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Chemoenzymatic synthesis of the sialyl-α-(2→3′)-lactosamine trisaccharide with a 3-aminopropyl group as a spacer at the reducing end

Indrani Choudhury (Mukherjee), Norihiko Minoura, Hirotaka Uzawa*

Laboratory of Advanced Bioelectronics, National Institute of Advanced Industrial Science and Technology (AIST), Central 5, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8565, Japan

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Abstract

The trisaccharide, 3-aminopropyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid-(2→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside has been synthesized chemoenzymatically for the first time. First, the acceptor, 3-aminopropyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside was synthesized in a conventional chemical manner, and then it was coupled with CMP-sialic acid using α-(2→3)-(*N*)-sialyltransferase to afford the desired trisaccharide by an enzymatically stereocontrolled manner.

Keywords: Lactosamine; Sialic acid; Trisaccharide; Chemoenzymatic

1. Introduction

Sialo glycoproteins, glycolipids and sialo-oligosaccharides play crucial roles in biological functions.[1–3] They are found as excellent natural ligands for the selectin family,[1] pathogenic bacteria and viruses,[1] and other carbohydrate receptor proteins like lectins.[4–6] Recently, we have reported a rapid detection for Shiga toxins secreted from *Escherichia coli* O-157 by applying the artificial carbohydrate ligand.[7] Bacterial toxins and other species–specific proteins that recognize the specific sialo-oligosaccharides on their binding sites might have potential application for the detection of pathogens and also for new drug discovery associated with inflammation and cancer.[8] These important phenomena have provided the impetus for the scientist to synthesize sialo-oligosaccharides for more precise studies. Most of the naturally occurring sialo-oligosaccharides contain α-linked sialic acids. Both chemical[9,10] as well as chemoenzymatic[11] methods have been used for stereospecific synthesis of α-linked sialo-oligosaccharides. However, the chemoenzymatic method has been found more advantageous compared to its chemical counterpart in that it often minimizes the number of synthetic steps and provides high yields of products. There are a number of reports on the synthesis of Neu5Ac-α-(2→3′)-lactosamine glycoside carrying different groups at the reducing end using chemical[9] and chemoenzymatic[12–14] synthetic strategies. However, to the best of our knowledge, we report herein for the first time, the chemoenzymatic synthesis of sialyl-α-(2→3′)-lactosamine carrying a 3-aminopropyl group as the spacer at the reducing end so that the final compound can be utilized for further biological studies. It should be noted here that Nifantiev and co-workers[9,15] have used the 3-aminopropyl group as a spacer by a different chemical manipulation for their chemical synthesis of Neu5Ac-α-(2→6′)-lactosamine 3-aminopropyl glycoside,[15] Neu5Gc-α-(2→6′)-lactosamine 3-aminopropyl glycoside[15] and Neu5Gc-α-(2→3′)-lactosamine 3-aminopropyl glycoside.[9] However, their chemical approaches yielded a byproduct and poor yields during the coupling of sialic acid in the final step.[9] To improve

* Corresponding author. Tel.: +81-298-614431; fax: +81-298-614680.
E-mail address: h.uzawa@aist.go.jp (H. Uzawa).

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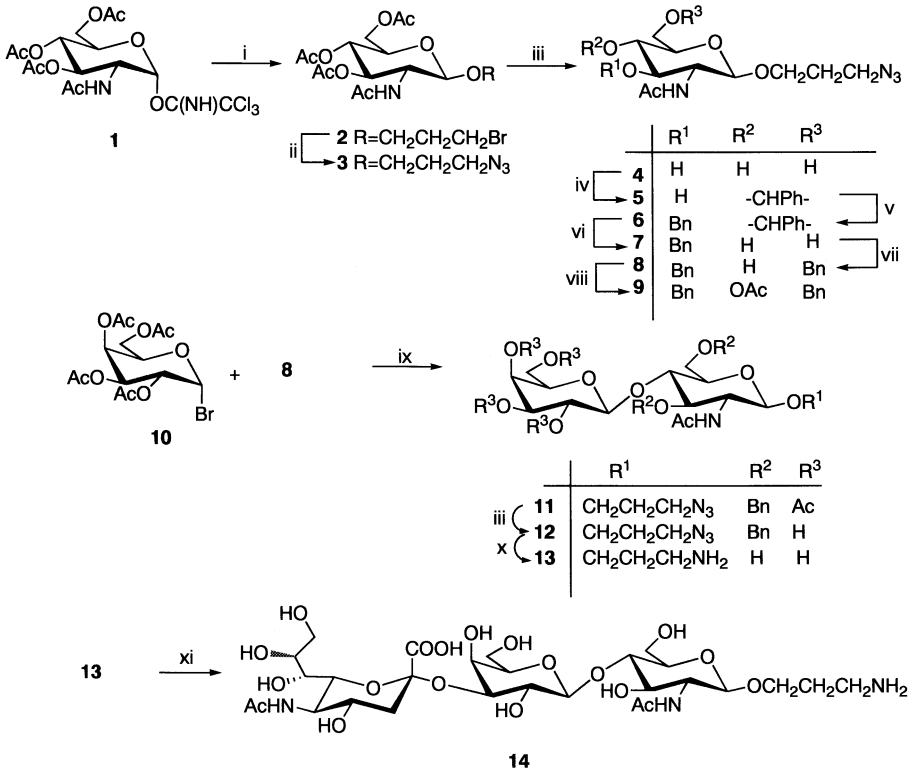
the yield of sialylation and to avoid the byproduct, we first chemically synthesized the disaccharide acceptor, lactosamine 3-aminopropyl glycoside (**13**), and then stereospecifically coupled it with CMP-sialic acid using α-(2→3)-(*N*)-sialyltransferase (E.C. 2.4.99.5) to afford the desired trisaccharide **14** in high yield. This chemoenzymatic synthesis provided a better yield of the desired trisaccharide **14** and also minimized the number of synthetic steps.

2. Results and discussion

The known 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl trichloroacetimidate[16] (**1**) was allowed (Scheme 1) to react with 3-bromo-1-propanol in the presence of trimethylsilyl trifluoromethanesulfonate[17] to obtain 3-bromopropyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (**2**) in 76% yield. Treatment of compound **2** with NaN_3 in *N*,*N*-dimethylformamide at 60 °C afforded 3-azidopropyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (**3**) in 94% yield. The β linkage at the anomeric position of the compound **3** was determined by its ^1H NMR spectrum, with H-1 appearing at δ 4.64 as a wide doublet (J 8.4 Hz) and by its ^{13}C NMR spectrum that showed C-1 at δ 101.0. Compound **3** was O-deacetylated with sodium methoxide[18] in methanol, and the resulting 3-azidopropyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**4**) was treated with benzaldehyde dimethylacetal in 1:1 acetonitrile–*N*,*N*-dimethylformamide in the presence of a catalytic amount of *p*-toluenesulfonic acid monohydrate to provide 3-azidopropyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (**5**) in 87% yield. To avoid N-benzylation, compound **5** was benzylated by using phase-transfer conditions in the presence of tetrabutylammonium bromide as a phase-transfer catalyst to obtain 3-azidopropyl 2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (**6**) in 84% yield. Removal of the benzylidene group of compound **6** with 80% aqueous acetic acid gave the diol derivative **7**. Selective benzylation[19] of compound **7** was performed via its stannylene derivative to afford 3-azidopropyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (**8**) in 52% yield. The presence of a free OH group at C-4 of **8** was confirmed by acetylation of **8** with acetic



Scheme 1. Reagents and conditions: (i) $Br(CH_2)_3OH$, Me_3SiOTf, CH_2Cl_2, 4 Å MS, 20 min, −10 °C; (ii) NaN_3, DMF, 3 h, 60 °C; (iii) NaOMe, MeOH; (iv) $PhCH(OMe)_2$, 1:1 CH_3CN–DMF, *p*-TsOH, 48 h; (v) BnBr, Bu_4NBr, 10% NaOH, CH_2Cl_2, 48 h, rt; (vi) 80% AcOH, 3 h, 80 °C; (vii) Bu_2SnO, toluene, BnBr, Bu_4NBr; (viii) Ac_2O, pyridine; (ix) AgOTf, CH_2Cl_2, 4 Å MS, −30→−10 °C; (x) H_2, 10% Pd–C, 2:1 MeOH–H_2O; (xi) CMP-β-D-sialic acid, disodium salt, rat liver α-(2→3)-(*N*)-sialyltransferase, calf intestine alkaline phosphatase, HEPES buffer.

anhydride and pyridine. In the ^{1}H NMR spectrum of the resulting acetate **9**, the signal of H-4 had shifted downfield to δ 5.00 compared with its position at δ 3.66 in the spectrum of **8**. Commercially available 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**10**) was used as donor and was reacted[20] with the acceptor **8** using AgOTf as activator in dichloromethane to afford the disaccharide 3-azidopropyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (**11**) in 76% yield. The β linkages of compound **11** were confirmed from its ^{1}H (δ 4.70, $J_{1,2}$ 5.6 Hz, H-1 and δ 4.50, $J_{1',2'}$ 8.8 Hz, H-1′) and ^{13}C NMR [100.2 (C-1), 99.8, (C-1′)] spectra. Deacetylation of compound **11** with sodium methoxide, followed by catalytic hydrogenolysis with Pd–C in MeOH–H_2O, afforded 3-aminopropyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (**13**) in 91% overall yield in two steps. Compound **13** was characterized from its ^{1}H NMR and FAB mass spectra (FABMS). Compound **13** was enzymatically[12] sialylated using commercially available cytidine-5′-monophospho-β-D-sialic acid (CMP-β-D-sialic acid), disodium salt as donor in *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) buffer in the presence of α-(2→3)-(*N*)-sialyltransferase, isolated from rat liver, and alkaline phosphatase (E.C. 3.1.3.1), isolated from calf intestine, to obtain exclusively 3-aminopropyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid-(2→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (**14**) in 78% yield. The structure of **14** was determined by ^{1}H and ^{13}C NMR spectroscopy. The characteristic peaks at δ 2.62 (H-3″e), 1.65 (H-3″a) in the ^{1}H NMR spectrum and peaks at δ 174.0 (C-1″), 72.3 (C-6″) in the ^{13}C NMR spectrum confirmed the α coupling of the Neu5Ac residue in trisaccharide **14**. The peak appearing at δ 78.5 (C-3′) in the ^{13}C NMR spectrum of compound **14** was clearly shifted towards downfield compared to other ring carbons [C-2′ (71.9), C-4′ (69.5) and C-6′ (61.2)] in the Gal residue, which are well consistent with the data reported for Neu5Gc-α-(2→3′)-lactosamine 3-aminopropyl glycoside.[9] In the ^{1}H NMR of **14**, the doublet of doublets peak appearing at δ 3.97 (H-3′) in the Gal moiety was also shifted downfield, supporting the α-(2→3′) regioselectivity. These results show that the Neu5Ac and Gal moieties are connected via the sialyl α-(2→3′)-Gal linkage.

In conclusion, we have chemoenzymatically synthesized the sialylated lactosamine 3-aminopropyl glycoside **14** in high yield, which is readily accessible for subsequent polymerization and immobilization for further biological studies with a surface plasmon resonance (SPR) technique and a quartz crystal microbalance (QCM) method.[7,21] The trisaccharide synthesized here is a promising candidate for ligands of, for instance, various sialo-oligosaccharides binding lectins like E, P, and L-selectins.[1,4] Application to these studies are in progress and will be reported in due course.

3. Experimental

3.1. General methods

All reactions were monitored by thin-layer chromatography (TLC) on Silica Gel 60 F_{254} (E. Merck Japan Ltd.) and 1:2:1 *n*-butanol–*n*-propanol–0.1 M aq HCl (BPH), 1:1:1 MeCN–MeOH–water (MMW) were used as elution solvents for the deblocked trisaccharide **14**. Column chromatography was performed on Silica Gel 60 N (Cica Reagent). Toyopearl HW-40S gel was purchased from Tosoh Corporation (Japan) and was used for gel-permeation chromatography. 2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl bromide was purchased from Aldrich Chemical Co. CMP-β-D-sialic acid, disodium salt, and α-(2→3)-(*N*)-sialyltransferase were purchased from Calbiochem (USA). Alkaline phosphatase was purchased from Wako Pure Chemical Industries Ltd. (Japan). Optical rotations were measured with a JASCO DIP-1000 digital polarimeter at 25 °C. NMR spectra were recorded on a Varian INOVA 400 MHz spectrometer. For the ^{1}H and ^{13}C NMR spectra, Me_4Si was used as the internal standard in $CDCl_3$ and CD_3OD solutions. *tert*-Butyl alcohol (δ 1.24 (^{1}H)) was used as the internal standard in D_2O solution for the NMR spectrum of compound **13**. The ^{13}C NMR spectrum of compound **14** in D_2O solution was measured by fixing HOD peak at δ 4.70 in the ^{1}H NMR spectrum followed by measuring ^{13}C NMR spectra, and thus ^{13}C chemical shifts in D_2O solution was reported in ppm without any reference standard. FABMS were recorded using a JEOL DX 303 mass spectrometer, and high-resolution EI mass spectra (EIMS) were recorded using a Hitachi M 80 mass spectrometer.

3.2. 3-Azidopropyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (3)

A solution of compound **1** (4.00 g, 8.13 mmol) and 3-bromo-1-propanol (7.4 mL, 81 mmol) in dry CH_2Cl_2 (20 mL) containing freshly activated 4 Å MS (400 mg) was stirred for 1 h at 25 °C under N_2. The reaction mixture was then cooled at −10 °C, and Me_3SiOTf (0.20 mL, 1.1 mmol) was added. The reaction mixture was stirred at −10 °C for 20 min and then filtered over a Celite bed. The organic layer was washed with satd aq $NaHCO_3$ and then water. The organic layer was dried (Na_2SO_4), filtered and concentrated. Column chromatography of the product on silica gel with 5:1 EtOAc–hexane afforded compound **2** as a white solid (2.90 g, 76%). 3-

Bromopropyl glycoside **2** (2.90 g, 6.19 mmol) was then dissolved in DMF (5 mL), and NaN_3 (2.01 g, 30.9 mmol) was added to it. The reaction mixture was stirred at 60 °C for 3 h, and it was then cooled at 25 °C. The insoluble material was filtered off. The organic layer was diluted with EtOAc, washed with brine solution, dried (Na_2SO_4), filtered and concentrated. Column chromatography of the product on silica gel with 2:1 EtOAc–hexane gave compound **3** as white foam (2.50 g, 94%): $[\alpha]_D$ −8.3° (*c* 0.6, $CHCl_3$); NMR ($CDCl_3$): ^1H, δ 5.81 (d, 1 H, $J_{N-H,2}$ 8.8 Hz, N*H*), 5.25 (dd, 1 H, $J_{2,3}$ 10.8, $J_{3,4}$ 9.6 Hz, H-3), 5.07 (t, 1 H, $J_{4,5}$ 9.6 Hz, H-4), 4.64 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.26 (dd, 1 H, $J_{6a,6b}$ 12.4, $J_{5,6a}$ 4.8 Hz, H-6a), 4.15 (dd, 1 H, $J_{5,6b}$ 2.4 Hz, H-6b), 3.99–3.89 (m, 2 H, H-5, OCH_2CH$_2$CH$_2$N$_3$), 3.74–3.69 (m, 1 H, OCH_2CH$_2$CH$_2$N$_3$), 3.64–3.57 (m, 1 H, H-2), 3.39–3.36 (m, 2 H, OCH$_2$CH$_2$CH_2N$_3$), 2.09, 2.04, 2.03 (3 s, 9 H, CH_3CO), 1.96 (s, 3 H, NHCOCH_3), 1.89–1.83 (m, 2 H, OCH$_2$CH_2CH$_2$N$_3$); ^{13}C, δ 171.0, 170.7, 170.3, 169.4 (4 *C*O), 101.0 (C-1), 72.4, 71.8, 68.6, 66.2, 62.1, 54.5, 48.0 (OCH$_2$CH$_2$*C*H$_2$N$_3$), 28.9 (OCH$_2$*C*H$_2$CH$_2$N$_3$), 23.3 (NHCO*C*H$_3$), 20.8, 20.7, 20.6 (3 *C*H$_3$CO). EIMS: Anal. Calcd for [$C_{17}H_{26}N_4O_9$ (430.1700)+H]$^+$, 431.1779. Found: *m*/*z* 431.1766.

3.3. 3-Azidopropyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (5)

Treatment of compound **3** (2.40 g, 5.58 mmol) with 0.1 M NaOMe for 2 h, followed by neutralization with Dowex 50W (H^+) resin, afforded compound **4** (1.60 g, 94%) as a white solid. To a solution of compound **4** (1.60 g, 5.26 mmol) in 1:1 CH_3CN–DMF (20 mL), benzaldehyde dimethylacetal (0.90 mL, 6.2 mmol) and a catalytic amount of *p*-TsOH were added. The reaction mixture was stirred at 25 °C for 48 h and then neutralized with Et_3N. The solution was concentrated under reduced pressure. Column chromatography on silica gel with EtOAc gave compound **5** as a white foam (1.80 g, 87%): $[\alpha]_D$ −35° (*c* 0.8, $CHCl_3$); NMR (1:1 CD_3OD–$CDCl_3$): ^1H, δ 7.65–7.34 (m, 5 H, aromatic protons), 5.58 (s, 1 H, PhC*H*), 4.53 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.36–4.32 (m, 1 H, H-6a), 3.96–3.91 (m, 1 H, OCH_2CH$_2$CH$_2$N$_3$), 3.83–3.73 (m, 3 H, H-4, H-6b, OCH_2CH$_2$CH$_2$N$_3$), 3.63–3.59 (m, 1 H, H-5), 3.55 (t, 1 H, $J_{2,3}=J_{3,4}=9.6$ Hz, H-3), 3.48–3.43 (m, 1 H, H-2), 3.42–3.36 (m, 2 H, OCH$_2$CH$_2$CH_2N$_3$), 2.01 (s, 3 H, NHCOCH_3), 1.84–1.82 (m, 2 H, OCH$_2$CH_2CH$_2$N$_3$); ^{13}C, 172.1 (*C*O), 136.8–125.7 (Ph), 101.3, 101.2 (C-1, Ph*C*H), 81.0, 70.6, 68.0, 65.7, 56.2, 48.0 (OCH$_2$CH$_2$*C*H$_2$N$_3$), 28.3 (OCH$_2$*C*H$_2$CH$_2$N$_3$), 21.7 (NHCO*C*H$_3$). EIMS: Anal. Calcd for [$C_{18}H_{24}N_4O_6$ (392.1696)+H]$^+$, 393.1775. Found: *m*/*z* 393.1746.

3.4. 3-Azidopropyl 2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (6)

A mixture of compound **5** (781 mg, 1.99 mmol), 10% aq NaOH (5 mL), and BnBr (0.35 mL, 3.0 mmol) in CH_2Cl_2 (8 mL) was stirred at 25 °C for 30 min, and then Bu_4NBr (126 mg, 0.391 mmol) was added. The reaction mixture was stirred at 25 °C for 48 h. The reaction mixture was then diluted with CH_2Cl_2. The organic layer was washed with water, dried (Na_2SO_4), filtered and concentrated. Column chromatography of the product on silica gel with 8:1 EtOAc–hexane gave **6** as white foam (810 mg, 84%): $[\alpha]_D$ −60.5° (*c* 0.6, $CHCl_3$); NMR ($CDCl_3$): ^1H, δ 7.49–7.31 (m, 10 H, aromatic protons), 5.56 (s, 1 H, PhC*H*), 5.54 (d, 1 H, $J_{N-H,2}$ 8.0 Hz, N*H*), 4.88 (dd, 2 H, *J* 11.6, $J_{1,2}$ 8.0 Hz, PhCH_2, H-1), 4.65 (d, 1 H, PhCH_2), 4.35 (dd, 1 H, $J_{6a,6b}$ 10.4, $J_{5,6a}$ 5.2 Hz, H-6a), 4.14 (t, 1 H, $J_{2,3}=J_{3,4}=9.2$ Hz, H-3), 3.94–3.89 (m, 1 H, OCH_2CH$_2$CH$_2$N$_3$), 3.78 (t, 1 H, $J_{5,6b}$ 10.3 Hz, H-6b), 3.68 (t, 1 H, $J_{4,5}$ 9.2 Hz, H-4), 3.61–3.55 (m, 1 H, H-5), 3.53–3.47 (m, 1 H, OCH_2CH$_2$CH$_2$N$_3$), 3.44–3.34 (m, 3 H, H-2, OCH$_2$CH$_2$CH_2N$_3$), 1.89 (s, 3 H, NHCOCH_3), 1.87–1.76 (m, 2 H, OCH$_2$CH_2CH$_2$N$_3$); ^{13}C, 170.4 (*C*O), 138.3–126.0 (Ph), 101.2, 100.8 (Ph*C*H, C-1), 82.7, 76.5, 74.4, 68.8, 66.4, 66.0, 57.3, 48.1 (OCH$_2$CH$_2$*C*H$_2$N$_3$), 29.0 (OCH$_2$*C*H$_2$CH$_2$N$_3$), 23.5 (NHCO*C*H$_3$). EIMS: Anal. Calcd for [$C_{25}H_{30}N_4O_6$ (482.2165)+H]$^+$, 483.2244. Found: *m*/*z* 483.2260.

3.5. 3-Azidopropyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (8)

A suspension of compound **6** (357 mg, 0.740 mmol) in 80% aq AcOH (2 mL) was heated at 80 °C for 3 h. The reaction mixture was then concentrated and flashed three times with toluene. The residue was dried overnight under vacuum to afford compound **7** (280 mg, 96%) as white foam. To a solution of **7** (280 mg, 0.710 mmol) in toluene, Bu_2SnO (199 mg, 0.799 mmol) was added, and the mixture was refluxed with azeotropic removal of water for 18 h. The reaction mixture was concentrated to dryness. Fresh toluene (3 mL), BnBr (0.40 mL, 3.6 mmol) and Bu_4NBr (114 mg, 0.354 mmol) were added, and the mixture was stirred overnight at 60 °C. The solvent was evaporated under reduced pressure, MeOH was added, and the mixture was cooled. Solids that separated were removed by filtration, and the filtrate was concentrated. Column chromatography of the product on silica gel with 5:1 EtOAc–hexane gave pure **8** as white foam (180 mg, 52%): $[\alpha]_D$ −12.2° (*c* 0.8, $CHCl_3$); NMR ($CDCl_3$): ^1H, δ 7.32–7.26 (m, 10 H, aromatic protons), 5.68 (d, 1 H, $J_{N-H,2}$ 8.0 Hz, N*H*), 4.79 (d, 1 H, *J* 12.0 Hz, PhCH_2), 4.72 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.69 (d, 1 H, PhCH_2), 4.57 (dd, 2 H, PhCH_2), 3.91–3.84 (m, 2 H, H-3, OCH_2CH$_2$CH$_2$N$_3$), 3.74–3.70 (m, 2 H, H-6), 3.66 (t, 1 H, $J_{3,4}=J_{4,5}=9.6$

Hz, H-4), 3.56–3.49 (m, 2 H, $OCH_2CH_2CH_2N_3$, H-5), 3.48–3.42 (m, 1 H, H-2), 3.36–3.30 (m, 2 H, $OCH_2CH_2CH_2N_3$), 1.89 (s, 3 H, $NHCOCH_3$), 1.87–1.76 (m, 2 H, $OCH_2CH_2CH_2N_3$); ^{13}C, 170.4 (*C*O), 138.4–127.7 (Ph), 100.2 (C-1), 80.5, 74.0, 73.8, 73.6, 73.0, 70.5, 66.0, 56.3, 48.1 ($OCH_2CH_2CH_2N_3$), 28.9 ($OCH_2CH_2CH_2N_3$), 23.5 ($NHCOCH_3$). FABMS: Anal. Calcd for $[C_{25}H_{32}N_4O_6\ (484)+H]^+$, 485. Found: *m*/*z* 485. Compound **8** (10 mg) was acetylated in the usual way with Ac_2O and Py to afford the acetate **9** (9 mg): NMR ($CDCl_3$): ^1H, δ 7.32–7.26 (m, 10 H, aromatic protons), 5.57 (d, 1 H, $J_{N-H,2}$ 7.6 Hz, N*H*), 5.00 (t, 1 H, $J_{3,4}$ 10.4, $J_{4,5}$ 9.6 Hz, H-4), 4.96 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.62 (d, 1 H, *J* 11.2 Hz, PhCH_2), 4.56 (d, 1 H, PhCH_2), 4.52 (s, 2 H, PhCH_2), 4.26 (t, 1 H, $J_{2,3}$ 9.2 Hz, H-3), 3.95–3.92 (m, 1 H, $OCH_2CH_2CH_2N_3$), 3.67–3.55 (m, 4 H, H-5, H-6, $OCH_2CH_2CH_2N_3$), 3.38–3.34 (m, 2 H, $OCH_2CH_2CH_2N_3$), 3.30–3.23 (m, 1 H, H-2), 1.90, 1.88 (2 s, 6 H, $COCH_3$, $NHCOCH_3$), 1.87–1.76 (m, 2 H, $OCH_2CH_2CH_2N_3$).

3.6. 3-Azidopropyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (11)

A solution of the acceptor **8** (50 mg, 0.10 mmol) and donor **10** (85 mg, 0.21 mmol) in CH_2Cl_2 (2 mL) containing 4 Å MS (218 mg) was stirred under N_2 for 2 h and cooled to −30 °C. AgOTf (81 mg, 0.32 mmol) was then added. Stirring was continued in the dark for 1 h at −30 °C and then for 2 h at −10 °C. The reaction mixture was diluted with CH_2Cl_2, filtered and washed with satd aq $NaHCO_3$ and water. The organic layer was dried (Na_2SO_4), filtered and concentrated. Column chromatography of the product on silica gel with 3:2 EtOAc–hexane afforded recovered acceptor **8** (8 mg, 16%) and disaccharide **11** (54 mg, 76%) as a white foam: $[\alpha]_D$ +16° (*c* 0.6, $CHCl_3$); NMR ($CDCl_3$): ^1H, δ 7.32–7.26 (m, 10 H, aromatic protons), 5.83 (d, 1 H, $J_{N-H,2}$ 8.0 Hz, N*H*), 5.32 (br d, 1 H, *J* 2.8 Hz, H-4′), 5.12 (dd, 1 H, $J_{2',3'}$ 10.4, $J_{1',2'}$ 8.0 Hz, H-2′), 4.91 (dd, 1 H, $J_{3',4'}$ 3.6 Hz, H-3′), 4.76 (d, 1 H, *J* 11.6 Hz, PhCH_2), 4.70 (d, 1 H, $J_{1,2}$ 5.6 Hz, H-1), 4.65 (dd, 2 H, PhCH_2), 4.50 (d, 1 H, $J_{1',2'}$ 8.8 Hz, H-1′), 4.47 (d, 1 H, PhCH_2), 4.15–3.47 (m, 11 H, H-2, H-3, H-4, H-5, H-6, H-5′, H-6′, $OCH_2CH_2CH_2N_3$), 3.37–3.28 (m, 2 H, $OCH_2CH_2CH_2N_3$), 2.11, 2.03, 2.01, 1.98, 1.93 (5 s, 15 H, $COCH_3$, $NHCOCH_3$), 1.87–1.76 (m, 2 H, $OCH_2CH_2CH_2N_3$); ^{13}C, 170.3, 170.2, 170.1, 170.0, 169.8 (*C*O), 138.4–127.7 (Ph), 100.2, 99.8 (C-1, C-1′), 75.2, 74.4, 73.6, 73.2, 70.7, 70.6, 69.3, 68.7, 66.8, 66.0, 60.8, 53.1, 48.2 ($OCH_2CH_2CH_2N_3$), 29.0 ($OCH_2CH_2CH_2N_3$), 23.4 ($NHCOCH_3$), 20.8, 20.6, 20.5 (CH_3CO). FABMS: Anal. Calcd for $[C_{39}H_{50}N_4O_{15}\ (814)+H]^+$, 815. Found: *m*/*z* 815.

3.7. 3-Aminopropyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (13)

Compound **11** (46 mg, 0.06 mmol) was deacetylated with 0.1 M NaOMe according to Zemplén[18] to give the deacetylated product **12**, which was then dissolved in 2:1 MeOH–H_2O (3 mL) and was stirred with 10% Pd–C (30 mg) under H_2 for 48 h at 25 °C. The reaction mixture was then filtered through Celite, and the pad was washed thoroughly with water. The combined filtrate and washings was concentrated to afford compound **13** as an amorphous powder (24 mg, 91%): $[\alpha]_D$ −6.7° (*c* 0.25, water); NMR (D_2O): ^1H, δ 4.38 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), 4.33 (d, 1 H, $J_{1',2'}$ 7.6 Hz, H-1′), 4.01–3.84 (m, 2 H, H-4′, $OCH_2CH_2CH_2NH_2$), 3.81–3.77 (m, 1 H, H-6a), 3.75–3.67 (m, 1 H, H-6b), 3.65–3.51 (m, 8 H, H-3, H-4, H-5, H-2′, H-3′, H-5′, H-6′), 3.48–3.43 (m, 1 H, $OCH_2CH_2CH_2NH_2$), 3.42–3.38 (m, 1 H, H-2), 3.00–2.93 (m, 2 H, $OCH_2CH_2CH_2NH_2$), 1.91 (s, 3 H, $NHCOCH_3$), 1.87–1.76 (m, 2 H, $OCH_2CH_2CH_2NH_2$); FABMS: Anal. Calcd for $[C_{17}H_{32}N_2O_{11}\ (440)+H]^+$, 441. Found: *m*/*z* 441.

3.8. 3-Aminopropyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid-(2→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (14)

The disaccharide acceptor **13** (3 mg, 0.007 mmol), CMP-β-D-sialic acid, disodium salt (4.6 mg, 0.007 mmol), alkaline phosphatase (17.6 U), rat liver α-(2→3)-(*N*)-sialyltransferase (18.6 mU) was incubated with HEPES buffer (1 mL, 50 mM, pH 7.4) at 35 °C for 5 days. On the third day of the incubation another portion of CMP-β-D-sialic acid, disodium salt (2.3 mg, 0.003 mmol) was added. On the fifth day TLC analysis (using 1:1 BPH–MMW as solvents) showed that all the donor was consumed. The reaction mixture was heated at 100 °C to deactivate the enzyme, and the crude product was subjected to a gel-permeation column (Toyopearl HW-40S, 1.5 × 50 cm), and the column was eluted with 0.1 M aq AcOH. The fractions containing the desired product were collected, combined and concentrated. The product was passed again through similar two Toyopearl HW-40S columns to obtain the desired trisaccharide **14** as an amorphous powder (4 mg, 78%): $[\alpha]_D$ −3.3° (*c* 0.25, water); NMR (D_2O): ^1H, δ 4.41 (d, 1 H, $J_{1',2'}$ 8 Hz, H-1′), 4.37 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), 3.97 (dd, 1 H, $J_{2',3'}$ 10, $J_{3',4'}$ 3.2 Hz, H-3′), 3.91–3.85 (m, 2 H, H-6a, $OCH_2CH_2CH_2NH_2$), 3.81 (d, 1 H, H-4′), 3.78–3.68 (m, 5 H, H-5, H-5′, H-5″, H-6b, H-8″), 3.62–3.54 (m, 5 H, H-2, H-3, H-4, H-4″, H-6″), 3.52–3.40 (m, 7 H, H-2′, H-6′, H-7″, H-9″, $OCH_2CH_2CH_2NH_2$), 3.00–2.87 (m, 2 H, $OCH_2CH_2CH_2NH_2$), 2.62 (dd, 1 H, *J* 12.8 Hz, $J_{3''e,4''}$ 4.8 Hz, H-3″$_e$), 1.89, 1.87 (2 s, 6 H, $NHCOCH_3$), 1.81–1.79 (m, 2 H, $OCH_2CH_2CH_2NH_2$), 1.65 (t, 1 H, H-3″$_a$);

^{13}C, 175.2, 174.9 (2 *C*O), 174.0 (C-1″), 102.8 (C-1′), 101.4 (C-1), 99.9 (C-2″), 78.5 (C-3′), 75.6 (C-5′), 75.4 (C-4), 74.9 (C-5), 73.1 (C-3), 72.3 (C-6″), 71.9 (C-8″, C-2′), 69.5 (C-4′), 68.3 (C-7″), 68.1 (C-4″), 67.6 (O*C*H_2CH_2-CH_2NH_2), 63.0 (C-9″), 61.2 (C-6′, C-6), 55.2 (C-2), 51.9 (C-5″), 40.0 (C-3″), 37.8 (OCH_2CH_2*C*H_2NH_2), 26.8 (OCH_2*C*H_2CH_2NH_2), 22.2 (NHCO*C*H_3). FABMS: Anal. Calcd for $[C_{28}H_{49}N_3O_{19}$ (731)+H$]^+$, 732. Found: *m*/*z* 732.

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