GLYCOCONJUGATES, PRODUCTS OF URIDINE DERIVATIVES
PHOSPHITYLATION AND OXIDATION AS GLYCOSYLTRANSFERASES
POTENTIAL INHIBITORS

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Abstract: The title compounds, variously protected 5'-uridine derivatives connected with 1-thiosugar with thio-
phosphoesters fragment (17-22) were synthesized in sequence of reactions: phosphitylation – reaction of 5'-
hydroxyl group of selectively protected nucleoside with a phosphitylating agent (N,N-diisopropyl chlorophos-
phoamidite), connection an phosphoroamidites with 2-bromoethanol or 3-bromopropanol and secondary oxi-
dation with sulfur presence and finally condensation reaction of obtained products with 1-thiosugar. Received
glycoconjugates (17-22) had a structure which mimic to structure of natural glycosyltransferases substrates.

Keywords: glycosyltransferases, glycoconjugates, phosphitylation, oxidation

Oligosaccharides located in a mammalian cell play a fundamental role in many important biological
processes, for example: immune recognition, cell-cell communication and initiation of microbial
pathogenesis and are commonly found as glycocon-
jugates (glycoproteins or glycolipids) (1). Enzymes
responsible for creating the glycosidic bonds in
nature belongs to the family of glycosyltransferases
(GTs). These enzymes catalyze the transfer of a
monosaccharide from a donor (usually a nucleotide-
sugar donor) to different acceptors (2). GTs are clas-
sified on the basis of the type of donor that is utilized
by the enzyme and the position and stereochemistry
of created glycosidic bond. These enzymes exhibit
strict regio- and stereospecificity, and can transfer a
sugar unit with either retention or inversion of con-
figuration at the anomeric carbon atom. GTs are
Golgi-localized enzymes and like the all such
enzymes share the common topology of type II
membrane proteins, consisting of a short N-terminal
cytoplasmic domain, a single transmembrane seg-
ment and a stem region of variable length followed by
a large C-terminal catalytic domain (3).

Enzymes are powerful tools in the synthesis
and modification of carbohydrates because most of
the relevant reactions require high degrees of chemoh-
regio- and stereo-selectivity (4). With regard to sig-
nificant role of glycoconjugates in function of mam-
alian cell, control studies of glycosyltransferases
activity are interesting. Compounds that can adjust
the biosynthesis of oligosaccharides may find appli-
cations as novel therapeutics for a wide range of dis-
eases as well as provide important tools for studying
biological functions of glycoconjugates (5).

The most important strategies looking for
inhibitors of GTs are the design for acceptor ana-
logues, for donor analogues and for transition state
mimetics including bisubstrate or trisubstrate ana-
logues (6). Despite an increasing number of synthe-
sized potential inhibitors, only a few of them have
exhibited significant activity. From these studies, a
new generation of inhibitors based on carbohydrate
mimetics has recently emerged (7). Compounds of
this family are structurally altered analogues of car-
bohydrates designed to simulate the shape and most
functionalities of the natural substrates with the goal
of modulating their biological activities. For exam-
ple, iminosugars have become a very important
source of new substrate analogues for the study of
glycosyltransferases (8). Some carbasugar
nucleotide analogues were potent sialyltransferase
inhibitors (9). Also C-glycosidic sugar nucleotide
analogues were found to be a good inhibitors of β-
galactosyltransferase from bovine milk (10).

Recently 5’-uridine derivatives connected with
5-amino-2-pyridyl-1-thio-β-D-glycosides via a suc-
cinic linker were proposed as analogues of GTs natural substrate with potential inhibition activity (11). Some of them were tested for ability to inhibit the propagation of classical swine fever virus (CSFV). Reduction of the number CSFV-infected cells was observed without significant toxicity for mammalian cells. Encouraged by these positive results new scale of compounds was made, in which variously protected 5'-uridine derivatives were connected with 1-thiosugar via thiophosphoesters fragments (Scheme 1).
EXPERIMENTAL

1H-NMR and 13C NMR spectra were recorded on a Varian spectrometer at 300 and 600 MHz, using TMS as an internal standard and CDC13 or DMSO-d6 as solvents. 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 a Perkin-Elmer 141 polarimeter using a sodium lamp (589.3 nm) at room temperature. Reactions were monitored by TLC on precoated plates of silica gel 60 F254 (Merck) developed with either toluene : Et3N (20:1 to 10:1, v/v) solvent system. Organic solvents were evaporated on a rotary evaporator under diminished pressure at 50°C.

Solution of N,N-diisopropylchlorophosphoamidite in benzene (1 mL of benzene solution containing 0.17 mmol of phosphitylating agent) (12), 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucose (1) (13), 2',3'-O-isopropylideneuridine (2) (14), 5'-O-tert-butylidimethylsilyl-uridine (3) (15), 2',3'-di-O-acetyl-5'-O-tert-butylidimethylsilyl-uridine (4) (16) were prepared according to the published procedures. 3-N-benzoyl-2',3'-di-O-benzoyl-5'-O-tert-butylidimethylsilyl-uridine (6) was obtained by benzoylation of 4 using procedure described earlier for another sugar derivatives (17). Compound 6 was isolated, but was subjected directly to deprotection. 2',3'-Di-O-acetyl-uridine (5) and 3-N-benzoyl-2',3'-di-O-benzoyl-uridine (7) were synthesized by selective removal of TBDMS group in 2',3'-di-O-acetyl-5'-O-tert-butylidimethylsilyl-uridine 4 and 3-N-benzoyl-2',3'-di-O-benzoyl-5'-O-tert-butylidimethylsilyl-uridine 6 in the presence of tetrafluoroboric acid in acetonitrile (17). Both reactions were led in room temperature for 24 h. Crude products were purified by column chromatography and 2',3'-di-O-acetyl-uridine 5 was received in 30% yield while 3-N-benzoyl-2',3'-di-O-benzoyl-uridine 7 was received in 54% yield. Structures of the products were confirmed by 1H NMR and 13C NMR spectra.

Compound 5

1H NMR (CDCl3, δ, ppm): 2.10, 2.15 (2s, 6H, (CH3)2CO), 2.95 (s, 1H, OH), 3.80–4.00 (m, 2H, H-5'a, H-5'b), 4.22 (d, 1H, J = 1.95 Hz, H-4'), 5.45–5.52 (m, 2H, H-2', H-3'), 5.80 (dd, 1H, J = 1.9 Hz, J = 8.1 Hz, H-5'w), 6.08 (m, 1H, H-1'), 7.76 (d, 1H, J = 8.1 Hz, H-6a), 9.13 (s, 1H, NH). 13C NMR (CDCl3, δ, ppm): 20.43, 20.64 (CH3,CO); 61.83 (C-5'); 71.19 (C-3'); 72.95 (C-2'); 83.45 (C-4'); 87.61 (C-1'); 103.24 (C-5p); 140.68 (C-6a); 150.45 (C-2w); 163.00 (C-4a); 169.75, 170.07 (CH3CO), [α]D = 7.7 (c = 2.2, MeOH), m.p. 112–115°C.

Compound 7

1H NMR (CDCl3, δ, ppm): 2.90 (bs, 1H, OH), 3.96–4.08 (m, 2H, H-5'a, H-5'b), 4.46 (d, 1H, J = 1.8 Hz, H-4'), 5.82 (dd, 1H, J = 5.9 Hz, J = 6.5 Hz, H-2'), 5.85 (dd, 1H, J = 2.4 Hz, J = 5.9 Hz, H-3'), 5.93 (d, 1H, J = 8.2 Hz, H-5w), 6.37 (d, 1H, J = 6.5 Hz, H-1'), 7.28–7.34 (m, 2H, H-Ph), 7.38–7.46 (m, 4H, H-Ph), 7.51 (m, 1H, H-Ph), 7.55–7.63 (m, 2H, H-Ph), 7.85–7.90 (m, 2H, H-Ph), 7.94–7.98 (m, 2H, H-Ph), 8.40 (C-4'), 8.74 (C-1'), 103.29 (C-5w), 128.42, 128.50, 128.63, 128.85, 129.14, 129.80, 129.88, 130.57, 131.32, 133.75, 135.10 (C-Ph), 140.36 (C-6w), 149.63 (C-2w), 161.98 (C-4w), 165.52, 165.67, (PhCOO), 168.41 (PhCON). [α]D = −113.3 (c = 0.6, CHCl3), m.p. 181–184°C.

General procedure for phosphitylation of protected 5'-uridine derivatives

To a protected 5'-uridine derivative 2, 5, or 7 (1 eqv.) solution of N,N-diisopropyl chlorophosphoamidite (2 eqv.) with N,N-diisopropylamine (10 eqv.) in benzene (phosphitylating agent) was added. The mixture was stirred for 24 h at room temperature under argon atmosphere. The reaction was monitored by TLC on silica gel plates using toluene : Et3N (10:1) solvent system. The reaction mixtures were concentrated and crude products were purified by column chromatography with toluene : Et3N (20:1 to 10:1, v/v) solvent system. Main products were stable in solution of toluene : Et3N (10:1, v/v) solvent system and under argon atmosphere.

Phosphoroamidite of 2',3'-O-isopropylideneuridine (8)

Uridine derivative 2 (150 mg, 0.53 mmol) and solution of N,N-diisopropyl chlorophosphoamide (1.06 mmol) and N,N-diisopropylamine (5.3 mmol) in benzene (total volume 6.2 mL) were submitted to the general procedure described above. Main product 8 (258 mg) was received in 95% yield as a colorless oil. 1H NMR (DMSO, δ, ppm): 1.06–1.14 (m, 2H, (CH3)2CH-N), 1.26, 1.46 (2s, 6H, (CH3)2C), 3.38–3.56 (m, 4H, (CH3)2CH-N), 3.58–3.72 (m, 2H,
H-5',a-H-5'-b), 4.13 (m, 1H, H-4'), 4.74 (dd, 1H, J = 3.8 Hz, J = 6.6 Hz, H-3'), 5.00 (dd, 1H, J = 2.2 Hz, J = 6.6 Hz, H-2'), 5.38 (d, 1H, J = 8.1 Hz, H-5''a); 5.76 (d, 1H, J = 2.0 Hz, H-1'), 7.64 (d, 1H, J = 8.1 Hz, H-6''), 11.38 (s, 1H, NH). 13C NMR (DMSO, δ ppm): 23.52, 23.60, 24.24, 24.35 (C(CH3)2CH-N), 25.06, 26.91 ((C(CH3)2CH-N), 43.79, 43.92 (C(CH3)2CH-N), 64.27 (d, J = 2.15 Hz, C-5'), 81.04 (C-3'), 83.50 (C-5'), 86.26 (d, J = 10.7 Hz, C-4'), 92.39 (C-1'), 101.52 (C-5'',a) 1.1300 (C(CH3)2), 142.58 (C-6''a), 150.23 (C-2'',a), 163.19 (C-4'',a).

Phosphoroamidite of 2',3'-di-O-acetyl-uridine (9)

Uridine derivative 5 (78 mg, 0.24 mmol) and solution of N,N-diisopropyl chorophosphoamidite (0.48 mmol) and N,N-diisopropylamine (2.4 mmol) in benzene (total volume 2.8 mL) were submitted to the general procedure described above. Main product 9 (131 mg) was received in 97% yield as a colorless oil. 1H NMR (DMSO, δ ppm): 1.08–1.19 (m, 2H, (CH2)2(NH)), 2.03, 2.08 (2s, 6H, (CH3)2CO), 3.43–3.57 (m, 4H, (CH3)2CH-N), 3.66–3.80 (m, 2H, H-5'a-H-5'b), 4.22 (dd, 1H, J = 4.3 Hz, J = 8.8 Hz, H-4'), 5.35 (dd, 1H, J = 4.3 Hz, J = 6.1 Hz, H-3'), 5.44 (dd-t, 1H, J = 6.0 Hz, J = 6.1 Hz, H-2'), 5.67 (d, 1H, J = 8.0 Hz, H-5'',a), 5.92 (d, 1H, J = 6.0 Hz, H-1'), 7.71 (d, 1H, J = 8.1 Hz, H-6''), 11.10 (bs, 1H, NH). 13C NMR (DMSO, δ ppm): 20.61, 20.71 (C(CH3)2), 24.17, 24.19, 24.22, 24.23, 24.65, 24.66, 24.70, 24.71 ((C(CH3)2CH-N), 44.40, 44.46, 44.49, 44.55 ((C(CH3)2CH-N), 63.98 (d, J = 20.8 Hz, C-5'), 70.78 (C-3'), 72.31 (C-2'), 81.83 (d, J = 11.6 Hz, C-4'), 87.31 (C-1'), 102.67 (C-5'',a), 141.31 (C-6''a), 150.77 (C-2'',a), 163.32 (C-4'',a), 167.71, 169.76 (C(CH3)2CO).

Phosphoroamidite of 3-N-benzoyl-2',3'-di-O-benzoyluridine (10)

Uridine derivative 7 (100 mg, 0.18 mmol) and N,N-diisopropyl chorophosphoamidite (0.36 mmol) and N,N-diisopropylamine (1.8 mmol) in benzene (total volume 2.1 mL) were submitted to the general procedure described above. Main product 10 (141 mg) was received in 97% yield as a colorless oil. 1H NMR (DMSO, δ ppm): 1.10–1.16 (m, 24H, (CH2)3CH-N), 3.42–3.57 (m, 4H, (CH3)2CH-N), 3.82–3.94 (m, 2H, H-5'a-H-5'b), 4.60 (dd, 1H, J = 4.6 Hz, J = 9.3 Hz, H-4'), 5.81 (dd, 1H, J = 4.9 Hz, J = 6.2 Hz, H-3'), 5.90 (dd-t, 1H, J = 5.8 Hz, H-2'), 6.06 (d, 1H, J = 8.1 Hz, H-5'',a), 6.25 (d, 1H, J = 5.3 Hz, H-1''), 6.38–7.42 (m, 2H, H-Ph), 7.46–7.50 (m, 2H, H-Ph), 7.57–7.64 (m, 3H, H-Ph), 7.66 (m, 1H, H-Ph), 7.76–7.82 (m, 3H, H-Ph), 7.92–8.02 (m, 4H, H-Ph), 8.08 (d, 1H, J = 8.3 Hz, H-6''). 13C NMR (DMSO, δ ppm): 24.17, 24.21, 24.25, 24.68, 24.74, (CH3)2CH-N), 44.42, 44.50, 44.58 ((CH3)2CH-N), 63.93 (d, J = 20.8 Hz, C-5'), 71.56 (C-3'), 73.86 (C-2'), 82.02 (d, J = 10.4 Hz, C-4'), 88.65 (C-1'), 102.41 (C-5'',a), 128.72, 128.74, 129.11, 129.18, 129.70, 129.73, 129.76, 129.92, 130.65, 131.38, 134.25, 134.38, 136.00 (C-Ph), 142.43 (C-6''a), 149.44 (C-2'',a), 162.05 (C-4'',a), 165.07, 165.10 (PhCOO), 169.40 (PhCON).

General procedure for connection of uridine’s phosphoamidites with alcohol and secondary oxidation with sulfur presence

To a solution of tetrazole in dry CH3CN (4 eqv., 0.45 mmol/1 mL) alcohol (2 eqv.) was added. A mixture of tetrazole and alcohol was added to uridine’s phosphoamidite 8, 9 or 10 (1 eqv.) at 0°C. The reaction was maintained for 15 min at 0°C. An intermediate of this reaction wasn’t isolated. Sulfur (1.2 eqv.) was dissolved in toluene (16 mg, 0.5 mmol/1 mL) with the addition of N,N-diisopropylamine (8 eqv.). The resulting mixture was poured into intermediate product. Reactions were performed for 24 h at room temperature under argon atmosphere and monitored by TLC on silica gel plates using toluene: AcOEt (1:1, v/v) solvent system. The reaction mixtures were concentrated and crude products were purified by column chromatography.

Compound 11

Phosphoroamidite of 2',3'-O-isopropylideneuridine 8 (270 mg, 0.53 mmol), solution of tetrazole (4.70 mL, 2.10 mmol) in dry CH3CN, 2-bromoethanol (0.07 mL, 1.06 mmol), solution of sulfur (20 mg, 0.63 mmol) in toluene (1.3 mL) and N,N-diisopropylamine (0.59 mL, 4.24 mmol) were used in reaction according to the general procedure described above. The reaction mixture was concentrated to give a crude product which was purified by column chromatography with CHCl3: acetone (100:1 to 10:1, v/v) solvent system to yield 11 as a solidifying liquid (157 mg, 50% yield). [α]20 = 2.1 (c = 1.1, CHCl3). 1H NMR (CDCl3, δ ppm): 1.36, 1.58 (2s, 6H, (CH3)2C), 3.50–3.60 (m, 4H, CH3Br), 4.28–4.41 (m, 7H, H-4', H-5',a-H-5',b, CH3O), 4.88 (dd, 1H, J = 3.5 Hz, J = 6.5 Hz, H-3'), 4.97 (dd, 1H, J = 2.4 Hz, J = 6.5 Hz, H-2'), 5.75 (d, 1H, J = 1.8 Hz, H-1'), 5.79 (d, 1H, J = 7.6 Hz, H-5'',a), 7.37 (d, 1H, J = 8.2 Hz, H-6''a), 9.39 (s, 1H, NH). 13C NMR (CDCl3, δ ppm): 25.31, 27.14 ((CH3)2C), 29.29 (d, J = 5.1 Hz, CH3Br), 29.35 (d, J = 6.2 Hz, CH3Br), 67.61 (d, J = 5.1 Hz, CH3O), 67.84 (d, J = 6.3 Hz, C-5'), 80.82 (C-3'), 84.49 (C-2'), 85.55 (d, J = 8.1 Hz, C-4'), 94.41 (C-1'), 102.81 (C-5'',a), 114.64...
Compound 12
Phosphoroamidite of 2',3'-O-isopropylideneuridine 8 (176 mg, 0.34 mmol), solution of tetrazole (3.04 mL, 1.37 mmol) in dry CH₂CN, 3-bromopropanol (0.06 mL, 0.68 mmol), solution of sulfur (13 mg, 0.41 mmol) in toluene (0.82 mL) and N,N-diisopropylamine (0.38 mL, 2.74 mmol) were used in reaction according to the general procedure described above. Reaction was monitored by TLC on silica gel plates using toluene : AcOEt (1:1, v/v) solvent system to yield a crude product which was purified by column chromatography with toluene : AcOEt (1:1, v/v) solvent system to yield **Compound 12** as a solidifying liquid (101 mg, 48% yield). [δ]₂₀ = 3.1 (c 1.3, CHCl₃). ¹H NMR (CDCl₃, δ ppm): 1.36, 1.58 (2s, 6H, (CH₃)₂C), 2.17-2.26 (m, 4H, CH₂), 3.59 (m, 4H, CH₂Br), 4.18-4.25 (m, 4H, CH₂O), 4.27-4.32 (m, 2H, H-5'a, H-5'b), 4.37 (m, 1H, H-4'), 4.87 (dd, 1H, J = 3.6 Hz, J = 5.4 Hz, H-3'), 4.97 (dd, 1H, J = 2.4 Hz, J = 6.6 Hz, H-2'), 5.73 (d, 1H, J = 1.8 Hz, H-1'), 5.76 (dd, 1H, J = 2.4 Hz, J = 8.4 Hz, H-5'a), 7.36 (d, 1H, J = 7.8 Hz, H-6'), 9.38 (s, 1H, NH). ¹³C NMR (CDCl₃, δ ppm): 25.31, 27.14 ((CH₃)₂C), 28.97 (d, J = 3.5 Hz, CH₃), 32.82 (d, J = 8.1 Hz, CH₂Br), 66.13 (d, J = 6.9 Hz, CH₂O), 67.57 (d, J = 4.6 Hz, C-5'), 80.86 (C-3'), 84.51 (C-2'), 85.72 (d, J = 8.1 Hz, C-4'), 94.54 (C-1'), 102.72 (C-5'a), 114.63 ((CH₃)₂C), 142.19 (C-6'), 150.03 (C-2'), 163.30 (C-4'a).

Compound 13
Phosphoroamidite of 2',3'-di-O-acetyl-uridine 9 (130 mg, 0.23 mmol), solution of tetrazole (2.07 mL, 0.93 mmol) in dry CH₂CN, 3-bromopropanol (0.04 mL, 0.47 mmol), solution of sulfur (9 mg, 0.28 mmol) in toluene (0.56 mL) and N,N-diisopropylamine (0.26 mL, 1.86 mmol) were used in reaction according to the general procedure described above. The reaction mixture was concentrated to give a crude product which was purified by column chromatography with toluene : AcOEt (6:1 to 1:1, v/v) solvent system to yield **Compound 13** as a solidifying liquid (101 mg, 48% yield). [δ]₂₀ = 3.1 (c 1.3, CHCl₃). ¹H NMR (CDCl₃, δ ppm): 1.36, 1.58 (2s, 6H, (CH₃)₂C), 1.97-2.06 (m, 4H, CH₂), 3.60-3.67 (m, 4H, CH₂O), 4.40-4.47 (m, 5H, H-5'a, CH₂O), 4.22-4.37 (m, 7H, H-4í, H-5íb, CH₂O), 5.62 (dd, 1H, J = 1.8 Hz, J = 8.4 Hz, H-5'a), 7.68 (d, 1H, J = 7.8 Hz, H-6'), 9.38 (s, 1H, NH). ¹³C NMR (CDCl₃, δ ppm): 22.41, 23.71 (2s, CH₃), 28.97 (d, J = 3.5 Hz, CH₃), 32.82 (d, J = 8.1 Hz, CH₂Br), 66.13 (d, J = 6.9 Hz, CH₂O), 67.57 (d, J = 4.6 Hz, C-5'), 80.86 (C-3'), 84.51 (C-2'), 85.72 (d, J = 8.1 Hz, C-4'), 94.54 (C-1'), 102.72 (C-5'a), 114.63 ((CH₃)₂C), 142.19 (C-6'), 150.03 (C-2'), 163.30 (C-4'a).

Compound 14
Phosphoroamidite of 2',3'-di-O-acetyl-uridine 9 (130 mg, 0.23 mmol), solution of tetrazole (2.07 mL, 0.93 mmol) in dry CH₂CN, 3-bromopropanol (0.04 mL, 0.47 mmol), solution of sulfur (9 mg, 0.28 mmol) in toluene (0.56 mL) and N,N-diisopropylamine (0.26 mL, 1.86 mmol) were used in reaction according to the general procedure described above. The reaction mixture was concentrated to give a crude product which was purified by column chromatography with toluene : AcOEt (1:1, v/v) solvent system to yield **Compound 14** as a solidifying liquid (101 mg, 69% yield). [δ]₂₀ = 3.1 (c 1.3, CHCl₃). ¹H NMR (CDCl₃, δ ppm): 0.97, 1.15 (2s, 6H, (CH₃)₂C), 2.15-2.25 (m, 4H, CH₂), 3.62-3.67 (m, 4H, CH₂O), 4.41-4.53 (m, 5H, H-5'a, CH₂O), 4.22-4.37 (m, 7H, H-4í, H-5íb, CH₂O), 5.62 (dd, 1H, J = 1.8 Hz, J = 8.4 Hz, H-5'a), 7.68 (d, 1H, J = 7.8 Hz, H-6'), 9.38 (s, 1H, NH). ¹³C NMR (CDCl₃, δ ppm): 20.36, 20.56 (CH₂O), 28.81 (d, J = 12.7 Hz, CH₂Br), 32.64 (d, J = 4.5 Hz, CH₃), 32.69 (d, J = 4.7 Hz, CH₂O), 66.37 (d, J = 4.5 Hz, CH₂O), 66.64 (d, J = 4.7 Hz, CH₂O), 66.74 (d, J = 4.7 Hz, C-5'), 70.59 (C-3'), 72.52 (C-2'), 80.99 (d, J = 9.4 Hz, C-4'), 86.24 (C-1'), 103.53 (C-5'a), 139.40 (C-6'), 150.37 (C-2'), 162.62 (C-4'a), 169.53, 169.70 (CH₂CO).

Compound 15
Phosphoroamidite of 3-N-benzoyl-2',3'-di-O-benzoyluridine 10 (140 mg, 0.18 mmol), solution of tetrazole (1.58 mL, 0.71 mmol) in dry CH₂CN, 2-bromoethanol (0.03 mL, 0.36 mmol), solution of sulfur (7 mg, 0.21 mmol) in toluene (0.43 mL) and N,N-diisopropylamine (0.2 mL, 1.43 mmol) were used in reaction according to the general procedure described above. The reaction mixture was concentrated to give a crude product which was purified by column chromatography with toluene : AcOEt (2:1 to 1:10, v/v) solvent system to yield **Compound 15** as a solidifying liquid (81 mg, 52% yield). [δ]₂₀ = 46.6 (c = 1.4, CHCl₃). ¹H NMR (CDCl₃, δ ppm): 3.59-3.62 (m, 4H, CH₂Br), 4.40-4.53 (m, 5H, H-5'a, CH₂O), 85.54-4.60 (m, 2H, H-5'], H-5'b), 5.62 (dd, 1H, J = 5.8 Hz, J = 6.6 Hz, H-2'), 5.83 (dd, 1H, J = 2.7 Hz, J = 5.7 Hz, H-3'), 6.01 (d, 1H, J = 8.4 Hz, H-5'a), 6.46 (d, 1H, J = 6.9 Hz, H-1'), 7.29-7.34 (m, 2H, CH₂)}
tetrazole (1.58 mL, 0.71 mmol) in dry CH$_3$CN, 3-$\delta$= 8.2 Hz, H-5 ur), 6.44 (d, 1H, J = 5.61 (dd-t, 1H, J = 6.5 Hz, H-1 í), 5.75 (dd, 1H, J = 2.2 Hz, H-6a glu), 7.50 (m, 2H, H-Ph), 7.55ñ7.63 (m, 2H, H-Ph), 7.83 (d, 1H, H-Ph), 7.96ñ8.10 (m, 2H, H-Ph). 13C NMR (CDCl$_3$, δ, ppm): 28.85 (d, J = 8.1 Hz, H-6b glu), 78.35 (d, J = 8.2 Hz, H-6b í), 7.85ñ7.88 (m, 2H, H-Ph), 7.91ñ7.94 (m, 2H, H-Ph), 7.97ñ8.10 (m, 2H, H-Ph). 13C NMR (CDCl$_3$, δ, ppm): 28.85 (d, J = 8.1 Hz, CH$_3$), 32.64 (d, J = 5.7 Hz, CH$_3$Br), 32.70 (d, J = 5.9 Hz, CH$_3$Br), 66.45 (d, J = 6.9 Hz, CH$_3$O), 66.48 (d, J = 6.9 Hz, CH$_3$O), 66.91 (d, J = 4.5 Hz, C-5 í), 71.55 (C-3 í), 73.62 (C-2 í), 81.59 (d, J = 9.2 Hz, C-4 í), 86.94 (C-1 í), 103.46 (C-5 íb), 128.14, 128.45, 128.50, 128.57, 128.96, 129.07, 129.73, 129.81, 130.42, 131.20, 133.76, 133.82, 135.01 (C-Pbi), 139.71 (C-6b í), 149.40 (C-2 ía), 161.57 (C-4 ía), 165.30, 165.34 (PbiCOO), 168.25 (PbiCON).

**Compound 16**

Phosphoroamidite of 3-N-benzoyl-2 í,3 í-di-O-benzoyluridine 10 (140 mg, 0.18 mmol), solution of tetrazole (1.58 mL, 0.71 mmol) in dry CH$_3$CN, 3-bromopropanol (0.03 mL, 0.36 mmol), solution of sulfur (7 mg, 0.21 mmol) in toluene (0.43 mL) and N,N-diisopropylamine (0.12 mL, 1.12 mmol) were used in reaction according to the general procedure described above. The reaction mixture was concentrated to give a crude product which was purified by column chromatography with hexane : CH$_2$Cl$_2$ (1:1 v/v) solvent system. After reaction was finished, inorganic salt was filtered off and the filtrate was concentrated to give a mixture of crude products which was purified by column chromatography. Product yields in brackets marked with asterisks are calculated after recycling of compounds 11-16.

**Glycoconjugate 17**

Compounds 11 (130 mg, 0.22 mmol) and 1 (39 mg, 0.11 mmol) were submitted to the general procedure described above. The resulting glycoconjugates 17 and 17a were purified on a column packed with silica gel using CHCl$_3$: acetone (100:1 to 10:1, v/v) solvent system to yield 17 (diastereomeric mixture) as solidifying liquid (51 mg, 26% /88%*) and 17a as white solid (20 mg, 8% /11%*). [α]$^2_0$ = −12.8 (c = 0.4, CHCl$_3$), m.p. 87ñ89 OC. 1H NMR (CDCl$_3$, δ, ppm): first diastereomer: 1.36, 1.58 (2s, 6H, (CH$_3$)$_2$C), 2.01, 2.03, 2.07, 2.10 (4s, 12H, CH$_2$CO), 2.85 (m, 1H, CH$_2$S), 3.04 (m, 1H, CH$_2$S), 4.45 (dd, 1H, J = 12.4 Hz, H-6a glu), 4.24 (m, 1H, H-6b glu), 4.25 (m, 1H, H-6b í), 4.25 (m, 2H, CH$_2$O), 4.30 (m, 2H, H-5 ía), 5.03 (dd, 1H, J = 9.4 Hz, J = 10.1 Hz, H-5 íb), 5.03 (dd, 1H, J = 9.4 Hz, J = 10.1 Hz, H-5 íb), 5.03 (dd, 1H, J = 9.4 Hz, J = 10.1 Hz, H-5 íb), 5.03 (dd, 1H, J = 9.4 Hz, J = 10.1 Hz, H-5 íb), 5.03 (dd, 1H, J = 9.4 Hz, J = 10.1 Hz, H-5 íb), 5.03 (dd, 1H, J = 9.4 Hz, J = 10.1 Hz, H-5 íb), 5.03 (dd, 1H, J = 9.4 Hz, J = 10.1 Hz, H-5 íb), 5.03 (dd, 1H, J = 9.4 Hz, J = 10.1 Hz, H-5 íb), 5.03 (dd, 1H, J = 9.4 Hz, J = 10.1 Hz, H-5 íb), 5.03 (dd, 1H, J = 9.4 Hz, J = 10.1 Hz, H-5 íb), 5.03 (dd, 1H, J = 9.4 Hz, J = 10.1 Hz, H-5 íb), 5.03 (dd, 1H, J = 9.4 Hz, J = 10.1 Hz, H-5 íb), 5.03 (dd, 1H, J = 9.4 Hz, J = 10.1 Hz, H-5 íb), 5.03 (dd, 1H, J = 9.4 Hz, J = 10.1 Hz, H-5 íb), 5.03 (dd, 1H, J = 9.4 Hz, J = 10.1 Hz, H-5 íb), 5.03 (dd, 1H, J

**General procedure for condensation of compounds 11-16 with 1-tiosugar 1**

To solution of 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucose 1 (0.5 eqv.) in acetone, compounds 11-16 (1 eqv.) and K$_2$CO$_3$ (2 eqv.) were added. The resulting mixture was stirred for 24 h at room temperature. The reactions were monitored by TLC on silica gel plates using toluene : AcOEt (v/v) solvent system. After reaction was finished, inorganic salt was filtered off and the filtrate was concentrated to give a mixture of crude products which was purified by column chromatography. Product yields in brackets marked with asterisks are calculated after recycling of compounds 11-16.

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20.82 (CH₃CO), 25.30, 27.13 ((CH₃)₂C), 29.40 (d, J = 8.5 Hz, CH₃Br), 29.65 (d, J = 7.5 Hz, CH₃S), 62.01 (C-6glu), 67.63 (d, J = 4.5 Hz, CH₃O), 67.65 (d, J = 5.4 Hz, C-5'), 67.67 (d, J = 5.0 Hz, CH₃O), 68.19 (C-4glu), 69.74 (C-2glu), 73.66 (C-3glu), 76.04 (C-5glu), 80.89 (C-1glu), 84.47 (C-2'), 85.70 (d, J = 8.0 Hz, C-4'), 94.77 (d, J = 8.5 Hz, C-1'), 102.75 (C-5'), 114.54 ((CH₃)₂C), 142.27 (C-6a), 149.97 (C-2'), 162.72 (C-4glu), 169.40, 169.50, 170.16, 170.66 (CH₃CO); second diastereoisomer: 20.59, 20.61, 20.73, 20.83 (CH₃CO), 25.29, 27.13 ((CH₃)₂C), 29.41 (d, J = 8.5 Hz, CH₃Br), 29.81 (d, J = 7.5 Hz, CH₃S), 62.07 (C-6glu), 67.48 (d, J = 4.5 Hz, CH₃O), 67.65 (d, J = 5.4 Hz, C-5'), 67.67 (d, J = 5.0 Hz, CH₃O), 68.21 (C-4glu), 69.78 (C-2glu), 73.70 (C-3glu), 76.04 (C-5glu), 80.89 (C-1glu), 84.47 (C-2'), 85.73 (d, J = 8.7 Hz, C-4'), 94.81 (C-1'), 102.76 (C-5'), 114.52 ((CH₃)₂C), 142.30 (C-6a), 149.99 (C-2'), 163.03 (C-4a), 169.44, 169.48, 170.16, 170.73 (CH₃CO).

**Compound 17a**

δ (ppm): 1.36, 1.56 (2s, 6H, (CH₃)₂C), 2.01, 2.03, 2.06, 2.09 (4s, 24H, CH₃CO), 2.76–2.90 (m, 2H, CH₃S), 2.96–3.10 (m, 2H, CH₃S), 3.75 (m, 2H, 2xH-5glu), 4.37 (m, 1H, H-4'), 4.54 (d, 1H, J = 10.0 Hz, H-1glu), 4.57 (d, 1H, J = 10.0 Hz, H-1glu), 4.88 (dd, 1H, J = 3.6 Hz, J = 6.4 Hz, H-3'), 4.98–5.16 (m, 5H, H-2'), 5.24 (dd-t, 2H, J = 9.3, 2xH-3glu), 5.65 (d, 1H, J = 1.7 Hz, H-1'), 5.74 (d, 1H, J = 1.8 Hz, H-5a), 7.34 (d, 1H, J = 8.1 Hz, H-6a), 9.00 (bs, 1H, NH).

**Glycoconjugate 18**

Compounds 12 (150 mg, 0.24 mmol) and 1 (43 mg, 0.12 mmol) were submitted to the general procedure described above. The resulting glycoconjugates 18 and 18a were purified on a column packed with silica gel using CHCl₃:acetone (100:1 to 10:1, v/v) solvent system to yield 18 (diastereoisomers mixture) as solidifying liquid (38 mg, 17%/46%*).

**Compound 18a**

δ (ppm): 1.36, 1.57 (2s, 6H, (CH₃)₂C), 1.90–2.10 (m, 4H, CH₂), 2.01, 2.03, 2.06, 2.08 (4s, 24H, CH₃CO), 2.64–2.88 (m, 4H, CH₃S), 3.74 (dd, 2H, J = 2.4 Hz, J = 4.9 Hz, H-3'), 4.07–4.13 (m, 10H, 2xH-6aglu, 2xH-6bglu, H-5'a, H-5'b, 2xCH₂O), 4.36 (m, 1H, H-4'), 4.51 (d, 1H, J = 9.9 Hz, H-1glu), 4.52 (d, 1H, J = 9.9 Hz, H-1glu), 4.87 (dd, 1H, J = 3.6 Hz, J = 6.3 Hz, H-3'), 4.94–5.16 (m, 5H, H-2', 2xH-2'glu) 2xH-4'glu, 5.24 (dd-t, 2H, J = 9.3, 2xH-3glu), 5.69 (d, 1H, J = 1.5 Hz, H-1'), 5.74 (dd, 1H, J = 1.9 Hz, J = 8.3 Hz, H-5a), 7.39 (d, 1H, J = 8.3 Hz, H-6a), 9.00 (bs, 1H, NH).

**Glycoconjugate 19**

Compounds 13 (91 mg, 0.14 mmol) and 1 (25 mg, 0.07 mmol) were submitted to the general procedure described above. The resulting glycoconjugates 19 and 19a were purified on a column packed with silica gel using CHCl₃:acetone (100:1 to 10:1, v/v) solvent system to yield 19 (diastereoisomers mixture) as a solidifying liquid (35 mg, 27%/33%*).

**Compound 19a**

δ (ppm): 2.01, 2.03, 2.06, 2.07, 2.09, 2.10, 2.14 (7s, 18H, CH₃CO), 2.87 (m, 1H, CH₂S), 3.06 (m, 1H, CH₃S), 3.52–3.62 (m, 2H, CH₃Br), 3.75 (m, 1H, H-5'sglu), 4.15–4.44 (m, 9H, H-4', H-5'a, H-5'b, H-6aglu, H-6bglu), 2xCH₂O), 4.57 (d, 0.5H, J = 10.0 Hz, H-5a).
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Compound 19a

H NMR (CDCl3, δ, ppm): 2.00, 2.01, 2.03, 2.06, 2.09, 2.10, 2.14 (8s, 30H, CH3(CO), 2.81–2.92 (m, 2H, CH2S), 3.00–3.10 (m, 2H, CH2S), 3.72–3.80 (m, 2H, 2xH-5glu), 4.18 (dd, 2H, J = 2.3 Hz, H-12 Hz, 2xH-6aglu), 4.21–4.44 (m, 9H, 2xH-6aglu, H-4’s, H-5’a, H-5’b, 2xCH2O), 4.58 (d, 1H, J = 10.0 Hz, H-1glu), 5.03 (dd-t, 2H, J = 9.4, J = 10.0 Hz, 2xH-6aglu), 5.10 (dd-t, 1H, J = 9.4, J = 10.0 Hz, H-4aglu), 5.11 (dd-t, 1H, J = 9.4, J = 10.0 Hz, H-4aglu), 5.25 (dd-t, 2H, J = 9.4 Hz, 2xH-3glu), 5.34 (dd-t, 1H, J = 5.9 Hz, H-2’), 5.44 (dd, 1H, J = 3.5 Hz, J = 5.9 Hz, H-3’); 5.82 (d, 1H, J = 8.2 Hz, H-6glu), 6.12 (d, 1H, J = 6.4 Hz, H-1’), 7.57 (d, 1H, J = 8.2 Hz, H-6aglu), 9.02 (s, 1H, NH).

Glycoconjugate 20

Compounds 14 (98 mg, 0.15 mmol) and 1 (27 mg, 0.075 mmol) were submitted to the general procedure described above. The resulting glycoconjugate 20 was purified on a column packed with silica gel using CHCl3 : acetone (100:1 to 10:1, v/v) solvent system to yield 20 as a solidifying liquid (22 mg, 21%/34%*) and 21a as a solidifying liquid (12 mg, 9%/15%*); [α]D23 = –23.6 (c = 0.3, CHCl3). H NMR (CDCl3, δ, ppm): 1.99, 2.00, 2.01, 2.04, 2.06, 2.08, 2.09, 2.14 (6s, 18H, CH3CO), 2.03–2.25 (m, 4H, CH3), 2.73 (m, 1H, CH2S), 2.82 (m, 1H, CH3S), 3.46–3.54 (m, 2H, CH2Br), 3.74 (m, 1H, H-5’glu), 3.98–4.39 (m, 9H, H-4’, H-5’a, H-5’b, H-6aglu, H-6aglu, 2xCH2O), 4.52 (d, 0.5H, J = 10.0 Hz, H-1glu), 4.53 (d, 0.5H, J = 10.0 Hz, H-1glu), 5.03 (dd-t, 0.5H, J = 9.4 Hz, J = 10.0 Hz, H-4aglu), 5.04 (dd-t, 0.5H, J = 9.4 Hz, J = 10.0 Hz, H-4aglu), 5.09 (dd-t, 1H, J = 9.4 Hz, J = 10.5 Hz, H-4aglu), 5.24 (dd-t, 1H, J = 9.4 Hz, J = 10.0 Hz, H-3glu), 5.31 (m, 1H, H-2’), 5.43 (m, 1H, H-3’), 5.82 (d, 0.5H, J = 8.2 Hz, H-5’aglu), 5.82 (d, 0.5H, J = 8.2 Hz, H-5’aglu), 6.15 (d, 0.5H, J = 6.5 Hz, H-5’aglu), 6.16 (d, 0.5H, J = 6.5 Hz, H-1’), 7.60 (d, 0.5H, J = 8.2 Hz, H-6glu), 7.58 (d, 0.5H, J = 8.2 Hz, H-6aglu), 9.33 (bs, 1H, NH). 13C NMR (CDCl3, δ ppm): 20.40, 20.45, 20.59, 20.60, 20.72, 20.78 (CH2CO), 26.11 (d, J = 12.8 Hz, CH3), 26.13 (d, J = 12.8 Hz, CH3), 28.91 (d, J = 8.0 Hz, CH2Br), 30.28 (d, J = 8.0 Hz, CH2Br), 32.71 (d, J = 8.3 Hz, CH3), 32.74 (d, J = 8.1 Hz, CH3), 62.12, 62.15 (C-6aglu), 66.36 (d, J = 4.5 Hz, C-5’), 66.41 (d, J = 4.7 Hz, CH2O), 68.55 (d, J = 4.7 Hz, CH2O), 68.33 (C-4aglu), 69.69 (C-2aglu), 70.64, 70.59 (C-3aglu), 72.60 (C-5’aglu), 73.79–73.73 (C-3’), 76.01, 76.07 (C-2’), 80.93 (d, J = 6.2 Hz, C-4’), 80.95 (d, J = 6.2 Hz, C-4’), 83.44, 83.51 (C-1’aglu), 86.55, 86.64 (C-1’), 103.53, 103.62 (C-5’aglu), 139.69 (C-6’aglu), 150.34 (C-2’aglu), 162.57 (C-4’aglu), 169.41, 169.45, 169.58, 169.60, 169.71, 170.17, 170.66 (CH2CO).

Glycoconjugate 21

Compound 15 (81 mg, 0.09 mmol) and 1 (16 mg, 0.045 mmol) were submitted to the general procedure described above. The resulting glycoconjugates 21 and 21a were purified on a column packed with silica gel using CHCl3 : acetone (100:1 to 10:1, v/v) solvent system to yield 21 as a solidifying liquid (22 mg, 21%/34%*) and 21a as a solidifying liquid (12 mg, 9%/15%*); [α]D23 = –23.6 (c = 0.3, CHCl3). H NMR (CDCl3, δ, ppm): 1.99, 2.00, 2.01, 2.04, 2.06, 2.08, 2.09, 2.14 (6s, 12H, CH3CO), 2.93 (m, 1H, CH2S), 3.11 (m, 1H, CH2S), 3.58–3.63 (m, 2H, CH2Br), 3.74 (m, 1H, H-5’aglu), 4.18 (dd, 0.5H, J = 2.4 Hz, J = 12.4 Hz, H-6aglu), 4.19 (dd, 0.5H, J = 2.4 Hz, J = 12.4 Hz, H-6aglu), 4.25 (dd, 0.5H, J = 2.8 Hz, J = 12.4 Hz, H-6aglu), 4.26 (dd, 0.5H, J = 2.9 Hz, J = 12.4 Hz, H-6aglu), 4.30–4.61 (m, 8H, H-4’, H-5’a, H-5’b, 2xCH2O, H-1glu), 5.04 (dd, 0.5H, J = 9.4 Hz, J = 10.0 Hz, H-2glu), 5.06 (dd, 0.5H, J = 9.4 Hz, J = 10.0 Hz, H-2glu), 5.10 (dd-t, 0.5H, J = 9.4 Hz, J = 10.0 Hz, H-2glu), 5.24 (dd-t, 0.5H, J = 9.4 Hz, J = 9.4 Hz, H-3glu), 5.25 (dd-t, 0.5H, J = 9.4 Hz, J = 9.4 Hz, H-3glu), 5.62 (dd, 0.5H, J = 5.9 Hz, J = 6.7 Hz, H-2’), 5.63 (dd, 0.5H, J = 6.0 Hz, J = 6.7 Hz, H-2’), 5.83 (dd, 1H, J = 3.9 Hz, J = 3.9 Hz, H-5’aglu), 6.01 (d, 0.5H, J = 8.2 Hz, H-5’aglu), 6.01
Compound 21a

¹H NMR (CDCl₃, δ ppm): 1.99, 2.00, 2.01, 2.04, 2.06, 2.08, 2.09 (7s, 24H, CH₃CO), 2.88–2.96 (m, 2H, CH₂S), 3.06–3.14 (m, 2H, CH₂S), 3.73–3.78 (m, 2H, 2xH-5ₙₐ), 4.18 (dd, 1H, J = 2.4 Hz, J = 12.5 Hz, H-6ₙₐ), 4.20 (dd, 1H, J = 2.4 Hz, J = 12.5 Hz, H-6ₙₐ), 4.25 (dd, 1H, J = 4.3 Hz, J = 12.5 Hz, H-6ₙₐ), 4.28–4.40 (m, 4H, H-5'ₐ, H-5'ₚ, CH₂), 4.44–4.54 (m, 2H, CH₂O), 4.58 (m, 1H, H-4'), 4.59 (d, 1H, J = 10.0 Hz, H-1ₙₐ), 4.60 (d, 1H, J = 10.1 Hz, H-1ₚₐ), 5.04 (dd, 1H, J = 9.4 Hz, J = 10.0 Hz, H-2ₙₐ), 5.05 (dd, 1H, J = 9.4 Hz, J = 10.1 Hz, H-2ₚₐ), 5.11 (dd-t, 1H, J = 9.5 Hz, J = 10.0 Hz, H-4ₙₐ), 5.12 (dd-t, 1H, J = 9.6 Hz, J = 10.1 Hz, H-4ₚₐ), 5.24 (dd-t, 1H, J = 9.4 Hz, J = 9.4 Hz, J = 10.0 Hz, H-3ₙₐ), 5.26 (dd-t, 1H, J = 9.4 Hz, J = 9.4 Hz, J = 10.0 Hz, H-3ₚₐ), 5.63 (dd, 1H, J = 5.9 Hz, J = 6.7 Hz, H-2'), 5.84 (dd, 1H, J = 2.8 Hz, J = 5.9 Hz, H-3'), 6.02 (d, 1H, J = 8.2 Hz, H-5ₚₐ), 6.48 (d, 1H, J = 6.7 Hz, H-1'), 7.30–7.34 (m, 2H, H-Ph), 7.39–7.45 (m, 4H, H-Ph), 7.52 (m, 1H, H-Ph), 7.56–7.63 (m, 2H, H-Ph), 7.86 (d, 1H, J = 8.2 Hz, H-6ₙₐ), 7.87–7.90 (m, 2H, H-Ph), 7.92–7.96 (m, 2H, H-Ph).

Glycoconjugate 22

Compounds 16 (83 mg, 0.093 mmol) and 1 (17 mg, 0.047 mmol) were submitted to the general procedure described above. The resulting glycoconjugates 22 and 22a were purified twice on a column packed with silica gel using toluene : AcOEt (8:1 to 1:1, v/v) solvent system to yield 22 as a solidifying liquid (15 mg, 14%/23%*). Product 22a wasn’t isolated after column chromatography as pure substance. Only TLC analysis indicated the presence of this compound in the reaction mixture. ¹H NMR (CDCl₃, δ ppm): 1.99, 1.991, 2.01, 2.014, 2.04, 2.05, 2.07, (7s, 12H, CH₃CO), 2.23–2.30 (m, 4H, CH₂), 2.78 (m, 1H, CH₂S), 2.85 (m, 1H, CH₂S), 3.52–3.56 (m, 2H, CH₃Br), 3.72 (m, 1H, H-5ₙₐ), 4.14 (dd, 0.5H, J = 2.4 Hz, J = 12.4 Hz, H-6ₙₐ), 4.15 (dd, 0.5H, J = 2.4 Hz, J = 12.4 Hz, H-6ₙₐ), 4.23 (dd, 0.5H, J = 1.0 Hz, J = 12.4 Hz, H-6ₙₐ), 4.26 (dd, 0.5H, J = 1.0 Hz, J = 12.4 Hz, H-6ₙₐ), 4.24–4.35 (m, 4H, H-5'ₐ, H-5'ₚ, CH₂O), 4.42–4.52 (m, 2H, CH₃O), 4.51 (d, 0.5H, J = 10.0 Hz, H-1ₙₐ), 4.52 (d, 0.5H, J = 10.0 Hz, H-1ₚₐ), 4.59 (m, 1H, H-4'), 5.02 (dd, 0.5H, J = 9.5 Hz, J = 10.0 Hz, H-2ₙₐ), 5.03 (dd-t, 0.5H, J = 9.5 Hz, J = 10.0 Hz, H-2ₚₐ), 5.07 (dd-t, 0.5H, J = 9.5 Hz, J = 10.0 Hz, H-4ₙₐ), 5.08 (dd-t, 0.5H, J = 9.5 Hz, J = 10.0 Hz, H-4ₚₐ), 5.22 (dd-t, 0.5H, J = 9.4 Hz, J = 9.4 Hz, H-3ₙₐ), 5.23 (dd-t, 0.5H, J = 9.4 Hz, J = 9.4 Hz, H-3ₚₐ), 5.61 (m, 1H, H-2'), 5.82 (dd, 1H, J = 2.7 Hz, J = 5.8 Hz, H-3'), 5.99 (d, 1H, J = 8.3 Hz, H-5ₚₐ), 6.46 (d, 0.5H, J = 6.2 Hz, H-1'), 6.47 (d, 0.5H, J = 6.2 Hz H-1'), 7.29–7.34 (m, 2H, H-Ph), 7.38–7.43 (m, 4H, H-Ph), 7.52 (m, 1H, H-Ph), 7.55–7.63 (m, 2H, H-Ph), 7.85 (d, 0.5H, J = 8.3 Hz, H-6ₙₐ), 7.85 (d, 0.5H, J = 8.3 Hz, H-6ₐ), 7.86–8.79 (m, 2H, H-Ph), 7.91–7.95 (m, 2H, H-Ph), 7.97–8.02 (m, 2H, H-Ph).

¹³C NMR (CDCl₃, δ ppm): 20.54, 20.56, 20.68, 20.75 (CH₃CO), 26.11 (d, J = 10.2 Hz, CH₂), 28.91 (d, J = 7.5 Hz, CH₂), 30.29 (d, J = 8.1 Hz, CH₃Br), 32.73 (d, J = 8.1 Hz, CH₂S), 32.77 (d, J = 8.1 Hz, CH₂S), 62.04 (C-6ₙₐ), 66.49 (d, J = 4.1 Hz, C-5'), 66.93 (d, J = 4.1 Hz, CH₂O), 67.30 (d, J = 4.1 Hz, CH₂O), 68.27 (C-4ₙₐ), 69.61 (C-4ₙₐ), 71.67 (C-3'), 73.68 (C-2'), 73.75 (C-3', C-5'), 75.93 (C-5ₘ), 81.72 (d, J = 6.1 Hz, C-4'), 83.61 (C-1ₙₐ), 86.64 (C-1'), 103.56 (C-5ₘ), 128.20, 128.21, 128.31, 128.49, 128.54, 128.59, 129.10, 129.79, 129.88, 130.49, 131.31, 133.78, 133.85, 134.99 (C-Ph), 139.36 (C-6ₚₐ), 149.49 (C-2ₚₐ), 161.60, 161.61 (C-4ₚₐ), 165.33, 165.37 (PhCOO), 168.20, 169.38, 169.42, 170.12, (CH₂CO), 170.56 (PhCON).

RESULTS AND DISCUSSION

Taking into account earlier mentioned requirements for GTs inhibitors and structural changes in known analogues of GTs natural substrates, new class of uridine derivatives connected with 1-thiosugar using thio phosphoesters fragments were synthesized.

The substrates for synthesis of final glycoconjugates were in the form of selectively protected
derivatives of uridine 2, 5, or 7 and 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucose 1. For protection of hydroxyl groups at 2’ and 3’-position of uridine, isopropylidene, acetyl or benzoyl groups were chosen, while for protection of nitrogen atom in uracil ring benzoyl group was employed. Uridine derivative 2 was obtained using procedure described by Cornia [14]. First step in synthesis of derivatives 5 and 7 was protection of 5’-OH group using tert-butyl-dimethylsilyl chloride/imidazole system and DMF as a solvent (15). This reaction proceeded in regioselective manner and as the only product tert-butyl-dimethylsilyl-uridine 3 was obtained. The next step was esterification of uridine 2’ and 3’-position by introduction of acyl groups (acetyl or benzoyl) using acetic anhydride and pyridine (16) or benzoyl chloride and pyridine (17) as acylating systems. These reactions led to products 4 and 6, respectively. Final removal of tert-butyl-dimethylsilyl group from 5’-position of uridine derivatives 4 or 6 was carried out according to procedure described for deprotection of primary hydroxyl group in 5-nitro-2-pyridyl-1-thio-glycosyl derivatives (17). During deprotection reactions of compounds 4 or 6 using HBF4 in acetonitrile, products 5 and 7 were obtained.

Selectively protected uridine derivatives were subjected to phosphitylation according to method described by Majumdar (18) with the use of solution of N,N-diisopropyl chlorophosphoamidite and N,N-diisopropylamine in benzene as phosphitylating agent. Phosphitylation of compounds 2, 5 and 7 was performed at room temperature under argon atmosphere for 24 hours. Reaction progress was monitored by TLC on silica gel plates using toluene : Et3N solvent system (20:1 to 10:1, v/v) and were received in almost quantitative yield. 1H NMR and 13C NMR spectra confirmed the structures of uridine phosphoroamidites 8-10. Isopropyl protons from phosphoroamidite’s fragment gave multiplets at approximately 1.06–1.19 ppm ((CH3)2CH-N) and at 3.38–3.57 ppm (CH3)2CH-N). Furthermore, absence of 5’-OH signal in 1H NMR spectra and the presence of doublets of C-4’ and C-5’ signals (resulting from coupling of P-C nucleus) also confirmed expected products structure. All phosphoroamidite products were unstable as solids so melting points and optical rotations were not measured. It is worth to mention that the least stable was derivative 10, while the most stable was phosphoroamidite 9. All products of phosphitylation were stable and preserved only in solution of toluene : Et3N (10:1, v/v) under argon atmosphere and were concentrated directly before the next reaction.

The next step in the synthesis of final glycoconjugates was the reaction of uridine phosphoroamidites with alcohol, followed by oxidation in the presence of sulfur. For substitution reaction, the procedure described by Majumdar (18) was used whereas oxidation was made according to the method described by Salomoficzky (19).

For reaction with uridine phosphoroamidites 8-10, such alcohols as 2-bromoethanol and 3-bromopropanol were selected. Such choice was dictated by the length of pyrophosphate bridge in natural GTs sugar donors – a junction between thiosugar and uridine in final glycoconjugates was expected to mimic the pyrophosphate bridge. For running substitution reaction, first, 2 molar equivalents of appropriate alcohol were added to a solution of tetrazole in dry CH3CN. The resulting mixture was poured to freshly concentrated uridine phosphoramidite 8, 9, or 10 at 0°C. Concentration of compounds 8-10 right before the reaction limited formation of by-products. Reactions were maintained for 15 min in temperature –5°C. According to data reported in the literature, substitution reactions of amine group in phosphoroamidite by alcohol in the presence of tetrazole at reduced temperature is completed in less that 60 s (20). Reaction mechanism assumes protonation of nitrogen atom of phosphoroamidite, nucleophilic attack of tetrazole’s nitrogen on phosphorous with formation of intermediate and secondary attack of oxygen from alcohol on phosphorous with concurrent breakdown of the intermediate. Products of substitution were not isolated but immediately submitted oxidation reaction. For the secondary oxidation, sulfur was dissolved in the mixture of toluene and N,N-diisopropylamine and subsequently added to the product of substitution. Reactions were performed for 48 h at room temperature under argon atmosphere and were monitored by TLC on silica gel plates using toluene : AcOEt (1:1, v/v). Reaction mixtures were concentrated and crude products were purified by column chromatography. Products 11-16 were received in good yields (Table 1). Due to the fact that products 11-16 were received as solidifying liquids, melting points were not determined and only optical rotations were measured (Table 1). Products structures were confirmed by 1H NMR and 13C NMR spectra. Signals of methylidyne protons of appropriate alcohol chains were observed at approximately 2.17–2.27 ppm (CH3), 3.42–3.64 ppm (CH2Br) and 4.18–4.60 ppm (CH2O),
Furthermore, an absence of isopropyl protons from phosphoramidite fragment signal confirmed products structure. Doublets (resulting from coupling P-C nucleus) of uridine C-4’, C-5’ and carbon atoms from alcohol chains observed on 13C NMR spectra also confirmed products structure.

The last step in glycoconjugates synthesis was the connection of compounds 11–16 with 1-thiosugar 1 in the presence of base such as K2CO3. Final products of this reactions were diastereoisomeric mixtures of monosubstituted uridine derivatives (17–22), which structures resembled natural GTs nucleotide-sugar donor and disubstituted derivatives (17a–22a). Disubstituted derivatives 17a–22a were treated as by-products due to their large structures, which could be a spatial hindrance in the enzyme’s active center.

Reaction conditions were selected using compound 11. For this purpose, synthesis with various molar ratio of uridine derivative 11 to thiosugar 1 (1:1, 1:0.75 and 1:0.5) were performed. As a base, in all attempts 2 molar equivalents of K2CO3 were used. The reactions were performed for 24 h at room temperature. Crude products of these reactions were purified by column chromatography. In case of the first reaction with 1:1 molar ratio of compounds 11 and 1 disubstituted derivative 17a was received as the main product in 51% yield, while monosubstituted compound 17 was received in only 8% yield. Both products were isolated and their structures were confirmed by 1H NMR and 13C NMR spectra. This procedure was repeated for 1:0.75 and 1:0.5 molar ratio of substrates 11 and 1, respectively. In the reaction of 1 molar equivalent of compound 11

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate I</th>
<th>Substrate II</th>
<th>Product</th>
<th>Yield [%]</th>
</tr>
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<td>1.</td>
<td>2</td>
<td>N,N-dimethylamine</td>
<td>8</td>
<td>95</td>
</tr>
<tr>
<td>2.</td>
<td>5</td>
<td>N,N-dimethylamine</td>
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<td>98</td>
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<td>3.</td>
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<td>N,N-dimethylamine</td>
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<td>8</td>
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<td>11</td>
<td>50</td>
</tr>
<tr>
<td>5.</td>
<td>8</td>
<td>3-bromopropanol/S</td>
<td>12</td>
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<tr>
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<td>1’</td>
<td>17</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>17a</td>
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<td>51</td>
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<tr>
<td>11.</td>
<td>11</td>
<td>1’</td>
<td>17</td>
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<td>17a</td>
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<tr>
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<td>1’</td>
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<td>26 (38)’</td>
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<td></td>
<td></td>
<td>17a</td>
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<td>8 (11)’</td>
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<td>1’</td>
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<td>1’</td>
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<td>1’</td>
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<td>1’</td>
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<tr>
<td></td>
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* Substrate I : Substrate II ratio 1:1; † Substrate I : Substrate II ratio 1:0.75; ‡ Substrate I : Substrate II ratio 1:0.5; ‘ yield in brackets was calculated after recycling of Substrate I, * only TLC analysis indicated the presence of this compound.

Table 1. Yields of the synthesized compounds.

* Substrate I : Substrate II ratio 1:1; † Substrate I : Substrate II ratio 1:0.75; ‡ Substrate I : Substrate II ratio 1:0.5; ‘ yield in brackets was calculated after recycling of Substrate I, * only TLC analysis indicated the presence of this compound.
with 0.75 molar equivalent of thiosugar 1, glycoconjugate 17a was received in 30% yield while monosubstituted compound 17 was received in 20% yield, whereas in reaction of 1 molar equivalent of compound 11 and 0.5 molar equivalent of thiosugar 1 monosubstituted glycoconjugate 17 was received as a main product in 26% yield, while disubstituted derivative 17a was received in 8% yield. Taking into consideration recycling of substrate 11, yields of products grew up to 38% and 11%, respectively. The obtained results indicated that the most desired product 17 could be isolated in the best yield when 1:0.5 substrates ratio was used. Therefore, in the next experiments with uridine derivatives 12–16 such substrates ratio was applied. Yields of obtained mono- and disubstituted products are presented in Table 1. Surprisingly, in case of reaction of uridine derivative 14 with thiosugar 1, formation of disubstituted compound 20a was not observed. In this case, product 20 was obtained in the highest yield among all prepared glycoconjugates 17–22. This result wasn’t repeated for use of uridine derivative 16 as a substrate. Changing of uridine protective groups from acetyl (in derivative 14) to benzoyl (in derivative 16) caused reduction of yield of final glycoconjugates 22 (only 14%). Additionally, formation of disubstituted product 22a was observed. Regrettably, pure product 22a could not be isolated even after repeated column chromatography.

The structures of glycoconjugates were confirmed by 1H NMR and 13C NMR spectra. The observed double carbon atoms signals in monosubstituted products spectra suggested that mixtures of diastereoisomers were received. Unfortunately, chromatographic attempts at diastereoisomers separations were unsuccessful.

Biological activity of received glycoconjugates 17–22 will be estimated in glycosylation reaction with participation of enzymes belonging to the family of glycosyltransferases (GTs).

Acknowledgment

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REFERENCES