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Synthesis of 2-deoxy-hexopyranosyl derivatives of uridine as donor substrate analogues for glycosyltransferases

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ABSTRACT

A series of 2-deoxy-hexopyranosyl derivatives of uridine have been synthesized as analogues of UDP-sugar. These compounds were tested as inhibitors against bovine β-1,4-galactosyltransferase I in fluorescent assays and showed no significant inhibition.

1. Introduction

Oligosaccharide chains in glycoconjugates play important roles in molecular recognition processes including adhesion in inflammation, bacterial or viral infection, tumor metastasis and immune response [1]. Construction of glycosidic linkages in the biosynthesis of oligosaccharides, glycolipids, glycoproteins and other glucoconjugates is catalysed by glycosyltransferases (GTs), usually through the Leloir pathway [2]. These enzymes catalyse the transfer of a monosaccharide unit from an activated nucleotide sugar donor to the hydroxyl group of an acceptor, which is a growing oligosaccharide, a lipid or a protein. Since GTs are responsible for the synthesis of glycoconjugates, modulation of their activities has potential for the control of certain cellular functions and make them desirable targets for inhibition. Identification of potent inhibitors has been developing very rapidly during the last two decades since the 3D structure of several GTs were determined [3–5] and catalytic mechanism proposed [6]. Different approaches based on analogies with donor substrates, acceptor substrates and transition state, respectively, have been used to design potent inhibitors of GTs [7–10]. Although many compounds have been designed and synthesized, only few of them exhibited significant activity against GTs. Among them the development of donor substrate analogues has received considerable attention. These compounds must display K_is value of the same order of magnitude as that of K_M. They feature some structural changes at the carbohydrate part or at the pyrophosphate linkage. Examples of inhibitors of one of the most extensively studied β-1,4-galactosyltransferase *I* (β4GalT I, K_M (UDP-Gal) = 44 μM [11a]) with modification of the carbohydrate part are: carbacyclic analogue of UDP-galactose (K_i 58 μM) [11b], UDP-2-deoxy-2-fluorogalactose (K_i 149 μM) [11c] or 2″-, 3″-, 4″- and 6″-methyl derivatives of UDP-galactose (K_i 44–270 μM) [11d]. Interestingly, UDP-fucose and UDP-mannose are also powerful inhibitors of β4GalT I (K_i 10.0 and 8.8 μM, respectively) [11d]. The pyrophoshpate group is known to bind strongly to a divalent cation, most commonly Mn^{2+}, in the enzyme active site. Some pyrophosphate analogues were obtained by varying the diphosphate moiety, resulting in higher stability towards enzymatic hydrolysis. The reported inhibitors of β4GalT I of that type are: glycosyl phosphonate [12a], methylenediphosphonate [12b] or C-glycosyl ethyl phosphonophosphate [13]. It is believed that replacement of pyrophosphate group is possible, for example natural glycosyltransferase inhibitor, such as tunicamycin is thought to use a sugar ring to mimic the pyrophosphate group [14]. Several synthetic analogues containing monosaccharide moieties acting as pyrophosphate-metal ion complex mimics were synthesized as GTs inhibitors [15–17]. Some examples with biological data are presented in Fig. 1.

Encouraged by the reports mentioned above, we made another attempt at using monosaccharide ring as pyrophosphate mimic in the synthesis of donor substrate analogues. In our study we report on efficient and stereoselective synthesis of 2-deoxy monosaccharides and disaccharides coupled with the uridine part, which according to our idea is treated as a necessary moiety for the recognition and proper binding in the active site. This concept is in accordance with saturation transfer difference (STD) NMR spectroscopy experiments applied to map the binding epitope of UDP-Gal and UDP-Glc bound to β4GalT I with atomic resolution [18]. These experiments revealed that binding of a donor substrate is essentially controlled by the uridine part. In both cases, the

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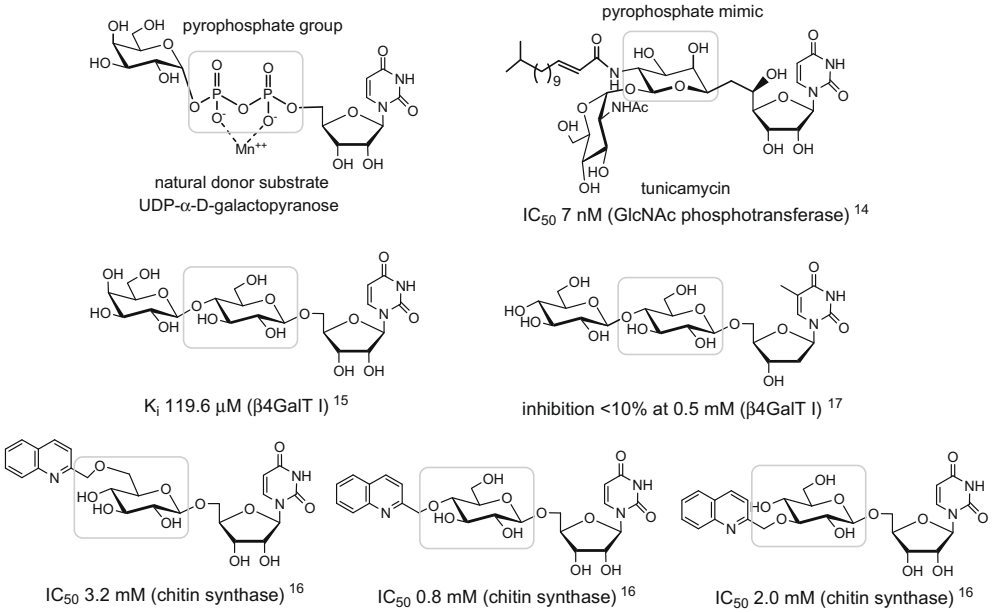


Fig. 1. Examples of glycosyltransferase inhibitors containing monosaccharide ring as a pyrophosphate mimic.

anomeric proton H-1 of the ribose ring and H-5 of the uracil received the largest amount of saturation transfer. This indicates that the uridine residue is in tight contact with the active site of the protein. The galactose protons show smaller STD effects, which suggests a minor role of this residue in the recognition step. For UDP-Glc in the presence of β4GalT I, STD effect was also observed, although only the uridine part received saturation transfer, the glucose protons give no STD responses. This is in accordance with the fact that β4GalT I does not process UDP-Glc at a significant rate [19]. Now, we propose compounds presented in Fig. 2 as potential inhibitors containing the uridine moiety, which is supposed to exhibit significant binding affinity for the enzyme. We hope that 2-deoxy-D-gluco- and 2-deoxy-D-galactopyranosyl residues can provide additional binding with protein, not necessary specific for glycosyl transfer, which is not expected.

2. Experimental

2.1. General

Melting points were determined on a Boetius apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer 141 polarimeter using a sodium lamp (589 nm) at room temperature. NMR spectra were recorded with a Varian spectrometer at a frequency of 600 MHz with Me_4Si as internal reference in CD_3OD as a solvent. Mass spectra were recorded in the positive mode on a Mariner (Perseptive Biosystem) detector using the electrospray-ionisation (ESI) technique.

Reactions were monitored by TLC on precoated plates of silica gel 60 F_{254} (Merck), and visualising using UV light (254 nm or 366 nm) and/or by charring with 10% sulphuric acid in ethanol.

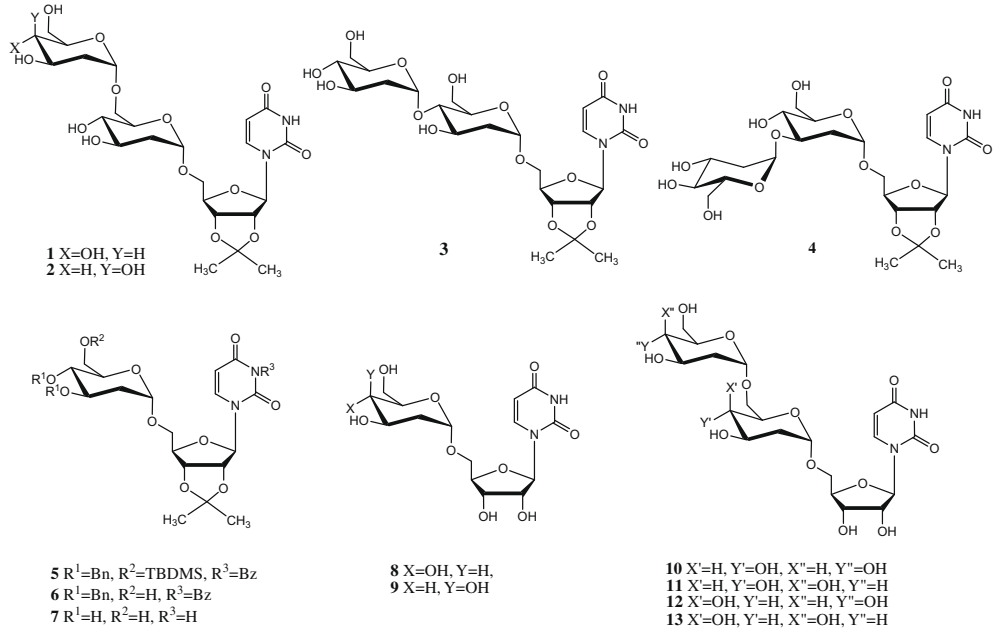


Fig. 2. 2-Deoxy-hexopyranosyl derivatives of uridine as donor substrate analogues for glycosyltransferases.

Column chromatography was performed on silica gel 60 (70–230 mesh, Merck) developed with one of the hexane/EtOAc or $CHCl_3$/MeOH solvent systems. All evaporations were performed under diminished pressure at 50 °C. Reversed phase HPLC analyses were performed using Perkin–Elmer Series 200 apparatus equipped with a reverse phase column (Aquapore OD-300, 7 μm, 4.6 × 250 mm; 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.

N^3,2′,3′-*O*-tris-(benzyloxycarbonyl)uridine (**18**) [20], 3,4-di-O-benzyl-6-O-*tert*-butyldimethylsilyl-D-glucal (**14**) and 3,4-di-O-benzyl-6-O-*tert*-butyldimethylsilyl-D-galactal (**15**) [21], 3,4,6-tri-*O*-benzyl-D-glucal (**16**) and 3,4,6-tri-*O*-benzyl-D-galactal (**17**) [22] were prepared according to the published procedures. Uridine 5′-diphosphogalactose disodium salt (UDP-Gal) and other chemicals were purchased from Aldrich and Fluka Chemical Companies and were used without purification. Bovine milk β-1,4-galactosyltransferase I (β4GalT I) was purchased from Sigma at 1 U/mg. Solvents were dried and stored over molecular sieves (4 Å) under an inert atmosphere.

2.2. General synthetic procedures

2.2.1. Procedure A

To a solution of glycal **14**, **15**, **16** or **17** (0.30 mmol) and uridine derivative **18**, **23** or **24** (0.30 mmol) in dry CH_2Cl_2 (5 mL) a catalytic amount of TPHB (10 mg, 0.03 mmol) was added. The mixture was kept at room temperature for 1–20 h. Then the reaction mixture was concentrated to give crude product purified directly by column chromatography with hexane/AcOEt 2:1 solvent system to yield adducts **19**, **20**, **21**, **22**, **25**, **26**, **27**, **28** as oils.

2.2.2. Procedure B

Desilylation of adduct **19** or **20** (0.20 mmol) was achieved with AcCl (16 μL, 0.20 mmol) in a MeOH/$CHCl_3$ 3:1 solvent system (8 mL) at room temperature within 15 min. The reaction mixture was neutralized with a basic resin, Amberlyst 21 (OH^-), filtered and concentrated to give crude product **23** or **24** as an oil.

2.2.3. Procedure C

A solution of **21**, **22**, **25, 26, 27** or **28** (0.10 mmol) in a 1:6:2 mixture of cyclohexene/EtOH/THF (10 mL) was heated under reflux in the presence of $Pd(OH)_2$/C (75 mg) for 30 min. After removal of the catalyst by filtration the reaction mixture was concentrated and crude product was purified by column chromatography with $CHCl_3$/MeOH 5:1 → 2:1 solvent system to yield final products **8**–**13** as white solids.

Compound **8** was synthesized in 60% overall yield and identified in our recent report [20].

2.3. 2-deoxy-α-D-galactopyranosyl – (1 → 5)- uridine (*9*)

Glycal **17** (125 mg, 0.30 mmol) and uridine derivative **18** (232 mg, 0.30 mmol) were submitted to general procedure A (reaction time 1 h). The resulting adduct **22** (230 mg, 0.20 mmol) was deprotected according to the general procedure C to yield **9** as a white solid (68 mg, 58% overall yield): mp 130–132 °C; $[\alpha]_D^{20}$ + 34.5 (MeOH, c 0.5); ^{1}H NMR: δ 1.79 (ddd, 1H, *J* 1.0, 13.1, 5.0 Hz, H-2″eq), 2.04 (ddd, 1H, *J* 3.7, 12.2, 13.1 Hz, H-2″ax), 3.66–3.80 (m, 5H, H-5′a, H-4″, H-5″, H-6″a,b), 3.89–3.95 (m, 2H, H-5′b, H-3″), 4.11–4.16 (m, 3H, H-2′, H-3′, H-4′), 5.01 (dd, 1H, *J* 1.1, 3.7 Hz, H-1″), 5.74 (d, 1H, *J* 8.1 Hz, H-5), 5.90 (d, 1H, *J* 3.9 Hz, H-1′), 7.98 (d, 1H, *J* 8.1 Hz, H-6); ^{13}C NMR: δ 33.72 (C-2″), 63.36 (C-6″), 66.74 (C-3″), 67.25 (C-5′), 69.57 (C-4″), 71.38 (C-3′), 73.00 (C-5″), 76.10 (C-2′), 84.47 (C-4′), 90.88 (C-1′), 99.15 (C-1″), 102.57 (C-5), 142.04 (C-6), 152.38 (C-2), 166.18 (C-4); ESI-HRMS: Calcd for $C_{15}H_{22}N_2O_{10}Na$ ([M + Na]$^+$): *m/z* 413.1167, found: *m/z* 413.1151.

2.4. 2-deoxy-α-D-glucopyranosyl-(1 → 6)-2-deoxy-α-D-glucopyranosyl-(1 → 5)- uridine (*10*)

Glycal **14** (132 mg, 0.30 mmol) and uridine derivative **18** (232 mg, 0.30 mmol) were submitted to general procedure A (reaction time 1 h). The resulting adduct **19** (273 mg, 0.24 mmol) was desilylating according to the general procedure B and submitted to the next addition step with glycal **16** (85 mg, 0.20 mmol) according to the general procedure A (reaction time 3 h). Deprotection of the resulting adduct **25** (165 mg, 0.12 mmol) according to the procedure C yielded **10** as a white solid (59 mg, 35% overall yield): mp 132–135 °C; $[\alpha]_D^{20}$ + 58.0 (MeOH, c 0.5); ^{1}H NMR: δ 1.61 (ddd, 1H, *J* 3.6, 11.7, 13.0 Hz, H-2‴ax), 1.71 (ddd, 1H, *J* 3.7, 11.8, 13.4 Hz, H-2″ax), 2.09 (ddd, 1H, *J* ∼0, 5.1, 13.4 Hz, H-2″eq), 2.11 (ddd, 1H, *J* ∼0, 5.1, 13.0 Hz, H-2‴eq), 3.27 (t, 1H, *J* 9.3 Hz, H-4‴), 3.33 (dd, 1H, *J* 9.7, 9.2 Hz, H-4″), 3.60–3.74 (m, 5H, H-5′a, H-5″, H-5‴, H-6″a, H-6‴a), 3.76–3.88 (m, 4H, H-3″, H-3‴, H-6″b, H-6‴b), 3.73 (dd, 1H, *J* 2.4, 11.1 Hz, H-5′b), 4.14 (dd, 1H, *J* 3.7, 4.4 Hz, H-2′), 4.15–4.19 (m, 2H, H-3′, H-4′), 4.95, 4.97 (2 dd, 2H, H-1″, H-1‴), 5.76 (d, 1H, *J* 8.1 Hz, H-5), 5.89 (d, 1H, *J* 3.7, Hz, H-1′), 7.98 (d, 1H, *J* 8.1 Hz, H-6); ^{13}C NMR: δ 38.82, 38.82 (C-2″, C-2‴), 62.82 (C-6‴), 66.98, 66.98 (C-5′, C-6″), 71.28 (C-3′), 70.01, 70.16, 72.99, 73.08, 73.28, 73.88 (C-3″, C-4″, C-5″, C-3‴, C-4‴, C-5″), 76.05 (C-2′), 84.31 (C-4′), 91.03 (C-1′), 98.49, 98.75 (C-1″, C-1‴), 102.59 (C-5), 142.05 (C-6), 152.24 (C-2), 166.17(C-4); ESI-HRMS: Calcd for $C_{21}H_{32}N_2O_{14}Na$ ([M + Na]$^+$): *m/z* 559.1746, found: *m/z* 559.1720.

2.5. 2-deoxy-α-D-galactopyranosyl-(1 → 6)-2-deoxy-α-D-glucopyranosyl-(1 → 5)- uridine (*11*)

Glycal **14** (132 mg, 0.30 mmol) and uridine derivative **18** (232 mg, 0.30 mmol) were submitted to general procedure A (reaction time 1 h). The resulting adduct **19** (273 mg, 0.24 mmol) was desilylating according to the general procedure B and submitted to the next addition step with glycal **17** (85 mg, 0.20 mmol) according to the general procedure A (reaction time 20 h). Deprotection of the resulting adduct **26** (141 mg, 0.10 mmol) according to the procedure C yielded **11** as a white solid (40 mg, 25% overall yield): mp 142–145 °C; $[\alpha]_D^{20}$ + 67.5 (MeOH, c 0.5); ^{1}H NMR: δ 1.71 (ddd, 1H, *J* 3.7, 11.8, 13.4 Hz, H-2″ax), 1.80 (ddd, 1H, *J* 0.8, 4.9, 12.7 Hz, H-2‴eq), 1.94 (ddd, 1H, *J* 3.7, 11.9, 12.7 Hz, H-2‴ax), 2.10 (ddd, 1H, *J* 1.0, 5.1, 13.4 Hz, H-2″eq), 3.32 (t, 1H, *J* 9.8 Hz, H-4″), 3.64 (ddd, 1H, *J* 2.0, 5.4 9.8 Hz, H-5″), 3.66–3.74 (m, 4H, H-5′a, H-6″a, H-6‴a, H-6‴b), 3.76–3.82 (m, 2H, H-3″, H-4‴), 3.83–3.88 (m, 2H, H-5‴, H-6″b), 3.96 (ddd, 1H, *J* 3.0, 4.9, 11.9 Hz, H-3‴), 3.90 (dd, 1H, *J* 2.5, 11.2, Hz, H-5′b), 4.14 (dd, 1H, *J* 3.7, 4.4 Hz, H-2′), 4.15–4.19 (m, 2H, H-3′, H-4′), 4.97, 4.98 (2 dd, 2H, H-1″, H-1‴), 5.75 (d, 1H, *J* 8.1 Hz, H-5), 5.88 (d, 1H, *J* 3.7, Hz, H-1′), 7.98 (d, 1H, *J* 8.1 Hz, H-6); ^{13}C NMR: δ 33.63 (C-2‴), 38.86 (C-2″), 63.19 (C-6‴), 66.58 (C-5′), 66.96 (C-3‴), 67.02 (C-6″), 69.66 (C-4‴), 70.12 (C-3″), 71.26 (C-3′), 72.33 (C-5‴), 73.04 (C-5″), 73.07 (C-4″), 76.06 (C-2′), 84.30 (C-4′), 91.06 (C-1′), 98.78, 98.81 (C-1″, C-1‴), 102.56 (C-5), 142.07 (C-6), 152.33 (C-2), 166.19 (C-4); ESI-HRMS: Calcd for $C_{21}H_{32}N_2O_{14}Na$ ([M + Na]$^+$): *m/z* 559.1746, found: *m/z* 559.1731.

2.6. 2-deoxy-α-D-glucopyranosyl-(1 → 6)-2-deoxy-α-D-galactopyranosyl-(1 → 5)-uridine (*12*)

Glycal **15** (132 mg, 0.30 mmol) and uridine derivative **18** (232 mg, 0.30 mmol) were submitted to general procedure A (reaction time 1 h). The resulting adduct **20** (217 mg, 0.20 mmol) was

desilylating according to the general procedure B and submitted to the next addition step with glycal **16** (70 mg, 0.17 mmol) according to the general procedure A (reaction time 20 h). Deprotection of the resulting adduct **27** (143 mg, 0.10 mmol) according to the procedure C yielded **12** as a white solid (48 mg, 30% overall yield): mp 134–136 °C; $[\alpha]_D^{20}$ + 52.7 (MeOH, c 0.5); ^{1}H NMR: δ 1.61 (ddd, 1H, *J* 3.7, 11.6, 13.0 Hz, H-2‴ax), 1.80 (ddd, 1H, *J* 1.0, 5.0, 13.0 Hz, H-2″eq), 2.03 (ddd, 1H, *J* 3.6, 12.2, 13.0 Hz, H-2″ax), 2.07 (ddd, 1H, *J* 1.3, 5.2, 13.0 Hz, H-2‴eq), 3.25 (dd, 1H, *J* 9.0, 9.8 Hz, H-4‴), 3.58–3.63 (m, 2H, H-5‴, H-6″a), 3.67–3.74 (m, 2H, H-5′a, H-6‴a), 3.77 (bd, 1H, *J* 3.0 Hz, H-4″), 3.79–3.85 (m, 3H, H-3‴, H-6″b, H-6‴b), 3.87–3.96 (m, 3H, H-3″, H-5′b, H-5″), 4.12–4.19 (m, 3H, H-2′, H-3′, H-4′), 4.94 (dd, 1H, *J* 1.3, 3.7 Hz, H-1‴), 4.99 (dd, 1H, *J* 1.0, 3.6 Hz, H-1″), 5.76 (d, 1H, *J* 8.1 Hz, H-5), 5.89 (d, 1H, *J* 4.0 Hz, H-1′), 7.98 (d, 1H, *J* 8.1 Hz, H-6); ^{13}C NMR: δ 33.68 (C-2″), 38.86 (C-2‴), 62.85 (C-6‴), 66.67 (C-3″), 67.15 (C-5′), 67.88 (C-6″), 69.50 (C-4″), 69.95 (C-3‴), 70.89 (C-5″), 71.36 (C-3′), 73.27 (C-4‴), 74.05 (C-5‴), 76.04 (C-2′), 84.34 (C-4′), 90.97 (C-1′), 98.69 (C-1‴), 98.99 (C-1″) 102.59 (C-5), 142.10 (C-6), 152.34 (C-2), 166.16 (C-4); ESI-HRMS: Calcd for $C_{21}H_{32}N_2O_{14}Na$ ([M + Na]$^+$): *m/z* 559.1746, found: *m/z* 559.1734.

2.7. 2-deoxy-α-D-galactopyranosyl-(1 → 6)-2-deoxy-α-D-galactopyranosyl-(1 → 5)-uridine (*13*)

Glycal **15** (132 mg, 0.30 mmol) and uridine derivative **18** (232 mg, 0.30 mmol) were submitted to general procedure A (reaction time 1 h). The resulting adduct **20** (217 mg, 0.20 mmol) was desilylating according to the general procedure B and submitted to the next addition step with glycal **17** (70 mg, 0.17 mmol) according to the general procedure A (reaction time 20 h). Deprotection of the resulting adduct **28** (108 mg, 0.08 mmol) according to the procedure C yielded **13** as a white solid (36 mg, 22% overall yield): mp 146–149 °C; $[\alpha]_D^{20}$ + 23.2 (MeOH, c 0.25); ^{1}H NMR: δ 1.75–1.82 (2 ddd, 2H, H-2″eq, H-2‴eq), 1.94 (ddd, 1H, *J* 3.7, 11.9, 12.7 Hz, H-2‴ax), 2.03 (ddd, 1H, *J* 3.6, 12.2, 13.0 Hz, H-2″ax), 3.57–3.63 (2 dd, 2H, H-6″a, H-6‴a), 3.66–3.76 (m, 2H, H-5′a, H-5″), 3.76–3.84 (m, 4H, H-4″, H-4‴, H-5‴, H-6″b), 3.87–3.96 (m, 4H, H-3″, H-3‴, H-5′b, H-6‴b), 4.12–4.19 (m, 3H, H-2′, H-3′, H-4′), 4.97 (bd, 1H, *J* 3.5 Hz, H-1″), 5.00 (bd, 1H, *J* 3.5 Hz, H-1‴), 5.75 (d, 1H, *J* 8.1 Hz, H-5), 5.89 (d, 1H, *J* 3.8 Hz, H-1′), 7.98 (d, 1H, *J* 8.1 Hz, H-6); ^{13}C NMR: δ 33.69, 33.69 (C-2″, C-2‴), 63.24 (C-6‴), 66.66, 66.69, 67.10, 67.88, 69.48, 69.67, 70.90, 71.37, 72.49 (C-3′, C-5′, C-3″, C-4″, C-5″, C-6″, C-3‴, C-4‴, C-5‴), 76.04 (C-2′), 84.35 (C-4′), 91.03 (C-1′), 99.01, 99,05 (C-1′, C-1‴), 102.58 (C-5), 142.08 (C-6), 152.34 (C-2), 166.15 (C-4); ESI-HRMS: Calcd for $C_{21}H_{32}N_2O_{14}Na$ ([M + Na]$^+$): *m/z* 559.1746, found: *m/z* 559.1737.

2.8. 6-N-(dansylamino)hexyl 2-acetamido-2-deoxy-β-D-glucopyranoside (β-GlcNAc-O-(CH$_2$)$_6$-dansyl)

6-N-(Benzyloxycarbonylamino)hexyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside [23] (232 mg, 0.40 mmol)) was deacetylated with 0.033 M solution of MeONa in methanol (30 mL) at room temperature within 20 h. The reaction mixture was neutralized with an acidic resin, Dowex 50WX8 (H$^+$), filtered and concentrated. The residue was dissolved in EtOH (10 mL) then cyclohexene (2 mL) and Pd(OH)$_2$/C (150 mg) were added. The solution was heated under reflux for 5 min. After removal of the catalyst by filtration the reaction mixture was concentrated and the residue was dissolved in 0.033 M solution of Na_2CO_3 in water (25 mL). To the resulting solution suspension of dansyl chloride (108 mg, 0.40 mmol) in aceton (8 mL) was added dropwise. The reaction mixture was stirred at room temperature for 1 h. Then the reaction mixture was passed through the column with macroreticular strong base anion exchange resin Amberlyst A-26 (10 mL). The resin was washed with methanol (180 mL) and drips containing product were concentrated. TLC analysis was performed in $CHCl_3$/MeOH 4:1 solvent system and product was visualised using UV light (366 nm): R_F = 0.4. The crude product was purified by column chromatography with $CHCl_3$/MeOH 25:1 → 10:1 solvent system to yield final product as a yellowish solid (116 mg, 52%): mp 86–89 °C; $[\alpha]_D^{20}$ − 3.1 (MeOH, c 0.5); ^{1}H NMR: δ 1.05–1.20 (m, 4H, $(CH_2)_2$); 1.23–1.39 (m, 4H, $(CH_2)_2$), 1.93 (s, 3H, Ac); 2.82 (t, 2H, *J* 6.8 Hz, CH_2NH); 2.85 (s, 6H, N$(CH_3)_2$); 3.25 (ddd, 1H, *J* 2.3, 5.7, 9.6 Hz, H-5); 3.31 (m, 2H, H-4, OCH$_2$a); 3.45 (dd, 1H, *J* 8.7, 10.2 Hz, H-3); 3.61 (dd, 1H, *J* 8.4, 10.2 Hz, H-2); 3.68 (dd, 1H, *J* 5.7, 11.9 Hz, H-6a); 3.75 (m, 1H, OCH$_2$b); 3.87 (dd, 1H, *J* 2.3, 11.9 Hz, H-6b); 4.34 (d, 1H, *J* 8.4 Hz, H-1); 7.25 (dd, 1H, *J* 0.7, 7.6 Hz, H-dansyl); 7.55 (dd, 1H, *J* 7.3, 8.5 Hz, H-dansyl); 7.57 (dd, 1H, *J* 7.6, 8.6 Hz, H-dansyl); 8.18 (dd, 1H, *J* 1.2, 7.3 Hz, H-dansyl); 8.36 (ddd, 1H, *J* 0.7, 1.2, 8.6 Hz, H-dansyl); 8.54 (d, 1H, *J* 8.5 Hz, H-dansyl); ^{13}C NMR: δ 23.08 (CH_3CO); 26.53, 27.19, 30,33, 30,57 (C-hexyl); 43.86 (CH_2NH); 45.85 (N$(CH_3)_2$); 57.89 (C-2); 62.81 (C-6); 70.34 (OCH$_2$); 72.16 (C-4); 76.06 (C-3); 77.87 (C-5); 102.65 (C-1); 116.42, 120.61, 124.31, 129.08, 130.11, 131,09 (C-dansyl), 130.99, 131.20, 137.20, 153.167 (C_q-dansyl), 173.62 CH_3CO); ESI-HRMS: Calcd for $C_{26}H_{40}N_3O_8S$ ([M + H]$^+$): *m/z* 554.2531, found: *m/z* 554.2545.

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

β4GalT I activity was assayed using UDP-Gal as glycosyl donor and β-GlcNAc-O-(CH$_2$)$_6$-dansyl as glycosyl acceptor as described previously [13]. Assays were performed in a total volume of 100 μl. The reaction mixtures contained reagents in the following final concentrations: 50 mM Hepes buffer (pH 7.4), 10 mM $MnCl_2$, 0.2 mg/ml BSA, 200 μM β-GlcNAc-O-(CH$_2$)$_6$-dansyl, 40 μM UDP-Gal and potential inhibitors **1**–**13** at a range of concentrations from 0 mM (control) to 2.4 mM. The enzymatic reactions were started by the addition of 0.2 mU β4GalT 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).

3. Results and discussion

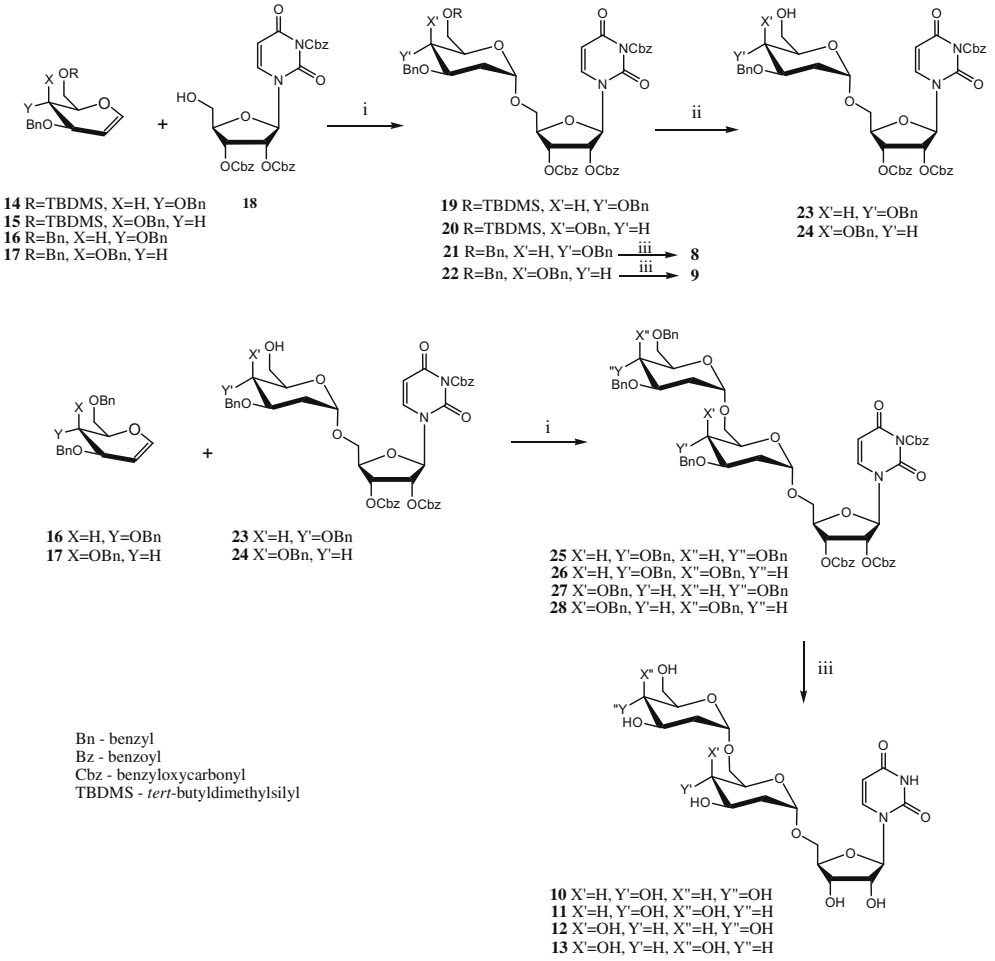
Recently, we have synthesized 2-deoxy-hexopyranosyl derivatives of 2,3-O-isopropylidene-uridine as donor substrate analogues of glycosyltransferases [24] (compounds **1–7**, Fig. 2). These compounds were composed of 2,3-O-isopropylidene-uridine and one or two residues of 2-deoxy-α-D-glucopyranose or 2-deoxy-α-D-galactopyranose. The central 2-deoxy-α-D-glucopyranose moiety replacing the key pyrophosphate group was linked with terminal 2-deoxy-O-glycosyl moiety through α-(1 → 3)-, α-(1 → 4)- or α-(1 → 6)-linked glycosidic linkage. Compounds **1**–**4** were synthesized in two sequential addition reactions of 2,3-O-isopropylidene-uridine acceptor to the double bond of glycal in a stereoselective manner using the Falck–Mioskowski protocol [25].

Synthesized compounds **1**–**7** feature an acetonide moiety as a protection of two hydroxyl groups on the ribose part. We originally attempted to synthesize totally deblocked structures. Unfortunately, acidic removal of the acetonide group occurred in low yield due to the poor stability of 2-deoxyglycosidic linkage in acid medium. There was a concern that acetonide derivatives will not fit into the active site of the enzyme owing to the steric hindrances created by the isopropylidene group. Therefore, we decided to improve our methodology by applying the another

way of protecting the uridine moiety. The second synthetic question was a kind of glycosidic linkage between two glycosyl units. Recently, we reported on molecular docking simulations aiming at investigating interactions between 2-deoxy-hexopyranosyl derivatives of uridine and the active site of β-1,4-galactosyltransferase *I* [26]. Structures composed of uridine and two glycosyl residues: 2-deoxy-D-glucopyranose and 2-deoxy-D-galactopyranose connected by different glycosidic linkages were analysed. Simulated binding modes of the top ranked ligands suggested that two hydroxyl groups: 2-OH and 3-OH in the ribose moiety as well as the uracil nitrogen exhibited interactions with the key amino acids residues similar to that of the natural substrate. Simulations showed that binding affinity of structures featuring α-1,6 glycosidic linkage between glycosyl units compared better with α-1,3 or α-1,4 analogues. Therefore, we wish to describe supplementary synthesis of 2-deoxy-hexopyranosyl derivatives of uridine **10**–**13** as totally deblocked compounds, which contained two residues of 2-deoxy sugars linked through α-1,6 glycosidic linkage (Fig. 2). In order to synthesize the target compounds we applied glycal derivatives **14**–**17** with benzyl protection of hydroxyl groups (Scheme 1). Unfortunately, N^3,2′,3′-*O*-tri-benzyl-uridine could not be applied due to a problem in the final N-debenzylation [27]. Our goal was to apply a protective group that could be removed in neutral conditions, preferably by catalytic hydrogenolysis, together with O-benzyl protection in 2-deoxy-hexopyranose part. Our previous experiments revealed beneficial influence of using a large group as uridine N-imide protection on α-selectivity in the addition of uridine derivatives to D-glucal [24]. Therefore, we used N^3,2′,3′-*O*-tris-(benzyloxycarbonyl)uridine (**18**) [20] as a substrate. Uridine derivative **18** enabled the addition reaction to 3,4-di-O-benzyl-6-O-*tert*-butyldimethylsilyl-D-glucal (**14**) and 3,4-di-O-benzyl-6-O-*tert*-butyldimethylsilyl-D-galactal (**15**) to be performed at 5-OH in the presence of TPHB as a catalyst in CH_2Cl_2 as a solvent (Scheme 1).

Removal of the orthogonal TBDMS protecting group in **19** and **20** was effected by treatment of AcCl in methanol [28]. Compounds **23** and **24**, which possessed free hydroxyl at C-6, were submitted without purification to the second addition step. In addition reaction to glycals **16** and **17**, respectively, we have synthesized four isomers as α-1,6-linked derivatives **25**–**28**. Simultaneous removal of benzyl and benzyloxycarbonyl groups in compounds **21**, **22**, **25**–**28** was accomplished by catalytic transfer hydrogenolysis on Pearlman's catalyst (palladium(II) hydroxide) [29] in the presence of cyclohexene [30] in reflux of ethanol affording the unprotected, new compounds **9**–**13** without side reactions. All new compounds 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, HMBC experiments and simulation analysis) and mass spectrometry analysis (for details see Sections 2.3–2.8).

The main goal of this work was to evaluate synthesized compounds **1**–**13** as galactosyltransferase inhibitors. Compounds **1–4** and **10**–**13** can be treated as UDP-sugar analogues, while compounds **5**–**9** as UDP analogues. Compounds **5** and **6** were included in examination due to their antiviral activity against classical swine fever virus, which can be associated with the inhibition of



Scheme 1. (i): TPHB (0.1 equiv.), CH_2Cl_2, rt., 1–20 h; (ii): AcCl (1.0 equiv.), MeOH, rt., 15 min.; and (iii): $Pd(OH)_2/C$, cyclohexene, EtOH/THF, reflux, 30 min.

glycosylation at the stage of glycan modification characteristic for mammalian cells [31].

Bovine milk β4GalT I was chosen as the target enzyme. It is one of the most well-studied enzyme among glycosyltransferases with available X-ray crystal structure analysis data [32] and commercial availability. B4GalT I is responsible for biosynthesizing N-acetyllactosamine by the transfer of galactose from UDP-Gal to the 4-OH group of N-acetylglucosamine of an acceptor sugar in glycoproteins or glycolipids with β-1,4-linkage.

Radioassays [12,33] and fluorescence assays [13,34] are routinely used to measure GTs activity. We have applied the fluorescence assay developed by Praly and co-workers [13]. β-GlcNAc-O-$(CH_2)_6$-dansyl was used instead of the natural acceptor substrate. Firstly, compounds **1**–**13** were tested as possible substrates. 1 mM sample of investigated compound and fluorescent acceptor substrate were incubated at 37 °C for 30 min in the presence of bovine milk β4GalT I. Reactions were monitored by TLC on Silica Gel $60F_{254}$ with UV detection. TLC analysis revealed that acceptor substrate was the only one bearing fluorescent label, this indicated that no reaction occurred between acceptor substrate and synthesized donor analogues.

All the synthesized compounds **1**–**13** were then tested as potential inhibitors in a competition assay against bovine milk β4GalT I using fluorescent acceptor β-GlcNAc-O-$(CH_2)_6$-dansyl as a substrate. None of the compounds presented in Fig. 2 displayed significant inhibitory activity against bovine milk β4GalT I at concentrations up to 2.4 mM. Very poor inhibition was observed for compound **9** (IC_{30} 2.4 mM). Our results suggest that derivatives of uridine connected with one or two 2-deoxy-hexopyranose rings in α-configuration of glycosidic linkages are not able to ensure binding into the enzyme active site with strength comparable to the natural donor substrate.

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