Supporting Information

Enhancing the Divergent Activities of Betulinic Acid via Neoglycosylation

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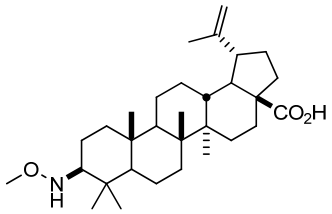
Contents

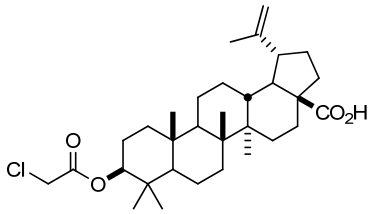
Materials and General Methods. Mass spectrometric data were obtained on either a Waters AutoSpec (Beverly, MA) spectrometer for electron ionization (EI; 70 eV) or a Waters LCT time-of-flight spectrometer for electrospray ionization (ESI). NMR spectra were obtained on a Varian ^{Unity}Inova 400 or 500 MHz instrument (Palo Alto, CA) using 99.8% $CDCl_3$ with 0.05% v/v TMS or 99.8% CD_3OD, 99.9% acetone-d_6, or 99.5% pyridine-d_5 in ampoules. ^{1}H and ^{13}C chemical shifts were referenced to nondeuterated solvent or TMS (where included). Multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). Chemical shift assignments for anomeric mixtures, where possible, are noted as α or β with the atom responsible for the shift. ^{1}H NMR characterization was supplemented with gCOSY for all neoglycoside library members as well as ^{13}C and gHSQC for pilot reactions. Tetrahydrofuran was dried using a column of activated alumina. All other solvents were used as provided by the

supplier. Reagents were obtained from Aldrich or Sigma and were used as received. Flash chromatography was performed using 40-63 μm particle sized silica gel. Thin layer chromatography was performed on aluminum-backed, 254 nm UV-active plates with a silica gel particle size of 60 μm.



5

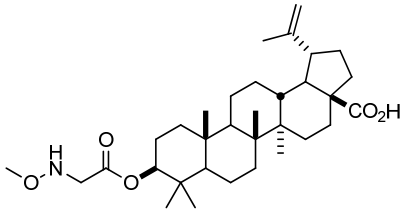
(3*S*)-*N*-Methoxyaminobetulinic acid (5). 3-*N*-Methoxyiminobetulinic acid (**4**, 660 mg, 1.36 mmol), which was prepared as described,[S1] was dissolved in a 1:2 solution of MeOH:*p*-dioxane (9 mL) and cooled to 0 °C. The reducing agent BH_3•*t*$BuNH_2$ was added followed by dropwise addition of 10% aqueous HCl (4 mL) over 5 m. After 1 h, the reaction was quenched with solid Na_2CO_3 (150 mg) and allowed to warm to room temperature. CH_2Cl_2 (30 mL) was added and the reaction mixture was washed with saturated aqueous $NaHCO_3$ (5 mL) and dried over Na_2SO_4. After solvent removal, the diastereomers were separated by column chromatography (SiO_2, EtOAc:Hex 1:5), yielding both as white solids (3*S*: 300 mg, 45%, R_f = 0.39 EtOAc:Hex 1:4; 3*R*: 114 mg, 17%, R_f = 0.47 EtOAc:Hex 1:4). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.74 (d, *J* = 1.6 Hz, 1 H), 4.62 (s, 1 H), 3.51 (s, 3 H), 3.01 (td, *J* = 10.7, 4.7 Hz, 1 H), 2.47 (dd, *J* = 11.7, 4.1 Hz, 1 H), 2.27 (dt, *J* = 12.6, 3.1 Hz, 1 H), 2.19 (td, *J* = 12.6, 3.5 Hz, 1 H), 2.04-1.85 (m, 2 H), 1.74-1.58 (m, 7 H), 1.55-1.32 (m, 6 H), 1.31-1.14 (m, 9 H), 1.11-1.04 (m, 2 H), 0.98 (s, 3 H), 0.93 (s, 3 H), 0.90-0.84 (m, 1 H), 0.82 (s, 3 H), 0.76-0.70 (m, 1 H), 0.67 (s, 3 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 182.56, 150.65, 109.89, 68.30, 61.86, 56.90, 56.64, 50.75, 49.49, 47.16, 42.64, 40.90, 38.90, 38.66, 37.50, 37.30, 36.92, 34.52, 32.41, 30.81, 29.91, 28.70, 25.73, 23.79, 20.99, 19.59, 18.31, 16.70, 16.29, 16.12, 14.91; HRMS (ESI) *m/z* for $C_{31}H_{50}NO_3$ ($[M-H]^-$) 484.3792, calc. 484.3791.



6

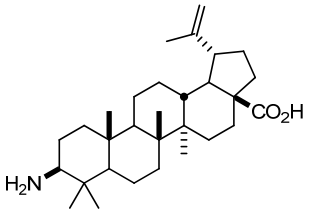
(**3*S*)-*O*-Chloroacetylbetulinic acid (6)**. Betulinic acid (**1**, 335 mg, 0.734 mmol) and DMAP (9 mg, 0.07 mmol) were dissolved in anhydrous THF (20 mL) under Ar. Diisopropylethylamine (190 μL, 1.09 mmol) was added followed by dropwise addition of chloroacetyl chloride (120 μL, 1.51 mmol), soon after which the reaction became cloudy. After stirring for 2 h, absolute ethanol (500 μL) was used to quench the reaction. The solvent was removed in vacuo and the resulting crude solid was adsorbed onto silica gel, after dissolving in CH_2Cl_2 (5 mL), then purified by column chromatography (SiO_2, EtOAc:Hex 1:5) to give the desired chloroacetate as a white solid (338 mg, 86%, R_f = 0.57 EtOAc:Hex 1:4). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.74 (d, *J* = 1.9 Hz, 1 H), 4.62 (t, *J* = 1.3 Hz, 1 H), 4.57 (m, 1 H), 4.05 (d, *J* = 2.4 Hz, 2 H), 3.01 (td, *J* = 10.9, 4.7 Hz, 1 H), 2.28 (dt, *J* = 12.5, 3.1, 1 H), 2.19 (td, *J* = 12.6, 3.3 Hz, 1 H), 2.03-1.94 (m, 2 H), 1.74-1.58 (m, 10 H), 1.55-1.47 (m, 3 H), 1.46-1.34 (m, 9 H), 1.33-1.25 (m, 2 H), 1.21-1.16 (m, 1 H), 0.98 (s, 3 H), 0.94 (s, 3 H), 0.87 (s, 3 h), 0.85 (s, 3 H), 0.82-0.78 (m, 1 H); ^{13}C NMR

($CDCl_3$, 100 MHz) δ 182.10, 167.36, 150.56, 109.98, 83.58, 56.60, 55.59, 50.60, 49.50, 47.16, 42.66, 41.47, 40.92, 38.62, 38.53, 38.24, 37.33, 34.42, 32.36, 30.77, 29.90, 28.15, 27.14, 25.63, 21.08, 19.56, 18.33, 16.62, 16.39, 16.25, 14.89; HRMS (ESI) ***m/z*** for $C_{32}H_{49}ClNaO_4$ ($[M+Na]^+$) 555.3209, calc. 555.3217.



7

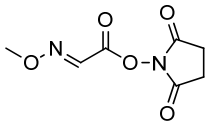
(**3*S*)-*O*-(*N*-Methoxyglycyl)betulinic acid (7)**. *Procedure A (<500 mg batches):* Chloroacetate **6** (177 mg, 0.333 mmol) was dissolved in absolute ethanol (16 mL) along with NaI (160 mg, 1.07 mmol) under Ar. After stirring at room temperature for 40 min, a solution of $MeONH_2$ in THF (1.7 *M*, 2 mL, 3.4 mmol; made by mixing $MeONH_3Cl$ in a NaOH/THF slurry for 16 h) was added, the inert gas line removed, and the reaction heated to 60 °C. After 14 h, and again at 16 h, another equivalent of $MeONH_2$ reagent was added. At 19 h total, the solvent was removed in vacuo and the crude solid was purified by column chromatography (SiO_2, EtOAc:Hex 1:3) to give the desired aglycon as a white sticky solid (120 mg, 67%, R_f = 0.26 EtOAc:Hex 1:3). *Procedure B (≥500 mg batches):* Chloroacetate **4** (1.20 g, 2.25 mmol) was dissolved in absolute ethanol (100 mL) along with NaI (1.01 g, 6.75 mmol) under Ar. After stirring at room temperature for 2 h, a solution of $MeONH_2$ in THF (2.4 *M*, 1.9 mL, 4.56 mmol) was added, the inert gas line removed, and the reaction heated to 60 °C. Two hours after base addition, the reaction was cooled to room temperature and another aliquot of $MeONH_2$ in THF (2 eq.) was introduced followed by reheating to 60 °C. This additive process was repeated roughly every 2 h until the reaction had progressed sufficiently (based upon TLC, EtOAc:Hex 1:3) which occurred after ~24 h of total reaction time. The solvent was removed and the product purified as described above (610 mg, 50%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.71 (s, 1 H), 4.58 (s, 1 H), 4.55 (m, 1 H), 3.60 (d, *J* = 4.4 Hz, 2 H), 3.51 (s, 3 H), 2.98 (td, *J* = 10.5, 4.4 Hz, 1 H), 2.25 (d, *J* = 2.7 Hz, 1 H), 2.16 (td, *J* = 12.5, 3.2 Hz, 1 H), 2.00-1.89 (m, 2 H), 1.72-1.53 (m, 11 H), 1.52-1.45 (m, 2 H), 1.44-1.32 (m, 6 H), 1.25-1.21 (m, 1 H), 1.18-1.12 (m, 1 H), 1.04-0.99 (m, 1 H), 0.95 (s, 3 H), 0.91 (s, 3 H), 0.83 (s, 6 H), 0.81 (s, 3 H), 0.80-0.75 (m, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.31, 170.97, 150.50, 109.78, 82.17, 61.52, 56.43, 55.47, 53.14, 50.48, 49.32, 46.99, 42.50, 40.78, 38.43, 37.96, 37.18, 37.14, 34.30, 32.26, 30.65, 29.78, 27.99, 25.52, 23.80, 20.95, 19.42, 18.23, 16.57, 16.25, 16.05, 14.74; HRMS (ESI) ***m/z*** for $C_{33}H_{53}NaNO_4$ ($[M+Na]^+$) 566.3820, calc. 566.3821.



3

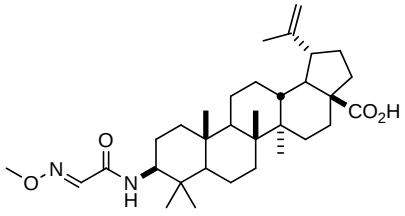
(3*S*)-Aminobetulinic acid (3). Betulonic acid[S1] (650 mg, 1.43 mmol) was dissolved in methanol (25 mL) with strong agitation. Ammonium acetate (1.11 g, 14.4 mmol) and $NaCNBH_3$ (61 mg, 0.97 mmol) were then combined to the reaction vessel. After 8 h, the clear reaction solution turned to a cloudy mixture, which remained until 40 h when the reaction was quenched with conc. HCl to a pH of 2. The methanol was removed in vacuo and the aqueous remainder

diluted with 25 mL of deionized water. The mixture was extracted with Et_2O (20 mL), resulting in an emulsion that required separation by centrifugation (4000 rpm, 4 m). After removal of the organic layer, the process of Et_2O extraction and centrifugation was repeated twice more. The pH of the combined aqueous layer and resulting solid mass was adjusted to 10 with KOH flakes, inducing the appearance of more white precipitate. Further centrifugation separated the desired product as a solid mass, which dried to a fluffy white powder (651 mg, >99%, R_f = 0.26 EtOAc:Hex 2:1). ^{1}H NMR (pyridine-d_5:acetone-d_6 9:1, 400 MHz) δ 4.96 (d, J = 1.9 Hz, 1 H), 4.79 (dd, J = 2.2, 1.3 Hz, 1 H), 3.54-3.48 (m, 1 H), 3.04 (dd, J = 11.7, 4.2 Hz, 1 H), 2.77-2.69 (m, 1 H), 2.64-2.58 (m, 1 H), 2.27-2.21 (m, 2 H), 2.02-1.93 (m, 2 H), 1.92-1.77 (m, 6 H), 1.74-1.68 (m, 1 H), 1.63-1.56 (m, 8 H), 1.32-1.19 (m, 3 H), 1.15 (s, 3 H), 1.13-1.05 (m, 8 H), 0.91 (s, 3 H), 0.90-0.87 (m, 1 H), 0.82 (s, 3 H); ^{13}C NMR (pyridine-d_5:acetone-d_6 9:1, 100 MHz) δ 179.04, 151.62, 110.07, 69.12, 56.85, 56.57, 51.31, 49.98, 47.97, 43.10, 41.37, 39.59, 38.82, 37.92, 37.79, 35.05, 33.09, 31.42, 30.50, 29.27, 26.36, 25.80, 21.39, 19.65, 18.90, 17.99, 16.80, 16.60, 15.10; HRMS (EI) *m/z* for $C_{30}H_{49}NO_4$ ($[M]^{+\bullet}$) 455.3750, calc. 455.3763.



8

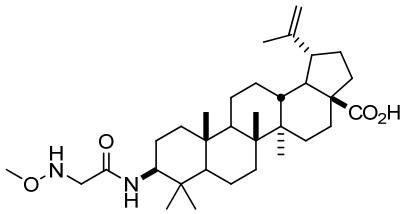
***N*'-Succinimidyl-*N*-methoxyiminoacetate (8)**. *N*-Methoxyiminoacetic acid[S2] (1.10 g, 10.7 mmol) was dissolved in 1:1 *p*-dioxane:CH_2Cl_2 (10 mL) followed by the addition of *N*-hydroxysuccinimide (1.35 g, 11.7 mmol). The reaction was cooled to 0 ^{o}C then 1,3-diisopropoylcarbodiimide (1.9 mL, 12.2 mmol) was added. After stirring for 30 min, the resulting suspension was cold-filtered and the solvent removed in vacuo. The white residue was dissolved in THF (5 mL) and passed through a silica gel plug with 1:1 EtOAc:Hex. The white solid (1.78 g, 83%, R_f = 0.73 EtOAc:Hex 1:1) was used without further purification. ^{1}H NMR (pyridine-d_5, 400 MHz) δ 7.95 (s, 1 H), 3.92 (s, 3 H), 2.88 (s, 4 H); ^{13}C NMR (pyridine-d_5, 100 MHz) δ 170.32, 158.62, 138.00, 64.53, 26.53; HRMS (ESI) *m/z* for $C_7H_9N_2O_5$ ($[M+H]^+$) 201.0526, calc. 201.0506.



9

(3*S*)-*N*-(*N*'-Methoxyiminoacetyl)aminobetulinic acid (9). (3*S*)-Aminobetulinic acid (**3**; 208 mg, 0.456 mmol) was dissolved in pyridine (20 mL) followed by addition of activated ester **8** (107 mg, 0.535 mmol). After 1 h, the solvent was removed in vacuo and the crude material was dissolved in a minimal volume of MeOH:CH_2Cl_2 1:1 and adsorbed onto silica gel. Subsequent flash chromatography (SiO_2, EtOAc:Hex 1:4) gave the desired purified product as a white amorphous solid (135 mg, 55%, R_f = 0.27 EtOAc:Hex 1:4). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.41 (s, 1 H), 6.41 (d, J = 10.3 Hz, 1 H), 4.75 (s, 1 H), 4.61 (s, 1 H), 3.96 (s, 3 H), 3.71 (td, J = 11.0, 5.4 Hz, 1 H), 3.07-2.99 (m, 1 H), 2.32-2.18 (m, 2 H), 2.04-1.94 (m, 2 H), 1.76-1.66 (m, 5 H), 1.65-1.29 (m, 15 H), 1.22-1.16 (m, 1 H), 1.11-1.05 (m, 1 H), 0.98 (s, 3 H), 0.94 (s, 3 H), 0.88 (s, 3 H), 0.86-0.85 (m, 1 H), 0.83 (s, 3 h), 0.80 (s, 3 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.70, 171.37, 161.44, 150.66, 109.82, 63.11, 60.57, 56.69, 56.17, 50.59, 49.41, 47.12, 42.59, 40.80, 39.25, 38.52, 38.17, 37.23, 34.37, 32.38, 30.77, 29.85, 28.59, 25.60, 22.95, 20.96, 19.49, 18.68,

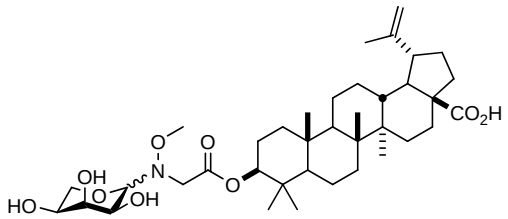
16.51, 16.24, 16.18, 14.78; HRMS (ESI) *m/z* for $C_{33}H_{52}N_2NaO_4$ ($[M+Na]^+$) 563.3809, calc. 563.3825.



10

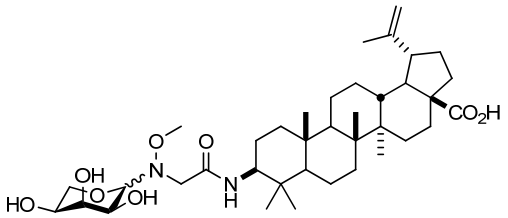
(3*S*)-*N*-(*N*'-Methoxyglycyl)aminobetulinic acid (10). Imine **9** (359 mg, 0.664 mmol) was dissolved in absolute ethanol (40 mL) and cooled to 0 ^oC. BH_3•Me_3N complex (484 mg, 6.63 mmol) was added in one aliquot and once fully dispersed, a 50% solution of HCl in absolute ethanol (1.11 mL, 6.71 mmol) was added, in dropwise fashion, over the course of five minutes. The reaction was allowed to warm to room temperature, dissolving the suspended material, and a second equal aliquot of ethanolic HCl was likewise added but at room temperature. After five hours, the reaction was quenched with saturated aqueous $NaHCO_3$ (20 mL) and extracted with CH_2Cl_2 (4 x 40 mL). The combined organic layers were washed with brine (20 mL) and dried over Na_2SO_4. Solvent removal yielded the aglycon as a flaky white solid (306 mg, 85%, R_f = 0.43 MeOH:CH_2Cl_2 5:95), which was used without further purification. ^1H NMR ($CDCl_3$, 500 MHz) δ 6.70 (d, *J* = 9.2 Hz, 1 H), 4.74 (s, 1 H), 4.60 (s, 1 H), 3.74-3.66 (m, 1 H), 3.56 (s, 5 H), 3.07-2.98 (m, 1 H), 2.32-2.19 (m, 2 H), 2.06-1.93 (m, 2 H), 1.76-1.65 (m, 5 H), 1.64-1.29 (m, 15 H), 1.20-1.14 (m, 1 H), 1.07-1.00 (m, 1 H), 0.97 (s, 3 H), 0.94 (s, 3 H), 0.88 (s, 3 H), 0.86 (s br, 1 H), 0.83 (s, 3 h), 0.79 (s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 180.98, 169.67, 150.59, 109.68, 61.99, 56.65, 56.50, 56.17, 55.11, 50.61, 49.40, 47.15, 42.61, 40.84, 39.30, 38.52, 37.93, 37.31, 37.29, 34.43, 32.46, 30.82, 29.90, 28.57, 25.68, 22.88, 21.03, 19.54, 18.75, 16.60, 16.31, 16.27, 14.86; HRMS (ESI) *m/z* for $C_{33}H_{55}N_2O_4$ ($[M+H]^+$) 543.4153, calc. 543.4156.

General procedure for neoglycoside library synthesis and purification. Aglycons **7** or **10** (typically 0.053-0.064 mmol) were added to 1 dram vials along with stir fleas and dissolved in CH_2Cl_2 (100 μL). The volumes were adjusted with MeOH (~600 μL) to provide the aglycon at a concentration of 90-100 mM. Reducing sugars (3 eq.) were added, the vials capped, and the vessels placed on a heating block/stir plate to react for 48 h at 40 ^oC. The vial caps were removed and the solvent evaporated by a Speedvac apparatus (55 ^oC, 3 h). Crude neoglycosides were suspended in MeOH:CH_2Cl_2 5:95 (250 μL) with sonication (5 min) and then purified via 2000 mg silica gel solid phase extraction (SPE) columns (Alltech, Deerfield, IL) prewashed with MeOH:CH_2Cl_2 5:95. The SPEs were eluted using a vacuum manifold, collecting fractions with a volume of approximately 1.5 mL. After the initial two fractions were obtained, eluting any unreacted aglycon, the isocratic separation was continued for pentoses and substituted hexoses while a step gradient of MeOH:$CHCl_3$ 15:85 was used for hexoses or MeOH:$CHCl_3$ 20:80 for glycuronosides. Typically, all neoglycoside was eluted by the seventh or eighth fraction leaving unreacted sugar on the SPE column. The fractions containing pure product were identified by TLC using *p*-anisaldehyde stain, then combined and dried. Compounds were assayed by ^1H and gCOSY NMR as well as high-resolution electrospray ionization mass spectrometry. Anomeric ratios were obtained by comparison of anomeric proton integration (see Table S2).



BA29

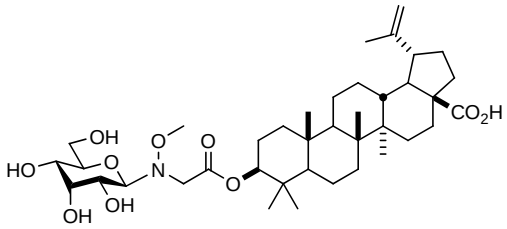
(3*S*)-*O*-(*N*-Methoxy-*N*-L-ribosylglycyl)betulinic acid (BA29)—Pilot reaction. Aglycon **7** (30 mg, 0.055 mmol) was placed into a 1 dram vial, dissolved in CH_2Cl_2 (100 μL), and the volume adjusted with methanol (600 μL). After adding L-ribose (41 mg, 0.27 mmol), the reaction was capped, warmed to 40 ^oC and allowed to stir for 2 d. Solvent was subsequently removed in vacuo and the resulting crude solid suspended in 5:95 methanol:CH_2Cl_2 (250 μL) by sonication. The mixture was purified by column chromatography (SiO_2, MeOH:CH_2Cl_2 5:95), providing the white solid neoglycoside as a mixture of anomers (18 mg, 49%, R_f = 0.23 MeOH:CH_2Cl_2 5:95). ^{1}H NMR (CD_3OD, 400 MHz) δ 4.71 (d, *J* = 1.9 Hz, 1 H), 4.61 (d, *J* = 3.8 Hz, 0.33 H, α-H1), 4.59 (s, 1 H), 4.55 (m, 1 H), 4.39 (d, *J* = 8.8 Hz, 0.67 H, β-H1), 4.12-4.09 (m, 1.34 H, 2β), 3.98 (t, *J* = 5.6 Hz, 0.33 H, α), 3.87 (td, *J* = 5.6, 3.7 Hz, 0.33 H, α), 3.78-3.75 (m, 0.33 H, α), 3.74-3.70 (m, 0.66 H, 2α), 3.69-3.65 (m, 0.67 H, β), 3.65 (s, 2 H), 3.61 (s, 3 H), 3.60-3.57 (m, 0.67 H, β), 3.52 (dd, *J* = 8.8, 2.9 Hz, 0.67 H, β), 3.03 (td, *J* = 10.7, 4.7 Hz, 1 H), 2.31 (td, *J* = 12.6, 3.4 Hz, 1 H), 2.23 (dt, *J* = 12.6, 3.2 Hz, 1 H), 1.99-1.87 (m, 2 H), 1.77-1.59 (m, 10 H), 1.57-1.50 (m, 2 H), 1.49-1.36 (m, 7 H), 1.32-1.27 (m, 1 H), 1.21-1.14 (m, 1 H), 1.08 (dd, *J* = 12.9, 4.4 Hz, 1 H), 1.02 (s, 3 H), 0.98 (s, 3 H), 0.90 (s, 3 H), 0.88 (s, 6 H), 0.84 (m, 1 H); ^{13}C NMR (CD_3OD, 100 MHz) δ 180.18, 172.61, 152.12, 110.35, 100.88 (α-C1), 91.67 (β-C1), 85.13 (α), 83.35, 73.36 (α), 72.54 (β), 72.30 (β), 69.01 (β), 68.64 (β), 66.00, 64.16 (α), 62.96 (α), 62.40, 57.63, 56.99, 55.31, 52.03, 50.58, 48.64, 43.77, 42.10, 39.77, 39.14, 38.45, 38.29, 35.63, 33.50, 31.86, 31.00, 28.61, 26.99, 24.86, 22.28, 19.72, 19.44, 17.16, 16.91, 16.79, 15.32; HRMS (ESI) *m/z* for $C_{38}H_{61}NNaO_9$ ($[M+Na]^+$) 698.4232, calc. 698.4239.



ABA4

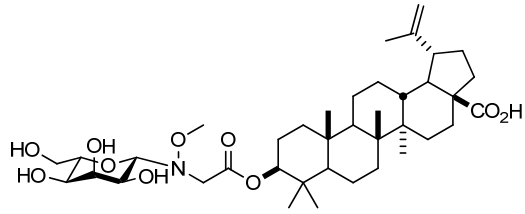
(3*S*)-*N*-(*N*'-Methoxy-*N*'-L-ribosylglycyl)betulinic acid (ABA4)—Pilot reaction. Using the same procedure as **BA29** but starting with aglycon **10** (46 mg, 0.085 mmol) yielded the anomeric mixture as a white solid (40 mg, 70%, $R_{f\,\alpha}$ = 0.50 $R_{f\,\beta}$ = 0.45 MeOH:CH_2Cl_2 10:90). ^{1}H NMR (CD_3OD:acetone-d_6 3:1, 500 MHz) δ 4.66 (s br, 1 H), 4.61 (d, *J* = 4.4 Hz, 0.33 H, α-H1), 4.54 (s br, 1 H), 4.35 (d, *J* = 9.0 Hz, 0.67 H, β-H1), 4.06 (s br, 0.67 H, β), 4.04-4.02 (m, 0.33 H, α), 3.98-3.96 (m, 0.33 H, α), 3.85-3.81 (m, 0.33 H, α), 3.65-3.62 (m, 0.67 H, β), 3.61-3.59 (m, 0.67 H, β), 3.58-3.54 (m, 2.33 H, α), 3.53 (s, 3 H), 3.51-3.49 (m, 0.67 H, β), 3.47-3.46 (m, 0.33 H, α), 3.44-3.42 (m, 0.67 H, β), 2.98 (td, *J* = 10.7, 4.8 Hz, 1 H), 2.27 (td, *J* = 12.8, 3.2 Hz, 1 H), 2.19-2.16 (m, 1 H), 1.90-1.83 (m, 2 H), 1.69-1.55 (m, 7 H), 1.52-1.30 (m, 12 H), 1.19 (dd, *J* = 12.5, 4.3 Hz, 1 H), 1.14-1.11 (m, 1 H), 1.04-1.01 (m, 1 H), 0.98 (s, 3 H), 0.92 (s, 3 H), 0.86 (s br, 1 H), 0.83 (s, 6 H), 0.78 (s, 3 H); ^{13}C NMR (CD_3OD:acetone-d_6 3:1, 125 MHz) δ 179.14, 171.90, 151.85, 110.14, 100.18 (α-C1), 91.18 (β-C1), 85.07 (α), 72.86 (α), 72.33 (β), 72.10 (α), 68.73 (β), 68.22 (β), 65.70, 63.65 (α), 62.58 (β), 61.85, 58.14, 57.35, 57.19, 51.70, 50.22, 48.29,

43.46, 41.75, 40.40, 39.39, 39.01, 38.17, 37.92, 35.33, 33.15, 31.58, 30.70, 29.04, 26.67, 23.40, 21.92, 19.55, 16.96, 16.64, 16.60, 15.13, 9.12; HRMS (ESI) *m/z* for $C_{38}H_{62}N_2O_8$ ($[M+H]^+$) 675.4594, calc. 675.4579.



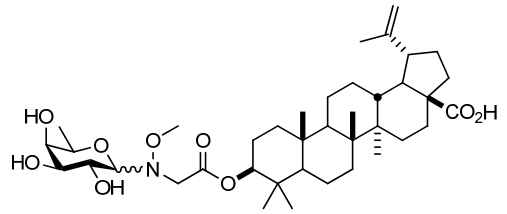
BA1

(3*S*)-*O*-(*N*-Methoxy-*N*-β-D-allosylglycyl)betulinic acid (BA1). Using aglycon **7** (31 mg, 0.057 mmol), the product was yielded as a white solid (9 mg, 23%, R_f = 0.35 MeOH:CH_2Cl_2 10:90). ^{1}H NMR (CD_3OD, 500 MHz) δ 4.75 (s br, 1 H), 4.63 (s br, 1 H), 4.62-4.59 (m, 1 H), 4.50 (d, *J* = 9.2 Hz, 1 H), 4.12 (t, *J* = 2.9 Hz, 1 H), 3.87-3.83 (m, 2 H), 3.72 (s, 2 H), 3.69 (s, 3 H), 3.60-3.56 (m, 1 H), 3.55-3.51 (m, 1 H), 3.50-3.46 (m, 1 H), 3.06 (td, *J* = 10.9, 4.7 Hz, 1 H), 2.36 (td, *J* = 12.7, 3.3 Hz, 1 H), 2.27 (dt, *J* = 12.7, 3.1 Hz, 1 H), 1.98-1.91 (m, 2 H), 1.80-1.63 (m, 10 H), 1.62-1.55 (m, 2 H), 1.53-1.39 (m, 8 H), 1.29-1.26 (m, 1 H), 1.12 (dd, *J* = 13.1, 4.2 Hz, 1 H), 1.06 (s, 3 H), 1.02 (s, 3 H), 0.94 (s, 3 H), 0.92 (s, 6 H), 0.91-0.87 (m, 1 H); HRMS (ESI) *m/z* for $C_{39}H_{63}NNaO_{10}$ ($[M+Na]^+$) 728.4365, calc. 728.4344.



BA2

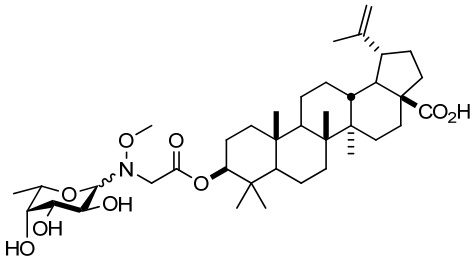
(3*S*)-*O*-(*N*-Methoxy-*N*-β-L-allosylglycyl)betulinic acid (BA2). Using aglycon **7** (31 mg, 0.057 mmol), the product was yielded as a white solid (8 mg, 20%, R_f = 0.35 MeOH:CH_2Cl_2 10:90). ^{1}H NMR (CD_3OD, 500 MHz) δ 4.74 (s br, 1 H), 4.63 (s br, 1 H), 4.62-4.56 (m, 1 H), 4.50 (d, *J* = 9.2 Hz, 1 H), 4.12 (t, *J* = 2.9 Hz, 1 H), 3.92-3.83 (m, 2 H), 3.72 (s, 2 H), 3.69 (s, 3 H), 3.60-3.56 (m, 1 H), 3.55-3.51 (m, 1 H), 3.50-3.46 (m, 1 H), 3.07 (td, *J* = 10.9, 4.7 Hz, 1 H), 2.36 (td, *J* = 12.6, 3.3 Hz, 1 H), 2.27 (dt, *J* = 12.6, 3.2 Hz, 1 H), 1.98-1.93 (m, 2 H), 1.80-1.63 (m, 10 H), 1.62-1.54 (m, 2 H), 1.53-1.39 (m, 8 H), 1.30-1.25 (m, 1 H), 1.14 (dd, *J* = 13.1, 4.2 Hz, 1 H), 1.06 (s, 3 H), 1.02 (s, 3 H), 0.94 (s, 3 H), 0.92 (s, 6 H), 0.91-0.87 (m, 1 H); HRMS (ESI) *m/z* for $C_{39}H_{63}NNaO_{10}$ ($[M+Na]^+$) 728.4382, calc. 728.4344.



BA8

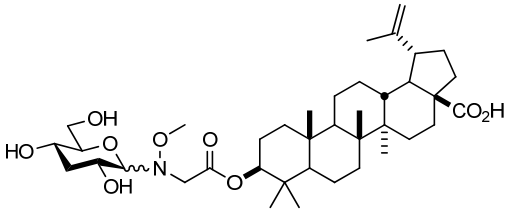
(3*S*)-*O*-(*N*-Methoxy-*N*-D-fucosylglycyl)betulinic acid (BA8). Using aglycon **7** (33 mg, 0.061 mmol), the anomeric mixture was yielded as a white solid (8 mg, 19%, R_f = 0.28 MeOH:CH_2Cl_2 5:95). ^{1}H NMR (CD_3OD, 500 MHz) δ 4.71 (s br, 1 H), 4.59 (s br, 1 H), 4.58-4.54 (m, 1 H), 4.48 (d, *J* = 5.2 Hz, 0.33 H, α-H1), 4.14 (t, *J* = 5.2 Hz, 0.33 H, α), 4.08 (d, *J* = 8.6 Hz, 0.67 H, β-H1),

3.94 (dd, *J* = 7.6, 6.2 Hz, 0.33 H, α), 3.78-3.72 (m, 1 H, α + β), 3.66 (s, 2 H), 3.64-3.62 (m, 0.33 H, α), 3.60 (s, 3 H), 3.59-3.58 (m, 0.67 H, β), 3.51-3.47 (m, 1.34 H, 2β), 3.02 (td, *J* = 10.7, 4.8 Hz, 1 H), 2.31 (td, *J* = 12.7, 3.3 Hz, 1 H), 2.23 (dt, *J* = 12.7, 3.2 Hz, 1 H), 1.95-1.87 (m, 2 H), 1.78-1.60 (m, 10 H), 1.59-1.50 (m, 2 H), 1.49-1.36 (m, 7 H), 1.31-1.28 (m, 1 H), 1.25 (d, *J* = 6.4 Hz, 2 H, β-H6), 1.23 (d, *J* = 6.6 Hz, 1 H, α-H6), 1.21-1.15 (m, 1 H), 1.09 (dd, *J* = 12.9, 4.4 Hz, 1 H), 1.02 (s, 3 H), 0.98 (s, 3 H), 0.91 (s, 3 H), 0.88 (s, 6 H), 0.87-0.83 (m, 1 H); HRMS (ESI) *m/z* for $C_{39}H_{63}NNaO_9$ ([M+Na]$^+$) 712.4409, calc. 712.4395.



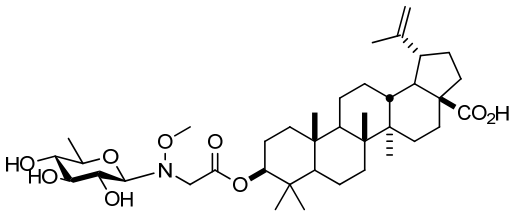
BA9

(3*S*)-*O*-(*N*-Methoxy-*N*-L-fucosylglycyl)betulinic acid (BA9). Using aglycon **7** (36 mg, 0.061 mmol), the anomeric mixture was yielded as a white solid (6 mg, 13%, R_f = 0.28 MeOH:CH_2Cl_2 5:95). ^{1}H NMR (CD_3OD, 500 MHz) δ 4.71 (s br, 1 H), 4.59 (s br, 1 H), 4.57-4.53 (m, 1 H), 4.51 (d, *J* = 5.2 Hz, 0.33 H, α-H1), 4.14 (t, *J* = 5.2 Hz, 0.33 H, α), 4.10 (d, *J* = 8.6 Hz, 0.67 H, β-H1), 3.94 (dd, *J* = 7.5, 6.3 Hz, 0.33 H, α), 3.78-3.72 (m, 1 H, α + β), 3.66 (s, 2 H), 3.64-3.62 (m, 0.33 H, α), 3.60 (s, 3 H), 3.59-3.58 (m, 0.67 H, β), 3.51-3.47 (m, 1.34 H, 2β), 3.02 (td, *J* = 10.7, 4.8 Hz, 1 H), 2.31 (td, *J* = 12.7, 3.2 Hz, 1 H), 2.23 (dt, *J* = 12.7, 3.2 Hz, 1 H), 1.95-1.86 (m, 2 H), 1.78-1.59 (m, 10 H), 1.59-1.50 (m, 2 H), 1.49-1.35 (m, 7 H), 1.31-1.27 (m, 1 H), 1.25 (d, *J* = 6.4 Hz, 2 H, β-H6), 1.23 (d, *J* = 6.6 Hz, 1 H, α-H6), 1.21-1.15 (m, 1 H), 1.07 (dd, *J* = 12.9, 4.4 Hz, 1 H), 1.02 (s, 3 H), 0.98 (s, 3 H), 0.90 (s, 3 H), 0.88 (s, 6 H), 0.87-0.83 (m, 1 H); HRMS (ESI) *m/z* for $C_{39}H_{61}NO_9$ ([M-H]$^-$) 688.4423, calc. 688.4430.



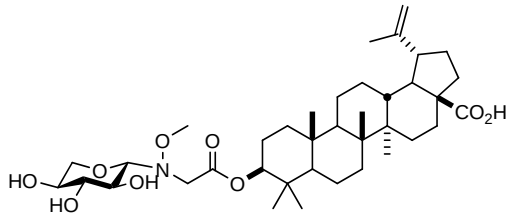
BA17

(3*S*)-*O*-(*N*-Methoxy-*N*-(3-deoxy-D-glucosyl)glycyl)betulinic acid (BA17). Using aglycon **7** (31 mg, 0.057 mmol), the anomeric mixture was yielded as a white solid (7 mg, 18%, R_f = 0.20 MeOH:CH_2Cl_2 5:95). ^{1}H NMR (CD_3OD, 500 MHz) δ 4.74 (s br, 1 H), 4.63 (s br, 1 H), 4.57 (dd, *J* = 11.3, 5.0 Hz, 1 H), 4.54 (d, *J* = 1.4 Hz, 0.25 H, α-H1), 4.42 (d, *J* = 5.7 Hz, 0.75 H, β-H1), 4.28-4.20 (m, 1 H, α + β), 4.09 (dd, *J* = 8.9, 2.6 Hz, 0.25 H, α), 3.88-3.84 (m, 0.25 H, α), 3.72 (s, 2 H), 3.70-3.65 (m, 1.5 H, 2β), 3.62-3.50 (m, 2 H, 2α + 2β), 3.07 (td, *J* = 10.9, 4.8 Hz, 1 H), 2.36 (td, *J* = 12.8, 3.3 Hz, 1 H), 2.27 (dt, *J* = 12.8, 3.2 Hz, 1 H), 2.21-2.15 (m, 1 H, α + β), 1.98-1.90 (m, 3 H), 1.80-1.63 (m, 10 H), 1.60-1.54 (m, 2 H), 1.53-1.39 (m, 7 H), 1.36-1.28 (m, 1 H), 1.24-1.18 (m, 1 H), 1.11 (dd, *J* = 13.1, 4.4 Hz, 1 H), 1.06 (s, 3 H), 1.02 (s, 3 H), 0.94 (s, 3 H), 0.92 (s, 6 H), 0.90-0.86 (m, 1 H); HRMS (ESI) *m/z* for $C_{39}H_{63}NNaO_9$ ([M+Na]$^+$) 712.4434, calc. 712.4395.



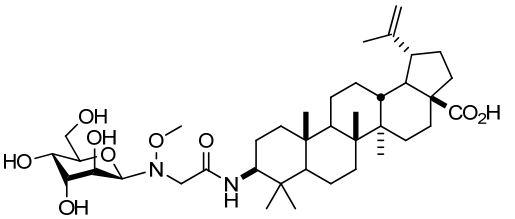
BA18

(3*S*)-*O*-(*N*-Methoxy-*N*-(6-deoxy-β-D-glucosyl)glycyl)betulinic acid (BA18). Using aglycon **7** (31 mg, 0.057 mmol), the product was yielded as a white solid (3 mg, 8%, R_f = 0.16 MeOH:CH_2Cl_2 5:95). ^{1}H NMR (CD_3OD, 500 MHz) δ 4.74 (s br, 1 H), 4.62 (s br, 1 H), 4.61-4.58 (m, 1 H), 4.12 (d, *J* = 8.9 Hz, 1 H), 3.72 (s, 2 H), 3.66 (s, 3 H), 3.39-3.35 (m, 2 H), 3.29 (dd, *J* = 9.3, 6.1 Hz, 1 H), 3.07 (td, *J* = 10.7, 4.7 Hz, 1 H), 3.00 (t, *J* = 9.1 Hz, 1 H), 2.40-2.33 (m, 1 H), 2.29-2.25 (m, 1 H), 1.98-1.90 (m, 2 H), 1.80-1.62 (m, 10 H), 1.60-1.54 (m, 2 H), 1.53-1.39 (m, 7 H), 1.36-1.32 (m, 1 H), 1.30 (d, *J* = 6.1 Hz, 3 H), 1.24-1.18 (m, 1 H), 1.11 (m, 1 H), 1.06 (s, 3 H), 1.02 (s, 3 H), 0.94 (s, 3 H), 0.92 (s, 6 H), 0.90-0.86 (m, 1 H); HRMS (ESI) ***m/z*** for $C_{39}H_{63}NNaO_9$ ($[M+Na]^+$) 712.4420, calc. 712.4395.



BA32

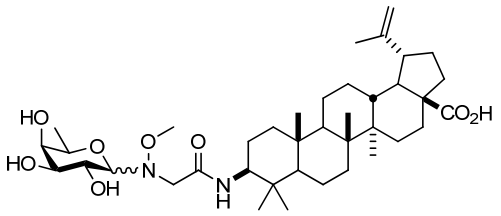
(3*S*)-*O*-(*N*-Methoxy-*N*-β-L-xylosylglycyl)betulinic acid (BA32). Using aglycon **7** (33 mg, 0.061 mmol), the product was yielded as a white solid (11 mg, 27%, R_f = 0.19 MeOH:CH_2Cl_2 5:95). ^{1}H NMR (CD_3OD, 500 MHz) δ 4.74 (s br, 1 H), 4.62 (s br, 1 H), 4.60-4.58 (m, 1 H), 4.11 (d, *J* = 8.3 Hz, 1 H), 3.91 (dd, *J* = 11.2, 5.4 Hz, 1 H), 3.78-3.76 (m, 1 H), 3.66 (s, 2 H), 3.64 (s, 3 H), 3.52-3.45 (m, 1 H), 3.38-3.36 (m, 1 H), 3.18 (t, *J* = 11.0 Hz, 1 H), 3.05 (td, *J* = 10.8, 4.6 Hz, 1 H), 2.33 (td, *J* = 12.7, 3.2 Hz, 1 H), 2.27 (dt, *J* = 12.7, 2.9 Hz, 1 H), 1.99-1.90 (m, 2 H), 1.80-1.61 (m, 10 H), 1.61-1.53 (m, 2 H), 1.52-1.37 (m, 7 H), 1.33-1.28 (m, 1 H), 1.24-1.17 (m, 1 H), 1.10 (dd, *J* = 13.3, 4.2 Hz, 1 H), 1.05 (s, 3 H), 1.00 (s, 3 H), 0.92 (s, 3 H), 0.90 (s, 6 H), 0.88-0.85 (m, 1 H); HRMS (ESI) ***m/z*** for $C_{39}H_{60}NO_9$ ($[M-H]^-$) 674.4273, calc. 674.4274.



ABA1

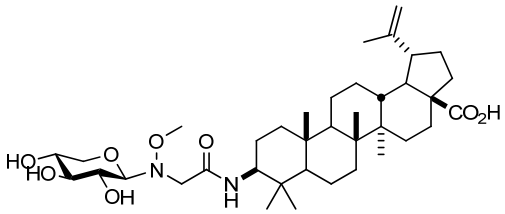
(3*S*)-*N*-(*N*'-Methoxy-*N*'-β-D-altrosylglycyl)betulinic acid (ABA1). Using aglycon **10** (47 mg, 0.087 mmol), the product was yielded as a white solid (12 mg, 20%, R_f = 0.25 MeOH:CH_2Cl_2 10:90). ^{1}H NMR (CD_3OD:acetone-d_6 3:1, 500 MHz) δ 4.74 (s br, 1 H), 4.63 (s br, 1 H), 4.55 (d, *J* = 4.6 Hz, 1 H), 4.22 (t, *J* = 5.9 Hz, 1 H), 4.17 (q, *J* = 5.0 Hz, 1 H), 3.98-3.93 (m, 2 H), 3.87-3.78 (m, 1 H), 3.76-3.68 (m, 3 H), 3.66 (s, 3 H), 3.07 (td, *J* = 10.7, 4.6 Hz, 1 H), 2.36 (td, *J* = 12.7, 3.1 Hz, 1 H), 2.27 (dt, *J* = 12.7, 3.2 Hz, 1 H), 1.98-1.91 (m, 2 H), 1.79-1.63 (m, 7 H), 1.62-1.40 (m, 12 H), 1.33-1.29 (m, 1 H), 1.24-1.21 (m, 1 H), 1.16-1.11 (m, 1 H), 1.07 (s, 3 H), 1.01 (s, 3 H),

0.95 (s br, 1 H), 0.92 (s, 6 H), 0.87 (s, 3 H); HRMS (ESI) *m/z* for $C_{39}H_{65}N_2O_9$ ($[M+H]^+$) 705.4686, calc. 705.4685.



ABA2

(3*S*)-*N*-(*N*'-Methoxy-*N*'-D-fucosylglycyl)betulinic acid (ABA2). Using aglycon **10** (45 mg, 0.083 mmol), the anomeric mixture was yielded as a white solid (6 mg, 11%, R_f = 0.42 MeOH:CH_2Cl_2 10:90). ^{1}H NMR (CD_3OD:acetone-d_6 3:1, 500 MHz) δ 4.74 (s br, 1 H), 4.63 (s br, 1 H), 4.51 (d, *J* = 4.7 Hz, 0.33 H, α-H1), 4.14 (d, *J* = 8.7 Hz, 0.67 H, β-H1), 4.13-4.12 (m, 0.33 H, α), 4.03 (t, *J* = 6.0 Hz, 0.33 H, α), 3.71-3.68 (m, 1 H, α + β), 3.66 (s, 2 H), 3.65-3.64 (m, 0.33 H, α), 3.61 (s, 3 H), 3.55 (dd, J = 9.4, 3.1 Hz, 0.67 H, β), 3.53-3.47 (m, 1.34 H, 2β), 3.07 (td, *J* = 10.7, 4.6 Hz, 1 H), 2.36 (td, *J* = 12.7, 3.2 Hz, 1 H), 2.27 (dt, *J* = 12.7, 3.1 Hz, 1 H), 1.99-1.90 (m, 2 H), 1.80-1.64 (m, 7 H), 1.63-1.38 (m, 12 H), 1.34-1.30 (m, 1 H), 1.28 (d, *J* = 6.4 Hz, 2 H, β-H6), 1.25 (d, *J* = 6.6 Hz, 1 H, α-H6), 1.24-1.22 (m, 1 H), 1.14-1.11 (m, 1 H), 1.07 (s, 3 H), 1.01 (s, 3 H), 0.94 (s br, 1 H), 0.92 (s, 3 H), 0.91 (s, 3 H), 0.87 (s, 3 H); HRMS (ESI) *m/z* for $C_{39}H_{65}N_2O_8$ ($[M+H]^+$) 689.4747, calc. 689.4735.



ABA5

(3*S*)-*N*-(*N*'-Methoxy-*N*'-β-D-xylosylglycyl)betulinic acid (ABA5). Using aglycon **10** (48 mg, 0.088 mmol), the product was yielded as a white solid (45 mg, 75%, R_f = 0.35 MeOH:CH_2Cl_2 5:95). ^{1}H NMR (CD_3OD:acetone-d_6 3:1, 500 MHz) δ 4.75 (s br, 1 H), 4.63 (s br, 1 H), 4.11 (d, *J* = 8.7 Hz, 1 H), 3.90 (dd, *J* = 11.1, 5.5 Hz, 1 H), 3.70-3.59 (m, 6 H), 3.51-3.46 (m, 1 H), 3.37 (t, *J* = 8.9 Hz, 1 H), 3.20 (t, *J* = 10.9 Hz, 1 H), 3.07 (td, *J* = 10.7, 4.6 Hz, 1 H), 2.36 (td, *J* = 12.7, 3.2 Hz, 1 H), 2.27 (dt, *J* = 12.7, 3.1 Hz, 1 H), 2.00-1.90 (m, 2 H), 1.80-1.63 (m, 7 H), 1.62-1.38 (m, 12 H), 1.34-1.26 (m, 1 H), 1.25-1.22 (m, 1 H), 1.14-1.10 (m, 1 H), 1.07 (s, 3 H), 1.01 (s, 3 H), 0.95 (s, 1 H), 0.92 (s, 3 H), 0.90 (s, 3 H), 0.87 (s, 3 H); HRMS (ESI) *m/z* for $C_{38}H_{61}N_2O_8$ ($[M-H]^-$) 673.4435, calc. 673.4433.

Cytotoxicity assays. Testing was performed by the Keck-UWCCC Small Molecule Screening Facility (Madison, WI). Carcinoma cell lines were maintained and harvested as previously reported, along with compound handling and assay set up.[S3] Cells were plated in 50 μL volumes in 384-well clear bottom tissue culture plates. Serial dilutions of 30 mM DMSO compound stock solutions were done in 96-well plates using a BioTek Precision XS liquid handler (Winooski, VT) to a concentration 100x greater than that of the most dilute assay. Final dilutions were performed in a 384-well plate in quadruplicate using a Beckman-Coulter Biomek FX liquid handler with a 384 channel pipetting head (Fullerton, CA) and were stored at -20 ^{o}C when not in use. Compounds were then added to the culture plates by the Biomek FX handler and were incubated at 37 ^{o}C for 72 h. The calcein AM reagent (acetoxymethyl ester; 30 μL, 10 μM) was

then added, the cells were incubated for 30 m at 37 ^{o}C, and plates were read for fluorescent emission (535 nm). Cell titer-glo reagent (15 μL; Promega Corp., Madison, WI) was added and the plates incubated for 10 m at room temperature with gentle agitation to lyse the cells. Each plate was reexamined for luminescence to verify inhibition. IC_{50} values for cytotoxicity were determined using XLfit 4.2 as previously reported.[S3]

Anti-HIV-1 assay. Testing was performed by Southern Research Institute (Frederick, MD) as previously described.[S4] HIV-1 virus (IIIB strain) was pre-titered with CEM-SS lymphocytes such that control wells exhibited 70 to 95% loss of cell viability six days after infection due to viral replication. Both cells and virus were mixed with compound (10 μM & 1 μM for **ABA1**-**5** and **10** or 10 μM for all others) in triplicate in 96-well plates and incubated for six days at 37 ^{o}C. Compound cytoprotection and cytotoxicity were evaluated using MTS tetrazolium dye (Promega Corp., Madison, WI) as previously reported.[S5] Each assay plate used the following controls: cells only, cells/virus, cells/compound, compound only. The reverse transcriptase inhibitor AZT and protease inhibitor Indinavir were included as positive controls.

Supplementary References

(S1) Kim, D. S. H. L.; Pezzuto, J. M.; Pisha, E. *Bioorg*. *Med*. *Chem*. *Lett*. **1998**, *8*, 1707-1712.

(S2) Ahmed, A.; Peters, N. R.; Fitzgerald, M. K.; Watson, J. A., Jr.; Hoffman, F. M.; Thorson, J. S. *J*. *Am*. *Chem*. *Soc*. **2006**, *128*, 14224-14225.

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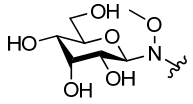
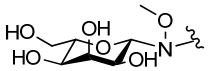
(S4) Ptak, R. G.; Gallay, P. A.; Jochmans, D.; Halestrap, A. P.; Ruegg, U. T.; Pallansch, L. A.; Bobardt, M. D.; de Bethune, M.-P.; Neyts, J.; De Clercq, E.; Dumont, J.-M.; Scalfaro, P.; Besseghir, K.; Wenger, R. M.; Rosenwirth, B. *Antimicrob*. *Agents Chemother*. **2008**, *52*, 1302-1317.

(S5) *CellTiter 96 Aqueous One Solution Cell Proliferation Assay*; Technical Bulletin TB245; Promega Corp.: Madison, WI, December 2007.

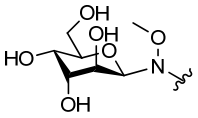
Figure S1. Betulinic acid neoglycoside library.



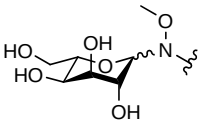
D-Alloside (**BA1**)



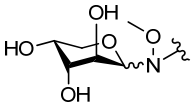
L-Alloside (**BA2**)



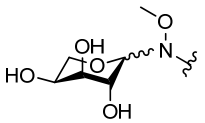
D-Altroside (**BA3**/**ABA1**)



L-Altroside (**BA4**)



D-Arabinoside (**BA5**)



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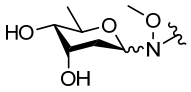
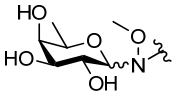
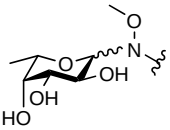


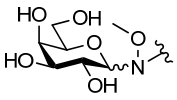
D-Digitoxoside (**BA7**)



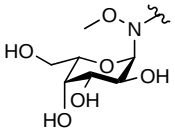
D-Fucoside (**BA8**/**ABA2**)



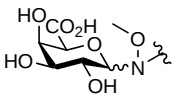
L-Fucoside (**BA9**)



D-Galactoside (**BA10**)



L-Galactoside (**BA11**)



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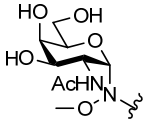
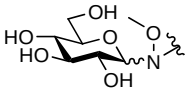
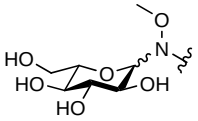


D-GalNAc (**BA13**)



D-Glucoside (**BA14**)



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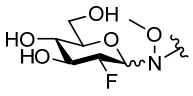
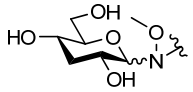
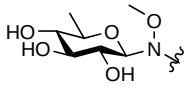


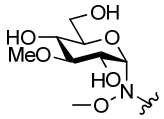
D-Glucoside, 2-fluoro (**BA16**)



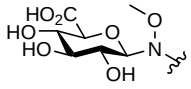
D-Glucoside, 3-deoxy (**BA17**)



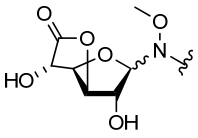
D-Glucoside, 6-deoxy (**BA18**)



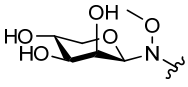
D-Glucoside, 3-*O*-methyl (**BA19**)



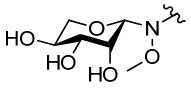
D-Glucuronide (**BA20**/**ABA3**)



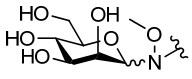
D-Glucurono-6,3-lactonide (**BA21**)



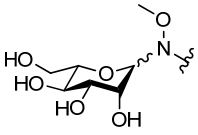
D-Lyxoside (**BA22**)



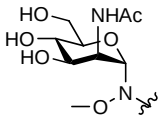
L-Lyxoside (**BA23**)



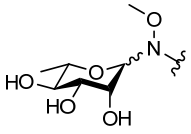
D-Mannoside (**BA24**)



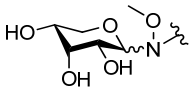
L-Mannoside (**BA25**)



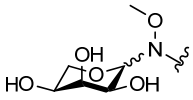
D-ManNAc (**BA26**)



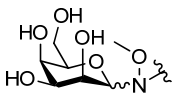
L-Rhamnoside (**BA27**)



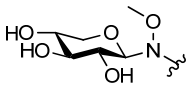
D-Riboside (**BA28**)



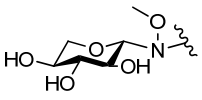
L-Riboside (**BA29**/**ABA4**)



D-Taloside (**BA30**)



D-Xyloside (**BA31**/**ABA5**)



L-Xyloside (**BA32**)

Table S1. Optimization of Neoglycosylation Conditions

entry	L-ribose (eq.)	solvent[a]	% yield
1	5	3:1 DMF:HOAc	36%
2	2	6:1 MeOH:CH_2Cl_2	17%
3	3	6:1 MeOH:CH_2Cl_2	48%
4	5	6:1 MeOH:CH_2Cl_2	49%

[a]Concentration of **7** at 90 mM.

Table S2. ^{1}H NMR Anomeric Proton and ESI-HRMS Characterization[a]

entry	neoglycoside	α-anomeric H1		β-anomeric H1		α:β	HRMS (ESI) m/z	
		δ (ppm)	*J* (Hz)	δ (ppm)	*J* (Hz)	ratio	measured	calculated
BA1	D-Alloside	not observed		4.50	9.2	β only	728.4365[b]	728.4344
BA2	L-Alloside	not observed		4.50	9.2	β only	728.4382[b]	728.4344
BA3	D-Altroside	4.21-4.16[c]	n/d[d]	4.57	4.4	n/d	728.4323[b]	728.4344
BA4	L-Altroside	4.25-4.16[c]	n/d	4.55	4.4	n/d	728.4364[b]	728.4344
BA5	D-Arabinoside	4.11	8.4	4.58	5.1	1:1	698.4227[b]	698.4239
BA6	L-Arabinoside	4.04	8.6	4.50	5.0	1:1	674.4244[e]	674.4274
BA7	D-Digitoxoside	4.66-4.64[f]				1 anomer	696.4484[b]	696.4446
BA8	D-Fucoside	4.48	5.2	4.08	8.6	1:2	712.4409[b]	712.4395
BA9	L-Fucoside	4.51	5.2	4.10	8.6	1:2	688.4423[e]	688.4430
BA10	D-Galactoside	4.60-4.53[f]				1 anomer	728.4329[b]	728.4344
BA11	L-Galactoside	4.55	5.4	not observed		α only	728.4343[b]	728.4344
BA12	D-Galacturonide	4.27-4.21[f]				1 anomer	742.4128[b]	742.4137
BA13	D-GalNAc	4.65	5.2	not observed		α only	769.4601[b]	769.4610
BA14	D-Glucoside	4.65-4.54[c]	n/d	4.17	8.6	n/d	728.4346[b]	728.4344
BA15	L-Glucoside	4.65-4.59[c]	n/d	4.19	8.8	n/d	728.4357[b]	728.4344
BA16	D-Glucoside, 2-fluoro	4.66-4.63[f]				1 anomer	730.4287[b]	730.4301
BA17	D-Glucoside, 3-deoxy	4.54	1.4	4.42	5.7	1:3	712.4434[b]	712.4395
BA18	D-Glucoside, 6-deoxy	not observed		4.12	8.9	β only	712.4420[b]	712.4395
BA19	D-Glucoside, 3-*O*-Me	4.67	3.0	not observed		α only	742.4504[b]	742.4501
BA20	D-Glucuronide	not observed		4.25	8.7	β only	742.4143[b]	742.4137
BA21	D-Glucuronolactonide	4.89-4.87[f]				1 anomer	724.4033[b]	724.4031
BA22	D-Lyxoside	not observed		4.46	8.7	β only	698.4268[b]	698.4239
BA23	L-Lyxoside	not observed		4.44	8.6	β only	698.4243[b]	698.4239
BA24	D-Mannoside	4.37	1.8	4.65-4.62[c]	n/d	n/d	728.4318[b]	728.4344
BA25	L-Mannoside	4.43	1.7	4.65-4.62[c]	n/d	n/d	728.4376[b]	728.4344
BA26	D-ManNAc	4.64	5.2	not observed		α only	769.4597[b]	769.4610
BA27	L-Rhamnoside	4.37	3.1	4.59	1.7	5:1	712.4376[b]	712.4395
BA28	D-Riboside	4.63	3.5	4.40	8.7	1:2	698.4232[b]	698.4239
BA29	L-Riboside	4.61	3.8	4.39	8.8	1:2	698.4232[b]	698.4239
BA30	D-Taloside	4.66-4.63[f]				1 anomer	706.4500[g]	706.4525
BA31	D-Xyloside	not observed		4.08	8.7	β only	698.4257[b]	698.4239
BA32	L-Xyloside	not observed		4.11	8.3	β only	674.4273[e]	674.4274
ABA1	D-Altroside	not observed		4.55	4.6	β only	705.4686[g]	705.4685
ABA2	D-Fucoside	4.51	4.7	4.14	8.7	1:2	689.4747[g]	689.4735
ABA3	D-Glucuronide	not observed		4.53	7.6	β only	719.4477[g]	719.4477
ABA4	L-Riboside	4.61	4.4	4.35	9.0	1:2	675.4594[g]	675.4579
ABA5	D-Xyloside	not observed		4.11	8.7	β only	673.4435[e]	673.4433

[a]Most-active neoglycosides in gray. [b]HRMS (ESI) m/z for $[M+Na]^+$. [c]Anomeric proton obscured by another peak. [d]Not determined. [e]HRMS (ESI) m/z for $[M-H]^-$. [f]Single anomeric proton signal detected but obscured by another peak. [g]HRMS (ESI) m/z for $[M+H]^+$.

Table S3. IC_{50} Cytotoxicity Data of Betulinic Acid Neoglycoside Library[ab]

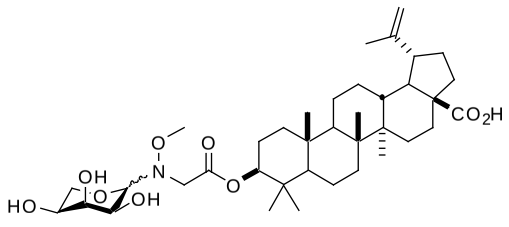
entry	neoglycoside	A549 lung	Du145 prostate	MCF7 breast	SKOV3 ovary	NCI H460 lung	NCI-ADR RES breast	HT-29 colorectal	HCT15 colorectal	SF-268 glioblastoma
BA1	D-Alloside	11.2±0.7[c]	13.7±0.6	9.2±0.6	n/d[d]	11.4±0.5	12±1	17.6±0.8	- - -	24.7±0.8
BA2	L-Alloside	>25	>25	>25	n/d	>25	>25	- - -	- - -	- - -
BA3	D-Altroside	8.4±0.4	9.0±0.3	8.6±0.2	n/d	10.1±0.4	7.6±0.2	11.1±0.4	- - -	17.6±0.6
BA4	L-Altroside	>25	>25	>25	n/d	>25	>25	- - -	- - -	- - -
BA5	D-Arabinoside	13.2±0.6	13.9±0.5	15.9±0.4	n/d	14.4±0.5	21.1±0.8	14.1±0.4	- - -	20.4±0.6
BA6	L-Arabinoside	>25	>25	>25	n/d	>25	>25	- - -	- - -	- - -
BA7	D-Digitoxoside	>25	>25	>25	n/d	>25	>25	- - -	- - -	- - -
BA8	D-Fucoside	>25	>25	>25	n/d	>25	>25	- - -	- - -	- - -
BA9	L-Fucoside	7.8±0.5	23±4	22±1	n/d	>25	>25	>25	- - -	>25
BA10	D-Galactoside	>25	21±1	>25	n/d	>25	>25	22±1	- - -	>25
BA11	L-Galactoside	n/d	>25	>25	n/d	>25	>25	- - -	- - -	- - -
BA12	D-Galacturonide	n/d	>25	>25	n/d	>25	>25	- - -	- - -	- - -
BA13	D-GalNAc	n/d	>25	>25	n/d	>25	>25	- - -	- - -	- - -
BA14	D-Glucoside	n/d	>25	>25	n/d	>25	>25	- - -	- - -	- - -
BA15	L-Glucoside	>25	>25	>25	n/d	12±1	11±2	22±3	- - -	n/d
BA16	D-Glc, 2-fluoro	n/d	>25	>25	n/d	>25	>25	- - -	- - -	- - -
BA17	D-Glc, 3-deoxy	n/d	>25	>25	n/d	>25	>25	- - -	- - -	- - -
BA18	D-Glc, 6-deoxy	>25	>25	18±1	n/d	>25	>25	18±1	- - -	>25
BA19	D-Glc, 3-*O*-Me	n/d	>25	>25	n/d	>25	>25	- - -	- - -	- - -
BA20	D-Glucuronide	15.7±0.9	19±1	23±1	n/d	19.8±0.6	20±2	18.8±0.4	- - -	23.7±0.2
BA21	D-Glc lactonide	>25	>25	>25	n/d	>25	>25	- - -	- - -	- - -
BA22	D-Lyxoside	>25	>25	>25	n/d	>25	>25	- - -	- - -	- - -
BA23	L-Lyxoside	>25	>25	>25	n/d	>25	>25	- - -	- - -	- - -
BA24	D-Mannoside	>25	>25	>25	n/d	>25	>25	- - -	- - -	- - -
BA25	L-Mannoside	>25	13±2	18.0±0.6	n/d	19.1±0.6	22±1	>25	- - -	n/d
BA26	D-ManNAc	>25	>25	>25	n/d	>25	>25	- - -	- - -	- - -
BA27	L-Rhamnoside	>25	>25	>25	n/d	>25	>25	- - -	- - -	- - -
BA28	D-Riboside	>25	>25	>25	n/d	>25	>25	- - -	- - -	- - -
BA29	L-Riboside	>25	>25	>25	n/d	>25	>25	- - -	- - -	- - -
BA30	D-Taloside	>25	>25	>25	n/d	>25	>25	- - -	- - -	- - -
BA31	D-Xyloside	10.5±0.5	11±2	11.8±0.4	>25	11.8±0.3	20.1±0.4	13.8±0.6	- - -	18.7±0.3
BA32	L-Xyloside	7.5±0.3	7.0±0.2	9.0±0.3	>25	8.4±0.3	13.3±0.4	8.8±0.3	- - -	11.8±0.2
2	Betulin	>25	>25	>25	>25	>25	>25	- - -	- - -	- - -
1	Betulinic acid	7.3±0.3	10.4±0.2	8.2±0.3	>25	7.8±0.6	7.5±0.3	12.7±0.3	- - -	10.7±0.4
7	BA aglycon	9.5±0.2	10.1±0.4	8.7±0.5	20±4	16.1±0.6	22±1	6.3±0.5	- - -	15.3±5
ABA1	D-Altroside	4.8±0.4	7.1±0.3	4.7±0.2	- - -	- - -	- - -	>25	12.1±0.5	- - -
ABA2	D-Fucoside	4.5±0.4	5.3±0.2	4.1±0.2	- - -	- - -	- - -	>25	9.9±0.4	- - -
ABA3	D-Glucuronide	>25	>25	>25	- - -	- - -	- - -	>25	>25	- - -
ABA4	L-Riboside	4.2±0.4	6.1±0.4	3.7±0.2	- - -	- - -	- - -	>25	10.0±0.4	- - -
ABA5	D-Xyloside	4.6±0.5	7.2±0.9	6±1	- - -	- - -	- - -	>25	11.0±0.4	- - -
10	ABA aglycon	12±1	10.3±0.9	8.2±0.7	- - -	- - -	- - -	>25	21±1	- - -

[a]Library and controls were found to be inactive against MDA-MB-231 breast cancer cell line. [b]Most-active neoglycosides in gray. [c]All values in μM. [d]No activity detected.

Table S4. CEM-SS Cytoprotection Data for Betulinic Acid Neoglycoside Library[a]

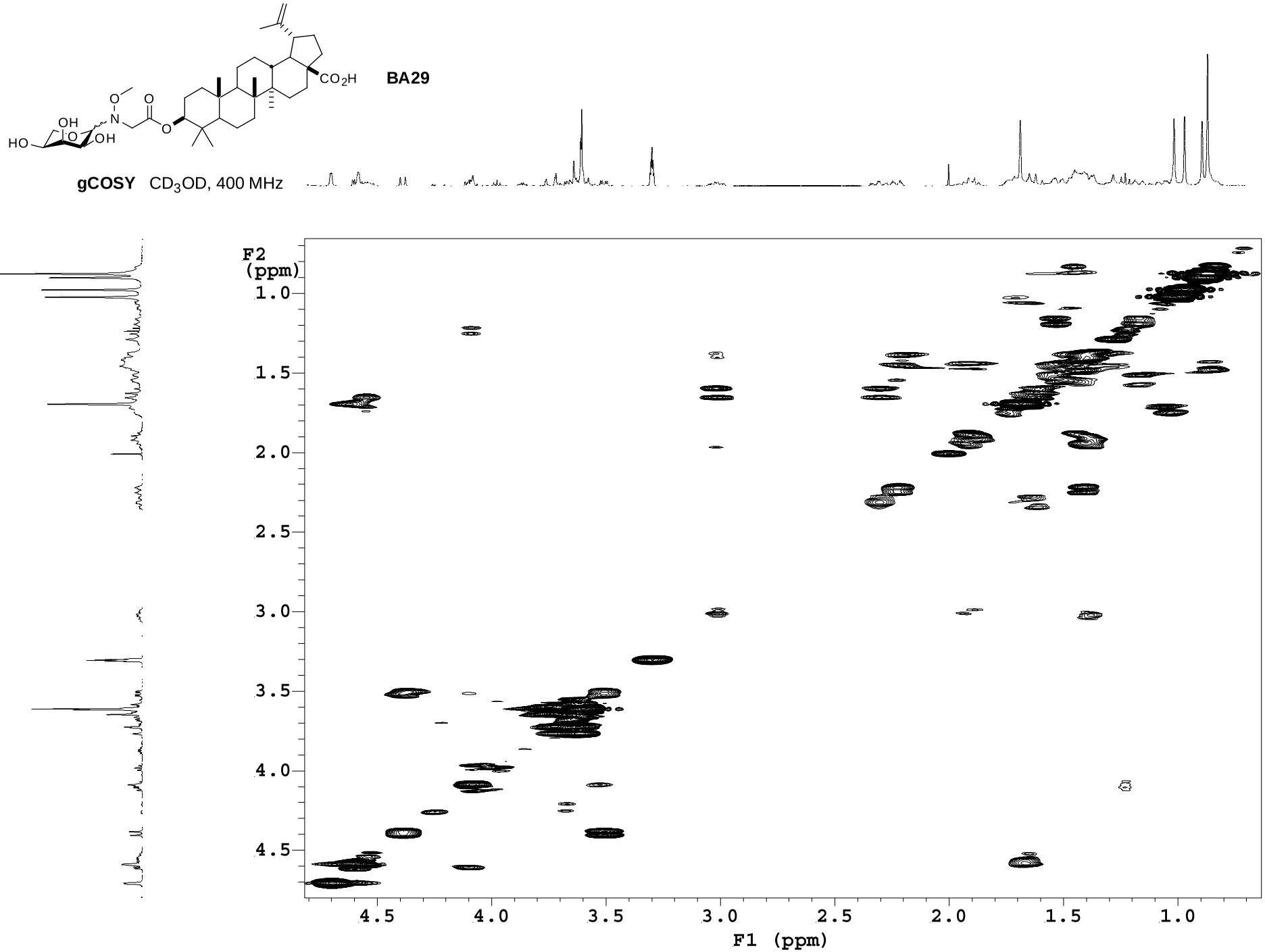
entry	neoglycoside	% increase in CPE[bc]	% cell viability
BA1	D-Alloside	29	100
BA2	L-Alloside	28	100
BA3	D-Altroside	6	99
BA4	L-Altroside	5	98
BA5	D-Arabinoside	12	100
BA6	L-Arabinoside	4	99
BA7	D-Digitoxoside	12	100
BA8	D-Fucoside	28	100
BA9	L-Fucoside	61	92
BA10	D-Galactoside	3	100
BA11	L-Galactoside	6	100
BA12	D-Galacturonide	1	100
BA13	D-GalNAc	n/d[d]	100
BA14	D-Glucoside	2	97
BA15	L-Glucoside	1	78
BA16	D-Glucoside, 2-fluoro	17	98
BA17	D-Glucoside, 3-deoxy	31	99
BA18	D-Glucoside, 6-deoxy	52	94
BA19	D-Glucoside, 3-*O*-methyl	23	92
BA20	D-Glucuronide	9	100
BA21	D-Glucuronolactonide	n/d	91
BA22	D-Lyxoside	6	90
BA23	L-Lyxoside	5	88
BA24	D-Mannoside	12	89
BA25	L-Mannoside	n/d	33
BA26	D-ManNAc	4	100
BA27	L-Rhamnoside	14	100
BA28	D-Riboside	13	100
BA29	L-Riboside	20	100
BA30	D-Taloside	5	99
BA31	D-Xyloside	4	100
BA32	L-Xyloside	43	99
2	Betulin	3	92
1	Betulinic acid	3	100
7	BA aglycon	1	100
ABA1	D-Altroside	n/d (n/d[e])	59 (100[f])
ABA2	D-Fucoside	n/d (12[e])	9 (100[f])
ABA3	D-Glucuronoside	n/d (32[e])	24 (100[f])
ABA4	L-Riboside	n/d (7[e])	97 (100[f])
ABA5	D-Xyloside	n/d (4[e])	52 (100[f])
10	3-Aminobet. acid aglycon	n/d (n/d[e])	73 (100[f])
- - -	AZT	18[g]	100
- - -	Indinavir	32[g]	100

[a]Most-active neoglcosides in gray. [b]Cytoprotective effect. [c]CPE at 10 μM unless otherwise indicated. [d]No activity detected. [e]CPE at 1 μM. [f]Cell viability at 1 μM. [g]CPE at 10 nM.



BA29

CD_3OD, 400 MHz


BA29
gCOSY CD3OD, 400 MHz
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F1 (ppm)
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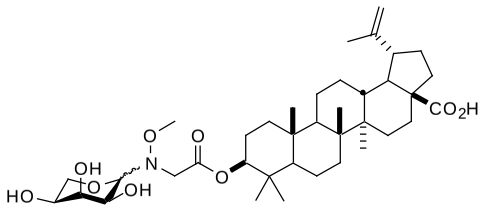
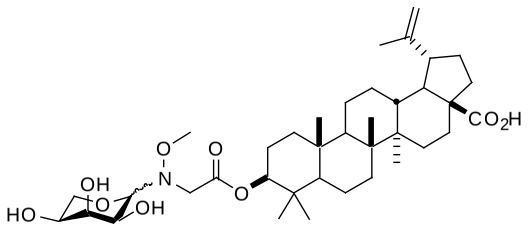
1.5
2.0
2.5
3.0
3.5
4.0
4.5



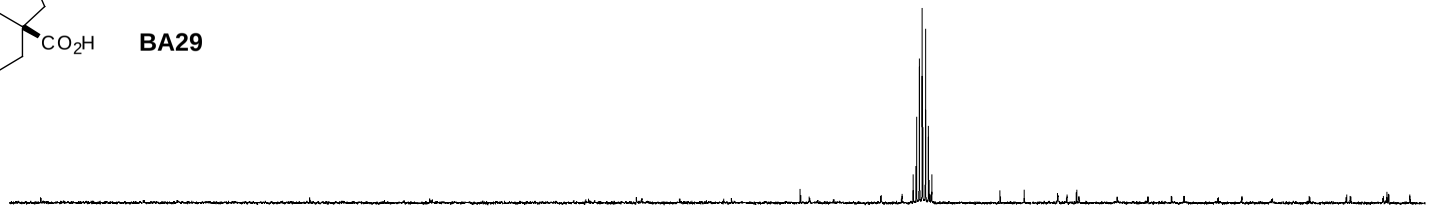
BA29

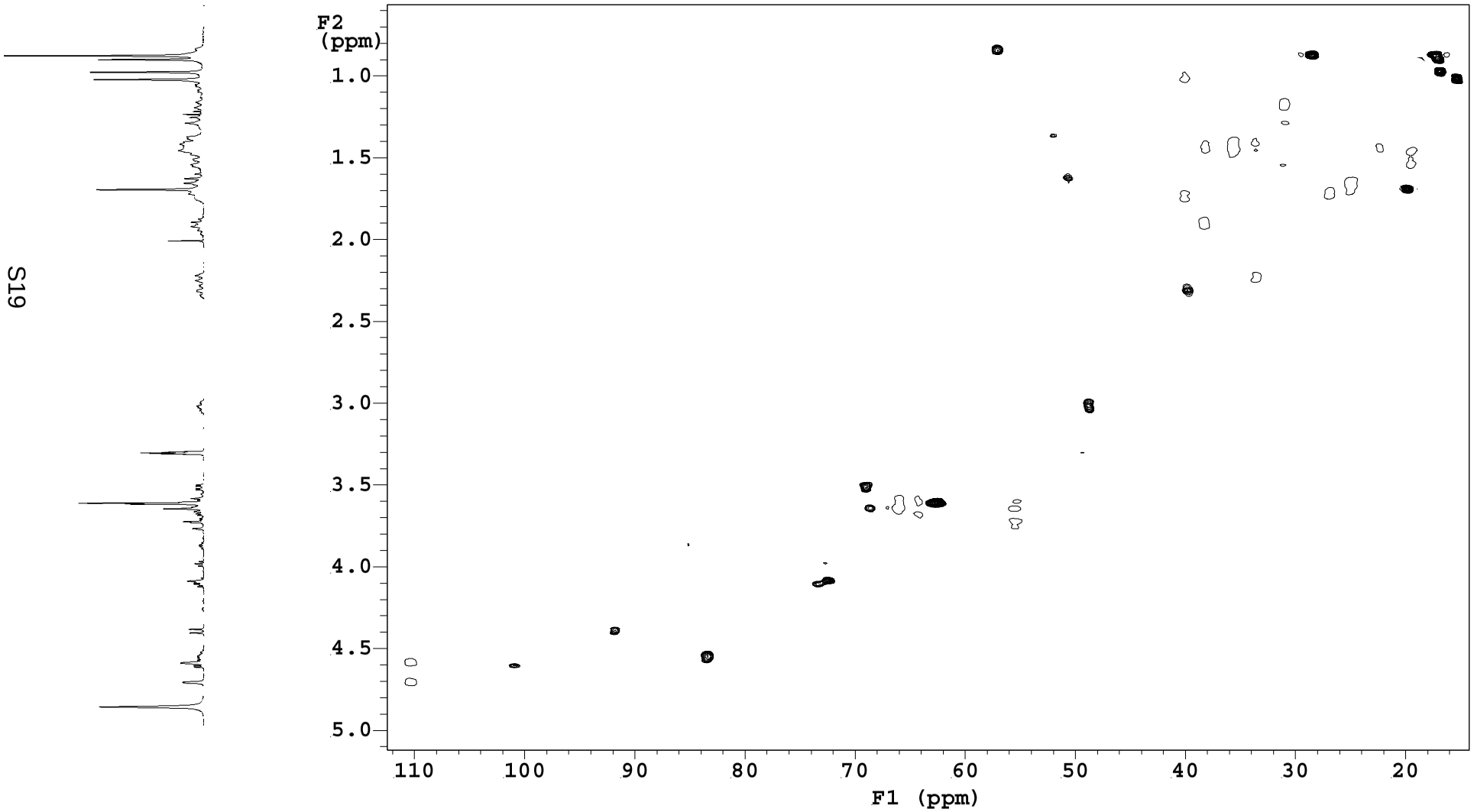
CD_3OD, 100 MHz

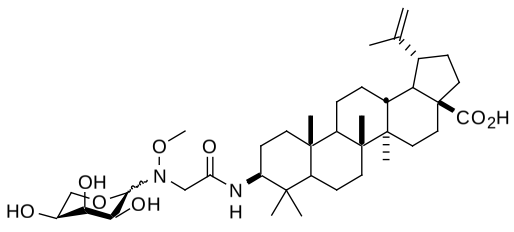


BA29

gHSQC CD_3OD, 100 MHz

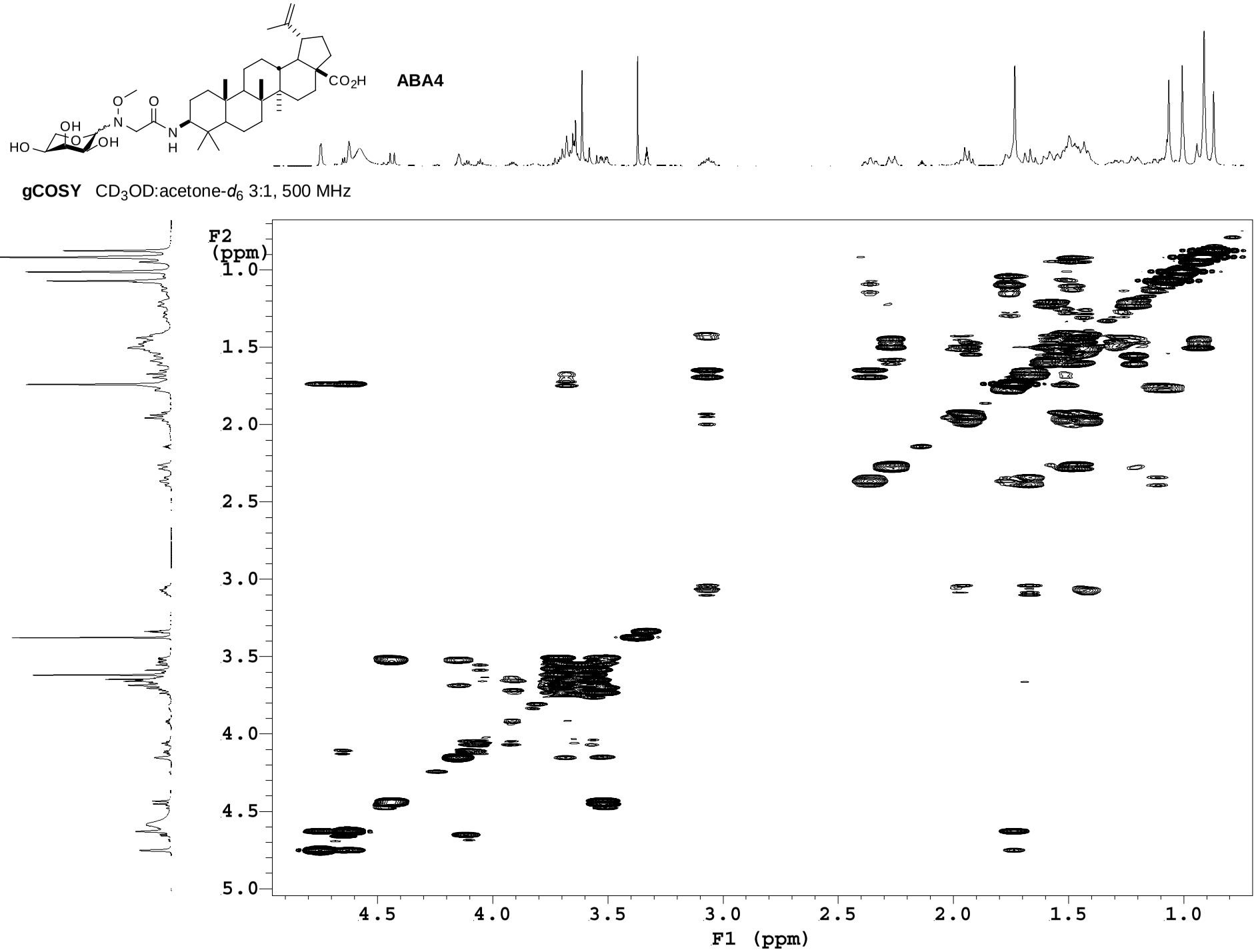






ABA4

CD_3OD:acetone-d_6 3:1, 500 MHz


ABA4
gCOSY CD3OD:acetone-d6 3:1, 500 MHz
F2 (ppm)
F1 (ppm)
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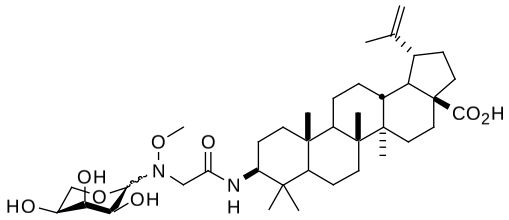
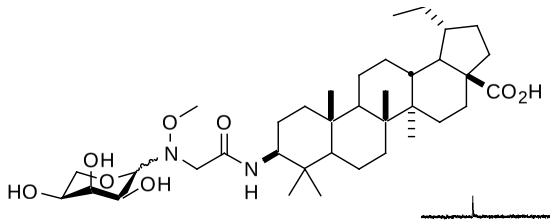
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0



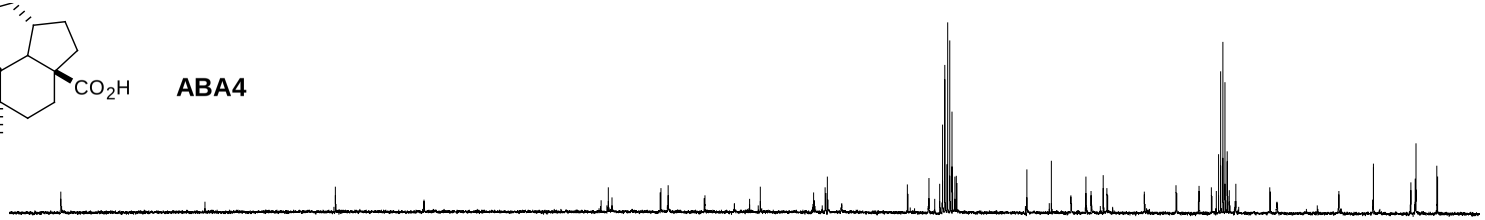
ABA4

CD_3OD:acetone-d_6 3:1, 125 MHz

S22



ABA4



gHSQC CD_3OD:acetone-d_6 3:1, 125 MHz