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Synthesis and binding affinity analysis of positional thiol analogs of mannopyranose for the elucidation of sulfur in different position

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ABSTRACT

Synthetic routes towards thio-α/β-D-mannose derivatives are presented. Double parallel or double serial inversion was successfully applied in the efficient synthesis of 2-thio- or 2,4-di-thio-mannoside derivatives. The protein recognition properties of the synthesized positional thiol analogs of mannose were then evaluated in a competition binding assay with the model lectin Concanavalin A (Con A), in order to investigate the roles of thiol group in the different position of the mannopyranose ring in binding affinity. Though the substitution of oxygen atom with sulfur atom in the methyl α-D-mannoside ring usually displayed low or no binding affinity towards Con A, it was a surprise finding that the methyl 2-thio-α-D-mannoside displayed four times higher inhibition than methyl α-D-mannoside, indicating the particular importance of 2-position for modification of α-D-mannoside. Methyl 3-thio-α-D-mannoside also displayed inhibition towards Con A, indicating that the *O*-atom at the C-3 position is less important in the binding site.

1. Introduction

Carbohydrate–protein recognitions are of great significance in many fundamental cellular processes such as adhesion, trafficking, communication, proliferation, and cell death.[1–5] The recognitions are involved in cancer and in the early stages of infection, playing roles of cell surface receptors enabling adherence of bacteria, parasites, and viruses.[6–9] New therapies and drugs would be able to be developed if these interactions are thoroughly understood and controlled. Thus, well-defined carbohydrate ligands need to be synthesized and further studied on their interactions to the corresponding proteins. Sulfur-containing glycosides are often applied in the synthesis of thio-oligosaccharides.[10–16] In comparison with nature O-linked ligands, studies have shown that various thio-oligosaccharides display increased conformational flexibility.[17–19] Generally these sulfur-containing analogs are more stable due to lower rates of both acid-catalyzed and enzymatic hydrolysis, and thus are potential candidates as glycosidase inhibitors and are of special interests in enzyme-inhibition studies.[20–26] In addition, sulfur-containing carbohydrates have special advantages in generating dynamic carbohydrate libraries based on easy oxidation of thiols to give disulfides.[27–31]

The lectins are another class of carbohydrate-specific proteins besides enzymes and antibodies. Carbohydrate-lectin interactions play important roles in cell-cell recognition.[32–34] Concanavalin A (Con A) is the most extensively studied member of the lectins and has a strong binding affinity to the α-linked mannose.[35–37] This binding to the mannose moiety of glycoproteins on cell surface is relate to the cell growth and death. For example, Con A has been found as potential *anti*-hepatoma therapeutic recently.[38] In early studies, by the analysis of affinity of various derivatives of D-mannose and other saccharides to Con A, Goldstein et al. suggested that *O*-atoms of the C-1, C-2 and C-3 hydroxyl groups and H-atoms of the C-4 and C-6 are involved in the H-bonding to the protein.[39–42] Recently, by the analysis of methyl α-mannoside-Con A complex crystal structure, it was suggested that 3-, 4- and 6-OHs of the mannoside provide the main contribution to affinity through H-bonding, whereas 2-OH and 1-OMe extend into solvent.[36,37] However, the complex in real solvent might not be exactly the same as its crystal structure. For example, as derivatives of 1- or 6-thio mannose are easy to synthesize, the affinities of 1-thio-α-D-mannose and methyl 6-thio-α-D-mannoside towards Con A have been measured.[30] Methyl 6-thio-α-D-mannoside shows no activity as expected. However, 1-thio-α-D-mannose shows lower activity compared to methyl α-D-mannoside, suggesting that 1-OMe group

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should be involved in pronounced interactions with the Con A lectin. Thus, synthesis of various derivatives of D-mannose modified at the different positions and further measurement of their affinity towards Con A in real solvent might provide additional information for the binding mechanism. Synthesises of 1- or 6-thio glycosides have been widely reported due to their distinctive reaction selectivities.[43–47] However, the synthesises of 2-, 3- or 4-thio glycosides are rarely reported likely due to the requirements of complicated multi-step reactions including selective protection/deprotection and epimerization.[48,49]

We have developed an efficient method for the synthesis of β-D-mannoside derivatives by double parallel or double serial inversion.[50] The strategy is based on multiple regioselective acylation via the respective stannylene intermediates,[51] followed by simultaneous inversion of both addressed hydroxyl groups or stepwise inversion of the hydroxyl groups. The double parallel or double serial inversion strategy was also applied in synthesis of orthogonally protected galactosamine thioglycoside building blocks.[52] Recently, we developed a multiple regioselective acetylation method using tetrabutylammonium acetate as a catalyst.[53,54] This method is more convenient and environmentally friendly than organotin method. In the present study, we developed synthetic routes towards positional thio-α/β-D-mannose derivatives (Fig. 1) using these methodologies. Especially, 2-thio- and 2,4-dithio-mannoside derivatives were efficient synthesized by the application of double parallel or double serial inversion, so as to avoid complicated multi-step reactions. The protein recognition properties of these positional thiol analogs of mannose were then evaluated in a competition binding assay with the model lectin Con A through Quartz Crystal Microbalance (QCM), in order to investigate the roles of thiol group in the different position of the mannopyranose ring in binding affinity.

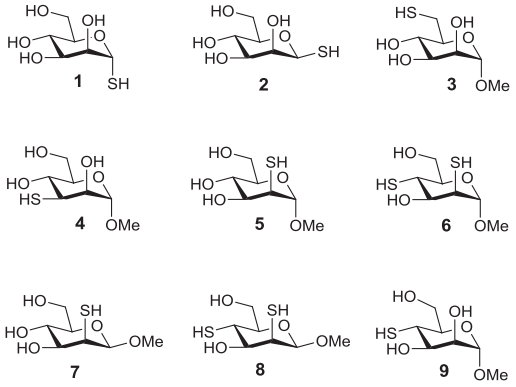
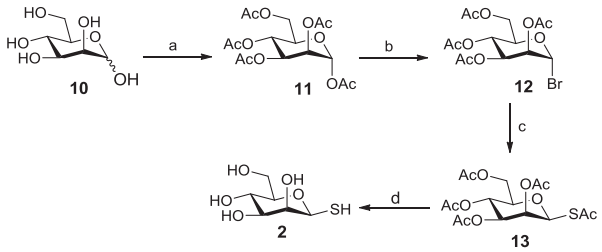


Fig. 1. The positional thio-α/β-D-mannose derivatives.

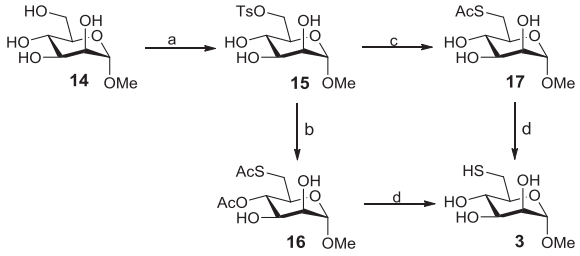
2. Results and discussion

The 1-thio-α-D-mannose compound **1** can be easily produced, starting from a fully acetylated α-D-mannose compound **11** followed by a substitution of thioacetate group at the anomeric center, in light of a reported method.[30] Synthesis of the 1-thio-β-D-mannose compound **2** has been reported in a quite low yield.[46] In the reported method, compound **11** was substituted by a bromide group at the anomeric center to form compound **12**, then treating compound **12** with KSAc in DMPU to form compound **13** in 45% yield. It is possible that the low yield of compound **13** was caused by partial formation of the five-membered acyloxonium ring arising from the 2-OAc group in the polar solvents.[55,56] In our method (Scheme 1), compound **12** was treated with TBASAc in no-polar solvent toluene for avoiding the neighboring group participation, leading to compound **13** in 85% yield.



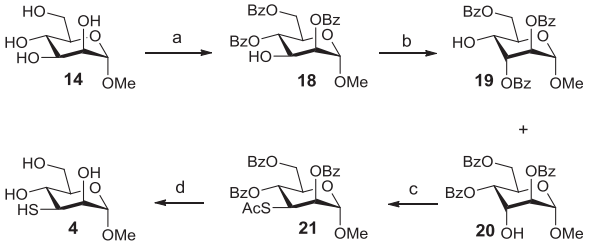
Scheme 1. Synthesis of 1-thio-α/β-D-mannopyranose **2**; (a) Ac_2O, pyridine, rt, 4h; (b) HOAc-HBr, $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0 °C to rt, 2h, 90%; (c) TBASAc, toluene, 5h, 85%; (d) NaOH, MeOH, rt, 2h, then with H^+ exchange resin, 95%.

Synthesis of methyl 6-thio-α-D-mannopyranoside **3** was reported to go through compound **15** and **16** starting from free methyl mannoside **14**.[30] In this method, tosylation of compound **14** formed compound **15**, and compound **15** reacted with 5 equiv of KSAc in DMF at 70 °C to form compound **16** in 60% yield. After careful scrutinize of this reaction, we found that compound **15** reacting with 1.5 equiv of KSAc at 35 °C led to compound **17** in 70% yield (Scheme 2). Treatment of compound **15** with 5 equiv of KSAc at 35 °C also formed compound **16**. However the yield was improved by 77% in this case.



Scheme 2. Synthesis of methyl 6-thio-α-D-mannopyranoside **3**; (a) TsCl, pyridine, 0 °C to rt, (70%); (b) KSAc, DMF, 35 °C, 6h, 77%; (c) i: KSAc, DMF, 35 °C, 6h; ii: KNO_2, DMF, 35 °C, 2h, 70%; (d) NaOH, MeOH, rt, 2h, then with H^+ exchange resin; DTT, H_2O, rt, 12h, 90%.

Synthesis of methyl 3-thio-α-D-mannopyranoside **4** has never been reported. The approach to this compound we developed is showed in Scheme 3. Starting from free methyl mannoside **14**, compound **18** was easily synthesized in 70% total yield, through organotin-mediated regioselective benzylation,[57,58] pyridine-mediated benzoylation and finally debenzylation by catalytic hydrogenation. However, compound **20** was only obtained in 33% yield when inversing compound **18** using nitrite-mediated epimerization methods, owing to the neighboring group participation.[55,56] Triflation of compound **20** followed by a substitution of thioacetate group led to compound **21** in 78% yield.



Scheme 3. Synthesis of methyl 3-thio-α-D-mannopyranoside **4**; (a) i: Bn_2SnO, toluene, reflux,4h; ii: BnBr, TBAB,100 °C, 8h; iii: BzCl, pyridine, 0 °C to rt, 12h; iiii: Pd–C, H_2, EtOH/AcOH (2:1), overnight, 60% total yield; (b) i: Tf_2O, pyridine, CH_2Cl_2, −30 to −10 °C, 4h; ii: KNO_2, DMF, 50 °C, 6h, 55% of **19** and 33% of **20**; (c) i: Tf_2O, pyridine, CH_2Cl_2, −30 to −10 °C, 4h; ii: TBASAc, toluene, rt, 5h, 78%; (d) NaOH, MeOH, rt, 4h, then with H^+ exchange resin; DTT, H_2O, rt, 12h, 90%.