ChemPubSoc Europe
DOI: 10.1002/open.201500197
Open Access ChemistryOPEN Full Papers

Nucleolipids of Canonical Purine ß-D-Ribo-Nucleosides: Synthesis and Cytostatic/Cytotoxic Activities Toward Human and Rat Glioblastoma Cells

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Dedicated to Prof. Dr. Jiri Žemlicka, Detroit, MI, USA.

We report on the synthesis of two series of canonical purine ß-D-ribonucleoside nucleolipids derived from inosine and adenosine, which have been characterized by elemental analyses, electrospray ionization mass spectrometry (ESI MS) as well as by ^{1}H and ^{13}C NMR, and pH-dependent UV/Vis spectroscopy. A selection of the novel nucleolipids with different lipophilic moieties were first tested on their cytotoxic effect toward human macrophages. Compounds without a significant inhibitory effect on the viability of the macrophages were tested on their cytostatic/cytotoxic effect toward human astrocytoma/oligodendroglioma GOS-3 cells as well as against the rat malignant neuroectodermal BT4Ca cell line. In order to additionally investigate the potential molecular mechanisms involved in the cytotoxic effects of the derivatives on GOS-3 or BT4Ca cells, we evaluated the induction of apoptosis and observed the particular activity of the nucleolipid ethyl 3-{4-hydroxymethyl-2-methyl-6-[6-oxo-1-(3,7,11-trimethyl-dodeca-2,6,10-trienyl)-1,6-dihydro-purin-9-yl]-tetrahydro-furo[3,4-d][1,3]dioxol-2-yl}propionate (**8c**) toward both human and rat glioblastoma cell lines in vitro.

Introduction

In a series of precedent publications we and others have demonstrated that the cancerostatic/cancerotoxic activity of pyrimidine ß-D-ribonucleoside antimetabolites such as 5-fluorouridine and 6-azauridine towards different human tumor cell lines[1,2] as well as neurobiological[3] and antiviral activities[4,5] can be significantly improved by lipophilization. Also, regular, canonical pyrimidine *ß*-D-ribonucleosides such as uridine and 5-methyluridine acquire a surprisingly high antitumor in vitro activity upon covalent hydrophobization.[6,7] The positioning and type of the lipophilic residues are hereby of decisive importance. It has been shown that, in particular, the introduction of an ethyl levulinate group at the *O*-2′,3′-hydroxyls in form of a cyclic ketal and, additionally, a farnesyl sesquiterpene moiety at N(3) leads to compounds with significant activity.[2]

In this manuscript, we extend our study to purine ß-D-ribonucleoside nucleolipids, particularly to inosine and adenosine derivatives. Again, a selection of the compounds was tested with respect to the viability/survival of phorbol 12-myristate 13-acetate (PMA)-differentiated human THP-1 macrophages when treated with these compounds.[2] Those which proved to be nontoxic for the immune cells were then further tested on their cytostatic/cytotoxic in-vitro activity towards human astrocytoma/oligodendroglioma GOS-3 cells, as well as against rat malignant neuroectodermal BT4Ca cells.

Results and Discussion

Synthesis

Starting from adenosine (**1**), its ethyl levulinate derivative **3** was prepared according to a well-known procedure (Scheme 1).[8] However, in contrast to older publications, we found that this ketal formation resulted in the formation of a diastereoisomeric mixture (1*R*)/(1*S*) with a ratio of about 10:1 in all cases.

Ketal formation of adenosine with nonadecan-10-one gave the nucleolipid **5**. Both *O*-2′,3′ ketals (**3** and **5**) were then submitted to an enzymatic deamination using adenosine deaminase (from calf intestine). It could be clearly shown that compound **3** could be deaminated within 72 h yielding the inosine

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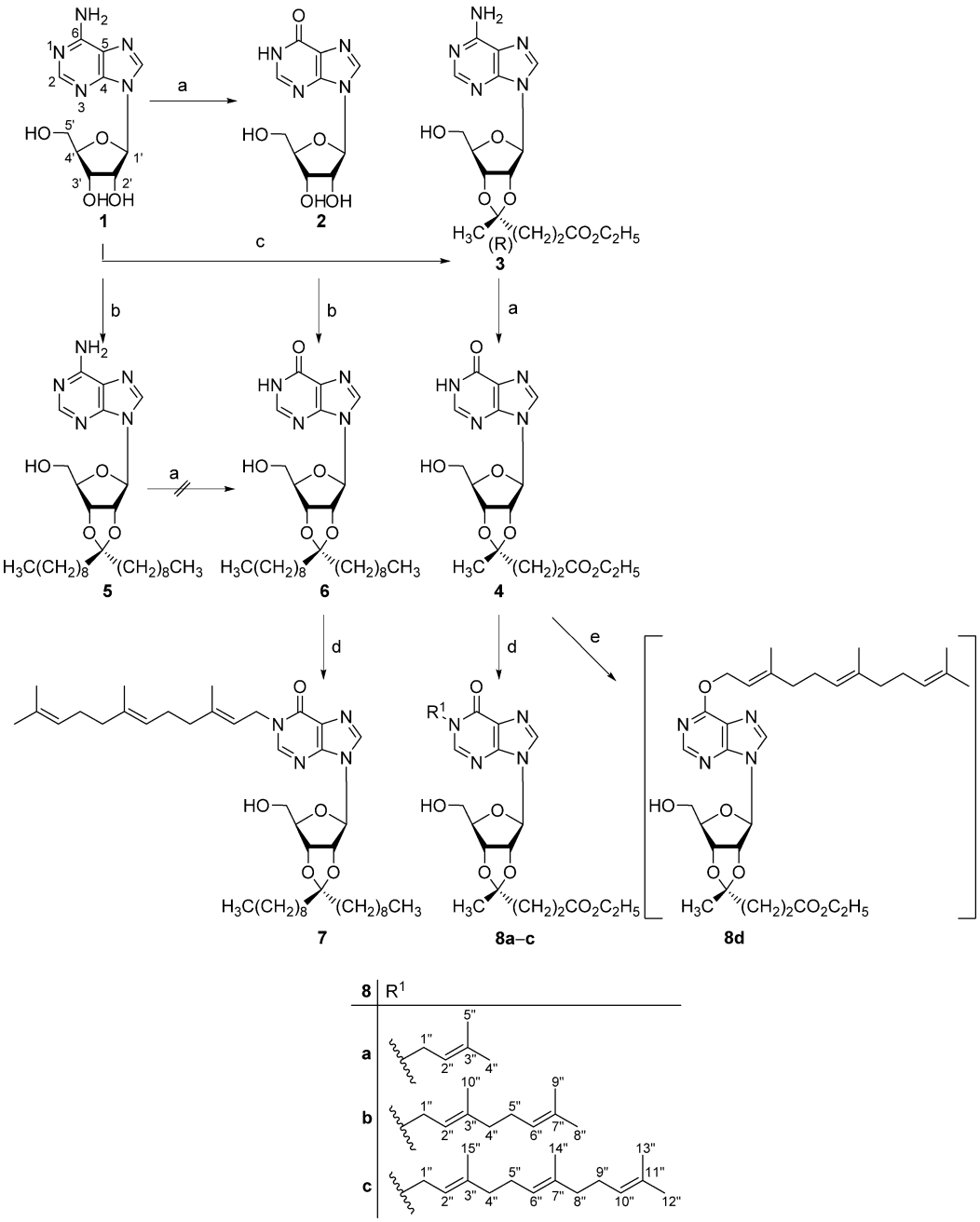
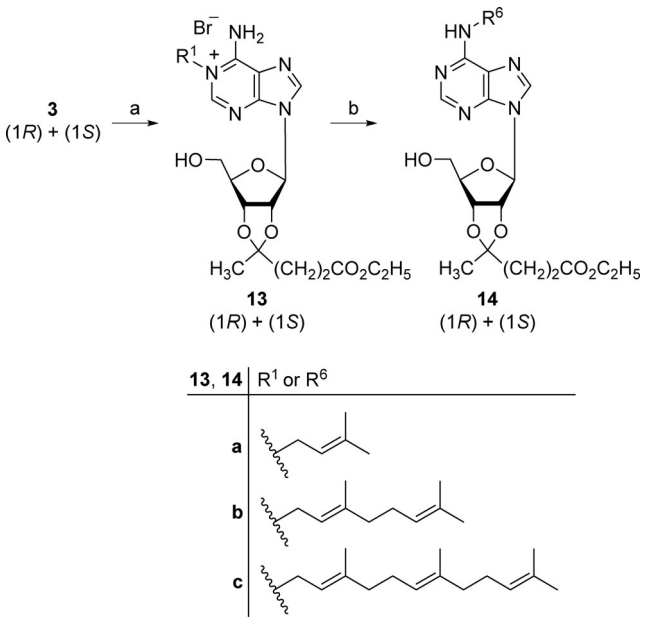
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/open.201500197.



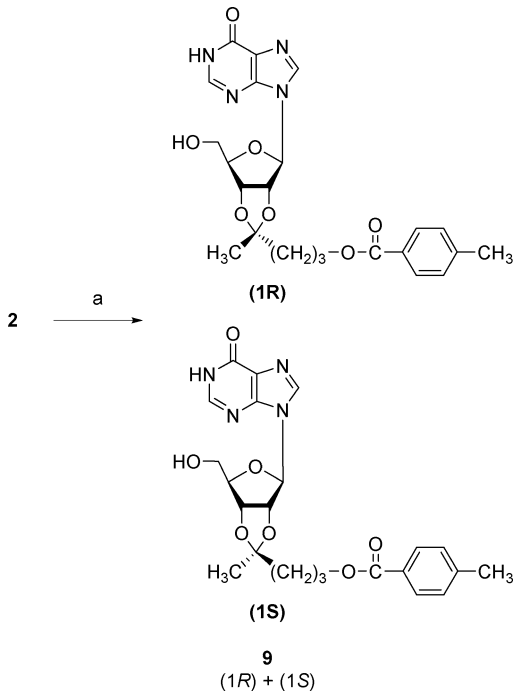
Scheme 1. Stepwise lipophilization of adenosine (**1**) and inosine (**2**) at their *O*-2′,3′-position and at the nucleobase. *Reagents and conditions:* a) adenosine deaminase, H_2O, rt, 24 h, 100%; b) $H_3C–(CH_2)_8–C(=O)–(CH_2)_8–CH_3$, $(EtO)_3CH$, 4 M HCl in 1,4-dioxane, DMF, rt, 24 h, **5**: 39% , **6**: 35%; c) $H_3C–C(=O)–(CH_2)_2–C(=O)OC_2H_5$, $(EtO)_3CH$, 4 M HCl in 1,4-dioxane, DMF, rt, 24 h, 71%; d) farnesyl bromide (for **7**), Δ^2-isopentenyl bromide (for **8a**), geranyl bromide (for **8b**), or farnesyl bromide (for **8c**), K_2CO_3, DMF, rt, 24 h, **7**:61%, **8a**: 48%, **8b**: 40%, **8c**: 57% ; e) farnesyl bromide, K_2CO_3, DMF, 30 °C, 30 min, then rt, 24 h, 37%.

derivative **4**,[9] while **5** could not be deaminated to **6**. The latter was obtained by direct ketal formation of inosine (**2**) with nonadecan-10-one.

All novel compounds were characterized by elemental analyses, high-resolution electrospray ionization mass spectrometry (HR ESI MS) as well as by ^{1}H and ^{13}C NMR, and pH-dependent UV/Vis spectroscopy. Assignment of ^{13}C NMR resonances was made with the help of DEPT-135 as well as by gradient-selected homo- and heteronuclear correlation spectroscopy (Bruker pulse programs, ^{1}H,^{13}C-HSQCETGP; ^{1}H,^{1}H-COSYGPSW). Careful inspection of the NMR spectra, particularly of the ^{13}C NMR spectra, revealed that compound **3** was formed as a diastereoisomeric (1*R*)/(1*S*)-mixture (for an example of the diastereoisomer structures, see Scheme 2), while the subsequent deamination product **4** proved to be the diastereoisomerically almost pure (1*R*) derivative. This might be traced back to the following reasons: 1) It has been shown earlier that the enzymatic deamination of adenosine which has been ketalized at the 2′,3′-O position with unsymmetrical ketones such as pentan-2-one[10] to the corresponding (1*R*) and (1*S*) 2′,3′-*O*-(1-methylbutylide-



Scheme 2. Dimroth rearrangement of compounds **13a–c**, prepared from compound **3**, yielding the N(6)-alkylated compounds **14a–c.** *Reagents and conditions*: a) Δ^2-isopentenyl bromide (for **13a**), geranyl bromide (for **13b**), or farnesyl bromide (for **13c**), DMF, $BaCO_3$, Ar atmosphere, rt, 24 h, **13a**: 63%, **13b**: 58%, and **13c**: 67%; b) $(CH_3)_2NH$ in H_2O (1 M), rt, 20 h, **14a**: 62%, **14b**: 23%, and **14c**: 38%.



Scheme 3. Ketal formation of inosine (**2**) with 4-oxopentyl 4-methylbenzoate yielding almost equimolar amounts of nonseparated (1*R*)-**9** (55%) and (1*S*)-**9** (45%) diastereoisomers.[12] *Reagents and conditions:* 4-oxopentyl 4-methylbenzoate, $(EtO)_3CH$, 4 M HCl in 1,4-dioxane, DMF, rt, 24 h.

ne)adenosines occurs with significantly different Michaelis–Menten kinetics; the (1*S*)-configured ketal is deaminated at an 8.5-fold lower v_{max} rate than the (1*R*)-configured isomer.[10] 2) The deamination product **4** was isolated by crystallization which might lead to a preferred precipitation of the corresponding (1*R*) product. In contrast to this, ketal formation of inosine (**2**) with ethyl levulinate in the presence of triethylorthoformate[9,11] leads to a diastereoisomeric mixture of compound **4** [(1*R*):(1*S*) ≈ 10:1] (Schemes 1 and 3).

Interestingly, the ^{13}C NMR spectrum of compound **4**, prepared by the latter method, exhibits characteristically increasing $\Delta\delta$ values [(1*R*)–(1*S*)] for the C(1′), C(4′), C(2′), and C(3′) carbons and a strong decrease again for C(5′) (Figure 1). The graph shown in Figure 1 demonstrates almost identical chemical shift differences of compound **4** as well as of the analogous compound **9**[12]—both adopting an *anti*-conformation at the *N*-glycosylic bond. The formation of an almost equimolar mixture of (1*R*) and (1*S*) diastereoisomers of a ketal formation reaction of inosine with 4-oxopentyl 4-methylbenzoate has been found already recently.[12] It was corroborated also for analogous reactions with other nucleosides which will be the subject of a forthcoming publication. The almost identical chemical shift differences, which have already been observed earlier for pyrimidine ß-D-ribonucleoside ketals[7] might be the result of the interworking of various C–O and C–C magnetic anisotropy effects[13] of ketal moiety bonds (Figure S1 in the Supporting Information), for example of {C(acetal)–O} and {CH_2(acetal)–CH_2(C=O)} bonds, within (1*R*)-*O*-2′,3′-[1-(2-carboxyethyl)ethyliden]adenosine.[14] Others such as {C(acetal)–CH_2(acetal)} anisotropy effects may be counterproductive.

In the following, we lipophilized the inosine nucleolipid **6** further at N(1) by farnesylation (dimethylformamide [DMF], K_2CO_3)[15] and obtained compound **7**. It could be shown that the alkylation occurs without 5′-OH protection which might be substantially advantageous for an antitumor activity as the latter requires probably an intracellular phosphorylation of the 5′-OH group. Because it had been recently shown that a lipophilization of pyrimidine ß-D-ribonucleosides at the *O*-2′,3′ position as well as at N(3) of the pyrimidine base leads to nucleolipids with a pronounced and selective cytostatic/cytotoxic in vitro activity toward various human tumor cell lines,[2] we now converted also the inosine derivative **4** [pure (1*R*)-diastereoisomer, prepared by enzymatic deamination from compound **3**] to the corresponding N(1)-prenylated inosine nucleolipids **8a–c**. In the case of the preparation of compounds **8a**,**b** by-products, such as probably formed O-alkylated compounds, were chromatographically removed and not further characterized. Only in the case of the farnesylation of compound **4**, the reaction was studied in more detail; it was performed at two different temperatures: 1) At room temperature (20 °C), the alkylation afforded the N(1)-prenylated derivatives **8c** (kinetic reaction control, see Experimental Section). 2) Alkylation at already slightly elevated temperatures (40–55 °C), however, gave, after work-up, the N(1)- as well as further O(4)-prenylated derivatives such as **8d** (thermodynamic reaction control) and another one, the structure of which proved to be more complicated. The general structure of the O-alkylated derivatives was corroborated by pH-dependent UV/Vis spectroscopy. Tentatively, we postulate that the O(6)-farnesylated nucleolipid **8d** reacts with

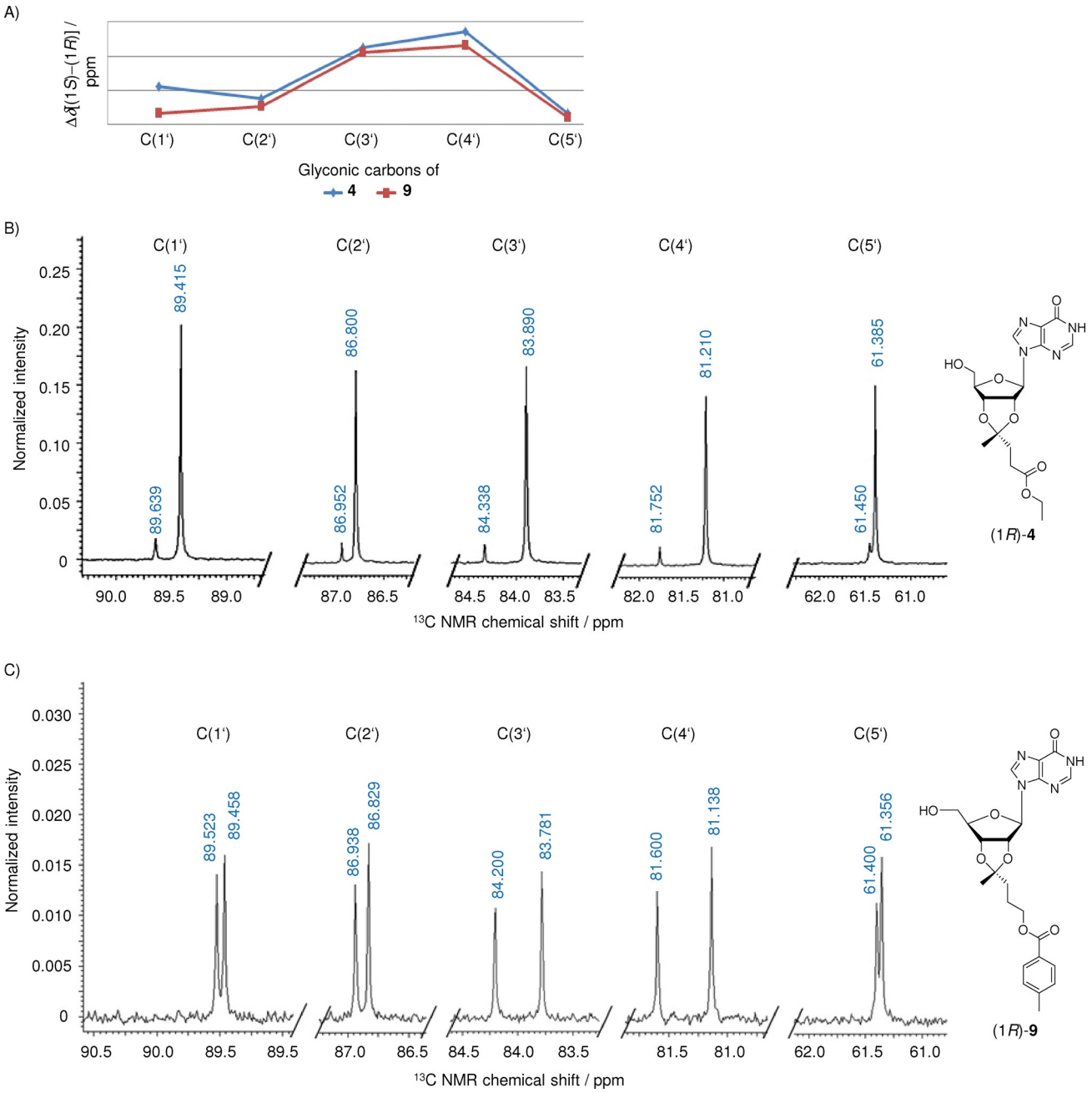
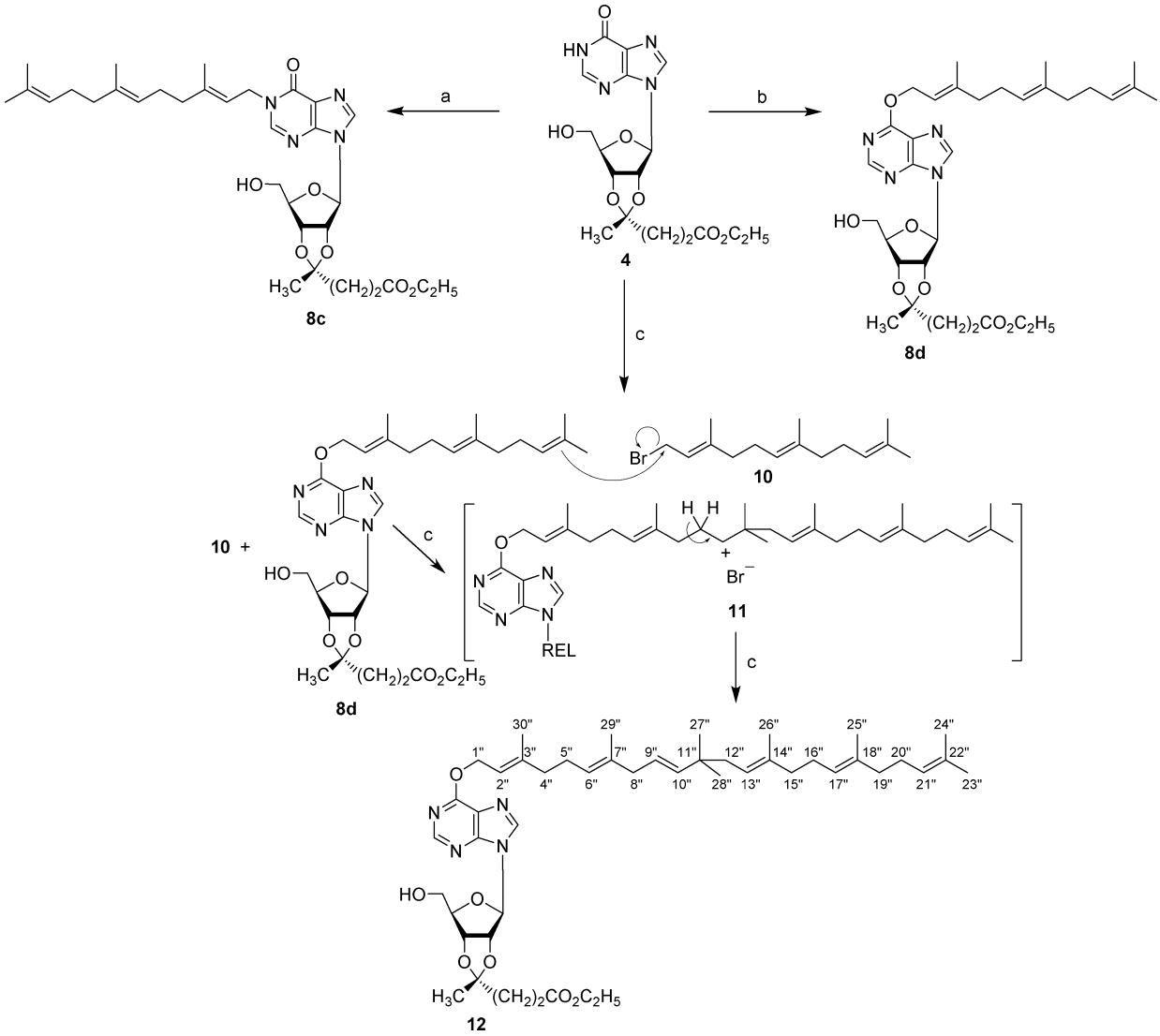


Figure 1. ^{13}C NMR chemical shift differences of the glyconic moiety resonances of compounds **4** and **9**, both as an unseparated diastereoisomeric mixture (A), and the corresponding (1*R*)-isomers are displayed (B and C).

an excess of farnesyl bromide (**10**) via the intermediate **11** to the *O*(6)-triterpenyl derivative **12** (Scheme 4)—an inosine nucleolipid carrying a squalene-analogous *N*(1)-side chain, which has been formed by a head–tail addition of two farnesyl residues. This is underlined by pH-dependent UV/Vis and NMR spectroscopy (^{1}H, ^{13}C NMR) as well as by HR ESI MS spectrometry.

Next, we alkylated the adenosine nucleolipid **3**[8] with three different prenyl bromides and obtained the corresponding N(1)-alkylated salts **13a–c** (Scheme 2). These were submitted to Dimroth rearrangements[16,17] with an aqueous dimethylamine solution[18] which yielded the adenosine nucleolipids **14a–c**. By this way, we were able to synthesize chain-extended analogues of the nucleoside antimetabolite *N*(6)-isopentenyladenosine {=N^6-(Δ^2-isopentenyl)adenosine}[19] carrying additionally an O-2′,3′-ethyllevulinate ketal group without saponification of the ester. In an earlier publication we have reported the synthesis of an *N*(6)-isopentenyladenosine derivative with an *O*-2′,3′-levulinate moiety using 1 N aq NaOH.[20] All compounds were characterized by HR ESI MS, elemental analyses, as well as by ^{1}H and ^{13}C NMR and UV/Vis spectroscopy.



Scheme 4. Side reactions occuring upon farnesylation of inosin-*O*-2′,3′-ketal **4**. *Reagents and conditions:* a) farnesyl bromide, K_2CO_3, DMF, rt, 24 h, 57 %; b) farnesyl bromide, K_2CO_3, DMF, 30 °C, 30 min, then r.t., 24 h; c) excess of farnesyl bromide, 24 h, 55 °C, **12**: 37 %. REL: ribosylethyllevulinate.

Lipophilic properties of purine ß-D-ribonucleosides and their nucleolipids

Applying the ALOGPS v.3.01 program,[21,22] the $^{10}\text{Log}P_{ow}$ values of the nucleolipids, as well as of their precursor molecules, were calculated; for this purpose we used the eadmet.com/de/physprop.php site with *ePhysChem* that contains the program mentioned. The results are shown in Figure 2 A,B. The figures clearly demonstrate the possibility of a fine tuning of the compounds' lipophilicity by the introduction of stepwise elongated prenyl side chains to both adenosine and inosine.

In addition to $^{10}\text{Log}P_{ow}$ calculations, we have also measured such data experimentally for those four compounds which have been tested biologically (see Experimental Section). A comparison of calculated and experimental data can be seen in Table 1.

Table 1. Translation of plain compound numbers into the NS/NL-nomenclature.[23]

Compound numbers	NS/NL code [a]	Compound numbers	NS/NL code[a]
1	NS_5.0.0.0	**9**	NL_6.6.0.0
2	NS_6.0.0.0	**10**	N.a.n.
3	NL_5.1.0.0	**11**	N.a.n.
4	NL_6.1.0.0	**12**	N.a.n.
5	NL_5.3.0.0	**13a**	NL_5.1.^{1}1.0
6	NL_6.3.0.0	**13b**	NL_5.1.^{1}2.0
7	NL_6.3.^{1}3.0	**13c**	NL_5.1.^{1}3.0
8a	NL_6.1.^{1}1.0	**14a**	NL_5.1.^{6}1.0
8b	NL_6.1.^{1}2.0	**14b**	NL_5.1.^{6}2.0
8c	NL_6.1.^{1}3.0	**14c**	NL_5.1.^{6}3.0
8d	NL_6.1.^{6}3.0		

[a] According to Ref. [23]. N.a.n.: not a number.

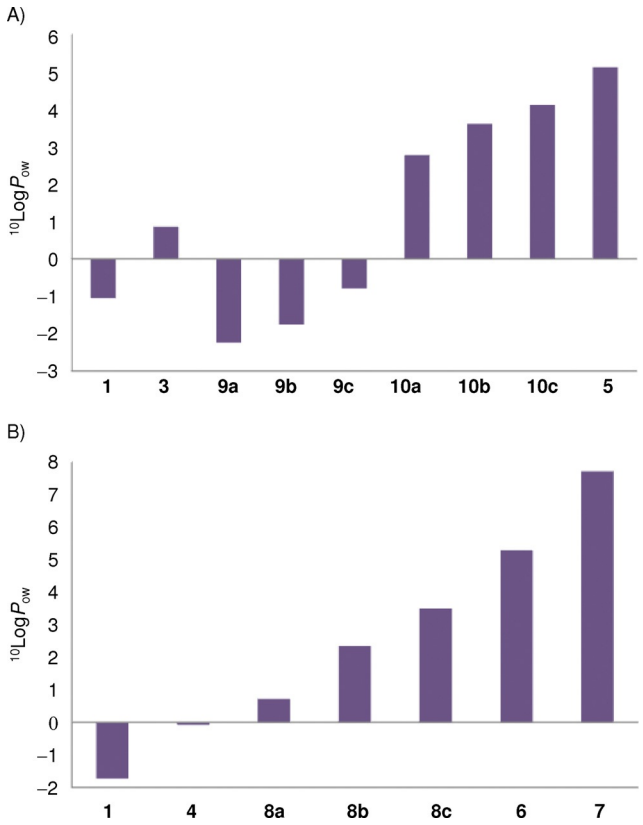


Figure 2. ^{10}LogP_{ow} values of A) purine ß-D-ribonucleosides adenosine (**5**) and B) inosine (**6**), as well as their nucleolipids.

Biological results

Using an in vitro model to differentiate between anticancer properties and side effects, we tested the cytotoxic effects on the viability of PMA-differentiated human THP-1 macrophages after treatment for 48 h. At concentrations of 6.25, 12.5, 25, or 50 μM 5-fluorouridine, there was a significant inhibition of the viability/survival by 11.9% ($p<0.001$), 8.4% ($p<0.05$), 13.7% ($p<0.001$), 12.14% ($p<0.05$) compared with negative control (Figure 3). The purine derivative **8c** did not exhibit any significant inhibitory effect on viability/survival of THP-1 macrophages (Figure 3). At a concentration of 50 μM, the derivatives **5** and **6**[23] revealed significantly ($p<0.001$) cytotoxic effects of 93.3% and 86.5%; additionally, the derivative **5** significantly ($p<0.001$) showed cytotoxic effects of 69.9% at 25 μM, in comparison with the control (Figure 3). Because the derivative **8c** revealed no or only marginal cytotoxic effects on differentiated human THP-1 macrophages, the effects of this substance—in comparison with the positive control (5-fluorouridine)—was tested in human astrocytoma/oligodendroglioma GOS-3 and rat malignant neuroectodermal BT4Ca cells. Incubation (48 h) of the human astrocytoma/oligodendroglioma GOS-3 cells with 5-fluorouridine, at concentrations of 1.56, 3.12, 6.25, 12.5, 25, or 50 μM, resulted in significant ($p<0.001$) 45.5, 54.4, 51.6, 54.0, 53.7, and 59.0% inhibitions of the viability/survival, whereas incubation with derivative **8c** at 25 μM and 50 μM significantly inhibited the viability/survival by 28.1% ($p<0.01$) and 91.2% ($p<0.001$), in comparison with the control (Figure 4).

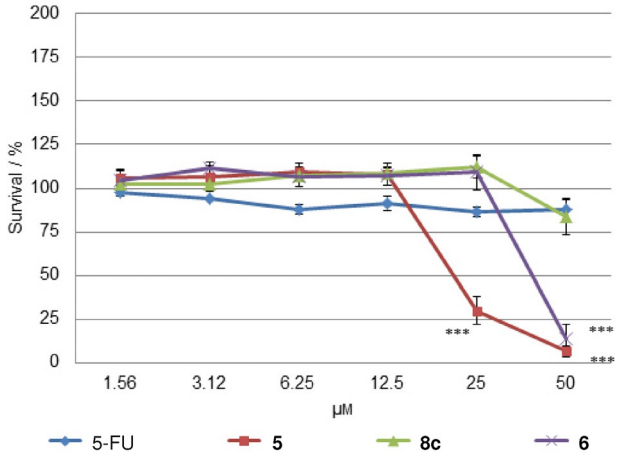


Figure 3. Viability/survival of differentiated human THP-1 macrophages after 48 h of incubation with 5-FU (5-fluorouridine), or its derivatives **5**, **6**, or **8c**. Values are given [in % survival of control (incubation with medium alone = 100% survival] as mean ± SEM; ***$p<0.001$ vs. negative control; $n=4$.

Moreover, incubation (48 h) of the rat malignant neuroectodermal BT4Ca cells with 5-fluorouridine (1.56, 3.12, 6.25, 12.5, 25, or 50 μM) resulted in significant ($p<0.001$), 60.3, 58.7, 65.1, 59.5, 58.8, and 68.1% inhibitions of the viability/survival when compared with the control. At concentrations of 25 μM and 50 μM, the derivative **8c** significantly ($p<0.001$) inhibited the viability/survival by 58.6% and 81.8%, and at a concentration of 12.5 μM, viability/survival was (not significantly) inhibited by 11.9%, in comparison with the control (Figure 5).

In order to investigate additionally the potential molecular mechanisms involved in the cytotoxic effects of the derivatives on GOS-3 or BT4Ca cells, induction of apoptosis was evaluated. At a concentration of 50 μM, the derivative 5-fluorouridine sig-

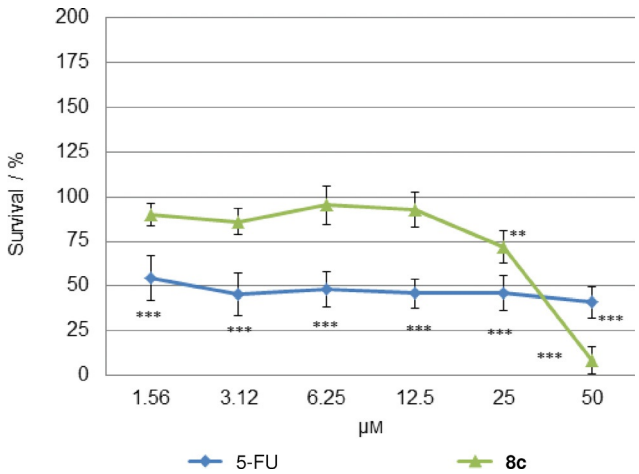


Figure 4. Viability/survival of human astrocytoma/oligodendroglioma GOS-3 cells after 48 h of incubation with 5-FU (5-fluorouridine, positive control) or its derivative **8c**. Values are given [in % survival of control (incubation with medium alone = 100% survival] as mean ± SEM; **$p<0.01$, ***$p<0.001$ vs. negative control (medium alone); $n=5$.

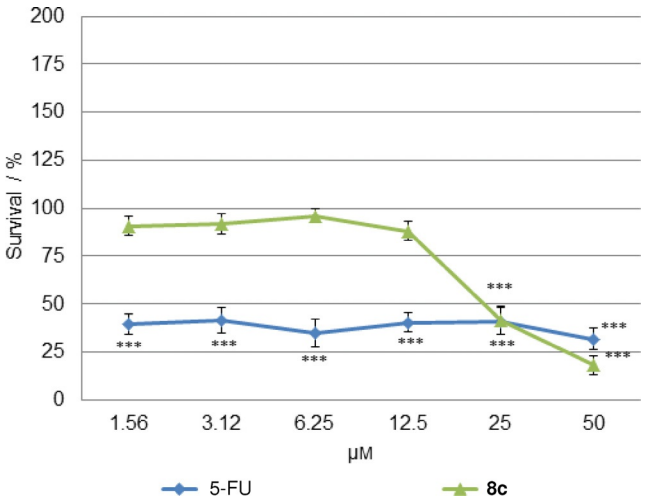


Figure 5. Viability/survival of rat malignant neuroectodermal BT4Ca cells after 48 h of incubation with 5-FU (5-fluorouridine), or its derivative **8c**. [in % survival of control (incubation with medium alone = 100% survival] as mean ± SEM; ***$p < 0.001$ vs. negative control; $n = 5$.

nificantly ($p < 0.01$) enhanced the percentage of apoptotic GOS-3 cells by 8.6%, when compared with the control (Figure 6 A,C). Moreover, a significant ($p < 0.05$) 63.6% and 62.0% decrease of the cell number was found using 25 μM and 50 μM of the derivative 5-fluorouridine (Figure 6 B,C). At a concentration of 50 μM, the derivative **8c** significantly ($p < 0.001$) induced apoptosis by 94.9% (Figure 6 A,C); additionally, a significant ($p < 0.05$ and $p < 0.001$) 45.7% and 82.4% decrease of the cell number was observed by 25 μM and 50 μM treatment, in comparison with the control (Figure 6 B,C).

Treatment of BT4Ca cells with 25 μM or 50 μM of 5-fluorouridine induced an increase of apoptosis by 5.2% ($p < 0.01$) and 14.7% ($p < 0.001$) (Figure 7 A,C), and decreased the cell number by 93.6% ($p < 0.001$) and 95.7% ($p < 0.001$) in comparison with the control (Figure 7 B,C). Treatment of BT4Ca cells with the derivative **8c** (50 μM) significantly ($p < 0.001$) enhanced the percentage of apoptosis by 95.7%, in comparison with the control (Figure 7 A,C). Moreover, treatment of BT4Ca cells with the derivative **8c** at 25 μM or 50 μM significantly ($p < 0.001$) decreased the cell number by 56.5% and 97.3%, in comparison with the control (~100% cells) (Figure 7 B,C).

Additional investigations of the protein expression (western blot) of the ubiquitin-binding autophagic adaptor p62/SQSMT1 (hereafter p62) showed a significant ($p < 0.01$) activation being 260% higher than the negative control (100%) after treatment with 25 μM **8c** (Figure 8 A,C). Proliferating cell nuclear antigen (PCNA) expression showed a significant ($p < 0.05$) inhibition by 84.4% or 73.1%, which was, however, not significant after treatment with 12.5 μM or 25 μM of 5-fluorouridine, respectively (Figure 8 A,C). Whereas treatment with 12.5 μM and 25 μM **8c** significantly ($p < 0.01$) inhibited the PCNA expression in GOS-3 cells by 76.6% and 96.8% (Figure 8 B,C). Furthermore, treatment of BT4Ca cells with 12.5 μM 5-fluorouridine showed a significant ($p < 0.01$) 67.7% inhibition, whereas the effect of 25 μM was nonsignificant when compared with the control. Treatment with 12.5 μM of **8c** showed a significant ($p < 0.01$)

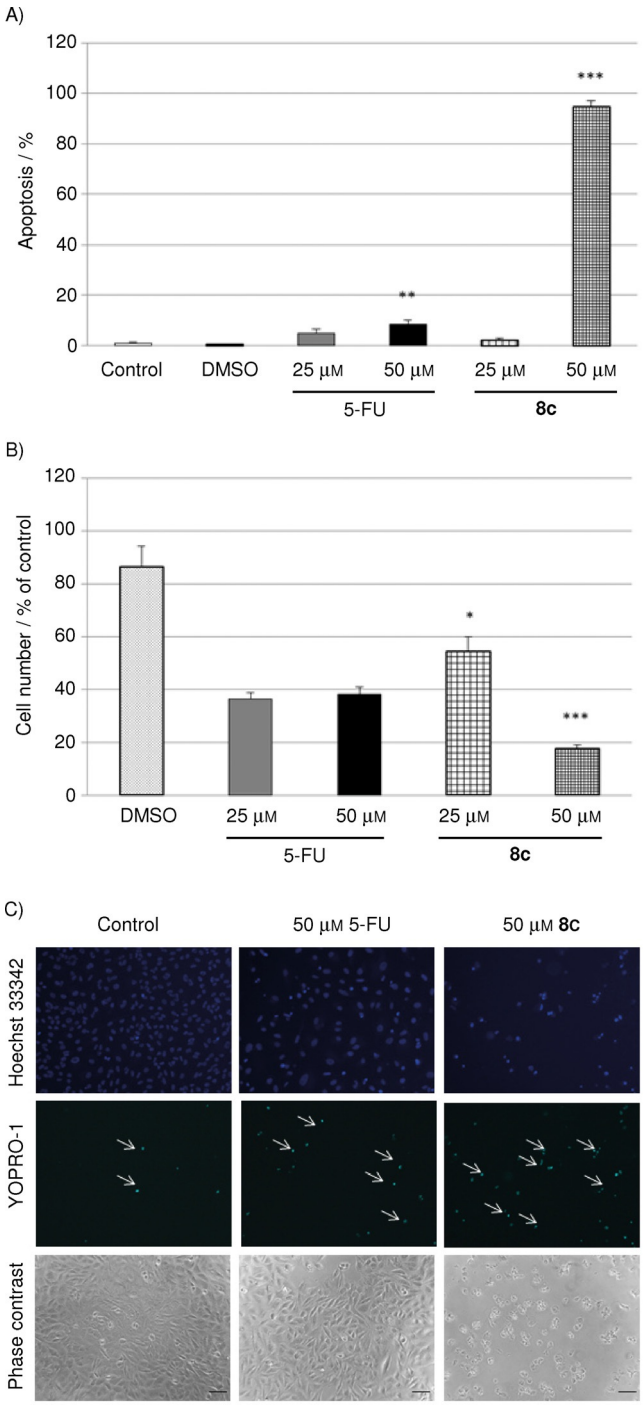


Figure 6. Treatment of human GOS-3 cells with 5-FU (5-fluorouridine), or its derivative **8c** after 48 h of incubation. Effects of 5-FU or its derivative **8c** on A) apoptosis rate, B) total cell number, C) morphological/quantitative changes of the apoptosis in GOS-3 cells observed by Hoechst 33342 (total cell count) or YOPRO-1 (apoptosis) staining, using a fluorescence microscope and phase contrast. Values in % apoptosis (A), or in % of control (100% of cell number) with medium alone (B), are given as mean ± SEM; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. negative control; $n = 4$. Scale bar: 50 μm; magnification × 100.

50.2% inhibition, but 25 μM increased significantly ($p < 0.05$) 49.5% the protein expression of p62/SQSMT1 when compared with the control (Figure 9 A,C). Furthermore, after treatment of

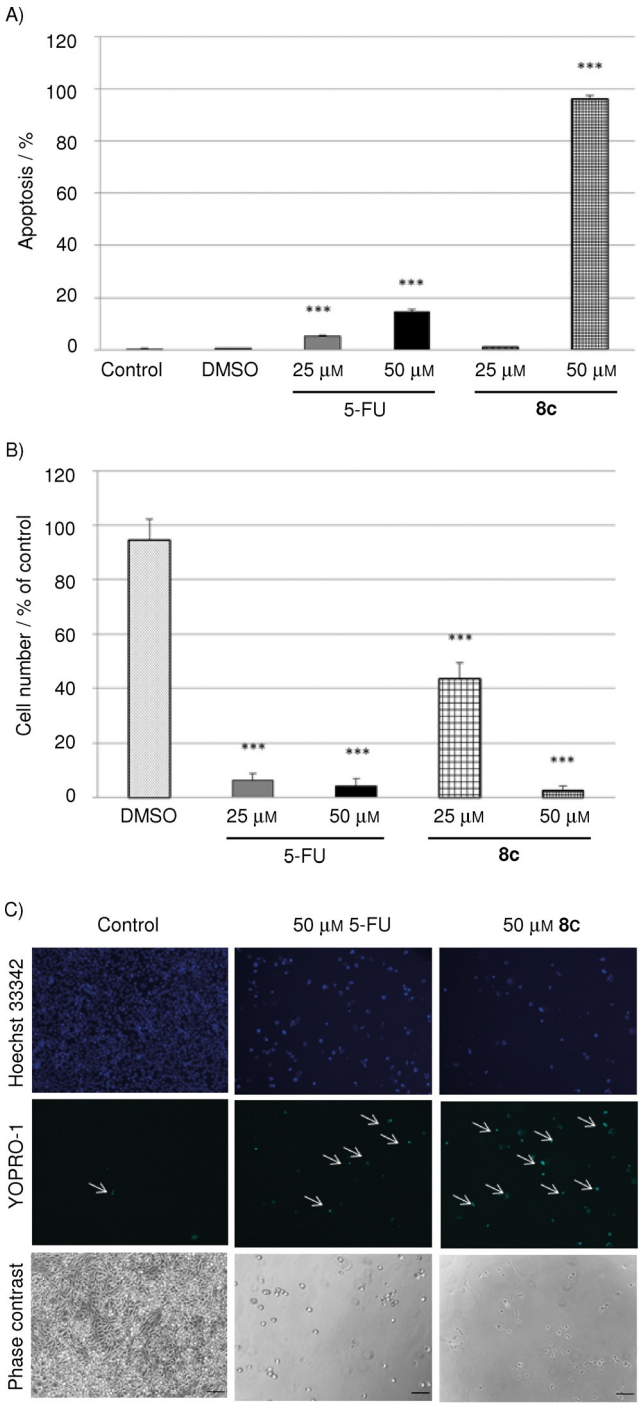


Figure 7. Treatment of rat BT4Ca cells with 5-FU (5-fluorouridine), or its derivative **8c** after 48 h of incubation. Effects of 5-FU or its derivative **8c** on A) apoptosis rate, B) total cell number, C) morphological/quantitative changes of the apoptosis in BT4Ca cells observed by Hoechst 33342 (total cell count) or YOPRO-1 (apoptosis) staining, using a fluorescence microscope and phase contrast. Values in % apoptosis are given as mean ± SEM. ***$p < 0.001$ vs. negative control (A); or in % of control (100 %) with medium alone (B); $n = 4$. Scale bar: 50 µm; magnification × 100.

BT4Ca cells with 12.5 µM or 25 µM 5-fluorouridine, we found an (insignificant) 54.9 % and 42.1 % inhibition of PCNA expression on protein level (Figure 9 B,C). When treated with 12.5 µM or 25 µM of the derivative **8c**, we found a significant ($p < 0.01$) 12.6 % or 80.4.3 % inhibition of the PCNA protein expression when compared with the control (Figure 9 B,C).

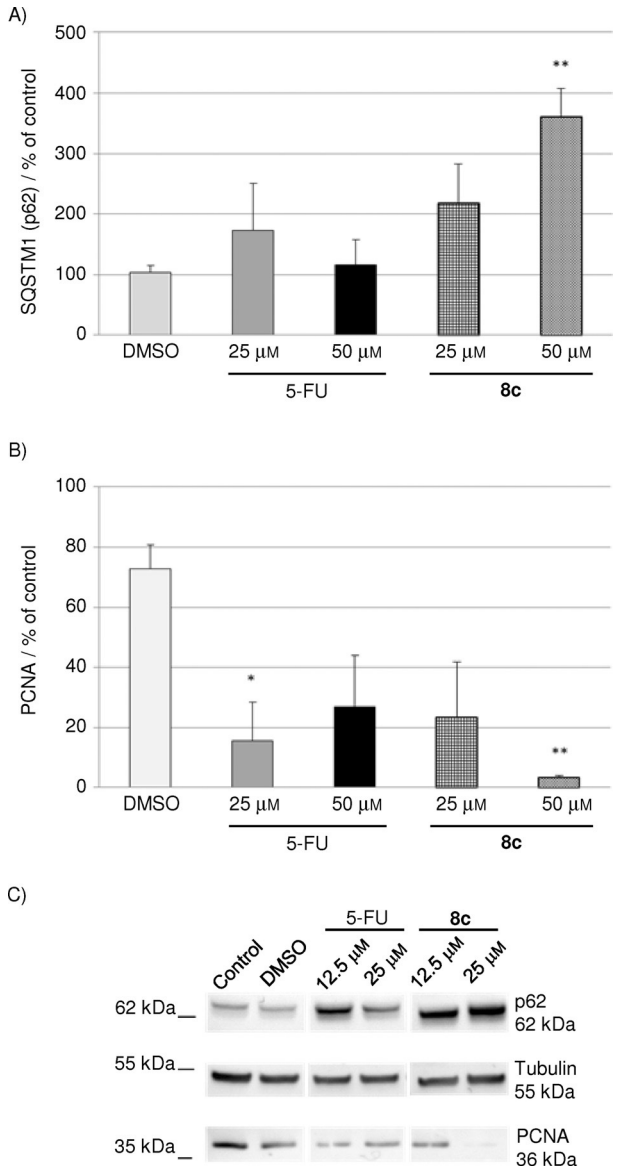


Figure 8. Effects of 48 h treatment of GOS-3 cells with 5-FU (5-fluorouridine), or its derivative **8c** shown as a western blot of A) autophagy (SQSTM1/p62) and B) proliferation (PCNA) markers quantified by densitometric analysis. Values expressed in % of negative control (100 %) are given as mean ± SEM; *$p < 0.05$ and **$p < 0.01$ vs. control; $n = 3$. C) Representative western blots of p62/SQSTM1 and PCNA are shown.

Experimental Section

Nomenclature

For the general numbering of nucleosides and nucleolipids, a novel denomination system was developed which allows an easy comparison of compound data among the various publications of our groups and which is disclosed in ref. [23]. Moreover, it was deposited sustainably in the repository of the library of the University

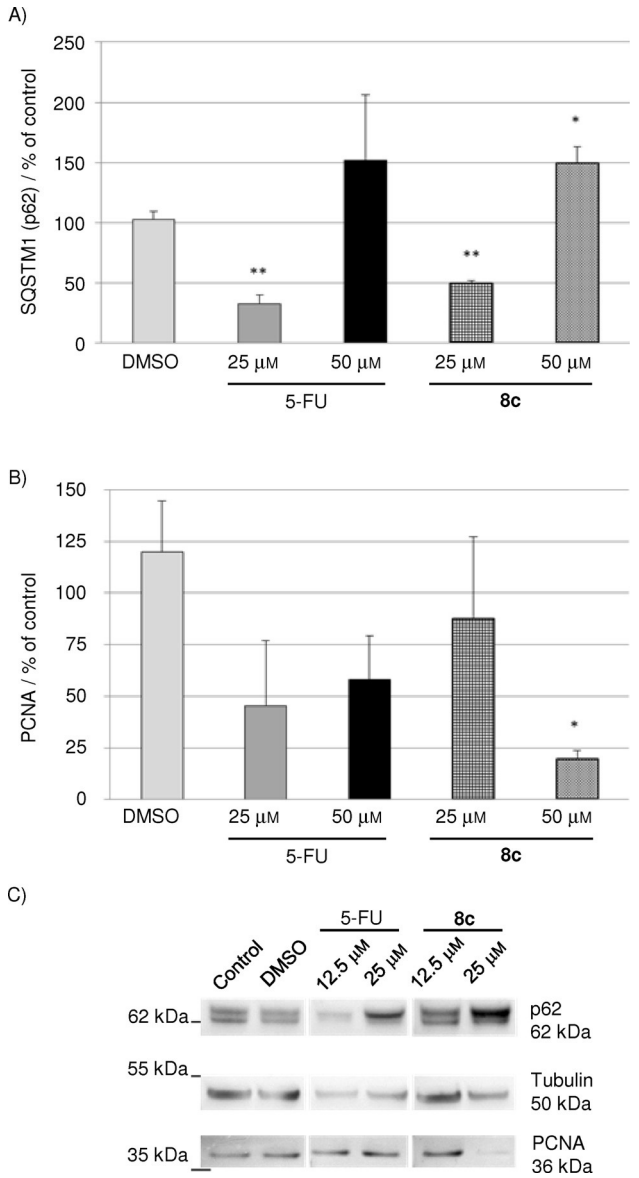


Figure 9. Effects of 48 h treatment of BT4Ca cells with 5-FU (5-fluorouridine), or its derivative 8c. Western blot of (A) autophagy [p62/SQSTM1] and (B) proliferation [PCNA] markers quantified by densitometric analysis. Values expressed in % of negative control (=100%) are given as mean+SEM. *$p<0.05$ and **$p<0.01$ (by T-TEST) significance vs. control. Representative western blots of p62/SQSTM1 and PCNA (C) are shown; $n=3$ independent experiments.

of Osnabrück under the following unique registration number (URN) and URL:

https://repositorium.uni-osnabrueck.de/handle/urn:nbn:de:gbv:700-2015110413639.

Nucleolipids are abbreviated by NL, nucleosides by NS. The first number refers to the nucleoside. The second number refers to the moiety at the 2′,3′-position at the glyconic ring; cyclic moieties are abbreviated by "cycl" before the number. The third number refers to the lipophilic moiety at the base [N(3) for pyrimidines, N(1) for purines]. The fourth number refers to a lipophilic moiety at the 5′-*O*-position. Identical residues carry the same number; "0" stands for a molecule without a residue at this position. For a translation of the NS/NL nomenclature to the plain compound numbers used throughout the text, schemes, figures see Table 1.

Chemistry

General Remarks: All chemicals were purchased from Sigma–Aldrich (Deisenhofen, Germany) or TCI-Europe (Zwijndrecht, Belgium). Solvents were of laboratory grade and were distilled before use. Column chromatography was performed on silica gel 60. Thin-layer chromatrography (TLC) was performed using aluminum sheets and silica gel 60 F_{254}; 0.2 mm layer (Merck, Germany). NMR spectra (^{1}H, ^{13}C, DEPT-135) were obtained using an AMX-500 instrument (Bruker, Rheinstetten, Germany); ^{1}H: 500.14 MHz, ^{13}C: 125.76 MHz; chemical shifts (δ) are reported in ppm referenced to an internal standard of residual proteosolvent [D$_6$]DMSO (2.50, 39.50 ppm, rel. to tetramethylsilane (TMS) as internal standard). Multiplicity is quoted as br (broad), s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), m (multiplet), and Ψt (pseudotriplet), dd (doublet of doublet), ddd (doublet of doublet of doublet). *J* values are reported in Hz. 2 D [^{1}H,^{1}H] and [^{1}H,^{13}C] correlation spectra (heteronuclear single quantum coherence, HSQC) and Cosy-Long-Range spectra (pulse program: cosygpmfph) were measured with the same instrument.

Sample preparation was performed as follows: an appropriate amount of compound (usually 20–25 mg) was dissolved in [D]$_6$DMSO (0.5 mL) and placed in the NMR quartz tube (diameter, 5 mm). Before measurement the solutions were degassed by ultrasonication for several minutes. Number of scans: ^{1}H: 64, ^{13}C: 12.000, DEPT-135: 5.000. ESI MS was performed using a Bruker Daltronics Esquire HCT instrument (Bruker Daltronics, Leipzig, Germany); ionization was performed with a 2% aq. formic acid (HCOOH) solution. UV/VIs spectra were obtained using a Cary 1E spectrophotometer (Varian, Darmstadt, Germany). Compound samples of about 1 mg were dissolved either in MeOH or an appropriate buffer solution (pH 3, 7, or 9, 100 mL each). Aliquots of 1 mL of the fully dissolved compounds (warming, ultrasonication) were subjected to UV/Vis spectrometry in MT4 quartz cuvettes (Hellma, Darmstadt, Germany). Elemental analyses (C, H, N) were performed on a VarioMICRO instrument (Fa. Elementar, Hanau, Germany). ^{10}LogP_{OW} values were calculated using the http://eadmet.com/de/physprop.php website with *ePhysChem* that contains ALOGPS v.3.0. Experimental determination of ^{10}LogP_{ow} values of compounds were performed as follows: samples of compounds 5-fluorouridine (NS_4.0.0.0), **6** (NL_5.3.0.0), **8c** (NL_6.1.^{1}3.0), **6** (NL_6.3.0.0) (2 mg, each) were dissolved in a heterogenic mixture of *n*-butanol (25 mL) and H_2O (25 mL) by ultrasonication (10 min) under slightly warming. After separation of the layers from each phase aliquots of 1 mL were withdrawn, and their UV spectra were run in 1 cm quartz cuvettes. From the ratio of maximal extinctions of both layers at λ_{max}, the corresponding ^{10}LogP_{ow} values were calculated and compared with increment-based calculations (Table 2).

General synthetic methods

The complete characterization data for all synthesized compounds mentioned below can be found in the Supporting Information.

Ethyl 3-[4-(6-amino-purin-9-yl)-6-hydroxymethyl-2-methyl-tetrahydro-furo[3,4-d][1,3]dioxol-2-yl]-propionate (3, NL_5.1.0.0,[8] diastereoisomeric mixture): The compound was prepared following a previously described procedure,[8] but with slight modifications and supported by further analytical data. To a solution of anhydrous adenosine (**1**, NS_5.0.0.0,[23] 1.38 g, 5 mmol) in dry and

Table 2. Comparison of calculated and UV/Vis-measured ^{10}LogP_{ow} values of 5-fluorouridine and compounds **5**, **6**, and **8c** used for in vitro tests.

Compound	^{10}LogP_{ow} measured	^{10}LogP_{ow} calculated[21]
5-Fluorouridine	−0.61	−1.3 ± 0.38
5	≥ 4.00	5.1 ± 0.74
6	≥ 3.50	5.3 ± 0.74
8c	≥ 3.74	3.5 ± 0.74

Values are for single measurements.

amine-free dimethylformamide (DMF, 10 mL), ethyl levulinate (1.42 mL, 10 mmol), triethylorthoformate (1.65 mL, 10 mmol) and 4 M HCl in 1,4-dioxane (2 mL) were added. After stirring for 27 h at ambient temperature, the reaction mixture was partitioned between CH_2Cl_2 (75 mL) and a saturated aqueous $NaHCO_3$ solution (30 mL). The aqueous phase was washed with CH_2Cl_2 (2×25 mL), and the combined organic layers were evaporated on a rotary evaporator. The resulting oil was co-evaporated repeatedly with CH_2Cl_2 in order to remove residual DMF. The product was precipitated by addition of dry diethyl ether (Et_2O), filtered, and dried in high vacuum overnight yielding the title compound as colorless crystals (1.24 g, 3.15 mmol, 71%; diastereoisomeric mixture, [1*R*]/[1*S*] = 12:1).

Ethyl 3-[4-hydroxymethyl-2-methyl-6-(6-oxo-1,6-dihydro-purin-9-yl)-tetrahydro-furo[3,4-d][1,3]dioxol-2-yl]-propionate (4, NL_6.1.0.0[9,11,23]): ***A) By enzymatic deamination of 3***: Compound **3** (0.94 g, 2.39 mmol) was dissolved in H_2O (27 mL), and adenosine deaminase (90 units, calf intestine)—dissolved in 0.9 mL of glycerol—was added. The reaction mixture was stirred at ambient temperature for 72 h (TLC monitoring). The resulting solution was evaporated on a rotary evaporator, leaving colorless crystals of the title compound as pure (1R)-**4** diastereoisomer (0.94 g, 2.39 mmol, 100%). ***B) By Direct Ketal Formation of Inosine***: Anhydrous inosine (**2**, 1.0 g; 3.73 mmol) was dissolved in dry DMF (18 mL). Then, ethyl levulinate (1.05 mL; 7.41 mmol), triethylorthoformate (0.92 mL; 5.60 mmol), and 4 M HCl in 1,4-dioxane (3.5 mL) were added, and the reaction mixture was stirred for 26 h at rt. The reaction mixture was partitioned between CH_2Cl_2 (175 mL) and a saturated conc. aq. $NaHCO_3$-solution (5 mL). The aqueous phase was washed twice with CH_2Cl_2 (80 mL, each). The combined organic layers were evaporated and repeatedly co-evaporated from CH_2Cl_2 to remove residual DMF. The oily residue was dried in high vacuum o/n at 40 °C and subsequently purified by column chromatography (SiO_2 60 H, column: 6.5×14 cm; CH_2Cl_2/MeOH; 85:15; *v*/*v*). From the main zone, the title compound was isolated as a white powder (diastereoisomeric mixture, 1.108 g, 2.81 mmol, 75%). The material proved to be identical with an authentic sample in all respects, except for the NMR data.[9,11]

6-(6-Amino-purin-9-yl)-2,2-dinonyl-tetrahydro-furo[3,4-d][1,3]dioxol-4-yl]-methanol (5, NL_5.3.0.0[23]): To a solution of anhydrous adenosine (1.0 g, 3.74 mmol) in dry DMF (15 mL) nonadecan-10-one (2.1 g, 7.48 mmol), triethylorthoformate (1.0 g, 5.61 mmol), and 4 M HCl in 1,4-dioxane (3.4 mL) were added. The reaction mixture was stirred for 48 h at rt and then partitioned between CH_2Cl_2 (175 mL) and a sat. aq. $NaHCO_3$ solution (50 mL). The organic layer was washed with H_2O (3×100 mL), and the combined aqueous layers were re-extracted with CH_2Cl_2 (25 mL). The combined organic phases were evaporated, and the oily residue was dried in high vacuum overnight and then submitted to column chromatography (SiO_2 60 H, column: 6×12 cm, CH_2Cl_2/MeOH 87:13). Evaporation of the main zone gave the title compound as a colorless oil (776 mg, 1.46 mmol, 39%).

9-(6-Hydroxymethyl-2,2-dinonyl-tetrahydro-furo[3,4-d][1,3-dioxol-4-yl)-1,9-dihydro-purin-6-one (6, NL_6.3.0.0): To a solution of anhydrous inosine (**2**, 1.0 g, 3.72 mmol) in dry DMF (15 mL), nonadecan-10-one (2.1 g, 7.44 mmol), triethylorthoformate (1.0 g, 5.58 mmol), and 4 M HCl in 1,4-dioxane (3.4 mL) were added. The reaction mixture was stirred for 48 h at ambient temperature under exclusion of moisture. Then, the mixture was partitioned between CH_2Cl_2 (350 mL) and a saturated aqueous $NaHCO_3$ solution (50 mL). The organic layer was washed with H_2O (3×100 mL), and the combined aqueous phases were re-extracted with CH_2Cl_2 (2×25 mL). The combined organic layers were dried (Na_2SO_4), filtered, and evaporated. The residue was dried o/n in high vacuum and then submitted to column chromatography (SiO_2 60 H, column: 6×14 cm, CH_2Cl_2/MeOH 9:1). Evaporation of the main zone afforded the title compound as a colorless oil (700 mg, 1.314 mmol, 35%).

9-(6-Hydroxymethyl-2,2-dinonyl-tetrahydro-furo[3,4-d][1,3]dioxol-4-yl)-1-(3,7,1)-trimethyl-dodeca-2,6,10-trienyl)-1,9-dihydro-purin-6-one (7, NL_6.3.3.0): To a solution of compound **6** (593 mg, 1.113 mmol) in dry DMF (6 mL), K_2CO_3, (390 mg, 2.88 mmol) was added, and the suspension was stirred for 30 min at ambient temperature. Thereupon, farnesyl bromide (0.35 mL, 1.17 mmol) was added dropwise under an N_2 atmosphere while stirring. After 24 h, K_2CO_3 was filtered off and washed repeatedly with CH_2Cl_2 (2×25 mL). The filtrate and washings were evaporated on a rotary evaporator; the resulting oil was dried in high vacuum for 24 h. The residue was submitted to column chromatography (SiO_2 60 H, column: 6×12 cm, CH_2Cl_2/MeOH 97:3). Evaporation of the main zone afforded the title compound as a colorless oil (499.2 mg, 0.677 mmol, 61%).

Ethyl 3-{4-hydroxymethyl-2-methyl-6-[1-(3-methyl-but-2-enyl)-6-oxo-1,6-dihydro-purin-9-yl]-tetrahydro-furo[3,4-d][1,3]dioxol-2-yl}-propionate (8a, NL_6.1.^{1}1.0,[23]): To a solution of anhydrous (dried at 80 °C overnight), stereochemically pure compound (1*R*)-**4** (0.35 g, 0.89 mmol) in dry DMF (10 mL), dry K_2CO_3 (0.49 g, 3.55 mmol) was added. The suspension was stirred for 30 min at ambient temperature. Subsequently, isopentenyl bromide (0.95 mmol) was added dropwise under Ar atmosphere and under exclusion of light and moisture. The reaction mixture was stirred for 60 h at rt. The salt was then filtered off and washed with CH_2Cl_2 (2×25 mL); the filtrate and washings were combined and partitioned between H_2O (30 mL) and CH_2Cl_2 (40 mL) in a separation funnel. The organic phase was pooled, and the aqueous was washed with CH_2Cl_2 (2×25 mL). The combined organic layers were dried (Na_2SO_4), filtered, and evaporated on a rotary evaporator. Residual DMF was removed by repeated co-evaporation with CH_2Cl_2 and subsequent drying in high vacuum. Column chromatography (SiO_2 60 H, column: 5×6.5 cm, CH_2Cl_2/MeOH 98:2) gave the title compound as a colorless oil (200 mg, 0.43 mmol, 48%)

Ethyl 3-{4-[1-(3,7-dimethyl-octa-2,6-dienyl)-6-oxo-1,6-dihydro-purin-9-yl]-6-hydroxymethyl- 2-methyl-tetrahydro-furo[3,4-d][1,3]dioxol-2-yl}-propionate (8b, NL_6.1.^{1}2.0,[23]): To a solution of anhydrous (dried at 80 °C overnight), stereochemically pure compound (1*R*)-**4** (0.35 g, 0.89 mmol) in dry DMF (10 mL), dry K_2CO_3 (0.49 g, 3.55 mmol) was added. The suspension was stirred for 30 min at ambient temperature. Subsequently, geranyl bromide (0.95 mmol) was added dropwise under Ar atmosphere and under exclusion of light and moisture. The reaction mixture was stirred for 60 h at rt. Then, the salt was filtered off and washed with CH_2Cl_2 (2×25 mL); the filtrate and washings were combined and

partitioned between H_2O (30 mL) and CH_2Cl_2 (40 mL) in a separation funnel. The organic phase was pooled, and the aqueous phase was washed with CH_2Cl_2 (2×25 mL). The combined organic layers were dried (Na_2SO_4), filtered, and evaporated on a rotary evaporator. Residual DMF was removed by repeated co-evaporation with CH_2Cl_2 and subsequent drying in high vacuum. Column chromatography (SiO_2 60 H, column: 5×6.5 cm, CH_2Cl_2/MeOH 95:5) gave the title compound as a colorless solid (140 mg, 0.25 mmol, 40%).

Ethyl 3-{4-hydroxymethyl-2-methyl-6-[6-oxo-1-(3,7,11-trimethyl-dodeca-2,6,10-trienyl)-1,6-dihydro-purin-9-yl]-tetrahydro-furo[3,4-d][1,3]dioxol-2-yl}propionate (8c, NL_6.1.^{1}3.0,[23]): To a solution of anhydrous, stereochemically pure compound (1*R*)-**4** (0.2 g, 0.51 mmol) in dry DMF (3 mL), K_2CO_3 (178 mg, 1.32 mmol) was added. The suspension was stirred at ambient temperature for 30 min. Then farnesyl bromide (0.16 mL, 0.56 mmol) was added dropwise under Ar atmosphere. The reaction mixture was stirred for 24 h at rt under exclusion of light and moisture. Thereupon, the suspension was filtered, and the salt was washed repeatedly with CH_2Cl_2 (2×25 mL). Filtrate and washings were evaporated on a rotary evaporator, and the oily residue was dried in high vacuum. The raw product was purified by column chromatography (SiO_2 60 H, column: 6.5×11 cm, CH_2Cl_2/MeOH 97:3). Evaporation of the main zone gave the title compound as a colorless oil (175.1 mg, 0.29 mmol, 57%).

Repetition of the formerly described experiment at elevated temperatures yielding 8c and 9-{2,3-O[(1R)-4-ethoxy-1-methyl-4-oxobutylidene]-ß-D-ribofuranosyl}-6-{[(2E,6E,9E,13E,17E)-3,7,11,11,14,18,22-heptamethyltricosa-2,6,9,13,17,21-hexaen-1-yl]oxy}-9H-purine (12, via nonisolated 8d): To a solution of anhydrous, stereochemically pure compound (1*R*)-**4** (0.15 g, 0.51 mmol) in dry DMF (4.8 mL), K_2CO_3 (140 mg, 1.32 mmol) was added. The suspension was heated to 50 °C for 30 min and then cooled to 40 °C. Then, farnesyl bromide (0.12 mL; 0.42 mmol) was added dropwise stirred at ambient temperature for 30 min. Subsequently, a second portion of farnesyl bromide (0.16 mL, 0.38 mmol) was added dropwise under Ar atmosphere. The reaction mixture was stirred for another 24 h at rt under exclusion of light and moisture. Then, CH_2Cl_2 (6 mL) was added; the suspension was filtered, and the salt was washed repeatedly with CH_2Cl_2 (2×25 mL). Filtrate and washings were evaporated on a rotary evaporator, and the oily residue was dried in high vacuum. The raw product was purified by column chromatography (SiO_2 60 H, column: 6.5×10 cm; 1. main zone, CH_2Cl_2/MeOH; 94:6; *v*/*v*; 2. main zone, MeOH, 0.3 bar). From the faster-migrating main zone, compound **8c** was isolated as a colorless oil (128 mg, 0.214 mmol, 56%). The compound was identical with an authentic sample of compound **8c** in all other respects (^{1}H NMR, ^{13}C NMR, HR ESI MS). From the slower-migrating zone, the title compound **12** was isolated as a colorless oil (115 mg, 0.142 mmol, 37%).

General procedure for the prenylation of the adenosine-*O*-2′,3′-ketal 3: Anhydrous compound **3** (0.59 g, 1.5 mmol) was dissolved in anhydrous and amine-free DMF (4 mL). To the solution was added $BaCO_3$ (0.89 g, 4.5 mmol). Under Ar atmosphere, isopentenyl bromide, geranyl bromide or farnesyl bromide were added dropwise (1.95 mmol each). The reaction mixtures were stirred o/n at rt under exclusion of moisture and light. Subsequently, the salt was removed by filtration through a Celite pad. The corresponding filtrates were evaporated on a rotary evaporator, and the residues were then dried in high vacuum to give a slightly red foam. Trituration with CH_2Cl_2 (50 mL) afforded an off-white solid which was isolated by filtration. Purification was performed by column chromatography (SiO_2 60 H, column: 5×8.5 cm or 6.5×10.5 cm, respectively, CH_2Cl_2/MeOH 9:1 or 95:5, respectively). Evaporation of the corresponding main zone gave the N(1)-prenylated salts **9a-c**.

6-Amino-9-[2-(2-ethoxycarbonyl-ethyl)-6-hydroxymethyl-2-methyl-tetrahydro-furo[3,4-d][1,3]dioxol-4-yl]-1-(3-methyl-but-2-enyl)-9H-purin-1-ium bromide (9a, NL_5.1.^{1}1.0,[23]): The product was obtained as a white solid (0.44 g, 0.95 mmol, 63%).

6-Amino-1-(3,7-dimethyl-octa-2,6-dienyl)-9-[2-(2-ethoxycarbonyl-ethyl)-6- hydroxymethyl-2-methyl-tetrahydro-furo[3,4-d][1,3]dioxol-4-yl]-9H-purin-1-ium bromide (9b, NL_5.1.^{1}2.0,[23]): The product was obtained as a slightly yellowish solid (0.78 g, 1.48 mmol, 58%).

6-Amino-9-[2-(2-ethoxycarbonyl-ethyl)-6-hydroxymethyl-2-methyl-tetrahydro-furo[3,4-d][1,3]dioxol-4-yl]-1-(3,7,11-trimethyl-dodeca-2,6,10-trienyl)-9H-purin-1-ium bromide (9c, NL_5.1.^{1}3.0,[23]): The product was obtained as a slightly orange oil (0.98 g, 1.64 mmol, 67%).

General Procedure for the Dimroth rearrangements of compounds 9a–c:[16,17] Compounds **9a–c** (0.7 mmol, each) were suspended in Me_2NH–MeOH (1 M, 4.5 mL) and stirred for 20 h at ambient temperature (TLC monitoring). Subsequently, the corresponding reaction mixtures, containing compounds **10a**, **10b**, or **10c**, were concentrated on a rotary evaporator and then in high vacuum. The residues were purified by column chromatography (SiO_2 60 H, columns: 5×7 cm, 6.5×12.5 cm, and 6.5×10.5 cm; solvent systems: CH_2Cl_2/MeOH, 98:2, CH_2Cl_2/MeOH, 95:5). The main fractions were pooled and evaporated to give slightly colored oils of **10a–c**.

Ethyl 3-{4-hydroxymethyl-2-methyl-6-[6-(3-methyl-but-2-enylamino)-purin-9-yl]-tetrahydro-furo[3,4-d][1,3]dioxol-2-yl}-propionate (10a, NL_5.1.^{6}1.0): The product was obtained as a colorless oil (0.21 g, 0.46 mmol, 62%).

3-{4-[6-(3,7-Dimethyl-octa-2,6-dienylamino)-purin-9-yl]-6-hydroxymethyl-2-methyl-tetrahydro-furo[3,4-d][1,3]dioxol-2-yl}-propionsäureethylester (10b, NL_5.1.^{6}2.0,[23]): The product was obtained as a colorless oil (0.18 g, 0.34 mmol, 23%).

Ethyl 3-{4-hydroxymethyl-2-methyl-6-[6-(3,7,11-trimethyl-dodeca-2,6,10-trienylamino)-purin-9-yl]-tetrahydro-furo[3,4-d][1,3]dioxol-2-yl}-propionate (10c, NL_5.1.^{6}3.0,[23]): The product was obtained as a colorless oil (0.47 g, 0.62 mmol, 38%).

Biological methods

Cell lines and culture conditions: In vitro experiments were performed using the human astrocytoma/oligodendroglioma GOS-3 cells (DSMZ GmbH, Braunschweig, Germany), the rat malignant neuro-ectodermal BT4Ca cells (a kind gift from Dr. Nadine John, Hannover Medical School, Hannover, Germany), as well as the human acute monocytic leukemia cell line THP-1 (DSMZ GmbH, Braunschweig, Germany). The cells were cultured in 90% RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 100 U mL^{-1} penicillin, and 0.1 mg mL^{-1} streptomycin, and were maintained at 37 °C in a humidified atmosphere (5% CO_2, 95% air) as described earlier.[1,2]

Determination of viability/survival of 5-fluorouridine and derivatives: 96-well plates (BD Falcon, Becton Dickinson GmbH, Heidelberg, Germany) were seeded with 1.5×10^4 GOS-3, 5×10^3 BT4Ca, or 3×10^4 THP-1 cells. After 24 h, the medium was changed and different concentrations of 5-fluorouridine or its derivatives **5**, **6**, **8c**, were tested at concentrations of 1.56, 3.12, 6.25, 12.5, 25, or 50 μM.

After incubation for 48 h, viability/cytotoxicity was measured using PrestoBlue reagent (Invitrogen-Life Technologies GmbH, Darmstadt, Germany) as described earlier.[1,2] PrestoBlue was added to the cells into the culture medium and 30 min, 1 h, 2 h, or 3 h after addition of PrestoBlue™, the optical density (OD) was measured at 570 nm and 600 nm (as reference) with a SUNRISE ELISA-reader (Tecan, Salzburg, Austria). Results are expressed in % survival (OD 570/600 nm of samples × 100 / OD 570/600 nm of control without substances). As control (100% viability = 0% cytotoxicity), cells were cultured with medium alone. To evaluate the cytotoxic effect on macrophages, this procedure was repeated with 5×10^3 per well of phorbol-12-myristate-13-acetate (PMA; 100 ng mL^{-1})-differentiated (48 h) human THP-1 macrophages, which were treated (48 h) with different concentrations of the substances under test.

Apoptosis assay: Human GOS-3 cells or rat BT4Ca were seeded in 96-well plates at a density of 1.5×10^4 and 5×10^3 per well, respectively. After 24 h, the medium was changed, and the substances under test were added at various concentrations as indicated. Apoptotic cells were identified by YO-PRO-1 (1 mM) in combination with the Hoechst 33342 nuclear staining dye (5 mg mL^{-1}; Mobitec Company, Göttingen, Germany). The total number of cells (Hoechst 33342$^+$ nuclei) and apoptotic cells (YO-PRO-1$^+$ nuclei) was counted using an inverse fluorescence microscope Eclipse TS100 (Nikon GmbH, Düsseldorf, Germany) equipped with a camera AxioCam MRc (Carl Zeiss Microscopy GmbH, Göttingen, Germany) and a computer-assisted morphometry system AxioVision 4 (Carl Zeiss Microscopy GmbH), and the percentage of apoptotic cells to the total cells was then calculated. Additionally the % of total cell count after 48 h treatment was calculated using the total number of cells containing Hoechst 33342$^+$ nuclei, considering the negative control without treatment as 100%.[24]

SDS-PAGE and western blotting: GOS-3 and BT4Ca cells were scraped off in radioimmunoprecipitation assay (RIPA) buffer pH 7.5 (Cell Signaling Technology Europa, Leiden, The Netherlands). An aliquot was used for protein quantification using Pierce™ BCA Protein Assay Kit (Pierce Biotechnology, Rockford, USA), and after addition of sample buffer, pH 8.3, boiled at 95 °C (10 min). Samples were stored at −20 °C. Afterwards, electrophoresis was done using NuPAGE Novex 4–12% Bis-Tris precast polyacrylamide gels (Life Technologies GmbH, Darmstadt, Germany) and a loading of 30 µg total protein per lane. Additionally, prestained peqGOLD ProteinMarker VI (PEQLAB Biotechnologie GmbH, Erlangen, Germany) was used. Blotting was performed with wet/tank blotting systems (Bio-Rad Laboratories GmbH, München, Germany) and nitrocellulose Amersham Hybond-ECL membranes (GE Healthcare Europe GmbH, Glattbrugg, Switzerland) for enhanced chemiluminescence (ECL). Protein transfer was done at 0.6 mA cm^{-2} (o/n, rt) with transfer buffer containing 10% MeOH. Nonspecific sites were blocked with Tween 20-supplemented Tris-buffered saline (TTBS) and 5% milk (150 mM NaCl, 10 mM Tris/HCl, pH 7.6; Tween-20 0.1%; 5% low-fat dried milk; 1 hour, rt). Rabbit polyclonal antibodies directed against p62/SQSTM1 (P0068, 1:500, Sigma–Aldrich); rabbit polyclonal antibodies against active caspase-3 (ab2302, 1:200, Abcam, Cambridge, UK); BAX (Ab7977, 1:1000, Abcam); PCNA (13110, 1:1000, New England Biolabs, UK); and α-tubulin (ab4074, 1:1000, Abcam) were used diluted in blocking buffer, o/n, 4 °C. An anti-rabbit IgG peroxidase conjugate (1:3000, GE Healthcare Europe GmbH, Germany) in blocking buffer; 1 h, rt) and the chemiluminescence ECL detection kit, AceGlow (PEQLAB Biotechnologie GmbH) were used for detection. A FUSION-FX7 system (PEQLAB Biotechnologie GmbH) served for documentation. Washing steps after every incubation period were performed using TTBS. Signals were quantified by computer software (Scion ImageJ, National Institutes of Health, Bethesda, USA).

Statistical analysis: Results are presented as means ± standard error of the mean (SEM). Depending on the mode of distribution, statistical procedures were performed by the Mann–Whitney U test or by the unpaired Student's *t*-test, using SigmaPlot software (Systat Software, Inc, Chicago, USA); $p<0.05$ was chosen for statistical significance.

Acknowledgements

The authors thank the following University of Osnabrück and Marburg members: Marianne Gather Steckhan for the NMR measurements, Dr. Stefan Walter for HR ESI MS, Anja Schuster for the elemental analyses, Petra Bösel for formulae drawings, Henning Eickmeier and Holger Heine for the preparation of Figure 3 (Univerity of Osnabrück); Andrea Cordes, Elke Völck–Badouin (University of Marburg); Diana Yudin and Anna Diestel (University of Osnabrück) for valuable technical assistance; as well as Ellen Essen and Gabriella Stauch (University of Marburg) for preparation of the manuscript. The authors also gratefully acknowledge financial support by the Bundesministerium für Wirtschaft, Germany (FKZ: KF 2369401 SB9 and FKZ 2369501 SB9). Moreover, the authors thank Prof. Dr. Uwe Beginn for excellent laboratory facilities at the University of Osnabrück.

Keywords: antitumor agents · cytotoxicity · glioblastomas · ketal lipophilicity · nucleolipids · prenylation

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Received: October 14, 2015
Published online on December 20, 2015

