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Adducts of uridine and glycals as potential substrates for glycosyltransferases

Ilona Wandzik *, Tadeusz Bieg

Silesian University of Technology, Faculty of Chemistry, Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, 44-100 Gliwice, Krzywoustego 4, Poland

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Abstract

We report on the synthesis of 2-deoxyglycosyl derivatives of uridine as potential donor substrates for glycosyltransferases. The totally stereoselective synthesis is accomplished by two sequential addition reactions of uridine derivatives to glycals promoted by triphenylphosphine-hydrogen bromide.

Keywords: GTs substrate analogues; Glycals; Stereoselective synthesis

1. Introduction

Glycosyltransferases (GTs) of the Leloir pathway [1] catalyse the transfer of a monosaccharide unit from an activated nucleotide sugar donor to the hydroxyl group of an acceptor with complete regio- and stereoselectivity. The molecules to which GTs transfer monosaccharide units include oligosaccharides, proteins or lipids. GTs are involved in several metabolic pathways and modulation of their activities by efficient inhibitors has potential for the control of certain cellular functions [2]. Compounds that can modulate the biosynthesis of glycoconjugates are of great interest as novel therapeutic agents. They may also find applications in the study of biological pathways and can be valuable tools in the preparative stereoselective synthesis of oligosaccharides [3,4]. The majority of GTs utilise donors containing uridine pyrophosphate leaving group (UDP). These enzymes can be

* Corresponding author.
E-mail address: ilona.wandzik@polsl.pl (I. Wandzik).

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grouped into two different structural superfamilies: GT-A and GT-B. Enzymes belonging to the GT-A superfamily employ a DxD motif, which is present on the N-terminal domain, to bind a divalent metal, most commonly Mn^{2+} cation. The metal ion is essential for catalysis since it interacts with the pyrophosphate group of the UDP-sugar donor in the enzyme active site.

Different strategies have been used in order to design potent inhibitors of GTs [5–8]. Identification of potent inhibitors has been developing very rapidly during the last two decades since the 3D structures of several GTs were found [9–11] and catalytic mechanism proposed [12]. Structures of GTs' inhibitors are based on analogies between donor substrates, acceptor substrates and transition state, respectively. Several inhibitors were designed as donor substrate analogues. They feature some structural changes at the carbohydrate part or at the pyrophosphate linkage. Among mimetics of carbohydrates, iminosugars and carbasugars are typical examples [6]. A variety of pyrophosphate analogues that are capable of mimicking pyrophosphate–metal interactions have been developed. A common approach is the substitution of a phosphoester oxygen atom by a carbon or sulphur atom, resulting in higher stability towards enzymatic hydrolysis [13]. It has been reported that monosaccharide units might act as pyrophosphate–metal ion complex mimetics [14–16]. It is likely that complexation with Mn^{2+} is feasible with two hydroxyl group of the sugar. Wang and co-workers reported on 5′-*O*-β-lactosyl-uridine which proved to be an inhibitor of the β-1,4-galactosyltransferase present in L1210 leukaemia ascites fluid (K_i 119.6 μM) [14]. Behr and co-workers prepared 3-, 4- and 6-hetaryl-glucose derivatives of uridine which were tested against chitin synthase (IC_{50} 0.8–3.2 mM) [15]. Ballell and co-workers demonstrated sceptical attitude in application of glycosyl units as pyrophosphate linkage mimetics [16]. Series of 5′-*O*-β-glycosyl-uridine and thymidine derivatives showed moderate inhibition of *Salmonella* dTDP-α-D-glucose 4,6-dehydratase and no significant inhibition of the bovine β-1,4-galactosyltransferase.

Being inspired by the reports mentioned above, we have synthesised 2-deoxy-hexopyranosyl derivatives of uridine as donor substrate analogues of gluco- and galactosyltransferases (Fig. 1). According to our knowledge, there is no report on application of 2-deoxy-α-D-glucosyl unit as a surrogate of the pyrophosphate linkage. Compounds **1–5** are composed of uridine and one or two residues of 2-deoxy-hexopyranose and can be synthesised in a totally stereoselective manner using the Falck–Mioskowski protocol [17]. In order to construct target compounds **1–5** we used orthogonally protected glycal substrates and uridine. In our study, the central 2-deoxy-α-D-glucopyranose moiety replacing the key pyrophosphate unit can be linked with terminal 2-deoxyglycosyl moiety through α-(1 → 3)-, α-(1 → 4)-and α-(1 → 6)-linked glycosidic linkages.

2. Experimental

2.1. General

NMR spectra were recorded with a Varian spectrometer at a frequency of 300 MHz with Me_4Si as internal reference. Optical rotations were measured with a Perkin–Elmer 141 polarimeter using a sodium lamp (589 nm) at room temperature. Mass spectra were recorded in the positive mode on a Mariner (Perseptive Biosystem) detector using the electrospray-ionisation (ESI) technique.

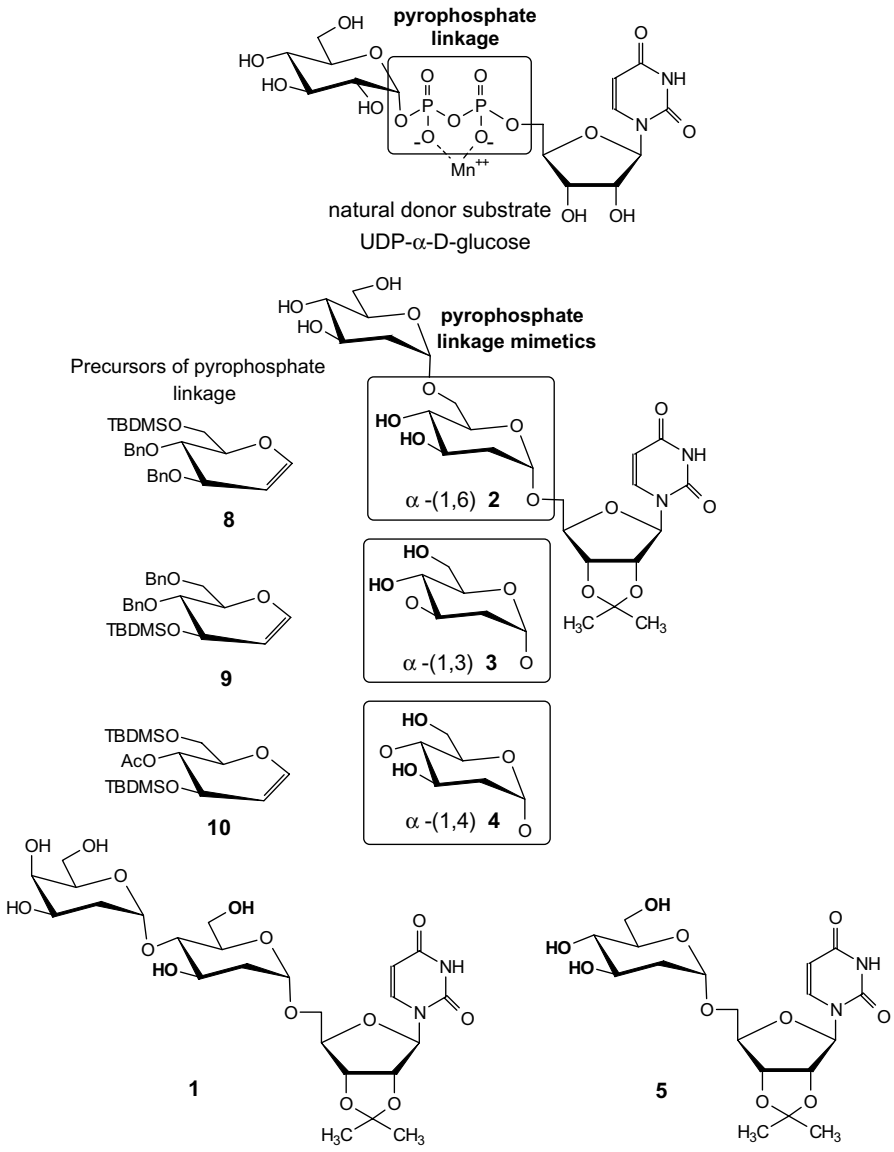


Fig. 1. 2-Deoxy-*O*-glycosyl rings as pyrophosphate linkage mimetics.

Reactions were monitored by TLC on precoated plates of silica gel G (Merck), components were detected under UV light or by charring with 10% sulphuric acid in ethanol. Column chromatography was performed on silica gel 60 (70–230 mesh, E. Merck) developed with one of the hexane/EtOAc or $CHCl_3$/MeOH solvent systems. All evaporations were performed under diminished pressure at 50 °C.

2′,3′-Isopropylidene-uridine (**13**) [18], 3-*N*-benzyl-2′,3′-isopropylidene-uridine (**14**) [19] and 3-*N*-benzoyl-2′,3′-isopropylidene-uridine (**15**) [20] were prepared according to the

published procedures. 4-*O*-acetyl-3,6-di-*O*-benzyl-D-glucal (**11**) was prepared by acetylation of 3,6-di-*O*-benzyl-D-glucal [21]. 3,4,6-Tri-*O*-benzyl-D-galactal (**6**) and 3,4,6-tri-*O*-benzyl-D-glucal (**7**) were prepared by benzylation of corresponding per-*O*-acetylated derivatives with benzyl bromide in NaOH/DMSO system. Pearlman catalyst $Pd(OH)_2$/C and other chemicals were purchased from Aldrich and Fluka Chemical Companies and were used without purification. Solvents were dried and stored over molecular sieves (4 Å) under an inert atmosphere.

*2.2. 3,4-Di-O-benzyl-6-O-tert-butyldimethylsilyl-D-glucal (**8**), 4,6-di-O-benzyl-3-O-tert-butyldimethylsilyl-D-glucal (**9**) and 4-O-benzyl-6-O-tert-butyldimethylsilyl-D-glucal (**10**)*

To a solution of D-glucal (15.0 g, 0.103 mol) in DMF (45 mL), imidazole (15.4 g, 0.227 mol) and TBDMSCl (16.9 g, 0.113 mol) were added. Reaction mixture was stirred at room temperature for 20 min and after this time was diluted with CH_2Cl_2 (400 mL), washed with water (2 × 150 mL), dried over anhydrous $MgSO_4$ and concentrated to give crude product. Crude product was subsequently purified by column chromatography with hexane/AcOEt 15:1 → 4:1 solvent system to yield 3,6-di-*O*-*tert*-butyldimethylsilyl-D-glucal (7.3 g, 19%) as white crystals and 6-*O*-*tert*-butyldimethylsilyl-D-glucal (14.5 g, 54%) as a colourless oil. 6-*O*-*tert*-butyldimethylsilyl-D-glucal (7.0 g, 0.028 mol) was dissolved in DMF (70 mL) and the solution was cooled to −5 °C. Then sodium hydride, 60% dispersion in oil (2.0 g) and benzyl bromide (6.6 mL, 0.056 mol) were added. After stirring for 4 h at room temperature, the solution was neutralized with 1 M HCl aqueous solution, diluted with hexane (500 mL) and washed with water (3 × 150 mL). The organic layer was then dried ($MgSO_4$) and the solvent removed under reduced pressure. The resulting crude product mixture was purified by column chromatography with hexane/AcOEt 20:1 → 10:1 solvent system to yield **8** (6.5 g, 53%) as a colourless oil, **9** (0.9 g, 8%) as a colourless oil and **10** (1.7 g, 17%) as white crystals. Glycal **8** was identified by comparison with literature data [22].

Compound **9**: $[\alpha]_D^{20}$ +13.0 ($CHCl_3$, c 1.0); ^{1}H NMR ($CDCl_3$): δ 0.08, 0.09 (2s, 6H, Si[C(CH_3)$_3$(CH_3)$_2$]), 0.89 (s, 9H, Si[C(CH_3)$_3$(CH_3)$_2$]), 3.66 (dd, 1H, *J* 5.9, 8.4 Hz, H-4), 3.70 (dd, 1H, *J* 2.6, 11.0 Hz, H-6a), 3.78 (dd, 1H, *J* 5.5, 11.0 Hz, H-6b), 4.07 (ddd, 1H, *J* 2.6, 5.5, 8.4 Hz, H-5), 4.34 (m, 1H, H-3), 4.56 (s, 2H, PhCH_2), 4.62, 4.82 (q_{AB}, 2H, *J* 11.3 Hz, PhCH_2), 4.64 (dd, 1H, *J* 2.7, 6.1 Hz, H-2), 6.34 (dd, 1H, *J* 1.2, 6.1 Hz, H-1), 7.23–7.33 (m, 10H, *Ph*CH_2); ^{13}C NMR ($CDCl_3$): δ −4.64, −4.36 (Si[C(CH_3)$_3$(*C*H_3)$_2$]), 17.98 (Si[*C*(CH_3)$_3$(CH_3)$_2$]), 25.82 (Si[C(*C*H_3)$_3$(CH_3)$_2$], 68.72, 69.07, 73.49, 73.96, 76.61, 76.81 (C-3, C-4, C-5, C-6, 2 Ph*C*H_2), 103.52 (C-2), 125.32–128.40 (*Ph*CH_2), 138.04, 138.19 (C_q, $PhCH_2$), 143.39 (C-1); ESI-HRMS: Calcd for $C_{26}H_{36}O_4SiNa$ ([M+Na]$^+$): *m*/*z* 463.2277. Found: *m*/*z* 463.2269.

Compound **10**: $[\alpha]_D^{20}$ +27.2 ($CHCl_3$, c 1.0); mp. 55–57 °C; ^{1}H NMR ($CDCl_3$): δ 0.09 (s, 6H, Si[C(CH_3)$_3$(CH_3)$_2$]), 0.92 (s, 9H, Si[C(CH_3)$_3$(CH_3)$_2$]), 2.31 (d, 1H, *J* 6.3 Hz, C-3OH), 3.68 (dd, 1H, *J* 6.2, 8.2 Hz, H-4), 3.86 (ddd, 1H, *J* 2.6, 2.6, 8.2 Hz, H-5), 3.95 (dd, 1H, *J* 2.9, 11.9 Hz, H-6a), 3.96 (dd, 1H, *J* 2.5, 11.9 Hz, H-6b), 4.29 (dddd, 1H, *J* 1.5, 2.8, 6.2, 6.3 Hz, H-3), 4.72 (dd, 1H, *J* 2.8, 6.1 Hz, H-2), 4.79 (s, 2H, PhCH_2), 6.36 (dd, 1H, *J* 1.5, 6.1 Hz, H-1), 7.26–7.37 (m, 5H, *Ph*CH_2); ^{13}C NMR ($CDCl_3$): δ −5.11, −5.42 (Si[C(CH_3)$_3$(*C*H_3)$_2$]), 18.37 (Si[*C*(CH_3)$_3$(CH_3)$_2$]), 25.91 (Si[C(*C*H_3)$_3$(CH_3)$_2$], 62.41 (C-6), 68.07 (C-3), 73.68 (Ph*C*H_2), 77.14 (C-4), 77.57 (C-5), 102.20 (C-2), 127.93, 127.95,

128.59 (*Ph*CH_2), 138.48 (C_q, $PhCH_2$), 144.54 (C-1); ESI-HRMS: Calcd for $C_{19}H_{30}O_4SiNa$ ($[M+Na]^+$): *m*/*z* 373.1806. Found: *m*/*z* 373.1809.

2.3. 3,4-Di-O-benzyl-6-O-tert-butyldimethylsilyl-2-deoxy-α-D-glucopyranosyl-(1 → 5)-2,3-O-isopropylideneuridine (16)

To a solution of glucal **8** (0.30 mmol) and uridine derivative **13** (85 mg, 0.30 mmol) in dry CH_2Cl_2 (15 mL), a catalytic amount of TPHB (10 mg, 0.03 mmol) was added. The mixture was stirred at room temperature for 24 h. Then the reaction mixture was concentrated to give crude product mixture purified directly by column chromatography with hexane/AcOEt 1:1 solvent system to yield **16** (54 mg, 25%) as a white foam; $[\alpha]_D^{20}$ +16.1 ($CHCl_3$, c 1.0); ^{1}H NMR ($CDCl_3$): δ 0.06, 0.07 (2s, 6H, Si[C(CH_3)$_3$(CH_3)$_2$]), 0.90 (s, 9H, (Si[C(CH_3)$_3$(CH_3)$_2$]), 1.35, 1.57 (2s, 6H, C(CH_3)$_2$), 1.69 (ddd, 1H, *J* 3.0, 11.4, 12.6 Hz, H-2″ax), 2.13 (ddd, 1H, $J \approx 0$, 4.5, 12.6 Hz, H-2″eq), 3.49–3.91 (m, 7H, H-5′a,b, H-3″, H-4″, H-5″, H-6″a,b), 4.34–4.38 (m, 1H, H-4′), 4.61–4.96 (m, 6H, H-2′, H-3′, 2 · PhCH_2), 4.92 (m, 1H, H-1″), 5.66 (dd, 1H, *J* 8.1, 2.0 Hz, H-5), 5.84 (d, 1H, *J* 2.0 Hz, H-1′), 7.22–7.38 (m, 10H, 2*Ph*CH_2), 7.41 (d, 1H, *J* 8.1 Hz, H-6), 9.77 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$): δ −5.35, −5.15 (Si[C(CH_3)$_3$(*C*H_3)$_2$]), 18.29 (Si[*C*(CH_3)$_3$(CH_3)$_2$]), 25.41, 27.25 (C(*C*H_3)$_2$), 25.89 (Si[C(*C*H_3)$_3$(CH_3)$_2$]), 35.33 (C-2″), 62.32 (C-5′), 66.83, 72.84, 76.83, 78.02 (C-3″, C-4″, C-5″, C-6″), 71.96, 75.12 (2Ph*C*H_2), 80.68 (C-3′), 85.10 (C-2′), 85.35 (C-4′), 92.94 (C-1′), 97.43 (C-1″), 101.95 (C-5), 114.30 (*C*(CH_3)$_2$), 127.75, 128.08, 128.45 (*Ph*CH_2), 138.23, 138.34 (C_q, $PhCH_2$), 140.60 (C-6), 149.80 (C-2), 162.76 (C-4); ESI-HRMS: Calcd for $C_{38}H_{52}N_2O_{10}SiNa$ ($[M+Na]^+$): *m*/*z* 747.3283. Found: *m*/*z* 747.3319.

2.4. 3,4-Di-O-benzyl-6-O-tert-butyldimethylsilyl-2-deoxy-α-D-glucopyranosyl (1 → 5)-3-N-benzyl-2,3-O-isopropylideneuridine (17)

Procedure A. To a solution of glucal **8** (132 mg, 0.30 mmol) and uridine derivative **14** (112 mg, 0.30 mmol, 1 equiv.) in dry CH_2Cl_2 (2 mL), a catalytic amount of TPHB (10 mg, 0.03 mmol, 0.1 equiv.) was added. The mixture was kept at room temperature for 1 h. Then the reaction mixture was concentrated to give crude product mixture purified directly by column chromatography with hexane/AcOEt 2:1 solvent system to yield **17** (161 mg, 66%) as a white foam; $[\alpha]_D^{20}$ +20.4 ($CHCl_3$, c 1.0); ^{1}H NMR ($CDCl_3$): δ 0.01, 0.05 (2s, 6H, Si[C(CH_3)$_3$(*C*H_3)$_2$]), 0.89 (s, 9H, (Si[C(*C*H_3)$_3$(CH_3)$_2$]), 1.35, 1.57 (2s, 6H, C(CH_3)$_2$), 1.68 (ddd, 1H, *J* 3.5, 11.5, 13.2 Hz, H-2″ax), 2.10 (ddd, 1H, $J \approx 0$, 4.9, 13.2 Hz, H-2″eq), 3.49–3.86 (m, 7H, H-5′a,b, H-3″, H-4″, H-5″, H-6″a,b), 4.36–4.41 (m, 1H, H-4′), 4.52–4.94 (m, 6H, H-2′, H-3′, 2 · PhCH_2), 4.90 (bd, 1H, *J* 3.5 Hz, H-1″), 5.04, 5.16 (q_{AB}, 2H, *J* 12.6 Hz, PhCH_2), 5.73 (d, 1H, *J* 8.1 Hz, H-5), 5.81 (d, 1H, *J* 2.4 Hz, H-1′), 7.21–7.50 (m, 15H, 3*Ph*CH_2), 7.42 (d, 1H, *J* 8.1 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ −5.34, −5.15 (Si[C(CH_3)$_3$(*C*H_3)$_2$]), 18.29 (Si[*C*(CH_3)$_3$(CH_3)$_2$]), 25.42, 27.25 (C(*C*H_3)$_2$), 25.89 (Si[C(*C*H_3)$_3$(CH_3)$_2$]), 35.19 (C-2″), 44.11 (N-*C*H_2Ph), 62.34, 66.83, 71.83, 72.91, 75.11, 76.81, 77.93 (C-5″, C-3″, C-4″, C-5″, C-6″, 2Ph*C*H_2), 80.68 (C-3′), 85.47 (C-2′), 85.55 (C-4′), 93.75 (C-1′), 97.45 (C-1″), 101.44 (C-5), 114.18 (*C*(CH_3)$_2$), 127.66–129.13 (*Ph*CH_2), 136.52 (C_q, $PhCH_2N$), 138.15, 138.22 (C_q, $PhCH_2$), 138.34 (C-6), 150.76 (C-2), 162.48 (C-4); ESI-HRMS: Calcd for $C_{45}H_{58}N_2O_{10}SiNa$ ($[M+Na]^+$): *m*/*z* 837.3753. Found: *m*/*z* 837.3745.

2.5. 3,4-Di-O-benzyl-6-O-tert-butyldimethylsilyl-2-deoxy-α-D-glucopyranosyl (1 → 5)-3-N-benzoyl-2,3-O-isopropylideneuridine (**18**)

Glucal **8** (528 mg, 1.20 mmol) and uridine derivative **15** (233 mg, 1.20 mmol) were submitted to general procedure A described above for the preparation of **17**. The resulting crude product was purified by column chromatography with hexane/AcOEt 2:1 solvent system to yield **18** (706 mg, 71%) as a white foam; $[\alpha]_D^{20}$ +18.8 ($CHCl_3$, c 2.0); ^{1}H NMR ($CDCl_3$): δ 0.01, 0.07 (2s, 6H, Si[C(CH_3)$_3$(CH_3)$_2$]), 0.90 (s, 9H, (Si[C(CH_3)$_3$(CH_3)$_2$]), 1.34, 1.54 (2s, 6H, C(CH_3)$_2$), 1.69 (ddd, 1H, J 3.7, 11.4, 12.5 Hz, H-2″ax), 2.14 (ddd, 1H, $J \approx 0$, 4.0, 12.5 Hz, H-2″eq), 3.47–3.92 (m, 7H, H-5′a,b, H-3″, H-4″, H-5″, H-6″a,b), 4.37–4.42 (m, 1H, H-4′), 4.68, 4.94 (q_{AB}, 2H, J 10.7 Hz, PhCH_2), 4.66 (s, 2H, PhCH_2), 4.74 (m, 1H, H-3′), 4.74 (dd, 1H, J 2.4, 6.1 Hz, H-2′), 4.90 (bd, 1H, J 3.7 Hz, H-1″), 5.77 (d, 1H, J 8.3 Hz, H-5), 5.82 (d, 1H, J 2.4 Hz, H-1′), 7.27–7.39 (m, 10H, 2*Ph*CH_2), 7.43–7.50 (m, 2H, Ph(m)), 7.54 (d, 1H, J 8.1 Hz, H-6), 7.59–7.65 (m, 1H, Ph(p)), 7.89–7.95 (m, 1H, Ph(o)); ^{13}C NMR ($CDCl_3$): δ −5.31, −5.11 (Si[C(CH_3)$_3$(*C*H_3)$_2$]), 18.33 (Si[*C*(CH_3)$_3$(CH_3)$_2$]), 25.44, 27.27 (C(*C*H_3)$_2$), 25.94 (Si[C(*C*H_3)$_3$(CH_3)$_2$]), 35.51 (C-2″), 62.36 (C-5′), 66.86, 72.92, 76.81, 78.04 (C-3″, C-4″, C-5″, C-6″), 71.92, 75.17 (2Ph*C*H_2), 80.76 (C-3′), 85.18 (C-2′), 85.68 (C-4′), 93.79 (C-1′), 97.53 (C-1″), 101.92 (C-5), 114.30 (*C*(CH_3)$_2$), 127.77, 127.79, 127.83, 128.13, 128.47, (*Ph*CH_2), 129.19, 130.50, 136.13, (*Ph*CO), 131.40, (C_q, PhCO), 138.33, 138.43, (C_q, PhCH_2), 140.48 (C-6), 149.10 (C-2), 161.94 (C-4), 168.32 (Ph*C*O); ESI-HRMS: Calcd for $C_{45}H_{56}N_2O_{11}SiNa$ ([M+Na]$^+$): m/z 851.3546. Found: m/z 851.3527.

2.6. 3,4-Di-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1 → 5)-3-N-benzoyl-2,3-O-isopropylideneuridine (**19**)

Procedure B. Desilylation of adduct **18** (650 mg, 0.79 mmol) was achieved with AcCl (62 μL, 0.79 mmol, 1 equiv.) in a solution of MeOH (15 mL) within 10 min. The reaction mixture was neutralized with a basic resin, Amberlyst 21 (OH^-), filtered and concentrated to give crude product mixture, which was subsequently purified by column chromatography with hexane/AcOEt 1:1 solvent system to yield **19** (465 mg, 83%) as a white foam; $[\alpha]_D^{20}$ +26.8 ($CHCl_3$, c 1.0); ^{1}H NMR ($CDCl_3$): δ 1.33, 1.54 (2s, 6H, C(CH_3)$_2$), 1.67 (ddd, 1H, J 3.3, 11.3, 13.2 Hz, H-2″ax), 2.03 (d, 1H, J 2.9 Hz, C-6OH), 2.18 (ddd, 1H, $J \approx 0$, 5.1, 13.2 Hz, H-2″eq), 3.45–3.82 (m, 6H, H-5′a,b, H-4″, H-5″, H-6″a,b), 3.90 (ddd, 1H, J 5.1, 8.3, 11.3 Hz, H-3″), 4.36 (m, 1H, H-4′), 4.61–4.97 (m, 4H, PhCH_2), 4.70 (dd, 1H, J 3.6, 6.1 Hz, H-3′), 4.80 (dd, 1H, J 2.2, 6.1 Hz, H-2′), 4.90 (bd, 1H, J 3.3 Hz, H-1″), 5.74 (d, 1H, J 2.2 Hz, H-1′), 5.78 (d, 1H, J 8.1 Hz, H-5), 7.24–7.39 (m, 10H, 2*Ph*CH_2), 7.42–7.50 (m, 2H, Ph(m)), 7.47 (d, 1H, J 8.1 Hz, H-6), 7.58–7.65 (m, 1H, Ph(p)), 7.89–7.95 (m, 1H, Ph(o)); ^{13}C NMR ($CDCl_3$): 25.39, 27.22 (C(*C*H_3)$_2$), 35.21 (C-2″), 62.08, 67.28, 71.71, 71.91, 75.15, 76.69, 77.99 (C-5′, C-3″, C-4″, C-5″, C-6″, 2Ph*C*H_2), 80.79 (C-3′), 84.91 (C-2′), 85.89 (C-4′), 94.44 (C-1′), 97.71 (C-1″), 102.01 (C-5), 114.35 (*C*(CH_3)$_2$), 127.69–128.53 (*Ph*CH_2), 129.20, 130.50, 135.21, (*Ph*CO), 131.33, (C_q, PhCO), 138.10, 138.30, (C_q, PhCH_2), 141.08 (C-6), 149.06 (C-2), 161.96 (C-4), 168.37 (Ph*C*O); ESI-HRMS: Calcd for $C_{39}H_{42}N_2O_{11}Na$ ([M+Na]$^+$): m/z 737.2681. Found: m/z 737.2680.

2.7. 4,6-Di-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1 → 5)-3-N-benzoyl-2,3-O-isopropylideneuridine (21)

Glucal **9** (300 mg, 0.68 mmol) and uridine derivative **15** (265 mg, 0.68 mmol) were submitted to general procedure A described above for the preparation of **17**. The resulting crude product was purified by column chromatography with hexane/AcOEt 3:1 solvent system to yield **20** (230 mg, 41%) as a white foam. Desilylation of adduct **20** according to the procedure B described above for the preparation of **18** yielded **21** (130 mg, 65%) in 1.5 h as a white foam; $[\alpha]_D^{20}$ +28.0 ($CHCl_3$, c 1.0) ^{1}H NMR ($CDCl_3$): δ 1.31, 1.53 (2s, 6H, $C(CH_3)_2$), 1.75 (ddd, 1H, *J* 3.7, 11.5, 13.1 Hz, H-2″ax), 2.02 (ddd, 1H, $J \approx 0$, 5.1, 13.1 Hz, H-2″eq), 2.62 (s, 1H, C-3OH), 3.46 (t, 1H, *J* 9.3 Hz, H-4″) 3.64 (m, 1H, H-5″), 3.76 (dd, 1H, *J* 3.8, 10.9 Hz, H-6″a), 3.83 (dd, 1H, *J* 3.7, 10.9 Hz, H-6″b), 3.95 (ddd, 1H, *J* 5.1, 9.3, 11.5 Hz, H-3″), 4.39 (m, 1H, H-4′), 4.49–4.72 ($2q_{AB}$, 4H, PhCH_2), 4.72 (dd, 1H, *J* 2.8, 6.1 Hz, H-3′), 4.82 (dd, 1H, *J* 2.2, 6.1 Hz, H-2′), 4.92 (bd, 1H, *J* 3.7 Hz, H-1″), 5.81 (d, 1H, *J* 2.2 Hz, H-1′), 5.81 (d, 1H, *J* 8.1 Hz, H-5), 7.21–7.35 (m, 10H, $2CH_2$*Ph*), 7.42–7.50 (m, 2H, Ph(m)), 7.59 (d, 1H, *J* 8.1 Hz, H-6), 7.58–7.65 (m, 1H, Ph(p)), 7.89–7.95 (m, 1H, Ph(o)); ^{13}C NMR ($CDCl_3$): 25.43, 27.27 ($C(CH_3)_2$), 37.32 (C-2″), 67.22, 68.73, 68.93, 71.38, 73.65, 74.97, 79.65 (C-5′, C-3″, C-4″, C-5″, C-6″, 2 PhCH_2), 80.75 (C-3′), 85.30 (C-2′), 85.97 (C-4′), 93.77 (C-1′), 97.82 (C-1″), 101.87 (C-5), 114.31 ($C(CH_3)_2$), 127.85–128.47, (*Ph*CH_2), 129.27, 130.60, 135.30, (*Ph*CO), 131.32 (C_q, PhCO), 137.87, 138.18, (C_q, PhCH_2), 140.88 (C-6), 149.12 (C-2), 162.16 (C-4), 168.67 (Ph*C*O); ESI-HRMS: Calcd for $C_{39}H_{42}N_2O_{11}Na$ ([M+Na]$^+$): *m*/*z* 737.2681. Found: *m*/*z* 737.2667.

2.8. 3,6-Di-O-benzyl-2-deoxy-D-glucopyranosyl-(1 → 5)-2,3-O-isopropylideneuridine (23)

Glucal **11** (250 mg, 0.68 mmol) and uridine derivative **15** (265 mg, 0.68 mmol) were submitted to general procedure A described above for the preparation of **17**. The resulting crude product **22** was purified by column chromatography with hexane/AcOEt 2:1 solvent system to yield **22** (298 mg, 58%) as an inseparable α,β-anomeric (6:1) mixture. Deacylation of adduct **22** was achieved with a 0.1 M solution of sodium methoxide in methanol (4 mL) in 4 days. The reaction mixture was neutralized with an acidic resin, Dowex 50WX8 (H^+), filtered, concentrated and purified by column chromatography with hexane/AcOEt 1:1 solvent system to yield **23** (180 mg, 75%) as a white foam; α:β = 7:1; ^{1}H NMR (CD_3OD) α anomer: δ 1.34, 1.54 (2s, 6H, $C(CH_3)_2$), 1.50–1.62 (m, 1H, H-2″ax), 2.06 (ddd, 1H, $J \approx 0$, 4.8, 12.9 Hz, H-2″eq), 3.36–3.86 (m, 6H, H-5′, H-3″, H-4″, H-5″, H-6″a,b), 4.44 (m, 1H, H-4′), 4.54–4.68 (m, 4H, PhCH_2), 4.68 (dd, 1H, *J* 2.4, 6.1 Hz, H-3′), 4.74 (dd, 1H, *J* 2.4, 6.1 Hz, H-2′), 4.91 (bd, 1H, *J* 2.5 Hz, H-1″), 5.66 (d, 1H, *J* 8.1 Hz, H-5), 5.77 (d, 1H, *J* 2.4 Hz, H-1′), 7.21–7.35 (m, 10H, $2CH_2$*Ph*), 7.63 (d, 1H, *J* 8.1 Hz, H-6), 7.90 (s, 1H, NH); ^{13}C NMR (CD_3OD) α anomer: 25.51, 27.53 ($C(CH_3)_2$), 36.01 (C-2″), 68.45, 70.95, 72.03, 72.77, 73.69, 74.56, 77.22 (C-5′, C-3″, C-4″, C-5″, C-6″, 2PhCH_2), 82.81 (C-3′), 86.88 (C-2′), 87.20 (C-4′), 95.29 (C-1′), 98.81 (C-1″), 102.08 (C-5), 114.79 ($C(CH_3)_2$), 128.69–129.41, (*Ph*CH_2), 139.70, 139.87, (C_q, PhCH_2), 143.06 (C-6), 152.03 (C-2), 166.31 (C-4). ESI-HRMS: Calcd for $C_{32}H_{38}N_2O_{10}Na$ ([M+Na]$^+$): *m*/*z* 633.2419. Found: *m*/*z* 633.2438.

2.9. 3,4,6-Tri-O-benzyl-2-deoxy-α-D-galactopyranosyl-(1 → 6)-3,4-di-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1 → 5)-3-N-benzoyl-2,3-O-isopropylideneuridine (**24**)

Glucal **6** (116 mg, 0.28 mmol) and uridine derivative **19** (200 mg, 0.28 mmol) were submitted to general procedure A described above for the preparation of **17**. Crude product **24** was purified by column chromatography with hexane/AcOEt 2:1 solvent system to yield **24** (155 mg, 49%) as a white foam; $[\alpha]_D^{20}$ +40.5 ($CDCl_3$, c 1.0); ^1H NMR ($CDCl_3$): δ 1.32, 1.53 (2s, 6H, $C(CH_3)_2$), 1.61–1.75 (m, 2H, H-2″ax, H-2‴eq), 2.12–2.27 (m, 2H, H-2″eq, H-2‴ax), 3.43–3.94 (m, 12 H, H-5′a,b, H-3″, H-4″, H-5″, H-6″a,b, H-3‴, H-4‴, H-5‴, H-6‴a,b), 4.35, 4.44 (q_{AB}, 2H, J 11.8 Hz, PhCH_2), 4.35 (m, 1H, H-4′), 4.51–4.95 (m, 8H, 4PhCH_2), 4.68 (dd, 1H, J 3.4, 6.1 Hz, H-3′), 4.77 (dd, 1H, J 2.2, 6.1 Hz, H-2′), 4.88 (bd, 1H, J 2.7 Hz, H-1″), 5.02 (bd, 1H, J 2.7 Hz, H-1‴), 5.75 (d, 1H, J 8.1 Hz, H-5), 5.75 (d, 1H, J 2.2 Hz, H-1′), 7.20–7.40 (m, 25H, 5CH_2*Ph*), 7.42–7.50 (m, 2H, Ph(m)), 7.45 (d, 1H, J 8.1 Hz, H-6), 7.55–7.65 (m, 1H, Ph(p)), 7.86–7.94 (m, 1H, Ph(o)); ^{13}C NMR ($CDCl_3$): δ 25.42, 27.24 ($C(CH_3)_2$), 30.99 (C-2‴), 35.08 (C-2″), 66.09, 67.02, 69.43, 70.10, 70.27, 71.17, 71.73, 72.92, 73.36, 74.28, 74.41, 75.11, 76.89, 78.09 (C-5′, C-3″, C-4″, C-5″, C-6″, C-3‴, C-4‴, C-5‴, C-6‴, 5Ph*C*H_2), 80.74 (C-3′), 85.07 (C-2′), 85.78 (C-4′), 94.21 (C-1′), 97.54 (C-1″), 98.30 (C-1‴), 101.90 (C-5), 114.31 (*C*$(CH_3)_2$), 127.34–128.47 (*Ph*CH_2), 129.19, 130.48, 135.18, (*Ph*CO), 131.32 (C_q, PhCO), 138.11, 138.15, 138.23, 138.39, 138.83 (C_q, PhCH_2), 140.79 (C-6), 149.02 (C-2), 161.92 (C-4), 168.30 (Ph*C*O); ESI-MS: Calcd for $C_{66}H_{70}N_2O_{15}Na$ ($[M+Na]^+$): *m*/*z* 1153.5. Found: *m*/*z* 1153.4.

2.10. 3,4,6-Tri-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1 → 6)-3,4-di-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1 → 5)-3-N-benzoyl-2,3-O-isopropylideneuridine (**25**)

Glucal **7** (116 mg, 0.28 mmol) and uridine derivative **19** (200 mg, 0.28 mmol) were submitted to general procedure A described above for the preparation of **17**. Crude product **25** was purified by column chromatography with hexane/AcOEt 2:1 solvent system to yield **25** (193 mg, 61%) as a white foam; $[\alpha]_D^{20}$ +43.7 ($CDCl_3$, c 1.0); ^1H NMR ($CDCl_3$): δ 1.32, 1.53 (2s, 6H, $C(CH_3)_2$), 1.61–1.75 (m, 2H, H-2″ax, H-2‴ax), 2.17, 2.30 (2dd, 2H, $J \approx 0$, 5.1, 12.5 Hz, H-2′eq, H-2‴eq), 3.43–3.94 (m, 12 H, H-5′a,b, H-3″, H-4″, H-5″, H-6″a,b, H-3‴, H-4‴, H-5‴, H-6‴a,b), 4.35 (m, 1H, H-4′), 4.38–5.00 (m, 10H, 5PhCH_2), 4.70 (dd, 1H, J 3.0, 6.1 Hz, H-3′), 4.78 (dd, 1H, J 2.1, 6.1 Hz, H-2′), 4.89 (bd, 1H, J 3.3 Hz, H-1″), 4.98 (bs, 1H, H-1‴), 5.76 (d, 1H, J 2.1 Hz, H-1′), 5.75 (d, 1H, J 8.1 Hz, H-5), 7.20–7.40 (m, 25H, 5*Ph*CH_2), 7.42–7.50 (m, 2H, Ph(m)), 7.45 (d, 1H, J 8.1 Hz, H-6), 7.55–7.65 (m, 1H, Ph(p)), 7.86–7.94 (m, 1H, Ph(o)); ^{13}C NMR ($CDCl_3$): δ 25.44, 27.25 ($C(CH_3)_2$), 35.06, 35.25 (C-2″, C-2‴), 65.80, 67.12, 68.76, 69.95, 70.89, 71.15, 71.66, 73.06, 73.42, 74.40, 74.88, 75.04, 76.01, 78.12 (C-5′, C-3″, C-4″, C-5″, C-6″, C-3‴, C-4‴, C-5‴, C-6‴, 5Ph*C*H_2), 80.76 (C-3′), 85.10 (C-2′), 85.86 (C-4′), 94.28 (C-1′), 97.57 (C-1″), 97.78 (C-1‴), 101.90 (C-5), 114.31 (*C*$(CH_3)_2$), 127.50–128.48 (*Ph*CH_2), 129.20, 130.49, 135.17 (*Ph*CO), 131.36 (C_q, PhCO), 138.13, 138.21, 138.23, 138.39, 138.83 (C_q, PhCH_2), 140.81 (C-6), 149.90 (C-2), 161.93 (C-4), 168.30 (Ph*C*O); ESI-MS: Calcd for $C_{66}H_{70}N_2O_{15}Na$ ($[M+Na]^+$): *m*/*z* 1153.5. Found: *m*/*z* 1153.4.

2.11. 3,4,6-Tri-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1 → 3)-4,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1 → 5)-3-N-benzoyl-2,3-O-isopropylideneuridine (**26**)

Glucal **7** (116 mg, 0.28 mmol) and uridine derivative **21** (200 mg, 0.28 mmol) were submitted to general procedure A described above for the preparation of **17**. Crude product **26** was purified by column chromatography with hexane/AcOEt 2:1 solvent system to yield **26** (152 mg, 48%) as a white foam; $[\alpha]_D^{20}$ +48.1 ($CDCl_3$, c 0.5); ^1H NMR ($CDCl_3$): δ 1.31, 1.53 (2s, 6H, $C(CH_3)_2$), 1.64–1.81 (m, 2H, H-2″ax, H-2‴ax), 2.17, 2.39 (2dd, 2H, $J \approx 0$, 5.1, 12.3 Hz, H-2′eq, H-2‴eq), 3.44–4.05 (m, 12 H, H-5′a,b, H-3″, H-4″, H-5″, H-6″a,b, H-3‴, H-4‴, H-5‴, H-6‴a,b), 4.29 (m, 1H, H-4′), 4.41–4.73 (m, 9H, PhCH_2), 4.69 (dd, 1H, J 2.2, 6.0 Hz, H-3′), 4.76 (dd, 1H, J 2.1, 6.2 Hz, H-2′), 4.79 (bd, 1H, J 2.7 Hz, H-1″), 4.86–4.92 (m, 1H, PhCH_2), 5.16 (bd, 1H, J 2.6 Hz, H-1‴), 5.89 (d, 1H, J 2.1 Hz, H-1′), 6.03 (d, 1H, J 8.2 Hz, H-5), 7.10–7.38 (m, 25H, 5 *Ph*CH_2), 7.40–7.48 (m, 2H, Ph(m)), 7.54 (d, 1H, J 8.2 Hz, H-6), 7.56–7.64 (m, 1H, Ph(p)), 7.86–7.94 (m, 1H, Ph(o)); ^{13}C NMR ($CDCl_3$): δ 25.49, 27.28 (C(*C*H_3)$_2$), 36.11, 36.74 (C-2″, C-2‴), 66.98, 68.64, 69.46, 71.18, 71.39, 71.78, 73.20, 73.54, 75.05, 75.19, 77.16, 77.25, 77.64, 78.43 (C-5′, C-3″, C-4″, C-5″, C-6″, C-3‴, C-4‴, C-5‴, C-6‴, 5Ph*C*H_2), 80.22 (C-3′), 84.82 (C-2′), 85.36 (C-4′), 92.30 (C-1′), 97.76 (C-1″), 99.77 (C-1‴), 102.82 (C-5), 114.52 (*C*$(CH_3)_2$), 127.47-128.51 (*Ph*CH_2), 129.15, 130.48, 135.05, (*Ph*CO), 131.40 (C_q, PhCO), 137.96, 138.04, 138.25, 138.29, 138.53 (C_q, $PhCH_2$), 140.41 (C-6), 149.09 (C-2), 161.98 (C-4), 168.47 (Ph*C*O); ESI-MS: Calcd for $C_{66}H_{70}N_2O_{15}Na$ ($[M+Na]^+$): m/z 1153.5. Found: m/z 1153.7.

2.12. 3,4,6-Tri-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1 → 4)-3,6-di-O-benzyl-2-deoxy-D-glucopyranosyl-(1 → 5)-2,3-O-isopropylideneuridine (**27**)

Glucal **7** (123 mg, 0.30 mmol) and uridine derivative **23** (180 mg, 0.30 mmol) were submitted to general procedure A described above for the preparation of **17**, reaction time 4.5 h. Crude product **27** was purified by column chromatography with hexane/AcOEt 1:1 solvent system to yield **27** (170 mg, 55%) as a white foam; $[\alpha]_D^{20}$ +26.0 ($CDCl_3$, c 0.5); ^1H NMR ($CDCl_3$)α,α-anomer : δ 1.37, 1.58 (2s, 6H, $C(CH_3)_2$), 1.58–1.76 (m, 2H, H-2″ax, H-2‴ax), 2.15, 2.22 (2dd, 2H, $J \approx 0$, 5.1, 13.2 Hz, H-2′eq, H-2‴eq), 3.40–3.92 (m, 12 H, H-5′a,b, H-3″, H-4″, H-5″, H-6″a,b, H-3‴, H-4‴, H-5‴, H-6‴a,b), 4.33-4.64 (m, 10H, H-4′, PhCH_2), 4.73 (dd, 1H, J 3.0, 6.2 Hz, H-3′), 4.77 (dd, 1H, J 2.2, 6.2 Hz, H-2′), 4.82–4.88 (m, 1H, PhCH_2), 4.94 (bd, 1H, J 2.5 Hz, H-1″), 5.47 (bd, 1H, J 2.4 Hz, H-1‴), 5.65 (dd, 1H, J 2.2, 8.1 Hz, H-5), 5.78 (d, 1H, J 2.2 Hz, H-1′), 7.13–7.36 (m, 25H, 5 *Ph*CH_2), 7.38 (d, 1H, J 8.1 Hz, H-6), 8.93 (bd, 1H, J 1.9 Hz); ^{13}C NMR ($CDCl_3$)α,α-anomer: δ 25.47, 27.26 (C(*C*H_3)$_2$), 34.40, 35.57 (C-2″, C-2‴), 67.22, 68.66, 69.61, 71.05, 71.15, 71.67, 71.80, 73.29, 73.46, 74.88, 75.64, 77.19, 77.33, 78.07 (C-5′, C-3″, C-4″, C-5″, C-6″, C-3‴, C-4‴, C-5‴, C-6‴, 5Ph*C*H_2), 81.03 (C-3′), 85.14 (C-2′), 85.54 (C-4′), 93.59 (C-1′), 97.32 (C-1″), 99.10 (C-1‴), 101.96 (C-5), 114.26 (*C*$(CH_3)_2$), 127.42-128.47 (*Ph*CH_2), 137.92, 138.11, 138.27, 138.56, 138.73 (C_q, $PhCH_2$), 140.88 (C-6), 149.88 (C-2), 162.89 (C-4); ESI-MS: Calcd for $C_{59}H_{66}N_2O_{14}Na$ ($[M+Na]^+$): m/z 1049.4. Found: m/z 1049.7.

2.13. 2-Deoxy-α-D-galactopyranosyl-(1 → 6)-2- deoxy-α-D-glucopyranosyl-(1 → 5)-2,3-O-isopropylideneuridine (**1**)

Procedure C. To a solution of **24** (115 mg, 0.10 mmol) in MeOH (5 mL) 25% aqueous NH_4OH (0.8 mL) was added and the mixture was stirred for 2.5 h at room temperature. The solution was concentrated under reduced pressure and the residue was dissolved in a 1:10:2 mixture of cyclohexene/EtOH/THF (6 mL). The resulting solution was heated under reflux in the presence of $Pd(OH)_2/C$ (75 mg) for 1 h. After removal of the catalyst by filtration, crude product was purified by column chromatography with $CHCl_3$/MeOH 10:1 → 5:1 solvent system to yield **1** (32 mg, 55%) as a white foam; $[\alpha]_D^{20}$ +67.3 (MeOH, c 0.5); ^{1}H NMR (CD_3OD): δ 1.36, 1.55 (2s, 6H, $C(CH_3)_2$), 1.62 (ddd, 1H, J 3.6, 11.7, 13.2 Hz, H-2″ax), 1.79 (ddd, 1H, $J \approx 0$, 5.1, 12.7 Hz, H-2‴eq), 1.95 (ddd, 1H, J 3.4, 12.0, 12.7 Hz, H-2‴ax), 2.01 (ddd, 1H, $J \approx 0$, 5.1, 13.2 Hz, H-2″eq), 3.28 (t, 1H, J 9.3 Hz, H-4″), 3.59–3.90 (m, 9H, H-5′a,b, H-5″, H-6″a,b, H-3″, H-5‴, H-6‴a,b), 3.64 (bd, 1H, J 2.9 Hz, H-4‴), 3.96 (ddd, 1H, J 2.9, 5.1, 12.0 Hz, H-3″), 4.40 (m, 1H, H-4′), 4.82 (dd, 1H, J 3.0, 6.2 Hz, H-3′), 4.91 (dd, 1H, J 2.2, 6.2 Hz, H-2′) 4.92 (bd, 1H, J 3.6 Hz, H-1″), 4.95 (bd, 1H, J 3.4 Hz, H-1‴), 5.72 (d, 1H, J 8.2 Hz, H-5), 5.84 (d, 1H, J 2.2 Hz, H-1′), 7.76 (d, 1H, J 8.2 Hz, H-5), 7.90 (s, 1H, NH); ^{13}C NMR (CD_3OD): δ 25.58, 27.57 ($C(CH_3)_2$), 33.62 (C-2‴), 38.70 (C-2″), 63.16, 66.96, 68.37 (C-5′, C-6″, C-6‴), 66.74 (C-3‴), 69.62, 69.99, 72.31 (C-3″, C-5″, C-5‴), 73.01, 73.09 (C-4″, C-4‴), 82.68 (C-3′), 86.50 (C-2′), 87.17 (C-4′), 94.85 (C-1′), 98.77 (C-1‴), 98.92 (C-1″), 102.38 (C-5), 115.06 ($C(CH_3)_2$), 143.48 (C-6), 152.00 (C-2), 166.27 (C-4); ESI-HRMS: Calcd for $C_{24}H_{36}N_2O_{14}Na$ ($[M+Na]^+$): m/z 599.2059. Found: m/z 599.2073.

2.14. 2-Deoxy-α-D-glucopyranosyl-(1 → 6)-2-deoxy-α-D-glucopyranosyl-(1 → 5)-2,3-O-isopropylideneuridine (**2**)

Deprotection of adduct **25** (115 mg, 0.10 mmol) according to the procedure C described above for the preparation of **1** yielded **2** (45 mg, 78%) as a white foam; $[\alpha]_D^{20}$ +50.4 (MeOH, c 0.5); ^{1}H NMR (CD_3OD): δ 1.36, 1.55 (2s, 6H, $C(CH_3)_2$), 1.60, 1.65 (2ddd, 2H, H-2″ax, H-2‴ax), 2.01, 2.09 (2ddd, 2H, H-2″eq, H-2‴eq), 3.23–3.31 (2t, 2H, H-4″, H-4‴), 3.59–3.90 (m, 10 H, H-5′a,b, H-3″, H-5″, H-6″a,b, H-3‴, H-5‴, H-6‴a,b), 4.40 (m, 1H, H-4′), 4.82 (dd, 1H, J 3.1, 6.1 Hz, H-3′), 4.88–4.94 (m, 3H, H-2′, H-1″, H-1‴), 5.72 (d, 1H, J 8.0 Hz, H-5), 5.84 (d, 1H, J 2.4 Hz, H-1′)), 7.75 (d, 1H, J 8.0 Hz, H-5), 7.90 (s, 1H, NH); ^{13}C NMR (CD_3OD): δ 25.60, 27.58 ($C(CH_3)_2$), 38.65, 38.79 (C-2″, C-2‴), 62.78 (C-6‴), 66.92 (C-6″), 68.37 (C-5′), 69.98, 70.04 (C-3″, C-3‴), 72.99, 73.08, 73.26, 73.82 (C-4″, C-5″, C-4‴, C-5‴), 82.63 (C-3′), 86.46 (C-2′), 87.12 (C-4′), 94.69 (C-1′), 98.50, 98.91 (C-1″, C-1‴), 102.44 (C-5), 115.07 ($C(CH_3)_2$), 143.40 (C-6), 152.01 (C-2), 166.23 (C-4); ESI-HRMS: Calcd for $C_{24}H_{36}N_2O_{14}Na$ ($[M+Na]^+$): m/z 599.2059. Found: m/z 599.2065.

2.15. 2-Deoxy-α-D-glucopyranosyl-(1 → 3)-2-deoxy-α-D-glucopyranosyl-(1 → 5)-2,3-O-isopropylideneuridine (**3**)

Deprotection of adduct **26** (115 mg, 0.10 mmol) according to the procedure C described above for the preparation of **1** yielded **3** (32 mg, 55%) as a white foam; $[\alpha]_D^{20}$ +122.3 (MeOH, c 0.5); ^{1}H NMR (CD_3OD): δ 1.36, 1.54 (2s, 6H, $C(CH_3)_2$), 1.58 (ddd, 1H, J

2.9, 11.7, 12.4 Hz, H-2‴ax), 1.65 (ddd, 1H, *J* 3.3, 11.7, 12.4 Hz, H-2″ ax), 2.17 (ddd, 1H, *J* 0.9, 5.1, 12.4 Hz, H-2‴eq), 2.21 (ddd, 1H, *J* 0.9, 5.1, 12.4 Hz, H-2″ eq), 3.27 (dd, 1H, *J* 9.0, 9.8 Hz, H-4″), 3.36 (dd, 1H, *J* 9.0, 9.9 Hz, H-4‴), 3.51 (ddd, *J* 2.2, 5.6, 9.9 Hz, H-5‴), 3.55 (ddd, *J* 2.4, 5.0, 9.8 Hz, H-5″) 3.69 (dd, *J* 3.0, 11.2 Hz, H-5′a), 3.56–3.85 (m, 6H, H-3″, H-6″a,b, H-3‴, H-6‴a,b), 3.88 (dd, *J* 3.9, 11.2 Hz, H-5′b), 4.41 (ddd, 1H, *J* 2.9, 3.0, 3.9 Hz, H-4′), 4.85 (dd, 1H, *J* 2.9, 6.2 Hz, H-3′), 4.92 (dd, 1H, *J* 2.5, 6.2 Hz, H-2′), 4.89 (bd, 1H, *J* 3.3 Hz, H-1″), 5.16 (bd, 1H, *J* 2.9 Hz, H-1‴), 5.83 (d, 1H, *J* 8.0 Hz, H-5), 5.85 (d, 1H, *J* 2.5 Hz, H-1′), 7.75 (d, 1H, *J* 8.0 Hz, H-5), 7.90 (s, 1H, NH); ^{13}C NMR (CD_3OD): δ 25.61, 27.60 ($C(CH_3)_2$), 37.75 (C-2″), 39.06 (C-2‴), 62.71, 62.72 (C-6″, C-6‴) 68.18 (C-5′), 69.85 (C-3‴), 71.85 (C-4‴), 73.22 (C-4″), 74.44, 74.61 (C-5″, C-5‴), 77.89 (C-3″), 82.51 (C-3′), 86.57 (C-2′), 87.09 (C-4′), 94.54 (C-1′), 98.86 (C-1″), 100.90 (C-1‴), 102.64 (C-5), 115.01 ($C(CH_3)_2$), 143.27 (C-6), 152.09 (C-2), 166.55 (C-4); ESI-HRMS: Calcd for $C_{24}H_{36}N_2O_{14}Na$ ($[M+Na]^+$): *m*/*z* 599.2059. Found: *m*/*z* 599.2055.

*2.16. 2-Deoxy-α-D-glucopyranosyl-(1 → 4)-2-deoxy-α-D-glucopyranosyl-(1 → 5)-2,3-O-isopropylideneuridine (**4**)*

A solution of **27** (105 mg, 0.10 mmol) in a 1:10:2 mixture of cyclohexene/EtOH/THF (6 mL) was heated under reflux in the presence of $Pd(OH)_2/C$ (75 mg) for 1 h. After removal of the catalyst by filtration, crude product was purified by column chromatography with $CHCl_3$/MeOH 10:1 → 5:1 solvent system to yield **4** (42 mg, 73%) as a white foam; $[\alpha]_D^{20}$ +151.5 (MeOH, c 0.5); ^{1}H NMR (CD_3OD): δ 1.36, 1.55 (2s, 6H, $C(CH_3)_2$), 1.57–1.71 (m, 2H, H-2″ax, H-2‴ax), 1.98 (ddd, 1H, $J \approx 0$, 5.1, 13.2 Hz, H-2″eq), 2.18 (ddd, 1H, $J \approx 0$, 4.6, 12.7 Hz, H-2‴eq), 3.18 (t, 1H, *J* 9.3 Hz, H-4‴), 3.49–3.98 (m, 11 H, H-5′a,b, H-3″, H-4″, H-5″, H-6″a,b, H-3‴, H-5‴, H-6‴a,b), 4.39 (m, 1H, H-4′), 4.82 (dd, 1H, *J* 2.9, 6.1 Hz, H-3′), 4.84–4.94 (m, 2H, H-2′, H-1″), 5.49 (bd, 1H, *J* 2.9 Hz, H-1‴), 5.72 (d, 1H, *J* 8.1 Hz, H-5), 5.83 (d, 1H, *J* 2.2 Hz, H-1′), 7.76 (d, 1H, *J* 8.2 Hz, H-5), 7.90 (s, 1H, NH); ^{13}C NMR (CD_3OD): δ 25.59, 27.57 ($C(CH_3)_2$), 39.01, 39.43 (C-2″, C-2‴), 62.48, 63.03, 68.29, 69.83, 70.66, 73.19, 73.49, 75.03, 77.58 (C-5′, C-3″, C-4″, C-5″, C-6″, C-3‴, C-4‴, C-5‴, C-6‴), 82.61 (C-3′), 86.50 (C-2′), 87.23 (C-4′), 94.83 (C-1′), 98.79 (C-1″), 99.71 (C-1′), 102.44 (C-5), 115.04 ($C(CH_3)_2$), 143.49 (C-6), 152.05 (C-2), 166.31 (C-4); ESI-HRMS: Calcd for $C_{24}H_{36}N_2O_{14}Na$ ($[M+Na]^+$): *m*/*z* 599.2059. Found: *m*/*z* 599.2084.

*2.17. 3,6-Di-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1 → 5)-2,3-O-isopropylideneuridine (**5**)*

Deprotection of adduct **19** (71 mg, 0.10 mmol) according to the procedure C described above for the preparation of **1** yielded **5** (36 mg, 84%) as a white foam, purification by column chromatography with $CHCl_3$/MeOH 10:1 → 5:1 solvent system; $[\alpha]_D^{20}$ +21.8 (MeOH, c 0.5); ^{1}H NMR (CD_3OD): δ 1.36, 1.55 (2s, 6H, $C(CH_3)_2$), 1.62 (ddd, 1H, *J* 3.4, 12.0, 13.2 Hz H-2″ax), 1.99 (ddd, 1H, *J* 1.0, 5.1, 13.2 Hz, H-2″eq), 3.23 (t, 1H, *J* 9.1 Hz, H-4″), 3.49 (ddd, 1H, *J* 2.2, 5.6, 9.7 Hz, H-5″), 3.62–3.72 (m, 2H, H-5′a, H-6″a), 3.76 (ddd, 1H, *J* 5.1, 9.1, 12.0 Hz, H-3″), 3.82 (dd, 1H, *J* 2.2, 11.7 Hz, H-6″b), 3.89 (dd, 1H, *J* 3.9, 11.0 Hz, H-5′b) 4.42 (m, 1H, H-4′), 4.83 (dd, 1H, *J* 2.9, 6.1 Hz, H-3′), 4.88 (dd, 1H, *J* 2.4, 6.1 Hz, H-2′), 4.93 (bd, 1H, *J* 3.4 Hz, H-1″), 5.71 (d, 1H, *J* 8.1 Hz, H-5), 5.85 (d, 1H, *J* 2.4 Hz, H-1′), 7.76 (d, 1H, *J* 8.1 Hz, H-5), 7.90 (s, 1H, NH); ^{13}C NMR (CD_3OD):

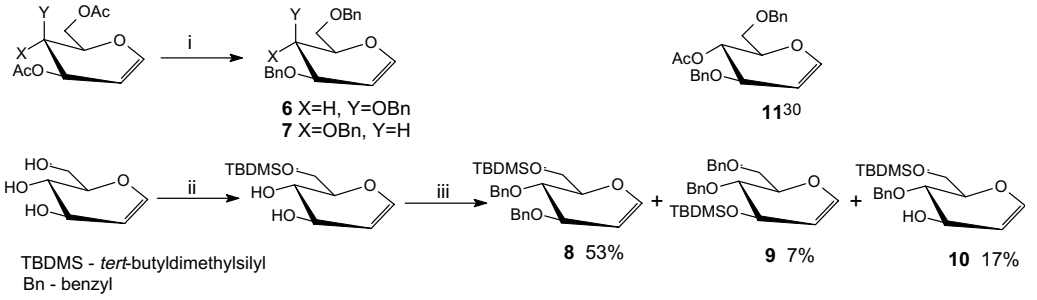
δ 25.57, 27.59 (C(CH_3)$_2$), 38.66 (C-2″), 62.79 (C-6″), 68.27 (C-5′), 69.85 (C-3″), 73.11 (C-4″), 74.57 (C-5″), 82.62 (C-3′), 86.57 (C-2′), 87.09 (C-4′), 94.59 (C-1′), 98.89 (C-1″), 102.39 (C-5), 115.00 (C(CH$_3$)$_2$), 143.22 (C-6), 152.06 (C-2), 166.25 (C-4); ESI-HRMS: Calcd for $C_{18}H_{26}N_2O_{10}Na$ ([M+Na]$^+$): m/z 453.1480. Found: m/z 599.2071.

3. Results and discussion

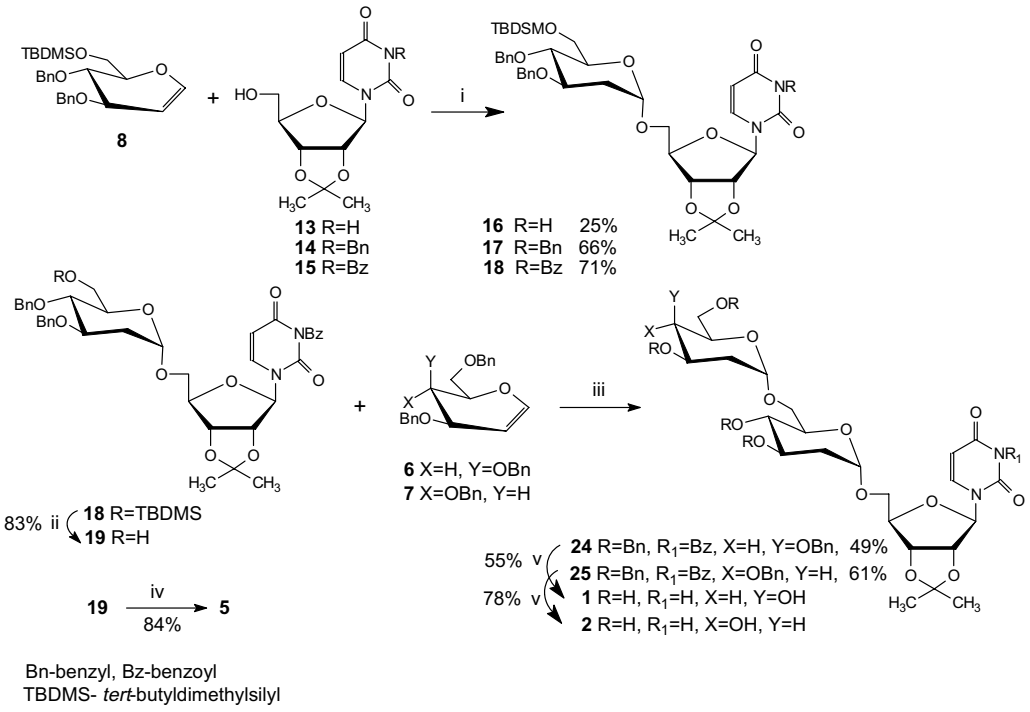
As a part of our current programme on the application of glycals in stereoselective synthesis of 2-deoxy-α-glycosides, we designed and synthesised their uridine derivatives as alternative substrates for GTs (Fig. 1). These compounds are composed of uridine and two 2-deoxy-hexopyranose units, one of which plays the role of mimetics of pyrophosphate linkage and the second acts as a glycosyl unit to be transferred. Stereoselective synthesis of the desired compounds can be accomplished in two successive addition reactions of uridine derivatives to the double bond of glycals.

One possible way of exploration of glycals as glycosylating agents is the direct addition of alcohols to the double bond of glycal. Successful direct regioselective addition can be accomplished in the presence of several catalysts, for instance, triphenylphosphine-hydrogen bromide (TPHB) [17], cation-exchange resin Dowex AG 50WX2 [23], BCl_3, BBr_3[24], CAN [25] or the $CeCl_3$–NaI reagent system [26]. Encouraged by the successful synthesis of α-1,6-linked di- and trisaccharides, derivatives of 2-deoxysugars from glycals [27,28] according to the Falck–Mioskowski procedure, we now report on the synthesis of adducts of glycals and uridine. Glycals are readily available building blocks, they compare well with fully oxygenated pyranose derivatives due to their relative ease in differentiation of hydroxyl group by selective protection. Benzyl protection of the hydroxyl group was chosen because of the mild conditions involved in its removal by catalytic hydrogenolysis. The synthesis of target compounds **1**–**4** required orthogonal protection at C-3, C-4 and C-6, respectively. *Tert*-butyldimethylsilyl ether (TBDMS) protecting groups provided orthogonal protection at C-6 and C-3 in glycals **8** and **9**, respectively, while acetyl ester (Ac) was used at C-4 in glucal **11** (Scheme 1).

We initially examined the addition of readily available 2,3-*O*-isopropylidene-uridine (**13**) to glucal **8** using the Falck–Mioskowski protocol [17] (Scheme 2). Reaction proceeded in the presence of a catalytic amount of TPHB (0.1 equiv) in CH_2Cl_2 at room temperature. We observed partial decomposition of glycal and low yield of desired product **16** due to

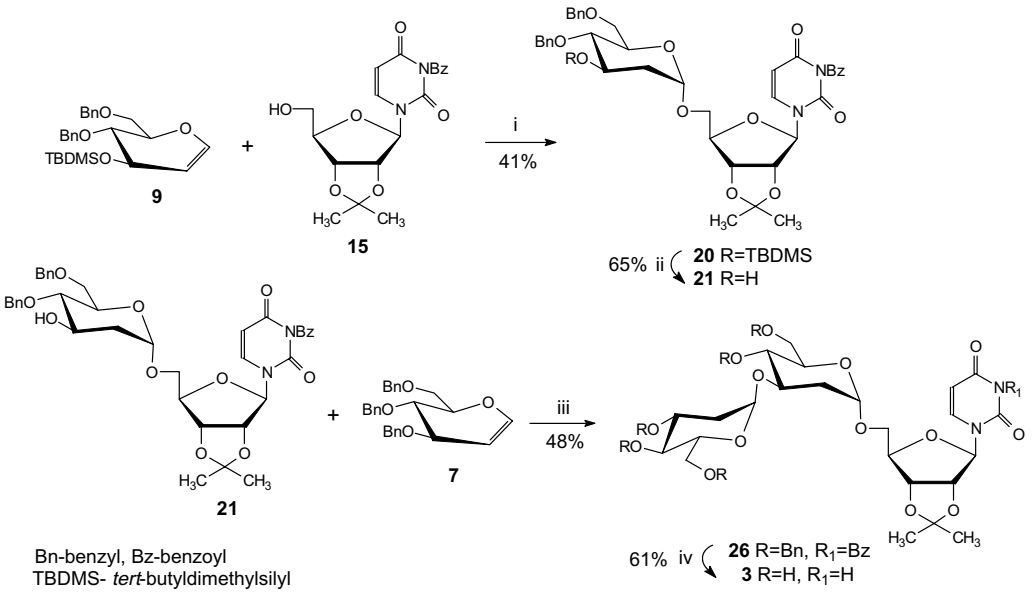


Scheme 1. (i) BnBr (3 equiv.), NaOH, DMSO, 0 °C → rt, 12 h; (ii) TBDMSCl (1.1 equiv.), imidazole (2.2 equiv.), DMF, rt, 2 h; (iii) BnBr (2 equiv.), NaH, DMF, −5 °C → rt, 4 h.

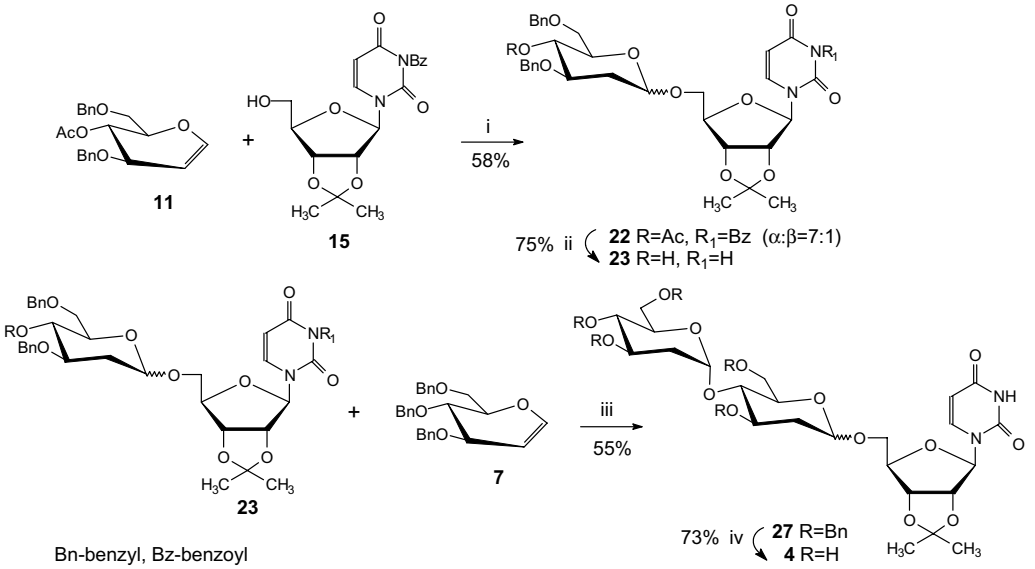


Scheme 2. (i) TPHB (0.1 equiv.), CH_2Cl_2, rt; (ii) AcCl (1.0 equiv.), MeOH, rt, 10 min.; (iii) **19** (1.0 equiv.), TPHB (0.1 equiv.), CH_2Cl_2, rt, 1 h; (iv) NH_4OH, MeOH, rt, 1 h, then $Pd(OH)_2$/C, cyclohexene, EtOH, reflux, 1 h; (v) NH_4OH, MeOH, rt, 2.5 h, then $Pd(OH)_2$/C, cyclohexene, EtOH/THF, reflux, 1 h.

limited solubility of uridine derivative **13**. Moreover, TBDMS group transfer from glucal **8** to C-5 hydroxyl group of uridine was observed. When acetonitrile or THF was used as solvents instead of CH_2Cl_2, the reaction yield increased but unfortunately a mixture of α and β isomers was formed. Therefore, in order to improve solubility of uridine in CH_2Cl_2, we employed protection of uracil nitrogen. At first we tried out benzyl protection of uracil nitrogen hoping that in the final step it would be cleaved simultaneously to benzyl protection of the hydroxyl groups in 2-deoxy-hexopyranose rings. Unfortunately, further experiments revealed that *N*-benzyl protection in uracil part was stable to catalytic transfer hydrogenolysis. Finally, benzoyl group as a protection of uracil nitrogen was studied. 3-*N*-benzoyl-2′,3′-isopropylidene-uridine (**15**) was readily soluble in CH_2Cl_2 and only trace amount of side products were formed as the result of transsilylation. In the course of our investigations, we have found that the presence of TBDMS as the hydroxyl group protection in glucals **8** and **9** beneficially led to total α-stereoselectivity. Adducts **18** and **20** were synthesised as pure α-anomers (Scheme 2, 3). In contrast, the addition of uridine derivative **15** to 4-*O*-acetyl-3,6-di-*O*-benzyl-D-glucal (**11**) led to adduct **22** which was contaminated with β-isomer (α:β = 7:1) (Scheme 4). The ratio of stereoisomers were determined by examination of the ^1H NMR spectra of isomer mixture. The equatorially oriented H-1α proton of the major α isomer gave the characteristic broad doublet at δ 4.91 ppm with $J_{1,2ax}$ 2.5 Hz, while the axially oriented H-1β proton was observed at δ 4.3 ppm as doublet of doublets with $J_{1,2eq}$ 2.2 Hz and $J_{1,2ax}$ 9.8 Hz.



Scheme 3. (i) TPHB (0.1 equiv.), CH_2Cl_2, rt, 1 h; (ii) AcCl (1.0 equiv.), MeOH, rt, 1.5 h; (iii) TPHB (0.1 equiv.), CH_2Cl_2, rt, 1 h; (iv) NH_4OH, MeOH, rt, 2.5 h, then $Pd(OH)_2/C$, cyclohexene, EtOH/THF, reflux, 1 h.



Scheme 4. (i) TPHB (0.1 equiv.), CH_2Cl_2, rt, 1 h; (ii) MeONa (1.0 equiv.), MeOH, rt, 4 days; (iii) TPHB (0.1 equiv.), CH_2Cl_2, rt, 1 h; (iv) $Pd(OH)_2/C$, cyclohexene, EtOH/THF, reflux, 1 h.

Target compounds **1**–**4** were synthesised by subsequent addition reactions to glycals **6** or **7** after partial deprotection of intermediate compounds **18**,**20** and **22**. Removal of the TBDMS protecting group in **18** and **20** by TBAF was unsuccessful, but deprotection could

be performed by treatment of AcCl in methanol [29]. This way, compounds **19** and **21**, which have a free hydroxyl at C-6 and C-3, respectively, were synthesised in good yields. Alternatively, saponification of the diacyl ester **22** using NaOMe in methanol gave compound **23**. Compounds obtained in this manner formed substrates for the second addition step to the double bond of galactal **6** or glucal **7** to form a new α-1,3; α-1,4 and α-1,6-glycosidic linkages. All adducts were isolated as pure α-anomers. Compounds **24**–**27** were easily deprotected by catalytic hydrogenolysis. Hydrogenolysis was performed by hydrogen transfer from cyclohexene [30] in the presence of Pearlman's catalyst (palladium(II) hydroxide) [31] in reflux of ethanol affording the unprotected target compounds **1**–**4**. All adducts were purified by column chromatography and their structures were elucidated by ^{1}H and ^{13}C NMR data (including DEPT; two-dimensional ^{1}H, ^{1}H COSY; ^{1}H, ^{13}C HETCOR experiments and simulation analysis) and mass spectrometry analysis (for details, see Section 2).

Synthesised adducts of uridine and glycals might serve as UDP-α-D-glucose and UDP-α-D-galactose analogues and might also be useful in the investigation of the effect of relative spatial arrangement of hydroxyl groups on the binding affinity of the corresponding **2-4** regioisomers. Biological evaluations are currently in progress, and the results will be presented soon.

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