Methylated glycans as conserved targets of animal and fungal innate defense

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Effector proteins of innate immune systems recognize specific non-self epitopes. Tectonins are a family of β-propeller lectins conserved from bacteria to mammals that have been shown to bind bacterial lipopolysaccharide (LPS). We present experimental evidence that two Tectonins of fungal and animal origin have a specificity for O-methylated glycans. We show that Tectonin 2 of the mushroom Laccaria bicolor (Lb-Tec2) agglutinates Gram-negative bacteria and exerts toxicity toward the model nematode Caenorhabditis elegans, suggesting a role in fungal defense against bacteria and nematodes. Biochemical and genetic analysis of these interactions revealed that both bacterial agglutination and nematotoxicity of Lb-Tec2 depend on the recognition of methylated glycans, namely O-methylated mannose and fucose residues, as part of bacterial LPS and nematode cell-surface glycans. In addition, a C. elegans gene, termed samt-1, coding for a candidate membrane transport protein for the presumptive donor substrate of glycan methylation, S-adenosyl-methionine, from the cytoplasm to the Golgi was identified. Intriguingly, limulus lectin L6, a structurally related antibacterial protein of the Japanese horseshoe crab Tachypleus tridentatus, showed properties identical to the mushroom lectin. These results suggest that O-methylated glycans constitute a conserved target of the fungal and animal innate immune system. The broad phylogenetic distribution of O-methylated glycans increases the spectrum of potential antagonists recognized by Tectonins, rendering this conserved protein family a universal defense armory.

Significance

Defense mechanisms against predators, parasites, and pathogens are a hallmark of all multicellular life forms. A conserved defense mechanism is the production of toxic proteins. Because of the limited number of innate defense effectors in a specific host organism, the target epitopes of such toxins are usually highly conserved or occur in different molecular contexts to cover a large spectrum of antagonists. Because glycan epitopes are part of different surface-displayed glycoconjugates in different organisms, carbohydrate-binding proteins (lectins) are the prevailing type of protein toxins in many multicellular organisms. Here we provide evidence that defense lectins can be specific for secondary glycan modifications, such as O-methylation, thereby broadening the range of target organisms.


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C. elegans gene coding for a member of the major facilitator superfamily conferred resistance to Lb-Tec2 and resulted in the lack of methylated N- and O-glycans. We hypothesize that the encoded protein, termed SAMT-1, is required for the transport of the donor substrate for glycan O-methylation, S-adenosyl-methionine (SAM), from the cytoplasm to the Golgi lumen.

Results

Tectonins from L. bicolar Are Related to Animal Proteins Involved in Innate Immunity Against Bacteria. The genome of the ectomycorrhizal mushroom L. bicolar encodes several predicted Tectonins (GenBank accession nos. XP_001877906.1, XP_001876432.1, XP_001876691.1, and XP_001875654.1). Of these, Tectonin 1 (Lb-Tec1) (XP_001877906.1) and Tectonin 2 (Lb-Tec2) (XP_001876432.1) each consists of six tandemly arranged Tectonin domains (Fig. 1B). Both proteins lack a signal sequence and are therefore predicted to be cytoplasmic. Transcription of the gene coding for Lb-Tec2 is 18-fold up-regulated in fruiting bodies compared with vegetative mycelium, whereas the expression of Lb-Tec1 is only 3-fold induced (14). In addition, transcription of Lb-Tec2 is up-regulated in L. bicolar vegetative mycelium upon challenge with several rhizobacteria (15). Similar expression patterns have been observed for lectins involved in fungal defense against predators and parasites (3).

Tectonins have been identified and characterized in slime molds as well as in animals ranging from invertebrates to humans (10), and genes coding for Tectonins have been discovered in the genomes of various, mostly multicellular (filamentous), bacteria. The L. bicolar Tectonins show highest homology to the predicted Tectonins of filamentous actinobacteria and to Tectonin I and II of the slime mold P. polycephalum (Fig. 1A). More distantly related homologs of fungal Tectonins are found in marine invertebrates (arthropods and sponges) as well as vertebrates (fishes, frogs, reptiles, and mammals). Among fungi, Tectonin-encoding genes occur mainly in the genomes of agaricomycetes, including the ectomycorrhizal species L. bicolor, Laccaria amethystina, Paxillus rubicundulus, Hebeloma cylindrosporum, Cortinarius glucopus, and the saprobic species Galerina marginata, but are also found in the genome of the glomeromycete Rhizophasis irregularis (Glomus intraradices), an arbuscular mycorrhizal species. No homologous genes have been identified in ascomycetes, zygomycetes, or chytrids to date. Sequencing of further fungal genomes will clarify the distribution of Tectonins within the fungal kingdom. Like the characterized Tectonins from slime mold, sponge, and horseshoe crab (5, 7–9, 13, 16) and the predicted Tectonins from filamentous bacteria, fungal Tectonins consist of multiple Tectonin domains and lack a signal sequence for secretion (Fig. 1B).

A multiple sequence alignment of an individual Tectonin domain (underlined by a green box in Fig. 1B) of each protein included in our phylogenetic analysis is shown in Fig. 1C. The conserved amino acid residues are in accordance with previous reports (5, 10). In contrast to their animal homologs, L. bicolar and most bacterial Tectonins do not contain any cysteine residues. The repetitive sequences of Tectonins are most closely related to WD proteins, suggesting that they form β-propeller structures (17). Accordingly, Lb-Tec2 is predicted by the structure prediction program Phyre2 (18) to adopt a six-bladed β-propeller. However, neither is a crystal structure of a Tectonin available nor has lectin function of such a protein unequivocally been demonstrated to date.

Lb-Tec2 Is Toxic Toward C. elegans, and This Toxicity Depends on Binding to N-Glycans. Lb-Tec2 shares several properties with fungal defense lectins, such as small size, predicted cytoplasmic localization, presumptive binding to carbohydrates, and induction in the fruiting body or upon bacterial challenge. We therefore assessed a potential function of Lb-Tec2 in fungal defense against insects and nematodes. For this purpose the corresponding cDNA was generated and cloned in an Escherichia coli expression vector, and soluble recombinant Lb-Tec2

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Fig. 1. Phylogenetic analysis of Tectonin proteins. (A) Phylogenetic tree of previously characterized Tectonins (marked with an asterisk) and selected animal, fungal, and bacterial homologs. GenBank identifiers of the analyzed protein sequences are listed in SI Appendix, Table S1. Numbers at the nodes indicate the level of confidence for the branches as determined by bootstrap analysis. Branches with confidence lower than 0.6 were collapsed. (B) Domain architecture of selected Tectonins predicted by SMART. Tectonin domains are depicted in green. Dark green boxes below the schemes indicate the domains used for the alignment in C. DysFC, Dysferlin domain, C-terminal region; DysFN, Dysferlin domain, N-terminal region. Pink box, low-complexity red box, signal sequence for the classical secretion pathway. (C) Multiple sequence alignment of an individual Tectonin domain of the proteins included in the phylogenetic tree in A. The location of the aligned domain within the protein is indicated by the positions of the flanking amino acids. The darkness of the color of specific amino acid residues indicates the degree of conservation.
was obtained upon expression in *E. coli* BL21 at 23 °C for 16 h. Attempts to purify His-tagged Lb-Tec2 via nickel-Sepharose revealed binding of the protein to the Sepharose CL-6B matrix, which is a cross-linked agar polysaccharide consisting of d-galactose β1,4-linked to 3,6-anhydro-d-galactose. Thus, Sepharose binding was a first indication of a carbohydrate-binding function of Lb-Tec2. Interestingly, binding to Sepharose CL-6B has also been reported for L6 and GBPs (7, 8, 16).

We next tested recombinant Lb-Tec2 for toxicity against *Drosophila melanogaster, Aedes aegypti*, and *C. elegans* using previously described assays (19–21). Recombinant Lb-Tec2 was not toxic for the tested insects (*SI Appendix, Fig. S1*) but displayed significant toxicity toward *C. elegans* N2, impairing the development of 100% of the larvae (Fig. 2D). In this assay, *C. elegans* L1 larvae (L) failed to develop to the L4 stage when fed *E. coli* BL21 expressing Lb-Tec2. To investigate whether the observed nematotoxicity was glycanc-dependent, we studied the effect of Lb-Tec2 on established *C. elegans* N-glycosylation mutants (Fig. 2A). *C. elegans* fut-8 (ok2558) as well as the double mutants fut-6(ok475)hut-1(ok892) and fut-6(ok475)hut-8(ok2558), lacking fucose residues linked to the GlcNAc units of the N-glycan core (22–24), were equally susceptible to Lb-Tec2 as the wild-type strain. We therefore concluded that α1,3- or α1,6-linked core fucose was not required for Lb-Tec2-induced nematotoxicity. Similarly, *C. elegans* bre-3 (ye26), lacking extended glycosylation modules (25), was susceptible to the toxin. Also, in a biochemical assay to assay intoxication by Lb-Tec2, indicating that glycolipids are not targets of fungal Tectonin. In contrast, the *C. elegans* triple mutant yu-14(id48)yg-12(id47)yj-13(ok712) and the single mutant aman-2(tm1078) were still susceptible to Lb-Tec2 but showed a considerably higher fraction of L4 staged larvae. In accordance with these results, the hex-3(tm2725); hex-2(tm2530) double mutant did not show any apparent susceptibility to Lb-Tec2 intoxication. Reduced toxicity toward these *C. elegans* mutants displaying altered structures of N-glycan antennae was evidence for a carbohydrate-dependent function of Lb-Tec2. To further establish whether Lb-Tec2 binds to additional glycosylation mutants of *C. elegans* with pmk-1 (km25) background. Similar to the N2 wild-type strain, *C. elegans* pmk-1 (km25), which was previously shown to be hypersensitive to different kinds of stress, including exposure to fungal lectins, due to a defective MAPK pathway (26), did not develop to the L4 stage when feeding on Lb-Tec2-expressing bacteria (Fig. 2B). As observed for the N2 background, Lb-Tec2 caused toxicity toward *C. elegans* fut-6(ok475)hut-1(ok892)pmk-1 (km25). In agreement with these results, a pmk-1 (km25);gal-1(op497) mutant lacking the Gal-1 β-galactosidase (PNGase) F is inhibited by α1,3-fucosylation of the N-glycan core (27) was also susceptible to the toxin. Finally, we tested *C. elegans* pmk-1 (km25);bre-1(op509) and ger-1(op499)pmk-1 (km25), two strains defective in the fusocyclosis biosynthesis pathway (28, 29). Both strains exhibited significant susceptibility toward Lb-Tec2, but this susceptibility was reduced compared with the pmk-1 (km25) strain, suggesting a role of fucose in nematotoxicity. As changes in fucosylation of the N-glycan core did not affect toxicity whereas the complete absence of fucose did reduce toxicity, we concluded that fucosylation of the antennae is at least partially required for susceptibility to Lb-Tec2. The enzyme transferring fucose to the antenna of N-glycans in *C. elegans*, however, is not known. Fucosyltransferases encoded by fut-2, fut-3, and fut-4 seem not to be involved in this process, as *C. elegans* single mutants in these genes were susceptible to Lb-Tec2 (*SI Appendix, Fig. S2*). However, redundancy of the enzymes cannot be excluded.

In addition to *C. elegans* toxicity assays with Lb-Tec2-expressing bacteria, we performed feeding experiments with purified, fluoro-resently labeled Lb-Tec2, 5(6)-Carboxytetramethylrhodamine (TAMRA)-Lb-Tec2 bound to the intestine of *C. elegans* N2 (*SI Appendix, Fig. S3*), displaying a staining pattern that was previously observed with several nematotoxic fungal lectins (20, 26). Unlike any other of these lectins, TAMRA–Lb-Tec2 also bound to the surface of adult *C. elegans*, lighting up specific structures, such as annuli and alae, of the nematode (Fig. 2C). Interestingly, surface staining correlated with impaired movement of the nematodes that was previously described as skiddy phenotype (30) (*Movies S1 and S2*).

**Deficiency in a Transporter of the Major Facilitator Superfamily Leads to Lb-Tec2 Resistance of *C. elegans***. To identify additional genes involved in the biosynthesis of the Lb-Tec2 target epitope in *C. elegans*, we performed a forward genetic screen for Lb-Tec2-resistant mutants. For this purpose, a Mos1-mediated transposition mutagenesis was carried out on a population of *C. elegans* pmk-1 (km25) and mutant nematodes were screened for absence of Lb-Tec2-induced skiddy movement. Indeed, one *C. elegans* mutant, carrying a Mos1 insertion (op532) in gene Y54G2A.4 (samt-1) encoding a protein of the major facilitator superfamily (MFS1), was unaffected in its movement by exposure to Lb-Tec2. In addition, this mutant was completely resistant for Lb-Tec2-induced toxicity (Fig. 2B) and was not stained with TAMRA-labeled Lb-Tec2 (Fig. 2C). We concluded that the *samt-1* gene product was necessary for the biosynthesis of the Lb-Tec2 target epitope on *C. elegans* N-glycan antennae. However, as the function of the identified MFS1 transporter was not known, the target epitope of Lb-Tec2 remained unclear.

**Lb-Tec2 Binds to N-Glycans of *C. elegans* Proteins In Vivo**. To confirm N-glycans as the possible target of Lb-Tec2 by other means, we used a similar biochemical approach. For this purpose, recombinant Lb-Tec2 was purified by affinity chromatography using Sepharose CL-6B as a matrix and 0.4 M GlcNAc as an eluent according to procedures that had previously been described for L6 and GBPs (7, 8, 16).

To assay binding of Lb-Tec2 to nematode N-glycoproteins, protein extracts from *C. elegans* pmk-1 (km25) and *samt-1* (op532) pmk-1 (km25) were probed with biotinylated Lb-Tec2 in a lectin blot (Fig. 3A). In this assay, Lb-Tec2 bound to several proteins from *C. elegans* pmk-1 (km25) migrating at around 250 kDa by SDS/PAGE. In contrast, no binding was observed in the case of proteins extracted from the resistant *samt-1* (op532) pmk-1 (km25) strain. A prominent band at around 73 kDa was also present in the negative control not incubated with the biotinylated lectin and therefore did not arise from Lb-Tec2 binding. Based on these results, we concluded that Lb-Tec2 recognizes a *samt-1*-dependent modification that is present on many *C. elegans* proteins. To confirm the genetic data suggesting N-glycans as targets of Lb-Tec2, we assessed binding to *C. elegans* proteins that had been deprived of N-glycans enzymatically. As peptidy1-N-glycosidase (PNGase) F is inhibited by α1,3-fucosylation of the N-glycan core, a modification highly abundant in nematodes, we took advantage of the *C. elegans* double mutant fut-6(ok475)hut-1(ok892) lacking α1,3-core fucosylation (20). In agreement with the genetic data, in vitro binding of Lb-Tec2 to *C. elegans* proteins was not affected by this alteration in N-glycan structure (Fig. 3B). However, de-N-glycosylation of *fut-6* (ok475)/ *fut-1* (ok892) proteins by PNGase F resulted in the loss of Lb-Tec2 binding. The absence of binding was not due to protein degradation, as a mock treatment of the protein extract without PNGase F did not affect binding of Lb-Tec2.

Despite the partial resistance of *C. elegans* N-glycosylation mutants to Lb-Tec2 (Fig. 2A), glycan array analysis of purified Lb-Tec2 did not reveal binding to any structure present on the mammalian glycan array of the Consortium for Functional Glycomics (*Dataset S1*). We concluded that Lb-Tec2 targeted a modification of N-glycans on *C. elegans* proteins that was not present on mammalian N-glycans and that the *samt-1* gene product was required for the biosynthesis of this modification.

*C. elegans* pmk-1 (km25)*samt-1* (op532) Mutant Lacks O-Methylation of Glycans. To identify this modification, we made use of the identified Lb-Tec2-resistant *C. elegans* mutant. First, we performed a structural analysis of N- and O-glycans of *C. elegans* pmk-1 (km25) and *samt-1* (op532) pmk-1 (km25) strains by MALDI-TOF-MS and TOF-TOF-MS/MS. To be able to detect glycan modifications, including the previously described O-methylation of *C. elegans*
were performed using the Mann–Whitney U test (**P < 0.01). (B) Development of C. elegans pmk-1(km25) strain (wild type) and various glycosylation mutants with pmk-1(km25) background feeding on Lb-Tec2-expressing E. coli (dark gray) or empty pET24 vector containing E. coli (light gray) (n = 5). Error bars indicate SD. Comparisons between Lb-Tec2 and vector control for each C. elegans strain were performed using the Mann–Whitney U test (**P < 0.01). (C) Development of C. elegans wild type (N2) and various glycosylation mutants feeding on Lb-Tec2-expressing E. coli (dark gray) or empty pET24 vector containing E. coli (light gray) (n = 5). Characteristics of the studied mutants are summarized in SI Appendix, Table S3. Error bars indicate SD. Comparisons between Lb-Tec2 and vector control for each C. elegans strain were performed using the Mann–Whitney U test (**P < 0.01).

To confirm the results, we performed a monosaccharide analysis of C. elegans N-glycans. N-glycans were released from trypticized protein extracts using PNGase F, and monosaccharide composition was determined by HPLC analysis in comparison with standard monosaccharides (Fig. S5 and SI Appendix, Fig. S4). We observed minimal amounts of 3-O-methyl-mannose in C. elegans. In addition, we detected small amounts of 2-O-methyl-fucose, as had previously been reported (31). Consistent with the results of the structural analysis, Lb-Tec2-resistant samt-1(op532)pmk-1(km25) did not contain any 3-O-methyl-mannose or 2-O-methyl-fucose. The increase of fucose in samt-1(op532)pmk-1(km25) compared with the wild-type strain was in accordance with the absence of the methylated monosaccharide, indicating that fucose biosynthesis itself was not affected by the mutation. Residual amounts of fucose were present in pmk-1(km25)bre-1(op590) and ger-1 (op499) pmk-1(km25), possibly due to residual gene function or utilization of fucose of bacterial origin in these mutants.

Taken together, these results strongly suggested that the nematotoxicity of Lb-Tec2 depended on the binding of the lectin to O-methylated mannose or fucose residues on the antennae of C. elegans N-glycans.

Lb-Tec2 Binds to 3-O-Methylated Mannose and 2-O-Methylated Fucose. To obtain direct evidence for Lb-Tec2 binding to methylated glycans, microcalorimetry with methylated allyl-monomosaccharides in comparison with nonmethylated monosaccharides was performed. Due to the low solubility (<350 μM) and low affinity of Lb-Tec2, titrations with low c values were performed. These titrations required a fixed N value and yielded reliable K_d values, whereas enthalpy and entropy values were of reduced reliability (32). Based on the six-bladed propeller structure predicted by Phyre2 and the fact that β-propeller lectins usually possess one binding site per blade, six binding sites were assumed for the lectin in a one-binding site model. Lb-Tec2 bound to allyl 3-O-methyl-mannoside (3) and allyl 2-O-methyl-fucoside (8) with an average K_d value of 21 and 4 mM, respectively (Fig. 6). No binding was observed to allyl mannoside (2), whereas low binding (K_d value of 75 mM) was detected to allyl fucoside (5). A fivefold higher K_d value was observed for allyl 3-O-methyl-fucoside (9) compared with allyl 2-O-methyl-fucoside (8), demonstrating a certain specificity of Lb-Tec2. The obtained microcalorimetry titration data are summarized in SI Appendix, Table S2.

Lb-Tec2 Agglutinates E. coli O8 Cells Comprising LPS with Terminal 3-O-Methylated Mannose. To further confirm the proposed carbohydrate specificity, we tested the lectin for its capacity to agglutinate a set of bacterial strains exhibiting defined LPS structures with regard to methylation (SI Appendix, Fig. S7). In many bacteria, the core oligosaccharide extending from lipid A is capped with a repeating unit glycan polymer known as the O-polysaccharide, or O-antigen. O-antigens are structures within a given species can vary significantly with regard to composition of...
Express cells showed significant toxicity toward Tec2. Due to solubility issues, we used the L6-coding region and expressed the protein in the E. coli strain Arcti-Tec2. For this purpose, we synthesized the L6-coding region out to test whether the distantly related Tectonin L6 from the C. elegans fut-6 (wild type) and pmk-1(km25) (samt-1) strains specifically bound to 3-O-methylated mannose. Therefore, we concluded that Lb-Tec2 specifically bound to 3-O-methylmannose.

**T. tridentatus L6 Has the Same Specificity as Lb-Tec2.** Finally, we set out to test whether the distantly related Tectonin L6 from the Japanese horseshoe crab T. tridentatus has the same specificity as Lb-Tec2. For this purpose, we synthesized the L6-coding region and expressed the protein in the E. coli cytoplasm analogous to Lb-Tec2. Due to solubility issues, we used the E. coli strain ArcticExpress and low temperature for expression. As a positive control and negative control, we expressed Lb-Tec2 under the same conditions. The E. coli agglutination assays were performed with soluble extracts instead of purified proteins because L6 could not easily be purified from the cytoplasmic fraction. Under the conditions used, both L6- and Lb-Tec2-containing extracts specifically agglutinated O8-K cells, whereas no agglutination was observed with extracts of empty vector-containing bacteria or any of the other E. coli strains (Fig. 7A). Accordingly, L6- and Lb-Tec2-expressing ArcticExpress cells showed significant toxicity toward C. elegans pmk-1 (km25) but not to pmk-1(op532)pmk-1(km25) (samt-1) mutant worms (Fig. 7B). These results implied that the animal Tectonin L6 had a similar activity and specificity as the fungal Tectonin Lb-Tec2.

### Discussion

The domain architecture of Tectonins is well-conserved throughout the different phyla (Fig. 1B), and Tectonins from both invertebrates and vertebrates have been associated with innate immunity. Lectin functions have been ascribed to this protein family, but this functionality was primarily based on Tectonin binding to complex glycans including Sepharose and LPS. A clear carbohydrate-binding specificity has been missing to date.

In this study, we demonstrate that two rather distantly related members of this protein family, Tectonin 2 of the mushroom *L. bicolor* and L6 of the horseshoe crab *T. tridentatus*, both bind specifically to O-methylated glycans. These results confirm the lectin function of this protein family and suggest that not only the domain architecture but also the carbohydrate-binding specificity is conserved among the different family members. This conservation can be explained by strong selection pressure or a rather recent horizontal transfer of the corresponding genes from animals to fungi (and bacteria). In agreement with the latter hypothesis, only a few fungal (and bacterial) genomes appear to contain Tectonin-encoding genes.

The relatively low affinity of Lb-Tec2 to allyl-3-O-methylmannose and allyl-2-O-methyl-fucose in vitro indicates that these monosaccharides are not sufficient for efficient binding of the lectin. The latter might be achieved by additional contacts of the carbohydrate-binding pocket of the lectin to subterminal glycan residues or by the presence of multiple methylated carbohydrate residues on the bound glycan. The importance of subterminal glycan residues has been demonstrated for many lectins, such as the fungal defense lectin CCL2, which was recently shown to bind with high affinity to the trisaccharide GlcNAc-β1,4-(Fucα1,3-) GlcNAc but had no detectable affinity to the monosaccharide fucose in isothermal titration calorimetry (20). Multivalency of the ligand, on the other hand, is highly relevant for the affinity of Tectonins to glycans, as these β-propeller lectins are expected to have multiple carbohydrate-binding sites. Accordingly, the affinity of another horseshoe crab β-propeller lectin, Tachylectin 2, which does not belong to the Tectonin superfamily, for BSA carrying multiple immobilized GlcNAc residues exceeded the affinity for free GlcNAc by three orders of magnitude (35, 36).

The lack of appropriate subterminal glycan residues might also explain the lack of Lb-Tec2 binding to PNGase F-treated *C. elegans* protein extracts (Fig. 3B) despite the presence of methylated O-glycans on these proteins (SI Appendix, Fig. S6). Alternatively, O-methylation of *C. elegans* O-glycans may occur on monosaccharides with a different configuration of the hydroxyl groups around the O-methylated position. In fact, 3-O-methylmannose and 2-O-methyl-fucose are very similar in this regard, which may explain why this lectin is able to bind to two different O-methylated monosaccharides. Testing of additional methylated monosaccharides, such as 4-O-methyl-mannose, for binding may reveal the pattern of hydroxyl groups recognized by Lb-Tec2. The molecular basis of the observed specificity should be resolved by the structural analysis of Lb-Tec2-β-propeller complexes. It will be interesting to see, for example, whether the coordination of this epitope occurs analogous to Anguilla anguilla agglutinin, where the methyl groups of either fucose or 3-O-methyl-galactose are bound via a hydrophobic pocket (37).

The predicted cytoplasmic localization of Lb-Tec2 is in accordance with its role in fungal defense, as fungivorous nematodes feed on the cytoplasm of fungal cells using a syringe-like stylet (38). Once ingested by the nematode, the lectin interacts with glycoproteins in the digestive tract of the fungivorous nematode. The relevance of the target glycan on the surface of the nematode is not clear, because the protein would have to be released from the fungal cytoplasm to bind to this target. The same issue applies to a possible role of Lb-Tec2 in fungal defense against bacteria. Thus far, this assumption is based on induction of Lb-Tec2 in vegetative mycelium of *L. bicolor* upon confrontation with an antagonistic soil bacterium and a mycorriza helper bacterium (15), agglutination of the E. coli O8:K strain.

**Fig. 3.** Binding of Lb-Tec2 to C. elegans N-glycans. (A) Silver-stained SDS/PAGE gel and lectin blot of protein extracts from C. elegans pmk-1(km25) (wild type) and pmk-1(op532)pmk-1(km25) (samt-1). (B) Silver-stained SDS/PAGE gel and lectin blot of protein extracts from C. elegans fut-6 (ok475)fur-1(ok892) after PNGase F digest. Dashes indicate negative controls not incubated with the lectin. Asterisks indicate unspecific bands.
by Lb-Tec2 (Fig. 7A), and bacterial agglutination and growth inhibition reported for animal Tectonins including limulus lectin L6 (7). Lb-Tec2 would certainly be in place for the interaction with bacteria invading the fungal cytoplasm. To agglutinate noninvasive bacteria, however, the protein would have to be released, for example upon lysis of the fungal cell by bacterial enzymes. In contrast, limulus lectin L6 is located in large granules of hemocytes in the horseshoe crab despite the lack of classical secretory signal in its protein sequence (7). Similar to the induction of Lb-Tec2 by bacteria, a fish Tectonin has been shown to be induced upon challenge with pathogenic bacteria (11).

The dual phenotype of C. elegans upon Lb-Tec2 treatment, skiddy movement and inhibition of larval development, is intriguing. The underlying mechanisms are unclear at the moment and were not addressed in this study. Based on preliminary results, we hypothesize that the skiddy phenotype is caused by cross-linking of the outer surface of the worm cuticle with the agar surface by the lectin. Movement of the worm then leads to stripping of the outermost layer of the cuticle, namely the surface coat, and concomitant loss of traction of the worm on the agar surface. Inhibition of larval development, on the other hand, is likely to be caused by cross-linking of glycoproteins on the luminal side of the intestinal epithelium, which leads to destruction of its microvillus structure and, as a consequence, loss of its function in nutrient uptake.

Our study sheds light not only on the function of Tectonins but also on the structure and biosynthesis of O-methylated glycans in nematodes. In C. elegans, O-methylated carbohydrate residues were shown to be present on N-glycans as 3- or 4-O-methylmannose and 2-O-methyl-fucose (31). We demonstrate that O-methylated carbohydrate residues are also present on O-glycans, although these residues were not bound by Lb-Tec2 (Fig. 3B and SI Appendix, Fig. S6). The physiological role of glycan methylation in nematodes is not known. The C. elegans samt-1(op532)pmk-1(km25) mutant lacking methylated N- and O-glycans did not display any phenotype under laboratory conditions. Preliminary results indicated a possible regulation of surface but not intestinal Lb-Tec2 target epitope expression upon developmental and environmental signals. It is therefore possible that the target epitopes on the cuticle and the intestine are different. Interestingly, methylated glycans of Toxocara canis have been associated with antigenicity of this parasitic nematode, as this epitope is absent from mammals. The excretory–secretory antigens of this parasitic nematode contain 2-O-methyl-fucose and 4-O-methyl-galactose (39).

Biosynthesis of methylated glycans is proposed to take place in the Golgi, where a methyl group is transferred from the substrate
SAM to the mature glycan. However, detailed studies of this process are missing. The products of this reaction are the O-methylated glycan and S-adenosyl-homocysteine. SAM, the donor substrate for various methylation reactions within the cell, is synthesized in the cytosol. Thus, availability of SAM in the lumen of the Golgi would require a specific transporter, as is the case for some nucleotide-activated monosaccharides. However, neither a Golgi SAM transporter nor a methyltransferase acting on glycans has been identified to date. A C. elegans forward genetic screen based on Lb-Tec2-induced skiddy movement and toxicity allowed for the identification of an MFS transporter required for methylation of glycans. The MFS family 1 is referred to as the sugar porter family, as substrates of many family members are mono- and disaccharides. However, also transporters of nonsugar molecules such as quinate, organocarboxylic acids, and inositol belong to this protein family (40). We propose that the identified transporter in C. elegans represents a Golgi SAM transporter. The respective gene was therefore named samt-1. Estereification of glucuronic acid residues with methyl groups in plant pectins is another SAM-dependent process taking place in the Golgi (41). Indeed, homologs of SAMT-1 are found encoded in plant genomes and may represent essential players for this common modification. SAM-dependent methyltransferases acting on animal glycans are still to be identified, as to date only a few bacterial and plant examples are known. The toxicity of methylation-specific Lb-Tec2 toward C. elegans may provide a tool for the identification of these enzymes in invertebrates.

O-methylated glycans have been reported in different phyla including bacteria (mainly as part of their LPS), fungi, amoebae, algae, plants, nematodes, and snails (42). Interestingly, methylated glycans have so far not been reported for insects or vertebrates. Methylation of glycans may, however, be more widespread than currently anticipated, as this modification cannot be detected when applying permethylation protocols during glycan analysis. Binding of Lb-Tec2 and its homologs to Sepharose is in accordance with reports of partially methylated galactose and galactans (2-O-, 4-O-, and 6-O-methyl-galactose residues as well as 3,6-anhydro-2-O-methyl-galactose) in this polysaccharide isolated from red seaweed (43).

So far, only a few proteins recognizing a specific modification of a glycan have been described, all of which have endogenous roles: P-type lectin recognizes terminal Man-6-P and has an important role in lysosomal targeting of glycoproteins (44), whereas Langerin binds to Gal-6-SO4 in sulfated proteoglycans and is involved in the modulation of innate immunity (45). In contrast, fungal Lb-Tec2 is a defense effector protein targeting a specific modification of exogenous glycans. The broad distribution of O-methylated glycans makes this epitope a perfect target of an innate immune system, as it allows a single type of effector to cope with a diversity of antagonists.

Materials and Methods

Strains and Cultivation Conditions. E. coli strain DH5α was used for cloning and plasmid amplification. For protein purification and biotization assays, proteins were expressed in E. coli strain BL21(DE3) and E. coli ArticExpress (DE3). E. coli strains O8:K+; O8:K–, O9a:K+, and O9a:K– were kindly provided by Chris Whitfield (University of Guelph, Guelph, Canada) (33, 34). E. coli was cultivated on LB medium as described (46). C. elegans strains were grown on nematode growth medium (NGM) plates or in liquid medium as described previously (47). Nematodes were harvested by sedimentation, washed with S-basal, and stored at –80 °C. The Bristol isolate N2 was used as the wild-type strain. Strains with genotypes pmk-1(km25), bre-3(e2e6), aman-2(tm1078), fut-1(ok892), fut-2(gk360), fut-2(gk509), fut-3(gk103), fut-4(gk111), fut-6(ok475), and fut-8(ok2558) were obtained from the Caenorhabditis Genetics Center at the University of Minnesota. The double mutants fut-6(ok475) fut-1 (ok892), fut-6(ok475) fut-8(ok2558), fut-1(ok892) fut-8(ok2558), and 3×3 (tm2725) hex-2(tm2530) were kindly provided by Iain Wilson (University of Natural Resources and Life Sciences [BOKU], Vienna). The triple mutant gly-14 (da48) gly-12 (da47) gly-13 (ok712) was kindly provided by Harry Schachter (University of Toronto, Toronto). The triple mutant fut-6(ok475) fut-1(ok892) pmk-1(km25) was generated by standard genetic crossing. Strains with genotype pmk-1(km25) bre-1(op509), ger-1(op499) pmk-1(km25), and pmk-1(km25) gait-1(op497) were generated by Maiz mutants generation of pmk-1(km25) worms as previously described (26). The samt-1(op532) pmk-1(km25) mutant was generated in this study by Maiz mutagenesis as previously described (26).

Cloning and Expression. The ORF encoding for Lb-Tec2 was amplified from genomic DNA of L. bicolor strain S23BN using the primers TectNdeIfwd 5′-GGGGCGGATATGGTGGAAATACTCGGC-3′ and TectSalIrev 5′-GGGCGGTGCAGCTTCACGAATTTCAACGGTGAATAACC-3′. The resulting fragment was ligated into the pET24a vector (Invitrogen) using the introduced restriction sites. For bacterial expression, E. coli BL21(DE3) transformed with the pET24-Lb-Tec2 construct was grown in LB medium supplemented with 50 μg/ml kanamycin. Expression was induced with 0.5 mM isopropyl-β-D-thiogalactoside (IPTG) at an OD600 of 1.0, and the culture was incubated at 33 °C for 16 h.

cDNA cloning for nematode lecin L 6 (7) was ordered from GenScript with two introduced restriction sites, an NotI site at the 5′ end and a NotI site at the 3′ end. Using these restriction sites, the cDNA was cloned into the pET24a expression vector (Invitrogen). For expression of proteins in E. coli ArticExpress (DE3) (Invitrogen), cells were transformed with either pET24a-Lb-Tec2 or pET24a-Lb-Tec2 and grown on LB medium according to the manufacturer's protocol. Expression was induced with 0.5 mM IPTG at an OD600 of 0.6 for 24 h at 10 °C. Expression and solubility of recombinant proteins were checked as previously described (19).

Purification of Recombinant Lb-Tec2. For purification of recombinant Lb-Tec2, induced cells were collected by centrifugation (15 min, 16,000 × g, 4 °C), washed with water, and shock-frozen in liquid nitrogen. The bacteria were resuspended in 0.1 M Hepes buffer (pH 7.5) containing 1 mM phenylmethylsulfonyl fluoride and lyzed in a French press (SLM Amino; SLM Instruments). The lysate was cleared by centrifugation (30 min, 38,000 × g, 4 °C) and the supernatant was applied to a Sepharose CL-6B (GE Healthcare) column equilibrated with 0.1 M Hepes (pH 7.5). After binding for 1 h at 4 °C, the flow-through was collected and the column was washed with 10 column volumes of 0.1 M Hepes (pH 7.5). Bound Lb-Tec2 was eluted with 0.4 M GlcNAC (Alfa Aesar) in Hepes buffer and desalted on a PD-10 column (Amersham Biosciences) or by dialysis. Purity of the protein was verified by SDS-PAGE, and the protein concentration was determined by measuring the absorbance at 280 nm, taking into account the extinction coefficient of Lb-Tec2. The purified protein was stable in Hepes (pH 7.5) and Tris (pH 8).

C. elegans Biotization Assays. Liquid toxicity assays with C. elegans feeding on E. coli BL21(DE3) or E. coli ArticExpress (DE3) expressing Lb-Tec2 or limulus lecin L6 or containing the empty vector were performed as described (20). Fractions were calculated from five independent experiments.

Wohlschlager et al.

PNAS Published online May 30, 2014 E2793
TAMRA Labeling of Lb-Tec2 and Fluorescence Microscopy of C. elegans. Purified Lb-Tec2 was labeled with TAMRA succinimidyl ester or Alexa Fluor 488 5-sulfodichlorophenol (SDP) ester (Molecular Probes) according to the manufacturer’s instructions and desalted on a PD-10 column (Amersham Biosciences). Nematodes were harvested from NGM plates, washed with S-basal (47), and incubated for 10 min in 50 μL S-basal containing 2 μg/mL TAMRA-labeled protein in the dark. Worms were washed three times with S-basal for 10 min each, anesthetized with levamisol (10 mM), and transferred to a glass slide coated with 1% agarose for microscopy. Phase-contrast and fluorescence images were acquired on a Zeiss Axiophot microscope using AxioVision 4.8 with a 10× or 20× objective.

Protein Extraction from C. elegans. Three hundred milligrams of nematodes was resuspended in 300 μL of ice-cold extraction buffer (PBS containing 1% Nonidet P-40 and Roche proteinase inhibitor mixture) in a 2-mL screw-cap tube. Two hundred microliters of acid-washed 0.5-mm glass beads (Sigma-Aldrich) was added and the worms were disrupted in a FastPrep FP120 (Thermo Savant) tissue and cell homogenizer (three times for 45 s at level 6, cooled on ice in-between).

Fig. 6. In vitro binding of Lb-Tec2 to O-methylated mannose and fucose. Indicated nonmethylated and O-methylated (Me) allyl(All)-mannoside (Man) and -fucoside (Fuc) were tested for binding to purified Lb-Tec2 by isothermal titration calorimetry. n.b., no binding observed. Errors indicate deviation of three independent titrations.
Enzymatic Removal of N-Glycans from *E. coli* ArcticExpress (DE3) expressing Lb-Tec2 or limulus lectin L6 or containing an empty vector pET24a plasmid were washed with 0.1 M Hepes buffer (pH 7.5) supplemented with 0.15 M NaCl and resuspended in 1 mL to an OD_{600} of 20. Cells were lysed with 1 g of beads in four consecutive homogenizations. A 1-h incubation at 4 °C. The homogenate was centrifuged at 12,000 × g for 30 min, and 40 μL of the supernatant was mixed with 10 μL bacteria. ArcticExpress (DE3) harboring an empty vector pET24a plasmid and 0.1 M Hepes buffer (pH 7.5) served as negative controls. Images were taken after a 1-h incubation at room temperature using a Leica MZ125 stereomicroscope.

**Microcalorimetry.** Isothermal titration calorimetry was performed on a MicroCal ITC200 (General Electric) and the data were analyzed using MicroCal Origin software. Lb-Tec2 (130–328 μM) in Hepes buffer (0.1 M Hepes, 150 mM NaCl, pH 7.5, 0.1% NaN₃) was placed in the sample cell at 25 °C. Details of the individual titrations are described in *SI Appendix.*

**Phylogenetic Analysis.** Amino acid sequences of *L. biour* Tectonin 2 homologs were retrieved from the National Center for Biotechnology Information. Sequences in were aligned using MUSCLE (49), multiple sequence alignments were curated by applying Gblocks (50), a phylogenetic tree was constructed with ProtDist/FastDist + Neighbor (PHYLIP) (51) with 1,000 bootstrap replicates under the Jones–Taylor–Thornton matrix, and tree rendering was performed with TreeDyn (52). All software was used at the Phylogeny.fr server (53). Domain prediction was performed with SMART (54, 55). Tectonin domains were aligned by MUSCLE, and the multiple sequence alignment was edited using Jalview (56). Structure prediction was performed on the Phyre2 server (18). Distribution of Tectonin proteins among fungi was analyzed using BLAST on the Joint Genome Institute fungal genome portal (57).

**Chemical Synthesis.** General and specific experimental details of the chemical synthesis of the various allyl-mannosides and -fucosides used in this study are described in *SI Appendix.* NMR spectra of synthetic glycosides are shown in Dataset S2.

**Statistical Analysis.** The statistical significance of the toxicity assays and the monosaccharide analysis was evaluated by pairwise comparisons using the nonparametric Mann–Whitney U test.

**ACKNOWLEDGMENTS.** We thank Chris Whitfield for providing *E. coli* strains O8′:K−, O8′:K−, O9a:K−′, and O9′:K− and Iain B.H. Wilson and Harry Schachter for providing the *C. elegans* strains. Simon Flückiger is acknowledged for *C. elegans* protein purification, and bacterial agglutination.

**Bacterial Agglutination Assay.** For assays shown in *SI Appendix, Fig. 57,* *E. coli* strains O8′:K−, O8′:K−, O9a:K−, and O9a:K−′ were grown in LB medium at 37 °C to an OD_{600} of 0.7–1, washed with PBS, and resuspended to an OD_{600} of 2.5. Agglutination assays were performed in a U-shaped 96-well microtiter plate. Ten microliters of bacteria was mixed with 40 μL Lb-Tec2 at a final protein concentration of 15–500 μg/mL. BSA was used as a negative control. Images were taken after a 10–20 min incubation at room temperature using a Leica MZ125 stereomicroscope.

For assays shown in Fig. 7, 50-μL cultures of *E. coli* ArcticExpress (DE3) expressing Lb-Tec2 or limulus lectin L6 or containing an empty vector pET24a plasmid were washed in 0.1 M Hepes buffer (pH 7.5) supplemented with 0.15 M NaCl and resuspended in 1 mL to an OD_{600} of 20. Cells were lysed with 1 g of beads in four consecutive homogenizations. A 1-h incubation at 4 °C. The homogenate was centrifuged at 12,000 × g for 30 min, and 40 μL of the supernatant was mixed with 10 μL bacteria. ArcticExpress (DE3) harboring an empty vector pET24a plasmid and 0.1 M Hepes buffer (pH 7.5) served as negative controls. Images were taken after a 1-h incubation at room temperature using a Leica MZ125 stereomicroscope.

**Structural Analysis of *C. elegans* N- and O-Glycans.** Details of the isolation and analysis of *C. elegans* N- and O-glycans by mass spectrometry are described in *SI Appendix.*

**Monosaccharide Analysis of *C. elegans* N-Glycans.** Details of the monosaccharide analysis of *C. elegans* N-glycans by HPLC are described in the *SI Appendix.*

***Microcalorimetry.*** Isothermal titration calorimetry was performed on a MicroCal ITC200 (General Electric) and the data were analyzed using MicroCal Origin software. Lb-Tec2 (130–328 μM) in Hepes buffer (0.1 M Hepes, 150 mM NaCl, pH 7.5, 0.1% NaN₃) was placed in the sample cell at 25 °C. Details of the individual titrations are described in *SI Appendix.*

**Phylogenetic Analysis.** Amino acid sequences of *L. biour* Tectonin 2 homologs were retrieved from the National Center for Biotechnology Information. Sequences in were aligned using MUSCLE (49), multiple sequence alignments were curated by applying Gblocks (50), a phylogenetic tree was constructed with ProtDist/FastDist + Neighbor (PHYLIP) (51) with 1,000 bootstrap replicates under the Jones–Taylor–Thornton matrix, and tree rendering was performed with TreeDyn (52). All software was used at the Phylogeny.fr server (53). Domain prediction was performed with SMART (54, 55). Tectonin domains were aligned by MUSCLE, and the multiple sequence alignment was edited using Jalview (56). Structure prediction was performed on the Phyre2 server (18). Distribution of Tectonin proteins among fungi was analyzed using BLAST on the Joint Genome Institute fungal genome portal (57).
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 \([\text{M+Na}]^+\) 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 glycobiinformatics 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 monosaccharides 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 (1H) or 101 MHz (13C). 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 1H, 1H-COSY, DEPT-135-edited 1H, 13C-HSQC and 1H, 13C-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% H₂SO₄) 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 CDCl₃ 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 (SiO₂/CH₂Cl₂/EtOH gradient of 3-20%) to give pure 2 (1.41 g, 58%). ¹H NMR (400 MHz, MeOH-d₄) δ 6.00-5.88 (m, 1H, allyl-CH), 5.33-5.26 (m, 1H, allyl-CH₂), 5.20-5.14 (m, 1H, allyl-CH₂), 4.80 (d, J = 1.7 Hz, 1H, H₁), 4.25-4.18 (m, 1H, allyl-CH₂), 4.05-3.95 (m, 1H, allyl-CH₂), 3.87-3.80 (m, 2H, H₂, H₆a), 3.75-3.68 (m, 2H, H₃, -6b), 3.62 (dd, J₁=J₂ = 9.5 Hz, 1H, H-4), 3.53 (ddd, J = 9.8, 5.7, 2.4 Hz, 1H, H-5). ¹³C NMR (101 MHz, MeOH-d₄) δ 135.39 (allyl-CH), 117.26 (allyl-CH₂), 100.60 (C₁), 74.60 (C₅), 72.54 (C₃), 72.09 (C-2), 68.77 (allyl-CH₂), 68.50 (C-4), 62.78 (C-6). HRMS: [C₉H₁₆O₆+Na]⁺ calcd: 243.08391 found: 243.08513. The ¹H 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-butylditinoxide (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, Mel (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 (SiO₂/CH₂Cl₂/EtOH gradient of 3-20%) to give pure 3 (267 mg, 40%) and recovered starting material 2 (305 mg, 49%). ¹H NMR (400 MHz, MeOH-d₄) δ 6.00-5.88 (m, 1H, allyl-CH), 5.35-5.26 (m, 1H, allyl-CH₂), 5.21-5.15 (m, 1H, allyl-CH₂), 4.83 (d, J = 1.8 Hz, 1H, H-1), 4.26-4.17 (m, 1H, allyl-CH₂), 4.05-3.97 (m, 2H, H-2, allyl-CH₂), 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, OCH₃), 3.37 (dd, J = 9.4, 3.3 Hz, 1H, H-3). ¹³C NMR (101 MHz, MeOH-d₄) δ 135.38 (allyl-CH), 117.43 (allyl-CH₂), 100.54 (C₁), 82.31 (C-3), 74.67 (C-5), 68.84 (allyl-CH₂), 67.91 (C-2), 67.38 (C-4), 62.83 (C-6), 57.35 (OCH₃). HRMS: [C₁₀H₁₈O₆+Na]⁺ calcd: 257.09956 found: 257.10046. The ¹H 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.

\[
\begin{align*}
\text{H NMR (400 MHz, MeOH-d4)} & \delta 6.02-5.90 \text{ (m, 1H, allyl-CH), 5.36-5.27 \text{ (m, 1H, allyl-CH}_2), 5.20-5.13 \text{ (m, 1H, allyl-CH}_2), 4.80 \text{ (d, J = 3.0 Hz, 1H, H-1), 4.20-4.13 \text{ (m, 1H, allyl-CH}_2), 4.06-3.99 \text{ (m, 1H, allyl-CH}_2), 3.95 \text{ (q, J = 6.9 Hz, 1H, H-5), 3.79-3.71 \text{ (m, 2H, H-2, H-3), 3.68-3.65 \text{ (m, 1H, H4), 1.21 \text{ (d, J = 6.6 Hz, 3H, H-6).}}}}
\end{align*}
\]

\[
\begin{align*}
\text{C NMR (101 MHz, MeOH-d4)} & \delta 135.73 \text{ (allyl-CH), 117.35 \text{ (allyl-CH}_2), 99.60 \text{ (C-1), 73.61 \text{ (C-4), 71.65 \text{ (C-2/3), 69.95 \text{ (allyl-CH}_2), 69.50 \text{ (C-2/3), 67.60 \text{ (C-5), 16.57 \text{ (C-6). HRMS: [C}_{9}\text{H}_{16}\text{O}_{5}\text{+Na}^+] \text{ calcd: 227.08899 found: 227.09026. The H NMR corresponds to the one reported by Unverzagt et al. (7), the C NMR corresponds to the selected signals reported in the same work.}}}}
\end{align*}
\]

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 NEt₃ (82 μl, 0.59 mmol) and the volatiles were removed in vacuo. Purification by column chromatography (SiO₂/petrol ether/EtOAc gradient of 3-40%) gave a endo-/exo-diastereomeric mixture of 6 (532 mg, 62%, d.r. 1:1). ¹H NMR (400 MHz, CDCl₃) 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-CH₂), 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, J₁ = J₂ = 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), 1.83 (d, J = 6.6 Hz, 3H, H-6), 1.37 (d, J = 6.7 Hz, 3H, H-6). ¹³C NMR (101 MHz, CDCl₃) 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-CH₂), 117.80 (allyl-CH₂), 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-CH₂), 68.67 (allyl-CH₂), 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, Mel (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 Mel (62 μl, 1.0 mmol) and the reaction was kept 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 NaHCO₃ (3 x 3 ml), dried over Na₂SO₄, 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. 1H NMR (400 MHz, CDCl₃) 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-CH₂), 5.28-5.15 (m, 2H, allyl-CH₂), 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), 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).

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 NaHCO₃ (15 ml) and extracted with EtOAc (5 x 10 ml). The combined organic layers were dried over Na₂SO₄, filtered and the volatiles were removed in vacuo. The residue (35.7 mg) was purified by column chromatography (SiO₂/petrol ether/EtOAc gradient of 35-75%) to give pure 8 (11.8 mg, 19% over 2 steps). 1H NMR (400 MHz, MeOH-d₄) δ 6.00-5.89 (m, 1H, allyl-CH), 5.36-5.28 (m, 1H, allyl-CH₂), 5.20-5.16 (m, 1H, allyl-CH₂), 4.99 (d, J = 3.8 Hz, 1H, H-1), 4.19-4.12 (m, 1H, allyl-CH₂), 4.05-3.98 (m, 1H, allyl-CH₂), 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) \(\delta\) 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-\(\alpha\)-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-butylditinoxide (329 mg, 1.32 mmol), a stirring bar and was dried under \textit{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, Mel (1.88 ml, 30.1 mmol) was added and the reaction was stirred for 66 h. The volatiles were removed \textit{in vacuo} and the residue was purified by column chromatography (SiO\(_2\)/CH\(_2\)Cl\(_2\)/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) \(\delta\) 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) \(\delta\) 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 \(\mu\)l), 19 injections of 2 \(\mu\)l 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).
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.
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 smt-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.

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

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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
_A
_d
). Individual titrations of Lb-Tec2 with O-methylated and unmethylated ligands are shown in Fig. 6.

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<td>6</td>
<td>346</td>
<td>-3.91</td>
<td>-0.45</td>
<td>2.89</td>
<td>0.104</td>
</tr>
<tr>
<td>average:</td>
<td>6</td>
<td>246</td>
<td>-4.21</td>
<td>-0.88</td>
<td>4.41</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>st.dev.:</td>
<td></td>
<td>78</td>
<td>0.34</td>
<td>0.45</td>
<td>1.49</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allyl 3-O-methyl-fucoside (9)</td>
<td>239</td>
<td>28</td>
<td>6</td>
<td>48.6</td>
<td>-4.47</td>
<td>-2.16</td>
<td>20.6</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>191</td>
<td>28</td>
<td>6</td>
<td>49</td>
<td>-3.79</td>
<td>-1.48</td>
<td>20.4</td>
<td>0.009</td>
</tr>
<tr>
<td>average:</td>
<td>6</td>
<td>48.6</td>
<td>-4.47</td>
<td>-2.16</td>
<td>20.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table S3. Characteristics of *C. elegans* glycosylation mutants used in this study.

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


Supporting Information

Wohlschlager et al. 10.1073/pnas.1401176111

**Movie S1.** Locomotion of *Caenorhabditis elegans pmk-1(km25)* on a lawn of Lb-Tec2–expressing *Escherichia coli* BL21(DE3) cells on nematode growth medium (NGM) agar.

**Movie S2.** Locomotion of *C. elegans samt-1(op532)pmk-1(km25)* on a lawn of Lb-Tec2–expressing *E. coli* BL21(DE3) cells on NGM agar.

**Dataset S1.** Carbohydrate-binding specificity of Tectonin 2 of the mushroom *Laccaria bicolor* as determined by glycan array analysis

The mammalian glycan array of the Consortium of Functional Glycomics was probed with 200 μg/mL Alexa Fluor 488-labeled Tectonin 2 of the mushroom *L. bicolor* (Lb-Tec2).

**Dataset S2.** NMR spectra of synthetic compounds 2–9
Other Supporting Information Files

SI Appendix (PDF)
120731DH48+48_DH116H2Fr2
DH116H2Fr2 AllMann
CARBON_296scales MeOD /DATA/topspin DH48 48

135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45

f1 (ppm)
120806DH48-11_DH120-2
DH120-2 All 2-Me-Fuc
CARBON_1024scans MeOD /DATA/topspin DH48 11