Teaming up synthetic chemistry and histochemistry for activity screening in galectin-directed inhibitor design

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Abstract A hallmark of endogenous lectins is their ability to select a few distinct glycoconjugates as counterreceptors for functional pairing from the natural abundance of cellular glycoproteins and glycolipids. As consequence, assays to assess inhibition of lectin binding should necessarily come as close as possible to the physiological situation, to characterize an impact of a synthetic compound on biorelevant binding with pharmaceutical perspective. We here introduce in a proof-of-principle manner work with sections of paraffin-embedded, fixed tissue (jejunum, epididymis) and labeled adhesion/growth-regulatory galectins, harboring one (galectins-1 and -3) or two (galectin-8) types of lectin domain. Six pairs of synthetic lactosides from tailoring of the headgroup (3'-O-sulfation) and the aglycone (β-methyl to aromatic S- and O-linked extensions) as well as three bi- to tetravalent glycoclusters were used as test compounds. Varying extents of reduction of staining intensity by synthetic compounds relative to unsubstituted/free lactose proved the applicability and sensitivity of the method. Flanking cytofluorimetric assays on lectin binding to native cells gave similar grading, excluding a major impact of fixation. The experiments revealed cell/tissue binding of galectin-8 preferentially via one domain, depending on the cell type so that the effect of an inhibitor in a certain context cannot be extrapolated to other cells/tissues. Moreover, the work with the other galectins attests that this assay enables comprehensive analysis of the galectin network in serial sections to determine overlaps and regional differences in inhibitory profiles.

Keywords Agglutinin · Glycocluster · Glycosylation · Glycoprotein · Lectin

Introduction

The glycan part of cellular glyoconjugates is increasingly considered to harbor physiologically relevant information (Gabius 2009, 2015; Gabius and Roth 2017). In this sense, the molecular characterization of its complexity and of the dynamics of regulation is a step toward elucidating the range of implied functions (Kopitz 2009, 2017; Zuber and Roth 2009; Corfield and Berry, 2015; Hennet and Cabalzar 2015; Ledeen and Wu 2015; Schengrund 2015; Colley 2017; Corfield 2017). Toward this end, the application of plant/fungal lectins for glycan profiling has considerably advanced our status of knowledge on glycan localization and sites of glycosylation (Roth 1978, 2011; Roth et al. 1983; Danguy et al. 1994; Manning et al. 2017). Although such work with a heterologous combination (plant lectin/mammalian tissue) is mostly of descriptive merit and cannot predict respective reactivity for tissue lectins, as recently emphasized (Topin et al. 2016), the broadly successful mapping with these tools provided the incentive to proceed to introduce tissue lectins to cyto- and histochemistry (for information on animal lectin classes, structure and functions, please see Solís et al. 2015; Colley 2017; Kaltner 2017; Lepenies 2017; Manning 2017). When done, this approach offers the potential to examine characteristics of the functional pairing *in situ* and strategies to block disease-associated binding.

A key finding from biochemical analysis of cellular binding partners of tissue lectins is that only a few glycoconjugates qualify as counterreceptors: when faced with the abundance of cell glycans, endogenous lectins have a particular selectivity among the diversity of glycoconjugates, engaging few distinct counterreceptors to complete their mission (Gabius et al. 2015, 2016; for examples and signaling routes triggered by this interaction, please see Kaltner et al. 2017). In addition to the structure of the target glycan(s), not yet fully delineated topological parameters of their presentation on the scaffold (glycoprotein, glycolipid or a complex thereof) or in a microdomain appear to matter (Murphy et al. 2013; Roy et al. 2016). As first critically discussed in the case of the selectins, common assay systems "can give a positive result that may not be relevant to the natural situation" (Varki 1994) so that the question of identifying the "real" ligands becomes highly relevant (Varki 1997). This said, cultured cells with their natural display of glycans are coming into focus as platform to examine lectin binding and then the potency of synthetic compounds to interfere with a functional pairing. In the quest of taking this testing even closer to the level of the in vivo situation, sections of tissues deserve attention and efforts. After all, the actual microenvironment is known to have a profound influence on cell properties. Having recently documented the graded capacity of bi- to tetravalent glycoclusters to reduce extent of binding

of two plant lectins to tissue sections (André et al. 2016), we here introduce this assay concept to human galectins and three types of synthetic compounds, i.e. free glycans with headgroup modification/aglyconic extension and glycoclusters. The tandem-repeat-type galectin-8 (Gal-8) is a challenging test case.

Structurally, a linker peptide connects the two different carbohydrate recognition domains (CRDs) of Gal-8 (Hadari et al. 1997; Zick et al. 2004). They have high affinity for either 3'-sialylated/sulfated lactosides (Gal-8N) or histo-blood group A/B epitopes (Gal-8C) and recognize N-acetyllactosamine (LacNAc) in high-density presentation, both CRDs being involved in letting the lectin associate to cells (Hirabayashi et al. 2002; Ideo et al. 2003, 2011; Carlsson et al. 2007; Stowell et al. 2008; Vokhmyanina et al. 2012; Yoshida et al. 2012; Ruiz et al. 2014, 2015). Thus, the individual contributions of each CRD to a binding process require to be elucidated in each case. Equally interesting with respect to the ligand side, binding 3'-sulfated lactose is also documented for galectins-1, -3 and -4 (Gals-1, -3, and -4) (Allen et al. 1998; Ideo et al. 2002). Shared reactivity, indeed, is of physiological relevance. The synthetic preparation of sulfated lactosides was conducive to delineate the cooperation of Gals-1 and -8 in differentiating B cells into plasma cells via blocking assays, illustrating functional overlap within the galectin network (Tsai et al. 2011; Tu et al. 2013). Such a cooperation (or also antagonism) is possible for other processes involving galectins such as pathogenesis of osteoarthritis or tumor growth regulation (Kopitz et al. 2001; Sanchez-Ruderisch et al. 2010; Toegel et al. 2014).

This collective evidence prompted us to give emphasis in this pilot study to Gal-8 and also consider Gals-1 and -3. In detail, we explored comparatively the effect of aglyconic extension of lactosides without/with 3'-O-sulfation on their ligand properties to these human galectins, first by screening in a solid-phase assay, then for selected cases in cell assays. The question whether and how staining of tissue sections by labeled Gal-8 is affected by such derivatives was then addressed next. Finally, three bi- to tetravalent glycoclusters, known to reduce Gal-1 and -3 binding in solid-phase and cell assays (Wang et al. 2012), were tested in the histochemical setting.

Materials and methods

General synthetic methods

All reactions in organic medium were performed in standard oven dried glassware under an inert atmosphere of nitrogen using freshly distilled solvents. Solvents and reagents were deoxygenated, when necessary by purging with nitrogen. All reagents were used as supplied without prior purification unless

otherwise stated, and obtained from Sigma-Aldrich Chemical Co. Ltd. Reactions were monitored by analytical thin-layer chromatography (TLC) using silica gel 60 F254 precoated plates (E. Merck) and compounds were visualized with a 254 nm UV lamp, a mixture of iodine/silica gel and/or mixture of ceric ammonium molybdate solution (100 mL H_2SO_4, 900 mL H_2O, 25 g $(NH_4)_6Mo_7O_{24}H_2O$, 10 g $Ce(SO_4)_2$) and subsequent spots development by gentle warming with a heat-gun. Purifications were performed by silica gel flash column chromatography using Silicycle (60 Å, 40-63 µm) with the indicated eluent. NMR spectroscopy was used to record ^{1}H NMR and ^{13}C NMR spectra at 300 or 600 MHz and at 75 or 150 MHz, respectively, on Bruker (300 MHz) and Bruker Avance III HD 600 MHz spectrometers. Proton and carbon chemical shifts (δ) are reported in ppm relative to the chemical shift of residual $CHCl_3$, which was set at 7.26 ppm (^{1}H) and 77.16 ppm (^{13}C). Coupling constants (*J*) are reported in Hertz (Hz), and the following abbreviations are used for peak multiplicities: singlet (s), doublet (d), doublet of doublets (dd), doublet of doublet with equal coupling constants (t_{ap}), triplet (t), multiplet (m). Analysis and assignments were made using COSY (COrrelated SpectroscopY) and HSQC (Heteronuclear Single Quantum Coherence) experiments. High-resolution mass spectrometry (HRMS) data were measured with a LC-MS-TOF (Liquid Chromatography-Mass Spectrometry-Time Of Flight; Agilent Technologies) in positive and/or negative electrospray mode(s) at the analytical platform of UQAM.

Synthesis of the ligand panel

General synthetic procedure A: phase-Transfer Catalysis (PTC) reaction. PTC reactions were performed following the previously established protocols (Roy 1997; Roy et al. 1997; Tropper et al. 1991, 1992a, b; Cao et al. 1994; Carrière et al. 2000) or under the slightly modified procedure as follows: to a solution of peracetylated lactosyl bromide **1** (Scheme 1; Shiao et al. 2014) (1 equiv.) in dichloromethane (6 mL) was added the corresponding aromatic alcohol (1 equiv.), tetrabutylammonium bromide (TBAB, 0.5 equiv.) and KOH (1M, 6 equiv.). The mixture was stirred at room temperature for two h 30 min and then washed successively with water and brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by silica gel column chromatography (Hex/AcOEt: 7/3) afforded the corresponding compounds **6-9** as yellow oil (yield 70-81%).

General synthetic procedure B: Zemplén transesterification reaction. To a solution of lactosides **6-9** in dry methanol was added a solution of sodium methoxide (1 M in MeOH, 0.1 equiv.). After stirring at room temperature for 1 h, the reaction was completed and then neutralized by addition of ion-exchange resin (Amberlite IR 120 H^+). The solution was filtered and evaporated *in vacuo* to afford the de-*O*-acetylated lactosides as white powders (yield 95%-quant.)

General synthetic procedure C: preparation of 3'-*O*-sulfated lactosides. A mixture of deacetylated lactosides (1 equiv.) and dibutyltin oxide (1.15 equiv.) in DMF/toluene (6 mL/3 mL) was stirred at 90 °C for six h. The solution was then concentrated and sulfur trioxide-trimethylamine complex ($Me_3N{\cdot}SO_3$) (1.3 equiv.) and dry DMF (6 mL) were added. After stirring at room temperature for 17 h, the reaction was quenched with water and evaporated under vacuum. The residue was purified through a column of DOWEX Marathon C (Na^+) and eluted with H_2O to obtain the pure 3'-*O*-sulfated lactosides as white powder after lyophilization (yields 80-84%).

2,3,6,2′,3′,4′,6′-Hepta-*O*-acetyl-β-D-lactopyranosyl bromide (1)

To a solution of per-*O*-acetylated lactose (Shiao et al. 2014) (14.2 g, 21 mmol) in anhydrous CH_2Cl_2 (63 mL) was added hydrobromic acid (33% in AcOH, 47.9 mL). The reaction mixture was stirred at room temperature for 1 h, then neutralized with saturated aqueous $NaHCO_3$ and washed with brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give lactosyl bromide **1** (13.6 g, 93%) as a white solid. Its spectrosopic data agreed well with those of the literature (Tropper at al. 1991; Šardzík et al. 2010; Shiao et al. 2014).

Methyl β-D-lactopyranoside (2)

To a solution of lactosyl bromide **1** (350 mg, 0.5 mmol, 1 equiv.) in dry methanol (10 mL) was added dropwise a solution of sodium methoxide (1 M in MeOH, 50 μL, 0.1 equiv.) during 10 min. After stirring at room temperature for one h, the reaction was completed and then neutralized by addition of ion-exchange resin (Amberlite IR 120 H^+). The solution was filtered and evaporated *in vacuo* to give methyl lactoside **2** (154 mg, 87%) as a white powder. R_f 0.33 (CH_2Cl_2/MeOH: 7/3). Spectrosopic data agrees with those described previously (Koto et al. 2004).

Methyl 3′-*O*-sulfo-β-D-lactopyranoside, sodium salt (3)

Following the general procedure C, compound **3** was obtained as a white powder; yield: 108 mg (80%), R_f 0.25 (CH_2Cl_2/MeOH: 7/3). ^{1}H NMR (300 MHz, MeOD): δ 4.53 (d, 1H, J = 7.77 Hz, H-1), 4.27 (m, 1H, H-3'), 4.26 (d, 1H, J = 7.83 Hz, H-1'), 4.24 (m, 1H, H-4'), 3.93 (m, 2H, H-6), 3.82-3.69 (m, 5H, H-6',2,2',5'), 3.64-3.61 (m, 1H, H-4), 3.59-3.56 (m, 1H, H-3), 3.55 (s, 3H, OMe), 3.47-3.44 (m, 1H, H-5). ^{13}C NMR (75 MHz, D_2O): δ 103.8 (C-1'), 103.6 (C-1), 80.2 (C-3'), 79.6 (C-4), 75.3 (C-5'), 75.0 (C-5), 74.9 (C-3), 73.3 and 69.4 (C-2,2'), 67.2 (C-4'), 61.1 (C-6'), 60.5 (C-6), 56.0 (OMe). ESI-HRMS: *m/z* calcd for $C_{13}H_{24}O_{14}S$, 436.0814; found 435.0811 $[M-H]^-$.

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl azide 4a. To a solution of 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-acetyl-α-D-glucopyranosyl bromide **1** (515 mg, 0.736 mmol, 1.0 equiv.) in ethyl acetate (1.0 mL/100 mg of sugar, 5.2 mL) was added sodium azide (NaN_3, 239 mg, 3.68 mmol, 5.0 equiv.), tetrabutylammonium hydrogen sulfate (TBAHS, 255 mg, 0.736 mmol, 1.0 equiv.) and a saturated aqueous solution of Na_2CO_3 (1.0

mL/100 mg of sugar, 5.2 mL). The mixture was vigorously stirred for two hours until disappearance of the starting material, as judged by TLC (hexane-EtOAc 2:3). The organic layer was separated, washed with a saturated aqueous $NaHCO_3$ solution (3 x 15 mL), H_2O (15 mL), brine (15 mL), then dried over Na_2SO_4, filtered and concentrated under reduced pressure. The white solid crude product was then purified by crystallization in a mixture of EtOH and petroleum ether at -20 ºC for 16 hours. The white solid was filtered and dried under vacuum to give the title compound **4a** (OAc) (453 mg, 93%). R_f = 0.34 hexanes/EtOAc (1:1); mp 73.5-75.0 ºC (EtOH- petroleum ether); $[\alpha]_D$ -19.4 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.33 (dd, 1H, $J_{3,4} = J_{4,5}$ 3.4 Hz, H-4'), 5.19 (dd, 1H, $J_{2,3} = J_{3,4}$ 9.3 Hz, H-3), 5.09 (dd, 1H, $J_{1,2}$ 7.9 Hz, $J_{2,3}$ 10.4 Hz, H-2'), 4.94 (dd, 1H, H-3'), 4.84 (dd, 1H, $J_{1,2}$ = 8.9 Hz, $J_{2,3}$ 9.4 Hz, H-2), 4.62 (d, 1H, H-1), 4.49 (dd, 1H, $J_{6a,6b}$ 12.1 Hz, $J_{5,6a}$ 2.1 Hz, H-6a), 4.47 (d, 1H, H-1'), 4.13-4.047 (m, 3H, H-6ª', H-6b and H-6b'), 3.86 (m, 1H, H-5'), 3.77 (dd, 1H, $J_{4,5}$ 9.2 Hz, H-4), 3.69 (ddd, 1H, $J_{5,6a}$ 2.1 Hz, , $J_{5,6b}$ 5.1 Hz, H-5), 2.13, 2.12, 2.06, 2.05, 2.03, 2.03 and 1.95 ppm (7s, 21H, $COCH_3$); ^{13}C NMR ($CDCl_3$) δ 170.3, 170.2, 170.1, 170.0, 169.6, 169.4, 169.0 (CO), 101.0 (C-1'), 87.6 (C-1), 75.7 (C-4), 74.7 (C-5), 72.4 (C-3), 70.9 (C-2), 70.9 (C-3'), 70.7 (C-5'), 69.0 (C-2'), 66.5 (C-4'), 61.7 (C-6), 60.7 (C-6'), 20.7, 20.7, 20.6, 20.6, 20.5, 20.5 and 20.4 ppm ($COCH_3$). IR ν_{max} ($CHCl_3$) cm^{-1}: 2120*s* (N_3). ESI-MS: $[M+NH_4]^+$ calcd for $C_{26}H_{39}N_4O_{17}$, 679.2305 ; found, 679.2316. The spectroscopic data agreed with those published (Tropper et al. **1992a**)

β-D-Lactopyranosyl azide **(4b)** was synthetized according to a literature procedure followed by the Zemplén transesterification procedure described above (procedure B) (Shiao et al. **2014**; Pintal et al. **2015**).

3′-*O*-Sulfo-β-D-lactopyranosyl azide, sodium salt (5)

Following the general procedure C, compound **5** was obtained as a white powder, ^{1}H NMR (600 MHz, D_2O): δ 4.77 (d, 1H, *J* = 8.8 Hz), 4.56 (d, 1H *J* = 7.8 Hz), 4.33 (ddd, 1H, *J* = 9.9, 3.3, 0.5 Hz), 4.28 (d, 1H, *J* = 3.3 Hz), 3.98 (dd, 1H, *J* = 12.3, 1.9 Hz), 3.84 (dd, 1H, *J* = 12.4, 4.6 Hz), 3.82-3.74 (m, 3H), 3.74-3.65 (m, 4H), 3.31 (t, 1H, *J* = 9.1 Hz). ^{13}C NMR (150 MHz, D_2O): δ 103.1, 90.6, 80.6, 8.3, 77.3, 75.6, 75.0, 73.2, 69.7, 67.5, 61.6, 60.5. ESI-HRMS: *m/z* calcd for $C_{12}H_{21}N_3O_{13}S$, 447.0722; found 446.0718 $[M-H]^-$.

2-Naphthyl 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-D-lactopyranoside (6)

Following the general procedure A, compound **6** was obtained as a yellow oil; yield: 556 mg (73%), R_f 0.5 (Hex/EtOAc: 1/1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.74-7.64 (m, 3H, Ar), 7.40-7.32 (m, 2H, Ar), 7.26 (d, 1H, Ar), 7.12-7.08 (dd, 1H, Ar), 5.37-5.36 (m, 1H, H-4'), 5.33-5.27 (m, 1H, H-3), 5.27-5.18 (m, 2H), 5.0-5.96 (dd, 1H), 4.55-4.52 (m, 2H), 4.20-4.07 (m, 3H), 3.96-3.85 (m, 3H), 2.17-1.98 (21H, 7OAc). Spectroscopic data agrees with those of the literature (Johnsson et al. 2005).

2-Naphthyl 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-1-thio-β-D-lactopyranoside (7)

Following the general procedure A, compound **7** was obtained as a yellow oil; yield: 650 mg (83%), R_f 0.52 (Hex/EtOAc: 1/1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.95 (d, 1H, *J* = 1.2 Hz, Ar), 7.81-7.73 (m, 3H, Ar), 7.53-7.45 (m, 3H, Ar), 5.31-5.28 (m, 1H, H-4'), 5.21 (t, 1H, H-3), 5.10-5.04 (dd, 1H, H-2'), 4.95-4.93 (m, 1H, H-3'), 4.90 (t, 1H, H-2), 4.75 (d, 1H, *J* = 10.0 Hz, H-1), 4.54-4.49 (m, 1H, H-6a), 4.47 (d, 1H, *J* = 7.8 Hz, H-1'), 4.12-4.10 (m, 1H, H-6b), 4.08-4.04 (m, 2H, H-6'ab), 3.85 (t, 1H, *J* = 6.7 Hz, H-5'), 3.72 (t, 1H, H-4), 3.69-3.65 (m, 1H, H-5), 2.13-1.93 (21H, 7OAc). Spectrocopic data agrees with those previously reported (Rodrigue et al. 2013).

4-Methylumbelliferyl 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-D-lactopyranoside (8)

This compound was prepared as already described in the literature (Wang et al. 1998).

Methyl 3-methoxy-4-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-D-lactopyranosyloxy)benzoate (9)

Following the general procedure A, compound **9** was obtained as a yellow oil; yield: 573 mg (71%), R_f 0.24 (Hex/EtOAc: 45/55). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.76-7.50 (m, 2H, Ar), 7.10 (d, 1H, *J* = 8.3 Hz, Ar), 5.38-5.37 (m, 1H, H-4'), 5.37-5.28 (m, 1H, H-3), 5.25-5.20 (m, 1H, H-2), 5.17-5.12 (m, 1H, H-2'), 5.05 (d, 1H, *J* = 7.4 Hz, H-1), 4.99-4.96 (m, 1H, H-3'), 4.52-4.51 (m, 2H, H-1',6a), 4.20-4.08 (m, 3H, H-6b, 6'ab), 3.94-3.88 (m, 2H, H-4, 5'), 3.92 (s, 3H, CO_2Me), 3.88 (s, 3H, OMe), 3.80-3.75 (m, 1H, H-5), 2.18 (s, 3H), 2.18-1.99 (21H, 7OAc). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.3, 170.2, 170.1, 170.0, 169.7, 169.5, 169.1, 166.5, 150.0, 149.8, 126.0, 122.9, 117.9, 113.4, 101.1 (C-1'), 99.6 (C-1), 76.1 (C-4), 72.9 (C-5), 72.4 (C-3), 71.3 (C-2), 70.9 (C-3'), 70.7 (C-5'), 69.0 (C-2'), 66.6 (C-4'), 61.9 (C-6), 60.8 (C-6'), 56.1 (OMe), 52.2 (CO_2Me), 20.8, 20.6, 20.5. ESI-HRMS: *m/z* calcd for $C_{35}H_{44}O_{21}$, 800.2375; found 818.2675 $[M+NH_4]^+$.

2-Naphthyl β-D-lactopyranoside (10)

Following the general procedure B, compound **10** was obtained as a white powder; yield: 209 mg (95%), R_f 0.55 (CH_2Cl_2/MeOH: 7/3). ^{1}H NMR (300 MHz, MeOD): δ 7.82-7.78 (m, 3H, Ar), 7.50-7.29 (m, 4H, Ar), 5.14 (d, 1H, *J* = 7.62 Hz, H-1), 4.45 (d, 1H, *J* = 7.5 Hz, H-1'), 4.02-3.90 (m, 2H, *J* = 10.86 Hz, H-6'), 3.86 (m, 1H, H-4'), 3.84-3.75 (m, 2H, *J* = 11.4 Hz, H-6), 3.73-3.71 (m, 3H, H-3,4,5'), 3.66 (m, 1H, *J* = 7.62 Hz, H-2), 3.64 (m, 1H, H-5), 3.60 (m, 1H, *J* = 7.5 Hz, H-2'), 3.55-3.50 (m, 1H, H-3'). ^{13}C NMR (75 MHz, MeOD): δ 155.3 (Cq Ar), 134.5 (Cq Ar), 129.9 (Cq Ar), 128.9, 127.2, 126.7, 125.9, 123.8, 118.5, 110.6, 103.6 (C-1'), 100.7 (C-1), 78.8 (C-5'), 75.7 (C-5), 75.3 and 74.9 (C-3,4), 73.4 (C-3'), 73.2 (C-2), 71.1 (C-2'), 68.9 (C-4'), 61.1 (C-6), 60.3 (C-6'). ESI-HRMS: *m/z* calcd for $C_{22}H_{28}O_{11}$, 468.1632; found 468.1608.

2-Naphthyl 3′-*O*-sulfo-β-D-lactopyranoside, sodium salt (11)

Following the general procedure C, compound **11** was obtained as a white powder; yield: 136 mg (80%), R_f 0.48 (CH_2Cl_2/MeOH: 7/3). ^{1}H NMR (300 MHz, D_2O): δ 7.85-7.77 (m, 3H, Ar), 7.5-7.42 (m, 3H, Ar), 7.27 (d, 1H, *J* = 9 Hz, Ar), 5.15 (d, 1H, *J* = 7.41 Hz, H-1), 4.51 (d, 1H, *J* = 7.7 Hz, H-1'), 4.28 (dd, 1H,

J = 3.3, 9.7 Hz, H-3'), 4.22 (m, 1H, *J* = 3.3 Hz, H-4'), 3.95 (d, 1H, *J* = 11.58 Hz, H-6a), 3.75 (d, 1H, *J* = 11.58 Hz, H-6b), 3.71-3.66 (m, 6H, H-3,4,5,5',6'ab), 3.64 (dd, 1H, *J* = 7.7, 9.7 Hz, H-2'), 3.59 (m, 1H, H-2). ^{13}C NMR (75 MHz, D_2O): δ 154.3 (Cq Ar), 133.8 (Cq Ar), 129.9, 129.7 (Cq Ar), 127.7, 127.1, 126.9, 124.9, 118.5, 110.7, 102.5 (C-1'), 99.9 (C-1), 80.0 (C-3'), 78.0 (C-5), 74.9 (C-3,5'), 74.1 (C-4), 72.6 (C-2), 69.1 (C-2'), 66.8 (C-4'), 60.8 (C-6'), 60.0 (C-6). ESI-HRMS: *m/z* calcd for $C_{22}H_{28}O_{14}S$, 548.1216; found 547.1144 $[M-H]^-$.

2-Naphthyl 1-thio-β-D-lactopyranoside (12)

Following the general procedure B, compound **12** was obtained as a white powder; yield: 401 mg (quant.), R_f 0.57 (CH_2Cl_2/MeOH: 7/3). ^{1}H NMR (300 MHz, MeOD): δ 8.10 (s, 1H, Ar), 7.85-7.80 (m, 3H, Ar), 7.68-7.65 (m, 1H, Ar), 7.53-7.48 (m, 2H, Ar), 4.76 (d, 1H, *J* = 9.0 Hz, H-1), 4.39 (d, 1H, *J* = 7.38 Hz, H-1'), 3.99-3.85 (m, 2H, H-6'), 3.82 (m, 1H, H-4'), 3.79-3.67 (m, 2H, H-6), 3.61-3.59 (m, 3H, H-3,4,5), 3.57 (m, 1H, H-2'), 3.53 (m, 1H, H-5'), 3.51-3.47 (m, 1H, H-3'), 3.37-3.36 (m, 1H, H-2). ^{13}C NMR (75 MHz, MeOD): δ 132.6 (Cq Ar), 130.9 (Cq Ar), 130.3 129.1 (Cq Ar), 129.0, 127.8, 127.2, 127.1, 126.1, 125.8, 103.5 (C-1'), 87.6 (C-1), 79.2 (C-5'), 78.7 (C-5), 76.6 and 75.7 (C-3,4), 73.4 (C-3'), 72.0 (C-2), 71.1 (C-2'), 68.9 (C-4'), 61.1 (C-6), 60.6 (C-6'). ESI-HRMS: *m/z* calcd for $C_{22}H_{28}O_{10}S$, 484.1403; found 484.1446.

2-Naphthyl 3′-*O*-sulfo-1-thio-β-D-lactopyranoside, sodium salt (13)

Following the general procedure C, compound **13** was obtained as a white powder; yield: 119 mg (84%), R_f 0.5 (CH_2Cl_2/MeOH: 7/3). ^{1}H NMR (300 MHz, D_2O): δ 7.92 (s, 1H, Ar), 7.79-7.72 (m, 3H, Ar), 7.5-7.4 (m, 3H, Ar), 4.74 (d, 1H, *J* = 8.82 Hz, H-1), 4.42 (d, 1H, *J* = 7.83 Hz, H-1'), 4.23 (dd, 1H, *J* = 3.2, 9.66 Hz, H-3'), 4.19 (t, 1H, *J* = 3.2 Hz, H-4'), 3.85 (d, 1H, *J* = 11.88 Hz, H-6a), 3.74 (d, 1H, *J* = 11.88 Hz, H-6b), 3.66 (s, 2H, H-6'ab), 3.63 (m, 1H, H-5'), 3.61 (d, 1H, *J* = 7.83 Hz, H-2'), 3.57-3.54 (m, 2H, H-3,4), 3.47 (s, 1H, H-5), 3.35 (m, 1H, H-2). ^{13}C NMR (75 MHz, D_2O): δ 133.2, 132.1, 130.2, 129.5, 128.7, 127.6, 127.4, 126.9, 126.7, 102.46 (C-1'), 87.0 (C-1), 80.0 (C-3'), 78.6 (C-5), 77.9 (C-3), 75.7 (C-4), 74.8 (C-5'), 71.5 (C-2), 69.0 (C-2'), 66.8 (C-4'), 60.8 (C-6'), 60.0 (C-6). ESI-HRMS: *m/z* calcd for $C_{22}H_{28}O_{13}S_2$, 564.0982; found 563.0909 $[M-H]^-$.

4-Methylumbelliferyl β-D-lactopyranoside (14)

Following the general procedure B, compound **14** was obtained as a white powder; ^{1}H NMR (600 MHz, DMSO-d_6+D_2O): δ 7.83- 7.52 (m, 1H), 7.17- 6.89 (m, 2H), 6.23 (d, 1H, *J* = 5.8 Hz), 5.12 (d, 1H, *J* = 7.8 Hz), 4.23 (d, 1H, *J* = 7.2 Hz), 3.82-3.44 (m, 8H), 3.41 (t, 1H, *J* = 9.1 Hz), 3.36-3.29 (m, 3H), 2.39 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.7, 160.3, 154.7, 154.0, 126.9, 114.6, 113.8, 112.0, 104.0, 103.5, 99.8, 80.1, 75.8, 75.3, 74.8, 73.3, 73.0, 70.8, 68.4, 60.8, 60.2, 18.5. ESI-HRMS: *m/z* calcd for $C_{22}H_{28}O_{13}$ 500.1530; found 523.1413 $[M+Na]^+$. Spectroscopic data agreed with those described in the literature (Wang et al. **1996**).

4-Methylumbelliferyl 3′-*O*-sulfo-β-D-lactopyranoside, sodium salt (15)

Following the general procedure C, compound **15** was obtained as a white powder; ^{1}H NMR (600 MHz, D_2O): δ 7.64 (d, 1H, *J* = 8.8 Hz), 7.16-6.88 (m, 2H), 6.17 (s, 1H), 5.21 (d, 1H, *J* = 7.7 Hz), 4.61 (d, 1H, *J* = 7.8 Hz), 4.35 (dd, 1H, *J* = 9.9, 3.2 Hz), 4.30 (d, 1H, *J* = 3.3 Hz), 4.08-3.98 (m, 1H), 3.94-3.74 (m, 7H), 3.70 (m, 2H), 2.35 (s, 3H). ^{13}C NMR (150 MHz, D_2O+MeOD): δ 165.4, 160.3, 157.1, 154.8, 127.6, 116.2, 114.8, 112.2, 104.5, 103.5, 100.5, 81.0, 78.8, 76.0, 75.9, 75.1, 73.4, 70.1, 67.8, 61.9, 60.7, 18.9. ESI-HRMS: *m/z* calcd for $C_{22}H_{28}O_{16}S$ 580.1171; found 581.1166 $[M+H]^+$. Spectroscopic data agreed with those described in the literature (Wang et al. **1998**).

Methyl 3-methoxy-4-(β-D-lactopyranosyloxy)benzoate (16)

Following the general procedure B, compound **16** was obtained as a white powder; yield: 283 mg (quant.), R_f 0.26 (CH_2Cl_2/MeOH: 75/25). ^{1}H NMR (300 MHz, D2O+DMSO-d_6): δ 7.76-7.74 (m, 2H, Ar), 7.31 (d, 1H, *J* = 9.2 Hz, Ar), 5.32 (d, 1H, *J* = 7.7 Hz, H-1), 4.53 (d, 1H, *J* = 7.4 Hz, H-1'), 4.07-3.90 (m, 9H, H-4',6',OMe and CO_2Me), 3.90-3.69 (m, 6H, H-3,4,5,5',6), 3.66 (m, 2H, H-2,3'), 3.55 (m, 1H, H-2'). ^{13}C NMR (75 MHz, D2O+DMSO-d_6): δ 157.3 (Cq Ar), 150.2 (Cq Ar), 148.5 (Cq Ar), 123.7, 114.8, 112.8, 103.4 (C-1'), 99.3 (C-1), 78.8; 75.6; 75.1 and 74.5 (C-3,4,5,5'), 72.9 (C-3'), 72.7 (C-2), 70.9 (C-2'), 68.5 (C-4'), 61.0 (C-6'), 60.0 (C-6), 56.1 (OMe), 52.7 (CO_2Me). ESI-HRMS: *m/z* calcd for $C_{21}H_{30}O_{14}$, 506.1635; found 529.1521 $[M+Na]^+$.

Methyl 3-methoxy-4-(3′-*O*-sulfo-β-D-lactopyranosyloxy)benzoate, sodium salt (17)

Following the general procedure C, compound **17** was obtained as a white powder; yield: 45 mg (81%), R_f 0.12 (CH_2Cl_2/MeOH: 75/25). ^{1}H NMR (300 MHz, MeOD): δ 7.65 (m, 2H, Ar), 7.26 (d, 1H, Ar), 5.13 (d, 1H, *J* = 7.7 Hz, H-1), 4.56 (d, 1H, *J* = 7.8 Hz, H-1'), 4.37-4.18 (m, 2H, H-3',4'), 3.98-3.87 (m, 8H, H-6,OMe and CO_2Me), 3.85-3.77 (m, 2H, H-6'), 3.75 (m, 1H, H-2'), 3.73-3.71 (m, 1H, H-4), 3.70-3.61 (m, 3H, H-3,5,5'), 3.61-3.57 (m, 1H, H-2). ^{13}C NMR (75 MHz, MeOD): δ 156.8 (Cq Ar), 150.6 (Cq Ar), 149.1 (Cq Ar), 123.1, 115.0, 112.7, 103.5 (C-1'), 100.2 (C-1), 80.3 (C-3'), 79.0 (C-4), 75.4 and 75.3 and 74.7 (C-3,5,5'), 73.0 (C-2), 69.5 (C-2'), 67.1 (C-4'), 61.0 (C-6'), 60.1 (C-6), 55.3 (OMe), 51.2 (CO_2Me). ESI-HRMS: *m/z* calcd for $C_{21}H_{30}O_{17}S$, 586.1204; found 585.1130 $[M-H]^-$.

Glycoclusters

The bi-, tri- and tetravalent glycoclusters with lactose (**18**,**19**) or 2-fucosyllactose (**20**) as headgroup (Scheme 2) were prepared using copper-catalyzed azide alkyne cycloaddition as described (Wang et al. 2012).

Galectins

Human galectins and their separate CRDs were purified after recombinant production by affinity chromatography as key step and rigorously checked for purity as described (André et al. 2008, 2014; Giguère et al. 2011). Labeling under activity-preserving conditions was carried out with the N-hydroxysuccinimide ester derivative of biotin (Sigma) or the fluorescent dyes fluorescein isothiocyanate or Alexa Fluor® 488 (Invitrogen, Darmstadt, Germany).

Solid-phase and cell assays

Binding of labeled galectins in the absence or presence of test compounds to surface-presented asialofetuin was monitored spectrophotometrically as described (André et al. 2008, 2014). Cytofluorimetric analysis of galectin binding to Chinese hamster ovary (CHO) wild-type (Pro^{-5}) as well as pancreatic (Capan-1) and colon (SW480) adenocarcinoma cells was performed as described (André et al. 2003, 2008; Amano et al. 2012). Experimental series with the synthetic test compounds and lactose as reference were routinely performed for each cell line with aliquots of the same cell suspension in parallel. Controls included monitoring of viability and assessment of lectin-independent staining. The quantitative data are expressed in the terms of percentage of positive cells/mean fluorescence intensity.

Galectin histochemistry

Tissue specimen of jejunum and epididymis of four six-week-old C57BL/6 mice were processed to obtain sections (about 5 µm) as described (André et al. 2016). Following saturation of sites for non-specific binding of proteins and systematic titrations to identify a concentration of biotinylated galectin yielding a strong signal with minimal background to be able to discern quantitative differences in the inhibition studies, incubation, visualization of bound probe, semi-quantitative grading of staining and recording of microphotographs were carried out as described (Kaltner et al. 2015, 2016; André et al. 2016)

Results and discussion

Design of the test compounds

Carbohydrate chemistry offers three possibilities to tune affinity and selectivity of lectin ligands, i.e. the structure of the headgroup including physiological and bio-inspired substitutions, the characteristics of the aglycone (spacer) and the properties of glycoclusters including valency and topology of ligand presentation (Roy et al. 2016). Our panel of test compounds covers examples for each case, starting from lactose as reference. The monovalent lactosides are arranged as pairs of unsubstituted and 3'-O-sulfated lactose (Scheme 1). Introduction of β-linked extensions from methyl and azide to four types of O- and S-conjugated aromatic substituents yielded a total of six pairs of lactosides (Scheme 1). Lactose as part of bi- and tetravalent glycoclusters (**18**, **19**) and 2'-fucosyllactose as part of a trivalent glycocluster **20** (Scheme 2) have already proven potent as inhibitor for Gal-3 in solid-phase and cell assays (Wang et al. 2012), thus serving here as internal control for the validity of the histochemical procedure.

The synthesis of the first part of the panel followed a strategy to combine deprotection of peracetylated glycosyl halide with inversion of the anomeric configuration of the halide under classical Zemplén transesterification conditions (NaOMe, MeOH) (Wang and Lee 1995). In detail, treatment of the known peracetylated lactoside bromide (**1**) (Shiao et al. 2014) under the conditions given above provided, directly and in one step, methyl lactoside **2** in 87% yield (Scheme 1). The 3'-O-sulfation was based on the regioselective enhancement of hydroxyl group nucleophilicity using well-established tin chemistry (Wang et al. 1996). Treatment of **2** with dibutyltin oxide followed by quenching of the labile tin acetal led to the desired 3'-O-sulfated methyl lactoside **3** in 80% yield. By stereoselective introduction of the aglycones under PTC conditions the synthesis of the six pairs shown in Scheme 1 was completed. For instance, peracetylated lactosyl azide **4a** and aryl derivatives **6-9** were obtained from peracetylated lactosyl bromide **1** by S_N2-type reaction conditions using our standard procedures. Compounds **4a** and **6-9** were obtained in yields ranging from 71 to 95%. All compounds were fully characterized by ^{1}H-NMR spectroscopy, mass spectrometry, and the data sets agreed with literature data when known (see experimental section). The peracetylated intermediates were de-*O*-acetylated under the Zemplén conditions described above in essentially quantitative yields in all cases to give free lactosides **10**, **12**, **14**, and **16**. Following sequential treatment of the polyols with the dibutyltin oxide reagent and sulfation with the commercially available sulfur trioxide-trimethylamine complex ($Me_3N{\cdot}SO_3$) the 3'-O-sulfated lactosides **11, 13, 15,** and **17** were obtained in good to excellent yields (Scheme 1). Positioning of the sulfate group was readily detectable on the basis of the ^{13}C-NMR chemical shift displacement of the 3'-carbon, which usually appeared 7 ppm downfield (~ δ 80 ppm) from the hydroxylated precursors at δ ~ 73 ppm.

Inhibition of galectin binding to glycoprotein/cells

Asialofetuin was selected as matrix in solid-phase assays, because the LacNAc termini of its three complex-type N-glycans are potent ligands for human galectins (Dam et al. 2005). As indicated previously for lactosides with aglyconic extensions (Giguère et al. 2011), extent of carbohydrate-dependent binding of labeled human Gal-8 to this surface-presented glycoprotein was responsive to both structural modifications in the compound panel of monovalent lactosides in solid-phase assays. The presence of aromatic aglycones increased the inhibitory capacity of the β-methyl derivative markedly in the enhancements, as reported previously for Gal-8N and spacered conjugation with fluorescein (Carlsson et al. 2007) and also for full-length Gal-8 and an isoxazole derivative (Giguère et al. 2011). The sensitivity of the reactivity was independent of the length of the linker peptide between the two CRDs arising from alternative splicing and resides mostly in the N-terminal CRD, when testing Gal-8N and -8C in parallel. 3'-*O*-Sulfation further enhanced extent of inhibition, as expected based on the respective literature listed in the introduction. Comparatively reduced but still notable extents of inhibition were observed for Gals-1 and -3, as expected. These results using an assay of a model glycoprotein adsorbed to a plastic surface thus ascertained activity of the compounds for galectin binding, competing with LacNAc, and confirmed the so far collected evidence on Gals-8, -1 and -3. Because Gal-8 binding to cells can involve both domains to varying extents by engaging different types of glycans from surface glycomes, we proceeded to assess the impact of presence of test compounds on galectin binding to cultured cells by cytofluorimetry.

The CHO wild-type line was tested as representative of a cell surface presenting α2,3-sialylated N-glycans, to which the N-terminal domain of Gal-8 binds well in a carbohydrate-dependent manner (Kaltner et al. 2009). Consequently, lactose (at 2 mM) gave a modest inhibition, which was enhanced by aglycone presence (**10** tested at 0.5 mM) (Fig. 1a). The 3'-sulfated compound **11** (at 0.1 mM) of this pair surpassed that level of inhibition (Fig. 1a). In order to examine inhibitory capacity of Gal-8 binding, when the degree of sialylation is reduced, we turned to a respective cell model, i.e. a pancreatic adenocarcinoma line with a tumor-suppressor-dependent decrease in this property (André et al. 2007; Amano et al. 2012).

In this case, surface staining of cells by Gal-8, too, was strong. Its sensitivity to presence of inhibitors with aglyconic extension and to 3'-O-sulfation, however, was reduced (Fig. 1b). This result indicates that a shift to involvement of the C-terminal domain in this situation is likely. Evidently, the susceptibility of inhibition of Gal-8 binding to cells can depend on the actual glycome profile of the target cells, which determines preferences for contact via the N- or the C-terminal domain. In contrast to the heterobivalent Gal-8, Gals-1 and -3 have only a single type of CRD in each case so that a synthetic compound will not face the problem to compete with the cellular glycan(s) at two structurally different lectin sites. Corroborating literature and solid-phase assay data, stepwise increase of inhibitory activity

by aglyconic extension and subsequent 3'-O-sulfation could be obtained, benzoate presence along with 3'-O-sulfation proving less favorable than other aromatic extensions (Fig. 1c-f). These data reveal that the presence of different CRDs in a galectin (here tandem-repeat-type Gal-8) can make the response to an inhibitor in the context of cell surface binding hardly predictable and that a cross-reactivity of the synthetic lactosides with other galectins is still possible. In order to be able to measure the effect of an inhibitor on galectin binding in the context of a tissue we proceeded to analyzing galectin-dependent staining in sections.

Inhibition of galectin binding to tissue sections

Building on the previous experience with two plant lectins and sections of murine jejunum/epididymis (André et al. 2016), probe-independent staining was rigorously excluded and a galectin concentration resulting in strong staining at low background was defined by systematic titrations in each case, i.e. Gal-8 at 0.5 µg/ml, Gals-1 and -3 at 1 µg/ml. Because generation of the signals could then be at least drastically impaired by high concentrations of lactose, as in the other assay types, direct comparisons between the reference with lactose and data obtained for each test compound in titrations were possible. To document this internal reference, the effect of presence of lactose in increasing concentrations on staining distribution and intensity in sections of jejunum is presented in Fig. 2a-d. The overall assessment by semiquantitative evaluation of the processed specimen (i.e. by grading staining into the categories from ++++ to -; for details, please see legend to Fig. 2) is given in the bottom left part of each microphotograph. Compound **11** (Fig. 2e-h) and also compound **13** (Fig. 2i-l) were more effective than lactose, as illustrated for compound **11** in Fig. 1a in cytofluorimetric analysis of cells with high-level α2,3-sialylation. Reinforcing the importance of the type of cell shown in Fig. 1a,b , processing of sections of epididymis yielded a different picture: lactose proved to be more active than compound **11** (Online Resource, Fig. 1). As with cells in culture (Fig. 1a,b), the activity profile of an inhibitor, mostly acting on the N-terminal domain, in this assay is governed by the nature of the contact sites in the tissue for tandem-repeat-type Gal-8.

When tested for Gal-3, the effect of lactose was evident (Fig. 3a,b). Compounds **16** and **17** were active in a markedly (**16**) or slightly better manner (**17**) (Fig. 3c,d). As in these two cases and Gal-3 (Fig. 1d,f), correspondence between cytofluorimetry and histochemistry data was revealed for Gal-1, too, when tested at identical concentration with Gal-1, compounds **14** and **15** proved to be more potent than lactose (Fig. 1c, Fig. 3e-h). For these two galectins, i.e. Gals-1 and -3, the presence of a single type of CRD makes inhibitory capacity less dependent on cell type than for the tandem-repeat-type protein. Having tested monovalent lactosides, we next explored the inhibitory activity of the glycoclusters **18-**

20, in one case with structural extension from lactose (**18**, **19**) to its 2-fucosyl derivative (**20**), the histo-blood group H (type II) trisaccharide.

Gal-3 is known to form aggregates upon complex formation with multivalent ligands due to intermolecular contacts via its N-terminal tail and CRD (Ahmad et al. 2004; Kopitz et al. 2014; Ippel et al. 2016). The presentation of ligands in spatial vicinity can thus convey strong inhibitory potency to glycoclusters, as shown in solid-phase and cell assay for compounds **18**-**20** (Wang et al. 2012). Because the degree of N-glycan branching of the tested glycoprotein can affect glycocluster efficiency (André et al. 2009), a monitoring in tissue sections can answer the question on relative inhibitory capacity. These three glycoclusters were thus tested in the histochemical assay. Staining profiles of sections of murine jejunum by labeled Gal-3 obtained by systematic titrations were examined comparatively and presented here for the concentrations of at 0.25 mM (Fig. 4a-d) and 0.5 mM (Fig. 4e-h) of lactose (free for conjugated). Compared to the effect of free sugar (Fig. 4a,e), the presence of the bivalent (**18** with lactose; Fig. 4b,f), trivalent (**20** with 2'-fucosyllactose; Fig. 4c,g) and tetravalent (**19** with lactose; Fig. 4d,h) glycoclusters reduced staining. Increase in valency and structural complexity led to increased inhibitory capacity, in full agreement to the solid-phase and cell assay data reported previously (Wang et al. 2012). Interestingly, staining by Gal-1 in the same setting was also very susceptible to inhibition in sections of jejunum (Fig. 4i-p). Although the homologues of Gal-1 tested previously were less responsive than Gal-3 in solid-phase assays (Wang et al. 2012), these results (please see Fig. 4m-p) advise caution and the avoidance of simple extrapolations when moving from an assay with a glycoprotein to that with tissue sections.

Conclusions and perspectives

The emerging wealth of data on the broad range of functions of endogenous lectins, as summarized in Table 5 in Manning et al. 2017, is teeming with information that could inspire novel ideas for a more thorough understanding of cell biological systems and routes for drug design, as indicated in this issue's introduction (Gabius and Roth 2017). In order to fulfill their missions these lectins appear to select distinct glycoconjugates as their physiological counterreceptors. When acting as effectors on different cell types, the biochemical nature of these target sites in terms of scaffold (protein or lipid) or glycan structure can differ between various cells. Also, due to their cooperation in a network (Kaltner et al. 2017), the response profile to a synthetic reagent may in vivo be complex. This situation calls for an assay that comes as close as possible to the natural profiles of glycans to assess the capacity of such a compound as physiologically relevant inhibitor in different cells/tissues and its range of side reactivities in the lectin network.

Toward this end, we have tested the hypothesis that lectin histochemistry with labeled human galectins and synthetic sugar derivatives/glycoclusters will be helpful. Compared to cells adapted to growth in culture, tissue sections present cells in their microenvironment, along with the extracellular matrix and stroma, albeit after tissue fixation and processing. As illustrated previously (Dawson et al. 2013; Kaltner et al. 2015, 2017), intracellular binding of the probes, too, can then be mapped in the same section and susceptibility of galectin binding at different sites to presence of synthetic compounds evaluated, and this on the level of the lectin network in serial sections. Viewed from the perspective of drug design, the resulting data can give incentive to iterative refinements of the structure of the test compound.

The presented evidence validates the hypothesis of the assay's applicability for human galectins. Especially the test case of the tandem-repeat-type Gal-8 highlights its practical value by revealing strong dependence of inhibitor activity on the tissue type. Obviously, caution needs to be exercised when considering extrapolations. The presence of the two different CRDs of Gal-8 equips this protein with capacity to let each CRD target certain counterreceptors, e.g. promatrix metalloproteinase-9 (Gal-8N) and CD11b (MAC-1, α_M-integrin) (Gal-8C) in the case of neutrophils (Nishi et al. 2003). On the grounds of differences in glycan reactivity between the two CRDs (Ideo et al. 2003; Carlsson et al. 2007; Stowell et al. 2008), our respective data attest that the inhibitory capacity of a blocking reagent against Gal-8 with preferential activity to one type of CRD cannot be simply calculated for a certain cell/tissue type, unless information on features of in situ contact partners are available. Equally important, the aspect of requirement of a network analysis is emphasized by revealing that inhibitors can interfere with binding of more than one galectin, a source of undesired side effects when envisioning the development of a galectin-based therapy (Smetana et al. 2013). Changing the backbone to type 1 LacNAc of a sulfated disaccharide and altering the site of sulfation to the 6-position of GlcNAc, for example, could reduce such side reactivity to Gal-1, while still exerting strong effects on Gal-8 (Carlsson et al. 2007; Tu et al. 2013). A switch back to 3'-O-sulfation and substituting GlcNAc by GalNAc to prepare sulfated CD176 (T(F) antigen) will direct respective compounds to Gal-4 (Ideo et al. 2002), the translator of glycan-encoded signals in apical and axonal transport of glycoproteins (Stechly et al. 2009; Velasco et al. 2013; Abad-Rodriguez 2017). Because galectins mediate cell adhesion and migration, for Gal-8 with potential biomedical relevance (Levy et al. 2001; Nagy et al. 2002; Friedel et al. 2016), the emerging possibility to program glycan presentation on surfaces of biomimetic vesicles (glycodendrimersomes) will make versatile test models for respective assays with such glycotopes available (Percec et al. 2013; Zhang et al. 2014, 2015a). Due to the feasibility of designing structural variants of galectins, e.g. by modular conjugations or transplantations (Vértesy et al. 2015; Zhang et al. 2015b; Ludwig et al. 2016), the lectin histochemical assay can complement structure-activity investigations, by studying the staining profiles

comparatively and by letting custom-made test compounds help characterize the molecular nature of binding sites.

In sum, using labeled tissue lectins as tools in histochemistry enables the mapping of profiles of accessible binding sites in sections, as reviewed by Kaltner et al. 2017. By maintaining aspects of the natural glycan complexity and mode of presentation in sections, this experimental platform has advantages relative to artificial systems, tissue processing and presence of lectins in situ notwithstanding.

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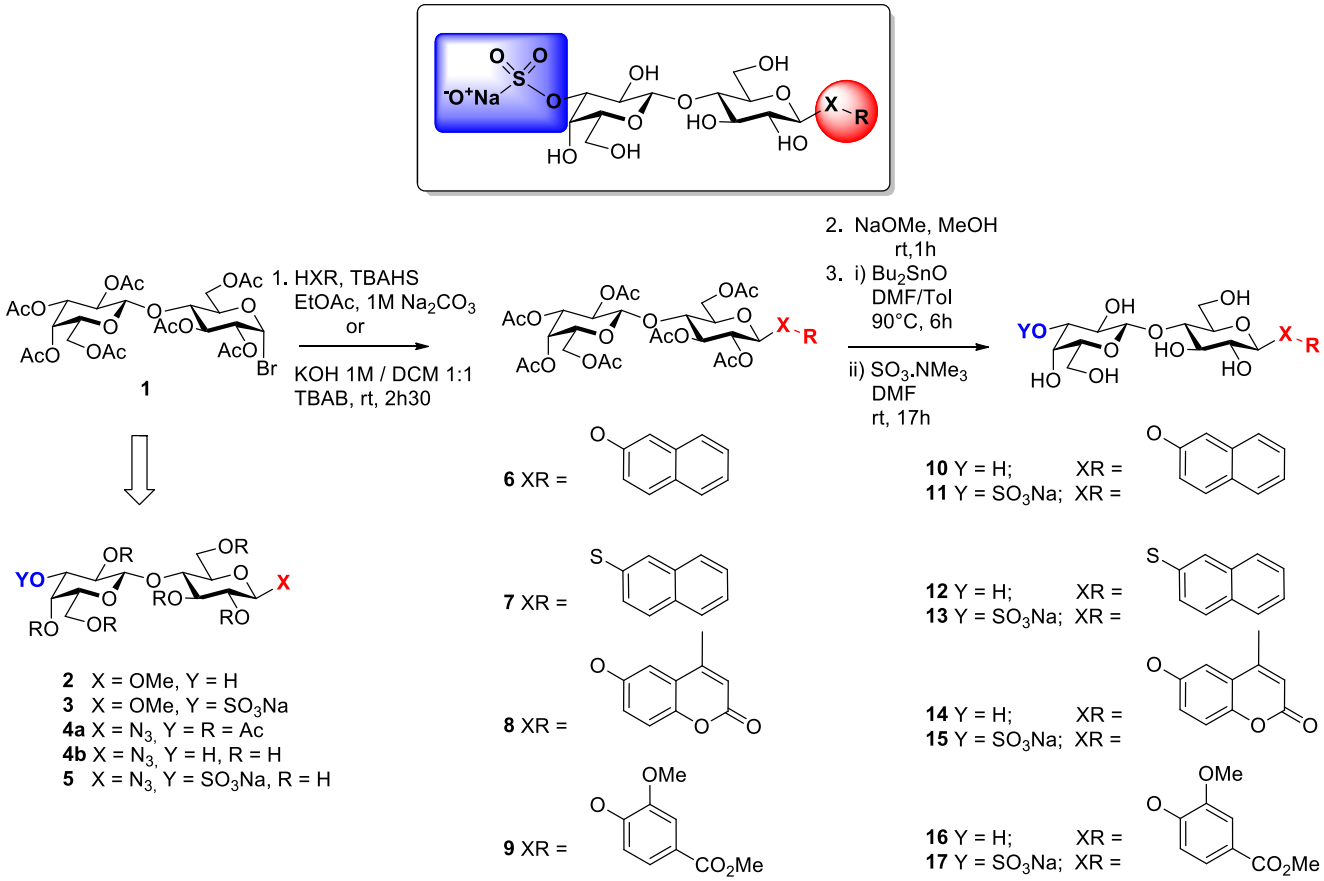
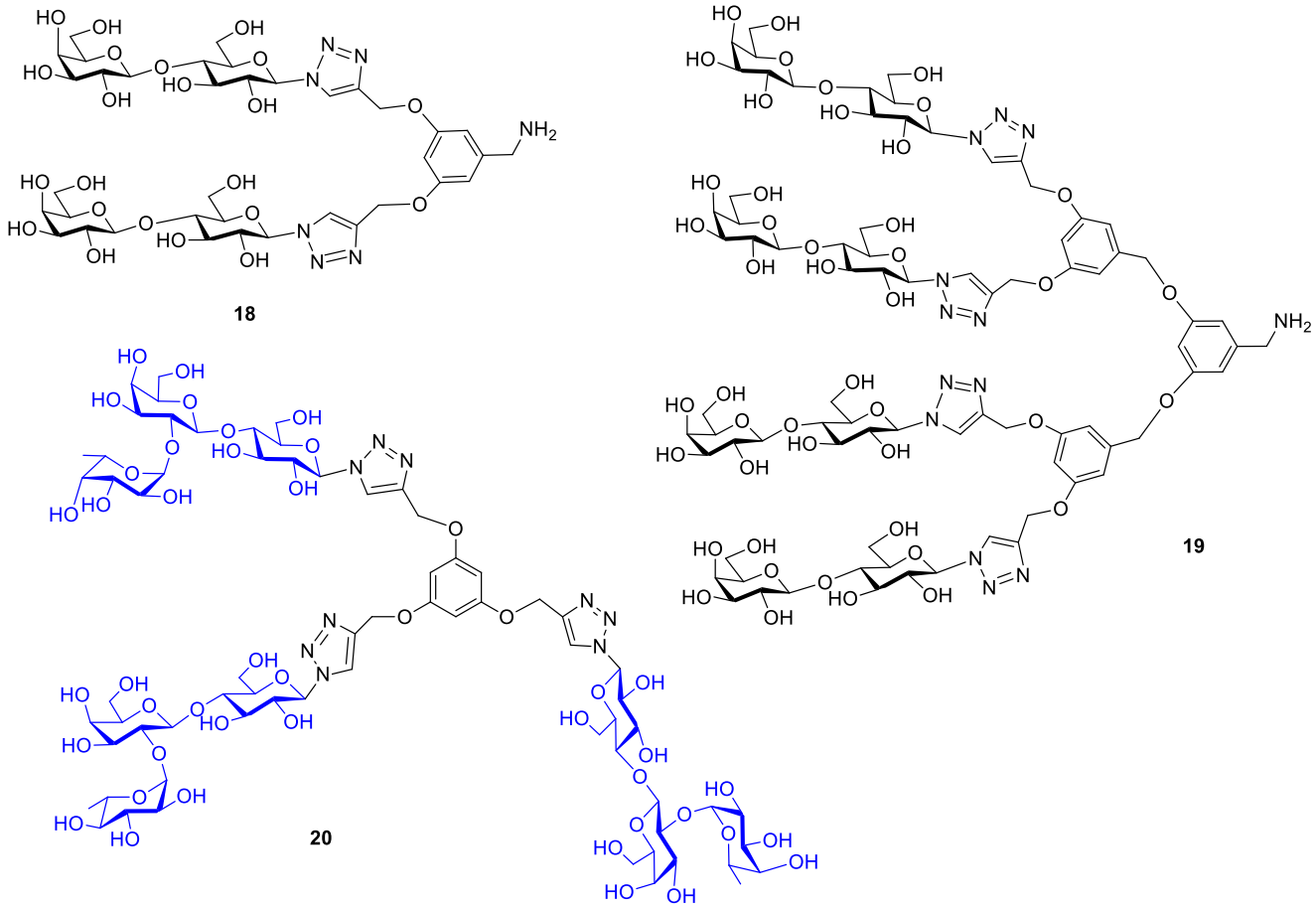


Scheme 1 The two sites of lactose modifications highlighted in blue and red (top) and the synthetic steps leading to lactoside derivatives with six types of aglyconic extension and their 3'-O-sulfated derivatives.



Scheme 2 Bi-, tri- and tetravalent glycoclusters with lactose (**18**, **19**) and 2'-fucosyllactose (**20**) as headgroups (Wang et al. 2012).