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Supporting Information

69451 Weinheim, Germany

SUPPORTING MATERIAL

Synthesis of the Xylose-Isomer of the Clinical Adjuvant QS-21. Establishing Immuno-Potentiating Activity of Synthetic QS-21 (sQS-21) with GD3-KLH Conjugate Melanoma Vaccine.

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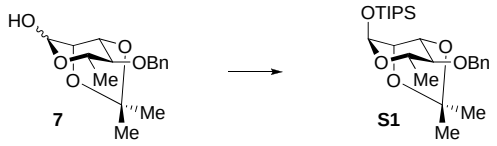
SYNTHESIS OF QS-21-XYL

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 and sulfoxide 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).

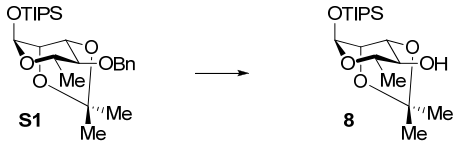
Materials. Lyophilized QS saponin Quil-A (batch L77-244) was obtained from Brenntag Biosector (Frederikssund, Denmark) via distribution by Accurate Chemical and Scientific Corporation (Westbury, NY). Dichloromethane, tetrahydrofuran, diethyl ether, and toluene were purified by passage through two packed 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. 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 (^{1}H NMR and ^{13}C NMR) spectra were recorded on a Varian 400, a Varian 500, a Varian Inova 500, or a Bruker Avance III instrument;

chemical shifts are expressed in parts per million (δ scale) downfield from tetramethylsilane and are referenced to the residual protium in the NMR solvent ($CHCl_3$: δ 7.26 for ^{1}H NMR, δ 77.16 for ^{13}C NMR). Data are presented as follows: chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, bd = broad doublet, t = triplet, q = quartet, m = multiplet and/or multiple resonances), coupling constant in Hertz (Hz), integration, assignment. 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 a wavelength of 214 nm.

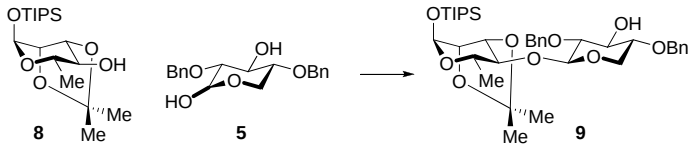


***O*-Triisopropyl 4-*O*-benzyl-2,3-di-*O*-isopropylidene-α-L-rhamnopyranoside (S1).** To a solution of rhamnopyranoside **7**[1] (6.00 g, 20.4 mmol, 1.00 equiv) in dichloromethane (100 mL) at 0 °C was added 2,6-lutidine (8.30 mL, 71.4 mmol, 3.50 equiv) and triisopropylsilyl trifluoromethanesulfonate (9.30 mL, 34.7 mmol, 1.70 equiv). The reaction was stirred at this temperature for 1 h and then at 23 °C for 3 h. Saturated aqueous $NaHCO_3$ (150 mL) was added, and the aqueous layer was extracted with dichloromethane (3 x 150 mL). The combined organic phase was washed with saturated aqueous NaCl (150 mL), dried (Na_2SO_4), filtered and concentrated. Silica gel chromatography (hexane/ethyl acetate 20:1) afforded **S1** (8.8 g, 20 mmol, 96% yield) as a colorless liquid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.39-7.33 (m, 4H, aromatic), 7.30-7.26 (m, 1H, aromatic), 5.38 (s, 1H, H-1), 4.93 (d, *J* = 10.2 Hz, 1H, PhC$\underline{H}_2$-), 4.65 (d, *J* = 10.2 Hz, 1H, PhC$\underline{H}_2$-), 4.32 (dd, *J* = 7.0, 5.6 Hz, 1H, H-3), 4.13 (d, *J* = 5.6 Hz, 1H, H-2), 3.95 (qd, *J* = 9.9, 6.2 Hz, 1H, H-5), 3.25 (dd, *J* = 9.9, 7.0 Hz, 1H, H-4), 1.53 (s, 3H, Me), 1.40 (s, 3H, Me), 1.29 (d, *J* = 6.3 Hz, 3H, Me), 1.18-1.08 (m, 21H, Si-*i*-Pr_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.51, 128.41, 128.17, 127.76, 109.31, 91.80, 81.56, 78.80, 78.15, 73.25, 64.54, 28.23, 26.66, 17.89, 17.81, 12.04; FTIR (neat film) 3032, 2941, 2896, 2868, 1463, 1382, 1370, 1243, 1220, 1081, 1058, 1020, 995, 883 cm^{-1}; HRMS (ESI) *m/z*: Calcd for $C_{25}H_{42}O_5SiNa$ ($M+Na^+$) 473.2699, found 473.2701.

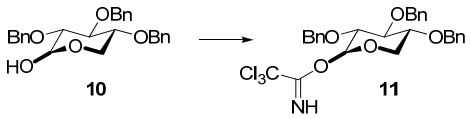


***O*-Triisopropyl 2,3-di-*O*-isopropylidene-α-L-rhamnopyranoside (8).** To a solution of **S1** (5.90 g, 13.1 mmol, 1.00 equiv) in methanol (100 mL) was added 10% (dry basis) palladium on carbon, wet, Degussa type E101 NE/W (1.4 g, 0.65 mmol, 0.050 equiv). The reaction mixture was vigorously stirred under hydrogen pressure (110 psi) for 7.5 h and was then filtered through a Celite 545 plug, which was rinsed with dichloromethane. The filtrate and rinsings were concentrated, and the residue was subjected to silica gel chromatography (hexane/ethyl acetate 3:1) to afford **8** (4.6 g, 1.3 mmol, 98% yield) as a colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz)

δ 5.34 (s, 1H, H-1), 4.16-4.10 (m, 2H, H-2 and H-3), 3.91 (qd, *J* = 8.8, 6.3 Hz, 1H, H-5), 3.42 (ddd, *J* = 11.6, 6.8, 4.8 Hz, 1H, H-4), 2.33 (d, *J* = 4.8 Hz, 1H, -OH), 1.53 (s, 3H, Me), 1.37 (s, 3H, Me), 1.28 (d, *J* = 6.3 Hz, 3H, Me), 1.18-1.05 (m, 21H, Si-*i*-Pr_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 109.5, 91.9, 78.2, 77.6, 74.4, 66.0, 28.0, 26.2, 17.8, 17.68, 17.66, 11.9; FTIR (neat film) 3463 (br), 2942, 2868, 1464, 1383, 1244, 1220, 1051, 1015, 883, 852, 807 cm^{-1}; HRMS (ESI) *m/z*: Calcd for $C_{18}H_{36}O_5SiNa$ ($M+Na^+$) 383.2230, found 383.2258.

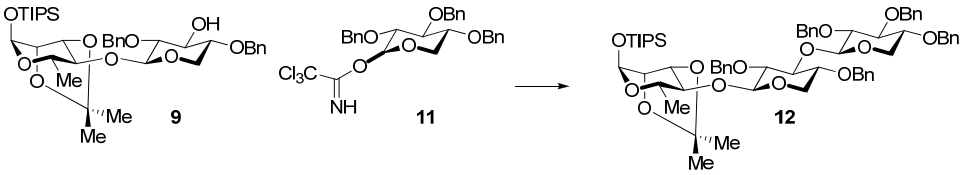


***O*-Triisopropylsilyl [2,4-di-*O*-benzyl-β-D-xylopyranosyl-(1→4)]-2,3-di-*O*-isopropylidene-α-L-rhamnopyranoside (9).** To a solution of xylopyranose **5** (140 mg, 0.424 mmol, 1.00 equiv), phenylsulfoxide (500 mg, 2.47 mmol, 5.83 equiv) and 2,4,6-tri-*t*-butylpyridine (604 mg, 2.44 mmol, 5.78 equiv) in dichloromethane (16 mL) at –78 °C was added trifluoromethanesulfonic anhydride (0.20 mL, 1.2 mmol, 2.8 equiv). After 15 min, a solution of rhamnopyranoside **8** (305 mg, 0.846 mmol, 2.00 equiv) in dichloromethane (5 mL) was added via cannula. The reaction mixture was stirred at –78 °C for 15 min, at –45 °C for 30 min, at 0 °C for 30 min, at 23 °C for 10 h, at 35 °C for 5 h, and finally at 23 °C for another 9 h. The reaction mixture was diluted with dichloromethane (100 mL) and washed with saturated aqueous $NaHCO_3$ (2 x 100 mL) and saturated aqueous NaCl (2 x 100 mL). The aqueous washings were extracted with dichloromethane (150 mL), and the combined organic phase was dried ($MgSO_4$), filtered, and concentrated to furnish a white amorphous solid. Silica gel chromatography (hexanes/ethyl acetate 7:3) afforded **9** (215 mg, 75% yield) as a white amorphous solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.40-7.27 (m, 10H), 5.37 (s, 1H), 4.94 (d, *J* = 11.5 Hz, 1H, PhC$\underline{H}_2$-), 4.92 (d, *J* = 7.2 Hz, 1H), 4.75 (d, *J* = 12.0 Hz, 1H, PhC$\underline{H}_2$-), 4.66 (d, *J* = 11.5 Hz, 1H, PhC$\underline{H}_2$-), 4.64 (d, *J* = 12.0 Hz, 1H, PhC$\underline{H}_2$-), 4.22 (dd, *J* = 7.0, 5.0 Hz, 1H), 4.06 (dd, *J* = 5.0, 0.5 Hz, 1H), 3.96 (dd, *J* = 11.5, 5.5 Hz, 1H), 3.87 (m, 1H), 3.72 (t, *J* = 9.0 Hz, 1H), 3.64 (dd, *J* = 10.0, 7.5 Hz, 1H), 3.53 (m, 1H), 3.24 (d, *J* = 10.0 Hz, 1H), 3.21 (t, *J* = 9.5 Hz, 2 H), 3.18 (d, *J* = 9.0 Hz, 1H), 1.51 (s, 3H), 1.36 (s, 3H), 1.26 (d, *J* = 6.0 Hz, 3H), 1.18-1.05 (m, 21H, Si-*i*-Pr_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.8, 138.7, 128.4, 128.2, 127.8, 109.2, 99.2, 97.9, 80.9, 78.6, 76.7, 76.4, 74.2, 73.1, 72.7, 70.0, 65.5, 28.2, 26.6, 21.0, 18.05, 18.00, 17.9, 16.5, 12.5; FTIR (neat film) 3483 (br), 3031, 2942, 2867, 1497, 1455, 1383, 1242, 1221, 1085, 1018, 883, 809, 735, 697 cm^{-1}; HRMS (ESI) *m/z*: Calcd for $C_{37}H_{56}O_9Si$ ($M+Na^+$) 695.3591, found 695.3594.

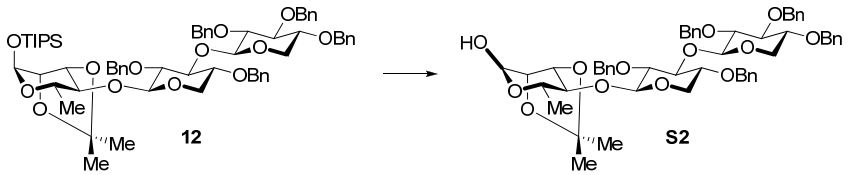


***O*-Trichloroacetimidoyl-2,3,4-tri-*O*-benzyl-D-xylopyranoside (11).** To a solution of hemiacetal **10** (31.4 mg, 0.0747 mmol, 1.00 equiv) in dichloromethane (2.0 mL) at 23 ºC was added trichloroacetonitrile (37 μL, 0.37 mmol, 5.0 equiv) and sodium hydride (60% dispersion in mineral oil, 3.0 mg, 0.075 mmol, 1.0 equiv). After 2 h, the reaction mixture was concentrated,

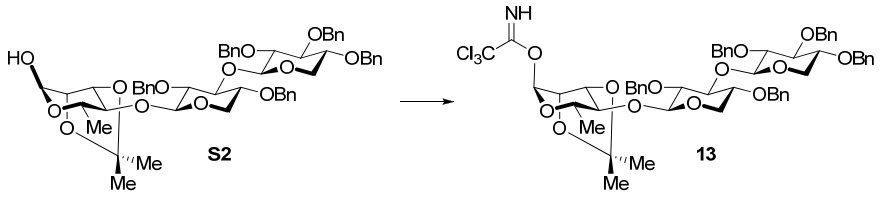
and the residue was purified by silica gel chromatography (hexane/ethyl acetate 3:1) to afford **11** (1.0:0.25 α:β, 34.6 mg, 0.0613 mmol, 82% yield) as a colorless oil. R_f = 0.65 (hexane/ethyl acetate 3:1); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.69 (s, 1Hβ), 8.57 (s, 1Hα), 7.37-7.25 (m, 15H), 6.35 (d, *J* = 3.5 Hz, 1Hα), 5.81 (s, *J* = 6.7 Hz, 1Hβ), 4.95-4.84 (m, 2H), 4.80-4.60 (m, 4H), 4.02 (m, 1Hβ), 3.98 (t, *J* = 9.1 Hz, 1Hα), 3.83-3.41 (m, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 175.26, 168.05, 161.64, 138.78, 138.16, 138.11, 128.64, 128.51, 128.48, 128.19, 128.08, 128.03, 127.88, 127.77, 127.72, 98.96, 94.83, 94.50, 93.13, 91.37, 82.99, 80.98, 80.14, 79.19, 77.28, 75.86, 73.90, 73.19, 62.61; FTIR (neat film) 3441 (br), 2925, 1669, 1616, 1497, 1490, 1256, 1072, 848, 825, 793, 739, 579 cm^{-1}.



O*-Triisopropylsilyl {[2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl-(1→3)]-2,4-di-*O*-benzyl-β-D-xylopyranosyl-(1→4)}-2,3-di-*O*-isopropylidene-α-L-rhamnopyranoside (12).** A solution of boron trifluoride diethyl etherate (23.5 μL, 0.187 mmol, 0.200 equiv) in dichloromethane (1.0 mL) was added to a solution of disaccharide **9** (630 mg, 0.936 mmol, 1.00 equiv) and *O*-trichloroacetimidoyl 2,3,4-tri-O***-benzyl-**D**-xylopyranoside (**11**) (1.06 g, 1.88 mmol, 2.00 equiv) in dichloromethane (60 mL) at –35 ºC. After 40 min at this temperature, triethylamine (0.3 mL) was added. The reaction mixture was concentrated, and the residue was purified by silica gel chromatography (hexane/ethyl acetate 4:1) to afford **12** (775 mg, 0.721 mmol, 77% yield) as a colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.44-7.28 (m, 25H), 5.42 (s, 1H), 5.04 (d, *J* = 11.5 Hz, 1H), 4.89-4.85 (m, 2H), 4.80 (d, *J* = 10.5 Hz, 1H, PhC$\underline{H}_2$-), 4.77 (d, *J* = 11.5 Hz, 1H, PhC$\underline{H}_2$-), 4.71 (d, *J* = 12.0 Hz, 1H, PhC$\underline{H}_2$-), 4.67 (d, *J* = 10.5 Hz, 1H, PhC$\underline{H}_2$-), 4.61 (d, *J* = 10.0 Hz, 1H, PhC$\underline{H}_2$-), 4.25 (dd, *J* = 7.0, 5.5 Hz, 1H), 4.10 (d, *J* = 5.5 Hz, 1H), 4.04 (t, *J* = 8.5 Hz, 1H), 4.01-3.94 (m, 2H), 3.92 (m, 1H), 3.71-3.65 (m, 2H), 3.63-3.57 (m, 2H), 3.44 (dd, *J* = 9.0, 8.0 Hz, 1H), 3.35 (dd, *J* = 8.5, 7.5 Hz, 1H), 3.30 (dd, *J* = 11.5, 10 Hz, 1H), 3.13 (dd, *J* = 11.5, 10.5 Hz, 1H), 1.55 (s, 3H), 1.39 (s, 3H), 1.32 (d, *J* = 6.0 Hz, 3H), 1.23-1.10 (m, 21H, Si-*i*-Pr$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.8, 138.6, 138.4, 138.3, 128.6, 128.5, 128.4, 128.3, 128.27, 128.03, 127.99, 127.9, 127.7, 127.66, 127.6, 127.5, 109.4, 103.3, 101.4, 91.7, 84.0, 82.7, 79.7, 78.3, 78.2, 78.1, 77.9, 77.3, 75.7, 75.5, 75.1, 74.6, 73.4, 73.2, 64.1, 63.9, 63.8, 27.9, 26.6, 17.85, 17.81, 17.75, 12.0; FTIR (neat film) 3063, 3030, 2939, 2897, 2866, 1455, 1383, 1369, 1084 cm^{-1}; HRMS (ESI) *m/z*: Calcd for $C_{63}H_{82}O_{13}SiNa$ (M+Na$^+$) 1097.5422, found 1097.5433.

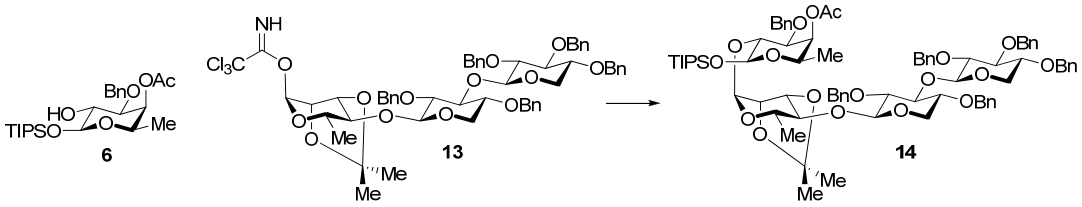


{[2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl-(1→3)]-2,4-di-*O*-benzyl-β-D-xylopyranosyl-(1→4)}-2,3-di-*O*-isopropylidene-L-rhamnopyranose (S2). To a solution of trisaccharide **12** (510 mg, 0.474 mmol, 1.00 equiv) in tetrahydrofuran (40 mL) at 0 ºC was added tetrabutylammonium fluoride solution (1.0 M in tetrahydrofuran, 0.52 mL, 0.52 mmol, 1.1 equiv). After 10 min, silica gel (100 mg) was added to the reaction mixture. The reaction mixture was concentrated, and the residue was directly purified by silica gel chromatography (hexane/ethyl acetate 1:1) to afford hemiacetal **S2** (434 mg, 0.472 mmol, >99% yield). ^{1}H NMR: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.44-7.25 (m, 25H), 5.40 (d, *J* = 3.5 Hz, 1H), 5.02 (d, *J* = 11.5 Hz, 1H), 4.88-4.83 (m, 2H), 4.79 (d, *J* = 10.5 Hz, 1H, PhC$\underline{H}_2$-), 4.76 (d, *J* = 12.0 Hz, 1H, PhC$\underline{H}_2$-), 4.71 (d, *J* = 12.0 Hz, 1H, PhC$\underline{H}_2$-), 4.67 (d, *J* = 11.5 Hz, 1H, PhC$\underline{H}_2$-), 4.59 (d, *J* = 10.5 Hz, 1H, PhC$\underline{H}_2$-), 4.25 (dd, *J* = 7.5, 6.0 Hz, 1H), 4.14 (d, *J* = 5.5 Hz, 1H), 4.04 (t, *J* = 8.5 Hz, 1H), 4.00-3.94 (m, 2H), 3.92 (m, 1H), 3.91 (m, 1H), 3.71-3.64 (m, 2H), 3.62-3.55 (m, 2H), 3.43 (dd, *J* = 9.0, 8.0 Hz, 1H), 3.34 (dd, *J* = 9.0, 7.5 Hz, 1H), 3.29 (dd, *J* = 11.5, 10.0 Hz, 1H), 3.22 (d, *J* = 3.5 Hz, 1H), 3.12 (dd, *J* = 11.5, 10.5 Hz, 1H), 1.55 (s, 3H), 1.39 (s, 3H), 1.32 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 138.8, 138.7, 138.6, 138.4, 138.2, 128.54, 128.51, 128.38, 128.33, 128.28, 128.05, 128.04, 128.00, 127.9, 127.71, 127.69, 127.65, 127.5, 109.4, 103.2, 101.7, 91.9, 83.9, 82.68, 82.65, 79.7, 78.2, 77.9, 77.8, 77.37, 76.2, 75.7, 75.5, 75.1, 74.6, 73.3, 73.1, 64.6, 63.9, 63.7, 27.8, 26.4, 17.8; FTIR (neat film) 3397 (br), 3063, 3031, 2983, 2933, 2903, 2873, 1454, 1382, 1370, 1242, 1220, 1163, 1130, 1072, 910, 733, 698 cm^{-1}; HRMS (ESI) *m/z*: Calcd for $C_{54}H_{62}O_{13}Na$ (M+Na$^+$) 941.4088, found 941.4080.

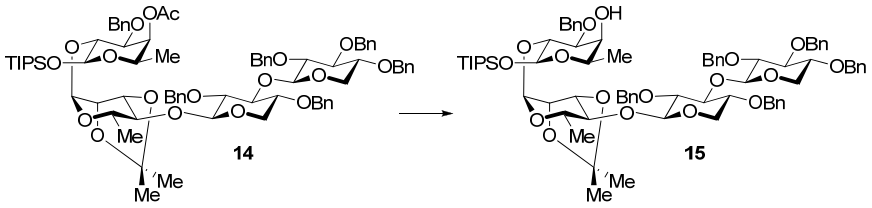


***O*- Trichloroacetimidoyl {[2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl-(1→3)]-2,4-di-*O*-benzyl-β-D-xylopyranosyl-(1→4)}-2,3-di-*O*-isopropylidene-α-L-rhamnopyranoside (13).** To a solution of hemiacetal **S2** (434 mg, 0.472 mmol, 1.00 equiv) in dichloromethane (20 mL) at 0 ºC was added trichloroacetonitrile (477 μL, 4.76 mmol, 10.1 equiv) and 1,8-diazabicyclo[5.4.0]undec-7-ene (142 μL, 0.950 mmol, 2.01 equiv). After 3 h at 0 ºC, triethylamine (100 μL) was added. The reaction mixture was concentrated, and the residue was purified by silica gel chromatography (hexane/ethyl acetate 3:1) to afford **13** (450 mg, 0.423 mmol, 90% yield). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.61 (s, 1H), 7.33-7.15 (m, 25H), 6.39 (s, 1H), 4.92 (d, *J* = 11.5 Hz, 1H, PhC$\underline{H}_2$-), 4.89-2.82 (m, 4H), 4.78-4.74 (m, 2H), 4.67 (d, *J* = 11.5 Hz, 1H, PhC$\underline{H}_2$-), 4.65 (d, *J* = 10.0 Hz, 1H, PhC$\underline{H}_2$-), 4.61 (d, *J* = 12.5 Hz, 1H, PhC$\underline{H}_2$-), 4.57 (d, *J* = 11.5 Hz, 1H, PhC$\underline{H}_2$-), 4.52 (d, *J* = 10.0 Hz, 1H, PhC$\underline{H}_2$-), 4.18-4.15 (m, 2H), 3.93 (t, *J* = 9.0 Hz, 1H), 3.90-3.84 (m, 2H), 3.76 (m, 1H), 3.64 (m, 1H), 3.57 (m, 1H), 3.52-3.46 (m, 2H), 3.33 (dd, *J* = 9.0, 8.0 Hz, 1H), 3.24 (dd, *J* = 9.0, 7.5 Hz, 1H), 3.19 (dd, *J* = 11.5, 10.0 Hz, 1H), 3.03 (dd, *J* = 11.5, 10.5 Hz, 1H), 1.47 (s, 3H), 1.31 (s, 3H), 1.25 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 160.3, 138.7, 138.5, 138.4, 138.2, 128.5, 128.4, 128.34, 128.31, 128.27, 128.01, 127.96, 127.86, 127.73, 127.67, 127.61, 127.52, 109.9, 103.3, 101.5, 95.3, 91.1, 84.0, 82.62, 82.59, 79.7, 78.2, 77.8, 77.3, 77.2, 75.6, 75.4, 75.1, 74.7, 74.6, 73.3, 73.1, 67.5, 63.9, 63.8,

27.7, 26.4, 17.7; FTIR (neat film) 3335, 3063, 3030, 2984, 2926, 2906, 2877, 2869, 1699, 1456, 1072, 796, 734, 697 cm^{-1}; HRMS (ESI) *m/z*: Calcd for $C_{56}H_{62}NO_{13}Cl_3Na$ ($M+Na^+$) 1084.3184, found 1084.3170.

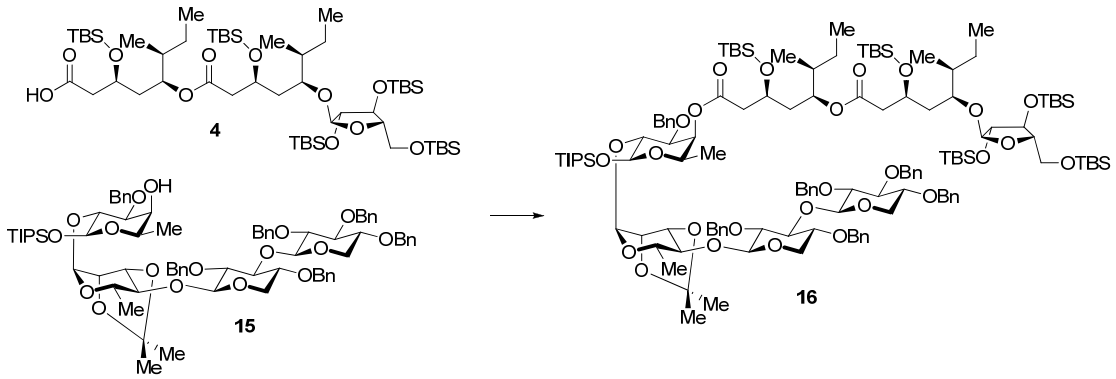


***O*-Triisopropylsilyl 4-*O*-acetyl-3-*O*-benzyl-({[2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl-(1→3)]-2,4-di-*O*-benzyl-β-D-xylopyranosyl-(1→4)}-2,3-di-*O*-isopropylidene-α-L-rhamnopyranosyl-(1→2))-β-D-fucopyranoside (14).** To a solution of trisaccharide imidate **13** (250 mg, 0.235 mmol, 1.00 equiv) and fucoside **6** (160 mg, 0.353 mmol, 1.50 equiv) in diethyl ether (40 mL) at 0 ºC was added a solution of trimethylsilyl trifluoromethanesulphonate (6.5 μL, 0.036 mmol, 0.15 equiv) in diethyl ether (100 μL). After 30 min at this temperature, triethylamine (0.2 mL) was added to the reaction, which was concentrated and purified by silica gel chromatography (hexane/ethyl acetate 3:1) to afford **14** (240 mg, 0.177 mmol, 75% yield). R_f = 0.42 (hexane/ethyl acetate 7:3); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.38-7.21 (m, 30H), 5.72 (s, 1H), 5.36 (d, *J* = 2.8 Hz, 1H), 5.00-4.83 (m, 5H), 4.82-4.75 (m, 3H), 4.72-4.56 (m, 4H), 4.57 (d, *J* = 7.5 Hz, 1H), 4.45 (d, *J* = 10.0 Hz, 1H, PhC$\underline{H}_2$-), 4.41 (d, *J* = 11.4 Hz, 1H, PhC$\underline{H}_2$-), 4.19 (dd, *J* = 7.4, 5.7 Hz, 1H), 4.08 (d, *J* = 5.6 Hz, 1H, PhC$\underline{H}_2$-), 4.00-3.85 (m, 4H), 3.86 (dd, *J* = 9.4, 7.5 Hz, 1H), 3.65-3.50 (m, 5H), 3.50 (t, *J* = 9.0 Hz, 1H), 3.39 (t, *J* = 7.8 Hz, 1H), 3.26-3.16 (m, 2H), 3.05 (t, *J* = 11.2 Hz, 1H), 2.19 (s, 3H), 1.53 (s, 3H), 1.40 (s, 3H), 1.28 (d, *J* = 6.2 Hz, 3H), 1.22 (d, *J* = 6.4 Hz, 3H), 1.15-0.90 (m, 21H, Si-*i*-Pr_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.1, 138.9, 138.8, 138.7, 138.5, 138.3, 137.3, 128.6, 128.51, 128.47, 128.4, 128.3, 128.24, 128.20, 128.10, 128.05, 128.01, 127.94, 127.88, 127.66, 127.64, 127.57, 127.52, 109.1, 103.1, 102.4, 96.9, 96.8, 83.9, 83.0, 82.8, 80.6, 79.9, 78.6, 78.30, 78.27, 77.3, 76.2, 75.6, 75.1, 74.4, 73.9, 73.3, 73.1, 71.0, 69.2, 69.1, 64.3, 64.0, 63.7, 27.9, 26.6, 21.0, 18.02, 17.98, 17.91, 16.3, 12.4; FTIR (neat film) 2901, 2866, 1744, 1513, 1402, 1273, 1088, 1071, 1047, 733 cm^{-1}; HRMS (ESI) *m/z*: Calcd for $C_{78}H_{100}O_{18}SiNa$ ($M+Na^+$) 1375.6577, found 1375.6561.



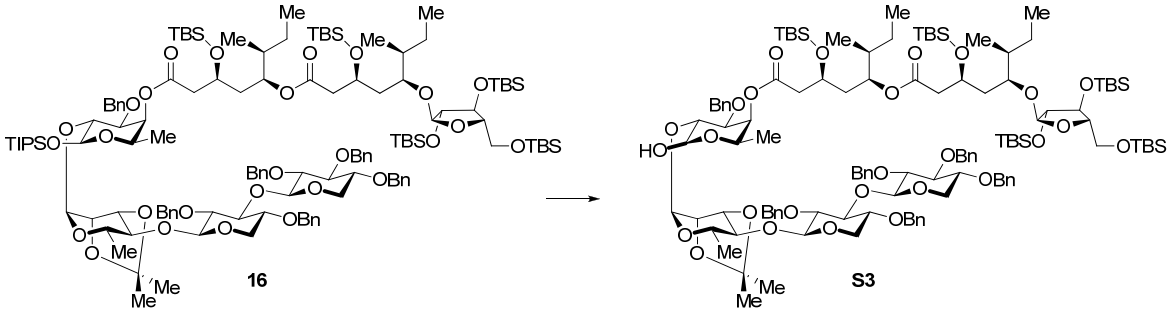
***O*-Triisopropylsilyl 3-*O*-benzyl-({[2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl-(1→3)]-2,4-di-*O*-benzyl-β-D-xylopyranosyl-(1→4)}-2,3-di-*O*-isopropylidene-α-L-rhamnopyranosyl-(1→2))-β-D-fucopyranoside (15)**. A solution of tetrasaccharide **14** (230 mg, 0.170 mmol, 1.00 equiv) in dichloromethane (60 mL) was cooled to –78 ºC, and a solution of diisobutylaluminium hydride (1.0 M in hexane, 0.34 mL, 0.34 mmol, 2.0 equiv) was added. After 15 min, ethyl acetate (0.1 mL, 1 mmol, 6 equiv) was added to quench excess diisobutylaluminium hydride.

After Celite (100 mg) and $Na_2SO_4 \cdot 10H_2O$ (100 mg) were added to the reaction at –78 ºC, the temperature was allowed to rise slowly to 23 °C. Following filtration and concentration, the residue was purified by silica gel chromatography (hexane/ethyl acetate 7:3) to provide tetrasaccharide **15** (184 mg, 0.140 mmol, 83% yield). R_f = 0.26 (hexane/ethyl acetate 7:3); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.41-7.25 (m, 30H), 5.70 (s, 1H), 4.97-4.87 (m, 4H), 4.82 (d, *J* = 12.0 Hz, 1H, PhC$\underline{H}_2$-), 4.81 (d, *J* = 11.0 Hz, 1H, PhC$\underline{H}_2$-), 4.79 (d, *J* = 12.0 Hz, 1H, PhC$\underline{H}_2$-), 4.72 (d, *J* = 11.0 Hz, 1H, PhC$\underline{H}_2$-), 4.69 (d, *J* = 12.0 Hz, 1H, PhC$\underline{H}_2$-), 4.67 (d, *J* = 12.0 Hz, 1H, PhC$\underline{H}_2$-), 4.62 (d, *J* = 11.5 Hz, 1H, PhC$\underline{H}_2$-), 4.55 (d, *J* = 11.5 Hz, 1H, PhC$\underline{H}_2$-), 4.52 (d, *J* = 7.5 Hz, 1H), 4.43 (d, *J* = 9.5 Hz, 1H, PhC$\underline{H}_2$-), 4.18 (dd, *J* = 7.5, 5.5 Hz, 1H), 4.07 (d, *J* = 6.0 Hz), 4.02-3.88 (m, 4H), 3.85 (dd, *J* = 9.5, 7.5 Hz, 1H), 3.81 (t, *J* = 2.5 Hz, 1H), 3.64-3.45 (m, 6H), 3.37 (dd, *J* = 9.0, 8.0 Hz, 1H), 3.28-2.19 (m, 2H), 3.02 (dd, *J* = 11.5, 10.5 Hz, 1H), 2.35 (d, *J* = 2.5 Hz, 1H), 1.53 (s, 3H), 1.39 (s, 3H), 1.34 (d, *J* = 7.0 Hz, 3H), 1.24 (d, *J* = 6.0 Hz, 3H), 1.10-0.88 (m, 21H, Si-*i*-Pr$_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 138.81, 138.79, 138.6, 138.5, 138.3, 137.1, 128.7, 128.5, 128.43, 128.36, 128.35, 128.29, 128.20, 128.16, 128.06, 128.02, 127.98, 127.8, 127.62, 127.60, 127.52, 127.49, 109.1, 103.1, 102.5, 96.69, 96.66, 83.9, 83.0, 82.7, 82.5, 79.8, 78.6, 78.3, 78.2, 77.3, 76.3, 75.61, 75.60, 75.1, 74.4, 73.36, 73.30, 73.0, 70.9, 70.2, 68.3, 64.3, 64.0, 63.7, 27.9, 26.5, 18.0, 17.9, 17.8, 16.3, 12.3; FTIR (neat film) 3509 (br), 2986, 2939, 2923, 2866, 2847, 1497, 1453, 1367, 1068, 730, 699 cm^{-1}; HRMS (ESI) *m/z*: Calcd for $C_{76}H_{98}O_{17}SiNa$ (M+Na$^+$) 1333.6471, found 1333.6461.

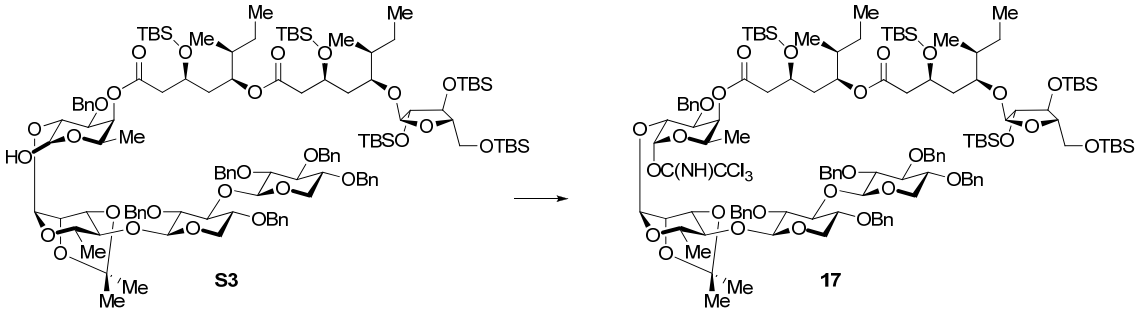


Intermediate 16. 2,4,6-Trichlorobenzoyl chloride (15 μL, 0.093 mmol, 1.4 equiv) was added to a solution of the glycosylated normonoterpene acyl chain **4** (90 mg, 0.084 mmol, 1.3 equiv) and triethylamine (14 μL, 0.10 mmol, 1.5 equiv) in toluene (6 mL) at 23 °C. After 1.5 h, a solution of tetrasaccharide **15** (88 mg, 0.067 mmol, 1.0 equiv) and 4-dimethylaminopyridine (15 mg, 0.12 mmol, 1.8 equiv) in toluene (2 mL) was added to the reaction flask. After 4 h the reaction was concentrated and purified by silica gel chromatography (benzene/ethyl acetate 19:1) to provide **16** (141 mg, 0.0598 mmol, 89% yield) as a colorless oil. R_f = 0.54 (benzene/ethyl acetate 9:1); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.34-7.02 (m, 30H), 5.63 (s, 1H), 5.31 (d, *J* = 2.8 Hz, 1H), 4.95 (m, 1H), 4.92-4.82 (m, 7H), 4.79-4.73 (m, 3H), 4.66 (d, *J* = 11.7 Hz, 1H, PhC$\underline{H}_2$-), 4.64-4.60 (m, 2H), 4.56 (d, *J* = 11.7 Hz, 1H, PhC$\underline{H}_2$-), 4.48 (d, *J* = 7.4 Hz, 1H, PhC$\underline{H}_2$-), 4.38 (d, *J* = 10.0 Hz, 1H), 4.33 (d, *J* = 11.6 Hz, 1H, PhC$\underline{H}_2$-), 4.17 (m, 1H), 4.11 (dd, *J* = 7.4, 5.8 Hz, 1H), 4.05 (m, 1H), 4.03-3.99 (m, 2H), 3.97-3.83 (m, 6H), 3.77 (dd, *J* = 9.5, 7.5 Hz, 1H), 3.68-3.63 (m, 2H), 3.60-3.45 (m, 6H), 3.43 (t, *J* = 9.0 Hz, 1H), 3.32 (dd, *J* = 9.0, 8.0 Hz, 1H), 3.24-3.14 (m, 2H), 2.98 (t, *J* = 11.0 Hz, 1H), 2.63 (m, 1H), 2.53 (m, 1H), 2.50-2.37 (m, 2H), 2.32 (s,

1H), 1.78-1.48 (m, 8H), 1.46 (s, 3H), 1.40 (m, 1H), 1.33 (s, 3H), 1.22 (d, J = 6.1 Hz, 3H), 1.14 (d, J = 6.4 Hz, 3H), 1.10-0.70 (m, 80H), 0.12-0.00 (m, 30H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.85, 170.79, 138.80, 138.77, 138.6, 138.4, 138.2, 137.2, 128.9, 128.5, 128.45, 128.43, 128.40, 128.34, 128.26, 128.18, 128.13, 128.04, 127.99, 127.95, 127.87, 127.82, 127.6, 127.50, 127.46, 109.0, 107.2, 103.0, 102.4, 96.80, 96.77, 85.8, 84.1, 83.9, 83.0, 82.7, 80.3, 79.8, 79.1, 78.6, 78.27, 78.22, 77.4, 77.3, 76.2, 75.6, 75.1, 74.45, 74.36, 73.8, 73.3, 73.0, 70.9, 69.1, 68.9, 67.0, 66.2, 64.1, 63.9, 63.7, 63.1, 43.5, 42.7, 39.4, 38.47, 38.42, 36.8, 29.7, 27.9, 26.5, 26.0, 25.94, 25.92, 25.83, 25.80, 25.76, 24.8, 24.1, 18.4, 17.97, 17.95, 17.92, 17.90, 17.86, 16.4, 14.49, 14.36, 12.4, 12.2, 12.0, -4.1, -4.2, -4.4, -4.5, -4.6, -4.7, -4.9, -5.2, -5.3; FTIR (neat film) 2957, 2930, 2893, 2885, 2858, 1737, 1463, 1382, 1361, 1252, 1088, 1072, 837, 696 cm^{-1}.

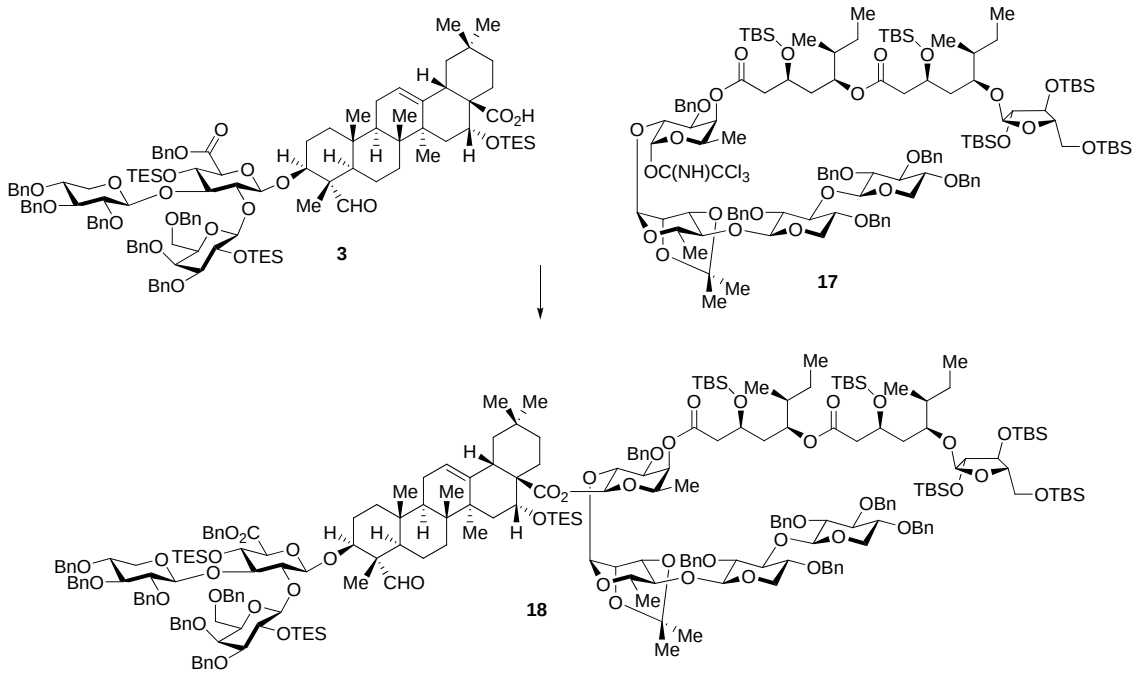


Hemiacetal S3. A solution of **16** (122 mg, 0.0517 mmol, 1.00 equiv) in tetrahydrofuran (20 mL) was cooled to 0 °C, and a solution of tetrabutylammonium fluoride (10 mM in tetrahydrofuran, 5.2 mL, 0.052 mmol, 1.0 equiv) was added. After 5.5 h at this temperature, a solution of acetic acid (10 mM in methanol, 4.4 mL, 0.044 mmol, 1.2 equiv) was added. The reaction was concentrated and purified by silica gel chromatography (hexane/ethyl acetate 4:1) to afford hemiacetal **S3** (75 mg, 0.034 mmol, 66% yield) and recovered starting material **16** (15 mg, 0.0064 mmol, 12% yield). R_f = 0.38 (benzene/ethyl acetate 9:1); characteristic resonances from ^{1}H NMR: ($CDCl_3$, 500 MHz) δ 7.37-7.23 (m, 30H), 3.36 (dd, J = 9.0, 7.5 Hz, 1H), 3.22 (dd, J = 11.5, 9.5 Hz, 1H), 3.04 (t, J = 10.5 Hz, 1H); *See appendix for proton NMR*; LRMS (ESI) *m/z*: Calcd for $C_{120}H_{188}O_{27}Si_5Na$ ($M+Na^+$) 2224.21, found 2223.88.



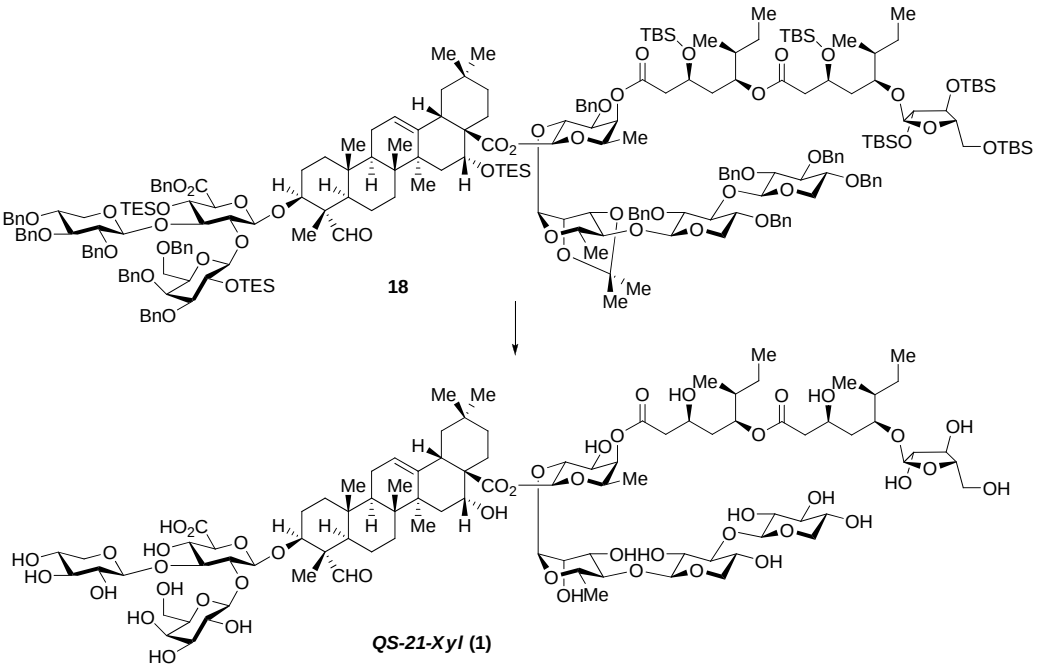
Trichloroacetimidate 17. To the solution of the hemiacetal **S3** (75 mg, 0.034 mmol, 1.0 equiv) in dichloromethane (15 ml) at 0 °C was added trichloroacetonitrile (683 μL, 6.81 mmol, 200 equiv) and 1,8-diazabicyclo[5.4.0]undec-7-ene (47 μL, 0.34 mmol, 10 equiv). After 3 h,

triethylamine (0.1 mL) was added, and the reaction was concentrated and purified by silica gel chromatography (benzene/ethyl acetate 19:1) to afford α-trichloroacetimidate **17** (58 mg, 0.025 mmol, 73% yield). R_f = 0.57 (benzene/ethyl acetate 9:1); characteristic resonances from ^{1}H NMR: ($CDCl_3$, 500 MHz) δ 8.45 (s, 1H), 7.37-7.22 (m, 30H), 6.37 (d, 1H, *J* = 3.5 Hz), 5.46 (m, 1 H), 5.20 (s, 1H), 4.80 (d, *J* = 12.0 Hz, 1H, PhC$\underline{H}_2$-), 4.78 (d, *J* = 12.0 Hz, 1H, PhC$\underline{H}_2$-), 4.64 (d, *J* = 12.0 Hz, 1H, PhC$\underline{H}_2$-), 4.60 (d, *J* = 12.0 Hz, 1H, PhC$\underline{H}_2$-), 3.46 (t, *J* = 8.5 Hz, 1H), 3.34 (dd, *J* = 9.0, 8.0 Hz, 1H), 3.02 (dd, *J* = 10.5, 9.5 Hz, 1H), 1.47 (s, 3H), 1.33 (s, 3H), 1.23 (d, *J* = 6.0 Hz, 3H), 1.17 (d, *J* = 6.5 Hz, 3H); *See appendix for proton NMR*; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.9, 170.7, 170.5, 161.1, 138.8, 138.7, 138.5, 138.3, 138.2, 137.6, 128.4, 128.32, 128.26, 128.20, 128.1, 127.99, 127.96, 127.82, 127.76, 127.61, 127.57, 127.46, 109.2, 107.2, 103.1, 101.8, 96.2, 91.2, 85.8, 84.1, 83.9, 82.7, 82.6, 79.7, 79.0, 78.2, 77.59, 77.26, 77.22, 77.01, 76.76, 75.6, 75.5, 75.0, 74.4, 74.3, 73.9, 73.3, 73.1, 71.5, 69.8, 67.7, 67.0, 66.2, 64.7, 63.9, 63.7, 63.1, 43.4, 42.8, 39.4, 38.4, 36.8, 27.7, 26.4, 25.98, 25.92, 25.88, 25.80, 25.75, 24.9, 24.0, 18.4, 18.0, 17.89, 17.87, 17.5, 16.4, 14.5, 14.3, 12.2, 11.9, -4.2, -4.3, -4.4, -4.5, -4.6, -4.7, -4.9, -5.2, -5.3.



Fully-protected QS-21-Xyl (18). A solution of boron trifluoride diethyl etherate (5.4 μL, 0.043 mmol, 1.0 equiv) in dichloromethane (100 μL) was added to a solution of α-trichloroacetimidate **17** (100 mg, 0.0426 mmol, 1.00 equiv) and trisaccharide-triterpene **3** (123 mg, 0.0637 mmol, 1.50 equiv) in dichloromethane (15 mL) and crushed 4Å molecular sieves (~50 mg) at –78 ºC. The temperature was allowed to gradually warm to 23 °C over 2 h, and triethylamine (100 μL) was added. The reaction was concentrated and purified by silica gel chromatography (benzene/ethyl acetate 19:1) to afford **18** (140 mg, 0.0340 mmol, 80% yield) as a clear film. R_f = 0.63 (benzene/ethyl acetate 9:1); characteristic resonances from ^{1}H NMR: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.46 (s, 1 H), 7.43-7.16 (m, 65H), 5.41 (d, *J* = 8.0 Hz, 1H), 5.38-5.34 (m, 2H), 5.28 (d, *J* = 12.0 Hz, 1H, PhC$\underline{H}_2$-), 5.22 (m, 1H), 5.17 (d, *J* = 12.0 Hz, 1H, PhC$\underline{H}_2$-),

5.04-4.89 (m, 11H), 4.89-4.76 (m, 6H), 4.76-4.60 (m, 9H), 4.56-4.50 (m, 2H), 4.47 (d, *J* = 12.0 Hz, 1H, PhC$\underline{H}_2$-), 4.44-4.36 (m, 3H), 4.27-4.20 (m, 2H), 4.20-4.09 (m, 3H), 4.07 (dd, *J* = 4.0, 2.0 Hz, 1H), 4.03-3.97 (m, 3H), 3.96-3.88 (m, 4H), 3.88-3.74 (m, 5H), 3.74-3.47 (m, 15H), 3.42-3.15 (m, 7H), 3.07 (t, *J* = 11.5 Hz, 1H), 2.94 (dd, *J* = 14.2, 3.2 Hz, 1H), 2.69 (m, 1H), 2.62-2.43 (m, 4H), 2.26 (t, *J* = 13.5 Hz, 1H), 1.95-1.82 (m, 5H), 1.82-1.68 (m, 6H), 1.66-1.50 (m, 7H), 1.47 (s, 3H), 1.36 (s, 3H), 1.30 (m, 1H), 1.26 (s, 3H), 1.24 (d, *J* = 6.0 Hz, 3H), 1.18 (d, *J* = 6.0 Hz, 3H), 1.13-0.97 (m, 25H), 0.97-0.82 (m, 72H), 0.82-0.53 (m, 23H), 0.17-0.06 (m, 30H); *See appendix for proton NMR*; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 212.3, 175.3, 171.0, 170.7, 168.4, 143.5, 139.3, 138.82, 138.80, 138.74, 138.69, 138.63, 138.59, 138.5, 138.4, 138.3, 137.3, 135.3, 128.54, 128.52, 128.50, 128.46, 128.43, 128.40, 128.36, 128.34, 128.29, 128.26, 128.24, 128.19, 128.17, 128.06, 128.02, 127.95, 127.88, 127.82, 127.77, 127.69, 127.64, 127.58, 127.50, 127.46, 127.42, 127.39, 127.2, 126.1, 109.5, 107.3, 103.4, 103.2, 102.2, 101.7, 101.0, 98.1, 93.9, 85.9, 85.6, 84.4, 84.2, 84.0, 82.80, 82.77, 88.65, 82.5, 80.0, 79.72, 79.70, 79.1, 78.65, 78.59, 78.30, 78.26, 77.4, 77.3, 76.6, 76.1, 75.9, 75.8, 75.7, 75.65, 75.63, 75.2, 75.06, 75.03, 74.7, 74.5, 74.3, 74.1, 73.3, 73.1, 72.7, 72.6, 72.4, 71.9, 71.0, 69.7, 69.1, 68.2, 67.07, 67.03, 66.9, 66.3, 63.9, 63.8, 63.7, 63.2, 53.9, 50.1, 49.0, 46.2, 43.5, 42.8, 41.6, 40.9, 39.8, 39.5, 38.8, 38.6, 36.9, 36.2, 32.8, 30.5, 27.6, 26.4, 26.06, 26.03, 25.91, 25.96, 25.92, 25.87, 25.83, 25.0, 24.4, 24.1, 20.3, 19.8, 18.5, 18.01, 17.97, 17.94, 17.85, 17.1, 16.3, 15.9, 14.6, 14.5, 12.3, 12.0, 11.7, 7.3, 7.2, 7.0, 5.4, 5.2, 5.1, 5.0, -4.2, -4.36, -4.45, -4.47, -4.51, -4.7, -4.8, -5.1, -5.2; FTIR (neat film) 2955, 2931, 2877, 2858, 1738, 1454, 1361, 1253, 1088, 1046, 837, 733, 697 cm^{-1}; LRMS (ESI) *m/z*: Calcd for $C_{234}H_{342}O_{46}Si_8Na_3$ (M+3Na$^+$) 1393.74, found 1394.85.



Synthetic QS-21-Xyl (1). Fully-protected QS-21-Xyl **18** (21.6 mg, 0.00525 mmol, 1.00 equiv) was partitioned into four 25-mL round bottom flasks with dichloromethane, and the solvent was removed by rotory evaporation so that starting material **21** formed a thin film inside each flask. A pre-cooled (0 °C) solution of trifluoroacetic acid (1.0 mL, TFA/water 4:1) was

added to each flask at 0 °C, and vigorous stirring dissolved the starting material within 20 min. After 65 min of stirring, the reaction solutions were concentrated *in vacuo* at 0 °C so that the solvent had visibly evaporated within 20 min, giving a clear solid residue that was pumped for a total of 75 min.

To avoid any unwanted reduction of the aldehyde within the triterpene during the next hydrogenolysis step, it was necessary to completely remove any trace quantities of trifluoroacetic acid from the partially-protected QS-21-Xyl intermediate. To this purpose, the reaction products in the four 25-mL round bottom flasks were consolidated in a single flask with toluene (4 mL), and the solvent was removed by rotory evaporation. Additional azeotropic removals of solvent were performed with toluene (4 mL) and tetrahydrofuran (3 x 4 mL).

The partially-protected QS-21-Xyl intermediate was then equally partitioned into four 30-mL vials with stirbars using a solution of 2:1 methanol/tetrahydrofuran (4 x 7.5 mL). The vials were charged with 10% (dry basis) palladium on carbon, wet, Degussa type E101 NE/W (4 x 5.5 mg, 0.010 mmol, 2.0 equiv), and the four reaction runs were stirred under hydrogen pressure (50 psi) for 17 h.

Each of the four reaction suspensions was filtered through a 0.45 μm polyvinylidene fluoride filter disk, which was then washed with methanol (5 mL). The filtrate and rinsing were concentrated and purified by four parallel RP-HPLC injections on an XBridge Prep BEH300 C18 column (5 μm, 10 x 250 mm) using a linear gradient of 35→45% acetonitrile (0.05% TFA) in water (0.05% TFA) over 30 min at a flow rate of 5 mL/min. The fractions containing the major peak (t_R = 27.2 min) were collected and lyophilized to dryness to afford synthetic QS-21-Xyl (**1**) (6.7 mg, 0.0034 mmol, 64% yield) as a white solid. $[\alpha]^{20}$ +2.6 (*c* 0.11 in methanol); LRMS (ESI) *m/z*: Calcd for $C_{92}H_{147}O_{46}$ ($M\text{-}H^+$) 1987.92, found 1987.58.

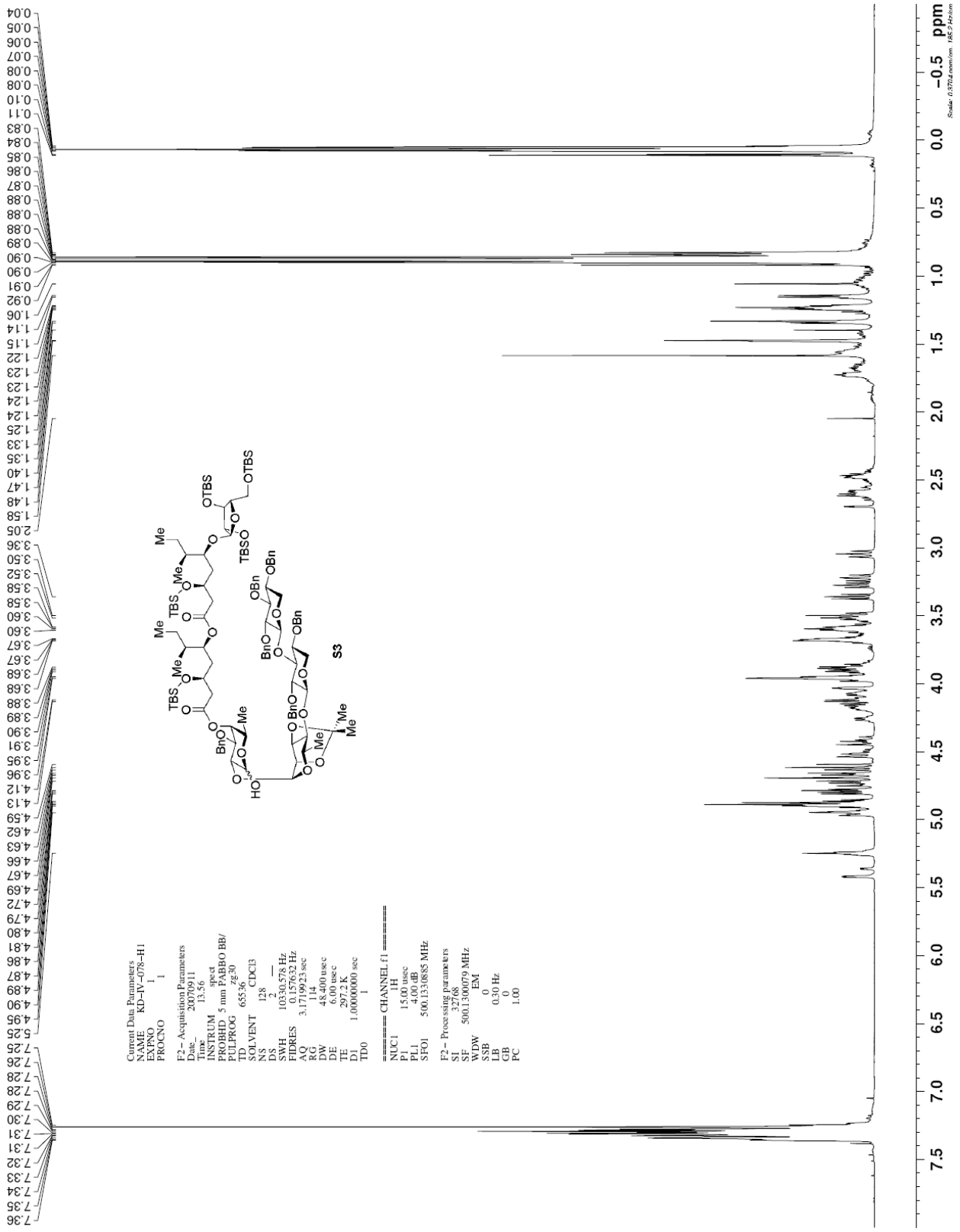
Natural QS-21-Xyl (1).[2,3] Brenntag Quil-A (batch L77-244) was fractionated by RP-HPLC on an XBridge Prep C18 OBD column (5 μm, 19 x 150 mm) using a linear gradient of 30→40% acetonitrile (0.05% TFA) in water (0.05% TFA) over 30 min at a flow rate of 15 mL/min, followed by isocratic 40% acetonitrile (0.05% TFA) in water (0.05% TFA). The QS-21 peak (t_R = 45.0 min) was collected and lyophilized. This crude QS-21 fraction was injected on an XBridge Prep BEH300 C18 column (5 μm, 10 x 250 mm) using a linear gradient of 35→45% acetonitrile in aqueous potassium phosphate monobasic-sodium hydroxide buffer (0.01 M, pH 7.0) over 30 min at a flow rate of 5 mL/min. The resulting resolution of QS-21-Xyl (**1**) from QS-21-Api (**2**) was modest: isomer **1** eluted as an early shoulder (t_R = 13.2 min) on the peak containing isomer **2** (t_R = 13.4 min). The fraction enriched in QS-21-Xyl (**1**) was further purified by RP-HPLC using the aqueous buffer (pH 7.0). Finally, the phosphate salts were removed from the naturally-isolated QS-21-Xyl (**1**) by a further injection of **1** on the XBridge Prep BEH300 C18 column (5 μm, 10 x 250 mm) using a linear gradient of 35→45% acetonitrile (0.05% TFA) in water (0.05% TFA) over 30 min at a flow rate of 5 mL/min. The fraction containing QS-21-Xyl (**1**) (t_R = 26.5 min) was collected and lyophilized to dryness to afford natural QS-21-Xyl (**1**) as a white solid with ~70% purity by ^{1}H NMR (7:3 D_2O:CD_3CN, 500 MHz). LRMS (ESI) *m/z*: Calcd for $C_{92}H_{147}O_{46}$ ($M\text{-}H^+$) 1987.92, found 1987.91.

*See appendix for spectroscopic and chromatographic comparison of synthetic vs. natural QS-21-Xyl (**1**).*

Preparation of GD3-KLH conjugate melanoma vaccine. GD3 was extracted from bovine buttermilk and conjugated to keyhole limpet hemocyanin (KLH) as described previously[4] with slight modifications. The principle involved in the conjugation procedure is cleavage of the double bond of GD3-ceramide by ozone, generation of an aldehyde group, and conjugation to ε-amino groups of lysine on carrier proteins by reductive amination. In brief, GD3 (5 mg) was dissolved in methanol (2 mL) 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 5 min. The reaction mixture was stirred for 2 min, and excess ozone was removed by bubbling N_2. Methyl sulfide (500 μL) was added, and the cleaved GD3 sample stirred at room temperature for 30 min. The sample was dried under a stream of N_2 and treated with *n*-hexane to remove free fatty aldehydes. Ten mg KLH (5 mg/ml in phosphate buffered saline) and sodium cyanoborohydride (2 mg) were added to cleaved GD3 and the mixture stirred for 5 min. The sample was filtered through a 0.22 μm filter under sterile conditions, placed in a sterile vial, capped and incubated at 37 °C for 48 h. Unreacted GD3 was removed by a molecular cut-off filter (MW 30,000, CentriPrep, Amicon Inc., Beverly, MA). Protein content was determined using the Bio-Rad dye-binding method according to the manufacturer's instructions and ganglioside content by estimating sialic acid as described by Svennerholm.[5] The epitope ratio of GD3-KLH was found to be 748/1.

Vaccination of mice. Three groups of five mice (C57BL/6J, female, 6 weeks of age) were vaccinated three times with GD3-KLH conjugate (10 μg equivalent of GD3) in 100 μL phosphate buffered saline either (1) alone without adjuvant, (2) with 10 μg of synthetic adjuvant sQS-21 (mixture of 65% QS-21-Api and 35% QS-21-Xyl), or (3) with 100 μg of semi-synthetic saponin adjuvant GPI-100. Vaccines were administered subcutaneously to each mouse on weeks 1, 2, and 3. Mice were bled 7 days after the third vaccination.

Measurement of antibody titers. The presence of antibodies was tested by an enzyme-linked immunosorbent assay (ELISA). ELISAs were performed to determine antibody response against GD3 and KLH as described previously.[4] In brief, ELISA plates were coated with either GD3 antigen at 0.1 μg/well in ethanol or KLH at 0.1 μg/well in carbonate buffer (pH 10). The GD3-coated plates were incubated overnight at room temperature, and KLH coated plates were incubated at 4 ºC overnight. ELISA plates were washed, blocked with 1% human serum albumin in phosphate-buffered saline containing 0.05% Tween 20. Serially diluted pre- and post-treatment sera in phosphate-buffered saline containing 1% human serum albumin 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 (SouthernBiotech, Alabama) was added to each well. Absorbance was measured at 405 nm. The titer was defined as the highest serum dilution that showed an absorbance 0.1 or greater over that of the pre-sera.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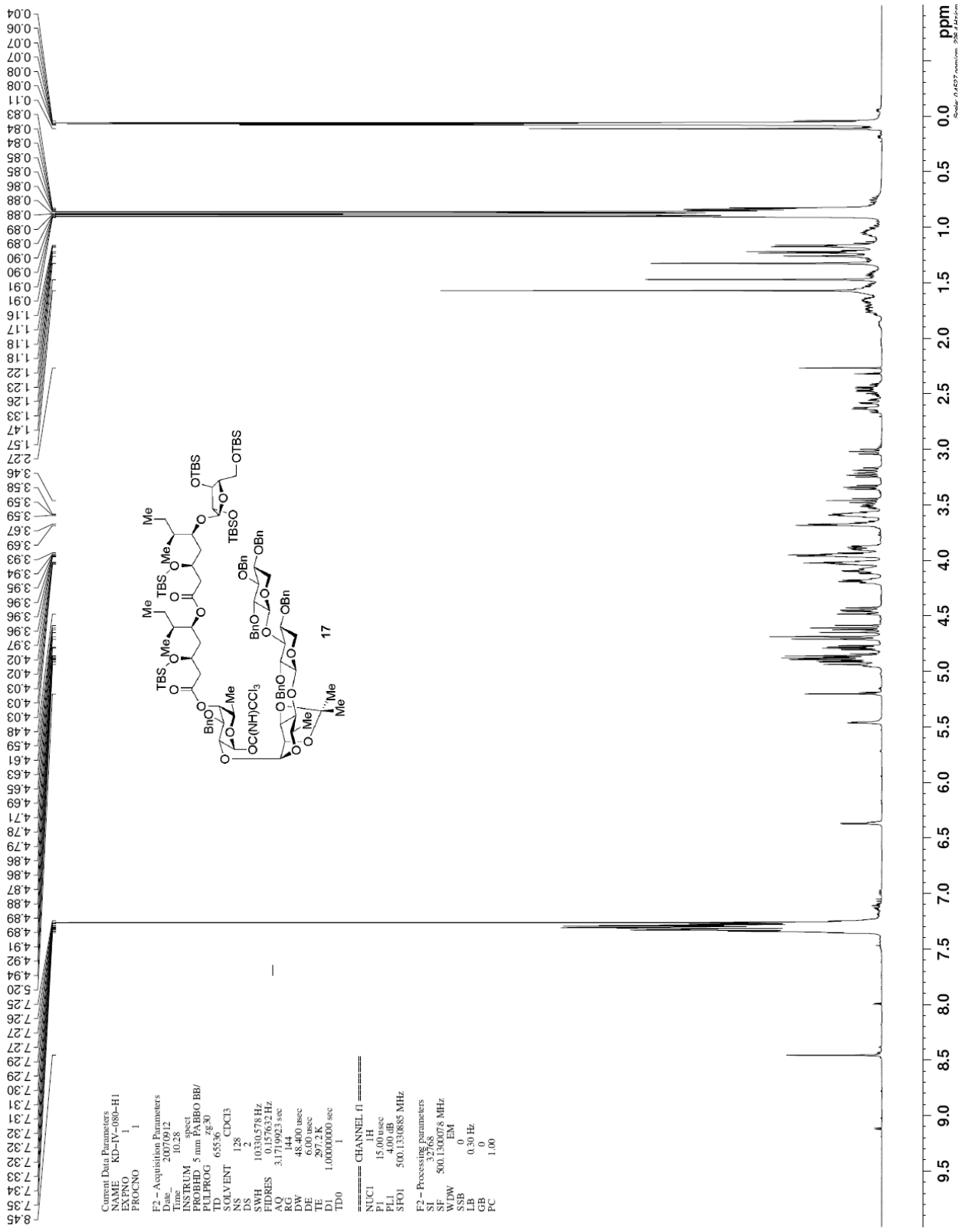
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TD0 1
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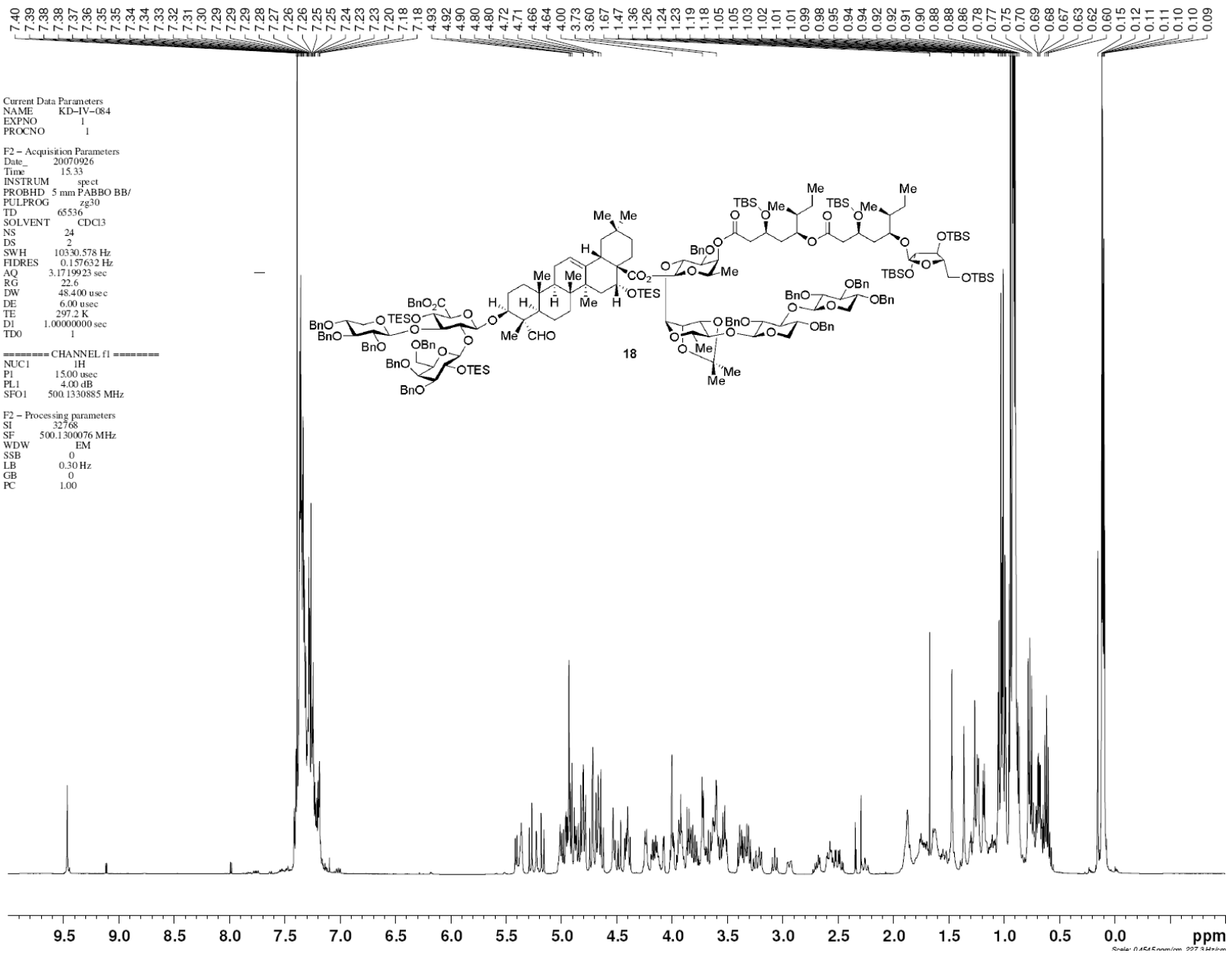
17
ppm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18
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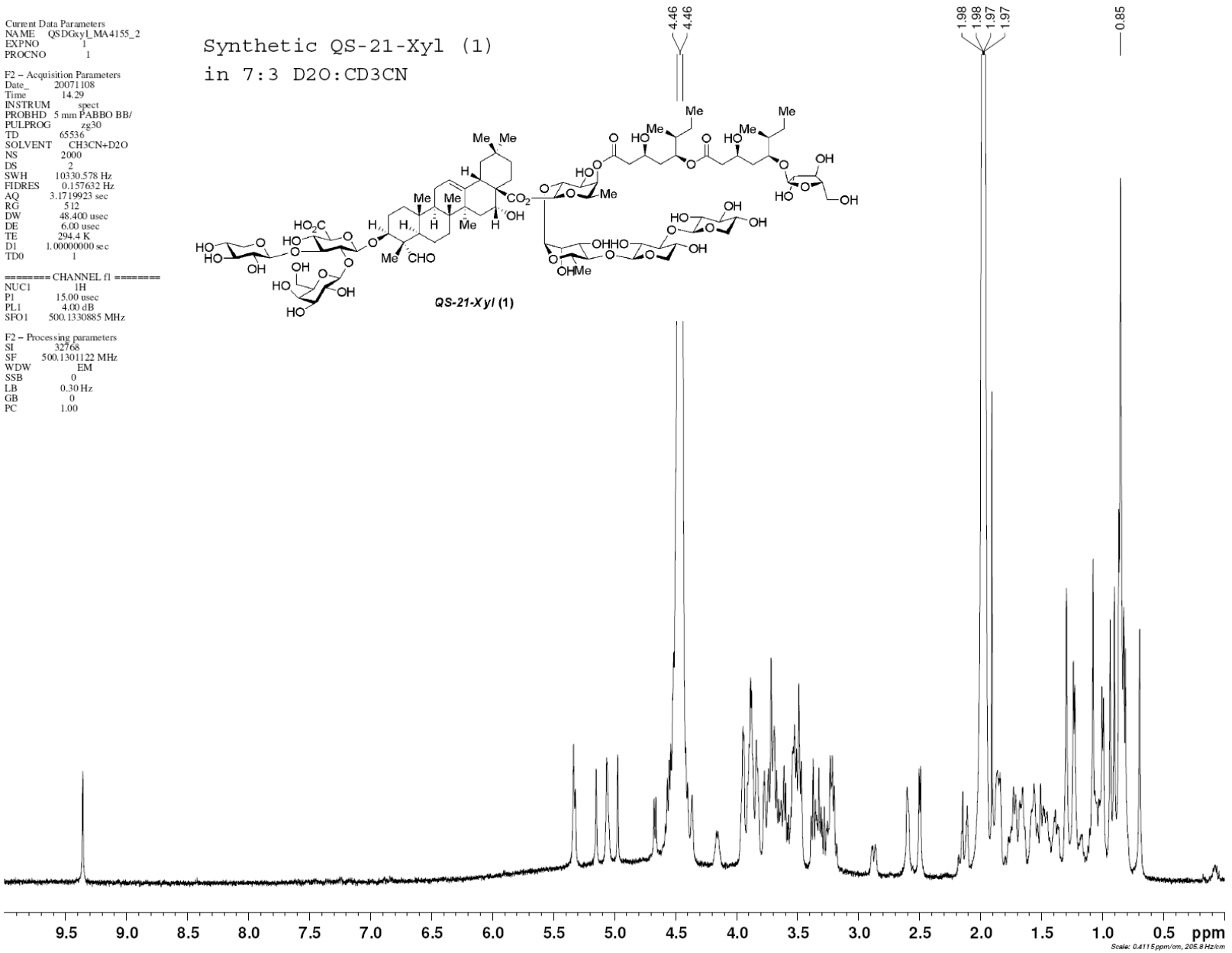
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TE 294.4 K
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PL1 4.00 dB
SFO1 500.1330885 MHz
F2 – Processing parameters
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WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
Synthetic QS-21-Xyl (1)
in 7:3 D2O:CD3CN
QS-21-Xyl (1)
4.46
4.46
1.98
1.98
1.97
1.97
0.85
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
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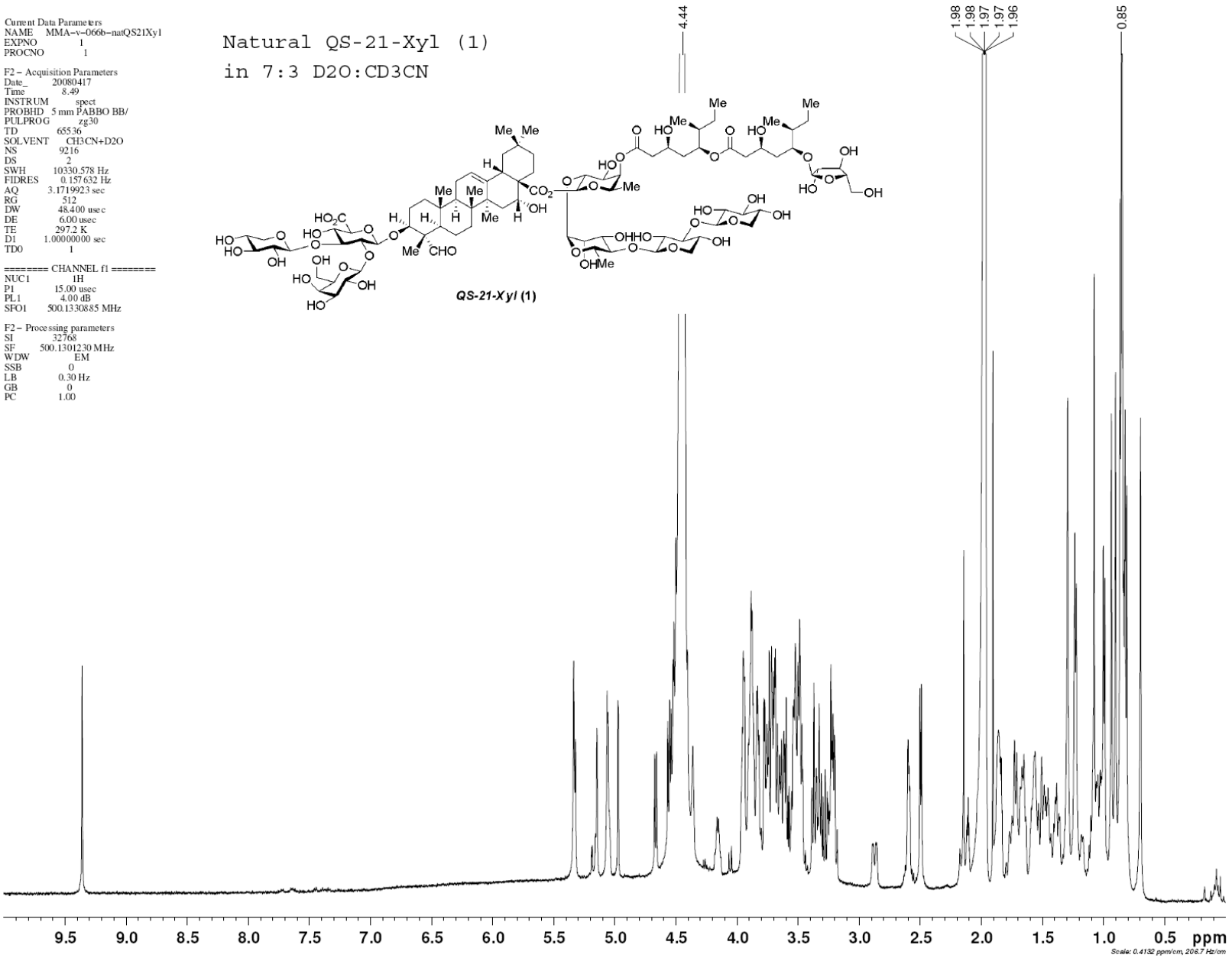
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TE 297.2 K
D1 1.00000000 sec
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======== CHANNEL f1 ========
NUC1 1H
P1 15.00 usec
PL1 4.00 dB
SFO1 500.1330885 MHz
F2 - Processing parameters
SI 32768
SF 500.1301230 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
Natural QS-21-Xyl (1)
in 7:3 D2O:CD3CN
QS-21-Xyl (1)
4.44
1.98
1.98
1.97
1.97
1.96
0.85
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
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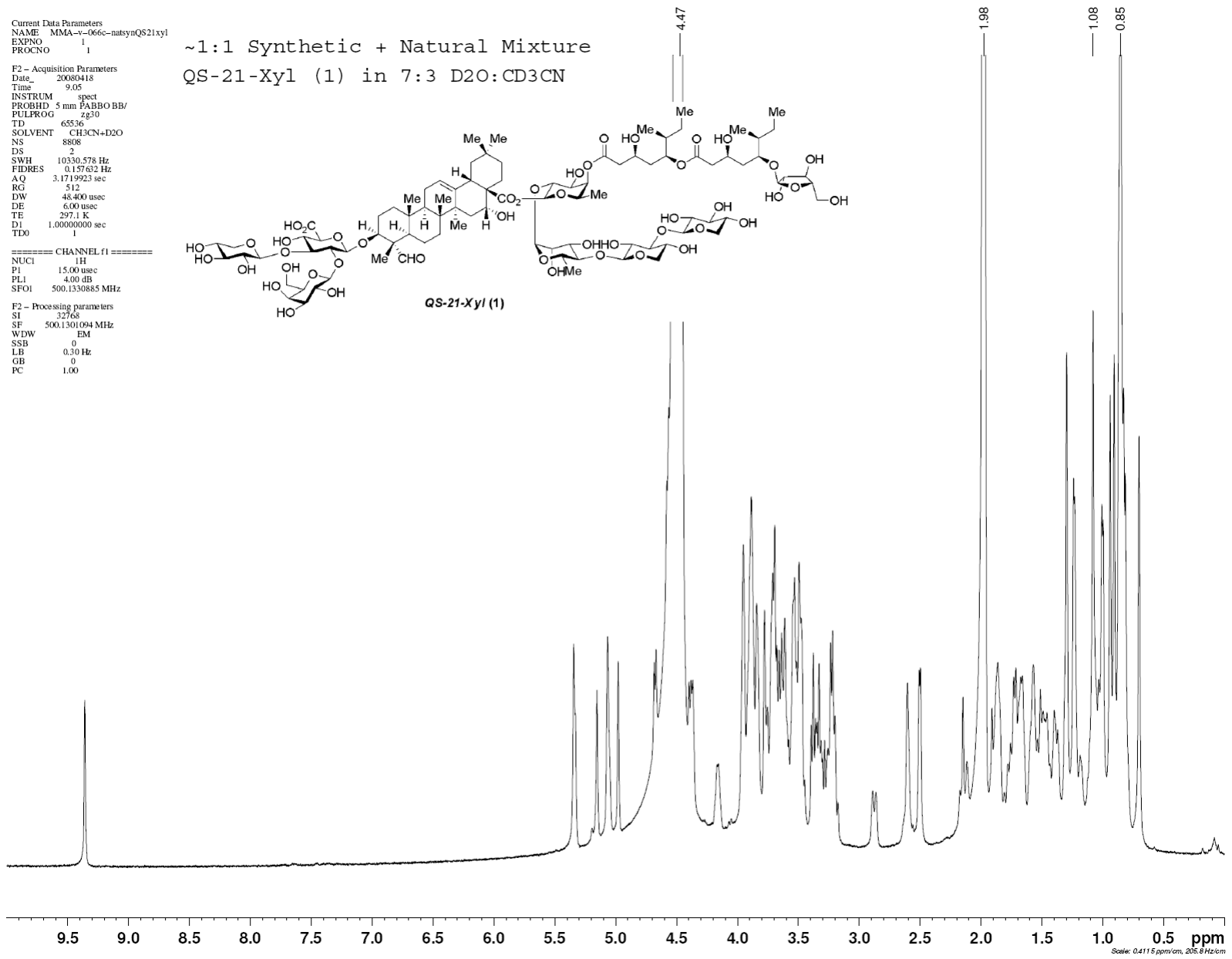
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FIDRES 0.157632 Hz
AQ 3.1719923 sec
RG 512
DW 48.400 usec
DE 6.00 usec
TE 297.1 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 15.00 usec
PL1 4.00 dB
SFO1 500.1330885 MHz
F2 - Processing parameters
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SF 500.1301094 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
~1:1 Synthetic + Natural Mixture
QS-21-Xyl (1) in 7:3 D2O:CD3CN
QS-21-Xyl (1)
4.47
1.98
1.08
0.85
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
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2.5
2.0
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ppm
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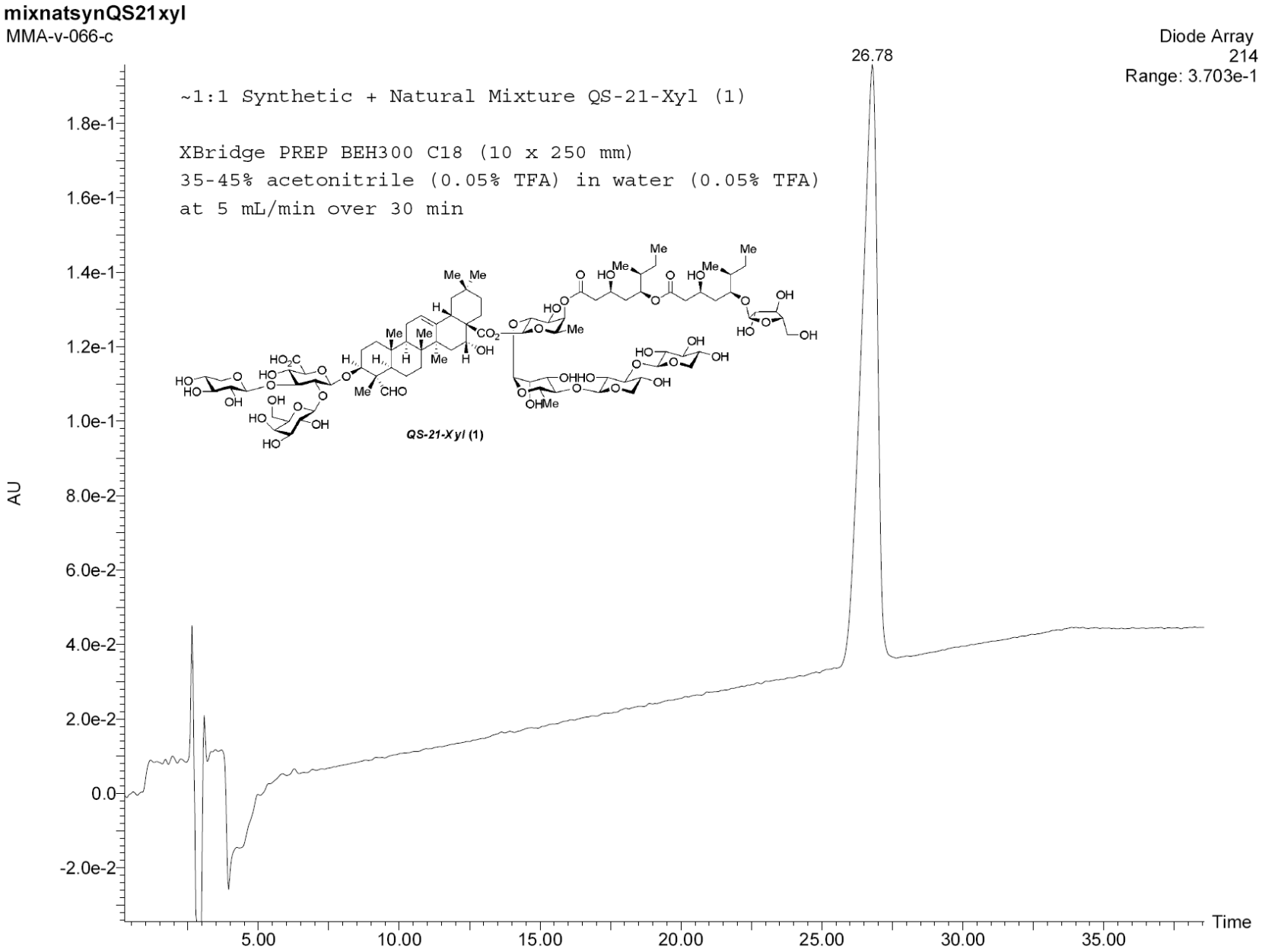
mixnatsynQS21xyl
MMA-v-066-c
Diode Array
214
Range: 3.703e-1
26.78
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XBridge PREP BEH300 C18 (10 x 250 mm)
35-45% acetonitrile (0.05% TFA) in water (0.05% TFA)
at 5 mL/min over 30 min
QS-21-Xyl (1)
AU
1.8e-1
1.6e-1
1.4e-1
1.2e-1
1.0e-1
8.0e-2
6.0e-2
4.0e-2
2.0e-2
0.0
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5.00
10.00
15.00
20.00
25.00
30.00
35.00
Time

[1] H. M. Nguyen, J. L. Poole, D. Y. Gin, *Angew. Chem. Int. Ed.* **2001**, *40*, 414-417.

[2] For other methods to resolve QS-21-Xyl (**1**) and QS-21-Api (**2**), see a) S. Soltysik, D. A. Bedore and C. R. Kensil, *Ann. N. Y. Acad. Sci*. **1993**, *690*, 392-395; C. A. Kensil, S. Soltysik and D. J. Marciani, "Methods for Enhancing Drug Delivery with Modified Saponins." US Pat. 5273965, 1993; and b) L. I. Nord and L. Kenne, *Carbohydr. Res.* **1999**, *320*, 70-81.

[3] For NMR characterizations of QS-21-Xyl (**1**), see a) N. E. Jacobsen, W. J. Fairbrother, C. R. Kensil, A. Lim, D. A. Wheeler and M. F. Powell, *Carbohydr. Res.* **1996**, *280*, 1-14; and b) L. I. Nord and L. Kenne, *Carbohydr. Res.* **1999**, *320*, 70-81.

[4] G. Ragupathi, M. Meyers, S. Adluri, L. Howard, C. Musselli and P. O. Livingston. *Int. J. Cancer* **2000**, *85*, 659-666.

[5] L. Svennerholm, *J. Neurochem*. **1963**, *10*, 613-623.