Synthesis of *N*-glycyl-β-glycopyranosylamines, derivatives of main human secreted oligosaccharide core structures*

L. M. Likhosherstov,[a]★ O. S. Novikova,[a] N. G. Kolotyrkina,[a] and V. E. Piskarev[b]

[a]*N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 47 Leninsky prosp., 119991 Moscow, Russian Federation. Fax: +7 (495) 135 5328. E-mail: likhosherstov@mail.ru*
[b]*A. N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, 28 ul. Vavilova, 119991 Moscow, Russian Federation*

β-Glycopyranosylamines were synthesized by the reaction of ammonium carbamate with di-, tetra-, and hexasaccharides, corresponding to the human milk and urine oligosaccharide core structures, in aqueous methanolic solution in the presence of NH_3. The *N*-acylation of these β-glycopyranosylamines with *N*-Boc-glycine *N*-hydroxysuccinimide ester followed by removal of the Boc group afforded *N*-glycyl-β-glycopyranosylamines of the corresponding oligosaccharides in yields of up to 60%.

Key words: oligosaccharides, human milk, human urine, β-glycopyranosylamines, *N*-acylation, *N*-glycyl-β-glycopyranosylamines.

Secreted oligosaccharides from human milk and urine include a number of core oligosaccharide structures. Human milk oligosaccharides[1] and most urine oligosaccharides[2—4] contain the lactose residue (-β-D-Gal*p*-(1→4)-D-Glc) at the reducing end; in some urine oligosaccharides,[5] this residue is replaced by *N*-acetyllactosamine (-β-D-Gal*p*-(1→4)-D-GlcNAc). Lactose and *N*-acetyllactosamine are building blocks of human milk and urine oligosaccharide core structures. The biosynthesis of more complex, both linear and branched, core oligosaccharides is accomplished *via* the addition of *N*-acetyllactosamine or lacto-*N*-biose I (β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc) residues to hydroxy groups at the C(3) atom or at the C(3) and C(6) atoms of a galactose residue in lactose.[6]

Apart from lactose, milk oligosaccharides comprise the following 12 core structures: two tetraoses (lacto-*N*-tetraose (LNT) and lacto-*N*-*neo*tetraose (LN*n*T)), four hexaoses (lacto-*N*-hexaose (LNH), lacto-*N*-*neo*hexaose (LN*n*H), *para*-lacto-*N*-hexaose (*p*LNH), and *para*-lacto-*N*-*neo*hexaose (*p*LN*n*H)), four octaoses (lacto-*N*-octaose (LNO), lacto-*N*-*neo*octaose (LN*n*O), *iso*-lacto-*N*-octaose (*i*LNO), and *para*-lacto-*N*-octaose (*p*LNO)), and two decaoses (lacto-*N*-decaose (LND) and lacto-*N*-*neo*decaose (LN*n*D)[7]); urine oligosaccharides include five core structures corresponding to lactose, *N*-acetyllactosamine, LNT, LNnT, and LNnH.[2—4] Complex milk and urine oligosaccharides are generated by fucosylation and/or sialylation of these core structures at hydroxy groups in different positions of monosaccharide residues, resulting in the formation of a mixture of hundreds of oligosaccharides with a molecular weight from 488 to 4000 Da;[8] however, just over a hundred such structures were characterized.[7] A certain number of nonglycosylated oligosaccharides corresponding to human milk or urine oligosaccharide core structures remain unmodified. Thus, oligosaccharide LNT is one of the major components of breast milk, with its content varying from 500 to 1000 mg L^{-1}.[9] A high LNT concentration in milk is due to its important biological function as one of main sources of lacto-*N*-biose I, which is liberated from LNT[10] and serves as a growth substrate for infant-associated bifidobacteria.[11] The contents of other nonglycosylated oligosaccharides (LN*n*T, LNH, LN*n*H, *i*LNO, and LND) corresponding to core structures are much lower.[12—14] The contents of more complex oligosaccharides are insignificant.[15]

Previously, we have developed an efficient method for the synthesis of mono- and oligosaccharide β-glycosylamines[16] using ammonium carbamate and prepared *N*-glycyl-β-glycopyranosylamines of fucosylated[17] and sialylated[18] human milk oligosaccharides. Microchips, sorbents, and nanoparticles based on these oligosaccharides were used to study carbohydrate specificity of lectins[19] and norovirus[20] and the bacteriostatic effect on a number of microorganisms.[21]

The goal of this work is to study amino-spacered oligosaccharide derivatives corresponding to main core structures found in human secreted oligosaccharides. An analysis of published data[6,7] shows that more than 90% (by weight) human milk oligosaccharides are glycosylation

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products of only five core structures: lactose (**1a**); LNT (β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc, **2a**); LN*n*T (β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc, **3a**); LNH (β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→3)-[β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→6)]-β-D-Gal*p*-(1→4)-D-Glc, **4a**); and LN*n*H (β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-[β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→6)]-β-D-Gal*p*-(1→4)-D-Glc, **5a**). Therefore, we used these oligosaccharides and *N*-acetyllactosamine (**6a**), which is an essential component of urine oligosaccharides, in the present work. It should be noted that lactose (**1a**), LNT (**2a**), and LN*n*T (**3a**) are also present in urine as nonglycosylated oligosaccharides.[2]

As described previously,[16] the treatment of oligosaccharides **1a**—**6a** with ammonium carbamate in aqueous MeOH in the presence of NH_3 gave the corresponding β-glycosylamines **1b**—**6b** containing up to 10% of the starting oligosaccharides as impurities. Since glycosylamines **1b**—**6b** are labile, they were not isolated in the individual state but were immediately *N*-acylated with *N*-Boc-glycine *N*-hydroxysuccinimide ester in aqueous DMF (Scheme 1). Taking into account the fact that glycopyranosylamines and the activated ester are gradually hydrolyzed in the presence of water, we performed the reaction using a threefold excess of the ester. After the removal of water, the reaction mixtures were diluted with Et_2O. The precipitates that formed were washed with an Et_2O—acetone mixture (1 : 1) and acetone and then chromatographed on octadecylsilane-modified silica gel (C_{18} silica gel). Impurities of starting oligosaccharides **1a**—**6a** were eluted with water, and *N*-acylated products **1c**—**6c** were eluted with aqueous MeOH. Products **1c**—**6c** were obtained in 60—65% yields and contained a small amount of *O*-acyl groups. The latter groups were removed by the treatment of the products with Et_3N in aqueous MeOH followed by C_{18} silica gel chromatography as described above. The purity of *N*-acylated glycosylamines **1c**—**6c** was checked by high-resolution mass spectrometry.

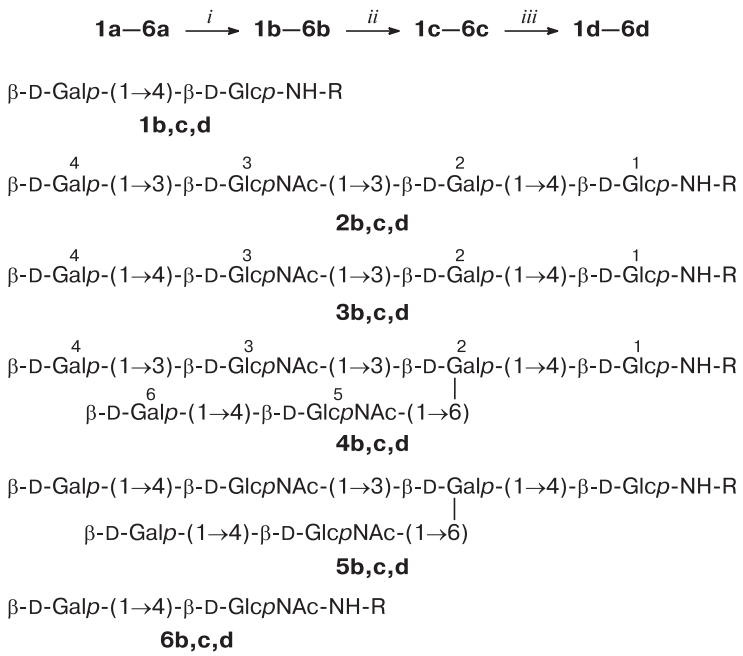
Boc groups in compounds **1c**—**6c** were removed using TFA followed by the treatment with an anion-exchange resin (OH^- form) as described previously[17] to prepare *N*-glycyl-β-glycopyranosylamines **1d**—**6d** in yields of up to 95%.

It should be noted that *N*-Boc-glycine *N*-hydroxysuccinimide ester in aqueous DMF is a more convenient reagent for *N*-acylation of glycosylamines compared to chloroacetic anhydride in anhydrous DMF[22] because it is more stable in aqueous organic solutions, where all oligosaccharide glycopyranosylamines are soluble.

The structures of compounds **1d**—**6d** were confirmed by ^1H NMR spectroscopy and high-resolution mass spectrometry.

Therefore, the method, which was developed previously for the synthesis of *N*-glycyl-β-glycopyranosylamines of

Scheme 1



R = H (**b**), $COCH_2NHBoc$ (**c**), $COCH_2NH_2$ (**d**)

Reagents and conditions: *i.* NH_2COONH_4—NH_3, MeOH—H_2O (2 : 1), 37 °C, 24 h; *ii.* 1) $BocNHCH_2COOSu$ (Su is succinimidyl), DMF—H_2O, 0 °C, 3 h, 2) 10% Et_3N in MeOH—H_2O (1 : 1), 20 °C, 3 h; *iii.* 1) TFA, ~3 min, 2) Dowex 1×8 (OH^-).

fucose-containing human milk oligosaccharides using *N*-Boc-glycine *N*-hydroxysuccinimide ester,[17] was extended to the synthesis of derivatives of a number of human milk and urine di-, tetra-, and hexasaccharides.

The presence of a primary amino group in compounds **1d—6d** makes it possible to perform the reaction with different carboxy- and aldehyde-containing compounds, immobilize them for use in affinity chromatography, and prepare neoglycoconjugates of polymers, proteins, and nanoparticles. These compounds may find use in various applications in glycobiology. Due to the presence of terminal D-galactopyranose residues with β-1→3 and β-1→4 linkages, compounds **1d—6d** can be employed to study fine specificity of various β-galactosidases from bifido- and lactobacteria. They are suitable acceptors for the assessment of activity of glycosyltransferases, in particular, α-sialyl-, α-fucosyl-, β-*N*-acetylglucosaminyl-, and α-*N*-acetylglucosaminyltransferases. Due to the presence of terminal β-D-galactopyranose residues, including cluster ones, these compounds can be employed in different test systems as mono- and divalent ligands for galactose-specific lectins, including galectins, an also as receptors for pathogenic bacteria and viruses.

Experimental

The ^{1}H NMR spectra were recorded in D_2O on a Bruker DRX 500 spectrometer (operating at 500.13 MHz) relative to the signals of acetone (δ_H 2.225) as the internal standard. High-resolution electrospray ionization mass spectra were obtained on a Bruker micrOTOF II spectrometer.[23] The *m/z* values of the most intense experimental peaks of isotopic clusters and the corresponding calculated *m/z* values are given. Solutions of the compounds in MeOH—H_2O mixture (1 : 1) were introduced by syringe injection. The optical rotation was measured on a Jasco P-2000 polarimeter (Japan). Lacto-*N*-hexaose (LNH) **4a** and lacto-*N*-*neo*hexaose (LN*n*H) **5a** were isolated previously[24] by gel permeation chromatography and HPLC. Lacto-*N*-tetraose (LNT) **2a** and lacto-*N*-*neo*tetraose (LN*n*T) **3a** were prepared in a similar way from the fraction F4 using reversed-phase HPLC. Chromatography was performed with a Gilson HPLC isocratic system (France) on 10×250 mm Kromasil NH_2 and Kromasil C_{18} columns (AkzoNobel, the Netherlands) in 60% aqueous MeCN and in H_2O, respectively. The elution of compounds **1c—6c** was monitored by UV absorbance at 210 nm on an Ultrospec 4050 spectrophotometer (LKB). The mixtures were concentrated at ~10 Torr, the bath temperature was ~30 °C. Lactose **1a**, *N*-acetyllactosamine **6a**, and other reagents purchased from Sigma were used.

β-Glycopyranosylamines 1b—6b (general procedure). A ~25% aqueous NH_3 solution (0.3 mL) and MeOH (0.6 mL) were added to a mixture of oligosaccharide **1a—6a** (0.03 mmol) and finely powdered ammonium carbamate (47 mg). The reaction mixture was kept for 24 h at 37 °C, diluted with MeOH (5 mL), and concentrated to ~0.3 mL at ~40 Torr. This procedure was repeated, on average, six times, the completeness of the removal of ammonium carbamate being monitored using an indicator paper placed over the solution of the compound in MeOH. Finally, the solution was concentrated at ~10 Torr. β-Glycopyranosylamines **1b—6b** containing the starting oligosaccharides as impurities in amounts of up to 10% were immediately subjected to *N*-acylation.

***N*-(*N*-Boc-glycyl)-β-glycopyranosylamines 1c—6c (general procedure).** β-Glycopyranosylamines **1b—6b** (~0.03 mmol) were dissolved in H_2O (0.06 mL) and cooled with ice. Then a solution of *N*-Boc-glycine *N*-hydroxysuccinimide ester (25 mg, 0.09 mmol) in DMF (0.25 mL) was added. The reaction mixture was stirred, kept for 3 h at 0 °C, diluted with MeOH (3 mL), and concentrated to ~0.2 mL. This procedure was repeated four times. Diethyl ether (5 mL) was added to the resulting stirred solution. After clarification, the liquid was decanted from oily precipitates. The precipitates were repeatedly washed with Et_2O (in portions of 2 mL) and an Et_2O—acetone mixture (1 : 1) and then dried. The residues were dissolved in water (0.5 mL) and chromatographed on columns packed with C_{18} silica gel (2 g) in H_2O. The columns were washed with water to minimum UV absorbance and then with 25% aqueous MeOH. The aqueous methanolic fractions containing products were combined and concentrated. The residues were dissolved in a 10% Et_3N solution in 50% aqueous MeOH (1 mL) and kept for 3 h at ~20 °C. The reaction mixtures were diluted with MeOH (2.5 mL) and concentrated. The procedure was repeated three times. The residues were dissolved in water and chromatographed on C_{18} silica gel columns as described above. The aqueous methanolic fractions containing the target products were combined and concentrated. The residues were dried, and compounds **1c—6c** were obtained in 60—65% yields. The purity of the reaction products was checked by mass spectrometry.

4-*O*-(β-D-Galactopyranosyl)-*N*-(*N*-*tert*-butyloxycarbonylglycyl)-β-D-glucopyranosylamine (1c). $[\alpha]_D^{20}$ +2.2 (*c* 0.8, H_2O). MS, found: *m/z* 499.2136 $[M + H]^+$. Calculated for $C_{19}H_{35}N_2O_{13}$: 499.2134. Found: *m/z* 516.2402 $[M + NH_4]^+$. Calculated for $C_{19}H_{38}N_3O_{13}$: 516.2399. Found: *m/z* 521.1954 $[M + Na]^+$. Calculated for $C_{19}H_{34}N_2NaO_{13}$: 521.1953. Found: *m/z* 537.1690 $[M + K]^+$. Calculated for $C_{19}H_{34}KN_2O_{13}$: 537.1692. ^{1}H NMR, δ: 1.42 (s, 9 H, CH_3); 3.38—3.49 (m, 1 H); 3.49—3.57 (m, 1 H); 3.61—3.88 (m, 8 H); 3.88—3.95 (m, 2 H, CH_2N); 4.44 (d, 1 H, H(1) Gal, *J* = 7.7 Hz); 5.00 (d, 1 H, H(1) Glc, *J* = 9.1 Hz).

4-*O*-[3-*O*-(2-Acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl)-β-D-galactopyranosyl]-*N*-(*N*-*tert*-butyloxycarbonylglycyl)-β-D-glucopyranosylamine (2c).[25] $[\alpha]_D^{20}$ +2.2 (*c* 0.8, H_2O). MS, found: *m/z* 864.3459 $[M + H]^+$. Calculated for $C_{33}H_{58}N_3O_{23}$: 864.3456. Found: *m/z* 886.3274 $[M + Na]^+$. Calculated for $C_{33}H_{57}N_3NaO_{23}$: 886.3275. Found: *m/z* 902.3007 $[M + K]^+$. Calculated for $C_{33}H_{57}KN_3O_{23}$: 902.3014. ^{1}H NMR, (δ: 1.42 (s, 9 H, CH_3); 2.00 (s, 3 H, NAc); 3.35—4.05 (m, 25 H); 4.13 (br.s, 1 H, H(4) Gal^2); 4.42 (d, 2 H, H(1) Gal^1 и Gal^2, *J* = 7.4 Hz); 4.71 (d, 1 H, H(1) GlcNAc, *J* = 8.0 Hz); 4.99 (d, 1 H, H(1) Glc, *J* = 9.1 Hz).

4-*O*-[3-*O*-(2-Acetamido-2-deoxy-4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl)-β-D-galactopyranosyl]-*N*-(*N*-*tert*-butyloxycarbonylglycyl)-β-D-glucopyranosylamine (3c). $[\alpha]_D^{24}$ +5.9 (*c* 0.5, H_2O). MS, found: *m/z* 864.3469 $[M + H]^+$. Calculated for $C_{33}H_{58}N_3O_{23}$: 864.3456. Found: *m/z* 886.3274 $[M + Na]^+$. Calculated for $C_{33}H_{57}N_3NaO_{23}$: 886.3282. Found: *m/z* 902.3007 $[M + K]^+$. Calculated for $C_{33}H_{57}N_3KO_{23}$: 902.3015.

4-*O*-{3-*O*-[2-Acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-6-*O*-[2-acetamido-2-deoxy-4-*O*-(β-

D-galactopyranosyl)-β-D-glucopyranosyl]-β-D-galactopyranosyl}-*N*-(*N*-*tert*-butyloxycarbonylglycyl)-β-D-glucopyranosylamine (4c). $[\alpha]_D^{21}$ −7.0 (*c* 0.4, H_2O). MS, found: *m/z* 1229.4791 $[M + H]^+$. Calculated for $C_{47}H_{81}N_4O_{33}$: 1229.4777. Found: *m/z* 637.2273 $[M + Na]^{2+}$. Calculated for $0.5C_{47}H_{80}N_4NaO_{33}$: 637.2245.

4-*O*-{3-*O*-[2-Acetamido-2-deoxy-4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-6-*O*-[2-acetamido-2-deoxy-4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-β-D-galactopyranosyl}-*N*-(*N*-*tert*-butyloxycarbonylglycyl)-β-D-glucopyranosylamine (5c). $[\alpha]_D^{21}$ −4.0 (*c* 0.4, H_2O). MS, found: *m/z* 1229.4793 $[M + H]^+$. Calculated for $C_{47}H_{81}N_4O_{33}$: 1229.4777. Found: *m/z* 637.2270 $[M + Na]^{2+}$. Calculated for $0.5C_{47}H_{80}N_4NaO_{33}$: 637.2245.

2-Acetamido-2-deoxy-4-*O*-(β-D-galactopyranosyl)-*N*-(*N*-*tert*-butyloxycarbonylglycyl)-β-D-glucopyranosylamine (6c). $[\alpha]_D^{24}$ +6.7 (*c* 0.4, H_2O). MS, found: *m/z* 540.2392 $[M + H]^+$. Calculated for $C_{21}H_{38}N_3O_{13}$: 540.2399. Found: *m/z* 562.2213 $[M + Na]^+$. Calculated for $C_{21}H_{37}N_3NaO_{13}$: 562.2219. ^{1}H NMR, δ: 1.45 (s, 9 H, CH_3); 2.02 (s, 3 H, NAc); 3.54 (t, 1 H, *J* = 10.0 Hz); 3.64—3.70 (m, 2 H); 3.70—3.97 (m, 11 H); 4.49 (d, 1 H, H(1) Gal, *J* = 7.8 Hz); 5.117 (d, 1 H, H(1) GlcNAc, *J* = 9.6 Hz).

***N*-Glycyl-β-glycopyranosylamines 1d—6d (general procedure).** Trifluoroacetic acid (0.5 mL) was added to compound **1c—6c** (~0.02 mmol). The reaction mixture was stirred at 20—25 °C for about 3 min until dissolution. The solution was concentrated at ~10 Torr. Then toluene (5 mL) was added, the solution was concentrated, MeOH (3 mL) was added to the residue, the mixture was stirred until dissolution, toluene (3 mL) was added, and the mixture was concentrated. This procedure was repeated five times, the completeness of the removal of the acid being monitored using an indicator paper placed over the solution of the compound in MeOH. The residue was dissolved in MeOH (3 mL). The solution was concentrated and dried to obtain trifluoroacetates of compounds **1d—6d**, which were dissolved in water (1 mL). Then dry Dowex 1×8 (OH^-) ion-exchange resin (50 mg) was added, and the solution was stirred for 25 min. The resin was filtered off and washed with water (4×0.5 mL). The solution was concentrated to ~1 mL, filtered through a membrane filter (0.45 μm), and again concentrated. The residues were dried, and compounds **1d—6d** were obtained as amorphous powders in ~95% yields.

4-*O*-(β-D-Galactopyranosyl)-*N*-glycyl-β-D-glucopyranosylamine (1d). $[\alpha]_D^{22}$ +3.1 (*c* 1, H_2O) (*cf.* Ref. 26: $[\alpha]_D^{20}$ +3.0 (*c* 1, H_2O)). MS, found: *m/z* 399.1615 $[M + H]^+$. Calculated for $C_{14}H_{27}N_2O_{11}$: 399.1609. Found: *m/z* 421.1429 $[M + Na]^+$. Calculated for $C_{14}H_{26}N_2NaO_{11}$: 421.1429. ^{1}H NMR, δ: 3.38 (d, 2 H, CH_2N, *J* = 4.0 Hz); 3.41—3.49 (m, 1 H); 3.49—3.55 (m, 1 H); 3.60—3.83 (m, 8 H); 3.87—3.95 (m, 2 H); 4.43 (d, 1 H, H(1) Gal, *J* = 7.8 Hz); 5.00 (d, 1 H, H(1) Glc, *J* = 9.2 Hz).

4-*O*-[3-*O*-(2-Acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl)-β-D-galactopyranosyl]-*N*-glycyl-β-D-glucopyranosylamine (2d).[26] $[\alpha]_D^{21}$ +2.0 (*c* 1, H_2O). MS, found: *m/z* 764.2923 $[M + H]^+$. Calculated for $C_{28}H_{50}N_3O_{21}$: 764.2931. Found: *m/z* 786.2724 $[M + Na]^+$. Calculated for $C_{28}H_{49}N_3NaO_{21}$: 786.2751. Found: *m/z* 802.2493 $[M + K]^+$. Calculated for $C_{28}H_{49}KN_3O_{21}$: 802.2490. ^{1}H NMR, δ: 2.03 (s, 3 H, NAc); 3.40 (s, 2 H, CH_2N); 3.41—3.84 (m, 19 H); 3.87—3.96 (m, 4 H); 4.16 (br.s, 1 H, H(4) Gal^2); 4.44 (d, 1 H, H(1) Gal, *J* = 7.5 Hz); 4.45 (d, 1 H, H(1) Gal, *J* = 7.4 Hz); 4.74 (d, 1 H, H(1) GlcNAc, *J* = 8.5 Hz); 5.04 (d, 1 H, H(1) Glc, *J* = 9.3 Hz).

4-*O*-[3-*O*-(2-Acetamido-2-deoxy-4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl)-β-D-galactopyranosyl]-*N*-glycyl-β-D-glucopyranosylamine (3d). $[\alpha]_D^{24}$ +5.3 (*c* 0.4, H_2O). MS, found: *m/z* 764.2906 $[M + H]^+$. Calculated for $C_{28}H_{50}N_3O_{21}$: 764.2931. Found: *m/z* 786.2722 $[M + Na]^+$. Calculated for $C_{28}H_{49}N_3NaO_{21}$: 786.2751. Found: *m/z* 802.2453 $[M + K]^+$. Calculated for $C_{28}H_{49}KN_3O_{21}$: 802.2490. ^{1}H NMR, δ: 2.04 (s, 3 H, NAc); 3.40 (s, 2 H, CH_2N); 3.41—3.87 (m, 20 H); 3.91—3.98 (m, 3 H); 4.16 (br.s, 1 H, H(4) Gal^2); 4.45 (d, 1 H, H(1) Gal, *J* = 7.8 Hz); 4.48 (d, 1 H, H(1) Gal, *J* = 7.8 Hz); 4.71 (d, 1 H, H(1) GlcNAc, *J* = 8.3 Hz); 5.04 (d, 1 H, H(1) Glc, *J* = 9.2 Hz).

4-*O*-{3-*O*-[2-Acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-6-*O*-[2-acetamido-2-deoxy-4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-β-D-galactopyranosyl}-*N*-glycyl-β-D-glucopyranosylamine (4d). $[\alpha]_D^{20}$ −7.9 (*c* 0.3, H_2O). MS, found: *m/z* 1129.4291 $[M + H]^+$. Calculated for $C_{42}H_{73}N_4O_{31}$: 1129.4253. Found *m/z* 565.2173 $[M + H]^{2+}$. Calculated for $0.5C_{42}H_{74}N_4O_{31}$: 565.2163. Found *m/z* 576.2081 $[M + Na]^{2+}$. Calculated for $0.5C_{42}H_{73}N_4NaO_{31}$: 576.2073. ^{1}H NMR, δ: 2.03 (s, 3 H, NAc, Glc^5NAc); 2.06 (s, 3 H, NAc, Glc^3NAc); 3.44—4.02 (m, 37 H); 4.15 (d, 1 H, H(4) Gal^2, *J* = 3.0 Hz); 4.44 (d, 2 H, H(1) Gal^2 and Gal^6, *J* = 7.7 Hz); 4.48 (d, 1 H, H(1) Gal^4, *J* = 7.8 Hz); 4.64 (d, 1 H, H(1) Glc^3NAc, *J* = 7.7 Hz); 4.74 (d, 1 H, H(1) Glc^5NAc, *J* = 8.5 Hz); 5.06 (d, 1 H, H(1) Glc, *J* = 9.2 Hz).

4-*O*-{3-*O*-[2-Acetamido-2-deoxy-4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-6-*O*-[2-acetamido-2-deoxy-4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-β-D-galactopyranosyl}-*N*-glycyl-β-D-glucopyranosylamine (5d). $[\alpha]_D^{21}$ −4.5 (*c* 0.8, H_2O). MS, found: *m/z* 1129.4215 $[M + H]^+$. Calculated for $C_{42}H_{73}N_4O_{31}$: 1129.4253. Found: *m/z* 1151.4018 $[M + Na]^+$. Calculated for $C_{42}H_{72}N_4NaO_{31}$: 1151.4073. Found: *m/z* 565.2181 $[M + H]^{2+}$. Calculated for $0.5C_{42}H_{74}N_4O_{31}$: 565.2163. Found: *m/z* 576.2094 $[M + Na]^{2+}$. Calculated for $0.5C_{42}H_{73}N_4NaO_{31}$: 576.2073. ^{1}H NMR, δ: 2.03 (s, 3 H, NAc, Glc^5NAc); 2.06 (s, 3 H, NAc, Glc^3NAc); 3.44—4.02 (m, 37 H); 4.151 (d, 1 H, H(4) Gal^2, *J* = 3.0 Hz); 4.44 (d, 1 H(1) Gal^2, *J* = 7.8 Hz); 4.46—4.50 (m, 2 H, H(1) Gal^4 and Gal^6); 4.64 (d, 1 H, H(1) Glc^3NAc, *J* = 7.6 Hz); 4.71 (d, 1 H, H(1) Glc^5NAc, *J* = 8.3 Hz); 5.04 (d, 1 H, H(1) Glc, *J* = 9.2 Hz).

2-Acetamido-2-deoxy-4-*O*-(β-D-galactopyranosyl)-*N*-glycyl-β-D-glucopyranosylamine (6d). $[\alpha]_D^{20}$ +7.5 (*c* 0.3). MS, found: *m/z* 440.1859 $[M + H]^+$. Calculated for $C_{16}H_{30}N_3O_{11}$: 440.1875. Found *m/z* 462.1672 $[M + Na]^+$. Calculated for $C_{16}H_{29}N_3NaO_{11}$: 462.1694. ^{1}H NMR, δ: 2.00 (s, 3 H, NAc); 3.37 (s, 2 H, CH_2N); 3.52 (t, 1 H, *J* = 10.0 Hz); 3.60—3.98 (m, 11 H); 4.42 (d, 1 H, H(1) Gal, *J* = 7.8 Hz); 5.10 (d, 1 H, H(1) GlcNAc, *J* = 9.6 Hz).

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