Synthesis of bi- and tervalent ligands of galectins, derivatives of β-lactosylamine, with the amino group in the terminal position of the spacer

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A method for the synthesis of glycoclusters by *N*-alkylation of *N*-glycyl-β-lactosylamine with *N*-chloroacetyl derivatives of β-lactosylamine and *N*,*N*′-iminodiacetyldilactosylamine has been developed. The glycoclusters obtained with two and three lactosylamine residues with the amino group in the spacer were found to be suitable for further conjugation with carboxy-containing physiologically active compounds.

Key words: glycoclusters, polyvalent ligands, *N*-glycyl-β-lactosylamine, *N*-chloroacetyl-β-lactosylamine.

Glycosylamines have found evergrowing application in the preparation of glycoconjugates that are used in various biochemical investigations associated, for example, with the directed transport of physiologically active compounds (PAC) to the target cells[1,2] or with the quest of vaccines against the human immunodeficiency virus.[3] The synthesis of glycoconjugates most often consists in N-acylation of glycosylamine, which leads either to the target products or to derivatives with functional groups suitable for further conjugation. From *N*-acyl derivatives, *N*-glycyl-β-glycosylamines derivatives from mono- and oligosaccharides, the synthesis of which has been described earlier (see Ref. 4 and references cited therein), are suitable for the preparation of glycoconjugates. These compounds are used for both the direct synthesis of glycoconjugates[4—9] and the preliminary modification of the spacer, *i.e.*, a bridge connecting a carbohydrate and PAC. A spacer can be varied in length and hydrophilicity[10] with subsequent yield of glycoconjugates.[11] As follows from the literature data,[12,13] the carbohydrate fragments of glycoconjugates with the optimally selected spacer can possess significantly higher binding ability to the receptors of a target cell and, therefore, enhanced efficiency of delivery of PAC. Yet higher efficiency of interaction of glycoconjugates with receptors can be usually achieved by multiple binding of glycoconjugates containing few identical carbohydrate residues, clusters (the so-called multivalent or cluster effect). Multivalent glycoconjugates are obtained from compounds containing several reaction centers, for example, by the reaction of *N*-glycyl-β-glycosylamines, derivatives of oligosaccharides, with activated polyacrylic esters.[6] For the preparation of multivalent glycoconjugates from PAC with one reaction center, a necessity arises in a preliminary synthesis of a glycocluster with a functional group in the spacer appropriate for the conjugation.

In continuation of our research on the synthesis of glycoconjugates of polyhedral boron compounds (see Ref. 11 and references cited therein), the present work deals with the development of a method for the synthesis of glycoclusters containing up to three residues of β-lactosylamine with the amino group in the terminal position of the spacer. The selection of the disaccharide lactose with β-galactose at the nonreducing end in the glycocluster structures is connected with the hyperexpression on the surface of tumor cells of galectins, *i.e.*, proteins, specifically binding the terminal β-galactose residues.[14] One can expect that the conjugates of the glycoclusters with PAC will efficiently bind to these cells.

The preparation of the glycoclusters includes a stepwise introduction of lactosylamine residues by the iteration of the N-alkylation reactions of the amino group in *N*-glycyl-β-lactosylamine followed by the N-acylation of the formed imino groups (Scheme 1). A similar approach was described earlier for the synthesis of the so-called *N*-glycopeptoids in which the residues of N-substituted glycine are present (see Ref. 8 and references cited therein). The major differences of our synthetic scheme consists in the use of unprotected carbohydrates and in that both components of the N-alkylation contain carbohydrate residues.

N-Glycyl-β-lactosylamine (**1**) and *N*-chloroacetyl-β-lactosylamine (**2**) were used as the starting compounds for the synthesis of the bivalent ligand (see Scheme 1).

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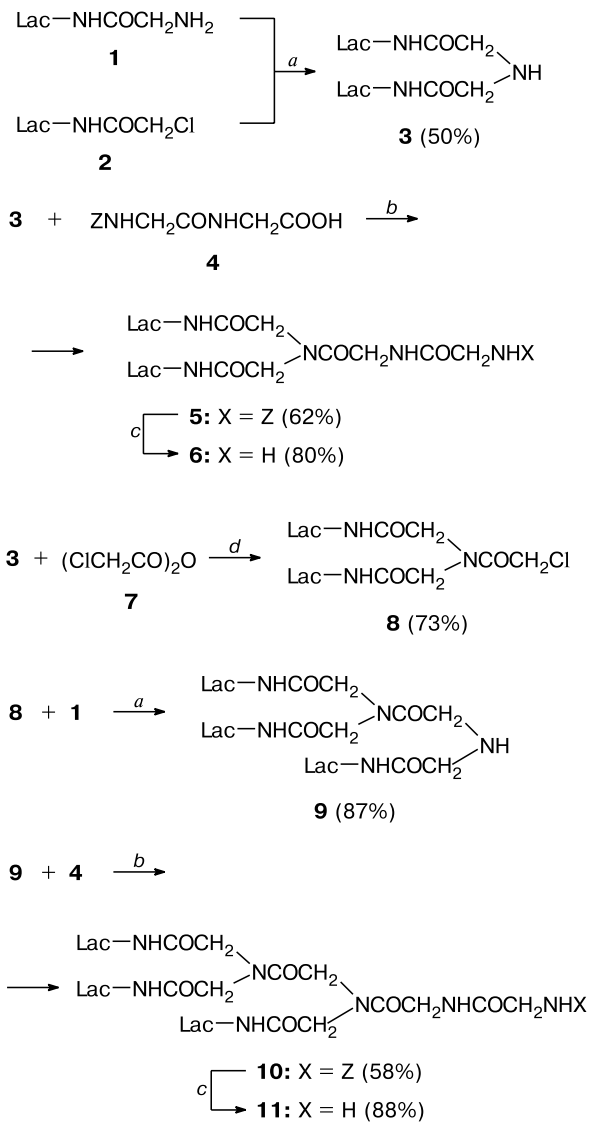
Scheme 1



Lac = D-Gal*p*(β1—4)D-Glc*p*β

Z = $PhCH_2OCO$

Reagents and conditions: *a*. Pr^i_2EtN, MeOH—H_2O (3 : 2); *b*. DCC, NHS, DMSO—DMF (10 : 1); *c*. H_2, Pd/C, H_2O; *d*. DMF.

These have been obtained by us earlier[4,15] from β-lactosylamine, the synthesis of which in the present work was carried out by a new and more efficient method.[16] The N-alkylation of amino compound **1** with chloroacetamide **2** in aqueous MeOH in the presence of Pr^i_2EtN leads to *N,N*′-iminodiacetyldilactosylamine (**3**). Imino compound **3** was further used for both the elongation of the spacer and the synthesis of the tervalent ligand. The elongation of the spacer was carried out by the condensation of compound **3** with a twofold excess of *N*-benzyloxycarbonylglycylglycine (**4**) and *N,N*′-dicyclohexylcarbodiimide (DCC) in the presence of *N*-hydroxysuccinimide (NHS) in DMSO—DMF (10 : 1) at 10 °C. Small amount of *O*-acyl groups in the N-acylated product **5** was removed by treatment with Et_3N in aq. MeOH. After removal of the N-protecting group (hydrogenolysis over Pd/C) in compound **5**, the bivalent ligand **6** with the elongated (as compared to **1**) spacer and the terminal amino group was obtained.

For the synthesis of a precursor of the tervalent ligand, N-chloroacetylation of imino compound **3** with chloroacetic anhydride (**7**) (a twofold excess) in anhydrous DMF was carried out. Small amount of *O*-acyl groups in the N-acylated product was removed by treatment with Et_3N in aq. MeOH to obtain compound **8**. The N-alkylation of amino compound **1** with *N*-chloroacetyl compound **8** led to imino compound **9** with three lactosylamide residues. The elongation of the spacer in compound **9** was carried out as described above for compound **3** to obtain compound **10**, hydrogenolysis of which afforded the target tervalent ligand **11**.

Structures of the synthesized compounds were confirmed by the elemental analysis and data from NMR spectroscopy. In the ^1H NMR spectra of all the compounds with two and three lactosylamine residues, signals for the protons H(2)—H(6) of the galactose and glucose residues in the region of δ 3.40—4.00 are present, which we did not study in detail. Some variations should be noted in the number and character of the observed H(1) proton signals in the spectra. Thus for compound **5** the signals for the protons H(1) of the β-galactose residue manifest themselves as two close doublets (Δδ 0.01 ppm), while for compounds **6** and **11** as broadened doublets. More noticeable distinctions in the signals for the protons H(1) are observed for the β-glucose residues of all the compounds, except for compound **3**. In the spectra of compounds **5**, **6**, and **8** containing two β-glucose residues two doublets each with Δδ from 0.04 to 0.06 ppm are present. In the spectra of compounds containing three β-glucose residues a doublet and a doublet with a twofold larger intensity with Δδ 0.03 ppm (compound **9**) or multiplets with Δδ up to 0.12 ppm (compounds **10** and **11**) are present.

In the spectra of the synthesized compounds, the signals for the protons of the CH_2 groups of the spacer are found in a broad region (δ_H 3.39—5.20). A part of these signals (mainly in the form of broadened singlets) in the spectra of compounds **3**, **5**, **6**, **8**, and **9** were assigned to particular CH_2 groups (see Experimental) based on both the literature data[4,11,15] and the appearance or disappearance of signals as a result of certain reactions. No assignment of signals for particular CH_2 groups in compounds **10** and **11** were made (except for the CH_2Ph group) since the greater part of the protons for the CH_2 groups gave complicated spectra with numerous signals, while the lesser part of the protons was hidden among the

proton signals of lactosylamide residues. The presence in the spectra of compounds **10** and **11** of numerous signals for the protons of the CH_2 groups and multiplets for the protons H(1) of the glucose residues, apparently, is caused by the formation of rotamers due to the presence of two disubstituted amide groups (see Ref. 8 and references cited therein).

The ^{13}C NMR spectra of the target glycoclusteres **6** and **11** gave additional confirmation of their structures. In the spectra of these compounds, signals for 12 carbon atoms of the galactose and glucose residues are present the chemical shifts of which are practically identical for both compounds **6** and **11** and compounds with one lactosylamide residue.[11] In addition, signals of low intensity of the C atoms of the CH_2 and CO groups are present in these spectra: three signals for four CH_2 groups for compound **6**, four signals for six CH_2 groups for compound **11**, three signals for four CO groups, and five signals for six CO groups for compounds **6** and **11**, respectively.

In conclusion, we have developed a method of the synthesis of bi- and tervalent ligands of galectins, derivatives of β-lactosylamine, without the use of the *O*-protecting groups. The elongation of the spacer by the introduction of a diglycine and the presence of the terminal amino group make the synthesized compounds suitable for the conjugation with carboxy-containing PAC. The synthesized glycoclusters allow us to continue our further research on the synthesis of new polyvalent glycoconjugates of polyhedral boron compounds, which are of interest as potential agents for the boron neutron capture cancer therapy.

Experimental

^1H and ^{13}C NMR spectra were recorded on a Bruker WM-250 spectrometer in D_2O at 24 °C (250 MHz for ^1H and 62.7 MHz for ^{13}C) with acetone as the external standard. Optical rotation was determined on a PU-07 polarimeter (Russia). The course of the reactions and control over isolation of products were carried out by electrophoresis (30 V cm^{-1}, 1 h) on Filtrak FN1 paper in a pyridinium acetate buffer (0.05 *M* with respect to Py, pH 4.5). Substances were visualized by the ninhydrin reagent and by the sequence of reagents KIO_4—$AgNO_3$—KOH.[17] Determination of water was performed by the Fischer method. Monitoring of the elution of compounds during chromatography on silica gel C-18 was performed by the UV absorption at 206 or 260 nm.

***N*-Di{*N*-[4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl]carbamoylmethyl}amine (3).** A mixture of 4-*O*-(β-D-galactopyranosyl)-*N*-glycyl-β-D-glucopyranosylamine (**1**) (2.46 g, 6.2 mmol) and 4-*O*-(β-D-galactopyranosyl)-*N*-chloroacetyl-β-D-glucopyranosylamine (**2**) (2.58 g, 6.2 mmol) in 60% aq. MeOH (18 mL) and Pr^i_2EtN (1.12 mL, 6.6 mmol) was heated in a screw-capped test tube for 20 h at 70 °C. The reaction mixture was diluted with MeOH (4 mL) and kept for 5 h at 20 °C. A gel-like pricipitate formed was filtered off and washed with 70% aq. MeOH. The precipitate was dissolved in H_2O (10 mL), MeOH (23 mL) was added with stirring, and the mixture was kept for 16 h at 5 °C. The pricipitate that formed was filtered off, washed with 70% aq. MeOH, MeOH, and Et_2O, and dried to give amorphous compound **3** (1.58 g). The mother liquor and a solution after washing with 70% aq. MeOH were combined and concentrated nearly to dryness. The residue was dissolved in H_2O (30 mL), anionite Dowex 1w×8 (OH^-) (30 mL) was added, the mixture was stirred for 10 min and diluted with H_2O (100 mL). The formed Pr^i_2EtN was evaporated as an azeotrope with H_2O, concentrating the suspension to 60 mL. The resin was filtered off and washed with H_2O (300 mL). The filtrate and washings were concentrated to 100 mL, cationite Dowex 50w×8 (H^+) (15 mL) was added, and the mixture was stirred for 1 h. The resin was filtered off, washed with H_2O (150 mL) and 0.5 *M* Py (110 mL). The latter elutate was concentrated to dryness. The residue was dissolved in H_2O (2.3 mL), MeOH (5 mL) was added with stirring and the mixture was kept for 16 h at 5 °C. The pricipitate that formed was filtered off, washed with 70% aq. MeOH, MeOH, and Et_2O, and dried to give additionally amorphous compound **3** (0.38 g, total yield 50%), $[\alpha]_D^{25}$ +5.0 (*c* 0.5, H_2O). Found (%): C, 42.09; H, 6.24; N, 5.08; H_2O, 2.58. $C_{28}H_{49}N_3O_{22}\cdot H_2O$. Calculated (%): C, 42.15; H, 6.44; N, 5.27; H_2O, 2.26. ^1H NMR, δ: 3.43 (s, 4 H, 2 CH_2N); 3.45—3.60 (m, 4 H); 3.62—3.84 (m, 16 H); 3.88—3.94 (m, 4 H); 4.45 (d, 2 H, 2 H(1) Gal, *J* = 7.5 Hz); 5.02 (d, 2 H, 2 H(1) Glc, *J* = 9.0 Hz).

***N*-(*N*-Benzyloxycarbonyldiglycyl)-*N*-di{*N*-[4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl]carbamoylmethyl}amine (5).** A mixture of *N*-di{*N*-[4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl]carbamoylmethyl}amine (**3**) (0.45 g, 0.56 mmol), *N*-benzyloxycarbonylglycylglycine (**4**) (0.315 g, 1.18 mmol), and NHS (0.162 g, 1.4 mmol) were dissolved with heating at 50 °C in anhydrous DMSO (2 mL) and DMF (0.2 mL). The solution was cooled to 10 °C and DCC (0.313 g, 1.52 mmol) was added. The reaction mixture was stirred for 45 min and kept for 70 h at 18 °C. A pricipitate of *N*,*N*'-dicyclohexylurea was filtered off and washed with DMSO (2×0.5 mL). The filtrate was added with stirring to toluene (40 mL) and the liquid after clearing was separated by decanting from the oily pricipitate. The pricipitate was triturated several times with toluene (10 mL each time) until a viscous substance was obtained, which further was treated several times with hot acetone (10 mL each time) until a powder was obtained. The powder was filtered off, washed with Et_2O, dried, and dissolved in a mixture of H_2O (2.5 mL) and MeOH (2 mL), Et_3N (0.5 mL) was added to the mixture and it was kept for 3 h at 20 °C. The solution was diluted with MeOH (15 mL) and concentrated. The procedure was repeated twice and concentrated to dryness. The residue was washed with hot Pr^iOH (4×7 mL) and Et_2O and dried. The residue was dissolved in H_2O (10 mL), cationite Dowex 50w×2 (H^+) (8 mL) was added to the mixture and it was stirred for 1 h. The resin was filtered off and washed with H_2O (90 mL). The solution was concentrated to 5 mL and passed through a filter with silica gel C-18 (10 g) in H_2O. Silica gel was washed with H_2O (200 mL), 10% aq. MeOH (80 mL), and 20% aq. MeOH (200 mL). The fractions containing the target product were concentrated to 4 mL and liophilized to obtain amorphous compound **5** (0.38 g, 62%), $[\alpha]_D^{25}$ +5.0 (*c* 0.5, H_2O). Found (%): C, 46.15; H, 6.34; N, 6.14; H_2O, 5.37. $C_{40}H_{61}N_5O_{26}\cdot 3H_2O$. Calculated (%): C, 44.40; H, 6.24; N, 6.47; H_2O, 5.00. ^1H NMR, δ: 3.43—3.60 (m, 4 H); 3.61—3.86 (m, 16 H); 3.86—4.01 (m, 6 H); 4.14 (br.s, 2 H, CH_2N); 4.17 (br.s, 2 H, CH_2N); 4.36 (br.s, 2 H, CH_2N); 4.44 (d, 1 H, H(1) Gal, *J* = 7.5 Hz); 4.45 (d, 1 H, H(1) Gal, *J* = 7.5 Hz); 5.02 (d, 1 H, H(1) Glc, *J* = 9.0 Hz); 5.08 (d, 1 H, H(1) Glc, *J* = 9.0 Hz); 5.16 (s, 2 H, CH_2Ph); 7.44 (br.s, 5 H, Ph).

***N*-Di{*N*-[4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-carbamoylmethyl}-*N*-(diglycyl)amine (6).** 10% Palladium on activated carbon (0.18 g) was added to a solution of compound **5** (0.35 g, 0.32 mmol) in H_2O (9 mL) under argon and hydrogenation was carried out in a weak flow of H_2 under vigorous stirring for 8 h at 20 °C. The catalyst was filtered off and washed with H_2O (3×5 mL). The solution was concentrated to 7 mL and filtered (0.45 μm filter), cationite Dowex 50w×2 (H^+) (3 mL) was added and the mixture was stirred for 1 h. The resin was filtered off, washed with H_2O (40 mL) and 0.5 *M* NH_4OH (30 mL). The alkaline fraction was concentrated to 5 mL, MeOH (20 mL) was added, and this was concentrated to 5 mL, the resulting solution with a formed precipitate was kept for 16 h at 5 °C. A gel-like precipitate was filtered off, washed with 90% aq. MeOH, MeOH, and Et_2O, and dried to give amorphous compound **6** (0.24 g, 80%), $[\alpha]_D^{25}$ +6.4 (*c* 0.5, H_2O). Found (%): C, 41.49; H, 6.55; N, 7.44; H_2O, 3.28. $C_{32}H_{55}N_5O_{24}\cdot 2H_2O$. Calculated (%): C, 41.33; H, 6.40; N, 7.53; H_2O, 3.87. ^{1}H NMR, δ: 3.39 (br.s, 2 H, CH_2NH_2); 3.43—3.58 (m, 4 H); 3.61—3.85 (m, 16 H); 3.88—3.96 (m, 4 H); 4.14, 4.17, 4.36 (all br.s, 2 H each, CH_2NH, 2 CH_2N); 4.44 (br.d, 2 H, 2 H(1) Gal, J = 7.5 Hz); 5.00 (d, 1 H, H(1) Glc, J = 9.0 Hz); 5.06 (d, 1 H, H(1) Glc, J = 9.0 Hz). ^{13}C NMR, δ: 41.9 (CH_2NH_2); 44.2 (CH_2NH); 52.9 (br., CH_2NCH_2); 60.9 (C(6)Glc); 62.1 (C(6)Gal); 69.6 (C(4)Gal): 72.0 (C(2)Gal); 72.5 (C(2)Glc); 73.5 (C(3)Gal); 76.0 (C(3)Glc); 76.4 (C(5)Gal); 77.5 (C(5)Glc); 78.8 (C(4)Glc); 80.2 (C(1)Glc); 103.9 (C(1)Gal); 172.6; 173.1; 175.9 (CO).

***N*-Di{*N*-[4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-carbamoylmethyl}-*N*-(chloroacetyl)amine (8).** A suspension of compound **3** (0.6 g, 0.75 mmol) in anhydrous DMF (2.8 mL) was cooled in ice followed by the addition of chloroacetic anhydride (**7**) (0.154 g, 0.9 mmol) and stirring for for 4 h at 0 °C. Another portion of chloroacetic anhydride (0.13 g, 0.76 mmol) was added to the reaction mixture with stirring, and the mixture was kept for 16 h at 5 °C. The obtained suspension was diluted with MeOH (5 mL), a gel-like precipitate was filtered off, washed several times with MeOH (until neutral pH) and Et_2O and dried. The residue was dissolved in H_2O (2.5 mL) followed by addition of MeOH containing 20% of Et_3N (2.5 mL), and kept for 3 h at 20 °C. The precipitate that formed was filtered off, washed with 70% aq. MeOH, then MeOH and Et_2O and dried to give amorphous compound **8** (0.5 g, 73%), $[\alpha]_D^{25}$ +6.0 (*c* 0.5, H_2O). Found (%): C, 39.39; H, 6.50; Cl, 4.60; N, 4.67; H_2O, 5.37. $C_{30}H_{50}ClN_3O_{23}\cdot 3H_2O$. Calculated (%): C, 39.58; H, 6.20; Cl, 3.90; N, 4.62; H_2O, 5.94. ^{1}H NMR, δ: 3.40—3.61 (m, 4 H); 3.61—3.86 (m, 16 H); 3.86—3.98 (m, 4 H); 4.20 (br.s, 2 H, CH_2Cl); 4.37 (s, 2 H, CH_2N); 4.40 (s, 2 H, CH_2N); 4.45 (d, 2 H, 2 H(1) Gal, J = 7.5 Hz); 5.01 (d, 1 H, H(1) Glc, J = 9.0 Hz); 5.05 (d, 1 H, H(1) Glc, J = 9.0 Hz).

***N*-{*N*-[4-*O*-(β-D-Galactopyranosyl)-β-D-glucopyranosyl]carbamoylmethyl}-*N*-(*N*-{*N*-bis[4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl]carbamoylmethyl}carbamoylmethyl)amine (9).** A mixture of 4-*O*-(β-D-galactopyranosyl)-*N*-glycyl-β-D-glucopyranosylamine (**1**) (0.545 g, 1.37 mmol), chloroacetyl compound **8** (0.63 g, 0.69 mmol) in anhydrous DMSO (4 mL) and Pr^i_2EtN (0.117 mL, 0.69 mmol) was heated in a screw-capped test tube for 13 h at 70 °C. The reaction mixture was diluted with Et_2O (50 mL) with stirring and the liquid after clearing was decanted from the oily precipitate. The precipitate was triturated several times with Et_2O (10 mL each time) until a viscous substance was obtained, which was dissolved in H_2O (45 mL). Cationite Dowex 50w×2 (H^+) (30 mL) was added to the solution followed by stirring for 1 h. The resin was filtered off, washed with H_2O (300 mL) and 0.5 *M* Py (250 mL). The latter eluate (150 mL) containing the target product was concentrated to dryness. The residue was dissolved in H_2O (10 mL), the solution was filtered (0.45 μm filter) and concentrated to dryness. The residue was three times treated with boiling MeOH (5 mL each time) and Et_2O and dried to obtain amorphous compound **9** (0.73 g, 87%), $[\alpha]_D^{25}$ +7.8 (*c* 0.5, H_2O). Found (%): C, 43.06; H, 6.51; N, 5.44. $C_{44}H_{75}N_5O_{34}$. Calculated (%): C, 43.38; H, 6.21; N, 5.75. ^{1}H NMR, δ: 3.44 (s, 2 H, CH_2NH); 3.44—3.61 (m, 8 H); 3.61—3.86 (m, 24 H); 3.90—4.00 (m, 6 H); 4.18 (br.s, 2 H, CH_2N); 4.30 (s, 2 H, CH_2N); 4.45 (d, 3 H, 3 H(1) Gal, J = 7.5 Hz); 5.01 (d, 2 H, 2 H(1) Glc, J = 9.0 Hz); 5.04 (d, 1 H, H(1) Glc, J = 9.0 Hz).

***N*-(*N*-Benzyloxycarbonyldiglycyl)-*N*-{*N*-[4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl]carbamoylmethyl}-*N*-(*N*-{*N*-bis[4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl]carbamoylmethyl}-carbamoylmethyl)amine (10)** was obtained as described for compound **5**, from compound **9** (0.23 g, 0.19 mmol), dipeptide **4** (0.1 g, 0.38 mmol), NHS (0.046 g, 0.4 mmol), and DCC (0.087 g, 0.42 mmol) in anhydrous DMSO (1.5 mL) and anhydrous DMF (0.15 mL). The yield of amorphous compound **9** was 0.16 g (58%), $[\alpha]_D^{25}$ + 4.8 (*c* 0.5, H_2O). Found (%): C, 42.88; H, 6.51; N, 6.35; H_2O, 5.06. $C_{56}H_{87}N_7O_{38}\cdot 5H_2O$. Calculated (%): C, 43.21; H, 6.28; N, 6.30; H_2O, 5.79. ^{1}H NMR, δ: 3.44—3.54 (m, 3 H); 3.54—3.60 (m, 3 H); 3.61—3.86 (m, 24 H); 3.88—3.98 (m, 9 H); 4.07 (br.s, 1 H); 4.13 (br.s, 1 H); 4.17 (br.s, 1 H); 4.21 (br.s, 1 H); 4.22—4.41 (m, 4 H); 4.42—4.59 (m, 4 H); 4.98—5.10 (m, 3 H, 3 H(1) Glc); 5.18 (s, 2 H, CH_2Ph); 7.46 (br.s, 5 H, Ph).

***N*-{*N*-[4-*O*-(β-D-Galactopyranosyl)-β-D-glucopyranosyl]carbamoylmethyl}-*N*-(*N*-{*N*-bis[4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl]carbamoylmethyl}carbamoylmethyl)-*N*-(diglycyl)-amine (11).** 10% Palladium on activated carbon (0.12 g) was added to a solution of compound **10** (0.234 g, 0.16 mmol) in H_2O (7 mL) under argon and hydrogenation was carried out in a weak flow of H_2 under vigorous stirring for 8 h at 20 °C. The catalyst was filtered off and washed with H_2O (3×5 mL). The solution was concentrated to 3.5 mL and passed through a filter with C-18 silica gel (2 g) in H_2O. The silica gel was washed with H_2O (25 mL) and the solution was concentrated. Methanol (5 mL) was added to the residue, the formed precipitate was filtered off, washed with MeOH and Et_2O and dried to give amorphous compound **11** (0.19 g, 88%), $[\alpha]_D^{25}$ + 5.2 (*c* 0.5, H_2O). Found (%): C, 42.30; H, 6.52; N, 7.38; H_2O, 1.96. $C_{48}H_{81}N_7O_{36}\cdot H_2O$. Calculated (%): C, 42.70; H, 6.20; N, 7.26; H_2O, 1.33. ^{1}H NMR, δ: 3.43—3.51 (m, 5 H); 3.52—3.59 (m, 3 H); 3.61—3.84 (m, 26 H); 3.88—3.96 (m, 7 H); 4.05—4.55 (m, 9 H); 4.44 (d, 3 H, 3 H(1) Gal, J = 7.5 Hz); 4.97— 5.07 (m, 3 H, 3 H(1) Glc). ^{13}C NMR, δ: 41.7 (CH_2NH_2); 43.9 (CH_2NH); 52.2 (CH_2NCH_2); 52.6 (CH_2NCH_2); 60.9 (C(6)Glc); 62.0 (C(6)Gal); 69.5 (C(4)Gal): 71.9 (C(2)Gal); 72.5 (C(2)Glc); 73.5 (C(3)Gal); 76.0 (C(3)Glc); 76.3 (C(5)Gal); 77.4 (C(5)Glc); 78.8 (C(4)Glc); 80.2 (C(1)Glc); 103.9 (C(1)Gal); 172.1; 172.5; 172.8; 173.2; 175.0 (CO).

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References

1. M. Monsigny, A.-C. Roche, P. Midoux, R. Mayer, *Adv. Drug Deliv. Rev.*, 1994, **14**, 1.
2. M. Monsigny, C. Quetard, S. Bourgerie, D. Delay, C. Pichon, P. Midoux, R. Mayer, A.-C. Roche, *Biochimie*, 1998, **80**, 99.

3. W. Wang, in *Carbohydrate Drug Design*, Eds A. A. Klyosov, Z. J. Witczak, D. Platt, Am. Chem. Soc., Washington, 2006, p. 149—155.
4. L. M. Likhosherstov, O. S. Novikova, A. O. Zheltova, V. N. Shibaev, *Izv. Akad. Nauk, Ser. Khim.*, 2000, 1461 [*Russ. Chem. Bull.*, 2000, **49**, 1454 (Engl. Transl.)].
5. (a) I. D. Manger, T. W. Rademacher, R. A. Dwek, *Biochemistry*, 1992, **31**, 10724; (b) I. D. Manger, S. Y. C. Wong, T. W. Rademacher, R. A. Dwek, *Biochemistry*, 1992, **31**, 10733.
6. (a) A. B. Tuzikov, A. S. Gambaryan, I. R. Juneja, N. V. Bovin, *J. Carbohydr. Chem.*, 2000, **19**, 1191; (b) N. V. Shilova, O. E. Galanina, T. V. Pochechueva, A. A. Chinarev, V. A. Kadykov, A. B. Tuzikov, N. V. Bovin, *Glycocon. J.*, 2005, **22**, 43.
7. (a) U. K. Saha, R. Roy, *Tetrahedron Lett.*, 1995, **36**, 3635; (b) U. K. Saha, R. Roy, *J. Chem. Soc., Chem. Commun.*, 1995, 2571.
8. U. K. Saha, R. Roy, *Tetrahedron Lett.*, 1997, **38**, 7697.
9. A. V. Orlova, N. N. Kondakov, A. I. Zinin, B. G. Kimel´, L. O. Kononov, I. B. Sivaev, V. I. Bregadze, *Bioorg. Khim.*, 2006, **32**, 632 [*Russ. J. Bioorg. Chem.*, 2006, **32**, 568].
10. L. M. Likhosherstov, O. S. Novikova, L. O. Kononov, V. N. Shibaev, *Izv. Akad. Nauk, Ser. Khim.*, 2006, 1214 [*Russ. Chem. Bull., Int. Ed.*, 2006, **55**, 1262].
11. L. M. Likhosherstov, O. S. Novikova, L. O. Kononov, A. V. Orlova, I. B. Sivaev, V. I. Bregadze, *Izv. Akad. Nauk, Ser. Khim.*, 2007, 2033 [*Russ. Chem. Bull., Int. Ed.*, 2007, **56**, 2105].
12. S. D. Abbott, L. Gagnon, M. Lagraoui, S. Kadhim, G. Attardo, B. Zacharie, C. L. Penney, *J. Med. Chem.*, 1998, **41**, 1909.
13. S. Svedhem, L. Ohberg, S. Borrelli, R. Valiokas, M. Andersson, S. Oscarson, S. C. T. Svensson, B. Liedberg, P. Konradsson, *Langmuir*, 2002, **18**, 2848.
14. R. S. Bresalier, P.-S. Yan, J. C. Byrd, R. Lotan, A. Raz, *Cancer*, 1997, **80**, 776.
15. L. M. Likhosherstov, O. S. Novikova, V. N. Shibaev, N. K. Kochetkov, *Izv. Akad. Nauk, Ser. Khim.*, 1996, 1848 [*Russ. Chem. Bull.*, 1996, **45**, 1760 (Engl. Transl.)].
16. L. M. Likhosherstov, O. S. Novikova, V. N. Shibaev, *Dokl. Akad. Nauk*, 2002, **383**, 500 [*Dokl. Chem.*, 2002, **383**, 89 (Engl. Transl.)].
17. A. I. Usov, M. A. Rekhter, *Zh. Obshch. Khim.*, 1969, **39**, 912 [*J. Gen. Chem. USSR*, 1969, **39** (Engl. Transl.)].

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