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Supporting Information

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Reactivity-Based One-Pot Synthesis of Tumor-Associated Antigen N3 Minor Octasaccharide for the Development of DIOS-MS Sugar Array

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General. All chemicals were purchased as reagent grade and used without further purification. Dichloromethane (CH_2Cl_2) and acetonitrile (CH_3CN) were distilled over calcium hydride. Tetrahydrofuran (THF) was distilled over sodium metal/benzophenone ketyl. Anhydrous DMF was purchased from a commercial source. Molecular Sieves (MS) for glycosylation were AW-300 (Aldrich). Reactions were monitored with analytical thin-layer chromatography (TLC) in EM silica gel 60 F254 plates and visualized under UV (254 nm) and/or by staining with acidic ceric ammonium molybdate or ninhydrin. ^{1}H NMR spectra were recorded on a Bruker DRX-500 (500 MHz) or DRX-600 (600 MHz) spectrometer at 20 ºC. Chemical shifts (in ppm) were assigned according to the internal standard signal of tetramethylsilane in $CDCl_3$ (δ = 0 ppm). ^{13}C NMR spectra were obtained with Bruker DRX-500 or DRX-600 spectrometer and were calibrated with $CDCl_3$ (δ = 77.00 ppm). Coupling constants (*J*) are reported in hertz (Hz) Splitting patterns are described by using the following abbreviations: s, singlet; brs, broad singlet, doublet; t, triplet; m, multiplet. Phosphorus-doped, *n*-type silicon single crystal with 0.5–2×10^{-2} Ω**.**cm tesistioity, were obtained from the Silicon Quest International (Santa Clara, CA). Deionized water used for all procedures was purified with a Milli-Q water system (Millipore, Milford, MA, USA).

Instrumentation and Mass Spectrometry.

Mass Spectra were obtained with Agilent ESI-TOF mass spectrometer (ESI-TOF) and Applied Biosystems Voyager-STR mass spectrometer (MALDI-TOF). DIOS-MS measurements were performed with an Applied Biosystems (Framingham, MA) Voyager STR or a Waters Micromass (Milford, MA) M@LDI-R time-of-flight reflectron mass spectrometer. The DIOS chips were directly attached to a modified MALDI target plate using Scotch Crystal Clear tape. To obtain consistent and accurate data, each spectra was calibrated prior to acquisition. The standard used to calibrate each spectrum is des-Bradykinin, a peptide, which has a *m/z* value of 904.4648 and ionizes very well in MALDI-type of analysis. Bradykinin mixed with alpha-cyano matrix was spotted directly next to the sample of interest. The reason this was done was to ensure that when the DIOS surface was mounted on to our modified MALDI plate it would not be affected by any variations in surface height, which can have a detrimental effect on the accuracy of the values within the spectra. The samples were irradiated with a 337-nm nitrogen laser operated at 5 Hz (3-ns pulse duration) and attenuated with a neutral density filter. A delayed extraction period of 10–250 ns was used to minimize the energy spread of the ions for optimum resolution, after which the ions were acceleration by a 20-kV pulse. The laser intensity was adjusted to optimize the signal-to-noise ratio obtained for the mass spectra data.

Preparation of a porous silicon wafer

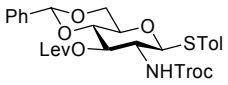
The details of DIOS chip preparation have been described elsewhere.[1] Briefly, pieces (~3.5 × 3.5 cm) were cut out from low-resistivity (0.005–0.02 Ω•cm) n-type Si-(100) wafers (500–550 μM thickness) from Silicon Quest International (Santa Clara, CA). The silicon chips were washed with ethanol and dried under N_2 gas before being placed on top of a gold foil (anode) in a Teflon chamber. A platinum wire (cathode) was placed in the cavity, and the chamber with the chip in the bottom was filled with 10 mL of 25% v/v hydrofluoric acid (Fisher Scientific)/ethanol. The silicon chip was subsequently electrochemically etched (2 min) with a current of 5.6 mA under white light illumination from a fiber-optic light source hosting a 250-W quartz/halogen lamp (model I-250, CUDA Fiberoptics) and placed above a transparent film with a printed pattern and a series of lenses focusing the pattern on the chip.[1] The printed pattern was made in

Adobe Photoshop (San Jose, CA) by fitting 10 x 10 circles (covering ~2 × 2 cm), the circles were made with black. The mask was subsequently printed printed on a standard laser printable transparency film. After etching, the chips were rinsed with ethanol and sried under N_2 gas.

The H-terminated porous silicon surface was next oxidized by exposure to ozone (flow rate 0.5 g/h) from an ozone generator Expotech (Huston, TX) for 30 s.

Preparation of the silylated amino surface 20.

The silylation reaction was performed by adding 15 μL of the (3-aminopropyl)dimethylethoxysilane (APDMES) on the oxidized DIOS chip, which was placed in a glass Petri dish and incubated in an oven at 70 °C for 30 min. The surface was washed with excess toluene, followed by isopropyl alcohol and methanol and was dried in a steam of N_2.

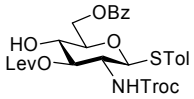


Compound 11. To a solution of **10** (22.1 g, 37.7 mmol) in MeOH (30 mL) was added a 25% solution of NaOMe in MeOH (3.0 mL) at 0 °C under argon. After 1.5 h, the reaction was neutralized with acidic resin and filtered. The filtrate was concentrated to give the deacetylated product.

Catalytic amount of CSA (1.7 g, 7.32 mmol) was added to a solution of the resulting tri-OH and $PhCH(OMe)_2$ (11.3 mL, 75.3 mmol) in CH_3CN (170 mL) at room temperature. After stirring overnight, the reaction was neutralized with triethylamine and concentrated under reduced pressure. The residue was filtered and washed with cold Hexane/Et_2O = 1/1 to yield the white solid product (16.6 g, 80%).

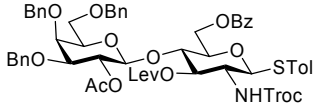
To a mixture of the resulting benzylidene thioglycoside (16.6 g, 30.2 mmol) and levulinic acid (9.3 mL, 90.8 mmol) in CH_2Cl_2 (80 mL) was added a solution of DCC (9.3 g, 45.1 mmol) and DMAP (3.7 g, 30.3 mmol) in CH_2Cl_2 (40 mL) at 0 °C under argon. The reaction was warmed up to room temperature and kept stirring overnight. The mixture was filtered and the filtrate was concentrated. The residue was recrystallized in EtOAc/Hexane to yield **11** (18.6 g, 95%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44–7.32 (m, 7H), 7.13 (d, J = 8.1 Hz, 1H), 5.49 (s, 1H), 5.44 (d, J = 9.2 Hz, 1H), 5.37 (t, J = 9.9 Hz, 1H), 4.86 (d, J = 10.3 Hz, 1H), 4.85 (d, J = 12.1 Hz, 1H), 4.74 (d, J = 12.1 Hz, 1H), 4.31

(dd, J = 10.3, 4.8 Hz, 1H), 3.77 (d, J = 9.9 Hz, 1H), 3.72–3.63 (m, 2H), 3.52 (td, J = 9.6, 4.8 Hz, 1H), 2.78-2.51 (m, 4H), 2.35 (s, 3H), 2.12 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.2, 172.6, 154.1, 138.6, 136.8, 133.5, 129.8, 128.2, 126.1, 101.3, 87.7, 78.4, 74.6, 72.5, 70.5, 68.4, 55.7, 37.9, 29.7, 27.9, 21.2; HRMS (ESI-TOF, MNa^+) calcd for $C_{28}H_{30}Cl_3NO_8SNa$ 668.0650, found 668.0654.



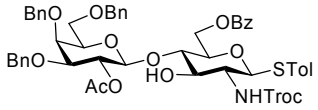
Compound 9. A solution of TFA (8 ml) in H_2O (2 mL) was added to a solution of **11** (5.0 g, 7.73 mmol) in CH_2Cl_2 (20 mL) at 0 °C. The ice-bath was removed and stirred at room temperature for 1 h. The reaction was neutralized with saturated $NaHCO_{3(aq)}$ at 0 °C and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure.

To a solution of the 4,6-diOH in pyridine/CH_2Cl_2 (1/1, 70 mL) was added dropwise BzCl (0.99 mL, 8.53 mmol) at –20 °C. After 2 h, the reaction mixture was quenched with MeOH and evaporated under reduced pressure. The residue was recrystallized in EtOH to yield **9** (3.76 g, 73 % in 2 steps). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.04 (d, J = 7.4 Hz, 2H), 7.61 (t, J = 7.4 Hz, 1H), 7.46 (t, J = 7.4 Hz, 2H), 7.36 (d, J = 7.9 Hz, 1H), 6.90 (t, J = 7.9 Hz, 2H), 5.63 (d, J = 9.2 Hz, 1H), 5.12 (t, J = 9.7 Hz, 1H), 4.77-4.71 (m, 4H), 4.56 (dd, J = 11.8, 5.7 Hz, 1H), 3.81–3.66 (m, 4H), 2.81–2.69 (m, 2H), 2.58–2.44 (m, 2H), 2.25 (s, 3H), 2.12 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 208.0, 173.3, 166.5, 154.2, 137.9, 133.2, 132.8, 129.8, 129.7, 129.5, 128.6, 128.4, 128.3, 95.6, 86.5, 77.5, 76.7, 74.4, 69.2, 63.8, 54.4, 38.2, 29.7, 28.1, 21.1; HRMS (ESI-TOF, MNa^+) calcd for $C_{28}H_{30}Cl_3NO_9SNa$ 684.0599, found 684.0614.



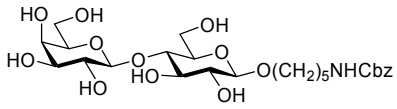
Compound 12. A solution of donor **8** (50 mg, 83.51 μmol), acceptor **9** (50 mg, 75.42 μmol), BSP (9.5 mg, 45.39 μmol) and molecular sieves AW-300 (0.6 g) in CH_2Cl_2 (2.0 mL) was stirred at room temperature for 1 h. The solution was cooled to –70 °C and Tf_2O (9.0 μL) was added. Then the mixture was warmed up from –70 °C to –10 °C gradually (ca. 2 h). Triethylamine (18.0 μL) was added and the mixture was filtered through celite.

The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography (EtOAc/Hex = 1/2) to give **12** (47 mg, 54%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (d, *J* = 7.7 Hz, 2H), 7.60 (t, *J* = 7.7 Hz, 1H), 7.44 (t, *J* = 7.7 Hz, 2H), 7.37-7.21 (m, 17H), 6.88 (d, *J* = 8.1 Hz, 2H), 5.32 (d, *J* = 9.6 Hz, 1H), 5.21 (dd, *J* = 9.9, 8.1 Hz, 1H), 5.12 (d, *J* = 9.2 Hz, 1H), 4.88 (d, *J* = 11.4 Hz, 1H), 4.84 (d, *J* = 12.1 Hz, 1H), 4.73–4.68 (m, 3H), 4.61 (d, *J* = 12.1 Hz, 1H), 4.48–4.42 (m, 4H), 4.35–4.32 (m, 2H), 3.90 (d, *J* = 2.6 Hz, 1H), 3.80-3.67 (m, 3H), 3.59 (t, *J* = 8.5 Hz, 1H), 3.55–3.52 (m, 1H), 3.45–3.44 (m, 1H), 3.41 (dd, *J* = 10.3, 2.6 Hz, 1H), 2.64–2.56 (m, 1H), 2.51–2.32 (m, 3H), 2.23 (s, 3H), 1.984 (m, 3H), 1.980 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.5, 172.4, 169.3, 165.9, 154.1, 138.4, 138.1, 137.72, 137.65, 133.3, 133.2, 129.7, 129.5, 128.52, 128.46, 128.41, 128.38, 128.3, 128.21, 128.18, 128.1, 128.0, 127.9, 127.7, 127.5, 127.3, 127.2, 101.0, 95.5, 86.9, 80.4, 74.9, 74.59, 74.55, 73.7, 73.5, 72.4, 72.0, 71.6, 67.9, 63.0, 55.0, 37.7, 29.6, 27.8, 21.1, 20.9; HRMS [ESI-TOF, $(M+Cl)^-$] calcd for $C_{57}H_{60}Cl_4NO_{15}S$ 1170.2443, found 1170.2419.



Compound 6. To a solution of compound **12** (144 mg 0.13 mmol) in pyridine (1.5 mL), was added a 1 M solution of hydrazine hydrate in pyridine/AcOH (1.3 mL, 1.30 mmol) at room temperature. After 2 h, The reaction was quenched with penta-2,4-dione and concentrated. The residue was dissolved in CH_2Cl_2 and washed with 0.1 M $HCl_{(aq)}$. The organic phase was dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc/Hex = 1/2) to afford **6** (116 mg, 88%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.01 (d, *J* = 7.7 Hz, 2H), 7.61 (t, *J* = 7.7 Hz, 1H), 7.46 (t, *J* = 7.7 Hz, 2H), 7.37–7.23 (m, 17H), 6.91 (d, *J* = 7.7 Hz, 2H), 5.38 (dd, *J* = 9.9, 8.1 Hz, 1H), 5.25 (d, *J* = 8.1 Hz, 1H), 4.89 (d, *J* = 11.8 Hz, 1H), 4.85 (d, *J* = 10.3 Hz, 1H), 4.82 (d, *J* = 12.1 Hz, 1H), 4.74 (s, 1H), 4.70 (d, *J* = 12.1 Hz, 1H), 4.62 (d, *J* = 12.1 Hz, 1H), 4.58 (d, *J* = 10.6 Hz, 1H), 4.52 (d, *J* = 11.8 Hz, 1H), 4.48 (d, *J* = 12.1 Hz, 1H), 4.45 (d, *J* = 11.8 Hz, 1H), 4.40 (d, *J* = 8.1 Hz, 1H), 4.36 (d, *J* = 11.8 Hz, 1H), 4.27 (dd, *J* = 11.8, 5.9 Hz, 1H), 3.86 (t, *J* = 9.0 Hz, 1H), 3.81 (d, *J* = 2.6 Hz, 1H), 3.73-3.70 (m, 1H), 3.69–3.59 (m, 2H), 3.49–3.41 (m, 3H), 3.39–3.35 (m, 1H), 2.25 (s, 3H), 2.04 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.6, 166.0, 154.0, 138.0, 137.8, 137.4, 137.2, 133.3, 133.1,

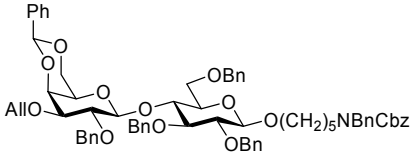
129.8, 129.7, 129.5, 128.5, 128.42, 128.38, 128.28, 128.25, 128.1, 127.92, 127.89, 127.8, 127.4, 102.1, 95.5, 86.1, 82.4, 80.2, 75.7, 74.5, 74.4, 74.1, 73.62, 73.56, 72.3, 72.0, 71.0, 68.5, 63.4, 56.0, 21.1, 20.8; HRMS [ESI-TOF, $(M+Cl)^-$] calcd for $C_{52}H_{54}Cl_4NO_{14}S$ 1072.2075, found 1072.2068.



Compound 13. To a solution of lactose octaacetate (20.0 g, 29.5 mmol) in AcOH/Ac_2O (1/1, 40 mL) was added 33 % HBr in AcOH (50 mL) and the mixture was stirred at room temperature for 5 h. The solution was then poured into H_2O and extracted with CH_2Cl_2. The organic layer was washed with saturated $NaHCO_{3(aq)}$, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give the lactosyl bromide.

To a mixture of *N*-(5-hydroxypentyl)-carbamate (14.0 g, 59.0 mmol), Ag_2CO_3 (9.7 g, 35.2 mmol), I_2 (374 mg, 1.47 mmol), and MS AW-300 (20.6 g) in CH_2Cl_2 (80 mL) was added a solution of lactosyl bromide (20.6 g, 29.5 mmol) in CH_2Cl_2 (60 mL) at room temperature under argon. After 24 h, the reaction was quenched with $Na_2S_2O_{3(aq)}$ and filtered through celite. The filtrate was concentrated and the residue was dried under vacuum.

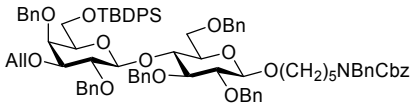
The residue was dissolved in MeOH (60 mL) and a 25% solution of NaOMe in MeOH (0.9 mL) was added. The reaction was stirred at room temperature for 2 h and neutralized with acidic resin. The mixture was filtered and the filtrate was concentrated in vacuo. The residue was purified by column chromatography (MeOH/$CHCl_3$ = 1/3) to provide **13** (7.6 g, 46% over 3 steps). ^{1}H NMR (500 MHz, D_2O) δ 7.35–7.32 (m, 5H), 5.01 (s, 1H), 4.35 (t, J = 8.1 Hz, 2H), 3.88–3.78 (m, 3H), 3.71–3.62 (m, 4H), 3.58–3.43 (m, 6H), 3.20 (t, J = 7.6 Hz, 1H), 3.03-3.01 (m, 2H), 1.54–1.51 (m, 2H), 1.42–1.39 (m, 2H), 1.28–1.26 (m, 2H); ^{13}C NMR (125 MHz, D_2O) δ 158.8, 137.0, 129.1, 128.7, 128.0, 103.3, 102.4, 78.7, 75.7, 75.1, 74.8, 73.2, 72.9, 71.3, 70.8, 68.9, 67.1, 61.4, 60.4, 40.6, 28.9, 28.7, 22.6; HRMS [ESI-TOF, MH^+] calcd for $C_{25}H_{40}NO_{13}$ 562.2494, found 562.2488.



Compound 14. A suspension of **13** (1.45 g, 2.58 mmol) and Bu_2SnO (0.77 g, 3.09 mmol) in benzene (64 mL) was refluxed for 24 h in a flask equipped with a Dean-Stark separator. Allyl bromide (4.2 mL, 48.3 mmol) and tetrabutylammonium iodide (0.48 g, 1.30 mmol) were added and the mixture was refluxed for another 16 h. After evaporation of solvent, the residue was purified by column chromatography (MeOH/$CHCl_3$ = 1/7 to 1/6) to yield the O3′-allyl product (1.11 g, 71%).

To a solution of the O3′-allyl product (1.11 g, 1.84 mmol) and $PhCH(OMe)_2$ (0.55 mL, 3.66 mmol) in CH_3CN was added a catalytic amount of CSA (43 mg, 18.5 μmol) at room temperature. After stirring overnight, the reaction was neutralized with triethylamine and concentrated under reduced pressure.

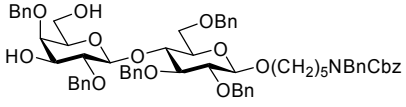
Sixty percent NaH (0.44 g, 11.0 mmol) was added to a solution of the resulting benzylinede lactoside and benzyl bromide (1.3 mL, 10.9 mmol) in DMF (12 mL) at 0 °C. The solution was allowed to warm to room temperature and stirred overnight. The reaction was quenched with MeOH and the solvent was evaporated in vacuo. The residue was diluted with CH_2Cl_2, and the organic layer was washed with H_2O, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc/Hex = 1/2) to give **14** (1.59 g, 76% in 2 steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51–7.49 (m 2H), 7.46–7.44 (m, 2H), 7.32–7.14 (m, 31H), 5.98–5.90 (m, 1H), 5.50 (s, 1H), 5.30 (dd, *J* = 17.3, 1.9 Hz, 1H), 5.19–5.14 (m, 4H), 4.87 (t, *J* = 9.8 Hz, 1H), 4.80 (t, *J* = 11.8 Hz, 2H), 4.723 (d, *J* = 11.0 Hz, 1H), 4.717 (d, *J* = 11.0 Hz, 1H), 4.54 (d, *J* = 12.1 Hz, 1H), 4.49–4.44 (m, 3H), 4.36–4.33 (m, 2H), 4.23–4.17 (m, 3H), 4.13–4.09 (m, 1H), 3.96 (dd, *J* = 9.6, 9.2 Hz, 1H), 3.89–3.85 (m, 3H), 3.73–3.70 (m, 2H), 3.62 (t, *J* = 9.2 Hz, 1H), 3.47-3.32 (m, 4H), 3.23-3.15 (m, 2H), 3.01 (s, 1H), 1.66–1.49 (m, 4H), 1.33–1.23 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.7, 138.9, 138.8, 138.6, 138.5, 138.0, 137.9, 135.1, 128.8, 128.5, 128.4, 128.20, 128.16, 128.1, 128.04, 128.00, 127.9, 127.8, 127.7, 127.5, 127.45, 127.39, 127.3, 127.2, 126.5, 117.0, 103.5, 102.8, 101.3, 83.0, 81.8, 79.6, 78.7, 77.7, 76.9, 75.7, 75.2, 75.0, 74.9, 73.9, 72.9, 71.1, 69.7, 68.9, 68.3, 67.1, 66.3, 60.3, 50.4, 50.2, 47.1, 46.1, 29.4, 27.9, 27.5, 23.3, 21.0; HRMS [ESI-TOF, MNa^+] calcd for $C_{70}H_{77}NO_{13}Na$ 1162.5287, found 1162.5274.



Compound 15. A solution of TFA (1.2 ml) in H_2O (0.3 mL) was added to a solution of **14** (0.73 g, 0.64 mmol) in CH_2Cl_2 (3 mL) at 0 °C. The ice-bath was removed and stirred at room temperature for 1 h. The reaction was neutralized with saturated $NaHCO_{3(aq)}$ at 0 °C and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure.

To a solution of the 4,6-diOH in CH_2Cl_2 (6.5 mL) were added TBDPSCl (0.18 mL, 0.70 mmol) and Et_3N (0.12 mL, 0.86 mmol). The mixture was stirred at room temperature overnight and the solvent was removed under reduced pressure. The residue was diluted with CH_2Cl_2, and the organic layer was washed with H_2O, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc/Hex = 2/7) to afford O6′-TBDPS product (0.64 g, 76% in 2 steps).

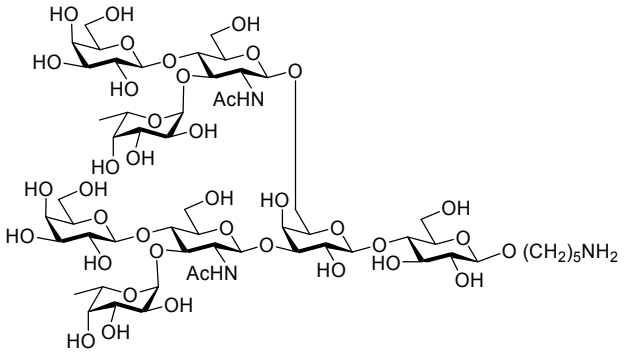
Sixty percent NaH (24 mg, 0.60 mmol) was added to a solution of the resulting O6′-TBDPS lactoside (0.64 g, 0.50 mmol) and benzyl bromide (72 μL, 0.60 mmol) in DMF (6.4 mL) at 0 °C. The solution was allowed to warm to room temperature and stirred overnight. The reaction was quenched with MeOH and the solvent was evaporated in vacuo. The residue was diluted with CH_2Cl_2, and the organic layer was washed with H_2O, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc/Hex = 2/7) to give **15** (0.47 g, 69%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.56 (d, *J* = 6.8 Hz, 2H), 7.53 (d, *J* = 6.8 Hz, 2H), 7.40-7.14 (m, 38H), 7.02 (t, *J* = 7.4 Hz, 1H), 6.93 (t, *J* = 7.4 Hz, 2H), 6.00–5.94 (m, 1H), 5.35 (dd, *J* = 17.3, 1.5 Hz, 1H), 5.21–5.14 (m, 3H), 5.05 (d, *J* = 11.0 Hz, 1H), 4.93 (d, *J* = 10.3 Hz, 1H), 4.80–4.71 (m, 3H), 4.65 (d, *J* = 11.0 Hz, 1H), 4.61–4.53 (m, 3H), 4.47–4.44 (m, 2H), 4.39-4.35 (m, 2H), 4.31–4.22 (m, 3H), 4.04 (d, *J* = 2.2 Hz, 1H), 3.87–3.79 (m, 4H), 3.70–3.63 (m, 3H), 3.49–3.14 (m, 8H), 1.68–1.49 (m, 4H), 1.36–1.23 (m, 2H), 1.04 (s, 9H); HRMS [ESI-TOF, MNa^+] calcd for $C_{86}H_{97}NO_{13}SiNa$ 1402.6621, found 1402.6609.



Compound 7. Compound **15** (0.26g, 0.19 mmol) was dissolved in THF (2.5 mL) and a 1 M solution of TBAF in THF (0.38 mL, 0.38 mmol) was added at 0 °C. The ice bath was removed and the reaction was kept stirring at room temperature for 3 h. The solvent was evaporated and the residue was diluted with CH_2Cl_2. The organic layer was washed with H_2O, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc/Hex = 1/1) to provide O6′-OH product (0.16 g, 74%).

A mixture of the resulting product (0.16 g, 0.14 mmol), $Rh(PPh_3)_3Cl$ (13 mg, 14.0 μmol) and DBU (1 μL, 6.7 μmol) in EtOH/toluene/H_2O (8/3/1, 6 mL) was stirred at 100 °C for 6 h. The mixture was concentrated and the residue was diluted with CH_2Cl_2. The organic layer was washed with H_2O, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was used for next step directly without further purification.

The residue was dissolved in an acetone/1 M $HCl_{(aq)}$ mixture and stirred at room temperature for 3 h. The solvent was removed and the residue was diluted with CH_2Cl_2. The organic layer was washed with saturated $NaHCO_{3(aq)}$, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc/Hex = 3/2) to give **7** (143 mg, 93%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37–7.14 (m, 35H), 5.15 (d, *J* = 10.6 Hz, 2H), 4.99 (d, *J* = 10.7 Hz, 1H), 4.89–4.83 (m, 1H), 4.83 (d, *J* = 11.8 Hz, 1H), 4.77 (d, *J* = 11.8 Hz, 1H), 4.76 (d, *J* = 10.7 Hz, 1H), 4.72 (d, *J* = 11.0 Hz, 1H), 4.66 (d, *J* = 11.4 Hz, 1H), 4.48–4.42 (m, 3H), 4.37 (d, *J* = 7.0 Hz, 1H), 4.34–4.32 (m, 1H), 3.91–3.82 (m, 2H), 3.76 (d, *J* = 2.6 Hz, 2H), 3.64 (d, *J* = 2.2 Hz, 1H), 3.58–3.35 (m, 8H), 3.27–3.16 (m, 3H), 2.27 (brs, 1H), 2.02 (brs, 1H), 1.63–1.49 (m, 4H), 1.41–1.24 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.1, 138.8, 138.5, 138.3, 138.2, 138.1, 137.9, 136.8, 128.48, 128.46, 128.4, 128.28, 128.26, 128.0, 127.9, 127.88, 127.83, 127.8, 127.6, 127.53, 127.50, 127.4, 127.2, 127.1, 103.5, 102.7, 82.5, 81.6, 80.2, 77.1, 75.9, 75.5, 75.3, 75.0, 74.94, 74.88, 74.8, 74.3, 73.2, 69.7, 68.3, 67.1, 61.8, 50.5, 50.2, 47.1, 46.1, 29.4, 27.9, 27.5, 23.4; HRMS (ESI-TOF, MNa^+) calcd for $C_{67}H_{75}NO_{13}Na$ 1124.5130, found 1124.5120.

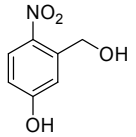


Compound 3. A solution of fucosyl donor **5** (61 mg, 0.11 mmol), lactosylamine acceptor **6** (105 mg, 0.10 mmol), BSP (13 mg, 0.06 mmol) and molecular sieves AW-300 (0.6 g) in CH_2Cl_2 (2.0 mL) was stirred at room temperature for 1 h. The solution was cooled to -70 °C and Tf_2O (12 μL, 0.07 mmol) was added. Then the mixture was warmed up gradually from –70 °C to 0 °C. After the donor **5** was consumed, the lactoside **7** (43 mg, 0.04 mmol) in CH_2Cl_2 (1.0 mL), NIS (31 mg, 0.14 μmol) and 0.5 M TfOH in Et_2O (39 μL, 0.02 mmol) were added at 0 °C sequentially. The reaction was allowed to stand at room temperature for another 5 h and then quenched with saturated $NaHCO_{3(aq)}$ and $Na_2S_2O_{3(aq)}$. The mixture was filtered through celite and the filtrate was extracted with CH_2Cl_2. The organic phase was dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc/Hex/CH_2Cl_2 = 1/3/1) to provide the mixture of desired octasaccharide and the unknown byproduct (64 mg). HRMS [MALDI-TOF, MNa^+] calcd for $C_{211}H_{223}Cl_6N_3O_{47}Na$ 3783, found 3783.

The mixture of the desired octasaccharide and the unknown byproduct (64 mg) was dissolved in acetic acid (2.0 mL) and activated Zn nanoparticles (200 mg) were added at room temperature. The reaction was kept stirring overnight and filtered through celite. The solvent was removed under reduced pressure and the residue was dried under vacuum. The resulting amino groups were acetylated with acetic anhydride (1.5 mL) and pyridine (3.0 mL) in the presence of catalytic amount of DMAP (2.0 mg). After 12 h, the solvent was removed and the residue was diluted with CH_2Cl_2. The organic layer was washed with H_2O, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc/Hex/CH_2Cl_2 = 2/3/1) to furnish the corresponding –NHAc derivative. HRMS [MALDI-TOF, MNa^+] calcd for $C_{209}H_{225}N_3O_{45}Na$ 3520, found 3519.

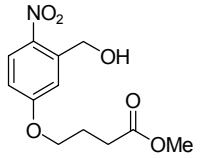
To a solution of the –NHAc derivative in CH_2Cl_2/MeOH (2.0 mL, 1:1) was added a 25% solution of NaOMe in MeOH (0.1 mL) at room temperature under argon. After 15 h, the reaction was neutralized with acidic resin and filtered. The filtrate was concentrated to give the deacylated product. HRMS [MALDI-TOF, MNa^+] calcd for $C_{191}H_{213}N_3O_{41}Na$ 3227, found 3227.

The deacylated product was dissolved in 5% formic acid/methanol solution and Pd-black was added (80 mg). The reaction mixture was stirred under $H_{2(g)}$ (1 atm) at room temperature overnight. The reaction was neutralized with NH_4OH (28–30 wt% in H_2O) and filtered through celite. The solvent was removed under reduced pressure and the residue was purified on a C-18 reverse phase column (LiChroprep RP-18, EM Science) with H_2O/MeOH elution (0% MeOH gradient to 25% MeOH) to afford **3** (6 mg, 11% from one-pot glycosylation) as white solid. ^{1}H NMR (600 MHz, D_2O) δ 4.98 (d, *J* = 3.7 Hz, 1H), 4.96 (d, *J* = 3.7 Hz, 1H), 4.56 (d, *J* = 8.5 Hz, 1H), 4.49 (d, *J* = 7.8 Hz, 1H), 4.35 (d, *J* = 8.1 Hz, 1H), 4.32 (d, *J* = 7.3 Hz, 1H), 4.31 (d, *J* = 7.4 Hz, 1H), 4.28 (d, *J* = 7.7 Hz, 1H), 4.00 (d, *J* = 3.5 Hz, 1H), 3.87-3.34 (m, 44H), 3.17 (t, *J* = 10.7 Hz, 1H), 2.87 (t, *J* = 9.0 Hz, 1H), 1.91 (s, 3H), 1.88 (s, 3H), 1.58–1.50 (m, 4H), 1.35–1.30 (m, 2H), 1.03 (d, *J* = 7.4 Hz, 6H); ^{13}C NMR (125 MHz, D_2O) δ 175.5, 175.1, 103.9, 103.3, 102.7, 102.64, 102.56, 101.6, 99.44, 99.41, 82.6, 79.9, 76.2, 75.9, 75.71, 75.65, 75.52, 75.48, 75.3, 74.2, 74.1, 73.8, 73.6, 73.24, 73.23, 72.7, 71.8, 70.9, 70.6, 70.00, 69.97, 69.6, 69.1, 68.5, 67.53, 67.48, 62.3, 60.8, 60.5, 60.4, 56.7, 56.4, 47.4, 40.1, 28.9, 27.2, 23.3, 23.0, 22.9; HRMS [ESI-TOF, MH^+] calcd for $C_{57}H_{100}N_3O_{39}$ 1450.5928, found 1450.5925.

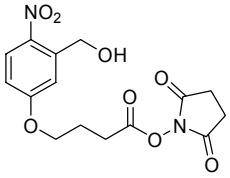


Compound 17. Sodium borohydride (1.4 g, 37.84 mmol) was added to a solution of compound **16** (4.07 g, 24.35 mmol) in methanol (30 mL) at 0 °C, then the ice-bath was removed and the reaction mixture was stirred at room temperature for 1 h. The solvent was removed under reduced pressure and the residue was recrystallized with MeOH to give **17** (4.0 g, 97%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.98 (d, *J* = 9.0 Hz, 1H), 6.78 (d, *J* = 2.5 Hz, 1H), 6.35 (dd, *J* = 9.0 Hz, J = 2.5 Hz, 1H), 4.95 (s, 2H). ^{13}C NMR (100 MHz,

CD_3OD) δ 178.5, 143.96, 133.26, 130.45, 118.76, 118.72, 63.73. HRMS [ESI-TOF, M^+] calcd. for $C_7H_7NO_4$ 169.0375, found 169.0377.



Compound 18. 1 M solution of TBAF in THF (8.7 mL, 8.7 mmol) was added to a mixture of compound **17** (0.98 g, 5.80 mmol) and methyl 4-bromobutyrate (1.26 g, 6.96 mmol) at room temperature. The reaction was kept stirring at room temperature for 12 h and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography (EtOAc/Hex = 1/2) to afford the **18** (1.37 g, 88%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15 (d, *J* = 9.0 Hz, 1H), 7.24 (d, *J* = 2.5 Hz, 1H), 6.87 (dd, *J* = 9.0 Hz, *J* = 2.5 Hz, 1H), 4.99 (s, 2H). 4.13 (t, *J* = 6.5 Hz, 2H), 3.70 (s, 3H), 2.54 (t, *J* = 6.5 Hz, 2H), 2.20–2.12 (m, 2H), ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.36, 163.36, 140.50, 140.10, 127.85, 114.25, 113.38, 67.43, 62.66, 51.69, 30.17, 24.23. HRMS [ESI-TOF, M^+] calcd. for $C_{12}H_{15}NO_6$ 269.0899, found 269.0898.



Compound 19. To a solution of compound **18** (0.55 g, 2.0 mmol) in a mixed solvent ($MeOH/H_2O$ = 4/1, 10 mL) was added LiOH (0.1g, 4.0 mmol) at 0 °C, then the reaction was allowed to react at room temperature. After 6 h, 1N $HCl_{(aq)}$ was added to neutralize the mixture, and the solvent was eavproated in vacuo. The residue was purified by column chromatography (EtOAc/Hex = 3/1) to provide the corresponding acid (0.48g, 94%). ^{1}H NHR (CD_3OD, 400 MHz) δ 7.95 (d, *J* = 7.2 Hz, 1H), 7.20 (d, *J* = 2.0 Hz, 1H), 6.77 (dd, *J* = 7.2 Hz, *J* = 2.0 Hz, 1H), 4.80 (s, 2H), 4.00 (t, *J* = 4.8 Hz, 2H), 2.34 (t, 6.0 Hz, 2H), 1.98–1.91 (m, 2H). ^{13}C NMR (CD_3OD, 100 MHz) δ 164.9, 164.5, 143.0, 141.1, 128.6, 114.3, 113.9, 68.9, 62.4, 31.6, 25.7. HRMS [ESI-TOF, MH^+] calcd. for $C_{11}H_{14}NO_6$ 256.0821, found 256.0819.

A mixture of acid (0.15 g, 0.56 mmol), DCC (0.20 g, 0.97 mmol), and *N*-hydroxysuccinimide (0.12 g, 0.97 mmol) in THF (5 mL) was stirred at room temperature

for 24 h. The solvent was removed and the residue was purified by column chromatography (EtOAc/Hex = 3/1) to give **19** (0.17g, 87 %). ^{1}H NHR (CD_3OD + $CDCl_3$ (1 : 1), 400 MHz) δ 8.16 (d, *J* = 9.0 Hz, 1H), 7.42 (d, *J* = 2.5 Hz, 1H), 6.94 (dd, *J* = 9.0 Hz, *J* = 2.5 Hz, 1H), 5.02 (s, 2H), 4.24 (t, *J* = 6.0 Hz, 2H), 2.89 (t, *J* = 7.0 Hz, 2H), 2.88 (s, 4H), 2.30–2.26 (m, 2H). ^{13}C NMR (CD_3OD + $CDCl_3$ (1:1), 100 MHz) δ 169.64, 167.91, 162.80, 141.31, 139.22, 126.95, 112.47, 112.39, 66.13, 60.73, 33.01, 26.77, 24.85. HRMS [ESI-TOF, MH^+] calcd. for $C_{15}H_{17}N_2O_8$ 353.0985, found 353.0986.

Synthesis of porous silicon plate with silylated hydroxyl surface. The porous silicon plate with silylated amino surface **20** was kept in a solution of compound **19** (0.10g, 0.28 mmol) in 200 μL toluene and 30 μL triethylamine at room temperature. After shaking slowly for 2 h, the surface was washed with HPLC grade ethyl acetate 3 times and then dried with high purity-nitrogen gas to get the porous silicon plate with silylated hydroxyl surface.

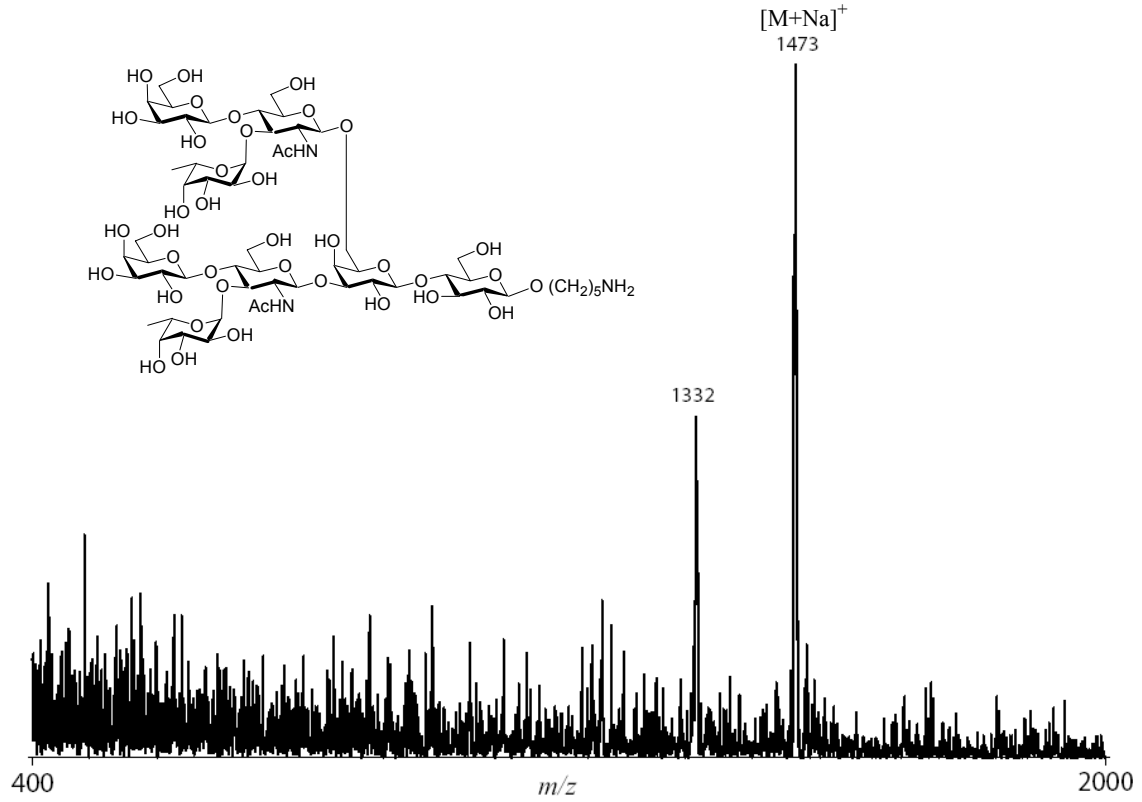
Synthesis of porous silicon plate with carbamate surface 21. The porous silicon plate with silylated hydroxyl surface was kept in a solution of *N*,*N*′-disuccinimidyl carbonate (90 mg, 0.45 mmol) in 500 μL of anhydrous acetonitrile at room temperature. 50 μL triethylamine was then added and kept shaking slowly for 5 h. The surface was sequentially washed with HPLC grade ethyl acetate (3 times) and 20 % ethanol solution (twice). Then the surface was dried with high purity-nitrogen gas to get the porous silicon plate with carbamate surface **21**.

Preparation of photocleavable N3 minor sugar array on porous silicon. N3 minor **3** (200 μM in H_2O, 400 μL) was added to the carbamate surface **21** at room temperature. 10 μL triethylamine was then added and the mixture was shaken slowly for 2 h, additional N3 minor **3** (200 μM in H_2O, 400 μL) was added and shaken for 2 h. The surface was sequentially washed with HPLC grade MeOH (3×) and pure water (3×). Then the surface was dried with high purity-nitrogen gas to get the sugar array, which was subjected to matrix-assisted laser desorption/ionization (MALDI) without adding additional matrix to obtain the expected mass spectrum (*m/z* = 1473 $(M+Na)^+$).

Control experiments:

N3 minor **3** (200 μM in H_2O, 400 μL) was added to the silylated amino surface **20** at room temperature. The surface was dried at room temperature after 1 h, which was subjected to matrix-assisted laser desorption/ionization (MALDI) without adding additional matrix to obtain the mass spectrum of N3 minor **3** (*m/z* = 1473 $(M + Na)^+$). Then the surface was sequentially washed with HPLC grade MeOH (3 times) and pure water (3×) and it was subjected to matrix-assisted laser desorption/ionization (MALDI) without adding additional matrix. No any obvious signal was found (Figure 1).

A)



B)

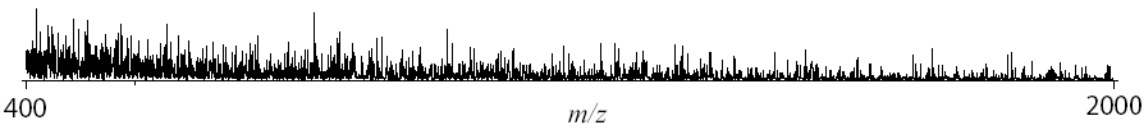


Figure 1. A) The DIOS mass spectrum of nonconvalently deposited N3 minor 3 on the silylated amino surface **20,** showed the expected *m/z* = 1473 ($[M+Na]^+$). B) No signal was shown after it had been washed by MeOH (3×) and H_2O (3×).

Reference

1) Z. Shen, J. J. Thomas, C. Averbuj. K. M. Broo, M. Engelhard, J. E. Crowell, M. G. Finn, G. Siuzdak, *Anal. Chem.* **2001**, *98*, 4932–4937.