

SYNTHESIS OF FUCOSYLATED URIDINE CONJUGATES AS POTENTIAL
GLYCOSYLTRANSFERASE INHIBITORSKATARZYNA KOMOR^{1*}, WIESŁAW SZEJA¹, ROMAN KOMOR¹, GABRIELA PASTUCH-
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Martin-Luther-King-Platz 6, Germany**Keywords:** glycosyltransferase inhibitor, L-fucose, glycosylation

The role glycans play in protein function, receptor binding and cell adhesion has spawned a great deal of research toward the identification of molecules that alter their structures. The development of improved methods for carbohydrate synthesis and particularly glycosidic bond formation is therefore crucial (1). The key role played by L-fucose in glycoprotein and cellular function has prompted significant research toward identifying recombinant and biochemical approaches for blocking its incorporation into proteins and membrane structures (2, 3). Also deoxyfucose analogues such as L-rhodinose (2,3,6-trideoxy-L-*threo*-hexopyranose), and L-oliose (2,6-dideoxy-L-*lyxo*-hexopyranose), occur as structural moieties of many bioactive natural products and often play an important role in their mechanism of action, in particular for DNA interaction of anti-cancer drugs (4, 5). Therefore, utilization of L-fucal derivatives as building blocks for the total synthesis of various deoxy glycosides is of great interest in bioorganic and medicinal chemistry.

In connection with our own studies in this area (6–9) we became interested in the prospect of developing a simple method that would lead to various L-fucosyl derivatives of uridine as a potential GTs inhibitors (10). Glycosyltransferases (GTs) are family of enzymes that are responsible for the biosynthesis of glycoconjugates such as glycolipids and glycoproteins as well as oligo- and polysaccharides which are crucial factors in bacterial and viral infections (11). Thus, the development of new selective

inhibitors is of great importance in dealing with bacterial and fungal diseases (12, 13).

EXPERIMENTAL

NMR spectra were recorded on Agilent spectrometer 400 MHz and Bruker AV spectrometer 400 MHz using TMS as internal standard and CDCl₃ as a solvent. NMR solvent was purchased from ACROS Organics (Geel, Belgium). Chemical shifts (δ) are expressed in ppm and coupling constants (J) in Hz. Optical rotations were measured with a JASCO 2000 polarimeter using a sodium lamp (589.3 nm) at room temperature. Electrospray-ionization mass spectrometry was performed on a 4000 QTrap (Applied Biosystems/MDS Sciex) mass spectrometer. Reactions were monitored by TLC on precoated plates of silica gel 60 F254 (Merck). TLC plates were inspected under UV light ($\lambda = 254$ nm) or charring after spraying with 10% sulfuric acid in ethanol. Crude products were purified using column chromatography performed on silica gel 60 (70–230 mesh, Fluka) developed with hexane/EtOAc and CHCl₃/MeOH as solvent systems. Organic solvents were evaporated on a rotary evaporator under diminished pressure at 40°C.

All selectively protected substrates: 4-*O*-benzyl-L-fucal **1** (14), 2',3'-bis-*O*-(*tert*-butyldimethylsilyl)uridine **3** (15) and 2',3'-*O*-isopropylidene-uridine-5'-carboxylic acid **11** (16) were prepared according to published procedures. Other chemicals were purchased from Acros and POCh Chemical Companies and were used without purification.

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3-O-[4-O-Benzyl-2,3,6-trideoxy- α -L-threo-hex-2-enopyranosyl]-1,5-anhydro-4-O-benzyl-2,6-dideoxy-2,6-dideoxy-L-lyxo-hex-1-enitol (2)

To a solution of the sugar **1** (1 g, 4.5 mmol) in toluene (40 mL) sat. aq. NaOH (4 mL) was added. The mixture was stirred for 20 min, then a catalytic amount of Bu₄N⁺I⁻ (84 mg, 0.23 mmol) and *p*TsCl (438 mg, 2.3 mmol) were added. The reaction was stirred at room temperature until TLC (hexane : ethyl acetate, 3 : 1, v/v) showed complete consumption of the starting material. The solution was then diluted with toluene (50 mL) and quenched with H₂O (100 mL). The aqueous layer was extracted with toluene (60 mL). The combined organic phases were washed with brine (2 × 100 mL), dried over anhydrous MgSO₄ and concentrated to give a crude residue, that was purified by column chromatography on silica gel with hexane : EtOAc (20 : 1, v/v) to give disaccharide **2 $\alpha\beta$** as a colorless syrup (α : β 4 : 1), yield 645 mg (65%). Diastereoisomer α was separated by crystallization from diethyl ether-hexane. Disaccharide **2 α** was obtained as white solid in 40% yield, m.p. 106–108 C, = 228.1 (c 0.4, CHCl₃). ¹H-NMR (400 MHz, CDCl₃, δ , ppm): 7.40–7.23 (m, 10H, H_{arom}), 6.33 (dd, *J* = 6.2, 1.6 Hz, 1H, H-1), 6.13 (ddd, *J* = 10.0, 5.2, 0.7 Hz, 1H, H-3'), 5.97 (dd, *J* = 10.1, 3.0 Hz, 1H, H-2'), 5.23 (d, *J* = 2.9 Hz, 1H, H-1'), 4.84, 4.67 (qAB, *J* = 11.9 Hz, 2H, 4-O-Bn), 4.76 (ddd, *J* = 6.2, 3.1, 1.1 Hz, 1H, H-2), 4.66, 4.57 (qAB, *J* = 12.0 Hz, 2H, 4'-O-Bn), 4.52 (ddd, *J* = 5.7, 2.9, 1.4 Hz, 1H, H-3), 4.23 (qd, *J* = 6.6, 2.6 Hz, 1H, H-5'), 4.14 (dq~q, *J* = 6.1 Hz, 1H, H-5), 3.72 (dd, *J* = 2.9, 1.3 Hz, 1H, H-4), 3.52 (dd, *J* = 5.3, 2.6 Hz, 1H, H-4'), 1.36 (d, *J* = 6.6 Hz, 3H, 6'-CH₃), 1.29 (d, *J* = 6.7 Hz, 3H, 6-CH₃); ¹³C-NMR (101 MHz, CDCl₃, δ , ppm): 144.2 (C1), 138.6 (C_{IV}-Ph), 138.4 (C_{IV}-Ph), 129.4 (C-2'), 128.4, 128.4, 128.05, 127.8, 127.7, 127.6 (10 C_{arom}), 127.4 (C-3'), 101.2 (C-2), 94.4 (C-1' α), 73.6 (CH₂Ph), 73.5 (C-4), 72.5 (C-5), 70.9 (CH₂Ph), 70.2 (C-3), 69.4 (C-4'), 66.4 (C-5'), 16.4 (C-6'), 16.0 (C-6).

Addition reaction of uridine derivative 3 to unsaturated disaccharide 2

To the mixture of disaccharide **2** (100 mg, 0.237 mmol) and the uridine derivative **3** (123 mg, 0.260 mmol) in dry CH₂Cl₂ (5 mL) molecular sieves 4Å were added and the mixture was cooled to 0°C in ice-water (Table 1, entry 2). Then, a catalytic amount (8 mg, 0.024 mmol) of triphenylphosphine hydrobromide (TPHB) in CH₂Cl₂ (1 mL) was added dropwise *via* syringe. The reaction was monitored by TLC (hexane : AcOEt, 2 : 1, v/v) and finally finished by addition of Et₃N (0.05 mL). Molecular sieves were filtered off and solvents were evaporated under reduced pressure. The residue was purified by column chromatography (hexane : AcOEt, 4 : 1, v/v) to yield the glycoconjugates: **4** (55 mg, 26%), **5** (46 mg, 29%) and **6** (10%, in a mixture with uridine **3**).

Glycoconjugate 4

Colorless syrup, α : β = 5 : 1; selected NMR data: ¹H-NMR (400 MHz, CDCl₃, δ , ppm): 8.34 (s, 1H, NH_{ur}), 8.15 (d, *J* = 8.2 Hz, 1H β , H-6_{ur} β -isomer), 7.80 (d, *J* = 8.2 Hz, 1H, H-6_{ur} α -isomer), 7.41–7.23 (m, 10H, H_{arom}), 6.15 (dd, *J* = 10.0, 5.3 Hz, 1H, H-3'), 5.98–5.92 (m, 2H, H-2', H-5_{ur}), 5.75 (d, *J* = 1.6 Hz, 1H, H-1'_{ur}), 5.22 (d, *J* = 2.8 Hz, 1H, H-1' α -isomer), 5.08 (d, *J* = 2.9 Hz, 1H β , H-1' β -isomer), 4.96 (d, *J* = 3.5 Hz, 1H, H-1 α), 4.87, 4.64 (qAB, *J* = 11.7 Hz, 2H, CH₂Ph), 4.67, 4.57 (qAB, *J* = 11.9 Hz, 2H, CH₂Ph), 4.25–4.14 (m, 2H, H-5', H-4'_{ur}), 4.11–4.02 (m, 3H, H-3, H-2'_{ur}, H-3'_{ur}), 4.04 (dd, *J* = 11.8, 2.7 Hz, 1H, H-5a'_{ur}), 3.80 (q, *J* = 6.5 Hz, 1H, H-5), 3.58 (dd, *J* = 5.4, 2.7 Hz, 1H, H-4'), 3.57–3.53 (m, 1H, H-4), 3.48 (dd, *J* = 11.8, 2.6 Hz, 1H, H-5b'_{ur}), 2.32–2.21 (m, 1H, H-2_{eq}), 1.98 (dd, *J* = 12.9, 5.0 Hz, 1H, H-2_{ax}), 1.32 (d, *J* = 6.7 Hz, 3H, 6'-CH₃), 1.18 (d, *J* = 6.5 Hz, 3H, 6-CH₃), 0.91, 0.90 (2 × s, 18H, 2 × C(CH₃)₃), 0.16, 0.10, 0.08, 0.07 (4 × s, 12H, 2 × Si(CH₃)₂). ¹³C-NMR (101 MHz, CDCl₃, δ , ppm): 162.69 (C=O), 149.92 (C=O), 139.51 (C-6_{ur}), 138.68, 138.49 (2C_{IV}), 129.31 (C-2'), 128.45,

Table 1. Reaction conditions for addition of uridine **1** to unsaturated disaccharide **4**.

Entry	Reaction	Temp. conditions	Time [°C]	Products / Yield [%]		
				4	5	6*
1.	TPHB, CH ₂ Cl ₂	10	10 min	11	49	~20
2.		0 3	0 min	26	29	~10
3.		-20	4 h	13**	12**	–

* in a mixture with **3**; ** consumption rate of **2** only 38%

128.34, 128.32, 128.28, 127.88, 127.81, 127.72, 127.68, 127.56 (C_{arom}, C-3'), 102.27 (C-5_{ur}), 98.58 (C-1), 93.47 (C-1'), 90.16 (C-1'_{ur}), 81.67 (C-4'_{ur}), 76.04 (C-4), 75.63, 73.45, 70.33 (C-3, C-2'_{ur}, C-3'_{ur}), 74.82, 70.23 (2 × CH₂Ph), 69.12 (C-4'), 67.09 (C-5'), 66.93 (C-5), 65.06 (C-5'_{ur}), 31.88 (C-2), 25.85, 25.78 (2 × C(CH₃)₃), 18.11, 18.03 (2 × C(CH₃)₃), 17.29 (6-CH₃), 16.38 (6'-CH₃), -0.00, -4.17, -4.46, -4.85, -5.00 (2 × Si(CH₃)₂).

Glycoconjugate 5

Colorless syrup, $\alpha : \beta = 10 : 1$; selected NMR data: ¹H-NMR (400 MHz, CDCl₃, δ , ppm): 8.91 (br.s, 1H, NH_{ur}), 8.15 (d, $J = 8.2$ Hz, 1H, H-6_{ur} α -isomer), 7.80 (d, $J = 8.2$ Hz, 1H β , H-6_{ur} β -isomer), 7.41–7.28 (m, 5H, H_{arom}), 6.20 (dd, $J = 10.3, 5.1$ Hz, 1H, H-3), 5.95 (dd, $J = 10.1, 3.0$ Hz, 1H, H-2), 5.75 (d, $J = 1.6$ Hz, 1H, H-1'_{ur} β -isomer), 5.72 (d, $J = 1.5$ Hz, 1H, H-1'_{ur} α -isomer), 5.48 (dd, $J = 8.2, 2.3$ Hz, 1H, H-5_{ur}), 5.08 (br.d, $J = 2.9$ Hz, 1H, H-1 α), 4.98–4.95 (m, 1H β , H-1 β) 4.66, 4.59 (qAB, $J = 11.9$ Hz, 2H, CH₂Ph), 4.27–4.17 (m, 2H, H-3'_{ur}, H-5a'_{ur}), 4.10–4.00 (m, 1H, H-2'_{ur}, H-4'_{ur}), 4.03 (dq, $J = 6.6, 2.6$ Hz, 1H, H-5), 3.55–3.52 (m, 1H, H-5b'_{ur}), 3.51 (dd, $J = 5.4, 2.7$ Hz, 1H, H-4), 1.36 (d, $J = 6.6$ Hz, 3H, 6-CH₃), 0.91, 0.90 (2 × s, 18H, 2 × C(CH₃)₃), 0.17, 0.09, 0.08, 0.06 (4 × s, 12H, 2 × Si(CH₃)₂); ¹³C-NMR (101 MHz, CDCl₃, δ , ppm): 163.37 (C=O), 150.11 (C-6_{ur}), 140.56 (C_{IV arom}), 138.26, 128.49, 128.44, 128.13, 127.81 (C_{arom}, C-2, C-3), 101.01 (C-5_{ur}), 94.08 (C-1), 90.16 (C-1'_{ur}), 81.82 (C-3'_{ur}), 76.15 (C-2_{ur}), 71.23 (CH₂), 70.10 (C-4'_{ur}), 68.94 (C-4), 66.97 (C-5), 64.49 (C-5'_{ur}), 25.83, 25.80 (2 × C(CH₃)₃), 18.11, 18.04 (2 × C(CH₃)₃), 16.36 (6-CH₃), -4.23, -4.48, -4.91, -5.09 (2 × Si(CH₃)₂); LRMS (ESI): calcd. for C₃₄H₅₄N₂O₈Si₂Na ([M + Na]⁺): m/z 697.9619; found: m/z 697.7.

Glycoconjugate 6

Compound **6** (α -isomer, 16 mg, 10%) was isolated as an inseparable mixture (27 mg) with substrate **3** (mol ratio 1:1 – according to ¹H-NMR); ¹H-NMR (400 MHz, CDCl₃, δ , ppm): 8.93 (s, 1H, NH_{ur}), 8.88 (s, 1H, NH_{ur}), 7.92 (d, $J = 8.2$ Hz, 1H, H-6_{ur}), 7.61 (d, $J = 8.1$ Hz, 1H, H-6_{ur}), 7.41–7.28 (m, 5H, H_{arom}), 5.75 (dd, $J = 8.2, 2.1$ Hz, 1H, H-5_{ur}), 5.72 (dd, $J = 8.3, 2.4$ Hz, 1H, H-5_{ur}), 5.71 (d, $J = 2.1$ Hz, 1H, H-1'_{ur}), 5.47 (d, $J = 5.3$ Hz, 1H, H-1'_{ur}), 4.95 (d, $J = 3.0$ Hz, 1H, H-1 α), 4.84, 4.69 (qAB, $J = 11.6$ Hz, 1H), 4.58–4.53 (m, 1H, H-2'_{ur}), 4.22–4.15 (m, 2H, H-3'_{ur}, H-4'_{ur}), 4.11–4.07 (m, 2H, H-2'_{ur}, H-4'_{ur}), 4.05 (dd, $J = 12.1, 2.3$ Hz, 1H, H-5a'_{ur}), 4.04 (dd, $J = 7.3, 4.0$ Hz, 1H, H-3'_{ur}), 3.94 (dd, $J = 12.3, 2.1$ Hz, 1H, H-5a'_{ur}), 3.93–3.87 (m, 1H, H-3), 3.85 (dq, $J =$

13.5, 6.8 Hz, 1H, H-5), 3.72 (br.dd, $J = 12.2, 1.3$ Hz, 1H, H-5b'_{ur}), 3.55 (d, $J = 2.8$ Hz, 1H, H-4), 3.47 (dd, $J = 11.7, 2.4$ Hz, 1H, H-5b'_{ur}), 3.02 (br.s, $J = 15.6$ Hz, 1H, 3-OH), 1.96 (dd, $J = 12.1, 3.5$ Hz, 1H, H-2_{eq}), 1.87 (dd, $J = 12.8, 5.1$ Hz, 1H, H-2_{ax}), 1.73–1.55 (m, 1H, OH), 1.28 (d, $J = 6.6$ Hz, 3H, H-6), 0.91, 0.89, 0.88 (3 × s, 36H, 2 × C(CH₃)₃), 0.17, 0.10, 0.09, 0.07, 0.07, 0.05, 0.04 (7 × s, 24H, 4 × Si(CH₃)₂); ¹³C-NMR (101 MHz, CDCl₃, δ , ppm): 163.24, 163.08, 150.26, 149.97 (4 × C=O), 143.03, 139.84 (2 × C-6_{ur}), 138.13 (C_{IV arom}), 128.61, 128.08, 128.07 (C_{arom}), 102.11, 101.53 (2 × C-5_{ur}), 98.40 (C-1), 94.02, 90.34 (C-1), 86.16 81.66 (2 × C-4'_{ur}), 79.18 (C-4), 76.00 (C-2'_{ur}), 75.89 (CH₂Ph), 73.56 (C-2'_{ur}), 71.71, 70.03 2 × (C-3'_{ur}), 67.06 (C-5), 66.19 (C-3), 65.04, 61.67 (2 × C-5'_{ur}), 33.61 (C-2), 25.81, 25.80, 25.78 (4 × C(CH₃)₃), 18.06, 18.05, 18.02, 17.94 (4 × C(CH₃)₃), 17.31 (6-CH₃), -4.19, -4.44, -4.45, -4.63, -4.80, -4.87, -4.93, -5.11 (4 × Si(CH₃)₂).

General procedure for deprotection of *tert*-butyldimethylsilyl groups

To the solution of anhydrous Bu₄NF (87 mg, 0.340 mmol) in CH₃CN (1 mL) the solution of glycoconjugate **4** or **5** (0.034 mmol) in CH₃CN (1 mL) was added. The reaction mixture was stirred at room temperature for 30 min and monitored by TLC (CHCl₃ : MeOH, 10 : 1, v/v). Then, the mixture was dissolved in chloroform (15 mL) and washed with saturated aq. solution of NaHCO₃ (20 mL) and water (20 mL). The organic phase was dried over anhydrous MgSO₄ and concentrated to give a crude residue, that was purified by column chromatography on silica gel with CHCl₃ : MeOH (25 : 1, v/v) elution system to give corresponding products **7** and **8** (isolated only the α anomers).

Glycoconjugate 7

Colorless syrup, 67% yield, α -isomer, = 30.5 (c 0.4, MeOH). ¹H-NMR (400 MHz, CDCl₃, δ , ppm): 9.35 (s, 1H, NH_{ur}), 7.73 (d, $J = 8.1$ Hz, 1H, H-6_{ur}), 7.40–7.23 (m, 10H, H_{arom}), 6.15 (dd, $J = 10.3, 4.9$ Hz, 1H, H-3'), 5.91 (dd, $J = 10.1, 3.0$ Hz, 1H, H-2'), 5.82 (dd, $J = 8.2, 1.4$ Hz, 1H, H-5_{ur}), 5.78 (d, $J = 3.6$ Hz, 1H, H-1'_{ur}), 5.13 (d, $J = 2.9$ Hz, 1H, H-1'), 4.96 (d, $J = 3.2$ Hz, 1H, H-1 α), 4.85, 4.61 (qAB, $J = 11.6$ Hz, 2H, CH₂Ph), 4.65, 4.57 (d, $J = 11.9$ Hz, 2H, CH₂Ph), 4.29–4.11 (m, 4H, H-5', H-2'_{ur}, H-3'_{ur}, H-4'_{ur}), 4.01 (m, 1H, H-3), 3.95 (dd, $J = 11.7, 2.4$ Hz, 1H, H-5a'_{ur}), 3.77 (q, $J = 6.6$ Hz, 1H, H-5), 3.56–3.49 (m, 3H, H-4, H-4', H-5b'_{ur}), 3.27 (s, 1H, OH), 2.23 (ddd, $J = 12.7, 3.8$ Hz, 1H, H-2_{eq}), 1.86 (dd, $J = 12.9, 4.7$ Hz, 1H, H-2_{ax}), 1.67 (s, 1H, OH),

1.31 (d, $J = 6.7$ Hz, 3H, 6'-CH₃), 1.18 (d, $J = 6.5$ Hz, 3H, 6-CH₃). ¹³C-NMR (101 MHz, CDCl₃, δ , ppm): 182.67 (C=O), 163.11 (C=O), 139.58 (C-6_{ur}), 138.44, 138.43 (2 C_{IV} arom), 129.20 (C-2'), 128.39, 128.31, 128.27, 128.10, 127.79, 127.70, 127.68 (C_{arom}, C-3'), 102.20 (C-5_{ur}), 98.95 (C-1), 93.53 (C-1'), 91.64 (C-1'_{ur}), 84.46 (C-4'_{ur}), 77.20, 76.00, 75.78, 74.82 (C-3, C-4, C-2'_{ur}, C-3'_{ur}), 73.20, 70.81 (2 \times CH₂), 69.38 (C-4'), 67.32 (C-5'), 66.89 (C-5), 66.69 (C-5'_{ur}), 31.95 (C-2), 17.27 (6-CH₃), 16.44 (6'-CH₃); LRMS (ESI): calcd. for C₃₅H₄₂N₂O₁₁Na ([M + Na]⁺): m/z 689.7045; found: m/z 689.8.

Glycoconjugate 8

Colorless syrup, 85% yield, α -isomer, = 163.3 (c 0.73, MeOH); ¹H-NMR (400 MHz, CDCl₃, δ , ppm): 10.10 (br.s, 1H, NH_{ur}), 8.00 (d, $J = 8.2$ Hz, 1H, H-6_{ur}), 7.37–7.25 (m, 5H, H_{arom}), 6.18 (dd, $J = 10.1$, 5.1 Hz, 1H, H-3), 5.98 (dd, $J = 10.1$, 2.9 Hz, 1H, H-2), 5.86 (d, $J = 2.1$ Hz, 1H, H-1'_{ur}), 5.56 (dd, $J = 8.2$, 1.2 Hz, 1H, H-5_{ur}), 5.11 (d, $J = 2.8$ Hz, 1H, H-1), 4.65, 4.57 (qAB, $J = 11.8$ Hz, 2H, CH₂Ph), 4.25–4.12 (m, 3H, H-2'_{ur}, H-3'_{ur}, H-5a'_{ur}), 4.03 (qd, $J = 6.5$, 2.6 Hz, 1H, H-5), 3.65 (dd, $J = 11.2$, 2.0 Hz, 1H, H-5b'_{ur}), 3.55–3.46 (m, 1H, H-4'_{ur}), 3.51 (dd, $J = 5.2$, 2.6 Hz, 1H, H-4), 1.78 (s, 1H, OH), 1.36 (d, $J = 6.6$ Hz, 3H, H-6). ¹³C-NMR (101 MHz, CDCl₃, δ , ppm): 163.83 (C=O), 151.09 (C=O), 140.52 (C-6_{ur}), 138.24 (C_{IV} arom), 128.64 (C-2), 128.42, 127.98, 127.80 (C_{arom}, C-3), 101.82 (C-5_{ur}), 94.54 (C-1), 90.63 (C-1'_{ur}), 83.42 (C-3'_{ur}), 75.29 (C-2'_{ur}), 71.15 (CH₂), 69.76 (C-4'_{ur}), 69.03 (C-4), 66.91 (C-5), 65.90 (C-5'_{ur}), 16.35 (6-CH₃); LRMS (ESI): calcd. for C₂₂H₂₆N₂O₈Na ([M + Na]⁺): m/z 469.4402; found: m/z 469.6.

(3-Azidopropyl) 3-O-[4-O-benzyl-2,3,6-trideoxy- α -L-threo-hex-2-enopyranosyl]-4-O-benzyl-2,6-dideoxy-L-lyxo-hexopyranoside (9)

To the mixture of disaccharide **2** (100 mg, 0.237 mmol) and 3-azidopropane-1-ol (0.02 mL, 0.284 mmol) in dry CH₂Cl₂ (3 mL) molecular sieves 4Å were added and the mixture was cooled to –10°C. Then, a catalytic amount (8 mg, 0.024 mmol) of triphenylphosphine hydrobromide (TPHB) in CH₂Cl₂ (1 mL) was added dropwise *via* syringe. After 15 min, the mixture was allowed to warm up to room temperature. The reaction was monitored by TLC (hexane : AcOEt, 2 : 1, v/v) and finally finished by addition of Et₃N (0.05 mL). Molecular sieves were filtered and solvent was evaporated under reduced pressure. The residue was purified by column chromatography (hexane : AcOEt, 10 : 1, v/v) to yield glycoside **9** (49 mg,

40%) as a colorless syrup; α : β 5 : 1; selected NMR data for the major α -isomer: ¹H-NMR (400 MHz, CDCl₃, δ , ppm): 7.42–7.23 (m, 10H, H_{arom}), 6.14 (ddd, $J = 10.1$, 5.3, 0.8 Hz, 1H, H-3'), 6.09–5.93 (m, 2H β , H-2', H-3' β -isomer), 5.91 (dd, $J = 10.1$, 3.1 Hz, 1H, H-2'), 5.20 (d, $J = 3.0$ Hz, 1H, H-1'), 5.16 (dd, $J = 3.2$, 1.5 Hz, 1H, H-1' β -isomer), 4.91 (d, $J = 3.2$ Hz, 1H, H-1 α), 4.85, 4.64 (qAB, $J = 11.7$ Hz, 1H), 4.65, 4.57 (qAB, $J = 11.9$ Hz, 1H), 4.22 (ddd, $J = 12.3$, 4.8, 2.7 Hz, 1H, H-3), 4.17 (dq, $J = 6.6$, 2.7 Hz, 1H, H-5'), 3.81 (q, $J = 6.6$ Hz, 1H, H-5), 3.73–3.66 (m, 1H, CHHO), 3.56–3.50 (m, 2H, H-4, H-4'), 3.42 (ddd, $J = 7.8$, 6.5, 5.1 Hz, 1H, CHHO), 3.37 (ddd~td, $J = 6.7$, 1.7 Hz, 2H, CH₂N₃), 2.20 (ddd~td, $J = 12.4$, 3.8 Hz, 1H, H-2_{eq}), 1.88 (ddd, $J = 12.7$, 5.0, 1.1 Hz, 1H, H-2_{ax}), 1.87–1.75 (m, 2H, CH₂), 1.35 (d, $J = 6.7$ Hz, 3H, 6'-CH₃), 1.18 (d, $J = 6.5$ Hz, 3H, 6-CH₃). ¹³C-NMR (101 MHz, CDCl₃, δ , ppm): 138.70, 138.58 (2 C_{IV} arom), 129.41 (C-2'), 128.31, 128.25, 128.18, 128.00, 127.67, 127.59, 127.53 (C_{arom}, C-3'), 97.88 (C-1), 92.86 (C-1'), 75.74 (C-4), 74.62 (CH₂Ph), 72.94 (CH₂Ph), 70.78 (C-3), 69.33 (C-4'), 66.66 (C-5), 66.52 (C-5'), 63.82 (CH₂O), 48.53 (CH₂N₃), 32.09 (C-2), 28.94 (CH₂), 17.30 (6-CH₃), 16.30 (6'-CH₃).

(3-Aminopropyl) 3-O-[4-O-benzyl-2,3,6-trideoxy- α -L-threo-hexopyranosyl]-4-O-benzyl-2,6-dideoxy-L-lyxo-hexopyranoside (10)

A solution of **9** (49 mg, 0.095 mmol) in methanol (4 mL) was hydrogenated in a Paar shaker flask at 1.5 atm. over 10% Pd/C (10 mg) for 30 min. After removal of the catalyst by filtration, the solvent was evaporated under reduced pressure. The crude product **10** was used in the next step without further purification.

Glycoconjugate 12

To the solution of 3-aminopropyl glycoside **10** (29 mg, 0.040 mmol) and the uridine derivative **11** (18 mg, 0.060 mmol) in dry THF (2 mL), 2-chloro-4,6-dimethoxy-1,3,5-triazine (11 mg, 0.060 mmol) was added. After 1 min, 4-methylmorpholine (0.009 mL, 0.080 mmol) was added and the reaction mixture was stirred for 30 min at room temperature. The reaction was monitored by TLC (CHCl₃ : MeOH, 20 : 1, v/v). After completion, the reaction mixture was concentrated under reduced pressure, remaining solid was dissolved in CH₂Cl₂ (15 mL) and washed with sat. aq. NaHCO₃ (10 mL) and brine (10 mL). The organic layer was dried over anhydrous MgSO₄, the adsorbent was filtered off and the filtrate was concentrated to give a crude product. This was purified by column chromatography (CHCl₃ : MeOH,

100 : 1, v/v) to give **12** as a colorless syrup in 83% yield; α : β 4 : 1. Selected NMR data for the major α -isomer: $^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ , ppm): 9.28 (s, 1H $_{\beta}$, NH $_{\text{ur}\beta}$), 9.20 (s, 1H, NH $_{\text{ur}}$), 7.43 (d, J = 8.1 Hz, 1H, H-6 $_{\text{ur}}$), 7.40–7.19 (m, 10H, H $_{\text{arom}}$), 6.81 (t, J = 5.4 Hz, 1H, CH $_2$ NH), 5.75–5.71 (m, 1H $_{\beta}$, H-5 $_{\text{ur}\beta}$), 5.68 (dd, J = 8.0, 2.0 Hz, 1H, H-5 $_{\text{ur}}$), 5.51 (s, 1H, H-1' $_{\text{ur}}$), 5.31 (dd, J = 6.3, 2.2 Hz, 1H, H-3' $_{\text{ur}}$), 5.23 (dd, J = 6.3, 2.6 Hz, 1H $_{\beta}$, H-2' $_{\text{ur}}$ β -isomer), 5.17 (dd, J = 6.3, 1.2 Hz, 1H, H-2' $_{\text{ur}}$), 5.13–5.09 (m, 1H, H-1'), 4.87, 4.64 (qAB, J = 11.6 Hz, 2H, CH $_2$ Ph), 4.84 (d, J = 3.2 Hz, 1H, H-1), 4.68, 4.44 (qAB, J = 12.1 Hz, 2H, CH $_2$ Ph), 4.57 (d, J = 2.2 Hz, 1H, H-4' $_{\text{ur}}$), 4.11 (ddd, J = 12.2, 4.6, 2.5 Hz, 1H, H-3), 4.02 (dq, J = 6.6, 1.3 Hz, 1H, H-5'), 3.78–3.71 (m, 1H, H-5), 3.68–3.58 (m, 1H, CHHO), 3.51 (br. s, 1H, H-4), 3.45–3.33 (m, 2H, CHHO, CHHNH), 3.29 (br.s, 1H, H-4'), 3.27–3.16 (m, 1H, CHHNH), 2.20 (ddd~td, J = 12.5, 3.8 Hz, 1H, H-2 $_{\text{eq}}$), 2.15–1.64 (m, 5H, H-2 $_{\text{ax}}$, H-2', H-3', CH $_2$), 1.53, 1.35 (2 \times s, 6H, C(CH $_3$) $_2$), 1.29 (d, J = 6.5 Hz, 3H $_{\beta}$, 6'-CH $_3$ β -isomer), 1.20 (d, J = 6.6 Hz, 3H, 6'-CH $_3$), 1.15 (d, J = 6.5 Hz, 3H, 6-CH $_3$), 1.07 (d, J = 6.5 Hz, 3H $_{\beta}$, 6-CH $_3$ β -isomer). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3 , δ , ppm): 168.98 (CONH), 162.88 (C=O), 150.01 (C=O), 144.32 (C-6 $_{\text{ur}}$), 138.77, 138.54 (2 \times C $_{\text{IP}}$ Ph), 128.70, 128.23, 128.16, 128.12, 128.10, 127.87, 127.75, 127.55, 127.51, 127.45, 127.35 (C $_{\text{arom}}$), 113.62 (C(CH $_3$) $_2$), 102.51 (C-5 $_{\text{ur}}$), 98.28 (C-1' $_{\text{ur}}$), 97.85 (C-1), 96.65 (C-1'), 88.21 (C-4' $_{\text{ur}}$), 83.68 (C-3' $_{\text{ur}}$), 83.31 (C-2' $_{\text{ur}}$), 76.19 (C-4), 74.57 (C-3), 73.55 (CH $_2$ Ph), 73.48 (C-4'), 70.85 (CH $_2$ Ph), 66.98 (C-5), 66.80 (C-5'), 66.06 (CH $_2$ O), 37.66 (CH $_2$ NH), 31.76 (C-2), 28.72 (CH $_2$), 26.71, 24.92 (2 \times C(CH $_3$) $_2$),

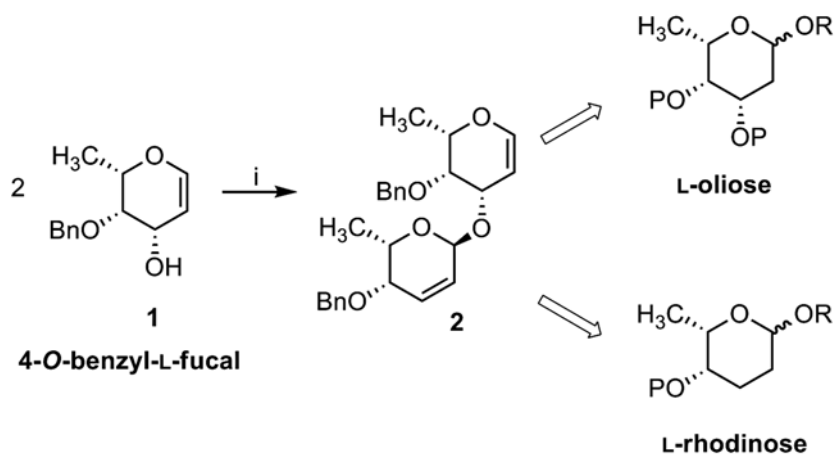
24.54, 21.24 (C-2', C-3'), 17.34 (6-CH $_3$), 17.18 (6'-CH $_3$); LRMS (ESI): calcd. for C $_{41}$ H $_{53}$ N $_3$ O $_{12}$ Na ([M + Na] $^+$): m/z 802.8622; found: m/z 802.8.

RESULTS AND DISCUSSION

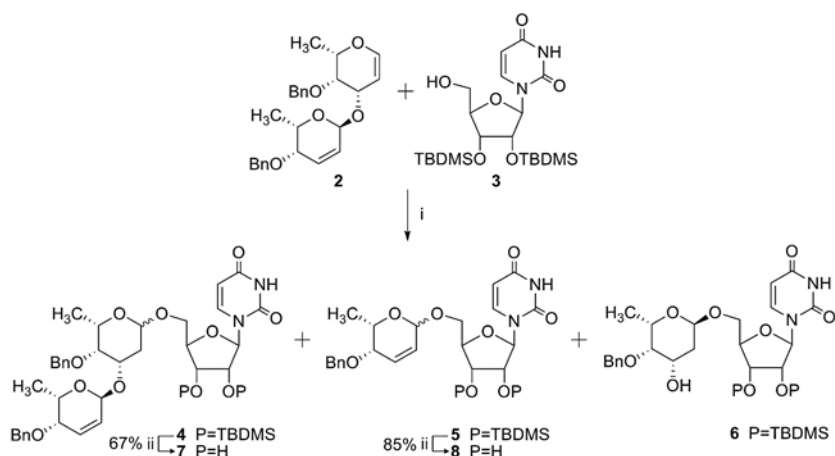
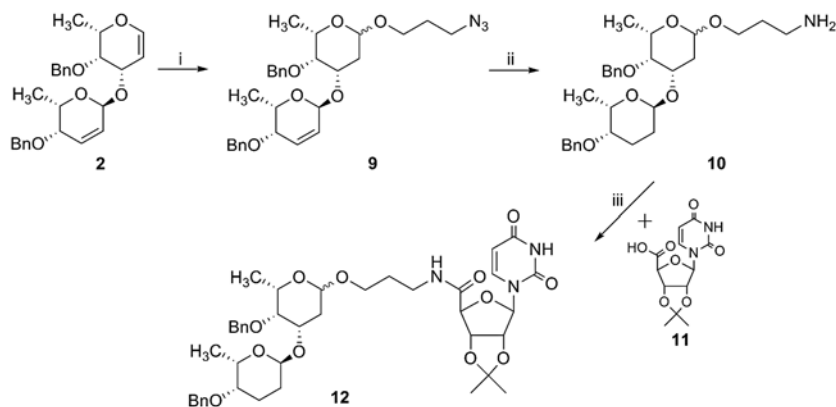
This contribution focuses on a design of potential GT inhibitors, mainly based on modifications of central building blocks of their natural donors. In our research, we decided to use L-fucal as glycosyl donor, which easily allows to obtain a number of 2,6-dideoxy-glycosides, by simple addition to 1,2-unsaturated bond. Deoxysaccharides ubiquitously occur in a number of structural fragments of natural products having biological activity and often play a key role in their mechanism of action.

We recently developed a novel approach for the synthesis of new doubly unsaturated disaccharides (17). This approach utilizes *in situ* generation of an active glycosyl donor *via* Ferrier-type rearrangement under phase-transfer conditions and subsequent reaction with a nucleophile. This concept was put into practice employing 4-*O*-benzyl-L-fucal **1** and afforded a disaccharide product containing 1,2- and 2,3-unsaturated hexoses **2** (Scheme 1). This moiety can be easily functionalized to give various deoxy saccharide derivatives such as L-oliose (2-deoxy-L-fucose) and L-rhodinose (2,3-dideoxy-L-fucose). These deoxy sugar units can be found in the structure of many biologically active natural products such as anthracycline antibiotics, cardiac glycosides, and aminoglycosides (18).

One of the most convenient methods for obtaining 2-deoxy-glycosides under mild conditions



Scheme 1. Reagents and conditions: i) *p*TsCl, toluene, sat. aq NaOH, Bu $_4$ NI, 40%

Scheme 2. Reagents and conditions: i) TPBH, CH₂Cl₂; ii) Bu₄NF, CH₃CNScheme 3. Reagents and conditions: i) TPBH, CH₂Cl₂, r.t., 40%, α : β 5 : 1; ii) H₂, Pd/C, methanol; iii) DMTMM, THF, r.t., 83%

is addition of a nucleophile to the 1,2-unsaturated sugar bond in the presence of the triphenylphosphine hydrobromide (TPHB) (**19**). The addition of the uridine derivative **3** to the disaccharide glycal **2** was carried out in anhydrous CH₂Cl₂ in an ice-bath (Scheme 2, Table 1). The reaction gave a mixture of three glycoconjugates **4**, **5** and **6**. It can be assumed that products **5** and **6** are subsequent cleavage products of 2,3-unsaturated sugar unit from **4** and/or substrate **2**, and their reaction with another molecule of uridine derivative **3**. Probably the observed results can be explained by the high reactivity of the glycosidic bond of the disaccharide. The addition was carried out under different temperature conditions (Table 1). Evidently, with diminishing temperature an increase of the stability of the disaccharide is observed. However, with a substantial reduction of

temperature (to -20°C) the reaction rate was significantly reduced resulting in incomplete conversion of the substrate. After deprotection of *tert*-butyldimethylsilyl groups the final uridine glycoconjugates **7** and **8** were isolated.

In order to obtain an amide bond with an uridine unit it was decided to attach it to the sugar using a three-carbon linker. Addition of 3-azidopropanol to the unsaturated disaccharide **2** and follow-up reduction led to obtain (3-aminopropyl) 3-*O*-[4-*O*-benzyl-α-*L*-rhodinosyl]-4-*O*-benzyl-2-deoxy-*L*-fucopyranoside **10**. The last step was condensation of **10** with uridine acid derivative **11** in the presence of (4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) generated *in situ* (**20**, **21**) This final glycoconjugate **12** was obtained in excellent yield of 83%.

This novel approach provides ample opportunities for facile syntheses to various glycoconjugates as well as derivatives of natural biologically active products containing labile, multi-functional natural compounds as acceptors. All novel glycoconjugates are presently subject of further studies to evaluate their activity in enzymatic reactions of GTs.

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