Development of a Minimal Saponin Vaccine Adjuvant based on QS-21

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I. SUPPLEMENTARY RESULTS

SUPPLEMENTARY FIGURES 1–10

Supplementary Figure 1. Aryl iodide saponin 8 lacking the linear tetrasaccharide domain exhibits poor adjuvant activity in a preclinical mouse vaccination model. (a) Synthesis of negative control saponin 8 (SQS-0-3-7-18): (i) SOCl₂, pyridine, CH₂Cl₂/DMF, 21 °C, 2 h, 91%; (ii) 1. H₂ (50 psi), Pd/C (Degussa), THF/EtOH (1:1), 21 °C, 24 h; 2. TFA/H₂O (4:1), 0 °C, 3.3 h, RP-HPLC, 50% (2 steps); (iii) 4, Et₃N, DMF, 21 °C, 3 h, RP-HPLC, 68%; (iv) 5, Et₃N, DMF, 21 °C, 2.5 h, RP-HPLC, 53%. (b) Biological
evaluation of aryl iodide saponin 8 with OVA antigen. Mice were vaccinated with OVA (20 µg) according to the general procedure (see Materials and Methods in the manuscript). Median titers represented as red horizontal bars. Statistical significance compared to SQS-21 was assessed using two-tailed unpaired Student's t-test with CI = 95%. * = 0.01 ≤ p ≤ 0.05 (significant).
Supplementary Figure 2. Radioiodinated saponin \([^{131}\text{I}]\)-6 localizes to and is retained at the lymph nodes and injection site in mice. Extended biodistribution of (a) active radioiodinated saponin \([^{131}\text{I}]\)-6 and (b) inactive radioactive saponin \([^{131}\text{I}]\)-8 with OVA antigen at 24, 72, and 96 h post-administration. Significantly higher radioactivity was recovered in the lymph nodes and at the injection site with \([^{131}\text{I}]\)-6 across all three timepoints while radioactivity in other organs where a large fold-difference was initially observed (muscle, bone, skin) decreased rapidly at the later timepoints; the increase in recovery from the thyroid at later timepoints is commonly observed for all radioiodinated tracers due to deiodination of the tracer. Statistical significance for \([^{131}\text{I}]\)-6 compared to \([^{131}\text{I}]\)-8 in each organ at each timepoint assessed using two-tailed unpaired Student’s \(t\)-test with CI = 95%. At 24 h: \(* = 0.01 \leq p \leq 0.05\) (significant): liver, muscle, lymph node, skin, thyroid; \(** = 0.001 < p < 0.01\) (very significant): blood, lungs, spleen, kidneys, bone, injection site; \(*** = p < 0.001\) (extremely significant): heart. At 72 h: \(* = 0.01 \leq p \leq 0.05\) (significant): spleen, thymus, lymph nodes, skin, bone; \(** = 0.001 < p < 0.01\) (very significant): heart, lungs, liver, kidneys, ovaries, thyroid; \(*** = p < 0.001\) (extremely significant): blood, injection site. At 96 h: \(* = 0.01 \leq p \leq 0.05\) (significant): bone, ovaries, thymus, skin, thyroid; \(** = 0.001 < p < 0.01\) (very significant): lungs, spleen, stomach, kidney, lymph nodes, injection site; \(*** = p < 0.001\) (extremely significant): blood, heart, liver.
Supplementary Figure 3. Biodistribution of radioiodinated saponins $[^{131}I]$-6 and $[^{131}I]$-8 is not perturbed by the absence of OVA antigen. Biodistribution of (a) active adjuvant $[^{131}I]$-6 ($[^{131}I]$-SQS-0-0-5-18) and (b) attenuated adjuvant $[^{131}I]$-8 ($[^{131}I]$-SQS-0-3-7-18). Comparison of radioactivity recovered in (c) the lymph nodes and (d) at the injection site, where significantly higher radioactivity was recovered with $[^{131}I]$-6 across all three timepoints while radioactivity in other organs where a large fold-difference was initially observed (muscle, bone, skin) decreased at the later timepoints. Statistical significance for
[\textsuperscript{131}I]-6 compared to [\textsuperscript{131}I]-8 in each organ at each timepoint assessed using two-tailed unpaired Student’s \(t\)-test with CI = 95%, not shown graphically in parts (a) and (b) for clarity. At 24 h: \(* = 0.01 \leq p \leq 0.05\) (significant): muscle, bone, ovaries, injection site, skin; \(** = 0.001 < p < 0.01\) (very significant): lungs, liver, spleen, kidneys, thymus; \(*** = p < 0.001\) (extremely significant): blood, heart. At 72 h: \(* = 0.01 \leq p \leq 0.05\) (significant): spleen, thymus, lymph node, injection site; \(** = 0.001 < p < 0.01\) (very significant): blood, lungs, liver, muscle, bone, thyroid; \(*** = p < 0.001\) (extremely significant): heart, kidney, ovaries, skin. At 96 h: \(* = 0.01 \leq p \leq 0.05\) (significant): heart, lungs, liver, spleen, kidney, thymus, lymph node, skin; \(** = 0.001 < p < 0.01\) (very significant): blood, injection site.

Supplementary Figure 4. Biodistribution of radioiodinated ovalbumin ([\textsuperscript{131}I]-OVA) indicates rapid deiodination. Biodistribution at 24, 72, and 96 h post-administration in the (a) presence (20 \(\mu\)g) and (b) absence of active adjuvant 6 (SQS-0-0-5-18). Statistical significance for vaccination with 6 compared to without 6 in each organ at each timepoint assessed using two-tailed unpaired Student’s \(t\)-test with CI = 95%, not shown graphically for clarity. At 24 h: \(* = 0.01 \leq p \leq 0.05\) (significant): heart, skin; \(** = 0.001 < p < 0.01\) (very significant): thymus. At 72 h: \(* = 0.01 \leq p \leq 0.05\) (significant): stomach, lymph node, skin. At 96 h: \(* = 0.01 \leq p \leq 0.05\) (significant): blood, lungs, stomach, kidney, bone; \(** = 0.001 < p < 0.01\) (very significant): spleen, ovaries, thymus.
Supplementary Figure 5. Fluorescein-labeled active adjuvant 3 is retained at the injection site. Whole mouse images with fluorescent saponin 3 (SQS-0-0-5-12) and amine-containing inactive adjuvant 2 (SQS-0-0-5-11) for comparison to Figure 2b of the manuscript.

Supplementary Figure 6. Synthesis of aryl iodide and aryl tin variants derived from oleanolic acid (18 [SQS-1-7-5-18] and S9). (i) 1. TESOTf, 2,6-lutidine, CH₂Cl₂, 0 °C, 1 h; 2. BF₃·OEt₂, 4 Å M.S., CH₂Cl₂, −50 °C, 20 min, 21 °C, 2 min [two temperature cycles], 54% (2 steps); (ii) 1. PhSeH, Et₃N, 38 °C, 8 h; 2. HO₂C(CH₂)₅NHBoc (14), EtOCOCl, Et₃N, THF, 0 °C, 2.5 h, [acid preactivation], then, 0 °C, 1.5 h, 77% (2 steps); (iii) 1. H₂ (50 psi), Pd/C (Degussa), THF/EtOH (1:1), 21 °C, 24 h; 2. TFA/H₂O (4:1), 0 °C,
2 h, RP-HPLC, 44% (2 steps); (iv) 4, Et₃N, DMF, 21 ºC, 2 h, RP-HPLC; 63%; (v) 5, Et₃N, DMF, 21 ºC, 1.5 h, RP-HPLC, 63%; (vi) [¹³¹I]-NaI, Chloramine-T, MeOH, 21 ºC, 1 min, RP-HPLC, 55%.

Supplementary Figure 7. Synthesis of aryl iodide saponin adjuvant 19 (SQS-1-11-5-18). (i) NaBH₄, MeOH, 21 ºC, 3 h, >99%; (ii) 1. H₂ (1atm), Pd/C (Degussa), EtOH/THF (1:1), 21 ºC, 12 h; 2. TFA/H₂O (3:1), 0 ºC, 1.25 h, RP-HPLC, 70% (2 steps); (iii) 4, Et₃N, DMF, 21 ºC, 3 h, RP-HPLC, 65%.
Supplementary Figure 8. Synthesis of the protected triterpene building blocks. (i) TESOTf, 2,6-lutidine, CH$_2$Cl$_2$, 0 ºC, 1 h; S14: 94%; S17: 65%; S18: 81%; (ii) 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO), N-chlorosuccinimide (NCS), tetrabutylammonium chloride hydrate (TBACl·H$_2$O), CH$_2$Cl$_2$/NaHCO$_3$ 0.5 M/ K$_2$CO$_3$ 0.05 M, 21 ºC, 2 h, 72%.

Supplementary Figure 9. Synthesis of additional aryl iodide variants lacking the branched trisaccharide domain, 20 (SQS-1-8-5-18), 21 (SQS-1-9-5-18), and 22 (SQS-1-10-5-18). (i) S14 or S17 or S18, BF$_3$·OEt$_2$, 4 Å M.S., CH$_2$Cl$_2$, −35 ºC, 30 min; S19: 80%; S20: 70%; S21: 71%; (ii) 1. PhSeH, Et$_3$N, 38 ºC, 8 h; 2. HO$_2$C(CH$_2$)$_5$NHBoc (14), EtOCCl, Et$_3$N, THF, 0 ºC, 2.5 h, [acid preactivation], then, 0 ºC, 1.5 h; S22: 73% (2 steps); S23: 62% (2 steps); S24: 74%; (iii) 1. H$_2$ (1 atm), Pd/C (Degussa), THF/EtOH (1:1), 21 ºC, 12 h; 2. TFA/H$_2$O (3:1), 0 ºC, 1.25 h, RP-HPLC, S25: 53% (2 steps); S26: 82% (2 steps); S27: 66%; (iv) 4, Et$_3$N, DMF, 21 ºC, 3 h, RP-HPLC; 20: 80%; 21: 56%; 22: 57%.
Supplementary Figure 10. Complete data for evaluation of triterpene variants 19–22 in a preclinical mouse vaccination mode. Biological evaluation of 19 (SQS-1-11-5-18), 20 (SQS-1-8-5-18), 21 (SQS-1-9-5-18), and 22 (SQS-1-10-5-18) at 20 µg and 50 µg doses with a four-component vaccine.
(MUC1-KLH, OVA, GD3-KLH) for (a) anti-KLH (IgG), (b) anti-MUC1 (IgG), (c) anti-OVA (IgG), (d) anti-GD3 (IgM), and (e) anti-GD3 (IgG) titers. Median titers values represented as red horizontal bars. Statistical significance is compared to no-adjuvant control and was assessed using two-tailed unpaired Student’s t-test with CI = 99%: * = 0.01 ≤ p ≤ 0.05 (significant), ** = 0.001 < p < 0.01 (very significant), *** = p < 0.001 (extremely significant). (e) Toxicity assessment of 19–22 based on median percent weight loss over one week after first vaccine injection.
II. SUPPLEMENTARY METHODS

A. MATERIALS AND METHODS

**General Procedures.** Reactions were performed in flame-dried sealed-tubes or modified Schlenk (Kjeldahl shape) flasks fitted with a glass stopper under a positive pressure of argon, unless otherwise noted. Air- and moisture-sensitive liquids and solutions were transferred via syringe. The appropriate carbohydrate reagents were dried via azeotropic removal of water with toluene. Molecular sieves were activated at 350 °C and were crushed immediately prior to use, then flame-dried under vacuum. Organic solutions were concentrated by rotary evaporation below 30 °C. Flash column chromatography was performed employing 230–400 mesh silica gel. Thin-layer chromatography was performed using glass plates pre-coated to a depth of 0.25 mm with 230–400 mesh silica gel impregnated with a fluorescent indicator (254 nm).

**Materials.** Dichloromethane, tetrahydrofuran, diethyl ether, and toluene were purified by passage through two packed columns of neutral alumina under an argon atmosphere. Methanol was distilled from magnesium at 760 Torr. Trifluoromethanesulfonic anhydride was distilled from phosphorus pentoxide at 760 Torr. Triethylamine and boron trifluoride diethyl etherate was distilled from calcium hydride at 760 Torr. All other chemicals were obtained from commercial vendors and were used without further purification unless noted otherwise.

**Instrumentation.** Infrared (IR) spectra were obtained using a Perkin Elmer Spectrum BX spectrophotometer or a Bruker Tensor 27. Data are presented as the frequency of absorption (cm⁻¹). Proton and carbon-13 nuclear magnetic resonance (¹H NMR and ¹³C NMR) spectra were recorded on a Bruker Avance III instrument; chemical shifts are expressed in parts per million (δ scale) downfield from tetramethylsilane and are referenced to residualproton in the NMR solvent (CDCl₃: δ 7.26 for ¹H NMR, δ 77.00 for ¹³C NMR; C₆D₆: δ 7.16 for ¹H NMR, δ 128.06 for ¹³C NMR; CD₃OD: δ 3.31 for ¹H NMR, δ 49.15 for ¹³C NMR; CD₃CN: δ 1.94 for ¹H NMR, δ 1.32 for ¹³C NMR; D₂O: δ 4.79 for ¹H NMR). Data are presented as follows: chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet and/or multiple resonances), coupling constant in Hertz (Hz), integration. RP-HPLC purification and analyses were carried out on a Waters 2545 binary gradient HPLC system equipped with a Waters 2996 photodiode array detector, and absorbances were monitored at wavelengths of 210–600 nm.
B. SYNTHESIS OF IODINATED AND RADIOLABELED SAPONIN ADJUVANTS

1. Synthesis of Initial Variants 6 (SQS-0-0-5-18) and 8 (SQS-0-3-7-18)

SQS-0-0-5-18 (6). (EC-V-056) To a solution of amine 2 (9.0 mg, 6.0 µmol, 1.0 equiv) in N,N'-dimethylformamide (2.0 mL), triethylamine (50 µL, 0.36 mmol, 60 equiv) was injected and the mixture stirred at 21 °C for 50 min. Aryl iodide 4 (20 mg, 60 µmol, 10 equiv) in N,N'-dimethylformamide (0.6 mL) was then added dropwise and the reaction stirred at 21 °C for 1 h. The contents were diluted with 20% acetonitrile/water (10 mL) and directly purified by RP-HPLC on an XBridge Prep BEH300 C18 column (5 µm, 10 × 250 mm) using a linear gradient of 20–70% acetonitrile/water, over 30 min, at a flow rate of 5 mL/min. SQS-0-0-5-18 (6) (5.4 mg, 52% yield) was obtained as a white powder after lyophilization.

HPLC: $t_{ret} = 19.50$ min, $\lambda_{max} = 251$ nm. $^1$H NMR (600 MHz, 1:1 CD$_3$CN/D$_2$O) characteristic resonances: $\delta$ 9.49 (s, 1H), 7.99–7.93 (m, 2H), 7.66–7.60 (m, 2H), 5.43 (t, $J = 3.3$ Hz, 1H), 5.40 (d, $J = 8.0$ Hz, 1H), 5.30 (d, $J = 1.5$ Hz, 1H), 4.80 (d, $J = 7.7$ Hz, 1H), 4.68 (d, $J = 7.8$ Hz, 1H), 4.59 (d, $J = 7.8$ Hz, 1H), 4.52 (d, $J = 6.7$ Hz, 1H), 4.05–3.96 (m, 3H), 3.94–3.81 (m, 5H), 3.70 (m, 5H), 3.66–3.54 (m, 6H), 3.53–3.28 (m, 7H), 3.28–3.20 (m, 2H), 2.99 (dd, $J = 14.1$, 4.3 Hz, 1H), 1.43 (s, 3H), 1.36 (d, $J = 6.2$ Hz, 3H), 1.21 (s, 3H), 1.05 (s, 3H), 1.01 (s, 3H), 0.97 (s, 3H); see below for proton NMR. $^{13}$C NMR (151 MHz, CD$_3$OD) $\delta$ 211.17, 178.37, 177.09, 169.51, 144.98, 139.05, 135.54, 130.18, 123.30, 107.26, 105.11, 104.84, 103.92, 101.65, 99.18, 95.60, 86.74, 84.52, 78.36, 78.19, 76.84, 76.44, 76.33, 75.53, 75.41, 75.10, 75.00, 74.73, 73.73, 72.40, 72.08, 71.13, 70.96, 69.05, 67.47, 67.32, 62.30, 61.87, 56.42, 52.69, 50.15, 48.15, 48.09, 42.89, 42.40, 41.20, 41.09, 39.36, 37.25, 36.66, 33.65, 33.54, 32.26, 31.48, 30.29,
27.67, 27.34, 26.95, 25.91, 24.96, 24.63, 21.63, 18.53, 17.86, 16.52, 11.13; see below for carbon NMR. **HRMS** (ESI) m/z: Calcd for C\textsubscript{77}H\textsubscript{115}IN\textsubscript{2}O\textsubscript{34}Na (M+Na)+ 1761.6274, found 1761.6359.

**Aryl tin precursor to [\textsuperscript{131}I]-SQS-0-0-5-18 (7).** (EC-V-052) To a solution of amine 22 (2.0 mg, 1.3 µmol, 1.0 equiv) in N,N’-dimethylformamide (0.9 mL) triethylamine (10 µL, 72 µmol, 55 equiv) was injected and the mixture stirred at 21 °C for 50 min. Aryl tin 5\textsuperscript{4} (2.0 mg, 5.2 µmol, 4.0 equiv) in N,N’-dimethylformamide (0.2 mL) was then added dropwise and the reaction stirred at 21 °C for 1 h. After this time, the contents were diluted with 20% acetonitrile/water (10 mL), and directly purified by RP-HPLC on an XBridge Prep BEH300 C18 column (5 µm, 10 × 250 mm) using a 20–70% acetonitrile/water linear gradient, over 30 min, at a flow rate of 5 mL/min. Saponin 7 (1.8 mg, 78% yield) was obtained as a white powder after lyophilization.

**HPLC:** \( t_{\text{ret}} = 21.93 \text{ min, } \lambda_{\text{max}} = 239 \text{ nm.} \) **\textsuperscript{1}H NMR** (600 MHz, CD\textsubscript{3}OD) characteristic resonances: \( \delta \) 9.43 (s, 1H), 7.78–7.72 (m, 2H), 7.65–7.53 (m, 2H), 5.37 (d, \( J = 1.4 \text{ Hz, } 1H \)), 5.34 (d, \( J = 7.4 \text{ Hz, } 1H \)), 5.30 (t, \( J = 3.5 \text{ Hz, } 1H \)), 4.80 (d, \( J = 7.3 \text{ Hz, } 1H \)), 4.64–4.59 (m, 2H), 4.50 (s, 1H), 4.47 (d, \( J = 7.7 \text{ Hz, } 1H \)), 4.37 (d, \( J = 6.9 \text{ Hz, } 1H \)), 4.33 (d, \( J = 2.8 \text{ Hz, } 1H \)), 2.93 (dd, \( J = 14.3, 3.7 \text{ Hz, } 1H \)), 1.39 (s, 3H), 1.15 (s, 3H), 1.00 (s, 3H), 0.94 (s, 3H), 0.88 (s, 3H), 0.75 (s, 3H), 0.31 (s, 9H); see below for proton NMR. **HRMS** (ESI) m/z: Calcd for C\textsubscript{80}H\textsubscript{125}N\textsubscript{2}O\textsubscript{34}Sn (M+H)+ 1777.7057, found 1777.7140.
**Fully protected aminoacyl prosapogenin S3.** (EC-V-191) A solution of acid S1\(^5\) (50 mg, 24 µmol, 1.0 equiv) in dichloromethane (1.56 mL) and pyridine (40 µL, 0.50 mmol, 20.5 equiv) was cooled in an ice bath. After stirring for 5 min, thionyl chloride (20 µL, 0.28 mmol, 11.5 equiv) was injected followed by addition of \(N,N'\)-dimethylformamide (6.25 µL, 0.081 mmol, 3.4 equiv) and stirred at 21 °C for 1.5 h. The resulting clear-yellow solution was concentrated to afford an amorphous white solid that was then redissolved in dichloromethane (1.6 mL) containing pyridine (40 µL, 0.50 mmol, 20.5 equiv). To the solution was injected S2 (0.1 mL, 0.62 mmol, 25.8 equiv), which caused an orange tint to form. After 30 min, the reaction was diluted with CH\(_2\)Cl\(_2\) (30 mL) and washed with saturated sodium bicarbonate (30 mL). The aqueous phase was extracted with CH\(_2\)Cl\(_2\) (2 × 30 mL) and the combined organic phases were dried over Na\(_2\)SO\(_4\), filtered, and evaporated to dryness to give a bright yellow oil. Purification by silica gel chromatography (4:1 hexanes/EtOAc) afforded S3 (48 mg, 91% yield) as a glassy solid.

**TLC:** \(R_f\) 0.22 (4:1 hexanes/EtOAc).  
**IR** (neat film) cm\(^{-1}\) 2953, 2876, 1718, 1458, 1239, 1102, 1006, 823, 737.  
**\(^1\)H NMR** (600 MHz, C\(_6\)D\(_6\)) characteristic resonances: \(\delta\) 9.73 (s, 1H), 6.47 (t, \(J = 4.2\) Hz, 1H), 5.50 (s, 1H), 5.20 (d, \(J = 12.4\) Hz, 1H), 5.03 (d, \(J = 12.4\) Hz, 1H), 5.00 (d, \(J = 7.3\) Hz, 1H), 4.92 (s, 1H), 4.77 (d, \(J = 7.3\) Hz, 1H), 4.65 (d, \(J = 6.9\) Hz, 1H), 4.41 (t, \(J = 9.1\) Hz, 1H), 4.20 (d, \(J = 9.4\) Hz, 1H), 4.16–4.11 (m, 1H), 4.08 (dd, \(J = 11.2, 4.9\) Hz, 1H), 4.03 (dd, \(J = 9.2, 7.5\) Hz, 1H), 3.98 (dd, \(J = 9.5, 5.5\) Hz, 1H), 3.65 (t, \(J = 8.0\) Hz, 1H), 3.52 (t, \(J = 10.8\) Hz, 1H), 2.62 (t, \(J = 13.1\) Hz, 1H), 2.36 (d, \(J = 14.2\) Hz, 1H), 2.04 (td, \(J = 12.7, 4.2\) Hz, 1H), 1.97 (dd, \(J = 13.7, 3.7\) Hz, 1H), 1.65 (s, 3H), 1.55 (s, 3H), 1.53 (s, 3H), 1.44 (s, 9H); **see below for proton NMR.**  
**\(^{13}\)C NMR** (151 MHz, C\(_6\)D\(_6\)) \(\delta\) 209.36, 177.86, 170.06, 168.83, 156.75, 155.80, 144.75, 135.91, 128.71, 128.35, 127.62, 123.10, 102.73, 101.71, 101.59, 83.52, 79.72, 79.42, 78.90, 78.76, 78.14, 77.16, 76.96, 76.71, 76.47, 75.94, 73.31, 73.18, 72.47, 71.86, 67.08, 66.15, 61.64, 60.08, 54.74, 49.13, 48.98, 47.69, 46.70, 42.00, 41.88, 41.36, 40.85, 40.02, 38.11, 36.12, 35.99, 34.98, 34.69, 33.07, 32.35, 32.19, 31.98, 30.92, 30.23, 29.43, 28.53, 28.30, 26.72, 25.64, 25.56, 24.71, 23.76, 23.07, 20.90, 20.59, 20.56, 19.04, 18.95, 16.93, 15.88, 14.41, 14.37, 14.23, 11.91, 11.68, 7.98, 7.80, 7.67, 7.64, 7.61, 7.50, 7.43, 7.24, 7.22, 6.46, 6.24, 6.12, 6.02, 5.97, 5.84, 5.78, 5.45, 4.93; **see below for carbon NMR.**  
**HRMS (ESI) m/z:** Calcd for C\(_{115}\)H\(_{218}\)N\(_2\)O\(_{21}\)Na (M+Na\(^+\)) 2238.3873, found 2238.3887.
Aminoacyl prosapogenin S4. (EC-IV-187) In a 10 mL round-bottom flask, S3 (24 mg, 10.8 µmol, 1.0 equiv) was dissolved in tetrahydrofuran/ethanol (5 mL, 1:1) and 10% (dry basis) palladium on carbon, wet, Degussa type E101 NE/W (63 mg, 29.6 µmol, 2.7 equiv) was added. The reaction was stirred under hydrogen pressure (50 psi) at 21 ºC for 24 h. The resulting crude mixture of partially desilylated products was filtered through a 0.45 µm nylon syringe filter, rinsed with methanol (20 mL), CH2Cl2 (10 mL), and methanol again (5 mL), and the clear filtrate was evaporated to dryness. Successful debenzylation is assessed by the disappearance of aromatic resonances by 1H NMR in CD3OD. The resulting mixture was then subjected to trifluoroacetic acid/water (2 mL, 4:1) for 3.3 h in an ice bath and then evaporated to dryness to afford a pink solid. The crude obtained was purified by RP-HPLC on an XBridge Prep BEH300 C18 column (5 µm, 10 × 250 mm) using a linear gradient of 20–95% acetonitrile/water (0.05% TFA), over 20 min, at a flow rate of 5 mL/min. Saponin S4 (5.4 mg, 50% yield) eluted as a broad single peak and existed as a white powder after lyophilization.

**HPLC:** $t_{ret} = 11.70$ min, $\lambda_{max} = 210$ nm. **1H NMR** (600 MHz, 1:1 CD3CN/D2O) characteristic resonances: δ 9.49 (s, 1H), 7.30 (t, $J = 5.4$ Hz, 1H), 5.57 (s, 1H), 4.79 (d, $J = 7.8$ Hz, 1H), 4.67 (d, $J = 7.7$ Hz, 1H), 4.53 (d, $J = 7.7$ Hz, 1H), 4.34 (s, 1H), 2.90 (d, $J = 13.4$ Hz, 1H), 2.33 (t, $J = 13.4$ Hz, 1H), 1.42 (s, 3H), 1.21 (s, 3H), 1.06 (s, 3H), 1.03 (s, 3H), 0.98 (s, 3H), 0.82 (s, 3H); see below for proton NMR. **13C NMR** (151 MHz, 1:1 CD3CN/D2O) δ 210.91, 180.62, 172.33, 161.92, 161.69, 143.15, 123.12, 117.76, 103.06, 102.53, 102.21, 84.74, 84.41, 76.58, 76.06, 75.42, 75.16, 74.22, 73.44, 73.32, 71.75, 70.09, 69.27, 65.41, 61.13, 55.31, 48.74, 47.36, 46.69, 46.46, 41.46, 40.66, 39.73, 39.12, 37.70, 37.34, 35.61, 34.81, 34.37, 32.17, 31.56, 29.84, 26.43, 24.39, 24.33, 23.17, 19.95, 16.52, 15.29, 9.60; see below for carbon NMR. **HRMS** (ESI) m/z: Calcd for C49H79N2O19 (M+H)+ 999.5277, found 999.5283.
SQS-0-3-7-18 (8). (EC-IV-194) To a solution of S4 (7.1 mg, 7.1 µmol, 1.0 equiv) in N,N’-dimethylformamide (0.4 mL) was injected triethylamine (20 µL, 0.14 mmol, 20 equiv), followed by dropwise addition of 4 (14 mg, 40.6 µmol, 5.7 equiv) in N,N’-dimethylformamide (0.4 mL). After stirring for 3 h, the contents were diluted with 10 mL water (0.05% TFA) and purified by RP-HPLC on an XBridge Prep BEH300 C18 column (5 µm, 10 x 250 mm) using a linear gradient of 30–80% acetonitrile/water (0.05% TFA), over 30 min, at a flow rate of 5 mL/min. SQS-0-3-7-18 (8) (5.9 mg, 68% yield) eluted as a single peak and existed as a white powder after lyophilization.

**HPLC:** $t_{\text{ret}} = 10.73$ min, $\lambda_{\text{max}} = 253$ nm. **$^1$H NMR** (600 MHz, CD$_3$OD) characteristic resonances: δ 9.42 (s, 1H), 7.90–7.80 (m, 2H), 7.65–7.57 (m, 2H), 5.40 (t, $J = 3.4$ Hz, 1H), 4.80 (d, $J = 7.0$ Hz, 1H), 4.57 (d, $J = 7.7$ Hz, 1H), 4.45 (d, $J = 7.4$ Hz, 1H), 4.33 (s, 1H), 3.90 (dd, $J = 11.4$, 5.4 Hz, 1H), 2.84 (dd, $J = 13.7$, 3.5 Hz, 1H), 2.33 (t, $J = 13.3$ Hz, 1H), 1.35 (s, 3H), 1.14 (s, 3H), 0.93 (s, 3H), 0.87 (s, 3H), 0.84 (s, 3H), 0.61 (s, 3H); see below for proton NMR. **$^{13}$C NMR** (151 MHz, CD$_3$OD) δ 210.82, 181.02, 172.12, 169.50, 145.12, 139.22, 134.69, 130.28, 129.13, 128.95, 128.80, 105.10, 104.68, 103.95, 99.79, 86.72, 86.52, 78.38, 78.27, 76.55, 75.54, 75.40, 73.70, 71.49, 71.14, 70.92, 67.32, 62.41, 56.32, 50.00, 48.04, 42.90, 41.09, 40.75, 39.23, 37.11, 36.06, 33.40, 33.15, 31.42, 27.39, 25.80, 25.43, 24.50, 21.36, 17.82, 16.50, 10.98; see below for carbon NMR. **HRMS** (ESI) $m/z$: Calcd for C$_{56}$H$_{81}$IN$_2$O$_{20}$Na (M+Na)$^+$ 1251.4325, found 1251.4359.
**Aryl tin precursor to [131I]-SQS-0-3-7-18 (9).** (EC-IV-193) To S4 (3.6 mg, 3.6 µmol, 1.0 equiv) dissolved in N,N’-dimethylformamide (0.2 mL) with triethylamine (20 µL, 0.14 mmol, 40 equiv) was added dropwise a solution of 5 (5 mg, 13.1 µmol, 3.6 equiv) in N,N’-dimethylformamide (0.1 mL). After stirring for 2.5 h, the contents were diluted with water (4 mL) and purified via RP-HPLC on an XBridge Prep BEH300 C18 column (5 µm, 10 x 250 mm) using a 20–95% acetonitrile/water linear gradient, over 30 min, at a flow rate of 5 mL/min. Saponin 9 (2.4 mg, 53% yield) eluted as a single peak and was obtained as a white powder after lyophilization.

**HPLC:** $t_{ret} = 9.90$ min, $\lambda_{max} = 241$ nm. $^1$H NMR (600 MHz, CD$_3$OD) $\delta$ 9.39 (s, 1H), 7.85–7.75 (m, 2H), 7.67–7.54 (m, 2H), 5.38 (t, $J = 3.5$ Hz, 1H), 4.79 (d, $J = 7.1$ Hz, 1H), 4.60 (d, $J = 7.7$ Hz, 1H), 4.38–4.30 (m, 2H), 3.88 (dd, $J = 11.4$, 5.4 Hz, 1H), 3.75 (dd, $J = 11.3$, 6.7 Hz, 1H), 3.71 (dd, $J = 11.4$, 5.9 Hz, 1H), 2.84 (dd, $J = 13.4$, 3.7 Hz, 1H), 2.34 (t, $J = 13.2$ Hz, 1H), 2.01 (dd, $J = 13.6$, 3.6 Hz, 1H), 1.35 (s, 3H), 1.09 (s, 3H), 0.95 (s, 3H), 0.87 (s, 3H), 0.75 (s, 3H), 0.60 (s, 3H), 0.32 (s, 9H); see below for proton NMR. **HRMS** (ESI) m/z: Calcd for C$_{50}$H$_{90}$N$_2$O$_{20}$SnNa (M+Na)$^+$ 1289.5007, found 1288.5051.
2. Synthesis of Variant Lacking the Branched Trisaccharide Domain (16)

**Bis(silyl ether) of quillaic acid (11).** (AFT-II-040) A suspension of quillaic acid 10 (200 mg, 0.41 mmol, 1.0 equiv) in CH$_2$Cl$_2$ (20 mL) was cooled in an ice bath and 2,6-lutidine (0.48 mL, 4.1 mmol, 10 equiv) and triethylsilyl trifluoromethanesulfonate (0.46 mL, 2.06 mmol, 5.0 equiv) were injected. After stirring for 1 h, the contents were washed with saturated NaHCO$_3$ (10 mL), the aqueous phase was extracted with CH$_2$Cl$_2$ ($2 \times 15$ mL) and the combined organics were dried over Na$_2$SO$_4$, filtered, and concentrated. The crude product was purified by silica gel chromatography (hexanes to 4:1 hexanes/EtOAc) to afford 11 (235 mg, 80% yield).

**TLC:** $R_f$ 0.30 (9:1 hexanes/ethyl acetate). **IR** (neat film) cm$^{-1}$ 2952, 2878, 1736, 1694, 1462, 1111, 1007, 725. **$^1$H NMR** (500 MHz, CDCl$_3$) $\delta$ 9.32 (s, 1H), 5.33 (t, $J$ = 3.0 Hz, 1H), 4.54 (s, 1H), 2.95 (dd, $J$ = 11.2, 4.4 Hz, 1H), 2.21 (t, $J$ = 13.6 Hz, 1H), 1.93–0.45 (m, 68H); **$^{13}$C NMR** (126 MHz, CDCl$_3$) $\delta$ 207.64, 183.08, 143.40, 122.37, 74.99, 73.46, 56.17, 48.78, 48.01, 46.73, 46.34, 41.44, 40.27, 39.76, 38.29, 36.03, 35.24, 34.79, 32.83, 32.44, 31.76, 30.65, 26.94, 26.65, 24.40, 23.43, 20.72, 17.17, 15.79, 9.62, 7.25, 6.96, 5.23, 5.15. **HRMS** (ESI) m/z: Calcd for C$_{42}$H$_{75}$O$_5$Si$_2$ (M+H) 715.5153, found 715.5161.

** Protected quillaic acid saponin azide S28.** (AFT-I-165) To a solution of 11 (38 mg, 49 µmol, 1.05 equiv) and imidate 12 (52 mg, 47 µmol, 1.0 equiv) in CH$_2$Cl$_2$ (7 mL) 80 mg powdered 4 Å molecular sieves was added and the mixture was stirred at 21 °C for 30 min. The reaction schlenk was then cooled to -35 °C and boron trifluoride diethyletherate (1.2 µL, 9.0 µmol, 0.2 equiv) was injected. The mixture was stirred for 0.5 h at this temperature, quenched with 0.2 mL of triethylamine and concentrated. Purification of the residue by silica gel chromatography (0.2%
triethylamine in benzene to 97:3 benzene/EtOAc) gave a colorless oil that was further chromatographed to afford the desired product S28 (56 mg, 72% yield) as a white solid.

**TLC:** $R_f$ 0.62 (9:1 benzene/EtOAc). **IR** (neat film) cm$^{-1}$ 2951, 2876, 2107, 1734, 1497, 1161. **$^1$H NMR** (500 MHz, CDCl$_3$) $\delta$ 9.31 (s, 1H), 5.37–5.30 (m, 2H), 5.18 (d, $J = 1.7$ Hz, 1H), 4.92–4.79 (m, 5H), 4.75–4.56 (m, 6H), 4.52 (s, 2H), 4.48 (s, 1H), 4.15 (dd, $J = 6.1$, 1.9 Hz, 1H), 4.13–4.08 (m, 1H), 4.03 (d, $J = 2.8$ Hz, 1H), 3.96–3.87 (m, 2H), 3.80 (dd, $J = 10.8$, 4.7 Hz, 1H), 3.69–3.58 (m, 6H), 3.55–3.48 (m, 3H), 3.31 (t, $J = 8.2$ Hz, 1H), 3.24–3.16 (m, 1H), 2.89 (dd, $J = 14.2$, 3.7 Hz, 1H), 2.21 (t, $J = 13.6$ Hz, 1H), 1.43 (s, 3H), 1.36 (s, 3H), 1.25 (s, 3H), 1.18 (d, $J = 6.2$ Hz, 3H), 1.05 (s, 3H), 0.87 (s, 3H), 0.72 (s, 3H); see below for proton NMR. **$^{13}$C NMR** (126 MHz, C$_6$D$_6$) $\delta$ 205.71, 175.28, 143.71, 139.76, 139.65, 139.11, 138.23, 137.76, 130.25, 128.48, 128.36, 128.16, 127.97, 122.71, 122.52, 109.73, 103.19, 103.00, 99.05, 98.84, 94.60, 94.38, 84.41, 82.73, 80.72, 78.94, 78.65, 76.35, 76.02, 75.88, 75.69, 75.56, 74.84, 74.67, 73.71, 73.03, 72.40, 72.31, 72.24, 67.50, 64.30, 63.99, 59.53, 56.16, 49.75, 48.39, 47.59, 47.13, 42.09, 41.46, 40.51, 38.56, 36.14, 35.85, 35.55, 33.07, 31.50, 30.87, 27.91, 27.37, 26.90, 24.58, 23.85, 21.13, 18.51, 17.78, 16.13, 10.15, 7.46, 7.10, 5.51, 5.45; see below for carbon NMR. **HRMS** (ESI) $m/z$: Calcd for C$_{97}$H$_{135}$N$_3$O$_{17}$Si$_2$Na (M+Na)$^+$ 1692.9235, found 1692.9228.

Protected quillaic acid saponin amine 13. (AFT-I-167) To S28 (62 mg, 37 µmol, 1.0 equiv) dissolved in triethylamine (28 mL) was added a freshly prepared solution of phenyl selenol (0.11 mmol, 30 equiv) via cannula. Upon addition of phenyl selenol a white precipitate was formed and the solution became bright yellow. The reaction was stirred for 8 h at 38 °C and the solution was then concentrated to afford a yellow-white solid. The crude mixture was purified by silica gel chromatography (90:10 to 85:15 benzene/EtOAc to afford the amine 13 (49 mg, 80% yield) as a glassy solid.

**TLC:** $R_f$ 0.20 (9:1 benzene/EtOAc). **IR** (neat film) cm$^{-1}$ 2951, 2911, 2876, 1734, 1497. **$^1$H NMR** (500 MHz, CDCl$_3$) characteristic resonances: $\delta$ 9.31 (s, 1H), 5.38 (d, $J = 7.9$ Hz, 1H), 5.32 (t, $J = 3.2$ Hz, 1H), 5.21 (s, 1H), 4.91–4.80 (m, 4H), 4.72 (d, $J = 11.7$ Hz, 1H), 4.68–4.60 (m, $J = ...$)
Fully protected aminoacyl quillaic acid saponin S10. (AFT-I-169) To a clear, colorless solution of 6-((t-butoxycarbonyl)-amino)hexanoic acid (14) (45 mg, 0.20 mmol, 11.5 equiv) in tetrahydrofuran (2.5 mL) at 0 °C was added triethylamine (213 µL, 1.53 mmol, 90 equiv) followed by ethyl chloroformate (16.0 µL, 0.17 mmol, 10.0 equiv). The turbid, white solution was stirred for 2.5 h at 0 °C and then added via cannula to amine 13 (28 mg, 17.0 µmol, 1.0 equiv) at 0 °C. The reaction mixture was stirred at this temperature for 1.5 h and then quenched with water (0.2 mL) to give a clear solution. The contents were diluted with saturated NaHCO3 (30 mL), and the aqueous phase was extracted with CH2Cl2 (3 × 25 mL). The combined organics were dried (Na2SO4), filtered, and evaporated to dryness. Purification by silica gel chromatography (2:1 hexanes/EtOAc with 0.2% triethylamine) afforded S10 (28 mg, 88% yield) as a white glassy solid.

**TLC:** \( R_f \) 0.18 (7:3 hexanes/EtOAc). **IR** (neat film) cm\(^{-1}\) 3030, 2950, 2876, 2360, 2341, 1717, 1684, 1456, 1365, 1166, 1088, 737. \(^1\)H NMR (600 MHz, CDCl3) characteristic resonances: \( \delta \) 9.31 (s, 1H), 5.65 (br s, 1H), 5.39 (d, \( J = 6.9 \) Hz, 1H), 5.33–5.27 (m, 1H), 5.22 (d, \( J = 1.4 \) Hz, 1H), 4.55–4.47 (m, 4H), 4.18–4.11 (m, 2H), 3.93 (dd, \( J = 11.6, 4.4 \) Hz, 1H), 3.85–3.76 (m, 2H), 3.71–3.49 (m, 8H), 3.36–3.28 (m, 2H), 3.23–3.17 (m, 1H), 2.90 (dd, \( J = 14.1, 3.8 \) Hz, 1H), 2.21 (t, \( J = 13.5 \) Hz, 1H), 1.44 (s, 3H), 1.37 (s, 3H), 1.26 (s, 3H), 1.18 (d, \( J = 6.2 \) Hz, 3H), 1.04 (s, 3H), 0.87 (s, 3H), 0.74 (s, 3H); see below for proton NMR. \(^{13}\)C NMR (151 MHz, C\(_6\)D\(_6\)) \( \delta \) 205.84, 175.27, 170.05, 143.88, 139.75, 139.63, 139.11, 138.86, 138.34, 128.77, 128.64, 128.58, 128.51, 128.46, 127.68, 127.62, 127.56, 122.48, 109.61, 103.14, 98.90, 95.06, 84.28, 82.66, 81.51, 79.02, 78.52, 76.47, 76.02, 75.63, 75.56, 74.91, 74.57, 73.50, 73.42, 73.02, 71.32, 69.06, 67.25, 64.14, 60.07, 56.14, 49.71, 49.04, 48.46, 47.54, 47.13, 42.10, 41.43, 40.48, 38.47, 36.10, 35.88, 35.45, 33.16, 33.03, 31.57, 30.90, 27.99, 27.34, 26.86, 26.36, 24.67, 23.86, 21.03, 20.56, 18.63, 17.60, 16.00, 14.22, 10.08, 7.54, 7.17, 5.49, 5.45; see below for carbon NMR. **HRMS** (ESI) \( m/z \): Calcd for C\(_{97}\)H\(_{138}\)NO\(_{17}\)Si\(_2\) (M+H)+ 1644.9503, found 1644.9528.
1H), 4.90 (d, J = 11.1 Hz, 1H), 4.88–4.80 (m, 4H), 4.78 (d, J = 10.9 Hz, 1H), 4.72 (d, J = 11.7 Hz, 1H), 4.67 (d, J = 11.1 Hz, 1H), 4.62 (d, J = 11.7 Hz, 1H), 4.54–4.41 (m, 5H), 4.17–4.13 (m, 1H), 4.11 (dd, J = 6.0, 1.7 Hz, 1H), 3.93 (dd, J = 11.7, 4.5 Hz, 1H), 3.81–3.76 (m, 2H), 3.70 (br s, 1H), 3.66–3.58 (m, 4H), 3.55–3.46 (m, 3H), 3.30 (dd, J = 9.0, 7.7 Hz, 1H), 3.23–3.17 (m, 1H), 3.06–2.97 (m, 2H), 2.88 (dd, J = 14.2, 4.0 Hz, 1H), 2.20 (t, J = 13.6 Hz, 1H), 2.17–2.11 (m, 2H), 1.46 (s, 3H), 1.44 (s, 9H), 1.37 (s, 3H), 1.25 (s, 3H), 1.15 (d, J = 6.2 Hz, 3H), 1.04 (s, 3H), 0.87 (s, 3H), 0.72 (s, 3H); see below for proton NMR. 13C NMR (151 MHz, CDCl3) δ 207.43, 175.13, 172.77, 155.91, 143.63, 138.73, 138.60, 138.28, 137.63, 137.39, 128.42, 128.33, 128.32, 128.28, 128.26, 127.96, 127.93, 127.86, 127.81, 127.78, 127.76, 127.58, 127.52, 121.40, 109.41, 102.29, 97.75, 83.79, 81.99, 79.03, 78.38, 78.11, 77.94, 76.06, 75.55, 75.26, 74.86, 74.72, 73.47, 73.20, 72.82, 71.56, 68.29, 66.56, 63.74, 55.97, 49.05, 47.75, 46.79, 46.44, 45.95, 41.51, 40.48, 40.32, 39.79, 38.18, 36.58, 35.76, 35.18, 34.51, 32.68, 32.33, 30.84, 30.43, 29.76, 28.41, 27.57, 26.76, 26.32, 26.28, 25.99, 25.30, 24.28, 23.34, 20.57, 17.72, 17.06, 15.81, 9.51, 7.11, 6.81, 5.03, 4.89; see below for carbon NMR. HRMS (ESI) m/z: Calcd for C108H157N2O20Si2 (M+H)+ 1858.0868, found 1858.0833.

Aminoacyl quillaic acid saponin 15. (AFT-I-204) In a 50 mL round-bottom flask, S10 (68 mg, 36.6 µmol, 1.0 equiv) was dissolved in tetrahydrofuran/ethanol (20 mL, 1:1) and 10% (dry basis) palladium on carbon, wet, Degussa type E101 NE/W (390 mg, 0.18 mmol, 5.0 equiv) was added. The reaction was stirred under hydrogen pressure (50 psi) at 21 °C for 24 h, and the suspension was filtered through a 0.45 µm nylon syringe filter, washed with methanol (3 × 30 mL) and concentrated. Successful debenzylation is assessed by the disappearance of aromatic resonances by 1H NMR in CD3OD. The crude mixture was then dissolved in a solution of trifluoroacetic acid (8 mL, TFA/H2O 3:1) and stirred for 2 h in an ice bath. The reaction was evaporated to dryness to afford a white solid that was dissolved in 20% acetonitrile/water (20 mL) and purified via RP-HPLC on an XBridge Prep BEH300 C18 column (5 µm, 10 × 250 mm) using a linear gradient of 30–70% acetonitrile/water (0.05% TFA), over 15 min, at a flow rate of 5 mL/min.
The aminoacyl quillaic acid saponin 15 eluted as a single peak and was obtained as a white powder (28 mg, 74% yield) after lyophilization.

**HPLC:** \( t_{\text{ret}} = 7.08 \text{ min} \), \( \lambda_{\text{max}} = 210 \text{ nm} \). \(^1\text{H NMR} \) (600 MHz, CD\textsubscript{3}OD) characteristic resonances: \( \delta \)

- 9.31 (s, 1H),
- 5.43 (d, \( J = 1.5 \text{ Hz} \), 1H),
- 5.34 (d, \( J = 8.1 \text{ Hz} \), 1H),
- 5.31 (t, \( J = 3.5 \text{ Hz} \), 1H),
- 4.51–4.48 (m, 2H),
- 4.36 (dd, \( J = 4.6, 1.6 \text{ Hz} \), 1H),
- 3.96 (dd, \( J = 9.4, 4.7 \text{ Hz} \), 1H),
- 3.91–3.80 (m, 5H),
- 3.80–3.75 (m, 1H),
- 3.72 (td, \( J = 6.5, 1.3 \text{ Hz} \), 1H),
- 3.59–3.50 (m, 2H),
- 3.49–3.41 (m, 2H),
- 3.24–3.17 (m, 2H),
- 2.96–2.90 (m, 3H),
- 2.41–2.28 (m, 3H),
- 2.00–1.90 (m, 4H),
- 1.41 (s, 3H),
- 1.34 (d, \( J = 6.2 \text{ Hz} \), 3H),
- 1.06 (dd, \( J = 12.9, 3.2 \text{ Hz} \), 1H),
- 1.02 (s, 3H),
- 1.00 (s, 3H),
- 0.95 (s, 3H),
- 0.88 (s, 3H),
- 0.77 (s, 3H); see below for proton NMR. \(^{13}\text{C NMR} \) (151 MHz, CD\textsubscript{3}OD) \( \delta \)

- 208.89,
- 177.90,
- 176.99,
- 163.46,
- 163.23,
- 163.00,
- 144.96,
- 123.31,
- 107.05,
- 101.14,
- 95.68,
- 83.97,
- 78.30,
- 76.46,
- 76.26,
- 75.37,
- 74.78,
- 73.76,
- 72.97,
- 72.31,
- 72.02,
- 71.22,
- 68.97,
- 67.48,
- 61.85,
- 57.77,
- 57.62,
- 57.48,
- 56.94,
- 52.72,
- 50.15,
- 50.00,
- 48.20,
- 48.14,
- 42.94,
- 42.50,
- 41.23,
- 40.74,
- 39.66,
- 37.14,
- 36.71,
- 36.32,
- 33.78,
- 33.51,
- 32.18,
- 31.46,
- 28.46,
- 27.33,
- 27.15,
- 26.91,
- 26.49,
- 24.93,
- 24.60,
- 22.04,
- 18.48,
- 17.83,
- 17.69,
- 17.57,
- 17.44,
- 17.31,
- 16.47,
- 9.64; see below for carbon NMR. \(^{1}\text{HRMS} \) (ESI) \( m/z \): Calcd for C\textsubscript{53}H\textsubscript{87}N\textsubscript{2}O\textsubscript{18} (M+H\textsuperscript{+}) 1039.5954, found 1039.5952.

SQS-1-0-5-18 (16). (AFT-I-300) To a solution of 15 (2.1 mg, 2.0 \( \mu \)mol, 1.0 equiv) in \( N,N' \)-dimethylformamide (0.4 mL) was added triethylamine (11 \( \mu \)L, 0.08 mmol, 40 equiv) followed by dropwise addition of 4 (4.0 mg, 10 \( \mu \)mol, 5.8 equiv) in \( N,N' \)-dimethylformamide (0.2 mL). After stirring for 2 h, the contents were diluted with 30% acetonitrile/water (2.3 mL) and purified by RP-HPLC on an XBridge Prep BEH300 C18 column (5 \( \mu \)m, 10 x 250 mm) using a linear gradient of 30–70% acetonitrile/water (0.05% TFA), over 15 min, at a flow rate of 5 mL/min. SQS-1-0-5-18 (16) (1.7 mg, 67% yield) was obtained as a white powder after lyophilization.
**HPLC**: \( t_{\text{ret}} = 12.87 \text{ min}, \lambda_{\text{max}} = 251 \text{ nm} \). **\textit{H} NMR** (600 MHz, CD\(_3\)OD) characteristic resonances: \( \delta 9.31 \text{ (s, 1H)}, 7.87–7.81 \text{ (m, 2H)}, 7.60–7.54 \text{ (m, 2H)}, 5.40 \text{ (s, 1H)}, 5.34 \text{ (d, } J = 7.4 \text{ Hz, 1H)}, 5.30 \text{ (d, } J = 3.3 \text{ Hz, 1H)}, 4.52–4.45 \text{ (m, 2H)}, 4.33 \text{ (d, } J = 3.0 \text{ Hz, 1H}), 3.98–3.89 \text{ (m, 3H)}, 3.87–3.74 \text{ (m, 4H)}, 3.70 \text{ (t, } J = 6.5 \text{ Hz, 1H}), 3.55 \text{ (t, } J = 9.5 \text{ Hz, 1H)}, 3.53–3.44 \text{ (m, 2H)}, 3.41 \text{ (dd, } J = 11.5, 6.7 \text{ Hz, 1H)}, 3.37 \text{ (t, } J = 7.1 \text{ Hz, 2H)}, 3.24–3.17 \text{ (m, 2H)}, 2.93 \text{ (dd, } J = 14.3, 4.0 \text{ Hz, 1H)}, 2.40–2.26 \text{ (m, 3H)}, 1.41 \text{ (s, 3H)}, 1.31 \text{ (d, } J = 6.1 \text{ Hz, 3H}), 1.02 \text{ (s, 3H)}, 1.00 \text{ (s, 3H)}, 0.94 \text{ (s, 3H)}, 0.88 \text{ (s, 3H)}, 0.77 \text{ (s, 3H)}; \textit{see below for proton NMR}. **\textit{C} NMR** (151 MHz, CD\(_3\)OD) \( \delta 208.83, 178.23, 169.36, 144.78, 138.90, 135.39, 130.04, 123.19, 116.96, 101.24, 99.03, 95.55, 84.03, 78.15, 76.32, 76.14, 74.92, 74.65, 74.22, 72.84, 72.19, 71.87, 71.07, 68.84, 67.33, 61.72, 57.62, 57.48, 57.33, 52.53, 50.00, 49.85, 49.57, 49.43, 49.28, 49.14, 49.00, 48.86, 48.72, 48.57, 48.02, 48.00, 42.78, 42.37, 42.10, 40.95, 39.57, 39.49, 39.31, 38.38, 36.51, 36.48, 33.62, 33.39, 31.99, 31.33, 30.14, 27.51, 27.19, 26.99, 26.79, 24.84, 24.46, 21.89, 18.32, 17.74, 17.41, 17.28, 17.16, 17.03, 16.32, 9.49; \textit{see below for carbon NMR}. **HRMS** (ESI) \( m/z \): Calcd for C\(_{60}\)H\(_{89}\)N\(_2\)O\(_{19}\)INa (M+Na)+ 1291.5002, found 1291.5034.

**Aryl tin precursor to \([^{131}\text{I}]\)-SQS-1-0-5-18 (17).** (EC-V-227) To **15** (0.65 mg, 0.63 \( \mu \)mol, 1.0 equiv) dissolved in \( N,N' \)-dimethylformamide (0.2 mL) was added triethylamine (10 \( \mu \)L, 72 \( \mu \)mol, 114 equiv) and **5** (1.0 mg, 2.6 \( \mu \)mol, 4.1 equiv). After stirring for 1.5 h the reaction was diluted with water (4 mL) and purified via RP-HPLC on an XBridge Prep BEH300 C18 column (5 \( \mu \)m, 10 x 250 mm) with a 35–95% acetonitrile/water linear gradient, over 30 min, at a flow rate of 5 mL/min. Saponin **17** (0.6 mg, 75% yield) eluted as a single peak and was obtained as a white powder after lyophilization.

**HPLC**: \( t_{\text{ret}} = 18.00 \text{ min}, \lambda_{\text{max}} = 237 \text{ nm} \). **\textit{H} NMR** (500 MHz, CD\(_3\)OD) characteristic resonances: \( \delta 9.31 \text{ (s, 1H)}, 7.77–7.72 \text{ (m, 2H)}, 7.64–7.52 \text{ (m, 2H)}, 5.39 \text{ (d, } J = 1.2 \text{ Hz, 1H)}, 5.34 \text{ (d, } J = 7.2 \text{ Hz, 1H)}, 5.31 \text{ (t, } J = 2.9 \text{ Hz, 1H)}, 4.52–4.45 \text{ (m, 2H)}, 4.33 \text{ (d, } J = 2.7 \text{ Hz, 1H)}, 3.88–3.73 \text{ (m, 4H)}, 3.70–3.64 \text{ (m, 4H)}, 3.55 \text{ (t, } J = 9.5 \text{ Hz, 1H)}, 3.53–3.44 \text{ (m, 2H)}, 3.41 \text{ (dd, } J = 11.5, 6.7 \text{ Hz, 1H)}, 3.37 \text{ (t, } J = 7.1 \text{ Hz, 2H)}, 3.24–3.17 \text{ (m, 2H)}, 2.93 \text{ (dd, } J = 14.3, 4.0 \text{ Hz, 1H)}, 2.40–2.26 \text{ (m, 3H)}, 1.41 \text{ (s, 3H)}, 1.31 \text{ (d, } J = 6.1 \text{ Hz, 3H}), 1.02 \text{ (s, 3H)}, 1.00 \text{ (s, 3H)}, 0.94 \text{ (s, 3H)}, 0.88 \text{ (s, 3H)}, 0.77 \text{ (s, 3H)}; \textit{see below for proton NMR}. **\textit{C} NMR** (151 MHz, CD\(_3\)OD) \( \delta 208.83, 178.23, 169.36, 144.78, 138.90, 135.39, 130.04, 123.19, 116.96, 101.24, 99.03, 95.55, 84.03, 78.15, 76.32, 76.14, 74.92, 74.65, 74.22, 72.84, 72.19, 71.87, 71.07, 68.84, 67.33, 61.72, 57.62, 57.48, 57.33, 52.53, 50.00, 49.85, 49.57, 49.43, 49.28, 49.14, 49.00, 48.86, 48.72, 48.57, 48.02, 48.00, 42.78, 42.37, 42.10, 40.95, 39.57, 39.49, 39.31, 38.38, 36.51, 36.48, 33.62, 33.39, 31.99, 31.33, 30.14, 27.51, 27.19, 26.99, 26.79, 24.84, 24.46, 21.89, 18.32, 17.74, 17.41, 17.28, 17.16, 17.03, 16.32, 9.49; \textit{see below for carbon NMR}. **HRMS** (ESI) \( m/z \): Calcd for C\(_{60}\)H\(_{89}\)N\(_2\)O\(_{19}\)INa (M+Na)+ 1291.5002, found 1291.5034.
4H), 3.69 (t, $J = 6.6$ Hz, 1H), 3.56 (d, $J = 9.5$ Hz, 1H), 3.24–3.16 (m, 3H), 2.93 (d, $J = 14.4$ Hz, 1H), 1.41 (s, 3H), 1.32 (d, $J = 6.3$ Hz, 3H), 1.05 (dd, $J = 13.4$, 3.6 Hz, 1H), 1.02 (s, 3H), 1.00 (s, 3H), 0.95 (s, 3H), 0.88 (s, 3H), 0.77 (s, 3H), 0.31 (s, 9H); see below for proton NMR. **HRMS (ESI)** $m/z$: Calcd for C$_{63}$H$_{98}$N$_2$NaO$_{19}$Sn (M+Na)$^+$ 1329.5683, found 1329.5737.
3. Synthesis of Variants with Modifications in the Triterpene Domain (18–22)

**Silyl ether of oleanolic acid (S29).** (AFT-I-137) To a solution of oleanolic acid S5 (250 mg, 0.55 mmol, 1.0 equiv) in CH₂Cl₂ (10 mL) at 0°C, 2,6-lutidine (0.38 mL, 3.28 mmol, 6.0 equiv), and triethylsilyl trifluoromethanesulfonate (0.37 mL, 1.64 mmol, 3.0 equiv) were added and the mixture was stirred for 1 h. The contents were quenched with 0.5 N HCl (10 mL), and the aqueous phase was extracted with CH₂Cl₂ (2 × 15 mL). The combined organics were dried over Na₂SO₄, filtered, concentrated and finally purified by silica gel chromatography (hexanes to 4:1 hexanes/EtOAc) to afford S29 (250 mg, 80% yield).

**TLC:** Rₗ 0.20 (9:1 hexanes/EtOAc). **¹H NMR** (600 MHz, CDCl₃) δ 5.26 (t, J = 3.5 Hz, 1H), 3.20 (dd, J = 11.4, 4.5 Hz, 1H), 2.81 (dd, J = 13.6, 4.2 Hz, 1H), 1.97 (td, J = 13.6, 4.0 Hz, 1H), 1.93–0.45 (m, 58H). **¹³C NMR** (151 MHz, CDCl₃) δ 184.61, 143.73, 122.84, 79.67, 55.49, 47.80, 46.68, 46.00, 41.65, 40.98, 39.48, 39.40, 38.59, 37.11, 33.92, 33.23, 32.76, 32.57, 30.82, 28.60, 27.85, 27.79, 26.12, 23.72, 23.55, 23.00, 18.59, 17.31, 16.16, 15.49, 7.21, 6.74, 5.91, 5.41. **HRMS (ESI) m/z:** Calcd for C₃₆H₆₂O₃SiNa (M+Na)+ 593.4366, found 593.4348.

**Protected oleanolic acid saponin azide S6.** (EC-V-215) To a solution of S29 (50 mg, 87 µmol, 1.0 equiv) and imidate 12 (97 mg, 87 µmol, 1.0 equiv) in CH₂Cl₂ (8.0 mL) was added 120 mg powdered 4 Å molecular sieves and the mixture was stirred at 21 °C for 1 h. The reaction schlenk was then transferred to a −78 °C bath and boron trifluoride diethyletherate (8.8 µL, 70 µmol, 0.8 equiv) was injected. The reaction was stirred at −50 °C for 20 min, at 21 °C for 1 min, cooled back to −50 °C, stirred for 20 min and finally again at 21 °C for 1 min. The mixture was then quenched with triethylamine (0.1 mL) at −50 °C and passed through a plug of silica gel. The
resulting filtrate was concentrated, and purified by silica gel chromatography (hexanes to 5:1 hexanes/EtOAc) to afford S6 (89 mg, 68% yield) as a glassy solid.

**TLC:** $R_f$ 0.48 (3:1 hexanes/EtOAc).  **IR** (neat film) cm$^{-1}$ 3064, 3031, 2948, 2875, 2107, 1751, 1497, 1455, 1365, 1240, 1222, 1096.  **$^1$H NMR** (600 MHz, C$_6$D$_6$) characteristic resonances: $\delta$ 5.88 (s, 1H), 5.69 (d, $J = 8.1$ Hz, 1H), 5.53–5.47 (m, 1H), 5.21 (d, $J = 11.0$ Hz, 1H), 5.15 (d, $J = 7.3$ Hz, 1H), 4.90 (d, $J = 11.5$ Hz, 1H), 4.56–4.51 (m, 1H), 4.39 (t, $J = 8.7$ Hz, 1H), 4.34 (d, $J = 12.0$ Hz, 1H), 4.14 (d, $J = 11.6$ Hz, 1H), 4.01 (dd, $J = 9.6$, 8.0 Hz, 1H), 3.46–3.42 (m, 1H), 3.38 (dd, $J = 9.3$, 3.5 Hz, 1H), 3.24 (dd, $J = 11.4$, 4.3 Hz, 1H), 1.65 (d, $J = 6.2$ Hz, 3H), 1.19 (s, 3H), 1.15 (s, 3H), 0.85 (s, 3H); see below for proton NMR.  **$^{13}$C NMR** (151 MHz, C$_6$D$_6$) $\delta$ 175.94, 143.76, 139.73, 139.54, 139.11, 138.30, 137.54, 128.84, 128.70, 128.56, 128.44, 127.81, 127.17, 127.53, 123.08, 109.52, 103.77, 98.46, 94.26, 84.19, 82.77, 81.91, 79.96, 79.71, 79.00, 78.45, 76.66, 75.55, 75.01, 73.69, 73.05, 72.95, 72.43, 71.91, 68.38, 66.45, 64.04, 59.29, 55.84, 48.24, 47.15, 46.54, 42.26, 42.06, 40.02, 39.71, 38.82, 37.26, 34.98, 34.89, 34.19, 33.49, 33.21, 32.26, 31.97, 30.78, 28.91, 28.39, 28.26, 27.97, 26.32, 25.94, 25.65, 23.94, 23.70, 23.07, 20.90, 19.05, 18.38, 17.57, 16.61, 15.79, 14.38, 7.42, 5.76; see below for carbon NMR.  **HRMS** (ESI) m/z: Calcd for C$_{91}$H$_{123}$N$_3$O$_{15}$SiNa (M+Na)$^+$ 1548.8621, found 1548.8668.

![Chemical structure of S6 and S30](image)

**Protected oleanolic acid saponin amine S30.** (EC-V-216) To S6 (44 mg, 29 µmol, 1.0 equiv) dissolved in triethylamine (12 mL) was added a freshly prepared solution of phenyl selenol (0.44 mmol, 15 equiv) via cannula transfer. Upon addition of phenyl selenol a white precipitate was formed and the mixture became bright yellow. After stirring at 38 ºC for 8 h, the solution was concentrated to give a yellow-white solid, which was purified by silica gel chromatography (5:1 hexanes/EtOAc to 2% triethylamine in EtOAc) to afford S30 (41 mg, 95% yield) as a white solid.

**TLC:** $R_f$ 0.07 (3:1 hexanes/EtOAc).  **IR** (neat film) cm$^{-1}$ 3010, 2948, 2874, 1751, 1497, 1454, 1367, 1240, 1222, 1093.  **$^1$H NMR** (600 MHz, C$_6$D$_6$) characteristic resonances: $\delta$ 5.96 (s, 1H), 5.75 (d, $J = 8.2$ Hz, 1H), 5.51 (t, $J = 3.4$ Hz, 1H), 5.24 (d, $J = 10.9$ Hz, 1H), 5.19 (d, $J = 7.2$ Hz,
$	ext{H},$ 4.91 (d, $J = 11.5$ Hz, 1H), 4.61–4.56 (m, 1H), 4.50 (d, $J = 5.8$ Hz, 1H), 4.18 (d, $J = 11.6$ Hz, 1H), 4.09 (d, $J = 11.6$ Hz, 1H), 4.05 (dd, $J = 9.8, 7.7$ Hz, 1H), 3.84 (dd, $J = 11.6, 5.3$ Hz, 1H), 3.80 (dd, $J = 9.7, 6.7$ Hz, 1H), 3.71–3.66 (m, 1H), 3.13 (dd, $J = 3.7, 1.2$ Hz, 1H), 1.73 (d, $J = 6.2$ Hz, 3H), 1.50 (s, 3H), 1.30 (s, 3H), 1.21 (s, 3H), 1.15 (s, 3H), 0.86 (s, 3H); see below for proton NMR. $^{13}$C NMR (151 MHz, C$_6$D$_6$) $\delta$ 176.25, 144.21, 140.08, 139.87, 139.46, 139.31, 138.51, 129.09, 128.95, 128.90, 128.85, 128.78, 127.87, 123.35, 109.79, 104.24, 98.64, 95.11, 84.55, 83.29, 83.15, 80.31, 80.21, 79.38, 78.80, 77.21, 75.89, 75.40, 75.12, 73.83, 73.29, 73.13, 71.12, 69.48, 66.42, 64.41, 56.19, 49.27, 48.60, 47.47, 46.93, 42.63, 42.43, 40.36, 40.06, 39.17, 37.60, 34.59, 33.86, 33.56, 32.67, 31.15, 29.26, 28.78, 28.61, 28.39, 26.77, 26.30, 24.30, 24.08, 19.43, 18.89, 17.93, 16.94, 16.12, 7.76, 6.11; see below for carbon NMR. HRMS (ESI) m/z: Calcd for C$_{91}$H$_{126}$NO$_{15}$Si (M+H)$^+$ 1500.8897, found 1500.8925.

Fully protected aminoacyl oleanolic acid saponin S7. (EC-V-217) To a solution of 14 (63 mg, 0.27 mmol, 10 equiv) in tetrahydrofuran (2.6 mL) was added triethylamine (365 µL, 2.6 mmol, 96 equiv) at 0 °C. To the clear, colorless solution was injected ethyl chloroformate (23 µL, 0.25 mmol, 9.0 equiv), which turned the solution turbid white. The acid activation was allowed to proceed at 0 °C for 2.5 h before the entire solution was cannula transferred into a schlenck containing amine S30 (41 mg, 27 µmol, 1.0 equiv). The reaction mixture was stirred at 0 °C for 1.5 h and then quenched with water (90 µL), at which point the solution turned from turbid, white to clear. The contents were then evaporated to dryness and purified by silica gel chromatography (5:1 hexanes/EtOAc) to afford S7 (40 mg, 81% yield) as a white glassy solid.

**TLC:** $R_f$ 0.64 (1:1 hexanes/EtOAc). IR (neat film) cm$^{-1}$ 3339, 2947, 1749, 1701, 1508, 1456, 1366, 1265, 1170, 1068, 862, 831, 737. $^1$H NMR (600 MHz, C$_6$D$_6$) characteristic resonances: $\delta$ 6.03 (s, 1H), 5.73 (d, $J = 8.2$ Hz, 1H), 5.52 (t, $J = 3.7$ Hz, 1H), 5.17 (d, $J = 7.0$ Hz, 1H), 4.60–4.55 (m, 1H), 4.13 (t, $J = 8.6$ Hz, 1H), 4.05–3.99 (m, 1H), 3.86 (dd, $J = 11.5, 5.2$ Hz, 1H), 3.41 (dd, $J = 9.3, 4.2$ Hz, 1H), 3.25 (dd, $J = 11.5, 4.4$ Hz, 1H), 2.96 (td, $J = 13.1, 6.3$ Hz, 1H), 2.81
Aminoacyl oleanolic acid saponin S8. (EC-V-218) In a 25 mL round-bottom flask containing S7 (10 mg, 5.8 µmol, 1.0 equiv) was added tetrahydrofuran/ethanol (2 mL, 1:1) followed by 10% (dry basis) palladium on carbon, wet, Degussa type E101 NE/W (14.0 mg, 6.5 µmol, 1.1 equiv). The reaction was stirred under hydrogen pressure (50 psi) at 21 ºC for 24 h and then filtered through a 0.45 mm nylon syringe filter, washed with methanol (20 mL), CH₂Cl₂ (10 mL), and methanol again (5 mL) to thoroughly wash the palladium. The clear filtrate was evaporated to dryness. Successful debenzylation was assessed by the disappearance of aromatic resonances by ¹H NMR in CD₃OD. The mixture was then dissolved in a solution of trifluoroacetic acid/water (2 mL, 4:1) and stirred for 2 h in an ice bath. After this time, the reaction was evaporated to dryness to give a white solid that was purified by RP-HPLC using a 30–80% acetonitrile/water (0.1% TFA) linear gradient, over 20 min, at a flow rate of 5 mL/min. The desired product S8 (2.6 mg, 44% yield) was obtained as a white powder after lyophilization.

HPLC: tₚₐₗ = 10.28 min, λₘₐₓ = 210 nm. ¹H NMR (600 MHz, CD₃OD) characteristic resonances: δ 5.44 (d, J = 1.2 Hz, 1H), 5.41 (d, J = 8.0 Hz, 1H), 5.24 (t, J = 3.3 Hz, 1H), 4.42 (d, J = 7.7 Hz, 1H), 4.37 (dd, J = 4.5, 1.3 Hz, 1H), 3.98 (dd, J = 9.5, 4.7 Hz, 1H), 3.95 (dd, J = 2.9, 1.7 Hz, 1H), 3.94–3.89 (m, 1H), 3.73 (td, J = 6.7, 1.1 Hz, 1H), 2.80 (dd, J = 13.7, 3.8 Hz, 1H), 2.07 (td, J =
13.5, 3.3 Hz, 3H), 1.31 (d, $J = 6.2$ Hz, 3H), 1.16 (s, 3H), 0.99 (s, 3H), 0.95 (s, 3H), 0.94–0.90 (m, 6H), 0.78 (s, 3H); see below for proton NMR. $^{13}$C NMR (151 MHz, CD$_3$OD) $\delta$ 177.78, 177.77, 163.31, 163.08, 162.85, 144.76, 123.69, 107.60, 101.30, 95.50, 85.00, 79.76, 78.32, 76.27, 76.23, 75.21, 74.03, 72.26, 71.84, 71.05, 68.70, 67.31, 61.68, 57.76, 57.61, 57.47, 57.33, 56.79, 52.60, 49.85, 48.11, 47.29, 43.06, 42.95, 40.71, 40.58, 39.92, 39.89, 38.17, 36.18, 34.87, 34.12, 33.47, 33.01, 31.53, 29.33, 28.82, 28.31, 27.88, 26.80, 26.37, 26.14, 24.55, 24.05, 23.93, 19.56, 18.25, 17.75, 17.54, 17.41, 17.29, 17.16, 17.03, 16.36, 16.06; see below for carbon NMR. HRMS (ESI) $m/z$: Calcd for C$_{53}$H$_{89}$N$_2$O$_{16}$ (M+H)$^+$ 1009.6212, found 1009.6224.

SQS-1-7-5-18 (18). (EC-V-221) Amine S8 (1.3 mg, 1.3 $\mu$mol, 1.0 equiv) was dissolved in N,N'-dimethylformamide (0.2 mL) and triethylamine (3.6 $\mu$L, 25.6 $\mu$mol, 20 equiv) was injected. To this solution, 4 (2.5 mg, 7.3 $\mu$mol, 5.7 equiv) was added and the reaction mixture was stirred at 21 ºC for 2 h. After this time, the contents were diluted with 3 mL water (0.05% TFA) and directly purified by HPLC using a 30–80% acetonitrile/water (0.05% TFA) linear gradient, over 20 min, at a flow rate of 5 mL/min to afford SQS-1-7-5-18 (18) (1.0 mg, 63% yield) as a white powder after lyophilization.

HPLC: $t_{ret} = 14.28$ min, $\lambda_{max} = 251$ nm. $^1$H NMR (600 MHz, CD$_3$OD) characteristic resonances: $\delta$ 7.88–7.80 (m, 2H), 7.60–7.54 (m, 2H), 5.42 (d, $J = 1.4$ Hz, 1H), 5.40 (d, $J = 7.6$ Hz, 1H), 5.24 (t, $J = 3.4$ Hz, 1H), 4.42 (d, $J = 7.7$ Hz, 1H), 4.37–4.30 (m, 1H), 3.71 (td, $J = 6.9$, 1.4 Hz, 1H), 3.14 (dd, $J = 11.5$, 4.5 Hz, 1H), 2.81 (dd, $J = 13.8$, 4.0 Hz, 1H), 2.40–2.32 (m, 2H), 2.11–2.01 (m, 1H), 1.29 (d, $J = 6.2$ Hz, 3H), 1.16 (s, 3H), 0.99 (s, 3H), 0.95 (s, 3H), 0.93–0.90 (m, 6H), 0.80–0.76 (m, 6H); see below for proton NMR. $^{13}$C NMR (151 MHz, CD$_3$OD) $\delta$ 178.24, 177.82, 169.34, 163.30, 163.07, 162.84, 144.74, 138.89, 135.40, 130.04, 107.54, 102.48, 101.65, 101.47, 100.42, 99.02, 95.53, 84.96, 79.79, 78.29, 76.28, 76.25, 74.95, 74.46, 72.27, 71.86, 71.08, 68.71, 67.31, 61.70, 57.75, 57.62, 57.47, 57.33, 57.19, 56.80, 52.57, 49.85, 49.57, 48.09, 47.29, 43.03,
42.96, 40.95, 40.73, 39.93, 39.89, 39.15, 38.16, 36.53, 34.85, 34.11, 33.50, 33.01, 31.54, 30.14, 29.28, 29.26, 28.87, 28.81, 27.89, 27.52, 26.81, 26.17, 24.57, 24.09, 23.92, 19.56, 18.27, 17.79, 17.54, 17.42, 17.29, 17.16, 17.04, 16.36, 16.07; see below for carbon NMR. HRMS (ESI) m/z: Calcd for C₆₀H₉₁N₂O₁₇INa (M+Na)+ 1261.5260, found 1261.5309.

**Aryl tin precursor to SQS-1-7-5-18 (S9).** (EC-V-222) To a solution of amine S8 (1.3 mg, 1.3 µmol, 1.0 equiv) in N,N'-dimethylformamide (0.2 mL) triethylamine (3.6 µL, 25.6 µmol, 20 equiv) was added followed by 5 (2.6 mg, 6.8 µmol, 5.2 equiv). After stirring at 21 °C for 1.5 h, the reaction was diluted with 3 mL water and directly purified by RP-HPLC using a linear gradient of 30–90% acetonitrile/water, over 30 min, at a flow rate of 5 mL/min to afford S9 (1.0 mg, 62% yield) as a white powder after lyophilization.

**HPLC:** \( t_{\text{ret}} = 22.28 \text{ min}, \lambda_{\text{max}} = 235 \text{ nm}. \) **¹H NMR** (600 MHz, CD₃OD) characteristic resonances: \( \delta \) 7.78–7.71 (m, 2H), 7.64–7.53 (m, 2H), 5.42 (d, \( J = 1.4 \text{ Hz, 1H} \)), 5.40 (d, \( J = 7.7 \text{ Hz, 1H} \)), 5.24 (d, \( J = 3.4 \text{ Hz, 1H} \)), 4.42 (d, \( J = 7.7 \text{ Hz, 1H} \)), 4.34 (d, \( J = 2.2 \text{ Hz, 1H} \)), 4.01–3.90 (m, 3H), 3.89–3.78 (m, 3H), 3.70 (t, \( J = 7.2 \text{ Hz, 1H} \)), 3.54–3.36 (m, 6H), 3.22–3.11 (m, 4H), 2.81 (dd, \( J = 13.7, 3.9 \text{ Hz, 1H} \)), 2.41–2.31 (m, 2H), 2.10–2.01 (m, 1H), 1.30 (d, \( J = 6.2 \text{ Hz, 3H} \)), 1.16 (s, 3H), 0.98 (s, 3H), 0.95 (s, 3H), 0.93–0.89 (m, 6H), 0.81–0.76 (m, 6H), 0.37–0.25 (m, 9H); see below for proton NMR. **¹³C NMR** (151 MHz, CD₃OD) \( \delta \) 176.85, 176.38, 169.03, 161.64, 161.42, 147.06, 143.31, 135.45, 134.05, 126.01, 122.27, 106.11, 100.05, 94.10, 83.54, 78.36, 76.86, 74.84, 73.50, 73.09, 70.85, 70.43, 69.64, 67.28, 65.87, 60.26, 56.18, 56.30, 55.90, 55.37, 51.13, 48.42, 48.14, 46.66, 45.88, 41.60, 41.51, 39.41, 39.30, 38.50, 38.46, 36.73, 35.12, 33.42, 32.69, 32.08, 30.11, 28.80, 27.83, 27.39, 26.45, 26.13, 25.42, 24.74, 23.14, 22.67, 22.49, 18.13, 16.86, 15.99, 15.86, 15.73, 14.93, 14.64, 0.06, 0.04, 0.00, -0.06; see below for carbon NMR. **¹HRMS** (ESI) m/z: Calcd for C₆₃H₁₀₀N₂O₁₇Sn (M+Na)+ 1299.5942, found 1299.5938.
Fully protected aminoacyl caulophylogenin saponin S11. (AFT-I-243). To a solution of S10 (10 mg, 5.4 µmol) in methanol (1.0 mL) NaBH₄ was added, and the reaction was stirred at 21 ºC for 3 h. The mixture was then diluted with acetone (2 mL), concentrated, and purified by silica gel chromatography (85:15 benzene/EtOAc) to afford S11 (10 mg, > 99% yield).

**TLC:** \( R_f \) 0.48 (4:1 benzene/EtOAc).  **IR** (neat film) cm⁻¹ 2949, 2875, 2360, 2341, 1686, 1455, 1365, 1167, 1090, 737.  **¹H NMR** (600 MHz, CDCl₃) characteristic resonances: δ 5.64 (br s, 1H), 5.40 (d, \( J = 7.0 \) Hz, 1H), 5.32–5.27 (m, 1H), 5.24 (s, 1H), 4.91 (d, \( J = 11.1 \) Hz, 1H), 4.79 (d, \( J = 10.9 \) Hz, 1H), 4.72 (d, \( J = 11.7 \) Hz, 1H), 4.67 (d, \( J = 11.1 \) Hz, 1H), 4.62 (d, \( J = 11.7 \) Hz, 1H), 4.19–4.10 (m, 2H), 3.93 (dd, \( J = 11.7, 4.2 \) Hz, 1H), 3.78 (t, \( J = 6.0 \) Hz, 1H), 3.35–3.26 (m, 2H), 2.88 (dd, \( J = 14.2, 3.9 \) Hz, 1H), 2.23–2.11 (m, 1H), 1.46 (s, 3H), 1.35 (s, 3H), 1.27 (s, 3H), 1.16 (d, \( J = 6.2 \) Hz, 3H), 0.86 (s, 3H); see below for proton NMR.  **¹³C NMR** (151 MHz, CDCl₃) δ 175.23, 172.80, 155.91, 143.55, 138.74, 138.62, 138.23, 137.68, 137.41, 128.43, 128.33, 128.29, 128.27, 127.99, 127.93, 127.86, 127.83, 127.79, 127.76, 127.58, 127.53, 121.66, 109.42, 102.31, 97.73, 83.81, 82.02, 79.03, 78.42, 78.13, 77.95, 76.14, 75.57, 75.37, 75.15, 74.94, 74.74, 73.49, 73.20, 72.84, 71.56, 68.32, 67.84, 66.52, 63.75, 49.11, 47.99, 46.82, 46.49, 45.96, 42.70, 41.48, 40.44, 40.33, 39.49, 38.26, 36.69, 36.59, 35.21, 34.58, 32.77, 32.68, 30.89, 30.44, 29.77, 28.42, 27.60, 27.25, 26.36, 26.29, 26.02, 25.32, 24.30, 23.37, 18.20, 17.74, 17.09, 16.14, 12.24, 7.14, 6.96, 5.32, 4.92; see below for carbon NMR.  **HRMS** (ESI) \( m/z \): Calcd for C₁₀₂H₁₅₈N₂₀₂O₂₀Si₂Na (M+Na)⁺ 1882.0844, found 1882.0817.
Aminoacyl caulophylogenin saponin S12. (AFT-I-244) In a 25 mL round-bottom flask, S11 (15 mg, 8.1 µmol, 1.0 equiv) was dissolved in 4.0 mL tetrahydrofuran/ethanol (1:1) and 10% (dry basis) palladium on carbon, wet, Degussa type E101 NE/W (85 mg, 0.04 mmol, 5.0 equiv) was added. The reaction was stirred under hydrogen atmosphere (balloon) at 21 ºC for 12 h, and the suspension was filtered through a 0.45 µm nylon syringe filter, thoroughly washed with methanol (4 × 20 mL) and concentrated. Successful debenzylation is assessed by the disappearance of aromatic resonances by 1H NMR in CD$_3$OD. The crude mixture was then dissolved in a pre-cooled (0 ºC) solution of trifluoroacetic acid (3.2 mL, TFA/H$_2$O 3:1) and stirred at 0 ºC for 1.25 h. The reaction was evaporated to dryness, and the crude product was dissolved in 20% acetonitrile/water (8 mL) and purified via RP-HPLC on an XBridge Prep BEH300 C18 column (5 µm, 10 × 250 mm) using a linear gradient of 20–70% acetonitrile/water (0.05% TFA), over 20 min, at a flow rate of 5 mL/min. The desired product S12 was obtained as a white powder (5.8 mg, 70% yield) after lyophilization.

**HPLC:** $t_{ret} = 12.28$ min, $\lambda_{max} = 210$ nm. **$^1$H NMR** (600 MHz, CD$_3$OD) $\delta$ 5.44 (d, $J = 1.5$ Hz, 1H), 5.35 (d, $J = 8.1$ Hz, 1H), 5.30 (t, $J = 3.4$ Hz, 1H), 4.52–4.47 (m, 2H), 4.36 (dd, $J = 4.7, 1.5$ Hz, 1H), 3.97 (dd, $J = 9.5, 4.7$ Hz, 1H), 3.74–3.69 (m, 1H), 3.61 (dd, $J = 11.5, 4.7$ Hz, 1H), 3.48 (ddd, $J = 10.4, 8.9, 5.4$ Hz, 1H), 3.43 (dd, $J = 11.5, 6.8$ Hz, 1H), 3.26 (dd, $J = 9.1, 7.7$ Hz, 1H), 3.23–3.18 (m, 1H), 2.96–2.89 (m, 3H), 2.42–2.27 (m, 3H), 1.80–1.73 (m, 1H), 1.39 (s, 3H), 1.35 (d, $J = 6.2$ Hz, 3H), 0.99 (s, 3H), 0.94 (s, 3H), 0.88 (s, 3H), 0.77 (s, 3H), 0.71 (s, 3H); see below for proton NMR. **$^{13}$C NMR** (151 MHz, CD$_3$OD) $\delta$ 177.94, 177.06, 162.86, 144.93, 123.55, 107.28, 101.27, 95.64, 84.35, 78.36, 76.42, 76.24, 75.38, 74.83, 74.00, 72.39, 72.04, 71.26, 68.98, 67.47, 67.40, 64.90, 61.83, 52.72, 50.18, 50.00, 49.72, 48.21, 48.18, 43.48, 42.94, 42.48, 40.87, 40.73, 39.78, 38.08, 36.76, 36.71, 36.34, 33.96, 33.52, 32.23, 31.46, 28.45, 27.64, 27.37, 26.94, 26.51, 25.40, 24.96, 24.64, 19.37, 18.50, 17.91, 16.67, 12.95; see below for carbon NMR. **HRMS** (ESI) $m/z$: Caled for C$_{53}$H$_{89}$N$_2$O$_{18}$ (M+H)$^+$ 1041.6110, found 1041.6155.
SQS-1-11-5-18 (19). (AFT-I-245) To a solution of S12 (7.0 mg, 6.7 µmol, 1.0 equiv) in N,N’-dimethylformamide (1.3 mL) was injected triethylamine (20 µL, 0.13 mmol, 20 equiv) followed by dropwise addition of 4 (11.6 mg, 33.6 µmol, 5.0 equiv) in N,N’-dimethylformamide (0.7 mL). After stirring for 3 h, the contents were diluted with 25% acetonitrile/water (10 mL) and purified by RP-HPLC on an XBridge Prep BEH300 C18 column (5 µm, 10 x 250 mm) using a linear gradient of 30–70% acetonitrile/water (0.05% TFA), over 15 min, at a flow rate of 5 mL/min. SQS-1-11-5-18 (19) (5.5 mg, 65% yield) was obtained as a white powder after lyophilization.

**HPLC**: $t_{ret} = 15.03$ min, $\lambda_{max} = 251$ nm. \(^1\)H NMR (600 MHz, CD$_3$OD) characteristic resonances: δ 7.87–7.80 (m, 2H), 7.60–7.53 (m, 2H), 5.42 (s, 1H), 5.35 (d, $J = 6.7$ Hz, 1H), 5.31–5.27 (m, 1H), 4.52–4.45 (m, 2H), 4.36–4.30 (m, 1H), 3.98–3.90 (m, 3H), 3.70 (t, $J = 6.4$ Hz, 1H), 3.61 (dd, $J = 11.0$, 4.5 Hz, 1H), 3.28–3.24 (m, 1H), 3.24–3.18 (m, 1H), 2.96–2.89 (m, 1H), 2.40–2.26 (m, 3H), 1.39 (s, 3H), 1.32 (d, $J = 5.8$ Hz, 3H), 0.99 (s, 3H), 0.94 (s, 3H), 0.88 (s, 3H), 0.77 (s, 3H), 0.70 (s, 3H); see below for proton NMR. \(^{13}\)C NMR (151 MHz, CD$_3$OD) δ 178.38, 177.14, 169.50, 144.91, 139.04, 135.54, 130.19, 123.56, 107.31, 101.48, 99.17, 95.66, 84.47, 78.35, 76.43, 76.27, 75.14, 74.84, 74.48, 74.03, 72.41, 72.05, 71.27, 68.99, 67.48, 67.43, 61.85, 52.70, 50.19, 50.00, 49.72, 48.21, 48.17, 43.48, 42.93, 42.48, 41.10, 40.88, 39.78, 38.08, 36.67, 33.96, 33.55, 32.20, 31.48, 30.29, 27.67, 27.37, 26.95, 25.01, 24.65, 19.38, 18.49, 17.96, 16.69, 12.95; see below for carbon NMR. **HRMS** (ESI) m/z: Calcd for C$_{60}$H$_{91}$N$_2$O$_{19}$INa (M+Na)$^+$ 1293.5159, found 1293.5168.
Bis(silyl ether) of echinocystic acid (S14). (AFT-I-206) Echinocystic acid S13 (18 mg, 38 \( \mu \)mol, 1.0 equiv) was suspended in CH\(_2\)Cl\(_2\) (10 mL) and cooled in an ice bath. 2,6-lutidine (71 \( \mu \)L, 0.61 mmol, 16 equiv) was then added followed by triethylsilyl trifluoromethanesulfonate (69 \( \mu \)L, 0.31 mmol, 8.0 equiv) and the reaction mixture was stirred at 0 °C for 1 h. After this time, the contents were washed with saturated NaHCO\(_3\) (5 mL) and the aqueous phase was extracted with CH\(_2\)Cl\(_2\) (2 \( \times \) 10 mL). The combined organics were dried over Na\(_2\)SO\(_4\), filtered, and concentrated. The crude product was purified by silica gel chromatography (hexanes to 9:1 hexanes/EtOAc) to afford S14 (25 mg, 94% yield).

TLC: \( R_f \) 0.25 (9:1 hexanes/ethyl acetate). IR (neat film) cm\(^{-1}\) 2950, 2876, 2360, 2341, 1699, 1458, 1239, 1110, 1005, 737. \(^1\)H NMR (600 MHz, CDCl\(_3\)) \( \delta \) 5.32 (t, \( J = 3.5 \) Hz, 1H), 4.55 (s, 1H), 3.20 (dd, \( J = 11.4, 4.4 \) Hz, 1H), 2.95 (dd, \( J = 14.3, 4.1 \) Hz, 1H), 2.21 (t, \( J = 13.6 \) Hz, 1H), 1.90–1.80 (m, 4H), 1.79–1.70 (m, 2H), 1.65–1.50 (m, 4H), 1.50–1.41 (m, 2H), 1.37–1.32 (m, 4H), 1.32–1.22 (m, 3H), 1.17–1.10 (m, 1H), 1.07–1.02 (m, 1H), 1.02–0.93 (m, 29H), 0.92–0.89 (m, 6H), 0.88 (s, 3H), 0.74 (s, 3H), 0.70–0.64 (m, 10H), 0.62–0.56 (m, 10H). \(^{13}\)C NMR (151 MHz, CDCl\(_3\)) \( \delta \) 183.04, 143.18, 122.54, 79.52, 75.01, 55.39, 48.61, 46.63, 46.27, 41.17, 40.02, 39.32, 39.25, 38.50, 36.93, 35.12, 34.68, 32.90, 32.70, 31.63, 30.48, 28.43, 27.70, 26.48, 24.23, 23.29, 18.42, 17.05, 16.01, 15.41, 7.11, 7.04, 6.58, 5.75, 5.25, 4.98. LRMS (ESI) m/z: Calcd for C\(_{42}\)H\(_{76}\)O\(_4\)Si\(_2\)Na (M+Na) 723.52, found 723.70.

Protected echinocystic acid saponin azide S19. (AFT-I-212) A solution of S14 (25 mg, 36 \( \mu \)mol, 1.0 equiv) and imidate 12\(^2\) (50 mg, 45 \( \mu \)mol, 1.25 equiv) in CH\(_2\)Cl\(_2\) (5 mL) with 40 mg powdered 4 Å molecular sieves was cooled to –45 °C and boron trifluoride diethyletherate (0.9 \( \mu \)L, 7 \( \mu \)mol, 0.2 equiv) was added. The mixture was stirred at this temperature for 0.5 h min,
quenched with 0.2 mL of triethylamine and concentrated. Purification of the residue by silica gel chromatography (0.2% triethylamine in benzene to 97:3 benzene/EtOAc) gave S19 (48 mg, 80% yield) as a white solid.

**TLC:** \( R_f \) 0.67 (9:1 benzene/EtOAc).  
**IR** (neat film) cm\(^{-1}\) 2950, 2875, 2361, 2342, 2107, 1750, 1507, 1221, 1097, 735.  
**\(^1\)H NMR** (600 MHz, CDCl\(_3\)) \( \delta \) 7.38–7.23 (m, 25H), 5.34 (d, \( J = 8.0 \) Hz, 1H), 5.32 (t, \( J = 3.4 \) Hz, 1H), 5.20 (d, \( J = 2.2 \) Hz, 1H), 4.89 (d, \( J = 11.1 \) Hz, 1H), 4.87–4.80 (m, 3H), 4.74–4.69 (m, 2H), 4.66 (d, \( J = 11.1 \) Hz, 1H), 4.62 (d, \( J = 11.8 \) Hz, 1H), 4.58 (d, \( J = 11.3 \) Hz, 1H), 4.54–4.47 (m, 3H), 4.16 (dd, \( J = 6.2, \) 2.3 Hz, 1H), 4.13–4.10 (m, 1H), 4.03 (d, \( J = 3.6 \) Hz, 1H), 3.70–3.63 (m, 3H), 3.62–3.57 (m, 2H), 3.56–3.49 (m, 3H), 3.31 (dd, \( J = 9.0, \) 7.6 Hz, 1H), 3.22–3.16 (m, 2H), 2.88 (dd, \( J = 14.1, \) 4.0 Hz, 1H), 2.21 (t, \( J = 13.6 \) Hz, 1H), 1.88–1.74 (m, 5H), 1.69–1.59 (m, 3H), 1.55–1.52 (m, 1H), 1.50–1.45 (m, 3H), 1.43 (s, 1H), 1.35–1.32 (m, 3H), 1.31–1.22 (m, 7H), 1.20 (d, \( J = 6.2 \) Hz, 3H), 1.13–1.09 (m, 1H), 1.06–1.00 (m, 1H), 1.00–0.93 (m, 18H), 0.91 (s, 1H), 0.89 (s, 1H), 0.88 (s, 1H), 0.85 (s, 1H), 0.73 (s, 1H), 0.71 (s, 1H), 0.69–0.62 (m, 7H), 0.61–0.55 (m, 6H); see below for proton NMR.  
**\(^{13}\)C NMR** (151 MHz, CDCl\(_3\)) \( \delta \) 175.16, 143.08, 138.75, 138.62, 138.22, 137.47, 136.98, 128.56, 128.50, 128.44, 128.29, 128.23, 128.11, 128.10, 127.99, 127.92, 127.85, 127.80, 127.77, 127.53, 122.18, 120.32, 109.49, 102.34, 97.98, 93.73, 83.80, 81.96, 80.67, 79.50, 78.62, 78.22, 77.95, 75.82, 75.56, 75.09, 74.65, 74.53, 73.59, 73.22, 72.51, 71.85, 67.56, 67.18, 63.75, 58.93, 55.40, 48.95, 46.67, 46.63, 42.55, 41.36, 40.66, 39.46, 39.31, 38.61, 36.89, 35.19, 34.84, 33.08, 32.74, 30.81, 30.43, 29.69, 28.44, 27.73, 27.52, 26.33, 25.84, 24.28, 23.34, 18.49, 17.76, 17.05, 16.09, 15.55, 7.14, 7.05, 5.26, 4.89; see below for carbon NMR.  
**HRMS** (ESI) \( m/z \): Calcd for C\(_{97}\)H\(_{137}\)N\(_3\)O\(_{16}\)Si\(_2\)Na (M+Na\(^+\)) 1678.9435, found 1678.9487.

Protected echinocystic acid saponin amine S31. (AFT-I-214)  To S19 (52 mg, 31 \( \mu \)mol, 1.0 equiv) dissolved in triethylamine (25 mL) was added a freshly prepared solution of phenyl selenol (0.94 mmol, 30 equiv) via cannula. The reaction was stirred at 38 °C for 8 h, and the solution was then concentrated to afford a yellow-white solid. The crude mixture was purified
by silica gel chromatography (9:1 to 4:1 toluene/EtOAc) to afford the amine S31 (42 mg, 83% yield) as a glassy solid.

**TLC:** $R_f$ 0.43 (4:1 toluene/EtOAc). **IR** (neat film) cm$^{-1}$ 2952, 2912, 2877, 1737, 1499, 1457, 1383, 1243, 1076, 914, 737. **$^1$H NMR** (600 MHz, CDCl$_3$) characteristic resonances: δ 5.39 (d, $J = 7.9$ Hz, 1H), 5.32 (t, $J = 3.5$ Hz, 1H), 5.24 (s, 1H), 4.72 (d, $J = 11.7$ Hz, 1H), 3.93 (dd, $J = 11.6$, 4.0 Hz, 1H), 3.83 (t, $J = 8.5$ Hz, 1H), 3.35 (d, $J = 3.6$ Hz, 1H), 3.31 (t, $J = 7.9$ Hz, 1H), 2.90 (dd, $J = 14.2$, 3.8 Hz, 1H), 2.21 (t, $J = 13.5$ Hz, 1H), 1.35 (s, 3H), 1.20 (d, $J = 6.2$ Hz, 3H), 0.92 (s, 3H), 0.89 (s, 3H); see below for proton NMR. **$^{13}$C NMR** (151 MHz, CDCl$_3$) δ 175.28, 143.33, 138.75, 138.61, 138.22, 137.93, 137.48, 128.60, 128.51, 128.42, 128.28, 128.23, 127.96, 127.92, 127.79, 127.76, 127.74, 127.52, 121.98, 109.37, 102.31, 97.87, 94.25, 83.81, 81.98, 81.24, 79.50, 78.54, 78.22, 77.95, 75.95, 75.55, 75.27, 74.69, 74.63, 73.72, 73.39, 73.20, 71.58, 68.09, 66.71, 63.75, 55.39, 53.42, 49.03, 48.60, 46.78, 46.60, 41.40, 40.58, 39.47, 39.30, 38.61, 36.88, 35.23, 34.74, 33.04, 32.73, 30.87, 30.45, 29.69, 28.44, 27.72, 27.60, 26.31, 25.99, 24.33, 23.35, 18.49, 17.85, 17.07, 16.08, 15.57, 7.15, 7.04, 5.26, 4.91, 1.01; see below for carbon NMR. **HRMS** (ESI) $m/z$: Calcd for C$_{97}$H$_{140}$NO$_{16}$Si$_2$ (M+H)$^+$ 1630.9711, found 1630.9741.

**Fully protected aminoacyl echinocystic acid saponin S22.** (AFT-I-215) To a clear, colorless solution of 6-((t-butoxycarbonyl)-amino)hexanoic acid (14) (44 mg, 0.19 mmol, 11.5 equiv) in tetrahydrofuran (2 mL) at 0 °C was added triethylamine (208 µL, 1.49 mmol, 90 equiv) followed by ethyl chloroformate (16.0 µL, 0.17 mmol, 10.0 equiv). The turbid, white solution was stirred at 0 °C for 2.5 h and then added via cannula to amine S31 (27 mg, 16.6 µmol, 1.0 equiv) at 0 °C. The reaction mixture was stirred at this temperature for 1.5 h and then quenched with water (0.2 mL) and concentrated. Purification by silica gel chromatography (9:1 to 5:1 benzene/EtOAc with 0.2% triethylamine) afforded S22 (27 mg, 88% yield) as a white glassy solid.
TLC: $R_f$ 0.13 (9:1 benzene/EtOAc). IR (neat film) cm$^{-1}$ 3031, 2950, 2875, 2360, 2341, 1750, 1686, 1499, 1455, 1366, 1242, 1167, 1075, 912, 735. $^1$H NMR (600 MHz, CDCl$_3$) characteristic resonances: $\delta$ 5.64 (br s, 1H), 5.40 (d, $J = 6.7$ Hz, 1H), 5.30 (t, $J = 3.6$ Hz, 1H), 5.24 (s, 1H), 4.91 (d, $J = 11.0$ Hz, 1H), 4.89–4.77 (m, 5H), 4.72 (d, $J = 11.7$ Hz, 1H), 4.68 (d, $J = 11.0$ Hz, 1H), 4.62 (d, $J = 11.7$ Hz, 1H), 4.55–4.41 (m, 5H), 4.19–4.10 (m, 2H), 3.93 (dd, $J = 11.6$, 4.0 Hz, 1H), 3.78 (t, $J = 6.0$ Hz, 1H), 3.31 (t, $J = 8.1$ Hz, 1H), 3.06–2.97 (m, 2H), 2.90–2.80 (m, 1H), 2.20 (t, $J = 13.9$ Hz, 1H), 2.14 (t, $J = 7.4$ Hz, 1H), 1.36 (s, 3H), 1.16 (d, $J = 6.2$ Hz, 3H), 0.92 (s, 3H), 0.90 (s, 3H), 0.86 (s, 6H), 0.73 (s, 6H); see below for proton NMR. $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 175.27, 172.81, 155.91, 143.54, 138.75, 138.63, 138.24, 137.67, 137.43, 128.43, 128.36, 128.33, 128.29, 128.27, 127.99, 127.93, 127.85, 127.79, 127.76, 127.58, 127.53, 121.78, 109.42, 102.29, 97.74, 83.83, 82.03, 79.43, 79.03, 78.41, 78.14, 77.96, 76.17, 75.58, 75.45, 75.05, 74.75, 73.51, 73.21, 72.85, 71.55, 68.28, 66.51, 63.76, 55.31, 49.14, 46.86, 46.51, 45.96, 41.45, 40.45, 40.33, 39.51, 39.31, 38.58, 36.86, 36.60, 35.23, 34.59, 33.00, 32.69, 30.89, 30.45, 29.77, 28.43, 27.70, 27.60, 26.31, 26.02, 25.32, 24.31, 23.39, 18.51, 17.73, 17.13, 16.10, 15.67, 7.14, 7.05, 5.26, 4.93, 1.02; see below for carbon NMR. HRMS (ESI) m/z: Calcd for C$_{108}$H$_{159}$N$_2$O$_{19}$Si$_2$ (M+H)$^+$ 1844.1076, found 1844.1154.

Aminoacyl echinocystic acid saponin S25. (AFT-I-216) In a 25 mL round-bottom flask containing S22 (24 mg, 13 µmol, 1.0 equiv) was added tetrahydrofuran/ethanol (6 mL, 1:1) and 10% (dry basis) palladium on carbon, wet, Degussa type E101 NE/W (138 mg, 65 µmol, 5.0 equiv). The reaction was stirred under hydrogen atmosphere (balloon) at 21 ºC for 12 h, and then filtered through a 0.45 µm nylon syringe filter, washed with methanol (3 × 10 mL), and concentrated. Successful debenzylation is assessed by the disappearance of aromatic resonances by $^1$H NMR in CD$_3$OD. The crude mixture was then dissolved in a pre-cooled (0 ºC) solution of trifluoroacetic acid (4 mL, TFA/H$_2$O 3:1) and stirred for 1.25 h in an ice bath. The reaction was evaporated to dryness to afford a white solid that was dissolved in 25% acetonitrile/water (12 mL) and purified via RP-HPLC on an XBridge Prep BEH300 C18 column (5 µm, 10 × 250 mm) using a linear gradient of 30–70% acetonitrile/water (0.05% TFA), over 15 min, at a flow rate of
5 mL/min. The aminoacyl echinocystic acid saponin S25 eluted as a single peak and was obtained as a white powder (7.0 mg, 53% yield) after lyophilization.

**HPLC:** $t_{\text{ret}} = 6.32$ min, $\lambda_{\text{max}} = 210$ nm. **1H NMR** (600 MHz, CD$_3$OD) characteristic resonances: $\delta$ 5.43 (d, $J = 1.6$ Hz, 1H), 5.34 (d, $J = 8.1$ Hz, 1H), 5.30 (t, $J = 3.5$ Hz, 1H), 4.52–4.48 (m, 2H), 4.37 (dd, $J = 4.7$, 1.5 Hz, 1H), 3.97 (dd, $J = 9.5$, 4.7 Hz, 1H), 3.93–3.81 (m, 5H), 3.72 (td, $J = 6.6$, 1.5 Hz, 1H), 3.59–3.51 (m, 2H), 3.48 (ddd, $J = 10.4$, 8.9, 5.4 Hz, 1H), 3.43 (dd, $J = 11.5$, 6.8 Hz, 1H), 3.26 (dd, $J = 9.1$, 7.8 Hz, 1H), 3.23–3.18 (m, 1H), 3.15 (dd, $J = 11.4$, 4.6 Hz, 1H), 2.95–2.89 (m, 3H), 2.41–2.27 (m, 3H), 1.38 (s, 3H), 1.35 (d, $J = 6.2$ Hz, 3H), 0.99 (s, 3H), 0.96 (s, 3H), 0.94 (s, 3H), 0.88 (s, 3H), 0.78 (s, 3H), 0.77 (s, 3H); see below for proton NMR. **13C NMR** (151 MHz, CD$_3$OD) $\delta$ 177.92, 177.04, 163.29, 144.87, 123.58, 107.14, 101.22, 95.67, 84.10, 79.89, 78.29, 76.42, 76.28, 75.34, 74.84, 73.94, 72.34, 72.03, 71.27, 68.99, 67.48, 61.83, 57.05, 52.71, 50.18, 50.00, 49.72, 48.27, 48.20, 42.86, 42.50, 40.91, 40.75, 40.55, 40.13, 40.03, 38.32, 36.71, 36.34, 34.47, 33.52, 32.17, 31.46, 28.94, 28.50, 28.06, 27.30, 26.93, 26.50, 24.96, 24.64, 19.75, 18.50, 17.89, 16.52, 16.30; see below for carbon NMR. **HRMS** (ESI) m/z: Calcd for C$_{53}$H$_{89}$N$_2$O$_{17}$ (M+H)$^+$ 1025.6161, found 1025.6188.

S25 [SQS-1-8-5-18] (20). (AF-I-223) S25 (7.0 mg, 6.8 µmol, 1.0 equiv) was dissolved in $N,N'$-dimethylformamide (2 mL) in a 25 mL round-bottom flask and triethylamine (20 µL, 0.14 mmol, 20 equiv) was injected. A solution of 4 (11.8 mg, 34 µmol, 5.0 equiv) in $N,N'$-dimethylformamide (1.5 mL) was added dropwise via syringe and the reaction was stirred at 21 °C in the dark. After 3 h, the contents were diluted with 25% acetonitrile/water (9 mL) and purified via RP-HPLC on an XBridge Prep BEH300 C18 column (5 µm, 10 x 250 mm) using a linear gradient of 30–70% acetonitrile/water (0.05% TFA), over 15 min, at a flow rate of 5 mL/min. SQS-1-8-5-18 (20) (6.8 mg, 80% yield) eluted as a single peak and was obtained as a white powder after lyophilization.
HPLC: \( t_{\text{ret}} = 14.08 \text{ min} \), \( \lambda_{\text{max}} = 251 \text{ nm} \). \(^1\)H NMR (600 MHz, CD\(_3\)OD) characteristic resonances:
\[
\begin{align*}
\delta &
7.87–7.80 (m, 2H), 7.60–7.54 (m, 2H), 5.41 (d, \( J = 1.6 \text{ Hz, } 1H \)), 5.35 (d, \( J = 7.5 \text{ Hz, } 1H \)), 5.30 (t, \( J = 3.5 \text{ Hz, } 1H \)), 4.52–4.47 (m, 2H), 4.34 (dd, \( J = 4.2, 1.7 \text{ Hz, } 1H \)), 3.98–3.90 (m, 3H), 3.89–3.79 (m, 3H), 3.70 (td, \( J = 6.7, 1.4 \text{ Hz, } 1H \)), 3.56 (t, \( J = 9.5 \text{ Hz, } 1H \)), 3.53–3.45 (m, 2H), 3.41 (dd, \( J = 11.5, 6.9 \text{ Hz, } 1H \)), 3.39–3.32 (m, 3H), 3.25 (dd, \( J = 9.1, 7.7 \text{ Hz, } 1H \)), 3.23–3.18 (m, 1H), 2.30 (t, \( J = 13.6 \text{ Hz, } 1H \)), 1.38 (s, 3H), 1.33 (d, \( J = 6.2 \text{ Hz, } 3H \)), 0.98 (s, 3H), 0.96 (s, 3H), 0.94 (s, 3H), 0.88 (s, 3H), 0.78 (s, 3H), 0.77 (s, 3H); see below for proton NMR. \(^{13}\)C NMR (151 MHz, CD\(_3\)OD) \( \delta \)
178.37, 177.13, 169.49, 144.85, 139.04, 135.54, 130.19, 123.59, 107.20, 101.45, 99.17, 95.68, 84.29, 79.92, 78.29, 76.44, 76.30, 75.08, 74.86, 74.48, 72.37, 72.04, 71.28, 69.00, 67.50, 61.85, 57.06, 52.68, 50.19, 50.00, 49.72, 48.28, 48.19, 42.85, 42.51, 41.10, 40.93, 40.14, 40.03, 38.31, 36.67, 34.46, 33.55, 32.14, 31.48, 30.30, 28.95, 28.07, 27.67, 27.31, 26.95, 25.01, 24.66, 19.75, 18.50, 17.94, 16.52, 16.32; see below for carbon NMR. HRMS (ESI) \( m/z \): Calcd for C\(_{60}\)H\(_{91}\)N\(_2\)O\(_{18}\)INa (M+Na)\(^+ \) 1277.5209, found 1277.5273.

Gypsogenin (S16). (AFT-I-218) In a 25 mL roundbottom flask, hederagenin S15 (45 mg, 95 \( \mu \)mol, 1.0 equiv) was suspended in CH\(_2\)Cl\(_2\) (3.5 mL), and an aqueous solution (3.5 mL) of 0.5 M NaHCO\(_3\) (147 mg), 0.05 M K\(_2\)CO\(_3\) (24.2 mg), and tetrabutylammonium chloride hydrate (28 mg, 95 \( \mu \)mol, 1.0 equiv) was then added. To the vigorously stirred mixture, TEMPO (14.8 mg, 95 \( \mu \)mol, 1.0 equiv) was added followed by \( N \)-chlorosuccinimide (38.0 mg, 0.29 mmol, 3.0 equiv) and the reaction was stirred for 2 h in the dark. The contents were partitioned in a separation funnel and extracted with CH\(_2\)Cl\(_2\) (3 \( \times \) 10 mL). The combined organic extracts were dried over Na\(_2\)SO\(_4\), filtered, and concentrated to give a crude product that was purified by silica gel chromatography (hexanes/EtOAc, 7:3) to afford the desired gypsogenin triterpene S16 (32 mg, 72% yield).

TLC: \( R_f 0.30 \) (7:3 hexanes/ethyl acetate). IR (neat film) \( \text{cm}^{-1} \) 3421, 2360, 2341, 1647. \(^1\)H NMR (500 MHz, CDCl\(_3\)) characteristic resonances:
\[
\begin{align*}
\delta &
9.41 (s, 1H), 5.29 (t, \( J = 3.4 \text{ Hz, } 1H \)), 3.77 (dd, \( J = 11.5, 4.4 \text{ Hz, } 1H \)), 2.82 (dd, \( J = 13.7, 3.9 \text{ Hz, } 1H \)), 0.96 (s, 3H), 0.93 (s, 3H), 0.91 (s, 3H), 0.75 (s, 3H). \quad \text{HRMS (ESI) } m/z:\text{ Calcd for C}_{30}\text{H}_{45}\text{O}_{4} (M-H) 469.3318, \text{ found 469.3300.}
\end{align*}
\]

Silyl ether of gypsogenin (S17). (AFT-I-219) A suspension of gypsogenin S16 (32 mg, 68 \( \mu \)mol, 1.0 equiv) in CH\(_2\)Cl\(_2\) (10 mL) was cooled in an ice bath and 2,6-lutidine (63 \( \mu \)L, 0.54 mmol, 8.0 equiv) and triethylsilyl trifluoromethanesulfonate (62 \( \mu \)L, 0.27 mmol, 4.0 equiv)
were injected. After stirring for 1 h, the contents were washed with saturated NaHCO₃ (7 mL) and the aqueous phase was extracted with CH₂Cl₂ (3 × 10 mL). The combined organics were dried (Na₂SO₄), filtered, and concentrated. The crude product was purified several times by silica gel chromatography (hexanes to 4:1 hexanes/EtOAc) to afford S17 (26 mg, 65% yield).

**TLC:** \(R_f 0.43\) (4:1 hexanes/ethyl acetate). \(\text{IR} \) (neat film) cm⁻¹ 2949, 2876, 1733, 1696, 1458, 1240, 1113, 1009, 734. \(\text{¹H NMR} \) (600 MHz, CDCl₃) characteristic resonances: \(\delta\) 9.32 (s, 1H), 5.27 (t, \(J = 3.4\) Hz, 1H), 3.80 (dd, \(J = 11.3, 4.4\) Hz, 1H), 2.81 (dd, \(J = 13.6, 3.9\) Hz, 1H), 1.96 (td, \(J = 13.7, 4.0\) Hz, 1H), 0.94 (s, 4H), 0.92 (s, 3H), 0.89 (s, 3H), 0.72 (s, 3H). \(\text{¹³C NMR} \) (151 MHz, CDCl₃) \(\delta\) 207.65, 183.21, 143.61, 122.31, 73.28, 55.99, 53.42, 47.81, 47.52, 46.45, 45.76, 41.61, 40.98, 39.56, 38.03, 35.86, 33.72, 33.05, 32.38, 31.99, 30.66, 27.57, 26.74, 25.96, 23.54, 23.33, 22.76, 20.57, 17.03, 15.55, 9.48, 6.81, 5.03. \(\text{LRMS} \) (ESI) \(m/z\) Calcd for C₃₆H₆₀O₄SiNa (M+Na) 607.43, found 607.50.

**Protected gypsogenin saponin azide S20.** (AF-I-224) A solution of S17 (26 mg, 44 µmol, 1.0 equiv) and imidate 12 (55 mg, 49 µmol, 1.1 equiv) in CH₂Cl₂ (6 mL) with 40 mg powdered 4 Å molecular sieves was stirred at 21 °C for 30 min and then cooled to –45 °C before injecting boron trifluoride diethyl etherate (1.1 µL, 9 µmol, 0.2 equiv). The reaction was stirred at this temperature for 0.5 h, quenched with triethylamine (0.2 mL) and concentrated. Purification by silica gel chromatography (benzene to 97:3 benzene/EtOAc) gave desired product plus some impure mixture that was further chromatographed to afford S20 (48 mg, 70% yield) as a glassy solid.

**TLC:** \(R_f 0.67\) (9:1 benzene/EtOAc). \(\text{IR} \) (neat film) cm⁻¹ 2934, 2874, 2360, 2341, 2106, 1734, 1507, 1221, 1092, 911, 735. \(\text{¹H NMR} \) (600 MHz, CDCl₃) characteristic resonances: \(\delta\) 9.22 (s, 1H), 5.45 (s, 1H), 5.28 (t, \(J = 3.4\) Hz, 1H), 5.24 (t, \(J = 3.4\) Hz, 1H), 4.90–4.84 (m, 2H), 4.83–4.78 (m, 3H), 4.75–4.68 (m, 3H), 4.65–4.53 (m, 4H), 4.51 (s, 2H), 4.18 (dd, \(J = 7.3, 5.8\) Hz, 1H), 4.06–4.00 (m, 2H), 3.93 (dd, \(J = 11.7, 4.3\) Hz, 1H), 3.75 (dd, \(J = 11.2, 4.4\) Hz, 1H), 3.32–3.28 (m, 1H), 3.19 (dd, \(J = 11.5, 9.4\) Hz, 1H), 2.73 (dd, \(J = 13.8, 4.0\) Hz, 1H), 1.94 (td, \(J = 13.8, 3.5\) Hz, 1H), 1.70 (td, \(J = 14.0, 4.3\) Hz, 1H), 1.47 (s, 3H), 1.31 (s, 3H), 1.23 (d, \(J = 6.2\) Hz, 3H), 0.91 (s, 3H), 0.68 (s, 3H); see below for proton NMR. \(\text{¹³C NMR} \) (151 MHz, CDCl₃) \(\delta\) 207.19, 175.95, ...
143.45, 138.72, 138.36, 138.23, 138.21, 137.51, 136.61, 128.55, 128.53, 128.49, 128.43, 128.32, 128.29, 128.25, 128.23, 128.20, 128.15, 127.99, 127.97, 127.95, 127.91, 127.85, 127.77, 127.75, 127.73, 127.54, 121.83, 109.28, 102.99, 97.60, 93.79, 83.74, 82.06, 81.50, 78.90, 78.41, 77.91, 75.85, 75.59, 74.82, 73.58, 73.10, 73.04, 72.06, 67.57, 65.60, 63.65, 58.79, 55.95, 47.73, 47.46, 46.50, 46.03, 41.73, 41.40, 39.57, 38.08, 35.73, 33.79, 32.97, 32.95, 31.41, 30.57, 29.69, 27.73, 27.51, 26.73, 26.15, 25.52, 23.58, 23.33, 22.98, 20.51, 17.68, 16.95, 15.62, 9.42, 6.82, 5.04; see below for carbon NMR.

**HRMS (ESI) m/z:** Calcd for C₉₁H₁₂₁N₃O₁₆SiNa (M+Na)⁺ 1562.8414, found 1562.8368.

**Protected gypsogenin saponin amine S32.** (AF-I-225) To a solution of S20 (50 mg, 32 µmol, 1.0 equiv) in triethylamine (27 mL) was added a freshly prepared solution of phenyl selenol (1.07 mmol, 32 equiv) via cannula. After stirring at 38 ºC for 8 h, the solution was concentrated to give a yellow-white solid, which was purified by silica gel chromatography (9:1 to 8:2 toluene/EtOAc) to afford S32 (35 mg, 72% yield) as a white solid.

**TLC:** Rf 0.19 (9:1 benzene/EtOAc). **IR** (neat film) cm⁻¹ 2949, 2875, 1751, 1735, 1497, 1455, 1368, 1241, 1221, 1091, 911, 735. **¹H NMR** (500 MHz, CDCl₃) characteristic resonances: δ 9.23 (s, 1H), 5.49 (s, 1H), 5.34 (d, J = 8.1 Hz, 1H), 5.25 (t, J = 3.4 Hz, 1H), 4.92–4.78 (m, 4H), 4.71 (d, J = 11.7 Hz, 1H), 4.68–4.58 (m, 3H), 4.57–4.50 (m, 3H), 4.17–4.10 (m, 2H), 4.00–3.90 (m, 2H), 3.70–3.64 (m, 1H), 3.38 (d, J = 3.3 Hz, 1H), 3.31 (t, J = 3.3 Hz, 1H), 3.21 (d, J = 3.3 Hz, 1H), 3.21 (dd, J = 26.1, 17.9, 7.4 Hz, 1H), 1.24 (d, J = 6.2 Hz, 3H), 0.71 (s, 3H); see below for proton NMR.

**¹³C NMR** (151 MHz, CDCl₃) δ 207.19, 175.93, 143.57, 138.70, 138.33, 138.21, 137.93, 137.24, 128.96, 128.52, 128.39, 128.29, 128.26, 128.24, 127.98, 127.96, 127.93, 127.74, 127.72, 127.70, 127.51, 121.72, 109.19, 102.99, 97.36, 94.19, 83.71, 82.09, 82.04, 78.91, 78.12, 77.88, 75.98, 75.56, 74.81, 73.86, 73.34, 73.07, 73.02, 71.76, 71.05, 68.05, 65.30, 63.62, 55.91, 48.41, 47.72, 47.43, 46.46, 46.06, 41.73, 41.35, 39.55, 38.05, 35.70, 33.82, 32.96, 32.04, 31.47, 30.56, 27.76, 27.50, 26.71, 26.19, 25.51, 23.59, 23.31, 23.07, 20.50, 17.69, 16.95, 15.60, 9.40, 6.80, 5.01; see below for carbon NMR. **HRMS (ESI) m/z:** Calcd for C₉₁H₁₂₄NO₁₆Si (M+H)⁺ 1514.8689, found 1514.8751.
Fully protected aminoacyl gypsogenin saponin S23. (AFT-I-226) To a solution of 14 (61 mg, 0.27 mmol, 11.5 equiv) in tetrahydrofuran (3 mL) at 0 ºC was added triethylamine (290 µL, 2.1 mmol, 90 equiv) followed by ethyl chloroformate (22 µL, 0.23 mmol, 10 equiv), which turned the clear solution turbid white. The acid activation was allowed to proceed for 2.5 h at 0 ºC and the entire solution was cannula transferred into a schlenck containing amine S32 (35 mg, 23 µmol, 1.0 equiv). The reaction mixture was stirred at 0º C for 1.5 h and then quenched with water (90 µL), at which point the solution turned from turbid, white to clear. The contents were then evaporated to dryness and purified by silica gel chromatography (9:1 to 5:1 benzene/EtOAc with 0.2 % triethylamine) to afford S23 (34 mg, 86% yield) as a white glassy solid.

TLC: Rf 0.22 (9:1 benzene/EtOAc). IR (neat film) cm⁻¹ 3337, 2949, 2875, 2360, 2341, 1749, 1717, 1508, 1456, 1365, 1242, 1169, 1089, 1067, 911, 862, 734. ¹H NMR (600 MHz, CDCl₃) characteristic resonances: δ 9.22 (s, 1H), 5.66 (d, J = 9.8 Hz, 1H), 5.50 (s, 1H), 5.36 (d, J = 7.6 Hz, 1H), 5.25 (t, J = 3.4 Hz, 1H), 4.90–4.75 (m, 6H), 4.71 (d, J = 11.8 Hz, 1H), 4.65–4.57 (m, 3H), 4.52 (d, J = 12.0 Hz, 1H), 4.49–4.41 (m, 2H), 4.15–4.08 (m, 2H), 3.91 (dd, J = 11.7, 4.3 Hz, 1H), 3.82 (t, J = 6.3 Hz, 1H), 3.66 (dd, J = 8.8, 4.2 Hz, 1H), 3.30 (dd, J = 8.8, 4.2 Hz, 1H), 3.21–3.15 (m, 1H), 2.93 (td, J = 13.3, 6.7 Hz, 1H), 2.74 (dd, J = 13.7, 4.1 Hz, 1H), 2.64–2.50 (m, 2H), 1.96 (td, J = 13.7, 3.4 Hz, 1H), 1.71 (td, J = 13.9, 4.3 Hz, 1H), 1.49 (s, 3H), 1.46–1.41 (m, 10H), 1.33 (s, 3H), 1.23 (d, J = 6.2 Hz, 3H), 1.02 (s, 3H), 0.88 (s, 3H), 0.86 (s, 3H), 0.69 (s, 3H); see below for proton NMR. ¹³C NMR (151 MHz, CDCl₃) δ 207.27, 175.77, 172.90, 155.96, 143.54, 138.70, 138.31, 138.23, 137.71, 137.18, 128.56, 128.42, 128.40, 128.35, 128.32, 128.29, 128.27, 127.99, 127.88, 127.85, 127.77, 127.73, 127.54, 127.52, 121.77, 109.25, 102.97, 97.42, 94.31, 83.75, 82.10, 79.76, 78.96, 78.88, 78.04, 77.88, 75.96, 75.61, 74.91, 73.42, 73.22, 73.12, 73.08, 72.30, 71.29, 68.16, 65.23, 63.66, 55.88, 47.75, 47.41, 46.53, 46.11, 46.07, 41.77, 41.32, 40.32, 39.55, 38.05, 36.61, 35.73, 33.84, 32.93, 32.04, 31.50, 30.56, 29.74, 28.43, 27.75, 27.47,
Aminoacyl gypsogenin saponin S26. (AF-I-227) In a 25 mL round-bottom flask, S23 (27 mg, 15.5 µmol, 1.0 equiv) was dissolved in 6 mL tetrahydrofuran/ethanol (1:1) and 10% (dry basis) palladium on carbon, wet, Degussa type E101 NE/W (166 mg, 78 µmol, 5 equiv) was added. The reaction was stirred under hydrogen atmosphere (balloon) at 21 ºC for 12 h. After this time, the mixture was filtered through a 0.45 mm nylon syringe filter, washed with methanol (20 mL) and concentrated. Successful debenzylation was assessed by the disappearance of aromatic resonances by 1H NMR in CD3OD. The residue was then dissolved in a pre-cooled (0 ºC) solution of trifluoroacetic acid/water (4 mL, 3:1) and stirred for 1.25 h in an ice bath. After this time, the reaction was evaporated to dryness to give a white solid that was purified by RP-HPLC using a 30–70% acetonitrile/water (0.05% TFA) linear gradient, over 15 min, at a flow rate of 5 mL/min. The desired product S26 (13 mg, 82% yield) was obtained as a white powder after lyophilization.

**HPLC:** t_{ret} = 8.07 min, λ_{max} = 210 nm. **1H NMR** (600 MHz, CD3OD) characteristic resonances: δ 9.31 (s, 1H), 5.43 (d, J = 1.1 Hz, 1H), 5.40 (d, J = 8.0 Hz, 1H), 5.25 (t', J = 3.5 Hz, 1H), 4.41 (d, J = 7.7 Hz, 1H), 4.38–4.34 (m, 1H), 3.98 (dd, J = 9.4, 4.7 Hz, 1H), 3.95–3.88 (m, 2H), 3.87–3.80 (m, 3H), 3.86–3.80 (m, 1H), 3.79–3.74 (m, 1H), 3.73 (t, J = 6.5 Hz, 1H), 3.55–3.40 (m, 4H), 3.20–3.12 (m, 2H), 2.92 (t, J = 7.7 Hz, 2H), 2.81 (dd, J = 13.6, 3.8 Hz, 1H), 2.42–2.31 (m, 2H), 2.11–2.03 (m, 1H), 1.96–1.90 (m, 2H), 1.30 (d, J = 6.2 Hz, 3H), 1.19 (s, 3H), 1.02 (s, 3H), 0.99 (s, 3H), 0.79 (s, 3H); see below for proton NMR. **13C NMR** (151 MHz, CD3OD) δ 208.91, 177.94, 177.87, 144.98, 123.58, 107.63, 101.39, 95.66, 85.03, 78.49, 76.41, 75.33, 74.06, 72.95, 72.40, 71.95, 71.13, 68.84, 67.43, 61.85, 56.92, 52.75, 49.72, 48.22, 47.41, 43.28, 43.10, 41.18, 40.74, 39.59, 37.14, 36.34, 34.99, 33.61, 33.57, 33.11, 31.68, 29.47, 28.46, 27.13, 26.94, 26.52,
26.34, 24.66, 24.19, 24.04, 21.99, 18.38, 16.38, 9.64; see below for carbon NMR. HRMS (ESI) m/z: Calcd for C_{53}H_{87}N_{2}O_{17} (M+H)^{+} 1023.6005, found 1023.6053.

SQS-1-9-5-18 (21). (AF-I-230) In a 25 mL round-bottom flask, amine S26 (6.6 mg, 6.5 µmol, 1.0 equiv) was dissolved in N,N'-dimethylformamide (2.0 mL) and triethylamine (18 µL, 0.13 mmol, 20 equiv) was injected. To this solution, 4 (11.1 mg, 32 µmol, 5.0 equiv) dissolved in N,N'-dimethylformamide (1.5 mL) was added dropwise and the reaction mixture was stirred at 21 ºC for 3 h in the dark. After this time, the contents were diluted with 9 mL 25% acetonitrile/water (0.05% TFA) and directly purified by RP-HPLC using a 30–70% acetonitrile/water (0.05% TFA) linear gradient, over 15 min, at a flow rate of 5 mL/min. SQS-1-9-5-18 (21) (4.5 mg, 56% yield) was obtained as a white powder after lyophilization.

HPLC: t_{ret} = 14.50 min, λ_{max} = 251 nm. ^1H NMR (600 MHz, CD_{3}OD) characteristic resonances: δ 9.31 (s, 1H), 7.84 (d, J = 8.5 Hz, 2H), 7.57 (d, J = 8.5 Hz, 2H), 5.42 (s, 1H), 5.40 (d, J = 7.5 Hz, 1H), 5.25 (t, J = 3.5 Hz, 1H), 4.41 (d, J = 7.7 Hz, 1H), 4.34 (s, 1H), 4.00–3.90 (m, 3H), 3.86–3.73 (m, 4H), 3.71 (t, J = 6.6 Hz, 1H), 3.53–3.39 (m, 4H), 3.37 (t, J = 7.1 Hz, 2H), 3.20–3.13 (m, 2H), 2.81 (dd, J = 13.7, 4.2 Hz, 1H), 2.40–2.31 (m, 2H), 2.06 (dd, J = 15.1, 11.8 Hz, 1H), 1.28 (d, J = 6.3 Hz, 3H), 1.18 (s, 3H), 1.01 (s, 3H), 0.99 (s, 3H), 0.78 (s, 3H); see below for proton NMR. ^13C NMR (151 MHz, CD_{3}OD) δ 208.91, 178.38, 177.93, 169.49, 144.96, 139.04, 135.55, 130.19, 123.59, 107.58, 101.56, 99.17, 95.69, 85.00, 78.47, 76.41, 75.08, 74.49, 72.97, 72.41, 71.98, 71.16, 68.85, 67.45, 61.87, 56.92, 52.72, 50.00, 49.72, 48.20, 47.42, 43.25, 43.10, 41.21, 41.09, 39.60, 37.13, 36.65, 34.96, 33.64, 33.11, 31.70, 30.28, 29.38, 27.67, 27.14, 26.96, 26.37, 24.68, 24.24, 24.03, 21.99, 18.39, 17.90, 16.39, 9.64; see below for carbon NMR. HRMS (ESI) m/z: Calcd for C_{60}H_{89}N_{2}O_{18}INa (M+Na)^{+} 1275.5053, found 1275.5088.
Bis(silyl ether) of hederagenin (S18). (AFT-I-228) Hederagenin S15 (35 mg, 74 µmol, 1.0 equiv) was suspended in CH$_2$Cl$_2$ (15 mL) and cooled in an ice bath. 2,6-lutidine (138 µL, 1.18 mmol, 16 equiv) was then added followed by triethylsilyl trifluoromethanesulfonate (134 µL, 0.59 mmol, 8.0 equiv) and the reaction mixture was stirred at 0 ºC for 1 h. After this time, the contents were washed with saturated NaHCO$_3$ (10 mL) and the aqueous phase was extracted with CH$_2$Cl$_2$ (2 × 15 mL). The combined organics were dried (Na$_2$SO$_4$), filtered, and concentrated. The crude product was purified several times by silica gel chromatography (hexanes to 4:1 hexanes/EtOAc) to afford S18 (45 mg, 81% yield).

TLC: $R_f$ 0.35 (4:1 hexanes/ethyl acetate). IR (neat film) cm$^{-1}$ 2951, 2876, 2360, 2341, 1696, 1458, 1238, 1101, 1008, 822, 740. $^1$H NMR (600 MHz, CDCl$_3$) δ 5.27 (t, $J$ = 3.4 Hz, 1H), 3.72 (dd, $J$ = 11.6, 4.5 Hz, 1H), 3.41 (d, $J$ = 9.7 Hz, 1H), 3.19 (d, $J$ = 9.6 Hz, 1H), 2.80 (dd, $J$ = 13.1, 3.4 Hz, 1H), 1.97 (td, $J$ = 13.5, 3.8 Hz, 1H), 1.11 (s, 1H), 0.96 (d, $J$ = 3.0 Hz, 2H), 0.93 (s, 1H), 0.89 (s, 1H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 183.28, 143.53, 122.74, 71.81, 64.24, 47.61, 46.52, 45.98, 45.86, 43.21, 41.60, 40.95, 39.19, 38.07, 36.52, 33.30, 32.35, 32.04, 30.64, 29.69, 27.62, 27.24, 25.71, 23.54, 23.40, 22.96, 17.94, 17.21, 15.67, 12.57, 7.08, 7.03, 6.58, 5.76, 5.41, 4.48. LRMS (ESI) m/z: Calcd for C$_{42}$H$_{76}$O$_4$Si$_2$Na (M+Na) 723.53, found 723.70.

Protected hederagenin saponin azide S21. (AFT-I-232) A solution of S18 (28 mg, 40 µmol, 1.1 equiv) and imidate 12 (41 mg, 36.7 µmol, 1.0 equiv) in CH$_2$Cl$_2$ (5 mL) with 35 mg powdered 4 Å molecular sieves was cooled to −45 ºC and boron trifluoride diethyl etherate (0.9 µL, 7 µmol, 0.2 equiv) was added. The mixture was stirred at this temperature for 0.5 h, quenched with 0.2 mL of triethylamine and concentrated. Purification of the residue by silica gel
chromatography (0.2% triethylamine in benzene to 98:2 benzene/EtOAc) afforded S21 (43 mg, 71% yield) as a glassy solid.

**TLC:** $R_f$ 0.72 (9:1 benzene/EtOAc). **IR** (neat film) cm$^{-1}$ 3030, 2951, 2875, 2106, 1749, 1456, 1364, 1239, 1093, 911, 822, 735. **$^1$H NMR** (600 MHz, CDCl$_3$) characteristic resonances: $\delta$ 5.40–5.35 (m, 2H), 5.27 (t, $J = 3.4$ Hz, 1H), 4.92–4.80 (m, 4H), 4.74–4.70 (m, 2H), 4.57 (d, $J = 11.4$ Hz, 1H), 4.15 (dd, $J = 5.9$, 1.3 Hz, 1H), 4.14–4.10 (m, 1H), 4.06–4.03 (m, 1H), 4.00–3.96 (m, 1H), 3.93 (dd, $J = 11.6$, 4.4 Hz, 1H), 3.38 (d, $J = 9.8$ Hz, 1H), 3.30 (dd, $J = 8.8$, 7.7 Hz, 1H), 3.23–3.17 (m, 1H), 3.15 (d, $J = 9.6$ Hz, 1H), 2.76 (dd, $J = 13.7$, 4.0 Hz, 1H), 1.75 (td, $J = 14.0$, 4.3 Hz, 1H), 1.47 (s, 3H), 1.24 (d, $J = 6.2$ Hz, 3H), 1.06 (s, 3H), 0.73 (s, 3H); see below for proton NMR. **$^{13}$C NMR** (151 MHz, CDCl$_3$) $\delta$ 176.21, 143.11, 138.76, 138.57, 138.21, 137.51, 136.79, 128.58, 128.51, 128.48, 128.46, 128.41, 128.31, 128.24, 128.14, 128.03, 127.97, 127.96, 127.91, 127.88, 127.77, 127.74, 127.73, 127.53, 127.48, 122.45, 109.25, 102.48, 97.88, 93.68, 83.79, 81.92, 81.29, 78.40, 78.06, 77.97, 75.80, 75.53, 74.61, 73.59, 73.43, 73.16, 72.20, 71.88, 71.75, 67.62, 66.03, 64.22, 63.73, 58.75, 47.56, 46.68, 45.95, 43.19, 41.75, 41.51, 39.33, 38.13, 36.43, 33.84, 33.02, 32.27, 31.56, 30.57, 27.65, 27.54, 27.23, 26.05, 25.38, 23.59, 23.39, 23.19, 18.06, 17.79, 17.14, 15.76, 12.57, 7.07, 7.05, 5.42, 4.48; see below for carbon NMR. **HRMS** (ESI) $m/z$: Caled for C$_{97}$H$_{137}$N$_3$O$_{16}$Si$_2$Na (M+Na)$^+$ 1678.9435, found 1678.9386.

**Protected hederagenin saponin amine S33.** (AFT-I-233) To S21 (44 mg, 26.5 µmol, 1.0 equiv) dissolved in triethylamine (24 mL) was added a freshly prepared solution of phenyl selenol (0.80 mmol, 30 equiv) via cannula. The reaction was stirred at 38 ºC for 8 h and the solution was then concentrated to afford a yellow-white solid. The crude mixture was purified by silica gel chromatography (9:1 to 4:1 toluene/EtOAc) to afford the amine S33 (34.5 mg, 80% yield) as a glassy solid.

**TLC:** $R_f$ 0.29 (85:15 benzene/EtOAc). **IR** (neat film) cm$^{-1}$ 3031, 2950, 2875, 2360, 2341, 1749, 1497, 1456, 1383, 1240, 1092, 911, 823, 741. **$^1$H NMR** (600 MHz, CDCl$_3$) characteristic resonances: $\delta$ 5.45–5.40 (m, 2H), 5.27 (t, $J = 3.4$ Hz, 1H), 4.93–4.80 (m, 4H), 4.72 (d, $J = 11.7$ Hz, 1H), 3.92–3.87 (m, 1H), 3.83–3.78 (m, 4H), 3.67 (s, 3H), 3.30 (s, 3H), 2.76 (d, $J = 11.7$ Hz, 1H), 1.74 (s, 3H), 1.18 (s, 3H), 0.88 (s, 3H); see below for proton NMR. **$^{13}$C NMR** (151 MHz, CDCl$_3$) $\delta$ 176.21, 143.11, 138.76, 138.57, 138.21, 137.51, 136.79, 128.58, 128.51, 128.48, 128.46, 128.41, 128.31, 128.24, 128.14, 128.03, 127.97, 127.96, 127.91, 127.88, 127.77, 127.74, 127.73, 127.53, 127.48, 122.45, 109.25, 102.48, 97.88, 93.68, 83.79, 81.92, 81.29, 78.40, 78.06, 77.97, 75.80, 75.53, 74.61, 73.59, 73.43, 73.16, 72.20, 71.88, 71.75, 67.62, 66.03, 64.22, 63.73, 58.75, 47.56, 46.68, 45.95, 43.19, 41.75, 41.51, 39.33, 38.13, 36.43, 33.84, 33.02, 32.27, 31.56, 30.57, 27.65, 27.54, 27.23, 26.05, 25.38, 23.59, 23.39, 23.19, 18.06, 17.79, 17.14, 15.76, 12.57, 7.07, 7.05, 5.42, 4.48; see below for carbon NMR. **HRMS** (ESI) $m/z$: Caled for C$_{97}$H$_{137}$N$_3$O$_{16}$Si$_2$Na (M+Na)$^+$ 1678.9435, found 1678.9386.
Hz, 1H), 4.68–4.61 (m, 3H), 3.97–3.89 (m, 1H), 3.55 (dd, \(J = 9.1, 3.9\) Hz, 1H), 3.31 (dd, \(J = 8.9, 7.7\) Hz, 1H), 3.16 (d, \(J = 9.6\) Hz, 1H), 2.78 (dd, \(J = 13.7, 4.1\) Hz, 1H), 1.96 (dt, \(J = 19.1, 5.1\) Hz, 1H), 1.78 (td, \(J = 14.0, 4.4\) Hz, 1H), 1.48 (s, 3H), 1.29 (s, 3H), 1.24 (d, \(J = 6.1\) Hz, 3H), 1.07 (s, 3H), 0.75 (s, 3H), 0.53 (s, 3H); see below for proton NMR. ¹³C NMR (151 MHz, CDCl₃) \(\delta\) 176.25, 143.31, 138.76, 138.21, 137.96, 137.31, 128.97, 128.50, 128.40, 128.23, 127.98, 127.95, 127.87, 127.85, 127.76, 127.71, 127.51, 127.46, 122.29, 109.16, 102.46, 97.65, 94.13, 83.79, 81.95, 81.84, 79.03, 78.36, 78.08, 77.97, 75.97, 75.52, 74.63, 73.76, 73.42, 73.40, 73.14, 71.74, 71.22, 68.17, 65.68, 64.21, 63.73, 48.41, 47.54, 46.67, 46.07, 45.93, 43.18, 41.79, 41.45, 39.33, 38.12, 36.42, 33.90, 33.01, 32.25, 31.64, 30.56, 27.70, 27.51, 27.22, 26.16, 25.37, 23.61, 23.40, 23.37, 18.07, 17.82, 17.18, 15.76, 12.56, 7.07, 7.04, 5.41, 4.47; see below for carbon NMR. HRMS (ESI) \(m/z\): Calcd for C₉₇H₁₄₀NO₁₆Si₂ (M+H)+ 1630.9711, found 1630.9722.

Fully protected aminoacyl hederagenin saponin S24. (AFT-I-234) To a solution of 6-((t-butoxycarbonyl)-amino)hexanoic acid (14) (56 mg, 0.24 mmol, 11.5 equiv) in tetrahydrofuran (2.5 mL) at 0 ºC was added triethylamine (263 µL, 1.89 mmol, 90 equiv) followed by ethyl chloroformate (20.0 µL, 0.21 mmol, 10.0 equiv). The turbid, white solution was stirred at 0 ºC for 2.5 h and then added via cannula to amine S33 (34.5 mg, 21.0 µmol, 1.0 equiv) at 0 ºC. The reaction mixture was stirred at this temperature for 1.5 h and then quenched with water (0.2 mL) and concentrated. Purification by silica gel chromatography (9:1 to 5:1 benzene/EtOAc with 0.2% triethylamine) afforded S24 (36.5 mg, 92% yield) as a white solid.

TLC: \(R_f\) 0.29 (85:15 benzene/EtOAc). IR (neat film) cm⁻¹ 3031, 2951, 2875, 2360, 2341, 1749, 1685, 1508, 1456, 1365, 1241, 1169, 1091, 911, 822, 735. ¹H NMR (600 MHz, CDCl₃) characteristic resonances: δ 5.70 (d, \(J = 9.3\) Hz, 1H), 5.45 (d, \(J = 7.1\) Hz, 1H), 5.39 (s, 1H), 5.25 (t, \(J = 3.4\) Hz, 1H), 4.78 (d, \(J = 10.9\) Hz, 1H), 4.71 (d, \(J = 11.7\) Hz, 1H), 4.66 (d, \(J = 11.0\) Hz, 1H), 4.61 (d, \(J = 11.7\) Hz, 1H), 3.92 (dd, \(J = 11.7, 4.3\) Hz, 1H), 3.82 (t, \(J = 5.8\) Hz, 1H), 3.78 (t, \(J = 7.7\) Hz, 1H), 3.38 (d, \(J = 9.8\) Hz, 1H), 3.29 (t, \(J = 8.2\) Hz, 1H), 3.15 (d, \(J = 9.6\) Hz, 1H), 2.75
(dd, $J = 13.6, 4.1$ Hz, 1H), 2.18–2.11 (m, 2H), 1.75 (td, $J = 13.9, 4.1$ Hz, 1H), 1.47 (s, 3H), 1.22 (d, $J = 6.0$ Hz, 3H), 1.07 (s, 3H), 0.74 (s, 3H); see below for proton NMR. $^{13}$C NMR (151 MHz, CDCl$_3$) δ 176.16, 172.83, 155.93, 143.44, 138.77, 138.56, 138.23, 137.71, 137.27, 128.44, 128.42, 128.33, 128.28, 128.26, 128.02, 127.90, 127.88, 127.77, 127.73, 127.58, 127.49, 122.21, 110.24, 97.62, 83.83, 82.01, 79.34, 78.98, 78.31, 77.99, 76.02, 75.56, 74.72, 73.52, 73.17, 72.85, 71.71, 71.39, 68.42, 65.58, 64.21, 63.76, 47.50, 46.74, 46.14, 45.91, 43.20, 41.86, 41.37, 40.33, 39.35, 38.12, 36.62, 36.42, 33.93, 32.98, 32.23, 31.66, 30.57, 29.76, 28.43, 27.71, 27.47, 27.21, 26.21, 25.36, 23.59, 23.44, 18.09, 17.83, 17.25, 15.82, 12.59, 7.08, 7.05, 5.42, 4.48; see below for carbon NMR. HRMS (ESI) m/z: Calcd for C$_{108}$H$_{158}$N$_2$O$_{19}$Si$_2$Na (M+Na)$^+$ 1866.0895, found 1866.0935.

Aminoacyl hederagenin saponin S27. (AFT-I-235) In a 25 mL round-bottom flask, S24 (30 mg, 16.3 µmol, 1.0 equiv) was dissolved in tetrahydrofuran/ethanol (7 mL, 1:1) and 10% (dry basis) palladium on carbon, wet, Degussa type E101 NE/W (173 mg, 81 µmol, 5.0 equiv) was added. The reaction was stirred under hydrogen atmosphere (balloon) at 21 ºC for 12 h, and then filtered through a 0.45 µm nylon syringe filter, washed with methanol (3 × 10 mL) and concentrated. Successful debenzylation is assessed by the disappearance of aromatic resonances by $^1$H NMR in CD$_3$OD. The crude mixture was then dissolved in a pre-cooled (0 ºC) solution of trifluoroacetic acid (4 mL, TFA/H$_2$O 3:1) and stirred for 1.25 h in an ice bath. The reaction was evaporated to dryness and the white solid was dissolved in 30% acetonitrile/water (0.05% TFA) (14 mL) and purified via RP-HPLC on an XBridge Prep BEH300 C18 column (5 µm, 10 × 250 mm) using a linear gradient of 30–70% acetonitrile/water (0.05% TFA), over 15 min, at a flow rate of 5 mL/min. The desired product S27 was obtained as a white powder (11.0 mg, 66% yield) after lyophilization.

HPLC: $t_{ret} = 5.75$ min, $\lambda_{max} = 210$ nm. $^1$H NMR (600 MHz, CD$_3$OD) characteristic resonances: δ 5.46 (s, 1H), 5.40 (d, $J = 8.0$ Hz, 1H), 5.24 (t, $J = 3.4$ Hz, 1H), 4.42 (d, $J = 7.6$ Hz, 1H), 4.36 (dd, $J = 4.5, 1.3$ Hz, 1H), 3.98 (dd, $J = 9.5, 4.7$ Hz, 1H), 3.73 (t, $J = 6.5$ Hz, 1H), 3.61 (dd, $J = 11.7, 8.0$ Hz, 1H), 3.47 (dd, $J = 11.7, 4.5$ Hz, 1H), 3.24 (d, $J = 13.6$ Hz, 1H), 2.18–2.11 (m, 2H), 1.75 (td, $J = 13.9, 4.1$ Hz, 1H), 1.47 (s, 3H), 1.22 (d, $J = 6.0$ Hz, 3H), 1.07 (s, 3H), 0.74 (s, 3H); see below for proton NMR. 13C NMR (151 MHz, CDCl$_3$) δ 176.16, 172.83, 155.93, 143.44, 138.77, 138.56, 138.23, 137.71, 137.27, 128.44, 128.42, 128.33, 128.28, 128.26, 128.02, 127.90, 127.88, 127.77, 127.73, 127.58, 127.49, 122.21, 110.24, 97.62, 83.83, 82.01, 79.34, 78.98, 78.31, 77.99, 76.02, 75.56, 74.72, 73.52, 73.17, 72.85, 71.71, 71.39, 68.42, 65.58, 64.21, 63.76, 47.50, 46.74, 46.14, 45.91, 43.20, 41.86, 41.37, 40.33, 39.35, 38.12, 36.62, 36.42, 33.93, 32.98, 32.23, 31.66, 30.57, 29.76, 28.43, 27.71, 27.47, 27.21, 26.21, 25.36, 23.59, 23.44, 18.09, 17.83, 17.25, 15.82, 12.59, 7.08, 7.05, 5.42, 4.48; see below for carbon NMR. HRMS (ESI) m/z: Calcd for C$_{108}$H$_{158}$N$_2$O$_{19}$Si$_2$Na (M+Na)$^+$ 1866.0895, found 1866.0935.
4.5 Hz, 1H), 3.25–3.20 (m, 1H), 2.91–2.86 (m, 2H), 2.81 (dd, \( J = 13.9, 4.0 \) Hz, 1H), 2.41–2.31 (m, 2H), 2.07 (td, \( J = 13.6, 3.3 \) Hz, 1H), 1.31 (d, \( J = 6.2 \) Hz, 3H), 1.17 (s, 3H), 0.98 (s, 3H), 0.93–0.90 (m, 2H), 0.78 (s, 3H), 0.70 (s, 3H); see below for proton NMR. \(^{13}\)C NMR (151 MHz, CD\(_3\)OD) \( \delta \) 177.95, 144.99, 123.80, 107.78, 101.44, 95.65, 85.25, 78.55, 76.36, 75.44, 74.07, 73.85, 72.47, 71.99, 71.23, 68.78, 67.42, 67.15, 61.85, 52.79, 50.00, 49.72, 48.29, 47.42, 43.52, 43.29, 43.05, 40.81, 39.73, 38.07, 36.40, 35.02, 33.82, 33.62, 33.17, 31.68, 29.60, 27.62, 27.04, 26.61, 26.35, 24.71, 24.19, 24.01, 19.30, 18.39, 17.93, 16.57, 12.96; see below for carbon NMR. HRMS (ESI) \( m/z \): Calcd for C\(_{53}\)H\(_{89}\)N\(_2\)O\(_{17}\) (M+H\(^+\)) 1025.6161, found 1025.6149.

SQS-1-10-5-18 (22). (AF-I-236) S27 (6.0 mg, 5.8 \( \mu \)mol, 1.0 equiv) was dissolved in \( N,N' \)-dimethylformamide (2.5 mL) in a 25 mL round-bottom flask and triethylamine (16.3 \( \mu \)L, 0.12 mmol, 20 equiv) was injected. A solution of 4 (10.1 mg, 29 \( \mu \)mol, 5.0 equiv) in \( N,N' \)-dimethylformamide (1.5 mL) was added dropwise via syringe and the reaction was stirred at 21 °C for 3 h. After this time, the contents were diluted with 25% acetonitrile/water (9 mL) and purified via RP-HPLC on an XBridge Prep BEH300 C18 column (5 \( \mu \)m, 10 x 250 mm) using a linear gradient of 30–70% acetonitrile/water (0.05% TFA), over 15 min, at a flow rate of 5 mL/min. SQS-1-10-5-18 (22) (4.2 mg, 57% yield) was obtained as a white powder after lyophilization.

HPLC: \( t_{\text{ret}} = 15.62 \) min, \( \lambda_{\text{max}} = 251 \) nm. \(^{1}\)H NMR (600 MHz, CD\(_3\)OD) characteristic resonances: \( \delta \) 7.87–7.80 (m, 2H), 7.60–7.53 (m, 2H), 5.45 (d, \( J = 1.5 \) Hz, 1H), 5.40 (d, \( J = 7.6 \) Hz, 1H), 5.24 (t, \( J = 3.5 \) Hz, 1H), 4.42 (d, \( J = 7.7 \) Hz, 1H), 4.36–4.32 (m, 1H), 4.00–3.92 (m, 1H), 3.87–3.79 (m, 3H), 3.71 (t, \( J = 7.2 \) Hz, 1H), 3.60 (dd, \( J = 11.7, 4.6 \) Hz, 1H), 3.22 (dd, \( J = 9.0, 7.8 \) Hz, 1H), 3.18 (t, \( J = 11.0 \) Hz, 1H), 2.81 (dd, \( J = 13.8, 4.2 \) Hz, 1H), 2.40–2.31 (m, 2H), 2.10–2.02 (m, 1H), 1.93–1.86 (m, 2H), 1.29 (d, \( J = 6.2 \) Hz, 3H), 1.17 (s, 3H), 0.98 (s, 3H), 0.94–0.87 (m, 2H), 0.78
(s, 3H), 0.70 (s, 3H); see below for proton NMR. $^{13}$C NMR (151 MHz, CD$_3$OD) $\delta$ 178.39, 177.98, 169.48, 144.98, 139.04, 135.55, 130.18, 123.79, 107.72, 101.57, 99.17, 95.67, 85.20, 78.52, 76.42, 76.36, 75.22, 74.42, 73.90, 72.48, 72.01, 71.24, 68.79, 67.43, 67.21, 61.86, 52.76, 50.00, 49.72, 48.27, 47.44, 43.50, 43.26, 43.04, 41.09, 40.82, 39.73, 38.07, 36.65, 35.01, 33.81, 33.65, 33.16, 31.68, 30.28, 29.53, 27.67, 27.62, 26.96, 26.38, 24.72, 24.23, 24.00, 19.30, 18.40, 17.96, 16.58, 12.95; see below for carbon NMR. HRMS (ESI) m/z: Calcd for C$_{60}$H$_{91}$N$_2$O$_{18}$INa (M+Na)$^+$ 1277.5209, found 1277.5261.
C. BIOLOGICAL EVALUATION OF SAPONINS IN PRECLINICAL MOUSE VACCINATION MODEL

Preparation of GD3 KLH conjugate. GD3 was extracted from bovine buttermilk and conjugated to keyhole limpet hemocyanin (KLH) as described previously\textsuperscript{6}. The double bond of GD3–ceramide was converted to aldehyde by ozonolysis and the aldehyde group was conjugated to ε-amino groups of lysine on KLH by reductive amination. Briefly, conjugation method is as follows, 50 mg of GD3 was dissolved in 5 mL methanol and cooled in an ethanol-dry ice bath. Ozone was generated by an ozone generator (Del Industries, San Luis Obispo, CA) and passed through the sample for 20 min. Methyl sulfide (1 mL) was added, and the sample was stirred at room temperature for 60 min. After this time, the sample was dried under reduced pressure. The free fatty aldehydes were removed by treating sample with n-hexane. 100 mg KLH and 20 mg of sodium cyanoborohydride were added to GD3-aldehyde and the mixture was incubated at 37 °C for 48 h. Unreacted GD3 was removed by a molecular cut-off filter Centriprep 30 (MW 30000, Millipore, Billerica, MA). KLH content was determined using the Bio-Rad dye-binding method according to the manufacturer’s instructions and GD3 content by estimating sialic acid as described by Svennerholm\textsuperscript{7}. The epitope ratio of GD3-KLH was found to be 849/1.

Preparation of MUC1 KLH conjugate. MUC1 peptide containing 33 amino acids: CHGVTSAPDTAPGSTAPHAHVTSAPDTAP–OH, (synthesized at MSKCC’s Microchemistry Core facility) was covalently conjugated to KLH (Sigma Chemical Co., St Louis, MO) using an MBS (m-maleimidobenzoyl-N-hydroxysuccinimide ester) linker as previously described\textsuperscript{8}. Briefly, 5 mg MBS in 70 µl dimethylformamide (Sigma Chemical Co., St Louis, MO) was added to 9 mg KLH in 1 ml 0.01 M phosphate buffer, pH 7.0. After an hour incubation at room temperature, the MBS activated KLH was separated using a Sephadex G 15 column equilibrated with 0.1 M phosphate buffer (pH 6.0), and stirred with 5 mg MUC1 peptide for 2 h at room temperature. The unconjugated peptide was separated using a Centriprep 30. The epitope ratio of MUC1:KLH was 1367:1 (calculated based on the initial amount of peptide and KLH, the amount of unconjugated peptide in the filtrate, and a KLH molecular weight of 8.6×10\textsuperscript{6} Da).

Vaccination of mice. Groups of five mice (C57BL/6J, female, 6-8 weeks old) were vaccinated three times with indicated combinations of GD3–KLH conjugate (5 µg equivalent of GD3), MUC1-KLH (2.5 µg equivalent of MUC1), and/or OVA (20 µg, Sigma Chemical Co., St Louis, MO) in 100 µL phosphate buffered saline either alone (without adjuvant), with natural QS-21 adjuvant (NQS-21), with synthetic QS-21 (SQS-21), or other saponins at indicated doses. Vaccines were administered subcutaneously to each mouse on days 0, 7, 14, and 65. Mice were bled 7 days after the third and fourth vaccinations.

Measurement of immunological response. The presence of antibodies was tested by an enzyme-linked immunosorbent assay (ELISA). ELISAs were performed to determine antibody response against GD3, MUC1, OVA, and/or KLH as described previously\textsuperscript{13}. The ELISA plates were coated with either GD3 antigen at 0.2 µg/well in ethanol, MUC1 antigen at 0.1 µg/well in carbonate buffer (pH 10), or KLH at 0.1 µg/well in carbonate buffer (pH 10). The GD3-coated plates were kept overnight at room temperature to evaporate ethanol, while MUC1 or KLH coated plates were incubated at 4°C overnight. ELISA plates were washed, blocked with 1%
human serum albumin (HSA) in phosphate-buffered saline containing 0.05% Tween 20. Serially
diluted pre- and postvaccination sera in PBS with 1% HSA were added to wells of the coated
plate with appropriate controls and incubated for 1 h at room temperature. After wash, goat anti-
mouse IgM or IgG conjugated with alkaline phosphatase (Southern Biotechnology Associates,
Inc., Birmingham, AL) was added to each well. Absorbance was measured at 405 nm. The titer
was defined as the highest serum dilution that showed an absorbance of 0.1 or greater over that
of the pre-sera.

**IgG subclass.** IgG subclasses were determined by using subclass-specific antibodies conjugated
with alkaline phosphatase (AP) (Southern Biotech, Birmingham, AL, USA). Flat-bottomed
ELISA plates (96 wells, Nunc, Rochester, NY, USA) were pre-coated with MUC1 or OVA
antigens, respectively, at 0.1 µg per well in carbonate buffer. Mouse sera diluted at 1:80 in 1%
human serum albumin (HSA, Grifols Biologicals, Los Angeles, CA, USA) were added with
positive and negative controls. These wells were probed using class-specific IgG antibodies such
as IgG1, IgG2a, IgG2b and IgG3, conjugated with AP at 1:400 dilutions, respectively. The
samples were measured at the end of 30 minutes at 405 nm followed by substrate solution.
Samples were considered positive if the absorbance was above 0.1 OD at 405nm.
D. Radiolabeling of Saponin Adjuvants for Biodistribution Studies

Method for Radiolabeling of Saponin Adjuvants. An aryl tin-halide exchange protocol using NaI as iodide source and the mild oxidant Chloramine-T was employed for the radiolabeling of the saponin adjuvants. To test the iodination conditions, aryl tin precursors were first reacted with the stable iodide isotope, iodide-127 (from NaI) and Chloramine-T, to give corresponding aryl iodides without observed oxidation of the free hydroxyl groups in >99% yield (as assessed by HPLC). The successful protocol was then adapted for the radioiodination of all the saponin variants with β-emitter, iodide-131 (from NaI) for biodistribution studies.

Scheme 1. Method of radioiodination of saponin adjuvants for biodistribution studies
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F. \(^1\)H NMR AND \(^{13}\)C NMR SPECTRA

Synthesis of Iodinated and Radiolabeled Saponin Adjuvants

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Supplementary Information
