New Methods for the Generation of Carbohydrate Arrays on Glass Slides and Their Evaluation

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Glycosides, having spacers functionalized with an aldehyde or a carboxylic group, were immobilized through reductive amination or amidation, respectively, onto amino-functionalized glass slides. Hybridization experiments with lectins exhibited very little nonspecific protein binding, hence precluding the necessity for the blocking of unreacted functional groups on the glass slide. The covalency and the concentration dependency of the sugar ligation to the glass slide were demonstrated; the reversibility and the selectivity of lectin–carbohydrate interactions were shown.

Introduction

Many lipids and more than 50% of all proteins are glycosylated, a fact that indicates the importance of posttranslational protein modification.[1] The great structural diversity of the carbohydrate residues often precludes clear answers as to their biological functions. Many carbohydrate epitopes are known to be specifically recognized by proteins (the "lectins")[2] and carbohydrate–carbohydrate recognition has also been observed.[3–7] However, the whole field of carbohydrate recognition is still in its infancy because the many diverse carbohydrate structures required for this work are not yet available in pure form. Hence, the characterization of the great variety of carbohydrate receptors meets with difficulties. Obviously, this multitude of epitope–receptor recognition events requires fast methods in order to decipher the information stored in the carbohydrate structures. This can best be achieved by carbohydrate arrays that consider this structural diversity.

Some methods have already been developed for the fabrication of carbohydrate arrays.[8–14] Several groups have used noncovalent ligation,[15–19] which relies on nonspecific adhesion phenomena between the carbohydrate epitope and the array. Methods for the covalent immobilization have also been employed. Houseman and Mrksich[20] used the Diels–Alder reaction mediated immobilization of carbohydrate–cyclopentadiene conjugates on to a monolayer that contains benzoquinone groups bound to a gold surface. Park and Shin[21] reported on the attachment of maleinimide-linked carbohydrates to a glass slide coated with thiol groups. A related method has been also employed by Mrksich and co-workers[22] and also by Seeberger and co-workers.[23] Schwarz et al.[24] used plastic surfaces containing amino groups on which the carbohydrate arrays were constructed by use of spacers and cyanuric chloride. Recently, Waldmann and co-workers[25] demonstrated that Staudinger ligation is a particularly efficient and versatile method for the generation of carbohydrate chips. Carbohydrate arrays as reported by these groups have found their first applications.[26–28]

The goal of our work for the generation of carbohydrate chips is 1) to use the commercially available functionalized glass slides employed in DNA-array fabrication,[29] 2) to use readily available saccharides carrying a complementary functional group at the reducing end, 3) to implement a robust and efficient ligation method, and 4) to perform only simple blocking of unreacted functional groups or, better, to employ no blocking at all. In this way, the amount of nonspecific binding of analytes to the surface should be minimized and the reliability of the results should be maximized. In this paper we report on a convenient carbohydrate-array generation methodology, based on the attachment of carbohydrate residues to amino-group- or formyl-group-functionalized glass slides through nondestructive reductive amination or amide-bond formation, respectively. The success of this approach is monitored by fluorescence measurements of labeled carbohydrates and lectins.

Results and Discussion

Immobilization of sugar residues bearing a fluorescence label

The immobilization of carbohydrates on commercially available functionalized glass slides by reductive amination was investigated. This type of reaction proceeds under mild conditions and tolerates a variety of functionalities, particularly the presence of the sugar hydroxy groups. To this end, glucopyranosides **1a**,**b** (Scheme 1) were prepared containing a triethylene glycol spacer ending in a formylmethyl or an aminopropyl

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*Supporting Information for this article is available on the WWW under http://www.chembiochem.org or from the author. It contains experimental details for compounds **2**, **3a–c**, **4a**, **b**, **5a**, **b**, **7a–c**, **10**, **11**, and **14**, as well as ^{1}H and ^{13}C NMR spectra of all new compounds.*

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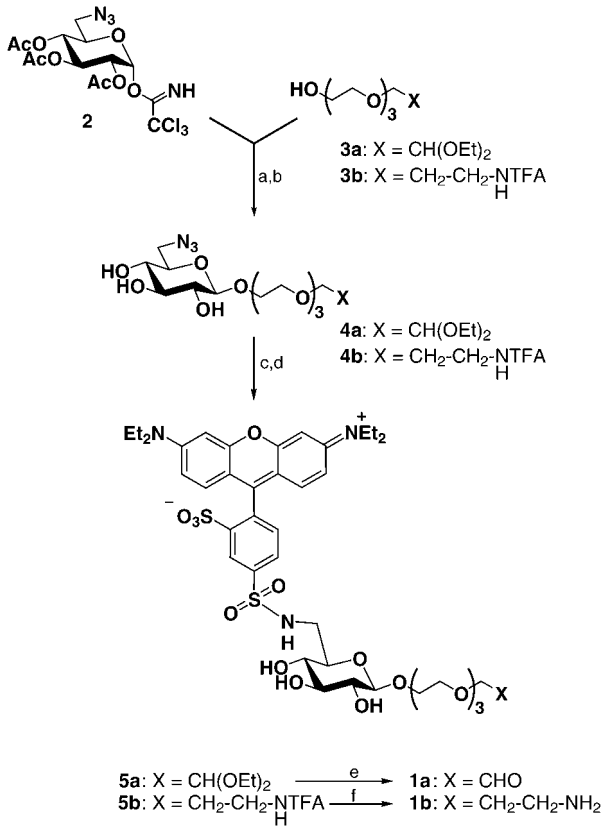


Scheme 1. Synthesis of **1a** and **1b**: a) Lewis acid, CH_2Cl_2, 0 °C, 15 min; b) K_2CO_3, MeOH, RT, 30–45 min, **4a** 60% over two steps, **4b** 61% over two steps; c) $Pd(OH)_2/C$, H_2, CH_2Cl_2/MeOH/H_2O, RT, 1.0–1.5 h; d) sulforhodamine-B sulfonyl chloride, DMF, NEt_3, RT, 4 h, **5a** 60% over two steps, **5b** 50% over two steps; e) CF_3COOH, H_2O/CH_3CN, 45 °C, 6 h, 44%; f) LiOH, H_2O/CH_3CN, RT, 45 min; ion-exchange DOWEX H^+ form, 52%. DMF = *N*,*N*-dimethylformamide.

moiety, respectively. All the compounds carried lissamine (sulforhodamine-B) as a fluorescence label at the 6-position. The 6-azido-6-deoxy-D-glucopyranosyl donor **2** (Scheme 1) was readily obtained from known *O*-acetylated 6-azido-6-deoxy-D-glucose.[30] The acceptors **3a**,**b** were prepared by monoalkylation of triethylene glycol with bromoacetaldehyde diethylacetal or 3-azido-1-propyl *p*-toluenesulfonate[31] and subsequent standard transformations. Glycosylation of acceptors **3a**,**b** with **2** as the donor in dichloromethane in the presence of catalytic amounts of Lewis acid afforded the β-glycosides in good yields; subsequent *O*-deacetylation in methanol in the presence of potassium carbonate led to compounds **4a**,**b**. Hydrogenolysis with palladium hydroxide on carbon liberated the 6-amino group to which the fluorescence label was attached by using sulforhodamine-B sulfonyl chloride in DMF as the solvent and with triethylamine as the base, thus affording compounds **5a**,**b**. Treatment of **5a** in acetonitrile/water with trifluoroacetic acid led to the desired aldehyde **1a**, and treatment of **5b** in acetonitrile/water with lithium hydroxide furnished the desired amine **1b**.

Immobilization of amine **1b** onto aldehyde-functionalized glass slides was performed by reductive amination with sodium cyanoborohydride as the reducing agent at pH 6.8. After this ligation, excess reagent was removed by suction and washing with aqueous 0.2% sodium dodecylsulfate (SDS) and water. The same immobilization procedure was applied to aldehyde **1a** with amino-functionalized glass slides. Measurement of the resulting fluorescence intensities at different concentrations was performed with the aid of an array scanner (Figure 1). The observed fluorescence intensities exhibited the expected concentration dependency down to a concentration of 10^{-8} mol L^{-1}. At higher concentrations (10^{-5}–10^{-4} mol L^{-1}), a plateau effect was almost reached. Slightly better results were obtained for the ligation to amino-group-functionalized glass slides; therefore, this ligation was preferred in the lectin stud-

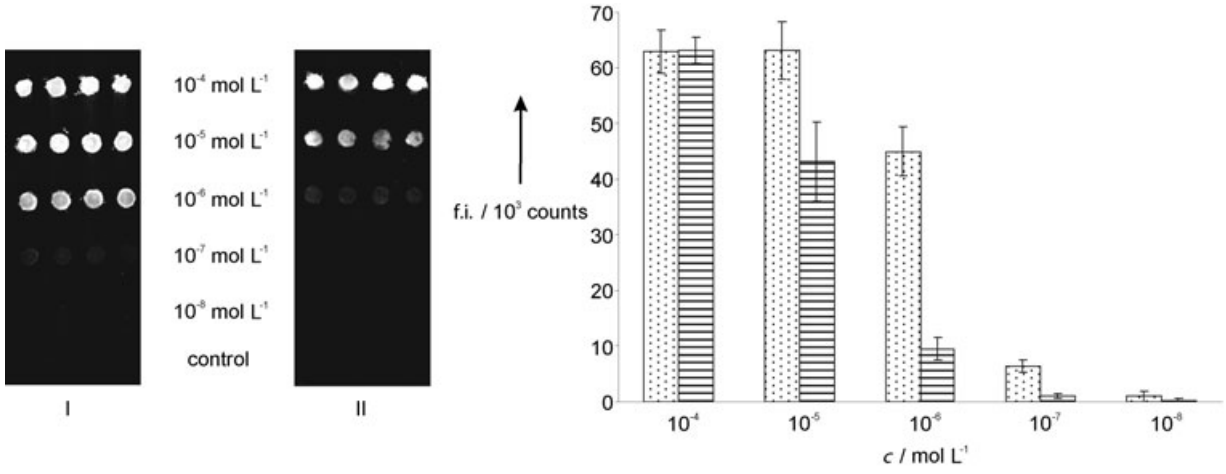


Figure 1. Immobilization of compounds **1a** and **1b** on glass slides by reductive amination. **I**: compound **1a** immobilized on an amino-functionalized glass slide; **II**: compound **1b** immobilized on an aldehyde-functionalized glass slide. In both experiments the same concentrations are spotted in one row and concentrations of spotted carbohydrate compound decrease tenfold from top to bottom. The diagram shows the concentration dependency of measured fluorescence intensity (f.i.). Left bars correspond to experiment **I** and right bars to experiment **II**; mean values from four experiments are depicted and the indicated error bars show the standard deviation.

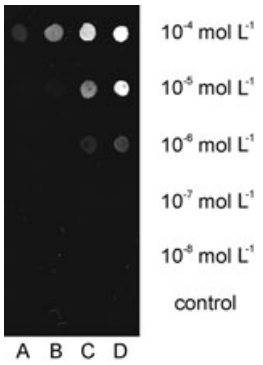


Figure 2. Control experiments with compounds **5b** and **1b** on an aldehyde-functionalized glass slide. Lane A: compound **5b** spotted without reducing agent; lane B: compound **5b** spotted with reducing agent; lane C: compound **1b** spotted without reducing agent; lane D: compound **1b** spotted with reducing agent. The same concentrations are spotted in one row and concentrations of spotted carbohydrate compound decrease tenfold from top to bottom.

ies (see below). These experiments with fluorescence-labeled carbohydrates clearly exhibit the covalent linkage to the glass support. The observed detection limit is in accordance with the results for the covalent immobilization of fluorescent dyes as reported by Park and Shin.[21]

Some control experiments with compounds **5b** and **1b** (shown in Figure 2) led to the expected results: *N*-Trifluoroacetyl-protected compound **5b** showed only low binding to the aldehyde surface (Figure 2, lane A). This low binding is presumably due to partial hydrolytic cleavage of the trifluoroacetyl group, which is increased when the reducing agent is present (lane B). Addition of **1b** without reducing agent led to imine formation and thus to covalent ligation (lane C); however, due to equilibration and/or hydrolytic lability, binding occurred to a lesser extent than that observed for reductive amination (lane D).

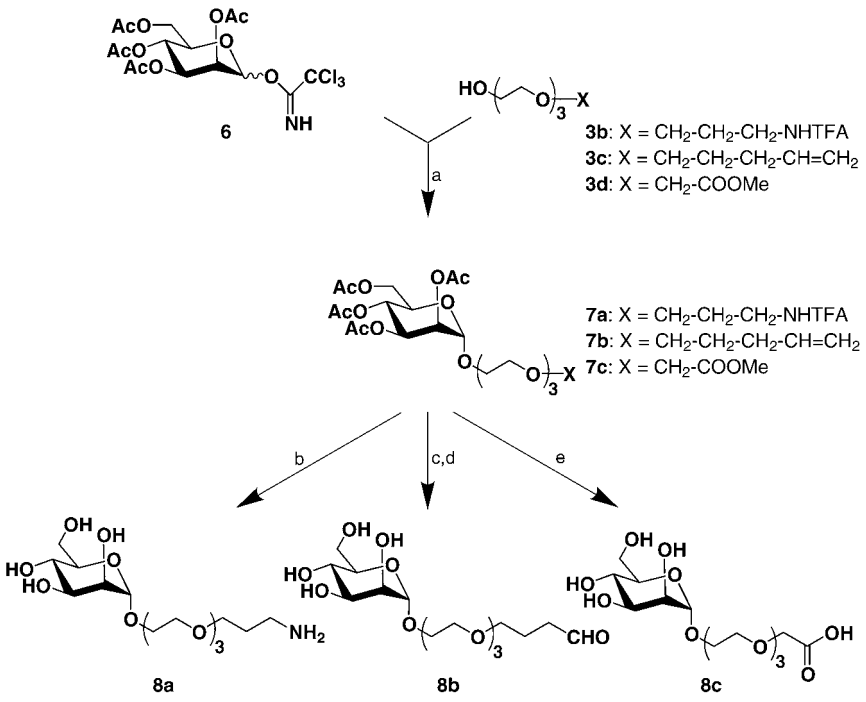
remaining functional groups with polyethylene glycol 2000 dialdehyde and reductive amination (Figure 3, lane A: **8a**; lane B: **8b**). Incubation of these carbohydrate arrays with excess rhodamine-labeled ConA (1 $\mu g\,mL^{-1}$) clearly showed that only aldehyde **8b** was bound to the glass surface. It was observed that this procedure for carbohydrate-array fabrication leads to very little nonspecific protein binding, even when the blocking procedure was omitted. Studies of **8a** and **8b** with and without reducing agent (Figure 3, lanes C–F) and subsequent incubation with ConA showed that only **8b** in the presence of reducing agent leads to firm covalent binding to the glass surface (lane F). Imine formation without reductive amination seems to result in lower loading (as previously observed in the case of the labeled D-glucose compounds, Figure 2); in experiments omitting the reducing agent (lane E), a lower affinity of ConA is observed than after reductive amination (lane F). The hybridization of immobilized **8b** with ConA shows that carbohydrates can be clearly detected down to concentrations of $10^{-4}\,mol\,L^{-1}$ in the ligation process. This is comparable to results obtained from experiments utilizing different immobilization strategies.[20,21] These findings are in accordance with typical association constants found for monovalent carbohydrate–lectin interactions in aqueous solution, which are commonly found to be in the millimolar range;[36] here values for methyl α-D-mannopyranoside–ConA interactions of $\approx 8\times10^{-4}\,mol\,L^{-1}$ are observed.[37]

As an alternative to the above-described immobilization procedure, α-connected mannopyranoside **8c** bearing a carboxylate group at the spacer end was linked in different concentrations to an amino-functionalized glass slide with the help of

Carbohydrate–lectin interactions

The well-investigated interaction of the lectin concanavalin A (ConA) with α-linked mannopyranosides was selected for the first studies on glass slides.[32] The required compounds **8a–c** were prepared similarly to **1a,b** (Scheme 2). To this end, triethylene glycol was monoalkylated with 3-azido-1-propyl *p*-toluenesulfonate,[31] 1-bromopent-4-ene, and allyl bromide; compounds **3b–d**[33,34] were obtained after subsequent standard reactions. Reaction of these acceptors with tetra-*O*-acetylmannopyranosyl trichloroacetimidate[35] as the donor afforded the α-mannosides **7a–c**, which were transformed by solvolysis and/or ozonolysis into the target compounds **8a–c**.

Immobilization of **8a** and **8b** on amino-functionalized glass slides by reductive amination was followed by blocking of the



Scheme 2. Synthesis of mannose derivatives **8a–c**: a) Lewis acid, CH_2Cl_2, −10 °C, 15 min, **7a** 61%, **7b** 79%, **7c** 52%; b) 1. LiOH, H_2O/dioxane, RT, 45 min, 2. ion-exchange DOWEX H^+ form, 86%; c) Na in MeOH, RT, 90 min, quantitative; d) 1. O_3, MeOH, −78 °C; 2. $P(OMe)_3$, MeOH, RT, 40%; e) LiOH, H_2O/dioxane, RT, 45 min, 97%.

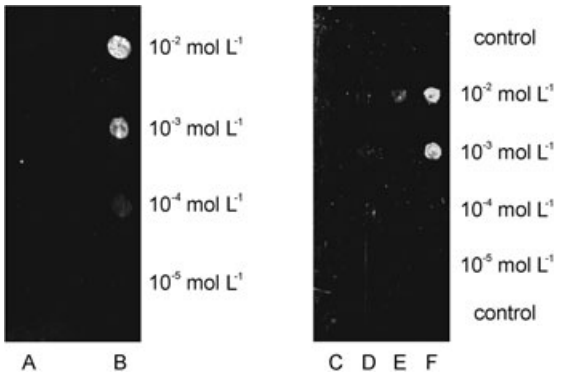


Figure 3. Carbohydrate arrays obtained by reductive amination on amino-functionalized glass slides. Lane A: compound **8a** spotted with reducing agent; lane B: compound **8b** spotted with reducing agent; lane C: compound **8a** spotted without reducing agent; lane D: compound **8a** spotted with reducing agent; lane E: compound **8b** spotted without reducing agent; lane F: compound **8b** spotted with reducing agent. The same concentrations are spotted in one row and concentrations of spotted carbohydrate compound decrease tenfold from top to bottom. Hybridization was performed with rhodamine-labeled ConA (1 $\mu g\,mL^{-1}$).

(benzotriazol-1-yloxy)-tripyrrolidinophosphonium hexafluorophosphate (PyBOP) as a mild condensing agent (Figure 4). Monitoring the result with rhodamine-labeled ConA showed that covalent ligation of the sugar residue to the glass surface occurred only after amide coupling (lanes A and D). Investigations concerning the time dependency of the coupling reaction were performed (data not shown). After two hours significant fluorescence intensities were found, after five hours signal intensities of more than 50% of the maximum intensity (reached after 12 h) were detected. Therefore, chemoselective amide-bond formation, as introduced by Waldmann and coworkers,[25] is of great value.

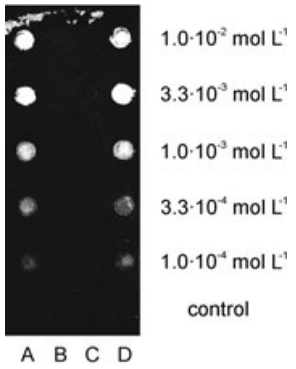


Figure 4. A carbohydrate array obtained by amide-bond formation on an amino-functionalized glass slide. Lanes A & D: compound **8c** spotted with condensing agent, lanes B & C: compound **8c** spotted without condensing agent. The same concentrations are spotted in one row and concentrations of spotted carbohydrate compound decrease tenfold from top to bottom. Hybridization was performed with rhodamine-labeled ConA (1 $\mu g\,mL^{-1}$).

Reversibility and selectivity

The reversibility of lectin binding to the immobilized carbohydrate residue was investigated with methyl α-D-mannopyranoside as a competitor (Figure 5). After 10 h of treatment almost all ConA was removed, and further elution leads to no significant reduction of the fluorescence intensities measured. This slow release of ConA could be due to multivalent interactions on the glass slide, as previously proposed,[21] or to adsorption of the lectin on to the surface once it is bound to the sugar residue. Rehybridization under the same conditions as previously employed is possible (Figure 5, VII). However, the measured fluorescence intensity does not reach the same value as that observed with a freshly prepared slide.

Additional evidence for the reversible and specific binding of the lectin to the immobilized sugar moiety can be inferred from comparison of the results of noncompetitive washing experiments with those of competitive elution (Figure 6). To this end, the time dependency of the measured fluorescence intensities of glass slides prepared in the same manner was monitored during washing and elution processes. As these results clearly show, the residual fluorescence intensity decreases faster when methyl α-mannopyranoside is available as a competitor in the washing solution. These investigations show the reversibility of the binding event on glass slides for the first time.

To study the selectivity of the molecular recognition of lectins, the *N*-acetyl glucosamine derivative **12** and the lactose derivative **15** were synthesized (Scheme 3). These compounds were prepared by glycosylation of the fully *O*-acetylated trichloroacetimidates **9**[38] and **13**[39,40] to the pentenyl-bearing acceptor **3c**. Subsequently, these β-glycosides were transformed into the desired aldehydes **12** and **15** by hydrolysis of the protecting groups and ozonolysis followed by reductive workup, which in the case of the glucosamine derivative is preceded by the conversion of the trichloroethoxycarbamate **10** into the *N*-acetyl glucosamine derivative **11** (Scheme 3).

The selectivity of lectin binding was investigated with compounds **8b**, **12**, and **15**, which should be recognized by ConA, wheat germ agglutinin (WGA), or peanut agglutinin (PNA), respectively.[32] Three carbohydrate arrays, with the control on lane A and compounds **8b**, **12**, and **15** immobilized on lanes B–D in different concentrations, were incubated with these lectins (Figure 7). As expected, the known specific recognition of **8b**, **12**, and **15** by the lectins was observed, thus showing that this method has promise for extending the research towards complex saccharide structures.

Conclusion

In conclusion, this convenient covalent ligation of carbohydrates on to functionalized glass slides led to stable arrays that can be used for the detection of specific carbohydrate–protein recognition events. The advantage of this ligation process lies in its simplicity: 1) commercially available glass slides can be used, 2) standard reagents (PyBOP or $NaCNBH_3$) are employed to couple simple functional groups, and 3) a variety of other functionalities are tolerated. The reported procedure showed little or no nonspecific protein binding to amino-functionalized glass slides, even when the blocking process was omitted, thus making these additional steps unnecessary. The observed sen-

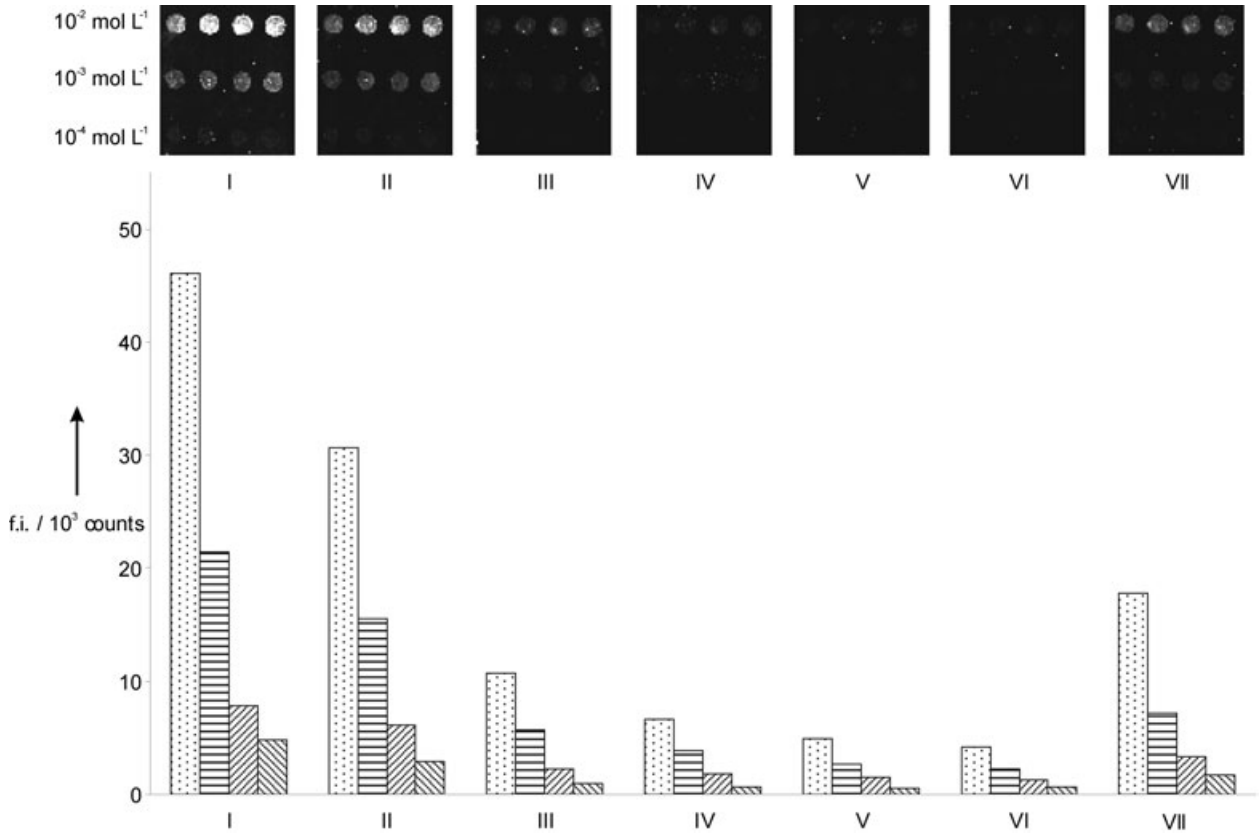


Figure 5. Reversibility of carbohydrate–lectin interactions. Compound **8b** immobilized on an amino-functionalized glass slide; the same concentrations are spotted in one row and concentrations of spotted carbohydrate compound decrease tenfold from top to bottom. Results after hybridization with rhodamine-labeled ConA (1 μg mL^{-1}; **I**), after elution with methyl α-D-mannopyranoside (200 mmol L^{-1} in phosphate-buffered saline (PBS) buffer) for 1 h (**II**), for 2 h (**III**), for 5 h (**IV**), for 10 h (**V**), or for 24 h (**VI**), and after rehybridization with rhodamine-labeled ConA (1 μg mL^{-1}; **VII**). Bars from left to right correspond to spotted concentrations of compound **8b** of 10^{-2}, 10^{-3}, and 10^{-4} mol L^{-1} and to the background intensity; mean values from four experiments are depicted.

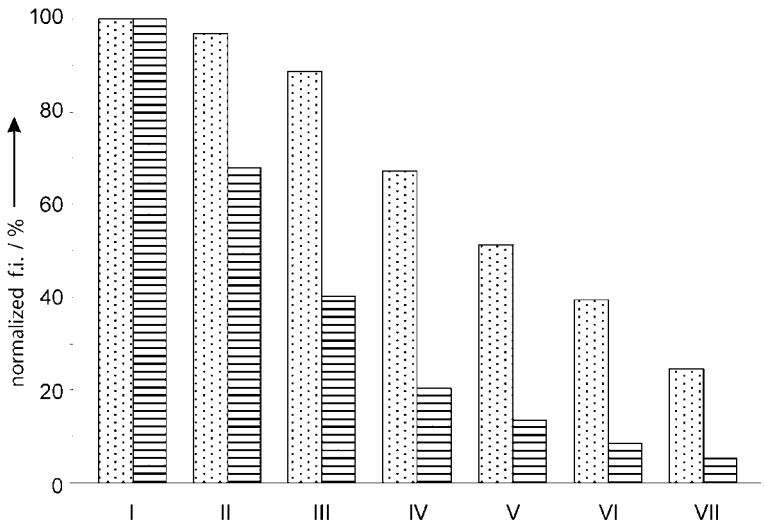


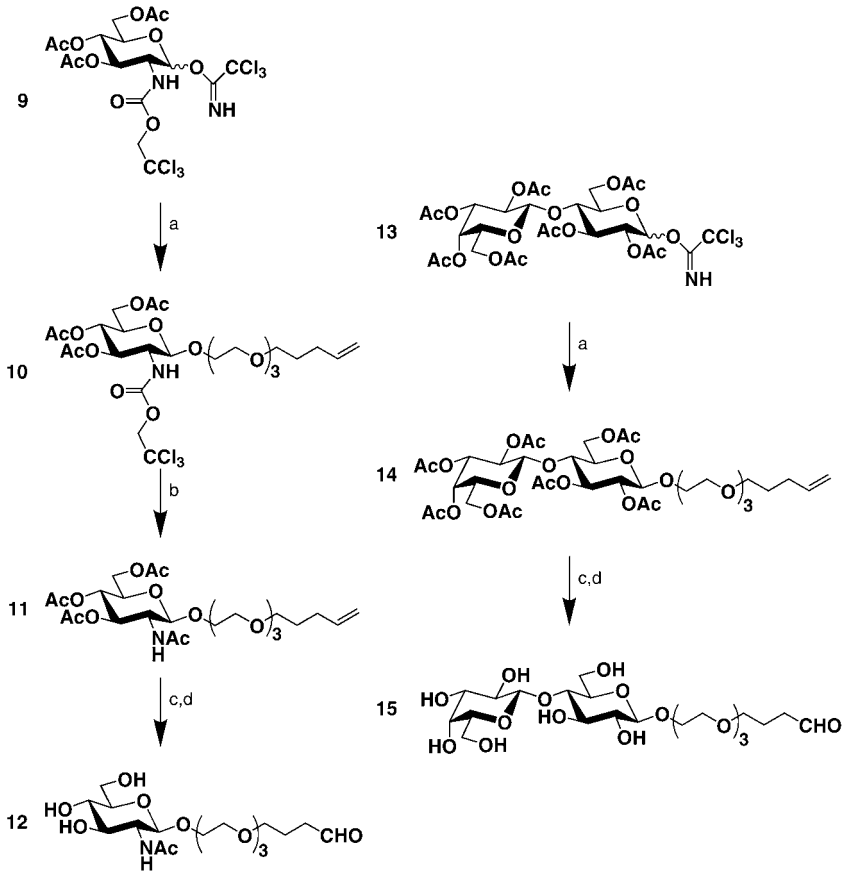
Figure 6. Washing versus elution. Compound **8b** (10^{-2} mol L^{-1}) immobilized on amino-functionalized glass slides. Results after hybridization with rhodamine-labeled ConA (1 μg mL^{-1}; **I**) and after treatment for 1 h (**II**), for 2 h (**III**), for 5 h (**IV**), for 10 h (**V**), for 20 h (**VI**), or for 40 h (**VII**). Left bars correspond to washing with PBS buffer and right bars to elution with methyl α-D-mannopyranoside (200 mmol L^{-1} in PBS buffer). Mean values from four experiments are depicted. Measured fluorescence intensities are normalized with regard to intensity after hybridization separately for washing or elution experiments.

sitivity of the ConA interaction with immobilized α-mannopyranosides is in accordance with values found for interactions with monosaccharides in aqueous solution, as well as with results reported for different immobilization methods.

In addition, evidence for the reversibility of the carbohydrate–lectin binding event on the glass slide could be elaborated. Investigations of this kind are of interest because the fabrication of carbohydrate microarrays will involve the use of precious oligosaccharides, therefore the reuse of these arrays is desirable. The transfer of the selective binding of different lectins to their immobilized carbohydrate ligands was principally achieved. For possible applications, miniaturization and automation studies for the preparation of arrays that bear a variety of complex saccharides are under way. Further sensitivity improvements are a major task as well.

Experimental Section

General methods: Solvents were purified by distillation and dried by normal procedures. Boiling range of the petroleum ether: 35–70 °C. Organic HPLC solvents were purchased from Roth. Thin-layer chromatography (TLC)



Scheme 3. Synthesis of β-glycosides **12** and **15**: a) **3c**, Lewis acid, CH_2Cl_2, −10 °C, 15 min, **10** 85%, **14** 70%; b) Zn, HOAc, ultrasonic agitation, RT, 30–45 min, 86%; c) Na in MeOH, RT, 90 min, quantitative; d) 1. O_3, MeOH, −78 °C; 2. $P(OMe)_3$, MeOH, RT, **12** 16%, **15** 36%.

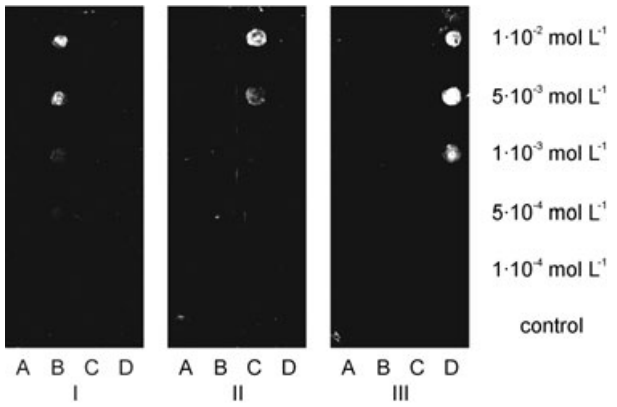


Figure 7. Selectivity of carbohydrate–lectin interactions. Lane A: control; lane B: compound **15** immobilized; lane C: compound **12** immobilized; and lane D: compound **8b** immobilized (amino-functionalized glass slide used). Hybridization was performed with rhodamine-labeled PNA (**I**), with rhodamine-labeled WGA (**II**), or with rhodamine-labeled ConA (**III**).

was performed on Merck silica gel $60F_{254}$ plates (0.2 mm) and Merck RP-18 F_{254} plates (0.2 mm). The plates were visualized by immersion in the appropriate stain (10% H_2SO_4 (200 mL), $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (10 g), $Ce(SO_4)_2$) (200 mg), ninhydrin solution (1% in EtOH), H_2SO_4 (10% in water), or $KMnO_4$ solution (1% in water, 1% $NaHCO_3$)) followed by heating. Preparative flash chromatography was carried out on Macherey–Nagel silica gel 60 (43–60 μm) at a pressure of 0.02–0.04 MPa. Preparative HPLC was performed with a system consisting of a low-pressure gradient mixer, a Shimadzu LC-8A pump, Knauer columns (RP-18 Eurosphere 5 μm; 16×250 mm), and a Dynamax UV1 detector (monitoring at $\lambda = 554$ nm) or a Shimadzu RID-10 detector. All separations were performed with a continous flow rate of 10 mL min^{-1} by using the following conditions: System I: solvent A: 10% acetonitrile/0.2% trifluoroacetic acid in water, solvent B: 60% acetonitrile/0.2% trifluoroacetic acid in water, linear gradient 0% B→95% B over 25 min; System II: solvent A: 15% acetonitrile in water, solvent B: 85% acetonitrile in water, linear gradient 0% B→95% B over 35 min; system III: solvent: acetonitrile in water, isocratic. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AC 250, Jeol LA 400, or Bruker DRX 600 instruments. Proton chemical shifts are reported in ppm relative to the signal from residual solvent protons or to Me_4Si as an internal standard. Assignments of proton and carbon signals were carried out with the aid of COSY, HMQC, and ROESY experiments. Measurements of optical rotations were performed on a Perkin–Elmer 241 MC polarimeter (1 dm cell). MALDI mass spectra were obtained on a Kratos Analytical Kompact Maldi II instrument with 2,5-dihydroxybenzoic acid (DHB) or α-cyano-4-hydroxy-cinnamic acid (CHCA) as the matrix (positive mode). Elemental analyses were performed by the microanalytical facilities at the Universität Konstanz. Fluorescence measurements were performed with a GenePix series 4000 array scanner from Axon Instruments.

General procedure I (GP I): Preparation of 6-azido-6-deoxy-D-glucose derivatives bearing linker moieties:

1) Under a nitrogen atmosphere, the glycosyl donor **2** and the triethylene glycol derivative **3** (1.1 equiv) were dissolved in dry dichloromethane (0.75 mL per mmol of **2**) and cooled to 0 °C, then Lewis acid (see individual experimental details) was added. The reaction mixture was stirred at 0 °C for 15 min and subsequently allowed to come to room temperature. After neutralization with triethylamine, the reaction mixture was diluted with ethyl acetate (15 mL) and water (15 mL) was added. After separation of the phases, the aqueous phase was extracted with ethyl acetate (2× 20 mL) and the combined organic phases were dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, toluene/acetone) to afford the acetylated precursors with yields of 60–70%.

2) The substance from step (1) was dissolved in methanol (2 mL) and potassium carbonate (1 equiv) was added. The reaction mix-

ture was stirred for 30–45 min, the potassium carbonate was removed by filtration, and the solvent was removed under reduced pressure. The partially deprotected azido compounds were obtained with yields greater than 90% and were sufficiently pure for the following reaction. Analytical samples were purified by flash column chromatography (silica gel, toluene/acetone).

General procedure II (GP II): **Preparation of lissamine-labeled glucose derivatives from azido precursors**:

1) A mixture of the azido precursor **4a** or **4b** (25 mg, ≈50 μmol) and Pearlman's catalyst[41] (≈5 mg) in dichloromethane/methanol/water (20:20:1, 2.5 mL) was degassed and saturated with hydrogen several times; it was subsequently vigorously stirred under a hydrogen atmosphere for 60–90 min. The catalyst was removed by filtration and the solvent was evaporated under diminished pressure at room temperature. The compounds bearing a free amino group were obtained in quantitative yields and immediately used in the next step.

2) The substance from step (1) was dissolved in dry DMF (5 mL) and sulforhodamine-B sulfonyl chloride (32 mg, 55 μmol, 1.1 equiv) and dry triethylamine (5 μL, ≈60 μmol, 1.2 equiv) were added. After 4 h, the reaction mixture was concentrated, and the residue was purified by RP-18 flash chromatography followed by RP-18 HPLC to afford the labeled compounds in yields of 50–60%.

General procedure III (GP III): **Preparation of fully *O*-acetylated carbohydrate derivatives bearing linker moieties**: The glycosyl donor and the triethylene glycol derivative were dissolved in dry dichloromethane (0.75 mL per mmol of donor) under a nitrogen atmosphere and cooled to −10 °C, then Lewis acid (see individual experimental details) was added. The reaction mixture was stirred at −10 °C for 30 min and subsequently allowed to come to room temperature. After neutralization with triethylamine, the reaction mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography to afford the acetylated products in yields of 60–85%.

10-Formyl-3,6,9-trioxadecyl 6-deoxy-6-(sulforhodamine-B-sulfonamido)-β-D-glucopyranoside (1a): Compound **5a** (10 mg, 10.3 μmol) was dissolved in water/acetonitrile (2:1, 2 mL) and trifluoroacetic acid (100 μL) was added. The reaction mixture was stirred at 45 °C for 6 h, then concentrated under reduced pressure. The residue was purified by RP-18 flash chromatography (water/acetonitrile 2:1) and subsequent RP-18 HPLC (system I; t_R = 20.1 min). Title compound **1a** was obtained as a bright red lyophilisate (4 mg, 4.5 μmol, 44%): TLC: R_f = 0.51 (RP-18 silica gel, water/acetonitrile 1:1); ^{1}H NMR (600 MHz, [D$_6$]-dimethylsulfoxide ([D$_6$]DMSO), 20 °C): δ = 1.19 (t, ^3J = 6.6 Hz, 12H; 4×NCH$_2$C*H*$_3$), 2.80–2.83 (m, 1H; *H*-6a), 2.87 (dd, $^3J_{3,4}$ = 8.8, $^3J_{4,5}$ = 9.1 Hz, 1H; *H*-4), 2.93 (dd, $^3J_{1,2}$ = 7.7, $^3J_{2,3}$ = 8.3 Hz, 1H; *H*-2), 3.02–3.08 (m, $^3J_{4,5}$ = 9.1 Hz, 1H; *H*-5), 3.07 (dd, $^3J_{2,3}$ = 8.3, $^3J_{3,4}$ = 8.8 Hz, 1H; *H*-3), 3.20–3.88 (m, 20H; *H*-1′a, 2×*H*-2′, 2×*H*-4′, 2×*H*-5′, 2×*H*-7′, 2×*H*-8′, 4×NC*H*$_2$CH$_3$, *H*-6b), 3.98–4.01 (m, 1H; *H*-1′b), 4.04 (d, $^3J_{1,2}$ = 7.7 Hz, 1H; *H*-1), 4.17 (s, 2H; 2*H*-10′), 6.90–7.10 (m, 6H; H_{ar}), 7.46 (d, ^3J = 7.9 Hz, 1H; H_{ar}), 7.94 (dd, ^3J = 7.9, ^3J = 1.4 Hz, 1H; H_{ar}), 8.07 (brs, 1H; CH$_2$N*H*SO$_2$), 8.43 (d, ^3J = 1.4 Hz, 1H; H_{ar}), 9.54 ppm (s, 1H; C*H*O); ^{13}C NMR (150.8 MHz, [D$_6$]DMSO): δ = 12.49 (4C, 4×NCH$_2$CH$_3$), 44.45 (1C, *C*-6), 45.28 (4C, 4×NCH$_2$CH$_3$), 67.8–70.2 (6C, *C*-1′, *C*-2′, *C*-4′, *C*-5′, *C*-7′, *C*-8′), 71.42 (1C, *C*-4), 73.29 (1C, *C*-2), 74.52 (1C, *C*-5), 76.07 (1C, *C*-10′), 76.36 (1C, *C*-3), 95.39 (2C, C_{ar}), 102.90 (1C, *C*-1), 113.48 (2C, C_{ar}), 113.74 ($C_{ar,q}$), 125.73 (1C, C_{ar}), 126.58 (1C, C_{ar}), 130.63 (1C, C_{ar}), 132.72 (2C, C_{ar}), 133.00 ($C_{ar,q}$), 142.05 ($C_{ar,q}$), 147.98 ($C_{ar,q}$), 155.05 ($C_{ar,q}$), 157.13 ($C_{ar,q}$), 157.52 ($C_{ar,q}$), 201.56 ppm (1C, CHO); $C_{41}H_{55}N_3O_{15}S_2$ (894.0): MALDI MS (pos. mode, CHCA): [*M*+H]$^+$ calcd: 894.3; found: 894.0; [*M*+Na]$^+$ calcd: 916.9; found: 916.6; [*M*+K]$^+$ calcd: 932.4; found: 931.8.

12-Amino-3,6,9-trioxadodecyl 6-deoxy-6-(sulforhodamine-B-sulfonamido)-β-D-glucopyranoside (1b): Compound **5b** (15 mg, 14.9 μmol) was dissolved in water/acetonitrile (2:1, 2 mL), and lithium hydroxide solution (1 mol L^{-1}, 100 μL) was added. The reaction mixture was stirred at room temperature for 45 min. The reaction mixture was neutralized with ion-exchange resin (DOWEX 50 H$^+$ form) and concentrated, then the residue was purified by RP-18 flash chromatography (water/acetonitrile 2:1) and subsequent RP-18 HPLC (system I; t_R = 19.6 min). Title compound **1b** was obtained as a bright red lyophilisate (7 mg, 7.7 μmol, 52%): TLC: R_f = 0.50 (RP-18 silica gel, water/acetonitrile 1:1); ^{1}H NMR (600 MHz, [D$_6$]DMSO, 20 °C): δ = 1.19 (t, ^3J = 6.6 Hz, 12H; 4×NCH$_2$C*H*$_3$), 1.76 (q, ^3J = 6.4 Hz, 2H; 2*H*-11′), 2.80–2.86 (m, 3H; *H*-6a, 2H-12′), 2.88 (dd, $^3J_{3,4}$ = 9.1, $^3J_{4,5}$ = 9.2 Hz, 1H; *H*-4), 2.94 (dd, $^3J_{1,2}$ = 7.8, $^3J_{2,3}$ = 8.2 Hz, 1H; *H*-2), 3.02–3.05 (m, $^3J_{4,5}$ = 9.2 Hz, 1H; *H*-5), 3.07 (dd, $^3J_{2,3}$ = 8.2, $^3J_{3,4}$ = 9.1 Hz, 1H; *H*-3), 3.35–3.70 (m, 22H; *H*-1′a, 2*H*-2′, 2*H*-4′, 2*H*-5′, 2*H*-7′, 2*H*-8′, 2*H*-10′, 4×NC*H*$_2$CH$_3$, *H*-6b), 3.97–4.01 (m, 1H; *H*-1′b), 4.06 (d, $^3J_{1,2}$ = 7.8 Hz, 1H; *H*-1), 6.90–7.08 (m, 6H; H_{ar}), 7.47 (d, ^3J = 7.9 Hz, 1H; H_{ar}), 7.65 (brs, 2H; N*H*$_2$), 7.94 (d, ^3J = 7.9 Hz, 1H; H_{ar}), 8.07 (brs, 1H; CH$_2$N*H*SO$_2$), 8.43 ppm (s, 1H; H_{ar}); ^{13}C NMR (150.8 MHz, [D$_6$]DMSO): δ = 12.52 (4C, 4NCH$_2$CH$_3$), 27.10 (1C, *C*-11′), 37.10 (1C, *C*-12′), 44.48 (1C, *C*-6), 45.34 (4C, 4NCH$_2$CH$_3$), 66.53 (1C, *C*-10′), 67.9–69.80 (6C, *C*-1′, *C*-2′, *C*-4′, *C*-5′, *C*-7′, *C*-8′), 71.48 (1C, *C*-4), 73.34 (1C, *C*-2), 74.56 (1C, *C*-5), 76.43 (1C, *C*-3), 95.45 (2C, C_{ar}), 102.87 (1C, *C*-1), 113.50 (2C, C_{ar}), 113.77 ($C_{ar,q}$), 125.76 (1C, C_{ar}), 126.73 (1C, C_{ar}), 130.72 (1C, C_{ar}), 132.69 (2C, C_{ar}), 133.10 ($C_{ar,q}$), 142.08 ($C_{ar,q}$), 147.92 ($C_{ar,q}$), 155.09 ($C_{ar,q}$), 157.18 ($C_{ar,q}$), 157.41 ppm ($C_{ar,q}$); $C_{42}H_{60}N_4O_{14}S_2$ (909.1); MALDI MS (pos. mode, CHCA): [*M*+H]$^+$ calcd: 909.4; found: 909.1; [*M*+Na]$^+$ calcd: 931.4; found: 931.0; [*M*+K]$^+$ calcd: 947.5; found: 946.9.

12-Amino-3,6,9-trioxadodecyl α-D-mannopyranoside (8a): Compound **7a** (280 mg, 440 μmol) was dissolved in water/dioxane (1:1, 5 mL), and lithium hydroxide solution (saturated, 100 μL) was added. The reaction mixture was stirred at room temperature for 45 min. The reaction mixture was acidified with diluted hydrochloric acid and concentrated, then the residue was purified by ion-exchange chromatography (DOWEX 50 H$^+$ form, elution with acetonitrile/0.5% aqueous ammonia (1:1 v/v)). Upon lyophilization, the free amine **8a** (140 mg, 380 μmol, 86%) was obtained as a colorless oil: TLC: R_f = 0.05 (dichloromethane/methanol 5:1); $[\alpha]_D^{20}$ = +30.8 (*c* = 1.0, methanol); ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 1.79 (q, $^3J_{10',11'} \approx {}^3J_{11',12'}$ = 7.4 Hz, 2H; 2*H*-11′), 2.80 (t, $^3J_{11',12'}$ = 7.4 Hz, 2H; 2*H*-12′), 3.24–3.69 (m, 20H; *H*-2, *H*-3, *H*-4, *H*-5, *H*-6a, *H*-6b, 2*H*-1′, 2*H*-2′, 2*H*-4′, 2*H*-5′, 2*H*-7′, 2*H*-8′, 2*H*-10′), 4.61 (d, $^3J_{1,2}$ = 1.4 Hz, 1H; *H-1*), 7.52 ppm (brs, 2H; N*H*$_2$); ^{13}C NMR (150.8 MHz, [D$_6$]DMSO): δ = 26.15, 36.54, 61.35, 65.38, 66.77, 67.08, 69.12, 69.38 (2C), 70.00 (2C), 70.08, 70.77, 73.92, 100.02 ppm; $C_{15}H_{31}NO_9$ (369.4); MALDI MS (pos. mode, DHB): [*M*+H]$^+$ calcd: 370.4; found: 370.5; [*M*+Na]$^+$ calcd: 392.4; found: 392.5; [*M*+K]$^+$ calcd: 408.5; found: 408.4.

12-Formyl-3,6,9-trioxadodecyl α-D-mannopyranoside (8b): Compound **7b** (390 mg, 690 μmol) was dissolved in methanol (10 mL), and potassium carbonate (380 mg, 2.8 mmol, 4.0 equiv) was added. The reaction mixture was stirred at room temperature for 90 min. The reaction mixture was filtered and concentrated to obtain the deprotected compound, which was not further purified. A small portion of this crude product (40 mg, 105 μmol) was dissolved in methanol (4 mL) and cooled to −78 °C, then ozone was bubbled through the reaction mixture for 15 min. Excess ozone was removed by passing an oxygen stream through the reaction mixture and subsequently trimethylphosphite (20 μL, 165 μmol,

1.5 equiv) was added, and the reaction mixture was allowed to come to room temperature. After removal of the solvent under diminished pressure and purification of the residue by RP-18 HPLC (system III; 16% H_3CCN, t_R = 6.2 min), the free aldehyde **8b** (11 mg, 29 μmol, 28%) was obtained as a colorless oil: TLC: R_f = 0.05 (dichloromethane/methanol 5:1); $[\alpha]_D^{30}$ = +34.1 (c = 0.6, DMSO); ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 1.74 (tt, $^3J_{10',11'}$ = 6.6, $^3J_{11',12'}$ = 7.2 Hz, 2H; 2*H*-11′), 2.44 (dt, $^3J_{11',12'}$ = 7.2, $^3J_{12',13'}$ = 1.5 Hz, 2H; 2*H*-12′), 3.38 (t, $^3J_{10',11'}$ = 6.6 Hz, 2H; 2*H*-10′), 3.50–3.70 (m, 18H; *H*-2, *H*-3, *H*-4, *H*-5, *H*-6a, *H*-6b, 2*H*-1′, 2*H*-2′, 2*H*-4′, 2*H*-5′, 2*H*-7′, 2*H*-8′), 4.44 (t, $^3J_{OH,6a} \approx {}^3J_{OH,6b}$ = 6.0 Hz, 1H; 6-O*H*), 4.58 (d, $^3J_{OH,CH}$ = 5.7 Hz, 1H; O*H*), 4.61 (d, $^3J_{1,2}$ = 1.3 Hz, 1H; *H*-1), 4.69–4.75 (m, 2H; 2O*H*), 9.65 ppm (t, $^3J_{12',13'}$ = 1.5 Hz, 1H; *H*-13′); ^{13}C NMR (150.8 MHz, [D$_6$]DMSO): δ = 21.88, 39.85, 61.05, 65.50, 66.73, 69.25, 69.30, 69.57, 69.60, 69.61, 70.08, 70.72, 73.75, 99.74, 203.06 ppm; $C_{16}H_{30}O_{10}$ (382.4); MALDI MS (pos. mode, DHB): [*M*+Na]$^+$ calcd: 405.4; found: 405.6; [*M*+K]$^+$ calcd: 421.5; found: 421.6.

10-Carboxyl-3,6,9-trioxadecyl α-D-mannopyranoside (8c): Compound **7c** (850 mg, 1.54 mmol) was dissolved in water/dioxane (3:1, 30 mL), and lithium hydroxide solution (1 mol L^{-1}, 100 μL) was added. The reaction mixture was stirred at room temperature for 90 min. The reaction mixture was neutralized with ion-exchange resin (DOWEX H$^+$ form), filtered, and concentrated. Upon lyophilization, the free acid **8c** (550 mg, 1.49 mmol, 97%) was obtained as an amorphous solid: TLC: R_f = 0.07 (trichloromethane/methanol 2:1); $[\alpha]_D^{20}$ = +39.4 (c = 1.0, methanol); ^{1}H NMR (250 MHz, [D$_4$]methanol): δ = 3.55–3.89 (m, 18H; *H*-2, *H*-3, *H*-4, *H*-5, *H*-6a, *H*-6b, 2*H*-1′, 2*H*-2′, 2*H*-4′, 2*H*-5′, 2*H*-7′, 2*H*-8′), 4.11 (s, 2H; 2*H*-10′), 4.80 ppm (d, $^3J_{1,2}$ = 1.7 Hz, 1H; *H*-1); ^{13}C NMR (62.8 MHz, [D$_4$]methanol): δ = 63.38, 68.22, 69.06, 69.87, 71.82, 71.95, 72.01 (2C), 72.10, 72.54, 73.00, 75.04, 102.18, 175.11 ppm; $C_{14}H_{26}O_{11}$ (370.3); MALDI MS (pos. mode, DHB): [*M*+Li]$^+$ calcd: 377.2; found: 377.1; [*M*+Na]$^+$ calcd: 393.4; found: 393.0; [*M*+K]$^+$ calcd: 409.5; found: 409.0.

12-Formyl-3,6,9-trioxadodecyl 2-deoxy-2-acetylamino-β-D-glucopyranoside (12): Compound **12** was prepared from the fully *O*-acetylated precursor **11** as described for compound **8b**. After removal of the solvent under diminished pressure and purification of the residue by RP-18 HPLC (system III; 12% H_3CCN, t_R = 9.9 min), the free aldehyde **12** (12 mg, 28 μmol, 16%) was obtained as a colorless oil: TLC: R_f = 0.58 (dichloromethane/methanol 2:1); $[\alpha]_D^{20}$ = −22.1 (c = 0.4, DMSO); ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 1.74 (tt, $^3J_{10',11'}$ = 6.6, $^3J_{11',12'}$ = 7.2 Hz, 2H; *H*-11′), 1.78 (s, 3H; NAc), 2.44 (dt, $^3J_{11',12'}$ = 7.2, $^3J_{12',13'}$ = 1.5 Hz, 2H; *H*-12′), 3.02–3.10 (m, 2H; *H*-4, *H*-5), 3.24–3.55 (m, 16H; *H*-2, *H*-3, *H*-6a, *H*-1′a, 2*H*-2′, 2*H*-4′, 2*H*-5′, 2*H*-7′, 2*H*-8′, 2*H*-10′), 3.64–3.68 (m, 1H; *H*-6b), 3.76–3.81 (1 m, 1H; *H*-1′b), 4.31 (d, $^3J_{1,2}$ = 8.3 Hz, 1H; *H*-1), 4.51 (br s, 1H; 6-O*H*), 4.91 (br s, 1H; O*H*), 4.99 (br s, 1H; O*H*), 7.63 (d, $^3J_{NH,2}$ = 8.0 Hz, 1H; N*H*), 9.64 ppm (t, $^3J_{12',13'}$ = 1.5 Hz, 1H; *H*-13′); ^{13}C NMR (150.8 MHz, [D$_6$]DMSO): δ = 22.05, 23.03, 39.92, 55.35, 61.04, 67.76, 68.74, 69.42, 69.53, 69.71, 69.80, 69.83, 70.62, 74.33, 77.02, 101.00, 169.08, 203.17 ppm; $C_{18}H_{33}NO_{10}$ (423.5); MALDI MS (pos. mode, DHB): [*M*+Na]$^+$ calcd: 446.5; found: 446.5; [*M*+K]$^+$ calcd: 462.6; found: 462.5.

12-Formyl-3,6,9-trioxadodecyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (15): Compound **15** was prepared from the fully *O*-acetylated precursor **14** as described for compound **8b**. After removal of the solvent under diminished pressure and purification of the residue by RP-18 HPLC (system III; 15% H_3CCN, t_R = 6.3 min), the free aldehyde **15** (11 mg, 20 μmol, 36%) was obtained as a colorless oil: TLC: R_f = 0.20 (dichloromethane/methanol 3:1); $[\alpha]_D^{20}$ = −13.6 (c = 0.4, DMSO); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 1.74 (tt, $^3J_{10'',11''}$ = 6.6, $^3J_{11'',12''}$ = 7.2 Hz, 2H; 2*H*-11″), 2.45 (dt, $^3J_{11'',12''}$ = 7.4, $^3J_{12'',13''}$ = 1.5 Hz, 2H; 2*H*-12″), 3.00 (dd, $^3J_{1',2'} \approx {}^3J_{2',3'}$ = 7.1 Hz, 1H; *H*-2′), 3.25–3.85 (m, 25H; *H*-2, *H*-3, *H*-4, *H*-5, *H*-6a, *H*-6b, *H*-3′, *H*-4′, *H*-5′, *H*-6′a, *H*-6′b, 2*H*-1″, 2*H*-2″, 2*H*-4″, 2*H*-5″, 2*H*-7″, 2*H*-8″, 2*H*-10″), 4.21 (2×d, 2H; $^3J_{1,2}$ = 7.8, $^3J_{1',2'}$ = 7.2 Hz, *H*-1, *H*-1′), 4.58 (br s, 1H; O*H*), 4.67 (br s, 1H; O*H*), 5.10 (br s, 1H; O*H*), 9.65 ppm (t, $^3J_{12'',13''}$ = 1.5 Hz, 1H; *H*-13″); ^{13}C NMR (100.5 MHz, [D$_6$]DMSO): δ = 21.67, 39.66, 60.00, 60.01, 67.63, 67.74, 69.06, 69.28, 69.34, 69.41, 70.13, 72.68, 72.72, 72.84, 74.44, 74.60, 75.14, 80.35, 80.60, 102.28, 103.47, 202.92 ppm; $C_{22}H_{40}O_{15}$ (544.2); MALDI MS (pos. mode, DHB): [*M*+Na]$^+$ calcd: 567.2; found: 567.8; [*M*+K]$^+$ calcd: 583.3; found: 583.9.

Protocol for the immobilization of carbohydrates onto glass surfaces by reductive amination: From stock solutions of carbohydrate compound (aldehyde- or amino-derivatized), dilution series were prepared in β-morpholinoethanesulfonic acid (MES) buffer (0.1 mol L^{-1}, pH 6.8) containing the carbohydrate in a 1.25-fold excess of the desired concentration. Immediately before usage sodium cyanoborohydride solution (100 mg in 50 mL of water) was added (1:4 v/v) to the dilution series.[42]

Glass slides bearing amino- or aldehyde-derivatized glass surfaces (commercially available from Genetix) were rinsed with acetone and patted dry with tissues. Press-to-Seal silicon isolators (Schleicher & Schuell, no. 10485006) were affixed to the cleaned glass slide and pressure was applied to ensure the removal of air between glass slide and isolator. The prepared solutions were spotted onto the slide (4 μL per well), which was then sealed with a microscope slide and placed in a humidity chamber at room temperature. After 12–16 h, the slide was taken from the humidity chamber, the microscope slide was removed, and the spotting solutions were removed from the wells by suction. Subsequently, the wells were rinsed with SDS solution (0.2% in water, 2×5 μL). The silicon isolator was removed, and the whole slide was subjected to ultrasonic agitation in SDS solution (0.2% in water) for ten minutes, rinsed with distilled water, and dried with tissues.

Protocol for the immobilization of carbohydrates onto glass surfaces by amide-bond formation: From stock solutions, three dilution series were prepared in DMF: A contained the carbohydrate compound (carboxylate-derivatized) in a 3-fold excess of the desired concentration, B contained PyBOP (in a 3.3-fold excess), and C contained Hünig's Base (in 9-fold excess), respectively. Immediately before usage solutions A–C were mixed for each desired concentration (1:1:1 v/v).[42]

Glass slides bearing amino-derivatized glass surfaces (commercially available from Genetix) were rinsed with acetone and patted dry with tissues. Press-to-Seal silicon isolators (Schleicher & Schuell, no. 10485006) were affixed to the cleaned glass slide and pressure was applied to ensure the removal of air between glass slide and isolator. The prepared solutions were spotted onto the slide (4 μL per well), which was then sealed with a microscope slide and placed in a sealed environment at room temperature. After 12 h, the microscope slide was removed and the spotting solutions were removed from the wells by suction. Subsequently the wells were rinsed with SDS solution (0.2% in water, 2×5 μL). The silicon isolator was removed, and the whole slide was subjected to ultrasonic agitation in SDS solution (0.2% in water) for 10 min, rinsed with distilled water, and dried with tissues.

Protocol for hybridization experiments with fluorescence-labeled lectins: For hybridization experiments, rhodamine-labeled lectins were purchased from Vector Laboratories. The hybridization solutions were prepared by diluting the stock solutions to a concentration of 1 μg mL^{-1} with PBS buffer (pH 7.5; 1 mmol L^{-1} $CaCl_2$, 1 mmol L^{-1} $MnCl_2$, 0.1% Tween 20). Prepared glass slides were fully

submerged in the lectin solution and gently shaken for 1 h. After hybridization, the slide was washed in PBS buffer containing no lectin with gentle shaking for 10–30 min, rinsed with distilled water, and patted dry with tissues.

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Keywords: arrays · carbohydrates · covalent ligation · fluorescent probes · lectins

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