Synthesis of mono- and di-α-L-fucosylated 2-acetamido-2-deoxy-*N*-glycyl-β-D-glucopyranosylamines, spacered fragments of glycans of *N*-glycoproteins*

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α-L-Fuc*p*-(1→3)-D-GlcNAc, α-L-Fuc*p*-(1→6)-[α-L-Fuc*p*-(1→3)]-D-GlcNAc, and β-D-Gal*p*-(1→3)-[α-L-Fuc*p*-(1→4)]-D-GlcNAc were converted into corresponding β-glycopyranosylamines by action of ammonium carbamate in aqueous boric acid, or in aqueous methanol in case of α-L-Fuc*p*-(1→6)-D-GlcNAc. *N*-Acylation of these fucooligosaccharides with *N*-Z-glycine *N*-hydroxysuccinimide ester (Z is benzyloxycarbonyl) followed by hydrogenolytic removal of Z-group afforded corresponding *N*-glycyl-β-glycopyranosylamines of these fucooligosaccharides; three of them model carbohydrate-peptide region of *N*-glycoproteins, and the forth is an amino-spacered Le^a-antigen.

Key words: α-L-fucose, 2-acetamido-2-deoxy-β-D-glucopyranose, oligosaccharides, *N*-glycyl-β-glucopyranosylamines, *N*-acylation, carbohydrate-peptide bond, *N*-glycoproteins.

α-L-Fucopyranose (Fuc) is an important component of many glycoconjugates, and in particular, glycoproteins and glycolipids. In these biopolymers, as a rule, Fuc is linked to 2-nd position of β-D-galactopyranose (Gal) or to 3-, 4- or 6-th positions of 2-acetamido-2-deoxy-β-D-glucopyranose (GlcNAc) (see Ref. 1) as a terminal moiety. Syntheses of fucooligosaccharides as free oligosaccharides, *O*-glycosides or *O*-spacered derivatives are described in the literature (see, for example, review[2]). Data on *N*-spacered fucooligosaccharides is not so plentiful[2,3a] and it is often referred to *N*-asparagine derivatives.[3b] One type of *N*-spacered oligosaccharides used for preparation of conjugates are *N*-glycyl-β-glycopyranosylamines (see Ref. 4 and references cited therein). Earlier, we reported synthesis of derivatives of this type from oligosaccharides of breast milk.[4,5] Some of these compounds were used in our research[6] aimed at natural inhibitors of two norovirus strains, which belong to the *Caliciviridae* family of RNA viruses, which are the leading cause of epidemics of acute gastroenteritis worldwide.[7]

Biosynthesis of N-glycoproteins in eukaryotic cells can be accompanied by formation of considerable quantities of N-glycans, which differ in monosaccharide composition and molecular weight; however, being different as they are, all of them are linked to the polypeptide chains through the D-GlcNAcβ1-*N*-Asn fragment. In some types of N-glycans the GlcNAc (GlcNAc-1) residue is modified with Fuc at 6- or 3-positions, and in some cases at both of them. The first type of modification was found in many animals, for example, mammals and birds, the second type was observed in plants, insects, helminths, and slime mold, and the third type was found in insects and some helminths.[8—10] *N*-Glycans, which comprise fucosylated GlcNAc-1, show pronounced biological activity. For example, it was shown, that the presence of α-1-6-fucose residue decreases cytotoxicity of *N*-glycoproteins,[11] thus providing for monitoring of the efficacy of therapeutic antibodies of G-type.[12] α-1-3-Fucosylated *N*-glycans are highly immunogenic for mammals and are one of the causes of allergic reactions to food from plant sources and to insect stings in humans.[13]

In the present work we report the application of the carbamate involving procedure for the synthesis of β-glycopyranosylamines,[14a,b] which was elaborated in our group earlier, to alkali labile fucooligosaccharides with GlcNAc on the reducing end followed by preparation of *N*-glycyl-β-glycopyranosidic derivatives thereof. Di- and trisaccharides α-L-Fuc*p*-(1→3)-D-GlcNAc (**1a**), α-L-Fuc*p*-(1→3)-[α-L-Fuc*p*-(1→6)]-D-GlcNAc (**2a**), β-D-Gal*p*-(1→3)-[α-L-Fuc*p*-(1→4)]-D-GlcNAc (**3a**) and α-L-Fuc*p*-(1→6)-D-GlcNAc (**4a**), which are the fragments

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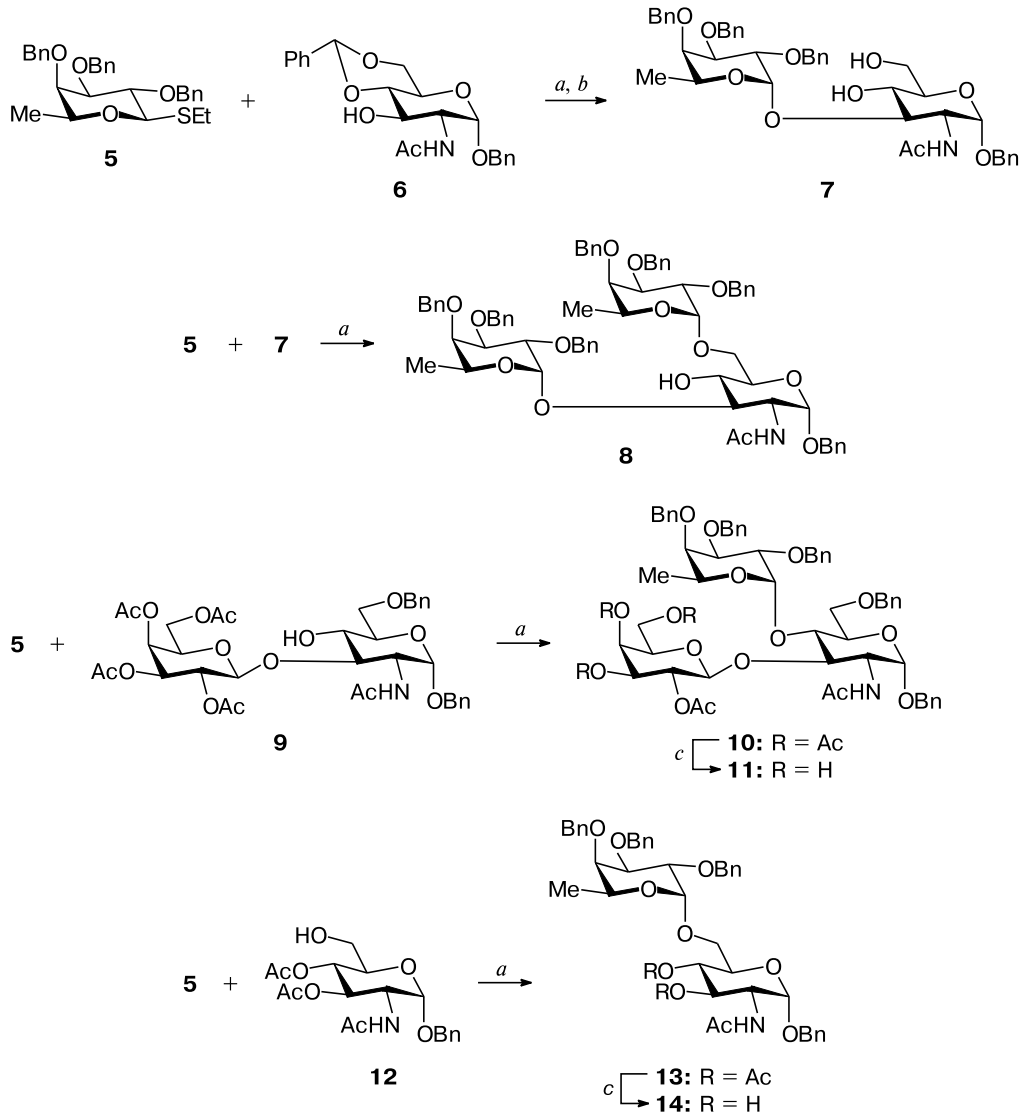
of physiologically important natural glycoconjugates, were used in this research. Disaccharide **1a** is a fragment of the aforementioned carbohydrate-peptide linking unit in *N*-glycoproteins of plants, insects, helminths, and slime mold.[9,10] Besides, it is a part of carcinoembryonic antigen Le^x and its derivatives ($SiaLe^x$, $SulfoLe^x$, VIM-2, LDNF), which were found in many glycoconjugates. Trisaccharide **2a** is a part of a carbohydrate—peptide linking unit in *N*-glycoproteins of insects and some helminths.[9,10] Trisaccharide **3a** is a carcinoembryonic antigen Le^a, which was found in many animal glycoconjugates. As a part of complex *N*-glycans in plant glycoproteins, Le^a is an antigen determinant, and it is highly immunogenic for animals, being one of the causes of allergic reaction to plants in humans.[13] Disaccharide **4a** is a part of a carbohydrate—peptide linking unit in N-glycoproteins found in many species of animals, including mammals and humans.[8]

Syntheses of oligosaccharides **1a**,[15] **3a**,[16] and **4a**[17] were described earlier, and trisaccharide **2a** was synthesized as an allyl *O*-glycoside[18] and a part of tetra- and octasaccharides.[19] Syntheses of oligosaccharides **1a**—**4a** were performed by our group (Schemes 1 and 2) with the use of earlier described approaches and procedures except that we chose to use ethyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside (**5**) in the presence of Et_4NBr and $CuBr_2$[20] for fucosylation. Herewith we have found, that increased quantity of Et_4NBr (3.5 molar excess instead of 1.5) results in considerable improvement of stereoselectivity of

Scheme 1



Reagents and conditions: *a*. Et_4NBr, $CuBr_2$, CH_2Cl_2, DMF, ~20 °C, 64 h; *b*. 90% aqueous TFA, $CHCl_3$, 27 °C, 30 min; *c*. MeONa, MeOH, ~20 °C, 5 h.

α-L-fucosylation (α : β ratio of anomers was ~9 : 1 instead of ~4 : 1). It should be noted, that we performed hydrogenolytic removal of benzyl groups (with Pd/C as a catalyst) at higher temperature, namely, ~50 °C, without addition of acetic acid or increasing the pressure of H_2.

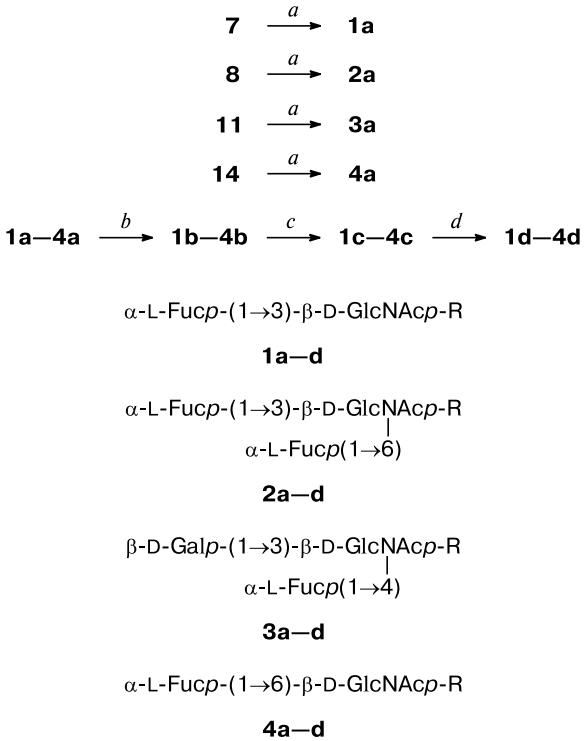
On the basis of literature data we assumed that oligosaccharides **1a**—**4a** may have different resistance to alkali. It is known[21] that 2-acetamido-2-deoxyhexoses are more alkali-labile than the corresponding hexoses. The presence of a monosaccharide residue at position 6 of 2-acetamido-2-deoxyhexoses has no effect on its resistance to alkaline conditions, and the presence of the substituent at position 4 considerably enhances the resistance of the oligosaccharide. Substituent at position 3 dramatically decreases the stability of 2-acetamido-2-deoxyhexose facilitating β-elimination of the substituent and formation of so called chromogens[21] along with 3,6-anhydro-2-acetamido-2-deoxyhexoses.[22]

The conditions, which we reported[7] earlier for the synthesis of β-glycopyranosylamines of alkali-labile oligosaccharides with a glucose residue on the reducing end (saturated solution of ammonium carbamate in ~90% aqueous MeOH, ~20 °C, 100 h), were found to be efficient only for disaccharide **4a**. Under these conditions, oligosaccharides **1a**—**3a** were found to decompose, especially oligosaccharides **1a** and **2a**. According to data of electrophoresis analysis, the reaction conditions result in transformation of oligosaccharides **1a**, **2a** or **3a** into one neutral and at least two positively charged products. The neutral product may be a mixture of the starting oligosaccharide and decomposition products which are disaccharide **1a** and 3,6-anhydro-2-acetamido-2-deoxy-D-hexofuranose,[22] together with oligosaccharides **1a**—**3a** and corresponding chromogens.[21] Two positively charged products have mobility, which is close to both β-glycopyranosylamines of di- and trisaccharides (β-glycopyranosylamines of lactose and 2′-fucosyllactose were used as reference compounds) and to β-glycopyranosylamines of monosaccharides (β-galactopyranosylamine was used as a reference compound). Presumably, the former are β-glycopyranosylamines of oligosaccharides **1b**—**3b**, and the latter are β-glycopyranosylamines of fucose or galactose, which are formed as a result of β-elimination of oligosaccharides **1a**—**2a** or trisaccharide **3a**, respectively.

In connection with the obtained data, the research work was carried out aimed at optimization of procedure for preparation of β-glycopyranosylamines, which decreases decomposition of oligosaccharides **1a**—**3a**. It was found, that the use of ammonium bicarbonate[23] instead of ammonium carbamate decreased the formation of decomposition products only to a small extent. Further investigation showed that addition of H_3BO_3 to the saturated aqueous ammonium carbamate decreases the decomposition of oligosaccharides **1a**—**3a**.

It is known,[24] that H_3BO_3 and its salts form negatively charged complexes with sugars. Presumably, formation of these complexes slows down β-elimination of fucose and galactose residues from the position 3 of oligosaccharides **1a**—**3a** (the effect was especially pronounced in case of oligosaccharide **1a**), and results in the increase of yields of β-glycopyranosylamines **1b**—**3b**. β-Glycopyranosylamines **1b**—**4b** were not isolated from the reaction mixtures as pure compounds as they are labile and can easily undergo different transformations. β-Glycopyranosylamines were transformed into stable *N*-acylated derivatives without purification using *N*-Z-glycine *N*-hydroxysuccinimide ester as described in Ref. 7 (see Scheme 2). *N*-Acyl-β-glycopyranosylamines **1c**—**4c** were isolated using chromatography on reversed phase silica gel (for **1c**, **3c**, and **4c**), and for **2c**, additionally, adsorption chromatography on silica gel was used. Compounds **1c**, **2c**, **3c**, and **4c** were obtained with yields 47, 17, 64, and 72%, respectively. It should be mentioned, that although boric acid was added to the saturated aqueous solution of ammonium carbamate, preparation of β-glycosylamine **2b** from 3,6-difu-

Scheme 2



R = OH (**1a**—**4a**), NH_2 (**1b**—**4b**), $NHCOCH_2NHZ$ (**1c**—**4c**), $NHCOCH_2NH_2$ (**1d**—**4d**)
Z = $PhCH_2OCO$

Reagents and conditions: *a*. H_2, Pd/C, 50 °C, EtOH, 48 h; *b*. NH_2COONH_4, H_3BO_3 (for **1b**—**3b**), H_2O or MeOH + H_2O (for **4a**), ~20 °C, 96 h; *c*. $ZNHCH_2COOSu$ (Su — succinimidyl), DMF, H_2O, 0 °C, 3 h; *d*. H_2, Pd/C, MeOH, H_2O, ~20 °C, 7 h.

cosylated GlcNAc **2a** was accompanied by substantial decomposition and the yield of *N*-acylated derivative **2c** is still low.

After removal of protective groups by hydrogenolysis of **1c**—**4c** using the procedure earlier developed by our group,[7] *N*-glycyl derivatives **1d**—**4d** were obtained with yields ~95%.

Structures of the obtained compounds **1a**,**b**,**c**,**d**—**4a**,**b**,**c**,**d**, **7**, **8**, **11**, **13**, **14** were confirmed using the high resolution mass- and ^{1}H and ^{13}C NMR spectrometry (for compounds **2a**,**c**,**d**).

In this way, the procedure suggested[14] by our group earlier, which assumes the use of ammonium carbamate for preparation of β-glycopyranosylamines of mono- and oligosaccharides, after modification (addition of H_3BO_3) can be applied to the synthesis of β-glycopyranosylamines **1b**—**3b** of known alkali-labile oligosaccharides **1a**—**3a** with Fuc or Gal at position 3 of reducing GlcNAc. The developed earlier procedure[5] of *N*-acylation of β-glycopyranosylamines **1b**—**4b** with activated ester of *N*-Z-glycine provided for *N*-acylated compounds **1c**—**4c**. Removal of the protective group afforded *N*-glycyl-β-glycopyranosylamines of four fucooligosaccharides; three of them (**1d**, **2d**, and **4d**) are models of carbohydrate—peptide linking unit in *N*-glycoproteins, and **3d** is an amino spacered determinant of carcinoembryonic antigen Le^a.

Amino-spacered compounds **1d**—**4d** can be used for immobilization on different carriers for different types of ELISA and for affinity chromatography of fucolectins. The primary amino group of glycine provides for formation of conjugates with different physiologically active compounds, (bio)polymers or nanoparticles in mild conditions. Glycoconjugates of this type can be used in different test systems, for example, for investigation of specificity of fucolectins with affinity to core Fuc, one of them is α-L-Fuc*p*-(1→6)-D-GlcNAc-specific lectin from *Aspergillus oryzae*,[25] and also for study of Fuc-specific adhesins of bacteria and viruses.

Fragments of α-1-3-fucosylated *N*-glycans can be used as immunogenic ligands for formulation of synthetic vaccines for treatment of helminthoses.[26] It was shown, that vaccination of sheep with glycoprotein H(11) isolated from nematodes *Haemonchus contortus*, which cause parasitic infections in some domestic animals, mounted immune response to other sheep parasites (see review[27]). Glycoproteins *H. contortus* include considerable amount of core antigen structures α-L-Fuc*p*-(1→3)-D-GlcNAc (**1a**) and α-L-Fuc*p*-(1→3)-[α-L-Fuc*p*-(1→6)]-D-GlcNAc (**2a**), and the immune response caused by the vaccination is basically connected to the presence of these motifs.[28] In this aspect, amino-synthons **1d** and **2d** can be conjugated to a polymer carrier (protein, water-soluble protein, or a nanoparticle) with the view to application as potential candidates for vaccination of mammals and humans against parasitic worms nematodes and trematodes.

Experimental

^{1}H and ^{13}C NMR spectra were recorded in D_2O on a Bruker AM 300 spectrometer (operating frequencies 300.13 and 75.47 MHz) relative to the signals of acetone δ_H 2.225, δ_C 31.45 (internal standard). Only characteristic proton signals are given. High resolution mass spectra were recorded on Bruker micrOTOF II mass spectrometer with electrospray ionization.[15] The mass spectra were registered in the positive- and negative-ion modes (capillary voltage was −4500 and 3200 V, respectively). Experimental *m/z* values are given for the most abundant signals of isotopic clusters together with the corresponding calculated *m/z* values. Solutions of samples in aqueous methanol or in MeOH—H_2O (1 : 1), which were diluted with MeCN or MeCN—H_2O (1 : 1), were introduced by the syringe injection. Optical rotation was measured on polarimeter PU-07 (Russia).

Electrophoresis (10 V cm^{-1}, 1 h) was carried out on a Filtrak FN1 paper in 4% aqueous HCOOH solution, using EFA-1 instrument (USSR). The spots of compounds were visualized with the sequence of reagents KIO_4—$AgNO_3$—KOH (see Ref. 16). Molecular sieves were activated *in vacuo* at ~0.5 Torr, 200 °C, 2 h. Solutions were concentrated at ~10 Torr, bath temperature was ~30 °C. Detection of all compounds during chromatography on Silica gel 100 C_{18} Reversed phase (Fluka) was carried out using UV-absorption at 206 nm.

Benzyl 2-acetamido-3-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-2-deoxy-α-D-glucopyranoside (7). Ethyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside[31] (**5**) (956 mg, 2 mmol), Et_4NBr (1.470 g, 7 mmole), $CuBr_2$ (535 mg, 2.4 mmol) and powdered molecular sieves 3 Å (2.4 g) in the mixture of anhydrous CH_2Cl_2 (20 ml) and DMF (4 ml) were stirred for 4 h at 21 °C. Benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside[32] (**6**) (800 mg, 2 mmol) was added to the suspension, and it was stirred for 64 h at 21 °C. The reaction mixture was diluted with $CHCl_3$ (125 mL), filtered through Celite, and Celite was washed with $CHCl_3$ until filtrate became colorless. The solution was concentrated to ~120 mL and washed with 1 *M* aqueous $Na_2S_2O_3$ until the color diappeared, then washed with H_2O (3×30 mL) and the solvent was removed. The residue was dissolved in benzene and subjected to chromatography on silica gel (benzene → benzene—acetone (7 : 3)). Fractions were concentrated until dryness and analyzed with TLC using benzene—acetone (7 : 3) as an eluent. Fractions with nearly homogenious product were combined to yield 881 mg of benzyl 2-acetamido-4,6-*O*-benzylidene-3-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-2-deoxy-α-D-glucopyranoside which was without further purification dissolved in 6 mL of $CHCl_3$, then 0.9 mL of 90% aqueous TFA was added and the mixture was kept for 30 min at 27 °C. The reaction mixture was diluted with 6 mL of $CHCl_3$, cooled with an ice bath and added 1.8 mL of Et_3N and 20 mL of Pr^{i}OH. The solution was concentrated until dryness. The residue was triturated with petroleum ether, dissolved in 15 mL of $CHCl_3$. The solution was washed with H_2O (5×2 mL), diluted with 10 mL of Pr^{i}OH and concentrated to dryness. The residue was subjected to column chromatography on silica gel using CH_2Cl_2—Et_2O (1 : 1) as an eluent. Fractions with homogeneous product were combined and the solvents were removed to obtain 653 mg of disaccharide **7** (yield 45%) which can be crystallized from MeOH, m.p. 204—205 °C (needles), $[\alpha]_D^{21}$ +26.5 (*c* 1, $CHCl_3$); compared to Ref.[15a] data: m.p. 178—180 °C, $[\alpha]_D^{25}$ +26.4 (*c* 1, $CHCl_3$). MS (positive ions), *m/z*: 728.3415,

750.3235, 766.2973, $C_{42}H_{49}NO_{10}$, calc., : 728.3429 $[M + H]^+$, 750.3249 $[M + Na]^+$, 766.2988 $[M + K]^+$. ^1H NMR, δ ($CDCl_3$): 1.17 (d, 3 H, CH_3, Fuc, J = 6.5 Hz); 1.40 (s, 3 H, NAc); 5.23 (d, 1 H, H(1), GlcNAc, J = 3.3 Hz); 5.99 (d, 1 H, NH, J = 7.6 Hz); 7.16—7.48 (m, 20 H, 4 Ph).

Benzyl 2-acetamido-3,6-di-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-2-deoxy-α-D-glucopyranoside (8) was obtained from disaccharide **7** (291 mg, 0.4 mmol) and thioglycoside **5** (191 mg, 0.4 mmol) as described for disaccharide **7**. Chromatography on silica gel (benzene→ benzene—acetone (8 : 2)) gave 247 mg (54%) of amorphous trisaccharide **8**, $[\alpha]_D^{20}$ −18.8 (*c* 1, $CHCl_3$). MS (positive ions), *m/z*: 1166.5232, $C_{69}H_{77}NO_{14}$, calc. 1166.5236 $[M + Na]^+$. ^1H NMR, δ ($CDCl_3$): 1.04 (d, 3 H, CH_3, Fuc1→3, J = 6.5 Hz); 1.17 (d, 3 H, CH_3, Fuc1→6, J = 6.5 Hz); 1.46 (s, 3 H, NAc); 3.56 (br.s, 1 H, H(4), Fuc1→3); 5.22 (d, 1 H, H(1), GlcNAc, J = 3.0 Hz); 5.99 (d, 1 H, NH, J = 6.6 Hz); 7.19—7.46 (m, 35 H, 7 Ph).

Benzyl 2-acetamido-6-*O*-benzyl-4-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-3-*O*-(β-D-galactopyranosyl)-2-deoxy-α-D-glucopyranoside (11) was obtained from benzyl 2-acetamido-6-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-deoxy-α-D-glucopyranoside[16a] (**9**) (292.5 mg, 0.4 mmol) and ethyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside[31] (**5**) (191 mg, 0.4 mmol) as described for disaccharide **7**. After chromatography on silica gel (ethyl acetate—hexane (1 : 1) → ethyl acetate—hexane (3 : 1)) 238.6 mg (52%) of benzyl 2-acetamido-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside[16a] (**10**) was obtained, which was dissolved in 4 mL of anhydrous MeOH and 0.4 mL of 1 *M* MeONa in anhydrous MeOH was added. The solution was kept for 5 h at 20 °C. Then the solution was diluted with 4 mL of MeOH, 75 mg of Amberlyst 15 (H^+) was added, the reaction mixture was stirred for 1 h, the resin was filtered off and washed with MeOH. The solution was concentrated to dryness to obtain 170 mg (82%) of trisaccharide **11**, which is crystallized from EtOH, m.p. 189—191 °C, $[\alpha]_D^{26}$ −14.3 (*c* 1, $CHCl_3$); compare to Ref. 16a: m.p. 180—181 °C from the mixture ethyl acetate—ether, $[\alpha]_D$ −15 (*c* 1, $CHCl_3$). MS (positive ions), *m/z*: 980.4421, 1002.4251, 1018.3987, $C_{55}H_{65}NO_{15}$, calc. 980.4427 $[M + H]^+$; 1002.4246 $[M + Na]^+$; 1018.3986 $[M + K]^+$. MS (negative ions), *m/z*: 978.4261. $C_{55}H_{65}NO_{15}$, calc. 978.4281 $[M - H]^-$. ^1H NMR, δ ($CDCl_3$): 1.12 (d, 3 H, CH_3, Fuc, J = 6.5 Hz); 1.95 (s, 3 H, NAc); 5.09 (d, 1 H, H(1), GlcNAc, J = 3.2 Hz); 5.84 (d, 1 H, NH, J = 7.7 Hz); 7.20—7.46 (m, 25 H, 5 Ph).

Benzyl 2-acetamido-3,4-di-*O*-acetyl-6-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-2-deoxy-α-D-glucopyranoside (13) was obtained from benzyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside[17b] (**12**) (158 mg, 0.4 mmol) and thioglycoside **5** (191 mg, 0.4 mmol) as desribed for disaccharide **7**. Chromatography on silica gel (benzene→ benzene—acetone (8 : 2)) gave 175 mg (54%) of amorphous disaccharide **6**, $[\alpha]_D^{21}$ +24.1 (*c* 1, $CHCl_3$). MS (positive ions), *m/z*: 834.3473, 850.3211, $C_{46}H_{53}NO_{12}$, calc., 834.3460 $[M + Na]^+$; 850.3199 $[M + K]^+$. ^1H NMR, δ ($CDCl_3$): 1.17 (d, 3 H, CH_3, Fuc, J = 6.5 Hz); 1.95 (s, 3 H, NAc); 2.03 (s, 3 H, Ac); 2.07 (s, 3 H, Ac); 5.70 (d, 1 H, NH, J = 9.4 Hz); 7.20—7.40 (m, 20 H, 4 Ph).

Benzyl 2-acetamido-6-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-2-deoxy-α-D-glucopyranoside (14). Disaccharide **13** (162 mg, 0.2 mmol) was dissolved in 4 mL of anhydrous MeOH and 0.4 mL of 1 *M* MeONa in anhydrous MeOH was added, and the solution was kept for 5 h at 20 °C. Then the solution was diluted with 4 mL of MeOH, 75 mg of Amberlyst 15 (H^+) was added, the reaction mixture was stirred for 1 h, the resin was filtered off and washed with MeOH. The solution was concentrated to dryness. The residue was subjected to column chromatography on silica gel in CH_2Cl_2→ CH_2Cl_2—Et_2O (1 : 1). Fraction with homogeneous product were combined and concentrated to dryness to obtain 113 mg (78%) of amorphous disaccharide **14**, $[\alpha]_D^{21}$ +6.5 (*c* 1, $CHCl_3$). MS (positive ions), *m/z*: 728.3441, 750.3254, 766.2994, $C_{42}H_{49}NO_{10}$, calc., 728.3429 $[M + H]^+$; 750.3249 $[M + Na]^+$; 766.2988 $[M + K]^+$. ^1H NMR, δ ($CDCl_3$): 1.13 (d, 3 H, CH_3, Fuc, J = 6.4 Hz); 2.00 (s, 3 H, NAc); 5.78 (d, 1 H, NH, J = 8.2 Hz); 7.23—7.45 (m, 20 H, 4 Ph).

Hydrogenolysis of 7, 8, 11 и 14 (general procedure). The suspension of 10% Pd/C (75 mg, the catalyst) in 1.5 mL of EtOH was stirred under H_2 (30 min at ~50 °C). The solution of compounds **7**, **8**, **11** or **14** (150 mg) in 12 mL of EtOH was heated to ~50 °C and added to the suspension at ~50 °C, and hydrogenation was performed upon stirring under a weak flow of H_2 for 24 h at ~50 °C. Then 1.5 mL of H_2O and 75 mg of 10% Pd/C was added to the suspension, and the hydrogenation was conducted for another 24 h at ~50 °C. The reaction mixture was diluted with 8 mL of 50% aqueous MeOH, the catalyst was removed by centrifugation, washed with 50% aqueous MeOH and the solution was concentrated to dryness. The residue was dissolved in 1 mL of H_2O and applied onto the C_{18}-reversed phase silica gel column (0.6×4.5 см). The product was eluted with 30 mL of H_2O, the solution was concentrated and dried. Oligosaccharides **1a**—**4a** were obtained with yield ~95%.

2-Acetamido-2-deoxy-3-*O*-(α-L-fucopyranosyl)-D-glucose (1a), m.p. 218—220 °C from EtOH, $[\alpha]_D^{22}$ −84.9 (after 15 min) → −102.2 (after 24 h) (*c* 1, H_2O); compare with Ref.: m.p. 218—220 °C from EtOH—MeOH—H_2O, $[\alpha]_D^{20}$ −60 → −74 (*c* 0.83, H_2O)[15a]; $[\alpha]_D^{22}$ −66.6[15b]. MS (positive ions), *m/z*: 368.1554, 390.1372, 406.1001, $C_{14}H_{25}NO_{10}$, calc., 368.1551 $[M + H]^+$; 390.1371 $[M + Na]^+$; 406.1110 $[M + K]^+$. ^1H NMR, δ 1.18 (d, 3 H, CH_3, Fuc, J = 6.5 Hz); 2.04 (s, 3 H, NAc); 4.35 (m, 1 H, H(5), Fuc); 4.73 (d, H(1), β-GlcNAc, J = 8.3 Hz); 5.00 and 5.03 (both d, H(1), Fuc, J = 3.9 Hz); 5.15 (d, H(1), α-GlcNAc, J = 3.4 Hz).

2-Acetamido-2-deoxy-3,6-di-*O*-(α-L-fucopyranosyl)-D-glucose (2a), $[\alpha]_D^{21}$ −93.4 (*c* 1, H_2O). MS (positive ions), *m/z*: 536.1941, $C_{20}H_{35}NO_{14}$, calc., 536.1950 $[M + Na]^+$. ^1H NMR ,δ: 1.17 (m, 3 H, CH_3, Fuc1→3); 1.22 (m, 3 H, CH_3, Fuc1→6); 2.02 (s, 3 H, NAc); 4.12 (m, 1 H, H(5), Fuc1→6); 4.33 (m, 1 H, H(5), Fuc1→ 3); 4.73 (d, H(1), β-GlcNAc, J = 8.2 Hz); 4.92 (m, 1 H, H(1), Fuc1→6); 4.98, 5.02 (both d, H(1), Fuc1→3); 5.14 (d, H(1), α-GlcNAc, J = 3.4 Hz). ^{13}C NMR, δ: 16.49 (2 C(6), 2 Fuc); 23.23 (CH_3CO, α-GlcNAc); 23.48 (CH_3CO, β-GlcNAc); 54.84 (C(2), α-GlcNAc); 57.63 (C(2), β-GlcNAc); 67.97 (C(5), Fuc1→6); 68.14 (C(5), Fuc1→3); 68.59 (C(6), β-GlcNAc); 68.97(C(6), α-GlcNAc); 69.24 (C(2), Fuc1→3); 69.47 (C(2), Fuc1→6); 69.89 (C(4), GlcNAc); 70.79 (2 C(3), 2 Fuc); 72.25 (C(5), α-GlcNAc); 73.08 (2 C(4), 2 Fuc); 76.24 (C(5), β-GlcNAc); 79.27 (C(3), α-GlcNAc); 81.75 (C(3), β-GlcNAc); 92.29 (C(1), α-GlcNAc); 96.01 (C(1), β-GlcNAc); 100.32, 100.67 (C(1), Fuc1→6); 101.01, 101.24 (C(1), Fuc1→3); 175.75 (C=O); 176.02 (C=O).

2-Acetamido-2-deoxy-4-*O*-(α-L-fucopyranosyl)-3-*O*-(β-D-galactopyranosyl)-D-glucose (3a), m.p. 168—170 °C from EtOH, $[\alpha]_D^{21}$ −48.2 (after 15 min) → −53.9 (after 24 h) (*c* 1, H_2O); compare to Ref.: $[\alpha]_D$ −44.5 (*c* 1, H_2O)[16a,c]; $[\alpha]_D^{25}$ −45.1

(*c* 1, H_2O)[16b]. MS (positive ions), *m/z*: 552.1890, $C_{20}H_{35}NO_{15}$, calc., 552.1899 $[M + Na]^+$. ^1H NMR, δ: 1.18 (d, 3 H, CH_3, Fuc, J = 6.5 Hz); 2.02 (s, 3 H, NAc); 4.47 and 4.50 (both d, H(1), Gal, J = 7.5 Hz); 4.71 (d, H(1), β-GlcNAc, J = 8.2 Hz); 4.88 (m, 1 H, H(5), Fuc); 5.02 (m, 1 H, H(1), Fuc); 5.12 (d, H(1), α-GlcNAc, J = 3.2 Hz).

2-Acetamido-2-deoxy-6-*O*-(α-L-fucopyranosyl)-D-glucose (4a), $[\alpha]_D^{22}$ −61.2 (*c* 1, H_2O); compare with Ref.: $[\alpha]_D^{25}$ −65 (*c* 1.05, H_2O)[17a,b]; $[\alpha]_D$ −61.2 → −63.2 (*c* 1, H_2O)[17c]. MS (positive ions), *m/z*: 368.1550, 390.1367, 406.1088, $C_{14}H_{25}NO_{10}$, calc., 368.1551 $[M + H]^+$; 390.1371 $[M + Na]^+$; 406.1110 $[M + K]^+$. ^1H NMR, δ : 1.21 (d, 3 H, CH_3, Fuc, J = 6.5 Hz); 2.02 (s, 3 H, NAc); 4.11 (m, 1 H, H(5), Fuc); 4.71 (d, H(1), β-GlcNAc, J = 8.4 Hz); 4.92 (m, 1 H, H(1), Fuc); 5.19 (d, H(1), α-GlcNAc, J = 3.4 Hz).

β-Glycopyranosylamines 1b—3b. Oligosaccharide **1a**, **2a** or **3a** (0.1 mmol) was dissolved in 1 mL of H_2O, boric acid was added (93 mg, 1.5 mmol) and powdered ammonium carbamate (468 mg, 6 mmol), stirred (~15 min) and kept for 4 days at ~20 °C. The residue was filtered off, washed with MeOH and the solution was concentrated to ~0.3 mL at ~40 Torr. MeOH (7mL) was added to the residue and the solution was concentrated ~0.3 mL at ~40 Torr. This procedure was repeated several times until total removal of ammonium carbamate, which was detected with a pH paper placed over the solution of the compound in methanol, andfinally concentrated to dryness at ~10 Torr. The residues were dried and the obtained β-glycopyranosylamines **1b—3b** were *N*-acylated without further purification or storage.

β-Glycopyranosylamine 4b. Oligosaccharide **4a** (36.7 mg, 0.1 mmol) was dissolved in 0.12 mL of H_2O, the 0.88 mL of MeOH and powdered ammonium carbamate (188 mg, 2.4 mmol) were added, the reaction mixture was stirred for ~15 min and kept for 4 days at ~20 °C. The reaction mixture was diluted with 7 mL of MeOH, the solution was concentrated and the excess of ammonium carbamate was removed with MeOH as described earlier. The residue was dried to obtain β-glycopyranosylamine **4b** (36 mg) contaminated with (up to 10%) of starting oligosaccharide **4a** (as estimated by the visual analysis of the intensity of spots on the electropherogram).

***N*-(*N*-Z-Glycyl)-β-glycopyranosylamines 1c—4c.** β-Glycopyranosylamines **1b—4b**, obtained from 0.1 mmol of oligosaccharides **1a—4a**, without further purification were dissovled in 0.15 mL of H_2O, cooled with ice, 0.85 mL of DMF and 112 mg (0.3 mmol) of *N*-Z-glycine *N*-hydroxysuccinimide ester were added and stirred for 3 h at ~0 °C. The reaction mixture was diluted with 10 mL of MeOH, concentrated to ~1 mL, and this procedure was repeated 2 times. To the stirred residue (~0.8 mL) 20 mL of Et_2O was added. After the liquid became clear, it was decanted from the oily residue. The residue was several times washed with Et_2O (5 mL), and the mixture Et_2O—acetone (1 : 1), and dried. The residue was dissolved in 1 mL of H_2O and subjected to column chromatography on C_{18}-reversed phase silica gel in H_2O in proportion 10 mg of the compound to 1 g (~3 mL) of the phase. The elution was proceeded until the absence of UV-absorption, and then the product was eluted with 25% aqueous MeOH. The water—methanol fractions, which contained compounds **1c—4c**, were combined and concentrated until dryness. The residue was dissolved in 3 mL of 10% Et_3N in 50% aqueous MeOH and kept for 3 h at ~20 °C. The reaction mixture was diluted with 10 mL of MeOH, concentrated to dryness, and this procedure was repeated twice. The residue was dissolved in H_2O subjected to column chromatography on C_{18}-reversed phase silica gel in the described conditions. The methanol—water fractions, which contained homogeneous compound (TLC, EtOAc—MeOH (2.5 : 1)), were combined and concentrated to dryness. The residue was dried to yield 26 mg (47%) of compound **1c**, 46 mg (64%) of compound **3c**, 40.2 mg (72%) of compound **4c**. Compound **2c** (16 mg) was additionally chromatographed on silica gel (acetone—MeOH (9 : 1) → acetone—MeOH (4 : 1)) to obtain 12 mg (17%) of compound **2c**.

2-Acetamido-*N*-(*N*-benzyloxycarbonylglycyl)-2-deoxy-3-*O*-(α-L-fucopyranosyl)-β-D-glucopyranosylamine (1c), $[\alpha]_D^{21}$ −53.8 (*c* 1, H_2O). MS (positive ions), *m/z*: 558.2284, 580.2103, 596.1844, $C_{24}H_{35}N_3O_{12}$, calc. 558.2294 $[M + H]^+$; 580.2113 $[M + Na]^+$; 596.1852 $[M + K]^+$. ^1H NMR, δ: 1.18 (d, 3 H, CH_3, Fuc, J = 6.5 Hz); 2.00 (s, 3 H, NAc); 4.35 (qd, 1 H, H(5), Fuc, J = 6.5 Hz); 5.03 (d, 1 H, H(1), Fuc, J = 3.8 Hz); 5.10—5.26 (m, 3 H, 1 H, H(1), GlcNAc, 2 H, CH_2Ph); 7.35—7.48 (m, 5 H, Ph).

2-Acetamido-*N*-(*N*-benzyloxycarbonylglycyl)-2-deoxy-3,6-di-*O*-(α-L-fucopyranosyl)-β-D-glucopyranosylamine (2c), $[\alpha]_D^{22}$−78.6 (*c* 1, H_2O). MS (positive ions), *m/z*: 726.2685, $C_{30}H_{45}N_3O_{16}$, calc.: 726.2692 $[M + Na]^+$. MS (negative ions), *m/z*: 702.2715, $C_{30}H_{45}N_3O_{16}$, calc.: 702.2727 $[M - H]^-$. ^1H NMR, δ: 1.18 (d, 3 H, CH_3, Fuc1→3, J = 6.6 Hz); 1.21 (d, 3 H, CH_3, Fuc1→6, J = 6.6 Hz); 2.00 (s, 3 H, NAc); 4.12 (qd, 1 H, H(5), Fuc1→6, J = 6.6 Hz); 4.34 (qd, 1 H, H(5), Fuc1→ 3, J = 6.6 Hz); 4.91 (d, 1 H, H(1), Fuc1→6, J = 3.6 Hz); 5.02 (d, 1 H, H(1), Fuc1→3, J = 3.6 Hz); 5.09 (d, 1 H, H(1), GlcNAc, J = 10.1 Hz); 5.11, 5.17 (AB-system, 2 H, CH_2Ph, J = 12.6 Hz); 7.35—7.48 (m, 5 H, Ph). ^{13}C NMR, δ: 16.41 (C(6), Fuc1→3); 16.54 (C(6), Fuc1→6); 23.31 (CH_3CO); 44.98 (CH_2N); 54.99 (C(2), GlcNAc); 67.98 (C(5), Fuc1→6); 68.18 (C(5), Fuc1→3); 68.58 (CH_2Ph); 68.72 (C(6), GlcNAc); 69.18 (C(2), Fuc1→3); 69.43 (C(2), Fuc1→6); 69.70 (C(4), GlcNAc); 70.74 (2 C(3), 2 Fuc); 73.07 (2 C(4), 2 Fuc); 78.05 (C(5), GlcNAc); 79.66 (C(1), GlcNAc); 81.85 (C(3), GlcNAc); 100.45 (C(1), Fuc1→6); 101.21 (C(1), Fuc1→3); 129.03; 129.68; 130.03 (6 C, C_6H_5); 174.21 (C=O); 175.91 (C=O).

2-Acetamido-*N*-(*N*-benzyloxycarbonylglycyl)-2-deoxy-4-*O*-(α-L-fucopyranosyl)-3-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosylamine (3c), $[\alpha]_D^{21}$ −40.6 (*c* 1, H_2O). MS (positive ions), *m/z*: 720.2822, 742.2634, 758.2351, $C_{30}H_{45}N_3O_{17}$, calc. 720.2822 $[M + H]^+$; 742.2641 $[M + Na]^+$; 758.2381 $[M + K]^+$. MS (negative ions), *m/z*: 718.2675, $C_{30}H_{45}N_3O_{17}$, calc. *m/z*: 718.2676 $[M - H]^-$. ^1H NMR, δ: 1.18 (d, 3 H, CH_3, Fuc, J = 6.5 Hz); 2.00 (s, 3 H, NAc); 4.50 (d, 1 H, H(1), Gal, J = 7.6 Hz); 4.88 (qd, 1 H, H(5), Fuc, J = 6.5 Hz); 5.04 (d, 1 H, H(1), Fuc, J = 3.6 Hz); 5.11 m, 1 H, 1(H), GlcNAc); 5.12, 5.17 (AB-system, 2 H, CH_2Ph, J = 12.5 Hz); 7.33—7.49 (m, 5 H, Ph).

2-Acetamido-*N*-(*N*-benzyloxycarbonylglycyl)-2-deoxy-6-*O*-(α-L-fucopyranosyl)-β-D-glucopyranosylamine (4c), $[\alpha]_D^{21}$ −28.3 (*c* 1, H_2O). MS (positive ions), *m/z*: 558.2302, 580.2116, 596.1853, $C_{24}H_{35}N_3O_{12}$, calc. 558.2294 $[M + H]^+$; 580.2113 $[M + Na]^+$; 596.1852 $[M + K]^+$. MS (negative ions), *m/z*: 556.2160. $C_{24}H_{35}N_3O_{12}$, calc. 556.2148 $[M - H]^-$. ^1H NMR, δ : 1.19 (d, 3 H, CH_3, Fuc, J = 6.3 Hz); 2.00 (s, 3 H, NAc); 4.10 (m, 1 H, H(5), Fuc); 4.89 (d, 1 H, H(1), Fuc, J = 3.5 Hz); 5.06 (d, 1 H, H(1), GlcNAc, J = 9.8 Hz); 5.09, 5.15 (AB-csystem, 2 H, CH_2Ph, J = 12.6 Hz); 7.30—7.48 (m, 5 H, Ph).

***N*-Glycyl-β-glycopyranosylamines 1d—4d.** Compounds **1c**, **3c**, **4c** (0.03 mmol) and compound **2c** (0.01 mmol) were dissolved in 2.5 mL (0.8 mL for **2c**) of 25% aqueous MeOH, then 10% Pd/C (half the weight of compounds **1c—4c**) under Ar and

Table 1. ^{1}H NMR data (characteristic signals) for compound **1d**—**4d**

Residue	Proton	δ (*J*/Гц)			
		1d	**2d**	**3d**	**4d**
GlcNAc	H(1)	5.13 (*J* = 9.8)	5.13 (*J* = 10.0)	5.13 (*J* = 9.6)	5.09 (*J* = 9.7)
	NAc	2.00	2.00	2.00	2.00
Gal	H(1)	—	—	4.50 (*J* = 7.6)	—
Gly	CH_2N	3.41	3.41	3.41	3.43
Fuc(1→3)	H(1)	5.03 (*J* = 3.8)	5.01 (*J* = 3.8)	—	—
	H(5)	4.37 (*J* = 6.5)	4.33 (*J* = 6.6)	—	—
	CH_3	1.18 (*J* = 6.5)	1.17 (*J* = 6.6)	—	—
Fuc(1→6)	H(1)	—	4.91 (*J* = 3.8)	—	4.90 (*J* = 3.5)
	H(5)	—	4.12 (*J* = 6.6)	—	4.11 (*J* = 6.3)
	CH_3	—	1.22 (*J* = 6.6)	—	1.20 (*J* = 6.3)
Fuc(1→4)	H(1)	—	—	5.04 (*J* = 3.6)	—
	H(5)	—	—	4.88	—
	CH_3	—	—	1.18 (*J* = 6.5)	—

the hydration was performed upon stirring under a weak flow of H_2 for 7 h at ~20 °C. The reaction mixture was diluted with 25% aqueous MeOH, the catalyst was removed by centrifugation, washed with 25% aqueous MeOH and the solution was concentrated to dryness. The residue was dissolved in 1 mL of H_2O and applied onto the C_{18}-reversed phase silica gel column (0.6×3 cm). The product was eluted with 20 mL of H_2O, the solution was concentrated and dried. Oligosaccharides **1d**—**4d** were obtained with yield 95% as amorphous powder.

2-Acetamido-2-deoxy-3-*O*-(α-L-fucopyranosyl)-*N*-glycyl-β-D-glucopyranosylamine (1d), $[\alpha]_D^{22}$ −75.1 (*c* 1, H_2O). MS (positive ions), *m/z*: 424.1907, 446.1731, 462.1471, $C_{16}H_{29}N_3O_{10}$, calc. *m/z*: 424.1926 $[M + H]^+$; 446.1745 $[M + Na]^+$; 462.1485 $[M + K]^+$. MS (negative ions), *m/z*: 422.1793, $C_{16}H_{29}N_3O_{10}$, calc. 422.1780 $[M - H]^-$.

2-Acetamido-2-deoxy-3,6-di-*O*-(α-L-fucopyranosyl)-*N*-glycyl-β-D-glucopyranosylamine (2d), $[\alpha]_D^{21}$ −107.0 (*c* 1, H_2O). MS (positive ions), *m/z*: 570.2508, 592.2328, 608.2062, $C_{22}H_{39}N_3O_{14}$, calc. 570.2505 $[M + H]^+$; 592.2324 $[M + Na]^+$; 608.2064 $[M + K]^+$. MS (negative ions), *m/z*: 568.2345, $C_{22}H_{39}N_3O_{14}$, calc. 568.2359 $[M - H]^-$. ^{13}C NMR, δ: 16.41 (C(6), Fuc1→3); 16.54 (C(6), Fuc1→6); 23.28 (CH_3CO); 44.39 (CH_2N); 55.14 (C(2), GlcNAc); 67.99 (C(5), Fuc1→6); 68.19 (C(5), Fuc1→3); 68.67 (C(6), GlcNAc); 69.17 (C(2), Fuc1→3); 69.42 (C(2), Fuc1→6); 69.68 (C(4), GlcNAc); 70.69 (2 C(3), 2 Fuc); 73.07 (2 C(4), 2 Fuc); 78.06 (C(5), GlcNAc); 79.50 (C(1), GlcNAc); 81.92 (C(3), GlcNAc); 100.39 (C(1), Fuc1→6); 101.24 (C(1), Fuc1→3); 176.00 (C=O); 176.18 (C=O).

2-Acetamido-2-deoxy-4-*O*-(α-L-fucopyranosyl)-3-*O*-(β-D-galactopyranosyl)-*N*-glycyl-β-D-glucopyranosylamine (3d), $[\alpha]_D^{23}$ −62.5 (*c* 1, H_2O). MS (positive ions), *m/z*: 586.2459, 608.2278, 624.1996, $C_{22}H_{39}N_3O_{15}$, calc. *m/z*: 586.2454 $[M + H]^+$; 608.2273 $[M + Na]^+$; 624.2013 $[M + K]^+$. MS (negative ions), *m/z*: 584.2312, $C_{22}H_{39}N_3O_{15}$, calc. *m/z*: 584.2308 $[M - H]^-$.

2-Acetamido-2-deoxy-6-*O*-(α-L-fucopyranosyl)-*N*-glycyl-β-D-glucopyranosylamine (4d), $[\alpha]_D^{20}$ −58.0 (*c* 1, H_2O). MS (positive ions), *m/z*: 424.1926, 446.1745, 462.1481, $C_{16}H_{29}N_3O_{10}$, calc. 424.1926 $[M + H]^+$; 446.1745 $[M + Na]^+$; 462.1485 $[M + K]^+$. MS (negative ions), *m/z*: 422.1787, $C_{16}H_{29}N_3O_{10}$, calc. 422.1780 $[M - H]^-$.

^{1}H NMR data for compounds **1d**—**4d** is given in Table 1.

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