Synthesis of spacered derivatives of β-lactosylamine with an amino function at the terminal position of an aglycon*

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A set of *N*-acyllactosylamine derivatives containing a terminal amino group in aglycons of various lengths (up to 16 atoms) and various hydrophilicities were synthesized. The aglycons were represented by di-, tri-, and pentapeptides containing glycine and serine residues and by aglycons containing fragments of tartaric acid or a tertiary amine.

Key words: lactosylamine, *N*-acyllactosylamines, aglycon-spacer, neoglycoconjugates.

An increase in the selectivity of the delivery of physiologically active substances (FAS), in particular, drugs, to the target cells is a topical problem of the modern drug therapy. The use of targeting is a promising approach to addressing this problem. In the case where this delivery is based on carbohydrate—protein interactions, this is called carbohydrate-mediated targeting (glycotargeting).[1] On the surface of many animal and human normal and tumor cells, proteins (the so-called lectins) are situated, which function as carbohydrate-specific receptors and as mediators in the carbohydrate-specific endocytosis of glucoconjugates.[1a] The malignant transformation of cells often results in changes in the lectin composition of the cell surface and is accompanied by hyperexpression of certain lectins.[1b,2] The choice of appropriate oligosaccharide "vectors" determining the targeting selectivity is based on the knowledge of carbohydrate-binding specificity of the target cells, which is determined by the lectin composition. For example, hyperexpression of galectins, *i.e.*, proteins capable of selective binding to the terminal β-galactose residue located on the non-reducing end of oligosaccharide chains, takes place on the surface of many tumor cells.[3] This feature of tumor cells was successfully used, for example, for targeted carbohydrate-mediated delivery of neoglycoconjugates to breast carcinoma cells.[2] Presumably, the selectivity of neoglycoconjugate binding to target cells is determined by the efficiency of interaction of the oligosaccharide ligand with lectin, which is facilitated by introduction of a spacer between the carbohydrate fragment and the FAS. It is believed that a spacer should be long enough, so that the pharmacophore fragment did not hamper the interaction of the carbohydrate ligands with lectins, which usually bind to the terminal (non-reducing) end of an oligosaccharide.[4] It is also known that the chemical nature of the spacer can influence the lectin interaction with neoglycoconjugates. For example, varying the spacer length and nature (oligomethylene chain with aromatic fragments) allowed modulation of the activity of neoglycoconjugates containing immunostimulating dipeptides over a broad range.[5] By means of self-assembling monolayers on the gold surface, it was convincingly demonstrated that the spacer chosen in the design of neoglycoconjugates dictates the mode of arrangement (presentation) of the carbohydrate ligands on the surface, which is reflected in the efficiency and the specificity of their interaction with the antibodies acting against these carbohydrate fragments.[6] Therefore, it comes as no surprise that immunogenicity of neoglycoconjugates (their ability to induce the production of carbohydrate-specific antibodies in the body) also depends appreciably (up to complete suppression) on the nature of the spacer used to attach the carbohydrate hapten to the carrier protein.[7]

Thus, the data available from the literature suggest that the efficiency of interaction of the carbohydrate residue of a neoglycoconjugate with a lectin may be substantially increased by selecting the optimal spacer connecting the carbohydrate fragment with the FAS.

The disaccharide lactose containing the β-galactose terminal residue is one of the simplest ligands for galectins. Therefore, lactose-based neoglycoconjugates with various FAS may prove to be most readily available and promising for FAS delivery into target cells. Previously, we have prepared lactose neoglycoconjugates by condensation of *N*-glycyl-β-lactosylamine with tartaric acid[8] and carboxy-containing boron compounds, which are potential agents for boron neutron capture therapy for cancer treatment.[9] The present study describes the synthesis of a

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† Deceased.

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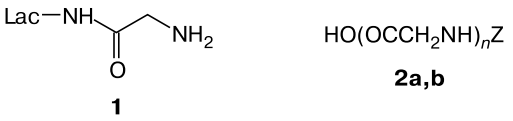
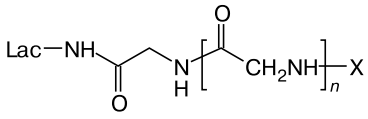
series of peptide *N*-lactosylamides of various lengths and hydrophilicities containing a terminal amino group for the subsequent preparation of novel neoglycoconjugates with polyhedral boron compounds, which would permit modulation of the selectivity of tumor cell targeting.

As the starting compound for the synthesis of most lactosylamides, we used *N*-glycyl-β-lactosylamine (**1**)[8] prepared from β-lactosylamine; the latter was synthesized by a new, more efficient method.[10] Elongation of the aglycon chain in lactosylamide **1** from four to sixteen atoms was carried out by condensation with *N*-benzyloxycarbonylglycine (**2a**) or *N*-benzyloxycarbonylglycylglycine (**2b**) followed by hydrogenolysis of the *N*-protective group (Scheme 1) and by repeating these reactions with the newly formed lactosylamides and compound **2b** or *N*-benzyloxycarbonyl-L-serine (**5**) (Scheme 1).

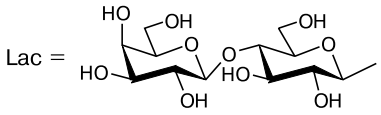


1

2a,b

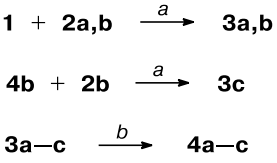


3a—c, 4a—c



Z = $PhCH_2OCO$, X = Z (**3a—c**); H (**4a—c**);
n = 1 (**2a**, **3a**, **4a**), 2 (**2b**, **3b**, **4b**), 4 (**3c**, **4c**)

Scheme 1

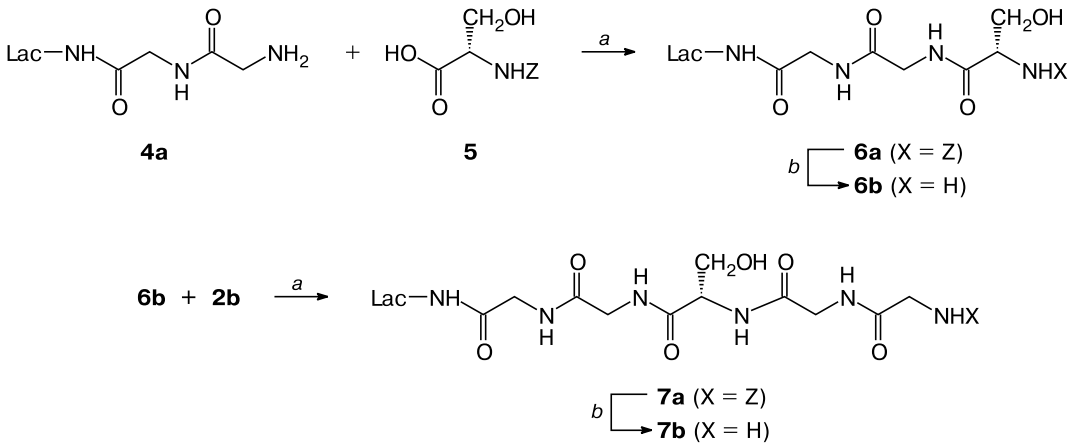


Reagents and conditions: *a*. DCC, NHS, DMSO; *b*. H_2, Pd/C.

Yields: 86% (**3a**), 70% (**3b**), 86% (**4a**), 88% (**4b**), 80% (**4c**).

The condensation of lactosylamide **1** with compounds **2a** or **2b** was carried out using DCC with *N*-hydroxysuccinimide (NHS) in DMSO at 15 °C for 24 h (see Scheme 1). During this period, the starting lactosylamide **1** was consumed almost completely (monitoring by paper electrophoresis). The isolation of *N*-(*N*-benzyloxycarbonyldiglycyl)-β-lactosylamine (**3a**) and *N*-(*N*-benzyloxycarbonyltriglycyl)-β-lactosylamine (**3b**) and subsequent hydrogenolysis of the *N*-protective group yielded *N*-(diglycyl)- (**4a**) and *N*-(triglycyl)-β-lactosylamine (**4b**), respectively. Similarly, lactosylamide **4b** and compound **2b** were converted into *N*-(*N*-benzyloxycarbonylpentaglycyl)-β-lactosylamine (**3c**) and, after deprotection, *N*-(pentaglycyl)-β-lactosylamine (**4c**) was obtained. The reaction of lactosylamide **4a** with *N*-benzyloxycarbonyl-L-serine (**5**) gave protected compound **6a**, which was converted without isolation into *N*-(L-seryldiglycyl)-β-lactosylamine (**6b**). Condensation of this product with **2b** afforded *N*-(*N*-benzyloxycarbonyldiglycyl-L-seryldiglycyl)-β-lactosylamine (**7a**) and, after deprotection, *N*-(diglycyl-L-seryldiglycyl)-β-lactosylamine (**7b**) (see Scheme 2).

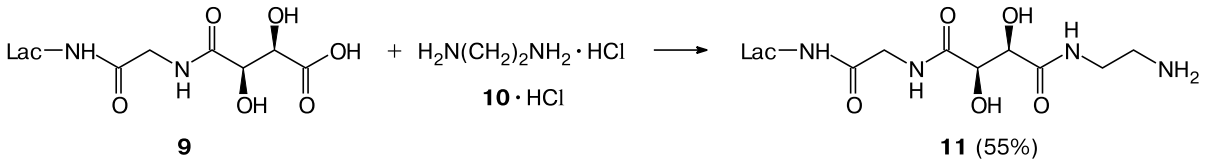
Scheme 2



Z = $PhCH_2OCO$

Reagents, conditions, and yields: *a*. DCC, NHS, DMSO; *b*. H_2, Pd/C; yield 69% (**6b**), 77% (**7b**).

Scheme 3



Reagents and conditions: DCC, NHS, DMSO—DMF—H_2O.

The next stage of the work was to prepare *N*-lactosylglycylamide derivatives containing simultaneously hydrophilic (hydroxy groups or a fragment of a tertiary amine hydrochloride) and hydrophobic (methylene) groups.

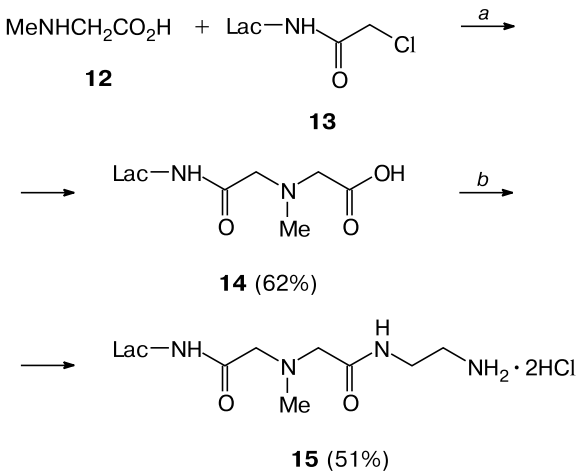
Previously,[8] by acylation of *N*-glycyl-β-lactosylamine (**1**) with *O*,*O*′-dibenzoyl-L-tartaric anhydride (**8**) followed by debenzoylation, we have synthesized acid **9** (Scheme 3). Condensation of this acid with ethylenediamine (**10**) in the presence of DCC and NHS gave the derivative with the terminal amino group needed in this study. In order to reduce the amount of *N*,*N*′-diacylated ethylenediamine formed as a by-product, we used a twofold excess of ethylenediamine hydrochloride (**10**·HCl). The reaction was carried out at 10 °C in a DMSO—DMF—H_2O mixture (10 : 1.3 : 1), which, first, ensured solubility of all reactants and second, maintained DMSO in the unfrozen state. After 24 h, the reaction mixture still contained a substantial amount of acid **9** (paper electrophoresis data). Therefore, to complete the reaction, additional amount of DCC was added and the mixture was kept for an additional 22 h at 10 °C. The reaction product, lactosylamide **11**, was isolated as the base by a combination of anion- and cation-exchange chromatography.

The preparation of *N*-lactosyl-*N*′-methylglycylamide derivative was accomplished by *N*-alkylation of *N*-methylglycine (**12**) with *N*-chloroacetyl-β-lactosylamine (**13**) (see Ref. 11) in aqueous MeOH in the presence of Et_3N (Scheme 4) under the conditions we described previously for *N*-chloroacetyl-β-galactopyranosylamine.[12] The resulting amino acid **14** was condensed with ethylenediamine dihydrochloride (**10**·2 HCl, a fourfold excess) using DCC and NHS in 92% aqueous DMSO at 15 °C. After 4 h, an additional amount of DCC was added, and the mixture was kept for an additional 24 h at 15 °C to complete conversion of amino acid **14**. The reaction product, lactosylamide **15**, was isolated as the dihydrochloride by gel-chromatography on a Sephadex G-15 column.

The structures of the obtained compounds were confirmed by elemental analysis and ^1H NMR spectroscopy.

Thus, we synthesized *N*-β-lactosylamides of various lengths, di-, tri-, and pentapeptide derivatives containing glycine and L-serine residues and glycine derivatives containing a tertiary amine residue and an L-tartaric acid residue. Due to the presence of the amino group at the end of the *N*-lactoside aglycon, the lactosylamides prepared are suitable for conjugation with carboxyl-containing physiologically active compounds and, in particular, with polyhedral boron compounds. The variation of the nature of the aglycon in the resulting set of lactosylamides would help to choose neoglycoconjugates with the optimal spacer for cell targeting and exhibiting the biological activity, for example, for boron neutron capture therapy of cancer.[13]

Scheme 4



Reagents and conditions: *a*. Et_3N, MeOH—H_2O; *b*. **10**·2 HCl, DCC, NHS, DMSO—H_2O.

Experimental

^1H NMR spectra were recorded in D_2O at 24 °C on a Bruker WM-250 spectrometer (operating at 250 MHz) (acetone as the external standard). Optical rotation was measured on a PU-07 polarimeter (Russia). Electrophoresis (30 V cm^{-1}, 1 h) was carried out on Filtrak FN 1 paper in a pyridinium acetate buffer (0.05 *M* with respect to Py, pH 4.5). The compounds were visualized by ninhydrin and by successive treatment with KIO_4—$AgNO_3$—KOH. Water of crystallization was determined by the Fischer method. The elution of compounds during gel chromatography on a Sephadex G-15 column was monitored by UV absorption at 206 nm.

***N*$^{\omega}$-Benzyloxycarbonyllactosylamides 3a—c, 6a, 7a (general procedure).** A mixture of the amino component (lactosylamides **1**, **4a**, **4b**,or **6b**) (1 mmol), carboxyl component (*N*-benzyloxy-

carbonylglycine (**2a**), *N*-benzyloxycarbonylglycylglycine (**2b**), or *N*-benzyloxycarbonyl-L-serine (**5**)) (1.1 mmol), and NHS (0.126 g, 1.1 mmol) was dissolved with heating to 40 °C in dry DMSO (4 mL). The solution was cooled to 15 °C, and DCC (0.247 g, 1.2 mmol) was added. The reaction mixture was stirred for 1 h and kept for 23 h at 15 °C until lactosylamide was converted almost completely (monitoring by electrophoresis). The precipitate of *N*,*N*′-dicyclohexylurea was filtered off and washed with DMSO (0.5 mL). The filtrate was added with stirring to Et_2O (45 mL), and, when the solution became clear, the liquid was decanted from the oily precipitate. The precipitate was triturated several times with Et_2O (each portion 10 mL) until a thick paste formed; this was triturated several times with acetone (each portion 5 mL) until a powder formed. The powder was filtered off, washed with acetone (2×5 mL) and Et_2O, and dried. The resulting compounds **3a—c** and **7a** were purified as described below for each particular case, and compound **6a** was used without purification

Lactosylamides 4a—c, 6b, 7b (general procedure). *N*-Benzyloxycarbonyllactosylamide (0.5 mmol) was dissolved in water with heating to 40 °C (**3a**, **3b** and **6a**, in 8 mL of water, and **3c** and **7a** in 30 mL of water). The solution was cooled to 20 °C, 10% Pd/C (its mass was half that of lactosylamide) was added under argon, and the mixture was hydrogenated with vigorous stirring in a gentle flow of H_2 for 8—10 h (monitoring by paper electrophoresis). The catalyst was filtered off and washed with water containing 10% MeOH (2×6 mL). The solution was twice concentrated and filtered (0.45 μm filter). Subsequent isolation of lactosylamides **4a—c, 6b**, **7b** is described below separately for each product.

4-*O*-(β-D-Galactopyranosyl)-*N*-(*N*-benzyloxycarbonyldiglycyl)-β-D-glucopyranosylamine (3a) was obtained from 4-*O*-(β-D-galactopyranosyl)-*N*-glycyl-β-D-glucopyranosylamine (**1**) (0.4 g, 1 mmol) (see Ref. 8) and *N*-benzyloxycarbonylglycine (**2a**) (0.23 g, 1.1 mmol). The crude product **3a** was dissolved with heating to 40 °C in a mixture of water (1.1 mL) and MeOH (20 mL), then Pr^iOH (4 mL) was added with stirring, and the mixture was kept for 16 h at 5 °C. The gel-like precipitate was filtered off, washed with MeOH and Et_2O, and dried to give 0.46 g of pure compound **3a**. The mother liquor was concentrated to dryness to give additional 0.06 g of amorphous compound **3a** in a similar way (total yield 86%), $[\alpha]_D^{18}$ +1.5 (*c* 1, H_2O). Found (%): C, 46.97; H, 6.31; N, 6.96; H_2O, 3.07. $C_{24}H_{35}N_3O_{14}\cdot H_2O$. Calculated (%): C, 47.44; H, 6.14; N, 6.92; H_2O, 2.97. ^{1}H NMR, δ: 3.40—3.60 (m, 2 H); 3.60—4.04 (m, 14 H); 4.45 (d, 1 H, H(1) Gal, *J* = 7.5 Hz); 5.02 (d, 1 H, H(1) Glc, *J* = 9.0 Hz); 5.17 (s, 2 H, CH_2O); 7.44 (br.s, 5 H, Ph).

4-*O*-(β-D-Galactopyranosyl)-*N*-diglycyl-β-D-glucopyranosylamine (4a) was obtained by hydrogenation of compound **3a** (0.3 g, 0.5 mmol). The solution was concentrated to 0.5 mL, and then MeOH (2 mL) was added with stirring. The mixture was kept for 16 h at 5 °C. The precipitate was filtered off, washed with MeOH and Et_2O, and dried to give 0.2 g (86%) of amorphous compound **4a**, $[\alpha]_D^{18}$ +3.6 (*c* 1, H_2O). Found (%): C, 41.22; H, 6.59; N, 8.77; H_2O, 1.88. $C_{16}H_{29}N_3O_{12}\cdot 0.5\ H_2O$. Calculated (%): C, 41.38; H, 6.51; N, 9.05; H_2O, 1.94. ^{1}H NMR, δ: 3.45 (br.s, 2 H, $C\underline{H}_2NH_2$); 3.46—3.60 (m, 2 H); 3.60—3.85 (m, 8 H); 3.85—3.94 (m, 2 H); 4.02 (br.s, 2 H, $C\underline{H}_2$ NH); 4.45 (d, 1 H, H(1) Gal, *J* = 7.5 Hz); 5.02 (d, 1 H, H(1) Glc, *J* = 9.0 Hz).

4-*O*-(β-D-Galactopyranosyl)-*N*-(*N*-benzyloxycarbonyltriglycyl)-β-D-glucopyranosylamine (3b) was obtained from compound **1** (0.4 g, 1 mmol) and *N*-benzyloxycarbonylglycylglycylglycine (**2b**) (0.29 g, 1.1 mmol). The crude product **3b** was dissolved in water (1.5 mL), Pr^iOH was added with stirring until the solution became turbid, and the mixture was kept for 16 h at 5 °C. The gel-like precipitate was filtered off, washed with 20% aq. Pr^iOH, acetone, and Et_2O, and dried to give 0.46 g (70%) of amorphous compound **3b**, $[\alpha]_D^{25}$ +2.7 (*c* 1, H_2O). Found (%): C, 47.19; H, 6.21; N, 8.43; H_2O, 3.03. $C_{26}H_{38}N_4O_{15}\cdot H_2O$. Calculated (%): C, 46.99; H, 6.07; N, 8.43; H_2O, 2.71. ^{1}H NMR, δ: 3.46—3.61 (m, 2 H); 3.61—3.86 (m, 8 H); 3.91 (br.s, 5 H); 3.98 (br.s, 3 H); 4.45 (d, 1 H, H(1) Gal, *J* = 7.5 Hz); 5.02 (d, 1 H, H(1) Glc, *J* = 9.0 Hz); 5.16 (s, 2 H, CH_2O); 7.44 (br.s, 5 H, Ph).

4-*O*-(β-D-Galactopyranosyl)-*N*-triglycyl-β-D-glucopyranosylamine (4b) was obtained by hydrogenation of compound **3b** (0.33 g, 0.5 mmol). The solution was concentrated to 0.5 mL, MeOH was added with stirring until turbidity appeared, and the mixture was left for 16 h at 5 °C. The precipitate was filtered off, washed with MeOH and Et_2O, and dried to give 0.22 g (88%) of amorphous compound **4b**, $[\alpha]_D^{18}$ +3.0 (*c* 1, H_2O). Found (%): C, 41.92; H, 6.48; N, 10.39. $C_{18}H_{32}N_4O_{13}$. Calculated (%): C, 42.19; H, 6.29; N, 10.93. ^{1}H NMR, δ: 3.46 (br.s, 2 H, $C\underline{H}_2NH_2$); 3.47—3.61 (m, 2 H); 3.61—3.86 (m, 8 H); 3.86—3.98 (m, 2 H); 4.01 (br.s, 4 H, 2 $C\underline{H}_2$ NH); 4.45 (d, 1 H, H(1) Gal, *J* = 7.5 Hz); 5.02 (d, 1 H, H(1) Glc, *J* = 9.0 Hz).

4-*O*-(β-D-Galactopyranosyl)-*N*-(*N*-benzyloxycarbonylpentaglycyl)-β-D-glucopyranosylamine (3c) was obtained from compound **4b** (0.51 g, 1 mmol) and *N*-benzyloxycarbonylglycylglycine (**2b**) (0.29 g, 1.1 mmol). The crude product **3c** was treated with water (25 mL) at 60 °C and the precipitate was filtered off. The filtrate was concentrated to 10 mL, then Pr^iOH (8 mL) was added with stirring, and the mixture was kept for 16 h at 5 °C. The gel-like precipitate was filtered off, washed with 80% aq. Pr^iOH, acetone, and Et_2O, and dried to give 0.47 g (59%) of amorphous compound **3c,** $[\alpha]_D^{25}$ +2.0 (*c* 0.5, H_2O). Found (%): C, 45.58; H, 6.16; N, 10.66; H_2O, 4.86. $C_{30}H_{44}N_6O_{17}\cdot 2\ H_2O$. Calculated (%): C, 45.22; H, 6.07; N, 10.55; H_2O, 4.52. ^{1}H NMR, δ: 3.46—3.61 (m, 2 H); 3.61—3.86 (m, 8 H); 3.91 (br.s, 5 H); 3.98 (br.s, 7 H); 4.45 (d, 1 H, H(1) Gal, *J* = 7.5 Hz); 5.02 (d, 1 H, H(1) Glc, *J* = 9.0 Hz); 5.17 (s, 2 H, CH_2O); 7.44 (br.s, 5 H, Ph).

4-*O*-(β-D-Galactopyranosyl)-*N*-pentaglycyl-β-D-glucopyranosylamine (4c) was prepared by hydrogenation of compound **3c** (0.4 g, 0.5 mmol). The solution was concentrated to 2.5 mL and kept for 16 h at 5 °C. The precipitate was filtered off, washed with cold water, MeOH, and Et_2O, and dried to give 0.26 g (80%) of amorphous **4c,** $[\alpha]_D^{25}$ +3.1 (*c* 0.5, H_2O). Found (%): C, 40.79; H, 6.35; N, 13.40; H_2O, 2.90. $C_{22}H_{38}N_6O_{15}\cdot H_2O$. Calculated (%): C, 40.99; H, 6.26; N, 13.04; H_2O, 2.80. ^{1}H NMR, δ: 3.43 (br.s, 2 H, $C\underline{H}_2NH_2$); 3.45—3.61 (m, 2 H); 3.61—3.85 (m, 8 H); 3.86—3.96 (m, 2 H); 4.02 (br.s, 8 H, 4 $C\underline{H}_2$ NH); 4.45 (d, 1 H, H(1) Gal, *J* = 7.5 Hz); 5.02 (d, 1 H, H(1) Glc, *J* = 9.0 Hz).

4-*O*-(β-D-Galactopyranosyl)-*N*-(*N*-benzyloxycarbonyl-L-seryldiglycyl)-β-D-glucopyranosylamine (6a) was obtained from compound **4a** (0.464 g, 1 mmol) and *N*-benzyloxycarbonyl-L-serine (**5**) (0.263 g, 1.1 mmol). The crude product (yield 0.64 g, 94%) was used without purification for the synthesis of compound **6b**.

4-*O*-(β-D-Galactopyranosyl)-*N*-(L-seryldiglycyl)-β-D-glucopyranosylamine (6b) was obtained by hydrogenation of compound **6a** (0.34 g, 0.5 mmol). The solution was concentrated to

5 mL, the cation-exchange resin Dowex 50w×8 (H^+) (3 mL) was added, the mixture was stirred for 30 min, and the resin was filtered off and washed with water (30 mL) and 1 *M* aq. NH_4OH (30 mL). The alkaline fraction was concentrated to 2 mL, and the product was precipitated by adding EtOH. The precipitate was filtered off, washed with EtOH and Et_2O, and dried to give 0.19 g (69%) of amorphous compound **6b**, $[\alpha]_D^{25}$ +6.8 (*c* 1, H_2O). Found (%): C, 41.43; H, 6.54; N, 9.63; H_2O, 1.84. $C_{19}H_{34}N_4O_{14}\cdot 0.5\ H_2O$. Calculated (%): C, 41.38; H, 6.40; N, 10.16; H_2O, 1.63. ^{1}H NMR, δ: 3.45—3.59 (m, 2 H); 3.62—3.85 (m, 11 H); 3.89—3.97 (m, 2 H); 4.02 (s, 2 H, CH_2); 4.03 (s, 2 H, CH_2); 4.45 (d, 1 H, H(1) Gal, J = 7.5 Hz); 5.02 (d, 1 H, H(1) Glc, J = 9.0 Hz).

4-*O*-(β-D-Galactopyranosyl)-*N*-(*N*-benzyloxycarbonyldiglycyl-L-seryldiglycyl)-β-D-glucopyranosylamine (7a) was obtained from compound **6b** (0.55 g, 1 mmol) and *N*-benzyloxycarbonylglycylglycine **2b** (0.29 g, 1.1 mmol). Crude **7a** was dissolved in water (20 mL) with heating to 40 °C, the solution was cooled to room temperature, the cation-exchange resin Dowex 50w×8 (H^+) (2 mL) was added, the mixture was stirred for 30 min, and the resin was filtered off and washed with water (25 mL). The combined filtrate was concentrated to dryness. The residue was dissolved in boiling MeOH, and the solution was cooled to 5 °C. The precipitate was filtered off, washed with MeOH and Et_2O, and dried to give 0.62 g (78%) of amorphous **7a**, $[\alpha]_D^{20}$ −7.0 (*c* 1, H_2O). Found (%): C, 46.17; H, 5.75; N, 10.59; H_2O, 0.86. $C_{31}H_{46}N_6O_{18}\cdot 0.5\ H_2O$. Calculated (%): C, 46.56; H, 5.92; N, 10.51; H_2O, 1.13. ^{1}H NMR, δ: 3.46—3.61 (m, 2 H); 3.61—4.10 (m, 20 H); 4.43—4.55 (m, 2 H); 5.02 (d, 1 H, H(1) Glc, J = 9.0 Hz); 5.17 (s, 2 H, CH_2O); 7.44 (br.s, 5 H, Ph).

4-*O*-(β-D-Galactopyranosyl)-*N*-(diglycyl-L-seryldiglycyl)-β-D-glucopyranosylamine (7b) was obtained by hydrogenation of compound **7a** (0.4 g, 0.5 mmol). The solution was concentrated to 5 mL, the cation-exchange resin Dowex 50w×8 (H^+) (3 mL) was added, the mixture was stirred for 30 min, and the resin was filtered off and washed with water (30 mL) and 1 *M* aq. NH_4OH (40 mL). The alkaline fraction was concentrated to a syrup, which was dissolved in MeOH (15 mL), and the solution was kept for 16 h at 5 °C. The precipitate was filtered off, washed with MeOH and Et_2O, and dried to give 0.26 g (77%) of amorphous compound **7b**, $[\alpha]_D^{25}$ −10.7 (*c* 1, H_2O). Found (%): C, 40.86; H, 6.00; N, 11.93; H_2O, 3.05. $C_{23}H_{40}N_6O_{16}\cdot H_2O$. Calculated (%): C, 40.95; H, 6.27; N, 12.46; H_2O, 2.67. ^{1}H NMR, δ): 3.43 (s, 2 H, C$\underline{H}_2$NH$_2$); 3.44—3.59 (m, 2 H); 3.61—3.88 (m, 8 H); 3.89—3.97 (m, 4 H); 4.01 (br.s, 4 H, 2 CH_2); 4.06 (s, 2 H, CH_2); 4.45 (d, 1 H, H(1) Gal, J = 7.5 Hz); 4.52 (t, 1 H, C$\underline{H}$NH, J = 4.5 Hz); 5.02 (d, 1 H, H(1) Glc, J = 9.0 Hz).

4-*O*-(β-D-Galactopyranosyl)-*N*-(*N*-{(*R*,*R*)-2,3-dihydroxy-3-[*N*-(2-aminoethyl)carbamoyl]propionyl}glycyl)-β-D-glucopyranosylamine (11). A mixture of 4-*O*-(β-D-galactopyranosyl)-*N*-{*N*-[(*R*,*R*)-2,3-dihydroxy-3-carboxypropionyl]glycyl}-β-D-glucopyranosylamine (**9**)[8] (0.212 g, 0.4 mmol), ethylenediamine dihydrochloride (**10**·2 HCl) (0.08 g, 0.6 mmol), a solution of ethylenediamine (**10**) (0.0132 g, 0.22 mmol) in water (0.3 mL), NHS (0.051 g, 0.44 mmol), DMSO (1.8 mL), and DMF (0.4 mL) was dissolved with heating to 45 °C. The solution was cooled to 10 °C, a solution of DCC (0.105 g, 0.51 mmol) in DMSO (1.1 mL) was added with stirring, and the mixture was left for 26 h at 10 °C. A solution of DCC (0.052 g, 0.25 mmol) in DMSO (0.5 mL) was added to the reaction mixture and the mixture was left at 10 °C for additional 24 h. The precipitated *N*,*N*′-dicyclohexylurea was filtered off and washed with DMSO (0.5 mL). The filtrate was added with stirring to Et_2O (50 mL), and when the solution became clear, the solution was decanted off. The liquid residue was treated with an Et_2O—acetone mixture (2 : 1, 3×10 mL), an Et_2O—acetone mixture (1 : 1, 3×10 mL), and acetone (3×10 mL). The oily precipitate was dissolved in water (6 mL) and passed through a column (0.55×8.5 cm) with the anion-exchange resin Dowex 1w×8 (OH^-) for 20 min. The column was washed with water (10 mL) and the eluate was concentrated to dryness. The residue was dissolved in water (10 mL) and the solution was concentrated to dryness; this operation was repeated once more. The residue was dissolved in water (3 mL), the cation-exchange resin Dowex 50w×8 (H^+) (0.9 mL) was added, and the mixture was stirred for 30 min. The resin was filtered off and washed with water (15 mL) and 1 *M* aq. NH_4OH (15 mL). The alkaline fraction was concentrated to 0.5 mL, then MeOH (5 mL) and Pr^iOH (10 mL) were added, and the mixture was concentrated to 1.5 mL. The precipitate was filtered off, washed with a MeOH—Pr^iOH mixture (1 : 2) and Et_2O, and dried to give 0.13 g (55%) of amorphous compound **11**, $[\alpha]_D^{25}$ +51.5 (*c* 1, H_2O). Found (%): C, 40.70; H, 6.34; N, 9.28; H_2O, 2.38. $C_{20}H_{36}N_4O_{15}\cdot H_2O$. Calculated (%): C, 40.68; H, 6.48; N, 9.49; H_2O, 3.05. ^{1}H NMR, δ: 2.82 (m, 2 H, C$\underline{H}_2$NH$_2$); 3.38 (m, 2 H, NHC$\underline{H}_2$CH$_2$); 3.43—3.60 (m, 2 H); 3.61—3.87 (m, 8 H); 3.88—3.98 (m, 2 H); 4.02, 4.09 (AB system, 2 H, $COCH_2$, J = 16.0 Hz); 4.45 (d, 1 H, H(1) Gal, J = 7.5 Hz); 4.59, 4.63 (both br.s, 1 H each, CHCH); 5.02 (d, 1 H, H(1) Glc, J = 9.0 Hz).

4-*O*-(β-D-Galactopyranosyl)-*N*-(*N*-(carboxymethyl-*N*-methylglycyl)-β-D-glucopyranosylamine (14). A mixture of 4-*O*-(β-D-galactopyranosyl)-*N*-chloroacetyl-β-D-glucopyranosylamine monohydrate (**12**) (0.426 g, 1 mmol) (see Ref. 11), *N*-methylglycine (**13**) (0.178 g, 2 mmol), and Et_3N (0.42 mL, 3 mmol) in 70% aq. MeOH (4 mL) was kept for 4 h at 70 °C in a tube with a screw cap. The reaction mixture was concentrated to dryness. The residue was dissolved in H_2O (3 mL), then MeOH (0.15 mL) and Ac_2O (1 mL) were added, and the mixture was stirred until a homogeneous solution formed and then kept for 16 h at 20 °C. The reaction mixture was diluted with a 5 : 1 MeOH—H_2O mixture (12 mL) and concentrated to dryness (the operation was repeated twice). The residue was dissolved in water (10 mL), the cation-exchange resin Dowex 50W×8 (H^+) (15 mL) was added and the mixture was stirred for 1 h. The resin was filtered off and washed with H_2O (150 mL) and 1.5 *M* aq. NH_4OH (150 mL). The alkaline fraction was concentrated to 5 mL, filtered (0.45 μm), and concentrated to 0.5 mL. Methanol was added with stirring until an oily precipitate formed. The mixture was heated until the precipitate dissolved, and the solution was kept for 2 h at 20 °C. The precipitate was filtered off, washed with MeOH and Et_2O, and dried to give 0.31 g (62%) of compound **14**, $[\alpha]_D^{25}$ +3.9 (*c* 1, H_2O). Found (%): C, 42.62; H, 7.19; N, 5.54. $C_{17}H_{30}N_2O_{13}\cdot CH_3OH$. Calculated (%): C, 43.03; H, 6.82; N, 5.56. ^{1}H NMR, δ: 3.00 (s, 3 H, CH_3N); 3.34 (s, 3 H, CH_3OH); 3.40—3.60 (m, 2 H); 3.61—3.86 (m, 10 H); 3.90—3.98 (m, 2 H); 4.16 (br.s, 2 H, $COCH_2$); 4.45 (d, 1 H, H(1) Gal, J = 7.5 Hz); 5.02 (d, 1 H, H(1) Glc, J = 9.0 Hz).

4-*O*-(β-D-Galactopyranosyl)-*N*-{*N*-methyl-*N*-[*N*-(2-aminoethyl)carbamoylmethyl]glycyl}-β-D-glucopyranosylamine dihydro-

chloride (15). Dimethyl sulfoxide (3 mL), compound **14** (0.2 g, 0.4 mmol), and NHS (0.0506 g, 0.44 mmol) were added to a solution of ethylenediamine dihydrochloride (0.213 g, 1.6 mmol) in water (0.32 mL) and dissolved with heating to 45 °C. The solution was cooled to 15 °C, DCC (0.091 g, 0.44 mmol) in DMSO (0.7 mL) were added with stirring, and the mixture was kept for 4 h at 15 °C. An additional portion of DCC (0.027 g, 0.12 mmol) in DMSO (0.3 mL) was added, and the mixture was kept for 24 h at 15 °C. The precipitated *N*,*N*′-dicyclohexylurea was filtered off and washed with DMSO (0.4 mL). The filtrate was added with stirring to Et_2O (50 mL), and, when the solution became clear, the solvent was decanted off. The liquid residue was treated with a 2 : 1 Et_2O—acetone mixture (3×10 mL), a 1 : 1 Et_2O—acetone mixture (3×10 mL), and acetone (3×10 mL). The oily precipitate was dissolved in 0.025 *M* AcOH (6 mL) and the solution was chromatographed on a Sephadex G-15 column (5×90 cm) in 0.025 *M* AcOH. The fractions containing the target product were combined and concentrated to dryness, and the traces of AcOH were distilled off *in vacuo* with a H_2O—MeOH—toluene mixture (2 : 15 : 5). The residue was dissolved in water (0.5 mL), and MeOH (10 mL) was slowly added with stirring. The precipitate was filtered off, washed with MeOH and Et_2O, and dried to give 0.13 g (51%) of amorphous **15**, $[\alpha]_D^{25}$ +2.8 (*c* 1, H_2O). Found (%): C, 35.50; H, 7.18; Cl, 10.61; N, 8.79; H_2O, 7.73. $C_{19}H_{38}Cl_2N_4O_{12} \cdot 3\,H_2O$. Calculated (%): C, 35.69; H, 6.94; Cl, 11.09; N, 8.76; H_2O, 8.45. ^{1}H NMR, δ: 2.75 (s, 3 H, CH_3); 3.26 (m, 2 H, CH_2); 3.30—4.04 (m, 18 H); 4.45 (d, 1 H, H(1) Gal, J = 7.5 Hz); 5.02 (d, 1 H, H(1) Glc, J = 9.0 Hz).

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