Thermodynamic Analysis of Interactions between *N*-linked Sugar Chains and Fbs1

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Supporting Information

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General Procedures. Reagents and solvents were purchased from standard suppliers and used without further purification. Reactions were monitored with TLC plates precoated with Merck silica gel 60 F254. Merck silica gel-60 was used for silica gel flash chromatography. ^{1}H and ^{13}C NMR spectra were measured on a JEOL EX-400 spectrometer. Coupling constants (J values) are represented in hertz. MALDI-TOF MS was recorded in the positive ion mode on a AXIMA-CFR (Shimazu) equipped with a nitrogen laser with an emission wavelength of 337 nm.

Compound 12

To a solution of acceptor **10** (1.6 g, 2.7 mmol), donor **11** (1.8 g, 2.8 mmol), and NIS (3.0 g, 13.3 mmol) in dry dichloromethane (60 ml) containing MS4A (20 g), TfOH (0.3 ml, 3.4 mmol) was added dropwise under Ar. The mixture was stirred at –78℃ for 1 h, diluted with AcOEt, and filtered through Celite. The filtrate was washed with 10% Na_2SO_3, $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was purified by silica gel column chromatography (hexane:AcOEt, 3:1) to afford **12** (2.9 g, 98%): ^{1}H NMR (400 Hz, $CDCl_3$) □ = 7.68-6.79 (m, 33 H, Ar), 5.31 (d, 1 H, J = 8.4 Hz), 5.13 (t, 1 H, J = 9.2 Hz), 4.93 (d, 1 H, J = 8.0 Hz), 4.79 (d, 1 H, J = 12.8 Hz), 4.66 (d, 1 H, J = 12.4 Hz), 4.58 (d, 1 H, J = 12.4 Hz), 1.90 (s, 3 H); MALDI-TOFMS $C_{65}H_{60}N_2NaO_{14}$ calcd: 1115.39, found: 1114.68 $(M + Na)^+$.

Compound 13

To a solution of **12** (6.3 g, 5.8 mmol) in THF (20 ml) containing 30% H_2O_2 (20 ml), LiOH (0.2 g, 8.7 mmol) was added at 0℃. The mixture was stirred at 0℃ for 12 h, diluted with AcOEt, and washed with H_2O and brine. The organic layer was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was purified by silica gel column chromatography (hexane:AcOEt, 2:1) to give compound **13** (5.5 g, 90%): ^{1}H NMR (400 Hz, $CDCl_3$) □ = 7.69-6.80 (33 H, Ar), 5.30 (d, 1 H, J = 8.0 Hz), 4.94 (d, 1 H, J = 8.0 Hz), 4.78 (d, 1H, J = 12.0 Hz), 4.76 (d, 1 H. J = 12.0 Hz), 4.68 (d, 1 H, J = 12.0 Hz), 3.81 (t, 1 H, J = 8.8 Hz), 3.70 (dd, 1 H, J = 10.0 and 4.4 Hz), 3.54 (dd, 2 H, J = 10.0 and 6.4 Hz), 3.43 (dd, 1 H, J = 11.2 and 3.6 Hz); MALDI-TOFMS $C_{63}H_{58}N_2NaO_{13}$ calcd: 1073.38, found: 1073.67 $(M + Na)^+$.

Compound 15

With cooling in ice-water, DDQ (1.1 g, 4.8 mmol) was added to a stirred mixture of **13** (4.0 g, 3.8 mmol), **14** (3.0 g, 4.6 mmol), and molecular sieves 4A (3 g) in CH_2Cl_2 (20 ml) under Ar. The mixture was stirred at room temperature for 3 h, quenched with an aqueous solution of ascorbic acid (0.7%)-citric acid (1.3%)-NaOH (0.9%), stirred for 5 min, diluted with AcOEt, and filtered through Celite. The filtrate was washed with $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was mixed with 2,6-di-*tert*-butyl-4-methylpyridine (3.1 g, 15.2 mmol) and molecular sieves 4A (6 g) in CH_2ClCHH_2Cl (50 ml) and stirred at room temperature. Then, a solution of MeOTf in CH_2ClCH_2Cl (1 M, 3.8 ml) was added at 0℃. The mixture was stirred at 45℃ for 12 h, quenched with Et_3N, diluted with EtOAc , and filtered through Celite. The filtrate was washed with water, $NaHCO_3$, and brine, dried over Na_2SO_4, and evaporated *in vacuo*. The residue was purified by silica gel column chromatography (hexane:AcOEt, 3:1) to afford **15** (5.0 g, 87%): ^{1}H NMR (400 MHz, $CDCl_3$) □ = 7.76-6.64 (43 H, Ar), 5.13 (d, 1 H, J = 8.4 Hz), 4.85 (d, 1H, J = 8.4 Hz), 4.74 (d, 1 H, J = 12.4 Hz), 4,72 (d, 1 H, J = 12.4 Hz), 4.60 (d, 1 H, J = 12.4 Hz), 4.45 (s, 1 H), 4.43 (d, 1 H, J = 12.4 Hz), 4.36 (d, 1 H, J = 12.4 Hz), 4.30 (d, 1 H, J = 12.4 Hz), 4.28 (d, 1 H, J = 12.4 Hz), 4.24 (dd, 1 H, J = 10.8 and 8.4 Hz), 2.65 (m, 1 H), 1.03 (s, 9H); MALDI-TOFMS $C_{91}H_{94}N_2NaO_{18}Si$ calcd: 1553.62, found: 1553.60 $(M + Na)^+$; Anal. ($C_{91}H_{94}N_2O_{18}Si$) calcd: C, 70.97; H, 6.15; N, 1.78; found: C, 71.07; H, 6.13; N, 1.57.

Compound 16

To a solution of **15** (470 mg, 0.30 mmol) in pyridine (2 ml), acetic anhydride (1 ml) was added under ice-cooling. The mixture was stirred at room temperature for 1 h and evaporated under reduced pressure. The residue was dissolved with CH_3CN, and *p*-TsOH (143 mg, 0.75 mmol) was added. The mixture was stirred at room temperature over night, diluted with AcOEt, and washed with $NaHCO_3$ and brine. The extract was evaporated *in vacuo*. The residue was dissolved in DMF (1 mL) containing 10% HF/pyridine (Aldrich). It was compressed to 1.0 GPa and left at 30℃ for 12 h and the resulting mixture was diluted

with AcOEt and washed with $NaHCO_3$ and brine, successively. The organic layer was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was purified by silica gel column chromatography (hexane:AcOEt, 10:1–1:2) to afford 260 mg (70%) of compound **16**; ^{1}H NMR (400 Hz, $CDCl_3$) δ = 7.83-6.72 (33 H, Ar), 5.25 (d, 1 H, J = 8.0 Hz), 5.16 (d, 1 H, J = 3.2 Hz), 4.93 (d, 1 H, J = 8.0 Hz), 4.88 (d, 1 H, J = 12.4 Hz), 4.82 (d, 1 H, J = 12.0 Hz) 4.69 (bs, 1 H), 4.68 (d, 1 H, J = 12.4 Hz), 4.58 (d, 1 H, J = 12.0 Hz), 4.50 (s, 2 H), 4.48 (d, 1 H, J = 12.0 Hz), 4.47 (d. 1 H, J = 12.4 Hz), 4.42 (d, 1 H, J = 12.4 Hz), 4.35 (d, 1 H, J = 12.0 Hz), 3.09 (m, 1 H), 2.13 (s, 3 H); ^{13}C NMR ($CDCl_3$) (anomeric carbons) δ = 98.08, 97.00, 96.87; MALDI-TOFMS $C_{71}H_{70}N_2NaO_{19}$ calcd: 1277.45, found: 1278.04 $(M + Na)^+$; Anal. ($C_{71}H_{70}N_2O_{19}$) calcd: C, 67.93; H, 5.62; N, 2.23; found: C, 67.45; H, 5.92; N, 2.02.

Compound 18, 19 and 20

A mixture of AgOTf (88 mg, 340 μmol) and molecular sieves (500 mg) in dry toluene (1 ml) was stirred at 0°C for 30 min and then cooled at –30°C. A solution of donor **8** (80 mg, 160 μmol) and acceptor **7** (126 mg, 100 μmol) in dry CH_2ClCH_2Cl (4 ml) was added dropwise over 5 min. The reaction mixture was stirred at –30°C (1h) and at ambient temperature (12 h). The reaction was quenched with Et_3N (0.1 ml). The mixture was diluted with EtOAc and filtered through Celite. The filtrate was washed with $NaHCO_3$ and brine. The solution was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/AcOEt) to afford the compounds **18** (74 mg, 34%), **19** (27 mg, 16%), and **20** (40 mg, 23%), and the remaining acceptor **16** (27 mg, 22%);

Compound **18**; ^{1}H NMR (400 Hz, $CDCl_3$) δ = 7.87-6.69 (63 H, Ar), 5.39 (bs, 1 H), 5.33 (d, 1 H, J = 2.8 Hz), 5.27 (bs, 1 H), 5.20 (d, 1 H, J = 8.4 Hz), 5.19 (bs, 1 H), 4.90 (d, 1 H, J = 8.4 Hz), 2.10 (s, 3 H), 2.05 (s, 3 H), 1.95 (s, 3 H); ^{13}C NMR ($CDCl_3$) (anomeric carbons) δ = 99.2, 99.0, 97.7, 96.9, 96.8; MALDI-TOF MS $C_{129}H_{130}N_2NaO_{31}$ calcd: 2225.86, found: 2226.16 $(M + Na)^+$; Anal. ($C_{129}H_{130}N_2O_{31}$) calcd: C, 70.29; H, 5.94; N, 1.27; found: C, 70.5; H, 5.77; N, 1.21.

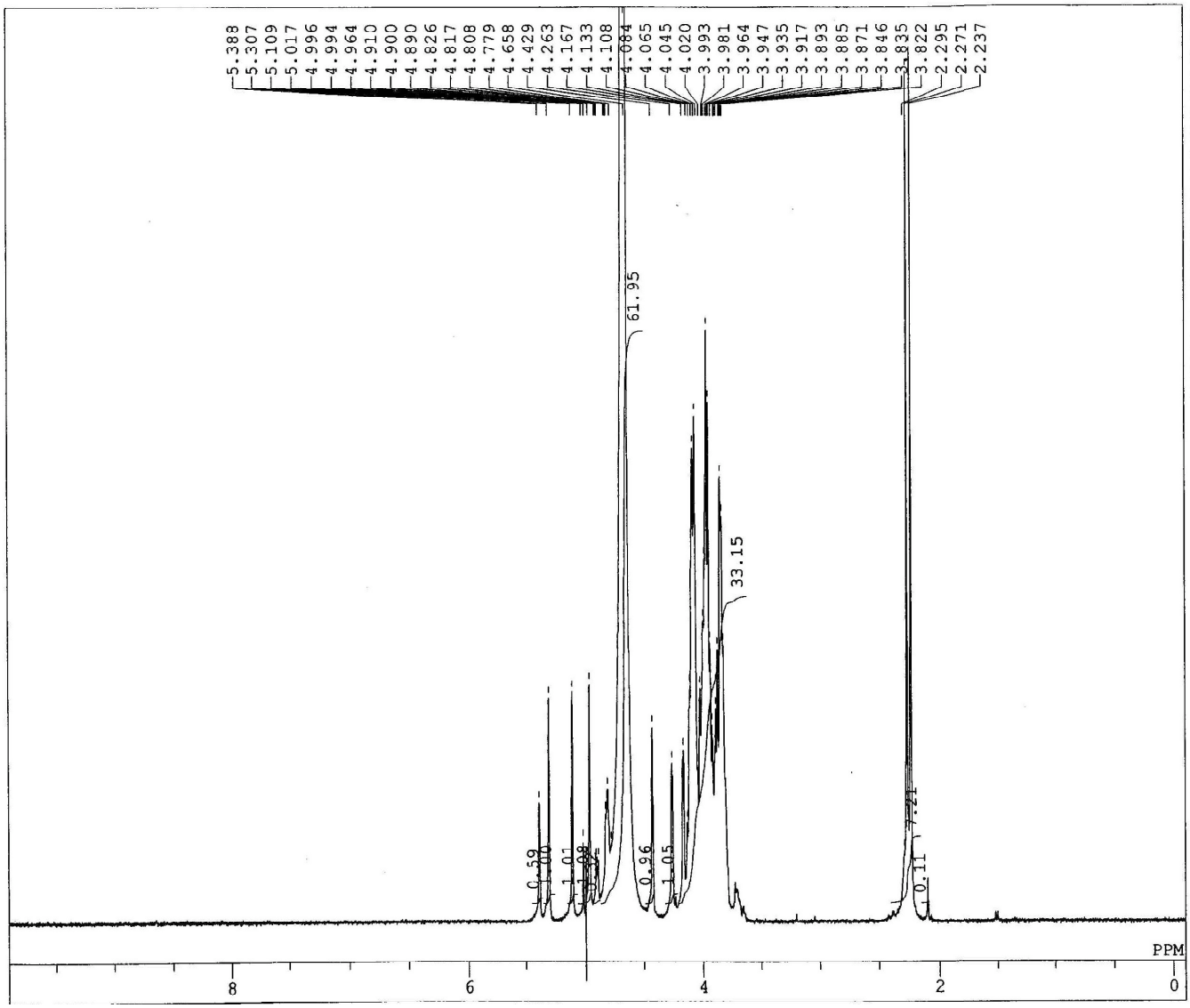
Compound **19**; ^{1}H NMR (400 Hz, $CDCl_3$) δ = 7.84-6.73 (48 H, Ar), 5.32 (bs, 1 H), 5.24 (d, 1 H, J = 8.0 Hz), 5.21 (bs, 1 H), 5.12 (d, 1 H, 3.0 Hz), 4.93 (d, 1 H, J = 8.4 Hz), 2.13 (s, 3 H), 2.11 (s, 3 H); ^{13}C NMR ($CDCl_3$) (anomeric carbons) δ = 98.4, 98.3, 97.0, 96.9; MALDI-TOFMS $C_{100}H_{100}N_2NaO_{25}$ calcd: 1751.65, found: 1752.25 $(M + Na)^+$; Anal. ($C_{100}H_{100}N_2O_{25}$) calcd: C, 69.43; H, 5.83; N, 1.62; found: C, 69.13; H, 5.61; N, 1.55.

Compound **20**; ^{1}H NMR (400 Hz, $CDCl_3$) δ = 7.78-6.71 (48 H, Ar), 5.25 (bs, 1 H), 5.21 (d, 1 H, J = 8.0 Hz), 5.17 (bs, 1 H), 4.91 (d, 1 H, J = 8.4 Hz), 2.99 (m, 1 H), 2.11 (s, 3 H), 2.04 (s, 3 H), 1.97 (s, 3 H); ^{13}C NMR ($CDCl_3$) (anomeric carbons) δ = 98.14, 98.13, 97.02, 96.92; MALDI-TOFMS $C_{100}H_{100}N_2NaO_{25}$ calcd: 1751.65, found: 1751.90 $(M + Na)^+$; Anal. ($C_{100}H_{100}N_2O_{25}$) calcd: C, 69.43; H, 5.83; N, 1.62; found: C, 69.14; H, 5.70; N, 1.57.

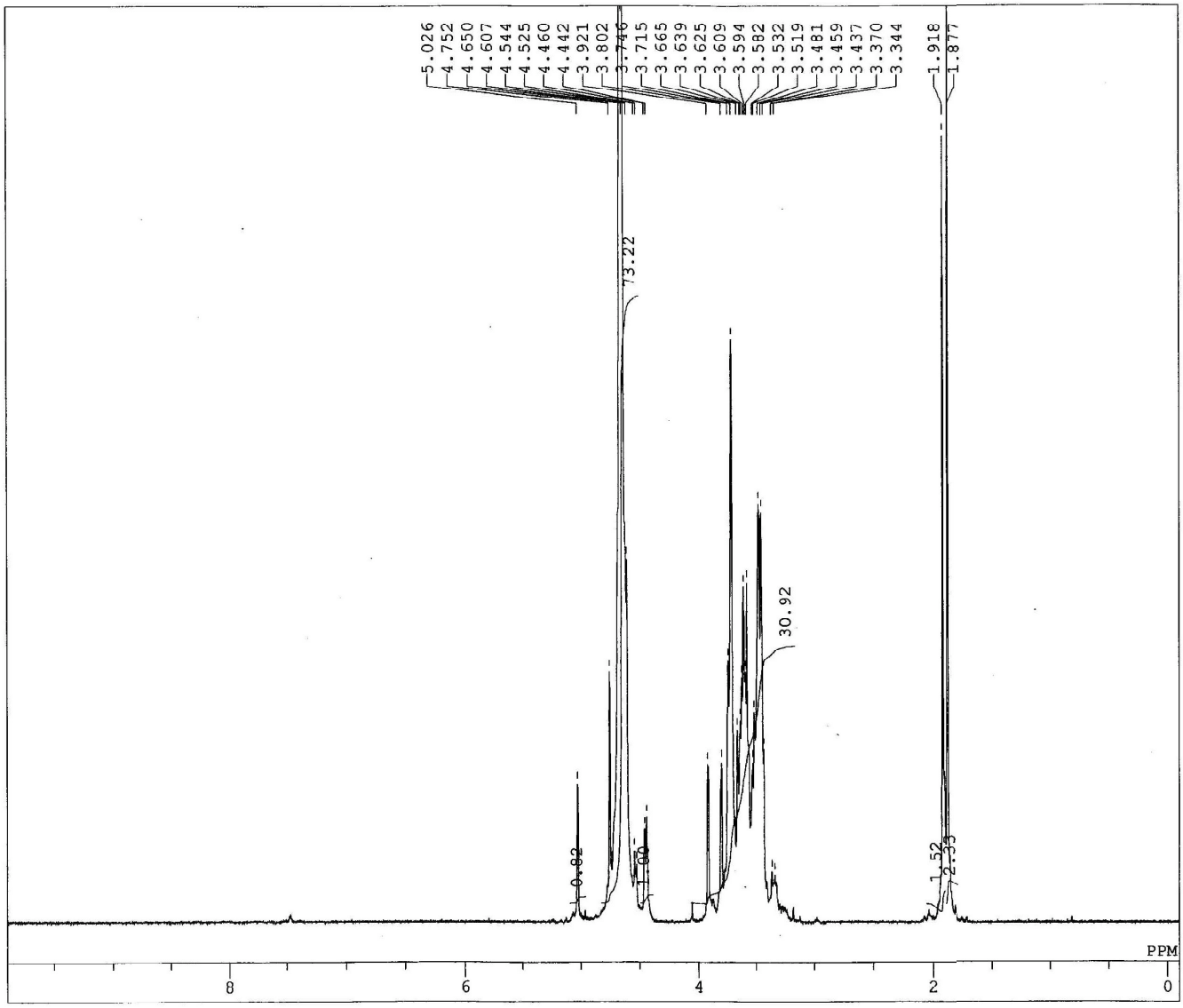
Deprotection procedure

A solution of protected oligosaccharide (200 mg) in *n*-buthanol (2 ml) containing ethylenediamine (0.5 ml) was stirred at 90℃ for 15 h. Volatiles were removed by evapotarion *in vacuo* and the residue was dissolved in pyridine (2 ml) and Ac_2O (1 ml) was added. The solution was stirred for 3 h and evaporated *in vacuo*. The residue was dissolved in MeOH (5 ml) and 1 N NaOMe (0.1 ml) was added. The mixture was stirred at 60℃ for 12 h, neutralized with Amberlyst 15 (H^+) resin, filtered through Celite, and evaporated. The residue was hydrogenated in the presence of $Pd(OH)_2$ in 50% aq. MeOH at room temperature for 12 h. The mixture was filtered through Celite and the filtrate was concentrated *in vacuo*. The residue was purified with a Sep-Pak 18C cartridge (Waters) to afford free oligosaccharides.

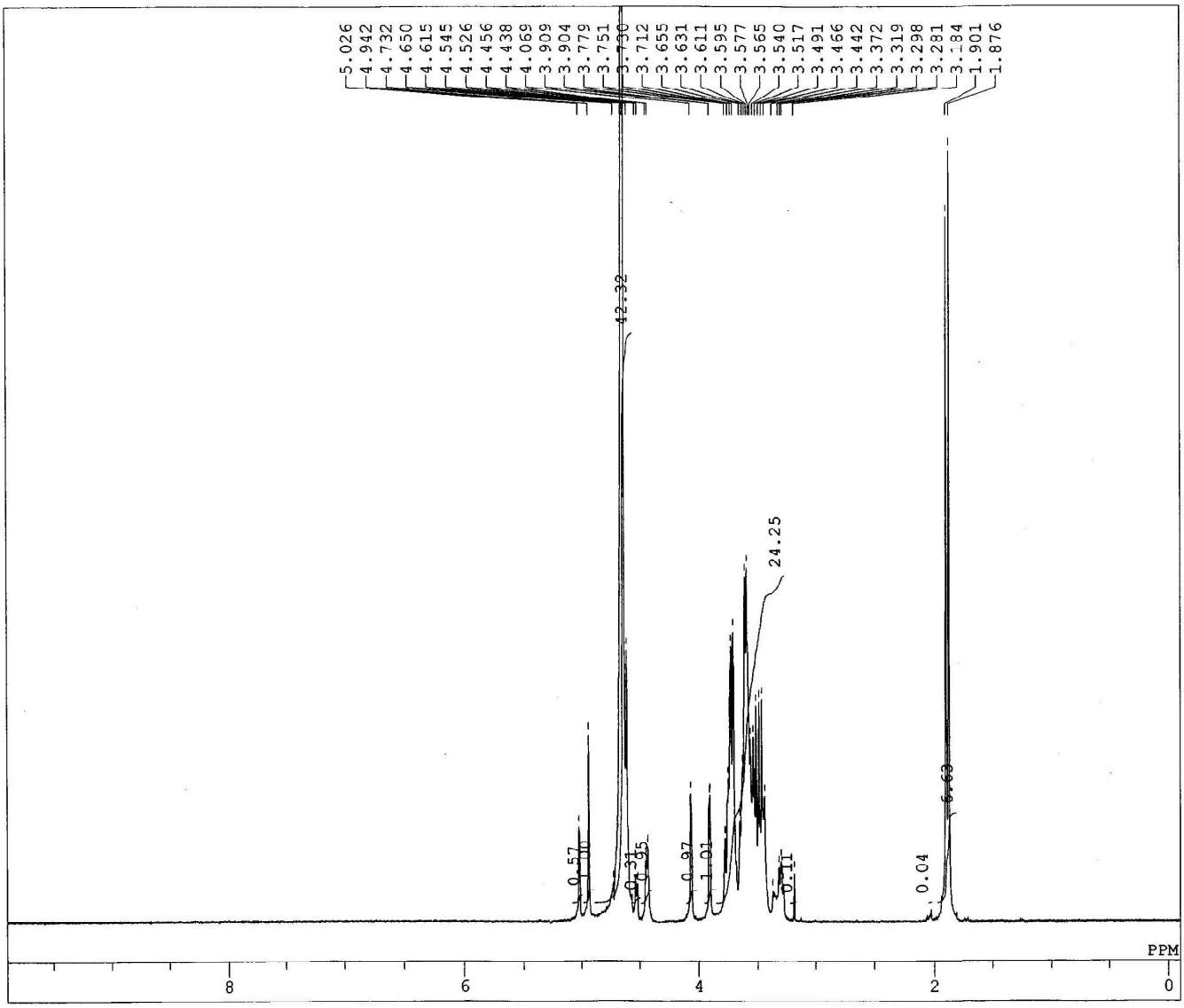
$Man_3GlcNAc_2$ (4); ^1H NMR (400 Hz, D_2O) □ = 5.39 (s, 1 H), 5.31 (s, 1 H), 5.11 (s, 1H), 4.96 (s, 1 H), 4.90 (m, 1 H), 4.82 (m, 1 H), 2.27 (s, 3 H), 2.24 (s, 3 H); MALDI-TOFMS: $C_{34}H_{58}N_2NaO_{26}$ calcd: 933.32; found: 933.14 $(M + Na)^+$.

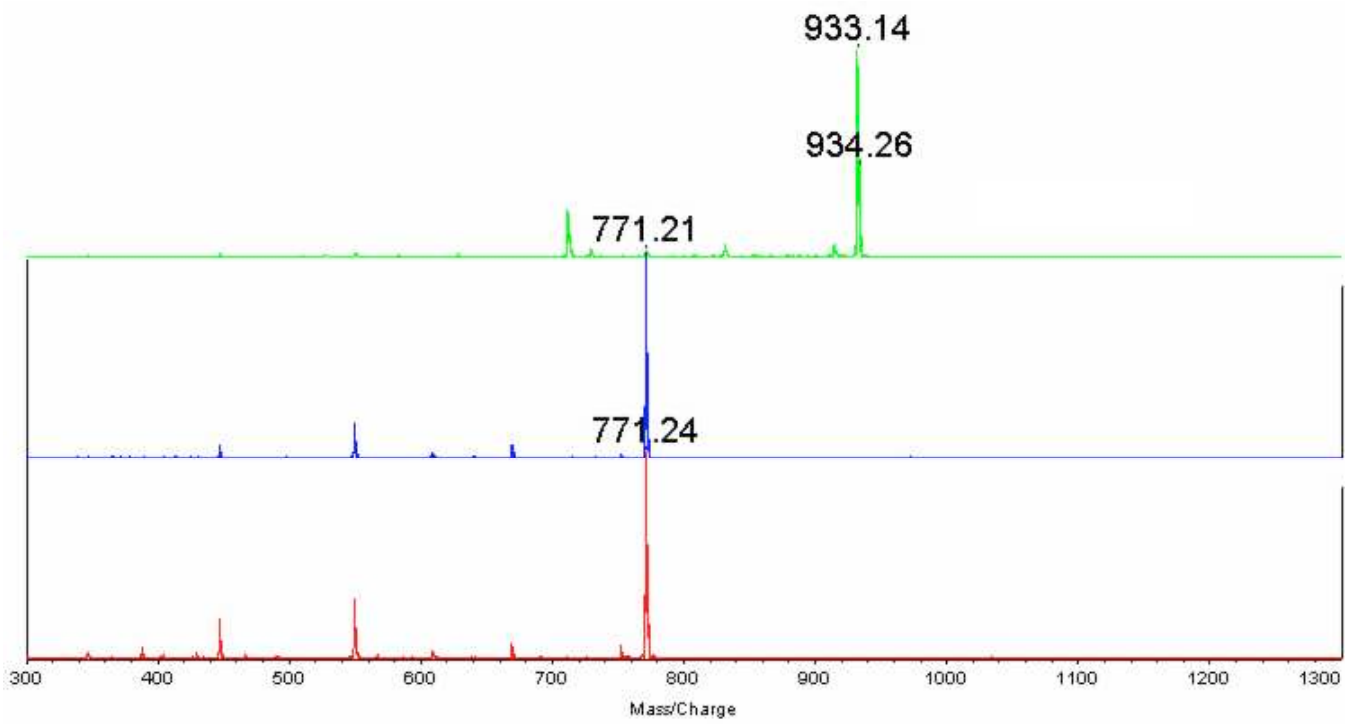


Man1-6ManGlcNAc$_2$ (5); ^{1}H NMR (400 Hz, D_2O) = 5.02 (s, 1 H), 4.75 (s, 1 H), 4.61 (s, 1 H), 4.53 (m, 1 H), 4.45 (m, 1 H), 1.92 (s, 3 H), 1.88 (s, 3 H); MALDI-TOFMS $C_{28}H_{48}N_2NaO_{21}$ calcd: 771.26, found: 771.21 $(M + Na)^+$.



Man1-3ManGlcNAc$_2$ (6); 1H NMR (400 Hz, D_2O) = 5.03 (s, 1 H), 4.94 (s, 1 H), 4.62 (s, 1 H), 4.54 (m, 1 H), 4.45 (m, 1 H), 1.90 (s, 3 H), 1.88 (s, 3 H); MALDI-TOFMS $C_{28}H_{48}N_2NaO_{21}$ calcd: 771.26, found: 771.24 $(M + Na)^+$.





MALDI-TOF MS for $Man_3GlcNAc_2$ (upper), Man1-6$GlcNAc_2$(middle), and Man1-3$GlcNAc_2$.

Compound	^{1}H NMR	^{13}C NMR	MALDI-TOF MS	Elemental analysis
12	√		√	
13	√		√	
15	√		√	√
16	√	√	√	√
18	√	√	√	√
19	√	√	√	√
20	√	√	√	√
4	√		√	
5	√		√	
6	√		√	

Isothermal titration calorimetry (ITC). The experiment was conducted by adding 6 □l of synthetic oligosaccharides (300 □M) every 4 min into a PBS buffer (10 mM, pH 7.2) containing NaCl (100 mM) and Fbs1 (0 or 20 □M) at 20 ℃. Heats of dilution were measured in blank titrations by injecting oligosaccharides into the buffer and were subtracted from the binding heats. Thermodynamic parameters were determined by the nonlinear least-squares methods, using routines included in the Origin software package (MicroCal, version 7.0).

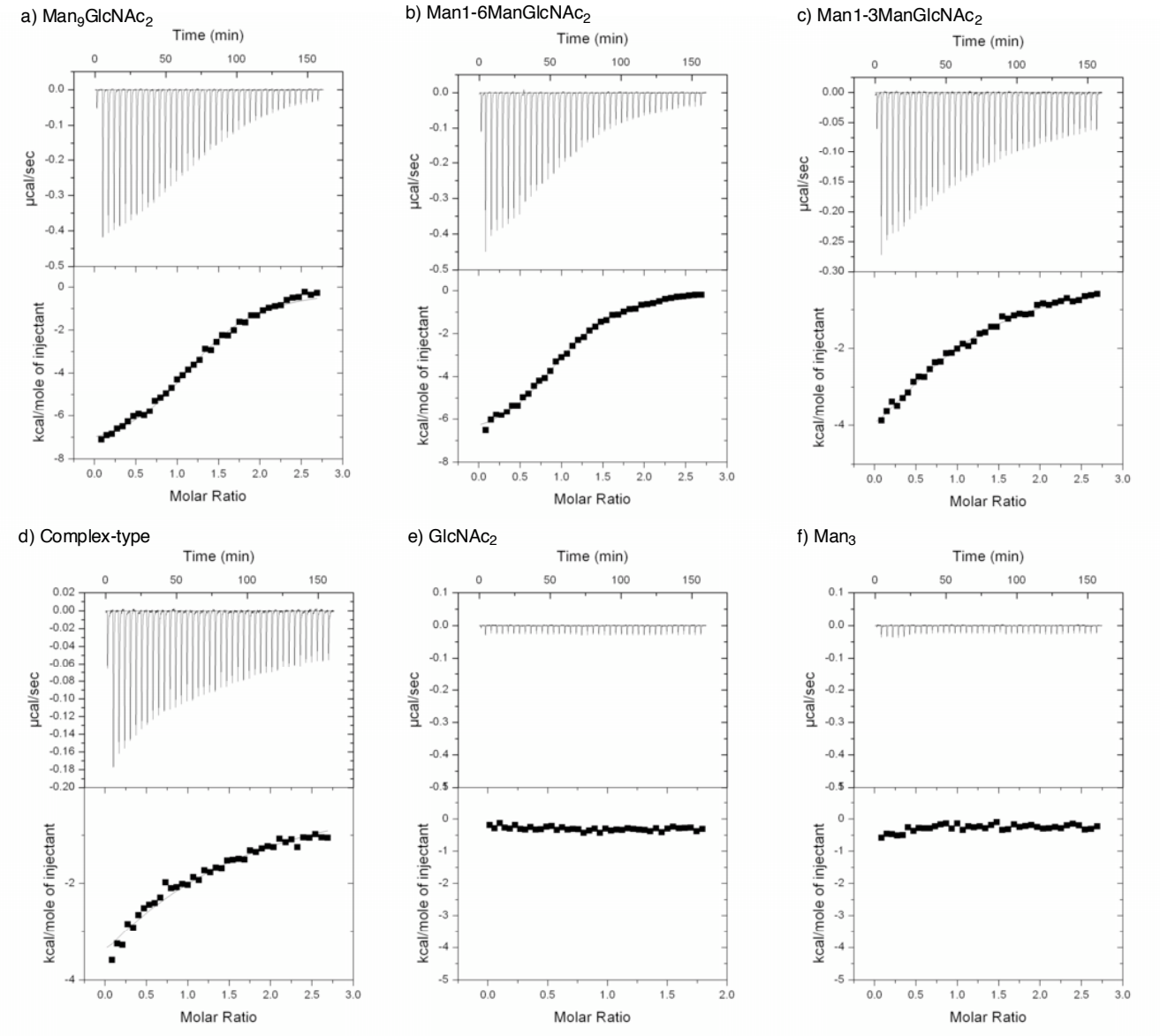


Figure. Isothermal titration calorimetric analysis for the binding of Fbs1 to a) $Man_9GlcNAc_2$, b) Man1-6ManGlcNAc$_2$, c) Man1-3ManGlcNAc$_2$, d) diantennary complex-type N-glycan, e) $GlcNAc_2$, and f) Man_3. Titration was conducted by injecting 6 μl of oligosaccharide (300 μM in PBS buffer) every 4 min into buffer solution (PBS) containing Fbs1 (20 μM) at 20.Upper; Law ITC data, lower; Molar heat values plotted as a function of the molar ratio ([oligosaccharide]/[Fbs1]). The solid line represents the best-fit binding isotherm. The data were fitted using a single site model.