

Supporting Information

Molecular-Scale Features that Govern the Effects of O-Glycosylation on a Carbohydrate-Binding Module

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I. MATERIALS AND METHODS

All commercial reagents and solvents were used as received. Unless otherwise noted, all reactions and purifications were performed under air atmosphere at room temperature. All LC-MS analyses were performed using a Waters Acquity™ Ultra Performance LC system equipped with Acquity UPLC® BEH 300 C4, 1.7μm, 2.1 x 100 mm column at flow rates of 0.3 and 0.5 mL/min. The mobile phase for LC-MS analysis was a mixture of H₂O (0.1% formic acid, v/v) and acetonitrile (0.1% formic acid, v/v). All preparative separations were performed using a LabAlliance HPLC solvent delivery system equipped with a Rainin UV-1 detector and a Varian Microsorb 100-5, C18 250x21.4 mm column at a flow rate of 16.0 mL/min. The mobile phase for HPLC purification was a mixture of H₂O (0.05% TFA, v/v) and acetonitrile (0.04% TFA, v/v). A Waters SYNAPT G2-S system was used mass spectrometric analysis. All circular dichroism (CD) spectra were obtained using an Applied Photophysics Chirascan™-plus CD spectrometer.

II. SYNTHESIS OF GLYCOAMINO ACIDS

The glycoamino acid building blocks Fmoc-Ser(Ac₄Manα1)-OH (**35**), Fmoc-Ser(Ac₄Manα1-2Ac₃Manα1)-OH (**36**), Fmoc-Thr(Ac₄Manα1)-OH (**37**), and Fmoc-Thr(Ac₄Manα1-2Ac₃Manα1)-OH (**53**) were prepared according to the previously reported method¹. Glycoamino acid building block **44** was purchased from AnaSpec. All the other building blocks, **38-43**, **45-52**, and **54** are prepared as described below. The spectroscopic characterizations (¹H NMR, ¹³C NMR, IR, and high-resolution MS) of all new compounds are reported.

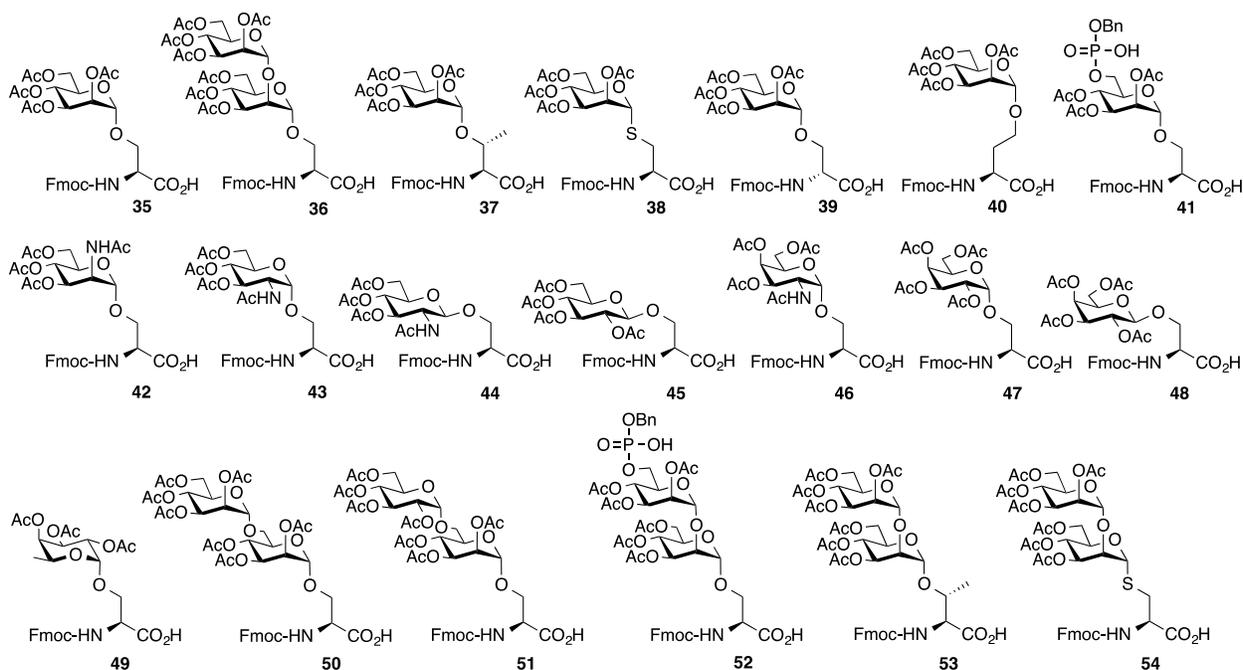
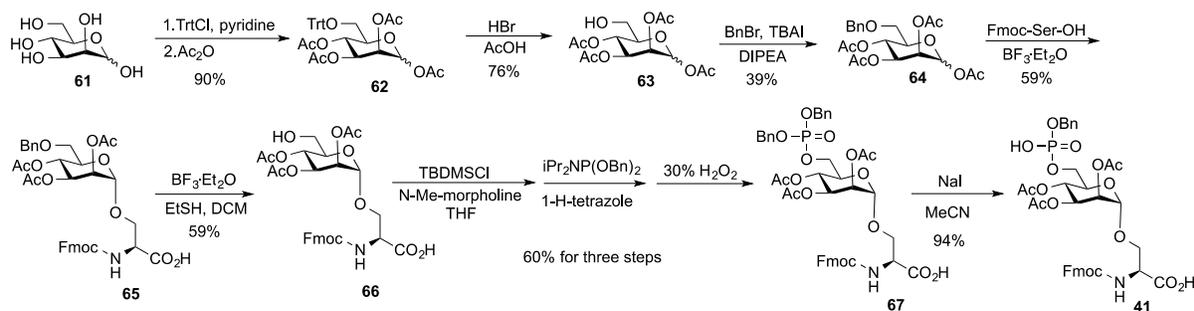


Figure S1. O-linked glycoamino acid building blocks used for the synthesis of CBM glyco-variants.

concentrated under reduced pressure. The residue was purified by flash chromatography on a silica gel column (Hex/EtOAc = 2:1→1:1) to give **60** (474 mg, 26%) as a white foam.

60 (470 mg, 0.65 mmol) was dissolved in a solution of TFA/H₂O (95:1, 8.0 ml) and stirred at room temperature for 2.5 h. The solvent was removed under reduced pressure by co-evaporation with toluene and the remaining residue was purified by flash chromatography on a silica gel column (Hex/EtOAc/AcOH = 1:1:0→1:1:0.2) to give **40** (400 mg, 92%) as a white foam. ¹H NMR (400 MHz, CDCl₃) δ 7.82 – 7.75 (m, 2H), 7.62 (d, *J* = 7.5 Hz, 2H), 7.46 – 7.37 (m, 2H), 7.33 (td, *J* = 7.4, 1.3 Hz, 2H), 5.81 (d, *J* = 6.6 Hz, 1H), 5.38 – 5.24 (m, 2H), 5.21 (s, 1H), 4.81 (s, 1H), 4.50 (d, *J* = 5.7 Hz, 1H), 4.43 (d, *J* = 7.1 Hz, 2H), 4.36 – 4.27 (m, 1H), 4.23 (t, *J* = 6.7 Hz, 1H), 4.12 (d, *J* = 12.5 Hz, 1H), 4.01 (d, *J* = 8.9 Hz, 1H), 3.90 (s, 1H), 3.53 (dd, *J* = 10.7, 5.3 Hz, 1H), 2.35 – 2.20 (m, 2H), 2.15 (s, 3H), 2.11 (s, 3H), 2.07 (d, *J* = 1.6 Hz, 3H), 2.01 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 171.37, 170.80, 170.37, 169.80, 155.82, 143.68, 141.31, 127.77, 127.08, 125.08, 125.03, 120.02, 120.00, 97.55, 69.63, 69.47, 68.92, 66.98, 65.65, 63.84, 62.51, 51.35, 47.18, 31.33, 20.87, 20.79, 20.75, 20.71. IR (NaCl, film): 3341, 3066, 2952, 1748, 1522, 1451, 1371, 1228, 1138, 1117, 1049, 980 cm⁻¹. HRMS (ESI) Calcd. for C₃₃H₃₇NNaO₁₄ [M + Na]⁺ requires 694.2107, Found: 694.2103.



Synthesis of glycoamino acid 41. To a stirred solution of D-(+)-mannose **61** (5 g, 27.8 mmol) in pyridine (25 ml) trityl chloride (TrtCl) (8.5 g, 30.55 mmol) was added and the resulting mixture was stirred for 1.5 h at 40 °C. After cooling to 0 °C, Ac₂O (15 ml) was added to the mixture and the resulting solution was stirred overnight at room temperature. The reaction mixture was poured into ice water and extracted with DCM. The organic phase was dried over anhydrous Na₂SO₄, filtered and the filtrate was concentrated under reduced pressure. The resulting oil was purified by flash chromatography on a silica gel column (Hex/EtOAc = 5:1) to give **62** (14.71 g, 90%) as a white foam. Product matched the previously known spectra of **62**³.

To a stirred solution of **62** (3 g, 5.08 mmol) in AcOH (10 ml) was added 33% HBr in AcOH (1.0 ml). The resulting mixture was stirred for 1 min. The Ph₃CBr formed was immediately removed by suction filtration. The filtrate was diluted with cold water and extracted with DCM. The organic layer was dried over Na₂SO₄, filtered and the filtrate was concentrated under reduced pressure. The resulting oil was purified by flash chromatography on a silica gel column (Hex/EtOAc = 2:1→1:1) to give **63** (1.34 g, 76%) as a white foam. Product matched the

previously known spectra of **63**³.

To a flask with **63** (23 g, 66 mmol) and tetra-*n*-butylammonium iodide (TBAI) (7.3 g, 19.8 mmol) was added DIPEA (45 ml, 264 mmol) and BnBr (31.6 ml, 264 mmol). The resulting mixture was stirred at 90 °C for 4 h. The reaction was diluted with DCM and washed with water. The organic phase was dried over Na₂SO₄, filtered and the filtrate was concentrated under reduced pressure. The resulting oil was purified by flash chromatography on a silica gel column (Hex/EtOAc =4:1→2:1) to give **64** (11.16 g, 39%) as an oil ($\alpha/\beta=2:1$). ¹H-NMR (400 MHz, CDCl₃) δ 7.27-7.36 (m, 7.5H, H-Ph), 6.10 (d, $J = 2.0$ Hz, 1H, H-1 α), 5.85 (d, $J = 1.2$ Hz, 0.5H, H-1 β), 5.47 (dd, $J = 3.2$ Hz, 1.2 Hz, 0.5H, H-2 β), 5.31-5.41 (m, 2.5H, H-3 α , H-4 α , H-4 β), 5.25 (dd, $J = 3.2$ Hz, 2.0 Hz, 1H, H-2 α), 5.11 (dd, $J = 10.0$ Hz, 3.2 Hz, 0.5H, H-3 β), 4.46-4.59 (m, 3H, CH₂-Bn), 3.98-4.02 (m, 1H, H-5 α), 3.73-3.77 (m, 0.5H, H-5 β), 3.57-3.60 (m, 3H, H-6 α , H-6 β), 2.21 (s, 1.5H, CH₃-Ac β), 2.162 (s, 3H, CH₃-Ac α), 2.155 (s, 3H, CH₃-Ac α), 2.09 (s, 1.5H, CH₃-Ac β), 2.002 (s, 3H, CH₃-Ac α), 1.997 (s, 1.5H, CH₃-Ac β), 1.92 (s, 3H, CH₃-Ac α), 1.90 (s, 1.5H, CH₃-Ac β). ¹³C-NMR (100 MHz, CDCl₃) δ 170.3, 170.1, 169.9, 169.8, 169.62, 169.58, 168.4, 168.2, 137.7, 137.6, 128.6, 128.4, 128.0, 127.9, 127.8, 127.7, 90.7, 90.5, 77.2, 74.5, 73.67, 73.61, 72.0, 70.9, 68.9, 68.8, 68.6, 68.4, 68.3, 66.4, 66.3, 20.9, 20.82, 20.79, 20.74, 20.68, 20.57. IR (NaCl, film): 3064, 3031, 2937, 2870, 1761, 1454, 1432, 1370, 1237, 1149, 1055, 972, 738, 701 cm⁻¹. HRMS (ESI) Calcd. for C₂₁H₂₆NaO₁₀ [M + Na]⁺ requires 461.1419, Found: 461.1417.

To the solution of **64** (400 mg, 0.91 mmol) and Fmoc-Ser-OH (448 mg, 1.37 mmol) in MeCN (22 mL), BF₃·OEt₂ (0.42 ml, 2.74 mmol) was added¹. The resulting mixture was stirred at room temperature for 24 h under argon. The solvent was removed under reduced pressure and the residue was diluted with EtOAc and washed with water. The organic layer was dried over Na₂SO₄, filtered and the filtrate was concentrated under reduced pressure. The resulting oil was purified by flash chromatography on a silica gel column (Hex/EtOAc/AcOH =2:1:0.3) to give **65** (379 mg, 59%) as a white foam. ¹H-NMR (400 MHz, CDCl₃) δ 7.74 (d, $J = 7.6$ Hz, 2H, H-Fmoc), 7.56-7.58 (m, 2H, H-Fmoc), 7.24-7.38 (m, 9H, H-Fmoc, H-Ph), 6.54 (d, $J = 8.8$ Hz, 1H, H-NH), 5.33-5.43 (m, 3H, H-2, H-3, H-4), 4.87 (s, 1H, H-1), 4.68 (brs, 1H, H- α), 4.49 (dd, $J = 43.2$ Hz, 12.0 Hz, 2H, CH₂-Bn), 4.03-4.44 (m, 6H, H-5, CH₂- β , CH₂-Fmoc, CH-Fmoc), 3.51 (s, 2H, H-6), 2.14 (s, 3H, CH₃-Ac), 2.00 (s, 3H, CH₃-Ac), 1.84 (s, 3H, CH₃-Ac). ¹³C-NMR (100 MHz, CDCl₃) δ 170.7, 170.3, 169.8, 156.1, 143.8, 141.27, 141.24, 137.5, 128.8, 128.3, 127.92, 127.86, 127.7, 127.1, 125.3, 119.9, 98.3, 73.5, 70.1, 69.5, 68.4, 67.3, 66.7, 47.1, 43.9, 20.9, 20.8, 20.7. IR (NaCl, film): 3339, 3065, 1754, 1707, 1370, 1224, 1050, 740, 700 cm⁻¹. HRMS (ESI) Calcd. for C₃₇H₃₉NNaO₁₃ [M + Na]⁺ requires 728.2314, Found: 728.2304.

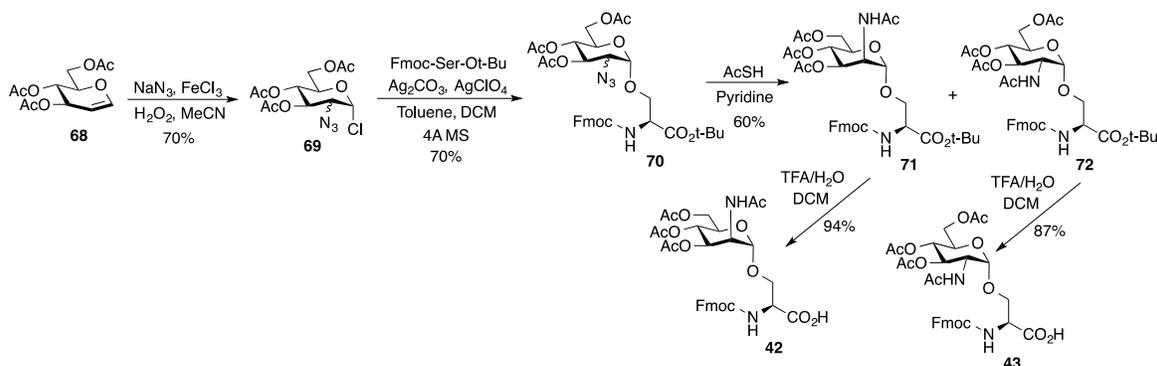
The mixture of **65** (300 mg, 0.42 mmol), EtSH (2.1 ml) and BF₃·OEt₂ (523 μ l, 3.40 mmol) in DCM (4.2 ml) was stirred for 6 h at room temperature under argon. The reaction was quenched with water and extracted with EtOAc. The organic phase was washed with brine, dried (Na₂SO₄), filtered and the filtrate was concentrated under reduced pressure. The resulting oil was purified

by flash chromatography on a silica gel column (Hex/EtOAc/AcOH =3:2:0.5→1:1:0.2) to give **66** (152 mg, 59%) as an oil. ¹H-NMR (400 MHz, CD₃OD) δ 7.82 (d, *J* = 7.6 Hz, 2H, H-Fmoc), 7.72-7.74 (m, 2H, H-Fmoc), 7.39-7.43 (m, 2H, H-Fmoc), 7.32-7.36 (m, 2H, H-Fmoc), 5.23-5.36 (m, 3H, H-2, H-3, H-4), 4.91 (s, 1H, H-1), 3.89-4.48 (m, 7H, H-5, H-α, CH₂-β, CH₂-Fmoc, CH-Fmoc), 3.66 (dd, *J* = 12.4 Hz, 2.4 Hz, 1H, H-6), 3.56 (dd, *J* = 12.4 Hz, 5.2 Hz, 1H, H-6), 2.12 (s, 3H, CH₃-Ac), 1.97 (s, 3H, CH₃-Ac), 1.95 (s, 3H, CH₃-Ac). ¹³C-NMR (400 MHz, CD₃OD) δ 170.22, 170.17, 170.1, 157.0, 144.1, 143.9, 141.18, 141.12, 127.34, 127.32, 126.82, 126.79, 125.1, 124.9, 119.47, 119.45, 98.1, 71.0, 69.6, 69.5, 68.5, 66.8, 66.2, 60.5, 19.21, 19.19. IR (NaCl, film): 1755, 1706, 1370, 1227, 1084, 1047, 760, 740 cm⁻¹. HRMS (ESI) Calcd. for C₃₀H₃₃NNaO₁₃ [M + Na]⁺ requires 638.1845, Found: 638.1844.

To a solution of **66** (150 mg, 0.24 mmol) in THF (1.5 ml) were added N-methyl-morpholine (27 μl, 0.24 mmol, dissolved in 0.4 ml THF) and tert-Butyldimethylchlorosilane (TBDMSCl) (36 mg, 0.24 mmol, dissolved in 0.5 ml THF)⁴. After stirring for 30 minutes, 1H-tetrazole (2.5 ml, 1.13 mmol, 0.45M in CH₃CN) and dibenzyl N,N-diisopropylphosphoramidite [iPr₂NP(OBn)₂] (166 μl, 0.5 mmol) were added. The reaction mixture was stirred for 3 h at room temperature, cooled to 0 °C, and then 30% H₂O₂ (aq., 64 μl, 0.64 mmol) was added. The resulting mixture was slowly warmed to room temperature over 30 minutes, saturated Na₂SO₃ (1.5 ml) was then added. After stirring vigorously for 30 minutes, the mixture was diluted with saturated Na₂SO₃, extracted with EtOAc. The organic layer was dried over Na₂SO₄, filtered and the filtrate was concentrated under reduced pressure. The resulting oil was purified by flash chromatography on a silica gel column (Hex/EtOAc/AcOH =2:1:0.3→3:2:0.5) to give **67** (127 mg, 60%) as a white foam. ¹H-NMR (400 MHz, CD₃OD) δ 7.81 (d, *J* = 7.2 Hz, 2H, H-Fmoc), 7.69 (d, *J* = 7.6 Hz, 2H, H-Fmoc), 7.30-7.42 (m, 14H, H-Fmoc, H-Ph), 5.33-5.38 (m, 2H, H-3, H-4), 5.26 (d, *J* = 2.0 Hz, 1H, H-2), 5.04-5.12 (m, 4H, CH₂-Bn), 4.90 (s, 1H, H-1), 4.40-4.48 (m, 2H, H-α, CH-Fmoc), 4.23-4.31 (m, 2H, CH₂-Fmoc), 4.05-4.17 (m, 4H, H-5, H-6, CH₂-β), 3.90 (dd, *J* = 10.4 Hz, 6.0 Hz, 1H, H-6), 2.01 (s, 3H, CH₃-Ac), 1.96 (s, 3H, CH₃-Ac), 1.95 (s, 3H, CH₃-Ac). ¹³C-NMR (400 MHz, CD₃OD) δ 170.04, 170.00, 169.91, 144.0, 143.8, 141.2, 135.8, 128.32, 128.30, 128.28, 127.81, 127.75, 127.4, 126.83, 126.80, 125.0, 119.5, 98.3, 69.55, 69.52, 69.49, 69.45, 69.4, 69.2, 68.4, 66.8, 65.6, 65.3, 54.7, 19.21, 19.16, 19.15. ³¹P-NMR (400 MHz, CD₃OD) δ -1.77. IR (NaCl, film): 3065, 3035, 2360, 2343, 1756, 1718, 1521, 1371, 1246, 1220, 1011, 882, 740, 698 cm⁻¹. HRMS (ESI) Calcd. for C₄₄H₄₆NNaO₁₆P [M + Na]⁺ requires 898.2447, Found: 898.2438.

67 (120 mg, 0.14 mmol) was dissolved in CH₃CN (1.5 ml). To this solution was added NaI (42 mg, 0.28 mmol)⁵. The reaction was stirred at 45 °C for 12 h under argon. The reaction mixture was concentrated and dissolved in small amount EtOAc. Hexanes was added until white solid formed. The suspension was centrifuged and the resulting solid was dissolved in H₂O/CH₃CN=1:1. The resulting solution was frozen and lyophilized to give **41** (103 mg, 94%) as a white solid. ¹H-NMR (400 MHz, CD₃OD) δ 7.80 (d, *J* = 7.6 Hz, 2H, H-Fmoc), 7.69-7.72 (m, 2H, H-Fmoc), 7.22-7.41 (m, 9H, H-Fmoc, H-Ph), 5.26-5.36 (m, 3H, H-2, H-3, H-4), 4.91-4.95

(m, 2H, CH₂-Bn), 4.85 (d, *J* = 1.6 Hz, 1H, H-1), 4.41-4.46 (m, 1H, CH-Fmoc), 4.22-4.29 (m, 3H, H- α , CH₂-Fmoc), 3.83-4.13 (m, 5H, H-5, H-6, CH₂- β), 2.09 (s, 3H, CH₃-Ac), 1.95 (s, 3H, CH₃-Ac), 1.90 (s, 3H, CH₃-Ac). ¹³C-NMR (400 MHz, CD₃OD) δ 170.3, 170.2, 170.1, 156.9, 144.1, 143.9, 141.17, 141.13, 138.5, 138.4, 128.2, 127.9, 127.3, 127.1, 126.9, 126.8, 125.1, 124.9, 119.5, 98.0, 69.8, 69.7, 69.5, 68.7, 66.79, 66.73, 66.1, 63.77, 63.72, 56.1, 19.30, 19.27, 19.24. ³¹P-NMR (400 MHz, CD₃OD) δ 0.31. IR (NaCl, film): 3405, 3065, 2952, 1753, 1613, 1524, 1452, 1416, 1371, 1228, 1138, 1048, 868, 761, 740, 699 cm⁻¹. HRMS (ESI) Calcd. for C₃₇H₄₀NNaO₁₆P [M + Na]⁺ requires 808.1977, Found: 808.1980.



Synthesis of glycoamino acid 42 and 43. To a solution of Tri-O-acetyl-D-glucal **68** (2.3 g, 8.43 mmol) in MeCN (38 mL) at -30 °C was added FeCl₃·6H₂O (2.50 g, 9.27 mmol), NaN₃ (602.5 mg, 9.27 mmol) and H₂O₂ (30%, aq., 1.26 ml, 12.65 mmol) and the reaction was stirred at -30 °C for 6 h⁶. The mixture was diluted with Et₂O and washed with H₂O, NaHCO₃ (sat.), and brine. The organic layer was dried with Na₂SO₄ and concentrated under reduced pressure to give **69** (2.06 g, 70%) as a viscous oil. The product was used directly without further purification.

To a solution of Fmoc-Ser-OH (1.0 g, 3.05 mmol) in EtOAc (15.3 ml) was slowly added a solution of tert-Butyl 2,2,2-trichloroacetimidate (TBTA) (918 mg, 5.13 mmol) in cyclohexane (6.1 ml) over the course of 15 minutes with stirring at room temperature⁷. The mixture was allowed to stir at room temperature for 5 hours, then quenched with NaHCO₃ (sat., aq.) and extracted with EtOAc. The organic layers were combined, washed with H₂O, then brine, dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography on a silica gel column (Hex/EtOAc = 10:1→2:1) to yield Fmoc-Ser-Ot-Bu (643.6 mg, 55%) as a white solid.

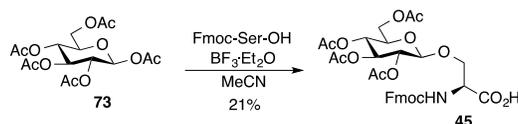
A solution of Fmoc-Ser-Ot-Bu (2.03 g, 5.31 mmol), Ag₂CO₃ (2.44 g, 8.85 mmol), 4A MS (3.5 g) in toluene (12 ml) and DCM (18 ml) was stirred at 0 °C for 30 min⁶. Then, a solution of AgClO₄ (306.8 mg, 1.48 mmol) in toluene (6 mL) was added dropwise at 0 °C. Subsequently, a solution of **69** (2.06 g, 5.9 mmol) in a mixture of DCM (12 ml) and toluene (12 mL) was very slowly added dropwise at 0 °C. The mixture was stirred, in the dark, at room temperature for 24 h. The reaction was diluted with EtOAc, filtered through Celite and washed with H₂O then NaHCO₃ (sat.). The organic layer was dried with Na₂SO₄ and concentrated under reduced pressure. The

residue was purified by flash chromatography on a silica gel column (PE/EtOAc = 5:1→2:1) to give **70** (2.86 g, 70%) as a white foam.

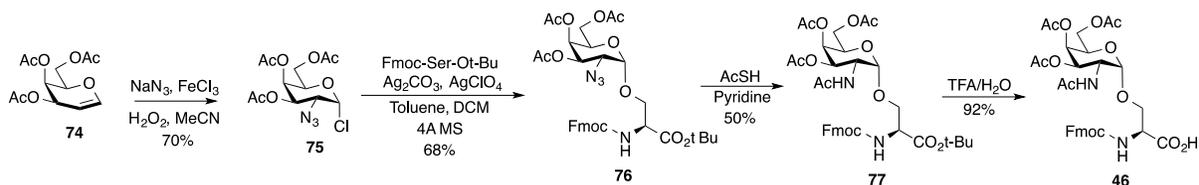
70 (2.6 g, 3.73 mmol) was dissolved in a solution of pyridine (4 ml) and AcSH (8 ml) and stirred at room temperature for 40 hours⁸. The mixture was diluted with EtOAc and washed with HCl (1M, aq.), and NaHCO₃ (sat., aq.). The organic layers were dried with Na₂SO₄ and concentrated under reduced pressure. The residue was purified by flash chromatography on a silica gel column (DCM/EtOAc = 3:2→1:1→0:1) to give **71** (780 mg, 29%) and **72** (830 mg, 31%) as white foams.

71 (780 mg, 1.09 mmol) was dissolved in a solution of TFA (4 ml), H₂O (0.2 mL) and DCM (4 ml) and stirred at room temperature for 3 h. The solvent was removed under reduced pressure by co-evaporation with toluene and the remaining residue was purified by flash chromatography on a silica gel column (DCM:MeOH = 15:1→10:1) to give **42** (680 mg, 94%) as a white foam.

72 (830 mg, 1.16 mmol) was dissolved in a solution of TFA (4 ml), H₂O (0.2 ml) and DCM (3 ml) and stirred at room temperature for 3 h. The solvent was removed under reduced pressure by co-evaporation with toluene and the remaining residue was purified by flash chromatography on a silica gel column (DCM:MeOH = 15:1→10:1) to give **43** (670 mg, 87%) as a white foam.



Synthesis of glycoamino acid 45. To the solution of penta-O-acetyl- β -D-glucopyranose **73** (1.0 g, 2.56 mmol) and Fmoc-Ser-OH (1.0 g, 3.07 mmol) in MeCN (30 ml), BF₃·OEt₂ (1.0 mL, 7.68 mmol) was added. The resulting mixture was stirred at room temperature for 24 h under argon¹. The solvent was removed under reduced pressure, and the resulting residue was diluted with EtOAc then washed with water. The organic layer was dried over Na₂SO₄, filtered and the filtrate was concentrated under reduced pressure. The resulting oil was purified by flash chromatography on a silica gel column (Hex/EtOAc/AcOH = 4:1:0.5→3:1:0.4→2:1:0.3) to give **45** (361 mg, 21%) as a white foam. Product matched the previously known spectra of **45**⁹.



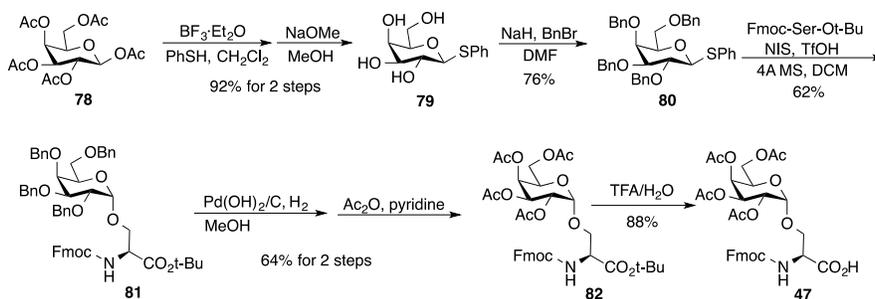
Synthesis of glycoamino acid 46. To a solution of 3,4,6-Tri-O-acetyl-D-galactal **74** (1.0 g, 3.67 mmol) in MeCN (30 ml) at -30 °C was added FeCl₃·6H₂O (0.79 g, 2.94 mmol), NaN₃ (263 mg, 4.04 mmol) and H₂O₂ (30%, aq., 0.42 ml, 4.04 mmol) and the reaction was stirred at -30 °C for 6 h⁶. The mixture was diluted with Et₂O and washed with H₂O, NaHCO₃, and brine. The organic layer was dried with Na₂SO₄ and concentrated under reduced pressure to give **75** (900 mg, 70%)

as a viscous oil. The product was used directly without further purification.

A solution of Fmoc-Ser-Ot-Bu (560 mg, 1.46 mmol), Ag₂CO₃ (600 mg, 2.19 mmol), 4A MS (1.7 g) in toluene (5 ml) and DCM (7.5 ml) was stirred at 0 °C for 30 minutes. Then, a solution of AgClO₄ (75 mg, 0.37 mmol) in toluene (2 ml) was added dropwise at 0°C. Subsequently, a solution of **75** (900 mg, 2.58 mmol) in a mixture of DCM (3.75 ml) and toluene (3.75 ml) was very slowly added dropwise at 0 °C. The mixture was stirred, in the dark, at room temperature for 19 h. The reaction was diluted with DCM, filtered through Celite and washed with H₂O then NaHCO₃ (sat.). The organic layer was dried with Na₂SO₄ and concentrated under reduced pressure. The residue was purified by flash chromatography on a silica gel column (Tol/EtOAc = 10:1) to give **76** (692 mg, 68%) as a white foam.

76 (500 mg, 0.72 mmol) was dissolved in a solution of pyridine (0.8 ml) and AcSH (1.6 ml) and stirred at room temperature for 24 h. The mixture was diluted with DCM and washed with H₂O, HCl (1M, aq.), and NaHCO₃ (sat., aq.). The organic layers were dried with Na₂SO₄ and concentrated under reduced pressure. The residue was purified by flash chromatography on a silica gel column (Hex/EtOAc = 1:1→0:1) to give **77** (253 mg, 50%) as a white foam.

77 (240 mg, 0.34 mmol) was dissolved in a solution of TFA/H₂O (95:5, 4.0 ml) and stirred at room temperature for 2 h. The solvent was removed under reduced pressure by co-evaporation with toluene and the remaining residue was suspended in MeCN/H₂O (1:1), frozen and lyophilized to give a white solid. The solid was dissolved in DCM and purified by flash chromatography on a silica gel column (DCM:MeOH = 10:1→5:1) to give **46** (206 mg, 92%) as a white foam.



Synthesis of glycoamino acid 47. To a solution of β-D-galactose pentaacetate **78** (20.0 g, 51.3 mmol) in CH₂Cl₂ (100 ml) was added thiophenol (7.3 mL, 72.0 mmol). The resulting mixture was cooled to 0 °C. BF₃·Et₂O (7.7 ml, 61.5 mmol) was then added dropwise and the reaction mixture was allowed to warm to room temperature. After being stirred at room temperature for 2 h, the mixture was diluted with CH₂Cl₂ (100 ml), washed with 2 M NaOH solution, H₂O, dried over MgSO₄ and concentrated under reduced pressure. The residue was dissolved in MeOH (100 ml) and MeONa (138 mg, 2.56 mmol) was added to the solution. The reaction was stirred at room temperature overnight, then neutralized with Amberlite IR-120 resin, filtered and

concentrated to give **79** (12.8 g, 92%) as a white foam¹⁰.

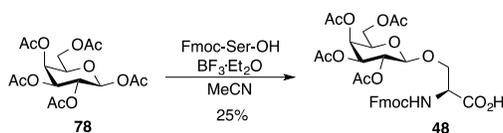
To a suspension of NaH (9.4 g, 235 mmol, 60% in mineral oil) in DMF (150 mL) at 0 °C was added dropwise a solution of **79** (12.8 g, 47.0 mmol) in DMF (70 ml), which was followed by the addition of a solution of BnBr (27.8 mL, 235 mmol) in DMF (80 mL). The resulting mixture was stirred at room temperature overnight, then diluted with EtOAc, washed with H₂O, and concentrated under reduced pressure. The oily residue was purified by flash chromatography on a silica gel column (Hex/EtOAc = 6:1) to afford **80** (22.6 g, 76%) as a white solid¹⁰.

To a solution of **80** (632 mg, 1.00 mmol) and Fmoc-Ser-Ot-Bu (421 mg, 1.10 mmol) in DCM (15 ml) at -30 °C were added 4A MS (300 mg), N-iodosuccinimide (NIS) (470 mg, 2.00 mmol) and trifluoromethanesulfonic acid (TfOH) (9 µL, 0.10 mmol). The resulting mixture was stirred at -30 °C for 10 min under argon before it was quenched with Na₂SO₃ (sat., aq.). The mixture was diluted with water and extracted with EtOAc. The organic layer was dried over Na₂SO₄, filtered and the filtrate was concentrated under reduced pressure. The resulting oil was purified by flash chromatography on silica gel (Hex/EtOAc = 5:1) to give **81** (561 mg, 62%) as a white foam. ¹H NMR (400 MHz, CDCl₃) δ 7.79 (d, *J* = 7.6 Hz, 2H), 7.63 (dd, *J* = 7.5, 4.0 Hz, 2H), 7.50 – 7.17 (m, 24H), 6.29 (d, *J* = 8.5 Hz, 1H), 4.99 (d, *J* = 11.3 Hz, 1H), 4.90 – 4.82 (m, ¹*J*_{CH} = 167.2 Hz, 3H), 4.79 (d, *J* = 11.7 Hz, 1H), 4.72 (d, *J* = 11.9 Hz, 1H), 4.62 (d, *J* = 11.4 Hz, 1H), 4.53 (d, *J* = 12.0 Hz, 1H), 4.49 – 4.40 (m, 2H), 4.40 – 4.33 (m, 2H), 4.29 – 4.19 (m, 2H), 4.11 (dd, *J* = 10.1, 3.7 Hz, 1H), 4.06 (t, *J* = 6.5 Hz, 1H), 4.01 (d, *J* = 2.8 Hz, 1H), 3.95 (dd, *J* = 10.1, 2.7 Hz, 1H), 3.87 (dd, *J* = 11.2, 3.0 Hz, 1H), 3.64 (dd, *J* = 9.3, 6.1 Hz, 1H), 3.55 (dd, *J* = 9.2, 6.6 Hz, 1H), 1.51 (s, 9H). ¹³C NMR (101 MHz, CDCl₃) δ 169.15, 156.14, 143.97, 143.95, 141.28, 138.71, 138.59, 138.57, 137.98, 128.42, 128.39, 128.37, 128.29, 127.96, 127.76, 127.71, 127.67, 127.64, 127.58, 127.51, 127.10, 125.27, 119.95, 99.52, 82.36, 78.74, 74.96, 74.80, 73.48, 73.41, 73.09, 70.73, 70.02, 68.93, 67.06, 55.24, 47.16, 28.04. IR (NaCl, film): 3340, 3064, 3031, 2978, 2929, 1725, 1497, 1453, 1369, 1347, 1248, 1155, 1100, 1057, 739, 697 cm⁻¹. HRMS (ESI) Calcd. for C₅₆H₅₉NNaO₁₀ [M + Na]⁺ requires 928.4032, Found: 928.4026.

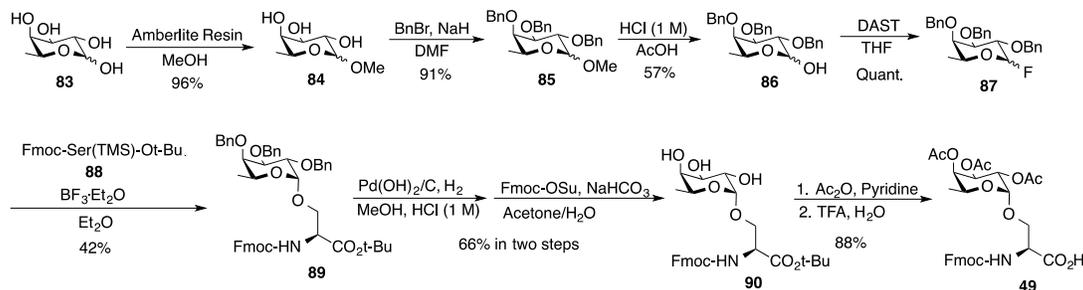
A solution of **81** (420 mg, 0.46 mmol) in MeOH (8 ml) was stirred with Pearlman's catalyst [Pd(OH)₂/C, 50 mg] under a hydrogen atmosphere at room temperature for 24 h. The reaction was filtered through Celite and the filtrate was concentrated under reduced pressure. The residue was dissolved in pyridine (2 ml) and Ac₂O (2 ml) was added dropwise. The resulting mixture was stirred at room temperature under argon overnight. The mixture was poured into ice-water and extracted with EtOAc. The organic layer was dried over Na₂SO₄, filtered and the filtrate was concentrated under reduced pressure. The resulting oil was purified by flash chromatography on silica gel column (Hex/EtOAc = 2:1) to give **82** (212 mg, 64%) as a white foam. ¹H NMR (400 MHz, CDCl₃) δ 7.75 (d, *J* = 7.5 Hz, 2H), 7.62 (d, *J* = 6.8 Hz, 2H), 7.39 (t, *J* = 7.3 Hz, 2H), 7.35 – 7.28 (m, 2H), 5.83 (d, *J* = 8.0 Hz, 1H), 5.45 (d, *J* = 2.4 Hz, 1H), 5.30 (dd, *J* = 10.9, 3.2 Hz, 1H), 5.15 (dd, *J* = 10.9, 3.5 Hz, 1H), 5.04 (d, *J* = 3.5 Hz, 1H), 4.46 – 4.35 (m, 3H), 4.24 (t, *J* = 7.1 Hz,

1H), 4.19 (t, $J = 6.6$ Hz, 1H), 4.10 – 4.02 (m, 2H), 3.98 (dd, $J = 10.4, 2.8$ Hz, 1H), 3.92 (dd, $J = 10.6, 2.9$ Hz, 1H), 2.13 (s, 3H), 2.05 (s, 3H), 1.99 (s, 3H), 1.95 (s, 3H), 1.48 (s, 9H). ^{13}C NMR (101 MHz, CDCl_3) δ 170.42, 170.31, 170.17, 169.97, 168.61, 155.84, 143.83, 141.29, 127.77, 127.11, 125.13, 120.02, 96.96, 82.82, 69.31, 67.98, 67.81, 67.40, 67.26, 66.82, 61.74, 54.74, 47.08, 28.00, 20.76, 20.68, 20.65, 20.61. IR (NaCl, film): 3357, 3066, 2979, 1751, 1519, 1451, 1371, 1229, 1156, 1065, 761, 741 cm^{-1} . HRMS (ESI) Calcd. for $\text{C}_{36}\text{H}_{43}\text{NNaO}_{14}$ $[\text{M} + \text{Na}]^+$ requires 736.2576, Found: 736.2579.

Compound **82** (120 mg, 0.17 mmol) was dissolved in a TFA-water mixture (95:5, 1 ml) and stirred at room temperature for 2 h. The solvent was evaporated and the residue was co-evaporated with toluene to afford **47** (98 mg, 88%) as a white foam. ^1H NMR (400 MHz, CDCl_3) δ 7.76 (d, $J = 7.5$ Hz, 2H), 7.61 (t, $J = 7.2$ Hz, 2H), 7.40 (t, $J = 7.5$ Hz, 2H), 7.31 (t, $J = 7.4$ Hz, 2H), 6.50 (d, $J = 8.7$ Hz, 1H), 5.50 – 5.39 (m, 2H), 5.17 (d, $^1J_{\text{CH}} = 167.2$ Hz, $J = 3.5$ Hz, 1H), 5.09 – 5.03 (m, 1H), 4.66 (d, $J = 8.3$ Hz, 1H), 4.39 (d, $J = 7.1$ Hz, 2H), 4.24 (d, $J = 6.5$ Hz, 2H), 4.12 – 4.01 (m, 2H), 4.01 – 3.91 (m, 2H), 2.14 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 1.91 (s, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 172.46, 170.79, 170.64, 170.35, 156.10, 143.82, 143.69, 141.29, 127.81, 127.08, 125.12, 125.05, 120.05, 96.73, 77.26, 69.28, 68.25, 67.94, 67.89, 67.38, 66.75, 61.89, 54.16, 47.03, 20.80, 20.64, 20.54, 20.52. IR (NaCl, film): 3336, 3067, 2954, 1750, 1527, 1451, 1372, 1229, 1153, 1063, 761, 741 cm^{-1} . HRMS (ESI) Calcd. for $\text{C}_{32}\text{H}_{36}\text{NNaO}_{14}$ $[\text{M} + \text{Na}]^+$ requires 658.2131, Found: 658.2144.



Synthesis of glycoamino acid 48. To the solution of β -D-Galactose pentaacetate **78** (1.0 g, 2.56 mmol) and Fmoc-Ser-OH (1.0 g, 3.07 mmol) in MeCN (30 ml), $\text{BF}_3\cdot\text{OEt}_2$ (1.0 ml, 7.68 mmol) was added. The resulting mixture was stirred at room temperature for 24 h under argon. The solvent was removed under reduced pressure, and the resulting residue was diluted with EtOAc then washed with water. The organic layer was dried over Na_2SO_4 , filtered and the filtrate was concentrated under reduced pressure. The resulting oil was purified by flash chromatography on a silica gel column (Hex/EtOAc/AcOH = 4:1:0.5 \rightarrow 3:1:0.4 \rightarrow 2:1:0.3) to give **48** (417.1 mg, 25%) as a white foam. Product matched the previously known spectra of **48**⁹.



Synthesis of glycoamino acid 49. To a solution of L-fucose **83** (3.3 g, 20.1 mmol) in MeOH (33 ml) was added Amberlite IR120 Resin (MeOH pre-treated H⁺ form, 5.3 g). The mixture was heated to reflux and allowed to stir at reflux for 3 h. The reaction was then filtered and concentrated under reduced pressure. The residue was purified by recrystallization from EtOH to give **84** (3.41 g, 96%) as an off-white solid¹¹.

To a solution of **84** (3.41 g, 19.1 mmol) in DMF (85 ml) was slowly added NaH (60% in oil, 5.04 g, 126.06 mmol) over the course of 20 min with stirring. The mixture was allowed to stir for 1 h before BnBr (6.77 ml, 57.3 mmol) was added. The mixture was stirred at room temperature overnight. The reaction was quenched by the slow addition of H₂O and extracted with EtOAc. The organic layers were combined, washed with NaHCO₃ (sat.) and brine, dried over Na₂SO₄ and concentrated under reduced pressure. The residue was purified by flash chromatography on a silica gel column (Hex/EtOAc = 12:1→6:1) to give **85** (7.84 g, 91%) as an oil¹¹.

85 (7.81 g, 17.4 mmol) was dissolved in AcOH (112.75 mL) and heated to 95 °C with stirring. HCl (aq., 1 M, 31.3 mL) was added and the mixture was allowed to stir at 95 °C for 2 h. The reaction was cooled to room temperature and extracted with CHCl₃. The organic layers were combined, washed first with ice-cold NaHCO₃ (sat.) until neutral, then brine, dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography on a silica gel column (Hex/EtOAc = 4:1→3:1) to give **86** (4.3 g, 57%) as a thick syrup¹¹.

86 (1.17 g, 2.68 mmol) was dissolved in THF (30 ml) and cooled to -30 °C. (Diethylamino)sulfur trifluoride (DAST) (0.37 mL, 2.81 mmol) was added at -30 °C and stirred for 5 min. The reaction was quenched with H₂O (4 ml) at -30 °C and stirred another 5 min, after which the cooling bath was removed and the reaction was diluted with EtOAc. The organic layer was washed with brine, dried over Na₂SO₄, filtered and concentrated under reduced pressure to almost-dryness. The residue was quickly purified by flash chromatography on a short silica gel column (Hex/EtOAc = 6:1) to give **87** (1.2 g, quant.) as a white oil¹².

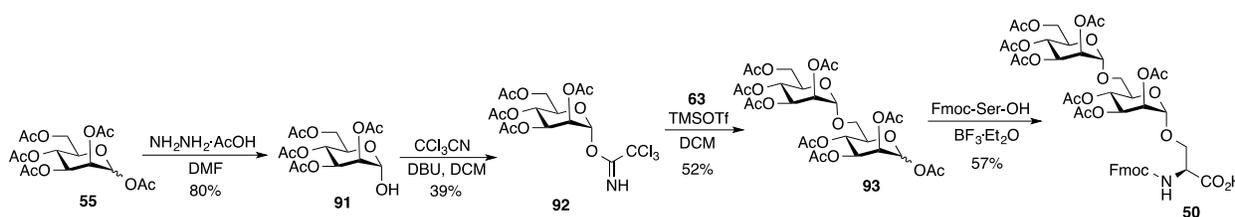
To a solution of Fmoc-Ser-Ot-Bu (643.6 mg, 1.68 mmol) in DMF (3.5 ml) was added imidazole (456.8 mg, 6.71 mmol) and trimethylchlorosilane (TMSCl) (0.426 ml, 3.36 mmol). The mixture was allowed to stir at room temperature for 5 h. The reaction was quenched with brine and extracted with EtOAc. The organic layers were combined, washed with brine, dried over Na₂SO₄, filtered and concentrated under reduced pressure to give **88** (712.3 mg, 93%) as a thick oil. The product was used directly without further purification.

This and subsequent steps of the synthesis of compound **49** are based on a previously published procedure¹³. **87** (670.5 mg, 1.53 mmol) and **88** (698.9 mg, 1.53 mmol) were dissolved in Et₂O (23 ml) and cooled to -20 °C. BF₃·OEt₂ (57 μl, 0.46 mmol) was added to the reaction and the

solution was allowed to stir at -20 °C for 1 h, after which the reaction was allowed to warm to 0 °C and stirred for another hour. The cooling bath was then removed and the reaction was stirred at room temperature for an additional 3 h. The reaction was quenched with NaHCO₃ (sat., aq.). The aqueous layer was extracted with Et₂O. The organic layers were then combined, washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure. The residue was purified by flash chromatography on a silica gel column (Hex/EtOAc = 4:1) to give **89** (511.1 mg, 42%) as a hard white foam.

A solution of **89** (479.4 mg, 0.6 mmol) in EtOH (17 ml) and HCl (aq., 1M, 0.5 ml) was stirred with Pearlman's catalyst [Pd(OH)₂/C, 120 mg] under a hydrogen atmosphere at room temperature for 3 h. The reaction was filtered through Celite and the filtrate was neutralized with NaHCO₃ (sat., aq.) then concentrated under reduced pressure. The resulting residue was dissolved in H₂O (20 ml) and NaHCO₃ (200 mg), acetone (40 ml) and Fmoc-OSu (202 mg, 0.6 mmol) were added with vigorous stirring. The reaction was allowed to stir at room temperature for 1 h. The reaction was concentrated under reduced pressure, diluted with H₂O and extracted with CHCl₃. The organic layers were combined, dried with Na₂SO₄ and concentrated under reduced pressure. The residue was purified by flash chromatography on a silica gel column (DCM/MeOH = 50:1→25:2) to give **90** (210.6 mg, 66% in two steps from **89**) as a hard, white foam.

90 (201.4 mg, 0.38 mmol) was dissolved in a mixture of Ac₂O (1 ml) and pyridine (0.8 ml) and allowed to stir at room temperature for 17 h. The reaction was concentrated under reduced pressure and the residue was co-evaporated with toluene three times before being dissolved in a solution of TFA and H₂O (95:5, 1 ml) and stirred for an additional hour at room temperature. Solvent was removed by co-evaporating the reaction mixture with toluene under reduced pressure and the resulting residue was purified by flash chromatography on a silica gel column (CHCl₃/MeOH = 40:1→20:1) to give an oil, which was then dissolved in H₂O/CH₃CN=1:1. The solution was frozen and lyophilized to give **49** (184.2 mg, 88% over two steps from **90**) as a white solid. Product matched the previously known spectra of **49**¹⁴.



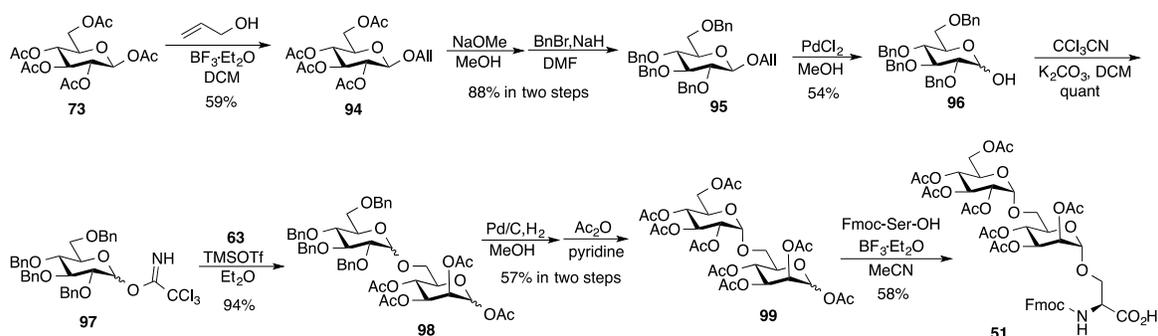
Synthesis of glycoamino acid 50. To a stirred solution of **55** (6.6 g, 16.92 mmol) in DMF (66 ml) was added N₂H₄·AcOH (1.87 g, 20.30 mmol). The resulting mixture was stirred for 4 h at room temperature under Ar. The reaction mixture was diluted with EtOAc, washed by water and brine. The organic layer was dried over Na₂SO₄, filtered and the filtrate was concentrated under reduced pressure. The resulting oil was purified by flash chromatography on a silica gel column

(Hex/EtOAc =1:1) to give **91** (4.69 g, 80%) as a syrup¹⁵.

To a stirred solution of **94** (4.6 g, 13.21 mmol) in DCM (60 ml), CCl₃CN (13.21 ml, 132.10 mmol) and DBU (3.95 ml, 24.43 mmol) were added. After stirring overnight, the solvent was evaporated under reduced pressure and the residue was purified by flash chromatography on a silica gel column (Hex/EtOAc =1:1) to give **92** (2.54 g, 39%) as a syrup¹⁵.

A solution of **63** (500 mg, 1.43 mmol) and **92** (776 mg, 1.58 mmol) in DCM (20 ml) was stirred with 4A molecular sieves (450 mg) under argon for 15 min. Then, a solution of Trimethylsilyl triflate (TMSOTf) (113 μ l, 0.40 mmol) in DCM (2 ml) was added dropwise. The resulting mixture was stirred for 4 h at room temperature under argon. The reaction mixture was diluted with DCM and washed with sat. aq. NaHCO₃. The organic layer was dried over Na₂SO₄, filtered and the filtrate was concentrated under reduced pressure. The resulting oil was purified by flash chromatography on a silica gel column (Hex/EtOAc =2:1→3:2→1:1) to give **93** (504 mg, 52%) as a white foam.

To the solution of **93** (500 mg, 0.73 mmol) and Fmoc-Ser-OH (363 mg, 1.11 mmol) in MeCN (18 ml), BF₃OEt₂ (0.34 ml, 2.22 mmol) was added. The resulting mixture was stirred at room temperature for 24 h under argon. The solvent was removed under reduced pressure, and the resulting residue was diluted with EtOAc then washed with water. The organic layer was dried over Na₂SO₄, filtered and the filtrate was concentrated under reduced pressure. The resulting oil was purified by flash chromatography on a silica gel column (Hex/EtOAc/AcOH = 2:1:0.3→3:2:0.5→1:1:0.2) to give **50** (390 mg, 57%) as a white foam. ¹H-NMR (400 MHz, Acetone-d₆) δ 7.86 (d, J = 7.6 Hz, 2H, H-Fmoc), 7.74 (d, J = 7.6 Hz, 2H, H-Fmoc), 7.42 (t, J = 7.2 Hz, 2H, H-Fmoc), 7.34 (t, J = 7.4 Hz, 2H, H-Fmoc), 5.22-5.37 (m, 6H, H-2, H-3, H-4, H-2', H-3', H-4'), 4.96 (s, $^1J_{CH}$ = 176 Hz, H-1), 4.94 (s, $^1J_{CH}$ = 172 Hz, H-1'), 4.03-4.55 (m, 10H, H- α , CH₂- β , CH-Fmoc, CH₂-Fmoc, H-5, H-5', H-6'), 3.69-3.83 (m, 2H, H-6), 2.13 (s, 3H, CH₃-Ac), 2.10 (s, 3H, CH₃-Ac), 2.02 (s, 6H, CH₃-Ac), 2.01 (s, 3H, CH₃-Ac), 1.953 (s, 3H, CH₃-Ac), 1.947 (s, 3H, CH₃-Ac). ¹³C-NMR (100 MHz, Acetone-d₆) δ 169.9, 169.5, 169.4, 169.3, 144.3, 144.2, 141.18, 141.15, 127.6, 127.1, 125.4, 125.3, 119.9, 98.2, 97.5, 69.4, 69.2, 68.6, 66.6, 65.9, 62.3, 47.1, 19.86, 19.83, 19.80, 19.76. IR (NaCl, film): 3361, 2954, 1751, 1371, 1225, 1138, 1087, 1047, 761, 741 cm⁻¹. HRMS (ESI) Calcd. for C₄₄H₅₁NNaO₂₂ [M + Na]⁺ requires 968.2795, Found: 968.2784.



Synthesis of glycoamino acid 51. **73** (5 g, 12.80 mmol) was dissolved in DCM (30 ml) under argon. The stirred solution was cooled to 0 °C and $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (2.44 ml, 19.22 mmol) was added by syringe. After stirring for 10 min at 0 °C, allyl alcohol (1.31 ml, 19.22 mmol) was added. The ice bath was removed after completion of the addition and the reaction stirred at room temperature for overnight. The reaction was then cooled to 0 °C and quenched with NaHCO_3 (sat. aq.). After dilution with water, the organic layer was separated and aqueous layer was extracted with DCM. The combined organic layer was washed with brine, dried over Na_2SO_4 , filtered and concentrated under reduced pressure. The product was purified by flash chromatography on a silica gel column (Hex/EtOAc =4:1→3:1→2:1) to give **94** (2.92 g, 59%) as a white solid¹⁶.

To a stirred solution of **94** (2.9 g, 7.47 mmol) in MeOH (30 ml) was added NaOMe (20.5 mg, 0.38 mmol). After stirring for 1 h, the reaction was neutralized with Dowex H^+ and then filtered. The solvent was concentrated under reduced pressure to give a white foam (1.68g, 7.47 mmol). The product was dissolved in DMF (5 ml). The resulting solution was used directly in the next step without purification. To a suspension of NaH (60% in oil, 1.52 g, 38 mmol) in DMF (25 ml) was added dropwise the above solution at 0 °C under argon. The resulting mixture was stirred at 0 °C for 30 min and then BnBr (4.5 ml, 38 mmol) was added dropwise. The reaction was allowed to warm up to room temperature and stirred overnight. With caution, the reaction was quenched with water at 0°C. The resulting mixture was diluted with EtOAc and washed with water then brine. The organic layer was dried over Na_2SO_4 , filtered and the filtrate was concentrated under reduced pressure. The resulting oil was purified by flash chromatography on a silica gel column (Hex/EtOAc =20:1→15:1→10:1) to give **95** (3.8 g, 88% over two steps) as a white solid¹⁷.

A solution of **95** (1.8 g, 3.10 mmol) and PdCl_2 (109 mg, 0.62 mmol) in MeOH (20 ml) was stirred vigorously at room temperature overnight. The reaction was diluted with diethyl ether and filtered through Celite. The solvent was concentrated under reduced pressure and the residue was purified by flash chromatography on a silica gel column (Hex/EtOAc =4:1→2:1) to give **96** (900 mg, 54%) as a white solid¹⁷.

A solution of **96** (900 mg, 1.66 mmol), CCl_3CN (1.72 ml, 21.6 mmol) and K_2CO_3 (1.15 g, 8.3 mmol) in DCM (22 ml) was stirred vigorously at room temperature under argon overnight. The reaction was filtered through Celite and the filtrate was concentrated under reduced pressure to

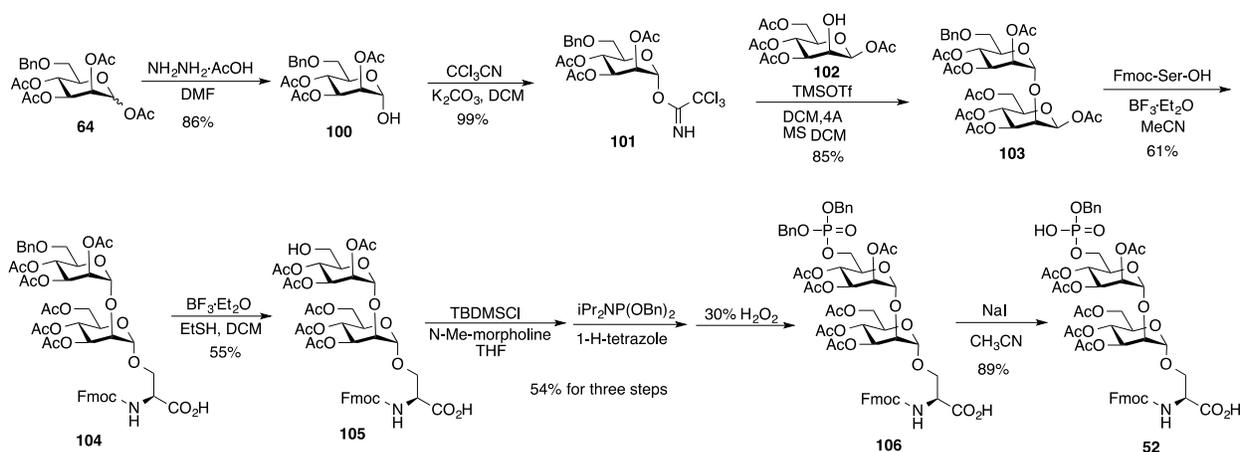
give **97** (1.21 g, 100%) as a white foam. The product was used directly in the next step without purification.

A solution of **63** (293 mg, 0.84 mmol) and **97** (690 mg, 1.01 mmol) in diethyl ether (20 ml) was stirred with 4A MS (700 mg) under argon for 1 h. The reaction was cooled to -40 °C and TMSOTf (76 µl, 0.42 mmol) was added. The resulting mixture was allowed to warm up to room temperature slowly and stirred for 4 h. The reaction was quenched with Et₃N (500 µl) and stirred for 10 additional minutes, then filtered. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography on a silica gel column (Hex/EtOAc =5:1→2:1→1:1) to give **98** (688 mg, 94%) as a white foam.

A solution of **98** (680 mg, 0.78 mmol) in MeOH (25 ml) was stirred at room temperature under a hydrogen atmosphere for 24 h in the presence of 10% Pd/C (200 mg). The reaction was filtered through Celite and the filtrate was concentrated under reduced pressure. The residue was dissolved in pyridine (3 ml) and Ac₂O (1 ml) was added dropwise. The resulting mixture was stirred at room temperature under argon overnight. The mixture was slowly poured into ice-water and extracted with DCM. The organic layer was dried over Na₂SO₄, filtered and the filtrate was concentrated under reduced pressure. The resulting oil was purified by flash chromatography on a silica gel column (Hex/EtOAc =2:1→3:2) to give first **99** (304 mg, 57%). ¹H-NMR (400 MHz, CDCl₃) δ 6.06 (d, *J* = 2.0 Hz, 1H, H-1), 5.47 (dd, *J* = 10.0 Hz, 9.2 Hz, 1H, H-3'), 5.38 (dd, *J* = 10.0 Hz, 3.2 Hz, H-3), 5.31 (t, *J* = 10.0 Hz, 1H, H-4), 5.27 (dd, *J* = 3.6 Hz, 2.0 Hz, 1H, H-2), 5.07-5.12 (m, 2H, H-1', H4'), 4.86 (dd, *J* = 10.0 Hz, 3.6 Hz, 1H, H-2'), 4.26-4.30 (m, 1H, H-6'), 4.09-4.13 (m, 2H, H-5', H-6'), 4.03-4.07 (m, 1H, H-5), 3.79 (dd, *J* = 11.2 Hz, 6.0 Hz, H-6), 3.62 (dd, *J* = 10.8 Hz, 2.8 Hz, H-6), 2.21 (s, 3H, CH₃-Ac), 2.20 (s, 3H, CH₃-Ac), 2.11 (s, 3H, CH₃-Ac), 2.102, (s, 3H, CH₃-Ac), 2.099 (s, 3H, CH₃-Ac), 2.07 (s, 3H, CH₃-Ac), 2.04 (s, 3H, CH₃-Ac), 2.03 (s, 3H, CH₃-Ac). ¹³C-NMR (100 MHz, CDCl₃) δ 170.7, 170.2, 169.98, 169.97, 169.8, 169.7, 169.6, 168.1, 95.3, 90.3, 71.1, 70.9, 69.9, 68.8, 68.30, 68.27, 67.3, 67.1, 66.3, 61.8, 20.84, 20.75, 20.72, 20.69, 20.66, 20.62. IR (NaCl, film): 1751, 1370, 1223, 1149, 1040, 977 cm⁻¹. HRMS (ESI) Calcd. for C₂₈H₃₈NaO₁₉ [M + Na]⁺ requires 701.1900, Found: 701.1902.

To the solution of **99** (400 mg, 0.59 mmol) and Fmoc-Ser-OH (290 mg, 0.88 mmol) in MeCN (15 mL), BF₃·OEt₂ (0.27 ml, 1.77 mmol) was added. The resulting mixture was stirred at room temperature for 24h under argon. The solvent was removed under reduced pressure. The resulting residue was diluted with EtOAc, and then washed with water. The organic layer was dried over Na₂SO₄, filtered and the filtrate was concentrated under reduced pressure. The resulting oil was purified by flash chromatography on a silica gel column (Hex/EtOAc/AcOH =2:1:0.3→3:2:0.5→1:1:0.2) to give **51** (321 mg, 58%) as a white foam. ¹H-NMR (400 MHz, CDCl₃) δ 7.76 (d, *J* = 7.6 Hz, 2H, H-Fmoc), 7.63 (d, *J* = 6.4 Hz, 2H, H-Fmoc), 7.40 (t, *J* = 7.4 Hz, 2H, H-Fmoc), 7.31 (t, *J* = 7.6 Hz, 2H, H-Fmoc), 5.92 (d, *J* = 8.4 Hz, 1H, H-NH), 5.44 (t, *J* = 9.8 Hz, 1H, H-3'), 5.34 (dd, *J* = 10.0 Hz, 3.6 Hz, 1H, H-3), 5.27-5.29 (m, 1H, H-2), 5.18 (t, *J* =

10.2 Hz, 1H, H-4), 5.10 (d, $J = 4.0$ Hz, 1H, H-1'), 5.03 (t, $J = 9.4$ Hz, 1H, H-4'), 4.86 (dd, $J = 10.0$ Hz, 3.6 Hz, 1H, H-2'), 4.83 (s, 1H, H-1), 4.67-4.72 (m, 1H, H- α), 4.42 (d, $J = 7.6$ Hz, 2H, CH₂-Fmoc), 4.25 (t, $J = 7.0$ Hz, 1H, CH-Fmoc), 4.07-4.15 (m, 5H, H- β , H-5, H-5', H-6'), 3.90-3.94 (m, 1H, H- β), 3.79 (dd, $J = 10.8$ Hz, 6.8 Hz, 1H, H-6), 3.53 (dd, $J = 10.8$ Hz, 2.8 Hz), 2.14 (s, 3H, CH₃-Ac), 2.08 (s, 3H, CH₃-Ac), 2.06 (s, 3H, CH₃-Ac), 2.05 (s, 3H, CH₃-Ac), 2.01 (s, 3H, CH₃-Ac), 2.00 (s, 3H, CH₃-Ac), 1.99 (s, 3H, CH₃-Ac). ¹³C-NMR (100 MHz, CDCl₃) δ 170.77, 170.75, 170.6, 170.3, 170.0, 169.9, 167.7, 156.1, 143.81, 143.77, 141.3, 127.7, 127.10, 127.08, 125.2, 120.0, 98.0, 95.4, 77.2, 70.7, 70.4, 69.9, 69.2, 69.0, 68.5, 68.4, 67.4, 67.3, 67.1, 66.6, 61.9, 54.1, 47.1, 20.80, 20.79, 20.77, 20.74, 20.70, 20.64, 20.58. IR (NaCl, film): 3355, 3065, 2952, 1751, 1521, 1452, 1370, 1223, 1139, 1042, 763, 741 cm⁻¹. HRMS (ESI) Calcd. for C₄₄H₅₁NNaO₂₂ [M + Na]⁺ requires 968.2795, Found: 968.2793.



Synthesis of glycoamino acid 52. To a solution of **64** (4 g, 9.13 mmol) in DMF (37 ml) was added N₂H₄·AcOH (1.0 g, 10.96 mmol). The resulting mixture was stirred for 4 h at room temperature under argon. The reaction was diluted with EtOAc and washed with water and brine. The organic layer was dried over anhydrous Na₂SO₄, filtered and the filtrate was concentrated under reduced pressure. The resulting oil was purified by flash chromatography on a silica gel column (Hex/EtOAc = 2:1 → 3:2) to give **100** (3.11 g, 86%) as a white foam. ¹H-NMR (400 MHz, CDCl₃) δ 7.27-7.36 (m, 5H, H-Ph), 5.40 (dd, $J = 10.0$ Hz, 3.2 Hz, 1H, H-3), 5.19-5.26 (m, 3H, H-1, H-2, H-4), 4.54 (dd, $J = 25.2$ Hz, 12.0 Hz, 2H, CH₂-Bn), 4.17-4.22 (m, 1H, H-5), 3.57 (dd, $J = 10.8$ Hz, 6.4 Hz, 1H, H-6), 3.49 (dd, $J = 10.4$ Hz, 2.4 Hz, 1H, H-6), 3.27 (brs, 1H, H-OH), 2.14 (s, 3H, CH₃-Ac), 1.99 (s, 3H, CH₃-Ac), 1.93 (s, 3H, CH₃-Ac). ¹³C-NMR (100 MHz, CDCl₃) δ 170.2, 170.0, 169.9, 137.5, 128.4, 128.1, 127.8, 92.1, 73.6, 70.0, 69.6, 69.1, 68.8, 66.9, 20.9, 20.72, 20.69. IR (NaCl, film): 3423, 2937, 2871, 1751, 1454, 1433, 1372, 1227, 1078, 1051, 739, 701 cm⁻¹. HRMS (ESI) Calcd. for C₁₉H₂₄NaO₉ [M + Na]⁺ requires 419.1313, Found: 419.1320.

100 (3.1 g, 7.83 mmol) in DCM (100 ml) was stirred vigorously with CCl₃CN (8.10 ml, 101.73 mmol) and K₂CO₃ (5.40 g, 39.13 mmol) at room temperature under argon overnight. The reaction mixture was filtered through celite and the filtrate was concentrated under reduced pressure to give **101** (4.39 g, 99%) as a syrup. The product was used directly to the next step

without further purification. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.75 (s, 1H, H-NH), 7.27-7.35 (m, 5H, H-Ph), 6.29 (d, $J = 2.0$ Hz, 1H, H-1), 5.38-5.50 (m, 3H, H-2, H-3, H-4), 4.54 (dd, $J = 40.4$ Hz, 12.0 Hz, 2H, $\text{CH}_2\text{-Bn}$), 4.14-4.18 (m, 1H, H-5), 3.60 (d, $J = 4.0$ Hz, 2H, H-6), 2.18 (s, 3H, $\text{CH}_3\text{-Ac}$), 2.00 (s, 3H, $\text{CH}_3\text{-Ac}$), 1.93 (s, 3H, $\text{CH}_3\text{-Ac}$). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ 169.9, 169.8, 169.6, 137.7, 128.3, 127.9, 127.7, 94.7, 73.5, 72.5, 69.0, 68.5, 67.9, 66.1, 20.8, 20.69, 20.65. IR (NaCl, film): 3323, 2917, 2869, 1751, 1678, 1454, 1432, 1369, 1246, 1159, 1089, 1050, 976, 944, 836, 798, 737, 701 cm^{-1} . HRMS (ESI) Calcd. for $\text{C}_{21}\text{H}_{24}\text{Cl}_3\text{NNaO}_9$ $[\text{M} + \text{Na}]^+$ requires 562.0409, Found: 562.0406.

The 1,3,4,6-Tetra-O-acetyl- β -D-mannopyranose **102** was prepared as reported in the literature¹. A solution of **102** (1.46 g, 4.20 mmol) and **101** (3 g, 5.58 mmol) in DCM (45 ml) was stirred with 4A MS (1.5 g) under argon for 30 min. The reaction was cooled to -30 $^\circ\text{C}$ and a solution of TMSOTf (234 μl , 1.26 mmol) in DCM (2 ml) was added dropwise. After stirring at -30 $^\circ\text{C}$ for 15 min, the reaction mixture was allowed to warm up to room temperature slowly and stirred for 5 h. The reaction was quenched with Et_3N (3 ml), stirred for an additional 10 minutes and then filtered. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography on a silica gel column (Hex/EtOAc = 3:2 \rightarrow 1:1) to give **103** (2.6 g, 85%) as a white foam. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.27-7.35 (m, 5H, H-Ph), 5.78 (d, $J = 1.2$ Hz, 1H, H-1), 4.99 (dd, $J = 10.0$ Hz, 3.2 Hz, 1H, H-3'), 5.29-5.41 (m, 3H, H-2', H-4, H-4'), 5.12 (dd, $J = 9.6$ Hz, 2.8 Hz, 1H, H-3), 5.00 (d, $J = 2.0$ Hz, 1H, H-1'), 4.50 (dd, $J = 34.0$ Hz, 8.0 Hz, 2H, $\text{CH}_2\text{-Bn}$), 4.36-4.41 (m, 1H, H-5'), 4.26 (dd, $J = 12.4$ Hz, 4.8 Hz, 1H, H-6), 4.16-4.20 (m, 2H, H-2, H-6), 3.76-3.80 (m, 1H, H-5), 3.55 (d, $J = 4.0$ Hz, 2H, H-6'), 2.14 (s, 3H, $\text{CH}_3\text{-Ac}$), 2.12 (s, 3H, $\text{CH}_3\text{-Ac}$), 2.10 (s, 3H, $\text{CH}_3\text{-Ac}$), 2.06 (s, 3H, $\text{CH}_3\text{-Ac}$), 2.03 (s, 3H, $\text{CH}_3\text{-Ac}$), 2.02 (s, 3H, $\text{CH}_3\text{-Ac}$), 1.92 (s, 3H, $\text{CH}_3\text{-Ac}$). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ 171.0, 170.3, 169.92, 169.85, 169.6, 169.3, 168.7, 137.6, 128.4, 127.9, 127.8, 98.4, 90.9, 74.6, 73.7, 73.1, 72.2, 70.2, 70.0, 69.3, 68.4, 67.1, 65.8, 61.8, 21.0, 20.77, 20.74, 20.70, 20.6, 20.5. IR (NaCl, film): 3064, 2939, 2869, 1751, 1454, 1433, 1370, 1221, 1055, 933, 737, 702 cm^{-1} . HRMS (ESI) Calcd. for $\text{C}_{33}\text{H}_{42}\text{NaO}_{18}$ $[\text{M} + \text{Na}]^+$ requires 749.2264, Found: 749.2261.

To the solution of **103** (2.5 g, 3.44 mmol) and Fmoc-Ser-OH (1.69 g, 5.16 mmol) in MeCN (80 mL), $\text{BF}_3\cdot\text{OEt}_2$ (1.6 ml, 10.33 mmol) was added. The resulting mixture was stirred at room temperature for 24 h under argon. The solvent was removed under reduced pressure; the resulting residue was diluted with EtOAc and washed with water. The organic layer was dried (Na_2SO_4), filtered and the filtrate was concentrated under reduced pressure. The resulting oil was purified by flash chromatography on a silica gel column (Hex/EtOAc/AcOH = 2:1:0.3 \rightarrow 3:2:0.5) to give **104** (2.10 g, 61%) as a white foam. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.77 (d, $J = 7.6$ Hz, 2H, H-Fmoc), 7.64 (d, $J = 7.2$ Hz, 2H, H-Fmoc), 7.41 (t, $J = 7.4$ Hz, 2H, H-Fmoc), 7.30-7.36 (m, 7H, H-Fmoc, H-Ph), 5.81 (d, $J = 8.0$ Hz, 1H, H-NH), 5.22-5.40 (m, 4H, H-2', H-3, H-3', H-4'), 5.09 (t, $J = 10.0$ Hz, 1H, H-4), 5.02 (d, $J = 0.8$ Hz, 1H, H-1), 4.98 (d, $J = 1.6$ Hz, 1H, H-1'), 4.65 (dd, $J = 56.4$ Hz, 12.4 Hz, 2H, $\text{CH}_2\text{-Bn}$), 4.50-4.52 (m, 1H, H- α), 3.90 (d, $J = 6.8$ Hz, 2H, $\text{CH}_2\text{-}$

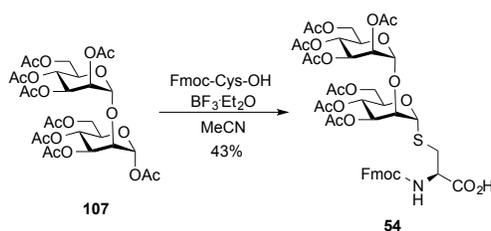
Fmoc), 3.96-4.27 (m, 8H, H-2, H-5, H5', H-6, CH-Fmoc, CH₂-β), 3.66 (dd, $J = 10.4$ Hz, 7.2 Hz, 1H, H-6'), 3.56 (dd, $J = 10.4$ Hz, 2.8 Hz, 1H, H-6'), 2.12 (s, 6H, CH₃-Ac), 2.09 (s, 3H, CH₃-Ac), 2.02 (s, 3H, CH₃-Ac), 2.00 (s, 3H, CH₃-Ac), 1.93 (s, 3H, CH₃-Ac). ¹³C-NMR (100 MHz, CDCl₃) δ 171.0, 170.7, 170.4, 170.0, 169.8, 169.4, 155.9, 143.79, 143.76, 141.3, 136.0, 128.6, 128.5, 128.4, 127.8, 127.1, 125.20, 125.17, 120.0, 100.2, 98.2, 74.5, 73.8, 70.23, 70.17, 70.15, 69.7, 69.6, 69.0, 68.0, 67.4, 67.3, 66.7, 62.1, 54.7, 47.1, 20.9, 20.69, 20.67, 20.65. IR (NaCl, film): 3338, 3065, 2952, 1751, 1521, 1452, 1370, 1225, 1136, 1047, 760 cm⁻¹. HRMS (ESI) Calcd. for C₄₉H₅₅NNaO₂₁ [M + Na]⁺ requires 1016.3159, Found: 1016.3148¹.

The mixture of **104** (1.9 g, 1.91 mmol), EtSH (7.5 ml) and BF₃·OEt₂ (2.42 ml, 15.30 mmol) in DCM (15 ml) was stirred for 6 h at room temperature under argon. The reaction was quenched with water and extracted with EtOAc. The organic phase was washed with brine, dried (Na₂SO₄), filtered and the filtrate was concentrated under reduced pressure. The resulting oil was purified by flash chromatography on a silica gel column (Hex/EtOAc/AcOH = 1:1:0.2 → 2:3:0.5) to give **105** (945 mg, 55%) as a white foam. ¹H-NMR (400 MHz, CD₃OD) δ 7.82 (d, $J = 7.6$ Hz, 2H, H-Fmoc), 7.71-7.74 (m, 2H, H-Fmoc), 7.39-7.43 (m, 2H, H-Fmoc), 7.32-7.36 (m, 2H, H-Fmoc), 5.42-5.41 (m, 5H, H-2', H-3, H-3', H-4, H-4'), 5.15 (d, $J = 2.0$ Hz, 1H, H-1), 5.00 (d, $J = 1.6$ Hz, 1H, H-1'), 4.47 (dd, $J = 10.0$ Hz, 6.4 Hz, 2H, CH₂-Fmoc), 3.93-4.34 (m, 9H, H-2, H-5, H-5', H-6, H-α, CH₂-β, CH-Fmoc), 3.66 (dd, $J = 12.4$ Hz, 2.4 Hz, 1H, H-6'), 3.59 (dd, $J = 12.0$ Hz, 5.6 Hz, 1H, H-6'), 2.14 (s, 3H, CH₃-Ac), 2.11 (s, 3H, CH₃-Ac), 2.052 (s, 3H, CH₃-Ac), 2.046 (s, 3H, CH₃-Ac), 2.00 (s, 3H, CH₃-Ac), 1.97 (s, 3H, CH₃-Ac). ¹³C-NMR (100 MHz, CD₃OD) δ 171.3, 170.5, 170.22, 170.20, 170.1, 170.0, 157.0, 144.0, 143.8, 141.19, 141.15, 127.41, 127.38, 126.84, 126.80, 125.0, 124.8, 119.53, 119.51, 99.2, 99.0, 76.9, 71.6, 70.3, 69.7, 69.0, 68.8, 68.4, 66.8, 66.4, 66.1, 54.8, 19.4, 19.29, 19.27, 19.23, 19.18. IR (NaCl, film): 3350, 3065, 1751, 1371, 1229, 1046, 740 cm⁻¹. HRMS (ESI) Calcd. for C₄₂H₄₉NNaO₂₁ [M + Na]⁺ requires 926.2690, Found: 926.2693.

To a solution of **105** (850 mg, 0.94 mmol) in THF (5.875 ml) were added N-methyl-morpholine (106 μl, 0.94 mmol, dissolved in 1.57 ml THF) and TBDMSCl (141 mg, 0.94 mmol, dissolved in 1.96 ml THF). After stirring for 30 minutes, 1H-tetrazole (9.79 ml, 4.42 mmol, 0.45M in CH₃CN) and iPr₂N(OBn)₂ (650 μl, 1.98 mmol) were added. The reaction mixture was stirred for 3 h at room temperature, cooled to 0 °C, and then 30% H₂O₂ (aq., 250 μl, 2.49 mmol) was added. The resulting mixture was slowly warmed to room temperature over 30 min, saturated Na₂SO₃ (6 ml) was then added. After stirring vigorously for 30 min, the mixture was diluted with saturated Na₂SO₃, extracted with EtOAc. The organic layer was dried over Na₂SO₄, filtered and the filtrate was concentrated under reduced pressure. The resulting oil was purified by flash chromatography on a silica gel column (Hex/EtOAc/AcOH = 1:1:0.2) to give **106** (586 mg, 54%) as a white foam. ¹H-NMR (400 MHz, CD₃OD) δ 7.81 (d, $J = 7.6$ Hz, 2H, H-Fmoc), 7.69-7.72 (m, 2H, H-Fmoc), 7.31-7.42 (m, 14H, H-Fmoc, H-Ph), 5.27-5.41 (m, 5H, H-2', H-3, H-3', H-4, H-4'), 5.04-5.13 (m, 5H, H-1, CH₂-Bn), 4.94 (d, $J = 1.2$ Hz, 1H, H-1'), 4.04-4.45 (m, 12H, H-2, H-

5, H-5', H-6, H-6', H- α , CH₂- β , CH₂-Fmoc, CH-Fmoc), 3.92 (dd, J = 10.4 Hz, 6.0 Hz, 1H, H-6), 2.09 (s, 3H, CH₃-Ac), 2.04 (s, 6H, CH₃-Ac), 2.00 (s, 6H, CH₃-Ac), 1.97 (s, 3H, CH₃-Ac). ¹³C-NMR (100 MHz, CD₃OD) δ 171.2, 170.4, 170.03, 170.99, 169.91, 169.86, 158.3, 157.0, 144.0, 143.8, 141.19, 141.14, 135.74, 135.67, 128.39, 128.36, 128.33, 128.31, 127.9, 127.8, 127.39, 127.37, 126.84, 126.80, 125.0, 124.9, 119.5, 99.1, 98.8, 77.1, 70.2, 69.57, 69.55, 69.51, 69.50, 69.45, 68.8, 68.4, 66.8, 65.93, 65.85, 65.80, 65.6, 61.7, 19.4, 19.28, 19.25, 19.20, 19.19, 19.13. ³¹P-NMR (400 MHz, CD₃OD) δ -1.51. IR (NaCl, film): 2956, 1750, 1452, 1370, 1225, 1046, 740 cm⁻¹. HRMS (ESI) Calcd. for C₅₆H₆₂NNaO₂₄P [M + Na]⁺ requires 1186.3292, Found: 1186.3287⁴.

106 (480 mg, 0.41 mmol) was dissolved in CH₃CN (4.4 ml). To this solution was added NaI (124 mg, 0.82 mmol). The reaction was stirred at 45 °C for 12 h under argon. The reaction mixture was concentrated and dissolved in small amount EtOAc. Hexane was added until white solid formed. The suspension was centrifuged and the resulting solid was dissolved in H₂O/CH₃CN=1:1. The resulting solution was frozen and lyophilized to give **52** (392 mg, 89%) as a white solid. ¹H-NMR (400 MHz, CD₃OD) δ 7.81 (d, J = 7.6 Hz, 2H, H-Fmoc), 7.71-7.74 (m, 2H, H-Fmoc), 7.22-7.42 (m, 9H, H-Fmoc, H-Ph), 5.24-5.40 (m, 5H, H-2', H-3, H-3', H-4, H-4'), 5.14 (d, J = 1.6 Hz, 1H, H-1), 4.93-5.00 (m, 2H, CH₂-Bn), 4.86 (d, J = 2.0 Hz, 1H, H-1'), 3.93-4.47 (m, 13H, H-2, H-5, H-5', H-6, H-6', H- α , CH₂- β , CH₂-Fmoc, CH-Fmoc), 2.13 (s, 3H, CH₃-Ac), 2.10 (s, 3H, CH₃-Ac), 2.02 (s, 3H, CH₃-Ac), 1.99 (s, 3H, CH₃-Ac), 1.98 (s, 3H, CH₃-Ac), 1.97 (s, 3H, CH₃-Ac). ¹³C-NMR (100 MHz, CD₃OD) δ 171.3, 170.3, 170.2, 170.1, 170.0, 143.9, 141.2, 127.9, 127.3, 127.0, 126.9, 126.8, 119.46, 119.43, 99.0, 98.9, 76.7, 70.4, 70.2, 69.6, 69.0, 68.6, 66.8, 66.7, 66.2, 66.1, 63.9, 61.7, 60.2, 55.4, 19.4, 19.28, 19.27, 19.25, 19.22, 19.15. ³¹P-NMR (400 MHz, CD₃OD) δ 0.28. IR (NaCl, film): 3431, 2954, 1749, 1623, 1452, 1370, 1227, 1081, 1046, 740 cm⁻¹. HRMS (ESI) Calcd. for C₄₉H₅₆NNaO₂₄P [M + Na]⁺ requires 1096.2822, Found: 1096.2830⁵.



Synthesis of glycoamino acid 54. **107** was prepared as reported in the literature¹. To the solution of **107** (678 mg, 1.0 mmol) and Fmoc-Cys-OH (514 mg, 1.5 mmol) in MeCN (20 ml), BF₃·OEt₂ (0.46 mL, 3.0 mmol) was added¹⁸. The resulting mixture was stirred at room temperature for 19 h under argon. The solvent was removed under reduced pressure, and the resulting residue was diluted with EtOAc then washed with water. The organic layer was dried (Na₂SO₄), filtered and the filtrate was concentrated under reduced pressure. The resulting oil was purified by flash chromatography on a silica gel column (Hex/EtOAc/AcOH = 2:1:0.3→3:2:0.5) to give **54** (414 mg, 43%) as a white foam. ¹H-NMR (400 MHz, CDCl₃) δ 7.76 (d, J = 7.6 Hz,

2H, H-Fmoc), 7.61 (d, $J = 7.2$ Hz, 2H, H-Fmoc), 7.40 (t, $J = 7.4$ Hz, 2H, H-Fmoc), 7.32 (t, $J = 7.4$ Hz, 2H, H-Fmoc), 6.05 (d, $J = 7.2$ Hz, 1H, NH), 5.48 (s, 1H, H-1), 5.40 (dd, 3H, $J = 10.0, 2.7$ Hz, H-3'), 5.31-5.36 (m, 2H, H-4, H-4'), 5.17-5.23 (m, 2H, H-2', H-3), 4.92 (d, $J = 1.2$ Hz, 1H, H-1'), 4.64 (s, 1H, H- α), 4.27-4.45 (m, 5H, H-5', H-6, CH₂-Fmoc), 4.22 (t, 1H, $J = 7.0$ Hz, CH-Fmoc), 4.13-4.17 (m, 4H, H-2, H-5, H-6'), 3.24 (dd, 2H, $J = 66.0, 13.6$ Hz, CH₂- β), 2.14 (s, 3H, CH₃-Ac), 2.13 (s, 3H, CH₃-Ac), 2.12 (s, 3H, CH₃-Ac), 2.08 (s, 3H, CH₃-Ac), 2.04 (s, 3H, CH₃-Ac), 2.02 (s, 3H, CH₃-Ac), 2.01 (s, 3H, CH₃-Ac). ¹³C-NMR (100 MHz, CDCl₃) δ 171.9, 171.1, 170.3, 169.8, 169.7, 169.6, 169.4, 155.8, 143.74, 143.72, 141.3, 127.8, 127.1, 125.1, 120.0, 99.2, 83.6, 78.4, 77.2, 70.2, 69.62, 69.56, 69.1, 68.4, 67.3, 66.6, 66.2, 62.4, 62.1, 53.6, 47.1, 33.6, 20.9, 20.8, 20.67, 20.65, 20.63. IR (NaCl, film): 3350, 2956, 1749, 1370, 1228, 1045, 741 cm⁻¹. HRMS (ESI) Calcd. for C₄₄H₅₁NNaO₂₁S [M + Na]⁺ requires 984.2567, Found: 984.2556.

III. SYNTHESIS AND CHARACTERIZATION OF CBM GLYCOFORMS

General procedure for the synthesis of unglycosylated CBM variants. The crude peptide was prepared using the previously reported protocol¹⁸. 16 mg of the crude peptide was dissolved in 80 ml of folding buffer (0.2 M Tris-acetate, 0.33 mM oxidized glutathione, 2.6 mM reduced glutathione, pH 8.2) and stirred at room temperature for 12 h under a helium atmosphere. The solution was then concentrated to a small volume (around 6 ml) using 3 kDa cut-off centrifugal filter units (Amicon) before RP-HPLC purification. The RP-HPLC purification was performed on a Versagrad Preparation-HPLC system using a semi-preparative C18 column. The products were detected by UV absorption at 275 nm. After HPLC purification with a linear gradient of 20→40% MeCN in H₂O over 30 min, the fractions were collected and checked by ESI+ MS. The pure fractions were combined and lyophilized to give the desired product as a white solid.

General procedure for the synthesis of glycosylated CBM variants. The crude glycopeptide was prepared using the previously reported protocol¹⁸. 16 mg of the crude peptide was dissolved in 1 ml of hydrazine solution (hydrazine/H₂O, 5/100, v/v) and stirred at room temperature for 30 min under helium. The reaction was quenched with 2 ml of acetic acid solution (AcOH/H₂O, 5/100, v/v). The resulting mixture was diluted to 80 mL with folding buffer (0.2 M Tris-acetate, 0.33 mM oxidized glutathione, 2.6 mM reduced glutathione, pH 8.2, 80 ml) and stirred at room temperature for 12 h under a helium atmosphere. The solution was then concentrated to a small volume (around 6 ml) using 3 kDa cut-off centrifugal filter units (Amicon) before RP-HPLC purification. The RP-HPLC purification was performed on a Versagrad Preparation-HPLC system using a semi-preparative C18 column. The products were detected by UV absorption at 275 nm. After HPLC purification with a linear gradient of 20→40% MeCN in H₂O over 30 min, the fractions were collected and checked by ESI+ MS. The pure fractions were combined and lyophilized to afford the desired product as a white solid.

LC-MS analysis of purified CBM variants. LC-MS was performed under two flow rates with C4 column: (1) 0.5 ml/min with a linear gradient of 15% to 35% acetonitrile in water over 3 min and (2) 0.3 ml/min with a linear gradient of 15% to 35% acetonitrile in water over 5 min.

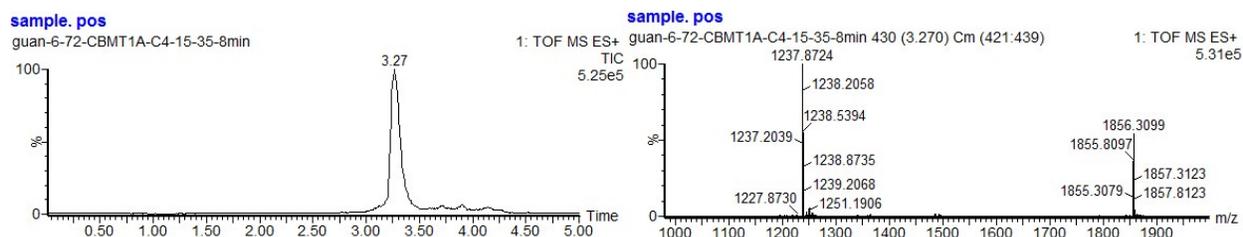


Figure S2. LC-MS trace and ESI-MS of purified CBM **4** (yield: 28%). LC-MS condition: 0.3 mL/min, 15%-35% MeCN in H₂O over 5 min. MS (ESI) Calcd for **4** C₁₅₈H₂₃₃N₄₃O₅₃S₄ [M+2H]²⁺ m/z = 1855.2949, [M+3H]³⁺ m/z = 1237.1992.

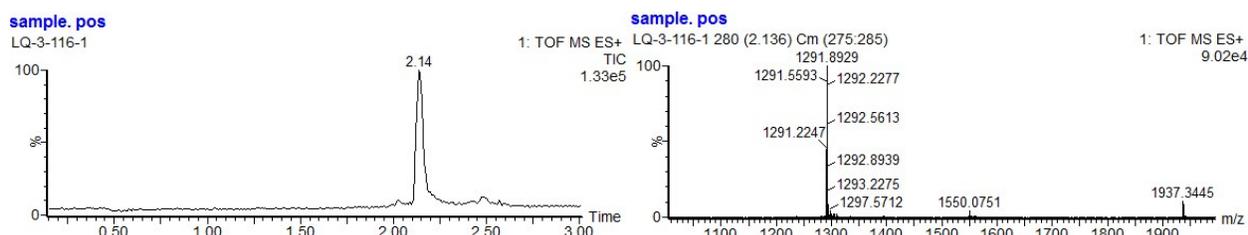


Figure S3. LC-MS trace and ESI-MS of purified CBM **5** (yield: 18%). LC-MS condition: 0.5 mL/min, 15%-35% MeCN in H₂O over 3 min. MS (ESI) Calcd for **5** C₁₆₄H₂₄₃N₄₃O₅₈S₄ [M+2H]²⁺ m/z = 1936.3135, [M+3H]³⁺ m/z = 1291.2090.

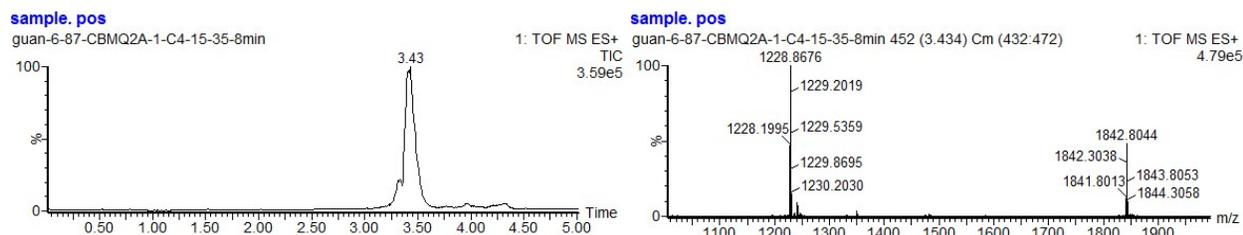


Figure S4. LC-MS trace and ESI-MS of purified CBM **6** (yield: 30%). LC-MS condition: 0.3 mL/min, 15%-35% MeCN in H₂O over 5 min. MS (ESI) Calcd for **6** C₁₅₇H₂₃₂N₄₂O₅₃S₄ [M+2H]²⁺ m/z = 1841.7894, [M+3H]³⁺ m/z = 1228.1955.

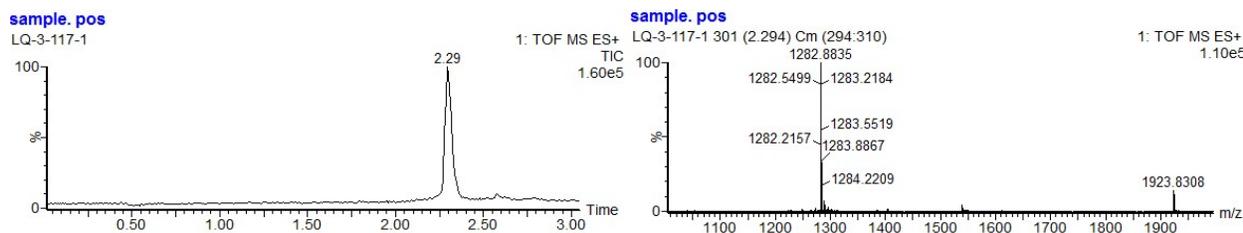


Figure S5. LC-MS trace and ESI-MS of purified CBM **7** (yield: 17%). LC-MS condition: 0.5 mL/min, 15%-35% MeCN in H₂O over 3 min. MS (ESI) Calcd for **7** C₁₆₃H₂₄₂N₄₂O₅₈S₄ [M+2H]²⁺ m/z = 1922.8081, [M+3H]³⁺ m/z = 1282.2054.

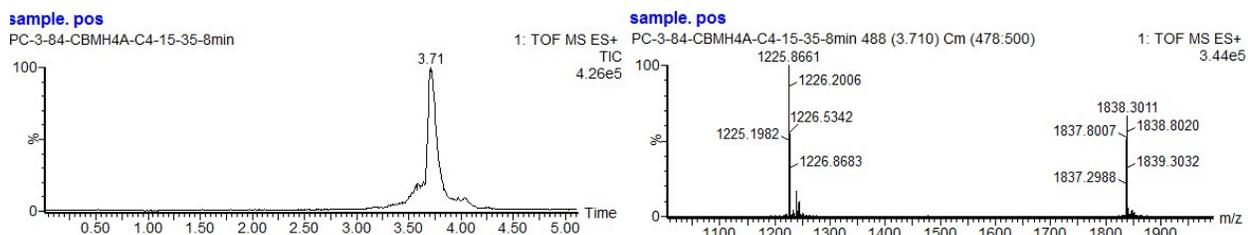


Figure S6. LC-MS trace and ESI-MS of purified CBM **8** (yield: 15%). LC-MS condition: 0.3 ml/min, 15%-35% MeCN in H₂O over 5 min. MS (ESI) Calcd for **8** C₁₅₆H₂₃₃N₄₁O₅₄S₄ [M+2H]²⁺ m/z = 1837.2893, [M+3H]³⁺ m/z = 1225.1954.

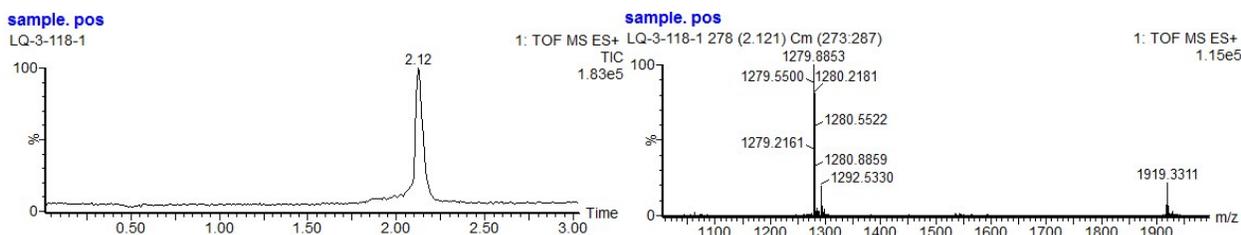


Figure S7. LC-MS trace and ESI-MS of purified CBM **9** (yield: 13%). LC-MS condition: 0.5 ml/min, 15%-35% MeCN in H₂O over 3 min. MS (ESI) Calcd for **9** C₁₆₂H₂₄₃N₄₁O₅₉S₄ [M+2H]²⁺ m/z = 1918.3079, [M+3H]³⁺ m/z = 1279.2053.

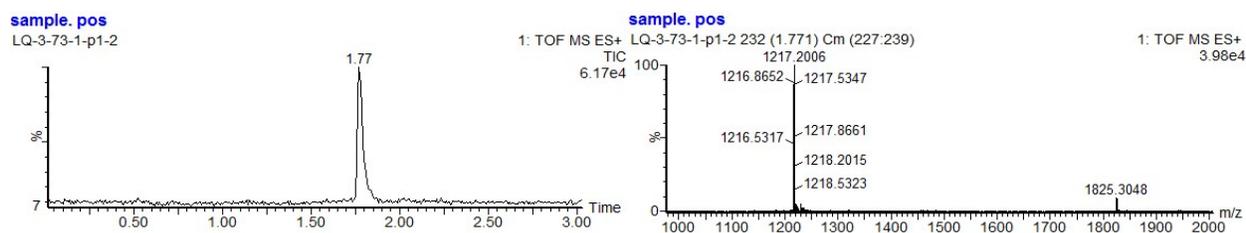


Figure S8. LC-MS trace and ESI-MS of purified CBM **10** (yield: 12%). LC-MS condition: 0.5 ml/min, 15%-35% MeCN in H₂O over 3 min. MS (ESI) Calcd for **10** C₁₅₃H₂₃₁N₄₃O₅₃S₄ [M+2H]²⁺ m/z = 1824.2793, [M+3H]³⁺ m/z = 1216.5195.

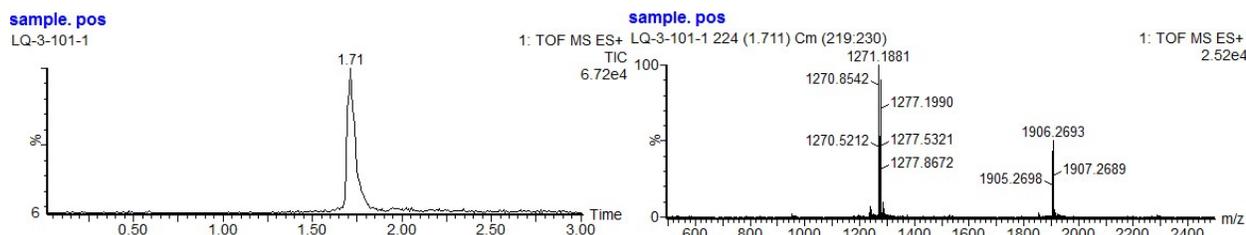


Figure S9. LC-MS trace and ESI-MS of purified CBM **11** (yield: 13%). LC-MS condition: 0.5 ml/min, 15%-35% MeCN in H₂O over 3 min. MS (ESI) Calcd for **11** C₁₅₉H₂₄₁N₄₃O₅₈S₄ [M+2H]²⁺ m/z = 1905.3057, [M+3H]³⁺ m/z = 1270.5371.

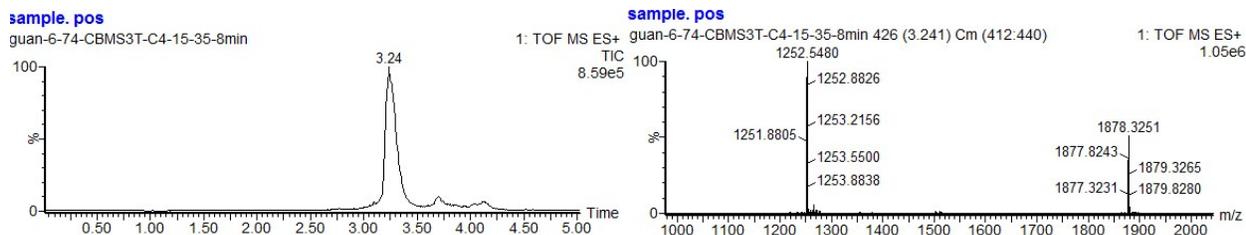


Figure S10. LC-MS trace and ESI-MS of purified CBM **12** (yield: 23%). LC-MS condition: 0.3 ml/min, 15%-35% MeCN in H₂O over 5 min. MS (ESI) Calcd for **12** C₁₆₀H₂₃₇N₄₃O₅₄S₄ [M+2H]²⁺ m/z = 1877.3080, [M+3H]³⁺ m/z = 1251.8746.

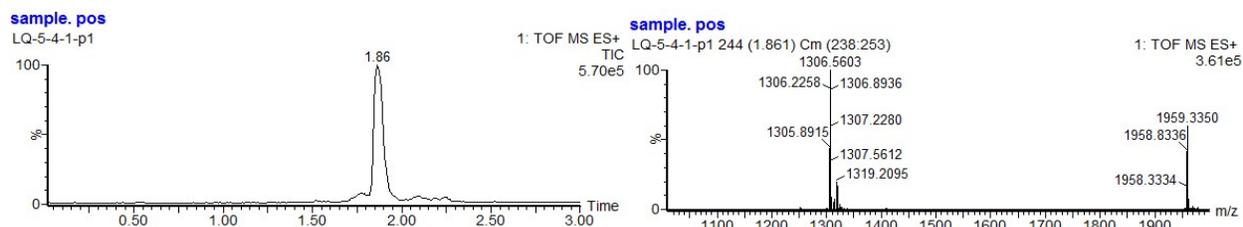


Figure S11. LC-MS trace and ESI-MS of purified CBM **13** (yield: 16%). LC-MS condition: 0.5 ml/min, 15%-35% MeCN in H₂O over 3 min. MS (ESI) Calcd for **13** C₁₆₆H₂₄₇N₄₃O₅₉S₄ [M+2H]²⁺ m/z = 1958.3266, [M+3H]³⁺ m/z = 1305.8844.

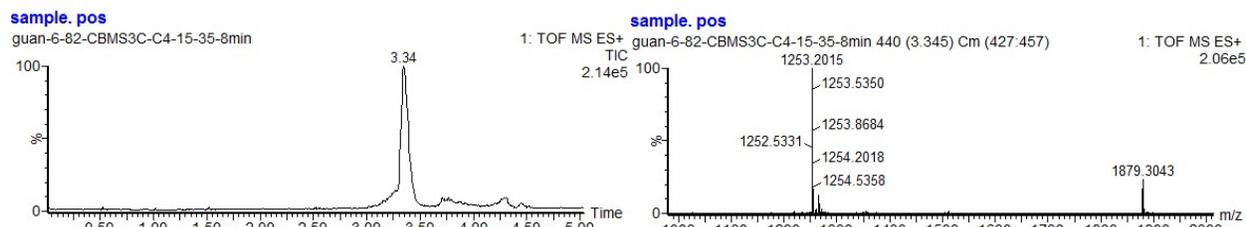


Figure S12. LC-MS trace and ESI-MS of purified CBM **14** (yield: 13%). LC-MS condition: 0.3 ml/min, 15%-35% MeCN in H₂O over 5 min. MS (ESI) Calcd for **14** C₁₅₉H₂₃₅N₄₃O₅₃S₅ [M+2H]²⁺ m/z = 1878.2887, [M+3H]³⁺ m/z = 1252.5284.

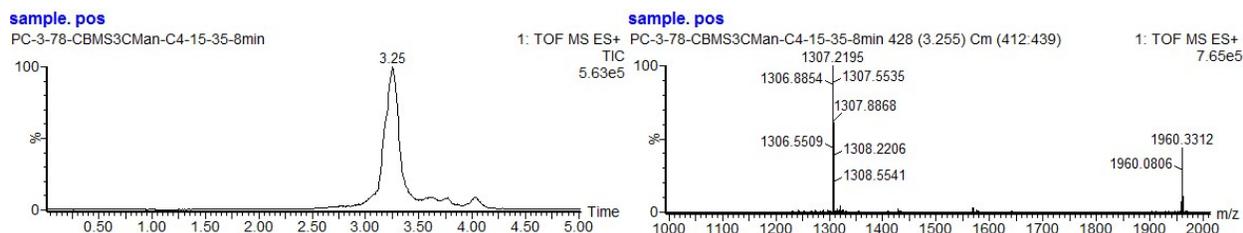


Figure S13. LC-MS trace and ESI-MS of purified CBM **15** (yield: 16%). LC-MS condition: 0.3 ml/min, 15%-35% MeCN in H₂O over 5 min. MS (ESI) Calcd for **15** C₁₆₅H₂₄₅N₄₃O₅₈S₅ [M+2H]²⁺ m/z = 1959.3151, [M+3H]³⁺ m/z = 1306.5460.

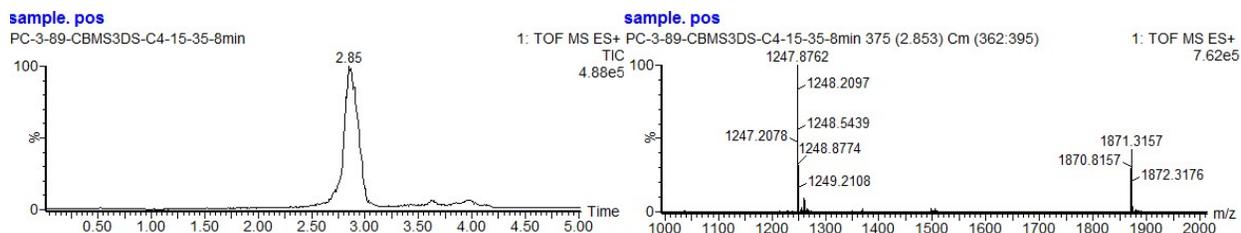


Figure 14. LC-MS trace and ESI-MS of purified CBM **16** (yield: 22%). LC-MS condition: 0.3 ml/min, 15%-35% MeCN in H₂O over 5 min. MS (ESI) Calcd for **16** C₁₅₉H₂₃₅N₄₃O₅₄S₄ [M+2H]²⁺ m/z = 1870.3002, [M+3H]³⁺ m/z = 1247.2027.

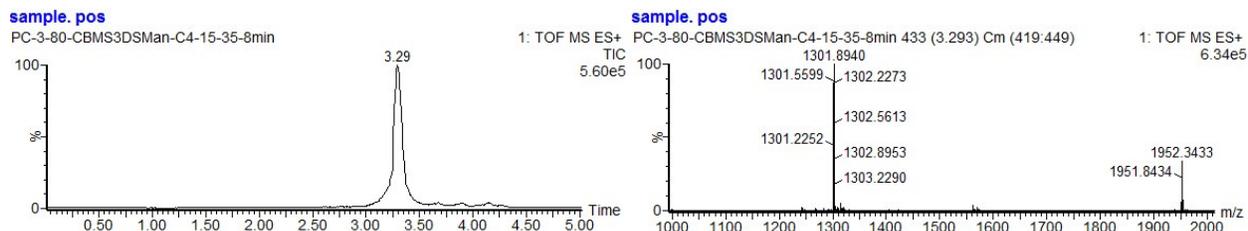


Figure S15. LC-MS trace and ESI-MS of purified CBM **17** (yield: 8%). LC-MS condition: 0.3 mL/min, 15%-35% MeCN in H₂O over 5 min. MS (ESI) Calcd for **17** C₁₆₅H₂₄₅N₄₃O₅₉S₄ [M+2H]²⁺ m/z = 1951.3188 [M+3H]³⁺ m/z = 1301.2125.

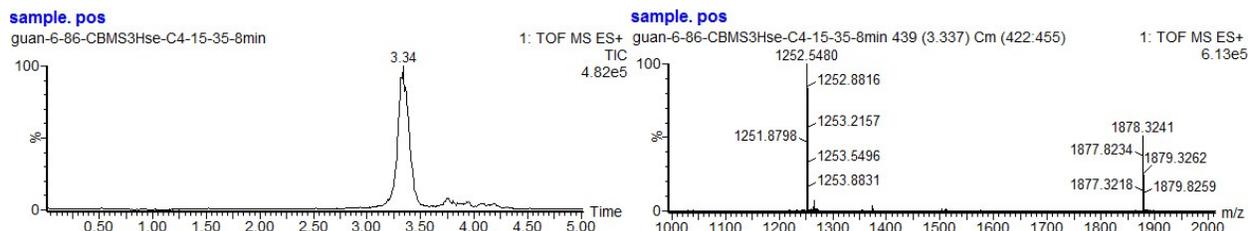


Figure S16. LC-MS trace and ESI-MS of purified CBM **18** (yield: 27%). LC-MS condition: 0.3 ml/min, 15%-35% MeCN in H₂O over 5 min. MS (ESI) Calcd for **18** C₁₆₀H₂₃₇N₄₃O₅₄S₄ [M+2H]²⁺ m/z = 1877.3080, [M+3H]³⁺ m/z = 1251.8746.

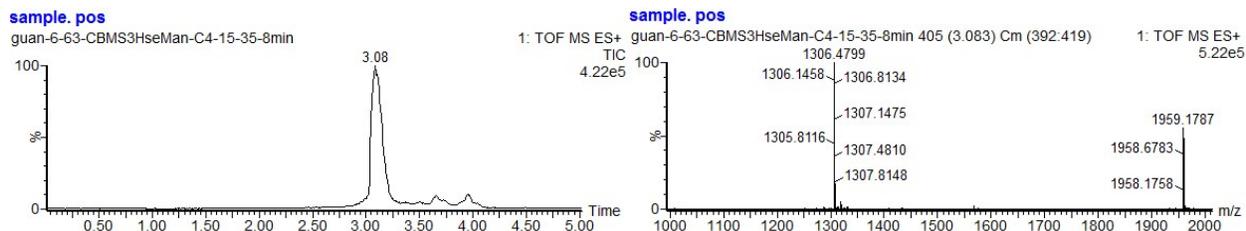


Figure S17. LC-MS trace and ESI-MS of purified CBM **19** (yield: 28%). LC-MS condition: 0.3 ml/min, 15%-35% MeCN in H₂O over 5 min. MS (ESI) Calcd for **19** C₁₆₆H₂₄₇N₄₃O₅₉S₄ [M+2H]²⁺ m/z = 1958.3344, [M+3H]³⁺ m/z = 1305.8922.

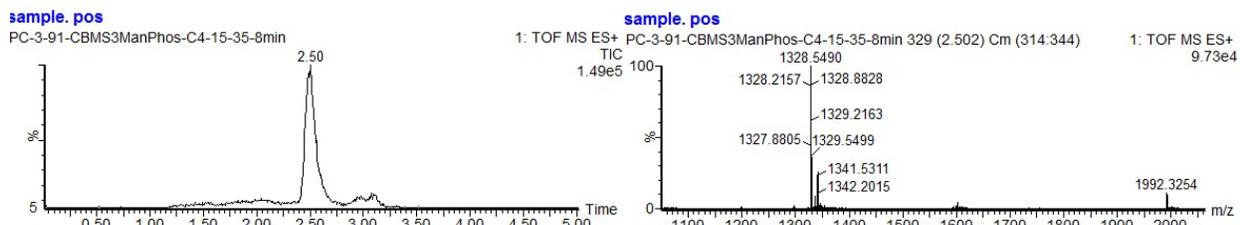


Figure S18. LC-MS trace and ESI-MS of purified CBM **20** (yield: 6%). LC-MS condition: 0.3 ml/min, 15%-35% MeCN in H₂O over 5 min. MS (ESI) Calcd for **20** C₁₆₅H₂₄₆N₄₃O₆₂PS₄ [M+2H]²⁺ m/z = 1991.3097, [M+3H]³⁺ m/z = 1327.8758.

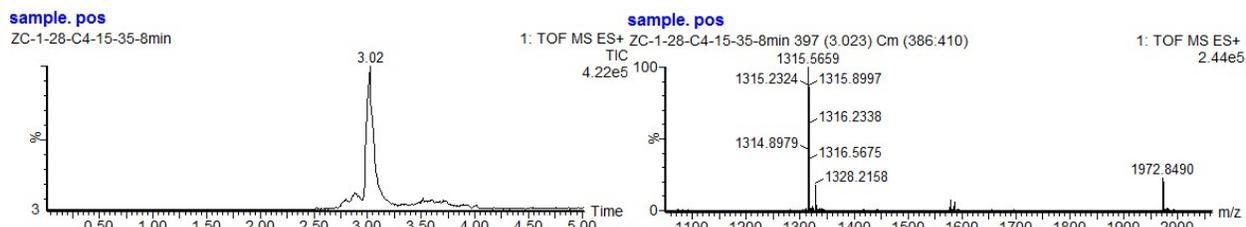


Figure S19. LC-MS trace and ESI-MS of purified CBM **21** (yield: 16%). LC-MS condition: 0.3 ml/min, 15%-35% MeCN in H₂O over 5 min. MS (ESI) Calcd for **21** C₁₆₇H₂₄₈N₄₄O₅₉S₄ [M+2H]²⁺ m/z = 1971.8321, [M+3H]³⁺ m/z = 1314.8880.

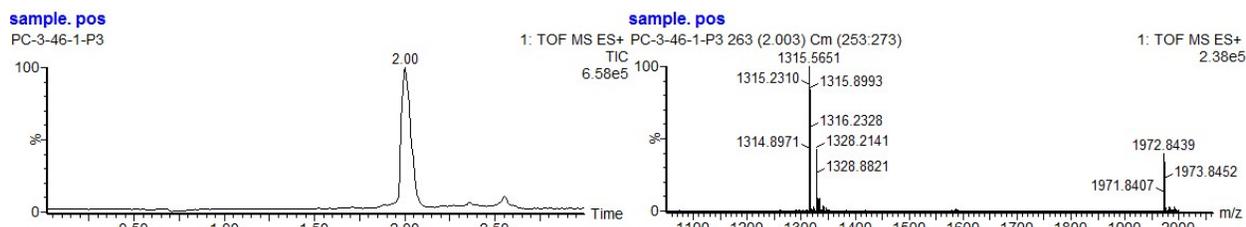


Figure S20. LC-MS trace and ESI-MS of purified CBM **22** (yield: 18%). LC-MS condition: 0.3 ml/min, 15%-35% MeCN in H₂O over 5 min. MS (ESI) Calcd for **22** C₁₆₇H₂₄₈N₄₄O₅₉S₄ [M+2H]²⁺ m/z = 1971.8321, [M+3H]³⁺ m/z = 1314.8880.

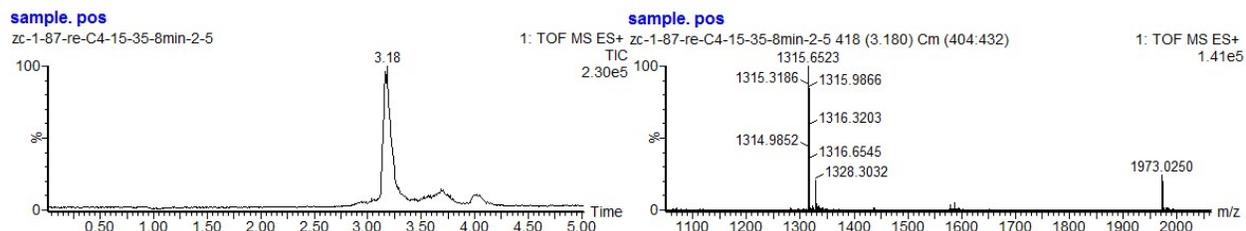


Figure S21. LC-MS trace and ESI-MS of purified CBM **23** (yield: 7%). LC-MS condition: 0.3 ml/min, 15%-35% MeCN in H₂O over 5 min. MS (ESI) Calcd for **23** C₁₆₇H₂₄₈N₄₄O₅₉S₄ [M+2H]²⁺ m/z = 1971.8321, [M+3H]³⁺ m/z = 1314.8880.

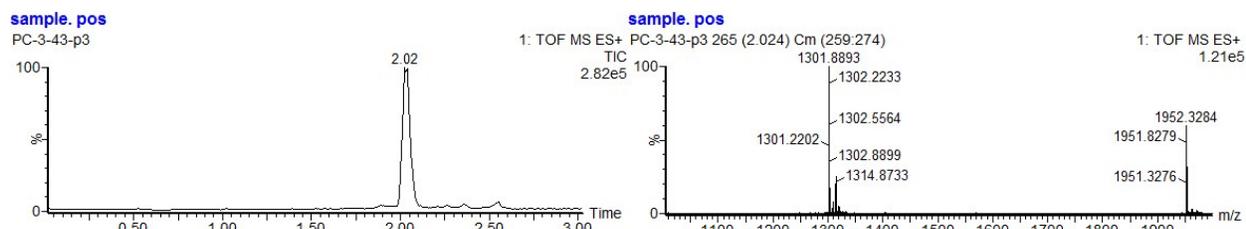


Figure S22. LC-MS trace and ESI-MS of purified CBM **24** (yield: 15%). LC-MS condition: 0.5 ml/min, 15%-35% MeCN in H₂O over 3 min. MS (ESI) Calcd for **24** C₁₆₅H₂₄₅N₄₃O₅₉S₄ [M+2H]²⁺ m/z = 1951.3188, [M+3H]³⁺ m/z = 1301.2125.

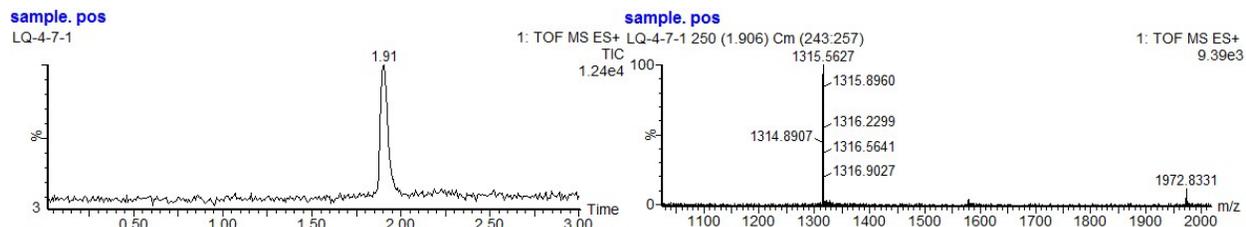


Figure S23. LC-MS trace and ESI-MS of purified CBM **25** (yield: 20%). LC-MS condition: 0.5 ml/min, 15%-35% MeCN in H₂O over 3 min. MS (ESI) Calcd for **25** C₁₆₇H₂₄₈N₄₄O₅₉S₄ [M+2H]²⁺ m/z = 1971.8321, [M+3H]³⁺ m/z = 1314.8880.

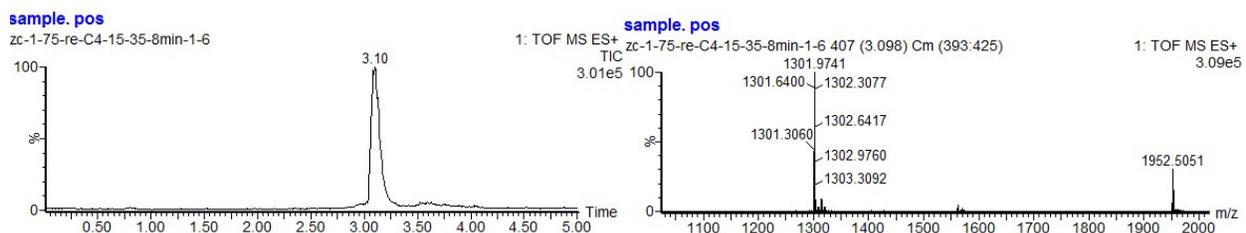


Figure S24. LC-MS trace and ESI-MS of purified CBM **26** (yield: 13%). LC-MS condition: 0.3 ml/min, 15%-35% MeCN in H₂O over 5 min. MS (ESI) Calcd for **26** C₁₆₅H₂₄₅N₄₃O₅₉S₄ [M+2H]²⁺ m/z = 1951.3188, [M+3H]³⁺ m/z = 1301.2125.

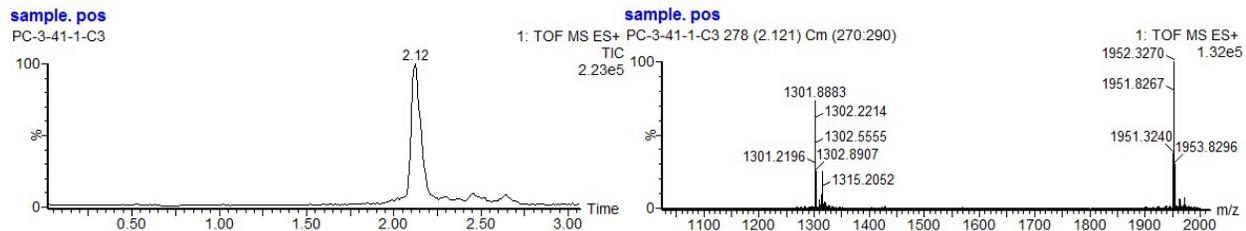


Figure S25. LC-MS trace and ESI-MS of purified CBM **27** (yield: 24%). LC-MS condition: 0.5 ml/min, 15%-35% MeCN in H₂O over 3 min. MS (ESI) Calcd for **27** C₁₆₅H₂₄₅N₄₃O₅₉S₄ [M+2H]²⁺ m/z = 1951.3188, [M+3H]³⁺ m/z = 1301.2125.

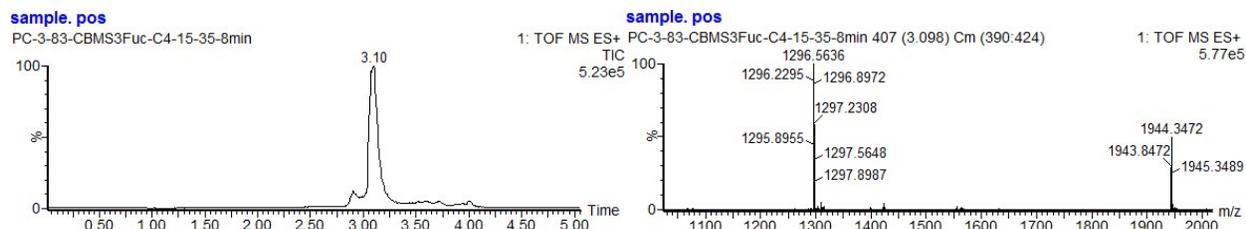


Figure S26. LC-MS trace and ESI-MS of purified CBM **28** (yield: 26%). LC-MS condition: 0.3 ml/min, 15%-35% MeCN in H₂O over 5 min. MS (ESI) Calcd for **28** C₁₆₅H₂₄₅N₄₃O₅₈S₄ [M+2H]²⁺ m/z = 1943.3213, [M+3H]³⁺ m/z = 1295.8809.

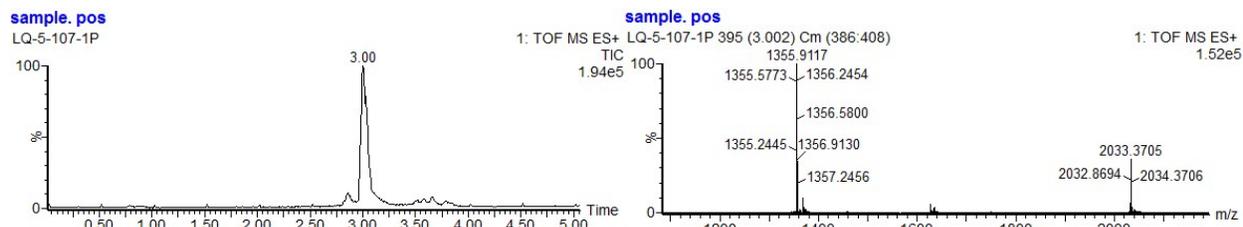


Figure S27. LC-MS trace and ESI-MS of purified CBM **29** (yield: 22%). LC-MS condition: 0.3 ml/min, 15%-35% MeCN in H₂O over 5 min. MS (ESI) Calcd for **29** C₁₇₁H₂₅₅N₄₃O₆₄S₄ [M+2H]²⁺ m/z = 2032.3530, [M+3H]³⁺ m/z = 1355.2379.

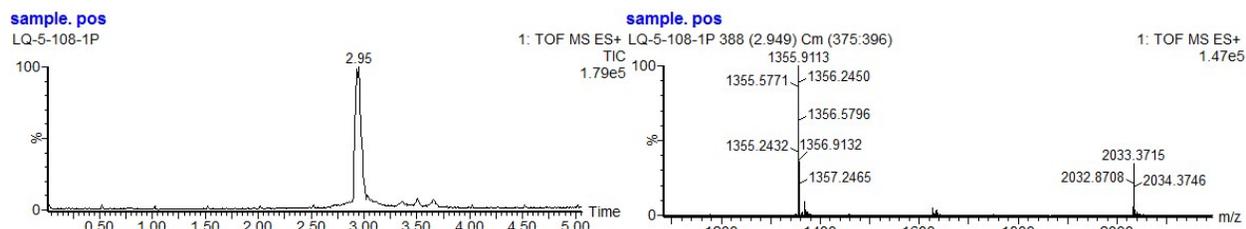


Figure S28. LC-MS trace and ESI-MS of purified CBM **30** (yield: 19%). LC-MS condition: 0.3 ml/min, 15%-35% MeCN in H₂O over 5 min. MS (ESI) Calcd for **30** C₁₇₁H₂₅₅N₄₃O₆₄S₄ [M+2H]²⁺ m/z = 2032.3530, [M+3H]³⁺ m/z = 1355.2379.

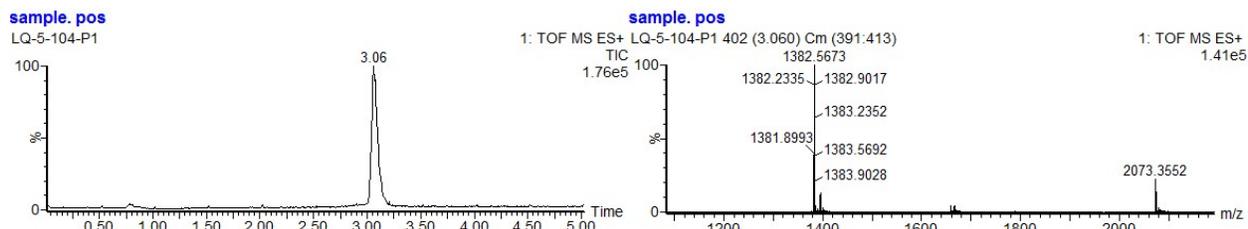


Figure S29. LC-MS trace and ESI-MS of purified CBM **31** (yield: 11%). LC-MS condition: 0.3 ml/min, 15%-35% MeCN in H₂O over 5 min. MS (ESI) Calcd for **31** C₁₇₁H₂₅₆N₄₃O₆₇PS₄ [M+2H]²⁺ m/z = 2072.3284, [M+3H]³⁺ m/z = 1381.8856.

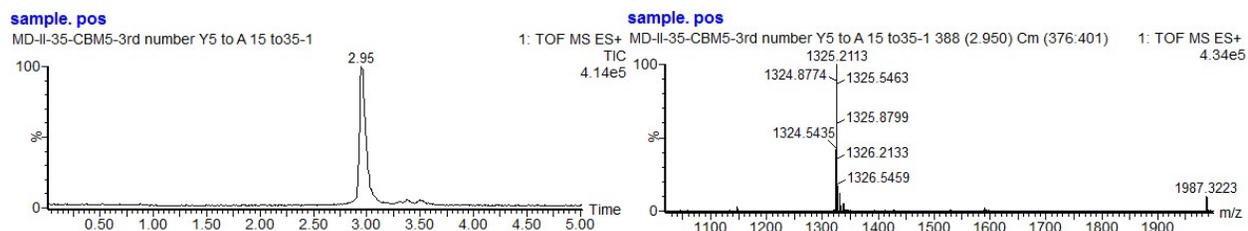


Figure S30. LC-MS trace and ESI-MS of purified CBM **32** (yield: 6%). LC-MS condition: 0.3 ml/min, 15%-35% MeCN in H₂O over 5 min. MS (ESI) Calcd for **32** C₁₆₅H₂₅₁N₄₃O₆₃S₄ [M+2H]²⁺ m/z = 1986.3321, [M+3H]³⁺ m/z = 1324.5547.

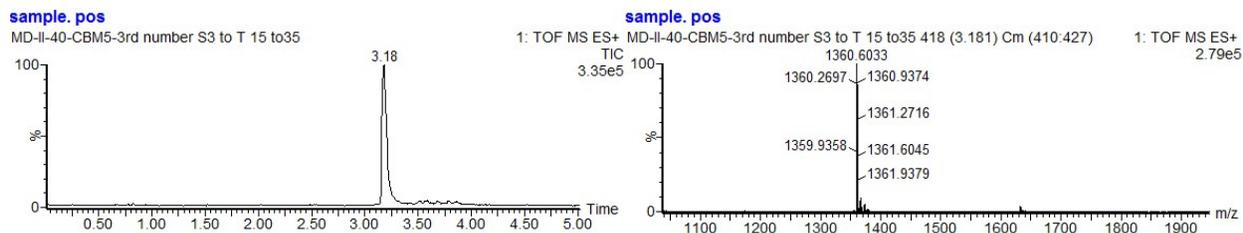


Figure S31. LC-MS trace and ESI-MS of purified CBM **33** (yield: 7%). LC-MS condition: 0.3 ml/min, 15%-35% MeCN in H₂O over 5 min. MS (ESI) Calcd for **33** C₁₇₂H₂₅₇N₄₃O₆₄S₄ [M+2H]²⁺ m/z = 2039.3530, [M+3H]³⁺ m/z = 1359.9020.

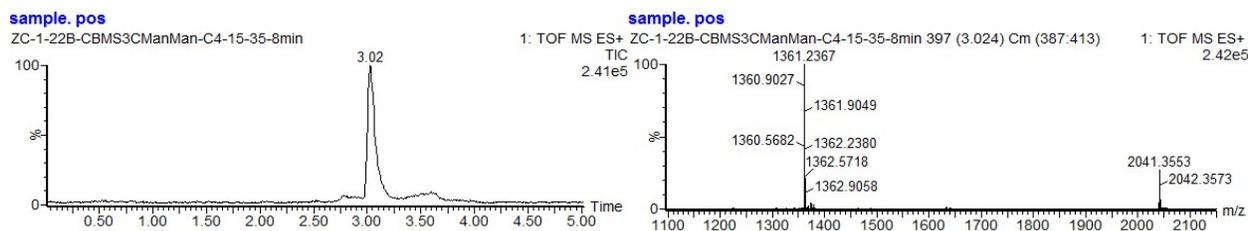


Figure S32. LC-MS trace and ESI-MS of purified CBM **34** (yield: 14%). LC-MS condition: 0.3 ml/min, 15%-35% MeCN in H₂O over 5 min. MS (ESI) Calcd for **34** C₁₇₁H₂₅₅N₄₃O₆₃S₅ [M+2H]²⁺ m/z = 2040.3338, [M+3H]³⁺ m/z = 1360.5558.

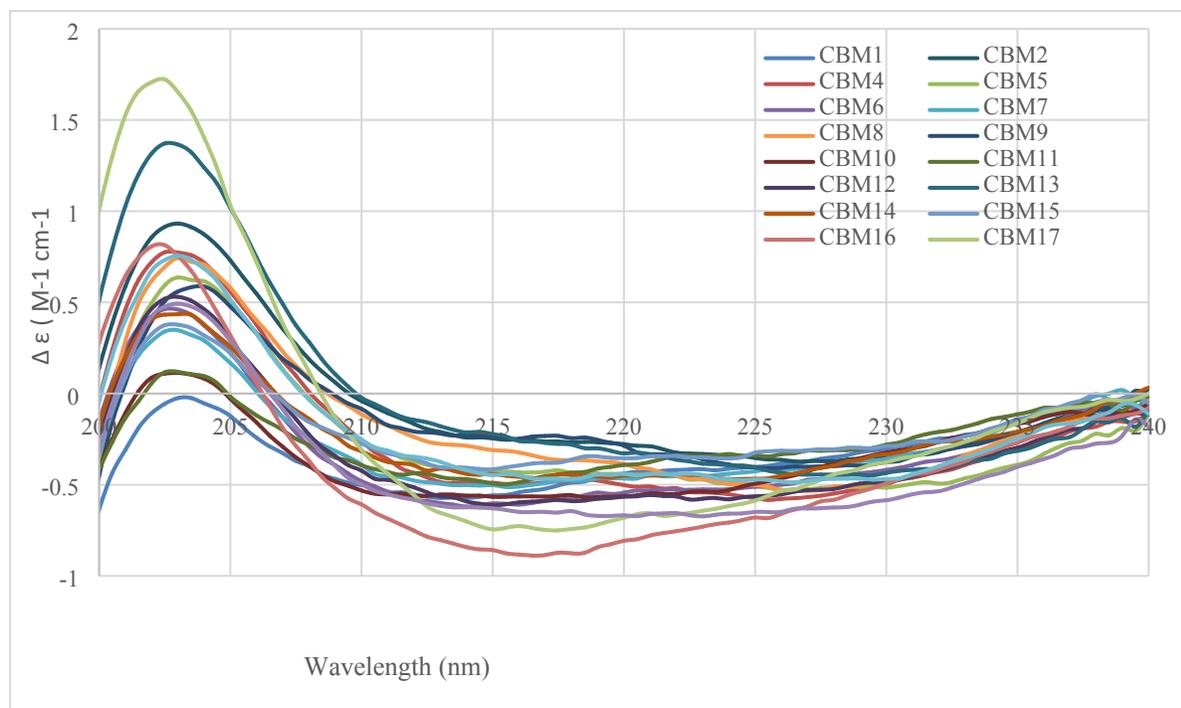


Figure S33. The CD spectra of CBM variants **1**, **2**, and **4-19**. The spectra were acquired in the same way as previously reported¹⁸.

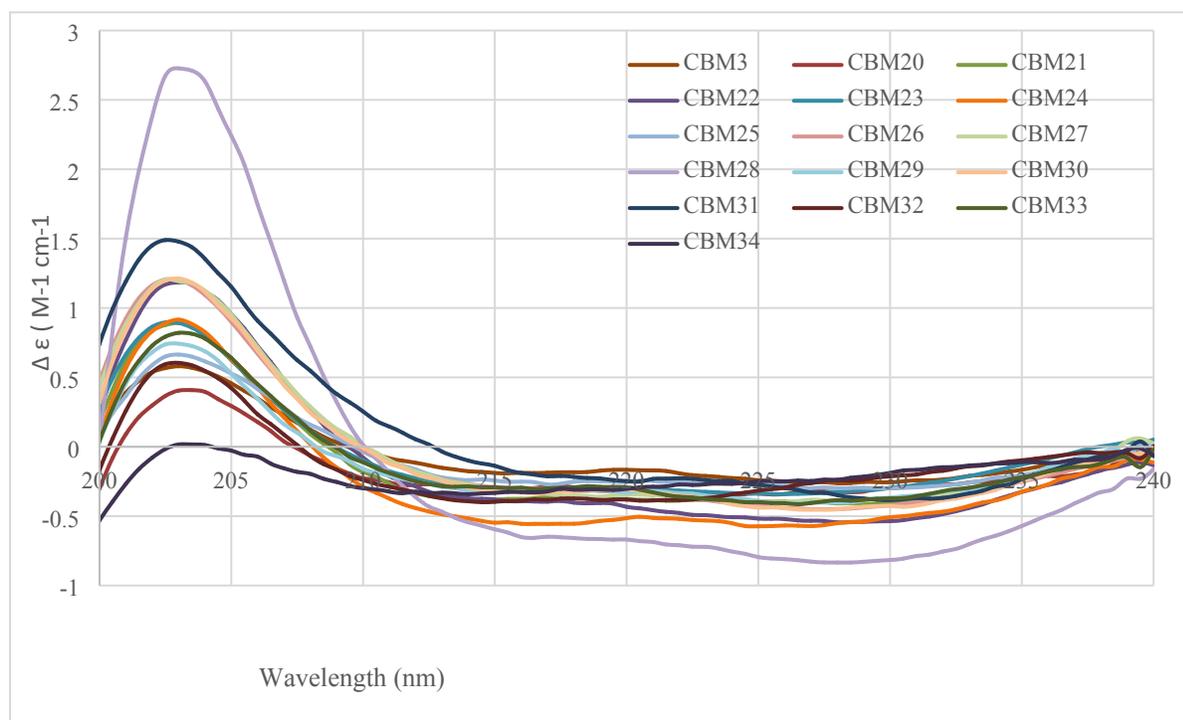
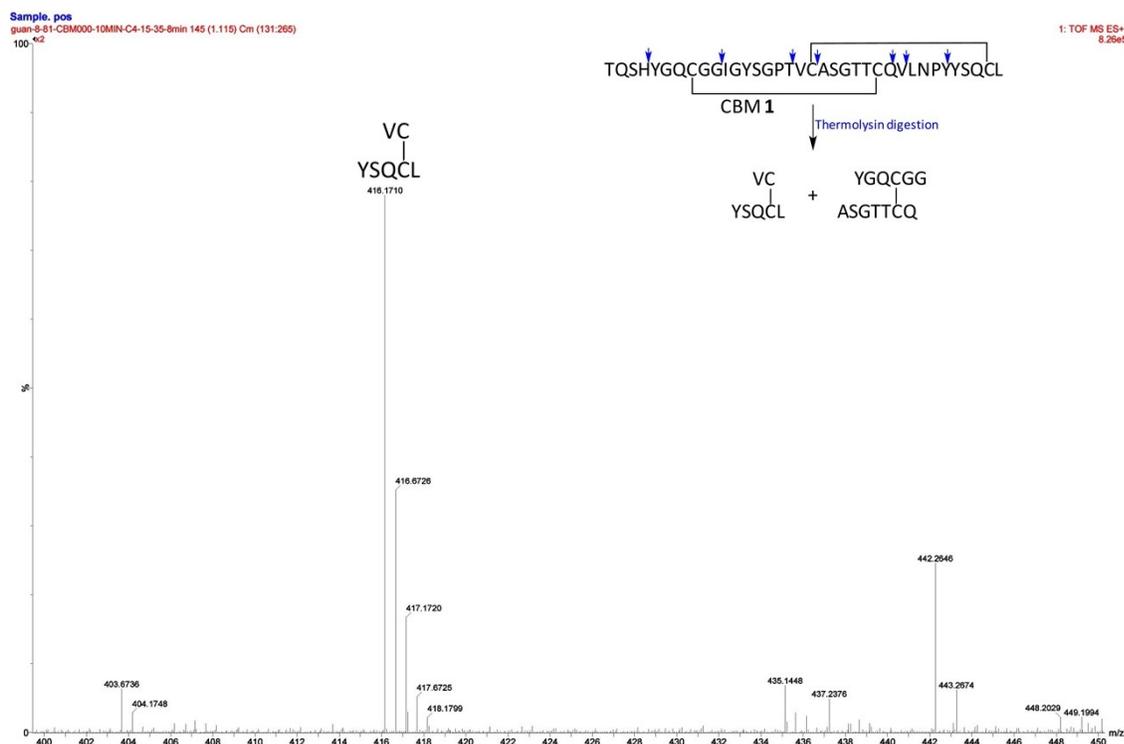


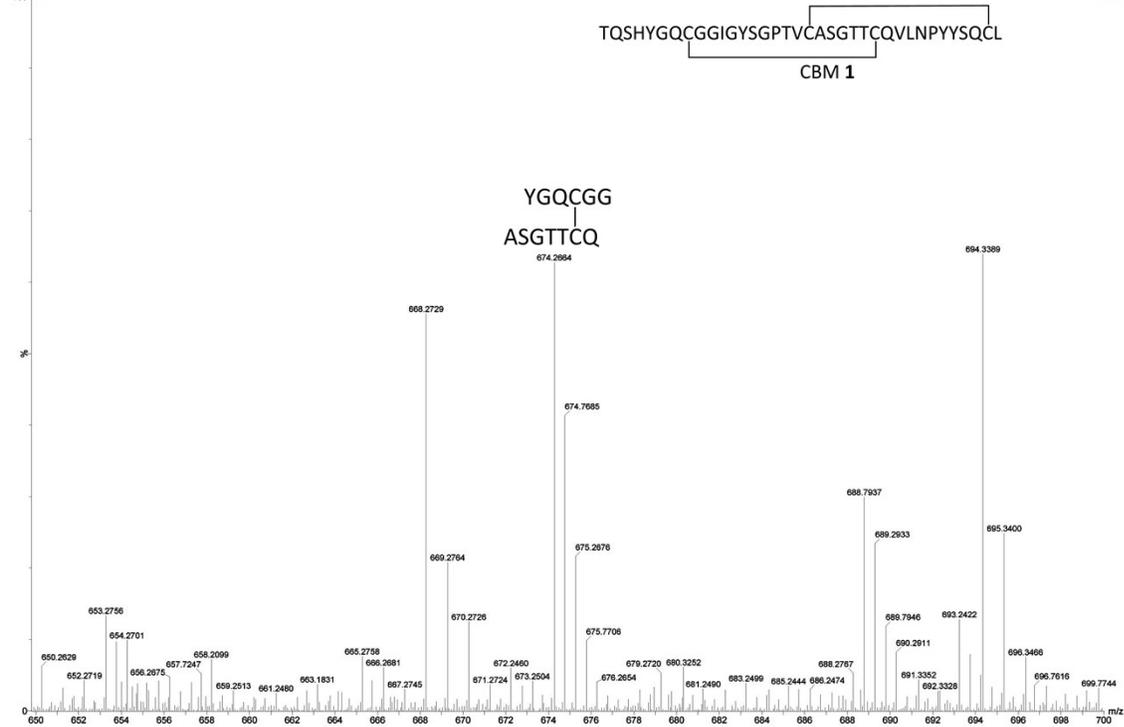
Figure S34. The CD spectra of CBM variants **3** and **20-34**. The spectra were acquired in the same way as previously reported¹⁸.

Confirming disulfide linkages. The folding of the CBMs was confirmed as described in our previous report.¹⁸ After HPLC purification, the UPLC-MS trace of the folded CBMs showed a single peak, which indicated the homogeneity of the product. The observed mass loss of 4 Da is consistent with the formation of two disulfide bridges. The far-ultraviolet CD spectra of the CBMs were very similar to previously obtained spectrum of the unglycosylated CBM, which suggested that the synthetic peptide adopted the appropriate secondary structure upon folding. Moreover, the UPLC-MS analysis revealed that the thermolysin digestion of CBMs produces two fragments that contain two short peptide chains, VC/YSQCL and YGQCGG/ASGTTCQV (or AGQCGG/ASGTTCQV for CBM 11). These short peptide chains are connected by disulfide linkages, which clearly confirmed the correct disulfide connectivity (Fig. S35).



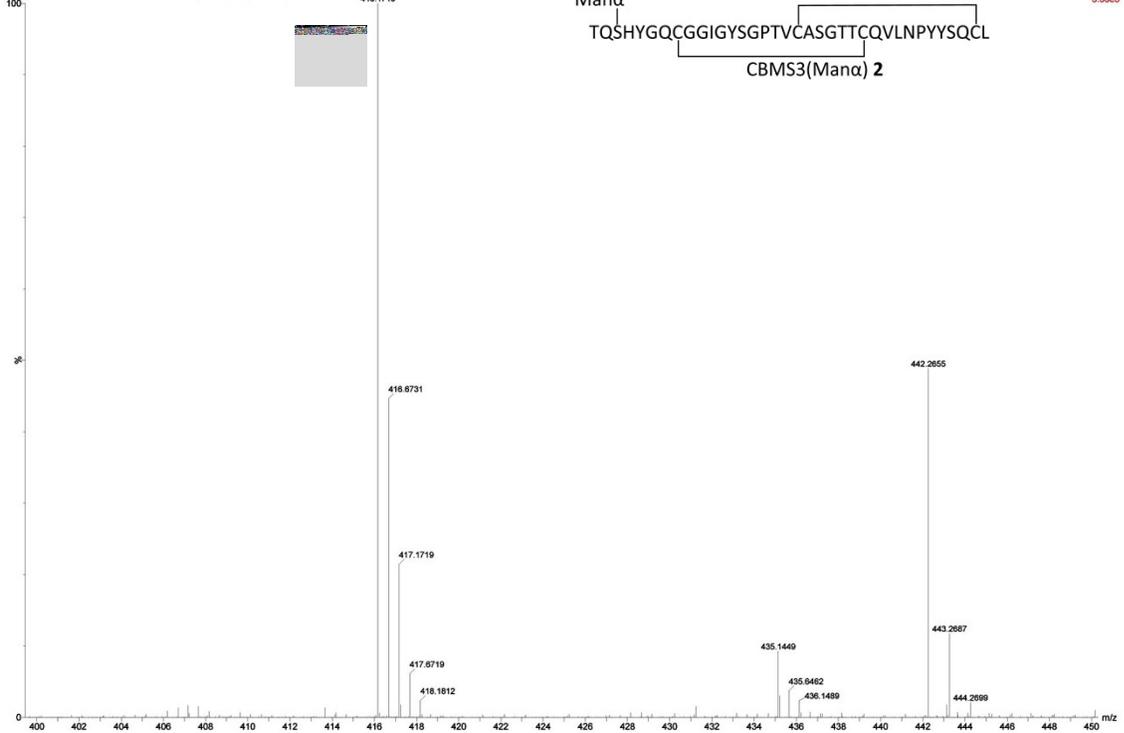
Sample_pos
guan-8-81-CBM000-10MIN-C4-15-35-8min 145 (1.115) Cm (134:161)
100

1: TOF MS ES+
3.81e5



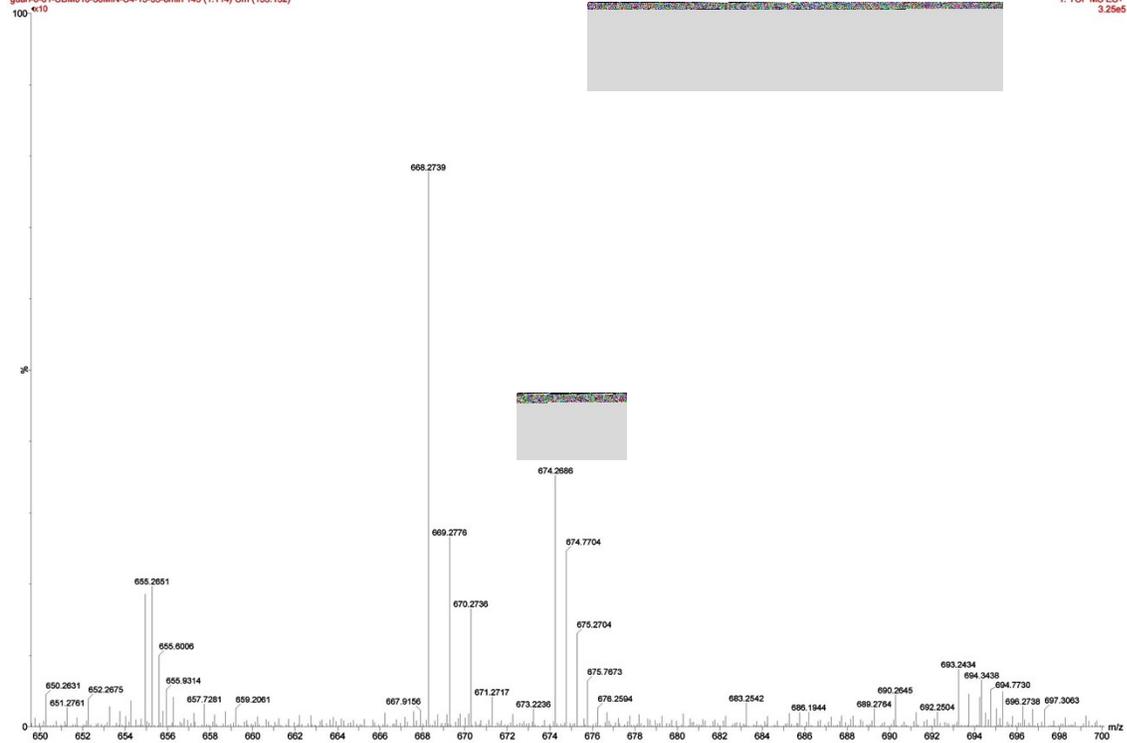
Sample_pos
guan-8-81-CBM10-30MIN-C4-15-35-8min 145 (1.114) Cm (133:150)

1: TOF MS ES+
3.33e5



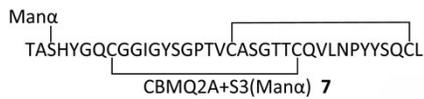
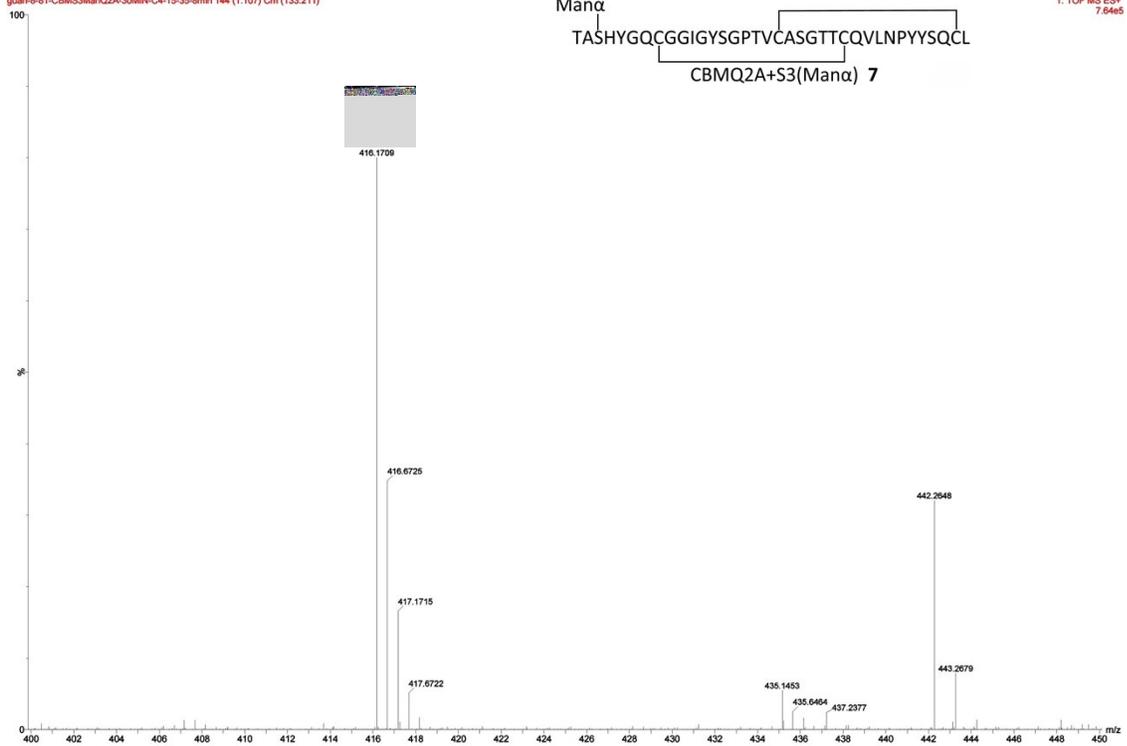
Sample_pos
guan-8-81-CBM010-30MIN-C4-15-35-8min 145 (1.114) Cm (135.152)

1: TOF MS ES+
3.25e5



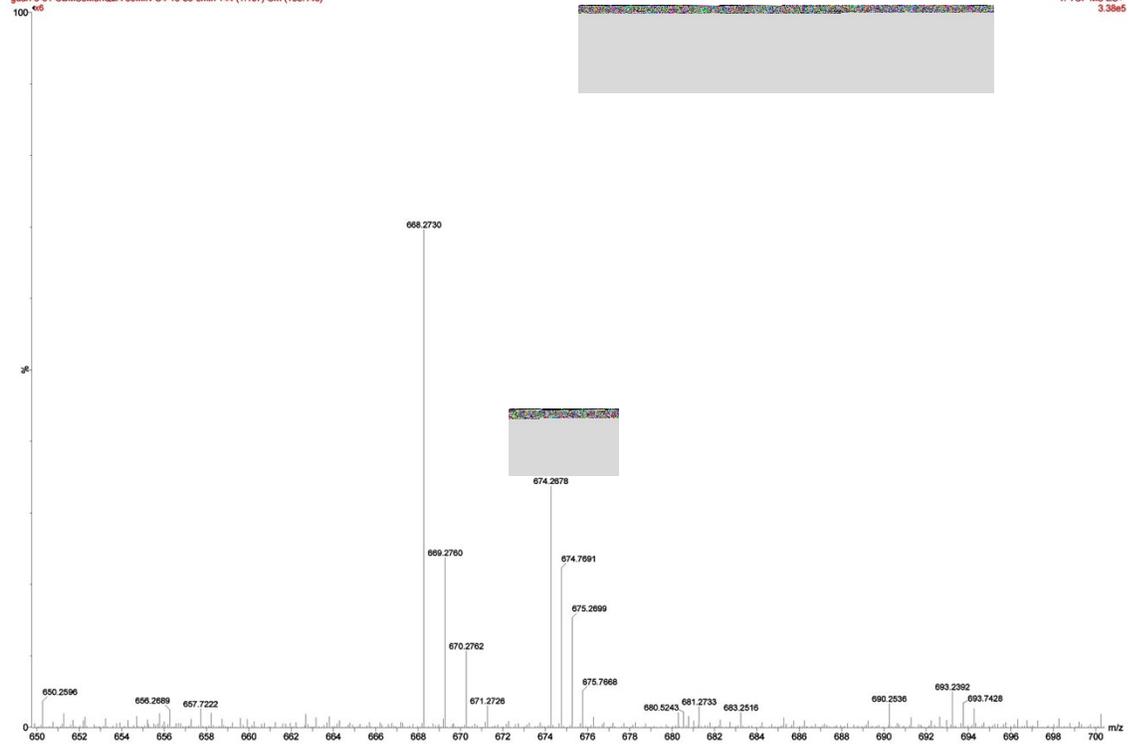
Sample_pos
guan-8-81-CBM3MarQ2A-30MIN-C4-15-35-8min 144 (1.107) Cm (133.211)

1: TOF MS ES+
7.54e5



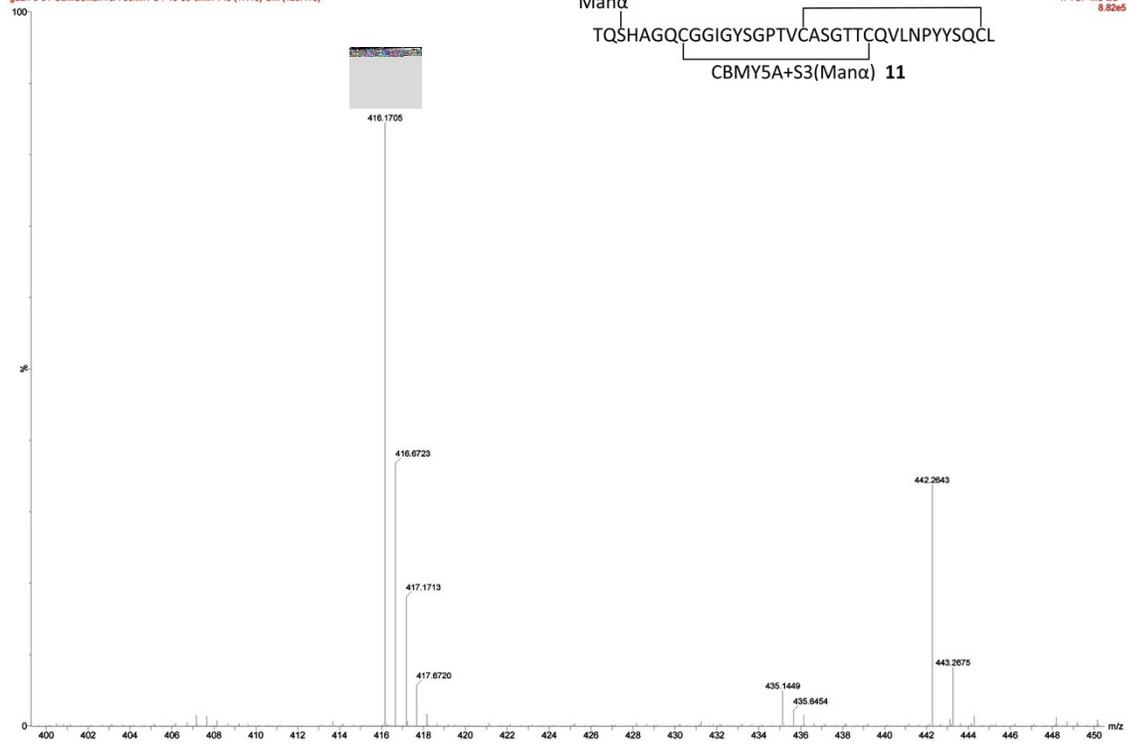
Sample_pos
guan-8-81-CBMS3ManQ2A-30MIN-C4-15-35-8min 144 (1.107) Cm (133:146)

1: TOF MS ES+
3.38e5



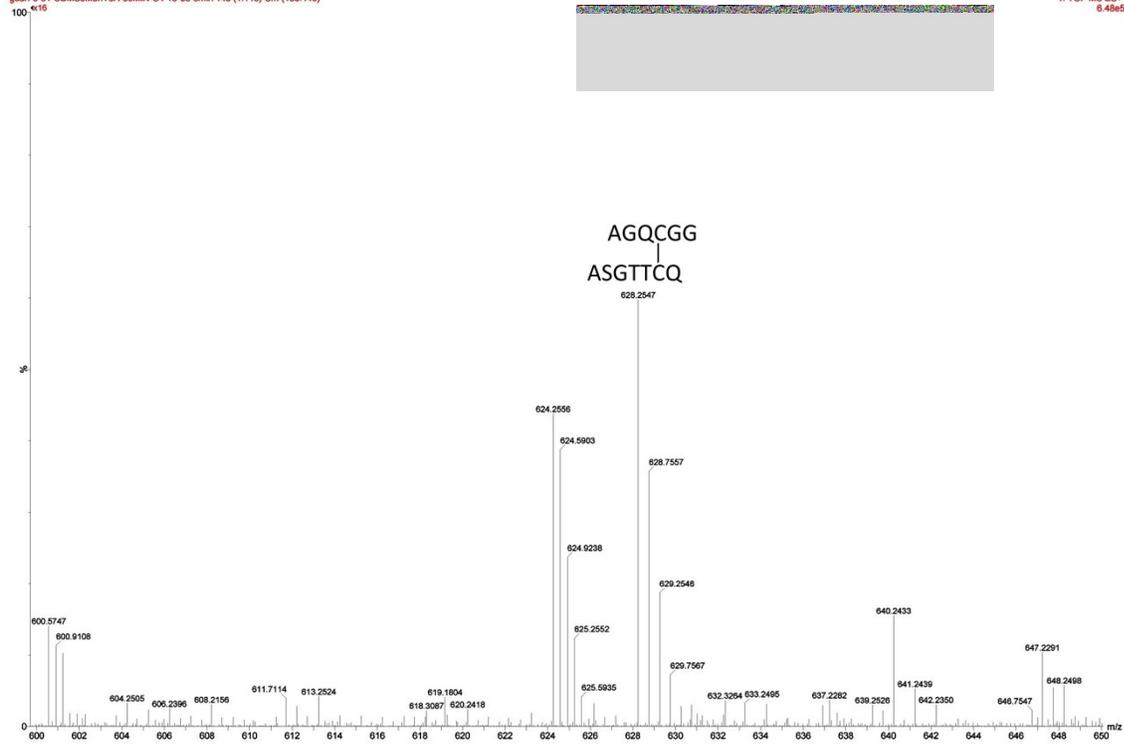
Sample_pos
guan-8-81-CBMS3ManY5A-30MIN-C4-15-35-8min 145 (1.115) Cm (129:173)

1: TOF MS ES+
6.92e5



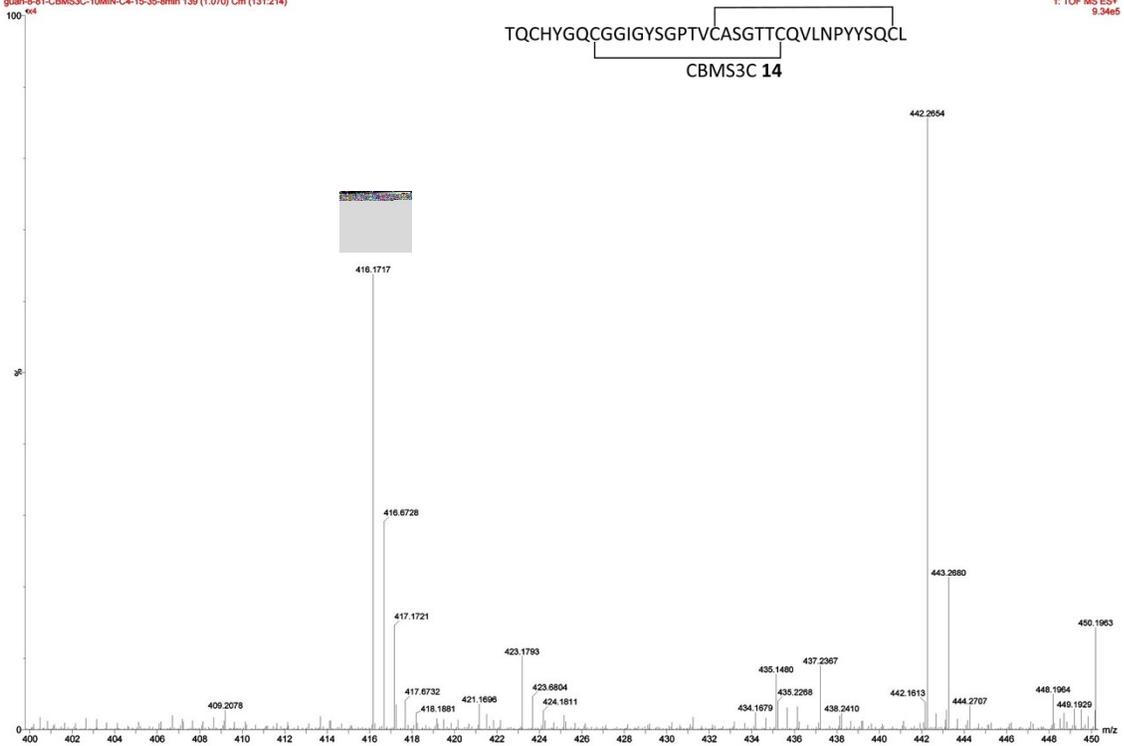
Sample_pos
guan-8-81-CBMS3ManYSA-30MIN-C4-15-35-8min 145 (1.115) Cm (133:149)

1: TOF MS ES+
6.48e5



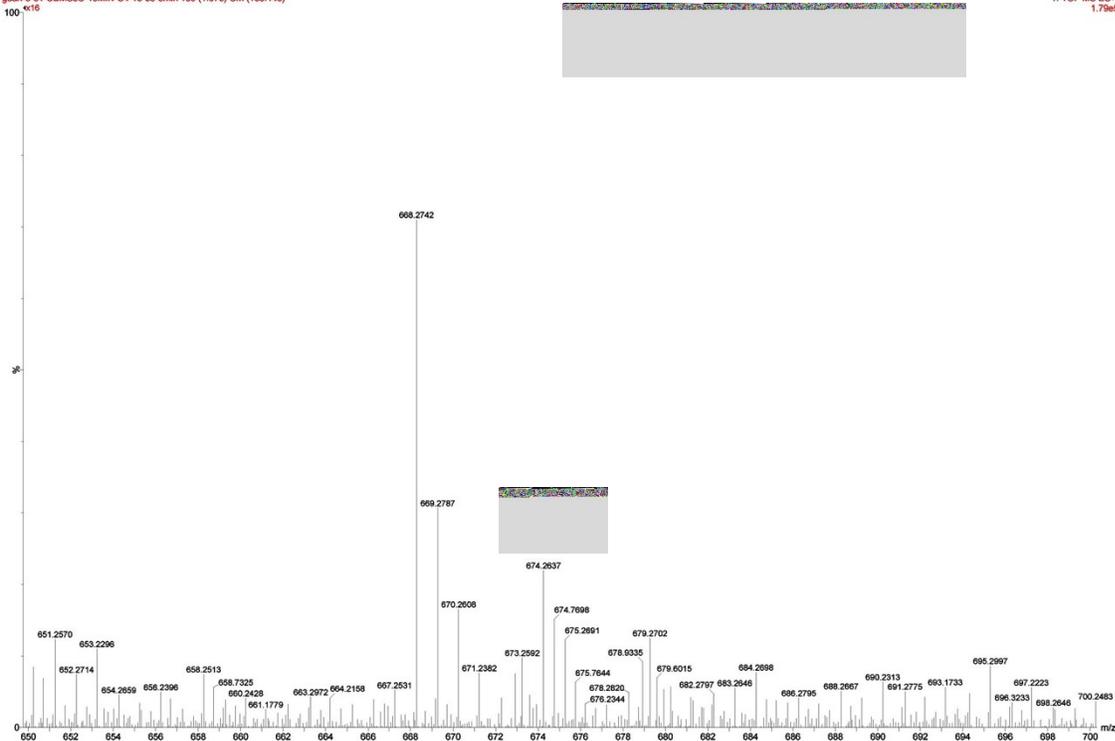
Sample_pos
guan-8-81-CBMS3C-10MIN-C4-15-35-8min 139 (1.070) Cm (131:214)

1: TOF MS ES+
9.34e5



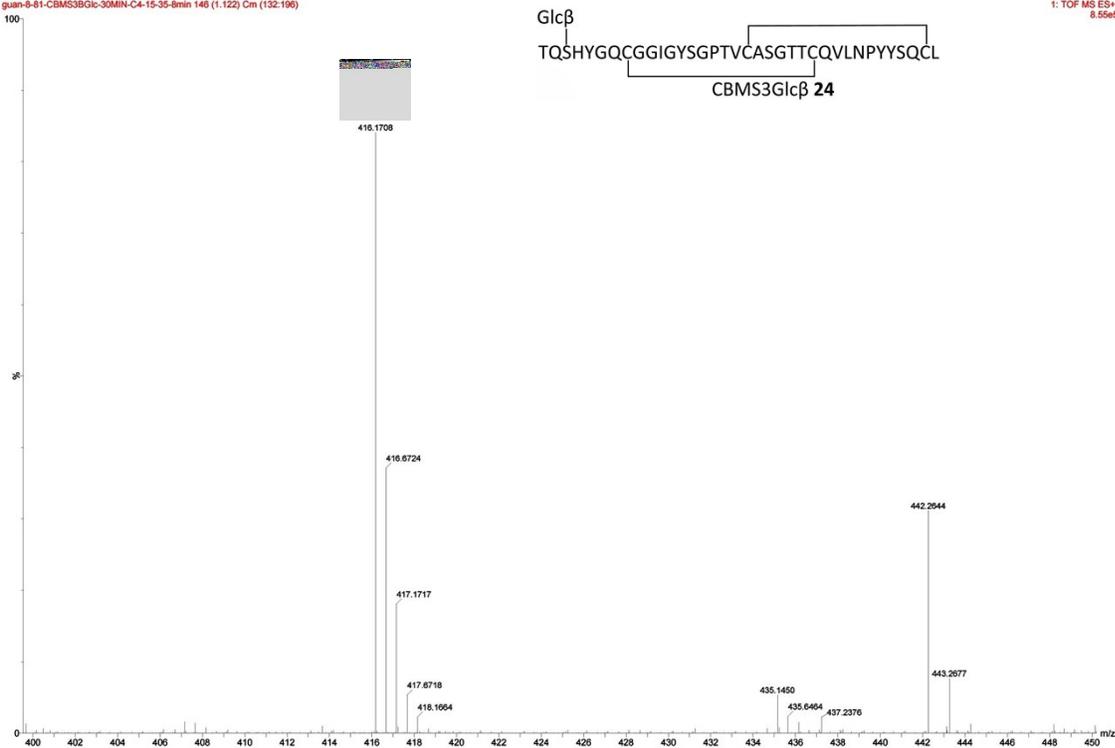
Sample_pos
 guan-8-81-CBMS3C-10MIN-C4-15-35-8min 139 (1.070) Cm (133:145)

1: TOF MS ES+
 1.79e5



Sample_pos
 guan-8-81-CBMS3Glc-30MIN-C4-15-35-8min 146 (1.122) Cm (132:196)

1: TOF MS ES+
 8.55e5



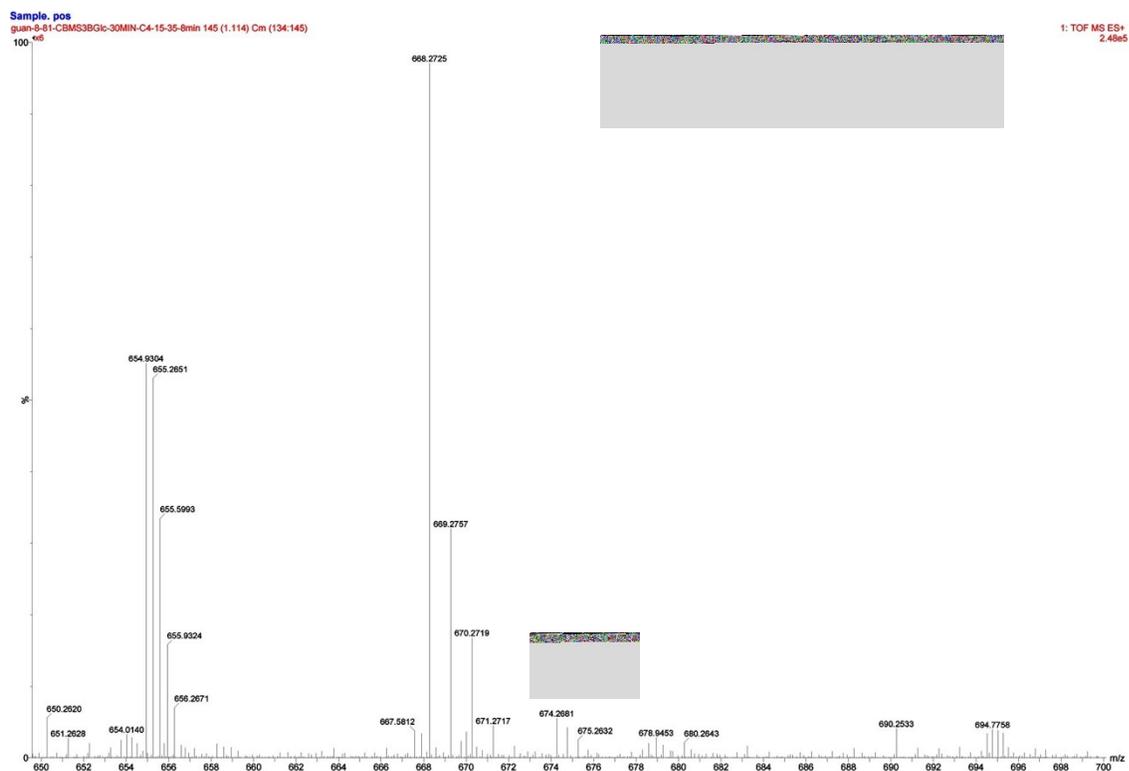


Figure S35. Confirming disulfide linkages of representative CBM variants by thermolysin digestion. MS (ESI) Calcd for VC/YSQCL $C_{34}H_{54}N_8O_{12}S_2$ $[M+2H]^{2+}$ $m/z = 416.1730$; MS (ESI) Calcd for YGQCGG/ASGTTCQV $C_{52}H_{82}N_{16}O_{22}S_2$ $[M+2H]^{2+}$ $m/z = 674.2693$; MS (ESI) Calcd for AGQCGG/ASGTTCQV $C_{46}H_{78}N_{16}O_{21}S_2$ $[M+2H]^{2+}$ $m/z = 628.2562$.

Table S1. Summary of CBM proteolytic stability, thermostability, and adsorption to crystalline cellulose that were determined in accordance with our procedures as described previously¹⁸.

| CBM Variant | Half-Life to Thermolysin Degradation (h) | T _m (°C) | K _{ads} (μM ⁻¹) | B _{max} (μmol/g) |
|-------------|--|---------------------|--------------------------------------|---------------------------|
| 1 | 0.23 ± 0.02 | 62.2 ± 0.6 | 0.0894 ± 0.0007* | 24 ± 5* |
| 2 | 1.09 ± 0.01 | 70.1 ± 0.9 | 0.4 ± 0.2 | 6 ± 1.3 |
| 3 | 2.13 ± 0.06 | 73.2 ± 0.7 | 0.21 ± 0.05 | 3.6 ± 0.8 |
| 4 | 0.16 ± 0.02 | 64.2 ± 0.6 | 0.20 ± 0.11 | 8.1 ± 1.1 |
| 5 | 0.74 ± 0.12 | 69.2 ± 0.9 | 0.20 ± 0.02 | 13 ± 2 |
| 6 | 0.16 ± 0.04 | 63.4 ± 0.7 | 0.40 ± 0.2 | 16 ± 3 |
| 7 | 0.27 ± 0.05 | 63.5 ± 0.7 | 0.32 ± 0.18 | 11 ± 2 |
| 8 | 0.22 ± 0.06 | 61.2 ± 0.3 | 0.53 ± 0.12 | 5.7 ± 0.7 |
| 9 | 0.65 ± 0.05 | 65.6 ± 0.3 | 0.73 ± 0.18 | 8.7 ± 1.0 |
| 10 | 0.08 ± 0.02 | 61.1 ± 1.0 | ~0** | ~0** |
| 11 | 0.18 ± 0.03 | 61.3 ± 0.5 | ~0** | ~0** |
| 12 | 0.36 ± 0.02 | 61.8 ± 1.6 | 0.18 ± 0.04 | 14 ± 4 |
| 13 | 1.14 ± 0.18 | 69.3 ± 0.4 | 0.30 ± 0.19 | 9.2 ± 1.6 |
| 14 | 0.18 ± 0.04 | 53.2 ± 0.7 | 0.32 ± 0.15 | 24 ± 4 |
| 15 | 0.20 ± 0.03 | 61.3 ± 0.9 | 0.22 ± 0.08 | 12 ± 1 |
| 16 | 0.19 ± 0.04 | 62.6 ± 1.6 | ~0** | ~0** |
| 17 | 0.17 ± 0.02 | 63.6 ± 1.3 | ~0** | ~0** |
| 18 | 0.30 ± 0.01 | 62.7 ± 1.7 | 0.17 ± 0.04 | 16 ± 2 |
| 19 | 0.34 ± 0.07 | 63.6 ± 0.7 | 0.20 ± 0.11 | 10 ± 1 |
| 20 | 0.57 ± 0.06 | 65.9 ± 0.3 | 0.28 ± 0.07 | 16 ± 1 |
| 21 | 0.69 ± 0.09 | 68.4 ± 1.4 | 0.19 ± 0.04 | 6.8 ± 1.3 |
| 22 | 0.40 ± 0.01 | 67.5 ± 0.4 | 0.40 ± 0.27 | 10 ± 3 |
| 23 | 0.32 ± 0.01 | 63.0 ± 0.3 | 0.12 ± 0.01 | 13 ± 1 |
| 24 | 0.28 ± 0.03 | 64.2 ± 0.1 | 0.14 ± 0.08 | 7.5 ± 2.4 |
| 25 | 0.38 ± 0.05 | 67.3 ± 1.7 | 0.52 ± 0.13 | 18 ± 2 |
| 26 | 0.50 ± 0.05 | 68.2 ± 0.9 | 0.17 ± 0.08 | 14 ± 4 |
| 27 | 0.29 ± 0.02 | 63.8 ± 0.5 | 0.18 ± 0.01 | 10 ± 1 |
| 28 | 0.62 ± 0.11 | 66.5 ± 0.2 | 0.25 ± 0.04 | 11 ± 1 |
| 29 | 0.86 ± 0.03 | 68.5 ± 1.2 | 0.25 ± 0.04 | 16 ± 2 |
| 30 | 0.78 ± 0.11 | 68.4 ± 0.7 | 0.51 ± 0.06 | 10 ± 1 |
| 31 | 0.43 ± 0.05 | 67.4 ± 0.8 | 0.25 ± 0.07 | 11 ± 1 |
| 32 | 0.10 ± 0.02 | 61.5 ± 1.1 | ~0** | ~0** |
| 33 | 1.56 ± 0.40 | 71.9 ± 0.4 | 0.51 ± 0.04 | 4.7 ± 0.2 |
| 34 | 0.18 ± 0.02 | 60.2 ± 1.2 | 0.15 ± 0.05 | 18 ± 3 |

The results are presented as mean of three trials ± SD. *Denotes an averaged value of four trials ± SD. **Weak affinity to cellulose noted, no K_{ads} and B_{max} value could be obtained.

Table S2. The change in half-life and melting temperature from the corresponding unglycosylated CBMs.

| Calculation | Change in Half-Life to Thermolysin Degradation (h) | Change in melting temperature (°C) | Change in binding affinity (μM^{-1}) |
|-------------|--|------------------------------------|---|
| 2-1 | 0.86 | 7.9 | 0.31 |
| 3-1 | 1.90 | 11.0 | 0.12 |
| 5-4 | 0.58 | 5.0 | 0 |
| 7-6 | 0.11 | 0.1 | -0.08 |
| 9-8 | 0.43 | 4.4 | 0.2 |
| 11-10 | 0.10 | 0.2 | 0 |
| 13-12 | 0.78 | 7.5 | 0.12 |
| 15-14 | 0.02*** | 8.1*** | -0.10*** |
| 17-16 | -0.02 | 1.0 | 0 |
| 19-18 | 0.04 | 0.9 | 0.03 |
| 20-1 | 0.34 | 3.7 | 0.19 |
| 21-1 | 0.46 | 6.2 | 0.10 |
| 22-1 | 0.17 | 5.3 | 0.31 |
| 23-1 | 0.09 | 0.8 | 0.03 |
| 24-1 | 0.05 | 2.0 | 0.05 |
| 25-1 | 0.15 | 5.1 | 0.43 |
| 26-1 | 0.27 | 6.0 | 0.08 |
| 27-1 | 0.06 | 1.6 | 0.09 |
| 28-1 | 0.39 | 4.3 | 0.16 |
| 29-1 | 0.63 | 6.3 | 0.16 |
| 30-1 | 0.55 | 6.2 | 0.42 |
| 31-1 | 0.20 | 5.2 | 0.16 |
| 32-10 | 0.02 | 0.4 | 0 |
| 33-12 | 1.2 | 10.1 | 0.33 |
| 34-14 | 0*** | 7.0*** | -0.17*** |

*** Not used in the correlation plot.

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