Supplementary Methods

Preparation of GnTs recombinant enzymes. The putative catalytic domain of GnT-I to -V was expressed as a secreted protein with a FLAG peptide in human embryonic kidney (HEK) 293T cells. PCR primer sets for amplification of each gene were as follows: GnT-I, 5’- caccACGCGCCCAGCACCTGGGCAGGC -3’ and 5’- CTAAATCCAGCTAGGATCATAGCCCTCCA -3’, GnT-II, 5’- caccAGCAGCAATGGGCGACAAGGAA -3’ and 5’- CTAGACTTCCGCCTCGTGTTT -3’, GnT-III, 5’- caccACTTCTCTCAAGACCTCCTGCTTCA -3’ and 5’- CTAGACTTCCGCCTCGTGTTT -3’, GnT-IVa, 5’- caccACTACATGGCAGGAAATGGGA -3’ and 5’- TCAGTTGGTGGCTTTTTTATATGA -3’, GnT-V, 5’- caccACCATCCAGCAGCAGAATCGCC -3’ and 5’- CTATAGAGCTCTTTTGAGAGAG -3’. Small letters correspond to nucleotides involved in the subcloning reactions. Each amplified fragment was subcloned into pENTR/D-TOPO to construct the entry clone for Gateway™ Cloning Technology (Invitrogen) according to the manufacturer’s instructions. Each gene was transferred from entry vector to Gateway™ destination vector, pFLAG-CMV-3-DEST for mammalian expression according to the manufacturer’s instructions (Invitrogen). Expression and preparation of each enzyme was described previously.

Preparation of β4GalTs recombinant enzymes. The putative catalytic domain of β4GalT-I to -V was expressed as a secreted protein with a FLAG peptide in human embryonic kidney (HEK) 293T cells. PCR primer sets for amplification of each gene were as follows: β4GalT-I, 5’- ggggacaagtttgttacaaaaaagagcttcCGCGACCTGAGCGCGTGGGCCTGCC-3’ and 5’- ggggacaagtttgttacaaaaaagagcttcCTAGCTCGGTGTCGGGATGTCCTCAGACT-3’, β4GalT-II, 5’- ggggacaagtttgttacaaaaaagagcttcAGCAGCTCGGTGTCGGGATGTCCTCAGACT-3’, β4GalT-III, 5’- ggggacaagtttgttacaaaaaagagcttcAGCAGCTCGGTGTCGGGATGTCCTCAGACT-3’, β4GalT-IV, 5’- caccGAAGTCTCAGTGCCCTATTTG -3’ and 5’- GGTAGACAGGAGGAGGAGTCA-3’, β4GalT-IV, 5’- GGTAGACAGGAGGAGGAGTCA-3’, β4GalT-IV, 5’-
caccGGTGCCATTCAAGAGATTCC -3' and 5'- CCAGGGTCATGCACCAAAC -3',
β4GalT-V, 5'- ggggacaagtgtgacaagaaaaagcagtcTGCAAGCACAAGGCATTCTGATC
-3' and 5'- ggggaccactttgacaagaaagctgggcTCAGTACTCGTCCACCTGAGCCA-3'.
Small letters correspond to nucleotides involved in the subcloning reactions. Each
amplified fragment was subcloned into pDONR™201 or pENTR/D-TOPO to construct
the entry clone for Gateway™ Cloning Technology (Invitrogen) according to the
manufacturer’s instructions. The Gateway™ destination vector for expression in
mammalian cells, pFLAG-CMV-3-DEST was constructed by insertion of Gateway™
conversion cassette (Invitrogen) into the EcoRV site of pFLAG-CMV-3 (Sigma-
Aldrich). Each gene was transferred from entry vector to pFLAG-CMV-3-DEST
according to the manufacturer’s instructions (Invitrogen). Expression and preparation
of each enzyme was described previously.

Preparation of the galactosylated products for Fig. 3. 25 mM HEPES buffer (pH
7.0) containing 2.5 μM acceptor substrate (1 - 4; Fig. 2), 10 mM MnCl₂ and 2.5 μM
UDP-Gal was used. A purified β4GalT-I solution for 20 μL of each reaction mixture
was added and incubated at 37 °C for 1 h. The reaction was terminated by boiling the
mixture prior to HPLC separation.

Preparation of UDP-¹³C₆-GlcNAc. Treatment of ¹³C₆-N-acetylglucosamine with
acetic anhydride in pyridine, followed by regioselective deacetylation at O-1 with
benzylamine gave the acetylated 2-acetamido-2-deoxy-D-glucopyranose.
Phosphorylation of the reducing sugar was achieved using diphenyl chlorophosphate
and 4-dimethylpyridine (DMAP). The glycosyl phosphate was purified by conventional
chromatography on a column of silica gel. Deprotection of the obtained phosphate
derivative by hydrogenolysis in ethanol in the presence of platinum catalyst, followed
by deacetylation with sodium methoxide in methanol gave the natural glycosyl
monophosphate in a form suitable for reaction with activated nucleotide monophosphate.
Finally, reaction of ¹³C₆-N-acetylglucosaminyl phosphate with uridine 5’-
monophosphomorpholidate (UMP-morpholidate) and 1H-tetrazole in pyridine gave
UDP-¹³C₆-GlcNAc. The product was purified on Dowex 1 column and Bio-Gel P-2
column.
Preparation of UDP-$^{13}$C$_6$-GlcNAc

References
1. $^{13}$C$_6$-$\text{D-}$N-acetylglucosamine was purchased from Omicron Biochemicals, Inc., USA