SUPPLEMENTAL FIGURE LEGENDS

Supplemental Fig. 1.

Varying concentrations of 5′-CMP or 5′-UMP were sialylated with ST3GalII (2.0 mU) using 0.25 mM sialyl donor **[9-^{3}H[3]]** for 4h at 37^{o}C. The product CMP-[9-^{3}H]NeuAc or UMP-[9-^{3}H] NeuAc and the unused **[9-^{3}H[3]]** were separated and quantitated by Dowex-1-Formate method. The sialyl donor **[9-^{3}H[3]]** eluted from Dowex-1 Formate column at 0.1 M NaCl whereas CMP-[9-^{3}H]NeuAc was completely recovered by eluting with 0.4 M NaCl. Δ Formation of CMP-[9-^{3}H]NeuAc; ▲Utilization of **[9-^{3}H[3]]** by 5′-CMP; ○ Formation of UMP-[9-^{3}H]NeuAc; ● Utilization of **[9-^{3}H[3]]** by 5′-UMP.

Supplemental Fig. 2.

Reversible sialyltransferase activity at different incubation periods. The transfer of [9-^{3}H]NeuAc from 0.15mM **[[9-^{3}H[1]]** to 2.5mM D-Fucβ1,3GalNAcα-O-Bn **[5]** in the presence of 0.8mM 5′-CMP and 0.5mU ST3Gal-II was studied at 37ºC for varying times using Sep-Pak C_{18} method. The reaction reached equilibrium by 4h.

Supplemental Fig. 3.

Effect of pH on ST3Gal-II catalytic activity measured over a range of buffer pH using: Na acetate (▲, pH=3.6-5.6), Hepes-NaOH (●, pH=6.0-8.4) and Tris-Maleate (■) buffers: **a.** Sialylation of 5′-CMP (2.0mM) by ST3Gal-II (0.15mU) using donor [9-^{3}H]NeuAcα2,3Galβ1,3(4-F-GlcNAcβ1,6)GalNAcα-O-Bn **[9-^{3}H[2]]** was optimum in pH range 4.8-6.4. **b.** Direct Sialylation of 1.0mM Galβ1,3GalNAcα-O-Bn **[16]** by 0.15mU ST3Gal-II using 0.1mM [9-^{3}H] CMP-NeuAc as donor was optimum in the pH range 5.2-7.2. **c.** Reversible sialylation of 6.0mM D-Fucβ1,3GalNAcα-O-Bn **[5]** by 0.15mU ST3Gal-II using the donor 0.15mM **[9-^{3}H[3]]** and 2.0mM 5′-CMP displayed a sharp optimum at pH5.6. C18 cartridge was used to quantify extent of all reactions since compounds with benzyl group at anomeric position but not

[9-^{3}H]CMP-NeuAc binds C18. All reactions were carried out at 37°C for 4h. In panels b and c Na acetate and Hepes-NaOH buffers were used respectively for pH3.6-5.6 and pH6.0-8.4.

Supplemental Fig. 4.

Catalytic activity of ST3Gal-II towards macromolecules following reaction scheme-II. **a.** Transfer of [9-^{3}H]NeuAc from 0.15mM **[[9-^{3}H][1]]** to 2mg Galβ1,3GalNAcα-O-Al/AA-CP using 3mU ST3Gal-II in the presence of: i) 0.7mM 5′-CMP, ii) 0.7mM 5′-UMP, and to 2mg asialo CGM (porcine Cowper's Gland Mucin) in the presence of iii) 0.7mM 5′-CMP and iv) 0.7mM 5′-UMP. **b.** Transfer of [9-^{3}H]NeuAc to 2mg bovine submaxillary mucin (BSM, panel i) and its asialo derivative (2mg asialo BSM, panel ii) from donor 0.15mM **[[9-^{3}H][1]]** by the cloned 3mU ST3Gal-II using 0.7mM 5′-CMP. Reactions took place for 18 h. at 37°C under conditions described in Fig. 1, and products were isolated using Biogel P2 chromatography for all data in a. and b. In each case, the first peak that appears with the void volume (V_0=35mL) corresponds to the sialylated macromolecule, the second peak is the unused donor and the third CMP- or UMP- [9-^{3}H]NeuAc formed in the reaction. In all panels, donors are depicted in red, acceptor in green and nucleotide phosphate in blue. **c.** Transfer of [9-^{3}H]NeuAc to 0.6mg Fetuin Triantennary asialo glycopeptide (asialo FTG) from donor **[[9-^{3}H][2]]** by the cloned 0.75mU ST6Gal-I and 0.75mU ST3Gal-II using 1.2mM 5′-CMP. Reaction was carried out for 19h at 37ºC. Following this, the reaction mixture was diluted with 1.0ml of 10mM Hepes pH7.5 containing $CaCl_2$ and $MnCl_2$ and subjected to SNA-agarose affinity chromatography to bind α2,6 linked NeuAc. Bound product was released by 0.5M β-lactose at fraction 10 and radioactivity was measured. 57% of [9-^{3}H]NeuAc was bound to column indicating the formation of α2,6 sialylated macromolecule.

Supplemental Fig. 5.

Transfer of [9-^{3}H]NeuAc to 5′-CMP from the donor [9-^{3}H] sialylated CGM. [9-^{3}H] sialylated CGM was first isolated by incubating asialo CGM with CMP-[9-^{3}H]NeuAc and clonal ST3Gal-II for 20h at 37ºC

and then purification of macromolecule using Biogel P2 chromatography. This isolated [9-^{3}H] sialyl CGM (1mg) was subsequently incubated with 1.0mM 5′-CMP and 1.5mU clonal ST3Gal-II for 20h at 37ºC and then the reaction mixture was subjected to Biogel P2 chromatography. About 43% of [9-^{3}H]NeuAc was identified with the peak of CMP-NeuAc. Thus, the clonal ST3Gal-II was capable of synthesizing CMP-NeuAc from 5′-CMP by utilizing α2,3 sialyl T-hapten units of CGM.

Supplemental Fig. 6.

Scheme-II using acceptor Fetuin O-glycosidic asialo GP (asialo FOG). **a.** When 0.4mg asialo FOG was [9-^{3}H]sialylated with 2.0mM CMP-[9-^{3}H]NeuAc and 10mU ST6Gal-I (Chicken) at 37ºC for 20h, more than 90% of the radioactivity bound PNA-agarose suggesting that the product was α2,6 sialylated. **b.** Incubation with Gal3ST-II, instead of ST6Gal-I, however, resulted in product that did not bind PNA agarose. **c.** When 2.0mg asialo FOG was incubated with 2.0mM CMP, donor 0.15mM [9-^{3}H]NeuAcα2,3Galβ1,3(4-F GlcNAcβ1,6)GalNAcα-O-Bn and two sialyltrasferases (4.0mU ST3Gal-II and 10.0mU ST6GalNAc I) for at 37ºC 20h. (panel c), more than 90% of the product bound PNA-agarose. The results indicate that 5′-CMP was converted to CMP-NeuAc by ST3Gal-II and this intermediate was utilized by ST6GalNAc I to synthesize [9-^{3}H]NeuAcα2,6(Galβ1,3)GalNAcα-O-Ser/Thr units. **d.** Transfer of sialic acid from Fetuin O-glycosidic sialo GP (FOG) to D-Fucβ1,3GalNAcα-O-Bn was assessed using mass spectroscopy. First α2,3[9-^{3}H]FOG was synthesized by incubating 0.4mg asialo-FOG with 1.0mM CMP-[9-^{3}H]NeuAc and 1.5mU cloned ST3Gal-II, and separating product using Biogel P2 column. 6μ mol D-Fucβ1,3GalNAcα-O-Bn, 20μ mol CMP, 4mg (3μmol) FOG containing the above isolated α2,3[9-^{3}H]FOG and 80mU/mL ST3Gal-II were then incubated at 37˚C for 16h. The reaction mixture was fractionated on Biogel P2 column to separate the radioactive product arising from D-Fucβ1,3GalNAcα-O-Bn from the unused radioactive Fetuin GP. The product arising from D-Fucβ1,3GalNAcα-O-Bn was identified by Mass Spectral analysis as NeuAcα2,3D-Fucβ1,3GalNAcα-O-Bn (Theoretical M.W. 748.7). The yield of this product was 0.63μmol since 20% of radioactivity from FOG was transferred.

Supplemental Figures

Fig. 1

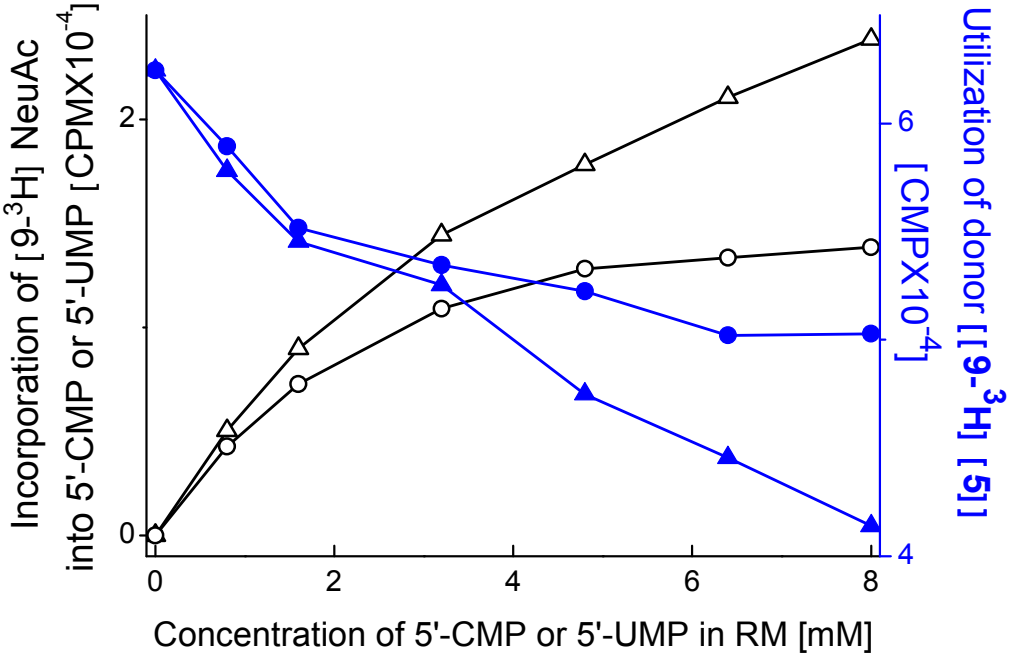


Fig. 2

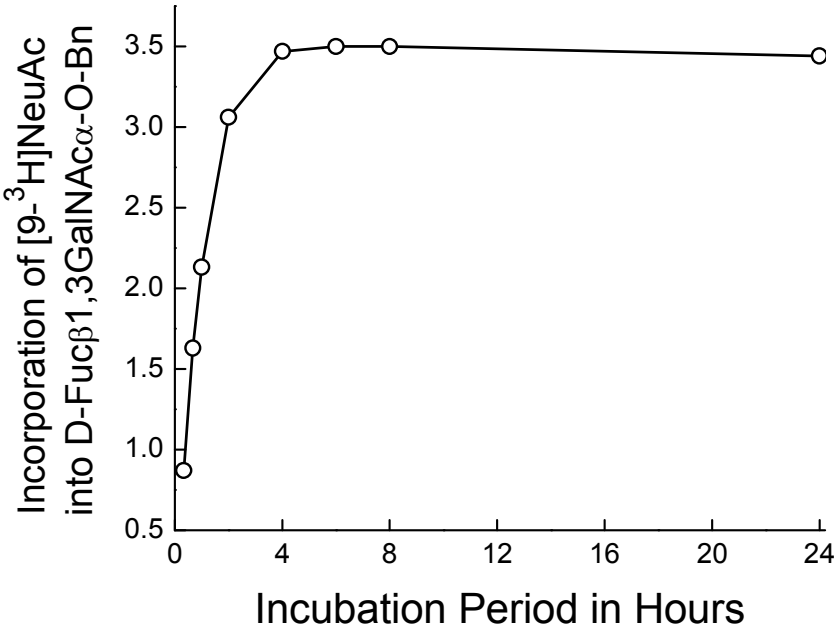

Incorporation of [9-3H]NeuAc into D-Fucβ1,3GalNAcα-O-Bn
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
4
8
12
16
20
24
Incubation Period in Hours

Fig. 3

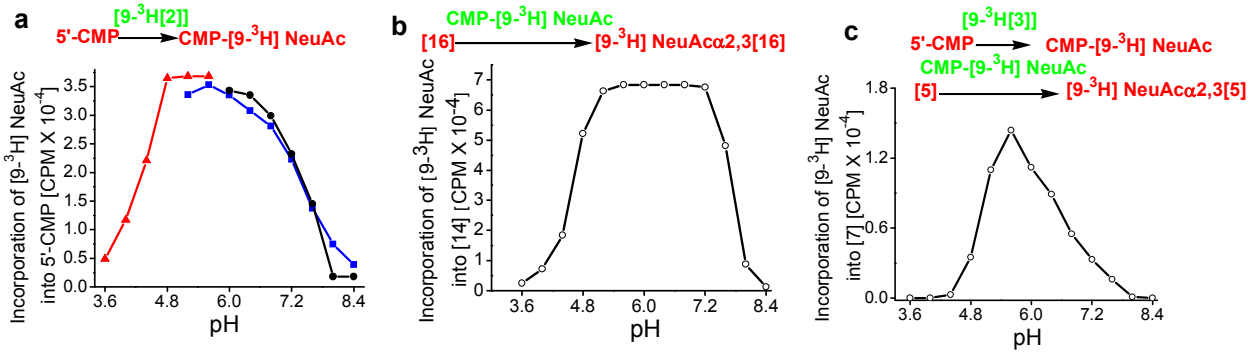

a
[9-3H[2]]
5'-CMP
CMP-[9-3H] NeuAc
Incorporation of [9-3H] NeuAc into 5'-CMP [CPM X 10-4]
pH
b
CMP-[9-3H] NeuAc
[16]
[9-3H] NeuAcα2,3[16]
Incorporation of [9-3H] NeuAc into [14] [CPM X 10-4]
pH
c
[9-3H[3]]
5'-CMP
CMP-[9-3H] NeuAc
CMP-[9-3H] NeuAc
[5]
[9-3H] NeuAcα2,3[5]
Incorporation of [9-3H] NeuAc into [7] [CPM X 10-4]
pH

Fig. 4

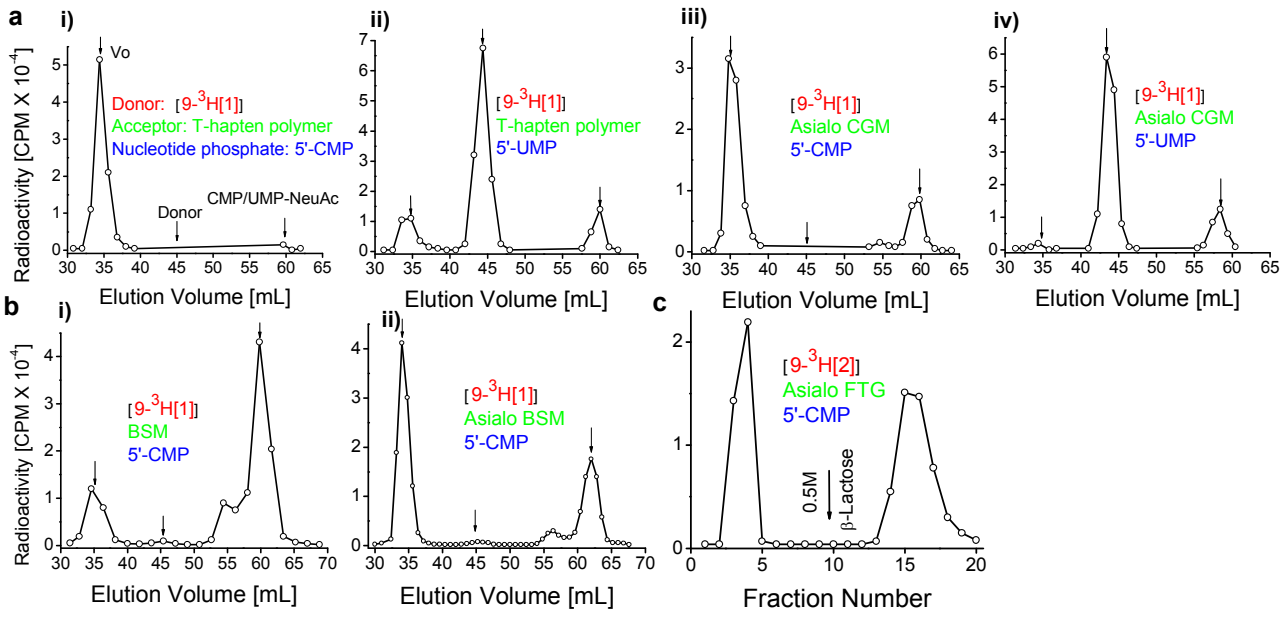

a
i)
Vo
Donor: [9-3H[1]]
Acceptor: T-hapten polymer
Nucleotide phosphate: 5'-CMP
Donor
CMP/UMP-NeuAc
Radioactivity [CPM X 10-4]
Elution Volume [mL]
ii)
[9-3H[1]]
T-hapten polymer
5'-UMP
Elution Volume [mL]
iii)
[9-3H[1]]
Asialo CGM
5'-CMP
Elution Volume [mL]
iv)
[9-3H[1]]
Asialo CGM
5'-UMP
Elution Volume [mL]
b
i)
[9-3H[1]]
BSM
5'-CMP
Radioactivity [CPM X 10-4]
Elution Volume [mL]
ii)
[9-3H[1]]
Asialo BSM
5'-CMP
Elution Volume [mL]
c
[9-3H[2]]
Asialo FTG
5'-CMP
0.5M
β-Lactose
Fraction Number

Fig. 5

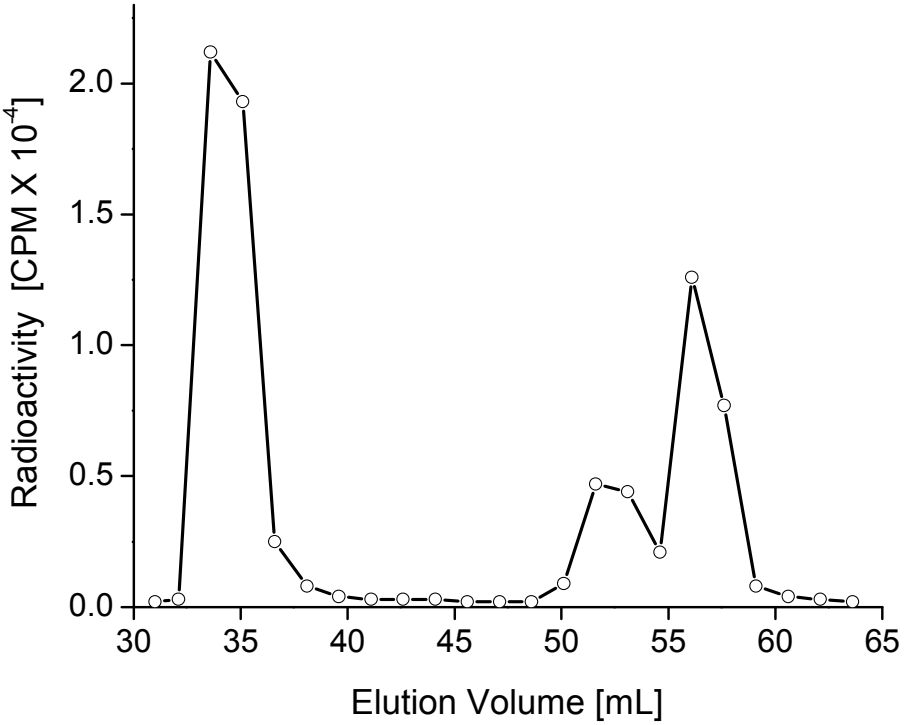
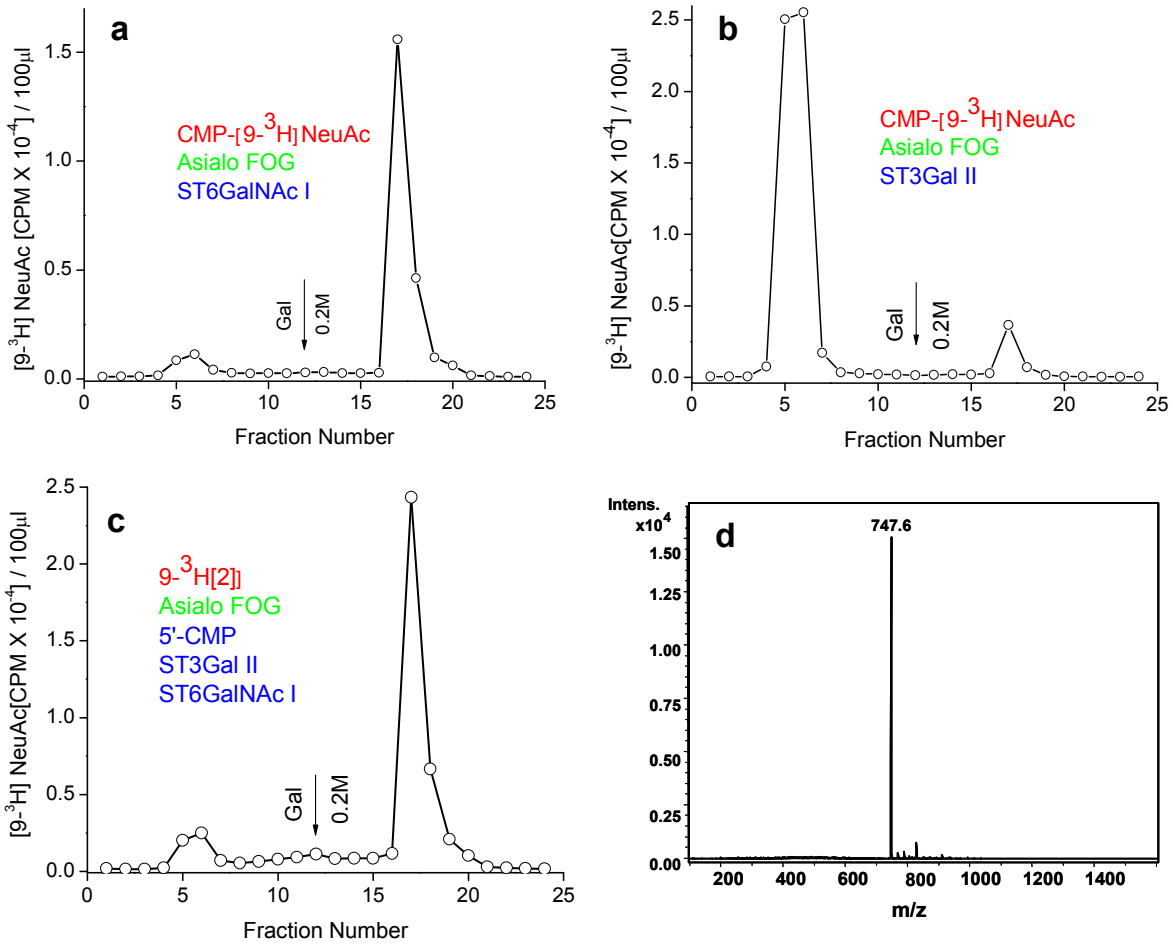

Radioactivity [CPM X 10^{-4}]
2.0
1.5
1.0
0.5
0.0
30
35
40
45
50
55
60
65
Elution Volume [mL]

Fig. 6


a
CMP-[9-3H] NeuAc
Asialo FOG
ST6GalNAc I
Gal
0.2M
[9-3H] NeuAc [CPM X 10-4] / 100µl
Fraction Number
b
CMP-[9-3H] NeuAc
Asialo FOG
ST3Gal II
Gal
0.2M
[9-3H] NeuAc[CPM X 10-4] / 100µl
Fraction Number
c
9-3H[2]
Asialo FOG
5'-CMP
ST3Gal II
ST6GalNAc I
Gal
0.2M
[9-3H] NeuAc[CPM X 10-4] / 100µl
Fraction Number
d
Intens.
x10^4
747.6
m/z