Supporting Information
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Supporting Information

Stereoselective glycosylation of 3-deoxy-D-manno-2-octulosonic acid (Kdo) with batch and microfluidic methods

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Experimental Procedures

General procedures: $^1$H NMR spectra were recorded in indicated solvents by using a JEOL JNM-LA 500, or a JEOL ECA 500 spectrometers. The chemical shifts in CDCl$_3$ are given in δ values from tetramethylsilane (TMS) as an internal standard. Mass spectrometry was carried out using Applied Biosystem Mariner™ Biospectrometry Workstation (ESI-TOF) or Micromass Q-Tof micro™ (ESI-QTOF). Silica-gel column chromatography was carried out using Kieselgel 60 (Merck, 0.040-0.063 mm). TLC analysis was performed on Silica-gel 60 F$_{254}$ (Merck) and compound visualized by UV (254 nm), phosphomolybdic acid solution (5.0% in EtOH), 0.03% p-methoxybenzaldehyde in EtOH-conc.H$_2$SO$_4$-acetic acid buffer or 0.2% ninhydrin in EtOH-collidine-acetic acid buffer. MS4A were activated by heating at 250 °C in vacuo for 3 h before use. Unless otherwise stated all reactions were performed at room temperature. Non-aqueous reactions were carried out under argon atmosphere unless otherwise noted. TfOH was purchased from Sigma-Aldrich Corporation, USA. Anhydrous CH$_2$Cl$_2$ was distilled from calcium hydride. Anhydrous CPME and 1,4-dioxane, were purchased from Kanto Chemicals, Tokyo, Japan. Anhydrous CH$_3$CN, acetone, EtOAc, toluene and DMF was purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan. Anhydrous benzene was purchased from NACALAI TESQUE INC., Kyoto, Japan. All other reagents and solvents used were also purchased from commercial sources.
Disaccharide (3b)

To a mixture of donor 1b (10.0 mg, 11.0 µmol), acceptor 2 (2.2 mg, 3.66 µmol) and MS4A in anhydrous CH$_3$CN (65 µL) was added a solution of TfOH (0.032 µL, 0.366 µmol) in CH$_3$CN (10 µL) at 0 °C under Ar atmosphere, and the reaction mixture was stirred 15 min. After addition of Et$_3$N, the mixture was concentrated in vacuo. The residue was purified by silica-gel column chromatography (toluene/EtOAc=100/1→50/1→20/1) to give 3b as a colorless solid (4.2 mg, 91% from acceptor).

$^1$H NMR (500 MHz, CDCl$_3$); δ = 7.31-7.21 (m, 25 H, -CH$_2$-C$_6$H$_5$), 5.88-5.76 (m, 1 H, -OCH$_2$-CH=CH$_2$), 5.15-5.10 (m, 3 H, -OCH$_2$-CH=CH$_2$), 5.02 (d, J = 9.5 Hz, 1 H, H-1), 4.78-4.64 (m, 7 H, C$_6$H$_5$-CH$_2$-, -COO-CH$_2$-C$_6$H$_5$), 4.53-4.42 (m, 3 H, C$_6$H$_5$-CH$_2$-, -COO-CH$_2$-CCl$_3$), 4.16-4.06 (m, 2 H, H-4', H-5', -OCH$_2$-CH=CH$_2$), 4.04-3.97 (m, 4 H, -OCH$_2$-CH=CH$_2$, H-2, H-6', H-8'a), 3.76-3.59 (m, 5 H, H-3, H-5, H-6a, H-8b'), 3.16 (t, J = 9.5 Hz, 1 H, H-4), 2.15 (t, J = 11.8 Hz, 1 H, H-3a'), 0.87 (d, J = 4.5 Hz, 18 H, t-BuSi), 0.08 (s, 3 H, CH$_3$Si), 0.06 (s, 3 H, CH$_3$Si), 0.04 (s, 6 H, CH$_3$Si); HRMS (ESI-QTOF, positive) Calcd. for C$_{67}$H$_{88}$Cl$_3$NO$_{14}$Si$_2$ [M+Na]$^+$: 1314.4707, Found 1314.4681.

Trisaccharide (6a)

To a mixture of imidate donor 1a (12.0 mg, 16.7 µmol), acceptor 5 (3.6 mg, 3.34 µmol) and MS4A in dry CH$_3$CN (90 µL) was added a solution of TfOH (0.028 µL, 0.334 µmol) in dry CH$_3$CN (20 µL) at 0 °C and the mixture was stirred under Ar atmosphere for 15 min. After addition of Et$_3$N, the mixture was concentrated in vacuo. The residue was purified by silica-gel flash column chromatography (toluene/EtOAc=10/1→5/1→1/1) to give 6a as a white solid (5.4 mg, quant.).

$^1$H NMR (500 MHz, CDCl$_3$); δ = 7.31-7.17 (m, 32 H, C$_6$H$_5$-CH$_2$-, CH$_3$O-C$_6$H$_4$-CH$_2$-, C$_6$H$_5$-CH$_2$-OCO-), 6.83 (d, J = 8.5 Hz, 2 H, CH$_3$O-C$_6$H$_4$-CH$_2$-), 5.85 (ddd, J = 22.6, 10.7, 5.6 Hz, 1 H, -OCH$_2$-CH=CH$_2$ of Alloc group), 5.75 (ddd, J = 22.5, 10.9, 5.7 Hz, 1 H, -OCH$_2$-CH=CH$_2$ of allyl group), 5.25 (dd, J = 17.2, 1.3 Hz, 1 H, -OCH$_2$-CH=CH$_2$ of Alloc group), 5.20-5.11 (m, 6 H, -OCH$_2$-CH=CH$_2$ of Alloc and allyl groups, C$_6$H$_5$-CH$_2$-OCO-), 5.05 (dd, J = 10.5, 9.3 Hz, 1 H, H-3), 5.01-4.98 (m, 2 H, 2-NH, C$_6$H$_5$-CH$_2$-OCO-), 4.89 (brs, 1 H, 2'-NH), 4.73 (d, J$_{gem}$ = 11.2 Hz, 1 H, C$_6$H$_5$-CH$_2$-), 4.71 (d, J = 2.7 Hz, 1 H, H-1), 4.68 (d, J$_{gem}$ = 10.8 Hz, 1 H, C$_6$H$_5$-CH$_2$-), 4.66 (d, J$_{gem}$ = 9.0 Hz, 1 H, CH$_3$O-C$_6$H$_4$-CH$_2$-), 4.63 (d, J$_{gem}$ = 11.3 Hz, 2 H, C$_6$H$_5$-CH$_2$-), 4.58 (d, J$_{gem}$ = 11.0 Hz, 1 H, CH$_3$O-C$_6$H$_4$-CH$_2$-), 4.57 (d, J$_{gem}$ = 12.6 Hz, 2 H, C$_6$H$_5$-CH$_2$-), 4.51-4.44
(m, 6 H, C₆H₅-CH₂-, -OCH₂-CH=CH₂ of Alloc group, -COO-CH₂-CI₃), 4.41-4.36 (m, 2 H, H-1’, H-4’’), 4.33 (dd, J = 7.0, 1.3 Hz, 1 H, H-5’”), 4.06 (d, J = 10.5 Hz, 1 H, H-7’’), 4.01 (dd, J = 13.0, 5.2 Hz, 1 H, -OCH₂-CH=CH₂ of allyl group), 3.98 (dt, J = 22.6, 10.7, 5.6 Hz, 1 H, -OCH₂-CH=CH₂ of Alloc group), 3.79-3.65 (m, 10 H, H-3’, H-8”a, H-6b, H-4’, H-6’a, H-4, -OCH₂-CH=CH₂ of allyl group), 3.59 (dd, J = 13.5, 3.2 Hz, 1 H, H-6”b), 3.37 (t, J = 9.2 Hz, 1 H, H-5’”), 3.34-3.28 (m, 2 H, H-6'b, H-2’), 2.57 (dd, J = 14.9, 5.0 Hz, 1 H, H-3’’a), 1.90 (dd, J = 14.9, 5.0 Hz, 1 H, H-3’’b), 1.33 (s, 3 H, methyl group of isopropylidene), 1.27 (s, 3 H, methyl group of isopropylidene); ESI-MS (positive): m/z = 1625.46 [M+Na]+; HRMS (ESI-QTOF, positive) Calcd. for C₈₄H₉₃Cl₃N₂O₂₃ [M+Na]+: 1625.5132, Found 1625.5127.

Trisaccharide (6b)

To a mixture of imidate donor 1b (10.0 mg, 11.2 µmol), acceptor 5 (2.4 mg, 2.20 µmol) and MS4A in dry CH₃CN (70 µL) was added a solution of TfOH (0.020 µL, 0.22 µmol) in dry CH₃CN (10 µL) at 0 °C and the mixture was stirred under Ar atmosphere for 15 min. After addition of Et₃N, the mixture was concentrated in vacuo.

The residue was purified by silica-gel flash column chromatography (toluene/EtOAc=20/1→10/1) to give 6b as a colorless solid (3.8 mg, 95%).

¹H NMR (500 MHz, CDCl₃); δ = 7.39-7.15 (m, 32 H, C₆H₅-CH₂-, CH₃O-C₆H₄-CH₂-, C₆H₅-CH₂-OCO-), 6.84 (d, J = 8.5 Hz, 2 H, CH₃O-C₆H₄-CH₂-), 5.86 (ddd, J = 22.6, 10.7, 5.6 Hz, 1 H, -OCH₂-CH=CH₂ of Alloc group), 5.77 (ddd, J = 22.3, 10.9, 5.8 Hz, 1 H, -OCH₂-CH=CH₂ of allyl group), 5.25 (dd, J = 17.2, 1.4 Hz, 1 H, -OCH₂-CH=CH₂ of Alloc group), 5.20-5.00 (m, 9 H, -OCH₂-CH=CH₂ of Alloc and allyl groups, C₆H₅-CH₂-OCO-, H-3, 2’-NH), 4.89 (brs, 1 H, 2’-NH), 4.78 (d, J = 3.5 Hz, 1 H, H-1), 4.71-4.39 (m, 15 H, C₆H₅-CH₂-, -OCH₂-CH=CH₂ of Alloc group, -COO-CH₂-CI₃, H-1’), 4.14-4.02 (m, 4 H, -OCH₂-CH=CH₂ of allyl group, H-4’’, H-5’’, H-6’’), 3.98-3.91 (m, 3 H, H-2, H-5, H-7’’), 3.88-3.56 (m, 12 H, -OCH₂-CH=CH₂ of allyl group, H-4, H-6a, H-6b, H-3’, H-3’, H-4’, H-6’a, H-8”a, H-8”b, CH₃O-C₆H₄-CH₂-), 3.41-3.28 (m, 3 H, H-2’, H-5’, H-6’b), 2.13 (t, J = 11.9 Hz, 1 H, H-3’’a), 1.93 (dd, J = 12.7, 3.9 Hz, 1 H, H-5’), 0.89 (d, J = 4.5 Hz, 18 H, t-BuSi-), 0.06 (s, 3 H, CH₃Si-), 0.02 (s, 3 H, CH₃Si-), 0.01 (s, 3 H, CH₃Si-); HRMS (ESI-QTOF, positive) Calcd. for C₉₀H₁₁₇Cl₃N₂O₂₃Si₂ [M+Na]⁺: 1813.6549, Found 1813.6672.

Disaccharide (7a)

To a mixture of donor 1a (12.0 mg, 16.8 µmol), acceptor 4 (3.0 mg, 5.60 µmol)
and MS4A in anhydrous CH$_3$CN (90 µL) was added a solution of TfOH (0.050 µL, 0.56 µmol) in CH$_3$CN (20 µL) at 0 ºC under Ar atmosphere, and the reaction mixture was stirred 15 min. After addition of Et$_3$N, the mixture was concentrated in vacuo. The residue was purified by silica-gel column chromatography (toluene/EtOAc=20/1→10/1→5/1→1/1) to give 7a as a white solid (2.8 mg, 92% from acceptor).

$^1$H NMR (500 MHz, CDCl$_3$); $\delta$ = 7.37-7.17 (m, 30 H, -CH$_2$-C$_6$H$_5$), 5.74-5.66 (m, 1 H, -OCH$_2$-CH=CH$_2$), 5.14 (d, $J_{gem}$ = 12.2 Hz, 1 H, -COOCH$_2$-C$_6$H$_5$), 5.07 (d, $J_{gem}$ = 12.2 Hz, 2 H, -COOCH$_2$-C$_6$H$_5$), 5.08-5.04 (m, 1 H, -OCH$_2$-CH=CH$_2$), 4.97 (dd, $J$ = 10.4, 1.5 Hz, 1 H, -OCH$_2$-CH=CH$_2$), 4.82 (d, $J$ = 12.3 Hz, 1 H, --COOCH$_2$-C$_6$H$_5$), 4.70 (d, $J_{gem}$ = 11.3 Hz, 1 H, C$_6$H$_5$-CH$_2$-), 4.63 (d, $J_{gem}$ = 11.2 Hz, 2 H, C$_6$H$_5$-CH$_2$-), 4.59 4.70 (d, $J_{gem}$ = 10.8 Hz, 1 H, C$_6$H$_5$-CH$_2$-), 4.55-4.50 (m, 4 H, C$_6$H$_5$-CH$_2$-), 3.97-3.94 (m, 1 H, H-7), 3.92 (ddd, $J = 6.9, 4.8, 2.3$ Hz, 1 H, H-7’), 3.87 (s, 1 H, H-5), 3.83-3.80 (m, 2 H, H-6, H-8a), 3.68 (ddd, $J = 10.5, 4.8, 2.2$ Hz, 1 H, H-8b, H-8'b), 2.91 (dd, $J = 15.4, 3.8$ Hz, 1 H, H-3’a), 2.36 (d, $J = 1.3$ Hz, 1 H, -OH), 2.14 (dd, $J = 13.0, 5.1$ Hz, 1 H, H-3a), 2.05 (t, $J = 11.8$ Hz, 1 H, H-3b), 1.85 (dd, $J = 15.3, 2.6$ Hz, 1 H, H-3’b), 1.26 (s, 3 H, CH-CH$_3$), 1.24 (s, 3 H, CH-CH$_3$); HRMS (ESI-TOF, positive) Calcd. for C$_{64}$H$_{70}$O$_{15}$ [M+Na]$^+$: 1101.4612, Found: 1101.4609.