ARTICLES

Carbamate-Linked Lactose: Design of Clusters and Evidence for Selectivity to Block Binding of Human Lectins to (Neo)Glycoproteins with Increasing Degree of Branching and to Tumor Cells

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Various pathogenic processes are driven by protein(lectin)-glycan interactions, especially involving β -galactosides at branch ends of cellular glycans. These emerging insights fuel the interest to design potent inhibitors to block lectins. As a step toward this aim, we prepared a series of ten mono- to tetravalent glycoconjugates with lactose as a common headgroup. To obtain activated carbonate for ensuing carbamate formation, conditions for the facile synthesis of pure isomers from anomERICALLY unprotected lactose were identified. To probe for the often encountered intrafamily diversity of human lectins, we selected representative members from the three subgroups of adhesion/growth-regulatory galectins as receptors. Diversity of the glycan display was accounted for by using four (neo)glycoproteins with different degrees of glycan branching as matrices in solid-phase assays. Cases of increased inhibitory potency of lactose clusters compared to free lactose were revealed. Extent of relative inhibition was not directly associated with valency in the glycoconjugate and depended on the lectin type. Of note for screening protocols, efficacy of blocking appeared to decrease with increased degree of glycan branching in matrix glycoproteins. Binding to tumor cells was impaired with selectivity for galectins-3 and -4. Representative compounds did not impair growth of carcinoma cells up to a concentration of 5 mM of lactose moieties (valence-corrected value) per assay. The reported bioactivity and the delineation of its modulation by structural parameters of lectins and glycans set instructive examples for the further design of selective inhibitors and assay procedures.

INTRODUCTION

A salient route of biological information transfer starts from glycan determinants of cellular glycoconjugates (1). These sugar-encoded signals act as ligands for target-specific receptors (lectins) (1, 2). Physiologically, this interaction is involved in disease manifestation, e.g., during chronic inflammation, heart failure, or malignancy. Thus, the design of glycoclusters blocking target recognition of lectins has become an active research area (2–4). In view of their role as markers for unfavorable prognosis in human tumors (5–7), we focus here on the family of adhesion/growth-regulatory galectins.

Galectins share the specificity for β -galactosides at branch ends of glycans so that glycoclusters can conveniently be loaded with lactose as a common headgroup (8). Of note, the spatial presentation of lectin sites differs within this family (2). Thus, it is mandatory to examine at least one member of each subgroup

in a comparative study. Hence, the following representatives were chosen: the homodimeric (prototype) galectin-1, the chimera-type galectin-3 with the ability for pentamerization in the presence of suitable ligands, the tandem-repeat-type galectin-4 with its two domains connected by a linker, and galectin-5 with its low degree of oligomer formation. Aside from the panel of lectins, it is equally important to take account of the local density of ligand. As work with different (neo)glycoproteins has revealed (9, 10), lectin affinity and selectivity can be modulated by glycan substitutions and branching.

In detail, four different types of binding partner for the lectins were thus tested: lactosylated bovine serum albumin (LacBSA), a neoglycoprotein with rather random and widely spaced presentation of lactose units, and three glycoproteins with either biantennary (serum amyloid P component: SAP), triantennary (asialofetuin: ASF), or tetra-antennary N-glycans (α_1 -acid glycoprotein: AGP). The listed (neo)glycoproteins were adsorbed to microtiter plate wells to serve as lectin binders. With these surfaces, solid-phase assays were carried out to determine inhibition of binding of the lectins to the surfaces by lactose or synthetic lactose clusters. Natural ligands on cell surfaces are known to be present in more complex form than these lactose derivatives (11, 12). Since it is an open question whether the glycoclusters are capable of impairing the binding of lectins to cell surfaces, we performed fluorimetric binding assays with

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human tumor cells. In addition to the galectins, a toxic plant lectin akin to ricin, i.e., the galactoside-specific mistletoe lectin (*Viscum album* agglutinin, VAA) with β -trefoil folding and two fully accessible lectin sites 87 Å apart in the dimer (13), was also tested.

The lactose derivatives that were tested for their potency to inhibit the lectin–matrix interaction varied in valency from one to four lactose moieties per inhibitor. However, the valency of the ligands is not the only factor that determines avidity. Case studies on selectins with a lipid anchor (i.e., 2-tetradecylhexadecyl) attached to the glycan, the cholera toxin B pentamer with spaced *p*-nitrophenyl galactopyranoside, and galectins with thiourea-linked ligands on calix[n]arenes showed that the spacer portion can notably contribute to the inhibitor's performance (14–16). Variations in this part can add to selectivity, because the vicinity of the lectin sites is quite divergent in galectins (12, 17). Therefore, we employed two series of lactose clusters differing in the absence or presence of a butene diol linker leading to varied lactose spacing. Lactosyl carbamates were conveniently synthesized by coupling an activated lactosyl carbonate to a set of amines. Therefore, we first had to resolve the problem of finding conditions to produce the glycosyl carbonate from anomerically deprotected lactose as pure isomer.

Our report, combining synthesis of ten mono- to tetravalent lactose derivatives with biochemical/cell biological testing on plant/human lectins, will answer the following questions: (1) Which conditions are suitable to gain fast and easy access to pure isomers of activated lactosyl carbonates? (2) Will the inhibitory potency of conjugated lactose relative to free lactose be enhanced? (3) How will the structure and valency of the lactose clusters and the degree of glycan branching in the (neo)glycoprotein matrix influence inhibition in solid-phase assays? (4) Will lectin binding to cells be inhibited by the lactose derivatives? (5) Will potent lactose derivatives lack cytotoxicity?

EXPERIMENTAL PROCEDURES

General Methods. Analytical thin layer chromatography was performed using silica gel 60 F₂₅₄ aluminum sheets from Merck (Darmstadt, Germany); compound spots were visualized by quenching of fluorescence and/or by charring after treatment with cerium molybdophosphate. Preparative flash column chromatography (FC) was carried out on Merck silica gel 60 (40–63 μ m). ¹H NMR and ¹³C NMR spectra were recorded on a Bruker Avance DRX 600 instrument at a temperature of 300 K. ¹H Chemical shifts are referenced to residual protic solvent (CDCl₃, $\delta_{\text{H}} = 7.26$; D₂O, $\delta_{\text{H}} = 4.63$). ¹³C Chemical shifts are referenced to the solvent signal (CDCl₃, $\delta_{\text{C}} = 77.0$) or in the case of D₂O to internal standard CD₃CN ($\delta_{\text{C}} = 1.32$ ppm). Assignments of proton and carbon signals were carried out by the aid of DQF-COSY, HMQC, and ROESY experiments. MALDI mass spectra were obtained on a Bruker Biflex III instrument with α -cyano-4-hydroxycinnamic acid as the matrix (positive mode). Elemental analyses were performed by the microanalytical facility at the Universität Konstanz.

4-Nitrophenyl-[2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-acetyl- α -D-glucopyranosyl] Carbonate (18) (α -3). Lactose derivative **1** (19) (100 mg, 0.16 mmol) was dissolved in CH₃CN (3.5 mL). A solution of *p*-nitrophenyl chloroformate **2** (48 mg, 0.24 mmol) in CH₃CN (0.5 mL) and 4-(dimethylamino)-pyridine (29 mg, 0.24 mmol) was added and the mixture was stirred for 30 min. The solvent was evaporated, and the remainder was dissolved in ethyl acetate and washed with water, 1 M HCl, and brine. The organic layer was dried (Na₂SO₄), concentrated, and purified by FC (petroleum ether/ethyl acetate 1:1 $\rightarrow$ 1:2) to yield α -3 (122 mg, 95%) as a white solid: $R_{\text{f}} = 0.45$ (petroleum ether/ethyl acetate 1:2). ¹H NMR (600 MHz, CDCl₃): $\delta = 8.30$ – 8.28 (m, 2 H, Ar), 7.43–7.42

(m, 2 H, Ar), 6.20 (d, $J = 3.6$ Hz, 1 H, H-1^{Glc}), 5.54 (dd, $J = 10.1$ Hz, $J = 9.5$ Hz, 1 H, H-3^{Glc}), 5.35 (dd, $J = 3.3$ Hz, $J = 0.7$ Hz, 1 H, H-4^{Gal}), 5.12 (dd, $J = 10.4$ Hz, $J = 7.9$ Hz, 1 H, H-2^{Gal}), 5.07 (dd, $J = 10.3$ Hz, $J = 3.6$ Hz, 1 H, H-2^{Glc}), 4.96 (dd, $J = 10.4$ Hz, $J = 3.5$ Hz, 1 H, H-3^{Gal}), 4.53–4.51 (m, 2 H, H-6^{Glc}, H-1^{Gal}), 4.17–4.06 (m, 4 H, H-5^{Glc}, H-6^{Glc}, H-6^{Gal}, H-6^{Gal}), 3.91–3.87 (m, 2 H, H-4^{Glc}, H-5^{Gal}), 2.15 (s, 3 H, C(O)CH₃), 2.13 (s, 3 H, C(O)CH₃), 2.07 (s, 3 H, C(O)CH₃), 2.05 (s, 3 H, C(O)CH₃), 2.04 (s, 3 H, C(O)CH₃), 1.95 (s, 3 H, C(O)CH₃). ¹³C NMR (150 MHz, CDCl₃): $\delta = 170.2$, 170.1, 170.0, 170.0, 169.7, 169.4, 168.9 (C(O)CH₃), 154.9 (OC(O)O), 150.7, 145.6, 125.4, 121.4 (Ar), 101.0 (C-1^{Gal}), 94.0 (C-1^{Glc}), 75.2 (C-4^{Glc}), 71.3 (C-5^{Glc}), 70.9 (C-3^{Gal}), 70.7 (C-5^{Gal}), 79.3 (C-2^{Glc}), 69.1 (C-3^{Glc}), 69.0 (C-2^{Gal}), 66.5 (C-4^{Gal}), 61.1 (C-6^{Glc}), 60.7 (C-6^{Gal}), 20.7–20.4 (C(O)CH₃). MALDI-MS: calcd m/z 824.2 [M + Na⁺], 840.2 [M + K⁺], found 824.0, 839.9. Anal. calcd for C₃₃H₃₉NO₂₂: C 49.44, H 4.90, N 1.75, found: C 49.24, H 4.98, N 1.76.

4-Nitrophenyl-[2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-acetyl- β -D-glucopyranosyl] Carbonate (18) (β -3). Lactose derivative **1** (19) (8.6 g, 13.5 mmol) was dissolved in CH₃CN (430 mL) and cooled to 0 °C. A solution of *p*-nitrophenyl chloroformate **2** (2.89 g, 14.3 mmol) in CH₃CN (20 mL) and 2,6-lutidine (1.66 mL, 14.3 mmol) was added, and the mixture was stirred overnight. The solvent was evaporated, and the remainder was dissolved in ethyl acetate and washed with water, 1 M HCl, and brine. The organic layer was dried (Na₂SO₄), concentrated, and crystallized from ethyl acetate/petroleum ether to yield β -3 (5.40 g, 50%) as a white solid: $R_{\text{f}} = 0.47$ (petroleum ether/ethyl acetate 1:2). ¹H NMR (600 MHz, CDCl₃): $\delta = 8.30$ – 8.28 (m, 2 H, Ar), 7.42–7.40 (m, 2 H, Ar), 5.63 (d, $J = 7.8$ Hz, 1 H, H-1^{Glc}), 5.35 (dd, $J = 3.4$ Hz, $J = 0.8$ Hz, 1 H, H-4^{Gal}), 5.26 (t, $J = 9.0$ Hz, 1 H, H-3^{Glc}), 5.15–5.10 (m, 2 H, H-2^{Glc}, H-2^{Gal}), 4.96 (dd, $J = 10.4$ Hz, $J = 3.2$ Hz, 1 H, H-3^{Gal}), 4.53–4.49 (m, 2 H, H-6^{Glc}, H-1^{Gal}), 4.16–4.06 (m, 3 H, H-6^{Glc}, H-6^{Gal}, H-6^{Gal}), 3.94–3.88 (m, 2 H, H-4^{Glc}, H-5^{Gal}), 3.83–3.81 (m, 1 H, H-5^{Glc}), 2.16 (s, 3 H, C(O)CH₃), 2.13 (s, 3 H, C(O)CH₃), 2.09 (s, 3 H, C(O)CH₃), 2.07 (s, 3 H, C(O)CH₃), 2.05 (s, 3 H, C(O)CH₃), 2.04 (s, 3 H, C(O)CH₃), 1.97 (s, 3 H, C(O)CH₃). ¹³C NMR (150 MHz, CDCl₃): $\delta = 170.4$, 170.2, 170.1, 169.6, 169.4, 169.0 (C(O)CH₃), 154.7 (OC(O)O), 150.7, 145.7, 125.4, 121.6 (Ar), 101.0 (C-1^{Gal}), 95.7 (C-1^{Glc}), 75.3 (C-4^{Glc}), 73.7 (C-5^{Glc}), 72.6 (C-3^{Glc}), 71.0 (C-3^{Gal}), 70.7 (C-5^{Gal}), 70.2 (C-2^{Glc}), 69.0 (C-2^{Gal}), 66.5 (C-4^{Gal}), 61.4 (C-6^{Glc}), 60.8 (C-6^{Gal}), 20.8–20.5 (C(O)CH₃). MALDI-MS: calcd m/z 824.2 [M + Na⁺], 840.2 [M + K⁺], found 824.1, 840.0. Anal. calcd for C₃₃H₃₉NO₂₂: C 49.44, H 4.90, N 1.75, found: C 48.98, H 5.22, N 1.82.

4-Hydroxy-(*Z*)-but-2-enyl 2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-acetyl- β -D-glucopyranoside (6). Under argon Ag₂CO₃ (2.7 g, 9.7 mmol), CaSO₄·0.5 H₂O (6.5 g, 45 mmol), an iodine crystal, and (*Z*)-but-2-ene-1,4-diol **5** (26 mL, 322 mmol) were stirred for 15 min at room temperature. Acetobromolactose **4** (20, 21) (3 g, 4.3 mmol) was added and the mixture was stirred for further 24 h. After addition of CH₂Cl₂ (100 mL), the mixture was filtered through Celite. To the biphasic filtrate water (100 mL) was added, and the aqueous phase was separated and extracted with CH₂Cl₂ (50 mL). The combined organic layers were washed with water (3 $\times$ 100 mL) and brine and dried over Na₂SO₄. The solvent was evaporated and the remainder purified by FC (petroleum ether/ethyl acetate 1:2) to give **6** (1.85 g, 61%): $R_{\text{f}} = 0.25$ (petroleum ether/ethyl acetate 1:2). ¹H NMR (600 MHz, CDCl₃): $\delta = 5.85$ – 5.81 (m, 1 H, H^{vinyl}), 5.63–5.59 (m, 1 H, H^{vinyl}), 5.34 (d, $J = 3.0$ Hz, 1 H, H-4^{Gal}), 5.19 (d, $J = 9.3$ Hz, 1 H, H-3^{Glc}), 5.10 (dd, $J = 10.4$ Hz, $J = 7.9$ Hz, 1 H, H-2^{Gal}), 4.95 (dd, $J = 10.4$ Hz, $J = 3.5$ Hz, 1 H, H-3^{Gal}), 4.90 (dd, $J = 9.4$ Hz, $J = 8.0$ Hz, 1 H,

H-2^{Glc}), 4.53–4.50 (m, 2 H, H-1^{Glc}, H-6^{Glc}), 4.49 (d, $J = 7.9$ Hz, 1 H, H-1^{Gal}), 4.28 (ddd, $J = 19.9$ Hz, $J = 12.6$ Hz, $J = 6.7$ Hz, 2 H, H^{allyl}), 4.18 (bd, $J = 6.2$ Hz, 2 H, H^{allyl}), 4.14–4.06 (m, 3 H, H-6^{Glc}, H-6^{Gal}, H-6^{Glc}), 3.87 (bt, $J = 6.7$ Hz, 1 H, H-5^{Gal}), 3.79 (t, $J = 9.5$ Hz, 1 H, H-4^{Glc}), 3.61 (ddd, $J = 9.8$ Hz, $J = 5.0$ Hz, $J = 2.0$ Hz, 2 H, H-5^{Glc}), 2.14 (s, 3 H, C(O)CH₃), 2.13 (s, 3 H, C(O)CH₃), 2.05 (s, 3 H, C(O)CH₃), 2.04 (s, 9 H, C(O)CH₃), 1.96 (s, 3 H, C(O)CH₃). ¹³C NMR (150 MHz, CDCl₃): $\delta = 170.4, 170.3, 170.1, 170.0, 169.7, 169.7, 169.0$ (C(O)CH₃), 133.3, 126.8 (CH^{olefin}), 125.3, 121.7 (Ar), 101.0 (C-1^{Gal}), 99.1 (C-1^{Glc}), 76.2 (C-4^{Glc}), 72.8 (C-3^{Glc}), 72.7 (C-5^{Glc}), 71.6 (C-2^{Glc}), 71.0 (C-3^{Gal}), 70.7 (C-5^{Gal}), 69.1 (C-2^{Gal}), 66.6 (C-4^{Gal}), 64.3 (CH₂^{allyl}), 61.9 (C-6^{Glc}), 60.8 (C-6^{Gal}), 58.5 (CH₂^{allyl}), 20.8–20.5 (C(O)CH₃).

4-(*p*-Nitrophenoxy carbonyloxy)-(Z)-but-2-enyl 2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-acetyl- β -D-glucopyranoside (7). Compound **6** (1.72 g, 2.43 mmol) was dissolved in CH₃CN (80 mL). A solution of *p*-nitrophenyl chloroformate **2** (0.59 g, 2.92 mmol) in CH₃CN (5 mL) and pyridine (0.24 mL, 2.92 mmol) were added, and the mixture was stirred for 1 h. The solvent was evaporated and the remainder was dissolved in ethyl acetate and washed with water, 1 M HCl, and brine. The organic layer was dried (Na₂SO₄), concentrated, and purified by FC (petroleum ether/ethyl acetate 1:1 $\rightarrow$ 1:2) to yield **7** (1.84 g, 87%) as a white solid: $R_f = 0.50$ (petroleum ether/ethyl acetate 1:2). ¹H NMR (600 MHz, CDCl₃): $\delta = 8.29$ – 8.28 (m, 2 H, Ar), 7.39–7.37 (m, 2 H, Ar), 5.83–5.78 (m, 2 H, H^{vinyl}), 5.34 (d, $J = 3.0$ Hz, 1 H, H-4^{Gal}), 5.19 (d, $J = 9.3$ Hz, 1 H, H-3^{Glc}), 5.09 (dd, $J = 10.4$ Hz, $J = 7.9$ Hz, 1 H, H-2^{Gal}), 4.95 (dd, $J = 10.4$ Hz, $J = 3.4$ Hz, 1 H, H-3^{Gal}), 4.90 (dd, $J = 9.4$ Hz, $J = 8.0$ Hz, 1 H, H-2^{Glc}), 4.87–4.79 (m, 2 H, H^{allyl}), 4.52 (d, $J = 7.8$ Hz, 1 H, H-1^{Glc}), 4.51 (dd, $J = 11.8$ Hz, $J = 2.1$ Hz, 1 H, H-6^{Glc}), 4.48 (d, $J = 7.9$ Hz, 1 H, H-1^{Gal}), 4.34 (ddd, $J = 18.6$ Hz, $J = 13.3$ Hz, $J = 4.2$ Hz, 2 H, H^{allyl}), 4.14–4.05 (m, 3 H, H-6^{Glc}, H-6^{Gal}, H-6^{Glc}), 3.86 (bt, $J = 6.9$ Hz, 1 H, H-5^{Gal}), 3.80 (t, $J = 9.4$ Hz, 1 H, H-4^{Glc}), 3.60 (ddd, $J = 9.8$ Hz, $J = 4.7$ Hz, $J = 2.0$ Hz, 1 H, H-5^{Glc}), 2.14 (s, 3 H, C(O)CH₃), 2.11 (s, 3 H, C(O)CH₃), 2.05 (s, 3 H, C(O)CH₃), 2.04 (s, 3 H, C(O)CH₃), 2.03 (s, 3 H, C(O)CH₃), 2.01 (s, 3 H, C(O)CH₃), 1.96 (s, 3 H, C(O)CH₃). ¹³C NMR (150 MHz, CDCl₃): $\delta = 170.3, 170.3, 170.1, 170.0, 169.7, 169.6, 169.0$ (C(O)CH₃), 155.4 (OC(O)O), 152.3, 145.5 (Ar), 130.8, 126.0 (Olefin CH), 125.3, 121.7 (Ar), 101.1 (C-1^{Gal}), 99.2 (C-1^{Glc}), 76.2 (C-4^{Glc}), 72.9 (C-3^{Glc}), 72.7 (C-5^{Glc}), 71.5 (C-2^{Glc}), 70.9 (C-3^{Gal}), 70.7 (C-5^{Gal}), 69.1 (C-2^{Gal}), 66.5 (C-4^{Gal}), 64.5, 64.4 (CH₂^{allyl}), 61.8 (C-6^{Glc}), 60.7 (C-6^{Gal}), 20.8–20.5 (C(O)CH₃). MALDI-MS: calcd m/z 894.2 [M + Na⁺], 910.2 [M + K⁺], found 894.0, 910.0. Anal. calcd for C₃₇H₄₅NO₂₃: C 50.98, H 5.20, N 1.61, found: C 50.80, H 5.23, N 1.61.

General Procedure 1 (GP 1) for Coupling of *p*-Nitrophenyl Carbonates to Primary Amines. The amine and *i*-Pr₂NEt (1 equiv per amino group) were added to a solution of activated carbonate β -3 or **7** (1.1–1.3 equiv per amino group) in dry CH₂Cl₂ (3–10 mL per mmol of activated carbonate). After complete reaction (usually after few minutes), the solvent was removed under reduced pressure and the product was purified by FC.

General Procedure 2 (GP 2) for Deacetylation with NaOMe/MeOH. The acetylated compound was dissolved in MeOH (10–30 mL per mmol) and a 0.5 M solution of NaOMe in MeOH (0.05–0.25 mL per mL of reaction mixture) was added. After complete reaction, any precipitate formed was dissolved by addition of water or MeOH. The solution was neutralized by addition of acidic ion-exchange resin (Dowex 50W-X8 or Amberlite IRC-86, H⁺ form) and filtered. The filtrate was concentrated and lyophilized.

General Procedure 3 (GP 3) for Deacetylation with EtNMe₂/MeOH. The acetylated compound was stirred with a 1:5 mixture of EtNMe₂/MeOH (10–30 mL per mmol). After complete reaction, the mixture was concentrated and the remaining product was then dried under vacuum and lyophilized.

(2,3,4,6-Tetra-*O*-acetyl- β -D-galactopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-acetyl- β -D-glucopyranosyloxycarbonyl)benzylamine (Ac-A). According to GP 1, β -3 (115 mg, 0.15 mmol) and benzylamine **8** (14 mg, 0.13 mol) were reacted in CH₂Cl₂ (3 mL). Purification by FC (petroleum ether/ethyl acetate 1:1) gave Ac-A (100 mg, 99%) as a white foam: $R_f = 0.45$ (petroleum ether/ethyl acetate 1:2). ¹H NMR (600 MHz, CDCl₃): $\delta = 7.35$ – 7.27 (m, 5 H, Ar), 5.67 (d, $J = 8.3$ Hz, 1 H, H-1^{Glc}), 5.35 (d, $J = 2.3$ Hz, 1 H, H-4^{Gal}), 5.25 (t, $J = 9.0$ Hz, 1 H, H-3^{Glc}), 5.17–5.10 (m, 2 H, H-2^{Gal}, NHC(O)), 5.02 (t, $J = 8.7$ Hz, 1 H, H-2^{Glc}), 4.95 (dd, $J = 10.4$ Hz, $J = 3.4$ Hz, 1 H, H-3^{Gal}), 4.48–4.45 (m, 2 H, H-6^{Glc}, H-1^{Gal}), 4.38–4.36 (m, 2 H, CH₂^{Bn}), 4.17–4.07 (m, 3 H, H-6^{Glc}, H-6^{Gal}, H-6^{Glc}), 3.88–3.83 (m, 2 H, H-4^{Glc}, H-5^{Gal}), 3.78–3.76 (m, 1 H, H-5^{Glc}), 2.16 (s, 3 H, C(O)CH₃), 2.12 (s, 3 H, C(O)CH₃), 2.07 (s, 3 H, C(O)CH₃), 2.05 (s, 6 H, C(O)CH₃), 2.02 (s, 3 H, C(O)CH₃), 1.97 (s, 3 H, C(O)CH₃), 1.97 (s, 3 H, C(O)CH₃). ¹³C NMR (150 MHz, CDCl₃): $\delta = 170.3, 170.2, 170.1, 169.7, 169.5, 169.0$ (C(O)CH₃), 153.7 (OC(O)N), 137.4, 128.8, 127.8, 127.6 (Ar), 101.0 (C-1^{Gal}), 92.8 (C-1^{Glc}), 75.8 (C-4^{Glc}), 73.3 (C-5^{Glc}), 72.6 (C-3^{Glc}), 71.0 (C-3^{Gal}), 70.7 (C-5^{Gal}), 70.6 (C-2^{Glc}), 69.0 (C-2^{Gal}), 66.6 (C-4^{Gal}), 61.8 (C-6^{Glc}), 60.8 (C-6^{Gal}), 45.2 (CH₂^{Bn}), 20.9–20.5 (C(O)CH₃). MALDI-MS: calcd m/z 792.2 [M + Na⁺], 808.2 [M + K⁺], found 792.4, 808.4. Anal. calcd for C₃₄H₄₃NO₁₉: C 53.05, H 5.63, N 1.82, found: C 53.03, H 5.66, N 1.83.

(β -D-Galactopyranosyl-(1 $\rightarrow$ 4)- β -D-glucopyranosyloxycarbonyl)benzylamine (A). According to GP 2, Ac-A (100 mg, 0.13 mmol) was deacetylated with 0.5 M NaOMe solution (0.25 mL) in MeOH (3 mL) to give A (61 mg, 99%) as a white solid. ¹H NMR (600 MHz, D₂O): $\delta = 7.33$ – 7.25 (m, 5 H, Ar), 5.36 (d, $J = 8.2$ Hz, 1 H, H-1^{Glc}), 4.35 (d, $J = 7.9$ Hz, 1 H, H-1^{Gal}), 4.26 (m, 2 H, CH₂^{Bn}), 3.86–3.82 (m, 2 H, H-4^{Gal}, H-6^{Glc}), 3.73–3.56 (m, 8 H, H-3^{Glc}, H-3^{Gal}, H-4^{Glc}, H-5^{Glc}, H-5^{Gal}, H-6^{Glc}, H-6^{Gal}, H-6^{Glc}), 3.47–3.40 (m, 2 H, H-2^{Glc}, H-2^{Gal}). ¹³C NMR (150 MHz, D₂O): $\delta = 156.6$ (OC(O)N), 138.2, 128.8, 127.5, 127.1 (Ar), 102.9 (C-1^{Gal}), 94.8 (C-1^{Glc}), 77.8, 75.4, 75.3, 74.1, 72.5 (C-3^{Glc}, C-3^{Gal}, C-4^{Glc}, C-5^{Glc}, C-5^{Gal}), 71.7 (C-2^{Glc}), 70.9 (C-2^{Gal}), 68.6 (C-4^{Gal}), 61.0 (C-6^{Glc}), 59.8 (C-6^{Gal}), 44.2 (CH₂^{Bn}). MALDI-MS: calcd m/z 498.2 [M + Na⁺], 514.2 [M + K⁺], found 498.1, 514.1.

***N,N'*-Bis-(2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-acetyl- β -D-glucopyranosyloxycarbonyl)-*m*-xylylene-diamine (Ac-B).** According to GP 1, β -3 (123 mg, 0.15 mmol) and α,α' -diamino-*m*-xylene **9** (9.5 mg, 0.07 mmol) were reacted in CH₂Cl₂ (3 mL). Purification by FC (petroleum ether/ethyl acetate 1:2) gave Ac-B (102 mg, 99%) as a white foam: $R_f = 0.18$ (petroleum ether/ethyl acetate 1:2). ¹H NMR (600 MHz, CDCl₃): $\delta = 7.27$ – 7.25 (m, 1 H, Ar), 7.15–7.11 (m, 3 H, Ar), 5.62 (m, 4 H, H-1^{Glc}, NHC(O)), 5.33 (bs, 2 H, H-4^{Gal}), 5.23 (t, $J = 9.1$ Hz, 2 H, H-3^{Glc}), 5.09 (t, $J = 9.4$ Hz, 2 H, H-2^{Gal}), 5.00 (t, $J = 8.7$ Hz, 2 H, H-2^{Glc}), 4.94 (bd, $J = 10.2$ Hz, 2 H, H-3^{Gal}), 4.49–4.38 (m, 6 H, H-6^{Glc}, H-1^{Gal}, CH₂^{Bn}), 4.26 (dd, $J = 14.9$ Hz, $J = 4.5$ Hz, 2 H, CH₂^{Bn}), 4.18–4.03 (m, 6 H, H-6^{Glc}, H-6^{Gal}, H-6^{Glc}), 3.88–3.86 (m, 4 H, H-4^{Glc}, H-5^{Gal}), 3.73–3.72 (m, 2 H, H-5^{Glc}), 2.14 (s, 6 H, C(O)CH₃), 2.04 (s, 6 H, C(O)CH₃), 2.03 (s, 18 H, C(O)CH₃), 2.00 (s, 6 H, C(O)CH₃), 1.95 (s, 6 H, C(O)CH₃). ¹³C NMR (150 MHz, CDCl₃): $\delta = 170.4, 170.3, 170.1, 170.0, 169.8, 169.5, 169.0$ (C(O)CH₃), 153.9 (OC(O)N), 138.4, 129.0, 126.7, 125.7 (Ar), 100.9 (C-1^{Gal}), 92.8 (C-1^{Glc}), 75.7 (C-4^{Glc}), 73.4 (C-5^{Glc}), 72.7 (C-3^{Glc}), 71.0 (C-3^{Gal}), 70.6 (C-5^{Gal}), 70.6 (C-2^{Glc}), 69.0 (C-2^{Gal}), 66.5 (C-4^{Gal}), 61.6 (C-6^{Glc}),

60.6 (C-6^{Gal}), 44.8 (CH₂^{Bn}), 20.7–20.5 (C(O)CH₃). MALDI-MS: calcd *m/z* 1483.4 [M + Na⁺], 1499.4 [M + K⁺], found 1483.4, 1499.7. Anal. calcd for C₆₂H₈₀N₂O₃₈: C 50.96, H 5.52, N 1.92, found: C 50.80, H 5.70, N 1.88.

N,N'-Bis-(β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyloxycarbonyl)-m-xylenediamine (B). According to GP 2, Ac-B (102 mg, 0.07 mmol) was deacetylated with 0.5 M NaOMe solution (0.5 mL) in MeOH (2 mL) to give **B** (61 mg, quant) as a white solid. ¹H NMR (600 MHz, D₂O): δ = 7.30–7.28 (m, 1 H, Ar), 7.18–7.16 (m, 3 H, Ar), 5.35 (d, *J* = 8.2 Hz, 2 H, H-1^{Glc}), 4.36 (d, *J* = 7.7 Hz, 2 H, H-1^{Gal}), 4.25 (bs, 4 H, CH₂^{Bn}), 3.85–3.82 (m, 4 H, H-4^{Gal}, H-6^{Glc}), 3.72–3.60 (m, 14 H, H-3^{Glc}, H-4^{Glc}, H-5^{Glc}, H-5^{Gal}, H-6^{Glc}, H-6^{Gal}, H-6^{Gal}), 3.57–3.55 (m, 2 H, H-3^{Gal}), 3.46–3.40 (m, 4 H, H-2^{Glc}, H-2^{Gal}). ¹³C NMR (150 MHz, D₂O): δ = 156.6 (OC(O)N), 138.6, 129.1, 126.1, 126.0 (Ar), 102.9 (C-1^{Gal}), 94.9 (C-1^{Glc}), 77.8, 75.4, 75.4, 74.1, 72.5 (C-3^{Glc}, C-3^{Gal}, C-4^{Glc}, C-5^{Glc}, C-5^{Gal}), 71.7 (C-2^{Glc}), 71.0 (C-2^{Gal}), 68.6 (C-4^{Gal}), 61.1 (C-6^{Gal}), 59.8 (C-6^{Glc}), 44.0 (CH₂^{Bn}). MALDI-MS: calcd *m/z* 895.3 [M + Na⁺], 911.3 [M + K⁺], found 895.2, 911.1.

1,4-Bis-(3-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxycarbonylaminopropoxy)butane (Ac-C). According to GP 1, β-3 (148 mg (0.188 mmol) and 1,4-bis-(3-aminopropoxy)butane **11** (16 μL, 0.075 mmol) were reacted in CH₂Cl₂ (4 mL). Purification by FC (petroleum ether/ethyl acetate 1:3, then ethyl acetate) gave Ac-C (114 mg, 99%) as a white solid: *R*_f = 0.38 (ethyl acetate). ¹H NMR (600 MHz, CDCl₃): δ = 5.62 (d, *J* = 8.1 Hz, 2 H, H-1^{Glc}), 5.36–5.34 (m, 4 H, H-4^{Gal}, NHC(O)), 5.25 (t, *J* = 9.2 Hz, 2 H, H-3^{Glc}), 5.09 (t, *J* = 8.1 Hz, 2 H, H-2^{Gal}), 5.00 (t, 2 H, *J* = 8.8 Hz, H-2^{Glc}), 4.94 (dd, 2 H, *J* = 10.1 Hz, *J* = 2.5 Hz, H-3^{Gal}), 4.48–4.43 (m, 4 H, H-1^{Gal}, H-6^{Glc}), 4.15–4.05 (m, 6 H, H-6^{Glc}, H-6^{Gal}, H-6^{Gal}), 3.87–3.83 (m, 4 H, H-4^{Glc}, H-5^{Gal}), 3.75 (m, 2 H, H-5^{Glc}), 3.47 (bs, 4 H, OCH₂CH₂), 3.41 (bs, 4 H, CH₂CH₂O), 3.28 (bs, 4 H, CH₂CH₂NH), 2.15 (s, 6 H, C(O)CH₃), 2.11 (s, 6 H, C(O)CH₃), 2.06 (s, 6 H, C(O)CH₃), 2.04 (s, 12 H, C(O)CH₃), 2.02 (s, 6 H, C(O)CH₃), 1.96 (s, 6 H, C(O)CH₃), 1.76 (bs, 4 H, CH₂CH₂CH₂), 1.59 (bs, 4 H, CH₂CH₂O). ¹³C NMR (150 MHz, CDCl₃): δ = 170.3, 170.1, 170.0, 169.7, 169.6, 169.0 (C(O)CH₃), 153.6 (OC(O)N), 100.9 (C-1^{Gal}), 92.6 (C-1^{Glc}), 75.7 (C-4^{Glc}), 73.2 (C-5^{Glc}), 72.8 (C-3^{Glc}), 71.0 (C-3^{Gal}), 70.8 (CH₂CH₂O), 70.7 (C-5^{Gal}), 70.6 (C-2^{Glc}), 69.0 (C-2^{Gal}), 68.9 (OCH₂CH₂), 66.6 (C-4^{Gal}), 61.8 (C-6^{Glc}), 60.8 (C-6^{Gal}), 39.4 (CH₂CH₂NH), 29.3 (CH₂CH₂CH₂), 26.3 (CH₂CH₂O), 20.9–20.5 (C(O)CH₃). MALDI-MS: calcd *m/z* 1551.5 [M + Na⁺], 1567.5 [M + K⁺], found 1551.2, 1567.1. Anal. calcd for C₆₄H₉₂N₂O₄₀: C 50.26, H 6.06, N 1.83, found: C 49.88, H 6.05, N 1.90.

1,4-Bis-(3-(β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyloxycarbonylaminopropoxy)butane (C). According to GP 2, Ac-C (93 mg, 0.061 mmol) was deacetylated with 0.5 M NaOMe solution (0.1 mL) in MeOH (2 mL) to give **C** (58 mg, quant) as a white solid. ¹H NMR (600 MHz, D₂O): δ = 5.31 (d, *J* = 8.2 Hz, 2 H, H-1^{Glc}), 4.35 (d, *J* = 7.8 Hz, 2 H, H-1^{Gal}), 3.85–3.81 (m, 4 H, H-4^{Gal}, H-6^{Glc}), 3.74–3.60 (m, 14 H, H-3^{Glc}, H-4^{Glc}, H-5^{Glc}, H-5^{Gal}, H-6^{Glc}, H-6^{Gal}, H-6^{Gal}), 3.55 (dd, *J* = 10.0 Hz, *J* = 3.3 Hz, 2 H, H-3^{Gal}), 3.46–3.38 (m, 12 H, H-2^{Glc}, H-2^{Gal}, OCH₂CH₂), 3.16–3.10 (m, 4 H, CH₂CH₂NH), 1.71–1.66 (m, 4 H, CH₂CH₂CH₂), 1.51 (bs, 4 H, OCH₂CH₂). ¹³C NMR (150 MHz, D₂O): δ = 156.4 (OC(O)N), 102.9 (C-1^{Gal}), 94.7 (C-1^{Glc}), 77.8, 75.4, 75.3 (C-4^{Glc}, C-5^{Glc}, C-5^{Gal}), 74.1 (C-3^{Glc}), 72.5 (C-3^{Gal}), 71.7 (C-2^{Glc}), 71.0 (C-2^{Gal}), 70.4 (OCH₂CH₂), 68.6 (C-4^{Gal}), 67.7 (OCH₂CH₂), 61.1 (C-6^{Gal}), 59.8 (C-6^{Glc}), 37.6 (CH₂CH₂NH), 28.6 (CH₂CH₂CH₂), 25.3 (OCH₂CH₂). MALDI-MS: calcd *m/z* 963.4 [M + Na⁺], 979.3 [M + K⁺], found 963.4, 979.1.

N,N'-Bis-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxycarbonyl)-4,7,10-trioxa-1,13-tridecanediamine (Ac-D). According to GP 1, β-3 (150 mg (0.190 mmol) and 4,7,10-trioxa-1,13-tridecanediamine **12** (17 μL, 0.078 mmol) were reacted in CH₂Cl₂ (4 mL). Purification by FC (ethyl acetate) gave Ac-D (106 mg, 89%) as a white solid. *R*_f = 0.36 (ethyl acetate). ¹H NMR (600 MHz, CDCl₃): δ = 5.62 (d, *J* = 8.2 Hz, 2 H, H-1^{Glc}), 5.54 (t, *J* = 5.3 Hz, 2 H, NHC(O)), 5.35 (bs, 2 H, H-4^{Gal}), 5.24 (t, *J* = 9.4 Hz, 2 H, H-3^{Glc}), 5.10 (t, *J* = 8.2 Hz, 2 H, H-2^{Gal}), 5.00 (t, 2 H, *J* = 8.8 Hz, H-2^{Glc}), 4.94 (dd, 2 H, *J* = 10.0 Hz, *J* = 2.9 Hz, H-3^{Gal}), 4.48–4.44 (m, 4 H, H-1^{Gal}, H-6^{Glc}), 4.15–4.07 (m, 6 H, H-6^{Glc}, H-6^{Gal}, H-6^{Gal}), 3.88–3.84 (m, 4 H, H-4^{Glc}, H-5^{Gal}), 3.76–3.75 (m, 2 H, H-5^{Glc}), 3.61 (bs, 4 H, OCH₂CH₂O), 3.58 (bs, 4 H, OCH₂CH₂O), 3.54 (bs, 4 H, OCH₂CH₂), 3.29 (bs, 4 H, CH₂CH₂NH), 2.15 (s, 6 H, C(O)CH₃), 2.11 (s, 6 H, C(O)CH₃), 2.06 (s, 6 H, C(O)CH₃), 2.04 (s, 12 H, C(O)CH₃), 2.03 (s, 6 H, C(O)CH₃), 1.96 (s, 6 H, C(O)CH₃), 1.79 (bs, 4 H, CH₂CH₂CH₂). ¹³C NMR (150 MHz, CDCl₃): δ = 170.3, 170.1, 170.1, 169.7, 169.6, 169.0 (C(O)CH₃), 153.7 (OC(O)N), 100.9 (C-1^{Gal}), 92.5 (C-1^{Glc}), 75.7 (C-4^{Glc}), 73.2 (C-5^{Glc}), 72.8 (C-3^{Glc}), 71.0 (C-3^{Gal}), 70.7 (C-5^{Gal}), 70.6 (C-2^{Glc}), 70.5 (OCH₂CH₂O), 70.2 (OCH₂CH₂O), 69.4 (OCH₂CH₂), 69.0 (C-2^{Gal}), 66.6 (C-4^{Gal}), 61.8 (C-6^{Glc}), 60.8 (C-6^{Gal}), 39.3 (CH₂CH₂NH), 29.1 (CH₂CH₂CH₂), 20.8–20.5 (C(O)CH₃). MALDI-MS: calcd *m/z* 1567.5 [M + Na⁺], 1583.5 [M + K⁺], found 1567.9, 1583.9. Anal. calcd for C₆₄H₉₂N₂O₄₁: C 49.74, H 6.00, N 1.81, found: C 49.58, H 6.18, N 1.81.

N,N'-Bis-(β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyloxycarbonyl)-4,7,10-trioxa-1,13-tridecanediamine (D). According to GP 2, Ac-D (100 mg, 0.065 mmol) was deacetylated with 0.5 M NaOMe solution (0.5 mL) in MeOH (2 mL) to give **D** (58 mg, 94%) as a white solid. ¹H NMR (600 MHz, D₂O): δ = 5.33 (d, *J* = 8.3 Hz, 2 H, H-1^{Glc}), 4.36 (d, *J* = 7.8 Hz, 2 H, H-1^{Gal}), 3.86–3.83 (m, 4 H, H-4^{Gal}, H-6^{Glc}), 3.74–3.56 (m, 24 H, H-3^{Glc}, H-3^{Gal}, H-4^{Glc}, H-5^{Glc}, H-5^{Gal}, H-6^{Glc}, H-6^{Gal}, H-6^{Gal}, OCH₂CH₂O, OCH₂CH₂O), 3.51–3.44 (m, 6 H, H-2^{Gal}, OCH₂CH₂), 3.40 (t, *J* = 8.6 Hz, 2 H, H-2^{Glc}), 3.15 (bs, 4 H, CH₂CH₂NH), 1.73–1.69 (m, 4 H, CH₂CH₂CH₂). ¹³C NMR (150 MHz, D₂O): δ = 156.4 (OC(O)N), 102.9 (C-1^{Gal}), 94.7 (C-1^{Glc}), 77.8, 75.4, 75.3, 74.1, 72.5 (C-3^{Glc}, C-3^{Gal}, C-4^{Glc}, C-5^{Glc}, C-5^{Gal}), 71.7 (C-2^{Glc}), 70.9 (C-2^{Gal}), 69.6 (OCH₂CH₂O), 69.3 (OCH₂CH₂O), 68.6 (C-4^{Gal}), 68.3 (OCH₂CH₂), 61.1 (C-6^{Gal}), 59.8 (C-6^{Glc}), 37.6 (CH₂CH₂NH), 28.5 (CH₂CH₂CH₂). MALDI-MS: calcd *m/z* 979.4 [M + Na⁺], 995.4 [M + K⁺], found 979.4, 995.3.

1,7-Bis-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxycarbonyl)diethylenetriamine (Ac-E). According to GP 1, β-3 (202 mg, 0.252 mmol) and diethylenetriamine **13** (11 μL, 0.102 mmol) were reacted in CH₂Cl₂ (2 mL). Purification by FC (CH₂Cl₂/MeOH 15:1) gave Ac-E (105 mg, 71%) as a colorless syrup. ¹H NMR (600 MHz, CDCl₃): δ = 5.76 (t, *J* = 5.1 Hz, 2 H, NHC(O)), 5.58 (d, *J* = 8.3 Hz, 2 H, H-1^{Glc}), 5.31 (d, *J* = 2.8 Hz, 2 H, H-4^{Gal}), 5.21 (t, *J* = 9.1 Hz, 2 H, H-3^{Glc}), 5.06 (dd, *J* = 9.9 Hz, *J* = 8.3 Hz, 2 H, H-2^{Gal}), 4.98 (t, *J* = 8.9 Hz, 2 H, H-2^{Glc}), 4.91 (dd, *J* = 10.4 Hz, *J* = 2.9 Hz, 2 H, H-3^{Gal}), 4.48–4.42 (m, 4 H, H-1^{Gal}, H-6^{Glc}), 4.11–4.09 (m, 2 H, H-6^{Glc}, H-6^{Gal}), 4.05–4.02 (m, 2 H, H-6^{Gal}), 3.86–3.84 (m, 4 H, H-4^{Glc}, H-5^{Gal}), 3.73–3.71 (m, 2 H, H-5^{Glc}), 3.40–3.18 (m, 4 H, CH₂CH₂NH), 2.76–2.71 (m, 4 H, CH₂CH₂NH), 2.11 (s, 6 H, C(O)CH₃), 2.07 (s, 6 H, C(O)CH₃), 2.02 (s, 6 H, C(O)CH₃), 2.01 (s, 6 H, C(O)CH₃), 2.00 (s, 6 H, C(O)CH₃), 1.99 (s, 6 H, C(O)CH₃), 1.95 (s, 6 H, C(O)CH₃). ¹³C NMR (150 MHz, CDCl₃): δ = 170.3, 170.2, 170.0, 170.0, 169.5, 168.9 (C(O)CH₃), 154.0 (OC(O)N), 100.8 (C-1^{Gal}), 92.6 (C-1^{Glc}), 75.5 (C-4^{Glc}), 73.2 (C-5^{Glc}), 72.6 (C-3^{Glc}), 70.9 (C-3^{Gal}), 70.5 (C-5^{Gal}), 70.5 (C-2^{Glc}), 68.9 (C-2^{Gal}), 66.5 (C-4^{Gal}), 61.6

(C-6^{Glc}), 60.6 (C-6^{Gal}), 48.0 (CH₂CH₂NH), 40.3 (CH₂CH₂NH), 20.8–20.5 (C(O)CH₃). MALDI-MS: calcd *m/z* 1450.5 [M + Na⁺], 1466.5 [M + K⁺], found 1449.6, 1466.0.

1,7-Bis-(β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyloxy-carbonyl)diethylenetriamine (E). According to GP 3, Ac-E (67 mg, 0.047 mmol) was deacetylated in EtNMe₂/MeOH 1:5 (2.4 mL) to give **E** (39 mg, 99%) as a white solid. ¹H NMR (600 MHz, D₂O): δ = 5.34 (d, *J* = 8.2 Hz, 2 H, H-1^{Glc}), 4.35 (d, *J* = 7.5 Hz, 2 H, H-1^{Gal}), 3.85–3.82 (m, 4 H, H-4^{Gal}, H-6^{Glc}), 3.72–3.54 (m, 16 H, H-3^{Glc}, H-3^{Gal}, H-4^{Glc}, H-5^{Gal}, H-5^{Glc}, H-6^{Glc}, H-6^{Gal}), 3.45–3.39 (m, 4 H, H-2^{Gal}, H-2^{Glc}), 3.20–3.13 (bs, 4 H, CH₂CH₂NH), 2.78 (bs, 4 H, CH₂CH₂NH). ¹³C NMR (150 MHz, D₂O): δ = 156.6 (OC(O)N), 102.9 (C-1^{Gal}), 94.8 (C-1^{Glc}), 77.8, 75.4, 75.3, 74.1 (C-3^{Glc}, C-4^{Glc}, C-5^{Glc}, C-5^{Gal}), 72.6 (C-3^{Gal}), 71.6 (C-2^{Glc}), 71.0 (C-2^{Gal}), 68.6 (C-4^{Gal}), 61.1 (C-6^{Gal}), 59.7 (C-6^{Glc}), 47.2 (CH₂CH₂NH), 38.9 (CH₂CH₂NH).

Tris-(2-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxycarbonylamino)ethyl)amine (Ac-F). According to GP 1, β-3 (144 mg, 0.18 mmol) and tris-(2-aminoethyl)amine **14** (8 μL, 0.053 mmol) were reacted in CH₂Cl₂ (4 mL). Purification by FC (petroleum ether/ethyl acetate 1:1, then ethyl acetate) gave Ac-F (111 mg, 97%) as a white solid. *R*_f = 0.32 (ethyl acetate). ¹H NMR (600 MHz, CDCl₃): δ = 5.61 (d, *J* = 8.0 Hz, 3 H, H-1^{Glc}), 5.49 (bs, 3 H, NHC(O)), 5.34 (s, 3 H, H-4^{Gal}), 5.25 (t, *J* = 9.1 Hz, 3 H, H-3^{Glc}), 5.10 (t, *J* = 9.2 Hz, 3 H, H-2^{Gal}), 5.03–4.94 (m, 6 H, H-2^{Glc}, H-3^{Gal}), 4.50–4.45 (m, 6 H, H-1^{Gal}, H-6^{Glc}), 4.15–4.05 (m, 9 H, H-6^{Glc}, H-6^{Gal}, H-6^{Glc}), 3.89–3.84 (m, 6 H, H-4^{Glc}, H-5^{Gal}), 3.75 (m, 3 H, H-5^{Glc}), 3.33 (bs, 3 H, CH₂CH₂NH), 3.23 (bs, 3 H, CH₂CH₂NH), 2.77 (bs, 6 H, CH₂CH₂NH), 2.15 (s, 9 H, C(O)CH₃), 2.11 (s, 18 H, C(O)CH₃), 2.06 (s, 9 H, C(O)CH₃), 2.05 (s, 9 H, C(O)CH₃), 2.04 (s, 9 H, C(O)CH₃), 1.96 (s, 9 H, C(O)CH₃). ¹³C NMR (150 MHz, CDCl₃): δ = 170.4, 170.1, 170.1, 169.8, 169.6, 169.0 (C(O)CH₃), 154.0 (OC(O)N), 100.9 (C-1^{Gal}), 92.7 (C-1^{Glc}), 75.6 (C-4^{Glc}), 73.3 (C-5^{Glc}), 72.7 (C-3^{Glc}), 71.0 (C-3^{Gal}), 70.7 (C-5^{Gal}), 70.6 (C-2^{Glc}), 69.0 (C-2^{Gal}), 66.6 (C-4^{Gal}), 61.8 (C-6^{Glc}), 60.7 (C-6^{Gal}), 48.2 (CH₂CH₂NH), 40.6 (CH₂CH₂NH), 20.9–20.5 (C(O)CH₃). MALDI-MS: calcd *m/z* 2155.7 [M + Na⁺], found 2155.2. Anal. calcd for C₈₇H₁₂₀N₄O₅₇: C 48.97, H 5.67, N 2.63, found: C 49.14, H 5.84, N 2.59.

Tris-(2-(β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyloxy-carbonylamino)ethyl)amine (F). According to GP 3, Ac-F (67 mg, 0.031 mmol) was deacetylated in EtNMe₂/MeOH 1:5 (1.8 mL) to give **F** (38 mg, 97%) as a white solid. ¹H NMR (600 MHz, D₂O): δ = 5.33 (d, *J* = 8.2 Hz, 3 H, H-1^{Glc}), 4.34 (d, *J* = 7.8 Hz, 3 H, H-1^{Gal}), 3.85–3.81 (m, 6 H, H-4^{Gal}, H-6^{Glc}), 3.72–3.60 (m, 21 H, H-3^{Glc}, H-4^{Glc}, H-5^{Glc}, H-5^{Gal}, H-6^{Glc}, H-6^{Gal}), 3.55 (dd, *J* = 10.0 Hz, *J* = 3.4 Hz, 3 H, H-3^{Gal}), 3.43 (dd, *J* = 9.7 Hz, *J* = 8.1 Hz, 3 H, H-2^{Gal}), 3.40 (t, *J* = 8.1 Hz, 3 H, H-2^{Glc}), 3.20–3.13 (bs, 6 H, CH₂CH₂NH), 2.63 (bs, 6 H, CH₂CH₂NH). ¹³C NMR (150 MHz, D₂O): δ = 156.4 (OC(O)N), 102.9 (C-1^{Gal}), 94.7 (C-1^{Glc}), 77.7, 75.4, 75.3, 74.1 (C-3^{Glc}, C-4^{Glc}, C-5^{Glc}, C-5^{Gal}), 72.5 (C-3^{Gal}), 71.6 (C-2^{Glc}), 70.9 (C-2^{Gal}), 68.5 (C-4^{Gal}), 61.0 (C-6^{Gal}), 59.7 (C-6^{Glc}), 52.5 (CH₂CH₂NH), 38.1 (CH₂CH₂NH). MALDI-MS: calcd *m/z* 1273.4 [M + Na⁺], 1289.4 [M + K⁺], found 1273.4, 1289.4.

N,N'-Bis-(4-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxy)-(Z)-but-2-enyloxycarbonyl)-*m*-xylylenediamine (Ac-G). According to GP 1, **7** (171 mg, 0.196 mmol) and α,α'-diamino-*m*-xylene **9** (12 mg, 0.088 mmol) were reacted in CH₂Cl₂ (3 mL). Purification by FC (petroleum ether/ethyl acetate 1:2 → 1:3) gave Ac-G (135 mg, 95%) as a white foam. *R*_f = 0.15 (petroleum ether/ethyl acetate 1:2). ¹H NMR (600 MHz, CDCl₃): δ = 7.33–7.30 (m, 1 H, Ar), 7.21–7.18 (m, 3 H, Ar), 5.75–5.64 (m, 4 H, H^{vinyl}), 5.35 (dd, *J* = 3.3 Hz, *J* = 0.7 Hz, 2 H, H-4^{Gal}), 5.20 (bs,

2 H, NHC(O)), 5.16 (t, *J* = 9.3 Hz, 2 H, H-3^{Glc}), 5.10 (dd, *J* = 10.4 Hz, *J* = 7.9 Hz, 2 H, H-2^{Gal}), 4.95 (dd, *J* = 10.4 Hz, *J* = 3.5 Hz, 2 H, H-3^{Gal}), 4.89 (dd, *J* = 9.4 Hz, *J* = 8.0 Hz, 2 H, H-2^{Glc}), 4.69 (dd, *J* = 13.0 Hz, *J* = 6.9 Hz, 2 H, H^{allyl}), 4.57 (dd, *J* = 13.1 Hz, *J* = 5.7 Hz, 2 H, H^{allyl}), 4.52–4.50 (m, 4 H, H-1^{Glc}, H-6^{Glc}), 4.48 (d, *J* = 7.9 Hz, 2 H, H-1^{Gal}), 4.35 (d, *J* = 5.9 Hz, 4 H, H^{benzyl}), 4.31 (d, *J* = 6.2 Hz, 4 H, H^{allyl}), 4.14–4.11 (m, 2 H, H-6^{Gal}), 4.09–4.06 (m, 4 H, H-6^{Glc}, H-6^{Gal}), 3.87 (bt, *J* = 6.8 Hz, 2 H, H-5^{Gal}), 3.79 (t, *J* = 9.5 Hz, 2 H, H-4^{Glc}), 3.61–3.59 (m, 2 H, H-5^{Glc}), 2.15 (s, 6 H, C(O)CH₃), 2.11 (s, 6 H, C(O)CH₃), 2.06 (s, 6 H, C(O)CH₃), 2.04 (s, 6 H, C(O)CH₃), 2.04 (s, 6 H, C(O)CH₃), 2.02 (s, 6 H, C(O)CH₃), 1.96 (s, 6 H, C(O)CH₃). ¹³C NMR (150 MHz, CDCl₃): δ = 170.4, 170.3, 170.1, 170.0, 169.8, 169.7, 169.1 (C(O)CH₃), 156.2 (OC(O)N), 137.1, 129.1 (Ar), 128.9, 128.5 (CH^{olefin}), 128.5, 126.6 (Ar), 101.1 (C-1^{Gal}), 99.0 (C-1^{Glc}), 76.3 (C-4^{Glc}), 72.9 (C-3^{Glc}), 72.6 (C-5^{Glc}), 71.6 (C-2^{Glc}), 71.0 (C-3^{Gal}), 70.7 (C-5^{Gal}), 69.1 (C-2^{Gal}), 66.6 (C-4^{Gal}), 64.3 (CH₂^{allyl}), 62.0 (C-6^{Glc}), 60.8 (C-6^{Gal}), 60.5 (CH₂^{allyl}), 44.9 (CH₂^{benzyl}), 20.9–20.5 (C(O)CH₃). MALDI-MS: calcd *m/z* 1623.5 [M + Na⁺], 1639.5 [M + K⁺], found 1623.6, 1639.6. Anal. calcd for C₇₀H₉₂N₂O₄₀: C 52.50, H 5.79, N 1.75, found: C 51.92, H 5.81, N 1.84.

N,N'-Bis-(4-(β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyloxy)-(Z)-but-2-enyloxycarbonyl)-*m*-xylylenediamine (G). According to GP 2, Ac-G (130 mg, 0.081 mmol) was deacetylated with 0.5 M NaOMe solution (0.5 mL) in MeOH (2 mL) to give **G** (81 mg, 98%) as a white solid. ¹H NMR (600 MHz, D₂O): δ = 7.29–7.27 (m, 1 H, Ar), 7.14–7.12 (m, 3 H, Ar), 5.73–5.71 (m, 4 H, H^{vinyl}), 4.60–4.54 (m, 4 H, H^{allyl}), 4.38–4.28 (m, 8 H, H-1^{Glc}, H-1^{Gal}, H^{allyl}), 4.20 (s, 4 H, H^{benzyl}), 3.84–3.82 (m, 4 H, H-4^{Gal}, H-6^{Glc}), 3.70–3.60 (m, 8 H, H-5^{Gal}, H-6^{Glc}, H-6^{Gal}), 3.57–3.48 (m, 6 H, H-3^{Glc}, H-3^{Gal}, H-4^{Glc}), 3.45–3.42 (m, 4 H, H-2^{Gal}, H-5^{Glc}), 3.20 (t, *J* = 8.3 Hz, 2 H, H-2^{Glc}). ¹³C NMR (150 MHz, D₂O): δ = 158.4 (OC(O)N), 139.2, 129.0 (Ar), 128.9, 128.5 (CH^{olefin}), 125.9, 125.3 (Ar), 102.9 (C-1^{Gal}), 100.9 (C-1^{Glc}), 78.3 (C-4^{Glc}), 75.4 (C-5^{Gal}), 74.8 (C-5^{Glc}), 74.4 (C-3^{Glc}), 72.8 (C-3^{Gal}), 72.6 (C-2^{Glc}), 71.0 (C-2^{Gal}), 68.6 (C-4^{Gal}), 64.8 (CH₂^{allyl}), 61.0 (C-6^{Gal}), 61.0 (CH₂^{allyl}), 60.0 (C-6^{Glc}), 43.9 (CH₂^{Bn}). MALDI-MS: calcd *m/z* 1035.4 [M + Na⁺], found 1035.4.

N,N'-Bis-(4-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxy)-(Z)-but-2-enyloxycarbonyl)-*p*-xylylenediamine (Ac-H). According to GP 1, **7** (157 mg, 180 mmol) and α,α'-diamino-*p*-xylene **10** (12 mg, 0.088 mmol) were reacted in CH₂Cl₂ (3 mL). Purification by FC (petroleum ether/ethyl acetate 1:2 → 1:3) gave Ac-H (130 mg, 92%) as a white foam. *R*_f = 0.10 (petroleum ether/ethyl acetate 1:2). ¹H NMR (600 MHz, CDCl₃): δ = 7.27 (s, 4 H, Ar), 5.75–5.64 (m, 4 H, H^{vinyl}), 5.35 (d, *J* = 2.9 Hz, 2 H, H-4^{Gal}), 5.17 (t, *J* = 9.3 Hz, 2 H, H-3^{Glc}), 5.10 (dd, *J* = 10.4 Hz, *J* = 7.9 Hz, 2 H, H-2^{Gal}), 4.96 (dd, *J* = 10.4 Hz, *J* = 3.5 Hz, 2 H, H-3^{Gal}), 4.89 (dd, *J* = 9.4 Hz, *J* = 8.0 Hz, 2 H, H-2^{Glc}), 4.68 (dd, *J* = 12.6 Hz, *J* = 7.1 Hz, 2 H, H^{allyl}), 4.57 (dd, *J* = 13.1 Hz, *J* = 5.8 Hz, 2 H, H^{allyl}), 4.51–4.48 (m, 6 H, H-1^{Glc}, H-1^{Gal}, H-6^{Glc}), 4.35 (d, *J* = 5.9 Hz, 4 H, H^{benzyl}), 4.32 (d, *J* = 6.3 Hz, 4 H, H^{allyl}), 4.15–4.12 (m, 2 H, H-6^{Gal}), 4.10–4.07 (m, 4 H, H-6^{Glc}, H-6^{Gal}), 3.87 (bt, *J* = 6.7 Hz, 2 H, H-5^{Gal}), 3.79 (t, *J* = 9.5 Hz, 2 H, H-4^{Glc}), 3.62–3.60 (m, 2 H, H-5^{Glc}), 2.15 (s, 6 H, C(O)CH₃), 2.11 (s, 6 H, C(O)CH₃), 2.06 (s, 6 H, C(O)CH₃), 2.05 (s, 6 H, C(O)CH₃), 2.04 (s, 6 H, C(O)CH₃), 2.03 (s, 6 H, C(O)CH₃), 1.97 (s, 6 H, C(O)CH₃). ¹³C NMR (150 MHz, CDCl₃): δ = 170.3, 170.3, 170.1, 170.0, 169.7, 169.6, 169.0 (C(O)CH₃), 156.7 (OC(O)N), 136.9 (Ar), 128.8, 128.5 (CH^{olefin}), 127.7 (Ar), 101.1 (C-1^{Gal}), 98.9 (C-1^{Glc}), 76.3 (C-4^{Glc}), 72.9 (C-3^{Glc}), 72.6 (C-5^{Glc}), 71.6 (C-2^{Glc}), 71.0 (C-3^{Gal}), 70.7 (C-5^{Gal}), 69.1 (C-2^{Gal}), 66.6 (C-4^{Gal}), 64.7 (CH₂^{allyl}), 62.0 (C-6^{Glc}), 60.8 (C-6^{Gal}), 60.5 (CH₂^{allyl}), 44.3 (CH₂^{benzyl}), 20.8–20.5

(C(O)CH₃). MALDI-MS: calcd *m/z* 1623.5 [M + Na⁺], 1639.5 [M + K⁺], found 1623.4, 1639.4. Anal. calcd for C₇₀H₉₂N₂O₄₀: C 52.50, H 5.79, N 1.75, found: C 52.21, H 5.72, N 1.72.

***N,N'*-Bis-(4-(β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyloxy)-(Z)-but-2-enyloxycarbonyl)-*p*-xylylenediamine (H).** According to GP 2, Ac-H (125 mg, 0.078 mmol) was deacetylated with 0.5 M NaOMe solution (0.5 mL) in MeOH (2 mL) to give **H** (76 mg, 96%) as a white solid. ¹H NMR (600 MHz, D₂O): δ = 7.21 (s, 4 H, Ar), 5.74–5.72 (m, 4 H, H^{vinyl}), 4.60–4.53 (m, 4 H, H^{allyl}), 4.39–4.29 (m, 8 H, H-1^{Glc}, H-1^{Gal}, H^{allyl}), 4.20 (s, 4 H, H^{benzyl}), 3.85–3.83 (m, 4 H, H-4^{Gal}, H-6^{Glc}), 3.71–3.66 (m, 6 H, H-6^{Glc}, H-6^{Gal}, H-6^{Gal}), 3.65–3.61 (m, 2 H, H-5^{Gal}), 3.58–3.49 (m, 6 H, H-3^{Glc}, H-3^{Gal}, H-4^{Glc}), 3.46–3.43 (m, 4 H, H-2^{Gal}, H-5^{Glc}), 3.21 (t, *J* = 8.5 Hz, 2 H, H-2^{Glc}). ¹³C NMR (150 MHz, D₂O): δ = 158.4 (OC(O)N), 135.4 (Ar), 128.9, 128.5 (CH^{olefin}), 127.9 (Ar), 102.9 (C-1^{Gal}), 100.9 (C-1^{Glc}), 78.3 (C-4^{Glc}), 75.4 (C-5^{Gal}), 74.8, 74.4, 72.8, 72.6, 71.0, 68.6, (C-2^{Glc}, C-2^{Gal}, C-3^{Gal}, C-3^{Glc}, C-4^{Gal}, C-5^{Glc}), 64.7 (CH₂^{allyl}), 61.0 (C-6^{Gal}), 61.0 (CH₂^{allyl}), 60.0 (C-6^{Glc}), 43.8 (CH₂^{Bn}). MALDI-MS: calcd *m/z* 1035.4 [M + Na⁺], found 1034.9.

***N,N'*-Bis-(4-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxy)-(Z)-but-2-enyloxycarbonyl)-4,7,10-trioxa-1,13-tridecanediamine (Ac-I).** According to GP 1, **7** (140 mg, 0.178 mmol) and 4,7,10-trioxa-1,13-tridecanediamine **12** (16 μL, 0.073 mmol) were reacted in CH₂Cl₂ (3 mL). Purification by FC (petroleum ether/ethyl acetate 1:3, then ethyl acetate) gave Ac-I (120 mg, 98%) as a white solid. *R*_f = 0.27 (ethyl acetate). ¹H NMR (600 MHz, CDCl₃): δ = 5.72–5.62 (m, 4 H, H^{vinyl}), 5.35 (dd, *J* = 3.4 Hz, *J* = 0.8 Hz, 2 H, H-4^{Gal}), 5.18 (t, *J* = 9.3 Hz, 2 H, H-3^{Glc}), 5.10 (dd, *J* = 10.4 Hz, *J* = 7.9 Hz, 2 H, H-2^{Gal}), 4.95 (dd, *J* = 10.4 Hz, *J* = 3.5 Hz, 2 H, H-3^{Gal}), 4.89 (dd, *J* = 9.5 Hz, *J* = 8.0 Hz, 2 H, H-2^{Glc}), 4.63–4.47 (m, 10 H, H-1^{Glc}, H-1^{Gal}, H-6^{Glc}, H^{allyl}), 4.33–4.26 (m, 4 H, H^{allyl}), 4.15–4.12 (m, 2 H, H-6^{Gal}), 4.13–4.06 (m, 4 H, H-6^{Glc}, H-6^{Gal}), 3.87 (bt, *J* = 6.8 Hz, 2 H, H-5^{Gal}), 3.79 (t, *J* = 9.5 Hz, 2 H, H-4^{Glc}), 3.65–3.59 (m, 10 H, H-5^{Glc}, OCH₂CH₂, OCH₂CH₂), 3.55 (t, *J* = 5.8 Hz, 4 H, OCH₂CH₂CH₂), 3.29–3.26 (m, 4 H, CH₂CH₂NH), 2.15 (s, 6 H, C(O)CH₃), 2.12 (s, 6 H, C(O)CH₃), 2.06 (s, 6 H, C(O)CH₃), 2.05 (s, 6 H, C(O)CH₃), 2.04 (s, 12 H, C(O)CH₃), 1.96 (s, 6 H, C(O)CH₃), 1.79–1.75 (m, 4 H, CH₂CH₂CH₂). ¹³C NMR (150 MHz, CDCl₃): δ = 170.4, 170.3, 170.1, 170.0, 169.8, 169.7, 169.1 (C(O)CH₃), 156.2 (OC(O)N), 128.8, 128.5 (CH^{olefin}), 101.1 (C-1^{Gal}), 99.1 (C-1^{Glc}), 76.3 (C-4^{Glc}), 72.9 (C-3^{Glc}), 72.6 (C-5^{Glc}), 71.6 (C-2^{Glc}), 71.0 (C-3^{Gal}), 70.7 (C-5^{Gal}), 70.5 (OCH₂CH₂O), 70.2 (OCH₂CH₂O), 69.6 (OCH₂CH₂), 69.1 (C-2^{Gal}), 66.6 (C-4^{Gal}), 65.5 (Allyl CH₂), 62.0 (C-6^{Glc}), 60.8 (C-6^{Gal}), 60.2 (Allyl CH₂), 39.3 (CH₂CH₂NH), 29.4 (CH₂CH₂CH₂), 20.9–20.5 (C(O)CH₃). MALDI-MS: calcd *m/z* 1707.6 [M + Na⁺], 1723.6 [M + K⁺], found 1707.2, 1723.2. Anal. calcd for C₇₂H₁₀₄N₂O₄₃: C 51.30, H 6.22, N 1.66, found: C 51.38, H 5.96, N 1.83.

***N,N'*-Bis-(4-(β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyloxy)-(Z)-but-2-enyloxycarbonyl)-4,7,10-trioxa-1,13-tridecanediamine (I).** According to GP 2, Ac-I (114 mg, 0.068 mmol) was deacetylated with 0.5 M NaOMe solution (0.5 mL) in MeOH (2 mL) to give **I** (73 mg, 99%) as a white solid. ¹H NMR (600 MHz, D₂O): δ = 5.72 (bs, 4 H, H^{vinyl}), 4.60–4.53 (m, 4 H, H^{allyl}), 4.41 (d, *J* = 8.0 Hz, 2 H, H-1^{Glc}), 4.38–4.29 (m, 6 H, H-1^{Gal}, H^{allyl}), 3.87 (dd, *J* = 12.4 Hz, *J* = 1.5 Hz, 2 H, H-6^{Glc}), 3.82 (d, *J* = 2.9 Hz, H-4^{Gal}), 3.73–3.61 (m, 8 H, H-5^{Gal}, H-6^{Glc}, H-6^{Gal}, H-6^{Gal}), 3.56–3.52 (m, 14 H, H-3^{Gal}, OCH₂CH₂, OCH₂CH₂, OCH₂CH₂O), 3.49–3.42 (m, 8 H, H-2^{Gal}, H-3^{Glc}, H-4^{Glc}, H-5^{Glc}), 3.22 (t, *J* = 8.4 Hz, 2 H, H-2^{Glc}), 3.01 (t, *J* = 6.2 Hz, 4 H, CH₂CH₂NH), 1.69 (p, *J* = 6.3 Hz, 4 H, CH₂CH₂CH₂). ¹³C NMR (150 MHz, D₂O): δ = 158.2 (OC(O)N), 128.8, 128.6 (CH^{olefin}), 102.9 (C-1^{Gal}), 101.0 (C-1^{Glc}), 78.3 (C-4^{Glc}), 75.4 (C-5^{Gal}), 74.8, 74.5, 72.8, 72.6, 71.0, 69.6 68.6 (C-

2^{Glc}, C-2^{Gal}, C-3^{Gal}, C-3^{Glc}, C-5^{Glc}, OCH₂CH₂), 68.4 (C-4^{Gal}), 68.3 (OCH₂CH₂), 64.8 (CH₂^{allyl}), 61.0 (C-6^{Gal}), 61.0 (CH₂^{allyl}), 60.8, 60.0 (C-6^{Glc}), 37.6 (CH₂CH₂NH), 28.7 (CH₂CH₂CH₂). MALDI-MS: calcd *m/z* 1119.4 [M + Na⁺], 1135.4 [M + K⁺], found 1119.3, 1135.3.

Tetrakis-(4-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxy)-(Z)-but-2-enyloxycarbonylaminomethyl)methane (Ac-J). According to GP 1, **7** (240 mg, 0.275 mmol) and tetra(aminomethyl)methane **15** (22) (8 mg, 0.056 mmol) were reacted in CH₂Cl₂ (2 mL). Purification by FC (petroleum ether/ethyl acetate 1:2 → 1:3 → 1:4) gave Ac-J (70 mg, 53%) as a white solid. *R*_f = 0.20 (petroleum ether/ethyl acetate 1:3). ¹H NMR (600 MHz, CDCl₃): δ = 6.36 (t, *J* = 6.6 Hz, 4 H, NH), 5.73–5.65 (m, 8 H, H^{vinyl}), 5.34 (d, *J* = 3.3 Hz, 4 H, H-4^{Gal}), 5.18 (d, *J* = 9.3 Hz, 4 H, H-3^{Glc}), 5.09 (dd, *J* = 10.4 Hz, *J* = 7.9 Hz, 4 H, H-2^{Gal}), 4.95 (dd, *J* = 10.4 Hz, *J* = 3.4 Hz, 4 H, H-3^{Gal}), 4.89 (dd, *J* = 9.4 Hz, *J* = 8.0 Hz, 4 H, H-2^{Glc}), 4.62–4.56 (m, 8 H, H^{allyl}), 4.51–4.48 (m, 12 H, H-1^{Glc}, H-1^{Gal}, H-6^{Glc}), 4.34 (dd, *J* = 12.8 Hz, *J* = 5.2 Hz, 4 H, H^{allyl}), 4.22 (dd, *J* = 12.8 Hz, *J* = 6.2 Hz, 4 H, H^{allyl}), 4.14–4.06 (m, 12 H, H-6^{Glc}, H-6^{Gal}, H-6^{Gal}), 3.87 (bt, *J* = 6.9 Hz, 4 H, H-5^{Gal}), 3.80 (t, *J* = 9.4 Hz, 4 H, H-4^{Glc}), 3.60 (ddd, *J* = 9.7 Hz, *J* = 4.9 Hz, *J* = 2.0 Hz, 4 H, H-5^{Glc}), 2.81 (d, *J* = 4.2 Hz, 8 H, CCH₂NH), 2.15 (s, 12 H, C(O)CH₃), 2.12 (s, 12 H, C(O)CH₃), 2.06 (s, 12 H, C(O)CH₃), 2.04 (s, 24 H, C(O)CH₃), 2.03 (s, 12 H, C(O)CH₃), 1.96 (s, 12 H, C(O)CH₃). ¹³C NMR (150 MHz, CDCl₃): δ = 170.3, 170.3, 170.1, 170.0, 169.7, 169.6, 169.0 (C(O)CH₃), 157.8 (OC(O)N), 128.7, 128.2 (CH^{olefin}), 101.1 (C-1^{Gal}), 99.4 (C-1^{Glc}), 76.2 (C-4^{Glc}), 72.9 (C-3^{Glc}), 72.7 (C-5^{Glc}), 71.6 (C-2^{Glc}), 71.0 (C-3^{Gal}), 70.7 (C-5^{Gal}), 69.1 (C-2^{Gal}), 66.6 (C-4^{Gal}), 64.7 (CH₂^{allyl}), 62.0 (C-6^{Glc}), 61.0 (CH₂^{allyl}), 60.8 (C-6^{Gal}), 39.8 (CCH₂NH), 20.8–20.5 (C(O)CH₃). MALDI-MS: calcd *m/z* 3084.0 [M + Na⁺], 3100.0 [M + K⁺], found 3083.9, 3099.9. Anal. calcd for C₁₂₉H₁₇₆N₄O₈₀: C 50.59, H 5.79, N 1.83, found: C 50.77, H 5.94, N 1.79.

Tetrakis-(4-(β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyloxy)-(Z)-but-2-enyloxycarbonylaminomethyl)methane (J). According to GP 2, Ac-J (78 mg, 0.025 mmol) was deacetylated with 0.5 M NaOMe solution (0.5 mL) in MeOH (2 mL) to give **J** (48 mg, quant) as a white solid. ¹H NMR (600 MHz, D₂O): δ = 5.77–5.72 (m, 8 H, H^{vinyl}), 4.61–4.56 (m, 8 H, H^{allyl}), 4.43–4.30 (m, 16 H, H-1^{Glc}, H-1^{Gal}, H^{allyl}), 3.88 (d, *J* = 8.3 Hz, 4 H, H-6^{Glc}), 3.83 (d, *J* = 3.0 Hz, 4 H, H-4^{Gal}), 3.73–3.62 (m, 16 H, H-5^{Gal}, H-6^{Glc}, H-6^{Gal}, H-6^{Gal}), 3.58–3.53 (m, 12 H, H-3^{Glc}, H-3^{Gal}, H-4^{Glc}), 3.49–3.43 (m, 8 H, H-2^{Gal}, H-5^{Glc}), 3.23 (t, *J* = 8.3 Hz, 4 H, H-2^{Glc}), 2.95 (bs, 8 H, CCH₂NH). ¹³C NMR (150 MHz, D₂O): δ = 158.3 (OC(O)N), 128.8, 128.5 (CH^{olefin}), 102.9 (C-1^{Gal}), 100.9 (C-1^{Glc}), 78.4 (C-4^{Glc}), 75.4 (C-5^{Gal}), 74.8 (C-5^{Glc}), 74.4 (C-3^{Glc}), 72.8 (C-3^{Gal}), 72.5 (C-2^{Glc}), 71.0 (C-2^{Gal}), 68.5 (C-4^{Gal}), 64.7 (CH₂^{allyl}), 61.1 (CH₂^{allyl}), 61.0 (C-6^{Gal}), 60.0 (C-6^{Glc}), 38.8 (CCH₂NH). MALDI-MS: calcd *m/z* 1907.7 [M + Na⁺], found 1908.4.

Lectins and (Neo)glycoproteins. Purification of VAA and of the galectins (human galectins-1, -3, and -4 as well as rat galectin-5) started from extracts of dried leaves and bacteria harboring the respective expression vector. One-step isolation used lactosylated Sepharose 4B, which was prepared after activation by divinyl sulfone and elution with 0.2 M lactose (23). The purity of the proteins was tested by one- and two-dimensional gel electrophoresis on 10% running gels and mass spectrometry by nanoESI-MS/MS on a QTOF instrument Q-ToF2 (Waters Micromass, Manchester, UK) (24). Their quaternary structure was analyzed on a Superose 12 HR 10/30 column (GE Healthcare, Munich, Germany), and lectin activity was tested in 2-fold dilutions by hemagglutination with trypsinized, glutaraldehyde-fixed rabbit erythrocytes (25–27). Biotinylation with the *N*-hydroxysuccinimide ester derivative

of biotin (Sigma, Munich, Germany) under activity-preserving conditions followed by mass spectrometric product analysis to quantify label incorporation was performed as described (24). Proteolytic truncation of human galectin-3 required 300 μ g collagenase D from *Clostridium histolyticum* (Roche Diagnostics, Mannheim, Germany) in Tris-buffered saline with 10 mM CaCl_2 at pH 7 for 2 h at 37 °C and 1 mg lectin as substrate (24). The neoglycoprotein with average ligand density of 28 units attached as *p*-isothiocyanatophenyl derivatives was based on bovine serum albumin as carrier; the glycoproteins serum amyloid P component, asialofetuin, and α_1 -acid glycoprotein were prepared as outlined (28–30).

Inhibition Assays. Adsorption of (neo)glycoproteins to the surface of microtiter plate wells from phosphate-buffered saline (50 μ L) at 4 °C overnight established the ligand-bearing matrix. Residual sites for binding proteins were saturated with bovine serum albumin free of contaminating glycoproteins (Biomol, Hamburg, Germany; 100 μ L, 1% w/v) for 1 h at 37 °C. The incubation with lectin-containing solution in the absence and presence of lactose and the glycoproteins lasted 1 h at 37 °C, and the extent of bound lectin was determined after thorough washing steps with aliquots of the indicator conjugate strept-avidin–peroxidase (0.5 μ g mL^{-1} ; Sigma, Munich, Germany) and then with *o*-phenylenediamine (1 mg mL^{-1})/ H_2O_2 (1 μ L mL^{-1}) as chromogenic substrates (31). Cell assays were performed with the human B-lymphoblastoid line Croco II, the T-lymphoblast line MOLT-4 (T-ALL), the human pancreatic carcinoma line Capan-1, with reconstituted expression of the tumor suppressor p16^{INK4a}, which enhances galectin-binding capacity linked to susceptibility to anoikis (32; kindly provided by K. M. Detjen, Berlin, Germany), and the colon adenocarcinoma line SW480 in Dulbecco's phosphate-buffered saline (9.2 mM Na_2HPO_4 , 1.5 mM KH_2PO_4 , 137 mM NaCl, and 2.7 mM KCl at pH 7.4) using standard conditions with lectin incubation for 30 min at 4 °C to minimize uptake and streptavidin/R-phycoerythrin as fluorescent indicator (1:40; Sigma, Munich, Germany). FACS analysis and processing of data from fluorescence detection yielded the percentage of positive cells and the mean fluorescence intensity (15, 32). Cell proliferation in the absence/presence of test compound in aliquots of cell suspension was monitored after 48 h by using the blue chromogen MTT (5 mg mL^{-1} ; 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide) (33).

RESULTS AND DISCUSSION

Synthesis of the Glycocompounds. A convergent strategy with lactose derivatives activated as *p*-nitrophenyl carbonates was set up, taking advantage of (a) the fast reaction of *p*-nitrophenyl carbonates with amines, (b) the high yields of resulting carbamates, and (c) the versatile nature of the reaction with a variety of amino-substituted scaffolds (34, 35). To delineate any influence of sugar spacing on lectin binding, we synthesized two series of ligands with the carbamate either directly connected to the anomeric center (*O*-glycosyl carbamates **A–F**) or separated from the pyranose ring by a butene diol linker (**G–J**). The synthesis of *O*-glycosyl carbamates by addition of anomeric unprotected carbohydrates to isocyanates is, with few exceptions (36), not stereoselective (37–39) and requires extensive product purification and identification, especially if several carbohydrate moieties are attached in one step. Activated glycosyl carbonates and 1-imidazolylcarbonyl glycosides, on the other hand, are excellent precursors of *O*-glycosyl carbamates—provided they are available as pure isomers—because they react under retention of configuration at the anomeric center. However, the preparation of glycosyl carbonates and 1-imidazolylcarbonyl glycosides from anomeric unprotected carbohydrates usually leads to α/β mixtures,

Scheme 1. Synthesis of Lactosyl Carbonate 3

Table 1. Synthesis of lactosyl carbonate **3** under various reaction conditions according to Scheme 1

solvent	base	α/β ratio ^a	yield (%) ^b
toluene	K_2CO_3	1:2	80
	NaH	1:1	60
	Et_3N	1:1	70
	pyridine	1:4	80
	2,6-lutidine	1:1	85
CH_2Cl_2	K_2CO_3	n.d. ^c	<10
	NaH	1:1.5	80
	Et_3N	1:1	80
	pyridine	2:1	80
	DMAP	2:1	80
THF	2,6-lutidine	1:4	90
	K_2CO_3	n.d.	<10
	Et_3N	1:2.5	80
	pyridine	1:1	80
	DMAP	2.5:1	80
CH_3CN	2,6-lutidine	1:7	30
	K_2CO_3	1:1	90
	Et_3N	1.5:1	90
	pyridine	3:1	90
	KOt-Bu	1:2.5	35
	DMAP	>19:1	95 ^c
	2,6-lutidine	1:>10	50 ^d

^a Determined by ^1H NMR spectroscopy of crude product.

^b Determined by ^1H NMR spectroscopy of crude product unless otherwise stated. ^c Yield of isolated α -**3**. ^d Yield of isolated β -**3**. ^e n.d. = not determined.

too (18, 40–45). Leenders et al. reported high β -selectivity by hydrolysis of an intermediate anomeric silyl ether and immediate condensation of the resulting β -hydroxy group with succinimidyl chloroformate in an overall yield of 38% (46). When we applied this strategy to lactosyl carbonates, we also experienced a low overall yield (30–40%). Therefore, we searched for conditions that gain fast and easy access to pure isomers of activated lactosyl carbonates to answer the first question given in the Introduction.

Scheme 1 and Table 1 summarize our results for the stereoselective preparation of lactosyl carbonate **3**. From anomeric *O*-alkylation reactions, it is well-established that solvent and base have a tremendous influence on the selectivity at the anomeric center (47, 48). To explore the effect of these parameters on stereoselective *O*-acylation, we reacted anomeric unprotected lactose (**1**) and *p*-nitrophenyl chloroformate (**2**) in different base/solvent combinations. With K_2CO_3 , NaH, Et_3N , and pyridine, only modest degrees of selectivity were observed regardless of the solvent. Similar results had been reported with CH_2Cl_2 /pyridine ($\alpha/\beta = 2/1$, 72%) and dimethoxyethane/NaH ($\alpha/\beta = 1/1$, 55%) (18). In acetonitrile, however, 4-(dimethylamino)pyridine (DMAP) led to a remarkable α/β -selectivity of >19:1, and it was possible to isolate α -**3** in a yield of 95%. In contrast, the sterically hindered base 2,6-lutidine gave an α/β -mixture of 1:>10, from which β -**3** could be purified in a yield of 50%. Attempts to improve the yield with

Scheme 2. Synthesis of Activated Carbonate 7

conservation of the β -selectivity by prolonging reaction times, however, were unsuccessful. We also observed that the concentration of the reaction mixture is crucial for product generation. Increasing the concentration from 0.03 M (the concentration at which all reactions had been carried out) to 0.4 M gave quantitative turnover, but with complete loss of selectivity ($\alpha/\beta = 1/1$). At lower concentrations (0.003 M) no products were found.

The synthesis of spacer-separated carbonate **7** is depicted in Scheme 2. Acetobromolactose **4** (20, 21) and excess (Z)-butenediol **5** were reacted under Koenigs–Knorr conditions to give the β -glycoside **6**. Treatment with chloroformate **2** and pyridine in acetonitrile resulted in *p*-nitrophenyl carbonate **7** with 87% yield. The activated carbonates β -**3** and **7** were coupled to a series of amines shown in Figure 1 to give the peracetylated lactosides Ac-**A**–Ac-**J**. These were deacetylated to the mono- to tetravalent lactose derivatives **A**–**J** in a straightforward manner (Schemes 3 and 4), completing the synthesis. These compounds were next tested for their potency relative to lactose to inhibit the binding of labeled lectins to the (neo)glycoproteins.

Solid-Phase Inhibition Assays. Commonly, solid-phase inhibition assays are performed with a single type of matrix. To address the issue of whether the inhibitory activity of glycoclusters depends on local density of glycans in the (neo)glycoprotein matrix, we employed four types of matrices. The (neo)glycoproteins (LacBSA, SAP, ASF, and AGP, respectively) were adsorbed to the surface of plastic wells of microtiter plates. In each experimental series, the binding of the lectins to the (neo)glycoproteins was saturable and inhib-

itable by blocking the carbohydrate-binding site of the lectins. Their concentration was adjusted to yield signal intensity in the linear range in each case. To assess the inhibitory profiles lectin incubation was carried out in the presence of different concentrations of lactose and the synthesized lactose derivatives **A**–**J**, respectively. Figure 2 shows examples for inhibition of the binding of biotinylated VAA to AGP and human galectin-4 to LacBSA, respectively, by lactose and lactose derivatives **B**, **D**, and **F**. From these inhibition curves, we determined the concentration of inhibitor leading to inhibition of bound lectin of 50% (IC_{50}). All IC_{50} values of multivalent ligands were calculated per lactose moiety contained; i.e., they were valency-corrected, leading to lower inhibitory potencies of some compounds (for example, trivalent **F**, Figure 2) compared to free lactose. Relative inhibitory potencies compared to free lactose (set to 1) were plotted for each type of matrix, test substance, and lectin (Figure 3A–D). Only data for inhibitors with values above 1 are given.

The inhibition tests revealed that conjugation of lactose to an aglycon by carbamate formation can increase inhibitory potency. Some selectivity of the bivalent lactose derivatives for certain galectins could be detected. The highest relative inhibitory potency (65 per lactose moiety), e.g., was observed for inhibition of binding of galectin-1 to the LacBSA surface by bivalent **E**. The insertion of the spacer (direct comparison for the pairs **B/G** and **D/I**) had favorable consequences only rarely. On average, these glycoclusters were more active than lysine-based compounds and surpass the activity of Tris- or aspartate-glycine-based compounds with 6-aminohexyl spacers in several cases (31, 49).

Comparison of the different (neo)glycoprotein matrices revealed interesting insight into the dependence of the inhibitory activity of the lactose clusters on the local density of glycans in the matrix. For example, relative inhibition was generally lowered with increased degree of glycan branching, except for the chimera-type galectin-3 (Figure 3A–D). Since this lectin is a substrate for proteolytic processing by matrix metalloproteinases-3/-9 *in vivo* with impact for its activity in biosignaling (50, 51), we tested the truncated version along with full-length galectin-3. Conspicuous selectivity for full-length galectin-3 but not its proteolytically processed derivative was observed for several ligands (**B**, **C**, **E**, **F**) with short spacers when the degree of glycan branching on the matrix was high (Figure 3D). This feature resembles the activity of 1,3,5-triiodobenzene-based inhibitors with rigidified 2-propynyl lactoside with its 7.6-fold enhancement relative to lactose and of an octavalent poly(amidoamine) starburst glycodendrimer (52, 53). Under this special condition, even the monovalent compound **A** showed high activity with remarkable selectivity for galectin-3 (Figure 3D). As detailed comparison among the four panels A–D reveals, relative potency is a factor of ligand display on the matrix (see **E** in Figure 3A–D as instructive example). Inhibitory capacity should thus not be regarded as an absolute measure. Its emerging context-dependent nature confers some uncertainty to predictions for the impact on binding under physiological conditions, i.e., to cell surfaces. It is thus an obligatory step to determine whether the lactose derivatives can interfere with the binding of lectins to cells, using established tumor cells as model.

Cell-Binding Inhibition Assays. In these assays, biotinylated lectins were incubated with cells of different lines. The extent of binding of lectins to cells was measured by using fluorescently labeled streptavidin as second-step reagent. As described in the preceding part, concentration dependence of lectin binding was first determined to plot the linear range and plateau levels in each combination. Then, sugar specificity and constant reactivity of cells were ascertained in each case. An example for the carbohydrate dependence of lectin binding is given in Figure

Figure 1. Amines used for preparation of **A**–**J**.

Scheme 3. Synthesis of Lactosyl Carbamates A–F^a

^a Method A: NaOMe, MeOH (used for **A–D**). Method B: EtNMe₂, MeOH (used for **E**, **F**).

Scheme 4. Synthesis of Lactosyl Carbamates G–J

4. To preclude an impact of any variability in the cells' reactivity due to length of culture time during the passage, comparative measurements were performed with aliquots of cell batches in

the same series. The obtained data, expressed in percentage of positive cells that show fluorescence above the control and mean fluorescence intensity (*F*), confirms that the test substances can

Figure 2. Inhibition of binding of biotinylated VAA (7.5 $\mu\text{g mL}^{-1}$; left) and human galectin-4 (5 $\mu\text{g mL}^{-1}$; right) to glycans of a (neo)glycoprotein-presenting matrix by a glycocompound and free lactose for comparison. The matrix presenting the lectin ligands was AGP (0.5 $\mu\text{g/well}$; left) and the neoglycoprotein LacBSA (0.25 $\mu\text{g/well}$; right). Lactose ($\blacktriangle$) was used as control, and the bivalent compounds **D** ($\circ$) and **B** ($\diamond$) and the trivalent compound **F** ($\bullet$) as inhibitors. The concentration of inhibitor is calculated with respect to its lactose contents present in the inhibition assay to normalize data.

Figure 3. Relative potency of the panel of the synthetic lactose-containing compounds A–J (for structures, see Schemes 3 and 4; designations of lectins are given in the top panel) as inhibitor of lectin binding. The IC_{50} values are based on the lactose concentration with free lactose as standard (set to 1) and they were determined in each case by systematic inhibition assays (for an example, see Figure 2). Experiments were repeated up to six times; the standard deviation did not exceed 11%. All cases better than free lactose are given. The optimal conditions were as follows: (A) 0.25 $\mu\text{g}/\text{well}$ neoglycoprotein (lactosylated bovine serum albumin: LacBSA) and 0.5 $\mu\text{g mL}^{-1}$ VAA, 30 $\mu\text{g mL}^{-1}$ galectin-1, 15 $\mu\text{g mL}^{-1}$ galectin-3 (both versions), 5 $\mu\text{g mL}^{-1}$ galectin-4, and 35 $\mu\text{g mL}^{-1}$ galectin-5; (B) 0.5 $\mu\text{g}/\text{well}$ serum amyloid P component (SAP) and 2 $\mu\text{g mL}^{-1}$ VAA, 2.5 $\mu\text{g mL}^{-1}$ galectin-1, 5 $\mu\text{g mL}^{-1}$ full-length galectin-3, 30 $\mu\text{g mL}^{-1}$ truncated galectin-3, 5 $\mu\text{g mL}^{-1}$ galectin-4, and 15 $\mu\text{g mL}^{-1}$ galectin-5; (C) 0.25 $\mu\text{g}/\text{well}$ asialofetuin (ASF) and 0.5 $\mu\text{g mL}^{-1}$ VAA, 5 $\mu\text{g mL}^{-1}$ galectin-1, full-length galectin-3 and galectin-4, 30 $\mu\text{g mL}^{-1}$ truncated galectin-3, and 10 $\mu\text{g mL}^{-1}$ galectin-5; (D) 0.5 $\mu\text{g}/\text{well}$ α -acid glycoprotein (AGP) and 7.5 $\mu\text{g mL}^{-1}$ VAA, 30 $\mu\text{g mL}^{-1}$ galectin-1, 10 $\mu\text{g mL}^{-1}$ full-length galectin-3, 60 $\mu\text{g mL}^{-1}$ truncated galectin-3, 20 $\mu\text{g mL}^{-1}$ galectin-4, and 25 $\mu\text{g mL}^{-1}$ galectin-5.

Figure 4. Semilogarithmic representation of the fluorescent surface staining of T-lymphoblast cells MOLT-4 (T-ALL) by labeled full-length galectin-3 ($10 \mu\text{g mL}^{-1}$). The control value representing staining by the second-step reagent in the absence of lectin is given as the shaded area; the peak for lectin binding in the absence of any inhibitor as the black line. Quantitative data on the percentage of positive cells that show fluorescence above the control and mean fluorescence intensity are given. The left diagram illustrates the inhibition curves obtained by increasing concentrations of lactose of 1 mM, 2 mM, 4 mM, 8 mM, and 10 mM. Given values are for the control (background staining without lectin), 2 mM lactose, and maximal lectin binding without inhibitor. Right diagram: inhibition curves obtained by glycocompounds **E**, **B**, and **D** at a concentration of 2 mM of lactose residues (i.e., a concentration of bivalent inhibitors of 1 mM).

act as inhibitors of lectin binding to cells. As shown in Figure 4, compound **E** at a concentration of 2 mM of lactose residues (i.e., a concentration of bivalent **E** of 1 mM) reduces binding of galectin-3 to the MOLT-4 T-lymphoblast cells from 92% (F 39.9) to 8% (F 5.0). In comparison, a 2 mM solution of free lactose leads to a reduction to 50% (F 9.4). This compound distinguishes the full-length lectin from its truncated derivative and is only rather weakly reactive with galectins-1 and -5. In general, none of the lactose clusters surpasses lactose as inhibitor for galectin-1. Compound **D**, at a concentration of 2 mM in lactose moieties reduces binding of truncated galectin-3 to MOLT-4 T-lymphoblasts from 53% (F 9.0) to 28% (F 9.0) and binding of galectin-5 to the colon adenocarcinoma line SW480 from 89% (F 50.8) to 60% (F 16.7). A 2 mM solution of lactose correspondingly leads to a reduction to 32% (F 7.6) and 69% (F 20.2), respectively. Binding of galectin-4 to the p16^{INK4a}-expressing pancreatic carcinoma line Capan-1 is impaired best by the bivalent compound **B**, a strongly active reagent in the solid-phase assays, with a reduction from 60% (F 47.0) to 16% (F 7.6) at a concentration of lactose residues of 4 mM. Here, even a 20 mM lactose solution results in a reduction to only 44% (F 24.5). To examine the influence of cell type, we tested galectin-3 binding also with pancreatic carcinoma cells. In this system, compound **F** was the best inhibitor with a nearly 4-fold enhancement relative to lactose. Similar results on influence of cell type were obtained with the plant toxin, with compound **D** yielding the best results at a nearly 2-fold increase (not shown).

Having delineated the activity of the lactose derivatives in lectin binding to tumor cells, any further consideration for potential applications will crucially depend on their lack of cytotoxicity. To answer this last question, we monitored cell proliferation over a period of 48 h in the presence of lactose as control and lactose clusters selected to represent the scaffolds all at the same concentration of sugar in parallel. Compounds **D**, **F**, **G**, and **I** did not influence the growth of SW480 colon adenocarcinoma cells at a concentration of lactose residues of 2 mM; compound **F** was also tested on Capan-1 pancreatic carcinoma cells at 5 mM without influence on proliferation.

CONCLUSIONS

In summary, we investigated inhibition of binding of adhesion/growth-regulatory galectins and a plant toxin to a series of immobilized (neo)glycoproteins and to tumor cells by mono- to tetravalent lactose derivatives. Our experiments enabled us to answer the five questions given at the end of the Introduction:

(1) Synthetically, we developed conditions to stereoselectively produce activated lactosyl carbonates from the anomerically unprotected disaccharide. These derivatives have general application for the attachment of lactosyl residues to amino-substituted scaffolds yielding lactosyl carbamates. (2) In systematic solid-phase screening, bioactivity was found to increase by more than 1 order of magnitude relative to free lactose (valence-corrected values) for galectins. (3) Individual scaffold structure was more important than valency of lactose in the glycoconjugates, and introduction of butene diol to extend the spacer's length led to enhancements only rarely. However, the inhibitory activity showed selectivity for lectin type (plant toxin vs galectins) and among galectins. In addition, the nature of the matrix glycans ranging from bi- to tetraantennary N-glycans and including the neoglycoprotein LacBSA also had a strong impact on inhibitory potency and selectivity. Interestingly, rather low activity was noted with the AGP matrix having the highest degree of branching except for the chimera-type galectin-3, an effector in cardiac dysfunction (54). This is why test series should routinely include cell assays to gauge potential for lectin blocking in a physiological context. (4) Strong inhibition of cell binding was detected for galectins-3 and -4 with distinct lactose-bearing compounds, and (5) we could show that representative examples of our compounds are not cytotoxic, emphasizing the suitability of carbamate-linked lactose derivatives in a lectin blocking approach.

Evidently, it is a reasonable long-term aim to tailor scaffolds adorned with lectin ligands for the ability to distinguish lectin groups or even members of a family based on reactivity. Due to arising evidence for functional antagonism among lectins, e.g., for galectins-1 and -3 in tumor growth regulation (50), such a discriminatory capacity is desirable. However, the impact of glycan branching revealed herein precludes immediate extrapolations from data in a single system. The measured relative reduction in inhibitory potency in cell assays could be compensated by tailoring the glycan part to the lectin. Extended structures such as histo-blood group ABH epitopes or other natural ligands will enhance affinity and selectivity (9, 11, 55–57).

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