Synthesis and affinities of C3-symmetric thioglycoside-containing trimannosides

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ARTICLE INFO

Article history:

Received 23 March 2015

Received in revised form

6 May 2015

Accepted 7 May 2015

Available online 16 May 2015

Keywords:

Thiooligosaccharides

Glucocluster

Mutivalent effect

Con A

Hemagglutination

ABSTRACT

Thioglycoside-containing trimannose analogs were designed and prepared to mimic the natural N-glycan core trisaccharide α -D-Man-(1 $\rightarrow$ 3)-[α -D-Man-(1 $\rightarrow$ 6)]-D-Man. (1 $\rightarrow$ 6)-S-Linked trimannoside **1** and its trivalent cluster **2** were synthesized in 11 and 15 steps, respectively, taking advantages of the armed mannopyranosyl trichloroacetimidate as glycosyl donor. Hemagglutination inhibition of the two new thiomannotriose analogs was preliminarily examined. Comparing to the parent trimannoside α -D-Man-(1 $\rightarrow$ 3)-[α -D-Man-(1 $\rightarrow$ 6)]-D-Man-OMe, the cluster mannotriose **2** presented a comparable binding affinity to Con A, while the monomer 6-S-trimannoside **1** exhibited a slightly lower inhibition ability.

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1. Introduction

Carbohydrate-protein interaction is one of the most important pathways for biological information exchange between cells and cell substrates. On cell surface, oligosaccharides contribute significant binding sites for inflammation, viral infections, and cancer metastasis.^{1–4} As a counterpart protein in this interaction, lectins mediate attachment and binding of bacteria and viruses accordingly, and also mediate the first-line defense against invading microorganisms with the mannan-binding lectin in the innate immune system.^{5,6} It is hypothesized that some hepatitis C viral glycoproteins attach to C-type lectins on the host liver cell surface for infection. Lectin activities may be inhibited through binding specific sugar structures and in turn prevent their further attachment to the carbohydrates within the host cell membrane.⁷ Con A, one of the most frequently studied member of the legume lectin family, are believed to bind specifically to certain structures found in various sugars, glycoproteins, and glycolipids, mainly internal and non-reducing terminal α -D-mannosyl residues. Due to its

commercial availability and a wide variety of sugar specificity, Con A has been used widely as a model system to understand the molecular basis of how proteins recognize carbohydrates.^{5–9}

The low affinity in carbohydrate-protein interaction limited their potential medicinal application. But, some proteins, such as lectin, contains typically two or more carbohydrate-combining sites, i.e., they are di- or polyvalent. Multivalent interactions were thus proposed to achieve effective adhesion through many individual binding events.^{10,11} There has been considerable reports in designing multivalent or clustered carbohydrate analogs for high-affinity binding.^{12–14} For instance, Bundle and co-workers reported that the in vitro inhibitory activity of decameric STARFISH for Shiga-like toxins was 10⁶-fold higher than that of the monomeric species.¹⁵ Recently, multivalent mannoside and trimannoside analogs have been synthesized and evaluated in order to obtain increased affinity for lectins.^{16,17} The isothermal titration microcalorimetry data indicated that tri- and tetravalent trimannoside analogs showed 11- and 35-fold higher K_a values for Con A, respectively, in comparison with the parent trimannoside.¹⁶ We have also reported a synthesis of C₃-symmetric (1 $\rightarrow$ 6)-N-acetyl- β -D-glucosamine octadecasaccharide with enhanced antitumor activity against H₂₂ in the preliminary mice tests.¹⁸

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To improve the chemical and biological stability against enzymatic cleavage and acid/base hydrolysis, the anomeric oxygen of O-glycosides were replaced by sulfur or carbon atom generating unnatural S-linked or C-linked glycosides, which kept the similar conformation to their natural O-glycosides and applied as potential probes for enzyme-inhibition studies.^{19–23} Jiménez-Barbero and co-workers reported that C-glycosyl analog of methyl 3,6-di-O-(α -D-mannopyranosyl)- α -D-mannopyranoside [abbreviated as Man(3,6)Man-OMe] displayed an enhanced flexibility and could be recognized by Con A in the similar binding way as the natural trimannose.²⁴ Based on these supporting results, we here report our design and synthesis of two new mannotriose analogs, 6-thio-linked mannotriose **1** and its trivalent cluster **2** (Fig. 1). The binding affinities of these thiooligosaccharides toward Con A were evaluated by the hemagglutination assay, and the trivalent cluster **2** showed a comparable binding ability to Con A versus trisaccharide Man(3,6)Man-OMe.

2. Results and discussion

Cluster compound **2** could be prepared from the condensation of 6-thio-trimannoside **1**, a bifunctional amino ethanethiol linker **3**, and 1,3,5-benzenetricarbonyl chloride **4**, as depicted in Scheme 1.

The key 6-thiolated trisaccharide monomer **1** could be acquired from glycosylation of mannosyl trichloroacetimidate donor **5** and the 6-thio-mannoside acceptor **6**.

We started from the synthesis of a suitable mannopyranoside acceptor, which contains a thiol on 6-position. Thus, D-mannose was treated with AlIOH in the presence of catalytic amount of AcCl (Scheme 2), followed by regioselective tosylation of the primary hydroxyl group with a slight excess of *p*-toluenesulfonyl chloride in pyridine, to give tosylate **8** in 72% yield over 2 steps. Regioselective silylation of **8** with TBSCl using imidazole as base in DMF provided 2,4-diol **9** in a good yield of 71%.²⁵ Acetylation of **9** with acetic anhydride in pyridine to block the free OHs, followed by introduction of the thioacetate group at the 6-position using potassium thioacetate and TBAI under reflux, to afford desired 6-thioacetate derivative **10** in 96% yield.²⁶ Several mild reaction conditions, such as sodium thiomethoxide,²⁷ hydrazine hydrate,²⁸ and cysteamine,²⁹ were tried to selective convert AcS- to HS- in the presence of acetates. All efforts either gave disulfide **14** as the main product or gave very low yields of expected compound **12**. Fortunately, we found that removal of acetyl from AcS- under 0.034 M of NaOMe in MeOH accomplished **12** in 70% isolated yield, as well as the disulfide **14** in 21% yield under optimized reaction conditions. Building block **13** was obtained in two ways, i.e. treatment of **12** with HF-pyridine complex in DCM (79% yield), or

Fig. 1. Structures of methyl 3,6-di-O-(α -D-mannopyranosyl)- α -D-mannopyranoside, 6-thio-trimannoside **1**, and the trivalent cluster **2**.

Scheme 1. Retrosynthetic analysis of 6-thio-trimannoside **1** and cluster compound **2**.

Scheme 2. Synthesis of acceptors. Reagents and conditions: a) AlOH , AcCl , 80°C ; b) TsCl , pyridine, 80°C , 65% for 2 steps; c) TBSCl , imidazole, DMF, rt, 71%; d) Ac_2O , pyridine, rt, 95%; e) KSac , NBu_4I , acetone, reflux, 96%; f) NaOMe (0.034 M in MeOH), 70% for **12**; 21% for disulfide **14**; g) HF -pyridine, CH_2Cl_2 , rt, 79%.

transformation of **14** with $n\text{-Bu}_3\text{P}$ ³⁰ and then HF -pyridine in one-pot two-step reaction (70% overall yield).

With the acceptors **12** and **13** in hand, we first examined the glycosylation of **13** with acetylated mannosyl trichloroacetimidate **15**, expecting to afford thiomannotrioxide in one-pot. However, the reaction was sluggish and no satisfactory result was obtained. Therefore, the coupling of **15** with acceptor **12** was investigated (Scheme 3). Treatment of **15** with **12** (1.5 equiv) in the presence of catalytic amount of TMSOTf (0.1 equiv) and 4 Å molecular sieves in dry dichloromethane at -78°C afforded (1→6)-*S*-linked disaccharide **16** and its β -isomer **18** ($\alpha/\beta=8/1$) in 89% total yield. The chemical structure and the isomeric ratio of the disaccharides were determined based on 2D NMR spectra analyses. When the reaction was carried out at higher temperature, such as 0°C , the α/β ratio was swift to 3/1 with a total yield of 92%. Removal of *t*-butyldimethylsilyl group from **16** using HF -pyridine furnished disaccharide acceptor **17**

in good yield without the migration of acetyl group.³¹ However, coupling of disaccharide acceptor **17** with donor **15** was really frustrating, as some components in the reaction mixture gave the same R_f value on TLC, arising difficulties in products separation. Alternatively, glycosylation of benzoylated trichloroacetimidate donor **19**³² with disaccharide **17** was explored. The desired trisaccharide **20** was afforded in 66% yield under the promotion of TMSOTf at -78°C . Removal of the TBS with HF -pyridine in DCM ($\rightarrow$ **21**) and the acyl groups with NaOMe in MeOH produced 6-thio-trimannoside **1** in excellent yield over two steps.

To synthesize cluster **2**, (see Scheme 4) cysteamine and 1,3,5-benzenetricarbonyl were chosen as the linker and the core, respectively. Radical addition of the Boc-protected aminoethanethiol **3** to the allyl glycoside **20** was carried out smoothly using α -azobisisobutyronitrile (AIBN) as a radical initiator to furnish linker-attached trisaccharide **22**.³³ Deprotection of TBS ($\rightarrow$ **23**), followed by in situ

Scheme 3. Synthesis of trisaccharide. Reagents and conditions: a) TMSOTf, 4 Å MS, CH₂Cl₂, −78 °C, 89%, $\alpha/\beta=8/1$, 79% for **16**; 66% for **20**; b) HF-pyridine, CH₂Cl₂, rt, 83% for **17**; 87% for **21**; c) NaOMe (1 M), MeOH, quantitative.

Scheme 4. Synthesis of cluster compound. Reagents and conditions: a) HSCH₂CH₂NHBoc, AIBN, toluene, 85 °C, 89%; b) HF-pyridine, CH₂Cl₂, rt; c) Ac₂O, pyridine, rt, 74% for 2 steps; d) TFA, CH₂Cl₂, rt, 82%; e) 1,3,5-benzenetricarbonyl trichloride, NEt₃, CH₂Cl₂, 0 °C, 67%; f) NaOMe (1M), MeOH, quantitative.

Table 1

Con A mediated hemagglutination inhibition for monomer **1** and trivalent **2** on Rabbit Erythrocytes

Inhibitor	Minimum inhibitory concentration ^a
Man(3,6)Man-OMe ^b	18.8 μ M
Man(3,6)Man-OAll ^c	35.8 μ M
1	67.7 μ M
2	18.9 μ M

^a Minimum inhibitory concentrations were obtained by averaging the results of three independent experiments. The error associated with the dose determination is a factor of 2, as dictated by the 2-fold dilutions of the assay.

^b Man(3,6)Man-OMe: methyl 3,6-di-O-(α -D-mannopyranosyl)- α -D-mannopyranoside.

^c Man(3,6)Man-OAll: allyl 3,6-di-O-(α -D-mannopyranosyl)- α -D-mannopyranoside.

acetylation provided the compound **24**, which was subjected to TFA in DCM afforded glycosyl amine **25** in good yield. Condensation of amine **25** with 1,3,5-benzenetricarbonyl trichloride **4** gave C₃-symmetric product **26** in the presence of TEA³⁴ in 67% yield, which was further deacylated to achieve the cluster compound **2** in a quantitative yield.

To fulfill our interests regarding the recognition of S-glycosyl mannatriose binding to Con A, as well as the cluster efficacy of the trivalent oligosaccharide **2**, we performed a hemagglutination assay on compounds **1** and **2** to determine the relative affinities toward Con A.^{35,36} The binding activities of 6-thio-trimannoside monomer **1** and the trivalent derivative **2** with Con A was tested and showed in Table 1 with Man(3,6)Man-OMe as a control. Our results showed that the trivalent derivative **2** presented a comparable minimum inhibitory concentration to that of natural trimannose Man(3,6)Man-OMe, while 6-thio-trimannoside **1** showed a 3.6-fold loss in relative affinity.

To get a further insight on intermolecular interaction between trimannose and Con A, computational study was performed with Surflex, which was implemented in the Sybyl software. The 3D structure of Con A was downloaded from PDB entry 1ONA. Three inhibitors were docked into the same active site of 1ONA. To verify the docking parameters, the trimannose ligand Man(3,6)Man-OMe of 1ONA structure was removed from the binding site and re-docked into the sugar binding site. The root mean square deviation (RMSD_ISO) between the best docking conformation of the trimannose and the X-ray crystallographic conformation was 0.53 Å, which indicated that the docking result was reliable and the parameters used here were adequate.

As shown in Fig. 2, all the three trimannoses could be docked into the active site of Con A. Consistent with the previous reports,^{37–39} the binding interactions of Man(3,6)Man-OMe, Man(3,6)Man-OAll, and compound **1** with Con A were quite similar, mainly contributed from the formation of hydrogen bonds with residues (PRO13, ASN14, THR15, ASP16, LEU99, TYR100, ASP208, and ARG228) around the active site (Table 2). The scores

indicated that they showed comparable bioactivities. Furthermore, crystal water molecule (WAT 259) in the active site connected the core mannose unit with the Con A residues ASN14, ASP16, and ARG228 (A and C in Fig. 2).

3. Conclusion

In summary, we have successfully synthesized a trimannoside monomer **1**, which contains 6-thio-mannosyl residue in 11 convergent steps from D-mannose with 11% overall yield. Using cysteamine and 1,3,5-benzenetricarbonyl as the linker and the core, a trivalent oligosaccharide **2** was accomplished in a further 6 steps and in 36.2% overall yield from the corresponding fully protected trisaccharide intermediate **20**. The bioassay revealed that the trivalent **2** possessed the similar inhibitory potency as that of the parent O-trimannoside, but 6-thio-trimannoside **1** showed a slight loss with respect to binding affinity to Con A. Computational results suggested that replacing of O-glycoside with S-glycoside in trimannose structure had very little effect on binding abilities between mannosyl residues and Con A.

4. Experimental

4.1. General methods

All chemicals were obtained from commercial suppliers and used without further purification unless stated. All moisture sensitive reactions were carried out in a flame dried flask under a nitrogen atmosphere. Anhydrous solvents were purified according to standard procedures before use. Column chromatography was carried out using silica gel (100–200 mesh). Reactions were monitored by silica gel 60 F₂₅₄ TLC plates and/or by charring following immersion in a 30% sulfuric acid in methanol. ¹H and ¹³C NMR spectra were measured with a Bruker AVANCE-III 400 MHz (100 MHz for ¹³C NMR) spectrometer. High resolution mass spectra (HRMS) were recorded with a Bruker microTOF Q II mass spectrometer. Optical rotations were measured with a Rudolph Research Analytical Autopol[®] VI automatic digital polarimeter.

4.2. Allyl 6-O-tosyl- α -D-mannopyranoside (**8**)

D-Mannose (20 g, 111 mmol) was refluxed for 4 h in AlOH (200 mL) in the presence of AcCl (0.42 mL, 5.6 mmol). After neutralization with NEt₃, the allyl alcohol was removed by evaporation, and the residue was subjected to flash column chromatography (ethyl acetate/MeOH=10:1) to afford allyl mannopyranoside as a syrup. To the above syrup in pyridine (200 mL) was added slowly *p*-TsCl (22.6 g, 118 mmol) in several portions at 0 °C. The reaction mixture was stirred at rt for 18 h. The solvent was removed by evaporation, and column chromatography of the residue on silica gel (EtOAc/MeOH, 100:1) afforded **8** (26.9 g, 65% over two

Table 2

Hydrogen bonds and total scores for the combination of Con A and trimannose residues

	Man(3,6)Man-OMe	Man(3,6)Man-OAll	1
α -(1,6) Man	LEU99 N–H...O1; ARG228 N–H...O–H3, ASN14H–N–H...O–H4; TYR100 N–H...O–H6; ASP208 O=C=O...H–O6, O=C=O...H–O4;	LEU99 N–H...O1; ARG228 N–H...O–H3, ASN14H–N–H...O–H4; TYR100 N–H...O–H6; ASP208 O=C=O...H–O6, O=C=O...H–O4;	LEU99 N–H...O1; ARG228 N–H...O–H3, ASN14H–N–H...O–H4; TYR100 N–H...O–H6; ASP208 O=C=O...H–O6, O=C=O...H–O4;
Core man	ASP16 O=C=O...H–O2;	ASP16 O=C=O...H–O2;	ASP16 O=C=O...H–O2;
α -(1,3) Man	ASP16 N–H...O–H4; PRO13C=O...H–O3; THR15 N–H...O–H3	ASP16 N–H...O–H3; PRO13C=O...H–O2;	ASP16 N–H...O–H4; PRO13C=O...H–O3; THR15 N–H...O–H3
Total score	11.22	10.92	11.10

Fig. 2. Structures of Con A combining with trimannoses derived from Surflex-docking: (A) Con A & Man(3,6)Man-OMe; (B) Con A & Man(3,6)Man-OAll; (C) Con A & compound **1**. The hydrogen bond interactions of trimannoses (cyan stick) with residues (green stick) and water molecule (in A and C) in Con A were labeled in red dot line (for more details about hydrogen bond information, see Table 2). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

steps) as a syrup. $[\alpha]_D^{25} +30.1$ (c 2.1, CH₃OH); ¹H NMR (400 MHz, CDCl₃): δ 7.79 (d, $J=8.0$ Hz, 2H), 7.31 (d, $J=8.0$ Hz, 2H), 5.84–5.80 (m, 1H, CH₂CH=CH₂), 5.23 (dd, $J=17.2$, 1.4 Hz, 1H, CH₂CH=CH₂), 5.15 (dd, $J=10.4$, 1.4 Hz, 1H, CH₂CH=CH₂), 4.80 (s, 1H, H-1), 4.41 (d, $J=5.2$ Hz, 1H, H-2), 4.33 (dd, $J=10.0$, 3.6 Hz, 1H, H-3), 4.28 (t, $J=10.4$ Hz, 1H, H-4), 4.20 (s, 1H, OH), 4.07 (dd, $J=12.8$, 5.2 Hz, 1H, CH₂CH=CH₂), 3.94–3.87 (m, 3H, H-5, OH, CH₂CH=CH₂), 3.80 (s, 1H, OH), 3.77–3.71 (m, 2H, H-6a,b), 2.41 (s, 3H) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 145.1, 133.9, 133.0, 129.7(2), 127.7(2), 116.1, 99.3, 71.1, 70.9, 70.5, 70.0, 67.6, 66.8, 20.2 ppm; HRMS (ESI) m/z calcd for C₁₆H₂₂O₈SiNa [M+Na]⁺ 397.0933, found m/z 397.0950.

4.3. Allyl 3-O-*t*-butyldimethylsilyl-6-O-tosyl- α -D-mannopyranoside (**9**)

To a mixture of compound **8** (4 g, 10.7 mmol) in N,N-dimethylformamide (10 mL) was added TBSCl (1.62 g, 10.7 mmol) and imidazole (0.801 g, 11.77 mmol) at 0 °C. The reaction mixture was stirred under this condition for 30 min, and kept at rt overnight, then diluted with water (50 mL) and extracted with EtOAc (3 × 100 mL). The organic phase was dried over anhydrous Na₂SO₄ and concentrated. Purification of the residue by column chromatography (petroleum ether/EtOAc=5/1) afforded **9** (3.7 g, 71%) as colorless oil. $[\alpha]_D^{25} +41.9$ (c 2.4, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ 7.78 (d, $J=8.0$ Hz, 2H), 7.31 (d, $J=8.0$ Hz, 2H), 5.85–5.81 (m, 1H, CH₂CH=CH₂), 5.22 (dd, $J=17.2$, 1.2 Hz, 1H, CH₂CH=CH₂), 5.16 (dd, $J=10.4$, 1.2 Hz, 1H, CH₂CH=CH₂), 4.81 (s, 1H, H-1), 4.32–4.24 (m, 2H, H-2, H-4), 4.08 (dd, $J=13.2$, 5.2 Hz, 1H, CH₂CH=CH₂), 3.90 (dd, $J=12.8$, 6.0 Hz, 1H, CH₂CH=CH₂), 3.83 (dd, $J=8.4$, 3.2 Hz, 1H, H-3), 3.76–3.65 (m, 3H, H-5, H-6a,b), 2.55 (s, 1H, OH), 2.42 (s, 3H, OAc), 2.35 (d, $J=4.0$ Hz, 1H, OH), 0.89 (s, 9H, C(CH₃)₃), 0.11 (s, 3H, Si(CH₃)₂), 0.05 (s, 3H, Si(CH₃)₂) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 144.8, 133.5, 132.9, 129.8(2), 128.0(2), 117.6, 98.2, 73.0, 71.3, 69.9, 69.4, 68.0, 67.3, 25.8(3), 21.6, 18.1, –4.5, –4.7 ppm; HRMS (ESI) m/z calcd for C₂₂H₃₆O₈SiNa [M+Na]⁺ 511.1798, found m/z 511.1815.

4.4. Allyl 2,4-di-O-acetyl-3-O-*t*-butyldimethylsilyl-6-O-tosyl- α -D-mannopyranoside (**10**)

To a solution of **9** (3.0 g, 6.15 mmol) in pyridine (10 mL) was added Ac₂O (1.34 mL, 14.1 mmol). The reaction mixture was stirred at rt for 2 h and then evaporated under vacuo to give a yellow oil. It was purified by silica gel column chromatography (petroleum ether/EtOAc=6/1) to give **10** (3.33 g, 95%) as colorless oil. $[\alpha]_D^{25} +29.6$ (c 2.1, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ 7.78 (d,

$J=8.0$ Hz, 2H), 7.34 (d, $J=8.0$ Hz, 2H), 5.89–5.85 (m, 1H, CH₂CH=CH₂), 5.27 (d, $J=17.2$ Hz, 1H, CH₂CH=CH₂), 5.22 (d, $J=10.0$ Hz, 1H, CH₂CH=CH₂), 5.06 (d, $J=1.6$ Hz, 1H, H-2), 4.98 (t, $J=9.6$ Hz, 1H, H-4), 4.72 (s, 1H, H-1), 4.15–4.05 (m, 4H, CH₂CH=CH₂, H-3, H-6a,b), 3.96–3.90 (m, 2H, H-5, CH₂CH=CH₂), 2.44 (s, 3H, CH₃), 2.08 (s, 3H, OAc), 2.03 (s, 3H, OAc), 0.80 (s, 9H, C(CH₃)₃), 0.05 (s, 3H, Si(CH₃)₂), 0.03 (s, 3H, Si(CH₃)₂) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 170.2, 169.9, 144.9, 133.1, 132.8, 129.8(2), 128.1(2), 118.2, 96.6, 71.7, 69.4, 69.1, 68.8, 68.4, 68.1, 25.4(3), 21.7, 21.0, 20.9, 17.7, –4.8, –5.1 ppm; HRMS (ESI) m/z calcd for C₂₆H₄₀O₁₀SiNa [M+Na]⁺ 595.2009, found 595.2020.

4.5. Allyl 2,4-di-O-acetyl-3-O-*t*-butyldimethylsilyl-6-*S*-acetyl- α -D-mannopyranoside (**11**)

A solution of **10** (2.5 g, 4.37 mmol), KSAc (2.7 g, 23.5 mmol), and a catalytic amount of NBu₄I in acetone (20 mL) was refluxed for 6 h. At the end of which time, the mixture was diluted with EtOAc (40 mL) and washed with H₂O (2 × 25 mL). The organic layer was dried over Na₂SO₄, concentrated in vacuo, and the syrup was purified by flash column chromatography (petroleum ether/EtOAc=12/1), to yield **11** (2 g, 96%) as light red oil. $[\alpha]_D^{25} +34.4$ (c 1.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ 5.90–5.85 (m, 1H, CH₂CH=CH₂), 5.28 (dd, $J=17.2$, 1.2 Hz, 1H, CH₂CH=CH₂), 5.22 (dd, $J=10.4$, 1.2 Hz, 1H, CH₂CH=CH₂), 5.09–5.05 (m, 2H, H-2, H-4), 4.74 (s, 1H, H-1), 4.16 (dd, $J=12.8$, 5.2 Hz, 1H, CH₂CH=CH₂), 4.07 (dd, $J=9.2$, 3.6 Hz, 1H, H-3), 3.96 (dd, $J=12.8$, 6.0 Hz, 1H, CH₂CH=CH₂), 3.72 (dt, $J=9.2$, 2.4 Hz, 1H, H-5), 3.27 (dd, $J=14.0$, 2.8 Hz, 1H, H-6a), 2.89 (dd, $J=14.0$, 8.8 Hz, 1H, H-6b), 2.34 (s, 3H, OAc), 2.13 (s, 3H, OAc), 2.11 (s, 3H, OAc), 0.81 (s, 9H, C(CH₃)₃), 0.05 (s, 3H, Si(CH₃)₂), 0.04 (s, 3H, Si(CH₃)₂) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 195.0, 170.3, 170.1, 133.2, 118.1, 96.6, 71.9, 71.8, 70.3, 68.2, 68.1, 30.7, 30.5, 25.4(3), 21.2, 21.0, 17.7, –4.8, –5.1 ppm; HRMS (ESI) m/z calcd for C₂₁H₃₆O₈SiNa [M+Na]⁺ 499.1798, found 499.1768.

4.6. Allyl 2,4-di-O-acetyl-3-O-*t*-butyldimethylsilyl-6-thio- α -D-mannopyranoside (**12**)

To a stirred solution of sodium methoxide (0.034 M in MeOH, 85 mL) was added **11** (1.38 g, 2.90 mmol) under nitrogen protection. The reaction mixture was stirred at rt for 2 h and amberlite IR-120 (H⁺) was added to neutralize when **11** was consumed completely (TLC), filtered and evaporated in vacuo. The mixture was purified by flash chromatography (petroleum ether/EtOAc=15/1) to yield **12** (881 mg, 70%) and disulfide **14** (263 mg, 21%). For **12**, $[\alpha]_D^{25} +47.1$ (c

2.3, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ 5.91–5.87 (m, 1H, CH₂CH=CH₂), 5.28 (dd, *J*=17.2, 1.4 Hz, 1H, CH₂CH=CH₂), 5.21 (dd, *J*=10.4, 1.4 Hz, 1H, CH₂CH=CH₂), 5.08 (dd, *J*=3.6, 1.6 Hz, 1H, H-2), 5.03 (t, *J*=9.6 Hz, 1H, H-4), 4.77 (d, *J*=1.2 Hz, 1H, H-1), 4.23 (dd, *J*=12.8, 5.2 Hz, 1H, CH₂CH=CH₂), 4.07 (dd, *J*=9.6, 3.6 Hz, 1H, H-3), 4.00 (dd, *J*=12.8, 6.0 Hz, 1H, CH₂CH=CH₂), 3.69 (dt, *J*=9.2, 2.4 Hz, 1H, H-5), 2.71–2.64 (m, 1H, H-6a), 2.55–2.48 (m, 1H, H-6b), 2.09 (s, 3H, OAc), 2.05 (s, 3H, OAc), 1.75 (dd, *J*=10.4, 6.0 Hz, 1H, SH), 0.79 (s, 9H, C(CH₃)₃), 0.04 (s, 3H, Si(CH₃)₂), 0.03 (s, 3H, Si(CH₃)₂) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 170.3, 169.9, 133.3, 118.0, 96.5, 72.1, 71.9, 71.5, 68.3, 68.2, 26.4, 25.4(3), 21.1, 20.9, 17.7, –4.8, –5.1 ppm; HRMS (ESI) *m/z* calcd for C₁₉H₃₄O₇SSiNa [M+Na]⁺ 457.1692, found 457.1709. For **14**, [α]_D²⁵ +102.8 (c 1.2, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ 5.91–5.87 (m, 1H, CH₂CH=CH₂), 5.31 (dd, *J*=17.2, 1.4 Hz, 2H, CH₂CH=CH₂), 5.22 (dd, *J*=10.0, 1.4 Hz, 2H, CH₂CH=CH₂), 5.10 (dd, *J*=3.6, 1.2 Hz, 2H, H-2), 5.02 (t, *J*=9.6 Hz, 2H, H-4), 4.77 (d, *J*=1.2 Hz, 2H, H-1), 4.22 (dd, *J*=12.8, 5.2 Hz, 2H, CH₂CH=CH₂), 4.10 (dd, *J*=9.6, 3.6 Hz, 2H, H-3), 3.99 (dd, *J*=12.8, 6.4 Hz, 2H, CH₂CH=CH₂), 3.92 (dt, *J*=9.6, 2.4 Hz, 2H, H-5), 2.86 (dd, *J*=13.6, 9.6 Hz, 2H, H-6a), 2.77 (dd, *J*=13.6, 2.4 Hz, 2H, H-6b), 2.10 (s, 6H, OAc), 2.09 (s, 6H, OAc), 0.81 (s, 18H, C(CH₃)₃), 0.06 (s, 6H, Si(CH₃)₂), 0.05 (s, 6H, Si(CH₃)₂) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 170.3(2), 170.1(2), 133.2(2), 118.2(2), 96.5(2), 72.2(2), 71.9(2), 68.8(2), 68.3(4), 40.8(2), 25.4(6), 21.2(2), 20.9(2), 17.7(2), –4.8(2), –5.1(2) ppm; HRMS (ESI) *m/z* calcd for C₃₈H₆₆O₁₄S₂Si₂Na [M+Na]⁺ 889.3330, found 866.3297.

4.7. Allyl 2,4-di-O-acetyl-3-O-*t*-butyldimethylsilyl-6-*S*-(2,3,4,6-tetra-O-acetyl-α-*D*-mannopyranosyl)-α-*D*-mannopyranoside (**16**)

A mixture of **12** (700 mg, 1.61 mmol) and freshly dried 4 Å molecular sieves (400 mg) in anhydrous dichloromethane (4 mL) was stirred for 10 min under nitrogen at –78 °C, and TMSOTf (30 μL, 0.16 mmol) was added quickly. Then a solution of **15** (1.2 g, 2.4 mmol) in dry CH₂Cl₂ (3 mL) was added dropwise. The reaction mixture was stirred at –78 °C until TLC indicated complete conversion of the acceptor **12** and then quenched with Et₃N (0.1 mL). Solid was collected and wash with dichloromethane, and the filtrate was concentrated in vacuo to obtain an oily residue that was purified by silica gel column chromatography (petroleum ether/EtOAc=4/1) to provide the major α-isomer **16** (971 mg, 79%) as a colorless syrup, as well as the β-isomer **18** (123 mg, 10%). For **16**, [α]_D²⁵ +55.1 (c 2.45, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ 5.91–5.87 (m, 1H, CH₂CH=CH₂), 5.30–5.24 (m, 5H, H-1', H-2', H-3', H-4', CH₂CH=CH₂), 5.21 (dd, *J*=10.4, 1.2 Hz, 1H, CH₂CH=CH₂), 5.10–5.06 (m, 2H, H-2, H-4), 4.75 (d, *J*=1.2 Hz, 1H, H-1), 4.38–4.33 (m, 1H, H-5'), 4.29 (dd, *J*=12.4, 4.8 Hz, 1H, H-6a'), 4.19 (dd, *J*=12.8, 4.8 Hz, 1H, CH₂CH=CH₂), 4.09–4.04 (m, 2H, H-3, H-6b'), 3.99 (dd, *J*=12.8, 6.0 Hz, 1H, CH₂CH=CH₂), 3.81 (dt, *J*=10.0, 2.8 Hz, 1H, H-5), 2.75 (dd, *J*=14.0, 3.2 Hz, 1H, H-6a), 2.68 (dd, *J*=14.0, 8.0 Hz, 1H, H-6b), 2.15 (s, 3H, OAc), 2.10 (s, 6H, OAc), 2.05 (s, 3H, OAc), 2.03 (s, 3H, OAc), 1.97 (s, 3H, OAc), 0.80 (s, 9H, C(CH₃)₃), 0.05 (s, 3H, Si(CH₃)₂), 0.03 (s, 3H, Si(CH₃)₂) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 170.7, 170.4, 169.9, 169.8, 169.7(2), 133.2, 117.9, 96.6, 82.3, 71.8, 71.7, 70.8, 69.5, 69.4, 69.0, 68.3, 68.1, 66.2, 62.2, 32.1, 25.4(3), 21.1, 20.9(2), 20.8, 20.7, 20.6, 17.7, –4.8, –5.1 ppm; HRMS (ESI) *m/z* calcd for C₃₃H₅₂O₁₆SSiNa [M+Na]⁺ 787.2643, found 787.2648. For **18**, [α]_D²⁵ +11.7 (c 1.1, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ 5.91–5.87 (m, 1H, CH₂CH=CH₂), 5.52 (d, *J*=3.2 Hz, 1H, H-2'), 5.31 (dd, *J*=17.2, 1.2 Hz, 1H, CH₂CH=CH₂), 5.27–5.22 (m, 2H, H-4', CH₂CH=CH₂), 5.11 (dd, *J*=3.6, 1.6 Hz, 1H, H-2), 5.07–5.01 (m, 2H, H-3', H-4), 4.95 (s, 1H, H-1'), 4.78 (s, 1H, H-1), 4.31–4.22 (m, 2H, CH₂CH=CH₂, H-6a'), 4.15–4.11 (m, 1H, H-6b'), 4.07 (dd, *J*=9.2, 3.6 Hz, 1H, H-3), 4.00 (dd, *J*=13.2, 6.0 Hz, 1H, CH₂CH=CH₂), 3.79 (dt, *J*=9.6, 2.4 Hz, 1H, H-5), 3.72–3.67 (m, 1H, H-5'), 2.87 (dd, *J*=14.0, 9.6 Hz, 1H, H-6a), 2.77 (dd,

J=14.0, 2.4 Hz, 1H, H-6b), 2.17 (s, 3H, OAc), 2.13 (s, 3H, OAc), 2.10 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.04 (s, 3H, OAc), 1.97 (s, 3H, OAc), 0.81 (s, 9H, C(CH₃)₃), 0.06 (s, 3H, Si(CH₃)₂), 0.04 (s, 3H, Si(CH₃)₂) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 170.7, 170.2, 170.1(2), 170.0, 169.6, 133.3, 118.0, 96.6, 83.4, 76.5, 73.2, 72.4, 71.9, 71.8, 71.4, 70.4, 68.4, 68.1, 65.7, 62.8, 32.9, 25.4(3), 21.1, 21.0, 20.8, 20.7, 20.6, 17.7, –4.8, –5.1 ppm; HRMS (ESI) *m/z* calcd for C₃₃H₅₂O₁₆SSiNa [M+Na]⁺ 787.2643, found 787.2650.

4.8. Allyl 2,4-di-O-acetyl-6-*S*-(2,3,4,6-tetra-O-acetyl-α-*D*-mannopyranosyl)-α-*D*-mannopyranoside (**17**)

To a stirred solution of **16** (637 mg, 0.83 mmol) in anhydrous CH₂Cl₂ (10 mL) was added HF-pyridine (1.25 mL) at 0 °C. The mixture was stirred at room temperature for 1 h and the reaction was monitored by TLC (Petroleum ether/EtOAc=1:1). When the starting material had disappeared, saturated aqueous sodium hydrogencarbonate was added carefully. The aqueous layer was extracted with CH₂Cl₂ (4×15 mL). The combined organic phase was dried over Na₂SO₄ and concentrated to a residue, which was subjected to silica gel column chromatography (petroleum ether/EtOAc=1.5/1) to afford **17** (448 mg, 83%) as a colorless syrup. [α]_D²⁵ +63.3 (c 1.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ 5.91–5.87 (m, 1H, CH₂CH=CH₂), 5.33–5.31 (m, 3H, H-1', H-2', H-4'), 5.29–5.24 (m, 2H, H-3', CH₂CH=CH₂), 5.22 (d, *J*=10.4 Hz, 1H, CH₂CH=CH₂), 5.08–5.03 (m, 2H, H-2, H-4), 4.86 (s, 1H, H-1), 4.37–4.28 (m, 2H, H-5', H-6a'), 4.19 (dd, *J*=13.2, 5.2 Hz, 1H, CH₂CH=CH₂), 4.10–4.05 (m, 2H, H-3, H-6b'), 4.00 (dd, *J*=13.2, 5.6 Hz, 1H, CH₂CH=CH₂), 3.91 (dt, *J*=10.0, 2.4 Hz, 1H, H-5), 2.84 (dd, *J*=13.6, 2.4 Hz, 1H, H-6a), 2.69 (dd, *J*=13.6, 7.2 Hz, 1H, H-6b), 2.20 (br, 1H, OH), 2.15 (s, 6H, OAc), 2.12 (s, 3H, OAc), 2.10 (s, 3H, OAc), 2.04 (s, 3H, OAc), 1.98 (s, 3H, OAc) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 171.2, 170.6(2), 169.9, 169.8, 169.7, 133.1, 118.0, 96.2, 82.5, 72.4, 72.0, 70.8, 69.3, 69.2, 69.1, 68.4, 68.3, 66.1, 62.2, 31.9, 21.0, 20.9(2), 20.8, 20.7, 20.6 ppm; HRMS (ESI) *m/z* calcd for C₂₇H₃₈O₁₆SNa [M+Na]⁺ 673.1778, found 673.1798.

4.9. Allyl 2,4-di-O-acetyl-3-O-(2,3,4-tri-O-benzoyl-6-O-*t*-butyldimethylsilyl-α-*D*-mannopyranosyl)-6-*S*-(2,3,4,6-tetra-O-acetyl-α-*D*-mannopyranosyl)-α-*D*-mannopyranoside (**20**)

A mixture of **17** (334 mg, 0.51 mmol) and freshly dried 4 Å molecular sieves (400 mg) in anhydrous dichloromethane (4 mL) was stirred for 10 min under nitrogen at –78 °C, and TMSOTf (14 μL, 0.08 mmol) was added quickly. Then a solution of compound **19** (824 mg, 1.1 mmol) in dry CH₂Cl₂ (3 mL) was added dropwise. The reaction was stirred at –78 °C until TLC indicated complete conversion of the acceptor **17** and then quenched with Et₃N (5 drops). Solid was filtered and washed with dichloromethane. The filtrate was concentrated in vacuo to obtain an oily residue that was purified by silica gel column chromatography (petroleum ether/EtOAc=3/1) to furnish **20** (420 mg, 66%) as a colorless syrup. [α]_D²⁵ +4.4 (c 2.2, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ 8.09 (dd, *J*=8.4, 1.2 Hz, 2H), 7.97 (dd, *J*=8.4, 1.6 Hz, 2H), 7.85 (dd, *J*=8.4, 1.2 Hz, 2H), 7.59 (tt, *J*=7.2, 1.2 Hz, 1H), 7.53–7.43 (m, 3H), 7.41–7.36 (m, 3H), 7.28–7.24 (m, 2H), 6.03 (t, *J*=10.0 Hz, 1H, H-4'), 5.92–5.88 (m, 1H, CH₂CH=CH₂), 5.67 (dd, *J*=10.0, 3.6 Hz, 1H, H-3'), 5.42 (dd, *J*=3.2, 1.6 Hz, 1H, H-2'), 5.37–5.35 (m, 3H, H-2, H-2'', CH₂CH=CH₂), 5.33 (s, 1H, H-1''), 5.31–5.30 (m, 3H, H-3'', H-4, H-4''), 5.27 (d, *J*=1.6 Hz, 1H, H-1'), 5.24 (dd, *J*=10.4, 1.2 Hz, 1H, CH₂CH=CH₂), 4.86 (d, *J*=1.6 Hz, 1H, H-1), 4.43–4.39 (m, 1H, H-5'), 4.35–4.29 (m, 2H, H-3, H-6a''), 4.25–4.18 (m, 2H, H-5', CH₂CH=CH₂), 4.13–4.08 (m, 1H, H-6b''), 4.04 (dd, *J*=12.8, 6.0 Hz, 1H, CH₂CH=CH₂), 3.93–3.87 (m, 1H, H-5), 3.85–3.78 (m, 2H, H-6a', 6b'), 2.85–2.75 (m, 2H, H-6a,b), 2.30 (s, 3H, OAc), 2.23 (s, 3H, OAc), 2.17 (s, 3H, OAc), 2.14 (s, 3H, OAc),

2.06 (s, 3H, OAc), 1.99 (s, 3H, OAc), 0.88 (s, 9H, C(CH₃)₃), 0.01 (s, 3H, Si(CH₃)₂), −0.02 (s, 3H, Si(CH₃)₂) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 170.7, 170.5, 170.4, 169.9, 169.7(2), 165.6, 165.4, 165.3, 133.5, 133.2, 133.0, 129.9(2), 129.8(2), 129.7(2), 129.5, 129.3, 129.2, 128.5(2), 128.4(2), 128.3(2), 118.4, 98.9, 96.3, 82.4, 74.4, 72.1, 71.0, 70.9, 70.8(2), 70.7, 69.9, 69.8, 69.4, 69.1, 68.3, 66.3, 66.2, 62.3, 61.8, 32.1, 25.9(3), 21.1, 21.0, 20.9, 20.8, 20.7, 20.6, 18.3, −5.5, −5.6 ppm; MALDI-TOF MS *m/z* calcd for C₆₀H₇₄O₂₄SSiNa [M+Na]⁺ 1261.4, found 1261.3.

4.10. Allyl 2,4-di-O-acetyl-3-O-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-6-S-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (21)

To a stirred solution of **20** (140 mg, 0.11 mmol) in anhydrous CH₂Cl₂ (2 mL) and pyridine (0.5 mL) was added HF-pyridine (1.0 mL) at 0 °C. The mixture was stirred at room temperature for 8 h and the reaction process was monitored by TLC (Petroleum ether/EtOAc=1:1). When the starting material had disappeared, saturated aqueous sodium bicarbonate was added carefully. The aqueous layer was extracted with CH₂Cl₂ (4×15 mL). The combined organic phase was dried and concentrated to a syrup that was purified by silica gel column chromatography (petroleum ether/EtOAc=2/1) to give **21** (122 mg, 87%) as a colorless syrup. [α]_D²⁵ +3.4 (c 1.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ 8.07 (d, *J*=7.2 Hz, 2H), 7.99 (d, *J*=7.8 Hz, 2H), 7.82 (d, *J*=7.2 Hz, 2H), 7.60 (t, *J*=7.2 Hz, 1H), 7.54–7.45 (m, 3H), 7.43–7.37 (m, 3H), 7.25 (t, *J*=7.8 Hz, 2H), 5.93–5.78 (m, 3H, CH₂CH=CH₂, H-3', H-4'), 5.45 (t, *J*=2.0 Hz, 1H, H-2'), 5.37–5.27 (m, 8H, H-2, H-4, H-1', H-1'', H-2'', H-3'', H-4'', CH₂CH=CH₂), 5.22 (d, *J*=10.4 Hz, 1H, CH₂CH=CH₂), 4.84 (s, 1H, H-1), 4.41–4.38 (m, 1H, H-5'), 4.34–4.27 (m, 2H, H-3, H-6a''), 4.22 (dd, *J*=12.8, 5.2 Hz, 1H, CH₂CH=CH₂), 4.14–4.08 (m, 2H, H-5', H-6b''), 4.02 (dd, *J*=12.8, 6.0 Hz, 1H, CH₂CH=CH₂), 3.91 (dt, *J*=7.2, 2.8 Hz, 1H, H-5), 3.83–3.78 (m, 1H, H-6a'), 3.74–3.70 (m, 1H, H-6b'), 2.84 (dd, *J*=14.0, 2.8 Hz, 1H, H-6a), 2.76 (dd, *J*=14.0, 7.6 Hz, 1H, H-6b), 2.59 (t, *J*=6.0 Hz, 1H, OH), 2.27 (s, 3H, OAc), 2.24 (s, 3H, OAc), 2.16 (s, 3H, OAc), 2.13 (s, 3H, OAc), 2.05 (s, 3H, OAc), 1.99 (s, 3H, OAc) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 170.7(2), 170.4, 169.9, 169.7(2), 166.5, 165.6, 165.2, 133.7, 133.2, 132.9, 130.0(2), 129.9(2), 129.7(2), 129.1(2), 128.7, 128.6(2), 128.5(2), 128.3(2), 118.3, 99.2, 96.4, 82.4, 75.1, 71.8, 70.9, 70.8(3), 70.3, 69.8, 69.4, 69.2, 69.0, 68.4, 66.9, 66.2, 62.3, 61.2, 32.0, 21.1, 20.9, 20.8, 20.7(2), 20.6 ppm; MALDI-TOF MS *m/z* calcd for C₅₄H₆₀O₂₄SSiNa [M+Na]⁺ 1147.3, found 1147.3.

4.11. Allyl α-D-mannopyranosyl-(1→3)-[6-S-α-D-mannopyranosyl-(1→6)]-α-D-mannopyranose (1)

To a solution of **21** (122 mg, 0.11 mmol) in anhydrous methanol (10 mL) under nitrogen was added a 1 M solution of sodium methoxide in MeOH until pH 9–10 was reached. The reaction mixture was stirred at rt for 10 h. Upon completion (TLC), the reaction was neutralized with amberlite IR-120 (H⁺) and filtered, solvent was evaporated to dryness. The residue was purified with Sephadex G-10 and desired fractions were combined and lyophilized to furnish compound **1** (58.3 mg, 96%) as white foam. [α]_D²⁵ +91.6 (c 0.68, H₂O); ¹H NMR (400 MHz, CDCl₃): δ 5.92–5.87 (m, 1H, CH₂CH=CH₂), 5.34–5.28 (m, 2H, H-1'', CH₂CH=CH₂), 5.18 (dd, *J*=10.4, 1.2 Hz, 1H, CH₂CH=CH₂), 5.06 (d, *J*=1.6 Hz, 1H, H-1'), 4.74 (d, *J*=1.6 Hz, 1H, H-1), 4.23 (ddt, 2H, *J*=13.2, 5.2, 1.2 Hz, CH₂CH=CH₂), 4.05–3.99 (m, 2H, H-2, CH₂CH=CH₂), 3.96 (dd, *J*=3.2, 1.6 Hz, 1H, H-2'), 3.94–3.65 (m, 13H, H-3, H-5, H-3', H-4', H-5', H-6a', H-6b', H-2'', H-3'', H-4'', H-5'', H-6a'', H-6b''), 3.61 (t, *J*=9.6 Hz, 1H, H-4), 3.15 (dd, *J*=14.0, 2.0 Hz, 1H, H-6a), 2.75 (dd, *J*=14.0, 3.6 Hz, 1H, H-6b) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 137.9, 120.2, 106.5, 103.3, 89.0, 82.9, 77.6, 77.5, 76.3, 76.2, 75.7, 75.0, 74.6,

74.0, 72.7, 71.5, 71.4, 71.3, 65.4, 65.3, 35.7 ppm; HRMS (ESI) *m/z* calcd for C₂₁H₃₆O₁₅SSiNa [M-H][−] 559.1697, found 559.1715.

4.12. 3-((2-t-Butoxycarbonylamino)ethylthio)propyl 2,4-di-O-acetyl-3-O-(2,3,4-tri-O-benzoyl-6-O-t-butylidimethylsilyl-α-D-mannopyranosyl)-6-S-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (22)

A solution of **20** (208 mg, 0.16 mmol), 2-t-butylcarbamate-ethanethiol **3** (170 mg, 0.96 mmol) and AIBN (13.8 mg, 0.08 mmol) in toluene (10 mL) was degassed with N₂ and refluxed for 4 h. The solution was cooled and the solvent removed in vacuo. The remaining oily residue was then purified by silica gel column chromatography (petroleum ether/EtOAc=2/1) to give **22** (211 mg, 89%) as a colorless syrup. [α]_D²⁵ +6.7 (c 2.7, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ 8.08 (dd, *J*=6.8, 1.2 Hz, 2H), 7.95 (dd, *J*=7.2, 1.6 Hz, 2H), 7.83 (dd, *J*=7.2, 1.2 Hz, 2H), 7.58 (t, *J*=7.2 Hz, 1H), 7.51–7.41 (m, 3H), 7.39–7.35 (m, 3H), 7.24 (t, *J*=6.8 Hz, 2H), 6.03 (t, *J*=10.0 Hz, 1H, H-4'), 5.66 (dd, *J*=10.4, 3.2 Hz, 1H, H-3'), 5.42 (dd, *J*=3.2, 1.6 Hz, 1H, H-2'), 5.35 (dd, *J*=2.8, 1.2 Hz, 1H, H-2), 5.34–5.27 (m, 5H, H-1', H-1'', H-3'', H-4, H-4''), 5.26 (d, *J*=1.6 Hz, 1H, H-2''), 5.05 (br, 1H, NH), 4.79 (d, *J*=1.2 Hz, 1H, H-1), 4.42–4.38 (m, 1H, H-5''), 4.31 (dd, *J*=12.4, 5.2 Hz, 1H, H-6a''), 4.23 (dd, *J*=9.6, 3.2 Hz, 1H, H-3) 4.18 (dt, *J*=10.0, 2.4 Hz, 1H, H-5'), 4.11–4.07 (dd, *J*=12.4, 6.0 Hz, 1H, H-6b''), 3.90–3.83 (m, 2H, H-5, OCH₂CH₂), 3.80–3.76 (m, 2H, H-6a', 6b'), 3.51 (dt, *J*=10.0, 6.0 Hz, 1H, OCH₂CH₂), 3.30 (d, *J*=6.0 Hz, 2H, CH₂NH), 2.82 (dd, *J*=14.0, 2.8 Hz, 1H, H-6a), 2.75 (dd, *J*=14.0, 8.0 Hz, 1H, H-6b), 2.65–2.58 (m, 4H, CH₂SCH₂), 2.28 (s, 3H, OAc), 2.23 (s, 3H, OAc), 2.16 (s, 3H, OAc), 2.13 (s, 3H, OAc), 2.04 (s, 3H, OAc), 1.98 (s, 3H, OAc), 1.93–1.84 (m, 2H, CH₂CH₂CH₂), 1.41 (s, 9H, NH(CH₃)₃), 0.87 (s, 9H, C(CH₃)₃), −0.01 (s, 3H, Si(CH₃)₂), −0.04 (s, 3H, Si(CH₃)₂) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 170.7, 170.5, 170.4, 169.9, 169.8, 169.7, 165.6, 165.3, 165.2, 155.8, 133.5, 133.2, 133.0, 129.9(2), 129.7(2), 129.6(2), 129.5, 129.2(2), 128.5(2), 128.4(2), 128.3(2), 99.0, 97.4, 82.3, 79.3, 74.8, 72.1, 70.9, 70.8(3), 70.5, 69.9, 69.8, 69.5, 69.1, 66.5, 66.2, 62.3, 61.7, 39.7, 32.1, 32.0, 29.0, 28.4(3), 28.2, 25.9(3), 21.1, 20.9, 20.8, 20.7(2), 20.6, 18.3, −5.5, −5.7 ppm; MALDI-TOF MS *m/z* calcd for C₆₇H₈₉NO₂₆S₂SiNa [M+Na]⁺ 1438.5, found 1438.5.

4.13. 3-((2-t-Butoxycarbonylamino)ethylthio)propyl 2,4-di-O-acetyl-3-O-(2,3,4-tri-O-benzoyl-6-O-acetyl-α-D-mannopyranosyl)-6-S-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (24)

To a stirred solution of **22** (200 mg, 0.14 mmol) in anhydrous CH₂Cl₂ (2 mL) and pyridine (0.5 mL) was added HF-pyridine (0.5 mL) at 0 °C. The mixture was stirred at rt for 8 h and monitored by TLC (Petroleum ether/EtOAc=1:1). When the starting material had disappeared, saturated aqueous sodium bicarbonate was added carefully. The aqueous layer was extracted with CH₂Cl₂ (4×15 mL). The combined organic phase was dried and then concentrated to afford a syrup. To a solution of the syrup in pyridine (10 mL) was added Ac₂O (0.03 mL, 0.28 mmol). The reaction mixture was stirred at rt for 4 h and then evaporated under vacuo to give a yellow oil. Purification by silica gel column chromatography (petroleum ether/EtOAc=1.5/1) give **24** (139 mg, 74%) as a colorless syrup. [α]_D²⁵ +8.9 (c 2.5, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ 8.06 (d, *J*=7.2 Hz, 2H), 7.96 (d, *J*=7.2 Hz, 2H), 7.81 (d, *J*=7.2 Hz, 2H), 7.59 (t, *J*=7.2 Hz, 1H), 7.51–7.44 (m, 3H), 7.41–7.34 (m, 3H), 7.24 (t, *J*=7.6 Hz, 2H), 5.91 (t, *J*=10.0 Hz, 1H, H-4'), 5.70 (dd, *J*=10.0, 2.8 Hz, 1H, H-3'), 5.42 (dd, *J*=2.8, 2.0 Hz, 1H, H-2'), 5.35–5.26 (m, 7H, H-1', H-2, H-4, H-1'', H-2'', H-3'', H-4''), 5.06 (br, 1H, NH), 4.81 (s, 1H, H-1), 4.39–4.37 (m, 2H, H-5', H-5''), 4.34–4.28 (m, 2H, H-6a', H-6a''), 4.25–4.21 (m, 2H, H-6b', H-3) 4.11–4.07 (m, 1H, H-6b''), 3.89–3.82

(m, 2H, H-5, OCH₂CH₂), 3.51 (dt, *J*=9.6, 6.0 Hz, 1H, OCH₂CH₂), 3.28 (d, *J*=5.6 Hz, 2H, CH₂NH), 2.82 (dd, *J*=14.0, 2.4 Hz, 1H, H-6a), 2.75 (dd, *J*=14.0, 7.6 Hz, 1H, H-6b), 2.63–2.57 (m, 4H, CH₂SCH₂), 2.28 (s, 3H, OAc), 2.23 (s, 3H, OAc), 2.15 (s, 3H, OAc), 2.12 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.04 (s, 3H, OAc), 1.97 (s, 3H, OAc), 1.91–1.83 (m, 2H, CH₂CH₂CH₂), 1.40 (s, 9H, NH(CH₃)₃) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 170.7, 170.6, 170.5, 170.4, 169.9, 169.8, 169.7, 165.5, 165.4, 165.2, 155.8, 133.7, 133.5, 133.2, 129.9(2), 129.8(2), 129.7(2), 129.1, 129.0, 128.9, 128.6(2), 128.5(2), 128.3(2), 98.8, 97.3, 82.4, 79.3, 74.9, 70.8(2), 70.6, 70.4, 69.9, 69.5, 69.4, 69.2, 69.1, 66.6, 66.5, 66.2, 62.6, 62.3, 39.7, 32.1, 31.9, 29.0, 28.4(3), 28.2, 21.0, 20.9, 20.8, 20.7(2), 20.6(2) ppm; MALDI-TOF MS *m/z* calcd for C₆₃H₇₇NO₂₇S₂Na [M+Na]⁺ 1366.4, found 1366.4.

4.14. 3-(2-Aminoethylthio)propyl 2,4-di-O-acetyl-3-O-(2,3,4-tri-O-benzoyl-6-O-acetyl-α-D-manno pyranosyl)-6-S-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (25)

To a solution of **24** (139 mg, 0.10 mmol) in CH₂Cl₂ (2 mL) was added TFA (0.06 mL, 0.54 mmol). The mixture was stirred at room temperature for 6 h, and when the starting material had disappeared, The reaction mixture was then concentrated under reduced pressure to a residue that was purified by silica gel column chromatography (EtOAc/MeOH=10/1) to afford **25** (102 mg, 82%) as a colorless syrup. [α]_D²⁵ +11.8 (c 1.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ 8.15 (br, 2H, NH), 8.08 (d, *J*=8.0 Hz, 2H), 7.97 (d, *J*=8.0 Hz, 2H), 7.83 (d, *J*=8.0 Hz, 2H), 7.61 (t, *J*=7.6 Hz, 1H), 7.53–7.46 (m, 3H), 7.44–7.37 (m, 3H), 7.26 (t, *J*=6.8 Hz, 2H), 5.94 (t, *J*=10.0 Hz, 1H, H-4'), 5.70 (dd, *J*=10.0, 3.2 Hz, 1H, H-3'), 5.43 (s, 1H, H-2'), 5.36 (d, *J*=3.2 Hz, 1H, H-2''), 5.34–5.27 (m, 5H, H-1', H-2, H-4, H-1'', H-4''), 5.21 (dd, *J*=10.0, 3.2 Hz, 1H, H-3''), 4.81 (s, 1H, H-1), 4.40–4.36 (m, 2H, H-5', H-5''), 4.32–4.28 (m, 3H, H-6a', H-6a'', H-6b''), 4.23 (dd, *J*=10.0, 3.2 Hz, 1H, H-3), 4.11 (d, *J*=11.6 Hz, 1H, H-6b'), 3.90–3.83 (m, 2H, H-5, OCH₂CH₂), 3.54 (dt, *J*=9.6, 6.0 Hz, 1H, OCH₂CH₂), 3.15 (t, *J*=6.0 Hz, 2H, CH₂NH), 2.83–2.78 (m, 4H, H-6a, H-6b, SCH₂CH₂NH₂), 2.60 (t, *J*=6.8 Hz, 2H, CH₂CH₂CH₂S), 2.29 (s, 3H, OAc), 2.24 (s, 3H, OAc), 2.15 (s, 3H, OAc), 2.13 (s, 3H, OAc), 2.05 (s, 6H, OAc), 1.98 (s, 3H, OAc), 1.90–1.87 (m, 2H, CH₂CH₂CH₂) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 170.8, 170.7, 170.5, 170.4, 170.2, 170.1, 169.7, 165.5, 165.4, 165.2, 133.7, 133.5, 133.2, 129.8(4), 129.6(2), 129.1, 129.0, 128.9, 128.6(2), 128.5(2), 128.3(2), 98.8, 97.4, 82.2, 74.7, 70.8(2), 70.6, 70.3, 70.1, 69.6, 69.4, 69.2, 69.1, 66.6, 66.5, 66.1, 62.6, 62.3, 38.8, 31.8, 28.7(2), 28.1, 21.0, 20.8, 20.7, 20.6, 20.5(2), 20.4 ppm; MALDI-TOF MS *m/z* calcd for C₅₈H₆₉NO₂₅S₂Na [M+Na]⁺ 1266.3, found 1266.4.

4.15. Tri-[[2,4-di-O-acetyl-3-O-(2,3,4-tri-O-benzoyl-6-O-acetyl-α-D-mannopyranosyl)-6-S-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyloxypropylthio]ethyl]benzene 1,3,5-tricarboxamide (26)

To a solution of compound **25** (102 mg, 0.08 mmol) and the acid chloride **4** (7.1 mg, 0.03 mmol) in CH₂Cl₂ (2 mL) was added NEt₃ (11 μg, 0.08 mmol) slowly at 0 °C. The mixture was stirred at 0 °C for 30 min, then the reaction mixture was concentrated under reduced pressure to give a residue that was subjected to silica gel column chromatography (EtOAc/Petroleum ether=3/1) to afford **26** (70 mg, 67%) as a colorless syrup. [α]_D²⁵ +9.0 (c 2.1, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ 8.36 (s, 3H), 8.07 (d, *J*=7.2 Hz, 6H), 7.96 (d, *J*=7.6 Hz, 6H), 7.83 (d, *J*=7.2 Hz, 6H), 7.60 (t, *J*=7.6 Hz, 3H), 7.53–7.44 (m, 9H), 7.42–7.36 (m, 9H), 7.26 (t, *J*=7.6 Hz, 6H), 7.15 (br, 3H, NH), 5.92 (t, *J*=10.0 Hz, 3H, H-4'), 5.71 (dd, *J*=10.0, 3.2 Hz, 3H, H-3'), 5.44 (d, *J*=2.8 Hz, 3H, H-2'), 5.37 (d, *J*=2.8 Hz, 3H, H-2''), 5.35–5.25 (m, 18H, H-1', H-2, H-4, H-1'', H-3'', H-4''), 4.83 (s, 3H, H-1), 4.41–4.38 (m, 6H, H-5', H-5''), 4.35–4.29 (m, 6H, H-6a', H-6a''), 4.27–4.22 (m, 6H, H-6b'', H-3) 4.10 (dd, *J*=12.4, 1.6 Hz, 3H, H-6b'), 3.91–3.86 (m,

6H, H-5, OCH₂CH₂), 3.62 (dd, *J*=13.2, 7.2 Hz, 6H, CH₂NH), 3.54 (dt, *J*=10.0, 6.0 Hz, 6H, OCH₂CH₂), 2.83 (dd, *J*=14.0, 2.8 Hz, 3H, H-6a), 2.78 (d, *J*=8.0 Hz, 3H, H-6b), 2.74 (t, *J*=6.8 Hz, 6H, SCH₂CH₂NH), 2.64 (t, *J*=6.8 Hz, 6H, CH₂CH₂CH₂S), 2.30 (s, 9H, OAc), 2.23 (s, 9H, OAc), 2.15 (s, 9H, OAc), 2.13 (s, 9H, OAc), 2.07 (s, 9H, OAc), 2.05 (s, 9H, OAc), 1.97 (s, 9H, OAc), 1.94–1.86 (m, 6H, CH₂CH₂CH₂) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 170.7(3), 170.6(3), 170.4(3), 170.1(3), 170.0(3), 169.7(6), 165.6(3), 165.5(3), 165.4(3), 165.2(3), 135.0(3), 133.7(3), 133.5(3), 133.2(3), 129.9(6), 129.8(6), 129.7(6), 129.2(3), 129.1(3), 128.9(3), 128.6(6), 128.5(6), 128.4(3), 128.3(6), 98.8(3), 97.4(3), 82.4(3), 74.8(3), 70.9(3), 70.8(3), 70.7(3), 70.4(3), 70.0(3), 69.5(3), 69.4(3), 69.2(3), 69.1(3), 66.6(3), 66.5(3), 66.2(3), 62.6(3), 62.3(3), 39.4(3), 32.0(3), 31.3(3), 28.9(3), 28.2(3), 21.1(3), 20.9(3), 20.8(3), 20.7(6), 20.6(6) ppm; MALDI-TOF MS *m/z* calcd for C₁₈₃H₂₀₇N₃O₇₈S₆Na [M+Na]⁺ 3910.5, found *m/z* 3910.5.

4.16. Tri-[[3-O-(α-D-mannopyranosyl)-6-S-(α-D-mannopyranosyl)-α-D-mannopyranosyloxypropylthio]ethyl]benzene 1,3,5-tricarboxamide (2)

To a solution of **26** (56 mg, 0.014 mmol) in anhydrous methanol (3 mL) was added 1M solution of sodium methoxide in MeOH until pH 9–10 was reached. The reaction mixture is stirred at rt for 10 h. Upon completion (TLC), more MeOH (5 mL) was added, and the solution was neutralized with amberlite IR-120 (H⁺). The filtrate was concentrated in vacuo in a rotary evaporator, and the residue was purified with Sephadex G-25. The desired fractions were lyophilized to give **2** (29 mg, quantitative) as white foam. [α]_D²⁵ +118 (c 0.75, H₂O); ¹H NMR (400 MHz, D₂O): δ 8.27 (s, 3H, Ph), 5.28 (s, 3H, H-1''), 5.08 (s, 3H, H-1'), 4.77 (s, 3H, H-1), 4.06–4.02 (m, 9H, H-2, H-2', H-2''), 3.97–3.60 (m, 48H), 3.57 (dd, *J*=9.6, 4.8 Hz, 3H, OCH₂CH₂), 3.12 (dd, *J*=13.2, 4.0 Hz, 3H, H-6a), 2.85 (t, *J*=6.0 Hz, 6H, SCH₂CH₂NH), 2.78–2.71 (m, 9H, H-6b, CH₂CH₂CH₂S), 1.99–1.89 (m, 6H, CH₂CH₂CH₂) ppm; ¹³C NMR (100 MHz, D₂O): δ 168.5(3), 134.9(3), 129.0(3), 102.2(3), 99.7(3), 84.6(3), 77.9(3), 73.2(3), 73.2(3), 71.7(3), 71.4(3), 71.1(3), 70.3(3), 70.0(3), 69.6(3), 68.7(3), 67.0(3), 66.7(3), 66.1(3), 61.0(3), 60.8(3), 39.3(3), 31.7(3), 30.6(3), 28.4(3), 28.0(3) ppm; MALDI-TOF MS *m/z* calcd for C₇₈H₁₂₉N₃O₄₈S₆Na [M+Na]⁺ 2090.6, found 2090.6.

4.17. Hemagglutination inhibition assays³⁶

Con A (5 mg) was dissolved in 10 mL of HEPES buffer with 100 mM CaCl₂, pH 8.5, and dialyzed into tris buffered saline (TBS), then phosphate buffered saline (PBS) for the assay. Protein solution concentration was estimated from its UV absorbance at 280 nm. Rabbit red blood cells were stored as a 10% suspension in Alsever's solution and washed with PBS 3 times before the assay. The cells were re-suspended in PBS with 0.5% BSA. The minimum concentration of inhibitor required to inhibit 8 units Con A was determined as follows: Serial two fold dilutions of Con A in assay buffer were incubated with 50 μL of the blood solution and examined for agglutination after 2 h at 23 °C. The minimum concentration causing complete agglutination was defined as 1 unit. Then 8 unit Con A was incubated with serial two fold dilutions of the inhibitors for 3 h at 23 °C. Then 50 μL of the blood solution was added and the samples were mixed and incubated for 2 h at 23 °C. The inhibiting dosage was determined as the minimum concentration of inhibitor at, which no agglutination is observed.

4.18. Computational details

To gain a deep insight into the complex structures of Con A and trimannoside, we carried out docking studies on them with Surflex-Dock algorithm^{40,41} of Sybyl-X software.⁴² The structure of Con A

was downloaded from Protein Data Bank (PDB entry code 1ONA, A chain; PDB extracted from the Brookhaven Protein Database <http://www.rcsb.org/pdb>) and used in initial docking studies. The structure of trisaccharide Man(3,6)Man-OMe is extracted from crystal structure 1ONA, while the structures of Man(3,6)Man-OAll and **1** were built by modifying Man(3,6)Man-OMe with Sybyl software. All the trimannosides were minimized with Powell method with default parameters provided by Sybyl. All hydrogen atoms were added, and Gasteiger-Huckel charges were calculated for ligand and protein. To generate an idealized protocol that represents the binding site, threshold was set to 0.5 Å and bloat was set to 5 Å. During the docking, most of the parameters were the default values, except for post-docking minimization. The bindings of trimannosides and Con A were estimated using a variety of scoring functions within the consensus score (CScore). Pictures were drawn with PyMol software (<http://www.pymol.org/>).

Acknowledgements

This work was supported in partial by Strategic Priority Research Program of CAS (Grant No. XDB14040201), the MOST of China (Projects 2012ZX09502001, 2012CB822101), and NNSF of China (Projects 21372254, 21321004). We thank Dr. Min Li for mass spectra performance and Dr. Feifei Cheng for the suggestions in HA bioassay.

Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.carres.2015.05.001>.

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