Synthesis of a Mannose-Bearing Disaccharide with a Thiol Spacer at the Reducing End

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Abstract: The synthesis of a mannose-bearing disaccharide containing a thiol spacer at the reducing end was carried out to provide a tethered sugar suitable for attaching to gold nanoparticles. An array of such sugars is designed to mimic carbohydrates involved in cell-surface interactions. The molecule was constructed *via* Schmidt glycosylation of an appropriately protected glycosyl donor and an acceptor, followed by removal of protective groups and reductive amination to introduce a protected thiol spacer at the reducing end of the glycan. A new finding that the anomeric reductive amination is capable of concomitantly removing a neighboring *N*-acetyl group was also observed. Subsequent removal of the thiol protective group and purification of the product gave the target disaccharide in a satisfactory yield.

Keywords: Carbohydrate, oligosaccharide, glycosylation, mannose, nanoparticle, thiol.

INTRODUCTION

Oligosaccharides on cell surfaces play a key role in cell adhesion and recognition events *via* attractive forces that exist between oligosaccharides and specific proteins (termed lectins) [1-3]. Especially important are the class of calcium-binding lectins, called "C-lectins" that are involved in mammalian cell-surface recognition [4]. Since the interactions between lectins and oligosaccharides are essential for higher order living systems, a fundamental understanding of the structure of the oligosaccharide and how it affects cell recognition will have an important impact in many fields such as immunology, virology, developmental biology, and biomineralization [5]. Potential clinical applications include diagnostic devices, biomedical imaging, as well as nanoparticles that are tissue-specific drug delivery agents [6-8]. However, the main challenge in quantitatively studying these carbohydrate–lectin interactions is devising methods to deal with the extremely low affinity of binding. To address this problem, the presentation of oligosaccharides on cell surfaces has to be multivalent [9]. In addition to multivalency, factors such as sugar structure (mono-, di- and higher oligosaccharides and linear vs. branched oligosaccharides) and carbohydrate density are important factors in binding.

In recent years, there has been a steadily growing interest in designing multivalent carbohydrate analogues that will mimic the natural cell surface and bind to lectins with high affinity. Synthetic oligosaccharides, elongated with suitable spacer groups, have been conjugated to polystyrenes [7], dendrimers [8], and carrier proteins [9]. More recently, water-soluble gold glyconanoparticles have been successfully applied as multivalent tools for transmission electron microscopic (TEM) studies on the mannose binding to *E. coli* FimH adhesin [10] and the self-recognition of Le^x antigen [11]. The results suggest that the globular shape and the multivalent presentation of oligosaccharides on the particle surface make gold glyconanoparticles an extremely suitable tool to overcome the low binding affinity of monomeric oligosaccharides to lectins [12, 13]. In addition, gold glyconanoparticles were found to be of exceptionally low cytotoxicity in some bio-assays, both *in vitro* and *in vivo* [14]. Moreover, gold nanodots, multivalently coated with thiol-containing saccharides linked *via* covalent linkages of sulfur to gold, were found to be stable probes for studying carbohydrate–protein interactions [15, 16].

Herein, we report the synthesis of a mannose-bearing disaccharide, derivatized with a thiol spacer at the reducing end, which is a part of the synthetic work of an ongoing project on the construction of nanoparticles. According to the planning of the project, the synthesized disaccharide will be attached to the gold nanoparticles (termed m-AuNP) to provide a tool for quantitative study of the complex carbohydrate-involved cell–cell interactions, and ultimately to give insights for the development of new entities that have potential as pharmaceuticals, diagnostic agents, and anti-bioterrorism devices.

RESULTS AND DISCUSSION

Synthetic Approaches

Thiol-terminated disaccharides are constructed by introducing a thiol group onto a disaccharide backbone that is made up of one or more sugar units constructed by glycosylation procedures, including the Schmidt-type glycosidation [17]. For the examples in this report, thiol introduction is carried out by reductive amination using an

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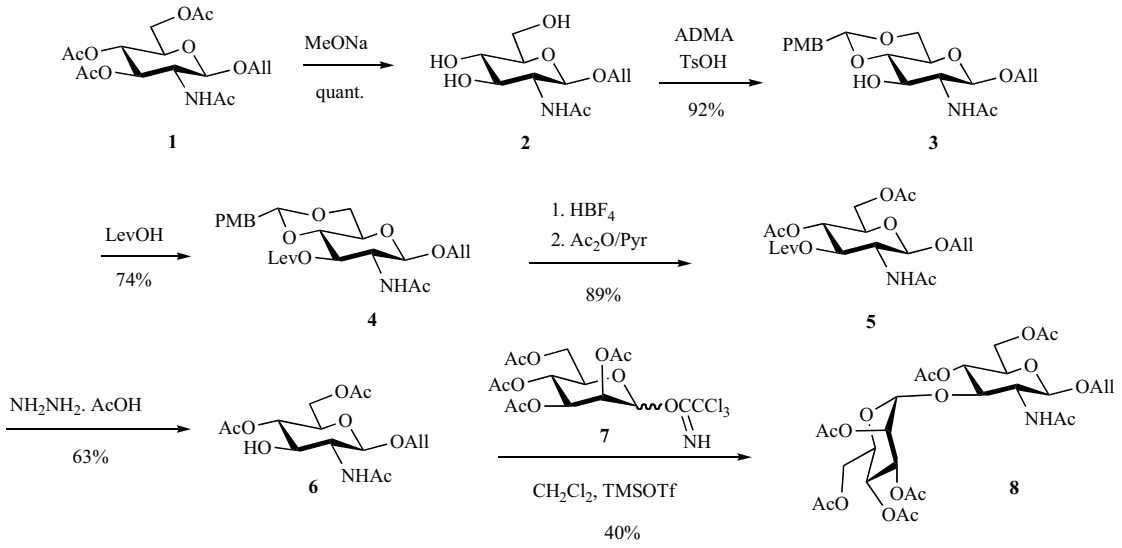


Scheme 1. Synthesis of disaccharide **8**.

S-protected 2-aminoethanethiol reagent that was chosen for its robust chemistry that allows for applicaton to various substrates under mild reaction conditions that produce stable compounds [14, 18]. The chemistry for thiol introduction as outlined is crucial in subsequent gold nanoparticle development to ensure smoothly operating procedures that produce good yields on minute amounts of substrates. The synthesis of the thiol-terminated disaccharide was carried out as presented in the sections that follow.

Synthesis of Disaccharide 8

Allyl β-D-glycoside **1** was prepared by a procedure repoorted in the literature [19]. The acetyl-protected sugar **1** (Scheme **1**) gave the triol **2** under Zemplén deacetylation conditions in quantitative yield. Triol **2** [20] was then converted to its 4,6-*O*-(*p*-methoxybenzylidene)-protected compound **3** by reaction with anisaldehyde dimethyl acetal (ADMA) under mildly acidic conditions [21]. Compound **3** was found to have a surprisingly low solubility in solvents such as CH_2Cl_2 that are commonly used in glycosylation reactions. This low solubility might be attributed to the rigidity of the fused-ring structure; therefore, an alternative acceptor with a less rigid structure (compound **6**) was called for.

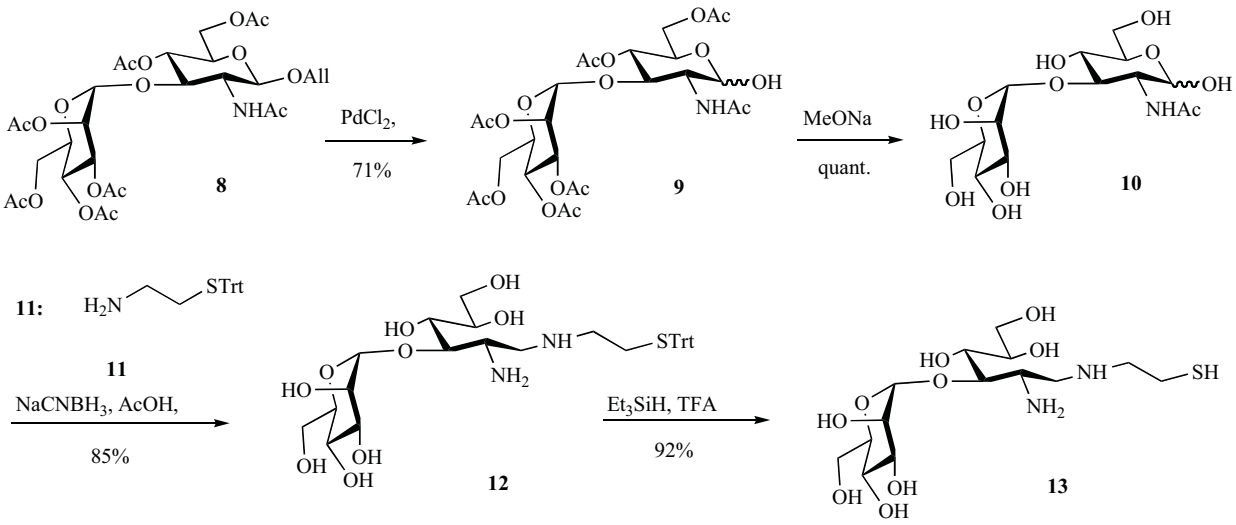
The free 3-OH group of **3** was protected by a levulinoyl group that was formed by reaction of **3** with levulinic acid activated by 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) under catalysis by 4-(dimethylamino)pyridine (DMAP). 4,6-*O*-Benzylidene ring opening was accomplished with tetrafluoroboric acid, and subsequent acetylation of the diol with acetic anhydride–pyridine gave **5**. Delevulinoylation of **5** was effected by hydrazine acetate [22] to give the acetylated acceptor **6**, which demonstrated significantly improved solubility in glycosylating solvents. The peracetylated mannosyl trichloroacetimidate donor **7** was synthesized the procedure of Tosin and Murphy [23]. With both acceptor **6** and donor **7** in hand, Schmidt glycosylation [17] was conducted to give the acetyl-protected disaccharide **8**. The addition of 4 Å molecular sieves was found necessary in the Schmidt glycosylation before the moisture-sensitive catalyst (TMSOTf) was added. After several failures by using beads of 4 Å molecular sieves, we found that pulverizing the beads in a mortar and pestle and heat activation of the resulting powder at 250 °C under vacuum was essential for the success of this reaction. However, unsatisfactorily low yields for reasons not fully understood have continually plagued this reaction. A possible explanation is that the 2-acetamido group of **6** reacts with the trichloroacetimidate donor **7** to form an imidate side product, thus lowering the yield of the O-glycosylation reaction [24]. By making use of the helpful premix–predry technique, the yield for the glycosylation was improved to a modest 40%.

Deprotection and Installation of Thiol Terminus

Anomeric deallylation of protected disaccharide **8** was carried out under the catalysis of $PdCl_2$ [25]. Compared with the conventional use of an iridium catalyst [26], the palladium chloride procedure was simpler; more importantly, the use of iodine during the workup was avoided, which kept the 1,3-*O*-glycosidic bond intact throughout the reaction. Subsequent removal of all protective *O*-acetyl groups of **9** under Zemplén conditions quantitatively yielded the free disaccharide **10**, which was directly used in the next step without further purification.

Following Kamerling's procedure [14, 18], the cysteamine (2-aminoethanethiol) moiety was introduced to the reducing end of the free disaccharide **10**. Prior to reductive amination, the thiol functional group of cysteamine was protected with a trityl group to avoid possible side reactions. Reaction of sugar **10** with trityl-protected cysteamine **11** smoothly produced **12**. With evidence from NMR and mass spectrometry[1], the *N*-acetyl group of **10** was

[1]The absence of singlet at δ 2.06 in the ^{1}H NMR and δ 21.85 in ^{13}C NMR spectra of **10** indicated the disappearance of the methyl of *N*-acetyl group; plus *m/z* 645.2875 of the hydrogen adduct of **12** in HRESIMS reconfirmed that the product structure had no *N*-acetyl group attached.



Scheme 2. Deprotection and installation of thiol terminus.

found to be concomitantly removed, presumably due to the rather harsh reducing conditions. This finding could be novel in that N-deacetylation occurred under acidic conditions in the presence of $NaCNBH_3$, which was in contrast to the previously reported basic conditions that used $NaBH_4$ [27]. Moreover, the report of this finding could hopefully pique interests in mechanism studies and possibly reveal new methods for N-deacetylation reactions.

The highly polar product **12** was then purified by a two-stage column protocol: Diaion HP-20 reversed-phase chromatography, followed by a fine separation from impurities on silica gel column chromatography. After the removal of the trityl protective group by ionic hydrogenation [19], the target compound **13** was produced in high yield (92%).

EXPERIMENTAL

General Methods

^1H (250 MHz, 300 MHz and 600 MHz) and ^{13}C (62.5 MHz, 75 MHz and 150 MHz) NMR spectra were recorded at 25 ^oC with a Bruker AC 250, a Varian Mercury 300 or a Varian Inova 600 instrument. Chemical shifts are given in δ-units (ppm) relative to an internal standard of TMS for ^1H, and relative to the signal for $CDCl_3$ (δ 77.0) or DMSO-d_6 (δ 39.5) for ^{13}C, unless otherwise stated. Apparent first-order multiplicities are indicated by s, singlet; d, doublet; dd, doublet of doublets; t, triplet; dt, doublet of triplets; q, quartet; m, multiplet. All two-dimensional experiments (gCOSY, gHSQC and TOCSY) were recorded with a Varian Inova 600 instrument. Column chromatography was performed on 60 Å (40–63 μm) silica gel (Sorbent Technologies), and fractions were monitored by TLC on Silica Gel 60 F_{254} (0.2-mm aluminum-backed plates, E. Merck) by detection with 254 nm UV light and then spray–heat development using a *p*-anisaldehyde–sulfuric acid reagent [28]. High-resolution ESI mass spectra (HRESIMS) were obtained on a quadrupole time-of-flight instrument (Q-Star-TOF) in the electrospray-ionization mode. High-resolution DART mass spectra (HRESIMS–DART) were obtained on a JEOL AccuTOF-DART workstation with an ESI source. All solvents were dried and distilled by standard methods unless otherwise stated.

*Allyl 2-acetamido-2-deoxy-β-*D*-glucopyranoside (2)*

To a solution of **1** (14.6 g, 37.7 mmol) in dry CH_2Cl_2 (60 mL) and dry MeOH (300 mL) was added NaOMe (25% in MeOH, 0.25 mL). The reaction was stirred at r.t. for 2 h, after which time the reaction was neutralized with Dowex 50 x 2-100 (H^+ form). The suspension was filtered and evaporated to afford triol **2** as a white solid (10.3 g, quant.), which was used in the next step without further purification. ^1H NMR (300 MHz, CD_3OD): δ 5.79 (m, 1H), 5.17 (d, J = 17.4 Hz, 1H), 5.04 (d, J = 10.2 Hz, 1H), 4.34 (d, J = 8.4 Hz, 1H), 4.24 (dd, J = 13.2 Hz, J = 4.8 Hz, 1H), 3.98 (dd, J = 13.5 Hz, J = 6.0 Hz, 1H), 3.79 (dd, J = 12.0 Hz, J = 2.1 Hz, 1H), 3.61-3.55 (m, 2H), 3.36 (t, J = 10.2 Hz, 1H), 1.90 (s, 3H). The NMR data matched those reported in the literature [23].

*Allyl 2-acetamido-2-deoxy-4,6-O-*p*-methoxybenzylidene-β-*D*-glucopyranoside (3)*

A mixture of triol **2** (26 g, 100 mmol), ADMA (80 mL, 470 mmol), TsOH (200 mg, 1.05 mmol) and DMF (100 mL) was stirred at r.t. for 24 h, after which time 1 mL of TEA was added, and the solution was poured into a well-stirred mixture of water (1.5 L) and hexane (300 mL). The precipitate was collected and washed several times with water and hexane to give **3** (34.9 g, 92%) as a white solid. ^1H NMR (300 MHz, DMSO-d_6): δ 7.82 (d, J = 8.7 Hz, 1H), 7.35 (d, J = 9.0 Hz, 2H), 6.91 (d, J = 8.4 Hz, 2H), 5.82 (m, 1H), 5.53 (s, 1H), 5.27 (d, J = 4.5 Hz, 1H), 5.23 (d, J = 17.1 Hz, 1H), 5.11 (d, J = 10.5 Hz, 1H), 4.48 (d, J = 7.8 Hz, 1H), 4.22-4.14 (m, 2H), 3.99 (dd, J = 13.5 Hz, J = 5.7 Hz, 2H), 3.74 (s, 3H), 3.69 (t, J = 9.9 Hz, 1H), 3.59-3.51 (m, 2H), 1.80 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 169.13, 159.54, 134.61, 130.13, 127.69, 116.17, 113.32, 101.09, 100.63, 81.24, 70.37, 68.96, 67.83, 65.99, 56.11, 55.12, 23.08. HRESIMS: m/z $[M + H]^+$ calcd for $C_{19}H_{26}NO_7$: 380.1709; found: 380.1710.

Allyl 2-acetamido-2-deoxy-3-O-levulinoyl-4,6-O-p-methoxybenzylidene-β-D-glucopyranoside (4)

To a suspension of **3** (8.0 g, 21 mmol) in dry CH_2Cl_2 (25 mL) were added levulinic acid (3.66 g, 31.7 mmol), EDCI (5.28 g, 27.5 mmol), and a catalytic amount of DMAP. The reaction was stirred at r.t. overnight, and then it was quenched with water and extracted with CH_2Cl_2. The organic phase was dried with anhyd $MgSO_4$ and evaporated to dryness. The residue was submitted to silica gel column chromatography to give compound **4** as a white solid (7.5 g, 74%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.37 (d, *J* = 8.4 Hz, 2H), 6.86 (d, *J* = 8.7 Hz, 2H), 6.03 (d, *J* = 9.0 Hz, 1H), 5.84 (m, 1H), 5.45 (s, 1H), 5.37 (t, *J* = 9.6 Hz, 1H), 5.25 (d, *J* = 17.4 Hz, 1H), 5.17 (d, *J* = 10.5 Hz, 1H), 4.68 (d, *J* = 8.7 Hz, 1H), 4.28 (t, *J* = 10.8 Hz, 1H), 4.26 (t, *J* = 9.6 Hz, 1H), 3.98 (dd, *J* = 12.6 Hz, *J* = 6.0 Hz, 1H), 3.91 (t, *J* = 10.2 Hz, 1H), 3.78 (s, 3H), 3.76 (t, *J* = 10.2 Hz, 1H), 3.66 (t, *J* = 9.3 Hz, 1H), 3.54 (m, 1H), 2.74 (m, 2H), 2.57 (m, 2H), 2.14 (s, 3H), 1.97 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 206.36, 172.75, 170.65, 160.03, 133.62, 129.48, 127.42, 117.33, 113.49, 101.23, 100.71, 78.73, 71.78, 70.16, 68.54, 66.06, 55.20, 55.01, 37.91, 29.64, 28.06, 23.26. HRESIMS–DART: *m/z* $[M + H]^+$ calcd for $C_{24}H_{32}NO_9$: 478.2077; found: 478.2067.

Allyl 2-acetamido-4,6-di-O-acetyl-2-deoxy-3-O-levulinoyl-β-D-glucopyranoside (5)

To a solution of **4** (621 mg, 1.30 mmol) in MeOH (25 mL) and CH_2Cl_2 (5 mL) were added two drops of HBF_4. The reaction was stirred at r.t. for 2 h. When TLC showed complete consumption of the starting material and formation of a new spot, the reaction was quenched with TEA and evaporated to dryness. The resulting diol was re-dissolved in dry pyridine (5 mL) and treated with Ac_2O (5 mL) at r.t. for 10 h, after which time the reaction mixture was diluted with CH_2Cl_2 and poured into ice water. The mixture was then extracted with 3 × 10 mL of CH_2Cl_2, and the combined organic phase was washed successively with dilute aq HCl and satd aq $NaHCO_3$. The organic phase was dried with anhyd $MgSO_4$ and evaporated to dryness. The residue was submitted to silica gel column chromatography to give compound **5** as a white solid (495 mg, 89%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.87 (m, 1H), 5.74 (d, *J* = 8.7 Hz, 1H), 5.37 (t, *J* = 9.3 Hz, 1H), 5.28 (d, *J* = 15.9 Hz, 1H), 5.20 (d, *J* = 9.0 Hz, 1H), 5.07 (t, *J* = 9.9 Hz, 1H), 4.86 (d, *J* = 8.4 Hz, 1H), 4.34 (dd, *J* = 12.9 Hz, *J* = 5.1 Hz, 1H), 4.27 (dd, *J* = 12.0 Hz, *J* = 4.5 Hz, 1H), 4.13 (dd, *J* = 12.3 Hz, *J* = 2.4 Hz, 1H), 4.09 (t, *J* = 6.3 Hz, 1H), 3.76-3.68 (m, 2H), 2.77-2.72 (m, 2H), 2.51-2.45 (m, 2H), 2.16 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 1.95 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 206.62, 172.31, 170.65, 169.67, 137.95, 133.54, 117.85, 99.42, 72.18, 71.72, 70.08, 68.30, 62.11, 55.35, 37.80, 29.61, 28.00, 23.35, 20.77, 20.70. HRESIMS–DART: *m/z* $[M + H]^+$ calcd for $C_{20}H_{30}NO_{10}$: 444.1864; found: 444.1893.

Allyl 2-acetamido-4,6-di-O-acetyl-2-deoxy-β-D-glucopyranoside (6)

To a solution of **5** (559 mg, 1.26 mmol) in 95% EtOH (45 mL) and toluene (15 mL) was added hydrazine acetate (110 mg, 1.22 mmol). The reaction was stirred at r.t. for overnight, after which time it was concentrated, and the residue was submitted to silica gel column chromatography to give compound **6** as a white solid (273 mg, 63%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.88 (m, 1H), 5.76 (d, *J* = 5.1 Hz, 1H), 5.28 (d, *J* = 15.9 Hz, 1H), 5.23 (d, *J* = 10.5 Hz, 1H), 4.92 (t, *J* = 9.0 Hz, 1H), 4.62 (d, *J* = 8.4 Hz, 1H), 4.36 (dd, *J* = 12.2 Hz, *J* = 5.1 Hz, 1H), 4.29 (dd, *J* = 12.3 Hz, *J* = 4.8 Hz, 1H), 4.12 (d, *J* = 12.2 Hz, 1H), 4.08 (t, *J* = 6.3 Hz, 1H), 3.95 (t, *J* = 9.0 Hz, 1H), 3.62 (m, 1H), 3.47 (m, 1H), 2.08 (s, 3H), 2.07 (s, 3H), 2.03 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.27, 170.78, 170.38, 133.25, 118.55, 98.97, 72.86, 72.00, 71.31, 70.03, 62.33, 58.77, 23.58, 20.90, 20.79. HRESIMS–DART: *m/z* $[M + H]^+$ calcd for $C_{15}H_{24}NO_8$: 346.1502; found: 346.1487.

Allyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl-(1→3)-2-acetamido-4,6-di-O-acetyl-2-deoxy-β-D-gluco-pyranoside (8)

To a stirred suspension of acceptor **6** (244 mg, 0.707 mmol), 2,3,4,6-tetra-*O*-acetyl-α-D-manno-pyranosyl trichloroacetimidate **7** (1.44 g, 2.93 mmol) and powdered 4 Å molecular sieves (400 mg, activated at 250 °C) in dry CH_2Cl_2 (10 mL) was added TMSOTf (20 μL) in CH_2Cl_2 (0.3 mL) solution at -30 ^{o}C. The reaction was stirred under N_2 for 1 h and warmed to r.t.. After 7 h the reaction was quenched with TEA, filtered through Celite, concentrated and purified by silica gel column chromatography to give **8** (191 mg, 40%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.22 (d, *J* = 8.4 Hz, 1H), 5.85 (m, 1H), 5.73 (d, *J* = 6.9 Hz, 1H), 5.43 (d, *J* = 2.4 Hz, 1H), 5.25 (d, *J* = 17.4 Hz, 1H), 5.17 (d, *J* = 7.2 Hz, 3H), 4.86 (t, *J* = 9.3 Hz, 1H), 4.53 (d, *J* = 2.4 Hz, 1H), 4.46 (t, *J* = 9.3 Hz, 1H), 4.30 (dd, *J* = 12.6 Hz, *J* = 5.1 Hz, 1H), 4.22 (t, *J* = 7.8 Hz, 1H), 4.19 (d, *J* = 7.8 Hz, 1H), 4.12-4.03 (m, 3H), 3.67-3.64 (m, 2H), 2.91 (dd, *J* = 15.6 Hz, *J* = 7.8 Hz, 1H), 2.13 (s, 3H), 2.05 (s, 12H), 2.02 (s, 3H), 1.96 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.05, 170.77, 170.55, 170.12, 169.89, 169.58, 169.43, 133.74, 125.04, 117.80, 98.16, 96.91, 71.72, 71.62, 71.41, 70.41, 69.80, 69.62, 65.33, 62.46, 62.25, 58.26, 25.83, 23.62, 21.04, 20.93, 20.76, 20.66, 20.62. HRESIMS: *m/z* $[M + Na]^+$ calcd for $C_{29}H_{41}NO_{17}Na$: 698.2267; found: 698.2311.

2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl-(1→3)-2-acetamido-4,6-di-O-acetyl-2-deoxy-α,β-D-glucopyranose (9)

To a solution of **8** (123 mg, 0.182 mmol) in dry MeOH (4 mL) and CH_2Cl_2 (4mL) was added a catalytic amount of $PdCl_2$ (10 mg). The reaction was stirred at r.t. for 7 h, after which time it was filtered through Celite, concentrated and purified by silica gel column chromatography to give compound **9** (82 mg, 71%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.03 (d, *J* = 8.4 Hz, 1H), 5.33 (d, *J* = 6.3 Hz, 1H), 5.28-5.20 (m, 2H), 5.13 (t, *J* = 9.6 Hz, 1H), 5.03 (dd, *J* = 11.1 Hz, *J* = 3.3 Hz, 1H), 4.94 (d, *J* = 1.8 Hz, 1H), 4.29-4.20 (m, 3H), 4.15-4.08 (m, 4H), 3.65 (m, 1H), 2.11 (s, 3H), 2.09 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.96 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.05, 170.99, 170.09, 170.01, 169.81, 169.71, 169.59, 98.90, 94.00, 71.96, 71.31, 70.40, 69.80, 69.53, 68.25, 66.25, 62.53, 62.39, 31.55, 25.22, 22.62, 20.90, 20.84, 20.78, 20.69, 20.59. HRESIMS: *m/z* $[M + H]^+$ calcd for $C_{26}H_{38}NO_{17}$: 636.2134; found: 636.2119.

α*-D-*Mannopyranosyl-(1→3)-2-acetamido-2-deoxy-α,β*-D-*glucopyranose (10)

To a solution of **9** (82 mg, 0.129 mmol) in dry CH_2Cl_2 (0.5 mL) and dry MeOH (2.5 mL) was added NaOMe (25% in MeOH, 4 drops). The reaction was stirred at r.t. for 4 h, after which time the reaction was neutralized with Dowex 50 x 2-100 (H^+ form). The suspension was filtered and evaporated to afford **10** (48 mg, quant.) as a white solid that was used in the next step without further purification. ^{1}H NMR (300 MHz, D_2O): δ 5.47 (d, *J* = 4.5 Hz, 1H), 5.01 (d, *J* = 1.8 Hz, 1H), 4.73 (d, *J* = 1.2 Hz, 1H), 3.76 (m, 2H), 3.72 (m, 1H), 3.68 (t, *J* = 3.0 Hz, 1H), 3.66 (d, *J* = 3.3 Hz, 1H), 3.62 (d, *J* = 3.9 Hz, 1H), 3.58 (dd, *J* = 6.3 Hz, *J* = 2.1 Hz, 1H), 3.55 (d, *J* = 2.7 Hz, 1H), 3.51 (t, *J* = 5.1 Hz, 1H), 3.46 (m, 1H), 3.40 (t, *J* = 9.6 Hz, 1H), 3.22 (m, 1H), 2.06 (s, 3H). ^{13}C NMR (75 MHz, D_2O): δ 134.58, 100.37, 94.18, 76.31, 73.19, 72.54, 71.36, 70.83, 70.37, 67.02, 66.99, 66.75, 61.11, 21.85. HRESIMS: *m/z* $[M + Na]^+$ calcd for $C_{14}H_{25}NO_{11}Na$: 406.1320; found: 406.1331.

α*-D-*Mannopyranosyl-(1→3)-2-amino-1,2-deoxy-1-(2-triphenylmethylthioeth-2-ylamino)*-D-*glucitol (12)

Free glycan **10** (28.0 mg, 73.1 μmol) and 2-(triphenylmethylthio)ethanamine (**11**) (47.0 mg, 146 mmol) were dissolved in a 5% solution of glacial HOAc in DMSO for a saccharide concentration of ~30 mM. Sodium cyanoborohydride (92.1 mg, 1.46 mmol) was added, and the reaction was stirred under N_2 at 65 °C for 4 h, after which time the mixture was diluted with water (5 mL) and loaded onto a Diaion HP-20 reversed-phase column (15 × 2.5 cm), and the unreacted saccharide was eluted with water. Subsequent methanol elution gave a mixture of the product and the unreacted amine. The methanol fractions were then concentrated and purified by silica gel column chromatography (3:2 CH_2Cl_2:MeOH–100% MeOH) to give **12** (40 mg, 85%) as a white solid. ^{1}H NMR (300 MHz, CD_3OD): δ 7.40 (m, 6H), 7.31 (m, 9H), 5.00 (d, *J* = 1.9 Hz, 1H), 3.95-3.47 (m, 12H), 2.90–2.32 (m, 6H), 1.91 (brs, 1H). ^{13}C NMR (300 MHz, CD_3OD): δ 144.85, 129.57, 127.86, 126.78, 102.61, 80.46, 74.26, 71.50, 71.28, 70.96, 70.80, 69.38, 67.45, 66.85, 63.87, 61.68, 50.78, 30.29. HRESIMS: Calcd for $C_{33}H_{44}N_2O_9S$ *m/z* 644.2762; $[M + H]^+$ *m/z* 645.2874 (calcd 645.2846).

α*-D-*Mannopyranosyl-(1→3)-2-amino-1,2-deoxy-1-(2-thioeth-2-ylamino)*-D-*glucitol (13)

To a solution of **12** (46 mg, 71 μmol) in CH_2Cl_2 (1 mL) and water (1 mL) were added triethylsilane (30 μL, 0.20 mmol) and TFA (3 mL). The two-phase system was vigorously stirred at r.t. for 40 min, after which time the mixture was evaporated to dryness. The residue was re-dissolved in water and submitted to a Diaion HP-20 reversed-phase gel column chromatography. Water elution and subsequent lyophilization gave **13** (26 mg, 92%) as a white solid. ^{1}H NMR (600 MHz, D_2O): δ 5.00 (d, *J* = 1.2 Hz, 1H), 4.02 (m, 2H), 3.85 (m, 1H), 3.82 (m, 1H), 3.78 (m, 3H), 3.71-3.74 (m, 5H), 3.51 (dd, *J* = 7.6 Hz, *J* = 9.9 Hz, 1H), 3.36 (dd, *J* = 2.8 Hz, *J* = 12.7 Hz, 1H), 3.27 (t, *J* = 6.4 Hz, 2H), 3.19 (dd, *J* = 10.5 Hz, *J* = 13.0 Hz, 1H), 2.84 (m, 2H). ^{13}C NMR (600 MHz, D_2O): δ 102.82, 80.97, 74.71, 71.48, 71.19, 70.80, 70.75, 67.71, 63.76, 61.94, 61.72, 50.23, 50.22, 39.38. HRESIMS: Calcd for $C_{14}H_{30}N_2O_9S$ *m/z* 402.1667; $[M + H]^+$ *m/z* 403.1759 (calcd 403.1745).

CONCLUSIONS

The synthesis of a mannose-capped disaccharide with a thiol spacer was carried out to provide a tethered sugar suitable for attaching to gold nanoparticles that would serve to mimic carbohydrate-involved cell-surface interactions. The molecule was synthesized *via* Schmidt glycosylation using a properly protected glycosyl donor and acceptor, followed by deprotection and thiol introduction using a robust reductive amination. After removal of the thiol protective group and the application of a facile purification protocol, the target disaccharide was produced in good yield.

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