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Synthesis of β-(1→6)-linked glucosamine oligosaccharides corresponding to fragments of the bacterial surface polysaccharide poly-*N*-acetylglucosamine

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Dedicated to the memory of Professor Nikolay K. Kochetkov

Abstract—A series of 3-β-acetamidopropyl oligo-β-(1→6)-glucosamines consisting of 5, 7, 9 and 11 glucosamine residues, and a series of corresponding per-N-acetylated derivatives were synthesized using a convergent blockwise approach. These compounds represent fragments of a bacterial surface polysaccharide produced by numerous bacterial pathogens, including *Staphylococcus aureus*, and will be used as models for its biochemical and immunological properties.

Keywords: *Staphylococcus aureus*; Poly-β-(1→6)-*N*-acetyl glucosamine; PNAG; Oligoglucosamines; Convergent synthesis

1. Introduction

In modern medicine many bacterial species are capable of causing diseases in hospitalized patients, often due to their ability to bind, to adhere, and to form biofilms on indwelling medical devices. Among the most prominent ones are the staphylococci, which cause a wide range of diseases in animals and humans. *Staphylococcus aureus* and coagulase-negative staphylococci, most notably *S. epidermidis*, are important causes of infection related to colonization of medical implants and formation of biofilms.[1] The importance of biofilm production for the virulence of *S. aureus* and *S. epidermidis* has been shown by several clinical and animal studies.[2] Numerous components of these extracellular biofilm layers have been identified, but one of the major components is poly-β-(1→6)-*N*-acetyl glucosamine (PNAG).[3,4] It has also been shown that this antigen has a good potential for use as an effective immunotherapeutic agent, but its immunogenicity depends on the degree of N-acetylation.[5] However, finding the optimal form for eliciting high titers of protective antibody is a difficult task. When PNAG is isolated from bacterial sources, a heterogeneous mixture of poly- and oligosaccharides with different immunological activities is obtained. Therefore, synthetic oligoglucosamines with glucosamine units bearing N-acetylated and free amino groups in defined positions are needed to determine the structure of active epitopes. As a first goal, we set out to synthesize oligoglucosamines, composed of glucosamine residues wherein either all of the amino groups were unprotected or N-acetylated.

To date, only a few papers dealing with the syntheses of oligo-β-(1→6)-glucosamine derivatives, including O-substituted oligomers,[6] have been published. Different approaches have been used such as solid-phase synthesis (trisaccharide),[6] multi-component coupling using solution and polymer support technology (trisaccharide),[7] ring-opening polymerization using 1,6-anhydro derivatives (trisaccharide),[8] one-pot synthesis (tetrasaccharide),[9] and block synthesis (penta-,[6] hexa-,[10] and nonasaccharide[11]). Some of these methods resulted in

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acceptable yields of oligosaccharides, which were mainly prepared as substituted, but not the de-blocked derivatives that are needed for biochemical investigations. The synthesis of both forms of oligo-β-(1→6)-glucosamines (N-unsubstituted and N-acetylated) in relation to PNAG studies has not been published so far.

Our first approach to the synthesis of these structures involved the reaction of terminated oligomerization, which has previously proven to be effective for the assembly of some other oligo- and polysaccharides.[12,13] However, the results of our experiments revealed a low efficiency of this method for the preparation of oligo-β-(1→6)-glucosamines.[14] Therefore, blockwise assembling of oligosaccharide chains was thought to potentially be a more reliable and scalable strategy based on the known examples of the syntheses of oligo-β-(1→6)-glucosamines and our experiments using terminated oligomerization.

To determine the sufficient length of oligosaccharide ligands for effective binding to antibodies to PNAG, a representative series of oligoglucosamines with a different number of glucosamine units were needed; therefore, oligoglucosamines with 5, 7, 9, and 11 monosaccharide residues, containing either unsubstituted (**15**–**18**) or N-acetylated (**19**–**22**) amino groups, were selected as the target structures. These compounds were prepared from a series of selectively substituted precursors (**8**, **10**, **12**, **13**) carrying Z-amidopropyl aglycon suitable for further functionalization and glycoconjugate design, which is the goal in the next step of our project.

2. Results and discussion

Although attempts to prepare oligoglucosamines by terminated oligomerization failed,[14] many synthetic instruments elaborated within this work were widely used in the block synthesis presented below. These included protecting group strategies (acetyl and benzoyl as temporary and permanent O-protecting groups, and phthaloyl and benzyloxycarbonyl for protection of glucosamine and spacer-arm amino groups, respectively), mono- and disaccharide building blocks **1**–**4** (Scheme 1), and optimal glycosylation conditions.

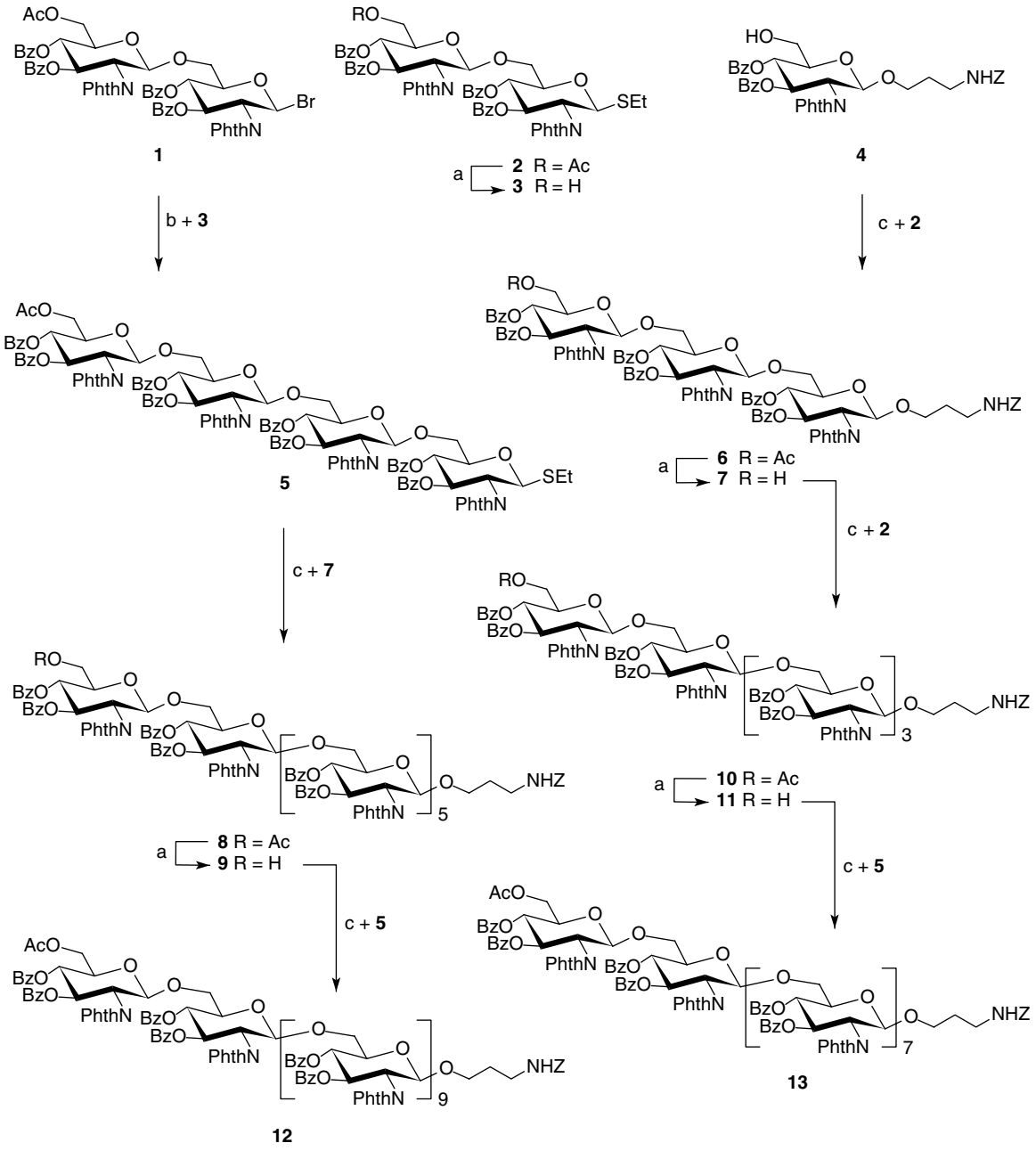
For the synthesis of target oligosaccharides, we employed a convergent approach based on the use of a minimal number of relatively large building blocks. The tetrasaccharide thioglycoside **5** was proposed as a key glycosylating agent. It was prepared in 76% yield by glycosylation of thioglycoside **3** with bromide **1** (Scheme 1) under Helferich conditions. Since the resulting tetrasaccharide **5** differed sufficiently in the molecular mass from the starting materials, it could be readily isolated by means of gel-permeation chromatography on the hydrophobic gel Bio-Beads SX-3 (exclusion limit 2000). This simple and convenient method of isolation and purification also enabled us to avoid silica gel chromatography in all further glycosylation steps.

For the synthesis of acceptor blocks bearing a protected 3-aminopropyl moiety at the reducing terminal sugar, we started from compound **4** described in our previous work.[14] The reaction of **4** with disaccharide **2** furnished compound **6** in 94% yield. Selective removal of the sole *O*-acetyl group by treatment with HCl–MeOH resulted in almost quantitative yield of the trisaccharide acceptor **7**. The presence of a free hydroxyl group at C-6 was confirmed by the shifts of the signal for C-6 and C-5 of the terminal glucosamine residue in the ^{13}C NMR spectrum from δ 62.40 (C-6^{III}-OAc) to δ 61.21 (C-6^{III}-OH) and from δ 71.74 (C-5^{III}-OAc) to δ 74.20 (C-5^{III}-OH). Subsequent coupling between thioglycoside **2** and acceptor **7** produced one of the target compounds, pentasaccharide **10**, in 90% yield, which was deacetylated in a similar way to give pentasaccharide acceptor block **11**. The signals for each type of related protons (carbon atoms) in the NMR spectra of pentasaccharide **10** and all higher oligomers appeared as complex multiplets, resulting from superposition of signals of very close but not completely equivalent protons (carbon atoms) belonging to individual glucosamine residues. The length of the oligosaccharide chains was revealed from the ratios between protons of the spacer-arm moiety and protons at the C-3 atoms.

The reaction of tetrasaccharide donor **5** with acceptor **7** furnished the desired spacer-armed heptasaccharide **8** in 74% yield. Selective deacetylation of **8** clearly produced **9**, but longer reaction times were required (2 days compared to 16 h for **6**). Purification of heptasaccharide **8** and longer oligosaccharides was performed using a Bio-Beads SX-1 gel with an exclusion limit of 14,000.

Similarly, the target nonasaccharide **13** was obtained in 60% yield by the reaction of thioglycoside **5** with pentasaccharide acceptor **11**. Glycosylation of heptasaccharide acceptor **9** with donor **5** was carried out under the same conditions as previous glycosylation reactions but resulted in only 50% yield of the undecasaccharide **12**, with 40% of the unreacted **9** recovered. Tetrasaccharide glycal **14** [δ 6.75 (s, 1H, H-1), δ 5.85 (d, 1H, J 3.7, H-3), δ 105.33 (C-1)] was also isolated as a main side product (Fig. 1). Attempts at reducing the formation of **14** by the use of non-basic molecular sieves AW-300 failed and gave rise to the formation of remarkably low yield of product **12**. The relatively low reactivity of acceptor **9** can be probably explained by its bulky structure.

As the next step in the preparation of target compounds **15**–**22**, Z-groups in the protected precursors **8**, **10**, **12**, and **13** were replaced by an acetyl (Scheme 2) followed by total N,O-deprotection of glucosamine residues by treatment with hydrazine hydrate in boiling EtOH. Free oligosaccharides **15**–**18** formed in the yields of ~85% were purified by gel-permeation chromatography.



Scheme 1. Synthesis of protected oligoglucosamines. Reagents and conditions: (a) AcCl, MeOH; (b) $HgBr_2$, $Hg(CN)_2$, CH_3CN; (c) NIS, TfOH, MS 4 Å, CH_2Cl_2.

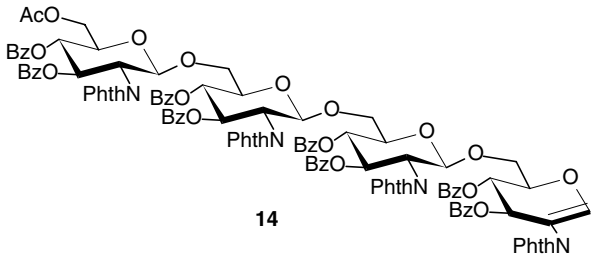
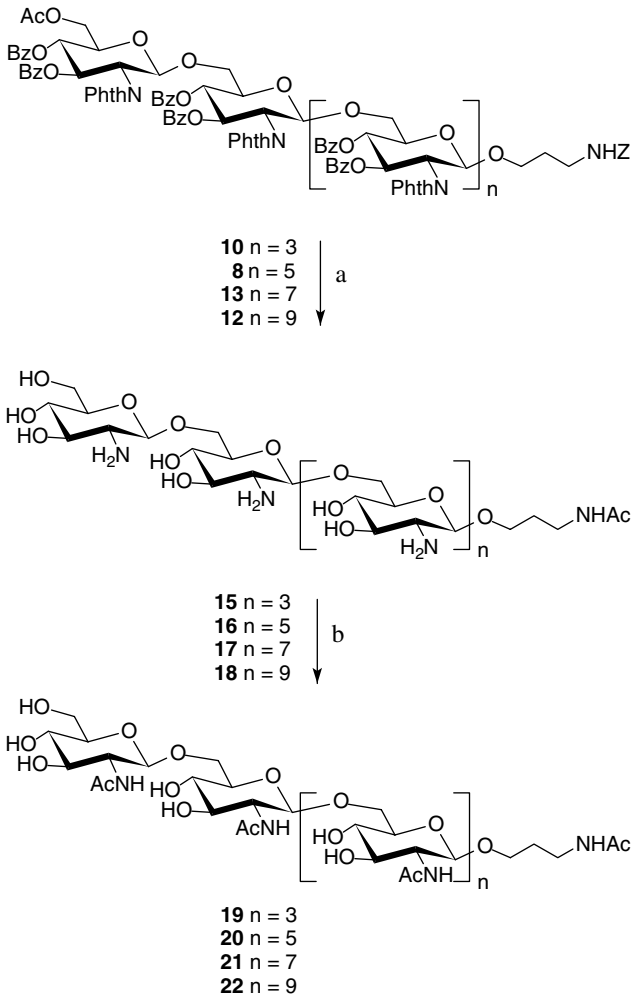


Figure 1.

^{1}H NMR spectra of compounds **15**–**18** represent a number of broad multiplets (vide supra), from which only signals of the aglycon moiety [δ 1.81 (m, $OCH_2CH_2CH_2N$), 1.90 (s, N–$COCH_3$), 3.20; 3.30 (2m, $OCH_2CH_2CH_2N$)] and some signals of glucosamines [δ 2.90 (m, H-2), 4.23 (m, H-6 of glycosylated residues), and 4.70 (m, H-1)] can be assigned unambiguously. The length of the oligosaccharide chain was confirmed by the ratio of integral intensities of spacer protons and protons at C-2 and the data of ESI mass-spectra.

To obtain the fully N-acetylated compound, pentasaccharide **15** was treated with an excess of acetic anhydride



Scheme 2. Reagents and conditions: (a) H_2, $Pd(OH)_2$, MeOH–THF; Ac_2O, Py; $N_2H_4{\cdot}H_2O$, EtOH; (b) Ac_2O, MeOH–H_2O.

in aqueous methanol. Product **19** was isolated in almost quantitative yield. The completeness of N-acetylation was deduced from the absence of the signal at δ ~2.90 characteristic for H-2 of the glucosamine residue with a free amino group and the upfield shift for the signal for H-1 from δ 4.70 (free amines) to δ 4.50 (NH–Ac). When applied to heptasaccharide **16**, these conditions did not result in complete N-acetylation; repeated treatment in the presence of solid $NaHCO_3$ was necessary to complete the reaction. Oligo-*N*-acetyl glucosamines **21** and **22** were prepared in a similar way. It should be noted that all N-acetylated oligosaccharides were completely soluble in water at 0.5 mg/mL concentration except undecasaccharide **22**, which was remarkably less water-soluble and gave an opalescent solution with the partial formation of a sediment. The lower solubility of undecasaccharide **22** could potentially be accounted for by the appearance of helical conformational regularity in its chain or by its ability to self-aggregate into macro-complexes through inter-molecular interactions of acetamido-groups.

In conclusion, a series of oligo-β-(1→6)-glucosamines comprising 5, 7, 9, and 11 monosaccharide residues with either all free or all N-acetylated amino groups have been efficiently prepared. Biochemical and immunological investigations of the properties of these synthetic oligoglucosamines, as well as their theoretical (MM3 calculations) and experimental (NOE-NMR, $^3J_{C,H}$-NMR) conformational analysis, are in progress.

3. Experimental

3.1. General methods

NMR spectra were recorded on Bruker DRX-500 and Bruker AM-300 instruments. The spectra of protected oligosaccharides were measured for solutions in $CDCl_3$, and ^1H NMR chemical shifts were referenced to a residual solvent signal. NMR spectra of free oligosaccharides were measured for solutions in D_2O. Optical rotation values were measured on a JASCO DIP-360 polarimeter at 22 ± 2 °C. TLC was performed on Silica Gel 60 F_{254} plates (E. Merck), and visualization was accomplished using UV light or by charring with 10% H_3PO_4 in ethanol. Column chromatography was carried out on Silica gel 60 (40–63 μm, E. Merck). Gel-permeation chromatography of protected oligosaccharides was performed on columns with Bio-Beads SX-3 (15 × 500 mm) or Bio-Beads SX-1 (20 × 600 mm) (Bio-Rad Laboratories). Gel-permeation chromatography of free oligosaccharides was performed on a column with TSK HW-40 (S) (25 × 400 mm) in 0.1 M AcOH. ESIMS spectra were recorded with a Micromass Quattro system. All reactions involving air- or moisture-sensitive reagents were carried out using dry solvents under dry argon.

3.2. General procedure for Helferich glycosylation

To a solution of a glycosyl acceptor (1 mmol), $Hg(CN)_2$ (1.2 mmol), and $HgBr_2$ (0.36 mmol) in anhyd CH_3CN (1 mL) was added a solution of glycosyl bromide (1.2 mmol) in anhyd CH_3CN (0.5 mL) at rt under dry argon. The mixture was stirred for 30 min, then diluted with $CHCl_3$ and poured into a mixture of 1 M aq KI and $NaHCO_3$, then extracted with $CHCl_3$. The organic extract was dried over anhyd Na_2SO_4 and concentrated. The residue was subjected to chromatography on a Bio-Beads SX-3 column in toluene to give the desired product.

3.3. General procedure for selective deacetylation in the presence of benzoyl groups

A solution of a 6-*O*-acetyl derivative (1 mmol) in CH_2Cl_2 (6 mL) was diluted with abs MeOH (20 mL) and then AcCl (0.2 mL) was added under cooling with an ice-bath. The mixture was kept for 16 h at rt and then

concentrated. The residue was purified by flash chromatography on silica gel (EtOAc–toluene) to give the product with a free terminal 6-OH group.

3.4. General procedure for NIS–TfOH-catalyzed glycosylation

A solution of an ethyl thioglycoside (0.1 mmol) and a glycosyl acceptor (0.09 mmol) in freshly distilled anhydrous CH_2Cl_2 (1 mL) containing 4 Å MS (130 mg) was stirred at rt under argon for 1 h and then cooled to −20 °C. NIS (0.2 mmol) and TfOH (0.04 mmol) were successively added and the resulting mixture was stirred for an additional 30 min at −20 °C. The reaction was quenched with a drop of pyridine, allowed to attain rt, diluted with CH_2Cl_2, and filtered through Celite. The filtrate was washed with 1 M $Na_2S_2O_3$, then with water, and dried with Na_2SO_4, and concentrated. The residue was purified by chromatography on the Bio-Beads SX-1 column in toluene.

3.5. General procedure for total deprotection of oligoglucosamines and subsequent N-acetylation

A protected oligosaccharide (0.01 mmol) was dissolved in 1:1 MeOH–THF (1.5 mL) and $Pd(OH)_2$/C (50 mg) was added. The resulting mixture was stirred under an H_2 atmosphere (1 atm) overnight, then filtered through Celite and the filtrate was concentrated. The product of hydrogenolysis was dissolved in pyridine (0.5 mL) and treated with Ac_2O (0.2 mL) and the resultant mixture was kept for 1 h at rt and concentrated. The residue was dissolved in EtOH (10 mL) and $N_2H_4{\cdot}H_2O$ (1 mL) was added. The mixture was heated under reflux for 3 h, then concentrated and co-concentrated several times with toluene under diminished pressure. The residue was purified on a TSK HW-40S column in 0.1 M AcOH to give a pure oligo-β-(1→6)-glucosamine. The latter was N-acetylated by treatment with Ac_2O (50 μL) in 50% aqueous MeOH for 16 h at rt. The reaction mixture was neutralized with solid $NaHCO_3$ and an additional portion of Ac_2O (50 μL) was added. After 1 h the solvent was evaporated and the crude product was subjected to gel chromatography on TSK HW-40S in 0.1 M AcOH to provide an oligo-β-(1→6)-*N*-acetylglucosamine oligosaccharide.

3.6. Ethyl 6-*O*-acetyl-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (5)

Compound **2**[14] (300 mg, 0.27 mmol) was dissolved in 2 mL of anhyd CH_2Cl_2 and a solution of Br_2 (15 μL, 0.29 mmol) in 0.1 mL of CH_2Cl_2 was added. The mixture was kept in the dark for 1 h at rt and then evaporated to give bromide **1**. Glycosyl bromide **1** (300 mg, 0.26 mmol) was reacted with **3** (210 mg, 0.20 mmol) as described in Section 3.2 to give **5** (315 mg, 76%) as a colorless foam: $[\alpha]_D$ +13 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.15 (t, 3H, *J* 7.8, SCH_2CH_3), 1.95 (s, 3H, $COCH_3$), 2.5–2.8 (m, 2H, SCH_2CH_3), 3.58 (m, 1H, H-6), 3.75 (m, 3H, H-5, 2H-6), 3.87 (m, 1H, H-6), 3.95–4.10 (m, 4H, 2H-5, 2H-6), 4.15–4.35 (m, 4H, H-2, H-5, 2H-6), 4.45–4.6 (m, 3H, 3H-2), 5.05 (t, 1H, *J* 9.7, H-4), 5.20 (t, 1H, *J* 9.5, H-4), 5.35–5.50 (m, 3H, 2H-1, H-4), 5.55 (m, 2H, H-1, H-4), 5.69 (d, 1H, *J* 8.4, H-1^{IV}), 6.10 (m, 2H, 2H-3), 6.22 (t, 1H, *J* 9.7, H-3), 6.32 (t, 1H, *J* 9.7, H-3^{IV}), 7.15–7.55 (m, 24H, aromatics), 7.60–7.90 (m, 32H, aromatics); ^{13}C NMR: δ 14.92, 20.60 ($COCH_3$), 23.76, 53.84 (4C-2), 54.61, 54.78, 62.55, 67.18, 67.82, 69.92, 70.14, 70.52, 70.90, 71.08, 71.73, 72.05, 72.67, 73.11, 77.36, 80.70 (C-1^I), 97.07 (1C, C-1), 97.98 (2C, C-1), 123.52, 123.69, 125.28, 128.18, 128.37, 128.68, 128.90, 129.01, 129.29, 129.74, 129.79, 131.19, 131.63, 133.10, 133.29, 133.86, 134.00, 134.28, 164.82, 164.97, 165.17, 165.23, 165.43, 165.57, 167.10, 167.84, 170.61. Anal. Calcd for $C_{116}H_{92}N_4O_{33}S$: C, 66.28; H, 4.41; N, 2.67. Found: C, 65.99; H, 4.30; N, 2.64.

3.7. 3-(Benzyloxycarbonylamino)propyl 6-*O*-acetyl-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (6)

Glycosyl donor **2** (200 mg, 0.18 mmol) was reacted with acceptor **4** (120 mg, 0.17 mmol) as described in Section 3.4 to give **6** (280 mg, 94%) as a colorless foam: $[\alpha]_D$ +14.6 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.60 (m, 2H, $OCH_2CH_2CH_2N$), 2.00 (s, 3H, $COCH_3$), 3.05 (m, 2H, $OCH_2CH_2CH_2N$), 3.45 (m, 1H, $OCH_2CH_2CH_2N$), 3.65–3.80 (m, 3H, $OCH_2CH_2CH_2N$, 2H-6), 3.85 (m, 1H, H-5), 3.90 (m, 3H, H-5, 2H-6), 4.08 (m, 1H, H-5^{III}), 4.2 (d, 1H, *J* 12.1, H-6C), 4.27 (dd, 1H, *J* 5.3, H-6^{III}), 4.40 (m, 2H, 2H-2), 4.50 (dd, 1H, *J* 10.0 and 8.5, H-2^{III}), 5.00 (s, 2H, CH_2Ph), 5.10 (m, 1H, NH), 5.21 (t, 1H, *J* 9.7, H-4), 5.30 (t, 1H, *J* 9.7, H-4), 5.38 (d, 1H, *J* 8.3, H-1), 5.45 (d, 1H, *J* 8.4, H-1), 5.53 (t, 1H, *J* 10.0, H-4^{III}), 5.62 (d, 1H, *J* 8.5, H-1^{III}), 6.10 (m, 2H, 2H-3), 6.23 (t, 1H, *J* 10.0, H-3^{III}), 7.10–7.50 (m, 27H, aromatics), 7.60–7.90 (m, 20H, aromatics); ^{13}C NMR: δ 20.58 ($COCH_3$), 29.44 ($OCH_2CH_2CH_2N$), 37.96 ($OCH_2CH_2CH_2N$), 54.69 (3C-2), 62.53 (C-6C), 66.27 (CH_2Ph), 67.13 ($OCH_2CH_2CH_2N$), 67.44 (C-6), 67.71 (C-6), 69.91 (C-4^{III}), 70.17 (C-4), 70.24 (C-4), 70.92 (C-3), 71.05 (C-3^{III}), 71.20 (C-3), 71.86 (C-5^{III}), 73.23 (C-5), 73.30 (C-5), 97.49 (C-1), 97.95 (2C-1), 123.48, 125.24, 127.86, 127.93, 128.66, 128.97, 131.36, 131.54, 133.06, 133.25, 133.95, 134.07, 156.35 (OC(O)NH),

164.96, 165.17, 165.55, 167.41, 170.553, 185.02. Anal. Calcd for $C_{97}H_{80}N_4O_{28}$: C, 66.59; H, 4.61; N, 3.20. Found: C, 66.40; H, 4.63; N, 3.22.

3.8. 3-(Benzyloxycarbonylamino)propyl 3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (7)

Compound **6** (280 mg, 0.16 mmol) was deacetylated as described in Section 3.3 to give **7** (260 mg, 95%) as a colorless foam: ^{1}H NMR ($CDCl_3$): δ 1.60 (m, 2H, $OCH_2CH_2CH_2N$), 3.10 (m, 3H, $OCH_2CH_2CH_2N$, OH), 3.50 (m, 1H, $OCH_2CH_2CH_2N$), 3.65 (m, 1H, H-6^{III}), 3.70–3.85 (m, 4H, $OCH_2CH_2CH_2N$, 3H-6), 3.88 (m, 1H, H-5^{III}), 3.95 (m, 3H, 2H-5, H-6), 4.05 (m, 1H, H-6), 4.35 (dd, 1H, *J* 8.4 and 10.6, H-2^{III}), 4.45 (m, 2H, 2H-2), 5.02 (s, 2H, CH_2Ph), 5.18 (m, 1H, NH), 5.30–5.40 (m, 3H, 3H-4), 5.44 (d, 1H, *J* 8.4, H-1), 5.52 (d, 1H, *J* 8.4, H-1), 5.57 (d, 1H, *J* 8.4, H-1^{III}), 6.10 (m, 2H, 2H-3), 6.24 (t, 1H, *J* 10.0, H-3^{III}), 7.10–7.50 (m, 22H, aromatics), 7.55–7.92 (m, 25H, aromatics); ^{13}C NMR: δ 29.43 ($OCH_2CH_2CH_2N$), 37.94 ($OCH_2CH_2CH_2N$), 54.59 (C-2), 54.65 (C-2), 54.73 (C-2), 61.26 (C-6^{III}), 66.30 (CH_2Ph), 67.32 ($OCH_2CH_2CH_2N$), 67.75 (C-6), 67.86 (C-6), 69.82 (C-4), 70.30 (C-4), 70.70 (C-4), 71.06 (2C-3), 71.19 (C-3), 72.59 (C-5), 73.34 (C-5), 74.33 (C-5^{III}), 97.55 (C-1^{III}), 97.75 (C-1), 98.04 (C-1), 123.46, 125.27, 127.89, 127.95, 128.37, 128.44, 128.62, 128.71, 128.89, 128.95, 128.99, 130.36, 131.33, 131.55, 133.12, 133.286, 133.345, 134.027, 134.12, 136.86, 156.32, 165.09, 165.46, 165.55, 167.50. Anal. Calcd for $C_{95}H_{78}N_4O_{27}$: C, 66.82; H, 4.60; N, 3.28. Found: C, 66.62; H, 4.62; N, 3.26.

3.9. 3-(Benzyloxycarbonylamino)propyl 6-*O*-acetyl-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (8)

Glycosylation of **7** (100 mg, 0.06 mmol) with thioglycoside **5** (150 mg, 0.07 mmol) was accomplished as described in Section 3.4 to give **8** (165 mg, 74%) as a colorless foam: $[\alpha]_D$ +16 (*c* 1, $CHCl_3$); Selected ^{1}H NMR data ($CDCl_3$): δ 3.00 (m, 2H, $OCH_2CH_2CH_2N$), 6.10 (m, 6H, 6H-3), 6.25 (t, 1H, *J* 10, H-3^{VII}); Selected ^{13}C NMR ($CDCl_3$): δ 62.56 (C-6^{VII}), 71.74 (C-5^{VII}), 97.25, 97.51, 97.82, 97.95 (C-1). Anal. Calcd for $C_{209}H_{164}N_8O_{60}$: C, 66.98; H, 4.41; N, 2.99. Found: C, 66.77; H, 4.29; N, 2.97.

3.10. 3-(Benzyloxycarbonylamino)propyl 3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (9)

Compound **8** (82 mg, 0.02 mmol) was deacetylated as described in Section 3.3 to give **9** (78 mg, 96%) as a colorless foam: $[\alpha]_D$ +10 (*c* 1, $CHCl_3$); Selected ^{13}C NMR ($CDCl_3$): δ 61.30 (C-6^{VII}), 74.21 (C-5^{VII}), 97.33, 97.52, 97.82, 98.03 (C-1). Anal. Calcd for $C_{207}H_{162}N_8O_{59}$: C, 67.10; H, 4.41; N, 3.02. Found: C, 66.95; H, 4.43; N, 3.01.

3.11. 3-(Benzyloxycarbonylamino)propyl 6-*O*-acetyl-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (10)

The reaction of disaccharide **2** (96 mg, 0.086 mmol) with acceptor **7** (130 mg, 0.076 mmol) as described in Section 3.4 resulted in the formation of **10** (188 mg, 90%) as a colorless foam: $[\alpha]_D$ +13 (*c* 1, $CHCl_3$); Selected ^{1}H NMR data ($CDCl_3$): δ 3.00 (m, 2H, $OCH_2CH_2CH_2N$), 6.08 (m, 4H, 4H-3), 6.26 (t, 1H, *J* 9, H-3^{V}); Selected ^{13}C NMR ($CDCl_3$): δ 62.55 (C-6^{V}), 71.74 (C-5^{V}), 97.42, 97.54, 97.76, 97.95 (C-1). Anal. Calcd for $C_{153}H_{122}N_6O_{44}$: C, 66.86; H, 4.47; N, 3.06. Found: C, 66.74; H, 4.42; N, 3.05.

3.12. 3-(Benzyloxycarbonylamino)propyl 3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (11)

Compound **10** (180 mg, 0.02 mmol) was deacetylated as described in Section 3.3 to give **11** (164 mg, 93%) as a

colorless foam: $[\alpha]_D$ +5 (*c* 1, $CHCl_3$); Selected ^{13}C NMR ($CDCl_3$): δ 61.27 (C-6^{V}), 74.20 (C-5^{V}), 97.46, 97.58, 97.73, 97.98 (C-1). Anal. Calcd for $C_{151}H_{120}N_6O_{43}$: C, 67.01; H, 4.47; N, 3.10. Found: C, 66.88; H, 4.36; N, 3.10.

3.13. 3-(Benzyloxycarbonylamino)propyl 6-*O*-acetyl-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (12)

Glycosylation of **9** (80 mg, 0.022 mmol) with thioglycoside **5** (60 mg, 0.028 mmol) was accomplished as described in Section 3.4 to give **12** (63 mg, 51%) as a colorless foam. $[\alpha]_D$ +17 (*c* 1, $CHCl_3$); Selected ^{1}H NMR data ($CDCl_3$): δ 3.00 (m, 2H, $OCH_2CH_2CH_2N$), 6.08 (m, 10H, 10H-3), 6.25 (t, 1H, *J* 9, H-3^{XI}); Selected ^{13}C NMR ($CDCl_3$): δ 62.65 (C-6^{XI}), 71.79 (C-5^{XI}), 97.28, 97.65, 97.89 (C-1). Anal. Calcd for $C_{321}H_{248}N_{12}O_{92}$: C, 67.10; H, 4.35; N, 2.93. Found: C, 66.99; H, 4.38; N, 2.95.

3.14. 3-(Benzyloxycarbonylamino)propyl 6-*O*-acetyl-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (13)

The reaction of tetrasaccharide **5** (56 mg, 0.027 mmol) with acceptor **11** (60 mg, 0.022 mmol) as described in Section 3.4 resulted in the formation of **13** (63 mg, 60%) as a colorless foam: $[\alpha]_D$ +16 (*c* 1, $CHCl_3$); Selected ^{1}H NMR data ($CDCl_3$): δ 3.03 (m, 2H, $OCH_2CH_2CH_2N$), 6.12 (m, 8H, 8H-3), 6.27 (t, 1H, *J* 9, H-3^{IX}); Selected ^{13}C NMR ($CDCl_3$): δ 62.59 (C-6^{IX}), 97.24, 97.61, 97.86, 97.98 (C-1). Anal. Calcd for $C_{256}H_{206}N_{10}O_{76}$: C, 67.06; H, 4.37; N, 2.95. Found: C, 67.10; H, 4.40; N, 2.92.

3.15. 3-Acetamidopropyl 2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranoside (15)

Compound **11** (50 mg, 0.018 mmol) was deprotected as described in Section 3.5 to give **15** (12 mg, 71%) $[\alpha]_D$ −23 (*c* 0.5, H_2O); Selected ^{1}H NMR data (D_2O): δ 1.8 (m, 2H, $OCH_2CH_2CH_2N$), 1.95 (s, 3H, $COCH_3$), 3.0 (m, 5H, 5H-2), 3.2 (m, 1H, $OCH_2CH_2CH_2N$), 3.29 (m, 1H, $OCH_2CH_2CH_2N$), 4.23 (m, 4H, 4H-6), 4.70 (m, 5H, 5H-1); Selected ^{13}C NMR (D_2O): δ 23.13 ($NCOCH_3$), 29.64 ($OCH_2CH_2CH_2N$), 37.30 ($OCH_2CH_2CH_2N$), 56.89 (C-2), 61.58 (C-6^{V}), 69.77 (C-6^{I-IV}), 100.53, 100.88, 101.35 (C-1). ESI(+)-MS: calcd for $C_{35}H_{66}N_6O_{22}$: 922.42 [M]; found 923.42 $[M+H]^+$.

3.16. 3-Acetamidopropyl 2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranoside (16)

Compound **8** (53 mg, 0.014 mmol) was deprotected as described in Section 3.5 to give **16** (16 mg, 93%) $[\alpha]_D$ −22 (*c* 0.5, H_2O); Selected ^{1}H NMR data (D_2O): δ 1.8 (m, 2H, $OCH_2CH_2CH_2N$), 2.95 (m, 7H, 7H-2). Selected ^{13}C NMR (D_2O): δ 23.14 ($NCOCH_3$), 29.63 ($OCH_2CH_2CH_2N$), 37.32 ($OCH_2CH_2CH_2N$), 57.06 (C-2), 61.65 (C-6^{VII}), 69.69 (C-6^{I-VI}), 101.00, 101.47, 102.14 (C-1). ESI(+)-MS: calcd for $C_{47}H_{88}N_8O_{30}$: 1244.56 [M]; found 1245.57 $[M+H]^+$.

3.17. 3-Acetamidopropyl 2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranoside (17)

Compound **13** (50 mg, 0.011 mmol) was deprotected as described in Section 3.5 to give **17** (15.8 mg, 95%) $[\alpha]_D$ −27 (*c* 1, H_2O); Selected ^{1}H NMR data (D_2O): δ 1.70 (m, 2H, $OCH_2CH_2CH_2N$), 2.90 (m, 9H, 9H-2). Selected ^{13}C NMR (D_2O): δ 23.62 ($NCOCH_3$), 29.73 ($OCH_2CH_2CH_2N$), 37.35 ($OCH_2CH_2CH_2N$), 56.88 (C-2), 61.62 (C-6^{IX}), 69.88 (C-6^{I-VIII}), 100.63, 101.05 (C-

1). ESI(+)-MS: calcd for $C_{59}H_{110}N_{10}O_{38}$: 1566.70 [M]; found 784.36 $[M+2H]^{2+}$.

3.18. 3-Acetamidopropyl 2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranoside (18)

Compound **12** (50 mg, 0.009 mmol) was deprotected as described in Section 3.5 to give **18** (12 mg, 75%) $[\alpha]_D$ −28 (*c* 1, H_2O); Selected ^1H NMR data (D_2O): δ 1.80 (m, 2H, $OCH_2CH_2CH_2N$), 3.00 (m, 11H, 11H-2). Selected ^{13}C NMR (D_2O): δ 23.07 ($NCOCH_3$), 29.57 ($OCH_2CH_2CH_2N$), 37.24 ($OCH_2CH_2CH_2N$), 56.88 (C-2), 61.53 (C-6^{XI}), 69.60 (C-$6^{I–X}$), 100.56, 100.93, 101.25, 101.63 (C-1).

3.19. 3-Acetamidopropyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranoside (19)

Compound **15** (6.5 mg, 0.007 mmol) was N-acetylated as described in Section 3.5 to form **19** (7.5 mg, 95%): $[\alpha]_D$ −30 (*c* 0.5, H_2O); Selected ^1H NMR data (D_2O): δ 1.75 (m, 2H, $OCH_2CH_2CH_2N$), 4.50 (m, 5H, 5H-1); Selected ^{13}C NMR (D_2O): δ 23.11, 23.35, 23.52 ($NCOCH_3$), 29.42 ($OCH_2CH_2CH_2N$), 37.61 ($OCH_2CH_2CH_2N$), 56.66 (C-2), 61.97 (C-6^{V}), 69.77 (C-$6^{I–IV}$), 102.26, 102.72 (C-1). ESI(+)-MS: calcd for $C_{45}H_{76}N_6O_{27}$: 1132.47 [M]; found 586.22 $[M+H+K]^{2+}$.

3.20. 3-Acetamidopropyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranoside (20)

Compound **16** (7.5 mg, 0.006 mmol) was N-acetylated as described in Section 3.5 to form **20** (7 mg, 76%); Selected ^1H NMR data (D_2O): δ 1.75 (m, 2H, $OCH_2CH_2CH_2N$), 4.50 (m, 7H, 7H-1). ESI(+)-MS: calcd for $C_{61}H_{102}N_8O_{37}$: 1538.63 [M]; found 789.31 $[M+H+K]^{2+}$.

3.21. 3-Acetamidopropyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranoside (21)

Compound **17** (8.8 mg, 0.006 mmol) was N-acetylated as described in Section 3.5 to form **21** (7.5 mg, 75%): $[\alpha]_D$ −31 (*c* 0.5, H_2O); Selected ^1H NMR data (D_2O): δ 1.75 (m, 2H, $OCH_2CH_2CH_2N$), 4.50 (m, 9H, 9H-1). Selected ^{13}C NMR (D_2O): δ 23.17, 23.28, 23.44 ($NCOCH_3$), 56.75 (C-2), 61.88 (C-6^{IX}), 101.92 102.28, 102.64 (C-1). ESI(+)-MS: calcd for $C_{77}H_{128}N_{10}O_{47}$: 1944.79 [M]; found 992.41 $[M+H+K]^{2+}$.

3.22. 3-Acetamidopropyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranoside (22)

Compound **18** (5 mg, 0.003 mmol) was N-acetylated as described in Section 3.5 to form **21** (4.8 mg, 94%); Selected ^1H NMR data (D_2O): δ 1.75 (m, 2H, $OCH_2CH_2CH_2N$), 4.50 (m, 11H, 11H-1). ESI(+)-MS: calcd for $C_{93}H_{154}N_{12}O_{57}$: 2350.95 [M]; found 1176.97 $[M+2H]^{2+}$.

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