Supporting Information

Materials and Methods

Structural analysis of *C. elegans* N- and O-glycans

Processing of samples to obtain N- and O-glycans. Extracts of about 600 mg of *C. elegans pmk-1(km25)* and *C. elegans samt-1(op532)pmk-1(km25)* were analyzed. Both samples were subjected to reduction, carboxymethylation, and tryptic digestion: they were reduced in 1 ml of 50 mM Tris-HCl buffer, pH 8.5, containing 2 mg/ml dithiothreitol. Reduction was performed at 37 °C in a water bath for 1 h. Carboxymethylation was carried out by the addition of iodoacetic acid (5-fold molar excess over dithiothreitol), and the reaction was allowed to proceed at room temperature in the dark for 1.5 h. Carboxymethylation was terminated by dialysis against 4 times 4.5 liters of 50 mM ammonium bicarbonate, pH 8.5, at 4 °C for 48 h. After dialysis, the samples were lyophilized. The reduced carboxymethylated proteins were then digested with N-p-tosyl-l-phenylalanine chloromethyl ketone-pretreated bovine pancreas trypsin (Sigma) for 16 h at 37 °C in 50 mM ammonium bicarbonate buffer, pH 8.4. The products were purified by C18 Sep-Pak® (Waters) as described previously (1).

N-Glycans were enzymatically released from the peptide backbone by sequential digestion with PNGase F and PNGase A. PNGase F (Roche Applied Science) digestion was carried out in 50 mM ammonium hydrogen carbonate, pH 8.5, for 24 h at 37 °C with 5 units of enzyme. The reaction was terminated by lyophilization, and the products were purified using a propanol, 5% (v/v) acetic acid reverse-phase C18 Sep-Pak system (Waters Corp.). Glycopeptides remaining after the PNGase F digestion were further digested with 0.2 milliunits of PNGase A (Roche Applied Science) for 24 h at 37 °C, and products were purified on a C18 Sep-Pak (Waters Corp.) as described previously (1). The released N-glycans were purified from glycopeptides and peptides by chromatography on a Sep-Pak C18 cartridge (Waters Corp., Milford, MA).

Reductive elimination of O-glycans was performed as explained previously (2). Four hundred microliters of 0.1 M potassium hydroxide (Sigma-Aldrich, UK) containing potassium borohydride (54 mg/ml) (Sigma-Aldrich, UK) was added to dried samples and incubated at 45 °C for 14 to 16 h. The reaction was terminated by adding a few drops of 5% (v/v) acetic acid followed by purification with Dowex 1-X8 desalting column (Sigma-Aldrich, UK). The columns were first washed with 15 ml of 5% (v/v) acetic acid. Next, the samples were loaded and eluted with 5 ml of 5% (v/v) acetic acid. The volume of the eluents was reduced with a Savant SpeedVac followed by lyophilization for 16 h. Excess borates in the samples were removed by co-evaporating with 10% (v/v) acetic acid in methanol (4 times 0.5 ml) under a stream of nitrogen at room temperature.

The purified N- and O-glycans were subsequently deuteromethylated using the sodium hydroxide permethylation procedure as described previously (3). Briefly, 5 to 7 NaOH pellets were ground to fine powder and mixed with 2 to 3 ml anhydrous dimethylsulfoxide (Romil) before adding to each dried sample. This was followed by the addition of 0.6 ml of d3-methyl iodide (Sigma-Aldrich) and vigorous shaking at room temperature for 15 min. Deuteropermethylated glycans were extracted with chloroform and then purified by using Sep-Pak C18 cartridges. The cartridges were successively conditioned with methanol (5 ml), water (5 ml), acetonitrile (5 ml) and water (15 ml). Each sample was dissolved in 200 µl of methanol:water (1:1) solution before loading onto the cartridges. The cartridges were washed with 5 ml of water and then eluted sequentially with 3 ml of each 15%, 35%, 50% and 75% acetonitrile solution in water (v/v). 35%, 50% and 75% acetonitrile/water fractions were collected and then concentrated with a Savant SpeedVac and subsequently lyophilized.

MS and MS/MS analyses of permethylated glycans. MALDI-TOF data were acquired on a Voyager-DE STR mass spectrometer (Applied Biosystems, Foster City, CA) in the reflectron mode with delayed extraction. Permethylated samples were dissolved in 10 µl of 70% (v/v) aqueous methanol, and 1 µl of dissolved sample was premixed with 1 µl of matrix (20 mg/ml 2,5-dihydroxybenzoic acid in 80% (v/v) aqueous methanol), spotted onto a target plate, and dried under vacuum. Further MS/MS analyses of peaks observed in the MS spectra were carried out using a 4800 MALDI-TOF/TOF (Applied Biosystems) mass spectrometer in the positive ion mode

producing $[M+Na]^+$ molecular ions. The collision energy was set to 1 kV, and argon was used as collision gas. Samples were dissolved in 10 µl of methanol, and 1 µl was mixed at a 1:1 ratio (v/v) with 2,5-dihydroxybenzoic acid (20 mg/ml in 70% methanol in water) as matrix.

Analyses of MALDI data. The MS and MS/MS data were processed using Data Explorer 4.9 Software (Applied Biosystems). The mass spectra were baseline corrected (default settings) and noise filtered (with correction factor of 0.7), and then converted to ASCII format. The processed spectra were then subjected to manual assignment and annotation with the aid of a glycobioinformatics tool known as GlycoWorkBench (4). Peak picking was done manually, and proposed assignments for the selected peaks were based on molecular mass composition of the 12C isotope together with knowledge of the biosynthetic pathways. Some of the proposed structures were then confirmed by data obtained from MS/MS experiment.

Monosaccharide analysis of *C. elegans* N-glycans

Proteins were extracted from 150 mg of nematodes with 150 µl of extraction buffer as described above. To precipitate proteins trichloroacetic acid was added to a final concentration of 10%. Samples were incubated for 5 min on ice before 5 min centrifugation at 20000 x g at 4 °C. The pellet was washed with acetone (-20 °C) twice, dissolved in 700 µl of PBS pH 7.4 and proteins were digested with 1 mg/ml trypsin (Sigma) at 37 °C for 16 h shaking. For release of N-glycans samples were acidified with sodium acetate buffer to pH 5 to 6. Three microliters of PNGase A (Roche Diagnostics) were added and samples were incubated at 37 °C for 16 h shaking. For purification of glycans a C18 cartridge (C18 Sep Pack, Waters) was placed on top of a column packed with 250 µl of ENVI-Carb 120/400 resin (Sigma-Aldrich). The combined columns were washed with 5 ml of methanol, 5 ml of acetonitrile, 5 ml of 50% acetonitrile (in water), and equilibrated with 10 ml of 2% acetonitrile. The sample was adjusted to 2% acetonitrile and loaded onto the columns. Columns were washed with 10 ml of 2% acetonitrile and glycans were eluted twice with 750 µl of 25% acetonitrile. The eluate was collected in a 1.5 ml screw-cap tube and the solvent was evaporated under vacuum. The dried pellet was resuspended in 100 µl of ultra-pure water and 100 µl of freshly prepared 5 M trifluoroacetic acid (TFA) were added. The tube was sealed with teflon tape, wrapped

with aluminum foil, and incubated on an Eppendorf Thermomixer (100 °C; 750 rpm) for 5 h. The solution was transferred to a new 1.5 ml screw-cap tube and TFA was evaporated under a stream of air at 45 °C. The residue was dissolved in 50 µl of 1% NaOAc and 50 µl of 2-AA labeling mix (30 mg/ml 2-aminobenzoic acid, 20 mg/ml sodium cyanoborohydride, 2.4% NaOAC, 2% boric acid in methanol) was added. The tube was sealed with teflon tape and wrapped with aluminum foil. After incubation on an Eppendorf Thermomixer (80 °C; 750 rpm) for 1 h, the sample was cooled to room temperature, diluted to 1 ml with eluent A (0.3% 1-amino butane, 0.5% phosphoric acid, 1% tetrahydrofuran in water) and passed through a 0.45 µm filter. Samples were finally diluted 20-fold in eluent A and 90 µl were loaded on a C18 column (YMC, C18 ODS-A, 5 µm particle size, 12 nm pore size, 46x150 mm). The following elution program was used: 0-35 min 6% eluent B (50% eluent A, 50% acetonitrile), 35-90 min linear gradient 6-25% B; 90-115 min 100% B; 115-130 min 6% B; detection parameters were: excitation 360 nm, emission 425 nm. Hundred µl of a solution of standard monosaccharides (concentration of each monosaccharide was 10 mM) were treated with TFA, labelled with 2-AA, and diluted 100-fold; 50 µl of the resulting solution were analysed by HPLC as described. Retention times of the standard monosacharides were used to identify the respective monosaccharides in the test samples. The abundance of individual monosaccharides was calculated as follows: the corresponding peak area in the HPLC chromatogram was determined using the software Chromeleon (Dionex) and divided by the total peak area of all monosaccharides identified. Ratios were calculated from four independent experiments.

Chemical synthesis

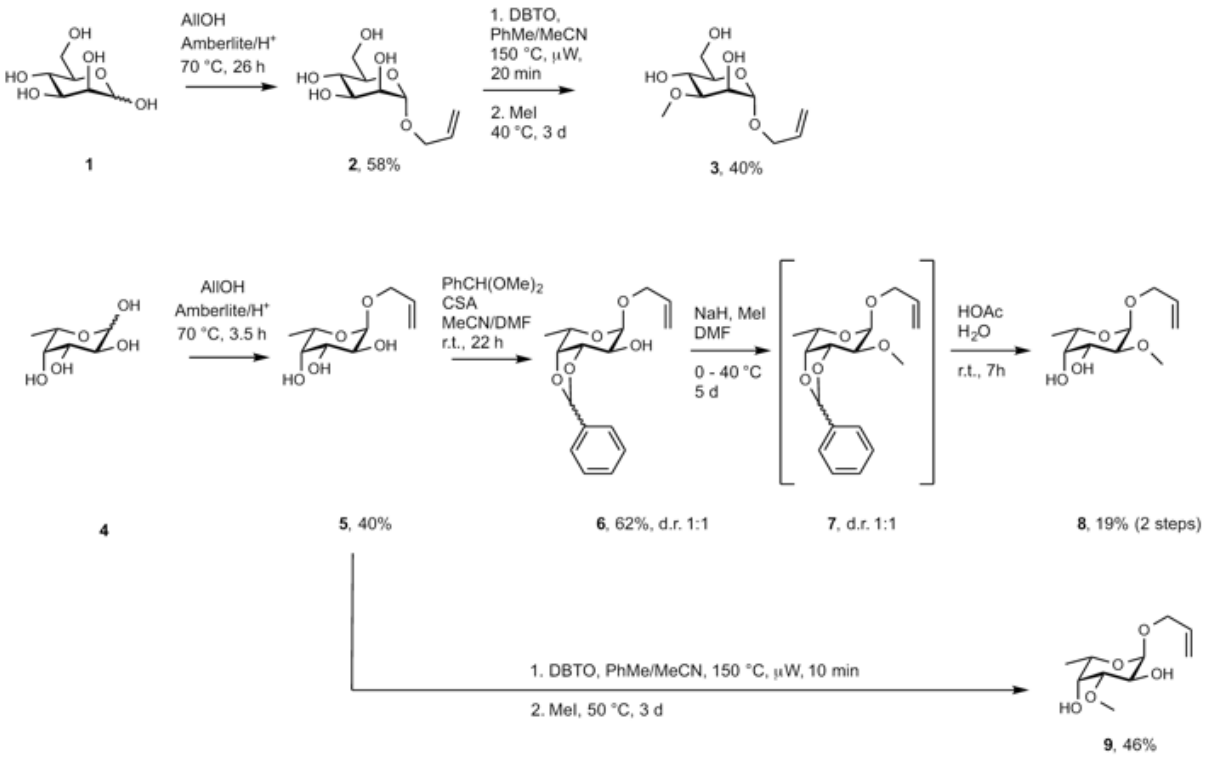
General Experimental Details

Nuclear magnetic resonance (NMR) spectroscopy was performed on a Bruker Avance III 400 UltraShield spectrometer at 400 MHz (^{1}H) or 101 MHz (^{13}C). Chemical shifts are given in ppm and were calibrated on residual solvent peaks as internal standard. Multiplicities were specified as s (singlet), m (multiplet) or interpreted according to 1st order where possible. ArH denotes aromatic protons and ArC or ArCH denotes aromatic quaternary carbons or aromatic CH carbon atoms. The signals were assigned with the help of ^{1}H,^{1}H-COSY, DEPT-135-edited ^{1}H,^{13}C-HSQC and ^{1}H,^{13}C-HMBC experiments. Spectra are supplied as Dataset S2. High

resolution mass spectra were obtained on an ESI Bruker micrOTOF II spectrometer. Data were analyzed using DataAnalysis from Bruker. Thin layer chromatography (TLC) was performed using silica gel 60 coated aluminum sheets containing fluorescence indicator (Merck KGaA, Darmstadt, Germany) using UV light (254 nm) and by charring in a molybdate solution (a 0.02 M solution of ammonium cerium sulfate dihydrate and ammonium molybdate tetrahydrate in aqueous 10% H_2SO_4) with heating. The microwave reactions were carried out in a Biotage Initiator Microwave Synthesizer. Medium pressure liquid chromatography (MPLC) was performed on a Teledyne Isco Combiflash Rf200 system using pre-packed silica gel 60 columns from Teledyne Isco, SiliCycle or Macherey-Nagel. Commercial chemicals and solvents were used without further purification. D-mannose and L-fucose was purchased from Dextra Laboratories (Reading, UK). MeOH-d4 and $CDCl_3$ was purchased from Eurisotop (Saarbrücken, Germany).

Overview

Chemical synthesis scheme of allyl 3-O-methyl α-D-mannopyranoside (**3**), allyl 2-O-methyl α-L-fucopyranoside (**8**) and allyl 3-O-methyl α-L-fucopyranoside (**9**).



Allyl α-D-mannopyranoside (**2**) was synthesized by Fischer glycosylation of D-mannose (**1**). **1** (2.0 g, 11.1 mmol) was suspended in allyl alcohol (18 ml) and Amberlite IR120/H^+ (910 mg) was added under argon. The mixture was heated to 70 °C for 26 h and filtered hot through a pad of celite. The volatiles were removed *in vacuo* and the residue was purified by column chromatography (SiO_2/CH_2Cl_2/EtOH gradient of 3-20%) to give pure **2** (1.41 g, 58%). ^{1}H NMR (400 MHz, MeOH-d4) δ 6.00-5.88 (m, 1H, allyl-CH), 5.33-5.26 (m, 1H, allyl-CH_2), 5.20-5.14 (m, 1H, allyl-CH_2), 4.80 (d, J = 1.7 Hz, 1H, H-1), 4.25-4.18 (m, 1H, allyl-CH_2), 4.05-3.95 (m, 1H, allyl-CH_2), 3.87-3.80 (m, 2H, H-2,-6a), 3.75-3.68 (m, 2H, H-3, -6b), 3.62 (dd, J^1=J^2 = 9.5 Hz, 1H, H-4), 3.53 (ddd, J = 9.8, 5.7, 2.4 Hz, 1H, H-5). ^{13}C NMR (101 MHz, MeOH-d4) δ 135.39 (allyl-CH), 117.26 (allyl-CH_2), 100.60 (C-1), 74.60 (C-5), 72.54 (C-3), 72.09 (C-2), 68.77 (allyl-CH_2), 68.50 (C-4), 62.78 (C-6). HRMS: $[C_9H_{16}O_6+Na]^+$ calcd: 243.08391 found: 243.08513. The ^{1}H NMR corresponds to the one reported by Winnik *et al*. (5).

Allyl 3-O-methyl-α-D-mannopyranoside (**3**) was synthesized from **2** in analogy to Liao *et al*. (6). A microwave tube was charged with **2** (627 mg, 2.85 mmol), di-n-butyltinoxide (780 mg, 3.13 mmol), a stirring bar and was dried under *vacuo*. The vial was flushed with argon, dry PhMe (5.8 ml) and dry MeCN (1.2 ml) was added and the vial was sealed with a rubber septum cap. The suspension was heated to 150 °C (2 x 10 min) under microwave irradiation. After cooling to room temperature, MeI (4.5 ml, 71 mmol) was added to the clear solution, which was then stirred for 72 h at 40 °C. The volatiles were removed *in vacuo* and the residue was purified by column chromatography (SiO_2/CH_2Cl_2/EtOH gradient of 3-20%) to give pure **3** (267 mg, 40%) and recovered starting material **2** (305 mg, 49%). ^{1}H NMR (400 MHz, MeOH-d4) δ 6.00-5.88 (m, 1H, allyl-CH), 5.35-5.26 (m, 1H, allyl-CH_2), 5.21-5.15 (m, 1H, allyl-CH_2), 4.83 (d, J = 1.8 Hz, 1H, H-1), 4.26-4.17 (m, 1H, allyl-CH_2), 4.05-3.97 (m, 2H, H-2, allyl-CH_2), 3.83 (dd, J = 11.8, 2.4 Hz, 1H, H-6a), 3.76-3.63 (m, 2H, H-4, -6b), 3.55 (ddd, J = 9.9, 5.8, 2.3 Hz, 1H, H-5), 3.45 (s, 3H, OCH_3), 3.37 (dd, J = 9.4, 3.3 Hz, 1H, H-3). ^{13}C NMR (101 MHz, MeOH-d4) δ 135.38 (allyl-CH), 117.43 (allyl-CH_2), 100.54 (C-1), 82.31 (C-3), 74.67 (C-5), 68.84 (allyl-CH_2), 67.91 (C-2), 67.38 (C-4), 62.83 (C-6), 57.35 (OCH_3). HRMS: $[C_{10}H_{18}O_6+Na]^+$ calcd: 257.09956 found: 257.10046. The ^{1}H NMR corresponds to the selected signals reported by Liao *et al*. (6).

Allyl α-L-fucopyranoside (**5**) was synthesized by Fischer glycosylation of L-fucose (**4**) according to Unverzagt *et al*. (7). **4** (2.0 g, 12.2 mmol) was suspended in allyl alcohol (24 ml) and Amberlite IR120/H^+ (1.0 g) was added under argon. The mixture was heated to 70 °C for 3.5 h and filtered hot through a pad of celite. Upon cooling of the reaction mixture, pure **5** (986 mg, 40%) was obtained by crystallization. Concentration of the mother liquor and recrystallization from allyl alcohol (5 ml) yielded additional **5** which was contaminated with the β-anomer and furanosides. ^{1}H NMR (400 MHz, MeOH-d4) δ 6.02-5.90 (m, 1H, allyl-CH), 5.36-5.27 (m, 1H, allyl-CH_2), 5.20-5.13 (m, 1H, allyl-CH_2), 4.80 (d, J = 3.0 Hz, 1H, H-1), 4.20-4.13 (m, 1H, allyl-CH_2), 4.06-3.99 (m, 1H, allyl-CH_2), 3.95 (q, J = 6.9 Hz, 1H, H-5), 3.79-3.71 (m, 2H, H-2, -3), 3.68-3.65 (m, 1H, H4), 1.21 (d, J = 6.6 Hz, 3H, H-6). ^{13}C NMR (101 MHz, MeOH-d4) δ 135.73 (allyl-CH), 117.35 (allyl-CH_2), 99.60 (C-1), 73.61 (C-4), 71.65 (C-2/3), 69.95 (allyl-CH_2), 69.50 (C-2/3), 67.60 (C-5), 16.57 (C-6). HRMS: $[C_9H_{16}O_5+Na]^+$ calcd: 227.08899 found: 227.09026. The ^{1}H NMR corresponds to the one reported by Unverzagt *et al*. (7), the ^{13}C NMR corresponds to the selected signals reported in the same work.

Allyl 3,4-O-benzylidene-α-L-fucopyranoside (**6**). Fucoside **5** (600 mg, 2.94 mmol) and camphorsulfonic acid monohydrate (73 mg, 0.29 mmol) was dissolved in dry DMF (6 ml) under argon. $PhCH(OMe)_2$ (1.32 ml, 8.81 mmol) was added dropwise under stirring at room temperature and the mixture was stirred for 22 h. The reaction was neutralized with NEt_3 (82 µl, 0.59 mmol) and the volatiles were removed *in vacuo*. Purification by column chromatography (SiO_2/petrol ether/EtOAc gradient of 3-40%) gave a endo-/exo-diastereomeric mixture of **6** (532 mg, 62%, d.r. 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56-7.51 (m, 2H, ArH), 7.48-7.44 (m, 2H, ArH), 7.41-7.34 (m, 6H, ArH), 6.18 (s, 1H, $PhCH(OR)_2$), 5.99-5.88 (m, 2H, allyl-CH), 5.89 (s, 1H, $PhCH(OR)_2$), 5.39-5.15 (m, 4H, allyl-CH_2), 4.96 (d, J = 4.0 Hz, 1H, H-1), 4.91 (d, J = 3.9 Hz, 1H, H-1), 4.45 (dd, J = 6.8, 5.8 Hz, 1H, H-3), 4.38 (dd, $J^1 = J^2$ = 6.3, 1H, H-3), 4.33-4.19 (m, 3H), 4.19-4.13 (m, 2H), 4.13-4.02 (m, 3H), 3.95 (dd, J = 6.9, 4.0 Hz, 1H, H-2), 3.90 (dd, J = 6.0, 4.0 Hz, 1H, H-2), 1.38 (d, J = 6.6 Hz, 3H, H-6), 1.37 (d, J = 6.7 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.21 (ArC), 137.43 (ArC), 133.90 (allyl-CH), 133.73 (allyl-CH), 129.53, 129.12, 128.51, 128.49, 126.96, 126.25 (10C, ArCH), 117.98 (allyl-CH_2), 117.80 (allyl-CH_2), 104.10 ($PhCH(OR)_2$), 103.22 ($PhCH(OR)_2$), 96.93 (C-1), 96.58 (C-1), 77.51, 76.27, 76.10 (4C, C-3/4), 69.26 (C-2), 68.76 (allyl-CH_2), 68.67 (allyl-CH_2), 67.75 (C-2), 64.22 (C-5), 64.17 (C-5), 16.44 (C-6), 16.36 (C-6).

Allyl 2-O-methyl-3,4-O-benzylidene-α-L-fucopyranoside (**7**). Benzylidene **6** (194 mg, 0.66 mmol) was dissolved in dry DMF (3 ml) under argon and cooled to 0 °C. NaH (48 mg, 1.2 mmol, 60% in mineral oil) was added and subsequently, MeI (124 µl, 2.0 mmol) was added dropwise. After stirring at 0 °C for 1 h, the reaction was heated to 40 °C and stirred for 4 d. Then, NaH (24 mg, 0.6 mmol, 60% in mineral oil) was added followed by MeI (62 µl, 1.0 mmol) and the reaction was keep at 40 °C for further 24 h. Then, the reaction was cooled to 0 °C, quenched with EtOH (1 ml) and diluted with EtOAc (10 ml). The organic layer was washed with aqueous $NaHCO_3$ (3 x 3 ml), dried over Na_2SO_4, filtered and the volatiles removed *in vacuo*. The diastereomeric mixture (1:1) of crude **7** (179 mg, 88%) which contained 15% **6** as judged by 1H NMR, was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58-7.49 (m, 2H, ArH), 7.49-7.43 (m, 2H, ArH), 7.43-7.31 (m, 6H, ArH), 6.19 (s, 1H, $PhCH(OR)_2$), 6.01-5.88 (m, 2H, allyl-CH), 5.92 (s, 1H, $PhCH(OR)_2$), 5.40-5.28 (m, 2H, allyl-CH_2), 5.28-5.15 (m, 2H, allyl-CH_2), 5.04 (d, J = 3.6 Hz, 1H, H-1), 4.95 (d, J = 3.5, 1H, H-1), 4.56 (dd, J = 8.0, 5.3, 1H, H-3), 4.41 (dd, J = 7.6, 6.0 Hz, 1H, H-3), 4.27-4.17 (m, 3H), 4.17-3.97 (m, 5H), 3.57 (s, 3H, OCH_3), 3.53 (dd, J = 8.0, 3.6 Hz, 1H, H-2), 3.44 (s, 3H, OCH_3), 3.38 (dd, J = 7.6, 3.6 Hz, 1H, H-2), 1.42 (d, J = 6.7 Hz, 3H, H-6), 1.37 (d, J = 6.7 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.45 (ArC), 138.01 (ArC), 133.80 (allyl-CH), 133.72 (allyl-CH), 129.26, 129.05, 128.49, 128.46, 128.43, 126.94, 126.72, 126.25 (10C, ArCH), 118.15 (allyl-CH_2), 118.08 (allyl-CH_2), 103.81 ($PhCH(OR)_2$), 102.62 ($PhCH(OR)_2$), 95.66 (C-1), 95.36 (C-1), 79.68 (C-2), 78.72, 77.04, 76.70, 76.12, 75.63 (5C, C-2, 2 x C-3, 2 x C-4), 68.51 (2C, allyl-CH_2), 63.30 (C-5), 63.23 (C-5), 58.66 (OCH_3), 58.59 (OCH_3), 16.54 (C-6), 16.36 (C-6).

Allyl 2-O-methyl-α-L-fucopyranoside (**8**). Crude **7** (75.4 mg) was dissolved in aqueous HOAc (60%, 5 ml) and stirred at room temperature for 7 h. The reaction was neutralized with saturated aqueous $NaHCO_3$ (15 ml) and extracted with EtOAc (5 x 10 ml). The combined organic layers were dried over Na_2SO_4, filtered and the volatiles were removed *in vacuo*. The residue (35.7 mg) was purified by column chromatography (SiO_2/petrol ether/EtOAc gradient of 35-75%) to give pure **8** (11.8 mg, 19% over 2 steps). ^{1}H NMR (400 MHz, MeOH-d4) δ 6.00-5.89 (m, 1H, allyl-CH), 5.36-5.28 (m, 1H, allyl-CH_2), 5.20-5.16 (m, 1H, allyl-CH_2), 4.99 (d, J = 3.8 Hz, 1H, H-1), 4.19-4.12 (m, 1H, allyl-CH_2), 4.05-3.98 (m, 1H, allyl-CH_2), 3.93 (dq, J = 6.6, 0.9 Hz, 1H, H-5), 3.82 (dd, J = 10.1, 3.4 Hz, 1H, H-3), 3.65 (dd, J = 3.5, 1.2 Hz, 1H, H-4),

3.47 (dd, J = 10.0, 3.8 Hz, 1H, H-2), 3.46 (s, 3H, OMe), 1.21 (d, J = 6.6 Hz, 3H, H-6).^{13}C NMR (101 MHz, MeOH-d4) δ 135.59 (allyl-CH), 117.56 (allyl-CH_2), 96.81 (C-1), 79.32 (C-2), 73.63 (C-4), 70.80 (C-3), 69.28 (allyl-CH_2), 67.44 (C-5), 58.49 (OCH_3), 16.54 (C-6). HRMS: $[C_{10}H_{18}O_5+Na]^+$ calcd: 241.10464 found: 241.10553. The ^{1}H NMR corresponds to the selected signals reported by Takeo *et al*. (8).

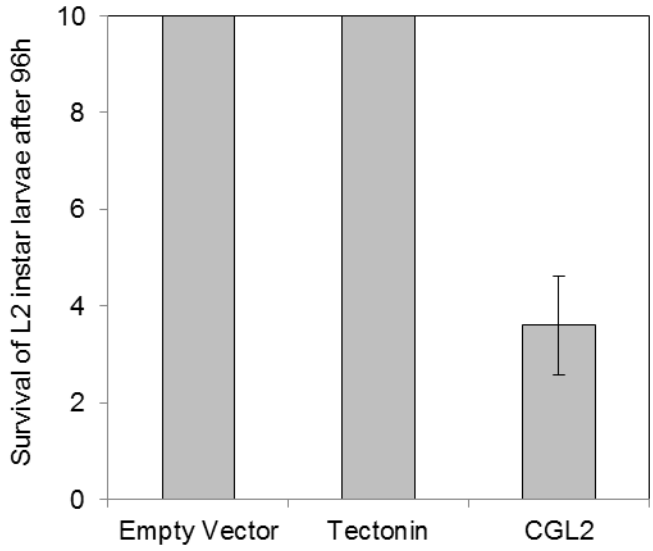
Allyl 3-O-methyl-α-L-fucopyranoside (**9**) was synthesized from **5** in analogy to the synthesis of **3**. A microwave tube was charged with **5** (245 mg, 1.20 mmol), di-n-butyltinoxide (329 mg, 1.32 mmol), a stirring bar and was dried under *vacuo*. The vial was flushed with argon, dry PhMe (2.5 ml) and dry MeCN (0.5 ml) was added and the vial was sealed with a rubber septum cap. The suspension was heated to 150 °C (2 x 5 min) under microwave irradiation. The vial was moved to an oil bath at 50 °C, MeI (1.88 ml, 30.1 mmol) was added and the reaction was stirred for 66 h. The volatiles were removed *in vacuo* and the residue was purified by column chromatography (SiO_2/CH_2Cl_2/EtOH gradient of 3-20%) to give pure **9** (121 mg, 46%) and recovered starting material **5** (74 mg, 30%). ^{1}H NMR (400 MHz, MeOH-d4) δ 6.02-5.90 (m, 1H, allyl-CH), 5.37-5.28 (m, 1H, allyl-CH_2), 5.19-5.13 (m, 1H, allyl-CH_2), 4.79 (d, J = 4.0 Hz, 1H, H-1), 4.20-4.13 (m, 1H, allyl-CH_2), 4.06-4.00 (m, 1H, allyl-CH_2), 3.94 (q, J = 6.8 Hz, 1H, H-5), 3.89 (d, J = 3.0 Hz, 1H, H-4), 3.82 (dd, J = 10.1, 4.0 Hz, 1H, H-2), 3.45 (s, 3H, OMe), 3.42 (dd, J = 10.1, 3.2 Hz, 1H, H-3), 1.22 (d, J = 6.6 Hz, 3H, H-6).^{13}C NMR (101 MHz, MeOH-d4) δ 135.71 (allyl-CH), 117.38 (allyl-CH_2), 99.49 (C-1), 81.23 (C-3), 69.49 (allyl-CH_2), 69.43 (C-4), 68.88 (C-2), 67.46 (C-5), 57.17 (OCH_3), 16.63 (C-6). HRMS: $[C_{10}H_{18}O_5+Na]^+$ calcd: 241.10464 found: 241.10562.

Microcalorimetry titrations

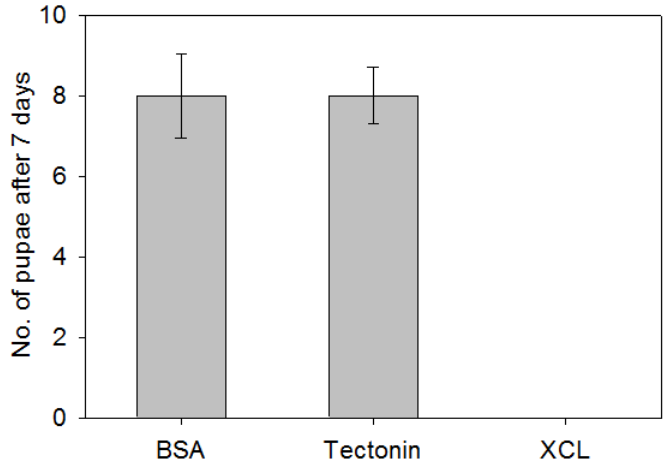
The titration was performed with a solution of **2**, **3**, **5**, **8** or **9** (12 - 28 mM) in the same buffer. After one preinjection (0.2 µl), 19 injections of 2 µl and 4 s each were performed with a spacing of 240 s. At least two independent titrations were run. Heats of dilution of the ligands (**2**, **3**, **5**, **8** or **9**) were measured by titrating the ligand into buffer and were insignificant.

Figure S1. Toxicity assays of Lb-Tec2 (Tectonin) towards insects. The assays against *Aedes aegypti (A) and Drosophila melanogaster (B*) were performed as described previously (9, 10). The fungal lectins CGL2 and XCL were used as positive and vector-containing bacteria (Empty vector: BL21(DE3)/pET24) and bovine serum albumin (BSA) as negative controls, respectively. The protein concentration in the *D. melanogaster* assays was 100 µg/ml. Error bars indicate the standard deviations (N=5).

A



B



Figures S2. Toxicity of Lb-Tec2 towards *C. elegans* fucosylation mutants. Development of *C. elegans* wild-type (N2), *pmk-1(km25), pmk-1(km25)samt-1(op532)* and various fucosylation mutants (the specificities of the encoded fucosyltransferases are not known yet) feeding on *E. coli* BL21(DE3) containing the empty vector (pET24) or expressing Lb-Tec2 (*n* = 5). Error bars indicate the standard deviations.

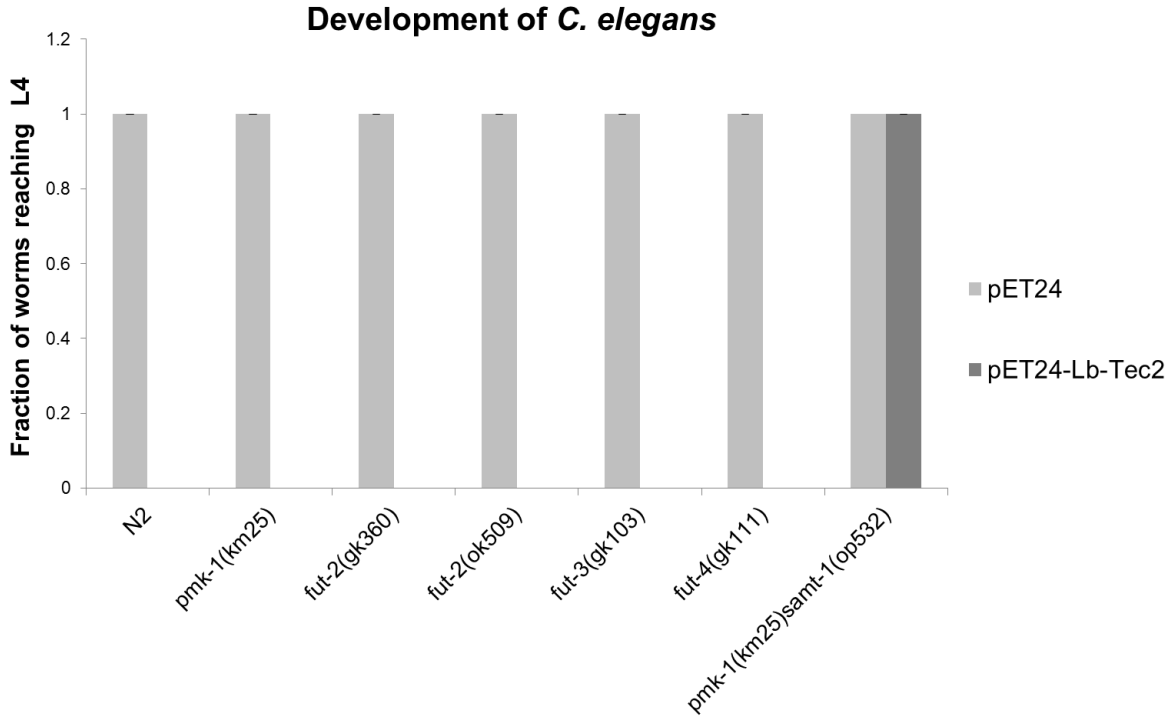


Figure S3. Binding of TAMRA-labeled Lb-Tec2 to *C. elegans* intestine. Lb-Tec2 was labeled with TAMRA and fed to larvae of *C. elegans* strain *pmk-1(km25)* as previously described (11). The picture shows an overlay of a phase contrast and fluorescent micrograph which were acquired as described in Materials and Methods.

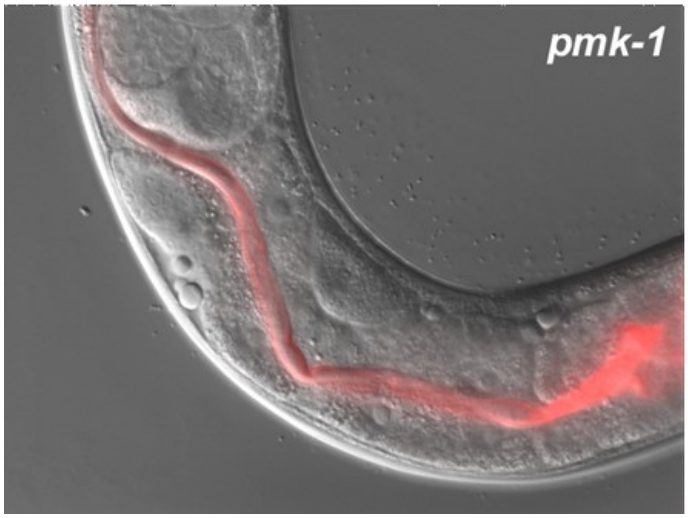


Figure S4. Chromatogram of 2-AA labeled monosaccharides of *C. elegans* N-glycans and monosaccharide standards. Proteins were extracted from *C. elegans pmk-1(km25)* and *samt-1(op532)pmk-1(km25)* and digested with trypsin. N-glycans were released with PNGase A, purified, and hydrolyzed. Monosaccharides were labeled with 2-AA and separated by reversed-phase HPLC. Elution profiles were recorded with a fluorescence detector. The retention times of monosaccharides were determined by comparison to standards. 1, GlcNAc; 2, GalNAc; 3, galactose; 4, mannose; 5, glucose; 6, xylose; 7, glucuronic acid; 8, fucose; 9, 3-O-methyl-galactose; 10, 3-O-methyl-mannose; 11, 2-O-methyl-fucose; the asterisk indicates label peaks.

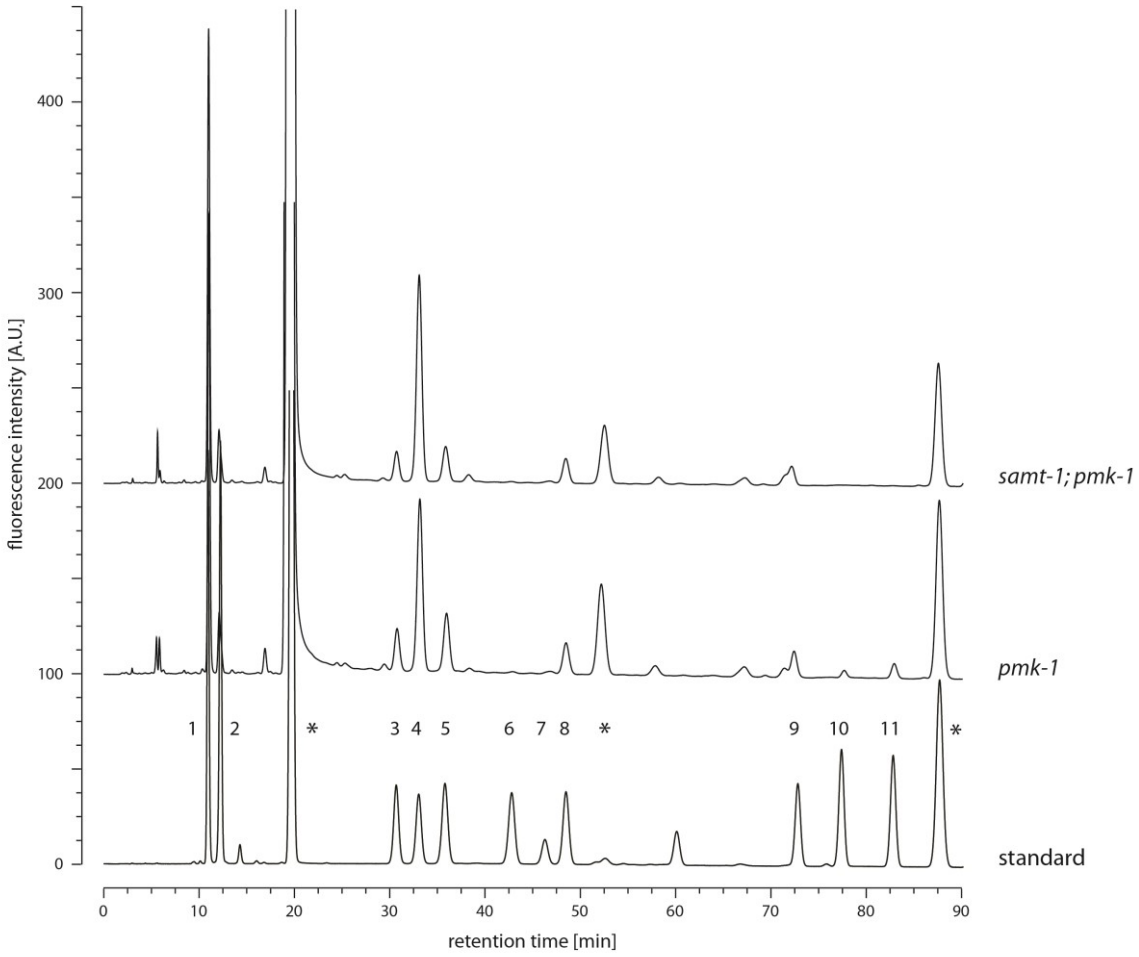
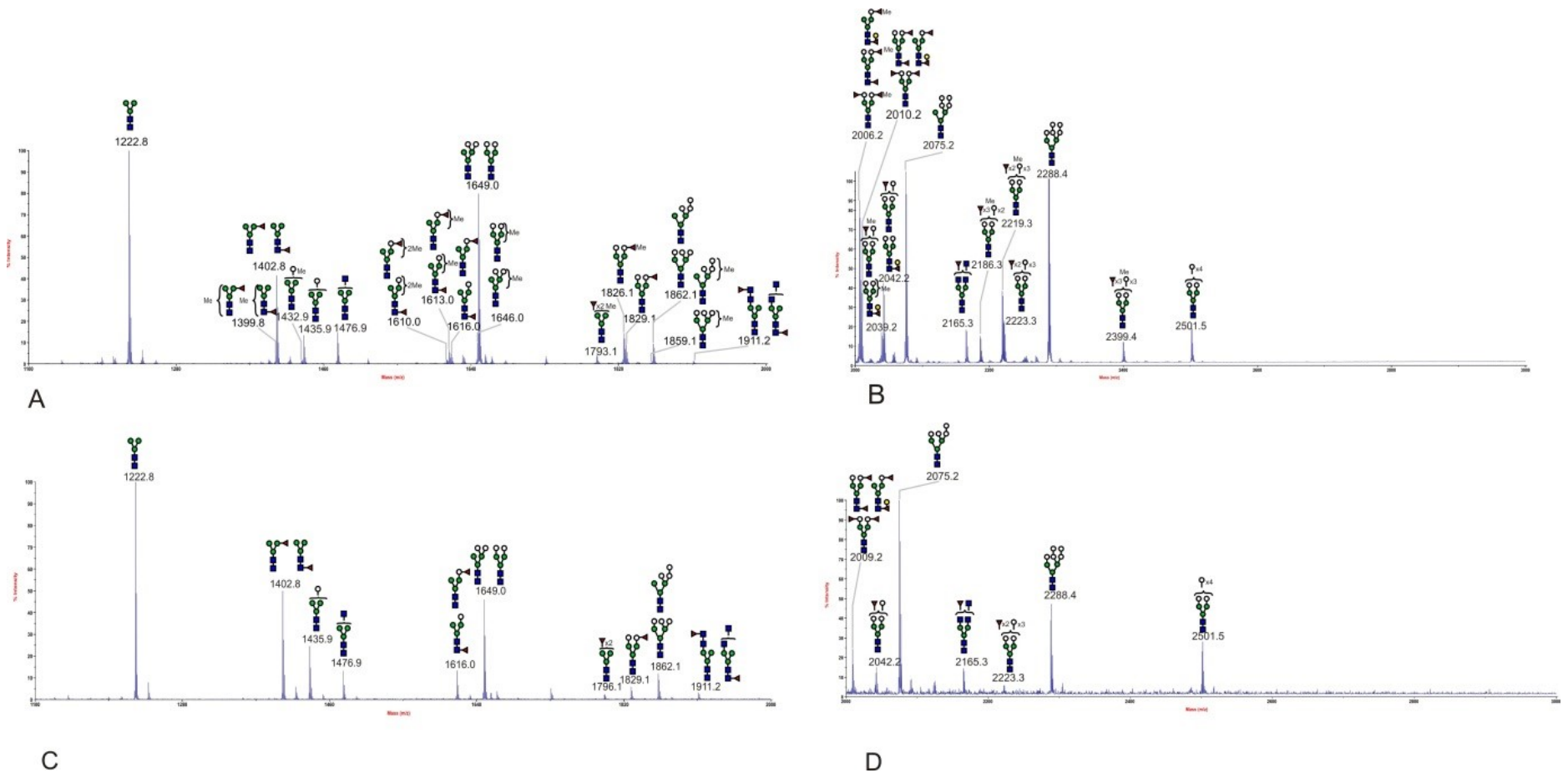
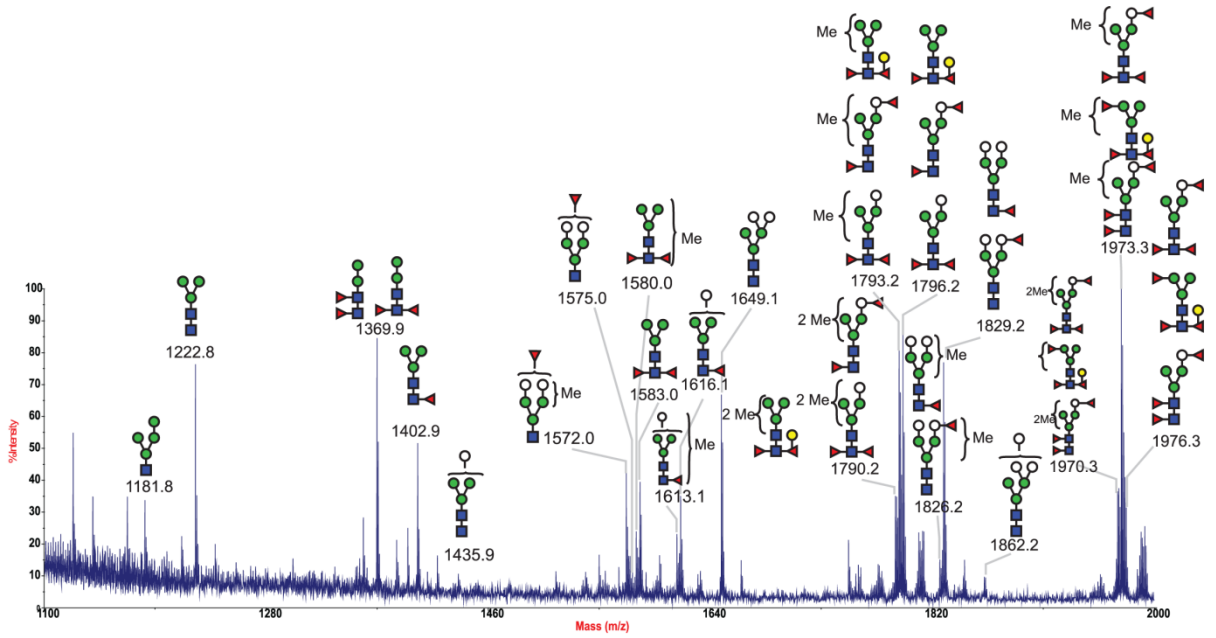
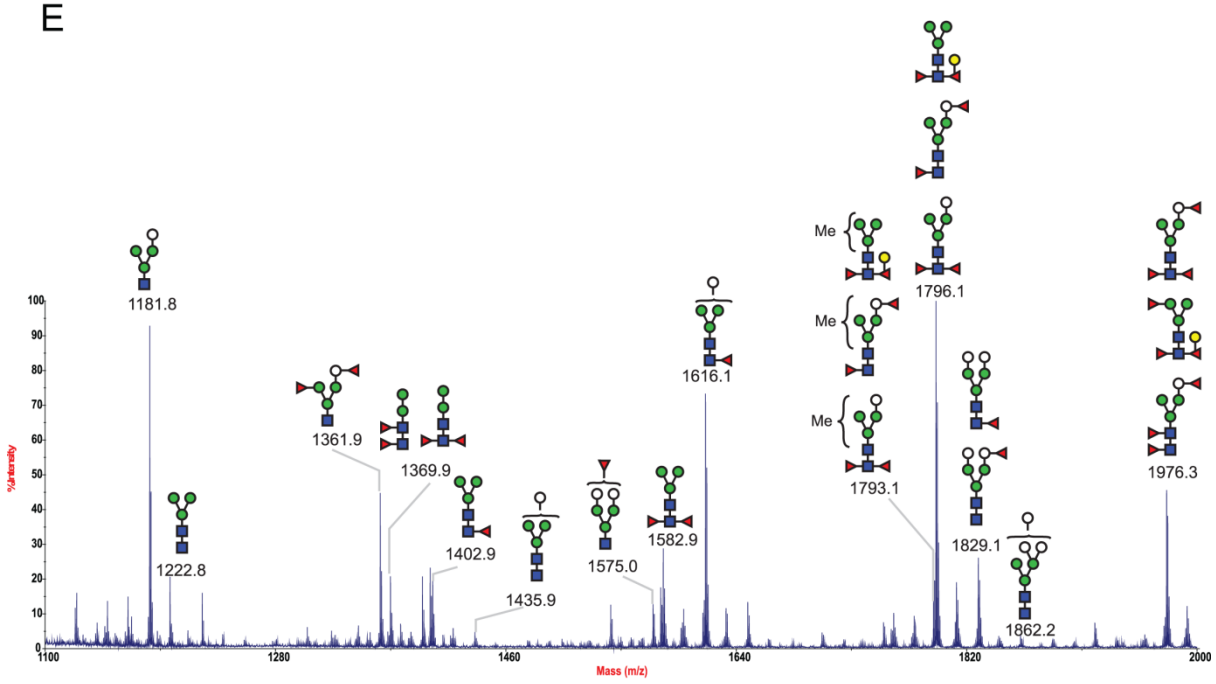


Figure S5. MALDI-TOF spectra of deuteromethylated wild-type and mutant *C. elegans* N-glycans released by PNGase digestion. N-Glycans were released from *C. elegans* tryptic glycopeptides by PNGase F and subsequent PNGase A digestion. Released glycans were deuteromethylated prior to analysis, all molecular ions are $[M+Na]^+$. Structural assignments are based on monosaccharide composition, MS/MS fragmentation analyses and knowledge of the glycan biosynthetic pathways. (A) N-glycans released from *C. elegans pmk-1(km25)*, m/z 1000-2000 by initial PNGase F digestion. (B) N-glycans released from *C. elegans pmk-1(km25)* by initial PNGase F digestion, m/z 2000-3000. (C) N-glycans released from *C. elegans samt-1(op532)pmk-1(km25)* by initial PNGase F digestion, m/z 1000-2000. (D) N-glycans released from *C. elegans samt-1(op532)pmk-1(km25)* by initial PNGase F digestion, m/z 2000-3000. (E) N-glycans released from *C. elegans pmk-1(km25)* by PNGase A after initial PNGase F digestion, m/z 1100-2000. (F) N-glycans released from *C. elegans samt-1(op532)pmk-1(km25)* by PNGase A after initial PNGase F digestion, m/z 1100-2000.


1222.8
1649.0
1402.8
1399.8
1432.9
1435.9
1476.9
1610.0
1613.0
1616.0
1646.0
1826.1
1829.1
1793.1
1862.1
1859.1
1911.2
A
2006.2
2010.2
2075.2
2288.4
2219.3
2186.3
2042.2
2039.2
2165.3
2223.3
2399.4
2501.5
B
1222.8
1402.8
1435.9
1476.9
1649.0
1616.0
1796.1
1829.1
1862.1
1911.2
C
2075.2
2009.2
2288.4
2042.2
2165.3
2223.3
2501.5
D
Hexose
Galactose
Mannose
N-acetylglucosamine
N-acetylgalactosamine
Fucose
Me Methyl group



E



F

Hexose Galactose Mannose N-acetylglucosamine N-acetylgalactosamine Fucose **Me** Methyl group

Figure S6. MALDI-TOF spectra of deuteromethylated wild-type and mutant *C. elegans* O-glycans released by reductive elimination. O-glycans were released from *C. elegans* tryptic glycopeptides by reductive elimination after release of N-glycans by digestion with PNGase F and PNGase A. (A) O-glycans released from *C. elegans pmk-1(km25)*, m/z 500-2000. (B) O-glycans released from *C. elegans samt-1(op532)pmk-1(km25)*, m/z 500-2000.

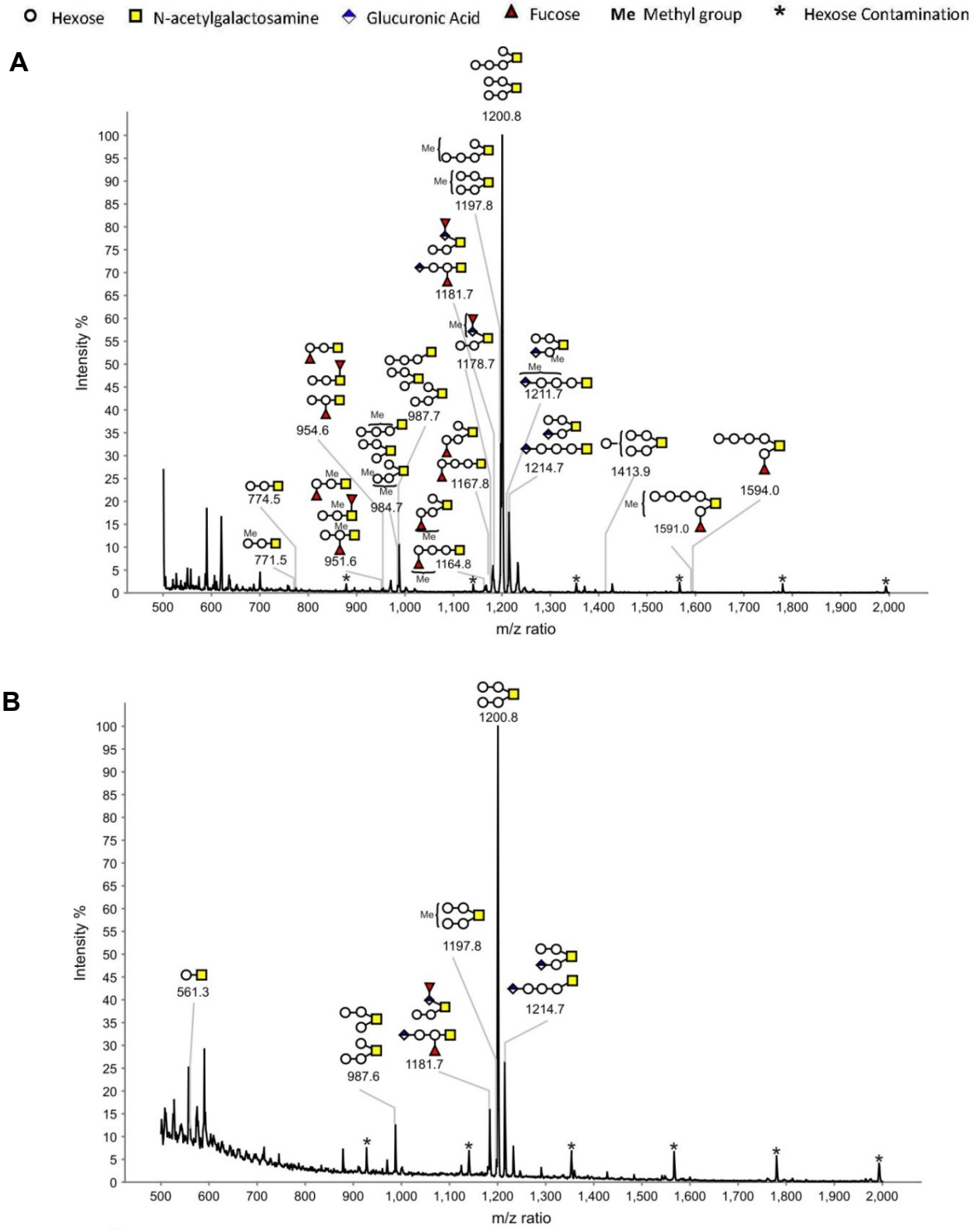


Figure S7. O-methylation-dependent agglutination of *E. coli* by recombinant Lb-Tec2. Purified Lb-Tec2 at the final concentration indicated was added to *E. coli* O8:K$^-$, *E. coli* O8$^-$:K$^-$ and *E. coli* O9a:K$^-$ in a 96 well plate. BSA was used as a negative control.

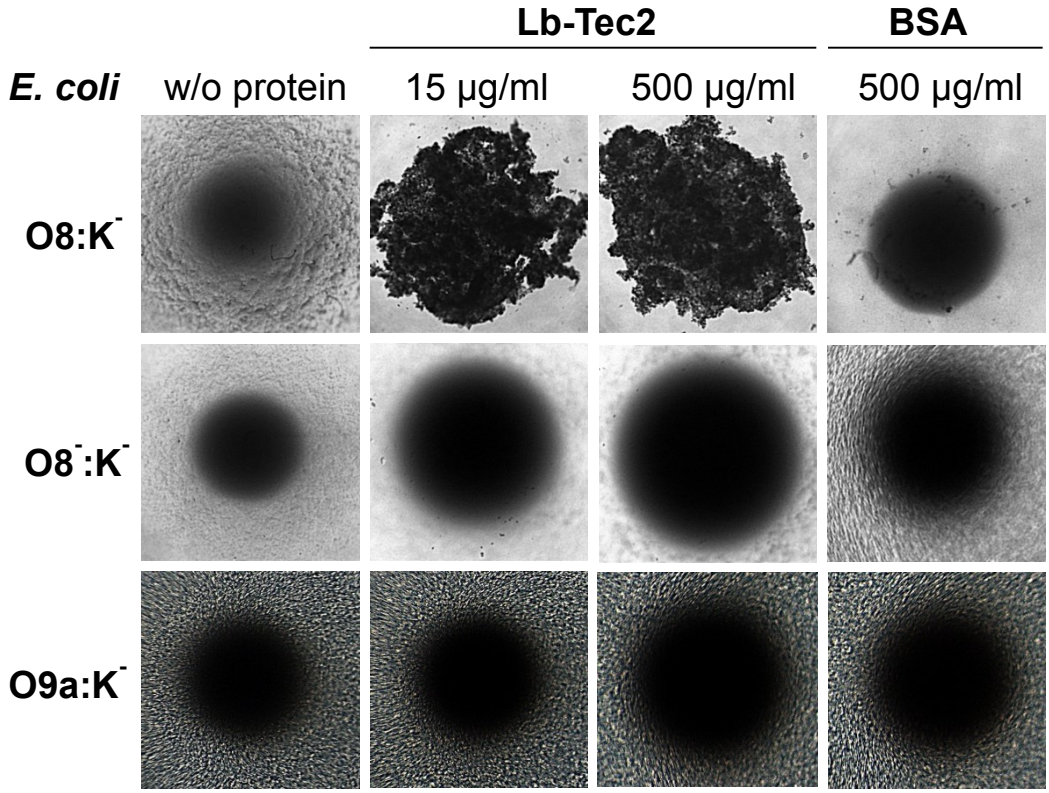


Table S1. Genbank Identifiers (GI) of analyzed Tectonin sequences shown in Fig 1.

Designation in Fig. 1	GI
L.bicolor_Tec2	170090420
L.bicolor_Tec1	170093369
N.dassonvillei	297560837
S.roseosporus	493083551
S.zinciresistens	494763290
Frankia_sp.	497423668
P.polycephalum_II	8134732
P.polycephalum_I	8134731
E.fluviatilis	83715952
T.tridentatus_L6	8134531
C.rotundicauda	55775500
T.tridentatus_TLP	6778723
T.tridentatus_GBP	8347764
S.domuncula	34368388
C.carpio	55976183
O.fasciatus	375331860
X.laevis	379046837
H.sapiens_Leuko	223016919
C.mydas	465976228
H.sapiens_TCPR1	32698704

Table S2. Microcalorimetry titration data for the binding of Lb-Tec2 to allyl monosaccharides. The stoichiometry was fixed (N = 6), due to the low solubility of Lb-Tec2 and the low affinity nature of its interaction with the ligands tested. Therefore, the thermodynamic parameters entropy (S) and enthalpy (H) should be considered with care (12) and are completely omitted for c-values below 0.01, (c = [protein]/K_d). Individual titrations of Lb-Tec2 with O-methylated and unmethylated ligands are shown in Fig. 6.

	Lb-Tec2 [µM]	**ligand [mM]**	**N**	**K_a (M^{-1})**	**H (kcal/mol)**	**TdS (kcal/mol)**	**K_d (mM)**	**c**
Allyl mannoside (**2**)	134	10	6		no binding			
	134	28	6		no binding			
Allyl 3-O-methyl mannoside (**3**)	134	28	6	52.0	-5.54	-3.19	19.2	0.007
	300	28	6	45.7	-5.63	-3.37	21.9	0.014
	40	28	6	48.6	-	-	20.6	0.002
		average:	**6**	**48.8**	**-5.59**	**-3.28**	**20.6**	
		st.dev.:		1.7	-	-	0.8	
Allyl fucoside (**5**)	134	28	6	10.0	-	-	100.2	0.001
	328	28	6	20.2	-	-	49.5	0.007
		average:	**6**	**15.1**	**-**	**-**	**74.9**	
Allyl 2-O-methyl-fucoside (**8**)	134	28	6	248	-4.13	-0.86	4.03	0.033
	252	28	6	234	-4.58	-1.34	4.27	0.059
	300	12	6	346	-3.91	-0.45	2.89	0.104
	40	28	6	155	-	-	6.45	0.006
		average:	**6**	**246**	**-4.21**	**-0.88**	**4.41**	
		st.dev.:		78	0.34	0.45	1.49	
Allyl 3-O-methyl-fucoside (**9**)	239	28	6	48	-5.14	-2.83	20.75	0.012
	191	28	6	49	-3.79	-1.48	20.41	0.009
		average:	**6**	**48.6**	**-4.47**	**-2.16**	**20.6**	

Table S3. Characteristics of *C. elegans* glycosylation mutants used in this study.

Designation	Characteristics	Reference
Bristol N2	wildtype	(13)
fut-8(ok2558)	Defective in α1,6- fucosylation of proximal core GlcNAc residue in N-glycans	(14)
fut-1(ok892);fut-8(ok2558)	Defective in α1,3- and α1,6-fucosylation of proximal core GlcNAc residue in N-glycans	(14)
fut-6(ok475);fut-8(ok2558)	Defective in α1,3-fucosylation of distal and α1,6-fucosylation of proximal core GlcNAc residue in N-glycans	(14)
bre-3(ye26)	Defective in β1,4-mannosylation of core glucose in arthroseries of glycosphingolipids (Egghead activity)	(15)
gly-14(id48);gly-12(id47)gly-13(ok712)	Defective in β1,4-GlcNAcylation of α1,3-branch of N-glycans (GNTI-activity) and thus the buildup of complex N-glycans	(16)
aman-2(tm1078)	Defective in removing α1,3- and α1,6-linked mannoses from the α1,6-branch of N-glycans (Golgi-mannosidase II activity) and thus the buildup of complex N-glycans	(17)
hex-3(tm2725);hex-2(tm2530)	Defective in removing GlcNAc from α1,3-branch of N-glycans (Hexosaminidase activity) and hypersensitive to lectins targeting N-glycan core modifications	(18)
pmk-1(km25)	Defective in p38 MAPK pathway and hypersensitive to many abiotic and biotic stresses	(19)
samt-1(op532)pmk-1(km25)	Defective in hypothetical Golgi-SAM transporter necessary for O-methylation of glycans in *pmk-1(km25)* background	This study
fut-6(ok475)fut-1(ok892);pmk-1(km25)	Defective in α1,3- fucosylation of proximal and distal core GlcNAc residue in N-glycans in *pmk-1(km25)* background	(14, 20)
ger-1(op499);pmk-1(km25)	Defective in the conversion of GDP-mannose to GDP-fucose in *pmk-1(km25)* background	(11, 21)
pmk-1(km25)bre-1(op509)	Defective in the conversion of GDP-mannose to GDP-fucose in *pmk-1(km25)* background	(21, 22)
pmk-1(km25);galt-1(op497)	Defective in the β1,4-galactosylation of the α1,6-linked fucose on the proximal core GlcNAc of N-glycans in *pmk-1(km25)* background	(11, 23)

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