Supporting information for:

# Synthetic UDP-galactofuranose analogs reveal critical enzyme–substrate interactions in GlfT2-catalyzed mycobacterial galactan assembly

Myles B. Poulin[§], Ruokun Zhou[§], and Todd L. Lowary*

Alberta Glycomics Centre and Department of Chemistry,

The Gunning–Lemieux Chemistry Center University of Alberta,

Edmonton, AB T6G 2G2, Canada.

[§] These authors contributed equally to this work

* Corresponding Author. Email: tlowary@ualberta.ca

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**General Methods.** All reagents were purchased from commercial sources without further purification, while solvents were purified using a PURESOLV-400 system (Innovative Technology Inc., Newburyport, MA). Reactions were carried out in oven-dried glassware. Unless stated otherwise, all reactions were carried out at room temperature under a positive pressure of argon and were monitored by TLC on silica gel G-25 (0.25 mm, Macherey–Nagel). Spots were detected under UV light or by charring with acidified ethanolic anisaldehyde. Unless otherwise indicated, column chromatography was performed on silica gel 60 (40–60 μM) where the ratio of silica gel and crude product ranged from 100:1 to 20:1 (w/w). Organic solutions were concentrated under vacuum at < 40 °C (bath). Optical rotations were measured at 22 ± 2 °C on a Perkin–Elmer 241 polarimeter with a sodium D line (589 nm) and are given in units of (°·mL)/(dm·g). $^{1}$H NMR spectra were recorded at 400 MHz, 500 MHz, 600 MHz or 700 MHz and chemical shifts are referenced to either TMS (0.0, $CDCl_3$) or HOD (4.78, $CD_3OD$; 4.67, $D_2O$). $^{13}$C NMR spectra were recorded at 100 MHz, 125 MHz or 175 MHz, and $^{13}$C chemical shifts were referenced to internal $CDCl_3$ (77.23, $CDCl_3$), $CD_3OD$ (48.9, $CD_3OD$). Electrospray mass spectra were recorded on samples suspended in $CH_3Cl$ or $CH_3OH$ and added NaCl.

**General procedure for glycosylation with dibenzylphosphate.** To a solution of methyl glycoside (0.15 mM, 1 equiv) in $CH_2Cl_2$ at 0 °C was added 33% HBr in AcOH (~1.0 mL) while keeping the temperature below 0 °C. After 3 h, the reaction mixture was diluted with toluene and concentrated. Without purification, the residue was resuspended in toluene (0.2 mM). To this was added dibenzylphosphate (1.08 eq) and $Et_3N$ (1 mL). The reaction was carried out at room temperature for 1 h. The reaction mixture was then

filtered to remove $Et_3NHBr$ salts and the filtrate was concentrated to give a crude oil that was purified by column chromatography (6:1 hexanes–EtOAc).

**General deprotection procedure.** To a solution of protected Gal*f*-1P analog (0.15 mM, 1 equiv) in EtOAc was added 10% Pd–C (15% by weight) and $Et_3N$ (6 equiv). The reaction mixture was stirred under $H_2$ (1 atm) for 16 h, and then the catalyst was removed by filtration and the filtrate evaporated. The resulting residue was dissolved in a 10:2:1 solution of $CH_3OH$–$H_2O$–$Et_3N$ (0.05 mM) and stirred at ambient temperature for 6 days until TLC showed complete consumption of the starting material. The solvent was removed by evaporation, and the product was purified using reversed phase $C_{18}$ column chromatography eluting with water.

**Methyl 5,6-*O*-isopropylidine-α-D-galactofuranoside (21)** and **Methyl 5,6-*O*-isopropylidine-β-D-galactofuranoside (22).** The galactofuranose methyl glycoside **20** was prepared as previously described.[1,2] To a solution of **20** (2.78 g, 14.3 mmol) in dry acetone (60 mL) was added (±)-camphor-sulfonic acid (162 mg, 0.05 mmol) and 2,2-dimethyoxypropane (2.7 mL, 1.5 mmol). After 2 h, the solution was quenched by the addition of $Et_3N$ (2 mL), and concentrated to afford a syrup that was purified by column chromatography (1:2 hexane–EtOAc) to give **21** (0.76 g, 22%) and **22** (2.05 g, 61%) as oils. (**21**): $R_f$ 0.36 (20:1 $CH_2Cl_2$–$CH_3OH$); $[\alpha]_D$ –84.0 (*c* 0.8, $CH_2Cl_2$); $^1$H NMR (500 MHz, $CDCl_3$, $\delta_H$) 4.9 (s, 1 H, H-1), 4.33 (dt, 1 H, *J* = 7.2, 1.8 Hz, H-5), 4.15–3.95 (m, 6 H, H-2, H-3, H-4, 2 × H-6, O*H*), 3.39 (s, 3 H, OC*H*$_3$), 3.01 (d, 1 H, *J* = 10.8 Hz, O*H*), 1.41 (s, 3 H, $O_2C(CH_3)_2$), 1.39 (s, 3 H, $O_2C(CH_3)_2$); $^{13}$C NMR (125 MHz, $CDCl_3$, $\delta_C$), 110.1 ($O_2C(CH_3)_2$), 109.6 (C-1), 85.5 (C-2), 78.5 (C-3/C-4), 78.3 (C-4/C-3), 75.7 (C-5), 65.7 (C-6), 55.0 ($OCH_3$), 25.6 (2 × $O_2C(CH_3)_2$); HRMS (ESI) *m/z* Calc. for (M + Na) $C_{10}H_{18}O_6Na$: 257.0998. Found: 257.0996.

(**22**): $R_f$ 0.25 (20:1 $CH_2Cl_2$–$CH_3OH$); $[\alpha]_D$ +6.7 (*c* 3.1, $CH_3OH$); $^1H$ NMR (600 MHz, $CDCl_3$, $\delta_H$) 4.84 (d, 1 H, *J* = 4.5 Hz, H-1), 4.19 (t, 1 H, *J* = 6.6 Hz, H-5), 4.10–4.00 (m, 3 H, H-2, H-3, H-6), 3.93 (dd, 1 H, *J* = 8.5, 6.9 Hz, H-6), 3.83 (t, 1 H, *J* = 6.6 Hz, H-4), 3.49 (s, 3 H, OC*H*$_3$), 2.64–2.60 (m, 2 H, 2 × O*H*), 1.47 (s, 3 H, $O_2C(CH_3)_2$), 1.40 (s, 3 H, $O_2C(CH_3)_2$); $^{13}C$ NMR (125 MHz, $CDCl_3$, $\delta_C$) 109.7 ($O_2C(CH_3)_2$), 101.9 (C-1), 81.3 (C-4), 78.1 (C-2/C-3), 77.2 (C-5), 76.7 (C-3/C-2), 64.9 (C-6), 55.6 (O*C*H$_3$), 25.5 ($O_2C(CH_3)_2$), 25.2 ($O_2C(CH_3)_2$); HRMS (ESI) *m/z* Calc. for (M + Na) $C_{10}H_{18}O_6Na$: 257.0994. Found: 257.0995.

**Methyl 2,3-anhydro-5,6-*O*-isopropylidene-β-D-galactofuranoside (23).** To a solution of **21** (1.97 g, 8.4 mmol) in THF (60 mL) at 0 °C was added $PPh_3$ (2.87 g, 10.9 mmol) and DIAD (2.11 mL, 10.9 mmol). The solution was stirred at room temperature for 1 h, and then concentrated by evaporation under reduced pressure. The resulting crude residue was dissolved in $Et_2O$ and cooled to precipitate the solid $Ph_3P$=O. After filtration, the supernatant was concentrated, and the resulting pale yellow syrup was purified by column chromatography (6:1 hexane–EtOAc) to yield **70** (1.91 g, 100%) as an oil. $R_f$ 0.29 (5:1 hexane–EtOAc); $[\alpha]_D$ –51.9 (*c* 1.3, $CH_2Cl_2$); $^1H$ NMR (500 MHz, $CDCl_3$, $\delta_H$) 4.95 (s, 1 H, H-1), 4.25 (ddd, 1 H, *J* = 7.0, 6.6, 6.5 Hz, H-5), 4.08 (dd, 1 H, *J* = 8.3, 6.5 Hz, H-6), 3.99 (d, 1 H, *J* = 7.0 Hz, H-4), 3.88 (dd, 1 H, *J* = 8.3, 6.6 Hz, H-6), 3.61 (d, 1 H, *J* = 2.8 Hz, H-2), 3.59 (dd, 1 H, *J* = 7.0, 2.8 Hz, H-3), 3.42 (s, 3 H, OC*H*$_3$) 1.45 (s, 3 H, $O_2C(CH_3)_2$), 1.35 (s, 3 H, $O_2C(CH_3)_2$); $^{13}C$ NMR (125 MHz, $CDCl_3$, $\delta_C$) 109.8 ($O_2C(CH_3)_2$), 102.4 (C-1), 77.3 (C-4), 77.2 (C-5), 65.7 (C-6), 55.8 (C-2), 55.6 (C-3), 53.3 (O*C*H$_3$), 26.6 ($O_2C(CH_3)_2$), 25.2 ($O_2C(CH_3)_2$); HRMS (ESI) *m/z* Calc. for (M + Na) $C_{10}H_{16}O_5Na$: 239.0890. Found: 239.0890.

**Methyl 3-*O*-benzyl-5,6-*O*-isopropylidene-β-D-galactofuranoside (24).** Compound **23** (8.0 g, 37 mmol) was dissolved in a solution of 1 M NaOBn in BnOH (100 mL). The solution was heated to reflux for 12 h. Reduced pressure distillation was applied to remove BnOH, and the resulting residue was diluted with EtOAc (60 mL), washed with distilled water (30 mL × 3), brine (30 mL) and dried over $Na_2SO_4$. The solution was concentrated to give a pale yellow syrup that was purified by column chromatography (2:1 hexane–EtOAc) to yield **24** (9.5 g, 79%) as an oil. $R_f$ 0.31 (2:1 hexane–EtOAc); $[\alpha]_D$ –102.1 (*c* 2.0, $CH_2Cl_2$); $^1$H NMR (600 MHz, $CDCl_3$, $\delta_H$) 7.39–7.25 (m, 5 H, Ar), 4.91 (s, 1 H, H-1), 4.72 (d, 1 H, *J* = 12.3 Hz, PhC*H*$_2$) 4.54 (d, 1 H, *J* = 12.3 Hz, PhC*H*$_2$), 4.14–4.10 (m, 3 H, H-2, H-4, H-5), 4.10–3.95 (m, 2 H, H-6a/H-6b), 3.82 (d, 1 H, *J* = 2.8 Hz, H-3), 3.41 (s, 3 H, OC*H*$_3$), 3.83 (d, 1 H, *J* = 10.8 Hz, O*H*), 1.41 (s, 3 H, $O_2C(CH_3)_2$), 1.36 (s, 3 H, $O_2C(CH_3)_2$); $^{13}$C NMR (125 MHz, $CDCl_3$, $\delta_C$) 137.6 (Ar), 128.5 (2 × Ar), 127.9 (Ar), 127.9 (2 × Ar), 110.6 (C-1), 109.9 ($O_2C(CH_3)_2$), 85.7 (C-3), 82.8 (C-5), 77.6 (C-2/C-4), 76.3 (C-4/C-2), 72.1 (Ph*C*H$_2$), 65.6 (C-6), 55.3 (O*C*H$_3$), 25.8 ($O_2C(CH_3)_2$), 25.6 ($O_2C(CH_3)_2$); HRMS (ESI) *m/z* Calc. for (M + Na) $C_{17}H_{24}O_6Na$: 347.1467. Found: 347.1465.

**Methyl 3-deoxy-5,6-*O*-isopropylidene-3-thiotolyl-β-D-galactofuranoside (25).** To a solution of **23** (1.38 g, 6.38 mmol) in DMF (65 mL) at 0 °C was added 60% NaH (382 mg, 9.57 mmol) and thiocresol (2.38 g, 19.1 mmol). After 10 min, the solution was heated to 90 °C, and stirred for 1.5 h. After cooling the solution to room temperature, excess NaH was quenched by adding $CH_3OH$. The solvent was evaporated, and the resulting residue was dissolved in EtOAc (100 mL). The solution was washed with distilled water (50 mL × 2), brine (30 mL), dried ($Na_2SO_4$), and filtered. After evaporating the solvent, the crude compound was purified by column chromatography

(3:1 hexane–EtOAc) to give **25** (1.86 g, 87%) as an oil. $R_f$ 0.27 (3:1 hexane–EtOAc); $[\alpha]_D$ –98.0 (*c* 2.4, $CH_2Cl_2$); $^1$H NMR (500 MHz, $CDCl_3$, $\delta_H$) 7.35–7.31 (m, 2 H, Ar), 7.18–7.13 (m, 2 H, Ar), 4.94 (s, 1 H, H-1), 4.18–4.12 (m, 3 H, H-2, H-4, H-6), 4.07–4.00 (m, 2 H, H-5, H-6), 3.47 (d, 1 H, *J* = 10.4 Hz, O*H*), 3.44 (d, 1 H, *J* = 4.2 Hz, H-3), 3.40 (s, 3 H, OC*H*$_3$), 2.33 (s, 3 H, SPhC*H*$_3$), 1.42 (s, 3 H, $O_2C(CH_3)_2$), 1.38 (s, 3 H, $O_2C(CH_3)_2$); $^{13}$C NMR (125 MHz, $CDCl_3$, $\delta_C$) 137.1 (Ar), 131.7 (Ar), 130.9 (2 × Ar), 129.9 (2 × Ar), 110.5 (C-1), 109.9 ($O_2C(CH_3)_2$), 83.3 (C-4), 80.2 (C-2), 76.6 (C-5), 65.6 (C-6), 55.1 ($OCH_3$), 54.0 (C-3), 25.8 ($O_2C(CH_3)_2$), 25.5 ($O_2C(CH_3)_2$), 21.0 ($SPhCH_3$); HRMS (ESI) *m/z* Calc. for (M + Na) $C_{17}H_{24}O_5SNa$: 363.1235. Found: 363.1236.

**Methyl 5,6-*O*-isopropylidene-3-*O*-methyl-β-D-galactofuranoside (26).** To a solution of **23** (70 mg, 0.32 mmol) in DMF (3.0 mL) at 0 °C was added $CH_3OH$ (0.26 mL, 6.48 mmol) and NaH (129 mg, 3.24 mmol), and the solution was then heated to 90 °C. After 1 h, the solution was cooled to room temperature, and $CH_3OH$ (1 mL) was added to quench the excess NaH. DMF was removed by evaporation to afford a crude residue that was dissolved in EtOAc (10 mL), washed with distilled water (2 × 10 mL), brine (10 mL), dried ($MgSO_4$), and filtered. The filtrate was concentrated to afford a crude syrup that was purified by column chromatography (3:2 hexane–EtOAc) to yield **26** (50 mg, 63%) as an oil. $R_f$ 0.33 (1:1 hexane–EtOAc); $[\alpha]_D$ –107.3 (*c* 1.0, $CH_2Cl_2$); $^1$H NMR (500 MHz, $CDCl_3$, $\delta_H$) 4.91 (s, 1 H, H-1), 4.29 (dt, 1 H, *J* = 7.2, 2.1 Hz, H-5), 4.12–4.00 (m, 4 H, H-2, H-4, H-6a/H-6b), 3.68 (d, 1 H, *J* = 2.5 Hz, H-3), 3.45 (d, 1 H, *J* = 11.0 Hz, O*H*), 3.42 (s, 3 H, OC*H*$_3$), 3.39 (s, 3 H, OC*H*$_3$), 1.42 (s, 3 H, $O_2C(CH_3)_2$), 1.39 (s, 3 H, $O_2C(CH_3)_2$); $^{13}$C NMR (125 MHz, $CDCl_3$, $\delta_C$) 110.7 (C-1), 109.9 ($O_2C(CH_3)_2$), 88.4 (C-3), 82.7 (C-4), 76.5 (C-2/C-5), 76.4 (C-5/C-2), 65.7 (C-6), 58.1 ($OCH_3$), 55.5

($OCH_3$), 25.7 ($O_2C(CH_3)_2$), 25.6 ($O_2C(CH_3)_2$); HRMS (ESI) *m/z* Calc. for (M + Na) $C_{11}H_{20}O_6Na$: 271.1150. Found: 271.1152.

**Methyl 3-*O*-benzyl-2-*O*-(methylthio)thiocarbonyl-5,6-*O*-isopropylidene-β-D-galactofuranoside (27).** To a solution of **24** (100 mg, 0.37 mmol) in THF (4 mL) was added 60% NaH (31 mg, 0.78 mmol). The reaction mixture was stirred for 30 min at room temperature, then cooled to 0 °C followed by the addition of $CS_2$ (47 μL, 0.78 mmol). After 3 h, the excess NaH was quenched by the addition of $CH_3OH$ (2 mL). The solvent was removed by evaporation under reduced pressure, and the resulting residue was purified by column chromatography (9:1 hexane–EtOAc) to give **27** (105 mg, 88%) as an oil. $R_f$ 0.25 (9:1 hexane–EtOAc); $[\alpha]_D$ –81.5 (*c* 0.7, $CH_2Cl_2$); $^1$H NMR (500 MHz, $CDCl_3$, $\delta_H$) 7.39–7.28 (m, 5 H, Ar), 5.88 (s, 1 H, H-2), 5.02 (s, 1 H, H-1), 4.76 (d, 1 H, *J* = 11.9 Hz, PhC*H*$_2$), 4.54 (d, 1 H, *J* = 11.9 Hz, PhC*H*$_2$), 4.18 (ddd, 1 H, *J* = 6.7, 6.6, 6.5 Hz, H-5), 4.15 (dd, 1 H, *J* = 6.5, 5.4 Hz, H-4), 3.86 (d, 1 H, *J* = 5.4 Hz, H-3), 3.84 (dd, 1 H, *J* = 8.5, 6.6 Hz, H-6), 3.75 (dd, 1 H, *J* = 8.5, 6.7 Hz, H-6), 3.43 (s, 3 H, OC*H*$_3$), 2.60 (s, 3 H, SC*H*$_3$), 1.41 (s, 3 H, $O_2C(CH_3)_2$), 1.38 (s, 3 H, $O_2C(CH_3)_2$); $^{13}$C NMR (125 MHz, $CDCl_3$, $\delta_C$) 214.5 (*C*=S), 136.9 (Ar), 128.5 (2 × Ar), 128.3 (2 × Ar), 128.1 (Ar), 109.9 ($O_2C(CH_3)_2$), 106.6 (C-1), 88.6 (C-2), 84.4 (C-4), 82.9 (C-3), 75.9 (C-5), 72.7 (Ph*C*H$_2$), 65.3 (C-6), 55.1 ($OCH_3$), 26.4 ($O_2C(CH_3)_2$), 25.3 ($O_2C(CH_3)_2$), 19.3 ($SCH_3$); HRMS (ESI) *m/z* Calc. for (M + Na) $C_{19}H_{26}O_6S_2Na$: 437.1065. Found: 437.1063.

**Methyl 3-*O*-benzyl-2-deoxy-5,6-*O*-isopropylidene-β-D-*lyxo*-hexofuranoside (28).** To a solution of **27** (97 mg, 0.23 mmol) in dry toluene (5 mL) at 50 °C was added AIBN (46 mg, 0.28 mmol) and *n*-$Bu_3SnH$ (0.55 mL, 1.56 mmol), followed by continued heating to 110 °C. AIBN (46 mg, 0.28 mmol) was added in portions every 30 min. After 2 h, the solution was cooled to room temperature, concentrated, and the resulting residue

was purified by column chromatography (6:1 hexane–EtOAc) to give **28** (41 mg, 59%) as an oil. $R_f$ 0.25 (6:1 hexane–EtOAc); $[\alpha]_D$ –101.8 (*c* 1.6, $CH_2Cl_2$); $^1$H NMR (500 MHz, $CDCl_3$, $\delta_H$) 7.38–7.42 (m, 5 H, Ar), 5.09 (s, 1 H, H-1), 4.60 (d, 1 H, *J* = 12.2 Hz, PhC*H*$_2$), 4.45 (d, 1 H, *J* = 12.2 Hz, PhC*H*$_2$), 4.10–4.05 (m, 2 H, H-4, H-5), 3.92–3.50 (m, 3 H, H-3, H-6), 3.40 (s, 3 H, OC*H*$_3$), 2.25–2.02 (m, 2 H, H-2a/H-2b), 1.40 (s, 3 H, $O_2C(CH_3)_2$), 1.33 (s, 3 H, $O_2C(CH_3)_2$); $^{13}$C NMR (125 MHz, $CDCl_3$, $\delta_C$) 137.8 (Ar), 128.4 (2 × Ar), 127.9 (2 × Ar), 127.8 (Ar), 109.5 ($O_2C(CH_3)_2$), 105.3 (C-1), 82.9 (C-4), 78.8 (C-3), 76.6 (C-5), 71.6 (Ph*C*H$_2$), 65.6 (C-6), 55.2 (O*C*H$_3$), 38.7 (C-2), 26.3 ($O_2C(CH_3)_2$), 25.5 ($O_2C(CH_3)_2$); HRMS (ESI) *m/z* Calc. for (M + Na) $C_{17}H_{24}O_5Na$: 331.1517. Found: 331.1516.

**Methyl 3,5,6-tri-*O*-benzoyl-2-deoxy-β-D-*lyxo*-hexofuranoside (29).** To a solution of **28** (271 mg, 0.88 mmol) in $CH_2Cl_2$ (15 mL) was added 10% HCl in $CH_3OH$ (1 mL). The reaction mixture was stirred for 1 h at room temperature, neutralized by adding $Et_3N$ (2 mL), and concentrated to give a crude compound that was purified by a column chromatography (1:2 hexane–EtOAc) to produce a pale yellow syrup. To the syrup was added a 3:1 EtOAc–$CH_3OH$ solution (16 mL) and 10% Pd–C (30 mg), and the mixture was stirred under $H_2$ (1 atm) for 12 h. After the waste Pd–C was filtered, the solution was concentrated to give a pale yellow syrup. The syrup was dissolved in pyridine (20 mL), followed by the addition of benzoyl chloride (0.4 mL, 3.2 mmol) dropwise under 0 °C. The solution was slowly warmed to room temperature. After 12 h, the solution was concentrated under reduced pressure. The resulting residue was dissolved in EtOAc (30 mL), washed with 1 M HCl (20 mL), satd aq. $NaHCO_3$ solution (20 mL), brine (20 mL), dried ($Na_2SO_4$), filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography (7:1 hexane–

EtOAc) to yield **29** (325 mg, 84%) as a solid. $R_f$ 0.5 (4:1 hexane–EtOAc); $[\alpha]_D$ –85.0 (*c* 1.3, $CH_2Cl_2$); $^1$H NMR (500 MHz, $CDCl_3$, $\delta_H$) 8.10–7.98 (m, 6 H, Ar), 7.60–7.18 (m, 9 H, Ar), 5.92 (ddd, *J* = 7.2, 4.5, 3.6 Hz, H-5), 5.42 (ddd, 1 H, *J* = 8.3, 3.6, 2.1 Hz, H-3), 5.26 (d, 1 H, *J* = 5.4 Hz, H-1), 4.72 (dd, 1 H, *J* = 11.8, 4.5 Hz, H-6), 4.69 (dd, 1 H, *J* =11.8, 7.2 Hz, H-6), 4.63 (t, 1 H, *J* = 3.6 Hz, H-4), 3.41 (s, 3 H, OC*H*$_3$) 2.51 (ddd, *J* = 14.5, 8.3, 5.4 Hz, H-2), 2.16 (dd, 1 H, *J* = 14.5, 2.1 Hz, H-2); $^{13}$C NMR (125 MHz, $CDCl_3$, $\delta_C$) 166.2 (*C*=O), 166.1 (*C*=O), 165.6 (*C*=O), 133.3 (Ar), 133.1 (Ar), 133.0 (Ar), 129.8 (2 × Ar), 129.7 (2 × Ar), 129.7 (2 × Ar), 129.6 (2 × Ar), 129.5 (Ar), 128.4 (Ar), 128.4 (Ar), 128.3 (2 × Ar), 128.3 (2 × Ar), 105.1 (C-1), 81.5 (C-4), 74.7 (C-3), 71.1 (C-5), 63.5 (C-6), 55.1 (O*C*H$_3$), 39.3 (C-2); HRMS (ESI) *m/z* Calc. for (M + Na) $C_{28}H_{26}O_8Na$: 513.1518. Found: 513.1520.

**Methyl 3-*O*-benzyl-5,6-*O*-isopropylidene-2-*O*-methyl-β-D-galactofuranoside (31).** To a solution of **24** (1.15 g, 3.55 mmol) in DMF (35 mL) under 0 °C was added NaH (284 mg, 1.10 mmol). After 5 min, $CH_3I$ (0.33 mL, 5.33 mmol) was slowly added. This reaction mixture was stirred at room temperature for 6 h, and then concentrated. The crude compound was dissolved in EtOAc (60 mL), washed with water (30 mL × 2), brine (30 mL) and dried ($Na_2SO_4$). After concentration, the crude residue was purified by column chromatography (6:1 hexane–EtOAc) to give **31** (1.15 g, 95%) as an oil. $R_f$ 0.30 (6:1 hexane–EtOAc); $[\alpha]_D$ –102.5 (*c* 2.1, $CH_2Cl_2$); $^1$H NMR (500 MHz, $CDCl_3$, $\delta_H$) 7.39–7.28 (m, 5 H, Ar), 4.90 (s, 1 H, H-1), 4.65 (d, 1 H, *J* = 12.0 Hz, PhC*H*$_2$), 4.49 (d, 1 H, *J* = 12.0 Hz, PhC*H*$_2$), 4.15 (q, 1 H, *J* = 6.5 Hz, H-5), 4.02 (t, 1 H, *J* = 6.5 Hz, H-4), 3.84 (dd, 1 H, *J* = 8.4, 6.5 Hz, H-6), 3.80–3.76 (m, 2 H, H-2, H-6), 3.67 (dd, 1 H, *J* = 6.7, 2.3 Hz, H-3), 3.44 (s, 3 H, OC*H*$_3$), 3.38 (s, 3 H, OC*H*$_3$), 1.41 (s, 3 H, $O_2C(CH_3)_2$), 1.38 (s, 3 H, $O_2C(CH_3)_2$); $^{13}$C NMR (125 MHz, $CDCl_3$, $\delta_C$) 137.4 (Ar), 128.4 (2 × Ar), 128.1 (2 × Ar),

127.9 (Ar), 109.7 ($O_2C(CH_3)_2$), 106.8 (C-1), 89.8 (C-2), 83.7 (C-3), 82.3 (C-4), 76.4 (C-5), 72.2 ($PhCH_2$), 65.3 (C-6), 57.6 ($OCH_3$), 54.9 ($OCH_3$), 26.5 ($O_2C(CH_3)_2$), 25.3 ($O_2C(CH_3)_2$); HRMS (ESI) *m/z* Calc. for (M + Na) $C_{18}H_{26}O_6Na$: 361.1620. Found: 361.1621.

**Methyl 3,5,6-tri-*O*-benzoyl-2-*O*-methyl-β-D-galactofuranoside (32)**. To **31** (1.16 g, 3.44 mmol) in a solution of 3:1 EtOAc–$CH_3OH$ (40 mL) was added 20% $Pd(OH)_2$–C (116 mg). The mixture was stirred under $H_2$ (1 atm) for 12 h, and the $Pd(OH)_2$ was removed by filtration. The filtrate was concentrated to give a syrup that was dissolved in a solution of $CH_2Cl_2$–$CH_3OH$ (40 mL). To the solution was added 10% HCl in $CH_3OH$ (1 mL), and the solution was stirred at room temperature for 1 h. The solvent was evaporated to afford a crude residue that was then dissolved in dry pyridine (35 mL). Benzoyl chloride (1.55 mL, 12.4 mmol) was added dropwise at under 0 °C. The solution was kept for 12 h at room temperature, then concentrated under reduced pressure. The resulting residue was dissolved in EtOAc (60 mL), washed with 1 M HCl (30 mL × 2), satd aq. $NaHCO_3$ solution (30 mL), brine (30 mL), dried ($Na_2SO_4$), filtered, and the solvent was evaporated. The crude residue was purified by column chromatography (6:1 hexane–EtOAc) to give **32** (1.56 g, 84%) as a foam. $R_f$ 0.4 (4:1 hexane–EtOAc); $[\alpha]_D$ –74.4 (*c* 1.2, $CH_2Cl_2$); $^1$H NMR (500 MHz, $CDCl_3$, $\delta_H$) 8.15–7.95 (m, 6 H, Ar), 7.61–7.35 (m, 9 H, Ar), 5.90 (ddd, 1 H, *J* = 7.0, 4.4, 3.8 Hz, H-5), 5.42 (d, 1 H, *J* = 4.7 Hz, H-3), 5.09 (s, 1 H, H-1), 4.74 (dd, 1 H, *J* = 11.8, 4.4 Hz, H-6a), 4.68 (dd, 1 H, *J* = 11.8, 7.0 Hz, H-6b), 4.60 (dd, 1 H, *J* = 4.7, 3.8 Hz, H-4), 3.89 (s, 1 H, H-2), 3.43 (s, 3 H, $OCH_3$), 3.37 (s, 3 H, $OCH_3$); $^{13}$C NMR (125 MHz, $CDCl_3$, $\delta_C$) 166.1 (*C*=O), 165.8 (*C*=O), 165.7 (*C*=O), 133.3 (Ar), 133.1 (Ar), 133.0 (Ar), 129.9 (2 × Ar), 129.8 (2 × Ar), 129.7 (2 × Ar), 129.6 (2 × Ar), 129.3 (Ar), 128.3 (2 × Ar), 128.3 (2 × Ar), 128.2 (2 × Ar), 107.4 (C-1),

89.1 (C-2), 81.3 (C-4), 76.9 (C-3), 70.6 (C-5), 63.4 (C-6), 57.6 (O*C*H$_3$), 54.9 (O*C*H$_3$); HRMS (ESI) *m/z* calc. for (M + Na) $C_{29}H_{28}O_9Na$: 543.1625. Found: 543.1625.

**Dibenzyl 3,5,6-tri-*O*-benzoyl-2-*O*-methyl-α-D-galactofuranosyl-1-phosphate (33).** Compound **32** (497 mg, 0.95 mmol) was treated following the general procedure for glycosylation with dibenzylphosphate to give **33** (334 mg, 46%) as a colorless oil. $R_f$ 0.19 (2:1 hexane–EtOAc); $[\alpha]_D$ +18.7 (*c* 0.6, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$, $\delta_H$) 8.10–7.90 (m, 6 H, Ar), 7.56–7.23 (m, 19 H, Ar), 6.15 (dd, 1 H, *J* = 5.9, 4.2 Hz, H-1), 5.89 (dd, 1 H, *J* = 7.8, 7.3 Hz, H-3), 5.74 (ddd, 1 H, *J* = 6.0, 4.5, 4.5 Hz, H-5), 5.18–5.07 (m, 4 H, 2 × OC*H*$_2$Ph), 4.71 (dd, 1 H, $J_{6a,6b}$ = 11.9, 4.5 Hz, H-6a), 4.60–4.55 (m, 2 H, H-4, H-6b), 4.27 (ddd, 1 H, $J_{2,3}$ = 7.8, 4.2, 1.9 Hz, H-2), 3.48 (s, 3 H, OC*H*$_3$); $^{13}$C NMR (100 MHz, $CDCl_3$, $\delta_C$) 165.8 (*C*=O), 165.6 (*C*=O), 165.4 (*C*=O), 135.7 (d, *J* = 7.8 Hz, Ar), 135.5 (d, *J* = 7.7 Hz, Ar), 133.5 (Ar), 133.1 (Ar), 133.0 (Ar), 130.0 (2 × Ar), 129.8 (2 × Ar), 129.7 (2 × Ar), 129.6 (Ar), 129.5 (Ar), 128.9 (Ar), 128.5 (2 × Ar), 128.4 (Ar), 128.4 (4 × Ar), 128.3 (Ar), 128.3 (2 × Ar), 128.2 (2 × Ar), 127.9 (2 × Ar), 127.8 (2 × Ar), 97.9 (d, *J* = 5.0 Hz, C-1), 84.2 (d, *J* = 5.9 Hz, C-2), 80.1 (C-4), 74.2 (C-3), 70.8 (C-5), 69.5 (d, *J* = 5.6 Hz, Ph*C*H$_2$), 69.3 (d, *J* = 5.5 Hz, Ph*C*H$_2$), 62.7 (C-6), 58.8 (O*C*H$_3$); HRMS (ESI) *m/z* Calcd. for (M + Na) $C_{42}H_{39}O_{12}NaP$: 789.2071. Found: 789.2071.

**2-*O*-Methyl-α-D-galactofuranosyl-1-phosphate triethylammonium acetate salt (13).** Compound **33** (302 mg, 0.39 mmol) was deprotected following the general deprotection procedure. The desired fractions were pooled and lyophilized to give **13** (112 mg, 60%)as a colorless oil. $R_f$ 0.27 (10:2:1 $CH_3OH$–$NH_4OH$–$H_2O$); $[\alpha]_D$ +47.8 (*c* 0.3, $H_2O$); $^1$H NMR (400 MHz, $D_2O$, $\delta_H$) 5.63 (dd, 1 H, *J* = 4.2, 4.2 Hz, H-1), 4.26 (dd, 1 H, *J* = 8.2, 7.2 Hz, H-3), 3.86 (ddd, 1 H, *J* = 8.2, 4.2, 2.2 Hz, H-2), 3.78 (dd, 1 H, *J* = 7.2,

4.5 Hz, H-4), 3.70 (dt, 1 H, *J* = 7.2, 4.5 Hz, H-5), 3.65 (dd, 1 H, *J* = 11.6, 4.5 Hz, H-6a), 3.59 (dd, 1 H, *J* = 11.6, 7.2 Hz, H-6b), 3.45 (s, 3 H, OC*H*$_3$), 3.16 (q, 10 H, *J* = 7.3, 1.6 × ($CH_3CH_2$)$_3$N), 1.25 (t, 15 H, *J* = 7.3, 1.6 × ($CH_3CH_2$)$_3$N); $^{13}$C NMR (100 MHz, $D_2O$, $\delta_c$) 95.8 (d, *J* = 5.7 Hz, C-1), 85.9 (d, *J* = 7.9 Hz, C-2), 82.3 (C-4), 73.6 (C-3), 72.6 (C-5), 63.2 (C-6), 58.7 (O*C*H$_3$), 47.4 (($CH_3CH_2$)$_3$N), 9.1 (($CH_3CH_2$)$_3$N); HRMS (ESI) *m/z* Calcd. for ($M^-$) $C_7H_{14}O_9P$: 273.0370. Found: 273.0377.

**Methyl 3-deoxy-5,6-*O*-isopropylidene-β-D-*xylo*-hexofuranoside (34).** To a solution of **25** (1.86 g, 5.46 mmol) in toluene (55 mL) at 50 °C was added AIBN (0.90 g, 5.46 mmol) and *n*-$Bu_3SnH$ (8.80 mL, 32.8 mmol). The solution was heated to 110 °C, and AIBN (0.90 g, 5.46 mmol) was added every 30 min. After 2 h, the solution was concentrated to afford a crude compound that was then purified by column chromatography (3:1 hexane–EtOAc) to give **34** (957 mg, 81%) as an oil. $R_f$ 0.31 (2:1 hexane–EtOAc); $[\alpha]_D$ –83.2 (*c* 2.0, $CH_2Cl_2$); $^1$H NMR (500 MHz, $CDCl_3$, $\delta_H$) 4.86 (s, 1 H, H-1), 4.20 (dt, 1 H, *J* = 9.2, 2.5 Hz, H-4), 4.14–4.06 (m, 3 H, H-5, 2 × H-6), 4.03 (dd, 1 H, *J* = 11.2, 5.5 Hz, H-2), 3.88 (d, 1 H, *J* = 11.2 Hz, OH), 3.33 (s, 3 H, OC*H*$_3$), 2.45 (ddd, 1 H, *J* = 13.8, 9.2, 5.5 Hz, H-3), 1.81 (dd, 1 H, *J* = 13.8, 2.5 Hz, H-3); 1.46 (s, 3 H, $O_2C(CH_3)_2$), 1.40 (s, 3 H, $O_2C(CH_3)_2$); $^{13}$C NMR (125 MHz, $CDCl_3$, $\delta_C$), 109.9 (C-1), 109.7 ($O_2C(CH_3)_2$), 77.6 (C-5), 76.0 (C-4), 73.7 (C-2), 65.7 (C-6), 54.5 (O*C*H$_3$), 34.4 (C-3), 25.7 ($O_2C(CH_3)_2$), 25.6 ($O_2C(CH_3)_2$); HRMS (ESI) *m/z* Calc. for (M + Na) $C_{10}H_{18}O_5Na$: 241.1047. Found: 241.1046.

**Methyl 3-deoxy-2,5,6-tri-*O*-benzoyl-β-D-*xylo*-hexofuranoside (35)**. To a solution of **34** (957 mg, 4.39 mmol) in $CH_2Cl_2$ (50 mL) was added 10% HCl in $CH_3OH$ (1 mL). The solution was stirred at room temperature for 1 h, and then neutralized by adding $Et_3N$ (1 mL). After concentrating the reaction mixture, the resulting syrup was

purified by column chromatography (1:2 hexane–EtOAc). The intermediate was dissolved in 3:1 EtOAc–$CH_3OH$ (40 mL), and 10% Pd–C (80 mg) was added. The mixture was stirred under $H_2$ (1 atm) for 12 h, followed by removal of the catalyst by filtration. Evaporation of the solvent afforded a pale yellow syrup that was then dissolved in dry pyridine (40 mL). Benzoyl chloride (1.98 mL, 15.8 mmol) was added dropwise at under 0 °C. After 12 h, the pyridine was removed by evaporation, and the resulting residue was dissolved in EtOAc (40 mL), washed with 1 M HCl (30 ml), satd aq. $NaHCO_3$ solution (30 mL), brine (30 mL), dried ($MgSO_4$), and filtered. The filtrate was concentrated to give a pale yellow syrup that was purified by column chromatography (7:1 hexane–EtOAc) to yield **35** (1.80 g, 84%) as a foam. $R_f$ 0.5 (4:1 hexane–EtOAc); $[\alpha]_D$ –14.9 (*c* 3.2, $CH_2Cl_2$); $^1$H NMR (500 MHz, $CDCl_3$, $\delta_H$) 8.13–7.92 (m, 6 H, Ar), 7.56–7.30 (m, 9 H, Ar), 5.76 (ddd, 1 H, *J* = 7.2, 3.9, 3.2 Hz, H-5), 5.36 (dd, 1 H, *J* = 7.0, 1.8 Hz, H-2), 5.17 (s, 1 H, H-1), 4.76 (dd, 1 H, *J* = 12.0, 3.9 Hz, H-6), 4.69 (dd, 1 H, *J* = 12.0, 7.2 Hz, H-6), 4.62 (ddd, 1 H, *J* = 7.5, 6.5, 3.2 Hz, H-4), 3.41 (s, 3 H, OC*H*$_3$), 2.70 (ddd, 1 H, *J* = 14.6, 7.5, 7.0 Hz, H-3), 2.08 (ddd, 1 H, *J* = 14.6, 6.5, 1.8 Hz, H-3); $^{13}$C NMR (125 MHz, $CDCl_3$, $\delta_C$) 166.1(7) (*C*=O), 166.1(2) (*C*=O), 165.9(2) (*C*=O), 133.2 (Ar), 133.1 (Ar), 133.1 (Ar), 129.9 (2 × Ar), 129.6 (5 × Ar), 129.6 (Ar), 129.4 (2 × Ar), 128.4 (5 × Ar), 107.2 (C-1), 77.9 (C-2), 76.3 (C-4), 72.0 (C-5), 63.8 (C-6), 54.7 (O*C*H$_3$), 32.1 (C-3); HRMS (ESI) *m/z* Calc. for (M + Na) $C_{28}H_{26}O_8Na$: 513.1521. Found: 513.1520.

**Dibenzyl 2,5,6-tri-*O*-benzoyl-3-deoxy-α-D-*xylo*-hexofuranosyl-1-phosphate (36).** Prepared from compound **35** (183 mg, 0.37 mmol) using the general glycosylation procedure with dibenzyl phosphate to give **36** (95 mg, 35%) as a colorless oil. $R_f$ 0.20 (2:1 hexane–EtOAc); $[\alpha]_D$ +11.0 (*c* 0.4, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$, $\delta_H$) 8.18–

8.02 (m, 6 H, Ar), 7.61–7.16 (m, 19 H, Ar), 6.20 (dd, 1 H, $J$ = 5.2, 4.0 Hz, H-1), 5.69 (ddd, 1 H, $J$ = 6.5, 4.0, 4.0 Hz, H-5), 5.43 (dddd, 1 H, $J$ = 11.3, 7.5, 4.0, 2.0 Hz, H-2), 5.11 (dd, 1 H, $J$ = 11.8, 7.4 Hz, PhC$H_2$), 5.04 (dd, 1 H, $J$ = 11.8, 8.0 Hz, PhC$H_2$), 5.03 (dd, 1 H, $J$ = 11.8, 6.7 Hz, PhC$H_2$), 4.95 (dd, 1 H, $J$ = 11.8, 7.8 Hz, PhC$H_2$), 4.75 (dd, 1 H, $J$ = 11.9, 4.0 Hz, H-6a), 4.73–4.68 (m, 1 H, H-4), 4.61 (dd, 1 H, $J$ = 11.9, 6.5 Hz, H-6b), 2.67 (ddd, 1 H, $J$ = 12.0, 7.5, 6.5 Hz, H-3a), 2.37–2.27 (m, 1 H, H-3b); $^{13}$C NMR (100 MHz, $CDCl_3$, $\delta_C$) 166.0 (*C*=O), 165.9 (*C*=O), 165.7 (*C*=O), 135.6 (d, $J$ = 7.5 Hz, Ar), 135.5 (d, $J$ = 7.8 Hz, Ar), 133.3 (Ar), 133.2 (Ar), 133.2 (Ar), 129.9 (2 × Ar), 129.8 (2 × Ar), 129.6 (2 × Ar), 129.5 (Ar), 129.4 (Ar), 128.9 (Ar), 128.4 (6 × Ar), 128.3 (2 × Ar), 128.3 (2 × Ar), 128.2 (Ar), 128.2 (Ar), 127.7 (2 × Ar), 127.5 (2 × Ar), 97.8 (d, $J$ = 5.1 Hz, C-1), 77.0 (C-4), 72.8 (d, $J$ = 7.0 Hz, C-2), 72.3 (C-5), 69.2 (d, $J$ = 5.3 Hz, Ph*C*H$_2$), 69.1 (d, $J$ = 5.3 Hz, Ph*C*H$_2$), 63.1 (C-6), 28.3 (C-3); HRMS (ESI) *m/z* Calcd. for (M + Na) $C_{41}H_{37}O_{11}NaP$: 759.1966. Found: 759.1970.

**3-deoxy-α-D-*xylo*-hexofuranosyl-1-phosphate triethylammonium acetate salt (14).** Compound **36** (90 mg, 0.12 mmol) was deprotected using the general deprotection procedure and lyophilized to give **14** (33.7 mg, 58%) as a colorless oil. $R_f$ 0.21 (10:2:1 $CH_3OH$–$NH_4OH$–$H_2O$); $[\alpha]_D$ +33.5 (*c* 0.19, $H_2O$); $^1$H NMR (500 MHz, $D_2O$, $\delta_H$) 5.45 (dd, 1 H, $J$ = 5.0, 4.1 Hz, H-1), 4.28 (dddd, 1 H, $J$ = 10.7, 7.4, 4.1, 2.0 Hz, H-2), 4.10–4.04 (m, 1 H, H-4), 3.70–3.62 (m, 2 H, H-5, H-6a), 3.57–3.53 (m, 1 H, H-6b), 3.18 (q, 8 H, $J$ = 7.3, 1.3 × ($CH_3CH_2$)$_3$N), 2.27 (ddd, 1 H, $J$ = 11.8, 7.4, 6.6 Hz, H-3a), 1.86–1.78 (m, 1 H, H-3b), 1.26 (t, 12H, $J$ = 7.3, 1.3 × ($CH_3CH_2$)$_3$N); $^{13}$C NMR (125 MHz, $D_2O$, $\delta_C$) 97.4 (d, $J$ = 5.9 Hz, C-1), 79.1 (C-4), 75.2 (C-5), 72.6 (d, $J$ = 7.7 Hz, C-2), 63.4 (C-6),

47.5 (1.3 × $(CH_3CH_2)_3N$), 31.7 (C-3), 9.1 (1.3 × $(CH_3CH_2)_3N$); HRMS (ESI) *m/z* Calcd. for ($M^-$) $C_6H_{12}O_8P$: 243.0264. Found: 243.0263.

**Methyl 2,5,6-tri-*O*-benzoyl-3-*O*-methyl-β-D-galactofuranoside (37).** To a solution of **26** (550 mg, 2.22 mmol) in 1:1 $CH_2Cl_2$–$CH_3OH$ (30 mL) was added 10% HCl in $CH_3OH$ (0.5 mL). The solution was stirred at room temperature for 1 h, and then concentrated. The resulting residue was dissolved in dry pyridine (30 mL) and benzoyl chloride (1.0 mL, 8.0 mmol) was added dropwise at under 0 °C. After 12 h, the pyridine was removed by evaporation, and the resulting residue was dissolved in EtOAc (50 mL). The solution was washed with 1 M HCl (30 mL), satd aq. $NaHCO_3$ solution (30 mL), brine (30 mL), dried ($MgSO_4$), filtered, and concentrated to afford a yellow syrup. The syrup was purified by column chromatography (6:1 hexane–EtOAc) to give **37** (1.0 g, 83%) as a white foam. $R_f$ 0.4 (4:1 hexane–EtOAc); $[\alpha]_D$ –11.4 (*c* 1.6, $CH_2Cl_2$); $^1$H NMR (500 MHz, $CDCl_3$, $\delta_H$) 8.08–7.88 (m, 6 H, Ar), 7.56–7.29 (m, 9 H, Ar), 5.87 (ddd, 1 H, *J* = 7.5, 4.7, 3.5 Hz, H-5), 5.30 (s, 1 H, H-2), 5.14 (s, 1 H, H-1), 4.73 (dd, 1 H, *J* = 11.7, 4.7 Hz, H-6), 4.69 (dd, 1 H, *J* = 11.7, 7.5 Hz, H-6), 4.44 (dd, 1 H, *J* = 5.5, 3.5 Hz, H-4), 3.90 (d, 1 H, *J* = 5.5 Hz, H-3), 3.51 (s, 3 H, OC*H*$_3$), 3.44 (s, 3 H, OC*H*$_3$); $^{13}$C NMR (125 MHz, $CDCl_3$, $\delta_C$) 166.1 (*C*=O), 166.0 (*C*=O), 165.3 (*C*=O), 133.3 (Ar), 133.2 (Ar), 133.1 (Ar), 129.8 (2 × Ar), 129.7 (4 × Ar), 129.6 (Ar), 129.5 (Ar), 129.1 (Ar), 128.4 (4 × Ar), 128.3 (2 × Ar), 107.1 (C-1), 86.0 (C-3), 81.6 (C-4), 80.9 (C-2), 70.5 (C-5), 63.5 (C-6), 58.8 (O*C*H$_3$), 54.9 (O*C*H$_3$); HRMS (ESI) *m/z* Calc. for (M + Na) $C_{29}H_{28}O_9Na$: 543.1625. Found: 543.1625.

**Dibenzyl 2,5,6-tri-*O*-benzoyl-3-*O*-methyl-α-D-galactofuranosyl-1-phosphate (38).** Prepared from compound **37** (162 mg, 0.31) as described in the general glycosylation procedure to provide **38** (114 mg, 41%) as a colorless oil. $R_f$ 0.30 (2:1

hexane–EtOAc); $[\alpha]_D$ +55.7 (*c* 0.3, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$, $\delta_H$) 8.16–7.98 (m, 6 H, Ar), 7.55–7.05 (m, 19 H, Ar), 6.23 (dd, 1 H, *J* = 5.7, 4.4 Hz, H-1), 5.80 (ddd, 1 H, *J* = 7.0, 4.6, 4.2 Hz, H-5), 5.39 (ddd, 1 H, *J* = 7.7, 4.4, 2.1 Hz, H-2), 4.99 (dd, 1 H, *J* = 11.7, 7.3 Hz, PhC$H_2$), 4.92 (dd, 1 H, *J* = 11.8, 8.3 Hz, PhC$H_2$), 4.91 (dd, 1 H, *J* = 11.7, 6.8 Hz, PhC$H_2$), 4.80 (dd, 1 H, *J* = 11.8, 7.8 Hz, PhC$H_2$), 4.69 (dd, 1 H, *J* = 11.8, 4.6 Hz, H-6a), 4.58 (dd, 1 H, *J* = 11.8, 7.0 Hz, H-6b), 4.43 (dd, 1 H, *J* = 7.4, 4.2 Hz, H-4), 4.35 (dd, 1 H, *J* = 7.7, 7.4 Hz, H-3), 3.50 (s, 3 H, OC$H_3$); $^{13}$C NMR (100 MHz, $CDCl_3$, $\delta_C$) 166.0 (*C*=O), 166.0 (*C*=O), 165.4 (*C*=O), 135.5 (d, *J* = 7.4 Hz, Ar), 135.4 (d, *J* = 7.8 Hz, Ar), 133.6 (Ar), 133.3 (Ar), 133.2 (Ar), 130.1 (2 × Ar), 129.8 (2 × Ar), 129.7 (2 × Ar), 129.6 (Ar), 129.3 (Ar), 128.8 (Ar), 128.5 (2 × Ar), 128.5 (2 × Ar), 128.4 (2 × Ar), 128.4 (2 × Ar), 128.3 (2 × Ar), 128.3 (Ar), 128.2 (Ar), 127.8 (2 × Ar), 127.6 (2 × Ar), 97.8 (d, *J* = 5.3 Hz, C-1), 80.2 (C-3), 79.7 (C-4), 77.9 (d, *J* = 7.0 Hz, C-2), 70.8 (C-5), 69.2 (d, *J* = 5.3 Hz, Ph*C*$H_2$), 69.1 (d, *J* = 5.1 Hz, Ph*C*$H_2$), 62.9 (C-6), 58.8 (O*C*$H_3$); HRMS (ESI) *m/z* Calcd. for (M + Na) $C_{42}H_{39}O_{12}NaP$: 789.2071. Found: 789.2079.

**3-*O*-Methyl-α-D-galactofuranosyl-1-phosphate triethylammonium acetate salt (15).** Compound **38** (111 mg, 0.145 mmol) was deprotected following the general deprotection procedure and lyophilized to yield **15** (37 mg, 54%) as a colorless oil: $R_f$ 0.28 (10:2:1 $CH_3OH$–$NH_4OH$–$H_2O$); $[\alpha]_D$ +33.2 (*c* 0.2, $H_2O$); $^1$H NMR (500 MHz, $D_2O$, $\delta_H$) 5.49 (dd, 1 H, *J* = 4.5, 4.5 Hz, H-1), 4.23 (ddd, 1 H, *J* = 7.4, 4.5, 2.0 Hz, H-2), 4.01 (dd, 1 H, *J* = 7.4, 6.5 Hz, H-3), 3.85 (dd, 1 H, *J* = 6.5, 5.0 Hz, H-4), 3.77 (ddd, 1 H, *J* = 7.0, 5.0, 4.5 Hz, H-5), 3.67 (dd, 1 H, *J* = 11.8, 4.5 Hz, H-6a), 3.59 (dd, 1 H, *J* = 11.8, 7.0 Hz, H-6b), 3.52 (s, 3 H, OC$H_3$), 3.18 (q, 9 H, *J* = 7.3, 1.5 × $(CH_3CH_2)_3N$), 1.26 (t, 13.5 H, *J* = 7.3 Hz, 1.5 × $(CH_3CH_2)_3N$); $^{13}$C NMR (125 MHz, $D_2O$, $\delta_c$) 97.9 (d, *J* = 5.7 Hz, C-1),

84.5 (C-3), 81.6 (C-4), 77.4 (d, $J$ = 7.7 Hz, C-2), 73.3 (C-5), 63.2 (C-6), 58.8 (O*C*H$_3$), 47.5 (1.5 × (CH$_3$*C*H$_2$)$_3$N), 9.1 (1.5 × (*C*H$_3$CH$_2$)$_3$N); HRMS (ESI) *m/z* Calcd. for (M$^-$) $C_7H_{14}O_9P$: 273.0370. Found: 273.0372.

**Methyl 2,3-di-*O*-benzyl-α-D-galactofuranoside (39).** To a solution of **22** (511 mg, 2.18 mmol) in DMF (20 mL) at 0 °C was added BnBr (0.65 mL, 5.46 mmol) and 60% NaH (262 mg, 6.54 mmol). After 12 h, the solution was quenched by the addition of $CH_3OH$ (3 mL), and then concentrated. The resulting residue was dissolved in a solution of 1:1 $CH_2Cl_2$–$CH_3OH$ (30 mL). To this mixture was added 10% HCl in $CH_3OH$ (1 mL). The solution was stirred at room temperature for 1 h before being neutralized with $Et_3N$ (2 mL). The solution was concentrated and the resulting crude residue was dissolved in EtOAc (30 mL), washed with distilled water (20 mL), brine (20 mL), dried ($MgSO_4$), filtered, and concentrated. The resulting syrup was purified by column chromatography to give **39** (709 mg, 87%) as an oil. $R_f$ 0.46 (25:1 $CH_2Cl_2$–MeOH); $[\alpha]_D$ +49.9 (*c* 1.6, $CH_3OH$); $^1$H NMR (500 MHz, $CDCl_3$, $\delta_H$) 7.40–7.24 (m, 10 H, Ar), 4.78–4.60 (m, 4 H, 2 × PhC*H*$_2$), 4.23 (s, 1 H, H-1), 4.35 (dd, 1 H, $J$ = 7.2, 6.3 Hz, H-3), 4.07 (dd, 1 H, $J$ = 7.2, 4.4 Hz, H-2), 4.03 (dd, 1 H, $J$ = 6.3, 3.9 Hz, H-4), 3.68–3.60 (m, 3 H, H-5, H-6a/H-6b), 3.42 (s, 3 H, OC*H*$_3$), 2.82 (d, 1 H, $J$ = 6.8 Hz, O*H*), 2.16 (d, $J$ = 12.1 Hz, O*H*); $^{13}$C NMR (125 MHz, $CDCl_3$, $\delta_C$) 137.7 (Ar), 137.3 (Ar), 128.5 (2 × Ar), 128.4 (2 × Ar), 128.2 (2 × Ar), 128.1 (Ar), 127.9 (Ar), 127.8 (2 × Ar), 102.0 (C-1), 84.1 (C-2), 81.9 (C-4), 81.1 (C-3), 72.8 (Ph*C*H$_2$), 72.5 (Ph*C*H$_2$), 72.2 (C-5), 64.2 (C-6), 56.0 (O*C*H$_3$); HRMS (ESI) *m/z* Calc. for (M + Na) $C_{21}H_{26}O_6Na$: 397.1621. Found: 397.1621.

**Methyl 2,3-di-*O*-benzyl-6-*O*-trityl-α-D-galactofuranoside (40).** To a solution of **39** (101 mg, 0.27 mmol) in dry pyridine (3 mL) was added trityl chloride (102 mg, 0.36 mmol) and DMAP (11 mg, 0.06 mmol). The solution was stirred at 40 °C for 12 h, and

then concentrated to afford a crude compound that was dissolved in EtOAc (10 mL). This solution was washed with 1 M HCl (5 ml), satd aq. $NaHCO_3$ solution (5 mL), brine (5 mL), dried ($MgSO_4$), and filtered. The filtrate was concentrated and the resulting syrup purified by column chromatography (6:1 hexane–EtOAc) to give **40** (163 mg, 98%) as an oil. $R_f$ 0.39 (4:1 hexane–EtOAc); $[\alpha]_D$ +27.7 (*c* 2.1, $CH_2Cl_2$); $^1$H NMR (500 MHz, $CDCl_3$, $\delta_H$) 7.50–7.20 (m, 25 H, Ar), 4.70–4.49 (m, 4 H, 2 × PhC*H*$_2$), 4.69 (d, 1 H, *J* = 4.4 Hz, H-1), 4.33 (dd, 1 H, *J* = 7.1, 6.0 Hz, H-3), 4.20 (dd, 1 H, *J* = 6.0, 3.8 Hz, H-4), 4.18 (dd, 1 H, *J* = 7.1, 4.4 Hz, H-2), 3.71 (ddd, 1 H, *J* = 6.5, 6.2, 3.8 Hz, H-5), 3.36 (s, 3 H, OC*H*$_3$), 3.24 (dd, 1 H, *J* = 9.4, 6.5 Hz, H-6a), 3.18 (dd, 1 H, *J* = 9.4, 6.2 Hz, H-6b); $^{13}$C NMR (125 MHz, $CDCl_3$, $\delta_C$) 143.9 (3 × Ar), 138.0 (Ar), 137.5 (Ar), 128.7 (5 × Ar), 128.5 (2 × Ar), 128.4 (2 × Ar), 128.2 (2 × Ar), 128.0 (Ar), 127.9 (Ar), 127.9 (Ar), 127.7 (5× Ar), 127.7 (3 × Ar), 126.9 (3 × Ar), 101.8 (C-1), 86.8 (Ph$_3$*C*), 84.8 (C-2), 81.8 (C-4), 81.5 (C-3), 72.7 (Ph*C*H$_2$), 77.6 (Ph*C*H$_2$), 71.1 (C-5), 64.6 (C-6), 55.9 (O*C*H$_3$); HRMS (ESI) *m/z* Calc. for (M + Na) $C_{40}H_{40}O_6Na$: 639.2714. Found: 639.2717.

**Methyl 2,3-di-*O*-benzyl-5-*O*-methyl-6-*O*-trityl-α-D-galactofuranoside (41).** To a solution of **40** (2.93 g, 4.75 mmol) in DMF (50 mL) at 0 °C was slowly added 60% NaH (380 mg, 9.50 mmol). After 5 min, $CH_3I$ (354 μL, 5.70 mmol) was added to the solution, which was stirred for 3 h at room temperature, and quenched by the addition of $CH_3OH$ (5 mL). The reaction mixture was then concentrated to afford a crude compound that was dissolved in EtOAc (100 mL), washed with distilled water (60 mL × 2), brine (60 mL), dried ($MgSO_4$), filtered, and the organic layer was concentrated. The resulting syrup was purified by column chromatography (6:1 hexane–EtOAc) to give **41** (2.84 g, 97%) as an amorphous solid. $R_f$ 0.44 (4:1 hexane–EtOAc); $[\alpha]_D$ +47.5 (*c* 0.4, $CH_2Cl_2$); $^1$H NMR (500 MHz, $CDCl_3$, $\delta_H$) 7.47–7.07 (m, 25 H, Ar), 4.73 (d, 1 H, *J* = 4.2 Hz, H-1),

4.63–4.25 (m, 4 H, 2 × PhC*H*$_2$), 4.06 (dd, 1 H, *J* = 7.2, 6.7 Hz, H-3), 4.01 (dd, 1 H, *J* = 7.2, 4.2 Hz, H-2), 3.86 (dd, 1 H, *J* = 6.7, 6.7 Hz, H-4), 3.57 (s, 3 H, OC*H*$_3$), 3.42 (ddd, 1 H, *J* = 6.7, 6.7, 3.5 Hz, H-5), 3.37 (s, 3 H, OC*H*$_3$), 3.26 (dd, 1 H, *J* = 10.4, 3.5 Hz, H-6a), 3.22 (dd, 1 H, *J* = 10.4, 6.7 Hz, H-6b); $^{13}$C NMR (125 MHz, $CDCl_3$, $\delta_C$) 144.1 (3 × Ar), 138.2 (Ar), 137.6 (Ar), 128.8 (6 × Ar), 128.4 (2 × Ar), 128.3 (2 × Ar), 128.2 (2 × Ar), 128.0 (Ar), 127.8 (6 × Ar), 127.6 (2 × Ar), 127.5 (Ar), 126.9 (3 × Ar), 101.3 (C-1), 87.0 (Ph$_3$*C*), 84.4 (C-2), 82.8 (C-5), 81.9 (C-3), 80.5 (C-4), 72.5 (Ph*C*H$_2$), 72.2 (Ph*C*H$_2$), 63.9 (C-6), 59.6 (O*C*H$_3$), 54.9 (O*C*H$_3$); HRMS (ESI) *m/z* Calc. for (M + Na) $C_{41}H_{42}O_6Na$: 653.2875. Found: 653.2874.

**Methyl 2,3,6-tri-*O*-benzoyl-5-*O*-methyl-α-D-galactofuranoside (42).** To **41** (2.81 g, 4.45 mmol) in a solution of 3:1 EtOAc–$CH_3OH$ (50 mL) was added 20% $Pd(OH)_2$ (280 mg). The solution was stirred under $H_2$ (1 atm) for 12 h. The catalyst was removed by filteration, and the filtrate concentrated to afford a syrup that was dissolved in 1:1 $CH_2Cl_2$–$CH_3OH$ (50 mL). To the solution was added 10% HCl in $CH_3OH$ (0.5 mL) and the mixture was stirred at room temperature for 1 h. The reaction mixture was neutralized by adding $Et_3N$ (2 mL), and concentrated to afford a crude compound that was dissolved in pyridine (60 mL) at 0 °C. To the solution was added benzoyl chloride (2.24 mL, 16.02 mmol) dropwise. The reaction mixture was stirred for 12 h while warming to room temperature. The excess benzoyl chloride was quenched by adding $CH_3OH$ and the solvent evaporated to give a residue that was dissolved in EtOAc (100 mL), washed with 1 M HCl (50 mL), satd aq. $NaHCO_3$ (50 mL), brine (50 mL), dried ($MgSO_4$), and filtered. After the filtrate was concentrated, the resulting syrup was purified by column chromatography (6:1 hexane–EtOAc) to give **42** (2.03 g, 88%) as an amorphous solid. $R_f$ 0.36 (4:1 hexane–EtOAc); $[\alpha]_D$ +120.4 (*c* 0.6, $CH_2Cl_2$); $^1$H NMR

(600 MHz, $CDCl_3$, $\delta_H$), 8.14–7.94 (m, 6 H, Ar), 7.63–7.34 (m, 9 H, Ar), 6.23 (dd, 1 H, *J* = 7.3, 6.0 Hz, H-3), 5.45 (dd, 1 H, *J* = 7.3, 4.5 Hz, H-2), 5.34 (d, 1 H, *J* = 4.5 Hz, H-1), 4.60 (dd, 1 H, *J* = 12.0, 4.2 Hz, H-6a), 4.51 (dd, 1 H, *J* = 12.0, 5.0 Hz, H-6b), 4.37 (t, 1 H, *J* = 6.0 Hz, H-4), 3.80 (ddd, 1 H, *J* = 6.0, 5.0, 4.2 Hz, H-5), 3.66 (OC*H*$_3$), 3.47 (OC*H*$_3$); $^{13}$C NMR (125 MHz, $CDCl_3$, $\delta_C$) 166.1 (*C*=O), 165.9 (*C*=O), 165.6 (*C*=O), 133.7 (Ar), 133.3 (Ar), 132.9 (Ar), 130.1 (Ar), 130.0 (2 × Ar), 129.8 (2 × Ar), 129.7 (Ar), 129.6 (2 × Ar), 129.1 (2 × Ar), 128.4 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (2 × Ar), 100.9 (C-1), 79.7 (C-4/C-5), 79.6 (C-5/C-4), 77.6 (C-2), 74.5 (C-3), 63.0 (C-6), 59.2 (O*C*H$_3$), 55.5 (O*C*H$_3$); HRMS (ESI) *m/z* Calc. for (M + Na) $C_{29}H_{28}O_9Na$: 543.1627. Found: 543.1626.

**Dibenzyl 2,3,6-tri-*O*-benzoyl-5-*O*-methyl-α-D-galactofuranosyl-1-phosphate (43).** Prepared from compound **42** (149 mg, 0.29 mmol) as described in the general glycosylation procedure above to give **43** (89 mg, 41%) as a colorless oil. $R_f$ 0.22 (2:1 hexanes–EtOAc); $[\alpha]_D$ +53.4 (*c* 0.2, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$, $\delta_H$) 8.08–7.93 (m, 6 H, Ar), 7.53–7.13 (m, 19 H, Ar), 6.30 (dd, 1 H, *J* = 5.5, 4.4 Hz, H-1), 6.26 (dd, 1 H, *J* = 8.0, 7.0 Hz, H-3), 5.68 (ddd, 1 H, *J* = 8.0, 4.4, 2.3 Hz, H-2), 5.11 (dd, 1 H, *J* = 10.6, 5.9 Hz, PhC*H*$_2$), 5.06 (dd, 1 H, *J* = 10.7, 6.4 Hz, PhC*H*$_2$), 4.97 (dd, 1 H, *J* = 11.7, 6.8 Hz, PhC*H*$_2$), 4.90 (dd, 1 H, *J* = 11.7, 7.6 Hz, PhC*H*$_2$), 4.59 (dd, 1 H, *J* = 12.0, 4.6 Hz, H-6a), 4.47 (dd, 1 H, *J* = 12.0, 5.2 Hz, H-6b), 4.46 (dd, 1 H, *J* = 7.0, 5.1 Hz H-4), 3.80 (app. q, 1 H, *J* = 4.9 Hz, H-5), 3.58 (s, 3 H, OC*H*$_3$); $^{13}$C NMR (100 MHz, $CDCl_3$, $\delta_C$) 166.0 (*C*=O), 165.6 (2 × *C*=O), 135.8 (d, *J* = 8.0 Hz, Ar), 135.5 (d, *J* = 7.8 Hz, Ar), 133.6 (2 × Ar), 133.0 (Ar), 130.0 (2 × Ar), 129.9 (2 × Ar), 129.7 (Ar), 129.6 (2 × Ar), 128.8 (Ar), 128.7 (Ar), 128.5 (2 × Ar), 128.5 (4 × Ar), 128.4 (2 × Ar), 128.3 (4 × Ar), 127.7 (2 × Ar), 127.6 (2 × Ar), 97.4 (d, $J_{1,P}$ = 2.3 Hz, C-1), 81.0 (C-4), 78.5 (C-5), 76.6 (d, $J_{2,P}$ = 7.6 Hz,

C-2), 73.1 (C-3), 69.2 (d, $J$ = 5.6 Hz, O*C*H$_2$Ph), 69.2 (d, $J$ = 5.3 Hz, O*C*H$_2$Ph), 63.0 (C-6), 59.3 (d, $J$ = 2.4 Hz, O*C*H$_3$); HRMS (ESI) *m/z* Calcd. for (M + Na) $C_{42}H_{39}O_{12}NaP$: 789.2071. Found: 789.2082.

**5-*O*-methyl-α-D-galactofuranosyl-1-phosphate triethylammonium acetate salt (17).** Deprotected compound **43** (88 mg, 0.12 mmol) as described in the general procedure above. The desired fractions were lyopilized to give **17** (33 mg, 64%) as a colorless oil. $R_f$ 0.19 (10:2:1 $CH_3OH$–$NH_4OH$–$H_2O$); $[\alpha]_D$ +31.5 (*c* 0.3, $H_2O$); $^1$H NMR (500 MHz, $D_2O$, $\delta_H$) 5.52 (dd, 1 H, $J$ = 6.3, 4.1 Hz, H-1), 4.14 (dd, 1 H, $J$ = 8.2, 7.2 Hz, H-3), 4.10 (ddd, 1 H, $J$ = 8.2, 4.1, 1.4 Hz, H-2), 3.87–3.82 (m, 2H, H-4, H-6a), 3.65 (dd, 1 H, $J$ = 12.4, 5.7 Hz, H-6b), 3.54–3.50 (m, 1H, H-5), 3.53 (s, 3 H, OC*H*$_3$), 3.19 (q, 8 H, $J$ = 7.3 Hz, 1.3 × ($CH_3CH_2$)$_3$N), 1.27 (t, 12 H, $J$ = 7.3 Hz, 1.3 × ($CH_3CH_2$)$_3$N); $^{13}$C NMR (125 MHz, $D_2O$, $\delta_c$) 97.7 (d, $J$ = 5.1 Hz, C-1), 83.6 (C-5), 81.5 (C-4), 77.4 (d, $J$ = 6.8 Hz, C-2), 74.9 (C-3), 60.3 (C-6), 59.2 (O*C*H$_3$), 47.6 (1.3 × ($CH_3CH_2$)$_3$N), 9.1 (1.3 × ($CH_3CH_2$)$_3$N); HRMS (ESI) *m/z* Calcd. for ($M^-$) $C_7H_{14}O_9P$: 273.0370. Found: 273.0372.

**Methyl 2,3,5-tri-*O*-benzyl-6-*O*-trityl-β-D-galactofuranoside (44).** To a solution of **20** (2.20 g, 11.4 mmol) in dry pyridine (70 mL) was added trityl chloride (3.8 g, 13.6 mmol) and DMAP (140 mg, 1.2 mmol). The solution was stirred for 12 h at 40 °C and quenched with the addition of $CH_3OH$ (5 mL). After concentration, the resulting residue was dissolved in dry DMF (60 mL) at 0 °C and 60% NaH (1.63 g, 41.0 mmol) was added. After 15 min, benzyl bromide (4.8 mL, 41.0 mmol) was added and the solution was stirred at room temperature for 12 h. Then, MeOH was added to quench any excess NaH. The solvent was evaporated to give a crude compound that was dissolved in EtOAc (100 mL), washed with distilled water (40 mL × 2) and brine (40 mL), dried ($MgSO_4$), filtered, and the solvent was evaporated to afford a syrup. The syrup was purified by column

chromatography (9:1 hexane–EtOAc) to give **44** (3.74 g, 46%, α:β 0.3:1) as a foam. $R_f$ 0.5 (6:1 hexane–EtOAc); $^1$H NMR (500 MHz, $CDCl_3$, $\delta_H$) 7.50–7.10 (m, 39 H, Ar), 4.93 (s, 1 H, H-1β), 4.88–4.26 (m, 8.22 H, 3 × PhC$H_2$-α, 3 × PhC$H_2$-β), 4.72 (d, 0.37 H, H-1α), 4.20–4.13 (m, 1.37 H, H-3α, H-4β), 4.02 (dd, 0.37 H, $J$ = 7.4, 4.3 Hz, H-2α), 3.98–3.94 (m, 2.37 H, H-4α, H-3β, H-2β), 3.74–3.70 (m, 1.37 H, H-5α, H-5β), 3.45 (dd, 1 H, $J$ = 9.9, 6.6 Hz, H-6aβ), 3.40–3.32 (m, 4.74 H, 2 × H-6aα, H-6bβ, OC$H_3$-β), 3.32 (s, 3 H, OC$H_3$-α); $^{13}$C NMR (125 MHz, $CDCl_3$, $\delta_C$) 144.1 (3 × Ar), 144.0 (3 × Ar), 138.9 (Ar), 138.4 (Ar), 138.2 (Ar), 137.9 (Ar), 137.7 (Ar-β), 137.6 (Ar), 129.1 (Ar), 128.8 (Ar), 128.7 (Ar), 128.4 (Ar), 128.3(8) (Ar), 128.3(2) (Ar), 128.2(8) (Ar), 128.2(5) (Ar), 128.2(4) (Ar), 128.2(1) (Ar), 128.1 (Ar), 127.9 (Ar), 127.8(5) (Ar), 127.8(3) (Ar), 127.8(0) (Ar), 127.7(6) (Ar), 127.7(0) (Ar), 127.6(4) (Ar), 127.6(2) (Ar), 127.5(2) (Ar), 127.4(7) (Ar), 127.4(3) (Ar), 127.3 (Ar), 127.1 (Ar), 127.0 (Ar), 106.9 (C-1β), 101.1 (C-1α), 88.4 (C-2β), 87.0 (2 × $Ph_3C$), 84.1 (C-2α), 82.9 (C-3β), 81.5 (C-3α), 81.1 (C-4β), 80.5 (C-4α), 80.5 (C-5α), 76.9 (C-5β), 73.6 (Ph$C$H$_2$-α), 73.3 (Ph$C$H$_2$-β), 72.5 (Ph$C$H$_2$-α), 72.2 (Ph$C$H$_2$-α), 72.1 (Ph$C$H$_2$-β), 71.8 (Ph$C$H$_2$-β), 63.2 (C-6α), 63.8 (C-6β), 54.9 (OC$H_3$-α), 54.7 (OC$H_3$-β); HRMS (ESI) *m/z* Calc. for (M + Na) $C_{47}H_{46}O_6Na$: 729.3184. Found: 729.3186.

**Methyl 2,3,5-tri-*O*-benzyl-β-D-galactofuranoside (45).** To **44** (1.22 g, 1.73 mmol) in a solution of 1:1 $CH_3OH$–$CH_2Cl_2$ (30 mL) was added 10% HCl in $CH_3OH$ (1 mL). The solution was stirred at room temperature for 1 h, and then $Et_3N$ (2 mL) was added to neutralize the solution. The reaction mixture was concentrated and the resulting residue was dissolved in EtOAc (60 mL). The solution was washed with water (40 mL), brine (40 mL), dried ($MgSO_4$), and filtered. The filtrate was concentrated to give a syrup that was purified by column chromatography (5:2 hexane–EtOAc) to yield **45** (701 mg,

87%, α:β = 0.3:1) as a solid. $R_f$ 0.74 (1:1 hexane–EtOAc); $^1$H NMR (500 MHz, $CDCl_3$, $\delta_H$) 7.42–7.24 (m, 19.5 H, Ar), 4.90 (s, 1 H, H-1β), 4.82–4.42 (m, 9.22 H, H-1α, 3 × PhC*H*$_2$-α, 3 × PhC*H*$_2$-β), 4.31–4.20 (m, 1.38 H, H-3α, H-4β), 4.10–3.98 (m, 2.74 H, H-2α, H-4α, H-2β, H-3β), 3.80–3.64 (m, 3.37 H, H-5β, H-6aα, H-6aβ/H-6bβ), 3.63–3.53 (m, 0.74 H, H-5α, H-6bα), 3.39 (s, 3 H, OC*H*$_3$-β), 3.38 (s, 1.11 H, OC*H*$_3$-α); $^{13}$C NMR (125 MHz, $CDCl_3$, $\delta_C$) 138.4 (Ar), 138.2 (Ar), 137.7 (Ar), 137.4 (2 × Ar), 137.3 (Ar), 128.5 (3 × Ar), 128.4 (Ar), 128.4 (6 × Ar), 128.3 (2 × Ar), 128.1 (2 × Ar), 128.1 (2 × Ar), 128.1 (Ar), 128.0 (2 × Ar), 127.9 (3 × Ar), 127.9 (2 × Ar), 127.9 (Ar), 127.8 (2 × Ar), 127.7 (2 × Ar), 127.7 (Ar), 107.0 (C-1β), 101.4 (C-1α), 87.7 (C-2β), 83.9 (C-2α), 83.1 (C-3β), 82.1 (C-4β), 81.1 (C-3α), 80.8 (C-4α/C-5α), 80.8 (C-5α/C-4α), 78.2 (C-5β), 72.8 (PhC*H*$_2$-α), 72.8 (PhC*H*$_2$-β), 72.6 (PhC*H*$_2$-α), 72.3 (PhC*H*$_2$-α), 72.2 (PhC*H*$_2$-β), 72.0 (PhC*H*$_2$-β), 62.2 (C-6β), 61.5 (C-6α), 55.3 (O*C*H$_3$-α), 54.8 (O*C*H$_3$-β); HRMS (ESI) *m/z* Calc. for (M + Na) $C_{28}H_{32}O_6Na$: 487.2092. Found: 487.2091.

**Methyl 2,3,5-tri-*O*-benzyl-6-*O*-methyl-β-D-galactofuranoside (46).** To a solution of **45** (914 mg, 1.97 mmol) in DMF (20 mL) at 0 °C was added 60% NaH (160 mg, 3.96 mmol). After 10 min, $CH_3I$ (160 μL, 2.56 mmol) was added to the solution. The reaction mixture was stirred at room temperature for 3 h, and $CH_3OH$ was added to quench the excess NaH. DMF was removed by evaporation, and the resulting crude compound was dissolved in EtOAc (50 mL), which was washed with water (30 mL × 2), brine (30 mL × 2), dried ($MgSO_4$), filtered, and concentrated to give a syrup that was purified by column chromatography (7:1 hexane–EtOAc) to give **46** (930 mg, 98%, α:β = 0.3:1) as a foam. $R_f$ 0.34 (6:1 hexane–EtOAc); $^1$H NMR (500 MHz, $CDCl_3$, $\delta_H$) 7.41–7.24 (m, 19.5 H, Ar), 4.98 (s, 1 H, H-1β), 4.78–4.30 (m, 8.1 H, H-1α, 3 × PhC*H*$_2$-α, 3 × PhC*H*$_2$-β), 4.32 (dd, 0.3 H, *J* = 7.5, 7.3 Hz, H-3α), 4.13 (dd, 1 H, *J* = 6.8, 3.5 Hz, H-4β),

4.06 (dd, 0.3 H, $J$ = 7.5, 4.3 Hz, H-2α), 4.01 (dd, 1 H, $J$ = 6.8, 2.8 Hz, H-3β), 4.00–3.97 (m, 1.3 H, H-4α, H-2β), 3.74 (ddd, 1 H, $J$ = 6.4, 4.9, 3.5 Hz, H-5β), 3.63–3.43 (m, 2.9 H, H-5α, H-6aα/H-6bα, H-6aβ/H-6bβ), 3.38 (s, 3 H, OC$H_3$-β), 3.36 (s, 3.9 H, OC$H_3$-α, OC$H_3$-β), 3.31 (s, 0.9 H, OC$H_3$-α); $^{13}$C NMR (125 MHz, $CDCl_3$, $\delta_C$) 138.8 (Ar), 138.5 (Ar), 138.2 (Ar), 137.8 (Ar), 137.7 (Ar), 137.6 (Ar), 128.4(4) (2 × Ar), 128.4(0) (2 × Ar), 128.3 (2 × Ar), 128.2(4) (3 × Ar), 128.2(0) (4 × Ar), 128.0(2) (2 × Ar), 128.0(0) (2 × Ar), 127.8(3) (3 × Ar), 127.7(8) (2 × Ar), 127.7(6) (Ar), 127.7(3) (2 × Ar), 127.7(0) (Ar), 127.6 (3 × Ar), 127.4 (Ar), 106.9 (C-1β), 101.0 (C-1α), 88.2 (C-4β), 84.2 (C-2α), 82.7 (C-3β), 81.3 (C-2β), 80.7 (C-3α/C-4α), 80.4 (C-4α/C-3α), 79.1 (C-5α), 76.4 (C-5β), 73.3 (C-6β/Ph$C$H$_2$-β), 73.2 (Ph$C$H$_2$-β/C-6β), 72.9 (Ph$C$H$_2$-α), 72.6 (2 × C, C-6α, Ph$C$H$_2$-α), 72.1 (Ph$C$H$_2$-α), 71.8 (Ph$C$H$_2$-β), 59.1(4) (O$C$H$_3$-β), 59.1(2) (O$C$H$_3$-α), 55.0 (O$C$H$_3$-α), 54.7 (O$C$H$_3$-β); HRMS (ESI) *m/z* Calc. for (M + Na) $C_{29}H_{34}O_6Na$: 501.2250. Found: 501.2248.

**Methyl 2,3,5-tri-*O*-benzoyl-6-*O*-methyl-β-D-galactofuranoside (47).** To a solution of **46** (2.81 g, 5.90 mmol) in 3:1 EtOAc–MeOH (60 mL) was added 20% $Pd(OH)_2$ (350 mg). The solution was stirred under $H_2$ (1 atm) for 12 h. The catalyst was removed by filtration, and the filtrate was concentrated to afford a syrup that was then dissolved in pyridine (60 mL) and cooled to 0 °C. Benzoyl chloride (2.67 mL, 21.2 mmol) was slowly added into the solution. After 12 h, $CH_3OH$ (5 mL) was added to quench the excess benzoyl chloride, and the solvent was evaporated. The resulting residue was dissolved in EtOAc (100 mL), and washed with 1 M HCl solution (50 mL), satd. aq. $NaHCO_3$ solution (50 mL), brine (50 mL) and dried ($MgSO_4$). The dried organic layer was concentrated to give a syrup that was purified by column chromatography (6:1 hexane–EtOAc) to yield **47** (2.76 g, 85%, α:β = 0.3:1) as a foam. $R_f$ 0.25 (6:1 hexane–

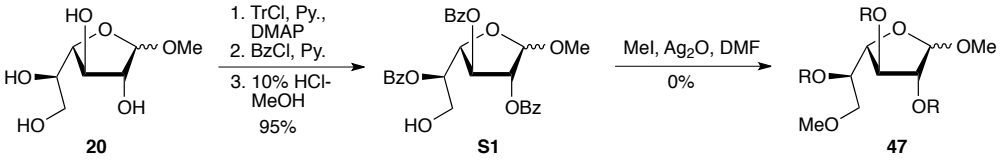
EtOAc); $^{1}$H NMR (500 MHz, $CDCl_3$, $\delta_H$) 8.20–7.95 (m, 7.8 H, Ar), 7.61–7.25 (m, 11.7 H, Ar), 6.20 (dd, 0.3 H, *J* = 7.4, 6.2 Hz, H-3α), 5.78 (ddd, 1 H, *J* = 6.0, 5.9, 4.0 Hz, H-5β), 5.60 (d, 1 H, *J* = 5.5 Hz, H-3β), 5.54 (m, 0.3 H, H-5α), 5.46 (m, 1.3 H, H-2α, H-2β), 5.36 (d, 0.3 H, *J* = 4.6 Hz, H-1α), 5.20 (s, 1 H, H-1β), 4.66 (dd, 1 H, *J* = 5.5, 4.0 Hz, H-4β), 4.61 (t, 0.3 H, *J* = 6.2 Hz, H-4α), 3.84 (dd, 1 H, *J* = 10.2, 6.0 Hz, H-6aβ), 3.82-3.77 (m, 1.3 H, H-6aα, H-6bβ), 3.74 (dd, 0.3 H, *J* = 10.4, 5.3 Hz, H-6bα), 3.50 (s, 3 H, OC*H*$_3$-β), 3.44 (s, 0.9 H, OC*H*$_3$-α), 3.38 (s, 3 H, OC*H*$_3$-β), 3.27 (s, 0.9 H, OC*H*$_3$-α); $^{13}$C NMR (125 MHz, $CDCl_3$, $\delta_C$) 165.9 (*C*=O), 165.9 (*C*=O), 165.8 (*C*=O), 165.6 (*C*=O), 165.5 (*C*=O), 165.4 (*C*=O), 133.4 (2 × Ar), 133.3(7) (Ar), 133.3(3) (Ar), 133.1 (Ar), 133.0 (Ar), 130.0 (2 × Ar), 129.9 (6 × Ar), 129.8(5) (2 × Ar), 129.8(3) (4 × Ar), 129.2(6) (Ar), 129.2(1) (2 × Ar), 129.1(7) (Ar), 129.1(1) (2 × Ar), 128.4(3) (4 × Ar), 128.4(0) (4 × Ar), 128.3 (4 × Ar), 106.6 (C-1β), 101.1 (C-1α), 82.4 (C-2β), 80.8 (C-4β), 78.4 (C-4α), 77.8 (C-2α), 77.5 (C-3β), 74.5 (C-3α), 73.1 (C-5α), 71.1 (C-5β), 70.9 (C-6β), 70.4 (C-6α), 59.2 (2 × C, O*C*H$_3$-α, O*C*H$_3$-β), 55.6 (O*C*H$_3$-α), 54.8 (O*C*H$_3$-β); HRMS (ESI) *m/z* Calc. for (M + Na) $C_{29}H_{28}O_9Na$: 543.1623. Found: 543.1625.

**Dibenzyl 2,3,5-tri-*O*-benzoyl-6-*O*-methyl-α-D-galactofuranosyl-1-phosphate (48).** Prepared from compound **47** (207 mg, 0.40 mmol) following the general glycosylation procedure with dibenzyl phosphate to give **48** (129 mg, 43%) as a colorless oil. $R_f$ 0.31 (2:1 hexanes–EtOAc); $[\alpha]_D$ +47.8 (*c* 0.3, $CHCl_3$); $^{1}$H NMR (400 MHz, $CDCl_3$, $\delta_H$) 8.16–7.99 (m, 6 H, Ar), 7.59–7.07 (m, 19 H, Ar), 6.37 (dd, 1 H, *J* = 5.7, 4.5 Hz, H-1), 6.20 (dd, 1 H, *J* = 7.6, 6.7 Hz, H-3), 5.75 (ddd, 1 H, *J* = 7.6, 4.5, 2.0 Hz, H-2), 5.59 (ddd, 1 H, *J* = 6.2, 5.0, 5.0 Hz, H-5), 5.06 (dd, 1 H, *J* = 11.8, 7.2 Hz, PhC*H*$_2$), 4.98 (dd, 1 H, *J* = 11.8, 6.3 Hz, PhC*H*$_2$), 4.97 (dd, 1 H, *J* = 11.8, 8.0 Hz, PhC*H*$_2$), 4.89 (dd, 1 H, *J* = 11.7, 7.6 Hz, PhC*H*$_2$), 4.75 (dd, 1 H, *J* = 6.7, 5.0 Hz, H-4), 3.76 (dd, 1 H, *J* = 10.1,

5.1 Hz, H-6a), 3.70 (dd, 1 H, *J* = 10.1, 6.2 Hz, H-6b), 3.27 (s, 3 H, OC*H*$_3$); $^{13}$C NMR (100 MHz, $CDCl_3$, $\delta_C$) 165.7 (*C*=O), 165.5 (*C*=O), 165.4 (*C*=O), 135.4 (d, *J* = 7.6 Hz, Ar), 135.4 (d, *J* = 8.1 Hz, Ar), 133.5 (Ar), 133.5 (Ar), 133.1 (Ar), 130.0 (2 × Ar), 129.9 (2 × Ar), 129.8 (2 × Ar), 129.5 (Ar), 128.8 (Ar), 128.6 (Ar), 128.4 (4 × Ar), 128.3 (6 × Ar), 128.3 (2 × Ar), 127.7 (2 × Ar), 127.6 (2 × Ar), 97.6 (d, *J* = 5.1 Hz, C-1), 79.8 (C-4), 76.6 (d, *J* = 7.7 Hz, C-2), 73.1 (C-3), 71.8 (C-5), 69.8 (C-6), 69.2 (d, *J* = 5.6 Hz, Ph*C*H$_2$), 69.1 (d, *J* = 5.5 Hz, Ph*C*H$_2$), 59.1 (O*C*H$_3$); HRMS (ESI) *m/z* Calcd. for (M + Na) $C_{42}H_{39}O_{12}NaP$: 789.2071. Found: 789.2081.

**6-*O*-Methyl-α-D-galactofuranosyl-1-phosphate triethylammonium acetate salt (19).** Compound **46** (123 mg, 0.16 mmol) was treated as described in the general deprotection procedure and lyophilized to give **19** (48 mg, 63%) as a colorless oil. $R_f$ 0.20 (10:2:1 $CH_3OH$–$NH_4OH$–$H_2O$); $[\alpha]_D$ +33.4 (*c* 0.2, $H_2O$); $^1$H NMR (400 MHz, $D_2O$, $\delta_H$) 5.49 (dd, 1 H, *J* = 4.7, 4.3 Hz, H-1), 4.20 (dd, 1 H, *J* = 8.3, 7.3 Hz, H-3), 4.10 (ddd, 1 H, *J* = 8.3, 4.3, 2.0 Hz, H-2), 3.86 (ddd, 1 H, *J* = 7.2, 5.2, 4.1 Hz, H-5), 3.76 (dd, 1 H, *J* = 7.3, 5.2 Hz, H-4), 3.59 (dd, 1 H, *J* = 10.7, 4.1 Hz, H-6a), 3.51 (dd, 1 H, *J* = 10.7, 7.2 Hz, H-6b), 3.38 (s, 3 H, OC*H*$_3$), 3.18 (q, 9 H, *J* = 7.3 Hz, 1.5 × ($CH_3CH_2$)$_3$N), 1.26 (t, 13.5 H, *J* = 7.3 Hz, 1.5 × (C*H*$_3$CH$_2$)$_3$N); $^{13}$C NMR (125 MHz, $D_2O$, $\delta_c$) 97.5 (d, *J* = 5.7 Hz, C-1), 82.5 (C-4), 77.6 (d, *J* = 7.5 Hz, C-2), 74.7 (C-3), 73.7 (C-6), 71.0 (C-5), 59.3 (O*C*H$_3$), 47.5 (1.5 × ($CH_3CH_2$)$_3$N), 9.1 (1.5 × (*C*H$_3$CH$_2$)$_3$N); HRMS (ESI) *m/z* Calcd. for ($M^-$) $C_7H_{14}O_9P$: 273.0370. Found: 273.0368.

1 A. K. Pathak, G. S. Besra, D. Crick, J. A. Maddry, C. B. Morehouse, W. J. Suling and R. C. Reynolds, *Bioorg. Med. Chem.*, 1999, 7, 2407-2413.
2 G. C. Completo and T. L. Lowary, *J. Org. Chem.*, 2008, 73, 4513-4525.




**Scheme S1.** Original proposed synthetic route to **47**.

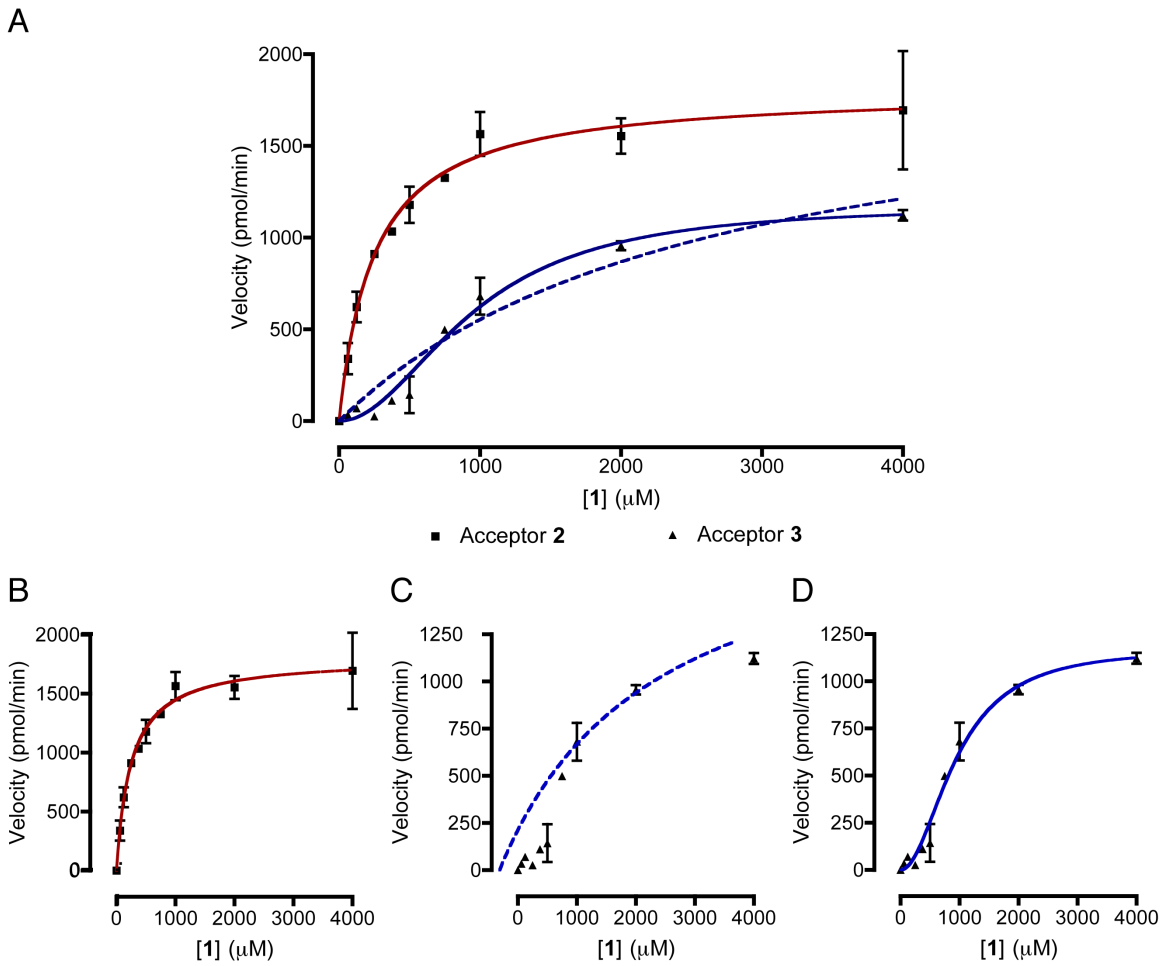



**Figure S1.** GlfT2 donor kinetics data with **1** and acceptor **2** or **3** (A). The donor kinetics data for acceptor **2** (B) and acceptor **3** (C) is shown fit to the Michealis–Menten equation ($r^2$ = 0.95 and 0.91, respectively). A better fit ($r^2$ = 0.96) was found with the Michealis–Menten equation containing a Hill-slope factor in the case of acceptor **3** (D).

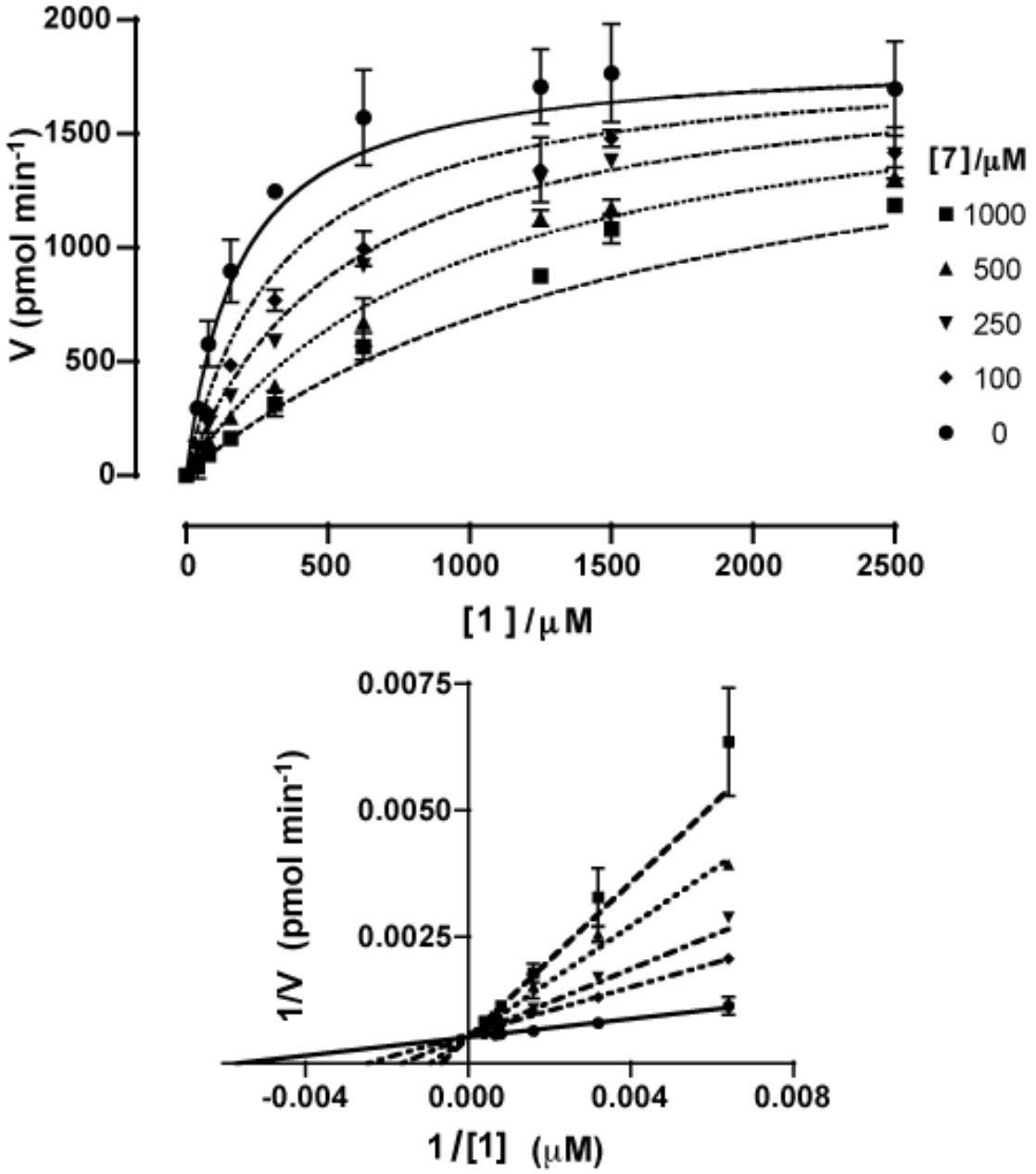



**Figure S2.** Inhibition of GlfT2 with **7** shows competitive inhibition kinetics.

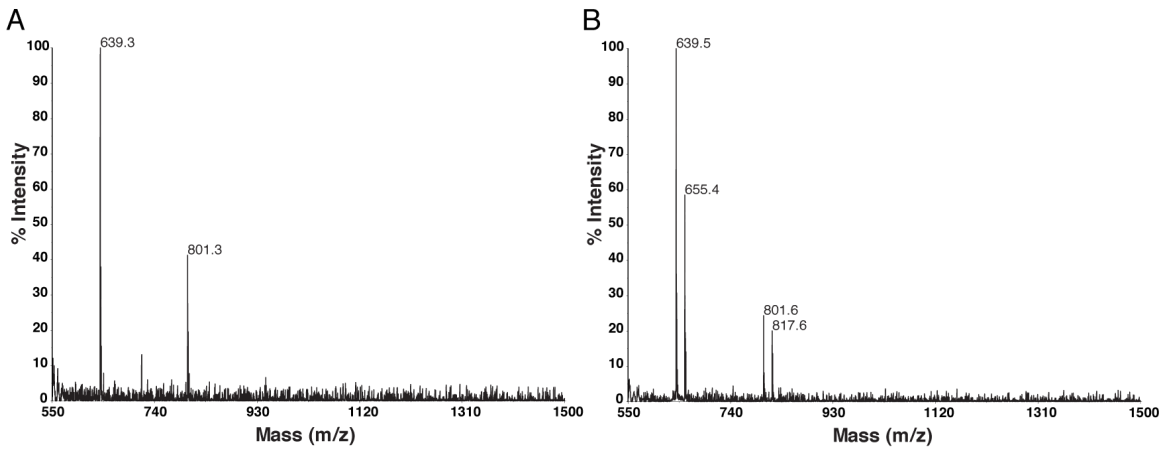



**Figure S3.** Incubations of **2** with UDP-Gal*f* or TDP-Gal*f* produce the same product. Mass spectra are shown from MALDI MS analysis of incubations of **2** with **1** (A) or **11** (B) and GlfT2. The peaks at *m/z* = 639 and 655 correspond to the $[M + Na]^+$ and $[M + K]^+$ adducts of acceptor trisaccharide **2**, respectively. The peaks *m/z* = 801 and 817 correspond to the $[M + Na]^+$ and $[M + K]^+$ adducts, respectively, of trisaccharide **2** plus one additional Gal*f* residue.

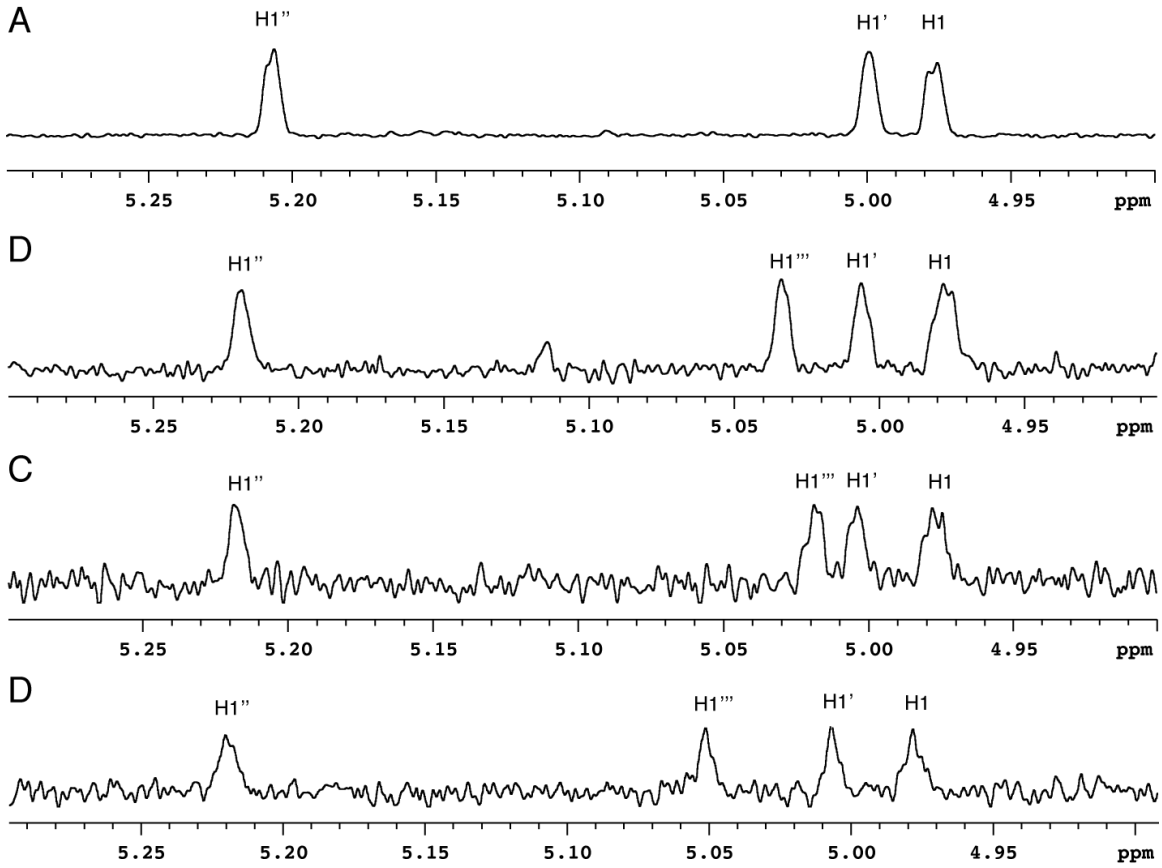



**Figure S4.** Partial $^{1}$H NMR spectra of trisaccharide acceptor **2** (A) and the product resulting from the incubation of acceptor **2** with donor analog **4** (B), **5** (C), or **6** (D) and GlfT2. The major signals corresponding to the anomeric hydrogens are labeled. In all cases the chemical shift for the H1′′′ proton signal indicates the new residue is added in a $\beta$-(1→6)-glycosidic linkage for **4** and **5**, and a $\alpha$-(1→6)-glycosidic linkage for **6**.

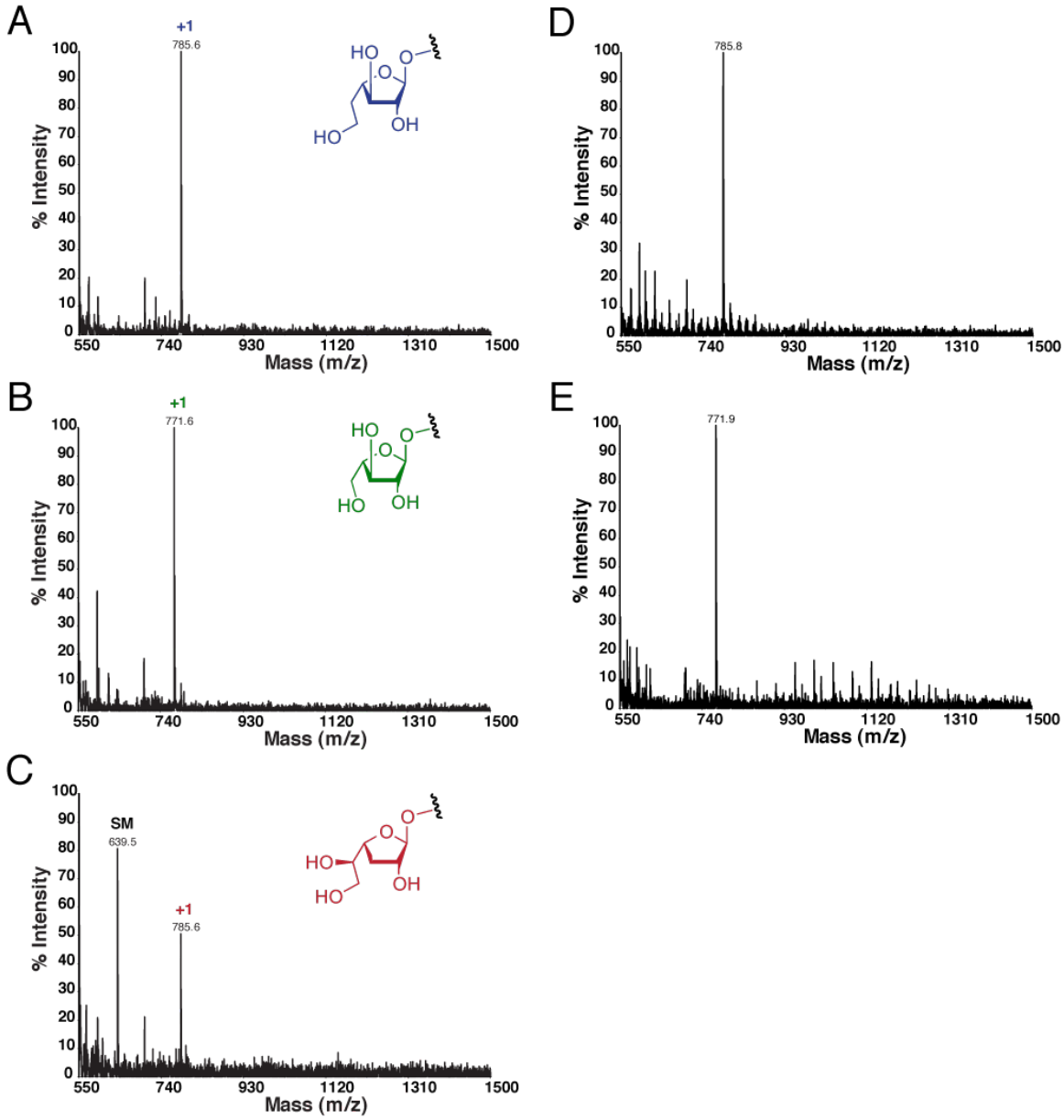



**Figure S5.** Incubations with donor analogs produce only tetrasaccharide products. Mass spectra are shown from MALDI MS analysis of incubations of **2** with **5** (A), **6** (B), or **7** (C) and GlfT2. The only product peaks observed correspond to tetrasaccharides with a single additional donor analog residue (peaks at *m/z* = 785, 771 and 785, respectively). The tetrasaccharide products isolated from incubations of **5** and **6** and then further incubated with donor **1** and GlfT2 produce no further products, (D) and (E), for **5** and **6**, respectively.

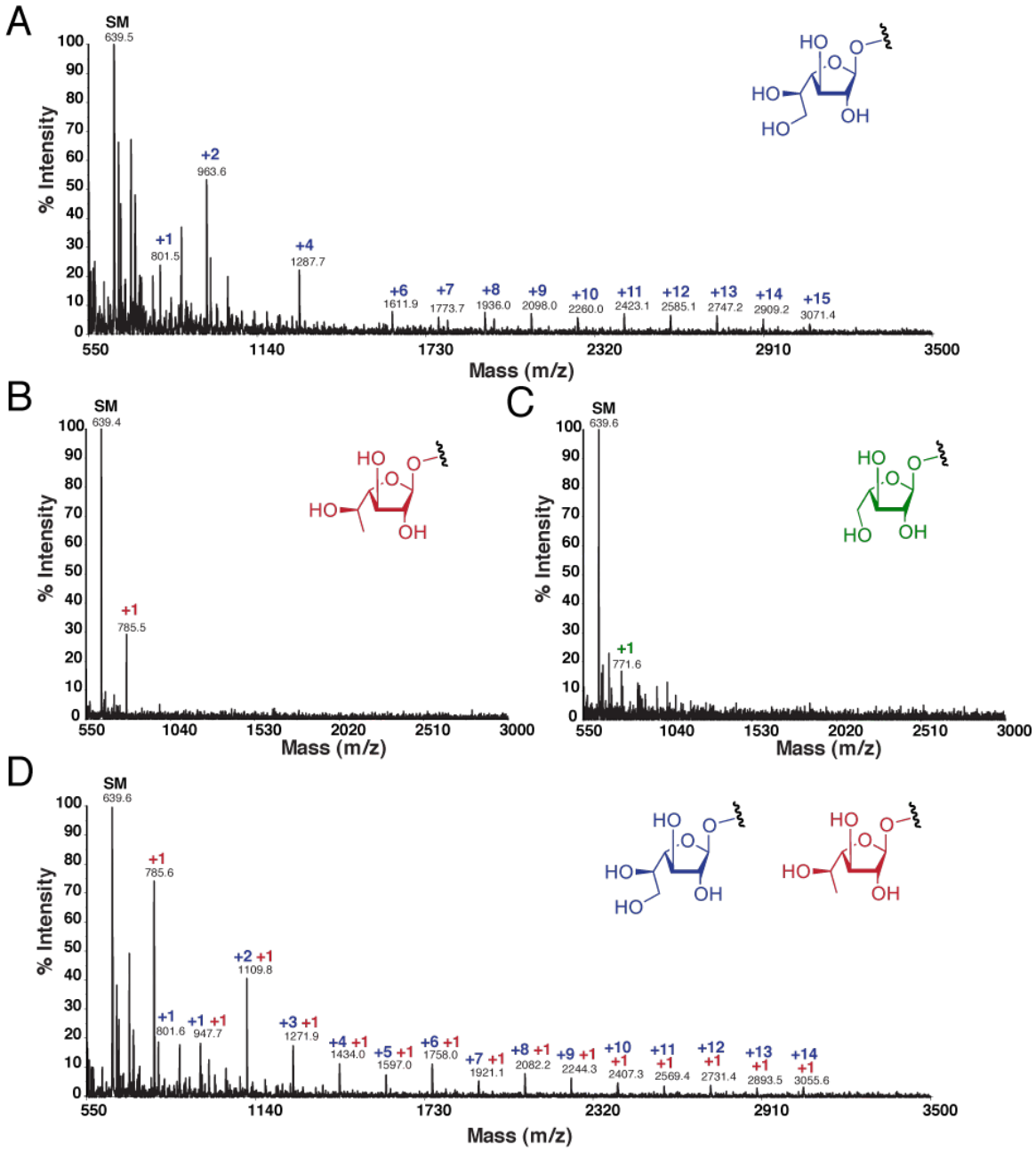
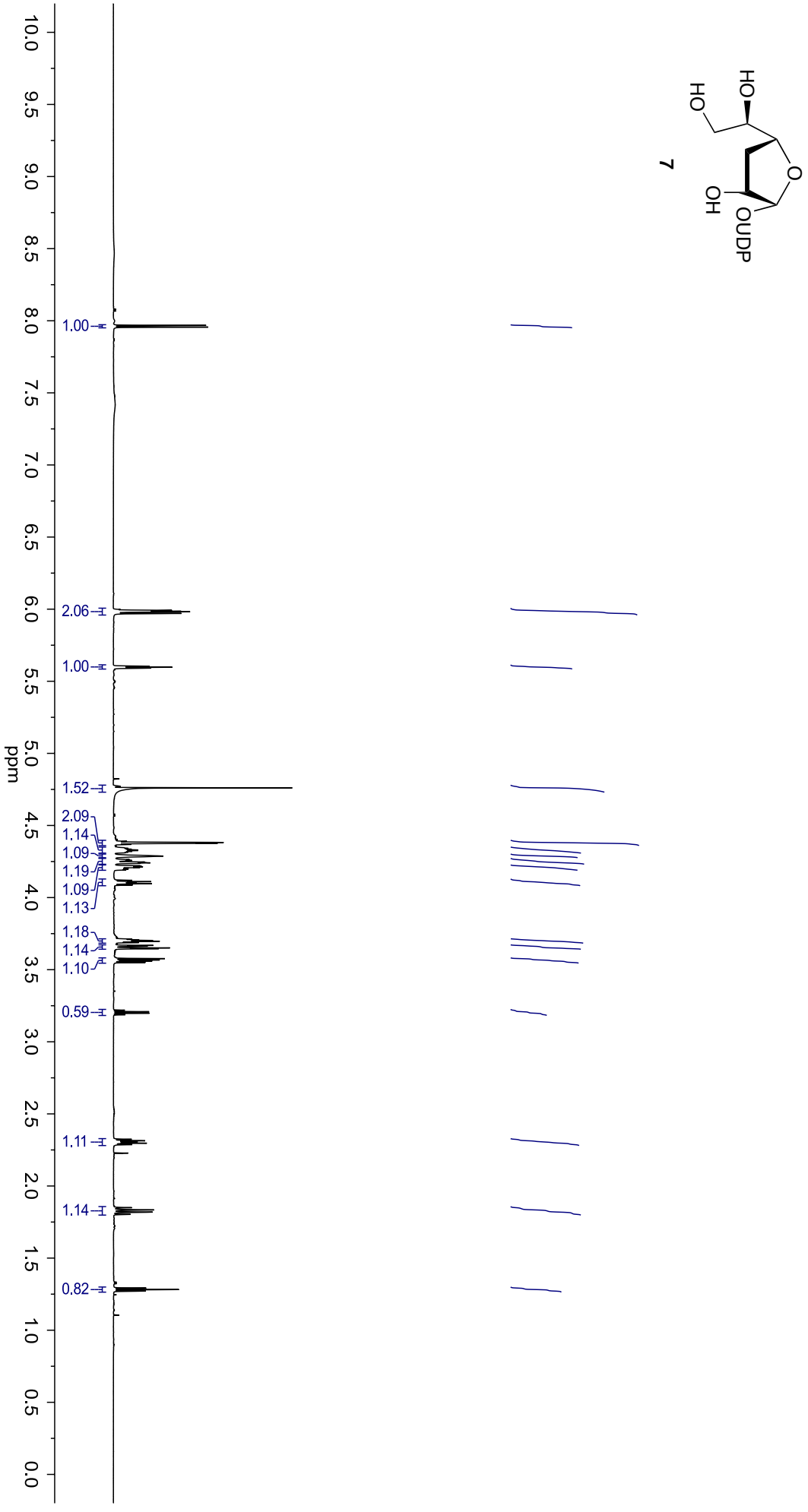



**Figure S6.** Incubations of GlfT2 using synthetic trisaccharide **3** as the acceptor substrate produce similar results to incubations using trisaccharide **2** as the acceptor. Mass spectra are shown from MALDI MS analysis of incubations of GlfT2 with acceptor **3**. Numbers shown indicate the number of additional Gal*f* (blue), 6-deoxy-Gal*f* (red), or Ara*f* (green) residues are observed. In all cases, a substantial amount of trisaccharide **3** (*m/z* = 639) remains, even after a five-day incubation. GlfT2 incubated with acceptor **3** and donor **1** resulted in polymers containing up to an additional 15 Gal*f* residues (A). For incubations of GlfT2 and **3** with donors **4** (B) or **6** (C), only tetrasaccharide products were observed (*m/z* = 785 and 771, respectively). Acceptor **3** and GlfT2 co-incubated with **1** and **4** resulted in a series of polymers containing a single 6-deoxy-Gal*f* and between 0–14 Gal*f* residues.







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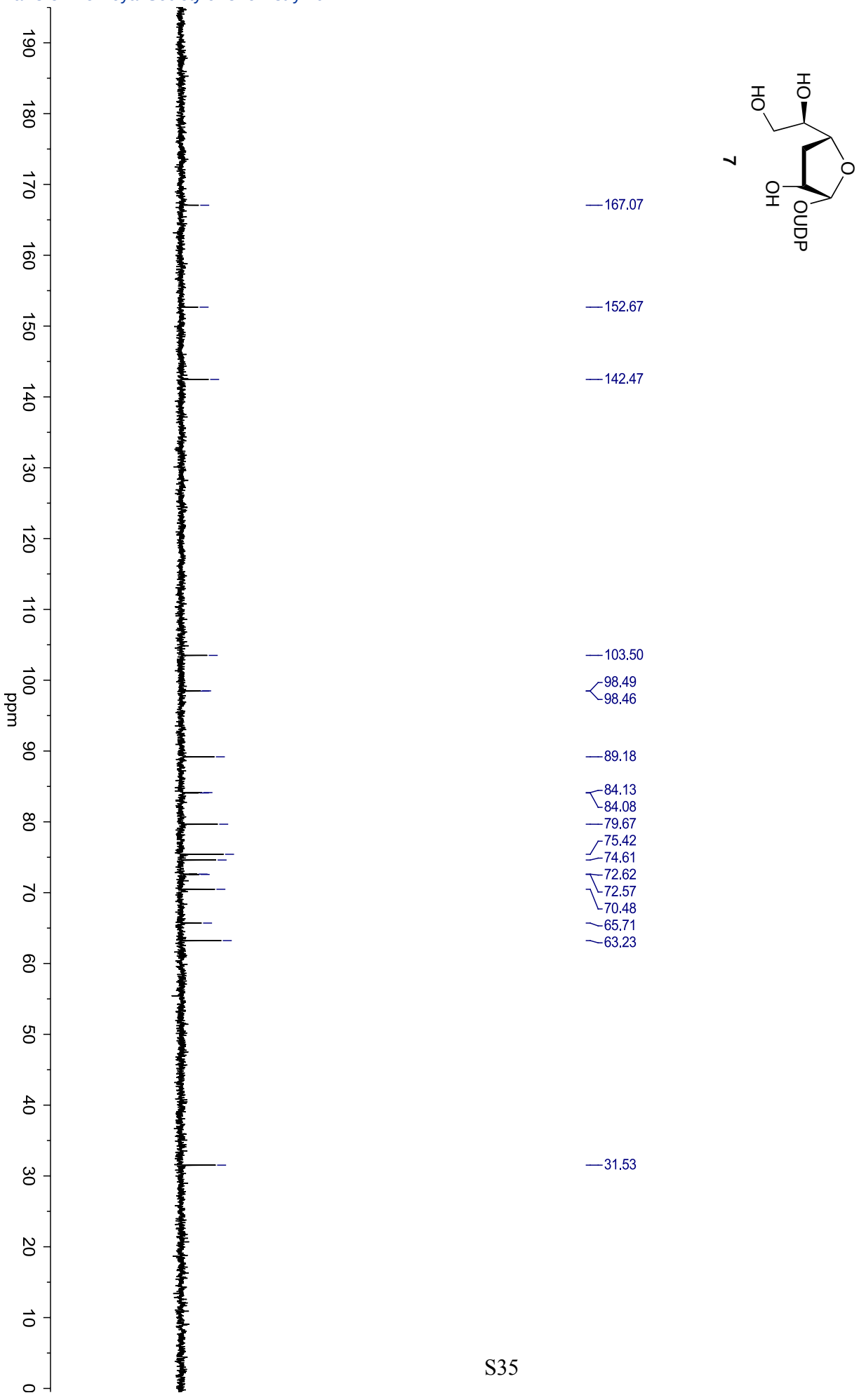

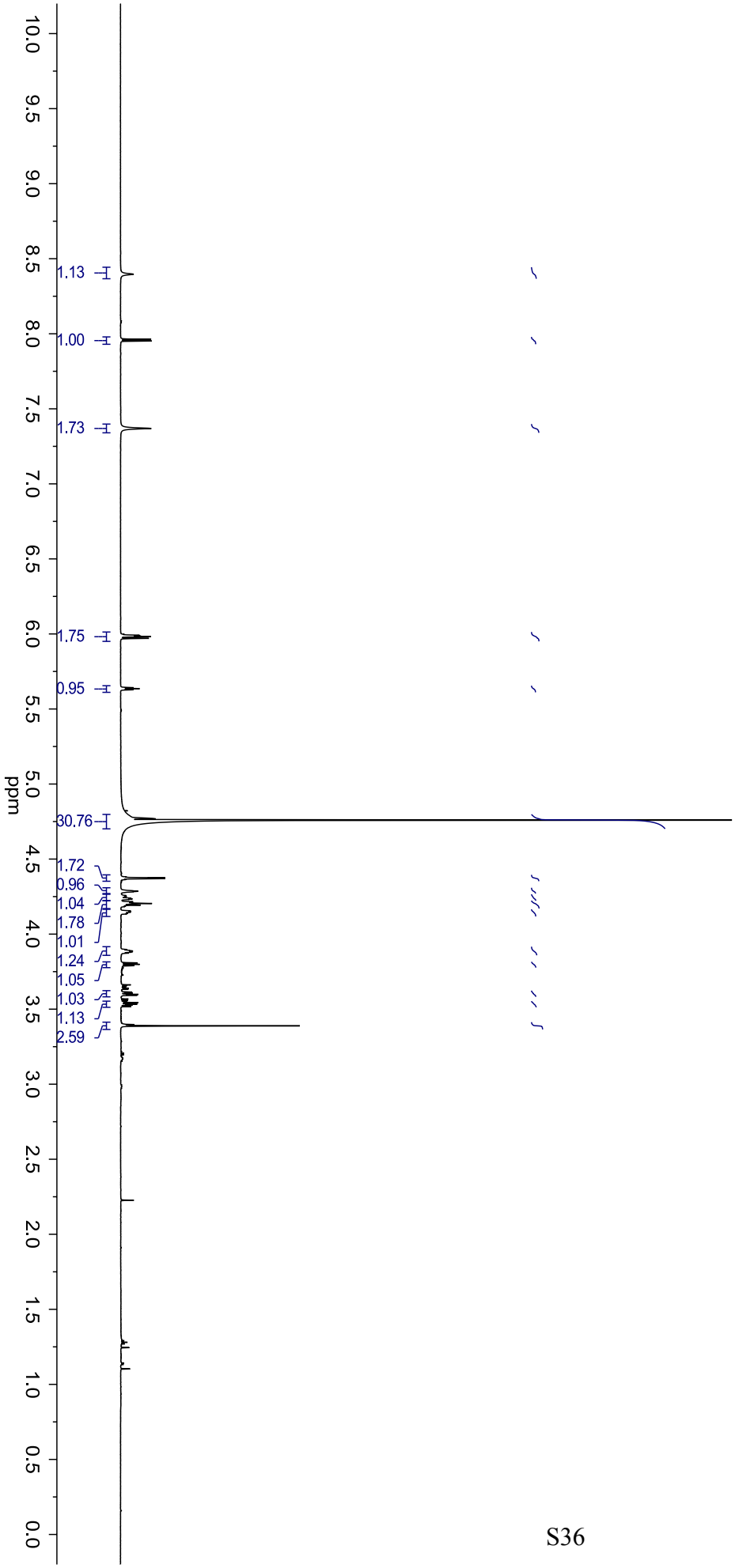


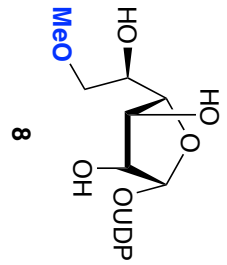


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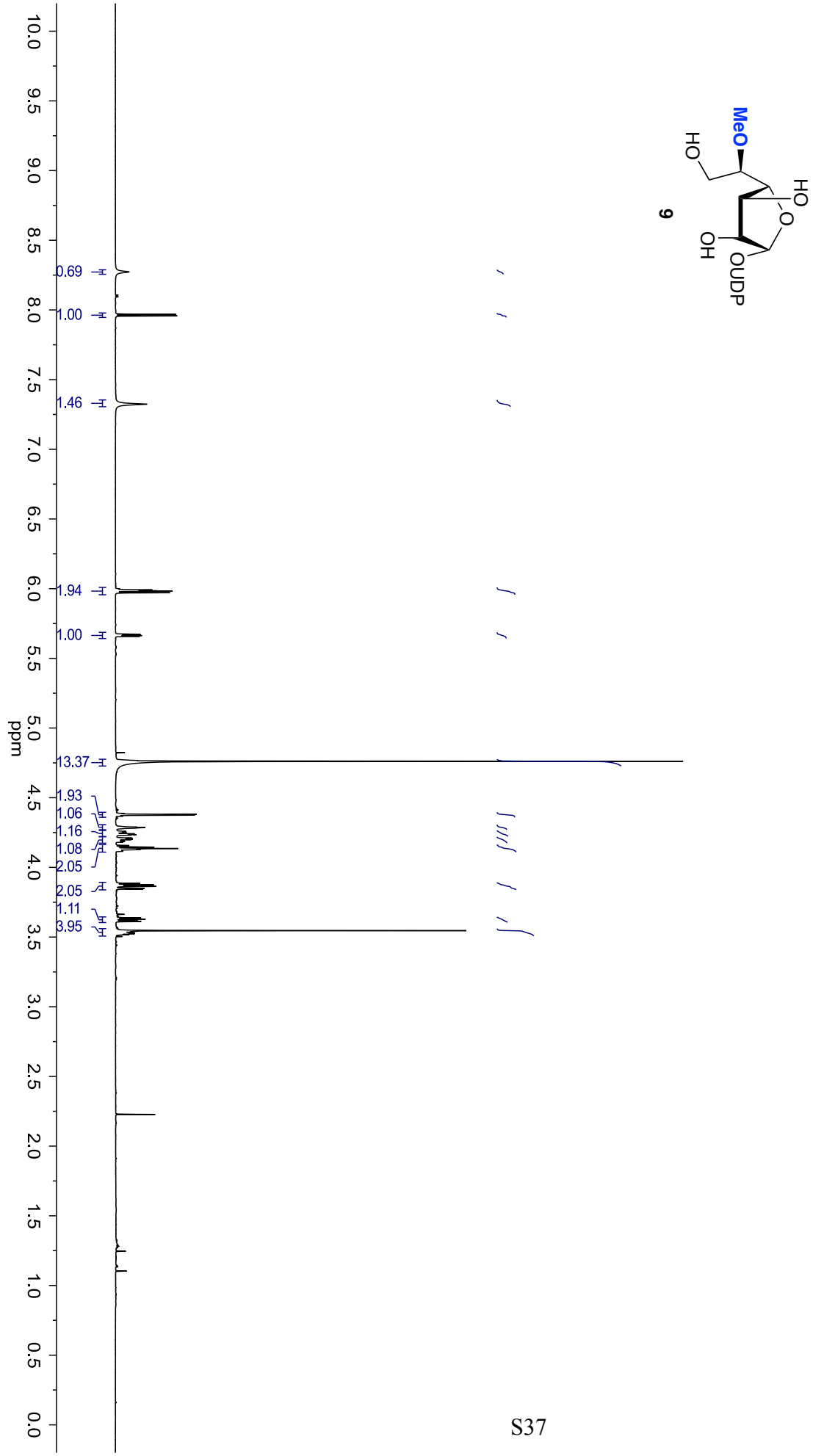

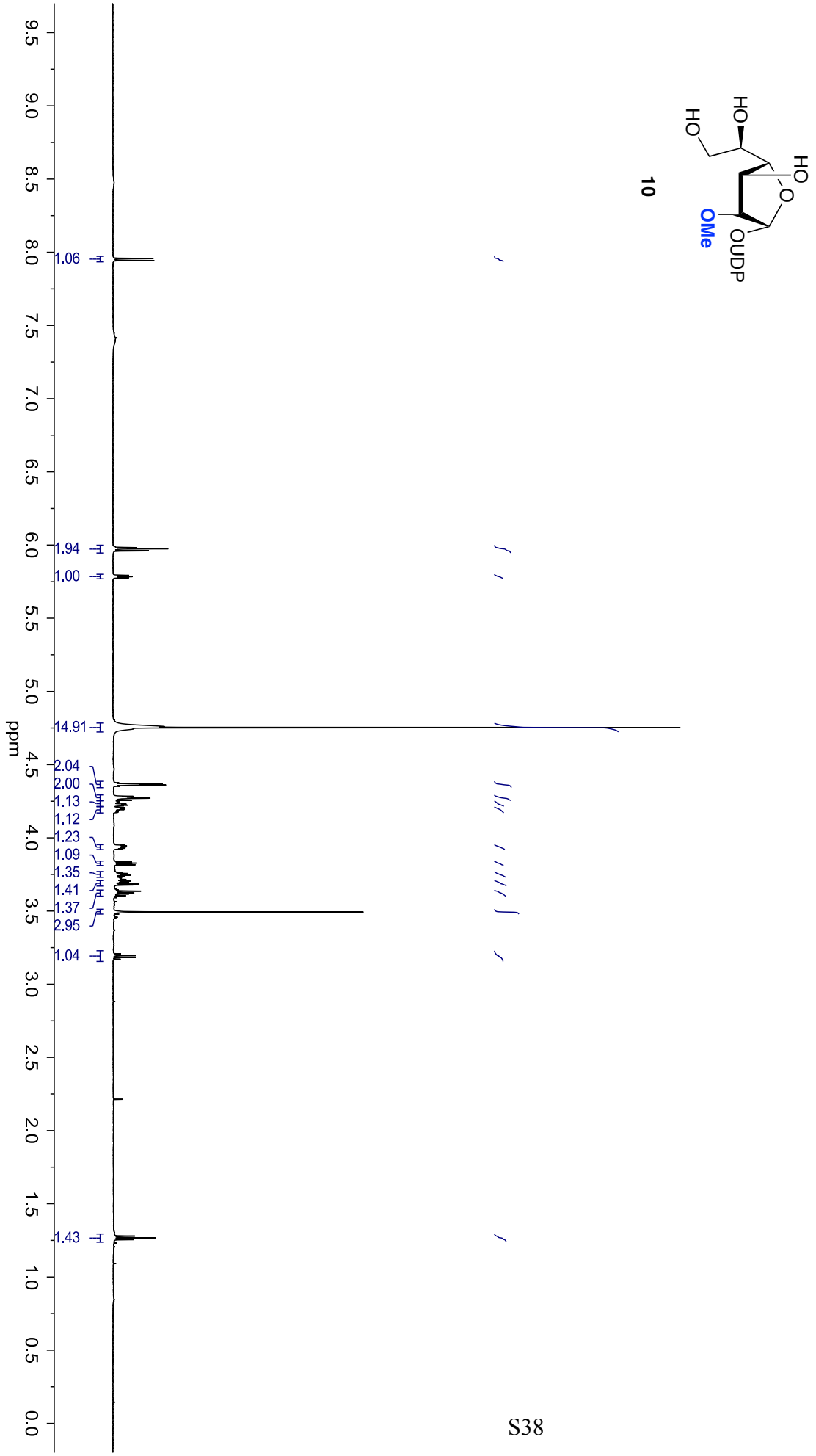





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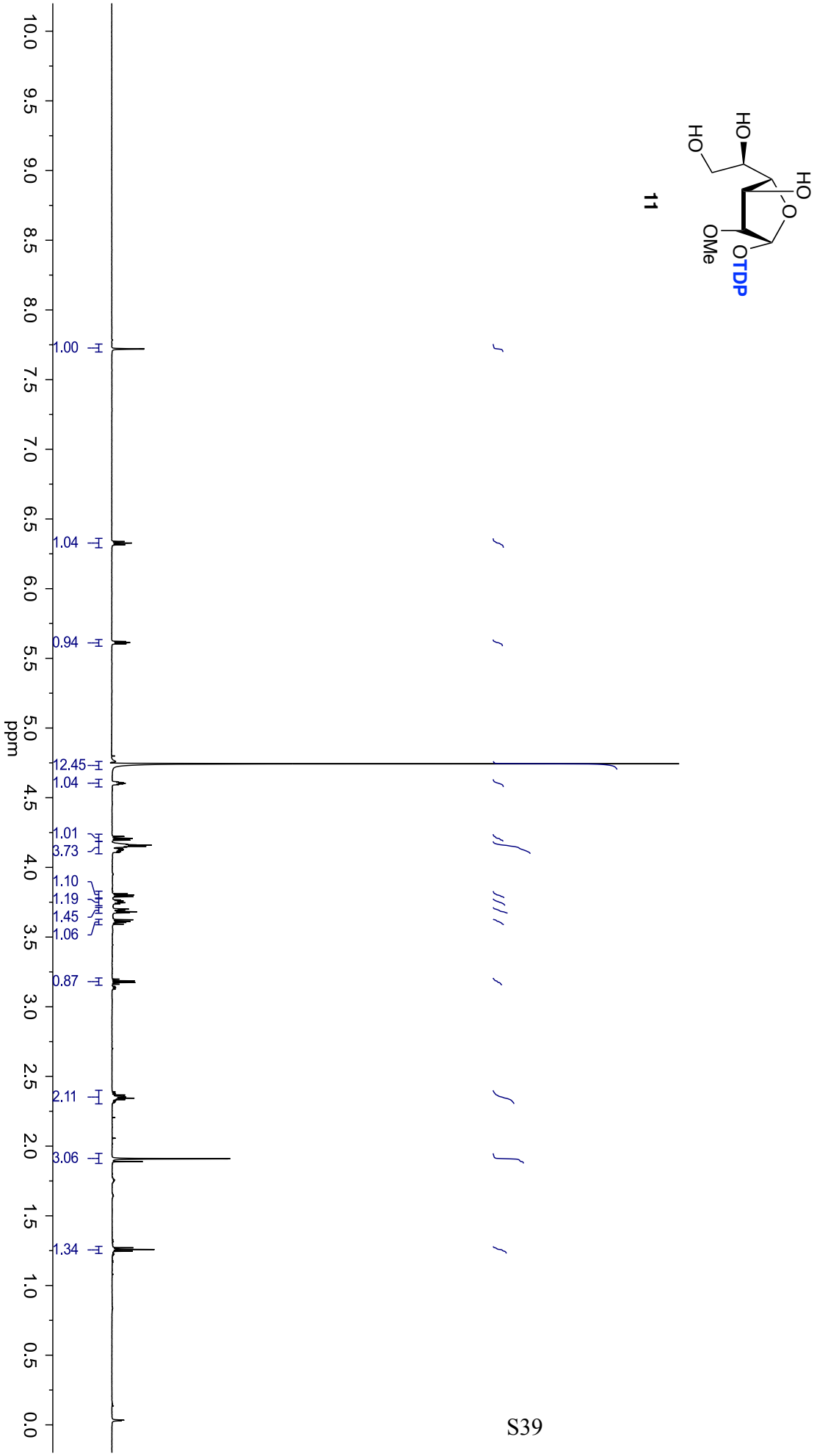





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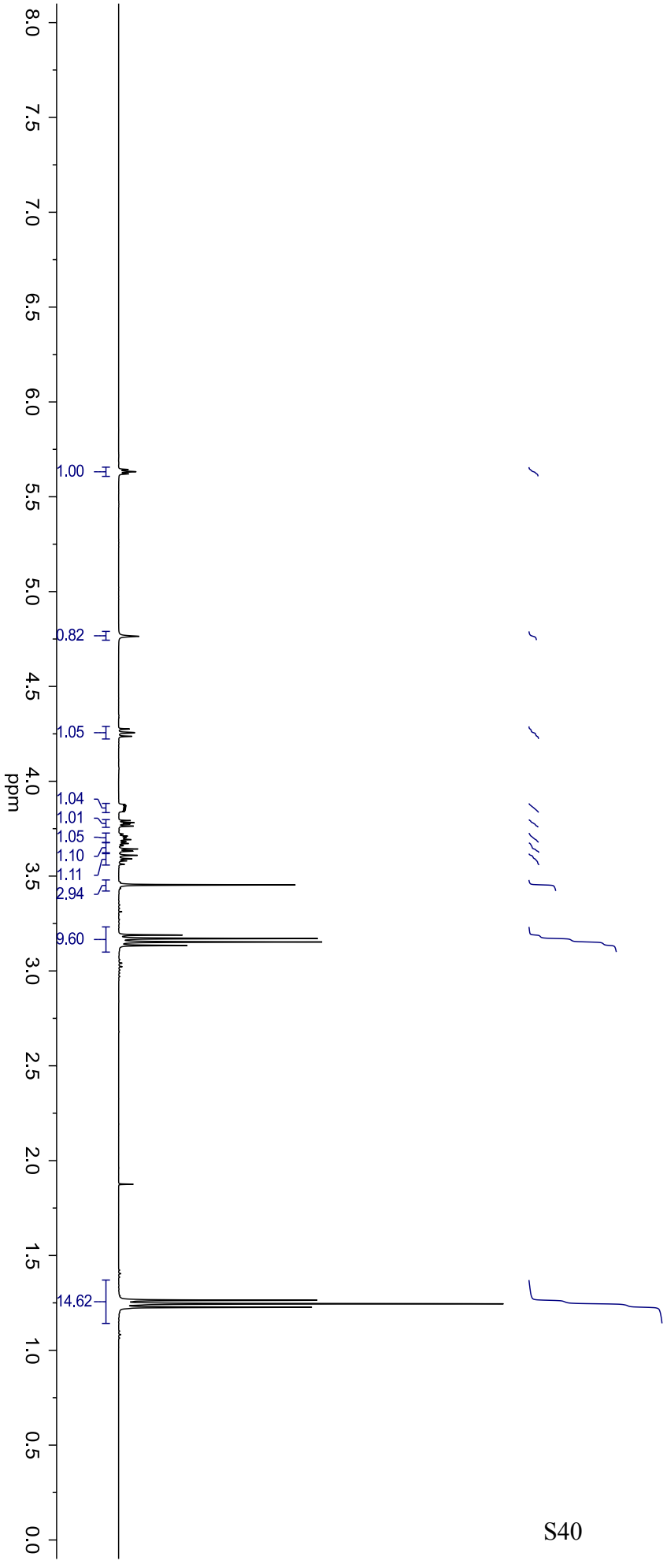


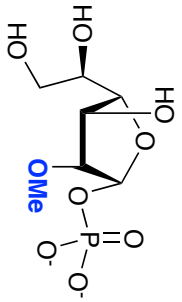


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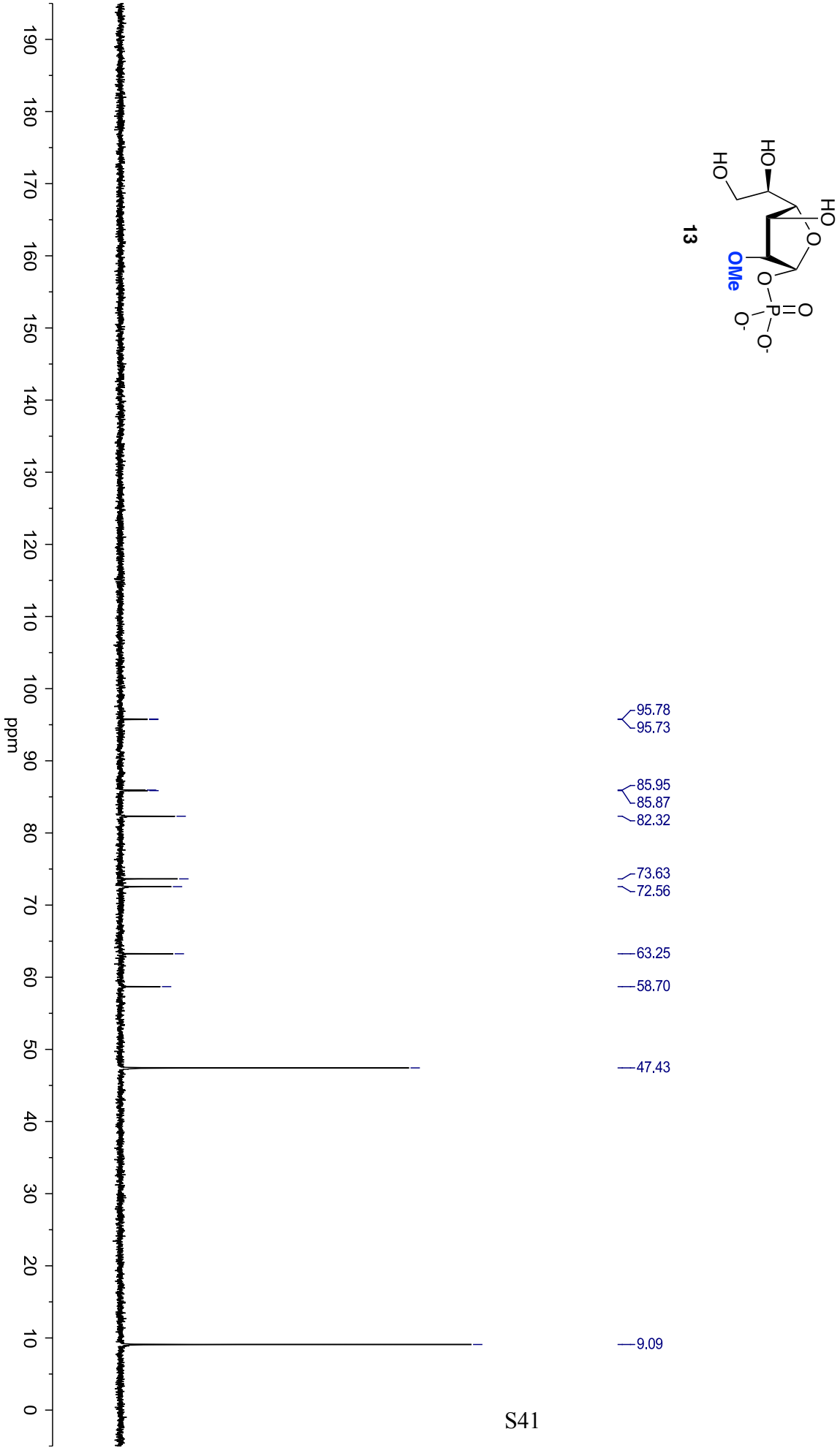


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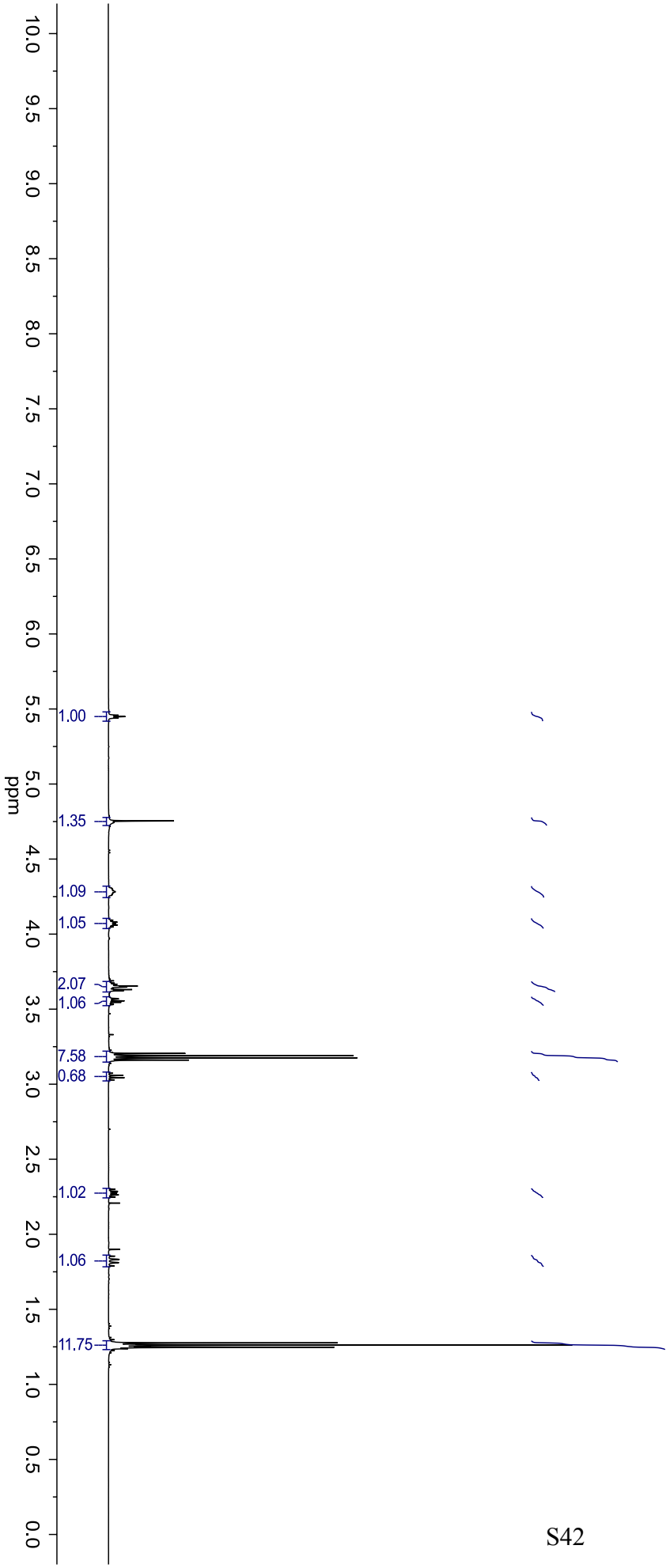


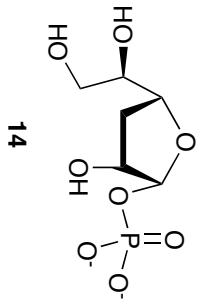


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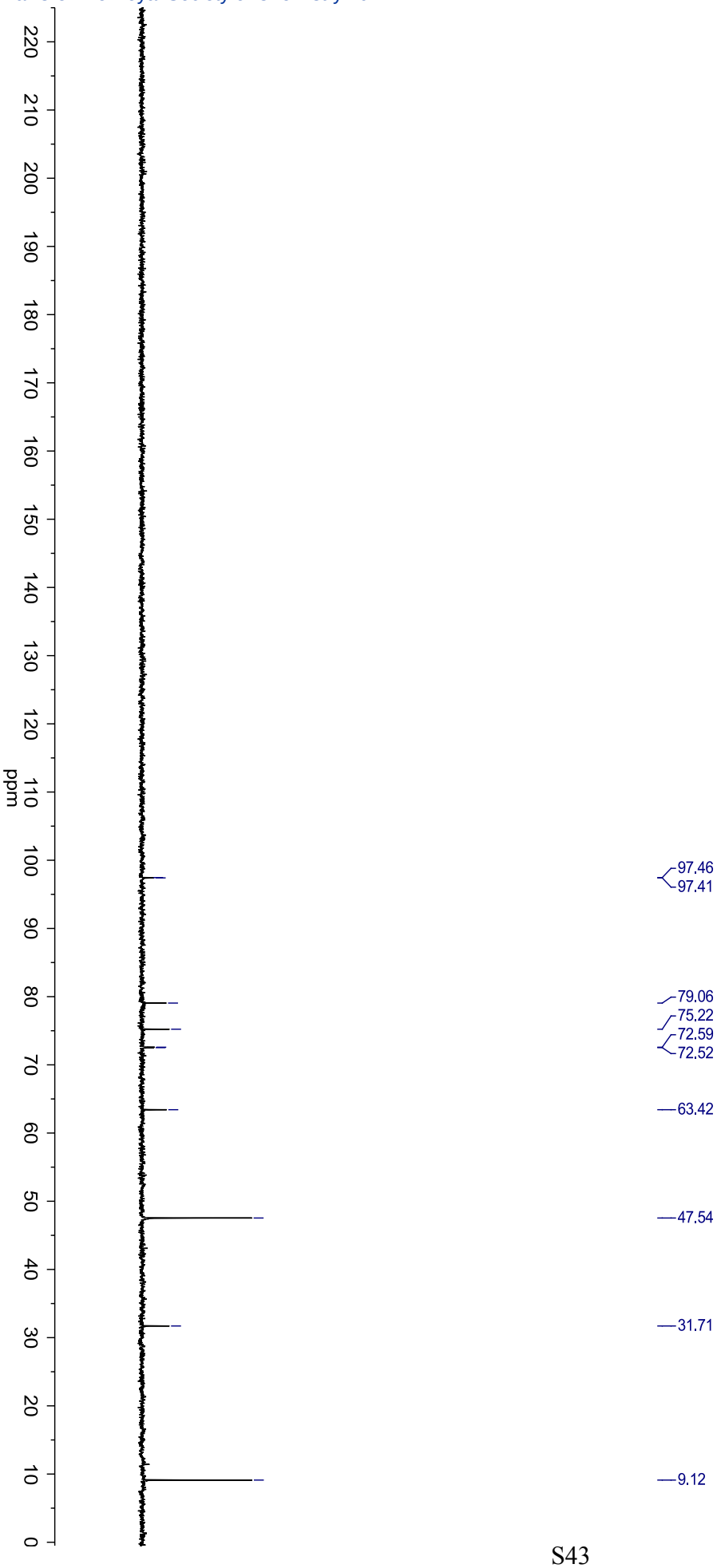


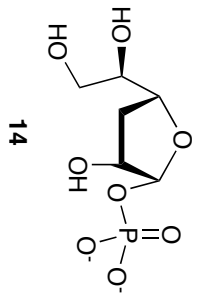


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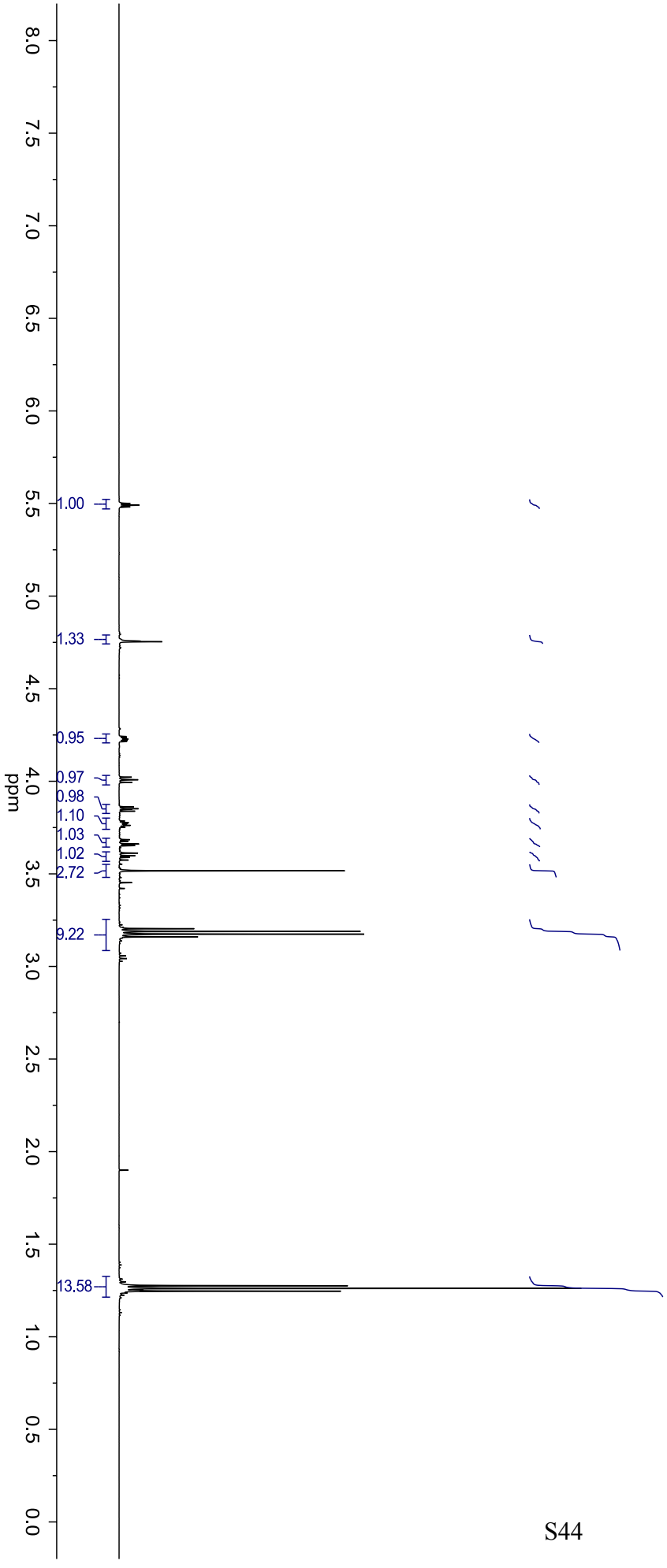


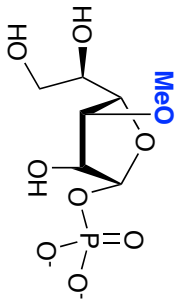


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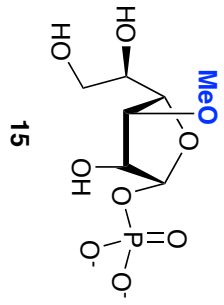


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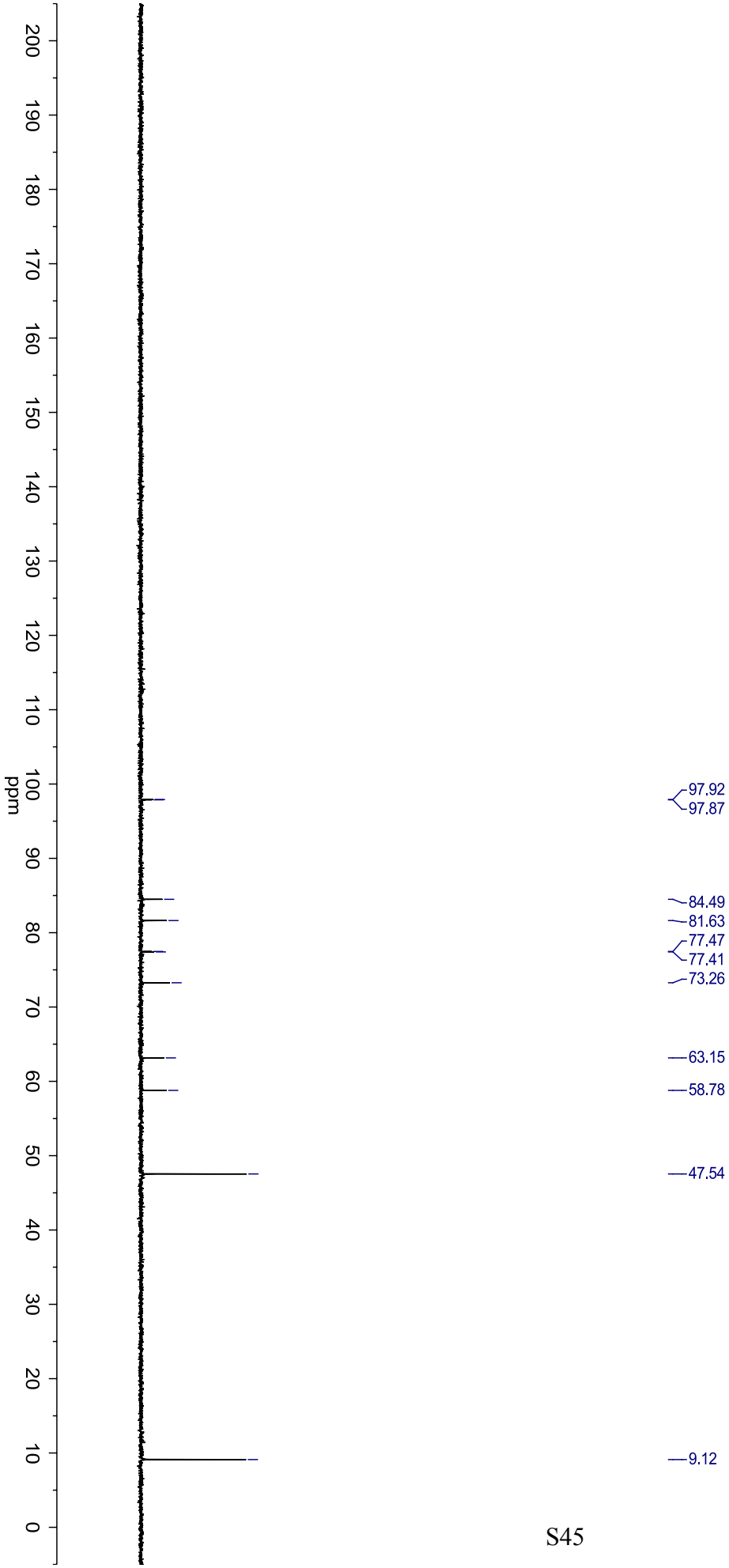

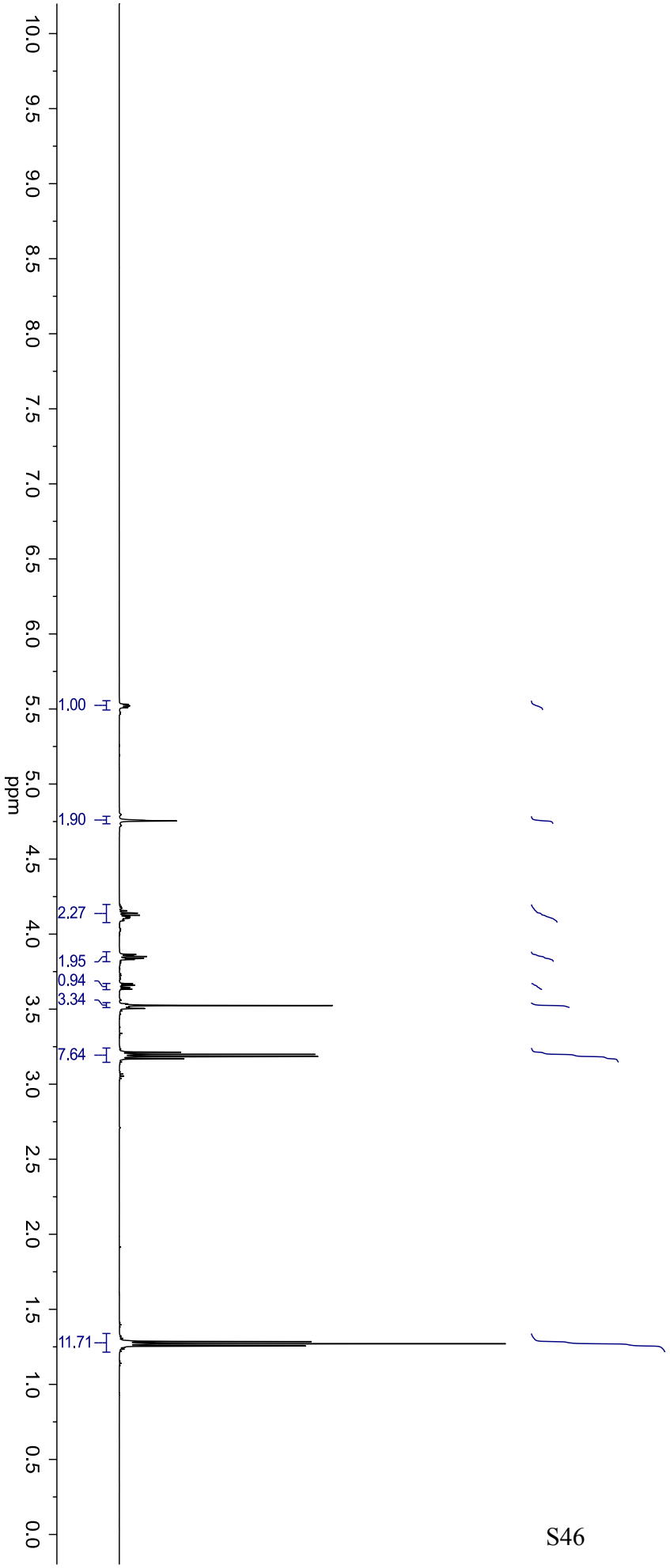


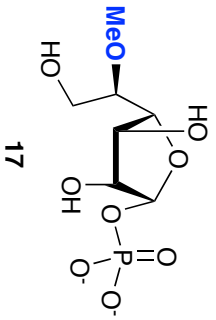


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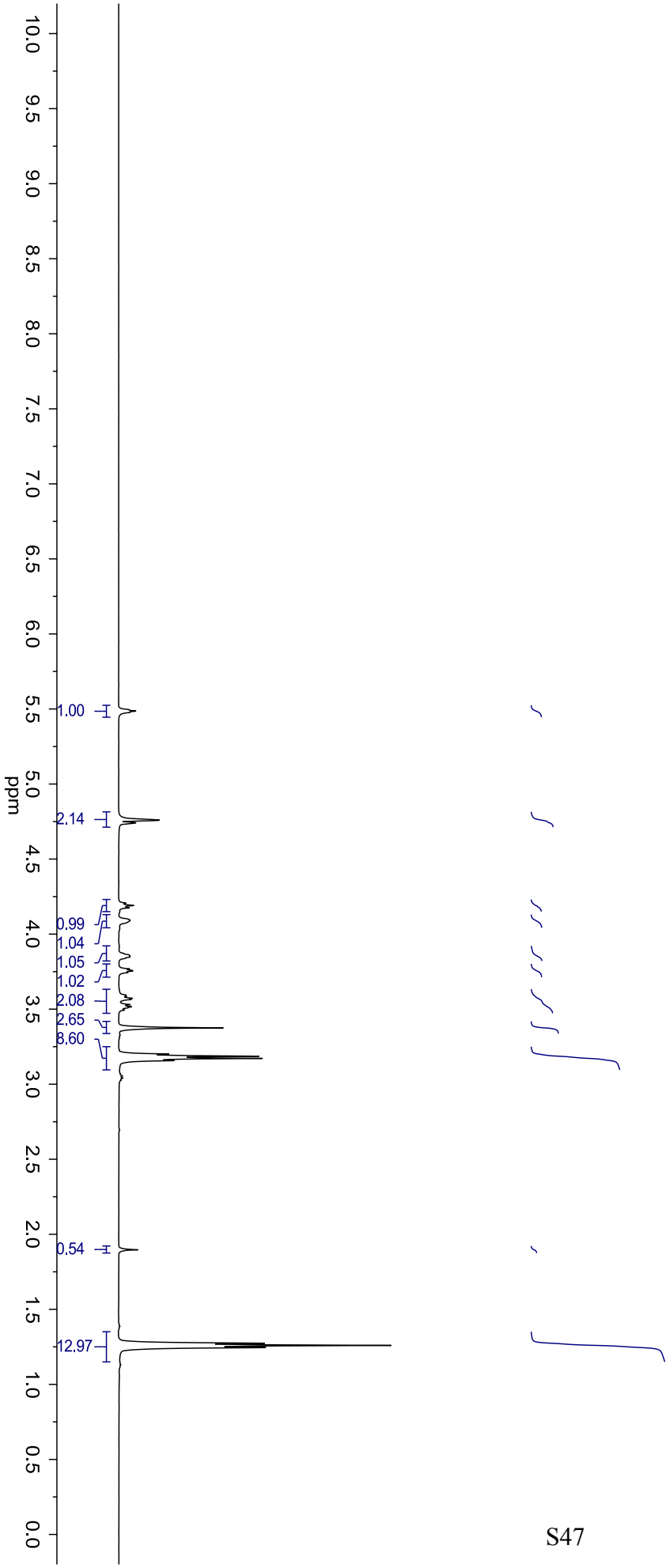


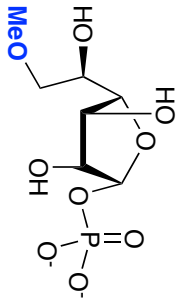


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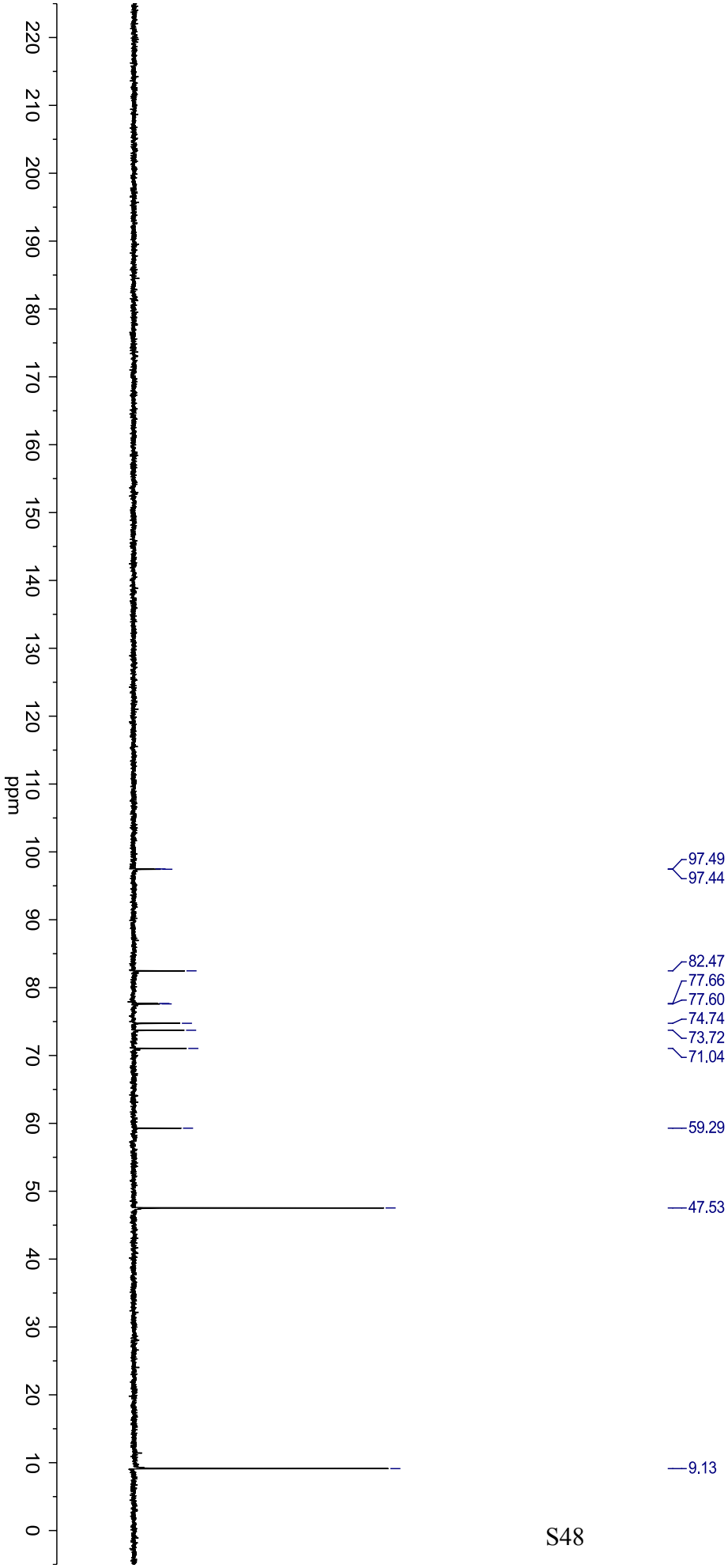


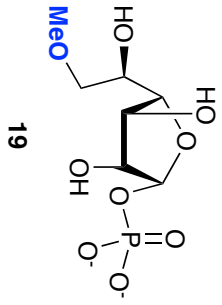


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