CHEMBIOCHEM

Supporting Information

Glycan Sequence-Dependent Nod2 Activation Investigated by Using a Chemically Synthesized Bacterial Peptidoglycan Fragment Library

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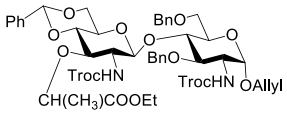
Supporting Information

Experimental Procedure of Synthesis / Spectroscopic Data

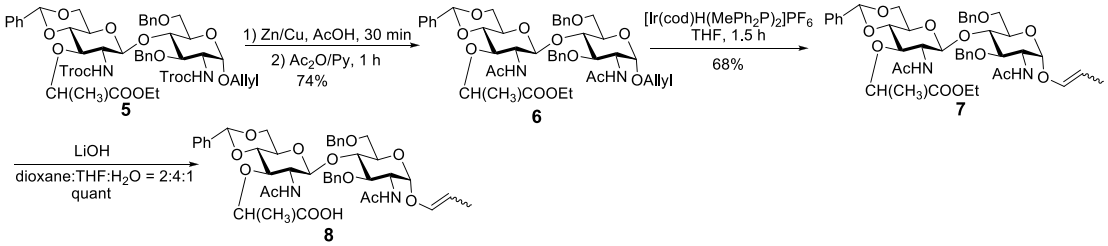
General procedures: ^{1}H NMR spectra were recorded in indicated solvents by using a JEOL ECA 400, or a JEOL JNM-LA 500, or a JEOL ECA 500, or a Varian INOVA 600 spectrometers. The chemical shifts in $CDCl_3$ are given in δ values from tetramethylsilane (TMS) as an internal standard. For the measurement in D_2O, HDO signal (4.718 ppm at 30 °C) was used as a reference. High resolution mass spectrometry was measured by using Bruker micrOTOFQII (ESI-QTOF). Silica-gel column chromatography was carried out using Kieselgel 60 (Merck, 0.040-0.063 mm) or Silica Gel 60 N (Kanto Chemical Co., spherical, neutral, 0.040-0.050 mm) at medium pressure (2-4 kg/cm2). Gel permeation chromatography (GPC) was carried out using Sephadex LH20 at atmospheric pressure. Precoated Kieselgel 60 F 254 (Merck Co., 0.5 mm) was used for preparative thin layer chromatography. 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_2SO_4-acetic acid buffer or 0.2% ninhydrin in EtOH-collidine-acetic acid buffer. MS4A was 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. Anhydrous CH_2Cl_2 was distilled from calcium hydride. Anhydrous THF was purchased from Kanto Chemicals, Tokyo, Japan. Anhydrous DMF was purchased from NACALAI TESQUE INC., Kyoto, Japan. Distilled water purchased from Otsuka (Tokyo, Japan) or prepared by a combination of Arium® 611 UV (Sautorius) or Toray Pure LV-308 (Toray) and GSL-200 (Advantec, Tokyo, Japan). All other reagents and solvents used were also purchased from commercial sources.



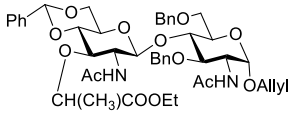
Allyl 3,6-di-*O*-benzyl-2-deoxy-4-*O*-[4,6-*O*-benzylidene-2-deoxy-3-*O*-{(*R*)-1-(ethoxycarbonyl)ethyl}-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (**5**)

To a mixture of the imidate **3** (411 mg, 0.59 mmol), the acceptor **4** (282 mg, 0.49 mmol), and MS4A in dry CH_2Cl_2 (20 mL) at -15 °C was added TMSOTf (9 μL, 0.05 mmol). After being stirred at the same temperature for 20 min, the reaction was quenched with chilled saturated aqueous $NaHCO_3$ (10 mL), and the mixture was extracted with $CHCl_3$ (50 mL). The organic layer was washed with sat. $NaHCO_3$ aq (20 mL) and brine (20 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel chromatography (60 g, toluene : EtOAc = 10 : 1) to

give **5** as a white solid (453 mg, 84%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42-7.24 (m, 15H), 6.61 (brs, 1H), 6.07 (brs, 1H), 5.86 (m, 1H), 5.62 (d, J = 7.5 Hz, 1H), 5.41 (s, 1H), 5.27-5.20 (m, 2H), 5.00 (d, J = 9.0 Hz, 1H), 4.93-4.88 (m, 3H), 4.78 (d, J = 11.5 Hz, 1H), 4.72-4.60 (m, 4H), 4.46-4.42 (m, 2H), 4.24-4.19 (m, 3H), 4.10-4.09 (m, 2H), 4.01-3.94 (m, 3H), 3.87 (d, J = 10.5 Hz, 1H), 3.67 (m, 1H), 3.61-3.49 (m, 4H), 3.40 (m, 1H), 3.26 (t, J = 10.5 Hz, 1H), 3.06 (m, 1H), 1.37 (d, J = 7.0 Hz, 3H), 1.30-1.27 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.8, 163.4, 155.3, 154.2, 138.9, 137.9, 137.1, 133.3, 129.0, 128.7, 128.5, 128.3, 128.2, 127.4, 127.3, 125.9, 118.2, 102.2, 101.0, 96.6, 95.4, 82.3, 78.1, 78.1, 77.1, 75.1, 74.6, 74.4, 73.5, 70.6, 68.5, 67.6, 65.6, 61.1, 57.4, 54.7, 18.8, 14.2; HRMS (ESI-QTOF) Anal. Calcd for $C_{47}H_{54}Cl_6N_2O_{15}Na$ $[M+Na]^+$: 1119.1553, found: 1119.1559.

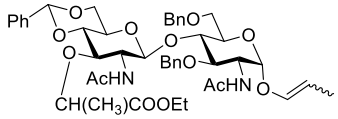


Scheme S1. Preparation of Disaccharide 8



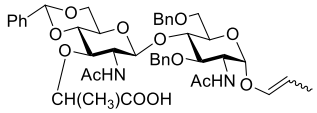
Allyl 2-acetylamino-4-*O*-[2-acetylamino-4,6-*O*-benzylidene-2-deoxy-3-*O*-{(*R*)-1-(ethoxy carbonyl)-ethyl}-β-D-glucopyranosyl]-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranos ide (6)

To a solution of **5** (50 mg, 1.9 mmol) in AcOH (2 mL) was added Zn-Cu (prepared from 300 mg of Zn), the mixture was stirred at room temperature for 30 min. The insoluble materials were filtered off and the filtrate was concentrated in vacuo. The residue solvent was removed by coevaporation with toluene (10 mL). The residue was dissolved in pyridine (2 mL) and acetic anhydride (2 mL) and the solution was stirred at room temperature for 1 h. The solution was removed by concentration with toluene (10 mL). The residue was purified by silica-gel chromatography (5 g, $CHCl_3$: acetone = 9 : 1) to give **6** as a white solid (28 mg, 74%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43-7.26 (m, 15H), 6.15 (d, J = 7.5 Hz, 1H), 5.84 (m, 1H), 5.44 (s, 1H), 5.28 (d, J = 9.0 Hz, 1H), 5.23-5.19 (m, 2H), 4.86-4.84 (m, 2H), 4.77 (d, J = 12.0 Hz, 1H), 4.58 (d, J = 12.0 Hz, 1H), 4.48-4.39 (m, 3H), 4.19-4.11 (m, 5H), 3.98-3.72 (m, 5H), 3.62-3.60 (m, 2H), 3.54-3.47 (m, 2H), 3.37 (t, J = 10.0 Hz, 1H), 3.13 (m, 1H), 2.00 (s, 3H), 1.84 (s, 3H), 1.37 (d, J = 7.0 Hz, 3H), 1.27 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.6, 172.3, 170.3, 148.7, 140.2, 139.2, 138.1, 137.2, 133.6, 129.0, 128.5, 128.2, 128.1, 127.7, 127.3, 125.8, 125.6, 117.8, 102.1, 101.9, 96.2, 82.5, 77.7, 74.9, 74.0, 73.5, 70.9, 68.5, 68.3, 67.7, 75.7, 61.1, 55.7, 52.1, 23.5, 23.3, 18.8, 14.1; HRMS (ESI-QTOF) Anal. Calcd for $C_{45}H_{57}N_2O_{13}$ $[M+H]^+$: 833.3861, found: 833.3839.



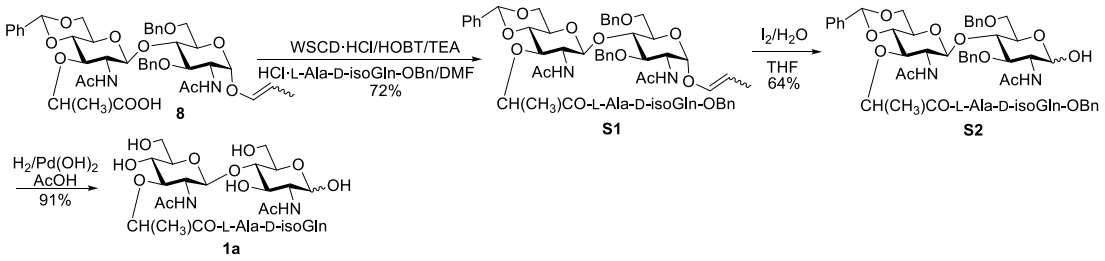
Prop-1-enyl 2-acetylamino-4-*O*-[2-acetylamino-4,6-*O*-benzylidene-2-deoxy-3-*O*-{(*R*)-1-(ethoxy carbonyl)-ethyl}-β-D-glucopyranosyl]-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (7)

To a solution of **6** (50 mg, 0.06 mmol) in THF (2 mL) was added H_2-activated $[Ir(cod)(MePh_2P)_2]PF_6$ (2.5 mg, 0.003 mmol) in dry THF (1 mL). After being stirred under an argon atmosphere at room temperature for 1.5 h, the reaction mixture was quenched with sat. $NaHCO_3$ aq (10 mL) and the mixture was extracted with AcOEt (20 mL × 2). The organic layer was washed with sat. $NaHCO_3$ aq (20 mL) and brine (20 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel chromatography (5 g, $CHCl_3$: acetone = 10 : 1) to give **7** as a white solid (34 mg, 68%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44-7.26 (m, 15H), 6.14 (d, J = 7.6 Hz, 1H), 6.09 (dd, J = 12.3 Hz, J = 1.6 Hz, 1H), 5.45 (s, 1H), 5.26 (d, J = 8.5 Hz, 1H), 5.12-5.08 (m, 1H), 5.04 (d, J = 3.8 Hz, 1H), 4.88 (d, J = 12.2 Hz, 1H), 4.76 (d, J = 12.0 Hz, 1H), 4.61 (d, J = 12.2 Hz, 1H), 4.48-4.39 (m, 3H), 4.23-4.16 (m, 4H), 4.01 (t, J = 9.0 Hz, 1H), 3.79-3.76 (m, 2H), 3.69 (d, J = 15.0 Hz, 1H), 3.63 (d, J = 3.9 Hz, 1H), 3.59-3.53 (m, 2H), 3.48 (d, J = 5.1 Hz, 1H), 3.39 (t, J = 10.0 Hz, 1H), 3.15 (m, 1H), 1.97 (s, 3H), 1.81 (s, 3H), 1.53 (d, J = 6.4 Hz, 3H), 1.37(d, J = 7.0 Hz, 3H), 1.28 (t, J = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.3, 171.6, 169.9, 142.8, 139.2, 138.2, 137.3, 129.0, 128.6, 128.3, 128.3, 128.2, 128.1, 127.9, 127.4, 125.9, 104.7, 102.2, 101.0, 96.9, 82.6, 77.4, 77.4, 74.9, 74.3, 73.6, 71.2, 68.6, 67.5, 65.7, 61.0, 55.6, 51.9, 23.5, 23.3, 18.9, 14.2, 12.4; HRMS (ESI-QTOF) Anal. Calcd for $C_{45}H_{56}N_2O_{13}Na$ $[M+Na]^+$: 855.3680, found: 855.3613.

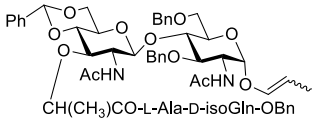


Prop-1-enyl 2-acetylamino-4-*O*-[2-acetylamino-4,6-*O*-benzylidene-2-deoxy-3-*O*-{(*R*)-1-carboxyethyl}-β-D-glucopyranosyl]-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (8)

To a solution of **7** (23 mg, 0.03 mmol) in dioxane : THF : H_2O (2 : 4 : 1, 4.0 mL) was added LiOH (4 mg, 0.17 mmol) and stirred at room temperature for 1 h. The solution was neutralized with Dowex H^+ (Dowex 50W × 8, 200-400 mesh H form, DowChemicals) and then applied to an HP-20 column (2 cm × 10 cm). Organic and inorganic salts were removed by elution with H_2O (160 mL), then eluted with MeOH and concentrated in vacuo to give a disaccharide with a free lactic acid moiety **8** as a white solid (22 mg, quant). HRMS (ESI-QTOF) Anal. Calcd for $C_{43}H_{52}N_2O_{13}Na$ $[M+Na]^+$: 827.3367, found: 827.3291.

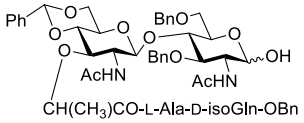


Scheme S2. Preparation of 1a



Prop-1-enyl 2-acetylamino-4-*O*-[[2-acetylamino-4,6-*O*-benzylidene-2-deoxy-3-*O*-[(*R*)-propionyl-{benzyl-(L-alanyl-D-isoglutaminate)}]-β-D-glucopyranosyl]]-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**S1**)

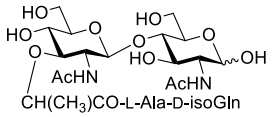
To a solution of **8** (20 mg, 0.025 mmol), HCl·L-Ala-D-isoGln-OBn (25 mg, 0.073 mmol), and HOBt (6 mg, 0.044 mmol) in DMF (3 mL) were added WSCD·HCl (6 mg, 0.037 mmol) and triethylamine (11 μL, 0.079 mmol) at 0 °C and the mixture was stirred at rt overnight. The mixture was concentrated and the residue was dissolved in $CHCl_3$. The $CHCl_3$ solution was washed with citric acid (1 M, 20 mL), H_2O (20 mL), sat. $NaHCO_3$ aq (20 mL), and brine (20 mL). The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel chromatography (5 g, $CHCl_3$: MeOH = 20 : 1) to give **S1** as a white solid (20 mg, 72%). ^{1}H NMR (500 MHz, $CDCl_3$: CD_3OD = 4 : 1) δ 7.84 (m, 1H), 7.72 (m, 1H), 7.38-7.17 (m, 24H), 6.13 (d, J = 12.5 Hz, 1H), 5.45 (s, 1H), 5.09-5.03 (m, 3H), 5.02 (d, J = 3.5 Hz, 1H), 4.87 (d, J = 12.0 Hz, 1H), 4.75 (d, J = 12.0 Hz, 1H), 4.65-4.59 (m, 2H), 4.53-4.39 (m, 4H), 4.25-4.15 (m, 3H), 4.07-4.01 (m, 2H), 3.86-3.80 (m, 2H), 3.72-3.66 (m, 3H), 3.54-3.52 (m, 2H), 2.22-2.17 (m, 2H), 2.00-1.91 (m, 5H), 1.85 (s, 3H), 1.56-1.54 (m, 3H), 1.43-1.40 (m, 3H), 1.34-1.31 (m, 3H); ^{13}C NMR (150 MHz, $CDCl_3$: CD_3OD = 4 : 1) δ 174.2, 173.5, 173.4, 173.0, 172.0, 171.4, 143.0, 139.1, 138.1, 137.2, 135.7, 129.3, 128.8, 128.6, 128.5, 128.5, 128.4, 128.4, 128.2, 127.9, 127.8, 127.7, 126.1, 105.0, 101.5, 101.1, 97.0, 81.5, 79.2, 76.7, 74.3, 73.9, 71.6, 68.7, 68.3, 67.0, 66.8, 65.9, 56.3, 52.5, 52.3, 30.6, 27.0, 23.2, 22.8, 17.6, 17.1, 12.4; HRMS (ESI-QTOF) Anal. Calcd for $C_{58}H_{71}N_5O_{16}Na$ $[M+Na]^+$: 1116.4794, found: 1116.4772.



2-Acetylamino-4-*O*-[[2-acetylamino-4,6-*O*-benzylidene-2-deoxy-3-*O*-[(*R*)-propionyl-{benzyl-(L-alanyl-D-isoglutaminate)}]-β-D-glucopyranosyl]]-3,6-di-*O*-benzyl-2-deoxy-D-glucopyranoside (**S2**)

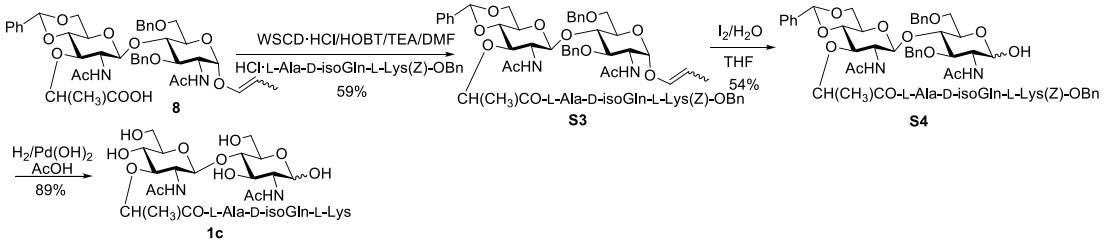
To a solution of **S1** (20 mg, 0.018 mmol) in THF (5 mL), iodine (10 mg, 0.039 mmol)

and water (0.5 mL) were added and the reaction mixture was stirred for 2 h. The reaction was quenched by the addition of $Na_2S_2O_3$ aq (5%, 10 mL). The mixture was then extracted with $CHCl_3$ (20 mL). The organic layer was washed with $Na_2S_2O_3$ aq (5%, 10 mL × 2), sat. $NaHCO_3$ aq (20 mL × 2), and brine (20 mL), dried over Na_2SO_4, and then concentrated in vacuo. The residue was purified by silica-gel chromatography (5 g, $CHCl_3$: MeOH = 10 : 1) to give 1-liberated **S2** as a white solid (12 mg, 64%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65 (m, 1H), 7.35-7.24 (m, 19H), 5.39 (s, 1H), 5.03 (m, 3H), 4.73 (d, J = 11.5 Hz, 1H), 4.62-4.53 (m, 3H), 4.42 (d, J = 11.5 Hz, 1H), 4.32 (brs, 1H), 4.10-4.01 (m, 4H), 3.93 (m, 1H), 3.83-3.63 (m, 4H), 3.48-3.39 (m, 4H), 2.42-2.36 (m, 2H), 2.11 (m, 1H), 1.88-1.81 (m, 7H), 1.25-1.19 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.9, 173.7, 173.0, 171.9, 171.2, 138.6, 137.6, 136.9, 135.4, 129.0, 128.4, 128.1, 128.1, 128.0, 128.0, 127.9, 127.5, 127.4, 125.8, 101.1, 100.7, 90.5, 87.8, 81.2, 78.6, 76.4, 73.7, 73.6, 70.9, 68.6, 68.3, 66.5, 65.7, 55.9, 52.2, 51.9, 30.2, 27.0, 22.9, 22.6, 19.0, 16.6; HRMS (ESI-QTOF) Anal. Calcd for $C_{55}H_{67}N_5O_{16}Na$ $[M+Na]^+$: 1076.4481, found: 1076.4492.

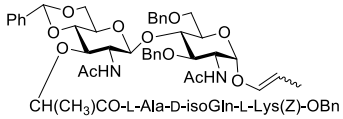


2-Acetylamino-4-*O*-[2-acetylamino-2-deoxy-3-*O*-{(*R*)-propionyl-(L-alanyl-D-isoglutamine)}-β-D-glucopyranosyl]-2-deoxy-D-glucopyranoside (1a)

To a solution of **S2** (11 mg, 0.01 mmol) in AcOH (4 mL) was added palladium hydroxide (60 mg) in AcOH and stirred under H_2 (2 MPa) for 1 d. The reaction was monitored by TLC analysis and the hydrogenolysis was continued until deprotection was completed. The Pd catalyst was filtered off by celite, and the filtrate was concentrated. The residue was lyophilized from acetonitrile-H_2O to give **1a** (6.5 mg, 91%) as a white solid. ^{1}H NMR (500 MHz, D_2O) δ 5.11 (d, J = 2.6 Hz, 1H), 4.50 (d, J = 8.6 Hz, 1H), 4.26-4.15 (m, 3H), 3.87-3.44 (m, 12H), 2.26 (dd, J = 7.8 Hz, J = 7.0 Hz, 2H), 2.06-1.87 (m, 8H), 1.36 (d, J = 7.2 Hz, 3H), 1.30 (d, J = 6.7 Hz, 3H); ^{13}C NMR (125 MHz, D_2O) δ 177.0, 176.2, 175.8, 175.4, 175.1, 174.7, 102.0, 95.5, 91.1, 83.1, 79.9, 78.9, 76.3, 75.2, 73.2, 70.7, 69.9, 69.4, 61.2, 60.8, 60.7, 56.8, 55.7, 54.3, 53.9, 50.4, 33.3, 27.9, 22.9, 22.6, 19.4, 17.3; HRMS (ESI-QTOF) Anal. Calcd for $C_{27}H_{44}N_5O_{16}Na_2$ $[M+2Na-H]^+$: 740.2578, found: 740.2542.

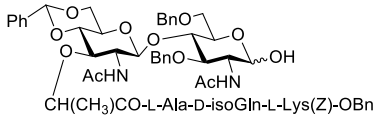


Scheme S3. Preparation of 1c



Prop-1-enyl 2-acetylamino-4-*O*-[[2-acetylamino-4,6-*O*-benzylidene-2-deoxy-3-*O*-[(*R*)-propionyl-{benzyl-(L-alanyl-D-isoglutamyl-ε-*N*-benzyloxycarbonyl-L-lysinate)}]-β-D-glucopyranosyl]]-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**S3**)

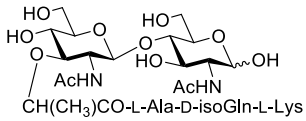
To a solution of **8** (14 mg, 0.017 mmol), HCl·L-Ala-D-isoGln-L-Lys-OBn (14 mg, 0.023 mmol), and HOBt (6 mg, 0.044 mmol) in DMF (1 mL) were added WSCD·HCl (6 mg, 0.037 mmol) and triethylamine (10 μL, 0.079 mmol) at 0 °C and the mixture was stirred at rt overnight. The mixture was concentrated and the residue was dissolved in $CHCl_3$. The $CHCl_3$ solution was washed with citric acid (1 M, 20 mL), H_2O (20 mL), sat. $NaHCO_3$ aq (20 mL), and brine (20 mL). The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel chromatography (5 g, $CHCl_3$: MeOH = 18 : 1) to give **S3** as a white solid (14 mg, 59%). ^{1}H NMR (500 MHz, $CDCl_3$: CD_3OD = 4 : 1) δ 7.79 (m, 1H), 7.44-7.29 (m, 28H), 6.13 (d, J = 13.5 Hz, 1H), 5.58 (m, 1H), 5.45 (s, 1H), 5.20-5.02 (m, 7H), 4.87 (d, J = 11.5 Hz, 1H), 4.73 (m, 1H), 4.62-4.59 (m, 2H), 4.60-4.45 (m, 4H), 4.28-4.00 (m, 5H), 3.88-3.67 (m, 5H), 3.54-3.52 (m, 2H), 3.13-3.11 (m, 2H), 2.31-2.27 (m, 2H), 2.11-2.09 (m, 1H), 1.93 (s, 4H), 1.85 (s, 4H), 1.73 (m, 1H), 1.55-1.54 (m, 3H), 1.48-1.42 (m, 4H), 1.36-1.26 (m, 6H); HRMS (ESI-QTOF) Anal. Calcd for $C_{72}H_{89}N_7O_{19}Na$ $[M+Na]^+$: 1378.6111, found: 1378.6103.



2-Acetylamino-4-*O*-[[2-acetylamino-4,6-*O*-benzylidene-2-deoxy-3-*O*-[(*R*)-propionyl-{benzyl-(L-alanyl-D-isoglutamyl-ε-*N*-benzyloxycarbonyl-L-lysinate)}]-β-D-glucopyranosyl]]-3,6-di-*O*-benzyl-2-deoxy-D-glucopyranoside (**S4**)

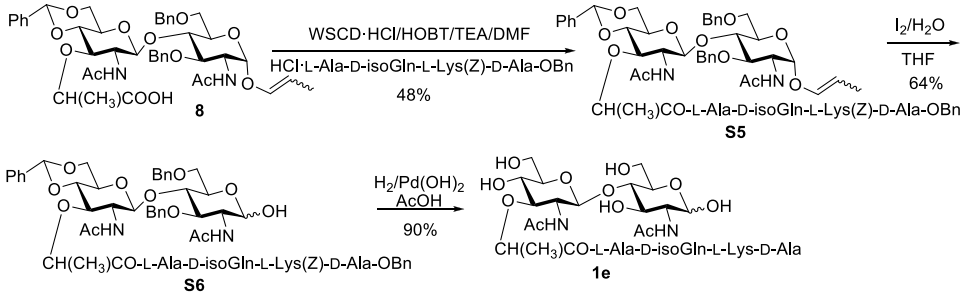
To a solution of **S3** (14 mg, 0.010 mmol) in THF (5 mL), iodine (10 mg, 0.039 mmol) and water (0.5 mL) were added and the reaction mixture was stirred for 2 h. The reaction was quenched by the addition of $Na_2S_2O_3$ aq (5%, 10 mL). The mixture was then extracted with $CHCl_3$ (20 mL). The organic layer was washed with $Na_2S_2O_3$ aq (5%, 10 mL × 2), sat. $NaHCO_3$ aq (20 mL × 2), and brine (20 mL), dried over Na_2SO_4, and then concentrated in vacuo. The residue was purified by silica-gel chromatography (5 g, $CHCl_3$: MeOH = 10 : 1) to give 1-liberated **S4** as a white solid (7 mg, 54%). ^{1}H NMR (500 MHz, DMF-d7) δ 8.18 (d, J = 8.2 Hz, 2H), 7.87 (m, 1H), 7.68 (m, 1H), 7.54-7.34 (m, 19H), 7.08 (brs, 1H), 6.89 (d, J = 4.4 Hz, 1H), 5.76 (s, 1H), 5.24-4.94 (m, 4H), 4.79-4.70 (m, 3H), 4.44-4.42 (m, 2H), 4.24 (m, 1H), 4.15-3.93 (m, 7H), 3.87-3.83 (m, 2H), 3.76-3.68 (m, 2H), 3.40-3.36 (m, 4H), 2.50-2.40 (m, 2H), 2.19 (m, 1H), 2.03 (s, 3H), 1.97 (s, 4H), 1.54-1.34 (m, 8H), 1.18 (t, J = 7.1 Hz, 2H), 1.08 (t, J = 7.1 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d6) δ 173.0, 172.0, 171.9, 171.8, 171.5, 169.5, 169.1, 156.0, 139.3, 138.4, 137.5, 137.2, 135.9, 128.7, 128.3, 128.3, 128.2, 128.0, 127.9, 127.9, 127.8, 127.7, 127.6, 127.6, 127.4,

127.3, 127.2, 127.1, 127.1, 127.0, 125.8, 100.0, 90.6, 79.9, 78.7, 77.5, 77.3, 76.6, 73.3, 71.9, 69.7, 67.7, 65.7, 65.6, 65.0, 55.6, 52.7, 52.1, 48.0, 31.4, 30.4, 28.9, 27.8, 23.0, 22.6, 22.5, 18.8, 18.2; HRMS (ESI-QTOF) Anal. Calcd for $C_{69}H_{85}N_7O_{19}Na$ $[M+Na]^+$: 1338.5798, found: 1338.5793.

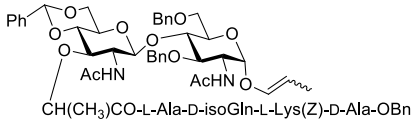


2-Acetylamino-4-*O*-[2-acetylamino-3-*O*-{(*R*)-propionyl-(L-alanyl-D-isoglutamyl-L-lysine)}-2-deoxy-β-D-glucopyranosyl]-2-deoxy-D-glucopyranoside (**1c**)

To a solution of **S4** (5 mg, 0.01 mmol) in AcOH (4 mL) was added palladium hydroxide (17 mg) in AcOH and stirred under H_2 (2 MPa) for 1 d. The reaction was monitored by TLC analysis and the hydrogenolysis was continued until deprotection was completed. The Pd catalyst was filtered off by celite and the filtrate was concentrated. The residue was lyophilized from acetonitrile-H_2O to give **1c** (3 mg, 89%) as a white solid. ^{1}H NMR (500 MHz, D_2O) δ 5.11 (d, J = 2.6 Hz, 1H), 4.49 (d, J = 8.5 Hz, 1H), 4.27 (dd, J = 9.5 Hz, J = 4.5 Hz, 1H), 4.22-4.13 (m, 2H), 3.87-3.44 (m, 14H), 3.32-3.26 (m, 3H), 2.47-2.32 (m, 2H), 2.15-1.88 (m, 8H), 1.36-1.29 (m, 7H), 1.11-1.00 (m, 5H); ^{13}C NMR (150 MHz, D_2O) δ 178.9, 175.9, 175.6, 175.1, 174.5, 174.2, 174.0, 101.3, 94.8, 90.4, 82.5, 79.7, 78.2, 75.6, 78.2, 75.6, 74.6, 72.5, 70.0, 69.3, 68.7, 61.3, 60.6, 60.1, 54.9, 53.6, 49.7, 39.3, 31.9, 31.1, 27.0, 26.3, 27.0, 26.3, 22.2, 22.1, 21.9, 18.8, 16.6; HRMS (ESI-QTOF) Anal. Calcd for $C_{33}H_{57}N_7O_{17}Na$ $[M+Na]^+$: 846.3709, found: 846.3697.



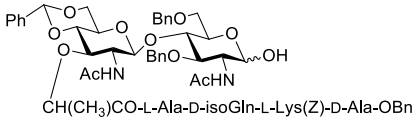
Scheme S4. Preparation of 1e



Prop-1-enyl 2-acetylamino-4-*O*-[[2-acetylamino-4,6-*O*-benzylidene-2-deoxy-3-*O*-[(*R*)-propionyl-{benzyl-(L-alanyl-D-isoglutamyl-ε-*N*-benzyloxycarbonyl-L-lysyl)-D-alaninate}]-β-D-glucopyranosyl]]-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**S5**)

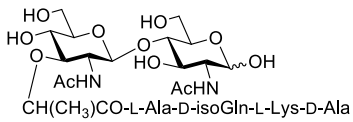
To a solution of **8** (10 mg, 0.012 mmol), HCl·L-Ala-D-isoGln-L-Lys(Z)-D-Ala-OBn (12 mg, 0.019 mmol), and HOBt (6 mg, 0.044 mmol) in DMF (3 mL) were added WSCD·HCl (6 mg, 0.037 mmol) and triethylamine (6 μL, 0.04 mmol) at 0 °C and the

mixture was stirred at rt overnight. The mixture was concentrated and the residue was dissolved in $CHCl_3$. The $CHCl_3$ solution was washed with citric acid (1 M, 20 mL), H_2O (20 mL), sat. $NaHCO_3$ aq (20 mL), and brine (20 mL). The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel chromatography (5 g, $CHCl_3$: MeOH = 20 : 1) to give **S5** as a white solid (8.8 mg, 48%). ^{1}H NMR (400 MHz, DMF-d7) δ 8.30 (d, J = 7.2 Hz, 1H), 8.17-8.14 (m, 3H), 7.91 (d, J = 7.2 Hz, 1H), 7.59 (m, 1H), 7.49-7.28 (m, 26H), 7.11-7.07 (m, 2H), 6.30 (m, 1H), 5.69 (s, 1H), 5.16-5.02 (m, 6H), 4.89 (m, 1H), 4.74-4.60 (m, 3H), 4.44-4.35 (m, 4H), 4.19-3.54 (m, 14H), 3.31 (m, 1H), 3.08-3.05 (m, 2H), 2.32-2.30 (m, 2H), 2.19 (m, 1H), 1.96-1.75 (m, 7H), 1.61-1.20 (m, 18H); ^{13}C NMR (100 MHz, DMF-d7) δ 174.1, 173.2, 172.7, 170.8, 170.2, 157.1, 139.7, 138.7, 138.5, 137.1, 129.4, 129.1, 129.0, 128.9, 128.7, 128.6, 128.4, 128.3, 128.1, 128.1, 128.0, 127.9, 127.9, 127.7, 126.7, 102.1, 101.3, 98.0, 81.5, 80.1, 78.8, 78.7, 74.5, 73.0, 69.3, 66.7, 66.0, 56.9, 53.9, 53.1, 49.8, 48.8, 41.2, 32.7, 32.6, 29.5, 29.0, 23.6, 22.7, 19.4, 18.1, 17.3, 12.4; HRMS (ESI-QTOF) Anal. Calcd for $C_{75}H_{94}N_8O_{20}Na$ $[M+Na]^+$: 1449.6482, found: 1449.6439.



2-Acetylamino-4-*O*-[[2-acetylamino-4,6-*O*-benzylidene-2-deoxy-3-*O*-[(*R*)-propionyl-{benzyl-(L-alanyl-D-isoglutamyl-ε-*N*-benzyloxycarbonyl-L-lysyl)-D-alaninate}]-β-D-glucopyranosyl]]-3,6-di-*O*-benzyl-2-deoxy-D-glucopyranoside (**S6**)

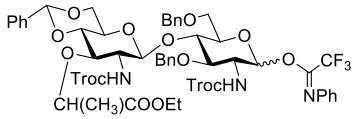
To a solution of **S5** (4.5 mg, 0.004 mmol) in THF (2 mL), iodine (5 mg, 0.02 mmol) and water (0.5 mL) were added and the reaction mixture was stirred for 2 h. The reaction was quenched by the addition of $Na_2S_2O_3$ aq (5%, 10 ml). The mixture was then extracted with $CHCl_3$ (20 mL). The organic layer was washed with $Na_2S_2O_3$ aq (5%, 10 mL × 2), sat. $NaHCO_3$ aq (20 mL × 2), and brine (20 mL), dried over Na_2SO_4, and then concentrated in vacuo. The residue was purified by silica-gel chromatography (5 g, $CHCl_3$: MeOH = 10 : 1) to give 1-liberated **S6** as a white solid (2.8 mg, 64%). HRMS (ESI-QTOF) Anal. Calcd for $C_{72}H_{90}N_8O_{20}Na$ $[M+Na]^+$: 1409.6169, found: 1409.6152.



2-Acetylamino-4-*O*-[2-acetylamino-3-*O*-{(*R*)-propionyl-(L-alanyl-D-isoglutamyl-L-lysiyl-D-alanine)}-2-deoxy-β-D-glucopyranosyl]-2-deoxy-D-glucopyranoside (**1e**)

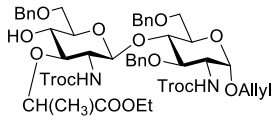
To a solution of **S6** (1.6 mg, 0.001 mmol) in AcOH (1 mL) was added palladium hydroxide (10 mg) in AcOH and stirred under H_2 (2 MPa) for 1 d. The reaction was monitored by TLC analysis and the hydrogenolysis was continued until deprotection was completed. The Pd catalyst was filtered off by celite and the filtrate was concentrated. The residue was lyophilized from acetonitrile-H_2O to give **1e** (0.9 mg, 90%) as a white solid. ^{1}H NMR (500 MHz, D_2O) δ 5.06 (d, J = 3.0 Hz, 1H), 4.44 (d, J

= 8.0 Hz, 1H), 4.20-4.02 (m, 5H), 3.82-3.38 (m, 12H), 2.86 (t, *J* = 7.5 Hz, 2H), 2.31-2.28 (m, 2H), 2.04 (m, 1H), 1.91 (s, 3H), 1.86-1.82 (m, 4H), 1.74-1.62 (m, 4H), 1.32-1.30 (m, 5H), 1.25 (d, *J* = 6.3 Hz, 3H), 1.19 (d, *J* = 7.3 Hz, 3H); HRMS (ESI-QTOF) Anal. Calcd for $C_{36}H_{63}N_8O_{18}$ $[M+H]^+$: 895.4260, found: 895.4213.



3,6-Di-*O*-benzyl-2-deoxy-4-*O*-[4,6-*O*-benzylidene-2-deoxy-3-*O*-{(*R*)-1-(ethoxycarbonyl)ethyl}-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranosyl (*N*-phenyl)trifluoroacetimidate (10b)

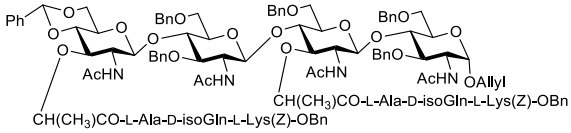
To a solution of **5** (0.87 g, 0.79 mmol), the solution of $[Ir(cod)(MePh_2P)_2]PF_6$ (32 mg, 0.04 mmol) activated with H_2 in dry THF (1 mL) was added. After being stirred at room temperature for 1.5 h, iodine (400 mg, 1.6 mmol) and water (2 mL) were added and the reaction mixture was stirred for additional 30 min. To the reaction mixture was rapidly added $Na_2S_2O_3$ aq (5%, 100 mL). The mixture was then extracted with EtOAc (50 mL). The organic layer was washed with $Na_2S_2O_3$ aq (5%, 50 mL × 2), sat. $NaHCO_3$ aq (100 mL × 2), brine (50 mL), dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica-gel chromatography (80 g, toluene : EtOAc = 5 : 1) to give 1-liberated-disaccharide (677 mg, 81%) as a pale yellow solid. HRMS (ESI-QTOF) Anal. Calcd for $C_{44}H_{50}Cl_6N_2O_{15}K$ $[M+K]^+$: 1095.0979, found: 1095.0933. To a solution of 1-liberated-disaccharide (329 mg, 0.31 mmol) in acetone (10 mL) at 0 °C was added Na_2CO_3 (987 mg, 9.3 mmol) and *N*-phenyl-2,2,2-trifluoroacetimidoyl chloride (0.1 mL, 0.47 mmol). After being stirred for 3 d at rt, insoluble materials were filtered off through celite and the filtrate was concentrated. The residue was purified by silica-gel chromatography (30 g, toluene : EtOAc = 12 : 1) to give **10b** as a pale yellow solid (290 mg, 76%).



Allyl 3,6-di-*O*-benzyl-2-deoxy-4-*O*-[6-*O*-benzyl-2-deoxy-3-*O*-{(*R*)-1-(ethoxycarbonyl)ethyl}-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (11)

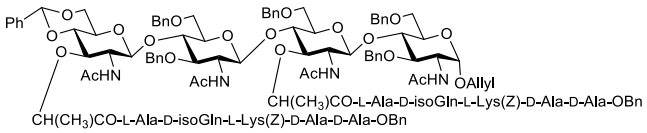
To a solution of **5** (138 mg, 0.125 mmol) and trimethylamine-borane (10 mg, 0.138 mmol) in dry CH_3CN (15 mL) at 0 °C was added boron trifluoride diethyl etherate (53 mg, 0.376 mmol) dropwise and the mixture was stirred at rt for 1 h. The reaction was then quenched with ice and sat. $NaHCO_3$ aq (20 mL) and the mixture was extracted with EtOAc (50 mL × 2). The organic layer was washed with citric acid (1 M, 15 mL × 4), sat. $NaHCO_3$ aq (50 mL), and brine (50 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel chromatography (30 g, toluene : AcOEt = 5 : 1) to give **37** as a colorless solid (115 mg, 83%). ^1H NMR (500

MHz, $CDCl_3$) δ 7.39-7.26 (m, 15H), 5.84 (m, 1H), 5.73 (d, J = 6.5 Hz, 1H), 5.26-5.18 (m, 2H), 4.94-4.87 (m, 4H), 4.76 (d, J = 12.0 Hz, 1H), 4.71-4.61 (m, 5H), 4.43 (d, J = 11.9 Hz, 1H), 4.33 (s, 2H), 4.45-4.18 (m, 3H), 4.09-4.07 (m, 1H), 3.96-3.85 (m, 4H), 3.66 (d, J = 10.0 Hz, 1H), 3.59-3.55 (m, 3H), 3.52-3.50 (m, 3H), 3.26 (m, 1H), 3.15 (m, 2H), 1.38 (d, J = 6.9 Hz, 3H), 1.29 (t, J = 7.2 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.6, 155.3, 154.2, 139.1, 137.9, 137.2, 133.3, 128.6, 128.5, 128.9, 127.9, 127.6, 127.4, 127.0, 118.1, 102.0, 96.6, 95.9, 79.7, 78.0, 75.4, 74.5, 74.3, 74.1, 73.7, 73.4, 71.8, 70.5, 68.4, 67.6, 61.2, 56.0, 54.7, 19.0, 14.2; HRMS (ESI-QTOF) Anal. Calcd for $C_{47}H_{56}Cl_6N_2O_{15}Na$ $[M+Na]^+$: 1121.1710, found: 1121.1663.



Tetrasaccharide tripeptide backbone (15)

To a solution of **13** (17 mg, 0.011 mmol), HCl·L-Ala-D-isoGln-L-Lys(Z)-OBn (26 mg, 0.044 mmol), and HOBt (5 mg, 0.033 mmol) in DMF (3 mL) were added WSCD·HCl (6 mg, 0.037 mmol) and triethylamine (10 μL, 0.04 mmol) at 0 °C and the mixture was stirred at rt overnight. The mixture was concentrated, and the residue was dissolved in $CHCl_3$. The $CHCl_3$ solution was washed with citric acid (1 M, 20 mL), H_2O (20 mL), sat. $NaHCO_3$ aq (20 mL), and brine (20 mL). The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel chromatography (5 g, $CHCl_3$: MeOH = 12 : 1) to give **15** as a white solid (15 mg, 53%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.52-7.26 (m, 50H), 5.83 (m, 1H), 5.45 (s, 1H), 5.15-5.07 (m, 8H), 4.82-3.12 (m, 54H), 2.28-2.17 (m, 4H), 2.04-1.74 (m, 16H), 1.43-1.25 (m, 24H); HRMS (ESI-QTOF) Anal. Calcd for $C_{141}H_{174}N_{14}O_{37}Na_2$ $[M+2Na]^{2+}$: 1350.5980, found: 1350.5985.



Tetrasaccharide pentapeptide backbone (17)

To a solution of **13** (15 mg, 0.006 mmol) and HCl·L-Ala-D-isoGln-L-Lys(Z)-D-Ala-D-Ala-OBn (22 mg, 0.019 mmol) in DMF (3 mL) were added HATU (11 mg, 0.019 mmol) and triethylamine (8 μL, 0.039 mmol) at 0 °C and the mixture was stirred at rt overnight. The mixture was concentrated and the residue was dissolved in $CHCl_3$. The $CHCl_3$ solution was washed with citric acid (1 M, 20 mL), H_2O (20 mL), sat. $NaHCO_3$ aq (20 mL), and brine (20 mL). The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel chromatography (5 g, $CHCl_3$: MeOH = 15 : 1) to give **17** as a white solid (20 mg, 71%). HRMS (ESI-QTOF) Anal. Calcd for $C_{153}H_{194}N_{18}O_{41}Na_2$ $[M+2Na]^{2+}$: 1492.6722, found: 1492.6753.