Syntheses of two trimannose analogs each containing *C*-mannosyl or 5-thio-*C*-mannosyl residue: their affinities to concanavalin A

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Abstract

C-Mannosyl residue-containing trimannose Man*C*α(1,6){Manα(1,3)Man} (**2**) and 5-thio-*C*-mannosyl residue-containing trimannose 5SMan*C*α(1,6){Manα(1,3)Man} (**3**) were synthesized via a glycosyl radical addition to enone derivative of mannose (**6**). Dissociation constants for the binding of these trisaccharides to concanavalin A (ConA) were determined by a fluorescence anisotropy inhibition assay: K_d = 198 and 31 μM, respectively. The unexpectedly large K_d value for the compound **2** compared with the compound **3** and the natural trimannose **1** demonstrates a characteristic of *C*-glycoside. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Carbohydrates; Glycosides; Lectins

Concanavalin A (ConA) is the most extensively studied member of the lectin family, and the crystal structure of a ConA-trimannose complex[1] and energetics of the binding between ConA and oligosaccharides[2,3] have been determined. Moreover, a number of effective multivalent ligands of ConA have been synthesized.[4,5] Neverthless, little is known about how monovalent trimannose analogs would behave in binding to ConA, despite the potency of this kind of information in designing novel ligands. In this regard, we are interested in the effect of slight modification for the natural trimannose, Manα(1,6){Manα(1,3)Man} (**1**), in the binding to ConA, and we have recently synthesized 5-thiomannose-containing

trisaccharides and examined their affinities to ConA.[6] As a result, we found the large retardation of the binding when the two non-reducing mannose units were replaced with 5-thiomannose. Here we describe the syntheses of C-glycoside-containing trimannose analogs, ManCα(1,6){Manα(1,3)Man} (2) and 5SManCα(1,6){Manα(1,3)Man} (3), and their affinities to ConA, as a part of the studies of the trimannoside analogs. It has been shown that the analog with two C-mannosyl residues attached to an oxanorbornene derivative has the similar binding ability to ConA as O-mannosides.[7,8] However, this compound is regarded as a bivalent ligand with only the non-reducing ends mimicked, rather than a trimannose analog.


1: X = O, Y = O
2: X = O, Y = CH₂
3: X = S, Y = CH₂
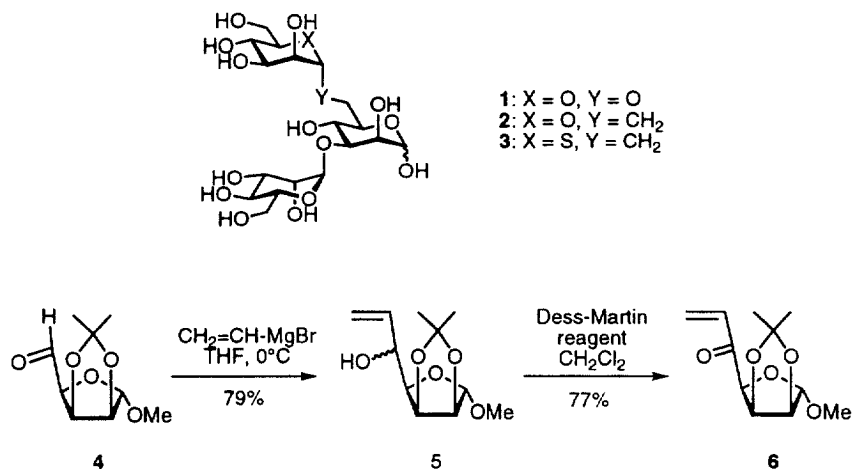
Scheme 1.

In view of synthesizing 5-thio-C-mannoside, a glycosyl radical addition to an enone derivative[9] was adopted for the C-glycoside formation, because the same type of reaction has been achieved for a 5-thio-C-glucoside synthesis.[10] The enone derivative 6 was synthesized from the known methyl 2,3-O-isopropylidene-α-D-lyxo-pentodialdofuranoside (4),[11] via addition of a vinyl anion to the aldehyde group of 4 and oxidation of the hydroxyl group of compound 5 (Scheme 1). Mannosyl bromide (7) and 5-thiomannosyl bromide (8) were used as glycosyl donors for the radical C-glycosidation to give the C-disaccharides 9 and 10, respectively, in moderate yields (Scheme 2). The reduction of the compounds 9 and 10 with Zn(BH₄)₂ mainly afforded the desired compounds 11 and 12, which have D-manno configuration at the aglycon, while Na(BH₄) and LiAl(OtBu)₃H gave the opposite selectivity (D-manno : L-gulo = 1 : 4). Anti-Cram selectivity for the former is explained by a chelation control, in which the carbonyl and the ring oxygen atoms coordinate to the zinc ion and the hydride attacks the carbonyl group from the less hindered side.

Deacetylation, benzylation, and deisopropylidenation of the compounds 11 and 12 gave the compounds 13 and 14, respectively (Scheme 3). Since the conditions for the deisopropylidenation imposed the hydrolysis of the reducing end, the methyl glycoside was reconstructed by the subsequent glycosidation. Regioselective mannosylation of 3-OH of the

compounds **13** and **14** was carried out by using tetra-*O*-acetyl-mannopyranosyl trichloroacetimidate (**15**) as a glycosyl donor to give the trisaccharide derivatives **16** and **17**. After debenzylation of the compounds **16** and **17**, the deprotected compounds were acetylated for purification by silica gel column chromatography to give compounds **18** and **19**.[1] Deacetylation and partial hydrolysis of the compounds **18** and **19** afforded the desired trisaccharide analogs **2** and **3**.[2]
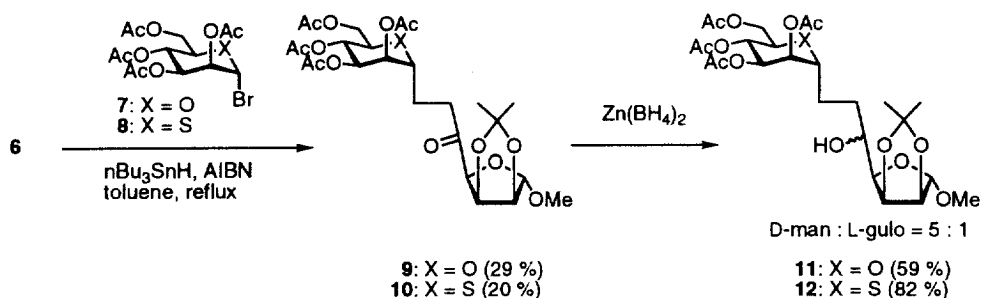
Scheme 2.
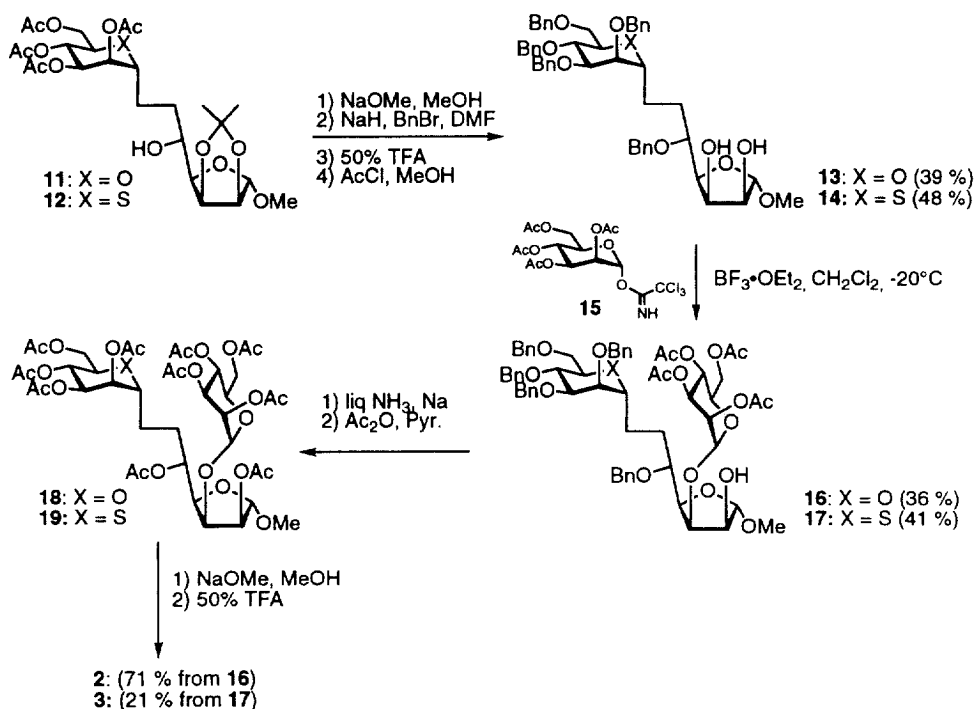
Scheme 3.

[1] **18**: ^{1}HNMR (CDCl$_3$, 400 MHz) δ 5.08 (1H, dd, $J_{1,2}$ 1.5, $J_{2,3}$ 5.0 Hz, H-2), 4.68 (1H, dd, $J_{3,4}$ 6.3 Hz, H-3), 3.96 (1H, m, H-1'), **19**: ^{1}HNMR (CDCl$_3$, 270 MHz) δ 4.68 (1H, dd, $J_{2,3} = J_{3,4}$ 5.6 Hz, H-3), 3.07 (1H, m, H-1').
[2] **2**: FAB Mass (m/z), 525.1 (M + Na); **3**: ESI Mass (m/z), 541.3 (M + Na).

The K_d values, determined by fluorescence anisotropy inhibition assay[6] for the binding of the synthesized trimannose analogs **2** and **3** to ConA, were 198 and 31 µM, respectively. The K_d values for the corresponding trimannose, which have methyl glycosides at the reducing ends, have been determined to be 3 and 18 µM. Influence of *C*-glycosidic linkage to the affinity to ConA is eminent for the compound **2** and the loss of binding free energy amounts to 2.5 kcal/mol, six times larger than that for 5-thio-*C*-mannoside **3** (0.4 kcal/mol). The unexpectedly large retardation of the affinity by a slight modification of the natural trisaccharide has also been observed for 5-thiomannose-containing disaccharide **5SManα(1,3)Man** (2.1 kcal/mol) and trisaccharide **5SManα(1,6){5SManα(1,3)Man}** (2.8 kcal/mol).[6] These large retardation could not be simply explained by a loss of some attractive forces such as hydrogen bonds, because, as shown in the small affinity retardation for 5-thio-*C*-mannoside **3**, the same modification does not always result in a serious loss of the affinity. Difference in the conformational behavior is a plausible explanation for these large losses of affinity. Indeed, recent study on *C*-lactose has indicated that β-linked *C*-glycosides are more flexible than *O*-glycosides and ricin-B lectin selects different conformers of *C*-lactose and its *O*-analog.[12] Though the conformational analysis for compounds **2** and **3** is desirable to withdraw some information regarding the above anomalies, it appeared impossible due to complex higher order paterns of ¹H NMR signals.[13]

Acknowledgments

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