Bioorganic Chemistry 58 (2015) 18–25

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Synthesis and biological evaluation of 5′-glycyl derivatives of uridine as inhibitors of 1,4-β-galactosyltransferase

Jadwiga Paszkowska [a], Katarzyna Kral [a], Tadeusz Bieg [a], Karolina Żaba [a], Katarzyna Węgrzyk [a], Natalia Jaśkowiak [a], Antonio Molinaro [b], Alba Silipo [b], Ilona Wandzik [a,*]

[a] Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, Silesian University of Technology, B. Krzywoustego 4, 44-100 Gliwice, Poland
[b] Department of Chemical Sciences, University of Naples Federico II, Via Cintia 4, 80126 Naples, Italy

ARTICLE INFO

Article history:
Received 15 August 2014
Available online 10 November 2014

Keywords:
Uridine
Peptidyl nucleoside
Glycosyltransferase inhibitors

ABSTRACT

New 5′-glycyl derivatives of uridine containing fragments of varying lipophilicity were synthesized as analogues of natural peptidyl antibiotics. One of the studied compounds, 5′-O-(N-succinylglycyl)-2′,3′-O-isopropylideneuridine (**A4**), showed moderate inhibition against 1,4-β-galactosyltransferase. However, additional studies showed that the observed inhibitory effect was due to binding to bovine serum albumin, which was used in assays as a stabilizer.

1. Introduction

Among numerous types of nucleoside antibiotics found in nature, peptidyl pyrimidine-containing antibiotics have been shown to exhibit activity against a broad variety of microbes [1,2]. There is a large group of uridyl peptide antibiotics, like mureidomycins, pacidamycins, liposidomycins, muramycins, caprazamycins and capuramycin, targeting the bacterial enzyme translocase, MraY, which is involved in the lipid-linked cycle of peptidoglycan biosynthesis [3,4]. Tunicamycin exhibits antimicrobial activity, and is a well-known inhibitor of replication in yeast, fungi, protozoa and enveloped virus [5]. Unfortunately, tunicamycin is also a potent inhibitor of mammalian phospho-GlcNAc transferase, which precludes its use as a candidate for drug development [6]. The other examples of peptidyl antibiotics are polyoxins and nikkomycins, which are competitive inhibitors of chitin synthases that demonstrate antifungal, insecticidal and acarcidal activities [7].

Despite favorable activity *in vitro*, these inhibitors are not good drug candidates, because they are charged, and do not readily transport across the cell membrane. The design of cell-permeable inhibitors of protein glycosylation is therefore of great interest. The common motif of the aforementioned natural products is uridine nucleoside. Because glycosyltransferases (GTs) are usually targets of these natural antibiotics, it can be hypothesized that the uridine component is essential for recognition and proper binding at the active site of the enzymes, similar to the recognition and proper binding of natural substrates like uridinediphospho-sugars (UDP-sugar).

Several unnatural uridine conjugates were designed and synthesized as analogues of natural active compounds or substrate analogues of GTs [8–11], but only a few of conjugates were strong inhibitors of GTs. Among the latter, derivatives featuring modification of uridine at the C-5 position inhibit galactosyl transfer by several different galactosyltransferases. For example, incorporation of a 5-formylthien-2-yl group onto the uracil of UDP-Gal yielded an efficacious micromolar inhibitor of GTB and α-1,3- and α-1,4-GalT [12,13]. Similarly, introducing a trifluoromethyl group at the C-5 position in the uracil part of UDP-Glc and UDP-Gal led to discovery of inhibitors of β-1,4-GalT and LgtC [14]. The other examples of well-studied β-1,4-GalT inhibitors are compounds with modification of the carbohydrate part of UDP-Gal. The strongest inhibitors in this group contain a naphthalene substituent linked with 6-O-galactose (K_i 1.86 μM) [15] or a carbacyclic analogue of galactose [16]. An example of a pyrophosphate analogue as a micromolar inhibitor is C-glycosyl ethyl phosphonophosphate [17]. Some potent inhibitors were designed as bisubstrate analogues. Interesting examples include the first tricomponent inhibitor with robust inhibition of β-1,4-GalT [18].

Several uridine derivatives were synthesized and tested against chitin synthase, because this fungal glycosyltransferase is absent in mammalian cells and is, therefore, a valuable therapeutic target [19]. The 1,2,3-triazoyl-linked uridine derivatives, synthesized by click reaction approach, were identified as inhibitors of chitin synthase activities, with some having inhibition strength comparable with that of nikkomycin [20]. Other strong inhibitors include

* Corresponding author.
E-mail address: ilona.wandzik@polsl.pl (I. Wandzik).

http://dx.doi.org/10.1016/j.bioorg.2014.11.001
0045-2068/

nikkomycin Z analogues that were synthesized by condensation of uridinyl aldehyde with various carboxylic acids and isocyanides in a combinatorial synthesis approach [21] or by introducing the aromatic groups at the terminal amino acid starting from uracil polyoxin C [22].

In this report, we describe the synthesis and biological evaluation of a series of 5′-O-glycyl-and 5′-N-glycyl uridine derivatives (Fig. 1). These compounds were tested against commercially available β-1,4-GalT, which plays a role in the biosynthesis of blood group antigens and is involved in some pathological conditions, such as arthritis and cancer.

2. Results and discussion

2.1. Chemistry

Amino acids can act as ligands in metal complexes, and incorporation of amino acid residues into nucleosides have been reported. There are many studies describing the synthesis and biological evaluation of uridine-containing analogues [23–28]. With the goal of broadening the library of uridine derivatives that could be active against glycosyltransferases, we have synthesized new 5′-glycyl derivatives of uridine, in which the glycine is attached via an ester or an amide linkage to the 5′-position of uridine (series A and B respectively, Fig. 1).

Relevant lipophilicity is a crucial parameter for transport across the cell membrane, and some highly polar compounds cannot be used in therapeutic applications because they cannot cross the cell membrane. We have designed compounds that are significantly less polar than peptidyl antibiotics, according to calculated XLogP3 parameter [29], [data not shown]. This algorithm has shown considerable agreement with experimentally determined log*P* values in the library of uridine derivatives [30]. Hydrophobic fragments, like benzyloxycarbonyl or benzoyl groups, were incorporated into target structures to ensure higher lipophilicity.

The proposed amino acid derivatives of uridine are divided into two series according to (1) the type of linkage between uridine and glycine and (2) the compounds in series A, which contain an isopropylidene group as a protecting group of C-2′ and C-3′ hydroxyls of a ribose unit, and compounds in series B, which contain a totally deprotected uridine unit. Although compounds **A1**–**A4** can be easily synthesized from 2′,3′-isopropylideneuridine (**3**) by coupling with a relevant glycine derivative, these compounds are acid sensitive, limiting their biological applicability. Moreover, deprotection of the acetonide group can lead to rupture of the ester bond. We, therefore, synthesized amide analogs **B1**–**B4**, which are more resistant to hydrolysis. The synthetic strategy relies on the coupling of relevant uridine substrates **3** [31] or **9** [27] with commercially available glycine derivatives **1** and **2** (Schemes 1 and 2, respectively). This approach has been demonstrated in works of Alargov et al. [25], who used dicyclohexylcarbodiimide (DCC) as the condensing agent. We used diisopropylcarbodiimide (DIC) as the condensing agent because, unlike DCC, DIC is believed to be non-allergenic. All reactions were carried out in the presence of 4-dimethylaminopyridine (DMAP) as a catalyst, affording products **A1**, **A2** and **B1**, **B2** in excellent yields. No side reactions (e.g., acylation of uracil or cyclization caused by the Michael addition of a 5′-amino group to the double bond of uracil) were observed. To obtain compounds **A3**, **A4** and **B3**, **B4**, intermediate substrates **6** and **14** were synthesized by removal of N-benzyloxycarbonyl groups of glycine in **A1** and **B1**, respectively, which was accomplished by catalytic hydrogenation with cyclohexene as the hydrogen donor and $Pd(OH)_2/C$ as the catalyst. The next step was coupling of compounds **6** and **14** with benzoyl chloride or succinyl anhydride in the presence of base (NEt_3 or pyridine) without any coupling agent. Reactions proceeded smoothly to give new compounds containing the ester (**A3**, **A4**) or amide (**B3**, **B4**) linkages. The latter were subjected to deprotection of ribose 2′ and 3′ hydroxyls. Attempts to remove the acetonide group in the ester series in the presence of acetic acid, trifluoroacetic acid, Amberlyst 15 or AcCl failed due to the acid hydrolysis of the ester linkage. Removal of tert-butyldimethylsilyl (TBDMS) groups from derivatives **B2** and **B4** was achieved by treatment with tetrabutylammonium fluoride in THF as a solvent, whereas deprotection of N-acetyl derivatives **B1** and **B3** was accomplished by treatment with AcCl in methanol.

All new compounds from series A and B were purified by column chromatography, and their structures were elucidated with the aid of ^1H and ^{13}C NMR spectroscopy data (including two-dimensional DQCOSY, HMQC and HMBC experiments and simulation analysis) and mass spectrometry analysis (for details see Section 4 and Supplementary materials).

2.2. Biological evaluation

The important part of this work was the biological evaluation of the synthesized compounds from series A and B. Bovine milk β-1,4-GalT I was chosen as the target enzyme. It is one of the most well

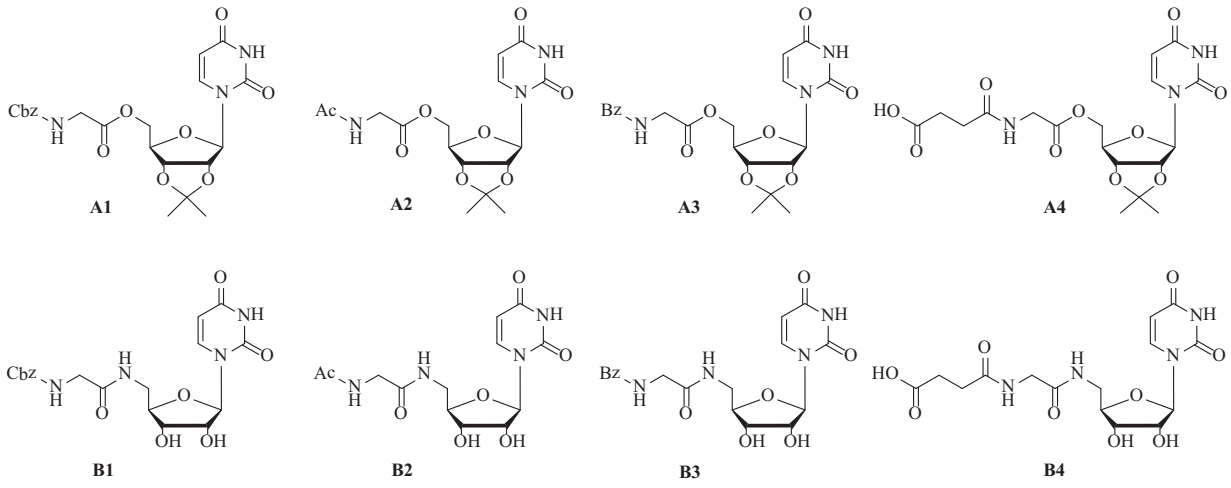
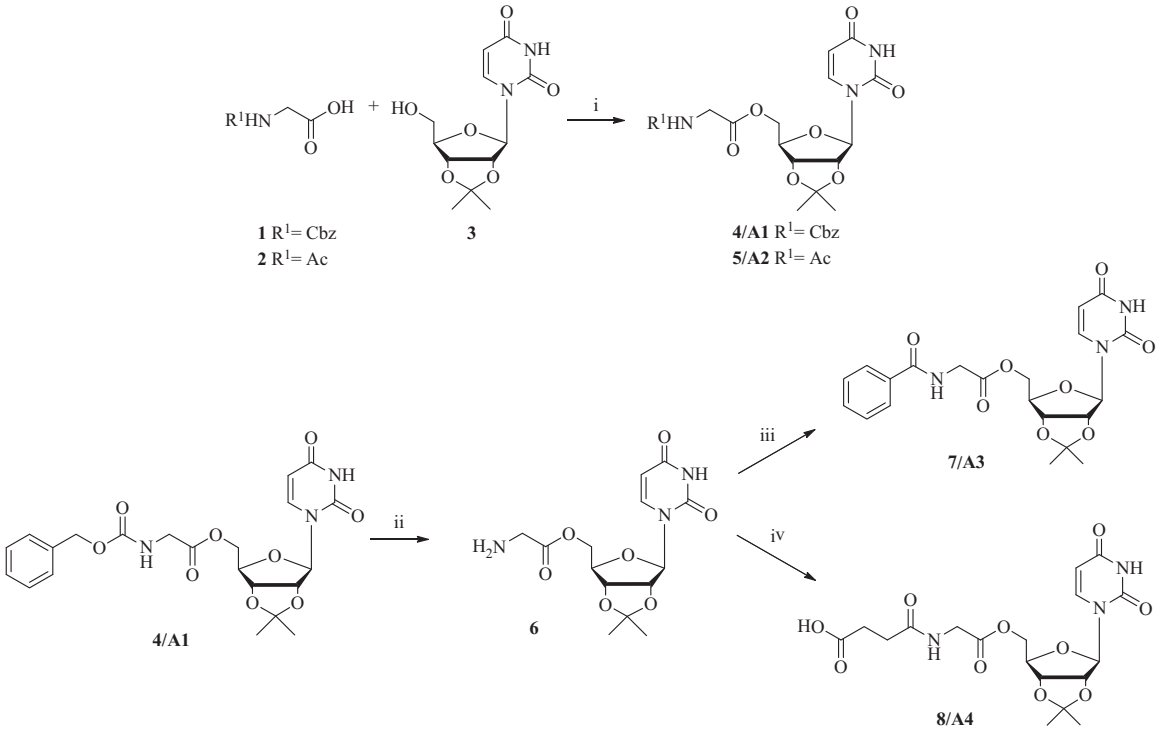
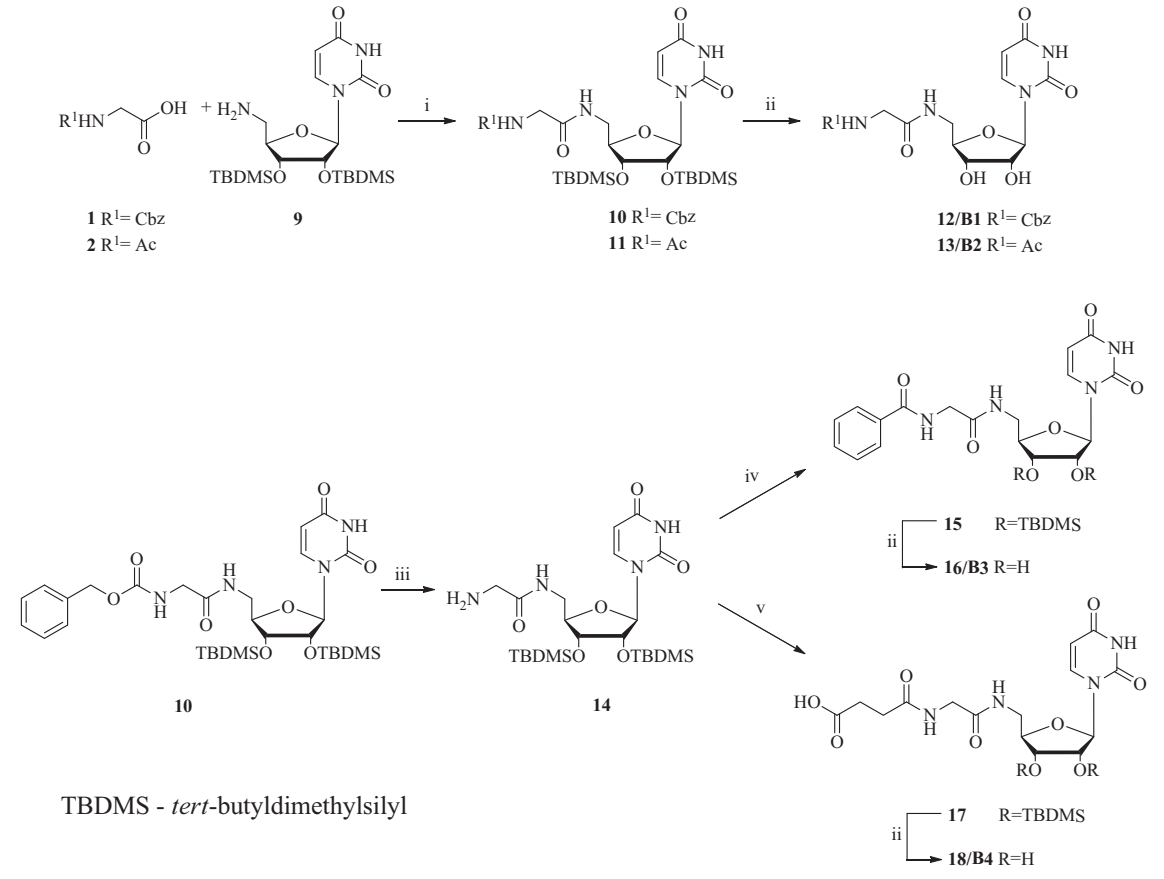


Fig. 1. C-5′-glycyl derivatives of uridine: series A and B.



Scheme 1. Synthesis of 5′-O-glycyl derivatives of uridine. Reagents and conditions: (i) DIC (1.5 equiv.), DMAP (0.1 equiv.), DMF, 0 °C → rt, 24 h; (ii) 20% $Pd(OH)_2/C$, cyclohexene, MeOH, reflux, 20–30 min, (iii) BzCl (1.0 equiv.), pyridine (10 equiv.), DMF, rt, 1 h; (iv) succinyl anhydride (1.0 equiv.), THF, rt, 4 h.



Scheme 2. Synthesis of 5′-N-glycyl derivatives of uridine. Reagents and conditions: (i) DIC (1.5 equiv.), DMAP (0.1 equiv.), CH_2Cl_2, 0 °C → rt, 15 min – 3 h; (ii) AcCl (2.0 equiv.), MeOH, rt, 5–24 h; or TBAF (1 M solution in THF, 3.0 equiv.), THF, rt, 1.5–3.0 h; (iii) 20% $Pd(OH)_2/C$, cyclohexene, MeOH, reflux, 4 h; (iv) BzCl (1.1 equiv.), Et_3N (1.1 equiv.), CH_2Cl_2, 0 °C, 10 min; (v) succinyl anhydride (1.0 equiv.), Et_3N (1.0 equiv.), THF, rt, 30 min.

Table 1
Inhibition of serum albumins denaturation by 5′-glycyl derivatives of uridine at 1.8 mM and aspirin at 1.1 mM at 70 °C in 30 min.

Protein	Buffer	Inhibition of denaturation (%)			
		A1	**A4**	**B1**	Aspirin
BSA	50 mM Hepes (pH 5.3)	35	68	42	76
	100 mM Phosphate (pH 5.3)	61	36	25	<20
HSA	50 mM Hepes (pH 5.3)	57	69	57	>97
	100 mM Phosphate (pH 5.3)	86	78	84	83

studied enzymes among glycosyltransferases with commercial availability. We used the fluorescence competition assay with β-1,4-GalT I, using the fluorescent acceptor β-GlcNAc-O-$(CH_2)_6$-dansyl as a substrate [17]. Among the tested compounds, activity was observed only for derivative **A4** (IC_{50} = 2.0 mM). However, additional experiments showed that the observed inhibitory effect was not due to direct interaction of compound **A4** with β-1,4-GalT I. It was observed that some compounds had an antidenaturation effect on bovine serum albumin (BSA), which was used in assays as a stabilizer. According to the procedure, when the reaction was completed, proteins were denatured in a boiling water bath. No denaturation was observed when compound **A4** was present in the reaction mixture. It was hypothesized that binding of some compounds to BSA disrupted stabilization of target β-1,4-GalT I. To confirm this theory, studies on the antidenaturation activity of the examined compounds from series A and B were carried out in the presence of BSA or human serum albumin (HSA) at varying pH at 70 °C. Samples of 0.2% albumins (control) and albumins with studied compounds were incubated for 30 min. Next, turbidity was measured spectrophotometrically at 660 nm, and percent of inhibition of protein denaturation was calculated [32,33]. Samples were incubated in various buffers in different pH. The strongest inhibition of denaturation was observed at pH 5.3. Three compounds, **A1**, **A4** and **B1**, showed significant antidenaturation activity against HSA and BSA in 1.8 mM concentration (Table 1). The stronger effect was observed when HSA was used, regardless of the buffer applied. The highest BSA antidenaturation activity was exhibited with compound **A4** in (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES) buffer. Antidenaturation activity of some fatty acids [34] and nonsteroidal anti-inflammatory drugs [32,33,35] was reported. For comparison, effect of known anti-inflammatory drug aspirin at 1.1 mM was included in our experiments as a positive control.

The higher antidenaturation effect of examined compounds towards HSA than to BSA suggests that binding specificities of these proteins are different. Serum albumins serve as carriers for molecules of low water solubility and possess many binding pockets. In addition, the distribution of BSA and HSA binding sites is not the same [36–38].

BSA is essential for predictive and repeatable activity of β-1,4-GalT I. We observed a twofold decrease of activity when enzymatic reactions were carried out without BSA. However, the use of BSA as a stabilizer in enzymatic assays should be treated with caution, because some compounds may bind to BSA, decreasing the activity of a specific enzyme and leading to false positive results.

To further examine the interaction, the binding of **A4** to BSA was investigated using STD-NMR spectroscopy [39]. STD-NMR has been previously proven to be extremely informative in the examination of protein-substrate interactions since it allows mapping of ligand epitopes in close contact with proteins [40]. A qualitative comparison of the ^{1}H NMR spectrum of **A4** and the STD spectrum (Fig. 2) using a saturation time of 2 s revealed changes in the relative areas of signals. A quantification of STD signals allowed to define the epitope mapping. Thus, beside the interacting proton signals from the lateral chain, the region comprising ribose and uracil were both in contact to the BSA and contributed to the overall binding.

The observed ability of some compounds to inhibit thermally induced protein denaturation is of interest, because denaturation of proteins is a well-documented cause of inflammation. Compounds that inhibit denaturation can be useful in studying

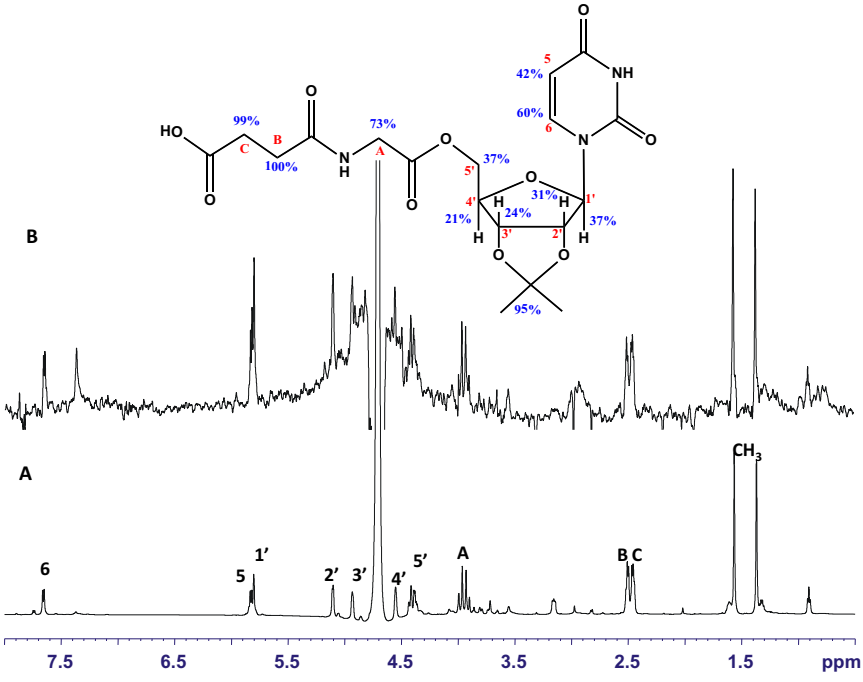


Fig. 2. NMR analysis of the binding of **A4** to BSA. (A) ^{1}H NMR spectrum of **A4** in the presence of BSA. Signals are indicated as reported in the figure. (B) STD spectrum. An on-resonance irradiation frequency at 0 ppm and a saturation time of 2 s was used, with a 1:75 BSA-ligand ratio.

anti-inflammation processes, similar to known nonsteroidal anti-inflammatory drugs [32,33].

3. Conclusions

Two series of compounds, 5′-O-glycyl and 5′-N-glycyl derivatives of uridine, were synthesized in simple coupling reactions of relevant uridine (**3** and **9**) and glycine (**1** and **2**) substrates followed by incorporation of lipophilic (benzoyl, benzyloxycarbonyl) or hydrophilic (succinyl) groups at the terminal nitrogen of glycine. Compounds were tested as β-1,4-GalT I inhibitors, but only compound **A4** exhibited moderate activity (IC_{50} = 2.0 mM). Accurate assays proved that with the conditions used, the inhibition effect was caused by binding of **A4** to BSA and subsequent lack of enzyme stabilization. Moreover it was observed that compounds **A1**, **A4** and **B1** exhibit a dose-dependent ability to inhibit thermally induced BSA and HSA denaturation. Binding of **A4** into BSA was additionally confirmed by STD NMR experiment, which showed that the highest saturation received lipophilic parts of the compound: ethylene and isopropylidene groups.

4. Experimental

4.1. General methods

Optical rotations were measured with a JASCO P-2000 polarimeter using a sodium lamp (589 nm) at room temperature. NMR spectra were recorded with a Varian spectrometer at a frequency of 600 MHz. Mass spectra were recorded with a WATERS LCT Premier XE system (high resolution mass spectrometer with TOF analyzer) using electrospray-ionization (ESI) technique. Reactions were monitored by TLC on precoated plates of silica gel 60 F254 (Merck) and visualized using UV light (254 nm). Crude products were purified using column chromatography performed on silica gel 60 (70–230 mesh, Fluka) or flash chromatograph (Isolera™, Biotage) equipped with UV detector (254 nm, flow rate 10–25 mL/min). Hexane/EtOAc or $CHCl_3$/MeOH were used as solvent systems. All evaporation were performed under diminished pressure at 50 °C. Reversed phase HPLC analyses were performed using JASCO LC 2000 apparatus equipped with a reverse column (Nucleosil 100 C18, 5 μm, 25 × 0.4 cm; mobile phase: H_2O/MeCN 73:27, flow rate 1 mL/min) with a fluorescence detector. Fluorescence of acceptor substrate and product was read at 385 nm excitation/540 nm emission.

Bovine milk β-1,4-galactosyltransferase I (β-1,4-GalT I, 1 U/mg), BSA (A7638), HSA (A1653) and other chemicals were purchased from Sigma–Aldrich Co. LLC. and were used without purification.

2′,3′-O-isopropylideneuridine (**3**) was prepared according to the published procedure [31]. 5′-amino-5′-deoxy-2′,3′-bis-O-(*tert*-butyldimethylsilyl)-uridine (**9**) was prepared in three step synthesis by direct conversion of unprotected uridine to 5′-azido-5′-deoxyuridine [41], followed by TBDMS protection and hydrogenation [27]. 5′-(N-(benzyloxycarbonyl)-glycyl)-2′,3′-O-isopropylideneuridine (**4**/**A1**) was prepared by coupling of N-benzyloxycarbonyl-glycine (**1**) with 2′,3′-O-isopropylideneuridine (**3**) according to procedure described by Algarov et al. [25] with slight modification: as coupling agent DIC was used instead of DCC.

4.2. Synthesis of 5′-O-glycyl uridine derivatives (*A2–A4*)

4.2.1. 5′-O-(N-acetyl-glycyl)-2′,3′-O-isopropylideneuridine (*5/A2*)

A cooled suspension of N-acetyl-glycine **2** (0.351 g, 3.0 mmol) and DIC (0.235 mL, 1.5 mmol) in DMF (4 mL) was stirred for 30 min at 0 °C. Then **3** (0.284 g, 1.0 mmol) and DMAP (0.012 g, 0.1 mmol) were added and the mixture was stirred at rt for 24 h. After completion of reaction the mixture was concentrated. Crude product was purified by flash chromatography ($CHCl_3$ → $CHCl_3$:MeOH 100:1 → 20:1) to obtain **5/A2** (0.374 g, 98%) as a white foam. $[\alpha]_D^{20} = 13.2$ ($CHCl_3$, c = 1.00); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.35 (s, 3H, C(C<u>H</u>$_3$)$_2$), 1.56 (s, 3H, C(C<u>H</u>$_3$)$_2$), 2.04 (s, 3H, CH_3), 4.04, 4.09 (q_{AB}d, 2H, J_{AB} = 18.3 Hz, J = 5.4 Hz, NH–C<u>H</u>$_2$–C(O)), 4.31 (ddd, 1H, J = 3.7, J = 4.3, J = 5.9 H-4′), 4.38 (dd, 1H, J = 5.9, J = 11.9 Hz, H-5′b), 4.45 (dd, 1H, J = 3.7, J = 11.9 Hz, H-5′a), 4.88 (dd, 1H, J = 4.3, J = 6.4 Hz, H-3′), 5.08 (dd, 1H, J = 1.8, J = 6.4 Hz, H-2′), 5.56 (d, 1H, J = 1.8 Hz, H-1′), 5.74 (dd, 1H, J = 1.7, J = 8.0 Hz, H-5), 6.42 (t, 1H, J = 5.4 Hz, C(O)N<u>H</u>CH$_2$), 7.27 (d, 1H, J = 8.0 Hz, H-6), 9.67 (s, 1H, NH_{ur}), ^{13}C NMR (150 MHz, $CDCl_3$): δ 22.82 (<u>C</u>H$_3$C(O)), 25.37 (C(<u>C</u>H$_3$)$_2$), 27.09 (C(<u>C</u>H$_3$)$_2$), 41.33 (NH–<u>C</u>H$_2$–C(O)), 64.46 (C-5′), 80.81 (C-3′), 84.26 (C-2′), 85.34 (C-4′), 95.57 (C-1′), 102.60 (C-5), 114.60 (<u>C</u>(CH$_3$)$_2$), 142.95 (C-6), 149.99 (C-2), 163.50 (C-4), 169.83 (C(O)), 170.57 (C(O)); ESI-LRMS: calcd for $C_{16}H_{21}N_3O_8Na$ ($[M+Na]^+$): m/z 406.1, found: 406.2.

4.2.2. 5′-O-(N-benzoyl-glycyl)-2′,3′-O-isopropylideneuridine (*7/A3*)

A cooled suspension of N-benzyloxycarbonyl-glycine **1** (0.627 g, 3 mmol) and DIC (0.235 mL, 1.5 mmol) in DMF (4 mL) was stirred for 30 min at 0 °C. Then **3** (0.284 g, 1 mmol) and DMAP (0.012 g, 0.1 mmol) were added and the mixture was stirred at rt for 24 h. After completion of reaction the mixture was diluted with AcOEt (20 mL), washed with 5% solution of $NaHCO_3$ (15 mL) and water (2 × 15 mL). Organic layer was dried ($MgSO_4$) and concentrated. Crude product was purified by flash chromatography ($CHCl_3$ → $CHCl_3$:MeOH 24:1) to obtain **4/A1** (0.461 g, 97%) as a white foam. In the next step **4** (0.461 g, 0.97 mmol) was dissolved in MeOH (11 mL). Then 20% Pd(OH)$_2$/C (0.092 g) and cyclohexene (2.2 mL) were added and the mixture was heated for 30 min. After removal of the catalyst by filtration the reaction mixture was concentrated to give **6**. Crude product **6** was dissolved in DMF (10 mL) and BzCl (0.113 mL, 0.97 mmol) and pyridine (0.783 mL, 9.7 mmol) were added. The reaction mixture was stirred for 1 h at rt, diluted with CH_2Cl_2 (50 mL) and washed with H_2O (3 × 50 mL). Organic layer was dried ($MgSO_4$) and concentrated. Crude product was purified by flash chromatography (hexane:AcOEt 1:9) to obtain **7/A3** (0.145 g, 32% overall yield) as a white foam; $[\alpha]_D^{20} = 1.00$ ($CHCl_3$, c = 1.00); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.32 (s, 3H, CH_3), 1.55 (s, 3H, CH_3), 4.24–4.28 (q_{AB}d, 2H, J_{AB} = 18.2, J = 5.3 Hz, NH–C<u>H</u>$_2$–C(O)), 4.32 (ddd, 1H, J = 3.8, J = 4.3, J = 5.7 Hz, H-4′), 4.39 (dd, 1H, J = 5.7, J = 11.9 Hz, H-5′b), 4.49 (dd, 1H, J = 3.8, J = 11.9 Hz, H-5′a), 4.87 (dd, 1H, J = 4.3, J = 6.5 Hz, H-3′), 5.03 (dd, 1H, J = 1.9, J = 6.5 Hz, H-2′), 5.56 (d, 1H, J = 1.9 Hz, H-1′), 5.70 (d, 1H, J = 8.1 Hz, H-5), 7.03 (t, 1H, J = 5.3 Hz, <u>N</u>H–CH$_2$), 7.26 (d, 1H, J = 8.1 Hz, H-6), 7.42 (m, 2H, $H_{ar\text{-}m}$), 7.50 (tt, 1H, J = 1.3, J = 7.4 Hz, 1H, $H_{ar\text{-}p}$), 7.82 (dd, 2H, J = 1.3, J = 8.3 Hz, $H_{ar\text{-}o}$), 9.56 (s, 1H, NH_{ur}); ^{13}C NMR (150 MHz, $CDCl_3$): δ 25.20 (CH_3), 27.09 (CH_3), 41.82 (NH–<u>C</u>H$_2$–C(O)), 64.47 (C-5′), 80.78 (C-3′), 84.23 (C-2′), 85.24 (C-4′), 95.35 (C-1′), 102.65 (C-5), 114.66 (<u>C</u>(CH$_3$)$_2$), 127.14 (2 $C_{ar\text{-}o}$), 128.60 ($C_{ar\text{-}p}$), 131.88 (2 $C_{ar\text{-}m}$), 133.52 ($C_{ar\text{-}IV}$), 142.82 (C-6), 150.03 (C-2), 163.45 (C-4), 167.68, 169.82 (2 C(O)); ESI-HRMS: Calcd for $C_{21}H_{24}N_3O_8$ ($[M+H]^+$): m/z 446.1563, found: 446.1562.

4.2.3. 5′-O-(N-succinyl-glycyl)-2′,3′-O-isopropylideneuridine (*8/A4*)

Crude product **6** (synthesised as described in Section 4.2.2) was dissolved in THF (18 mL), succinyl anhydride (0.097 g, 0.97 mmol) was added and the mixture was stirred for 4 h at rt. Then the mixture was concentrated and crude product was purified by flash chromatography ($CHCl_3$:MeOH 100:1 → 25:1) to obtain **8/A4** (0.171 g, 38% overall yield) as yellowish foam; $[\alpha]_D^{20} = 2.13$ (MeOH, c = 1.00); ^{1}H NMR (600 MHz, CD_3OD): δ 1.35 (s, 3H, CH_3), 1.54 (s, 3H, CH_3), 2.53, 2.59 (q_{AB}, 2H, J_{AB} = 12.5 Hz, CH_2–C<u>H</u>$_2$), 2.53, 2.59 (q_{AB}, 2H, J_{AB} = 8.7 Hz, C<u>H</u>$_2$–CH_2), 3.95, 3.97 (q_{AB}, 2H, J_{AB} = 17.6 Hz, NH–C<u>H</u>$_2$–C(O)), 4.29–4.39 (m, 3H, H-4′, H-5′a, H-5′b), 4.86

(m, 1H, H-3′), 5.05 (dd, 1H, J = 2.2, J = 6.4 Hz, H-2′), 5.71 (d, 1H, J = 8.0 Hz, H-5), 5.77 (d, 1H, J = 2.2 Hz, H-1′), 7.62 (d, 1H, J = 8.0 Hz, H-6); ^{13}C NMR (150 MHz, CD_3OD): δ 24.12 (CH_3), 26.06 (CH_3), 29.04, 30.00 ($\underline{C}H_2$–$\underline{C}H_2$), 40.70 (NH–$\underline{C}H_2$–C(O)), 64.20 (C-5′), 80.97 (C-3′), 84.20 (C-2′), 84.69 (C-4′), 93.90 (C-1′), 101.48 (C-5), 114.06 ($\underline{C}(CH_3)_2$), 143.07 (C-6), 150.50 (C-2), 164.76 (C-4), 169.63, 173.77 (2 C(O)), 175.16 (COOH); ESI-HRMS: Calcd for $C_{18}H_{22}N_3O_{10}$ ([M−H]$^-$): m/z 440.1305, found: 440.1296.

4.3. Synthesis of 5′-N-glycyl uridine derivatives (**B1**–**B4**)

4.3.1. 5′-(N-benzyloxycarbonyl-glycyl)-5′-amino-5′-deoxyuridine (**12/B1**)

A cooled suspension of N-benzyloxycarbonyl-glycine (**1**) (0.627 g, 3.0 mmol) and DIC (0.235 mL, 1.5 mmol) in CH_2Cl_2 (14 mL) was stirred for 30 min at 0 °C. Then **9** (0.471 g, 1.0 mmol) and DMAP (0.012 g, 0.1 mmol) were added and the mixture was stirred at rt for 15 min. After completion of reaction the mixture was diluted with CH_2Cl_2 (70 mL), washed with 5% solution of $NaHCO_3$ (80 mL) and water (2 × 80 mL). Organic layer was dried ($MgSO_4$) and concentrated. Crude product was purified by flash chromatography ($CHCl_3$:MeOH 100:1 → 96:1) to obtain **10** (0.635 g, 96%) as a white foam. For desilylation, derivative **10** (0.635 g, 0.96 mmol) was dissolved in MeOH (13 mL) and AcCl (0.137 mL, 1.92 mmol) was added. The reaction mixture was stirred at rt for 24 h. After completion of reaction the mixture was neutralised by Amberlyst 21 and concentrated. Crude product was purified by column chromatography ($CHCl_3$:MeOH 20:1 → 13:1) to obtain **12/B1** (0.288 g, 69%) as a white foam; $[\alpha]_D^{20} = -9.33$ (MeOH, c = 1.00); ^{1}H NMR (600 MHz, CD_3OD) δ 3.52 (dd, 1H, J = 4.3, J = 14.2 Hz, H-5′a), 3.56 (dd, 1H, J = 5.6, J = 14.2 Hz, H-5′b), 3.77, 3.78 (q_{AB}, 2H, J_{AB} = 16.9 Hz, NH–C$\underline{H}_2$–C(O)), 3.98 (ddd, 1H, J = 4.3, J = 5.4, J = 5.6 Hz, H-4′), 4.01 (dd,1H, J = 5.4, J = 5.6 Hz, H-3′), 4.21 (dd, 1H, J = 4.3, J = 5.6 Hz, H-2′), 5.09, 5.10 (q_{AB}, 2H, J_{AB} = 12.4 Hz, C$\underline{H}_2$-Ph), 5.71 (d, 1H, J = 8.1 Hz, H-5), 5.75 (d, 1H, J = 4.3 Hz, H-1′), 7.26–7.36 (m, 5H, H_{ar}), 7.65 (d, 1H, J = 8.1 Hz, H-6); ^{13}C NMR (150 MHz, CD_3OD) δ 41.82 (C-5′), 45.01 (NH–$\underline{C}H_2$–CO), 67.87 ($\underline{C}H_2$-Ph), 83.92, 74.61, 72.10 (C-2′, C-3′, C-4′), 92.24 (C-1′), 103.01 (C-5), 138.05 ($C_{ar\text{-}IV}$), 128.89–129.47 (C_{ar}), 143.36 (C-6), 152.30 (C-2), 159.06 (C(O)), 166.05 (C-4), 172.70 (C(O)); ESI-HRMS: Calcd for $C_{19}H_{22}N_4O_8Na$ ([M+Na]$^+$): m/z 457.1335, found: 457.1326.

4.3.2. 5′-(N-acetyl-glycyl)-5′-amino-5′-deoxyuridine (**13/B2**)

A cooled suspension of N-acetyl-glycine (**2**) (0.351 g, 3.0 mmol) and DIC (0.235 mL, 1.5 mmol) in CH_2Cl_2 (14 mL) was stirred for 30 min at 0 °C. Then **9** (0.471 g, 1.0 mmol) and DMAP (0.012 g, 0.1 mmol) were added and the mixture was stirred at rt for 3 h. After completion of reaction the mixture was diluted with CH_2Cl_2 (80 mL), washed with 5% solution of $NaHCO_3$ (80 mL) and water (2 × 80 mL). Organic layer was dried ($MgSO_4$) and concentrated. Crude product was purified by flash chromatography ($CHCl_3$:MeOH 100:1 → 90:1) to obtain **11** (0.520 g, 91%) as a white foam. For desilylation, derivative **11** (0.520 g, 0.91 mmol) was dissolved in THF (27 mL) and TBAF (2.73 mL, 1 M solution in THF, 3 equiv.) was added. The reaction mixture was stirred at rt for 1.5 h. After completion of reaction Amberlyst 15 was added and the mixture was left to stir for 3 h at rt. Then the mixture was filtered and concentrated. Crude product was purified by column chromatography ($CHCl_3$:MeOH 5:1) to obtain **13/B2** (0.104 g, 30% overall yield); $[\alpha]_D^{20} = 3.53$ (DMSO, c = 1.00); ^{1}H NMR (600 MHz, DMSO): δ 1.85 (s, 3H, C$\underline{H}_3$), 3.28 (ddd, 1H, J = 5.9, J = 6.3, J = 14.0 Hz, H-5′b), 3.39 (ddd, 1H, J = 4.5, J = 5.9, J = 14.0 Hz, H-5′a), 3.66, 3.65 (q_{AB}d, 2H, J_{AB} = 16.7, J = 5.9 Hz, NH–C$\underline{H}_2$–C(O)), 3.80 (ddd, 1H, J = 4.5, J = 4.5, J = 6.3 Hz, H-4′), 3.87 (dd, 1H, J = 4.5, J = 5.5 Hz, H-3′), 4.04 (dd, 1H, J = 5.5, J = 5.6 Hz, H-2′), 5.59 (d, 1H, J = 8.0 Hz, H-5), 5.70 (d, 1H, J = 5.6 Hz, H-1′), 7.61 (d, 1H, J = 8.0 Hz, H-6), 8.08 (t, 1H, J = 5.9 Hz, CH_3–C(O)–N$\underline{H}$–CH_2), 8.16 (t, 1H, J = 5.9 Hz, CH_2–C(O)–N$\underline{H}$–CH_2); ^{13}C NMR (150 MHz, DMSO): δ 22.96 (CH_3), 41.20 (C-5′), 42.54 (NH–$\underline{C}H_2$–C(O)), 71.17 (C-3′), 72.96 (C-2′), 82.78 (C-4′), 88.99 (C-1′), 102.49 (C-5), 141.55 (C-6), 151.98 (C-2), 164.70 (C-4), 169.79 (CH_2–$\underline{C}$(O)–NH), 170.04 (CH_3–$\underline{C}$(O)–NH); ESI-HRMS: Calcd for $C_{13}H_{18}N_4O_7Na$ ([M+Na]$^+$): m/z 365.1073, found: 365.1079.

4.3.3. 5′-(N-benzoyl-glycyl)-5′-amino-5′-deoxyuridine (**16/B3**)

Compound **10** (0.635 g, 0.96 mmol, synthesised as described in Section 4.3.1) was dissolved in MeOH (45 mL). Then 20% $Pd(OH)_2$/C (0.092 g) and cyclohexene (1.55 mL) were added and the mixture was heated for 4 h. After removal of the catalyst by filtration the reaction mixture was concentrated. Crude product was purified by flash chromatography ($CHCl_3$:MeOH 100:1 → 75:1) to obtain **14** (0.400 g, 79%). In the next step **14** (0.400 g, 0.76 mmol) was dissolved in CH_2Cl_2 (23 mL) and the solution was cooled in ice bath to 0 °C. Then BzCl (0.097 mL, 0.84 mmol) and Et_3N (0.116 mL, 0.84 mmol) were added and the mixture was stirred at 0 °C for 10 min and concentrated. Crude product was purified by column chromatography ($CHCl_3$ → $CHCl_3$:MeOH 20:1) to obtain **15** (0.460 g, 96%). For desilylation, derivative **15** (0.460 g, 0.728 mmol) was dissolved in MeOH (9 mL) and AcCl (0.104 mL, 1.456 mmol) was added. The reaction mixture was stirred at rt for 5 h. After completion of reaction the mixture was neutralised by Amberlyst 21 and concentrated. Crude product was purified by column chromatography ($CHCl_3$:MeOH 20:1 → 10:1) to obtain **16/B3** (0.144 g, 49%); $[\alpha]_D^{20} = -27.5$ (MeOH, c = 1.00); ^{1}H NMR (600 MHz, DMSO): δ 3.30 (ddd, 1H, J = 5.9, J = 6.4, J = 14.1 Hz, H-5′b), 3.43 (ddd, 1H, J = 4.5, J = 5.9, J = 14.1 Hz, H-5′a), 3.83 (ddd, 1H, J = 4.5, J = 5.7, J = 6.4 Hz, H-4′), 3.86–3.91 (m, 3H, NH–C$\underline{H}_2$–CO, H-3′), 4.05 (ddd, 1H, J = 5.7, J = 5.7, J = 5.7 Hz, H-2′), 5.15 (d, 1H, J = 5.2 Hz, 3′-OH), 5.36 (d, 1H, J = 5.7 Hz, 2′-OH), 5.58 (d, 1H, J = 8.1 Hz, H-5), 5.73 (d, 1H, J = 5.7 Hz, H-1′), 7.47 (m, 2H, $H_{ar\text{-}m}$), 7.54 (tt, 1H, J = 1.3, J = 7.5 Hz, $H_{ar\text{-}p}$), 7.66 (d, 1H, J = 8.1 Hz, H-6), 7.88 (m, 2H, $H_{ar\text{-}o}$), 8.09 (t, 1H, J = 5.9 Hz, C(O)–N$\underline{H}$), 8.73 (t, 1H, J = 5.9 Hz, C(O)N$\underline{H}$CH$_2$ C(O)); 11.33 (s, 1H, NH_{ur}), ^{13}C NMR (150 MHz, DMSO): δ 41.24 (C-5′), 43.13 (NH–$\underline{C}H_2$–C(O)), 71.10 (C-3′), 72.84 (C-2′), 83.04 (C-4′), 88.51 (C-1′), 102.44 (C-5), 127.80 ($C_{ar\text{-}o}$), 128.69 ($C_{ar\text{-}m}$), 131.77 ($C_{ar\text{-}p}$), 134.47 ($C_{ar\text{-}IV}$), 141.74 (C-6), 151.20 (C-2), 163.45 (C-4), 166.96 ($C(O)_{Bz}$), 169.77 (CH_2–$\underline{C}$(O)–NH); ESI-HRMS: Calcd for $C_{18}H_{20}N_4O_7Na$ ([M+Na]$^+$): m/z 427.1230, found: 427.1236.

4.3.4. 5′-(N-succinyl-glycyl)-5′-amino-5′-deoxyuridine (**18/B4**)

Compound **14** (0.400 g, 0.76 mmol, synthesised as described in Section 4.3.3) was dissolved in THF (15 mL) and succinyl anhydride (0.758 g, 0.76 mmol) and Et_3N (0.105 mL, 0.76 mmol) were added. The mixture was stirred at rt for 30 min and concentrated. Crude product was purified by flash chromatography ($CHCl_3$:MeOH 20:1) to obtain **17** (0.213 g, 45%). For desilylation, derivative **17** (0.213 g, 0.34 mmol) was dissolved in THF (10 mL) and TBAF (1.02 mL, 1 M solution in THF, 3.0 equiv.) was added. The reaction mixture was stirred at rt for 3 h. After completion of reaction Amberlyst 15 was added and the mixture was left to stir for 3 h at rt. Then the mixture was filtered and concentrated. Crude product was purified by column chromatography ($CHCl_3$:MeOH 2:1) to obtain **18/B4** (0.046 g, 33%); $[\alpha]_D^{20} = -6.73$ (MeOH, c = 1.00); ^{1}H NMR (600 MHz, DMSO): δ 2.37 (m, 2H, CH_2–CH_2), 2.42 (m, 2H, CH_2–CH_2), 3.26 (ddd, 1H, J = 5.9, J = 6.2, J = 13.9 Hz, H-5′b), 3.39 (ddd, 1H, J = 4.6, J = 5.9, J = 13.9 Hz, H-5′a), 3.67 (d, 2H, J = 5.8 Hz, NH–C$\underline{H}_2$–C(O)), 3.81 (ddd, 1H, J = 4.3, J = 4.6, J = 6.2 Hz, H-4′), 3.86 (dd, 1H, J = 4.3, J = 5.4 Hz, H-3′), 4.04 (dd, 1H, J = 5.4, J = 5.6 Hz, H-2′), 5.63 (d, 1H, J = 8.1 Hz, H-5), 5.71 (d, 1H, J = 5.7 Hz, H-1′), 7.66 (d, 1H, J = 8.1 Hz, H-6), 7.98 (t, 1H, J = 5.8 Hz, C(O)–N$\underline{H}$–CH_2–C(O)), 8.14 (t, 1H, J = 5.9 Hz, C(O)–N$\underline{H}$), 11.33 (s, 1H, NH_{ur}); ^{13}C

NMR (150 MHz, DMSO): δ 22.22, 22.18 ($\underline{C}H_2$–$\underline{C}H_2$), 40.69 (NH–$\underline{C}H_2$–C(O)), 64.10 (C-5′), 81.13, 72.64, 69.68 (C-2′, C-3′, C-4′), 88.36 (C-1′), 102.14 (C-5), 140.71 (C-6), 150.63 (C-2), 163.00 (C-4), 169.87 (C(O)); ESI-HRMS: Calcd for $C_{15}H_{19}N_4O_9$ ($[M-H]^-$): m/z 399.1152, found: 399.1147.

4.4. Biological evaluation

4.4.1. Bovine milk β-1,4-galactosyltransferase I assay

β-1,4-GalT I activity was assayed using UDP-Gal as glycosyl donor and β-GlcNAc-O-$(CH_2)_6$-dansyl as glycosyl acceptor [17]. Assays were performed in a total volume of 100 μL. The reaction mixtures contained reagents in the following final concentrations: 50 mM Hepes buffer, 10 mM $MnCl_2$, 2.0 mg/mL BSA, 200 μM β-GlcNAc-O-$(CH_2)_6$-dansyl, 40 μM UDP-Gal and compounds **A1**–**A4** and **B1**–**B4** at a range of concentrations from 0 mM (control) to 2.0 mM. The enzymatic reactions were started by the addition of 0.2 mU β-1,4-GalT I and incubated at 30 °C for 14 min. Inactivation was quickly done by immersion of the reaction solutions for 2 min in a boiling water bath. The solutions were diluted with water (200 μL) and centrifuged for 10 min, and the supernatant was injected into RP-HPLC system. The percentage of inhibition was evaluated from the fluorescence intensity of the peaks referring to product (Galβ-1,4-GlcNAcβ-O-$(CH_2)_6$-dansyl).

4.4.2. In vitro antidenaturation studies

Assays were performed in 96-well microtiter plates (Costar 3596, Corning) in total volume of 100 μL. The reaction mixtures contained reagents in the following final concentrations: 0.2% W/V of BSA or HSA, 100 mM phosphate buffer (pH 5.3) or 50 mM Hepes buffer (pH 5.3) and compounds **A1**–**A4** and **B1**–**B4** at a range of concentrations from 0 mM (control) to 2.0 mM. The plates were heated at 70 °C for 30 min and then cooled for 10 min. The absorbance of these solutions was determined by using spectrophotometer at a wavelength of 660 nm. The %inhibition of precipitation (denaturation of the protein) was determined on *a*% basis relative to the control using the following formula:

$$\%\text{Inhibition of denaturation} = (A_{\text{control}} - A_{\text{treated}})/A_{\text{control}} \times 100$$

4.4.3. STD NMR experiments

Experiments were recorded on a Bruker 600-MHz DRX equipped with triple resonance (1H, 13C and 15N) probe head with a cryo probe at 303 K. Spectra were calibrated with internal [D4](trimethylsilyl)propionic acid sodium salt (TSP, 10 μM). All the samples were dissolved in deuterated PBS buffer at pH 6.5. For the acquisition of STD NMR spectra, the protein–ligand molar saturation times at 2 s was used; a T1ρ filter (50 ms spin-lock pulse) to eliminate the unwanted broad resonance signals of the protein was used. The on resonance frequency was set at 0 ppm and the off-resonance frequency was maintained at 40 ppm. The on resonance irradiation frequency of 0 ppm falls in a region in which ligand signals are completely absent, as necessary to avoid false positives in the STD spectrum. A train of 40 Gaussian-shaped pulses of 50 ms each (1 ms delay between pulses, field strength of 21 Hz) was employed. Saturated and reference spectra were acquired simultaneously by creating a pseudo-2D experiment. Reference experiments were carried out to assure the absence of direct irradiation of the ligand. STD spectra were performed with 16 K and 32 K data points. The original FID was zero-filled to 64 k and Fourier transformation with use of an exponential window function was applied. In order to determine the magnitude of the STD effects, the intensity of the signal in the STD NMR spectrum were compared with the signal intensities of a reference spectrum (off-resonance). The STD signal with the highest intensity was set to 100% and the others were normalized to this signal. Data acquisition and processing were performed with TOPSPIN software.

Acknowledgments

Publication part-financed by the European Union within the European Regional Development Fund (POIG. 01.01.02-14-102/09). KK received scholarships under the project 'DoktoRIS-Scholarship Program for Innovative Silesia'. AS and IW acknowledge the Executive Programme of Scientific and Technological Cooperation between the Italian Republic and the Replubic of Poland, project code: PL13MO6.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bioorg.2014.11.001.

References

[1] K. Isono, J. Antibiot. 41 (1988) 1711–1739.
[2] K. Isono, Pharmacol. Therapeut. 52 (1991) 269–286.
[3] M. Winn, R.J. Goss, K. Kimura, T.D. Bugg, Nat. Prod. Rep. 27 (2010) 279–304.
[4] C.T. Walsh, W. Zhang, ACS Chem. Biol. 6 (2011) 1000–1007.
[5] A. Varki, R.D. Cummings, J.D. Esco, H.H. Freeze, P. Stanley, C.R. Bertozzi, G.W. Hart, M.E. Etzler, Essentials of Glycobiology, second ed., CSHL Press, 2009.
[6] A. Heifetz, R.W. Keenan, A.D. Elbein, Biochemistry 18 (1979) 2186–2192.
[7] K.E. Jackson, P.K. Pogula, S.E. Patterson, Heterocycl. Commun. 19 (2013) 375–386.
[8] T.M. Gloster, D.J. Vocadlo, Nat. Chem. Biol. 8 (2012) 683–694.
[9] R. Roychoudhury, N.L.B. Pohl, Curr. Opin. Chem. Biol. 14 (2010) 168–173.
[10] T. Kajimoto, M. Node, Synthesis (2009) 3179–3210.
[11] S. Wang, S. Vidal, Recent Design of Glycosyltransferase Inhibitors, in: A.P. Rauter, T. Lindhorst (Eds.), Carbohydrate Chemistry, vol. 39, RSC Publishing, London, 2013, pp. 78–101.
[12] K. Descroix, T. Pesnot, Y. Yoshimura, S.S. Gehrke, W. Wakarchuk, M.M. Palcic, G.K. Wagner, J. Med. Chem. 55 (2012) 2015–2024.
[13] T. Pesnot, R. Jørgensen, M.M. Palcic, G.K. Wagner, Nat. Chem. Biol. 6 (2010) 321–323.
[14] A. Evitt, L.M. Tedaldi, G.K. Wagner, Chem. Commun. 48 (2012) 11856–11858.
[15] K. Takaya, N. Nagahori, T. Furuike, N. Miura, K. Monde, Y.C. Lee, S.-I. Nishimura, J. Med. Chem. 48 (2005) 6054–6065.
[16] H. Yuasa, M.M. Palcic, O. Hindsgaul, Can. J. Chem. 73 (1995) 2190–2195.
[17] S. Vidal, I. Bruyère, A. Malleron, C. Augé, J.-P. Praly, Bioorg. Med. Chem. 14 (2006) 7293–7301.
[18] H. Hashimoto, T. Endo, Y. Kajihara, J. Org. Chem. 62 (1997) 1914–1915.
[19] J.-B. Behr, Chimia 65 (2011) 1–5.
[20] P.M. Chaudhary, S.R. Chavan, F. Shirazi, M. Razdan, P. Nimkar, S.P. Maybhate, A.P. Likhite, R. Gonnade, B.M. Hazara, M.V. Deshpande, S.R. Deshpande, Bioorg. Med. Chem. 17 (2009) 2433–2440.
[21] A. Suda, A. Ohta, M. Sudoh, T. Tsakuda, N. Shimma, Heterocycles 55 (55) (2001) 1023–1028.
[22] K. Obi, J.-I. Uda, K. Iwase, O. Sugimoto, H. Ebisu, A. Matsuda, Bioorg. Med. Chem. Lett. 10 (2000) 1451–1454.
[23] D.K. Alargov, Z. Naydenova, K. Grancharov, P.S. Denkova, E.V. Golovinsky, Monatsh. Chem. 128 (1997) 725–732.
[24] D.K. Alargov, Z. Naydenova, K. Grancharov, P.S. Denkova, E.V. Golovinsky, Monatsh. Chem. 129 (1998) 755–760.
[25] D.K. Alargov, R.G. Gugova, P.S. Denkova, G. Müller, E.V. Golovinsky, Monatsh. Chem. 130 (1999) 937–943.
[26] N.I. Howard, T.D.H. Bugg, Bioorg. Med. Chem. 11 (2003) 3083–3099.
[27] A.E. Trunkfield, S.S. Gurcha, G.S. Besra, T.D.H. Bugg, Bioorg. Med. Chem. 18 (2010) 2651–2663.
[28] K. Vembaiyan, J.A. Pearcey, M. Bhasin, T.L. Lowary, W. Zou, Bioorg. Med. Chem. 19 (2011) 58–66.
[29] I.V. Tetko, J. Gasteiger, R. Todeschini, A. Mauri, D. Livingstone, P. Ertl, V.A. Palyulin, E.V. Radchenko, N.S. Zefirov, A.S. Makarenko, V.Y. Tanchuk, V.V. Prokopenko, J. Comput. Aid. Mol. Des. 19 (2005) 453–463 (VCCLAB, Virtual Computational Chemistry Laboratory <http://www.vcclab.org>).
[30] J. Paszkowska, B. Kania, I. Wandzik, J. Liq. Chromatogr. RT 35 (2012) 1202–1212.
[31] A.M. Bello, E. Poduch, M. Fujihashi, M. Amani, Y. Li, I. Crandall, R. Hui, P.I. Lee, K.C. Kain, E.F. Pai, L.P. Kotra, A. Potent, J. Med. Chem. 50 (2007) 915–921.
[32] Y. Mizushima, M. Kobayashi, J. Pharm. Pharmacol. 20 (1968) 169–173.
[33] L. Saso, G. Valentini, M.L. Casini, E. Grippa, M.T. Gatto, M.G. Leone, B. Silvestrini, Arch. Pharm. Res. 24 (2001) 150–158.
[34] P.D. Boyer, F.G. Lum, G.A. Ballou, J.M. Luck, R.G. Rice, J. Biol. Chem. 162 (1946) 181–198.

[35] L.V. Nargund, G.R. Reddy, V. Hariprasad, J. Pharm. Sci. 83 (1994) 246–248.
[36] J. Ghuman, P.A. Zunszain, I. Petitpas, A.A. Bhattacharya, M. Otagiri, S. Curry, J. Mol. Biol. 353 (2005) 38–52.
[37] P. Bourassa, S. Dubeau, G.M. Maharvi, A.H. Fauq, T.J. Thomas, H.A. Tajmir-Riahi, Eur. J. Med. Chem. 46 (2011) 4344–4353.
[38] B. Sekula, K. Zielinski, A. Bujacz, Int. J. Biol. Macromol. 60 (2013) 316–324.
[39] F. Squeglia, R. Marchetti, A. Ruggiero, R. Lanzetta, D. Marasco, J. Dworkin, M. Petoukhov, A. Molinaro, R. Berisio, A. Silipo, J. Am. Chem. Soc. 133 (2011) 20676–20679.
[40] B. Meyer, T. Peters, Angew. Chem. Int. Ed. Engl. 42 (2003) 864–890.
[41] J. Park, S. Bhuniya, H. Lee, Y.-W. Noh, Y.T. Lim, J.H. Jung, K.S. Hong, J.S. Kim, Chem. Commun. 48 (2012) 3218–3220.