SUPPLEMENTARY INFORMATION

Carbohydrate microarrays for screening functional glycans

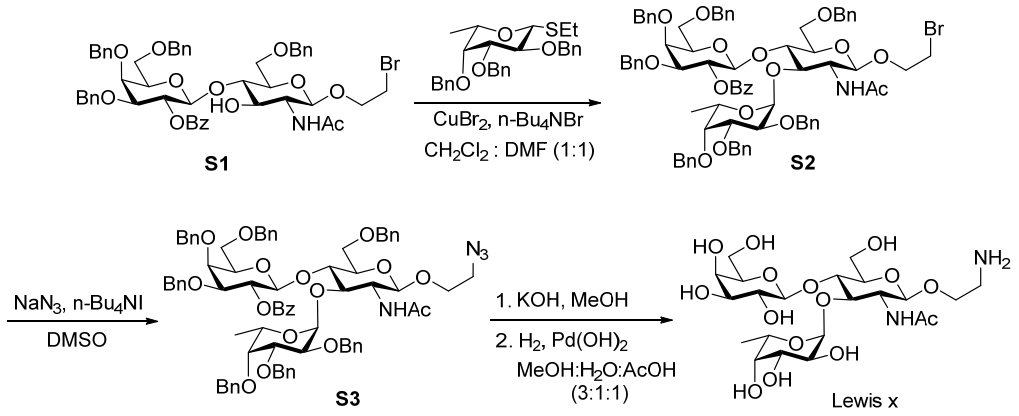
Jaeyoung Pai,[1,§] Ji Young Hyun,[1,§] Jieun Jeong,[1] Sohee Loh,[2] Eun-Hee Cho,[2] Young-Sun Kang,[2] and Injae Shin[1,*]

[1]*Center for Biofunctional Molecules, Department of Chemistry, Yonsei University, Seoul* 03722, *Korea*
[2]*Department of Biomedical Science and Technology, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Medicine, Konkuk University, Seoul 05029, Korea*

[§]These two authors equally contributed to this work.

SUPPLEMETAL PROCEDURE

Synthesis of Lewis x



2-Bromoethyl 2-acetamido-4-O-(2-O-benzoyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl) -6-O-benzyl-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-2-deoxy-β-D-glucopyranoside (S2). To a mixture of **S1** (132 mg, 0.14 mmol), ethyl 2,3,4-tri-*O*-benzyl-1-thio-*β*-L-fucopyranoside (93 mg, 0.19 mmol), $CuBr_2$ (93 mg, 0.42 mmol), n-Bu_4NBr (138 mg, 0.43 mmol) and freshly activated molecular sieve 4 Å (250 mg) was added a mixture of anhydrous CH_2Cl_2 and DMF (1:1, 4 mL) at room temperature under argon atmosphere. After stirring for 24 h, the reaction mixture was filtered through Celite® pad. The filtrate was diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$, water and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography (hexane : EtOAc = 3:1) to afford **S2** as a white amorphous solid in 78% yield: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92 (d, 2 H, *J* = 7.0 Hz), 7.59 (t, 1H), 7.44 (t, 2 H), 7.37-7.15 (m, 35 H), 5.72 (d, 1 H, *J* = 7.5 Hz), 5.52 (q, 1 H), 5.02 (d, 1 H, *J* = 4.0 Hz), 4.97 (d, 1 H, *J* = 11.0 Hz), 4.76 (t, 2 H), 4.69-4.64 (m, 5H), 4.58 (q, 3 H), 4.52 (q, 2 H), 4.44-4.41 (m, 3 H), 4.34 (d, 1 H, *J* = 12.0 Hz), 4.14-4.10 (m, 2 H), 4.06 (d, 1 H, *J* = 2.5 Hz), 4.00 (dd, 1 H, *J* = 14.0, 6.0 Hz), 3.96 (d, 1 H, *J* = 8.0 Hz), 3.87-3.82 (m, 2 H), 3.78 (t, 1 H), 3.69 (d, 1 H, *J* = 4.0 Hz), 3.67 (d, 1 H), 3.57-3.53 (m, 1 H), 3.50 (dd, 1 H, *J* = 13.0, 7.5 Hz), 3.43-3.38 (m, 2 H), 3.30-3.26 (m, 4 H), 1.72 (s, 3 H), 1.19 (d, 3 H, *J* = 6.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.3, 165.1, 139.3, 139.1, 139.0, 138.7, 138.2, 137.8, 133.3, 129.8, 128.9, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 127.2, 99.7, 99.6, 97.3, 79.9, 79.8, 78.3, 76.1, 75.5, 75.0, 74.8, 73.6, 73.5, 73.4, 73.3, 73.0, 72.9, 72.4, 72.1, 71.8, 69.0, 68.4, 67.8, 66.7, 30.3, 23.4, 16.5; MALDI-TOF-MS calcd for $C_{78}H_{84}BrNO_{16}Na$ $[M + Na]^+$ 1392.4 and 1394.4, found 1392.4 and 1394.4 (1:1).

2-Azidoethyl 2-acetamido-4-O-(2-O-benzoyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-6-O-benzyl-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-2-deoxy-β-D-glucopyranoside (S3). To a stirred solution of **S2** (121 mg, 0.09 mmol) in DMSO (1.5 mL) was treated with NaN_3 (29 mg, 0.4 mmol) and n-Bu_4NI (65 mg, 0.18 mmol) at room temperature under

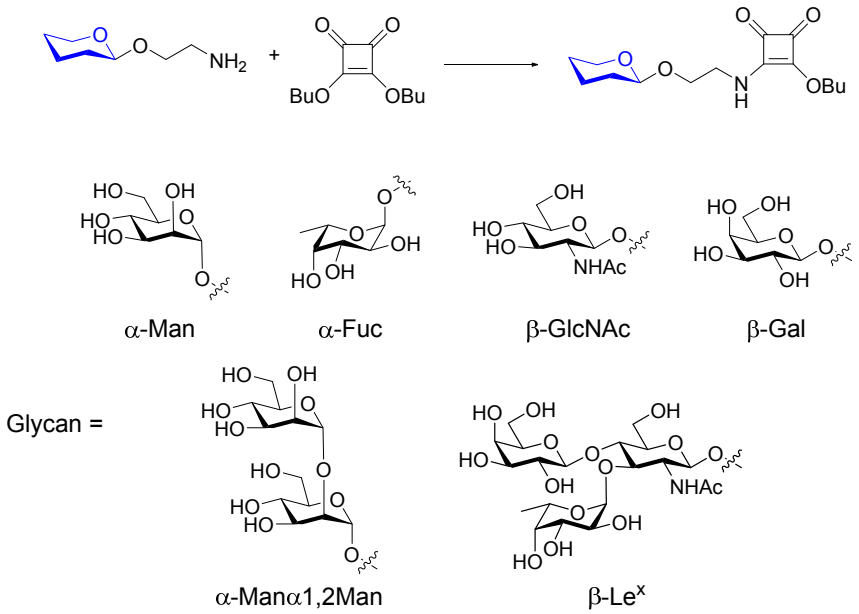
nitrogen atmosphere. After stirring for 24 h, the reaction mixture was poured into H_2O (200 mL) and extracted with CH_2Cl_2 (15 mL) several times. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography (hexane : EtOAc = 2.5:1) to afford **S3** as a white amorphous solid in 84% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, 2 H, J = 7.6 Hz), 7.59 (t, 1 H), 7.44 (t, 2 H), 7.37-7.12 (m, 35 H), 5.65 (d, 1 H, *J* = 7.2 Hz), 5.51 (q, 1 H), 4.96-4.93 (m, 2H), 4.88 (d, 1 H, *J* = 7.6 Hz), 4.80 (d, 1 H, *J* = 12.0 Hz), 4.70-4.65 (m, 4 H), 4.62 (d, 1 H, *J* = 9.2 Hz), 4.58 (dd, 2 H, *J* = 7.6, 3.6 Hz), 4.55 (d, 1 H, *J* = 9.2 Hz), 4.52-4.48 (m, 3 H), 4.41 (s, 2 H), 4.34 (d, 1 H, *J* = 12.0 Hz), 4.17 (t, 1 H), 4.11-4.07 (m, 2 H), 4.00 (dd, 1 H, *J* = 14.0, 6.8 Hz), 3.95 (d, 1 H, *J* = 8.0 Hz), 3.85-3.81 (m, 2 H), 3.77 (d, 1 H, *J* = 8.4 Hz), 3.70-3.67 (m, 2 H), 3.53-3.47 (m, 2 H), 3.44-3.39 (m, 2 H), 3.33-3.24 (m, 4 H), 3.14-3.08 (m, 1 H), 1.66 (s, 3 H), 1.19 (d, 3 H, *J* = 6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 165.1, 139.3, 139.1, 139.0, 138.7, 138.2, 137.8, 137.7, 133.2, 129.9, 129.0, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.3, 99.6, 99.5, 97.5, 80.0, 78.3, 76.0, 75.5, 75.0, 74.8, 73.8, 73.6, 73.5, 73.2, 73.1, 73.0, 72.2, 72.1, 71.7, 68.3, 68.2, 67.8, 66.7, 50.5, 23.4, 16.5; MALDI-TOF-MS calcd for $C_{78}H_{84}N_4O_{16}Na$ $[M + Na]^+$ 1355.5, found 1355.5.

2-Aminoethyl 2-acetamido-4-O-(β-D-galactopyranosyl)-3-O-(α-L-fucopyranosyl)-2-deoxy-β-D-glucopyranoside (Le^x). To a stirred solution of **S3** (99 mg, 0.07 mmol) in CH_2Cl_2 (1 mL) was added 3% KOH in MeOH (5 mL) at room temperature. After stirring for 10 h, the reaction mixture was diluted with CH_2Cl_2 and washed with H_2O. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography (hexane : EtOAc = 2:1) to afford a debenzoylated product as a white amorphous solid in 77% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39-7.22 (m, 35 H), 5.69 (d, 1 H, *J* = 6.4 Hz), 5.10 (d, 1 H, *J* = 7.6 Hz), 4.96 (d, 1 H, *J* = 2.8 Hz), 4.87 (q, 2 H), 4.76 (d, 1 H, *J* = 12.0 Hz), 4.70-4.60 (m, 6 H), 4.48 (t, 3 H), 4.42-4.38 (m, 3 H), 4.32 (t, 1 H), 4.10 (d, 1 H, *J* = 11.6 Hz), 4.01-3.93 (m, 5 H), 3.87 (d, 1 H, *J* = 9.6 Hz), 3.78 (d, 2 H, *J* = 10.0 Hz), 3.71-3.55 (m, 4 H), 3.43-3.39 (m, 1 H), 3.34-3.27 (m, 3 H), 3.22-3.10 (m, 2 H), 1.60 (s, 3 H), 1.01 (d, 3 H, *J* = 6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170. 8, 139.2, 138.9, 138.8, 138.3, 138.2, 137.9, 128.8, 128.6, 128.5, 128.4, 128.2, 128.1, 127.9, 127.8, 127.7, 127.6, 127.5, 127.3, 127.2, 101.6, 99.4, 97.8, 82.2, 80.0, 78.1, 76.2, 75.4, 75.0, 74.8, 74.2, 74.1, 73.4, 73.1, 72.7, 72.6, 72.1, 71.5, 68.5, 68.3, 67.8, 66.5, 58.9, 50.6, 29.8, 23.3, 16.5; MALDI-TOF-MS calcd for $C_{71}H_{80}N_4O_{15}Na$ $[M + Na]^+$ 1251.5, found 1251.5.

A mixture of an above compound (70 mg, 0.06 mmol) and $Pd(OH)_2$ (210 mg) in MeOH-H_2O-HOAc (3:1:1, 5 mL) was stirred for 27 h at room temperature under hydrogen atmosphere. The reaction mixture was filtered through Celite® pad. The filtrate was concentrated under reduced pressure at 15 ^{o}C and the remaining volatile materials were removed by lyophilization to afford Le^x as a white solid with quantitative yield; ^{1}H NMR (400 MHz, D_2O) δ 5.11 (d, 1 H, *J* = 4.0 Hz), 4.59 (d, 1 H, *J* = 8.4 Hz), 4.45 (d, 1 H, *J* = 8.0 Hz), 4.08-4.02 (m, 1 H), 3.97 (t, 2 H), 3.93-3.85 (m, 6 H), 3.79 (d, 1 H, *J* = 2.8 Hz), 3.77-3.72 (m, 3 H), 3.71-3.68 (m, 1 H), 3.66 (d, 1 H, *J* = 3.6 Hz), 3.64-3.58 (m, 2 H), 3.50 (q, 1 H),

3.28-3.22 (m, 2 H), 2.04 (s, 3 H), 1.18 (d, 3 H, J = 6.8 Hz); ^{13}C NMR (100 MHz, D_2O) δ 174.6, 101.8, 100.7, 98.6, 75.3, 74.9, 73.2, 72.5, 71.9, 69.2, 68.3, 67.6, 66.7, 65.7, 61.5, 59.6, 55.5, 39.4, 22.2, 21.4, 15.3; HR ESI-MS calcd for $C_{22}H_{40}N_2O_{15}$ $[M + Na]^+$ 595.2321, found 595.2323.

Preparation of squarate-activated carbohydrates.



A solution of 3,4-dibutyl-3-cyclobutene-1,2-dione (5 μL, dibutyl squarate) and triethylamine (0.3 μL) in methanol (80 μL) was added to 2-aminoethylated glycans (2 mg, α-Man, α-Fuc, β-GlcNAc, β-Gal, α-Manα1,2Man, β-Le^x), which were prepared according to a known procedure,[1] in water (5 μL). After stirring for 5 min, methanol was quickly removed by a flow of air. The residue was diluted with water (100 μL) and purified by C18 reversed-phase column chromatography according to the known procedure.[2] The appropriate fractions were combined and freeze-dried to give the squarate-activated carbohydrates as a hygroscopic solid in 80-90% yields.

Squarate activated α-Man; ESI-MS calculated for $C_{16}H_{25}NO_9$ $[M + H]^+$ 376, found 376.
Squarate activated α-Fuc; ESI-MS calculated for $C_{16}H_{25}NO_8$ $[M + H]^+$ 360, found 360.
Squarate activated β-GlcNAc; ESI-MS calculated for $C_{18}H_{28}N_2O_9$ $[M + H]^+$ 417, found 417.
Squarate activated β-Gal; ESI-MS calculated for $C_{16}H_{25}NO_9$ $[M + H]^+$ 376, found 376.
Squarate activated α-Manα1,2Man; ESI-MS calculated for $C_{22}H_{35}NO_{14}$ $[M + H]^+$ 538, found 538.
Squarate activated β-Le^x; ESI-MS calculated for $C_{30}H_{48}N_2O_{18}$ $[M + H]^+$ 725, found 725.

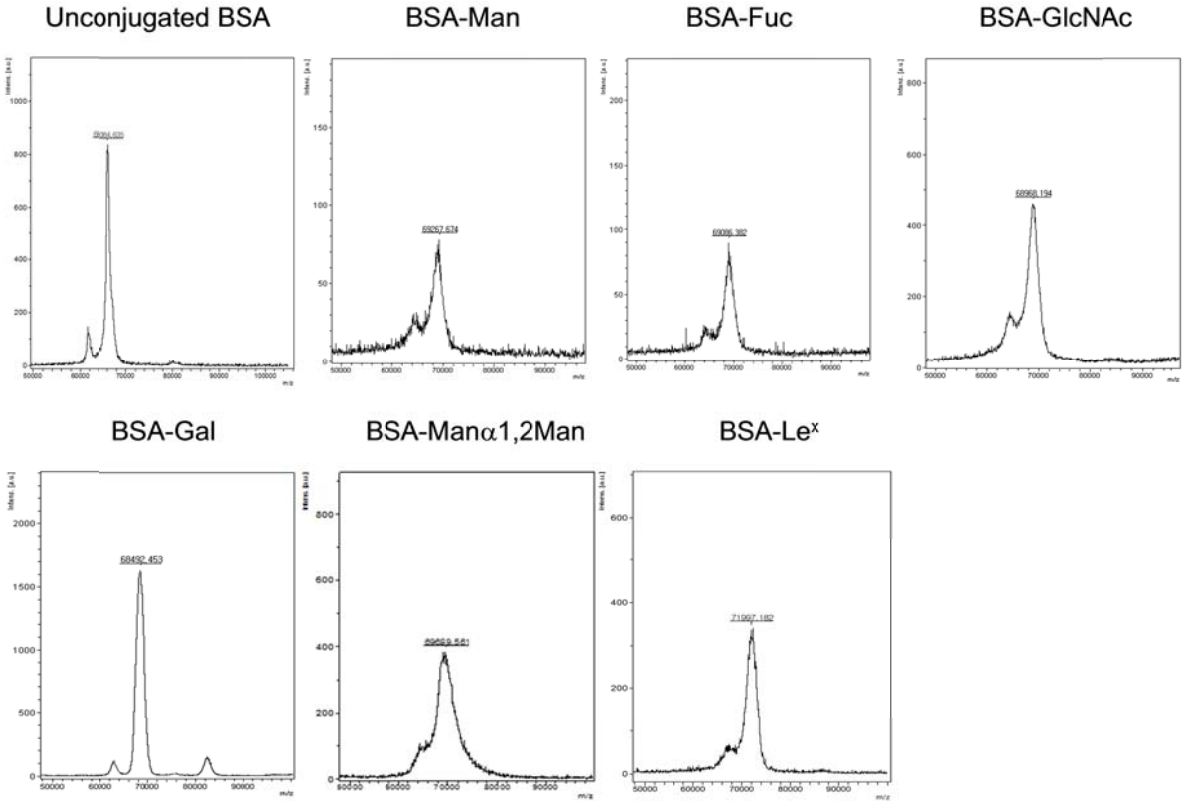
Preparation of BSA-glycan conjugates.

Squarate-activated carbohydrate (1.13 μmol) was added to BSA (5 mg) in 0.5 M borate buffer (pH 9). The mixture was gently stirred for 3 days. Low-molecular mass material was removed by using an Amicon centrifugal filter device (cutoff: 30 KDa) and the BSA-glycan conjugates were washed with aqueous 10 mM $(NH_4)_2CO_3$. Glycan conjugation ratios to BSA were determined by MS analysis.

<Glycan conjugation ratios to BSA>

Glycoconjugates	BSA-$(Man)_n$	BSA-$(Fuc)_n$	BSA-$(GlcNAc)_n$	BSA-$(Gal)_n$	BSA-$(Man\alpha 1,2Man)_n$	BSA-$(Le^x)_n$
n	10	9.9	8	7.6	7.5	8.9

<MALDI-TOF-MS data of BSA-glycan conjugates>



Glycoconjugates	Unconjugated BSA	BSA-Man	BSA-Fuc	BSA-GlcNAc	BSA-Gal	BSA-Manα1,2Man	BSA-Le^x
Mass	66084	69267	69086	68968	68492	69699	71997

Supplementary References

1. (a) S. Park, and I. Shin, *Org. Lett.,* 2007, **9**, 1675-1678; (b) J. Angulo, I. Diaz, J. J. Reina, G. Tabarani, F. Fieschi, J. Rojo, and P. M. Nieto, *ChemBioChem,* 2008, **9,** 2225-2227.

2. (a) A. Chernyak, S. Oscarson, D. Turek, *Carbohyd. Res.,* 2000, **329**, 309-316; (b) H. Yan, A. L. Aguilar, Y. Zhao, *Bioorg. Med. Chem. Lett.,* 2007, **17,** 6535-6538.

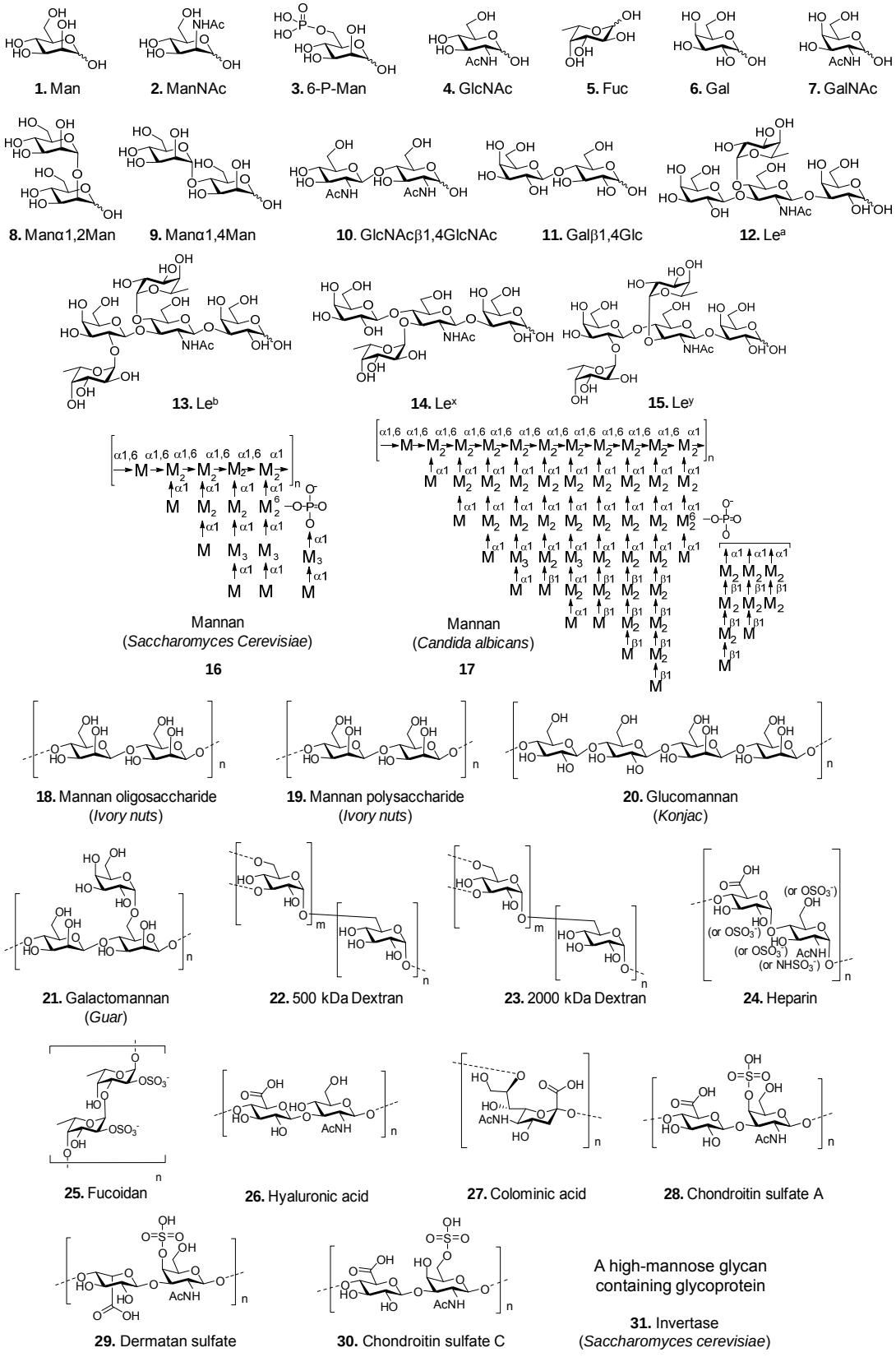


Figure S1. Structure of glycans shown in Table 1 (**16** and **17**, M = mannose).

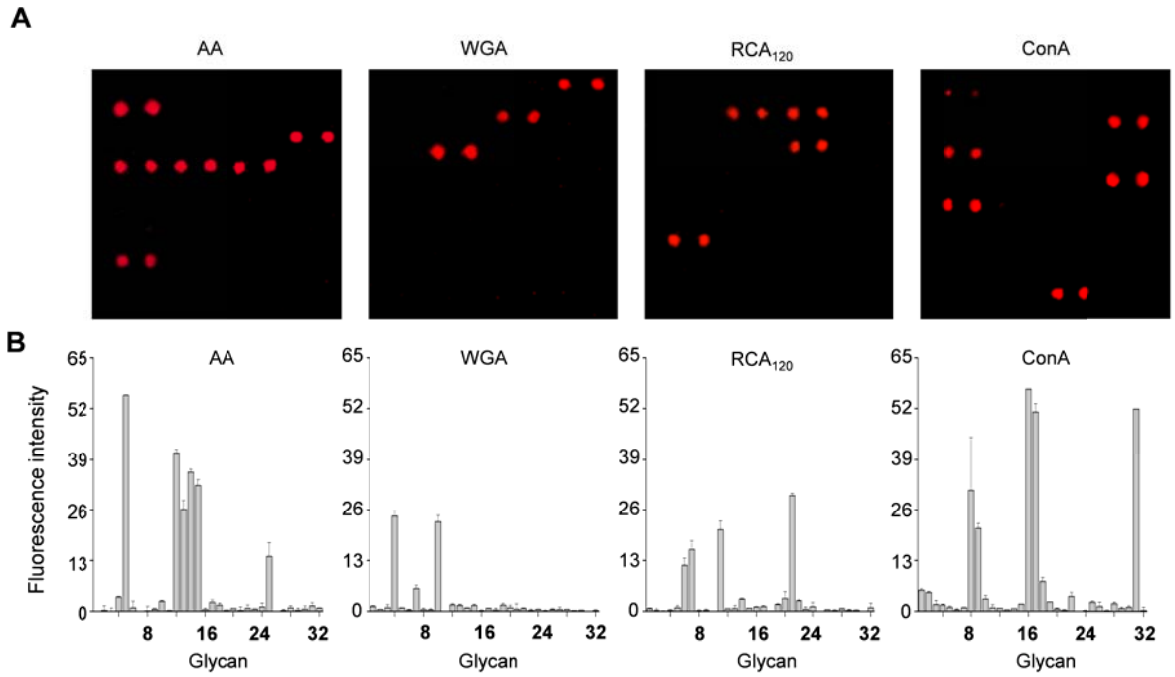


Figure S2. (A) Fluorescence images of carbohydrate microarrays after probing with Cy3-AA, Cy3-WGA, Cy3-RCA_{120} and Cy3-ConA, fluorescence intensity (FI, arbitrary unit) of carbohydrate microarrays in (A) (error bar: mean ± s.d., n = 3).

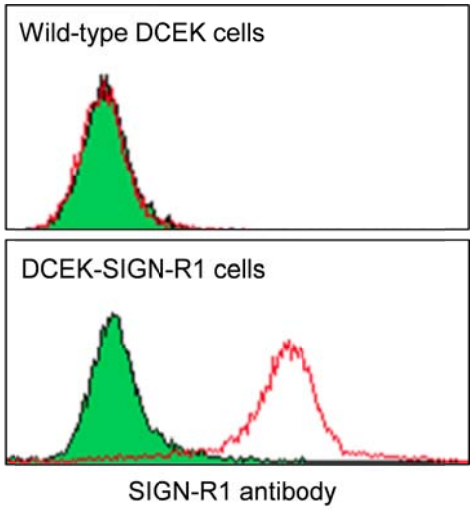


Figure S3. DCEK cells express mouse SIGN-R1 lectin after stable transfection.

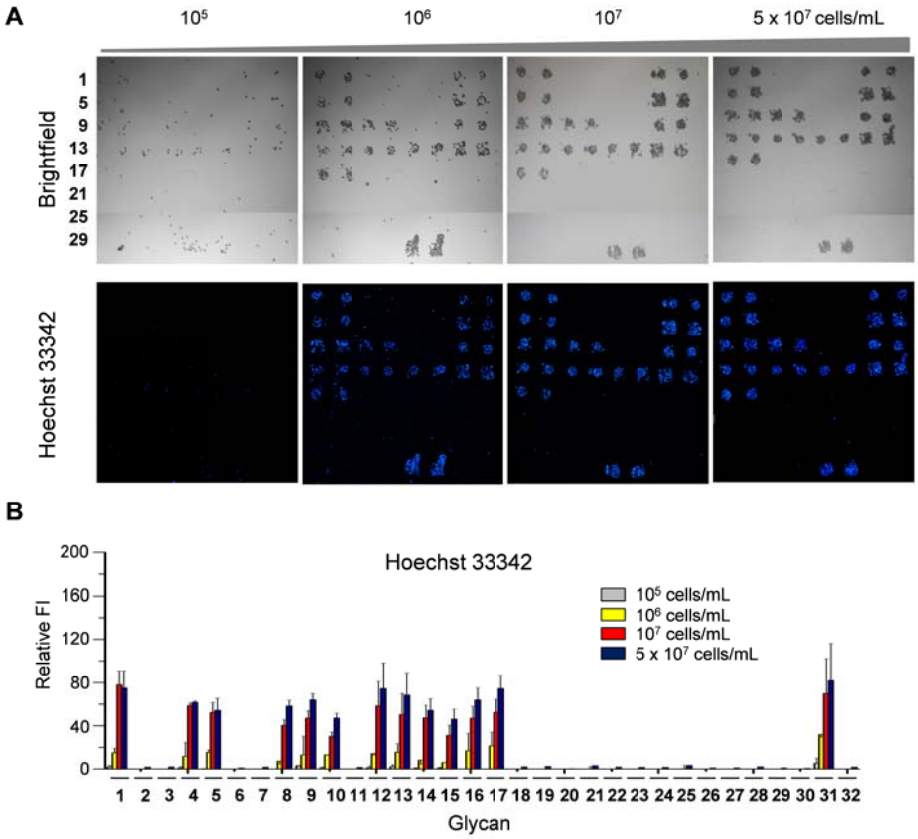


Figure S4. (A) DCEK-SIGN-R1 cells (10^5–5 x 10^7 cells per mL) cultured under non-adherent conditions were treated with 1 μM Hoechst 33342 and then applied to carbohydrate microarrays. After 1 h incubation, images were obtained by using a microscopy (a distance between centers of adjacent spots: 350 μm). (B) Quantitative analysis of fluorescence intensity of Hoechst 33342 in (A) (error bar: mean ± s.d., n = 3).

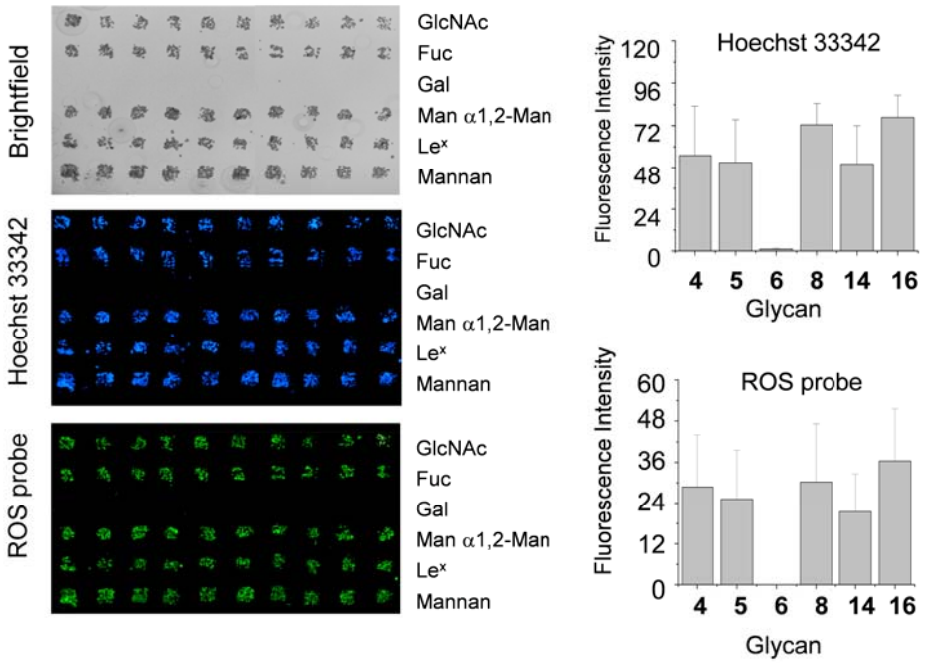


Figure S5. Carbohydrate microarrays containing 10 spots of each of six glycans were incubated for 1 h with DCEK-SIGN-R1 cells pretreated with 50 μM PF1 and 1 μM Hoechst 33342. After washing, images were obtained by using a microscopy (a distance between centers of adjacent spots: 350 μm). Graphs show quantitative fluorescence intensity of PF1 and Hoechst 33342 (error bar: mean ± s.d., n = 3).

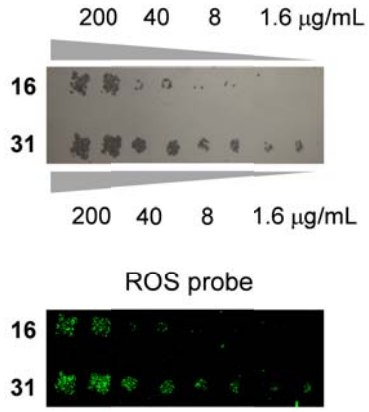


Figure S6. Glycan immobilization concentration-dependent study. Carbohydrate microarrays constructed by immobilizing various concentrations of *S. cerevisiae* mannan (**16**) and invertase (**31**) were incubated with DCEK-SIGN-R1 cells (10^7 cells/mL) treated with 50 μM PF1 for 1 h. After incubation for 1 h, the bound cells were analyzed by using a microscopy and/or a microarray scanner.

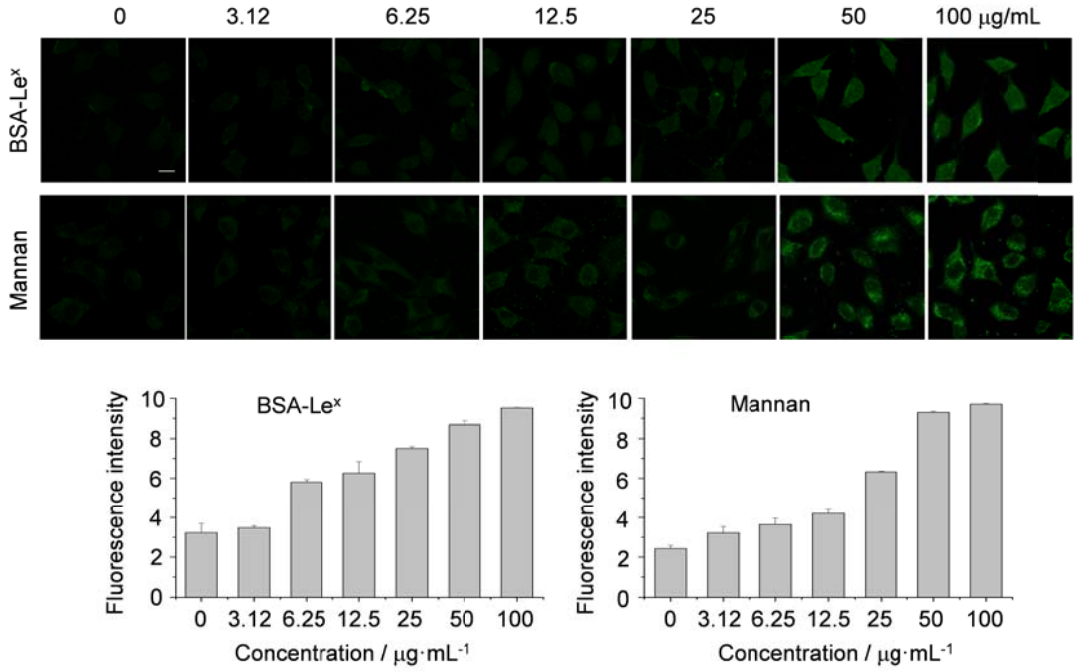


Figure S7. DCEK-SIGN-R1 cells pretreated with 50 μM PF1 for 1 h were incubated with BSA-Le^x and *S. cerevisiae* mannan at various concentrations for 2 h. After washing, images were obtained by using confocal microscopy (scale bar: 10 μm). Graphs show quantitative fluorescence intensity of PF1 in glycan treated cells (error bar: mean ± s.d., n = 3).

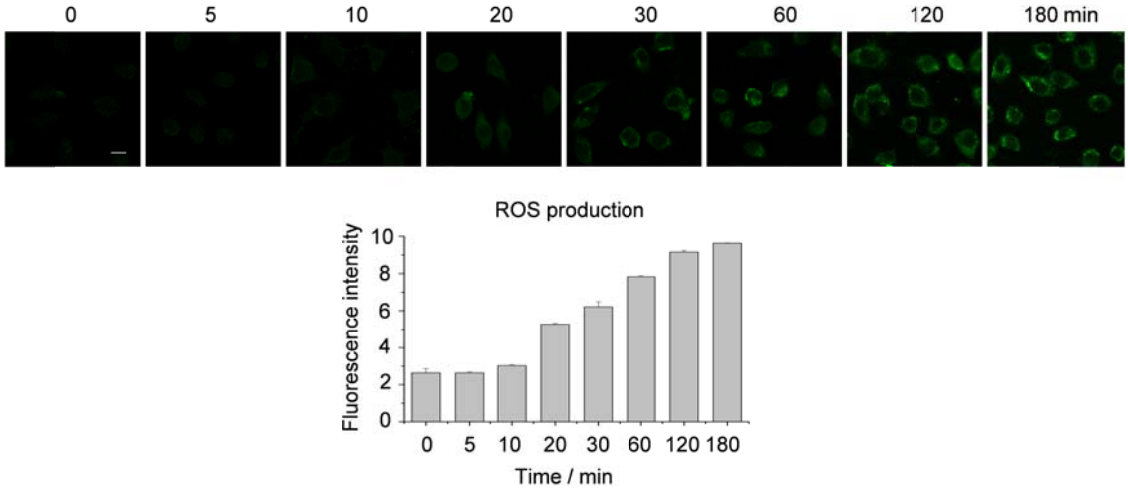


Figure S8. DCEK-SIGN-R1 cells pretreated with 50 μM PF1 for 1 h were incubated with 50 mg/mL of *S. cerevisiae* mannan during different time periods. After washing, images were obtained by using confocal microscopy (scale bar: 10 μm). Graph shows quantitative fluorescence intensity of PF1 in glycan treated cells (error bar: mean ± s.d., n = 3).

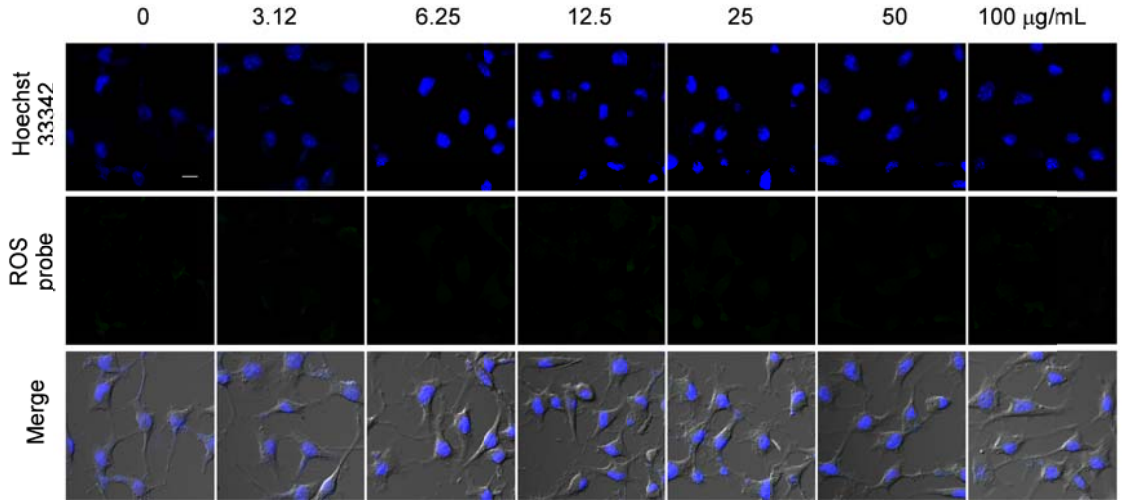


Figure S9. DCEK-SIGN-R1 cells pretreated 50 μM PF1 for 1 h were incubated with incubated with unconjugated BSA at various concentrations for 2 h. After washing, images were obtained by using confocal microscopy. The nucleus of cells was stained with 1 μM Hoechst 33342 (scale bar: 10 μm).

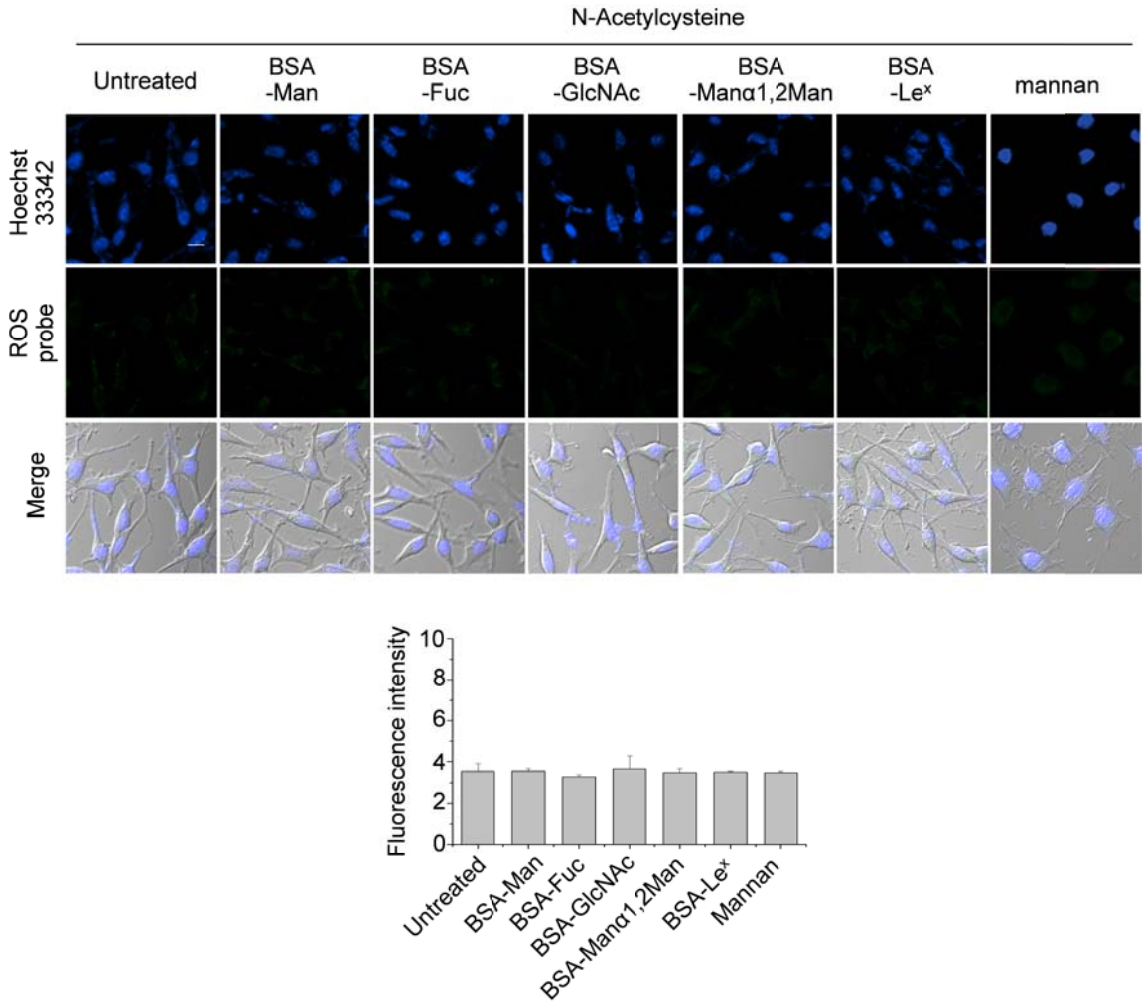


Figure S10. DCEK-SIGN-R1 cells pretreated with 30 mM *N*-acetylcysteine and 50 μM PF1 for 1 h were incubated with 50 μg/mL of each glycoconjugate or *S. cerevisiae* mannan for 2 h. Glycan untreated cells were used as a negative control. After washing, images were obtained by using confocal microscopy. The nucleus of cells was stained with 1 μM Hoechst 33342 (scale bar: 10 μm). Graph shows quantitative fluorescence intensity of PF1 in treated cells (error bar: mean ± s.d., n = 3).

NMR spectra of Le^x