Wedgelike Glycodendrimers as Inhibitors of Binding of Mammalian Galectins to Glycoproteins, Lactose Maxiclusters, and Cell Surface Glycoconjugates

Sabine André,[b] Roland J. Pieters,*[a] Ioannis Vrasidas,[a] Herbert Kaltner,[b] Ichiro Kuwabara,[c] Fu-Tong Liu,[c] Rob M. J. Liskamp,[a] and Hans-Joachim Gabius[b]

Galectins are mammalian carbohydrate-binding proteins that are involved in cell – cell and cell – matrix adhesion, cell migration, and growth regulation with relevance to inflammation and tumor spread. These important functions account for the interest to design suitable low molecular weight inhibitors that match the distinct modes of presentation of the carbohydrate recognition domains of the different galectin subfamilies. Using 3,5-di-(2-aminoethoxy)benzoic acid as the branching unit, wedgelike glycodendrimers with two, four, and eight lactose moieties (G1 – G3) were synthesized. They were tested in solid-phase competition assays with lactose maxiclusters and various N*-glycan branching profiles (miniclusters) as the matrix and also in cell assays. Prototype galectins-1 and -7, chimera-type galectin-3, a plant $(AB)_2$ toxin, and a lactose-binding immunoglobulin G fraction from human serum were the carbohydrate-binding targets. Potent inhibition and remarkable cluster effects were seen for the homodimeric galectin-1, especially in combination with biantennary* N*-glycans as the matrix. Remarkably, for the tetravalent G2 glycodendrimer, the inhibitory potency of each lactose unit reached a maximum value of 1667 relative to free lactose. In haemagglutination experiments as a model for cell adhesion, galectin-3 was markedly sensitive to increased sugar valency and a relative potency per lactose of 150 was reached. The spatial orientation of the carbohydrate recognition domains of the endogenous lectins and the branching pattern of the carbohydrates of the glycoprotein matrices used are both important factors in the design and synthesis of glycodendrimers with galectin-selective properties.*

KEYWORDS:

cell recognition · dendrimers · drug research · glycoconjugates · lectins

Introduction

When examining cell surface properties and characteristics of serum proteins, a major common characteristic observed is the presence of glycan chains. Initially primarily considered to impart physicochemical attributes, such as increased solubility, or to protect protein parts from proteolysis, their role in biological information transfer is increasingly emerging. Knowledge accrued over the last decade convincingly underscores the relationship of glycan presentation and disturbed glycan chain assembly and turnover with various diseases.[1] This is leading to the timely concept to count custom-made carbohydrates among pharmaceuticals of clinical potential.[2] Fittingly, the incentive to chemically synthesize glycoconjugates, termed neoglycoproteins when linked to carriers such as albumin or neoglycoconjugates when linked to a non-protein backbone, has shifted from their application to raise carbohydrate-specific antibodies to the generation of reagents for research on lectin – carbohydrate recognition.[3] In this area, polymers containing crucial sugar ligands have served as tools in drug targeting and histochemistry.[4] The inherent drawback of the polymers is their low potential for systemic distribution. Better results can be expected by attaching carbohydrate derivatives to small linker molecules such as 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris)[*] or peptides containing amino acids such as aspartic acid or lysine as the aglyconic core.[5] When the synthetic assembly proceeds in an iterative fashion with a common branching point,

[*] *A list of abbreviations can be found in ref. [47].*

[a] *Dr. R. J. Pieters, Dipl.-Chem. I. Vrasidas, Prof. Dr. R. M. J. Liskamp*
Utrecht Institute for Pharmaceutical Sciences
Department of Medicinal Chemistry
Utrecht University
P.O. Box 80082, 3508 TB Utrecht (The Netherlands)
Fax: (+31) 30-2536655

[b] *Dr. S. André, Priv.-Doz. Dr. H. Kaltner, Prof. Dr. H.-J. Gabius*
Institut für Physiologische Chemie
Tierärztliche Fakultät
Ludwig-Maximilians-Universität München
Veterinärstrasse 13, 80539 München (Germany)

[c] *Dr. I. Kuwabara, Prof. Dr. F.-T. Liu*
La Jolla Institute for Allergy and Immunology
Division of Allergy
10355 Science Center Drive, San Diego, CA 92121 (USA)

  1439-4227/01/02/11 $ 17.50+.50/0 CHEMBIOCHEM **2001**, 2, 822 – 830

dendrimers will result with a regular increase in the number of attachment points in each new generation.[6] To what extent these glycodendrimers will compete with natural ligands for lectins in clinically relevant biorecognition—for example, interference with undesired lectin-dependent cell adhesion in early stages of infection, tumor spread or inflammation,[7, 8]—is likely to depend on several factors.

Factors to take into account for optimal inhibitor design include the carbohydrate ligand presentation. In principle, ligands can be part of a branched glycan in so-called miniclusters or be more widely separated on a protein surface in maxiclusters.[9] Other factors include the ways how carbohydrate recognition domains (CRD) of lectins are arranged and home in onto their ligands with distinct topology.[7] To experimentally assess the potency of the glycodendrimers, assays have to be the selected in which they are exposed to several topological contexts. The positioning of the CRDs differs between lectin families and even within a certain lectin family,[7, 8] and opens the possibility for selective binding. To illustrate this specific CRD arrangement, the endocytic C-type lectin of hepatocytes is known to form regular complexes in the membrane, and bacterial AB_5 toxins also contain a well-defined orientation of binding sites in solution. These rigid geometries have served as templates for elegant drug design.[7, 9, 10] The situation is more difficult for soluble lectins in mammals, because in general they are not likely to assemble in high-order complexes in a membrane. Despite this inherent challenge, the potential for blocking endogenous β-galactoside-specific lectins by efficient low molecular weight inhibitors affords a promising medical perspective. The medical perspective for galectins, which are endogenous Ca^{2+}-independent β-galactoside-specific lectins that all share a jelly-roll-like folding pattern and comprise an important target group in our studies, is their role in cell – matrix and cell – cell adhesion, cell migration and communication with relevance to proliferation. In addition to the analysis of the basic folding pattern, knowledge about arrangement modes of their CRDs is available. In the galectins forming homodimeric molecules (the so-called prototype galectins, for example, galectins-1 and -7) the CRDs can be found at opposing ends, whereas the only chimeric family member exists as a monomeric molecule in solution (galectin-3).[8] In addition to the three mammalian galectins, we included a tetrameric plant lectin (the AB toxin *Viscum album* agglutinin (VAA)), and the lactose-specific immunoglobulin G (IgG α^-/β^+) fraction from human serum to extend the examination of the topological aspect.

In this study, we assessed the capacity of glycodendrimers with 3,5-di-(2-aminoethoxy)benzoic acid as the branching unit of the backbone[11] to serve as inhibitors of sugar receptor binding. Three generations of the dendrimer backbone were used which resulted in glycodendrimers containing two, four, and eight lactose units (Figure 1). Their relative inhibitory activities were determined in a solid-phase inhibition assay involving binding of the above-mentioned lectins to a matrix of glycosylated proteins. Potencies were studied as a function of carbohydrate presentation of the matrix glycoproteins. Several glycosylated proteins were used as the matrix. We used a neoglycoprotein and a lactosylated albumin (as an example of a maxicluster) and deliberately selected natural glycoproteins carrying bi- to triantennary glycan chains or a heterogeneous profile as examples of miniclusters. The potency of inhibition of galectin binding to surfaces of erythrocytes as a physiological ligand panel was also determined in haemagglutination experiments.

Results and Discussion

Structures of glycodendrimers, sugar receptors, and glycans on the tested glycoproteins

The glycoinhibitors all contained a constant ligand structure (i.e. lactose) that interferes with lectin binding. In the inhibition study, structural variations in each of the three components in the solid-phase competition assay were examined. The three components were: 1) the glycodendrimer as the inhibitor, interfering with the binding of 2) the carbohydrate-binding protein to 3) the immobilized glycoprotein.

Firstly, glycodendrimer variation was achieved by linking lactose to three generations of a dendrimer based on the 3,5-di-(2-aminoethoxy)benzoic acid branching unit, which resulted in glycodendrimers **2** – **4** containing 2, 4, and 8 lactose units (G1 – G3), respectively (Figure 1). To ensure that the dendrimer itself had a negligent protein-binding capacity, competitive inhibition assays with haptenic sugar were regularly performed (see below). Independently, the glycodendrimers were shown to be devoid of nonspecific binding activity in inhibition assays involving the mannose-specific plant lectin concanavalin A and yeast mannan. The control experiments showed that the interactions of the headgroups in the dendrimers with their cognate receptor could thus be safely examined.

The second element in the study is the carbohydrate-binding protein or lectin. The arrangement of CRDs can differ even within a lectin family, as indicated in the Introduction section. Therefore, it is not justified to simply extrapolate from one family member to the other. Here, the structural features of the representative galectins are presented. The prototype mammalian galectin-1 and its homologues from other animals form homodimers containing a 22-strand antiparallel β sandwich, placing the two lactose-binding sites at opposing sides of the dimer at a distance of about 46 Å.[12] Galectin-7 belongs to the prototype galectin subgroup and has been reported to be monomeric in solution while crystallizing as a dimer.[13] Among the galectins, the chimera-type galectin-3 is unique in having eight to twelve collagenase-sensitive proline-, glycine-, and tyrosine-rich tandem repeats of the length of a nonapeptide as a short N-terminal stalk.[8] Although it lacks a typical dimer interface, this does not mean that it will necessarily remain monomeric when associating to a glycan surface. In fact, primarily the N-terminal section of galectin-3 promotes aggregate assembly on a surface, which can even lead to positive cooperativity of laminin binding.[14] Besides the galectins, the topological effects were further studied by using a β-galactoside-binding immunoglobulin G fraction from human serum

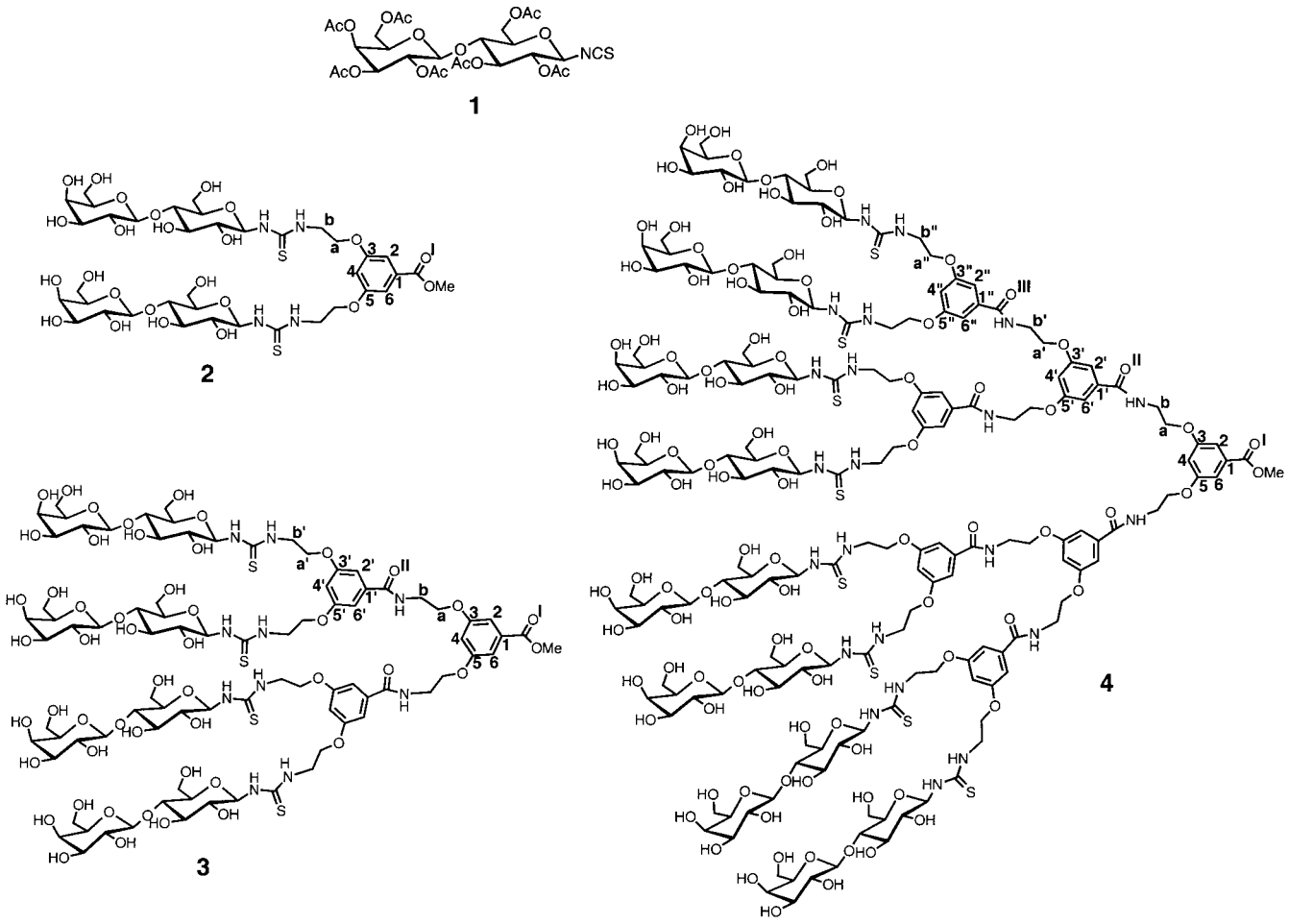

Figure 1. *Structures of the glycodendrimers used in this study (**2** – **4**) displaying two (**2** = G1), four (**3** = G2), or eight (**4** = G3) lactose units. They were obtained by coupling the protected lactose isothiocyanate **1** to the amino groups of three generations of dendrimers based on the 3,5-di-(2-aminoethoxy)benzoic acid branching unit.*

(IgG α^-/β^+) and a potent plant haemagglutinin, the mistletoe lectin (VAA) with two binding sites per galactoside-binding B chain in the $[AB]_2$ tetramer.[15]

As already referred to in the introduction, the third topological parameter concerns the display of the carbohydrates on glycosylated proteins. As a model matrix with maxiclusters, we used lactosylated BSA (LacBSA), a neoglycoprotein with an average of 28 sugar moieties as *p*-isothiocyanatophenyl derivatives. To infer the importance of the branching modality of *N*-glycan antennae (miniclusters), four types of natural glycoproteins with a well-documented glycosylation status were selected. These glycoproteins contain either di- and triantennary glycans or they cover the range of a complex heterogeneous *N*-glycan profile. In detail, the human pentraxin serum amyloid P component (SAP) carries a single complex-type biantennary *N*-glycan exclusively at Asn 32 of each monomer.[16] The glycan population of asialofetuin (ASF) consists of three glycosylation sites with complex-type triantennary chains either containing an *N*-acetyllactosamine terminus (74%) or the Gal-β-(1 $\rightarrow$3)-GlcNAc isomer in the outer Man-α-(1 $\rightarrow$3) arm (9%) and a biantennary chain (17%), as well as three *O*-linked chains containing primarily Gal-β-(1 $\rightarrow$3)-GalNAc-α disaccharide;[17] however, only the *N*-glycan chains participate in carbohydrate-dependent crosslinking of ASF by sugar receptors.[18] Since galectin functions include mediation of (tumor) cell adhesion to the extracellular matrix with laminin as a popular target molecule,[8] this glycoprotein was further added to the test panel. Purified from Engelbreth – Holm – Swarm (EHS) tumor, the murine laminin contains up to 27% carbohydrate in its three protein chains. Glycan attachment is distributed over a total of 73 potential *N*-glycosylation sites with especially bi- and triantennary chains in the complex profile including galectin-reactive poly(*N*-acetyllactosamine) elongations.[19] Another physiological ligand reported for galectin-3, which is also called Mac-2 antigen based on its immunodetection on activated but not resident peritoneal macrophages,[20] is the Mac-2-binding protein (Mac-2 BP).[20] Its *N*-glycosylation of seven potential sites accounts for about 30% of the molecular weight of the gene product based on the shift of electrophoretic mobility from 90 to 60 kDa after treatment with *N*-glycosidase F.[21] If, and to what extent, other galectins besides galectin-3 bind to this glycoprotein is another question of interest to be answered by the assays.

Solid-phase inhibition assays

To prevent any negative influence on the lectin structure by immobilizing them on a plastic surface and also to mimic a cell surface with glycan ligands, we chose to attach the (neo)-glycoproteins to the surface of microtiter plate wells. The coating density was adjusted to yield a comparable high signal intensity ($OD_{490} = 0.8–1.5$) in the assays for all tested matrix constituents. Under these conditions, non-carbohydrate-dependent background binding of lectins was rather low. This observation corroborated the already mentioned controls excluding non-carbohydrate-dependent binding of the synthesized dendrimers. Routinely, total binding was nonetheless reduced in each case by the residual carbohydrate-independent signal intensity. This difference was defined as 100%. As a measure of relative potency of the inhibitors to interfere with binding, the concentration that reduces the original signal by 50% (IC_{50} value) was determined. Exemplary illustrations of the shape of inhibition curves when adding increasing concentrations of glycodendrimer are given in Figure 2. With respect to using ASF as an inhibitor for comparison, it should be kept in mind that the *N*-acetyllactosamine disaccharide present in the terminal position of ASF's *N*-glycan chains surpasses lactose as an inhibitor for galectins-1 and -3 (by a factor of 3.5 – 11.3 in inhibitory potency), while the effect of the penultimate sugar unit is less conspicuous with only a factor of 1.6 for the plant lectin, as also reflected in

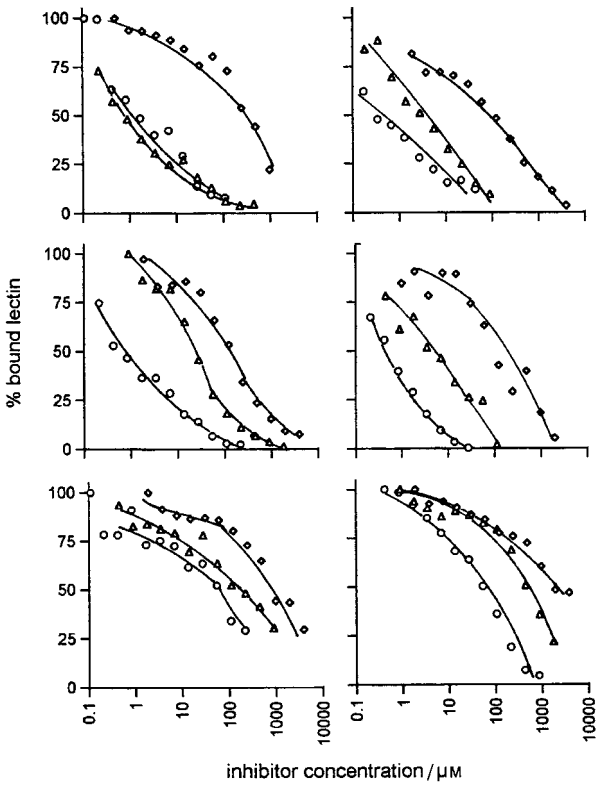

Figure 2. *Inhibition by glycodendrimers G1 (◇), G2 (△), and G3 (○) of the binding of lectins to immobilized glycoproteins. The following combinations of lectin and glycoprotein matrix were used: top panel, left: VAA and LacBSA; right: VAA and laminin; middle panel, left: galectin-3 and LacBSA; right: galectin-3 and MAC-2 BP; bottom panel, left: galectin 1 and laminin; right: galectin-7 and SAP.*

ΔG values derived from isothermal titration calorimetry.[22] No precipitate formation of sugar receptor – glycodendrimer complexes was observed during the incubation period under the given experimental conditions, similar to studies with starburst glycodendrimers and cluster glycosides.[23] Thus, this potential source of error due to the inherent change of the concentrations of the reactants in solution could be excluded. This is a relevant observation because isothermal titration calorimetry studies have shown that receptor cross-linking and/or precipitation seems to be a determining factor for enhanced multivalent binding. However, those studies were conducted at receptor concentrations of at least one order of magnitude higher than our highest working concentration.[24]

When the five different (neo)glycoproteins were tested as matrix, all of them fulfilled the expectation to be a galactose-dependent binding partner. A brief survey of the data readily indicates that the topology of glycan presentation and not just the mere presence of galactosides on the glycopoteins is the important factor (Table 1). As shown in Figure 2, the sugar content of the dendrimer is often positively related to the inhibitory capacity. To infer the actual average potency of a lactose unit beyond the algebraic increase in sugar density, Table 1 also presents the normalized data with respect to carrier-free lactose (set to 1).

Especially the G2 glycodendrimer was an effective inhibitor in two structural contexts, reaching normalized potency values of 1875 for inhibiting the binding of plant lectin VAA to the ASF matrix and 1667 for the galectin-1 and SAP combination. It is noteworthy that calculations of the distances between the sugar residues in the biantennary *N*-glycan chain and the dendrimers for adjacent lactose units revealed them to be remarkably similar, with values close to or about 20 Å. A further increase in the valency to eight lactose units in G3 in these cases clearly reduced the extent of inhibitory capacity. This result indicates that optimal inhibition does not simply correlate with high valency, but requires a distinct mode of presentation. The inhibition experiments also showed a remarkable dependence on the matrix structure. Whereas the G2 glycodendrimer has the above-mentioned relative potency of 1667 in the inhibition of galectin-1 binding to the biantennary glycan SAP, this number is only 76 with the maxiclusters of LacBSA as matrix. Several examples of this phenomenon can be observed in this table. A conspicuous cluster effect (relative potencies of 463 and 250 at best) was also seen for the IgG fraction, with preference for the minicluster matrix display of SAP and ASF (relative potencies up to 463, right column of the first three blocks of Table 1).

Between the different galectins-1, -3, and -7 it is seen that, irrespective of the matrix, the best inhibition is observed for galectin-1 followed by galectin-3 and galectin-7. We also investigated how the noncovalent association in the chimeric galectin-3 affects the inhibition. This was probed by trimming galectin-3 proteolytically (using a collagenase) up to the C-terminal CRD. The reduced aggregation of the galectin reduced the binding to the matrices more than the binding to the glycodendrimer and resulted in a drop in the IC_{50} value to 4.3 μM in the case of G3 with ASF as the matrix. Relative to lysine-based cluster glycosides,[23b] the glycodendrimers used here

Table 1. *Determination of the IC_{50} values and the relative inhibitory capacity (relative potency, rel. pot.) of wedge-like glycodendrimers (G1 – G3) and a glycoprotein (ASF) in a solid-phase assay with surface-immobilized (neo)glycoproteins and five different labeled sugar receptors in solution.*[a]

Matrix:		0.05 μg LacBSA		1 μg LacBSA		0.25 μg LacBSA		0.05 μg LacBSA		0.05 μg LacBSA	
Probe:		VAA (1.5 μg mL^{-1})		galectin-1 (10 μg mL^{-1})		galectin-3 (5 μg mL^{-1})		galectin-7 (30 μg mL^{-1})		IgG(α^-/β^+) (1 μg mL^{-1})	
Inhibitor	Lac units per molecule	IC_{50} [μM]	rel. pot.	IC_{50} [μM]	rel. pot.	IC_{50} [μM]	rel. pot.	IC_{50} [μM]	rel. pot.	IC_{50} [μM]	rel. pot.
D-Gal	1	2500	0.4	40000	0.03	60000	0.01	35000	0.14	100000	0.03
lactose	1	1000	1	1100	1	500	1	5000	1	3000	1
ASF	9	0.8	1205 (133)	2.6	423 (47)	9.4	53 (5.9)	13	385 (43)	31	96 (11)
G1 (**2**)	2	360	2.8 (1.4)	50	22 (11)	140	3.6 (1.8)	300	17 (8.5)	50	60 (30)
G2 (**3**)	4	0.7	1429 (357)	3.6	306 (76)	20	25 (6.3)	135	37 (9.3)	18	167 (42)
G3 (**4**)	8	1.5	667 (83)	1.7	647 (81)	26	19 (2.4)	56	89 (11)	17	176 (22)
Matrix:		0.5 μg SAP		0.5 μg SAP		0.5 μg SAP		0.5 μg SAP		0.5 μg SAP	
Probe:		VAA (1.5 μg mL^{-1})		galectin-1 (15 μg mL^{-1})		galectin-3 (10 μg mL^{-1})		galectin-7 (30 μg mL^{-1})		IgG(α^-/β^+) (5 μg mL^{-1})	
Inhibitor	Lac units per molecule	IC_{50} [μM]	rel. pot.	IC_{50} [μM]	rel. pot.	IC_{50} [μM]	rel. pot.	IC_{50} [μM]	rel. pot.	IC_{50} [μM]	rel. pot.
D-Gal	1	20000	0.38	200 mM (=20% inhib.)	–	30000	0.03	95000	0.06	200000	1
lactose	1	7500	1	180000	1	800	1	6000	1	200000	1
ASF	9	8.4	893 (99)	6.3	28571 (3175)	42	19 (2.1)	19	316 (35)	11	18182 (2020)
G1 (**2**)	2	no inhib. up to 4 mM	–	120	1500 (750)	20	40 (20)	1900	3.2 (1.6)	4 mM (=20% inhib.)	–
G2 (**3**)	4	1350	5.6 (1.4)	27	6667 (1667)	4	200 (50)	450	13 (3.3)	1800	111 (28)
G3 (**4**)	8	430	17 (2.2)	34	5294 (662)	1.7	470 (59)	54	111 (14)	54	3704 (463)
Matrix:		1 μg ASF		1 μg ASF		1 μg ASF		1 μg ASF		1 μg ASF	
Probe:		VAA (1.5 μg mL^{-1})		galectin-1 (10 μg mL^{-1})(5 μg mL^{-1})		galectin-3 (30 μg mL^{-1})		galectin-7 (10 μg mL^{-1})		IgG(α^-/β^+)	
Inhibitor	Lac units per molecule	IC_{50} [μM]	rel. pot.	IC_{50} [μM]	rel. pot.	IC_{50} [μM]	rel. pot.	IC_{50} [μM]	rel. pot.	IC_{50} [μM]	rel. pot.
D-Gal	1	5000	0.6	70000	0.06	55000	0.02	4500	0.18	55000	1.82
lactose	1	3000	1	4000	1	1000	1	800	1	100000	1
ASF	9	2.6	1154 (128)	1.2	3200 (354)	6.3	159 (17.6)	21	38 (4.2)	0.6	158730 (17544)
G1 (**2**)	2	200	15 (7.5)	240	17 (8.5)	260	3.8 (1.9)	1100	0.7 (0.36)	200	500 (250)
G2 (**3**)	4	0.4	7500 (1875)	45	89 (22)	23	43 (11)	270	3.0 (0.75)	153	654 (163)
G3 (**4**)	8	2.2	1364 (170)	13	308 (39)	22	45 (5.7)	108	7.4 (0.93)	86	1163 (145)
Matrix:		0.5 μg laminin		0.5 μg laminin		0.5 μg laminin		0.5 μg laminin		0.5 μg laminin	
Probe:		VAA (3 μg mL^{-1})		galectin-1 (10 μg mL^{-1})(20 μg mL^{-1})		galectin-3 (30 μg mL^{-1})		galectin-7 (15 μg mL^{-1})		IgG(α^-/β^+)	
Inhibitor	Lac units per molecule	IC_{50} [μM]	rel. pot.	IC_{50} [μM]	rel. pot.	IC_{50} [μM]	rel. pot.	IC_{50} [μM]	rel. pot.	IC_{50} [μM]	rel. pot.
D-Gal	1	900	0.56	200 mM (=6% inhib.)	–	15000	0.03	15000	0.13	100 mM (=45% inhib.)	–
lactose	1	500	1	300000	1	400	1	2000	1	no inhib. up to 200 mM	–
ASF	9	0.34	1471 (163)	6.3	47619 (5291)	2	200 (22)	15	133 (15)	1.1	–
G1 (**2**)	2	100	5 (2.5)	900	333 (167)	200	2 (1)	4 mM (=27% inhib.)	–	no inhib. up to 4 mM	–
G2 (**3**)	4	2.7	185 (46)	162	1852 (463)	32	12.5 (3.1)	450	4.4 (1.1)	no inhib. up to 1.8 mM	–
G3 (**4**)	8	0.09	5556 (694)	56	5357 (670)	8.6	46.5 (5.8)	151	13 (1.7)	no inhib. up to 0.9 mM	–
Matrix:		0.25 μg Mac-2 BP		0.25 μg Mac-2 BP		0.25 μg Mac-2 BP		0.25 μg Mac-2 BP			
Probe:		VAA (2.5 μg mL^{-1})		galectin-1 (15 μg mL^{-1})		galectin-3 (10 μg mL^{-1})		galectin-7 (25 μg mL^{-1})			
Inhibitor	Lac units per molecule	IC_{50} [μM]	rel. pot.	IC_{50} [μM]	rel. pot.	IC_{50} [μM]	rel. pot.	IC_{50} [μM]	rel. pot.		
D-Gal	1	12000	0.5	200 mM (=8% inhib.)	–	12000	0.025	120000	0.01		
lactose	1	6000	1	200 mM (=12% inhib.)	–	300	1	1500	1		
ASF	9	4.2	1429 (159)	0.4	–	17	17.6 (2)	5	300 (33)		
G1 (**2**)	2	4000	1.5 (0.75)	no inhib. up to 4 mM	–	100	3 (1.5)	800	1.9 (0.9)		
G2 (**3**)	4	900	6.7 (1.7)	no inhib. up to 1.8 mM	–	3.6	83 (12)	1.8 mM (=24% inhib.)	–		
G3 (**4**)	8	86	70 (8.7)	no inhib. up to 0.9 mM	–	0.7	429 (54)	65	23 (2.9)		

[a] The numbers in parentheses express the relative potency of each lactose unit in the more than univalent inhibitor compared to carrier-free lactose. The results present average values from at least four independent experiments.

were significantly more active for galectins. A similar order of potency has been observed for starburst glycodendrimers with identical sugar content.[23a]

In addition to the neoglycoprotein and the two model glycoproteins, we used two selected components of the extracellular matrix. The results are compiled in the last two blocks of Table 1 with laminin and Mac-2 BP, respectively, as the matrix. Galectin-1 proved to be a strong binding partner for both Mac-2 BP and laminin, requiring lactose concentrations of over 100 mM to reach inhibition. Large multivalency effects with G2/G3 (463-fold and 670-fold increases, respectively) were observed for the laminin matrix, but for Mac-2 BP the inhibitory capacity of the glycodendrimers (up to the milimolar range) was insufficient. Interestingly, Mac-2 BP had a strong affinity not only for galectin-3,[25] but also for galectin-7 (see last block of Table 1). With the lectin in solution, sugar units in G3 proved to be 54-fold more active than free lactose in the case of galectin-3 (Table 1). As already outlined, these last two glycoproteins have been included to mimic cell surface properties. In the next step, we tested the three glycodendrimers as inhibitors of lectin-mediated cell interactions.

Cell assays

Agglutination of erythrocytes by lectins is a classical activity test.[26] It simulates the mediation of cell interactions by lectins. In contrast to binding to a surface, here the lectin connects two opposing surfaces, adding a new topological constraint. With standard trypsinized, glutaraldehyde-fixed rabbit erythrocytes, all lectins were active as erythrocyte adhesion molecules. After routinely normalizing inhibitory capacity to free lactose, the strongest multivalency effects were seen for galectin-3 and the plant lectin VAA (Table 2). The enhancement of inhibitory capacity of each sugar unit in the G3 glycodendrimer relative to lactose free in solution was 144-fold for galectin-3 and 446-fold for VAA. In view of a potential role of galectin-3 in the metastatic cascade, this finding has potential practical relevance. As described in the Introduction, it was an aim of this study to find inhibitors sensitive to the CRD presentation. The haemagglutination experiment represents a comparison between the crosslinker galectin-1 with binding sites on opposite ends to an aggregate of monovalent galectin-3 molecules. The differences in the relative inhibitory potencies (Table 2) show the preference of the glycodendrimers for the latter.

For VAA, the strong inhibitory effect of G3 was also seen in other cell adhesion assay systems (data not shown). Consistently, blocking of tumor cell (SW480) binding to nitrocellulose-immobilized lectin was observed. In a flow cytofluorometric assay with two human tumor cell lines of different histogenetic origin, that is, B-lymphoblastoid and colon adenocarcinoma cells, we also observed an enhanced potency of G3. Trends of potency with galectin-3 were also reproduced in the flow cytometric assay. Importantly, the glycodendrimers did not reduce the viability of the cells upon exposure.

Conclusions

Matching optimal topological features in protein – carbohydrate interactions with appropriate ligand clustering is a central aim in the design of small soluble compounds capable of targeting drugs or interfering with adhesion processes. The simple expectation that any form of clustering will necessarily lead to affinity enhancements has not been borne out by several experiments involving plant lectins or glycosyltransferases,[27] influenza virus strains,[28] or bacteria.[29] For inhibition of influenza virus haemagglutination the comb-branched or dendrigraft modes of ligand presentation were found to be superior to other types of dendritic polymeric inhibitors.[28] Spatial vicinity of sugar residues slowing the dissociation rate appears to have to meet further requirements which are even less predictable for both soluble and membrane-associated lectins. Naturally, the choice of the assay and its inherent conditions, for example, time scale, ligand presentation and static or dynamic set-up, will have an influence on the outcome, as shown recently for multivalent concanavalin A ligands by cell agglutination or surface plasmon resonance spectroscopic detection.[30] To mimic the situation in which a pharmaceutical compound comes into contact with the lectin prior to the lectin encountering a natural target, for example, on endothelial cells, we preincubated lectin and dendrimer for 15 min before adding this mixture to the test surface (microtiter plate well, cell suspension).

The experiments clearly prove that the known difference in binding-site presentation in the galectin subfamilies is of importance for the effective design of glycodendrimer inhibitors. Equally important is the nature of the matrix ligands. Relating our data to the biological roles of the galectins, it is likely that the differences in matrix and glycodendrimer preference of the various galectins point toward different functions of these

Table 2. *Determination of the minimal inhibitory concentration (MIC) and the relative inhibitory capacity (relative potency, rel. pot.) of wedge-like glycodendrimers (G1 – G3) and a glycoprotein (ASF) in lectin-mediated haemagglutination.[a]*

Probe:		VAA (0.2 μg mL^{-1})		galectin-1 (2 μg mL^{-1})		galectin-3 (50 μg mL^{-1})		galectin-7 (2 μg mL^{-1})	
Inhibitor	Lac units	MIC [μM]	rel. pot.	MIC [μM]	rel. pot.	MIC [μM]	rel. pot.	MIC [μM]	rel. pot.
D-Gal	1	50000	0.25	12500	0.1	50000	0.03	50000	0.015
lactose	1	12500	1	1250	1	1500	1	750	1
ASF	9	10.5	1190 (132)	8.4	149 (16.5)	42	35.7 (4.0)	10.5	71.4 (7.9)
G1 (**2**)	2	58.8	213 (106)	98.0	12.8 (6.4)	49	30.6 (15.3)	490	1.5 (0.8)
G2 (**3**)	4	53.8	232 (58.0)	44.8	27.9 (7.0)	5.4	278 (69.4)	112	6.7 (1.7)
G3 (**4**)	8	3.5	3571 (446)	10.8	116 (14.5)	1.3	1154 (144)	29.1	25.8 (3.2)

[a] The numbers in parentheses express the relative potency of each lactose unit in the more than univalent inhibitor compared to carrier-free lactose. The results present average values from at least two independent experiments.

homologous proteins. Expression of more than one galectin by a cell, as shown by RT-PCR fingerprinting for 61 human tumor lines and biochemical and immunological assays,[31] is not simply a case of genetic redundancy. Hence, it is desirable to introduce selectivity of binding when, for example, trying to block galectin-dependent cell adhesion in order to avoid side effects. In this context we showed that our glycodendrimers appear to be especially effective for the homodimeric (prototype) galectin-1 based on the solid-phase assay. However, in the context of the haemagglutination experiments a preference for the chimeric galectin-3 was observed. Before considering further tests for application, the concern about side reactivity to endocytic *C*-type asialoglycoprotein receptors will have to be addressed to avoid rapid clearance from the serum. Interestingly, lysine-based cluster glycosides, which have high affinity for these lectins on hepatocytes and macrophages,[32] had only minor inhibitory efficiency for galectins.[23b] Tailoring the carbohydrate structure to the preference of the target lectin will further contribute to the selectivity of new inhibitors. To this end, the recently developed strategies for establishing and screening carbohydrate libraries,[33] for preparing glycopeptides efficiently,[34] and for spectroscopically determining the saccharide topology when they are bound to a galectin, will be helpful.[35] It is worth noting that the development of these galectin-selective probes will also find application in cyto- and histochemistry.[36]

Experimental Section

Reagents: Galectin-1 was purified from bovine heart, murine galectin-3 and human galectin-7 after recombinant expression, using the plasmids prCBP35s, kindly provided by J. L. Wang (East Lansing, MI, USA), and *E. coli* JA221 cells or pQE-60/hGal-7 (establishment of an *Nco*I cleavage site required to switch from Ser 2 to Ala 2 in the protein sequence) and *E. coli* M15[pREP4] cells obtained from Qiagen (Hilden, Germany), respectively; the plant lectin from commercially available dried mistletoe leaves and the lactoside-binding immunoglobulin G fraction, further subfractionated by removing any α-galactoside-specific activity (α^-/β^+), by affinity chromatography on lactosylated Sepharose 4B, derived from ligand coupling to divinyl-sulfone-activated resin, as crucial step, as described previously.[37] Digestion of the collagenase-sensitive N-terminal domain of galectin-3 was performed with an equal amount of collagenase D (Roche Diagnostics, Mannheim, Germany) overnight at 4 °C in a buffer solution containing 75 mM Tris-HCl, pH 7.0, 75 mM NaCl, and 10 mM $CaCl_2$, as described previously.[38] Biotinylation of the carbohydrate-binding proteins was carried out with the *N*-hydroxysuccinimide ester derivative (Sigma, Munich, Germany) under activity-preserving conditions.[15a, 23] The neoglycoprotein with an average content of 28 lactoside moieties attached as *p*-isothiocyanato derivatives per molecule of carbohydrate-free bovine serum albumin was synthesized following an established protocol.[3c] Serum amyloid P component was purified from human serum by affinity chromatography on mannosylated Sepharose 4B and was subjected to neuraminidase treatment,[39] while commercial fetuin was chemically desialylated,[40] as routinely checked by mobility shifting in gel electrophoretic analysis. Murine EHS laminin[19b] and recombinant human Mac-2 BP produced in human embryonic kidney cells[21e] were kindly provided by R. Timpl (Munich, Germany). The glycodendrimers were synthesized as described,[41] coupling protected lactose isothiocyanate **1** (see Figure 1) to our dendrimer scaffolds based on 3,5-di-(2-aminoethoxy)benzoic acid repeating units carrying 2, 4, and 8 amino groups, respectively. The acetate-protected lactose isothiocyanate **1** was prepared starting from lactose following an established method.[42] Covalent coupling of **1** to the polyamino dendrimer scaffolds was achieved in CH_2Cl_2 in the presence of iPr$_2$NEt. Basic deprotection of the carbohydrate hydroxy groups resulted in the lactose dendrimers **2** – **4**, which were characterized by NMR spectroscopy and MS.

2: ^{13}C NMR (5 % CD_3OD/D_2O, 75.4 MHz): $\delta = 184.2$ (C=S), 169.0 (C=O^{I}), 160.0 (C$^{3/5}$), 132.2 (C^{1}), 109.3, 107.6 (C$^{2/6}$ + C^{4}), 104.0 (C$^{Gal}_{anom.}$), 84.2 (C$^{Glc}_{anom.}$), 79.0, 77.0, 76.3, 76.1, 73.5, 72.8, 71.9, 69.6 (8 CH^{Lac}), 67.3 (CH$_2^a$), 62.0 (CH$_2^{Glc}$), 61.0 (CH$_2^{Gal}$), 53.8 (CH_3O), 44.8 (CH$_2^b$); MS (ESI): *m/z*: 1021.3 [*M*+H]$^+$.

3: ^{13}C NMR (5 % CD_3OD/D_2O, 75.4 MHz): $\delta = 184.3$ (C=S), 169.7, (C=O^{II}), 168.5 (C=O^{I}), 160.1 (C$^{3/5}$ + C$^{3'/5'}$), 136.4 (C$^{1'}$), 132.0 (C^{1}), 107.2, 106.0 (C$^{2/6}$ + C$^{2'/6'}$ + C^{4} + C$^{4'}$), 104.0 (C$^{Gal}_{anom.}$), 84.3 (C$^{Glc}_{anom.}$), 79.1, 76.9, 76.3, 73.5, 72.8, 71.9, 69.5 (all CH^{Lac}), 67.2 (CH$_2^a$ + CH$_2^{a'}$), 62.0 (CH$_2^{Glc}$), 61.0 (CH$_2^{Gal}$), 53.7 (CH_3O), 44.7 (CH$_2^b$), 40.4 (CH$_2^{b'}$); MS (ESI): *m/z*: 1057.4 [*M* − Glc+2 Na]$^{2+}$, 1116.9 [*M*+2 H]$^{2+}$, 1127.4 [*M*+H+Na]$^{2+}$, 1138.9 [*M*+2 Na]$^{2+}$, 2091.7 [*M* − Glc+Na]$^+$, 2253.8 [*M*+Na]$^+$.

4: ^{13}C NMR (5 % CD_3OD/D_2O, 75.4 MHz): $\delta = 184.3$ (C=S), 169.8 (br, all C=O), 160.2 (C$^{3/5}$ + C$^{3'/5'}$ + C$^{3''/5''}$), 136.8 (br, C^{1} + C$^{1'}$ + C$^{1''}$), 107.3 (br, C$^{2/6}$ + C$^{2'/6'}$ + C$^{2''/6''}$ + C^{4} + C$^{4'}$ + C$^{4''}$), 104.0 (C$^{Gal}_{anom.}$), 84.3 (C$^{Glc}_{anom.}$), 79.1, 76.3, 73.5, 72.8, 71.9, 69.5 (all CH^{Lac}), 67.2 (br, CH$_2^a$ + CH$_2^{a'}$ + CH$_2^{a''}$), 62.0, 61.1 (2 CH$_2^{Lac}$), 54.0 (CH_3O), 44.9, 40.4 (br, CH$_2^b$ + CH$_2^{b'}$ + CH$_2^{b''}$); MS (ESI): *m/z*: 1465 [*M* − 2 Glc+3Na]$^{3+}$, 1472 [*M* − 2 Glc+4 Na − H]$^{3+}$, 1480 [*M* − 2 Glc+5 Na − 2 H]$^{3+}$, 1519 [*M* − Glc+3 Na]$^{3+}$, 1526 [*M* − Glc+4 Na − H]$^{3+}$, 1534 [*M* − Glc+5 Na − 2 H]$^{3+}$, 1574 [*M*+3 Na]$^{3+}$, 1581 [*M*+4 Na − H]$^{3+}$, 1588 [*M*+5 Na − 2 H]$^{3+}$, 2207 [*M* − 2 Glc+4 Na − 2 H]$^{2+}$, 2289 [*M* − Glc+4 Na − 2 H]$^{2+}$, 2371 [*M*+4 Na − 2 H]$^{2+}$.

Solid-phase assay: Attachment of the (neo)glycoproteins to the surface of microtiter plate wells proceeded from 50 µL per well at 4 °C overnight from phosphate-buffered saline solution (concentrations for each matrix type are given in Table 1), and residual protein-binding sites were blocked by an incubation step using 100 µL of this buffer containing 1 % carbohydrate-free bovine serum albumin for 1 h at 37 °C. Following thorough washes, the mixture containing lectin and any tested glycoinhibitor which had been preincubated for 15 min at room temperature was added to a well and kept for 1 h at 37 °C. Omission of labeled probe, of any inhibitor, and inhibition with 0.5 mg mL^{-1} ASF plus 75 mM lactose were routinely carried out in parallel assays to determine blank and 100 % values. Following thorough washes to remove unbound probe, signal generation to determine the extent of protein – carbohydrate binding was performed using streptavidin – peroxidase (Sigma, Munich, Germany; 0.5 µg mL^{-1}) and *o*-phenylenediamine (1 mg mL^{-1})/H_2O_2 (1 µL mL^{-1}) as indicator substances, as described previously.[23] The concentration of inhibitor required for 50 % inhibition (IC_{50}) was determined to compare the potencies of the tested substances.

Cell assays: Haemagglutination with trypsin-treated, glutaraldehyde-fixed rabbit erythrocytes to determine the minimal inhibitory concentration of a glycosubstance at a constant lectin concentration (see Table 2) was performed as described previously.[43] For the fluorometric analysis cell batches of identical passages of the human colon adenocarcinoma line SW480, obtained from the American Type Culture Collection (Rockville, MD, USA), and the human B-lymphoblastoid line Croco II, established and maintained as described,[44] were carefully washed with Dulbecco's phosphate-buffered saline solution containing 0.1 % carbohydrate-free bovine serum albumin to remove any trace of glycoproteins originating

from the serum of the culture medium. Following the incubation step with biotinylated lectin and inhibitor for 30 min at 4 °C (4×10^5 cells per assay) and thorough washing steps to remove unbound probe, automated flow cytofluorometric analysis in a FACScan instrument (Becton – Dickinson, Heidelberg, Germany) with the fluorescent marker streptavidin – R-phycoerythrin (Sigma, Munich, Germany; used at 1:4 dilution) was performed as described previously.[45] Interference of tumor cell adhesion to VAA immobilized onto nitrocellulose squares (Schleicher and Schuell, Dassel, Germany; 0.2 μm) by glycodendrimer was determined in assays with 7×10^5 tumor cells per 200 μL and incubation periods of 1.5 or 3 h at 37 °C, as described previously.[46]

We are indebted to Dr. J. Tukker (Utrecht, The Netherlands) for catalyzing the establishment of the cooperation, to B. Hofer and L. Mantel for excellent technical assistance, to the Euroworkshop program and the DAAD/NSF cooperation program for travel grants, and to the Wilhelm-Sander-Stiftung (Munich, Germany) for generous financial support. Furthermore, the Royal Netherlands Academy of Arts and Sciences (KNAW) is gratefully acknowledged for a fellowship to R.J.P. I.V. is thankful to The Netherlands Organization for International Cooperation in Higher Education (NUFFIC) for a Huygens fellowship.

[1] a) A. Varki, *Glycobiology* **1993**, *3*, 97 – 130; b) *Glycoproteins and Disease*, *New Comprehensive Biochemistry, Vol. 30* (Eds.: J. Montreuil, J. F. G. Vliegenthart, H. Schachter), Elsevier, Amsterdam, **1996**; c) N. Sharon, H. Lis in *Glycosciences: Status and Perspectives* (Eds.: H.-J. Gabius, S. Gabius), Chapman & Hall, London, **1997**, pp. 133 – 162; d) I. Brockhausen, J. Schutzbach, W. Kuhns, *Acta Anat.* **1998**, *161*, 36 – 78; e) A. Etzioni, C. M. Doerschuk, J. M. Harlan, *Blood* **1999**, *94*, 3281 – 3288; f) G. Keir, B. G. Winchester, P. Clayton, *Ann. Clin. Biochem.* **1999**, *36*, 20 – 36; g) G. Reuter, H.-J. Gabius, *Cell. Mol. Life Sci.* **1999**, *55*, 368 – 422; h) *Biochim. Biophys. Acta* **1999**, *1455* (2-3), 61 – 418 (special issue); i) H.-J. Gabius, *Naturwissenschaften* **2000**, *87*, 108 – 121.

[2] a) H.-J. Gabius, *Angew. Chem.* **1988**, *100*, 1321 – 1330; *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 1267 – 1276; b) R. L. Schnaar, *Adv. Pharmacol. (San Diego)* **1992**, *23*, 35 – 84; c) M. Petitou in *Synthetic Oligosaccharides. Indispensable Probes for the Life Sciences* (Ed.: P. Kovác), ACS, Washington, DC, **1994**, pp. 19 – 35; d) *Carbohydrates in Drug Design* (Eds.: Z. J. Witczak, K. A. Nieforth), Dekker, New York, **1997**; e) H.-J. Gabius, *Pharm. Res.* **1998**, *15*, 23 – 30; f) D. J. Lefer, *Annu. Rev. Pharmacol. Toxicol.* **2000**, *40*, 283 – 294; g) H. Rüdiger, H.-C. Siebert, D. Solís, J. Jiménez-Barbero, A. Romero, C.-W. von der Lieth, T. Díaz-Mauriño, H.-J. Gabius, *Curr. Med. Chem.* **2000**, *7*, 389 – 416.

[3] a) W. F. Göbel, O. T. Avery, *J. Exp. Med.* **1929**, *50*, 521 – 531; b) O. T. Avery, W. F. Göbel, *J. Exp. Med.* **1929**, *50*, 533 – 550; c) C. R. McBroom, C. H. Samanen, I. J. Goldstein, *Methods Enzymol.* **1972**, *28*, 212 – 219; d) Y. C. Lee, R. T. Lee in *Lectins and Cancer* (Eds.: H.-J. Gabius, S. Gabius), Springer, Heidelberg, **1991**, pp. 53 – 69; e) H.-J. Gabius, S. Gabius, *Adv. Lectin Res.* **1992**, *5*, 123 – 157; f) M. Mammen, S. K. Choi, G. M. Whitesides, *Angew. Chem.* **1998**, *110*, 2908 – 2953; *Angew. Chem. Int. Ed.* **1998**, *37*, 2754 – 2794; g) L. L. Kiessling, J. E. Gestwicki, L. E. Strong, *Curr. Opin. Chem. Biol.* **2000**, *4*, 696 – 703.

[4] a) M. M. Ponpipom, R. L. Bugianesi, T. Y. Shen, *Can. J. Chem.* **1980**, *58*, 214 – 220; b) L. Fiume, C. Busi, A. Mattioli, *FEBS Lett.* **1983**, *153*, 6 – 10; c) H.-J. Gabius, *Cancer Invest.* **1987**, *5*, 39 – 46; d) K. G. Rice in *Glycosciences: Status and Perspectives* (Eds.: H.-J. Gabius, S. Gabius), Chapman & Hall, London, **1997**, pp. 471 – 483; e) N. Yamazaki, S. Kojima, N. V. Bovin, S. André, S. Gabius, H.-J. Gabius, *Adv. Drug Delivery Rev.* **2000**, *43*, 225 – 244.

[5] a) Y. C. Lee, *Carbohydr. Res.* **1978**, *67*, 509 – 514; b) R. T. Lee, P. Lin, Y. C. Lee, *Biochemistry* **1984**, *23*, 4255 – 4261; c) R. T. Lee, Y. C. Lee, *Glycoconjugate J.* **1987**, *4*, 317 – 328; d) R. T. Lee, Y. C. Lee in *Lectins and Glycobiology* (Eds.: H.-J. Gabius, S. Gabius), Springer, Heidelberg, **1993**, pp. 9 – 22; e) M. A. Findeis, *Int. J. Pept. Protein Res.* **1994**, *43*, 477 – 485; f) A. Kichler, F. Schuber, *Glycoconjugate J.* **1995**, *12*, 275 – 281.

[6] a) T. K. Lindhorst, *Nachr. Chem. Tech. Lab.* **1996**, *44*, 1073 – 1079; b) R. Roy, *Trends Glycosci. Glycotechnol.* **1996**, *8*, 79 – 99; c) R. Roy, *Top. Curr. Chem.* **1997**, *187*, 241 – 274; d) N. Jayaraman, S. A. Nepogodiev, J. F. Stoddart, *Chem. Eur. J.* **1997**, *3*, 1193 – 1199; e) M. Fischer, F. Vögtle, *Angew. Chem.* **1999**, *111*, 934 – 955; *Angew. Chem. Int. Ed.* **1999**, *38*, 884 – 905; f) P. Veprek, J. Jezek, *J. Peptide Sci.* **1999**, *5*, 5 – 23.

[7] a) H.-J. Gabius, *Eur. J. Biochem.* **1997**, *243*, 543 – 576; b) H. Lis, N. Sharon, *Chem. Rev.* **1998**, *98*, 637 – 674.

[8] a) F. L. Harrison, C. J. Chesterton, *FEBS Lett.* **1980**, *122*, 157 – 165; b) H.-J. Gabius, *In Vivo* **1987**, *1*, 75 – 84; c) M. Caron, D. Bladier, R. Joubert, *Int. J. Biochem.* **1991**, *22*, 1379 – 1385; d) S. H. Barondes, D. N. W. Cooper, M. A. Gitt, H. Leffler, *J. Biol. Chem.* **1994**, *269*, 20807 – 20810; e) *Trends Glycosci. Glycotechnol.* **1997**, *9*, 1 – 180 (special issue); f) H. Kaltner, B. Stierstorfer, *Acta Anat.* **1998**, *161*, 162 – 179; g) S. André, S. Kojima, N. Yamazaki, C. Fink, H. Kaltner, K. Kayser, H.-J. Gabius, *J. Cancer Res. Clin. Oncol.* **1999**, *125*, 461 – 474; h) G. A. Rabinovich, *Cell Death Differ.* **1999**, *6*, 711 – 721.

[9] a) R. T. Lee, Y. C. Lee in *Neoglycoconjugates. Preparation and Applications* (Eds.: Y. C. Lee, R. T. Lee), Academic Press, San Diego, **1994**, pp. 23 – 50; b) R. T. Lee, Y. C. Lee in *Glycosciences: Status and Perspectives* (Eds.: H.-J. Gabius, S. Gabius), Chapman & Hall, London, **1997**, pp. 55 – 77.

[10] a) E. Fan, Z. Zhang, W. E. Minke, Z. Hou, C. M. L. J. Verlinde, W. G. J. Hol, *J. Am. Chem. Soc.* **2000**, *122*, 2663 – 2664; b) P. I. Kitov, J. M. Sadowska, G. Mulvey, G. D. Armstrong, H. Ling, N. S. Pannu, R. J. Read, D. R. Bundle, *Nature* **2000**, *403*, 669 – 672.

[11] a) S. J. E. Mulders, A. J. Brouwer, P. G. J. van der Meer, R. M. J. Liskamp, *Tetrahedron Lett.* **1997**, *38*, 631 – 634; b) S. J. E. Mulders, A. J. Brouwer, R. M. J. Liskamp, *Tetrahedron Lett.* **1997**, *38*, 3085 – 3088; c) A. J. Brouwer, S. J. E. Mulders, R. M. J. Liskamp, *Eur. J. Org. Chem.* **2001**, 1903 – 1915.

[12] a) D.-I. Liao, G. Kapadia, H. Ahmed, G. R. Vasta, O. Herzberg, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 1428 – 1432; b) Y. Bourne, B. Bolgiano, D.-I. Liao, G. Strecker, P. Cantau, O. Herzberg, T. Feizi, C. Cambillau, *Nat. Struct. Biol.* **1994**, *1*, 863 – 870; c) T. Shirai, C. Mitsuyama, Y. Niwa, Y. Matsui, H. Hotta, T. Yamane, H. Kamiya, C. Ishii, T. Ogawa, K. Muramoto, *Structure* **1999**, *7*, 1223 – 1233; d) P. F. Varela, D. Solís, T. Díaz-Mauriño, H. Kaltner, H.-J. Gabius, A. Romero, *J. Mol. Biol.* **1999**, *294*, 537 – 549.

[13] a) P. M. Madsen, H. H. Rasmussen, T. Flint, P. Gromov, T. A. Kruse, B. Honoré, H. Vorum, J. E. Celis, *J. Biol. Chem.* **1995**, *270*, 5823 – 5829; b) D. D. Leonidas, E. H. Vatzaki, H. Vorum, J. E. Celis, P. Madsen, K. R. Acharya, *Biochemistry* **1998**, *37*, 13930 – 13940.

[14] a) D. K. Hsu, R. I. Zuberi, F.-T. Liu, *J. Biol. Chem.* **1992**, *267*, 14167 – 14174; b) S. M. Massa, D. N. W. Cooper, H. Leffler, S. H. Barondes, *Biochemistry* **1993**, *32*, 260 – 267; c) J. Ochieng, D. Platt, L. Tait, V. Hogan, T. Raz, P. Carmi, A. Raz, *Biochemistry* **1993**, *32*, 4455 – 4460; d) B. Mehul, S. Bawumia, S. R. Martin, R. C. Hughes, *J. Biol. Chem.* **1994**, *269*, 18250 – 18258; e) S. Kuklinski, R. Probstmeier, *J. Neurochem.* **1998**, *70*, 814 – 823; f) R.-Y. Yang, P. N. Hill, D. K. Hsu, F.-T. Liu, *Biochemistry* **1998**, *37*, 4086 – 4092; g) E. A. M. Barboni, S. Bawumia, R. C. Hughes, *Glycoconjugate J.* **1999**, *16*, 365 – 373.

[15] a) H.-J. Gabius, H. Walzel, S. S. Joshi, J. Kruip, S. Kojima, V. Gerke, H. Kratzin, S. Gabius, *Anticancer Res.* **1992**, *12*, 669 – 676; b) S. Eschenburg, R. Krauspenhaar, A. Mikhailov, S. Stoeva, C. Betzel, W. Voelter, *Biochem. Biophys. Res. Commun.* **1998**, *247*, 367 – 372; c) E. C. Sweeney, A. G. Tonevitsky, R. A. Palmer, H. Niwa, U. Pfüller, J. Eck, H. Lentzen, I. I. Agapov, M. P. Kirpichnikov, *FEBS Lett.* **1998**, *431*, 367 – 370; d) E. Kunze, H. Schulz, M. Adamek, H.-J. Gabius, *J. Cancer Res. Clin. Oncol.* **2000**, *126*, 125 – 138.

[16] M. B. Pepys, T. W. Rademacher, S. Amatayakul-Chantler, P. Williams, G. E. Noble, W. L. Hutchinson, P. N. Hawkins, S. R. Nelson, J. R. Gallimore, J. Herbert, T. Hutton, R. A. Dwek, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 5602 – 5606.

[17] a) R. G. Spiro, *J. Biol. Chem.* **1960**, *235*, 2860 – 2869; b) A. S. B. Edge, R. G. Spiro, *J. Biol. Chem.* **1987**, *262*, 16135 – 16141; c) E. D. Green, A. Gabriela, J. U. Baenziger, S. Wilson, H. van Halbeek, *J. Biol. Chem.* **1988**, *263*, 18253 – 18268; d) M. G. Yet, C. C. Q. Chin, F. Wold, *J. Biol. Chem.* **1988**, *263*, 111 – 117; e) K. G. Rice, N. B. N. Rao, Y. C. Lee, *Anal. Biochem.* **1990**, *184*, 249 – 258; f) A. Guttman, *Electrophoresis* **1997**, *18*, 1136 – 1141.

[18] a) D. K. Mandal, C. F. Brewer, *Biochemistry* **1992**, *31*, 8465 – 8472; b) D. Gupta, H. Kaltner, X. Dong, H.-J. Gabius, C. F. Brewer, *Glycobiology* **1996**, *6*, 843 – 849.

[19] a) R. G. Arumugham, T. C.-Y. Hsieh, M. L. Tanzer, R. A. Laine, *Biochim. Biophys. Acta* **1986**, *883*, 112 – 126; b) S. Fujiwara, H. Shinkai, R.

Deutzmann, M. Paulsson, R. Timpl, *Biochem. J.* **1988**, *252*, 453 – 461; c) M. Sasaki, H. K. Kleinman, H. Huber, R. Deutzmann, Y. Yamada, *J. Biol. Chem.* **1988**, *263*, 16536 – 16544; d) R. Timpl, *Eur. J. Biochem.* **1989**, *180*, 487 – 502; e) M. Paulsson, *Crit. Rev. Biochem. Mol. Biol.* **1992**, *27*, 93 – 127.

[20] M. K. Ho, T. A. Springer, *J. Immunol.* **1982**, *128*, 1221 – 1228.

[21] a) I. Rosenberg, B. J. Cherayil, K. J. Isselbacher, S. Pillai, *J. Biol. Chem.* **1991**, *266*, 18731 – 18736; b) K. Koths, E. Taylor, R. Halenbeck, C. Casipit, A. Wang, *J. Biol. Chem.* **1993**, *268*, 14245 – 14249; c) H. Inohara, A. Raz, *Biochem. Biophys. Res. Commun.* **1994**, *201*, 1366 – 1375; d) H. Inohara, S. Akahani, K. Koths, A. Raz, *Cancer Res.* **1996**, *56*, 4530 – 4534; e) T. Sasaki, C. Brakebusch, J. Engel, R. Timpl, *EMBO J.* **1998**, *17*, 1606 – 1613.

[22] a) H. Leffler, S. H. Barondes, *J. Biol. Chem.* **1986**, *261*, 10119 – 10126; b) C. P. Sparrow, H. Leffler, S. H. Barondes, *J. Biol. Chem.* **1987**, *262*, 7383 – 7390; c) W. M. Abbott, E. F. Hounsell, T. Feizi, *Biochem. J.* **1988**, *252*, 283 – 287; d) R. T. Lee, Y. Ichikawa, H. J. Allen, Y. C. Lee, *J. Biol. Chem.* **1990**, *265*, 7864 – 7871; e) R. T. Lee, H.-J. Gabius, Y. C. Lee, *J. Biol. Chem.* **1992**, *267*, 23722 – 23727; f) R. Ramkumar, A. Surolia, S. K. Podder, *Biochem. J.* **1995**, *308*, 237 – 241; g) D. Gupta, M. Cho, R. D. Cummings, C. F. Brewer, *Biochemistry* **1996**, *35*, 15236 – 15243; h) O. E. Galanina, H. Kaltner, L. S. Khraltsova, N. V. Bovin, H.-J. Gabius, *J. Mol. Recognit.* **1997**, *10*, 139 – 147; i) F. P. Schwarz, H. Ahmed, M. A. Bianchet, L. M. Amzel, G. R. Vasta, *Biochemistry* **1998**, *37*, 5867 – 5877; j) S. Bharadwaj, H. Kaltner, E. Y. Korchagina, N. V. Bovin, H.-J. Gabius, A. Surolia, *Biochim. Biophys. Acta* **1999**, *1472*, 191 – 196.

[23] a) S. André, P. J. C. Ortega, M. A. Perez, R. Roy, H.-J. Gabius, *Glycobiology* **1999**, *9*, 1253 – 1261; b) S. André, B. Frisch, H. Kaltner, D. L. Desouza, F. Schuber, H.-J. Gabius, *Pharm. Res.* **2000**, *17*, 985 – 990.

[24] a) S. M. Dimick, S. C. Powell, S. A. McMahon, D. N. Moothoo, J. H. Naismith, E. J. Toone, *J. Am. Chem. Soc.* **1999**, *121*, 10286 – 10296; b) K. Tagawa, N. Sendai, K. Ohno, T. Kawaguchi, H. Kitano, *Bioconjugate Chem.* **1999**, *10*, 354 – 360; c) T. K. Dam, R. Roy, S. K. Das, S. Oscarson, C. F. Brewer, *J. Biol. Chem.* **2000**, *275*, 14223 – 14230; d) J. B. Corbell, J. J. Lundquist, E. J. Toone, *Tetrahedron: Asymmetry* **2000**, *11*, 95 – 111.

[25] N. Tinari, I. Kuwabara, M. E. Huflejt, P. F. Shen, S. Iacobelli, F.-T. Liu, *Int. J. Cancer* **2001**, *91*, 167 – 172.

[26] a) S. W. Mitchell, *Smithson. Contrib. Knowl.* **1860**, *12*, 89 – 90; b) S. W. Mitchell, E. T. Reichert, *Smithson. Contrib. Knowl.* **1886**, *26*, 155; c) H. Stillmark, *Ueber Ricin, ein giftiges Ferment aus den Samen von Ricinus comm. L. und einigen anderen Euphorbiaceen*, Schnakenburg's Buchdruckerei, Dorpat, **1888**.

[27] a) D. Zanini, R. Roy, *Bioconjugate Chem.* **1997**, *8*, 187 – 192; b) D. Zanini, R. Roy, *J. Am. Chem. Soc.* **1997**, *119*, 2088 – 2095; c) P. R. Ashton, E. F. Hounsell, N. Jayaraman, T. M. Nilsen, N. Spencer, J. F. Stoddart, M. Young, *J. Org. Chem.* **1998**, *63*, 3429 – 3437; d) B. T. Houseman, M. Mrksich, *Angew. Chem.* **1999**, *111*, 876 – 880; *Angew. Chem. Int. Ed.* **1999**, *38*, 782 – 785.

[28] J. D. Reuter, A. Myc, M. M. Hayes, Z. Gan, R. Roy, D. Qin, R. Yin, L. T. Piehler, R. Esfand, D. A. Tomalia, J. R. Baker, Jr., *Bioconjugate Chem.* **1999**, *10*, 271 – 278.

[29] D. Yi, R. T. Lee, P. Longo, E. T. Boger, Y. C. Lee, W. A. Petri, Jr., R. L. Schnaar, *Glycobiology* **1998**, *8*, 1037 – 1043.

[30] a) M. Kanai, K. H. Mortell, L. L. Kiessling, *J. Am. Chem. Soc.* **1997**, *119*, 9931 – 9932; b) D. A. Mann, M. Kanai, D. J. Maly, L. L. Kiessling, *J. Am. Chem. Soc.* **1998**, *120*, 10575 – 10582.

[31] H. Lahm, S. André, A. Hoeflich, J. R. Fischer, B. Sordat, H. Kaltner, E. Wolf, H.-J. Gabius, *J. Cancer Res. Clin. Oncol.* **2001**, *127*, 375 – 386.

[32] a) K. Ozaki, R. T. Lee, Y. C. Lee, T. Kawasaki, *Glycoconjugate J.* **1995**, *12*, 268 – 274; b) A. Kichler, F. Schuber, *J. Drug Target.* **1998**, *6*, 201 – 205.

[33] a) P. Arya, R. N. Ben, *Angew. Chem.* **1997**, *109*, 1335 – 1337; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1280 – 1282; b) M. J. Sofia, *Mol. Diversity* **1998**, *3*, 75 – 94; c) F. Schweizer, O. Hindsgaul, *Curr. Opin. Chem. Biol.* **1999**, *3*, 291 – 298; d) O. Ramström, J.-M. Lehn, *ChemBioChem* **2000**, *1*, 41 – 48; e) P. M. St. Hilaire, M. Meldal, *Angew. Chem.* **2000**, *112*, 1210 – 1228; *Angew. Chem. Int. Ed.* **2000**, *39*, 1162 – 1179.

[34] O. Seitz, *ChemBioChem* **2000**, *1*, 214 – 246.

[35] a) H.-C. Siebert, M. Gilleron, H. Kaltner, C.-W. von der Lieth, T. Kozár, N. V. Bovin, E. Y. Korchagina, J. F. G. Vliegenthart, H.-J. Gabius, *Biochem. Biophys. Res. Commun.* **1996**, *219*, 205 – 212; b) J. L. Asensio, J. F. Espinosa, H. Dietrich, F. J. Cañada, R. R. Schmidt, M. Martín-Lomas, S. André, H.-J. Gabius, J. Jiménez-Barbero, *J. Am. Chem. Soc.* **1999**, *121*, 8995 – 9000; c) H.-C. Siebert, S. André, J. L. Asensio, F. J. Cañada, X. Dong, J. F. Espinosa, M. Frank, M. Gilleron, H. Kaltner, T. Kozár, N. V. Bovin, C.-W. von der Lieth, J. F. G. Vliegenthart, J. Jiménez-Barbero, H.-J. Gabius, *ChemBioChem* **2000**, *1*, 181 – 195.

[36] a) H.-J. Gabius, A. Bardosi, *Prog. Histochem. Cytochem.* **1991**, *22*, 1 – 66; b) A. Danguy, *Eur. J. Histochem.* **1995**, *39*, 5 – 14; c) H.-J. Gabius, K. Kayser, S. Gabius, *Naturwissenschaften* **1995**, *82*, 533 – 543; d) S. Kannan, M. K. Nair in *Glycosciences: Status and Perspectives* (Eds.: H.-J. Gabius, S. Gabius), Chapman & Hall, London, **1997**, pp. 563 – 583; e) A. Zschäbitz, *Acta Anat.* **1998**, *161*, 254 – 274; f) K. Kayser, H.-J. Gabius, *Cell Tissue Res.* **1999**, *296*, 443 – 455; g) S. E. Baldus, C. Wickenhauser, E. Dittmar, A. Stefanovic, J. Thiele, *Histochem. J.* **2000**, *32*, 27 – 32.

[37] a) H.-J. Gabius, R. Engelhardt, F. Cramer, R. Bätge, G. A. Nagel, *Cancer Res.* **1985**, *45*, 253 – 257; b) H.-J. Gabius, *Anal. Biochem.* **1990**, *189*, 91 – 94; c) X. Dong, S. André, B. Hofer, K. Kayser, H.-J. Gabius, *Int. J. Oncol.* **1997**, *10*, 709 – 719; d) H. Kaltner, K. S. Lips, G. Reuter, S. Lippert, F. Sinowatz, H.-J. Gabius, *Histol. Histopathol.* **1997**, *12*, 945 – 960.

[38] N. Agrwal, Q. Sun, S.-Y. Wang, J. L. Wang, *J. Biol. Chem.* **1993**, *268*, 14932 – 14939.

[39] a) F. C. De Beer, M. B. Pepys, *J. Immunol. Methods* **1982**, *50*, 17 – 31; b) K. Kayser, U. Biechele, H. Dienemann, S. André, N. V. Bovin, H.-J. Gabius, *J. Surg. Oncol.* **1998**, *69*, 137 – 146.

[40] A. DeWaard, S. Hickman, S. Kornfeld, *J. Biol. Chem.* **1976**, *251*, 7581 – 7587.

[41] I. Vrasidas, N. J. de Mol, R. M. J. Liskamp, R. J. Pieters, *Eur. J. Org. Chem.*, in press.

[42] T. K. Lindhorst, C. Kieburg, *Synthesis* **1995**, *10*, 1228 – 1230.

[43] a) H.-J. Gabius, R. Engelhardt, S. Rehm, F. Cramer, *J. Natl. Cancer Inst.* **1984**, *73*, 1349 – 1357; b) H. Ahmed, H.-J. Gabius, *J. Biol. Chem.* **1989**, *264*, 18673 – 18678.

[44] S. Gabius, S. S. Joshi, H.-J. Gabius, J. G. Sharp, *Anticancer Res.* **1991**, *11*, 793 – 800.

[45] a) S. André, C. Unverzagt, S. Kojima, X. Dong, C. Fink, K. Kayser, H.-J. Gabius, *Bioconjugate Chem.* **1997**, *8*, 845 – 855; b) S. Kojima, S. André, E. Y. Korchagina, N. V. Bovin, H.-J. Gabius, *Pharm. Res.* **1997**, *14*, 879 – 886.

[46] a) S. Gabius, R. Wawotzny, S. Wilholm, U. Martin, B. Wörmann, H.-J. Gabius, *Int. J. Cancer* **1993**, *54*, 1017 – 1021; b) R. Wawotzny, S. André, X. Dong, S. S. Joshi, H.-J. Gabius, *Anticancer Res.* **1995**, *15*, 169 – 174.

[47] Abbreviations: ASF = asialofetuin, BSA = bovine serum albumin, EHS tumor = transplantable Engelbreth – Holm – Swarm sarcoma producing extracellular matrix of basement membrane, G1 – G3 = generation of dendrimer growth, IgG = immunoglobulin G, IgG (α^-/β^+) = β-galactoside-binding IgG fraction depleted of any α-galactoside-specific activity by affinity chromatography, LacBSA = lactosylated BSA (a neoglycoprotein with an average of 28 sugar moieties as *p*-isothiocyanatophenyl derivatives), Mac-2 BP = binding protein for Mac-2 antigen (= galectin-3), SAP = serum amyloid P component, Tris = 2-amino-2-(hydroxymethyl)-1,3-propanediol, VAA = *Viscum album* L. agglutinin.

Received: February 20, 2001
Revised version: April 30, 2001 [F205]