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5-NITRO-2-PYRIDYL-1-THIOGLUCOSIDES: APPLICATION IN SYNTHESIS OF ANALOGUES OF GLYCOSYLTRANSFERASES NATURAL SUBSTRATES

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Abstract: 5-Nitro-2-pyridyl-1-thioglucosides were used in synthesis of complex uridine derivatives (**13**-**16**) in two different sequences of reactions. In one route, the first step was glycosylation of selectively protected 5-nitro-2-pyridyl-1-thioglucoside **1** with two different glycosyl donors (**5** or **6**), next, the nitro group in aglycone of obtained disaccharides **7** or **8** was reduced and then obtained products **9** or **10** were condensed with uridine derivatives **3** or **4** using DMT-MM as condensing agent under microwave irradiation. In the second route, condensation and glycosylation reactions were applied in reverse order. As it turned up, a sequence of reactions affected the yield of final glycoconjugates **13**-**16** and depended on the type of uridine derivatives used.

Keywords: glycosyltransferases, glycoconjugates, uridine derivatives, 1-thioglucosides

Glycoconjugates play a key role in cell-cell recognition and interaction processes. They are also responsible for such events as inflammation, tumor metastasis, bacterial or viral infection or activation of immune system (1). In synthesis of glycoconjugates in biological systems, enzymes such as glycosidases and glycosyltransferases are involved. Glycosyltransferases of the Leloir pathway are key enzymes responsible for synthesis of most cell-surface glcoconjugates in mammalian systems. They catalyze the transfer of a sugar moiety from an activated nucleotide sugar to the hydroxyl group of an acceptor which may be a growing oligosaccharide, a lipid or a protein (2). Inhibition of these enzymes leads to the modulation of oligosaccharide biosynthesis and enables recognition of their biological functions. Therefore, some of such inhibitors might be of therapeutic interest.

Designing of glycosyltransferases inhibitors are generally based on analogies between the three different moieties composing NDP sugar natural substrates, mimicking either the carbohydrate part, the diphosphate linkage, the nucleoside moiety or combination of all of these. The diphosphate linkage plays a key role in most GTs activities. It interacts with metallic cations (e.g., Mn^{2+}) coordinated with two aspartate residues in an active site of enzyme (3, 4). Development of diphosphate analogues that are capable of mimicking this interaction could provide a new group of potent GTs inhibitors. Numerous analogs of pyrophosphate linker have been proposed, however, almost none of the proposed potential inhibitors exhibited significant activity (5–8).

Recently, synthesis of a new kind of sugar nucleotides analogues, which were designed to act as glycosyltransferases inhibitors, particularly donor substrate analogues, was presented (9). In these glycoconjugates heteroaryl 1-thioglycosides derivatives of D-glucose or D-galactose were connected to selectively protected uridine by amide bond with a succinic spacer. Studies of biological properties of these compounds revealed that some of them exhibited antiviral activity against classical swine fever virus (CSFV) (10).

As it turned out, the compounds reported appeared to be quite active towards the target virus. Thus, the following study was the synthesis of several structural variations of already received 1-thioglycosyl uridine derivatives with one more sugar unit incorporated. Synthesis of such models would complete the already existing structures library and biological evaluations would be enriched in information concerning the biochemical relevance of size and structure of sugar part of obtained glycoconjugates.

EXPERIMENTAL

^{1}H-NMR and ^{13}C NMR spectra were recorded for solutions in $CDCl_3$ or DMSO-d_6 with Varian

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spectrometer at 300 MHz or 600 MHz, using TMS as the internal standard. NMR solvents were purchased from ACROS Organics (Geel, Belgium). Chemical shifts (δ) are expressed in ppm and coupling constants (*J*) in Hz. The following abbreviations were used to explain the observed multiplicities: s, singlet; d, doublet; dd, doublet of doublets; ddd, doublet of doublet of doublets; t, triplet; dd~t, doublet of doublets looking as triplet; m, multiplet; b, broad. 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. Microwave reactions were carried out in Discover® BenchMate™ (CEM) microwave instrument, equipped with 10 mL vessels. Reactions were monitored by TLC on aluminium sheets coated with silica gel 60 F_{254} (Merck). TLC plates were inspected under UV light (λ = 254 nm) and developed by charring after spraying with 10% H_2SO_4 in EtOH. Column chromatography was performed on Silica Gel 60 (70–230 mesh, Fluka) developed with either toluene : Et_3N, toluene : AcOEt or $CHCl_3$: MeOH solvent systems. Organic solvents were evaporated on a rotary evaporator under diminished pressure at 50°C.

5-Nitro-2-pyridyl-2,3,6-tri-*O*-benzoyl-1-thio-β-D-glucopyranoside (**1**) (11), succinic acid mono-2',3'-isopropylidene-uridin-5'-yl ester (**3**) (9), 2',3'-*O*-isopropylideneuridine-5'-carboxylic acid (**4**) (12), methyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (**5**) (13) and 3,4-di-*O*-acetyl-2-iodo-2,6-dideoxy-1-*O*-*tert*-butyldimethylsilyl-α-L-mannopyranoside (**6**) (14) were prepared according to the published procedures. 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium chloride (DMT-MM) was synthesized according to the procedure described by Kunishima (15). Other chemicals were purchased from Sigma-Aldrich, Fluka and Acros Chemical Companies and were used without purification. Solvents were dried and stored over molecular sieves (4 Å).

General procedures

Procedure A: glycosylation reactions of compound 5 and glycosyl acceptors

Glycosyl acceptor **1**, **11** or **12** (1 eqv.) and methyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside **5** (1.1 eqv.) were dissolved in CH_2Cl_2 and then molecular sieves 4Å were added. The mixture was stirred at a room temperature for 30 min then NIS (1.1 eqv.) and $AgBF_4$ (0.4 eqv.) were added. The mixture was stirred again at room temperature. The reaction was monitored by TLC on silica gel plates using toluene : AcOEt (1:1, v/v) solvent system. After reaction was completed, molecular sieves were filtered off, the reaction mixture was diluted with CH_2Cl_2 and washed with brine (2×10 mL) and then with 1% water solution of $Na_2S_2O_3$ (2×10 mL). The organic layer was dried over anhydrous $MgSO_4$, the adsorbent was filtered off and the filtrate was concentrated to give crude product which was purified by column chromatography with toluene : AcOEt (15:1 to 1:1, v/v) solvents system.

Procedure B: glycosylation reactions of compound 6 and glycosyl acceptors

Glycosyl acceptor **1**, **11** or **12** (1 eqv.) and 3,4-di-*O*-acetyl-2-iodo-2,6-dideoxy-1-*O*-*tert*-butyldimethylsilyl-α-L-mannopyranoside **6** (1.1 eqv.) were dissolved in dry CH_3CN and then molecular sieves 4Å were added. The mixture was stirred at a room temperature for 30 min. After this time, TMSOTf (1.1 eqv.) was added. The mixture was stirred at 0°C and the reaction was monitored by TLC on silica gel plates using toluene : AcOEt (1:1, v/v) (for product **8**) or $CHCl_3$: MeOH (10:1, v/v) (for products **14** and **16**) solvent systems. When the reaction was completed, molecular sieves were filtered off, reaction mixture was diluted with CH_2Cl_2 and washed with brine (2×10 mL). The organic layer was dried over anhydrous $MgSO_4$, the adsorbent was filtered off and the filtrate was concentrated to give crude product which was purified by column chromatography.

Procedure C: reduction of nitro group in 1-thioglycosides aglycone

Compounds **1**, **7** or **8** (1 eqv.) were dissolved in CH_2Cl_2. To the resulting solution acetic acid and zinc powder (8 eqv.) were added. The whole mixture was stirred at room temperature. The reaction was monitored by TLC on silica gel plates using toluene : AcOEt (2:1, v/v) solvent system. After completion of reaction, zinc was filtered off, the reaction mixture was diluted with CH_2Cl_2 and washed with brine (3×10 mL). The organic layer was dried over anhydrous $MgSO_4$, the adsorbent was filtered off and the filtrate was concentrated to give crude product which was purified by column chromatography.

Procedure D: condensation reactions of amines with uridine derivatives 3 or 4

To the solutions of compounds **2**, **9** or **10** (1 eqv.) and uridine derivatives **3** or **4** (1 eqv.) in dry

THF (2 mL), DMT-MM (1 eqv.) was added. The mixtures were prepared in special tubes applied in microwave reactor. The tube was put in the microwave reactor and reaction was carried out at program Standard at 50°C. The reaction was monitored by TLC on silica gel plates using CH_3OH : MeOH (10:1, v/v) solvent system. After completion of reaction, the reaction mixture was concentrated under reduced pressure, remaining solid was dissolved in CH_2Cl_2 and washed with brine (2×10 mL). The organic layer was dried over anhydrous $MgSO_4$, the adsorbent was filtered off and the filtrate was concentrated to give crude product which was purified by column chromatography.

5-Amino-2-pyridyl-2,3,4-tri-*O*-benzoyl-1-thio-β-D-glucopyranoside (2)

5-Nitro-2-pyridyl-2,3,6-tri-*O*-benzoyl-1-thio-β-D-glucopyranoside **1** (562 mg, 0.89 mmol) in CH_2Cl_2 (36 mL) with addition of acetic acid (8.4 mL) and zinc powder (490 mg, 7.12 mmol) were submitted to the general procedure **C** described above. Reaction time was 30 min. Product **2** (352 mg, 66%) was obtained as a light yellow solid after purification by column chromatography with toluene : AcOEt (4:1 to 1:2, v/v) solvent system. $[\alpha]_D^{20}$ = 55.9 (c = 0.1, $CHCl_3$), m.p. 90–98°C. ^{1}H NMR ($CDCl_3$, δ, ppm): 3.72 (dd, 1H, *J* = 5.6 Hz, *J* = 12.7 Hz, H-6a), 3.80 (dd, 1H, *J* = 1.9 Hz, *J* = 12.7 Hz, H-6b), 3.94 (ddd, 1H, *J* = 1.9 Hz, *J* = 5.6 Hz, *J* = 9.9 Hz, H-5), 3.82–4.04 (bs, 2H, NH_2), 5.49 (dd~t, 1H, *J* = 9.8 Hz, *J* = 9.8 Hz, H-4), 5.56 (dd, 1H, *J* = 8.9 Hz, *J* = 10.1 Hz, H-2), 5.62 (d, 1H, *J* = 10.0 Hz, H-1), 5.99 (dd~t, 1H, *J* = 9.1 Hz, *J* = 9.1 Hz, H-3), 6.85 (dd, 1H, *J* = 2.9 Hz, *J* = 8.4 Hz, H-4_{pyr}), 7.08 (d, 1H, *J* = 8.4 Hz, H-3_{pyr}), 7.11–7.54 (m, 9H, H-Ph), 7.78–7.96 (m, 6H, H-Ph), 8.05 (dd, 1H, *J* = 0.3 Hz, *J* = 2.7 Hz, H-6_{pyr}). ^{13}C NMR ($CDCl_3$, δ, ppm): 61.52 (C-6), 69.51, 70.73, 74.36, 79.22 (C-2, C-3, C-4, C-5), 83.96 (C-1), 122.83 (C-4_{pyr}), 126.64 (C-3_{pyr}), 128.31, 128.35, 128.50, 128.75, 128.91, 129.71, 129.91, 133.23, 133.30, 133.59 (C-Ph), 137.88 (C-5_{pyr}), 141.39 (C-2_{pyr}), 141.89 (C-6_{pyr}), 165.31, 165.78, 165.82 (Ph<u>C</u>OO).

5-Nitro-2-pyridyl-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1-6)-2,3,4-tri-*O*-benzoyl-1-thio-β-D-glucopyranoside (7)

5-Nitro-2-pyridyl-2,3,6-tri-*O*-benzoyl-1-thio-β-D-glucopyranoside **1** (122 mg, 0.193 mmol), methyl-2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside **5** (82 mg, 0.212 mmol), NIS (48 mg, 0.212 mmol) and $AgBF_4$ (11 mg, 0.077 mmol) in CH_2Cl_2 (4 mL) were submitted to the general procedure **A** described above. Reaction time: 24 h. Product **7** (105 mg, 50%) was obtained as a white solid. $[\alpha]_D^{20}$ = 35.4 (c = 1.1, $CHCl_3$), m.p. 145–148°C. ^{1}H NMR ($CDCl_3$, δ, ppm): 1.96, 1.97, 1.98, 2.00 (4s, 12H, CH_3CO), 3.59 (ddd, 1H, *J* = 2.2 Hz, *J* = 4.8 Hz, *J* = 9.8 Hz, H-5'$_{glu}$), 3.80 (dd, 1H, *J* = 6.6, Hz, *J* = 11.6 Hz, H-6a$_{glu}$), 3.98–4.07 (m, 2H, H-6b$_{glu}$, H-6'b$_{glu}$), 4.18 (dd, 1H, *J* = 4.8 Hz, *J* = 12.3 Hz, H-6'a$_{glu}$), 4.24 (ddd, 1H, *J* = 1.6 Hz, *J* = 6.6 Hz, *J* = 9.7 Hz, H-5$_{glu}$), 4.56 (d, 1H, *J* = 7.8 Hz, H-1'$_{glu}$), 4.90–5.04 (m, 3H, H-2'$_{glu}$, H-3'$_{glu}$, H-4'$_{glu}$), 5.51 (dd~t, 1H, *J* = 9.7 Hz, *J* = 9.7 Hz, H-4$_{glu}$), 5.71 (dd~t, 1H, *J* = 9.9 Hz, *J* = 9.9 Hz, H-2$_{glu}$), 6.04 (dd~t, 1H, *J* = 9.4 Hz, *J* = 9.4 Hz, H-3$_{glu}$), 6.17 (d, 1H, *J* = 10.3 Hz, H-1$_{glu}$), 7.22–7.56 (m, 10H, H-Ph, H-3$_{pyr}$), 7.78–7.96 (m, 6H, H-Ph), 8.29 (dd, 1H, *J* = 2.6 Hz, *J* = 8.8 Hz, H-4$_{pyr}$), 9.30 (d, 1H, *J* = 2.6 Hz, H-6$_{pyr}$). ^{13}C NMR ($CDCl_3$, δ, ppm): 20.53, 20.65 (<u>C</u>H$_3$CO), 61.80 (C-6'$_{glu}$), 67.72 (C-6$_{glu}$), 68.31, 69.22, 70.01, 70.99, 71.87, 72.62, 74.10, 78.40 (C-2$_{glu}$, C-3$_{glu}$, C-4$_{glu}$, C-5$_{glu}$, C-2'$_{glu}$, C-3'glu, C-4'$_{glu}$, C-5'$_{glu}$), 81.43 (C-1$_{glu}$), 100.36 (C-1'$_{glu}$), 122.20 (C-3$_{pyr}$), 128.23, 128.39, 128.51, 128.55, 128.64, 128.70, 129.70, 129.83, 129.87 (C-Ph), 131.40 (C-4$_{pyr}$), 133.33, 133.54, 133.67 (C-Ph); 142.11 (C-5$_{pyr}$), 145.21 (C-6$_{pyr}$), 163.66 (C-2$_{pyr}$), 165.17, 165.22, 165.67, 169.13, 169.32, 170.14, 170.57 (CH$_3$<u>C</u>OO, Ph<u>C</u>OO).

5-Nitro-2-pyridyl-3,4-di-*O*-acetyl-2-iodo-2,6-dideoxy-α-L-mannopyranosyl-(1-6)-2,3,4-tri-*O*-benzoyl-1-thio-β-D-glucopyranoside (8)

5-Nitro-2-pyridyl-2,3,6-tri-*O*-benzoyl-1-thio-β-D-glucopyranoside **1** (138 mg, 0.22 mmol), 3,4-di-*O*-acetyl-2-iodo-2,6-dideoxy-1-*O*-*tert*-butyldimethylsilyl-α-L-mannopyranoside **6** (114 mg, 0.242 mmol) and TMSOTf (48 mL, 0.242 mmol) in CH_3CN (4 mL) were submitted to the general procedure **B** described above. Reaction time: 2 h. Product **8** (134 mg, 63%) was obtained as a white solid after purification by column chromatography with toluene : AcOEt (30:1 to 20:1, v/v) solvent system. $[\alpha]_D^{20}$ = 71.7 (c = 1.2, $CHCl_3$), m.p. 114–118°C. ^{1}H NMR ($CDCl_3$, δ, ppm): 1.12 (d, 3H, *J* = 6.3 Hz, $CH_3(6)_{man}$), 2.06, 2.07 (2s, 6H, CH_3CO), 3.74 (dd, 1H, *J* = 7.2 Hz, *J* = 12.4 Hz, H-6a$_{glu}$), 3.82 (dd, 1H, *J* = 2.4 Hz, *J* = 12.4 Hz, H-6b$_{glu}$), 3.90 (dq, 1H, *J* = 6.3 Hz, *J* = 9.5 Hz, H-5$_{man}$), 4.25 (ddd, 1H, *J* = 2.4 Hz, *J* = 7.2 Hz, *J* = 9.8 Hz, H-5$_{glu}$), 4.39 (dd, 1H, *J* = 1.1 Hz, *J* = 4.4 Hz, H-2$_{man}$), 4.46 (dd, 1H, *J* = 4.4 Hz, *J* = 9.5 Hz, H-3$_{man}$), 5.06 (dd~t, 1H, *J* = 9.5 Hz, *J* = 9.5 Hz, H-4$_{man}$), 5.09 (bs, 1H, H-1$_{man}$), 5.51 (dd~t, 1H, *J* = 9.8 Hz, *J* = 9.8 Hz, H-4$_{glu}$), 5.72 (dd, 1H, *J* = 9.4 Hz, *J* = 10.5 Hz, H-2$_{glu}$), 6.07 (dd~t, 1H, *J* = 9.4 Hz, *J* = 9.4 Hz, H-3$_{glu}$), 6.21 (d, 1H, *J* = 10.5

Hz, H-1_{glu}), 7.22–7.58 (m, 10H, H-Ph, H-3_{pyr}), 7.80–7.99 (m, 6H, H-Ph), 8.27 (dd, 1H, *J* = 2.7 Hz, *J* = 8.8 Hz, H-4_{pyr}), 9.29 (dd, 1H, *J* = 0.8 Hz, *J* = 2.7 Hz, H-6_{pyr}). ^{13}C NMR ($CDCl_3$, δ, ppm): 17.67 (CH_3-6_{man}), 20.97, 21.06 ($\underline{C}H_3CO$), 30.35 (C-2_{man}), 67.02 (C-6_{glu}), 67.44 (C-5_{man}), 69.15 (C-3_{man}), 69.59, 70.06 (C-2_{glu}, C-4_{glu}), 72.58, 74.21 (C-3_{glu}, C-5_{glu}), 79.29 (C-4_{man}), 81.43 (C-1_{glu}), 102.29 (C-1_{man}), 122.56 (C-3_{pyr}), 128.47, 128.59, 128.67, 128.75, 128.81, 128.87, 129.96, 130.10, 130.16 (C-Ph); 131.48 (C-4_{pyr}), 133.61, 133.81, 133.97 (C-Ph), 142.58 (C-5_{pyr}), 145.51 (C-6_{pyr}), 163.87 (C-2_{pyr}), 165.46, 165.54, 165.92, 169.93, 170.24 ($CH_3\underline{C}OO$, Ph$\underline{C}$OO).

5-Amino-2-pyridyl-3,4-di-*O*-acetyl-2-iodo-2,6-dideoxy-α-L-mannopyranosyl-(1-6)-2,3,4-tri-*O*-benzoyl-1-thio-β-D-glucopyranoside (9)

Compound **7** (98 mg, 0.1 mmol) in CH_2Cl_2 (5 mL) with addition of acetic acid (0.5 mL) and zinc powder (53 mg, 0.8 mmol) were submitted to the general procedure **C** described above. Reaction time: 75 min. Product **9** (58 mg, 63%) was obtained as a light yellow solid after purification by column chromatography with toluene : AcOEt (8:1 to 1:2, v/v) solvent system. $[\alpha]_D^{20}$ = 11.2 (c = 0.4, $CHCl_3$), m.p. 191–195°C. ^{1}H NMR ($CDCl_3$, δ, ppm): 2.01, 2.03, 2.07 (3s, 12H, CH_3CO), 3.33 (ddd, 1H, *J* = 2.2 Hz, *J* = 4.9 Hz, *J* = 9.5 Hz, H-5'$_{glu}$), 3.70–4.28 (m, 7H, H-5_{glu}, H-$6a_{glu}$, H-$6b_{glu}$, H-6'b_{glu}, H-6'a_{glu}, NH_2), 4.70 (d, 1H, *J* = 8.1 Hz, H-1'$_{glu}$), 4.89 (dd~t, 1H, *J* = 8.7 Hz, *J* = 8.7 Hz, H-2'$_{glu}$), 4.98 (dd~t, 1H, *J* = 9.4 Hz, *J* = 9.4 Hz, H-4'$_{glu}$), 5.06 (dd~t, 1H, *J* = 9.3 Hz, *J* = 9.3 Hz, H-3'$_{glu}$), 5.38 (dd~t, 1H, *J* = 9.9 Hz, *J* = 9.9 Hz, H-4_{glu}), 5.60 (dd~t, 1H, *J* = 9.9 Hz, *J* = 9.9 Hz, H-2_{glu}), 5.79 (d, 1H, *J* = 10.5 Hz, H-1_{glu}), 5.97 (dd~t, 1H, *J* = 9.5 Hz, *J* = 9.5 Hz, H-3_{glu}), 6.96 (dd, 1H, *J* = 2.8 Hz, *J* = 8.5 Hz, H-4_{pyr}), 7.12 (d, 1H, *J* = 8.5 Hz, H-3_{pyr}), 7.23–7.58 (m, 9H, H-Ph), 7.76–7.98 (m, 6H, H-Ph), 8.13 (d, 1H, *J* = 2.8 Hz, H-6_{pyr}).

5-Amino-2-pyridyl-3,4-di-*O*-acetyl-2-iodo-2,6-dideoxy-α-L-mannopyranosyl-(1-6)-2,3,4-tri-*O*-benzoyl-1-thio-β-D-glucopyranoside (10)

Compound **8** (134 mg, 0.14 mmol) in CH_2Cl_2 (10 mL) with addition of acetic acid (1 mL) and zinc powder (80 mg, 0.12 mmol) were submitted to the general procedure **C** described above. Reaction time: 30 min. Product **10** (42 mg, 31%) was obtained as a light yellow solidifying oil after purification by column chromatography with toluene : AcOEt (10:1 to 2:1, v/v) solvent system. $[\alpha]_D^{20}$ = 60.7 (c = 0.6, $CHCl_3$). ^{1}H NMR ($CDCl_3$, δ, ppm): 1.08 (d, 3H, *J* = 6.3 Hz, $CH_3(6)_{man}$), 2.04, 2.08 (2s, 6H, CH_3CO), 3.60–3.94 (bs, 2H, NH_2), 3.69 (dd, 1H, *J* = 6.8 Hz, *J* = 12.0 Hz, H-$6a_{glu}$), 3.80 (dd, 1H, *J* = 2.0 Hz, *J* = 12.0 Hz, H-$6b_{glu}$), 3.86 (dq, 1H, *J* = 6.3 Hz, *J* = 9.7 Hz, H-5_{man}), 4.11 (ddd, 1H, *J* = 2.0 Hz, *J* = 6.8 Hz, *J* = 9.5 Hz, H-5_{glu}), 4.41 (dd, 1H, *J* = 1.0 Hz, *J* = 4.5 Hz, H-2_{man}), 4.48 (dd, 1H, *J* = 4.5 Hz, *J* = 9.5 Hz, H-3_{man}), 5.06 (dd~t, 1H, *J* = 9.5 Hz, *J* = 9.5 Hz, H-4_{man}), 5.08 (bs, 1H, H-1_{man}), 5.49 (dd~t, 1H, *J* = 9.8 Hz, *J* = 9.8 Hz, H-4_{glu}), 5.62 (dd, 1H, *J* = 9.3 Hz, *J* = 10.3 Hz, H-2_{glu}), 5.80 (d, 1H, *J* = 10.3 Hz, H-1_{glu}), 5.98 (dd~t, 1H, *J* = 9.4 Hz, *J* = 9.4 Hz, H-3_{glu}), 6.91 (dd, 1H, *J* = 2.9 Hz, *J* = 8.8 Hz, H-4_{pyr}), 7.15 (d, 1H, *J* = 8.8 Hz, H-3_{pyr}), 7.16–7.58 (m, 9H, H-Ph), 7.80–7.98 (m, 6H, H-Ph), 8.05 (d, 1H, *J* = 2.9 Hz, H-6_{pyr}). ^{13}C NMR ($CDCl_3$, δ, ppm): 17.63 (CH_3-6_{man}), 21.02, 21.70 ($\underline{C}H_3CO$), 30.27 (C-2_{man}), 67.04 (C-6_{glu}), 67.15 (C-5_{man}), 69.43 (C-3_{man}), 69.91, 70.69 (C-2_{glu}, C-4_{glu}), 72.79, 74.59 (C-3_{glu}, C-5_{glu}), 78.52 (C-4_{man}); 83.48 (C-1_{glu}), 102.22 (C-1_{man}), 123.47 (C-4_{pyr}), 125.40 (C-3_{pyr}), 128.74, 128.97, 129.11, 129.97, 130.10, 130.20, 133.45, 133.81, 137.87 (C-Ph), 138.11 (C-5_{pyr}), 141.45 (C-6_{pyr}), 142.66 (C-2_{pyr}), 165.51, 165.54, 166.02, 169.98, 170.16 ($CH_3\underline{C}OO$, Ph$\underline{C}$OO).

Glycoconjugate (11)

5-Amino-2-pyridyl-2,3,4-tri-*O*-benzoyl-1-thio-β-D-glucopyranoside **2** (100 mg, 0.16 mmol), uridine derivative **3** (62 mg, 0.16 mmol) and DMT-MM (45 mg, 0.16 mmol) in THF (2 mL) were submitted to the general procedure **D** described above. Reaction time: 6 h. Product **11** (79 mg, 51%) was obtained as a white solid after purification by column chromatography with toluene : AcOEt (10:1 to 1:2, v/v) and then $CHCl_3$: MeOH (80:1 to 30:1, v/v) solvent systems. $[\alpha]_D^{20}$ = 64.6 (c = 0.8, $CHCl_3$), m.p. 181–183°C. ^{1}H NMR ($CDCl_3$, δ, ppm): 1.29, 1.53 (2s, 6H, $(CH_3)_2C$), 2.58–2.78 (m, 4H, CH_2), 3.67–3.87 (m, 2H, H-$6a_{glu}$, H-$6b_{glu}$), 4.04 (ddd, 1H, *J* = 2.5 Hz, *J* = 5.6 Hz, *J* = 10.0 Hz, H-5_{glu}), 4.31–4.42 (m, 3H, H-4', H-5'a, H-5'b), 4.84 (dd, 1H, *J* = 3.7 Hz, *J* = 6.6 Hz, H-3'), 5.04 (dd, 1H, *J* = 1.7 Hz, *J* = 6.6 Hz, H-2'), 5.53 (dd~t, 1H, *J* = 9.8 Hz, *J* = 9.8 Hz, H-4_{glu}), 5.61 (d, 1H, *J* = 1.7 Hz, H-1'); 5.62–5.71 (m, 2H, H-5_{ur}, H-2_{glu}), 5.89 (d, 1H, *J* = 10.3 Hz, H-1_{glu}), 6.05 (dd~t, 1H, *J* = 9.3 Hz, *J* = 9.3 Hz, H-3_{glu}), 7.13 (d, 1H, *J* = 8.8 Hz, H-3_{pyr}), 7.23–7.55 (m, 10H, H-Ph, H-6_{ur}), 7.79–7.98 (m, 7H, H-Ph, H-4_{pyr}), 8.48 (d, 1H, *J* = 2.2 Hz, H-6_{pyr}), 8.78 (s, 1H, NH), 10.02 (bs, 1H, NH). ^{13}C NMR ($CDCl_3$, δ, ppm): 25.73 (($\underline{C}H_3)_3C$), 25.21, 27.09 (($\underline{C}H_3)_2C$), 29.18, 29.70 (CH_2), 61.53 (C-6_{glu}), 64.15 (C-5'), 69.49, 70.44, 74.19, 79.06 (C-2_{glu}, C-3_{glu}, C-4_{glu}, C-5_{glu}), 80.84, 82.82, 84.50, 85.38 (C-1_{glu}, C-2', C-3', C-4'); 95.22 (C-1'), 102.26 (C-

5_{ur}), 114.44 ($(CH_3)_2\underline{C}$), 124.23 (C-3_{pyr}), 128.32, 128.39, 128.51 (C-Ph), 128.65 (C-4_{pyr}), 128.33, 128.91, 129.71, 129.88, 129.93, 133.27, 133.39, 133.51 (C-Ph), 133.63 (C-5_{pyr}), 140.99 (C-6_{pyr}), 142.86 (C-6_{ur}), 148.84 (C-2_{pyr}), 150.19 (C-2_{ur}), 158.41 (C-4_{ur}), 164.05, 165.32, 165.79, 170.59, 172.70 ($CH_3\underline{C}OO$, Ph$\underline{C}OO$).

Glycoconjugate (12)

5-Amino-2-pyridyl-2,3,4-tri-*O*-benzoyl-1-thio-β-D-glucopyranoside **2** (152 mg, 0.246 mmol), uridine derivative **4** (56 mg, 0.246 mmol) and DMT-MM (70 mg, 0.32 mmol) in THF (4 mL) were submitted to the general procedure **D** described above. Reaction time: 4 h. Product **12** (72 mg, 33%) was obtained as a white solid after purification by column chromatography with toluene : AcOEt (8:1 to 1:1, v/v) solvent system. $[\alpha]_D^{20}$ = 117.4 (c = 0.4, $CHCl_3$), m.p. 171–175°C. ^{1}H NMR ($CDCl_3$, δ, ppm): 1.35, 1.57 (2s, 6H, $(CH_3)_2C$), 3.46 (bs, 1H, OH), 3.71 (dd, 1H, *J* = 6.6 Hz, *J* = 12.6 Hz, H-6a$_{glu}$), 3.78 (dd, 1H, *J* = 2.0 Hz, *J* = 12.6 Hz, H-6b$_{glu}$), 3.99 (ddd, 1H, *J* = 2.0 Hz, *J* = 6.6 Hz, *J* = 9.9 Hz, H-5$_{glu}$), 4.71 (d, 1H, *J* = 2.3 Hz, H-4'), 5.24 (dd, 1H, *J* = 1.5 Hz, *J* = 6.4 Hz, H-2'), 5.31 (dd, 1H, *J* = 2.3 Hz, *J* = 6.4 Hz, H-3'), 5.48 (s, 1H, H-1'), 5.48 (dd~t, 1H, *J* = 9.8 Hz, *J* = 9.8 Hz, H-4$_{glu}$), 5.62 (dd~t, 1H, *J* = 9.9 Hz, *J* = 9.9 Hz, H-2$_{glu}$), 5.69 (d, 1H, *J* = 7.3 Hz, H-5$_{ur}$), 5.89 (d, 1H, *J* = 10.3 Hz, H-1$_{glu}$), 6.02 (dd~t, 1H, *J* = 9.4 Hz, *J* = 9.4 Hz, H-3$_{glu}$), 7.10–7.58 (m, 11H, H-Ph, H-6$_{ur}$, H-3$_{pyr}$), 7.75–8.00 (m, 6H, H-Ph), 8.02 (d, 1H, *J* = 8.4 Hz, H-4$_{pyr}$), 8.39 (d, 1H, *J* = 2.4 Hz, H-6$_{pyr}$), 8.70 (s, 1H, NH), 9.88 (bs, 1H, NH). ^{13}C NMR ($CDCl_3$), δ, ppm): 24.90, 26.86 ($(\underline{C}H_3)_2C$), 61.67 (C-6$_{glu}$), 69.67, 70.48, 74.20 (C-4$_{glu}$, C-2$_{glu}$, C-3$_{glu}$), 79.12 (C-5$_{glu}$), 82.72, 82.92, 83.84, 87.85 (C-1$_{glu}$, C-2', C-3', C-4'), 99.61 (C-1'), 103.09 (C-5$_{ur}$), 114.36 ($(CH_3)_2\underline{C}$), 124.33 (C-3$_{pyr}$), 128.30, 128.38, 128.52, 128.68, 128.87 (C-Ph), 128.93 (C-4$_{pyr}$), 129.04, 129.71, 129.89, 129.94 (C-Ph), 132.43 (C-5$_{pyr}$), 133.23, 133.38, 133.60 (C-Ph), 141.39, 144.19 (C-6$_{pyr}$, C-6$_{ur}$), 149.82 (C-2$_{pyr}$), 150.65 (C-2$_{ur}$), 157.98 (C-4$_{ur}$), 165.31, 165.77, 165.81, 168.16 ($CH_3\underline{C}OO$, Ph$\underline{C}OO$).

Glycoconjugate (13)

Route I: Compound **9** (18 mg, 0.02 mmol), uridine derivative **3** (8 mg, 0.02mmol) and DMT-MM (6 mg, 0.02 mmol) in THF (2 mL) were submitted to the general procedure **D** described above. Reaction time: 8 h. Product **13** (1 mg, 4%) was obtained after purification by column chromatography with toluene : AcOEt (10:1 to 1:2, v/v) and then $CHCl_3$: MeOH (100:1 to 40:1, v/v) solvent systems.

Route II: Glycoconjugate **11** (40 mg, 0.042 mmol), methyl-2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside **5** (19 mg, 0.046 mmol), NIS (10 mg, 0.046 mmol) and $AgBF_4$ (0.3 mg, 0.002 mmol) in CH_2Cl_2 (2 mL) were submitted to the general procedure **A** described above. Reaction time: 1 hour. Product **13** (18 mg, 32%) was obtained as a white solid after purification by column chromatography with toluene : AcOEt (10:1 to 1:2, v/v) and then $CHCl_3$: MeOH (100:1 to 40:1, v/v) solvents systems. $[\alpha]_D^{20}$ = 43.5 (c = 0.3, $CHCl_3$), m.p. 129–131°C. ESI-MS: calcd. for $C_{62}H_{64}N_4O_{25}SNa$ ($[M + Na]^+$): m/z 1319.35; found: m/z 1319.5. ^{1}H NMR ($CDCl_3$, δ, ppm): 1.31, 1.55 (2s, 6H, $(CH_3)_2C$), 1.95, 2.03, 2.07, 2.08 (4s, 12H, CH_3CO), 2.60–2.79 (m, 4H, CH_2); 3.80 (d, 1H, *J* = 12.8 Hz, H-6a$_{glu}$), 4.02 (dd, 1H, *J* = 7.6 Hz, *J* = 12.8 Hz, H-6b$_{glu}$), 4.03 (dd, 1H, *J* = 1.8 Hz, *J* = 12.2 Hz, H-6'a$_{glu}$), 4.10 (dd, 1H, *J* = 4.8 Hz, *J* = 12.2 Hz, H-6'b$_{glu}$), 4.17 (dd, 1H, *J* = 7.6 Hz, *J* = 9.9 Hz, H-5$_{glu}$), 4.22–4.42 (m, 4H, H-4', H-5'a, H-5'b, H-5'$_{glu}$), 4.78 (d, 1H, *J* = 7.3 Hz, H-1'$_{glu}$), 4.82 (dd, 1H, *J* = 3.7 Hz, *J* = 6.3 Hz, H-3'), 4.84–4.97 (m, 3H, H-2'$_{glu}$, H-3'$_{glu}$, H-4'$_{glu}$), 5.01 (dd, 1H, *J* = 1.8 Hz, *J* = 6.3 Hz, H-2'), 5.38 (dd~t, 1H, *J* = 9.9 Hz, *J* = 9.9 Hz, H-4$_{glu}$), 5.59–5.65 (m, 2H, H-2$_{glu}$, H-1'), 5.72 (d, 1H, *J* = 8.1 Hz, H-5$_{ur}$), 6.02 (dd~t, 1H, *J* = 9.4 Hz, *J* = 9.4 Hz, H-3$_{glu}$), 6.04 (d, 1H, *J* = 10.4 Hz, H-1$_{glu}$), 7.19 (d, 1H, *J* = 8.7 Hz, H-3$_{pyr}$), 7.22–7.56 (m, 10H, H-Ph, H-6$_{ur}$), 7.77–7.98 (m, 6H, H-Ph), 8.12 (dd, 1H, *J* = 2.4 Hz, *J* = 8.7 Hz, H-4$_{pyr}$), 8.32 (s, 1H, NH), 8.53 (d, 1H, *J* = 2.4 Hz, H-6$_{pyr}$), 8.82 (bs, 1H, NH). ^{13}C NMR ($CDCl_3$, δ, ppm): 20.48, 20.75, 20.78, 20.84 ($\underline{C}H_3CO$), 25.23, 27.12 ($(\underline{C}H_3)_2C$), 28.91, 31.37 (CH_2), 61.62 (C-6'$_{glu}$), 64.10, 66.35, 68.12, 69.26, 69.91, 71.49, 71.63, 73.30, 74.11 (C-6$_{glu}$, C-5', C-5'$_{glu}$, C-2$_{glu}$, C-3$_{glu}$, C-4$_{glu}$, C-2'$_{glu}$, C-3'$_{glu}$, C-4'$_{glu}$), 79.79, 80.89, 81.76, 84.49, 85.24 (C-1$_{glu}$, C-5$_{glu}$, C-2', C-3', C-4'); 94.99 (C-1'); 98.51 (C-1'$_{glu}$), 102.55 (C-5$_{ur}$), 123.32 (C-3$_{pir}$), 128.30, 128.38, 128.48, 128.65, 128.75, 128.90 (C-Ph), 129.12 (C-4$_{pyr}$), 129.69, 129.86, 129.91, 132.96, 133.28, 133.41 (C-Ph), 133.60 (C-5$_{pyr}$), 141.58, 142.31 (C-6$_{pyr}$, C-6$_{ur}$), 149.82, 149.96 (C-2$_{ur}$, C-2$_{pyr}$), 162.89 (C-4$_{ur}$), 165.30, 165.43, 165.76, 169.38, 169.56, 170.14, 170.69, 171.44, 172.27 ($CH_3\underline{C}OO$, Ph$\underline{C}OO$).

Glycoconjugate (14)

Route I: Compound **10** (21 mg, 0.022 mmol), uridine derivative **3** (9 mg, 0.022 mmol) and DMT-MM (6 mg, 0.022 mmol) in THF (2 mL) were submitted to the general procedure **D** described above. Reaction time: 6 h. Product **14** (2 mg, 6%) was obtained after purification by column chromatogra-

phy with toluene : AcOEt (10:1 to 1:2, v/v) and then $CHCl_3$: MeOH (100:1 to 60:1, v/v) solvent systems. Route II: Glycoconjugate **11** (39 mg, 0.041 mmol), 3,4-di-*O*-acetyl-2-iodo-2,6-dideoxy-1-*O*-*tert*-butyldimethylsilyl-α-L-mannopyranoside **6** (22 mg, 0.045 mmol), TMSOTf (9 mL, 0.045 mmol) in CH_3CN (2 mL) were submitted to the general procedure **B** described above. Reaction time: 10 min. Product **14** (16 mg, 29%) was obtained as a white solid after purification by column chromatography with toluene : AcOEt (10:1 to 1:2, v/v) and then $CHCl_3$: MeOH (100:1 to 60:1, v/v) solvent systems. $[\alpha]_D^{20}$ = 46.2 (c = 0.4, $CHCl_3$), m.p. 138–142°C. ESI-MS: calcd. for $C_{59}H_{63}IN_4O_{21}SNa$ ($[M + Na]^+$): m/z 1345.26; found: m/z 1329.4. ^{1}H NMR ($CDCl_3$, δ, ppm): 1.03 (d, 3H, *J* = 6.3 Hz, $CH_3(6)_{man}$), 1.31, 1.55 (2s, 6H, $(CH_3)_2C$), 2.07, 2.12 (2s, 6H, CH_3CO), 2.61–2.82 (m, 4H, CH_2), 3.66 (dd, 1H, *J* = 7.6 Hz, *J* = 12.0 Hz, H-6a$_{glu}$), 3.78 (dd, 1H, *J* = 1.5 Hz, *J* = 12.0 Hz, H-6b$_{glu}$), 3.85 (dq, 1H, *J* = 6.3 Hz, *J* = 9.7 Hz, H-5$_{man}$), 4.19 (ddd, 1H, *J* = 1.7 Hz, *J* = 7.6 Hz, *J* = 9.3 Hz, H-5$_{glu}$), 4.29–4.41 (m, 3H, H-4', H-5'a, H-5'b), 4.43 (dd, 1H, *J* = 0.7 Hz, *J* = 4.6 Hz, H-2$_{man}$), 4.49 (dd, 1H, *J* = 4.6 Hz, *J* = 9.3 Hz, H-3$_{man}$), 4.82 (dd, 1H, *J* = 3.7 Hz, *J* = 6.6 Hz, H-3'), 5.01 (dd, 1H, *J* = 1.9 Hz, *J* = 6.6 Hz, H-2'), 5.06 (bs, 1H, H-1$_{man}$), 5.07 (dd~t, 1H, *J* = 9.7 Hz, *J* = 9.7 Hz, H-4$_{man}$), 5.48 (dd~t, 1H, *J* = 9.9 Hz, *J* = 9.9 Hz, H-4$_{glu}$), 5.63 (d, 1H, *J* = 1.9 Hz, H-1'), 5.68 (dd, 1H, *J* = 9.3 Hz, *J* = 10.5 Hz, H-2$_{glu}$), 5.72 (dd, 1H, *J* = 1.5 Hz, *J* = 7.6 Hz, H-5$_{ur}$), 6.03 (d, 1H, *J* = 10.5 Hz, H-1$_{glu}$), 6.04 (dd~t, 1H, *J* = 9.4 Hz, *J* = 9.4 Hz, H-3$_{glu}$), 7.14 (d, 1H, *J* = 8.6 Hz, H-3$_{pyr}$), 7.22–7.58 (m, 10H, H-Ph, H-6$_{ur}$), 7.79–7.98 (m, 6H, H-Ph), 8.08 (dd, 1H, *J* = 2.4 Hz, *J* = 8.6 Hz, H-4$_{pyr}$), 8.28 (s, 1H, NH), 8.45 (d, 1H, *J* = 2.4 Hz, H-6$_{pyr}$); 8.91 (bs, 1H, NH). ^{13}C NMR ($CDCl_3$, δ, ppm): 17.28 (CH_3-6$_{man}$), 20.80, 21.35 ($\underline{C}H_3CO$), 25.25, 27.15 (($\underline{C}H_3)_2C$), 29.13, 29.47 (CH_2), 31.66 (C-2$_{man}$), 64.20 (C-5'), 66.67, 66.80 (C-6$_{glu}$, C-5$_{man}$), 69.69 (C-3$_{man}$), 70.25 (C-4$_{glu}$), 72.41, 74.36 (C-2$_{glu}$, C-4$_{man}$), 77.38, 78.48 (C-3$_{glu}$,C-5$_{glu}$), 80.89, 82.02, 84.46, 85.23 (C-1$_{glu}$, C-2', C-3', C-4'), 94.95 (C-1'), 101.76 (C-1$_{man}$), 102.58 (C-5$_{ur}$), 114.64 ($(CH_3)_2\underline{C}$), 123.29 (C-3$_{pyr}$), 128.33, 128.38, 128.53, 128.71, 128.83, 128.97, 129.75, 129.84, 129.93 (C-Ph, C-4$_{pyr}$), 132.71 (C-5$_{pyr}$), 133.28, 133.37, 133.62 (C-Ph), 141.15 (C-6$_{pyr}$), 142.33 (C-6$_{ur}$), 149.52 (C-2$_{pyr}$), 149.84 (C-2$_{ur}$), 162.94 (C-4$_{ur}$), 165.32, 165.80, 169.76, 169.86, 170.75, 172.38 ($CH_3\underline{C}OO$, Ph$\underline{C}OO$).

Glycoconjugate (15)

Route I: Compound **9** (40 mg, 0.044 mmol), uridine derivative **4** (13 mg, 0.044 mmol) and DMT-MM (12 mg, 0.044 mmol) in THF (2 mL) were submitted to the general procedure **D** described above. Reaction time: 9 h. Product **15** (23 mg, 43%) was obtained after purification by column chromatography with toluene : AcOEt (10:1 to 1:2, v/v) solvents system.

Route II: Glycoconjugate **12** (36 mg, 0.041 mmol), methyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside **5** (19 mg, 0.045 mmol), NIS (10 mg, 0.045 mmol) and $AgBF_4$ (0.3 mg, 0.002 mmol) in CH_2Cl_2 (2 mL) were submitted to the general procedure **A** described above. Reaction time: 3 h. Product **13** (3 mg, 7%) was obtained as a white solid after purification by column chromatography with toluene : AcOEt (10:1 to 1:2, v/v) and then toluene : AcOEt (4:1 to 3:1, v/v) solvent systems. $[\alpha]_D^{20}$= 23 (c = 0.8, $CHCl_3$), m.p. 130–134°C. ESI-MS: calcd. for $C_{58}H_{58}N_4O_{23}SNa$ ($[M + Na]^+$): m/z 1233.31; found: m/z 1233.5. ^{1}H NMR ($CDCl_3$, δ, ppm): 1.37, 1.59 (2s, 6H, $(CH_3)_2C$), 1.91, 1.97, 1.98, 2.01 (4s, 12H, CH_3CO), 3.54 (ddd, 1H, *J* = 2.2 Hz, *J* = 4.9 Hz, *J* = 9.1 Hz, H-5$_{glu}$), 3.81 (dd, 1H, *J* = 7.3 Hz, *J* = 11.7 Hz, H-6a$_{glu}$), 3.96 (dd, 1H, *J* = 1.3 Hz, *J* = 10.7 Hz, H-6b$_{glu}$), 4.03 (dd, 1H, *J* = 2.2 Hz, *J* = 12.1 Hz, H-6'a$_{glu}$), 4.18 (dd, 1H, *J* = 5.0 Hz, *J* = 12.0 Hz, H-6'b$_{glu}$), 4.19 (m, 1H, H-5$_{glu}$), 4.59 (d, 1H, *J* = 7.2 Hz, H-1'$_{glu}$), 4.74 (d, 1H, *J* = 2.3 Hz, H-4'), 4.98–5.06 (m, 3H, H-2'$_{glu}$, H-3'$_{glu}$, H-4'$_{glu}$), 5.24 (dd, 1H, *J* = 1.8 Hz, *J* = 6.3 Hz, H-2'), 5.32 (dd, 1H, *J* = 2.3 Hz, *J* = 6.3 Hz, H-3'), 5.43 (dd~t, 1H, *J* = 9.8 Hz, *J* = 9.8 Hz, H-4$_{glu}$), 5.52 (d, 1H, *J* = 1.8 Hz, H-1'), 5.59–5.65 (dd~t, 1H, *J* = 9.9 Hz, *J* = 9.9 Hz, H-2$_{glu}$), 5.77 (d, 1H, *J* = 7.9 Hz, H-5$_{ur}$), 5.97 (d, 1H, *J* = 10.6 Hz, H-1$_{glu}$), 5.98 (dd~t, 1H, *J* = 9.5 Hz, *J* = 9.5 Hz, H-3$_{glu}$), 7.13–7.55 (m, 11H, H-Ph, H-3$_{pyr}$, H-6$_{ur}$), 7.76–7.96 (m, 6H, H-Ph), 8.01 (dd, 1H, *J* = 2.5 Hz, *J* = 8.6 Hz, H-4$_{pyr}$), 8.48 (d, 1H, *J* = 2.5 Hz, H-6$_{pyr}$), 8.60 (s, 1H, NH), 9.55 (bs, 1H, NH). ^{13}C NMR ($CDCl_3$, δ, ppm): 20.47, 20.57, 20.66 ($\underline{C}H_3CO$), 24.94, 26.87 (($\underline{C}H_3)_2C$), 61.89 (C-6'$_{glu}$), 68.09, 68.42, 69.44, 70.16, 71.13, 71.76, 72.94, 74.26 (C-6$_{glu}$, C-2$_{glu}$, C-4$_{glu}$, C-4$_{glu}$, C-2'$_{glu}$,C-3'$_{glu}$, C-4'$_{glu}$, C-5'$_{glu}$), 78.17, 82.47, 83.06, 83.80, 87.85 (C-2', C-3', C-4', C-5$_{glu}$, C-1$_{glu}$), 99.37 (C-1'), 100.20 (C-1'$_{glu}$), 103.24 (C-5$_{ur}$), 114.39 ($\underline{C}(CH_3)_2$), 123.44 (C-3$_{pyr}$), 128.23, 128.30, 128.35, 128.50, 128.69, 128.80, 128.96, 129.69, 129.83, 129.88 (C-Ph, C-4$_{pyr}$), 132.27 (C-5$_{pyr}$), 133.25, 133.36, 133.60 (C-Ph), 141.51, 143.74 (C-6$_{ur}$, C-6$_{pyr}$), 150.27, 150.68 (C-2$_{ur}$, C-2$_{pyr}$), 162.69 (C-4$_{ur}$), 165.28, 165.35, 165.74, 167.92, 169.51, 170.17, 170.50, 170.76 ($CH_3\underline{C}OO$, Ph$\underline{C}OO$).

Glycoconjugate (16)

Route I: Compound **10** (21 mg, 0.022 mmol), uridine derivative **4** (7 mg, 0.022 mmol) and DMT-MM

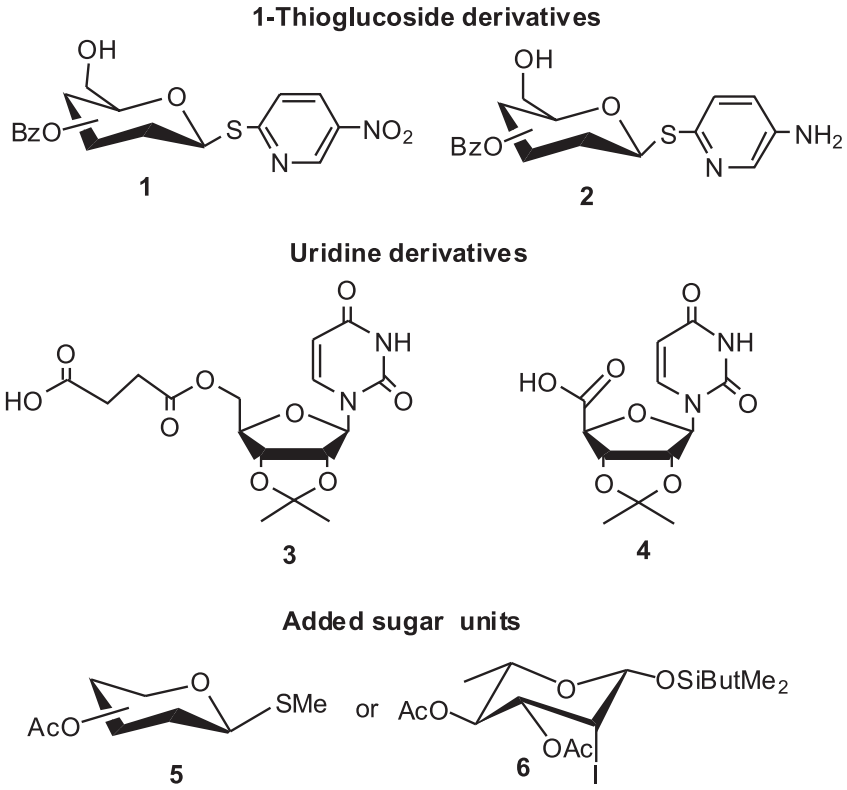
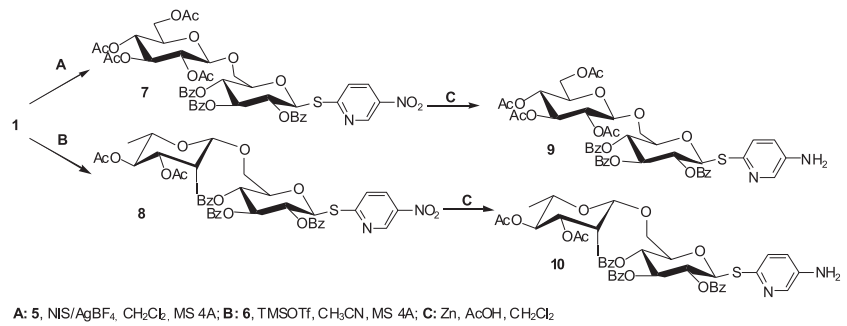


Figure 1. Substrates for glycoconjugates synthesis



Scheme 1. Synthesis of disaccharides derivatives of 5-amino-2-pyridyl-1-thioglucoside

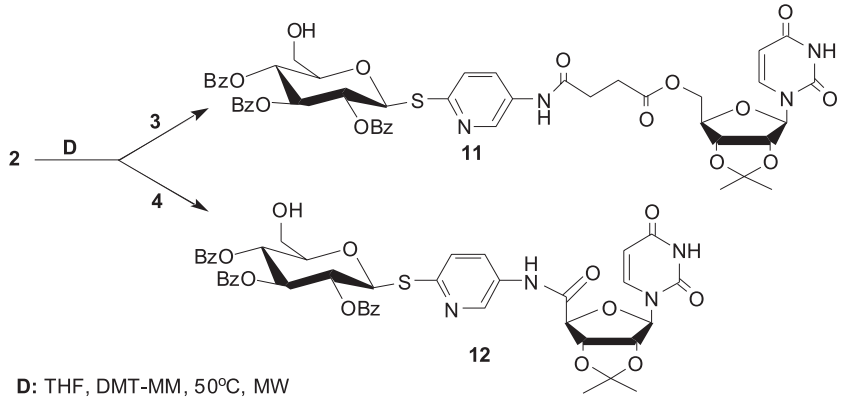
(6 mg, 0.022 mmol) in THF (2 mL) were submitted to the general procedure **D** described above. Reaction time: 6 h. Product **16** (6 mg, 22%) was obtained after purification by column chromatography with toluene : AcOEt (10:1 to 1:2, v/v) solvents system.

Route II: Glycoconjugate **12** (36 mg, 0.041 mmol), 3,4-di-*O*-acetyl-2-iodo-2,6-dideoxy-1-*O*-*tert*-butyldimethylsilyl-α-L-mannopyranoside **6** (22 mg, 0.045 mmol), TMSOTf (9 μL, 0.045 mmol) in CH_3CN (2 mL) were submitted to the general procedure **B** described above. Reaction time: 15 min. Product **16** (38 mg, 75%) was obtained as a white solid after purification by column chromatography with toluene : AcOEt (10:1 to 1:2, v/v) solvent system. $[\alpha]_D^{20} = 21.2$ (c = 0.34, $CHCl_3$), m.p. 135-139°C. ESI-MS: calcd. for $C_{54}H_{53}IN_4O_{19}SNa$ ($[M + Na]^+$): m/z 1243.20; found: m/z 1243.3. ^1H NMR ($CDCl_3$, δ, ppm): 1.05 (d, 3H, J = 6.3 Hz, $CH_3(6)_{man}$), 1.36, 1.56

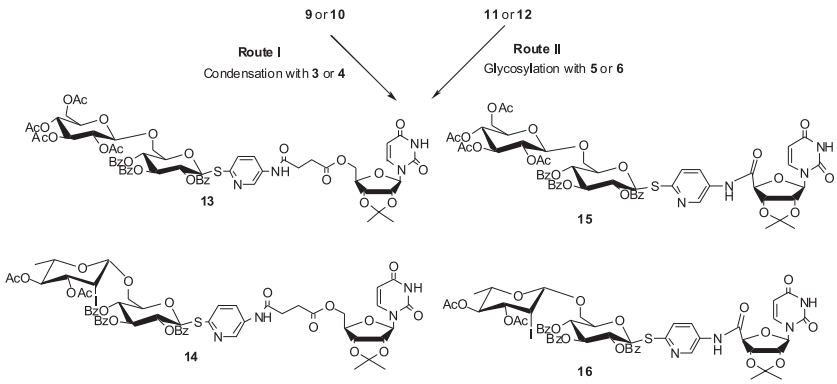
(2s, 6H, $(CH_3)_2C$), 2.04, 2.08 (2s, 6H, CH_3CO), 3.73 (dd, 1H, *J* = 6.9 Hz, *J* = 12.2 Hz, H-6a$_{glu}$), 3.78 (dd, 1H, *J* = 1.7 Hz, *J* = 12.2 Hz, H-6b$_{glu}$), 3.83 (dq, 1H, *J* = 6.3 Hz, *J* = 9.7 Hz, H-5$_{man}$), 4.21 (ddd, 1H, *J* = 1.7 Hz, *J* = 6.9 Hz, *J* = 9.4 Hz, H-5$_{glu}$), 4.49 (d, 1H, *J* = 4.6 Hz, H-2$_{man}$), 4.52 (dd, 1H, *J* = 4.6 Hz, *J* = 9.3 Hz, H-3$_{man}$); 4.70 (d, 1H, *J* = 2.5 Hz, H-4'); 5.05 (dd~t, 1H, *J* = 9.5 Hz, *J* = 9.5 Hz, H-4$_{man}$), 5.13 (s, 1H, H-1$_{man}$), 5.23 (dd, 1H, *J* = 2.1 Hz, *J* = 6.3 Hz, H-2'), 5.25 (dd, 1H, *J* = 2.5 Hz, *J* = 6.3 Hz, H-2'), 5.47 (d, 1H, *J* = 2.1 Hz, H-1'), 5.50 (dd~t, 1H, *J* = 9.8 Hz, *J* = 9.8 Hz, H-4$_{glu}$), 5.66 (dd~t, 1H, *J* = 9.9 Hz, *J* = 9.9 Hz, H-2$_{glu}$), 5.77 (d, 1H, *J* = 7.9 Hz, H-5$_{ur}$), 5.97 (d, 1H, *J* = 10.4 Hz, H-1$_{glu}$), 6.01 (dd~t, 1H, *J* = 9.5 Hz, *J* = 9.5 Hz, H-3$_{glu}$), 7.12–7.56 (m, 11H, H-Ph, H-3$_{pyr}$, H-6$_{ur}$), 7.79–7.98 (m, 6H, H-Ph), 8.04 (dd, 1H, *J* = 2.5 Hz, *J* = 8.7 Hz, H-4$_{pyr}$), 8.44 (d, 1H, *J* = 2.5 Hz, H-6$_{pyr}$), 8.73 (s, 1H, NH), 9.65 (bs, 1H, NH). ^{13}C NMR ($CDCl_3$, δ, ppm): 17.34 (CH_3-6$_{man}$), 20.79, 21.04 ($\underline{C}H_3CO$), 25.02, 27.00 (($\underline{C}H_3)_2C$), 29.71 (C-2$_{man}$), 66.33 (C-6$_{glu}$), 66.92 (C-5$_{man}$), 69.42 (C-3$_{man}$), 69.58 (C-4$_{glu}$), 70.14 (C-2$_{glu}$), 72.49 (C-4$_{man}$), 74.29 (C-3$_{glu}$), 78.56 (C-5$_{glu}$), 82.33 (C-1$_{glu}$), 82.64, 83.60 (C-2', C-3'), 87.37 (C-4'), 99.44 (C-1'), 101.62 (C-1$_{man}$), 103.34 (C-5$_{ur}$), 114.75 (($CH_3)_2\underline{C}$), 123.38 (C-3$_{pyr}$), 128.31, 128.36, 128.39, 128.53, 128.69, 128.82, 128.99, 129.74, 129.87, 129.91 (C-Ph, C-4$_{pyr}$), 132.26 (C-5$_{pyr}$), 133.25, 133.36, 133.60 (C-Ph); 141.33 (C-6$_{pyr}$); 143.77 (C-6$_{ur}$), 150.24 (C-2$_{ur}$), 150.72 (C-2$_{pyr}$), 162.63 (C-4$_{ur}$), 165.29, 165.77, 167.64, 169.84, 170.89 ($CH_3\underline{C}OO$, Ph$\underline{C}OO$).

RESULTS AND DISCUSSION

Taking into consideration earlier reported structure of potential GTs inhibitors as well as abovementioned structural requirements for analogues of GTs natural substrates, new group of glycoconjugates with one more sugar unit added to earlier obtained 1-thioglucosyl uridine derivatives were



Scheme 2. Synthesis of selectively protected glycoconjugates **11** and **12**



Scheme 3. Two different routes for synthesis of glycoconjugates **13**-**16**

Table 1. Reactions conditions and yields of the synthesized compounds.

Entry	Substrate I	Substrate II	Reaction conditions	Product	Yield [%]
1.	**1**	-	Zn, AcOH, CH_2Cl_2, 30 min.	**2**	66
2.	**1**	**5**	NIS/$AgBF_4$, CH_2Cl_2, MS 4Å, 24 h	**7**	50
3.	**1**	**6**	TMSOTf, CH_3CN, MS 4Å, 2 h	**8**	63
4.	**7**	-	Zn, AcOH, CH_2Cl_2, 75 min.	**9**	63
5.	**8**	-	Zn, AcOH, CH_2Cl_2, 30 min.	**10**	31
6.	**2**	**3**	THF, DMT-MM, 50°C, MW, 6 h	**11**	51
7.	**2**	**4**	THF, DMT-MM, 50°C, MW, 4 h	**12**	33
8.	**9**	**3**	THF, DMT-MM, 50°C, MW, 8 h	**13**	4
9.	**10**	**3**	THF, DMT-MM, 50°C, MW, 6 h	**14**	6
10.	**9**	**4**	THF, DMT-MM, 50°C, MW, 9 h	**15**	43
11.	**10**	**4**	THF, DMT-MM, 50°C, MW, 6 h	**16**	22
12.	**11**	**5**	NIS/$AgBF_4$, CH_2Cl_2, MS 4Å, 1 h	**13**	32
13.	**11**	**6**	TMSOTf, CH_3CN, MS 4Å, 10 min.	**14**	29
14.	**12**	**5**	NIS/$AgBF_4$, CH_2Cl_2, MS 4Å, 3 h	**15**	7
15.	**12**	**6**	TMSOTf, CH_3CN, MS 4Å, 15 min.	**16**	75

synthesized. As an additional sugar unit derivatives of glucose or 2-iodo-2-deoxy mannose were chosen (Fig. 1).

Extension of sugar part of glycoconjugates could be obtained in two different procedures. One of them would be glycosylation using selectively protected 5-nitro-2-pyridyl-1-thioglucoside **1** as acceptor and donors **5** or **6**. Next, the nitro group in aglycone of obtained disaccharides **7** or **8** could be reduced (Scheme 1) and finally reduced products **9** or **10** could be used in condensation reaction with uridine derivatives **3** or **4**. Second way would be glycosylation reaction of previously obtained selectively protected glycoconjugates **11** or **12** (Scheme 2) used as glycosyl acceptors with compounds **5** or **6** as glycosyl donors (Scheme 3).

For synthesis of disaccharides **7** or **8** selectively protected 5-nitro-2-pyridyl-1 thioglucoside **1** was selected as glycosyl acceptor. Simple and efficient synthesis of this compound was described a few years ago [11]. Glycosylation conditions were adjusted to type of used glycosyl donor. So, for glycosylation with methyl-1-thioglucoside **5** as glycosyl donor NIS/$AgBF_4$ system was applied as a promoter. According to the recently reported data, 1-thioglycosides could be activated by this system and coupling reactions proceeded very rapidly and provided the corresponding disaccharide in minutes (16). For activation of 3,4-di-*O*-acetyl-2-iodo-2,6-dideoxy-1-*O*-*tert*-butyldimethylsilyl-α-L-mannopyranoside **6** TMSOTf was marked out and glycosylation was performed in CH_3CN as a solvent (14). Application of such reaction conditions allowed to obtain expected disaccharides **7** and **8** in proper yields 50% and 63%, respectively. For reduction of a nitro group in aglycone of these two disaccharides, reduction procedure with zinc powder/acetic acid system in CH_2Cl_2, previously described by Roy and co-workers for 4-nitrophenyl-1-thioglycosides (17) and recently utilized for other derivatives of 5-nitro-2-pyridyl-1-thioglycosides (9), was applied. Reaction proceeded at room temperature and 5-amino-2-pyridyl-1-thioglycosides **9** and **10** were obtained with 63% and 31% yield, respectively. Both obtained compounds were used for condensation reactions with uridine derivatives **3** and **4**. Amide bond formation in reaction between amine and carboxylic acid becomes feasible at high temperature or in the presence of a wide range of condensing agents (18). Kaminski and co-workers showed the efficiency of 2-chloro-4,6-disubstituted-1,3,5-triazines in the presence of tertiary amine (e.g., N-methylmorpholine) in formation of the peptide bond. So-called "superactive ester" containing an excellent leaving group was formed in this reaction (19). 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 of amides and esters (20). Successful application of DMT-MM as condensing agent for preparation of glycoconjugates derivatives of uridine **3** was described last year (9). The only drawback of this method was a long reaction time, even two days. In order to avoid such long reaction time in amide bond formation, microwave irradiation could be applied (21, 22). For condensation of amine group in aglycone of disaccharides **9** and **10** with carboxylic group in uridine derivatives **3** and **4,** DMT-MM was applied as condensing agent in combination with microwave irradiation (Standard program, 50^{o}C). As a result of these reactions, glycoconjugates **13**-**16** were obtained. Yields of these products are presented in Table 1.

In the second procedure, glycoconjugates **11** and **12** were obtained first with application of the same condensation conditions as described above. Then, these compounds were used in glycosylation reactions with glycosyl donors **5** or **6**. Also in this case, the expected glycoconjugates **13**-**16** were obtained. As it turned out, both reactions sequences led to the expected products. It is worth mentioning that final condensation of disaccharides **9** or **10** with uridine derivative **3** allow to obtain glycoconjugates **13** and **14** with rather poor yield of 4% and 6%, respectively). The same route in the case of uridine derivatives **4** supplied glycoconjugates **15** and **16** with considerably better yields.

All products were purified by column chromatography and their structure was determined by ^{1}H and ^{13}C NMR spectroscopy. Final uridine derivatives of 1-thioglycosides **13**-**16** were additionally characterized on the basis of mass spectrometry. The **o**btained spectra proved the structure of glycoconjugates **13**-**16**. New singlet of amide proton origin appeared in ^{1}H NMR spectra of obtained glycoconjugates, whereas a broad signal from two amine protons present in spectra of the substrates used disappeared in the product's spectra.

Biological activity of glycoconjugates **13**-**16** will be tested, paying special attention to a possible inhibition of enzymes from glycosyltransferases group, especially 1,4-β-galactosyltransferase.

Acknowledgment

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