ELSEVIER

www.elsevier.nl/locate/carres

Carbohydrate Research 329 (2000) 717–730

CARBOHYDRATE RESEARCH

Synthesis of 3-*O*-sulfoglucuronyl lacto-*N*-neotetraose 2-aminoethyl glycoside and biotinylated neoglycoconjugates thereof☆

Andrei V. Kornilov, Andrey A. Sherman, Leonid O. Kononov, Alexander S. Shashkov, Nikolay E. Nifant'ev *

N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninsky prospect 47, Moscow 117913, Russia

Received 14 April 2000; accepted 15 August 2000

Abstract

The 2-aminoethyl glycoside of pentasaccharide 3-*O*-sulfo-GlcA(β-1→3)Gal(β-1→4)GlcNAc(β-1→3)Gal(β-1→4)Glc(β (**1**) and its conjugates with biotin and biotinylated polyacrylic acid were synthesized as molecular probes to investigate the recognition of the HNK-1 epitope containing carbohydrates by proteins. Key steps in the first of two investigated schemes for the preparation of the target compound **1** were (a) assembling of the pentasaccharide backbone (compound **10**) by glycosylation of selectively substituted allyl glycoside of the trisaccharide GlcNAc(β-1→3)Gal(β-1→4)Glc(β with glucuronyl-galactose glycosyl donor, (b) transformation of the allyl aglycon in **10** into 2-azidoethyl one (to give **11**), (c) selective deprotection of the OH group at C-3 of the GlcA residue in **11** via saponification, intramolecular formation of 6,3-lacton (**13**) and its methanolysis, and (d) subsequent O-sulfation. The alternative scheme with the use of 2-azido-ethyl glycoside of the trisaccharide GlcNAc(β-1→3)Gal(β-1→4)Glc(β instead of the allyl glycoside **6** was less effective due to smaller yield at the step of pentasaccharide synthesis. Additionally to **1** the 2-aminoethyl glycosides of the oligosaccharides GlcA(β-1→3)Gal(β-1→4)GlcNAc(β-1→3)Gal(β-1→4)Glc(β, 3-*O*-sulfo-GlcA(β-1→3)Gal(β, and GlcA(β-1→3)Gal(β were also synthesized.

Keywords: HNK-1; Neoglycoconjugates; Glucuronic acid; Lactonization

1. Introduction

Natural carbohydrates that have the HNK-1 epitope participate in neurite outgrowth and in neural cells adhesion. They are the ligands for L- and P-selectins, and play important roles in other developmental processes of mammalian nervous system (see reviews [2–5]). This epitope is presented in glycolipids [6] with penta- or heptasaccharide chains 3-*O*-sulfo-GlcA(β1-[3)Gal(β-1→4)GlcNAc(β1-]$_n$3)-Gal(β-1→4)Glc(β ($n=1$, 2) and on several glycoproteins and proteoglycans [5] of neural tissues. In the case of glycoproteins the antigenic structure is variable and has been precisely described only for the carbohydrate chains of bovine peripheral nerve glycoprotein P0 [7] in which the trisaccharide fragment 3-*O*-sulfo-GlcA(β-1→3)Gal(β-1→4)GlcNAc is attached to bisected mannose core.

☆ Synthesis of oligosaccharides related to HNK-1 antigen, Part 3. For Part 2, see Ref. [1].

* Corresponding author. Fax: +7-95-1358784.
E-mail address: nen@ioc.ac.ru (N.E. Nifant'ev).

0008-6215/00/$ - see front matter
PII: S0008-6215(00)00258-5

In order to prepare the tools for investigation of proteins which recognize HNK-1 carbohydrates, we have performed systematic synthesis of related oligosaccharides and neoglycoconjugates. In this paper we describe the synthesis of di- and pentasaccharide 2-aminoethyl glycosides **1**–**4**, and of the conjugates of **1** with biotin (**24**) and biotinylated polyacrylic acid (**27**). The pentasaccharide 3-*O*-sulfo-GlcA(β-1→3)Gal(β-1→4)GlcNAc(β-1→3)Gal(β-1→4)Glc was previously synthesized as ceramide [8–10] and propyl [1] glycosides.

[3 - *O* - sulfo - GlcA(β - 1→3)Gal(β - 1→4)GlcNAc(β - 1→3)Gal(β - 1→4)Glc(β1 - $O(CH_2)_2NH_2$]$^-$ Na_2^+ **1**

[GlcA(β - 1→3)Gal(β - 1→4)GlcNAc(β - 1→3)-Gal(β-1→4)Glc(β1-$O(CH_2)_2NH_2$]$^-$ Na^+ **2**

[3 - *O* - sulfo - GlcA(β - 1→3)Gal(β1 - $O(CH_2)_2NH_2$]$^-$ Na_2^+ **3**

[GlcA(β-1→3)Gal(β1-$O(CH_2)_2NH_2$]$^-$ Na^+ **4**

2. Results and discussion

Preparation of the target pentasaccharide **1** was performed by two synthetic schemes with the use of disaccharide glycosyl donor **9** [1] and trisaccharide glycosyl acceptor blocks **6** [1] and **8** as main precursors (Scheme 1). They were selected according to retrosynthetic analysis of structure **1** which revealed the efficiency of the [2 + 3]. The strategy used to build the pentasaccharide backbone was also used by Hasegawa and co-workers [9].

The synthesis of pentasaccharide **1** (Scheme 1) included the selective deprotection of the OH group at C-3 of the glucuronic acid (GlcA) residue via saponification and the 6,3-lactonization–methanolysis procedure [1,11] (**12**→**13**→**14**) prior to O-sulfation. This key feature of the strategy allowed us to use the readily available disaccharide trichloroacetimidate **9** [1] which does not contain *any* temporary protecting group at *O*-3 of the GlcA residue (instead of much less available selectively protected derivatives which were used in first syntheses of HNK-1 related oligosaccharides [8,9]).

The second distinct feature in the synthesis of target compound **1** was the use of the allyl aglycon as the pre-spacer which provided good flexibility during assembling of the pentasaccharide backbone and permitted introduction and removal of necessary protecting groups (Scheme 1). The 2-azidoethyl group is known [12] to be a direct precursor of the 2-aminoethyl spacer. To transform the allyl group into 2-azidoethyl we used a convenient procedure what included sequences ozonolysis→reduction→sulfonylation [13] and sulfonate→azide [14] according to known procedures which we adopted to the cases of glucuronic and sialic acid derivatives [15].

Along these lines we investigated two ways (Routes A and B in the Scheme 1) for the preparation of substituted pentasaccharide 2-azidoethyl glycoside **11** which was a direct precursor of target compound **1**. In Route **A** the pentasaccharide allyl glycoside **10** was prepared first and then transformed into **11**. In Route **B** functionalization of allyl aglycon was performed at an earlier stage during the synthesis of trisaccharide block **8**.

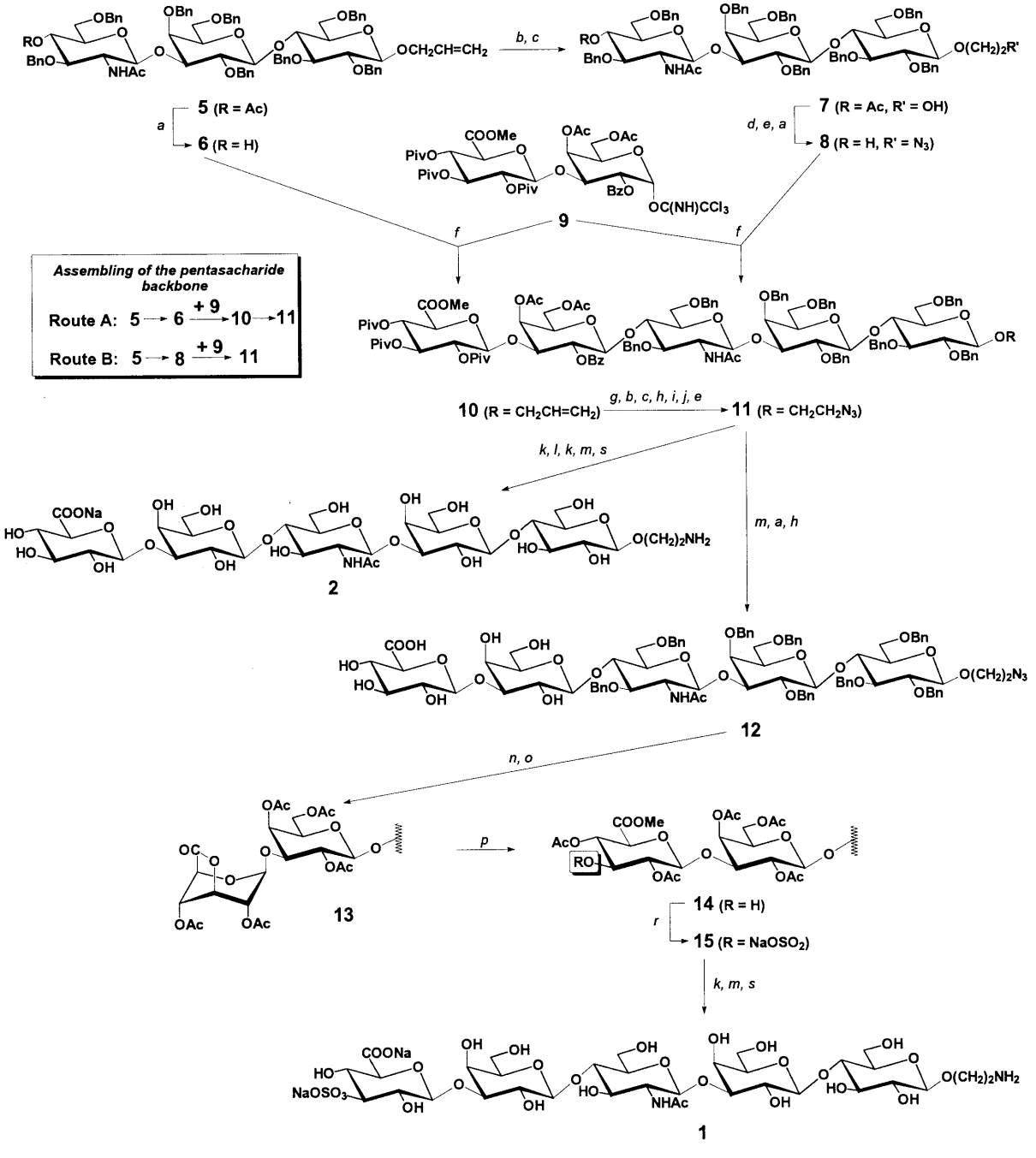
Ozonolysis of trisaccharide allyl glycoside **5** [1] and subsequent reduction of ozonides with excess of sodium borohydride afforded 2-hydroxyethyl glycoside **7**. Its mesylation, treatment with NaN_3 and O-deacetylation afforded to the corresponding 2-azidoethyl glycoside **8** in the overall yield of 77%.

Coupling of the trichloroacetimidate **9** with the trisaccharides **6** [1] and **8** in dichloromethane under promotion with trimethylsilyltriflate (TMSOTf) afforded the pentasaccharide allyl and 2-azidoethyl glycosides **10** (identical to the previously obtained compound [1]) and **11** in 82 and 62% yields, respectively. The β configuration of the newly formed glycosidic bonds was deduced from the characteristic values of the respective spin–spin coupling constants $J_{1,2}$ (8.0 and 7.4 Hz) in the ^{1}H NMR spectra of **10** and **11**.

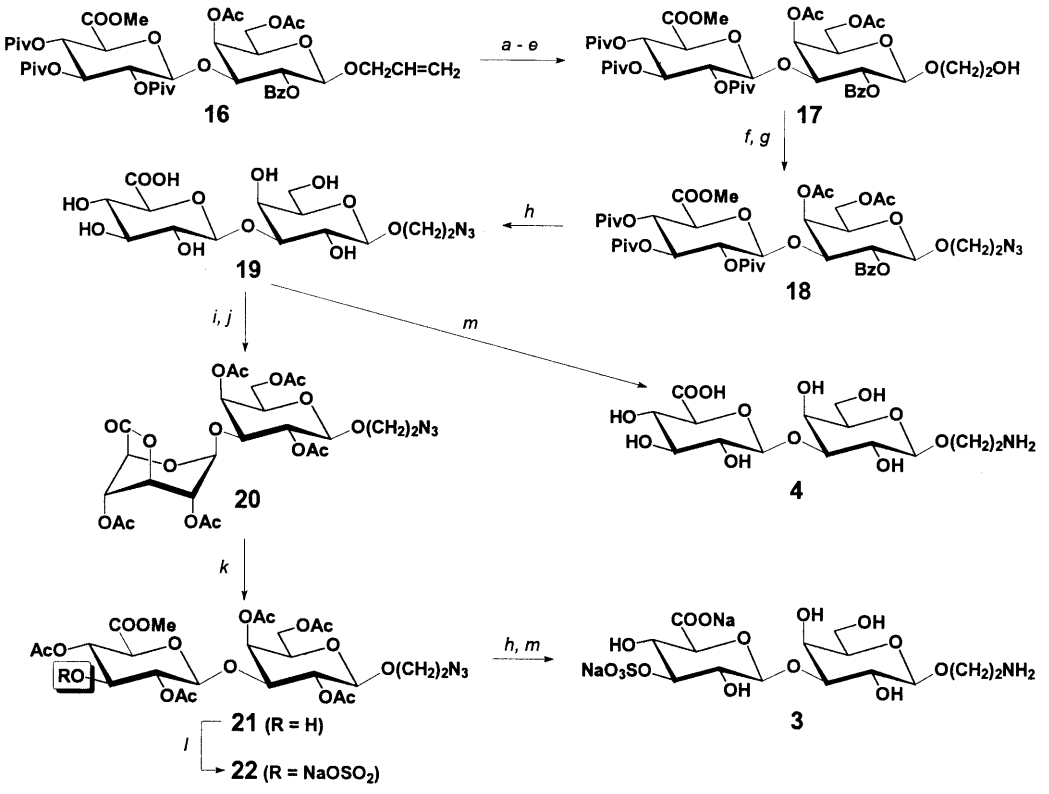
Before transforming the allyl aglycon in **10** into 2-azidoethyl group, the CO_2Me group in pentasaccharide **10** was converted into its lithium salt under treatment with LiI in Py [16] in order to avoid the undesired reduction of C-6 in GlcA residue during the treatment of ozonides with sodium borohydride (Scheme

1). The salt was subjected to ozonolysis, reduction, O-methylation with CH_2N_2, tosylation, and subsequent treatment with NaN_3 to give **11** in 53% yield over nine steps along Route A. A shorter Route **B** gave a comparable but slightly lower overall yield (49%) of **11** for six steps from **5**. The diminished yield in the latter case is due to less effective glycosylation by imidate **9** of 2-azidoethyl glycoside **8** than of respective allyl glycoside **6**.

Selective deprotection of the OH group at C-3 of the GlcA residue in pentasaccharide **11** was the next step of the preparation of target compound **1**. It was achieved using saponification of **11** and further 6,3-lactonization, exhaustive O-acetylation and subsequent



Scheme 1. Reagents and conditions: (a) MeONa in MeOH; (b) O_3, −78 °C, CH_2Cl_2–MeOH; (c) $NaBH_4$, −78 °C; (d) MsCl, Et_3N; (e) NaN_3, 18-crown-6, DMF; (f) TMSOTf, CH_2Cl_2, MS 4 Å, −30 °C, 100 h; (g) LiI, Py, reflux; (h) treatment with 1 M H_2SO_4; (i) CH_2N_2, 0 °C, CH_2Cl_2; (j) TsCl, Py; (k) H_2, PdO/C; (l) CF_3COOEt, Et_3N; (m) LiOH in aq THF; (n) Ac_2O, 80 °C; (o) Ac_2O, Py, DMAP, rt; (p) MeOH, anhyd AcONa; (r) SO_3·Py, DMF; (s) MeONa in aq MeOH.



Scheme 2. Reagents and conditions: (a) LiI, Py, reflux; (b) O_3, −78 °C, CH_2Cl_2–MeOH; (c) $NaBH_4$, −78 °C; (d) treatment with 1 M H_2SO_4; (e) CH_2N_2, 0 °C, CH_2Cl_2; (f) MsCl, Et_3N; (g) NaN_3, 18-crown-6, DMF, rt; (h) LiOH in aq THF, NaOH in aq MeOH; (i) Ac_2O, 80 °C; (j) Ac_2O, Py, DMAP, rt; (k) MeOH, AcONa; (l) SO_3·Py, DMF; (m) H_2, PdO/C.

opening of lacton ring by methanolysis under treatment with sodium acetate and methanol (**11** → **12** → **13** → **14**). For this sequence of reactions we used an experimental procedure, which was previously applied [1] for the transformation of pentasaccharide allyl glycoside **10** and gave its 3‴′-hydroxy derivative in 74% overall yield [1].

Thus pentasaccharide **11** was saponified first to give the acid **12**, which was lactonized under treatment with acetic anhydride at 70 °C and then O-acetylated by addition of Py and a catalytic amount of 4-(*N*,*N*-dimethylamino)pyridine added to the reaction mixture. The thus formed crude lactone **13** was subjected to methanolysis to give the target monohydroxy derivative **14** in 35% overall yield from **11**. The presence of the free OH group at C-3 of GlcA residue in **14** followed from the high-field position of H-3‴′ resonance (δ 3.70 ppm) and the low-field values of the chemical shifts of H-2‴′ and H-4‴′ protons (δ 4.81 and 5.14 ppm, respectively) in the ^{1}H NMR spectrum.

Treatment of the monohydroxy derivative **14** with SO_3·Py in DMF and subsequent gel filtration on an LH-20 column in methanol gave the O-sulfated compound **15** in 95% yield (Scheme 1). Location of the sulfate at C-3 of the GlcA residue in **15** was confirmed by characteristic low-field shift of the H-3 resonance (δ 3.70 → 4.62 ppm) in the ^{1}H NMR spectrum when compared with the data for alcohol **14**. The sulfate **15** was subjected to hydrogenolysis and subsequent saponification to give the target pentasaccharide **1** (67%) which was purified by gel chromatography. Characteristic low-field positions of H-3‴′ resonance (δ 4.33 ppm) in the ^{1}H NMR and of C-3‴′ resonance (δ 84.8 ppm) in the ^{13}C NMR spectra of **1** confirmed the location of the sulfate group at C-3 of the GlcA residue.

In a similar way to the preparation of **11**, **15** and **1**, the disaccharide allyl glycoside **16** [17] was transformed into its 2-azidoethyl glycoside **18** in 65% overall yield in six steps, monohydroxy derivative **21** (53%), its sulfate **22** (85%) and target 2-aminoethyl glycosides **3**

and **4** (Scheme 2). Compound **4** as the Na salt has been previously obtained [18].

Aiming to obtain **2** we tried to perform hydrogenolysis of the pentasaccharide **11**. It was unsuccessful in different mixtures of solvents (THF–AcOH (1%); EtOH–AcOH (1%)) and even under heating to 35–45 °C. This problem could be connected with catalyst passivation due to interaction with the aminoethyl fragment formed. Finally the hydrogenolysis was carried out in two steps: after the completing of the reduction of N_3 group in **11** the mixture was treated with ethyl trifluoroacetate. The N-trifluoroacetylated product formed was separated by flash column chromatography and subjected to further hydrogenolysis and subsequent saponification to give spacered pentasaccharide **2** which was purified by gel chromatography (Scheme 1).

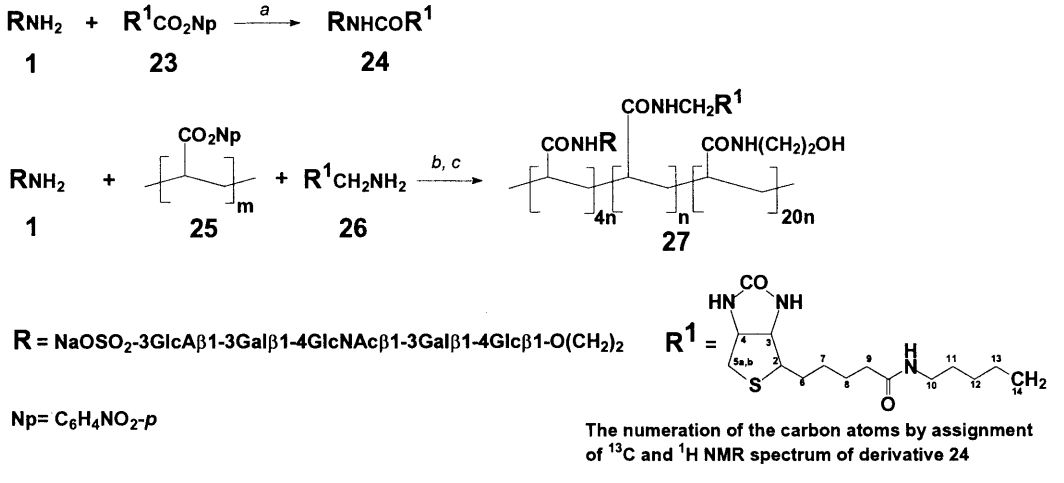
Condensation of the spacer-armed pentasaccharide **1** with the *p*-nitrophenyl ester of ω-*N*-biotinyl capronic acid **23** gave quantitatively the monovalent biotinylated glycoconjugate **24** (Scheme 3) which was purified by gel chromatography. The polyvalent biotinylated conjugate (**27**) of spacered pentasaccharide **1** was synthesized according to the original procedure by Bovin et al. [19] by treatment of poly(*p*-nitrophenyl acrylate) **25** with 0.2 equiv of **1** and 0.05 equiv of *N*-biotinyl hexamethylendiamine **26** followed by capping the unreacted activated carboxylic groups of the polyacrylic backbone with an excess of 2-aminoethanol (Scheme 3).

The structure of conjugates **24** and **27** was confirmed by ^{1}H and ^{13}C NMR spectroscopy. Particularly, the location of the sulfate group at C-3 of the GlcA residue was confirmed by the characteristic values of H-3'''' resonances in ^{1}H NMR spectra (δ 4.26 and 4.28 ppm for compounds **24** and **27**, respectively). The saccharide patterns in the ^{13}C NMR spectra of conjugates **24** and **27** agreed well with those of the ^{13}C NMR spectra of starting material **1**. The only marked difference occurred in the case of conjugate **27** for C-1 of the Glc residue which was observed as a broad signal due to the complex surrounding the matrix.

In conclusion, we have accomplished the first synthesis of a spacer-armed oligosaccharides **1**–**4** together with mono- and polyvalent biotinylated neoglycoconjugates **24** and **27**. These compounds were used in the investigations of a mechanism of long-term regulation of perisomatic inhibition in the hyppocampus and assessment of selectins [20] and anti-CD57 Abs [21] carbohydrate specificities.

3. Experimental

NMR spectra were recorded with Bruker DRX-500 and Bruker AM-300 instruments, assignments were made by 2D correlation spectroscopy and polarization-transfer experiments, and only characteristic signals are included in Section 3. For solutions in D_2O, *tert*-BuOH served as the reference at 1.23 and



Scheme 3. Reagents and conditions: (a) DMSO, Et_3N; (b) **1** (0.2 equiv), $R^1CH_2NH_2$ (0.05 equiv), Et_3N, 1:1 DMF–DMSO (v/v), 40 °C, 24 h; (c) $HO(CH_2)_2NH_2$ (excess), Et_3N, 24 h.

31.1 ppm for ^1H and ^{13}C NMR, respectively. Mass spectra were recorded using matrix-assisted laser-desorption ionization time-of-flight (MALDI-TOF) on a Vision 2000 mass spectrometer. The optical rotation values were measured on a JASCO DIP-360 digital polarimeter at 22–26 °C. TLC was performed on Kieselgel 60 plates (Merck, Germany); the spots were visualized by spraying with 10% H_3PO_4 in EtOH and subsequent heating to ~150 °C. Column chromatography was carried out on a Silica Woelm 32–63 μm chromatograph (Woelm Pharma) using the solvent system specified. Ion-exchange chromatography was performed on a DEAE-Spheron 25–40 μm (Chemapol) column (2 × 15 cm) using a gradient elution from water to 20% AcOH at a flow rate of 3 mL/min. Gel filtration was performed on a TSK-HW40s (TOYO SODA) column (1.5 × 90 cm) by elution with 0.1 M aq AcOH at a flow rate of 0.8 mL/min, a Sephadex G-10 column (2 × 100 cm) by elution with water at a flow rate 2 mL/min, and a Sephadex LH-20 column (2 × 40 cm) by elution with either MeOH or 1:1 CH_3CN–water at a flow rate of 1 mL/min. HPLC was performed on a 7 μm Silica Gel column (1 × 20 cm) at a flow rate of 4 mL/min. The hydrogenolysis was performed over 10% PdO/C (E. Merck) at atmospheric pressure and 35 °C. Biotin derivatives **23** and **26** and poly(*p*-nitrophenyl acrylate) **25** were obtained from Syntesome GmbH (Muenchen).

*2-Hydroxyethyl (2-acetamido-4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-*D*-glucopyranoside* (**7**).—Ozone was bubbled through a stirred solution of **6** [1] (476 mg, 0.35 mmol) in 2:1 anhyd MeOH–CH_2Cl_2 (30 mL) at −78 °C until a blue color persisted, the excess of ozone was removed with a stream of Ar for 15 min at −78 °C, and $NaBH_4$ (200 mg, 13.3 mmol) was added. The solution was allowed to attain −5 °C over a period of 1.5 h, and the next portion of $NaBH_4$ (133 mg, 3.5 mmol) was added. The mixture was stirred for 1 h at 5–10 °C, neutralized with AcOH (1 mL), and concd to dryness. The solution of the residue in CH_2Cl_2 was washed with 1 M H_2SO_4 (15 mL) and brine (20 mL), filtered through cotton wool and concd. Column chromatography (3:2 EtOAc–petroleum ether) of the residue gave **7** as a white foam (423 mg, 90%): $[\alpha]_D$ +6° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.07 (dd, 1 H, $J_{3'',4''}$, $J_{4'',5''}$ 10.0 Hz, H-4″), 5.02 (d, 1 H, $J_{1'',2''}$ 8.9 Hz, H-1″), 4.39 (d, 1 H, $J_{1,2}$ 9.2 Hz, H-1), 4.33 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.06 (dd, 1 H, $J_{2'',3''}$, $J_{3'',4''}$ 10.0 Hz, H-3″), 4.00 (d, 1 H, $J_{3',4'}$ 3.2 Hz, H-4′), 3.89 (dd, 1 H, $J_{3,4}$, $J_{4,5}$ 9.2 Hz, H-4), 3.71 (dd, 1 H, $J_{1',2'}$ 9.3 Hz, $J_{2',3'}$ 10.5 Hz, H-2′), 3.71 (dd, 1 H, H-5″), 3.69 (m, 2 H, H-6a, H-6b), 3.61 (dd, 1 H, H-3′), 3.56 (b dd, 1 H, H-2″), 3.55 (dd, 1 H, H-3, $J_{2,3}$ 10.1 Hz, H-3), 3.43 (dd, 1 H, H-2), 3.36 (m, 1 H, H-5); ^{13}C NMR ($CDCl_3$): δ 62.41 (CH_2OH), 57.02 (C-2″), 23.08 (CH_3CONH), 20.88 (CH_3COO); MALDI-TOF-MS: Calcd for $[M+Na]^+$: 1374.6. Found 1375.2. Anal. Calcd for $C_{80}H_{89}NO_{18}$: C, 71.04; H, 6.63. Found: C, 71.25; H, 6.81.

*2-Azidoethyl (2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-*D*-glucopyranoside* (**8**).—To a solution of **7** (737 mg, 0.55 mmol) and Et_3N (0.4 mL, 5.50 mmol) in anhyd CH_2Cl_2 (10 mL) was added dropwise methanesulfonyl chloride (MsCl) (0.09 mL, 1.12 mmol) at −10 °C. The reaction mixture was diluted with CH_2Cl_2 and washed with satd aq $NaHCO_3$ (20 mL) and brine (20 mL), filtered through cotton wool, concd and filtered through a pad of Silica Gel (EtOAc–petroleum ether, 1:1). To the solution of the residue (744 mg) in anhyd DMF (5 mL), 18-crown-6 (150 mg, 0.56 mmol) and NaN_3 (432 mg, 6.60 mmol) were added and the reaction mixture was stirred for 50 h at rt. EA (50 mL) was added and the solution was washed with water (2 × 30 mL), filtered through cotton wool, concd and filtered through a pad of Silica Gel (EtOAc–petroleum ether, 1:1). To the solution of the crude mesylate thus obtained (720 mg) in anhyd MeOH (10 mL) MeONa (80 mg, 1.48 mmol) was added and the mixture was kept for 2 h at rt, neutralized with KU-2 (H^+) cation-exchange resin, filtered and concd. Column chromatography (2:3 EtOAc–petroleum ether) gave amorphous **8** (545 mg, 79%): $[\alpha]_D$ −6° (*c* 3, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 4.87 (d, 1 H, $J_{1'',2''}$ 8.1 Hz, H-1″), 4.45 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.41 (d, 1 H,

$J_{1,2}$ 7.5 Hz, H-1), 4.03 (m, 1 H, $OCH_2CH_2N_3$), 3.99 (dd, 1 H, $J_{3,4}$, $J_{4,5}$ 9.5 Hz, H-4), 3.97 (b d, 1 H, $J_{3',4'}$ 3.5 Hz, H-4′), 3.74 (b dd, 1 H, $J_{2'',3''}$ 10.5 Hz, H-2″), 3.73 (dd, 1 H, $J_{1',2'}$ 8.9 Hz, $J_{2',3'}$ 10.0 Hz, H-2′), 3.68-3.79 (m, 2 H, H-6a, H-6b), 3.71 (m, 1 H, $OCH_2CH_2N_3$), 3.65 (dd, 1 H, H-3′), 3.58 (dd, 1 H, $J_{2,3}$ 9.5 Hz, H-3), 3.55–3.41 (m, 2 H, $OCH_2CH_2N_3$), 3.46 (dd, 1 H, H-2), 3.35 (m, 1 H, H-5); ^{13}C NMR ($CDCl_3$): δ 55.69 (C-2″), 50.90 (CH_2N_3), 23.07 (CH_3CONH); MALDI-TOF-MS: Calcd for $[C_{78}H_{86}N_4O_{16} + Na]^+$: 1357.6. Found 1358.6.

*2-Azidoethyl (methyl 2,3,4-tri-*O*-pivaloyl-β-*D*-glucopyranosyluronate)-(1→3)-(4,6-di-*O*-acetyl-2-*O*-benzoyl-β-*D*-galactopyranosyl)-(1→4)-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-*D*-glucopyranoside* (**11**)

Method A. A solution of **10** [1] (116 mg, 0.05 mmol) and anhyd LiI (50 mg, 0.37 mmol) in dry Py (4 mL) was refluxed for 2 h under dry Ar and concd. A solution of the residue in CH_2Cl_2 (20 mL) was washed with 1 M H_2SO_4 (10 mL), satd aq $NaHCO_3$ (10 mL), and satd aq NaCl (10 mL), filtered through cotton wool and concd. The crude salt thus obtained was dissolved in a 2:1 mixture of anhyd MeOH–CH_2Cl_2 (7.5 mL) and ozone was bubbled through the solution at −78 °C until a blue color persisted. The excess of ozone was removed with a stream of Ar for 15 min at −78 °C, $NaBH_4$ (76 mg, 2.0 mmol) was added, and the mixture was allowed to attain −5 °C over a 1 h period. Another portion of $NaBH_4$ (38 mg, 1.0 mmol) was added, the mixture was kept for 1 h at −5 °C, neutralized with AcOH, and concd to dryness. A solution of the residue in CH_2Cl_2 (10 mL) was filtered, diluted with CH_2Cl_2 (10 mL), washed with 1 M H_2SO_4 (15 mL), satd aq NaCl (20 mL), filtered through cotton wool and concd. The residue was dissolved in 1:5 anhyd MeOH–CH_2Cl_2 (6 mL) and a solution of CH_2N_2 in Et_2O was added at 0 °C until a yellow color persisted. The mixture was neutralized with AcOH (1 mL), concd and dried in vacuo. The monohydroxy derivative thus obtained was dissolved in dry Py, TosCl (95 mg, 0.5 mmol) was added, the mixture was kept for 20 h at rt, MeOH (3 mL) was added for 1 h and the reaction mixture was coevaporated with toluene (5 mL). Chromatography on a short column of Silica Gel (1:1 benzene–EtOAc) gave the crude tosylate (65 mg, 52%) and the corresponding 2-hydroxyethyl glycoside (21 mg, 18%), which was recovered and recycled. The tosylate thus obtained (86 mg) was stirred with NaN_3 (32 mg, 0.5 mmol) and 18-crown-6 (20 mg) in DMF (2 mL) for 72 h at rt, then diluted with EtOAc (15 mL), washed with water (10 mL), concd and coevaporated with toluene (10 mL). Column chromatography of the residue (1:1 benzene–EtOAc) gave the syrup **11** (74 mg, 64%).

Method B. A solution of **9** [1] (130 mg, 0.10 mmol) and **8** (135 mg, 0.14 mmol) in anhyd CH_2Cl_2 (3 mL) was stirred over powdered molecular sieves (800 mg) under dry Ar at rt for 1 h and was then cooled to −30 °C. Trimethylsilyl triflate (8 μL, 44 μmol) was added and the reaction mixture was kept at −30 °C for 80 h. Triethylamine (0.1 mL) was added, the reaction mixture was diluted with CH_2Cl_2 (20 mL), filtered through a pad of Celite, washed with satd aq $NaHCO_3$ (15 mL) and water, filtered through a cotton wool and concd. HPLC (1:20 acetone–toluene) afforded **11** (132 mg, 62%) as a white foam: $[\alpha]_D$ −11° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.45 (d, 1 H, $J_{3''',4'''}$ 3.5 Hz, H-4‴), 5.35 (dd, 1 H, $J_{1''',2'''}$ 8.2 Hz, $J_{2''',3'''}$ 10.3 Hz, H-2‴), 5.26 (dd, 1 H, $J_{3'''',4''''}$, $J_{4'''',5''''}$ 9.7 Hz, H-4⁗), 5.16 (dd, 1 H, $J_{2'''',3''''}$ 9.7 Hz, H-3⁗), 4.92 (dd, 1 H, $J_{1'''',2''''}$ 7.3 Hz, H-2⁗), 4.70 (d, 1 H, H-1⁗), 4.54 (d, 1 H, $J_{1''',2'''}$ 8.2 Hz, H-1‴), 4.51 (d, 1 H, $J_{1'',2''}$ 7.8 Hz, H-1″), 4.38 (d, 1 H, $J_{1,2}$ 7.0 Hz, H-1), 4.36 (d, 1 H, $J_{1',2'}$ 8.6 Hz, H-1′), 4.12 (dd, 1 H, $J_{2''',3'''}$ 7.9 Hz, H-3‴), 4.00 (d, 1 H, H-5⁗), 3.89 (dd, 1 H, $J_{3,4}$, $J_{4,5}$ 9.6 Hz, H-4), 3.80 (s, 3 H, $COOCH_3$), 3.79 (d, 1 H, $J_{3',4'}$ 2.8 Hz, H-4′), 3.62 (dd, 1 H, $J_{2',3'}$ 9.6 Hz, H-2′), 3.54 (dd, 1 H, $J_{2,3}$ 9.6 Hz, H-3), 3.42 (dd, 1 H, H-2), 3.11 (dd, 1 H, H-3′); ^{13}C NMR ($CDCl_3$): δ 61.44 (C-6‴), 53.65 (C-2″), 52.74 ($COOCH_3$), 50.93 (CH_2N_3); MALDI-TOF-MS: Calcd for $[M + Na]^+$: 2149.9. Found: 2151.6. Anal. Calcd for $C_{117}H_{138}N_4O_{33}$: C, 66.03; H, 6.54; N, 2.63. Found: C, 66.09; H, 6.56; N, 2.61.

*2-Azidoethyl (β-*D*-glucopyranosyluronic acid)-(1→3)-(β-*D*-galactopyranosyl)-(1→4)-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-*D*-*

glucopyranosyl)-(1→3)-(2,4,6-tri-O-*benzyl-β*-D-*galactopyranosyl)-(1→4)-2,3,6-tri*-O-*benzyl-β*-D-*glucopyranoside* (**12**).—Lithium hydroxide (1 M aq, 0.34 mL) was added to a solution of **11** (101 mg, 47 μmol) in 8:1 THF–water (9 mL) at −10 °C, the mixture was allowed to attain slowly rt, and was kept overnight. Acetic acid (0.02 mL) was added, and the reaction mixture was concd. The residue was coevaporated with toluene (3 × 10 mL), dissolved in MeOH (3 mL), MeONa (40 mg, 0.74 mmol) was added, and the mixture was kept for 100 h at rt, neutralized with AcOH (0.1 mL) and concd. Toluene (20 mL) was added, the solids were filtered off through a pad of Celite and the pad was washed with toluene (20 mL). The filtrate was concd and the resulting sodium salt of **12** was converted into the free acid by treatment with 90% aq TFA (0.3 mL) in CH_2Cl_2 (7 mL) for 1 h at rt. Coevaporation with toluene (3 × 20 mL) and MeOH (3 × 15 mL) followed by gel filtration (Sephadex, LH-20) in MeOH gave **12** (79 mg, 99%) as a white solid: $[\alpha]_D$ −5° (*c* 1, MeOH); ^{13}C NMR ($CDCl_3$ + two drops CD_3OD): δ 104.0, 103.3, 102.2, 102.0 and 102.2 (five anomeric carbons), 50.8 (CH_2N_3), 22.4 ($NHCOCH_3$). Anal. Calcd for $C_{90}H_{104}N_4O_{27}\cdot 3\,H_2O$: C, 62.56; H, 6.42; N, 3.24. Found: C, 62.24; H, 6.08; N, 2.97.

2-Azidoethyl (methyl 2,4-di-O-*acetyl-β*-D-*glucopyranosyluronate)-(1→3)-(2,4,6-tri*-O-*acetyl-β*-D-*galactopyranosyl)-(1→4)-(2-acetamido-3,6-di*-O-*benzyl-2-deoxy-β*-D-*glucopyranosyl)-(1→3)-(2,4,6-tri*-O-*benzyl-β*-D-*galactopyranosyl)-(1→4)-2,3,6-tri*-O-*benzyl-β*-D-*glucopyranoside* (**14**).—A solution of **12** (63 mg, 38 μmol) in Ac_2O (6 mL) was kept at 70 °C for 2 h, then cooled to rt, and Py (2 mL) together with a catalytic amount of DMAP were added. After 48 h the reaction mixture was coevaporated with toluene (2 × 5 mL) at 30 °C. MeOH (10 mL) was added and the mixture was kept overnight at rt, quenched with AcOH (1 mL) and concd at rt. Column chromatography (1:4 acetone–toluene) gave **14** (25 mg, 35%) as a white solid: $[\alpha]_D$ −7° (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.35 (d, 1 H, $J_{3''',4'''}$ 3.1 Hz, H-4‴), 5.14 (dd, 1 H, $J_{3'''',4''''}$, $J_{4'''',5''''}$ 9.4 Hz, H-4⁗), 5.13 (dd, 1 H, $J_{1''',2'''}$ 8.4 Hz, $J_{2''',3'''}$ 9.5 Hz, H-2‴), 4.85 (d, 1 H, $J_{1'',2''}$ 7.1 Hz, H-1″), 4.81 (dd, 1 H, $J_{1'''',2''''}$ 9.2 Hz, H-2⁗), 4.67 (d, 1 H, H-1⁗), 4.48 (d, 1 H, $J_{1''',2'''}$ 8.6 Hz, H-1‴), 4.43 (d, 1 H, $J_{1',2'}$ 7.6 Hz, H-1′), 4.37 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 4.03 (m, 1 H, $OCH_2CH_2N_3$), 4.01 (1 H, H-4″), 3.97 (d, 1 H, H-5⁗), 3.95 (d, 1 H, $J_{3',4'}$ 2.8 Hz, H-4′), 3.94 (dd, 1 H, $J_{3,4} = J_{4,5}$ 8.6 Hz, H-4), 3.91 (1 H, H-2″), 3.89 (m, 1 H, $OCH_2CH_2N_3$), 3.80 (s, 3 H, $COOCH_3$), 3.77 (1 H, H-3‴), 3.77 (2 H, H-6a″, H-6b″), 3.72 (1 H, H-6b), 3.71 (1 H, H-2′), 3.70 (1 H, H-3⁗), 3.70 (1 H, H-3″), 3.63 (m, 2 H, $OCH_2CH_2N_3$), 3.62 (1 H, H-5‴), 3.62 (1 H, H-6a), 3.60 (dd, 1 H, $J_{2',3'}$ 9.4 Hz, H-3′), 3.59 (1 H, H-5″), 3.53 (dd, 1 H, $J_{2,3}$ 8.6 Hz, H-3), 3.41 (dd, 1 H, H-2), 3.30 (b dd, 1 H, H-5); ^{13}C NMR ($CDCl_3$): δ 61.53 (C-6‴), 54.27 (C-2″), 52.82 ($COOCH_3$), 50.90 (CH_2N_3). MALDI-TOF-MS: Calcd for $[M + Na]^+$: 1919.8. Found: 1920.0. Anal. Calcd for $C_{101}H_{116}N_4O_{32}$: C, 63.91; H, 6.16; N, 2.95. Found: C, 64.27; H, 6.74; N, 2.50.

2-Azidoethyl (methyl 2,4-di-O-*acetyl-3*-O-*sulfo-β*-D-*glucopyranosyluronate)-(1→3)-(2,4,6-tri*-O-*acetyl-β*-D-*galactopyranosyl)-(1→4)-(2-acetamido-3,6-di*-O-*benzyl-2-deoxy-β*-D-*glucopyranosyl)-(1→3)-(2,4,6-tri*-O-*benzyl-β*-D-*galactopyranosyl)-(1→4)-2,3,6-tri*-O-*benzyl-β*-D-*glucopyranoside*, *sodium salt* (**15**).—A solution of **14** (37 mg, 0.02 mmol) in DMF (0.5 mL) was treated with $SO_3\cdot$Py (17 mg, 0.11 mmol) for 1 h at rt, then quenched with $NaHCO_3$ (80 mg), and stirred for 1 h. The solid was filtered and washed with MeOH (10 mL). The filtrate was treated with KU-2 (Na^+) cation-exchange resin for 20 min, the resin was filtered off, and the filtrate was concd. Gel chromatography on the Sephadex LH-20 column in MeOH afforded amorphous **15** (36 mg, 95%): $[\alpha]_D$ −17° (*c* 1.5, MeOH); ^{1}H NMR (CD_3OD): δ 5.33 (d, 1 H, $J_{3''',4'''}$ 3.4 Hz, H-4‴), 5.06 (dd, 1 H, $J_{3'''',4''''}$ 9.3 Hz, $J_{4'''',5''''}$ 10.1 Hz, H-4⁗), 5.06 (dd, 1 H, $J_{1''',2'''}$ 8.4 Hz, $J_{2''',3'''}$ 9.5 Hz, H-2‴), 4.85 (dd, 1 H, $J_{1'''',2''''}$, $J_{2'''',3''''}$ 9.3 Hz, H-2⁗), 4.79 (d, 1 H, H-1″), 4.76 (d, 1 H, $J_{1'''',2''''}$ 9.3 Hz, H-1⁗), 4.62 (dd, 1 H, H-3⁗), 4.56 (d, 1 H, $J_{1''',2'''}$ 8.8 Hz, H-1‴), 4.38 (d, 1 H, H-1′), 4.37 (d, 1 H, $J_{1,2}$ 8.7 Hz, H-1), 4.21 (d, 1 H, H-5⁗), 3.74 (s, 3 H, $COOCH_3$). ^{13}C NMR (CD_3OD): δ 63.02 (C-6‴), 56.87 (C-2″), 53.23 ($COOCH_3$), 52.17 (CH_2N_3);

MALDI-TOF-MS: Calcd for $[C_{101}H_{115}N_4NaO_{35}S + Na]^+$: 2021.7. Found: 2023.6. Calcd for $[M + K]^+$: 2038.6. Found: 2039.0.

*2-Hydroxyethyl (methyl 2,3,4-tri-*O*-pivaloyl-β-*D*-glucopyranosyluronate)-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-β-*D*-galactopyranoside* (**17**).—A solution of **16** [1] (851 mg, 1.0 mmol) and anhyd LiI (1.0 g, 5.82 mmol) in 20 mL of dry Py was refluxed for 5 h under dry Ar, and then concd. A solution of the residue in CH_2Cl_2 (30 mL) was washed with 1 M H_2SO_4 (15 mL), satd aq $NaHCO_3$ (15 mL), and satd aq NaCl (15 mL), filtered through cotton wool and concd. The salt thus obtained (908 mg) was dissolved in 2:1 anhyd MeOH–CH_2Cl_2 (30 mL) and ozone was bubbled through this solution at −78 °C until a blue color persisted. The excess of ozone was removed with a stream of Ar for 15 min at −78 °C, and $NaBH_4$ (784 mg, 20.8 mmol) was added. The mixture was then allowed to attain −5 °C over a 1 h period. Another portion of $NaBH_4$ (340 mg, 8.95 mmol) was added, the mixture was kept for 1 h at −5 °C, neutralized with AcOH (3 mL), and concd to dryness. A solution of the residue in CH_2Cl_2 (15 mL) was filtered, diluted with CH_2Cl_2 (50 mL), washed with 1 M H_2SO_4 (20 mL), satd aq NaCl (30 mL), filtered through cotton wool and concd. The residue was dissolved in 2:5 anhyd MeOH–CH_2Cl_2 (7 mL) and a solution of CH_2N_2 in Et_2O was added at 0 °C until the yellow color persisted. The mixture was neutralized with AcOH (1 mL), and concd to dryness. Column chromatography (3:2 EtOAc–petroleum ether) of the residue on Silica Gel gave **17** (813 mg, 95%) as a white foam, $[\alpha]_D$ +12° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.49 (d, 1 H, $J_{3,4}$ 3.2 Hz, H-4), 5.41 (dd, 1 H, $J_{1,2}$ 8.1 Hz, $J_{2,3}$ 9.8 Hz, H-2), 5.23 (dd, 1 H, $J_{3',4'}$, $J_{4',5'}$ 9.5 Hz, H-4′), 5.13 (dd, 1 H, $J_{2',3'}$ 9.5 Hz, H-3′), 4.93 (dd, 1 H, $J_{1',2'}$ 7.5 Hz, H-2′), 4.72 (d, 1 H, H-1′), 4.55 (d, 1 H, H-1), 4.27 (dd, 1 H, H-3), 4.22 (dd, 1 H, $J_{5,6b}$ 4.8 Hz, $J_{6a,6b}$ 11.4 Hz, H-6b), 4.10 (dd, 1 H, $J_{5,6a}$ 7.8 Hz, H-6a), 3.97 (m, 1 H, H-5), 3.96 (d, 1 H, H-5′), 3.79 (m, 2 H, OCH_2CH$_2$OH), 3.76 (s, 3 H, COOCH_3), 3.63 (m, 2 H, OCH$_2$CH_2OH), 2.15 and 2.09 (2s, 2 × 3 H, 2 × COCH_3), 1.10, 1.09 and 0.99 (3s, 3 × 9 H, 3 × COC(CH_3)$_3$); ^{13}C NMR ($CDCl_3$): δ 62.31 (CH$_2$OH), 62.01 (C-6), 52.76 (COO*C*H$_3$); MALDI-TOF-MS: Calcd for $[M + Na]^+$: 877.4. Found 876.9, 878.0. Anal. Calcd for $C_{41}H_{58}O_{19}$: C, 57.60; H, 6.84. Found: C, 57.68; H, 7.06.

*2-Azidoethyl (methyl 2,3,4-tri-*O*-pivaloyl-β-*D*-glucopyranosyluronate)-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-β-*D*-galactopyranoside* (**18**).—To a solution of **17** (737 mg, 0.55 mmol) and Et_3N (0.4 mL, 5.50 mmol) in anhyd CH_2Cl_2 (10 mL) was added dropwise MsCl (0.09 mL, 1.12 mmol) at −10 °C over a 10 min period. The reaction mixture was diluted with CH_2Cl_2, washed with satd aq $NaHCO_3$ (20 mL) and satd aq NaCl (20 mL), filtered through cotton wool, concd, and filtered through a pad of Silica Gel (1:1 EtOAc–petroleum ether). To the solution of the crude mesylate thus obtained (744 mg) in anhyd DMF (5 mL), 18-crown-6 (150 mg, 0.56 mmol) and NaN_3 (432 mg, 6.60 mmol) were added and the reaction mixture was stirred for 50 h at rt, then diluted with EtOAc (50 mL), washed with water (2 × 30 mL), filtered through cotton wool and concd. Column chromatography (3:2 EtOAc–petroleum ether) of the residue gave **18** (423 mg, 90%) as a white foam, $[\alpha]_D$ −1° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.49 (d, 1 H, $J_{3,4}$ 3.2 Hz, H-4), 5.41 (dd, 1 H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 9.0 Hz, H-2), 5.22 (dd, 1 H, $J_{3',4'}$, $J_{4',5'}$ 9.5 Hz, H-4′), 5.10 (dd, 1 H, $J_{2',3'}$ 9.5 Hz, H-3′), 4.92 (dd, 1 H, $J_{1',2'}$ 7.6 Hz, H-2′), 4.70 (d, 1 H, H-1′), 4.59 (d, 1 H, H-1), 4.26 (dd, 1 H, H-3), 4.18 (dd, 1 H, $J_{5,6b}$ 5.7 Hz, $J_{6a,6b}$ 11.5 Hz, H-6b), 4.12 (dd, 1 H, $J_{5,6a}$ 6.9 Hz, H-6a), 3.93 (m, 1 H, H-5), 3.93 (d, 1 H, H-5′), 3.92 (m, 1 H, OCH_2CH$_2$N$_3$), 3.75 (s, 3 H, COOCH_3), 3.61 (m, 1 H, OCH_2CH$_2$N$_3$), 3.35 (m, 1 H, OCH$_2$CH_2N$_3$), 3.28 (m, 1 H, OCH$_2$CH_2N$_3$); ^{13}C NMR ($CDCl_3$): δ 62.04 (C-6), 52.70 (COO*C*H$_3$), 50.71 (*C*H$_2$N$_3$); MALDI-TOF-MS: Calcd for $[M + Na]^+$: 902.4. Found 902.2. Anal. Calcd for $C_{41}H_{57}N_3O_{18}$: C, 55.97; H, 6.53. Found: C, 55.83; H, 6.39.

*2-Azidoethyl (β-*D*-glucopyranosyluronic acid)-(1→3)-β-*D*-galactopyranoside* (**19**).—To a solution of **18** (162 mg, 0.18 mmol) in a 10:1 mixture of THF–water (7.5 mL), LiOH (1 M aq, 0.5 mL) was added at −15 °C and the mixture was allowed to attain rt slowly and was kept overnight. The reaction mixture was

Table 1
^{1}H NMR data for compounds **1**–**4** and **19** (δ, ppm; J, Hz; D_2O, 293–315 K) [a]

Compound	Residue	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6a}$	$J_{5,6b}$	$J_{6a,6b}$
1	Glc	4.54	3.38	3.68	3.66	3.64	3.98	3.80	7.6	7.4					
	Gal	4.44	3.62	3.72	4.15	3.60	3.86	3.74	7.6		2.8				
	GlcNAc	4.72	3.79	3.74–3.76	3.74–3.76	3.61	3.94	3.86	8.1						
	Gal *	4.54	3.70	3.82	4.18	3.74	3.73–3.75	3.73–3.75	7.6		2.8				
	GlcA	4.74	3.60	4.33	3.71	3.77			7.7	9.0	9.0				
2	Glc	4.52	3.36	3.63	3.62	3.61	3.79	3.96	7.7	9.3					12.1
	Gal	4.41	3.56	3.71	4.14	3.62	3.70–3.75	3.70–3.75	7.9		3.2				
	GlcNAc	4.68	3.79	3.72	3.73	3.58	3.94	3.82	8.3				4.7		12.4
	Gal *	4.51	3.67	3.79	4.17	3.72	3.70–3.75	3.70–3.75	7.7		3.1				
	GlcA	4.65	3.39	3.49–3.51	3.49–3.51	3.70			7.8	8.2		9.8			
3	Gal	4.51	3.71	3.83	4.19	3.72	3.75	3.75	7.9	9.5	3.0				
	GlcA	4.76	3.62	4.33	3.72	3.80			8.0	8.5	9.0	9.0			
4	Gal	4.47	3.71	3.79	4.18	3.71	3.75–3.78	3.75–3.78	7.8	9.9	2.8				
	GlcA	4.67	3.41	3.50–3.53	3.50–3.53	3.75			7.8						
19	Gal	4.47	3.71	3.79	4.13	3.68	3.76	3.69	7.9	9.5	2.7				
	GlcA	4.75	3.44	3.55	3.61	4.01			7.9	8.3	9.0	9.4			

[a] Other signals: $OCH_2CH_2NH_2$ m, 1 H, δ 4.11–4.12 and m, 1 H, δ 3.92–3.97; $OCH_2CH_2NH_2$ t, 2 H, δ 3.25–3.27 ppm (for **1**–**4**). $OCH_2CH_2N_3$ m, 1 H, δ 3.76 and m, 1 H, δ 3.95; $OCH_2CH_2N_3$ t, δ 3.46 ppm (for **19**).

* Gal residue from the GlcA-Gal block.

Table 2
^{13}C NMR data for compounds **1**–**4** and **19** (δ, ppm; D_2O, 293–315 K) [a]

Compound	Residue	C-1	C-2	C-3	C-4	C-5	C-6
1	Glc	103.0	73.8	75.3	79.5	75.9	61.1
	Gal	104.0	71.1	83.1	69.4	76.0	62.1 [b]
	GlcNAc	103.7	56.3	73.3	79.5	75.7	61.1
	Gal *	103.6	71.2	83.3	69.2	76.2	62.0 [b]
	GlcA	104.2	73.1	84.8	71.5	77.2	175.8
2	Glc	102.6	73.3	74.9	79.0	75.5	60.7
	Gal	103.6	70.6	82.7	68.9	75.7 [c]	61.6
	GlcNAc	103.2	55.9	72.8	79.0	75.3	60.7
	Gal *	103.3	70.8	82.8	68.8	75.9 [c]	61.6
	GlcA	104.2	73.7	75.9	72.3	76.3	175.4
3	Gal	103.0	70.4	83.2	68.8	75.6	61.7
	GlcA	103.8	72.7	84.3	71.1	76.8	176.0
4	Gal	103.0	70.4	83.1	68.7	75.6	61.7
	GlcA	104.2	73.8	75.9	72.3	76.6	176.0
19	Gal	103.9	71.1	83.8	69.5	76.1	62.2
	GlcA	104.9	74.4	76.1	72.8	76.5	

[a] Other signals: $OCH_2CH_2NH_2$ 66.4–67.0; $OCH_2CH_2NH_2$ 40.1–40.6 (for compounds **1**–**4**). $OCH_2CH_2N_3$ 69.5; $OCH_2CH_2N_3$ 51.8 (for **19**).
[b] Signals can be interchanged.
[c] Signals can be interchanged.
* Gal residue from the GlcA-Gal block.

concd, dissolved in MeOH (10 mL) and treated with aq NaOH (2.5 M, 0.8 mL) at −5 °C for 48 h. The reaction mixture was then neutralized with KU-2 (H^+) cation-exchange resin, the resin was filtered off, and the filtrate was evaporated. Ion-exchange chromatography and subsequent freeze-drying afforded amorphous **19** (63 mg, 82%), $[\alpha]_D$ −24° (*c* 1, MeOH); for ^1H NMR data see Table 1, and for ^{13}C NMR data see Table 2; MALDI-TOF-MS: Calcd for $[C_{14}H_{23}N_3O_{12} + Na]^+$: 448.1. Found 448.2.

*2-Azidoethyl (methyl 2,4-di-*O*-acetyl-β-*D*-glucopyranosyluronate) - (1→3) - 2,4,6 - tri - O-acetyl-β-*D*-galactopyranoside* (**21**).—A suspension of **19** (83 mg, 0.20 mmol) in Ac_2O (5 mL) was kept at 80 °C for 1 h, then cooled to rt, and Py (3 mL) and a catalytic amount of DMAP (10 mg) were added. After 72 h the reaction mixture was coevaporated with toluene (2 × 5 mL) at 30 °C, dissolved in benzene, washed with satd aq NaCl and water, filtered through cotton wool, concd, and dried in vacuo. A solution of the crude lactone thus obtained was treated with anhyd AcONa (16 mg, 0.24 mmol) in anhyd MeOH (5 mL) overnight at rt, then quenched with KU-2 (H^+) cation-exchange resin. The resin was then filtered off, and the filtrate was concd. Column chromatography (1:3 acetone–toluene) of the residue on Silica Gel gave **21** (85 mg, 65%) as a white solid, $[\alpha]_D$ −27° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.37 (sd, 1 H, $J_{3,4}$ 3.5 Hz, H-4), 5.21 (dd, 1 H, $J_{1,2}$ 9.5 Hz, $J_{2,3}$ 11.0 Hz, H-2), 5.09 (t, 1 H, $J_{3',4'} = J_{4',5'}$ 10.9 Hz, H-4′), 4.70 (dd, 1 H, $J_{1',2'}$ 9.2 Hz, $J_{2',3'}$ 10.5 Hz, H-2′), 4.59 (d, 1 H, H-1′), 4.45 (d, 1 H, H-1), 4.16 (dd, 1 H, $J_{5,6b}$ 6.8 Hz, $J_{6a,6b}$ 13.6 Hz, H-6b), 4.05 (dd, 1 H, $J_{5,6a}$ 8.5 Hz, H-6a), 4.00 (m, 1 H, $OCH_2CH_2N_3$), 3.91 (d, 1 H, H-5′), 3.89 (dd, 1 H, H-3), 3.84 (m, 1 H, H-5), 3.76 (s, 3 H, $COOCH_3$), 3.69 (dd, 1 H, H-3′), 3.66 (m, 1 H, $OCH_2CH_2N_3$), 3.48 (m, 1 H, $OCH_2CH_2N_3$), 3.29 (m, 1 H, $OCH_2CH_2N_3$); ^{13}C NMR ($CDCl_3$): δ 100.64 (C-1), 100.28 (C-1′), 75.71 (C-3), 61.96 (C-6), 52.63 ($COOCH_3$), 50.42 (CH_2N_3); MALDI-TOF-MS: Calcd for $[M + Na]^+$: 672.2. Found 671.9. Anal. Calcd for $C_{25}H_{35}N_3O_{17}$: C, 46.23; H, 5.43. Found: C, 46.52; H, 5.31.

2-Azidoethyl (methyl 2,4-di-O-acetyl-3-O-sulfo-β-D-glucopyranosyluronate)-(1→3)-2,4,6-tri-O-acetyl-β-D-galactopyranoside, sodium salt (**22**).—A solution of **21** (76 mg, 0.12 mmol) in DMF (1 mL) was treated with SO_3·Py (90 mg, 0.57 mmol) for 2 h at rt, then quenched with $NaHCO_3$ (80 mg) and stirred for 1 h. The solid was filtered off, washed with MeOH (10 mL), and the filtrate was treated with KU-2 (Na^+) cation-exchange resin for 20 min. The resin was filtered off, and the filtrate was concd. Column chromatography (1:10 MeOH–CH_2Cl_2) of the residue on Silica Gel afforded amorphous **22** (75 mg, 85%), $[\alpha]_D$ −8° (*c* 1, $CHCl_3$); ^{1}H NMR (CD_3OD): δ 5.44 (d, 1 H, $J_{3,4}$ 3.3 Hz, H-4), 5.10 (dd, 1 H, $J_{1,2}$ 8.3 Hz, $J_{2,3}$ 9.9 Hz, H-2), 5.02 (dd, 1 H, $J_{3',4'}$, $J_{4',5'}$ 9.7 Hz, H-4′), 4.85 (d, 1 H, $J_{1',2'}$ 8.2 Hz, H-1′), 4.82 (dd, 1 H, $J_{2',3'}$ 8.2 Hz, H-2′), 4.62 (d, 1 H, H-1), 4.61 (dd, 1 H, H-3′), 4.21 (d, 1 H, H-5′), 4.16 (dd, 1 H, $J_{5,6b}$ 8.7 Hz, $J_{6a,6b}$ 14.4 Hz, H-6b), 4.12 (dd, 1 H, H-3), 4.03 (m, 2 H, H-6a, H-5), 3.98 (m, 1 H, $OCH_2CH_2N_3$), 3.74 (s, 3 H, $COOCH_3$), 3.71 (m, 1 H, $OCH_2CH_2N_3$), 3.45 (m, 1 H, $OCH_2CH_2N_3$), 3.33 (m, 1 H, $OCH_2CH_2N_3$), 2.12–2.03 (5 s, 15 H, 5 × $COCH_3$); ^{13}C NMR (CD_3OD): δ 78.9 (C-3′), 78.2 (C-3), 63.4 (C-6), 53.2 ($COOCH_3$), 51.8 (CH_2N_3). Anal. Calcd for $C_{25}H_{34}N_3NaO_{20}S$: C, 39.95; H, 4.56. Found: C, 40.02; H, 4.50.

2-Aminoethyl (3-O-sulfo-β-D-glucopyranosyluronic acid)-(1→3)-(β-D-galactopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside, disodium salt (**1**).—A solution of **15** (40 mg, 0.02 mmol) in 10:1 MeOH–water (8 mL) and 0.1 mL AcOH was hydrogenolyzed for 19 h over Pd–C. The catalyst was filtered using a pad of Celite, the pad was washed with a linear gradient from MeOH to water (50 mL), and the filtrate was concd to a volume of 3 mL. 1 M aq LiOH (2.2 mL) was added at −10 °C, and the mixture was allowed to attain rt over a 4 h period, then neutralized with AcOH. The reaction mixture was concd, diluted with water (1.5 mL) and MeONa (12 mg, 0.20 mmol) was added. The mixture was kept for 48 h at rt, neutralized with AcOH (0.1 mL) and concd to a volume of 0.5 mL. Gel filtration (TSK-HW40s) and subsequent freeze-drying yielded amorphous **1** (14 mg, 67%), $[\alpha]_D$ −8° (*c* 0.4, water); for ^{1}H NMR data see Table 1, and for ^{13}C NMR data see Table 2; MALDI-TOF-MS (negative mode): Calcd for $[C_{34}H_{56}Na_2N_2O_{30}S - 2\,Na + H]^-$: 1005.3. Found 1005.8.

2-Aminoethyl (β-D-glucopyranosyluronic acid)-(1→3)-(β-D-galactopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside, sodium salt (**2**).—A solution of **11** (92 mg, 0.04 mmol) in MeOH (2 mL) and 0.01 mL AcOH was hydrogenated for 2 h over Pd–C. The catalyst was filtered through a pad of Celite, the pad was washed thoroughly with MeOH (20 mL), and the filtrate was concd and dried in vacuo. The residue was dissolved in anhyd MeOH (2 mL), and CF_3COOEt (25 μL, 21 mmol) and Et_3N (20 μL) were added. The mixture was evaporated and the residue was filtered through a short column of Silica Gel by elution with EtOAc to give a mixture (60 mg) of partially debenzylated 2-trifluoroacetamidoethyl glycosides. This mixture was hydrogenolyzed in EtOH (1.5 mL) for 72 h over Pd–C. The catalyst was filtered off through a pad of Celite, the pad was washed with EtOH (20 mL), and the filtrate was concd. A solution of the residue in 10:1 THF–water (11 mL) was treated with 1 M aq LiOH (0.2 mL) at −10 °C for 2 h, stirred overnight at rt, and then concd. The residue was dissolved in 5:3 MeOH–water (8 mL) and treated with 2.5 M aq NaOH (0.4 mL) for 48 h at rt. The solution was neutralized with AcOH (0.1 mL), concd, followed by gel filtration (Sephadex G-10) and subsequent freeze-drying yielded amorphous **2** (20 mg, 49%), $[\alpha]_D$ −5° (*c* 1, water); for ^{1}H NMR data see Table 1, and for ^{13}C NMR data see Table 2; MALDI-TOF-MS: Calcd for $[C_{34}H_{57}NaN_2O_{27} + H]^+$: 949.3. Found 949.5. Calcd for $[M + Na]^+$: 971.3. Found 971.4. Calcd for $[M + K]^+$: 988.2. Found 987.2.

2-Aminoethyl (3-O-sulfo-β-D-glucopyranosyluronic acid)-(1→3)-β-D-galactopyranoside, disodium salt (**3**).—2 M aq LiOH (0.2 mL) was added to a solution of **22** (59 mg, 0.08 mmol) in 10:1 THF–water (5.5 mL) at −10 °C. The mixture was allowed to attain rt over a 2 h period, it was neutralized with AcOH, and then it was concd. To a solution of the residue in MeOH (1.5 mL) and water (0.5 mL) MeONa

(35 mg, 0.65 mmol) was added. The mixture was kept at rt for 48 h, neutralized with AcOH and concd. A solution of the residue in water (2 mL) was hydrogenated for 1 h over Pd–C. The catalyst was filtered off through a pad of Celite, the pad was washed with water (20 mL), and the filtrate was concd to a volume of 0.5 mL. Gel-filtration (TSK HW-40s) and subsequent freeze-drying yielded amorphous **3** (27 mg, 67%), $[\alpha]_D$ −7° (*c* 0.8, water); for ^{1}H NMR data see Table 1, and for ^{13}C NMR data see Table 2; MALDI-TOF-MS (negative mode): Calcd for $[C_{14}H_{23}NNa_2O_{15}S - 2\ Na + H]^-$: 478.1. Found 478.4; Calcd for $[M - Na]^-$: 500.1. Found 500.5.

2-Aminoethyl (β-D-glucopyranosyluronic acid)-(1→3)-β-D-galactopyranoside (**4**).—A solution of **19** (48 mg, 0.11 mmol) in water (2 mL) was hydrogenated for 19 h over Pd–C. The catalyst was filtered off through a pad of Celite, the pad was washed with water (20 mL), and the filtrate was concd to a volume of 0.5 mL. Gel-filtration (Sephadex G-10) and subsequent freeze-drying yielded amorphous **4** (40 mg, 89%), $[\alpha]_D$ −15° (*c* 2, water); for ^{1}H NMR data see Table 1, and for ^{13}C NMR data see Table 2; MALDI-TOF-MS: Calcd for $[C_{14}H_{25}NO_{12} + H]^+$ 400.1. Found 399.9; Calcd. for $[M + Na]^+$: 422.1. Found 421.9.

*2-(6-*N*-Biotinylamido)hexanamidoethyl (3-O-sulfo-β-D-glucopyranosyluronic acid)-(1→3)-(β-D-galactopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside, bis-triethylammonium salt* (**23**).—To a solution of **1** (6.5 mg, 6.2 μmol) and *p*-nitrophenyl(6-*N*-biotinylamido)capronoate (3.6 mg, 7.5 μmol) in 0.5 mL DMSO was added Et_3N (0.01 mL) and the reaction mixture was kept at rt for 24 h. Gel-filtration (Sephadex LH-20) in 1:1 CH_3CN–water and subsequent concentration and freeze-drying gave **23** (9.2 mg, 94%) as a white powder, $[\alpha]_D$ 9° (*c* 0.6, water); ^{1}H NMR (D_2O, see Scheme 3 for the numeration of atoms in the aglycon): δ (for aglycon) 4.62 (dd, 1 H, $J_{3,4}$ 7.7 Hz, $J_{4,5a}$ 5.1 Hz, H-4), 4.43 (dd, 1 H, $J_{2,3}$ 4.6 Hz, H-3), 3.99 and 3.81 (2m, 2 H, OCH_2CH_2NH), 3.45 (m, 2 H, OCH_2CH_2N), 3.35 (m, 1 H, H-2), 3.19 (t, 2 H, $J_{10,11}$ 6.6 Hz, H-10a, H-10b), 3.12 (q, 12 H, $2 \times NH(CH_2CH_3)_3$), 3.01 (dd, 1 H, $J_{5a,5}$ 13.0 Hz, H-5a), 2.80 (d, 1 H, H-5b), 2.27 (m, 4 H, H-9a, H-9b, H-14a, H-14b), 1.73 (m, 1 H, H-6a), 1.66 (m, 2 H, H-8a, H-8b), 1.62 (m, 2 H, H-13a, H-13b), 1.60 (m, 1 H, H-6b), 1.53 (m, 2 H, H-11a, H-11b), 1.43 (m, 2 H, H-7a, H-7b), 1.34 (m, 2 H, H-12a, H-12b), 1.20 (t, 18 H, $2 \times NH(CH_2CH_3)_3$). Other signals were very similar to those for **1** (see Table 1). ^{13}C NMR (D_2O, see Scheme 3 for the numeration of atoms in the aglycon): δ (for aglycon) 62.9 (C-3), 61.1 (C-4), 56.2 (C-2), 48.0 ($NH(CH_2CH_3)_3$), 40.5 (C-5), 39.9 (C-10), 36.4 (C-9, C-14), 28.8 (C-11), 28.6 (C-7), 28.5 (C-6), 26.4 (C-12), 26.0 (C-8), 25.8 (C-13), 9.5 ($NH(CH_2CH_3)_3$). Other signals were very similar to those for **1** (see Table 2). MALDI-TOF-MS (negative mode): Calcd for $[C_{62}H_{113}N_7O_{33}S_2 - 2(Et_3NH) + H]^-$: 1344.4. Found 1344.8. Calcd for $[M - 2(Et_3NH) + Na]^-$: 1366.4. Found 1366.8.

Synthesis of biotinylated PAA-neoglycoconjugate (**27**).—To a solution A of **1** (4.0 mg, 3.7 μmol) in Me_2SO (0.2 mL), solution B (178 μL, 18.4 μmol of **25**), solution C (42 μL, 0.92 μmol of **26**) and Et_3N (13 μL) were added. Solution B: poly(*p*-nitrophenyl acrylate) **25** (14.2 mg) in DMF (1.0 mL). Solution C: 6-(biotinylamido)hexamethylenamine **26** (1.0 mg) in Me_2SO (0.1 mL). The reaction mixture was kept at 40 °C for 24 h then 2-ethanolamine (43 μL) was added and the mixture was kept at rt for 24 h. Gel chromatography (Sephadex LH-20) in 1:1 CH_3CN–water and subsequent concentration and freeze-drying gave **25** as a white powder (6.2 mg, 90%).

Acknowledgements

This work was supported, in part, by Syntesome GmbH, Muenchen and the Russian Foundation for Basic Research (Project No. 00-03-32815a). We thank E.A. Gordeeva (M.M. Shemyakin and Y.A. Ovchinnikov Institute of Bioorganic Chemistry, RAS) for the kind assistance in the preparation of the polymeric conjugate **27**.

References

[1] L.O. Kononov, A.V. Kornilov, A.A. Sherman, E.V. Zyryanov, G.V. Zatonsky, A.S. Shashkov, N.E. Nifant'ev, *Bioorg. Khim.*, 24 (1998) 608–622.

[2] M. Schachner, in G. Back, S. Harnett (Eds.), *Carbohydrate Recognition in Cellular Function*, *Ciba Fnd. Symp.*, Vol. 145, Wiley, Chichester, UK, 1989, pp. 56–172.

[3] R.D. Fields, K. Itoh, *Trends Neurosci.*, 19 (1996) 473–480.

[4] F.B. Jungalwala, *Neurochem. Res.*, 19 (1994) 945–957.

[5] M. Schachner, R. Martini, *Trends Neurosci.*, 18 (1995) 183–191.

[6] D.K.H. Chou, A.A. Ilyas, J.E. Evans, C. Costello, R.H. Quarles, F.B. Jungalwala, *J. Biol. Chem.*, 261 (1986) 11717–11725.

[7] H. Voshol, C.W.E.M. van Zyelen, G. Orberger, J.F.G. Vliegenthart, M. Schachner, *J. Biol. Chem.*, 271 (1996) 22957–22960.

[8] T. Nakano, Y. Ito, T. Ogawa, *Carbohydr. Res.*, 243 (1993) 43–69.

[9] Y. Isogai, T. Kawase, H. Ishida, M. Kiso, A. Hasegawa, *J. Carbohydr. Chem.*, 15 (1996) 1001–1023.

[10] R. Chevalier, J.-M. Mallet, J. Aubry, P. Sinay, *Abstracts of Papers*, XV International Symposium on Glycoconjugates, The International Glycoconjugate Organisation, Tokyo, August 1999, 4pP # 445.

[11] A.Ya Chernyak, L.O. Kononov, N.K. Kochetkov, *Carbohydr. Res.*, 216 (1991) 381–398 and Refs. cited therein.

[12] A.Ya. Chernyak, G.V.M. Sharma, L.O. Kononov, P. Radha Krishna, A.B. Levinsky, N.K. Kochetkov, A.V. Rama Rao, *Carbohydr. Res.*, 223 (1992) 303–309.

[13] J. Lehmann, M. Schmidt-Schuchardt, *Carbohydr. Res.*, 276 (1995) 43–55 and Refs. cited therein

[14] S. Hanessian, A. Benalil, C. Laferriere, *J. Org. Chem.*, 60 (1995) 4786–4797.

[15] L.O. Kononov, A.A. Sherman, A.S. Shashkov, A.V. Kornilov, E.V. Zyryanov, G.V. Zatonsky, O.N. Judina, N.E. Nifant'ev, *Proceedings of the XVIII International Carbohydrate Symposium*, Milan, 1996, p. 417.

[16] J. Mc Murry, *Org. React.*, 24 (1976) 187–224.

[17] A.V. Kornilov, L.O. Kononov, G.V. Zatonsky, A.S. Shashkov, N.E. Nifant'ev, *Bioorg. Khim.*, 23 (1997) 655–666.

[18] J. Tamura, Y. Miura, H.H. Freeze, *J. Carbohydr. Chem.*, 18 (1999) 1–14.

[19] N.V. Bovin, E.Yu. Korchagina, T.V. Zemlyanukhina, N.E. Byramova, O.E. Galanina, A.E. Zemlyakov, A.E. Ivanov, V.P. Zubov, L.V. Mochalova, *Glycoconjugate J.*, 10 (1993) 142–151.

[20] N.P. Debus, M. Steinach, D. Stibenz, B. Hofmann, S. Sydow, C. Buehrer, N.E. Nifant'ev, N.V. Bovin, W. Semmler, *Abstracts of Papers*, 27th Annual Conference of The Society for Glycobiology, San Francisco, CA, October–November 1999, 162 (*Glycobiology*, 9 (1999) 1144).

[21] T.V. Pochechueva, O.E. Galanina, N.E. Nifant'ev, A. Kornilov, A.B. Tuzikov, N.V. Bovin, *Leucoc. Typing*, in press.