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The Synthesis of Linear Trilactosamine

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Abstract—TrilactosamineGalβ1–4GlcNAcβ1–3Galβ1–4GlcNAcβ1–3Galβ1–4GlcNAcβ-sp, where sp = $O(CH_2)_3NH_2$ is a spacer, was synthesized. The tetrasaccharide fragment Galβ1–4GlcNAcβ1–3Galβ1–4GlcNAcβ-sp was obtained by successive glycosylation using elongation by one monosaccharide residue at a time; and the tetrasaccharide was then transformed into a hexasaccharide with a disaccharide glycosyl donor. A 2,2,2-trichloroethoxycarbonyl group was used for the protection of the glucosamine amino group.

Key words: N-acetyllactosamine, oligosaccharide synthesis, polylactosamine

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INTRODUCTION

Oligolactosamine and polylactosamine fragments are specific structural elements of complex *N*- and O-chains of glycoproteins and some glycolipids [1, 2].[2] One of the functions of oligolactosamines is specific interaction with proteins of the galectine family [3]. The topicality of detailed studies of galectine carbohydrate specificity is explained by their involvement in various biological processes, for example, they mediate cell adhesion, proliferation, and apoptosis [4–7]. For studying galectine specificity directly on the cell surface we needed to have oligolactosamines and fluorescein-labeled glycoconjugates on their basis. Although chemical and enzymatic synthesis of oligolactosamines was described earlier [8–11], we propose another method providing intermediate properly protected derivatives for the synthesis of substituted oligolactosamines, such as sulfated and fucosylated oligosaccharides.

Earlier, we reported the synthesis of short and branched oligolactosamines [12]. In this work, we describe the synthesis of linear hexasaccharide, which is currently considered to be one of the most potent affinity ligands of most human galectines [13].

RESULTS AND DISCUSSION

The main goal of this work was the preparation of trilactosamine in the form of a 3-aminopropyl derivative suitable for preparation of a fluorescein-labeled probe. *N*-Trichloroethoxycarbonyl (Troc) derivatives of lactosamine or glucosamine were used as glycosyl donors because the *N*-Troc protection provided β-stereoorientation of glycosylation, but at the same time transformation of the Troc-NH fragment into the target Ac-NH did not affect other protective groups [14]. The presence of 6-OH benzyl groups in the intermediate products increased their solubility in organic solvents, made chromatographic separation easier and, what is most important, enabled the use of other protective groups for release of this or that hydroxyl group.

Glycosylation of glucosamine derivative (**II**) [15] at the 4-OH group with glycosyl bromide (**I**) [16] yielded 61% of disaccharide (**III**) (Scheme). The formation of a β-glycoside bond was supported by a characteristic value of $J_{1b,2b}$ 7.9 Hz.

Successive deacetylation of lactosamine derivative (**III**), orthoether protection, acetylation, and opening of the orthoether cycle [17] rsulted in disaccharide (**IV**) in a total yield of 59%. The presence of an OH group at the C3 galactose fragment was supported by a downfield shift of the H3b resonance (δ 3.54–3.68) if compared with acetylated analogue (**III**) (δ 4.870 ppm, H3b).

Trisaccharide (**IV**) was obtained in a yield of 65% by glycosylation of disaccharide (**IV**) with bromide (**V**). In addition, the corresponding α anomer at the terminal residue was isolated (15%). In the presence of zinc in a mixture of acetic acid and acetic anhydride, the Troc protection of compound (**VI**) was substituted by a *N*-acetyl group to give 77% of derivative (**VII**). The glycosylation site and β configuration of the glucosamine terminal residue was confirmed by ^{1}H NMR spectroscopy (δ 3.69 ppm, H3b, $J_{1c,2c}$ 7.9 Hz). Trisaccharide (**VII**) was deacetylated, benzylidenated, and acetylated (see the scheme) to obtain derivative (**VIII**) in a total yield of 70%. The use of sodium cyanoboro-

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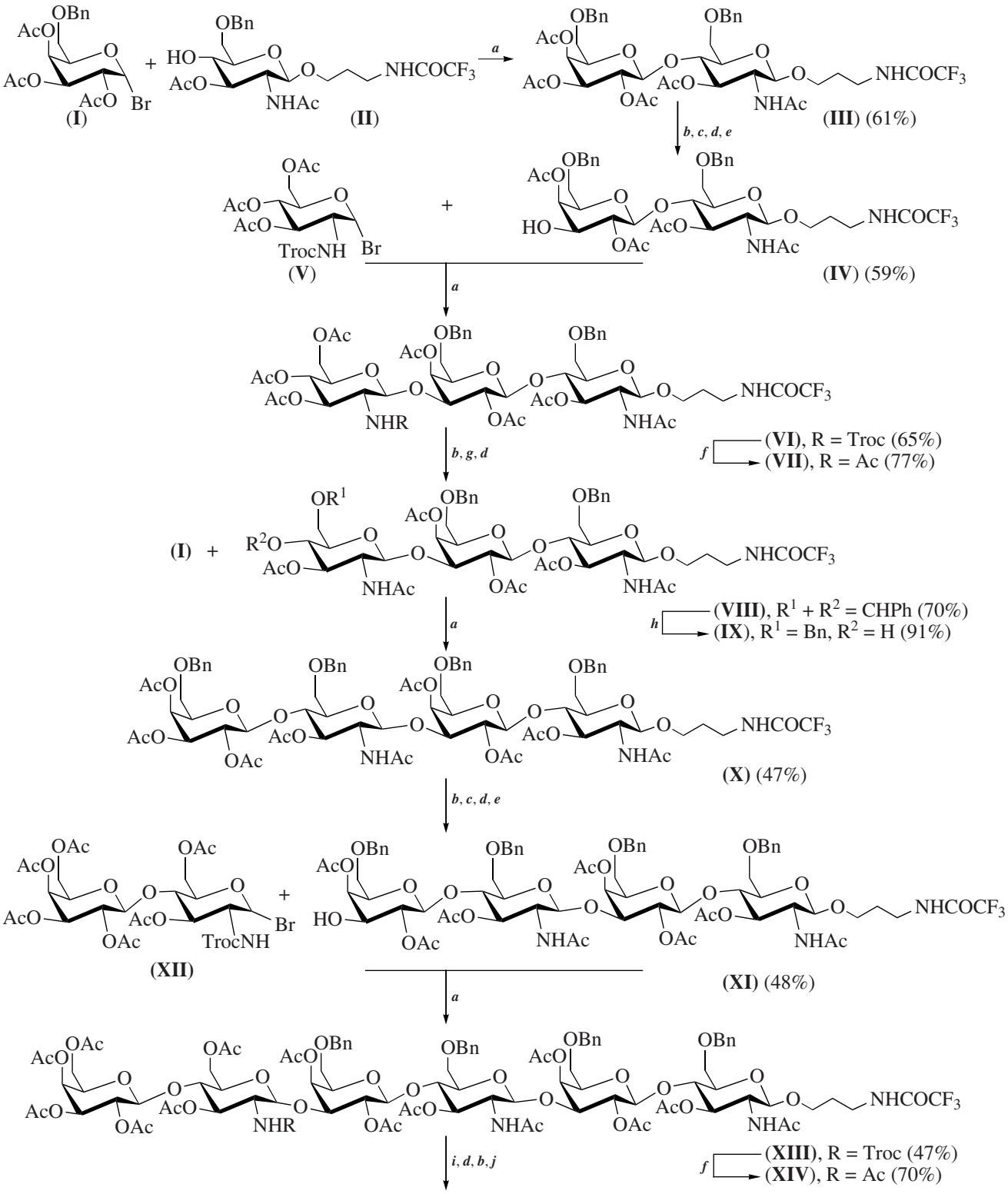
[2] Abbreviations: Bn, benzyl; LacNAc, *N*-acetyllactosamine; MS, mass-spectrum; AgOTf, silver triflate; TMM, tetramethylurea; Troc, 2,2,2-trichlorethoxycarbonyl.



Galβ1–4GlcNAcβ1–3Galβ1–4GlcNAcβ1–3Galβ1–4GlcNAcβ-O$(CH_2)_3NH_2$ (XV) (89%)

Scheme. ***a*****:** AgOTf, TMM, MS-4 Å, CH_2Cl_2; ***b***: MeONa/MeOH; ***c***: $MeC(OEt)_3$, TsOH, MeCN; ***d***: Ac_2O/Py; ***e***: 80% aqueous AcOH; ***f***: AcOH, Ac_2O, Zn, NEt_3; ***g***: $PhCH(OMe)_2$, TsOH, MeCN; ***h***: $NaCNBH_3$, THF, HCl/Et_2O; ***i***: 10% Pd/C, H_2, MeOH; ***j***: NaOH/H_2O.

hydride [18] provided selective opening of the benzylidene cycle to give 91% oligosaccharide (**IX**) bearing an OH group at C4c and a benzyl group at C6c, which was supported by correlation of proton resonances in the ^{1}H NMR spectra at C4c (δ 3.58–3.70 ppm) and C6c (δ 3.872 and 4.027 ppm) with proton resonances at C4 and C6 (δ 3.70–3.81 ppm) of compound (**II**).

Tetrasaccharide (**X**) in 47% yield was prepared by glycosylation of trisacchardie (**IX**) with 6-OBn-acetobromogalactose (**I**). The formation of a β1–4 bond followed from the ^{1}H NMR data: $J_{1d,2d}$ 7.9 Hz; proton resonances of C4c and C4a (δ 3.885 and 3.902 ppm), C5c and C5a (δ 3.50–3.60 ppm), as well as C6c and C6a (δ 3.66–3.74 ppm).

Derivative (**XI**) with one OH group in the terminal galactose residue (δ 3.708 3.708 ppm, H3d) was obtained in a total yield of 48% by successive deacetylation, introduction of the orthoether protection, acetylation, and opening of the orthoether cycle of dilactosamine derivative (**XI**). Glycosylation of the latter with glycosyl bromide of *N*-Troc lactosamine derivative (**XII**) resulted in 47% hexasaccharide (**XIII**). The Troc protective group was substituted by an *N*-acetyl one as described above to obtain derivative (**XIV**) in a yield of 70% ($J_{1e,2e}$ ~ 8 Hz).

Trilactosamine peracetate was obtained in 80% yield by hydrogenation of derivative (**XIV**) followed by acetylation. Its deacetylation and removal of the *N*-trifluoroacetyl group resulted in 89% of target trilactosamine (**XV**). The analysis of ^{13}C NMR spectral data [19] indicated the presence of an aminopropyl spacer (δ 26.96, 37.60, and 67.96 ppm) and nonglycosylated C6-OH groups (δ 59.85–61.03 ppm). The lack of upfield resonances of methylene groups (δ 68–69 ppm) unambiguously indicated the absence of 1–6 glycoside bonds. The presence of a double-carbon resonance (δ 82.08 ppm) confirmed the presence of two β1–3 glycosylated galactose residues and the presence of a group of signals (δ 78.15, 78.17, and 78.47 ppm), of three β1–4 glycosylated glucosamine residues. The presence in the anomeric area (δ 101.16–102.91 ppm) of three resonance groups of six anomeric carbon atoms completely corresponded to the proposed structure containing three β-galactose residues, one terminal and two internal residues of β-glucosamine. The obtained ^{13}C NMR data and their comparison with the published data for trilactosamine [11] and dilactosamine derivative [9] unequivocally confirmed the structure of the synthesized hexasaccharide (**XV**).

The structures of the prepared compounds were confirmed by mass spectral data and ^{1}H NMR data based on 2D-^{1}H and^{1}H-COSY experiments.

The proposed synthetic scheme enables the control of regio- and stereoselectivity of successive elongation of the oligosaccharide chain and the use of intermediate compounds for the synthesis of more complex oligolactosamine derivatives, particularly:

(1) sulfated at C-6 galactose or glucosamine residues;

(2) sulfated at C-3 galactose residues;

(3) α-fucosyl and α-galactosyl derivatives at galactose positions 3 and 6;

(4) oligolactosamines branched at C-6 galactose residue.

Hexasaccharide (**XV**) was converted into a fluorescein-labeled polyacrylamide derivative (OS-PAA-fluo) with a molecular mass of ~30 kDa using the earlier described procedure [20]. This derivative as well a series of analogous probes obtained from simpler oligolactosamines were used for studying specificity and functioning of galectines-1 and -3 composing a cell membrane [21].

EXPERIMENTAL

Optical rotation was measured on a digital polarimeter Jasco DIP-360 at 25°C. ^{1}H NMR spectra (in $CDCl_3$ if not stated otherwise) were registered on a Bruker WM spectrometer 500 MHz; ^{13}C NMR on a Bruker 600 MHz at 303K. Chemical shifts (δ for characteristic proton resonances are given in ppm and coupling constants *J*, in Hz. The resonances in ^{1}H NMR spectra were assigned using a technique of spin–spin decoupling (double resonance) and 2D-^{1}H,^{1}H- COSY experiments. The resonances in ^{13}C NMR spectra were assigned using DEPT-135 experiments. Mass spectra were registered on a MALDI-TOF Vision-2000 spectrometer (Thermo Bioanalysis Corp., England). Dihydroxybenzoic acid was used as a template. TLC was performed on Kieselgel-60 plates (Merck, Germany); the compounds were developed with 5% orthophosphoric acid at 150°C (carbohydrates) or ninhydrin solution (3 g/l in a 30 : 1 butanol–acetic acid mixture) (amines). Column chromatography was performed on Silica gel 60 (0.040–0.063 mm, Merck, Germany); gel-chromatography, on Sephadex LH-20 columns (Pharmacia, Sweden). Solvents were evaporated in vacuum at 30–40°C. Solvents for glycoside syntheses were dried and stored with molecular sieves; solid reagents were dried for two hours in vaccum (0.1 mm Hg) at 20–40°C. For the syntheses of bromides (**V**) and (**XII**), ethyl-3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamido)-1-thio-β-*D*-glucopyranoside and ethyl-3,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamido)-4-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-1-thio-β-*D*-glucopyranoside obtained by acetylation of commercial ethylthioglycosides of *N*-Troc substituted glucosamine and lactosamine (GlycoRex, Sweden), respectively, were used.

Deacetylation was carried out using the Zemplen reaction in dry methanol by adding 2 M sodium methylate in methanol to the acyl derivative up to pH 8–9. After the reaction completion Na^+ ions were removed on a resin Dowex 50X-400 (H+) (Acros, Belgium) and the solution was evaporated.

Glycosyl bromides (I), (V), and (XII). A solution of the corresponding thioglycoside (270 mg, 0.332 mmol) in dry dichloromethane (10 ml) was cooled to 0°C and bromine (18 μl, 0.37 mmol) was added. The reaction mixture was kept for 1 h at 0°C, evaporated, coevaporated with toluene, and used without additional purification.

General glycosylation procedure. A solution of glycosyl acceptor (0.1 mmol), silver triflate (0.2 mmol), tetramethylurea (0.2 mmol), and freshly calcinated molecular sieves 4 Å in dry dichloromethane (5 ml) were stirred at room temperature and in darkness for 30 min. Another portion of sieves 4 Å (100 mg) was added, and a solution of glycosyl bromide (0.2 mmol) in dry dichloromethane (2 ml) was added. The mixture was filtered in 15–20 h, the solvent was evaporated, and the product was isolated by silica gel chromatography.

Acetylation was performed with a 2 : 1 pyridine–acetic anhydride mixture at 20°C for 12–24 h; the reagents were coevaporated with toluene.

Hydrogenolysis was carried out over 10% Pd/C (Merck, Germany) at a substrate–catalyst ratio of 1 : 1 (w/w) in methanol at atmospheric pressure for 1–3 h.

Removal of the Troc group followed by N-acetylation. Freshly activated Zn dust (4 g) and then triethylamine (0.5 ml) were added to a solution of Troc derivative (0.5 mmol) in acetic acid (40 ml) and acetic anhydride (2 ml). In 20–30 h the mixture was filtered, evaporated, and chromatographed on a LH-20 column eluted with a 1 : 1 chloroform–methanol mixture.

Deacetylation and removal of N-trifluoroacetyl protection. A solution of 2 M sodium methylate in methanol (50 μl) was added to a solution of oligosaccharide peracetate (0.05 mmol) in dry methanol (2 ml), the solution was evaporated in 1 h, water (2 ml) was added, the solution was kept for 3 h and chromatographed on a Dowex-H^+ column (elution with 1 M ammonia). The eluate was evaporated and lyophilized.

(3-Trifluoroacetamidopropyl)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-4-*O*-(2,3,4-tri-*O*-acetyl-6-*O*-benzyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside (III). Glycosylation of glucosamine derivative (**II**) (2.6 g, 5.14 mmol) with glycosyl bromide (**I**) obtained from the corresponding thioglycoside (4.52 g, 10.28 mmol) gave after twofold purification by silica gel chromatography (elution with 0–6% MeOH in chloroform, then 4 : 2 : 1 hexane–chloroform–isopropanol) the starting monosaccharide (**II**) (409 mg, 16%) and disaccharide (**III**) (2.78 g, 61%), R_f 0.4 (10% MeOH in chloroform)), $[\alpha]_D$ –48.7 (*c*1, $CHCl_3$), ^{1}H NMR: 1.840 (2 H, m, CH_2), 1.900, 1.959, 1.972, 1.995, 2.019, 2.029, 2.053, and 2.060 (5 × 3 H, 5 c, 5 Ac), 3.260 (1 H, m, CHN), 3.303 (1 H, ddd, $J_{2,NH}$ 7.9, $J_{1,2}$ 7.9, $J_{2,3}$ 10.3, H2c), 3.439 (1 H, dd, $J_{6',6''}$ 9.0, $J_{5,6''}$ 7.9 H6''b), 3.489 (2 H, m, H5a, H6'b), 3.559 (1 H, m, CHN), 3.613 (2 H, m, CHO, H5b), 3.65–3.74 (4 H, H6'a, H6''a, H3b, H5c), 3.917 (2 H, m, CHO, H4a), 4.032 (1 H, ddd, $J_{1,2}$ 7.9, $J_{2,NH}$ 8.6, $J_{2,3}$ 9.7, H2a), 4.144 (1 H, dd, $J_{6',6''}$ 11.9, $J_{5,6''}$ 4.2, H6''c), 4.311 (1 H, dd, $J_{6',6''}$ 11.9, $J_{5,6''}$ 2.2, H6'c), 4.345 and 4.361 (2 × 2 1 H, 2 d, $J_{1,2}$ 7.9, H1a, H1b), 4.468, 4.490, 4.514, and 4.703 (4 × 1 H, 4 d,$J_{A,B}$ 12.0, 2 × CH_2Ph), 4.939 (1 H, dd, $J_{1,2}$ 7.9, $J_{2,3}$ 10.3, H2b), 4.980 (1 H, dd ≈ t, $J_{3,4}$ 8.8, $J_{2,3}$ 9.7, H3a), 5.000 (1 H, d, $J_{1,2}$ 7.9, H1c), 5.029 (1 H, dd ≈ t, $J_{3,4}$ 9.5, $J_{4,5}$ 9.9, H4c), 5.393 (1 H, dd ≈ d, $J_{3,4}$ 3.4, $J_{4,5}$ < 1, H4b), 5.421 (1 H, d, $J_{2,NH}$ 7.9, N*H*Ac-c), 5.508 (1 H, dd ≈ t, $J_{2,3}$ 10.3, $J_{3,4}$ 9.5, H3c), 5.794 (1 H, d, $J_{2,NH}$ 8.8, N*H*Ac-a), 7.25–7.39 (10 H, m, 2 × Ph), 7.491 (1 H, m, $NHCOCF_3$).

(3-Trifluoroacetamidopropyl)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-4-*O*-(2,4-di-*O*-acetyl-6-*O*-benzyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside (IV). Disaccharide obtained by Zemplen deacetylation of lactosamine derivative (**III**) (2.74 g, 3.1 mmol) was dissolved in dry acetonitrile (50 ml). Triethylorthoacetate (0.6 ml, 4.57 mmol) and toluenesulfonic acid (80 mg) were added, and the mixture was stirred at room temperature for 1 h. After neutralization with pyridine and evaporation the resulting orthoacetate was acetylated with a 2 : 1 mixture of pyridine and acetic anhydride (30 ml) and 80% acetic acid (40 ml) was added. The mixture was kept for 2 h and coevaporated with toluene. After silica gel chromatography (elution with 100 : 5 : 0.5 chloroform–isopropanol–pyridine) lactosamine derivative (**IV**) (1.53 g, 59%) was isolated; R_f 0.37 (10% isopropanol in chloroform), $[\alpha]_D$ –54.9 (*c*1, $CHCl_3$); MS, *m*/*z*: 865 (842 + 23) (M^+ + Na^+). ^{1}H NMR: 1.845 (2 H, m, CH_2), 1.965, 1.984, 2.036, and 2.080 (4 × 3 H, 4 s, 4 Ac), 2.416 (1 H, s, OH), 3.265 (1 H, m, CHN), 3.441 (1 H, dd, $J_{6',6''}$ 9.2, $J_{5,6''}$ 7.2, H6''b), 3.515 (2 H, m, $J_{6',6''}$ 9.2, $J_{5,6'}$ 5.5, H6'b, H5a), 3.54–3.68 (4 H, m, CHN, CHO, H5b, H3b), 3.713 (1 H, dd, $J_{6',6''}$ 10.7, $J_{5,6''}$ 2.0, H6''a), 3.768 (1 H, dd, $J_{6',6''}$ 10.7, $J_{6',5}$ 4.0, H6'a), 3.929 (2 H, m, CHO, $J_{3,4}$ 8.8, $J_{4,5}$ 8.6, H4a), 4.039 (1 H, ddd, $J_{2,NH}$ 8.8, $J_{1,2}$ 8.1, $J_{2,3}$ 9.7, H2a), 4.384 (1 H, d, $J_{1,2}$ 8.1, H1a), 4.407 (1 H, d, $J_{1,2}$ 7.9, H1b), 4.443, 4.494, 4.524, and 4.693 (4 × 1 H, 4 d, $J_{A,B}$ 11.9, 2 × CH_2Ph), 4.785 (1 H, dd, $J_{1,2}$ 7.9, $J_{2,3}$ 10.1, H2b), 5.000 (1 H, dd ≈ t, $J_{2,3}$ 9.7, $J_{3,4}$ 8.8, H3a), 5.335 (1 H, dd ≈ d, $J_{3,4}$ 3.5, $J_{4,5}$ < 1, H4b), 5.824 (1 H, d, $J_{2,NH}$, 8.8, N*H*Ac), 7.28–7.39 (10 H, m, 2 × Ph), 7.470 (1 H, m, $NHCOCF_3$).

(3-Trifluoroacetamidopropyl)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-4-*O*-[2,4-di-*O*-acetyl-6-*O*-benzyl–3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy)-β-*D*-glucopyranosyl]-β-*D*-glucopyranoside (VII). Lactosamine derivative (**IV**) (1.53 g, 1.82 mmol) was glycosylated with glycosyl bromide (**V**) obtained from the corresponding thioglycoside (1.98 g, 3.64 mmol), the mixture was chromatographed on silica gel (elution with 3 ⟶ 10%) isopropanol in chloroform) to give starting disaccharide (**IV**) (310 mg, 20%), α-glycosylation product (355 mg, 15%), and trisaccharide (**VI**) (1.53 g, 65%), R_f 0.28 (10% isopropanol in chloroform). Troc derivative (**VI**) was treated with zinc in acetic acid and the product was chromatographed on silica gel (elution with 10 ⟶ 20% isopropanol in

chloroform) to give *N*-acetyl derivative (**VII**) (1.06 g, 77%), R_f 0.23 (10% methanol in chloroform), $[\alpha]_D$ –21.2 (*c*1, $CHCl_3$); MS, *m/z*: 1194 (1171 + 23) (M^+ + Na^+). ^{1}H NMR: 1.840 (2 H, m, CH_2), 1.900, 1.959, 1.972, 1.995, 2.019, 2.029, 2.053, and 2.060 (8 × 3 H, 8 s, 8 Ac), 3.260 (1 H, m, CHN), 3.303 (1 H, ddd, $J_{2,NH}$ 7.9, $J_{1,2}$ 7.9, $J_{2,3}$ 10.3, H2c), 3.439 (1 H, dd, $J_{6',6''}$ 9.7, $J_{5,6''}$ 6.2, H6''b), 3.489 (2 H, m, H5a, H6'b), 3.559 (1 H, m, CHN), 3.613 (2 H, m, CHO, H5b), 3.65–3.74 (4 H, H6'a, H6''a, H3b, H5c), 3.917 (2 H, m, CHO, H4a), 4.032 (1 H, ddd, $J_{2,NH}$ 8.8, $J_{1,2}$ 7.9, $J_{2,3}$ 9.1, H2a), 4.144 (1 H, dd, $J_{6',6''}$ 11.9, $J_{5,6''}$ 4.2, H6''c), 4.311 (1 H, dd, $J_{6',6''}$ 11.9, $J_{5,6'}$ 2.2, H6'c), 4.345, and 4.361 (2 × 1 H, 2 d, $J_{1,2}$ 7.9, H1a, H1b), 4.468, 4.490, 4.514, and 4.703 (4 × 1 H, 4 d, $J_{A,B}$ 12.0, 2 × CH_2Ph), 4.939 (1 H, dd, $J_{1,2}$ 7.9, $J_{2,3}$ 10.3, H2b), 4.980 (1 H, dd ≈ t, $J_{3,4} \approx J_{2,3} \approx$ 9.1, H3a), 5.000 (1 H, d, $J_{1,2}$ 7.9, H1c), 5.029 (1 H, dd ≈ t, $J_{3,4}$ 9.5, $J_{4,5}$ 9.9, H4c), 5.393 (1 H, dd ≈ d, $J_{3,4}$ 3.5, $J_{4,5} < 1$, H4b), 5.421 (1 H, d, $J_{2,NH}$ 7.9, N*H*Ac-c), 5.508 (1 H, dd ≈ t, $J_{2,3}$ 10.3, $J_{3,4}$ 9.5, H3c), 5.794 (1 H, d, $J_{2,NH}$ 8.8, N*H*Ac-a), 7.25–7.39 (10 H, m, 2 × Ph), 7.491 (1 H, m, $NHCOCF_3$).

(3-Trifluoroacetamidopropyl)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-4-*O*-[2,4-di-*O*-acetyl-6-*O*-benzyl-3-*O*-(2-acetamido-3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy)-β-*D*-glucopyranosyl]-β-*D*-galactopyranosyl]-β-*D*-glucopyranoside VIII. To trisaccharide obtained by the Zemplen deacetylation of compound (**VII**) (1.06 g, 0.905 mmol) dry acetonitrile (20 ml), α,α-dimethoxymethylbenzene (235 μl, 1.57 mmol), and toluene sulfonic acid (35 mg) were added, and the mixture was stirred for 3 h at room temperature. The mixture was neutralized with pyridine, evaporated, and the resulting benzylidene derivative was acetylated with a 2 : 1 pyridine–acetic anhydride mixture (18 ml). After silica gel chromatography (elution with 7–15% isopropanol in ethyl acetate) derivative (**VIII**) (780 mg, 70%) was isolated, R_f 0.42 (10% isopropanol in ethyl acetate), $[\alpha]_D$ –55.2 (*c*1, $CHCl_3$); MS, *m/z*: 1175 (1175) (M^+). ^{1}H NMR: 1.839 (2 H, m, CH_2), 1.916, 1.961, 1.974, 2.003, 2.059, and 2.091 (6 × 3 H, 6 s, 6 Ac), 3.259 (1 H, m, CHN), 3.433 (1 H, dd, $J_{6',6''}$ 9.4, $J_{5,6''}$ 6.4, H6''b), 3.47–3.74 (11 H, CHN, CHO, H5a, H6'a, H6''a, H3b, H5b, H6'b, H2c, H5c, and H6''c), 3.829 (1 H, dd ≈ t, $J_{3,4} \approx J_{4,5} \approx$ 10.5, H4c), 3.922 (2 H, m, CHO, H4a), 4.041 (1 H, ddd, $J_{2,NH}$ 8.8, $J_{1,2}$ 8.3, $J_{2,3}$ 9.7, H2a), 4.356 (3 H, m, H1a, H1b, H6'c), 4.461, 4.494, 4.517, and 4.699 (4 × 1 H, 4 d, $J_{A,B}$ 12.0, 2 × CH_2Ph), 4.742 (1 H, d, $J_{1,2}$ 7.9, H1c), 4.969 (2 H, m, H3a, H2b), 5.365 (1 H, dd ≈ t, $J_{2,3} \approx J_{3,4} \approx$ 9.9, H3c), 5.399 (2 H, m, H4b, N*H*Ac-c), 5.513 (1 H, s, C*H*Ph), 5.828 (1 H, d, $J_{2,NH}$ 8.8, N*H*Ac-a), 7.29–7.45 (15 H, m, 3 × Ph), 7.493 (1 H, m, $NHCOCF_3$).

(3-Trifluoroacetamidopropyl)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-4-*O*-[2,4-di-*O*-acetyl-6-*O*-benzyl-3-*O*-(2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-*D*-glucopyranosyl)-β-*D*-galactopyranosyl]-β-*D*-glucopyranoside (IX). A mixture of benzylidene derivative (**VIII**) (780 mg, 0.664 mmol) and freshly calcinated molecular sieves 3 Å (2.5 g) in dry tetrahydrofuran (20 ml) was stirred for 30 min. Another portion of sieves (1 g) and sodium cyanoborohydride (420 mg, 6.67 mmol) in portions were added and then saturated HCl in dry Et_2O up to pH 2–3 was added for 1 h. After 1 h the mixture was neutralized with triethylamine, filtered, the residue was washed with chloroform and the united filtrate was evaporated. The dry residue was dissolved in chloroform (50 ml), washed with water (2 × 50 ml), dried with sodium sulfate, and evaporated. Trisaccharide (**IX**) (714 mg, 91%) was isolated by silica gel chromatography (elution with 10% isopropanol in ethyl acetate), R_f 0.30 (elution with 10% isopropanol in ethyl acetate); $[\alpha]_D$ –24.8 (*c*0.5, $CHCl_3$–CH_3OH); MS, *m/z*: 1177 (1201 + 23) (M^+ + Na^+). ^{1}H NMR (3 : 1 $CDCl_3$–CD_3OD): 1.962 (2 H, m CH_2), 2.006, 2.076, 2.093, 2.151, 2.199, and 2.205 (6 × 3 H, 6 s, 6 Ac), 3.395 (1 H, m, CHN), 3.564 (1 H, dd, $J_{6',6''}$ 9.7, $J_{5,6''}$ 6.4, H6''b), 3.58–3.70 (7 H, m, CHN, CHO, H5a, H6'b, H2c, H4c, H5c), 3.719 (1 H, ddd ≈ t, $J_{5,6'}$ 5.9, $J_{5,6''}$ 6.4, $J_{4,5} < 1$, H5b), 3.814 (1 H, dd, $J_{2,3}$ 10.0, $J_{3,4}$ 3.5, H3b), 3.852 (2 H, m, H6'a, H6''a), 3.872 (1 H, dd, $J_{6',6''}$ 10.8, $J_{5,6''}$ 6.1, H6''c), 4.027 (3 H, m, CHO, H4a, H6'c), 4.085 (1 H, dd, $J_{1,2}$ 8.4, $J_{2,3}$ 10.3, H2a), 4.417 (1 H, d, $J_{1,2}$ 8.1, H1b), 4.544 (1 H, d, $J_{1,2}$ 8.3, H1a), 4.593, 4.603, 4.639, 4.729, 4.775, and 4.857 (6 × 1 H, 6 d, $J_{A,B}$ 11.9, 3 × CH_2Ph), 4.785 (1 H, d, $J_{1,2}$ 8.3, H1c), 4.986 (1 H, dd, $J_{1,2}$ 8.1, $J_{2,3}$ 10.0, H2b), 5.121 (1 H, dd, $J_{3,4}$ 9.2, $J_{2,3}$ 10.3, H3a), 5.215 (1 H, dd, $J_{2,3}$ 10.6, $J_{3,4}$ 8.6, H3c), 5.616 (1 H, dd ≈ d, $J_{3,4}$ 3.5, $J_{4,5} < 1$, H4b), 7.462 (15 H, m, 3 × Ph).

(3-Trifluoroacetamidopropyl)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-4-*O*-{2,4-di-*O*-acetyl-6-*O*-benzyl-3-*O*-[2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-4-*O*-(2,3,4-tri-*O*-acetyl-6-*O*-benzyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranosyl]-β-*D*-galactopyranosyl}-β-*D*-glucopyranoside (X). Trisaccharide (**IX**) (709 mg, 0.60 mmol) was glycosylated with glycosyl bromide (**I**) obtained from the corresponding thioglycoside (528 mg, 1.20 mmol) [16], the mixture was chromatographed on silica gel (elution with 10 ⟶ 20%) isopropanol in ethyl acetate) to give tetrasaccharide (**X**) (442 mg, 47%), R_f 0.50 (10% isopropanol in ethyl acetate), $[\alpha]_D$ –31.9 (*c*1, $CHCl_3$); MS, *m/z*: 1555 (1579 + 23) (M^+ + Na^+). ^{1}H NMR: 1.841 (2 H, m, CH_2), 1.900, 1.961 (×5), 1.986, 2.032, and 2.077 (9 × 3 H, 9 s, 9 Ac), 3.258 (1 H, m, CHN), 3.36–3.75 (16 H, m, CHN, CHO, H5a, H6'a, H6''a, H3b, H5b, H6'b, H6''b, H2c, H5c, H6'c, H6''c, H5d, H6'd, and H6''d), 3.86–3.95 (3 H, m, CHO, H4a and H4c), 4.037 (1 H, ddd, $J_{1,2}$ 7.9, $J_{2,3}$ 9.2, $J_{2,NH}$ 8.8, H2a), 4.301 (1 H, d, $J_{1,2}$ 7.9, H1b), 4.347 (1 H, d, $J_{1,2}$ 7.9, H1a), 4.395, 4.457, 4.476, 4.501, 4.521, 4.545, 4.681, and 4.725 (8 × 1 H, 8 d, $J_{A,B}$ 12.0, 4 × CH_2Ph), 4.485 (1 H, d, $J_{1,2}$ 7.9, H1d), 4.577 (1 H, d, $J_{1,2}$ 7.9, H1c), 4.913 (1 H, dd, $J_{2,3}$ 10.5, $J_{3,4}$ 3.5, H3d), 4.946 (1 H, dd, $J_{1,2}$ 7.9,

$J_{2,3}$ 9.7, H2b), 4.964 (1 H, dd ≈ t, $J_{2,3} \approx J_{3,4} \approx 9.2$, H3a), 5.019 (1 H, dd, $J_{1,2}$ 7.9, $J_{2,3}$ 10.5, H2d), 5.104 (1 H, dd, $J_{2,3}$ 9.7, $J_{3,4}$ 8.4, H3c), 5.357 (1 H, d, $J_{2,NH}$ 8.4, N*H*Ac-c), 5.423 (1 H, dd ≈ d, $J_{3,4}$ 3.5, $J_{4,5} < 1$, H4b), 5.460 (1 H, dd ≈ d, $J_{3,4}$ 3.5, $J_{4,5} < 1$, H4d), 5.817 (1 H, d, $J_{2,NH}$ 8.8, N*H*Ac-a), 7.27–7.39 (20 H, m, 4 × Ph), 7.506 (1 H, m, $NHCOCF_3$).

(3-Trifluoroacetamidopropyl)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-4-*O*-{2,4-di-*O*-acetyl-6-*O*-benzyl-3-*O*-[2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-4-*O*-(2,4-di-*O*-acetyl-6-*O*-benzyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranosyl]-β-*D*-galactopyranosyl}-β-*D*-glucopyranoside (XI). To tetrasaccharide obtained by deacetylation by the Zemplen method of dilactosamine derivative (**X**) (442 mg, 0.284 mmol), dry acetonitrile (20 ml), triethyl orthoacetate (0.1 ml, 0.53 mmol), and toluene sulfonic acid (30 mg) were added, and the mixture was stirred for 3 h at room temperature. The mixture was neutralized with pyridine, evaporated, and the resulting orthoacetate was acetylated with a 2 : 1 pyridine–acetic anhydride mixture (6 ml). After 80% acetic acid (10 ml) was added, the mixture was kept for 1 h and coevaporated with toluene. Silica gel chromatography (elution with 8 : 1 : 0.05 ⟶ 5 : 1 : 0.05 chloroform–isopropanol–pyridine) allowed derivative (**XI**) (205 mg, 48%), R_f 0.39 (10% isopropanol in ethyl acetate), $[\alpha]_D$ –25.8 (*c*1, $CHCl_3$); MS, *m*/*z*: 1537 (1513 + 23) ($M^+ + Na^+$). ^{1}H NMR: 1.844 (2 H, m, CH_2), 1.903, 1.952 (×2), 1.965, 1.995, 2.017, and 2.075 (×2) (8 × 3 H, 8 c, 8 Ac), 3.272 (1 H, m, CHN), 3.38–3.80 (17 H, m, CHN, CHO, H5a, H6'a, H6"a, H3b, H5b, H6'b, H6"b, H2c, H5c, H6'c, H6"c, H3d, H5d, H6'd, and H6"d), 3.900 (3 H, m, CHO, H4a and H4c), 4.052 (1 H, ddd, H2a), 4.302 (1 H, d, $J_{1,2}$ 8.0, H1b), 4.396 (1 H, d, $J_{1,2}$ 7.8, H1a), 4.40–4.58 (7 H, 7 d, $J_{A,B} \approx 12$, 3 × C*H*$_2$Ph, H1d), 4.597 (1 H, d, $J_{1,2}$ 7.8, H1c), 4.685, 4.796 (2 H, 2 d, $J_{A,B}$ 12.1, C*H*$_2$Ph), 4.796 (1 H, dd, $J_{1,2}$ 8.0, $J_{2,3}$ 9.8, H2b), 4.939 (1 H, dd ≈ t, $J_{2,3} \approx J_{3,4} \approx 8.8$, H3a), 4.996 (1 H, dd ≈ t, $J_{1,2} \approx J_{2,3} \approx 9.0$, H2d), 5.128 (1 H, dd ≈ t, $J_{2,3} \approx J_{3,4} \approx 9.2$, H3c), 5.351 (1 H, dd ≈ d, $J_{3,4}$ 3.5, $J_{4,5} < 1$, H4b), 5.467 (1 H, dd ≈ d, $J_{3,4}$ 3.5, $J_{4,5} < 1$, H4d), 5.529 (1 H, d, $J_{2,NH}$ 8.9, N*H*Ac-c), 6.055 (1 H, d, $J_{2,NH}$ 8.5, N*H*Ac-a), 7.30 (20 H, m, 4 × Ph), 7.585 (1 H, m, $NHCOCF_3$).

(3-Trifluoroacetamidopropyl)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-4-*O*-{2,4-di-*O*-acetyl-6-*O*-benzyl-3-*O*-[2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-4-*O*-(2,4-di-*O*-acetyl-6-*O*-benzyl-3-*O*-{2-acetamido-3,6-di-*O*-acetyl-2-deoxy-4-*O*-2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranosyl]-β-*D*-galactopyranosyl}-β-*D*-glucopyranoside (XIV)**.** Tetrasaccharide (**XI**) (203 mg, 0.134 mmol) was glycosylated with glycosyl bromide (**XII**) obtained from the corresponding thioglycoside, the mixture was chromatographed on silica gel (elution with 8 ⟶ 15%) isopropanol in chloroform) to give hexasaccharide (**XIII**) (141 mg, 47%), R_f 0.50 (10% isopropanol in ethyl acetate); MS, *m*/*z*: 2155 (2131 + 23) (M^++Na^+). ^{1}H NMR: 1.836 (2 H, m, CH_2), 1.894, 1.907, 1.941, 1.958 (×2), 1.978, 1.983, 2.003, 2.059 (×2), 2.067 (×2), 2.073 (×2), 2.154 (15 × 3 H, 15 c, 15 Ac), 3.258 (1 H, m, CHN), 3.37–3.75 (19 H, m, CHN, CHO, H5a, H6'a, H6"a, H3b, H5b, H6'b, H6"b, H2c, H5c, H6'c, H6"c, H3d, H5d, H6'd, H6"d, H2e, and H5e), 3.791 (1 H, dd ≈ t, $J_{3,4} \approx J_{4,5} \approx 8.9$, H4e), 3.891 (4 H, m, CHO, H4a, H4c, and H5f), 4.037 (2 H, m, H2a and H6'e), 4.114 (2 H, m, H6'f and H6"f), 4.298 (1 H, d, $J_{1,2}$ 7.8, H1b), 4.345 (1 H, d, $J_{1,2}$ 7.6, H1a), 4.390 (1 H, d, $J_{1,2}$ 8.1, H1d), 4.42–4.59 (8 H, 8 d, 3 × C*H*$_2$Ph, H1c, H1f), 4.63–4.77 (4 H, m, C*H*$_2$Ph, H1e and H6'e), 4.960 (4 H, m, H3a, H2b, H2d, and H3f), 5.078 (1 H, dd ≈ t, $J_{2,3} \approx J_{3,4} \approx 9.2$, H3c), 5.122 (1 H, dd, $J_{1,2}$ 7.9, $J_{2,3}$ 10.3, H2f), 5.195 (1 H, dd ≈ t, $J_{2,3} \approx J_{3,4} \approx 9.1$, H3e), 5.34–5.41 (4 H, m, H4d, H4f, N*H*Ac-c, N*H*Ac-e), 5.456 (1 H, dd ≈ d, $J_{3,4}$ 3.5, $J_{4,5} < 1$, H4b), 5.825 (1 H, d, $J_{2,NH}$ 8.7, N*H*Ac-a), 7.27–7.39 (20 H, m, 4 × Ph), 7.509 (1 H, m, $NHCOCF_3$).

(3-Aminopropyl)-2-acetamido-2-deoxy-4-*O*-{3-*O*-[2-acetamido-2-deoxy-4-*O*-(3-*O*-{2- acetamido-2-deoxy-4-*O*-[β-*D*-galactopyranosyl]-β-*D*-glucopyranosyl}-β-*D*-galactopyranosyl)-β-*D*-glucopyranosyl]-[β-*D*-galactopyranosyl}-β-*D*-glucopyranoside (XV). Hydrogenolysis of hexasaccharide (**XIV**) (93 mg, 0.044 mmol) followed by acetylation with a pyridine–acetic anhydride mixture (2 : 1. 3) and silica gel chromatography (elution with 10–25% isopropanol in chloroform) gave 68 mg (80%) of trilactosamine peracetate, R_f 0.17 (10% isopropanol in ethyl acetate). *O*-Deacetylation of the latter and removal of *N*-trifluoroacetamide protection yielded 36.4 mg (89%) of hexasaccharide (**XV**), R_f 0.46 (4 : 1 : 1 : 1 ethanol–water–pyridine–acetic acid), $[\alpha]_D$ –5.0 (*c*0.5, H_2O), MS, *m*/*z*: 1194 (1171 + 23) ($M^+ + Na^+$). ^{1}H NMR (D_2O): 1.966 (2 H, m, CH_2), 2.050 (×2), 2.062 (3 × 3 H, 3 c, 3 Ac), 3.101 (1 H, m, CH_2N), 3.53–3.64 (6 H, m, H2b, H2d, H2f, H5a, H5c, and H5e), 3.66–3.83 (25 H, m), 3.93–4.07 (5 H, m, CHO, H4f, H6'a, H6'c, and H6'e), 4.172 (2 H, m, H4b and H4d), 4.476, 4.485, and 4.497 (3 H, 3 d, $J_{1,2}$ 7.8, H1b, H1d, and H1f), 4.531 (1 H, d, $J_{1,2}$ 8.0, H1a), 4.723 (2 H, 2 d, $J_{1,2}$ 8.3, H1e, H1c). ^{13}C NMR (D_2O): 26.96 ($OCH_2CH_2CH_2NH$), 37.6 (CH_2N), 55.00, 55.14, and 55.18 (3 C2, GlcN), 59.85, and 59.98 (2 C6, GlcN), 60.95, and 61.03 (2 C6, Gal), 67.96 (OCH_2), 68.28, 68.30, and 68.54 (3 C4, Gal), 69.94 (2 C2, Gal), 70.96 (C2, Gal), 72.16, 72.18, and 72.20 (3 C3, GlaN), 72.50 (C3f), 74.54 (2 C5, GlcN), 74.71 (C5, GlcN), 74.87 (2 C5, Gal), 75.35 (C5, Gal), 78.15, and 78.17 (2 C4, GlcN), 78.47 (C4a), 82.08 (2 C3, Gal), 101.16 (C1a), 102.75 (2 C1, GlcN), 102.86, 102.89, and 102.91 (3 C1, Gal).

Fluorescein-labeled glycoconjugate [19]. A solution of amino spacer-bearing fluorescein (A-10466, Molecular Probes, United States) (0.112 mg, 0.171 mmol) in DMSO (50 μl), a solution of polynitrophenyl acrylate (3.299 mg, 17.08 μg-eq) in DMSO (165 μl),

and triethylamine (2 μl, 2 vol %) were added to a solution of hexasaccharide (**XIV**) (4 mg, 3.416 μl) in DMSO (350 μl). The mixture was kept for 25 h at 40°C, cooled to room temperature, and ethanolamine (57 μl, 10 vol %) was added, the mixture was kept for 24 h at room temperature, and the solution was loaded onto a Sephadex LH-20 column. The conjugate was eluted with a 1 : 1 acetonitrile–water, the target fractions were evaporated, and the residue was lyophilized from water to give glycoconjugate (5.66 mg, 97%).

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