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Antitumour tiazofurin analogues embedded with an amide moiety at the C-2′ position

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ABSTRACT

Synthesis of four new tiazofurin analogues has been accomplished starting from D-glucose. The key step of the synthesis was the three-step cascade that enabled an efficient hydrogen sulfide mediated one-pot conversion of 2-azido-3-*O*-acyl-ribofuranosyl cyanides to the corresponding 2-alkylamido ribofuranosyl thiocarboxamides. The resulting key intermediates were first converted to protected tiazofurin derivatives by cyclocondensation with ethyl bromopyruvate, and finally to target C-nucleosides by treatment with ammonia in methanol. In vitro cytotoxicities of tiazofurin analogues against a number of human tumour cell lines were recorded and compared with those observed for the parent molecule (tiazofurin), as well as the commercial antitumour agent doxorubicin (DOX). Analogues **2b**–**d** have shown a potent in vitro cytotoxic activity against human myelogenous leukaemia K562. Among solid tumour cell lines, HT29 was sensitive only to **2d**, while HeLa cells were sensitive to **2a**, **2b** and **2d**. Only analogue **2a** was highly cytotoxic against MCF-7 cells. No tiazofurin analogue exhibits any significant cytotoxicity towards normal foetal lung MRC-5 cells. Downregulation of Bcl-2, activation of caspase-3 and presence of cleavage product of PARP suggest that the cytotoxic effects of tiazofurin analogues **2a**–**d** in K562 might be mediated by apoptosis in a caspase-dependent way. On the contrary, tiazofurin did not induce apoptosis of K562 cells, which suggests a different mechanism of action, most probably through the inhibition of IMPDH. Flow cytometry and Western blot analysis data agreed well with the results of MTT assay, and enabled identification of analogue **2c** as the most promising antitumour agent that preferentially target cancer cells over normal cells and thus represents a new lead for further optimization.

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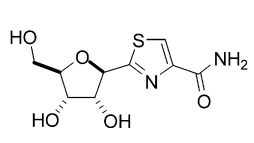
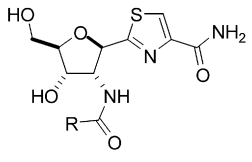
1. Introduction

Among the many classes of nucleoside analogues, C-nucleosides represent popular synthetic targets due to their potential value as therapeutic agents and biochemical probes.[1] Remarkable among them is tiazofurin (**1**, Fig. 1), a synthetic[2] C-nucleoside that shows potent antitumour activity in a variety of tumour systems.[3] Tiazofurin has been extensively studied both in preclinical[4] and clinical studies,[5] and has been approved as an orphan drug for the treatment of patients with acute myeloid leukaemia in blast crisis.[3] It exhibits at least two different mechanisms of action. The first one is the non-competitive inhibition of inosine 5′-monophosphate dehydrogenase, a rate-limiting enzyme for guanylate synthesis,[6] and the second is the induction of apoptosis.[4,7] Despite the remarkable efficacy of tiazofurin, lack of specificity and a significant neurotoxicity[3] limits widespread use of this drug and it is not currently marketed. In the search for new antineoplastic agents with improved therapeutic effects, many tiazofurin derivatives have been synthesized, including a number of those with a modified sugar



Tiazofurin (**1**)



2a R = C_5H_{11}
2b R = C_7H_{15}
2c R = C_9H_{19}
2d R = $C_{11}H_{23}$

Fig. 1. Structures of tiazofurin (**1**) and analogues **2a**–**d**.

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segment.[8] However, some of these compounds did not show favourable biological properties, although a number of recently synthesized analogues have not been assayed for their antitumour activity. We have recently reported the synthesis of a number of tiazofurin analogues (TAs) that showed increased antitumour activities with respect to the lead compound **1**.[9] Most of these analogues were devoid of any toxicity towards normal mammalian cells. We have also found that some tiazofurin derivatives induced apoptosis in the C6 rat glioma cells.[10] Our previous findings[11] that the incorporation of 2′-nitrogen functionalities into the tiazofurin sugar moiety may result in an improvement of the lead compounds cytotoxity against some neoplastic cells, prompted us to study other variations in the structure of the C-2′ portion of the tiazofurin sugar moiety in order to determine the structure–activity relationships associated with this portion of the molecule, as well as to possibly get a better insight into the mechanism of action of TAs in certain tumour cell lines. In particular, we wished to investigate the biological activity of TAs bearing a saturated amide function at the C-2′ position.

Herein, we disclose in detail the synthesis of new TAs **2a**–**d**, with hexan-, octan-, decan- and dodecanamido functions at C-2′, along with their effects on the proliferation of a number of malignant cell lines.[12] In addition, the purpose of the present study was to examine the apoptotic signalling induced by tiazofurin and analogues in the human K562 malignant cell line.

2. Results and discussion

2.1. Chemical synthesis

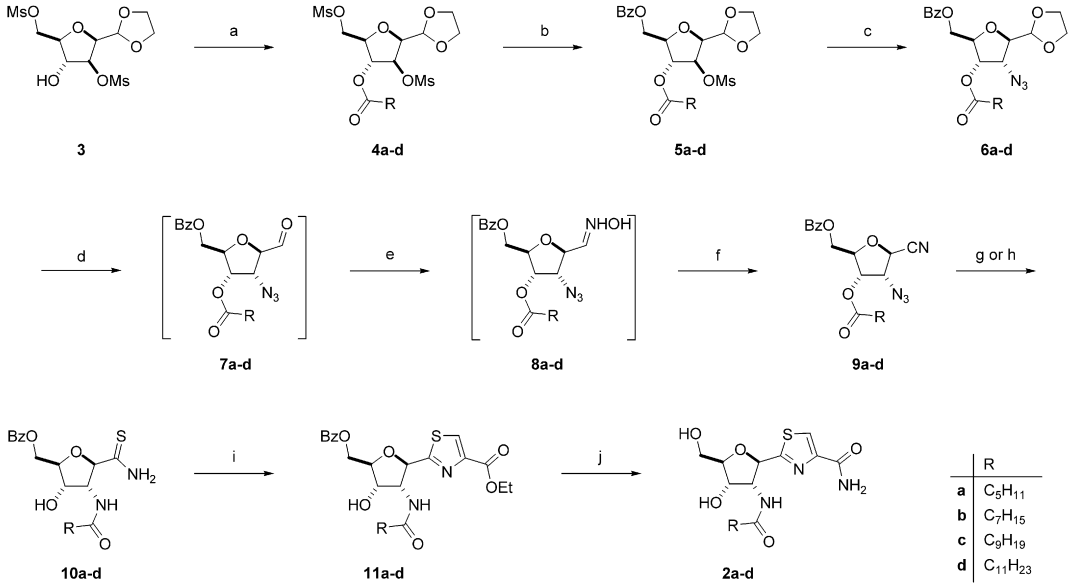
Our strategy for the synthesis of target C-nucleosides of type **2** is based on initial multi-step chemical transformations of D-glucose to the ribofuranosyl-thioamides **10a**–**d** (Scheme 1), followed by their subsequent cyclocondensation with ethyl bromopyruvate to form the thiazole ring. The 2,5-anhydro-D-glucose derivative **3**, readily available from D-glucose[13] was used as a convenient starting material in this work. The synthesis commenced with a three-step sequence for the conversion of **3** to the key azido intermediates **6a**–**d**.

Thus, by use of the appropriate acyl chlorides, the corresponding 4-*O*-acyl derivatives **4a**–**d** were first prepared from the common precursor **3**, as shown in Scheme 1 and Table S1 in the Supplementary data. The corresponding 4-*O*-alcanoyl derivatives **4a**–**d** were thus obtained in 87, 93, 69 and 99% respective yields. Regioselective displacement of the primary mesyloxy groups of **4a**–**d** with potassium benzoate in DMF then produced the 6-*O*-benzoyl derivatives **5a**–**d**, in 86, 70, 86 and 77% yields, respectively. Subsequent treatment of **5a**–**d** with sodium azide in DMSO proceeded with configurational inversion at C-3 to afford moderate yields of **6a**–**d**. Presumably the lower yields in these reactions are due to a number of side processes, that may give elimination and/or neighbouring group participation products, as observed in our preceding work.[14] However, in the present work neither of the side-products could be isolated in pure form, due to their similar chromatographic properties.

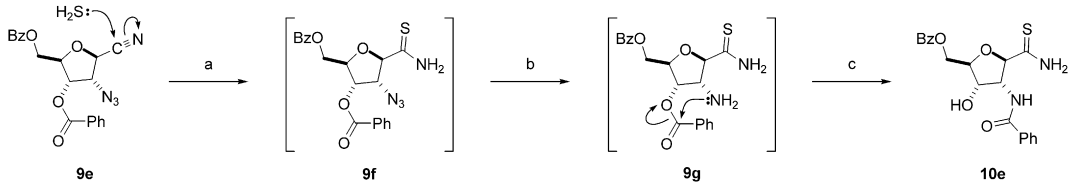
In the next stage of the synthesis the protected 2,5-anhydro-3-azido-3-deoxy-derivatives **6a**–**d** were converted to the corresponding glycosyl cyanides **9a**–**d**. Hydrolytic removal of the dioxolane protective group in **6a**–**d** was achieved in a mixture of trifluoroacetic acid and 6 M hydrochloric acid at +4 °C. The resulting unstable aldehydes **7a**–**d** were not purified, but were immediately treated with hydroxylamine hydrochloride to yield the corresponding oximes **8a**–**d** as mixtures of the corresponding *E*- and *Z*-isomers. An attempted separation of *E*-**6a** and *Z*-**6a** by flash column chromatography provided only pure *E*-**6a** (35%), while the corresponding *Z*-isomer could not be obtained free of *E*-**6a**. At this point we decided to avoid the purification step of the remaining oximino derivatives **8b**–**d**. Accordingly, the mixtures of *E*- and *Z*-isomers **8b**–**d** were not separated but were further treated with mesyl chloride in pyridine. The expected ribofuranosyl cyanides **9a**–**d** were thus obtained in respective overall yields of 46, 57, 47 and 36% (from three synthetic steps).

It was assumed that the 2-azido-2-deoxy-D-ribofuranosyl cyanides **9a**–**d** may be converted to the corresponding thioamides through the one-step H_2S-mediated cascade that was recently used for the conversion of 2-azido ribofuranosyl cyanide **9e** to the ribofuranosyl thioamide **10e**[11] (Scheme 2).

This efficient one-pot process is comprised of an initial addition of hydrogen sulfide to the nitrile group, followed by the azide reduction and spontaneous *O*,*N*-shift of the acyl group. Depending on the reaction conditions and reagents used, the major product **10e** was accompanied with a variable amount of 2-azido ribofuranosyl



Scheme 1. Reagents and conditions: (a) RCOCl, Py, CH_2Cl_2, rt; (b) KOBz, DMF, 100 °C; (c) NaN_3, DMSO, 110–112 °C; (d) 6 M HCl, TFA, +4 °C; (e) NaOAc, $HONH_2\times HCl$, EtOH, CH_2Cl_2, rt; (f) MsCl, Py, 0 °C to rt; (g) H_2S, Py, rt; (h) H_2S, DMAP, EtOH, rt; (i) $BrCH_2COCO_2Et$, EtOH, reflux; (j) NH_3, MeOH, rt.



Scheme 2. Conversion of 2-azido ribofuranosyl cyanide **9e** to the thioamide **10e**: (a) addition of H_2S to the nitrile function to give thioamide **9f**; (b) H_2S reduction of azide **9f** to amine **9g**; (c) *O*,*N*-shift of the acyl group to give amide **10e**.

thioamide **9f**. Thus, when **9e** was reacted with hydrogen sulfide in dry pyridine for 1.5 h at room temperature both **10e** and **9f** were obtained in 71% and 25% yields, respectively. However, on prolonging the reaction time to 8 h, the benzamido derivative **10e** was obtained as the only reaction product, but in somewhat lower yield (69%). This result indicates that the addition of hydrogen sulfide to the nitrile function in **9e** precedes the reduction of the azido group. An alternative conversion of **9e** was carried out with hydrogen sulfide in ethanol and in the presence of DMAP. The reaction also proceeds cleanly at room temperature for 8 h to afford **10e** as the only reaction product in 82% yield.

As both of these H_2S-mediated transformations were previously carried out only with the aromatic ester derivative **9e**, in the present work we wanted to extend the application of this procedure to the saturated esters **9a**–**d**. Hence, the ribofuranosyl cyanides **9a**–**c** were treated with hydrogen sulfide in pyridine, under the conditions presented in Scheme 1 and Table S1 in the Supplementary data, to provide the corresponding thioamides **10a**–**c** in 99, 92 and 87% yields, respectively. Although the nitrile **9d** may also be converted to the thioamide **10d** under the same reactions conditions (H_2S, Py, rt), at this point we wanted to explore alternative reagent system for the preparation of **10d** (H_2S, DMAP, EtOH, rt), which was also successfully applied in the aromatic ester series.[11] Accordingly, the nitrile **9d** was treated with hydrogen sulfide gas and DMAP in ethanol for 8 h at room temperature, whereby the desired thioamide **10d** was obtained in 92% yield.

The synthesis of the final products **2a**–**d** began with the modified Hantzsch thiazole synthesis.[15] Accordingly, the intermediates **10a**–**d** were treated with ethyl bromopyruvate in refluxing ethanol to give the protected thiazoles **11a**–**d** in 54, 60, 47 and 56% yield, respectively. Final exposure of **11a**–**d** to methanolic ammonia provided the tiazofurin analogues **2a**–**d** in 66, 49, 81 and 80% respective yields.

2.2. In vitro antitumour activity

After completion of the synthesis, tiazofurin analogues **2a**, **2b**, **2c** and **2d** were evaluated for their in vitro cytotoxic activity against a panel of human malignant cell lines, including chronic myelogenous leukaemia (K562), promyelocytic leukaemia (HL-60), Burkitt's lymphoma (Raji), colon adenocarcinoma (HT-29), breast adenocarcinoma (MCF-7), cervix carcinoma (HeLa), as well as normal foetal lung fibroblasts (MRC-5). Cytotoxic activity was evaluated by using the standard MTT assay, after exposure of cells to the tested compounds for 72 h. Both tiazofurin (**1**) and the commercial antitumour agent doxorubicin (DOX) were used as reference compounds. The results are presented in Table 1.

According to the resulting IC_{50} values of the cytotoxic assay (Table 1) the synthesized tiazofurin analogues **2a**, **2b**, **2c** and **2d** exhibited the similar growth inhibition pattern as tiazofurin (**1**) against human K562, HL-60, Raji and HeLa malignant cells, with IC_{50} values in the range 0.15–6.94 μM. The most active compounds against HL-60 cell line were analogues **2b** and **2d**, which demonstrated almost the same potency as doxorubicin (DOX), although tiazofurin remains the most potent agent towards these malignant cells. Analogues **2a**–**d** exhibited notable antiproliferative effects on Raji cells, with IC_{50} values in the low, micro-molar range. The most active molecules against this cell line were analogues **2a** and **2b** that displayed the similar activities as both control compounds (**1** and DOX). Analogues **2a**, **2b** and **2c** showed a weak activity against HT-29 cells. Meanwhile, compound **2d** exhibited a strong antiproliferative activity against this cell line being 47-fold more potent than the parent compound **1**. In the same time, analogue **2d** demonstrated 19-fold higher potency than DOX in the same cell line. Only moderate to weak activities of analogues **2b**, **2c** and **2d** were recorded in MCF-7 tumour cell line. However, compound **2a** exhibited 20- and 3-fold higher potency in this cell line with respect to tiazofurin (**1**) and DOX, respectively. The most active molecules against HeLa cells were analogues **2a**, **2b** and **2d** that exhibited 1.5-fold higher potency when compared to the control compound **1**. The most interesting results were obtained in experiments with K562 cells, whereupon all tiazofurin analogues **2a**–**d** exhibited remarkable antiproliferative effects with IC_{50} values in the range 0.15–2.69 μM. The most active molecule against this cell line was derivative **2c** being 14-fold more potent than tiazofurin (**1**), while analogues **2b** and **2d** demonstrated 2-fold grater cytotoxicity than the parent compound **1**. Moreover, it is noteworthy that compound **2c** displayed 2.5-fold higher potency than the commercial cytotoxic agent doxorubicin (DOX) in the same cell line. Unfortunately, SAR for the inhibition of tumour cells growth does not indicate a correlation between the length of carbon chain in the amide functionality and the corresponding inhibitory potencies.

Table 1
In vitro cytotoxicity of tiazofurin (**1**), analogues **2a**–**d** and DOX

Compd	IC_{50} (μM)[a]						
	K562	HL-60	Raji	HT-29	MCF-7	HeLa	MRC-5
1	2.06	0.67	4.61	0.56	2.03	3.26	0.33
2a	2.69	3.64	3.96	89.34	0.099	1.99	>100
2b	1.02	1.02	3.54	93.67	16.78	2.11	>100
2c	0.15	5.97	6.87	91.34	69.64	6.94	>100
2d	0.94	0.87	5.34	0.012	78.37	2.06	>100
DOX	0.37	1.06	3.09	0.23	0.32	0.12	0.23

[a] IC_{50} is the concentration of compound required to inhibit the cell growth by 50% compared to an untreated control. Values are means of three independent experiments. Coefficients of variation were less than 10%.

When the cytotoxic effect of analogues **2a**–**d** was expressed in relation to tiazofurin cytotoxicity, it was obvious that analogue **2c** was the most active towards K562 cells in the range of concentrations from 10^{-8} M to 10^{-4} M, while other tiazofurin mimetics showed similar or smaller activity compared to tiazofurin (Fig. 2).

These results indicate that the introduction of 2′-alkylamido functions into the tiazofurin sugar moiety may improve antiproliferative effects of the resulting analogues against some human neoplastic cells. Remarkably, the synthesized tiazofurin mimics **2a**–**d** are fully non-toxic towards human normal MRC-5 cells while lead compound **1**, as well as commercial antitumour agent DOX exhibited sub-micromolar toxicity to both malignant and normal cells.

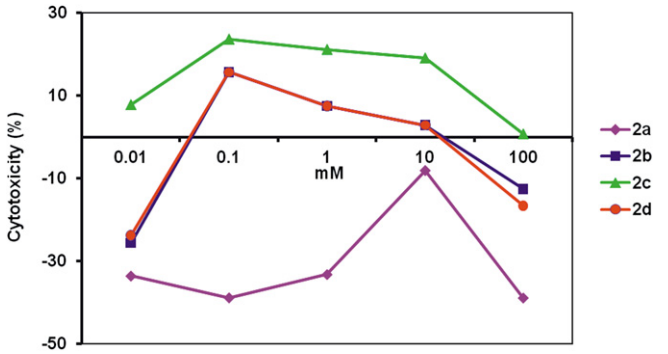


Fig. 2. Cytotoxicity (%) of tiazofurin mimics **2a**–**d** against K562 cells compared to tiazofurin. K562 cells were treated with **2a**–**d** in a concentration range from 10^{-8} M to 10^{-4} M. Cytotoxicity was evaluated after 72 h by MTT assay. Each point represents a mean value of quadruplicate from two independent experiments.

2.3. Detection of apoptosis

Intact apoptosis pathways are critical for chemotherapy-induced cytotoxicity. The majority of chemotherapeutic agents trigger the mitochondrial (intrinsic) pathway, but the cell-death receptor dependent (extrinsic) pathway is also involved in chemotherapy-induced apoptosis. Recent findings that tiazofurin induces apoptosis in SiHa, Hep2 and Ca Ski cancer cells[7a] encouraged us to examine the possible apoptotic signalling induced by tiazofurin and analogues in the K562 cell line. The type of cell death induced by tiazofurin (**1**), and analogues **2a**–**d** in K562 human leukaemia cells, was determined by flow cytometric analysis after annexin V-FITC and propidium iodide staining. Flow cytometric analysis clearly differentiates normal, living cells (low Annexin and low PI staining), apoptotic cells (high Annexin and low PI staining), and necrotic cells (low Annexin and high PI staining). Translocation of phosphatidylserine from the inner part of the plasma membrane to the outer layer occurs in the early stages of apoptosis.[16] Necrotic cells also expose PS according to the loss of membrane integrity. Annexin V is a Ca^{2+}-dependent phospholipid-binding protein with high affinity for PS. As Annexin-V-Fluos stains both apoptotic and necrotic cells, simultaneous application of PI that stains DNA of leaky necrotic cells only, allows us to discriminate truly necrotic cells from the Annexin V positively stained cells. Cells were treated with **1** and TAs (**2a**–**d**) for 72 h and then stained with the components of the Annexin-V-FLUOS Staining Kit and analyzed by flow cytometry (for representative dot plots see Fig. S1 in the Supplementary data).

Exposure of K562 cells to tiazofurin at the concentrations of 8.7 and 2 μM for 24 h and 72 h, respectively, did not induce apoptosis compared to control (untreated cells). This is in accordance with the results of De Abreu et al.[17] who found that tiazofurin, at similar concentrations, does not induce apoptosis in the Molt-4 cell line within the first 24 h. Longer cell treatment resulted in a slight increase of Annexin-V positive cells. These findings do suggest that tiazofurin, after initial treatment for 24 h at the IC_{50} concentration, mainly acts as an IMPDH inhibitor. Apoptotic response presented as a percentage of specific apoptosis (Fig. 3) showed that all tiazofurin analogues after 24 or 72 h induced several-fold more Annexin-V positive K562 cells compared to the parent compound. Among them, analogue **2c** was the most active in both time points, and percentage of specific apoptosis was doubled (increased 2-fold) after longer treatment. This compound was also the most active after 72 h of cell treatment in the MTT assay, when compared to both tiazofurin and other analogues (Table 1). Compound **2d** also induced a higher specific apoptotic response after 72 h-treatment (4.11% vs 14.76% after 24 and 72 h, respectively). Compounds **2a** and **2b** caused a similar level of specific apoptotic response after 24 h-treatment, but their activity was significantly lower after longer cell treatment.

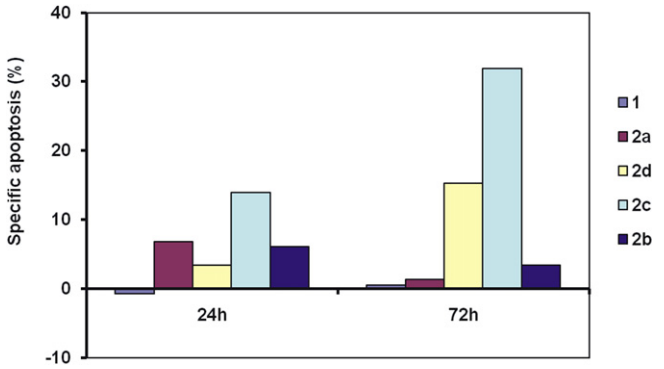


Fig. 3. Percentage of specific apoptosis of K562 cells induced by tiazofurin and analogues **2a**–**d** after 24 and 72 h-treatment. Cells were stained with Annexin-V-FLUOS and propidium iodide and analyzed by flow cytometry: Percentage of specific apoptosis of tiazofurin (**1**) and tiazofurin mimetics (**2a**–**d**) was calculated according to ref 20.

To elucidate the mechanisms underlying the TAs-induced apoptosis, we investigated how compounds **2a**–**d** modulate expression of Bcl-2, Bax, caspase-3 and Poly (ADP-ribose) polymerase (PARP), which are critically involved in the apoptosis pathway. Members of the Bcl-2 protein family are the key regulators of apoptosis. Apoptosis depends on the balance between pro- and anti-apoptotic proteins of the Bcl-2 family. Anti-apoptotic member Bcl-2 plays a crucial role in apoptosis and conferring cancer cell drug resistance.[18] It interferes with cytochrom C release and suppresses apoptosis progression. Bax, the pro-apoptotic protein of the Bcl-2 family, undergoes conformational changes in response to apoptotic stimuli, and thus counteracts Bcl-2 activity, stimulates the release of cytochrom *c* and other apoptogenic proteins into the cytoplasm, and ultimately initiates apoptosis. Western blot analysis (Fig. 4) revealed that analogues **2a**–**d** reduced expression of Bcl-2 compared to control and tiazofurin suggesting downregulation of the anti-apoptotic Bcl-2 gene. Compound **2c** induced the highest reduction of Bcl-2 expression. Bax is commonly over-expressed in apoptotic process initiated by various chemotherapeutics. Tiazofurin analogues **2a**–**d** did not significantly influence Bax protein expression. Several literature reports also showed that apoptosis in various human solid tumours can be induced in the absence of any change in Bax protein level.[19] However, activation of Bax may occur before its upregulation.[20]

The expression level of the precursor and active subunit of effector caspase-3 in the cells exposed to tiazofurin and analogues were measured in order to determine if TAs-induced apoptosis is associated with the activation of caspases. Caspases are important mediators in initiation and execution of apoptotic signal.[21] However, apoptotic morphology can be achieved either by activation of caspases or by other families of proteases.[21b] Cell death induced by antitumour agents is commonly associated with an increase in apoptosis by the caspase-dependent pathway, but caspase-independent pathway can be also involved,[18d,22] in other words apoptosis can be lethal without caspase activation, and caspase activation does not necessarily cause cell death.[23] Western blot data showed that caspase-3 precursor protein was expressed in TAs-treated K562 cells; however, expression level of caspase-3 active subunit was different and depended on the particular compound. Caspase-3 activation is followed by cleavage of different downstream targets including Poly (ADP-ribose) polymerase (PARP)

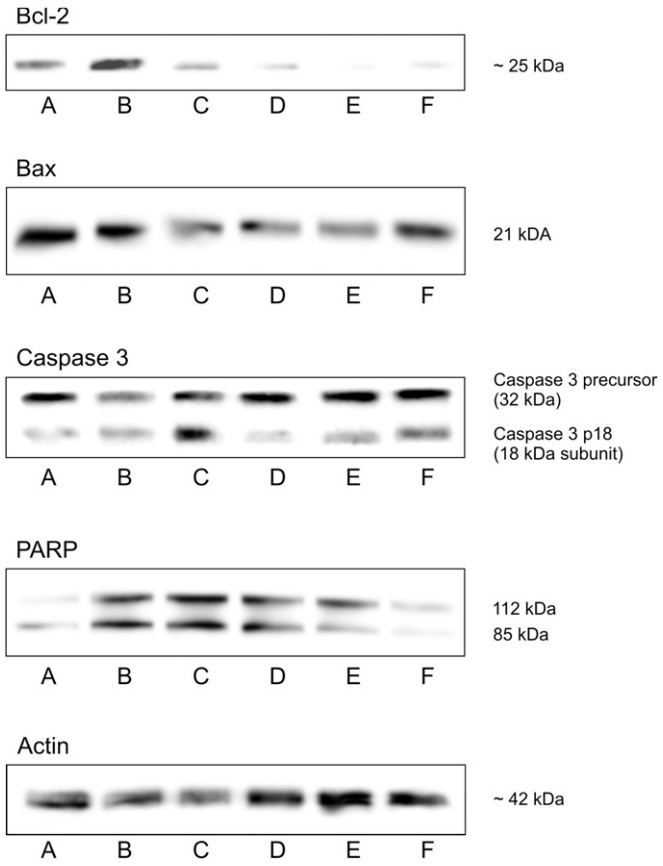


Fig. 4. Western blot analysis of the protein expression of Bcl-2, Bax, caspase-3, and PARP after 72 h-treatment: control (A), tiazofurin (B), analogue **2a** (C), analogue **2d** (D), analogue **2c** (E), analogue **2b** (F).

protein. The enzyme PARP is involved in short-patch base excision repair and its cleavage is one of the biochemical hallmarks of apoptosis.[24] Western blot analysis showed proteolysis cleavage of PARP in K562 cells after treatment with tiazofurin mimics (**2a**–**d**) but it depended on the particular compound. Compared to other analogues, **2b** induced the weakest cleavage of PARP.

3. Conclusion

In conclusion, four new tiazofurin mimics bearing a hexan- (**2a**), octan- (**2b**), decan- (**2c**) and dodecanamido functionality (**2d**) in the C-2′ position, were synthesized starting from D-glucose. The key intermediates for this approach, that is, 2-amido-D-ribofuranosyl thiocarboxamides **10a**–**d** were easily accessible from the 2-azido-2-deoxy-D-ribofuranosyl cyanides **9a**–**d**, through the one-step H_2S-mediated cascade, comprised of an initial addition of hydrogen sulfide to the nitrile group, followed by the azide reduction and spontaneous *O*,*N*-acyl shift. This efficient one-pot sequence represents a general and particularly versatile approach to a variety of 2-amido-D-ribofuranosyl thiocarboxamides, which can be easily converted to the corresponding ribofuranosyl thiazole-4-carboxamides, and finally to a series of new tiazofurin mimics for biological testing.

Analogues **2b**–**d** have shown potent in vitro cytotoxic activity against human myelogenous leukaemia K562. Among solid tumour cell lines, HT29 was sensitive only to **2d**, while HeLa cells were sensitive to **2a**, **2b** and **2d**. Only analogue **2a** was highly cytotoxic against MCF-7 cells. Neither analogues exhibit any significant cytotoxicity towards normal foetal lung MRC-5 cells. Downregulation of Bcl-2, activation of caspase-3, and presence of cleavage product of PARP suggest that cytotoxic effects of tiazofurin analogues **2a**–**d** in K562 might be mediated by apoptosis mostly in caspase-dependent way. On the contrary, tiazofurin did not induce apoptosis of K562 cells after 24 h, which suggests a different mechanism of its action, most probably through the inhibition of IMPDH. Flow cytometry and Western blot analysis data agreed well with the results of MTT assay and enabled identification of analogue **2c** as the most promising antitumour agent. These results, along with our previous findings[11] confirmed that the introduction of 2′-nitrogen functionalities into the tiazofurin sugar moiety may provide analogues of significant antitumour activity, but without any significant cytotoxicity towards normal cells. Therefore we believe that this approach may be of use in the search for new, more potent and selective anticancer agents derived from lead **1**.

4. Experimental section

4.1. General

Melting points were determined on a Büchi 510 apparatus and were not corrected. Optical rotations were measured on P 3002 (Krüss) and Polamat A (Carl–Zeiss) polarimeters at room temperature. NMR spectra were recorded on a Bruker AC 250 E instrument and chemical shifts are expressed in ppm downfield from TMS. IR spectra were recorded with an FTIR Nexus 670 spectrophotometer (Thermo-Nicolet). Low resolution mass spectra (CI) were recorded on Finnigan-MAT 8230 and on an Agilent Technologies HPLC/MS 3Q system (ESI), series 1200/6410. High resolution mass spectra (ESI) of synthesized compounds were acquired on a Agilent Technologies 1200 series instrument equipped with Zorbax Eclipse Plus C18 (100 mm×2.1 mm i.d. 1.8 μm) column and DAD detector (190–450 nm) in combination with a 6210 time-of-flight LC/MS instrument (ESI) in the positive ion mode. Column chromatography was performed on Kieselgel 60 (<0.063 mm, E. Merck). Flash column chromatography was performed using Kieselgel 60 (0.040–0.063, E. Merck). Self-made preparative TLC plates were prepared using Kieselgel 60 G (E. Merck) with Fluorescent Indicator F_{254} as additive. The corresponding bands were scraped and eluted with EtOAc. All organic extracts were dried with anhydrous Na_2SO_4. Organic solutions were concentrated in a rotary evaporator under reduced pressure at a bath temperature below 35 °C.

4.2. General procedure for the preparation of ester derivatives 4a–d

To a cooled (0 °C) and stirred solution of **3** (1 equiv) in dry CH_2Cl_2 (0.2 M) were added anhydrous pyridine (9 equiv) and the appropriate acyl chloride (1.5–2 equiv). The mixture was first stirred at 0 °C for 15 min and then at room temperature until the starting materials were consumed (TLC). The mixture was poured onto ice and acidified with 6 M HCl (to pH 1–2) and the resulting suspension was extracted with CH_2Cl_2 (4×50 mL). The combined extracts were washed with water (40 mL), saturated aq $NaHCO_3$ (40 mL) and again with water (40 mL), dried and evaporated. The residue was then purified by silica gel column chromatography and/or by crystallization from the appropriate solvents.

*4.2.1. 2,5-Anhydro-4-O-hexanoyl-3,6-di-O-methanesulfonyl-D-glucose ethylene acetal (**4a**).* Alcohol **3** (4.8 g, 13.2 mmol) was converted into crude **4a** after treatment with hexanoyl chloride (3.8 mL, 27 mmol) for 24 h at room temperature according to the above general procedure. Double crystallization from MeOH gave pure **4a** (5.3 g, 87%) as colourless crystals, mp 94 °C; $[\alpha]_D^{20}$ +33.8 (*c* 1.3, $CHCl_3$); IR (KBr): ν_{max} 1724, 1369, 1179; ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.86 (t, 3H, *J*=7.1 Hz, CH_3CH_2), 1.28 (m, 4H, 2× CH_2), 1.59

(m, 2H, CH_2), 2.32 (t, 2H, CH_2CO), 3.06 and 3.14 (2× s, 3H each, 2× CH_3SO_2), 3.84–4.04 (m, 4H, 2× CH_2–dioxolane), 3.93 (dd, 1H, $J_{1,2}$=6.3, $J_{2,3}$=3.6 Hz, H-2), 4.12 (m, 1H, H-5), 4.43 (pseudo d, 2H, $J_{5,6}$=4.6 Hz, 2× H-6), 5.07 (dd, 1H, $J_{2,3}$=3.7, $J_{3,4}$=1 Hz, H-3), 5.10 (d, 1H, $J_{1,2}$=6.3 Hz, H-1), 5.20 (dd, 1H, $J_{3,4}$=1, $J_{4,5}$=2.6 Hz, H-4); ^{13}C (62.9 MHz, $CDCl_3$): δ 13.65 (CH_3CH_2), 22.0, 24.1 and 30.9 (3× CH_2), 33.6 (CH_2CO), 37.5 and 38.4 (2× CH_3SO_2), 65.2 and 65.3 (2× CH_2–dioxolane), 67.8 (C-6), 77.6 (C-4), 80.8 (C-2), 81.9 (C-3), 82.4 (C-5), 100.9 (C-1), 172.4 ($C_5H_{11}CO$). Anal. Calcd for $C_{16}H_{28}O_{11}S_2$: C, 41.73; H, 6.13; S, 13.93. Found: C, 41.42; H, 6.52; S, 13.97.

*4.2.2. 2,5-Anhydro-4-O-octanoyl-3,6-di-O-methanesulfonyl-D-glucose ethylene acetal (**4b**).* Alcohol **3** (2.08 g, 5.7 mmol) was converted into crude **4b** after treatment with octanoyl chloride (1.5 mL, 8.8 mmol) for 72 h at room temperature according to the above general procedure. Flash column chromatography (97:3, 19:1, to 9:1 CH_2Cl_2/EtOAc) followed by recrystallization from MeOH gave pure **4b** (2.61 g, 93%) as colourless crystals, mp 58–59 °C; $[\alpha]_D^{20}$ +24.6 (*c* 7.5, $CHCl_3$); IR (KBr): ν_{max} 1744, 1360, 1178; ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.87 (t, 3H, *J*=6.8 Hz, CH_3CH_2), 1.27 (m, 8H, 4× CH_2), 1.60 (m, 2H, CH_2), 2.34 (t, 2H, CH_2CO), 3.08 and 3.17 (2× s, 3H each, 2× CH_3SO_2), 3.87–4.05 (m, 5H, H-2 and 2× CH_2–dioxolane), 4.14 (m, 1H, H-5), 4.47 (d, 2H, $J_{5,6}$=4.4 Hz, 2× H-6), 5.10 (dd, 1H, $J_{2,3}$=4, $J_{3,4}$=1 Hz, H-3), 5.13 (d, 1H, $J_{1,2}$=6.4 Hz, H-1), 5.23 (dd, 1H, $J_{3,4}$=1, $J_{4,5}$=2.6 Hz, H-4); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 14.0 (CH_3CH_2), 22.5, 24.5, 28.7, 28.8 and 31.5 (5× CH_2), 33.7 (CH_2CO), 37.6 and 38.5 (2× CH_3SO_2), 65.3 and 65.4 (2× CH_2–dioxolane), 67.7 (C-6), 77.6 (C-4), 80.9 (C-2), 82.0 (C-3), 82.5 (C-5), 101.0 (C-1), 172.5 ($C_7H_{15}CO$); LRMS (ESI): *m*/*z* 489 (M^++H). Anal. Calcd for $C_{18}H_{32}O_{11}S_2$: C, 44.25; H, 6.60; S, 13.13. Found: C, 44.08; H, 6.44; S, 13.42.

*4.2.3. 2,5-Anhydro-4-O-decanoyl-3,6-di-O-methanesulfonyl-D-glucose ethylene acetal (**4c**).* Alcohol **3** (4.02 g, 11.1 mmol) was converted into crude **4c** after treatment with decanoyl chloride (4.7 mL, 22.7 mmol) for 48 h at room temperature according to the above general procedure. Flash column chromatography (19:1, to 23:2 CH_2Cl_2/EtOAc) followed by recrystallization from MeOH gave pure **4c** (3.93 g, 69%) as colourless crystals, mp 55–57 °C; $[\alpha]_D^{20}$ +20.5 (*c* 1.3, $CHCl_3$); IR (KBr): ν_{max} 1742, 1360, 1177; ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.86 (t, 3H, *J*=6.8 Hz, CH_3CH_2), 1.15–1.67 (m, 14H, 7× CH_2), 2.33 (t, 2H, CH_2CO), 3.08 and 3.16 (2× s, 3H each, 2× CH_3SO_2), 3.85–4.06 (m, 5H, 2× CH_2–dioxolane and H-2), 4.14 (m, 1H, H-5), 4.46 (pseudo d, 2H, $J_{5,6}$=4.5 Hz, H-6), 5.07 (dd, 1H, $J_{2,3}$=4.0, $J_{3,4}$=0.8 Hz, H-3), 5.12 (d, 1H, $J_{1,2}$=6.4 Hz, H-1), 5.22 (dd, 1H, $J_{4,5}$=2.2, $J_{3,4}$=0.8 Hz, H-4); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 14.0 (CH_3CH_2), 22.5, 24.5, 28.9, 29.0, 29.1 29.2, and 31.4 (7× CH_2), 33.8 (CH_2CO), 37.6 and 38.5 (2× CH_3SO_2), 65.3 and 65.4 (2× CH_2–dioxolane), 67.7 (C-6), 77.6 (C-4), 80.9 (C-2), 82.0 (C-3), 82.5 (C-5), 101.0 (C-1), 172.4 ($C_9H_{19}CO$); LRMS (CI): *m*/*z* 517 (M^++H). Anal. Calcd for $C_{20}H_{36}O_{11}S_2$: C, 46.50; H, 7.02; S, 12.41. Found: C, 46.77; H, 7.19; S, 12.63.

*4.2.4. 2,5-Anhydro-4-O-dodecanoyl-3,6-di-O-methanesulfonyl-D-glucose ethylene acetal (**4d**).* Alcohol **3** (3.3 g, 9.2 mmol) was converted into crude **4d** after treatment with dodecanoyl chloride (4.2 mL, 18.4 mmol) for 22 h at room temperature according to the above general procedure. Silica gel column chromatography (7:3, to 3:2, toluene/EtOAc) gave pure **4d** (4.96 g, 99%) as a colourless syrup, $[\alpha]_D^{20}$ +11.8 (*c* 1.0, $CHCl_3$); IR (neat): ν_{max} 1747, 1360, 1178; ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.83 (t, 3H, CH_3CH_2), 1.16–1.68 (m, 18H, 9× CH_2), 2.35 (t, 2H, CH_2CO), 3.09 and 3.17 (2× s, 3H each, 2× CH_3SO_2), 3.89–4.07 (m, 5H, 2× CH_2–dioxolane, and H-2), 4.15 (m, 1H, H-5), 4.47 (d, 2H, $J_{5,6}$=4.5 Hz, 2× H-6), 5.12 (d, 1H, $J_{2,3}$=4.5 Hz, H-3), 5.14 (d, 1H, $J_{1,2}$=6.5 Hz, H-1), 5.24 (d, 1H, $J_{4,5}$=2.7 Hz, H-4); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 13.9 (CH_3CH_2), 22.4, 24.4 28.8, 29.0, 29.1, 29.2, 29.4, 31.7 and 33.6 (10× CH_2), 37.4 and 38.3 (2× CH_3SO_2), 65.2 and 65.3 (2× CH_2–dioxolane), 67.7 (C-6), 77.5 (C-4), 80.7 (C-2), 81.9 (C-3), 82.4 (C-5), 100.9 (C-1), 172.4 ($C_{11}H_{23}CO$); LRMS (CI): *m*/*z* 545 (M^++H). Anal. Calcd for $C_{22}H_{40}O_{11}S_2$: C, 48.51; H, 7.40; S, 11.77. Found: C, 48.52; H, 7.43; S, 11.50.

4.3. General procedure for the synthesis of 6-*O*-benzoyl ester derivatives 5a–d

To a solution of disulfonate **4** (1 equiv) in DMF (0.1–0.15 M) was added KOBz (1.2–4.2 equiv), and the reaction mixture was heated at 100 °C until no further reaction was evident by TLC (12 h for **4a**–**c**, 8 h for **4d**). The mixture was allowed to cool down to room temperature, poured into cold water and extracted with a 1:1 mixture of benzene/light petroleum. The combined extracts were washed with water, dried and evaporated. The residue was then purified by silica gel column chromatography.

*4.3.1. 2,5-Anhydro-6-O-benzoyl-4-O-hexanoyl-3-O-methanesulfonyl-D-glucose ethylene acetal (**5a**).* Disulfonate **4a** (4.75 g, 10.3 mmol) was treated with KOBz (7.1 g, 44.2 mmol) according to the above general procedure to give crude **5a** as an oil. Pure **5a** (4.32 g, 86%) was obtained by flash column chromatography (2:1 cyclohexane/Me_2CO) followed by recrystallization from MeOH, as colourless crystals, mp 98–99 °C; $[\alpha]_D^{20}$ +34.3 (*c* 2.0, $CHCl_3$); IR (neat): ν_{max} 1743, 1719, 1353, 1185; ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.89 (t, 3H, *J*=7.1 Hz, CH_3CH_2), 1.22–1.38 (m, 4H, 2× CH_2), 1.62 (m, 2H, CH_2), 2.34 (m, 2H, CH_2CO), 3.10 (s, 3H, CH_3SO_2), 3.88–4.08 (m, 5H, H-2 and 2× CH_2–dioxolane), 4.26 (m, 1H, H-5), 4.56 (dd, 1H, $J_{5,6a}$=4.3, $J_{6a,6b}$=12.1 Hz, H-6a), 4.62 (dd, 1H, $J_{5,6b}$=4.7, $J_{6a,6b}$=12.1 Hz, H-6b), 5.15 (d, 1H, $J_{2,3}$=3.7 Hz, H-3), 5.18 (d, 1H, $J_{1,2}$=6.3 Hz, H-1), 5.35 (d, 1H, $J_{4,5}$=3.1 Hz, H-4), 7.38–8.15 (m, 5H, Ph); ^{13}C (62.9 MHz, $CDCl_3$): δ 13.8 (CH_3CH_2), 22.2, 24.3 and 31.1 (3× CH_2), 33.77 (CH_2CO), 38.5 (CH_3SO_2), 63.5 (C-6), 65.4 and 65.5 (2× CH_2–dioxolane), 78.5 (C-4), 80.8 (C-2), 82.6 (C-5), 82.9 (C-3), 101.2 (C-1), 128.3, 129.6, 129.8 and 133.1 (Ph), 166.2 (PhC=O), 172.4 ($C_5H_{11}CO$); LRMS (CI): *m*/*z* 545 (M^++H). Anal. Calcd for $C_{22}H_{30}O_{10}S$: C, 54.31; H, 6.22; S, 6.59. Found: C, 54.10; H, 6.46; S, 6.97.

*4.3.2. 2,5-Anhydro-6-O-benzoyl-3-O-methanesulfonyl-4-O-octanoyl-D-glucose ethylene acetal (**5b**).* Disulfonate **4b** (2.76 g, 5.3 mmol) was treated with KOBz (1.06 g, 6.6 mmol) according to the above general procedure to give crude **5b** as an oil. Pure **5b** (1.9 g, 70%) was obtained after purification on a column of flash silica (9:1 toluene/EtOAc), followed by recrystallization from MeOH, as colourless crystals, mp 88–89 °C; $[\alpha]_D^{20}$ +32.4 (*c* 1.0, $CHCl_3$); IR (neat): ν_{max} 1750, 1724, 1367, 1180; ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.87 (t, 3H, *J*=6.8 Hz, CH_3CH_2), 1.27 (m, 8H, 4× CH_2), 1.60 (m, 2H, CH_2), 2.34 (t, 2H, CH_2CO), 3.08 (s, 3H, CH_3SO_2), 3.81–4.07 (m, 5H, H-2 and 2× CH_2–dioxolane), 4.24 (m, 1H, H-5), 4.53 (dd, 1H, $J_{5,6a}$=4.9, $J_{6a,6b}$=11.5 Hz, H-6a), 4.59 (dd, 1H, $J_{5,6b}$=4.7, $J_{6a,6b}$=11.8 Hz, H-6b), 5.12 (d, 1H, $J_{2,3}$=3.7 Hz, H-3), 5.15 (d, 1H, $J_{1,2}$=6.4 Hz, H-1), 5.33 (d, 1H, $J_{4,5}$=2.7 Hz, H-4), 7.34–8.12 (m, 5H, Ph); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 13.9 (CH_3CH_2), 22.4, 24.5, 28.6, 28.7 and 31.4 (5× CH_2), 33.64 (CH_2CO), 38.3 (CH_3SO_2), 63.4 (C-6), 65.2 and 65.3 (2× CH_2–dioxolane), 78.3 (C-4), 80.7 (C-2), 82.5 (C-5), 82.8 (C-3), 101.0 (C-1), 128.2, 129.4, 129.7 and 133.0 (Ph), 166.0 (PhC=O), 172.3 ($C_7H_{15}CO$); LRMS (ESI): *m*/*z* 537 (M^++Na). Anal. Calcd for $C_{24}H_{34}O_{10}S$: C, 56.02; H, 6.66; S, 6.23. Found: C, 55.88; H, 6.60; S, 6.10.

*4.3.3. 2,5-Anhydro-6-O-benzoyl-4-O-decanoyl-3-O-methanesulfonyl-D-glucose ethylene acetal (**5c**).* Disulfonate **4c** (1.9 g, 3.7 mmol) was treated with KOBz (4.7 g, 4.6 mmol) according to the above general procedure to give crude **5c** as an oil. After purification on a column of flash silica (9:1 toluene/EtOAc), followed by

preparative TLC (4:1 toluene/EtOAc) of several impure fractions pure **5c** (1.72 g, 86%) was isolated as a colourless oil, $[\alpha]_D^{20}$ +32.3 (*c* 1.1, $CHCl_3$); IR (neat): ν_{max} 1750, 1724, 1367, 1180; ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.88 (t, 3H, *J*=6.8 Hz, CH_3CH_2), 1.26 (m, 12H, 6× CH_2), 1.61 (m, 2H, CH_2), 2.34 (t, 2H, *J*=7.7 Hz, CH_2CO), 3.11 (s, 3H, CH_3SO_2), 3.88–4.08 (m, 5H, 2× CH_2–dioxolane and H-2), 4.26 (m, 1H, H-5), 4.56 (dd, 1H, $J_{5,6a}$=4.8, $J_{6a,6b}$=11.8 Hz, H-6a), 4.62 (dd, 1H, $J_{5,6b}$=4.8, $J_{6a,6b}$=11.8 Hz, H-6b), 5.15 (d, 1H, $J_{2,3}$=3.8 Hz, H-3), 5.18 (d, 1H, $J_{1,2}$=6.3 Hz, H-1), 5.35 (d, 1H, $J_{4,5}$=3.0 Hz, H-4), 7.40–8.14 (m, 5H, Ph); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 13.9 (CH_3CH_2), 22.4, 24.5, 28.8, 28.98, 29.0, 29.1 and 31.6 (7× CH_2), 33.68 (CH_2CO), 38.3 (CH_3SO_2), 63.4 (C-6), 65.2 and 65.3 (2× CH_2–dioxolane), 78.3 (C-4), 80.7 (C-2), 82.5 (C-5), 82.3 (C-3), 101.1 (C-1), 128.2, 129.5, 129.7 and 133.0 (Ph), 166.0 (PhC=O), 172.2 ($C_9H_{19}CO$); LRMS (CI): *m*/*z* 543 (M^++H). Anal. Calcd for $C_{26}H_{38}O_{10}S$: C, 57.55; H, 7.06; S, 5.91. Found: C, 57.28; H, 7.28; S, 5.97.

*4.3.4. 2,5-Anhydro-6-O-benzoyl-4-O-dodecanoyl-3-O-methanesulfonyl-D-glucose ethylene acetal (**5d**).* Disulfonate **4c** (4.9 g, 9 mmol) was treated with KOBz (6.2 g, 38.7 mmol) according to the above general procedure to give crude **5d** as an oil. Pure **5d** (3.97 g, 77%) was obtained after purification on a column of silica gel (17:3 toluene/EtOAc) as a colourless oil, $[\alpha]_D^{20}$ +24.8 (*c* 1.2, $CHCl_3$); IR (neat): ν_{max} 1724, 1369, 1179; ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.86 (t, 3H, CH_3CH_2), 1.24 (br s, 16H, 8× CH_2), 1.59 (m, 2H, CH_2CH_2CO), 2.32 (t, 2H, CH_2CO), 3.09 (s, 3H, CH_3SO_2), 3.84–4.06 (m, 5H, 2× CH_2–dioxolane, and H-2), 4.24 (m, 1H, H-5), 4.54 (dd, 1H, $J_{5,6a}$=4.9, $J_{6a,6b}$=11.9 Hz, H-6a), 4.60 (dd, 1H, $J_{5,6b}$=4.8, $J_{6a,6b}$=11.9 Hz, H-6b), 5.13 (dd, 1H, $J_{2,3}$=3.7, $J_{3,4}$=0.6 Hz, H-3), 5.16 (d, 1H, $J_{1,2}$=6.3 Hz, H-1), 5.34 (dd, 1H, $J_{3,4}$=0.6, $J_{4,5}$=3.0 Hz, H-4), 7.34–8.11 (m, 5H, Ph); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 13.9 (CH_3CH_2), 22.5, 24.5, 28.8, 29.0, 29.1, 29.2, 29.4 and 31.7 (9× CH_2), 33.7 (CH_2CO), 38.4 (CH_3SO_2), 63.4 (C-6), 65.26 and 65.3 (2× CH_2–dioxolane), 78.4 (C-4), 80.7 (C-2), 82.5 (C-5), 82.3 (C-3), 101.1 (C-1), 128.2, 129.5, 129.7 and 133.0 (Ph), 166.0 (PhC=O), 172.3 ($C_{11}H_{23}CO$); LRMS (CI): *m*/*z* 571 (M^++H). Anal. Calcd for $C_{28}H_{42}O_{10}S$: C, 58.93; H, 7.42; S, 5.62. Found: C, 58.56; H, 7.27; S, 5.63.

4.4. General procedure for the preparation of azido derivatives 6a–d

To a solution of sulfonate **5** (1 equiv) in DMSO (0.1 M) was added NaN_3 (8.5–10 equiv), and the reaction mixture was heated at 110 °C until no further reaction was evident by TLC (24 h for **6a**, 30 h for **6b**, 27 h for **6c**, 70 h for **6d**). The mixture was allowed to cool down to room temperature, poured into cold water and extracted with a 1:1 mixture of benzene/light petroleum. The combined extracts were washed with water, dried and evaporated. The residue was purified by flash column chromatography.

*4.4.1. 2,5-Anhydro-3-azido-6-O-benzoyl-3-deoxy-4-O-hexanoyl-D-allose ethylene acetal (**6a**).* Sulfonate **5a** (3.2 g, 6.5 mmol) was treated with NaN_3 (3.6 g 55.4 mmol) according to the above general procedure to give crude **6a** as a yellow oil. After purification on a column of flash silica (19:1 toluene/EtOAc), followed by preparative TLC (19:1 toluene/EtOAc) of several impure fractions, pure **6a** (1.33 g, 47%) was isolated as a colourless oil, $[\alpha]_D^{20}$ +12.9 (*c* 7.5, $CHCl_3$); IR (neat): ν_{max} 2111, 1724, 1602; ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.87 (t, 3H, *J*=7.1 Hz, CH_3CH_2), 1.23–1.36 (m, 4H, 2× CH_2), 1.63 (m, 2H, CH_2), 2.38 (m, 2H, CH_2CO), 3.81–4.05 (m, 4H, 2× CH_2–dioxolane), 4.08 (dd, 1H, $J_{1,2}$=2.8 Hz, $J_{2,3}$=5.1 Hz, H-2), 4.20 (t, 1H, *J*=5.7 Hz, H-3), 4.31 (m, 1H, H-5), 4.38 (dd, 1H, $J_{5,6a}$=4.5, $J_{6a,6b}$=11.8 Hz, H-6a), 4.58 (dd, 1H, $J_{5,6b}$=3.6, $J_{6a,6b}$=11.8 Hz, H-6b), 4.99 (d, 1H, $J_{1,2}$=2.8 Hz, H-1), 5.27 (t, 1H, *J*=5.8 Hz, H-4), 7.38–8.08 (m, 5H, Ph); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 13.8 (CH_3CH_2), 22.1, 24.2 and 31.0 (3× CH_2), 33.6 (CH_2CO), 60.5 (C-3), 63.6 (C-6), 65.3 and 65.5 (2× CH_2–dioxolane), 73.5 (C-4), 79.2 (C-5), 82.1 (C-2), 102.1 (C-1), 128.3, 129.6, and 133.0 (Ph), 166.0 (PhC=O), 172.9 ($C_5H_{11}CO$); LRMS (CI): *m*/*z* 434 (M^++H). Anal. Calcd for $C_{21}H_{27}N_3O_7$: C, 58.19; H, 6.28; N, 9.69. Found: C, 58.41; H, 6.33; N, 10.01.

*4.4.2. 2,5-Anhydro-3-azido-6-O-benzoyl-3-deoxy-4-O-octanoyl-D-allose ethylene acetal (**6b**).* Sulfonate **5b** (1.37 g, 2.7 mmol) was treated with NaN_3 (1.51 g, 23.2 mmol) according to the above general procedure to give crude **6b** as a yellow oil. Pure **6b** (0.49 g, 40%) was isolated by flash column chromatography (17:3 cyclohexane/Me_2CO) as a colourless oil, $[\alpha]_D^{20}$ +1.6 (*c* 0.6, $CHCl_3$); IR (neat): ν_{max} 2110, 1726, 1604; ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.88 (t, 3H, *J*=6.8 Hz, CH_3CH_2), 1.29 (m, 8H, 4× CH_2), 1.65 (m, 2H, CH_2), 2.40 (m, 2H, CH_2CO), 3.84–4.07 (m, 4H, 2× CH_2–dioxolane), 4.10 (dd, 1H, $J_{1,2}$=2.8, $J_{2,3}$=5.2 Hz, H-2), 4.22 (t, 1H, $J_{3,4}$=5.8 Hz, H-3), 4.28–4.46 (m, 2H, H-5 and H-6a), 4.56 (dd, 1H, $J_{5,6b}$=3.5, $J_{6a,6b}$=11.9 Hz, H-6b), 5.02 (d, 1H, $J_{1,2}$=2.8 Hz, H-1), 5.28 (t, 1H, $J_{3,4}$=5.8 Hz, H-4), 7.40–8.11 (m, 5H, Ph); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 13.9 (CH_3CH_2), 22.4, 24.6, 28.7, 28.8 and 31.5 (5× CH_2), 33.64 (CH_2CO), 60.5 (C-3), 63.6 (C-6), 65.3 and 65.5 (2× CH_2–dioxolane), 73.5 (C-4), 79.3 (C-5), 82.1 (C-2), 102.1 (C-1), 128.3, 129.6 and 133.0 (Ph), 166.0 (PhC=O), 172.9 ($C_7H_{15}CO$); HRMS (ESI): *m*/*z* 462.2222 (M^++H), calcd for $C_{23}H_{32}N_3O_7$: 462.2235.

*4.4.3. 2,5-Anhydro-3-azido-6-O-benzoyl-4-O-decanoyl-3-deoxy-D-allose ethylene acetal (**6c**).* Sulfonate **5c** (1.74 g, 3.2 mmol) was treated with NaN_3 (1.81 g, 27.8 mmol) according to the above general procedure to give crude **6c** as a yellow oil. After purification on a column of flash silica (17:3 cyclohexane/Me_2CO), followed by preparative TLC (17:3 cyclohexane/Me_2CO) of several impure fractions, pure **6c** (0.76 g, 49%) was isolated as a colourless oil, $[\alpha]_D^{20}$ +13.3 (*c* 0.9, $CHCl_3$); IR (neat): ν_{max} 2110, 1750, 1725, 1617; ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.87 (t, 3H, *J*=6.8 Hz, CH_3CH_2), 1.25 (m, 12H, 6× CH_2), 1.64 (m, 2H, CH_2), 2.38 (m, 2H, CH_2CO), 3.82–4.05 (m, 4H, 2× CH_2–dioxolane), 4.09 (dd, 1H, $J_{1,2}$=2.7, $J_{2,3}$=5.1 Hz, H-2), 4.20 (t, 1H, $J_{3,4}$=5.8 Hz, H-3), 4.33 (m, 1H, H-5), 4.39 (dd, 1H, $J_{5,6a}$=4.5, $J_{6a,6b}$=11.8 Hz, H-6a), 4.55 (dd, 1H, $J_{5,6b}$=3.6, $J_{6a,6b}$=11.8 Hz, H-6b), 5.00 (d, 1H, $J_{1,2}$=2.7 Hz, H-1), 5.28 (t, 1H, *J*=5.8 Hz, H-4), 7.39–8.09 (m, 5H, Ph); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 14.0 (CH_3CH_2), 22.5, 24.6, 29.0, 29.1, 29.3 and 31.7 (7× CH_2), 33.7 (CH_2CO), 60.6 (C-3), 63.6 (C-6), 65.4 and 65.5 (2× CH_2–dioxolane), 73.6 (C-4), 79.3 (C-5), 82.2 (C-2), 102.2 (C-1), 128.28, 129.6 and 133.0 (Ph), 166.01 (PhC=O), 172.9 ($C_9H_{19}CO$); LRMS (CI): *m*/*z* 490 (M^++H). Anal. Calcd for $C_{25}H_{35}N_3O_7$: C, 61.33; H, 7.21; N, 8.58. Found: C, 61.70; H, 7.44; N, 8.35.

*4.4.4. 2,5-Anhydro-3-azido-6-O-benzoyl-3-deoxy-4-O-dodecanoyl-D-allose ethylene acetal (**6d**).* Sulfonate **5d** (3.8 g, 6.7 mmol) was treated with NaN_3 (4.3 g, 66.6 mmol) according to the above general procedure to give crude **6d** as a yellow oil. Pure **6d** (1.5 g, 44%) was isolated after column chromatography on silica gel (19:1, 4:1 toluene/EtOAc) as a colourless oil, $[\alpha]_D^{20}$ +7.5 (*c* 1.1, $CHCl_3$); IR (neat): ν_{max} 2110, 1735; ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.86 (t, 3H, CH_3CH_2), 1.08–1.72 (m, 18H, 9× CH_2), 2.38 (m, 2H, CH_2CO), 3.81–4.03 (m, 4H, 2× CH_2–dioxolane), 4.08 (dd, 1H, $J_{1,2}$=2.8, $J_{2,3}$=5.2 Hz, H-2), 4.20 (dd, 1H, $J_{2,3}$=5.2, $J_{3,4}$=5.6 Hz, H-3), 4.25 (m, 1H, H-5), 4.38 (dd, 1H, $J_{5,6a}$=4.5, $J_{6a,6b}$=11.9 Hz, H-6a), 4.53 (dd, 1H, $J_{5,6b}$=3.7, $J_{6a,6b}$=11.9 Hz, H-6b), 4.99 (d, 1H, $J_{1,2}$=2.8 Hz, H-1), 5.28 (dd, 1H, $J_{3,4}$=5.6, $J_{4,5}$=5.9 Hz, H-4), 7.37–8.10 (m, 5H, Ph); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 13.9 (CH_3CH_2), 22.5, 24.6, 28.9, 29.0, 29.1, 29.3, 29.4 and 31.7 (9× CH_2), 33.6 (CH_2CO), 60.5 (C-3), 63.6 (C-6), 65.3 and 65.4 (2× CH_2–dioxolane), 73.5 (C-4), 79.2 (C-5), 82.1 (C-2), 102.1 (C-1), 128.2, 129.55, 129.6, and 133.0 (Ph), 165.9 (PhC=O), 172.8 ($C_{11}H_{23}CO$);

LRMS (CI): m/z 518 (M^++H). Anal. Calcd for $C_{27}H_{39}N_3O_7$: C, 62.65; H, 7.59; N, 8.12. Found: C, 62.95; H, 7.74; N, 8.43.

4.5. General procedure for the synthesis of ribofuranosyl cyanides 9a–d

A solution of acetal **6** (1 equiv) in a 4:1 mixture of TFA/6 M HCl (0.2 M) was kept at +4 °C for 5–9 days. The mixture was concentrated to a third of the initial volume and poured into saturated aq $NaHCO_3$. The aqueous solution was rendered alkaline with solid $NaHCO_3$ to pH 8–9 and extracted with CH_2Cl_2. The combined extracts were washed successively with saturated aq $NaHCO_3$ and water, dried and evaporated. The remaining crude aldehyde **7** was immediately dissolved in a mixture of EtOH/CH_2Cl_2 (~0.2 M) and treated with NaOAc (~3 equiv) and $NH_2OH\times HCl$ (~2 equiv) while stirring at room temperature for 2–4 h. The mixture was evaporated and the residue distributed between water and CH_2Cl_2. The organic layer was separated and the aqueous phase extracted with CH_2Cl_2. The combined organic solutions were washed with water, dried and evaporated to afford crude oxime **8** as a mixture of the corresponding *E*- and *Z*-isomers. To a cooled (−15 °C) and stirred solution of **8** in anhydrous pyridine (~0.2 M) was added dropwise during 0.5 h a cold solution of MsCl (~4 equiv) in dry pyridine (1 M). The mixture was first stirred at 0 °C for 15 min, then at room temperature for 2 h and then poured into a 1:1 mixture of ice and concentrated HCl (pH ~2). The emulsion was extracted with CH_2Cl_2, the combined extracts were washed with water, saturated aq $NaHCO_3$ and again with water. The extract was dried and evaporated, and the residue was purified by column chromatography.

*4.5.1. 2,5-Anhydro-3-azido-6-O-benzoyl-3-deoxy-4-O-hexanoyl-D-allononitrile (**9a**).* Acetal **6a** (1.31 g, 3 mmol) was converted into crude aldehyde **7a** after treatment with a mixture TFA (11 mL) and 6 M HCl (2.7 mL) for 5 days according to the above general procedure. Crude **7a** was treated with a mixture of NaOAc (0.87 g, 10.6 mmol) and $NH_2OH\times HCl$ (0.49 g, 7 mmol) in EtOH (9 mL) for 2.5 h at room temperature and then evaporated. Flash column chromatography of the residue (9:1 toluene/EtOAc) gave pure *E*-**8a** (0.4 g) that was characterized by NMR spectral data: ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.89 (t, 3H, J=7.1 Hz, CH_3CH$_2$), 1.23–1.36 (m, 4H, 2× CH_2), 1.61 (m, 2H, CH_2), 2.41 (m, 2H, CH_2CO), 4.17 (dd, 1H, $J_{2,3}$=6.8, $J_{3,4}$=6.0 Hz, H-3), 4.35 (m, 1H, H-5), 4.43 (dd, 1H, $J_{6a,6b}$=12.0, $J_{5,6a}$=4.2 Hz, H-6a), 4.50–4.59 (m, 2H, H-2 and H-6b), 5.35 (dd, 1H, $J_{3,4}$=6.0, $J_{4,5}$=5.6 Hz, H-4), 7.40–7.50 (m, 3H, H-1 and 2H from Ph), 7.50–8.09 (m, 3H, Ph), 8.79 (br s, 1H, NOH); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 13.7 (*C*H$_3$CH$_2$), 22.1, 24.2 and 31.0 (3× CH_2), 33.7 (*C*H$_2$CO), 62.6 (C-3), 63.7 (C-6), 73.4 (C-4), 78.4 (C-2), 80.2 (C-5), 128.4, 129.2, 129.5 and 133.2 (Ph), 147.6 (C-1), 166.1 (Ph*C*=O), 173.0 (C$_5$H$_{11}$*CO*). An inseparable mixture of *E*- and *Z*-**8a** was also isolated, the total yield of purified **8a** was 0.77 g (68% on the basis of the recovered **6a**). A minor amount of unreacted starting compound **6a** (0.1 g, 8%) was recovered. Purified **8a** (0.77 g, 1.9 mmol) was converted into crude **9a** by treatment with MsCl (0.6 mL, 7.5 mmol) in dry pyridine (8.5 mL) for 2 h, according to the above general procedure. Pure **9a** (0.5 g, 68%) was isolated by flash chromatography (9:1 toluene/EtOAc) as a colourless oil, $[\alpha]_D^{20}$ −14.9 (*c* 3.3, $CHCl_3$); IR (neat): ν_{max} 2230, 2118, 1724; ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.89 (t, 3H, J=7.1 Hz, CH_3CH$_2$), 1.23–1.66 (m, 6H, 3× CH_2), 2.40 (m, 2H, CH_2CO), 4.41 (dt, 1H, $J_{5,6}$=4.3, $J_{4,5}$=6.1 Hz, H-5), 4.50–4.65 (m, 4H, H-2, H-3 and 2× H-6), 5.45 (dd, 1H, $J_{3,4}$=4.8, $J_{4,5}$=5.7 Hz, H-4), 7.41–8.12 (m, 5H, Ph); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 13.7 (*C*H$_3$CH$_2$), 22.1, 24.2 and 31.0 (3× CH_2), 33.5 (*C*H$_2$CO), 62.7 (C-6), 64.4 (C-3), 69.1 (C-2), 72.6 (C-4), 80.6 (C-5), 115.9 (C-1), 128.4, 129.1, 129.6 and 133.4 (Ph), 165.9 (Ph*C*=O), 172.6 (C$_5$H$_{11}$*CO*); LRMS (CI): m/z 387 (M^++H). Anal. Calcd for $C_{19}H_{22}N_4O_5$: C, 59.06; H, 5.74; N, 14.50. Found C, 58.96; H, 5.67; N, 14.31.

*4.5.2. 2,5-Anhydro-3-azido-6-O-benzoyl-3-deoxy-4-O-octanoyl-D-allononitrile (**9b**).* Acetal **6b** (0.91 g, 2 mmol) was converted into crude **9b** according to the above general procedure. Pure **9b** (0.43 g, 57% from three steps) was isolated by flash column chromatography (99:1, 9:1 toluene/EtOAc) as a colourless oil, $[\alpha]_D^{20}$ +5.6 (*c* 5.4, $CHCl_3$); IR (neat): ν_{max} 2280, 2117, 1727; ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.86 (t, 3H, J=6.8 Hz, CH_3CH$_2$), 1.25 (m, 8H, 4× CH_2), 1.61 (m, 2H, CH_2), 2.37 (m, 2H, CH_2CO), 4.30–4.50 (m, 3H, H-5 and 2× H-6), 4.57 (d, 1H, $J_{2,3}$=5.3 Hz, H-2), 4.60 (t, 1H, $J_{3,4}$=5.4 Hz, H-3), 5.46 (t, 1H, $J_{4,5}$=5.4 Hz, H-4), 7.37–8.09 (m, 5H, Ph); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 13.7 (*C*H$_3$CH$_2$), 22.2, 24.3, 28.5, 28.6 and 31.3 (5× *C*H$_2$), 33.4 (*C*H$_2$CO), 62.6 (C-6), 64.2 (C-3), 68.9 (C-2), 72.4 (C-4), 80.5 (C-5), 115.9 (C-1), 128.2, 129.0, 129.4 and 133.1 (Ph), 165.7 (Ph*C*=O), 172.4 (C$_7$H$_{15}$*CO*); LRMS (CI): m/z 415 (M^++H); HRMS (ESI): m/z 415.1967 (M^++H), calcd for $C_{21}H_{27}N_4O_5$: 415.1976. Anal. Calcd for $C_{21}H_{26}N_4O_5$: C, 60.86; H, 6.32; N, 13.52. Found: C, 60.68; H, 6.30; N, 13.12. A minor amount of unreacted starting compound **6b** (0.075 g, 8%) was recovered.

*4.5.3. 2,5-Anhydro-3-azido-6-O-benzoyl-4-O-decanoyl-3-deoxy-D-allononitrile (**9c**).* Acetal **6c** (0.89 g, 1.8 mmol) was converted into crude **9c** according to the above general procedure. Pure **9c** (0.25 g, 47% from three steps) was isolated by flash column chromatography (99:1, 9:1 toluene/EtOAc) as a colourless oil, $[\alpha]_D^{20}$ −4.9 (*c* 1.1, $CHCl_3$); IR (neat): ν_{max} 2282, 2116, 1725, 1602; ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.88 (t, 3H, J=6.8 Hz, CH_3CH$_2$), 1.27 (m, 12H, 6× CH_2), 1.64 (m, 2H, CH_2), 2.42 (m, 2H, CH_2CO), 4.38–4.70 (m, 5H, H-2, H-3, H-5 and 2× H-6), 5.45 (m, 1H, H-4), 7.42–8.15 (m, 5H, Ph); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 14.1 (*C*H$_3$CH$_2$), 22.6, 24.6, 29.0, 29.1, 29.2, 29.3 and 31.8 (7× CH_2), 33.6 (*C*H$_2$CO), 62.7 (C-6), 64.5 (C-3), 69.2 (C-2), 72.6 (C-4), 80.6 (C-5), 115.9 (C-1), 128.5, 129.0, 129.7 and 133.5 (Ph), 166.0 (Ph*C*=O), 172.7 (C$_9$H$_{19}$*CO*); LRMS(CI): m/z 443 (M^++H). Anal. Calcd for $C_{23}H_{30}N_4O_5$: C, 62.43; H, 6.83; N, 12.66. Found: C, 62.59; H, 7.19; N, 12.24. A minor amount of unreacted starting compound **6c** (0.04 g, 5%) was recovered.

*4.5.4. 2,5-Anhydro-3-azido-6-O-benzoyl-3-deoxy-4-O-dodecanoyl-D-allononitrile (**9d**).* Acetal **6d** (1.34 g, 2.6 mmol) was converted into crude **9d** according to the above general procedure. Pure **9d** (0.41 g, 36% from three steps) was isolated by silica gel column chromatography (97:3 toluene/EtOAc) as a colourless oil, $[\alpha]_D^{20}$ −7.3 (*c* 1.0, $CHCl_3$); IR (neat): ν_{max} 2300, 2130, 1740; ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.88 (t, 3H, CH_3CH$_2$), 1.16–1.72 (m, 18H, 9× CH_2), 2.41 (t, 2H, CH_2CO), 4.42 (m, 1H, H-5), 4.48 (dd, 1H, $J_{5,6a}$=3.7, $J_{6a,6b}$=12.2 Hz, H-6a), 4.56 (dd, 1H, $J_{5,6b}$=3.7, $J_{6a,6b}$=12.2 Hz, H-6b), 4.58 (m, 2H, H-2 and H-3), 5.46 (dd, 1H, $J_{3,4}$=4.9, $J_{4,5}$=5.5 Hz, H-4), 7.46–8.12 (m, 5H, Ph); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 14.1 (*C*H$_3$CH$_2$), 22.6, 24.6, 29.0, 29.1, 29.2, 29.3, 29.4, 29.5, 31.8 and 33.6 (10× CH_2), 62.8 (C-6), 64.5 (C-3), 69.2 (C-2), 72.6 (C-4), 80.7 (C-5), 115.9 (C-1), 128.5, 129.5, 129.7 and 133.5 (Ph), 166.0 (Ph*C*=O), 172.7 (C$_{11}$H$_{23}$*CO*); LRMS (CI): m/z 471 (M^++H). Anal. Calcd for $C_{25}H_{34}N_4O_5$: C, 64.81; H, 7.28; N, 11.91. Found: C, 64.59; H, 7.07; N, 11.86. A minor amount of unreacted starting compound **6d** (0.09 g, 7%) was recovered.

4.6. General procedure for the one-pot conversion of 2-azido-3-*O*-acyl-ribofuranosyl cyanides 9a–c into the corresponding 2-alkylamido-ribofuranosyl thiocarboxamides 10a–c

Through a solution of nitrile **9** (1 mmol) in anhydrous pyridine (0.2 M) was passed H_2S gas at room temperature. The mixture was evaporated and the residue was purified on a column of silica gel.

*4.6.1. 2,5-Anhydro-6-O-benzoyl-3-deoxy-3-hexanamido-D-allono-thioamide (**10a**).* Nitrile **9a** (0.5 g, 1.3 mmol) was converted into crude thioamide **10a** after treatment with H_2S for 12 h according to the above general procedure. Pure **10a** (0.5 g, 99%) was isolated by column chromatography (11:9 toluene/EtOAc) as a colourless oil $[\alpha]_D^{20}$ +29.0 (*c* 5.7, $CHCl_3$); IR (neat): ν_{max} 3300, 1716, 1651, 1540, 1531, 1315, 1275, 1178; ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.84 (t, 3H, J=7.1 Hz, CH_3CH$_2$), 1.17–1.72 (m, 6H, 3× CH_2), 2.24 (m, 2H, CH_2CO), 4.05 (ddd, 1H, $J_{2,3}$=9.1, $J_{3,NH}$=5.4, $J_{3,4}$=5.2 Hz, H-3), 4.29 (br s, 1H, exchangeable with D_2O, OH), 4.36 (m, 1H, H-5), 4.45 (dd, 1H, $J_{5,6a}$=3.1, $J_{6a,6b}$=12.1 Hz, H-6a), 4.50–4.62 (m, 2H, H-4 and H-6b), 4.68 (d, 1H, $J_{2,3}$=9.1 Hz, H-2), 6.86 (d, 1H, exchangeable with D_2O, $J_{3,NH}$=5.4 Hz, N*H*COC$_5$H$_{11}$), 7.38–8.16 (m, 5H, Ph), 8.24 and 8.47 (2× br s, 1H each, exchangeable with D_2O, $CSNH_2$); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 13.8 (*C*H$_3$CH$_2$), 22.2, 25.0 and 31.2 (3× CH_2), 36.5 (*C*H$_2$CO), 59.0 (C-3), 64.7 (C-6), 71.8 (C-4), 84.0 (C-5), 84.6 (C-2), 128.5, 129.2, 129.6 and 133.5 (Ph), 166.9 (Ph*C*=O), 174.7 (NH*C*OC$_5$H$_{11}$), 203.5 ($CSNH_2$); LRMS (CI): *m*/*z* 395 (M^++H). Anal. Calcd for $C_{19}H_{26}N_2O_5S$: C, 57.85; H, 6.64; N, 7.10; S, 8.13. Found: C, 57.85; H, 6.83; N, 7.36; S, 8.24.

*4.6.2. 2,5-Anhydro-6-O-benzoyl-3-deoxy-3-octanamido-D-allono-thioamide (**10b**).* Nitrile **9b** (0.32 g, 0.8 mmol) was converted into crude thioamide **10b** after treatment with H_2S for 120 h according to the above general procedure. Pure **10b** (0.3 g, 92%) was isolated by flash column chromatography (3:2 toluene/EtOAc) as a colourless oil, $[\alpha]_D^{20}$ +14.2 (*c* 3.0, $CHCl_3$); IR (neat): ν_{max} 3300, 1717, 1652, 1533, 1315, 1274, 1177; ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.85 (t, 3H, J=6.4 Hz, CH_3CH$_2$), 1.25 (m, 8H, 4× CH_2), 1.61 (m, 2H, CH_2), 2.25 (m, 2H, CH_2CO), 3.00 (br s, 1H, exchangeable with D_2O, OH), 4.02 (ddd, 1H, $J_{2,3}$=9.0, $J_{3,NH}$=5.1, $J_{3,4}$=5.2 Hz, H-3), 4.38 (m, 1H, H-5), 4.48 (dd, 1H, $J_{5,6a}$=3.0, $J_{6a,6b}$=12.1 Hz, H-6a), 4.52–4.60 (m, 2H, H-4 and H-6b), 4.65 (d, 1H, $J_{2,3}$=9.0 Hz, H-2), 7.81 (d, 1H, exchangeable with D_2O, $J_{3,NH}$=5.1 Hz, N*H*COC$_7$H$_{15}$), 7.37–8.08 (m, 5H, Ph), 8.40 and 8.45 (2× br s, 1H each, exchangeable with D_2O, $CSNH_2$); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 14.0 (*C*H$_3$CH$_2$), 22.6, 25.5, 29.0, 29.2 and 31.6 (5× CH_2), 36.6 (*C*H$_2$CO), 58.8 (C-3), 64.9 (C-6), 71.9 (C-4), 84.1 (C-5), 84.8 (C-2), 128.6, 129.2, 129.7 and 133.5 (Ph), 167.0 (Ph*C*=O), 174.9 (NH*C*OC$_7$H$_{15}$), 203.7 ($CSNH_2$); HRMS (ESI): *m*/*z* 423.1954 (M^++H), calcd for $C_{21}H_{31}N_2O_5S$: 423.1948.

*4.6.3. 2,5-Anhydro-6-O-benzoyl-3-decanamido-3-deoxy-D-allono-thioamide (**10c**).* Nitrile **9c** (0.25 g, 0.6 mmol) was converted into crude thioamide **10c** after treatment with H_2S for 96 h according to the above general procedure. Pure **10c** (0.23 g, 87%) was isolated by flash column chromatography (3:2 toluene/EtOAc) as a colourless oil, $[\alpha]_D^{20}$ +15.0 (*c* 1.8, $CHCl_3$); IR (neat): ν_{max} 3295, 1716, 1648, 1532, 1315, 1275, 1177; ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.86 (t, 3H, J=6.8 Hz, CH_3CH$_2$), 1.12–1.72 (m, 14H, 7× CH_2), 2.25 (t, 2H, J=7.1 Hz, CH_2CO), 3.97 (ddd, 1H, $J_{2,3}$=9.2, $J_{3,NH}$=4.7, $J_{3,4}$=5.4 Hz, H-3), 4.03 (br s, 1H, exchangeable with D_2O, OH), 4.38 (m, 1H, H-5), 4.48 (dd, 1H, $J_{5,6a}$=3.1, $J_{6a,6b}$=12.1 Hz, H-6a), 4.50–4.62 (m, 2H, H-4 and H-6b), 4.65 (d, 1H, $J_{2,3}$=9.2 Hz, H-2), 6.81 (d, 1H, $J_{3,NH}$=4.7 Hz, exchangeable with D_2O, N*H*COC$_9$H$_{19}$), 7.37–8.04 (m, 5H, Ph), 8.07 and 8.47 (2× br s, 2H, partially exchangeable with D_2O, $CSNH_2$); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 14.0 (*C*H$_3$CH$_2$), 22.6, 25.4, 29.17, 29.2, 29.3 and 31.7 (7× CH_2), 36.59 (*C*H$_2$CO), 59.1 (C-3), 64.7 (C-6), 71.9 (C-4), 84.1 (C-5), 84.6 (C-2), 128.5, 129.1, 129.6 and 133.5 (Ph), 166.9 (Ph*C*=O), 174.8 (NH*C*OC$_9$H$_{19}$), 203.7 (C-1); HRMS (ESI): *m*/*z* 451.2278 (M^++H), calcd for $C_{23}H_{35}N_2O_5S$: 451.2261.

*4.6.4. 2,5-Anhydro-6-O-benzoyl-3-deoxy-3-dodecanamido-D-allono-thioamide (**10d**).* Through a solution of **9d** (0.1 g, 0.2 mmol) and DMAP (0.005 g, 0.04 mmol) in absolute EtOH (2 mL) was passed H_2S gas for 8 h at room temperature. The mixture was evaporated and the residue was purified on a column of silica gel (7:3, 3:2 toluene/EtOAc) to afford pure **10d** (0.1 g, 92%) as a colourless oil. Crystallization from CH_2Cl_2/hexane gave colourless crystals, mp 110.5–111 °C; $[\alpha]_D^{20}$ +15.4 (*c* 1.3, $CHCl_3$); IR (neat): ν_{max} 3300, 1730, 1630, 1550, 1540, 1320, 1275, 1140; ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.87 (t, 3H, J=6.7 Hz, CH_3CH$_2$), 1.24 (br s, 16H, 8× CH_2), 1.60 (m, 2H, CH_2CH$_2$CO), 2.24 (dd, 2H, J=7.3, J=8.2 Hz, CH_2CO), 4.06 (ddd, 1H, $J_{2,3}$=9.0, $J_{3,4}$=5.2, $J_{3,NH}$=5.5 Hz, H-3), 4.13 (br s, 1H, exchangeable with D_2O, OH), 4.36 (m, 1H, H-5), 4.41–4.61 (m, 3H, H-4 and 2× H-6), 4.65 (d, 1H, $J_{2,3}$=9.0 Hz, H-2), 6.85 (d, 1H, $J_{3,NH}$=5.5 Hz, N*H*COC$_{11}$H$_{23}$), 7.36–8.04 (m, 5H, Ph), 8.21 and 8.47 (br s, 2H, partially exchangeable with D_2O, $CSNH_2$); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 14.0 (*C*H$_3$CH$_2$), 22.6, 25.4, 29.2, 29.22, 29.4, 29.5, 29.52, 29.6 and 31.8 (9× CH_2), 36.6 (*C*H$_2$CO), 59.0 (C-3), 64.8 (C-6), 71.9 (C-4), 84.0 (C-5), 84.6 (C-2), 128.50, 129.2, 129.6 and 133.4 (Ph), 166.9 (Ph*C*=O), 174.7 (NH*C*OC$_{11}$H$_{23}$), 203.6 (C-1); LRMS (CI): *m*/*z* 479 (M^++H), 477 (M^+–H). Anal. Calcd for $C_{25}H_{38}N_2O_5S$: C, 62.73; H, 8.00; N, 5.85; S, 6.70. Found: C, 62.59; H, 7.84; N, 6.16; S, 6.52.

4.7. General procedure for the synthesis of protected thiazole derivatives 11a–d

To a solution of thioamide **10** (1 equiv) was added ethyl bromopyruvate (1.8 equiv) in absolute EtOH (0.1 M) and the mixture was stirred under reflux for 50–180 min. The solvent was evaporated in vacuum and the residue was purified on a column of silica gel.

*4.7.1. Ethyl 2-(5-O-benzoyl-2-deoxy-2-hexanamido-β-D-ribofuranosyl)thiazole-4-carboxylate (**11a**).* Thioamide **10a** (0.49 g, 1.3 mmol) was converted into crude thiazole **11a** after treatment with ethyl bromopyruvate (0.3 mL, 2.2 mmol) for 1 h according to the above general procedure. Pure **11a** (0.325 g, 54%) was isolated by flash column chromatography (1:1 toluene/EtOAc) as a colourless oil, $[\alpha]_D^{20}$ –25.0 (*c* 1.7, $CHCl_3$); IR (neat): ν_{max} 3355, 1722, 1652, 1538, 1272; ^{1}H NMR (250 MHz, 1:1 $CDCl_3$/benzene-d_6): δ 0.87 (t, 3H, J=6.4 Hz, CH_3CH$_2$CH$_2$), 1.05 (t, 3H, J=7.1 Hz, CO$_2$CH$_2$CH_3), 1.20–1.65 (m, 6H, 3× CH_2), 2.22 (m, 2H, CH_2CO), 4.05 (q, 2H, CO$_2$CH_2CH$_3$), 4.35 (dd, 1H, $J_{4',5a'}$=3.2, $J_{5a',5b'}$=12.1 Hz, H-5a′), 4.47 (m, 1H, H-4′), 4.58 (dd, 1H, $J_{2',3'}$=4.9, $J_{3',4'}$=1.7 Hz, H-3′), 4.72 (dd, 1H, $J_{4',5b'}$=3.7, $J_{5a',5b'}$=12.1 Hz, H-5b′), 4.94 (m, 1H, H-2′), 5.50 (d, 1H, $J_{1',2'}$=9.1 Hz, H-1′), 7.18–8.41 (m, 7H, Ph, N*H*COC$_5$H$_{11}$ and OH), 7.38 (s, 1H, H-5); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 13.8 (*C*H$_3$CH$_2$CH$_2$), 14.2 (CO$_2$CH$_2$*C*H$_3$), 22.3, 25.0 and 31.3 (3× *C*H$_2$), 36.14 (*C*H$_2$CO), 59.6 (C-2′), 61.6 (CO$_2$*C*H$_2$CH$_3$), 64.6 (C-5′), 72.4 (C-3′), 79.6 (C-1′), 84.3 (C-4′), 128.3 (C-5), 128.9, 129.4, 129.7 and 133.2 (Ph), 146.3 (C-4), 161.4 (C-2), 166.3 (Ph*C*=O), 172.0 (*C*O$_2$Et), 174.4 (NH*C*OC$_5$H$_{11}$); LRMS (CI): *m*/*z* 491 (M^++H). Anal. Calcd for $C_{24}H_{30}N_2O_7S$: C, 58.76; H, 6.16; N, 5.71; S, 6.54. Found: C, 59.03; H, 5.81; N, 6.03; S, 6.25.

*4.7.2. Ethyl 2-(5-O-benzoyl-2-deoxy-2-octanamido-β-D-ribofuranosyl)thiazole-4-carboxylate (**11b**).* Thioamide **10b** (0.3 g, 0.7 mmol) was converted into crude thiazole **11b** after treatment with ethyl bromopyruvate (0.2 mL, 1.3 mmol) for 3 h according to the above general procedure. Pure **11b** (0.18 g, 60%) was isolated by flash column chromatography (1:1 toluene/EtOAc) as a colourless oil: $[\alpha]_D^{20}$ –4.1 (*c* 1.0, $CHCl_3$); IR (neat): ν_{max} 3363, 1721, 1651, 1534, 1272; ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.85 (t, 3H, J=6.4 Hz, CH_3CH$_2$CH$_2$), 1.38 (t, 3H, J=7.1 Hz, CO$_2$CH$_2$CH_3), 1.12–1.88 (m, 10H, 5× CH_2), 2.17 (m, 2H, CH_2CO), 3.91 (br s, 1H, OH), 4.39 (q, 2H, CO$_2$CH_2CH$_3$), 4.45–4.73 (m, 5H, H-2′, H-3′, H-4′, and 2× H-5′), 5.50 (d, 1H, $J_{1',2'}$=8.9 Hz, H-1′), 7.02 (d, 1H, $J_{2',NH}$=7.3 Hz, N*H*COC$_7$H$_{15}$), 7.41–8.10 (m, 5H, Ph), 8.11 (s, 1H, H-5); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 13.9 (*C*H$_3$CH$_2$CH$_2$), 14.1 (CO$_2$CH$_2$*C*H$_3$), 22.5, 25.3, 28.9, 29.2 and 31.3 (5× CH_2), 36.15 (*C*H$_2$CO), 59.5 (C-2′), 61.5 (CO$_2$*C*H$_2$CH$_3$), 64.6 (C-5′), 72.6 (C-3′), 79.5 (C-1′), 84.4 (C-4′), 128.4, 129.4, 129.6 and 133.2 (Ph and C-5), 146.1 (C-4), 161.4 (C-2), 166.3 (Ph*C*=O), 172.1

(CO_2Et), 174.2 (NHCOC_7H_{15}); HRMS (ESI): *m*/*z* 519.2157 (M^++H), calcd for $C_{26}H_{35}N_2O_7S$: 519.2160.

*4.7.3. Ethyl 2-(5-O-benzoyl-2-decanamido-2-deoxy-β-D-ribofuranosyl)thiazole-4-carboxylate (**11c**).* Thioamide **10c** (0.17 g, 0.4 mmol) was converted into crude thiazole **11c** after treatment with ethyl bromopyruvate (0.1 mL, 0.7 mmol) for 50 min according to the above general procedure. Pure **11c** (0.1 g, 47%) was isolated by flash column chromatography (1:1 toluene/EtOAc) as a colourless oil, $[\alpha]_D^{20}$ +4.7 (*c* 1.0, $CHCl_3$); IR (neat): ν_{max} 3353, 1724, 1652, 1533, 1272; ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.83 (t, 3H, *J*=6.8 Hz, C*H*$_3$CH$_2$CH$_2$), 1.17 (m, 12H, 6× CH_2), 1.34 (t, 3H, *J*=7.1 Hz, CO_2CH$_2$C*H*$_3$), 1.50 (m, 2H, CH_2), 2.12 (m, 2H, C*H*$_2$CO), 4.33 (q, 2H, *J*=7.1 Hz, CO_2C*H*$_2$CH$_3$), 4.41–4.69 (m, 6H, H-2′, H-3′, H-4′, H-5a′, H-5b′ and OH), 5.29 (d, 1H, $J_{1'2'}$=8.9 Hz, H-1′), 7.30 (d, 1H, $J_{2',NH}$=8.1 Hz, N*H*COC$_9H_{19}$), 7.36–8.11 (m, 5H, Ph), 8.08 (s, 1H, H-5); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 14.0 (*C*H$_3$CH$_2$CH$_2$), 14.2 (CO_2CH$_2$*C*H$_3$), 22.5, 25.3, 29.2, 29.26, 29.3 and 31.7 (7× CH_2), 36.1 (*C*H$_2$CO), 59.5 (C-2′), 61.6 (CO_2CH$_2$CH$_3$), 64.6 (C-5′), 72.5 (C-3′), 79.4 (C-1′), 84.3 (C-4′), 128.4, 129.3, 129.7 and 133.2 (C-5 and Ph), 146.1 (C-4), 161.4 (C-2), 166.3 (Ph*C*=O), 172.2 (*C*O$_2$Et), 174.3 (NH*C*OC$_{11}H_{23}$); LRMS (CI): *m*/*z* 547 (M^++H). Anal. Calcd for $C_{28}H_{38}N_2O_7S$: C, 61.52; H, 7.01; N, 5.12; S, 5.87. Found: C, 61.48; H, 6.93; N, 4.89; S, 6.01.

*4.7.4. Ethyl 2-(5-O-benzoyl-2-deoxy-2-dodecanamido-β-D-ribofuranosyl)thiazole-4-carboxylate (**11d**).* Thioamide **10d** (0.14 g, 0.3 mmol) was converted into crude thiazole **11d** after treatment with ethyl bromopyruvate (0.05 mL, 0.4 mmol) for 50 min according to the above general procedure. Pure **11d** (0.09 g, 56%) was isolated by column chromatography (4:1, 7:3, to 3:2 toluene/EtOAc) as a bright yellow solid. Recrystallization from a mixture of CH_2Cl_2/hexane gave an analytical sample **11d** as pale yellow needles, mp: 101–102 °C, $[\alpha]_D^{20}$ −23.3 (*c* 1.1, $CHCl_3$); IR (neat): ν_{max} 3452, 1713, 1650, 1546, 1278; ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.87 (t, 3H, C*H*$_3$CH$_2$CH$_2$), 1.10–1.62 (m, 18H, 9× CH_2), 1.37 (t, 3H, *J*=7.0 Hz, CO_2CH$_2$C*H*$_3$), 2.15 (t, 2H, C*H*$_2$CO), 4.36 (q, 2H, CO_2C*H*$_2$CH$_3$), 4.43–4.71 (m, 6H, H-2′, H-3′, H-4′, 2× H-5′ and OH), 5.30 (d, 1H, $J_{1',2'}$=8.2 Hz, H-1′), 7.22 (d, 1H, $J_{2',NH}$=7.9 Hz, N*H*COC$_{11}H_{23}$), 7.39–8.12 (m, 5H, Ph), 8.09 (s, 1H, H-5); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 14.0 (*C*H$_3$CH$_2$CH$_2$), 14.2 (CO_2CH$_2$*C*H$_3$), 22.6, 25.4, 29.29, 29.3, 29.4, 29.44, 29.56, 29.6, 31.8 and 36.2 (10× CH_2), 59.7 (C-2′), 61.6 (CO_2CH$_2$CH$_3$), 64.63 (C-5′), 72.6 (C-3′), 79.5 (C-1′), 84.34 (C-4′), 128.4 (C-5), 128.44, 129.5, 129.7 and 133.2 (Ph), 146.3 (C-4), 161.4 (C-2), 166.3 (Ph*C*=O), 172.1 (*C*O$_2$Et), 174.4 (NH*C*OC$_{11}H_{23}$); LRMS (CI): *m*/*z* 575 (M^++H). Anal. Calcd for $C_{30}H_{42}N_2O_7S$: C, 62.69; H, 7.37; N, 4.87; S, 5.58. Found: C, 62.89; H, 7.25; N, 5.08; S, 5.28.

4.8. General procedure for the preparation of C-nucleosides 2a–d

A solution of protected thiazole **11** (1 mmol) in saturated methanolic ammonia (0.05 M) was kept at room temperature for 7 days, then evaporated and the residue was purified on a column of silica gel or by preparative TLC.

*4.8.1. 2-(2-Deoxy-2-hexanamido-β-D-ribofuranosyl)thiazole-4-carboxamide (**2a**).* Protected thiazole **11a** (0.28 g, 0.6 mmol) was converted into crude **2a** after treatment with saturated methanolic ammonia (12 mL) according to the above general procedure. Pure **2a** (0.13 g, 66%) was isolated by silica gel column chromatography (9:1, 4:1, to 7:3 EtOAc/^{i}PrOH) as a white solid. Recrystallization from a mixture of MeOH/^{i}Pr$_2$O gave an analytical sample **2a** as colourless crystals, mp 155–156 °C; $[\alpha]_D^{20}$ −16.4 (*c* 1.1, MeOH); IR (KBr): ν_{max} 3288, 1653, 1271; ^{1}H NMR (250 MHz, methanol-d_4): δ 0.85 (t, 3H, C*H*$_3$CH$_2$CH$_2$), 1.14–1.64 (m, 6H, 3× CH_2), 2.25 (t, 2H, C*H*$_2$CO), 3.74 (d, 2H, $J_{4',5'}$=4.5 Hz, 2× H-5′), 4.13 (m, 1H, H-4′), 4.25 (dd, 1H, $J_{2',3'}$=5.4, $J_{3',4'}$=2.5, Hz, H-3′), 4.51 (dd, 1H, $J_{1',2'}$=8.9, $J_{2',3'}$=5.4 Hz, H-2′), 5.10 (d, 1H, $J_{1',2'}$=8.9 Hz, H-1′), 7.53 and 7.56 (2× br s, 0.2H, the minor NH_2 signal remained after partial exchange with methanol-d_4), 8.18 (d, 0.2H, the minor NH_2 signal remained after partial exchange with methanol-d_4), 8.22 (s, 1H, H-5); ^{13}C NMR (62.9 MHz, methanol-d_4): δ 14.3 (*C*H$_3$CH$_2$CH$_2$), 23.4, 26.6 and 32.3 (3× CH_2), 36.9 (CO*C*H$_2$), 60.0 (C-2′), 63.4 (C-5′), 72.7 (C-3′), 80.6 (C-1′), 88.8 (C-4′), 126.3 (C-5), 150.3 (C-4), 165.7 (C-2), 172.2 and 176.8 ($CONH_2$ and NHCOC$_5H_{11}$); LRMS (CI): *m*/*z* 358 (M^++H). Anal. Calcd for $C_{15}H_{23}N_3O_5S$: C, 50.41; H, 6.49; N, 11.76; S, 8.97. Found: C, 50.10; H, 6.40; N, 11.47; S, 8.64.

*4.8.2. 2-(2-Deoxy-2-octanamido-β-D-ribofuranosyl)thiazole-4-carboxamide (**2b**).* Protected thiazole **11b** (0.12 g, 0.2 mmol) was converted into crude **2b** after treatment with saturated ammonia in MeOH (5 mL) according to the above general procedure. Pure **2b** (0.05 g, 49%) was isolated by preparative TLC (9:1 EtOAc/^{i}PrOH). Recrystallization from a mixture of EtOAc/^{i}Pr$_2$O gave pure **2b** as colourless crystals, mp 162 °C; $[\alpha]_D^{20}$ −24.7 (*c* 0.6, MeOH); IR (KBr): ν_{max} 3319, 1649, 1540; ^{1}H NMR (250 MHz, methanol-d_4): δ 0.88 (t, 3H, *J*=7.1 Hz, CH_3CH_2), 1.16–1.66 (m, 10H, 5× CH_2), 2.25 (t, 2H, *J*=7.7 Hz, C*H*$_2$CO), 3.73 (d, 2H, $J_{4',5'}$=4.5 Hz, 2× H-5′), 4.11 (m, 1H, H-4′), 4.24 (dd, 1H, $J_{2',3'}$=5.4, $J_{3',4'}$=2.4 Hz, H-3′), 4.52 (dd, 1H, $J_{1',2'}$=8.7, $J_{2',3'}$=5.4 Hz, H-2′), 5.08 (d, 1H, $J_{1',2'}$=8.7 Hz, H-1′), 8.20 (s, 1H, H-5); ^{13}C NMR (62.9 MHz, methanol-d_4): δ 15.4 (CH_3CH_2), 24.6, 28.0, 31.1, 31.2, and 33.8 (5× CH_2), 38.0 (*C*H$_2$CO), 61.0 (C-2′), 64.5 (C-5′), 73.7 (C-3′), 81.8 (C-1′), 89.9 (C-4′), 127.1 (C-5), 151.6 (C-4), 166.6 (C-2), 173.3 and 177.5 ($CONH_2$ and NHCOC$_7H_{15}$); HRMS (ESI): *m*/*z* 386.1758 (M^++H), calcd for $C_{17}H_{28}N_3O_5S$: 386.1744.

*4.8.3. 2-(2-Decanamido-2-deoxy-β-D-ribofuranosyl)thiazole-4-carboxamide (**2c**).* Protected thiazole **11c** (0.09 g, 0.2 mmol) was converted into crude **2c** after treatment with saturated methanolic ammonia (3.4 mL) according to the above general procedure. Pure **2c** (0.05 g, 81%) was isolated by preparative TLC (9:1, 4:1 EtOAc/^{i}PrOH, two successive developments). An analytical sample **2c** was obtained after recrystallization from a mixture of EtOAc/^{i}Pr$_2$O as colourless crystals, mp 138 °C; $[\alpha]_D^{20}$ −15.7 (*c* 0.8, MeOH); IR (KBr): ν_{max} 3321, 1654, 1541; ^{1}H NMR (250 MHz, methanol-d_4): δ 0.89 (t, 3H, *J*=6.8 Hz, C*H*$_3$CH$_2$), 1.26 (m, 12H, 6× CH_2), 1.56 (m, 2H, CH_2), 2.25 (t, 2H, *J*=7.4 Hz, C_8H_{17}C*H*$_2$CO), 3.73 (d, 2H, $J_{4',5'}$=4.5 Hz, 2× H-5′), 4.11 (m, 1H, H-4′), 4.23 (dd, 1H, $J_{2',3'}$=5.4, $J_{3',4'}$=2.6 Hz, H-3′), 4.51 (dd, 1H, $J_{1',2'}$=8.5, $J_{2',3'}$=5.5 Hz, H-2′), 5.09 (d, 1H, $J_{1',2'}$=8.6 Hz, H-1′), 8.21 (s, 1H, H-5); ^{13}C NMR (62.9 MHz, methanol-d_4): δ 15.0 (*C*H$_3$CH$_2$), 24.3, 27.5, 30.8, 30.9, 31.0, 31.1 and 33.6 (7× CH_2), 37.5 (CH_2CO_2), 60.6 (C-2′), 64.0 (C-5′), 73.2 (C-3′), 81.2 (C-1′), 89.3 (C-4′), 126.6 (C-5), 151.0 (C-4), 166.0 (C-2), 172.9 and 177.1 ($CONH_2$ and NHCOC$_9H_{19}$); LRMS (CI): *m*/*z* 414 (M^++H). Anal. Calcd for $C_{19}H_{31}N_3O_5S$: C, 55.18; H, 7.56; N, 10.16; S, 7.75. Found: C, 55.22; H, 7.53; N, 10.02; S, 7.83.

*4.8.4. 2-(2-Deoxy-2-dodecanamido-β-D-ribofuranosyl)thiazole-4-carboxamide (**2d**).* Protected thiazole **11d** (0.06 g, 0.1 mmol) was converted into crude **2d** after treatment with saturated methanolic ammonia (3 mL) according to the above general procedure. Pure **2d** (0.04 g, 80%) was isolated by silica gel column chromatography (9:1 EtOAc/^{i}PrOH) as a white solid. Recrystallization from a mixture of MeOH/^{i}Pr$_2$O gave an analytical sample **2d** as colourless crystals, mp 133.5–134.5 °C; $[\alpha]_D^{20}$ −17.0 (*c* 0.9, MeOH); IR (KBr): ν_{max} 3396, 1655, 1541, 1288; ^{1}H NMR (250 MHz, methanol-d_4): δ 0.87 (t, 3H, *J*=7.0 Hz, C*H*$_3$CH$_2$), 1.18–1.62 (m, 18H, 9× CH_2), 2.25 (t, 2H, *J*=7.3 Hz, C*H*$_2$CO), 3.74 (d, 2H, $J_{4',5'}$=4.5 Hz, 2× H-5′), 4.12 (m, 1H, H-4′), 4.26 (dd, 1H, $J_{2',3'}$=5.1, $J_{3',4'}$=2.2 Hz, H-3′), 4.52 (dd, 1H, $J_{1',2'}$=8.7, $J_{2',3'}$=5.1 Hz, H-2′), 5.09 (d, 1H, $J_{1',2'}$=8.7 Hz, H-1′), 8.21 (s, 1H, H-5); NOE contact: H-5 and H-5′; ^{13}C NMR (62.9 MHz, methanol-d_4): δ 14.4 (*C*H$_3$CH$_2$), 23.6, 26.9, 30.1, 30.3, 30.5, 30.6 and 32.9 (9× CH_2),

36.9 (CH_2CO), 59.9 (C-2′), 63.4 (C-5′), 72.6 (C-3′), 80.7 (C-1′), 88.7 (C-4′), 126.3 (C-5), 150.4 (C-4), 165.6 (C-2), 172.2 and 176.7 ($NHCOC_{11}H_{23}$ and $CONH_2$); LRMS (CI): m/z 442 (M^++H). Anal. Calcd for $C_{21}H_{35}N_3O_5S$: C, 57.12; H, 7.99; N, 9.52; S, 7.26. Found: C, 57.53; H, 7.99; N, 9.24; S, 7.21.

4.9. Biological materials

Rhodamine B, RPMI 1640 medium, foetal calf serum, 3-(4,5-dimethylthiazol)-2,5-diphenyltetrazolium bromide, propidium iodide and RNase A, were purchased from Sigma (St. Louis, MO, USA). Penicillin and streptomycin were purchased from ICN Galenika (Belgrade, Serbia). Annexin V–FLUOS apoptosis detection kit was purchased from BD Biosciences Pharmingen (Belgium). Proteins were detected by Western blotting using the following monoclonal: the antibodies against human Bcl-2 and Caspase-3 were obtained from R&D Systems (Minneapolis, MN). anti-Poly (ADP-ribose) polymerase (PARP) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Enhanced chemiluminescence (ECL Plus) kit and Hyperfilm were purchased from Amersham Biosciences (Arlington Heights, IL). All other chemicals used in the experiments were commercial products of reagent grade. Stock solution (10 mM) was prepared in DMSO and diluted to various concentrations with serum-free culture medium.

4.10. Cell lines

Human chronic myelogenous leukaemia (K562), promyelocytic leukaemia (HL-60) and Burkitt's lymphoma (Raji) were grown in RPMI 1640 while colon adenocarcinoma (HT-29), breast adenocarcinoma (MCF-7), cervix carcinoma (HeLa) malignant cells, and normal foetal lung fibroblasts (MRC-5) were grown in DMEM medium. Both media were supplemented with 10% of foetal calf serum (FTS, NIVNS) and antibiotics (100 IU/mL of penicillin and 100 μg/mg of streptomycin). Cell lines were cultured in flasks (Costar, 25 mL) at 37 °C in the atmosphere of 100% humidity and 5% of CO_2 (Heraeus). Exponentially growing viable cells were used throughout the assays.

4.11. Cells treatment

The cells were seeded in six-well plates at a concentration of 5×10^5 cells/well. Cells were treated for 72 h with tiazofurin (**1**) and TAs (**2a**–**d**) at their IC_{50}/72 h concentrations. Untreated cells were used as control. Viable cells of treated and control samples were used for apoptosis detection and Western blot analysis. Viability was determined using trypan blue dye-exclusion assay.

4.12. MTT assay

Cells were harvested, counted by trypan blue and plated into 96-well microtitar plates (Costar) at optimal seeding density of 5×10^3 cells per well to assure logarithmic growth rate throughout the assay period. Viable cells were placed in a volume of 90 μL per well, and preincubated in complete medium at 37 °C for 24 h to allow cell stabilization prior to the addition of substances. Tested substances, at 10-fold the required final concentration, were added (10 μL/well) to all wells except to the control ones and microplates were incubated for 72 h. The wells containing cells without tested substances were used as control. 3 h before the end of incubation period MTT solution (10 μL) was added to all wells. MTT was dissolved in medium at 5 mg/mL and filtered to sterilize and remove a small amount of insoluble residue present in some batches of MTT. Acidified 2-propanol (100 μL of 0.04 M HCl in 2-propanol) was added to all wells and mixed thoroughly to dissolve the dark blue crystals of formazan. After a few minutes at room temperature, to ensure that all crystals were dissolved, the plates were read on a spectrophotometer plate reader (Multiscan MCC340, Labsystems) at 540 and 690 nm. The wells without cells containing complete medium and MTT acted as blank.

4.13. Flow cytometry

Apoptosis of K562 cells was evaluated with an Annexin V-FITC detection kit. Cells from each sample were collected (800 rpm/5 min, Megafuge 1.0 R, Heraeus, Thermo Fisher Scientific) and pellet was re-suspended in 1 mL of phosphate buffer (PBS, pH 7.2). K562 cells were washed twice with cold PBS and then re-suspended in binding buffer to reach the concentration of 1×10^6 cells/mL. The cell suspension (100 μL) was transferred to 5 mL culture tubes and mixed with Annexin V (5 μL) and propidium iodide (5 μL). The cells were gently vortexed and incubated for 15 min at 25 °C. After incubation, 400 μL of binding buffer was added to each tube and suspension was analyzed after 1 h on FACS Calibur E440 (Becton Dickinson) flow cytometer. Results were presented as percent of Annexin V positive gated cells. Percentage of specific apoptosis was calculated according to Bender et al.[20]

4.14. Western blot

For the Western blot, 50 μg of proteins per sample were separated by electrophoresis and electro-transferred to a PVDF membrane Hybond-P and then blotted with primary antibodies (Bcl-2, Caspase-3 and PARP). Proteins were detected by an enhanced chemiluminescence (ECL Plus) kit that includes peroxidase-labelled donkey anti-rabbit and sheep anti-mouse secondary antibodies. Blots were developed with an ECL Plus detection system and recorded on the Amersham Hyperfilm.

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Supplementary data

Additional details of experimental procedures, FACS analysis of apoptotic K562 cells, copies of ^1H, and ^{13}C NMR spectra of selected key compounds. Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tet.2011.06.090.

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