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A convergent strategy for the preparation of N-glycan core di-, tri-, and pentasaccharide thioaldoses for the site-specific glycosylation of peptides and proteins bearing free cysteines

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Abstract—Mammalian glycoprotein biosynthesis produces heterogeneous ranges of proteins that possess the same peptide backbone but differ in the nature and site of glycosylation. This feature has frustrated efforts to develop therapeutic glycoproteins as well as the elucidation of biological functions of individual glycoforms. We have developed an attractive approach to well-defined glycoforms of glycoproteins by oxidative coupling of thioaldoses to cysteine-containing peptides and proteins to give disulfide-linked neoglycoconjugates. To this end, the chemical synthesis di-, tri-, and pentasaccharide N-glycan thioaldoses was undertaken. A convergent approach was used for the preparation of the pentasaccharide containing a 'synthetically difficult' β-mannoside linkage. This linkage was installed by forming initially the corresponding β-glucoside-containing pentasaccharide, followed by inversion of configuration at C-2. This approach exploited a levulonyl ester at C-2 of a glucosyl donor, which directed the coupling to give the β-glucoside exclusively and could be removed selectively using hydrazine acetate without affecting other base-labile functionalities. The resulting alcohol was converted into a triflate, which was displaced by tri-*n*-butylammonium acetate to give a β-mannosidic linkage. The trisaccharide N-glycan was prepared in a similar manner. Thioaldoses were prepared by displacing the peracetylated α-glycosyl chlorides with thioacetate to give the peracetylated β-thioacetates, which upon saponification gave the desired compounds. The incubation of molar excesses of chitobiose thioaldose with cysteine-containing glutathione and BSA resulted in the site-specific formation of a disulfide-linked neoglycopeptide and neoglycoprotein, respectively.

Keywords: Carbohydrates; Glycosylation; Glycoprotein; Glycopeptide; Ligation

1. Introduction

Protein glycosylation is an important co-translational modification, which ranges from monosaccharides on nuclear and cytoplasmic proteins to enormously complex structures on extracellular N- or O-linked glycoproteins and proteoglycans.[1–6] At the cellular level, N- and O-glycans have been shown to contribute to a myriad of functions, including cell-adhesion events during immune surveillance, inflammatory reactions, hormone action, arthritis, and viral and bacterial infections.[7–9] The cell- and tissue-specific changes in cell-surface oligosaccharides during development have indicated that these structures may be involved in cell adhesion and migration events during embryogenesis. Alterations in the branching and extension of N-glycans have also been found on the surfaces of cells that have undergone oncogenic transformation, and these changes correlate with alterations in cell adhesion, invasiveness, and metastasis of transformed cells.[10–12]

Protein-bound oligosaccharides are assembled by a large number of glycosyltransferases that catalyze the transfer of monosaccharide residues from nucleoside mono- or diphosphate sugars to growing oligosaccharide chains. Protein glycosylation is not under direct

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genetic control, and therefore results in the formation of a number of glycoforms that possess the same peptide backbone but differ in the nature and site of glycosylation.[13] It has been suggested that each glycoform possess a unique set of biological activities. The few studies that have compared single glycoforms have required abundant sources, extensive enzymatic processing, and difficult chromatographic separations. Obviously, there is an urgent need for more efficient approaches for preparing homogeneous forms of glycoproteins. Such preparations will not only be important to study biological properties of individual glycoforms, but will also be of critical importance for the development of therapeutic glycoproteins.

The de novo synthesis of glycoproteins offers a promising approach to well-defined glycoforms, and several different ways have been explored each representing unique and difficult challenges.[14] The chemical synthesis of glycoproteins using standard peptide synthesis is only feasible for peptides under 30 amino acids in length. Small glycoproteins can be obtained by 'the state of the art' native ligation techniques,[15] which allow efficient combining of peptide fragments.[16–20] A more direct approach to well-defined glycoproteins involves the use of synthetic oligosaccharides that are functionalized in such a way that they react in a site-specific manner with a unique functionality within the protein structure. One innovative idea involves the coupling of a sugar bearing an iodoacetamide group with a thiol of a free cysteine moiety.[21,22] This method was, for example, applied to the in vitro glycosylation of a human erythropoietin cysteine mutant.[23]

We have developed a general approach for site-selective glycosylation of proteins,[24] whereby an electrophilic sulfur-containing nitropyridinesulfenyl thioglycoside is reacted with the thiol group of a free cysteine residue of a peptide or protein. This approach is advantageous in that the resulting disulfide linkage between the oligosaccharide and the protein can adopt a conformation similar to that of the amide linkage found in natural N-linked glycoproteins, a feature which may be important for reproducing the contact points between the glycan and the peptide chain.

Here we report a highly convergent approach for the synthesis of N-glycan core di-, tri-, and pentasaccharide thioaldoses and the glycosylation of cysteine-containing peptide, glutathione, and protein, bovine serum albumin (BSA). Difficulties were encountered in synthesizing nitropyridinesulfenyl thioglycosides of oligosaccharides; however, disulfide-linked neoglycopeptides and proteins could be obtained conveniently by coupling of thioaldoses directly with the thiol group of free cysteine-containing peptides or proteins. As a cysteine can be introduced at any point of a protein by site-directed mutagenesis, a protein can be glycosylated at a specific site.[14,25–27]

2. Results and discussion

It was envisaged that thioaldoses **1**, **2**, and **3** (Fig. 1), which are derived from the core region of N-linked oligosaccharides, would be ideally suited for site-specific glycosylation of proteins by disulfide formation. The preparation of compounds **2** and **3** is complicated by the presence of a β-linked mannoside and the necessity to introduce a β-thioaldose. β-Mannosides are difficult to synthesize because the axial C-2 hydroxyl group sterically blocks the β-face and exerts a strong α-directing anomeric effect. As a result, attempts to introduce these linkages by standard chemical glycosylation methods usually produce mixtures of anomers. Despite these difficulties, several elegant strategies to prepare β-mannosides have reported including the following: the use of insoluble silver salt promoters,[28] intramolecular glycosylations,[29–34] epimerization of a C-2 hydroxyl of a β-glucoside,[35–38] and the use of an α-mannosyl triflate for the introduction of a β-mannosidic linkage.[39] Elegant chemical preparations of the core N-glycan pentasaccharide or larger structures have been reported[40–49] employing these strategies. Glycosidases[50–52] and glycosyltransferases[53] have also been used for the synthesis of the β-mannosyl-linked core trisaccharide.

We have designed a synthetic route for thioaldoses **2** and **3** in which the β-mannosidic linkage is installed by the initial formation of a β-glucoside, followed by inversion of configuration of the C-2 hydroxyl of the latter moiety. This strategy is attractive since β-glucosides can be stereospecifically obtained by exploiting neighboring-group participation of a C-2 ester protecting group. Thus, coupling of trisaccharide **15**, which has

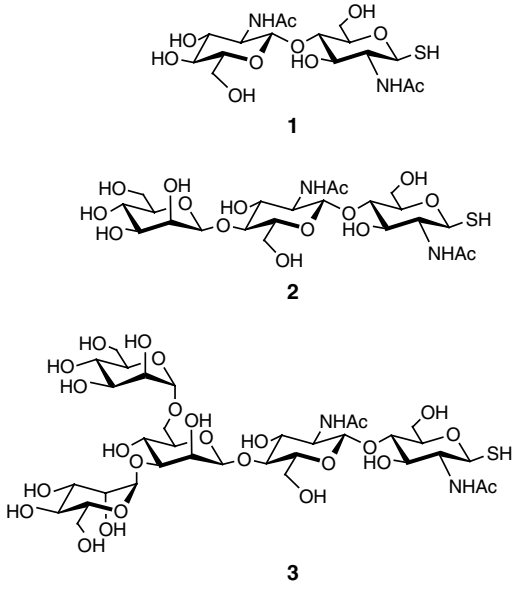
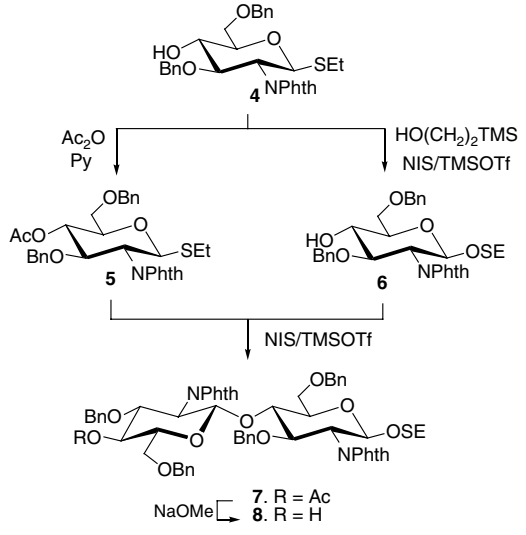


Figure 1. Target compounds.

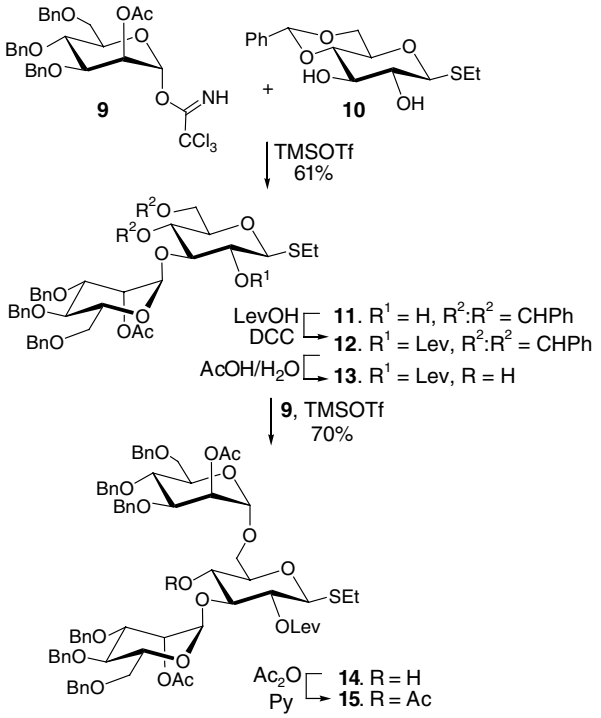
a levulonyl (Lev) ester at C-2, with disaccharide **8** should provide key pentasaccharide **19** (Scheme 3) in a convergent manner. An attractive feature of the Lev ester is that after the glycosylation, it can be removed under mild conditions using hydrazine acetate without affecting other ester functionalities. The resulting alcohol can then be converted to a triflate leaving group, which upon S_N2 displacement with acetate should yield the desired β-mannosidic linkage.

Glycosyl acceptor **8** was easily obtained by a two-directional glycosylation approach[54–57] in which thioglycosyl donor **4** was coupled with 2-trimethylsilylethanol using trimethylsilyl triflate (TMSOTf)/*N*-iodosuccinamide (NIS) as the promoter[58] to give **6**, which was immediately used in the next NIS/TMSOTf-mediated glycosylation and coupled with thioglycosyl donor **5** to give disaccharide **7** (Scheme 1). Deacetylation of **7** using NaOMe in methanol afforded the required glycosyl acceptor **8** in a yield of 82%.

The trisaccharide **15** was efficiently prepared by a chemoselective glycosylation strategy, which exploits the fact that trichloroacetimidates can be activated with TMSOTf[59] without affecting thioglycosides (Scheme 2). However, at a later stage of the synthesis a thioglycoside can act as a glycosyl donor in the presence of a thiophilic promoter, minimizing protecting-group manipulations at the anomeric center. TMSOTf-promoted glycosylation of mannosyl trichloroacetimidate **9** with thioglycosyl acceptor **10** gave, after selective crystallization, the (1→3)-linked disaccharide **11** in a yield of 61%. Compound **11** was the major regioisomer formed due to the higher reactivity of the C-3 hydroxyl of **10**. The remaining free hydroxyl of **11** was protected with a Lev group[60] by reaction with levulonic acid activated by DCC and 4-(*N*,*N*-dimethylamino)pyridine (DMAP) to



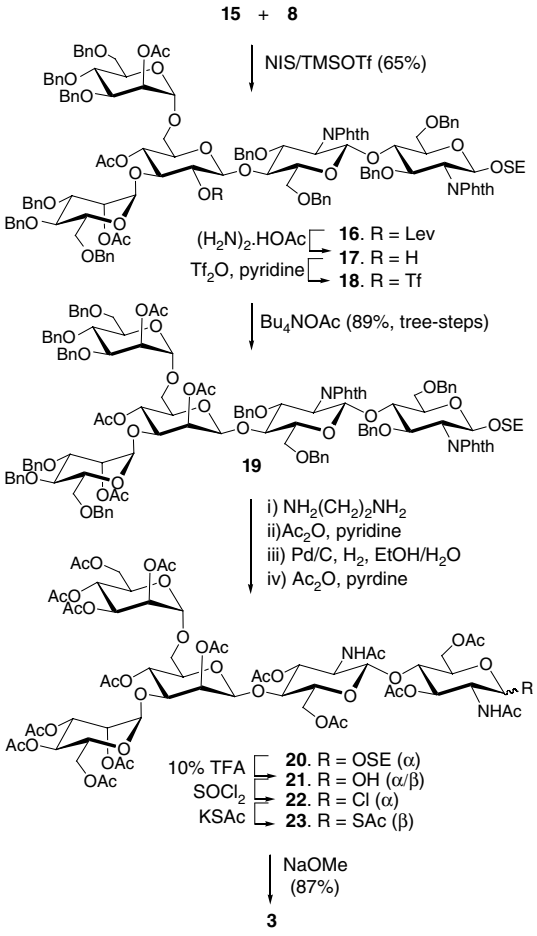
Scheme 1.



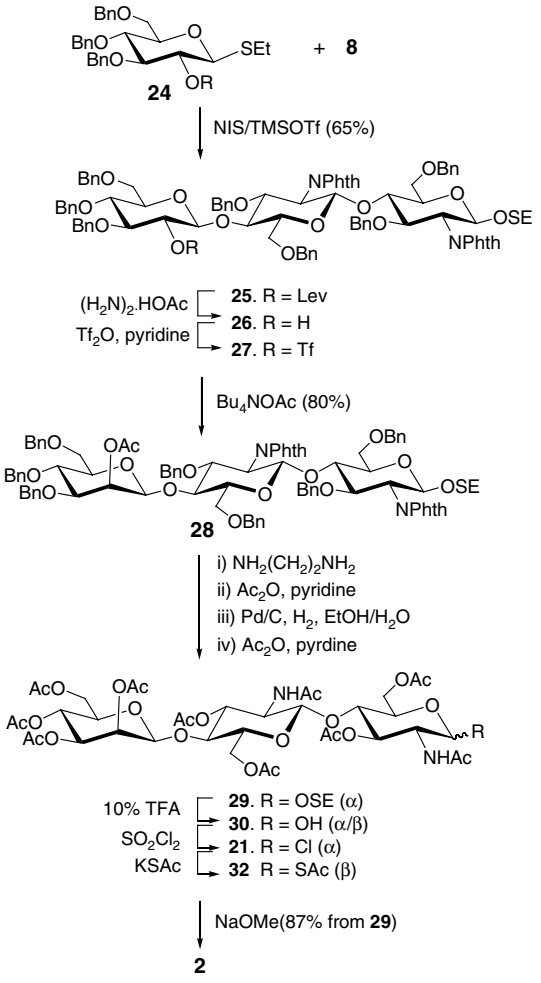
Scheme 2.

give fully protected **12**. Next, the benzylidene acetal of **12** was removed by hydrolysis using a mixture of acetic acid and water to give **13** in an overall yield of 95% (two steps). The resulting 4,6-diol acceptor **13** was coupled with mannosyl donor **9** in the presence of a catalytic amount of TMSOTf to give regioselectively the 3,6-O-branched trisaccharide **14** in 70% yield. This reaction exploits the higher reactivity of the primary sugar hydroxyls over secondary ones. Finally, glycosyl acceptor **15** was obtained by protection of the C-4 hydroxyl of **14** as an acetyl ester by reaction with acetic anhydride and pyridine.

Having glycosyl acceptor **8** and glycosyl donor **15** at hand, attention was focused on the preparation of pentasaccharide **19** (Scheme 3). Thus, coupling of **8** with **15** in the presence of NIS and TMSOTf afforded the β-glucosyl-linked pentasaccharide **16** in a yield of 65%. Next, selective removal of the levulonyl ester of **16** using hydrazine acetate gave compound **17**, with its central glucosyl residue C-2 hydroxyl unmasked, in 94% yield. The C-2-triflate **18**, which was obtained upon reaction of the foregoing compound with triflic anhydride in pyridine, was subjected to a nucleophilic substitution with tri-*n*-butylammonium acetate in toluene with sonication[48] to give the corresponding β-mannoside **19** in an overall yield of 89%. Analysis of the ^{1}H NMR spectrum of **19** revealed a singlet at 4.87 ppm corresponding to the β-mannosyl anomeric proton. The following protecting group manipulations were performed in good yields to give the peracetylated TMSEt glycoside **20**: treatment



Scheme 3.



Scheme 4.

with ethylenediamine removed the phthalamido groups,[61] and the resulting amines were acetylated with acetic anhydride in pyridine to give an N-acetylated compound. Next, debenzylation was accomplished by catalytic hydrogenation over 10% palladium-on-charcoal, and the resulting hydroxyl groups were acetylated using acetic anhydride and pyridine. The aglycone of **20** was cleaved by 10% trifluoroacetic acid in dichloromethane to afford hemiacetal **21**, which was treated with thionyl chloride to give the corresponding α-glycosyl chloride **22**, which, in turn, was reacted with potassium thioacetate in acetone to give the peracetylated thioaldose **23**, in 40% yield. The relatively low yield of this step was due to a side reaction, which led to the formation of an oxazoline-containing product. The core-pentasaccharide thioaldose **3** was obtained by deacetylation of **23** using sodium methoxide in methanol. The ^{1}H NMR spectrum of **3** showed the reducing-end anomeric proton at 4.52 ppm, which had a coupling constant of 11.0 Hz, demonstrating that the anomeric configuration of the thiol group was exclusively in the β-configuration.

Trisaccharide **2** was prepared by a similar strategy, but in this case chitobiose acceptor **8** was coupled with glucoside **24** using NIS/TMSOTf as the promoter to give trisaccharide **25** in 81% yield (Scheme 4). Selective removal of the Lev ester of **25** using standard conditions gave **26**, containing a C-2 hydroxyl, which was converted to a triflate-leaving group and subsequently subjected to nucleophilic displacement with tributylammonium acetate to give β-mannoside **28** in 82% yield. Using a similar procedure as described for **3**, compound **28** was deblocked and the thioaldose moiety installed to give target compound **2**.

Peracetylated chitobiose was converted into its anomeric chloride with thionyl chloride (details not shown), and subsequent treatment with potassium thioacetate was followed by base-catalyzed deacetylation, which gave thioaldose **1**.

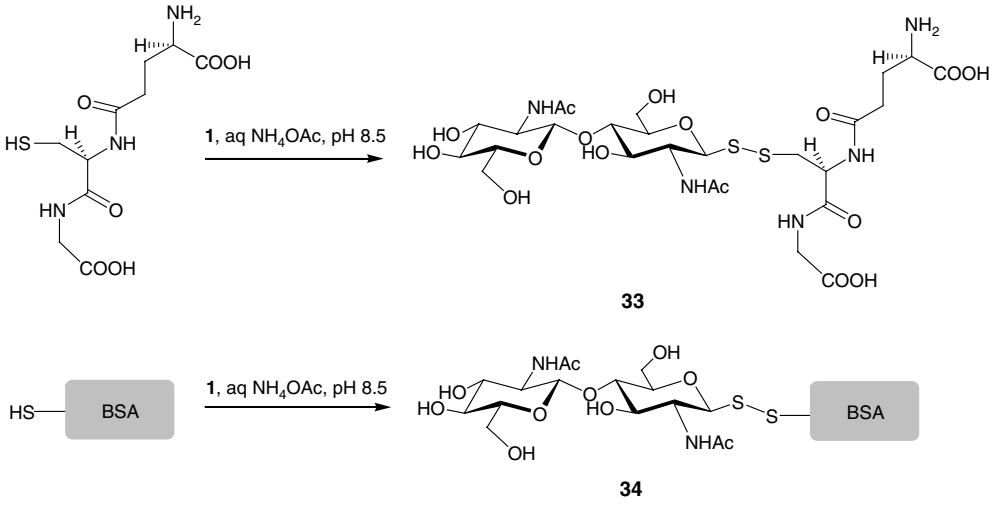
Having successfully prepared thioaldoses **1**–**3**, attention was focused on the site-specific glycosylation of peptides and proteins that have a cysteine moiety. Although the nitropyridinesulfenyl thioglycoside of GlcNAc could easily be obtained by reaction of **1** with 2,2′-dithiobis(5-nitropyridine) (DTNP) in acetic acid/

water,[24] the preparation of similar derivatives of the more complex thioaldoses **1**–**3** proved problematic, and mainly disulfide-linked dimers were obtained. It was anticipated that oxidative coupling of a thiol moiety of a cysteine residue with a thioaldose should offer an alternative approach to disulfide-linked neoglycoconjugates. It is well known that thiolates can easily be oxidized to disulfides, whereas analogous thiols are very slow to react. Thus, treatment of a cysteine-containing peptide or protein with an excess of a thioaldose at relatively high pH should lead to the formation of a mixed disulfide. Indeed, the combination of equimolar amounts of thioaldose **1** and glutathione in aqueous ammonium acetate at pH 8.5 yielded the disulfide-linked glycopeptide **33**, the thioaldose homodisulfide and glutathione disulfide in a statistical 2:1:1 ratio as determined by ^{1}H NMR spectroscopy (Scheme 5). The use of 2 equiv of the thioaldose afforded the products in ratio of 1:0.9:0.2, thus increasing the amount of the heterodisulfide compared to glutathione disulfide. When a larger excess of the thioaldose (5–20 equiv) was used only the sugar homodimer and glycopeptide were present, and the oxidized glutathione disulfide was not detected.

Bovine serum albumin (BSA) was chosen as a model protein on the merit of its availability and the convenience of having a free cysteine residue at Cys-58 of its sequence. After treatment of BSA with excess of **1** in aqueous ammonium acetate, the BSA-glycoconjugate **34** was isolated free from the excess amount of **1** and its homodimer by ultrafiltration using Centricon concentration devices fitted with 10-kDa membranes. Ellman type analysis, using dithiodipyridine,[62] of the isolated conjugate against a blank sample of nonreacted BSA gave a zero absorbance reading at 340 nm, showing the absence of free thiols, and indicating that the conjugation had gone to completion. Further spectroscopic corroboration of the conjugate came from MALDI-TOF mass spectrometry, which showed a mass increase for the conjugate over BSA sample of 477 Da (while the theoretical difference is 438 Da, indicating the incorporation of the thiochitobiose moiety). The attachment of the synthetic oligosaccharides to IgG fragments, engineered to have Cys-297 in place of glycan-linked Asn, has been achieved with thioaldoses, where peptide mapping indicated selective glycosylation of the free cysteine, and biological assays demonstrated improved effector functions. The results of these studies will be reported elsewhere.

3. Conclusion

Mammalian glycoprotein biosynthesis produces heterogeneous ranges of proteins that possess the same peptide backbone but differ in the nature and site of glycosylation. This feature has frustrated efforts to elucidate biological functions of glycoproteins, in particular in those cases in which the precise structure of a glycan determines biological activity. Furthermore, the inability to control N-glycan formation in cell culture is a major obstacle in the development of therapeutic glycoproteins. We have developed an attractive approach to well-defined glycoforms of glycoproteins by the chemical synthesis of a range of N-glycan thioaldoses, which can be attached to cysteine-containing peptides and proteins by an oxidative coupling to give disulfide-linked neoglycoconjugates. The synthetic oligosaccharides **2** and **3** were prepared by a strategy, whereby the β-mannosidic linkages were installed by the initial formation of the corresponding β-glucosides, followed by inversion of configuration at C-2. The anomeric thiols of **1**, **2**, and **3** were prepared by displacing the peracetylated α-glycosyl chlorides



Scheme 5.

with thioacetate to give the peracetylated β-thioacetates, whereupon saponification of the O- and S-acetates gave the desired compounds. Coupling studies involving the incubation of chitobiose thioaldose **1** in molar excess and the cysteine-containing peptide glutathione found that the disulfide-linked glycopeptide was formed along with the homodimer of **1**. Free cysteine-containing protein BSA, was incubated with **1** also to give a neoglycoprotein product, where MALDI-TOF mass spectrometry indicated the addition of thiochitobiose, and the free thiol concentration, determined using dithiodipyridine, was found to be 0. In addition, to providing well-defined glycoforms, in vitro glycosylation technology offers several unique advantages such as the quantitative incorporation of synthetic oligosaccharides that are of low natural abundance, but which are known to display exceptional bioactivities. Furthermore, unnatural oligosaccharides can be used that will give neoglycoproteins with improved biostabilities. Also, toxins or other reagents can be incorporated to give conjugates with novel effector functions.

4. Experimental

4.1. General methods

Chemicals were purchased from Acros or Aldrich, Sigma, and Fluka. Protein concentrations were determined using a Lowry protein concentration kit from Sigma, where UV absorption was recorded at 690 nm using a Finstruments 96 well ELISA-plate reader. Thiol concentrations were determined using purified *S*,*S*-dithiodipyridine as described by Grassetti and Murray,[62] and the absorption was recorded at 340 nm using a Finstruments ELISA-plate reader. Mass spectra were obtained using a Micromass Q-Tof-2 mass spectrometer and a Hewlett–Packard G2025A MALDI-TOF mass spectrometer in positive-ion mode using 2,5-dihydroxybenzoic acid as the matrix, unless stated otherwise. High-resolution mass spectra were obtained using a Voyager delayed extraction STR with 2,5-dihydroxybenzoic acid as an internal calibration matrix.

4.2. Trimethylsilylethyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalamido-β-D-glucopyranoside (6)

A suspension of compound **4**[63] (7.03 g, 13.18 mmol) and trimethylsilylethanol (28.2 mL, 19.77 mmol) containing activated 4 Å molecular sieves (10 g) in dry CH_2Cl_2 (30 mL) was stirred under an atmosphere of argon at room temperature for 30 min. After cooling to 0 °C, successively *N*-iodosuccinamide (3.85 g, 16.68 mmol) and trimethylsilyl triflate (260 μL, 1.32 mmol) were added, and the stirring was continued at 0 °C for 30 min. The reaction mixture was diluted with CH_2Cl_2 (200 mL), passed through a sintered glass filter, and the eluent was washed with 15% aq sodium thiosulfate (2×200 mL), then with satd aq $NaHCO_3$ (200 mL), followed by drying ($MgSO_4$), filtration and concentration in vacuo. Flash silica gel column chromatography of the residue using 9:1 toluene–EtOAc as the eluent afforded compound **6** (6.06 g, 78%) as a white foam. The ^{1}H NMR spectrum was identical to a preparation of **6** reported previously.[64]

4.3. Trimethylsilylethyl 4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-2-phthalamido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalamido-β-D-glucopyranoside (7)

A suspension of compound **5**[65] (8.00 g, 13.9 mmol) and **6** (6.06 g, 10.3 mmol) containing activated 4 Å molecular sieves (10 g) in dry CH_2Cl_2 (30 mL) was stirred under an atmosphere of argon at room temperature for 30 min. After cooling to 0 °C, successively *N*-iodosuccinamide (3.75 g, 16.68 mmol) and trimethylsilyl triflate (252 μL, 1.28 mmol) were added and stirring was continued at 0 °C for 15 min. The reaction mixture was diluted with CH_2Cl_2 (200 mL), passed through a sintered glass filter, and the eluent was washed with 15% sodium thiosulfate (2×200 mL), then with satd aq $NaHCO_3$ (200 mL) followed by drying ($MgSO_4$), filtration and concentration in vacuo. Flash silica gel column chromatography of the residue using 9:1 toluene–EtOAc as the eluent afforded compound **7** (10.02 g, 88%) as white foam. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.00–7.00 (m, 28H, Ar), 5.54 (d, 1H, $J_{1'-2'}$ 8.2 Hz, H-1′), 5.34 (dd, 1H, $J_{3'-4'}$ 9.1, $J_{4'-5'}$ 9.5 Hz, H-4′), 5.28 (d, 1H, J_{1-2} 7.4 Hz, H-1), 5.04 (d, 1H, J_{AB} 12.4 Hz, CH_2Ph), 4.83–4.61 (m, 7H, H-3′ and 6×CH_2Ph), 4.53 (d, 1H, J_{AB} 12.1 Hz, CH_2Ph), 4.50 (dd, 1H, $J_{2'-3'}$ 10.7 Hz, H-2′), 4.36 (m, 3H, H-2, H-3, and H-4), 3.99 (m, 1H, CH_2CH$_2$TMS), 3.80–3.45 (m, 7H, CH_2CH$_2$TMS, H-5, H-5′, H-6b, H-6a, H-6b′, and H-6a′), 2.11 (s, 3H, COCH$_3$), 0.90 (m, 2H, CH$_2$CH_2TMS), and 0.00 (s, 9H, TMS); MALDI-TOF MS: m/z 1124.9 (M+Na$^+$) and 1141.0 (M+K$^+$); Anal. Calcd for $C_{63}H_{66}N_2O_{14}Si$ (1103.3): C, 68.58; H, 6.03; N, 2.54. Found: C, 68.32; H, 6.13; N, 2.49.

4.4. Trimethylsilylethyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalamido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalamido-β-D-glucopyranoside (8)

To compound **7** (1.38 g, 1.26 mmol) in a mixture of 1:1 dry THF–dry MeOH (20 mL, 1/1, v/v) was added a freshly prepared solution of NaOMe in MeOH until the pH was 11, and the reaction mixture left for 6 h at room temperature. Neutralization of the reaction with Amberlite 650 cation-exchange resin [H$^+$-form] was followed by filtration and concentration of the filtrate in vacuo. Flash silica gel column chromatography of the residue (eluent: 9:1 toluene–EtOAc) afforded compound

8 (1.09 g, 82%) as white foam. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.95–7.00 (m, 28H, Ar), 5.55 (d, 1H, $J_{1'-2'}$ 8.2 Hz, H-1′), 5.18 (d, 1H, J_{1-2} 8.0 Hz, H-1), 5.00 (m, 2H, 2×CH_2Ph), 4.68 (m, 6H, 6×CH_2Ph), 4.44 (dd, 1H, J_{2-3} 10.7 Hz, H-2), 4.37 (m, 4H, H-2′, H-3, H-4, and H-3′), 4.02 (m, 1H, H-4′), 4.00 (m, 1H, CH_2CH$_2$TMS), 3.90 (dd, 1H, $J_{5'-6b'}$ 4.4, $J_{6a'-6b'}$ 10.2 Hz, H-6b′), 3.74 (m, 2H, H-6b, H-6a′), 3.66–3.38 (m, 4H, CH_2CH$_2$TMS, H-5, H-5′, and H-6a), 0.90 (m, 2H, CH$_2$CH_2TMS), and 0.00 (s, 9H, TMS); MALDI-TOF MS: m/z 1083.1 (M+Na$^+$) and 1099.0 (M+K$^+$). Anal. Calcd for $C_{61}H_{64}N_2O_{13}Si$ (1061.2): C, 69.04; H, 6.08; N, 2.64. Found: C, 68.28; H, 6.14; N, 2.55.

4.5. Ethyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-4,6-*O*-*p*-methoxybenzylidene-1-thio-β-D-glucopyranoside (11)

To a cooled (−60 °C) and stirred solution of trichloroacetimidate **9** (781 mg, 1.23 mmol) and compound **10** (350 mg, 1.03 mmol) in dry CH_2Cl_2 (5 mL) under an argon atmosphere was added trimethylsilyl triflate (11 μL, 62 μmol). The temperature was allowed to rise to 0 °C over a period of 2 h, whereafter, TLC (4:1 toluene–ethyl acetate) showed that a major and minor product had been formed. Et_3N (20 μL) was added to quench the reaction and after dilution with CH_2Cl_2 (20 mL) and washing with a satd aq $NaHCO_3$ (25 mL) the CH_2Cl_2 phase was dried ($MgSO_4$), filtered and concentrated in vacuo. Precipitation of the residue from hexane and HOAc afforded **11** (494 mg, 61%) as an amorphous material. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.32–6.77 (m, 19H, Ph), 5.43 (m, 2H, CHPhOCH$_3$ and H-2′), 5.17 (d, 1H, $J_{1'-2'}$ 1.4 Hz, H-1′), 4.76 (d, 1H, J_{AB} 10.7 Hz, CH_2Ph), 4.59 (d, 1H, J_{AB} 12.8 Hz, CH_2Ph), 4.49 (m, 4H, 4×CH_2Ph), 4.31 (d, 1H, J_{1-2} 9.9 Hz, H-1), 4.25 (dd, 1H, J_{5-6b} 5.0, J_{6a-6b} 10.4 Hz, H-6b), 4.15 (m, 1H, H-5′), 3.93 (dd, 1H, $J_{2'-3'}$ 3.3, $J_{3'-4'}$ 9.1 Hz, H-3′), 3.71 (m, 2H, H-3 and H-4′), 3.68 (s, 3H, PhOCH_3), 3.64 (m, 3H, H-6b′, H-6a′, and H-6a), 3.54 (t, 1H, J 9.3 Hz, H-4), 3.38 (m, 2H, H-2 and H-5), 2.96 (d, 1H, J_{2-2OH} 3.3 Hz, 2-OH), 2.61 (m, 2H, SCH_2CH$_3$), 2.02 (s, 3H, COCH$_3$), and 1.22 (t, 3H, SCH$_2$CH_3); MALDI-TOF MS: m/z 837.7 (M+Na$^+$) and 853.6 (M+K$^+$).

4.6. Ethyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-levulinoyl-1-thio-β-D-glucopyranoside (13)

Compound **11** (400 mg, 0.49 mmol) was dissolved in dry CH_2Cl_2 (4 mL) and levulinic acid (100 μL, 0.98 mmol), DCC (202 mg, 0.98 mmol), and DMAP (6 mg, 50 μmol) were added, and the reaction was stirred at room temperature for 2 h. Dilution with DCM (20 mL) was followed by washing with a satd aq $NaHCO_3$ (25 mL), the CH_2Cl_2 phase was dried ($MgSO_4$), filtered and concentrated in vacuo. The crude residual **12** was treated with 80% HOAc (15 mL) at room temperature for 20 h, at the end of which time the reaction mixture was concentrated in vacuo. Flash silica gel column chromatography of the residue using 4:1 toluene–EtOAc as the eluent afforded **13** (368 mg, 95%) as a syrup. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.38–7.10 (m, 15H, Ph), 5.28 (s, 1H, H-1′), 5.23 (dd, 1H, $J_{1'-2'}$ 1.9, $J_{2'-3'}$ 3.0 Hz, H-2′), 4.95 (dd, 1H, J_{1-2} 9.6, J_{2-3} 9.3 Hz, H-2), 4.84 (d, 1H, J_{AB} 11.3 Hz, CH_2Ph), 4.74 (d, 1H, J_{AB} 11.0 Hz, CH_2Ph), 4.54 (m, 4H, 4×CH_2Ph), 4.40 (d, 1H, H-1), 4.19 (d, 1H, $J_{4\text{-OH}}$ 5.2 Hz, 4-OH), 4.08 (m, 1H, H-5′), 3.93 (dd, 1H, $J_{3'-4'}$ 9.1 Hz, H-3′), 3.79 (m, 3H, H-3, H-4, and H-6b′), 3.68 (m, 1H, H-6b), 3.65–3.55 (m, 3H, H-6a, H-6a′, and H-4′), 3.25 (m, 1H, H-5), 2.62 (m, 6H, CH_2CH_2COCH$_3$ and SCH_2CH$_3$), 2.10 (m, 1H, 6-OH), 2.10 and 2.08 (2×s, 2×3H, CH$_2$CH$_2$COCH_3 and COCH$_3$), and 1.22 (t, 3H, SCH$_2$CH_3); MALDI-TOF MS: m/z 819.7 (M+Na$^+$) and 835.5 (M+K$^+$). Anal. Calcd for $C_{42}H_{52}O_{13}S$ (796.3): C, 63.30; H, 6.58. Found: C, 63.27; H, 6.61.

4.7. Ethyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-[2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-]-2-*O*-levulonyl-1-thio-β-D-glucopyranoside (14)

A cooled (−60 °C) and stirred solution of **9** (172 mg, 0.27 mmol) and disaccharide **13** (165 mg, 0.20 mmol) in dry CH_2Cl_2 (2 mL) under argon was treated with trimethylsilyl triflate (5 μL, 27 μmol). After stirring for 2 h, the reaction was quenched with Et_3N (8 μL) and after aqueous workup, the residue was subjected to flash silica gel column chromatography (eluent: 4:1 toluene–EtOAc) affording **14** (172 mg, 70%) as a syrup. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.38–7.05 (m, 30H, Ar), 5.36 (dd, 1H, $J_{1''-2''}$ 1.5, $J_{2''-3''}$ 2.9 Hz, H-2″), 5.19 (s, 1H, H-1′), 5.17 (dd, 1H, $J_{1'-2'}$ 2.0, $J_{2'-3'}$ 3.4 Hz, H-2′), 4.92 (dd, 1H, J_{1-2} 9.1, J_{2-3} 9.3 Hz, H-2), 4.84 (d, 1H, J_{AB} 11.2 Hz, CH_2Ph), 4.82 (d, 1H, J_{AB} 11.6 Hz, CH_2Ph), 4.81 (d, 1H, H-1″), 4.73 (d, 1H, J_{AB} 11.2 Hz, CH_2Ph), 4.70 (d, 1H, J_{AB} 11.2 Hz, CH_2Ph), 4.68 (d, 1H, J_{AB} 12.2 Hz, CH_2Ph), 4.53 (m, 3H, 3×CH_2Ph), 4.47 (m, 4H, 4×CH_2Ph), 4.34 (d, 1H, H-1), 4.21 (d, 1H, $J_{4\text{-OH}}$ 5.4 Hz, 4-OH), 4.03 (ddd, 1H, $J_{5''-6b''}$ 1.5, $J_{5''-6a''}$ 6.8, $J_{4''-5''}$ 10.3 Hz, H-5″), 3.92 (m, 2H, H-3′ and H-3″), 3.84 (m, 2H, H-4′ and H-5′), 3.76 (dd, 1H, $J_{6a''-6b''}$ 10.3 Hz, H-6b″), 3.74 (m, 4H, H-3, H-4, H-6b, and H-6b′), 3.67 (m, 1H, H-6a′), 3.64 (dd, 1H, $J_{3''-4''}$ 9.7 Hz, H-4″), 3.60 (dd, 1H, H-6a″), 3.51 (dd, 1H, J_{5-6a} 1.9, J_{6a-6b} 11.2 Hz, H-6a), 3.30 (ddd, 1H, J_{5-6b} 5.4, J_{4-5} 9.4 Hz, H-5), 2.60 (m, 6H, CH_2CH_2COCH$_3$ and SCH_2CH$_3$), 2.04, 2.10, and 2.08 (3×s, 3×3H, CH$_2$CH$_2$COCH_3 and 2×COCH$_3$) and 1.15 (t, 3H, SCH$_2$CH_3); MALDI-TOF MS: m/z 1295.3 (M+Na$^+$) and 1311.0 (M+K$^+$). Anal. Calcd for $C_{71}H_{82}O_{19}S$ (1271.5): C, 67.07; H, 6.50. Found: C, 67.28; H, 6.44.

4.8. Ethyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-[2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-]-4-*O*-acetyl-2-*O*-levulonyl-1-thio-β-D-glucopyranoside (15)

Compound **14** (172 mg, 0.14 mmol) was treated with pyridine (1 mL) and Ac_2O (0.5 mL) for 18 h, after which time the reaction was concentrated in vacuo and the residue was subjected to flash silica gel column chromatography (eluent: 4:1 toluene–EtOAc) to afford **15** (173 mg, 98%) as syrup. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.40–7.04 (m, 30H, Ar), 5.33 (dd, 1H, $J_{1''-2''}$ 2.1, $J_{2''-3''}$ 2.9 Hz, H-2″), 5.11 (dd, 1H, $J_{1'-2'}$ 1.5, $J_{2'-3'}$ 2.9 Hz, H-2′), 5.02 (d, 1H, H-1′), 5.01 (dd, 1H, J_{3-4} 9.3, J_{4-5} 9.8 Hz, H-4), 4.94 (dd, 1H, J_{1-2} 9.7, J_{2-3} 9.3 Hz, H-2), 4.84 (d, 1H, J_{AB} 10.7 Hz, CH_2Ph), 4.82 (d, 1H, H-1″), 4.80 (d, 1H, J_{AB} 11.2 Hz, CH_2Ph), 4.79 (d, 1H, J_{AB} 11.2 Hz, CH_2Ph), 4.70–4.60 (m, 4H, 4×CH_2Ph), 4.54 (d, 1H, J_{AB} 11.2 Hz, CH_2Ph), 4.49 (m, 3H, 3×CH_2Ph), 4.41 (d, 1H, J_{AB} 10.7 Hz, CH_2Ph), 4.33 (d, 1H, H-1), 3.93 (dd, 1H, $J_{3''-4''}$ 9.3 Hz, H-3″), 3.88 (m, 4H, H-3, H-3′, H-4′, and H-4″), 3.80 (m, 1H, H-5″), 3.75 (m, 4H, H-5′, H-6b′, H-6b″, and H-6b), 3.68 (dd, 1H, $J_{5''-6a''}$ 1.5 and $J_{6a''-6b''}$ 10.3 Hz, H-6a″), 3.62 (m, 1H, H-6a′), 3.51 (m, 2H, H-5 and H-6a), 2.59 (m, 6H, CH_2CH_2COCH$_3$ and SCH_2CH$_3$), 2.13, 2.12, 2.11, and 1.97 (4×s, 4×3H, CH$_2$CH$_2$COCH_3 and 3×COCH$_3$) and 1.14 (t, 3H, SCH$_2$CH_3); MALDI-TOF MS: m/z 1335.9 (M+Na$^+$) and 1352.1 (M+K$^+$). Anal. Calcd for $C_{73}H_{84}O_{19}S$ (1312.5): C, 66.75; H, 6.45. Found: C, 66.48; H, 6.54.

4.9. Trimethylsilylethyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-[2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-]-4-*O*-acetyl-2-*O*-levulonyl-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalamido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalamido-β-D-glucopyranoside (16)

A cooled (0 °C) and stirred solution of **15** (160 mg, 0.12 mmol) and acceptor **8** (155 mg, 0.15 mmol) in dry CH_2Cl_2 (2 mL) containing 4 Å molecular sieves (0.1 g) was treated with *N*-iodosuccinamide (31 mg, 0.13 mmol) and trimethylsilyl triflate (3 μL, 12 μmol). Stirring was continued at 0 °C for 15 min, after which time, the reaction was subjected to the same workup as described for the preparation of compound **7** to afford **16** (171 mg, 65%) as a white foam. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.00–6.90 (m, 58H, Ar), 5.49 (d, 1H, $J_{1'-2'}$ 7.8 Hz, H-1′), 5.43 (dd, 1H, $J_{1''''-2''''}$ 2.0, $J_{2''''-3''''}$ 4.9 Hz, H-2″″), 5.33 (dd, 1H, $J_{1'''-2'''}$ 2.0, $J_{2'''-3'''}$ 2.4 Hz, H-2‴), 5.30 (dd, 1H, $J_{3''-4''}$ 9.8 and $J_{4''-5''}$ 9.3 Hz, H-4″), 5.18 (d, 1H, H-1‴), 5.17 (m, 2H, H-2″ and H-1), 5.11 (d, 1H, J_{AB} 13.2 Hz, CH_2Ph), 5.04 (d, 1H, J_{AB} 10.7 Hz, CH_2Ph), 5.02 (d, 1H, J_{AB} 11.2 Hz, CH_2Ph), 4.96 (m, 1H, CH_2Ph), 4.94 (s, 1H, H-1″″), 4.83 (m, 3H, 3×CH_2Ph), 4.75–4.56 (m, 13H, 13×CH_2Ph), 4.72 (m, 1H, H-1″), 4.45 (dd, 1H, $J_{2''-3''}$ 8.3 Hz, H-3″), 4.38 (dd, 1H, $J_{2'-3'}$ 10.7 Hz, H-2′), 4.31 (m, 4H, H-2, H-3, H-4, and H-4′), 4.11 (m, 3H, H-3‴, H-5‴, and H-3″″), 4.00 (m, 7H, H-4‴, H-4″″, H-6b‴, H-6b″″, H-6b′, H-6a′, and CH_2CH$_2$TMS), 3.85 (m, 4H, H-3″, H-5″″, H-6a‴, and H-6a″″), 3.82 (dd, 1H, $J_{5''-6b''}$ 3.9, $J_{6a''-6b''}$ 11.2 Hz, H-6b″), 3.77 (m, 1H, H-5), 3.72 (dd, 1H, $J_{5''-6a''}$ 3.4 Hz, H-6a″), 3.67 (m, 1H, H-6b), 3.52 (m, 3H, H-6a, H-5′ and CH_2CH$_2$TMS), 3.46 (m, 1H, H-5″), 2.96 (m, 1H, CH_2CH$_2$COCH$_3$), 2.72 (m, 2H, CH$_2$CH_2COCH$_3$), 2.60 (m, 1H, CH_2CH$_2$COCH$_3$), 2.38, 2.35, and 2.18(×2) (4×s, 4×3H, CH$_2$CH$_2$COCH_3 and 3×COCH$_3$), 0.90 (m, 2H, CH$_2$CH_2TMS), and 0.00 (s, 9H, TMS); MALDI-TOF MS: m/z 2335.1 (M+Na$^+$) and 2351.9 (M+K$^+$).

4.10. Trimethylsilylethyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-[2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-]-2,4-di-*O*-acetyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalamido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalamido-β-D-glucopyranoside (19)

A solution of compound **16** (121 mg, 52 μmol) in MeOH (2 mL) containing hydrazine acetate (12 mg, 124 μmol) was stirred at room temperature for 1 h, then at 55 °C for 20 h. The reaction was concentrated in vacuo and partitioned between CH_2Cl_2 (20 mL) and water (20 mL), and the organic phase was dried ($MgSO_4$), filtered and concentrated in vacuo. Flash silica gel column chromatography (eluent: 4:1 toluene–EtOAc) of the residue afforded **17** (108 mg, 94%) as a white foam. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.00–6.90 (m, 58H, Ar), 5.46 (d, 1H, $J_{1'-2'}$ 8.8 Hz, H-1′), 5.44 (m, 1H, H-2″″), 5.34 (m, 1H, H-2‴), 5.22 (dd, 1H, $J_{3''-4''}$ 9.8 and $J_{4''-5''}$ 9.3 Hz, H-4″), 5.15 (d, 1H, J_{1-2} 7.8 Hz, H-1), 5.11 (d, 1H, H-1‴), 5.06 (m, 3H, 3×CH_2Ph), 5.06 (m, 1H, CH_2Ph), 5.00 (m, 1H, CH_2Ph), 4.97 (m, 1H, CH_2Ph), 4.96 (s, 1H, H-1″″), 4.89–4.62 (m, 14H, 14×CH_2Ph), 4.77 (m, 1H, H-1″), 4.55 (dd, 1H, $J_{2'-3'}$ 10.3, $J_{3'-4'}$ 8.8 Hz, H-3′), 4.48 (d, 1H, J_{AB} 9.7 Hz, CH_2Ph), 4.31 (m, 6H, H-2, H-3, H-4, H-2′, H-4′, and H-5″), 4.20 (dd, 1H, $J_{2'''-3'''}$ 2.9 and $J_{3'''-4'''}$ 9.3 Hz, H-3‴), 4.15 (s, 1H, 2″-OH), 4.11 (m, 1H, H-6b′), 4.07 (m, 2H, H-4‴ and H-3″″), 4.00 (t, 1H, J 9.3 Hz, H-4″″), 3.87 (m, 7H, H-5″″, H-6b‴, H-6b″″, H-6b″, H-6a′, CH_2CH$_2$TMS, and H-6a″″), 3.72 (m, 3H, H-6b, H-6a″, and H-6a‴), 3.64 (m, 2H, H-2″ and H-3″), 3.52 (m, 5H, H-5, H-6a, H-5′, H-5″, and CH_2CH$_2$TMS), 2.38 (×2) and 2.18 (3×s, 3×3H, 3×COCH$_3$), 0.90 (m, 2H, CH$_2$CH_2TMS), and 0.00 (s, 9H, TMS); MALDI-TOF MS: m/z 2237.2 (M+Na$^+$) and 2253.1 (M+K$^+$). The foregoing compound **17** (108 mg, 49 μmol) in CH_2Cl_2 (2 mL) was treated with pyridine (130 μL) and triflic anhydride (110 μL) at 0 °C. The reaction was stirred at this temperature for 2 h, after which time it was diluted with CH_2Cl_2 (50 mL), washed with satd aq $NaHCO_3$ (50 mL), dried ($MgSO_4$), filtered and concentrated in

vacuo. The residual **18** was taken up in dry toluene (4 mL) and treated with Bu_4NOAc (100 mg, 0.33 mmol) and the resulting suspension was subjected to sonication at room temperature under argon for 20 h. The reaction was subjected directly to flash-column chromatography (eluent: 9:1 toluene–EtOAc) affording **19** (98 mg, 89%) as a glass-like foam. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.00–6.90 (m, 58H, Ar), 5.54 (d, 1H, $J_{2''-3''}$ 2.9 Hz, H-2″), 5.45 (m, 1H, H-2‴), 5.36 (m, 2H, H-1′ and H-4″), 5.27 (m, 1H, H-2‴), 5.09 (d, 1H, J_{1-2} 7.8 Hz, H-1), 5.08 (d, 1H, H-1‴), 5.03–4.94 (m, 3H, 3×CH_2Ph), 4.96 (s, 1H, H-1⁗), 4.87 (s, 1H, H-1″), 4.84 (d, 1H, J_{AB} 12.2 Hz, CH_2Ph), 4.79–4.58 (m, 15H, 15×CH_2Ph), 4.54 (d, 1H, J_{AB} 10.7 Hz, CH_2Ph), 4.37 (dd, 1H, $J_{2'-3'}$ 10.4, $J_{3'-4'}$ 8.8 Hz, H-3′), 4.27 (m, 5H, H-2, H-3, H-4, H-2′, and H-4′), 4.09 (m, 2H, H-4‴ and H-5‴), 4.05 (dd, 1H, H-3⁗), 3.95 (m, 4H, H-3‴, H-4⁗, H-6b‴, and CH_2CH$_2$TMS), 3.92 (dd, 1H, $J_{5''''-6b''''}$ 4.4, $J_{6a''''-6b''''}$ 10.8 Hz, H-6b⁗), 3.86 (m, 2H, H-5⁗ and H-6a‴), 3.77 (m, 4H, H-3″, H-6b′, H-6b″, and H-6a⁗), 3.72 (dd, 1H, $J_{5''-6a''}$ 4.1, $J_{6a''-6b''}$ 11.2 Hz, H-6a″), 3.65 (m, 2H, H-6b and H-6a′), 3.59 (m, 2H, H-6a and CH_2CH$_2$TMS), 3.39 (m, 3H, H-5, H-5′, H-5″), 2.30, 2.27, 2.26, and 2.07 (4×s, 4×3H, 4×COCH$_3$), 0.90 (m, 2H, CH$_2$CH_2TMS), and 0.00 (s, 9H, TMS); MALDI-TOF MS: m/z 2278.8 (M+Na$^+$) and 2295.0 (M+K$^+$).

4.11. Trimethylsilylethyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)-]-2,4-di-*O*-acetyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-D-glucopyranose (20)

A stirred solution of compound **19** (98 mg, 43 μmol) in 1-butanol (12 mL) under argon was treated with ethylenediamine (2.40 mL) at 80 °C for 15 h. After 1 h at room temperature, the reaction mixture was concentrated in vacuo and co-evaporated with toluene three times. The resulting residue was taken up in pyridine (8 mL) and Ac_2O (4 mL) and after 48 h at room temperature the reaction was concentrated in vacuo. To the residue in 95% EtOH (25 mL) was added 10% palladium-on-charcoal (200 mg) and the resulting suspension stirred was vigorously under a slight over pressure of hydrogen gas for 48 h. After filtration through a plug of Celite, the filtrate was concentrated in vacuo and treated with pyridine (8 mL) containing Ac_2O (4 mL). After 48 h, the reaction was concentrated in vacuo and flash silica gel column chromatography (eluent: MeOH gradient in CH_2Cl_2) of the residue gave **20** (50 mg, 73%) as a syrup. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.05 (d, 1H, $J_{2\text{-NH}}$ 9.8 Hz, 2-NH), 5.65 (d, 1H, $J_{2'\text{-NH}}$ 8.8 Hz, 2′-NH), 5.38 (m, 1H, H-4⁗), 5.35 (m, 1H, H-2″), 5.33 (t, 1H, J 10.1 Hz, H-4‴), 5.27 (m, 2H, H-2⁗ and H-3⁗), 5.15 (dd, 1H, $J_{2'''-3'''}$ 3.1, $J_{3'''-4'''}$ 10.1 Hz, H-3‴), 5.11 (dd, 1H, $J_{2'-3'}$ 9.7, $J_{3'-4'}$ 10.1 Hz, H-3′), 5.07 (t, 1H, J 8.8 Hz, H-4″), 5.05 (dd, 1H, J_{2-3} 8.8, J_{3-4} 8.4 Hz, H-3), 4.99 (m, 2H, H-1‴ and H-2‴), 4.83 (s, 1H, H-1⁗), 4.65 (s, 1H, H-1″), 4.47 (d, 1H, $J_{1'-2'}$ 8.4 Hz, H-1′), 4.44 (d, 1H, J_{1-2} 7.5 Hz, H-1), 4.39 (dd, 1H, $J_{5'-6b'}$ 3.5, $J_{6a'-6b'}$ 12.9 Hz, H-6b′), 4.34 (dd, 1H, $J_{5'''-6b'''}$ 1.9, $J_{6a'''-6b'''}$ 11.0 Hz, H-6b‴), 4.31 (m, 1H, H-6b⁗), 4.29 (m, 2H, H-6b and H-6a‴), 4.24 (m, 1H, H-6a′), 4.20 (dd, 1H, $J_{5''''-6a''''}$ 2.0, $J_{6a''''-6b''''}$ 11.5 Hz, H-6a⁗), 4.16 (dd, 1H, J_{5-6a} 2.2, J_{6a-6b} 12.3 Hz, H-6a), 4.09 (m, 1H, H-5‴), 4.03 (m, 1H, H-5⁗), 3.99 (ddd, 1H, H-2), 3.90 (m, 2H, H-2′ and CH_2CH$_2$TMS), 3.89 (m, 1H, H-3″), 3.87 (dd, 1H, $J_{4'-5'}$ 9.7 Hz, H-4′), 3.83 (m, 1H, H-6b″), 3.77 (dd, 1H, J_{4-5} 7.9 Hz, H-4), 3.63 (m, 2H, H-5 and H-5′), 3.60 (dd, 1H, $J_{5''-6a''}$ 3.1, $J_{6a''-6b''}$ 11.0 Hz, H-6a″), 3.53 (m, 2H, H-5″ and CH_2CH$_2$TMS), 2.40–180 (16×s, 16×3H, 16×COCH$_3$), 0.90 (m, 2H, CH$_2$CH_2TMS), and 0.00 (s, 9H, TMS); MALDI-TOF MS: m/z 1622.6 (M+Na$^+$) and 1638.8 (M+K$^+$).

4.12. 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)]-2,4-di-*O*-acetyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-1-*S*-acetyl-3,6-di-*O*-acetyl-2-deoxy-1-thio-D-glucopyranose (23)

Compound **20** (41 mg, 26 μmol) in CH_2Cl_2 (0.5 mL) was treated with CF_3CO_2H (1 mL) for 45 min at room temperature. The mixture was concentrated in vacuo, and the crude hemiacetal **21** was taken up in 1:1 anhyd CH_2Cl_2–toluene (0.5 mL) and treated with thionyl chloride (1 mL) at room temperature for 2 h. After removal of the solvent and reagent in vacuo, the resulting α-glycosyl chloride **22** was treated with dry acetone (3 mL) containing potassium thioacetate (5.8 mg, 51 μmol). The resulting mixture was stirred at room temperature under argon for 18 h, and the solvent was removed under reduced pressure. Flash silica gel column chromatography of the residue using a 1–6% gradient of MeOH in CH_2Cl_2 afforded **23** (16.3 mg, 41%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.94 (d, 1H, $J_{2\text{-NH}}$ 8.8 Hz, 2-NH), 5.64 (d, 1H, $J_{2'\text{-NH}}$ 9.8 Hz, 2′-NH), 5.31 (t, 1H, J 9.8 Hz, H-4⁗), 5.30 (m, 1H, H-2″), 5.25 (t, 1H, J 10.3 Hz, H-4‴), 5.20 (m, 1H, H-2⁗) 5.15 (dd, 1H, $J_{2''''-3''''}$ 2.9 Hz, H-3⁗), 5.06 (m, 2H, H-3′ and H-3‴), 4.99 (t, 1H, $J_{3''-4''}$ 9.3, $J_{4''-5''}$ 11.2 Hz, H-4″), 4.97 (d, 1H, J_{1-2} 8.8 Hz, H-1), 4.91 (m, 3H, H-3, H-1‴, and H-2‴), 4.75 (s, 1H, H-1⁗), 4.57 (s, 1H, H-1″), 4.39 (d, 1H, $J_{1'-2'}$ 8.3 Hz, H-1′), 4.32 (dd, 1H, $J_{5'-6b'}$ 3.9, $J_{6a'-6b'}$ 12.2 Hz, H-6b′), 4.20 (m, 4H, H-2, H-6b, H-6b‴, and H-6b⁗), 4.16 (m, 1H, H-6a′), 4.12 (dd, 1H, $J_{5'''-6a'''}$ 2.4, $J_{6a'''-6b'''}$ 12.7 Hz, 6a‴), 4.08 (dd, 1H, $J_{5''''-6a''''}$ 2.0, $J_{6a''''-6b''''}$ 12.2 Hz, H-6a⁗), 4.01 (m, 2H, H-6a and H-5‴), 3.95 (ddd, 1H, $J_{5''''-6a''''}$ 4.4 Hz, H-5⁗), 3.81 (m, 2H, H-2′ and H-3″), 3.76 (m, 2H, H-4′ and H-6b″), 3.71 (m, 1H, H-4), 3.64 (m, 1H, H-5), 3.52 (m, 2H, H-5′

and H-6a″), 3.47 (m, 1H, H-5″), 2.28 (s, 3H, $COCH_3$), and 2.10–1.85 (m, 16×3H, 16×$COCH_3$); MALDI-TOF MS: m/z 1580.5 ($M+Na^+$) and 1596.5 ($M+K^+$).

4.13. α-D-Mannopyranosyl-(1→6)-[α-D-mannopyranosyl-(1→3)]-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-1-thio-D-glucopyranose (3)

Compound **23** (3.0 mg, 1.9 μmol) was treated with a 1 M solution NaOMe in MeOH (2 mL) at 0 °C for 30 min. After neutralization of the reaction mixture with Amberlite 650C ion-exchange resin, the solvent was removed in vacuo to give **3** (1.7 mg) in 96% yield. ^1H NMR (D_2O, 500 MHz): δ 4.92 (s, 1H, H-1‴), 4.72 (s, 1H, H-1⁗), 4.61 (s, 1H, H-1″), 4.52 (d, 1H, J_{1-2} 11.0 Hz, H-1), 4.44 (d, 1H, $J_{1'-2'}$ 7.7 Hz, H-1′), 4.08 (d, 1H, $J_{2''-3''}$ 3.4 Hz, H-2″), 3.89 (m, 1H, H-2‴), 3.79 (m, 1H, H-2⁗), 3.78–3.66 (m, 8H), 3.66–3.51 (m, 10H), 3.51–3.42 (m, 6H), 3.42–3.30 (m, 3H), 1.92 and 1.89 (2×s, 6H, 2×$COCH_3$); MALDI-TOF MS: m/z 949.9 ($M+Na^+$) and m/z 965.8 ($M+K^+$); HRMS (m/z): Calcd for $C_{34}H_{58}N_2NaO_{25}S$ 949.2898; found m/z 949.2947.

4.14. Trimethylsilylethyl 3,4,6-tri-*O*-benzyl-2-*O*-levulinoyl-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalamido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalamido-β-D-glucopyranoside (25)

To a solution of compound **24** (700 mg, 1.18 mmol) and disaccharide acceptor **8** (1.04 g, 0.98 mmol) in dry CH_2Cl_2 (9 mL) was added activated 4 Å molecular sieves (1 g). After stirring at room temperature for 30 min under argon, the mixture was cooled (0 °C) and *N*-iodosuccinamide (3.75 g, 16.68 mmol) and trimethylsilyl triflate (252 μL, 1.28 mmol) were added. Stirring was continued at 0 °C for 15 min, at the end of which time the reaction was subjected to the same workup as described for the preparation of compound **7** to afford **25** (1.27 g, 81%) as a white foam. ^1H NMR ($CDCl_3$, 300 MHz): δ 8.08–6.90 (m, 43H, Ar), 5.48 (d, 1H, $J_{1'-2'}$ 7.9 Hz, H-1′), 5.22 (dd, 1H, $J_{1''-2''}$ 8.4, $J_{2''-3''}$ 9.2 Hz, H-3″), 5.15 (d, 1H, J_{1-2} 7.9 Hz, H-1), 5.09 (m, 2H, 2×CH_2Ph), 4.96 (m, 2H, 2×CH_2Ph), 4.89 (m, 2H, 2×CH_2Ph), 4.78 (m, 2H, H-1″ and CH_2Ph), 4.75–4.60 (m, 7H, 7×CH_2Ph), 4.48 (dd, 1H, $J_{2'-3'}$ 10.6, $J_{3'-4'}$ 7.9 Hz, H-3′), 4.41 (dd, 1H, H-2′), 4.31 (m, 4H, H-2, H-3, H-4, and H-4′), 3.93 (m, 5H, CH_2CH$_2$TMS, H-4″, H-6b′, H-6b″, and H-6a″), 3.81 (dd, 1H, $J_{5'-6a'}$ 4.0, $J_{6a'-6b'}$ 11.0 Hz, H-6a′), 3.76 (dd, 1H, $J_{3''-4''}$ 8.8 Hz, H-2″), 3.70 (m, 1H, H-6b), 3.64–3.49 (m, 5H, CH_2CH$_2$TMS, H-5, H-5′, H-5″, and H-6a), 2.90 (m, 2H, COCH_2CH$_2$COCH$_3$), 2.60 (m, 2H, COCH$_2$CH_2COCH$_3$), 2.35 (s, 3H, COCH$_2$CH$_2$COCH_3), 0.90 (m, 2H, CH$_2$CH_2TMS), and 0.00 (s, 9H, TMS); MALDI-TOF MS: m/z 1613.3 ($M+Na^+$) and 1629.4 ($M+K^+$). Anal. Calcd for $C_{93}H_{98}N_2O_{20}Si$ (1591.8): C, 70.17; H, 6.21; N, 1.76. Found: C, 70.02; H, 6.17; N, 1.75.

4.15. Trimethylsilylethyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalamido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalamido-β-D-glucopyranoside (28)

A solution of compound **25** (1.20 g, 0.754 mmol) in MeOH (20 mL) was treated with hydrazine acetate (83 mg, 905 mmol) at room temperature for 2 h. The reaction was concentrated in vacuo and partitioned between CH_2Cl_2 (100 mL) and H_2O (100 mL), and the organic phase was dried ($MgSO_4$), filtered and concentrated in vacuo. Flash silica gel column chromatography (eluent: 9:1 toluene–EtOAc) of the residue afforded **26** (900 mg, 80%) as a white foam. ^1H NMR ($CDCl_3$, 600 MHz): δ 8.02–6.90 (m, 43H, Ar), 5.45 (d, 1H, $J_{1'-2'}$ 8.4 Hz, H-1′), 5.15 (d, 1H, J_{1-2} 7.9 Hz, H-1), 5.12 (d, 1H, J_{AB} 11.0 Hz, CH_2Ph), 5.12 (d, 1H, J_{AB} 13.2 Hz, CH_2Ph), 5.04 (d, 1H, J_{AB} 12.8 Hz, CH_2Ph), 5.00 (d, 1H, J_{AB} 11.0 Hz, CH_2Ph), 4.99 (d, 1H, J_{AB} 11.0 Hz, CH_2Ph), 4.80 (d, 1H, $J_{1''-2''}$ 7.7 Hz, H-1″), 4.78 (d, 1H, J_{AB} 12.4 Hz, CH_2Ph), 4.75 (d, 1H, J_{AB} 12.1 Hz, CH_2Ph), 4.71 (d, 1H, J_{AB} 11.3 Hz, CH_2Ph), 4.71 (d, 1H, J_{AB} 11.3 Hz, CH_2Ph), 4.70 (d, 1H, J_{AB} 12.5 Hz, CH_2Ph), 4.65 (d, 1H, J_{AB} 12.8 Hz, CH_2Ph), 4.65 (d, 1H, J_{AB} 12.8 Hz, CH_2Ph), 4.62 (d, 1H, J_{AB} 11.0 Hz, CH_2Ph), 4.61 (dd, 1H, $J_{2'-3'}$ 10.6, $J_{3'-4'}$ 8.4 Hz, H-3′), 4.60 (d, 1H, J_{AB} 12.5 Hz, CH_2Ph), 4.41 (m, 2H, H-2′ and H-3), 4.25 (dd, 1H, $J_{3'-4'}$ 8.4, $J_{4'-5'}$ 9.9 Hz, H-4′), 4.12 (m, 2H, H-2 and H-4), 4.02 (dd, 1H, $J_{5''-6b''}$ 1.9, $J_{6a''-6b''}$ 11.7 Hz, H-6b″), 3.97 (m, 1H, CH_2CH$_2$TMS), 3.86 (dd, 1H, $J_{5''-6a''}$ 1.8 Hz, H-6a″), 3.82 (m, 1H, H-6b′), 3.80 (m, 1H, H-4″), 3.76 (dd, 1H, $J_{5'-6a'}$ 4.4, $J_{6a'-6b'}$ 10.6 Hz, H-6a′), 3.74 (dd, 1H, J_{5-6b} 1.1, J_{6a-6b} 11.0 Hz, H-6b), 3.71 (m, 1H, H-2″), 3.68 (dd, 1H, $J_{2''-3''}$ 8.4, $J_{3''-4''}$ 9.2 Hz, H-3″), 3.60 (dd, 1H, J_{5-6a} 3.7 Hz, H-6a), 3.52 (m, 3H, CH_2CH$_2$TMS, H-5′, and H-5″), 3.48 (m, 1H, H-5), 0.85 (m, 2H, CH$_2$CH_2TMS), and 0.00 (s, 9H, TMS); MALDI-TOF MS: m/z 1516.1 ($M+Na^+$) and 1532.2 ($M+K^+$). The foregoing compound **26** (565 mg, 0.38 mmol) was taken up in CH_2Cl_2 (8 mL) and cooled to 0 °C before pyridine (91 μL, 1.13 mmol) and triflic anhydride (95 μL, 0.57 mmol) were added as described for compound **19**. The reaction was stirred at this temperature for 2 h, after which time it was diluted with CH_2Cl_2 (50 mL), washed with satd aq $NaHCO_3$ (50 mL), dried ($MgSO_4$), filtered and concentrated in vacuo. The residual **27** was taken up in dry toluene (18 mL) and Bu_4NOAc (684 mg, 2.27 mmol) was added, and the resulting suspension was subjected to sonication at room temperature under argon for 20 h. The reaction was subjected directly to flash column chromatography (eluent: 9:1 toluene–EtOAc) affording **28** (477 mg, 82%)

as a white foam. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.02–6.90 (m, 43H, Ar), 5.68 (d, 1H, $J_{2''-3''}$ 3.0 Hz, H-2″), 5.45 (d, 1H, $J_{1'-2'}$ 8.8 Hz, H-1′), 5.19 (d, 1H, J_{AB} 12.7 Hz, CH_2Ph), 5.17 (d, 1H, J_{1-2} 8.3 Hz, H-1), 5.04 (d, 1H, J_{AB} 13.7 Hz, CH_2Ph), 5.02 (d, 1H, J_{AB} 11.2 Hz, CH_2Ph), 4.90 (s, 1H, H-1″), 4.84 (d, 1H, J_{AB} 11.2 Hz, CH_2Ph), 4.84 (d, 1H, J_{AB} 12.2 Hz, CH_2Ph), 4.73–4.64 (m, 8H, 8×CH_2Ph), 4.56 (d, 1H, J_{AB} 11.2 Hz, CH_2Ph), 4.50 (dd, 1H, $J_{2'-3'}$ 10.7, $J_{3'-4'}$ 8.3 Hz, H-3′), 4.40 (dd, 1H, H-2′), 4.37 (m, 1H, H-3), 4.35 (dd, 1H, $J_{3'-4'}$ 8, $J_{4'-5'}$ 9.8 Hz, H-4′), 4.10 (m, 2H, H-2 and H-4), 3.97 (m, 1H, CH_2CH$_2$TMS), 3.95 (dd, 1H, $J_{3''-4''}$ 9.3, $J_{4''-5''}$ 9.8 Hz, H-4″), 3.90–3.80 (m, 4H, H-6b″, H-6b′, H-6a′, and H-6b), 3.74 (m, 1H, H-6a), 3.65 (dd, 1H, H-3″), 3.60 (dd, 1H, $J_{5''-6a''}$ 3.9, $J_{6a''-6b''}$ 10.7 Hz, H-6a″), 3.56–3.47 (m, 4H, CH_2CH$_2$TMS, H-5″, H-5′, and H-5), 2.39 (s, 3H, COCH$_3$), 0.90 (m, 2H, CH$_2$CH_2TMS), and 0.00 (s, 9H, TMS); MALDI-TOF MS: m/z 1558.0 (M+Na$^+$) and 1574.2 (M+K$^+$). Anal. Calcd for $C_{90}H_{94}N_2O_{19}Si$ (1535.8): C, 70.38; H, 6.17; N, 1.82. Found: C, 70.05; H, 6.19; N, 1.70.

4.16. Trimethylsilylethyl 2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-D-glucopyranose (29)

A stirred solution of compound **28** (107 mg, 70 μmol) in 1-butanol (14 mL) under argon was treated with ethylenediamine (2.85 mL) as described for the synthesis of compound **20**. The syrupy residue was taken up in 95% EtOH (18 mL) and 10% palladium-on-charcoal (120 mg) added. The suspension was stirred vigorously under a slight over pressure of hydrogen for 48 h, at the end of which time the mixture was filtered through a plug of Celite. The filtrate was concentrated in vacuo and dissolved in pyridine (5 mL) containing Ac_2O (2 mL) and left to stand at room temperature for 48 h. The reaction mixture was concentrated in vacuo, and flash silica gel column chromatography (eluent: MeOH gradient in CH_2Cl_2) gave **29** (55 mg, 77%) as a syrup. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.20 (d, 1H, $J_{2'\text{-NH}'}$ 9.3 Hz, N*H*Ac′), 5.94 (d, 1H, $J_{2\text{-NH}}$ 9.3 Hz, N*H*Ac), 5.37 (d, 1H, $J_{2''-3''}$ 3.0 Hz, H-2″), 5.18 (t, 1H, J 9.9 Hz, H-4″), 5.12 (m, 2H, H-3 and H-3′), 5.00 (dd, 1H, H-3″), 4.68 (s, 1H, H-1″), 4.53 (d, 1H, J_{1-2} 7.7 Hz, H-1), 4.47 (d, 1H, $J_{1'-2'}$ 8.2 Hz, H-1′), 4.30 (m, 5H, H-6b″, H-6b′, H-6b, H-6a′, and H-6a), 4.10 (dd, 1H, $J_{5''-6a''}$ 2.5, $J_{6a''-6b''}$ 12.4 Hz, H-6a″), 3.98–3.78 (m, 4H, CH_2CH$_2$TMS, H-2, H-2′, and H-4), 3.71 (dd, 1H, J_{3-4} 7.7, J_{4-5} 8.5 Hz, H-4), 3.68–3.48 (m, 4H, CH_2CH$_2$TMS, H-5, H-5′, and H-5″), 2.14, 2.12, 2.11, 2.09, 2.08, 2.03, 2.02, 1.97, 1.94 and 1.93 (10×s, 30H, 10×COCH$_3$), 0.90 (m, 2H, CH$_2$CH_2TMS), and 0.00 (s, 9H, TMS); MALDI-TOF MS: m/z 1044.9 (M+Na$^+$) and 1060.8 (M+K$^+$); HRMS (m/z): Calcd for $C_{43}H_{66}N_2O_{24}$ 1022.3775; found m/z 1022.3864.

4.17. 2,3,4,6-Tetra-*O*-acetyl-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-1-*S*-acetyl-3,6-di-*O*-acetyl-2-deoxy-1-thio-D-glucopyranose (32)

Compound **29** (55 mg, 54 μmol) in CH_2Cl_2 (0.5 mL) was treated with CF_3CO_2H (1 mL) for 45 min at room temperature. After concentration in vacuo, crude **30** was dissolved in 1:1 anhyd CH_2Cl_2–anhyd toluene (0.6 mL) and treated with thionyl chloride (0.8 mL) as described for **23**. Removal of the solvent and reagent under reduced pressure gave the α-glycosyl chloride **31**, which was subsequently dissolved in dry acetone (2 mL) containing potassium thioacetate (8.6 mg, 76 μmol) as described for **23** to afford **32** (22 mg, 41%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.88 (d, 1H, $J_{2'\text{-NH}'}$ 8.8 Hz, N*H*Ac′), 5.75 (d, 1H, $J_{2\text{-NH}}$ 9.8 Hz, N*H*Ac), 5.32 (d, 1H, $J_{2''-3''}$ 3.4 Hz, H-2″), 5.13 (t, 1H, J 9.8 Hz, H-4″), 4.98 (m, 3H, H-3, H-3′, and H-1), 4.92 (dd, 1H, H-3″), 4.60 (s, 1H, H-1″), 4.33 (d, 1H, $J_{1'-2'}$ 7.8 Hz, H-1′), 4.27–4.17 (m, 6H, H-2, H-6b″, H-6b′, H-6b, H-6a′, and H-6a), 4.04 (dd, 1H, $J_{5''-6a''}$ 2.4, $J_{6a''-6b''}$ 12.2 Hz, H-6a″), 3.87 (ddd, 1H, $J_{2'-3'}$ 8.8 Hz, H-2′), 3.75 (t, 1H, J 9.3 Hz, H-4′), 3.67 (m, 2H, H-4 and H-5), 3.55 (ddd, 1H, $J_{5''-6b''}$ 4.9 Hz, H-5″), 3.49 (dt, 1H, J 3.4 Hz, H-5′), 2.28, 2.08, 2.06, 2.06, 2.03, 2.02, 1.97, 1.95, 1.91, 1.88, and 1.86 (11×s, 33H, 11×COCH$_3$); MALDI-TOF MS: m/z 1002.2 (M+Na$^+$) and 1018.1 (M+K$^+$).

4.18. β-D-Mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-1-thio-D-glucopyranose (2)

Compound **32** (5.0 mg, 5.1 μmol) was treated with a 0.5 M solution NaOMe in MeOH (1.5 mL) as described for compound **3** to give **2** (3.0 mg, 100%). ^{1}H NMR (D_2O, 500 MHz): δ 4.60 (s, 1H, H-1″), 4.50 (d, 1H, J_{1-2} 10.3 Hz, H-1), 4.42 (d, 1H, $J_{1'-2'}$ 7.6 Hz, H-1′), 3.88 (d, 1H, $J_{2''-3''}$ 3.4 Hz, H-2″), 3.81 (dd, 1H, $J_{5''-6b''}$ 2.0, $J_{6a''-6b''}$ 12.2 Hz, H-6b″), 3.77 (dd, 1H, $J_{5'-6b'}$ 2.0, $J_{6a'-6b'}$ 12.3 Hz, H-6b′), 3.71 (dd, 1H, J_{5-6b} 2.0, J_{6a-6b} 12.3 Hz, H-6b), 3.69–362 (m, 5H, H-2, H-2′, H-3′, H-4′, and H-6a′), 3.61 (dd, 1H, $J_{5''-6a''}$ 6.8 Hz, H-6a″), 3.55–3.50 (m, 4H, H-3, H-4, H-6a, and H-3″), 3.50 (m, 1H, H-5′), 3.45 (dd, 1H, $J_{3''-4''}$ 9.3, $J_{4''-5''}$ 9.8 Hz, H-4″), 3.34 (m, 1H, H-5), 3.30 (m, 1H, H-5″), 1.94 and 1.93 (2×s, 6H, 2×COCH$_3$); MALDI-TOF MS: m/z 624.7 (M+Na$^+$) and 640.7 (M+K$^+$); HRMS (m/z): Calcd for $C_{22}H_{38}N_2NaO_{15}S$ 625.1855; found m/z 625.1891.

4.19. 2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-1-thio-β-D-glucopyranose-*S*′-*S*-glutathione disulfide (33)

A solution (20 mL) of **1** (5 mg, 11.0 μM) and glutathione (1.7 mg, 5.7 μM) in aq 25 mM NH_4OAc at pH 8.5 was

stirred at room temperature for 18 h. The reaction mixture was freeze-dried, and the disulfide-linked glycopeptide **33** (3.2 mg, 76%) was isolated by P-2 gel filtration (eluent: H_2O). ^1H NMR (D_2O, 500 MHz): δ 4.80 (dd, 1H, J 3.9 and 9.8 Hz, CH-Cys), 4.62 (d, 1H, $J_{1,2}$ 10.7 Hz, H-1), 4.58 (d, 1H, $J_{1',2'}$ 8.9 Hz, H-1′), 4.04 (dd, 1H, $J_{2,3}$ 9.8 Hz, H-2), 3.91 (dd, 1H, $J_{5',6b'}$ 1.9, $J_{6a',6b'}$ 11.7 Hz, H-6b′), 3.86 (dd, 1H, $J_{5,6b}$ 1.9, $J_{6a,6b}$ 11.7, H-6b), 3.69–3.81 (m, 6H, CH_2-Gly, CH-Glu, H-2′, H-3, and H-6a′), 3.66 (dd, 1H, $J_{5,6a}$ 5.9 Hz, H-6a), 3.65 (dd, 1H, $J_{3,4}$ 8.8, $J_{4,5}$ 9.8 Hz, H-4), 3.55 (dd, 1H, $J_{2',3'}$ 10.7, $J_{3',4'}$ 8.8 Hz, H-3′), 3.54 (m, 1H, H-5), 3.50 (m, 1H, H-5′), 3.46 (dd, 1H, $J_{4',5'}$ 9.7 Hz, H-4′), 3.36 (dd, 1H, J 3.9 and 14.6 Hz, CH_2-Cys), 2.99 (dd, 1H, J 9.8 and 14.6 Hz, CH_2-Cys), 2.52 and 2.15 (2×m, 4H, 2×CH_2-Glu), 2.06 and 2.01 (2×s, 6H, 2×CH_3CO); MALDI-TOF MS: m/z 746.2 ($M+H^+$), 768.2 ($M+Na^+$), 784.2 ($M+K^+$), and 789.2 ($M-H^++2Na^+$).

4.20. 2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-1-thio-β-D-glucopyranose-*S*′,*S*-BSA disulfide (34)

A solution of BSA (15 μM) and 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-1-thio-β-D-glucopyranose (**1**, 750 μM) in 0.25 M NH_4OAc at pH 8.5 (1 mL) was stirred at 30 °C. After 24 h the free thiol concentration, determined using *S*,*S*-dithiodipyridine, was very low. An aliquot (500 μL) was removed and subjected to ultrafiltration using Millipore Centricon devices fitted with 10 kDa cutoff membranes. The concentrate was washed three times with water (500 μL) and dissolved in water (300 μL) to give neoglycoprotein **34**, which was subjected to MALDI-TOF MS (with sinapinic acid as matrix); **34**: m/z 67,089 (BSA: m/z 66,612).

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