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Novel dodecyl-containing azido and glucuronamide-based nucleosides exhibiting anticancer potential

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Abstract: The synthesis and anticancer evaluation of new series of nucleosides constructed on 5/6-azidoglycosyl or glucuronamide moieties and containing an *O*- or an *N*-dodecyl chain, respectively, are disclosed. Based on our previous results, their structures were planned to preclude them to act *via* a similar metabolic pathway than that of clinically used nucleoside antimetabolites, against which cancer cells frequently acquire resistance. *Xylo* and *gluco*-configured 5/6-azido-1,2-di-*O*-acetyl furanosyl and pyranosyl donors containing a 3-*O*-dodecyl group were synthesized from diacetone-D-glucose and were subsequently coupled with silylated uracil or 2-acetamido-6-chloropurine. *N*-Dodecyl glucuronamide-based nucleosides were accessed from acetonide-protected glucofuranurono-6,3-lactone, which was converted in few steps into *O*-benzylated 1,2-di-*O*-acetyl furanuronamide or pyranuronamide derivatives to undergo further *N*-glycosylation. Both types of nucleosides demonstrated notorious antiproliferative effects in chronic myeloid leukemia (K562) and in breast cancer (MCF-7) cells. The most potent molecules were a 6′-azidoglucopyranosyl N^7-linked purine nucleoside and glucofuranuronamide derivatives comprising N^1-linked uracil and N^7-linked purine units with activities in the single-digit micromolar order of concentration against both cell lines. Their GI_{50} values in MCF-7 cells were similar or *ca.* 3-fold lower than that of the standard drug 5-fluorouracil. Cell cycle studies and immunoblotting analysis of apoptosis-associated proteins in treated K562 cells indicated that the antiproliferative effect of the most effective nucleosides is based on apoptosis induction.

Keywords: anticancer activity; apoptosis; azido nucleosides; glucuronamide-based nucleosides; ICS-29; nucleoside analogs.

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Introduction

Synthetic nucleosides and nucleotides, frequently referred as analogs to differentiate them from their physiological counterparts, are among the most relevant groups of chemotherapeutic agents. The clinically-used compounds of these types are mainly prodrugs that act as nucleic acid antimetabolites [1–3]. Their active forms, i.e. nucleosides mono, di- or triphosphates, are generated intracellularly by kinase-mediated phosphorylation and these metabolites may interfere with nucleic acid synthesis through inhibition of polymerases or of key enzymes involved in nucleotide biosynthetic pathways, or by incorporation into nucleic acids. These mechanisms of action lead ultimately to apoptosis or to the disruption of the viral replication cycle [2, 3]. With respect to the anticancer nucleos(t)ide analogs, there are currently 12 compounds approved by the FDA and more than 20 in clinical development [1]. Examples include the antileukemic drugs cytarabine [1], fludarabine 5′-monophosphate [4], clofarabine [5], cladribine [6] and the broad-spectrum anticancer agent gemcitabine [7]. These compounds are prodrugs that act mainly by inhibition of DNA polymerases and by incorporation into DNA through their corresponding nucleoside triphosphate (NTP) metabolites. Gemcitabine and cladribine also inhibit ribonucleotide reductase, a key enzyme for the access to 2′-deoxynucleotides for further DNA synthesis, through their nucleoside diphosphate and triphosphate metabolites, respectively [6, 8]. Some nucleoside analogs, such as floxuridin [1] and trifluorothymidine [9], exert their action through their monophosphate metabolites, namely by inhibiting thymidylate synthase, which results in the decrease on the level of thymidine monophosphate, a key nucleotide precursor for DNA synthesis. Incorporation into RNA or interference with DNA repair pathways are additional mechanisms that were described as playing a role in the anticancer effect of nucleos(t)ide analogs. The DNA is however their main target, whose damage triggers cell death. Due to the fact that cancer cells are actively replicating their genome, contrarily to normal cells, which are rather quiescent, a cancer cell-selective action can be reached by this effect [3].

Despite significant effectiveness in clinics, some disadvantages are associated with the use of nucleos(t)ide analogs, namely their low oral bioavailability and the acquired and intrinsic resistance exhibited by cancer cells. The mechanisms of acquired resistance include the decrease on the level of nucleoside transporters, hampering the cell uptake of the nucleos(t)ide, and events that disturb the metabolic pathways leading to their conversion into the active principles. Among them are the downregulation of nucleoside kinases, the overexpression of 5′-nucleotidases, which dephosphorylate the nucleoside 5′-monophosphate metabolites, and the increase in the activity of deaminases, which deactivate the cytosine/adenine nucleosides and also allow intrinsic resistance [2, 10, 11].

To overcome the issues of the nucleos(t)ide antimetabolites, the development and synthesis of new structures of nucleos(t)ide analogs that show suitable cell permeability and exhibit anticancer efficacy through different mechanisms of action is needed. Since other biological processes involving nucleotide-dependent events/enzymes, besides nucleic acid synthesis, are relevant or overactivated in cancer, such as various intracellular signaling pathways [12–16] or cell cycle regulation [17–21], the anticancer potential of these groups of compounds is not restricted to their ability to act as nucleic acid antimetabolites. Therefore, alternative mechanisms of anticancer action may be exhibited by distinct structures of nucleoside analogs.

We have previously reported the synthesis and the antiproliferative efficacy of 5′/6′-azidofuranosyl/pyranosyl and *N*-dodecyl glucopyranuronamide-containing purine nucleosides against chronic myeloid leukemia (K562) and breast cancer (MCF-7, BT474) cells [22, 23] (Fig. 1). Such molecules lack a free hydroxyl group at the terminal position of the nucleoside's sugar moiety (5′ or 6′), which preclude them to be activated by kinases and to undergo similar metabolic pathways inherent to the mechanisms of action of the known anticancer nucleos(t)ide antimetabolites. The compounds were active at micromolar order of concentration and the most potent glucuronamide-based nucleoside (**B**) exhibited a comparable GI_{50} value to that of the clinically used 5-fluorouracil in MCF-7 cells. The most active azido nucleoside (**A**) caused G2/M cell cycle arrest in K562 and MCF-7 cells [22]. The glucuronamide nucleoside **B** was shown to induce apoptosis in K562 cells through downregulation of the anti-apoptotic myeloid cell leukemia 1 (Mcl-1) protein and activation of caspases 3 and 7 [23], which are enzymes that play a crucial role in apoptosis [24]. Since cancer cells are frequently more resistant to apoptosis than normal ones and their resistance increases when subjected to chemotherapy,

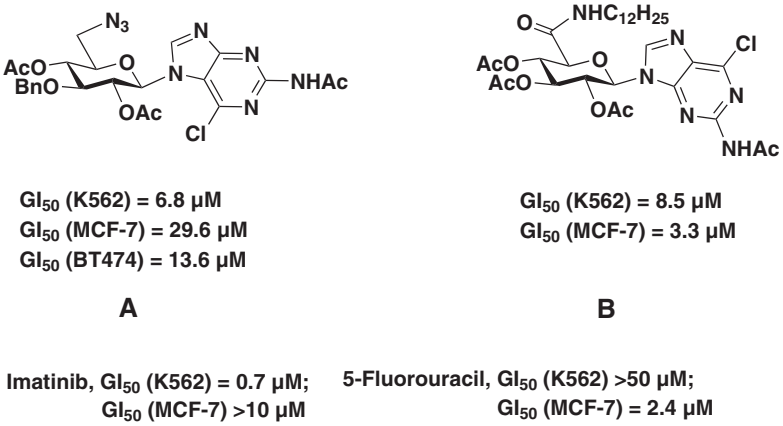


Fig. 1: Azido (**A**) and *N*-dodecyl glucuronamide-based nucleosides (**B**) possessing significant antiproliferative activities in cancer cells.

namely through overexpression of anti-apoptotic proteins [25, 26], the effects shown by nucleoside **B** make it a promissing lead anticancer molecule for overcoming chemotherapy resistance.

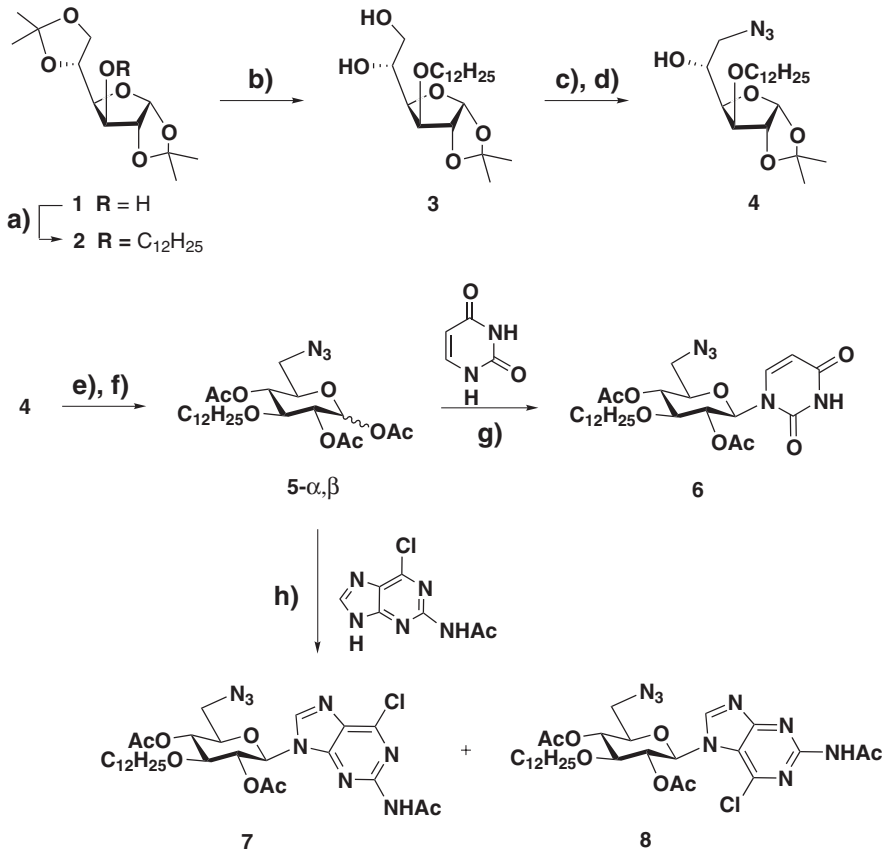
Based on these encouraging previous results and aiming at optimizing and further investigating the anticancer profile of these types of nucleosidic structures, we report herein on the synthesis of new furanosyl and pyranosyl analogs of compounds **A** and **B**. In view of the potent activity of nucleoside **B**, which possesses a *N*-dodecyl group, azido nucleoside counterparts of compound **A** containing an *O*-dodecyl chain instead of a *O*-benzyl group were accessed, aiming at improving the antiproliferative activities previously encountered for these types of nucleosides. Moreover, the introduction of such long hydrocarbon chain in the structures may turn them more able to penetrate into cells by passive diffusion and thus enable them to circumvent a main mechanism of resistance exhibited by cancer cells based on the decrease on the level of nucleoside transporters. Few reports showed the usefulness of this strategy to improve the cell penetrability and orally bioavailability of anticancer nucleoside analogs, as demonstrated by the cytarabine derivatives elacytarabine and sapacitabine [27, 28].

The inclusion in the molecules of both *O*-benzyl and *N*-dodecyl groups, which were present in the lead compounds **A** and **B**, respectively, was carried out leading to O-benzylated analogs of nucleoside **B**. This structural approach was undertaken not only to enhance the antiproliferative potential of the nucleosides, but also to increase their lipophilicity and hence to confer them a higher bioavailability. O-Acetylated derivatives were the final compounds for testing, since their hydroxylated couterparts would be less prompted for cell penetration, whereas the acetate groups may be intracellularly cleaved by esterases. Some examples from the literature support this prodrug strategy, showing that keeping ester protection in a nucleoside leads to an improvement on its bioavailability, cell penetrating ability or biological efficacy [29–31]. The evaluation of the antiproliferative effects of the molecules in cancer cells is presented.

Results and discussion

Chemistry

The synthesis of 3-*O*-dodecyl 5′- and 6′-azido nucleosides started from diacetone-D-glucose (**1**), which was converted into the 3-*O*-dodecyl derivative **2** by nucleophilic displacement with dodecyl bromide in the presence of sodium hydride (Scheme 1). Subsequent selective acid-mediated hydrolysis of the primary acetonide of **2** by treatment with aqueous acetic acid (70 %) afforded the 5,6-diol **3**. An azide moiety was installed at C-6 of diol **3** *via* generation of a good leaving group and further nucleophilic replacement by the azide ion, a convenient method for the synthesis of azido sugars [32, 33]. Thus, selective tosylation of diol **3** and



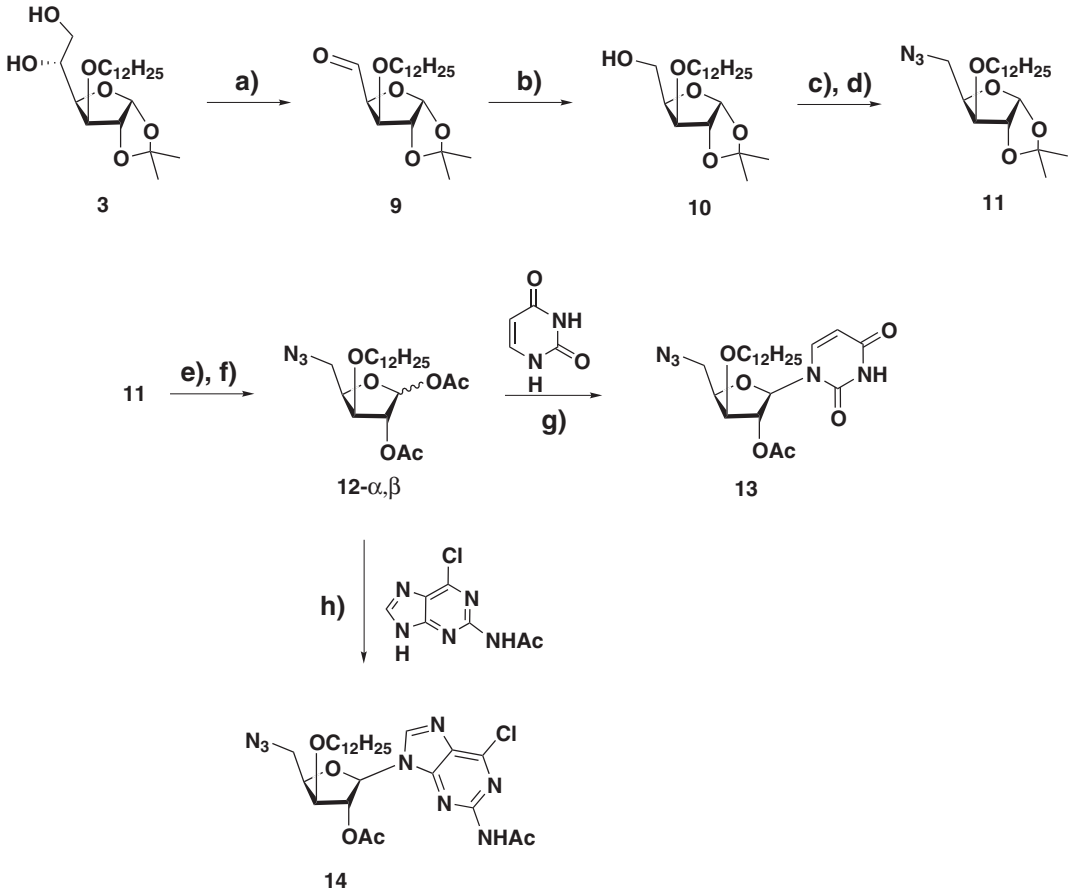
Scheme 1: Reagents and conditions: (a) $C_{12}H_{25}Br$, NaH, DMF, r.t, 17 h, 93 %; (b) AcOH (70 % aq. soln.), r. t., 5 d, 78 %; (c) TsCl, CH_2Cl_2/pyridine, r. t., 16 h; (d) NaN_3, DMF, r. t., 64 %, 2 steps; (e) TFA (60 % aq. soln.), r.t., 1 h; (f) Ac_2O, py, r. t., 1 h, 96 % (α/β = 1:0.6), 2 steps; (g) uracil, BSA, TMSOTf, CH_3CN, 65 °C, MW, max. 150 W, 10 min, 83 %; (h) 2-NHAc-6-Cl-purine, BSA, TMSOTf, CH_3CN, 65 °C, MW, max. 150 W, 20 min, 32 % (**7**), 41 % (**8**).

further substitution of the intermediate tosylate with sodium azide gave the 6-azidoglucofuranose derivative **4** in 64 % yield. Removal of the remaining isopropylidene group of **4** with aqueous trifluoroacetic acid (TFA, 60 %) and further acetylation (Ac_2O/pyridine) gave the tri-*O*-acetylated 6-azido-3-*O*-dodecyl glycosyl donor **5** as an anomeric mixture. The nucleosidation reactions with uracil and 2-acetamido-6-chloropurine, which were pre-activated by silylation with bis(trimethylsilyl)acetamide (BSA), were performed under microwave irradiation (150 W, Pmax 250 Psi) at 65 °C in the presence of trimethylsilyl triflate (TMSOTf). Hence, N-glycosylation of uracil with **5** led to the N^1-linked nucleoside **6** in 83 % yield, as confirmed by the key HMBC correlations between H-1′ with C-2 and C-6 of the uracil moiety. In the case of 2-acetamido-6-chloropurine, both the N^9-linked nucleoside **7** and the N^7 regioisomer **8** were obtained in 32 % and 41 % yields, respectively, whose regiochemistry of the nucleosidic linkage was distinguished on the basis on their $^1H/^{13}C$-NMR and HMBC spectra. Diagnostic spectral features were the higher chemical shift values of the signals for H-8, H-1′, C-4 and C-8 of the N^7-linked nucleoside **8**, while the signal for C-5 is deshielded in the N^9-regioisomer **7**, as well as a HMBC correlation between H-1′ and C-4 of the purine moiety in the case of **7**. The use of this purine derivative was motivated not only by the previous reported anticancer potency of nucleosides containing this nucleobase [22, 23, 34], notably compounds **A** and **B**, but also considering other benefits that the presence of such motif may confer to the molecules. The resulting nucleosides are less susceptible to undergo deamination, firstly due to the *N*-acetyl protection and secondly, due to the choro atom at C-6, similarly to the clinically used anticancer halopurine nucleosides clofarabine, fludarabine and cladribine [5] and may therefore overcome this natural mechanism of chemotherapy resistance. Moreover, since this nucleobase derivative is significantly less polar than the corresponding amino purine, it may increase the cell-penetrating ability of

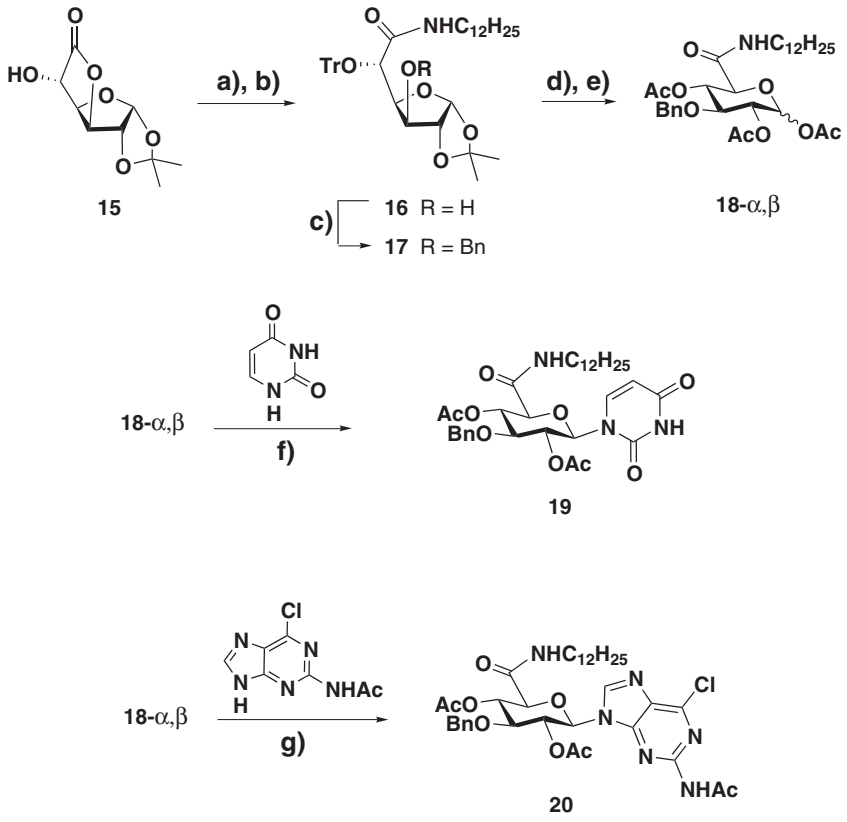
the molecules and eventually their oral bioavailability, while the acetamide moiety is prompted to be cleaved by intracellular amidases.

For the access to 5-azido pentofuranosyl nucleoside analogs of **6–8**, the diol **3** was converted into the xylofuranose precursor **10** *via* sodium periodate-mediated oxidative cleavage and subsequent reduction of the intermediate aldehyde **9** (Scheme 2). Instalation of an azido group at C-5 of **10** was then accomplished by tosylation followed by nucleophilic displacement with sodium azide. Removal of the 1,2-*O*-isopropylidene group of the 5-azido xylofuranose derivative **11** followed by acetylation afforded the corresponding glycosyl acetate **12**. Further MW-assisted *N*-glycosylation of silylated uracil, promoted by TMSOTf, with **12** led to the 5-azido xylofuranosyl nucleoside **13**, although in modest yield (13 %). In the case of 2-acetamido-6-chloropurine, the N^9-linked purine nucleoside **14** was obtained in moderate yield (35 %) as the virtually sole regioisomer, being identified based on similar spectral features as previously mentioned for **7**.

Based on the satisfactory antiproliferative activities of the 3-*O*-benzyl nucleoside lead compound **A** (Fig. 1), we were motivated to synthesize an analog of the glucuronamide-based nucleoside **B** containing a 3-*O*-benzyl group (Scheme 3). Hence, the acetonide-protected glucofuranurono-6,3-lactone **15** was firstly subjected to tritylation to protect the 5-hydroxyl group, which was followed by opening of the lactone moiety with dodecyl amine, enabling the access to the *N*-dodecyl glucuronamide derivative containing a unique free hydroxyl group at C-3 (**16**) for further benzylation. This strategy was employed since previous reported results [35] showed the unfeasibility to obtain a derivative regioselectively *O*-benzylated at C-3 by benzylation of *N*-dodecyl 1,2-di-*O*-isopropylidene-α-D-glucofuranuronamide (**21**, Scheme 4). Thus, treatment of **16**



Scheme 2: Reagents and conditions: (a) $NaIO_4$, THF (60 % aq. soln.), r. t., 2.5 h, 79 %; (b) $NaBH_4$, $EtOH/H_2O$, r. t., 1 h, 81 %; (c) TsCl, CH_2Cl_2/pyridine, r. t., 16 h; (d) NaN_3, DMF, 80 °C, 77 %, 2 steps; (e) TFA (60 % aq. soln.), r. t., 2 h; (f) Ac_2O, py, r. t., 1 h, 42 % (α/β = 1:0.3), 2 steps; (g) uracil, BSA, TMSOTf, CH_3CN, 65 °C, MW, max. 150 W, 20 min, 13 %; (h) 2-NHAc-6-Cl-purine, BSA, TMSOTf, CH_3CN, 65 °C, MW, max. 150 W, 20 min, 35 %.



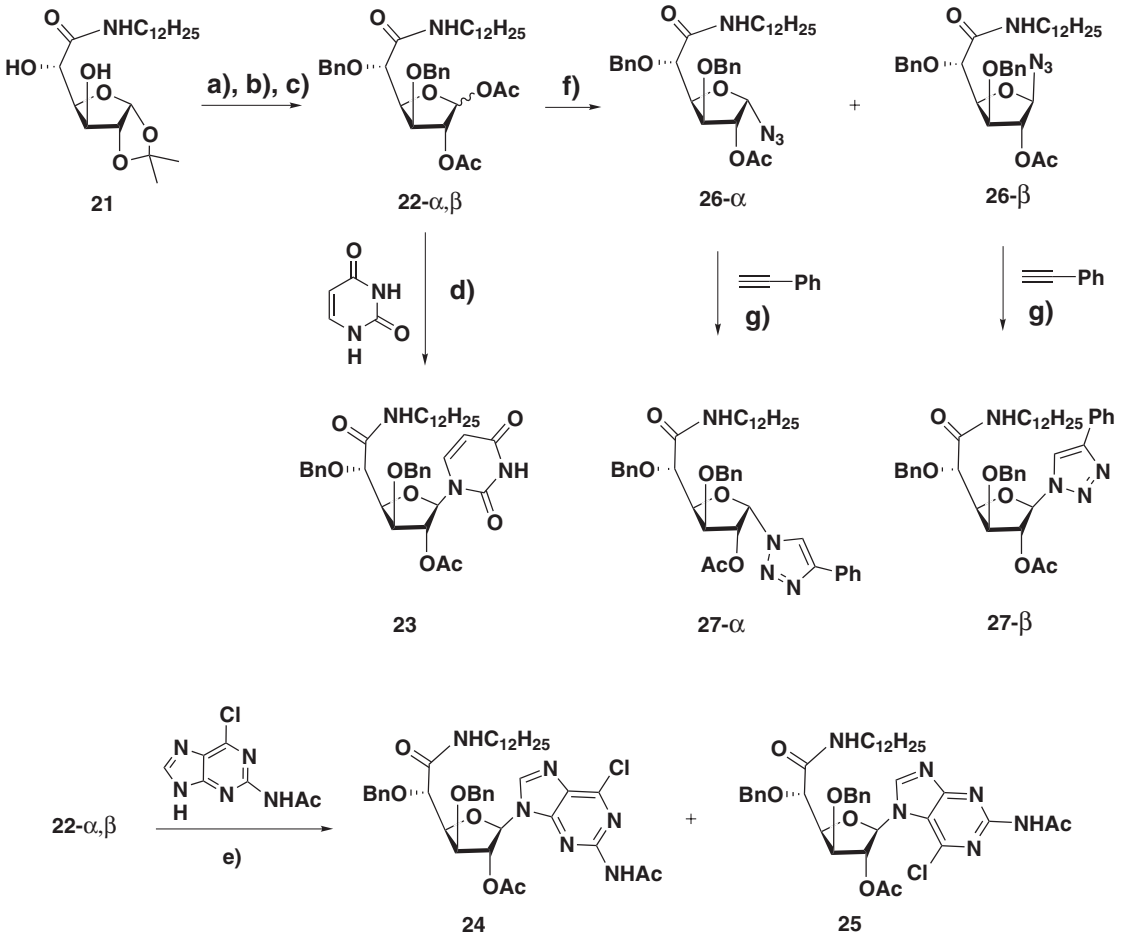
Scheme 3: Reagents and conditions: (a) TrCl, DMAP, CH_2Cl_2/pyridine, 40 °C, 16 h; (b) $C_{12}H_{25}NH_2$, CH_2Cl_2, r.t, 16 h, 45 %, 2 steps; (c) BnBr, NaH, DMF, r.t, 4 h, 38 %; (d) TFA (60 % aq. soln.), r.t., 2 h; (e) Ac_2O, py, r. t., 1 h, 94 % (α/β = 1:0.65), 2 steps; (f) uracil, BSA, TMSOTf, CH_3CN, 65 °C, MW, max. 150 W, 30 min, 58 %; (g) 2-NHAc-6-Cl-purine, BSA, TMSOTf, CH_3CN, 65 °C, MW, max. 150 W, 20 min, 47 %.

with benzyl bromide in the presence of sodium hydride furnished the 3-*O*-benzyl derivative **17** in 35 % yield. It was subjected to acid-mediated hydrolysis (TFA, 60 % aq. soln.) to effect both removal of the trityl and of the 1,2-*O*-isopropylidene groups, and further acetylation to give the glucopyranuronoamidyl donor **18.** Under similar *N*-glycosylation conditions as already mentioned, the TMSOTf-mediated coupling of **18** with uracil led to the N^1-linked nucleoside **19** in 58 % yield. The reaction with 2-acetamido-6-chloropurine afforded the N^9-linked nucleoside **20** in 47 % yield and only residual formation of the N^7 regioisomer was detected.

The synthesis of *N*-dodecyl glucuronamide-based nucleosides containg O-benzylated furanose moieties employed the 1,2-*O*-acetyl glucofuranuronoamide **22** as glycosyl donor (Scheme 4). This compound was accessed in three steps from the *N*-dodecyl furanuronamide derivative **21**, including benzylation, acetonide cleavage and acetylation, as previously described [35]. It was then converted into the uracil nucleoside **23** through the MW-assisted glycosylation method in 58 % yield. On the other hand, nucleosidation of **22** with 2-acetamide-6-chloropurine provided both N^9 and N^7-linked purine nucleosides **24** and **25**, in 26 % and 11 % yields, respectively. Glycosyl triazoles **27α** and **27β** were also included in the panel of compounds for antiproliferative evaluation. The synthesis of these triazole nucleosides was recently reported and involved the anomeric azidation of **22** with trimethylsilyl azide and 1,3-dipolar cycloaddition of the resulting 1-azido-glucofuranuronamides **26α** and **26β** with phenyl acetylene [35].

Biological evaluation

The antiproliferative effects of the newly synthesized azido and glucuronamide-based nucleosides were evaluated in chronic myeloid leukemia cell line K562, breast adenocarcinoma cell line MCF-7 and in normal



Scheme 4: Reagents and conditions: (a) BnBr/NaH, DMF, r.t, 16 h; (b) TFA (60 % aq. soln.), r.t., 1.5 h (c) Ac_2O, py, r.t., 1.5 h, 45 % (α/β = 1:0.8), 3 steps [35]; (d) uracil, BSA, TMSOTf, CH_3CN, 65 °C, MW, max. 150 W, 20 min, 52 %; (e) 2-NHAc-6-Cl-purine, BSA, TMSOTf, CH_3CN, 65 °C, MW, max. 150 W, 20 min, 26 % (**24**), 11 % (**25**); (f) $TMSN_3$, TMSOTf, CH_3CN, 65 °C, MW, max. 150 W, 70 min, **26-α** (29 %) and **26-β** (29 %) [35]; (g) phenylacetylene, CuI/Amberlyst A-21 (cat.), CH_2Cl_2, r.t., 72 h, 70 % (**27-α**), 82 % (**27-β**) [35].

human fibroblasts BJ (Table 1) using the rezasurin (Alamar Blue) assay. For an appropriate comparison, the effects of the previously reported anticancer nucleosides (**A**, **B**, Fig. 1) were also assessed by this method. The azido nucleosides (**6–8**, **13–14**) showed significant activities against both cancer cells, with slight better activity against K562 cells, whose GI_{50} values ranged from 13.7 μM to 3.2 μM. The replacement of an *O*-benzyl group by an *O*-dodecyl chain in the nucleoside **A** (Fig. 1) resulted in a significant improvement on the antiproliferative effects of the structure. Indeed, the 6′-azido 3-*O*-dodecyl pyranosyl N^7-linked purine nucleoside (**8**) was the most active compound of this group, with GI_{50} values of 3.2 μM in both cell lines, which is approximately 3-fold lower than that of the anticancer drug 5-fluorouracil in MCF-7 cells and 7-fold higher than the antileukemic agent imatinib in K562 cells. The N^7-nucleoside was ca. 4- to 5-fold more potent than its N^9 regioisomer (**7**) against the assayed cancer cell lines. However, it showed significant antiproliferative effects in BJ fibroblasts. The effects for the 6′-azido pyranosyl and the 5′-azido furanosyl nucleosides are not significantly different, considering both uracil (**6**, **13**) and N^9-linked purine (**7**, **14**) nucleosides. However, the furanosyl nucleosides **13–14** exhibited noticeably lower antiproliferative activities in BJ fibroblasts that their pyranosyl conterparts, with ca. 2.4–5.5-fold selectivity to the cancer cells. The most promising azido nucleoside balancing efficacy and cancer cell selectivity was the 5′-azido furanosyl N^9-linked purine derivative **14**, which is only ca. 2-fold less potent (GI_{50} = 18.5 μM) than 5-fluorouracil towards MCF-7 cells.

With respect to the *N*-dodecyl glucuronamide-based nucleosides, the 3′,5′-di-*O*-benzyl furanosyl derivatives (**23–25**) showed higher effects than their 3′-*O*-benzyl pyranosyl counterparts (**19–20**) in both cancer

Table 1: Antiproliferative activities of the azido and carboxamide nucleoside analogs.

Compound	GI_{50} (μM)[a]		
	K562	MCF-7	BJ confluent
A	8.8 ± 2.0	12.8 ± 3.6	NT
B	11.2 ± 1.8	7.2 ± 0.7	NT
5	26.0 ± 8.7	23.8 ± 2.9	62.9 ± 0.9
6	12.5 ± 0.7	24.1 ± 4.9	15.8 ± 0.2
7	13.7 ± 1.5	17.1 ± 5.0	31.3 ± 7.2
8	3.2 ± 1.1	3.2 ± 0.1	4.8 ± 0.3
12	66.7 ± 21.7	86.1 ± 3.8	>100
13	13.7 ± 0.1	21.7 ± 0.8	52.2 ± 2.1
14	10.2 ± 2.1	18.5 ± 2.6	55.7 ± 22.4
18	14.8 ± 3.7	13.3 ± 3.3	43.0 ± 2.2
19	>50	>50	>50
20	30.3 ± 11.1	15.9 ± 5.6	>50
22	23.9 ± 0.3	17.1 ± 2.3	>25
23	6.7 ± 1.1	8.6 ± 1.5	27.6 ± 3.6
24	7.4 ± 0.0	10.4 ± 1.8	9.6 ± 0.3
25	3.3 ± 0.7	3.3 ± 0.0	7.8 ± 0.4
26-α	>25	>25	>25
26-β	>25	>25	>25
27-α	>100	>100	>100
27-β	>25	>25	>25
Imatinib	0.47 ± 0.02	26.8 ± 2.0	>40
5-fluorouracil	>100	9.7 ± 0.0	>40

[a]All values were obtained at least from two determinations; NT-not tested.

cells. The N^9-linked purine nucleoside **20** was the significant compound comprising a pyranuronamide system, with a GI_{50} value in MCF-7 cells that is *ca*. 1.6 lower that 5-fluorouracil. This compound was however less active that lead compound **B** (Fig. 1), differing only in the 3′-*O*-substituent. Furanosyl uracil (**23**) and purine nucleosides (**24–25**) were active at micromolar order of concentration in K562 and/or MCF-7 cells. Their GI_{50} values in the breast cancer cell line were close or lower to that of the standard drug. The most active compound was the N^7-linked purine derivative **25**, with identical potency than compound **8** towards both cancer cell lines (GI_{50} = 3.3 μM). Its N^9 regioisomer **24** showed however antiproliferative activities in malignant cells similar to those shown in BJ fibroblasts. On the other hand, slight selectivity towards cancer cells was revealed by the uracil nucleoside **23** and purine nucleosides **20** and **25**. Triazole nucleosides **27-α** and **27-β** and their 1-azido glucuronamide precursors **26-α** and **26-β** were devoid of any noticeable activities in the range of concentrations that could be tested.

It is noteworthy to mention that the presence of the uracil and the purine moieties is important to confer activity to the 5′/6′-azido and glucofuranuronamide-based nucleosides, since their 1-*O*-acetyl glycosyl donor precursors **5**, **12** and **22** showed, in comparison, significantly lower effects. An exception to this trend is the activity of the uracil nucleoside **6** in MCF-7 cells, which is similar to that of the 6-azidoglucopyranosyl acetate **5**. In contrast, the *N*-dodecyl 3-*O*-benzyl glucopyranuronamide derivative **18** showed higher antiproliferative effects in both cell lines that its corresponding uracil nucleoside **19** and was *ca*. 2-fold more active than purine nucleoside **20** in K562 cells. In fact, compound **18** revealed antiproliferative activity in MCF-7 cells (GI_{50} = 13.3 μM) similar to that of 5-fluorouracil. The 6-azidoglycosyl acetate **5** and the *N*-dodecyl 3,5-*O*-benzyl glucofuranuronamide derivative **22** also demonstrated significant effects in MCF-7 cells, with GI_{50} values that are only *ca*. 2.5 and 1.8-fold lower than 5-fluorouracil, respectively. Moreover, the glycosyl acetates **5** and **18** showed some selectivity for cancer cells.

The active compounds were subjected to further assays in order to have insights into their mode of action, namely by evaluating their effect on the cell cycle and their ability to induce apoptosis.

Flow cytometry analyses of the cell cycle distributions of K562 cells and MCF-7 cells treated with the azido nucleosides **6–8** and **13–14**, indicated accumulation of sub-G1 cell phase populations (data not shown), which is a typical marker for cellular damage. This event was considerable more visible in K562 cells, especially upon treatment with the most active compound **8**.

The effect of nucleoside **8** on the expression of apoptosis-related proteins in K562 and in MCF-7 cells was then evaluated by imunoblotting. As shown in Fig. 2a, in treated K562 cells, a decreased level of zymogenes of caspase 7 at 5 μM as well as dose-dependent cleavage of substrate poly(ADP-ribose) polymerase-1 (PARP-1), which are typical indicative events of apoptosis [24, 36], were detected. The activation of caspases 3,7 in lysates of treated K562 cells was confirmed by an enzymatic activity assay using a fluorogenic substrate, with a maximum of activity at 16 h (Fig. 2b). Furthermore, a decrease on the expression of the anti-apoptotic short half-life protein Mcl-1 (contrary to stable Bcl-2) and on the expression of the X-linked inhibitor of apoptosis protein (XIAP) at 5 μM, was observed. These results, taken together, clearly suggest that compound **8** is able to induce apoptosis in K562 cells.

Similarly, when MCF-7 cells were treated with **8**, cleavage of PARP-1 was also observed, although in a lesser extend than that observed in K562 cells. Furthermore, no changes in the expression of anti-apoptotic protein XIAP were detected, but Mcl-1 level downregulated again (Fig. 2c).

Among *N*-dodecil glucuronamide-based nucleosides, the most active compound (**25**) was selected for complementary assays in K562 leukemia cells. Flow cytometry analysis of the cycle of cells treated with **25** (5 μM) showed a significant increase of sub-G1 population (cell debris) and continuous decrease of the population of actively replicating cells (BrdU-positive) in a time-dependent manner (Fig. 3a). Surprisingly, this observation was not consistent with the immunoblotting analysis and biochemical measurement of caspase activity, which revealed the maximum of expression level of active fragments of caspase 7 (accompanied by increased cleavage of PARP-1) (Fig. 3c) and caspase activity at 8 h after treatment (Fig. 3d). It seems that treatment of K562 cells with nucleoside **25** led to starting of apoptosis (as documented by activation of caspases) in early time points while in later times the alternative mechanisms of cell death were triggered, as observed for compound **8** at 24 h treatment (Fig. 2b). We can exclude autophagy, because the fragmentation of microtubule-associated protein 1-light chain 3 (LC3) (Fig. 3c) that is considered a marker of autophagic activity in cells did not occur. We further monitored the expression of anti-apoptotic proteins (such as Mcl-1, XIAP, Bcl-2), but no significant changes were observed (Fig. 3c). Analysis of the dose-dependent level of PARP-1 and caspase 7-zymogenes in K562 cells treated with compound **25** for 24 h, revealed a significant dowregulation

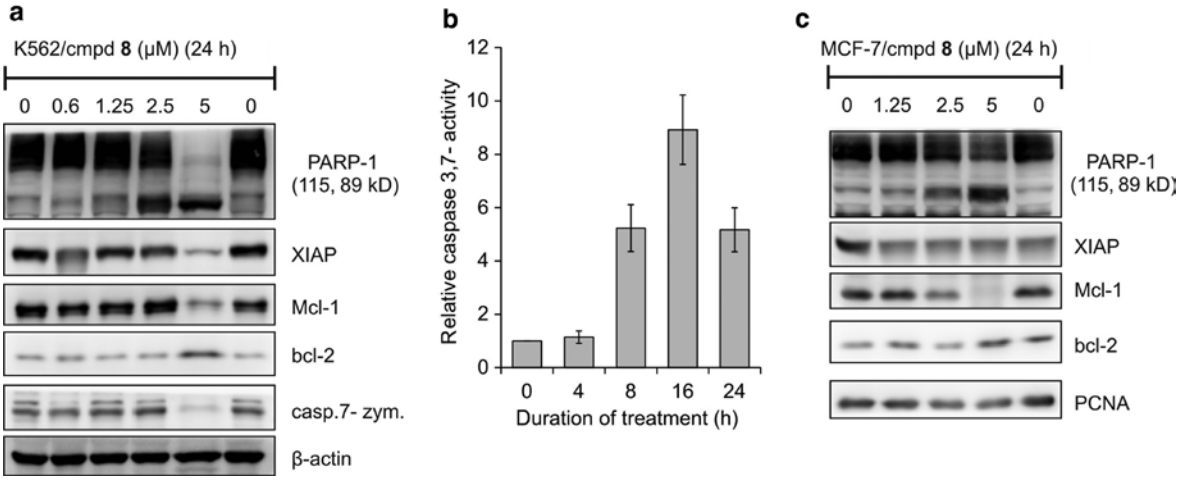


Fig. 2: (a, c) Immunoblotting analysis of apoptosis-related proteins in K562 and MCF-7 cells treated with compound **8** for 24 h. β-Actin and PCNA were used as loading controls. (b) Activity of caspases-3,7 in K562 cell lysates treated with 5 μM dose of compound **8** for indicated times. Caspase activities were measured using the fluorogenic substrate Ac-DEVD-AMC and normalized to an untreated control.

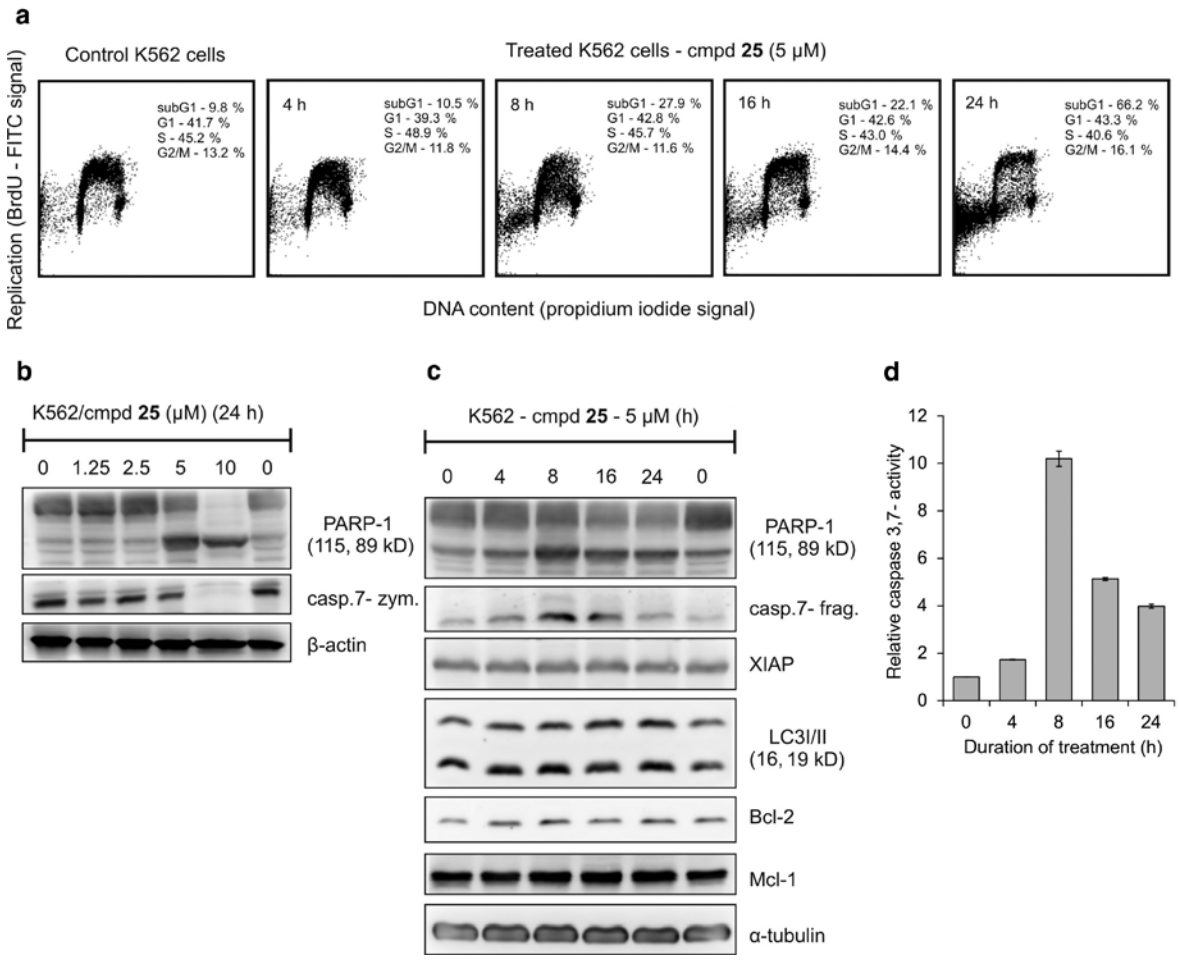


Fig. 3: The antiproliferative and pro-apoptotic activity of compound **25** in the K562 cell line. (a) Cell cycle analyses and replication potential of the cell upon treatment with 5 μM dose of compound **25** for indicated times. Flow cytometric analysis (10 000 counts) of DNA stained by propidium iodide and 5-bromo-2′-deoxyuridine. (b, c) Immunoblotting analysis of apoptosis-related proteins in K562 cells treated with compound **25** for 24 h and upon treatment with 5 μM dose of compound **25** for indicated times. β-Actin and α-tubulin were used as loading controls. (d) Activity of caspases-3,7 in cell lysates treated with 5 μM dose of compound **25** for indicated times.

of these proteins at 10 μM (Fig. 3b). By comparing these effects with those of nucleoside **8**, which provokes a decrease of PARP-1 and caspase 7-zymogenes levels already after treatment with a 5 μM dose, it appears that the nucleoside **25** is less potent than nucleoside **8** in the first 24 h of treatment, although their antiproliferative effects after the following 48 h are similar (Table 1).

Conclusions

Novel furanosyl/pyranosyl azido and glucuronamide-based nucleosides comprising uracil or 2-acetamido-6-chloropurine moieties and a dodecyl chain in the glycosyl unit were synthesized starting from diacetone-D-glucose or 1,2-*O*-isopropylidene glucofuranurono-6,3-lactone. Significant antiproliferative effects were revealed by these types of molecules. N^7-Linked purine nucleosides comprising a 6-azido-3-*O*-dodecyl glucopyranosyl and *N*-dodecyl 3,5-di-*O*-benzyl glucofuranuronamide units (**8** and **25**) were the most active compounds with GI_{50} values of ca. 3 μM in leukemic K562 and breast cancer MCF-7 cells. In particular, their

activity in MCF-7 cells was 3-fold higher that than of the anti-breast cancer drug 5-fluorouracil, which is a significant improvement relatively to the effect of the previously reported azido and glucuronamide-based nucleosides **A** and **B** (Fig. 1) that motivated this study. Uracil nucleosides also demonstrated satisfactory anticancer activities in both cancer cells, with GI_{50} values ranging from 24.1 to 12.5 μM among azido nucleosides. Concerning glucuronamide-based uracil derivatives, the active molecule was a furanosyl nucleoside (**23**) with GI_{50} values of 6.7 μM and 8.6 μM in K562 and MCF-7 cells, respectively, which in case of MCF-7 cells is similar to that of 5-fluorouracil, in addition to showing some cancer cell selectivity. Considering our previous reports [22, 23], in which azido and glucuronamide-based uracil nucleosides did not demonstrate significant anticancer profile, these results show that tuning the glycosyl moieties by simple structural modifications, such as changing the *O*-substituents or the glycosyl ring system, can lead to a considerable change, in this case to an improvement, in the activities of the nucleoside.

Moreover, the antiproliferative activities exhibited by 1-*O*-acetyl *N*-dodecyl glucuronamide derivatives **18** and **22** demonstrate the anticancer capability of glucuronamide derivatives. A noteworthy result is the effect of compound **18** in MCF-7 cells, which is close to that of 5-fluorouracil.

The complementary studies in K562 cells treated with the most potent nucleosides **8** and **25**, showing that both compounds induce or activate apoptosis and **8** leads to downregulation of anti-apoptotic proteins, which are valuable events to fight chemotherapy resistance, further reinforce the potential for nucleosides containing 5/6-azidoglycosyl and *N*-dodecyl glucuronamide moieties as anticancer agents.

Experimental section

Chemistry

General methods

The reactions were monitored by TLC using Merck 60 F_{254} silica gel aluminium plates with detection by UV light (254 nm) and/or by immersion in a 10 % H_2SO_4/EtOH solution of or with 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. Microwave experiments were carried out in 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 or 100.62 MHz for ^{13}C. $CDCl_3$ was used as solvent and chemical shifts are given in parts per million (ppm) relatively to internal TMS or to the residual $CHCl_3$ peak (7.26 ppm), in the case of ^{1}H NMR spectra, and to the $CDCl_3$ peak (77.16 ppm) for ^{13}C NMR spectra. Coupling constants (*J*) are given in hertz (Hz). Signals in the NMR spectra were assigned with the help of two-dimensional experiments (COSY, HSQC, HMBC). High-resolution mass spectra were acquired with a High Resolution QqTOF Impact II mass spectrometer equipped with an ESI ion source (Bruker Daltonics). Spectra were recorded in positive ESI mode. Samples were analyzed by flow injection analysis (FIA) using a isocratic gradient 50 A:50 B of 0.1 % formic acid in water (A) and 0.1 % of formic acid in acetonitrile (B), at a flow rate of 5 μL min^{-1} over 15 min. Calibration of the TOF analyzer was performed with a 10 mM solution of sodium formate. Optical rotations were measured on a Perkin–Elmer 343 polarimeter.

Synthesis of compounds **21**, **22**, **26** and **27** was previously described [23, 35].

3-*O*-Dodecyl-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (2)

To a solution of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**1**, 5.0 g, 19.2 mmol) in anhydrous DMF (35 mL) at 0 °C and under nitrogen atmosphere, NaH (2.35 g, 56.4 mmol) was added. The suspension was stirred at 0 °C for 10 min, whereupon dodecyl bromide (5.5 mL, 22.9 mmol) was added. The mixture was stirred for 17 h

at room temperature. It was then diluted with CH_2Cl_2, 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 residue was purified by column chromatography (EtOAc/cyclohexane, 1:20) to afford **2** (7.65 g, 93 %) as a colorless oil. NMR data were in agreement with the published data [37]. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 5.85$ (d, 1 H, H-1, $^3J_{1,2} = 3{,}6$ Hz), 4.50 (d, 1 H, H-2), 4.28 (ddd ~ q, 1 H, H-5), 4.10 (dd, 1H, H-4, $^3J_{3,4} = 3.1$ Hz, $^3J_{4,5} = 7.4$ Hz), 4.05 (dd, 1 H, part A of ABX system, H-6a, $J_{5,6a} = 6.3$, $J_{6a,6b} = 8.6$), 3.96 (dd, 1 H, part B of AB system, H-6b, $J_{5,6a} = 6.0$), 3.82 (d, 1 H, H-3), 3.61–3.53 (m, 1 H, H-7a), 3.52–3.43 (m, 1H, H-7b), 1.58–1.48 (m, 2 H, C*H*$_2$-8), 1.47 (s, 3 H, C*H*$_3$, *i*-Pr), 1.40 (s, 3 H, CH$_3$, *i*-Pr), 1.36–1.18 (m, 24 H, C*H*$_2$-9 to C*H*$_2$-17, 2×C*H*$_3$, *i*-Pr), 0.85 (t, 3 H, C*H*$_3$-18, $J = 6.8$ Hz) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 111.8$ (Cq, *i*-Pr), 108.9 (Cq, *i*-Pr), 105.4 (C-1), 82.6 (C-2), 82.2 (C-3), 81.3 (C-4), 72.6 (C-5), 70.8 (C-7′), 67.3 (C-6), 32.0, 29.8, 29.8, 29.7, 29.7, 29.7, 29.5, 29.5, 26.9, 26.9, 26.3, 26.2, 25.5 (C-8–C-16, 2×CH_3, *i*-Pr), 22.8 (C-17), 14.2 (C-18) ppm.

3-*O*-Dodecyl-1,2-*O*-isopropylidene-α-D-glucofuranose (3)

A solution of 3-*O*-dodecil-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (5.72 g, 13.3 mmol) in aq. acetic acid (70 % soln., 45 mL) was stirred at room temperature for 5 day. The solvents were co-evaporated with toluene and the residue was subjected to column chromatography (EtOAc/hexane, from 1:3, then 1:1, then EtOAc) to afford 3 (4.05 g, 78 %) as a colorless oil. $\left[\alpha\right]_D^{20} = -46$ (c = 1, in CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 5.91$ (d, 1 H, H-1, $J_{1,2} = 3.7$), 4.55 (d, 1 H, H-2), 4.13 (dd, 1 H, H-4, $J_{3,4} = 3.4$, $J_{4,5} = 7.2$), 4.02 (ddd, 1 H, H-5), 3.92 (d, 1 H, H-3, $J_{3,4} = 3.4$), 3.81 (dd, part A of AB system, H-6a, $J_{5,6a} = 3.7$, $J_{a,b} = 11.3$), 3.71 (dd, part A of AB system, H-6a, $J_{5,6a} = 5.5$), 3.68–3.59 (m, 1 H, H-7a), 3.51–3.43 (m, 1 H, H-7b), 1.61–1.52 (m, 2 H, C*H*$_2$-8), 1.48 (s, 3 H, C*H*$_3$, i-Pr), 1.36–1.19 (m, 21 H, C*H*$_2$-9–C*H*$_2$-17, C*H*$_3$, *i*-Pr), 0.88 (t, 3 H, C*H*$_3$-18, $J = 6.6$) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 111.8$ (Cq, *i*-Pr), 105.1 (C-1), 83.3 (C-3), 82.1 (C-2), 79.9 (C-4), 70.6 (C-7), 69.9 (C-5), 64.5 (C-6), 32.0, 29.9, 29.8, 29.8, 29.7, 29.7, 29.5, 29.5 (C-8–C-15), 26.9, 26.3 (2×CH_3, *i*-Pr), 26.2 (C-16), 22.8 (C-17), 14.3 (C-18) ppm. HRMS: calcd for $C_{21}H_{40}O_6$ $[M + H]^+$ 389.2898, found 389.2899.

6-Azido-6-deoxy-3-*O*-dodecyl-1,2-*O*-isopropylidene-α-D-glucofuranose (4)

To a solution of 3-*O*-dodecyl-1,2-*O*-isopropylidene-α-D-glucofuranose (**3**, 1.7 g, 4.38 mmol) in CH_2Cl_2 and pyridine (40 mL, 1:1) under nitrogen, tosyl chloride (0.96 g, 5 mmol) was added and the solution was stirred at room temperature overnight. Then, the solution was diluted with CH_2Cl_2, water was added and the mixture was washed with a aq. 1 N HCl soln. The aqueous phase was re-extracted with CH_2Cl_2 (2×). The combined organic layers were dried with anhydrous MgSO4. After filtration and evaporation of the solvent under vacuum, the residue was purified by column chromatography (EtOAc/petroleum ether, 1:2) to furnish the 6-*O*-tosylated derivative (1.95 g, 3.5 mmol). It was then dissolved in *N*,*N*-dimethylformamide (DMF, 43 mL) and sodium azide (860 mg, 13.2 mmol) was added. The mixture was stirred at room temperature overnight. Then, water was added and the mixture was extracted with EtOAc. The combined organic layers were washed with water and dried with anhydrous MgSO4. After filtration and evaporation of the solvent, the residue was subjected to column chromatography (EtOAc/petroleum ether, 1:4) to afford **4** (1.15 g, 64 %, 2 steps) as a colorless oil. $\left[\alpha\right]_D^{20} = -33$ (c = 1, in CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 5.89$ (d, 1 H, H-1, $J_{1,2} = 3.7$), 4.55 (d, 1 H, H-2), 5.15 (t, 0.7 H, H-2 β), 4.14–4.05 (m, 2 H, H-4, H-5), 3.98 (d, 1 H, H-3, $J_{3,4} = 2.2$), 3.69–3.53 (m, 2 H, H-6a, H-7a), 3.51–3.40 (m, 2 H, H-6b, H-7′b), 2.76 (br.s, 1 H, O*H*), 1.61–1.52 (m, 2 H, C*H*$_2$-8), 1.48 (s, 3 H, C*H*$_3$, i-Pr), 1.35–1.19 (m, 21 H, C*H*$_2$-9–C*H*$_2$-17, C*H*$_3$, *i*-Pr), 0.87 (t, 3 H, C*H*$_3$-18, $J = 6.6$) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 112.0$ (Cq, *i*-Pr), 105.2 (C-1), 83.0 (C-3), 82.0 (C-2), 79.9 (C-4), 70.6 (C-7), 69.1 (C-5), 54.6 (C-6), 32.0, 29.8, 29.7, 29.7, 29.7, 29.6, 29.5, 29.5 (C-8–C-15), 26.9, 26.4 (2×CH_3, *i*-Pr), 26.2 (C-16), 22.8 (C-17), 14.2 (C-18) ppm.

1,2,4-Tri-*O*-acetyl-6-azido-6-deoxy-3-*O*-dodecyl-α,β-D-glucopyranose (5-α,β)

A solution of 6-azido-6-deoxy-3-*O*-dodecyl-1,2-*O*-isopropylidene-α-D-glucofuranose (**4**, 1.1 g, 2.66 mmol) in aqueous trifluoroacetic acid (60 %, 14.5 mL) was stirred at room temperature for 1 h. The solvents were

co-evaporated with toluene and the residue was dried under vacuum. It was then treated with pyridine (10 mL) and acetic anhydride (7.7 mL) and the mixture was stirred at room temperature for 1 h. The solvents were co-evaporated with toluene and the residue was purified by flash column chromatography (EtOAc/petroleum ether, 1:2) to give **5-α,β** (1.27 g, 96 %, 2 steps, anomeric mixture, α/β ratio, 1:0.6) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 6.23$ (d, 1H, H-1α, $J_{1,2\,(\alpha)} = 3.7$), 5.58 (d, 0.6 H, H-1 β, $J_{1,2\,(\beta)} = 8.3$), 5.00 (t, 0.6 H, H-2 β), 4.97–4.84 (m, 2.6 H, H-2 α, H-4 α, H-4 β), 3.90 (ddd, 1 H, H-5 α, $J_{4,5\,(\alpha)} = 9.5$), 3.70 (t, H-3 α, $J_{2,3} \sim J_{3,4} \sim 9.7$), 3.64 (ddd, 0.6 H, H-5 β), 3.59–3.40 (m, 3.8 H, H-3 β, CH_2-7 α, CH_2-7 β), 3.32–3.17 (m, 3.2 H, CH_2-6 α, CH_2-6 β), 2.10, 2.04, 2.03, 2.02, 2.01, 1.98 (6 s, 9.6 H, 2 × CH_3, Ac, α, β), 1.45–1.34 (m, 3.2 H, CH_2-8, α, β), 1.26–1.11 (m, 28.8 H, CH_2-8–CH_2-17, α, β), 0.81 (t, 4.8 H, CH_3-18, α, β, $J = 6.7$) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 169.5$, 169.3, 169.3, 169.0, 169.0, 168.7 (*C*O, Ac, α, β), 91.7 (C1-β), 89.1 (C1-α), 80.0 (C-3 β), 76.7 (C-3 α), 74.1 (C-5 β), 73.1, 72.7 (C-7), 71.3, 71.3, 71.2 (C-5 α, C-2 α, C-2 β), 70.3, 70.3 (C-4 α, C-4 β), 58.0, 50.8 (C-6 α, C-6 β), 31.9 (C-7 α, β), 30.2, 30.1, 29.6, 29.5, 29.5, 29.5, 29.4, 29.4, 29.3, 25.9, 25.9, 22.6 (C-8–C-17, α, β), 20.8, 20.7, 20.7, 20.7 (*C*H$_3$, Ac, α, β), 14.0 (C-18 α, β) ppm. HRMS: calcd for $C_{24}H_{41}N_3O_8$ $[M+H]^+$ 500.2966, found 500.2966; calcd for $C_{24}H_{41}N_3O_8$ $[M+Na]^+$ 522.2786, found 522.2783.

General procedure for *N*-glycosylation of 2-acetamido-6-chloropurine or uracil with 1-*O*-acetyl glycosyl donors

To a suspension of the nucleobase (1.5 equiv.) in acetonitrile (1.8 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 (0.20 mmol) in acetonitrile (3.7 mL) was added to the previous solution, followed by dropwise addition of trimethylsilyl triflate (TMSOTf, 5.5–7.5 equiv.). The resulting mixture was stirred under microwave irradiation (150 W, P max = 250 Psi) at 65 °C for 10–30 min. 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.

1-(2,4-di-*O*-Acetyl-6-azido-1,6-dideoxy-3-*O*-dodecyl-β-D-glucopyranosyl)uracil (6)

Obtained according to the general procedure, starting from 1,2,4-tri-*O*-acetyl-6-azido-6-deoxy-3-*O*-dodecyl-α,β-D-glucopyranose (**5-α,β**, 101 mg, 0.2 mmol) and uracil (34 mg, 0.3 mmol) and using BSA (0.15 mL, 0.6 mmol) and TMSOTf (0.27 mL, 1.5 mmol). The reaction mixture was exposed to MW conditions for 10 min. Purification by column chromatography (EtOAc/hexane, 1:1) afforded **6** (93 mg, 83 %) as a pale-yellow oil. $[\alpha]_D^{20} = +50$ (c = 1, in CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 9.67$ (br. s, 1 H, N*H*), 7.35 (d, H-6, $J_{5,6} = 8.3$), 5.83–5.77 (m, 2 H, H-1, H-5), 5.12–5.02 (m, 2 H, H-2′, H-4′, $J_{1',2'} = J_{2',3'} = 9.3$, $J_{3',4'} = J_{4',5'} = 9.7$), 3.77 (ddd, 1 H, H-5′), 3.68 (t, 1 H, H-3′), 3.61–3.49 (m, 2 H, CH_2-7′), 3.40 (dd, part A of ABX system, 1 H, H-6′a, $J_{5',6'a} = 2.5$, $J_{6'a,6'b} = 13.7$), 3.25 (dd, part B of ABX system, 1 H, H-6′b, $J_{5',6'b} = 5.7$), 2.10, 2.03 (2 s, 2 × 3 H, CH_3, 2 × OAc), 1.51–1.40 (m, 2 H, CH_2-8′), 1.32–1.16 (m, 18 H, CH_2-9′–CH_2-17′), 0.85 (t, 3 H, CH_3-18′, $J = 6.6$) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 169.5$, 169.4 (2 × *C*O, Ac), 163.0 (C-4), 150.8 (C-2), 139.4 (C-6), 103.8 (C-5), 80.4 (C-1′), 80.4 (C-3′), 76.2 (C-5′), 73.6 (C-7′), 71.2, 70.1 (C-2′, C-4′), 50.8 (C-6′), 32.0, 30.2, 29.7, 29.7, 29.7, 29.6, 29.5, 29.4, 26.2, 22.7 (C-8′–C-17′), 20.9, 20.6 (2 × *C*H$_3$, 2 × OAc), 14.2 (C-18′) ppm. HRMS: calcd for $C_{24}H_{41}N_5O_8$ $[M+H]^+$ 552.3028, found 552.3027; calcd for $C_{24}H_{41}N_5O_8$ $[M+Na]^+$ 574.2847, found 574.2841.

2-Acetamido-9-(2,4-di-*O*-acetyl-6-azido-1,6-dideoxy-3-*O*-dodecyl-β-D-glucopyranosyl)-6-chloropurine (7) and 2-acetamido-7-(2,4-di-*O*-acetyl-6-azido-1,6-dideoxy-3-*O*-dodecyl-β-D-glucopyranosyl)-6-chloropurine (8)

Obtained according to the general procedure, starting from 1,2,4-tri-*O*-acetyl-6-azido-6-deoxy-3-*O*-dodecyl-α,β-D-glucopyranose (**5-α,β**, 138 mg, 0.28 mmol) and 2-acetamido-6-chloropurine (88 mg, 0.41 mmol) and

using BSA (0.2 mL, 0.83 mmol) and TMSOTf (0.3 mL, 1.7 mmol). The reaction mixture was exposed to MW conditions for 20 min. Purification by column chromatography (AcOEt/cyclohexane, from 1:4, then 1:1) afforded the N^9 nucleoside **7** (57 mg, 32 %) and the N^7 regioisomer **8** (73 mg, 41 %) as pale-yellow oils.

Data for 7

$[\alpha]_D^{20} = +10$ (c = 0.5, in CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 8.41$ (br.s, 1 H, N*H*), 8.20 (s, H-8), 7.38–7.20 (m, 5 H, Ph), 5.74 (d, 1 H, H-1′, $J_{1',2'} = 9.4$), 5.50 (t, 1 H, H-2′, $J_{1',2'} = J_{2',3'} = 9.4$), 5.16 (t, 1 H, H-4′, $J_{3',4'} = J_{4',5'} = 9.5$), 3.86 (ddd, 1 H, H-5′), 3.77 (t, 1 H, H-3′), 3.53 (t, 2 H, CH_2-7′, $J = 6.2$), 3.40 (dd, part A of ABX system, 1 H, H-6′a, $J_{5',6'a} = 2.2$, $J_{6'a,6'b} = 13.7$), 3.32 (dd, part B of ABX system, 1 H, H-6′b, $J_{5',6'b} = 5.9$), 2.54 (s, 3 H, CH_3, NHAc), 2.14, 1.85 (2 s, 2 × 3 H, CH_3, 2 × OAc), 1.53–1.41 (m, 2 H, CH_2-8′), 1.32–1.15 (m, 18 H, CH_2-9′ to CH_2-17′), 0.86 (t, 3 H, CH_3-18′, $J = 6.5$) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 170.6$ (*C*O, NHAc), 169.4 (*C*O, Ac-4), 169.0 (*C*O, Ac-2), 152.8 (C-4), 152.4, 151.2 (C-2, C-6), 142.4 (C-8), 127.9 (C-5), 80.9 (C-1′), 80.5 (C-3′), 76.5 (C-5′), 73.5 (C-7′), 71.7 (C-2′), 70.2 (C-4′), 50.9 (C-6′), 32.0, 30.3, 29.7, 29.7, 29.7, 29.5, 29.4, 26.1 (C-8′–C-16′), 25.3 (*C*H$_3$, NHAc), 22.8 (C-17′), 20.9, 20.5 (2 × *C*H$_3$, 2 × OAc), 14.2 (C-18′) ppm. HRMS: calcd for $C_{29}H_{43}ClN_8O_7$ $[M+H]^+$ 651.3016, found 651.3016; calcd for $C_{29}H_{43}ClN_8O_7$ $[M+Na]^+$ 673.2835, found 673.2835.

Data for 8

$[\alpha]_D^{20} = +2$ (c = 1, in CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 8.47$ (s, 1 H, H-8), 8.27 (br. s, 1 H, N*H*), 6.02 (br.s, 1 H, H-1′), 5.57 (br.t, 1 H, H-2′), 5.14 (t, 1 H, H-4′, $J_{3',4'} = J_{4',5'} = 9.5$), 3.88 (ddd, 1 H, H-5′), 3.76 (t, 1 H, H-3′, $J_{2',3'} \sim J_{3',4'}$), 3.59 (t, 2 H, CH_2-7′, $J = 6.0$), 3.43–3.30 (m, CH_2-6′), 2.59 (s, 3 H, CH_3, NHAc), 2.13 (s, 3 H, CH_3, OAc-4), 1.94 (s, 3 H, CH_3, OAc-2), 1.56–1.40 (m, 2 H, CH_2-8′), 1.34–1.13 (m, 18 H, CH_2-9′ to CH_2-17′), 0.86 (t, 3 H, CH_3-18′, $J = 6.5$) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 171.3$ (*C*O, NHAc), 169.4 (*C*O, Ac-4), 169.0 (*C*O, Ac-2), 163.5 (C-4), 153.0 (C-2 or C-6), 147.5 (C-8), 143.4 (C-2 or C-6), 118.6 (C-5), 82.6 (C-1′), 80.9 (C-3′), 76.6 (C-5′), 73.6 (C-7′), 71.2 (C-2′), 70.3 (C-4′), 60.0 (C-6′), 32.0, 30.3, 29.8, 29.7, 29.7, 29.7, 29.5, 29.4, 26.1 (C-8′–C-16′), 25.4 (*C*H$_3$, NHAc), 22.8 (C-17′), 20.9, 20.5 (2 × *C*H$_3$, 2 × OAc), 14.2 (C-18′). HRMS: calcd for $C_{29}H_{43}ClN_8O_7$ $[M+H]^+$ 651.3016, found 651.3019; calcd for $C_{29}H_{43}ClN_8O_7$ $[M+Na]^+$ 673.2835, found 673.2840.

3-*O*-Dodecyl-1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose (9)

To a solution of 3-*O*-dodecyl-1,2-*O*-isopropylidene-α-D-glucofuranose (**3**, 1.48 g, 3.8 mmol) in 60 % aq. THF (13 mL), at 0 °C, sodium metaperiodate (1.63 g, 7.6 mmol) was added. The mixture was stirred for 2.5 h at room temperature The mixture was diluted with EtOAc, washed with water and brine solution, and the aqueous phase was extracted with EtOAc (3×). The combined organic layers were dried with anhydrous $MgSO_4$. After filtration, evaporation of the solvent and drying under vacuum, **9** (1,073 g, 79 %) was obtained as a white hygroscopic solid. $[\alpha]_D^{20} = -55$ (c = 1.1, in CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 9.66$ (d, 1 H, C*H*O, $J = 1.5$), 6.09 (d, $^3J_{1,2} = 3.4$ Hz, 1 H, 1-H), 4.58 (d, 1 H, 2-H), 4.53 (dd, $^3J_{3,4} = 3.7$ Hz, $^3J_{4,5} = 1.4$ Hz, 1H, 4-H), 4.18 (d, 1 H, 3-H), 3.57-3.47 (m, 1 H, H-7a), 3.41–3.30 (m, 1 H, H-7b), 1.51–1.41 (m, 5 H, CH_3, *i*-Pr, CH_2-8), 1.35–1.16 (m, 21 H, CH_3, *i*-Pr, CH_2-9 to CH_2-17), 0.86 (t, 3 H, CH_3-18′, $J = 6.8$) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 200.2$ (*C*O), 112.6 (Cq, *i*-Pr), 106.4 (C-1), 84.8, 84.8 (C-3, C-4), 82.4 (C-2), 71.1 (C-7), 32.0, 29.8, 29.7, 29.7, 29.6, 29.6, 29.5, 29.4 (C-8–C-15), 27.1, 26.5 (2 × *C*H$_3$, *i*-Pr), 26.0 (C-16), 22.8 (C-17), 14.3 (C-18) ppm. HRMS: calcd for $C_{20}H_{36}O_5$ $[M+H]^+$ 357.2636, found 357.2636.

3-*O*-Dodecyl-1,2-*O*-isopropylidene-α-D-xylofuranose (10)

To a solution of 3-*O*-dodecyl-1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose (**9**, 1.03 g, 2.89 mmol) in EtOH/H_2O (15 mL, 2:1) at 0 °C, $NaBH_4$ (0.142 g, 3.75 mmol) was added. The mixture was stirred at room temperature for 1 h. Then, EtOAc and water were added, the phases were separated and the aqueous phase was extracted twice with EtOAc. The combined organic layers were dried with anhydrous $MgSO_4$ and concentrated under vacuum to afford **10** (0.84 g, 81 %) as a colorless oil. $[\alpha]_D^{20} = -35$ (c = 1.1, in CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz):

δ = 5.97 (d, $^3J_{1,2}$ = 3.8 Hz, 1 H, 1-H), 4.56 (d, 1 H, H-2), 4.27 (ddd ~ q, 1H, 4-H), 3.95 (dd, part A of ABX system, 1 H, H-5a, $^2J_{5a,5b}$ = 12.1 Hz, $^3J_{4,5a}$ = 4.7 Hz), 3.93–3.84 (m, 2 H, H-3, H-5b, $^3J_{3,4}$ = 3.5 Hz, $^3J_{4,5b}$ = 4.0 Hz), 3.68–3.58 (m, 1 H, H-7a), 3.47–3.37 (m, 1 H, H-7b), 2.44 (br.s, 1 H, 5-OH), 1.60–1.51 (m, 2 H, CH_2-8), 1.49 (s, 3 H, CH_3, *i*-Pr), 1.35–1.20 (m, 21 H, CH_3, *i*-Pr, CH_2-9 to CH_2-17), 0.87 (t, 3 H, CH_3-18, *J* = 6.8) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 111.7 (Cq, *i*-Pr), 105.1 (C-1), 84.2 (C-3), 82.4 (C-2), 80.0 (C-4), 70.6 (C-7), 61.1 (C-5), 32.0, 29.7, 29.7, 29.7, 29.6, 29.4, 29.4 (C-8–C-15), 26.9, 26.4 (2 × CH_3, *i*-Pr), 26.1 (C-16), 22.8 (C-17), 14.2 (C-18) ppm. HRMS: calcd for $C_{20}H_{38}O_5$ $[M+H]^+$ 359.2792, found 359.2802.

5-Azido-5-deoxy-3-*O*-dodecyl-1,2-*O*-isopropylidene-α-D-xylofuranose (11)

To a solution of 3-*O*-dodecyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**10**, 0.51 g, 1.42 mmol) in CH_2Cl_2 and pyridine (15 mL, 1:1) under nitrogen, tosyl chloride (0.53 g, 2.8 mmol) was added and the solution was stirred at room temperature overnight. Then, the solution was diluted with CH_2Cl_2, water was added and the mixture was washed with a aq. 1 N HCl soln. The aqueous phase was re-extracted with CH_2Cl_2 (2×). The combined organic layers were dried with anhydrous $MgSO_4$. After filtration and evaporation of the solvent under vacuum, the residue was purified by column chromatography (EtOAc/hexane, 1:6) to furnish the 6-*O*-tosylated derivative (0.642 g, 1.25 mmol) as a white solid. It was then dissolved in *N*,*N*-dimethylformamide (DMF, 15 mL) and sodium azide (244 mg, 3.75 mmol) was added. The mixture was stirred at 80 °C overnight. Then, water was added and the mixture was extracted with EtOAc. The combined organic layers were washed with water and dried with anhydrous MgSO4. After filtration and evaporation of the solvent, the residue was subjected to column chromatography (EtOAc/hexane, 1:9) to afford **11** (0.421 g, 77 %, 2 steps) as a colorless oil. $\left[\alpha\right]_D^{20} = -26$ (c = 1, in CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.89 (d, 1 H, H-1, $^3J_{1,2}$ = 3.8 Hz), 4.56 (d, 1 H, H-2), 4.28 (td, 1H, H-4, $J_{4,5}$ = 6.6, $J_{3,4}$ = 3.3), 3.65–3.55 (m, 1 H, H-7a), 3.51 (d, 2 H, CH_2-5, $^3J_{4,5}$ = 6.6 Hz), 1.61–1.51 (m, 2 H, CH_2-8), 1.49 (s, 3 H, CH_3, *i*-Pr), 1.36–1.19 (m, 21 H, CH_3, *i*-Pr, CH_2-9–CH_2-17), 0.87 (t, 3 H, CH_3-18, *J* = 6.8) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 111.9 (Cq, *i*-Pr), 105.2 (C-1), 82.2, 82.2 (C-2, C-3), 78.9 (C-4),70.5 (C-7), 49.1 (C-5), 32.1, 29.8, 29.8, 29.7, 29.7, 29.5, 29.5 (C-8–C-15), 27.0, 26.4 (2 × CH_3, *i*-Pr), 26.2 (C-16), 22.8 (C-17), 14.3 (C-18) ppm. HRMS: calcd for $C_{20}H_{37}N_3O_4$ $[M+Na]^+$ 406.2676, found 406.2674.

1,2-Di-*O*-acetyl-5-azido-5-deoxy-3-*O*-dodecyl-α,β-D-xylofuranose (12-α,β)

A solution of 5-azido-5-deoxy-3-*O*-dodecyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**11**, 275 mg, 0.72 mmol) in aqueous trifluoroacetic acid (60 %, 4.9 mL) was stirred at room temperature for 2 h. The solvents were co-evaporated with toluene and the residue was dried under vacuum. It was then treated with pyridine (3 mL) and acetic anhydride (2 mL) and the mixture was stirred at room temperature for 1 h. The solvents were co-evaporated with toluene and the residue was purified by flash column chromatography (EtOAc/petroleum ether, 1:3) to give **12-α,β** (129 mg, 42 %, two steps, anomeric mixture, α/β ratio, 1:0.3) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.37 (d, 1 H, H-1 α, $J_{1,2(\alpha)}$ = 4.5), 6.08 (br. s, 0.3 H, H-1 β), 5.21–5.15 (m, 1.3 H, H-2 α, H-2 β), 4.42–4.34 (m, 1.3 H, H-4 α, H-4 β), 4.12 (t, 1 H, H-3α, $J_{2,3} \sim J_{3,4} \sim 5.5$), 3.90 (br. d, 1 H, H-3 β), 3.68–3.31 (m, CH_2-5, CH_2-7, α, β), 2.08, 2.07, 2.06, 2.04 (4 s, 7.8 H, CH_3, OAc, α, β), 1.59–1.47 (m, 2.6 H, CH_2-8, α, β), 1.34–1.13 (m, 23.4 H, CH_2-9′–CH_2-17′, α, β), 0.85 (t, 3 H, CH_3-18, α, β, *J* = 6.5) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 169.7, 169.6, 169.4 (*C*O, Ac, α, β), 99.5 (C-1 β), 93.9 (C-1 α), 81.9 (C-4 β), 80.8 (C-3 β), 80.1 (C-3 α), 79.2 (C-2 β), 77.9 (C-4 α), 76.4 (C-2 α), 71.0, 70.6 (C-7), 50.4, 50.2 (C-5, α, β), 32.0, 29.8, 29.7, 29.7, 29.7, 29.6, 29.5, 29.4, 26.2, 26.1, 25.9, 22.8 (C-8 to C-17, α, β), 21.2, 21.0, 20.9, 20.6 (*C*H_3, Ac, α, β), 14.2 (C-18 α, β) ppm. HRMS: calcd for $C_{21}H_{37}N_3O_6$ $[M+H]^+$ 428.2755, found 428.2755; calcd for $C_{21}H_{37}N_3O_6$ $[M+Na]^+$ 450.2575, found 450.2579.

1-(2-*O*-Acetyl-5-azido-1,5-dideoxy-3-*O*-dodecyl-β-D-xylofuranosyl)uracil (13)

Obtained according to the general procedure for nucleosidation, starting from 1,2-di-*O*-acetyl-5-azido-5-deoxy-3-*O*-dodecyl-α,β-D-xylofuranose (**12-α,β**, 60 mg, 0.14 mmol) and uracil (24 mg, 0.21 mmol) and using BSA (0.1 mL, 0.42 mmol) and TMSOTf (0.19 mL, 1.05 mmol). The reaction mixture was exposed to MW conditions

for 20 min. Purification by column chromatography (EtOAc/hexane, 1:1) afforded **13** (9 mg, 13 %) as a yellow oil, along with the recovered starting material (17 mg, 28 %). $[\alpha]_D^{20} = -5$ (c = 0.4, in CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 8.63$ (br. s, 1 H, NH), 7.59 (d, 1 H, H-6, $J_{5,6} = 8.2$), 6.11 (br.s, 1 H, H-1′), 5.74 (d, 1 H, H-5), 5.09 (br.s, 1 H, H-2′), 4.25 (ddd, 1 H, H-4′), 3.81 (d, 1 H, H-3′, $J_{3',4'} = 2.8$), 3.76–3.61 (m, 3 H, CH_2-5, H-7′a), 3.53–3.45 (m, 1 H, H-7′b), 2.15 (s, 3 H, CH_3, OAc), 1.62–1.48 (m, 2 H, CH_2-8′), 1.38–1.17 (m, 18 H, CH_2-9′–CH_2-17′), 0.88 (t, 3 H, CH_3-18′, $J = 6.5$) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 169.7$ (*C*O, Ac), 162.8 (C-4), 150.0 (C-2), 140.3 (C-6), 102.8 (C-5), 88.9 (C-1′), 81.0 (C-4′), 80.7 (C-3′), 79.5 (C-2′), 70.7 (C-7′), 48.9 (C-5′), 32.1, 30.5, 29.8, 29.7, 29.5, 29.5, 26.3, 22.8 (C-8′ to C-17′), 20.9 (*C*H$_3$, Ac), 14.3 (C-18′) ppm. HRMS: calcd for $C_{23}H_{37}N_5O_6$ $[M+H]^+$ 480.2817, found 480.2826; calcd for $C_{23}H_{37}N_5O_6$ $[M+Na]^+$ 502.2636, found 502.2643.

2-Acetamido-9-(2-*O*-acetyl-5-azido-1,5-dideoxy-3-*O*-dodecyl-β-D-xylofuranosyl)-6-chloropurine (14)

Obtained according to the general procedure for nucleosidation, starting from 1,2-di-*O*-acetyl-5-azido-5-deoxy-3-*O*-dodecyl-α,β-D-xylofuranose (**12-α,β**, 69 mg, 0.16 mmol) and 2-acetamido-6-chloropurine (50 mg, 0.24 mmol) and using BSA (0.12 mL, 0.48 mmol) and TMSOTf (0.16 mL, 0.88 mmol). The reaction mixture was exposed to MW conditions for 20 min. Purification by column chromatography (EtOAc/cyclohexane, from 1:4, then 1:1) afforded **14** (33 mg, 35 %) as a colorless oil. $[\alpha]_D^{20} = +3$ (c = 0.14, in CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 8.30$ (s, 1 H, H-8), 8.11 (br.s, 1 H, N*H*), 6.22 (br.s, 1 H, H-1′), 5.37 (br.s, 1 H, H-2′), 4.41 (td, 1 H, H-4′, $J_{3',4'} = 3.6$, $J_{4',5'a} = J_{4',5'b} = 6.5$), 3.96 (d, 1 H, H-3′), 3.73–3.64 (m, 3 H, CH_2-5, H-7′a), 3.55–3.47 (m, 1 H, H-7′b), 2.54 (s, 3 H, CH_3, NHAc), 2.19 (s, 3 H, CH_3, OAc), 1.62–1.49 (m, 2 H, CH_2-8′), 1.36–1.17 (m, 18 H, CH_2-9′–CH_2-17′), 0.87 (t, 3 H, CH_3-18′, $J = 6.5$) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 170.8$ (*C*O, NHAc), 169.5 (*C*O, OAc), 152.3 (C-2 or C-6), 152.2 (C-4), 151.5 (C-2 or C-6), 142.1 (C-8), 128.0 (C-5), 88.9 (C-1′), 81.9 (C-4′), 80.8 (C-3′), 79.5 (C-2′), 71.2 (C-7′) 49.1 (C-5′), 32.1, 29.8, 29.7, 29.7, 29.6, 29.5, 29.5, 26.1 (C-8′ to C-16′), 25.3 (*C*H$_3$, NHAc), 22.8 (C-17′), 20.9 (*C*H$_3$, 2 × OAc), 14.3 (C-18′) ppm. HRMS: calcd for $C_{26}H_{39}ClN_8O_5$ $[M+H]^+$ 579.2805, found 579.2814; calcd for $C_{26}H_{39}ClN_8O_5$ $[M+Na]^+$ 601.2624, found 601.2629.

N-Dodecyl 1,2-di-*O*-isopropylidene-5-*O*-trityl-α-D-glucofuranuronamide (16)

To a solution of 1,2-di-*O*-isopropylidene-α-D-glucofuranurono-6,3-lactone (**15**, 1.2 g, 5.55 mmol) in pyridine/CH_2Cl_2 (12.5 mL, 2.5:1), under nitrogen, trityl chloride (2.3 g, 8.25 mmol) and 4-dimethylaminopyridine (DMAP, 135 mg, 1.11 mmol) were added. The solution was stirred overnight at 40 °C. 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 (12 mL) and dodecylamine (86 mg, 0.46 mmol) was added. The solution was stirred overnight at room temperature. The solvent was evaporated and the residue was subjected to column chromatography (EtOAc/hexane, 1:2) to afford **16** (1.61 g, 45 %, 2 steps) as a white foam. ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 7.44$–7.26 (m, 15 H, Ph), 6.90 (t, 1 H, N*H* = 5.8), 6.64 (d, 1 H, O*H*, $J = 6.8$), 5.74 (d, 1 H, H-1, $J_{1,2} = 3.4$), 4.62 (d, 1 H, H-5), 4.35 (d, 1 H, H-2), 3.90 (dd, 1 H, H-4, $J_{3,4} = 3.6$), 3.70 (dd, 1 H, H-3), 3.25–2.98 (m, 2 H, CH_2-7), 1.50–1.37 (m, 2 H, CH_2-8), 1.35–1.16 (m, 24 H, CH_2-9 to CH_2-17, 2 × CH_3, *i*-Pr), 0.88 (t, 3 H, CH_3-18, $J = 6.5$) ppm. HRMS: calcd for $C_{40}H_{53}NO_6$ $[M+Na]^+$ 666.3765, found 666.3776.

N-Dodecyl 3-*O*-benzyl-1,2-di-*O*-isopropylidene-5-*O*-trityl-α-D-glucofuranuronamide (17)

To a solution of *N*-dodecyl 1,2-di-*O*-isopropylidene-5-*O*-trityl-α-D-glucofuranuronamide (**16**, 615 g, 0.96 mmol) in anhydrous DMF (10 mL) at 0 °C and under nitrogen atmosphere, NaH (60 %, 100 mg, 2.5 mmol) was added. The suspension was stirred at 0 °C for 10 min, whereupon benzyl bromide (0.2 mL, 1.68 mmol) was added. The mixture was stirred for 4 h at room temperature it was then diluted with CH_2Cl_2, 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 residue was purified by column chromatography (EtOAc/petroleum ether, 1:3) to afford **17** (267 g, 38 %) as a colorless oil. $[\alpha]_D^{20} = -25$ (c = 1, in CH_2Cl_2).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.48–7.13 (m, 20 H, Ph), 5.91 (d, 1 H, H-1, $J_{1,2}$ = 3.7), 5.79 (t, 1 H, N*H* = 5.3), 4.62–4.53 (m, 2 H, H-2, H-a from C*H*$_2$Bn), 4.37 (d, 1 H, H-5, $J_{4,5}$ = 4.6), 4.26 (dd app. t, 1 H, H-4), 4.14 (d, part B of AB system, H-b from C*H*$_2$Ph, $J_{a,b}$ = 11.2), 3.77 (d, 1 H, H-3, $J_{3,4}$ = 3.4), 2.97–2.71 (m, 2 H, C*H*$_2$-7), 1.44 (s, 3 H, C*H*$_3$, *i*-Pr), 1.35–1.04 (m, 23 H, C*H*$_2$-8–C*H*$_2$-17, C*H*$_3$, *i*-Pr), 0.89 (t, 3 H, C*H*$_3$-18, *J* = 6.7) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 169.8 (*C*O), 141.4 (Cq, Ph from Tr), 137.5 (Cq, Ph), 129.3, 128.5, 128.0, 127.9, 127.4 (*C*H, Ph), 111.8 (Cq, *i*-Pr), 104.9 (C-1), 88.8 (Cq, Tr), 82.8 (C-3), 81.7 (C-2), 81.5 (C-4), 75.5 (C-5), 71.8 (*C*H$_2$, Bn), 39.2 (C-7), 32.0, 29.8, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 27.1, 27.0, 26.5 (C-8–C-16, 2 × *C*H$_3$, *i*-Pr), 22.8 (C-17), 14.3 (C-18) ppm. HRMS: calcd for $C_{47}H_{59}NO_6$ $[M + Na]^+$ 756.4235, found 756.4242.

N-Dodecyl 1,2,4-tetra-*O*-acetyl-3-*O*-benzyl-α,β-D-glucopyranuronamide (18-α,β)

A solution of *N*-dodecyl 3-*O*-benzyl-1,2-di-*O*-isopropylidene-5-*O*-trityl-α-D-glucofuranuronamide (**17**, 264 mg, 0.36 mmol) in aqueous trifluoroacetic acid (60 %, 2.5 mL) was stirred at room temperature for 2 h. The solvents were co-evaporated with toluene and the residue was dried under vacuum. It was then treated with pyridine (1.5 mL) and acetic anhydride (1 mL) and the mixture was stirred at room temperature for 1 h. The solvents were co-evaporated with toluene and the residue was purified by flash column chromatography (EtOAc/hexane, from 1:3 to 1:2) to give **18-α,β** (195 mg, 94 %, two steps, anomeric mixture, α/β ratio, 1:0.65) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.35–7.19 (m, 8.25 H, C*H*, Ph), 6.56 (t, 1 H, N*H* α, *J* = 5.4), 6.33 (t, 0.65 H, N*H* β, *J* = 5.5), 6.29 (d, 1 H, H-1 α, $J_{1,2\,\alpha}$ = 3.2), 5.68 (d, 0.65 H, H-1 β, $J_{1,2\,\beta}$ = 8.0), 5.26–5.15 (m, 1.65 H, H-4 α, H-4 β), 5.11 (t, 0.65 H, H-2 β, $J_{2,3\,\beta} \sim J_{1,2\,\beta}$), 5.00 (d, 1 H, H-2 α, $J_{1,2\,\alpha}$ = 3.5, $J_{2,3\,\alpha}$ = 9.0), 4.74–4.56 (m, 3.3 H, C*H*$_2$Ph, α, β), 4.25 (d, 1 H, H-5 α, $J_{4,5\,\alpha}$ = 9.1), 4.03–3.95 (m, 1.65 H, H-3 α, H-5 β, $J_{4,5\,\beta}$ = 9.1), 3.77 (t, 0.65 H, H-3 β, $J_{2,3\,\beta} \sim J_{3,4\,\beta} \sim$ 8.6), 3.29–3.07 (m, 2.6 H, C*H*$_2$-7), 2.14, 2.10, 2.06, 2.03, 2.01, 1.96 (8 s, C*H*$_3$, Ac, α, β), 1.51–1.40 (m, 3.3 H, C*H*$_2$-8), 1.32–1.18 (m, 29.7 H, C*H*$_2$-9–C*H*$_2$-17), 0.86 (t, 3.9 H, CH$_3$-18, *J* = 6.8) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 169.8, 169.7, 169.6, 169.3, 169.1, 169.1 (CO, α, β), 166.9, 166.5 (CO, amide, α, β), 137.8, 137.5 (Cq, Ph, α, β), 128.5, 128.5, 128.0, 127.9, 127.7 (*C*H, Ph), 91.7 (C-1 β), 88.6 (C-1 α), 79.0 (C-3 β), 75.9 (C-3 α), 74.7, 74.3 (*C*H$_2$Ph), 73.7 (C-5 β), 71.7 (C-5 α), 71.5 (C-2 β), 70.8 (C-4 β), 70.5 (C-2 α), 70.2 (C-4 α), 39.4, 39.3 (C-7 α, β), 32.0, 29.7, 29.7, 29.7, 29.6, 29.4, 29.3, 29.3, 29.2, 26.9, 26.9, 22.8 (C-8–C-17, α, β), 20.9, 20.9, 20.9, 20.8, 20.7, 20.7 (*C*H$_3$, Ac, α, β), 14.2 (C-18) ppm. HRMS: calcd for $C_{31}H_{47}NO_9$ $[M + Na]^+$ 600.3143, found 600.3156.

N-Dodecyl 2,4-di-*O*-acetyl-3-*O*-benzyl-1-deoxy-1-(uracil-1-yl)-β-D-glucopyranuronamide (19)

Obtained according to the general procedure for nucleosidation, starting from *N*-dodecyl 1,2,4-tetra-*O*-acetyl-3-*O*-benzyl-α,β-D-glucopyranuronamide (**18-α,β**, 35 mg, 0.061 mmol) and uracil (10 mg, 0.09 mmol) and using BSA (0.05 mL, 0.2 mmol) and TMSOTf (0.08 mL, 0.44 mmol). The reaction mixture was exposed to MW conditions for 30 min. Purification by column chromatography (EtOAc/hexane, 1:1) afforded **13** (22 mg, 58 %) as a colorless oil. $\left[\alpha\right]_D^{20}$ = +3 (c = 1, in CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.40 (br. s, 1 H, N^3*H*), 7.40–7.20 (m, 6 H, Ph, H-6), 6.14 (t, 1 H, N*H*, *J* = 5.3), 5.83 (d, 1 H, H-5, $J_{5,6}$ = 8.2), 5.80 (d, 1 H, H- 1′, $J_{1',2'}$ = 9.6), 5.29–5.12 (m, 2H, H-2′, H-4′), 4.70, 4.63 (2 d, AB system, C*H*$_2$Ph, $J_{a,b}$ = 11.9), 4.02 (d, 1 H, H-5′, $J_{4',5'}$ = 9.6 Hz), 3.90 (t, 1 H, H-3′, $J_{2',3'} = J_{3',4'}$ = 8.8), 3.24–3.14 (m, 2 H, C*H*$_2$-7′), 2.06, 1.92 (2 s, 4 H, 2 × C*H*$_3$, Ac), 1.52–1.41 (m, 2 H, C*H*$_2$-8′), 1.36–1.13 (m, 18 H, C*H*$_2$-9′ to C*H*$_2$-17′), 0.87 (t, 3 H, C*H*$_3$-18′, *J* = 6.7) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 169.7, 169.6 (2 × *C*O), 165.6 (CO, amide), 162.0 (C-4), 150.2 (C-2), 139.3 (C-6), 137.4 (Cq, Ph), 128.7, 128.3, 128.0 (*C*H, Ph), 104.0 (C-5), 80.6 (C-1′), 79.6 (C-3′), 75.5 (C-5′), 75.1 (*C*H$_2$Ph), 70.8, 70.4 (C-2′, C-4′), 39.5 (C-7′), 32.1, 29.8, 29.7, 29.7, 29.5, 29.4, 27.0, 22.8 (C-8′–C-17′), 21.0, 20.6 (2 × *C*H$_3$, Ac), 14.3 (C-18′) ppm. HRMS: calcd for $C_{33}H_{47}N_3O_9$ $[M + Na]^+$ 652.3205, found 652.3222.

N-Dodecyl 1-(2-acetamido-6-chloropurin-9-yl)-2,4-di-*O*-acetyl-3-*O*-benzyl-1-deoxy-β-D-glucopyranuronamide (20)

Obtained according to the general procedure for nucleosidation, starting from *N*-dodecyl 1,2,4-tetra-*O*-acetyl-3-*O*-benzyl-α,β-D-glucopyranuronamide (**18-α,β**, 58 mg, 0.10 mmol) and 2-acetamido-6-chloropurine (31 mg, 0.15 mmol) and using BSA (0.08 mL, 0.33 mmol) and TMSOTf (0.1 mL, 0.55 mmol). The reaction mixture was

exposed to MW conditions for 20 min. Purification by column chromatography (EtOAc/hexane, 1:1) afforded **20** (34 mg, 47 %) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.45 (s, 1 H, N*H*), 8.20 (s, 1 H, H-8), 7.37–7.20 (m, 5 H, Ph), 6.49 (br. t, 1H, N*H*-6′), 5.76 (d, 1H, H-1′, $J_{1',2'}$ = 9.4), 5.68 (t, 1H, H-2′, $J_{1',2'} \sim J_{2',3'}$), 5.36 (t, 1H, H-4′, $J_{3',4'} \sim J_{4',5}$), 4.77–4.60 (2 d, AB system, CH_2Ph, $J_{a,b}$ = 11.9), 4.16 (d, 1H, H-5′, $J_{4',5'}$ = 9.5), 3.99 (t, 1H, H-3′, $J_{2',3'} \sim J_{3',4'}$), 3.22–3.07 (m, 2 H, CH_2-7′), 2.48 (s, 3 H, CH_3, NHAc), 2.08, 1.74 (2 s, 4 H, 2×CH_3, Ac), 1.46–1.36 (m, 2 H, CH_2-8′), 1.30–1.15 (m, 18 H, CH_2-9′–CH_2-17′), 0.86 (t, 3 H, CH_3-18′, J = 6.8) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 170.0, 169.5, 169.0 (2×*C*O, OAc), 165.7 (*C*O, amide), 152.7 (C-4), 152.4, 151.8 (C-2, C-6), 142.4 (C-8), 137.3 (Cq, Ph), 128.6, 128.1, 127.9 (*C*H, Ph), 128.0 (C-5), 80.7 (C-1′), 79.4 (C-3′), 75.7 (C-5′), 70.9, 70.7 (C-2′, C-4′), 39.4 (C-7′), 31.9, 29.6, 29.6, 29.6, 29.5, 29.3, 29.2, 26.9, 22.7 (C-8′–C-17′), 25.2 (*C*H_3, NHAc), 20.9, 20.3 (2×*C*H_3, Ac), 14.1 (C-18′) ppm. HRMS: calcd for $C_{36}H_{49}ClN_6O_8$ [M + Na]$^+$ 751.3193, found 751.3193.

N-Dodecyl 2-*O*-acetyl-3,5-di-*O*-benzyl-1-deoxy-1-(uracil-1-yl)-β-D-glucofuranuronamide (23)

Obtained according to the general procedure for nucleosidation, starting from *N*-dodecyl 1,2-di-*O*-acetyl-3,5-di-*O*-benzyl-α,β-D-glucofuranuronamide (**22-α,β** [35], 109 mg, 0.174 mmol) and uracil (29 mg, 0.26 mmol) and using BSA (0.13 mL, 0.52 mmol) and TMSOTf (0.24 mL, 1.33 mmol). The reaction mixture was exposed to MW conditions for 20 min. Purification by column chromatography (EtOAc/cyclohexane, from 1:2 to 1:1) afforded **23** (61 mg, 52 %) as a colorless oil. $\left[\alpha\right]_D^{20}$ = +8 (c = 1, in CH_2Cl_2). H NMR ($CDCl_3$, 400 MHz): δ = 9.33 (br. s, 1 H, N*H*), 7.90 (d, 1 H, H-6, $J_{5,6}$ = 8.2), 7.41–7.13 (m, 10 H, Ph), 6.55 (t, 1 H, N*H*, J = 5.7), 6.04 (s, 1 H, H-1), 5.42 (d, 1 H, H-5), 5.23 (s, 1 H, H-2), 4.69 (d, 1 H, part A of AB system, H-a from CH_2Ph-3′, $J_{a,b}$ = 11.4), 4.58, 4.51 (2 d, AB system, CH_2Ph-5′, $J_{a,b}$ = 10.9), 4.47–4.36 (m, 3 H, H-4′, H-5′, H-b from CH_2Ph-3′), 4.07 (br.d, 1 H, H-3′, $J_{3',4'}$ = 3.5), 3.38–3.25 (m, 1 H, H-7′a), 3.17–3.05 (m, 1 H, H-7′b), 2.12 (s, CH_3, OAc), 1.47–1.34 (m, 2 H, CH_2-8′), 1.34–1.12 (m, 18 H, CH_2-9′–CH_2-17′), 0.88 (t, 3 H, CH_3-18′, J = 6.6) ppm. ^{13}C NMR ($CDCl_3$, 100MHz): δ = 169.5, 169.4 (*C*O, Ac, NHAc, amide), 163.5 (C-4), 150.3 (C-2), 140.9 (C-6), 136.6, 136.6 (2×Cq, Ph), 128.8, 128.7, 128.6, 128.3, 128.1, 127.8 (*C*H, Ph), 101.9 (C-5), 89.2 (C-1′), 82.7 (C-4′), 80.0 (C-3′), 78.7 (C-2′), 77.2 (C-5′), 74.1 (*C*H_2Ph-5′), 72.2 (*C*H_2Ph-3′), 39.3 (C-7′), 32.0, 29.7, 29.7, 29.7, 29.6, 29.4, 29.3, 27.0, 22.8 (C-8′ to C-17′), 20.9 (*C*H_3, OAc), 14.2 (C-18′) ppm. HRMS: calcd for $C_{38}H_{51}N_3O_8$ [M + H]$^+$ 678.3749, found 678.3744.

N-Dodecyl 1-(2-acetamido-6-chloropurin-9-yl)-2-*O*-acetyl-3,5-di-*O*-benzyl-1-deoxy-β-D-glucofuranuronamide (24) and *N*-dodecyl 1-(2-acetamido-6-chloropurin-7-yl)-2-*O*-acetyl-3,5-di-*O*-benzyl-1-deoxy-β-D-glucofuranuronamide (25)

Obtained according to the general procedure for nucleosidation, starting from *N*-dodecyl 1,2-di-*O*-acetyl-3,5-di-*O*-benzyl-α,β-D-glucofuranuronamide (**22-α,β** [35], 123 mg, 0.2 mmol) and 2-acetamido-6-chloropurine (63 mg, 0.3 mmol) and using BSA (0.15 mL, 0.61 mmol) and TMSOTf (0.2 mL, 1.1 mmol). The reaction mixture was exposed to MW conditions for 20 min. Purification by column chromatography (EtOAc/cyclohexane, from 1:4 to 2:1) afforded afforded the N^9 nucleoside **24** (40 mg, 26 %) and the N^7 regioisomer **25** (17 mg, 11 %) as pale-yellow oils.

Data for 24

$\left[\alpha\right]_D^{20}$ = +18 (c = 0.5, in CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.57 (s, H-8′), 8.14 (s, 1 H, N*H*), 7.39–7.31 (m, 3 H, Ph), 7.28–7.20 (m, 5 H, Ph), 7.11–7.05 (m, 2 H, Ph), 6.43 (t, 1 H, N*H*, J = 5.8), 6.19 (s, 1 H, H-1′), 5.54 (s, 1 H, H-2′), 4.64–4.46 (m, 5 H, H-4′, 2×CH_2Ph, $J_{a,b}$ = 11.6), 4.42 (d, 1 H, H-5′, $J_{4',5'}$ = 7.7), 4.23 (br.d, 1 H, H-3′, $J_{3',4'}$ = 4.1), 3.35–3.23 (m, 1 H, H-7′a), 3.18–3.06 (m, 1 H, H-7′b), 2.51 (s, 3 H, CH_3, NHAc), 2.17 (s, CH_3, OAc), 1.42–1.35 (m, 2 H, CH_2-8′), 1.33–1.17 (m, 18 H, CH_2-9′–CH_2-17′), 0.88 (t, 3 H, CH_3-18′, J = 6.6) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 169.6, 169.6 (*C*O, Ac, NHAc, amide), 137.5, 136.9 (2×Cq, 2×Ph), 152.0 (C-4), 151.9, 151.2 (C-2, C-6), 143.4 (C-8), 136.6, 136.5 (2×Cq, Ph), 128.9, 128.7, 128.6, 128.3, 127.6 (C-5, *C*H, Ph), 88.3 (C-1′), 83.3 (C-4′), 80.7 (C-3′), 78.5 (C-2′), 77.0 (C-5′), 74.0 (*C*H_2Ph-5′), 72.9 (*C*H_2Ph-3′), 39.3 (C-7′), 32.0, 29.8, 29.8, 29.7, 29.7, 29.6, 29.5, 29.4, 27.0, 22.8 (C-8′ to C-17′), 25.2 (*C*H_3, NHAc), 20.9 (*C*H_3, OAc), 14.3 (C-18′) ppm. HRMS: calcd for $C_{41}H_{53}ClN_6O_7$ [M + H]$^+$ 777.3737, found 777.3744.

Data for 25

$[\alpha]_D^{20} = +22$ (c = 0.5, in CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.92 (s, 1 H, H-8′), 8.21 (s, 1 H, N*H*), 7.42–7.32 (m, 3 H, Ph), 7.32–7.14 (m, 5 H, Ph), 7.13–7.05 (m, 2 H, Ph), 6.53 (t, 1 H, N*H*, J = 5.8), 6.51 (s, 1 H, H-1′), 5.42 (s, 1 H, H-2′), 4.62–4.49 (m, 5 H, H-4′, H-5′, CH_2Ph, H-a from CH_2Ph $J_{4',5'}$ = 7.7, $J_{a,b}$ = 11.2), 4.44 (d, 1 H, H-b from CH_2Ph, $J_{a,b}$ = 11.9), 4.18 (br.d, 1 H, H-3′, $J_{3',4'}$ = 3.5), 3.41–3.26 (m, 1 H, H-7′a), 3.21–3.09 (m, 1 H, H-7′b), 2.63 (s, 3 H, CH_3, NHAc), 2.20 (s, CH_3, OAc), 1.53–1.15 (m, 20 H, CH_2-8′–CH_2-17′), 0.88 (t, 3 H, CH_3-18′, J = 6.6) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 169.5, 169.4 (*C*O, Ac, NHAc, amide), 163.3 (C-4), 152.6 (C-2 or C-6), 148.8 (C-8), 142.5 (C-2 or C-6), 136.5, 136.4 (2 × Cq, 2 × Ph), 128.9, 128.7, 128.7, 128.3, 128.2, 127.9 (*C*H, Ph), 117.9 (C-5), 90.5 (C-1′), 83.8 (C-4′), 80.7 (C-3′), 79.2 (C-2′), 76.8 (C-5′), 74.0 (*C*H_2Ph-5′), 73.0 (*C*H_2Ph-3′), 39.3 (C-7′), 32.0, 29.8, 29.8, 29.8, 29.7, 297, 29.6, 29.5, 29.4, 27.0, 22.8 (C-8′ to C-17′), 25.4 (*C*H_3, NHAc), 20.8 (*C*H_3, OAc), 14.3 (C-18′) ppm. HRMS: calcd for $C_{41}H_{53}ClN_6O_7$ $[M+H]^+$ 777.3737, found 777.3725.

Biological assays

Cancer cell lines and cytotoxicity 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 mg/mL) at 37 °C in 5 % CO_2. For the cytotoxicity assays, cells were 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.

Immunoblotting

In brief, cellular lysates were prepared by harvesting cells in Laemmli sample buffer. 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), Cell Signaling (anti-PARP, clone 46D11; anti-XIAP; anti-Mcl-1, clone D35A5; anti-caspase-7) and Merck (anti-Bcl-2; anti-α-tubulin, clone DM1A; anti-LC3B and peroxidase-labeled secondary antibodies). Anti-PCNA (clone PC-10) was generously gifted by Dr. B. Vojtěšek (Masaryk Memorial Cancer Institute, Brno, Czech Republic).

Caspase activity assay

The cells were homogenized in an extraction buffer (10 mM KCl, 5 mM HEPES, 1 mM EDTA, 1 mM EGTA, 0.2 % CHAPS, inhibitors of proteases, pH 7.4) on ice for 20 min. The homogenates were clarified by centrifugation at 10 000 × *g* for 30 min at 4 °C, and then the proteins were quantified and diluted to equal concentrations. Lysates were then incubated for 4 h with 100 mM Ac-DEVD-AMC as a substrate of caspases 3 and 7 in the assay buffer (25 mM PIPES, 2 mM EGTA, 2 mM $MgCl_2$, 5 mM DTT, pH 7.3). The fluorescence of the product was measured using a Fluoroskan Ascent microplate reader (Labsystems) at 355/460 nm (excitation/emission).

Cell cycle analysis

Asynchronous cells were treated with different concentrations of compound for the indicated time. The cultures were fed and pulse-labeled with 10 μM 5-bromo-2′-deoxyuridine (BrdU) for 30 min at 37 °C before harvesting. The cells were trypsinized, washed with phosphate-buffered saline containing 1% bovine serum albumin (PBS/BSA), fixed with ice-cold 70% ethanol, incubated on ice for 30 min, washed with PBS/BSA again and resuspended in 2N HCl for 30 min at room temperature in order to denature their DNA. Following neutralization with 0.1 M $Na_2B_4O_7$, the cells were harvested by centrifuging and washed with PBS/BSA containing 0.5% Tween-20. They were then stained with anti-BrdU fluorescein-labeled antibody (1:50, eBioscience) for 30 min at room temperature in the dark. The cells were then washed with PBS, incubated with propidium iodide for 1 h at room temperature in the dark, and finally analyzed by flow cytometry using a 488 nm laser (BD FACS Verse with software BD FACSuite, version 1.0.6.). Cell cycle distribution was analyzed using ModFit LT (Verity Software House, version 4.1.7).

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