-iodination, which is described to occur in CuI-catalyzed azide-alkyne cycloadditions in the presence of organic bases [31,32], and also previously observed by us when using the CuI/Amberlyste A-21-catalysed cycloaddition method [33].



Scheme 3. Reactions and conditions: (a) CuI/Amberlyste A21, CH_3CN, r. t., 6 d, 29% (**20**), 23% (**21**), 27% (**22**), 9% (**23**).

The synthesis of dodecyl-containing glucuronamide-based nucleosides comprising a 1,2,3-triazole between the sugar moiety and the purine unit was based on the 1,3-dipolar cycloaddition between 1-azido glucuronamide derivatives and N-propargyl-6-chloropurine (Scheme 4). The glycosyl azides **25**, **26** were synthesized in moderate to good yields (46%, 72%) via anomeric azidation of *N*-dodecyl tetra-*O*-acetylglucuronamide (**24**), accessible from glucuronolactone **1** as previously reported [12], and of *N*-propargyl 1,2,4-tri-*O*-acetyl-3-*O*-dodecylglucuronamide (**12**), respectively, with trimethylsilyl azide. The α-glucuronoamidyl azides **25α**, **26α**, which could be isolated from the respective anomeric mixtures, were then subjected to cycloaddition with N^9 and N^7-propargyl-6-chloropurines (**28**, **29**), which were obtained in a 3:1 ratio (57% total yield) via nucleophilic substitution with propargyl bromide in the presence of potassium carbonate. The *N*-dodecyl glycosyl azide **25α** was subjected to both CuI/A21-mediated cycloaddition and thermal cycloaddition in refluxing toluene. The catalysed method provided higher yields for the expected N^9-linked (**30**, 56%**)** or N^7-linked (**32**, 63%) 4-(6-choloropurinyl)methyl triazole nucleosides than the thermal protocol, which led to **30** and to in modest yields of 35% and 23%. The CuI/A-21 methodology in the case when N^9-propargyl-6-chloropurine was used also led to the 6-dimethylaminopurine derivative **31** in 18% yield. Key spectral differences between nucleosides 6-cloropurine **30** and 6-dimethylaminopurine derivatives **31,** besides the appearance of the proton and ^{13}C signals for the dimethylamino group in **31**, include the chemical shift values for purine H-2 and H-8, which are the higher in **30** (δ 8.76 and 8.38 ppm , respectively) than in **31**, and the lower chemical shift of C-8 in 31 (δ 138.2 ppm) than in 30 (δ 145.2 ppm). The CuI/A21 catalytic method was employed for the cycloadditions between the *N*-propargyl 3-*O*-dodecyl glycosyl azide **26α**. Similarly as for **25α**,

coupling of **26α** with N^9-propargyl-6-chloropurine (**28**) furnished both (6-choloropurinyl)methyl triazole nucleoside **33** and its 6-dimethylaminopurine counterpart **34**, in 23% and 46% yields, respectively, while when using N^7-propargyl-6-chloropurine (**29**) only the 6-chloropurine-containing nucleoside **35** was the sole product obtained in 51% yield. Is appears therefore that chlorine replacement by the dimethylamino group is sterically hindered in the N^7-linked purine derivatives.



Scheme 4. Reactions and conditions: (a) $C_{12}H_{25}NH_2$, CH_2Cl_2, r.t., 16 h, 83%; (b) TFA (60% aq.), r.t., 1.5 h; (c) Ac_2O, py, r. t., 15 min, 85% (α/β = 1:0.3), two steps [12]; (d) $TMSN_3$, TMSOTf, CH_3CN, 65 °C, MW, max. 150 W, 50 min, 72% (**25**, α/β = 1: 0.6) [12] or reflux, 24 h, 46% (**26**, α/β = 1:0.6); (e) propargyl bromide, K_2CO_3, r. t., 48 h 43% (**28**), 14% (**29**); (f) CuI/Amberlyste A21 (cat.), CH_2Cl_2, r.t., 48 h, 56% (**30**), 18% (**31**), 63% (**32**), 23% (**33**), 46% (**34**), 51% (**35**); (g) toluene, reflux, 24 h, 35% (**30**), 23% (**32**).

2.2. Biological Evaluation

The nucleosides **4-6**, **14-16**, **18**, the oxazole derivative **11**, the (purinyl)methyltriazole nucleosides **30-35** and the (triazolyl)methyl amide-linked pseudodisaccharide nucleosides **20-22** were subjected to antiproliferative assays in chronic myeloid leukemia (K562) and breast adenocarcinoma (MCF-7) cell lines as well as in normal human fibroblasts BJ (Table 1) by the rezasurin (Alamar Blue) assay.

Table 1. Compounds' antiproliferative activities in K562, MCF-7 and BJ cell lines.

Compound	**GI_{50} (μM)***		
	K562	**MCF-7**	**BJ**
N-Propargyl glucuronamide-based nucleosides			
4	>100	>100	n.d.
5	>12.5	>12.5	n.d.
6	>100	>100	n.d.
14	9.6 ± 0.7	11.9 ± 1.1	>25
15	11.6 ± 0.6	12.4 ± 0.1	>25
16	>12.5	>12.5	n.d.
18	>100	>100	n.d.
5-C-[5'-methyloxazol-2'-yl]xylofuranose derivative			
11	>12.5	>12.5	n.d.
(Triazolyl)methyl amide-linked pseudodisaccharide nucleosides			
20	>100	>100	n.d.
21	>100	>100	n.d.
22	>100	>100	n.d.
Glucuronamide-based (purinyl)methyltriazole nucleosides			
30	>12.5	12.2 ± 0.4	>25
31	>25	>25	>25
32	19.2 ± 7	23.4 ± 0.5	25 ± 0.0
33	11.8 ± 5.1	17.6 ± 4.9	23.8 ± 1.7
34	>25	>25	>25
35	8.0 ± 1.3	26.3 ± 1.0	23.6 ± 5.1
imatinib	0.30 ± 0.2	29.7 ± 8.2	53.5 ± 9.5

**tested at least in duplicate.*
n.d.: not determined

The significantly active compounds were glucuronamide-based nucleosides comprising one dodecyl group (N- or O-linked) and a 6-chloropurine moiety. Among the *N*-propargyl glucuronamide-based nucleosides, only the 6-chloropurine nucleosides having a 3-*O*-dodecyl group (**14-15**) showed relevant antiproliferative effects in both cancer cells. The GI_{50} values of the N^7-nucleoside (**15**) were identical in both cells (12 μM) and similar to

that of the N^9-nucleoside (**14**) in breast cancer MCF-7 cells. The activity of **14** was slightly better in K562 leukemia cells (GI_{50} = 9.6 μM) than that exhibited by the N^7 isomer **15**. These nucleosides showed some selectivity for cancer cells relatively to normal fibroblasts with GI_{50} values in the cancer cells assayed that are at least *ca.* 2-fold lower than those in BJ cells. The tested di-*N*,*O*-dodecylated *N*-propargyl glucuronamide nucleosides (**16** and **18**) did not show significant effects most probably due to their higher lipophilicity and consequent higher membrane retention. With respect to glucuronamide-based (purinyl)methyltriazole nucleosides, the *N*-propargyl-3-*O*-dodecyl derivative comprising a N^7-linked 6-chloropurine moiety (**35**) showed the higher antiproliferative effects in K562 cells among the panel of compounds tested, with activity in the single digit micromolar concentration (GI_{50} = 8 μM), which is ca. 3.4 fold-lower than that in MCF-7 cells (GI_{50} = 26.3 μM). Its N^9-linked purine-containing counterpart (**33**) showed a ca. 1.5-fold higher effect against MCF-7 cells (GI_{50} = 17.6 μM), but a slight lower activity towards K562 cells (GI_{50} = 11.8 μM). The related nucleosides comprising a *N*-dodecyl glucuronamide moiety (**30** and **32**) showed also significant effects against both cells. The N^7-linked 6-chloropurine derivative (**32**) was less active against the K562 cells (GI_{50} = 19.2 μM) than the analogous nucleoside containing a *N*-propargyl 3-*O*-dodecylglucuronamide moiety (**35**). The N^9-linked purine counterpart (**30**) was more active towards the MCF-7 cells (GI_{50} = 12.2 μM) than its N^7-linked isomer and than its *N*-propargyl 3-*O*-dodecylglucuronamide-containing analogue (**33**). Replacing the chlorine atom of N^9-linked 6-cloropurine-containing triazole nucleosides **30**, **33** by a dimethylamino group (nucleosides **31**, **34**, respectively) leads to a decrease on the nucleosides' antiproliferative effects in both cancer cells indicating therefore that the presence of the chlorine atom at purine C-6 is relevant for the bioactivity. The pseudodisaccharide nucleosides **20**-**21** were virtually inactive while the oxazole derivative **11** did not show significant activity.

The *N*-propargyl 3-*O*-dodecyl glucuronamide-based nucleoside **14** as one of the most active compounds of the series was subjected to complementary assays in leukemia cells aiming at providing insights about its mode of action (Figure 2). To assess the effect of **14** on the K562 cell cycle, flow cytometry analyses was performed, revealing an accumulation of sub-G1/apoptotic cell population upon treatment with the compound at 25 μM, which is an indicator of apoptosis. Immunoblotting of lysates of K562 cells treated with increasing concentrations of compound **14** during 24 h was performed to monitor changes on the expression level of apoptosis-associated proteins. A dose-dependent cleavage of PARP-1 (poly(ADP-ribose) polymerase-1), a substrate of caspases, with maximum cleavage at 25 μM, consistent with maximum expression level of caspase 7 active fragments at 25 μM, were detected, both of which are considered hallmarks of apoptosis [34,35]. No significant changes in the expression of the anti-apoptotic half-life protein Mcl-1 and of the X-linked inhibitor of apoptosis protein (XIAP) were detected.

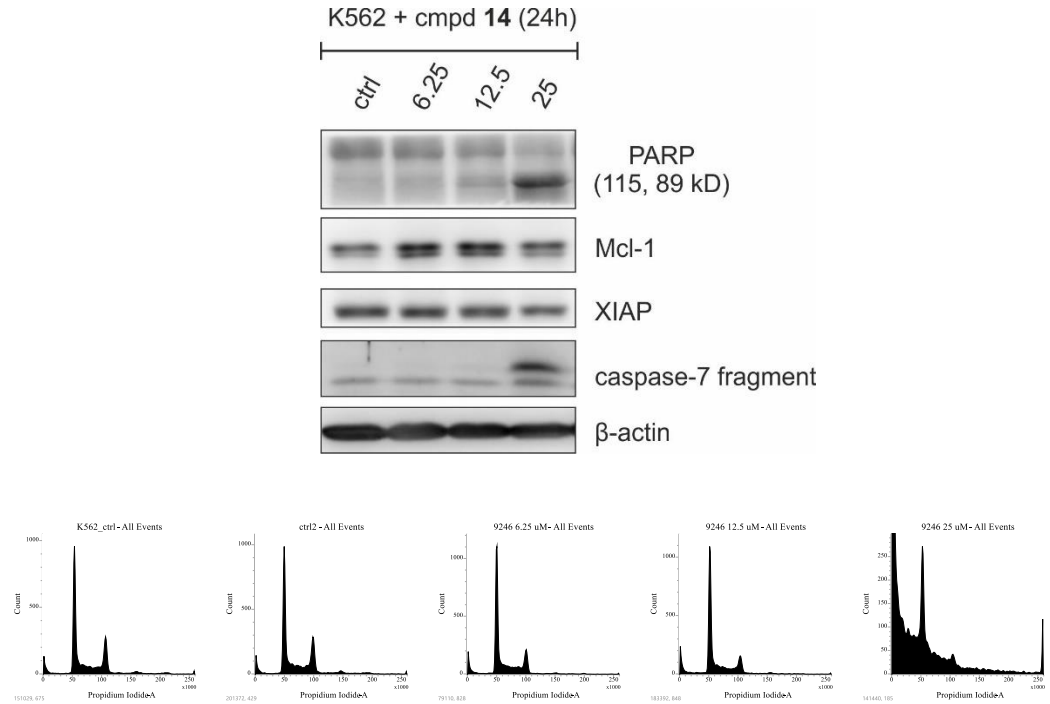


Figure 2: Top: Immunoblotting analysis of apoptosis-related proteins in K562 cells treated with nucleoside **14** for 24 h. β-Actin was used as control for equal loading. Below: Cell cycle distributions of K562 cells upon treatment with increasing concentrations of compound **14** (6.25 µM, 12.5 µM and 25 µM). Flow cytometric analysis of DNA stained by propidium iodide

3. Conclusions

A variety of D-glucuronamide-based nucleosides having different structural skeletons were synthesized in few steps starting from D-glucofuranurolactone, including a previously unreported type of D-glucuronamide-based nucleosidic framework possessing a N-anomerically-linked 4-(6-chloropurinyl)methyl triazole moiety. From the series of compounds subjected to antiproliferative evaluation, the monododecylated nucleosides encompassed within *N*-propargyl 3-*O*-dodecyl glucuronamide-based 6-cloropurine nucleosides (**14**-**15**) and related (6-chloropurinyl)methyltriazole nucleosides (**30**, **32**, **33**, **35**) were the relevant molecules. All of them showed significant activities towards breast cancer MCF-7 cells (GI_{50} values ranging from 26.3 µM to 11.9 µM**)** and five of them were active against leukemia K562 cells (GI_{50} values ranging from 19.2 µM to 8 µM**)**, including two nucleosides with single-digit micromolar activities (**14** and **35**). These results reinforce the importance of the presence of the lipophilic dodecyl moiety on the activity, most likely facilitating cell penetration by the nucleoside, and this effect is independent of whether the dodecyl unit is a *N*-substituent of the carbohydrate amide function or is *O*-linked to the sugar. The obtained findings demonstrate also the interest of including the 6-chloropurine moiety in D-glucuronamide-based nucleosides for the search for new antiproliferative molecules. Moreover, the inclusion of a (triazolyl)methylene system between the D-glucuronamide unit and the 6-chloropurine motif, also led to nucleoside analogues with promising antiproliferative effects, with GI_{50}

values are in the same order of magnitude than those of their analogues in which the 6-chloropurine is *N*-anomerically-linked to the glucuronamide moiety. The anticancer potential of such types of nucleosides was further demonstrated by the additional studies showing the ability of compound **14** to induce apoptosis.

4. Experimental Section

4.1. Chemistry

4.1.1. General Methods

Chemicals were purchased from *Sigma-Aldrich*, *Acros Organics*, *Alfa Aesar and Carbosynt*. The progress of the reactions was monitored by TLC (Merck 60 F_{254} silica gel aluminium plates) with visualization by UV light (254 nm) and/or staining using a 10% (v/v) ethanolic H_2SO_4 solution or a solution of cerium (IV) sulfate (0.2% w/v) and ammonium molybdate (5% w/v) in H_2SO_4 (6% aq.), followed by charring (200 ºC). Microwave-assisted reactions were carried out using a CEM Discover SP Microwave Synthesizer. The operating conditions were power = 150 W, pressure = 250 Psi and T = 65 °C with stirring in high speed. Flash column chromatography was performed on silica gel 60 G (0.040–0.063 mm, E. Merck). NMR experiments were carried out using a BRUKER Avance 400 spectrometer operating at 400.13 MHz (for ^{1}H NMR) and 100.62 MHz (for ^{13}C NMR) and spectra were referenced relatively to internal TMS, in the case of ^{1}H NMR spectra in $CDCl_3$, or to the respective solvent peak. Chemical shifts are given in parts per million and coupling constants (J) are reported in Hertz (Hz). High-resolution mass spectrometry (HRMS) data were obtained on a High Resolution QqTOF Impact II mass spectrometer from Bruker Daltonics equipped with an electrospray ion source (ESI). Spectra were recorded in positive mode with external calibration. Melting points were determined with a Stuart SMP30 instrument. Optical rotations (589 nm, sodium D line, 20 ºC) were measured using an Anton Paar Modular compact polarimeter MCP-100.

Synthesis of compounds **2-3**, **6-7** [11], and **24-25** [12] was previously described.

4.1.2. General procedure for *N*-glycosylation of 6-chloropurine, 2-acetamido-6-chloropurine or uracil with peracetylated N-substituted glucuronamide derivatives

To a suspension of the nucleobase (1.5 equiv.) in acetonitrile (3 mL), *N*,*O*-bis(trimethylsilyl)acetamide (BSA, 3 equiv.) was added. The mixture was stirred at room temperature until a clear solution is obtained (ca. 20 min). A solution of 1-O-acetyl glycosyl donor (0.30 mmol) in acetonitrile (5 mL) was added to the previous solution, followed by dropwise addition of trimethylsilyl triflate (TMSOTf, 7.5–10 equiv.). The resulting mixture was stirred under microwave irradiation (150 W, P max = 250 Psi) at

65 °C for 1.5 h. The solution was then diluted with CH_2Cl_2 and it was neutralized with sat. sodium bicarbonate soln. The aqueous phase was extracted with dichloromethane (3 ×) and the combined organic phases were washed with brine and then dried with anhydrous $MgSO_4$. After filtration and concentration under vacuum, the residue was purified by column chromatography.

4.1.2.1. *N*-Propargyl 2,3,4-tri-*O*-acetyl-1-(6-chloropurin-9-yl)-1-deoxy-β-D-glucopyranuronamide (**4**) and *N*-propargyl 2,3,4-tri-*O*-acetyl-1-(6-chloropurin-7-yl)-1-deoxy-β-D-glucopyranuronamide (**5**)

Obtained according to the general procedure, starting from *N*-propargyl 1,2,3,4-tetra-*O*-acetyl-α,β-D-glucopyranuronamide (**3**, 230 mg, 0.58 mmol) and 6-chloropurine (134 mg, 0.87 mmol) and using BSA (0.4 mL, 1.74 mmol) and TMSOTf (0.8 mL, 4.3 mmol). The reaction mixture was exposed to MW irradiation for 1.5 h. Purification by column chromatography (AcOEt/hexane, from 1:1, then 2:1) afforded the N^9 nucleoside **4** (95 mg, 33 %) and the N^7 regioisomer **5** (97 mg, 34%) as white powders.

Data for **4**: $[\alpha]_D^{20} = -5.3$ (c = 0.6, in CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.81 (s, 1 H, H-2), 8.31 (s, 1 H, H-8), 6.89 (t, $J_{NH,7'a}$=$J_{NH,7'b}$ = 5.3 Hz, 1 H, N*H*), 5.99 (d, $J_{1',2'}$ = 9.6 Hz, 1H, H-1'), 5.79 (t, $J_{1',2'}$ = $J_{2',3'}$ = 9.0 Hz, 1 H, H-2'), 5.54-5,40 (m, 2 H, H-3', H-4'), 4.35 (d, $J_{4',5'}$ = 8.8 Hz, 1 H, H-5'), 4.06 (ddd, $J_{7'a,7'b}$ = 17.6 Hz, $J_{7'a,NH}$ = 5.3 Hz, $J_{7'a,9}$ =2.6 Hz, 1H, H-7'a), 3.98 (ddd, $J_{7'a,7'b}$ = 17.6 Hz, $J_{7'b,NH}$ = 5.3 Hz, $J_{7'b,9}$ =2.6 Hz, 1 H, H-7'b), 2.22 (t, $J_{7'a,9}$ = $J_{7'b,9}$ = 2.6 Hz, 1 H, H-9'), 2.13 (s, 3 H, CH_3, $^{3'/4'}$OAc), 2.06 (s, 3 H, C*H*$_3$, $^{3'/4'}$OAc), 1.82 (s, 3 H, C*H*$_3$, $^{2'}$OAc) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.8, 169.6, 169.0 (3 × *C*O, Ac), 165.3 (C-6'), 152.9 (C-2), 152.1 (C-6), 151.6 (C-4), 143.1 (C-8), 132.0 (C-5), 81.0 (C-1'), 78.6 (C-8'), 75.3 (C-5'), 72.4 (C-9'), 72.2 (C-3'), 69.64 (C-2'), 69.06 (C-4'), 29.16 (C-7'), 20.83 (*C*H$_3$, $^{3'/4'}$OAc), 20.6 (*C*H$_3$,$^{3'/4'}$OAc), 20.3 (C*H*$_3$, $^{2'}$OAc) ppm. HRMS: calcd for $C_{20}H_{20}ClN_5O_8$ $[M-H]^-$ 492.0928, found 492.0942.

Data for **5**: $[\alpha]_D^{20} = +5.7$ (c = 1.2, in CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.93 (s, 1 H, H-2), 8.57 (s, 1 H, H-8), 6.82 (t, $J_{NH,7'a}$=$J_{NH,7'b}$ = 5.3 Hz, 1 H, N*H*), 6.28 (br.s, 1 H, H-1'), 5.77 (t, $J_{1',2'}$ =$J_{2',3'}$ = 9.4 Hz, 2 H, H-2'), 5.54 (t, $J_{2',3'}$ = $J_{3',4'}$ = 9.4 Hz, 2 H, H-3'), 5.42 (t, $J_{3',4'}$ ~$J_{4',5'}$ ~ 9.6 Hz, 2 H, H-4'), 4.37 (d, $J_{4',5'}$ = 9.9 Hz, 1 H, H-5'), 4.05 (ddd, $J_{7'a,7'b}$ = 17.6 Hz, $J_{NH,7'a}$ =5.3 Hz, $J_{7'a,9}$ =2.6 Hz, 1 H, H-7'a), 3.97 (ddd, $J_{7'a,7'b}$ = 17.6 Hz, $J_{NH,7'b}$ = 5.3 Hz, $J_{7'b,9}$ = 2.6 Hz, 1 H, H-7'b), 2.21 (t, $J_{7'a,9}$ = $J_{7'b,9}$ = 2.6 Hz, 1 H, H-9'), 2.12 (s, 3 H, C*H*$_3$, Ac), 2.07 (s, 3 H, C*H*$_3$, Ac), 1.89 (s, 3 H, CH_3, Ac) ppm.^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.9 (*C*O, Ac), 169.7 (*C*O, Ac), 169.2 (C-6'), 165.0 (C-4), 162.6 (C-6)*, 153.4 (C-2), 146.3 (C-8)*, 122.2 (C-5), 78.6 (C-8'), 75.1 (C-5'), 72.4 (C-3', C-9'), 69.7 (C-2'), 68.8 (C-4'), 29.8 (C-7'), 20.8 (*C*H$_3$, Ac), 20.6 (*C*H$_3$, Ac), 20.4 (*C*H$_3$, Ac) ppm.

4.1.3. *N*-Propargyl 1,2-di-*O*-isopropylidene-5-*O*-trityl-α-D-glucofuranuronamide (**8**)

To a solution of 1,2-di-*O*-isopropylidene-α-D-glucofuranurono-6,3-lactone (1, 2.02 g, 9.34 mmol) in pyridine/CH_2Cl_2 (29 mL, 1:3), under nitrogen, trityl chloride (7.78 g, 27.9 mmol) and 4-dimethylaminopyridine (DMAP, 203 mg, 1.66 mmol) were added. The solution was stirred at 40 °C for 5 days. The solution was then diluted with CH_2Cl_2, washed with sat. aq. $NaHCO_3$ soln. and water and the aqueous phase was extracted with CH_2Cl_2 (3 ×). The combined organic layers were dried with $MgSO_4$, filtered and concentrated under vaccum. The obtained solid residue was then dissolved in CH_2Cl_2 (40 mL) and propargylamine (943 mg, 17.1 mmol) was added. The solution was stirred at room temperature for 4 days. The solvent was evaporated and the residue was subjected to column chromatography (EtOAc/hexane, 1:8) to afford **8** (3.14 g, 65%, 2 steps) as a yellow oil. m.p.: 119.8-121 ºC. $[\alpha]_D^{20}$ = + 4.5 (c = 1.1, in CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.45–7.27 (m, 15 H, Ph), 6.98 (t, $J_{NH,7a}$ = $J_{NH,\ 7b}$= 5.3 Hz, 1 H, N*H*), 5.96 (d, $J_{OH,\ 3}$ = 7.0 Hz, 1 H, O*H*), 5.75 (d, $J_{1,2}$ = 3.5 Hz, 1 H, H-1), 4.65 (s, 1 H, H-5), 4.35 (d, *J1,2* = 3.5 Hz, 1 H, H-2), 3.94 (d, $J_{3,4}$ = 3.7 Hz, 1H, H-4), 3.91-3.87 (m, $J_{7,\ NH}$ = 5.5, $J_{7,9}$ = 2.5 Hz, 2 H, CH_2-7), 3.73 (dd, $J_{3,OH}$ = 6.6, $J_{3,4}$ = 3.7 Hz, 1 H, H-3), 2.25 (t, $J_{7,9}$ = 2.5 Hz, 1 H, H-9), 1.33 (s, 3 H, CH_3, *i*-Pr), 1.24 (s, 3 H, CH_3, *i*-Pr) ppm. ^{13}C NMR ($CDCl_3$, 101 MHz): 171.3 (C-6), 142.9 (Cq-Ph), 128.8 (CH-ph), 128.6 (CH-Ph), 128.1 (CH-Ph), 111.9 (Cq, *i*-Pr), 105.0 (C-1), 90.1 (Cq-OTr), 85.8 (C-2), 83.2 (C-4), 78.5 (C-8), 75.2 (C-3), 74.0 (C-5), 72.3 (C-9), 29.3 (C-7), 27.3 (CH_3, *i*-Pr), 26.5 (CH_3, *i*-Pr) ppm. HRMS: calcd for $C_{31}H_{31}NO_6$ $[M + Na]^+$ 536.2044, found 536.2058.

4.1.4. *N*-Propargyl 3-*O*-dodecil-1,2-di-*O*-isopropylidene-5-*O*-trityl-α-D-glucofuranuronamide (**9**), *N*-dodecyl-*N*-propargyl 3-*O*-dodecyl-1,2-di-*O*-isopropylidene-5-*O*-trityl-α-D-glucofuranuronamide (**10**) and 3-*O*-dodecyl-1,2-*O*-isopropylidene-5-C-[5'-methyloxazol-2'-yl]-5-O-trityl-alpha-D-xylofuranose (**11**)

To a solution of *N*-propargyl 1,2-di-*O*-isopropylidene-5-*O*-trityl-α-D-glucofuranuronamide (**8**, 3.06 g, 6.01 mmol) in DMF (30 mL) at 0 °C and under nitrogen atmosphere, NaH (712 mg, 29.7 mmol) was added. The suspension was stirred at 0 °C for 10 min, whereupon dodecyl bromide (2.5 mL, 12.02 mmol) was added. The mixture was left stirring at room temperature for 17 h. It was then diluted with CH2Cl2 , washed with water and brine solution, and the aqueous phase was extracted with dichloromethane (3 ×). The combined organic layers were dried with anhydrous $MgSO_4$ and concentrated. The solvent was evaporated and the residue was subjected to column chromatography (EtOAc/hexane, from 1:12 to 1:8) to afford **9** (719 mg, 18%), **10** (1.939 g, 38%) and **11** (0.174 g, 4%).

Data for **9**: $[\alpha]_D^{20}$ = −16.8 (c = 1.1, in CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.59–7.56 (m, 6 H, Ph), 7.36–7.20 (m, 9 H, Ph), 5.99 (t, $J_{NH,7a}$ = $J_{NH,\ 7b}$ = 5.0 Hz, 1 H, N*H*),

5.94 (d, $J_{1,2}$ = 3.8 Hz, 1 H, H-1), 4.55–4.47 (m, H-2, H-5, 2 H), 4.30 (t, $J_{3,4}$ = $J_{4,5}$ = 4 Hz, 1 H, H-4), 4.28 (t, $J_{4,5}$ = $J_{3,4}$= 4.1 Hz, 1 H, H-4), 3.74 (ddd, $J_{7a,7b}$ = 17.6 Hz, $J_{7a,NH}$ = 6.0 Hz, $J_{,7a,9}$= 2.5 Hz, 1 H, H-7a), 3.33 (ddd, $J_{7a,7b}$ = 17.6 Hz, $J_{7b,NH}$ = 6.0 Hz, $J_{7b,9}$ = 2.5 Hz, 1H, H-7b), 3.63 (d, $J_{3,4}$ = 3.5 Hz, 1H, H-3), 3.57-3.47 (m, $J_{1'a,1'b}$ = 15.3 Hz, $J_{1'a,H2'}$ = 6.8 Hz, 1 H, H-1'), 3.31 (ddd, $J_{7a,7b}$= 17.6 Hz, $J_{7a,NH}$ = 4.2 Hz, $J_{7a,9}$ = 2.6 Hz, 1 H, H-7a), 3.18 (dd, $J_{1'a,1'b}$ = 15.3 Hz, $J_{1'b,2'}$ =6.8 Hz, 1H, H-2'b), 2.10 (t, $J_{7a,9}$ =$J_{7b,9}$ = 2.5 Hz, 1 H, H-9), 1,62 (m, 2 H, H-2'), 1.46 (s, 3 H, C*H*$_3$, *i*-Pr), 1.31 (s, 3 H, C*H*$_3$, *i*-Pr), 1.33-1.18 (m, 18 H, C*H*$_2$-3'– C*H*$_2$-11'), 0.88 (t, $J_{11',12'}$ = 6.7 Hz, 3 H, C*H*$_3$-12') ppm. ^{13}C NMR (101 MHz, $CDCl_3$) : δ = 169.8 (C-6), 144.0 (Cq, Ph), 129.4 (CH, Ph), 128.1 (CH, Ph), 128.0 (CH, Ph), 127.5 (CH, Ph), 111.8 (Cq, *i*-Pr), 104.9 (C-1), 88.7 (Cq, Tr), 83.1 (C-3), 81.5 (C-2), 81.1 (C-4), 79.5 (C-8), 73.5 (C-5), 71.6 (C-9), 70.2 (C-1'), 32.1, 30.0, 29.8, 29.8 , 29.7, 29.5 (C-2'–C-9'), 28.9 (C-7), 27.0 (C-10'), 26.5 (CH_3, *i*-Pr), 26.4 (CH_3, *i*-Pr), 22.8 (C-11'), 14.3 (C-12') ppm. HRMS: calcd for $C_{43}H_{55}NO_6$ $[M + H]^+$ 682.4102, found 682.4130. calcd for $C_{43}H_{55}NO_6$ $[M + Na]^+$ 704.3922, found 704.3948.

Data for **10**: $[\alpha]_D^{20}$ = −15,3 (c = 1.1, in CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.55–7.44 (m, 12 H, C*H*, Ph, both rotamers), 7.31–7.17 (m, 20 H, C*H*, Ph, both rotamers), 5.71, 5.65 (2 d, J_{H1-H2} = 3.7 Hz, 2 H, H-1, H-1′), 4.93 (d, J_{H3-H4} = 7.5 Hz, 1 H, H-3′), 4.91 (d, $J_{3,4}$ = 8.0 Hz, *1* H, H-3), 4.55 (dd, $J_{3,4}$ = 7.5, $J_{4,5}$ = 2.5 Hz, 1 H, H-4), 4.51-4.40 (m, 3 H, H-4′, H-2, H-2′), 4.12 (d, $J_{7a,7b}$ = 17.5 Hz, 1 H, H-7a one rotamer), 3.94 (br. s, 1 H, H-5), 3.86 (br. s, 1H, H-5′), 3.68-3.60 (t, *J* = 6.6 Hz, 2 H, C*H*$_2$-7 other rotamer), 3.54 (d, $J_{7a,7b}$ = 17.5 Hz, 1 H, H-7b one rotamer), 3.41–3.30 (m, 2 H, H-a from OC$\underline{H}_2$-dodec, both rotamers), 3.04-2.95 (m, 2 H, NC$\underline{H}_2$-dodec, one rotamer), 2.90-2.77 (m, 2 H, H-b from OC*H*$_2$-dodec, both rotamers), 2.68-2.52 (br.s, 2 H, NC$\underline{H}_2$-dodec, other rotamer), 2.08, 2.06 (2 t, $J_{7,9}$ = 2.3 Hz, H-9, H-9′), 1.53, 1.51 (2 s, 6 H, 2 × C$\underline{H}_3$, *i*-Pr, one rotamer), 1.38–1.11 (m, 86 H, $^{2\text{-}11}$C$\underline{H}_2$-Ododec, $^{2\text{-}11}$C$\underline{H}_2$-Ndodec, both rotamers, 2 × C$\underline{H}_3$, *i*-Pr, other rotamer), 0.94 – 0.81 (m, 12H, ^{12}C*H*$_3$-Ododec, ^{12}C*H*$_3$-Ndodec, both rotamers) ppm.
^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.9, 169.1 (C-6, C-6'), 144.5, 144.4 (Cq, Ph, both rotamers), 129.8, 129.7, 127.7, 127.3, 127.3 (*C*H, Ph, both rotamers), 111.9, 111.8 (Cq, *i*-Pr, both rotamers), 104.5, 104.4 (C-1, C-1'), 87.86 (Cq from Tr, both rotamers), 82.1 (C-5), 81.9, 81.9, 81.7 (C-5′, C-4, C-4′), 81.1 (C-2, C-2′), 79.3, 79.3 (C-8, C-8′), 71.6, 71.4 (C-9, C-9′), 69.6, 69.3 (O*C*H$_2$-dodec, both rotamers), 67.3 (C-3, C-3′), 46.2, 45.2 (NCH$_2$-dodec, both rotamers), 36.2, 33.5 (C-7, C-7′), 32.1, 29.9, 29.9, 29.8, 29.8, 29.8, 29.7, 29.6, 29.6, 29.5, 29.5, 29.5, 26.6, 27.2, 27.1, 27.0, 27.0, 26.7 26.4, 26.2, 26.2, 22.8 ($^{2\text{-}11}$*C*H$_2$-Ododec, $^{2\text{-}11}$*C*H$_2$-Ndodec, *C*H$_3$, *i*-Pr, both rotamers),14.26 (^{12}*C*H$_3$-Ododec, ^{12}*C*H$_3$-Ndodec, both rotamers) ppm. HRMS: calcd for $C_{55}H_{79}NO_6$ $[M + Na]^+$ 872.5800, found 872.5827.

Data for **11**: $[\alpha]_D^{20}$ = − 45.8 (c = 0.8, in CH_2Cl_2).^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37 (d, *J* = 6.9 Hz, 6 H, C*H*, Ph), 7.30 – 7.14 (m, 9 H, C*H*, Ph), 6.34 (br.s, 1 H, H-4"), 5.71(d, $J_{1,2}$ = 3.6 Hz, 1 H, H-1), 4.94 (d, $J_{4,5}$ = 8.9 Hz, 1 H, H-5), 4.85 (dd, $J_{4,5}$ = 8.9, $J_{3,4}$ = 2.8 Hz, 1

H, H-4), 4.47 (d, $J_{1,2}$ = 3.6 Hz, 1 H, H-2), 4.05 (d, $J_{3,4}$ = 2.8 Hz, 1 H, H-3), 3.32–3.23 (m, 1 H, H-1'a), 3.05-2.96 (m, 1 H, H-1'b), 2.13 (s, 3 H, CH_3), 1.58 (s, 3 H, CH_3-*i*-Pr), 1.37-1.00 (m, 23 H, CH_3-*i*-Pr, CH_2-2'–CH_2-11'), 0.87 (t, $J_{11',12'}$ = 7.0 Hz, 5 H, CH_3-12') ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 161.0 (C-2"), 148.1 (C-5")*, 143.9 (Cq, Tr), 129.4 (*C*H, Ph), 127.5 (*C*H, Ph), 127.0 (*C*H, Ph), 122.7 (C-4"), 112.0 (Cq-isop), 105.0 (C-1), 87.8 (Cq-OTr), 81.62 (C-2), 81.3 (C-4), 81.2 (C-3), 70.0 (C-1'), 65.6 (C-5), 32.1, 29.8, 29.8, 29.8, 29.7, 29.6, 29.5, 27.2 (C-2'–C10') , 26.6 (*C*H_3, *i*-Pr), 26.0 (*C*H_3, *i*-Pr), 22.8 (C-11'), 14.3 (C-12'), 10.9 (*C*H_3) ppm. HRMS: calcd for $C_{43}H_{55}NO_6$ [*M* + H]$^+$ 682.4102, found 682.4116. calcd for $C_{43}H_{55}NO_6$ [*M* + Na]$^+$ 704.3922, found 704.3925.
*inferred by HMBC.

4.1.5. *N*-Propargyl 1,2,4-tri-*O*-acetyl-3-*O*-dodecyl-α,β-D-glucopyranuronamide (**12**)

A solution of *N*-propargyl 3-*O*-dodecil-1,2-di-*O*-isopropylidene-5-*O*-trityl-α-D-glucofuranuronamide (**9**, 626 mg, 0.9 mmol) in aq. TFA (70%, 4.3 mL) was stirred at 40 ºC for 2h. The solvents were co-evaporated with toluene and the residue was dried under vacuum. Pyridine (7 mL) and acetic anhydride (5 mL) were the added to the crude and the resulting mixture was stirred at room temp. for 30 min. After co-evaporation with toluene, the residue was purified by column chromatography (EtOAc/hexane, from 1 : 3.5) to give **9** (343 mg, 75%, two steps, anomeric mixture, α/β ratio, 1 : 0.6) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.72 (t, $J_{NH,7a\alpha}$ = $J_{NH,7b\alpha}$ = 5.1 Hz, 1H, N*H*-α), 6.49 (t, $J_{NH,7a\beta}$ = $J_{NH,7b\beta}$ = 5.1 Hz, 0.6 H, N*H*-β), 6.29 (d, $J_{1,2\ \alpha}$ = 3.4 Hz, 1 H, H-1α), 5.68 (t, $J_{1,2\beta}$ = 6.3 Hz, 0.6 H, H-1β), 5.19-5.10 (m, 1.6 H, H-4α, H-4β), 5.06 (t, $J_{1,2\beta}$ = $J_{2,3\beta}$ = 8.1 Hz, 0.6 H, H-2β), 4.95 (dd, $J_{2,3\alpha}$ = 9.1, $J_{1,2\alpha}$ = 3.4 Hz, 1 H, H-2α), 4.26 (d, $J_{4,5\alpha}$ = 9.1 Hz, 1 H, H-5α), 4.11 – 4.00 (m, $J_{4,5\beta}$ = 9.0 Hz, 2.2 H, H-7a α,β, H-5β), 3.95 (ddd, $J_{7a(\alpha/\beta),7b(\alpha/\beta)}$ = 17.5, $J_{7a(\alpha/\beta),\ NH(\alpha/\beta)}$ = 5.0, $J_{7a(\alpha/\beta),\ 9(\alpha/\beta)}$ =2.5 Hz, 1.6 H, H-7b, α,β), 3.81 (t, $J_{2,3\alpha}$ = $J_{3,4\alpha}$ = 8.8 Hz, 1 H, H-3α), 3.64–3.49 (m, 3.8 H, H-1' α,β, H-3β), 2.24 (t, $J_{7(\alpha/\beta),\ 9(\alpha/\beta)}$ = 2.5 Hz, 1.6 H, H-9 α,β), 2.17 (s, 3 H, CH_3, Ac α), 2.14 (s, 1.8 H, CH_3, Ac, β), 2.12 (s, 3 H, CH_3, Ac, α), 2.11 (s, 1.8 H, CH_3, Ac, β), 2.08 (s, 4.8 H, CH_3, Ac, α, β), 1.52-1.42 (m, 3.2H, CH_2-2' α,β), 1.34 – 1.19 (m, 28.8 H, CH_2-3'–CH_2-11' α,β), 0.87 (t, $J_{11'(\alpha/\beta),12'(\alpha/\beta)}$ = 6.8 Hz, 4.8 H, CH_3-12' α,β) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.8, 169.7, 169.2, 169.2, 169.18 (*C*O, Ac, α,β), 166.8 (C-6α), 166.4 (C-6β), 91.7 (C-1β), 88.7 (C-1α), 79.5 (C-3β), 78.8 (C-8β), 78.8 (C-8α), 76.0 (C-3α), 73.7 (C-5β), 73.2 (C-1'α), 72.8 (C-1'β), 72.2 (C-9 α), 72.2 (C-9 β), 71.6 (C-5α), 71.4 (C-2β), 70.5 (C-2α), 70.4 (C-4β), 70.1 (C-4α), 32.0, 30.2, 30.2, 29.8, 29.8, 29.7, 29.6, 29.6, 29.5 (C-2'–C9' α,β), 29.1 (C-7α), 29.03 (C-7β), 26.1, 26.0, 22.8 (C-10'–C11' α,β), 21.02, 20.97, 20.89, 20.80 (*C*H_3, Ac, α,β), 14.27 (C-12', α,β) ppm. HRMS: calcd for $C_{27}H_{43}NO_9$ [*M* + H]$^+$ 526.3011, found 526.3023. calcd for $C_{27}H_{43}NO_9$ [*M* + Na]$^+$ 548.2830, found 548.2842.

4.1.6. *N*-Dodecyl-*N*-propargyl 1,2,4-tri-*O*-acetyl-3-*O*-dodecyl-α,β-D-glucopyranuronamide (**13**)

A solution of *N*-dodecyl-*N*-propargyl 1,2-di-*O*-isopropylidene-5-*O*-trityl-α-D-glucofuranuronamide (**10**, 182 mg, 0.214 mmol) in aq. TFA (60%, 11 mL) was stirred at room temperature for 16 h. The solvents were co-evaporated with toluene and the residue was dried under vacuum. Pyridine (2 mL) and acetic anhydride (1 mL) were added to the crude and the resulting mixture was stirred at room temp. for 30 min. After co-evaporation with toluene, the residue was purified by column chromatography (EtOAc/hexane, from 1:8) to give **9** (104 mg, 70%, two steps, anomeric mixture, α/β ratio, 1:0.7) as a yellowish solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.33, 6.29 (2 d, $J_{1,2}$ = 3.6 Hz, 2 H, H-1α, H-1′α), 5.61, 5.58 (2 d, 1.4 H, $J_{1,2\,\beta}$ = 8.2, H-1β, H-1′β), 5.56 – 5.43 (m, 3.4 H, H-4α, H-4′α, H-4β, H-4β′α), 5.15 (2 t, 1.4 H, H-2β, H-2′β), 5.02 (m, 2 H, H-2α, H-2′α), 4.63, 4.66 (2 d, $J_{4,5}$ = 10.0 Hz, 2 H, H-5α, H-5′α), 4.44-4.19 (m, 4.8 H, H-5 β, H-5′β, H-7a, H-7′a, α, H-7a, H-7′a, β), 4.12-4.01 (m, 1.4 H, H-7b, H-7′b, β), 3.92 – 3.74 (m, 4 H, H-3α, H-3′α, H-7b, H-7′b, α), 3.67 – 3.27 (m, 15 H, H-3β, H-3′β, NCH_2-dodec, OCH_2-dodec, α, β, both rotamers), 2.32, 2.31 (2 t, $J_{7.9}$ = 2.3 Hz, H-9, H-9′, β), 2.21 (s, 3 H, CH_3, Ac, α), 2.20-2.15 (m, 5 H, H-9, H-9′, α, CH_3, Ac, α), 2.12-2.01 (m, 24.6 H, CH_3, Ac, α, β, both rotamers), 1.69-1.42 (m, 13.6 H, ^{2}CH_2-Ododec, ^{2}CH_2-Ndodec, α, β, both rotamers), 1.37-1.15 (m, 122.4 H, $^{3\text{-}11}$CH_2-Ododec, $^{3\text{-}11}$CH_2-Ndodec, α, β, both rotamers), 0.87 (t, 20.4 H, ^{12}CH_3-Ododec, ^{12}CH_3-Ndodec, α, β, both rotamers) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.7, 169.6, 169.5, 169.2, 169.0, 168.6, 168.6, 168.5, 168.5 (CO, Ac, α, β, both rotamers), 165.2, 165.0 (C-6, C-6′, α), 164.5, 164.3 (C-6, C-6′, β), 92.6, 92.4 (C-1β, C-1′β), 90.0, 89.8 (C-1α, C-1′α), 80.4, 80.3 (C-3β, C-3′β), 79.0, 78.6 (C-8, C-8′, α, β), 77.1 (C-3α, C-3′α), 72.8, 72.7, 72.7, 72.6 (C-3β, C-3′β, C-9 β, C-9′ β), 72.4, 72.2, 72.1, 72.1, 71.9, 71.9 (OCH_2-dodec, both rotamers, α, β, C-5 β, C-5′β), 70.9, 70.8, 70.8, 70.7 (C-2α, C-2′α, C-2β, C-2′β), 70.1, 69.8, 69.8, 69.7 (C-4α, C-4′α, C-4β, C-4′β), 69.5, 69.3 (C-5α, C-5′α), 47.4, 47.1, 47.0, 46.9 (NCH_2-dodec, both rotamers, α, β), 36.8, 36.5, 34.9, 34.9 (C-7, C-7′, α, β), 32.1, 30.3, 30.3, 29.8, 29.8, 29.7, 29.7, 29.6, 29.5, 28.7, 27.3, 27.2, 27.0, 26.9, 26.9, 26.8, 26.1, 26.0, 26.2, 22.8 ($^{2\text{-}11}$CH_2-Ododec, $^{2\text{-}11}$CH_2-Ndodec, both rotamers, α, β), 21.2, 21.0, 21.0, 20.9, 20.8, 20.8 (CH_3, Ac, both rotamers, α, β), 14.3 (^{12}CH_3-Ododec, ^{12}CH_3-Ndodec, both rotamers, α, β) ppm.

4.1.7. *N*-Propargyl 2,4-di-*O*-acetyl-1-(6-cloropurin-9-yl)- 1-deoxy-3-*O*-dodecyl-β-D-glucopyranuronamide (**14**) and *N*-Propargyl 2,4-di-*O*-acetyl-1-(6-cloropurin-7-yl)- 1-deoxy-3-*O*-dodecyl-β-D-glucopyranuronamide (**15**)

Obtained according to the general procedure for nucleobase N-glycosylation, starting from *N*-propargyl 1,2,4-tri-*O*-acetyl-3-*O*-dodecyl-α,β-D-glucopyranuronamide (**12**, 152 mg, 0.29 mmol) and 6-chloropurine (69 mg, 0.45 mmol) and using BSA (0.22 mL, 0.90 mmol) and TMSOTf (0.4 mL, 2.21 mmol). The reaction mixture was exposed to MW irradiation for 1.5 h. Purification by column chromatography (AcOEt/hexane, 1:1)

afforded the N^9 nucleoside **14** (138 mg, 77%) and the N^7 regioisomer **15** (5 mg, 3%) as yellowish oils.

Data for **14**: $[\alpha]_D^{20} = -$ 1.7 (c = 1.2, in CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.72 (s, 1H, H-2), 8.42 (s, 1 H, H-8), 7.32 (br.s, 1 H, N*H*), 5.91 (d, $J_{1',2'}$ = 9.5 Hz, 1 H, H-1'), 5.62 (t, $J_{1',2'}$ = 9.5, $J_{2',3'}$ = 8.9 Hz, 1 H, H-2'), 5.35 (t, $J_{3',4'} \sim J_{4',5'} \sim$ 8.8 Hz, 1 H, H-4'), 4.27 (d, $J_{4',5'}$ = 9.0 Hz, 1 H, H-5'), 4.10-3.88 (m, $J_{7'a,7'b}$ = 17.4 Hz, 2 H, H-7'a, H-7'b), 3.81 (t, $J_{2',3'} \sim J_{3',4'} \sim$ 8.5 Hz, 1 H, H-3'), 3.68-3.50 (m, 2 H, CH_2-1''), 2.19 (t, $J_{7'a,9'} = J_{7'b,9'}$ = 2.4 Hz, 1 H, H-9'), 2.14 (s, 3 H, CH_3, $^{4'}$OAc), 1.82 (s, 3 H, CH_3, $^{2'}$OAc), 1.50-1.41 (m, 2 H, CH_2-2''), 1.20 (m, 18 H, CH_2-3''–CH_2-11''), 0.83 (t, $J_{11',12'}$ = 6.6 Hz, 3 H, CH_3-12') ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.4 (*C*O, $^{4'}$OAc), 168.9 (*C*O, $^{2'}$OAc), 166.0 (C-6'), 152.6 (C-2), 151.5 (C-4), 143.5 (C-8), 131.3 (C-5), 80.8 (C-1'), 80.02 (C-3'), 78.9 (C-8'), 75.6 (C-5'), 73.2 (C-1''), 72.1 (C-9'), 71.3 (C-2'), 70.5 (C-4'), 31.9, 30.1, 29.7, 29.7, 29.6, 29.5, 29.4, 29.0, 26.0, 22.7 (C-7', C-3''–C11''), 20.97 (*C*H_3, $^{4'}$OAc), 20.40 (*C*H_3, $^{2'}$OAc), 14.2 (C-12'') ppm. HRMS: calcd for $C_{30}H_{42}ClN_5O_7$ [*M* +H]$^+$ 620.2846, found 620.2862.

Data for **15**: $[\alpha]_D^{20}$ = + 16.4 (c = 1.2, in CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.92 (s, 1 H, H-2), 8.72 (s, 1 H, H-8), 6.77 (br.s, 1 H, N*H*), 6.17 (br.s, 1 H, H-1'), 5.69 (t, $J_{1',2'} = J_{2',3'}$ = 8.4 Hz, 1 H, H-2'), 5.34 (t, $J_{3',4'} \sim J_{4',5'} \sim$ 8.8 Hz, 1 H, H-4'), 4.26 (d, $J_{4',5'}$ = 9.2 Hz, 1 H, H-5'), 4.11-3.88 (m, $J_{7'a,7'b}$ = 17.4 Hz, 2 H, CH_2-7'), 3.84 (t, $J_{2',3'} \sim J_{3',4'} \sim$ 8.4 Hz, 1 H, H-3'), 3.70–3.56 (m, 2 H, CH_2-1''), 2.19 (br.s, 1 H, H-9'), 2.15 (s, 3 H, CH_3, Ac), 1.94 (s, 3 H, CH_3, Ac), 1.55–1.43 (m, 2 H, CH_2-2''), 1.35–1.09 (m, 18 H, CH_2-3''—CH_2-11''), 0.87 (t, $J_{11',12'}$ = 6.8 Hz, 3 H, CH_3-12') ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.6, 169.1 (2 ×*C*O, Ac), 165.6 (C-6'), 162.3 (C-4), 158.8 (C-6), 153.3 (C-2), 143.0 (C-8), 122.2 (C-5), 80.4 (C-3'), 78.6 (C-8'), 75.5 (C-5'), 73.6 (C-1''), 72.3 (C-9'), 70.7 (C-2'), 70.5 (C-4'), 32.0, 30.2, 29.8, 29.7, 29.7, 29.5, 29.5, 29.1, 26.0, 22.8 (C-7', C-3''–C11''), 21.0, 20.6 (2 × CH_3, Ac), 14.3 (C-12'') ppm. HRMS: calcd for $C_{30}H_{42}ClN_5O_7$ [*M* − H]$^-$ 618.2700, found 618.2708.

4.1.8. *N*-Dodecyl-*N*-Propargyl 1-(6-cloropurin-9-yl)-2,4-di-*O*-acetyl-1-deoxy-3-*O*-dodecyl-β-D-glucopyranuronamide (**16**) and *N*-dodecyl-*N*-propargyl 1-(6-chloropurin-7-yl)-2,4-di-*O*-acetyl-1-deoxy-3-*O*-dodecyl-β-D-glucopyranuronamide (**17**)

Obtained according to the general procedure for nucleobase N-glycosylation, starting from *N*-dodecyl-*N*-propargyl 1,2,4-tri-*O*-acetyl-3-*O*-dodecyl-α,β-D-glucopyranuronamide (**13**, 50 mg, 0.072 mmol) and 6-chloropurine (39 mg, 0.25 mmol) and using BSA (0.1 mL, 0.41 mmol) and TMSOTf (0.13 mL, 0.71 mmol). The reaction mixture was exposed to MW irradiation for 1.5 h. Purification by column chromatography (AcOEt/hexane, 1:8 to 1:4) afforded the N^9 nucleoside **16** (14 mg, 27%) and the N^7 regioisomer **17** (5 mg, 10%) as colorless oils.

Data for **16**: $[\alpha]_D^{20}$ = − 7.9 (c = 1.0, in CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.77, 8.55 (2 br.s, 1.8 H, H-2-purina), 8.40 (br.s., 1.8 H, H-8-purina) 5.92 (2 d, 1.8 H, $J_{1,2}$ = 9.3 Hz, 1.8 H, H-1, H-1′), 5.69-5.57 (m, 3.6 H, H-2, H-2′, H-4, H-4′), 4.53, 4.48 (2 d, $J_{4,5}$ = 9.6 Hz, 1.8 H, H-5, H-5′), 4.29, 4.24 (2 dd, 1.8 H, H-7a, H-7′a), 3.99, 3.92 (2 dd, $J_{7a,7b}$, = 17.4 Hz, $J_{7b,9}$ = 2.1 Hz, 1.8 H, H-7b, H-7′b), 3.85 (t, 1.8 H, H-3, H-3′), 3.65-3.55 (m, 3.6 H, OCH_2-dodec, both rotamers), 3.50-3.29 (m, 3.6 H, NCH_2-dodec, both rotamers), 2.16, 2.14 (2 t, $J_{7,9}$ = 2.1 Hz, 1.8 H, H-9, H-9′), 2.08, 1.86, 1.84 (3s, 10.8 H, CH_3, OAc, both rotamers), 1.75-1.40 (m, 7.2 H, ^{2}CH_2-Ododec, ^{2}CH_2-Ndodec, both rotamers), 1.35-1.15 (m, 64.8 H, $^{3\text{-}11}$CH_2-Ododec, $^{3\text{-}11}$CH_2-Ndodec, both rotamers), 0.87 (t, J = 6.8 Hz, 10.8 H, ^{12}CH_3-Ododec, ^{12}CH_3-Ndodec, both rotamers) ppm.
^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.0, 168.9, 168.7, 168.5 (CO, Ac, both rotamers), 164.2, 164.0 (C-6, C-6′), 152.5, 152.5 (C-2-purine, C-2′-purine), 143.4 (C-8-purin, C-8′-purin), 81.0, 81.1, 80.8 (C-1, C-1′,C-3, C-3′), 78.4, 78.2 (C-8, C-8′), 75.1, 74.1 (C-5, C-5′), 72.8, 72.3 (O*C*H$_2$-dodec, both rotamers), 72.9 (C-9, C-9′), 70.9 (C-2, C-2′), 70.0, 69.9 (C-4, C-4′), 47.3, 47.3 (N*C*H$_2$-dodec, both rotamers), 37.2, 35.0 (C-7, C-7′), 32.1, 30.3, 29.8, 29.8, 29.7, 29.7, 29.7, 29.6, 29.5, 29.4, 28.9, 27.0, 26.9, 26.9, 26.9, 26.1, 22.8 ($^{2\text{-}11}$CH$_2$-Ododec, $^{2\text{-}11}$*C*H$_2$-Ndodec, both rotamers), 21.0, 20.5 (*C*H$_3$, OAc, both rotamers), 14.3 (^{12}*C*H$_3$-Ododec, ^{12}*C*H$_3$-Ndodec, both rotamers) ppm. HRMS: calcd for $C_{42}H_{66}ClN_5O_7$ $[M + H]^+$ 788.4724, found 788.4737. calcd for $C_{42}H_{66}ClN_5O_7$ $[M + Na]^+$ 810.4543, found 810.4554.

Data for **17**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.93 (2 br.s, 1.8 H, H-2-purina), 8.63, 8.63 (br.s., 1.8 H, H-8-purina) 6.27, 6.24 (2 d, 1.8 H, $J_{1,2}$ = 9.3 Hz, 1.8 H, H-1, H-1′), 5.73-5.55 (m, 3.6 H, H-2, H-2′, H-4, H-4′), 4.58, 4.52 (2 d, $J_{4,5}$ = 9.5 Hz, 1.8 H, H-5, H-5′), 4.32, 4.21 (2 dd, 1.8 H, $J_{7a,7b}$, = 17.4, $J_{7a,9}$ = 2.1, H-7a, H-7′a), 3.99 (dd, 1.8 H, H-7b, H-7′b), 3.86 (t, 1.8 H, H-3, H-3′), 3.67-3.55 (m, 3.6 H, OCH_2-dodec, both rotamers), 3.52-3.26 (m, 3.6 H, NCH_2-dodec, both rotamers), 2.18, 2.13 (2 t, $J_{7,9}$ = 2.1 Hz, 1.8 H, H-9, H-9′), 2.08, 1.93, 1.92 (3s, 10.8 H, CH_3, OAc, both rotamers), 1.78-1.42 (m, 7.2 H, ^{2}CH_2-Ododec, ^{2}CH_2-Ndodec, both rotamers), 1.35-1.15 (m, 64.8 H, $^{3\text{-}11}$CH_2-Ododec, $^{3\text{-}11}$CH_2-Ndodec, both rotamers), 0.87 (t, J = 6.8, 10.8 H, ^{12}CH_3-Ododec, ^{12}CH_3-Ndodec, both rotamers) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 168.9, 168.9, 168.5, 168.3 (CO, Ac, both rotamers), 164.3, 164.1 (C-6, C-6′), 153.1 (C-2-purine, C-2′-purine), 147.6 (C-8-purin, C-8′-purin), 82.9 (C-1, C-1′), 80.9, 80.8 C-3, C-3′), 78.1 (C-8, C-8′), 74.6, 73.7 (C-5, C-5′), 73.0, 72.4 (O*C*H$_2$-dodec, both rotamers), 72.1 (C-9, C-9′), 70.8, 70.3, 70.1 (C-2, C-2′, C-4, C-4′), 47.5, 47.3 (N*C*H$_2$-dodec, both rotamers), 37.3, 35.2 (C-7, C-7′), 32.0, 30.3, 29.8, 29.8, 29.7, 29.7, 29.7, 29.6, 29.5, 28.9, 27.1, 26.9, 26.1, 22.8 ($^{2\text{-}11}$*C*H$_2$-Ododec, $^{2\text{-}11}$*C*H$_2$-Ndodec, both rotamers), 20.9, 20.6 (*C*H$_3$, OAc, both rotamers), 14.3 (^{12}*C*H$_3$-Ododec, ^{12}*C*H$_3$-Ndodec, both rotamers) ppm.

4.1.9. *N*-Dodecyl-*N*-propargyl 2,4-di-*O*-acetyl-3-*O*-dodecyl-1-deoxy-1-(uracil-1-yl)-β-D-glucopyranuronamide (**18**)

Obtained according to the general procedure for nucleobase N-glycosylation, starting from *N*-dodecyl-*N*-propargyl 1,2,4-tri-*O*-acetyl-3-*O*-dodecyl-α,β-D-glucopyranuronamide (**13**, 21 mg, 0.03 mmol) and uracil (9 mg, 0.08 mmol) and using BSA (0.03 mL, 0.12 mmol) and TMSOTf (0.06 mL, 0.33 mmol). The reaction mixture was exposed to MW irradiation for 1.5 h. Purification by column chromatography (AcOEt/hexane, 5:1) afforded **18** (12 mg, 52%) as colorless oil. $[\alpha]_D^{20}$ = + 2.6 (c = 0.8, in CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.67 (br.s, 0.8 H, N*H*), 8.54 (br.s., 1 H, N*H*) 7.41 (2 d, $J_{H5\text{-}H6}$ = 7.7 Hz, 1.8 H, H-6-urac, H-6′-urac), 5.87-5.74 (m, 3.6 H, H-1, H-1′, H-5-urac, H-5′-urac), 5.51 (2 t, $J_{3,4} \sim J_{4,5} \sim$ 9.3 Hz, 1.8 H, H-4, H-4′), 5.19, 5.17 (2 t, $J_{1,2} = J_{2,3}$ = 9.2 Hz, 1.8 H, H-2, H-2′), 4.44-4.29 (m, $J_{4,5}$ = 9.3 Hz, 2.8 H, H-5, H-5′, H-7a), 4.15 (dd, $J_{7'a,7'b}$ = 18.4, $J_{7'a,9'}$ = 2.3, 0.8 H, H-7'a), 4.03. 3.96 (2 dd, 1.8 H, H-7b, H-7′b), 3.73 (2 t, $J_{2,3} = J_{3,4}$ = 9.2 Hz, 1.8 H, H-3, H-3′), 3.69-3.50 (m, 3.6 H, OCH_2-dodec, both rotamers), 3.46-3.30 (m, 3.6 H, NCH_2-dodec, both rotamers), 2.28, 2.20 (2 t, $J_{H9\text{-}H7}$ = 2.3 Hz, 1.8 H, H-9, H-9′), 2.05 (s, 10.8 H, CH_3, OAc, both rotamers), 1.75-1.43 (m, 7.2 H, ^{2}CH_2-Ododec, ^{2}CH_2-Ndodec, both rotamers), 1.37-1.15 (m, 64.8 H, $^{3\text{-}11}$CH_2-Ododec, $^{3\text{-}11}$CH_2-Ndodec, both rotamers), 0.87 (t, J = 6.8, 10.8 H, ^{12}CH_3-Ododec, ^{12}CH_3-Ndodec, both rotamers) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.4, 169.4, 168.5, 164.4 (CO, Ac, both rotamers), 164.4, 164.3 (C-6, C-6′), 162.4, 162.2 (C-4-urac, C-4′-urac), 150.7, 150.4 (C-2-urac, C-2′-urac), 139.9, 138.8 (C-6-urac, C-6′-urac), 103.7, 103.6 (C-5-urac, C-5′-urac), 81.0, 80.9 (C-1, C-1′), 80.8, 80.7 (C-3, C-3′), 78.6, 78.3 (C-8, C-8′), 73.9, 73.2 (C-5, C-5′), 73.1, 73.1 (O*C*H$_2$-dodec, both rotamers), 72.9, 72.3 (C-9, C-9′), 70.3, 70.1 (C-2, C-2′), 70.0, 69.8 (C-4, C-4′), 47.3, 47.2 (N*C*H$_2$-dodec, both rotamers), 37.3, 35.0 (C-7, C-7′), 32.1, 30.3, 29.8, 29.8, 29.7, 29.6, 29.5, 26.9, 26.9, 26.1, 22.8 ($^{2\text{-}11}$CH$_2$-Ododec, $^{2\text{-}11}$*C*H$_2$-Ndodec, both rotamers), 21.0, 20.9, 20.7, 20.7 (*C*H$_3$, OAc, both rotamers), 14.3 (^{12}*C*H$_3$-Ododec, ^{12}*C*H$_3$-Ndodec, both rotamers) ppm. HRMS: calcd for $C_{41}H_{67}N_3O_9$ [*M* + H]$^+$ 746.4950, found 746.4973.

4.1.10. General procedure for "click"-1,3-dipolar cycloaddition between tri-*O*-acetylated *N*-propargyl 1-purinyl glucuronamide nucleosides and 6-azido-6-deoxy-α-D-glucopyranoside

To a solution of *N*-propargyl 1-purinyl-2,3,4-tri-*O*-acetyl-β-D-glucopyranuronamide (53 μmol) in acetonitrile (6 mL), methyl 6-azido-6-deoxy-α-D-glucopyranoside (**19**, 1,1 equiv.) and CuI/Amberlyste A21 [30] (0.5 mmol CuI.g^{-1}, 250 mg) were added. The suspension was gently stirred at room temperature for 6 days. The catalyst was filtered off and the solvent was evaporated. The residue was subjeted to column chromatography.

4.1.10.1. *N*-[1-(Methyl α-D-glucopyranosid-6-yl)-1*H*-1,2,3-triazol-4-yl]metyl 1-(6-dimethylaminopurin-9-yl)-2,3,4-tri-*O*-acetil-β-D-glucopyranuronamide (**20**) and N-[5-

iodo-1-(methyl-α-D-glucopyranosid-6-yl)-1*H*-1,2,3-triazol-4-yl]methyl 1-(6-dimethylaminopurin-9-yl)-2,3,4-tri-O-acetyl-β-D-glucopyranuronamide (**21**)

Obtained according to the general procedure starting from *N*-propargyl-1-(6-chloropurin-9-yl)-2,3,4-tri-*O*-acetyl-β-D-glucopyranuronamide (**4**, 75 mg, 0.15 mmol), methyl 2,3,4-O-acetyl-6-azido-6-deoxy-α-D-glucopyranoside (**19**, 35 mg, 0.16 mmol). Purification by column chromatography (AcOEt to AcOEt/methanol, 7:1) afforded **20** (31 mg, 29%) as yellow solid and its 5-iodo derivative **21** (29 mg, 23%) as yellow oil.

Data for **20**: m.p.: 132-134 ºC. $[\alpha]_D^{20}$ = + 42.9 (c = 0.8, in MeOH). ^{1}H NMR (400 MHz, MeOD): δ = 8.25 (s, 1 H, H-8), 8.23 (s, 1 H, H-2), 7.92 (s, 1 H, H-9'), 6.06 (d, $J_{1',2'}$ = 9.4 Hz, 1H, H-1'), 5.82 (t, $J_{1',2'}$ = $J_{2',3'}$ = 9.2 Hz, 1 H, H-2'), 5.57 (t, $J_{2',3'}$ = $J_{3',4'}$ = 9.2 Hz, 1 H, H-3'), 5.43 (t, $J_{3',4'}$ ~ $J_{4',5'}$ ~ 9.3 Hz, 1 H, H-4'), 4.77 (d, $J_{6a'',6b''}$ = 14.1 Hz, 1 H, H-6"a), 4.56 (d, $J_{1'',2''}$ = 3.6 Hz, 1 H, H-1"), 4.54-4.32 (m, 3 H, H-5', C*H*$_2$-7', H-6b"), 3.80 (t, $J_{4'',5''}$ = $J_{5'',6a''}$ =8.3 Hz, 1 H, H-5"), 3.59 (t, $J_{3'',4''}$ = $J_{2'',3''}$ = 9.2 Hz, 1 H, H-3"), 3.57-3.37 (m, 6 H, N(C*H*$_3$)$_2$), 3.33 (dd, 1 H, H-2"), 3.12–3.05 (m, 3 H, H-4", OC*H*$_3$), 2.02 (s, 6 H, 2 × C*H*$_3$, Ac), 1.74 (s, 3 H, C*H*$_3$, Ac) ppm. ^{13}C NMR (101 MHz, MeOD): δ = 171.3, 171.1, 170.5 (3 × *C*O, Ac), 168.2 (C-6'), 156.1 (C-6), 153.5 (C-2), 151.3 (C-4), 139.1 (C-8), 120.9 (C-5), 101.2 (C-1"), 81.80 (C-1'), 76.9 (C-5'), 74.9 (C-3"), 73.9 (C-3'), 73.3 (C-2"), 72.9 (C-4"), 71.6 (C-5"), 71.2 (C-2'), 70.7 (C-4'), 55.7 (O*C*H$_3$), 52.5 (C-6"), 39.1 (N(*C*H$_3$)$_2$), 35.6 (C-7'), 20.6, 20.5, 20.0 (3 × *C*H$_3$, Ac) ppm. HRMS: calcd for $C_{29}H_{39}N_9O_{13}$ [*M* + H]$^+$ 722.2740, found 722.2759.

Data for **21**: $[\alpha]_D^{20}$ = + 43.6 (c = 0.4, in MeOH). ^{1}H NMR (400 MHz, MeOD): δ = 8.27 (s, 1 H, H-8), 8.21 (s, 1 H, H-2), 6.03 (d, $J_{1',2'}$ = 9.5 Hz, 1 H, H-1'), 5.80 (t, $J_{1',2'}$ ~ $J_{2',3'}$ ~ 9.3 Hz, 1 H, H-2'), 5.56 (t, $J_{2',3'}$ ~ $J_{3',4'}$ ~ 9.3 Hz, 1 H, H-3'), 5.45 (t, $J_{3',4'}$ ~ $J_{4',5'}$ ~ 9.6 Hz, 1 H, H-4'), 4.77 (dd, $J_{6a'',6b''}$ = 14.2 Hz, $J_{6a'',5''}$ = 2.5 Hz, 1 H, H-6"a), 4.55–4.39 (m, 4 H, H-5', H-7'a, H-1", H-6"b), 4.35 (d, 1 H, H-7'b, $J_{7'a,7'b}$ = 15.2), 3.99 (td, $J_{4'',5''}$ = $J_{5'',6a''}$ = 9,3 Hz, $J_{5'',6b''}$ =$_=$ 2,7 Hz, 1 H, H-5"), 3.59 (t, $J_{3'',4''}$ ~$J_{2'',3''}$ ~ 9,3 Hz, 1 H, H-3"), 3.58-3.37 (m, 6 H, N(C*H*$_3$)$_2$), 3.35 (dd, $J_{2'',3''}$ = 9.7 Hz, $J_{1'',2''}$ =3.8 Hz, 1 H, H-2''), 3.26 (t, $J_{3'',4''}$ = $J_{4'',5''}$ = 9.3 Hz, 1 H, H-4"), 2.97 (s, 3 H, OC*H*$_3$), 2.05, 2.01, 1.75 (3 × s, 3× 3 H, 2 × C*H*$_3$, Ac) ppm. ^{13}C NMR (101 MHz, MeOD): δ = 171.4, 170.9, 170.5 (3 × *C*O, Ac), 168.3 (C-6'), 156.1 (C-6), 153.5 (C-2), 151.3 (C-4), 139.1 (C-8), 120.8 (C-5), 100.9 (C-1"), 83.6 (C-8'), 81.8 (C-1') 76.8 (C-5'), 74.9 (C-3"), 73.9 (C-3'), 73.6 (C-4"), 73.3 (C-2"), 71.6 (C-5"), 71.2 (C-2'), 70.7 (C-4'), 55.4 (O*C*H$_3$), 52.8 (C-6"), 38.7 (N(*C*H$_3$)$_2$)*, 36.1 (C-7'), 20.7, 20.5, 20.0 (3 × *C*H$_3$, Ac) ppm. HRMS: calcd for $C_{29}H_{38}IN_9O_{13}$ [*M* + H]$^+$ 848.1707, found 848.1722. *inferred by HSQC

4.1.10.2. *N*-[1-(Methyl α-D-glucopyranosid-6-yl)-1*H*-1,2,3-triazol-4-yl]metyl 1-(2-acetamido-6-dimethylaminopurin-9-yl)-2,3,4-tri-*O*-acetil-β-D-glucopyranuronamide (**22**) and N-[5-iodo-1-(methyl-α-D-glucopyranosid-6-yl)-1*H*-1,2,3-triazol-4-yl]methyl 1-

(2-acetamido-6-dimethylaminopurin-9-yl)-2,3,4-tri-O-acetyl-β-D-glucopyranuronamide (**23**)

Obtained according to the general procedure starting from *N*-propargyl-1-(2-acetamido-6-chloropurin-9-yl)-2,3,4-tri-*O*-acetyl-β-D-glucopyranuronamide (**6**, 29 mg, 0.052 mmol), methyl 2,3,4-O-acetyl-6-azido-6-deoxy-α-D-glucopyranoside (**19**, 13 mg, 0.06 mmol). Purification by column chromatography (AcOEt to AcOEt/methanol, 7:1) afforded **22** (11 mg, 27%) and its 5-iodo derivative **23** (4 mg, 9%) as yellow oils.

Data for **22**: $[\alpha]_D^{20}$ = + 27.4 (c = 1.1, in MeOH). ^{1}H NMR (400 MHz, MeOD): δ = 8.17 (s, 1 H, H-8), 7.82 (s, 1 H, H-9'), 6.23 (d, $J_{1',2'}$ = 9.1 Hz, 1 H, H-1'), 5.79 (t, $J_{1',2'}$ ~ $J_{2',3'}$ ~ 9.6 Hz, 1 H, H-2'), 5.56 (t, $J_{2',3'}$ ~ $J_{3',4'}$ ~ 9.5 Hz, 1 H, H-3'), 5.36 (t, $J_{3',4'}$ ~ $J_{4',5}$ ~ 9.8 Hz, 1 H, H-4'), 4.77 (dd, $J_{6''a,6''b}$ = 14.3, $J_{5'',6''a}$ = 2.2 Hz, 1 H, H-6"a), 4.63 (s, 2 H), 4.56 (d, J = 3.7 Hz, 1H, H-1"), 4.51-4.38 (m, 4 H, H-5', H-6"b, CH_2-7'), 3.79 (ddd, $J_{4",5"}$ = 9.9 Hz, $J_{5",6"a}$ = 2.2 Hz, $J_{5",6"b}$ = 8.3 Hz, 1 H, H-5"), 3.58 (t, $J_{2",3"}$ ~ $J_{3",4"}$ ~ 9.2 Hz, 1H, H-3"), 3.54-3.36 (m, 6 H, N(CH3)$_2$), 3.34 (dd, $J_{1",2"}$ = 3.7 Hz, $J_{2",3"}$ = 9.7 Hz, 1 H, H-2"), 3.12-3.04 (m, 4 H, OCH_3, H-4"), 2.29 (CH_3, NHAc), 2.03, 2.01, 1.77 (3 × s, 3× 3 H, 2 × CH_3, Ac) ppm. ^{13}C NMR (101 MHz, MeOD): δ = 171.3, 171.1, 170.7 (3 × *C*O, Ac), 168.7 (C-6') 156.2, 154.0 (C-2, C-6), 152.9 (C-4), 145.6 (C-8')*, 138.4 (C-8), 125.8 (C-9'), 117.7 (C-5), 101.2 (C-1"), 81.0 (C-1'), 76.4 (C-5'), 74.90 (C-3"), 73.9 (C-3'), 73.3 (C-2"), 72.9 (C-4"), 74.7 (C-5"), 71.1 (C-2'), 70.80 (C-4'), 55.63 (OCH_3), 52.4 (C-6"), 38.9 (N(CH_3)$_2$), 35.5 (C-7), 23.7 (NHCOCH_3), 20.6, 20.5, 20.1 (3 × CH_3, Ac) ppm. HRMS: calcd for $C_{31}H_{42}N_{10}O_{14}$ [M + H]$^+$ 779.2955, found 779.2956.
* inferred by HMBC

Data for **23**: $[\alpha]_D^{20}$ = + 32.1 (c = 0.3, in MeOH). ^{1}H NMR (400 MHz, MeOD): δ = 8.17 (s, 1 H, H-8), 6.20 (d, $J_{1',2'}$ = 9.4 Hz, 1 H, H-1'), 5.78 (t, $J_{1',2'}$ ~ $J_{2',3'}$ ~ 9.4 Hz, 1 H, H-2'), 5.54 (t, $J_{2',3'}$ ~ $J_{3',4'}$ ~ = 9.5 Hz, 1 H, H-3'), 5.39 (t, $J_{3',4'}$ ~ $J_{4',5'}$ ~ 9.7 Hz, 1 H, H-4'), 4.77 (dd, $J_{6''a,6''b}$ = 14.2 Hz, $J_{5'',6''a}$ = 2.7 Hz, 1 H, H-6"a), 4.54 – 4.39 (m, 5 H, H-1", H-6"b, H-5', H-7'a), 4.35 (d, 1 H, $J_{7'a,7'b}$ = 15.2 Hz, H-7'b), 3.99 (td, $J_{4",5"}$ = $J_{5",6"b}$ = 9.3 Hz, $J_{5",6"a}$ = 2.7 Hz, 1 H, H-5"), 3.59 (t, 1 H, $J_{2",3"}$ ~ $J_{3",4"}$ = 9.2 Hz, H-3"), 3.55-3.32 (m, 6 H, N(CH_3)$_2$), 3.35 (dd, $J_{1",2"}$ = 3.8 Hz, $J_{2",3"}$ = 9.5 Hz, 1 H, H-2"), 3.25 (dd, 1 H, $J_{3",4"}$ ~ $J_{4",5"}$ = 9.3 Hz, H-4''), 2.97 (s, 3 H, OCH_3), 2.32 (s, 3 H, CH_3, NHAc), 2.04, 2.01, 1.77 (3 × s, 3× 3 H, 2 × CH_3, Ac) ppm. ^{13}C NMR (101 MHz, MeOD): δ = 171.4, 171.0, 170.6 (3 × *C*O, Ac), 168.4 (C-6'), 152.9 (C-4), 148.4 (C-8'), 138.4 (C-8), 117.6 (C-5), 100.9 (C-1"), 83.1 (C-8'), 81.1 (C-1'), 76.4 (C-5'), 75.0 (C-3''), 74.1 (C-3'), 73.6 (C-2''), 73.4 (C-4''), 71.6 (C-5''), 71.2 (C-2'), 70.8 (C-4'), 55.4 (OC$\underline{H}_3$), 52.8 (C-6''), 36.1 (C-7), 24.8 (NHCO$\underline{C}$H$_3$), 20.7 (OCO$\underline{C}$H$_3$), 20.5 (OCO$\underline{C}$H$_3$), 20.1 (OCO$\underline{C}$H$_3$). HRMS: calcd for $C_{31}H_{41}IN_{10}O_{14}$ [M + H]$^+$ 905.1921, found 905.1937.

4.1.11. *N*-Propargyl 2,4-di-*O*-acetyl-1-azido-1-deoxy-3-*O*-dodecyl-α,β-D-glucopyranuronamide (**26α, β**)

To a solution of *N*-propargyl 1,2,4-tri-*O*-acetyl-3-*O*-dodecyl-α,β-D-glucopyranuronamide (12, 376 mg, 0.715 mmol) in acetonitrile (25 mL), and under nitrogen atmosphere, trimethylsilyl azide (0.85 mL, 6.4 mmol) was added, followed by dropwise addition of TMSOTf (1.04 mL, 5.75 mmol). The mixture was stirred under reflux for 24 h. It was then diluted with DCM and neutralized with sat. aq. $NaHCO_3$ soln. The aqueous phase was extracted with dichloromethane (3×) and the combined organic phases were dried over anhydrous $MgSO_4$. After filtration and concentration under vacuum, the residue was purified by column chromatography (EtOAc/hexane, 3:7) to give **26** (168 mg, 46%, α/β ratio, 1:0.6) as white solid.

Data for **26α**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.46 (t, $J_{NH\text{-}CH2}$ ~ 5.2 Hz, 1 H, N*H*), 5.64 (d, $J_{1',2'}$ = 4.3 Hz, 1 H, H-1), 5.01 (t, $J_{3,4}$ ~ $J_{4,5}$, 1 H, H-4), 4.79 (dd, $J_{2,3}$ = 9.8 Hz, 1 H, H-2), 4.29 (d, $J_{4,5}$ = 10.3 Hz, 1 H, H-5), 4.11 (ddd, $J_{7a,7b}$ = 17.6, $J_{7'a,NH}$ = 5.6, $J_{7'\text{-}a,9}$ = 2.6, 1 H, H-7a), 3.94 (ddd, $J_{7b,NH}$ = 4.8, $J_{7\text{-}b,9}$ = 2.5, H-7b, 1 H), 3.70 (t, $J_{2,3}$ ~ $J_{3,4}$ = 9.5 Hz, 1 H, H-3), 5.24 3.64-3.52 (m, 2 H, CH_2-1'), 2.26 (t, *J* = 2.6, H-9), 2.14, 2.11 (2 s, 2 × 3 H, 2 × C*H*$_3$, Ac), 1.52–1.41 (m, 2 H, CH_2-2'); 1.34-1.17 (m, 18 H, C*H*$_2$-3'—C*H*$_2$-11''), 0.87 (t, *J* = 6.6 Hz, 3 H, C*H*$_3$-12'). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.2, 169.7 (2 × *C*O, Ac), 166.7 (C-6'), 86.3 (C-1'), 78.8 (C-8), 76.2 (C-3), 73.7 (*C*H_2-1'), 72.3 (C-2), 71.2 (C-9), 70.8 (C-4), 70.5 (C-5), 32.1, 30.3, 29.8, 29.7, 29.6, 29.5, 27.0, 22.8 (C-2'—C9', C-7), 26.1 (C-10'), 22.8 (C-11'), 21.1, 20.9 (2 × *C*H_3, Ac), 14.3 (C-12') ppm. HRMS: calcd for $C_{25}H_{40}N_4O_7$ [M + H]+ 509.2970, found 509.2955. calcd for $C_{25}H_{40}N_4O_7$ [M + Na]+ 531.2789, found 531.2782. calcd for $C_{25}H_{40}N_4O_7$ [M + K]+ 547.2529, found 547.2516.

Data for **26β**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.56 (t, $J_{NH\text{-}CH2}$ ~ 5.1 Hz, 1 H, N*H*), 5.07 (t, $J_{3,4}$ ~ $J_{4,5}$ ~ 9.2, 1 H, H-4), 4.90 (t, $J_{2,3}$ ~ $J_{1,2}$ ~ 8.9 Hz, 1 H, H-2), 4.63 (d, $J_{1',2'}$ = 8.9 Hz, 1 H, H-1), 4.12 (ddd, $J_{7a,7b}$ = 17.5, $J_{7'a,NH}$ = 5.8, $J_{7'\text{-}a,9'}$ = 2.5, 1 H, H-7'a), 3.99-3.90 (m, $J_{4,5}$ = 9.6 Hz, $J_{7b,NH}$ = 5.0, $J_{7'\text{-}b,9'}$ = 2.6, 2 H, H-5, H-7b), 3.61-3.46 (m, 3 H, H-3, CH_2-1'), 2.26 (t, *J* = 2.5, H-9), 2.12, 2.10 (2 s, 2 × 3 H, 2 × C*H*$_3$, Ac), 1.50–1.40 (m, 2 H, CH_2-2'); 1.34-1.18 (m, 18 H, C*H*$_2$-3'—C*H*$_2$-11''), 0.87 (t, *J* = 6.7 Hz, 3 H, C*H*$_3$-12'). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.6, 169.2 (2 × *C*O, Ac), 166.2 (C-6'), 88.3 (C-1'), 79.7 (C-3), 78.7 (C-8), 74.8 (C-5), 73.0 (*C*H_2-1'), 72.2 (C-9), 71.7 (C-2), 70.7 (C-4), 32.0, 30.2, 29.8, 29.7, 29.7, 29.5, 29.5, 29.1, 27.7, 22.8 (C-2'—C9', C-7), 26.0 (C-10'), 22.8 (C-11'), 21.0, 20.9 (2 × *C*H_3, Ac), 14.3 (C-12') ppm. HRMS: calcd for $C_{25}H_{40}N_4O_7$ [M + H]+ 509.2970, found 509.2951. calcd for $C_{25}H_{40}N_4O_7$ [M + Na]+ 531.2789, found 531.2780. calcd for $C_{25}H_{40}N_4O_7$ [M + K]+ 547.2529, found 547.2515.

4.1.12. N^9-Propargyl-6-chloropurine (**28**) and N^7-propargyl-6-chloropurine (**29**)

To a solution of 6-chloropurine (500 mg, 3.24 mmol) in DMF (25 mL) under nitrogen atmosphere, potassium carbonate (450 mg, 3.24 mmol) was added, which was followed by dropwise addition of propargyl bromide (0.25 mL, 3.3 mmol). The mixture was stirred at room temperature for 48h. The solvent was evaporated under reduced pressure and the crude residue was subjected to column chromatography (AcOEt/hexane, from 3:7 to 1:1) to afford the N^9-propargyl derivative **28** (270 mg, 43%) and its N^7-regioisomer $_{29}$ (86 mg, 14%) as white solids.

NMR data for **28**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.76 (s, 1 H, H-2), 8.34 (s, 1 H, H-8), 5.07 (d, J = 2.6 Hz, 2 H, CH_2-1′), 2.59 (t, J = 2.5 Hz, 1 H, H-3′) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 152.3 (C-2), 151.4 (C-6), 144.4 (C-8), 131.7 (C-5), 76.0 (C-2′), 75.1 (C-3′), 33.8 (C-1′) ppm.

NMR data for **29:** ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.88 (s, 1 H, H-2), 8.49 (s, 1 H, H-8), 5.29 (d, J = 2.7 Hz, 2 H, CH_2-1′), 2.67 (t, J = 2.7 Hz, 1 H, H-3′) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 162.6 (C-4), 152.9 (C-2), 148.4 (C-8), 143.3 (C-6), 122.7 (C-5),* 77.3 (C-2′), 75.1 (C-3′), 37.4 (C-1′) ppm.
*Inferred by HMBC

4.1.13. General methods for the 1,3-dipolar cycloaddition between *N*-substituted 1-azido glucopyranuronamides with *N*9/*N*7-propargylpurine

Method A: To a solution of *N*-substituted 1-azido glucopyranuronamides (0.1 mmol) in dichloromethane (2 mL), N^9/N^7-propargyl-6-chloropurine (1.5 equiv.) and CuI/Amberlyste A21 (0.5 mmol CuI.g^{-1}, 30 mg)
[C. Girard, E. Önen, M. Aufort, S. Beauvire, E. Samson, J. Herscovici, *Org. Lett.* **2006**, *8* (8) 1689–1692.] (30 mg) were added. The suspension was gently stirred at room temperature for 48 h. The catalyst was filtered off and the solvent was evaporated. The residue was subjected to column chromatography.

Method B: A mixture of *N*-dodecyl 1-azido-2,3,4-tri-O-acetyl-1-deoxy-α-D-glucopiranuronamide (0,14 mmol) and N^9/N^7-propargyl-6-chloropurine (0.28 mmol) in toluene (2mL) was stirred under reflux and under nitrogen atmosphere for 24 h. The solvent was evaporated under vacum and the residue was subjected to column chromatography.

4.1.13.1. *N*-Dodecyl 2,3,4-tri-*O*-acetyl-1-[4-(6-chloropurin-9-yl)methyl-1*H*-1,2,3-triazol-1-yl]-1-deoxy-α-D-glucopyranuronamide (**30**) and *N*-Dodecyl 2,3,4-tri-*O*-acetyl-1-deoxy-1-[4-(6-dimethylaminopurin-9-yl)methyl-1*H*-1,2,3-triazol-1-yl]-α-D-glucopyranuronamide (**31**)

Obtained according to the general method A starting from *N*-dodecyl 2,3,4-tri-O-acetyl-1-deoxy-α-D-glucopyranuronamide (**25α**, 124 mg, 0.24 mmol), 6-chloro-9-propargylpurine (68 mg, 0.35 mmol), and using CuI/Amberlyste A21 (60 mg). Purification by column chromatography (AcOEt/hexane, 9:1) afforded **30** (91 mg, 56%) as white solid and its 6-dimethylamino derivative **31** (31 mg, 18%) as yellow oil.
Method B, starting from **25α** (73 mg, 0,14 mmol) and 6-chloro-9-propargylpurine (55 mg, 0.28 mmol) under refluxing toluene (2 mL), afforded only **30** (35 mg, 35%).

Data for **30**: m.p.: 81.3 – 82.9 ºC. $[\alpha]_D^{20}$ = + 61.2 (c = 1.0, in CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.76 (s, 1 H, H-2''), 8.38 (s, 1 H, H-8''), 7.88 (s, 1 H, H-5), 6.46 (d, $J_{1',2'}$ = 5.4 Hz, 1 H, H-1'), 6.16 (t, $J_{NH\text{-}CH2}$ = 6.0 Hz, 1 H, N*H*), 6.09 (t, $J_{3',4'}$ = 8.7 Hz, 1 H, H-3'), 5.62 (s, 2 H, CH_2N), 5.31 (t, $J_{3',4'} \sim J_{4',5'}$, 1 H, H-4'), 5.24 (dd, $J_{2',3'}$ = 9.1 Hz, 1 H, H-2'), 4.42 (d, $J_{4',5'}$ = 8.9 Hz, 1 H, H-5'), 3.26-3.06 (m, 2 H, CH_2-1'''), 2.08, 2.04, 1.85 (3 s, 3 × 3 H, 3 × CH_3, Ac), 1.48–1.39 (m, 2 H, CH_2-2'''); 1.35-1.15 (m, 18 H, CH_2-3'''−CH_2-11'''), 0.87 (t, *J* = 6.7 Hz, 3 H, CH_3-12'''). ^{13}C NMR (101 MHz, $CDCl_3$): δ =170.1, 169.8, 169.4 (3 × *C*O, Ac), 165.7 (C-6'), 152.2 (C-2''),151.6 (C-6''); 151.6 (C-4''), 145.2 (C-8''), 141.5 (C-4), 131.8 (C-5''), 125.0 (C-5), 81.3 (C-1'), 72.7 (C-5'), 69.3 (C-2'), 68.9 (C-3', C-4'), 39.52 (CH_2-1'''); 38,96 (*C*H_2N), 32.0, 29.8, 29.7, 29.5, 29.4, 27.0, 22.8 (C-3'''−C11'''), 20.9, 20.8. 20.5 (3 × *C*H_3, Ac), 14.3 (C-12''') ppm.
HRMS: calcd for $C_{32}H_{45}ClN_8O_8$ [M + H]+ 705.3122, found 705.3152

Data for **31**: $[\alpha]_D^{20}$ = + 79.5 (c = 1.0, in CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.32 (s, 1 H, H-2''), 7.92 (s, 1 H, H-8''), 7.83 (s, 1 H, H-5), 6.39 (d, $J_{1',2'}$ = 5.3 Hz, 1 H, H-1'), 6.18 (t, $J_{NH\text{-}CH2}$ = 5.5 Hz, 1 H, N*H*), 6.08 (t, $J_{3',4'} = J_{2',3'}$ = 8.7 Hz, 1 H, H-3'), 5.51, 5.47 (2 d, AB systems, $J_{a,b}$ = 16.4, 2 H, CH_2N), 5.32 (t, $J_{3',4'} \sim J_{4',5'}$ = 8.6, 1 H, H-4'), 5.20 (dd, 1 H, H-2'), 4.46 (d, $J_{4',5'}$ = 8.8 Hz, 1 H, H-5'), 3.81-3.24 (m, 6 H, N(C*H*3)$_2$), 3.23-3.06 (m, 2 H, CH_2-1'''), 2.07, 2.02, 1.82 (3 s, 3 × 3 H, 3 × CH_3, Ac), 1.49–1.37 (m, 2 H, CH_2-2'''); 1.33-1.14 (m, 18 H, CH_2-3'''—CH_2-11'''), 0.86 (t, *J* = 6.7 Hz, 3 H, CH_3-12''').
^{13}C NMR (101 MHz, $CDCl_3$) δ 170.0, 169.8, 169.3 (3 × *C*O, Ac), 165.8 (C-6'), 155.0 (C-6''), 152.5 (C-2''), 150.2 (C-4''), 142.8 (C-4), 138.2 (C-8''), 125.0 (C-5), 120.2 (C-5''), 81.1 (C-1'), 72.7 (C-5'), 69.2 (C-2'), 68.9 (C-3'), 68.8 (C-4'), 39.5 (C-1'''), 38.5 (*C*H_2N), 38.5 (N(*C*H_3)$_2$), 32.0, 29.7, 29.6, 29.5, 29.4, 29.3, 27.0, 22.8 (C-2'''−C11'''), 20.9, 20.8. 20.4 (3 × *C*H_3, Ac), 14.3 (C-12''') ppm. HRMS: calcd for $C_{34}H_{51}N_9O_8$ [M + H]+ 714.3933, found 714.3958.

4.1.13.2. *N*-Dodecyl 2,3,4-tri-*O*-acetyl-1-[4-(6-chloropurin-7-yl)methyl-1*H*-1,2,3-triazol-1-yl]-1-deoxy-α-D-glucopyranuronamide (**32**)

Obtained according to the general method A starting from *N*-dodecyl 2,3,4-tri-O-acetyl-1-deoxy-α-D-glucopyranuronamide (**25α**, 59 mg, 0.115 mmol), 6-chloro-7-propargylpurine (33 mg, 0.17 mmol), and using CuI/Amberlyste A21 (29 mg).

Purification by column chromatography (from AcOEt/hexane, 7:3 to AcOEt) afforded **32** (51 mg, 63%) as a yellow solid.

Method B, starting from **25 α** (59 mg, 0.115 mmol) and 6-chloro-7-propargylpurine (34 mg, 0.17 mmol) under refluxing toluene (3 mL), afforded **32** in 23% yield (19 mg). m.p.: 86.4 – 91.0 ºC. $[\alpha]_D^{20}$ = + 64.0 (c = 1.0, in CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.88 (s, 1 H, H-2''), 8.55 (s, 1 H, H-8''), 7.87 (s, 1 H, H-5), 6.50 (d, $J_{1',2'}$ = 5.3 Hz, 1 H, H-1'), 6.35 (t, $J_{NH\text{-}CH2}$ = 6.0 Hz, 1 H, N*H*), 6.07 (t, $J_{3',4'}$ = 8.7 Hz, 1 H, H-3'), 5.86 (s, 2 H, CH_2N), 5.32 (t, $J_{3',4'} \sim J_{4',5'}$, 1 H, H-4'), 5.26 (dd, $J_{2',3'}$ = 8.9 Hz, 1 H, H-2'), 4.46 (d, $J_{4',5'}$ = 8.9 Hz, 1 H, H-5'), 3.25-3.06 (m, 2 H, CH_2-1'''), 2.06, 2.03, 1.85 (3 s, 3 × 3 H, 3 × CH_3, Ac), 1.49–1.37 (m, 2 H, CH_2-2'''); 1.31-1.16 (m, 18 H, CH_2-3'''-CH_2—11'''), 0.86 (t, *J* = 6.7 Hz, 3 H, CH_3-12'''). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.9, 169.7, 169.5 (3 × *C*O, Ac), 165.6 (C-6'), 152.9 (C-2''), 149.2 (C-8''), 142.0 (C-4), 124.5 (C-5), 122.3 (C-5''), 81.4 (C-1'), 72.8 (C-5'), 69.2 (C-2'), 68.9 (C-3'), 68.9 (C-4'), 42.2 (*C*H_2N), 39.6 (C-1'''), 32.0, 29.7, 29.6, 29.5, 29.4, 27.0, 22.8 (C-2'''—C11'''), 20.8, 20.8. 20.5 (3 × *C*H_3, Ac), 14.3 (C-12''') ppm.

HRMS: calcd for $C_{32}H_{45}ClN_8O_8$ [M + H]+ 705.3122, found 705.3122.

4.1.13.3. *N*-Propargyl 2,4-di-*O*-acetyl-1-[4-(6-chloropurin-9-yl)methyl-1*H*-1,2,3-triazol-1-yl]-1-deoxy-3-*O*-dodecyl-α-D-glucopyranuronamide (**33**) and *N*- Propargyl 2,4-di-*O*-acetyl-1-deoxy-1-[4-(6-dimethylaminopurin-9-yl)methyl-1*H*-1,2,3-triazol-1-yl]-3-*O*-dodecyl-α-D-glucopyranuronamide (**34**)

Obtained according to the general method A starting from *N*-propargyl 2,4-di-*O*-acetyl-1-azido-1-deoxy-3-*O*-dodecyl-α-glucopyranuronamide (**26α**, 52 mg, 0,102 mmol, 6-chloro-9-propargylpurine (28 mg, 0.15 mmol), and using CuI/Amberlyste A21 (30 mg). Purification by column chromatography (from AcOEt/hexane, 3:1 to AcOEt/MeOH, 9:1) afforded **33** (16 mg, 23%) as white solid and its 6-dimethylamino derivative **34** (33.5 mg, 46%) as white solids.

Data for **33**: m.p.: 71.9 – 74.3 ºC. $[\alpha]_D^{20}$ = + 33.0 (c = 1.0, in CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.76 (s, 1 H, H-2''), 8.37 (s, 1 H, H-8''), 7.87 (s, 1 H, H-5), 6.49-6.40 (m, 1 H, H-1', N*H*), 5.61 (s, 2 H, CH_2N), 5.29 (t, $J_{3',4'} \sim J_{4',5'}$ = 7.6, 1 H, H-4'), 5.07 (dd, $J_{1',2'}$ = 5.0, $J_{2',3'}$ = 8.0 Hz, 1 H, H-2'), 4.48-4.39 (m, 2 H, H-3', H-5'), 4.06 (ddd, $J_{7a,7b}$ = 17.7, $J_{7'a,NH}$ = 5.9, $J_{7'\text{-}a,9'}$ = 2.5, 1 H, H-7'a), 3.90 (ddd, $J_{7b,NH}$ = 4.8, $J_{7'\text{-}b,9'}$ = 2.5, H-7'b, 1 H), 3.71-3.53 (m, 2 H, CH_2-1'''), 2.22 (t, *J* = 2.5, H-9'), 2.11, 1.87 (2 s, 2 × 3 H, 2 × CH_3, Ac), 1.53–1.43 (m, 2 H, CH_2-2'''); 1.33-1.17 (m, 18 H, CH_2-3'''—CH_2-11'''), 0.85 (t, *J* = 6.7 Hz, 3 H, CH_3-12''').

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.0, 169.8 (2 × *C*O, Ac), 166.1 (C-6'), 152.2 (C-2''), 151.6, 151.5 (C-4'', C-6''), 145.2 (C-8''), 141.4 (C-4), 131.7 (C-5''), 124.8 (C-5), 81.4 (C-1'), 78.7 (C-8'), 75.3 (C-3'), 73.3 (C-5'), 73.2 (*C*-1'''), 72.3 (C-9'), 70.9 (C-2'), 68.5 (C-4'), 39.0 (*C*H_2N), 32.0, 30.1, 29.8, 29.7, 29.6, 29.5, 29.1, 26.0, 22.8 (C-2'''—C11''', C-7'), 21.0, 20.6 (2 × *C*H_3, Ac), 14.3 (C-12''') ppm.

HRMS: calcd for $C_{33}H_{45}ClN_8O_7$ $[M + H]^+$ 701.3172, found 701.3180. calcd for $C_{33}H_{45}ClN_8O_7$ $[M + Na]^+$ 723.2992, found 723.3002.

Data for **34**: m.p.: 65.1 – 68.0 ºC. $[\alpha]_D^{20}$ = + 41.0 (c = 1.0, in CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = δ = 8.33 (s, 1 H, H-2''), 7.91 (s, 1 H, H-8''), 7.82 (s, 1 H, H-5), 6.48 (dd app. t, $J_{NH\text{-}CH2}$ = 5.1 Hz, 1 H, N*H*), 6.39 (d, $J_{1',2'}$ = 4.7 Hz, 1 H, H-1'), 5.48 (s, 2 H, CH_2N), 5.29 (t, $J_{3',4'} \sim J_{4',5'}$ = 7.6, 1 H, H-4'), 5.07 (dd, $J_{1',2'}$ = 5.0, $J_{2',3'}$ = 7.9 Hz, 1 H, H-2'), 4.48-4.39 (m, 2 H, H-3', H-5'), 4.04 (ddd, $J_{7a,7b}$ = 17.7, $J_{7'a,NH}$ = 5.1, $J_{7'\text{-}a,9'}$ = 2.4, 1 H, H-7'a), 3.91 (ddd, $J_{7b,NH}$ = 4.4, $J_{7'\text{-}b,9'}$ = 2.7, H-7'b, 1 H), 3.72-3.31 (m, 8 H, CH_2-1''', N(C$H3$)$_2$), 2.21 (t, *J* = 2.5, H-9'), 2.10, 1.86 (2 s, 2 × 3 H, 2 × CH_3, Ac), 1.57–1.40 (m, 2 H, CH_2-2'''), 1.33-1.13 (m, 18 H, CH_2-3'''—CH_2-11'''), 0.85 (t, *J* = 6.7 Hz, 3 H, CH_3-12'''). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.0, 169.8 (2 × *C*O, Ac), 166.2 (C-6'), 155.1 (C-6''), 152.6 (C-2''), 150.3 (C-4''), 142.8 (C-4), 138.1 (C-8''), 124.8 (C-5), 120.2 (C-5''), 81.2 (C-1'), 78.7 (C-8'), 75.2 (C-3'), 73.4 (C-5'), 73.2 (*C*-1'''), 72.3 (C-9'), 70.8 (C-2'), 69.4 (C-4'), 38.5 (*C*H_2N, N(*C*H_3)$_2$), 32.0, 30.1, 29.8, 29.7, 29.6, 29.5, 29.1, 26.0, 22.8 (C-2'''—C11''', C-7'), 21.0, 20.6 (2 × *C*H_3, Ac), 14.3 (C-12''') ppm. HRMS: calcd for $C_{35}H_{51}N_9O_7$ $[M + H]^+$ 710.3984, found 710.3993. calcd for $C_{33}H_{45}ClN_8O_7$ $[M + Na]^+$ 732.3804, found 732.3821. calcd for $C_{33}H_{45}ClN_8O_7$ $[M + K]^+$ 748.3543, found 748.3560.

4.1.13.4. *N*-Propargyl 2,4-di-*O*-acetyl-1-[4-(6-chloropurin-7-yl)methyl-1*H*-1,2,3-triazol-1-yl]-1-deoxy-3-*O*-dodecyl-α-D-glucopyranuronamide (**35**)

Obtained according to the general method A starting from *N*-propargyl 2,4-di-*O*-acetyl-1-azido-1-deoxy-3-*O*-dodecyl-α-glucopyranuronamide (**26α**, 52 mg, 0,102 mmol, 6-chloro-7-propargylpurine (28 mg, 0.15 mmol), and using CuI/Amberlyste A21 (30 mg). Purification by column chromatography (from AcOEt/hexane, 3:1 to AcOEt/MeOH, 9:1) afforded **35** (37 mg, 51%) as yellow solid. m.p.: 92.1 – 94.3 ºC. $[\alpha]_D^{20}$ = + 37.0 (c = 1.0, in CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.91 (s, 1 H, H-2''), 8.50 (s, 1 H, H-8''), 7.79 (s, 1 H, H-5), 6.54-6.42 (m, 1 H, H-1', N*H*), 5.85 (s, 2 H, CH_2N), 5.30 (t, $J_{3',4'} \sim J_{4',5'}$ = 7.4, 1 H, H-4'), 5.08 (dd, $J_{1',2'}$ = 4.8, $J_{2',3'}$ = 7.8 Hz, 1 H, H-2'), 4.47-4.41 (m, 2 H, H-3', H-5'), 4.07 (ddd, $J_{7a,7b}$ = 18.1, $J_{7'a,NH}$ = 6.2, $J_{7'\text{-}a,9'}$ = 2.7, 1 H, H-7'a), 3.93 (ddd, $J_{7b,NH}$ = 4.8, $J_{7'\text{-}b,9'}$ = 2.6, H-7'b, 1 H), 3.71-3.55 (m, 2 H, CH_2-1'''), 2.25 (t, *J* = 2.6, H-9'), 2.12, 1.89 (2 s, 2 × 3 H, 2 × CH_3, Ac), 1.57–1.43 (m, 2 H, CH_2-2'''), 1.35-1.14 (m, 18 H, CH_2-3'''—CH_2-11'''), 0.85 (t, *J* = 6.7 Hz, 3 H, CH_3-12'''). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.0, 169.8 (2 × *C*O, Ac), 166.2 (C-6'), 152.9 (C-2''), 149.7 (C-8''), 141.9 (C-4), 124.2 (C-5), 122.3 (C-5''), 81.4 (C-1'), 78.9 (C-8'), 75.2 (C-3'), 73.7 (C-5'), 73.2 (*C*-1'''), 72.2 (C-9'), 70.8 (C-2'), 69.4 (C-4'), 42.2 (*C*H_2N), 32.0, 30.1, 29.8, 29.7, 29.6, 29.5, 29.1, 26.0, 22.8 (C-2'''—C11''', C-7'), 21.0, 20.6 (2 × *C*H_3, Ac), 14.3 (C-12''') ppm. HRMS: calcd for $C_{33}H_{45}ClN_8O_7$ $[M + H]^+$ 701.3172, found 701.3218. calcd for $C_{33}H_{45}ClN_8O_7$ $[M + Na]^+$ 723.2992, found 723.3019.

4.2. Biological assays

4.2.1. Cell lines and antiproliferative assay

Human cancer cell lines were obtained from the American Type Culture Collection and were cultivated according to the provider's instructions. In brief, MCF-7 , K562 and BJ cell lines were maintained in DMEM medium supplemented with 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 µg/mL) at 37˚ C in 5% CO_2. For the cytotoxicity assays, cells were seeded into 96-well plates at the appropriate densities (5000 cell /well for MCF-7 and K562; 20000 cells/well for BJ) treated in triplicate with six different doses of each compound for 72 h. After treatment, the resazurin solution (Sigma Aldrich) was added for 4 h, and then the fluorescence of resorufin was measured at 544 nm / 590 nm (excitation / emission) using a Fluoroskan Ascent microplate reader (Labsystems). The GI_{50} value, the drug concentration lethal to 50% of the cells, was calculated from the dose response curves that resulted from the assays.

4.2.2. Flow cytometric analysis

After harvesting, the cells were then washed with PBS, fixed with 70% ethanol and kept frozen overnight. After washing with PBS and staining with propidium iodide (10 µg/mL), the cells were analysed by flow cytometry using a 488 nm laser (BD FACS Verse with BD FACSuite software, version 1.0.6.).

4.2.3. Immunoblotting

In brief, cellular lysates were prepared and denaturated in Laemmli sample buffer. Further, proteins were separated on SDS-polyacrylamide gels and electroblotted onto nitrocellulose membranes. After blocking, the membranes were incubated with specific primary antibodies overnight, washed and then incubated with peroxidase-conjugated secondary antibodies. Finally, peroxidase activity was detected with SuperSignal West Pico reagents (Thermo Scientific) using a CCD camera LAS-4000 (Fujifilm). Specific antibodies were purchased from Santa Cruz Biotechnology (anti β-actin, clone C4) and Cell Signaling (anti-PARP, clone 46D11; anti-XIAP; anti-Mcl-1, clone D35A5; anti-caspase-7).

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