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# SYNTHESIS OF 5-THIOMANNOSE-CONTAINING OLIGOMANNOSIDE MIMICS: BINDING ABILITIES TO CONCANAVALIN A

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**Abstract:** 5-Thiomannose-containing oligomannoside mimics, 5SManα(1,6)Man, 5SManα(1,-3)Man, 5SManα(1,6){Manα(1,3)Man}, Manα(1,6){5SManα(1,3)Man}, and 5SManα(1,6){5S-Manα(1,3)Man}, were synthesized. Dissociation constants for the binding of these mimics to concanavalin A (ConA) were determined by a fluorescence anisotropy inhibition assay. Comparison of these data with those of the natural oligomannosides and with a crystal structure of the trimannoside-ConA complex established that replacing a ring oxygen atom with a sulfur atom causes about 1 kcal/mol decrease in the binding free energy when the ring oxygen is recognized with a hydrogen bonding. 

5-Thio-analog of an aldohexopyranose is referred to as a 5-thiosugar, in which the ring oxygen is replaced with a sulfur atom. The glycosides of 5-thiosugars are glycosidase-resistant[1] and, depending on the structure, they behave as glycosidase inhibitors.[2] When 5-thiosugar is incorporated into an oligosaccharide,[1,3] the resulting mimic is a potential tool to investigate oligosaccharide-receptor interaction, even being hoped as a hydrolase-resistant drug. Such oligosaccharide mimics so far synthesized have shown equivocal effects of the ring sulfur in the binding to receptors; e.g., incorporation of 5-thiofucose into an H-type 2 trisaccharide in place of the fucose residue results in enhancement of binding to an antibody on the one hand, hampering a lectin binding on the other.[3a] This variation in the binding strengths may be due to the difference in the ring oxygen recognition pattern. A stacking interaction between an aromatic residue of the binding site and a sugar face may be strengthened by incorporation of a sulfur atom into the ring. On the other hand, hydrogen bonds involving the ring oxygen should be weakened by replacing it with a sulfur atom. Confirming these assumptions is important for the future finding of 5-thiosugar based drug candidates targeting specific receptors. To this end, required is a systematic investigation on an oligosaccharide-receptor interaction where the recognition pattern of the ring

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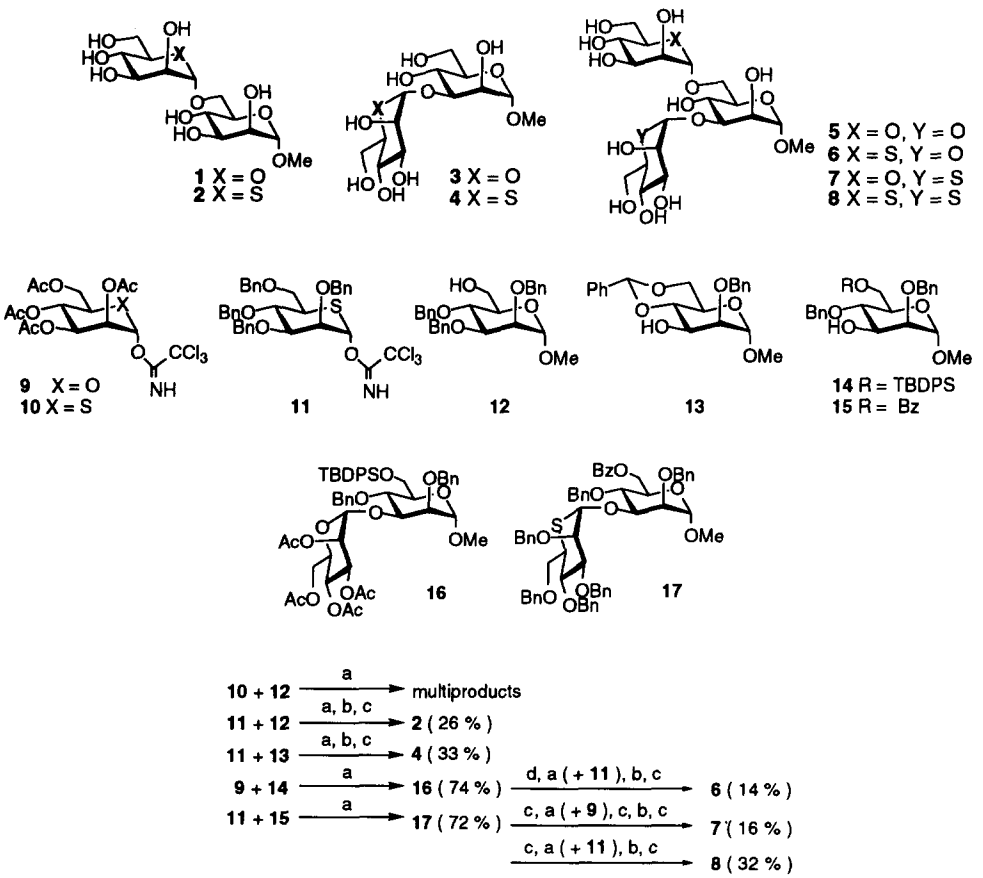
oxygen is known. Concanavalin A (ConA) meets this criterion; i.e., the crystal structure of the ConA-trimannoside (Manα(1,6){Manα(1,3)Man}) complex indicates a hydrogen bonding to the ring oxygen of the 1,6-mannose residue.[4] Therefore, by replacing the ring oxygen of the 1,6-mannose residue with sulfur, we will be able to estimate the effect of sulfur atom on a hydrogen bond. Moreover, it is interesting to investigate the effect of the ring sulfur atom on the 1,3-mannose residue, which is free from hydrogen bondings and stacking interactions. We thus synthesized 5-thiomannose containing oligomannoside mimics, 5SManα(1,6)Man **2**, 5SManα(1,3)Man **4**, 5SManα(1,6){Manα(1,3)Man} **6**, Manα(1,6){5SManα(1,3)Man} **7**, and 5SManα(1,6){5SManα(1,3)Man} **8**, and determined dissociation constants ($K_d$) of the binding of these mimics to ConA.




**Scheme 1.** (a) $BF_3{\cdot}OEt_2$, $CH_2Cl_2$. (b) Na-liq.$NH_3$, THF; $Ac_2O$-Py. (c) NaOMe. (d) $Bu_4NF$, THF.

First attempt of the synthesis of the disaccharide **2** was made by the glycosylation of the compound **12** with the per-O-acetyl-5-thiomannosyl trichloroacetimidate **10** as a glycosyl donor (Scheme 1). However, it ended in the formation of multiproducts, being also the case for other glycosyl acceptors. These results were unexpected

because 5-thioglucose has been incorporated into disaccharides with the same method.[3b] Only the difference in configuration at C-2 caused the dramatic change of reactivity. We reasoned that the stability of a 1,2-orthoester intermediate might be responsible for the result, and altered the all acetyl groups to benzyl ones. With the per-O-benzylated 5-thiomannosyl trichloroacetimidate **11** in hand, we were able to synthesize the desired 5-thiomannose-containing mimics[5] as shown in Scheme 1. In all glycosidation reactions, α-glycosides were stereoselectively obtained as a single isomer. The natural type oligomannosides **1**, **3**, and **5** were synthesized as reported.[6]

The $K_d$ values for the binding of the synthesized oligomannose derivatives (**1**-**8**) to ConA was determined by fluorescence anisotropy inhibition assay (Table 1).[7,8] The obtained $K_d$ values for the natural type oligomannosides **1**, **3**, **5** were in good accordance with those reported.[9] The all $K_d$ values for the mimics showed decreased affinities for ConA, in comparison with the corresponding natural type oligomannosides, the extent of which varies depending on the structures (see $\Delta\Delta G$). ConA has a single high-affinity site that binds the 1,6-linked mannose of the trimannosides with the aid of the hydrogen bonding to the ring oxygen.[4,9c] Therefore, the $\Delta\Delta G$ values of 1.0 kcal/mol for the trimannoside **6** and of 1.3 kcal/mol for the dimannoside **2** indicate a lessened hydrogen accepting ability of the ring sulfur. These magnitudes correspond to those for the substitution of a key hydroxyl group with a hydrogen atom.[10] Since ConA binds the 1,3-linked mannose at the extended site that includes no hydrogen bonds to the ring oxygen, the $\Delta\Delta G$ value of 0.5 kcal/mol for the trimannoside **7** implies that the ring sulfur atom is somewhat an obstruction for the binding. The unexpectedly large binding retardations of the disaccharide **4** and the trisaccharide **8** are difficult to interpret. These results exemplify that apparently small difference in the structure of a ligand saccharide sometimes affects the fitness for ConA to a large extent.

**Table 1.** Thermodynamic parameters for the binding of oligomannose derivatives to ConA at 25 °C.

| compound | structure | $K_d$ (μM) | $\Delta G$ (kcal/mol) | $\Delta\Delta G$ (kcal/mol) |
|---|---|---|---|---|
| **1** | Manα(1,6)Man | 150 | -5.2 | - |
| **2** | 5S Manα(1,6)Man | 1280 | -3.9 | 1.3[a] |
| **3** | Manα(1,3)Man | 49 | -5.9 | - |
| **4** | 5S Manα(1,3)Man | 1720 | -3.8 | 2.1[b] |
| **5** | Manα(1,6){Manα(1,3)Man} | 3 | -7.5 | - |
| **6** | 5S Manα(1,6){Manα(1,3)Man} | 18 | -6.5 | 1.0[c] |
| **7** | Manα(1,6){5S Manα(1,3)Man} | 7 | -7.0 | 0.5[c] |
| **8** | 5S Manα(1,6){5S Manα(1,3)Man} | 376 | -4.7 | 2.8[c] |

[a]Compared with **1**. [b]Compared with **3**. [c]Compared with **5**.

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5. Selected $^1$H ($D_2O$, 400 MHz) and $^{13}$C NMR ($D_2O$, 22.5 MHz) signals (δ ppm)—**2**. $^1$H: 4.67 (d, $J$ = 3.7 Hz, H-1'), 4.21 (dd, $J$ = 2.9, 3.7 Hz, H-2'), 3.06 (ddd, $J$ = 3.4, 6.7, 10.1 Hz, H-5'). $^{13}$C: 101.8 (C-1), 85.0 (C-1'), 44.6 (C-5'). **4**. $^1$H: 4.83 (d, $J$ = 3.6 Hz, H-1'), 4.62 (d, $J$ = 1.8 Hz, H-1), 4.19 (dd, $J$ = 2.3, 3.6 Hz, H-2'), 4.03 (dd, $J$ = 1.8, 3.4 Hz, H-2), 3.53 (ddd, $J$ = 2.1, 6.0, 9.9 Hz, H-5), 2.99 (ddd, $J$ = 3.2, 6.6, 9.9 Hz, H-5'). $^{13}$C: 101.6 (C-1), 87.1 (C-1'), 45.0 (C-5'). **6**. $^1$H: 3.04 (ddd, $J$ = 3.4, 6.6, 9.9 Hz, H-5"). $^{13}$C: 103.8, 102.4 (C-1, C-1'), 85.5 (C-1''), 45.1 (C-5"). **7**. $^1$H: 4.88 (d, $J$ = 3.7 Hz, H-1'), 4.27 (dd, $J$ = 2.5, 3.7 Hz, H-2'), 3.08 (ddd, $J$ = 3.4, 6.6, 9.9 Hz, H-5'). $^{13}$C: 101.8, 100.2 (C-1, C-1"), 87.1 (C-1'), 45.0 (C-5'). **8**. $^1$H: 4.79 (d, $J$ = 3.7 Hz, H-1"), 4.58 (d, $J$ = 3.8 Hz, H-1'). $^{13}$C: 101.8 (C-1), 87.1, 85.0 (C-1', C-1"), 45.0, 44.5 (C-5', C-5").
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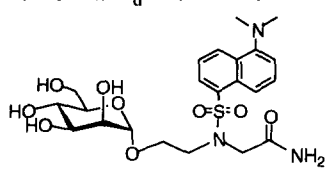
8. *N*-(α-D-Mannopyranosyloxyethyl)-*N*-(5-dimethylaminonaphthalene-1-sulfonyl)-glycinamide **18** was used as a fluorecent ligand. To a solution of **18** (7.5 μM) and ConA (70 μM) in 500 μL of HEPES (0.1 M, pH 7.2, containing 0.9 M NaCl, 1 mM $CaCl_2$, and 1 mM $MnCl_2$), was added small portions of the inhibitor solution: a 5-thiomannose containing mimic (ca 50 mM), **18** (7.5 μM), and ConA (70 μM) in 200 μL of HEPES. Suitable volume of each addition (1-20 μL) was determined after several examinations for each inhibitor. The anisotropy $r$ was measured 30 min after each addition of the inhibitor. The plots of the measured $r$ against the inhibitor concentrations were fitted to a competition binding equation[7] using a curve fitting program to give the dissociation constants $K_d$ listed in Table 1.




**18**

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