Supporting Information

Toward a PSA-Based Prostate Cancer Diagnostic Assay: Preparation of KLH-Conjugated Normal and Transformed PSA Fragments

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Experimental Section: Synthesis.

Reagents. All commercial materials were used as received unless otherwise noted. The following solvents were obtained from a dry solvent system and used without further drying: THF, diethyl ether, toluene, and DCM. Reagents were obtained from Aldrich or as noted, with the following exceptions: amino acids and resins for solid phase peptide synthesis were purchased from NovaBiochem; Biosynthesis grade DMF from EM Science; and other solvents from Fisher Scientific (HPLC grade).

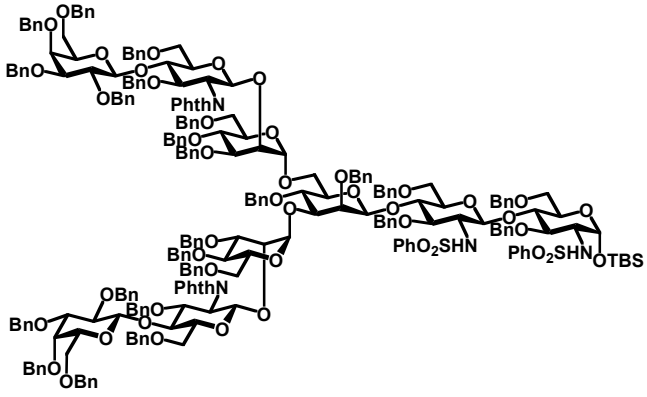
HPLC. All separations involved a mobile phase of 0.05% TFA (v/v) in water (solvent A)/0.0425% TFA in acetonitrile (solvent B). Preparative, semipreparative, and analytical HPLC separations were performed using a Rainin HXPL solvent delivery system equipped with a Rainin UV-1 detector and one of the following Dynamax-60Å C18 axial compression columns 250 mm in length equipped with a similarly packed guard column: 41.4 mm diameter (prep),

21.4 mm diameter (semiprep), or 4.6 mm diameter (analytical). Separations were performed at flow rates of 48 mL/min (prep), 16 mL/min (semiprep), or 1 mL/min (analytical), and were monitored at a wavelength between 214 and 230 nm, depending on column loading. LCMS chromatographic separations were performed using a Waters 2695 Separations Module and a Waters 996 Photodiode Array Detector equipped with a Varian Microsorb C18 2 × 150 mm column at a flow rate of 0.2 mL/min.

ESMS and LCMS. Electrospray mass spectroscopy and LCMS analyses were obtained on a Waters Micromass ZQ mass spectrometer in conjunction with the Waters HPLC apparatus described above.

NMR. ^{1}H and ^{13}C NMR spectra were recorded on Bruker instruments in $CDCl_3$, $C_6D_5CD_3$, D_2O at 400 or 500 MHz for ^{1}H and 100 or 125 MHz for ^{13}C.

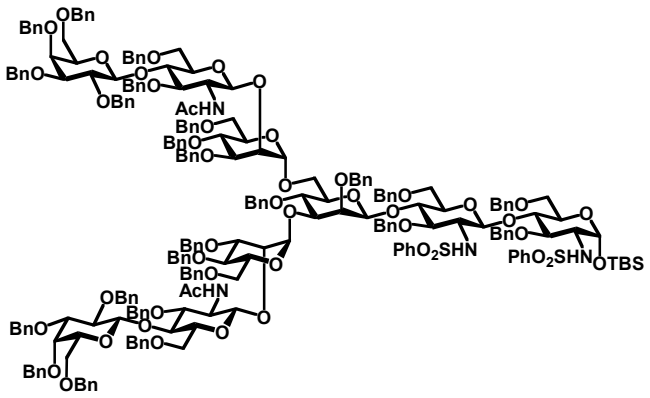
*Nonasaccharide **22a**.*



Saccharide **5** (120.6 mg, 52.3 μmol) and **21** (388.7 mg, 368.0 μmol, 7.03 equiv) were combined in an oven-dried 10 mL roundbottom flask and concentrated from dry toluene, then placed under high vacuum for 3 hr. The flask was then fitted with a stirbar and a rubber septum with an argon inlet. The material was dissolved in 2 mL dry dichloromethane; to the stirred solution were

added di-*tert*-butylpyridine (DTBP, 300 μL, 1.34 mmol, 25.5 equiv) and flame-dried 4Å molecular sieves (0.5 g). The suspension was stirred for 30 min under argon, then cooled to 0 °C over an ice bath; methyl triflate (120 μL, 1.06 mmol, 20.2 equiv) was added *via* plastic syringe. The reaction was allowed to warm slowly to room temperature. After 41.5 hr, the mixture was diluted with ethyl acetate, filtered through a plug of silica gel, and eluted with ethyl acetate. Some toluene was added and the solution concentrated almost to dryness; ethyl acetate was added, and the organics in a 60 mL separatory funnel were washed with saturated sodium bicarbonate, dried over magnesium sulfate, and concentrated to give 800 mg of an oily solid, yellow residue. The material was purified by column chromatography on silica gel, loaded with methylene chloride and eluted with 10 %→20 %→30 %→40 % ethyl acetate in hexanes. The fractions containing desired material were combined and concentrated, affording nonasaccharide **22a** as an amorphous white solid (135.6 mg, 31.6 μmol, 60 % yield). R_f = 0.32 (40 % ethyl acetate in hexanes); R_f = 0.71 (20 % ethyl acetate in toluene). $[\alpha]_D$=+0.5° (c=1.9, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$, selected signals), δ: 0.04 (s, 3H), 0.08 (s, 3H), 0.92 (s, 9H), 2.75 (br. d, *J*=9.3 Hz, 1H), 2.87 (dd, *J*=6.4, 10.7 Hz, 1H), 2.97 (d, *J*=10.6 Hz, 1H), 5.06 (d, *J*=2.2 Hz, 1H), 5.19 (d, *J*=7.1 Hz, 1H), 7.70 (d, *J*=7.6 Hz, 1H), 7.74 (d, *J*=7.5 Hz, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$), δ: -5.7, -4.5, 17.9, 25.7, 29.6, 55.5, 57.8, 58.3, 66.2, 67.7, 68.0, 68.1, 69.0, 69.4, 69.7, 69.9, 70.3 ,70.5, 71.7, 72.2, 72.3, 72.5, 72.8, 72.85, 72.9, 73.0, 73.6, 73.9, 74.0, 74.1, 74.3, 74.4, 74.5, 74.6, 74.8, 74.9, 75.0, 76.0, 76.4, 77.5, 77.6, 78.9, 79.4, 79.8, 80.8, 82.2, 82.3, 92.6, 95.9, 96.7, 97.9, 98.8, 100.6, 101.4, 102.7, 102.9, 123.0, 123.2, 126.1, 126.7, 127.0, 127.1-128.4, 128.7, 131.8, 132.1, 132.3, 133.4, 133.8, 137.4, 137.9, 138.0, 138.2, 138.3, 138.4, 138.42, 138.46, 138.5, 13836, 138.7, 138.8, 138.85, 138.9, 138.95, 139.0, 140.6, 141.1, 167.4, 168.2, 168.4. ESI-MS calcd for $C_{256}H_{268}N_4O_{50}S_2Si$ $[M+2H]^{2+}$ m/z = 2144.89, found: 2145.3.

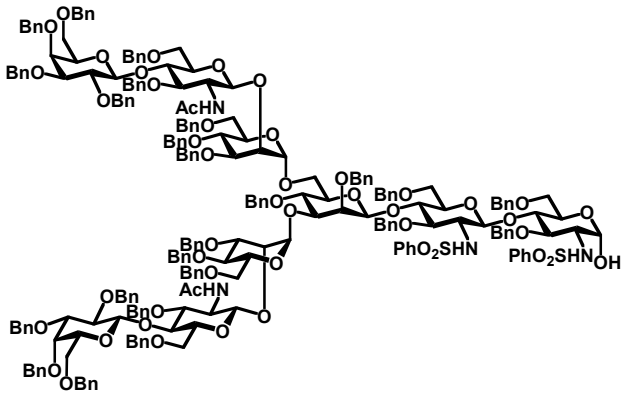
Di-N-*acetyl nonasaccharide* **22b**.



To nonasaccharide **22a** (178.1 mg, 41.5 μmol) in a 50 mL roundbottom flask with a stirbar were added fresh toluene (1 mL) and *n*-butanol (8 mL), and the contents were washed in with additional toluene (1 mL). The flask was briefly evacuated, then placed under argon using a septum with an argon needle inlet. Ethylenediamine (2.0 mL, 29.9 mmol) was added, and the reaction was heated to 90 °C and stirred for 40 hr. After cooling to room temperature, the stirbar was rinsed with toluene and removed, and the reaction mixture concentrated at low pressure, then concentrated twice more from toluene, affording 204 mg of the crude diamine (R_f = 0.29 in 5 % ethanol in toluene with 2 % triethylamine) as a pale yellow oil with some solid. Under argon, pyridine (3.0 mL, 37 mmol) and acetic anhydride (2.5 mL, 26.5 mmol) were added. After 17 hr of stirring at room temperature, the reaction mixture was concentrated, as before, 3 times from dry toluene, yielding 213 mg of a foam with some solid. Under argon, the material was dissolved in dry THF (2 mL) and dry methanol (5 mL). Sodium methoxide, 25 % by weight in methanol (100 μL, 437 μmol, 10.5 equiv) was added *via* micropipette. After stirring 45.5 hr, the reaction was quenched by the addition of solid ammonium chloride (82.7 mg, 1.55 mmol) all at once. The suspension was concentrated, then transferred to a 60 mL separatory funnel using ethyl acetate and water. The aqueous layer was removed and the organics were washed once

with saturated brine, dried with magnesium sulfate, filtered, and concentrated to give 172.0 mg of a crude oil. Purification by preparative TLC on 4 20×20 cm × 1 mm thickness PK6F plates developed with 15 % ethanol in toluene afforded desired nonasaccharide **22b** as a foam (122.8 mg, 29.8 μmol, 72 % yield). R_f = 0.35 (5 % ethanol in toluene). $[\alpha]_D$=+10.6° (*c* 3.5, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$, selected signals), δ: 0.07 (s, 3H), 0.10 (s, 3H), 0.94 (s, 9H), 1.71 (s, 3H), 1.74 (s, 3H), 5.05 (s, 1H), 5.11 (s, 1H), 5.23 (d, *J*=6.8 Hz, 1H), 5.59 (d, *J*=6.9 Hz, 1H), 7.69 (d, *J*=7.5 Hz, 2H), 7.77 (d, *J*=7.5 Hz, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$), δ: -5.7, -4.5, 18.0, 26.4, 25.8, 29.7, 56.8, 57.4, 58.0, 58.2, 66.6, 67.6, 68.2, 68.5, 69.1, 69.2, 69.4, 69.9, 70.0, 70.9, 71.8, 72.6, 72.9, 73.2, 73.3, 73.4, 73.7, 73.9, 74.0, 74.3, 74.6, 74.9, 75.0, 75.1, 76.1, 76.2, 76.3, 76.9, 77.2, 77.6, 77.7, 77.8, 78.2, 78.6, 79.8, 79.8, 79.9, 81.2, 82.4, 92.7, 98.1, 98.2, 98.4, 99.6, 100.6, 101.9, 102.7, 102.9, 126.2, 126.9-128.8, 132.2, 132.3, 137.6, 138.0, 138.05, 138.1, 138.3, 138.4, 138.45, 138.5, 138.7, 138.8, 139.0, 139.1, 139.4, 139.5, 140.6, 141.2, 170.6, 170.8. ESI-MS calcd for $C_{244}H_{266}N_4O_{48}S_2SiNa_2$ $[M+2Na]^{2+}$ *m*/*z* = 2078.88, found: 2079.3.

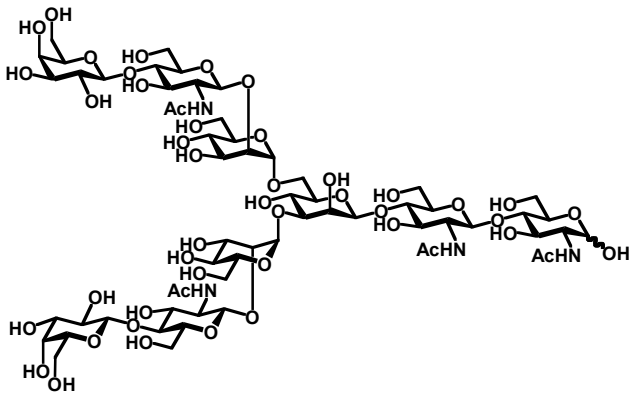
Reducing nonasaccharide **22**.



To nonasaccharide **22b** (122.8 mg, 29.8 μmmol) under argon in a 50 mL roundbottom flask fitted with a stirbar and a septum with an argon inlet was added acetic acid, 1.0 M in THF

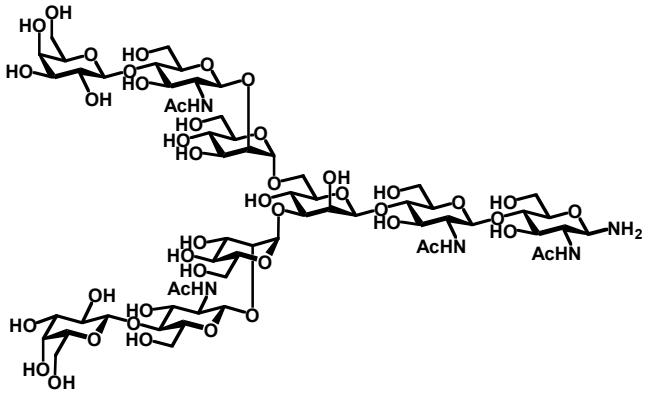
(2.0 mL, 2.0 mmol). Over an ice-water bath, TBAF, 1.0 M in THF (0.8 mL, 0.8 mmol, 27 equiv) was added, then the ice-bath was removed. After stirring for 21 hr at ambient temperature, the stirbar was rinsed and removed and the reaction mixture was concentrated at low pressure. The residue was transferred to a 60 mL separatory funnel with 30 mL ethyl acetate, washed with 2 × 10 mL saturated sodium bicarbonate and 1 × 10 mL saturated brine, dried over magnesium sulfate, filtered, and concentrated, yielding 138.1 mg crude product. Purification by preparative TLC on 4 20×20 cm × 1 mm thickness PK6F plates developed with 10 % ethanol in toluene afforded the desired nonasaccharide **22** as a foam (90.7 mg, 22.7 μmol, 76 % yield). R_f = 0.32 (5 % ethanol in toluene). $[\alpha]_D$=+4.0° (*c* 3.5, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$, selected signals), δ: 1.70 (s, 3H), 1.73 (s, 3H), 5.05 (s, 1H), 5.09 (s, 1H), 5.23 (d, *J*=7.0 Hz, 1H), 5.58 (d, *J*=6.7 Hz, 1H), 7.68 (d, *J*=7.4 Hz, 2H), 7.76 (d, *J*=7.6 Hz, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$), δ: 22.7, 22.9, 23.4, 23.7, 28.9, 29.3, 29.7, 30.3, 31.9, 38.7, 56.8, 57.0, 57.3, 58.4, 67.0, 67.8, 68.1, 68.2, 68.5, 68.8, 69.2, 69.5, 70.0, 70.8, 71.8, 72.6, 72.9, 73.2, 73.4, 73.7, 73.9, 74.0, 74.4, 74.6, 74.7, 75.0, 75.4, 76.0, 76.1, 77.2, 77.7, 78.1, 78.5, 79.5, 79.9, 81.3, 82.3, 82.4, 91.3, 98.2, 98.3, 99.6, 100.3, 101.8, 102.8, 102.9, 126.2, 127.0-128.8, 129.7, 130.8, 132.4, 132.5, 137.4, 138.0, 138.1, 138.2, 138.3, 138.4, 138.5, 138.7, 138.8, 138.9, 139.0, 139.4, 139.5, 140.2, 141.4, 170.6, 171.0. ESI-MS calcd for $C_{238}H_{252}N_4O_{48}S_2Na_2$ $[M+2Na]^{2+}$ *m*/*z* = 2021.83, found: 2021.7.

Deprotected nonasaccharide **23**.



(For preparation, see undecamer **37**). ^{1}H-NMR (400 MHz, D_2O, selected signals), δ: 2.02 (s, 3H), 2.03 (s, 3H), 2.04 (s, 3H), 2.11 (s, 3H), 4.10 (br. s, 1H), 4.18 (br. s, 1H), 4.24 (br. s, 1H), 4.46 (dd, *J*=2.3, 7.7 Hz, 1H), 4.58 (m, 3H), 4.91 (s, 1H), 5.10 (s, 1H), 5.17 (d, *J*=2.1 Hz, 1H). ESI-MS calcd for $C_{62}H_{104}N_4O_{46}Na$ $[M+Na]^+$ m/z = 1663.58, for $C_{62}H_{104}N_4O_{46}Na_2$ $[M+2Na]^{2+}$ m/z = 843.29, found: 1663.4, 843.3.

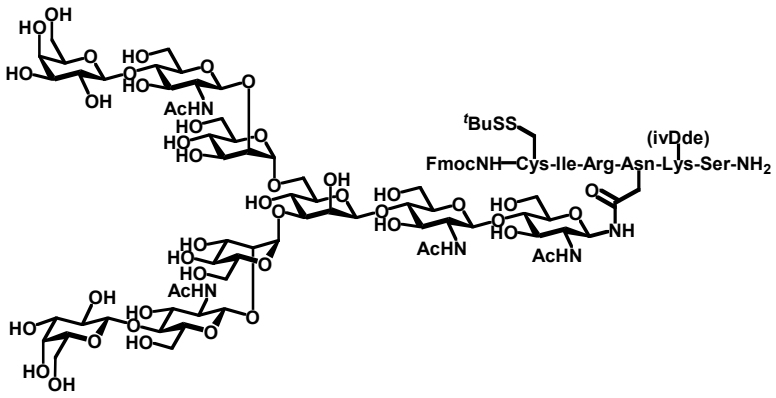
Glycosylamine **24**.



The starting material reducing saccharide **23** (20 mg, 12.2 μmol) in a 50 mL pear flask was dissolved in water (10 mL). To this clear, colorless solution was added solid ammonium hydrogencarbonate (6.28 g, 79.7 mmol); the suspension was stirred vigorously and heated

immediately to 40°C over a warm water bath. As the solution became almost clear, additional ammonium hydrogencarbonate was added at almost 2 hr., (2.99 g, 37.8 mmol) and at 8.5 hr. (5.00 g, 63.2 mmol). At 30 hr., the cloudy reaction mixture was allowed to cool, with stirring. The slurry was then transferred to a tared, 50 mL polypropylene conical tube, washed in with water to a total volume of 35 mL, immediately shell frozen, and lyophilized to give 650 mg white powder. This white powder was dissolved in 15 mL water, then immediately shell frozen and lyophilized again, affording 33.8 mg white powder. Similar lyophilization thrice more from 10 mL water (used to transfer the material to a 15 mL polypropylene conical tube) gave 28.0, 25.2, and 24.4 mg white or off-white solid. The ammonium bicarbonate seemed to be gone and the dry mass fairly constant when the lyophilized solid became relatively dense and granular. The crude solid was taken directly into the next reaction. ESI-MS calcd for $C_{62}H_{105}N_5O_{45}Na$ $[M+Na]^+$ m/z = 1662.60, for $C_{62}H_{105}N_5O_{45}Na_2$ $[M+2Na]^{2+}$ m/z = 842.79, found: 1662.5, 842.8.

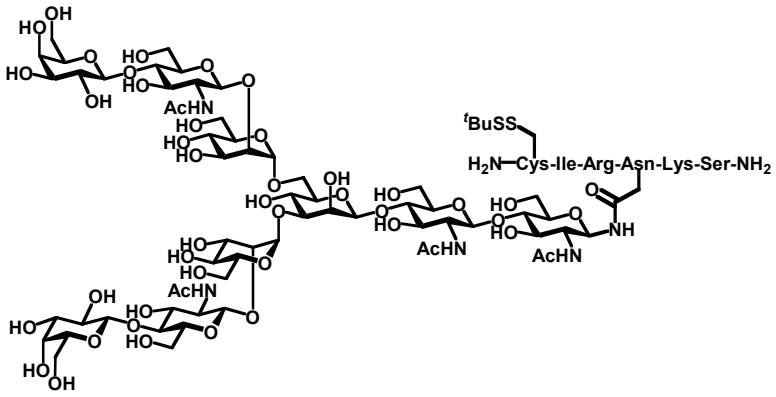
<u>*Nonasaccharide – hexapeptide* **26a**.</u>



To a 4 mL glass vial charged with a small stirbar, peptide **25** (44.4 mg, 35.9 μmol, 3.0 equiv) and HATU (27 mg, 71 μmol, 5.9 equiv) were added DMSO (100 μL), then diisopropylethylamine (9.0 μL, 52 μmol, 4.3 equiv), then DMSO (300 μL). With stirring, the solid dissolved in ~30 sec

to give an orange-brown solution. After 4 min, the solution was transferred *via* 500 μL syringe to a 15 mL polypropylene conical tube containing glycosylamine **24** (24.4 mg crude, 12 μmol). After swirling, the reaction mixture was centrifuged briefly, then stirred to dissolve all remaining solid over 5 min. To follow the reaction by LCMS, 1 μL samples were diluted with 20 μL DMSO and analyzed. The reaction had ceased, incomplete, by 3 hr, but was rejuvenated at 7 hr by addition of HATU (9.0 mg, 24 μmol, 2.0 equiv) and DIEA (1.0 μL, 5.7 μmol, 0.48 equiv), then addition of DIEA at 9 hr (3.7 μL, 21 μmol, 1.8 equiv) and at 10.5 hr (3.2 μL, 18 μmol, 1.5 equiv). Almost no glycosylamine remained by 11 hr. The entire reaction mixture was purified by semiprep HPLC (30-70 %B over 20 min). The combined fractions from 13.95 to 15.35 min were shell frozen and lyophilized in a 50 mL polypropylene conical tube, affording the desired **26a** as a white solid (21 mg, 7.3 μmol, 61 % yield). LCMS 30-70 %B over 20 min, RT 15.7 min. ESI-MS calcd for $C_{122}H_{194}N_{16}O_{57}S_2$ $[M+2H]^{2+}$ m/z = 1429.61, found: 1429.6

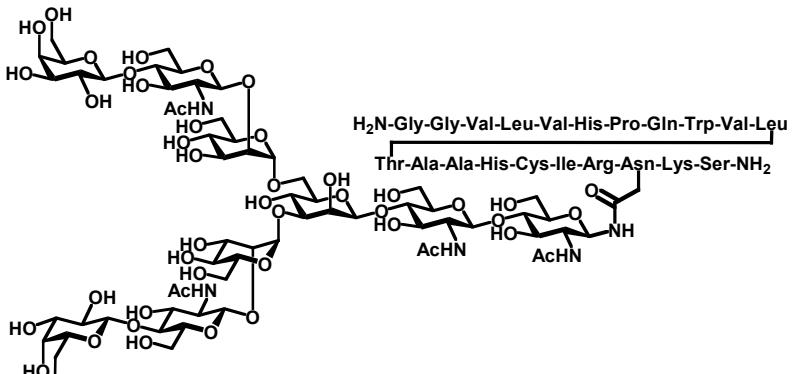
Deprotected glycopeptide **26**.



To protected glycopeptide **26a** (21 mg, 7.3 μmol) in a 50 mL polypropylene conical tube was added a cocktail (1 mL) consisting of hydrazine, piperidine, and DMF in a volume ratio of 5:15:80, respectively. After 30 min with occasional stirring, the reaction mixture was cooled

over an ice bath and acidified to pH ~3 with an ice-cold solution of TFA:water (1:10). The entire reaction mixture was purified by semiprep HPLC (5-15 %B over 20 min). The fractions from 16.9 to 17.8 min were combined and concentrated. The fractions eluting directly before and after the major fraction were repurified by HPLC as before. The fractions containing the desired **26** were combined and concentrated, then lyophilized, affording the desired **26** as a white solid (10.9 mg, 4.5 μmol, 62 % yield). ^{1}H-NMR (400 MHz, D_2O, selected signals), δ: 0.86 (t, J=7.5 Hz, 3H), 0.90 (d, J=6.8 Hz, 3H), 1.17 (m, 1H), 1.32 (s, 9H), 2.00 (s, 3H), 2.03 (br. s, 6H), 2.06 (s, 3H), 2.73 (dd, J=6.8, 16.2 Hz, 1H), 2.86 (dd, J=6.3, 16.2 Hz, 1H), 0.86 (br. t, J=7.6 Hz, 2H), 3.13-3.22 (m, 4H), 3.28 (dd, J=5.6, 14.6 Hz, 1H), 4.09 (d, J=3.1 Hz, 1H), 4.17 (d, J=2.2 Hz, 1H), 4.23-4.34 (m, 5H), 4.40 (t, J=5.4 Hz, 1H), 4.45 (d, J=7.7 Hz, 1H), 4.46 (d, J=7.8 Hz, 1H), 4.56-4.60 (m, 3H), 4.70 (t, J=6.4 Hz, 1H), 4.91 (br. s, 1H), 5.01 (d, J=9.6 Hz, 1H), 5.10 (br. s, 1H); ^{13}C-NMR (125 MHz, D_2O, selected signals), δ: 12.8, 17.4, 24.7, 24.9, 25.0, 25.1, 27.2, 27.4, 29.0, 30.9, 31.6, 33.0, 69.2, 39.3, 41.9, 43.3, 46.5, 47.3, 52.6, 55.4, 56.1, 56.4, 56.7, 57.6, 57.7, 58.2, 60.9, 62.7, 63.8, 64.4, 64.5, 68.5, 68.6, 70.0, 70.1, 71.3, 72.1, 72.9, 73.7, 74.7, 74.8, 75.2, 75.6, 76.3, 77.1, 77.4, 78.1, 79.0, 79.2, 81.0, 81.2, 81.4, 82.3, 83.1, 99.8, 102.1, 102.2, 102.3, 103.2, 104.1, 105.7, 107.7. LCMS 8-18 %B over 10 min, RT 9.4 min. ESI-MS calcd for $C_{94}H_{166}N_{16}O_{53}S_2$ $[M+2H]^{2+}$ m/z = 1215.51, found: 1215.6.

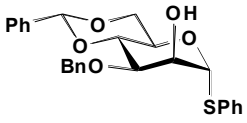
Nonasaccharide-uneicosapeptide glycoconjugate **1**.



To a 1 mL eppendorf tube charged with MES-Na (14.2 mg, 86.5 μmol, 38 equiv) was added aqueous phosphate buffered saline (1.0 mL, 0.2 M sodium chloride, 0.2 M phosphate, pH 7.4). The buffered solution was then added to a 100 mL roundbottom flask containing thioester **27** (5.8 mg, 3.5 μmol, 1.5 equiv) and glycopeptide **26** (5.5 mg, 2.3 μmol). The cloudy mixture was stirred over 2 hr, during which time acetonitrile (500 μL) was added, then placed under argon and stirred for a week to ensure complete destruction of the thioester, which co-eluted by HPLC with the desired material. The reaction was quenched by the addition of tris(carboxyethyl)phosphine (TCEP) (37.0 mg, 129 μmol, 56 equiv), giving a clear solution, which was stirred for 2.5 hr, acidified to pH ~2 with TFA, and purified by semiprep HPLC (20-40 %B over 20 min). The combined fractions from 11.2 to 12.0 min were shell frozen and lyophilized, affording the desired PSA fragment **1** as a white solid (1.5 mg, 0.38 μmol, 17 % yield). ^{1}H-NMR (400 MHz, D_2O, selected signals), δ: 1.19 (d, *J*=6.3 Hz, 3H), 1.32 (d, *J*=7.2 Hz, 3H), 1.35 (d, *J*=7.2 Hz, 3H), 2.88 (d, *J*=6.8 Hz, 2H), 2.97-3.07 (m, 4H), 4.26-4.35 (m, 5H), 4.37-4.41 (m, 2H), 4.45 (d, *J*=7.8 Hz, 2H), 4.50 (t, *J*=6.7 Hz, 1H), 4.56-4.60 (m, 3H), 4.91 (s, 1H), 4.94 (dd, *J*=5.5, 8.4 Hz, 1H), 5.01 (d, *J*=9.5 Hz, 1H), 5.10 (s, 1H), 7.10 (t, *J*=7.2 Hz, 1H), 7.18 (m, 2H), 7.23 (s, 1H), 7.28 (s, 1H), 7.42 (d, *J*=8.0 Hz, 1H), 7.58 (d, *J*=8.2 Hz, 1H), 8.53 (d, *J*=1.2

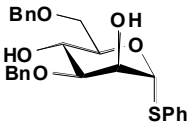
Hz, 1H), 8.60 (d, *J*=1.2 Hz, 1H). LCMS 25-35 %B over 10 min, RT 8.6 min. ESI-MS calcd for $C_{164}H_{270}N_{37}O_{70}S$ $[M+3H]^{3+}$ *m/z* = 1303.28, for $C_{164}H_{271}N_{37}O_{70}S$ $[M+4H]^{4+}$ *m/z* = 977.71, found: 1303.3, 977.8.

*Thiomannoside alcohol **29**.*



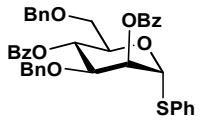
A suspension of diol **28** (2.52 g, 7.0 mmol) and dibutyltin oxide (1.867 g, 7.5 mmol) was refluxed in toluene (80 ml) with azeotropic removal of water for 3h. The resulting clear solution of the tin adduct was treated with benzyl bromide (1.25 ml, 10.5 mmol) and tetrabutylammonium iodide (544 mg, 1.5 mmol) and the reaction mixture was refluxed for 3 more hours. The reaction mixture was concentrated to ~ 20 ml volume, diluted with EtOAc, and washed with sat. aq. sodium bicarbonate solution. The organic layer was treated with Celite and sodium sulfate, filtered and concentrated to give the title alcohol (2.8 g, 89%) as a clear oil. $[\alpha]_D$ = +223.0° (c = 1.0, $CHCl_3$) ^{1}H-NMR ($CDCl_3$, 400MHz), δ: 2.6 (br.s, 1H), 3.90 (t, *J*=10.2 Hz, 1H), 4.00 (dd, *J*=3.5, 9.5 Hz, 1H), 4.19-4.27 (m, 2H), 4.31 (dd, *J*=1.5, 3.5 Hz, 1H), 4.38 (dt, *J*=5.0, 9.9 Hz, 1H), 4.78 (d, *J*=11.8 Hz, 1H), 4.94 (d, *J*=11.9 Hz, 1H), 5.63 (d, *J*=1.2 Hz, 1H), 5.66 (s, 1H), 7.29-7.58 (m, 15H). ^{13}C-NMR ($CDCl_3$, 100 MHz), δ: 64.8, 68.8, 71.7, 73.5, 76.0, 79.3, 88.1, 101.9, 126.4, 128.0, 128.2, 128.3, 128.5, 128.8, 129.3, 129.4, 132.0, 133.6, 137.7, 138.0. HRMS: Calcd. for $C_{26}H_{27}O_5S$ $[M+H]^+$ 451.1579, Found: 451.1570

*Thiomannoside diol **30a**.*



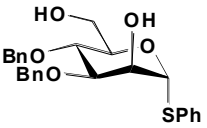
To a solution of the 4,6-*O*-benzylidene protected compound **29** (120 mg, 0.266 mmol) stirred in ice bath under Ar was added dropwise 1M HCl in ethyl ether until gas evolution ceased completely, and then another 0.1 ml. The reaction mixture was stirred in ice bath for another 2h and then at room temperature for 0.5 h before quenching with sat. aq. sodium bicarbonate solution. The residue was extracted with chloroform and isolated by preparative TLC (ethyl acetate/ hexanes 1/2) to give the title product (92 mg, 77%) as a colorless oil. $[\alpha]_D = +131.3°$ (c = 1.0, $CHCl_3$) ^{1}H-NMR ($CDCl_3$, 400MHz), δ: 2.73 (br.d, *J*=2.4 Hz 1H), 2.80 (br.s, 1H), 3.70 (dd, *J*=3.5, 9.2 Hz, 1H), 3.77 (dd, *J*=2.4, 4.0 Hz, 1H), 4.02 (br.t, *J*=9.2 Hz 1H), 4.24 (br.s, 1H), 4.27-4.32 (m, 1H), 4.52 (d, *J*=12.1 Hz, 1H), 4.59 (d, *J*=12.0 Hz, 1H), 4.69 (d, *J*=11.6 Hz, 1H), 4.75 (d, *J*=11.6 Hz, 1H), 5.59 (br.s, 1H), 7.23-7.52 (m, 15H). ^{13}C-NMR ($CDCl_3$, 100 MHz), δ: 68.5, 69.8, 70.4, 71.9, 72.4, 73.8, 79.9, 87.8, 127.8, 128.0, 128.1, 128.4, 128.6, 128.7, 129.0, 129.4, 131.9, 134.1, 137.9, 138.2. HRMS: Calcd. for $C_{26}H_{29}O_5S$ $[M+H]^+$ 453.1736, Found: 453.1741

*Thiomannoside donor **30**.*



The diol **30a** was benzoylated as described for **31** to give dibenzoyl protected material **30** in 98% yield. $[\alpha]_D$ = +13.1° (c = 1.0, $CHCl_3$) ^{1}H-NMR ($CDCl_3$, 400MHz), δ: 3.71-3.78 (m, 2H), 4.07 (dd, *J*=9.7, 3.0 Hz, 1H), 4.46-4.57 (m, 3H), 4.61-4.66 (m, 1H), 4.69 (d, *J*=12.5 Hz, 1H), 5.68 (br.s, 1H), 5.81 (t, *J*=9.8 Hz, 1H), 5.88 (dd, *J*=3.0, 1.5 Hz, 1H), 7.61-7.64 (m, 21H), 7.98-8.11 (m, 4H). ^{13}C-NMR ($CDCl_3$, 100 MHz), δ: 68.9, 69.7, 70.5, 71.3, 71.6, 73.8, 74.9, 86.6, 127.7, 127.9, 128.0, 128.2, 128.3, 128.5, 128.6, 128.7, 128.8, 129.4, 129.8, 129.9, 130.2, 130.3, 132.5, 133.4, 133.5, 133.6, 137.5, 138.3, 165.8, 166.0. HRMS: Calcd. for $C_{40}H_{37}O_7S$ $[M+H]^+$ 661.2260, Found: 661.2284

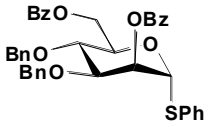
*Thiomannoside diol **31a**.*



Benzylidine-protected mannoside **29** (98.6 mg, 219 μmol) was concentrated twice from dry toluene in a 25 mL roundbottom flask, which was then fitted with an oven-dried stirbar and a dry argon inlet. The flask was almost entirely submerged in an ice-water bath for 10 min, at which point a 1.0 M solution of borane-THF in THF (1.55 mL, 1.55 mmol, 7.08 equiv) was added slowly *via* syringe down the sides of the flask. After stirring 15 min, a 1.0 M solution of dibutylboron triflate in dichloromethane (0.22 mL, 220 μmol, 1.0 equiv) was added *via* syringe dropwise. The resulting solution was stirred for 90 min at 0° C, at which point triethylamine (0.12 mL, 861 μmol, 3.9 equiv) was added *via* syringe, then methanol (10 mL) was added,

dropwise at first. The mixture was concentrated, then concentrated three more times from 10 mL methanol to give an oily residue. Column chromatography on silica gel eluted with 40 %→50 % ethyl acetate in hexanes afforded diol **30a** (99.5 mg, 217 μmol, 97 % yield) as a clear, colorless oil. R_f = 0.16 (50 % ethyl acetate in hexanes); R_f = 0.63 (100 % ethyl acetate). $[\alpha]_D$ = +200.0° (*c* 0.52, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$, selected signals), δ: 2.68 (br. s, 2H), 3.80 (d, *J*=3.1 Hz, 2H), 3.90 (dd, *J*=2.9, 9.1 Hz, 1H), 3.96 (t, *J*=9.3 Hz, 1H), 4.15 (dt, *J*=9.3, 3.0 Hz, 1H), 4.25 (t, *J*=1.5 Hz, 1H), 4.66 (d, *J*=10.9 Hz, 1H), 4.73 (s, 2H), 4.87 (d, *J*=10.9 Hz, 2H), 5.57 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$), δ: 61.7, 69.9, 72.2, 72.7, 74.0, 75.2, 80.0, 87.2, 127.7, 127.8, 128.0, 128.1, 128.4, 128.6, 129.1, 131.8, 133.3, 137.5, 138.1. ESI-MS calcd. for $C_{26}H_{28}O_5SNa$ $[M+Na]^+$ *m/z* = 475.16, found: 475.0. HRMS calcd. for $C_{26}H_{28}O_5SNa$ $[M+Na]^+$ *m/z* = 475.1555, found: 475.1543.

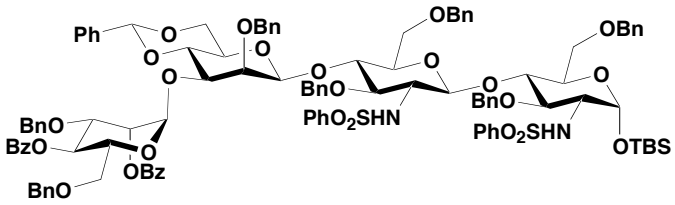
*Thiomannoside **31**.*



Diol **30a** (33.3 mg, 73.6 μmol) in a 50 mL pear-shaped flask was dissolved in 1 mL of freshly-dried dichloromethane. Pyridine (20.0 μL, 247 μmol, 3.36 equiv) and benzoyl chloride (26.0 μL, 224 μmol, 3.04 equiv) were added *via* micropipette, followed by DMAP (1.3 mg, 11 μmol, 14 mol %). After stirring for 26 hr at room temperature, a drop of water was added, and after an additional 10 min the mixture was concentrated at low pressure. Purification by preparative TLC on 2 20×20 cm × 250 μm thickness LK6F plates developed with 50 % ethyl acetate in hexanes afforded **30** (40.8 mg, 61.7 μmol, 84 % yield) as a colorless film. R_f = 0.33 (20 % ethyl acetate in hexanes); R_f = 0.63 (50 % ethyl acetate in hexanes). $[\alpha]_D$ = +92.1° (*c* 1.19,

$CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$, selected signals), δ: 4.06-4.13 (m, 2H), 4.53-4.67 (m, 5H), 4.83 (d, *J*=11.2 Hz, 1H), 4.93 (d, *J*=10.8 Hz, 1H), 8.00 (dd, *J*=0.9, 8.2 Hz, 2H), 8.04 (d, *J*=8.4 Hz, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$), δ: 63.5, 70.5, 70.9, 71.7, 73.9, 75.3, 78.6, 86.3, 127.8, 127.9, 128.2, 128.28, 128.33, 128.40, 128.42, 129.1, 129.66, 129.72, 129.8, 130.0, 131.9, 133.0, 133.3, 137.4, 137.7, 165.5, 166.1. ESI-MS calcd. for $C_{40}H_{36}O_7SNa$ $[M+Na]^+$ *m/z* = 683.20, found: 683.2. HRMS calcd. for $C_{40}H_{36}O_7SNa$ $[M+Na]^+$ *m/z* = 683.2079, found: 683.2087.

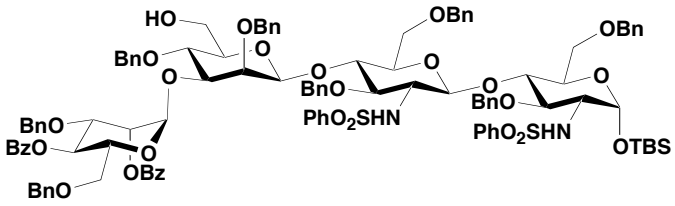
*Tetrasaccharide **32a**.*



A solution of thiomannoside donor **30** (180.0 mg, 0.27 mmol) in dry acetonitrile (1.75 ml) was stirred with 3A molecular sieves for 1 h and then cooled to 15 °C. The solution was then treated with solid promoter $(BrC_6H_4)_3NSbCl_6$ (210 mg, 0.34 mmol) followed by the trisaccharide acceptor **4** (129 mg, 0.09 mmol) in dry acetonitrile (0.75 ml). The reaction mixture was protected from light and stirred at 15 °C for 40 min before the addition of another portion of $(BrC_6H_4)_3NSbCl_6$ (48 mg, 0.08 mmol). Stirring was continued for another 12 h at room temperature at which point the reaction was quenched by addition of triethylamine, filtered through a Celite pad, and concentrated. The residue was purified by flash chromatography on silica gel (eluent: ethyl acetate/hexanes 1/2) and then by preparative TLC (eluent: ethyl acetate/toluene 1/5) to afford starting trisaccharide acceptor **4** (18 mg) and desired tetrasaccharide **32a** (127 mg, 71%, 83 % b.r.s.m.) as a colorless oil. $[\alpha]_D$ = –31.0° (c = 2.2, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400MHz), δ: 0.05 (s, 3H), 0.11 (s, 3H), 0.93 (s, 3H), 3.00 (dt, *J*=4.6,

9.8 Hz, 1H), 3.18 (dt, *J*=7.8, 3.6 Hz, 1H), 3.26-3.41 (m, 6H), 3.45 (dd, *J*=3.1, 11.0 Hz, 1H), 3.51 (dt, *J*=11.0, 2.1 Hz, 1H), 3.59-3.68 (m, 3H), 3.76-3.87 (m, 4H), 3.92-3.99 (m, 2H), 4.02-4.16 (m, 4H), 4.21 (d, *J*=12.0 Hz, 1H), 4.28 (d, *J*=12.0 Hz, 1H), 4.29-4.35 (m, 2H), 4.38 (d, *J*=12.8 Hz, 1H), 4.39 (d, *J*=10.8 Hz, 1H), 4.43-4.54 (m, 4H), 4.61 (d, *J*=12.5 Hz, 1H), 4.74 (d, *J*=11.5 Hz, 1H), 4.76 (d, *J*=12.0 Hz, 1H), 4.83-4.93 (m, 2H), 5.12 (d, *J*=1.5 Hz, 1H), 5.45 (d, *J*=1.3 Hz, 1H), 5.53 (s, 1H), 5.69 (t, *J*=10.0 Hz, 1H), 5.80 (dd, *J*=2.0, 3.0 Hz, 1H), 6.93-6.99 (m, 2H), 7.02-7.09 (m, 3H), 7.12-7.17 (m, 4H), 7.18-7.49 (m, 4H), 7.54-7.63 (m, 2H), 7.74-7.79 (m, 4H), 7.90-7.99 (m, 2H), 8.05-8.09 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$), δ: -5.7, -4.4, 18.0, 25.8, 58.0, 58.7, 67.1, 67.6, 68.1, 68.2, 68.6, 69.5, 69.7, 70.7, 71.1, 73.4, 73.5, 73.6, 73.7, 74.0, 74.6, 75.2, 75.3, 76.0, 76.3, 77.2, 78.2, 78.3, 80.0, 92.8, 98.9, 100.9, 101.0, 101.1, 125.8, 127.0, 127.1, 127.3, 127.4, 127.5, 127.6, 127.65, 127.7, 127.8, 127.9, 128.0, 128.03, 128.1, 128.14, 128.2, 128.25, 128.3, 128.33, 128.4, 128.45, 128.5, 128.7, 128.8, 128.9, 129.3, 129.5, 129.7, 129.8, 129.9, 132.2, 132.3, 133.1, 133.2, 137.1, 137.4, 137.6, 137.8, 137.9, 138.4, 138.5, 140.7, 141.5, 165.39, 165.42. HRMS: Calcd. for $C_{112}H_{120}N_2O_{25}S_2SiNa$ $[M+Na]^+$ 2007.7289, Found: 2007.7309

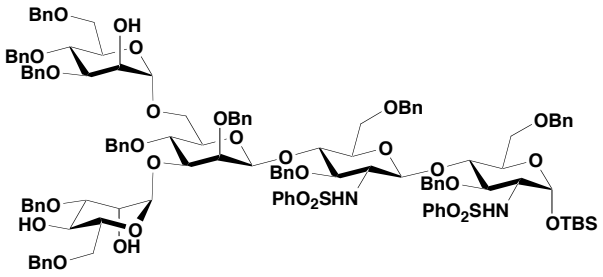
*Tetrasaccharide alcohol **32**.*



4,6-*O*-Benzylidene protected tetrasaccharide **32a** (100.0 mg, 0.05 mmol) was taken up in 1M solution of BH_3 in tetrahydrofuran (1.00 ml, 1.00 mmol) and the resulting mixture was stirred at 0 °C for 5 min before the addition of 1M Bu_2BOTf in dichloromethane (0.15 ml, 0.15 mmol). Stirring was continued at this temperature for 2 h and then the reaction was quenched by

sequential addition of triethylamine and methanol. The solution was concentrated and coevaporated with methanol 5 times. The residue was dried and subjected to preparative TLC (eluent: ethyl acetate/hexanes 1/2) to afford starting material **32a** (12 mg) and desired alcohol **32** (85 mg, 85 %, 96 % b.r.s.m.). $[\alpha]_D = -34.0°$ (c = 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ: 0.06 (s, 3H), 0.11 (s, 3H), 0.94 (s,9H), 2.10 (br.s, 1H), 3.01 (dq, *J*=9.0, 3.0 Hz, 1H), 3.22 (dd, *J*=11.2, 3.0 Hz, 1H), 3.27-3.34 (m, 4H), 3.37 (dd, *J*=7.8, 15.7 Hz, 2H), 3.41-3.50 (m, 4H), 3.56-3.62 (m, 3H), 3.64 (dd, *J*=3.0, 9.6 Hz, 1H), 3.72-3.79 (m, 2H), 3.90 (d, *J*=3.0 Hz, 1H), 3.96 (t, *J*=9.5 Hz, 1H), 4.02-4.10 (m, 2H), 4.20 (d, *J*=7.5 Hz, 1H), 4.22-4.29 (m, 3H), 4.31-4.35 (m, 2H), 4.35-4.52 (m, 7H), 4.57 (d, *J*=12.7 Hz, 1H), 4.62 (d, *J*=10.8 Hz, 1H), 4.64 (d, *J*=12.1 Hz, 1H), 4.74 (d, *J*=11.4 Hz, 1H), 4.76-4.84 (m, 3H), 4.99 (d, *J*=12.5 Hz, 1H), 5.13 (d, *J*=1.7 Hz, 1H), 5.34 (d, *J*=1.5 Hz, 1H), 5.67 (t, *J*=10.7 Hz, 1H), 5.70-5.73 (m, 1H), 6.95-7.46 (m, 52H), 7.55-7.64 (m, 2H), 7.72-7.80 (m, 4H), 7.82-7.85 (m, 2H), 8.06-8.10 (m, 2H); ^{13}C-NMR ($CDCl_3$, 100 MHz), δ: -5.7, -4.4, 18.0, 25.8, 57.9, 58.4, 61.3, 67.7, 68.4, 68.7, 68.8, 69.4, 69.9, 71.0, 71.1, 73.1, 73.4, 73.5, 73.6, 74.0, 74.1, 74.3, 74.6, 75.2, 76.0, 76.1, 76.9, 77.2, 77.5, 78.6, 79.7, 81.4, 92.8, 99.9, 100.8, 101.2, 126.6, 127.0, 127.06, 127.1, 127.2, 127.3, 127.34, 127.4, 127.44, 127.5, 127.6, 127.7, 127.75, 127.8, 127.87. 127.9, 128.0, 128.07, 128.1, 128.15, 128.2, 128.23, 128.26, 128.3, 128.4, 128.45, 128.5, 128.52, 128.62, 128.8, 129.0, 129.5, 129.6, 129.8, 130.0, 132.3, 132.4, 133.1, 133.2, 137.3, 137.7, 137.8, 137.9, 138.3, 138.4, 138.9, 140.7, 141.1, 165.3, 165.5. HRMS: Calcd. for $C_{112}H_{122}N_2O_{25}S_2SiNa$ $[M+Na]^+$ 2009.7445, Found: 2009.7450

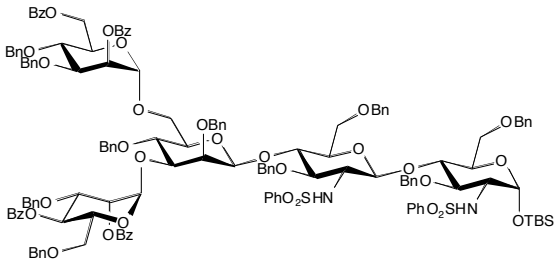
*Pentasaccharide **6**.*



Thiomannoside donor **20** (323 mg, 0.16 mmol) was coupled with tetrasaccharide acceptor **32** (323 mg, 0.05 mmol) following the procedure for the preparation of tetrasaccharide **32a**. Separation of reaction mixture using flash chromatography (eluent: ethyl acetate/hexanes 1/3 to 1/2) afforded pentasaccharide **33** (ESI-MS: Calcd. for $C_{146}H_{154}N_2O_{31}S_2SiNa$ $[M+Na]^+$ 2546.0, Found: 2546.0), containing trace amounts of impurities. This material was dissolved in dry methanol (10.0 ml) and treated with sodium methoxide (25 wt.% in methanol, 0.3 ml). The reaction mixture was stirred overnight at room temperature, quenched with ammonium chloride and concentrated. The dry residue was suspended in ethyl acetate, filtered, and concentrated. Purification by preparative TLC (eluent: ethyl acetate/hexanes 1/2) gave the desired triol **6** (255 mg, 72% for 2 steps from acceptor **32**). $[\alpha]_D$ = +6.6° (c = 1.3, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.05 (s, 3H), 0.09 (s, 3H), 0.92 (s, 9H), 2.30 (br. s, 3H), 3.10 (dq, *J*=9.3, 2.2 Hz, 1H), 3.17-3.36 (m, 7H), 3.39-3.72 (m, 12H), 3.73-3.91 (m, 8H), 3.95 (dd, *J*=2.0, 2.9 Hz, 1H), 4.11 (d, *J*=7.4 Hz, 1H), 4.20 (d, *J*=8.1 Hz, 1H), 4.23-4.34 (H, 4H), 4.37-4.60 (m, 13H), 4.65 (d, *J*=11.9 Hz, 1H), 4.70-4.81 (m, 3H), 4.83 (d, *J*=11.7 Hz, 1H), 4.88 (d, *J*=1.4 Hz, 1H), 4.93 (d, *J*=12.0 Hz, 1H), 5.11 (d, *J*=2.2 Hz, 1H), 5.12 (d, *J*=1.1 Hz, 1H), 7.10-7.35 (m, 57H), 7.37-7.44 (m, 4H), 7.71 (br. d, *J*=8.4 Hz, 1H), 7.76 (br. d, *J*=8.3 Hz, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$), δ: –5.65, –4.42, 18.03, 25.82, 58.0, 58.5, 66.3, 67.6, 67.7, 67.8, 67.9, 68.8, 69.0, 69.8, 70.4, 71.1, 71.4, 71.5,

72.0, 73.1, 73.2, 73.4, 73.5, 73.6, 74.2, 74.5, 74.6, 74.7, 74.8, 75.0, 75.7, 76.1, 77.2, 77.4, 78.6, 79.3, 79.5, 79.7, 80.9, 92.8, 100.0, 100.8, 101.2, 101.4, 127.0, 127.3, 127.4, 127.45, 127.5, 127.6, 127.65, 127.7. 127.76, 127.8, 127.86, 127.9, 128.1, 128.15, 128.2, 128.24, 128.3, 128.4, 128.46, 128.5, 128.6, 128.8, 128.9, 132.3, 132.34, 137.6, 137.7, 137.8, 137.86, 137.94, 138.0,138.2, 138.45, 138.5, 139.0, 140.7, 141.2. HRMS: Calcd. for $C_{125}H_{142}N_2O_{28}S_2SiNa$ $[M+Na]^+$ 2233.8858, Found: 2233.8859

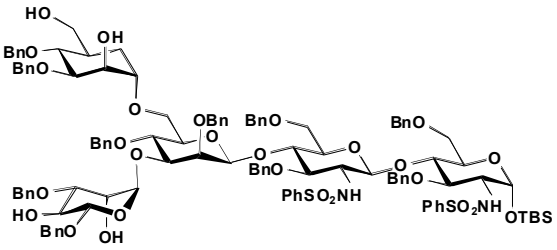
*Tetra-O-benzoate **34**.*



Tetrasaccharide acceptor **32** (259 mg, 0.13 mmol) was coupled with thiomannoside **31** (264 mg, 0.40 mmol) as described for the preparation of **32a**. Preparative TLC of the product mixture (eluent: ethyl acetate/toluene 1/10; in mixtures of ethyl acetate/hexanes the product and starting acceptor have identical R_f values) afforded the desired pentasaccharide **34** (244 mg, 74%). $[\alpha]_D$= – 8.6° (c = 1.22, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$), δ: -0.01 (s, 3H), 0.04 (s, 3H), 0.85 (s, 9H), 2.95-3.01 (m, 2H), 3.10 (br. d , *J*=10.3 Hz, 1H), 3.17-3.33 (m, 6H), 3.35-3.44 (m, 2H), 3.46-3.62 (m, 5H), 3.68-3.83 (m, 5H), 3.88-3.99 (m, 5H), 4.11 (dd, *J*=3.0, 9.6 Hz, 1H), 4.14-4.25 (m, 5H), 4.28 (d, *J*=10.0 Hz, 1H), 4.31-4.53 (m, 12H), 4.56 (d, *J*=12.0 Hz, 1H), 4.59-4.65 (m, 3H), 4.69 (d, *J*=11.4 Hz, 1H), 4.73 (d, *J*=11.2 Hz, 1H), 4.77-4.86 (m, 3H), 5.04-5.09 (m, 2H), 5.20 (d, *J*=1.2 Hz, 1H), 5.56 (br. t , *J*=2.2 Hz, 1H), 5.66-5.68 (m, 1H), 5.69 (t, *J*=10.0 Hz, 1H), 6.86-6.95 (m, 4H), 6.97-7.44 (m, 60H), 7.48-7.56 (m, 5H), 7.61 (br. d, *J*=8.0 Hz, 2H), 7.70

(br. d, *J*=8.0 Hz, 2H), 7.83 (br. d, *J*=8.0 Hz, 2H), 7.91-7.98 (m, 4H), 8.04 (br. d, *J*=8.3 Hz, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$), δ: -5.69, -4.51, 17.96, 25.76, 57.9, 58.8, 63.1, 66.3, 67.7, 68.3, 68.5, 68.7, 69.5, 69.7, 70.0, 70.9, 71.1, 71.2, 72.9, 73.2, 73.5, 73.6, 73.9, 74.3, 74.5, 74.7, 75.1, 75.9, 76.9, 77.2, 77.6, 78.4, 78.5, 79.1, 82.8, 92.8, 98.0, 100.0, 100.8, 101.9, 126.6, 126.9, 126.96, 127.0, 127.1, 127.2, 127.3, 127.4, 127.5, 127.6, 127.64, 127.7, 127.85, 127.9, 128.0, 128.1, 128.2, 128.25, 128.3, 128.34, 128.36, 128.4, 128.5, 128.6, 128.7, 129.0, 129.4, 129.55, 129.6, 129.64, 129.76, 129.82, 129.9, 130.0, 130.1, 132.1, 132.3, 132.9, 133.0, 133.1, 133.2, 137.4, 137.5, 137.6, 137.7, 137.74, 137.8, 137.9, 138.0, 138.3, 138.4, 139.1, 140.6, 141.1, 165.1, 165.4, 165.5, 166.0. ESI-MS: Calcd. for $C_{146}H_{152}N_2O_{32}S_2SiNa$ $[M+Na]^+$ 2559.9, Found: 2559.8

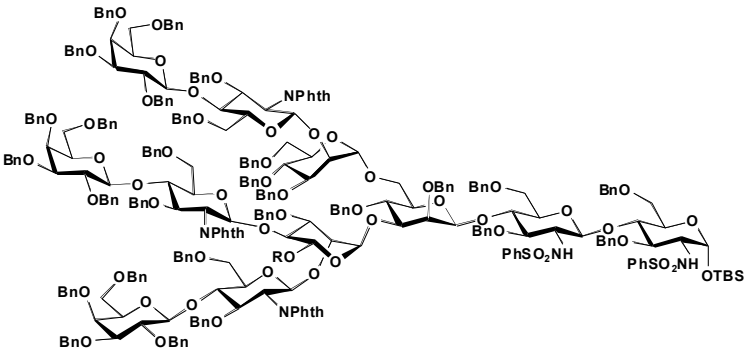
*Pentasaccharide tetraol **7**.*



Pentasaccharide **34** (244 mg. 0.096 mmol) was dissolved in dry methanol (20.0 ml) and treated with sodium methoxide (25 wt.% in methanol, 0.5 ml). The reaction mixture was stirred overnight at room temperature, quenched with ammonium chloride and concentrated. The dry residue was suspended in ethyl acetate, filtered, and concentrated. Purification by preparative TLC (eluent: ethyl acetate/hexanes 1/2) gave the desired tetraol **7** (187 mg, 92%). $[\alpha]_D$=+3.7° (c=1.87, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$), δ: 0.08 (s, 3H), 0.13 (s, 3H), 0.95 (s, 9H), 2.52 (s, 4H), 3.15 (br. d, *J*=10.4 Hz, 1H), 3.20-3.32 (m, 4H), 3.34-3.42 (m, 3H), 3.46-3.51 (m, 2H), 3.52-3.98 (m, 20H), 4.16 (d, *J*=7.6 Hz, 1H), 4.25-4.39 (m, 5H), 4.41-4.69 (m, 12H), 4.74 (d, *J*=12.0

Hz, 1H), 4.79 (d, *J*=12.0 Hz, 1H), 4.84-4.98 (m, 4H), 5.14 (d, *J*=2.6 Hz, 1H), 5.18 (br. s, 1H), 7.12-7.49 (m, 56H), 7.74 (br. d, *J*=8.2 Hz, 2H), 7.79 (br. d, *J*=8.0 Hz, 2H), 8.07 (d, *J*=7.8 Hz, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$), *δ*: -5.7, -4.5, 18.0, 25.7, 58.0, 58.4, 61.8, 66.1, 67.6, 67.7, 68.0, 68.7, 69.8, 70.3, 71.2, 71.6, 71.9, 72.0, 73.0, 73.3, 73.5, 73.6, 74.0, 74.4, 74.5, 74.7, 74.9, 75.0, 75.6, 76.0, 77.2, 78.5, 79.2, 79.8, 80.8, 92.8, 99.7, 100.7, 101.1, 101.4, 126.9, 127.0, 127.1, 127.2, 127.4, 127.5, 127.6, 127.7, 127.73, 127.8, 127.82, 127.86, 127.9, 128.0, 128.1, 128.2, 128.24, 128.3, 128.36, 128.4, 128.5, 128.7, 128.8, 128.84, 128.9, 129.5, 132.2, 132.3, 132.8, 137.5, 137.6, 137.7, 137.8, 137.9, 138.4, 138.43, 138.9, 140.6, 141.1. HRMS: Calcd. for $C_{118}H_{136}N_2O_{28}S_2SiNa$ $[M+Na]^+$ 2143.8388, Found: 2143.8383.

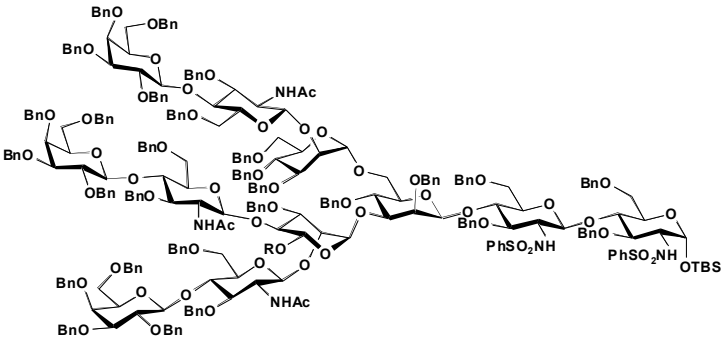
*Undecasaccharide **35**.*



Solutions of lactosamine donor **21** (528 mg, 0.500 mmol) and pentasaccharide triol **6** (100 mg, 0.045 mmol) in dry acetonitrile (3.0 ml and 3.0 ml) were stirred with 3A molecular sieves for 1h at room temperature. The solution of donor **21** was cooled to 10 °C and treated with solid promoter $(BrC_6H_4)_3NSbCl_6$ (255 mg, 0.41 mmol) followed by dropwise addition of the acceptor solution. The reaction mixture was stirred at this temperature for 40 min and another portion of promoter (84 mg, 0.14 mmol) was added. The reaction mixture was allowed to warm to room

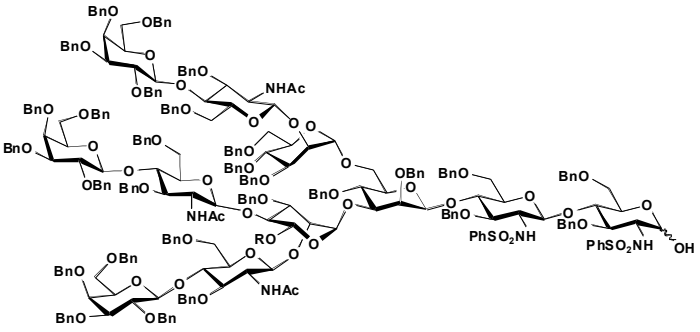
temperature and stirred for 20 h (the reaction was monitored by ESI-MS analysis) in absence of light and then quenched by the addition of triethylamine, filtered through a pad of Celite, and concentrated. ESI-MS of the crude reaction mixture showed presence of the product undecasaccharide and intermediate nonasaccharides, no starting acceptor or monoglycosylated compounds were found. The reaction mixture was subjected to column chromatography on silica gel (eluent: ethyl acetate/hexanes 1/4 to 1/2) and fractions containing glycosylated materials were combined and concentrated. The resulting mixture was further purified by preparative TLC (eluent: ethyl acetate/toluene 1/5) to give the mixture of nonasaccharides (45 mg) and desired undecasaccharide **35** (95 mg, 41%) yield. $[\alpha]_D$=+5.5° (c=1.0, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$, selected signals), δ: 0.13 (s, 3H), 0.17 (s, 3H), 1.01 (s, 9H), 2.73 (br. d, *J*=9.6 Hz, 1H), 2.96 (dd, *J*=5.0, 10.0 Hz, 1H), 3.03 (d, *J*=11.0 Hz, 1H), 4.02 (d, *J*=9.0 Hz, 1H), 5.18 (d, *J*=2.7 Hz, 1H), 5.25 (d, *J*=7.5 Hz, 1H), 5.45 (d, *J*=7.8 Hz, 1H), 6.70-6.78 (m, 2H), 7.79 (d, *J*=8.2 Hz, 1H), 7.85 (d, *J*=8.0 Hz, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$), δ: -5.7, -4.5, 18.0, 25.8, 55.6, 57.9, 58.3, 67.8, 68.0, 68.1, 68.2, 69.9, 71.7, 72.2, 72.4, 72.45, 52.5, 72.6, 72.8, 72.85, 73.0, 73.1, 73.3, 73.34, 73.6, 73.7, 73.9, 74.2, 74.3, 74.4, 74.5, 74.7, 74.9, 75.0, 75.7, 76.0, 76.4, 77.2, 77.8, 78.5, 79.3, 79.8, 79.85, 82.2, 82.4, 92.7, 96.1, 96.8, 97.9, 99.3, 100.7, 101.4, 102.5, 102.7, 102.8, 122.6, 122.9, 123.0, 125.3, 126.6, 126.9-128.6, 128.8, 129.0, 131.6, 131.8, 131.9, 132.3, 133.1, 133.2, 133.4, 137.4, 138.0, 138.1, 138.2, 138.3, 138.4-138.6, 138.7, 138.8, 138.9, 138.95, 139.0, 139.1, 140.7, 141.14, 166.9, 167.4, 167.8, 168.0, 168.2, 168.4. ESI-MS: Calcd. for $C_{311}H_{319}N_5O_{61}S_2SiNa_2$ $[M+2Na]^{2+}$ 2618.6, Found:2618.5

*Tri-N-Acetyl undecasaccharide **36a**.*



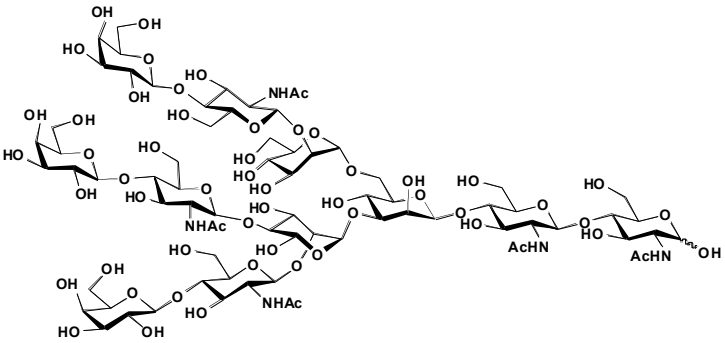
Tri-*N*-Acetyl undecasaccharide **36a** was obtained in 77% yield from **35** following phthalimide deprotection and acetylation as described for nonasaccharide **22a**. $[\alpha]_D$=+10.7° (c=1.3, $CHCl_3$); ^{1}H-NMR (400 MHz, $C_6D_5CD_3$, selected signals), δ: 0.24 (s, 3H), 0.26 (s, 3H), 1.04 (s, 9H), 1.70 (s, 3H), 1.76 (s, 6H), 2.70-2.77 (m, 1H), 2.87-2.96 (m, 1H), 3.15 (q, *J*=8.1 Hz, 1H), 5.39 (br. s, 1H), 5.45 (d, *J*=7.0 Hz, 1H), 5.50 (d, *J*=8.0 Hz, 1H), 5.57 (br. s, 1H), 6.0 (br. d, *J*=8.9 Hz, 1H), 6.76-6.83 (m, 2H), 6.87-6.93 (m, 2H), 7.74 (br. d, *J*=7.7 Hz, 3H), 7.78 (d, *J*=7.8 Hz, 2H), 7.87 (d, *J*=7.6 Hz, 2H); ^{13}C-NMR (100 MHz, $C_6D_5CD_3$), δ: -4.5, -3.7, 19.1, 24.2, 24.3, 26.8, 57.8, 58.9,59.7, 59.8, 67.5, 67.7, 69.1-69.7, 70.4, 70.6, 71.2, 71.4, 72.3, 72.7, 73.1, 73.5, 73.6, 73.8, 73.9-74.7, 75.1-75.5, 75.6-76.3, 76.5, 76.6, 77.5, 77.8, 78.0, 78.1, 78.3, 78.5, 78.8, 78.9, 79.3, 80.8, 81.0, 81.1, 81.2, 81.3, 81.4, 83.7, 83.8, 94.4, 99.2, 100.1, 100.4, 101.6, 102.2, 102.4, 103.8, 104.1, 104.15, 125.2, 125.4-126.4, 127.5-130.4, 137.8, 137.9, 138.4, 139.5, 139.55, 139.6, 139.7 139.8, 139.9, 139.93, 140.0, 140.06, 140.1, 140.3, 140.4, 140.5, 141.08, 141.13, 141.5, 142.2, 142.6, 170.8, 171.3. ESI-MS: Calcd. for $C_{293}H_{319}N_5O_{58}S_2SiNa_2$ $[M+2Na]^{2+}$ 2686.6, Found: 2686.7

*Reducing undecasaccharide **36**.*



Reducing undecasaccharide **36** was obtained from –TBS protected **36a** in 95% yield as described for nonasaccharide **22**. ^{1}H-NMR (400 MHz, $C_6D_5CD_3$, selected signals), δ: 1.73 (s, 6H), 1.77 (s, 3H), 2.84-3.06 (m, 2H), 3.13-3.24 (m, 1H), 5.40 (s, 1H), 5.45-5.53 (m, 2H), 6.77-6.83 (m, 2H), 6.87 (d, *J*=8.1 Hz, 1H), 7.69-7.75 (m, 2H), 7.80 (d, *J*=7.0 Hz, 2H), 7.86-7.93 (m, 2H). ESI-MS: Calcd. for $C_{287}H_{305}N_5O_{58}S_2Na_2$ $[M+2Na]^{2+}$ 2429.5, Found: 2429.6

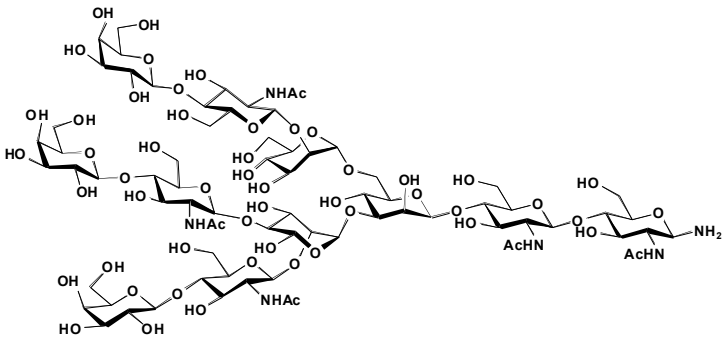
*Deprotected undecasaccharide **37**.*



Liquid NH_3 (15 mL) was condensed at –78° C into a 2-neck flask (25 mL) equipped with stirbar and Dewar-type condenser. Solid sodium (24 mg, 1.5 mmol) was added, and the resulting deep blue solution was stirred for 10 min. A solution of undecasaccharide **36** (23 mg, 0.0048 mol) in

THF (1.0 mL) was added, and the reaction was continued to be stirred for 120 min at –78° C. Upon removing the cold finger, solid NH_4Cl (100 mg) was added and the suspension was vigorously stirred until the blue color disappeared. The reaction vessel was subsequently removed from its cooling bath, warmed to 25° C, and the resulting white solid was dried for 3h. This residue was suspended in sat. aq. $NaHCO_3$ (4.0 ml), the mixture was cooled in an ice bath and treated with Ac_2O (0.2 ml). The reaction mixture was warmed to room temperature and stirred for 2h. The whole solution was then purified with Bio-Rad P-2 gel filtration column to afford the desired deprotected saccharide **37** as a mixture of α/β-anomers (8.9 mg, 94%). ^{1}H-NMR (400 MHz, D_2O), *δ*: 1.93-2.03 (m, 15H), 3.38-4.05 (m, 65H), 4.14 (br. s, 2H), 4.39 (br. d, *J*=7.5 Hz, 3H), 4.45-4.56 (m, 4H), 4.85 (s, 1H), 4.97 and 5.11 (d, *J*=9.4 Hz and s, 1H), 5.04 (s, 1H). ESI-MS: : Calcd. for $C_{76}H_{127}N_5O_{56}N_2Na$ $[M+2Na]^{2+}$ 1025.9, Found: 1026.0. Calcd. for $C_{76}H_{127}N_5O_{56}N_2Na_2[M+Na]^+$ 2028.7, Found: 2028.8

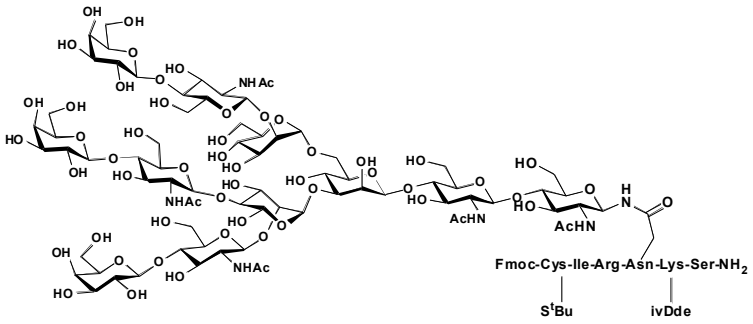
*Glycosylamine **38**.*



Glycosylamine **38** was prepared from reducing saccharide **37** by Kochetkov amination (see **24**) and used in the next step without further purification. ^{1}H-NMR (400 MHz, D_2O), *δ*: 1.93-2.05 (m, 15H), 3.34-4.08 (m, 66H), 4.14 (br. s, 2H), 4.39 (br. d, *J*=7.2 Hz, 3H), 4.45-4.55 (m, 4H), 4.85

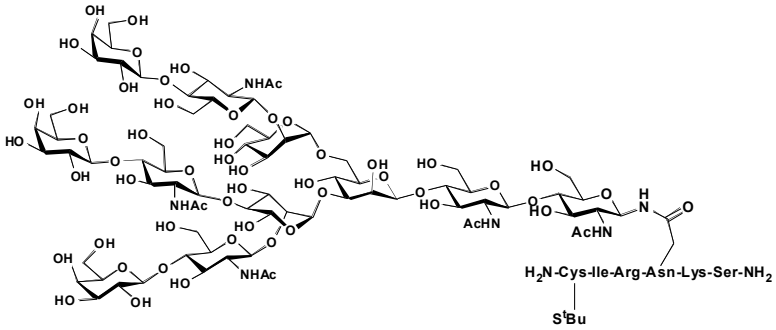
(s, 1H), 5.04 (s, 1H). ESI-MS: Calcd. for $C_{76}H_{128}N_6O_{55}N_2Na$ $[M+2Na]^{2+}$ 1025.4, Found: 1025.5 Calcd. for $C_{76}H_{128}N_6O_{55}N_2Na_2[M+Na]^+$ 2027.7, Found: 2027.8

*Glycopeptide **39a**.*



Glycosylamine **38** was aspartylated with hexapeptide **25** following the protocol described for the preparation of **26a** to provide **39a** in 25% yield (from the reducing saccharide **37**) after purification by HPLC and lyophilization. ESI-MS: Calcd. for $C_{76}H_{129}N_5O_{56}N_2$ $[M+2H]^{2+}$ 1025.9, Found: 1026.0 Calcd. for $C_{76}H_{130}N_5O_{56}N_2$ $[M+3H]^+$ 1075.1, Found: 1075.1

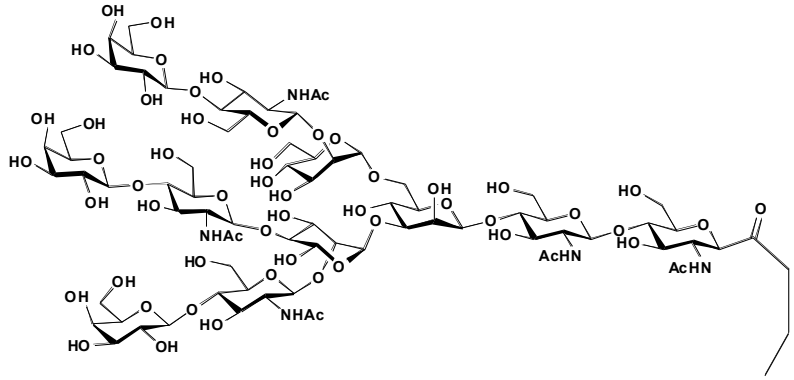
*Deprotected glycopeptide **39**.*



Fmoc and ivDde protections were removed from amino groups in **39a** by treatment with hydrazine/piperidine in DMF (see **26**) to give **24** in 66% yield. ^{1}H-NMR (400 MHz, D_2O, selected signals), δ: 0.76-0.78 (m, 6H), 1.05-1.16 (m, 1H), 1.26 (s, 9H), 1.92-2.03 (m, 15H), 2.68 (dd, *J*=6.3, 16.1 Hz, 1H), 2.80 (dd, *J*=6.4, 16.1 Hz, 1H), 2.93 (t, *J*=7.6 Hz, 2H), 3.06-3.25 (m,

5H), 4.04 (br. s, 1H), 4.12-4.16 (m, 2H), 4.19-4.29 (m, 5H), 4.35 (t, *J*=6.0 Hz, 1H), 4.40 (br. d, *J*=7.2 Hz, 3H), 4.45-4.55 (m, 4H), 4.85 (s, 1H), 4.95 (d, *J*=9.8 Hz, 1H), 5.05 (s, 1H). ESI-MS: Calcd. for $C_{108}H_{189}N_{17}O_{63}S_2$ $[M+2H]^{2+}$ 1398.1, Found: 1398.3

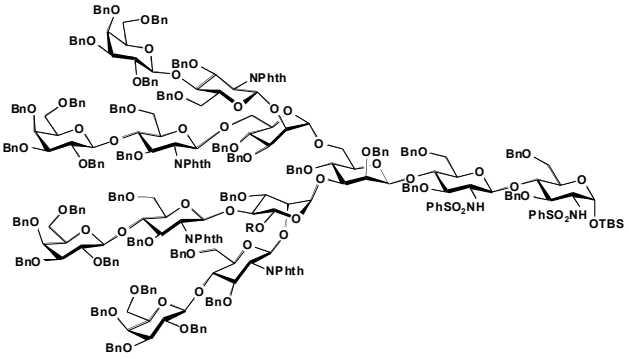
*Glycopeptide **2**.*



H_2N-Gly-Gly-Val-Leu-Val-His-Pro-Gln-Trp-Val-Leu-Thr-Ala-Ala-His-Cys-Ile-Arg-Asn-Lys-Ser-NH_2

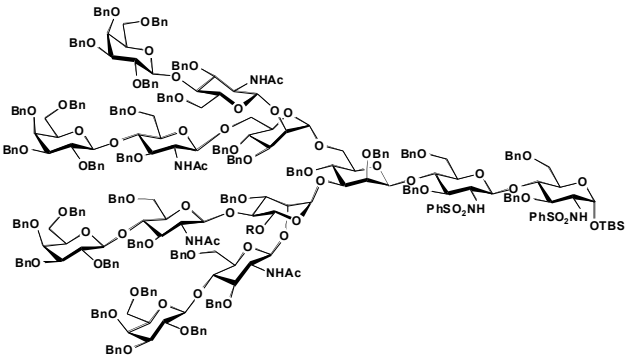
Glycopeptide **2** was produced by ligation of **39** and **27** as described for the preparation of **1**. ^{1}H-NMR (400 MHz, D_2O, selected signals), δ: 1.19 (d, *J*=6.6 Hz, 3H), 1.32 (d, *J*=7.1 Hz, 3H), 1.35 (d, *J*=7.1 Hz, 3H), 2.88 (d, *J*=7.0 Hz, 2H), 4.45 (br. d, *J*=7.4 Hz, 3H), 4.50 (t, *J*=6.5 Hz, 1H), 4.53-4.61 (m, 4H), 5.01 (d, *J*=10.0 Hz, 1H), 5.11 (br. s, 1H), 7.10 (t, *J*=7.4 Hz, 1H), 7.16-7.22 (m, 2H), 7.23 (s, 1H), 7.28 (s, 1H), 7.42 (d, *J*=8.2 Hz, 1H), 7.58 (d, *J*=8.0 Hz, 1H), 8.52 (br. s, 1H), 8.59 (br. s, 1H). ESI-MS: Calcd. for $C_{178}H_{293}N_{38}O_{80}S$ $[M+3H]^{3+}$ 1425.0, Found: 1424.9 Calcd. for $C_{178}H_{294}N_{38}O_{80}S$ $[M+4H]^{4+}$ 1069.0, Found: 1069.1

*Tridecasaccharide **40**.*



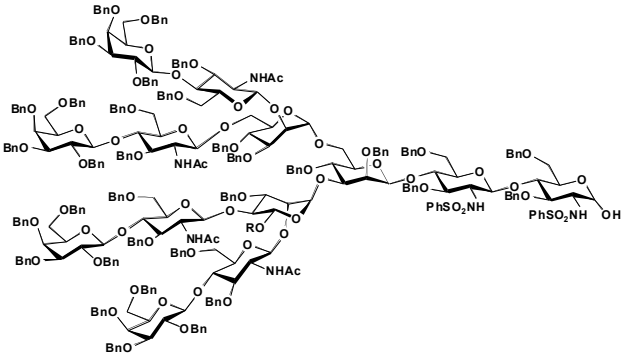
Tetraol acceptor **7** (93 mg, 0.044 mmol) was coupled with lactosamine donor **21** (480 mg, 0.455 mmol) using $(BrC_6H_4)_3NSbCl_6$ (308 mg, 0.5 mmol) as described in the preparation of undecamer **35** to give a mixture of intermediate undecamers and the desired tridecasaccharide **40** (51 mg, 19 %). $[\alpha]_D$=+0.6° (c=1.01, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$, selected signals), δ: 0.02 (s, 3H), 0.06 (s, 3H), 0.90 (s, 9H), 2.49-2.64 (m, 1H), 2.67-2.75 (m, 1H), 2.78-2.87 (m, 1H), 5.01-5.04 (m, 1H), 5.08 (br. s, 1H), 5.35 (br. d, *J*=6.3 Hz, 1H), 6.63-6.71 (m, 2H), 7.68 (d, *J*=7.8 Hz, 2H), 7.77 (d, *J*=7.4 Hz, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$), δ: -5.7, -4.5, 18.0, 25.8, 55.6, 58.0, 67.6, 67.7, 68.1-68.3, 70.1, 72.2, 72.3, 72.7-73.0, 73.2-73.4, 73.7, 73.8, 74.0, 74.2, 74.3-74.5, 74.7, 74.9, 75.0, 75.2, 76.0, 76.2, 77.2, 77.7, 78.0, 78.8, 79.8, 79.7, 80.0, 82.2, 82.3, 82.4, 82.5, 92.7, 99.2, 100.8-101.3, 102.4-103.1, 122.5-123.6, 126.4-129.2, 131.4-131.9, 132.4, 132.9-133.7, 137.6, 137.8-139.2, 140.8, 140.85, 166.0-168.4. ESI-MS: Calcd. for $C_{366}H_{372}N_6O_{72}S_2SiNa_2$ $[M+2Na]^{2+}$ 3070.2, Found: 3070.3. Calcd. for $C_{366}H_{372}N_6O_{72}S_2SiNa_3$ $[M+3Na]^{3+}$ 2054.5, Found: 2054.7

Tri-N-Acetyl tridecasaccharide ***40a***



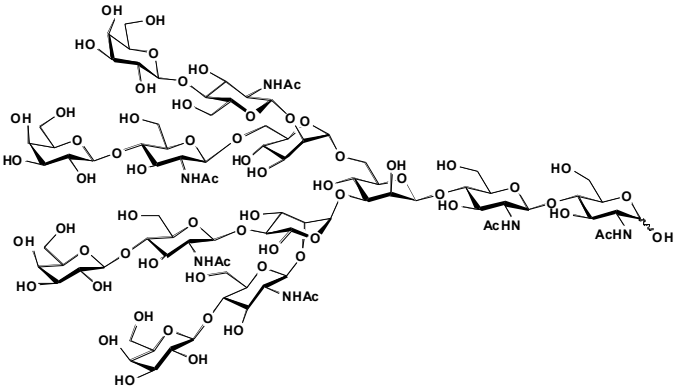
Tri-*N*-Acetyl tridecasaccharide **40a** was obtained in 79% yield from **40** following phthalimide deprotection and acetylation as described for nonasaccharide **22b**. ^{1}H-NMR (400 MHz, $CDCl_3$, selected signals), δ: 0.04 (s, 3H), 0.06 (s, 3H), 0.90 (s, 9H), 1.60 (s, 3H), 1.62 (s, 3H), 1.87 (s, 3H), 1.90 (s, 3H), 2.90-3.00 (m, 2H), 3.10 (d, J=8.8 Hz, 2H), 4.07 (d, J=12.0 Hz, 1H), 5.08 (d, J=1.8 Hz, 1H), 5.12-5.17 (m, 1H), 6.87-6.94 (m, 2H), 7.63 (d, J=7.6 Hz, 2H), 7.73 (d, J=7.8 Hz, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$), δ: -5.6, -4.4, 18.0, 25.8, 26.8, 27.2, 27.3, 67.9-68.3, 72.6, 72.7-73.0, 73.4, 73.5, 73.6, 74.0, 74.5-74.8, 75.0-75.3, 77.7, 77.8, 79.8, 79.9, 82.3, 82.34, 92.8, 100.6-100.8, 101.2-101.6, 102.7-103.0, 126.1, 126.7-129.0, 129.6, 130.5,130.9, 132.3, 132.4, 137.9-139.3, 139.8, 140.5, 141.3, 141.4, 170.0-171.0. ESI-MS: Calcd. for $C_{342}H_{372}N_6O_{68}S_2SiNa_2$ $[M+2Na]^{2+}$ 2894.3, Found: 2894.5. Calcd. for $C_{342}H_{372}N_6O_{68}S_2SiNa_3$ $[M+3Na]^{3+}$ 1937.2, Found: 1937.1

*Tridecasaccharide **41**.*



Reducing tridecasaccharide **41** was obtained from –TBS protected **40a** in 97% yield as described for nonasaccharide **22**. ^{1}H-NMR (400 MHz, $CDCl_3$, selected signals), δ: 1.59 (s, 3H), 1.63 (s, 3H), 1.86 (s, 3H), 1.89 (s, 3H), 2.84-2.91 (m, 1H), 2.91-3.02 (m, 3H), 5.01-5.06 (m, 1H), 5.10-5.18 (m, 1H), 6.88-6.95 (m, 2H), 7.66 (d, J=7.7 Hz, 2H), 7.73 (d, J=7.8 Hz, 2H). ESI-MS: Calcd. for $C_{336}H_{358}N_6O_{68}S_2Na_2$ $[M+2Na]^{2+}$ 2837.2, Found: 2837.4

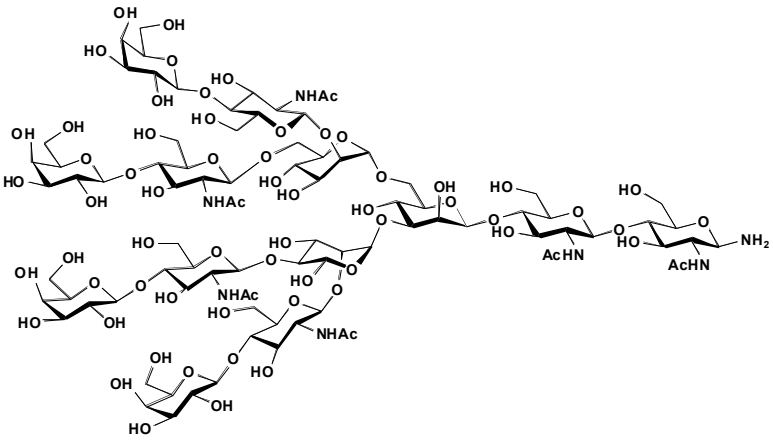
*Deprotected tridecasaccharide **42**.*



Reduction of **41** with sodium in liquid ammonia and acetylation (see **37**) afforded tridecasaccharide **42** in 81% yield. ^{1}H-NMR (400 MHz, D_2O, selected signals), δ: 1.93-2.05 (m, 18H), 3.23 (t, J=9.4 Hz, 1H), 4.02 (br. s, 1H), 4.09-4.16 (s, 3H), 4.36-4.43 (m, 4H), 4.45-4.56

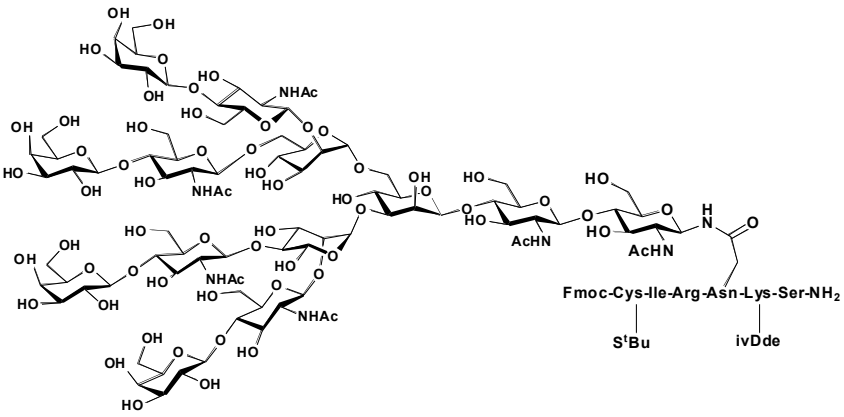
(m, 5H), 4.79 (s, 1H), 5.05 (s, 1H), 5.11 (s, 1H). ESI-MS: Calcd. for $C_{90}H_{150}N_6O_{66}Na_2$ $[M+2Na]^{2+}$ 1208.5, Found: 1208.5

*Glycosylamine **43**.*



Glycosylamine **43** was prepared from reducing saccharide **42** by Kochetkov amination (see **23**) and used in the next step without further purification. ESI-MS: Calcd. for $C_{90}H_{151}N_7O_{65}Na_2$ $[M+2Na]^{2+}$ 1208.0, Found: 1208.1. Calcd. for $C_{90}H_{151}N_7O_{65}Na$ $[M+Na]^+$ 2392.9, Found: 2393.0

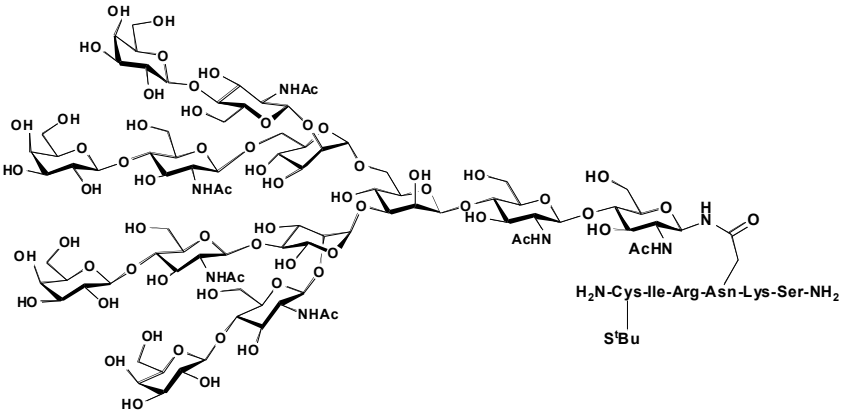
*Glycopeptide **44a**.*



Glycosylamine **43** was aspartylated with hexapeptide **25**, following the protocol described for the preparation of **26a**, to provide **44a**. This compound was used directly in the deprotection (see **44**)

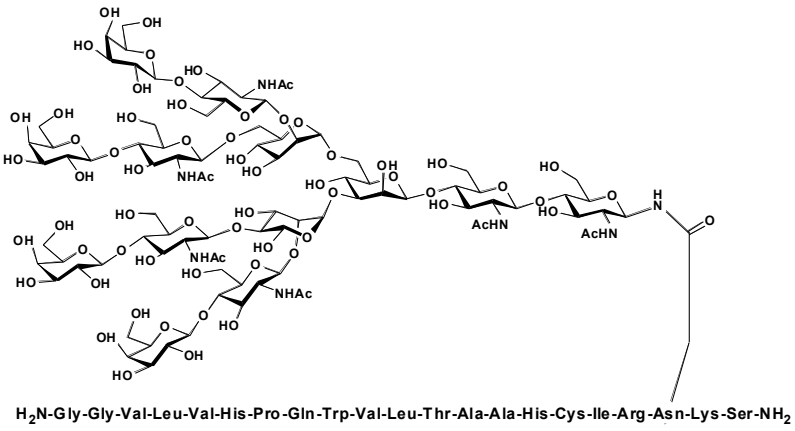
after HPLC purification and partial concentration from DMF (DMF was added to avoid foaming of the solution). ESI-MS: Calcd. for $C_{150}H_{241}N_{18}O_{77}S_2$ $[M+3H]^{3+}$ 1196.8, Found: 1196.8

*Deprotected glycopeptide **44**.*



Fmoc and ivDde protections were removed from amino groups in **44a** by treatment with hydrazine/piperidine in DMF (see **26**) to give **44** in 52% yield for three steps starting with the global deprotection/acetylation product (**42**). ^{1}H-NMR (400 MHz, D_2O, selected signals), δ: 0.78-0.87 (m, 6H), 1.05-1.16 (m, 1H), 1.26 (s, 9H), 1.93-2.03 (m, 18H), 2.91 (t, J=7.2 Hz, 2H), 3.33 (t, J=9.0 Hz, 1H), 4.01 (br. s, 1H), 4.10-4.16 (m, 3H), 4.17-4.29 (m, 5H), 4.34 (t, J=5.8 Hz, 1H), 4.37-4.43 (m, 4H), 4.45-4.55 (m, 5H), 4.64 (t, J=6.5 Hz, 1H), 4.79 (s, 1H), 4.95 (d, J=10.0 Hz, 1H), 5.06 (s, 1H). ESI-MS: Calcd. for $C_{122}H_{212}N_{18}O_{73}S_2$ $[M+2H]^{2+}$ 1580.7, Found: 1580.8 Calcd. for $C_{122}H_{213}N_{18}O_{73}S_2$ $[M+3H]^{3+}$ 1054.1, Found: 1054.2

*Tridecasaccharide – uneicosapeptide glycoconjugate **3**.*



Tridecasaccharide – uneicosapeptide glycoconjugate **3** was produced in 65% yield by ligation of **27** and **44** as described for the preparation of **1**. ^{1}H-NMR (400 MHz, D_2O, selected signals), δ: 1.16-1.22 (m, 2H), 1.28-1.42 (m, 6H), 2.96-3.05 (m, 2H), 4.11 (s, 1H), 4.45-4.52 (m, 4H), 4.53-4.64 (m, 5H), 5.02 (d, J=10.2 Hz, 1H), 5.14 (s, 1H), 7.04-7.12 (m, 1H), 7.15-7.33 (m, 4H), 7.38-7.46 (m, 1H), 7.51-7.60 (m, 1H), 8.47-8.65 (m, 2H). ESI-MS: Calcd. for $C_{192}H_{316}N_{39}O_{90}S$ $[M+3H]^{3+}$ 1546.7, Found: 1546.9. Calcd. for $C_{192}H_{317}N_{39}O_{90}S$ $[M+4H]^{4+}$ 1160.3, Found: 1160.3

Experimental Section: Immunological Studies.

***General procedure for KLH activation and glycopeptide conjugation*.** Lyophilized KLH (Pierce Biotechnology, Inc., Rockford, IL) (15 mg, 0.001875 μmol) was reconstitued in 1.5 mL degassed water and allowed to react 2 hours at room temperature with maleimidobutyric acid (0.515 mg, 2.81 μmol) in degassed sodium acetate buffer pH 7.0. The heterobifunctional linker LC-SMCC (Succinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxy-(6-amidocaproate)) (5.9 mg, 13.1 μmol) was solubilized in 200 μL DMSO and added to the reaction mixture. After 2 hours at room temperature, the activated protein was purified by size exclusion chromatography (10DG column, Biorad Laboratories, Hercules, CA) in degassed PBS containing 5 mM EDTA,

pH 6.4, and concentrated using Microcon devices of 50 kDa molecular weight cut-off (Millipore Corporation, Bedford, MA).

The 11+21 glycopeptide (1.05 mg, 0.246 μmol) was solublized in degassed water, and added to the activated KLH (1.4 mg, 0.000175 μmol) stored in PBS EDTA 5 mM. Sodium carbonate (200 mM) was added to adjust the pH to 6.8. The reaction was allowed to proceed over 18 hours at room temperature. Purification and concentration of the conjugate was performed as described above for the activated KLH.

***Immunization and hybridoma generation*.** A group of 5 BALB/c mice were immunized subcutaneously every two weeks for four weeks with the 11+21 glycopeptide conjugated to KLH. The animal which serum had the highest titer in antibodies recognizing the 11+21 glycopeptide was selected and subjected to a boost immunization. After two weeks, the animal was sacrificed and hybridomas were prepared according to standard protocols.

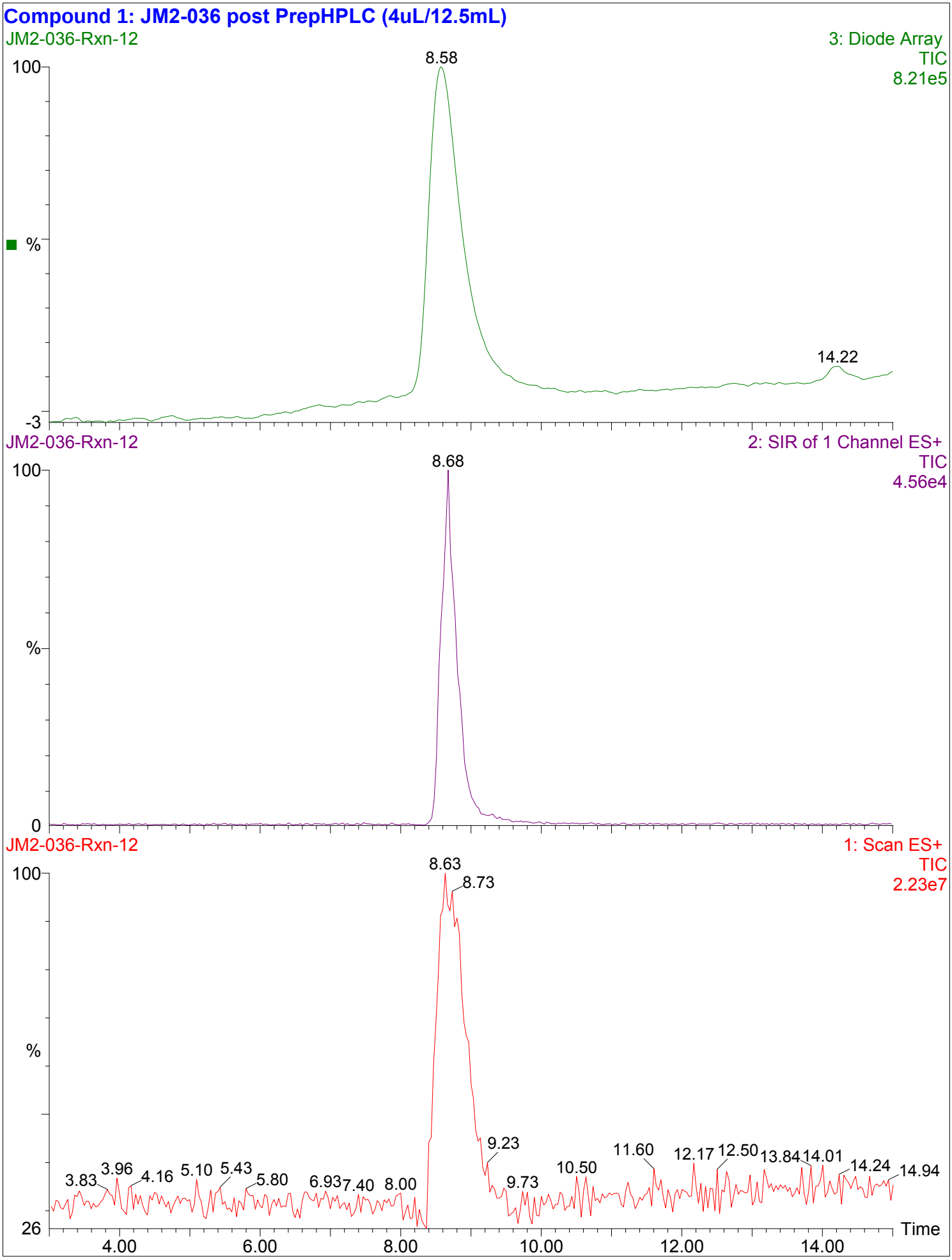
***Enzyme-linked immunosorbent assays*.** For screening of the hybridomas, a standard ELISA was conducted as follows. The 96-well plates (Nunc, Naperville, IL) were coated with 50 ng of 11+21 glycopeptide per well in PBS overnight at 4°C. The wells were washed 3 times with PBS/Tw (PBS containing 0.01% Tween 20) and once with PBS. The wells were then blocked with 300 µL 2% BSA in PBS for one hour at 37°C, and 50 µL hybridoma supernatant (1:50 dilution in 2% BSA PBS) was added to the wells. After one hour incubation at room temperature, the plates were again washed 3 times with PBS/Tw and once with PBS before adding 50 µL of a 1:1000 dilution of goat-antimouse immunoglobulin IgG and IgM antibody conjugated to alkaline phosphatase (Jackson ImmunoResearch,West Grove, PA). After washing, 50 µL of Turbo TMB (Pierce, Rockford, IL) was added per well, and the plates were read at 450

nm in an ELISA plate reader after stopping the reaction by adding 50 μL 1N H_2SO_4. A negative control consisted of wells treated according to the same protocol, but incubated with an unrelated hybridoma supernatant. Selected positive hybridoma supernatants were tested for recognition of the 9+21 glycopeptide using the same protocol.

For characterizing the reactivity of selected hybridoma supernatants towards tumor PSA, a sandwich ELISA was conducted as follows. Desialylation of LnCap PSA from cell culture supernatant was performed using sialidase A according to the suggested protocol from the manufacturer (Prozyme, San Leandro, CA). 5 ng LnCap PSA, pretreated or not with sialidase was added to 96-well plates precoated with rabbit anti-PSA (Alpco Diagnostics, Salem, NH). After one hour incubation at room temperature, the plates were washed 3 times with PBS/Tw and once with PBS before adding 50 μL undiluted hybridoma supernatant to the wells. The protocol was then identical to the standard ELISA described above. A negative control consisted of wells treated according to the same protocol, but incubated with an unrelated hybridoma supernatant. Positive control wells were incubated with H117 anti-PSA antibody.

References:

1. Leinonen, J., Wu, P., and Stenman, U.H. (2002). Epitope mapping of antibodies against prostate-specific antigen with use of peptide libraries. Clin Chem 48, 2208-2216.


Compound 1: JM2-036 post PrepHPLC (4uL/12.5mL)
JM2-036-Rxn-12
3: Diode Array
TIC
8.21e5
100
%
-3
8.58
14.22
JM2-036-Rxn-12
2: SIR of 1 Channel ES+
TIC
4.56e4
100
%
0
8.68
JM2-036-Rxn-12
1: Scan ES+
TIC
2.23e7
100
%
26
8.63
8.73
9.23
9.73
10.50
11.60
12.17
12.50
13.84
14.01
14.24
14.94
3.83
3.96
4.16
5.10
5.43
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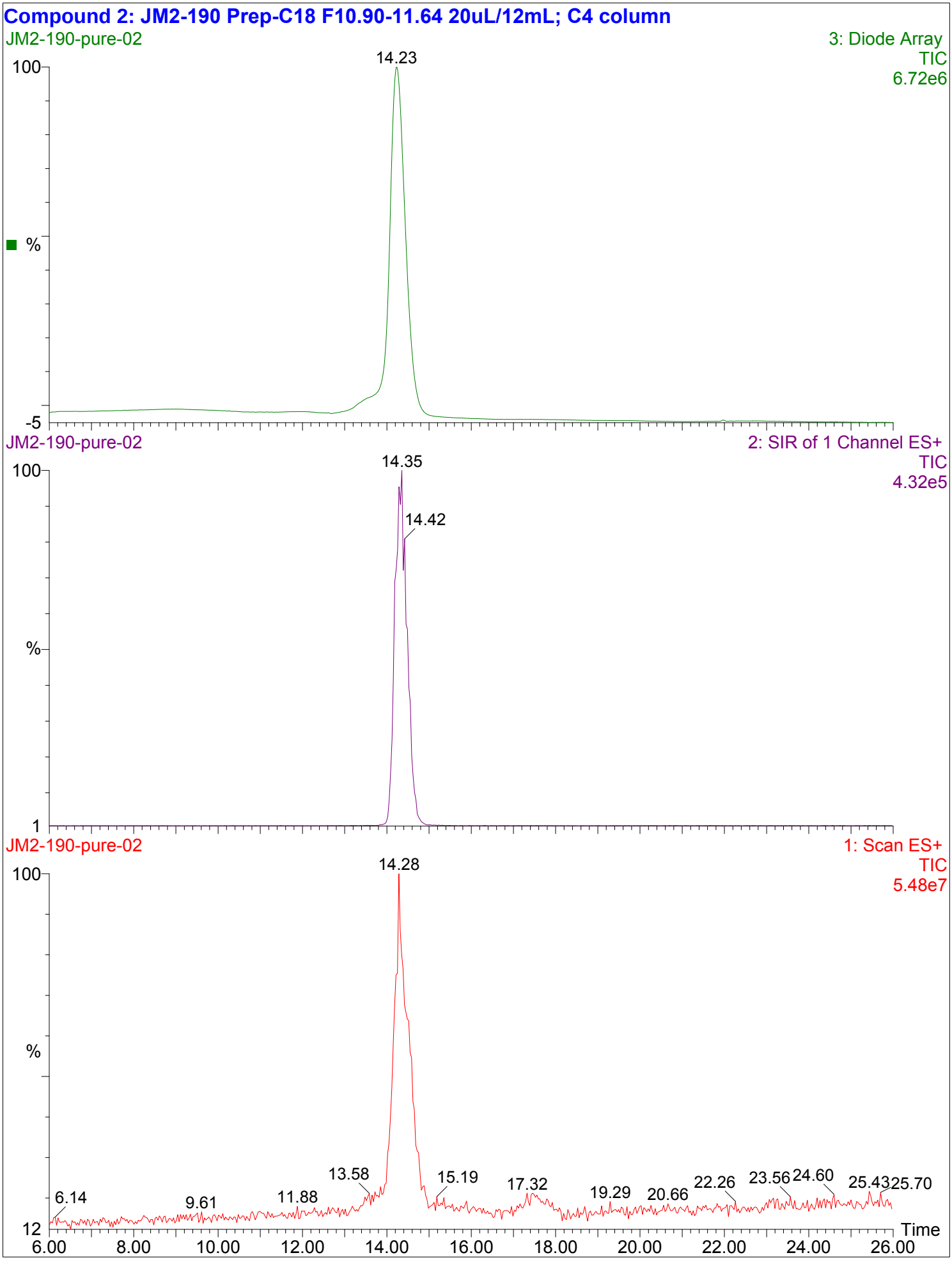
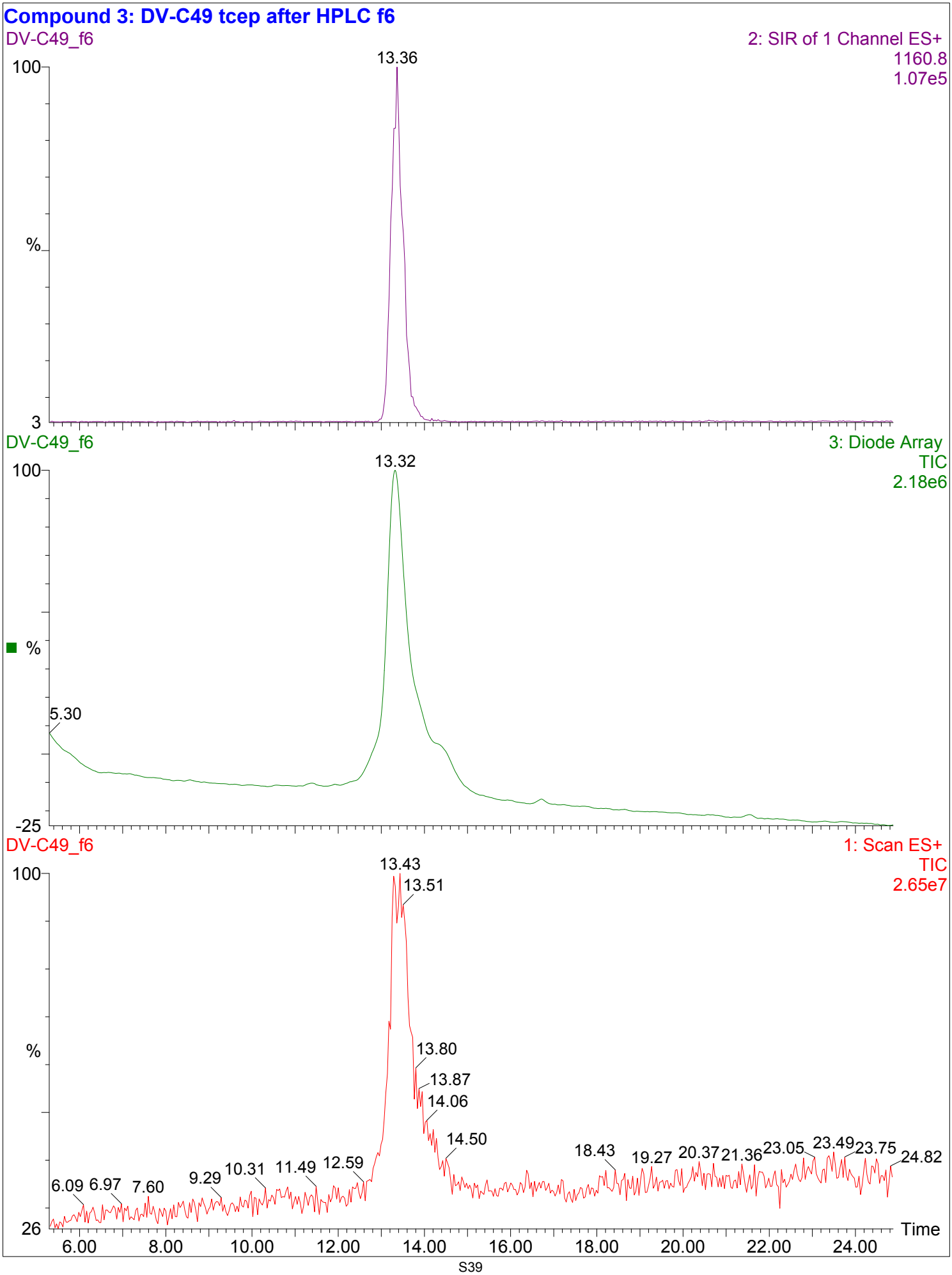
4.00
6.00
8.00
10.00
12.00
14.00
Time


Compound 2: JM2-190 Prep-C18 F10.90-11.64 20uL/12mL; C4 column
JM2-190-pure-02
3: Diode Array
TIC
6.72e6
14.23
100
%
-5
JM2-190-pure-02
2: SIR of 1 Channel ES+
TIC
4.32e5
14.35
14.42
100
%
1
JM2-190-pure-02
1: Scan ES+
TIC
5.48e7
14.28
100
%
12
6.14
9.61
11.88
13.58
15.19
17.32
19.29
20.66
22.26
23.56
24.60
25.43
25.70
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
Time


Compound 3: DV-C49 tcep after HPLC f6
DV-C49_f6
2: SIR of 1 Channel ES+
1160.8
1.07e5
100
%
3
13.36
DV-C49_f6
3: Diode Array
TIC
2.18e6
100
%
-25
13.32
5.30
DV-C49_f6
1: Scan ES+
TIC
2.65e7
100
%
26
13.43
13.51
13.80
13.87
14.06
14.50
6.09
6.97
7.60
9.29
10.31
11.49
12.59
18.43
19.27
20.37
21.36
23.05
23.49
23.75
24.82
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
Time