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Supporting Information

A Glycan Array-Based Assay for the Identification and Characterization of Plant Glycosyltransferases

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Contents

Abbreviations

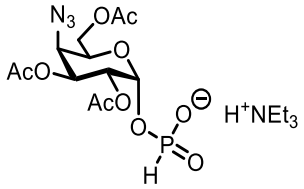
Ac: acetyl, COSY: correlation spectroscopy, *cyclo*Sal: *cyclo*-saligenyl, DCM: dichloromethane, DMF: dimethylformamide, ELSD: evaporative light scattering detector, ESI: electrospray ionization, FTIR: fourier transform infrared, hex: hexane, HPLC: high performance liquid chromatography, HRMS: high resolution mass spectrometry, HSQC: heteronuclear single quantum coherence spectroscopy, IR: infrared, LC: liquid chromatography, MS: mass spectrometry, NMR: nuclear magnetic resonance, QTOF: quadrupole time of flight, RP: reversed phase, rt: room temperature, *t*BuOOH: *tert*-butyl hydroperoxide, THF: tetrahydrofurane, TLC: thin layer chromatography, TOF: time of flight, Ts: *p*-toluenesulfonyl, UDP: uridine diphosphate, UMP: uridine monophosphate; UV: ultraviolet.

General Information

Advanced intermediates for the synthesis of sugar nucleotides 1,2,3,4-tetra-*O*-acetyl-4-azido-4-deoxy-D-galactopyranose[i], 2,3-di-*O*-acetyl-uridine[ii], 5-nitro-*cyclo*Sal phosphorochloridite[iii], 2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-α-D-galactopyranosyl phosphate[iv], and 2,3-di-*O*-acetyl-4-azido-4-deoxy-α-D-xylopyranoside[v] were synthesized as reported in literature. Solvents and reagents were used as supplied without any further purification. Anhydrous solvents were obtained from a dry solvent system (JC-Meyer Solvent Systems). Column chromatography was carried out using Fluka Kieselgel 60 (230-400 mesh). NMR spectra were recorded on a Varian 600 (600 MHz), a Bruker AVIII 400 (400MHz), or a Bruker AVIII 700 (700 MHz) spectrometer using solutions of the respective compound in $CDCl_3$, D_2O, or CD_3OD. NMR chemical shifts (δ) are reported in ppm and coupling constants (J) in Hz. Spectra recorded in $CDCl_3$ or CD_3OD used the solvent residual peak chemical shift as internal standard ($CDCl_3$: 7.26 ppm ^{1}H, 77.1 ppm ^{13}C; CD_3OD: 3.31 ppm ^{1}H, 49.0 ppm ^{13}C). The chemical shifts in ^{31}P NMR-spectra of compounds **1**, **2**, **3, 5**, and **6** were referenced based on the reported shifts for **1**[iv]. NMR signals of products and intermediates were assigned based on COSY and HSQC NMR experiments. Optical rotations were measured using a UniPol L1000 polarimeter (Schmidt&Haensch) with concentrations expressed as g/100 mL. IR spectra were recorded with a Spectrum 100 FTIR spectrophotometer (Perkin-Elmer). High resolution mass spectra were obtained using a Xevo J2-XGQTOF (Waters) mass spectrometer. Preparative HPLC was performed with an Agilent 1200 series using a semi-preparative Phenomenex Hydro RP18 column (250 x 10 mm). Ion exchange chromatography was performed on a Knauer Azura system using a GE HiTrap Q XL 5 mL column with a linear gradient of 100% aq. 0.05 M NH_4OAc (pH 6.8) buffer to 100% aq 0.5 M NH_4OAc (pH 6.8) buffer. Size exclusion chromatography was performed on a Knauer Azura system using a HiLoad Superdex 30 (16 x 600 mm) column and 5% ethanol in water as eluent.

Chemical Synthesis of Sugar Nucleotides

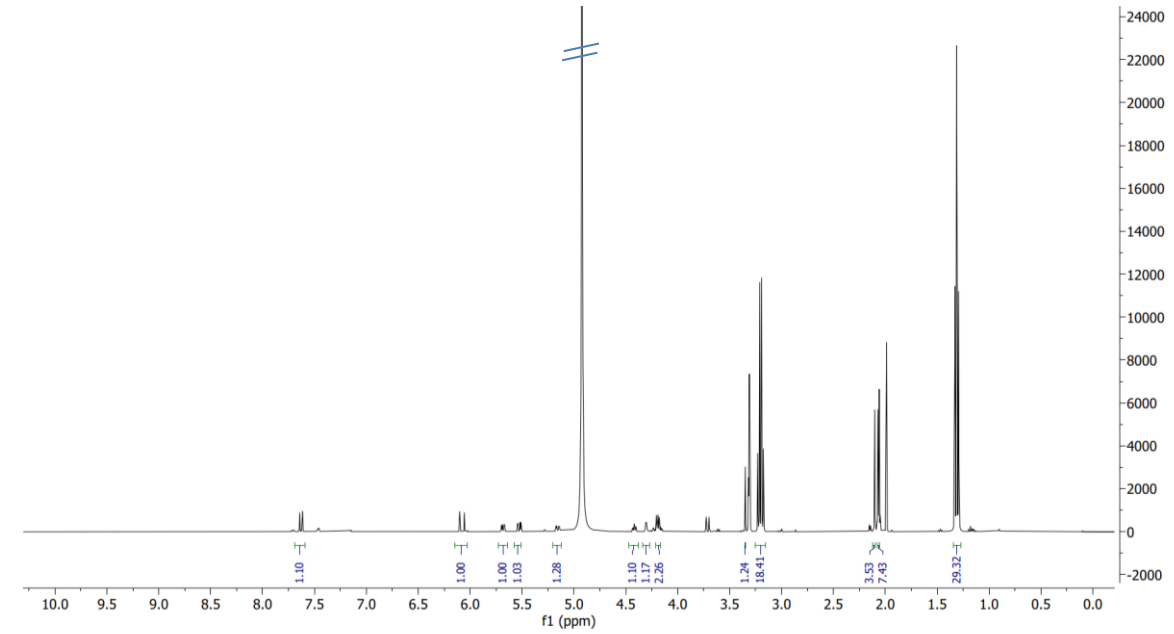
2,3,6-Tri-*O*-acetyl-4-azido-4-deoxy-α-D-galactopyranosyl phosphite (SI1)



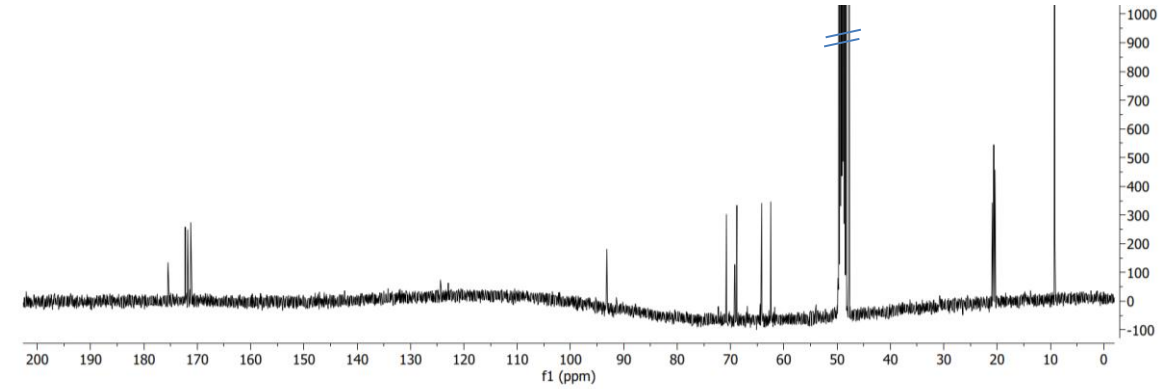
To a solution of 1,2,3,6-tetra-*O*-acetyl-4-azido-4-deoxy-α-D-galactopyranose[i] (150 mg, 0.402 mmol) in DMF (4 mL) was added hydrazine acetate (41.0 mg 0.446 mmol, 1.1 equiv) and the solution was stirred for 90 min at 50 °C. The reaction was subsequently diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give 2,3,6-tri-*O*-acetyl-4-azido-4-deoxy-α/β-D-galactopyranose (133 mg, 0.402 mmol) as a colorless oil. Without further purification, parts of the anomeric mixture (120 mg, 0.362 mmol, 1 equiv) were dissolved in anhydrous dioxane (5 mL) containing NEt_3 (120 µL, 0.861 mmol, 2.4 equiv) under argon atmosphere, and 2-chloro-4*H*-benzodioxaphosphorin-4-one (89 mg, 0.435 mmol, 1.2 equiv) was added to the mixture. The reaction mixture was stirred at rt for 30 min before water (0.200 mL) was added, and the reaction was stirred for another 5 min. After concentration of the reaction mixture *in vacuo,* the residue was dissolved in THF (2 mL). Upon solvation in THF a colorless precipitate formed within 2 min. The precipitate was filtered off and the filtrate was concentrated *in vacuo*. Purification of the residue by silica gel column chromatography (DCM/MeOH = 20:1 to 9:1 to DCM/MeOH/AcOH = 9:1:0.1) yielded 131 mg (0.265 mmol, 66%) of **SI1** as an anomeric mixture of the monotriethylamine salt. The anomeric mixture was dissolved in anhydrous MeCN (4 mL) and phosphorous acid (162 mg, 1.98 mmol, 6 equiv) was added. The mixture was stirred under argon atmosphere at rt overnight. The reaction mixture was neutralized by the addition of NEt_3 (275 µL, 1.98 mmol) and the solvent was removed *in vacuo*. The product was filtered through a short plug of silica (DCM/MeOH = 9:1), yielding 4-azido-α-galactosyl phosphite **SI1** (91.0 mg, 0.184 mmol, 64%) as slightly brown sticky oil.

$[\alpha]_D^{25}$ = +42.9 (*c* 0.5, $CHCl_3$) ^{1}H NMR (400 MHz, CD_3OD) δ = 5.68 (dd, *J* = 8.6, 3.6 Hz, 1H, H-1), 5.53 (dd, *J* = 10.7, 3.7 Hz, 1H, H-3), 5.16 (ddd, *J* = 10.7, 3.6, 1.6 Hz, 1H, H-2), 4.42 (td, *J* = 6.3, 1.7 Hz, 1H, H-5), 4.30 (dd, *J* = 3.7, 1.7 Hz, 1H, H-4), 4.19 (m, 2H, H-6), 2.10 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.06 (s, 3H, OAc) ppm. ^{13}C NMR (101 MHz, CD_3OD) δ = 172.2 (C=O), 171.8 (C=O), 171.2 (C=O), 93.2 (d, $J_{C\text{-}P}$ = 4.7 Hz, C-1), 70.7 (C-3), 69.2 (d, $J_{C\text{-}P}$ = 5.8 Hz, C-2), 68.8 (C-5), 64.1 (C-6), 62.4 (C-4) 20.6 (OAc), 20.6 (OAc), 20.4 (OAc) ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd for $C_{12}H_{18}N_3NaO_{10}P$: 418.0622, found: 418.0620. IR (neat) ν_{max}: 2115, 1744, 1214, 918 cm^{-1}.

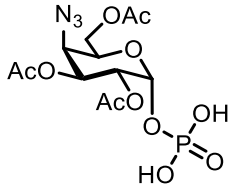
^{1}H NMR (400 MHz, CD_3OD):



^{13}C NMR (101 MHz, CD_3OD):



2,3,6-Tri-*O*-acetyl-4-azido-4-deoxy-α-D-galactopyranosyl phosphate (SI2)

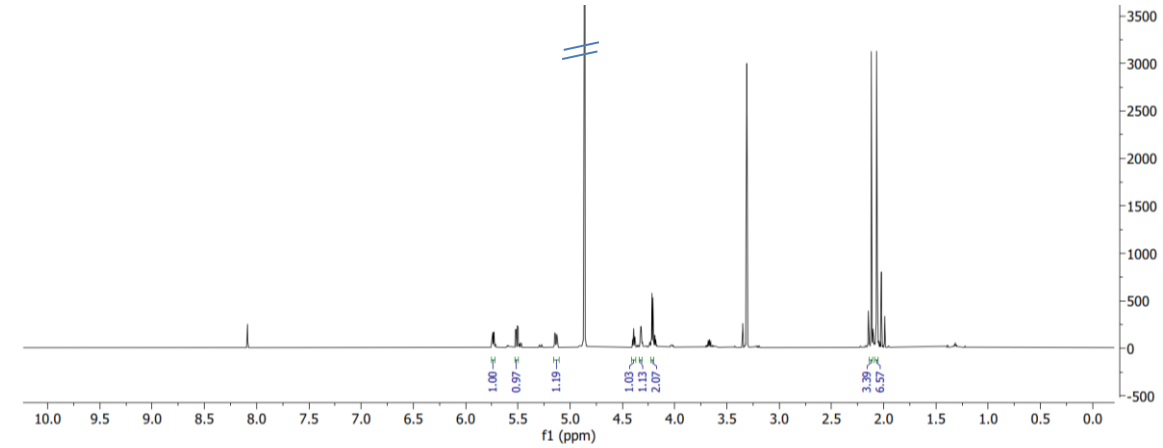


The mono-triethylammonium salt of phosphite **SI1** (46 mg, 0.0929 mmol) was dissolved in THF (5 mL) and passed through a 5 mL syringe filled with H^+-ion exchange resin (prewashed Amberlite IR-120). The column was flushed with additional THF (10 mL) and the eluent was concentrated *in vacuo* to approx. 5 mL. *t*BuOOH (22.3 µL, 5 M in decane, 0.111 mmol, 1.1 equiv) and a spatula tip of iodine were added. The resulting brown solution was stirred under Ar atmosphere at rt overnight. The reaction was neutralized by the addition of NEt_3 (28.0 µL 0.202 mmol) and concentrated *in vacuo* yielding **SI2**. The crude product was dissolved in water and

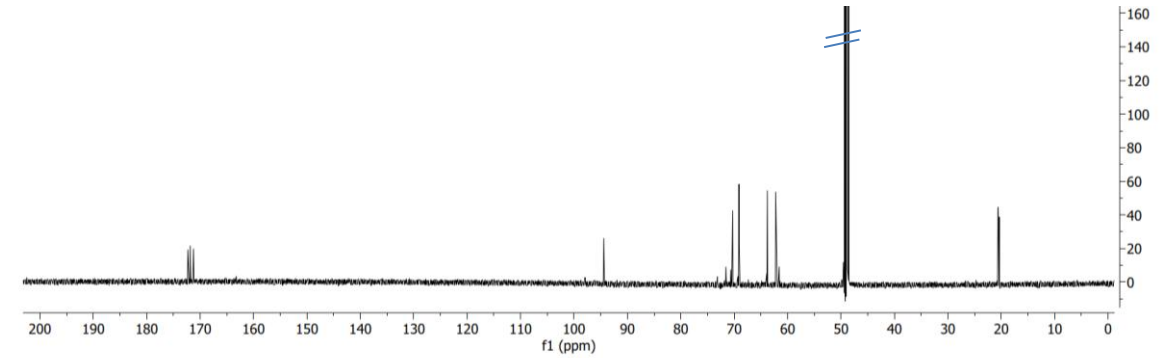
purified by HPLC using a semi-preparative Synergy C18 column. The product was eluted from the column with 31% MeCN in H_2O containing 0.1% formic acid in 33% yield (12.0 mg, 0.0292 mmol).

$[\alpha]_D^{25}$ = +64.9 (*c* 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, CD_3OD) δ = 5.73 (dd, *J* = 7.2, 3.6 Hz, 1H, H-1), 5.52 (dd, *J* = 10.7, 3.7 Hz, 1H, H-3), 5.13 (ddd, *J* = 10.7, 3.6, 2.6 Hz, 1H, H-2), 4.40 (m, 1H, H-5), 4.33 (dd, *J* = 3.7, 1.5 Hz, 1H, H-4), 4.21 (d, *J* = 6.1 Hz, 2H, H-6), 2.12 (s, 3H, OAc), 2.07, 2.05 (2 s, 6H, OAc). ^{13}C NMR (101 MHz, CD_3OD) δ = 172.3 (C=O), 171.9 (C=O), 171.2 (C=O), 94.3 (d, J_{C-P} = 4.9 Hz, C-1), 70.3 (C-3), 69.1 (d, J_{C-P} = 7.5 Hz, C-2), 69.0 (C-5), 63.8 (C-6), 62.2 (C-4), 20.6 (OAc), 20.6 (OAc), 20.4 (OAc). ESI-HRMS: m/z $[M+Na]^+$ calcd for $C_{12}H_{18}N_3NaO_{11}P$: 434.0571, found: 434.0579. IR (neat) ν_{max}: 2115, 1743, 1218, 943 cm^{-1}.

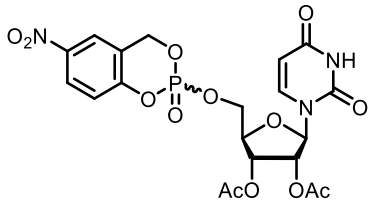
^{1}H NMR (400 MHz, CD_3OD):



^{13}C NMR (101 MHz, CD_3OD):



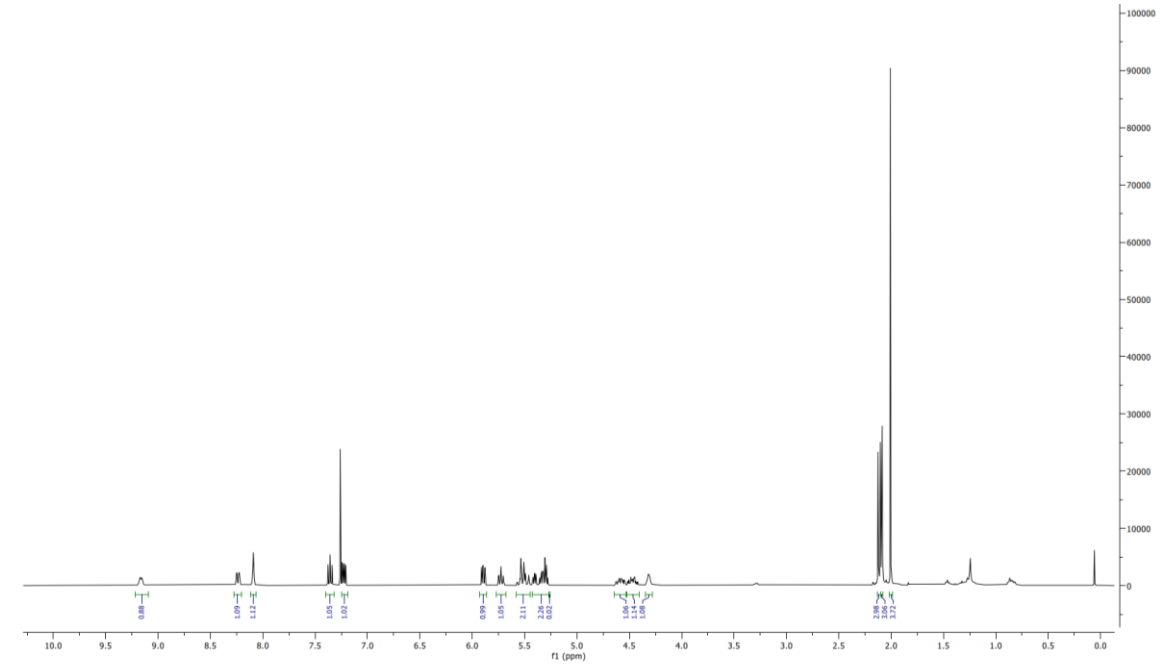
5-Nitro-*cyclo*Sal-2',3'-O-diacetyluridine monophosphate (SI3)[vi]



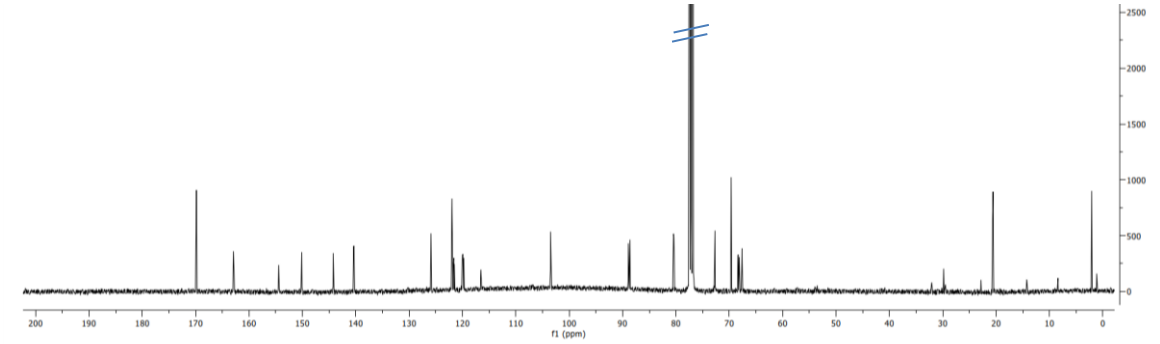
To a solution of 2,3-di-*O*-acetyl-uridine[ii] (100 mg, 0.305 mmol, 1 equiv) in MeCN (2 mL) was added NEt_3 (118 μL, 0.854 mmol, 2.8 equiv) at 0 °C. Subsequently, 5-nitro-*cyclo*Sal-chloro-phosphite[iii] (0.610 mmol. 2 equiv) dissolved in anhydrous MeCN (5 mL) was added. The solution turned bright yellow immediately upon addition of the phosphite. The solution was allowed to warm to rt and stirred until full consumption of the uracil starting material was detected by TLC. To the stirred solution at rt was added 0.464 g (3.05 mmol, 10 equiv) oxone dissolved in water (5 mL). After stirring for 30 min at rt, DCM was added and the organic phase washed with water three times. The organic phase was separated, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was subjected to silica gel column chromatography (hex/EtOAc/AcOH = 1:4:0.1), yielding a sticky oil that was dissolved in anhydrous MeCN and freeze-dried to give a diastereomeric mixture of **SI3** (65.0 mg, 0.120 mmol, 24%) as a colorless solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.16 (d, J = 7.4 Hz, 1H, Ar), 8.27 – 8.19 (m, 1H, Ar), 8.09 (td, J = 2.7, 1.3 Hz, 1H, Ar), 7.36 (dd, J = 8.2, 7.4 Hz, 1H, Ar), 7.23 (dd, J = 9.0, 4.8 Hz, 1H, Ar), 5.90 (dd, J = 7.8, 5.3 Hz, 1H), 5.58 – 5.45 (m, 2H), 5.43 – 5.27 (m, 2H), 4.64 – 4.54 (m, 1H), 4.52 – 4.41 (m, 1H), 4.35 – 4.28 (m, 1H), 2.12 (s, Hz, 1.5H), 2.10 (s, 1.5 H), 2.09 (s, 1.5 H) 2.09 (s, 1.5 H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 169.9, 169.8, 162.94, 162.90, 154.5, 154.43, 154.42, 154.3, 150.1, 150.0, 144.22, 144.19, 140.4, 140.3, 125.93, 125.91, 125.89, 122.0, 121.7, 121.65, 121.64, 121.5, 120.0, 119.9, 119.8, 119.7, 103.5, 103.4, 88.9, 88.6, 80.5, 80.4, 77.5, 77.2, 76.8, 72.8, 72.7, 69.7, 68.4, 68.3, 68.1, 68.0, 67.7, 67.6, 20.7, 20.6, 20.56, 20.53 ppm. ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{20}H_{20}N_3NaO_{13}P$: 564.0626, found: 564.0624

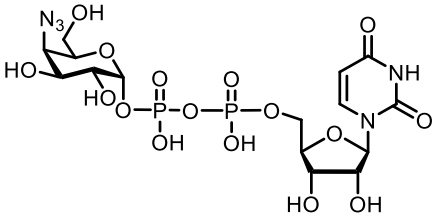
^{1}H NMR (400 MHz, $CDCl_3$):



^{13}C NMR (101 MHz, $CDCl_3$):



Uridine 5'-(4-azido-4-deoxy-α-D-galactopyranosyl) diphosphate (2)

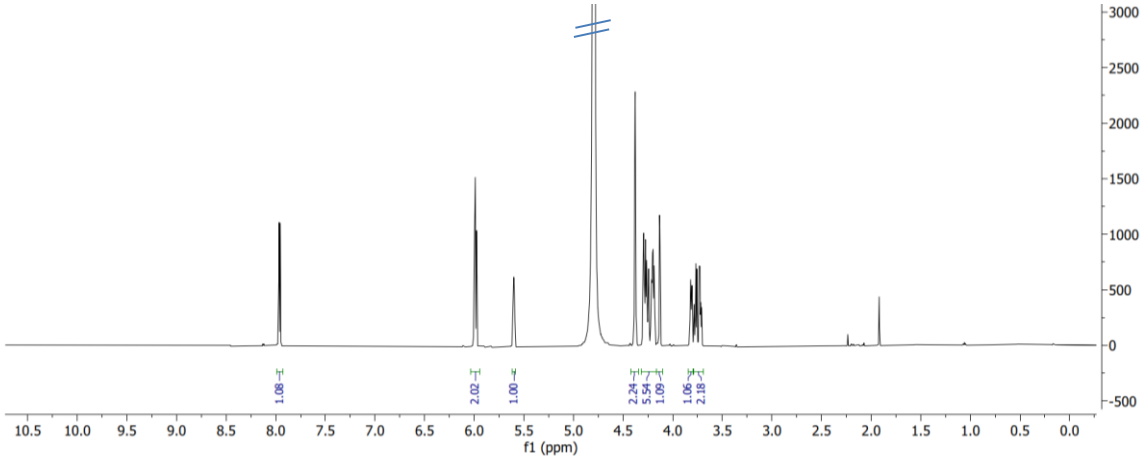


SI2 (10.0 mg, 0.0243 mmol, 1 equiv) was dissolved in anhydrous DMF (2 mL) and three spatula tips of activated powdered molecular sieves (4 Å) were added. After storage over activated molecular sieves for 1 h, 5-nitro-*cyclo*Sal-2',3'-*O*-diacetyluridine monophosphate (**SI3**)[vi] (17.1 mg, 0.0316 mmol, 1.3 equiv) was added and the reaction mixture was stirred at rt overnight. The reaction mixture was filtered through a short bed of celite and the solvent was removed *in vacuo*. The residue was dissolved in water and washed with EtOAc. The aqueous phase was concentrated *in vacuo*, yielding the crude intermediate, which was dissolved in

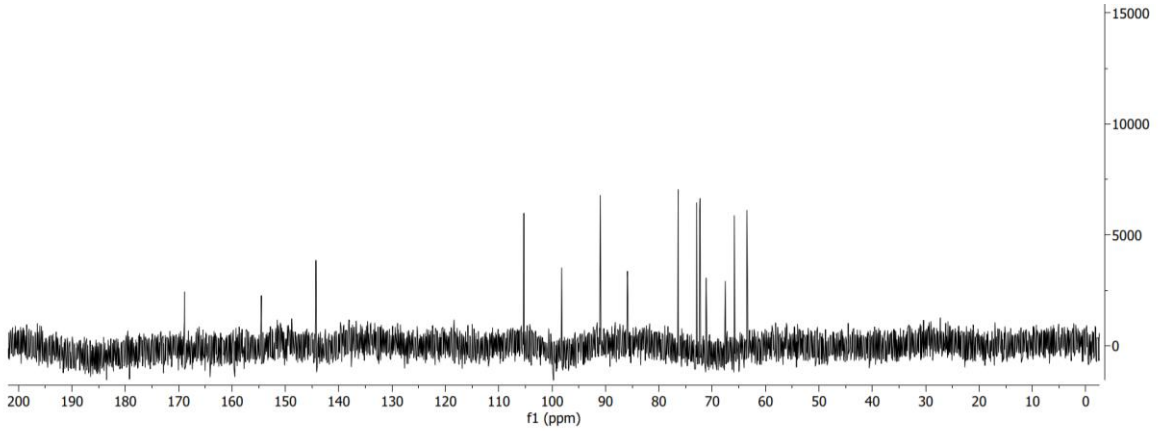
MeOH/H_2O/NEt_3 (7:3:1, 5.50 mL) and stirred at rt overnight. The solvents were removed *in vacuo* and the residue was taken up in 1.80 mL water. The resulting solution was subjected to ion exchange chromatography in two portions of 0.9 mL each. Fractions that still contained impurities after ion exchange chromatography were subsequently passed through a disposable Carbograph C18 column, from which the product eluted with 10% MeCN in water. In total, 2.10 mg (0.00355 mmol, 15%) of **2** were obtained.

^{1}H NMR (700 MHz, D_2O) δ 7.96 (d, *J* = 8.1 Hz, 1H, uracil), 6.02 – 5.96 (m, 2H, uracil, H-1 Rib), 5.60 (dd, *J* = 6.8, 4.0 Hz, 1H, H-1 Gal), 4.41 – 4.36 (m, 2H, H-2 Rib, H-3 Rib), 4.30 – 4.23 (m, 3H, H-4 Rib, H-5 Gal, H-5a Rib), 4.23 – 4.17 (m, 2H, H-3 Gal, H-5b Rib), 4.13 (d, *J* = 2.3 Hz, 1H, H-4 Gal), 3.81 (d, *J* = 10.4 Hz, 1H, H-2 Gal), 3.77 (dd, *J* = 11.7, 6.9 Hz, 1H, H-6a Gal), 3.72 (dd, *J* = 11.7, 5.5 Hz, 1H, H-6b Gal). ^{13}C NMR (176 MHz, D_2O) δ = 168.9 (C=O), 154.5 (C=O), 144.3 (uracil), 105.3 (uracil), 98.2 (d, J_{C-P} = 6.3 Hz, C-1 Gal), 91.0 (C-1 Rib), 85.9 (d, J_{C-P} = 9.1 Hz, C-4 Rib), 76.4 (C-3 Rib), 72.9 (C-5 Gal), 72.4 (C-3 Gal), 72.3 (C-2 Rib), 71.1 (d, J_{C-P} = 8.7 Hz, C-2 Gal), 67.6 (d, J_{C-P} = 5.5 Hz, C-5 Rib), 65.9 (C-4 Gal), 63.5 (C-6 Gal) ppm. ^{31}P NMR (243 MHz, D_2O) δ = -11.3 (d, *J* = 20.7 Hz), -13.0 (d, *J* = 20.7 Hz) ppm. ESI-HRMS: m/z $[M-H]^-$ calcd for $C_{15}H_{23}N_5O_{16}P_2$: 590.0542, found: 590.0552.

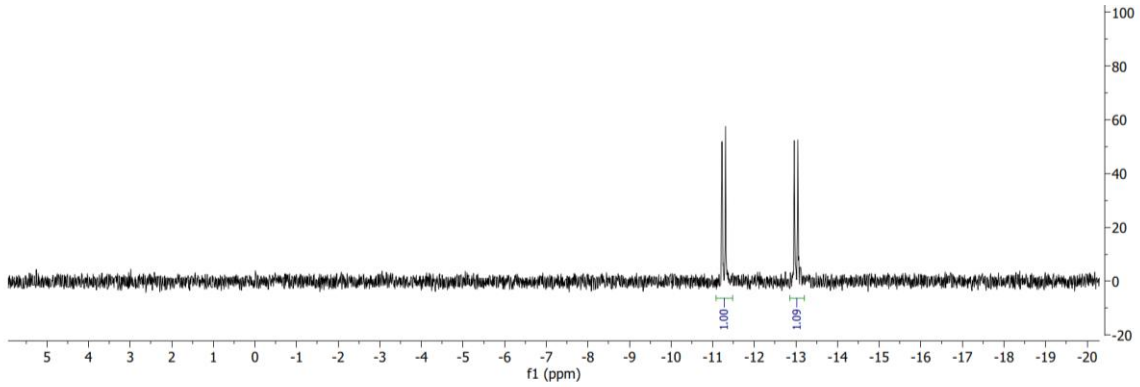
^{1}H NMR (700 MHz, D_2O):



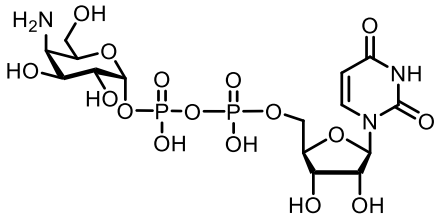
^{13}C NMR (176 MHz, D_2O):



^{31}P NMR (243 MHz, D_2O):



Uridine 5'-(4-amino-4-deoxy-α-D-galactopyranosyl) diphosphate (6)

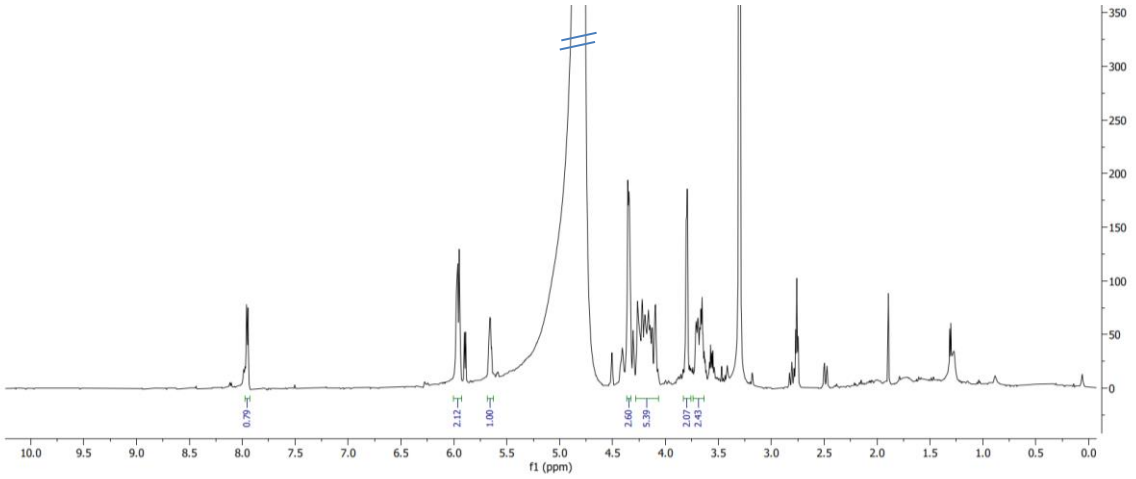


Azide-functionalized UDP-galactose **2** (0.4 mg, 0.64 µmol) was dissolved in 1.5 mL H_2O and Pd/C (10 wt% on activated carbon, 2.0 mg) was added. The solution was stirred under H_2 atmosphere for 30 min at rt before the catalyst was filtered off through a syringe filter. The solvent was removed using a lyophilizer, yielding 0.4 mg (0.64 µmol) of **6** contaminated with moderate amounts of a side product resulting from hydrogenation of the uracil moiety.

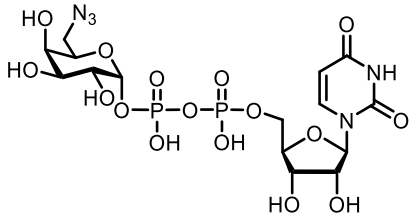
^{1}H NMR (600 MHz, D_2O) δ = 7.95 (d, *J* = 8.0 Hz, 1H, uracil), 5.99 – 5.93 (m, 2H, uracil, H-1 Rib), 5.68 – 5.64 (m, 1H, H-1 Gal), 4.35 (m, 2H), 4.28 – 4.06 (m, 5H), 3.80 (d, *J* = 5.2 Hz, 2H), 3.74 – 3.62 (m, 2H, H-6 Gal) ppm. Insufficient amounts for recording a ^{13}C-NMR spectrum of

material have been obtained. ESI-HRMS: m/z [M-H]$^-$ calcd for $C_{15}H_{24}N_3O_{16}P_2$: 564.0637, found: 564.0640.

^{1}H NMR (600 MHz, D_2O):



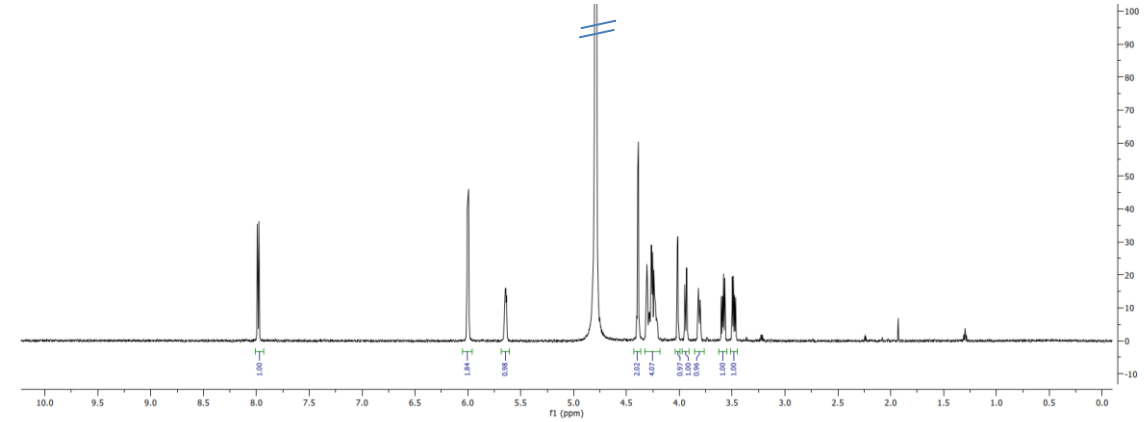
Uridine 5'-(6-azido-6-deoxy-α-D-galactopyranosyl) diphosphate (1)



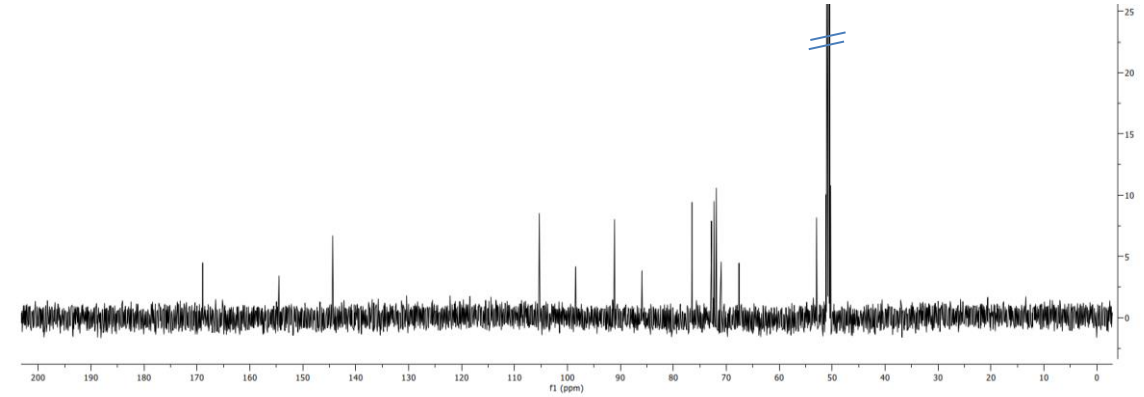
2,3,4-Tri-*O*-acetyl-6-azido-6-deoxy-α-D-galactopyranosyl phosphate[iv] (200 mg, 0.0486 mmol, 1.0 equiv) was dissolved under argon atmosphere in anhydrous DMF (3 mL) and 3 spatula tips of activated powdered molecular sieves (4 Å) were added. After 1 h storage over the activated molecular sieves, 5-nitro-*cyclo*Sal-2',3'-*O*-diacetyluridine monophosphate (**SI3**)[vi] (34.0 mg, 0.0628 mmol, 1.3 equiv) was added and the reaction mixture was stirred at rt overnight. The reaction mixture was subsequently filtered through a short bed of celite and the solvent was removed *in vacuo*. The residue was dissolved in water and washed with EtOAc. The aqueous phase was concentrated *in vacuo* yielding a crude intermediate (41.0 mg). The crude intermediate was dissolved in MeOH/H_2O/NEt_3 (7:3:1; 5.5 mL) and stirred at rt overnight. The solvent was removed *in vacuo*. The crude product was taken up in water (4.50 mL) and subjected to ion exchange chromatography in five portions of 900 µL each. Fractions that still contained impurities after ion exchange chromatography were subsequently passed through a Carbograph C18 column, from which the product eluted with 10% MeCN in water to obtain the azide-functionalized UDP-galactose derivative **1** in 25% yield (6.7 mg, 0.0114 mmol).

^{1}H NMR (600 MHz, D_2O) δ = 7.98 (d, *J* = 8.1 Hz, 1H), 6.05 – 5.96 (m, 2H), 5.64 (dd, *J* = 7.1, 3.6 Hz, 1H), 4.40 – 4.38 (m, 2H), 4.33 – 4.19 (m, 4H), 4.02 (d, *J* = 3.3 Hz, 1H), 3.94 (dd, *J* = 10.2, 3.3 Hz, 1H), 3.81 (dt, *J* = 10.2 Hz, 1H), 3.59 (dd, *J* = 12.7, 7.2 Hz, 1H), 3.48 (dd, *J* = 12.7, 6.0 Hz, 1H) ppm. ^{13}C NMR (151 MHz, D_2O) δ = 168.9, 154.5, 144.3, 105.3, 98.5 (d, J_{C-P} = 6.5 Hz), 91.1, 86.0 (d, J_{C-P} = 9.2 Hz), 76.5, 72.8, 72.3, 71.9 (d, J_{C-P} = 2.6 Hz), 71.0 (d, J_{C-P} = 8.1 Hz), 67.6, 53.0 ppm. ^{31}P NMR (243 MHz, D_2O) δ = -11.2 (d, *J* = 20.8 Hz), -12.9 (d, *J* = 20.8 Hz) ppm. ESI-HRMS: m/z $[M-H]^-$ calcd for $C_{15}H_{23}N_5O_{16}P_2$: 590.0542, found: 590.0550. The analytical data is in agreement with literature data.[iv]

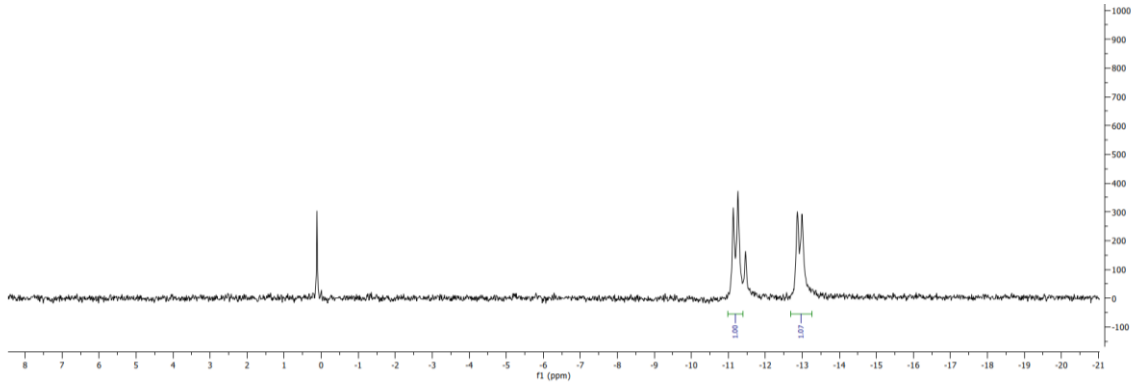
^{1}H NMR (600 MHz, D_2O):



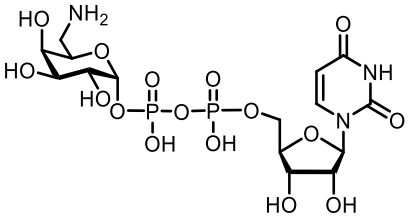
^{13}C NMR (151 MHz, D_2O):



^{31}P NMR (243 MHz, D_2O):



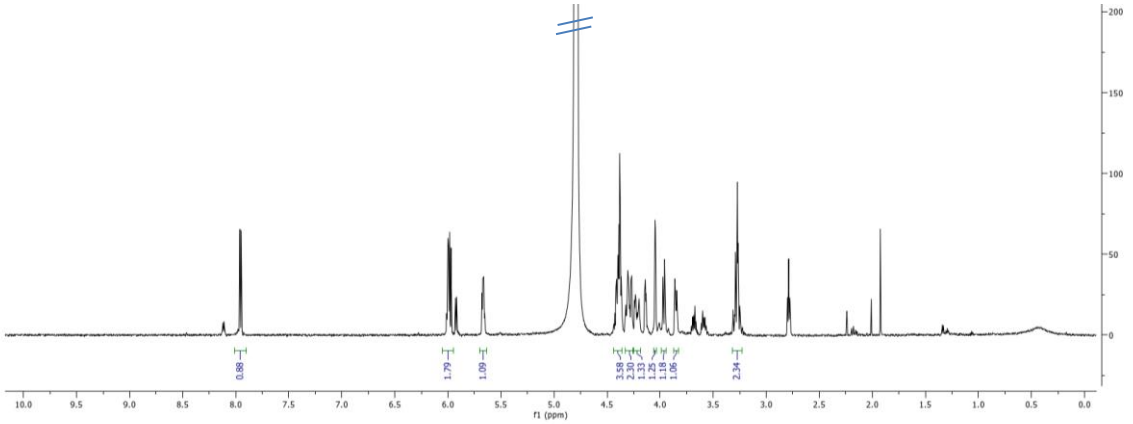
Uridine 5'-(6-amino-6-deoxy-α-D-galactopyranosyl) diphosphate (5)



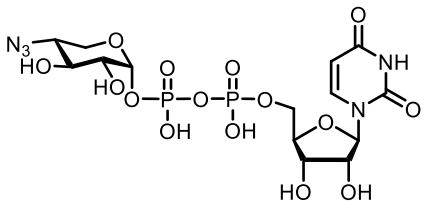
Following a procedure reported by Takaya et al.[iv], azide-functionalized UDP-galactose derivative **1** (0.5 mg, 0.85 µmol) was dissolved in H_2O (1 mL) and Pd/C (10 wt% on activated carbon, 2.0 mg) was added. The solution was stirred under H_2 atmosphere for 30 min at rt before the catalyst was filtered off through a RC-syringe filter. The solvent was removed *in vacuo* to yield 0.4 mg (0.71 µmol, 84%) of **5**.

^{1}H NMR (600 MHz, D_2O) δ = 7.95 (d, *J* = 8.2 Hz, 1H), 6.02 – 5.97 (m, 2H), 5.67 (dd, *J* = 6.5, 3.9 Hz, 1H), 4.42 – 4.36 (m, 3H), 4.33 – 4.26 (m, 2H), 4.25 – 4.18 (m, 1H), 4.05 (d, *J* = 3.3 Hz, 1H), 3.96 (dd, *J* = 10.3, 3.3 Hz, 1H), 3.87 – 3.83 (m, 1H), 3.32 – 3.24 (m, 2H) ppm. Insufficient amounts of material for recording a ^{13}C-NMR spectrum have been obtained. ESI-HRMS: m/z $[M-H]^-$ calcd for $C_{15}H_{24}N_3O_{16}P_2$: 564.0637, found: 564.0652. The analytical data is in agreement with literature data.[iv]

^{1}H NMR (600 MHz, D_2O):



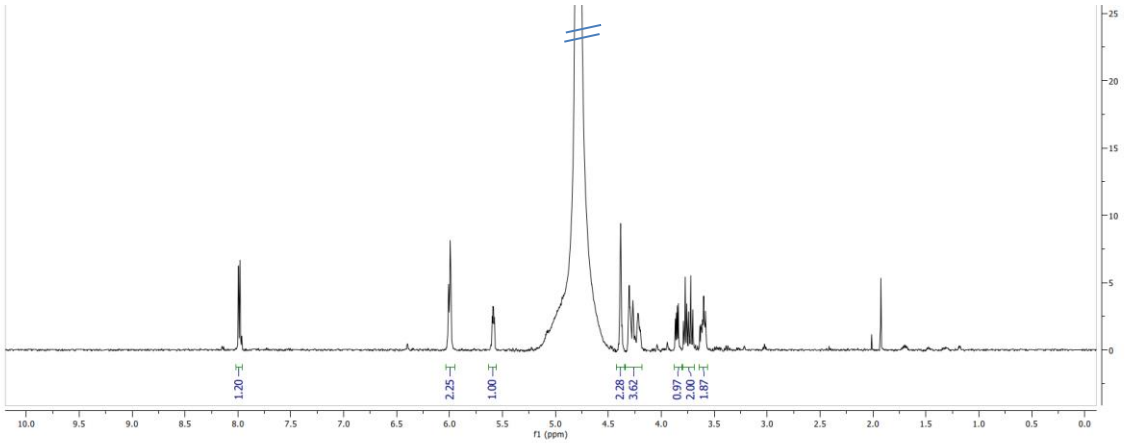
Uridine 5'-(4-azido-4-deoxy-α-D-xylopyranosyl) diphosphate (3)



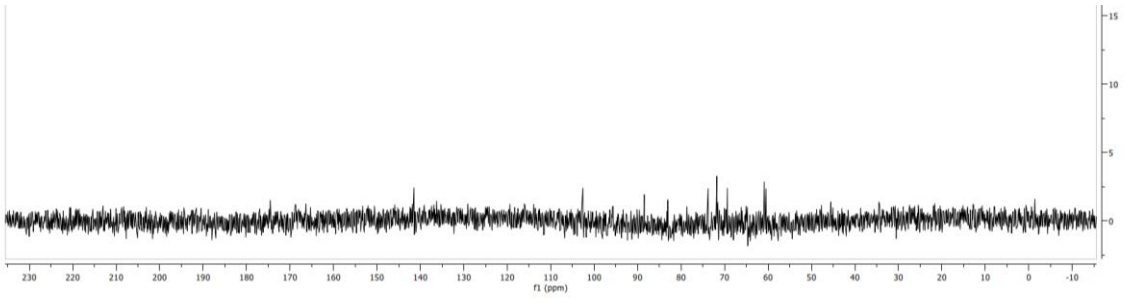
Diallylphosphoryloxy 2,3-di-*O*-acetyl-4-azido-4-deoxy-α-D-xylopyranoside[v] (12.0 mg, 0.0310 mmol, 1.0 equiv) was dissolved under argon atmosphere in anhydrous THF/MeOH (0.7 mL, 1:1 v/v) and NaOTs (11.1 mg, 0.0621 mmol, 2.0 equiv) and $Pd(PPh_3)_4$ (1.79 mg, 1.55 µmol, 0.05 equiv) were added. The reaction mixture was stirred at room temperature for 1.5 h, resulting in a pale yellow solution. The reaction mixture was concentrated *in vacuo* and co-evaporated with anhydrous toluene (3 times). The residue was re-dissolved in anhydrous DMF (2 mL) and 3 spatula tips of activated powdered molecular sieves (4 Å) were added. 5-Nitro-*cyclo*Sal-2',3'-*O*-diacetyluridine monophosphate (**SI3**)[vi] (21.8 mg, 0.0403 mmol, 1.3 equiv) was added over a time of 10 minutes and the reaction mixture was stirred at room temperature for 1 day. Subsequently, the mixture was filtered through a short bed of celite and the solvent was removed under reduced pressure. The residue was solubilized in water and extracted with ethyl acetate. The aqueous phase was concentrated *in vacuo*, yielding a crude intermediate. The crude intermediate was dissolved in $MeOH/H_2O/NEt_3$ (7:3:1; 5.5 mL) and stirred at room temperature for 18 h. The solvent was removed *in vacuo*. The crude product was taken up in water (1.80 mL) and subjected to ion exchange chromatography in two portions of 900 µL each. Fractions that still contained impurities after ion exchange chromatography (NH_4OAc in water, 0.5 M and 0.05 M, 5 mL/min) were subsequently passed through a Carbograph C18 column, from which the product eluted with 50% MeCN in water to obtain azide-functionalized UDP-xylose **3** in 6% yield (1.2 mg, 0.00178 mmol).

^{1}H NMR (700 MHz, D_2O) δ = 7.99 (d, *J* = 8.2 Hz, 1H), 6.06 – 5.94 (m, 2H), 5.59 (dd, *J* = 7.2, 3.5 Hz, 1H), 4.41 – 4.38 (m, 2H), 4.32 – 4.19 (m, 3H), 3.85 (dd, *J* = 11.4, 5.6 Hz, 1H), 3.81 – 3.69 (m, 2H), 3.64 – 3.51 (m, 2H) ppm. ^{13}C NMR (151 MHz, D_2O) δ = 167.9, 141.5, 102.6, 88.1, 83.1, 78.1, 73.4, 71.8, 69.4, 60.9, 60.5 ppm. Due to the small amounts obtained, not all ^{13}C-NMR peaks could be detected. ^{31}P NMR (243 MHz, D_2O) δ = -11.3 (d, *J* = 20.1 Hz), -13.1 (d, *J* = 20.1 Hz) ppm. ESI-MS: m/z $[M-H]^-$ 590.0. The analytical data is in agreement with literature data.[v]

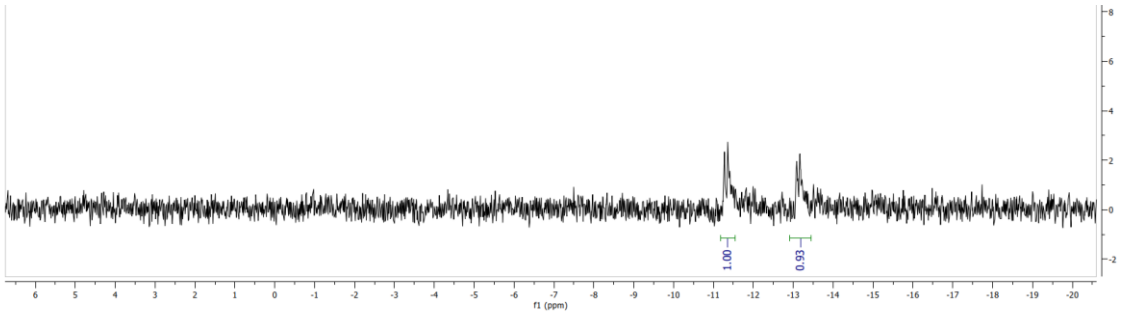
^{1}H NMR (700 MHz, D_2O):



^{13}C NMR (176 MHz, D_2O):

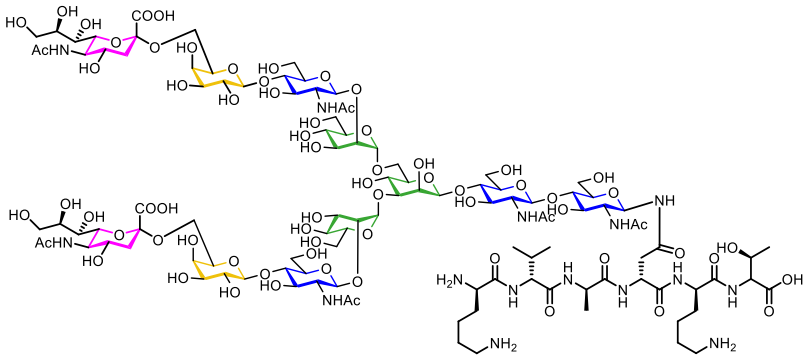


^{31}P NMR (243 MHz, D_2O):



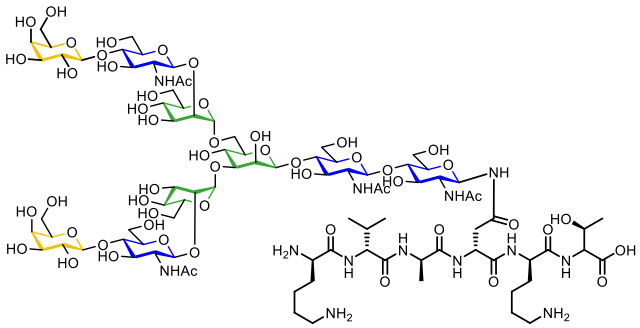
Preparation of Glycopeptides

Isolation of sialoglycopeptide P1 from egg yolk



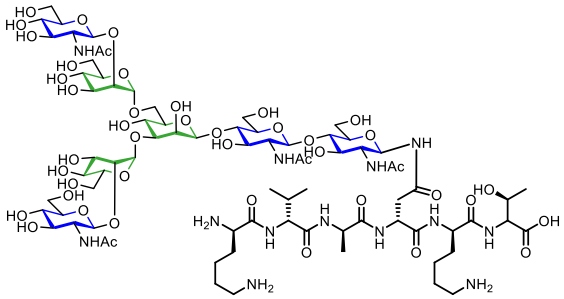
The sialoglycopeptide (SGP, **P1**) isolation procedure was slightly revised from the work of L. Liu[vii] and F. Tang[viii]. Egg yolk powder (1.5 kg) was mixed with tert-butyl methyl ether (4 liters, 1.5 h, twice) and 70% acetone (4 L, 1 h, twice). The mixture was filtered through a Buchner funnel to obtain the filter cake. The filter cake was mixed with 4 L of 40% acetone for 4 h and was filtered through a Buchner funnel to collect the filtrate. This procedure was repeated and combined to the first filtrate. The filtrate was concentrated to 1 L volume using rotary evaporator (R-210, Buchi, Switzerland) and filtered through an active carbon/celite (1:1) 200 g column (10 × 10 cm) prewashed with 50% acetonitrile and water. The column was washed with 2 L of water, 5% and 10% acetonitrile, and finally eluted with 4 L of 25% acetonitrile. The elution was concentrated using rotary evaporator and filtered through a regenerated cellulose membrane filter (RC58, GE Healthcare Life Sciences, Germany) to completely remove the carbon particle. The filtrate was lyophilized to obtain white powder of crude SGP. The crude SGP was dissolved with water and purified with sephadex G25 column (3.2 × 80 cm) on FPLC system (Azura Bio LC, Knauer, Germany). The fractions were examined with ESI-MS and the fractions with SGP were collected and lyophilized. The collected product was purified on HiLoad™ Superdex™ 30 (16 x 600 mm) column (GE Gealthcare Life Sciences, Germany) on a FPLC system and using 5% ethanol as eluent. The fractions were analyzed using ESI-MS and the fractions containing the product were collected and lyophilized to obtain white SGP (**P1**) powder.

Synthesis of asialoglycopeptide P2



The dried SGP (**P1**) was dissolved in a 50 mM sodium acetate buffer (pH 5.5) with 5 mM $CaCl_2$ and Neuraminidase from *Clostridium perfringens* (New England Biolabs, Germany) was added. The mixture was incubated at 37 °C with shaking until TLC and ESI indicated complete removal of the sialic acid. A portion of the reaction mixture was separated for further degalactosylation. The remainder desialylated product was purified using FPLC on Superdex™ 30 prep grade column with 5% ethanol. The fractions containing the product were confirmed using ESI-MS, collected , and lyophilized to give **P2**.

Synthesis for degalactosylated glycopeptide P3



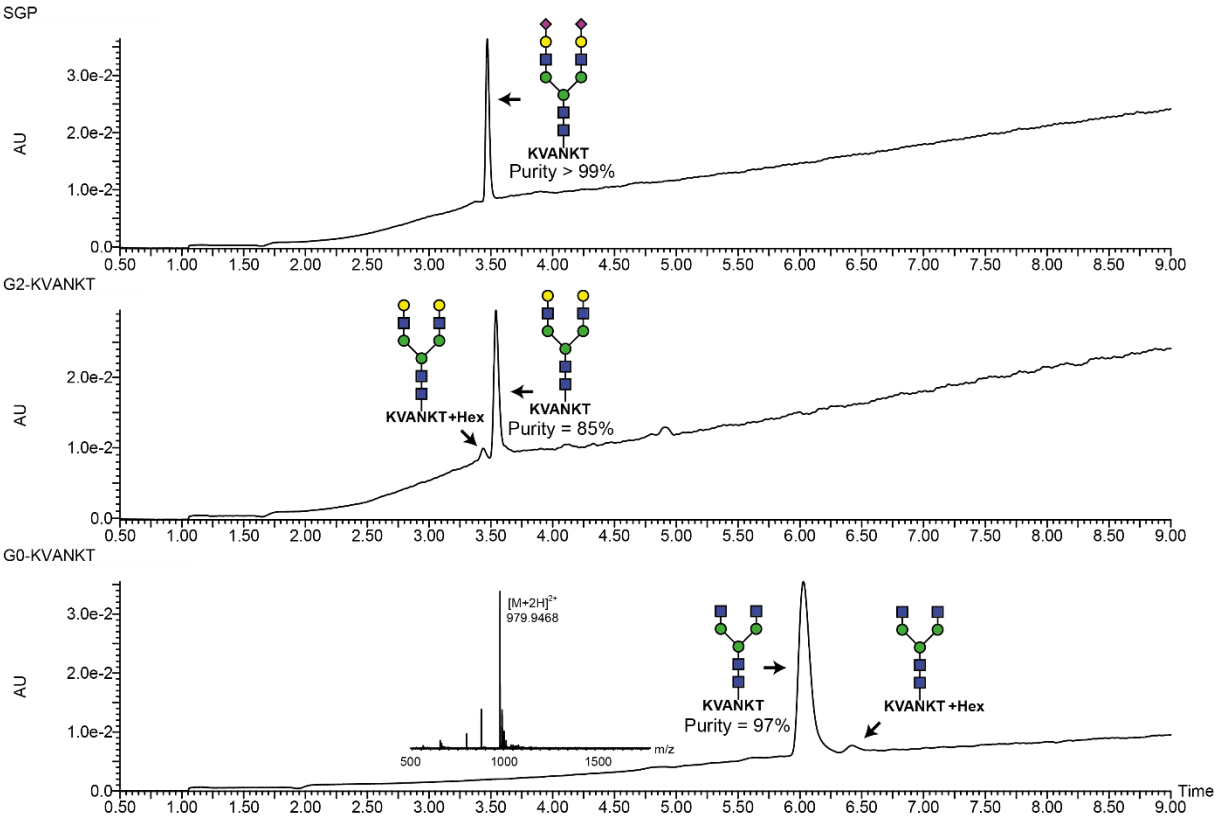
The pH of the crude mixture containing **P2** was set up to 4.5 using acetic acid and β-galactosidase from *Aspergillus niger* (Megazyme, Germany) was added. The reaction was incubated at 37 °C with shaking until complete removal of galactoses was observed by TLC and ESI. The reaction product was purified by FPLC using a HiLoad Superdex™ 30 (16 x 600 mm) column and 5% ethanol as eluent. The fractions containing the product (according to ESI-MS) were collected and lyophilized to obtain the glycopeptide **P3**.

Characterization of P1-3

Characterization was carried out using LC-MS on a Waters ACQUITY H-Class UPLC system connected to a Xevo G2-XS QTof. LC of **P1**, **P2** and **P3** was carried out on a ACQUITY UPLC BEH C18 column (2.1 × 50 mm, 1.7 µm), at 35 °C and were separated using a gradient of 0 to 5% of acetonitrile (0.1% TFA) in ater (0.1% TFA) in 9 minutes and 0.95 mL/min flow rate. UV

absorbance at 214 nm was recorded and used to calculate the purity. The ion source parameters of QTof mass spectrometry were set: capillary voltage 1.5 kV, sampling cone 80 V, source offset 80 V, source temperature 120°C, cone gas flow 80 L/h and desolvation gas 600 L/h. The MS spectra were taken in positive and resolution mode with the range 500-1800 m/z.

UPLC-QTof data



GT Expression in HEK293 cells

The GTs were heterologously expressed in HEK293 cells in its truncated form as soluble secreted protein. Individual GT catalytic domains were cloned into the pGEn2 vector that harbors chimeric *NH_2*-terminal fusion tags according to the methods reported by Moremen and coworkers.[ix] Specifically, truncated forms of the GTs, devoid of the NH_2-terminal cytoplasmic tails and transmembrane domains, were generated as fusion proteins containing an NH_2-terminal signal sequence followed by an 8xHis tag, an AviTag, “superfolder” GFP, the TEV protease recognition site, and the amino acid residues indicated in the construct table (Suppl. Table 1). The constructs were transfected into wild-type HEK293F (ThermoFisher) cells and cultured for several days while they express and secret the GT proteins into the growth medium. Expression and purification of 8xHis-tagged plant enzymes secreted into the culture medium was performed using HisTrap HP columns (GE Healthcare) according to the methods of Urbanowicz and coworkers.[x] To eliminate the possibility of protein contamination, purification of each enzyme was carried out on individual 1 ml HisTrap HP columns. Mammalian enzymes were

cloned, expressed, and purified according to the methods by Moremen and coworkers.[ix] Successful expression was verified using denaturing polyacrylamide gel electrophoresis. After exchanging the buffer into the GT reaction buffer (75 mM Hepes sodium salt, pH 6.8) via dialysis, the resulting enzyme solutions were used for the assays on the array.

Suppl. Table 1: List of primers used to amplify plant glycosyltransferase genes in this study. The primers were designed to amplify the amino acid (AA) residues according to the full-length peptide sequence indicated by the numbering in the primer name. The Locus identifiers are indicated below the common name. Underlined sequences denote the partial *att*B adapter sequences appended to the primers used in the first round of PCR amplification, and the bold sequence indicates the inserted STOP codon.

Gene	**Name**	**Primer Sequence 5'-3'**
AtFUT4 AT2G15390	AtFUT4_54F	AACTTGTACTTTCAAGGCAACGACGAATCCGAAACA
	AtFUT4_535R	ACAAGAAAGCTGGGTC**CTA**TAACTCATCAAAAAGCT
AtFUT6 AT1G14080	AtFUT6_42F	AACTTGTACTTTCAAGGCAACGACTTCAACAACCAAC
	AtFUT6_521R	ACAAGAAAGCTGGGTC**CTA**TAACTCATCAAATAGCTTA
AtFUT7 AT1G14070	AtFUT7_47F	AACTTGTACTTTCAAGGCCCGAGGGATAGACTGTTAG
	AtFUT7_509R	ACAAGAAAGCTGGGTC**CTA**TTTGGTATCATCAACTAG
XXT1 At3G62720	AtXXT1AA51F	AACTTGTACTTTCAAGGCATCGAGGAGATCCGTGAG
	AtXXT1 AA457R	ACAAGAAAGCTGGGTC**CTA**CGTACTAAGCTTGGCCG
PtGALS1 Potri.005G258900	PtGALS1 73F	AACTTGTACTTTCAAGGCTCGTTCACCAGTACCTCAT
	PtGALS1 495R	ACAAGAAAGCTGGGTC**CTA**TGAGTAAGCCTGTACATT
AtGALS1 AT2G33570	AtGALS1 47F	AACTTGTACTTTCAAGGCTCCACCGCTCGTCCCTGCT
	AtGALS1 495R	ACAAGAAAGCTGGGTC**CTA**GAAATTCTTCACATCCGT
AtGALS2 At5g44670	AtGALS2 52F	AACTTGTACTTTCAAGGCATCTCCGCTTCCGAACTC
	AtGALS2 519R	ACAAGAAAGCTGGGTC**CTA**TTGTCTCGTCCTAAGCAA
AtGAL31A AT1G32930	AtGALT31A 45F	AACTTGTACTTTCAAGGCGATGGCATTGAGAGAGCT
	AtGALT31A 399R	ACAAGAAAGCTGGGTC**CTA**GAAACTACTATGCCAAATT

Production of Glycan Microarray

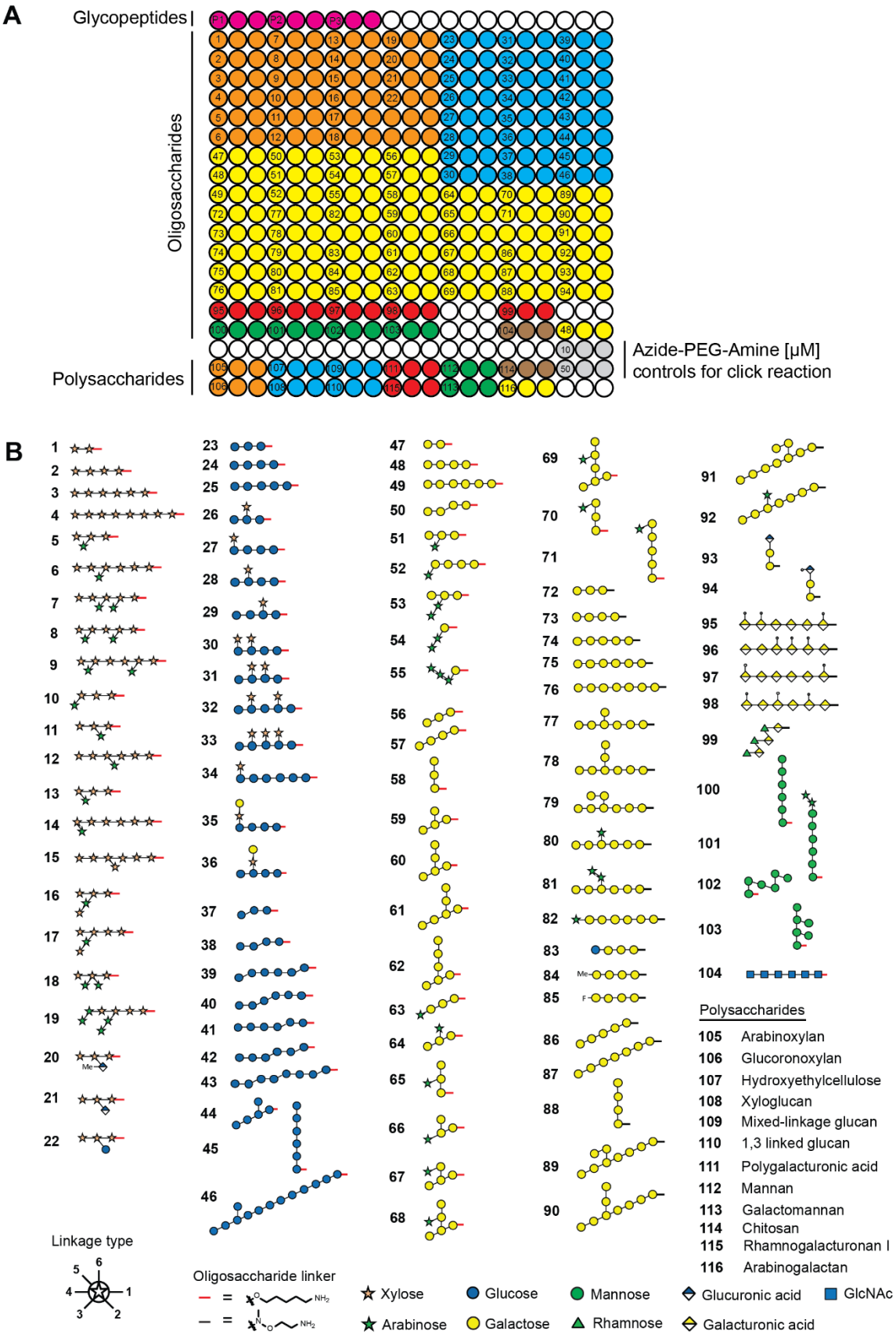
The synthetic plant glycan microarray was printed as described previously.[xi] The synthetic oligosaccharides were dissolved in the coupling buffer (200 µM, 80% 50 mM sodium phosphate, pH 8.5, 0.005% CHAPS, 20% PEG400 (Roth)) and printed in triplicates on CodeLink *N*-hydroxyl succinimide (NHS) ester-activated glass slides (SurModics Inc., USA) using a non-contact piezoelectric spotting device (S3; Scienion, Germany). This spotting device allows printing of ca. 0.5 nl oligosaccharide solutions resulting in spots with a diameter of 150-200 µm. Sixteen identical fields with all glycans in triplicates were printed on a glass slide. Additionally, amino-PEG3-azide (TCI Germany) was printed in different concentrations as controls. After printing, the microarray slides were quenched for 1 h at rt in 100 mM ethanolamine, 50 mM sodium phosphate, pH 9, and washed three times with deionized water.

Glycan Microarray Assay

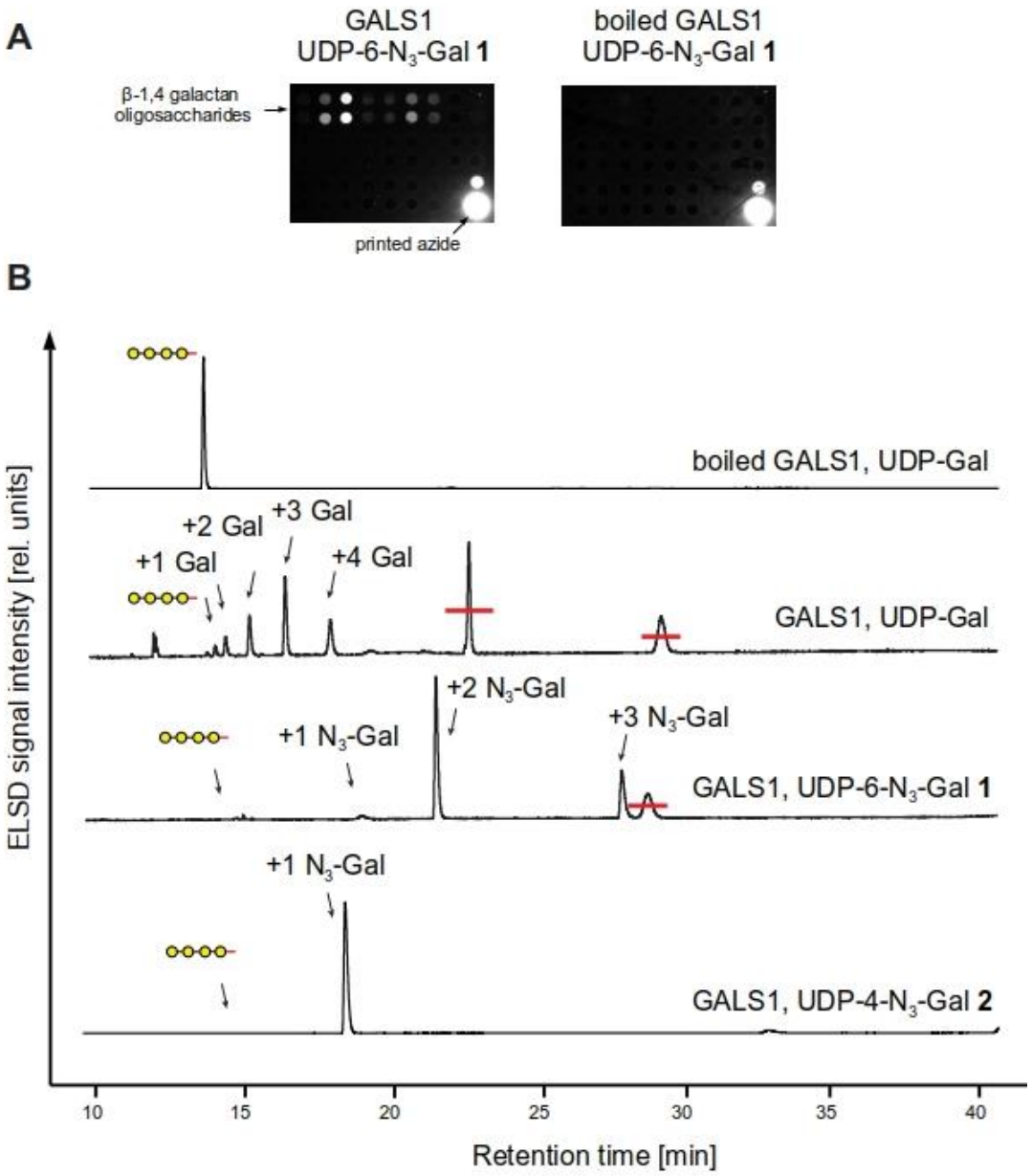
On one microarray slide, 16 different combinations of sugar nucleotide and putative GT were studied in individual wells of a FlexWell grid (Grace Biolabs) that is applied to the microarray slides. For each of the GT reactions different enzyme and donor concentrations were used, depending on the activity of the respective enzyme preparation and availability of the different donors: 0.037 mg/ml *At*GALS1 was used with 0.005 mM UDP-6-N_3-Gal **1**, 0.5 mM UDP-4-N_3-Gal **2**, 0.05 mM UDP-6-NH_2-Gal **5**, or 0.05 mM UDP-4-NH_2-Gal **6**; 0.17 mg/ml *At*GALS2 was used with 0.5 mM UDP-4-N_3-Gal **2**; 0.035mg/ml *Pt*GALS1 was used with 0.5 mM UDP-4-N_3-Gal **2**, 0.05 mg/ml *At*GALT31A was used with 0.5 mM UDP-6-N_3-Gal **1**; 1.1 mg/ml FUT4, 3.84 mg/ml FUT6, and 9.4 mg/ml FUT7 were each used with 0.02 mM GDP-6-N_3-Fuc **4**; 0.37 mg/ml *At*GALS1 was used with 0.5 mM UDP-4-N_3-Xyl **3**; 0.4 mg/ml *At*XXT1 was used with 0.5 mM UDP-6-N_3-Gal **1**; 0.25 mg/ml human FUT1, FUT6, and FUT7 were each used with 0.01 mM GDP-6-N_3-Fuc **4**; 0.25 mg/ml human B4GALT5 was used with 0.01 mM UDP-6-N_3-Gal **1**. All reactions were carried out for 16 h at room temperature in 50 mM HEPES buffer, pH 6.8, including 5 mM Mn^{2+} and Mg^{2+}. Subsequently, any transferred functionalized monosaccharide was visualized by reaction with an alkyne-functionalized Sulfo-Cy5 dye (Jena Biosciences). After drying, the array was treated with a 1:1 DMF:water mixture including the dye (2mM), premixed $CuSO_4$ (1mM) and THPTA (tris(3-hydroxypropyltriazolylmethyl)amine, a Cu(I) stabilizing ligand, 1 mM), and sodium ascorbate (10mM). After 1 h reaction, the array was first washed three times with 1% SDS in PBS for 20 min to remove unreacted dye, then two times with deionized water for 5 min to remove salts, and finally dried and scanned for fluorescence as described previously.[ix] The fluorescence of each printed oligosaccharide spot was quantified using GenePixPro software, which revealed any active combination of sugar nucleotide

donor, oligosaccharide acceptor, and GT. In case of the amino-functionalized monosaccharides, the array was first, before cycloaddition reaction, incubated for 1h with a solution of 1 mM azido-PEG4-NHS ester (Jena Biosciences) in 50 mM sodium phosphate buffer, pH 8.5, including 10% DMSO, and the unreacted azido-linker was removed by washing three times with 1% SDS in PBS for 20 min and two times with water for 5 min.

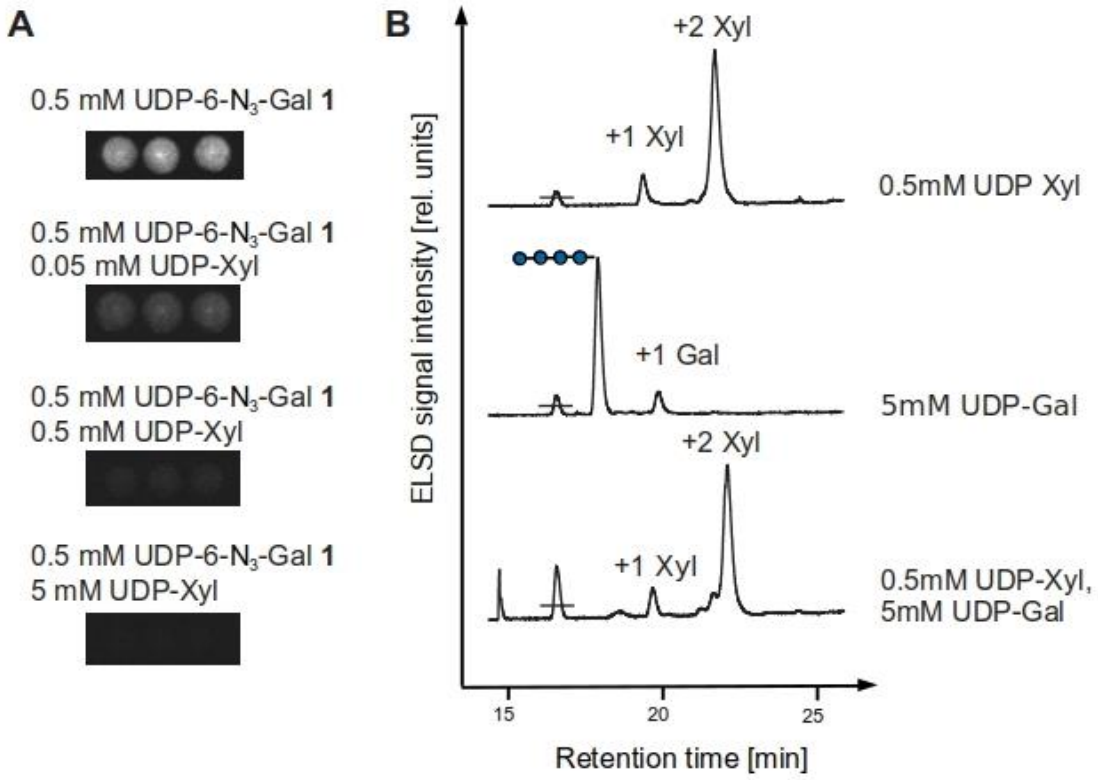
Supplementary Figures



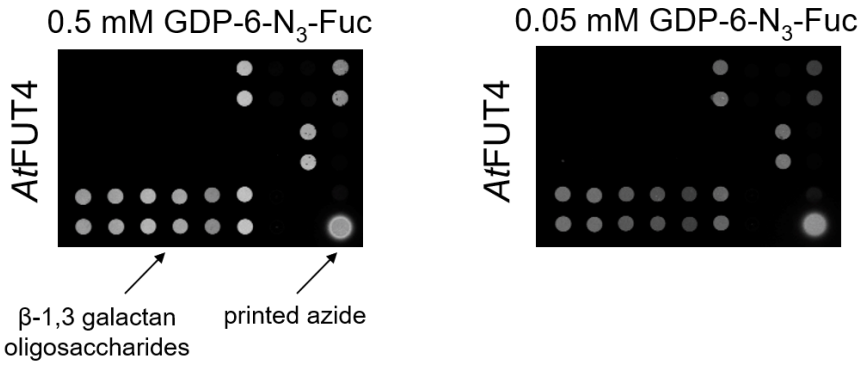
Supplementary Figure 1. A) Printing pattern for results presented in Figure 3. B) List of printed oligosaccharides.



Supplementary Figure 2. Transfer of UDP-Gal or UDP-N_3-Gal by GALS1. A) On-array controls showing that the observed incorporation of 6-N_3-Gal is specific to the GALS1 enzyme. B) HPLC traces of solution-phase experiments with GALS1. The GALS1 enzyme was incubated with 1 mM tetragalactoside acceptor and 2 mM of different UDP-Gal donors in 50 mM HEPES buffer, including 5 mM Mg^{2+} and Mn^{2+}, for 16h at room temperature. The reaction mixture was heated to 80°C for 5 min to terminate the enzyme reaction and subsequently injected into a LC-MS equipped with an evaporative light scattering detector (ELSD) as described previously.[XII] Peaks were annotated using the corresponding mass in the MS trace. Note the shift of oligosaccharide retention times due to the presence of the azides. Red bars denote peaks that do not correspond to oligosaccharides.



Supplementary Figure 3. On-array and in-solution competition experiments show that UDP-Xyl is the favored substrate of *At*XXT1 compared to UDP-Gal and UDP-6-N_3-Gal **1**. A) Glycan array experiment on a printed pentaglucoside using UDP-6-N_3-Gal **1** as the donor and different concentrations of UDP-Xyl as a competitor. B) Experiments in solution with 0.25 mM tetraglucoside acceptor and different concentrations of UDP-Xyl and UDP-Gal donors. In both experimental setups, the *At*XXT1 enzyme mixture was incubated in 50 mM HEPES buffer including 5 mM Mg^{2+} and Mn^{2+} for 16 h at room temperature. After incubation, the reaction mixture with soluble oligosaccharide acceptor was heated to 80°C for 5 min to terminate the reaction. Then, the samples were injected into a LC-MS equipped with an evaporative light scattering detector (ELSD) as described previously.[XII] The peaks were annotated using the corresponding mass in the MS trace. The incorporation of 6-N_3-Gal by *At*XXT1 was not detected, when UDP-Xyl was added to the reaction mixture (lower panel).



Supplementary Figure 4. Qualitatively observed effects of nucleotide sugar donor concentration on signal intensity as exemplified for *At*FUT4 mediated transfer of GDP-6-N_3-Fuc. Stronger signal intensities where observed throughout the panel of acceptor substrates when higher donor concentrations were used.

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