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5-Amino-2-pyridyl 1-thioglycosides in synthesis of analogs of glycosyltransferases substrates

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ABSTRACT

We present the synthesis of 1-thioglycosyl derivatives of uridine, which were designed to act as potential donor substrates for glycosyltransferases. We constructed such analogs using 5-amino-2-pyridyl 1-thioglycosides as glycosyl units which were connected to uridine via succinic linker. For preparation of the amide bonds we applied different condensation procedures.

1. Introduction

Modulation of biological adhesion processes has been a long standing goal of pharmaceutical invention in pathological processes ranging from inflammation, arteriosclerosis to viral infection and tumor metastasis [1]. In fact, cellular or cell-surface-associated cell adhesion molecules responsible for diseases are attractive drug targets because of their accessibility, which is evoked by the fact that the drug molecules no longer need to transfer through cell membranes [2]. Alternatively, enzymes utilizing such molecules or responsible for the biosynthesis of the recognition epitopes may also be targeted in order to alter the process of cell adhesion [3]. Actually, they should be chemically more tractable than the adhesion molecules that function in the targeted pathway.

Glycosylation catalyzed by a family of glycosyltransferases (GTs) is one of post-translational modification of peptides important in cell-adhesion [4]. GTs of the Leloir pathway [5] which catalyze the transfer of a sugar moiety from an activated nucleotide sugar to a hydroxyl group of an acceptor (a growing oligosaccharide, a lipid or a protein [6]) are responsible for synthesizing most of glycoproteins and other glycoconjugates in mammalian systems. Glycosyltransferases are usually metal ion dependent, where magnesium or manganese are the most typical metals found in the active site. The metal ion is essential for catalytic properties of GTs since it interacts with pyrophosphate group of the UDP-sugar donor in enzyme active site, thus binding it and forcing its correct orientation in space [7].

Affecting glycosylation processes might bring about various changes within the biological processes utilizing glycoproteins (e.g. enzyme activity or ligand binding). In some reported cases, absence of particular glycans or introduction of other particular glycans within the glycoprotein structure has been found to influence its biological activity [8]. Therefore, search for potent inhibitors of such processes is of grave importance.

Among known GT inhibitors there are such natural compounds as tunicamycin (a GlcNAc phosphotransferase inhibitor which may also act as GT inhibitor) [9], Nikkomycin Z and Polyoxin D (a chitin synthetase inhibitor) [10] or moenomycin A (a bacterial transglycosylase inhibitor) [11]. However, tunicamycin exhibits high cytotoxicity which prevents from its use in clinical therapy. Therefore, more selective inhibitors are needed.

Searching for carbohydrate mimetics-based GT inhibitors appears to be a task of substantial and still growing interest. Different strategies for designing new GTs inhibitors were applied [12]. Structures of proposed inhibitors are based on their structural analogies between donor substrates, acceptor substrates and transition state, respectively. In case of donor substrate analogs some structural changes concerned the carbohydrate part or pyrophosphate linkage [13].

Numerous analogs of pyrophosphate linker have been proposed, most of which would theoretically come into interactions with Mn^{2+} similarly to their natural counterparts. Some of the proposed structures bear carbon or sulfur atoms in the phosphoester structures instead of oxygen, which is expected to increase the overall stability against enzymatic hydrolysis [14]. Malonates, tartarates and even monosaccharide moieties have also been proposed as replacements for pyrophosphate linkage [15]. However, almost none of the proposed potential inhibitors exhibited significant activity.

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In our study, we focused on simple and efficient synthesis of carbohydrate derivatives conjoined with uridine part, which is responsible for the recognition and proper binding within the active site. The structures we proposed are believed to exhibit improved stability against hydrolytic cleavage.

Stability of the structure would be increased by replacing the oxygen atom between the linker and sugar moiety with sulfur. Thioglycosides are well tolerated by most of biological systems [16,17]. They are also known for their increased resistance against enzymatic degradation [18,19]. Stability of (5-nitro-2-pyridyl) 1-thioglycosides has been shown while utilizing their selectively protected derivatives as glycosyl acceptors in the presence of various acidic promoters [20]. That is the main reason for choosing such structures to be the sugar fragment analogs joined with uridine. Although all of the proposed structures have β-configuration which stays in contradiction to the naturally occurring GT substrates, there are reports stating that such sugar donor analog as 5′-O-β-lactosyl-uridine [15] exhibited biological activity despite having β-configuration at the anomeric carbon. Disodium uridin-5′-yl (2-acetamido-2-deoxy-β-D-glucopyranosylthiomethylphosphono) phosphate was also tested as inhibitor of UDP-GlcNAc 2-epimerase [19].

Within the structures we proposed, the pyrophosphate was replaced with succinic linkage. Choice of this particular fragment was based on the reports in which succinic derivatives were effectively employed to coordinate divalent metal ions [21,22]. The aforementioned studies proved that carbonyl oxygen atoms of the succinic fragment are responsible for the complexation process. Linker stability was additionally enhanced by introduction of amide bond between the linker and sugar in the reaction between the succinic carboxyl groups with primary amine present within the structure of sugar aglycone.

2. Experimental

2.1. General methods

NMR spectra were recorded for solutions in $CDCl_3$ or DMSO-d_6 using Varian spectrometer at a frequency of 300 MHz with TMS as internal reference. NMR solvents were purchased from ACROS Organics (Geel, Belgium). Chemical shifts (δ) are expressed in ppm and coupling constants (J) in Hz. Optical rotations were measured on Perkin–Elmer 141 polarimeter using a sodium lamp (589.3 nm) at room temperature. Mass spectra were measured in the positive mode with a Mariner (Perspective Biosystem) detector using the electrospray-ionization (ESI) technique.

Reactions were monitored by TLC on precoated plates of silica gel 60 F_{254} (Merck). TLC plates were inspected under UV light and developed by charring after spraying with 10% sulphuric acid in ethanol. Column chromatography was performed on Silica Gel 60 (70–230 mesh, Fluka) developed with either toluene–EtOAc or $CHCl_3$–MeOH solvent systems. Organic solvents were evaporated on a rotary evaporator under reduced pressure at 50 °C.

2′,3′-*O*-isopropylideneuridine (**3**) [23], 3-*N*-benzoyl-2′,3′-*O*-isopropylideneuridine (**4**) [24], 2′,3′-di-*O*-*tert*-butyldimethylsilyl-uridine (**5**) [25] were prepared according to the published procedures. (5-amino-2-pyridyl) 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (**1**) and (5-amino-2-pyridyl) 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (**2**) were synthesized by reduction of the corresponding (5-nitro-2-pyridyl) 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glycopyranosides [26] with zinc powder/acetic acid system in CH_2Cl_2; 3-*N*-benzoyl-2′,3′-di-*O*-*tert*-butyldimethylsilyl-uridine (**6**) was synthesized by benzoylation of **5** with benzoyl chloride/Et_3N system in CH_2Cl_2. 4-(4,6-Dimethoxy-(1,3,5)triazin-2-yl)-4-methyl-morpholinium chloride (DMT-MM) was synthesised according to a procedure described by Kunishima [27].

Other chemicals were purchased from Aldrich, Fluka and Acros Chemical Companies and were used without purification. Solvents were dried and stored over molecular sieves (4 Å).

2.2. General procedures for condensation of amines and uridine derivatives

2.2.1. Procedure A using DCC as a coupling agent

To a solution of amine **1** (0.25 mmol), DCC (0.25 mmol) and DMAP (0.25 mmol) in dry $CHCl_3$ (5 mL), an equimolar amount of uridine derivative **8** (0.25 mmol) in $CHCl_3$ (2 mL) was added. The mixture was stirred at room temperature for 48 h. The reaction mixture was concentrated to give a mixture of crude products which was purified directly by column chromatography with $CHCl_3$/MeOH 100:1 → 20:1 solvent system.

2.2.2. Procedure B using DCC as a coupling agent

To a solution of amine **1** or **2** (0.25 mmol) and uridine derivative **7, 8** or **9** (0.25 mmol) in dry pyridine (3 mL), DCC (51 mg, 0.25 mmol) and DMAP (31 mg, 0.25 mmol) were added. The mixture was stirred at room temperature for 17–24 h (appropriate reaction times are given in Table 1). After completion (TLC, 10:1 $CHCl_3$–MeOH or 1:1 toluene–AcOEt) the reaction mixture was concentrated with toluene (3 × 10 mL) in order to remove the whole amount of pyridine. The crude product was purified directly by column chromatography with an appropriate solvent system as indicated.

2.2.3. Procedure C using ethyl chloroformate as coupling agent

To a solution of ethyl chloroformate (0.25 mmol) in dry CH_2Cl_2 (4 mL) with an addition of Et_3N (0.25 mmol), a solution of uridine derivative **8** or **9** in CH_2Cl_2 (4 mL) was added in portions. Resulting reaction mixture was stirred at room temperature for 1.5 h and then the amine **1** (0.25 mmol) was added. The mixture was stirred at room temperature for 48 h. Finally, the reaction mixture was concentrated to give a crude product that was purified directly by column chromatography with an appropriate solvent system as indicated.

2.2.4. Procedure D using DMT-MM as coupling agent

To a solution of amine **1** or **2** (0.25 mmol) and uridine derivative **7, 8** or **9** (0.25 mmol) in dry THF (5 mL) with addition of MeOH (0.3 mL), an equimolar amount of DMT-MM (69 mg, 0.25 mmol) was added (Table 1). The mixture was stirred at room temperature for 24–48 h (appropriate reaction times are given in Table 1). After

Table 1
Condensation of amines **1**–**2** with uridine derivatives **7**–**9**.

Entry	Amine	Uridine derivative	Product	Reaction time (h)	Procedure	Yield (%)
1	**1**	**8**	**15**	48	**A**	34
			11			5
2	**1**	**7**	**10**	24	**B**	Traces[a]
3	**1**	**8**	**10**	17	**B**	23
4	**1**	**9**	**14**	20	**B**	14
5	**2**	**8**	**12**	19	**B**	10
6	**1**	**8**	**11**	48	**C**	40
7	**1**	**9**	**14**	48	**C**	27
8	**1**	**7**	**10**	48	**D**	53
9	**1**	**8**	**11**	48	**D**	42
10	**1**	**9**	**14**	48	**D**	51
11	**2**	**7**	**12**	24	**D**	39
12	**2**	**8**	**13**	48	**D**	37

[a] Only TLC analysis, product was not isolated by column chromatography.

completion (TLC, 10:1 $CHCl_3$–MeOH or 1:1 toluene–AcOEt) the reaction mixture was concentrated to give a crude product which was purified directly by column chromatography with an appropriate solvent system as indicated.

2.3. Compound (**10**)

2.3.1. Succinic acid mono-2′,3′-isopropylidene-uridin-5′-yl ester (**7**)

To a solution of 2′,3′-isopropylidene-uridine **3** (500 mg, 1.76 mmol) in a mixture of CH_2Cl_2 (7 mL) and pyridine (3 mL) succinic anhydride (176 mg, 1.76 mmol) and DMAP (215 mg, 1.76 mmol) were added. The resulting mixture was stirred at room temperature for 2 h. After completion (TLC, 10:1 $CHCl_3$–MeOH) the reaction mixture was concentrated with toluene (3 × 10 mL) in order to remove the whole amount of pyridine. The crude product was purified on a column of silica gel using $CHCl_3$–MeOH 10:1 solvent system as the eluent to yield **7** as a white powder (490 mg, 72%): mp 101–102 °C; $[\alpha]_D^{25}$ +3.1 (c 1.0, MeOH); ^{1}H NMR (DMSO-d_6): δ 12.24 (s, 1H, COOH), 11.44 (s, 1H, NH), 7.69 (d, 1H, *J* 8.1 Hz, H-6_{ur}), 5.81 (d, 1H, *J* 2.0 Hz, H-$1'_{ur}$), 5.65 (dd, 1H, *J* 2.0, 8.1 Hz, H-5_{ur}), 5.03 (dd, 1H, *J* 2.2, 6.4 Hz, H-$2'_{ur}$), 4.78 (dd, 1H, *J* 3.1, 6.4 Hz, H-$3'_{ur}$), 4.31–4.13 (m, 3H, H-$4'_{ur}$, H-5′a_{ur}, H-5′b_{ur}), 2.56–2.45 (m, 4H, 2 × CH_2), 1.49, 1.29 (2s, 6H, C(CH_3)$_2$); ^{13}C NMR (DMSO-d_6): δ 174.07 (COOH), 172.66 (CO), 163.91 (C-4_{ur}), 150.98 (C-2_{ur}), 143.30 (C-6_{ur}), 114.01 (*C*(CH_3)$_2$), 102.53 (C-5_{ur}), 92.97 (C-$1'_{ur}$), 84.80 (C-$2'_{ur}$), 84.31 (C-$3'_{ur}$), 81.27 (C-$4'_{ur}$), 64.65 (C-$5'_{ur}$), 29.29, 29.27 (2 × *C*H_2CO), 27.65, 25.85 (C(*C*H_3)$_2$).

2.3.2. Succinic acid mono-3-N-benzoyl-2′,3′-isopropylidene-uridin-5′-yl ester (**8**)

To a solution of 3-*N*-benzoyl-2′,3′-isopropylidene-uridine **4** (500 mg, 1.24 mmol) in CH_2Cl_2 (5 mL) succinic anhydride (124 mg, 1.24 mmol) and DMAP (152 mg, 1.24 mmol) were added. The resulting mixture was stirred at room temperature for 6 h. After completion (TLC, 10:1 $CHCl_3$–MeOH) the reaction mixture was concentrated. The crude product was purified on a column packed with silica gel using $CHCl_3$–MeOH 100:1 → 20:1 solvent systems as the eluent to yield **8** as a white powder (425 mg, 48%): mp 146–147 °C; $[\alpha]_D^{25}$ +6.3 (c 2.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.5 (bs, 1H, COOH), 7.98–7.93 (m, 2H, Ph(o)), 7.67 (m, 1H, Ph(p)), 7.55–7.45 (m, 2H, Ph(m)), 7.42 (d, 1H, *J* 8.1 Hz, H-6_{ur}), 5.87 (d, 1H, *J* 8.4 Hz, H-5_{ur}), 5.67 (d, 1H, *J* 1.8 Hz, H-$1'_{ur}$), 5.04 (dd, 1H, *J* 1.8, 6.1 Hz, H-$2'_{ur}$), 4.79 (dd, 1H, *J* 3.3, 6.1 Hz, H-$3'_{ur}$), 4.40–4.28 (m, 3H, H-$4'_{ur}$, H-5′a_{ur}, H-5′b_{ur}), 2.70–2.55 (m, 4H, CH_2), 1.55, 1.33 (2s, 6H, C(CH_3)$_2$); ^{13}C NMR ($CDCl_3$): δ 172.62, 171.76, 168.31 (CO), 161.94 (C-4_{ur}), 149.07 (C-2_{ur}), 141.89 (C-6_{ur}), 135.22, 131.16, 130.49, 129.16 (*Ph*CO), 114.48 (*C*(CH_3)$_2$), 102.41 (C-5_{ur}), 95.08 (C-$1'_{ur}$), 85.35 (C-$2'_{ur}$), 84.43 (C-$3'_{ur}$), 80.77 (C-$4'_{ur}$), 63.91 (C-$5'_{ur}$), 28.81, 28.65 (2 × *C*H_2CO), 27.01, 25.14 (C(*C*H_3)$_2$).

2.3.3. Procedure B using uridine derivative **7** for preparation of **10**

(5-Amino-2-pyridyl) tetra-*O*-acetyl-1-thio-β-D-glucopyranoside **1** (114 mg, 0.25 mmol) and uridine derivative **7** (96 mg, 0.25 mmol) were submitted to general procedure B described above. After 24 h TLC analysis indicated presence of substrates **1** and **7** and only traces of **10**. So, product **10** was not isolated from the reaction mixture.

2.3.4. Procedure B using uridine derivative **8** for preparation of **10**

(5-Amino-2-pyridyl) tetra-*O*-acetyl-1-thio-β-D-glucopyranoside **1** (114 mg, 0.25 mmol) and uridine derivative **8** (112 mg, 0.25 mmol) were submitted to general procedure B described above. The crude product was purified on a column packed with silica gel using $CHCl_3$/MeOH 30:1 solvent system as the eluent to yield **10** as a white solid (47 mg, 23%).

2.3.5. Procedure D using uridine derivative **7** for preparation of **10**

Compound **1** (114 mg, 0.25 mmol) and uridine derivative **7** (96 mg, 0.25 mmol) were submitted to general procedure D described above. The resulting crude product **10** was purified by column chromatography to yield **10** as a white solid (109 mg, 53%): mp 145–148 °C; $[\alpha]_D^{25}$ −5.4 (c 1.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 10.10 (bs, 1H, NH), 8.72 (s, 1H, NH), 8.52 (d, 1H, *J* 2.4 Hz, H-6_{pyr}), 7.98 (dd, 1H, *J* 2.4, 8.6 Hz, H-4_{pyr}), 7.35 (d, 1H, *J* 8.0 Hz, H-6_{ur}), 7.20 (d, 1H, *J* 8.6 Hz, H-3_{pyr}), 5.70 (d, 1H, *J* 8.0 Hz, H-5_{ur}), 5.65 (d, 1H, *J* 10.4 Hz, H-1_{glu}), 5.62 (d, 1H, *J* 1.5 Hz, H-$1'_{ur}$), 5.35 (dd ∼ t, 1H, *J* 9.3 Hz, H-3_{glu}), 5.18 (dd ∼ t, 1H, *J* 9.8 Hz, H-2_{glu}), 5.15 (dd ∼ t, 1H, *J* 9.5 Hz, H-4_{glu}), 5.05 (dd, 1H, *J* 1.6, 6.4 Hz, H-$2'_{ur}$), 4.85 (dd, 1H, *J* 3.3, 6.4 Hz, H-$3'_{ur}$), 4.40–4.32 (m, 3H, H-$4'_{ur}$, H-5′a_{ur}, H-5′b_{ur}), 4.26 (dd, 1H, *J* 4.4, 12.4 Hz, H-$6a_{glu}$), 4.10 (dd, 1H, *J* 3.3, 12.3 Hz, H-$6b_{glu}$), 3.88 (ddd, 1H, *J* 2.4, 4.2, 10.0 Hz, H-5_{glu}), 2.78–2.65 (m, 4H, 2 × CH_2), 2.04, 2.03, 2.01 (3s, 12H, 4 × CH_3CO), 1.55, 1.32 (2s, 6H, C(CH_3)$_2$); ^{13}C NMR ($CDCl_3$): δ 172.86, 171.02, 170.68, 170.41, 169.80, 169.71 (CO), 162.11 C-4_{ur}), 150.38 (C-2_{ur}), 149.43 (C-2_{pir}), 143.09 (C-6_{ur}), 141.27 (C-6_{pyr}), 133.55 (C-5_{pyr}),128.33 (C-4_{pyr}), 124.03 (C-3_{pyr}), 114.70 (*C*(CH_3)$_2$), 102.57 (C-5_{ur}), 95.45 (C-$1'_{ur}$), 85.56 (C-$4'_{ur}$), 84.67 (C-$2'_{ur}$), 82.70 (C-1_{glu}), 81.17 (C-$3'_{ur}$), 75.97 (C-5_{glu}), 74.25 (C-3_{glu}), 69.85 (C-2_{glu}), 68.47 (C-4_{glu}), 64.51 (C-$5'_{ur}$), 62.20 (C-6_{glu}), 31.71 (*C*H_2COO), 29.33 (*C*H_2CONH), 27.31, 25.44 (C(*C*H_3)$_2$), 20.95, 20.89, 20.82, 20.80 (4 × *C*H_3CO). ESI-MS: Calcd for $C_{35}H_{42}N_4O_{17}$SNa ([M+Na]$^+$): *m/z* 845.2. Found: *m/z* 845.2.

2.4. Compound (**11**)

2.4.1. Procedure A using uridine derivative **8** for preparation of **11** and (**15**)

(5-Amino-2-pyridyl) tetra-*O*-acetyl-1-thio-β-D-glucopyranoside **1** (114 mg, 0.25 mmol) and uridine derivative **8** (122 mg, 0.25 mmol) were submitted to general procedure A described above. Two compounds were isolated: product **11** (12 mg, 5%) as a white foam and byproduct **15** (41 mg, 34%) as a colorless oil; $[\alpha]_D^{25}$ +7.4 (c 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.98–7.91 (m, 4H, $Ph_{(o)}$), 7.70–7.62 (m, 2H, $Ph_{(p)}$), 7.55–7.46 (m, 4H, $Ph_{(m)}$), 7.43 (d, 2H, *J* 8.1 Hz, H-6_{ur}), 5.85 (d, 2H, *J* 8.1 Hz, H-5_{ur}), 5.68 (d, 2H, *J* 1.8 Hz, H-$1'_{ur}$), 5.04 (dd, 2H, *J* 1.8, 6.1 Hz, H-$2'_{ur}$), 4.79 (dd, 2H, *J* 3.3, 6.1 Hz, H-$3'_{ur}$), 4.40–4.24 (m, 6H, H-$4'_{ur}$, H-5′a_{ur}, H-5′b_{ur}), 2.68-2.56 (m, 8H, 4 × CH_2), 1.54, 1.33 (2s, 12H, C(CH_3)$_2$).

2.4.2. Procedure C using uridine derivative **8** for preparation of **11**

Compound **1** (114 mg, 0.25 mmol) and uridine derivative **8** (122 mg, 0.25 mmol) were submitted to general procedure C described above. The resulting crude product **11** was purified on a column packed with silica gel using $CHCl_3$/MeOH 30:1 solvent system as the eluent to yield **11** as a white solid (92 mg, 40%).

2.4.3. Procedure D using uridine derivatives **8** for preparation of **11**

Compound **1** (114 mg, 0.25 mmol) and uridine derivative **8** (122 mg, 0.25 mmol) were submitted to general procedure D described above. The resulting crude product **11** was purified by column chromatography to yield **11** as a white solid (97 mg, 42%): mp 142–143 °C; $[\alpha]_D^{25}$ +2.3 (c 1.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.49 (d, 1H, *J* 2.4 Hz, H-6_{pyr}), 8.02 (s, 1H, NH), 7.98–7.85 (m, 3H, $Ph_{(o)}$, H-4_{pyr}), 7.66 (m, 1H, $Ph_{(p)}$), 7.55–7.45 (m, 2H, $Ph_{(m)}$), 7.43 (d, 1H, *J* 8.1 Hz, H-6_{ur}), 7.18 (d, 1H, *J* 8.8 Hz, H-3_{pyr}), 5.84 (d, 1H, *J* 8.2 Hz, H-5_{ur}), 5.66 (d, 1H, *J* 10.4 Hz, H-1_{glu}), 5.62 (d, 1H, *J* 1.6 Hz, H-$1'_{ur}$), 5.33 (dd ∼ t, 1H, *J* 9.3 Hz, H-3_{glu}), 5.19 (dd ∼ t, 1H, *J* 10.4 Hz, H-2_{glu}), 5.15 (dd ∼ t, 1H, *J* 9.8 Hz, H-4_{glu}), 5.05 (dd, 1H, *J* 1.6, 6.2 Hz, H-$2'_{ur}$), 4.85 (dd, 1H, *J* 3.3, 6.2 Hz, H-$3'_{ur}$), 4.40–4.28 (m, 3H, H-$4'_{ur}$, H-5′a_{ur}, H-5′b_{ur}), 4.26 (dd, 1H, *J* 4.4, 12.4 Hz, H-$6a_{glu}$), 4.09 (dd, 1H, *J* 2.2, 12.4 Hz, H-$6b_{glu}$), 3.85 (ddd, 1H, *J* 2.2, 4.4, 10.1 Hz, H-5_{glu}), 2.70 (m, 2H, $NHCOCH_2$), 2.60 (m, 2H, CH_2COO), 2.04, 2.03, 2.02, 2.01 (4s, 12H, 4 × CH_3CO), 1.53, 1.28 (2s, 6H, C(CH_3)$_2$); ^{13}C

NMR ($CDCl_3$): δ 172.26, 170.75, 170.18, 170.11, 169.57, 169.48, 168.69 (CO), 162.08, (C-4_{ur}), 149.44, 149.16 (C-2_{pyr}, C-2_{ur}), 142.52 (C-6_{ur}), 141.01 (C-6_{pyr}), 135.54 (*Ph*$_{(p)}$CO), 132.69 (C-5_{pyr}), 131.11 (*Ph*CO), 130.57 (*Ph*$_{(o)}$CO), 129.35 (*Ph*$_{(m)}$CO), 128.11 (C-4_{pyr}), 123.66 (C-3_{pyr}), 114.58 (*C*$(CH_3)_2$), 102.38 (C-5_{ur}), 95.72 (C-$1'_{ur}$), 85.38 (C-$4'_{ur}$), 84.39 (C-$2'_{ur}$), 82.37 (C-1_{glu}), 80.50 (C-$3'_{ur}$), 75.83 (C-5_{glu}), 74.07 (C-3_{glu}), 69.53 (C-2_{glu}), 68.20 (C-4_{glu}), 63.85 (C-$5'_{ur}$), 61.92 (C-6_{glu}), 31.42 (*C*H_2COO), 29.12 (*C*H_2CONH), 27.10, 25.17 (C(*C*$H_3)_2$), 20.76, 20.70, 20.64, 20.61 (4 × *C*H_3CO). ESI-HRMS: Calcd for $C_{42}H_{46}N_4O_{18}$SNa ($[M+Na]^+$): *m/z* 949.2420. Found: *m/z* 949.2447.

2.5. Compound (**12**)

2.5.1. Procedure B using uridine derivative **8** for preparation of **12**

(5-Amino-2-pyridyl) tetra-*O*-acetyl-1-thio-β-D-galactopyranoside **2** (114 mg, 0.25 mmol) and uridine derivative **8** (122 mg, 0.25 mmol) were submitted to general procedure B described above. The crude product was purified on a column packed with silica gel using $CHCl_3$/MeOH 60:1 solvent system as the eluent to yield **12** as a white solid (21 mg, 10%).

2.5.2. Procedure D using uridine derivative **7** for preparation of **12**

Compound **2** (114 mg, 0.25 mmol) and uridine derivative **7** (96 mg, 0.25 mmol) were submitted to general procedure D described above. The resulting crude product **12** was purified by column chromatography to yield **12** as a white solid (81 mg, 39%): mp 115–118 °C; $[\alpha]_D^{25}$ +8.9 (c 1.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 9.95 (s, 1H, NH), 8.63 (s, 1H, NH), 8.49 (d, 1H, *J* 2.4 Hz, H-6_{pyr}), 8.01 (dd, 1H, *J* 2.4, 8.6 Hz, H-4_{pyr}), 7.34 (d, 1H, *J* 8.1 Hz, H-6_{ur}), 7.23 (d, 1H, *J* 8.6 Hz, H-3_{pyr}), 5.72 (dd, 1H, *J* 1.7, 8.1 Hz, H-5_{ur}), 5.64 (d, 1H, *J* 10.2 Hz, H-1_{gal}), 5.60 (d, 1H, *J* 1.5 Hz, H-$1'_{ur}$), 5.48 (d, 1H, *J* 3.3 Hz, H-4_{gal}), 5.38 (dd, 1H, *J* 10.2, *J* 9.9 Hz, H-2_{gal}), 5.19 (dd, 1H, *J* 3.3, 9.9 Hz, H-3_{gal}), 5.05 (dd, 1H, *J* 1.8, 6.3 Hz, H-$2'_{ur}$), 4.85 (dd, 1H, *J* 3.3, 6.3 Hz, H-$3'_{ur}$), 4.42–4.30 (m, 3H, H-$3'_{ur}$, H-5′a_{ur}, H-5′b_{ur}), 4.18–4.02 (m, 3H, H-5_{gal}, H-6a_{gal}, H-6a_{gal}), 2.81–2.64 (m, 4H, 2 × CH_2), 2.17, 2.03, 2.00 (3s, 12H, 4 × CH_3CO), 1.55, 1.32 (2s, 6H, C$(CH_3)_2$); ^{13}C NMR ($CDCl_3$): δ 172.70, 170.51, 170.44, 170.33, 170.10, 169.77 (CO), 163.83 (C-4_{ur}), 150.12 (C-2_{ur}), 149.58 (C-2_{pyr}), 142.92 (C-6_{ur}), 141.04 (C-6_{pyr}), 133.24 (C-5_{pyr}), 128.17 (C-4_{pyr}), 123.83 (C-3_{pyr}), 114.54 (*C*$(CH_3)_2$), 102.41 (C-5_{ur}), 95.42 (C-$1'_{ur}$), 85.40 (C-$4'_{ur}$), 84.45 (C-$2'_{ur}$), 83.01 (C-1_{gal}), 80.97 (C-$3'_{ur}$), 74.43 (C-5_{gal}), 72.05 (C-3_{gal}), 67.38 (C-4_{gal}), 67.02 (C-2_{gal}), 64.33 (C-$5'_{ur}$), 61.34 (C-6_{gal}), 31.58, 29.18 (*C*H_2CO), 27.11, 25.24 (C$(CH_3)_2$), 20.79, 20.68, 20.62 (4 × *C*H_3CO). ESI-MS: Calcd for $C_{35}H_{42}N_4O_{17}$SNa ($[M+Na]^+$): *m/z* 845.2. Found: *m/z* 845.2.

2.6. Compound (**13**)

2.6.1. Procedure D using uridine derivatives **8** for preparation of **13**

Compound **2** (114 mg, 0.25 mmol) and uridine derivative **8** (122 mg, 0.25 mmol) were submitted to general procedure D described above. The resulting crude product **13** was purified on a column packed with silica gel using $CHCl_3$/MeOH 60:1 solvent system as the eluent to yield **13** as a white solid (86 mg, 37%): mp 122–124 °C; $[\alpha]_D^{25}$ +6.8 (c 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.57 (s, 1H, NH), 8.53 (d, 1H, *J* 2.4 Hz, H-6_{pyr}), 8.01–7.90 (m, 3H, Ph$_{(o)}$, H-4_{pyr}), 7.65 (m, 1H, Ph$_{(p)}$), 7.54–7.45 (m, 2H, Ph$_{(m)}$), 7.44 (d, 1H, *J* 8.1 Hz, H-6_{ur}), 7.21 (d, 1H, *J* 8.8 Hz, H-3_{pyr}), 5.86 (d, 1H, *J* 8.1 Hz, H-5_{ur}), 5.66 (d, 1H, *J* 1.7 Hz, H-$1'_{ur}$), 5.62 (d, 1H, *J* 10.2 Hz, H-1_{gal}), 5.48 (d, 1H, *J* 3.2 Hz, H-4_{gal}), 5.39 (dd ∼ t, 1H, *J* 10.1 Hz, H-2_{gal}), 5.17 (dd, 1H, *J* 3.4, 9.8 Hz, H-3_{gal}), 5.05 (dd, 1H, *J* 1.7, 6.3 Hz, H-$2'_{ur}$), 4.78 (dd, 1H, *J* 3.4, 6.3 Hz, H-$3'_{ur}$), 4.39–4.25 (m, 3H, H-$3'_{ur}$, H-5'a_{ur}, H-5'b_{ur}), 4.15–4.04 (m, 3H, H-6a_{gal}, H-6b_{gal}, H-5_{gal}), 2.74-2.59 (m, 4H, CH_2), 2.17, 2.02, 2.00, 1.99 (4s, 12H, 4 × CH_3CO), 1.52, 1.28 (2s, 6H, C$(CH_3)_2$); ^{13}C NMR ($CDCl_3$): δ 172.22, 170.31, 170.19, 169.93, 169.61, 168.51 (CO), 162.05 (C-4_{ur}), 159.42 (CO), 149.31 (C-2_{pyr}), 149.05 (C-2_{ur}), 142.33 (C-6_{ur}), 140.93 (C-6_{pyr}), 135.35 (*Ph*$_{(p)}$CO), 133.09 (C-5_{pyr}), 130.99 (*Ph*CO), 130.41 (*Ph*$_{(o)}$CO), 129.19 (*Ph*$_{(m)}$CO), 127.94 (C-4_{pyr}), 123.53 (C-3_{pyr}), 114.40 (*C*$(CH_3)_2$), 102.20 (C-5_{ur}), 95.27 (C-$1'_{ur}$), 85.22 (C-$4'_{ur}$), 84.30 (C-$2'_{ur}$), 82.85 (C-1_{gal}), 80.47 (C-$3'_{ur}$), 74.31 (C-5_{gal}), 71.91 (C-3_{gal}), 67.18, 66.78 (C-2_{gal}, C-4_{gal}), 63.78 (C-$5'_{ur}$), 61.15 (C-6_{gal}), 31.17, 28.92 (2 × *C*H_2CO), 26.94, 25.03 (C(*C*$H_3)_2$), 20.64, 20.55, 20.52, 20.46 (4 × *C*H_3CO). ESI-HRMS: Calcd for $C_{42}H_{46}N_4O_{18}$SNa ($[M+Na]^+$): *m/z* 949.2420. Found: *m/z* 949.2438.

2.7. Compound (**14**)

2.7.1. Succinic acid 3-*N*-benzoyl-2′,3′-di-*O*-tert-butyldimethylsilyl-uridin-5′-yl ester (**9**)

To a solution of 2′,3′-*di*-O-*tert*-butyldimethylsilyl-uridine **6** (1.015 g, 1.76 mmol) in a mixture of CH_2Cl_2 (10 mL) and pyridine (3 mL) succinic anhydride (176 mg, 1.76 mmol) and DMAP (215 mg, 1.76 mmol) were added. The resulting mixture was stirred at room temperature for 6 h. After completion (TLC, 10:1 $CHCl_3$–MeOH) the reaction mixture was concentrated with toluene (3 × 10 mL) in order to remove the whole amount of pyridine. The crude product was purified on a column packed with silica gel using $CHCl_3$–MeOH 30:1 solvent system as the eluent to yield **9** as a white powder (1.048 g, 88%): mp 144–145 °C; $[\alpha]_D^{25}$ +38.9 (c 1.2, $CDCl_3$); ^{1}H NMR ($CDCl_3$): δ 10.38 (bs, 1H, COOH), 7.96–7.87 (m, 3H, Ph$_{(o)}$, H-6_{ur}), 7.64 (m, 1H, Ph$_{(p)}$), 7.52–7.44 (m, 2H, Ph$_{(m)}$), 5.97 (d, 1H, *J* 8.1 Hz, H-5_{ur}), 5.81 (d, 1H, *J* 3.9 Hz, H-$1'_{ur}$), 4.47 (m, 1H, H-5′a_{ur}), 4.31–4.18 (m, 3H, H-$2'_{ur}$, H-$3'_{ur}$, H-5′b_{ur}), 4.08 (m, 1H, H-$3'_{ur}$), 2.82–2.50 (m, 4H, CH_2), 0.89, 0.88 (2s, 18H, 2 × $(CH_3)_3$CSi), 0.06, 0.07, 0.09 (3s, 12H, CH_3Si); ^{13}C NMR ($CDCl_3$): δ 172.56 (COOH), 168.74 (CO), 162.18 (C-4_{ur}), 156.69 (CO), 149.20 (C-2_{ur}), 139.87 (C-6_{ur}), 134.99 (*Ph*$_{(p)}$CO), 131.42 (*Ph*CO), 130.45(*Ph*$_{(o)}$CO), 129.03 (*Ph*$_{(m)}$CO), 102.15 (C-5_{ur}), 89.59 (C-$1'_{ur}$), 81.42 (C-$4'_{ur}$), 75.40 (C-$2'_{ur}$), 70.86 (C-$3'_{ur}$), 62.48 (C-$5'_{ur}$), 28.85, 28.70 (2 × *C*H_2CO), 25.70 (2 × $(CH_3)_3$*C*Si), 17.92, 17.88 (2 × $(CH_3)_3$*C*Si), −4.41, −4.53, −4.87, −5.07 (4 × CH_3Si).

2.7.2. Procedure B using uridine derivative **9** for preparation of **14**

Compound **1** (114 mg, 0.25 mmol) and uridine derivative **9** (169 mg, 0.25 mmol) were submitted to general procedure B described above. The crude product was purified on a column of silica gel using $CHCl_3$–MeOH 60:1 solvent system as the eluent to yield **14** as a white solid (39 mg, 14%).

2.7.3. Procedure C using uridine derivative **9** for preparation of **14**

Compound **1** (114 mg, 0.25 mmol) and uridine derivative **9** (169 mg, 0.25 mmol) were submitted to general procedure C described above. The resulting crude product **14** was purified by column chromatography to yield **14** as a white solid (75 mg, 27%).

2.7.4. Procedure D using uridine derivatives **9** for preparation of **14**

Compound **1** (114 mg, 0.25 mmol) and uridine derivative **9** (169 mg, 0.25 mmol) were submitted to general procedure D described above. The resulting crude product **14** was purified by column chromatography to yield **14** as a white solid (142 mg, 51%): mp 143–144 °C; $[\alpha]_D^{25}$ +5.6 (c 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.49 (d, 1H, *J* 2.6 Hz, H-6_{pyr}), 7.97–7.88 (m, 3H, Ph$_{(o)}$, H-4_{pyr}), 7.82 (s, 1H, NH), 7.77(d, 1H, *J* 8.2 Hz, H-6_{ur}), 7.66 (m, 1H, Ph$_{(p)}$), 7.55–7.45 (m, 2H, Ph$_{(m)}$), 7.19 (d, 1H, *J* 8.6 Hz, H-3_{pyr}), 5.90 (d, 1H, *J* 8.2 Hz, H-5_{ur}), 5.68 (d, 1H, *J* 10.2 Hz, H-1_{glu}), 5.68 (d, 1H, *J* 3.3 Hz, H-$1'_{ur}$), 5.34 (dd ∼ t, 1H, *J* 9.2 Hz, H-3_{glu}), 5.18 (dd, 1H, *J* 9.3, 10.2 Hz, H-2_{glu}), 5.15 (dd ∼ t, 1H, *J* 9.6 Hz, H-4_{glu}), 4.47 (dd, 1H, *J* 3.9, 13.4 Hz, H-5′a_{ur}), 4.36–4.18 (m, 4H, H-$2'_{ur}$, H-$3'_{ur}$, H-6a_{glu}, H-5′b_{ur}), 4.14–4.04 (m, 2H, H-$3'_{ur}$, H-6b_{glu}), 3.86 (ddd, 1H, *J* 2.1, 4.3, 9.9 Hz, H-5_{glu}), 2.84–2.59 (m, 4H, 2 × CH_2), 2.04, 2.03, 2.02 (3s,

12H, 4 × CH_3CO), 0.89, 0.88 (2s, 18H, 2 × $(CH_3)_3CSi$), 0.10, 0.08, 0.07, 0.06 (4s, 12H, 4 × CH_3Si); ^{13}C NMR ($CDCl_3$): δ 172.22, 170.65, 170.12, 169.69, 169.50, 169.41, 168.72 (CO), 162.16 (C-4_{ur}), 149.67 (C-2_{pyr}), 149.04 (C-2_{ur}), 140.97 (C-6_{pyr}), 140.37 (C-6_{ur}), 135.23 ($Ph_{(p)}CO$), 132.69 (C-5_{pyr}), 131.28 ($PhCO$), 130.49 ($Ph_{(o)}$-CO), 129.16 ($Ph_{(m)}CO$), 128.11 (C-4_{pyr}), 123.61 (C-3_{pyr}), 102.03 (C-5_{ur}), 91.42 (C-$1'_{ur}$), 82.31 (C-1_{glu}), 81.31 (C-$4'_{ur}$), 75.82 (C-5_{glu}), 74.91 (C-$2'_{ur}$), 74.06 (C-3_{glu}), 70.87 (C-$3'_{ur}$), 69.50 (C-2_{glu}), 68.20 (C-4_{glu}), 62.86 (C-$5'_{ur}$), 61.89 (C-6_{glu}), 31.35 (CH_2COO), 28.96 (CH_2CONH), 25.72 ($(CH_3)_3CSi$), 20.70, 20.65, 20.58 (4 × CH_3CO), 17.96, 17.92 (2 × $(CH_3)_3CSi$), −4.34, −4.46, −4.81, −5.05 (4 × CH_3Si). ESI-MS: Calcd for $C_{51}H_{70}N_4O_{18}SSi_2Na$ ($[M+Na]^+$): *m/z* 1137.4. Found: *m/z* 1137.3.

3. Results and discussion

Taking into account previously mentioned structural and stability requirements for glycosyltransferases sugar donor analogs we would like to present here compounds consisting of variously protected 5'-uridine derivatives connected with (5-amino-2-pyridyl) 1-thio-β-D-glycosides **1** or **2** (Fig. 1) with a succinic linker, which together with the pyridyl ring is expected to mimic the pyrophosphate bridge (Scheme 1).

For the preparation of amine **1** or **2** (5-nitro-2-pyridyl) 1-thioglycosides were used as starting compounds and nitro group reduction procedure with zinc powder/acetic acid system in CH_2Cl_2 described by Roy and co-workers for 4-nitrophenyl 1-thioglycosides was applied [28]. (5-Amino-2-pyridyl) 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glycoside derivatives of D-glucose and D-galactose were obtained in a good yields (86% and 73%, respectively).

Uridine derivatives **7–9** served as the second structural component of glycoconjugates **10–14**. They were first selectively protected at 2′ and 3′-positions in ribose and at N^3-position in uracil ring. For protection of hydroxyl groups isopropylidene or *tert*-butyldimethylsilyl (TBDMS) groups were chosen, while for protection of uracil nitrogen benzoyl group was used. Having selectively protected uridine derivatives **3–6**, we converted them into 5-succinic acid monoesters by acylation reaction with succinic anhydride in dry pyridine or in CH_2Cl_2 (choice of solvent was dependent of substrate solubility). Reaction proceeded at room temperature in

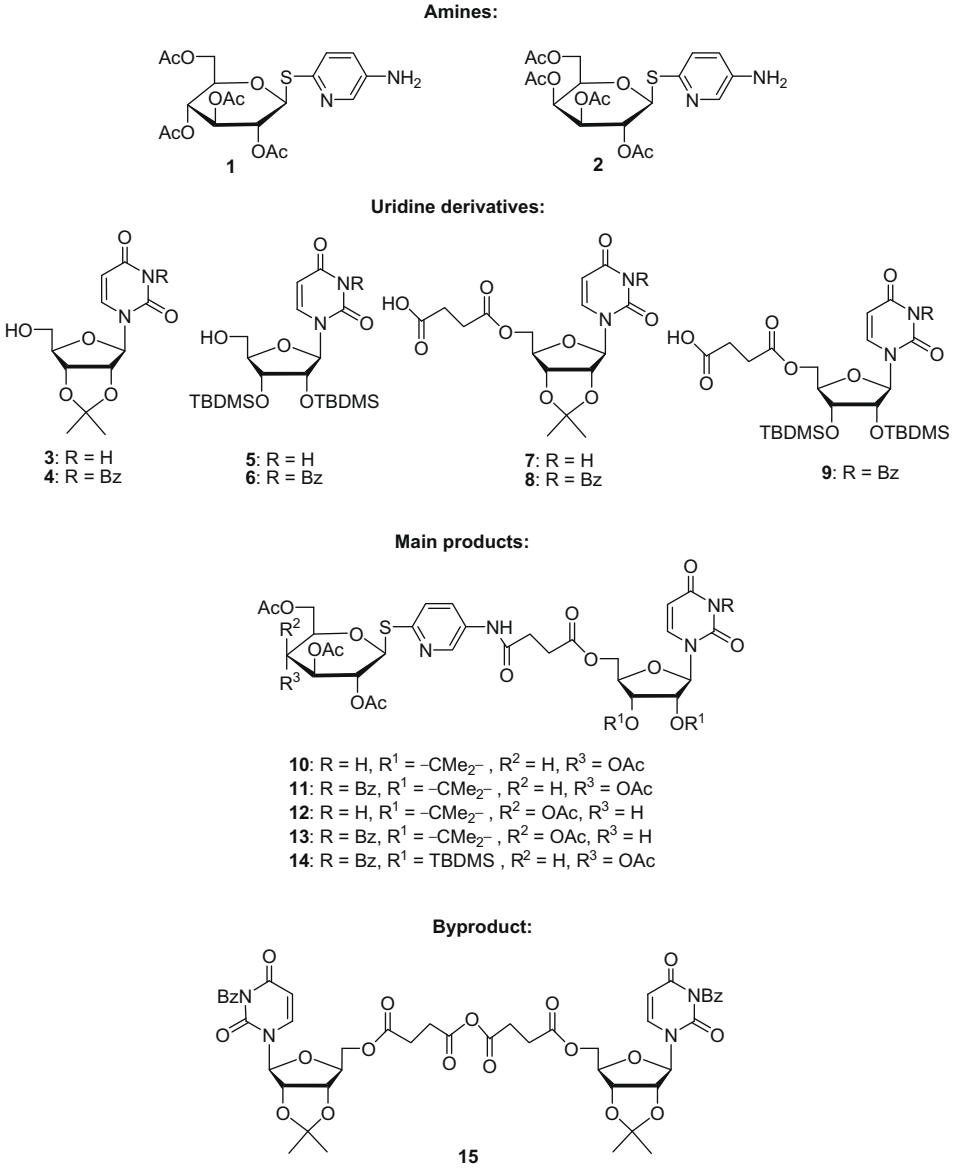
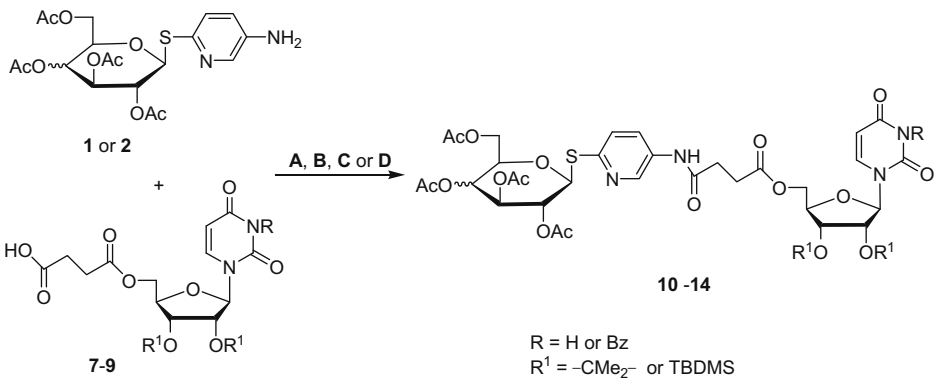


Fig. 1. Structures of reagents and products.



Scheme 1. Synthesis of compounds **11**–**14**.

the presence of DMAP [29]. ^{1}H NMR and ^{13}C NMR spectra proved the structures of uridine derivatives **7–9**. Succinyl methylene protons gave multiplets at approximately 2.50–2.80 ppm. Appearance of a broad signal corresponding to carboxylic proton also confirmed products structure. Desired products of esterification reactions **7**-**9** were obtained in satisfactory yields of 72%, 48% and 88%, respectively.

With the amines and uridine derivatives of succinic acid in hand, different methods for construction of an amide bond between these building blocks were tested next. Although amide bond formation between an acid and amine is formally a condensation, yet mixing an amine with a carboxylic acid results in an acid–base reaction and formation of a stable salt. The direct condensation becomes feasible at high temperature (above 150 °C), which may be troublesome when other sensitive functionalities are present within coupled compounds. Therefore, activation of carboxylic acid seems to be necessary [30]. There are numerous commercially available coupling reagents for construction of amide bond including carbodiimides alone [31] or plus additives such as HOBt or DMAP [32,33]. The carbodiimide reacts with the carboxylic acid to form O-acylisourea mixed anhydride which can react directly with amine to yield the desired amide. Unfortunately, isomerisation of reactive *O*-acylisourea into unreactive N-acylurea may also be observed. Addition of nucleophiles such as DMAP or HOBt hampers the side reaction [28]. We applied DCC/DMAP condensing system for coupling amine **1** with uridine derivative **8** with $CHCl_3$ as a solvent. Reaction was carried out at room temperature for 48 h (Table 1, procedure A). The desired product **11** was isolated in only 5% yield. We also isolated symmetrical anhydride **15** in a 34% yield. When $CHCl_3$ was replaced with pyridine (procedure B) the reaction yield increased but N-benzoyl protection in uracil ring was not stable enough under such conditions. Glycoconjugates **10** and **12** were isolated in a 23% and 10% yields, respectively (Table 1, entries 3 and 5). When uridine derivative of succinic acid **9** with silyl protective groups was used, debenzoylation of uracil nitrogen was no longer observed but the yield of glycoconjugate **14** was far from satisfactory (Table 1, entry 4). Interestingly, when uridine derivative **7** with unprotected uracil nitrogen was applied any significant amount of product could not be isolated. Only TLC analysis showed traces of product **10** (Table 1, entry 2) and finally, this experiment proved the necessity of uracil nitrogen protection.

Low yield of glycoconjugates induced us to search for another method of amide bond formation. We tried to apply uridine ethoxycarbonyl anhydride which can be generated using ethyl chloroformate [34]. Treatment of uridine derivatives of succinic acid **8** or **9** with 1 equivalent each of ethyl chloroformate and triethylamine in CH_2Cl_2 at room temperature gave the mixed anhydrides, which were condensed, without isolation, with amine **1** to give the corresponding amides **11** or **14** as white solids in a 40% and 27% yields, respectively (Table 1, entries 6 and 7).

Kaminski and co-workers showed the efficiency of 2-chloro-4,6-disubstituted-1,3,5-triazines in formation of the peptide bond [35]. Activated ester resulting from reaction of carboxylic acid with triazine derivative (“superactive ester”) contains an excellent leaving group which is displaced by the amine. Such reaction requires the presence of a tertiary amine in the reaction medium. A range of tertiary amines were tested and the best results were obtained when *N*-methylmorpholine (NMM) was applied [36]. Kunishima and co-workers found that 4-(4,6-dimethoxy-(1,3,5)triazin-2-yl)-4-methyl-morpholinium chloride (DMT-MM) can be formed and isolated in THF and then used as an efficient condensing agent facilitating formation amides and esters. They tested stability of this compound in different solvents because DMT-MM is susceptible to demethylation at the morpholinium nitrogen. They showed that only small amount of demethylation product was formed when THF or MeOH was used as a solvent [37]. Taking advantage of these information we performed condensation reactions amine **1** or **2** with uridine derivatives **7**–**9** using equimolar amount of DMT-MM as condensing agent. Reactions were carried out in THF at room temperature for 24–48 h. We obtained expected products **10**–**14** in yields superior to those obtained in case of using other condensing procedures (Table 1, entries 8-12). Additionally, no debenzoylation products were observed, so a mild and effective method for amide bond preparation in desired glycoconjugates was found. All glycoconjugates were purified by column chromatography and their structures were elucidated by ^{1}H and ^{13}C NMR data (including ^{1}H, ^{13}C HETCOR experiments and simulations analysis) and mass spectrometry analysis.

Biological evaluations of obtained glycoconjugates **10, 12** and **14** were performed by Szewczyk’s team [38,39]. These compounds were tested for the ability to inhibit the propagation of classical swine fever virus (CSFV). After adsorption with CSFV virus swine kidney cells SK6 were washed, replenished with medium containing varying amounts of glycoconjugates **10, 12** or **14** (10–140 μg/ml) and incubated for 2 days. The extent of CSFV infection in these cells was measured by immunoperoxidase Monolayer Assay. It was observed that compounds **10**, **12** and **14** reduced the number of CSFV-infected cells in dose-dependent manner without significant toxicity for mammalian cells. The best results were obtained for compound **14** (reduction of CSFV-infected cells was observed for dose 30 μg/ml of compound **14** at 82% survival of mammalian

cells) [40]. Detailed results of these investigations will be published after patent proceedings are completed.

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