Supporting Information for: Marlow, A. L.; Kiessling, L. L. "Improved Chemical Synthesis of UDP-Galactofuranose."

General methods. All reactions, unless otherwise specified, were performed under an inert atmosphere of nitrogen. Glassware used in reactions described was either flame-dried prior to use or heated overnight in an oven at 120 °C. *N*-Methylimidazole (MeIm) was purchased from Aldrich and used without further purification. Trifluoroacetic anhydride (TFAA) was purchased from Aldrich and distilled from phosphorus pentoxide. *N*,*N*-Dimethylaniline (DMA) was purchased from Aldrich and vacuum distilled from calcium hydride. Acetonitrile and triethylamine were distilled from calcium hydride. Toluene was distilled from Na/anthracene.

HPLC purifications were accomplished using a Dionex Carbopac PA-100 (9 x 250 mm) ion-exchange column with a Carbopac PA-100 guard column (4 x 50 mm), eluting isocratically with a solution of 250 mM aqueous ammonium acetate (pH 7) at a flow rate of 2.5 mL/min. The purification was monitored with an ultraviolet (UV) detector at λ = 254 nm. UV/vis absorption spectroscopy was performed on a Hewlett-Packard HP 8452 diode array spectrophotometer at 25 °C in water at λ = 262 nm, using 1 cm pathlength quartz cuvettes. NMR spectra were obtained on a Bruker AC-300 (300 MHz for ^{1}H, 75.41 MHz for ^{13}C and 121.39 MHz for ^{31}P). ^{1}H NMR spectra are reported in parts per million (δ) relative to trimethylsilane (0.00 ppm) for spectra run in $CDCl_3$, or relative to the non-deuterated solvent peak for spectra run in D_2O (4.63 ppm) or CD_3OD (3.30 ppm). ^{31}P NMR spectra are reported in δ relative to an external standard of 85% H_3PO_4 (0.00 ppm in D_2O).

Analytical thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} coated glass plates. Compounds were visualized by using a 254 nm UV lamp and/or para-anisaldehyde staining. Flash column chromatography was performed on silica gel 60 (230-400 mesh).

Cation-exchange resin Dowex 50WX8-200 (H^+ form, strongly acidic) was purchased from Aldrich and converted to the appropriate salt form prior to use. Uridine 5'-monophosphate (5'-UMP) disodium salt was purchased from Sigma and converted to the triethylammonium salt (1.4 eq by ^{1}H NMR) prior to coupling reactions by stirring with Dowex 50WX8-200 (NEt_3H^+ form) overnight. The resin was removed by filtration and washed with H_2O. Combined filtrates were evaporated to dryness under reduced pressure and then co-evaporated with dry toluene (3 x 10 mL) to remove residual water. α-D-galactopyranose 1-phosphate dipotassium salt was purchased from Sigma and converted to the tributylammonium salt (1.16 eq. by ^{1}H NMR) prior to coupling reactions as described for 5'-UMP using Dowex 50WX8-200 (NBu_3H^+ form). α-D-Galactofuranose 1-phosphate bisammonium salt **3** was synthesized as described[5a] and converted to the tributylammonium salt (1.16 eq. by ^{1}H NMR) prior to coupling reactions as described for 5'-UMP using Dowex 50WX8-200 (NBu_3H^+ form).

Synthesis of UDP-α-D-galactofuranose (2). Preparation of the activated UMP-*N*-methylimidazolide **4** was adapted from the original reference.[8] 5'-UMP (triethylammonium salt; 70 mg, 0.15 mmol) was suspended in a mixture of acetonitrile (500 µL), DMA (73 mg, 76 µL, 0.60 mmol) and triethylamine (15 mg, 21 µL, 0.15 mmol), cooled to 0 °C and stirred under N_2. In a separate flask, a solution of TFAA (158 mg, 106 µL, 0.75 mmol) in acetonitrile (150 µL) was cooled to 0 °C and added dropwise to the flask containing 5'-UMP. The reaction was allowed to stir for a few minutes at rt, after which time an orange-pink solution was obtained. Excess TFAA and trifluoracetic acid were removed from the reaction mixture by vacuum evaporation using aspirator pressure. The reaction solution was then cooled to 0 °C. In a separate flask, a mixture of MeIm (37 mg, 36 µL, 0.45 mmol) in acetonitrile (150 µL) and triethylamine (76 mg, 105 µL, 0.75 mmol) was prepared, cooled to 0 °C, and then added to the flask containing the mixed phosphoryl anhydride. The reaction was allowed to stir for 5-10 min at 0 °C, after which time a bright yellow solution was obtained. Preparation of the activated UMP-*N*-methylmidazolide **4** was confirmed by TLC (10:10:1 $CHCl_3$/MeOH, 1 M ammonium acetate pH 7), HPLC, and ^{31}P NMR spectroscopy.

The solution of UMP-*N*-methylimidazolide **4** was then added dropwise to a flask containing α-D-Gal*f* 1-phosphate (**3**) (tributylammonium salt; 60 mg, 0.13 mmol) and 4 Å molecular sieves (ca. 5-10) in acetonitrile (500 µL) at 0 °C. The reaction was stirred at 0 °C under N_2. The progress of the coupling reaction of **3** and **4** was monitored by ^{31}P NMR spectroscopy by removing 25 µL aliquots of the reaction mixture at time intervals and quenching with 700 µL of D_2O. All components of the reaction mixture were distinguishable from one another in the ^{31}P NMR spectrum.[11] The reaction was complete after 2 h, and was then quenched with cold 250 mM aqueous ammonium acetate, pH 7 (3 mL). The amines were extracted from the aqueous phase with $CHCl_3$ (3 mL). The organic layer was washed with cold 250 mM NH_4OAc (3 mL), and the combined aqueous layers were purified by ion-exchange HPLC. The appropriate fractions were pooled and lyophilized. Water was added to the residue which was lyophilized again to remove as much residual buffer as possible. The residue was then dissolved in D_2O (1 mL) for NMR analysis. ^{1}H, ^{13}C and ^{31}P NMR spectra were in agreement with those published.[5] Yields were determined by UV spectroscopy to ensure that no residual buffer was included in the final coupling yield (λ = 262 nm, ε = 10 mM^{-1}, cm^{-1}). This protocol afforded UDP-Gal*f* **2** as the bisammonium salt (25 mg, 0.042 mmol) in 35% yield.