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Interactions of aromatic mannosyl disulfide derivatives with Concanavalin A: synthesis, thermodynamic and NMR spectroscopy studies

Bandaru Narasimha Murthy [a], Sharmistha Sinha [b], Avadhesha Surolia [b,*], Narayanaswamy Jayaraman [a,*], László Szilágyi [c,*], Ildikó Szabó [c], Katalin E. Kövér [d,*]

[a] Department of Organic Chemistry, Indian Institute of Science, Bangalore 560 012, India
[b] National Institute of Immunology, Aruna Asif Ali Marg, New Delhi 110 067, India
[c] Department of Organic Chemistry, University of Debrecen, H-4010 Debrecen, pf. 20, Hungary
[d] Department of Inorganic Chemistry, University of Debrecen, H-4010 Debrecen, pf. 21, Hungary

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ABSTRACT

α-D-Mannopyranosyl units were attached to an aromatic scaffold through disulfide linkages to obtain mono- to trivalent glycosylated ligands for lectin binding studies. Isothermal titration calorimetric (ITC) measurements indicated that binding affinities of these derivatives to Concanavalin A (Con A) were comparable to or slightly higher than that of methyl α-D-mannopyranoside (K_a values in the range of 10^4 M^{-1}). The stoichiometries of the lectin-ligand complexes were in agreement with the formal valencies (1–3) of the respective ligands indicating cross-linking in interactions with the di- and trivalent derivatives. Multivalency effects could not, however, be observed with the latter. These ligands were shown to bind to the carbohydrate binding site of Con A using saturation transfer difference (STD) NMR competition experiments.

1. Introduction

Lectins are carbohydrate-binding proteins involved in a multitude of biological processes. To provide insight into the molecular basis of their biological activity, the binding of synthetic carbohydrate derivatives or glycoconjugates is currently studied with various techniques (for a recent survey, see Ref. 1). Discovering novel carbohydrate structures with lectin-binding properties may contribute to our understanding of the structure, energetics, and dynamics of lectin–saccharide complexes.

Replacing the interglycosidic oxygen in disaccharides by a disulfide bond has been proposed as a promising approach to obtain novel glycomimetics.[2,3] The disulfide bridge, featuring a three-bond distance between the anomeric carbon and the aglycon, provides a larger conformational space than the natural, two-bond glycosidic linkage,[4] and differences in the stereoelectronic properties between the O- and S-atoms may also play a role in interactions with proteins. Such studies have not, however, been reported until recently when it was shown[4–6] that appropriately positioned symmetric diglycosyl disulfides are capable to bind to various lectins, as judged from competition binding experiments. Importantly, inhibitory activities against an endogenous lectin were clearly demonstrated in vivo on human tumor cell lines. Based on these results disulfide-linked sugar derivatives were suggested 'as new substance platform for lectin-directed drug design'.[4] The binding affinities of the disulfide disaccharides with *manno* configurations were found to be comparable to that of methyl α-D-mannopyranoside in experiments with Concanavalin A.[6] The structures and thermodynamics of Con A in complexes with various mannopyranosides have previously been extensively investigated in solution and in the solid state.[7–12]

In the present study, we have chosen to attach one to three mannopyranosyl units to a benzene ring through disulfidomethylene linkages to prepare mono- to trivalent glycosyl disulfide derivatives. It is known that the binding of aromatic glycosides to Con A is stronger than aliphatic ones.[13] We desired to study interactions of these novel ligands with Con A to assess the binding affinities and thermodynamic parameters, and also to probe multivalency effects, if any.

2. Results and discussion

2.1. Syntheses of the ligands 1–6

The sodium salt of 1-thio-α-D-mannopyranose, obtained from 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranose[14,15] by treatment with sodium methoxide, was reacted directly with

* Corresponding authors. Fax: +36 52 453836 (L.S.).
E-mail address: lszilagyi@tigris.klte.hu (L. Szilágyi).

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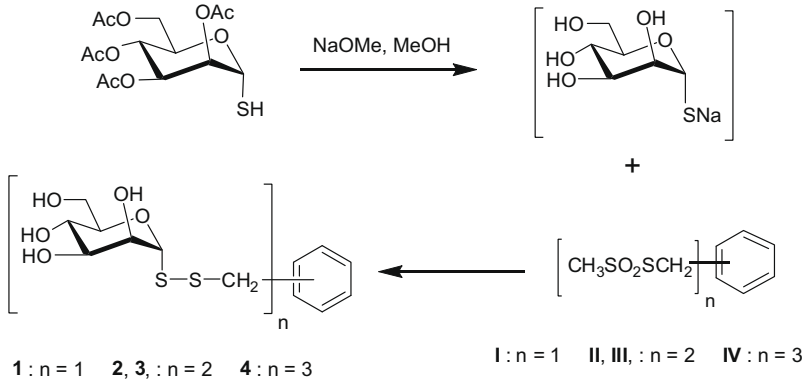
methanethiosulfonatomethylenebenzenes I to IV (Scheme 1) following a modified procedure.[16] The mannopyranosyl disulfide ligands **1**–**4** (Chart 1) were obtained after purification by column chromatography (see Section 3).

Compounds **5** and **6** were prepared by standard procedures as described in Section 3.

2.2. ITC studies

The binding potencies of **1–6** (Chart 1) were assessed first by ITC, which provides not only the binding constants but also the associated thermodynamic parameters, the free energy changes, the enthalpy, and the entropy changes.[17,18] The ITC measurements were conducted at pH 7.4, in HEPES buffer and the ligand and lectin concentrations ranged from 2.5 to 9 mM and from 0.2 to 0.4 mM, respectively. The Con A exists as a tetramer at this pH.[19]

Displayed in Figure 1 are representative raw and integrated plots for the calorimetric titration of the monovalent sugar disulfide ligand **1** and the divalent ligand **3**. The equilibrium constants, thermodynamic parameters, and stoichiometries for the interactions of ligands **1** to **6** with Con A are presented in Table 1. The binding parameters of the glycosyl disulfides were compared with methyl α-D-mannopyranoside (MeαMan), D-mannose (Man), thioglycoside **5**, and glycoside **6**. Compounds **5** and **6**



Scheme 1. Synthesis of glycosyl disulfide ligands **1**–**4**.

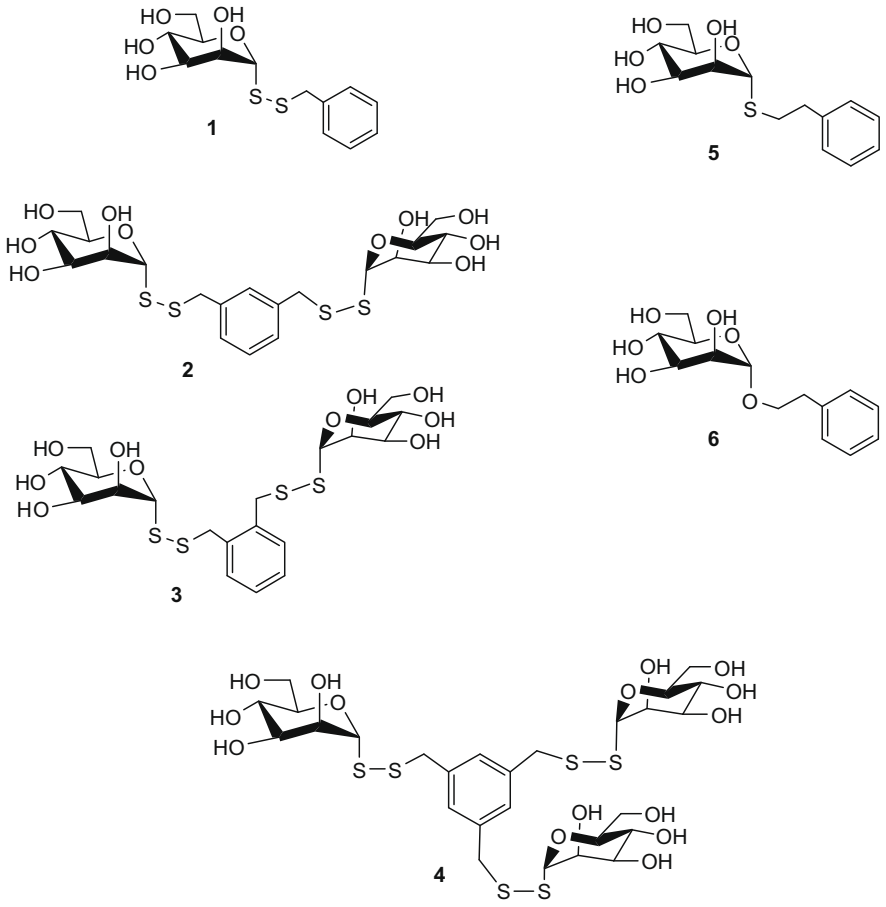


Chart 1. Structures of the mono-, di-, and trivalent mannopyranosyl ligands **1**–**6**.

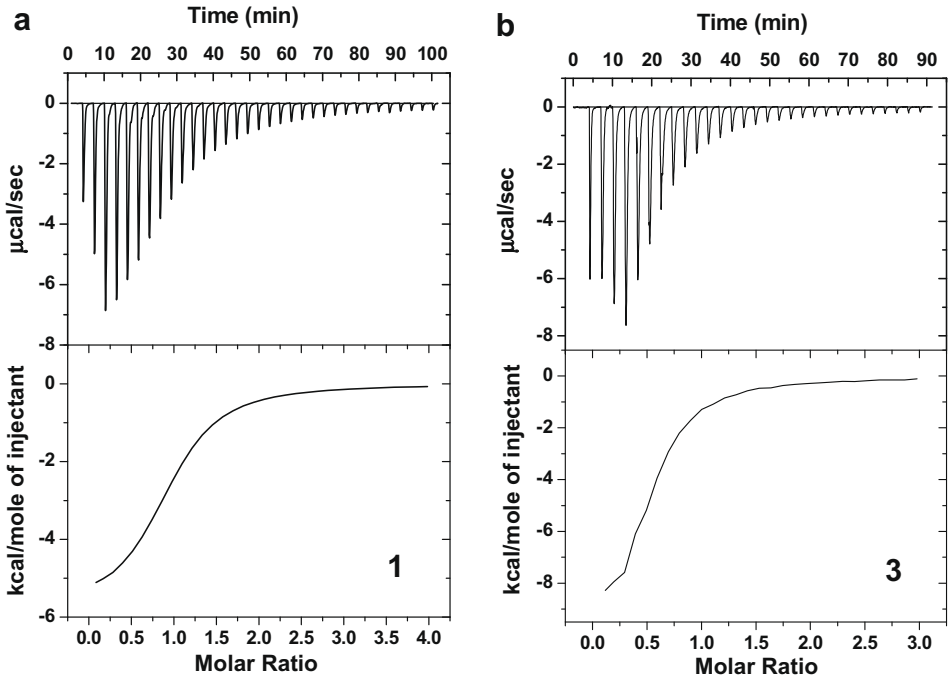


Figure 1. Raw and integrated data for the calorimetric titration of Con A with (a) monovalent sugar disulfide **1** and (b) divalent sugar disulfide **3**.

Table 1
Binding stoichiometries and thermodynamic parameters for various glycosyl disulfide ligands–lectin interactions[a]

Ligand	*N*	K_a	ΔG	ΔH	$T\Delta S$
1	0.96	$(9.4 \pm 0.3) \times 10^3$	−5.41	−5.83 ± 0.1	−0.42
2	0.56	$(2.11 \pm 0.2) \times 10^4$	−5.9	−8.73 ± 0.2	−2.83
3	0.54	$(3.71 \pm 0.2) \times 10^4$	−6.23	−10.25 ± 0.1	−4.02
4	0.39	$(3.16 \pm 0.2) \times 10^4$	−6.17	−12.7 ± 0.4	−6.53
5	1.1	$(8.6 \pm 0.08) \times 10^3$	−5.35	−7.21 ± 0.04	−1.86
6	1.03	$(2.23 \pm 0.02) \times 10^4$	−5.95	−9.32 ± 0.06	−3.37
MeαMan	1.04	$(7.9 \pm 0.04) \times 10^3$	−5.27	−7.83 ± 0.12	−2.56
Man	1.0	$(2.21 \pm 0.05) \times 10^3$	−4.42	−3.92 ± 0.2	0.67

[a] Thermodynamic parameters were derived from one-site binding model; K_a is in the unit of M^{-1}; ΔG, ΔH, and $T\Delta S$ are in the units of kcal mol^{-1}. Errors in ΔG are ~1–5%. Errors in $T\Delta S$ are in the range of 1–3%.

were included to investigate the role, if any, of the disulfide bond on the binding.

The *c* parameter, which is the product of the macromolecular concentration and the binding constant,[20,21] was above 1 for all the ligand–lectin complexations. As shown in Table 1, the α-configurated ligands **1**–**4** exhibited binding affinities comparable to or slightly better than the monovalent glycosides **5, 6**, or MeαMan. The association constant (K_a) values for the monovalent disulfide- and thioglycoside derivatives **1** and **5**, respectively, are practically identical with that of MeαMan. Interestingly, the O-glycoside **6** binds ca. three times stronger. The K_a enhancements observed for the di- and trivalent ligands **2** and **4**, respectively, may be ascribed to the binding of individual mannose units in these derivatives to independent lectin binding sites (vide infra). The binding of the divalent ligand **3** is, however, stronger than expected on a valency-corrected basis alone.

The binding process is favored by large enthalpic changes with all the ligands. The binding stoichiometry was close to 1 for all the monovalent derivatives, whereas it was close to 0.5 for the divalent ligands **2** and **3**. This is an indication of the functional divalency[22] of these derivatives, that is, binding of each of the sugar residues in **2** and **3** to separate lectin binding sites. Similarly, the *n*-value of 0.39 observed for **4** is an indication of approximate functional trivalency for this triglycoside-type ligand. In the absence of the possibility for an intramolecular complexation within the lectin tetramer, due to distance constraints, the observed functional valencies relate to intermolecular complexation, that is, the cross-linking of the lectin tetramers by the ligands. While the cross-linked complexes exist in solution at low molar ratios of ligand to lectin, at higher ratios the cross-linking between the ligand and the lectin becomes dense. As a result, a visible precipitation of the complex could be observed in the ITC cell when saturation of the binding sites was completed.

In order to elucidate whether the binding of disulfide ligands occurs at the carbohydrate binding site of the lectin, as opposed to non-specific binding, competition experiments were run, using saturation transfer difference (STD) NMR spectroscopy.[23,24] This method has found widespread application in the study of carbohydrate–protein recognition phenomena[25] and is suitable to investigate competition between two ligands for the same binding site on the protein. Changes in the STD signal intensities upon titration of a protein–ligand A complex with ligand B are indicative of a competition process between A and B.[24]

An example is shown in Figure 2. Traces (a) and (b) are the regular and STD ^{1}H NMR spectra, respectively, of **3** in the presence of Con A. The appearance of the ligand signals in (b) is indicative of the binding of ligand **3** to Con A. Saturation transfer resonances in spectrum (b) reveal contacts of sugar ring protons 2–6 (3.5–3.9 ppm) and aromatic protons (7.3–7.5 ppm) to the protein. The anomeric signal (4.7 ppm) is suppressed by the water suppression sequence which also reduces the intensities of the SCH_2 resonances at 4.12 and 4.24 ppm. Addition of increasing amounts of MeαMan to the sample causes to decrease the signal intensities of **3**. When signal intensities are plotted as a function of the concentration of the MeαMan competitor added to the Con A–**3** complex, displacement of **3** by MeαMan becomes evident (Fig. 3). This is an evidence to indicate that the binding of **3** takes place at the carbohydrate binding site of Con A.

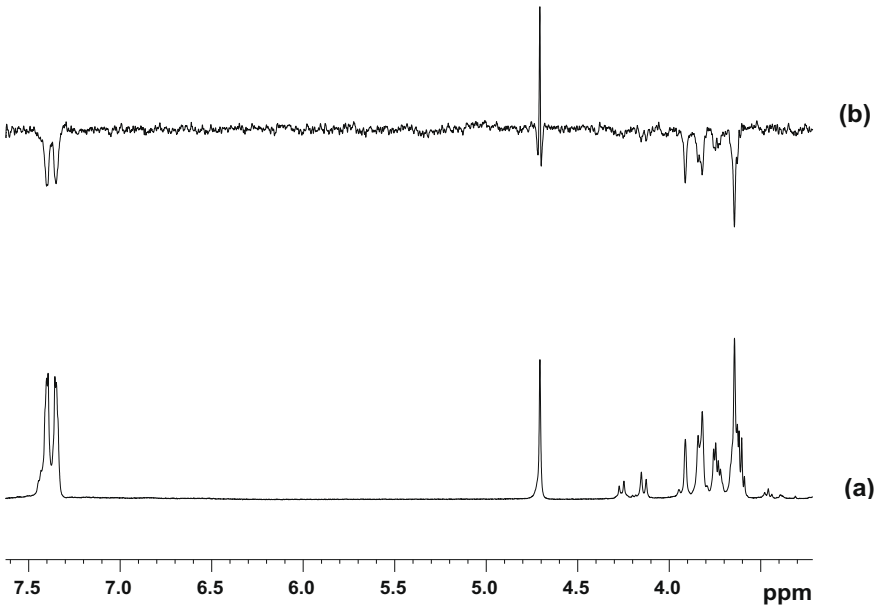


Figure 2. Binding of ligand **3** to Con A as seen in a ^{1}H NMR STD experiment. Sample conditions: Con A (80 μM), NH_4OAc buffer (100 mM, pH 7.2), NaCl (150 mM), $CaCl_2$ (2 mM) in 500 μL D_2O at 300 K. (a) ligand **3** (4 mM), standard ^{1}H NMR spectrum; (b) same sample, STD ^{1}H NMR spectrum. For the STD measurement conditions, see: Section 3.

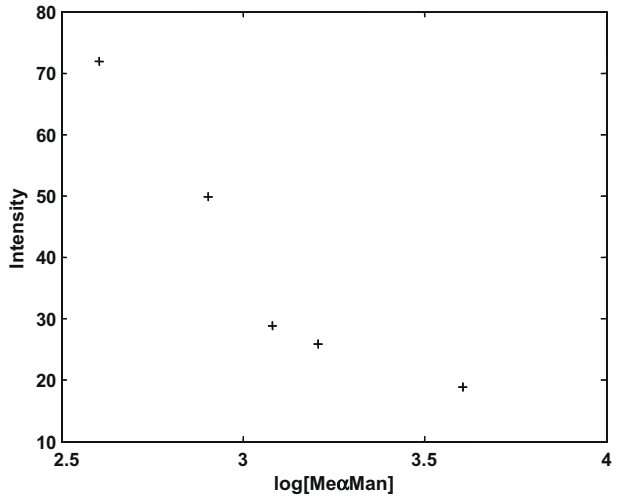


Figure 3. Titration data for the experiment in Figure 2. Changes of the aromatic signal intensities (~7.4 ppm) in the STD ^{1}H NMR spectra of ligand **3** as a function of the added MeαMan. The concentration (log[MeαMan] in μM) of the added MeαMan competitor was increased from 0.4 to 4 mM.

In summary, we have constructed novel glycosyl disulfide derivatives characterized by attachment of one to three α-D-mannopyranosyl units to a benzene ring through disulfidomethylene linkages. ITC measurements indicated binding of these derivatives to the lectin Con A with binding affinities comparable to or slightly higher than that of MeαMan. The stoichiometries of the lectin–ligand complexes were in agreement with the formal valencies (1–3) of the respective ligands therefore, cross-linking is likely to occur in interactions with the di- and trivalent derivatives (**2**–**4**). Multivalency (or cluster glycoside) effects could not, however, be observed with the latter. Saturation transfer difference (STD) NMR competition experiments indicated binding to the carbohydrate binding site of Con A. The present derivatives, together with recently reported diglycosyl-disulfides[4–6] represent novel carbohydrate structures with lectin-binding properties to study lectin–carbohydrate interactions. Further studies will be needed to assess the sugar-disulfide-based approach especially in view of increasing evidence that ligand efficiency does not depend only on valency but on a multitude of other factors such as the topology and architecture of the epitopes, their density, and/or the nature of the scaffolds.[†,12,26–30] On the other hand, the disulfide linkage is easily established and offers remarkable possibilities to manipulate the interactions via mild chemical transformations of this bond.[31–33]

3. Experimental

3.1. General methods

Chemicals were purchased from commercial sources and were used without further purifications. Solvents were dried and distilled according to literature procedures. Analytical TLC was performed on commercial Merck plates coated with Silica Gel GF254 (0.25 mm). Silica Gel Merck (100–200 mesh) was used for column chromatography. High-resolution mass spectra were recorded on a Bruker micrOTOF-Q instrument by electrospray ionization (ESI) technique. Lectin Con A (salt-free lyophilized powder) was purchased from Sigma.

3.2. ITC and NMR studies

Isothermal titrations were performed using a microcalorimeter Microcal VP-ITC. Aqueous solutions were prepared from doubly distilled water purified through a Milli Q-plus system to 18.2 MΩ resistance. All ligand–lectin binding experiments were performed in HEPES buffer (10 mM) (pH 7.4) containing NaCl (150 mM), $CaCl_2$ (1 mM), and $MnCl_2$ (1 mM). Buffer solution was filtered (0.2 μm) and thoroughly degassed. The concentration of Con A was determined spectrophotometrically at 280 nm using $A^{1\%}_{1cm} = 13.7$ at pH 7.4 and expressed in terms of monomer ($M_r = 25,\ 600$).[34] In individual titrations, injection of 6–10 μL of ligand was added from the computer-controlled 300 μL microsyringe at intervals of 3 min into the Con A solution dissolved in the same buffer as the ligand; the microsyringe stirring at 300 rpm. All measurements were

[†] We thank a referee for raising these points.

made at 25 °C. Control experiments were performed by injecting the ligand into a cell containing buffer with no protein, and the heats of dilution subtracted from those measured in the presence of Con A. The initial injection was discarded in order to remove the effect of titrant diffusion across the syringe tip during the equilibration process. The experimental data were fitted to a one site binding model, using a nonlinear least-squares procedure,[20] with ΔH, K_a (association constant), and n (number of binding sites for monomer), as adjustable parameters.

^{1}H and ^{13}C NMR spectral analyses were performed using a Bruker Avance DRX-500 spectrometer operating at 500 and 125 MHz, respectively, and equipped with a 5 mm *z*-gradient multinuclear proton detection (bbi) probe head. The residual solvent signal was used as the internal standard. For STD measurements the duration of the ^{1}H 90° pulse was 15.5 μs and semi-selective irradiation of Con A resonances was achieved by a train of Gaussian 90° pulses of 50 ms each. The residual water signal was suppressed by a WATERGATE sequence. All spectra were processed with XWINNMR 2.6. The samples for STD experiments contained 4 mM carbohydrate ligand in complex with 80 μM Con A in 500 μL D_2O with 150 mM NaCl and 2 mM $CaCl_2$ added in a 100 mM NH_4OAc buffer of pH 7.2 at 300 K.

3.3. Synthetic procedures

3.3.1. General procedure for the preparation of aromatic methanethiosulfonates I–IV

Sodium methanethiosulfonate was prepared from sodium sulfide and mesyl chloride as described.[35] Aromatic methanethiosulfonates **I**, **II**, and **III** were synthesized via reaction of sodium methanethiosulfonate with commercially available mono- and bis(bromomethyl)benzenes according to literature procedures.[36,37] 1,3,5-Tris(methanethiosulfonatomethylene)benzene **IV** was obtained via an analogous reaction starting from 1,3,5-tris(bromomethyl)benzene (89%), mp 100–102 °C; HRESIMS: calcd for $C_{12}H_{18}O_6S_6$ [M+Na]$^+$: 472.932; found: *m/z* 472.936. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.41 (s, 3H, Aryl-H), 4.38 (s, 6H, SCH_2), 3.12 (s, 9H, CH_3); ^{13}C NMR ($CDCl_3$ 125 MHz) δ 137.3 (C-1,-3,-5), 129.5 (C-2,-4,-6), 51.1 (CH_3), 39.9 (SCH_2).

3.3.2. General procedure for the syntheses of glycosyl disulfide ligands 1–4

2,3,4,6-Tetra-*O*-acetyl-1-thio-α-D-mannopyranose[14,15] (0.910 g, 2.5 mmol) in dry MeOH (10 mL) was treated with 1 M sodium methoxide in MeOH (2.6 mL, 2.6 mmol) at room temperature for 0.5 h. Water (3–4 mL) was added to dissolve the precipitated sodium D-mannopyranose 1-thiolate followed by the addition of the calculated amounts of methanethiosulfonates **I, II, III**, or **IV**. The reaction mixture was kept at rt until TLC (38:7:3 EtOAc–MeOH–water) indicated disappearance of the starting materials (ca. 1 h). After evaporation to dryness under diminished pressure, the crude products were purified by column chromatography on silica gel.

3.3.3. (α-D-Mannopyranosyldithiomethylene)benzene (1)

White amorphous solid, 0.690 g (81%), $[\alpha]_D^{22}$ +75.7 (*c* 0.34, MeOH); ^{1}H NMR (CD_3OD, 500 MHz): δ 7.45–7.48 (m, 5H, Aryl-H), 5.09 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1), 4.11 (d, 1H, $J_{S\text{-}CH_2a,b}$ 13.3 Hz, S–CH_{2a}), 4.05 (d, 1H, S–CH_{2b}), 3.96 (m, 1H, H-2), 3.90 (dd, 1H, $J_{5,6a}$ 2.4 Hz, $J_{6a,6b}$ 12.7 Hz, H-6a), 3.89 (m, 1H, H-5), 3.81 (dd, 1H, $J_{5,6b}$ 6.2 Hz, H-6b), 3.61–3.72 (m, 2H, H-3, H-4); ^{13}C NMR (CD_3OD 125 MHz): δ 138.8, 130.6, 129.7, 128.6 (Aryl-C), 94.6 (C-1), 76.5 (C-5), 73.1 (C-2, C-3), 68.9 (C-4) 62.9 (C-6), 44.5 (S-CH_2); HRESIMS: calcd for $C_{13}H_{18}O_5S_2$ [M+Na]$^+$: 341.0488; found: *m/z* 341.0500.

3.3.4. 1,3-Bis(α-D-mannopyranosyldithiomethylene)benzene (2)

White solid, 1.103 g (76%), mp 76–78 °C, $[\alpha]_D^{22}$ +114.7 (*c* 0.22, MeOH); ^{1}H NMR (CD_3OD, 500 MHz): δ 7.27–7.38 (m, 4H, Aryl-H), 5.11 (d, 2H, $J_{1,2}$ 1.6 Hz, H-1), 4.11 (d, 2H, $J_{S\text{-}CH_2a,b}$ 12.3 Hz, S–CH_{2a}), 4.02 (d, 2H, S–CH_{2b}), 3.96 (dd, 2H, $J_{2,3}$ 3.3 Hz, H-2), 3.88 (dd, 2H, $J_{5,6a}$ 2.3 Hz, $J_{6a,6b}$ 11.6 Hz, H-6a), 3.84 (m, 2H, H-5), 3.77 (dd, 2H, $J_{5,6b}$ 5.7 Hz, H-6b), 3.67 (t, 2H, $J_{4,5}$ 9.5 Hz, H-4), 3.58 (dd, 2H, $J_{3,4}$ 9.5 Hz, H-3); ^{13}C NMR (D_2O, 125 MHz): δ 138.6, 131.7, 130.3, 129.9 (Aryl-C), 93.0 (C-1), 75.6 (C-5), 72.4 (C-2, C-3), 67.8 (C-4), 61.9 (C-6), 43.9 (S–CH_2); HRESIMS: calcd for $C_{20}H_{30}O_{10}S_4$ [M+Na]$^+$: 581.0614; found: *m/z* 581.0630.

3.3.5. 1,2-Bis(α-D-mannopyranosyldithiomethylene)benzene (3)

Pale yellowish solid, 1.147 g (79%), mp 134–136 °C, $[\alpha]_D^{22}$ +63.9 (*c* 0.5, MeOH); ^{1}H NMR (CD_3OD, 500 MHz): δ 7.24–7.36 (m, 4H, Aryl-H), 5.13 (d, 2H, $J_{1,2}$ 1.5 Hz, H-1), 4.36 (d, 2H, $J_{S\text{-}CH_2a,b}$ 12.2 Hz, S-CH_{2a}), 4.18 (d, 2H, S-CH_{2b}), 3.95 (dd, 2H, $J_{2,3}$ 3.2 Hz, H-2), 3.87 (dd, 2H, $J_{5,6a}$ 2.2 Hz, $J_{6a,6b}$ 11.5 Hz, H-6a), 3.83 (m, 2H, H-5), 3.79 (dd, 2H, $J_{5,6b}$ 5.1 Hz, H-6b), 3.69 (t, 2H, $J_{4,5}$ 9.4 Hz, H-4), 3.60 (dd, 2H, $J_{3,4}$ 9.4 Hz, H-3); ^{13}C NMR (D_2O, 125 MHz) δ 136.2, 132.7, 129.5 (Aryl-C), 93.2 (C-1), 75.5 (C-5), 72.4 (C-2), 72.3 (C-3), 67.7 (C-4), 61.9 (C-6), 42.1 (S–CH_2); HRESIMS: calcd for $C_{20}H_{30}O_{10}S_4$ [M+Na]$^+$: 581.0614; found: *m/z* 581.0620.

3.3.6. 1,3,5-Tris(α-D-mannopyranosyldithiomethylene)benzene (4)

White solid, 1.190 g (58%), mp 153–155 °C, $[\alpha]_D^{22}$ +31.6 (*c* 0.5, MeOH); ^{1}H NMR (CD_3OD, 500 MHz): δ 7.29 (br s, 3H, Aryl-H), 5.12 (d, 3H, $J_{1,2}$ 1.5 Hz, H-1), 4.11 (d, 3H, $J_{S\text{-}CH_2a,b}$ 12.6 Hz, S–CH_{2a}), 4.03 (d, 3H, S–CH_{2b}), 3.96 (dd, 3H, $J_{2,3}$ 3.2 Hz, H-2), 3.90 (dd, 3H, $J_{5,6a}$ 2.3 Hz, $J_{6a,6b}$ 11.8 Hz, H-6a), 3.85 (m, 3H, H-5), 3.77 (dd, 3H, $J_{5,6b}$ 5.9 Hz, H-6b), 3.65 (t, 3H, $J_{4,5}$ 9.5 Hz, H-4), 3.58 (dd, 3H, $J_{3,4}$ 9.5 Hz, H-3); ^{13}C NMR (CD_3OD 125 MHz): δ 139.5, 130.8, (Aryl-C), 94.6 (C-1), 76.5 (C-5), 73.1 (C-2, C-3), 68.8 (C-4), 62.9 (C-6), 44.1 (S–CH_2); HRESIMS: calcd for $C_{27}H_{42}O_{15}S_6$ [M+Na]$^+$: 821.0740; found: *m/z* 821.0755.

3.3.7. 2-Phenylethyl 1-thio-α-D-mannopyranoside (5)

This compound was prepared as described[14] starting from 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranose. The syrup obtained after deacetylation could not be crystallized. Overall yield 0.476 g (59%); $[\alpha]_D^{22}$ +186 (*c* 0.4, MeOH), lit.[14] $[\alpha]_D^{27}$ +198 (*c* 0.9, MeOH). ^{1}H NMR (Me_2SO-d_6, 500 MHz) δ 7.34–7.12 (m, 5H, phenyl-H), 5.18 (br s, 1H, H-1), 3.69 (dd, 1H, H-2), 3.64 (m, 1H, H-5), 3.36–3.52 (m, 4H, H-3, H-4, H-6a,b), 2.86–2.80 (m, 4H, 2 × CH_2). ^{13}C NMR (Me_2SO-d_6, 125 MHz) δ 140.7, 128.7, 128.5, 126.3 (phenyl), 85.0 (C-1), 74.6, 72.0, 71.7, 67.5 (C-2 to C-5), 61.3 (C-6), 35.7, 31.7 (2 × CH_2). HRESIMS: calcd for $C_{14}H_{20}O_5S$ [M+Na]$^+$: 323.0929; found: *m/z* 323.0922.

3.3.8. 2-Phenylethyl α-D-mannopyranoside (6)

Dry D-mannose (1 g, 5.5 mM) and 2-phenylethanol (6.8 mL, 55 mM) were refluxed in dry MeCN (130 mL) in the presence of Amberlyst cation exchange resin for 5 h. After filtration, the reaction mixture was diluted with water, extracted with CH_2Cl_2, and the aqueous phase concentrated to dryness. The desired phenylethyl α-mannoside was isolated from the anomeric mixture through silica gel column chromatography (8:1.5:1 EtOAc–MeOH–water). Colorless glassy material, 0.392 g (23%), $[\alpha]_D^{22}$ +54.8 (*c* 0.5, MeOH). ^{1}H NMR (D_2O, 500 MHz): δ 7.35–7.20 (m, 5H, phenyl-H), 4.76 (br s, 1H, H-1), 3.87 (m, 1H, OCH_2(a)), 3.81 (dd, 1H, H-2), 3.76 (m, 1H, OCH_2(b)), 3.65 (dd, 1H, H-6a), 3.59 (dd, 1H, H-3), 3.54 (br.d, 1H, H-6b), 3.53 (t, 1H, H-4), 3.13 (m, 1H, H-5), 2.89–2.78 (m, 2H, Ph-CH_2). ^{13}C NMR (D_2O, 125 MHz): δ

139.1, 128.9, 128.5, 126.4 (phenyl), 99.3 (C-1), 72.4 (C-5), 70.4 (C-3), 69.9 (C-2), 67.9 (OCH_2), 66.3 (C-4), 60.5 (C-6), 34.9 (Ph-CH_2). HRESIMS: calcd for $C_{14}H_{20}O_6$ $[M+Na]^+$: 307.1158; found: *m/z* 307.1146.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2009.06.008.

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