The Pepper Mannose-Binding Lectin Gene CaMBL1 Is Required to Regulate Cell Death and Defense Responses to Microbial Pathogens

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Plant mannos-binding lectins (MBLs) are crucial for plant defense signaling during pathogen attack by recognizing specific carbohydrates on pathogen surfaces. In this study, we isolated and functionally characterized a novel pepper (Capsicum annuum) MBL gene, CaMBL1, from pepper leaves infected with Xanthomonas campestris pv. vesicatoria (Xcv). The CaMBL1 gene contains a predicted Galanthus nivalis agglutinin-related lectin domain responsible for the recognition of high-mannose N-glycans but lacks a middle S-locus glycoprotein domain and a carboxyl-terminal PAN-Apple domain. The CaMBL1 protein exhibits binding specificity for mannone and is mainly localized to the plasma membrane. Immunoblotting using a CaMBL1-specific antibody revealed that CaMBL1 is strongly expressed and accumulates in pepper leaves during avirulent Xcv infection. The transient expression of CaMBL1 induces the accumulation of salicylic acid (SA), the activation of defense-related genes, and the cell death phenotype in pepper. The G. nivalis agglutinin-related lectin domain of CaMBL1 is responsible for cell death induction. CaMBL1-silenced pepper plants are more susceptible to virulent or avirulent Xcv infection compared with unsilenced control plants, a phenotype that is accompanied by lowered reactive oxygen species accumulation, reduced expression of downstream SA target genes, and a concomitant decrease in SA accumulation. In contrast, CaMBL1 overexpression in Arabidopsis (Arabidopsis thaliana) confers enhanced resistance to Pseudomonas syringae pv. tomato and Alternaria brassicicola infection. Together, these data suggest that CaMBL1 plays a key role in the regulation of plant cell death and defense responses through the induction of downstream defense-related genes and SA accumulation after the recognition of microbial pathogens.

Plants protect themselves against a wide range of pathogens, such as bacteria, fungi, and viruses, with numerous defense mechanisms, including a complicated innate immune system against pathogens (Chisholm et al., 2006; Jones and Dangl, 2006). Plants can distinguish between self and nonself or detect specific pathogens by recognizing pathogen-associated molecular patterns mostly generated or secreted from pathogens. Defense responses mediated via signal transduction pathways lead to the reinforcement of plant cell walls, the production of antimicrobial metabolites (phytoalexins) and pathogenesis-related (PR) proteins, and the hypersensitive response (HR), a form of programmed cell death at infection sites that limits pathogen development (Dangl and Jones, 2001; Mur et al., 2008). Signaling molecules, such as reactive oxygen species (ROS), ethylene, salicylic acid (SA), and jasmonic acid, play important roles in the complex signaling networks that activate defense mechanisms (Hammond-Kosack and Parker, 2003). Although the regulation and execution of cell death associated with the HR are not fully understood, the production of ROS, ion fluxes, defense-related gene activation, and the induction of signaling molecules have been proposed to be implicated in the cell death process (Torres and Dangl, 2005).

Plant cell walls are dynamic structures whose composition and architecture change during growth, development, and defense responses. Performed physical and chemical barriers, such as cell walls, and constitutively produced antimicrobial compounds protect plants against invading pathogens. Proteins embedded in the cell wall and plasma membrane are involved in a monitoring system that is required for the recognition and transduction of environmental, developmental, and defense-associated signals (Garcia-Brugger et al., 2008).
lar glycans. The potato (*Solanum tuberosum*) interaction with cell wall carbohydrates or extracellular bacteria through an indirect mechanism, based on an 

Several plant lectins play a role in the defense against 

lectins recognize a carbohydrate that is a typical constituent of fungal cell walls (Broekaert et al., 1989). GNA was originally considered a Man-specific lectin that possesses three similar Man-binding sites per subunit (Van Damme et al., 2008). Many of them interact only weakly with Man but exhibit a strong affinity toward oligomannosides and high-Man N-glycans. A monomeric Man/Glc-binding lectin inhibits the germination of *Aspergillus flavus* and *Fusarium moniliforme* spores and hyphal growth in red cluster pepper (*Capsicum frutescens*) seed (Ngai and Ng, 2007). Some lectin-like receptor-like kinases (LecRLKs) have been implicated in plant defense, senescence, and wounding (Riou et al., 2002). RLKs containing an extracellular Man-binding lectin (MBL) have also been reported in rice (*Oryza sativa*; Chen et al., 2006). The MBL domain in a Ser/Thr RLK gene, *Pi-d2*, is required for *R* gene-mediated resistance to rice blast. However, the function of plant carbohydrate-binding proteins containing this domain is not fully understood. Although several R genes that encode RLKs have been cloned and characterized, none has been shown to have an extracellular lectin domain. There is convincing in vitro evidence that some plant MBLs exert a deleterious effect on fungal pathogens. Gastrodianin, which is a monomeric Man-binding protein homolog in *Gastrodia elata* and *Epipactis helleborine*, displays in vitro antifungal activity against *Rhizoctonia solani* and *Phytophthora nicotianae* (Wang et al., 2001). Gastrodianin-like proteins also inhibit the mycelial growth of *Botrytis cinerea*, *Giberella zeae*, *Ganoderma lucidum*, *R. solani*, and *Valsa* (Cox et al., 2006). *Dendrobium findlayanum* agglutinin inhibits the growth of *Alternaria alternata* (Sudmoon et al., 2008).

In this study, we isolated genes differentially expressed in pepper (*Capsicum annuum* ‘Nokwang’) during the incompatible interaction with *Xanthomonas campestris pv vesicatoria* (*Xcv*). Among them, the novel pepper pathogen-responsive gene *CaMBL1* encodes a putative glycoprotein with a GNA-related lectin domain that localizes to the plasma membrane. To our knowledge, this is the first report suggesting that glycoproteins with the GNA-related lectin domain play a crucial role for cell death and defense responses and the expression of defense-related genes in pepper plants during *Xcv* infection. We also report that overexpression of *CaMBL1* in Arabidopsis (*Arabidopsis thaliana*) enhances innate immunity against *Pseudomonas syringae* pv tomato DC3000 (*Pst DC3000*) and *Alternaria brassicicola*.
RESULTS

Isolation and Identification of *CaMBL1* cDNA

In a previous study, we constructed a cDNA library using RNA isolated from pepper leaves inoculated with the *Xcv* avirulent strain BV5-4a (Jung and Hwang, 2000). The full-length cDNA clone, designated *CaMBL1* and containing glycoprotein-homologous sequences, was isolated from this pepper cDNA library. The cloned *CaMBL1* cDNA had an insert of 1,160 bp containing an 894-bp coding region encoding a protein of 298 amino acids (Supplemental Fig. S1). The *CaMBL1* cDNA sequence was analyzed using the BLASTx program (http://www.ncbi.nlm.nih.gov/BLAST/), the ExPASy Proteomics Server (http://www.expasy.org), and the PLeCDom program (http://www.nipgr.res.in/plcedom.html). *CaMBL1* shares 47% to 55% amino acid sequence identities with an unknown grape (*Vitis vinifera*) protein (accession no. CAN75015), the sugar beet (*Beta vulgaris*) SIEP1L protein (accession no. CAA61158), the carrot (*Daucus carota*) cell attachment protein (accession no. BAD24818), the Arabidopsis curculin-like (Man-binding) lectin family protein (CLLFP; At1g78860; accession no. NP_178007), the flax (*Linum usitatissimum*) secreted glycoprotein (accession no. AAO15899), and another Arabidopsis CLLFP (At1g78830; accession no. NP_565191; Supplemental Fig. S2). A database search revealed that *CaMBL1* and related plant proteins have the GNA-related lectin domain commonly present in the plant lectin family. After the identification of GNA, similar lectins were isolated and characterized from many other plant species (Van Damme et al., 2007a). Previously, GNA and related lectins were classified into the so-called “monocot Man-binding lectins.” Therefore, the term bulb-type MBL domain can no longer be used and has been replaced by GNA domain (Van Damme et al., 2007a, 2008). Based on the BLAST search with *CaMBL1*, we revealed that *CaMBL1* contains the putative GNA-related lectin domain but lacks a middle S-locus glycoprotein domain (SLP) and a C-terminal PAN-Apple domain that are widespread in plant lectin proteins (Van Damme et al., 2008). Moreover, the GNA-SLP-PAN-type protein itself corresponds to the N-terminal half of the so-called receptor kinases, which constitute a transmembrane helix and a C-terminal protein kinase domain next to the GNA-SLP-PAN module. Notably, some S-locus receptor kinases that constitute these four domains were demonstrated in Brassicaceae species (Naithani et al., 2007). Thus, *CaMBL1* containing only the GNA-related lectin domain may be a truncated homolog of the GNA-SLP-PAN-type protein in pepper plants.

A phylogenetic tree was constructed with *CaMBL1* and homologous proteins retrieved from the GenBank database and the PLeCDom program (Supplemental Fig. S3), in which MBL family members are divided into two groups containing GNA-related lectin domain or kinase domain. MBL proteins in the first group contain the GNA-related lectin domain alone, and those in the second group contain GNA-related lectin and kinase domains. Pepper GNA-related lectins including *CaMBL1* were constructed in the phylogenetic tree with the GNA-related lectin members from sugar beet, grape, carrot, flax, and Arabidopsis (Supplemental Fig. S3). Some GNA-related lectins were identified in pepper, although the role of the GNA-related lectins remains unknown. *CaMBL1* was closely related to the pepper GNA-related lectin (TC7720) rather than other pepper GNA-related lectins.

Expression of the *CaMBL1* Gene in Pepper

To determine the expression profiles of *CaMBL1*, we performed RNA gel-blot and immunoblot analyses of pepper plant tissues (Fig. 1). The expression of *CaMBL1* was undetected or nearly so in leaves, stems, roots, flowers, and green and red fruits during normal growth and development (Fig. 1A). Northern analysis was also performed to determine whether *CaMBL1* expression is induced by the virulent (compatible) *Ds1* and avirulent (incompatible) *Bv5-4a* strains of *Xcv*. *CaMBL1* transcript accumulation in pepper leaves began 5 h after inoculation with the avirulent *Bv5-4a* strain, and it reached maximal levels from 5 to 25 h (Fig. 1A). Mock inoculation and virulent Xcv infection induced *CaMBL1* in pepper leaves in a similar manner. This indicates that infiltration by itself is sufficient to induce *CaMBL1*. Furthermore, infiltration may prime for enhanced expression of *CaMBL1* upon inoculation with avirulent Xcv. However, avirulent Xcv infection led to high levels of *CaMBL1* protein, as detected by western blotting. Treatments with SA, but not with methyl jasmonate, also significantly induced the *CaMBL1* gene in pepper leaves 5 to 25 h after exposure (Fig. 1B). After SA treatment, transcripts were detected within 5 h, reached their highest levels between 10 and 15 h, and gradually decreased by 20 h. A high level of *CaMBL1* protein expression was also detected 24 h after SA treatment. In contrast, treatment with methyl jasmonate did not induce *CaMBL1* protein expression. We tested whether the anti-*CaMBL1* antibody used in this study is specific to the corresponding *CaMBL1* protein (Fig. 1C). The anti-*CaMBL1* antibody raised in *Escherichia coli*.

Binding of *CaMBL1* to d-Man

*CaMBL1* was predicted to contain the GNA-related lectin domain. To determine whether it has an essential function as an MBL, we generated a series of *CaMBL1* deletion mutants (Fig. 2A) and expressed them in *E. coli* as fusion proteins with a 6×His tag at the N terminus (Fig. 2B, left panel). As a result, His-CaMBL1-1 that lacks the putative signal peptide region, His-CaMBL1-2 that contains a GNA-related
lectin domain, and His-CaMBL1-3 that contains only the C-terminal region were distinctly expressed in E. coli, followed by their purification using D-Man-agarose columns. However, full-length 6×His-tagged CaMBL1 was not expressed in E. coli (no arrowhead), even with the use of a GNA-related lectin domain and glutathione S-transferase-tagged ones (data not shown). This indicated that the presence of the signal peptide may block the expression of CaMBL1 in E. coli.

Immunoblot analysis of the purified CaMBL1 proteins using the anti-His antibody showed that protein bands from CaMBL1 deletion mutants that lack the signal peptide region but contain the GNA-related lectin domain region could be immunodetected by this antibody (Fig. 2B, right panel). These immunoblotting data indicate that the GNA-related lectin domain of CaMBL1 is essential for its binding to d-Man. The glycan array screening of CaMBL1 was performed by the Consortium for Functional Glycomics at the School of Medicine, Emory University. Binding levels of CaMBL1 to the various glycans on the arrays were measured by fluorescence scanning (Supplemental Fig. S5; Supplemental Table S1). These glycan array data indicate that CaMBL1 has affinity toward Manα and/or Manβ and GalNAc residues.

Subcellular Localization of CaMBL1

To identify the subcellular location of the CaMBL1 protein, and to determine whether it depends on the GNA-related lectin domain, soluble modified GFP (smGFP) was fused to the full-length CaMBL1 protein (CaMBL1:smGFP), the GNA-related lectin domain region (GNA domain:smGFP), and the C-terminal region (CaMBL1c:smGFP; Fig. 3A). The subcellular localization of these fusion proteins was visualized in onion (Allium cepa) epidermal cells by confocal microscopy. The merging of fluorescence and bright-field images revealed that both the full-length and GNA-related lectin domain fusion proteins localized to the plasma membrane, whereas the C-terminal region of CaMBL1 was expressed only in the cytosol (Fig. 3B). In contrast, the control smGFP was uniformly distributed throughout the cell. Onion epidermis was treated with
1 M NaCl to induce plasmolysis. The effect of plasmolysis on onion cells expressing full-length and GNA-related lectin domain fusion proteins also confirmed that CaMBL1 is localized to plasma membrane and that the GNA-related lectin domain is required for its localization (Fig. 3C). We also tested the subcellular localization of CaMBL1 in pepper leaves. Total proteins were extracted from the pepper leaves during Xcv infection. To separate soluble and microsomal fractions, total proteins were centrifuged at 92,000 g for 90 min. Immunoblotting data with anti-CaMBL1 antibody indicated that CaMBL1 was enriched in the microsomal fraction including the plasma membrane or the endoplasmic reticulum membrane from the leaves infected by the avirulent (incompatible) strain Bv5-4a of Xcv. However, CaMBL1 was slightly detected in the soluble fraction including secretory proteins (Fig. 3D). Collectively, these results indicate that CaMBL1 is a membrane-associated protein and requires the GNA-related lectin domain to localize to the plasma membrane.

Induction of Cell Death by Transient Expression of CaMBL1

To further test whether the CaMBL1 gene and the CaMBL1-mediated signaling pathway affect the induc-
toms appeared in pepper leaves 48 h after agroinfiltration. In contrast, infiltration with the Agrobacterium empty vector control did not induce a cell death response. Plant cells undergoing HR-like cell death accumulated autofluorescent compounds, which may contain cross-linked phenolics that could serve to strengthen the cell wall (Dixon and Paiva, 1995). As observed under UV illumination, autofluorescence was detected in pepper leaves as early as 2 d after infiltration with Agrobacterium (35S:CaMBL1). This result indicates that CaMBL1 transient expression triggers the accumulation of fluorescent phenolic compounds as a cell death-related defense reaction. An increased expression of c-Myc-tagged CaMBL1 was detected in leaf tissues 18, 36, and 60 h after infiltration with Agrobacterium (35S:CaMBL1), as shown by western-blot analysis (Fig. 4B). However, the protein expressed by 35S-c-Myc was not transiently induced in empty vector control leaves. Large clusters of dead cells that stained with trypan blue were seen in the Agrobacterium (35S:CaMBL1)-infiltrated leaf areas but not in leaves transfected with the empty vector (Fig. 4C). The severity of cell necrosis caused by membrane damage in leaves was estimated by measuring electrolyte leakage from leaf tissues. Pepper leaves that transiently expressed CaMBL1 exhibited drastic electrolyte leakage compared with empty vector control leaves 36 h after infiltration, when the cell death phenotype appeared (Fig. 4D). We used real-time reverse transcription (RT)-PCR to determine whether the HR-like cell death in leaves transiently expressing CaMBL1 is accompanied by the expression of defense-related genes (Fig. 4E). CaBPR1 (for basic pathogenesis-related gene) was selected as a marker gene responsible for the activation of plant defense responses. The transcript level of CaBPR1 increased significantly during the transient expression of CaMBL1, indicating that induction by CaMBL1 con-
tributes to the defense response in plants. However, the transcript level of CaDEF1 (for defensin) was not affected by CaMBL1 transient expression, except for that at the 12-h time point. As shown in Figure 4F, increased CaMBL1 expression led to the accumulation of free SA and total SA (free SA plus Glc-conjugated SA) in pepper leaves. In particular, SA levels 24 and 48 h after infiltration were significantly higher in leaves transiently expressing CaMBL1 than those in leaves transfected with the empty vector control. Increased SA levels in CaMBL1 transiently expressed leaves 24 h after agroinfiltration were higher than those 48 h after agroinfiltration. These increased SA levels correlated with the increased CaBPR1 expression levels during the transient expression of CaMBL1. Overall, these results indicate that the transient expression of CaMBL1 induces cell death in pepper leaves, which is supported by the expression of the defense-related gene CaBPR1 and SA accumulation.

The GNA-Related Lectin Domain of CaMBL1 Is Responsible for Cell Death Induction

Induction of CaMBL1 by Agrobacterium-mediated transient expression led to cell death, which was accompanied by defense responses, including the induction of defense-related genes and SA accumulation (Fig. 4). Within 36 to 48 h after agroinfiltration, hypersensitive cell death was seen in the infiltrated area of pepper leaves, which collapsed 3 to 4 d later (data not shown). To determine which region of the CaMBL1 gene is responsible for the induction of cell death in pepper leaves, we transformed the constructs pBIN:CaMBL1, pBIN:GNA domain, and pBIN:CaMBL1c, which contains only the C-terminal region, into Agrobacterium strain GV3101. As expected, the transient expression of 35S:CaMBL1 and of 35S:GNA domain induced much more hypersensitive cell death 4 d after agroinfiltration compared with that induced by 35S:CaMBL1c (Fig. 5A). All three proteins from these constructs were detected in leaf protein extracts by immunoblotting using an anti-cMyc antibody (Fig. 5B), showing that the constructs are transiently expressed in pepper leaves. The hypersensitive cell death responses were also well supported by ion conductivity data (Fig. 5C). Distinct electrolyte leakage, a measure of membrane damage, began within 12 h after agroinfiltration. Leaves transiently expressing 35S:CaMBL1 and 35S:GNA domain exhibited significantly enhanced electrolyte leakage compared with empty vector and CaMBL1c-expressing leaves 12, 24, and 36 h after agroinfiltration (Fig. 5C). Collectively, these data indicate that the GNA-related lectin domain of the CaMBL1 gene contributes to CaMBL1-specific cell death induction.

Enhanced Susceptibility of CaMBL1-Silenced Pepper to Xcv Infection

To characterize the loss of function of CaMBL1 in pepper plants, we used the virus-induced gene silencing (VIGS) system with tobacco rattle virus (TRV)-based vectors to knock down the expression of CaMBL1. CaMBL1-silenced pepper plants (TRV:CaMBL1) were

Figure 5. Agrobacterium-mediated transient expression of 35S:00 (empty vector), 35S:CaMBL1, 35S:GNA domain, and 35S:CaMBL1c in pepper leaves. A, Visible and UV light-illuminated phenotypes of the 35S:00 (empty vector), 35S:CaMBL1, 35S:GNA domain, and 35S:CaMBL1c-transiently expressing pepper leaves 4 d after agroinfiltration. B, Immunoblot analysis of 35S:8cMyc, 35S:CaMBL1:8cMyc, 35S:GNA domain:8cMyc, and 35S:CaMBL1c:8cMyc protein expression in pepper leaves using an anti-cMyc antibody. Coomassie Brilliant Blue (CBB) staining is shown for the 60-kD regions of protein extracts. C, Electrolyte leakage assay of 35S:00 (empty vector), 35S:CaMBL1, 35S:GNA domain, and 35S:CaMBL1c-transiently expressing pepper leaves at different times after agroinfiltration. All experiments were repeated three times with similar results. Values are presented as means ± sd. Different letters indicate significant differences as determined by Fisher's LSD test (P < 0.05).
Enhanced Resistance of CaMBL1-Overexpression Arabidopsis to P. syringae pv. tomato Infection

Because stable transformation of pepper plants is difficult, we constructed transgenic Arabidopsis plants constitutively expressing CaMBL1 under the control of the cauliflower mosaic virus 35S constitutive promoter to study the gain-of-function phenotype of CaMBL1 in heterologous plants during pathogen infection. When detected with the CaMBL1-specific antibody by western blotting, at least 10 independent transgenic lines were found to constitutively express the CaMBL1 protein, which was not detected in wild-type plants. Three transgenic lines with a single insertion of the CaMBL1 transgene, lines 2, 4, and 5, were selected for this study (Fig. 8A). Expression of the pathogenesis-related genes PRI and PDF was constitutively up-regulated in the three CaMBL1-overexpression (OX) transgenic lines compared with that in wild-type plants, although the levels of expression differed among these lines (Fig. 8A). CaMBL1-OX transgenic plants did not exhibit any apparent phenotypic abnormality compared with wild-type plants (data not shown). The three CaMBL1 transgenic Arabidopsis lines were tested for resistance against Pst DC3000 and the Pst DC3000 strain carrying avrRpm1 (Pst DC3000 avrRpm1). Five days after inoculation, the leaves of CaMBL1-OX plants exhibited mild chlorotic lesions compared with wild-type leaves (Fig. 8B). Overexpression of CaMBL1 significantly inhibited the growth of Pst DC3000 and Pst DC3000 avrRpm1 in Arabidopsis leaves (Fig. 8C). Reduction of disease symptoms was intimately correlated with the bacterial growth suppressed in the leaves of CaMBL1-OX plants.

We next examined the necrotizing process at the microscopic level by assessing H$_2$O$_2$ production after 3,3’-diaminobenzidine (DAB) staining (dark brown) and HR-like cell death after trypan blue staining (dark blue) of leaves infected by Pst DC3000 and Pst DC3000 avrRpm1 (Fig. 8D). DAB polymerizes instantly and locally upon contact with H$_2$O$_2$ to form reddish brown polymers. CaMBL1-OX plants, which were either uninoculated or inoculated with Pst DC3000 or Pst DC3000 avrRpm1, exhibited DAB-stained spots, indicating H$_2$O$_2$ accumulation to high levels. CaMBL1-OX plants exhibited significantly increased dark blue zones (HR-like cell death) compared with wild-type plants after inoculation with Pst DC3000 and Pst DC3000 avrRpm1. Cell death associated with the HR was quantified using the electrolyte leakage assay (Fig. 8E). CaMBL1-OX leaves exhibited higher levels of electrolyte leakage compared with wild-type leaves during Pst DC3000 and Pst DC3000 avrRpm1 infection. We next quantified H$_2$O$_2$ levels at different times after infection using the xylenol orange assay and also hypersensitive response (HR)-like cell death (Fig. 8F). H$_2$O$_2$ production was significantly promoted in CaMBL1-OX leaves during Pst DC3000 and Pst DC3000 avrRpm1 infection, as observed by DAB staining. Together, these results suggest that CaMBL1 overexpression in
Arabidopsis may contribute to both basal defense and HR-mediated resistance to hemibiotrophic Pst infection.

Role of CaMBL1 and the Arabidopsis Ortholog At1g78830 in Resistance to A. brassicicola Infection

To investigate whether the expression of CaMBL1 and the Arabidopsis ortholog At1g78830 affects fungal infection, a spore suspension of A. brassicicola was drop inoculated on leaves of wild-type (ecotype Columbia [Col-0]), CaMBL1-OX, and At1g78830-defective Arabidopsis plants (Fig. 9). The predicted amino acid sequence of At1g78830, which contains an MBL domain, is 48% identical to that of CaMBL1 (Supplemental Figs. S2 and S3). Two At1g78830 T-DNA insertion mutants were investigated, mbl1-1 (SALK_003821) and mbl1-2 (SALK_121641; Fig. 9A). RT-PCR analysis did not detect At1g78830 transcripts in mbl1-1 or mbl1-2 plants during Pst DC3000 avrRpm1 infection (Fig. 9B), indicating that the At1g78830 function is completely lost due to the T-DNA insertion. Typical spreading of susceptible lesions was observed in the leaves of mbl1-1 and mbl1-2 plants 5 d after inoculation with A. brassicicola (Fig. 9, C and E). However, wild-type and CaMBL1-OX leaves exhibited small necrotic lesions no larger than the initial inoculation droplet during the A. brassicicola infection. Trypan blue staining of infected leaf tissues showed restricted hyphal growth and cell death in wild-type and CaMBL1-OX leaves but extensive hyphal growth in At1g78830 mutant leaves (Fig. 9D). These data indicate that the Arabidopsis ortholog At1g78830 is required for resistance to A. brassicicola infection of Arabidopsis plants.

Distinct Responses of CaMBL1-OX and CaMBL1 Arabidopsis Ortholog, mbl1, Mutants to HyaIoperonospora arabidopsidis and P. syringae pv tomato Infection

To determine whether the Arabidopsis defense response to biotrophic oomycete infection is induced by CaMBL1 and the CaMBL1 Arabidopsis ortholog, we tested the responses of the wild type (Col-0), CaMBL1-OX Arabidopsis, and the CaMBL1 Arabidopsis ortholog At1g78830 mutant (mbl1-1 and mbl1-2) plants to the biotrophic oomycete H. arabidopsidis. Over 100 wild-type and CaMBL1-OX leaves exhibited small necrotic lesions no larger than the initial inoculation droplet during the A. brassicicola infection. Trypan blue staining of infected leaf tissues showed restricted hyphal growth and cell death in wild-type and CaMBL1-OX leaves but extensive hyphal growth in At1g78830 mutant leaves (Fig. 9D). These data indicate that the Arabidopsis ortholog At1g78830 is required for resistance to A. brassicicola infection of Arabidopsis plants.

leaf tissues at different time points after inoculation with Xcv strains. All experiments were repeated three times with similar results. Values are presented as means ± sd. Asterisks indicate significant differences between the means as determined by Student’s t test (P < 0.05).
Noco2. No differences were observed between wild-type and CaMBL1-OX plants in mycelial growth, sporulation, and sporangiophores, as observed by trypan blue staining. The formation of conidiospores was scored for 7 d (Supplemental Fig. S4B). Extensive sporulation occurred in wild-type and CaMBL1-OX plants. Five days after inoculation, H. arabidopsidis infection was evaluated by measuring asexual sporangiophores on Arabidopsis cotyledons (Supplemental Fig. S4C). CaMBL1-OX plants exhibited the formation of sporangiophores similar to that on the cotyledons of wild-type plants. These results indicate that the CaMBL1 gene does not confer enhanced defense response against the biotrophic oomycete H. arabidopsidis isolate Noco2. No significant defense responses to H. arabidopsidis isolate Noco2 and Pst DC3000 were observed in CaMBL1 Arabidopsis ortholog At1g78830 mutant plants (mbl1-1 and mbl1-2), as assayed by measuring sporangiophores and bacterial growth (Supplemental Fig. S4, D and E).

**DISCUSSION**

MBLs (recently called the GNA-related lectins) are involved in many biological processes, including cell-to-cell and host-pathogen interactions, by specifically binding to carbohydrates (Peumans and Van Damme, 1995; Lis and Sharon, 1998; Van Damme et al., 2008). Notably, MBLs play a crucial role in the innate immune response through binding to carbohydrates on the surface of a wide range of microbial pathogens (Vijayan and Chandra, 1999; Barre et al., 2001). In this study, we isolated and functionally characterized the pepper CaMBL1 gene, which was rapidly and strongly induced in pepper leaves during incompatible interactions with Xcv. The CaMBL1 protein contains the GNA-related lectin domain that is conserved in rice (Barre et al., 2001). Lectins known to be carbohydrate-binding proteins are ubiquitous in plants, and MBLs that contain the GNA-related lectin domain are representative of a new plant lectin family (Van Damme et al., 2008). Among the MBL genes, there are also LecRLK genes encoding receptor-like kinases with predicted extracellular bulb-type Man-specific B-lectin and intracellular Ser/Thr kinase domains (Riou et al., 2002). Some LecRLKs have been shown to be involved in Rhizobium symbiosis and in other cellular processes (Navarro-Gochicoa et al., 2003; Shiu et al., 2004). More recently, the rice LecRLK gene Pi-d2 with these domains was suggested to confer resistance to the Chinese blast strain ZB15 of Magnaporthe oryzae (Chen et al., 2006). Intriguingly, a specific feature of the CaMBL1 gene is that it contains only the GNA-related lectin domain, unlike these LecRLK genes, and phylogenetic analysis suggests that CaMBL1 is more closely related to the group having only the GNA-related lectin domain. Notably, some pepper GNA-related lectins fall into similar clusters with CaMBL1 in the phylogenetic tree.
It has been demonstrated that the consensus sequence motif (QxDxNxVxY) of GNA-related lectin domain is involved in α-D-Man recognition (Van Damme et al., 2008). However, CaMBL1 containing the GNA-related lectin domain lacks the putative Man-binding site sequence (QxDxNxVxY). Thus, we tested whether CaMBL1 has the Man-binding ability. Using D-Man-agarose (Sigma), we found that His-tagged CaMBL1 could bind to D-Man-agarose, suggesting that CaMBL1 may have Man-binding ability without the intact consensus sequence motif (QxDxNxVxY). The immunoblot analysis of purified CaMBL1 deletion mutants strongly supports the notion that its GNA-related lectin domain is responsible for binding D-Man, suggesting that pepper GNA-related lectin may recognize Man on the Xcv cell surface. However, we could not find any direct interaction (agglutination) between Xcv and purified CaMBP1 fusion protein (data not shown). Notably, the glycan array screening of CaMBL1 performed by the Consortium for Functional Glycomics also indicates that CaMBL1 has affinity toward Manα and/or...
Manβ and GalNAc residues. Tsutsui et al. (2006) revealed that only the first of the two consensus sequence motifs (QxDxNxVxY) is required for Man-binding activity of pufflectins identified in the skin mucus of the fugu (Takifugu rubripes). In that study, three mutants of the second motif (QxDxNxVxY) could bind to Man. These findings support the possibility that the consensus sequence motif (QxDxNxVxY) of the GNA-related lectin domain is not necessarily essential for Man-binding activity.

Animal MBLs bind Man-based carbohydrates on bacterial plasma membranes (Fujita et al., 2004). The finding that CaMBL1 encodes a GNA-related lectin domain is consistent with the observation that GFP-tagged CaMBL1 localizes to the plasma membrane of onion epidermal cells, suggesting that CaMBL1 does the same in its native pepper cells. We also tested the subcellular localization of CaMBL1 in pepper leaves. Indeed, CaMBL1 was mainly detected in the microsomal fraction including plasma membrane from pepper leaves during Xcp infection. In a subcellular localization experiment with a GNA domain:smGFP construct, we also confirmed that the GNA-related lectin domain is responsible for the localization of CaMBL1 to the plasma membrane. Together, these data suggest that the plasma membrane targeting of the CaMBL1 protein may be involved in the specific recognition of plant pathogens by GNA-related lectins. This proposal is well supported by the finding of Chen et al. (2006) that a B-lectin receptor kinase protein that triggers rice blast resistance is plasma membrane localized.

Most plant lectins may be involved in defense responses during pathogen infection by recognizing a wide range of pathogens (Peumans and Van Damme, 1995). Lectins are generally expressed at relatively low levels in a tissue- or organ-specific manner. In contrast, some lectins are induced to higher levels in plants following attack by other organisms or by stress. Plant lectins, including the GNA-related lectins, are believed to play a role in recognizing and binding high-Man glycans of foreign microorganisms or plant pathogens (Barre et al., 2001). GNA-related lectins are associated with specific resistance responses to herbivorous higher animals or phytophagous invertebrates (Peumans et al., 2002). In wheat (Triticum aestivum) resistant to the Hessian fly, induced GNA-related lectins recognize Man-rich glycans of microorganisms or plant predators, suggesting that this early recognition may play a significant role in defense responses (Subramanyam et al., 2008). However, with the exception of LecRLKs, the functions of plant lectins, such as those of GNA-related lectins in plant-pathogen interactions, are poorly understood. We found CaMBL1 expression to

Figure 9. Disease development on the leaves of wild-type (WT), CaMBL1-OX, and Arabidopsis ortholog mutant (mb1-1 and mb1-2) plants inoculated with A. brassicicola. Leaves of 4-week-old Arabidopsis plants were inoculated with 10-μl droplets of 5 × 10^4 spores mL⁻¹. A, Schematic representation of T-DNA insertion sites in mb1-1 (SALK_038821) and mb1-2 (SALK_121641) plants and the genomic structure of the At1g78830 gene. The At1g78830 exon is represented by the black box. B, RT-PCR analysis of expression of the mb1-1 and mb1-2 genes 15 h after inoculation with Pst DC3000 avrRpm1. C, Disease symptoms on leaves 5 d after inoculation. D, Microscopic images of fungal structures and damaged cells of infected leaves stained with trypan blue. Bars = 0.1 mm. E, Quantification of disease development based on the average diameter of lesions caused by A. brassicicola. The size of lesions is an average of 30 lesions per line. Values are presented as means ± SD. Different letters indicate significant differences from three independent experiments based on the LSD test (P < 0.05). [See online article for color version of this figure.]
be pathogen specific; however, the role of CaMBL1 as a defense protein in plants remains to be elucidated. In incompatible interactions with Xcv, CaMBL1 was strongly and rapidly induced compared with its response in compatible interactions. These results suggest that CaMBL1 is crucial in pepper plants for overall defense responses to pathogen invasion. Similarly, an MBL gene is expressed in maize (Zea mays) plants early during Colletotrichum graminicola infection (Sugui and Deising, 2002).

In plants, GNA-related lectins may be involved in pathogen recognition at an early stage of infection. The expression of CaMBL1 was induced rapidly and strongly during incompatible interactions with Xcv. Thus, its expression may lead to the activation of defense-related genes. Silencing of CaMBL1 resulted in enhanced disease susceptibility, increased bacterial growth, lowered accumulation of ROS, as well as reduced expression of PR genes in pepper leaves during virulent or avirulent Xcv infection. These findings support the notion that CaMBL1 expression during Xcv infection activates basal resistance in pepper plants, which is accompanied by the induction of PR genes. Reduced SA levels in CaMBL1-silenced plants impaired their ability to mount responses such as defense-related gene expression and HR-like cell death. Taken together, these data suggest that the CaMBL1 gene is a positive regulator of SA-dependent cell death and defense responses during Xcv infection.

Interestingly, CaMBL1 induced cell death responses in pepper leaves when it was transiently expressed by agroinfiltration, as evidenced by remarkably high levels of SA with concomitant PR1 activation in CaMBL1-overexpressing pepper leaves. More importantly, domain analyses of CaMBL1-induced cell death in pepper leaves revealed that the GNA-related lectin domain is responsible for this phenomenon. Collectively, these results suggest that induction of CaMBL1, as a positive regulator of cell death and defense responses, causes SA to accumulate in pepper leaf tissues and trigger the expression of downstream defense-related genes, such as CaBPR1 and CaSAR82A.

MBLs or GNA-related lectins may contribute to an innate immunity as a first line in plant defense (De Hoff et al., 2009). To determine whether overexpression of CaMBL1 confers defense response to heterologous plants, we generated CaMBL1-OX transgenic lines in Arabidopsis, which were resistant to virulent and avirulent P. syringae pv tomato infection. Resistance responses in the transgenic plants included ROS accumulation, cellular membrane damage, and necrotic disease symptoms. These findings suggest that overexpression of the CaMBL1 gene in Arabidopsis enhances basal or R gene-mediated resistance to bacterial infection. Inhibition of P. syringae pv tomato infection by overexpression of CaMBL1, which localizes to the plasma membrane, may be strongly supported by the activation of PR genes, induction of ROS accumulation, and cell death in Arabidopsis leaves following recognition of bacterial pathogens. However, there may be no correlation between CaMBL1 transcript and protein levels induced in the CaMBL1-OX Arabidopsis leaves by Pst DC 3000 infection. Furthermore, constitutively ectopic expression of the transgene CaMBL1 distinctly primes the induction of other defense-related genes, such as PRI, which may be dependent on the transgenic Arabidopsis lines. The susceptible response of Arabidopsis ortholog A11g78830 mutant plants to the fungal pathogen A. brassicicola also suggests that CaMBL1 is required for enhanced resistance to fungal pathogens in pepper plants.

GNA-related lectins constitute an extended superfamily of structurally and evolutionarily related proteins that play crucial roles for plant defenses. We have shown that CaMBL1 is required for enhanced resistance to the bacterial pathogen Xcv as well as to the fungal pathogen A. brassicicola. These results support the notion that CaMBL1 is effective for the recognition of bacterial and fungal pathogens on plant cell surfaces. Upon establishing contact with a host surface, pathogens release extracellular matrix-containing glycoproteins with Man residues (Sugui et al., 1998; Barre et al., 2001). Once GNA-related lectins recognize pathogens, their lectin domain could bind to Man or lipooligosaccharide residues on their surfaces. Therefore, expression of CaMBL1 may provide cues for the plant to specifically recognize pathogens and to promote further defense responses.

Taking these results together, we propose a working model for CaMBL1-mediated defense responses in plants (Fig. 10). Plants recognize a diversity of attacking pathogens, including virulent and avirulent Xcv.
most likely by interactions of GNA-related lectins with a subset of pathogen-associated molecular patterns in the host plasma membrane. In this study, we showed that pathogen recognition by CaMBL1 triggers the accumulation of ROS and of the defense signal molecule SA in pepper plants, which in turn induces the expression of defense-related genes such as CaBPR1, CaPOA1, CaDEF1, and CaSAR82A. The CaMBL1 transcript does not accumulate in the absence of pathogens, but as a result of rapid CaMBL1 gene activation in response to their presence, pepper plants exhibit enhanced resistance to pathogens. In particular, plasma membrane-localized CaMBL1 is strongly expressed at an early stage of infection. However, it remains unclear how the recognition by CaMBL1 can lead to hypersensitive cell death, which is inevitably associated with ROS and SA accumulation, the expression of defense-related genes, and enhanced resistance. The presence of the GNA-related lectin domain supports a direct role for CaMBL1 in pathogen recognition, despite the absence of intracellular Ser/Thr kinase domains. Furthermore, experimental data using CaMBL1-silenced pepper and CaMBL1-OX Arabidopsis plants also support the notion that recognition by CaMBL1 may play a significant role in specific bacterial pathogen-plant interactions. Pathogen-induced CaMBL1 gene expression may alert the plant to invading pathogens, ultimately leading to cell death and disease resistance responses. We propose that CaMBL1 expression also triggers regulatory mechanisms to control defense responses in plants. Further studies of the GNA-related lectin protein CaMBL1 in pepper plants are required to reveal how this is achieved.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Pepper plants (Capsicum annuum 'Nockwang') were used in this study. Pepper seeds were germinated at 27°C. Plants were raised in a plastic tray (55 × 35 × 15 cm) and were transplanted to pots at the two-leaf stage. All plants were cultivated in a soil mix (peat moss:vermiculite:perlite, 3:3:1, v/v/v) at 26°C under a 16-h-light/8-h-dark cycle.

Wild-type ecotype Col-0 and transgenic lines of Arabidopsis (Arabidopsis thaliana) were also studied. CaMBL1 Arabidopsis ortholog At1g78380 mutant seeds (mbnl-1 and mbnl-2) were obtained from the Salk Institute T-DNA insertion library database (http://signal.salk.edu/cgi-bin/tdnaexpress).

Prior to sowing on soil (peat moss:vermiculite:perlite, 1:1:0.5, v/v/v), seeds were vernalized at 4°C under low light for 2 d. Arabidopsis plants were raised in a plastic tray (20 × 10 × 8 cm) at 26°C for 2 weeks under a 16-h-light/8-h-dark cycle.

Pathogen Inoculation and Disease Rating

The virulent strain Ds1 and avirulent strain Bv5-4a of Xanthomonas campestris pv vesicatoria were used to inoculate pepper plants. To prepare bacterial inoculum, Xcv was grown on yeast-nutrient broth (5 g of yeast extract, 8 g of nutrient broth, and 1 L of water) at 28°C for 18 h. Pepper plants at the six-leaf stage were inoculated by infiltrating with a suspension (5 × 10^6 cfu mL^{-1}) of the virulent strain Ds1 or the avirulent strain Bv5-4a or were mock infiltrated with 10 mM MgCl2. The inoculated plants were incubated for 18 h in a moist chamber at 28°C and then transferred to a growth room. Infected leaves were sampled at various times after inoculation.

Pseudomonas syringae pv tomato DC3000 and DC3000 expressing avrRpm1 were grown overnight at 28°C on yeast-nutrient broth containing rifampicin (50 μg mL^{-1}). Arabidopsis plants were infiltrated with a 10^7 cfu mL^{-1} bacterial suspension in 10 mM MgCl2. To assess bacterial growth, infected leaves were collected 0 and 3 d after inoculation.

Hyaloperonospora arabidopsis isolate Noco2 was maintained on Arabidopsis Col-0 by weekly subculturing. To produce a large quantity of inocula, 7- to 10-d-old seedlings were inoculated with H. arabidopsis, and the spores produced on cotyledons were collected in water. The seedlings were sprayed inoculated with a suspension of avirulent inoculum (5 × 10^7 conidiosporangia mL^{-1}), covered with a transparent dome to maintain high humidity (80–100%), and incubated for 7 d at 17°C. Asexual sporulation of H. arabidopsis was assessed by using a light stereomicroscope to count the number of sporangiophores on both sides of the cotyledons 7 d after inoculation. The extent of disease was assigned to five classes based on the number of sporangiophores per cotyledon: zero to five, six to 15, 16 to 20, and over 20. For the spore-count assay, the infected cotyledons of each line were excised and shaken vigorously. Spores harvested from 20 cotyledons per replicate were counted using a hemacytometer.

Alternaria brassicicola was cultured on potato dextrose agar medium at 24°C for 10 d. Conidial concentration was determined using a hemacytometer and adjusted to 5 × 10^6 conidia mL^{-1}. A. brassicicola was inoculated by placing 10-μL droplets of suspension on leaves of 5-week-old plants. For mock treatment, 10-μL droplets of water were placed onto the leaves. Inoculated plants were kept at 100% relative humidity at 24°C. Four days after inoculation, lesion diameters were measured.

Treatment with SA and Methyl Jasmonate

The leaves of pepper plants at the six-leaf stage were treated with SA (5 μM) and methyl jasmonate (100 μM). SA and methyl jasmonate were sprayed onto pepper plants at the six-leaf stage. The pepper plants treated with methyl jasmonate were incubated in a vinyl bag. Control plants were sprayed with water. Pepper plants were sampled at various times after treatment, frozen in liquid nitrogen, and stored at −70°C for RNA isolation.

Isolation of the CaMBL1 Gene from a Pepper cDNA Library

A pathogen-induced cDNA library was prepared from total RNA extracted from pepper leaves 18 h after inoculation with Xcv using a AZAPLI cDNA library synthesis kit (Jung and Hwang, 2000). Differential hybridization was performed to isolate pathogen-induced cDNAs from the library using cDNA probes from uninoculated pepper leaves or leaves inoculated with the Xcv avirulent strain Bv5-4a.

Gene-specific RT-PCR was performed to confirm the differential expression of genes identified on an expression array. Digoxigenin-labeled single-stranded cDNAs were synthesized using total RNA from healthy and bacteria-infected pepper leaves. Total RNA (20 μg) from pepper was mixed with 5× reaction buffer (5× avian myeloblastosis virus [AMV] RT buffer), AMV reverse transcriptase (Roche), and oligo(dT)12 as a primer in diethyl pyrocarbonate water. To produce probes, RT-PCR was performed using RNA and primer mixes at 25°C for 10 min, 42°C for 60 min, and 99°C for 5 min. Equal amounts of the single-stranded cDNA probes from uninoculated control leaves and leaves inoculated with Xcv were hybridized to the two pepper cDNA expression arrays in a separate bottle for 18 h at 65°C in a mixture of 5× SSC, 0.1% sodium lauroylsarcosine, 0.02% SDS, and 10% blocking reagent. The hybridization membranes were washed twice in solution 1 (2× SSC and 0.1% SDS) for 10 min each at room temperature and then twice at 65°C for 5 min each in solution 2 (0.1× SSC and 0.1% SDS). The blotted membranes were exposed to x-ray film. The hybridization patterns produced by 96 samples each of uninoculated and inoculated samples were compared.

Several cDNA clones that were expressed in pepper leaves inoculated with the avirulent strain Bv5-4a of Xcv were isolated and sequenced with an ABI310 DNA sequencer (PE Biosystems) using PRISM BigDYE Terminator sequencing-ready reaction kits. The DNA sequences were verified and analyzed using BLAST network services at the National Center for Biotechnology Information and the ExPaSy Proteomics Server. A full-length cDNA clone encoding a pepper glycoprotein, designated CaMBL1, was isolated and analyzed.

Expression of the CaMBL1 Protein and Its Binding Assay with Man-Agarose

The CaMBL1 gene was cloned into the vector pET28a to generate pET28a::CaMBL1, which created a translational fusion of CaMBL1 to a His tag at the
N terminus. The CaMBL1 protein construct was expressed in *Escherichia coli* and purified on a Man-agarose column. The CaMBL1 deletion series was made by PCR with specific primers CaMBL1 forward (5′-GGATCCATGCTTCCTTTTCATTGACTTACTAC-3′) and CaMBL1 reverse (5′-CAAACTTTAATTCTCTTGTAAGTTAC-3′); CaMBL1-1 forward (5′-GGATCCATGCAAGTTCCAGGATTAGGTGCTG-3′) and CaMBL1-1 reverse (5′-GGATCCATGCAAGTTCCAGGATTAGGTGCTG-3′); CaMBL1-2 reverse (5′-TTAAATCTGCTGTGTAATTGACA-3′) and CaMBL1-2 reverse (5′-TTAAATCTGCTGTGTAATTGACA-3′); and CaMBL1-3 forward (5′-GGATCCATGCAAGTTCCAGGATTAGGTGCTG-3′) and CaMBL1-3 reverse (5′-AAAGCTTTTAAATCTGCTGTGTAATTGACA-3′). To express the CaMBL1 gene, pET28a:CaMBL1 was transformed into *E. coli* BL21 (DE3). Recombinant strains were grown in 50 mL of Luria-Bertani medium at 37°C to an optical density at 600 nm (OD600) of 0.4 to 0.5, induced with 1 mM isopropyl-β-D-thiogalactopyranoside for 3 h at 18°C, and harvested. Cells were collected by centrifugation, resuspended in 10 mL of Man-binding buffer (50 mM Tris, pH 7.5, 100 mM NaCl, and 5 mM CaCl2), and harvested. Cells were collected by centrifugation, resuspended in 10 mL of Man-binding buffer (50 mM Tris, pH 7.5, 100 mM NaCl, and 5 mM CaCl2), and disrupted by sonication. Cell debris was removed by centrifugation, and the supernatant was applied to a Man-agarose column (Sigma). The column was incubated in the presence of the protein extract for 30 min. After unbound proteins were washed out with Man-binding buffer, bound proteins were eluted from the column using Man-binding buffer containing 100 mM α-Man. Purified CaMBL1 was subjected to 15% SDS-PAGE. Protein bands were visualized by Coomassie blue staining (Carl Zeiss LSM 5 Exciter).

Subcellular Localization
Plasmids harboring *smGFP*, CaMBL1:smGFP, GNA domain:smGFP, and CaMBL1:smGFP were transformed into *Agrobacterium* epidermal cells by particle bombardment (Bio-Rad) using 5 μg of each plasmid DNA, which was precipitated onto gold particles. After bombardment, the onion epidermal cells on Murashige and Skoog agar plates were incubated for 18 h at 25°C in the dark. Subcellular localization of fused proteins was visualized in onion epidermal cells by confocal laser scanning fluorescence microscopy (Carl Zeiss LSM 5 Elevated).

Agrobacterium tumefaciens-Mediated Transient Expression
Constructs containing pBIN35S:CaMBL1 or 35S:CaMBL1:8Myc were introduced into the *Agrobacterium* strain GV3101 by direct transformation. Recombinant *Agrobacterium* was grown overnight at 28°C, collected by centrifugation, and resuspended to a final concentration of OD600 = 1.0 in induction medium (10 mM ethanesulfonic acid, pH 5.7, and 10 mM MgCl2). In experiments to test the efficacy of additives, acetosyringone was added to the liquid culture to a final concentration of 200 μM at pH 5.6. The cell suspensions were incubated at 28°C for 3 h before infiltration. *Agrobacterium* suspensions expressing CaMBL1 were injected at different concentrations into pepper leaves.

VIGS
TRV-based vectors (pTRV1/2) were used for VIGS of the CaMBL1 gene (Liu et al., 2002). An 861-bp fragment of the CaMBL1 cDNA was amplified by PCR to specifically silence CaMBL1. The resulting PCR product was cloned into pTRV2 to generate pTRV:CaMBL1. The TRV2 derivative pTRV:CaMBL1 was transformed into *Agrobacterium* strain GV3101, and transformed cells were selected on yeast extract peptone medium with 50 μg/mL kanamycin and 100 μg/mL rifampicin. *Agrobacterium* GV3101 cells containing pTRV1 and pTRV2:CaMBL1 VIGS vectors were coinfiltrated into fully expanded pepper cotyledons using a syringe (OD600 = 0.4). A positive control was infiltrated with a VIGS vector containing the *Phytophthora Dehiscence* gene. A negative control was infiltrated with an empty vector clone (TRV00). The inoculated plants were transferred to a growth chamber maintained at 17°C for 2 d with 80% relative humidity and then placed in a growth room at 28°C ± 2°C with a 16-h-light:8-h-dark cycle. Uninfected (TRV00) and CaMBL1-silenced (TRV: CaMBL1) plants were inoculated with the virulent Ds1 and avirulent Bv5-4a strains of *Xco* to examine gene expression (10 μg cDNA mL−1) and to assay bacterial growth (5 × 106 cfu mL−1). Bacterial cells were counted 0 and 3 d after inoculation.

Identification of T-DNA Insertion Mutant Lines
The T-DNA insertion lines (SALK_121641 and SALK_038821) of At1g78830 were obtained from the Arabidopsis Biological Resource Center (http://www.arabidopsis.org/abrc/) at Ohio State University. Homozygous mutant plants were identified by PCR using *T-DNA* and gene-specific primer sets as described on the T-DNA Express Web site (http://signal.salk.edu/tdnaprimers.html). Two sets of PCR were carried out using the three primers (Lp, left gene-specific primer; R, right gene-specific primer; Lb, left border primer of the T-DNA insertion) for each Salk line: LP primer (5′-GAGGCATTATTCACAAGTTGA-3′) and R primer (5′-TACATGCTTGAGCAACGGCCT-3′) for SALK_121641; LP primer (5′-AGCTGGAGCTTCAAGCACC3′) and R primer (5′-ATCCAAATCTTACCAAGGG-3′) for SALK_038821; and Lb1 primer (5′-TGTTCCAGTGGCGGCTACCG-3′).

Northern, RT-PCR, and Quantitative Real-Time PCR Analyses
Total RNA was isolated from pepper leaves, stems, roots, flowers, and fruits and from Arabidopsis plants using TRIzol (Invitrogen) according to the manufacturer’s instructions. Leaf samples were harvested and immersed in liquid nitrogen at different times after inoculation with *Xco*. Total RNA was extracted from the samples by the guanidinium thiocyanate-phenoil-chloroform extraction method (Chomczynski and Sacchi, 1987). The total RNA concentration and purity were estimated by spectrophotometry and by ribosomal RNA staining with ethidium bromide, respectively. Total RNAs (1 μg) were fractionated on 1.2% agarose gels containing 7% formaldehyde and transferred onto nylon membranes (Hybond+; Amersham) for 18 h followed by UV cross-linking. A biotin probe (14-dCTP-biotin) was prepared from the corresponding pepper cDNA, and CaMBL1 and CaBPR1 were amplified with the following specific primer sets: CaMBL1, 5′-AAAATCAACACACGAGAAGTTGACAGTATG-3′ (forward) and 5′-AATCCATTAATTCGACGTGCGGACCA-3′ (reverse); CaBPR1, 5′-ATGGGACACTCTAATATTGCC-3′ (forward) and 5′-GACATCAGTGGACACCT-3′ (reverse). After probe generation, membranes were prehybridized for 3 h at 65°C and then hybridized in 5% dextran sulfate, 0.25 xssodiumdiphosphate(pH7.2),7%SDS,and1mxeDTAat65°Covernight.Membraneswerewashedwith2xSSC,0.1%SDSfor10min each at room temperature and twice in 0.1xSSC, 0.1% SDS for 15 min each at 65°C. Membranes were exposed to x-ray film.

For RT-PCR analysis, first-strand cDNA synthesis was carried out in a reaction volume of 20 μL with 1 μg of RNA, 10 units μL−1 AMV reverse transcriptase (BIO BASIC), 2 μL of oligo(dT), 2 μL of deoxyribonucleotide triphosphates, and 2x AMV-RT buffer at 42°C for 60 min. PCR was performed in a final volume of 50 μL per reaction containing 0.5 μL of cDNA, 250 nM forward and reverse primers, 10x Taq reaction buffer (Takara), 0.2 μM deoxyribonucleotide triphosphates (Takara), and 0.5 units of Taq polymerase (Takara). Thirty cycles of amplification were preceded by an initial denaturation at 95°C for 5 min, with each amplification cycle including a 30-s denaturation at 95°C, 2 min annealing at 95°C to 58°C (primer pair-specific), and a 1-min extension at 72°C. After the amplification cycles, the samples were subjected to a 10-min extension at 72°C. Quantitative real-time PCR using the Bio-Rad iCycler System was performed to monitor levels of gene expression in plants. Each reaction (20 μL)
mix contained 10 μL of SYBR Green super mix (Bio-Rad) and 0.2 μM gene-specific primers. Thermal cycling conditions consisted of 2 min at 95°C, 10 min at 95°C, and 50 cycles of 15 s at 95°C and 1 min at 60°C. Data acquisition and analysis were performed by using iCycler Real-Time PCR Detection System software (Bio-Rad). Transcript levels were normalized to the expression of pepper 18S ribosomal RNA measured in the same samples.

Protein Extraction and Immunoblotting

For immunoblotting with the anti-c-Myc (Sigma) and anti-CaMBL1 antibodies, total protein was extracted from 0.5 g of leaf tissues homogenized in 1 mL of extraction buffer (50 mM HEPES, pH 7.4, 50 mM NaCl, 10 mM EDTA, 0.2% Triton X-100, and 1X proteinase inhibitor cocktail [Roche]). Crude protein extracts were immunoprecipitated on an anti-c-Myc agarose affinity gel (Sigma) overnight at 4°C. Protein extracts were separated by 12% SDS-PAGE and blotted onto Hybond-P membranes (GE Healthcare Bioscience). Fusion proteins were detected using a mouse anti-c-Myc antibody (Sigma) at 1:2,000 dilution. An anti-CaMBL1 antibody was raised in a rabbit against a synthetic peptide corresponding to C-terminal residues 284 to 297 of CaMBL1 (AbFrontier). Leaf protein extracts were immunoblotted using the anti-CaMBL1 antibody at 1:5,000 dilution, which was able to detect only CaMBL1 from the leaf extracts.

Measurement of SA

SA and SA glycoside were extracted and quantified according to the method described by Verberne et al. (2002). Leaf tissue samples (0.5 g) were frozen in liquid nitrogen, ground to a fine powder, and sequentially extracted with 90% and 100% methanol. As an internal standard for SA, 3-hydroxybenzoic acid (Sigma) was added at a mass ratio of 50 mg g⁻¹ fresh weight. SA was determined by fluorescence (excitation, 305 nm; emission, 355 nm) after separation on a C₁₈ reverse-phase HPLC column (Waters).

Measurement of Electrolyte Leakage

Pepper leaves were infiltrated with *Agrobacterium* strain GV3101 carrying the pBIn:CaMBL1 construct to determine electrolyte leakage. Leaf discs (1 cm in diameter) were washed in 20 mL of double distilled water for 30 min and transferred to 20 mL of double distilled water. Ion leakage was measured 0, 12, 24, and 36 h after infiltration. Conductance was measured with a conductivity meter (model sensION7; Hach). Electrical conductivities of the medium were expressed in μS cm⁻¹.

Quantification of H₂O₂

H₂O₂ production in plants was measured by ferrous oxidation using the xylenol orange assay (Choi et al., 2007). One milliliter of freshly prepared assay reagent (25 mM FeSO₄, and 25 mM [NH₄]₂SO₄ dissolved in 2.5 M H₂SO₄) was added to 100 μL of xylenol orange and 100 μL of sorbitol. Leaf discs (0.5 cm²) were floated for 10 min in 1 mL of distilled water, followed by centrifugation at 5,000g for 1 min. The supernatant (100 μL) was incubated for 30 min in 1 mL of xylenol orange reagent. H₂O₂ was determined at 560 nm using a standard H₂O₂ curve.

Staining with DAB and Trypan Blue

Infection and development of pathogens were assessed by staining inoculated plants with 1 mg mL⁻¹ DAB (Sigma D8001) and lactophenol-trypsin blue (10 mL of lactic acid, 10 mL of glycerol, 10 g of phenol, and 10 mg of trypsin blue, dissolved in 10 mL of distilled water). After overnight treatment with DAB, the stained leaves were cleared by boiling for 10 min in absolute ethanol and then destained overnight in absolute ethanol. The inoculated leaves were also boiled in trypan blue staining solution for 5 min and destained overnight in chloral hydrate (2.5 g of chloral hydrate dissolved in 1 mL of distilled water). The destained plant tissues were mounted in 70% glycerol for observation with a microscope.

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession numbers QG266892 (CaMBL1), AF503345 (CaBPR1), AF442388 (CaDEF1), AF442387 (CaPOA1), AF313766 (CaAR82A), DQ489711 (CaPO2), PR1 (At2g14610), PDF1.2 (At4g44420), NP_565191 (At1g78830), and UBQ5 (At3g62250).

Supplemental Data

The following materials are available in the online version of this article.

**Supplemental Figure S1.** Nucleotide and deduced amino acid sequences of pepper *CaMBL1* cDNA encoding a GNA-related lectin protein.

**Supplemental Figure S2.** Alignment of pepper *CaMBL1* with other GNA-related lectins.

**Supplemental Figure S3.** Phylogenetic tree analysis of *CaMBL1* with other GNA-related lectins.

**Supplemental Figure S4.** Disease responses of *CaMBL1-ox* and Arabidopsis ortholog mutants *mb1-1* and *mb1-2* to *H. arabidopsis* isolate Noco2 and *Pst DC3000* infection.

**Supplemental Figure S5.** Glycan-binding profile of *CaMBL1* as analyzed by the Consortium for Functional Glycomics.

**Supplemental Table S1.** The glycan binding data of *CaMBL1* as analyzed by the Consortium for Functional Glycomics.

ACKNOWLEDGMENTS

We thank Dr. S.P. Dinesh-Kumar (Yale University) for the pTRV1 and pTRV2 vectors and Dr. U. Bonas (Martin-Luther-Universität) for *Agrobacterium* strain GV3101.

Received August 26, 2010; accepted October 6, 2010; published January 3, 2011.

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Supplemental Figure S1. Nucleotide and deduced amino acid sequences of pepper CaMBL1 cDNA encoding a GNA-related lectin protein. The deduced amino acid sequences are given below the nucleotide sequences. The transcriptional initiation site is shown in bold type and the termination codon is marked by an asterisk (*). The putative GNA-related lectin is underlined. The signal peptide sequence is round-boxed. The putative N-glycosylation sites are indicated by white letters on a black background, and asparagines predicted to be N-glycosylated are indicated by the mark (+). The box indicates the polyadenylation sequence.
Supplemental Figure S2. Alignment of pepper (*Capsicum annuum*) CaMBL1 with other GNA-related lectins. Comparison of the deduced amino acid sequence of pepper CaMBL1 with an unknown grape (*Vitis vinifera*) protein (accession no. CAN75015), the sugar beet (*Beta vulgaris*) SIEP1L protein (accession no. CAA61158), the carrot (*Daucus carota*) cell attachment protein (accession no. BAD24818), the *Arabidopsis thaliana* curculin-like (mannose-binding) lectin family protein (CLLFP) (At1g78860, accession no. NP_178007), the flax (*Linum usitatissimum*) secreted glycoprotein (accession no. AAO15899) and another *Arabidopsis thaliana* CLLFP (At1g78830, accession no. NP_565191). The GNA-related lectin domain is boxed.
Supplemental Figure S3. Phylogenetic tree analysis of CaMBL1 with other GNA-related lectins. The neighbor-joining phylogenetic tree was constructed by using the GNA-related lectin sequences from the GenBank database and PlecDom program (http://www.nipgr.res.in/plecdom.html) for detection of plant lectin domain polypeptide or EST sequences. Values indicate the percent bootstrap for each branch. The horizontal scale shows the number of differences per 100 residues derived from the ClustalW alignment. Capsicum annuum GNA-related lectins (TC7720), Beta vulgaris SIEP1L protein (accession no. CAA61158), Vitis vinifera unknown (accession no. CAN75015), Capsicum annuum GNA-related lectins (TC7822_1), Daucus carota cell attachment protein (accession no. BAD24818), Linum usitatissimum secreted glycoprotein (accession no. AAO15899), Arabidopsis thaliana curculin-like (mannose-binding) lectin family protein (accession no. NP_565191, At1g78830), Arabidopsis thaliana curculin-like (mannose-binding) lectin family protein (accession no. NP_178003, At1g78820), Arabidopsis thaliana curculin-like (mannose-binding) lectin family protein (accession no. NP_178007, At1g78860), Arabidopsis thaliana curculin-like (mannose-binding) lectin family protein (accession no. NP_178006, At1g78850), Arabidopsis thaliana sugar binding protein (accession no. NP_001077549, At1g16905), Capsicum annuum GNA-related lectins (TC8218_1), Oryza sativa D-mannose-binding lectin family protein (accession no. ABA91378), Crinum asiaticum mannose-binding lectin gene (accession no. AY452806), Galanthus nivalis lectin (LECGNA 2) (accession no. M55556), Capsicum annuum GNA-related lectins (BM062519_1), Populus nigra PnLPK mRNA for lectin-like protein kinase, putative (accession no. AB030083), Arabidopsis thaliana lectin protein kinase, putative (accession no. NM_116475, At4g02420), Medicago truncatula lectin-like receptor kinase 7;2 (accession no. AAR11299), Arabidopsis thaliana lectin protein kinase family protein (accession no. NM_129932, At2g43700), Arabidopsis thaliana lectin protein kinase, putative (accession no. NM_129931, At2g43690), Arabidopsis thaliana receptor lectin kinase, putative (accession no. NM_115835, At3g59730), Arabidopsis thaliana receptor lectin kinase, putative (accession no. NM_115837, At3g59750), Arabidopsis thaliana ATHLECRK (ARABIDOPSIS THALIANA LECTIN-RECEPTOR KINASE) (accession no. NM_115832) and Arabidopsis thaliana receptor lectin kinase 3 (lecRK3) (accession no. NM_115836, At3g59740).
(A) Representative images of wild-type (WT) and #2 cotyledons infected with 35S::CaMBL1.

(B) Bar graph showing the percentage of cotyledons/class with sporangiophores/cotyledon for WT and 35S::CaMBL1 lines. The data are presented as mean ± standard deviation. Asterisks indicate significant differences between lines (ANOVA, Tukey's multiple comparison test, *p* < 0.05).

(C) Bar graph showing the percentage of cotyledons/class with sporangiophores/cotyledon for WT and mbl1-1, mbl1-2 lines. The data are presented as mean ± standard deviation. Asterisks indicate significant differences between lines (ANOVA, Tukey's multiple comparison test, *p* < 0.05).

(D) Bar graph showing the percentage of cotyledons/class with sporangiophores/cotyledon for WT and mbl1-1, mbl1-2 lines. The data are presented as mean ± standard deviation. Asterisks indicate significant differences between lines (ANOVA, Tukey's multiple comparison test, *p* < 0.05).

(E) Bar graph showing the change in Log cfu cm⁻² for WT, mbl1-1, mbl1-2 lines over time (0 and 3 days after inoculation). The data are presented as mean ± standard deviation.
Supplemental Figure S4. Disease responses of CaMBL1-OX plants and the Arabidopsis ortholog mutants mbl1-1 and mbl1-2 to Hyaloperonospora arabidopsis isolates Noco2 and Pseudomonas syringae pv. tomato (Pst) DC3000 infection. A, Disease reactions of 7-day-old cotyledons inoculated with an asexual spore suspension (5x 10^4 spores mL^-1) of H. arabidopsis isolates. Diseased cotyledons were photographed 7 days after inoculation and stained with trypan blue 5 days after inoculation. Bars= 0.5 mm. B, Sporulation on the infected cotyledons. The spores produced on at least 20 cotyledons were counted. C, Quantification of sporangiophore formation on cotyledons inoculated with H. arabidopsis isolate Noco2. Sporangiophores produced on at least 50 cotyledons were counted. D, Quantification of sporangiophore formation on cotyledons of the Arabidopsis ortholog mutants mbl1-1 and mbl1-2 inoculated with H. arabidopsis isolate Noco2. E. Bacterial growth in leaves of wild-type and Arabidopsis ortholog mutant (mbl1-1 and mbl1-2) plants 0 and 3 days after inoculation with Pst DC3000. Values are presented as means ± standard deviations. Different letters indicate significant differences using Fisher’s least significant difference test (P < 0.05) from three independent experiments.
Supplemental Figure S5. Glycan binding profile of CaMBL1, as analyzed by the Consortium for Functional Glycomics. CaMBL1 was used at concentration of 200 μg mL⁻¹ to determine the levels of glycan binding to CaMBL1 by fluorescence intensity. The printed array consists of 465 glycans in replicates of 6. The RFU (relative fluorescence units) is the mean of the 4 remaining values after removal of the highest and low values.
Supplemental Table 1. The glycan binding data of CaMBL1 (200 ug/mL), as analyzed by the Consortium for Functional Glycomics. The printed array data consist of 465 glycans in replicates of 6. Individual columns represent the glycan number, the structure or name, the average RFU (relative fluorescence units) value from the 6 replicates, the standard deviation, the standard error of the mean (used for the error bars) and %CV (%CV=100 X Std. Dev /Mean). The highest and lowest point from each set of six replicates has been removed, so that the average is of 4 values rather than 6. This eliminates some of the false hits that contain a single very high or low point. Thus, points with high %CV should be considered suspect.

| No. | Glycan Structure                                                                 | Average | SDDEV | SEM | %CV |
|-----|----------------------------------------------------------------------------------|---------|-------|-----|-----|
| 25  | [3OSO3]Galβ1-4[6OSO3]Glcβ-Sp0                                                   | 1021    | 65    | 33  | 6   |
| 378 | [GalNAcβ1-4GlcNAcβ1-2Manα1-6]GalNAcβ1-4GlcNAcβ1-2Manα1-3Manβ1-4GlcNAcβ1-4GlcNAcβ1-4GlcNAc-Sp12 | 573     | 39    | 20  | 7   |
| 350 | GlcNAcβ1-2Manα1-3(GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4(Fucα1-6)GlcNAcβ-Sp22     | 440     | 80    | 40  | 18  |
| 242 | Neu5Acα2-3Galβ1-3(Chi5Acα2-6)GalNAcα-Sp14                                      | 381     | 83    | 41  | 22  |
| 415 | Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ1-3GalNAcα-Sp14                                  | 317     | 60    | 30  | 19  |
| 340 | GlcNAcα1-4Galβ1-4GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ-Sp0 | 305     | 176   | 88  | 58  |
| 341 | GlcNAcα1-4Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp0                                  | 287     | 149   | 74  | 52  |
| 417 | GalNAcα1-3(Fucα1-2)Galβ1-4(Fucα1-3)GlcNAcβ1-3GalNAc-Sp14                        | 207     | 48    | 24  | 23  |
| 194 | Glcα1-4Glcα-Sp8                                                                | 141     | 63    | 32  | 45  |
| 423 | GalNAcα1-3(Fucα1-2)Galβ1-3GlcNAcβ1-3GalNAc-Sp14                                | 139     | 52    | 26  | 38  |
| 101 | Galα1-3(Fucα1-2)Galβ1-3GlcNAcβ-Sp0                                             | 137     | 20    | 10  | 14  |
| 352 | Galβ1-3GlcNAcβ1-2Manα1-3(Galβ1-3GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4(Fucα1-6)GlcNAcβ-Sp22 | 116     | 16    | 8   | 14  |
| 377 | Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3GalNAcα-Sp14                               | 115     | 32    | 16  | 28  |
| 449 | Galβ1-4(Fucα1-3)GlcNAcβ1-6GalNAc-Sp14                                          | 113     | 29    | 15  | 26  |
| 83  | GalNAcα1-3(Fucα1-2)Galβ1-4(Fucα1-3)GlcNAcβ-Sp0                                 | 112     | 32    | 16  | 29  |
| 351 | Galβ1-4GlcNAcβ1-2Manα1-3(Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4(Fucα1-6)GlcNAcβ-Sp22 | 98      | 17    | 9   | 18  |
| 12  | Galβ-Sp8                                                                       | 95      | 44    | 22  | 47  |
| 399 | Galβ1-4(Fucα1-3)GlcNAcβ1-3GalNAcα-Sp14                                         | 88      | 25    | 13  | 29  |
| 412 | Galα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-3GalNAcα-Sp14                                 | 87      | 15    | 7   | 17  |
| 60  | Fucα1-2Galβ1-3(Fucα1-4)GlcNAcβ-Sp8                                             | 87      | 18    | 9   | 21  |
| 342 | GlcNAcα1-4Galβ1-3GalNAc-Sp14                                                   | 86      | 94    | 47  | 109 |
| 291 | \(\text{Gal}\beta1-4\text{GlcNAc}\beta1-3\text{Gal}\beta1-3\text{GlcNAc}\beta-\text{Sp0} \) | 85 | 41 | 21 | 48 |
| 441 | \(\text{Neu5Aco2-3Gal}\beta1-4\text{GlcNAc}\beta1-3\text{Gal}\beta-\text{Sp8} \) | 84 | 21 | 10 | 25 |
| 347 | \(\text{Gal}\beta1-4\text{GlcNAc}\beta1-2\text{Mana1-3Man}\beta1-4\text{GlcNAc}\beta1-4\text{GlcNAc-Sp12} \) | 83 | 32 | 16 | 39 |
| 103 | \(\text{Gal-a1-3(Fuco1-2)Gal}\beta1-4(Fuco1-3)\text{GlcNAc}\beta-\text{Sp0} \) | 80 | 15 | 8 | 19 |
| 355 | \(\text{KDo}\beta2-3\text{Gal}\beta1-4(Fuco1-3)\text{GlcNAc-Sp0} \) | 79 | 7 | 4 | 9 |
| 58  | \(\text{Fuco1-2Gal}\beta1-3\text{GalNAc}\beta1-3\text{Galo-Sp9} \) | 79 | 16 | 8 | 20 |
| 138 | \(\text{Gal}\beta1-3\text{GalNAc-Sp14} \) | 76 | 8 | 4 | 11 |
| 97  | \(\text{GalNAc}\beta1-4(Fuco1-3)\text{GlcNAc-Sp0} \) | 75 | 6 | 3 | 8 |
| 416 | \(\text{Gal-a1-3(Fuco1-2)Gal}\beta1-4(Fuco1-3)\text{GlcNAc}\beta1-3\text{GalNAc-Sp14} \) | 73 | 22 | 11 | 30 |
| 238 | \(\text{Neu5Aco2-3Gal}\beta1-3(Fuco1-4)\text{GlcNAc}\beta1-3\text{Gal}\beta1-4(Fuco1-3)\text{GlcNAc-Sp0} \) | 72 | 3 | 2 | 4 |
| 349 | \(\text{Gal}\beta1-4\text{GlcNAc}\beta1-2\text{Mana1-3(Mana1-6)}\text{Man}\beta1-4\text{GlcNAc}\beta1-4\text{GlcNAc-Sp12} \) | 72 | 3 | 2 | 5 |
| 363 | \(\text{Mana1-3(Gal}\beta1-4\text{GlcNAc}\beta1-2\text{Mana1-6)}\text{Man}\beta1-4\text{GlcNAc}\beta1-4\text{GlcNAc-Sp12} \) | 71 | 14 | 7 | 19 |
| 434 | \(\text{Gal}\beta1-4\text{GlcNAc}\beta1-2\text{Mana1-3(GlcNAc}\beta1-4)\text{(Gal}\beta1-4\text{GlcNAc}\beta1-2)\text{Man}\beta1-4\text{GlcNAc}\beta1-4\text{GlcNAc-Sp21} \) | 67 | 18 | 9 | 26 |
| 364 | \(\text{Gal}\beta1-3(Fuco1-4)\text{GlcNAc}\beta1-2\text{Mana1-3(Gal}\beta1-3(Fuco1-4)\text{GlcNAc}\beta1-2\text{Mana1-6)}\text{Man}\beta1-4\text{GlcNAc}\beta1-4(Fuco1-6)\text{GlcNAc-Sp22} \) | 67 | 41 | 20 | 61 |
| 297 | \(\text{Gal}\beta1-3(\text{Neu5Aco2-3Gal}\beta1-4(Fuco1-3)\text{GlcNAc}\beta1-6)\text{GalNAc-Sp14} \) | 66 | 23 | 11 | 35 |
| 314 | \(\text{Mana1-2Mana1-2Mana1-6(Mana1-2Mana1-3(Mana1-6))Mana-Sp9} \) | 65 | 26 | 13 | 39 |
| 211 | \(\text{Mana1-3(Mana1-6)Mana-Sp9} \) | 64 | 20 | 10 | 31 |
| 57  | \(\text{Neu5Aco2-6Gal}\beta1-4\text{GlcNAc}\beta1-2\text{Mana1-3( Neu5Aco2-6Gal}\beta1-4\text{GlcNAc}\beta1-2\text{Mana1-6)}\text{Man}\beta1-4\text{GlcNAc}\beta1-4\text{GlcNAc-Sp13} \) | 64 | 21 | 10 | 32 |
| 290 | \(\text{Gal}\beta1-4(Fuco1-3)\text{GlcNAc}\beta1-3\text{Gal}\beta1-3(Fuco1-4)\text{GlcNAc-Sp0} \) | 64 | 25 | 12 | 38 |
| 2   | \(\text{Glco-Sp8} \) | 64 | 18 | 9 | 29 |
| 312 | \(\text{Mana1-6(Mana1-3)Mana1-6(Mana1-3)Man}\beta-\text{Sp10} \) | 63 | 28 | 14 | 45 |
| 313 | \(\text{Mana1-2Mana1-2Mana1-6(Mana1-2Mana1-3(Mana1-6))Mana-Sp9} \) | 63 | 12 | 6 | 19 |
| 197 | \(\text{Glc}\beta1-6\text{Glc}\beta-\text{Sp8} \) | 61 | 25 | 12 | 40 |
| 113 | \(\text{Gal}\beta1-3\text{Gal}\beta1-4(Fuco1-3)\text{GlcNAc-Sp8} \) | 60 | 13 | 6 | 21 |
| 388 | \(\text{Fuco1-2Gal}\beta1-3\text{GalNAco1-3(Fuco1-2)Gal}\beta1-4\text{Glc}\beta-\text{Sp0} \) | 60 | 26 | 13 | 44 |
| 429 | \(\text{Gal}\beta1-4\text{GlcNAc}\beta1-3\text{Gal}\beta1-4(Fuco1-3\text{GlcNAc}\beta1-6)\text{Gal}\beta1-4\text{Glc-Sp21} \) | 60 | 14 | 7 | 24 |
| 459 | \(\text{GalNAco1-3(Fuco1-2)Gal}\beta1-3\text{GlcNAc}\beta1-2\text{Mana1-6(GalNAco1-3(Fuco1-2)Gal}\beta1-3\text{GlcNAc}\beta1-2\text{Mana1-3)Man}\beta1-4\text{GlcNAc}\beta1-4(Fuco1-6)\text{GlcNAc-Sp22} \) | 60 | 38 | 19 | 64 |
| 361 | \(\text{Fuco1-2Gal}\beta1-4(Fuco1-3)\text{GlcNAc}\beta1-2\text{Mana1-3(Fuco1-2Gal}\beta1-4(Fuco1-3)\text{GlcNAc}\beta1-2\text{Mana1-6)Man}\beta1-4\text{GlcNAc}\beta1-4\text{GlcNAc-Sp20} \) | 60 | 14 | 7 | 24 |
| 209 | \(\text{Mana1-2Mana1-6(Mana1-3)Mana1-6(Mana1-2Mana1-2Mana1-3)Man}\beta1-4\text{GlcNAc}\beta1-4\text{GlcNAc-Sp12} \) | 60 | 26 | 13 | 44 |
|   | Chemical Structure                                                                 | Position | β1-4Galβ1-4(Fuco1-3)[6OSO3]Glc-Sp0 |
|---|------------------------------------------------------------------------------------|----------|-----------------------------------|
| 23|                                                                                   |          |                                   |
| 139| Galβ1-3GalNAc-Sp16                                                               |          |                                   |
| 115| Galα1-3Galβ1-4GlcNAcβ-Sp8                                                         |          |                                   |
| 167| Galβ1-4GlcNAcβ-Sp0                                                                |          |                                   |
| 14 | Manβ-Sp8                                                                         |          |                                   |
| 144| Galβ1-3Galβ-Sp8                                                                  |          |                                   |
| 195| Glc1-6Glc1-6Glcβ-Sp8                                                              |          |                                   |
| 168| Galβ1-4GlcNAcβ-Sp8                                                                |          |                                   |
| 443| [6OSO3]Galβ1-3GlcNAcβ-Sp0                                                         |          |                                   |
| 205| Manα1-2Manα1-2Manα1-3Manα-Sp9                                                     |          |                                   |
| 190| GlcNAcβ1-6GalNAc-Sp8                                                              |          |                                   |
| 124| Galβ1-2Galβ-Sp8                                                                  |          |                                   |
| 385| Galβ1-3GlcNAcβ1-3(Galβ1-3GlcNAcβ1-3Galβ1-4(Fuco1-3)GlcNAcβ1-6)Galβ1-4Glc-Sp21    |          |                                   |
| 433| GlcNAcβ1-4(GlcNAcβ1-2)Manα1-3(GlcNAcβ1-4)(GlcNAcβ1-6(GlcNAcβ1-2)Manα1-6)Galβ1-4GlcNAcβ1-4GlcNAc-Sp21 |          |                                   |
| 368| Galβ1-4GlcNAcβ1-2(Galβ1-4GlcNAcβ1-4)Manα1-3(Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc-Sp21 |          |                                   |
| 220| Fuc1-2[6OSO3]Galβ1-4[6OSO3]Glc-Sp0                                                |          |                                   |
| 438| Galα1-3Galβ1-4Glc-Sp10                                                            |          |                                   |
| 268| Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4(Fuco1-3)GlcNAcβ1-3Galβ1-4(Fuco1-3)GlcNAcβ-Sp0|          |                                   |
| 214| Manα1-6(Manα1-3)Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12                   |          |                                   |
| 435| Galβ1-4GlcNAcβ1-4(Galβ1-4GlcNAcβ1-2)Manα1-3(GlcNAcβ1-4)(Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc-Sp21 |          |                                   |
| 121| Galα1-4Galβ1-4Glcβ-Sp0                                                            |          |                                   |
| 149| Galβ1-4(Fuco1-3)GlcNAcβ-Sp0                                                       |          |                                   |
| 282| Neu5Gco2-3Galβ1-4Glcβ-Sp0                                                         |          |                                   |
| 170| Galβ1-4Glcβ-Sp8                                                                  |          |                                   |
| 164| Galβ1-4GlcNAcβ1-6(Galβ1-3)GalNAc-Sp8                                              |          |                                   |
| 357| KDNα2-3Galβ1-4Glc-Sp0                                                             |          |                                   |
| 309| GlcNAcβ1-4GlcNAcβ-Sp12                                                            |          |                                   |
| 114| Galα1-3Galβ1-3GlcNAcβ-Sp0                                                         |          |                                   |
| 180| GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp8                                                      |          |                                   |
| 260| Fuc1-2Galβ1-4[6OSO3]Glc-Sp0                                                       |          |                                   |
| 345| Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6Manβ1-4GlcNAcβ1-4GlcNAc-Sp12                   |          |                                   |
| Number | Structure                                                                 | n  | ω  | m  |
|--------|----------------------------------------------------------------------------|----|----|----|
| 325    | Fucα1-3(Galβ1-4)GlcNAcβ1-2Manα1-3(Fucoα1-3(Galβ1-4)GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp20 | 49 | 15 | 8  |
| 122    | Galα1-4GlcNAcβ-Sp8                                                        | 48 | 24 | 12 |
| 393    | Galα1-3Galβ1-3GalNAcβ1-2Manα1-3(Galα1-3Galβ1-3GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc-Sp19 | 48 | 22 | 11 |
| 165    | Galβ1-3(Galβ1-4GlcNAcβ1-6)GalNAcα-Sp8                                     | 48 | 1  | 1  |
| 321    | Neu5Gcβ2-6Galβ1-4GlcNAc-Sp8                                              | 48 | 13 | 6  |
| 199    | GlcAα-Sp8                                                                 | 48 | 27 | 13 |
| 212    | Mana1-3(Mana1-2Manα1-2Manα1-6)Manα-Sp9                                    | 47 | 24 | 12 |
| 217    | [3OSO3]Galβ1-4(Fucoα1-3)[6OSO3]GlcNAc-Sp8                                 | 47 | 13 | 7  |
| 96     | GalNAcβ1-3Galα1-4Galβ1-4GlcNAcβ-Sp0                                       | 47 | 12 | 6  |
| 465    | Glcα1-4Glcα1-4Glcβ-Sp10                                                   | 47 | 18 | 9  |
| 374    | Fucα1-2Galβ1-3(Fucoα1-4)GlcNAcβ1-2Manα1-3(Fucoα1-2)Galβ1-3(Fucoα1-4)GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp19 | 47 | 26 | 13 |
| 3      | Mana-Sp8                                                                  | 46 | 4  | 2  |
| 426    | Galα1-3(Fucoα1-2)Galβ1-4GlcNAcβ1-2Manα1-3(Galα1-3(Fucoα1-2)Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp22 | 46 | 9  | 4  |
| 439    | Galβ1-4Galβ-Sp10                                                          | 46 | 9  | 5  |
| 166    | Galβ1-3(Galβ1-4GlcNAcβ1-6)GalNAc-Sp14                                     | 46 | 19 | 10 |
| 188    | GlcNAcβ1-4GlcNAcβ1-4GlcNAcβ-Sp8                                           | 46 | 3  | 2  |
| 362    | Galα1-3Galβ1-4GlcNAcβ1-2Manα1-3(Galα1-3Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp20 | 46 | 23 | 12 |
| 409    | Neu5Aco2-6Galβ1-3GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Galβ1-4Glc-Sp21             | 46 | 16 | 8  |
| 440    | Galβ1-6Galβ-Sp10                                                          | 45 | 15 | 8  |
| 81     | Fucβ1-3GlcNAcβ-Sp8                                                        | 45 | 11 | 5  |
| 77     | Fucα1-2Galβ1-4Glcβ-Sp0                                                    | 45 | 12 | 6  |
| 339    | GlcNAcα1-4Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ-Sp0                                | 45 | 16 | 8  |
| 7      | Fuc-Sp9                                                                   | 45 | 19 | 10 |
| 153    | Galβ1-4[6OSO3]Glcβ-Sp0                                                    | 45 | 10 | 5  |
| 126    | Galβ1-3(Fucoα1-4)GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp0                              | 44 | 31 | 15 |
| 116    | Galα1-3Galβ1-4Glcβ-Sp0                                                    | 44 | 9  | 5  |
| 105    | Galα1-3(Fucoα1-2)Galβ1-4GlcNAc-Sp0                                        | 44 | 11 | 5  |
| 208    | Mana1-6((Mana1-2Manα1-3)Manα1-6((Mana1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12 | 44 | 8  | 4  |
| 79     | Fucα1-3GlcNAcβ-Sp8                                                        | 44 | 28 | 14 |
| Reaction  | Structure                                                | Reaction  | Structure                                                |
|-----------|-----------------------------------------------------------|-----------|-----------------------------------------------------------|
| 287       | Galβ1-3GlcNAcβ1-3Galβ1-3GlcNAcβ-Sp0                      | 44        | 13                                                       | 6   | 29 |
| 366       | Neu5Acα2-6GlcNAcβ1-4GlcNAcβ1-4GlcNAc-Sp21                 | 43        | 24                                                       | 12  | 55 |
| 4         | GalNAc-Sp8                                                | 43        | 8                                                        | 4   | 19 |
| 69        | Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ-Sp0 | 43        | 15                                                       | 7   | 34 |
| 127       | Galβ1-3(Fucα1-4)GlcNAcβ-Sp0                              | 42        | 12                                                       | 6   | 28 |
| 316       | Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12 | 42        | 11                                                       | 5   | 25 |
| 391       | Neu5Acα2-3(GalNAcβ1-4)Galβ1-4GlcNAcβ1-3GalNAc-Sp14       | 42        | 9                                                        | 5   | 22 |
| 106       | Galα1-3(Fucα1-2)Galβ1-4Glcβ-Sp0                          | 42        | 10                                                       | 5   | 24 |
| 382       | Galβ1-3GlcNAcβ1-3(Galβ1-4(Fucα1-3)GlcNAcβ1-6)Galβ1-4Glc-Sp21 | 42        | 32                                                       | 16  | 76 |
| 436       | Galβ1-4GlcNAcβ1-2Manα1-3(GlcNAcβ1-4)(Galβ1-4GlcNAcβ1-6(Galβ1-4GlcNAcβ1-2)Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc-Sp21 | 42        | 15                                                       | 7   | 36 |
| 192       | GlicNAcβ1-6Galβ1-4GlcNAcβ-Sp8                            | 42        | 11                                                       | 5   | 26 |
| 413       | GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-3GalNAc-Sp14          | 42        | 12                                                       | 6   | 28 |
| 367       | Fucα1-2Galβ1-3GlcNAcβ1-3(Galβ1-4(Fucα1-3)GlcNAcβ1-6)Galβ1-4Glc-Sp21 | 42        | 16                                                       | 8   | 38 |
| 446       | Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ1-2(Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ1-4)Manα1-3(Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12 | 42        | 22                                                       | 11  | 54 |
| 379       | Galβ1-3GalNAcα1-3(Fucα1-2)Galβ1-4Glc-Sp0                  | 42        | 19                                                       | 10  | 47 |
| 216       | Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAc-Sp0    | 42        | 12                                                       | 6   | 30 |
| 437       | Galβ1-4GlcNAcβ1-4(Galβ1-4GlcNAcβ1-2)Manα1-3(GlcNAcβ1-4)(Galβ1-4GlcNAcβ1-6(Galβ1-4GlcNAcβ1-2)Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc-Sp21 | 41        | 11                                                       | 6   | 28 |
| 99        | GalNAcβ1-4GlcNAcβ-Sp8                                     | 41        | 21                                                       | 10  | 51 |
| 387       | GlicNAcβ1-2(GlcNAcβ1-4)Manα1-3(GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc-Sp21 | 40        | 12                                                       | 6   | 30 |
| 289       | Galβ1-4(Fucα1-3)β6OSO3Glc-Sp0                             | 40        | 12                                                       | 6   | 29 |
| 56        | Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12 | 40        | 30                                                       | 15  | 74 |
| 141       | Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ-Sp0                  | 40        | 38                                                       | 19  | 96 |
| 67        | Fucα1-2Galβ1-3GlcNAcβ-Sp0                                 | 40        | 14                                                       | 7   | 35 |
| 111       | Galα1-3GalNAc-Sp16                                        | 40        | 10                                                       | 5   | 26 |
| 386       | Galβ1-4GlcNAcβ1-2(Galβ1-4GlcNAcβ1-4)Manα1-3(Galβ1-4GlcNAcβ1-2)Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp21 | 40        | 39                                                       | 19  | 98 |
| 76        | Fucα1-2Galβ1-4GlcNAcβ-Sp8                                 | 39        | 6                                                        | 3   | 15 |
| 119       | Galα1-4Galβ1-4GlcNAcβ-Sp0                                 | 39        | 17                                                       | 8   | 42 |
| 148       | Galβ1-3GlcNAcβ-Sp8                                        | 39        | 17                                                       | 8   | 43 |
|   |   |   |   |   |
|---|---|---|---|---|
| 288 | Galβ1-4(Fucα1-3)[6OSO3]GlcNAc-Sp0 | 39 | 23 | 12 | 60 |
| 189 | GlcNAcβ1-6(Galβ1-3)GalNAc-Sp8 | 39 | 21 | 11 | 55 |
| 140 | Galβ1-3GalNAcβ-Sp8 | 39 | 13 | 6 | 33 |
| 305 | GlcAB1-3GlcNAcβ-Sp8 | 38 | 12 | 6 | 32 |
| 71 | Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ-Sp0 | 38 | 10 | 5 | 26 |
| 431 | GlcNAcβ1-4(GlcNAcβ1-2)Manα1-3(GlcNAcβ1-4)(GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc-Sp21 | 38 | 17 | 9 | 45 |
| 369 | GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-2Manα1-3(GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc-Sp20 | 38 | 16 | 8 | 41 |
| 447 | Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)GalNAc-Sp14 | 38 | 9 | 4 | 23 |
| 129 | Galβ1-3(Fucα1-4)GlcNAc-Sp8 | 38 | 6 | 3 | 16 |
| 112 | Galect1-3GalNAcβ-Sp8 | 38 | 9 | 4 | 23 |
| 323 | Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc-Sp12 | 38 | 9 | 5 | 25 |
| 201 | GlcAB1-3Galβ-Sp8 | 37 | 13 | 6 | 34 |
| 32 | [3OSO3]Galβ1-4(Fucα1-3)GlcNAcβ-Sp8 | 37 | 9 | 4 | 23 |
| 331 | Galect1-4Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ-Sp0 | 37 | 15 | 7 | 40 |
| 428 | Fucα1-2Galβ1-3GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Galβ1-4Glc-Sp21 | 37 | 12 | 6 | 33 |
| 407 | GlcNAcβ1-6(GlcNAcβ1-3)GalNAc-Sp14 | 37 | 16 | 8 | 43 |
| 264 | Neu5Acα2-6GalNAcβ1-4GlcNAcβ-Sp0 | 37 | 5 | 3 | 15 |
| 5 | GalNAc-Sp15 | 37 | 9 | 4 | 24 |
| 61 | Fucα1-2Galβ1-3GalNAc-Sp8 | 37 | 25 | 13 | 68 |
| 450 | Galβ1-4GlcNAcβ1-2Manα-Sp0 | 37 | 13 | 6 | 34 |
| 455 | GalNAcβ1-4Galβ1-4Glc-b-sp0 | 37 | 21 | 11 | 58 |
| 154 | Galβ1-4[6OSO3]Glcβ-Sp8 | 36 | 12 | 6 | 33 |
| 215 | Manβ1-4GlcNAcβ-Sp0 | 36 | 8 | 4 | 23 |
| 299 | Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc-Sp12 | 36 | 11 | 5 | 30 |
| 128 | Galβ1-3(Fucα1-4)GlcNAcβ-Sp8 | 36 | 22 | 11 | 60 |
| 19 | Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)GalNAc-Sp8 | 36 | 13 | 6 | 36 |
| 92 | GalNAcα1-3Galβ-Sp8 | 36 | 15 | 8 | 43 |
| 36 | [3OSO3]Galβ1-4GlcNAcβ-Sp8 | 36 | 15 | 8 | 42 |
| 427 | Galβ1-3GlcNAcβ1-2Manα1-3(Galβ1-3GlcNAcβ1-2(Galβ1-3GlcNAcβ1-6)Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp19 | 36 | 15 | 8 | 43 |
| 13 | Glcβ-Sp8 | 36 | 19 | 10 | 53 |
|   |   |   |   |   |
|---|---|---|---|---|
| 408 | Galβ1-3(Fuco1-2)Galβ1-4(Fuco1-3)Glcβ-Sp21 | 36 | 9 | 4 | 24 |
| 203 | KDNo2-3Galβ1-3GlcNAcβ-Sp0 | 36 | 7 | 3 | 18 |
| 320 | Neu5Aco2-8Neu5Aco2-8Neu5Acoβ-Sp8 | 36 | 17 | 8 | 47 |
| 311 | Mana1-6Manβ-Sp10 | 36 | 9 | 4 | 24 |
| 147 | Galβ1-3GlcNAcβ-Sp0 | 36 | 9 | 4 | 24 |
| 62 | Fuco1-2Galβ1-3GalNAc-Sp14 | 36 | 9 | 4 | 24 |
| 262 | Neu5Aco2-3Galβ1-4Glcβ-Sp8 | 36 | 9 | 4 | 24 |
| 84 | [3OSO3]Galβ1-4(Fuco1-3)Glc-Sp0 | 37 | 3 | 20 |
| 135 | Galβ1-3(Neu5Aco2-6)GalNAc-Sp8 | 38 | 30 | 15 | 85 |
| 70 | Fuco1-2Galβ1-4(Fuco1-3)GlcNAcβ1-3Galβ1-4(Fuco1-3)GlcNAcβ1-3Galβ1-4(Fuco1-3)GlcNAcβ-Sp0 | 35 | 9 | 4 | 24 |
| 206 | Mana1-2Mana1-3(Mana1-2Mana1-6)Manβ-Sp9 | 35 | 18 | 9 | 52 |
| 85 | GalNAcβ1-3(Fuco1-2)Galβ1-4GlcNAcβ-Sp0 | 34 | 13 | 7 | 38 |
| 109 | Galα1-3(Galα1-4)Galβ1-4GlcNAcβ-Sp8 | 34 | 13 | 7 | 38 |
| 182 | GlcNAcβ1-3Galβ1-4Glcβ-Sp0 | 34 | 5 | 3 | 16 |
| 335 | Neu5Aco2-3-Galβ1-3(Galβ1-4(Fuco1-3)GlcNAcβ1-6)GalNAc-Sp14 | 34 | 13 | 7 | 38 |
| 40 | 6-H2PO3Manβ-Sp8 | 34 | 5 | 3 | 16 |
| 142 | Galβ1-3GlcNAcβ1-4(Neu5Aco2-3)Galβ1-4Glcβ-Sp0 | 34 | 12 | 6 | 37 |
| 333 | GalNAcβ1-3(Fuco1-2)Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp0 | 34 | 11 | 6 | 33 |
| 464 | Glcα1-6Glcα1-6Glcβ-Sp10 | 34 | 8 | 4 | 24 |
| 53 | Galβ1-4GlcNAcβ1-2Manα1-3(Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12 | 33 | 16 | 8 | 46 |
| 68 | Fuco1-2Galβ1-3GlcNAcβ-Sp8 | 33 | 12 | 6 | 35 |
| 131 | Galβ1-3(GlcNAcβ1-6)GalNAc-Sp8 | 33 | 36 | 18 | 107 |
| 304 | GalNAcβ1-3Galβ-Sp8 | 33 | 22 | 11 | 66 |
| 49 | Mana1-3(Mana1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12 | 33 | 10 | 5 | 29 |
| 411 | Neu5Aco2-3Galβ1-3GalNAcβ1-4(Neu5Aco2-8Neu5Aco2-3)Galβ1-4Glcβ-Sp0 | 33 | 27 | 14 | 83 |
| 265 | Neu5Aco2-6Galβ1-4[6OSO3]GlcNAcβ-Sp8 | 33 | 17 | 9 | 53 |
| 8 | Rhac-Sp8 | 33 | 49 | 24 | 149 |
| 329 | Neu5Aco2-3Galβ1-3(Fuco1-4)GlcNAcβ1-3Galβ1-3(Fuco1-4)GlcNAcβ-Sp0 | 33 | 23 | 12 | 71 |
| 137 | Galβ1-3GalNAc-Sp8 | 33 | 9 | 5 | 29 |
| 247 | Neu5Aco2-3Galβ1-3GlcNAcβ-Sp0 | 33 | 22 | 11 | 68 |
| 271 | Neu5Aco2-6Galβ1-4Glcβ-Sp8 | 32 | 17 | 9 | 54 |
| 73 | Fuco1-2Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp0 | 32 | 12 | 6 | 38 |
| 80 | Fuco1-4GlcNAcβ-Sp8 | 32 | 12 | 6 | 38 |
|   | Monosaccharides Description | Count | Count | Count | Count |
|---|-----------------------------|-------|-------|-------|-------|
| 59 | Fucα1-2Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ-Sp9 | 32    | 9     | 4     | 27    |
| 458 | Neu5Acoα2-6Gαlβ1-4GlcNAcβ1-6(Fucα1-2Galβ1-3GalNAcβ1-3)Galβ1-4Glc-Sp21 | 32    | 15    | 8     | 48    |
| 87  | GalNAcβ1-3(Fucα1-2)Galβ1-4Glcβ-Sp0 | 32    | 26    | 13    | 82    |
| 336 | GlcNAcα1-4Galβ1-4GlcNAcβ1-3Galβ1-1GlcNAcβ1-3Galβ1-4GlcNAcβ-SP0 | 32    | 11    | 6     | 35    |
| 383 | Fucα1-2Galβ1-3(Fucα1-4)GlcNAcβ1-3(Galβ1-1GlcNAcβ1-6)Galβ1-1Glc-Sp21 | 32    | 27    | 14    | 86    |
| 98  | GalNAcβ1-4GlcNAcβ-Sp0 | 32    | 26    | 13    | 82    |
| 422 | Galα1-3(Fucα1-2)Galβ1-3GlcNAcβ1-3GalNAc-Sp14 | 31    | 20    | 10    | 63    |
| 125 | Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-14(Fucα1-3)GlcNAcβ-Sp0 | 31    | 13    | 6     | 41    |
| 41  | [6OSO3]Galβ1-4Glcβ-Sp0 | 31    | 17    | 9     | 56    |
| 306 | GlcNAcβ1-2Manα1-3(Neu5Acoα2-6Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-1GlcNAcβ1-4GlcNAcβ-Sp12 | 31    | 25    | 13    | 82    |
| 187 | GlcNAcβ1-4GlcNAcβ-1-4GlcNAcβ1-4GlcNAcβ1-4GlcNAcβ-Sp8 | 31    | 6     | 3     | 19    |
| 237 | Neu5Acoα2-3Galβ1-3(Fucα1-4)GlcNAcβ-Sp8 | 31    | 12    | 6     | 40    |
| 1   | Galα-Sp8 | 31    | 11    | 6     | 37    |
| 94  | GalNAcβ1-3GalNAcα-Sp8 | 31    | 15    | 8     | 49    |
| 95  | GalNAcβ1-3(Fucα1-2)Galβ-Sp8 | 31    | 11    | 6     | 37    |
| 210 | Manα1-2Manα1-2Manα1-3(Manα1-2Manα1-6)Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12 | 31    | 17    | 8     | 54    |
| 365 | Neu5Acoα2-6GlcNAcβ1-4GlcNAc-Sp21 | 31    | 13    | 7     | 43    |
| 267 | Neu5Acoα2-6Galβ1-4GlcNAcβ-Sp8 | 30    | 28    | 14    | 93    |
| 34  | [3OSO3]Galβ1-4[6OSO3]GlcNAcβ-Sp8 | 30    | 36    | 18    | 120   |
| 337 | GlcNAcβ1-4Galβ1-4GlcNAcβ-Sp0 | 30    | 3     | 2     | 11    |
| 136 | Galβ1-3(Neu5Acoα2-6)GlcNAcβ1-4Galβ1-4Glcβ-Sp10 | 30    | 19    | 10    | 64    |
| 231 | Neu5Acoα2-3(GalNAcβ1-4)Galβ1-14Glcβ-Sp0 | 30    | 9     | 4     | 29    |
| 303 | Galβ1-4GlcNAcβ1-6Galβ1-4GlcNAcβ-Sp0 | 30    | 15    | 7     | 49    |
| 452 | Galα1-3(Fucα1-2)Galβ1-14GlcNAcβ1-3(Manα1-3Fucα1-2Galβ1-4GlcNAcβ1-6)GalNAc-Sp14 | 30    | 6     | 3     | 21    |
| 269 | Neu5Acoα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp0 | 30    | 19    | 9     | 63    |
| 301 | Galβ1-4GlcNAcβ1-3(Manα1-6)Galβ1-14GlcNAc-Sp0 | 30    | 7     | 4     | 25    |
| 107 | Galα1-3(Fucα1-2)Galβ-Sp8 | 29    | 5     | 2     | 17    |
| 381 | Galβ1-3GlcNAcβ1-3(Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ1-6)Galβ1-1Glcβ-Sp0 | 29    | 2     | 1     | 7     |
| 169 | Galβ1-4Glcβ-Sp0 | 29    | 18    | 9     | 61    |
| 162 | Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ-Sp0 | 29    | 9     | 5     | 32    |
| 253 | Neu5Acoα2-3Galβ1-14(Fucα1-3)GlcNAcβ-Sp8 | 29    | 22    | 11    | 75    |
| 277 | Neu5Acoβ2-6Galβ1-4GlcNAcβ-Sp8 | 29    | 12    | 6     | 42    |
| 445 | Fucα1-2Galβ1-4GlcNAcβ1-2(Fucα1-2Galβ1-4GlcNAcβ1-4)Manα1-3{Fucα1-2Galβ1-4} | 29    | 6     | 3     | 19    |
| GlcNAcβ1-2Mana1-6| Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12 | 82 | 29 | 17 | 9 | 61 |
|-----------------|-------------------------------|---|----|----|---|----|
| GlcNAcβ1-3(Fucα1-2)Galβ1-3GlcNAcβ-Sp0 | 177 | 29 | 19 | 9 | 66 |
| GlcNAcβ1-3GalNAc-Sp14 | 338 | 28 | 22 | 11 | 77 |
| Neu5Gcα2-3Galβ1-3GlcNAcβ-Sp0 | 279 | 28 | 3 | 1 | 10 |
| GlcNAcβ1-3GalNAcβ1-4GlcNAcβ-Sp0 | 179 | 28 | 14 | 7 | 51 |
| Galα1-3(Fucα1-2)Galβ1-3GalNAc-Sp8 | 462 | 28 | 19 | 9 | 67 |
| Neu5Aca2-8Neu5Aca2-3Galα1-4(Neu5Aca2-8Neu5Aca2-3)Galβ1-4Glcβ-sp0 | 454 | 28 | 7 | 3 | 24 |
| Galα1-4Galβ1-4GlcNAcβ-Sp8 | 120 | 28 | 5 | 2 | 17 |
| Galα1-3Galβ-Sp8 | 117 | 28 | 8 | 4 | 30 |
| GalNAcα1-3Fucα1-2Galβ1-4GlcNAcβ1-3(GalNAcα1-3Fucα1-2Galβ1-4GlcNAcβ1-6)GalNAc-Sp14 | 453 | 28 | 13 | 7 | 49 |
| KDNo2-3Galβ1-4GlcNAcβ-Sp0 | 204 | 27 | 15 | 7 | 53 |
| GalNAcβ1-3Galα1-6Galβ1-4Glcβ-Sp8 | 406 | 27 | 12 | 6 | 46 |
| Neu5Aca2-3Galα1-4(Fucα1-3)GlcNAcβ-Sp8 | 254 | 27 | 16 | 8 | 60 |
| GlcNAcβ1-3(GlcNAcβ1-6)Galβ1-4GlcNAcβ-Sp8 | 175 | 27 | 26 | 13 | 97 |
| Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ-Sp8 | 72 | 27 | 8 | 4 | 29 |
| Neu5Aca2-8Neu5Aca2-8Neu5Aca2-3Galβ1-4Glcβ-Sp0 | 225 | 27 | 10 | 5 | 37 |
| Fucα1-2Galβ-Sp8 | 78 | 27 | 28 | 14 | 106 |
| Neu5Aca-Sp8 | 9 | 27 | 7 | 4 | 28 |
| GalNAcβ1-2Mana1-3(GlcNAcβ1-2Mana1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12 | 51 | 27 | 13 | 6 | 48 |
| Neu5Aca2-3Galβ-Sp8 | 243 | 27 | 11 | 6 | 42 |
| Galα1-3GlcNAcβ1-3GalNAc-Sp14 | 390 | 27 | 13 | 7 | 50 |
| 6-H2PO3Glcβ-Sp10 | 296 | 27 | 12 | 6 | 44 |
| Neu5Gcα2-6GalNAc-Sp0 | 283 | 27 | 6 | 3 | 23 |
| Galβ1-3GalNAcβ1-4Galβ1-4Glcβ-Sp8 | 143 | 26 | 3 | 1 | 10 |
| KDNo2-6Galβ1-4GlcNAc-Sp0 | 356 | 26 | 19 | 10 | 73 |
| Galβ1-4(Fucα1-3)GlcNAcβ1-4Galβ1-4(Fucα1-3)GlcNAcβ-Sp0 | 151 | 26 | 18 | 9 | 70 |
| GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp0 | 181 | 26 | 7 | 4 | 27 |
| Galβ1-3GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAc-Sp0 | 380 | 26 | 3 | 2 | 13 |
| Galβ1-4(Fucα1-3)GlcNAcβ-Sp8 | 150 | 26 | 8 | 4 | 31 |
| GalNAcβ1-2Mana1-3(GlcNAcβ1-4)GlcNAcβ1-2Mana1-6)Manβ1-4GlcNAcβ1-4GlcNAc-Sp21 | 430 | 26 | 13 | 6 | 49 |
|   | Formula  |   |   |   |
|---|----------|---|---|---|
| 35 | GlcNAcβ1-4GlcNAcβ-Sp8 | 26 | 15 | 7 | 56 |
| 202 | GlcAβ1-6GlcAβ-Sp8 | 25 | 13 | 6 | 50 |
| 63 | Neu5Acβ1-2Galβ1-3GalNAcβ1-4(Neu5Acβ1-3)Galβ1-4Glcβ-Sp0 | 25 | 10 | 5 | 38 |
| 273 | Neu5Acβ1-4Glcβ-Sp8 | 25 | 5 | 2 | 20 |
| 401 | Galβ1-3GlcNAcβ1-2Manβ1-3(Galβ1-4GlcNAcβ1-2Manβ1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp19 | 25 | 16 | 8 | 62 |
| 425 | Neu5Acβ1-2GlcNAcβ1-3GalNAcβ1-4Glcβ-Sp0 | 25 | 11 | 6 | 45 |
| 414 | Neu5Acβ1-3GalNAcβ1-4Galβ1-4Glcβ-Sp8 | 25 | 8 | 4 | 31 |
| 410 | Neu5Acβ1-4GlcNAcβ1-3GalNAcβ-Sp8 | 25 | 8 | 4 | 31 |
| 418 | Neu5Acβ1-2Galβ1-3GalNAcβ-Sp0 | 25 | 11 | 6 | 47 |
| 453 | Neu5Acβ1-3GalNAcβ-Sp8 | 25 | 8 | 4 | 31 |
| 50 | Neu5Acβ1-2Manβ1-3(GlcNAcβ1-2Manβ1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp13 | 24 | 23 | 11 | 95 |
| 183 | Neu5Acβ1-4(GlcNAcβ1-6)GalNAcβ-Sp8 | 24 | 17 | 8 | 70 |
| 463 | Neu5Acβ1-3GlcNAcβ-Sp8 | 24 | 5 | 3 | 23 |
| 30 | Neu5Acβ1-3GlcNAcβ-Sp8 | 24 | 5 | 3 | 22 |
| 198 | Neu5Acβ1-3GlcNAcβ-Sp8 | 24 | 5 | 3 | 22 |
| 202 | Neu5Acβ1-2Manβ1-3(Galβ1-4GlcNAcβ1-2Manβ1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp19 | 23 | 14 | 7 | 61 |
| 318 | Neu5Acβ1-2Manβ1-3(GlcNAcβ1-2Manβ1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12 | 23 | 6 | 3 | 25 |
| 343 | Neu5Acβ1-2Manβ1-3(GlcNAcβ1-2Manβ1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12 | 23 | 6 | 3 | 25 |
| 275 | Neu5Acβ1-3GalNAcβ1-3GlcNAcβ-Sp0 | 23 | 5 | 3 | 23 |
| 322 | Neu5Acβ1-2Manβ1-3(Galβ1-4GlcNAcβ1-2Manβ1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp19 | 23 | 5 | 2 | 20 |
| 389 | Neu5Acβ1-2Manβ1-3GlcNAcβ-Sp8 | 23 | 6 | 3 | 28 |
| 400 | Neu5Acβ1-2GlcNAcβ1-3GalNAcβ1-4GlcNAcβ-Sp0 | 23 | 14 | 7 | 60 |
| 37 | Neu5Acβ1-4GlcAβ-Sp8 | 23 | 8 | 4 | 37 |
|   |   |   |   |   |
|---|---|---|---|---|
| 371 Galα1-3Galβ1-4(Fucoα1-3)GlcNAcβ1-2Manα1-3(Galα1-3Galβ1-4(Fucoα1-3)GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp20 | 23 | 24 | 12 | 107 |
| 16 GlcNAcβ-Sp0 | 23 | 4 | 2 | 19 |
| 75 Fucoα1-2Galβ1-4GlcNAcβ-Sp0 | 22 | 8 | 4 | 38 |
| 44 [6OSO3]Galβ1-4[6OSO3]Glcβ-Sp8 | 22 | 12 | 6 | 53 |
| 123 Galα1-6Glcβ-Sp8 | 22 | 6 | 3 | 28 |
| 298 Galβ1-3Galβ1-4GlcNAcβ-Sp8 | 22 | 13 | 6 | 58 |
| 346 Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manβ1-3Manβ1-4GlcNAcβ1-4GlcNAc-Sp12 | 22 | 14 | 7 | 63 |
| 266 Neu5Acα2-6Galβ1-4GlcNAcβ-Sp0 | 22 | 2 | 1 | 9 |
| 292 Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-3GlcNAcβ-Sp0 | 22 | 14 | 7 | 62 |
| 334 GalNAcα1-3(Fucoα1-2)Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp0 | 22 | 12 | 6 | 57 |
| 50 Manα1-3(Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp13 | 22 | 13 | 6 | 59 |
| 373 Galα1-3(Fuco1-2)Galβ1-3GlcNAcβ1-2Manα1-3(Galα1-3(Fuco1-2)Galβ1-3GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp20 | 22 | 13 | 7 | 61 |
| 229 Neu5Acα2-3(GalNAcβ1-4)Galβ1-4GlcNAcβ-Sp0 | 22 | 9 | 5 | 43 |
| 160 Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp0 | 22 | 8 | 4 | 39 |
| 65 Fuco1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ-Sp8 | 22 | 8 | 4 | 35 |
| 451 Fuco1-2Galβ1-4GlcNAcβ1-3(Fuco1-2Galβ1-4GlcNAcβ1-6)GalNAc-Sp14 | 22 | 3 | 1 | 13 |
| 240 Neu5Acα2-3Galβ1-3[6OSO3]GalNAcα-Sp8 | 22 | 8 | 4 | 38 |
| 108 Galα1-3(Fuco1-2)Galβ-Sp18 | 22 | 10 | 5 | 46 |
| 198 G-ol-Sp8 | 22 | 8 | 4 | 39 |
| 15 GalNAcβ-Sp8 | 22 | 2 | 1 | 8 |
| 384 Fuco1-2Galβ1-3(Fuco1-4)GlcNAcβ1-3Galβ1-4(Fucoα1-3)GlcNAcβ1-6)Galβ1-4Glc-Sp21 | 21 | 11 | 5 | 50 |
| 424 Galα1-3Galβ1-3GlcNAcβ1-3GalNAc-Sp14 | 21 | 8 | 4 | 37 |
| 392 GalNAcα1-3(Fuco1-2)Galβ1-3GalNAcα1-3(Fuco1-2)Galβ1-4GlcNAcβ-Sp0 | 21 | 21 | 11 | 101 |
| 130 Galβ1-4GlcNAcβ1-6GalNAc-Sp8 | 21 | 5 | 3 | 26 |
| 219 Fuco1-2Galβ1-4[6OSO3]GlcNAc-Sp8 | 21 | 6 | 3 | 28 |
| 91 GalNAcα1-3GalNAcβ-Sp8 | 21 | 11 | 5 | 51 |
| 200 GlcAβ-Sp8 | 21 | 15 | 8 | 74 |
| 26 [3OSO3]Galβ1-4[6OSO3]Glcβ-Sp8 | 21 | 13 | 6 | 60 |
| 55 Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp8 | 21 | 22 | 11 | 108 |
| 280 Neu5Gcoα2-3Galβ1-4(Fuco1-3)GlcNAcβ-Sp0 | 21 | 13 | 6 | 63 |
| 155 Galβ1-4GalNAcα1-3(Fuco1-2)Galβ1-4GlcNAcβ-Sp8 | 20 | 3 | 2 | 17 |
| 10 Neu5Acα-Sp11 | 20 | 31 | 15 | 150 |
| Number | Sequence                                                                 | Molarities |
|--------|---------------------------------------------------------------------------|------------|
| 241    | Neu5Acα2-3Galβ1-3(Neu5Acα2-6)GalNAcα-Sp8                                | 20 8 4 40  |
| 348    | Galβ1-4GlcNAcβ1-2Manα1-6Manβ1-4GlcNAcβ1-4GlcNAc-Sp12                      | 20 10 5 49 |
| 18     | GlcN(Gc)β-Sp8                                                            | 20 4 2 22  |
| 395    | Galβ1-4GlcNAcβ1-2Manα1-3(GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc-Sp12 | 20 10 5 50 |
| 376    | Neu5Acα2-6Galβ1-4GlcNAcβ1-3GalNAcα-Sp14                                  | 20 9 4 44  |
| 276    | Neu5Acβ2-6GalNAcα-Sp8                                                   | 20 4 2 21  |
| 315    | Neu5Acα2-3Galβ1-3(Neu5Acα2-3Galβ1-4GlcNAcβ1-6)GalNAcα-Sp14              | 20 13 6 63 |
| 93     | GalNAcα1-(Fucα1-2)Galβ1-4GlcNAcα-Sp8                                     | 20 13 6 64 |
| 307    | GlcNAcβ1-3Manα-Sp10                                                      | 20 14 7 71 |
| 394    | Galα1-3Galβ1-3(Fucα1-4)GlcNAcβ1-2Manα1-3(Galα1-3Galβ1-3(Fucα1-4)GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc-Sp19 | 20 12 6 59 |
| 24     | [3OSO3]Galβ1-4Glcβ-Sp8                                                   | 20 7 3 35  |
| 118    | Galα1-(Fucα1-2)Galβ1-4GlcNAcβ-Sp8                                       | 19 6 3 33  |
| 176    | GlcNAcβ1-3GalNAcα-Sp8                                                    | 19 18 9 91 |
| 110    | Galα1-3GalNAcα-Sp8                                                       | 19 4 2 20  |
| 457    | Galα1-3(Fucα1-2)Galβ1-3GlcNAcβ1-2Manα1-6(Galα1-3(Fucα1-2)Galβ1-3GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4(Fucα1-6)GlcNAcβ-Sp22 | 19 9 4 47 |
| 255    | Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp8                   | 19 7 4 39  |
| 405    | Galβ1-3GlcNAcα1-6Galβ1-4GlcNAcβ-Sp0                                      | 19 6 3 33  |
| 448    | Galβ1-4GlcNAcβ1-6GalNAcα-Sp14                                            | 19 14 7 74 |
| 64     | Fucα1-2Galβ1-3GalNAcβ1-4(Neu5Acα2-3)Galβ1-4Glcβ-Sp9                     | 19 11 5 58 |
| 359    | Fucα1-2Galβ1-3GlcNAcβ1-2Manα1-3(Fucα1-2Galβ1-3GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp20 | 19 13 6 66 |
| 163    | Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ-Sp8                                         | 19 6 3 33  |
| 185    | GlcNAcβ1-4Galβ1-4GlcNAcβ-Sp8                                            | 19 23 12 124|
| 33     | [3OSO3]Galβ1-4[6OSO3]GlcNAcβ-Sp0                                          | 18 19 9 102|
| 308    | GlcNAcβ1-4GlcNAcβ-Sp10                                                   | 18 13 6 69 |
| 420    | GlcNAcβ1-2Manα1-3(GlcNAcβ1-2(GlcNAcβ1-6)Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp19 | 18 9 5 50 |
| 354    | [6OSO3]GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp17                                      | 18 5 2 27  |
| 133    | Galβ1-3(Neu5Acα2-6)GalNAcα-Sp8                                          | 18 16 8 92  |
| 174    | GlcNAcβ1-3(GlcNAcβ1-6)GalNAcα-Sp8                                        | 18 10 5 58  |
| 285    | Neu5Acα-Sp8                                                              | 17 14 7 79  |
| 145    | Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp0                                       | 17 17 9 100|
| 319    | Neu5Acα2-8Neu5Acβ-Sp17                                                   | 17 10 5 61  |
| Line | Formula | Position |
|------|---------|----------|
| 310  | HOOC(CH₃)CH-3-O-GlcNAcβ1-4GlcNAcβ-Sp10 | 17 15 7 86 |
| 66   | Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ-Sp10 | 17 15 8 89 |
| 230  | Neu5Acα2-3(GalNAcβ1-4)Galβ1-4GlcNAcβ-Sp8 | 17 11 5 63 |
| 263  | Neu5Acα2-6GalNAcα-Sp8 | 17 21 10 123 |
| 270  | Neu5Acα2-6Galβ1-4Glcβ-Sp0 | 17 8 4 51 |
| 239  | Neu5Acα2-3Galβ1-3 Neu5Acα2-3Galβ1-4)GlcNAcβ-Sp8 | 16 27 14 166 |
| 191  | GlcNAcβ1-6GalNAcα-Sp1 | 16 12 6 75 |
| 281  | Neu5Gcα2-3Galβ1-4GlcNAcβ-Sp0 | 16 12 6 73 |
| 419  | Fucα1-2Galβ1-4GlcNAcβ1-2Manα1-3(Fucα1-2Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4(Fucα1-6)GlcNAcβ-Sp22 | 16 9 4 56 |
| 418  | Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-3(Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4(Fucα1-6)GlcNAcβ-Sp22 | 16 7 4 47 |
| 344  | Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc-Sp12 | 16 8 4 52 |
| 293  | Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-3GlcNAcβ-Sp0 | 16 9 4 57 |
| 444  | [6OSO₃]Galβ1-3[6OSO₃]GlcNAc-Sp0 | 16 28 14 178 |
| 161  | Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp0 | 16 12 6 76 |
| 284  | Neu5Gcα2-6Galβ1-4GlcNAcβ-Sp0 | 15 18 9 118 |
| 403  | Galα1-3Galβ1-4GlcNAcβ1-3GalNAcα-Sp14 | 15 10 5 67 |
| 274  | Neu5Acα2-8Neu5Acα2-3Galβ1-4Glcβ-Sp0 | 15 12 6 76 |
| 86   | GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAcβ-Sp8 | 15 27 13 175 |
| 244  | Neu5Acα2-3Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ-Sp0 | 15 20 10 130 |
| 17   | GlcNAcβ-Sp8 | 15 8 4 54 |
| 375  | Neu5Acα2-3Galβ1-4GlcNAcβ1-3GalNAc-Sp14 | 15 17 8 112 |
| 43   | [6OSO₃]Galβ1-4GlcNAcβ-Sp8 | 15 39 19 262 |
| 324  | Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12 | 15 7 4 50 |
| 100  | Galα1-2Galβ-Sp8 | 15 26 13 181 |
| 258  | Neu5Acα2-3Galβ1-4GlcNAcβ-Sp8 | 15 18 9 125 |
| 156  | Galβ1-4GlcNAcβ1-3(Fucα1-2)Galβ1-4GlcNAcβ-Sp8 | 14 12 6 81 |
| 332  | GalNAcβ1-3Galα1-4Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ-Sp0 | 14 11 5 76 |
| 207  | Mana1-2Mana1-3Manα-Sp9 | 14 9 4 62 |
| 228  | Neu5Acα2-3(6-O-Su)Galβ1-4(Fucα1-3)GlcNAcβ-Sp8 | 14 7 4 51 |
| 186  | GlcNAcβ1-4GlcNAcβ1-4GlcNAcβ1-4GlcNAcβ1-4GlcNAcβ1-4GlcNAcβ-Sp8 | 14 28 14 199 |
| 328  | Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-3GlcNAcβ-Sp0 | 13 9 5 69 |
| 157  | Galβ1-4GlcNAcβ1-3GalNAcα-Sp8 | 13 14 7 105 |
| Rank | Hexoses | Hexosamines | Sulfates | Other | Total |
|------|----------|-------------|---------|-------|-------|
| 302  | Galβ1-4GlcNAcα1-6Galβ1-4GlcNAcβ-Sp0 | 13 | 10 | 5 | 76 |
| 11   | Neu5Acβ-Sp8 | 13 | 14 | 7 | 106 |
| 404  | Galβ1-3GlcNAcβ1-6Galβ1-4GlcNAcβ-Sp0 | 13 | 13 | 6 | 100 |
| 358  | KDNα2-3Galβ1-3GalNAcβ-Sp14 | 13 | 12 | 6 | 95 |
| 221  | Neu5Acβ2-3Galβ1-3GalNAcβ-Sp8 | 13 | 20 | 10 | 157 |
| 183  | GlcNAcβ1-4-MDPlys | 13 | 12 | 6 | 98 |
| 278  | Neu5GcO2-3Galβ1-3(Fucα1-4)GlcNAcβ-Sp0 | 13 | 3 | 2 | 26 |
| 300  | Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Galβ1-4GlcNAc-Sp0 | 13 | 9 | 4 | 69 |
| 74   | Fucα1-2Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp0 | 13 | 21 | 10 | 165 |
| 421  | Fucα1-2Galβ1-3GlcNAcβ1-3GalNAc-Sp14 | 12 | 14 | 7 | 109 |
| 442  | GalNAcβ1-6GalNAcβ-Sp8 | 12 | 8 | 4 | 64 |
| 353  | Galβ1-3(Fucα1-4)GlcNAcβ1-2Manα1-3(Galβ1-3(Fucα1-4)GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp19 | 12 | 9 | 5 | 76 |
| 146  | Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ-Sp10 | 12 | 26 | 13 | 210 |
| 456  | GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-2Manα1-6(GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4(Fucα1-6)GlcNAcβ-Sp22 | 12 | 9 | 4 | 75 |
| 20   | GlcNAcβ1-3(GlcNAcβ1-4)(GlcNAcβ1-6)GlcNAc-Sp8 | 12 | 6 | 3 | 50 |
| 360  | Fucα1-2Galβ1-4GlcNAcβ1-2Manα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp20 | 12 | 5 | 2 | 42 |
| 246  | Fucα1-2[6OSO3]Galβ1-4Glc-Sp0 | 12 | 14 | 7 | 119 |
| 249  | Neu5Acα2-3Galβ1-4[6OSO3]GlcNAcβ-Sp8 | 12 | 15 | 8 | 131 |
| 257  | Neu5Acα2-3Galβ1-4GlcNAcβ-Sp0 | 11 | 45 | 22 | 396 |
| 370  | Galα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-2Manα1-3(Galα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp20 | 11 | 21 | 10 | 187 |
| 396  | GlcNAcβ1-2Manα1-3(Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc-Sp12 | 11 | 3 | 1 | 25 |
| 104  | Galα1-3(Fucα1-2)Galβ1-4(Fucα1-3)GlcNAcβ-Sp8 | 11 | 4 | 2 | 37 |
| 227  | Neu5Acα2-8Neu5Acα2-8Neu5Ac-Sp8 | 11 | 14 | 7 | 124 |
| 159  | Galβ1-4GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ-Sp0 | 11 | 21 | 10 | 191 |
| 460  | Galβ1-4GlcNAcβ1-6(Galβ1-4GlcNAcβ1-2Manα1-6)(Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp19 | 11 | 23 | 11 | 210 |
| 171  | GlcNAcα1-3Galβ1-4GlcNAcβ-Sp8 | 11 | 14 | 7 | 135 |
| 236  | Neu5Acα2-3Galβ1-3[6OSO3]GlcNAc-Sp8 | 10 | 16 | 8 | 152 |
| 235  | Neu5Acα2-3GalNAcβ1-4GlcNAcβ-Sp0 | 10 | 22 | 11 | 212 |
| 42   | [6OSO3]Galβ1-4Glcβ-Sp8 | 10 | 12 | 6 | 115 |
| 22   | [3OSO3][6OSO3]Galβ1-4GlcNAcβ-Sp0 | 10 | 5 | 2 | 45 |
| 397 | Neu5Aco2-3Galβ1-3GlcNAcβ1-3GalNAcα-Sp14 | 10 | 7 | 4 | 72 |
|-----|------------------------------------------|----|---|---|----|
| 28  | [3OSO3]Galβ1-3GalNAcα-Sp8               | 10 | 3 | 2 | 31 |
| 172 | GlcNAcα1-6Galβ1-4GlcNAcβ-Sp8             | 10 | 10 | 5 | 100 |
| 272 | Neu5Aco2-6Galβ-Sp8                      | 10 | 6 | 3 | 66 |
| 330 | Neu5Aco2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp0 | 9 | 15 | 7 | 156 |
| 89  | GalNAcα1-3(Fucα1-2)Galβ-Sp8              | 9 | 9 | 4 | 96 |
| 48  | [9NAc]Neu5Aco2-6Galβ1-4GlcNAcβ-Sp8       | 9 | 1 | 1 | 15 |
| 232 | Neu5Aco2-3(Neu5Aco2-3Galβ1-3GalNAcβ1-4)Galβ1-4Glcβ-Sp0 | 8 | 9 | 4 | 104 |
| 402 | Galα1-4Galβ1-4GlcNAcβ1-2Manα1-3(Galα1-4Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-LVANKT | 8 | 15 | 7 | 188 |
| 252 | Neu5Aco2-3Galβ1-4(Fucα1-3)GlcNAcβ-Sp0    | 8 | 19 | 10 | 242 |
| 46  | [6OSO3]GlcNAcβ-Sp8                      | 8 | 7 | 3 | 89 |
| 294 | [3OSO3][4OSO3]Galβ1-4GlcNAcβ-Sp0         | 7 | 7 | 4 | 103 |
| 250 | Neu5Aco2-3Galβ1-4(Fucα1-3)[6OSO3]GlcNAcβ-Sp8 | 5 | 7 | 3 | 123 |
| 88  | GlcNAcβ1-3Galβ1-3GalNAcα-Sp8             | 5 | 13 | 6 | 238 |
| 158 | Galβ1-4GlcNAcβ1-3GalNAcα-Sp14            | 5 | 6 | 3 | 107 |
| 295 | [6OSO3]Galβ1-4[6OSO3]GlcNAcβ-Sp0         | 5 | 20 | 10 | 389 |
| 222 | Neu5Aco2-3Galβ1-3GalNAcα-Sp14            | 5 | 6 | 3 | 120 |
| 259 | Neu5Aco2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp0 | 5 | 29 | 14 | 575 |
| 218 | Fucα1-2[6OSO3]Galβ1-4GlcNAc-Sp0          | 4 | 7 | 3 | 150 |
| 461 | Galβ1-4GlcNAcβ-(OCH2CH2)6NH2             | 4 | 15 | 7 | 353 |
| 102 | Galα1-3(Fucα1-2)Galβ1-3GlcNAcβ-Sp8       | 3 | 10 | 5 | 308 |
| 45  | Neu5Aco2-3[6OSO3]Galβ1-4GlcNAcβ-Sp8      | 3 | 8 | 4 | 258 |
| 38  | [4OSO3][6OSO3]Galβ1-4GlcNAcβ-Sp0         | 3 | 11 | 5 | 362 |
| 90  | GalNAcα1-3(Fucα1-2)Galβ-Sp18             | 3 | 7 | 4 | 284 |
| 39  | [4OSO3]Galβ1-4GlcNAcβ-Sp8               | 2 | 14 | 7 | 847 |
| 47  | [9NAc]Neu5Aco-Sp8                       | 2 | 14 | 7 | 847 |
| 372 | GalNAcα1-3(Fucα1-2)Galβ1-3GlcNAcβ1-2Manα1-3(GalNAcα1-3(Fucα1-2)Galβ1-3GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp20 | 1 | 6 | 3 | 670 |
| 248 | Neu5Aco2-3Galβ1-3GlcNAcβ-Sp8            | 0 | 6 | 3 | 1452 |
| 327 | Neu5Ac(9Ac)a2-3Galβ1-3GlcNAcβ-Sp0       | 0 | 8 | 4 | 3852 |
| 326 | Neu5Ac(9Ac)a2-3Galβ1-4GlcNAcβ-Sp0       | 0 | 13 | 6 | 1212 |
| 224 | Neu5Aco2-8Neu5Aco2-8Neu5Aco2-3(GalNAcβ1-4)Galβ1-4Glcβ-Sp0 | 0 | 15 | 7 | -3463 |
| 178 | GlcNAcβ1-3Galβ-Sp8                      | -2 | 29 | 14 | -1313 |
| Code   | Spacer Arm                                                                 |
|--------|---------------------------------------------------------------------------|
| Sp0    | -CH$_2$CH$_2$NH$_2$                                                       |
| Sp8    | -CH$_2$CH$_2$CH$_2$NH$_2$                                                 |
| Sp9    | -CH$_2$CH$_2$CH$_2$CH$_2$NH$_2$                                           |
| Sp10   | -NHCOCH$_2$NH                                                             |
| Sp11   | -OCH$_2$C$_6$H$_4$-p-NHCOCH$_2$NH                                          |
| Sp12   | Asparagine                                                                |
| Sp13   | Glycine                                                                   |
| Sp14   | Threonine                                                                 |
| Sp15   | Serine                                                                    |
| Sp16   | -PNP(OC$_3$H$_7$NH$_2$)                                                   |
| Sp17   | -OCH$_2$C$_6$H$_4$NH                                                        |
| Sp18   | -O(CH$_2$)$_3$NHCO(CH$_2$)$_2$NH                                        |
| Sp19   | EN or NK                                                                  |
| Sp20   | GENR                                                                      |
| Sp21   | -N(CH$_3$)$_2$-O-(CH$_2$)$_2$-NH$_2$                                      |
| Sp22   | NST                                                                       |

Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp0

[3OSO3]Galβ1-3(Fucoα1-4)GlcNAcβ-Sp8

Fuco-Sp8

Galβ1-4(Fucoα1-3)GlcNAcβ1-4Galβ1-4(Fucoα1-3)GlcNAcβ1-4Galβ1-4(Fucoα1-3)GlcNAcβ-Sp0

[3OSO3][6OSO3]Galβ1-4[6OSO3]GlcNAcβ-Sp0

Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp0

Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-N(LT)AVL

Galβ1-3(Neu5Acα2-3Galβ1-4GlcNAcβ1-6)GalNAcα-Sp14

[3OSO3]Galβ1-3GlcNAcβ-Sp0

Neu5Acα2-3(Neu5Acα2-6)GalNAcα-Sp8

[3OSO3]Galβ1-4(Fucoα1-3)GlcNAc-Sp0

Galβ1-3(Neu5Acα2-3Galβ1-4GlcNAcβ1-6)GalNAcα-Sp14

Neu5Acα2-3GalNAcα-Sp8
|          |               |
|----------|--------------|
| Sp23     | (OCH$_2$CH$_2$)$_6$NH$_2$ |
| MDPLys   | Mur-L-Ala-D-iGlnb-(CH$_2$)$_4$NH$_2$ |