Synthesis and screening of a small glycomimetic library for inhibitory activity on medically relevant galactoside-specific lectins in assays of increasing biorelevance†

Sabine André,[a] Denis Giguère,[b] Tarun K. Dam,[c] Fred Brewer,[c]
Hans-Joachim Gabius[a] and René Roy*[b]

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Synthetic introduction of aglyconic substitutions into carbohydrate ligands is an approach toward identifying potent inhibitors of medically relevant lectins. We tested a panel of 27 galactoside/lactoside derivatives harboring varying aglycone moieties together with some O-3/O-3′ functionality toward a biohazardous plant toxin and four human adhesion/growth-regulatory galectins. Differential sensitivity profiles of lectin binding with cases showing activity increase relative to galactose/lactose were revealed by systematic assessments using a solid-phase assay. Quantitative differences between the homologous human proteins could even be detected. Binding of substituted lactosides to galectins-1 and -3 was shown to be enthalpically driven. To determine the potential of substituted glycosides to protect cells from harmful lectin association, binding assays with human tumor cells were performed. Invariably, compounds were identified with increased potency relative to the unsubstituted parent sugars. However, aglyconic substitutions were shown to be able to convey cytotoxicity. This report directs further attention to examining additional 2′- and 3′-substitutions of the galactose core and the potential of ligand presentation in glycoclusters to enhance avidity and selectivity, continuing to use the herein applied strategic combination of a convenient biochemical test system with bioassays.

Introduction

Glycans have an exceptional capacity for coding information, hereby predestining oligosaccharides of cellular glycoconjugates to serve as versatile biochemical signals.[1] Acting as decoding device, a large panel of protein folds has developed which define the different families of lectins.[2] The growing awareness of the functional orchestration of glycan and lectin in human diseases, *e.g.* in inflammation or tumor suppression,[3] directs increasing attention to lectins as targets for drug design.[4] Two main synthetic approaches are taken: diverse types of ligand derivatives or mimetics and of modes of multimeric presentation, *e.g.* in glycoclusters such as glycodendrimers,[5] are being examined to identify potent and lectin-specific candidates for medical application. In addition to tailoring contacts in the primary binding sites, aglyconic extensions of ligand derivatives can prove to be effective to enhance their inhibitory potency, as revealed for selectins and a 2-tetradecylhexadecyl anchor or cholera toxin and non-spanning bivalent ligands.[6] In this report, we explore the impact of adding a spacered aglycone to galactose/lactose in O/S-glycosidic linkages on lectin reactivity for a library of 27 synthetic compounds in biochemical screening, flanked by experimental considerations on thermodynamics of binding and increasing biorelevance of the assay procedure for two classes of medically relevant lectins.

As test cases, a biohazardous plant toxin, *i.e. Viscum album* L. agglutinin (VAA), and four members of the family of adhesion/growth-regulatory human galectins are chosen.[7] The plant lectin has strong affinity for histo-blood group B and H constituents and tolerates α2,6-sialylation, whereas this modification precludes galectin binding, common to substituted β-galactosides with protein-type-dependent affinity differences.[8] Of particular note for galectins, target selection for distinct glycoconjugates can be specific, as illustrated for galectins-1, -3 and -9 when inducing activated T-cell or T helper cell apoptosis.[9] Observations on a beneficial effect of introducing a nitrophenyl group to galactose at the anomeric position for VAA and galectin-1 and also evidence from spacered derivatives using glycosyl triazoles formed the basis for the intended screening.[10] Since galectins constitute a family of homologous and functionally non-redundant proteins divided into three groups, it is essential to look at the reactivity profiles for several proteins.[7f,h] Thus, members of each galectin category, *i.e.* a homodimeric proto-type protein (galectin-1), the chimera-type galectin-3 and two tandem-repeat-type proteins (galectins-4 and -9), were included to infer indications for differential sensitivities despite the close structural homology. Of note, respective results can provide clues to define the structural basis for the functional divergence among these proteins and

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[a] *Institute of Physiological Chemistry, Faculty of Veterinary Medicine, Ludwig-Maximilians-University, Veterinärstr. 13, 80539 Munich, Germany*
[b] *PharmaQAM, Department of Chemistry, Université du Québec à Montréal, P. O. Box 8888, Succ. Centre-ville, Montreal (QC), Canada H3C 3P8. E-mail: roy.rene@uqam.ca; Fax: +1 514-987-4054; Tel: +1 514-987-3000 ext 2546*
[c] *Departments of Molecular Pharmacology, Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York 10461, USA*

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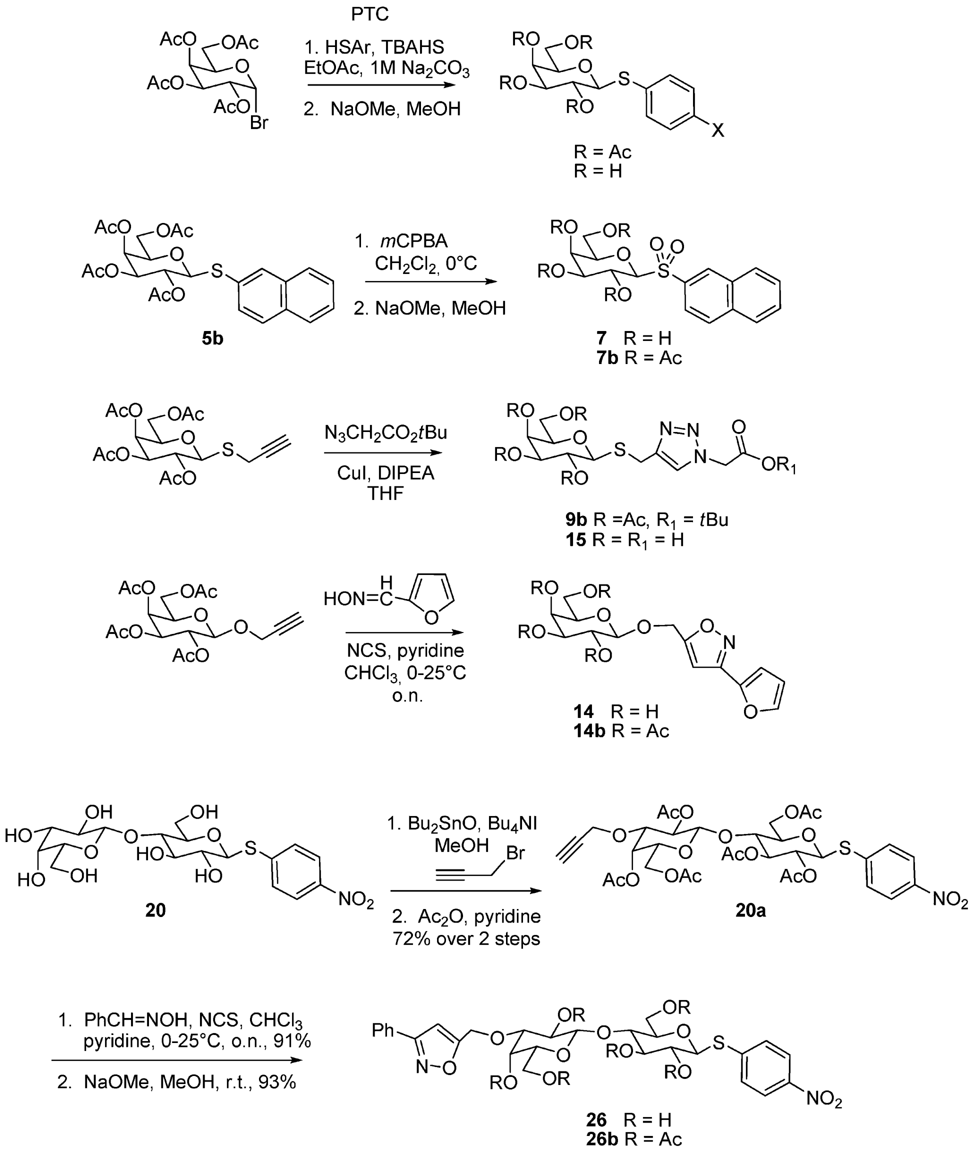
serve as input to computational docking. Although the intricate network of lectin-ligand contacts in crystals is known for the lectins, *e.g.* VAA and galectin-1,[11] the significant influence of ligand binding in solution on these two proteins' gyration radius will impede to precisely predict surface properties from modeling.[12] Thus, the reported comparative analyses can also be considered as an experimental surface mapping of the binding-site vicinity.

Technically, we performed a competitive solid-phase assay, in which extent of lectin binding to a surface-presented neoglycoprotein is monitored, for screening in a systematic manner. In order to provide insights into thermodynamics of binding of substituted lactosides, in relation to the disaccharide,[13] we then proceeded to perform isothermal titration microcalorimetry for selected cases. The biorelevance of screening is next increased by running inhibition assays with human tumor cells, simulating the scenario in medical application with the aim to protect cells from lectin binding. Finally, the so far unresolved issue is addressed as to whether substituted galactosides/lactosides can harbor cytotoxicity. Thus, our study strategically combines synthesis with biochemical and cell biological screening of the products.

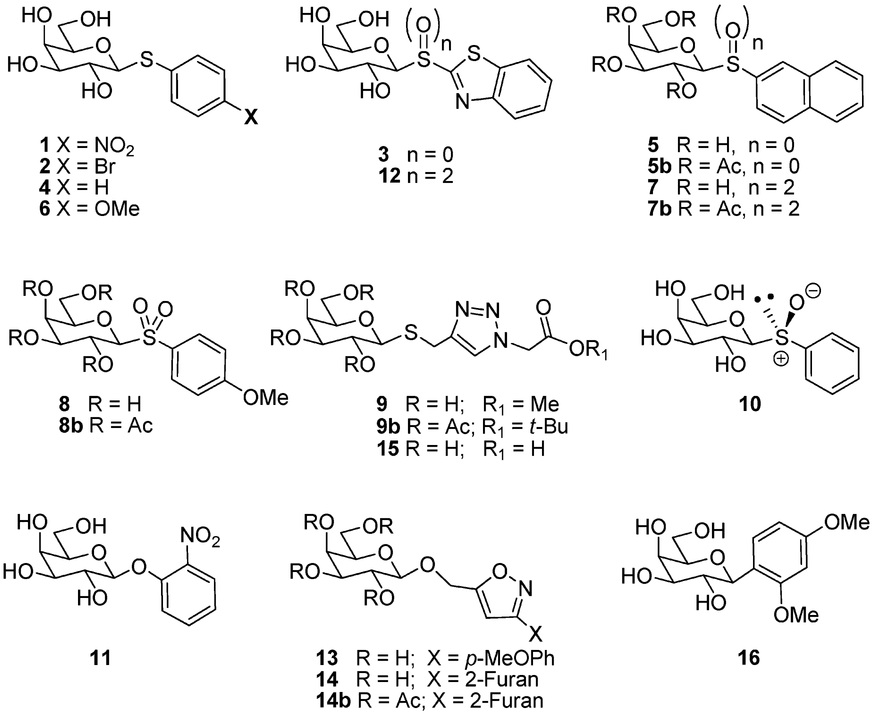
Results and discussion

Solid-phase inhibition assays

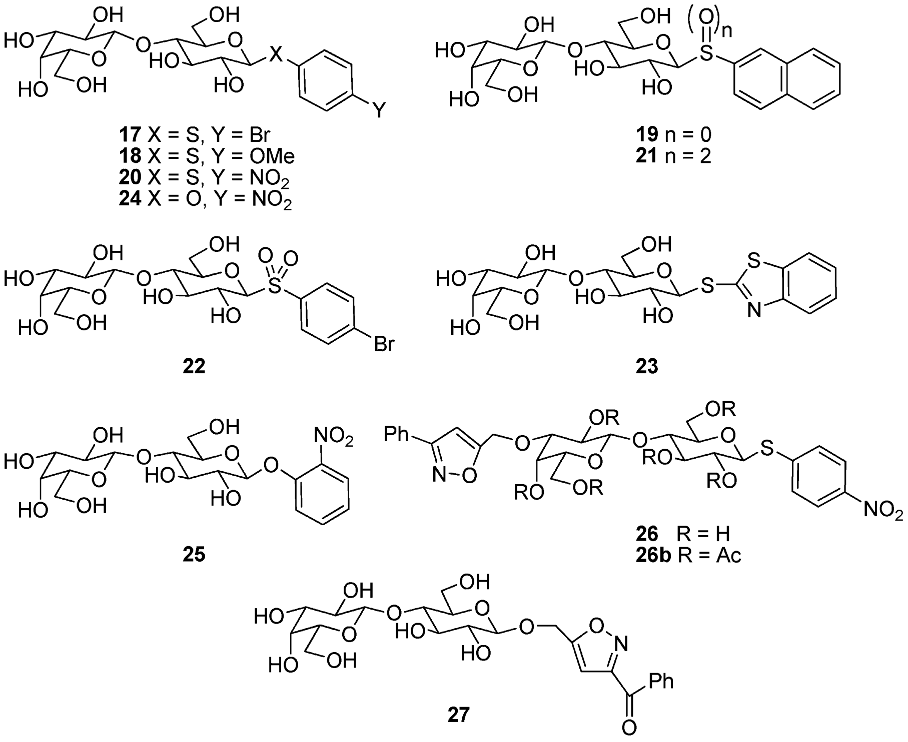
The test panel comprised 16 galactosides and 11 lactosides with different aglycones in O/S-glycosidic linkages, in one case harboring an additional 3′-substitution in the galactose moiety (Schemes 1–3). The synthetic and analytical data including assignment of compounds to numbers are given in the Experimental section. The size of the aglycone reached up to two ring systems with an isoxazole extended by furan (**14**) or phenylmethanone (**27**) or to a ring with an aliphatic chain. The experimental setting to systematically determine inhibitory potency was established in microtiter plate wells by the labeled lectin in solution and a surface-presented



Scheme 1 Representative synthetic procedures for new compounds.



Scheme 2 Structures of the β-D-galactopyranosides used in this study.



Scheme 3 Structures of the β-D-lactopyranosides used in this study.

glycan as its ligand. The effect of the series of compounds on the extent of binding was measured spectrophotometrically, with cognate/non-cognate mono- and disaccharides as controls. Due to the noted enhanced reactivity of the plant toxin and galectin-1 toward *p*-nitrophenyl derivatives, a neoglycoprotein presenting the *p*-isothiocyanatophenyl derivative of lactose was selected as binding partner to spot especially active compounds hence, considerably increasing the binding interactions to inhibit. For each lectin, the binding was saturable and inhibitable by the cognate sugar. Controls run parallel with mannose or cellobiose excluded non-specific effects.

The assay conditions are defined by two variables: ligand density on the surface was deliberately kept constant in all cases, and the concentration of each lectin was adjusted to yield signal intensity in the linear range. To assess the inhibitory profiles comparatively, the assays were run in the presence of increasing concentrations of test compound (up to eight different concentrations). The resulting effect on signal intensity is illustrated in Fig. 1. As a measure for comparison, we assessed the concentration of inhibitor at the 50%-level of inhibition (IC_{50}) from these curves. Relative inhibitory potency with respect to galactose/lactose, routinely set to 1, could then be plotted for each lectin. The detailed data for compounds with relative values above a tenth/fifth of this internal control for potency with the unsubstituted sugar are given in Fig. 2 and 3.

The results for the plant toxin answered the question on the impact of structural variations. In detail, they revealed a differential sensitivity of inhibitory properties for substitutions to the galactose core (Fig. 2A). 4-Nitrophenyl 1-thio-β-D-galactopyranoside (**1**), initially tested as further control due to the activity of the corresponding O-glycoside,[10c] proved active, demonstrating efficacy of the non-hydrolyzable S-glycoside (Fig. 2A). In negative terms, the presence of a carboxyl group in terminal position of a spacered triazole in compound **15** proved detrimental, underscoring its unfavorable effect noted for a short aliphatic chain previously.[10c] The two sulfones **7** and **8** were less reactive than unsubstituted galactose. In contrast, a benzothiazolyl (**3**) and a furan-substituted isoxazolyl (**14**) as substituents were notably active. These substituents will likely occupy the position of the glucose moiety of lactose in a favorable manner. To test the impact of substitutions beyond the disaccharide, 11 substituted lactosides were processed next. Invariably, the extensions to the lactose core conveyed no remarkable increase of inhibitory potency (Fig. 2B). These results are in accord with the galactose core as major contact site and a calorimetrically determined difference in ΔG of 0.4 kcal mol^{-1} between galactose and lactose to the lectin site around tyrosine residue 249 in VAA's subdomain 2γ.[14] As consequence, these results turn special attention to further substitutions at the galactose core, which can in parallel consider the 2′- and 3′-positions and combinations thereof. In fact, α1,2-fucosylation of galactose to establish the histo-blood group H-disaccharide and α1,3/4-galactosylation had yielded increased avidity.[8d] Thus, introducing a thio-linked substituent at the anomeric position and exploring the potential of derivatization at the indicated positions gives work on further enhancing avidity a clear direction.

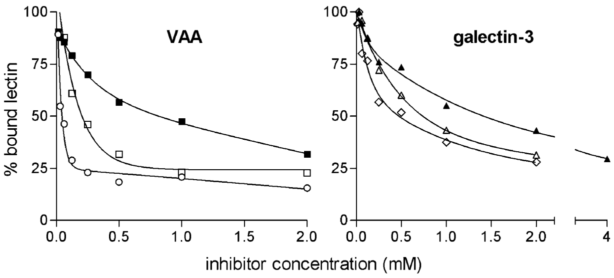


Fig. 1 Inhibition of the binding of biotinylated VAA (left) and human galectin-3 (right) to surface-immobilized lactosylated neoglycoprotein by ligand derivatives. Galactose (■) or lactose (▲) were used as control. Data on the following synthetic test substances are presented: compound **1** (□) and compound **14** (○) in the case of VAA, and compound **23** (Δ) and compound **26** (◇) in the case of galectin-3.

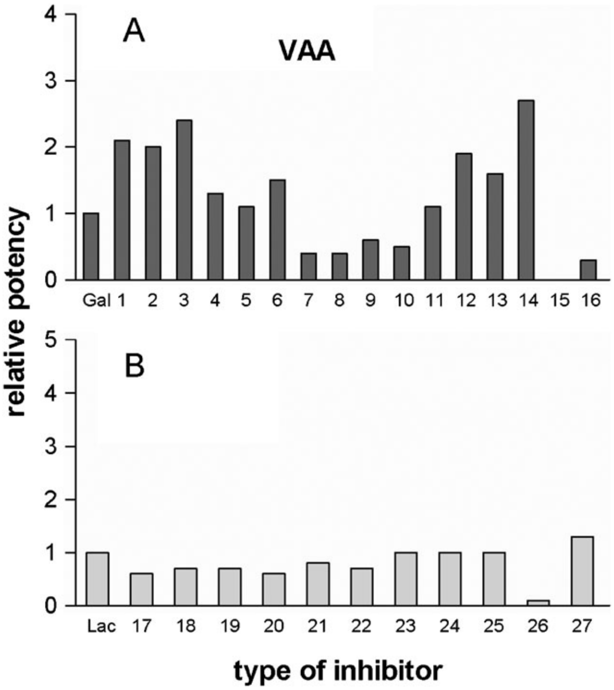


Fig. 2 Relative potency of the panel of synthetic derivatives of galactose (A) and lactose (B) for inhibition of binding of VAA to surface-immobilized lactosylated neoglycoprotein. The absolute IC_{50}-values were determined by systematic inhibition assays in each case, as shown in Fig. 1, and then set into relation to the data for galactose (Gal) and lactose (Lac) used for normalization to **1** (IC_{50} (Gal) = 0.6 mM, IC_{50} (Lac) = 0.29 mM). The connection between compound number (type of inhibitor) and the structure is given in the Experimental section together with the experimental details on which the upper limit of 15.4% for the standard deviation is based.

Turning next to galectins, here galactose is a much weaker inhibitor than lactose.[10a,b] The natural extension, bound as low-energy *syn* conformer,[15] is thus very suited for affinity generation. Fittingly, the substituted galactosides were mostly inactive or only rather weak inhibitors for the galectins. At best, the benzothiazolyl derivative **3** noted above reached an activity level comparable to lactose for galectins-3, -4 and -9. The reactivity profiles of the lactosides presented in Fig. 3 revealed differences in reactivity between the test compounds, with cases of relative enhancement in inhibition, and characteristic features for the intergalectin comparison. The attained selectivity levels yet appeared as quantitative with tangible cross-reactivity. Relative inhibitory potency was lowest for galectin-1, increased activity was seen for the other three tested proteins, with cross-reactivity of the library to different galectins reflecting the structural homology (Fig. 3). The tested 3′-substitution to the galactose moiety with a substituted isoxazole (compound **26**) yielded rather similar effects on the chimera- and tandem-repeat-type galectins, *i.e.*

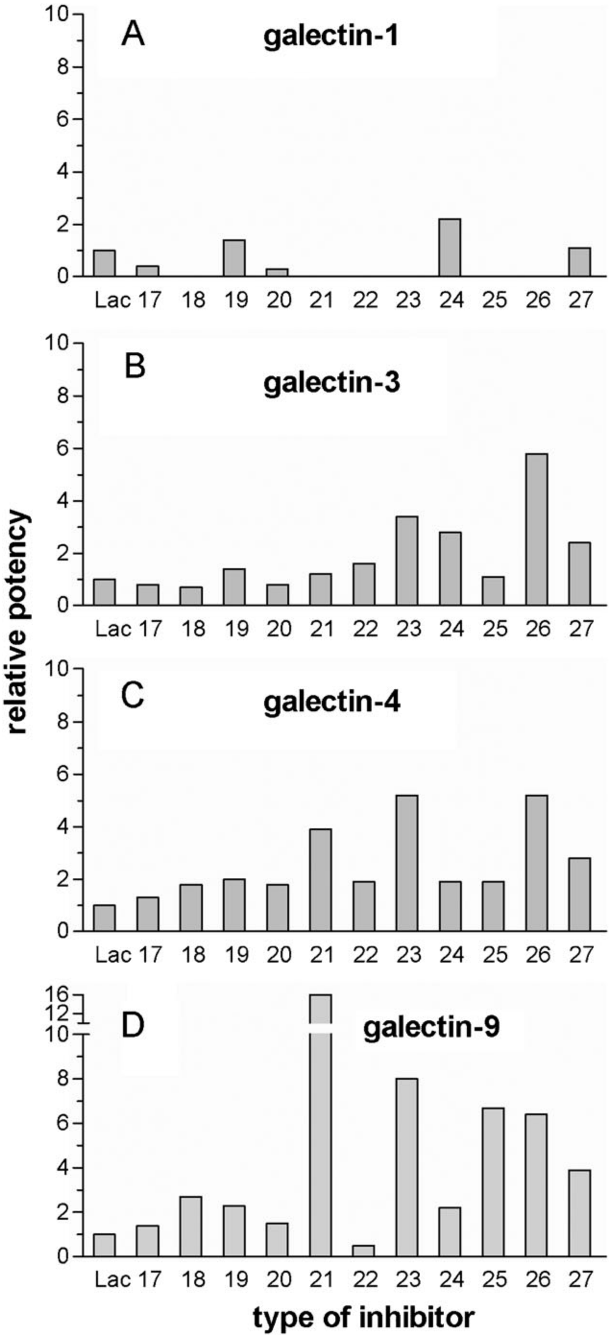


Fig. 3 Relative potency of the panel of synthetic derivatives of lactose for inhibition of human galectins, *i.e.* homodimeric proto-type galectin-1 (A, IC_{50} (Lac) = 0.7 mM), chimera-type galectin-3 (B, IC_{50} (Lac) = 2.25 mM) and the tandem-repeat-type galectins-4 (C; IC_{50} (Lac) = 3.88 mM) and -9 (D, IC_{50} (Lac) = 4 mM), to surface-immobilized lactosylated neoglycoprotein. The absolute IC_{50}-values were determined by systematic inhibition assays in each case, as shown exemplarily for galectin-3 in Fig. 1, and then set into relation to the data for lactose (Lac) used for normalization to **1**. The connection between compound number (type of inhibitor) and the structure is given in the Experimental section together with the experimental details on which the upper limit of 15.4% for the standard deviation is based.

galectins-3, -4, and -9, underscoring the challenge to refine target specificity. The combination of a sulfone with a distinct aromatic extension indicated potential toward this end (compounds **21** and **22**, Fig. 3). The β-naphthyl sulfone derivative (**21**) proved most potent on galectin-9 relative to lactose (Fig. 3D), with a graded activity also to galectin-4 and then galectin-3 (Fig. 3B and C). Its IC_{50}-value of about 300 μM compares favorably to 4-benzylaminocarbonyl-1*H*-[1,2,3]-triazol-1-yl mannoside at K_D = 540 μM, its galactoside (K_D = 670 μM) and *N*-acetyllactosamine (K_D = 500 μM).[10d] Evidently, despite global sequence homology structural differences in the vicinity of the contact site for the glucose moiety of lactose are inferable by the synthetic derivatives, giving direction to iterative refinements as noted above for VAA. In addition, modifying 2′- and 3′-positions at the galactose unit, hereby yielding closer glycomimetics of high-affinity histo-blood group ABH-oligosaccharide epitopes, is a promising means to combine avidity enhancements from three different subsites for generating optimal inhibitory potency and selectivity. This perspective warrants to consider further methodological transformations.

Technically, this solid-phase system as screening approach provides comparative data comprehensively in a simple setting which simulates lectin binding from solution to biological targets. As additional advantage, it requires rather low amounts of sugar analogs. An affinity determination by calorimetry would be technically more demanding and will require considerably more time and substance. This type of analysis will yet provide quantitative data on affinity and the major thermodynamic driving forces to ligand accommodation. Whereas it is known that this process for lactose and *N*-acetyllactosamine is enthalpically favored,[13] the issue is open for derivatives thio/sulfone-linked aglycones. Thus, we performed a series of titrations with galectin-3 and also galectin-1, a resulting binding profile is presented in Fig. 4. Since galectin-3 will only form aggregates in the presence of multivalent ligands,[16] the natural form can be used for titrations.

The experiments enabled us to calculate the entropic contribution and the binding affinity. The *n*-value of 1, representing one active lectin site per subunit, ensured full activity of the lectins. Lectin/ligand association was invariably enthalpically driven (Table 1). As compiled in Table 1, binding affinity of the glycomimetics showed differences of up to 1.8 kcal mol^{-1}, in this case with decrease in enthalpic contribution. These data were in line with the observed inhibitory activity, with an approximate correlation between data sets of IC_{50}-values from screening and affinity from calorimetry. Having herewith also supported applicability of the biochemical assay for screening, the matter of biorelevance remains a concern. It is obvious that any perspective for medical application will rest on the compounds' potency to block binding of lectin to physiologic ligands on cell surfaces and their lack of cytotoxicity. To address these issues we performed cell assays. The surface of cells presents an array of galactosides in contrast to the screening with the lactose derivative conjugated to a carrier protein. Moreover, the common glycan branching facilitates functional multivalency of glycoproteins with an inherent gradient of decreasing binding constants.[13] Hence, the IC_{50} data obtained with this cell assay is more closely representative of the *in vivo* situation, as opposed to other physical measurements.

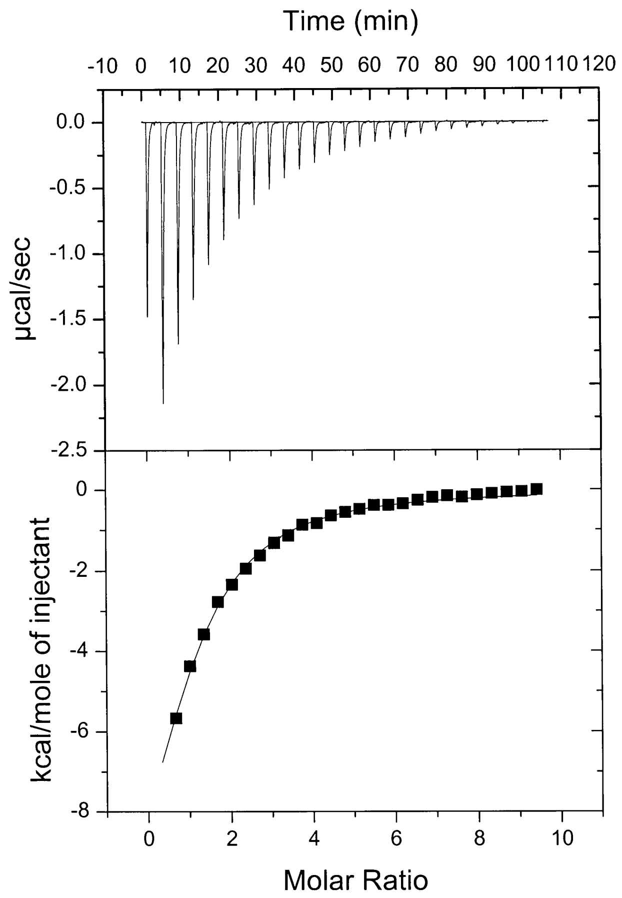


Fig. 4 Profile of isothermic titration calorimetry using human galectin-3 and increasing concentrations of compound **23** at 27 °C added in 4 μl injections (top) and the integrated curve showing experimental points (■) and the best fit (---) (bottom).

Table 1 Thermodynamic parameters of binding of synthetic compounds to human galectins-1 and -3 at 27 °C

Compounds	K_a/ $M^{-1} \times 10^{-3}$	$-\Delta G$/ kcal mol^{-1}	$-\Delta H$/ kcal mol^{-1}	$-T\Delta S$/ kcal mol	*n*
Galectin-3					
17	4.5	5.0	10.1	5.1	1.05
21	45.0	6.4	11.8	5.4	1.02
23	65.0	6.7	12.1	5.4	1.01
24	3.6	4.9	9.7	4.8	1.04
Galectin-1					
17	1.3	4.3	10.7	6.4	1.03
24	2.0	4.5	9.8	5.3	1.03

Errors in K_a range from 1–6%; errors in ΔG are less than 1%; errors in ΔH are 1% to 5%; errors in $T\Delta S$ are 1% to 7%; errors in *n* are less than 2%.

Cell-binding inhibition and cytotoxicity assays

Human tumor cells *in vitro* were used as model targets of the biotinylated lectins. Concentration dependence was routinely assessed first to determine linear range and plateau levels of signal generation. Since galectins are known to interact with peptide motifs and also protein-bound lipids besides carbohydrates, sugar-inhibitable binding was rigorously ascertained. Testing was performed in the linear range for each lectin/cell type pair with 5 × 10^4 cells per assay, using different concentrations of glycomimetics in each case. Monitoring was always run in parallel flanked by internal controls, *e.g.* to determine lectin- and carbohydrate-independent binding. The results with inhibitory compounds are documented in semilogarithmic representation, along with the control standard, to illustrate the level of attainable inhibition for the two quantitative parameters, *i.e.* percentage of positive cells and mean fluorescence intensity (Fig. 5). The numbers are given in each panel. Herein, the best case is illustrated, together with the sugar control and other representative results (Fig. 5).

The presented results for VAA revealed the activity of galactose and the superiority of the thio-linked compound **3** in blocking binding to colon cancer cells (Fig. 5A and B). Compound **14** was equally effective (not shown), and the lack of activity for compound **15** ascertained accordance to solid-phase data (Fig. 5B). The activity profile was not notably different in this case when a line of lymphoma cells was tested, and substituted lactosides did not yield an activity increase in accord with the screening data (Fig. 5C and D). In total, at this stage, the galactoside family is clearly superior to the lactosides with unmodified lactose being better within the latter in both assay settings. This situation is different for the galectins.

Compounds **24** and **21** were more active than lactose on galectins-1 and -3 or galectins-4 and -9, respectively (Fig. 5, third and fourth panels). Compounds **23** and **26** were rather similar in potency on galectin-3 (compound **23** given in Fig. 5E–H) so that no obvious advantage is achieved by this 3′-substitution, also considering its cross-reactivity to galectins-4 and -9 (Fig. 3C and D). Similarly reflecting the biochemical data, compounds **21**, **23** and **26** share a comparable activity level (no. **21** given in Fig. 5G), clearly above lactose. The extent of inhibitory potency and selectivity was enhanced for galectin-9, compound **21** at 0.25 mM significantly more active than compound **23** at 0.5 mM and lactose at 2 mM (Fig. 5H). The illustration of scans for weak inhibitors (compound **17**/galectin-3; compound **15**/galectin-4) attests an inherent specificity control, solidifying the fair degree of comparability. Since the nature of a galectin's binding partner and its spatial presentation can differ depending on the cell type, firm conclusions on inhibitor potency in cell assays should only be based on extensive testing of selected candidates with various cell types.

At this stage, it is appropriate to test the tacitly assumed lack of cytotoxicity of substituted galactosides/lactosides. Examined as control, no tested sugar (galactose, lactose, cellobiose) affected proliferation of the SW480 colon adenocarcinoma cells used in cell-binding assay up to a concentration of 10 mM. As models to examine cytotoxic activity we selected the nitrophenyl thiogalactoside (**1**), a sulfone (**12**) and an aromatic thiolactoside (**17**). All three substances negatively affected proliferation over the period of 48 h. Presence of compound **1** was a weak effector reducing cell growth by 20% at 10 mM, whereas the other two compounds had a strong impact already measurable at 2 mM with growth reduction by 10–15%, yielding an about 40% inhibition at 5 mM.

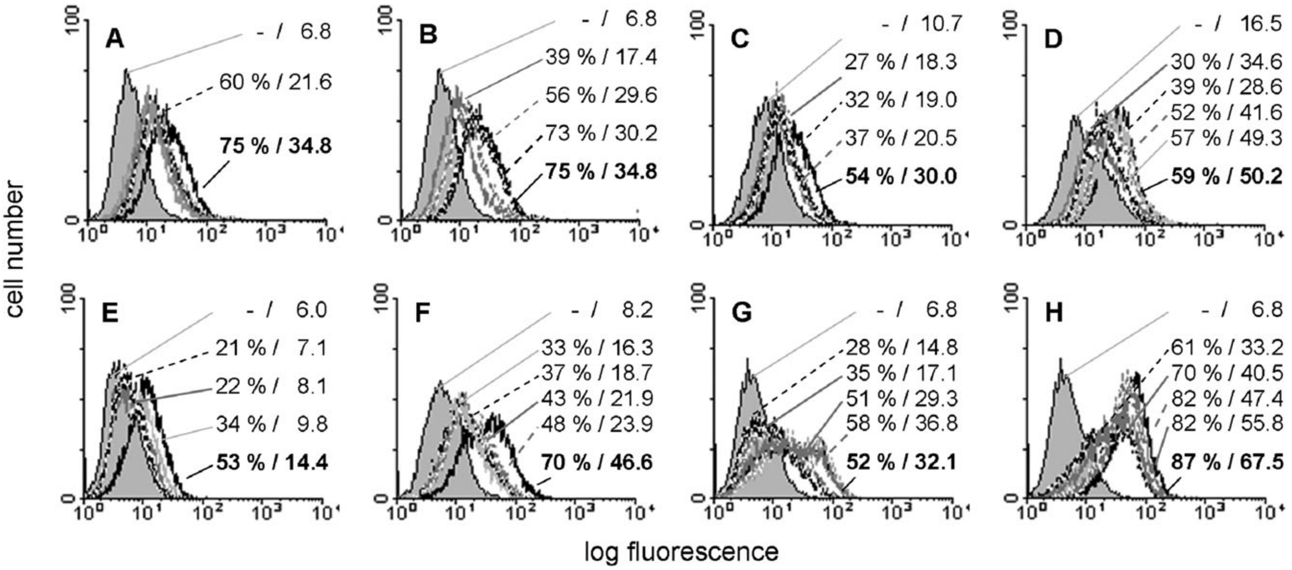


Fig. 5 Semilogarithmic representation of fluorescent surface staining of human tumor cells by labeled lectins. The control value representing marker-independent staining by incubation with the second-step reagent is given as shaded area, the 100%-value obtained by lectin binding in the absence of any test compound is shown as black line. Quantitative data on percentage of positive cells and mean fluorescence intensity are given in each panel (numbers of control printed in bold), and lines connect these data with the respective peak. The following pairs of biotinylated lectin/human tumor cell line were processed for the given set of compound/concentration: (A) 2 μg ml^{-1} VAA and the colon adenocarcinoma line SW480 with galactose concentrations of 1 mM, 2 mM, 4 mM and 8 mM, with quantitative data given at 2 mM galactose and in the absence of inhibitor; (B) respective results in the presence of the synthetic galactose derivatives **3**, **11** and **15** (listed from top to bottom) at the fixed concentration of 2 mM; (C) respective results in the presence of 1 mM lactose and the synthetic lactose derivatives **18** and **17**; (D) 0.2 μg ml^{-1} VAA and the B-lymphoblastoid cell line Croco II tested without marker, in the presence of compound **3**, compound **11**, galactose and compound **8** at the concentration of 2 mM as well as in the absence of test compound; (E) 10 μg ml^{-1} galectin-1 and the tumor suppressor p16$^{\mathrm{INK4a}}$-expressing pancreatic carcinoma line Capan-1 tested without marker, in the presence of compound **24**, lactose and galactose at the concentration of 2 mM as well as in the absence of test compound; (F) 10 μg ml^{-1} galectin-3 and the colon adenocarcinoma line SW480 tested without marker, in the presence of compound **23**, compound **24**, lactose and compound **17** at the concentration of 0.5 mM as well as in the absence of test compound; (G) 10 μg ml^{-1} galectin-4 and the tumor suppressor p16$^{\mathrm{INK4a}}$-expressing pancreatic carcinoma line Capan-1 tested without marker, with 0.4 mM compound **21**, 0.4 mM lactose, 2 mM compound **15** and 0.4 mM galactose as well as in the absence of test compound; (H) 5 μg ml^{-1} galectin-9 and the given pancreatic tumor line tested without a marker, in the presence of compound **21** at 0.25 mM, compound **23** at 0.5 mM, lactose at 2 mM and galactose at 4 mM as well as in the absence of test compound.

Conclusions

This report explores the potential of a panel of 27 synthetic galactosides/lactosides with O/S-linked extensions at the anomeric position to block glycan binding of two classes of medically relevant lectins. Solid-phase assays with the plant toxin revealed activity enhancement especially for substituted galactosides, directing further work to 2′- and 3′-modifications at the galactose core of a lactoside. Minor enhancements can arise from further extensions at the anomeric linkage. In contrast, these are sizeable when testing adhesion/growth-regulatory galectins. β-Naphthyl sulfone established remarkable potency in the solid-phase system for the two tandem-repeat-type galectins, maintained in cell-binding assays especially for galectin-9. The observed cross-reactivity among the four homologous proteins is, however, still reason for concern, as also previously seen for (glyco)peptides from one-bead-one-compound libraries.[17] Its level will need to be reduced by co-substitutions at the galactose core, *e.g.* mimicking ABH-epitopes or the pentasaccharide chain of ganglioside GM1.[8f,18] Toward this end, our combination of a simple and sensitive inhibition assay with low requirements for sample quantity with a cell-based assay to determine bioactivity and extent of cross-reactivity is helpful. Besides the further iterative synthetic refinements the design of glycoclusters can help reduce cross-reactivity. The presented data on cytotoxicity advise to run respective controls at an early stage of such efforts.

Experimental

General

All reactions involving water-sensitive chemicals were carried out in flamedried glassware with magnetic stirring under a nitrogen atmosphere. Anhydrous DCM was distilled from CaH_2 and anhydrous THF were distilled from Na/K prior to use. All non-aqueous reactions were carried out under anhydrous conditions within a nitrogen atmosphere in distilled solvents. All other solvents and reagents were used as received. TLC was performed on aluminium plates (silica gel 60 F254) with detection by UV or by coloration with ammonium molybdate in acid solution. Column chromatography was performed on silica gel (230–400 mesh) with the indicated eluent. ^{1}H and ^{13}C NMR spectra were recorded at 300 (75) MHz with a Variant apparatus. Chemical shifts (ppm) are reported relative to $CHCl_3$ (7.27) or D_2O or CD_3OD as internal standard. Optical rotations were measured on a Polarimeter JASCO P-1000 and melting point on a Fisher-Johns Melting Point Apparatus. ESI-MS analyses were carried out on a MICROMASS Quattro LC. High-resolution mass spectrometry (HRMS) was carried out on a LC-MSTOF (liquid chromatography mass spectrometry time of flight) model 6210 from Agilent Technologies.

Syntheses

Typical procedure for PTC glycosylation[19]. To a solution of glycosyl halides (1 equiv.) and tetrabutyl ammonium hydrogen sulfate (TBAHS) (1 equiv.) in ethyl acetate (1.0 ml per 100 mg of sugar) were added the nucleophile (2–3 equiv.) and 1 M sodium carbonate (1.0 ml per 100 mg of sugar). The reaction mixture was vigorously stirred at room temperature until the starting material was completely consumed (1–3 h) as judged by TLC monitoring using a mixture of ethyl acetate and hexane as eluent. The solution was then diluted with ethyl acetate and the organic phase was separated. The organic solution was washed with saturated sodium bicarbonate, water and brine, then dried over anhydrous sodium sulfate, filtered and concentrated. The crude residues were purified by silica gel column chromatography using a mixture of ethyl acetate and hexane as eluent. Pure glycosyl derivatives were obtained after silica gel column chromatography and usually recrystallised from ethanol.

Typical procedure for de-*O*-acetylation (Zemplén conditions). The acetyl protected glycoside (0.1 mmol) was dissolved into methanol (2 ml), to which was added a catalytic amount of sodium methoxide (until pH = 8). The solution was stirred at room temperature until disappearance of the starting material. After neutralization of sodium methoxide with Amberlite IR-120 (H^+) resin, the solution was filtered and removal of the methanol under reduced pressure afforded the fully deprotected glycoside.

Typical procedure for sulfone synthesis. The 1-thio-β-D-glycoside (1.0 mmol) was dissolved in dry dichloromethane at 0 °C under an atmosphere of nitrogen. *m*CPBA (3.5 mmol) was added by small portions and the reaction was monitored by TLC. When the reaction was finished the mixture was allowed to warm to room temperature and washed with saturated $NaHCO_3$, brine and water. The organic solution was dried over Na_2SO_4, filtered and concentrated under reduce pressure. The crude residues were purified by silica gel column chromatography using a mixture of ethyl acetate and hexane as eluent.

Compounds and characterisation

4-Nitrophenyl 1-thio-β-D-galactopyranoside (**1**),[19c] 4-bromophenyl 1-thio-β-D-galactopyranoside (**2**),[20] 2-benzothiazolyl 1-thio-β-D-galactopyranoside (**3**),[21] phenyl 1-thio-β-D-galactopyranoside (**4**),[22] β-naphthyl 1-thio-β-D-galactopyranoside (**5**),[22] 4-methoxyphenyl 1-thio-β-D-galactopyranoside (**6**),[23] phenyl 1-thio-β-D-galactopyranoside S-oxide (**10**),[24] *o*-nitrophenyl β-D-galactopyranoside (**11**),[25] β-D-galactopyranosyl 2-benzothiazolyl sulfone 2-benzothiazolyl 1-sulfonyl-β-D-galactopyranoside (**12**),[26] 3-(4-methoxyphenyl)-5-(1-deoxy-β-D-galactopyranosyl)-isoxazole (**13**),[27] 3-β-D-galactopyranosyl-2,5-dimethoxybenzene (**16**),[28] *p*-nitrophenyl 1-thio-β-D-lactoside (**20**)[19a] β-naphthyl 1-sulfonyl-β-D-lactoside (**21**),[27] *p*-nitrophenyl β-D-lactoside (**24**),[21] *o*-nitrophenyl β-D-lactoside (**25**),[29] and 3-(2-phenylmethanone)-5-(1-deoxy-β-D-lactopyranosyl)-isoxazole (**27**),[30] were obtained as previously described. The following known compounds gave the listed HRMS data: **20**: ESI$^+$-HRMS: $[M + Na]^+$ calcd for $C_{18}H_{25}NO_{12}SNa$: 502.0990; found: 502.0993, Δ 0.66 ppm; **21**: ESI$^+$-HRMS: $[M + NH_4]^+$ calcd for $C_{22}H_{32}NO_{12}S$: 534.1640; found: 534.1646, Δ −2.93 ppm; **24**: ESI$^+$-HRMS: $[M + Na]^+$ calcd for $C_{18}H_{25}NO_{13}$: 486.1218; found: 486.1221, Δ 0.63 ppm.

β-Naphthyl 1-sulfonyl-β-D-galactopyranoside (**7**) was obtained from peracetylated **7b** using the above general Zemplén de-*O*-acetylation procedure as a white powder (89%) and had mp 175–176 °C; IR: 3192 cm^{-1} (s, OH), 1104 cm^{-1} (s, O=S=O); ^{1}H NMR (CD_3OD) δ (ppm): 8.47 (1H, s, H_{AR}), 7.99–7.82 (4H, m, H_{AR}), 7.63–7.52 (2H, m, H_{AR}), 4.33 (1H, d, J = 9.3 Hz, H-1), 3.82 (1H, dd, J = 9.3 Hz, H-2), 3.71 (dd, J = 3.3 Hz, H-4), 3.51–3.23 (4H, m, H-3, H-5, H-6a, H-6b); ^{13}C NMR (CD_3OD) δ (ppm): 131.4, 129.4, 129.3, 128.6, 127.8, 127.4, 124.2 ($10C_{AR}$), 92.7 (C-1), 80.1 (C-5), 74.5 (C-3), 68.6 (C-4), 67.1 (C-6), 61.0 (C-2); ESI-MS calcd for $C_{16}H_{18}O_7S + (Na^+)$: 377.3; found: 377.1; ESI$^+$-HRMS: $[M + Na]^+$ calcd for $C_{16}H_{18}O_7SNa$: 377.0665; found: 377.0667, Δ −0.69 ppm.

β-Naphthyl 2,3,4,6-tetra-*O*-acetyl-1-sulfonyl-β-D-galactopyranoside (**7b**) was obtained from known **5b**[22] following the above general oxidation procedure as a white powder (91%) and had mp 145−146 °C; $[\alpha]_D^{25}$: −18.7 (c = 1, $CHCl_3$); IR: 1751 cm^{-1} (s, C=O), 1229 cm^{-1} (s, O=S=O); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.56 (1H, s, H_{AR}), 8.10–7.91 (4H, m, H_{AR}), 7.73–7.63 (2H, m, H_{AR}), 5.51 (1H, dd, J = 9.89 Hz, H-2), 5.27 (1H, dd, J = 3.02 Hz, H-4), 5.04 (1H, dd, J = 9.89, 3.29 Hz, H-3), 4.58 (1H, d, J = 9.62 Hz, H-1), 4.13–4.07 (1H, m, H-6a), 3.95–3.86 (2H, m, H-5, H-6b), 2.17, 1.96, 1.85, 1.63 (12H, 4s, $\underline{C}\underline{H}_3CO$); ^{13}C NMR ($CDCl_3$) δ (ppm): 170.2, 169.9, 169.7, 169.4 ($4CH_3\underline{C}\underline{O}$), 135.6, 132.9, 131.8, 131.6, 129.7, 128.5, 127.7, 127.5, 124.9 ($10C_{AR}$), 89.2 (C-1), 74.7 (C-5), 71.5 (C-3), 66.4 (C-4), 63.9 (C-2), 60.9 (C-6), 20.8, 20.5, 20.4, 19.9 ($4\underline{C}\underline{H}_3CO$); ESI-MS calcd for $C_{24}H_{26}O_{11}S + (Na^+)$: 545.5; found: 545.1; ESI$^+$-HRMS: $[M + NH_4]^+$ calcd for $C_{24}H_{30}NO_{11}S$: 540.1534; found: 540.1536, Δ 0.43 ppm.

4-Methoxyphenyl 1-sulfonyl-β-D-galactopyranoside (**8**) was obtained from **8b** under Zemplén conditions as a white powder (91%) and had mp 161-163 °C; $[\alpha]_D^{25}$: +1.0 (c = 1, MeOH); IR: 3377 cm^{-1} (s, O-H), 1268 cm^{-1} (s, O=S=O); ^{1}H NMR (CD_3OD) δ (ppm): 7.83 (2H, d, J = 8.7 Hz, H-*ortho*), 7.05 (2H, d, J = 8.7 Hz, H-*meta*), 4.25 (1H, d, J = 9.6 Hz, H-1), 3.85 (3H, s, OMe), 3.81–3.78 (2H, m, H-2, H-4), 3.60–3.58 (2H, m, H-3, H-6a), 3.52–3.45 (2H, m, H-5, H-6b); ^{13}C NMR (CD_3OD) δ (ppm): 165.8 (C-*para*), 133.1 (C-*ortho*), 129.3 (C-*ipso*), 115.2 (C-*meta*), 93.8 (C-1), 81.3 (C-5), 75.7 (C-3), 69.8 (C-4), 68.3 (C-6), 62.3 (C-2), 56.3 (OMe); ESI-MS calcd for $C_{13}H_{18}O_8S + (Na^+)$: 357.1; found: 357.3; ESI$^+$-HRMS:

$[M + NH_4]^+$ calcd for $C_{13}H_{22}NO_8S$: 352.1061; found: 352.1062, Δ 0.54 ppm.

4-Methoxyphenyl 2,3,4,6-tetra-*O*-acetyl-1-sulfonyl-β-D-galactopyranoside (**8b**) was obtained as above from peracetylated **6** as a white powder (89%) and had mp 115–1116 °C; $[\alpha]_D^{25}$: −58.2 (*c* = 1, $CHCl_3$); IR: 1750 cm^{-1} (s, C=O), 1273 cm^{-1} (s, O=S=O); ^1H NMR ($CDCl_3$) δ (ppm): 7.88 (2H, d, *J* = 8.5 Hz, H-*ortho*), 7.05 (2H, d, *J* = 8.5 Hz, H-*meta*), 5.42 (1H, dd, *J* = 9.8 Hz, H-2), 5.32 (1H, dd, *J* = 3.29 Hz, H-4), 5.04 (1H, dd, *J* = 3.0, 9.8 Hz, H-3), 4.47 (1H, d, *J* = 9.6 Hz, H-1), 4.11 (1H, dd, *J* = 9.0, 13.1 Hz, H-6a), 3.97–3.91 (2H, m, H-5, H-6b), 3.90 (3H, s, OMe), 2.15, 2.01, 1.98, 1.92 (12H, 4s, $\underline{CH_3}CO$); ^{13}C NMR ($CDCl_3$) δ (ppm): 170.2, 169.9, 169.7, 169.5 ($4CH_3\underline{CO}$), 164.5 (C-*para*), 132.9 (C-*ortho*), 125.9 (C-*ipso*), 113.9 (C-*meta*), 89.1 (C-1), 74.6 (C-5), 71.5 (C-3), 66.5 (C-4), 64.0 (C-2), 60.9 (C-6), 55.7 (OMe), 20.8, 20.6, 20.5, 20.3 ($4\underline{CH_3}CO$); ESI-MS calcd for $C_{21}H_{26}O_{12}S$ + (Na^+): 525.1; found: 525.3; ESI^+-HRMS: $[M + NH_4]^+$ calcd for $C_{21}H_{30}NO_{12}S$: 520.1483; found: 520.1482, Δ −0.15 ppm.

2-(4-(β-D-Galactopyranosylthiomethyl)-1*H*-1,2,3-triazol-1-yl)-acetate (**9**) was obtained as a yellow oil (81%) and had $[\alpha]_D^{25}$: −133.0 (*c* = 1, H_2O); ^1H NMR (CD_3OD) δ (ppm): 7.89 (1H, s, $H_{triazole}$), 5.18 (1H, s, $\underline{CH_2}CO_2CH_3$), 4.15 (1H, d, *J* = 9.6 Hz, H-1), 3.91 (2H, dd, *J* = 14.5, 61.8 Hz, $S\underline{CH_2}$), 3.73 (1H, d, *J* = 3.0 Hz, H-4), 3.67 (3H, s, $CH_2CO_2\underline{CH_3}$), 3.65–3.28 (5H, m, H-2, H-3, H-5, H-6a, H-6b); ^{13}C NMR (D_2O) δ (ppm): 173.1 ($\underline{CO}CH_3$), 144.9 ($C_{triazole}$), 125.4 ($CH_{triazole}$), 85.0 (C-1), 79.0 (C-5), 73.9 (C-3), 69.5 (C-4), 68.8 (C-2), 61.1 (C-6, $\underline{CH_2}CO_2Me$), 53.0 ($CO\underline{CH_3}$), 23.6 ($S\underline{CH_2}$).

tert-Butyl 2-(4-(β-D-galactopyranosylthiomethyl)-1*H*-1,2,3-triazol-1-yl)acetate (**9b**). To a solution of prop-2-ynyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside[23] (1.0 mmol) in THF (10 ml) was added CuI (0.1 mmol), DIPEA (2.0 mmol) and *tert*-butyl 2-azidoacetate (3 mmol). The reaction mixture was stirred at room temperature and stopped after 3 hours as judged by TLC. After this time, the mixture was evaporated, diluted with ethyl acetate, filtered through a pad of Celite and washed with a solution of HCl (10%). The organic solution was dried over Na_2SO_4, filtered and concentrated under reduce pressure. The crude residue was purified by silica gel column chromatography using a mixture of ethyl acetate and hexane (1 : 1) as eluent. Compound **9b** was obtained as a yellow oil (83%) and had $[\alpha]_D^{25}$: −66.5 (*c* = 1, $CHCl_3$); IR: 1757 cm^{-1} (s, C=O); ^1H NMR ($CDCl_3$) δ (ppm): 7.63 (1H, s, $H_{triazole}$), 5.36 (1H, dd, *J* = 3.3 Hz, H-4), 5.18 (1H, dd, *J* = 9.9 Hz, H-2), 5.12 (2H, s, $\underline{CH_2}CO_2t$-Bu), 4.97 (1H, dd, *J* = 3.3, 9.9 Hz, H-3), 4.52 (1H, d, *J* = 9.9 Hz, H-1), 4.14–3.98 (2H, m, H-6a, H-6b, $S\underline{CH_2}$), 3.92–3.84 (1H, m, H-5), 2.09, 1.97, 1.96, 1.90 (12H, 4s, $\underline{CH_3}CO$), 1.37 (9H, s, $CH_2CO_2C(\underline{CH_3})_3$); ^{13}C NMR ($CDCl_3$) δ (ppm): 169.9, 169.8, 169.5, 169.2 ($4CH_3\underline{CO}$), 165.0 ($CH_2\underline{CO_2}t$-Bu), 144.4 ($C_{triazole}$), 123.5 ($CH_{triazole}$), 83.3 (C-1), 82.7 ($\underline{C}(CH_3)_3$), 74.0 (C-5), 71.4 (C-3), 67.0 (C-4), 66.8 (C-2), 61.0 (C-6), 51.1 ($\underline{CH_2}CO_2t$-Bu), 29.3 ($S\underline{CH_2}$), 27.6 ($C(\underline{CH_3})_3$), 20.6, 20.4, 20.3 ($4\underline{CH_3}CO$); ESI^+-MS: $[M + Na]^+$ calcd for $C_{23}H_{33}N_3O_{11}SNa$: 582.17280; found: 582.17.

3-(2-Furan)-5-(1-deoxy-β-D-galactopyranosyl)-isoxazole (**14**) was obtained quantitatively from **14b** under Zemplén conditions as a clear oil and had ^1H NMR (CD_3OD) δ (ppm): 7.65 (1H, d, *J* = 1.6 Hz, H_{pyran}), 6.95 (1H, d, *J* = 3.6 Hz, H_{pyran}), 6.78 (1H, s, $H_{isoxazole}$), 6.57–6.56 (1H, m, H_{pyran}), 4.81 (2H, s, $O\underline{CH_2}$), 4.33 (1H, d, *J* = 7.7 Hz, H-1), 3.81–3.70 (2H, m, H-2, H-4), 3.54–3.32 (4H, m, H-3, H-5, H-6a, H-6b); ^{13}C NMR (CD_3OD) δ (ppm): 171.7, 164.5, 154.7, 144.5, 111.6, 110.7, 103.2 ($3C_{isoxazole}$, $4C_{pyrane}$), 100.8 (C-1), 75.7, 73.7, 71.2, 69.1, 61.3, 60.9 (C-2 to C-6, OCH_2); ESI-MS calcd for $C_{14}H_{17}NO_8$ + (Na^+): 350.3; found: 350.0; ESI^+-MS: $[M + Na]^+$ calcd for $C_{14}H_{17}NO_8Na$: 350.08464; found: 350.0.

3-(2-Furan)-5-(1-deoxy-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-isoxazole (**14b**). To a mixture a acetylenic galactoside[30] (0.89 mmol, 346 mg), NCS (1.43 mmol, 191 mg) and pyridine (1.34 mmol, 108 mg) in $CHCl_3$ (5 ml) was added dropwise 2-furan oxime (0.89 mmol, 99 mg) at 0 °C, then the reaction was stirred over night at room temperature. After this time the organic solution was washed with saturated $NaHCO_3$, brine and water, dried over Na_2SO_4, filtered and concentrated under reduce pressure. Flash chromatography using ethyl acetate: hexane (1 : 1) afforded pure **14b** (0.65 mmol, 324 mg) in 73% yield as a yellow oil and had $[\alpha]_D^{25}$: −1.1 (*c* = 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$) δ (ppm): 7.55 (1H, d, *J* = 1.2 Hz, H_{pyran}), 6.89 (1H, d, *J* = 2.3, H_{pyran}), 6.52–6.49 (1H, m, H_{pyran}), 6.46 (1H, s, $H_{isoxazole}$), 5.41 (1H, dd, *J* = 3.0 Hz, H-4), 5.24 (1H, dd, *J* = 9.8 Hz, H-2), 5.02 (1H, dd, *J* = 9.8, 3.3 Hz, H-3), 4.56 (1H, d, *J* = 9.6 Hz, H-1), 4.18–4.08 (3H, m, H-6a, H-6b, $O\underline{CH_2}$), 3.97, 3.90 (1H, m, H-5), 2.17, 2.04, 2.02, 1.96 ($4\underline{CH_3}CO$); ^{13}C NMR ($CDCl_3$) δ (ppm): 170.3, 170.1, 169.8, 169.6, 169.2, 154.8, 143.8, 111.7, 110.2 ($4CH_3\underline{CO}$, $3C_{isoxazole}$, $4C_{pyran}$); 100.4 (C-1), 82.7, 74.6, 71.5, 67.1, 66.9, 61.4 (C-2 to C-6, $O\underline{CH_2}$), 23.9, 20.6, 20.5, 20.4 ($4\underline{CH_3}CO$); ESI-MS calcd for $C_{22}H_{25}NO_{12}$ + (H^+): 496.4; found: 496.0; ESI^+-MS: $[M + Na]^+$ calcd for $C_{22}H_{26}NO_{12}$: 496.14495; found: 496.0.

2-(4-(β-D-Galactopyranosylthiomethyl)-1*H*-1,2,3-triazol-1-yl)-acetic acid (**15**). To a solution of **9** (1.0 mmol) in wet MeOH (5ml) was added LiOH (30 mg) and the reaction was stirred until disappearance of the starting materials as seen by TLC. After this time, Amberlite resin (H^+) was added until neutral pH and the mixture was filtered and concentrated under reduce pressure to give **15** as colorless oil in quantitative yield. ^1H NMR (D_2O) δ (ppm): 7.40 (1H, s, $H_{triazole}$), 7.73–4.47 (m, 5H, H-1, $\underline{CH_2}COOH$, $S\underline{CH_2}$), 3.72–2.88 (m, 6H, H-2, H-3, H-4, H-5, H-6a, H-6b); ^{13}C NMR (D_2O) δ (ppm): 173.3 ($\underline{COOH}$), 144.8 ($C_{triazole}$), 125.2 ($CH_{triazole}$), 86.6 (C-1), 80.1 (C-5), 76.2 (C-3), 70.4 (C-4), 69.6 (C-2), 61.2 (C-6, $\underline{CH_2}COOH$), 23.2 ($S\underline{CH_2}$); ESI^+-MS: $[M + Na]^+$ calcd for $C_{11}H_{17}N_3O_7SNa$: 358.06794; found: 358.07.

p-Bromophenyl 1-thio-β-D-lactoside (**17**) was obtained from acetobromolactose and *p*-bromothiophenol using the general PTC conditions described above (88%) followed by de-*O*-acetylation (quant.) as a white powder and had mp 207.0–208.0 °C; $[\alpha]_D^{25}$: −38.1 (*c* = 1, MeOH); IR: 3337 cm^{-1} (s, O-H); ^1H NMR (CD_3OD) δ (ppm): 7.36 (4H, m, H_{AR}), 4.49 (1H, d, *J* = 9.8 Hz, H-1′), 4.25 (1H, d, *J* = 7.5 Hz, H-1), 3.78–3.59 (6H, m, H-2, H-2′, H-3, H-3′, H-4′, H-6a), 3.51–3.21 (6H, m, H-4, H-5, H-5′, H-6a′, H-6b, H-6b′); ^{13}C NMR (CD_3OD) δ (ppm): 134.8 (C-*meta*), 134.2 (C-*ipso*), 132.9 (C-*ortho*), 122.4 (C-*para*), 104.9 (C-1′), 88.7 (C-1), 80.5 (C-4), 80.0 (C-3), 77.95 (C-5), 77.1 (C-2), 74.8 (C-3′), 73.3 (C-5′), 72.5 (C-2′), 70.3 (C-4′), 62.5 (C-6), 61.9 (C-6′); ESI-MS calcd for $C_{18}H_{25}O_{10}BrS(Na^+)$: 535.0; found: 535.2; ESI$^+$-HRMS: $[M + Na]^+$ calcd for $C_{18}H_{25}BrO_{10}SNa$: 535.0244; 537.0226; found: 535.0245; 537.0227, Δ 0.08 ppm.

p-Methoxyphenyl 1-thio-β-D-lactoside (**18**) was obtained from acetobromolactose and *p*-methoxythiophenol using the general PTC conditions described above (98%) followed by quantitative Zemplén deacetylation as a white powder and had mp 167.5–169.5 °C; $[\alpha]_D^{25}$: −24.3 (*c* = 1, MeOH); IR: 3373 cm^{-1} (s, O-H), 1246 cm^{-1} (s, C-O-Me); ^{1}H NMR (CD_3OD) δ (ppm): 7.42 (2H, d, *J* = 8.7 Hz, H-*ortho*), 6.75 (2H, d, *J* = 8.7 Hz, H-*meta*), 4.30 (1H, d, *J* = 9.6 Hz, H-1′), 4.22 (1H, d, *J* = 7.4 Hz, H-1), 3.79–3.50 (6H, m, H-2, H-2′, H-3, H-3′, H-4′, H-6a), 3.67 (3H, s, OMe), 3.46–3.04 (6H, m, H-4, H-5, H-5′, H-6a′, H-6b, H-6b′); ^{13}C NMR (CD_3OD) δ (ppm): 161.4 (C-*para*), 136.7 (C-*ortho*), 124.0 (C-*ipso*), 115.3 (C-*meta*), 104.9 (C-1′), 89.6 (C-1), 80.5 (C-4), 80.1 (C-3), 77.9 (C-5), 77.0 (C-2), 74.7 (C-3′), 73.1 (C-5′), 72.5 (C-2′), 70.3 (C-4′), 62.5 (C-6′), 62.0 (C-6), 55.7 (OMe); ESI-MS calcd for $C_{19}H_{28}O_{11}S(Na^+)$: 487.1; found: 487.3; ESI$^+$-HRMS: $[M + Na]^+$ calcd for $C_{19}H_{28}O_{11}SNa$: 487.1245; found: 487.1252, Δ −0.42 ppm.

β-Naphthyl 1-thio-β-D-lactoside (**19**)[27] was obtained as a white powder (93%) and had mp 217.5–218.2 °C; $[\alpha]_D^{25}$: −26.9 (*c* = 1, DMSO); IR: 3363 cm^{-1} (s, O-H); ^{1}H NMR (CD_3OD) δ (ppm): 8.08 (1H, s, H_{AR}), 7.80 (3H, m, H_{AR}), 7.64 (1H, d, *J* = 6.8 Hz, H_{AR}), 7.47 (2H, t, *J* = 3.8 Hz, H_{AR}), 4.73 (1H, d, *J* = 9.8 Hz, H-1′), 4.35 (1H, d, *J* = 7.1 Hz, H-1), 3.96–3.79 (2H, m, H-3, H-4′), 3.77–3.65 (4H, m, H-2′, H-2, H-3′, H-6a), 3.58–3.46 (6H, m, H-4, H-5, H-5′, H-6a′, H-6b, H-6b′); ^{13}C NMR (CD_3OD) δ (ppm): 131.7, 127.7, 130.4, 128.6, 128.5, 127.5, 127.2 ($10C_{AR}$), 104.9 (C-1′), 89.0 (C-1), 80.6 (C-4), 80.1 (C-3), 78.0 (C-5), 77.1 (C-2), 74.8 (C-3′), 73.4 (C-5′), 72.5 (C-2′), 70.3 (C-4′), 62.5 (C-6′), 62.0 (C-6); ESI-MS calcd for $C_{22}H_{28}O_{10}S\ (Na^+)$: 507.1; found: 507.3; ESI$^+$-HRMS: $[M + Na]^+$ calcd for $C_{22}H_{28}O_{10}SNa$: 507.1295; found: 507.1305, Δ 1.2 ppm.

p-Bromophenyl 1-sulfonyl-β-D-lactoside (**22**) was obtained from **17** using *m*CPBA as above (98%) as a white powder and had mp 174.5–175.5 °C; $[\alpha]_D^{25}$: −9.5 (*c* = 1, MeOH); IR: 3305 cm^{-1} (s, O-H); ^{1}H NMR (CD_3OD) δ (ppm): 7.76 (2H, d, *J* = 8.5 Hz, H-*ortho*), 7.68 (2H, d, *J* = 8.5 Hz, H-*meta*), 4.32 (1H, d, *J* = 8.7 Hz, H-1), 4.20 (1H, d, *J* = 7.1 Hz, H-1′), 3.67–3.31 (12H, m, H-2, H-2′, H-3, H-3′, H-4′, H-4′, H-5, H-5′, H-6a, H-6a′, H-6b, H-6b′); ^{13}C NMR (CD_3OD) δ (ppm): 137.3 (C-*ipso*), 133.3 (C-*meta*), 132.6 (C-*ortho*), 130.4 (C-*para*), 104.8 (C-1′), 92.7 (C-1), 81.1 (C-4), 78.9 (C-3), 77.3 (C-5), 77.1 (C-2), 74.7 (C-3′), 72.4 (C-5′), 71.1 (C-2′), 70.3 (C-4′), 62.5 (C-6), 61.3 (C-6′); ESI-MS calcd for $C_{18}H_{25}O_{12}BrS(NH_4^+)$: 562.0; found: 562.2; ESI$^+$-HRMS: $[M + Na]^+$ calcd for $C_{18}H_{25}BrO_{12}SNa$: 567.0142; 569.0124; found: 567.0145; 569.0124, Δ −0.47 ppm.

2-Benzothiazolyl 1-thio-β-D-lactoside (**23**) was obtained from acetobromolactose and 2-benzothiazole using the general PTC conditions described above (98%) followed by Zemplén deacetylation (77%) and was isolated as a white solid and had mp 95–96 °C; $[\alpha]_D^{25}$: −12 (*c* = 0.5, DMSO); ^{1}H NMR (DMSO) δ (ppm): 7.45 (1H, d, *J* = 6.8 Hz, H_{AR}), 7.29–7.17 (3H, m, H_{AR}), 4.57 (1H, d, *J* = 5.5 Hz, H-1′), 4.25 (1H, d, *J* = 7.4 Hz, H-1), 3.76–3.34 (12H, m, H-2 to H-6, H-2′ to H-6′); ^{13}C NMR (DMSO) δ (ppm): 164.2, 153.7, 137.0, 127.7, 126.4, 122.9, 122.4 ($7C_{AR}$), 105.1, 101.2 (C-1, C-1′), 80.8, 77.0, 74.8, 73.5, 73.2, 72.5, 71.8, 70.3, 62.4, 61.8 (C-2 to C-6, C-2′ to C-6′). ESI$^+$-HRMS: $[M + H]^+$ calcd for $C_{19}H_{26}NO_{10}S_2$: 492.0993; found: 492.0999, Δ 1.11 ppm.

Compound **26b**. To a solution of lactoside **20** in MeOH was added Bu_2SnO, Bu_4NI and propargyl bromide. The mixture was stirred under reflux for 7 hours, and then concentrated under reduced pressure. To the crude yellow residue dissolved in pyridine was added Ac_2O dropwise. The mixture was stirred 16 hours at room temperature and concentrated under reduced pressure. Flash chromatography using ethyl acetate: hexanes (3 : 2) afforded compound **20a**. This compound showed satisfactory physical properties and was converted into compound **26b**, in 91% yield, following the same procedure as for the synthesis of compound **14b**. Compound **26b** was isolated in a yellow oil and had $[\alpha]_D^{25}$: −0.3 (*c* = 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.12 (2H, d, *J* = 9.1 Hz, H_{AR}), 7.78–7.75 (2H, m, H_{AR}), 7.53 (2H, d, *J* = 8.8 Hz, H_{AR}), 7.45–7.43 (3H, m, H_{AR}), 6.51 (1H, s, $H_{isoxazole}$), 5.44 (1H, d, *J* = 3.0 Hz), 5.23 (1H, dd, *J* = 8.7 Hz), 5.03–4.91 (2H, m), 4.83 (1H, d, *J* = 10.1 Hz), 4.73 (1H, m), 4.57–4.40 (3H, m), 4.11 (1H, d, *J* = 6.3 Hz), 3.83–3.59 (6H, m), 2.15, 2.08, 2.06, 2.05, 2.02 ($6\underline{C}\underline{H}_3CO$); ^{13}C NMR ($CDCl_3$) δ (ppm): 170.6, 170.5, 170.3, 169.7, 169.4, 168.9 ($6CH_3\underline{C}\underline{O}$), 162.6 ($C_{isoxazole}$), 147.0, 142.2, 130.9, 130.4, 129.2, 128.8, 126.9, 124.0 ($12C_{AR}$), 101.7, 101.3 (C-1, C-1′), 84.1, 77.1, 76.8, 76.2, 73.8, 70.9, 70.4, 70.1, 65.3, 62.4, 62.3, 61.3, 21.0, 20.9, 20.8 ($6\underline{C}H_3CO$); ESI-MS calcd for $C_{40}H_{44}N_2O_{19}S(Na^+)$: 911.82; found: 911.22.

Compound **26** was obtained by de-*O*-acetylation of **26b** as a yellow powder and had mp 220–221 °C; $[\alpha]_D^{25}$: −45.2 (*c* = 0.8, DMSO); ^{1}H NMR (DMSO) δ (ppm): 8.06 (2H, d, *J* = 7.9 Hz, H_{AR}), 7.78 (2H, m, H_{AR}), 7.56 (2H, d, *J* = 8.2 Hz, H_{AR}), 7.44–7.03 (3H, m, H_{AR}), 4.95 (1H, d, *J* = 9.6 Hz, H-1′), 4.75 (1H, d, *J* = 8.5 Hz, H-1), 4.74–4.70 (2H, m, $O\underline{C}\underline{H}_2$), 3.87–3.28 (12H, m, H-2 to H-6, H-2′ to H-6′); ^{13}C NMR (DMSO) δ (ppm): 171.3, 162.3 ($2C_{isoxazole}$), 146.4, 145.5, 130.8, 129.8, 129.2, 128.5, 127.2, 127.1, 124.4, 124.3 ($10C_{AR}$), 104.1, 102.1 (C-1, C-1′), 85.2, 82.2, 80.2, 79.5, 76.8, 76.0, 72.8, 70.2, 65.3, 62.4, 60.8, 60.7 (C-2 to C-6, C-2′ to C-6′, $CH_{isoxazole}$, $O\underline{C}H_2$); ESI$^+$-MS: $[M + Na]^+$ calcd for $C_{28}H_{32}N_2O_{13}SNa$: 659.15173; found: 659.2.

Compound **26b** (to be published elsewhere) was obtained as a yellow oil and had $[\alpha]_D^{25}$: −0.3 (*c* = 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.12 (2H, d, *J* = 9.1 Hz, H_{AR}), 7.78–7.75 (2H, m, H_{AR}), 7.53 (2H, d, *J* = 8.8 Hz, H_{AR}), 7.45–7.43 (3H, m, H_{AR}), 6.51 (1H, s, $H_{isoxazole}$), 5.44 (1H, d, *J* = 3.0 Hz), 5.23 (1H, dd, *J* = 8.7 Hz), 5.03–4.91 (2H, m), 4.83 (1H, d, *J* = 10.1 Hz), 4.73 (1H, m), 4.57–4.40 (3H, m), 4.11 (1H, d, *J* = 6.3 Hz), 3.83–3.59 (6H, m), 2.15, 2.08, 2.06, 2.05, 2.02 ($6\underline{C}\underline{H}_3CO$); ^{13}C NMR ($CDCl_3$) δ (ppm): 170.6, 170.5, 170.3, 169.7, 169.4, 168.9 ($6CH_3\underline{C}\underline{O}$), 162.6 ($C_{isoxazole}$), 147.0, 142.2, 130.9, 130.4, 129.2, 128.8, 126.9, 124.0 ($12C_{AR}$), 101.7, 101.3 (C-1, C-1′), 84.1, 77.1, 76.8, 76.2, 73.8, 70.9, 70.4, 70.1, 65.3, 62.4, 62.3, 61.3, 21.0, 20.9, 20.8 ($6\underline{C}H_3CO$); ESI$^+$-MS: $[M + Na]^+$ calcd for $C_{40}H_{44}N_2O_{19}SNa$: 911.21512; found: 911.22.

Lectins

Purification of the plant toxin and the human lectins (galectins-1, -3, -4 and -9) started with extracts of dried leaves and pellets from bacteria harboring expression vector for the respective galectin and involved affinity chromatography on lactosylated Sepharose 4B, obtained after activation with divinyl sulfone, as crucial step.[3a,31] Purity controls by one- and two-dimensional gel electrophoresis and mass spectrometry by nanoESI-MS/MS on a QTOF instrument Q-Tof2 (Waters Micromass, Manchester, UK), determination of the quaternary structure by gel filtration on a Superose 12 HR 10/30 column (GE Healthcare, Munich, Germany) and activity assays in 2-fold serial dilutions with trypsinized, glutaraldehyde-fixed rabbit erythrocytes ensured the lectins' quality.[3a,32] Biotinylation was performed under activity-preserving conditions with the *N*-hydroxysuccinimide ester derivative of biotin (Sigma, Munich, Germany) followed by gel electrophoretic/mass spectrometric product analysis.[33]

Solid-phase inhibition assay. The neoglycoprotein with average ligand density of 26 sugar units per carrier protein (bovine serum albumin) was prepared using the *p*-isothiocyanatophenyl derivative and adsorbed to the surface of microtiter plate wells from a solution in 20 mM phosphate-buffered saline (PBS) (routinely using a total of 0.25 μg in 50 μl) at 4 °C overnight. Residual sites for binding proteins were saturated with albumin free of contaminating glycoproteins (Biomol, Hamburg, Germany; 100 μl, 1% w/v) for 1 h at 37 °C. Lectin-containing solution (routinely using 1.5 μg ml^{-1} VAA, 20 μg ml^{-1} galectin-1, 15 μg ml^{-1} galectins-3 and -9 and 3 μg ml^{-1} galectin-4 based on thorough titrations) in the absence (control) or presence of test compound (at up to eight different concentrations) was incubated in the processed microtiter plate wells for 1 h at 37 °C, and extent of bound lectin was quantitated spectrophotometrically at 490 nm. In detail, solutions of the indicator conjugate streptavidin-peroxidase (0.5 μg ml^{-1}; Sigma) and the signal-generating substrates *o*-phenylenediamine (1 mg ml^{-1})/H_2O_2 (1 μl ml^{-1}) were stepwisely applied after thorough washes. Assays were routinely done in triplicates for up to four independent series with standard deviations not exceeding 15.4%.

Isothermal titration microcalorimetry. These experiments were performed with purified galectins using a VP-ITC instrument from Microcal, Inc. (Northampton, USA) as described.[13] In detail, injections of 4 μl of solutions containing the synthetic were added from a computer-controlled microsyringe at an interval of 4 min into the sample solution containing the galectin (cell volume = 1.43 ml) with stirring at 350 rpm. Control experiments were performed by making identical injections of the carbohydrate derivative into a cell containing buffer. Titrations were done at pH 7.2 using PBS. The experimental data were fitted to a theoretical titration curve using software supplied by Microcal, with ΔH (binding enthalpy kcal mol^{-1}), K_a (association constant) and n (number of binding sites per monomer), as adjustable parameters. The instrument was calibrated using the calibration kit containing ribonuclease A (RNase A) and cytidine 2′-monophosphate supplied by the manufacturer. Thermodynamic parameters were calculated from the Gibbs Free Energy equation, $\Delta G = \Delta H - T\Delta S = -RT \ln K_a$, where ΔG, ΔH and ΔS are the changes in free energy, enthalpy, and entropy of binding, respectively. T is the absolute temperature and $R = 1.98$ cal mol^{-1} K^{-1}.

Cell-binding inhibition assay. Cells of the human colon adenocarcinoma line SW480, the B-lymphoblastoid line Croco II and the pancreatic carcinoma line Capan-1 reconstituted for expression of the tumor suppressor $\text{p16}^{\text{INK4a}}$ (kindly provided by K. M. Detjen, Berlin, Germany) were grown and processed as described, using a 30 min incubation step for labeling and fluorescent streptavidin/*R*-phycoerythrin (1 : 40; Sigma) as indicator in automated FACScan analysis, data processing yielding the percentage of positive cells and the mean fluorescence intensity.[3a] Controls included omission of the incubation step with labeled lectin and application of non-cognate sugar to detect any osmolarity effect. Assays were performed in triplicates with up to four independent series using aliquots of cell suspensions of the same or the next passage with standard deviations not exceeding 12% after normalization of data.

Cytotoxicity assay. Extent of cell proliferation in the absence (control) or presence of test compounds was measured after a period of 48 h using the blue chromogen 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (5 mg ml^{-1}; Sigma) as indicator for spectrophotometric determination. Assays were performed in triplicates (nine samples for the control) with aliquots of a cell suspension and three independent series at standard deviations of less than 14%.

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