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Carbohydrate Research 317 (1999) 29–38

CARBOHYDRATE RESEARCH

Synthesis of a sialyl-α-(2→6)-lactosamine trisaccharide with a 5-amino-3-oxapentyl spacer group at C-1[I]☆

Santiago Figueroa-Pérez, Vicente Vérez-Bencomo *

Laboratory of Synthetic Antigens, Facultad de Química, Universidad de La Habana, Ciudad, Havana 10400, Cuba

Received 6 July 1998; accepted 2 January 1999

Abstract

As part of a continuing study aimed to achieve improved monoclonal antibodies against carcinoembryonic antigen (CEA) carbohydrate fragments, the synthesis of a sialyl-(2→6)-lactosamine trisaccharide with a 5-amino-3-oxapentyl spacer group at C-1[I] has been developed. Two different routes to access this target are described. For this purpose 5-azido-3-oxapentyl 6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**4**) was selectively β-galactosylated in 81% yield using the crystalline 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-α-D-galactopyranosyl trichloroacetimidate as the donor, taking advantage of the bulky phthalimido group at C-2 of **4**. On the other hand, galactosylation of the suitable protected acceptor 5-azido-3-oxapentyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside with the crystalline 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-α-D-galactosyl bromide renders the corresponding disaccharide in a moderate 58% yield. Despite the fact that the first strategy, unlike the second one, requires a hydrazinolysis–acetylation reaction at the disaccharide stage, it was found to be more convenient to access the disaccharide acceptor. Sialylation was performed using a thiophenyl donor under an NIS–TfOH activation procedure in acetonitrile to give a mixture of α and β trisaccharides in 49 and 16% yields, respectively.

Keywords: Selective glycosylation; Trichloroacetimidates; Carcinoembryonic antigen; Sialyllactosamine

1. Introduction

Notwithstanding the discovery of many new tumour markers, carcinoembryonic antigen (CEA) is still one of the most widely used [1]. Its serum levels, currently measured by sandwich immunoassay, are used for monitoring surgery-treated patients with several types of adenocarcinomas [2].

CEA is a highly glycosylated protein containing an average of 25 N-linked oligosaccharides [3,4]. The high heterogeneity observed for all these chains prompted the selection of anti-CEA monoclonal antibodies by their protein specificity [5]. Two other proteins, non-specific cross-reacting antigen-2 (NCA-2) [6], and normal fecal antigen-2 (NFA-2) [7], have been found to be the same gene products as CEA, and largely cross-reacted with it.

A comparison between all these proteins [4] revealed that, among other distinctive features, in all the examined CEA samples were found the determinants Lewis[y], α-Neu5Ac-(1→6)-β-D-Gal-(1→4)-D-GlcNAc and β-D-Gal-(1→4)-D-GlcNAc6*S*, as common chain termini. Lewis[y] and α-Neu5Ac-(1→6)-β-D-Gal-(1→4)-D-GlcNAc haptens have already been employed for the detection of CEA, using either a monoclonal antibody [8] or the lectin [9,10] *Trichosanthes japonica* agglutinin-I.

☆ Presented at the IXth European Carbohydrate Symposium, Utrecht, The Netherlands, 1997.

* Corresponding author.

E-mail address: vicente@fq.oc.uh.cu (V. Vérez-Bencomo)

0008-6215/99/$ - see front matter

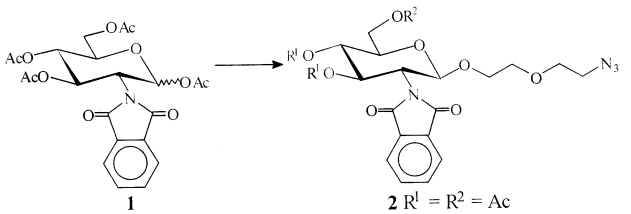
PII: S0008-6215(99)00022-1

As part of our ongoing study to develop an improved anti-CEA sandwich immunoassay by using monoclonal antibodies against synthetic carbohydrates as a complement for anti-protein antibodies, we have focused on these three distinct structures. The synthesis of Lewis^y neoglycoproteins [11] was the subject of a previous paper, whereas in the present study sialyllactosamine neoglycoprotein was chosen as the target.

Two different strategies were evaluated for the synthesis of the lactosamine acceptor **16**, which was further sialylated, deprotected, and hydrogenated to afford the trisaccharide **21** with a 5-amino-3-oxapentyl spacer group at C-1[I]. The amino group on the spacer linkage of **21** was used to attach the trisaccharide to bovine serum albumin (BSA) following a very efficient procedure previously described by us [12].

2. Results and discussion

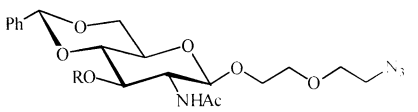
1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose (**1**) was condensed with 1-azido-3-oxapentanol in the presence of stannic chloride [13] to afford the glycoside **2** in an acceptable 62% yield. The signal for C-1 appeared at 98.0 ppm in the ^{13}C NMR spectra, while in the ^1H NMR spectra, H-1 showed a doublet at 5.46 ppm. The presence of the spacer arm was confirmed here and in all the following steps by the signal at 50.2 ppm in the ^{13}C NMR spectra, corresponding to the methylene group attached to the azido group. The diol acceptor **4** was obtained in 63% yield after deacetylation of **2** (→**3**) and further selective benzylation of the primary position using bis-tributyltin oxide. The ^{13}C NMR spectrum of compound **4** showed a deshielding for C-6 (61.1 → 68.7 ppm), that in conjunction with the unchanged shift of the signals for C-4 and C-3 was the main evidence that position 6 was selectively benzylated.



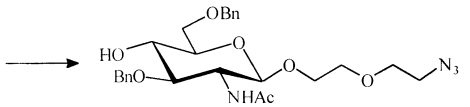
1

2 $R^1 = R^2 = Ac$
3 $R^1 = R^2 = H$
4 $R^1 = H$ $R^2 = Bn$

On the other hand, benzylation of the readily accessible benzylidene glycoside **5** (85%; C-3, 77.6 ppm) followed by reductive benzylidene opening gave acceptor **7** in moderate 60% yield. The shielding of the C-4 signal (→75.1 ppm), and the presence of the C-6 signal at 68.7 ppm, confirmed the expected transformation.

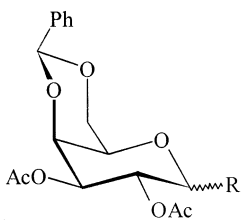


5 R = H
6 R = Bn

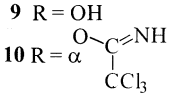


7

1,2,3-Tri-*O*-acetyl-4,6-*O*-benzylidene-D-galactopyranose (**8**) [11] was used as the starting material for the construction of the galactosyl donor. Selective deacetylation of **8** using 2-aminoethanol in ethyl acetate [14] afforded the anomeric mixture **9** (55%), which on treatment overnight with potassium carbonate and trichloroacetonitrile in dichloromethane [15], gave the crystalline α-imidate **10** in good 71% yield. The ^1H NMR spectra shows singlets at 8.65 and 5.57 ppm, corresponding to imidate NH and benzylidene protons, respectively, the doublet of the α-anomeric proton appeared at 6.71 ppm with a coupling constant of 3.5 Hz.



8 R = OAc
9 R = OH
10 R = α



11 R = βSBn
12 R = αBr

Selective galactosylation [16] of the diol **4** with donor **10**, in the presence of trimethylsilyl trifluoromethanesulfonate as catalyst, proceeded smoothly, giving disaccharide **13** in an excellent 81% yield, whereby no (1 → 3)-linked

product was detected. The regioselectivity of the reaction was confirmed in the ^{13}C NMR spectra by the deshielding of the signal assigned to C-4 (72.9 → 80.9 ppm), and in the ^{1}H NMR spectrum of the acetylated product **14** by the particular deshielding of the signal assigned to H-3 at 5.62 ppm. The galactose anomeric proton doublet at 4.48 ppm in the ^{1}H NMR spectrum of **13** had a J value of 8 Hz, thus proving the β-stereochemistry of the formed bond.

Galactosylation of acceptor **7** with donor **10** using boron trifluoride–diethyl ether complex as a catalyst [17] was not feasible in our hands. So, we decided to prepare the crystalline α-bromide **12** from the readily available thioglycoside **11** by reaction with bromine in dichloromethane [18].

Galactosylation of **7** with **12** was then performed under silver triflate promotion in dichloromethane, using Hünig's base to neutralize the acid, to afford the desired disaccharide **15** in a moderate 59% yield. In the ^{13}C NMR spectrum, the signal for C-4 was deshielded to 76.1 ppm owing to the substitution by the galactosyl moiety; the doublet at 4.46 ppm in the ^{1}H NMR with J 8.1 Hz corresponding to H-1′ ascertained the β-configuration at the galactose anomeric center.

At this point it seems to be clear that the selective glycosylation strategy was more advantageous mainly due to the shorter route to the acceptor and the faster, cheaper and higher-yielding galactosylation reaction. However, the phthalimido group of the disaccharide derivative has to be removed before sialylation, owing the incompatibility of the phthalimido and methyl ester group at the deprotection stage. Therefore, hydrazinolysis of compound **13** followed by acetylation and hydrolysis of the benzylidene ring afforded the disaccharide acceptor **17** in 73% yield, which represents an overall yield of 59% starting from diol acceptor **4**.

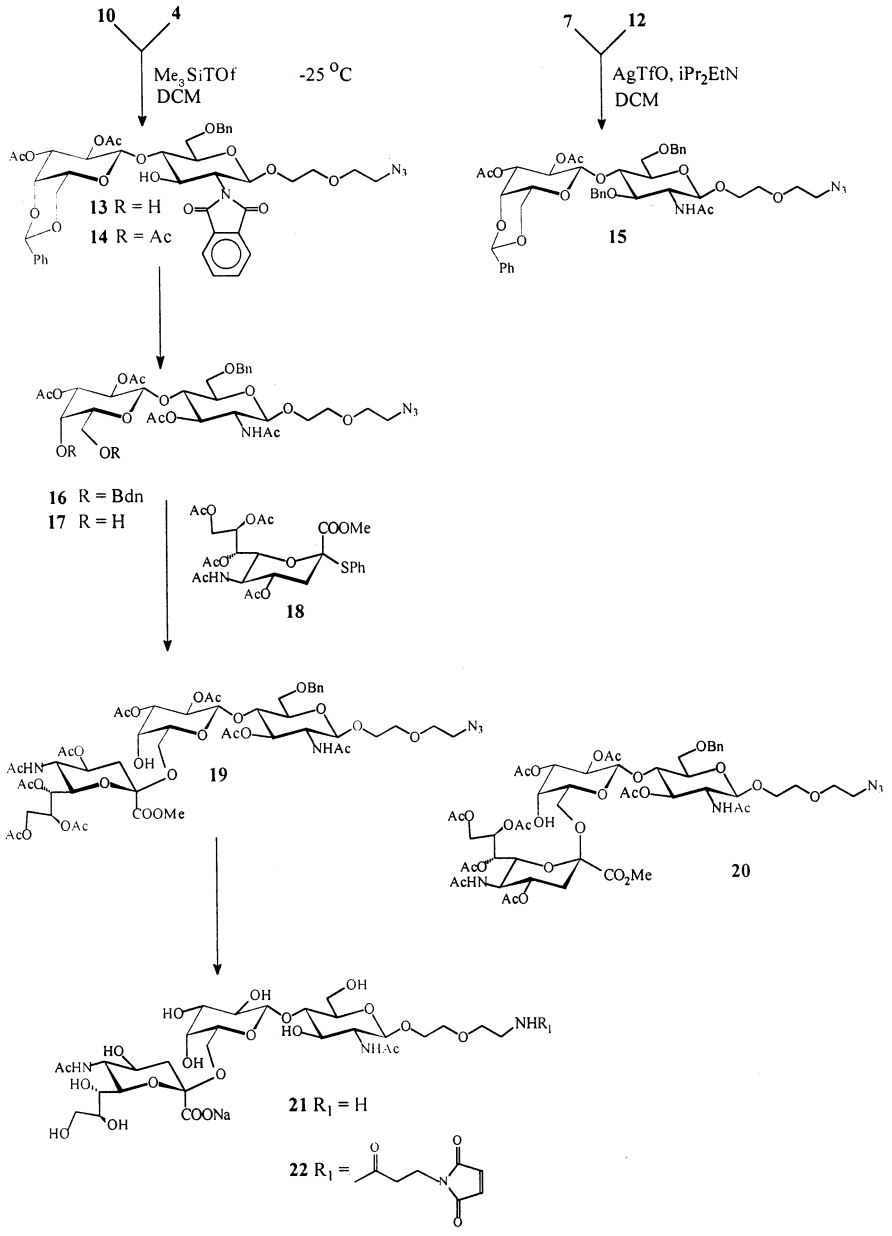
Though the two strategies could afford comparable yields of disaccharide acceptors, the selective galactosylation route is more convenient owing to: (1) the faster and cleaner galactosylation reaction; (2) the more complicated route to donor **12** and to acceptor **7**; and (3) the relative stability of imidate **10** (it can be stored at 4 °C for at least 4 months) while bromide **12** is particularly unstable.

Sialylation of **17** was conducted using the thiophenyl glycoside **18** [19] as the donor and using NIS/triflic acid as the activating system, in acetonitrile at −40 °C [20]. These conditions led to an α:β mixture (3:1) in a 67% overall yield; the α isomer **19** was recovered in 50.5% yield after a simple chromatographic purification. The stereochemistry of the glycosidic linkage was determined by the long-range coupling constant between C-1 and H-3ax [21]. These values were 6.6 Hz for the α isomer **19** and 1.5 Hz for the β isomer **20**. Other differences in the ^{1}H chemical shifts between the β and the α isomers are reported in Table 1.

Compound **19** was deacetylated and saponified in one step [22], then hydrogenolysed to give the free trisaccharide **21** in 91% overall yield. The triplet located at 3.19 ppm in the ^{1}H NMR spectrum and the signal at 41.5 ppm in the ^{13}C NMR spectrum, which represent the CH_2NH_2 group, are indicative of the transformation of the azido into an amino group.

Finally, compound **21** was treated with an excess of *N*-succinimidoxyl β-maleimidopropionate in *N*,*N*-dimethylformamide/PBS to functionalize the trisaccharide for conjugation to BSA. Compound **22** was obtained together with a small quantity of β-maleimidopropionic acid as a consequence of the partial hydrolysis of the active ester. ^{1}H NMR analysis of the mixture showed a deshielding of the triplet initially located at 3.19 to 3.32 ppm owing to acylation of the amino function. The singlet at 6.89 ppm and the triplet at 2.52 ppm confirmed the presence of the maleimidopropionamide moiety.

Crude compound **22** was directly condensed with previously thiolated BSA in an excellent 80% oligosaccharide-based yield affording a $(trisaccharide)_{14}$-BSA molecule. The use of this neoglycoprotein for the elucidation of the epitopes recognized by anti-CEA carbohydrate-specific monoclonal antibodies and also as immunogens for the preparation of monoclonal antibodies is now in progress (Scheme 1).



Scheme 1.

3. Experimental

General procedures.—Optical rotations were measured at 25 °C with a POLAMAT A automatic polarimeter, using a 5-cm 5-mL cell. NMR spectra were recorded at 25 °C with a Bruker AC-250F spectrometer. Chemical shifts (δ) are given in ppm relative to the signal for internal Me_4Si for ^1H; and indirectly to $CDCl_3$ (δ 77.03) for ^{13}C. ^1H, and ^{13}C assignments were made on the basis of homo- and heteronuclear correlation experiments. The following notation was used to define the NMR signals: ′ for Gal, and neu for neuramic acid unit.

All compounds were purified by column chromatography on Kieselgel 60 (Fluka, < 230 mesh ASTM) and fractions were monitored by TLC on Kieselgel $60F_{254}$ (E. Merck). Detection was effected by charring with aq 20% H_2SO_4 after examination under UV light. Evaporations were conducted under reduced pressure at 50 °C (bath).

5-Azido-3-oxapentyl 3,4,6-tri-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranoside* (**2**). —To a stirred solution of **1** (1 g, 2.1 mmol)

Table 1
^{1}H NMR data for α and β trisaccharides **19** and **20**

GlcNAc unit	H-1 $J_{1,2}$	H-2	H-3 $J_{2,3}$	H-4 $J_{3,4}$	H-5	H-6a	H-6b	NH $J_{NH,2}$		
19	4.53 (7.7)	4.06	5.02 (9.8)	3.92 (8.7)	3.50	3.72	3.9 4	6.08 (9.2)		
20	4.51 (7.6)	4.05	5.02 (9.5)	3.88 (8.3)	3.50	3.72	3.9 3	6.15 (9.5)		
Gal unit	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$							
19	4.46 (7.9)	5.14 (10.2)	4.79 (3.1)	4.03	3.73	3.51	3.92			
20	4.45 (7.9)	5.13 (10.2)	4.82 (3.0)	4.16	3.56	3.43	3.78			
Neu5Ac unit	H-3a $J_{3a,3e}$	H-3e $J_{3e,4}$	H-4 $J_{4,3}$	H-5 $J_{5,6}$	H-6 $J_{6,7}$	H-7 $J_{7,8}$	H-8 $J_{8,9a}$	H-9a $J_{9a,9b}$	H-9b $J_{NH,5}$	NH
19	1.97 (12.8)	2.57 (4.7)	4.90 (11.9)	4.05 (9.8)	4.15	5.31	5.32	4.37 (2.5)	4.15 (12.7)	5.42 (9.2)
20	1.82 (12.8)	2.48 (4.8)	5.31	3.81 (9.8)	4.25 (10.5)	5.34 (2.0)	5.23 (2.6)	4.73 (2.0)	4.18 (12.3)	6.00 (9.4)

and 5-azido-3-oxapentanol (0.8 mL, 4.2 mmol) in dry CH_2Cl_2 (5 mL) was added dropwise $SnCl_4$ (0.5 mL, 4.2 mmol). After being stirred overnight at room temperature (rt), CH_2Cl_2 was added (20 mL) and the solution was poured into ice-satd $NaHCO_3$ (40 mL). The resulting emulsion was filtered through Celite and the solid thoroughly rinsed with CH_2Cl_2. The organic layer was collected from the filtrate and washed with satd NaCl (20 mL), then dried and concentrated. Column chromatography of the residue (1:1 hexane–EtOAc) afforded pure **2** as a colorless syrup (690 mg, 62%); $[\alpha]_D + 41°$ (*c* 0.96, $CHCl_3$); R_f 0.35 (2:1 hexane–EtOAc); NMR ($CDCl_3$): ^{1}H, δ 7.85 and 7.75 (Phth), 5.83 (dd, 1 H, $J_{2,3}$ 10.7, $J_{3,4}$ 9.2 Hz, H-3), 5.46 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 5.19 (d, 1 H, $J_{4,5}$ 5.9 Hz, H-4), 4.20 (dd, 1 H, $J_{6a,6b}$ 12.3, $J_{5,6a}$ 2.3 Hz, H-6a), 3.33 (t, 2 H, *J* 4.2 Hz, CH_2N$_3$), 2.20–2.01 (9 H, CH_3COO); ^{13}C, δ 170.6, 170.0 and 169.4 (C=O), 134.2, 131.3 and 123.4 (Phth), 98.0 (C-1), 71.7 (C-3), 70.6 (C-5), 69.9, 69.7 (*C*H$_2$O), 68.9 (C-4 and *C*H$_2$O), 61.9 (C-6), 54.5 (C-2), 50.2 (*C*H$_2$N$_3$), 20.6, 20.5 and 20.3 (*C*H$_3$COO). Anal. Calcd for $C_{28}H_{43}N_4O_{11}$: C, 54.98; H, 7.09; N, 9.16. Found: C, 54.87; H, 7.18; N, 9.24.

*5-Azido-3-oxapentyl 6-*O*-benzyl-2-deoxy-2-phthalimido-β-*D*-glucopyranoside* (**4**).—To a stirred solution of **2** (0.69 g, 1.3 mmol) in a mixture of 1:1 dry MeOH–CH_2Cl_2 (10 mL), was added a freshly prepared solution of methanolic sodium methoxide (0.5 M, 1 mL). The reaction was monitored by TLC (1:1 hexane–EtOAc). When all the starting material was transformed (~ 30 min), the solution was neutralized with Amberlite IR-120 (H^+) resin, filtered, and evaporated to dryness to afford a slightly yellow syrup **3** (500 mg, 96%). Bis-(tri-*n*-butyl)tin oxide (0.64 mL) was added to a solution of compound **3** (0.8 g, 2 mmol) in toluene (10 mL) and the mixture was stirred with continuous azeotropic removal of H_2O. After 4 h, the mixture was concentrated to a volume of 3 mL, then benzyl bromide (0.8 mL, 6.7 mmol) and tetrabutylammonium bromide (0.32 g, 1 mmol) were added, and the mixture was stirred at 100 °C for 4 h. The solution was then diluted with EtOAc (15 mL), and aq 10% NaF (5 mL) was added to the solution. After 20 min of vigorous stirring, the insoluble material was removed by filtration over Celite, and the organic phase was collected from the filtrate, washed with satd

aq $NaHCO_3$ (5 mL) and H_2O (5 mL), then dried (Na_2SO_4), filtered, and concentrated. The syrupy residue was chromatographed (1:1 toluene–EtOAc) to give **4** as a colorless syrup (0.61 g, 63%); $[\alpha]_D - 22.7°$ (*c* 0.96, $CHCl_3$); R_f 0.37 (1:1 toluene–EtOAc); NMR ($CDCl_3$): ^1H, δ 7.85 and 7.75 (Phth), 7.30 (Ph), 5.21 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.58 (s, 2 H, CH_2Ph), 4.30 (dd, 1 H, $J_{2,3}$ 10.6, $J_{3,4}$ 7.9 Hz, H-3), 4.10 (dd, 1 H, H-2), 3.85 (m, 1 H, H-6a), 3.61 (m, 1 H, H-6b), 3.59 (m, 1 H, H-5), 3.53 (m, 1 H, H-4), 3.34 (t, 2 H, CH_2N_3); ^{13}C, δ 170.6 and 170.0 (C=O), 134.2, 131.3 and 123.4 (Phth), 128.9–127.7 (Ph), 98.2 (C-1), 74.3 (C-5), 72.9 (C-4), 71.4 (C-3), 69.9 and 69.6 (*C*H_2O), 68.7 (C-6), 56.3 (C-2), 50.3 (*C*H_2N_3). Anal. Calcd for $C_{29}H_{43}N_4O_8$: C, 60.51; H, 7.53; N, 9.73. Found: C, 60.35; H, 7.59; N, 9.65.

5-Azido-3-oxapentyl 2-acetamido-3-O-*benzyl-4,6*-O-*benzylidene-2-deoxy-β*-D-*glucopyranoside* (**6**).—To a solution of **5** (2.5 g, 5.35 mmol), BaO (4.1 g, 26.75 mmol) and $Ba(OH)_2{\cdot}8H_2O$ (740 mg, 2.3 mmol) in Me_2NCHO (20 mL) was added benzyl bromide (1.83 mL, 12.6 mmol) and the mixture was stirred for 15 min at rt. Then, CH_2Cl_2 (50 mL) was added and the resulting mixture was boiled under reflux for 1 h, and then filtered through Celite. The filtrate was washed with 2% HCl (25 mL), satd aq $NaHCO_3$ (25 mL) and H_2O (25 mL), then dried and concentrated. After the addition of toluene (10 mL) the solid was filtered, rinsed thoroughly with toluene and recrystallized from EtOAc to afford **6** (2.6 g, 85%): mp 203–205 °C; $[\alpha]_D$ $-18°$ (*c* 1.0, $CHCl_3$); R_f 0.69 (5:1 CH_2Cl_2–acetone); NMR ($CDCl_3$): ^1H, δ 7.5–7.2 (m, 10 H, Ph), 6.35 (d, 1 H, *J* 8.5 Hz, NH), 5.65 (s, 1 H, PhC*H*), 4.95 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 4.36 (dd, 1 H, $J_{5,6a}$ 4.1, $J_{6a,6b}$ 10.2 Hz, H-6a), 4.10 (t, 1 H, $J_{2,3}$ 9.3 Hz, H-3), 3.95 (m, 1 H, H-2), 3.73 (m, 2 H, H-4,6b), 3.50 (m, 1 H, H-5), 3.38 (t, 2 H, *J* 4.9 Hz, CH_2N_3), 1.95 (s, 3 H, CH_3CON); ^{13}C, (170.4 (C=O), 127.4–129.3 (Ph), 101.2 (Ph*C*H), 100.9 (C-1), 82.1 (C-4), 77.6 (C-3), 72.0 (*C*H_2Ph), 68.5 (C-6), 65.8 (C-5), 56.2 (C-2), 50.1 (*C*H_2N_3), 23.2 (*C*H_3CON). Anal. Calcd for $C_{27}H_{36}N_4O_7$: C, 61.35; H, 6.86; N, 10.60. Found: C, 61.24; H, 6.98; N, 10.51.

5-Azido-3-oxapentyl 2-acetamido-3,6-di-O-*benzyl-2-deoxy-β*-D-*glucopyranoside* (**7**).—A mixture of **6** (1 g, 1.7 mmol), $NaBH_3CN$ (1.07 g, 17 mmol) and 3 Å molecular sieves (2 g) in dry THF (20 mL) was stirred for 15 min at rt. Then, the mixture was cooled to 0 °C, and dry Et_2O saturated with HCl was added at 0 °C until the evolution of gas stopped. The cooling bath was then removed and the mixture was further stirred for 20 min. Cold H_2O (5 mL) was then added, and the suspension was diluted with CH_2Cl_2 (50 mL) and filtered through Celite. The filtrate was washed with aq 1% $KMnO_4$ (3 × 20 mL), satd aq $NaHCO_3$ (20 mL) and H_2O (20 mL), dried, and concentrated. Column chromatography (5:1 CH_2Cl_2–acetone) of the residue afforded **7** (600 mg, 60%) as a colorless solid. An analytical sample was obtained by recrystallization from EtOAc: mp 92–94 °C; $[\alpha]_D - 12.3°$ (*c* 1.0, $CHCl_3$); R_f 0.48 (4:1 CH_2Cl_2–acetone); ^1H NMR (C_6D_6): δ 7.45–7.35 (m, 10 H, Ph), 6.62 (d, 1 H, *J* 7.7 Hz, NH), 4.97 (AB pattern, 2 H, CH_2Ph), 4.69 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 4.41 (s, 2 H, PhCH_2), 4.15 (m, 1 H, H-2), 3.88 (m, 3 H, H-3,4,6a), 3.70 (m, 1 H, H-5), 3.45 (m, 1 H, H-6b), 3.23 (t, 2 H, CH_2CH_2N_3), 2.87 (t, 2 H, *J* 4.9 Hz, CH_2N_3) and 1.82 (s, 3 H, CH_3CON); ^{13}C NMR ($CDCl_3$): δ 170.4 (C=O), 129.6-127.7 (Ph), 101.7 (C-1), 83.0 (C-3), 75.1 (C-4), 73.6 and 72.8 (*C*H_2Ph), 72.3 (C-5), 68.7 (C-6), 55.6 (C-2), 50.7 (*C*H_2N_3), 23.3 (*C*H_3CON). Anal. Calcd for $C_{27}H_{38}N_4O_7$: C, 61.12; H, 7.22; N, 10.56. Found: C, 60.98; H, 7.30; N, 10.35.

2,3-Di-O-*acetyl-4,6*-O-*benzylidene*-D-*galactopyranose* (**9**).—To a solution of **8** (1 g, 2.5 mmol) in EtOAc (25 mL) was added 2-aminoethanol (0.3 mL, 5 mmol) and the mixture was stirred overnight at rt. Then the solution was washed with H_2O (5 mL) and brine (5 mL), dried (Na_2SO_4), filtered, and concentrated. Column chromatography (2:1 hexane–EtOAc) of the residue afford the anomeric mixture **9** as a white foam (0.49 g, 55%); R_f 0.36 (3:2 hexane–EtOAc); ^1H NMR [23]; ^{13}C NMR ($CDCl_3$): δ 170.6 and 170.4 (C=O), 137.4 (ipso Ph), 128.9–125.9 (Ph), 100.4 (Ph*C*H), 95.3 (C-1β), 90.5 (C-1α), 20.8–20.4 (*C*H_3COO). Anal. Calcd for $C_{17}H_{20}O_8$: C, 57.95; H, 5.72. Found: C, 57.79; H, 5.85.

*2,3-Di-*O*-acetyl-4,6-*O*-benzylidene-α-*D*-galactopyranosyl trichloroacetimidate* (**10**).—To a solution of **9** (500 mg, 1.42 mmol) and trichloroacetonitrile (0.7 mL, 7.09 mmol) in dry CH_2Cl_2 (2 mL) was added anhyd K_2CO_3 (500 mg, 3.55 mmol), and the mixture was stirred overnight at rt. Then, diethyl ether (2 mL) was added, the mixture was filtered over Celite, and the filtrate was concentrated. The syrupy residue was crystallized from Et_2O to give **10** as a white powder (503 mg, 71%): mp 174–175 °C; $[\alpha]_D + 176°$ (*c* 1.02, $CHCl_3$), lit + 152.5° [23]; R_f 0.46 (3:2 hexane–EtOAc); NMR ($CDCl_3$): ^{1}H, δ 8.65 (s, 1 H, NH), 7.55–7.45 (m, 5 H, Ph), 6.71 (d, 1 H, *J* 3.5 Hz, H-1), 5.60 (dd, 1 H, $J_{2,3}$ 10.9 Hz, H-2), 5.57 (s, 1 H, PhC*H*), 5.39 (dd, 1 H, $J_{3,4}$ 3.3 Hz, H-3), 4.62 (d, 1 H, H-4), 4.35 (dd, 1 H, $J_{5,6a}$ 1.3, $J_{6a,6b}$ 11.2 Hz, H-6a), 4.11 (m, 2 H, H-5,6b), 2.10 and 2.05 (2 s, each 3H, 2CH_3COO); ^{13}C, δ 171.1–170.6 (C=O), 137.4 (ipso Ph), 128.9–126.1 (Ph), 100.8 (Ph*C*H), 94.4 (C-1), 20.9 and 20.5 (2*C*H_3COO). Anal. Calcd for $C_{18}H_{20}Cl_3NO_8$: C, 44.60; H, 4.16; N, 2.89. Found: C, 44.47; H, 4.26; N, 2.79.

*2,3-Di-*O*-acetyl-4,6-*O*-benzylidene-α-*D*-galactopyranosyl bromide* (**12**).—A solution of bromine (61 μL, 1.1 mmol) in dry CH_2Cl_2 (0.6 mL) was added at 0 °C to a stirred solution of **11** (500 mg, 1.09 mmol) in dry CH_2Cl_2 (5 mL). After 20 min, tetraethylammonium bromide (100 mg) was added, and the mixture was further stirred for 3 h at rt. Allyl bromide (0.33 mL) was added to destroy the excess bromine. The decolorized mixture was successively washed with cold aq satd $NaHCO_3$ and H_2O, dried, and concentrated. Crystallization of the residue from Et_2O–hexane afforded **12** as a white powder (325 mg, 72%): mp 145–147 °C; $[\alpha]_D + 285°$ (*c* 1, $CHCl_3$); R_f 0.47 (3:2 hexane–EtOAc); NMR ($CDCl_3$): ^{1}H, δ 7.58–7.44 (m, 5 H, Ph), 6.82 (d, 1 H, $J_{1,2}$ 2.1 Hz, H-1), 5.53 (s, 1 H, PhC*H*), 5.45 (dd, 1 H, $J_{2,3}$ 10.4 Hz, H-2), 5.26 (dd, 1 H, $J_{3,4}$ 3.7 Hz, H-3), 4.61 (d, 1 H, H-4), 4.34 (d, 1 H, $J_{6a,6b}$ 11.2 Hz, H-6a), 4.13 (m, 1 H, H-6b), 2.10 and 2.05 (2 s, each 3H, 2CH_3COO); ^{13}C, δ 171.1–170.6 (C=O), 137.4 (ipso Ph), 128.9–126.1 (Ph), 100.8 (Ph*C*H), 89.8 (C-1), 20.9 and 20.5 (*C*H_3COO). Anal. Calcd for $C_{17}H_{19}BrO_7$: C, 49.17; H, 4.61. Found: C, 49.00; H, 4.67.

*5-Azido-3-oxapentyl (2,3-di-*O*-acetyl-4,6-O-benzylidene-β-*D*-galactopyranosyl)-(1→4)-6-*O*-benzyl-2-deoxy-2-phthalimido-β-*D*-glucopyranoside* (**13**).—A solution of **4** (600 mg, 1.7 mmol) and **10** (640 mg, 1.28 mmol) in dry CH_2Cl_2 (5 mL) containing 4 Å molecular sieves (1 g) was stirred under dry N_2 for 1 h at rt. Then the mixture was cooled to −25 °C and a solution of 0.1 M trimethylsilyl trifluoromethanesulfonate in CH_2Cl_2 (0.2 mL, 0.02 mmol) was added. When TLC (2:1 toluene–EtOAc) showed the complete transformation of the starting material, the mixture was diluted with CH_2Cl_2 (10 mL) and filtered over Celite. The filtrate was washed with satd aq $NaHCO_3$ (3 mL) and H_2O (3 mL), then dried (Na_2SO_4), filtered, and concentrated. Column chromatography (2:1 toluene–EtOAc) of the residue afforded **13** as a colorless syrup (803 mg, 81%); $[\alpha]_D + 15°$ (*c* 1.30, $CHCl_3$); R_f 0.33 (2:1 toluene–EtOAc); NMR ($CDCl_3$): ^{1}H, δ 7.84 and 7.73 (Phth), 7.39 (Ph), 5.43 (s, 1 H, PhC*H*), 5.33 (dd, 1 H, $J_{1',2'}$ 8, $J_{2',3'}$ 10.5 Hz, H-2′), 5.29 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.85 (dd, 1 H, $J_{3',4'}$ 3.6, H-3), 4.69 (AB, 2 H, *J* 12 Hz, CH_2Ph), 4.48 (d, 1 H, $J_{1',2'}$ 8 Hz, H-1′), 4.45 (dd, 1 H, $J_{2,3}$ 10.4, $J_{3,4}$ 8.1 Hz, H-3), 4.32 (d, 1 H, $J_{3',4}$ 3.6 Hz, H-4′), 4.22 (m, 1 H, H-6′a), 4.18 (dd, 1 H, H-2), 3.97 (m, 1 H, H-6′b), 3.91 (m, 1 H, H-6a), 3.75 (m, 1 H, H-4), 3.66 (m, 1 H, H-6b), 3.55 (m, 1 H, H-5), 3.45 (m, 1 H, H-5′), 3.05 (t, 2 H, CH_2N_3), 2.01 and 1.98 (2CH_3COO); ^{13}C, δ 170.6, 170.5, 170.0 and 168.9 (C=O), 137.9 and 137.1 (ipso Ar), 134.2, 131.3 and 123.4 (Phth), 128.9-127.7 (Ph), 101.0 (C-1′), 100.8 (*C*HPh), 98.1 (C-1), 80.9 (C-4), 74.1 (C-5), 73.5 (*C*H_2), 72.9 (C-4′), 71.5 (C-3′), 69.9 and 69.7 (*C*H_2O), 69.3 (C-3), 68.7 (C-6), 68.5 (C-2′), 68.2 (C-6′), 66.5 (C-5′), 55.9 (C-2), 50.3 (*C*H_2N_3), 20.7 and 20.3 (2*C*H_3COO). Anal. Calcd for $C_{47}H_{64}N_4O_{15}$: C, 61.03; H, 6.97; N, 6.06. Found: C, 60.89; H, 7.06; N, 5.97.

*5-Azido-3-oxapentyl (2,3-di-*O*-acetyl-4,6-O-benzylidene-β-*D*-galactopyranosyl)-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-*D*-glucopyranoside* (**14**).—Compound **13** (114 mg, 0.14 mmol) was treated with 1:1 Ac_2O–pyridine (3 mL) for 24 h, then concentrated and co-concentrated with toluene to

give 120 mg of **14**: mp 212–213 °C (EtOAc–hexane); $[\alpha]_D + 32.5°$ (*c* 2.4, EtOAc); R_f 0.45 (2:1 toluene–EtOAc); NMR ($CDCl_3$): ^{1}H, δ 7.84 and 7.73 (Phth), 7.39 (Ph), 5.62 (dd, 1 H, $J_{2,3}$ 9.7, $J_{3,4}$ 10.6 Hz, H-3), 5.42 (s, 1 H, PhC*H*), 5.40 (d, 1 H, $J_{1,2}$ 9.7 Hz, H-1), 5.18 (dd, 1 H, $J_{1',2'}$ 8, $J_{2',3'}$ 10.5 Hz, H-2′), 4.77 (dd, 1 H, $J_{3',4'}$ 3.6 Hz, H-3′), 4.65 (AB, 2 H, *J* 12 Hz, C*H*$_2$Ph), 4.51 (d, 1 H, $J_{1',2'}$ 8 Hz, H-1), 4.32 (d, 1 H, $J_{3',4'}$ 3.6 Hz, H-4′), 3.66 (m, 1 H, H-5), 3.28 (s, 1 H, H-5′), 3.10 (t, 2 H, C*H*$_2$N$_3$), 2.01, 1.99 and 1.98 (3C*H*$_3$COO); ^{13}C, δ 170.6, 170.5, 170.0 and 168.9 (C=O), 137.9 and 137.1 (ipso Ar), 134.2, 131.3 and 123.4 (Phth), 128.9–127.7 (Ph), 101.0 (*C*HPh), 99.9 (C-1′), 97.1 (C-1), 75.0 (C-4), 74.5 (C-5), 73.4 (*C*H$_2$), 73.0 (C-4′), 71.9 (C-3′), 70.5 (C-3), 69.9 (C-6), 69.7 (*C*H$_2$O), 68.9 (C-2′), 68.3 (C-6′), 65.9 (C-5′), 54.7 (C-2), 50.3 (*C*H$_2$N$_3$), 20.6, 20.5 and 20.3 (3*C*H$_3$COO). Anal. Calcd for $C_{49}H_{66}N_4O_{16}$: C, 60.86; H, 6.88; N, 5.79. Found: C, 60.79; H, 7.00; N, 5.61.

*5-Azido-3-oxapentyl (2,3-di-*O*-acetyl-4,6-*O*-benzylidene-β-*D*-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-*D*-glucopyranoside* (**15**).—A solution of **7** (100 mg, 0.17 mmol), silver trifuoromethanesulfonate (150 mg, 0.85 mmol) and ethyldiisopropylamine (32 μL, 0.17 mmol) in dry CH_2Cl_2 (1.5 mL) containing 4 Å molecular sieves (200 mg) was stirred under dry N_2 for 1 h at rt. Then, a solution of **12** (331 mg, 0.8 mmol) in dry CH_2Cl_2 was added and the mixture was stirred overnight. The mixture was diluted with CH_2Cl_2 (10 mL) and filtered over Celite. The filtrate was washed with aq 10% $Na_2S_2O_3$ (5 mL), satd aq $NaHCO_3$ (5 mL), and H_2O (5 mL), then dried (Na_2SO_4) and concentrated. The residue was chromatographed (6:1 CH_2Cl_2–acetone) to afford **15** (95 mg, 58%) as a slightly yellow syrup; $[\alpha]_D + 22°$ (*c* 1.0, $CHCl_3$); R_f 0.35 (5:1 CH_2Cl_2–acetone); NMR ($CDCl_3$): ^{1}H, δ 7.38–7.29 (Ph), 6.21 (d, 1 H, *J* 8.9 Hz, NH), 5.41 (s, 1 H, PhC*H*), 5.35 (dd, 1 H, $J_{1',2'}$ 8, $J_{2',3'}$ 10.5 Hz, H-2′), 4.87 (dd, 1 H, $J_{3',4'}$ 3.8 Hz, H-3′), 4.77 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.46 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1′), 4.43 (m, 1 H, H-2), 4.32 (m, 2 H, H-4,4′), 3.96 (m, 3 H, H-6a,3,6′b), 3.91 (m, 1 H, H-6a), 3.66 (m, 1 H, H-6b), 3.43 (m, 2 H, H-5,5′), 3.07 (t, 2 H, C*H*$_2$N$_3$), 2.01 (s, 3 H, CH_3CON), 1.98 (C*H*$_3$COO); ^{13}C, δ 170.3 and 169.4 (C=O), 137.7 and 137.3 (ipso Ar), 129.3–127.9 (Ph), 101.3 (C-1), 101.0 (*C*HPh), 100.2 (C-1′), 79.1 (C-3), 76.1 (C-4), 75.1 (C-5), 73.4 and 72.7 (*C*H$_2$Ph), 73.3 (C-4′), 71.4 (C-3′), 69.9 and 69.5 (*C*H$_2$O), 69.6 (C-2′), 68.5 (C-6), 68.2 (C-6′), 66.1 (C-5′), 52.8 (C-2), 50.4 (*C*H$_2$N$_3$), 22.7 (*C*H$_2$CON), 20.5 and 20.0 (*C*H$_3$COO). Anal. Calcd for $C_{45}H_{59}N_4O_{14}$: C, 61.42; H, 6.76; N, 6.37. Found: C, 61.54; H, 6.83; N, 6.21.

*5-Azido-3-oxapentyl (2,3-di-*O*-acetyl-4,6-*O*-benzylidene-β-*D*-galactopyranosyl)-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-*D*-glucopyranoside* (**16**).—To a solution of **13** (237 mg, 0.31 mmol) in EtOH (25 mL) was added hydrazine hydrate (1 mL, 20 mmol), and the solution was stirred for 12 h at 90 °C. Then, the solution was concentrated, and the residue was treated with 1:1 Ac_2O–pyridine (8 mL). After being stirred for 24 h at rt, the mixture was concentrated and dissolved in CH_2Cl_2 (10 mL), the resulting solution was successively washed with 3% HCl (2 × 3 mL), satd aq $NaHCO_3$ (5 mL) and H_2O (5 mL), dried (Na_2SO_4), and concentrated. Column chromatography of the residue (3:2 toluene–EtOAc) afforded **16** as a yellow syrup (177 mg, 72.8%); $[\alpha]_D + 13°$ (*c* 2.80, $CHCl_3$); R_f 0.33 (3:2 toluene–EtOAc); NMR ($CDCl_3$): ^{1}H, δ 7.39 (Ph), 6.55 (d, 1 H, *J* 9.5 Hz, N*H*), 5.43 (s, 1 H, PhC*H*), 5.13 (dd, 1 H, $J_{1',2'}$ 8, $J_{2',3'}$ 10.2 Hz, H-2′), 5.05 (dd, 1 H, $J_{2,3}$ 10.1, $J_{3,4}$ 9 Hz, H-3), 4.76 (dd, 1 H, $J_{3',4'}$ 4 Hz, H-3′), 4.61 (AB, 2 H, *J* 12.3 Hz, C*H*$_2$Ph), 4.52 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.45 (d, 1 H, $J_{1',2'}$ 8 Hz, H-1′), 4.32 (m, 2 H, H-4′,6′a), 4.14 (dd, 1 H, H-2), 3.95 (m, 3 H, H-6′b,6a,4), 3.70 (dd, 1 H, H-6b), 3.58 (t, 6 H, C*H*$_2$O), 3.46 (m, 1 H, H-5), 3.32 (t, 2 H, C*H*$_2$N$_3$), 3.24 (s, 1 H, H-5′), 2.21 (C*H*$_2$CON), 2.00 and 1.98 (C*H*$_3$COO); ^{13}C, δ 170.0 and 168.9 (C=O), 137.7 and 137.4 (ipso Ar), 128.9–127.7 (Ph), 101.4 (C-1), 100.9 (*C*HPh), 100.0 (C-1′), 74.6 (C-4), 74.6 (C-5), 73.4 (*C*H$_2$Ph), 73.0 (C-4′), 72.9 (C-3), 71.6 (C-3′), 70.2 and 69.7 (*C*H$_2$O), 68.8 (C-2′), 68.3 (C-6), 68.3 (C-6′), 65.8 (C-5′), 53.1 (C-2), 50.6 (*C*H$_2$N$_3$), 22.9 (*C*H$_2$CON), 20.7 and 20.3 (*C*H$_3$COO). Anal. Calcd for $C_{39}H_{51}N_4O_{15}$: C, 57.42; H, 6.30; N, 6.87. Found: C, 57.58; H, 6.45; N, 6.79.

*5-Azido-3-oxapentyl (2,3-di-*O*-acetyl-β-*D*-galactopyranosyl)-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-*D*-glucopyranoside* (**17**).—A solution of **16** (177 mg, 0.22 mmol) in aq 60% HOAc (2 mL) was stirred at 100 °C until TLC (2:1 CH_2Cl_2–acetone) showed the complete transformation of the starting material into a slower migrating product ($\sim$30 min). Then, the mixture was concentrated and the remaining acid was co-evaporated with toluene (3 × 3 mL). The residue was filtered through a short column of silica gel (1 g) using EtOAc as the solvent to give **17** as a foam (150 mg, 94%); NMR ($CDCl_3$): ^{1}H, δ 7.39 (Ph), 6.47 (d, 1 H, J 9.2 Hz, N*H*), 5.11 (dd, 1 H, $J_{1',2'}$ 8, $J_{2',3'}$ 10.2 Hz, H-2′), 5.01 (dd, 1 H, $J_{2,3}$ 10.1, $J_{3,4}$ 9 Hz, H-3), 4.69 (dd, 1 H, $J_{3',4'}$ 4 Hz, H-3′), 4.63 (AB, 2 H, J 12.3 Hz, C*H*$_2$Ph), 4.51 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.41 (d, 1 H, $J_{1',2'}$ 8 Hz, H-1′), 3.35 (t, 2 H, C*H*$_2$N$_3$), 2.21 (s, 3 H, C*H*$_3$CON), 2.00 and 1.98 (2 s, 6 H, C*H*$_3$COO); ^{13}C, δ 170.0 and 168.9 (C=O), 137.7 (ipso Ar), 128.9–127.7 (Ph), 101.1 (C-1), 99.9 (C-1′), 61.1 (C-6′), 55.1 (C-2), 50.4 (*C*H$_2$N$_3$), 22.9 (*C*H$_2$CON), 20.7 and 20.3 (*C*H$_3$COO). Anal. Calcd for $C_{32}H_{47}N_4O_{15}$: C, 52.81; H, 6.51; N, 7.70. Found: C, 52.68; H, 6.65; N, 7.57.

*5-Azido-3-oxapentyl (methyl [5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosyl]onate)-(2→6)-(2,3-di-*O*-acetyl-β-*D*-galactopyranosyl)-(1→4)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-*D*-glucopyranoside* (**19**) *and 5-azido-3-oxapentyl (methyl [5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero-β-*D*-galacto-2-nonulopyranosyl]onate)-(2→6)-(2,3-di-*O*-acetyl-β-*D*-galactopyranosyl)-(1→4)-2-acetamido-3-*O*-acetyl-2-deoxy-6-*O*-benzyl-β-*D*-glucopyranoside* (**20**).—A solution of **17** (62 mg, 0.09 mmol) and **18** [19] (103 mg, 0.18 mmol) in dry CH_3CN (1 mL) containing 3 Å molecular sieves (200 mg) was stirred for 1 h at rt. Then, the mixture was cooled to −40 °C, and *N*-iodosuccinimide (50 mg, 0.22 mmol) and a solution of trifluoromethanesulfonic acid in CH_3CN (0.22 M, 0.1 mL) were added under dry N_2. After being stirred for 4 h at −40 °C, the mixture was diluted with CH_2Cl_2 (10 mL), filtered through Celite, and the filtrate was washed with aq 10% $Na_2S_2O_3$ (3 mL), satd aq $NaHCO_3$ (3 mL) and H_2O (4 mL), then dried (Na_2SO_4), and concentrated. Column chromatography of the residue (50:1 $CHCl_3$–MeOH) afforded **19** (50.5 mg, 45%); $[\alpha]_D$ +35° (*c* 0.6, $CHCl_3$); R_f 0.35 (20:1 $CHCl_3$–MeOH); ^{13}C NMR ($CDCl_3$): δ 171.1–169.3 (6C=O), 167.8 ($J_{C\text{-}1neu,H\text{-}3a}$ 6.6 Hz, C-1neu), 137.7 (ipso Ar), 128.9–127.7 (Ph), 101.2 (C-1), 100.4 (C-1′), 98.8 (C-2neu), 62.3 and 62.1 (C-9neu,6′), 53.3 (C-2), 53.0 (O*C*H$_3$), 50.8 (*C*H$_2$N$_3$), 49.3 (C-5neu), 37.0 (C-3neu), 23.2 and 23.1 (*C*H$_3$CON), 20.9–20.7 (*C*H$_3$COO). Anal. Calcd for $C_{53}H_{76}N_5O_{26}$: C, 53.08; H, 6.39; N, 5.84. Found: C, 52.99; H, 6.51; N, 5.80 and **20** (16 mg, 16.5%); R_f 0.37 (20:1 $CHCl_3$–MeOH); ^{13}C NMR ($CDCl_3$): δ 171.7–169.4 (6C=O), 166.9 ($J_{C\text{-}1neu,H\text{-}3a}$ 1.7 Hz, C-1neu), 137.7 (ipso Ar), 128.9–127.7 (Ph), 101.2 (C-1), 100.5 (C-1′), 98.3 (C-2neu), 62.5 and 60.7 (C-9neu,6′), 53.2 (C-2), 52.9 (O*C*H$_3$), 50.8 (*C*H$_2$N$_3$), 49.6 (C-5neu), 37.3 (C-3neu), 23.1 (2*C*H$_3$CON), 21.0–20.8 (*C*H$_3$COO). Anal. Calcd for $C_{53}H_{76}N_5O_{26}$: C, 53.08; H, 6.39; N, 5.84. Found: C, 52.97; H, 6.53; N, 5.78.

*5-Amino-3-oxapentyl (sodium [5-acetamido-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosyl]onate)-(2→6)-(β-*D*-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-*D*-glucopyranoside* (**21**).—Compound **19** (60 mg, 51.9 μmol) was dissolved in a solution of methanolic NaOMe (0.05 M, 2 mL), and stirred overnight at rt, then H_2O (1 mL) was added and the mixture was stirred for 12 h at rt. Then, the pH was brought to 7 with Amberlite IR 120 (H^+) resin, and after filtration, aq 0.1 M NaOH (0.5 mL) was added to the solution. The solvents were evaporated in vacuo and the residue was chromatographed on a LH-20 column slurred with MeOH. Hydrogenolysis of the resulting foam in 2:1 MeOH–H_2O in the presence of 10% Pd/C under H_2 gas rendered after lyophilization **21** as a white powder (35.5 mg, 91%); R_f 0.17 (3:3:1:0.1 EtOAc–MeOH–H_2O–HOAc); NMR (D_2O): ^{1}H, δ 4.48 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.45 (d, 1 H, $J_{1',2'}$ 8.2 Hz, H-1′), 3.19 (t, 2 H, C*H*$_2$NH$_2$), 2.69 (dd, 1 H, J_{gem} 13.7, $J_{3e,4}$ 4.5 Hz, H-3$_e$neu), 2.04 and 2.02 (2 s, 6 H, C*H*$_3$CON), 1.73 (t, 1 H, H-3$_a$neu); ^{13}C, δ 105.1, 103.4 and 100.4 (C-1, 1′, 2neu), 78.4, 78.0 and 77.8, (C-4,3′,6neu), 64.7, 64.0 and 62.0 (C-6,6′,9neu), 57.9 and 54.0 (C-2,5neu), 42.4 (C-

3neu), 41.5 (CH_2NH_2), 24.8 and 24.2 (CH_3CON). Anal. Calcd for $C_{31}H_{55}N_3NaO_{19}$: C, 46.73; H, 6.96; N, 5.27. Found: C, 46.85; H, 7.09; N, 5.15.

*5-(β-Maleimidopropionamido)-3-oxapentyl (sodium [5-acetamido-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosyl]onate)-(2→6)-(β-*D*-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-*D*-glucopyranoside* (**22**).—To a solution of the free oligosaccharide **21** (5.2 mg, 4 μmol) in Me_2NCHO/PBS (0.5 mL) was added the *N*-hydroxysuccinimide derivative of β-maleimidopropanoic acid [24] (2.6 mg, 10 μmol). After 2 h, the solution was concentrated and dried in vacuo, the residue was resuspended in D_2O (0.5 mL, for the NMR analysis) and centrifuged to remove the excess of reagent. The maleimido derivative **22** could be stored in a lyophilized form at 0 °C or used directly in the coupling reaction; ^{1}H NMR (D_2O): δ 6.90 (s, 0.3 H, HC=CH β-maleimido-propionic acid), 6.89 (s, 2 H, HC=CH), 3.32 (t, 2 H, CH_2NH), 3.05 and 2.56 (2 t, 0.3 H each, CH_2 β-maleimido-propionic acid), 2.48 (t, 2 H, CH_2CO).

Coupling reaction between **22** *and thiolated BSA*.—Compound **22** was added to a solution [12] of BSA-SH_{30} in PBS (pH 7.2, 0.4 mL). After 2 h, the process was complete as evidenced by a negative Ellman test [25]. The resulting solution was dyafiltrated against PBS (pH 7.4). The protein and carbohydrate contents were determined by the Lowry [26] and phenol–sulfuric acid methods [27], respectively. Several assays gave a carbohydrate to protein ratio of 13–15 oligosaccharide units per BSA molecule.

Acknowledgements

The authors wish to thank the Ministry of Health for financial support.

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