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Synthesis of High-Mannose 1-Thio Glycans and Their Conjugation to Protein

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The oligosaccharides Man_4 and Man_5, substructures of the high-mannose glycans of HIV glycoprotein gp120, were synthesized with a terminal 1-thiomannopyranose residue. The anomeric thiol can be readily converted to an azidomethyl aglycone through reaction with dichloromethane and displacement with sodium azide. The resulting oligomannans were then conjugated to ubiquitin utilizing thiol alkylation or azide/alkyne reactive tethers of minimal length. By combining high efficiency conjugation reactions and a short tether, we sought to establish conjugation conditions that would permit high density clustering of oligomannans in conjugate vaccines that could produce antibodies able to bind gp120 and potentially neutralize virus. LC-UV-MS was used to separate, identify and quantify the ubiquitin glycoconjugates with differing degrees of oligomannan incorporation. Binding of the HIV protective monoclonal antibody 2G12 and concanavalin A to microtitre plates coated with glycoconjugates was measured by ELISA.

Introduction

The HIV-1 envelope glycoprotein gp120 is the primary focus of humoral responses against HIV-1 and is an important target for vaccine development against the virus. The surface of gp120 is heavily glycosylated, with approximately half its weight attributed to N-linked glycans.[1] These densely clustered glycans appear to play a protective role, shielding the protein surface of gp120 from immune recognition.[2, 3] The glycans themselves can also be immunological targets, as broadly neutralizing antibodies have been isolated from asymptomatic HIV-1 infected individuals that recognize carbohydrate epitopes, and the existence of these antibodies has been used by many as a rationale for the construction of glycoconjugate vaccines that mimic the glycan shield. Such broadly neutralizing antibodies include antibody (Ab) 2G12, which recognizes a unique cluster of high-mannose type oligosaccharides on gp120,[4-7] and the antibodies PG9 and PG16 and the PGT class of antibodies which recognize both glycan and protein elements of gp120.[8-11]

Synthetic glycoconjugate vaccines against HIV-1 have focused on clustering high mannose oligosaccharides that are recognized by Ab 2G12 onto various carrier scaffolds.[12-15] The oligomannosides utilized in these vaccines were the Man_9 and Man_4 substructures of $Man_9GlcNAc_2$ oligosaccharide (Figure 1), both of which have been implemented with or without the $GlcNAc_2$ chitobiose core. The conjugate vaccines reported to date have used a variety of linker chemistries to attach glycans to immunogenic carriers, either as prearranged clustered haptens or directly as univalent haptens. In nearly all of the above cited examples, the length of the linker that attaches glycan to immunogenic carrier ranged between 9 to greater than 20 atoms in length. Unfortunately these glycoconjugate vaccines did not induce protective antibodies against HIV-1. Accurate simulation of the crowded oligomannose glycan surface of gp120 is thought to be crucial to elicit protective anti-glycan antibodies by vaccination. Thus, appropriate glycan presentation, via choice of carrier, conjugation strategy, and glycan selection, may yield a more effective glycoconjugate vaccine. It seems reasonable to assume that the most relevant choice of glycan for use in an HIV-1 vaccine would be those found on the native virion.

Recent mass spectrometry analysis of the gp120 glycans isolated from HIV-1 pseudovirus and replication competent HIV-1 particles revealed a remarkably simple and conserved glycan profile compared to recombinant gp120 expressed in the same 293T cell line.[16-18] While recombinant gp120 displays over ~70% complex-type glycans,[16, 19-21] the glycans of gp120 derived from virions are predominantly of the high-mannose $Man_{5-9}GlcNAc_2$ glycan series. Glycans isolated from the envelope of various clades of infectious virus particles that were derived from peripheral blood mononuclear cells (PBMCs), the natural medium of HIV-1, displayed the same predominant $Man_{5-9}GlcNAc_2$ glycan profile. The apparent universal conservation and abundance of the high-mannose type glycans on virion-associated gp120 provides a prospective target for eliciting broadly neutralizing antibodies.

To achieve a high loading and dense packing of glycan on a carrier protein scaffold requires efficient, robust conjugation chemistry. Crosslinkers of moderate length are commonly preferred to alleviate steric hindrance at the glycan/carrier interface, but longer linkers add flexibility may impede adequate replication of the more rigid structural environment at the gp120 surface. Reducing the length of the tether could restrict the glycan flexibility which may better represent the naturally occurring glycans on gp120.

We set out to address the issue of maintaining conjugation efficiency as steric crowding increases during the course of glycoconjugate formation, while retaining structural rigidity through the use of a tethering group of the shortest possible length. To mimic the N-glycans of the crowded surface of gp120 we elected to synthesize the Man_5 epitope which appears with high frequency in clinical isolates,[16, 18] and the Man_4 epitope which is recognized by Ab 2G12.[22] To facilitate the conjugation of these oligosaccharides in high copy number to a carrier molecule, the Man_4 and Man_5

structures were synthesized as 1-thiopyranose derivatives. The nucleophilic sulfhydryl group is useful in multiple highly reactive conjugation reactions and it can also be readily converted to a short azidomethyl tether[23] for use in the copper(I)-catalyzed azide alkyne cycloaddition (CuAAC).

Here we describe the synthesis of the mannosyl thiols and their azidomethyl analogues and the corresponding thiol alkylation and CuAAC conjugations of oligosaccharide to ubiquitin. These methods are a prelude to high loading of the oligosaccharide on phage particles. Since phage particles permit conjugation of several thousand copies to a single virion, this may mimic the dense crowding of glycans found on gp120 and enable the use of such conjugates as a candidate HIV-1 vaccine.

Results and discussion

Retrosynthetic analysis

An anomeric thiol group was chosen to act as the shortest possible tether to conjugate Man_4 and Man_5 oligosaccharides to carrier molecules. Since thiols are highly reactive nucleophiles we anticipated that it would facilitate high efficiency conjugation under a variety of conditions and thereby achieve high density presentation of the target epitopes.

The synthesis of the tetra- and penta-mannopyranosyl thiol analogues **1** and **2** posed two synthetic challenges:

1. the installation and protection of the β-anomeric thiol
2. protection of a glycosyl acceptor for creating 3,6-branched mannosides.

Both the tetrasaccharide **1** and pentasaccharide **2** share common structural features that can be broken down into the monosaccharide building blocks **4** and **5**, and synthon **A** (Scheme 1). Well precedented chemistry has been reported for the synthesis of the glycosyl imidate **4**[24] and the orthogonally protected allyl mannoside **5**.[25, 26] These building blocks provide access to the trisaccharide donor **3** through the 3,6-deprotection of **5** followed by glycosylation with donor **4**. In addition, glycosyl donor **4** can be utilized to prepare α1,2-linked oligomannosides of the type found in tetrasaccharide **1**.

Synthesis of building blocks

For synthon A, we elected to introduce a protected anomeric thiol at the beginning of our synthetic route to **1** and **2**. An unsymmetrical *tert*-butyl disulfide appeared to be the protecting group most tolerant to protecting group manipulations and glycosylation conditions when evaluated with other anomeric thiol protecting groups.[27] Reaction of tetracetyl-1-*S*-acetyl-1-thio-β-D-mannopyranose **6**[28, 29] with diisopropyl-*N*-(*tert*-butylsulfanyl) hydrazodicarboxylate **7** gave **8** in 66% yield as a colourless syrup containing both β and α anomers in the ratio 22:1 (Scheme 2). Transesterification of **8** produced tetrol **9**, which was recrystallized as a white powder of anomers in the ratio 100:64 β/α. It was anticipated that separation of pure anomers would be most practical following selective protection.

Various protection strategies were explored to selectively protect the thiol mannopyranoses **9** for glycosylation at *O*-3 and *O*-6. Foremost amongst the approaches was the introduction of a trityl group at *O*-6 and a bulky silyl group at *O*-3 followed by benzoylation, as reported for allyl α-D-mannopyranoside **5**.[25] Application of this regioselective methodology to the anomeric mixture **9** afforded the 3,6-protected product **10** as an inseparable mixture of anomers (Scheme 2). Benzoylation of **10** produced the monobenzoate and dibenzoate **11** and **12**. The β-anomer of **10** was extremely resistant to 4-*O*-benzoylation, giving almost exclusively the monobenzoate product **10.** Only after 5 days at reflux with excess benzoyl chloride could appreciable amounts of the β-anomer of **12** be obtained. Since separation of these anomeric and incompletely benzoylated mixtures was impractical on a preparative scale, an alternate approach

was used. Reaction of *tert*-butylchlorodiphenylsilane with **8** gave an anomeric mixture of the 6-*O*-TBDPS ether **13** in 94% yield from which the pure β-anomer could be obtained in 71% yield by recrystallization (Scheme 2).

Conveniently, glycosylation of **13** by the donor **4** was selective for the formation of the *O*-3 linked disaccharide **14** with only trace amounts of *O*-2 or *O*-4 linked disaccharides (Scheme 3). Use of a 1.5-2 fold molar excess of donor **4** to drive the reaction to higher yields resulted in formation of glycosylated *N*-trichloroacetamide and limited the yield of **14** in the range 43-67%. A yield of 83% was eventually achieved through an inverse glycosylation procedure,[30] in which donor **4** was slowly added to a premixed solution of acceptor **13** and activator.

Disaccharide **14** is a convenient intermediate to access both tetrasaccharide **1** and pentasaccharide **2**. For use in the synthesis of tetrasaccharide **1**, the TBDPS group was first removed to give triol **15** followed by benzoylation to provide the protected disaccharide **16** (Scheme 3). The acetyl group was selectively removed using anhydrous HCl in methanol to give **17** in 78%. The reaction was carefully monitored and extended over 170 hours at -10°C to minimize loss of benzoates. For use in the synthesis of pentasaccharide **2**, disaccharide **14** was first perbenzoylated to give **18**. Removal of the silyl ether gave the selectively protected disaccharide alcohol **19**.

Assembly of tetrasaccharide 22

Glycosylation of acceptor **17** with donor **4** afforded trisaccharide **20** in high yield (Scheme 4). Following acid catalyzed transesterification, trisaccharide acceptor **21** was obtained in moderate yield together with quantitative recovery of unreacted trisaccharide **20**. The trisaccharide acceptor **21** was reacted with **4** to give the protected target tetrasaccharide **22** in 91% yield. The anomeric configuration of each newly introduced α-D-mannpyranosyl residue for the sequence **14**→**20**→**22** was unambiguously confirmed by heteronuclear $^{1}J_{C1,H1}$ coupling constants of 171 Hz.[31]

Assembly of Pentasaccharide 26

The pentasaccharide was assembled in a 3+2 fashion, requiring the synthesis of the branched trisaccharide imidate **3** for glycosylation to disaccharide **19**. The trisaccharide donor was prepared by selectively deprotecting **5**[25, 26] followed by glycosylation with donor **4** to afford trisaccharide **24**[26] in 93% yield (Scheme 5). The configuration of each newly formed α1,3 and α1,6 glycosidic linkages was confirmed by $^{1}J_{C1,H1}$ heteronuclear coupling constants of 171 and 175 Hz,[31] and the position of the linkages by the $^{3}J_{H1,C3}$ and $^{3}J_{H1,C6}$ correlations. Deallylation of **24** with $PdCl_2$ and NaOAc in aqueous acetic acid[32] produced hemiacetal **25** as an anomeric mixture along with a 2-oxopropyl side-product. The formation of a Wacker ketone byproduct during anomeric deallylation has been previously reported.[33-35] The hemiacetal and side-product were difficult to separate from each other by chromatography and the mixture was carried to the next step for separation. Reaction of **25** with trichloroacetonitrile furnished the trisaccharide imidate **3** in 52% yield from **24**.

Pentasaccharide **26** was obtained in 93% yield by glycosylation of **19** with **3**. The configuration of the newly formed α1,6-linkage was confirmed by the heteronuclear $^{1}J_{C1,H1}$ coupling constant of 175 Hz.[31]

Deprotection of tetrasaccharide 22 and pentasaccharide 26

Global deprotection of tetrasaccharide **22** and pentasaccharide **26** was achieved by first reducing the disulphide with trimethylphosphine (TMP) and then removing benzoates. This deprotection order allows for the chromatographic removal of excess reducing agent which was necessary to avoid the formation of a complex mixture of side products upon disulfide reduction.

Tetrasaccharide **22** was cleanly reduced to thiol **27** with TMP (Scheme 6). Subsequent transesterification was performed in deuterated solvents in order to monitor reaction progress by ^{1}H NMR. Anomerization was observed and

the formation of the unwanted α-anomer was found to be time rather than pH dependent, whereas deacylation was more dependent on pH than reaction time. Thus, the formation of the α-anomer could be kept to a minimum by increasing the amount of base. The 2-*O*-benzoate was extremely resistant to treatment with methoxide solution, even at 1 M concentrations. This benzoate was removed by saponification of mono-benzoylated intermediate with 1 M NaOD to produce the fully deprotected tetrasaccharide anomers in 70% yield as a 1:3 α/β anomeric mixture. The mixture could be cleanly separated via HPLC to produce tetrasaccharide **1** as the β-anomer, as verified by anomeric $^{1}J_{C1,H1}$ heteronuclear coupling constant of 153 Hz.[31]

Pentasaccharide **26** was deprotected in analogous fashion to tetrasaccharide **22** (Scheme 7). Treatment with TMP produced the thiol **28** in quantitative yield. Transesterification and saponification produced the fully deprotected pentasaccharide **2** in 63% yield with a 1:3 α/β ratio. The β-anomer of pentasaccharide **2** was obtained after HPLC and its anomeric configuration verified by a $^{1}J_{C1,H1}$ coupling constant of 158 Hz.[31]

Synthesis and deprotection of azidomethyl mannosides 33 and 34

Two additional reactive glycans were produced employing a convenient method to introduce a short azidomethyl tether.[23] The tetra- and pentasaccharides were converted into azidomethyl analogues through the reaction of **27** and **28** with dichloromethane in the presence of a hindered base to form the corresponding chloromethylthio glycosides **29** and **30** (Scheme 7). Displacement with sodium azide yielded the azidomethyl products **31** and **32**. Saponification yielded the deprotected tetra- and pentasaccharides **33** and **34**.

Glycoconjugation to ubiquitin

Ubiquitin is a non-glycosylated small molecular weight (8.5 kDa) protein with eight exposed amino groups available for conjugation. Glycoconjugates of ubiquitin were prepared by first functionalizing the amino groups by reaction with either *N*-succinimidyl iodoacetate **35** or *N*-succinimidyl 4-pentynoate **36** tethers (Scheme 8). In brief, 5 equivalents of activated tether per amine were reacted with ubiquitin in phosphate buffer, pH 8.3. After 3 hours, buffer and excess crosslinker were removed by size exclusion desalting chromatography.

The degree of tether conjugation was assessed by LC-UV-MS, where differentially conjugated ubiquitin species were separated by liquid chromatography (LC) and identified by mass spectroscopy (MS) (Figure 2). The identified conjugate species were quantified using PeakFit analysis of their UV absorbance at 210 nm. Using this method, an average labelling of 5.3 iodoacetyl and 6.1 pentynyl functionalities for **37** and **38** were observed. Approximately 71% of the iodoacetyl functionalized ubiquitin contained 1 or 2 internal crosslinks as indicated by the presence of mass+40 species, accounting for the lower linker loading of **37**.

Iodoacetyl functionalized ubiquitin **37** was then reacted with either the Man_4 or Man_5 1-thio-mannans **1** and **2** in phosphate buffer, pH 8.0 for 3 hours. Excess oligosaccharide was removed by size exclusion chromatography to obtain the desired glyconjugates **39** and **40**, with an average glycan loading of 5.3 Man_4 or Man_5 oligosaccharides per ubiquitin, as confirmed by LC-UV-MS (ESI). Similarly, the alkyne functionalized ubiquitin was reacted with the azidomethyl mannosides **33** and **34** under CuAAC conditions, to produce the **41** and **42** glycoconjugate products bearing an average of 4.9 Man_4 and 4.7 Man_5 oligisaccharides per ubiquitin.

ConA and Ab 2G12 binding to ubiquitin glycoconjugates

The ubiquitin glycoconjugates were analyzed by ELISA for their ability to bind concanavalin A (ConA), a lectin with specificity for terminal α-D-mannosyl groups,[36, 37] and Ab 2G12, which displays specificity for the Manα1→2Man-linked moiety.[7] The pentaaccharide glycoconjugates **40** and **42** exhibited higher relative avidity for

ConA than for its tetrasaccharide counterparts (Figure 3, left). This is reasonable since the pentasaccharide structure has three terminal α-D-mannose moieties compared to the one of the tetrasaccharide. There was no apparent preference in avidity with ConA between the ubiquitin conjugates with the iodoacetyl or alkynyl linkers despite differences in conjugation density. The choice of linker did not appear to affect the avidity of ConA to the hapten as indicated by the identical binding curves between the Man_4-bearing conjugates **39** and **41**, and the Man_5-bearing conjugates **40** and **42**.

Titration of Ab 2G12 against the ubiquitin glycoconjugates revealed a stark preference for the tetrasaccharide conjugates **39** and **41** over that of the pentasaccharide conjugates (Figure 3, right). This disparity can be attributed to differences in hapten structure, as the pentasaccharide does not contain any Manα1→2Man linkages. Interestingly, the binding preference of 2G12 is not equivalent for **39** and **41**, unlike with ConA. This may be due to differences in the linker structures, as the linker used in **41** is longer and more flexible, and contains a triazole moiety which could disfavorably orientate the glycan for 2G12 recognition. The glycan loading of ubiquitin may also be a factor as 2G12 is known to exhibit increased binding to glycans that are clustered.[22, 38] Thus, it could be expected that the more densely conjugated **39** would bind 2G12 with higher avidity. These observations in differences of avidity highlight the importance of correctly displaying glycans for immunological recognition.

Conclusions

Man_4 and Man_5 oligosaccharides terminated by a 1-thiomannopyranose residue are shown to be effective derivatives for efficient conjugation of iodoacetyl-functionalized proteins. The ready conversion of 1-thio derivatives to azidomethyl glycosides provides allows access to the highly effectual CuAAC click chemistry for conjugation. These conjugation strategies should facilitate the congested conjugation of oligomannose structures on phage particles with the objective of simulating the crowded glycan shield of gp120 for a potential HIV-1 vaccine. The conjugation to phage, characterization of the conjugates and their immunogenicity will be reported elsewhere.

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Experimental

General methods

All chemical reagents were of analytical grade and used as obtained from commercial sources unless otherwise specified. Solvents used for water sensitive reactions were distilled under an inert atmosphere. Unless otherwise noted, all reactions were carried out at room temperature and water sensitive reactions were performed under a positive pressure of argon. Solvents were removed under reduced pressure between 20-40 °C with a rotary evaporator unless otherwise indicated. Molecular sieves (4 Å) were stored in an oven at 500 °C and were cooled *in vacuo* prior to use. Analytical thin layer chromatography (TLC) was conducted on silica gel 60-F_{254} (Merck). TLC plates were visualized under UV light and an anisaldehyde stain composed of 4% H_2SO_4, 1.2% AcOH and 0.4% p-anisaldehyde in ethanol,

followed by heating. Amberlite® IR 120 resin (strongly acidic H^+ form) was used where H^+ resin is indicated. Medium pressure chromatography was conducted using silica gel (230-400 mesh, Silicycle, Montreal) or Fluoro*Flash*® silica gel (40 μm, Fluorous Technologies Incorporated) with flow rates between 5-10 mL/min. Following deprotection, final compounds were purified by HPLC conducted on a Waters Delta 600 system using a Waters 2996 Photodiode Array detector. Separations were performed on a TOSOH TSKgel Amide-80 column with a matched TSKgel Amide-80 column guard using a gradient of water and acetonitrile. NMR experiments were recorded on Varian INOVA 500 or 600 MHz spectrometers, or on Varian VNMRS 500 or 700 MHz spectrometers equipped with cryogenic probes. The 600 and 700 MHz spectrometers utilize inverse probes ($^1H\{^{13}C/^{15}N\}$) allowing for 1200:1 and 7000:1 ^1H sensitivity, respectively. ^{13}C NMR was recorded at approximately 125 MHz. ^1H and ^{13}C NMR chemical shifts, reported in δ (ppm), were referenced to internal residual protonated solvent signals or to external acetone (0.1% ext. acetone @ 2.225 ppm) in the case of D_2O. Electrospray ionization mass spectra were recorded on a Micromass Zabspec TOF mass spectrometer by the analytical services facility at the University of Alberta's Department of Chemistry. Optical rotations were determined with a Perkin-Elmer model 241 polarimeter at 22±2 °C using the sodium D-line and are reported in units of degree·mL·g^{-1}·dm^{-1}. Concentrations (c) are reported in mg/mL. Combustion analysis was performed by the analytical services facility at the University of Alberta's Department of Chemistry.

Procedure for ubiquitin conjugation

Linker addition: To a solution of ubiquitin (0.5 mg, 0.058 μmol) in sodium borate (0.5 mL, 50 mM, pH 8.3, 5mM EDTA) was added a solution of tether **35** (commercially available) or **36** (2.5 μmol in 30 μL DMSO, 85 mM). The reaction mixture was rotated at room temperature for 3 hours. The mixture was passed through a Zeba™ size exclusion column (7K MW cutoff) according to the manufacturer's guidelines. In brief, a 2.0 mL column was washed three times with water and 0.5 mL of protein solution loaded onto the resin. A stacker volume of 20 μL H_2O was added and the column centrifuged at 1,000 x g for 3.5 minutes. Approximately 98% of the linker addition products **37** and **38** were recovered, as determined by the UV absorption at 280 nm.

Thiol alkylation: To a solution of **37** (0.49 mg, 0.057 umol) in 0.55 mL H_2O was added 100 μL of 0.2 M sodium phosphate pH 8.0 (31 mM final conc.) and 1 mg of **1** (1.4 μmol) or **2** (1.2 μmol). The reaction mixture was rotated at room temperature for 3 hours and purified by size exclusion chromatography as previously described. Protein was quantified using a Pierce™ BCA Protein Assay Kit, yielding ~80% recovery of glycoconjugates **39** and **40**.

CuAAC: To a solution of **38** (0.27 mg, 0.032 μmol) in 0.3 mL H_2O was added 100 μL of 0.2 M sodium phosphate pH 7.2 (63 mM final conc.) and sparged with argon for 30 minutes. To this degassed solution was added 1 mg of **33** (1.3 μmol) or **34** (1.1 μmol), 2.5 μL of $CuSO_4$ (2.5 μL, 20 mM, 0.05 nmol), tris(3-hydroxypropyltriazolylmethyl)amine (THPTA)[39] (5 μL, 50 mM, in H_2O, 0.25 nmol), aminoguanidine hydrochloride (25 μL, 100 mM, in H_2O, 2.5 nmol), and sodium ascorbate (25 μL, 100 mM, in H_2O, 2.5 nmol). The reaction mixture was rotated at room temperature for 3 hours and purified by size exclusion chromatography as previously described. Protein was quantified using a Pierce™ BCA Protein Assay Kit, yielding 90% recovery of glycoconjugates **41** and **42**.

LC-UV-MS protocol

Reverse-phase HPLC-UV-MS was performed using an Agilent 1200 SL HPLC System with a ProSwift® RP-4H Analytical column (1×250 mm) (Dionex, USA), thermostated at 60°C, with a buffer gradient system composed of 0.1% formic acid in water as mobile phase A and 0.1% formic acid in acetonitrile as mobile phase B. Gradients were optimized separately for the linker labeled proteins and glycoconjugates.

An aliquot of 2 µL equivalent to an amount of 2 µg ubiquitin conjugate was loaded onto the column at a flow rate of 0.20 mL min^{-1} and an initial buffer composition of 95% mobile phase A and 5% mobile phase B. After injection, the column was washed using the initial loading conditions for 3 minutes to effectively remove salts. Elution of the protein-linker analytes was done by using a linear gradient from 5% to 15% mobile phase B over a period of 12 minutes, 15% to 50% mobile phase B over a period of 25 minutes and 50% to 85% mobile phase B over a period of 5 minutes. Elution of the protein-linker-oligosaccharide analytes was done by using a linear gradient from 5% to 18% mobile phase B over a period of 2 minutes, 18% to 38% mobile phase B over a period of 35 minutes and 38% to 85% mobile phase B over a period of 8 minutes.

UV absorbance was monitored at 210, 214, 254 and 280 nm. Mass spectra were acquired in positive mode of ionization using an Agilent 6220 Accurate-Mass TOF HPLC/MS system (Santa Clara, CA, USA) equipped with a dual sprayer electrospray ionization source with the second sprayer providing a reference mass solution. Mass spectrometric conditions were drying gas 9 L/min at 300°C, nebulizer 30 psi, mass range 100-3000 Da, acquisition rate of ~1.03 spectra/sec, fragmentor 175 V, skimmer 63 V, capillary 3200 V, instrument state 4 GHz High Resolution. Mass correction was performed for every individual spectrum using peaks at m/z 121.0509 and 922.0098 from the reference solution. Data acquisition was performed using the Mass Hunter software package (ver. B.02.01.) Analysis of the HPLC-UV-MS data was done using the Agilent Mass Hunter Qualitative Analysis software (ver. B.04.01).

Analysis of LV-UV-MS chromatograms

Peak regions within the TIC were extracted and deconvoluted using the Qualitative Analysis with BioConfirm software that is part of the Agilent MassHunter Workstation. The resulting peaks were matched with theoretical masses for each analyte. Miscellaneous peaks due to side reactions were also investigated. In-depth elution profiles for each analyte were then constructed using extracted-ion chromatograms (EICs) generated from the most intense isotope of the most intense charge state of each analyte. Since all other isotope peaks and other charge states were ignored, peak intensities and peak areas do not represent a real relative abundance of analytes in the sample, therefore quantitation was performed solely using the corresponding UV spectra. For illustrative purposes, signals for the least abundant analytes were magnified by 10-30%.

UV chromatograms were exported in excel format by use of the Agilent MassHunter Workstation before being imported into the PeakFit software (Version 4.12; SeaSolve Software, San Jose, USA) for deconvolution and processing. Peak simulations were obtained by use of the 4-parameter exponentially modified Gaussian function of the PeakFit software. Peaks were then manually adjusted to include additional peaks in areas of significant overlap as identified in the EIC elution profiles. Fitting was iterated until the correlation reached a stable maximum value. The integration results were used to determine the relative abundance of differentially conjugated ubiquitin.

Procedure for ELISA analysis

Maxisorp 96-well plates (Nunc) were incubated overnight at 4 °C with 10 µg per well of ubiquitin or ubiquitin conjugate in PBS. The plates were washed (Molecular Devices Skan Washer 400) 5 times with PBS containing 0.05% Tween-20 (PBST). A 1% solution of BSA in PBS was used to block plates by incubating for 30 m at room temperature. The plates were then washed 5 times with PBST. 2G12 antibody (produced in CHO cells, NIH AIDS Reagent Program Cat No: 1476) or biotinylated ConA were then titrated against ubiquitin or ubiquitin conjugates using $\sqrt{10}$ serial dilutions. After 2 h incubation at room temperature, the plate was washed 5 times with PBST and 100 µL per well of goat anti-human IgG conjugated to horseradish peroxidase (HRP) (Kirkegaard & Perry Laboratories, 1:5000 dilution in PBST) for 2G12 or streptavidin-HRP conjugate diluted 1:20,000 for biotinylated ConA added. After 40 min

incubation, plates were washed 5 times with PBST and developed for 15 minutes at room temperature with 100 µL per well of a 1:1 mixture of 3,3',5,5'-tetramethylbenzidine (0.4 g/L) and 0.002% H_2O_2 solution (Kirkegaard & Perry Laboratories). Reaction was quenched by addition 100 µL of 1 M phosphoric acid per well. Absorbance was read at 450 nm (Molecular Devices Spectra Max 190 plate reader).

Experimental procedures

α-D-Mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-1-thio-β-D-mannopyranose 1. Tetrasaccharide **27** (13.2 mg, 6.7 µmol) was dissolved in deuterated sodium hydroxide (1 mL, 0.5 M) and the reaction monitored by ^{1}H NMR. After 2 hours, the reaction was neutralized with H^+ resin, filtered, and concentrated under reduced pressure. The reaction was dissolved in H_2O and lyophilized to remove methyl benzoate. The crude powder was dissolved in deuterated sodium hydroxide (1 mL, 1 M) to remove the tenacious 2-*O*-Bz. After 2 hours, the reaction was neutralized with H^+ resin, filtered and lyophilized. Purification of the crude yellow product via HPLC to yield **1** (3.2 mg, 4.7 µmol, 70%) as a white powder.

^{1}H NMR (700 MHz, D_2O): □ 5.34 (br. s., 1H, H-1), 5.30 (s, 1H, H-1), 5.05 (s, 1H, H-1), 4.99 (s, 1H, H-1^a), 4.11 (br. s., 1H, H-2), 4.08 (br. s., 1H, H-2), 4.07 (br. s., 1H, H-2), 3.26-4.04 (m, 20H), 3.37-3.44 (m, 1H, H-5^a); Coupled HSQC (700 MHz, D_2O): □103.1/5.05 ($J_{C1/H1}$ 172 Hz), 101.7/5.30 ($J_{C1/H1}$ 173 Hz), 101.5/5.33 ($J_{C1/H1}$ 174 Hz), 82.5/5.00 ($J_{C1^a/H1^a}$ 153 Hz); Anal. calc for $C_{24}H_{42}O_{20}S$: HR ESIMS $[M+Na]^+$: 705.1882, found: 705.1881.

α-D-mannopyranosyl-(1→6)-[α-D-mannopyranosyl-(1→3)]-α-D-mannopyranosyl-(1→6)-[α-D-mannopyranosyl-(1→3)]-1-thio-β-D-mannopyranoside 2. Pentasaccharide **28** (54.6 mg, 22.6 µmol) was dissolved in deuterated sodium hydroxide (1 mL, 0.5 M) and the reaction monitored by ^{1}H NMR. After 2 hours, the reaction was neutralized with H^+ resin, filtered, and concentrated under reduced pressure. The reaction was dissolved in H_2O and lyophilized to remove methyl benzoate. The crude powder was dissolved in deuterated sodium hydroxide (1 mL, 1M) to remove the tenacious 2-*O*-Bz. After 2 hours, the reaction was neutralized with H^+ resin, filtered and lyophilized. Purification of the crude yellow product via HPLC to yield **2** (12.1 mg, 14.2 µmol, 63%) as a white powder.

^{1}H NMR (700 MHz, D_2O): δ 5.15 (s, 1H, H-1), 5.10 (s, 1H, H-1), 5.00 (s, 1H, H-1^a), 4.92 (d, 1H, $J_{1,2}$ 1.5 Hz, H-1), 4.87 (d, 1H, $J_{1,2}$ 1.5 Hz, H-1), 4.16 (dd, 1H, $J_{2,3}$ 3.2 Hz, $J_{1,2}$ 1.8 Hz, H-2), 3.64-4.10 (m, 29H); Coupled HSQC (700 MHz, D_2O): □103.3/5.10 ($J_{C1/H1}$ 172 Hz), 103.0/5.15 ($J_{C1/H1}$ 171 Hz), 100.4/4.87 ($J_{C1/H1}$ 172 Hz), 101.1/4.92 ($J_{C1/H1}$ 172 Hz), 92.1/5.00 ($J_{C1a/H1a}$ 158 Hz); Anal. calc for $C_{30}H_{52}O_{25}S$: HR ESIMS $[M+Na]^+$: 867.2411, found: 867.2411.

2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-[2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)]-2,4-di-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate 3. Trisaccharide **24** (0.73 g, 0.5 mmol) was dissolved in a solution of NaOAc (0.5 g, 6 mmol) in AcOH (6.65 mL) and H_2O (0.35 mL). $PdCl_2$ (0.54 g, 3.0 mmol) was added and the reaction mixture stirred for 10 hours. Palladium black precipitated from the solution over the course of the reaction. The reaction mixture was poured into saturated $NaHCO_3$ and the aqueous layer was extracted with EtOAc three times. The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. Purification of the crude product by chromatography (8.5:1.5 toluene/EtOAc) yielded trisaccharide **25** as a white powder that was inseparable from a trisaccharide byproduct. Trisaccharide **25** (~0.60 g), was dissolved in a solution of trichloroacetonitrile (0.5 mL, 5.0 mmol) in CH_2Cl_2 (3 mL) and a drop of DBU added. After 24 hours, the reaction mixture was diluted with toluene and concentrated under reduced pressure. Purification of the crude syrup by chromatography (8:2 toluene/EtOAc) yielded **3** (0.41 g, 0.26 mmol, 52% from 23) as a white foam.

$[\alpha]_D^{25}$ +6 (*c* 8.1, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.00 (s, 1H, NH), 7.27-8.28 (m, 40H, ArH), 6.56 (d, 1H, $J_{1,2}$ 1.4 Hz, H-1^a), 5.95 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 10.1 Hz, H-4^a), 5.90 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 10.1 Hz H-4^b), 5.87 (dd, 1H, $J_{2,3}$ 3.3 Hz, $J_{1,2}$ 2.0 Hz, H-2^a), 5.84 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.8 Hz, H-4^c), 5.81 (dd, 1H, $J_{3,4}$ 10.0 Hz, $J_{2,3}$ 3.3 Hz, H-3^b), 5.59 (dd, 1H,

$J_{3,4}$ 9.8 Hz, $J_{2,3}$ 3.3 Hz, H-3^{c}), 5.46 (dd, 1H, $J_{2,3}$ 3.2 Hz, $J_{1,2}$ 1.8 Hz, H-2^{b}), 5.24 (d, 1H, $J_{2,3}$ 1.7 Hz, H-1^{c}), 5.17 (dd, 1H, $J_{2,3}$ 2.9 Hz, $J_{1,2}$ 2.3 Hz, H-2^{c}), 4.95 (d, 1H, $J_{1,2}$ 1.4 Hz, H-1^{b}), 4.65 (dd, 1H, $J_{3,4}$ 9.7 Hz, $J_{2,3}$ 3.4 Hz, H-3^{a}), 4.42-4.50 (m, 5H, H-5^{a}, H-5^{b}, H-5^{c}, H-6^{b}, H-6^{c}), 4.38 (dd, 1H, J_{gem} 12.2 Hz, $J_{5,6}$ 3.2 Hz, H-6^{b}), 4.36 (dd, 1H, J_{gem} 12.4 Hz, $J_{5,6}$ 5.2 Hz, H-6^{c}), 4.09 (dd, 1H, J_{gem} 10.9 Hz, $J_{5,6}$ 6.2 Hz, H-6^{a}), 3.78 (dd, 1H, J_{gem} 10.9 Hz, $J_{5,6}$ 1.9 Hz, H-6^{a}), 2.10 (s, 3H, CH_3(C=O)), 1.91 (s, 3H, CH_3(C=O)); ^{13}C NMR (151 MHz, $CDCl_3$): δ 169.7 (C=O), 169.0 (C=O), 166.0 (2×C=O), 165.9 (C=O), 165.6 (C=O), 165.4 (C=O), 165.2 (C=O), 165.0 (C=O), 164.7 (C=O), 159.5 (C=NH), 133.8 (Ar), 133.6 (Ar), 133.3 (Ar), 133.3 (Ar), 133.1 (Ar), 133.0 (Ar), 133.0 (Ar), 132.9 (Ar), 130.2 (Ar), 130.0 (Ar), 129.9 (Ar), 129.8 (Ar), 129.8 (Ar), 129.7 (Ar), 129.6 (Ar), 129.6 (Ar), 129.6 (Ar), 129.4 (Ar), 129.3 (Ar), 129.2 (Ar), 128.9 (Ar), 128.9 (Ar), 128.8 (Ar), 128.7 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.3 (Ar), 128.3 (Ar), 128.3 (Ar), 128.2 (Ar), 99.7 (C-1^{b}), 97.2 (C-1^{c}), 94.3 (C-1^{a}), 90.7 (CCl_3), 76.1 (C-3^{a}), 72.2 (C-2^{a}), 70.5, 69.8, 69.8, 69.7, 69.6, 69.1, 68.7, 67.8 (C-2^{b}, C-2^{c}, C-3^{b}, C-3^{c}, C-4^{a}, C-5^{a}, C-5^{b}, C-5^{c}), 66.9, 66.6, 66.3 (C-4^{b}, C-4^{c}, C-6^{a}), 63.0, 62.7 (C-6^{b}, C-6^{c}), 20.7 (CH$_3$(C=O)), 20.4 (CH$_3$(C=O)); Anal. calc for $C_{80}H_{68}O_{26}NCl_3$: HR ESIMS [M+Na]$^{+}$: 1586.2987, found: 1586.3012; Elem. Anal: C, 61.37; H, 4.38; N, 0.89; found: C, 61.19; H, 4.54; N, 1.05.

Diisopropyl-*N*-(*tert*-butylsulfanyl)hydrazodicarboxylate 7. To a solution of 2-methyl-2-propanethiol (5.5 mL, 48.8 mmol) in CH_2Cl_2 (150 mL) was added diisopropyl azodicarboxylate (10.2 mL, 49.2 mmol) dropwise. The reaction orange mixture gradually turned yellow over the course of the reaction. After 1 week, the reaction mixture was concentrated under reduced pressure and the yellow syrup purified by chromatography (8:2 hexanes/EtOAc) to yield **15** (13.6 g, 46.3 mmol, 94%) as a colorless syrup.

$[\alpha]_D^{25}$ -0.1 (*c* 16.0 in $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$): δ 7.19-7.35 (br. d., 1H, NH), 4.90 (spt, 1H, *J* 6.2 Hz, C*H*(CH$_3$)$_2$), 4.86 (spt, 1H, *J* 6.1 Hz, C*H*(CH$_3$)$_2$), 1.27 (br. s., 9H, C(C*H*$_3$)$_3$), 1.21 (d, 3H, *J* 6.4 Hz, C(C*H*$_3$)$_2$), 1.19 (d, 3H, *J* 6.3 Hz, C(C*H*$_3$)$_2$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 157.3 (C=O), 155.3 (C=O), 72.2 (O*C*H(CH$_3$)$_2$), 70.0 (O*C*H(CH$_3$)$_2$), 49.9 (S*C*(CH$_3$)$_3$), 28.8 (SC(*C*H$_3$)$_3$), 22.0 (OCH(*C*H$_3$)$_2$), 21.8 (OCH(*C*H$_3$)$_2$); Anal. calc for $C_{12}H_{24}N_2O_4S$: HR ESIMS [M+Na]$^{+}$: 315.1349, found: 315.1349; Elem. Anal: C, 49.29; H, 8.27; N, 9.58; S, 10.97; found: C, 48.69; H, 7.61; N, 9.21; S, 10.56.

Mecapto-*tert*-butyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-mannopyranoside 8. Monosaccharide **6**[28, 29] (17.9 g, 44.1 mmol) was added to a solution of **7** (13.6 g, 46.3 mmol) in CH_2Cl_2 (300 mL) and cooled to 0 °C. Diethylamine (4.8 mL, 46 mmol) was added to the reaction mixture. After 96 hours, reaction was diluted with CH_2Cl_2 and washed with sat. NH_4Cl, sat. $NaHCO_3$, H_2O, and brine. The organic was dried over Na_2SO_4 and concentrated under reduced pressure. Purification of the crude product required two rounds of chromatographic purification (6:4 hexanes EtOAc, then 9:1 CH_2Cl_2/EtOAc). The bulk of the diisopropyl hydrazodicarboxylate byproduct can be removed through recrystallization (hexanes/EtOAc), yielding **8** (13.1 g, 29.0 mmol, 66%) as a colorless syrup in a 1:22 α/β ratio, containing trace diisopropyl hydrazodicarboxylate.

$[\alpha]_D^{25}$ +31 (*c* 24.3, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$): δ 5.62 (br. d, 1H, $J_{2,3}$ 2.6 Hz, H-2), 5.20 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 10.0 Hz, H-4), 5.03 (dd, 1H, $J_{3,4}$ 10.1 Hz, $J_{2,3}$ 3.5 Hz, H-3), 4.65 (d, 1H, $J_{1,2}$ 0.9 Hz, H-1), 4.25 (dd, 1H, J_{gem} 12.3 Hz, $J_{5,6}$ 6.1 Hz, H-6), 4.14 (dd, 1H, J_{gem} 12.3 Hz, $J_{5,6}$ 2.3 Hz, H-6), 3.67 (ddd, 1H, $J_{4,5}$ 9.9 Hz, $J_{5,6}$ 6.2 Hz, $J_{5,6}$ 2.3 Hz, H-5), 2.19 (s, 3H, CH_3(C=O)), 2.07 (s, 3H, CH_3(C=O)), 2.03 (s, 3H, CH_3(C=O)), 1.97 (s, 3H, CH_3(C=O)), 1.34 (s, 9H, C(CH$_3$)$_3$); ^{13}C NMR (176 MHz, $CDCl_3$): δ 170.6 (C=O), 170.0 (C=O), 170.0 (C=O), 169.6 (C=O), 92.1 (C-1), 76.9 (C-5), 71.7 (C-3), 70.5 (C-2), 65.6 (C-4), 62.8 (C-6), 47.8 (*C*(CH$_3$)$_3$), 29.9 (C(*C*H$_3$)$_3$), 20.7 (CH$_3$(C=O)), 20.7 (CH$_3$(C=O)), 20.6 (CH$_3$(C=O)), 20.5 (CH$_3$(C=O)); Anal. calc for $C_{18}H_{28}O_9S_2$: HR ESIMS [M+Na]$^{+}$: 475.1067, found: 475.1065; Elem. Anal: C, 47.77; H, 6.24; S, 14.17; found: C, 47.98; H, 6.21; S, 13.80.

Mecapto-*tert*-butyl 1-thio-D-mannopyranoside 9. Monosaccharide **8** (10.68 g, 23.6 mmol) was suspended in methanol (110 mL) and 1 M sodium methoxide (0.2 mL) was added. After 4 hours, reaction was neutralized with H^+ resin, filtered, and concentrated under reduced pressure. Purification of the crude product by recrystallization (hexanes/EtOAc) yielded **9** (6.13 g, 21.5 mmol, 91%) in a 64:100 α/β mixture as a white powder.

β-anomer: ^{1}H NMR (600 MHz, CD_3OD): δ 4.59 (d, 1H, $J_{1,2}$ 1.1 Hz, H-1), 4.05 (dd, 1H, $J_{2,3}$ 3.4 Hz, $J_{1,2}$ 1.0 Hz, H-2), 3.85 (dd, 1H, J_{gem} 11.9 Hz, $J_{5,6}$ 2.4 Hz, H-6), 3.74 (dd, 1H, J_{gem} 11.7 Hz, $J_{5,6}$ 5.5 Hz, H-6), 3.61 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.5 Hz, H-4), 3.45 (dd, 1H, $J_{3,4}$ 9.5, $J_{2,3}$ 3.3 Hz, H-3), 3.22 (ddd, 1H, $J_{4,5}$ 9.7 Hz, $J_{5,6}$ 5.1 Hz, $J_{5,6}$ 2.4 Hz, H-5), 1.36 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (126 MHz, CD_3OD): δ 94.4 (C-1), 81.4 (H-5), 74.7 (C-3), 72.4 (C-2), 66.5 (C-4), 61.3 (C-6), 46.6 (S*C*(CH$_3$)$_3$), 28.9 (SC(*C*H$_3$)$_3$).

α-anomer: ^{1}H NMR (600 MHz, CD_3OD): δ 5.10 (d, 1H, $J_{1,2}$ 1.8 Hz, H-1), 4.10 (dd, 1H, $J_{2,3}$ 3.1 Hz, $J_{1,2}$ 1.8 Hz, H-2), 3.80-3.83 (m, 1H, H-6), 3.75-3.79 (m, 1H, H-6), 3.73-3.76 (m, 1H, H-5), 3.72 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.7 Hz, H-4), 3.66 (m, 1H, H-3), 1.36 (s, 9H, C(CH_3)$_3$); Anal. calc for $C_{10}H_{20}O_5S_2$: HR ESIMS $[M+Na]^+$: 307.0644, found: 307.0641; Elem. Anal: C, 42.23; H, 7.09; S, 22.55; found: C, 42.07; H, 7.05; S, 22.54.

Mecapto-*tert*-butyl 3-*O*-thexyldimethylsilyl-1-thio-6-*O*-triphenylmethyl-β-D-mannopyranoside 10. Tetrol **9** (0.34 g, 1.2 mmol) and TrCl (0.32 g, 1.5 mmol) were dissolved in pyridine (10 mL) and stirred at room temperature. After 68 hours, solid $NaHCO_3$ was added and the reaction stirred until bubbling ceased. The reaction mixture was diluted with EtOAc and washed with 1 M $NaHCO_3$, H_2O, and brine. The organic was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was dissolved in a solution of imidazole (38 mg, 0.56 mmol) and DMF (3 mL). A solution of TDSCl (64 μL, 0.33 mmol) in DMF (1 mL) was added dropwise to the reaction mixture over 1 hour. After 24 hours, the reaction was diluted with EtOAc and washed with 1 M $NaHCO_3$, H_2O, and brine. The organic was dried over Na_2SO_4 and concentrated under reduced pressure. Purification of the crude yellow product by chromatography (7:3 hexanes/EtOAc) yielded **10** (0.17 g, 0.26 mmol, 92%) in a 64:100 α/β mixture as a white powder.

β-anomer: R_f 0.53 (8:2 hexanes/EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.43-7.47 (m, 6H, ArH), 7.28-7.33 (m, 6H, ArH), 7.22-7.26 (m, 3H, ArH), 4.52 (dd, 1H, $J_{1,2} \approx {}^4J_{1,OH}$ 1.5 Hz, H-1), 4.03 (ddd, 1H, $J_{2,3}$ 3.6 Hz, $J_{1,2}$ 1.6 Hz, $J_{2,OH}$ 1.5 Hz, H-2), 3.66 (ddd, 1H, $J_{3,4} \approx J_{4,5}$ 9.2 Hz, $J_{4,OH}$ 2.6 Hz, H-4), 3.57 (dd, 1H, $J_{3,4}$ 8.7 Hz, $J_{2,3}$ 3.6 Hz, H-3), 3.41-3.44 (m, 2H, H-6), 3.37 (m, 1H, H-5), 2.60 (dd, 1H, ${}^4J_{1,OH} \approx J_{2,OH}$ 1.6 Hz, 2-OH), 2.30 (d, 1H, $J_{4,OH}$ 2.7, 4-OH), 1.64 (spt., 1H, J 7.1 Hz, C*H*(CH$_3$)$_2$), 1.35 (s, 9H, C(CH$_3$)$_3$), 0.90 (d, 3H, J 6.8, CH(CH_3)$_2$), 0.89 (d, 3H, J 6.8 Hz, CH(CH_3)$_3$), 0.86 (s, 6H, C(CH$_3$)$_2$), 0.18 (s, 3H, SiCH$_3$), 0.17 (s, 3H, SiCH$_3$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 146.9 (Ar), 143.7 (Ar), 143.6 (Ar), 128.7 (Ar), 127.9 (Ar), 127.3 (Ar), 127.1 (Ar), 93.5 (C-1), 82.0 (*C*Ph$_3$), 78.6 (C-5), 76.0 (C-2), 72.7 (C-3), 69.6 (C-4), 64.9 (C-6), 47.5 (S*C*(CH$_3$)$_3$), 34.2 (*C*H(CH$_3$)$_2$), 30.1 (C(*C*H$_3$)$_3$), 25.0 (Si*C*(CH$_3$)$_2$), 20.5 (CH(*C*H$_3$)$_2$), 20.1 (CH(*C*H$_3$)$_2$), 18.7 (SiC(*C*H$_3$)$_2$), 18.5 (SiC(*C*H$_3$)$_2$), -2.5 (SiCH$_3$), -2.7 (SiCH$_3$); Anal. calc for $C_{37}H_{52}O_5S_2Si$: HR ESIMS $[M+Na]^+$: 691.2918, found: 691.2923.

α-anomer: R_f 0.50 (8:2 hexanes/EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.41-7.52 (m, 6H, ArH), 7.27-7.33 (m, 3H, ArH), 7.21-7.28 (m, 3H, ArH), 5.24 (d, 1H, $J_{1,2}$ 1.3 Hz, H-1), 4.03 (ddd, 1H, $J_{2,3}$ 3.3 Hz, $J_{1,2}$ 1.3 Hz, H-2), 3.97 (ddd, 1H, $J_{5,6}$ 9.4 Hz, $J_{4,5} \approx J_{5,6}$ 4.6 Hz, H-5), 3.83 (dd, 1H, $J_{3,4}$ 8.8 Hz, $J_{2,3}$ 3.3 Hz, H-3), 3.73 (ddd, 1H, $J_{4,5}$ 9.2 Hz, $J_{3,4} \approx J_{4,OH}$ 2.6 Hz, H-4), 3.40 (br. d, 2H, J 4.6 Hz, H-6), 2.67 (d, 1H, $J_{1,2}$ 1.3 Hz, 2-OH), 2.20 (d, 1H, $J_{4,OH}$ 2.9 Hz, 4-OH), 1.65 (spt, 1H, J 7.3 Hz, C*H*(CH$_3$)$_2$), 1.37 (m, 6H, C(CH$_3$)$_3$), 0.90 (d, 3H, J 6.8 Hz, CH(CH_3)$_2$), 0.89 (d, 3H, J 6.8 Hz, CH(CH_3)$_2$), 0.87 (s, 6H, C(CH_3)$_2$), 0.18 (br. s., 6H, Si(CH_3)$_2$).

Mercapto-*tert*-butyl 2,4-di-*O*-benzoyl-3-*O*-*tert*-butyldimethylsilyl-1-thio-6-*O*-triphenylmethyl-β-D-mannopyranoside 12. To a solution of diol **10** (0.32 g, 0.48 mmol) in pyridine (5 mL) was added benzoyl chloride (0.14 mL, 1.21 mmol) and 1-methylimidazole (1 drop). The reaction was heated to 60 °C for 120 hours, cooled and

diluted with EtOAc. The reaction mixture was washed with sat. $NaHCO_3$, H_2O and brine. The organic was dried over Na_2SO_4 and concentrated under reduced pressure. Purification of the crude product by chromatography (toluene) produced products **11** and **12** as an inseparable mixture (0.37 g, 0.38 mmol, 80%).

R_f 0.65 (toluene); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.16 (m, 2H, ArH), 7.79 (m, 2H, ArH), 7.58 (m, 1H, ArH), 7.54 (m, 1H, ArH), 7.40-7.45 (m, 8H, ArH), 7.38 (m, 2H, ArH) 7.14 (m, 6H, ArH), 7.09 (m, 3H, ArH), 5.84 (d, 1H, $J_{2,3}$ 2.8 Hz, H-2), 5.62 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 8.8 Hz, H-4), 4.81 (s, 1H, H-1), 4.03 (dd, 1H, $J_{2,3}$ 3.1 Hz, $J_{3,4}$ 9.1 Hz, H-3), 3.73 (m, 1H, H-5), 3.30 (dd, 2H, H-6), 1.32 (spt, 1H, J 7.1 Hz, C*H*$(CH_3)_2$), 0.55 (m, 6H, C$(CH_3)_2$), 0.54 (m, 6H, CH(C*H*$_3)_2$), 0.16 (s, 3H, $SiCH_3$), -0.15 (s, 3H, $SiCH_3$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 165.8 (C=O), 164.7 (C=O), 143.7 (Ar), 130.1 (Ar), 130.0 (Ar), 128.7 (Ar), 128.4 (Ar), 127.9 (Ar), 127.6 (Ar), 127.3 (Ar), 93.1 (C-1), 86.1 (*C*Ph_3) 78.9 (C-5), 73.9 (C-2), 72.5 (C-3), 69.7 (C-4), 63.1 (C-6), 47.6 (S*C*$(CH_3)_3$), 33.8 (*C*H$(CH_3)_2$), 30.1 (SC(*C*$H_3)_3$), 24.6 (Si*C*H$(CH_3)_2$), 19.9 (CH(*C*$H_3)_2$), 19.7 (SiC(*C*$H_3)_2$), 18.3 (SiC(*C*$H_3)_2$), -2.5 ($SiCH_3$), -3.0 ($SiCH_3$); Anal. calc for $C_{51}H_{60}O_7S_2Si$: HR ESIMS $[M+Na]^+$: 899.3442, found: 899.3443.

α-product of **12**: Mercapto-*tert*-butyl 2,4-di-*O*-benzoyl-3-*O*-*tert*-butyldimethylsilyl-1-thio-6-*O*-triphenylmethyl-α-D-mannopyranoside: R_f 0.68 (toluene); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.18 (d, 2H, J_{ortho} 8.4 Hz, ArH), 7.87 (d, 2H, J_{ortho} 7.1 Hz, ArH), 7.61 (t, 1H, J_{ortho} 7.5 Hz, ArH), 7.57 (t, 1H, J_{ortho} 7.5 Hz, ArH), 7.49 (t, 2H, J_{ortho} 7.7 Hz, ArH), 7.38-7.45 (m, 8H, ArH), 7.10-7.15 (m, 6H, ArH), 7.06 (m, 3H, ArH), 5.81 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.7 Hz, H-4), 5.63 (dd, 1H, $J_{2,3}$ 2.9 Hz, $J_{1,2}$ 2.0 Hz, H-2), 5.45 (d, 1H, $J_{1,2}$ 1.6 Hz, H-1), 4.31 (m, 1H, H-5), 4.29 (m, 1H, H-3), 3.33 (dd, 1H, J_{gem} 10.4 Hz, $J_{5,6}$ 2.2 Hz, H-6), 3.22 (dd, 1H, J_{gem} 10.4 Hz, $J_{5,6}$ 4.4 Hz, H-6), 1.38 (spt, 1H, J 7.1 Hz, C*H*$(CH_3)_2$), 0.62 (m, 6H, C(C*H*$_3)_2$), 0.59 (m, 6H, CH(C*H*$_3)_2$), 0.11 (s, 3H, $SiCH_3$), -0.11 (s, 3H, SiCH).

β-product of **11**: Mercapto-*tert*-butyl 2-*O*-benzoyl-3-*O*-*tert*-butyldimethylsilyl-1-thio-6-*O*-triphenylmethyl-β-D-mannopyranoside: R_f 0.32 (toluene); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.11 (m, 2H, ArH), 7.52-7.60 (m, 7H, ArH), 7.43 (m, 2H, ArH), 7.31-7.37 (m, 6H, ArH), 7.24-7.30 (m, 3H, ArH), 5.75 (d, 1H, $J_{1,2}$ 2.9 Hz, H-2), 4.71 (s, 1H, H-1), 3.97 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 8.7 Hz, H-4), 3.72 (dd, 1H, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 9.1 Hz, H-3), 3.56-3.63 (m, 1H, H-6), 3.44 (m, 1H, H-5), 3.42 (m, 1H, H-6), 1.96 (br. s, 1H, 4-OH), 1.51 (spt, 1H, J 6.8 Hz, C*H*$(CH_3)_2$), 1.37 (s, 9H, C$(CH_3)_3$), 0.75 (s, 3H, C$(CH_3)_2$), 0.74 (d, 3H, J 7.0 Hz, CH(C*H*$_3)_2$), 0.74 (d, 3H, J 7.0 Hz, C$(CH_3)_2$), 0.72 (m, 6H, CH(C*H*$_3)_2$), 0.21 (s, 3H, $SiCH_3$), 0.14 (s, 3H, $SiCH_3$).

α-product of **11**: Mercapto-*tert*-butyl 2-*O*-benzoyl-3-*O*-*tert*-butyldimethylsilyl-1-thio-6-*O*-triphenylmethyl-α-D-mannopyranoside: R_f 0.47 (toluene); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.07 (m, 2H, ArH), 7.58 (m, 1H, ArH), 7.52 (m, 6H, ArH), 7.38-7.48 (m, 2H, ArH), 7.22-7.31 (m, 9H, ArH), 5.53 (dd, 1H, $J_{1,2}$ 3.1 Hz, $J_{2,3}$ 1.8 Hz, H-2), 5.36 (d, 1H, $J_{1,2}$ 1.5 Hz, H-1), 4.10 (ddd, 1H, $J_{3,4} \approx J_{4,5}$ 9.3 Hz, $J_{4,OH}$ 2.7 Hz, H-4), 4.02 (m, 1H, H-5), 3.99 (m, 1H, H-3), 3.53 (dd, 1H, J_{gem} 10.3 Hz, $J_{5,6}$ 2.9 Hz, H-6), 3.40 (dd, 1H, J_{gem} 10.1 Hz, $J_{5,6}$ 3.8 Hz, H-6), 1.95 (d, 1H, $J_{4,OH}$ 2.7, 4-OH), 1.52 (spt, 1H, J 6.8 Hz, C*H*$(CH_3)_2$), 1.38 (s, 9H, C$(CH_3)_3$), 0.76 (s, 3H, C$(CH_3)_2$), 0.74 (s, 3H, C$(CH_3)_2$), 0.74 (d, 6H, J 6.4, CH(C*H*$_3)_2$), 0.15 (s, 6H, Si$(CH_3)_2$).

Mecapto-*tert*-butyl 6-*O*-*tert*-butyldiphenylsilyl-1-thio-β-D-mannopyranoside 13. The anomeric mixture **9** (2.38 g, 8.4 mmol) was dissolved in pyridine (40 mL) and TBDPSCl (2.4 mL, 9.4 mmol) was slowly added. After 48 hours, the reaction was diluted with toluene and extracted with 1 M $NaHCO_3$, H_2O, and brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. Purification of the crude product by chromatography (6:3 CH_2Cl_2/EtOAc) followed by recrystallization (heptane/EtOAc) yielded the β–product **13** (1.86 g, 3.57 mmol, 71%) a white crystalline solid.

$[\alpha]_D^{25}$ -35 (*c* 6.2, CH_2Cl_2); ^{1}H NMR (498 MHz, $CDCl_3$): δ 7.65-7.70 (m, 4H, ArH), 7.37-7.47 (m, 6H, ArH), 4.53 (s, 1H, H-1), 4.20 (dd, 1H, $J_{2,3} \approx J_{2,OH}$ 3.8 Hz, H-2), 3.96 (dd, 1H, J_{gem} 10.8 Hz, $J_{5,6}$ 4.8 Hz, H-6), 3.92 (dd, 1H, J_{gem} 10.8 Hz, $J_{5,6}$ 5.8 Hz, H-6), 3.87 (ddd, 2H, $J_{3,4} \approx J_{4,5}$ 9.2 Hz, $J_{4,OH}$ 1.6 Hz, H-4), 3.58 (ddd, 1H, $J_{3,4}$ 9.3 Hz, $J_{3,OH}$ 6.2 Hz, $J_{2,3}$

3.3 Hz, H-3), 3.36 (dt, 2H, $J_{4,5}$ 9.7 Hz, $J_{5,6}$ 5.0 Hz, H-5), 3.09 (d, 2H, $J_{4,OH}$ 1.8 Hz, 4-OH), 2.58 (d, 2H, $J_{3,OH}$ 6.4 Hz, 3-OH), 2.38 (d, 2H, $J_{2,OH}$ 4.8 Hz, 2-OH), 1.30 (s, 9H, $SC(CH_3)_3$), 1.06 (s, 9H, $SiC(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 135.6 (Ar), 135.6 (Ar), 132.6 (Ar), 132.5 (Ar), 130.0 (Ar), 127.9 (Ar), 92.3 (C-1), 78.7 (C-5), 74.9 (C-3), 71.4 (C-2), 70.3 (C-4), 65.1 (C-6), 47.7 ($SC(CH_3)_3$), 29.9 ($SC(CH_3)_3$), 26.8 ($SiC(CH_3)_3$), 19.2 ($SiC(CH_3)_3$); Coupled HSQC (500 MHz, $CDCl_3$): □92.3/4.53 ($J_{C1/H1}$ 158 Hz, C-1); Anal. calc for $C_{26}H_{38}O_5S_2Si$: HR ESIMS $[M+Na]^+$: 545.1822, found: 545.1818; Elem. Anal: C, 59.73; H, 7.33; S, 12.27; found: C, 59.12; H, 7.24; S, 12.34.

Mecapto-*tert*-butyl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-6-*O*-*tert*-butyldiphenylsilyl-1-thio-β-D-mannopyranoside 14. Acceptor **13** (0.32 g, 0.61 mmol) was dissolved in a solution of CH_2Cl_2 (8 mL) containing molecular sieves, cooled to 0 °C, and $BF_3{\cdot}OEt_2$ (12 μL, 96 μmol) added. A solution of donor **4** (0.42 g, 0.61 mmol) in CH_2Cl_2 (5 mL) was added dropwise over 30 minutes to the acceptor solution. After 1 hour, the reaction was quenched with 3 drops of TEA and concentrated under reduced pressure. Purification of the crude product by chromatography (9:1 toluene/EtOAc) yielded **14** (0.53 g, 0.51 mmol, 83%) as a white powder.

$[\alpha]_D^{25}$ +25 (*c* 2.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.01-8.05 (m, 2H, ArH), 7.95-7.99 (m, 2H, ArH), 7.87-7.91 (m, 2H, ArH), 7.70 (m, 4H, ArH), 7.33-7.56 (m, 15H, ArH), 5.90 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 10.1 Hz, H-4^b), 5.82 (dd, 1H, $J_{3,4}$ 10.7 Hz, $J_{2,3}$ 3.7 Hz, H-3^b), 5.62 (dd, 1H, $J_{2,3}$ 3.2 Hz, $J_{1,2}$ 1.9 Hz, H-2^b), 5.34 (d, 1H, $J_{1,2}$ 1.6 Hz, H-1^b), 4.63 (ddd, 1H, $J_{4,5}$ 9.8 Hz, $J_{5,6}$ 5.6 Hz, $J_{5,6}$ 3.1 Hz, H-5^b), 4.59 (dd, 1H, J_{gem} 11.9 Hz, $J_{5,6}$ 2.9 Hz, H-6^b), 4.53 (dd, 1H, J_{gem} 11.7 Hz, $J_{5,6}$ 5.7 Hz, H-6^b), 4.47 (s, 1H, H-1^a), 4.36 (dd, 1H, $J_{2,OH}$ 5.4 Hz, $J_{2,3}$ 3.4 Hz, H-2^a), 4.12 (ddd, 1H, $J_{3,4} \approx J_{4,5}$ 9.2 Hz, $J_{4,OH}$ 2.6 Hz, H-4^a), 3.94 (d, 2H, $J_{gem} \approx J_{5,6}$ 4.8 Hz, H-6^a), 3.67 (dd, 1H, $J_{3,4}$ 9.2 Hz, $J_{2,3}$ 3.2 Hz, H-3^a), 3.34 (ddd, 1H, $J_{4,5}$ 9.3 Hz, 2×$J_{5,6}$ 4.6 Hz, H-5^a), 2.90 (d, 1H, $J_{4,OH}$ 2.6 Hz, 4-OH), 2.50 (d, 1H, $J_{2,OH}$ 5.5 Hz, 2-OH), 2.14 (s, 3H, CH_3(C=O)), 1.29 (s, 9H, $SC(CH_3)_3$), 1.08 (s, 9H, $SiC(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 169.8 (C=O), 166.3 (C=O), 165.6 (C=O), 165.6 (C=O), 135.7 (Ar), 135.6 (Ar), 133.5 (Ar), 133.3 (Ar), 133.2 (Ar), 132.9 (Ar), 132.7 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7 (Ar), 129.1 (Ar), 128.9 (Ar), 128.5 (Ar), 128.4 (Ar), 127.9 (Ar), 99.3 (C-1^b), 92.7 (C-1^a), 82.9(C-3^a), 79.5 (C-5^a), 71.7 (C-2^a), 69.9, 69.8 (C-2^b, C-3^b), 69.4 (C-5^b), 68.2 (C-4^a), 67.2 (C-4^b), 64.7 (C-6^a), 63.7 (C-6^b), 47.6 ($SC(CH_3)_3$), 30.0 ($SC(CH_3)_3$), 26.9 ($SiC(CH_3)_3$), 20.8 (CH_3(C=O)), 19.3 ($SiC(CH_3)_3$); Coupled HSQC (500 MHz, $CDCl_3$): □99.3/5.34 ($J_{C1/H1}$ 177 Hz, C-1^b), 92.7/4.47 ($J_{C1/H1}$ 157 Hz, C-1^a); Anal. calc for $C_{55}H_{62}O_{14}S_2Si$: HR ESIMS $[M+Na]^+$: 1061.3246, found: 1061.3242; Elem. Anal: C, 63.56; H, 6.01; S, 6.17; found: C, 63.56; H, 6.29; S, 6.17.

Mecapto-*tert*-butyl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-1-thio-β-D-mannopyranoside 15. Disaccharide **13** (0.21 g, 0.17 mmol) was dissolved in a pyridine (0.25 mL) and hydrogen fluoride in pyridine (25 μL, 70% HF) added. The reaction was performed in a polypropylene flask. After 45 minutes, a suspension of $CaCO_3$ in 1 M $NaHCO_3$ (0.5 mL) was added and the reaction mixture stirred for 30 minutes. The slurry was filtered through a pad of Celite and the eluted solution concentrated under reduced pressure. Purification of the crude product by chromatography (9:1 toluene/EtOAc) yielded **15** (0.16 g, 0.16 mmol, 93%) as a white solid.

$[\alpha]_D^{25}$ +42 (*c* 11.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.01-8.05 (m, 2H, ArH), 7.89-7.93 (m, 2H, ArH), 7.76-7.81 (m, 2H, ArH), 7.51-7.56 (m, 1H, ArH), 7.38-7.48 (m, 3H, ArH), 7.27-7.35 (m, 3H, ArH), 7.09-7.15 (m, 2H, ArH), 5.95 (dd, 1H, $J_{3,4}$ 10.0 Hz, $J_{2,3}$ 3.3 Hz, H-3^b), 5.92 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.5 Hz, H-4^b), 5.64 (dd, 1H, $J_{2,3}$ 2.4 Hz, $J_{1,2}$ 1.8 Hz, H-2^b), 5.45 (d, 1H, $J_{1,2}$ 1.6 Hz, H-1^b), 4.76 (ddd, 1H, $J_{4,5}$ 8.9 Hz, $J_{5,6}$ 5.5 Hz, $J_{5,6}$ 3.1 Hz, H-5^b), 4.65 (br. s, 1H, 4-OH^a), 4.56 (dd, 1H, J_{gem} 12.1 Hz, $J_{5,6}$ 3.1 Hz, H-6^b), 4.52 (s, 1H, H-1^a), 4.52 (dd, 1H, J_{gem} 12.3 Hz, $J_{5,6}$ 5.5 Hz, H-6^b), 4.39 (dd, 1H, $J_{2,3}$ 5.9 Hz, $J_{2,OH}$ 3.3 Hz, H-2^a), 4.30 (ddd, 1H, $J_{3,4} \approx J_{4,5}$ 4.8 Hz, $J_{4,OH}$ 9.5 Hz, H-4^a), 4.16 (m, 1H, 3-OH^a), 3.98 (m, 2H, H-6^a), 3.74 (dd, 1H, $J_{3,4}$ 9.3 Hz, $J_{2,3}$ 3.3 Hz, H-3^a), 3.61 (br. s, 1H, 6-OH^a), 3.31 (ddd, 1H, $J_{4,5} \approx J_{5,6}$ 9.7, $J_{5,6}$ 2.6 Hz, H-5^a), 2.11 (s, 3H, CH_3(C=O)), 1.30 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.3 (C=O), 166.5 (C=O),

166.3 (C=O), 165.6 (C=O), 133.6 (Ar), 133.3 (Ar), 133.2 (Ar), 129.8 (Ar), 129.7 (Ar), 129.7 (Ar), 129.6 (Ar), 128.8 (Ar), 128.7 (Ar), 128.6 (Ar), 128.5 (Ar), 128.3 (Ar), 99.2 (C-1^b), 93.2 (C-1^a), 82.7 (C-3^a), 80.5 (C-5^a), 72.4(C-2^a), 70.3, 70.1 (C-2^b, C-3^b), 69.4 (C-5^b), 66.9 (C-4^b), 65.4 (C-4^a), 63.7 (C-6^b), 61.2 (C-6^a), 47.5 ($C(CH_3)_3$), 30.0 (C(CH_3)$_3$), 20.8 (CH_3(C=O)); Anal. calc for $C_{39}H_{44}O_{14}S_2$: HR ESIMS $[M+Na]^+$: 823.2065, found: 823.2053; Elem. Anal: C, 58.49 H, 5.54; S, 8.01; found: C, 57.97 H, 5.87; S, 7.75.

Mecapto-*tert*-butyl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-1-thio-β-D-mannopyranoside 16. Triol **15** (0.48 g, 0.60 mmol) was dissolved in pyridine (2.5 mL) and cooled to 0 °C (ice-water bath). Benzoyl chloride (2.5 mL, 21.6 mmol) was added in portions and after 1 hour the reaction was removed from the ice bath. After 24 hours total reaction time, the reaction mixture was diluted with toluene and washed with 1 M NaOH, H_2O, and brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. Purification of the crude product by chromatography (9:1 toluene/EtOAc) yielded **16** (0.53 g, 0.47 mmol, 87%) as a white powder.

$[\alpha]_D^{25}$ -39 (*c* 3.9, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.25-8.26 (m, 30H, Ar), 6.04 (dd, 1H, $J_{2,3}$ 3.5 Hz, $J_{1,2}$ 1.0 Hz, H-2a), 5.82 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.8 Hz, H-4^a), 5.79 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.8 Hz, H-4^b), 5.53 (dd, 1H, $J_{3,4}$ 9.7 Hz, $J_{2,3}$ 3.4 Hz, H-3^b), 5.09 (dd, 1H, $J_{2,3}$ 3.4 Hz, $J_{1,2}$ 2.0 Hz, H-2^b), 5.07 (d, 1H, $J_{1,2}$ 1.9 Hz, H-1^b), 4.80 (d, 1H, $J_{1,2}$ 1.2 Hz, H-1^a), 4.80 (ddd, 1H, $J_{4,5}$ 9.8 Hz, $J_{5,6}$ 4.7 Hz, $J_{5,6}$ 2.8 Hz, H-5^b), 4.68 (dd, 1H, J_{gem} 12.3 Hz, $J_{5,6}$ 2.5 Hz, H-6^b), 4.68 (dd, 1H, J_{gem} 12.1 Hz, $J_{5,6}$ 2.5 Hz, H-6^a), 4.53 (dd, 1H, J_{gem} 12.2 Hz, $J_{5,6}$ 4.6 Hz, H-6^b), 4.47 (dd, 1H, J_{gem} 12.2 Hz, $J_{5,6}$ 5.3 Hz, H-6^a), 4.33 (dd, 1H, $J_{3,4}$ 9.8 Hz, $J_{2,3}$ 3.5 Hz, H-3^a), 3.99 (ddd, 1H, $J_{4,5}$ 10.0 Hz, $J_{5,6}$ 5.3 Hz, $J_{5,6}$ 2.9 Hz, H-5^a), 1.78 (s, 3H, CH_3(C=O)), 1.32 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.6 (C=O), 166.5 (C=O), 166.4 (C=O), 165.5 (C=O), 164.9 (C=O), 133.79 (Ar), 133.77 (Ar), 133.70 (Ar), 133.66 (Ar), 133.60 (Ar), 133.50 (Ar), 133.34 (Ar), 133.32 (Ar), 133.26 (Ar), 133.22 (Ar), 133.21 (Ar), 130.63 (Ar), 133.61 (Ar), 130.5 (Ar), 130.18 (Ar), 130.10 (Ar), 130.08 (Ar), 130.06 (Ar), 129.99 (Ar), 129.95 (Ar), 129.2 (Ar) 128.8 (Ar) 128.61 (Ar), 127.8 (Ar), 99.4 (H-1^b), 92.6 (H-1^a), 77.3 (H-3^a), 76.9 (H-5^a), 72.9 (H-2^a), 69.9 (H-5^b), 69.5 (H-2^b), 69.27 (H-3^b), 69.21 (H-4^a), 67.2 (H-4^b), 63.52 (H-6^a), 63.4 (H-6^b), 48.0 ($C(CH_3)_3$), 30.1 (C(CH_3)$_3$), 20.4 (CH_3(C=O)); Anal. calc for $C_{60}H_{56}O_{17}S_2$: HR ESIMS $[M+Na]^+$: 1135.2851, found: 1135.2847; Elem. Anal: C, 64.74 H, 5.07; S, 5.76; found: C, 62.62 H, 5.02; S, 5.49.

Mecapto-*tert*-butyl 3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-1-thio-β-D-mannopyranoside 17. Trisaccharide **16** (0.36 g, 0.32 mmol) was dissolved in CH_2Cl_2 (1.5 mL) and MeOH (2.5 mL) and cooled to -10 °C (HAAKE Fisons chiller). A solution of acetyl chloride (0.25 mL, 2.9 mmol) in MeOH (1 mL) was added dropwise. The reaction was stirred at -10 °C for 170 hours, and quenched with 1 M $NaHCO_3$. The reaction mixture was extracted three times with EtOAc and the combined organic layer concentrated under reduced pressure. Purification of crude product by chromatography (9.4:0.6 toluene/EtOAc) yielded **17** (0.27 g, 0.25 mmol, 78%) as a white powder.

$[\alpha]_D^{25}$ -27 (*c* 3.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.28-8.27 (m, 30H, Ar), 6.05 (dd, 1H, $J_{2,OH}$ 3.4, $J_{1,2}$ 1.0 Hz, H-2^a), 5.85 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.8 Hz, H-4^b), 5.80 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.8 Hz, H-4^a), 5.43 (dd, 1H, $J_{3,4}$ 9.5 Hz, $J_{2,3}$ 3.1 Hz, H-3^b), 5.11 (d, 1H, $J_{1,2}$ 1.8 Hz, H-1^b), 4.87 (ddd, 1H, $J_{4,5}$ 9.9 Hz, $J_{5,6}$ 4.6 Hz, $J_{5,6}$ 2.8 Hz, H-5^b), 4.82 (d, 1H, $J_{1,2}$ 1.1 Hz, H-1^a), 4.66 (dd, 1H, J_{gem} 12.2 Hz, $J_{5,6}$ 2.7 Hz, H-6^b), 4.66 (dd, 2H, J_{gem} 12.2 Hz, $J_{5,6}$ 2.8 Hz, H-6^a), 4.56 (dd, 1H, J_{gem} 12.3 Hz, $J_{5,6}$ 4.8 Hz, H-6^b), 4.47 (dd, 4H, J_{gem} 12.3 Hz, $J_{5,6}$ 5.3 Hz, H-6^a), 4.38 (dd, 1H, $J_{3,4}$ 9.8 Hz, $J_{2,3}$ 3.4 Hz, H-3^a), 4.00 (ddd, 1H, $J_{4,5}$ 9.9 Hz, $J_{5,6}$ 5.3 Hz, $J_{5,6}$ 2.9 Hz, H-5^a), 3.95 (ddd, 1H, $J_{2,OH}$ 3.9 Hz, $J_{2,3}$ 3.2 Hz, $J_{1,2}$ 1.9 Hz, H-2^b), 1.92 (d, 1H, $J_{2,OH}$ 4.2 Hz, 2-OH), 1.32 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.3 (C=O), 166.2 (C=O), 166.0 (C=O), 165.5 (C=O), 165.1 (C=O), 164.8 (C=O), 133.7 (Ar), 133.5 (Ar), 133.3 (Ar), 133.1 (Ar), 133.1 (Ar), 133.0 (Ar), 130.3 (Ar), 129.9 (Ar), 129.8 (Ar), 129.8 (Ar), 129.7 (Ar), 129.4 (Ar), 129.3 (Ar), 129.2 (Ar), 128.7 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.4 (Ar), 128.3 (Ar), 101.3 (C-1^b), 92.3 (C-1^a), 77.2 (C-5^a), 76.6 (C-3^a), 72.8 (C-

2[a]), 71.8 (C-3[b]), 69.6 (C-5[b]), 69.3 (C-4[a]), 69.1 (C-2[b]), 66.8 (C-4[b]), 63.4 (C-6[b]), 63.3 (C-6[a]), 47.8 ($C(CH_3)_3$), 30.0 ($C(CH_3)_3$); Anal. calc for $C_{56}H_{54}O_{16}S_2$: HR ESIMS $[M+Na]^+$: 1093.2745, found: 1093.2742.

Mercapto-*tert*-butyl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-2,4-di-*O*-benzoyl-6-*O*-*tert*-butyldiphenylsilyl-1-thio-β-D-mannopyranose 18. Disaccharide **14** (0.75 g, 0.72 mmol) was dissolved in a solution of pyridine (0.75 mL), benzoyl chloride (0.25 mL, 2.16 mmol) and 1-methylimidazole (1 drop). After 48 hours, the reaction was diluted with CH_2Cl_2 and washed with 1 M NaOH, H_2O and brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. Purification of the crude product by chromatography (9.7:0.3 toluene/EtOAc) yielded **18** (0.69g, 0.52 mmol, 72%) as a white solid.

$[\alpha]_D^{25}$ -40 (*c* 8.1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.16-8.30 (m, 35H, ArH), 6.05 (d, 1H, $J_{2,3}$ 3.0 Hz, H-2[a]), 5.90 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.9 Hz, H-4[a]), 5.80 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.8 Hz, H-4[b]), 5.55 (dd, 1H, $J_{3,4}$ 9.7 Hz, $J_{2,3}$ 3.3 Hz, H-3[b]), 5.12 (dd, 1H, $J_{2,3}$ 3.0 Hz, $J_{1,2}$ 2.1 Hz, H-2[b]), 5.01 (d, 1H, $J_{1,2}$ 1.4 Hz, H-1[b]), 4.81 (m, 1H, H-5[b]), 4.79 (s, 1H, H-1[a]), 4.69 (dd, 1H, J_{gem} 12.2 Hz, $J_{5,6}$ 2.4 Hz, H-6[b]), 4.52 (dd, 1H, J_{gem} 12.2 Hz, $J_{5,6}$ 4.4 Hz, H-6[b]), 4.28 (dd, 1H, $J_{3,4}$ 9.9 Hz, $J_{2,3}$ 3.4 Hz, H-3[a]), 3.86 (m, 2H, H-6[a]), 3.68 (ddd, 1H, $J_{4,5}$ 9.9 Hz, 2×$J_{5,6}$ 2.9 Hz, H-5[a]), 1.79 (s, 3H, CH_3(C=O)), 1.36 (s, 9H, $SC(CH_3)_3$), 1.08 (s, 9H, $SiC(CH_3)_3$); ^{13}C NMR (151 MHz, $CDCl_3$): δ 168.7 (C=O), 166.2 (C=O), 166.2 (C=O), 165.4 (C=O), 164.7 (C=O), 164.5 (C=O), 135.8 (Ar), 135.6 (Ar), 133.4 (Ar), 133.3 (Ar), 133.1 (Ar), 133.1 (Ar), 133.0 (Ar), 132.8 (Ar), 130.4 (Ar), 130.0 (Ar), 129.9 (Ar), 129.7 (Ar), 129.5 (Ar), 129.5 (Ar), 129.4 (Ar), 129.3 (Ar), 129.2 (Ar), 129.1 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 127.6 (Ar), 127.5 (Ar), 99.1 (C-1[b]), 92.4 (C-1[a]), 79.7 (C-5[a]), 77.5 (C-3[a]), 72.7 (C-2[a]), 69.6, 69.5, 68.9, 68.4 (C-4[a], C-2[b],C-3[b], C-5[b]), 67.0 (C-4[b]), 63.1 (C-6[b]), 62.6 (C-6[a]), 47.7 ($SC(CH_3)_3$), 30.0 ($SC(CH_3)_3$), 26.7 ($SiC(CH_3)_3$), 20.3 (CH_3(C=O)), 19.2 ($SiC(CH_3)_3$); Anal. calc for $C_{69}H_{70}O_{16}S_2Si$: HR ESIMS $[M+Na]^+$: 1269.3767, found: 1269.3765; Elem. Anal: C, 66.43 H, 5.66; S, 5.14; found: C, 66.10; H, 5.61; S, 5.09.

Mercapto-*tert*-butyl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-2,4-di-*O*-benzoyl-1-thio-β-D-mannopyranose 19. Disaccharide **18** (0.21 g, 0.17 mmol) was dissolved in a pyridine (0.25 mL) and hydrogen fluoride in pyridine (25 µL, 70% HF) added. The reaction was performed in a polypropylene flask. After 45 minutes, a suspension of $CaCO_3$ in sat. $NaHCO_3$ (0.5 mL) was added and the reaction mixture stirred for 30 minutes. The slurry was filtered through a pad of Celite and the eluted solution concentrated under reduced pressure. Purification of the crude product by chromatography (9:1 toluene/EtOAc) yielded **19** (0.16 g, 0.16 mmol, 93%) as a white solid.

$[\alpha]_D^{25}$ -63 (*c* 7.2, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.25-8.28 (m, 25H, ArH), 6.05 (d, 1H, $J_{2,3}$ 3.2 Hz, H-2[a]), 5.82 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.8 Hz, H-4[b]), 5.58 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.8 Hz, H-4[a]), 5.50 (dd, 1H, $J_{3,4}$ 9.6 Hz, $J_{2,3}$ 3.3 Hz, H-3[b]), 5.13 (d, 1H, $J_{1,2}$ 1.5 Hz, H-1[b]), 5.09 (dd, 1H, $J_{2,3}$ 2.9 Hz, $J_{1,2}$ 2.1 Hz, H-2[b]), 4.80 (s, 1H, H-1[a]), 4.77 (ddd, 1H, $J_{4,5}$ 9.9 Hz, $J_{5,6}$ 4.1 Hz, $J_{5,6}$ 3.0 Hz, H-5[b]), 4.70 (dd, 1H, J_{gem} 12.2 Hz, $J_{5,6}$ 2.5 Hz, H-6[b]), 4.53 (dd, 1H, J_{gem} 12.2 Hz, $J_{5,6}$ 4.4 Hz, H-6[b]), 4.36 (dd, 1H, $J_{3,4}$ 9.7 Hz, $J_{2,3}$ 3.5 Hz, H-3[a]), 3.84 (ddd, 1H, J_{gem} 12.7 Hz, $J_{5,6}$ 9.5 Hz, $J_{5,6}$ 2.1 Hz, H-6[a]), 3.78 (dd, 1H, $J_{5,6}$ 12.8 Hz, $J_{5,6}$ 4.9 Hz, H-6[a]), 3.64 (ddd, 1H, $J_{4,5}$ 9.9 Hz, $J_{5,6}$ 4.7 Hz, $J_{5,6}$ 2.3 Hz, H-5[a]), 2.62 (dd, 1H, J_{gem} 9.4 Hz, $J_{5,6}$ 5.0 Hz, 6-OH), 1.80 (s, 3H, CH_3(C=O)), 1.38 (s, 9H, $SC(CH_3)_3$); ^{13}C NMR (151 MHz, $CDCl_3$): δ 168.8 (C=O), 166.2 (C=O), 166.1 (C=O), 166.0 (C=O), 165.4 (C=O), 164.6 (C=O), 133.7 (Ar), 133.6 (Ar), 133.4 (Ar), 133.1 (Ar), 132.9 (Ar), 130.3 (Ar), 129.9 (Ar), 129.7 (Ar), 129.5 (Ar), 129.3 (Ar), 129.1 (Ar), 129.0 (Ar), 128.7 (Ar), 128.5 (Ar), 128.5 (Ar), 128.4 (Ar), 128.2 (Ar), 99.2 (C-1[b]), 92.0 (C-1[a]), 79.5 (C-5[a]), 76.9 (C-3[a]), 72.5 (C-2[a]), 69.7, 69.4, 69.1, 68.9 (C-4[a], C-2[b], C-3[b], C-5[b]), 66.8 (C-4[b]), 63.1 (H-6[b]), 61.7 (H-6[a]), 47.8 ($SC(CH_3)_3$), 29.9 ($SC(CH_3)_3$), 20.2 (CH_3(C=O)O); Anal. calc for $C_{53}H_{52}O_{16}S_2$: HR ESIMS $[M+Na]^+$: 1031.2589, found: 1031.2591; Elem. Anal: C, 63.08; H, 5.19; S, 6.36; found: C, 63.07; H, 5.25; S, 6.35.

Mecapto-*tert*-butyl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-1-thio-β-D-mannopyranoside 20. Donor **4** (0.22 g, 0.33 mmol) and disaccharide acceptor **17** (0.27 g, 0.25 mol) were dissolved in dry CH_2Cl_2 (8 mL), activated molecular sieves added, and TMSOTf (4.6 µL, 25 µmol) was added at room temperature. After 1 hours the reaction was quenched with a drop of TEA and the reaction mixture concentrated under reduced pressure. The resulting slurry was purified by chromatography (6:4 heptane/EtOAc) to yield **20** (0.38 g, 0.24 mmol, 95%) as a white powder.

$[\alpha]_D^{25}$ -53 (*c* 6.0, $CHCl_3$); ^{1}H NMR (498 MHz, $CDCl_3$): δ 7.29-8.25 (m, 45H, Ar), 5.93 (dd, 1H, $J_{2,3}$ 3.5 Hz, $J_{1,2}$ 0.7 Hz, H-2^a), 5.88 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 10.0 Hz, H-4^b), 5.71 (dd, 1H, $J_{3,4}$ 9.9 Hz, $J_{2,3}$ 3.3 Hz, H-3^c), 5.64 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.8 Hz, H-4^a), 5.61 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.9 Hz, H-4^c), 5.51 (dd, 1H, $J_{3,4}$ 9.9 Hz, $J_{2,3}$ 3.3 Hz, H-3^b), 5.33 (d, 1H, $J_{1,2}$ 1.5 Hz, H-1^b), 5.28 (dd, 1H, $J_{2,3}$ 3.1 Hz, $J_{1,2}$ 2.2 Hz, H-2^c), 4.87 (ddd, 1H, $J_{4,5}$ 9.8 Hz, $J_{5,6}$ 5.0 Hz, $J_{5,6}$ 2.7 Hz, H-5^b), 4.71 (dd, 1H, J_{gem} 12.4 Hz, $J_{5,6}$ 5.2 Hz, H-6^b), 4.61 (dd, 1H, J_{gem} 12.3 Hz, $J_{5,6}$ 2.9 Hz, H-6^b), 4.57 (d, 1H, $J_{1,2}$ 0.9 Hz, H-1^a), 4.55 (dd, 1H, J_{gem} 12.1 Hz, $J_{5,6}$ 2.9 Hz, H-6^a), 4.37 (m, 1H, H-5^c), 4.35 (m, 1H, H-6^a), 4.34 (m, 1H, H-6^c), 4.21 (d, 1H, $J_{1,2}$ 1.8 Hz, H-1^c), 4.12 (m, 1H, H-6^c), 3.87 (dd, 1H, $J_{2,3}$ 3.4 Hz, $J_{1,2}$ 1.9 Hz, H-2^b), 3.83 (dd, 1H, $J_{3,4}$ 9.6 Hz, $J_{2,3}$ 3.4 Hz, H-3^a), 3.51 (ddd, 1H, $J_{4,5}$ 10.0 Hz, $J_{5,6}$ 5.3 Hz, $J_{5,6}$ 3.2 Hz, H-5^a), 1.95 (s, 3H, CH_3(C=O)), 1.34 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (126 MHz, $CDCl_3$): □ 169.1 (C=O), 166.30 (C=O), 166.29 (C=O), 166.25 (C=O), 165.9 (C=O), 166.30 (C=O), 166.28 (C=O), 166.26 (C=O), 166.24 (C=O), 165.15 (C=O), 133.8 (Ar), 133.79 (Ar), 133.78 (Ar), 133.27 (Ar), 133.26 (Ar), 133.24 (Ar), 133.0 (Ar), 132.78 (Ar), 132.73 (Ar), 130.63 (Ar), 130.61 (Ar), 130.56 (Ar), 130.26 (Ar), 130.23 (Ar), 130.14 (Ar), 130.11 (Ar), 130.10 (Ar), 130.09 (Ar), 130.08 (Ar), 130.01 (Ar), 129.61 (Ar), 129.60 (Ar), 129.58 (Ar), 129.57 (Ar), 129.4 (Ar), 129.3 (Ar), 128.85 (Ar), 128.83 (Ar), 128.56 (Ar), 128.55 (Ar), 128.54 (Ar), 128.3 (Ar), 102.8 (C-1^b), 99.7 (C-1^c), 92.5 (C-1^a), 78.8 (C-2^b), 77.4 (C-3^a), 76.6 (C-5^a), 73.0 (C-2^a), 70.0 (C-5^c), 69.8 (C-3^b), 69.78 (C-3^c), 69.74 (C-2^c), 69.6 (C-5^b), 67.5 (C-4^b), 5.65 (C-4^a), 5.62 (C-4^c), 64.53 (C-6^b), 63.80 (C-6^c), 63.5 (C-6^a), 48.0 (*C*(CH_3)$_3$), 30.3 (C(*C*H$_3$)$_3$), 20.7 (*C*H_3(C=O)); Coupled HSQC (700 MHz, D_2O): □102.8/5.33 ($J_{C1/H1}$ 174 Hz, C-1^b), 99.7/4.21 ($J_{C1/H1}$ 171 Hz, C-1^c), 92.5/4.57 ($J_{C1/H1}$ 158 Hz, C-1^a); Anal. calc for $C_{87}H_{78}O_{25}S_2$: HR ESIMS $[M+Na]^+$: 1609.4160, found: 1609.4160; Elem. Anal: C, 65.82 H, 4.95; S, 4.04; found: C, 65.51; H, 5.13; S, 3.92.

Mecapto-*tert*-butyl 3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-1-thio-β-D-mannopyranoside 21. Trisaccharide **20** (0.24 g, 0.15 mmol) was dissolved in CH_2Cl_2 (1 mL) and MeOH (1 mL) and cooled to -10 °C (HAAKE Fisons chiller). A solution of acetyl chloride (0.15 mL, 2.1 mmol) in MeOH (0.85 mL) was added dropwise. The reaction was stirred at -10 °C for 2 weeks, and quenched with 1 M $NaHCO_3$. The reaction mixture was extracted three times with EtOAc and the combined organic layer concentrated under reduced pressure. Purification of crude product by chromatography (9.5:0.5 toluene/EtOAc) yielded **21** (0.11 g, 71 mmol, 48%) as a white powder.

$[\alpha]_D^{25}$ -63 (*c* 10.4, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$): δ 7.30-8.26 (m, 45H, Ar), 5.94 (dd, 1H, $J_{2,3}$ 3.4, $J_{1,2}$ 0.9 Hz, H-2^a), 5.88 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 10.0 Hz, H-4^b), 5.67 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.9 Hz, H-4^a), 5.65 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.8 Hz, H-4^c), 5.58 (dd, 1H, $J_{3,4}$ 9.9 Hz, $J_{2,3}$ 3.1 Hz, H-3^c), 5.46 (dd, 1H, $J_{3,4}$ 9.9 Hz, $J_{2,3}$ 3.4 Hz, H-3^b), 5.36 (d, 1H, $J_{1,2}$ 1.5 Hz, H-1^b), 4.86 (ddd, 1H, J_{gem} 10.0 Hz, $J_{5,6}$ 4.8 Hz, $J_{5,6}$ 2.8 Hz, H-5^b), 4.69 (dd, 1H, J_{gem} 12.4 Hz, $J_{5,6}$ 4.9 Hz, H-6^b), 4.62 (dd, 1H, J_{gem} 12.3 Hz, $J_{5,6}$ 2.5 Hz, H-6^b), 4.59 (d, 1H, $J_{1,2}$ 1.0 Hz, H-1^a), 4.57 (dd, 1H, J_{gem} 12.2 Hz, $J_{5,6}$ 3.0 Hz, H-6^a), 4.39 (dd, 1H, J_{gem} 12.2 Hz, $J_{5,6}$ 5.2 Hz, H-6^a), 4.36 (m, 1H, H-5^c), 4.34 (m, 1H, H-6^c), 4.18 (d, 1H, $J_{1,2}$ 1.4 Hz, H-1^c), 4.12 (m, 1H, H-6^c), 4.09 (m, 1H, H-2^c), 3.90 (dd, 1H, $J_{2,3}$ 2.8 Hz, $J_{1,2}$ 1.4 Hz, H-2^b), 3.85 (dd, 1H, $J_{3,4}$ 9.7 Hz, $J_{2,3}$ 3.3 Hz, H-3^a), 3.56 (ddd, 1H, $J_{4,5}$ 9.8 Hz, $J_{5,6}$ 5.2 Hz, $J_{5,6}$ 3.1 Hz, H-5^a), 1.65 (d, 1H, $J_{2,OH}$ 4.8 Hz, 2-OH), 1.35 (s, 9H, C(C*H*$_3$)$_3$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.5 (C=O), 166.1 (C=O), 166.0 (C=O), 165.9 (C=O), 165.8 (C=O), 165.3 (C=O), 165.1 (C=O), 165.0 (C=O), 164.9 (C=O), 133.8 (Ar), 133.5 (Ar), 133.4 (Ar), 133.4 (Ar), 133.3 (Ar), 133.2 (Ar), 133.1

(Ar), 133.0 (Ar), 133.0 (Ar), 130.3 (Ar), 130.2 (Ar), 130.1 (Ar), 130.0 (Ar), 130.0 (Ar), 130.0 (Ar), 129.9 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7 (Ar), 129.7 (Ar), 129.3 (Ar), 129.3 (Ar), 129.3 (Ar), 129.1 (Ar), 128.9 (Ar), 128.8 (Ar), 128.7 (Ar), 128.7 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.4 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 101.7 (C-1^{c}), 100.1 (C-1^{b}), 92.2 (C-1^{a}), 78.5 (C-2^{b}), 77.4 (C-3^{a}), 76.4 (C-5^{a}), 72.9 (C-2^{a}), 72.1 (C-3^{c}), 70.0 (C-3^{b}), 69.7 (C-5^{c}), 69.4 (C-5^{b}), 69.1 (C-2^{c}), 68.9 (C-4^{a}), 67.2 (C-4^{b}), 66.6 (C-4^{c}), 64.1 (C-6^{b}), 63.7 (C-6^{a}), 63.3 (C-6^{c}), 47.8 (*C*(CH$_3$)$_3$), 30.1 (C(*C*H$_3$)$_3$); Anal. calc for $C_{85}H_{76}O_{24}S_2$: HR ESIMS [M+Na]$^+$: 1567.4060, found: 1567.4042.

Mecapto-*tert*-butyl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-1-thio-β-D-mannopyranoside 22. Donor **4** (45 mg, 66 μmol) and trisaccharide acceptor **21** (79 mg, 51 μmol) were dissolved in dry CH_2Cl_2 (0.6 mL), activated molecular sieves added, and the reaction mixture cooled to 0 °C (ice water bath). TMSOTf (1 μL, 5.5 μmol) was added and after 1.5 hours the reaction was quenched with a drop of TEA. The reaction mixture was concentrated under reduced pressure and the resulting slurry purified by chromatography (7:3 heptane/EtOAc) to yield **22** (95 mg, 46 μmol, 91%) as a white crystalline solid.

$[\alpha]_D^{25}$ -32 (*c* 43.2, Benzene); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.09-8.32 (m, 60H, Ar), 5.99 (d, 1H, $J_{1,2}$ 3.3 Hz, H-2^{a}), 5.89 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.9 Hz, H-4^{b}), 5.80 (dd, 1H, $J_{3,4}$ 10.1 Hz, $J_{2,3}$ 2.9 Hz, H-3^{c}), 5.76 (dd, 1H, $J_{3,4}$ 10.0 Hz, $J_{2,3}$ 3.4 Hz, H-3^{d}), 5.74 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 7.5 Hz, H-4^{c}), 5.71 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.7 Hz, H-4^{a}), 5.68 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 10.0 Hz, H-4^{d}), 5.63 (dd, 1H, $J_{2,3}$ 3.2 Hz, $J_{1,2}$ 1.9 Hz, H-2^{d}), 5.47 (dd, 1H, $J_{3,4}$ 9.9 Hz, $J_{2,3}$ 3.4 Hz, H-3^{b}), 5.37 (d, 1H, $J_{1,2}$ 1.2 Hz, H-1^{b}), 4.89 (ddd, 1H, $J_{4,5}$ 10.0 Hz, $J_{5,6}$ 4.7 Hz, $J_{5,6}$ 2.6 Hz, H-5^{b}), 4.86 (d, 1H, $J_{1,2}$ 1.3 Hz, H-1^{d}), 4.71 (dd, 1H, J_{gem} 12.1 Hz, $J_{5,6}$ 5.0 Hz, H-6^{b}), 4.62 (s, 1H, H-1^{a}), 4.62 (br. s., 1H, H-1^{c}), 4.63 (dd, 1H, J_{gem} 12.3 Hz, $J_{5,6}$ 2.6 Hz, H-6^{b}), 4.58 (dd, 1H, J_{gem} 12.2 Hz, $J_{5,6}$ 2.9 Hz, H-6^{a}), 4.40 (dd, 1H, J_{gem} 12.2 Hz, $J_{5,6}$ 5.2 Hz, H-6^{a}), 4.33 (ddd, 1H, $J_{4,5}$ 9.9 Hz, $J_{5,6}$ 6.0 Hz, $J_{5,6}$ 2.0 Hz, H-5^{c}), 4.26 (d, 1H, $J_{2,3}$ 1.8 Hz, H-2^{c}), 4.24 (dd, 1H, J_{gem} 12.1 Hz, $J_{5,6}$ 6.0 Hz, H-6^{c}), 4.13 (br. d, 1H, J_{gem} 12.2 Hz, H-6^{c}), 4.02 (m, 1H, H-5^{d}), 4.00 (dd, 1H, $J_{3,4}$ 10.4 Hz, $J_{2,3}$ 3.3 Hz, H-3^{a}), 3.98 (dd, 1H, J_{gem} 12.6 Hz, $J_{5,6}$ 3.8 Hz, H-6^{d}), 3.93 (dd, 1H, J_{gem} 12.3 Hz, $J_{5,6}$ 3.5 Hz, H-6^{d}), 3.90 (br. s., 1H, H-2^{b}), 3.53 (ddd, 1H, $J_{4,5}$ 9.6 Hz, $J_{5,6}$ 5.0 Hz, $J_{5,6}$ 3.3 Hz, H-5^{a}), 2.00 (s, 3H, CH$_3$(C=O)), 1.36 (s, 9H, C(CH$_3$)$_3$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 169.0 (C=O), 166.5 (C=O), 166.1 (C=O), 166.1 (C=O), 165.9 (C=O), 165.7 (C=O), 165.7 (C=O), 165.4 (C=O), 165.2 (C=O), 165.2 (C=O), 165.1 (C=O), 165.0 (C=O), 164.6 (C=O), 134.1 (Ar), 133.6 (Ar), 133.4 (Ar), 133.3 (Ar), 133.2 (Ar), 133.1 (Ar), 133.1 (Ar), 133.0 (Ar), 132.8 (Ar), 130.4 (Ar), 130.3 (Ar), 130.0 (Ar), 130.0 (Ar), 130.0 (Ar), 129.8 (Ar), 129.8 (Ar), 129.7 (Ar), 129.7 (Ar), 129.7 (Ar), 129.4 (Ar), 129.4 (Ar), 129.3 (Ar), 129.3 (Ar), 128.9 (Ar), 128.9 (Ar), 128.7 (Ar), 128.7 (Ar), 128.6 (Ar), 128.5 (Ar), 128.5 (Ar), 128.4 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 100.8 (C-1^{c}), 100.0 (C-1^{b}), 99.3 (C-1^{d}), 92.3 (C-1^{a}), 78.2 (C-2^{b}), 76.6 (C-3^{a}), 76.5 (C-5^{a}), 75.1 (C-2^{c}), 72.8 (C-2^{a}), 71.0 (C-3^{c}), 69.9 (C-5^{c}), 69.8 (C-3^{b}), 69.5 (C-2^{d}), 69.4 (C-5^{b}), 69.3 (C-3^{d}), 69.3 (C-5^{d}), 68.9 (C-4^{a}), 67.6 (C-4^{d}), 67.4 (C-4^{b}), 66.8 (C-4^{c}), 64.2 (C-6^{a}), 64.0 (C-6^{c}), 63.3 (C-6^{a}), 63.1 (C-6^{d}), 47.8 (*C*(CH$_3$)$_3$), 30.1 (C(*C*H$_3$)$_3$), 20.6 (*C*H$_3$(C=O)); Coupled HSQC (600 MHz, $CDCl_3$): 100.8/4.62 ($J_{C1/H1}$ 171 Hz, C-1^{c}), 100.0/5.37 ($J_{C1/H1}$ 175 Hz, C-1^{b}), 99.2/4.86 ($J_{C1/H1}$ 175 Hz, C-1^{d}), 92.2/4.62 ($J_{C1/H1}$ 156 Hz, C-1^{a}); Anal. calc for $C_{114}H_{100}O_{33}S_2$: HR ESIMS [M+Na]$^+$: 2083.5480, found: 2083.5457.

Allyl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-[2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)]-2,4-di-*O*-benzoyl-α-D-mannopyranoside 24. Donor **4** (1.55 g, 2.28 mmol) and acceptor **23** (0.47 g, 1.09mol) were dissolved in dry CH_2Cl_2 (18 mL), activated molecular sieves added, and the reaction mixture cooled to 0 °C (ice water bath). TMSOTf (14.5 μL, 73 μmol) was added and after 30 minutes the reaction was removed from the ice bath. Additional TMSOTf (8 μL, 27 μmol) was added after 2 hours total reaction time and the reaction was quenched 30 minutes later with the addition of 3 drops of TEA. The reaction mixture was concentrated under reduced pressure and the resulting slurry purified by chromatography (8:2 hexanes/EtOAc) to yield **24** (1.44 g, 0.99 mol, 93%) as a white glassy solid; Spectral data matches literature values.[26]

$[\alpha]_D^{25}$ -5 (*c* 4.3, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.26-8.25 (m, 40H, ArH), 5.95 (ddt, 1H, J_{trans} 16.8 Hz, J_{cis} 10.7 Hz, 2×*J* 6.0 Hz, Hb), 5.91 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.9 Hz, H-4c), 5.85 (dd, 1H, $J_{3,4}$ 10.3 Hz, $J_{2,3}$ 3.0 Hz, H-3^c), 5.83 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 10.1 Hz, H-4^a), 5.79 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.8 Hz, H-4^b), 5.68 (dd, 1H, $J_{2,3}$ 3.4 Hz, $J_{1,2}$ 1.6 Hz, H-2^a), 5.57 (dd, 1H, $J_{3,4}$ 9.7 Hz, $J_{2,3}$ 3.3 Hz, H-3^b), 5.50 (dd, 1H, $J_{2,3}$ 3.1 Hz, $J_{1,2}$ 1.8 Hz, H-2^c), 5.43 (dq, 1H, J_{trans} 17.2 Hz, $J_{gem} \approx 2\times{}^4J$ 1.3 Hz, Ha), 5.30 (dq, 1H, J_{cis} 10.4 Hz, $J_{gem} \approx 2\times{}^4J$ 1.2 Hz, Hc), 5.18 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1^b), 5.13 (s, 1H, H-1^a), 5.12 (dd, 1H, $J_{2,3}$ 2.8 Hz, $J_{1,2}$ 2.1 Hz, H-2^b), 4.97 (d, 1H, $J_{1,2}$ 1.3 Hz, H-1^c), 4.60 (dd, 1H, $J_{3,4}$ 9.7 Hz, $J_{2,3}$ 3.4 Hz, H-3^a), 4.52 (dd, 1H, J_{gem} 12.1 Hz, $J_{5,6}$ 2.5 Hz, H-6^b), 4.49 (dd, 1H, J_{gem} 11.9 Hz, $J_{5,6}$ 2.5 Hz, H-6^c), 4.45 (ddd, 1H, J_{gem} 9.9 Hz, $J_{5,6}$ 5.2 Hz, $J_{5,6}$ 2.6 Hz, H-5^c), 4.42 (ddd, 1H, J_{gem} 9.8 Hz, $J_{5,6}$ 4.3 Hz, $J_{5,6}$ 2.5 Hz, H-5^b), 4.36 (dd, 1H, J_{gem} 12.0 Hz, $J_{5,6}$ 4.1 Hz, H-6^b), 4.36 (dd, 1H, J_{gem} 11.9 Hz, $J_{5,6}$ 5.2 Hz, H-6^c), 4.34 (ddt, 1H, J_{gem} 12.8 Hz, ${}^3J$ 5.3 Hz, 2×${}^4J$ 1.2 Hz, Hd), 4.27 (ddd, 1H, $J_{4,5}$ 9.9 Hz, $J_{5,6}$ 6.4 Hz, $J_{5,6}$ 1.8 Hz, H-5^a), 4.16 (ddt, 1H, J_{gem} 12.8 Hz, ${}^3J$ 6.2 Hz, 2×${}^4J$ 1.1 Hz, Hd), 4.10 (dd, 1H, J_{gem} 10.6 Hz, $J_{5,6}$ 6.4 Hz, H-6^a), 3.72 (dd, 1H, J_{gem} 10.6 Hz, $J_{5,6}$ 1.9 Hz, H-6^a), 2.10 (s, 3H, CH_3(C=O)), 1.88 (s, 3H, CH_3(C=O)); ^{13}C NMR (151 MHz, $CDCl_3$): δ 169.7 (C=O), 169.0 (C=O), 166.1 (C=O), 166.1 (C=O), 166.0 (C=O), 165.5 (C=O), 165.5 (C=O), 165.2 (C=O), 165.1 (C=O), 164.6 (C=O), 133.5 (Ar), 133.4 (Ar), 133.4 (Ar), 133.2 (Ar), 133.1 (Ar), 133.1 (*C*H=CH_2), 133.0 (Ar), 132.9 (Ar), 132.9 (Ar), 130.1 (Ar), 129.9 (Ar), 129.9 (Ar), 129.9 (Ar), 129.8 (Ar), 129.8 (Ar), 129.6 (Ar), 129.6 (Ar), 129.6 (Ar), 129.3 (Ar), 129.3 (Ar), 129.2 (Ar), 129.0 (Ar), 128.9 (Ar), 128.9 (Ar), 128.8 (Ar), 128.4 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 118.7 (CH=*C*H_2), 99.5 (C-1^b), 97.3 (C-1^c), 96.5 (C-1^a), 76.3 (C-3^a), 72.0 (C-2^a), 69.83, 69.78, 69.71, 69.7, 69.6, 69.1, 68.8, 68.7, 68.6 (C-2^b, C-2^c, C-3^b, C-3^c, C-4^a, C-5^a, C-5^b, C-5^c, *C*H_2CH=CH_2), 66.9, 66.8, 66.8 (C-4^b, C-4^c, C-6^a), 63.1, 63.0 (C-6^b, C-6^c), 20.7 (*C*H_3(C=O)), 20.4 (*C*H_3(C=O)); Coupled HSQC (500 MHz, $CDCl_3$): □99.7/5.18 ($J_{C1/H1}$ 171 Hz, C-1^b), 97.6/4.97 ($J_{C1/H1}$ 175 Hz, C-1^c), 96.8/5.12 ($J_{C1/H1}$ 175 Hz, C-1^a); Anal. calc for $C_{87}H_{72}O_{26}$: HR ESIMS $[M+Na]^+$: 1483.4204, found: 1483.4169.

Mecapto-*tert*-butyl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-[2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)]-2,4-di-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-[2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)]-2,4-di-*O*-benzoyl-1-thio-β-D-mannopyranoside 26. Trisaccharide donor **3** (84.6 mg, 54 μmol) and disaccharide acceptor **19** (53 mg, 53 μmol) were dissolved in dry CH_2Cl_2 (0.7 mL), activated molecular sieves added, and the reaction mixture cooled to 0 °C (ice water bath). TMSOTf (1 μL, 5.5 μmol) was added and after 30 minutes the reaction was quenched with a drop of TEA. Reaction mixture was concentrated under reduced pressure and the resulting slurry purified by chromatography (9:1 toluene/EtOAc) to yield **26** (0.119 g, 49 μmol, 93%) as a white glassy solid.

$[\alpha]_D^{25}$ -10 (*c* 9.1, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$): δ 7.24-8.27 (m, 65H, ArH), 6.08 (dd, 1H, $J_{2,3}$ 3.5 Hz, $J_{1,2}$ 1.1 Hz, H-2^a), 5.95 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 10.0 Hz, H-4^c), 5.89 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 10.0 Hz, H-4^e), 5.84 (dd, 1H, $J_{3,4}$ 10.4 Hz, $J_{2,3}$ 3.1 Hz, H-3^e), 5.83 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.6 Hz, H-4^d), 5.82 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 10.0 Hz, H-4^b), 5.79 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 10.0 Hz, H-4^a), 5.76 (dd, 1H, $J_{2,3}$ 3.4 Hz, $J_{1,2}$ 1.6 Hz, H-2^c), 5.59 (dd, 1H, $J_{3,4}$ 9.8 Hz, $J_{2,3}$ 3.3 Hz, H-3^d), 5.54 (dd, 1H, $J_{3,4}$ 9.6 Hz, $J_{2,3}$ 2.9 Hz, H-3^b), 5.46 (dd, 1H, $J_{2,3}$ 3.1 Hz, $J_{1,2}$ 1.9 Hz, H-2^e), 5.23 (d, 1H, $J_{1,2}$ 1.9 Hz, H-1^d), 5.18 (dd, 1H, $J_{2,3}$ 3.1 Hz, $J_{1,2}$ 2.1 Hz, H-2^d), 5.12 (d, 1H, $J_{1,2}$ 1.2 Hz, H-1^c), 5.10 (br. s, 1H, H-1^b), 5.10 (dd, 1H, $J_{2,3}$ 3.3 Hz, $J_{1,2}$ 1.9 Hz, H-2^b), 4.83 (d, 1H, $J_{1,2}$ 1.2 Hz, H-1^a), 4.80 (d, 1H, $J_{1,2}$ 1.8 Hz, H-1^e), 4.80 (ddd, 1H, $J_{4,5}$ 9.6 Hz, $J_{5,6}$ 4.0 Hz, $J_{5,6}$ 3.0 Hz, H-5^b), 4.72 (dd, 1H, J_{gem} 12.2 Hz, $J_{5,6}$ 2.6 Hz, H-6^b), 4.64 (dd, 1H, $J_{3,4}$ 9.7 Hz, $J_{2,3}$ 3.5 Hz, H-3^c), 4.52 (dd, 1H, J_{gem} 12.2 Hz, $J_{5,6}$ 4.4 Hz, H-6^b), 4.51 (dd, 1H, J_{gem} 13.3 Hz, $J_{5,6}$ 4.0 Hz, H-6^d), 4.38 (dd, 1H, J_{gem} 11.9 Hz, $J_{5,6}$ 2.4 Hz, H-6^e), 4.36 (dd, 1H, $J_{3,4}$ 10.0 Hz, $J_{2,3}$ 3.7 Hz, H-3^a), 4.34-4.37 (m, 2H, H-5^d, H-6^a), 4.35 (ddd, 1H, $J_{4,5}$ 9.5 Hz, $J_{5,6}$ 4.8 Hz, $J_{5,6}$ 2.9 Hz, H-5^e), 4.28 (dd, 1H, J_{gem} 11.8 Hz, $J_{5,6}$ 5.1 Hz, H-6^e), 4.24 (ddd, 1H, $J_{4,5}$ 10.2 Hz, $J_{5,6}$ 5.1 Hz, $J_{5,6}$ 2.0 Hz, H-5^c), 4.18 (dd, 1H, J_{gem} 11.1 Hz, $J_{5,6}$ 6.1 Hz, H-6^a), 4.01 (dd, 1H, J_{gem} 10.8 Hz, $J_{5,6}$ 5.2 Hz, H-6^c), 3.92 (ddd, 1H, $J_{4,5}$ 10.0 Hz, $J_{5,6}$ 6.2 Hz, $J_{5,6}$ 1.8 Hz, H-5^a), 3.75 (dd, 1H, J_{gem} 11.1 Hz, $J_{5,6}$ 1.6 Hz, H-6^a), 3.49 (dd, 1H, J_{gem} 10.6 Hz, $J_{5,6}$ 1.9 Hz, H-6^c), 2.10

(s, 3H, CH_3(C=O)), 1.92 (s, 3H, CH_3(C=O)), 1.77 (s, 3H, CH_3(C=O)), 1.38 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (176 MHz, $CDCl_3$): δ 169.5 (C=O), 168.9 (C=O), 168.7 (C=O), 166.2 (C=O), 166.2 (C=O), 166.1 (C=O), 166.0 (C=O), 165.9 (C=O), 165.6 (C=O), 165.6 (C=O), 165.4 (C=O), 165.2 (2×C=O), 165.1 (C=O), 164.6 (C=O), 164.6 (C=O), 133.5 (Ar), 133.4 (Ar), 133.3 (Ar), 133.1 (Ar), 133.1 (Ar), 133.0 (Ar), 133.0 (Ar), 132.9 (Ar), 132.9 (Ar), 130.2 (Ar), 130.2 (Ar), 130.0 (Ar), 130.0 (Ar), 130.0 (Ar), 129.9 (Ar), 129.8 (Ar), 129.8 (Ar), 129.7 (Ar), 129.7 (Ar), 129.7 (Ar), 129.6 (Ar), 129.5 (Ar), 129.5 (Ar), 129.4 (Ar), 129.3 (Ar), 129.2 (Ar), 129.2 (Ar), 129.1 (Ar), 129.1 (Ar), 128.9 (Ar), 128.9 (Ar), 128.7 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.4 (Ar), 128.3 (Ar), 128.3 (Ar), 128.2 (Ar), 128.2 (Ar), 100.0 (C-1^d), 99.3 (C-1^b), 97.6 (C-1^c), 97.3 (C-1^e), 92.0 (C-1^a), 77.7, 77.4 (C-3^c, C-5^a), 77.4 (C-3^a), 72.6 (C-2^a), 71.9 (C-2^c), 70.1, 69.67, 2×69.67, 69.5, 2×69.4, 69.3, 69.0, 68.7, 68.6 (C-4^a, C-2^b, C-3^b, C-5^b, C-5^c, C-2^d, C-3^d, C-5^d, C-2^e, C-3^e, C-5^e), 67.9 (C-4^c), 66.9 (C-4^b), 66.74 (C-6^a), 66.66 (C-4^d, C-4^e), 66.0 (C-6^c), 63.1, 63.0, 62.8 (C-6^b, C-6^d, C-6^e), 47.9 ($C(CH_3)_3$), 29.9 (C(CH_3)$_3$), 20.7 (CH_3(C=O)), 20.4 (CH_3(C=O)), 20.2 (CH_3(C=O)); Coupled HSQC (700 MHz, $CDCl_3$): □ 100.0/5.23 ($J_{C1/H1}$ 174 Hz, C-1^d), 99.3/5.10 ($J_{C1/H1}$ 177 Hz, C-1^b), 97.6/5.12 ($J_{C1/H1}$ 175 Hz, C-1^c),-97.3/4.80 ($J_{C1/H1}$ 175 Hz, C-1^e), 92.0/4.83 ($J_{C1/H1}$ 157 Hz, C-1^a); Anal. calc for $C_{131}H_{118}O_{41}S$: HR ESIMS $[M+Na]^+$: 2433.6482, found: 2433.6434.

2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-1-thio-β-D-mannopyranose 27. Tetrasaccharide **22** (21.5 mg, 10 µmol) was dissolved in a 1 M solution of trimethylphosphine in THF (1 mL, 1 mmol) and a 1 M solution of $NaHCO_3$ (1 mL, pH 9) added and stirred vigourously. After 1.5 hours, the reaction mixture was neutralized with dilute AcOH and extracted with EtOAc three times and the organic layer concentrated under reduced pressure. Purification of the crude product by chromatography (1:1 heptane/EtOAc) yielded **27** (20.4 mg, 10 µmol, 99%) as a white powder.

$[\alpha]_D^{25}$ -45 (*c* 7.0, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$): δ 7.06-8.34 (m, 60H, Ar), 5.84 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.9 Hz, H-4^b), 5.81 (dd, 1H, $J_{2,3}$ 3.4 Hz, $J_{1,2}$ 0.9 Hz, H-2^a), 5.79 (dd, 1H, $J_{3,4}$ 10.2 Hz, $J_{2,3}$ 3.0 Hz, H-3^c), 5.78 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.9 Hz, H-4^a), 5.75 (dd, 1H, $J_{3,4}$ 9.9 Hz, $J_{2,3}$ 3.4 Hz, H-3^d), 5.75 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.9 Hz, H-4^c), 5.67 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 10.1 Hz, H-4^d), 5.62 (dd, 1H, $J_{2,3}$ 3.3, $J_{1,2}$ 1.8 Hz, H-2^d), 5.48 (dd, 1H, $J_{3,4}$ 9.8 Hz, $J_{2,3}$ 3.3 Hz, H-3^b), 5.35 (d, 1H, $J_{1,2}$ 1.5 Hz, H-1^b), 4.92 (ddd, 1H, $J_{4,5}$ 10.1 Hz, $J_{5,6}$ 5.8 Hz, $J_{5,6}$ 2.6 Hz, H-5^b), 4.86 (d, 1H, $J_{1,2}$ 1.5 Hz, H-1^d), 4.73 (dd, 1H, J_{gem} 12.2 Hz, $J_{5,6}$ 5.8 Hz, H-6^b), 4.67 (dd, 1H, $J_{1,SH}$ 10.1 Hz, $J_{1,2}$ 1.0 Hz, H-1^a), 4.63 (s, 1H, H-1^c), 4.61 (dd, 1H, J_{gem} 9.2 Hz, $J_{5,6}$ 2.6 Hz, H-6^b), 4.59 (dd, 1H, J_{gem} 12.2 Hz, $J_{5,6}$ 3.1 Hz, H-6^a), 4.34 (dd, 1H, J_{gem} 12.3 Hz, $J_{5,6}$ 4.4 Hz, H-6^a), 4.29 (ddd, 1H, $J_{4,5}$ 9.8, $J_{5,6}$ 5.9 Hz, $J_{5,6}$ 2.2 Hz, H-5^c), 4.25 (dd, 1H, $J_{2,3}$ 2.7, $J_{1,2}$ 2.1 Hz, H-2^c), 4.20 (dd, 1H, J_{gem} 12.1 Hz, $J_{5,6}$ 6.1 Hz, H-6^c), 4.11 (br. d, 1H, J_{gem} 11.9 Hz, H-6^c), 4.08 (dd, 1H, $J_{3,4}$ 9.9 Hz, $J_{2,3}$ 3.3 Hz, H-3^a), 4.02 (dt, 1H, $J_{4,5}$ 9.9 Hz, $J_{5,6}$ 4.0 Hz, H-5^d), 3.96 (d, 2H, $J_{5,6}$ 3.2 Hz, H-6^d), 3.88 (dd, 1H, $J_{2,3}$ 2.7 Hz, $J_{1,2}$ 1.5 Hz, H-2^b), 3.57 (dt, 1H, $J_{4,5}$ 10.0 Hz, 2×$J_{5,6}$ 3.7 Hz, H-5^a), 2.59 (d, 1H, $J_{1,SH}$ 10.1 Hz, SH), 1.99 (s, 3H, CH_3(C=O)O); ^{13}C NMR (126 MHz, $CDCl_3$): δ 168.9 (C=O), 166.4 (C=O), 166.1 (C=O), 166.0 (C=O), 165.9 (C=O), 165.7 (C=O), 165.7 (C=O), 165.4 (C=O), 165.3 (C=O), 165.1 (C=O), 165.1 (C=O), 165.0 (C=O), 164.7 (C=O), 134.1 (Ar), 133.8 (Ar), 133.4 (Ar), 133.3 (Ar), 133.3 (Ar), 133.2 (Ar), 133.2 (Ar), 133.1 (Ar), 133.1 (Ar), 133.0 (Ar), 133.0 (Ar), 132.9 (Ar), 132.9 (Ar), 132.8 (Ar), 130.3 (Ar), 130.3 (Ar), 130.0 (Ar), 130.0 (Ar), 130.0 (Ar), 130.0 (Ar), 129.9 (Ar), 129.9 (Ar), 129.9 (Ar), 129.8 (Ar), 129.8 (Ar), 129.7 (Ar), 129.7 (Ar), 129.7 (Ar), 129.4 (Ar), 129.4 (Ar), 129.3 (Ar), 129.3 (Ar), 129.3 (Ar), 129.1 (Ar), 128.9 (Ar), 128.9 (Ar), 128.9 (Ar), 128.8 (Ar), 128.6 (Ar), 128.6 (Ar), 128.5 (Ar), 128.5 (Ar), 128.4 (Ar), 128.4 (Ar), 128.4 (Ar), 128.3 (Ar), 128.3 (Ar), 128.2 (Ar), 100.7 (C-1^c), 100.0 (C-1^b), 99.3 (C-1^d), 78.1 (C-2^b), 76.9 (C-1^a), 76.7 (C-3^a), 76.6 (C-5^a), 75.1 (C-2^c), 74.1 (C-2^a), 71.0 (C-3^c), 69.9 (C-5^c), 69.78 (C-3^b), 69.74 (C-5^b), 69.4 (C-2^d), 69.3 (C-3^d), 69.3 (C-5^d), 68.5 (C-4^a), 67.6 (C-4^d), 67.4 (C-4^b), 66.7 (C-4^c), 64.4 (C-6^b), 63.9 (C-6^c), 63.07 (C-6^a), 63.03 (C-6^d), 20.6 (CH_3C=O); Coupled HSQC (700 MHz, $CDCl_3$): □100.7/4.63 ($J_{C1/H1}$ 175 Hz, C-1^c), 99.98/5.35 ($J_{C1/H1}$ 178 Hz, C-1^b),

99.3/4.85 ($J_{C1/H1}$ 176 Hz, C-1^{d}), 76.94/4.67 ($J_{C1/H1}$ 152 Hz, C-1^{a}); Anal. calc for $C_{110}H_{92}O_{33}S$: HR ESIMS $[M+Na]^+$: 1995.5134, found: 1995.5105.

2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-[2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)]-2,4-di-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-[2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)]-2,4-di-*O*-benzoyl-β-D-mannopyranosyl thiol 28. Pentasaccharide **26** (20.5 mg, 8.5 μmol) was dissolved in a 1 M solution of trimethylphosphine in THF (1 mL, 1 mmol) and a 1 M solution of $NaHCO_3$ (1 mL, pH 9) added and stirred vigourously. After 30 minutes, the reaction mixture was neutralized with dilute AcOH and extracted with EtOAc three times and the organic layer concentrated under reduced pressure. Purification of the crude product by chromatography (1:1 heptane/EtOAc) yielded **28** (19.7 mg, 8.5 μmol, 99%) as a white powder.

^{1}H NMR (700 MHz, $CDCl_3$): δ 7.26 (m, 65H, ArH), 5.87 (d, 1H, $J_{2,3}$ 2.4 Hz, H-2^{a}), 5.86 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 10.0 Hz, H-4^{e}), 5.85 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.4 Hz, H-4^{a}), 5.84 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.8 Hz, H-4^{d}), 5.83 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 10.0 Hz, H-4^{c}), 5.78 (dd, 1H, $J_{3,4}$ 10.1 Hz, $J_{2,3}$ 3.3 Hz, H-3^{e}), 5.73 (dd, 1H, $J_{2,3}$ 3.5 Hz, $J_{1,2}$ 1.7 Hz, H-2^{c}), 5.74 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.8 Hz, H-4^{b}), 5.57 (dd, 1H, $J_{3,4}$ 9.8 Hz, $J_{2,3}$ 3.2 Hz, H-3^{d}), 5.51 (dd, 1H, $J_{3,4}$ 9.6 Hz, $J_{2,3}$ 3.4 Hz, H-3^{b}), 5.35 (dd, 1H, $J_{2,3}$ 3.3 Hz, $J_{1,2}$ 1.8 Hz, H-2^{e}), 5.23 (d, 1H, $J_{1,2}$ 2.0 Hz, H-1^{d}), 5.18 (dd, 1H, $J_{2,3}$ 3.3 Hz, $J_{1,2}$ 2.0 Hz, H-2^{d}), 5.11 (d, 1H, $J_{1,2}$ 1.4 Hz, H-1^{c}), 5.10 (dd, 1H, $J_{2,3}$ 3.4 Hz, $J_{1,2}$ 1.9 Hz, H-2^{b}), 5.05 (d, 1H, $J_{1,2}$ 2.0 Hz, H-1^{b}), 4.88 (dd, 1H, $J_{1,SH}$ 10.4 Hz, $J_{1,2}$ 1.2 Hz, H-1^{a}), 4.85 (ddd, 1H, $J_{4,5}$ 10.0 Hz, $J_{5,6}$ 5.3 Hz, $J_{5,6}$ 2.5 Hz, H-5^{b}), 4.71 (d, 1H, $J_{1,2}$ 1.8 Hz, H-1^{e}), 4.65 (dd, 1H, J_{gem} 11.4 Hz, $J_{5,6}$ 2.5 Hz, H-6^{b}), 4.63 (dd, 1H, $J_{3,4}$ 9.9 Hz, $J_{2,3}$ 3.5 Hz, H-3^{c}), 4.58 (dd, 1H, J_{gem} 12.2 Hz, $J_{5,6}$ 5.3 Hz, H-6^{b}), 4.53 (dd, 1H, J_{gem} 13.0 Hz, $J_{5,6}$ 3.5 Hz, H-6^{d}), 4.40 (dd, 1H, J_{gem} 11.2 Hz, $J_{5,6}$ 2.5 Hz, H-6^{e}), 4.38 (dd, 1H, $J_{3,4}$ 9.9 Hz, $J_{2,3}$ 3.2 Hz, H-3^{a}), 4.36 (ddd, 1H, $J_{4,5}$ 9.8 Hz, $J_{5,6}$ 5.3 Hz, $J_{5,6}$ 2.4 Hz, H-5^{e}), 4.29-4.33 (m, 2H, H-5^{d}, H-6^{d}), 4.28 (dd, 1H, J_{gem} 11.8 Hz, $J_{5,6}$ 5.2 Hz, H-6^{e}), 4.20 (ddd, 1H, $J_{4,5}$ 10.2 Hz, $J_{5,6}$ 6.0 Hz, $J_{5,6}$ 2.0 Hz, H-5^{c}), 4.15 (dd, 1H, J_{gem} 11.2 Hz, $J_{5,6}$ 5.3 Hz, H-6^{a}), 3.94 (dd, 1H, J_{gem} 10.4 Hz, $J_{5,6}$ 5.8 Hz, H-6^{c}), 3.94 (ddd, 1H, $J_{4,5}$ 9.9 Hz, $J_{5,6}$ 5.1 Hz, $J_{5,6}$ 1.7 Hz, H-5^{a}), 3.79 (dd, 1H, J_{gem} 11.2 Hz, $J_{5,6}$ 1.9 Hz, H-6^{a}), 3.37 (dd, 1H, J_{gem} 10.5 Hz, $J_{5,6}$ 2.0 Hz, H-6^{c}), 2.70 (d, 1H, $J_{1,SH}$ 10.4 Hz, SH), 2.07 (s, 3H, CH_3(C=O)), 1.95 (s, 3H, CH_3(C=O)), 1.75 (s, 3H, CH_3(C=O)); ^{13}C NMR (126 MHz, $CDCl_3$): δ 169.6 (C=O), 169.0 (C=O), 168.6 (C=O), 166.3 (C=O), 166.2 (C=O), 166.1 (C=O), 166.0 (C=O), 166.0 (C=O), 165.6 (C=O), 165.6 (C=O), 165.5 (C=O), 165.2 (C=O), 165.2 (C=O), 165.1 (C=O), 164.6 (C=O), 164.6 (C=O), 133.7 (Ar), 133.5 (Ar), 133.5 (Ar), 133.4 (Ar), 133.2 (Ar), 133.1 (Ar), 133.0 (Ar), 132.9 (Ar), 132.9 (Ar), 130.2 (Ar), 130.2 (Ar), 130.0 (Ar), 130.0 (Ar), 130.0 (Ar), 129.9 (Ar), 129.8 (Ar), 129.8 (Ar), 129.7 (Ar), 129.7 (Ar), 129.7 (Ar), 129.6 (Ar), 129.6 (Ar), 129.5 (Ar), 129.4 (Ar), 129.4 (Ar), 129.4 (Ar), 129.2 (Ar), 129.1 (Ar), 129.1 (Ar), 129.0 (Ar), 128.9 (Ar), 128.9 (Ar), 128.8 (Ar), 128.7 (Ar), 128.5 (Ar), 128.5 (Ar), 128.4 (Ar), 128.4 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 128.2 (Ar), 100.1 (C-1^{d}), 99.3 (C-1^{b}), 97.6 (C-1^{c}), 97.1 (C-1^{e}), 77.9 (C-5^{a}), 77.8 (C-3^{c}), 77.7 (C-3^{a}), 77.5 (C-1^{a}), 74.2 (C-2^{a}), 71.9 (C-2^{c}), 70.0, 69.8, 69.7, 69.68, 69.62, 69.4, 69.35, 69.31, 68.9, 68.8, 68.3, 68.1, 67.1, 66.7, 66.6, 66.5, 66.2 (C-4^{a}, C-6^{a}, C-2^{b}, C-3^{b}, C-4^{b}, C-5^{b}, C-4^{c}, C-5^{c}, C-6^{c}, C-2^{d}, C-3^{d}, C-4^{d}, C-5^{d}, C-2^{e}, C-3^{e}, C-4^{e}, C-5^{e}), 63.3 (C-6^{b}), 63.0 (C-6^{e}), 62.8 (C-6^{d}), 20.7 (CH$_3$(C=O)), 20.5 (CH$_3$(C=O)), 20.2 (CH$_3$(C=O)); Coupled HSQC (700 MHz, $CDCl_3$): □ 100.1/5.23 ($J_{C1/H1}$ 175 Hz, C-1^{d}), 99.3/5.05 ($J_{C1/H1}$ 176 Hz, C-1^{b}), 97.6/5.11 ($J_{C1/H1}$ 176 Hz, C-1^{c}), 97.1/4.71 ($J_{C1/H1}$ 176 Hz, C-1^{e}), 77.9/4.88 ($J_{C1/H1}$ 163 Hz, C-1^{a}); Anal. calc for $C_{127}H_{110}O_{41}S$: HR ESIMS $[M+Na]^+$: 2345.6135, found: 2345.6132.

Chloromethyl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-1-thio-β-D-mannopyranoside 29. To a solution of tetrasaccharide **27** (20.4 mg, 10.3 μmol) in CH_2Cl_2 (5 mL) was added DBU (6 μL, 20 μmol). After 2 hours, reaction was diluted with toluene and concentrated under reduced pressure. Crude product **29** was sensitive to oxidation and unstable on silica gel and was used immediately for the next step.

$[\alpha]_D^{25}$ -70 (*c* 16.9, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$): δ 7.10-8.28 (m, 60H, Ar), 5.86 (d, 1H, $J_{2,3}$ 3.5 Hz, H-2^a), 5.85 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.5 Hz, H-4^b), 5.81 (dd, 1H, $J_{3,4}$ 10.1 Hz, $J_{2,3}$ 3.1 Hz, H-3^c), 5.76 (dd, 1H, $J_{3,4}$ 10.1 Hz, $J_{2,3}$ 3.6 Hz, H-3^d), 5.76 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.5 Hz, H-4^a), 5.72 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 10.1 Hz, H-4^c), 5.68 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.9 Hz, H-4^d), 5.63 (dd, 1H, $J_{2,3}$ 3.2 Hz, $J_{1,2}$ 1.9 Hz, H-2^d), 5.44 (dd, 1H, $J_{3,4}$ 9.9 Hz, $J_{2,3}$ 3.3 Hz, H-3^b), 5.39 (s, 1H, H-1^b), 5.05 (s, 1H, H-1^a), 5.04 (d, 1H, J_{gem} 11.8 Hz, SCH_2Cl), 4.86 (s, 1H, H-1^d), 4.77 (ddd, 1H, $J_{4,5}$ 9.8 Hz, $J_{5,6}$ 5.7 Hz, $J_{5,6}$ 2.6 Hz, H-5^b), 4.72 (dd, 1H, J_{gem} 12.2 Hz, $J_{5,6}$ 5.5 Hz, H-6^b), 4.70 (d, 1H, J_{gem} 11.6 Hz, SCH_2Cl), 4.59 (dd, 1H, J_{gem} 12.0 Hz, $J_{5,6}$ 2.8 Hz, H-6^a), 4.58 (dd, 1H, J_{gem} 12.0 Hz, $J_{5,6}$ 2.2 Hz, H-6^b), 4.56 (s, 1H, H-1^c), 4.41 (m, 1H, H-5^c), 4.40 (dd, 1H, J_{gem} 12.5 Hz, $J_{5,6}$ 5.7 Hz, H-6^a), 4.37 (dd, 1H, J_{gem} 11.7 Hz, $J_{5,6}$ 6.8 Hz, H-6^c), 4.26 (br. s., 1H, H-2^c), 4.20 (br. d, 1H, J_{gem} 11.6 Hz, H-6^c), 3.99 (dd, 1H, $J_{3,4}$ 9.3 Hz, $J_{2,3}$ 3.73 Hz, H-3^a), 3.98 (m, 1H, H-5^d), 3.95 (m, 1H, H-6^d), 3.92 (s, 1H, H-2^b), 3.89 (m, 1H, H-6^d), 3.50 (ddd, 1H, $J_{4,5}$ 9.6 Hz, $J_{5,6}$ 5.5 Hz, $J_{5,6}$ 3.0 Hz, H-5^a), 1.99 (s, 3H, CH_3(C=O)); ^{13}C NMR (176 MHz, $CDCl_3$): δ 168.9 (C=O), 166.4 (C=O), 166.0 (C=O), 165.9 (C=O), 165.6 (C=O), 165.6 (C=O), 165.4 (C=O), 165.1 (C=O), 165.1 (C=O), 164.9 (C=O), 164.6 (C=O), 134.2 (Ar), 133.6 (Ar), 133.4 (Ar), 133.3 (Ar), 133.2 (Ar), 133.2 (Ar), 133.1 (Ar), 133.1 (Ar), 132.9 (Ar), 132.8 (Ar), 130.2 (Ar), 130.0 (Ar), 130.0 (Ar), 129.9 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7 (Ar), 129.7 (Ar), 129.6 (Ar), 129.6 (Ar), 129.4 (Ar), 129.4 (Ar), 129.2 (Ar), 129.2 (Ar), 129.2 (Ar), 129.1 (Ar), 128.9 (Ar), 128.8 (Ar), 128.8 (Ar), 128.7 (Ar), 128.7 (Ar), 128.5 (Ar), 128.4 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 128.2 (Ar), 128.1 (Ar), 101.0 (C-1^c), 100.1 (C-1^b), 99.2 (C-1^d), 79.2 (C-1^a), 78.5 (C-2^b), 76.60 (C-3^a), 76.55 (C-5^a), 74.8 (C-2^c), 71.7 (C-2^a), 71.0 (C-3^c), 69.9 (C-5^c), 69.7 (C-3^b), 69.5 (C-5^b), 69.4 (C-2^d), 69.1 (C-3^d), 69.1 (C-4^a), 68.8 (C-5^d), 67.7 (C-4^d), 67.3 (C-4^b), 66.8 (C-4^c), 64.3 (C-6^b), 64.1 (C-6^c), 63.0 (C-6^a), 63.0 (C-6^d), 46.0 (S*C*H_2Cl), 20.5 (*C*H_3(C=O)); Anal. calc for $C_{111}H_{93}ClO_{33}S$: HR ESIMS $[M+Na]^+$: 2043.4901, found: 2043.4887.

Chloromethyl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-[2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)]-2,4-di-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-[2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)]-2,4-di-*O*-benzoyl-1-thio-β-D-mannopyranoside 30. To a solution of pentasaccharide **28** (20 mg, 8.6 μmol) in CH_2Cl_2 (5 mL) was added DBU (3 μL, 20 μmol). After 2.5 hours, reaction was diluted with toluene and concentrated under reduced pressure. Crude product **30** was sensitive to oxidation and unstable on silica gel and was used immediately for the next step.

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.26 (s, 65H, Ar), 6.00 (dd, 1H, $J_{2,3}$ 3.5 Hz, $J_{1,2}$ 0.9 Hz, H-2^a), 5.85 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.9 Hz, H-4^e), 5.85 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.6 Hz, H-4^c), 5.83 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.8 Hz, H-4^a), 5.81 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.8 Hz, H-4^b), 5.75 (dd, 1H, $J_{3,4}$ 9.9 Hz, $J_{2,3}$ 3.2 Hz, H-3^e), 5.76 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.9 Hz, H-4^d), 5.71 (dd, 1H, $J_{2,3}$ 3.5 Hz, $J_{1,2}$ 1.7 Hz, H-2^c), 5.57 (dd, 1H, $J_{3,4}$ 9.7 Hz, $J_{2,3}$ 3.3 Hz, H-3^b), 5.49 (dd, 1H, $J_{3,4}$ 9.6 Hz, $J_{2,3}$ 3.4 Hz, H-3^d), 5.36 (dd, 1H, $J_{2,3}$ 3.4 Hz, $J_{1,2}$ 1.7 Hz, H-2^e), 5.32 (d, 1H, $J_{1,2}$ 1.0 Hz, H-1^a), 5.21 (d, 1H, $J_{1,2}$ 2.1 Hz, H-1^b), 5.16 (dd, 1H, $J_{2,3}$ 3.3 Hz, $J_{1,2}$ 2.1 Hz, H-2^b), 5.14 (d, 1H, $J_{1,2}$ 1.8 Hz, H-1^c), 5.14 (d, 1H, J_{gem} 11.8 Hz, SCH_2Cl), 5.10 (d, 1H, $J_{1,2}$ 2.1 Hz, H-1^d), 5.08 (dd, 1H, $J_{2,3}$ 3.4 Hz, $J_{1,2}$ 2.0 Hz, H-2^d), 4.77 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1^e), 4.77 (d, 1H, J_{gem} 11.8 Hz, SCH_2Cl), 4.66 (ddd, 1H, $J_{4,5}$ 9.9 Hz, $J_{5,6}$ 4.8 Hz, $J_{5,6}$ 2.8 Hz, H-5^d), 4.63 (dd, 1H, J_{gem} 11.8 Hz, $J_{5,6}$ 2.9 Hz, H-6^d), 4.62 (dd, 1H, $J_{3,4}$ 9.9 Hz, $J_{2,3}$ 3.2 Hz, H-3^c), 4.50 (dd, 1H, J_{gem} 12.3 Hz, $J_{5,6}$ 3.1 Hz, H-6^b), 4.49 (dd, 1H, J_{gem} 12.1 Hz, $J_{5,6}$ 4.2 Hz, H-6^d), 4.45 (dd, 1H, $J_{3,4}$ 9.7 Hz, $J_{2,3}$ 3.4 Hz, H-3^a), 4.38 (dd, 1H, J_{gem} 11.7 Hz, $J_{5,6}$ 2.5 Hz, H-6^e), 4.33-4.36 (m, 1H, H-5^e), 4.32-4.35 (m, 1H, H-6^b), 4.30-4.34 (m, 1H, H-5^b), 4.28 (dd, 1H, $J_{4,5}$ 11.7 Hz, $J_{5,6}$ 5.2 Hz, H-6^e), 4.22 (ddd, 1H, $J_{4,5}$ 10.2 Hz, $J_{5,6}$ 5.7 Hz, $J_{5,6}$ 2.9 Hz, H-5^c), 4.17 (dd, 1H, J_{gem} 11.1 Hz, $J_{5,6}$ 6.1 Hz, H-6^a), 4.03 (ddd, 1H, $J_{4,5}$ 10.0 Hz, $J_{5,6}$ 6.3 Hz, $J_{5,6}$ 2.2 Hz, H-5^a), 3.98 (dd, 1H, J_{gem} 10.7 Hz, $J_{5,6}$ 5.4 Hz, H-6^c), 3.79 (dd, 1H, J_{gem} 11.4 Hz, $J_{5,6}$ 2.2 Hz, H-6^a), 3.50 (dd, 1H, J_{gem} 10.6 Hz, $J_{5,6}$ 2.8 Hz, H-6^c), 2.09 (s, 3H, CH_3(C=O)), 1.92 (s, 3H, CH_3(C=O)), 1.76 (s, 3H, CH_3(C=O)); ^{13}C NMR (176 MHz, $CDCl_3$): δ 169.5 (C=O), 168.9 (C=O), 168.7 (C=O), 166.1 (C=O), 166.1 (C=O), 166.1 (C=O), 165.9 (C=O), 165.9 (C=O), 165.6 (C=O), 165.5 (C=O), 165.3 (C=O), 165.2 (C=O), 165.2 (C=O), 165.1 (C=O), 164.6 (C=O), 164.5 (C=O), 133.6 (Ar), 133.5 (Ar), 133.5 (Ar), 133.3 (Ar), 133.3 (Ar), 133.3 (Ar), 133.1 (Ar), 133.0 (Ar), 133.0 (2×Ar),

132.9 (Ar), 132.9 (2×Ar), 130.1 (Ar), 130.1 (Ar), 130.0 (Ar), 130.0 (Ar), 129.9 (Ar), 129.9 (Ar), 129.7 (Ar), 129.7 (Ar), 129.7 (Ar), 129.6 (Ar), 129.5 (Ar), 129.5 (Ar), 129.3 (Ar), 129.3 (Ar), 129.3 (Ar), 129.1 (Ar), 129.0 (Ar), 129.0 (Ar), 128.9 (Ar), 128.9 (Ar), 128.9 (Ar), 128.6 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.4 (Ar), 128.3 (Ar), 128.3 (Ar), 128.2 (Ar), 128.2 (Ar), 128.1 (Ar), 100.0 (C-1^{d}), 99.4 (C-1^{b}), 97.4 (C-1^{c}), 97.3 (C-1^{e}), 80.5 (C-1^{a}), 77.9 (C-5^{a}), 77.7 (C-3^{a}), 77.1 (C-3^{b}), 71.9, 71.9 (C-2^{a}, C-2^{c}), 69.9, 69.7, 69.59, 69.57, 69.51, 2×69.3, 69.2, 68.9, 68.8, 68.6, 68.5 (C-4^{a}, C-2^{b}, C-3^{b}, C-5^{b}, C-4^{c}. C-5^{c}, C-2^{d}, C-3^{d}, C-5^{d}, C-2^{e}, C-3^{e}, C-5^{e}), 66.9 (C-6^{a}), 66.8, 66.7, 66.6 (C-4^{b}, C-4^{d}, C-4^{e}), 66.5 (C-6^{c}), 63.2, 62.9, 62.8 (C-6^{b}, C-6^{d}, C-6^{e}), 46.4 (SCH_2Cl), 20.7 (CH_3(C=O)), 20.4 (CH_3(C=O)), 20.2 (CH_3(C=O)); Anal. calc for $C_{128}H_{111}ClO_{41}S$: HR ESIMS $[M+Na]^+$: 2393.5902, found: 2393.5850.

Azidomethyl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-1-thio-β-D-mannopyranoside 31. Crude tetrasaccharide **29** (~10.3 μmol) was dissolved in acetone (3 mL) and 15 mg (0.23 mmol) of NaN_3 added. Reaction was heated to 60 °C and H_2O was added dropwise until the solution became homogenous. After 24 hours at 60 °C, the reaction mixture was cooled and diluted with EtOAc, washed with H_2O, then brine, and dried over Na_2SO_4. The organic layer was concentrated under reduced pressure and the crude yellow powder purified by chromatography (9.7:0.3 toluene/acetonitrile) to yield **31** (16 mg, 7.89 μmol, 77% from **27**) as a white solid.

$[\alpha]_D^{25}$ -70 (*c* 8.7, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$): δ 7.09-8.29 (m, 60H, ArH), 5.88 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 10.0 Hz, H-4^{b}), 5.86 (dd, 1H, $J_{2,3}$ 3.5, $J_{1,2}$ 0.5 Hz, H-2^{a}), 5.80 (dd, 1H, $J_{3,4}$ 10.2 Hz, $J_{2,3}$ 3.1 Hz, H-3^{c}), 5.79 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.8 Hz, H-4^{a}), 5.76 (dd, 1H, $J_{3,4}$ 10.1 Hz, $J_{2,3}$ 3.5 Hz, H-3^{d}), 5.74 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 10.1 Hz, H-4^{c}), 5.68 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.9 Hz, H-4^{d}), 5.62 (dd, 1H, $J_{2,3}$ 3.2 Hz, $J_{1,2}$ 1.8 Hz, H-2^{d}), 5.46 (dd, 1H, $J_{3,4}$ 9.8 Hz, $J_{2,3}$ 3.4 Hz, H-3^{b}), 5.39 (d, 1H, $J_{1,2}$ 1.5 Hz, H-1^{b}), 4.91 (d, 1H, $J_{1,2}$ 0.5 Hz, H-1^{a}), 4.86 (d, 1H, $J_{1,2}$ 1.4 Hz, H-1^{d}), 4.76 (ddd, 1H, $J_{4,5}$ 9.9 Hz, $J_{5,6}$ 4.9 Hz, $J_{5,6}$ 2.8 Hz, H-5^{b}), 4.69 (dd, 1H, J_{gem} 12.1 Hz, $J_{5,6}$ 4.9 Hz, H-6^{b}), 4.62 (br. s, 1H, H-1^{c}), 4.62 (dd, 1H, J_{gem} 12.2 Hz, $J_{5,6}$ 3.0 Hz, H-6^{b}), 4.61 (dd, 1H, J_{gem} 12.5 Hz, $J_{5,6}$ 2.8 Hz, H-6^{a}), 4.50 (d, 1H, J_{gem} 13.3 Hz, SCH_2N_3), 4.39 (dd, 1H, J_{gem} 12.2 Hz, $J_{5,6}$ 5.2 Hz, H-6^{a}), 4.38 (d, 1H, J_{gem} 13.1 Hz, SCH_2N_3), 4.35 (ddd, 1H, $J_{4,5}$ 10.0 Hz, $J_{5,6}$ 6.3 Hz, $J_{5,6}$ 2.2 Hz, H-5^{c}), 4.29 (dd, 1H, J_{gem} 12.0 Hz, $J_{5,6}$ 6.2 Hz, H-6^{c}), 4.26 (dd, 1H, $J_{2,3}$ 2.8 Hz, $J_{1,2}$ 1.9 Hz, H-2^{c}), 4.17 (br. d, 1H, J_{gem} 11.8 Hz, H-6^{c}), 4.04 (dd, 1H, $J_{3,4}$ 9.8 Hz, $J_{2,3}$ 3.2 Hz, H-3^{a}), 4.00 (dt, 1H, $J_{4,5}$ 9.9 Hz, 2×$J_{5,6}$ 4.1 Hz, H-5^{d}), 3.95 (dd, 1H, J_{gem} 12.1 Hz, $J_{5,6}$ 4.3 Hz, H-6^{d}), 3.92 (br. s., 1H, H-2^{b}), 3.91 (dd, 1H, J_{gem} 13.7 Hz, $J_{5,6}$ 3.2 Hz, H-6^{d}), 3.57 (ddd, 1H, $J_{4,5}$ 9.8 Hz, $J_{5,6}$ 5.0 Hz, $J_{5,6}$ 3.2 Hz, H-5^{a}), 2.00 (s, 3H, CH_3(C=O)); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.0 (C=O), 166.5 (C=O), 166.1 (C=O), 166.0 (C=O), 166.0 (C=O), 165.7 (C=O), 165.4 (C=O), 165.1 (C=O), 165.0 (C=O), 164.7 (C=O), 134.1 (Ar), 133.6 (Ar), 133.4 (Ar), 133.3 (Ar), 133.3 (Ar), 133.2 (Ar), 133.1 (Ar), 133.0 (Ar), 132.8 (Ar), 130.3 (Ar), 130.0 (Ar), 130.0 (Ar), 130.0 (Ar), 129.9 (Ar), 129.7 (Ar), 129.7 (Ar), 129.7 (Ar), 129.6 (Ar), 129.5 (Ar), 128.9 (Ar), 128.7 (Ar), 128.7 (Ar), 128.6 (Ar), 128.5 (Ar), 128.5 (Ar), 128.5 (Ar), 128.4 (Ar), 128.4 (Ar), 128.3 (Ar), 128.3 (Ar), 128.2 (Ar), 100.9 (C-1^{c}), 100.2 (C-1^{b}), 99.2 (C-1^{d}), 80.0 (C-1^{a}), 78.2 (C-2^{b}), 77.2 (C-3^{a}), 76.6 (C-5^{a}), 75.0 (C-2^{c}), 72.0 (C-2^{a}), 71.0 (C-3^{c}), 69.9 (C-5^{c}), 69.8 (C-3^{b}), 69.5 (C-5^{b}), 69.4 (C-2^{d}), 69.2 (C-3^{d}), 69.2 (C-4^{a}), 68.8 (C-5^{d}), 67.7 (C-4^{d}), 67.3 (C-4^{b}), 66.9 (C-4^{c}), 64.2 (C-6^{b}), 64.0 (C-6^{c}), 63.1 (C-6^{a},C-6^{d}), 51.8 (SCH_2N_3), 20.6 (CH_3(C=O)); Coupled HSQC (700 MHz, $CDCl_3$): □100.9/4.61 ($J_{C1/H1}$ 174 Hz, C-1^{c}), 100.1/5.39 ($J_{C1/H1}$ 177 Hz, C-1^{b}), 99.2/4.86 ($J_{C1/H1}$ 176 Hz, C-1^{d}), 80.0/4.91 ($J_{C1/H1}$ 156 Hz, C-1^{a}); Anal. calc for $C_{111}H_{93}N_3O_{33}S$: HR ESIMS $[M+Na]^+$: 2050.5304, found: 2050.5232.

Azidomethyl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-[2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)]-2,4-di-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-[2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)]-2,4-di-*O*-benzoyl-1-thio-β-D-mannopyranoside 32. Crude pentasaccharide **30** (~8.6 μmol) was dissolved in acetone (2 mL) and 3 mg (46 μmol) of NaN_3 added. Reaction was heated to 60 °C and H_2O was added dropwise until the solution became homogenous. After 20 hours at 60 °C, the reaction mixture was cooled and diluted with EtOAc and washed with H_2O, then brine, and dried over Na_2SO_4. The organic layer was concentrated under

reduced pressure and the crude yellow powder purified by chromatography (9.6:0.4 toluene/acetonitrile) to yield **32** (12 mg, 7.15 μmol, 83% from **28**) as a white solid.

$[\alpha]_D^{25}$ -49 (*c* 12.0 in $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$): δ 7.21-8.21 (m, 65H, Ar), 5.97 (dd, 1H, $J_{2,3}$ 3.5 Hz, $J_{1,2}$ 0.9 Hz, H-2^a), 5.84 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 10.0 Hz, H-4^c), 5.83 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 10.2 Hz, H-4^e), 5.80 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.6 Hz, H-4^b), 5.79 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.4 Hz, H-4^a), 5.76 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.8 Hz, H-4^d), 5.73 (dd, 1H, $J_{3,4}$ 10.1 Hz, $J_{2,3}$ 3.2 Hz, H-3^e), 5.68 (dd, 1H, $J_{2,3}$ 3.4 Hz, $J_{1,2}$ 1.7 Hz, H-2^c), 5.55 (dd, 1H, $J_{3,4}$ 9.7 Hz, $J_{2,3}$ 3.3 Hz, H-3^b), 5.47 (dd, 1H, $J_{3,4}$ 9.5 Hz, $J_{2,3}$ 3.4 Hz, H-3^d), 5.34 (dd, 1H, $J_{2,3}$ 3.2 Hz, $J_{1,2}$ 1.7 Hz, H-2^e), 5.19 (d, 1H, $J_{1,2}$ 1.9 Hz, H-1^b), 5.14 (d, 1H, $J_{1,2}$ 0.8 Hz, H-1^a), 5.14 (dd, 1H, $J_{2,3}$ 3.3 Hz, $J_{1,2}$ 1.9 Hz, H-2^b), 5.11 (d, 1H, $J_{1,2}$ 1.4 Hz, H-1^c), 5.08 (d, 1H, $J_{2,3}$ 1.9 Hz, H-1^d), 5.06 (dd, 1H, $J_{2,3}$ 3.4 Hz, $J_{1,2}$ 2.1 Hz, H-2^d), 4.76 (d, 1H, $J_{1,2}$ 1.5 Hz, H-1^e), 4.66 (s, 1H, H-5^d), 4.63-4.66 (m, 1H, H-6^d), 4.58-4.61 (dd, 1H, $J_{3,4}$ 9.7 Hz, $J_{2,3}$ 3.4 Hz, H-3^c), 4.56 (d, 1H, J_{gem} 13.4 Hz, SCH_2N_3), 4.47-4.50 (m, 1H, H-6^b), 4.47 (dd, 1H, J_{gem} 12.2 Hz, $J_{5,6}$ 4.5 Hz, H-6^d), 4.43 (d, 1H, J_{gem} 13.4 Hz, SCH_2N_3), 4.38-4.41 (dd, 1H, $J_{3,4}$ 9.7 Hz, $J_{2,3}$ 3.5 Hz, H-3^a), 4.33-4.36 (m, 1H, H-6^e), 4.32-4.34 (m, 1H, H-5^e), 4.31-4.34 (m, 1H, H-6^b), 4.29-4.32 (m, 1H, H-5^b), 4.24-4.27 (m, 1H, H-6^e), 4.20 (ddd, 1H, $J_{4,5}$ 10.1 Hz, $J_{5,6}$ 5.4 Hz, $J_{5,6}$ 2.9 Hz, H-5^c), 4.14 (dd, 1H, J_{gem} 11.4 Hz, $J_{5,6}$ 6.3 Hz, H-6^a), 3.97 (dd, 1H, J_{gem} 10.6 Hz, $J_{5,6}$ 5.4 Hz, H-6^c), 3.95 (ddd, 1H, $J_{4,5}$ 10.1 Hz, $J_{5,6}$ 6.3 Hz, $J_{5,6}$ 2.1 Hz, H-5^a), 3.75 (dd, 1H, J_{gem} 11.3 Hz, $J_{5,6}$ 1.8 Hz, H-6^a), 3.49 (dd, 1H, J_{gem} 10.6 Hz, $J_{5,6}$ 2.8 Hz, H-6^c), 2.07 (s, 3H, CH_3(C=O)), 1.91 (s, 3H, CH_3(C=O)), 1.74 (s, 3H, CH_3(C=O)); ^{13}C NMR (176 MHz, $CDCl_3$): δ 169.5 (C=O), 169.0 (C=O), 168.7 (C=O), 166.2 (C=O), 166.1 (C=O), 166.1 (C=O), 166.0 (C=O), 165.9 (C=O), 165.57 (C=O), 165.56 (C=O), 165.3 (C=O), 165.2 (C=O), 165.17 (C=O), 165.15 (C=O), 164.61 (C=O), 164.55 (C=O), 133.6 (Ar), 133.5 (Ar), 133.5 (Ar), 133.3 (Ar), 133.3 (Ar), 133.1 (Ar), 133.1 (Ar), 133.0 (Ar), 132.9 (Ar), 130.2 (Ar), 130.1 (Ar), 130.0 (Ar), 129.93 (Ar), 129.90 (Ar), 129.8 (Ar), 129.71 (Ar), 129.67 (Ar), 129.6 (Ar), 129.5 (Ar), 129.5 (Ar), 129.4 (Ar), 129.33 (Ar), 129.29 (Ar), 129.18 (Ar), 129.17 (Ar), 129.03 (Ar), 128.99 (Ar), 128.98 (Ar), 128.92 (Ar), 128.89 (Ar), 128.67 (Ar), 128.65 (Ar), 128.5 (Ar), 128.46 (Ar), 128.45 (Ar), 128.42 (Ar), 128.35 (Ar), 128.26 (Ar), 128.21 (Ar), 128.18 (Ar), 100.0 (C-1^d), 99.4 (C-1^b), 97.6 (C-1^c), 97.3 (C-1^e), 80.9 (C-1^a), 77.9 (C-5^a), 77.6 (C-3^a), 77.0 (C-3^c), 72.2, 71.9 (C-2^a, C-2^c), 70.0, 69.7, 2×69.6, 69.6, 69.3, 69.34, 69.33, 69.0, 68.9, 68.6, 68.5 (C-4^a, C-2^b, C-3^b, C-5^b, C-4^c C-5^c, C-2^d, C-3^d, C-5^d, C-2^e, C-3^e, C-5^e), 67.1 (C-6^a), 66.9, 66.7, 66.6 (C-4^b, C-4^d, C-4^e), 66.5 (C-6^c), 63.1, 63.0, 62.9 (C-6^b, C-6^d, C-6^e), 51.9 (SCH_2N_3), 20.7 (*C*H_3(C=O)), 20.5 (*C*H_3(C=O)), 20.2 (*C*H_3(C=O)); Coupled HSQC (700 MHz, D_2O): □100.0/5.08 ($J_{C1/H1}$ 175 Hz, C-1^d), 99.4/5.19 ($J_{C1/H1}$ 176 Hz, C-1^b), 97.6/5.11 ($J_{C1/H1}$ 175 Hz, C-1^c), 97.3/4.76 ($J_{C1/H1}$ 176 Hz, C-1^e), 80.9/5.14 ($J_{C1/H1}$ 155 Hz, C-1^a); Anal. calc for $C_{128}H_{111}N_3O_{41}S$: HR ESIMS $[M+Na]^+$: 2400.6306, found: 2400.6308.

Azidomethyl α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-1-thio-β-D-mannopyranoside 33. Tetrasaccharide **31** (14 mg, 6.9 μmol) was suspended in deuterated methanol (1 mL) and deuterated sodium methoxide (0.14 mL, 1 M) added. Reaction was monitored by ^{1}H NMR. After 20 hours, the reaction was neutralized with H^+ resin and the solvent removed under reduced pressure. The orange syrup was purified via HPLC to yield **33** (4.3 mg, 5.83 μmol, 84%) as a white powder.

$[\alpha]_D^{25}$ -10 (*c* 5.1, H_2O); ^{1}H NMR (700 MHz, D_2O): δ 5.37 (d, 1H, $J_{1,2}$ 1.5 Hz, H-1^b), 5.31 (d, 1H, $J_{1,2}$ 1.5 Hz, H-1^c), 5.06 (s, 1H, H-1^a), 5.05 (d, 1H, $J_{1,2}$ 1.5 Hz, H-1^d), 4.59 (d, 1H, J_{gem} 13.3 Hz, SCH_2N_3), 4.52 (d, 1H, J_{gem} 13.4 Hz, SCH_2N_3), 4.23 (d, 1H, $J_{2,3}$ 3.1 Hz, H-2^a), 4.11 (dd, 1H, $J_{2,3}$ 3.1 Hz, $J_{1,2}$ 1.9 Hz, H-2^c), 4.09 (dd, 1H, $J_{2,3}$ 3.2 Hz, $J_{1,2}$ 1.7 Hz, H-2^b), 4.07 (dd, 1H, $J_{2,3}$ 3.3 Hz, $J_{1,2}$ 1.9 Hz, H-2^d), 3.99 (dd, 1H, $J_{3,4}$ 9.6 Hz, $J_{2,3}$ 3.3 Hz, H-3^b), 3.96 (dd, 1H, $J_{3,4}$ 9.3 Hz, $J_{2,3}$ 3.3 Hz, H-3^c), 3.93 (dd, 1H, J_{gem} 12.5 Hz, $J_{5,6}$ 2.2 Hz, H-6^a), 3.90 (m, 1H, H-6^b), 3.87 (m, 1H, H-6^d), 3.86 (dd, 1H, J_{gem} 12.5 Hz, $J_{5,6}$ 1.7 Hz, H-6^c), 3.84 (dd, 1H, $J_{3,4}$ 9.5 Hz, $J_{2,3}$ 3.4 Hz, H-3^d), 3.78 (dd, 1H, $J_{3,4}$ 9.7 Hz, $J_{2,3}$ 3.2 Hz, H-3^a), 3.77 (dd, 1H, J_{gem} 11.7 Hz, $J_{5,6}$ 6.0 Hz, H-6^c), 3.74-3.78 (m, 2H, H-5^b, H-6^b), 3.75 (m, 1H, H-6^a), 3.75 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.4 Hz, H-4^a), 3.73-3.79 (m, 2H, H-5^d, H-6^d), 3.73 (m, 1H, H-5^c), 3.70 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.4 Hz, H-4^b), 3.70

(dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.4 Hz, H-4^c), 3.64 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.7 Hz, H-4^d), 3.49 (ddd, 1H, $J_{4,5}$ 9.1 Hz, $J_{5,6}$ 6.0 Hz, $J_{5,6}$ 2.1 Hz, H-5^a); ^{13}C NMR (126 MHz, D_2O): δ 102.2 (C-1^d), 100.7 (C-1^c), 100.7 (C-1^b), 83.2 (C-1^a), 81.3 (C-3^a), 80.4 (C-5^a), 78.6 (C-2^b), 78.5 (C-2^c), 73.4 (C-5^b), 73.2 (C-5^c), 73.2 (C-5^d), 71.5 (C-2^a), 70.3 (C-3^d), 70.1 (C-3^b), 70.0 (C-3^c), 70.0 (C-2^d), 67.0 (C-4^c), 66.9 (C-4^b), 66.8 (C-4^d), 65.9 (C-4^a), 61.1 (C-6^d), 61.0 (C-6^a, C-6^b, C-6^c), 52.2 (SCH_2N_3); Coupled HSQC (700 MHz, D_2O): □102.3/5.05 ($J_{C1/H1}$ 172 Hz, C-1^d), 100.70/5.30 ($J_{C1/H1}$ 173 Hz, C-1^c), 100.66/5.36 ($J_{C1/H1}$ 174 Hz, C-1^b), 83.2/5.06 ($J_{C1/H1}$ 156 Hz, C-1^a); Anal. calc for $C_{25}H_{43}N_3O_{20}S$: HR ESIMS $[M+Na]^+$: 760.2053, found: 760.2045.

Azidomethyl α-D-mannopyranosyl-(1→6)-[α-D-mannopyranosyl-(1→3)]-α-D-mannopyranosyl-(1→6)-[α-D-mannopyranosyl-(1→3)]-1-thio-β-D-mannopyranoside 34. Pentasaccharide **32** (15 mg, 6.3 μmol) was suspended in deuterated methanol (1 mL) and deuterated sodium methoxide (0.14 mL, 1 M) added. Reaction was monitored by ^{1}H NMR. After 20 hours, the reaction was neutralized with H^+ resin and the solvent removed under reduced pressure. The yellow syrup was purified via HPLC to yield **34** (4.0 mg, 4.45 μmol, 71%) as a white powder.

^{1}H NMR (700 MHz, D_2O): δ 5.15 (d, 1H, $J_{1,2}$ 1.4 Hz, H-1^d), 5.12 (d, 1H, $J_{1,2}$ 1.2 Hz, H-1^b), 5.07 (s, 1H, H-1^a), 4.91 (d, 1H, $J_{1,2}$ 1.3 Hz, H-1^e), 4.87 (d, 1H, $J_{1,2}$ 1.5 Hz, H-1^c), 4.60 (d, 1H, J_{gem} 13.4 Hz, SCH_2N_3), 4.49 (d, 1H, J_{gem} 13.4 Hz, SCH_2N_3), 4.27 (d, 1H, $J_{2,3}$ 3.5 Hz, H-2^a), 4.16 (dd, 1H, $J_{2,3}$ 3.2 Hz, $J_{1,2}$ 1.9 Hz, H-2^c), 4.09 (dd, 1H, $J_{2,3}$ 3.4 Hz, $J_{1,2}$ 1.7 Hz, H-2^b), 4.07 (dd, 1H, $J_{2,3}$ 3.4 Hz, $J_{1,2}$ 1.7 Hz, H-2^d), 3.99 (dd, 1H, $J_{2,3}$ 3.4 Hz, $J_{1,2}$ 1.7 Hz, H-2^e), 3.97-4.00 (m, 1H, H-6^c), 3.96 (dd, 1H, J_{gem} 11.3 Hz, $J_{5,6}$ 4.8 Hz, H-6^a), 3.93 (dd, 1H, $J_{3,4}$ 9.5 Hz, $J_{2,3}$ 3.5 Hz, H-3^c), 3.90 (s, 1H, H-6^b), 3.89 (dd, 1H, $J_{3,4}$ 9.5 Hz, $J_{2,3}$ 3.4 Hz, H-3^b), 3.89 (m, 1H, H-3^d), 3.91 (m, 1H, H-6^c), 3.88-3.91 (m, 1H, H-6^e), 3.89 (m, 1H, H-5^c), 3.87-3.89 (m, 1H, H-4^c), 3.87 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.8 Hz, H-4^a), 3.84 (dd, 1H, $J_{3,4}$ 9.2 Hz, $J_{2,3}$ 3.4 Hz, H-3^e), 3.81 (dd, 1H, $J_{3,4}$ 9.9 Hz, $J_{2,3}$ 3.5 Hz, H-3^a), 3.79 (br. s., 1H, H-6^a), 3.76 (dd, 1H, J_{gem} 12.4 Hz, $J_{5,6}$ 5.9 Hz, H-6^e), 3.75-3.77 (m, 1H, H-6^c), 3.75-3.80 (m, 2H, H-6^d, H-6^d), 3.68-3.71 (m, 1H, H-5^e), 3.66-3.70 (m, 1H, H-5^d), 3.68 (dd, 1H, $J_{3,4} \approx J_{4,5}$ 9.9 Hz, H-4^d), 3.64-3.68 (m, 1H, H-4^e), 3.65 (ddd, 1H, $J_{4,5}$ 9.9 Hz, $J_{5,6}$ 5.3 Hz, $J_{5,6}$ 1.9 Hz, H-5^a); ^{13}C NMR (176 MHz, D_2O): δ 103.3 (C-1^b), 103.1 (C-1^d), 100.4 (C-1^c), 100.1 (C-1^e), 84.7 (C-1^a), 82.4 (C-3^a), 79.4 (C-3^c), 79.2 (C-5^a), 74.2, 74.1, 73.5, 72.3, 71.7, 71.4, 2×71.2, 70.9, 70.85, 70.8, 70.3, 67.61, 2×67.56 (C-2^a, C-2^b, C-3^b, C-4^b, C-5^b, C-2^c, C-5^c, C-2^d, C-3^d, C-4^d, C-5^d, C-2^e, C-3^e, C-4^e, C-5^e), 66.6 (C-4^c), 66.5 (C-6a), 66.4 (C-4^a), 66.1 (C-6^c), 61.8, 2×61.8 (C-6^b. C-6^d, C-6^e), 53.3 (SCH_2N_3); Coupled HSQC (700 MHz, D_2O): □103.3/5.12 ($J_{C1/H1}$ 176 Hz, C-1^b), 103.1/5.15 ($J_{C1/H1}$ 175 Hz, C-1^d), 100.4/4.87 ($J_{C1/H1}$ 175 Hz, C-1^c), 100.0/4.91 ($J_{C1/H1}$ 174 Hz, C-1^e), 84.7/5.07 ($J_{C1/H1}$ 157 Hz, C-1^a); Anal. calc for $C_{31}H_{53}N_3O_{25}S$: HR ESIMS $[M+Na]^+$: 922.2581, found: 922.2573.

***N*-succinimidyl 4-pentynoate 36.** To a 0 °C (ice-water bath) solution of 4-pentynoic acid (0.5 g, 4.84 mmol) and *N*-hydroxysuccinimide (0.57 g, 4.84 mmol) in 1:1 EtOAc:1,2-dioxane (60 mL) was added DCC (1.01 g, 4.90 mmol) in a single portion. The reaction mixture was allowed to warm to room temperature overnight. After 20 hours, the mixture was filtered through Celite and the collected solution diluted with EtOAc and washed with H_2O and brine. The organic layer was concentrated under reduced pressure and recrystallized with hexanes/EtOAc to yield 54 (0.68 g, 3.49 mmol, 72%) as a white crystalline solid.

m.p.: 74.20 °C; ^{1}H NMR (498 MHz, $CDCl_3$): δ 2.88 (t, 2H, *J* 7.9 Hz, CH_2(C=O)O), 2.84 (br. s., 4H, CH_2(C=O)N), 2.62 (td, 4H, *J* 7.5, *J* 2.7 Hz, CH_2C≡C), 2.04 (t, 1H, *J* 2.7 Hz, C≡CH); ^{13}C NMR (126 MHz, $CDCl_3$): δ 168.9 (C=O), 167.0 (C=O), 80.8 (C≡C), 70.0 (C≡C), 30.3 (CH_2), 25.6 (CH_2), 14.1 (CH_2); Anal. calc for $C_9H_{11}NO_4$: HR ESIMS $[M+Na]^+$: 218.0424, found: 218.0424; Elem. Anal: , 55.39; H, 4.65; N, 7.18; found: C, C, 55.13; H, 4.65; N, 7.35.

Notes and references

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Electronic Supplementary Information (ESI) available: LC-UV-MS chromatograms and analysis. NMR spectra data available for compounds **1**, **2**, **16**, **17**, **21**, **22**, **24**, **26**, **27**, **28**, **29**, **31**, **32**, **33**, and **34**. See DOI: 10.1039/b000000x/

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Figure 1 Structure of the $Man_9GlcNAc_2$ oligosaccharide with the Man_4 and Man_5 substructures outlined in red and green.

Figure 2 Example LC-UV-MS analysis of **37**. Extracted-ion chromatograms (top) are used to identify the corresponding peaks in the UV trace (bottom).

Figure 3 ELISA titration curves of (left) ConA and Ab 2G12 against **39**:■, **40**:■, **41**:■, **42**:■, and ubiquitin:■. ELISAs were performed in triplicate.

Scheme 1 Retrosynthesis of tetrasaccharide **1** and pentasaccharide **2**. R and R' represent temporary orthogonal protecting groups.

Scheme 2 (a) **7**, DEA, CH_2Cl_2, 66%; (b) NaOMe, MeOH, 91% 100:64 α/β; (c) (i) TrCl, pyridine 81%, (ii) TDSCl, DMF, imidazole, 92%; (d) BzCl, 1-methylimidazole, pyridine, 80% for **11** (α/β mixture); (e) TBDPSCl, pyridine, 71%.

Scheme 3 (a) $BF_3{\cdot}OEt_2$, CH_2Cl_2, 0°C, 83%; (b) HF·pyridine (70%), pyridine, 93%; (c) BzCl, pyridine, 87%; (d) CH_2Cl_2/MeOH 2:3, AcCl, -10°C, 78%; (e) BzCl, pyridine, 1-methylimidazole, 72%, (f) HF·pyridine (70%), pyridine, 93%.

Scheme 4 (a) **4**, TMSOTf, CH_2Cl_2, rt, 95%; (b) CH_2Cl_2/MeOH 1:1, AcCl, -10°C, 48%; (c) **4**, TMSOTf, CH_2Cl_2, 0°C, 91%.

Scheme 5 (a) 90% TFA aq., Et_3SiH, 75%; (b) **4**, TMSOTf, CH_2Cl_2, 0°C, 93%; (c) $PdCl_2$, 90% AcOH aq., NaOAc; (d) Cl_3CCN, DBU, CH_2Cl_2, 52% (2 steps); (e) **19**, TMSOTf, CH_2Cl_2, 0°C, 93%.

Scheme 6 (a) TMP (1 M in THF), $NaHCO_3$ aq. (1 M), 99%; (b) (i) $NaOCD_3$ (0.5 M), (ii) NaOD (1 M), 70% 1:3 α/β for **1**, 63% 1:3 α/β for **2**.

Scheme 7 (a) DBU, CH_2Cl_2; (b) NaN_3, acetone, H_2O, reflux, 77% for **31**, 83 % for **32** (2 steps); (c) $NaOCD_3$ (0.14 M), 84% for **33**, 71% for **34**.

Scheme 8 (a) **35** or **36**, sodium borate, pH 8.3; (b) **1** or **2**, sodium phosphate, pH 8.0; (c) **33** or **34**, sodium phosphate, pH 7.2, $CuSO_4$, THPTA, amino guanidine, sodium ascorbate.