Chemistry of Natural Compounds and Bioorganic Chemistry

N-Glycyl-β-glycopyranosylamines, derivatives of mono- and disaccharides, and their use for the preparation of carboxylic acid glycoconjugates

L. M. Likhosherstov, O. S. Novikova, A. O. Zheltova, and V. N. Shibaev*

N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 47 Leninsky prosp., 117913 Moscow, Russian Federation. Fax: +7 (095) 135 5328. E-mail: shiba@ioc.ac.ru

A convenient preparative procedure was developed for the synthesis of *N*-glycyl-β-glycopyranosylamines, derivatives of monosaccharides (D-galactose, D-mannose, L-fucose, and *N*-acetyl-D-glucosamine) and disaccharides (lactose, melibiose, cellobiose, and maltose). These compounds were demonstrated to be useful for the preparation of glycoconjugates of biologically active compounds containing the carboxy group (nicotinic, orotic, kynurenic, and indoleacetic acids). Synthetic pathways were developed for conversions of *N*-glycyl-β-glycopyranosylamines into derivatives containing the carboxy group with the use of malonic and L-tartaric acid derivatives.

Key words: glycoconjugates, carboxylic acids, *N*-chloroacetylglycosylamines, *N*-glycylglycosylamines.

In recent years, the introduction of carbohydrate residues into physiologically active compounds has been extensively studied[1,2] in connection with the use of this modification for the directed transport of medicines to cells of definite types. Readily accessible *N*-chloroacetyl-β-glycopyranosylamines[3,4] are convenient starting compounds for the preparation of glycoconjugates. Previously, we have reported the use of these compounds for the addition of residues of mono- and disaccharides to biologically active amines[5] and amino acids.[6] As part of our continuing studies, in the present work we report the synthesis of glycoconjugates from biologically active carboxyl-containing compounds using *N*-glycyl-β-glycopyranosylamines as the starting compounds.

The first syntheses of *N*-glycylglycosylamines, derivatives of *N*-acetyl-β-D-glucosamine and α- and β-D-glucose, were carried out in connection with examinations of the properties of the *N*-glycosylamide carbohydrate—peptide bond in glycoproteins. In these studies, glycosylamines of per-*O*-acetates of the corresponding monosaccharides,[7—10] free β-D-glucopyranosylamine,[11] or its 4,6-*O*-benzylidene derivative[12] were subjected to *N*-acylation with *N*-benzyloxycarbonylglycine in the presence of *N,N'*-dicyclohexylcarbodiimide (DCC) followed by deprotection. More recently, it has been demonstrated that *N*-glycyl-β-glycosylamines can serve as the starting compounds for the preparation of labeled carbohydrates containing fluorophore groups or

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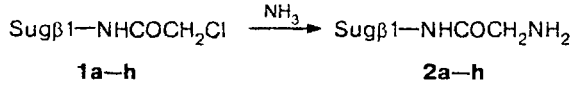
biotin residues.[3,13] This gave impetus to the development of new procedures for the synthesis of *N*-glycyl-β-glycosylamines based on reactions of *N*-chloroacetyl-β-glycosylamines with $(NH_4)_2CO_3$[3] or *N*-acylation of unprotected glycosylamines under the action of *N*-(9-fluorenylmethoxycarbonyl)glycine followed by treatment with piperidine.[14] These syntheses were carried out on a microscale using *N*-acetyl-D-glucosamine or oligosaccharides containing this monosaccharide residue at the reducing terminus of the chain.

The aim of this study was to develop a convenient preparative synthesis of *N*-glycyl-β-glycosylamines containing residues of different mono- and disaccharides and to study the possibility of the use of these compounds for the synthesis of glycoconjugates starting from a wide range of carboxyl-containing biologically active compounds. For the purpose of extending the use of *N*-glycyl-β-glycosylamines in the synthesis of glycoconjugates, we investigated also *N*-acylation of these compounds under the action of derivatives of dicarboxylic acids giving rise to compounds with a carboxyl-containing spacer.

We used the corresponding *N*-chloroacetyl-β-glycosylamines as the starting compounds for the synthesis of *N*-glycyl-β-glycosylamines (Scheme 1). Derivatives of D-galactose (**1a**), D-mannose (**1b**), L-fucose (**1c**), and lactose (**1d**) have been described previously.[4] Chloro derivatives of *N*-acetyl-D-glucosamine (**1e**), melibiose (**1f**), cellobiose (**1g**), and maltose (**1h**) were synthesized according to a general procedure by the reaction of $(ClCH_2CO)_2O$ with the corresponding glycosylamine in DMF.[4] In this case, disaccharide derivatives **1f—h** were prepared as oily substances containing up to 10% of the starting disaccharides and were used for conversions into *N*-glycyl derivatives without additional purification.

Scheme 1



1a—h **2a—h**

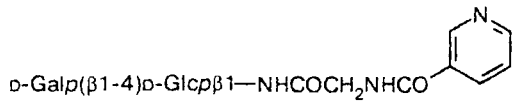
Sug = D-Gal*p* (**a**), D-Man*p* (**b**), L-Fuc*p* (**c**), D-Gal*p*(β1-4)D-Glc*p* (**d**), D-GlcNAc*p* (**e**), D-Gal*p*(α1-6)D-Glc*p* (**f**), D-Glc*p*(β1-4)D-Glc*p* (**g**), D-Glc*p*(α1-4)D-Glc*p* (**h**)

A concentrated aqueous solution of NH_3 proved to be the most convenient reagent for the replacement of the chlorine atom in *N*-chloroacetamides by the amino group.[15] Under the chosen conditions (a 0.04 *M* aqueous solution of the chloro derivative, 10 °C, 40 h), we observed (paper electrophoresis) *N*-glycylglycosylamines (the yields were ≥95%) and only traces of by-products, *viz.*, disubstituted amine and glycylamide (apparently, the ammonolysis product of the *N*-glycosylamide bond, whose lability in the glucose derivative has been mentioned previously[10]). The resulting *N*-glycylglycosylamines **2a—h** were purified with the use of ion-exchange resins, *viz.*, an anion-exchange resin for removal of Cl^- ions and a cation-exchange resin, which made it possible, in particular, to separate the reaction products from small amounts of neutral carbohydrates present in some starting *N*-chloroacetyl derivatives. After recrystallization, *N*-glycyl-β-glycosylamines **2a—h** were obtained in 55—70% yields.

Glycoconjugates of biologically active acids were synthesized primarily starting from compounds **2a,d,e** containing β-D-galactose or *N*-acetyl-β-D-glucosamine residues, which can bind to widespread lectins of animal cell surfaces.[1,16] The prospects for using derivatives of the above-mentioned carbohydrates for the directed transport of medicines have been demonstrated previously.[1,2]

Initially, we prepared glycoconjugates (**3—6**, Scheme 2) of a number of heterocyclic monocarboxylic acids (nicotinic, orotic, kynurenic, and indoleacetic acids), which exhibit pronounced biological activity and which are often used as medicines possessing various therapeutic actions.[17]

Scheme 2



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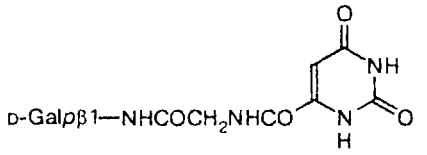

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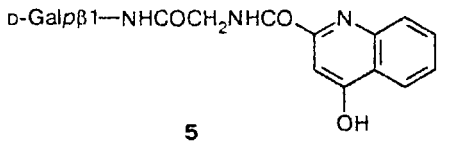

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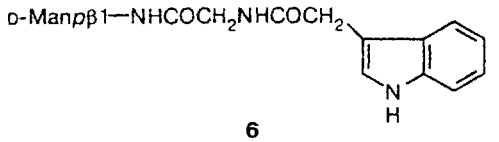

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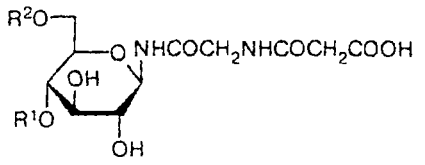
Condensation of the corresponding acids with *N*-glycylglycosylamines in the presence of DCC and *N*-hydroxysuccinimide[18] in DMSO proceeded smoothly to form the corresponding glycoconjugates (it is convenient to monitor the course of the reactions by paper

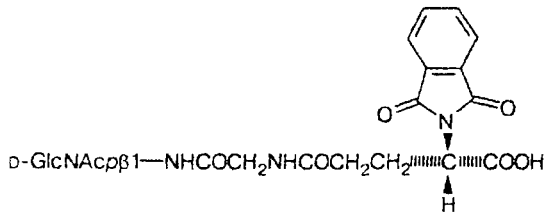
electrophoresis). After precipitation with toluene and purification by crystallization or gel chromatography on Sephadex G-15, products **3**—**6** were obtained in 45—65% yields.

With the aim of extending the potential of *N*-glycyl-β-glycosylamines in the synthesis of glycoconjugates, it was of interest to prepare derivatives whose aglyconic fragments contain a carboxy group, which can subsequently be used for conjugation to proteins or amine-containing polymeric carriers. In this connection, we studied the synthesis of this type of compounds by reactions of *N*-glycylglycosylamines with dicarboxylic acid derivatives (Scheme 3).

Scheme 3



7a: R^1 = D-Galpβ1, R^2 = H; **7b**: R^1 = H, R^2 = D-Galpα1;
7c: R^1 = D-Glcpβ1, R^2 = H; **7d**: R^1 = D-Glcpα1, R^2 = H



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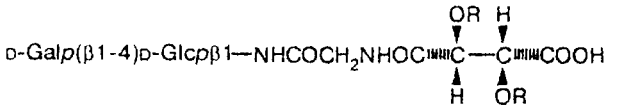

9a: R = PhCO, **9b**: R = H

Initially, acylation of disaccharide derivatives **2d,f—h** under the action of ethyl hydrogen malonate in the presence of DCC and *N*-hydroxysuccinimide has been studied.[18] The reaction products were subjected to saponification without isolation, giving rise to *N*-[*N*-(carboxyacetyl)glycyl]-β-glycosylamines, derivatives of lactose (**7a**), melibiose (**7b**), cellobiose (**7c**), and maltose (**7d**), in 46—65% yields. In the isolation of these compounds using KU-2 (H^+) cation-exchange resin, small amounts of neutral (paper electrophoresis) products (apparently, lactones) were obtained. The latter were separated from the target compounds by subsequent anion-exchange chromatography.

An alternative procedure for the preparation of these derivatives involves the reactions of *N*-glycyl-β-glycosylamines with acid anhydrides. The reaction of compound **1e** with readily accessible *N*-phthaloyl-L-glutamic anhydride (it is known[19] that the latter is opened at the γ-carboxy group to form amide) proceeded smoothly to form the corresponding *N*-[(*N*-phthaloyl-γ-L-glutamyl)glycyl]-β-glycosylamine (**8**). This derivative is of interest because it was found that the corresponding acid exhibits properties of a neuromediator analog of the mammalian central nervous system.

With the aim of preparing derivatives of *N*-glycylglycosylamines containing a spacer possessing hydrophilic properties, we studied *N*-acylation of compound **2d** with readily accessible[20] di-*O*-benzoyl-L-tartaric anhydride. The reaction in DMSO (1 h, 20 °C) afforded the corresponding dibenzoate (**9a**) in 67% yield. Debenzoylation of compound **9a** under the action of Et_3N in 50% aqueous MeOH followed by treatment with KU-2 (H^+) cation-exchange resin gave rise to the 2,3-dihydroxy-3-carboxypropionyl derivative (**9b**) in 87% yield.

The structures of the resulting compounds were confirmed by the data from elemental analysis and NMR spectroscopy.

A convenient procedure for the synthesis of *N*-glycyl-β-glycopyranosylamines described in the present work opens up a route to the wide use of these compounds for the preparation of glycoconjugates of different carboxylic acids and for the synthesis of derivatives with a carboxyl-containing spacer.

Experimental

The ^1H and ^{13}C NMR spectra were recorded on a Bruker WM-250 spectrometer (250 MHz for ^1H and 75 MHz for ^{13}C) in D_2O at 24 °C relative to residual protons of the solvent (internal standard) or relative to acetone (external standard). The optical rotation was measured on a Jasco DIP-360 polarimeter. Electrophoresis was carried out on Filtrak FN1 paper in a pyridinium—acetate buffer (0.05 *M* Py, pH 4.5, 30 V cm^{-1}, 1 h), a 2% solution of HCOOH (12 V cm^{-1}, 1 h), or a 0.4% solution of NH_4HCO_3 (pH ~8, 20 V cm^{-1}, 1 h). Ascending chromatography was performed using Filtrak FN15 paper and the 4 : 1.4 : 2.5 Bu^nOH—AcOH—H_2O solvent system. Compounds were detected using ninhydrin, KIO_4—$AgNO_3$—KOH reagents, or in UV light. Elution of compounds in gel chromatography was monitored by absorption at 206 nm. Water of crystallization was determined by the Fischer method.

2-Acetamido-*N*-chloroacetyl-2-deoxy-β-D-glucopyranosylamine (1e) was prepared from 2-acetamido-2-deoxy-β-D-glucopyranosylamine under the action of $(ClCH_2CO)_2O$ in DMF according to a procedure reported previously.[4] The yield was 69%, m.p. 223—225 °C (from a EtOH—EtOAc mixture, with decomp.), $[\alpha]_D^{20}$ +29.1° (*c* 1, H_2O). ^1H NMR, δ: 2.02 (s, 3 H, CH_3); 3.46—3.57 (m, 2 H, H(4), H(5)); 3.65 (t, 1 H, H(3), $J_{2,3} = J_{3,4} = 9$ Hz); 3.74—3.96 (m, 3 H, H(2), H(6a), H(6b)); 4.15 (br.s, 2 H, CH_2Cl); 5.11 (d, 1 H, H (1), $J = 9$ Hz); *cf.* lit. data[21]: m.p. 220—221 °C, $[\alpha]_D^{20}$ +28.2° (*c* 1, H_2O).

***N*-Chloroacetyl-6-*O*-(α-D-galactopyranosyl)-β-D-glucopyranosylamine (1f), *N*-chloroacetyl-4-*O*-(β-D-glucopyranosyl)-β-D-glucopyranosylamine (1g), and *N*-chloroacetyl-4-*O*-(α-D-glucopyranosyl)-β-D-glucopyranosylamine (1h)** were prepared from the corresponding glycosylamines[22] under the action of $(ClCH_2CO)_2O$ in DMF according to a known procedure.[4] The solvent was evaporated and the residue was triturated with acetone at 50 °C (2 times). Then the reaction products were extracted from the residue with 90% aqueous acetone at 50 °C. The aqueous-acetone extracts were concentrated and chloroacetamides **1f—h** were obtained in ~50% yields as oily products containing ~10% of the initial disaccharides (the data from paper chromatography). These products were used without additional purification for conversions into *N*-glycylglycosylamines.

Preparation of *N*-glycyl-β-glycopyranosylamines (2a—h) (general procedure). *N*-Chloroacetyl-β-D-glycopyranosylamines **1a—h** (4 mmol) were dissolved in a 30% aqueous solution of NH_3 (100 mL). The reaction solution was kept at ~10 °C for 40 h and concentrated to 20 mL. Then Dowex 1×8 (OH^-) anion-exchange resin (15 mL) was added and the mixture was stirred for 30 min. The resin was filtered off and washed with H_2O (150 mL). The filtrate and washings were concentrated to ~50 mL. Then AcOH was added to pH ~5, the reaction mixture was kept at 20 °C for 16 h, KU-2 (H^+) cation-exchange resin (30 mL) was added, and the mixture was stirred for 1 h. The resin was filtered off and washed with water (300 mL) and 0.5 *M* Py in water (300 mL). The product was eluted with 2 *M* NH_4OH (300 mL), the corresponding fractions (control by paper electrophoresis, pH 4.5) were concentrated to dryness, and the residue was crystallized.

***N*-Glycyl-β-D-galactopyranosylamine (2a).** The yield was 0.65 g (70%), m.p. 220—222 °C (from H_2O—MeOH), $[\alpha]_D^{20}$ +13.2° (*c* 1, H_2O). Found (%): C, 40.74; H, 6.99; N, 11.55. $C_8H_{16}N_2O_6$. Calculated (%): C, 40.68; H, 6.83; N, 11.86. ^1H NMR, δ: 3.46 (br.s, 2 H, NCH_2); 3.66—3.89 (m, 5 H, Gal); 4.04 (m, 1 H, H(4) Gal); 5.02 (d, 1 H, H(1) Gal, J = 9 Hz).

***N*-Glycyl-β-D-mannopyranosylamine (2b).** The yield was 0.6 g (65%), m.p. 200—202 °C (from propan-2-ol, with decomp.), $[\alpha]_D^{20}$ −41.2° (*c* 1, H_2O). Found (%): C, 40.74; H, 6.99; N, 11.49. $C_8H_{16}N_2O_6$. Calculated (%): C, 40.68; H, 6.83; N, 11.86. ^1H NMR, δ: 3.42 (br.s, 2 H, NCH_2); 3.48 (m, 1 H, H(5)); 3.61 (t, 1 H, H(4), $J_{3,4} = J_{4,5}$ = 9.7 Hz); 3.68—3.79 (m, 2 H); 3.87—3.99 (m, 2 H); 5.27 (br.s, 1 H, H(1)).

***N*-Glycyl-β-L-fucopyranosylamine (2c)** was prepared according to the general procedure, but the product was crystallized from a mixture of H_2O and MeOH after treatment of the mixture with Dowex 1×8 (OH^-). The yield was 0.6 g (68%), m.p. 219—221 °C, $[\alpha]_D^{18}$ −0.1° (*c* 1, H_2O). Found (%): C, 43.61; H, 7.60; N, 12.45. $C_8H_{16}N_2O_5$. Calculated (%): C, 43.63; H, 7.32; N, 12.72. ^1H NMR, δ: 1.30 (d, 3 H, CH_3, J = 6.5 Hz); 3.48 (br.s, 2 H, NCH_2); 3.68 (t, 1 H, H(2), $J_{1,2} = J_{2,3}$ = 8.5 Hz); 3.79 (m, 1 H, H(3)); 3.86 (m, 1 H, H(4)); 3.95 (m, 1 H, H(5)); 5.00 (d, 1 H, H(1), J = 8.5 Hz).

4-*O*-(β-D-Galactopyranosyl)-*N*-glycyl-β-D-glucopyranosylamine (2d), the yield was 1.1 g (70%), m.p. 246—248 °C (from H_2O—EtOH), $[\alpha]_D^{20}$ +3.0° (*c* 1, H_2O). Found (%): C, 42.37; H, 6.46; N, 7.06. $C_{14}H_{26}N_2O_{11}$. Calculated (%): C, 42.21; H, 6.58; N, 7.03. ^1H NMR, δ: 3.42 (br.s, 2 H, NCH_2); 3.45—3.60 (m, 2 H); 3.64—3.88 (m, 8 H); 3.92—3.99 (m, 2 H); 4.48 (d, 1 H, H(1) Gal, J = 8 Hz); 5.04 (d, 1 H, H(1) Glc, J = 10 Hz).

2-Acetamido-2-deoxy-*N*-glycyl-β-D-glucopyranosylamine (2e), the yield was 0.64 g (69%), m.p. 245—247 °C (from H_2O—EtOH), $[\alpha]_D^{20}$ +26.2° (*c* 1, H_2O); *cf.* lit. data[9]: m.p. 236—237 °C, $[\alpha]_D^{23}$ +23.54° (*c* 0.8, H_2O).

6-*O*-(α-D-Galactopyranosyl)-*N*-glycyl-β-D-glucopyranosylamine (2f) was purified by precipitation with ether from MeOH. The yield of the amorphous compound was 0.9 g (55%), $[\alpha]_D^{20}$ +66.3° (*c* 1, H_2O). Found (%): C, 41.36; H, 6.92; N, 7.22; H_2O, 2.70. $C_{14}H_{26}N_2O_{11} \cdot 1/2\ H_2O$. Calculated (%): C, 41.28; H, 6.68; N, 6.87; H_2O, 2.21. ^1H NMR, δ: 3.47 (br.s, 2 H, NCH_2); 3.38—4.04 (m, 12 H); 5.00 (d, 1 H, H(1) Gal, J = 4 Hz); 5.04 (d, 1 H, H(1) Glc, J = 9.5 Hz). ^{13}C NMR, δ: 44.7 (NCH_2); 62.3 (C(6) Gal); 67.2 (C(6) Glc); 69.7, 70.5, 70.6, 70.8, 72.1, 73.0, 77.4, 77.9 (8 C, Glc, Gal); 80.6 (C(1) Glc); 99.5 (C(1) Gal).

4-*O*-(β-D-Glucopyranosyl)-*N*-glycyl-β-D-glucopyranosylamine (2g). The yield was 1.15 g (71%), m.p. 246—247 °C (from H_2O—MeOH, with decomp.), $[\alpha]_D^{20}$ −17.1° (*c* 1, H_2O). Found (%): C, 42.37; H, 6.56; N, 7.15. $C_{14}H_{26}N_2O_{11}$. Calculated (%): C, 42.21; H, 6.58; N, 7.03. ^1H NMR, δ: 3.42 (br.s, 2 H, NCH_2); 3.30—3.60 (m, 5 H); 3.67—3.99 (m, 7 H); 4.53 (d, 1 H, H(1) *O*-Glc, J = 8 Hz); 5.04 (d, 1 H, H(1) *N*-Glc, J = 9.5 Hz).

4-*O*-(α-D-Glucopyranosyl)-*N*-glycyl-β-D-glucopyranosylamine (2h). The yield was 1 g (60%), m.p. 220—222 °C (from a H_2O—MeOH—EtOH mixture), $[\alpha]_D^{20}$ +75.2° (*c* 1, H_2O). Found (%): C, 41.29; H, 6.75; N, 6.81; H_2O, 2.25. $C_{14}H_{26}N_2O_{11} \cdot 1/2\ H_2O$. Calculated (%): C, 41.28; H, 6.68; N, 6.87; H_2O, 2.21. ^1H NMR, δ: 3.43 (br.s, 2 H, NCH_2); 3.40—3.98 (m, 12 H, Glc); 5.04 (d, 1 H, H(1) *N*-Glc, J = 9 Hz); 5.46 (d, 1 H, H(1) *O*-Glc, J = 4 Hz).

4-*O*-(β-D-Galactopyranosyl)-*N*-(*N*-nicotinoylglycyl)-β-D-glucopyranosylamine (3). A solution of compound **2d** (0.3 g, 0.75 mmol), *N*-hydroxysuccinimide (0.124 g, 1.08 mmol), and nicotinic acid (0.14 g, 1.14 mmol) in DMSO (2 mL) was cooled to −12 °C. An emulsion of DCC (0.24 g, 1.16 mmol) in DMSO (1.1 mL) was added over 3 min. Then the reaction mixture was stirred at 15 °C for 1 h and kept at 20 °C for 20 h. The precipitate was filtered off and washed with DMSO (2×0.5 mL). Toluene (55 mL) was added to the filtrate and the mixture was kept at −5 °C for 16 h. The solvent was decanted from the oily residue, the residue was washed with toluene (2×20 mL), and MeOH (15 mL) was added to the precipitate. The mixture was heated to ~50 °C. The precipitate that formed was filtered off, washed with MeOH (5×20 mL), and dried. Then the precipitate was recrystallized from water (4 mL) and washed with ice water and acetone to give compound **3** in a yield of 0.23 g (56%), m.p. 268—270 °C (with decomp.), $[\alpha]_D^{20}$ +2.5° (*c* 1, H_2O). Found (%): C, 44.32; H, 6.29; N, 7.86; H_2O, 6.64. $C_{20}H_{29}N_3O_{12} \cdot 2H_2O$. Calculated (%): C, 44.52; H, 6.16; N, 7.79; H_2O, 6.68. ^1H NMR, δ: 3.54—3.70 (m, 2 H); 3.71—3.97 (m, 8 H); 3.98—4.09 (m, 2 H); 4.31 (br.s, 2 H, NCH_2); 4.56 (d, 1 H, H(1) Gal, J = 8 Hz); 5.16 (d, 1 H, H(1) Glc, J = 9 Hz); 7.70 (m, 1 H, Ar); 8.34 (d, 1 H, Ar, J = 8 Hz); 8.80 (d, 1 H, Ar, J = 4 Hz); 9.02 (s, 1 H, Ar).

***N*-[*N*-Uracil-6-ylcarbonyl)glycyl]-β-D-galactopyranosylamine (4).** A solution of compound **2a** (235 mg, 1 mmol), orotic acid monohydrate (190 mg, 1.1 mmol), and *N*-hydroxysuccinimide (135 mg, 1.2 mmol) in DMSO (6 mL) was cooled to 15 °C. An emulsion of DCC (290 mg, 1.4 mmol) in DMSO (1 mL) was added over 3 min and the reaction mixture was stirred for 30 min and kept at 25 °C for 6 h. The precipitate was filtered off and washed with DMSO (2×0.5 mL). Toluene (80 mL) was added to the filtrate and the mixture was kept at −5 °C for 16 h. The solvent was decanted and the oily product was washed with toluene (3×10 mL) and dried. The residue was extracted with H_2O (3×10 mL) and the solution was concentrated to ~1 mL. The gel-like precipitate that formed was filtered off, washed with H_2O (4×0.5 mL), and dissolved in H_2O at ~70 °C. The hot solution was filtered and cooled to

20 °C. The precipitate that formed was filtered off, washed with H_2O and acetone, and dried to give compound **4** in a yield of 95 mg. The mother liquor was concentrated, the residue was dissolved in a 0.1 *M* NH_4OH solution in 10% aqueous MeOH (5 mL), and the solution was applied onto a column (4×100 cm) with Sephadex G-15. The column was washed with the same solvent system and the fractions containing product **4** (electrophoresis at pH 4.5 and ~8) were concentrated. Water (15 mL) was added to the residue and the solvent was evaporated. This treatment was repeated 5 times. The residue was washed with H_2O and acetone and then dried. Compound **4** was obtained in a yield of 105 mg. The total yield of compound **4** was 200 mg (51%), m.p. 258—260 °C (with decomp.), $[\alpha]_D^{20}$ +31.7° (*c* 1, DMSO). Found (%): C, 40.08; H, 4.86; N, 14.58; H_2O, 5.00. $C_{13}H_{18}N_4O_9 \cdot H_2O$. Calculated (%): C, 39.80; H, 5.14; N, 14.28; H_2O, 4.59. ^1H NMR, δ: 3.70—3.86 (m, 5 H); 4.01 (m, 1 H, H(4) Gal); 4.19 (br.s, 2 H, NCH_2); 5.00 (d, 1 H, H(1) Gal, J = 9 Hz); 6.30 (s, 1 H, C=CH). ^{13}C NMR, δ: 44.0 (NCH_2); 62.2 (C(6) Gal); 69.9 (C(4) Gal); 70.5 (C(2) Gal); 74.6 (C(3) Gal); 78.0 (C(5) Gal); 81.2 (C(1) Gal); 101.3 (C=CH); 151.4, 156.9, 165.3, 169.1, 173.2 (5 C).

***N*-[*N*-(4-Hydroxy-2-quinolylcarbonyl)glycyl]-β-D-galactopyranosylamine (5).** Compound **2a** (0.35 g, 1.5 mmol) was added to a solution of kynurenic acid monohydrate (0.33 g, 1.6 mmol), *N*-hydroxysuccinimide (0.2 g, 1.7 mmol), and DCC (0.39 g, 1.9 mmol) in DMSO (9 mL) and the reaction mixture was kept at ~20 °C for 24 h. Then DCC (0.21 g, 1 mmol) was added and the reaction mixture was kept at ~20 °C for 24 h. The precipitate of *N,N'*-dicyclohexylurea was filtered off and washed with DMSO (3×1 mL). Toluene (180 mL) was added to the filtrate and the mixture was kept at −5 °C for 16 h. The precipitate that formed was filtered off, washed with toluene (3×10 mL) and ether (3×10 mL), dried, and triturated with H_2O (15 mL) at 80 °C. An undissolved precipitate was filtered off and washed with H_2O (2×5 mL). The gel-like product that precipitated from the filtrate upon cooling was filtered off, washed with cold water (3×10 mL), MeOH (5×15 mL), and ether, and dried to give compound **5** in a yield of 0.28 g. After concentration of the mother liquor and the above-described trituration of the residue, the product was additionally obtained in a yield of 0.18 g. The total yield of compound **5** was 0.46 g (76%), m.p. 244—246 °C, $[\alpha]_D^{20}$ +27.8° (*c* 1, DMSO). Found (%): C, 52.94; H, 5.68; N, 10.28. $C_{18}H_{21}N_3O_8$. Calculated (%): C, 53.07; H, 5.20; N, 10.31. ^1H NMR (80 °C), δ: 3.74—3.95 (m, 5 H, Gal); 4.11 (br.s, 1 H, H(4) Gal); 4.36 (br.s, 2 H, NCH_2); 5.09 (d, 1 H, H(1) Gal, J = 7 Hz); 6.95 (s, 1 H, Ar); 7.63 (t, 1 H, Ar, J = 7.5 Hz); 7.90—7.96 (m, 2 H, Ar); 8.28 (d, 1 H, Ar, J = 8 Hz). ^{13}C NMR (80 °C), δ: 44.4 (NCH_2); 62.3 (C(6) Gal); 70.1 (C(4) Gal); 70.9 (C(2) Gal); 74.8 (C(3) Gal); 78.0 (C(5) Gal); 81.3 (C(1) Gal); 108.2, 120.9, 125.6, 126.6, 134.9 (5 CH, Ar).

***N*-{*N*-[(Indol-3-yl)acetyl]glycyl}-β-D-mannopyranosylamine (6).** *N*-Hydroxysuccinimide (0.21 g, 1.8 mmol) and 3-indoleacetic acid (0.315 g, 1.8 mmol) were added to a solution of compound **2b** (0.35 g, 1.4 mmol) in DMSO (4 mL). Then the reaction mixture was cooled to 15 °C, DCC (0.4 g, 1.9 mmol) was added, and the mixture was kept at ~20 °C for 24 h. The precipitate of *N,N'*-dicyclohexylurea was filtered off and washed with DMSO (2×0.5 mL). Then toluene (100 mL) was added to the filtrate. The mixture was kept at −5 °C for 16 h and an oily product was separated by decantation, washed with toluene (2×15 mL), and triturated with acetone (4×30 mL) upon heating. The resulting solid residue was filtered off, washed with acetone and ether, dried, and dissolved in 0.033 *M* AcOH. The reaction solution was applied onto a column (4×100 cm) with Sephadex G-15. The column was washed with 0.033 *M* AcOH (1200 mL) and the product was eluted with 10% aqueous MeOH. The fractions containing the product were concentrated, the residue was dissolved in MeOH (15 mL), propan-2-ol (20 mL) was added, and the solution was concentrated to 7 mL *in vacuo*. The precipitate that formed was filtered off, washed with propan-2-ol and ether, and dried to give an amorphous compound **6** in a yield of 0.36 g (62%), $[\alpha]_D^{20}$ −24.7° (*c* 1, H_2O). Found (%): C, 54.62; H, 6.24; N, 10.46. $C_{18}H_{23}N_3O_7$. Calculated (%): C, 54.96; H, 5.89; N, 10.68. ^1H NMR, δ: 3.50 (m, 1 H, H(4) Man); 3.63—4.02 (m, 7 H); 3.88 (br.s, 2 H, CH_2); 5.20 (br.s, 1 H, H(1) Man); 7.22—7.34 (m, 2 H, Ar); 7.37 (s, 1 H, Ar); 7.60 (d, 1 H, Ar, J = 8 Hz); 7.69 (d, 1 H, Ar, J = 8 Hz).

Preparation of *N*-[*N*-(carboxyacetyl)glycyl]-β-D-glycopyranosylamines (7a—d) (general procedure). An emulsion of DCC (0.38 g, 1.85 mmol) in DMSO (1.7 mL) was added to a cooled (12—15 °C) solution of compound **2d**, **2f**, **2g**, or **2h** (0.48 g, 1.2 mmol), *N*-hydroxysuccinimide (0.18 g, 1.6 mmol), and ethyl hydrogen malonate (0.24 g, 1.8 mmol) in DMSO (3 mL) over 5 min. The reaction mixture was stirred for 30 min and kept at 20 °C for 18 h. Then the mixture was diluted with toluene (50 mL) and kept at −5 °C for 5 h. The solvent was decanted and the precipitate was washed with toluene (5×15 mL) and dried *in vacuo*. Water (6 mL) was added to the precipitate and the mixture was stirred for 20 min and kept at 20 °C for 18 h. The precipitate of *N,N'*-dicyclohexylurea was filtered off and washed with H_2O. The filtrate and washings were concentrated. The oily residue (0.46 g) was dissolved in 50% aqueous MeOH (11 mL). Then Et_3N (1.1 mL) was added and the solution was kept at 15 °C for 24 h and concentrated. The residue was dissolved in H_2O (5 mL), the solution was stirred for 30 min with KU-2 (H^+) cation-exchange resin (3 mL), and the resin was filtered off and washed with H_2O (30 mL). The filtrate and washings were concentrated to ~5 mL, Dowex 1×8 (CO_3^{2-}) anion-exchange resin (2.5 mL) was added, and the mixture was stirred for 1 h. The resin was filtered off and washed with H_2O (30 mL) and the product was eluted with 0.5 *M* AcOH (60 mL). Fractions containing the target product (control by eletrophoresis at pH 4.5) were concentrated to ~5 mL, a 2 : 15 : 5 water—MeOH—toluene mixture was added, and the reaction mixture was concentrated to remove AcOH. The residue was dissolved in water (1 mL), a 1 : 1 MeOH—EtOH mixture was added, and the solution was concentrated until a precipitate formed. The precipitate was filtered off, washed with EtOH and ether, and dried.

***N*-[*N*-(Carboxyacetyl)glycyl]-4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosylamine (7a).** The yield of compound **7a** was 0.29 g (54%), m.p. 265—267 °C (from H_2O—MeOH, with decomp.), $[\alpha]_D^{20}$ +5.0° (*c* 1, H_2O). Found (%): C, 41.40; H, 6.03; N, 5.85; H_2O, 2.02. $C_{17}H_{28}N_2O_{14} \cdot 1/2\ H_2O$. Calculated (%): C, 41.38; H, 5.92; N, 5.67; H_2O, 1.82. ^1H NMR, δ: 3.46—3.86 (m, 12 H); 3.89—3.98 (m, 2 H); 4.05 (br.s, 2 H, NCH_2); 4.46 (d, 1 H, H(1) Gal, J = 8 Hz); 5.04 (d, 1 H, H(1) Glc, J = 10 Hz).

***N*-[*N*-(Carboxyacetyl)glycyl]-6-*O*-(α-D-galactopyranosyl)-β-D-glucopyranosylamine (7b).** The yield of compound **7b** was 0.27 g (46%), m.p. 197—199 °C (from H_2O—MeOH—EtOH, with decomp.), $[\alpha]_D^{20}$ +57.1° (*c* 1, H_2O). Found (%): C, 41.38; H, 6.00; N, 6.00; H_2O, 2.00. $C_{17}H_{28}N_2O_{14} \cdot 1/2\ H_2O$. Calculated (%): C, 41.38; H, 5.92; N, 5.67; H_2O, 1.82. ^1H NMR, δ: 3.44—3.65 (m, 4 H); 3.66—4.04 (m, 10 H); 4.06 (br.s, 2 H, NCH_2); 4.99 (d, 1 H, H(1) Gal, J = 3.5 Hz); 5.04 (d, 1 H, H(1) Glc, J = 9.5 Hz).

***N*-[*N*-(Carboxyacetyl)glycyl]-4-*O*-(β-D-glucopyranosyl)-β-D-glucopyranosylamine (7c).** The yield of amorphous compound **7c** was 0.28 g (50%), $[\alpha]_D^{20}$ −9.8° (*c* 1, H_2O). Found (%):

C, 41.68; H, 6.07; N, 5.94. $C_{17}H_{28}N_2O_{14}$. Calculated (%): C, 42.15; H, 5.83; N, 5.78. ^{1}H NMR, δ: 3.27—3.93 (m, 14 H); 4.04 (br.s, 2 H, NCH_2); 4.51 (d, 1 H, H(1) *O*-Glc, J = 8.5 Hz); 5.04 (d, 1 H, H(1) *N*-Glc, J = 9.5 Hz).

***N*-[*N*-(Carboxyacetyl)glycyl]-4-*O*-(α-D-glucopyranosyl)-β-D-glucopyranosylamine (7d)** was prepared analogously, but the compound was precipitated with ether. The yield of amorphous compound **7d** was 0.38 g (65%), $[\alpha]_D^{20}$ +67.9° (*c* 1, H_2O). Found (%): C, 41.73; H, 6.31; N, 5.81. $C_{17}H_{28}N_2O_{14}$. Calculated (%): C, 42.15; H, 5.83; N, 5.78. ^{1}H NMR, δ: 3.40—3.65 (m, 4 H); 3.66—3.95 (m, 10 H); 4.05 (br.s, 2 H, NCH_2); 5.04 (d, 1 H, H(1) *N*-Glc, J = 9.5 Hz); 5.45 (d, 1 H, H(1) *O*-Glc, J = 4 Hz).

2-Acetamido-2-deoxy-*N*-[(*N*-phthaloyl-γ-L-glutamyl)-glycyl]-β-D-glucopyranosylamine (8). A solution of compound **2e** (0.415 g, 1.5 mmol) in DMSO (5 mL) prepared with heating to 70 °C was cooled to 20 °C. Then *N*-phthaloyl-L-glutamic anhydride (0.623 g, 1.6 mmol) was added and the reaction mixture was kept at 20 °C for 24 h. Toluene (70 mL) was added and the reaction mixture was kept at −5 °C for 16 h. The solvent was decanted and the precipitate was washed with toluene (5×10 mL), filtered off, washed with hot acetone (7×15 mL) and ether (2×10 mL), and dried. The yield of amorphous compound **8** was 0.52 g (65%), $[\alpha]_D^{20}$ +15.9° (*c* 1, H_2O). Found (%): C, 51.20; H, 5.61; N, 10.59. $C_{23}H_{25}N_4O_{11}$. Calculated (%): C, 51.49; H, 5.26; N, 10.44. ^{1}H NMR, δ: 2.03 (s, 3 H, CH_3); 2.37—2.61 (m, 4 H, CH_2CH_2); 3.45—3.71 (m, 5 H); 3.72—3.97 (m, 3 H, GlcN); 4.96—5.08 (m, 2 H, H(1) GlcN, COCH); 7.88—8.00 (m, 4 H, Ar). ^{13}C NMR, δ: 23.0 (CH_3); 24.0 (CH_2); 33.1 (CH_2); 43.4 (NCH_2); 52.7 (CN); 54.9 (CN); 61.5 (C(6) GlcN); 70.5 (C(4) GlcN); 75.1 (C(3) GlcN); 78.6 (C(5) GlcN); 79.6 (C(1) GlcN); 124.8 (2 C, CH, Ar); 131.8 (2 C, C, Ar); 136.2 (2 C, CH, Ar); 170.4, 173.2, 173.8, 175.8, 176.2 (5 CO).

***N*-{*N*-[(*R*,*R*)-2,3-Dibenzoyloxy-3-carboxypropionyl]glycyl}-4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosylamine (9a).** A solution of compound **2d** (0.4 g) in DMSO (5 mL) was added with stirring to a cooled (12—15 °C) suspension of di-*O*-benzoyl-L-tartaric anhydride (0.62 g, 1.8 mmol)[20] in DMSO (1 mL) over 3 min. The reaction mixture was kept at 20 °C for 1 h, diluted with toluene (120 mL), and then kept at −5 °C for 16 h. The precipitate was separated and washed with toluene (5×5 mL). The residue was triturated with acetone (10 mL) and boiling acetone (3×2 mL). The acetone extracts were concentrated to dryness, the residue was dissolved in propan-2-ol (6 mL), and ether (30 mL) was added. The precipitate that formed was filtered off, washed with ether, and dried. The yield of amorphous compound **9a** was 0.5 g (67%), $[\alpha]_D^{20}$ −55.1° (*c* 1, MeOH). Found (%): C, 51.68; H, 5.43; N, 3.46. $C_{32}H_{38}N_2O_{18}$. Calculated (%): C, 52.03; H, 5.10; N, 3.79. ^{1}H NMR (CD_3OD), δ: 3.37—3.88 (m, 12 H); 3.97 (br.s, 2 H, NCH_2); 4.41 (d, 1 H, H(1) Gal, J = 8 Hz); 4.91 (d, 1 H, H(1) Glc, J = 9 Hz); 5.96 (br.s, 1 H, COCH); 6.06 (br.s, 1 H, COCH); 7.54 (m, 4 H, Ar); 7.68 (m, 2 H, Ar); 8.17 (m, 4 H, Ar).

***N*-{*N*-[3-Carboxy-(*R*,*R*)-2,3-dihydroxypropionyl]glycyl}-4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosylamine (9b).** Et_3N (1.2 mL) was added to a solution of dibenzoate **9a** (0.35 g, 0.47 mmol) in 50% aqueous MeOH (12 mL). The reaction mixture was kept at ~17 °C for 20 h and concentrated to ~2 mL. Then MeOH (2×15 mL) was added and the mixture was concentrated. The residue was dissolved in H_2O (8 mL), the solution was stirred with KU-2 (H^+) cation-exchange resin (4 mL) for 30 min, and the resin was filtered off and washed with H_2O (50 mL). Benzoic acid was removed from the filtrate and washings by extraction with ether (4×10 mL). The aqueous solution was concentrated to 5 mL and then EtOH (15 mL) was added. The precipitate that formed upon concentration of the solution was filtered off, washed with EtOH and ether, and dried. The yield of compound **9b** was 0.22 g (87%), m.p. 202—205 °C, $[\alpha]_D^{20}$ +35.7° (*c* 1, H_2O). Found (%): C, 40.41; H, 5.97; N, 5.31. $C_{18}H_{30}N_2O_{16}$. Calculated (%): C, 40.75; H, 5.70; N, 5.28. ^{1}H NMR, δ: 3.40—3.57 (m, 2 H); 3.61—3.84 (m, 8 H); 3.88—3.95 (m, 2 H); 4.07 (br.s, 2 H, NCH_2); 4.44 (d, 1 H, H(1) Gal, J = 8 Hz); 4.68 (br.s, 2 H, COCHCH); 5.02 (d, 1 H, H(1) Glc, J = 9 Hz).

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