*Supporting Information 2-1*

# $^{13}$C-NMR quantification of proton exchange at LewisX hydroxyl groups in water

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# Contents

## 1. General material and methods

LewisX trisaccharide **1** was chemically synthesized from glucosamine, fucose, and galactose building blocks. Deuterium oxide for NMR solvents was purchased from ISOTEC. For NMR experiments, a standard 5 mm tube (Shigemi, Japan) and microprobe tubes (Wilmad) were used.

## 2. Experimental procedures for NMR experiments

$^{1}$H and $^{13}$C-NMR spectra were recorded using DRX-600 (600 MHz), DRX-800 (800 MHz) (BrukerBiospin) and 900 MHz spectrometer (Varian), which equipped with 5 mm triple resonance inverse (TXI) and cryogenic-TXI probes. $^{1}$H and $^{13}$C chemical shifts on 1D experiments were given in ppm referenced with the external reference of 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) at 0.00 ppm, while indirect referencing was used for $^{13}$C chemical shifts on 2D according to the absolute frequency value. The LewisX was dissolved in 10 mM sodium acetate buffer composed with $D_2O/H_2O$ = 1/1 (v/v) and adjusted pH to 6.0 to be 40 mM. Probe temperature was set at the constant temperature (278 K) or desired temperatures. $^{1}$H-$^{13}$C 2D heteronuclear single-quantum correlation (HSQC) spectra were acquired in the phase sensitive mode using the echo/anti-echo gradient selection with decoupling during acquisition. $^{1}$H/$^{13}$C correlation was obtained via double INEPT transfer using trim pulse of 0.1 ms duration. The spectra were recorded with 256 experiments of 1024 data points. Data processing and analysis was performed by using XWIN-NMR ver. 3.5 (BrukerBiospin) and Delta ver. 4.3.6 (JEOL).

The proton exchanging rates at coalescence temperature ($T_c$) were obtained from Gutowski's equation (1),

$$k_{ex} = \pi(\Delta\nu)/\sqrt{2} \qquad (1)$$

in which $k_{ex}$ is the proton exchange rate ($s^{-1}$) at $T_c$ (K) and $\Delta\nu$ is the deuterium secondary isotope shift on $^{13}C$ signal (Hz).

The $k_{ex}$ values indicated in Table 1 include some degree of the errors mainly based on the accuracy of the coalescence temperature, because the temperature was increased in each 5 degrees. Additionally, digital resolutions of the $^{13}C$-NMR spectra (1~2 Hz/point) would also give influence to the accuracy. The errors in $k_{ex}$ are thus estimated to be at most 10 %.

### 3. Supplemental Figures

**Figure S1**. $^{1}H$-$^{13}C$ HSQC spectra of LewisX, (red), with 200 mM $CaCl_2$ (green), and 1.0 M $CaCl_2$ (blue). $CaCl_2$ titration broadly influences chemical-shift changes of LewisX trisaccharide because of the very weak binding affinity.

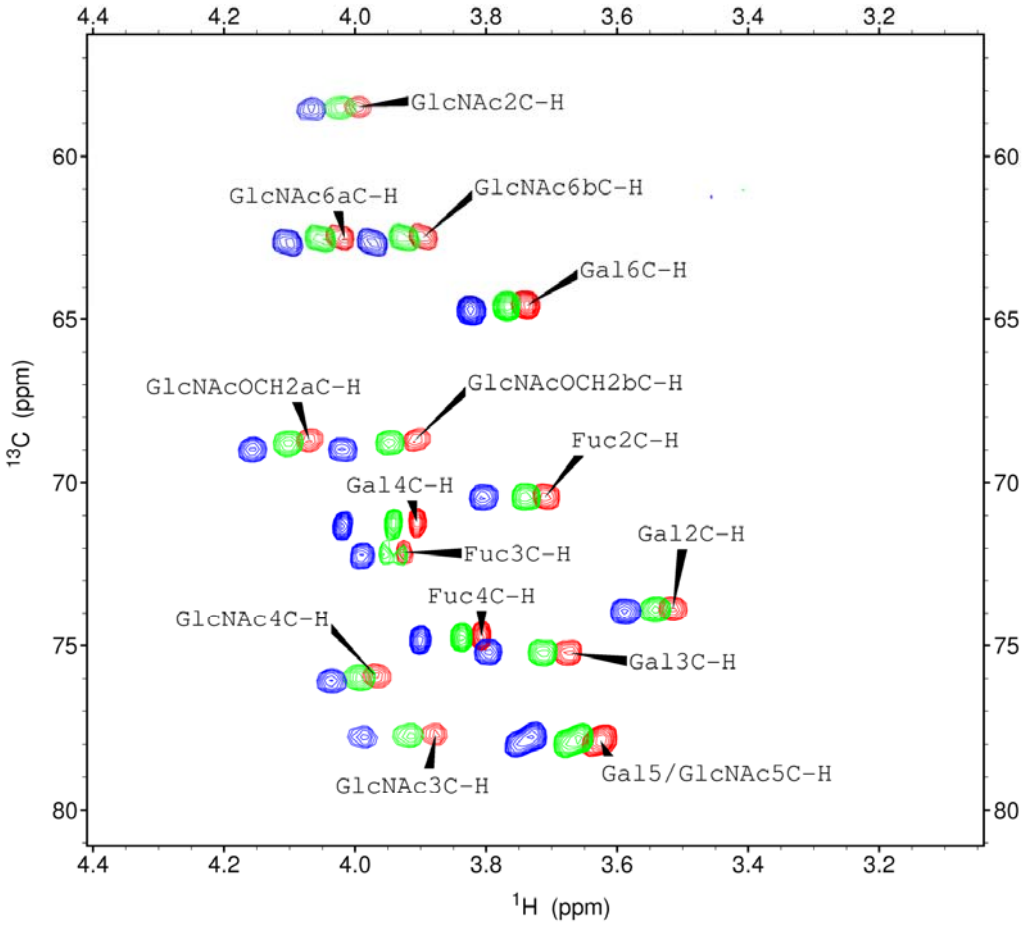


**Figure S2.** $^{1}H$-$^{13}C$ HSQC spectra of β-(OMe)-galactoside **2**, (red), with 200 mM $CaCl_2$ (green), and 1.0 M $CaCl_2$ (blue).

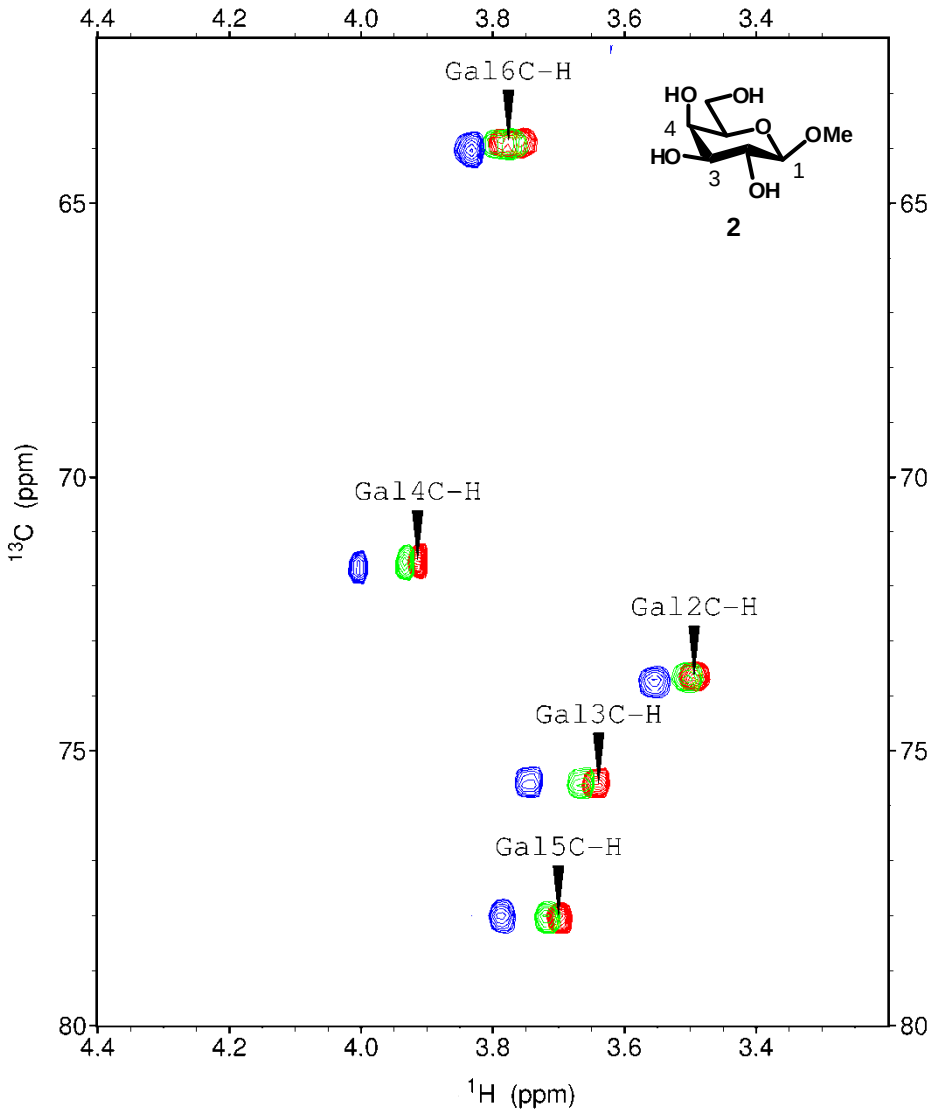


**Figure S3.** $^{1}H$-$^{13}C$ HSQC spectra of α-(OMe)-fucoside **3**, (red), with 200 mM $CaCl_2$ (green), and 1.0 M $CaCl_2$ (blue).

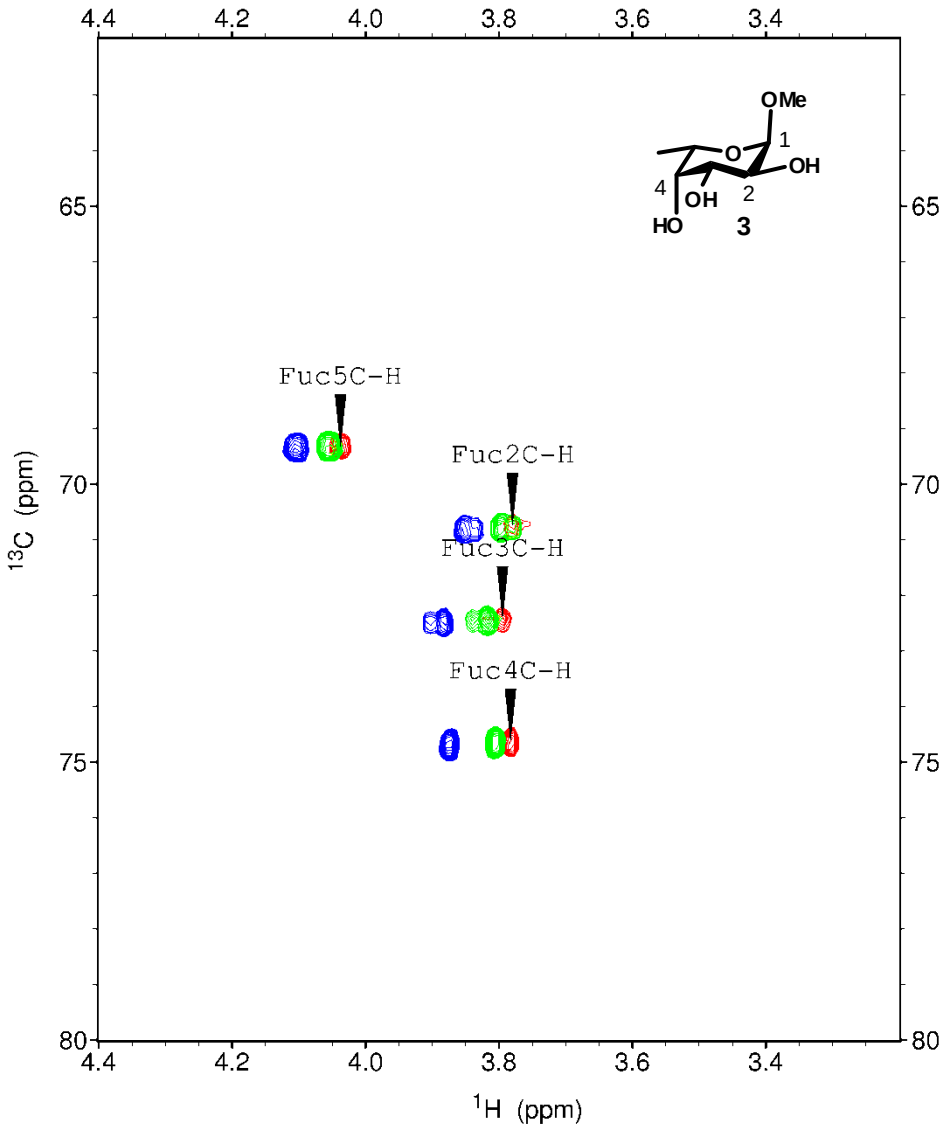


**Figure S4.** $^{13}$C-NMR spectra of LewisX **3** expanded in the area reporting on the C6 group of GlcNAc and Gal, and C2 group of GlcNAc in the presence or absence of $CaCl_2$ at 5 °C or −10 °C a); LewisX at 5 °C, b); LewisX with 1.0 M $CaCl_2$ at 5 °C, c) ; LewisX at −10 °C, d); LewisX with 1.0 M $CaCl_2$ at −10 °C. All spectra were collected in 10 mM sodium acetate buffer (pH 6.0) in $H_2O$:$D_2O$ = 50:50 using 900 MHz ($^1$H-resononce frequency) spectrometer with a 3 mm sample tube. The amide proton of GlcNAc C2NH is a doublet even at 5 °C, which is generally explained by its slow exchange. Gal6 and GlcNAc6 also showed doublet signals in the presence of $CaCl_2$ (b and d).

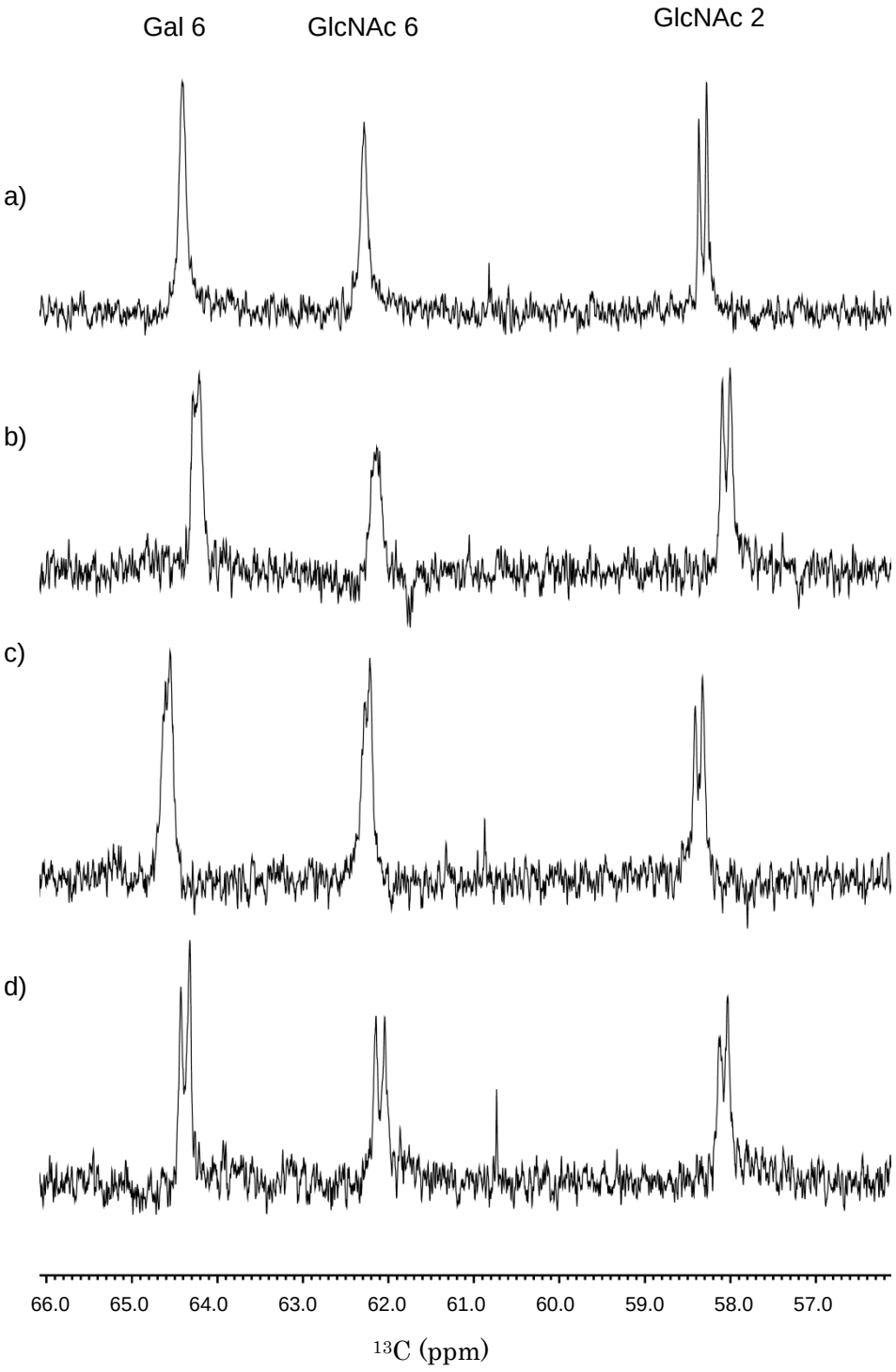
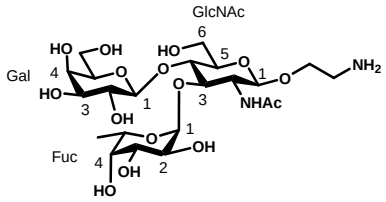

Gal 6
GlcNAc 6
GlcNAc 2
a)
b)
c)
d)
66.0
65.0
64.0
63.0
62.0
61.0
60.0
59.0
58.0
57.0
$^{13}$C (ppm)

**Table S1**. Assignment of $^{13}C$- and $^{1}H$-signals of LewisX trisaccharide **3** at 5 °C in 10 mM AcONa buffer (pH 6.0). The spectrum was collected on a 600 MHz instrument equipped with a TXI-probe. Chemical shifts of $^{1}H$- and $^{13}C$-axes are referred from DSS at 0 ppm. The full assignment was achieved through correlations with 2D $^{1}H$-$^{1}H$ DQF-COSY, 2D $^{1}H$-$^{1}H$ NOESY, 2D $^{1}H$-$^{13}C$ HSQC-TOCSY spectra (data not shown).




| Residue | $^{13}C$ (ppm) | $^{1}H$ (ppm) |
|---|---|---|
| Fuc1 | 101.7 | 5.13 |
| Fuc2 | 70.4 | 3.71 |
| Fuc3 | 72.1 | 3.93 |
| Fuc4 | 74.7 | 3.81 |
| Fuc5 | 69.7 | 4.87 |
| Fuc6 | 18.1 | 1.19 |
| Gal1 | 103.6 | 4.60 |
| Gal2 | 73.9 | 3.52 |
| Gal3 | 75.2 | 3.67 |
| Gal4 | 71.2 | 3.91 |
| Gal5 | 77.9 | 3.63 |
| Gal6 | 64.6 | 3.74 |
| GlcNAc1 | 104.6 | 4.47 |
| GlcNAc2 | 58.5 | 4.00 |
| GlcNAc3 | 77.7 | 3.88 |
| GlcNAc4 | 75.9 | 3.97 |
| GlcNAc5 | 77.9 | 3.63 |
| GlcNAc6 | 62.5 | 4.02 |
| | | 3.90 |
| $GlcNAcOCH_2$ | 68.7 | 4.07 |
| | | 3.91 |
| $NH_2CH_2$ | 42.4 | 3.21 |
| | | 3.24 |
| NHAc | 26.0 | 1.94 |

| | | |
|---|---|---|
| C=O | 171.1 | – |

**Figure S6**. $^{13}$C-NMR spectra of Methyl-β-O-galactoside **2** and Methyl-α-O-fucoside **3** **at** 5 °C in the presence or absence of 1.0 M $CaCl_2$ at pH 6.0 in 10 mM AcONa buffer. A; β-OMe-Gal **2** only, B; β-OMe-Gal **2** with 1.0 M $CaCl_2$, C; α-OMe-Fuc **3** only, D; α-OMe-Fuc **3** with 1.0 M $CaCl_2$. $Ca^{2+}$ hardly changed the chemical shifts, except for that of the C3 galactoside, and the cation did not change the signal shape. These data suggest that the proton-exchange rate of the monosaccharides is not significantly influenced by $Ca^{2+}$.

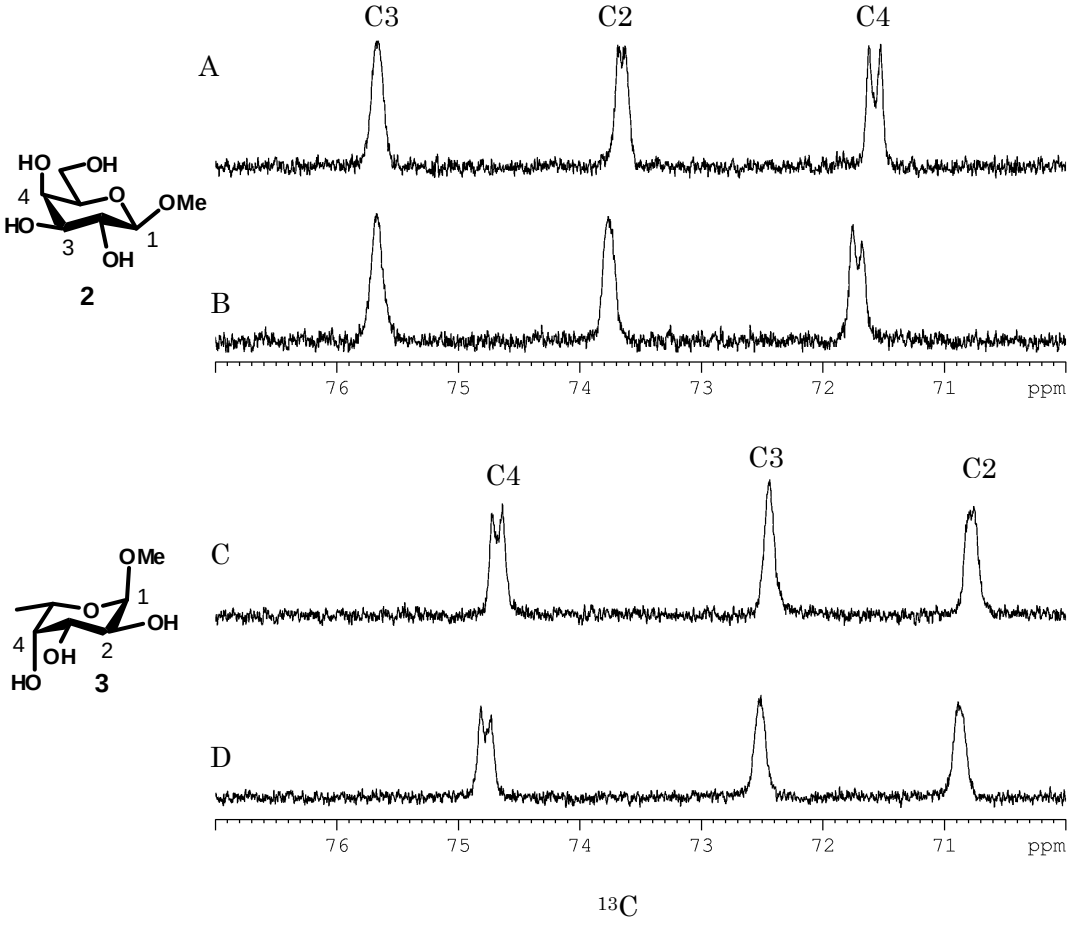
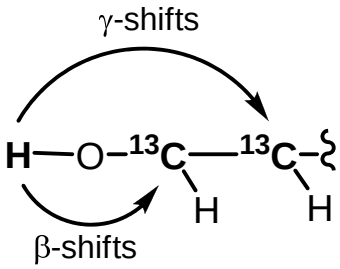
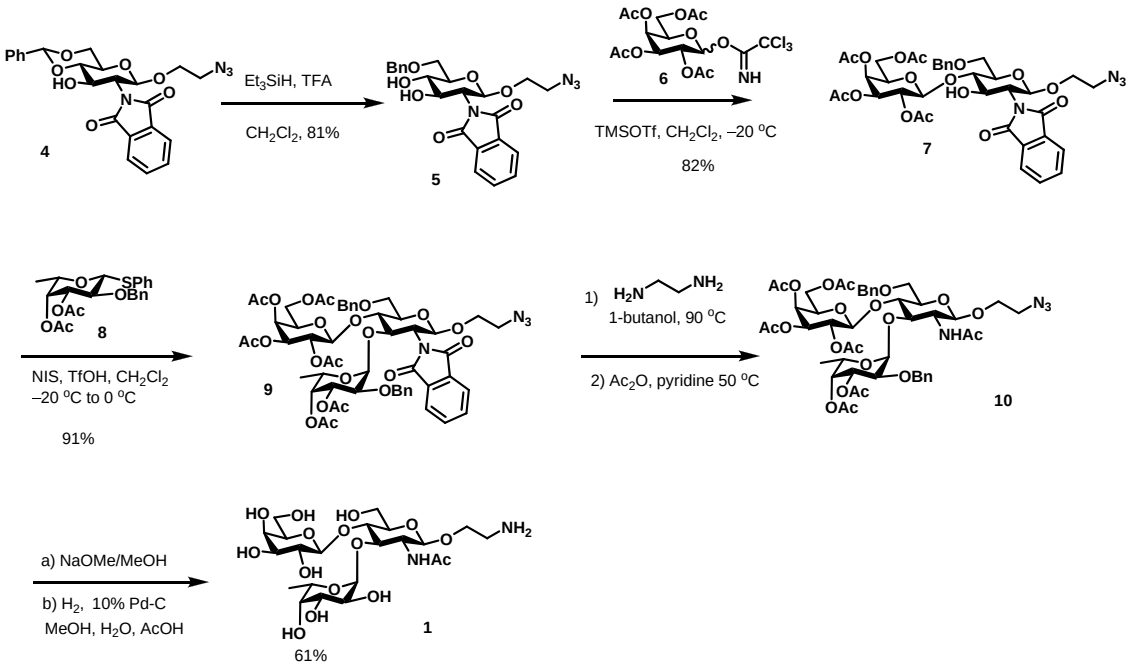


**Figure S7**. β-Shifts and γ-shifts on $^{13}$C-NMR isotope shifts.



## 4. Synthesis of LewisX trisaccharide 1.




**Scheme S1**. Synthesis of LewisX trisaccharide **1**.

LewisX trisaccharide **1** was synthesized as shown in Scheme S1. Initially, the benzylidene group of known GlcNAc derivative **4**[1] was submitted to reductive benzylidene opening using TFA and $Et_3SiH$ to give diol **5** in 76% yield. Coupling with galactose imidate **6**[2] in the presence of catalytic amount of TMSOTf at 0 °C produced disaccharide **7** in 60% yield.[3] Under these conditions, the bis-galactosylated byproduct was obtained in 12% yield. Then, the fucose moiety was introduced to **7** using phenylthio derivative **8**[4], which was activated by NIS and TfOH at −20 °C to produce the desired trisaccharide **9** in 91% yield The trisaccharide **9** was submitted to global deprotection in 3 steps to give LewisX trisaccharide **1**. The phthaloyl group was removed by treatment with ethylenediamine in 1-butanol at 90 °C, and re-acetylation by $Ac_2O$ and pyridine produced **10** in 72% yield. Acetyl groups on compound **10** were removed using Zemplen conditions, and final hydrogenolysis to remove the benzyl groups and reduction of an azide group produced desired LewisX trisaccharide **1** in 61%

yield.

## 5. Experimental procedures for the synthesis of LewisX 1.

**2-Azidoethyl 2-deoxy-2-phthalimidyl-6-*O*-benzyl-β-D-glucopyranoside** (**5**).

A solution of compound **4** (100 mg, 0.214 mmol) in $CH_2Cl_2$ (3 mL) was cooled in an ice-water bath, and TFA (90 μL, 1.2 mmol) and $Et_3SiH$ (180 μL, 1.1 mmol) were added. After stirring at room temperature for 1.5 h under an atmosphere of argon, the mixture was concentrated and co-evaporated with toluene. The residue was purified with silica-gel flash column chromatography (hexane:EtOAc = 2:1 to 1:1) to give **5** (74 mg, 0.16 mmol, 76%).

$[\alpha]_D^{23}$ −30 (*c* 0.4, chloroform);

$^1$H NMR (400 MHz, $CDCl_3$); δ 7.82 (m, 2H, PhH), 7.69 (m, 2H, PhH), 7.38-7.25 (m, 5H, PhH), 5.29 (d, 1H, *J* = 8.1 Hz, H-1), 4.64 (d, 1H, *J* = 12.1 Hz, Bn), 4.58 (d, 1H, *J* = 12.1 Hz, Bn), 4.31 (dd, 1H, *J* = 7.3, 7.3 Hz, H-3), 4.16 (dd, 1H, *J* = 7.3, 8.1 Hz, H-2), 3.96 (m, 1H, $OCH_2$), 3.79 (m, 2H, H-6), 3.64-3.58 (m, 3H, H-4, H-5, $OCH_2$), 3.36 (m, 1H, $CH_2N_3$), 3.16 (br s, 1H, -OH), 3.14 (m, 1H, -$CH_2N_3$), 2.68 (br s, 1H, -OH);

$^{13}$C NMR (100 MHz, $CDCl_3$); δ 168.4, 137.7, 134.0, 131.6, 128.4, 127.8, 123.3, 98.2 (C1), 74.4 (C5), 73.6 (Bn), 73.0 (C4), 71.6 (C3), 69.9 (C6), 68.3 ($OCH_2$), 56.2 (C2), 50.3 ($CH_2N_3$);

HR-ESI-MS: calcd for $C_{23}H_{24}N_4O_7Na$; 491.1543, found; m/z 491.1535 $[M+Na]^+$.

**2-Azidoethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl**

**(1-4) 2-deoxy-2-phthalimidyl-6-*O*-benzyl-β-D-glucopyranoside (7).**

A solution of diol **5** (66 mg, 0.142 mmol) and imidate **6** (102 mg, 0.207 mmol) in $CH_2Cl_2$ (3 mL) was cooled to 0 °C, and TMSOTf (3 μL) was added. The reaction mixture was stirred for 30 min at −10 °C under an atmosphere of argon, and then

neutralized with $Et_3N$. The reaction mixture was concentrated, and purified with size-exclusion chromatography (BioRad SX-3, toluene) and silica-gel chromatography (hexane:EtOAc = 4:1 to 2:1) to give disaccharide **7** (95 mg, 0.116 mmol, 82%).

$[\alpha]_D^{22}$ + 3 (*c* 0.4, chloroform);

$^1$H NMR (400 MHz, $CDCl_3$); δ 7.82 (m, 2H, PhH), 7.70 (m, 2H, PhH), 7.41-7.30 (m, 5H, PhH), 5.32 (d, 1H, *J* = 3.5 Hz, H-$4_{Gal}$), 5.28 (d, 1H, *J* = 8.5 Hz, H-$1_{GN}$), 5.18 (dd, 1H, *J* = 8.1, 10.5 Hz, H-$2_{Gal}$), 4.92 (dd, 1H, *J* = 3.5, 10.5 Hz, H-$3_{Gal}$), 4.74 (d, 1H, *J* = 12.1 Hz, Bn), 4.52 (d, 1H, *J* = 12.1 Hz Bn), 4.47 (d, 1H, *J* = 8.1 Hz, H-$1_{Gal}$), 4.40 (dd, 1H, *J* = 8.1, 10.7 Hz, H-$3_{GN}$), 4.19 (dd, 1H, *J* = 8.5, 10.7 Hz, H-2GN), 4.04 (d, 2H, *J* = 6.6 Hz, H-$6_{Gal}$), 4.02-3.97 (m, 2H, OH, $OCH_2$), 3.90 (dd, 1H, J = 6.6, 6.6 Hz, H-$5_{Gal}$), 3.75-3.69 (m, 3H, H-$4_{GN}$, H-$6_{GN}$), 3.66-3.60 (m, 2H, H-5GN, $OCH_2$), 3.39 (m, 1H, $CH_2N_3$), 3.17 (m, 1H, $CH_2N_3$), 2.11 (s, 3H, Ac), 1.99 (s, 3H, Ac), 1.97 (s, 3H, Ac), 1.92 (s, 3H, Ac);

$^{13}$C NMR (100 MHz, $CDCl_3$); δ 170.6, 170.2, 170.1, 169.3, 138.1, 134.1, 131.9, 128.7, 128.5, 128.0, 127.9, 127.7, 123.4, 123.3, 101.6 ($C1_{Gal}$), 98.4 ($C1_{GN}$), 82.0 ($C4_{GN}$), 74.3 ($C5_{GN}$), 73.8 (Bn), 71.3 ($C5_{Gal}$), 70.8 ($C3_{Gal}$), 69.7 ($C3_{GN}$), 68.8 ($C2_{Gal}$), 68.6 ($OCH_2$), 68.0 ($C6_{GN}$), 66.9 ($C4_{Gal}$), 61.5 ($C6_{Gal}$), 55.9 ($C2_{GN}$), 50.5 ($CH_2N_3$), 20.8, 20.7, 20.6, 20.4;

HR-ESI-MS: calcd for $C_{37}H_{42}N_4O_{16}Na$; 821.2494, found; m/z 821.2514 $[M+Na]^+$.

**2-Azidoethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl (1-4) {2-O-benzyl-3,4-di-O-acetyl-α-L-fucosyl-(1-3)}2-deoxy-2-phthalimidyl-6-*O*-benzyl-β-D-glucopyranoside (9).**

To a solution of disaccharide acceptor **7** (73 mg, 0.891 mmol) and fucose donor **8** (56 mg, 0.14 mmol) in $CH_2Cl_2$ (2 mL) MS 4Å (500 mg) was added and the mix stirred for

15 min at room temperature under an atmosphere of argon. After cooling to −20 °C, NIS (37 mg, 0.16 mmol) and TfOH (1.5 μL) was added to the reaction mixture and all was stirred for 6 h at −20 °C to 0 °C. Then, the mixture was neutralized with $Et_3N$, filtered through Celite, and the filtrate diluted with EtOAc. The organic phase was washed with 3% $Na_2S_2O_3$ solution, saturated $NaHCO_3$ and brine, dried over $Na_2SO_4$, filtered, and concentrated. The residue was purified by size exclusion chromatography (BioRad SX-3, toluene) and silica-gel flash chromatography produced **9** (92 mg, 0.082 mmol, 91%).

$[\alpha]_D^{23}$ − 30 (*c* 1.2, chloroform);

$^1$H NMR (400 MHz, $CDCl_3$); δ 7.78 (br s, 2H, PhH), 7.73 (br s, PhH), 7.43-7.32 (m, 5H, PhH), 7.20-7.12 (m, 3H, PhH), 6.90 (m, 2H, PhH), 5.28-5.26 (m, 2H, H-$4_{Fuc}$, H-$4_{Gal}$), 5.12 (d, 1H, *J* = 8.3 Hz, H-$1_{GN}$), 5.11 (dd, 1H, *J* = 3.3, 10.8 Hz, H-$3_{Fuc}$), 4.98 (dd, 1H, *J* = 8.4, 10.1 Hz, H-$2_{Gal}$), 4.96 (m, 1H, H-$5_{Fuc}$), 4.84 (d, 1H, *J* = 11.9 Hz, Bn), 4.77 (d, 1H, *J* = 4.1 Hz, H-$1_{Fuc}$), 4.75 (dd, 1H, *J* = 3.6, 10.5 Hz, H-$3_{Gal}$), 4.67 (dd, 1H, *J* = 9.2, 10.7 Hz, H-$3_{GN}$), 4.64 (d, 1H, *J* = 8.4 Hz, H-$1_{Gal}$), 4.47 (d, 1H, *J* = 12.2 Hz, Bn), 4.43 (dd, 1H, *J* = 8.3, 10.7 Hz, H-$2_{GN}$), 4.41 (dd, 1H, *J* = 3.3, 8.4 Hz, H-6a$_{Gal}$), 4.34 (d, 1H, *J* = 12.2 Hz, Bn), 4.27 (dd, 1H, *J* = 7.5, 11.7 Hz, H-6b$_{Gal}$), 4.17 (dd, 1H, *J* = 9.5, 9.5 Hz, H-$4_{GN}$), 4.08 (d, 1H, *J* = 12.6 Hz, Bn), 3.95 (m, 1H, $OCH_2$), 3.86 (dd, 1H, *J* = 2.7, 11.2 Hz, H-6a$_{GN}$), 3.77 (d, 1H, *J* = 11.2 Hz, H-6b$_{GN}$), 3.55 (dd, 1H, *J* = 3.7, 10.2 Hz, H-$2_{Fuc}$), 3.55-3.50 (m, 3H, H-$5_{Gal}$, H-$5_{GN}$, $OCH_2$), 3.31 (m, 1H, $CH_2N_3$), 3.16 (m, 1H, $CH_2N_3$), 2.11 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.01 (s, 3H, Ac) 1.95 (s, 3H, Ac), 1.93 (s, 3H, Ac), 1.76 (s, 3H, Ac), 1.14 (d, 3H, *J* = 6.3 Hz, H-$6_{Fuc}$);

$^{13}$C NMR (100 MHz, $CDCl_3$); δ 170.6, 170.1, 169.6, 169.0, 137.7, 137.5, 134.3, 128.8, 128.3, 128.1, 128.0, 127.6, 123.8, 99.7 (C$1_{Gal}$), 98.4 (C$1_{GN}$), 97.4 (C$1_{Fuc}$), 75.3 (C$5_{GN}$), 74.3 (C$4_{GN}$), 73.8 (Bn), 72.6 (Bn), 72.2 (C$3_{GN}$), 72.0 (C$^{4Fuc}$), 71.6 (C$2_{Fuc}$), 71.1 (C$3_{Gal}$),

71.0 ($C5_{Gal}$), 70.4 ($C3_{Fuc}$), 69.3 ($C2_{Gal}$), 68.1 ($OCH_2$), 67.4 ($C6_{GN}$), 67.0 ($C4_{Gal}$), 64.4 ($C5_{Fuc}$), 61.1 ($C6_{Gal}$), 56.1 ($C2_{GN}$), 50.5 ($CH_2N_3$), 21.0, 20.8, 20.74, 20.68, 20.6, 15.9 ($C6_{Fuc}$);

HR-ESI-MS: calcd for $C_{54}H_{62}N_4O_{22}Na$; 1141.3753, found; m/z 1141.3777 $[M+Na]^+$.

**2-Aminoethyl β-D-galactopyranosyl (1-4)**

**{α-L-fucosyl-(1-3)}2-deoxy-2-acetamido-β-D-glucopyranoside (1)**

To a suspension of trisaccharide **9** (82 mg, 0.073 mmol) in 1-BuOH (4 mL) ethylenediamine (1 mL) was added and the mix stirred at 85 °C for 18 h. After cooling to room temperature, the reaction mixture was concentrated, and co-evaporated with toluene/MeOH three times. Then, the crude mixture was dissolved in pyridine (3 mL) and $Ac_2O$ (1.5 mL) added dropwised in an ice-water bath. After stirring for 15 h at 50 °C, the mixture was cooled to room temperature, and evaporated. The residue was re-dissolved in excess EtOAc, and successively washed with 1 M HCl, $H_2O$, saturated $NaHCO_3$ solution, and brine. Then, the organic layer was dried with $Na_2SO_4$, filtered, and concentrated to give crude **10**. The crude compound **10** was then dissolved in 0.05 M NaOMe in MeOH (3 mL) with a portion of phenolphthalein as an indicator. After stirring for 12 h at room temperature, the mixture was acidified by a few drops of AcOH. Then, 10% Pd-C (42 mg) and $H_2O$ (1 mL) was added and the mix stirred for 18 h at room temperature under an atmosphere of hydrogen gas. After completion of the reaction, the mixture was filtered through Celite and stirred for 30 min under an atmosphere of nitrogen. The filtrate was concentrated, and purified by solid-phase extraction (SepPak C-18, MeOH/$H_2O$) and size exclusion column chromatography (Sephadex G-15, $H_2O$) to give desired trisaccharide **3** (29 mg, 0.047mmol, 64%).

Details of the assignment of $^{1}$H- and $^{13}$C-NMR data of the compound **1** are summarized in Table S1.

$[\alpha]_D^{24}$ − 58 (*c* 0.2, $H_2O$);

HR-ESI-MS: calcd for $C_{22}H_{40}N_2O_{15}Na$; 595.2326, found; m/z 595.2344 $[M+Na]^+$.

## 6. References

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