Supplementary Material

Synthesis of *anti*-inflammatory α-and β-linked acetamidopyranosides as inhibitors of toll-like receptor 4

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General. All glassware was dried in an oven at 140 °C for 2 h prior to use. All air and moisture-sensitive reactions were performed using syring-septum cap techniques under a dry N_2 or Ar atmosphere. 1,4-dioxane was distilled from sodium/benzophenone ketyl and degassed prior to use; aniline was distilled from KOH or CaH and stored over KOH; and toluene was purified by passage through an activated alumina filtration system. DMF was distilled and stored over 4Å molecular sieves. All other materials were obtained from commercial sources and used as received unless otherwise stated.
Reactions were monitored by thin-layer chromatography analysis using pre-coated silica gel 60 F_{254} plates (EMD, 250 μm thickness) and visualization was accomplished with a 254 nm UV light or by staining with a solution of $KMnO_4$ (1.5 g of $KMnO_4$, 10 g of K_2CO_3, and 2.5 mL of 5% aq. NaOH in 150 mL of H_2O). Flash chromatography was performed using SiO_2 (Silicycle, Silia-P Flash Silica Gel, 40-63 μm). Concentrating under reduced pressure refers to the use of a rotary evaporator connected to a membrane vacuum pump to remove solvent.
Melting points were determined using a Laboratory Devices Mel-Temp II in open capillary tubes and are uncorrected. Infrared spectra were determined as neat solids on a Smiths Detection IdentifyIR FT-IR spectrometer. Mass spectra were obtained on a Micromass Autospec double focusing instrument. ^1H and ^{13}C NMR spectra were obtained on a Bruker Avance 300 MHz or 400 MHz in DMSO-d_6 unless otherwise noted. Chemical shifts (δ) were reported in parts per million with the residual solvent peak used as an internal standard δ $^1H/^{13}C$ (Solvent); 2.50/39.52 (DMSO), 7.16/77.16 ($CDCl_3$); and are tabulated as follows: chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, app t = apparent triplet, q = quartet, sept = septet, m = multiplet), number of protons, and coupling constant(s). ^{13}C NMR spectra were obtained at 75 MHz or 100 MHz using a proton-decoupled pulse sequence and are tabulated by observed peak. $CDCl_3$ was filtered through dried basic alumina prior to sample preparation.

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Synthesis Information

***2-Acetamido-1,3,4,6-tetra-O-acetyl-ß-D-Galactopyranoside* (5)**.[1] D-(+)-Galactosamine hydrochloride (2.00 g, 9.28 mmol) was dissolved in anhydrous pyridine (20 mL), and acetic anhydride (10.5 mL, 111.3 mmol, 12 eq) was added. The reaction mixture was stirred at room temperature until disappearance of starting material, and poured in a beaker with ice-cold water (200 mL). A white solid precipitated was collected by vacuum filtration, washed with ice-cold water and co-evaporated with toluene (3×20 mL) to remove residual water to yield **5** (2.96 g, 82%) as a powdery solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.73 (d, *J* = 8.8 Hz, 1 H), 5.46 (d, *J* = 9.5 Hz, 1 H), 5.40 (d, *J* = 2.8 Hz, 1 H), 5.12 (dd, *J* = 3.3, 11.3 Hz, 1 H), 4.52-4.43 (m, 1 H), 4.24-4.11 (m, 2 H), 4.07-4.02 (m, 1 H), 2.20 (s, 3 H), 2.16 (s, 3 H), 2.08 (s, 3 H), 2.05 (s, 3 H), 1.97 (s, 3 H).
***2,3-Dihydrooxazole-3,4,6-tri-O-acetyl-α-D-galactopyranoside* (6)**.[2] A solution of **5** (400 mg, 1.03 mmol) in dichloroethane (28.6 mL) was treated with TMSOTf (0.200 mL, 1.08 mmol) at room temperature, heated at 50 °C for 1 h, cooled, and treated with NEt_3 (0.440 mL, 3.08 mmol). The mixture was stirred at room temperature for 10 min, passed through a short plug of SiO_2 and washed with ethyl acetate (25 mL) and dichloromethane (30 mL). The solvent was evaporated under reduced pressure and the crude oil was purified by chromatography on SiO_2 (100% EtOAc, SiO_2 was base-washed with 1% NEt_3 prior to use) to yield **5** as a clear slightly orange oil (304 mg, 0.923 mmol, 90%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.98 (d, *J* = 6.8 Hz, 1 H), 5.45 (d, *J* = 2.8 Hz, 1 H), 4.90 (dd, *J* = 3.2, 7.2 Hz, 1 H), 4.26-4.16 (m, 2 H), 4.10 (dd, *J* = 5.6, 11.2 Hz, 1 H), 3.98 (td, *J* = 1.2, 7.6 Hz, 1 H), 2.11 (s, 3 H), 2.06 (s, 6 H), 2.04 (d, *J* = 1.2 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 170.2, 169.8, 166.4, 101.5, 71.8, 69.5, 65.3, 63.6, 61.6, 20.8, 20.7, 20.6, 14.4; HRMS (ESI) *m/z* calcd for $C_{14}H_{20}NO_8$ $[M+H]^+$ 330.1189, found 330.1187.
Isopropyl *3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-ß-D-galactopyranoside* (7). A solution of **6** (0.133 g, 0.404 mmol) and anhydrous $CuCl_2$ in anhydrous $CHCl_3$ (0.76 mL) in a 2-5 mL conical sealed vessel under an atmosphere of Ar was treated with anhydrous 2-propanol (0.130 mL, 1.72 mmol). The reaction mixture was heated at 62 °C for 2 h, cooled to room temperature, diluted with acetone (15 mL) and sat. $NaHCO_3$ solution (7 mL) filtered through a short plug of Celite®, and concentrated. The residue was co-evaporated with toluene to remove residual water and shaken in $CHCl_3$ and weakly acidic ion-exchange resin (Amberlite IRC-86, ca 1.5 g). The solution was filtered, the solvent was removed under vacuum, and the residue was purified by chromatography on SiO_2 (75% EtOAc/hexanes) to give **7** (119 mg, 0.305 mmol, 75%) as a colorless solid: $[\alpha]_D$ -14.4 (*c* 1.0, CH_2Cl_2); Mp 188.5-189.5 °C; IR (ATR) 3264, 2980, 1735, 1648, 1568, 1380, 1256, 1232, 1215, 1124, 1072, 1053, 1023 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.57 (d, *J* = 7.8 Hz, 1 H), 5.42 (dd, *J* = 3.0, 11.1 Hz, 1 H), 5.35 (app d, *J* = 3.6 Hz, 1 H), 4.86 (d, *J* = 8.4 Hz, 1 H), 4.16 (dd, *J* = 6.6, 11.4 Hz, 1 H), 4.10 (dd, *J* = 7.2, 11.4 Hz, 1 H), 3.95-3.90 (m, 2 H), 3.77-3.73 (m, 1 H), 2.12 (s, 3 H), 2.03 (s, 3 H), 1.99 (s, 3 H), 1.94 (s, 3 H), 1.23 (d, *J* = 6.6 Hz, 3 H), 1.13 (d, *J* = 6.6 Hz, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.5,

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170.3, 99.4, 72.8, 70.4, 69.6, 66.8, 61.5, 52.4, 23.5, 23.3, 22.0, 20.7 (2 C); HRMS (ESI) *m/z* calcd for $C_{17}H_{28}NO_9$ $[M+H]^+$ 390.1764, found 390.1774.

***Cyclohexyl 3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-galactopyranoside* (8)**. A solution of **6** (0.135 g, 0.411 mmol) and anhydrous $CuCl_2$ (55.0 mg, 0.411 mmol) in anhydrous $CHCl_3$ (0.68 mL) in a 2-5 mL conical sealed vessel under an atmosphere of Ar was treated with cyclohexanol (0.170 mL, 1.66 mmol). The reaction mixture was heated at 62 °C for 2 h, cooled to room temperature, diluted with ethyl acetate (15 mL), washed with 1 N HCl (2 × 9 mL), sat. $NaHCO_3$ solution (1 × 10 mL), and brine (1 × 10 mL), dried ($MgSO_4$), evaporated, and purified by chromatography on SiO_2 (75% EtOAc/hexanes) to give **8** (141 mg, 0.328 mmol, 80%) as a colorless solid: $[\alpha]_D$ -14.6 (*c* 1.0, CH_2Cl_2); Mp 164.3-165.3 °C; IR (ATR) 3331, 2936, 2857, 1735, 1661, 1541, 1364, 1251, 1219, 1079, 1031, 984 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.48 (d, *J* = 8.5 Hz, 1 H), 5.41 (dd, *J* = 3.5, 11.5 Hz, 1 H), 5.35 (app. d, *J* = 3.5 Hz, 1 H), 4.89 (d, *J* = 8.5 Hz, 1 H), 4.17 (dd, *J* = 6.5, 11.5 Hz, 1 H), 4.10 (dd, *J* = 7.0, 11.0 Hz, 1 H), 3.91 (app t, *J* = 10.5 Hz, 1 H), 3.80-3.74 (m, 1 H), 3.64-3.59 (m, 1 H), 2.13 (s, 3 H), 2.03 (s, 3 H), 1.99 (s, 3 H), 1.94 (s, 3 H), 1.93-1.17 (m, 10 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4, 170.3 (3 C), 99.1, 78.0, 70.4, 69.6, 66.8, 61.4, 52.5, 33.3, 31.7, 25.5, 23.9, 23.8, 23.5, 20.7 (2 C); HRMS (ESI) *m/z* calcd for $C_{20}H_{32}NO_9$ $[M+H]^+$ 430.2077, found 430.2084.

***Geranyl 3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-galactopyranoside* (9)**. A solution of **6** (0.120 g, 0.364 mmol) and anhydrous $CuCl_2$ (49.0 mg, 0.364 mmol) in anhydrous $CHCl_3$ (0.84 mL) in a 2-5 mL conical sealed vessel under an atmosphere of Ar was treated with geraniol (0.270 mL, 1.47 mmol). The reaction mixture was heated at 62 °C for 2 h, cooled to room temperature, diluted with ethyl acetate (15 mL), washed with 1 N HCl (2 × 9 mL), sat. $NaHCO_3$ solution (1 × 10 mL), and brine (1 × 10 mL), dried ($MgSO_4$), evaporated, and purified by chromatography on SiO_2 (75% EtOAc/hexanes) to give **9** (149 mg, 0.308 mmol, 65%) as a colorless solid: $[\alpha]_D$ -17.0 (*c* 1.0, CH_2Cl_2); Mp 117.5-118.9 °C; IR (ATR) 3279, 2982, 2939, 1737, 1659, 1555, 1536, 1431, 1374, 1241, 1226, 1131, 1064, 1053, 1036, 1012, 997, 958 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.41 (d, *J* = 8.5 Hz, 1 H), 5.36-5.27 (m, 3 H), 5.09-5.06 (m, 1 H), 4.75 (d, *J* = 8.5 Hz, 1 H), 4.29 (dd, *J* = 6.5, 12.0 Hz, 1 H), 4.23-4.11 (m, 3 H), 4.94-3.88 (m, 2 H), 2.13 (s, 3 H), 2.12-2.00 (m, 4 H), 2.03 (s, 3 H), 1.99 (s, 3 H), 1.94 (s, 3 H), 1.68 (s, 3 H), 1.65 (s, 3 H), 1.60 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4 (2 C), 170.3, 170.2, 142.1, 131.8, 123.8, 119.3, 99.0, 70.6, 69.9, 66.9, 65.2, 61.6, 51.8, 39.6, 26.3, 25.7, 23.5, 20.7 (2 C), 17.7, 16.3; HRMS (ESI) *m/z* calcd for $C_{24}H_{37}NO_9Na$ $[M+H]^+$ 506.2366, found 506.2384.

***2-Acetamido-2-deoxy-1,3,4,6-tetra-O-acetyl-β-D-glucopyranose* (11)**.[3] Acetic anhydride (7.76 mL, 82.3 mmol) was cooled to 0 °C and treated sequentially over 15 min with *N*-acetyl-D-glucosamine **10** (0.692 g, 3.13 mmol) and montmorillonite K-10 (2.40 g). The reaction mixture was stirred at room temperature for 24 h, filtered through a pad of Celite®, and rinsed with methyl acetate (100 mL). The filtrate was concentrated and the resulting orange residue was recrystallized twice from hot methanol, filtered, and the crystals were washed with ice-cold diethyl ether (3 × 2 mL) to afford **11** as a colorless crystalline solid (350 mg, 0.900 mmol, 29%): ^1H NMR (400 MHz, $CDCl_3$) δ 5.69 (d, *J* = 8.8 Hz, 1 H), 5.41 (d, *J* = 9.6 Hz, 1 H), 5.17-5.09 (m, 2 H), 4.33-4.25 (m, 2 H), 4.13 (dd,

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J = 2.4, 12.6 Hz, 1 H), 3.78 (ddd, *J* = 2.4, 4.4, 9.6 Hz, 1 H), 2.12 (s, 3 H), 2.09 (s, 3 H), 2.05 (s, 3 H), 2.04 (s, 3 H).

***2,3-Dihydrooxazole-3,4,6-tri-O-acetyl-α-D-glucopyranoside* (12)**.[4] A solution of **11** (200 mg, 0.514 mmol) in 1,2-dichloroethane (14.3 mL) in a 50-mL round bottom flask was treated with TMSOTf (0.100 mL, 0.539 mmol). The reaction mixture was stirred at 50 °C for 35 min, cooled, and NEt_3 (0.220 mL, 1.54 mmol) was added. After stirring at room temperature for 10 min, the solution was passed through a short plug of SiO_2 and washed with dichloromethane (25 mL) and ethyl acetate (15 mL). The solvents were removed under reduced pressure and the crude orange oil was purified by chromatography on SiO_2 (100% EtOAc, SiO_2 was base-washed with 1% NEt_3) to give **12** (158 mg, 0.478 mmol, 93%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.97 (d, *J* = 7.6 Hz, 1 H), 5.27 (t, *J* = 2.4 Hz, 1 H), 4.93 (app. d, *J* = 9.2 Hz, 1 H), 4.18-4.13 (m, 3 H), 3.61 (quint., *J* = 4.4 Hz, 1 H), 2.12 (s, 3 H), 2.10 (s, 3 H), 2.09 (d, *J* = 1.6 Hz, 3 H), 2.08 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 169.5, 169.2, 166.7, 99.4, 70.4, 68.4, 67.5, 65.0, 63.3, 20.9, 20.8, 20.7, 14.0.

***Isopropyl 3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranoside* (13)**.[5] A solution of **12** (0.142 g, 0.424 mmol) and anhydrous $CuCl_2$ (58.0 mg, 0.431 mmol) in anhydrous $CHCl_3$ (0.82 mL) in a 2-5 mL conical sealed vessel under an atmosphere of Ar was treated with anhydrous 2-propanol (0.134 mL, 1.75 mmol). The reaction mixture was heated at 62 °C for 2 h. After cooling to room temperature, the mixture was diluted with acetone (15 mL) and sat. $NaHCO_3$ solution (7 mL), and filtered through a short plug of Celite® with acetone (20 mL). The filtrate was concentrated, co-evaporated with toluene and the crude residue was shaken with $CHCl_3$ and weakly acidic ion-exchange resin (Amberlite IRC-86, ca 1.5 g). The solution was filtered, the solvent was removed under vacuum, and the residue was purified by chromatography on SiO_2 (75% EtOAc/hexanes) to give **13** (146 mg, 0.375 mmol, 87%) as a colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.51 (d, *J* = 8.4 Hz, 1 H), 5.41 (dd, *J* = 9.2, 10.4 Hz, 1 H), 5.03, (app. t, *J* = 10.0 Hz, 1 H), 4.84 (d, *J* = 8.4 Hz, 1 H), 4.24 (dd, *J* = 5.2, 12.0 Hz, 1 H), 4.11 (dd, *J* = 2.4, 12.0 Hz, 1 H), 3.93 (sept, *J* = 6 Hz, 1 H), 3.73-3.60 (m, 2 H), 2.07 (s, 3 H), 2.02 (s, 3 H), 2.02 (s, 3 H), 1.94 (s, 3 H), 1.22 (d, *J* = 6.0 Hz, 3 H), 1.13 (d, *J* = 6.4 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 170.7, 170.2, 169.5, 99.1, 72.6, 72.1, 71.5, 68.9, 62.3, 55.5, 23.3, 23.2, 21.9, 20.8, 20.7, 20.6; HRMS (+ESI-TOF) calcd for $C_{17}H_{28}NO_9$ $[M+H]^+$ 390.1764, found 390.1742.

***Cyclohexyl 3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranoside* (14)**.[5,6] A solution of **12** (0.099 g, 0.301 mmol) and anhydrous $CuCl_2$ (41.0 mg, 0.301 mmol) in anhydrous $CHCl_3$ (0.68 mL) in a 2-5 mL conical sealed vessel under an atmosphere of Ar was treated with cyclohexanol (0.130 mL, 1.22 mmol). The reaction mixture was heated at 62 °C for 2.5 h, cooled to room temperature, diluted with ethyl acetate (15 mL), washed with 1 N HCl (2 × 9 mL), sat. $NaHCO_3$ solution (1 × 10 mL), and brine (1 × 10

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[6] Iglesias-Guerra, F.; Romero, I.; Alcudia, F.; Vega-Pérez, J. M. *Carbohydr. Res.* **1998**, *308* (1–2), 57-62.

mL), dried ($MgSO_4$), evaporated, and purified by chromatography on SiO_2 (75% EtOAc/hexanes) to give **14** (112 mg, 0.260 mmol, 86%) as a colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.53 (d, *J* = 8.4 Hz, 1 H), 5.40 (app. t, *J* = 8.9 Hz, 1 H), 5.04 (t, *J* = 9.6 Hz, 1 H), 4.86 (d, *J* = 8.4 Hz, 1 H), 4.26 (dd, *J* = 4.8 , 12.0 Hz, 1 H), 4.10 (dd, *J* = 2.4, 12.0 Hz, 1 H), 3.72-3.58 (m, 3 H), 2.07 (s, 3 H), 2.02 (s, 3 H), 2.01 (s, 3 H), 1.93 (s, 3 H), 1.92-1.18 (m, 10 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 170.7, 170.1, 169.5, 98.9, 77.7, 72.2, 71.6, 69.0, 62.3, 55.6, 33.3, 31.7, 25.5, 23.8, 23.7, 23.4, 20.8, 20.7 (2 C); HRMS (ESI) *m/z* calcd for $C_{20}H_{31}NO_9Na$ $[M+Na]^+$ 452.1897, found 452.1898.

***Geranyl 3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranoside* (15)**. A solution of **12** (0.120 g, 0.364 mmol) and anhydrous $CuCl_2$ (49.0 mg, 0.364 mmol) in anhydrous $CHCl_3$ (0.84 mL) in a 2-5 mL conical sealed vessel under an atmosphere of Ar was treated with geraniol (0.270 mL, 1.47 mmol). The reaction mixture was heated at 62 °C for 2 h, cooled to room temperature, diluted with ethyl acetate (15 mL), washed with 1 N HCl (2 × 9 mL), sat. $NaHCO_3$ solution (1 × 10 mL), and brine (1 × 10 mL), dried ($MgSO_4$), evaporated, and purified by chromatography on SiO_2 (75% EtOAc/hexanes) to give **15** (120 mg, 0.248 mmol, 68%) as a colorless solid: $[\alpha]_D$ -20.9 (*c* 1.0, CH_2Cl_2); Mp 104.8-105.8 °C; IR (ATR) 3282, 2930, 1743, 1735, 1650, 1569, 1431, 1368, 1223, 1128, 1077, 1029, 975 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.40 (d, *J* = 8.4 Hz, 1 H), 5.30-5.26 (m, 2 H), 5.08-4.98 (m, 2 H), 4.70 (d, *J* = 8.4 Hz, 1 H), 4.32-4.12 (m, 4 H), 3.82 (app. q, *J* = 8.4 Hz, 1 H), 3.69-3.63 (m, 1 H), 2.11-2.02 (m, 4 H), 2.07 (s, 3 H), 2.02 (s, 3 H), 2.02 (s, 3 H), 1.94 (s, 3 H), 1.69 (s, 3 H), 1.66 (s, 3 H), 1.60 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.9, 170.7, 170.1, 169.4, 142.2, 131.8, 123.8, 119.2, 98.8, 72.5, 71.9, 68.7, 65.1, 62.3, 54.9, 39.6, 26.3, 25.7, 23.4, 20.7 (2 C), 20.6, 17.7, 16.3; HRMS (ESI) *m/z* calcd for $C_{24}H_{37}NO_9Na$ $[M+Na]^+$ 506.2366, found 506.2360.

***Isopropyl 3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-α-D-glucopyranoside* (16**, **C34)**.[7] A solution of **11** (50.0 mg, 0.128 mmol) in a 5% HCl solution in 2-propanol (3.8 mL) was stirred at 65 °C for 1 h. The reaction mixture was evaporated to dryness, and the residue was dissolved in pyridine (0.48 mL) and treated with acetic anhydride (0.146 mL, 1.54 mmol). This mixture was stirred at room temperature for 2 d and then co-evaporated with toluene to give a brown oil which was a 3:1 mixture of α:β-isomers by NMR analysis. Purification by chromatography on SiO_2 (75% EtOAc/hexanes) gave **16** (30.4 mg, 0.0781 mmol, 61%) as a foaming colorless solid: IR (ATR) 3359, 2975, 1750, 1730, 1676, 1534, 1437, 1376, 1364, 1243, 1225, 1150, 1122, 1038, 1031, 997 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.62 (d, *J* = 9.6 Hz, 1 H), 5.19 (app t, *J* = 9.6 Hz, 1 H), 5.10 (app t, *J* = 9.6 Hz, 1 H), 4.92 (d, *J* = 3.8 Hz, 1 H), 4.30 (td, *J* = 3.6, 9.6 Hz, 1 H), 4.22 (dd, *J* = 4.8, 12.4 Hz, 1 H), 4.08 (dd, *J* = 2.4, 12.4 Hz, 1 H), 4.03-3.99 (m, 1 H), 3.88 (sept, *J* = 6.0 Hz, 1 H), 2.07 (s, 3 H), 2.02 (s, 3 H), 2.01 (s, 3 H), 1.93 (s, 3 H), 1.23 (d, *J* = 6.4 Hz, 3 H), 1.14 (d, *J* = 6.4 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 170.7, 169.8, 169.3, 95.7, 71.4, 71.0, 68.2, 67.7, 62.0, 51.8, 23.2, 23.1, 21.6, 20.7 (2 C), 20.6; HRMS (ESI) *m/z* calcd for $C_{17}H_{28}NO_9$ $[M+H]^+$ 390.1764, found 390.1790.

***Isopropyl 3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-α-D-galactopyranoside* (17)**.[7] A solution of **5** (50.0 mg, 0.128 mmol) in a 5% HCl solution in 2-propanol (3.8 mL) was stirred at 65 °C for 1 h. The reaction mixture was evaporated to dryness, and the residue was dissolved in pyridine (0.50 mL) and treated with acetic anhydride (0.146 mL, 1.54

[7] Lemieux, R. U.; James, K.; Nagabhushan, T. L.; Ito, Y. *Can. J. Chem.* **1973**, *51*, 33-41.

mmol). This mixture was stirred at room temperature for 2 d and then co-evaporated with toluene to give a brown oil that was a 3:1 mixture of α:β-isomers by NMR analysis. Purification by chromatography on SiO_2 (75% EtOAc/hexanes) gave **17** (28.6 mg, 0.0737 mmol, 57%) as a foaming colorless solid: IR (ATR) 3310, 2973, 2930, 1745, 1732, 1653, 1534, 1372, 1284, 1240, 1217, 1124, 1083, 1034, 992, 936, 878 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.54 (d, *J* = 9.6 Hz, 1 H), 5.35 (app. d, *J* = 2.8 Hz, 1 H), 5.13 (dd, *J* = 3.2, 11.2 Hz, 1 H), 4.95 (d, *J* = 4.0 Hz, 1 H), 4.56-4.50 (m, 1 H), 4.23 (app. t, *J* = 6.8 Hz, 1 H), 4.12-4.02 (m, 2 H), 3.88 (sept, *J* = 6.0 Hz, 1 H), 2.15 (s, 3 H), 2.03 (s, 3 H), 1.98 (s, 3 H), 1.95 (s, 3 H), 1.22 (d, *J* = 6.0 Hz, 3 H), 1.13 (d, *J* = 6.4 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 170.4 (2 C), 169.9, 96.3, 71.0, 68.5, 67.5, 66.7, 62.0, 47.8, 23.3, 23.1, 21.7, 20.8, 20.7, 20.6; HRMS (ESI) *m/z* calcd for $C_{17}H_{28}NO_9$ $[M+H]^+$ 390.1764, found 390.1776.

***Cyclohexyl 3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-α-D-glucopyranoside* (18)**.[8] A solution of **11** (50.0 mg, 0.128 mmol) in a 5% HCl solution in cyclohexanol (3.1 mL) was stirred at 65 °C for 1 h. The reaction mixture was evaporated to dryness, and the residue was dissolved in pyridine (0.50 mL, anhydrous) and treated with acetic anhydride (0.146 mL, 1.54 mmol). This mixture was stirred at room temperature for 31 h and co-evaporated with toluene to give a brown oil that was a 3:1 mixture of α:β-isomers by NMR analysis. Purification by chromatography on SiO_2 (75% EtOAc/hexanes) gave **18** (31.7 mg, 0.0738 mmol, 57%) as a foaming colorless solid: ^1H-NMR (400 MHz, $CDCl_3$) δ 5.63 (d, *J* = 9.6 Hz, 1 H), 5.19 (app. t, *J* = 10.2 Hz, 1 H), 5.08 (app. t, *J* = 10.8 Hz, 1 H), 4.96 (d, *J* = 3.6 Hz, 1 H), 4.32-4.26 (m, 1 H), 4.21 (dd, *J* = 4.8, 12.4 Hz, 1 H), 4.08 (dd, *J* = 2.4, 12.4 Hz, 1 H), 3.54 (sept, *J* = 4.0 Hz, 1 H), 2.07 (s, 3 H), 2.01 (s, 3 H), 2.00 (s, 3 H), 1.93 (s, 3 H), 1.91-1.20 (m, 10 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 170.7, 169.8, 169.3, 95.6, 71.4, 68.2, 67.7, 62.0, 51.9, 33.3, 31.5, 25.4, 24.1, 23.8, 23.2, 20.7 (2 C), 20.6; HRMS (ESI) *m/z* calcd for $C_{20}H_{32}NO_9$ ($[M+H]^+$ 430.2077, found 430.2086.

Assay Information

The small intestinal cell line rat-intestinal epithelial cell (IEC)-6 and mouse-RAW 264.7 macrophages were obtained from American Type Culture Collection (ATCC, Manassas, VA) and were cultured as recommended by ATCC. Nfkb-reporter mice (BALB/c-Tg(Rela-luc)31Xen) were purchased from Taconic and used for experiments in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Lipopolysaccharide (LPS) (Escherichia coli 0111:B4 purified by gel filtration chromatography >99% pure) was obtained from Sigma-Aldrich (St. Louis, MO). For *in vitro* experiments, both IEC6 and Raw 264.7 cells were plated overnight in 12-well plates (BD Biosciences) in growth media and pre-treated with compounds (5μg/mL, 30 min) before 6-hrs treatment with LPS (25 μg/mL for IEC6 cells, 10 ng/mg for Raw 264.7 cells). For *in vivo* experiments, Nfkb-reporter mice were pre-treated with compounds (2.5 mg/kg, 30 min) before 6-hrs treatment with LPS (2.5 mg/kg).

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[8] (a) Sauer, G.; Matsui, M.; Bloch, R.; Liang, J. S.; Fukushima, D. K. *J. Org. Chem.* **1969**, *34*, 3525-30. (b) Kadokawa, J.-i.; Nagaoka, T.; Ebana, J.; Tagaya, H.; Chiba, K. *Carbohydr. Res.* **2000**, *327*, 341-44.

Dosages of compounds and LPS were chosen based on preliminary experiments carried out to evaluate optimal time and dosages. Cells were harvested at the end of treatments for total RNA isolation using RNeasy mini kit (Qiagen) and thoroughly checked for concentration and purity by measuring the OD260 absorbance and OD260/280 ratio (BioTek Epoch Micro-Volume Spectrophotometer System) as well as by agarose gel electrophoresis. A 0.5 μg of total RNA were reverse transcribed using QuantiTect Reverse Transcription Kit (Qiagen) for cDNA synthesis and qRT-PCR assay. Gene specific amplification of transcripts was performed by quantitative PCR using IQ SYBR GRN SUPERMX and CFX96 real-time system (Bio-Rad, Hercules, CA). The primer sequences used for quantification of transcripts using delta delta CT method were: mouse/rat IL6: Fwd 5'-GGCTAAGGACCAAGACCATCCAA-3', Rev 5'-TCTGACCACAGTGAGGAATGTCCA (amplicon size =138bp); mouse IL1β: Fwd 5'-AGTGTGGATCCCAAGCAATACCCA-3', Rev 5'-TGTCCTGACCACTGTTGTTTCCCA-3' (amplicon size 175b); rat IL1β: Fwd 5'-TAGGAAACAGCAATGGTCGGGACA-3', Rev 5'-AGACCTGACTTGGCAGAGGACAAA-3' (amplicon size=167bp); mouse/rat TNFα: Fwd 5'- CATCTTCTCAAAATTCGAGTGACAA-3', Rev 5'-TGGGAGTAGACAAGGTACAACCC-3' (amplicon size=175bp); mouse/rat house keeping gene RPLO: Fwd 5'-GGCGACCTGGAAGTCCAACT-3', Rev 5'-CCATCAGCACCACAGCCTTC-3' (amplicon size = 143bp).