Supplementary Material (ESI) for Chemical Communications

Supplementary Methods

Synthesis of fluorescein-lactose conjugate. The succinimidyl ester of 5-(and-6)-carboxyfluorescein (mixed isomers, Molecular Probes) was reacted with *p*-aminophenyl-β-D-lactopyranoside (Toronto Research Chemicals) in 0.2M sodium bicarbonate buffer pH 8.3 according to the manufacturers instructions. The product was partially purified with a SepPak C_{18}Plus Cartridge (0.39 g, Waters) by washing with HPLC grade water (10 mL) and eluting with HPLC grade MeOH (10 mL). The lyophilized partially purified product was then subjected to a final purification by preparative HPLC using an Amide 80 column (Tosoh Bioscience) and a gradient elution (90:10 to 40:60 MeCN: H_2O over 45 minutes, then 40:60 MeCN: H_2O for 15 minutes). Fractions containing pure product were combined and lyophilized to yield an orange powder (40.8 mg, 89.6%). HR ESI-MS: *m/z* = 814.1960 $[M + Na]^+$ (expected for $C_{39}H_{37}NO_{17}Na^+$: *m/z* = 814.1959).

Cst II catalyzed glycosylation reactions. Fluorescein-lactose acceptor substrate (2 mM), *p*-nitrophenyl α-sialoside (6 mM), CMP (3 mM) and Cst II (~0.5 mg/mL) were incubated for 48 hours at room temperature in 20 mM HEPES buffer (pH 7.5) containing 10 mM $MgCl_2$. Upon completion, the reaction was purified with a SepPak C_{18}Plus Cartridge (0.39 g, Waters) followed by preparative HPLC as described above for the fluorescein-lactose conjugate. Fractions containing the purified product were combined and lyophilized to yield an orange powder (10.1 mg, 72.0%). HR ESI-MS: *m/z* = 1081.2931 $[M]^-$ (expected for $C_{50}H_{53}N_2O_{25}$: *m/z* = 1081.2937). Owing to the complexity of the product (an unprotected trisaccharide existing as a mixture of 5- and 6-

carboxy fluorescein regioisomers) the regiochemistry of the sialylated product could not be unambiguously deduced by ^{1}H or ^{13}C NMR spectroscopy. The regiochemistry was therefore confirmed by treatment of 5 nmol of the purified reaction product with a selective α-2,3-sialidase (New England Biolabs). Under a unit defined time condition, complete reversion of the product to the fluorescein-lactose conjugate was observed (Sup. Fig. 2).

LgtC catalyzed glycosylation reactions. Fluorescein-lactose acceptor substrate (2 mM), 2,4-dinitrophenyl β-galactoside (6 mM), UDP (3 mM) and LgtC (~0.5 mg/mL) were incubated for 96 hours at room temperature in 20 mM HEPES buffer (pH 7.5) containing 10 mM $MnCl_2$, and 10 mM DTT. The reaction mixture was partially purified with a SepPak C_{18}Plus Cartridge (0.39 g, Waters). Due to the high rate of spontaneous hydrolysis of the alternative donor 2,4-dinitrophenyl β-galactoside, detailed product analysis was not possible. Presumably due to the high rates of reaction, for the positive control reaction using excess UDP Gal, tetra- and penta-saccharide products were detected by TLC (Sup. Fig. 3) and their identities were confirmed by ESI MS (data not shown). HR ESI-MS: *m/z* = 954.2674 $[M + Na]^+$ (expected for $C_{45}H_{48}NO_{22}$: *m/z* = 954.2668).