Organic & Biomolecular Chemistry

Cite this: *Org. Biomol. Chem.*, 2011, **9**, 6579

www.rsc.org/obc

PAPER

2-Oxabutane as a substitute for internal monomer units of oligosaccharides to create lectin ligands†

Li-Ying Yang, Yuki Kawada, Lina Bai, Daijiro Kubota and Hideya Yuasa*

Received 18th May 2011, Accepted 7th July 2011
DOI: 10.1039/c1ob05775h

The synthesis of bioactive oligosaccharides is too tedious to scale up for commercialization. However, structurally simplified glycomimetics are commercializable, if they can be synthesized much more easily than the oligosaccharides while having a comparable bioactivity. In this study, we propose a 2-oxabutane (OB) structure as an imitation of the internal monosaccharide units in oligosaccharides. Two trimannoside and three pentamannoside OB-glycomimics were synthesized in remarkably short steps. Among them, Manα1-OB-2Man **10**, a trimannoside mimic, showed eight-fold affinity toward concanavalin A (ConA) relative to methyl mannoside in latex agglutination lectin assay and equilibrium dialysis assay (EDA), while the other mimics showed three- to four-fold affinities. EDA indicated that the bindings between each mimic molecule and a ConA subsite were all in one-to-one stoichiometry and thus these mimics were monovalent ligands, excluding multivalence effect for the high affinities. The strong affinity of **10** could be explained by the occupation of two mannose binding sites of a ConA subsite by its two mannose units. Mimic **10** proved to be even a better ligand for ConA than the natural disaccharide Manα1,2Man, while been much more easy to synthesize, thereby illustrating the potential of the approach here presented.

Introduction

Lectins,[1] a class of carbohydrate-binding proteins, play important roles in infectious diseases. For example, lectins on the surface of bacteria and viruses bind to specific oligosaccharide epitopes on the host cells prior to invasion, causing targeted infections.[2] A small molecule incorporating a structure of these oligosaccharide epitopes, therefore, could be a blocking agent against the infection. Lectin-oligosaccharide interactions are also involved in immune systems.[3] For example, selectins, the sialyl Lewis^a (sLe^a) and sialyl Lewis^x (sLe^x) binding lectins, mediate the interaction between leukocytes and the endothelial cells at the inflammatory sites, helping leukocytes reach the inflammatory sites.[4] Cancer cells in turn exploit this recognition system and pretend to be leukocytes, escaping from the immune system and metastasizing to other organs.[5] The antagonists of selectins, therefore, are potential anti-cancer agents. Another aspect of cancer cells perverting the immune system is the recruitment of macrophage 2, an atypical macrophage, to promote the growth of cancer tissues as opposed to the phagocytosis expected for a typical macrophage.[6] Macrophage 2 expresses high level of mannose receptor and therefore the mannose ligand conjugated with a fluorophore or photosensitizer is a potential agent for photodynamic diagnosis and therapy, respectively, of cancer tissues,[7] because the mannose ligand would be trapped by the macrophage 2 accumulated in cancer tissues.[8]

As mentioned above, lectin anatagonists have a potential of becoming new bioactive products. However, the synthesis of oligosaccharides is generally tedious. Although oligosaccharide mimics could be more easily synthesized than the original oligosaccarides, the design of them requires trial and error even with the aid of sophisticated computer modeling based on the crystal structures of lectin-oligosaccharide complexes.[9] Thus, efforts have been made for the facile syntheses of oligosaccharide mimics using linkers, *e.g.* disulfide, amide, and triazole segments, to connect monosaccharides.[10]

Here we propose the use of a 2-oxabutane-1,4-diyl (OB: -$CH_2OCH_2CH_2$-) unit as an imitation of inner monosaccharide units in oligosaccharides (Fig. 1). Since the OB linker can be a

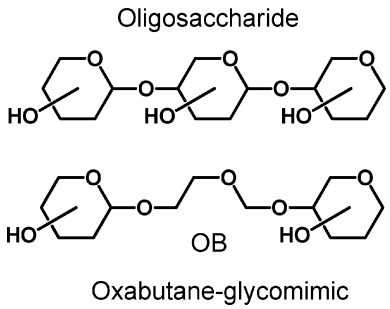


Fig. 1 A structure and synthetic plan of an oxabutane glycomimics.

Department of Life Science, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, J2-10, 4259 Nagatsutacho, Midoriku, Yokohama, 226-8501, Japan. E-mail: hyuasa@bio.titech.ac.jp; Fax: (+81)45-924-5850

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c1ob05775h

common imitation of internal glycosides, we would need no trial and error for the mimic design. We named these oligosaccharide mimics "oxabutane (OB)-glycomimics". In this study, we focused on OB-glycomimics for oligomannosides, since oligomannosides are recognized by a lot of biologically important lectins,[1] such as FimH of *Escherichia coli*,[11] mannose receptor on macrophages,[12] DC-SIGN of dendric cells,[13] and mannose-binding protein inducing complement activation.[14] The OB skeleton imitates the internal mannosides with 1→4 and 1→6 linkages and these mimicry linkages could be synthesized by the coupling reaction between a protected 2-methylthiomethoxyethyl mannoside and a partially protected mannoside; *e.g.*, MTM-Et-Man and 2-OH-Man (Fig. 2). In addition, the OB linker can afford a separation length similar to that of the internal mannosides with 1→2 linkages (the broken circle in Fig. 2), and thus we regard this as the mimic of 1→2 mannosides hereafter. The reaction center of this substitution reaction is a primary carbon atom as opposed to a secondary one in glycosidation reactions, which means that the coupling reaction is non-stereogenic and would probably proceed efficiently, solving some difficulties inherent in glycosidation reactions.

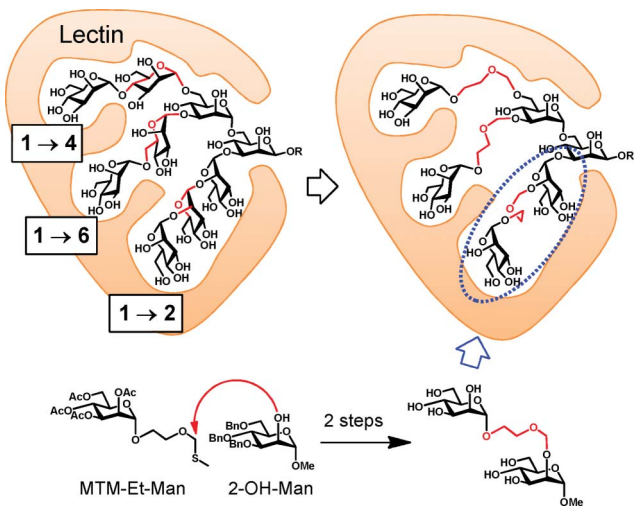
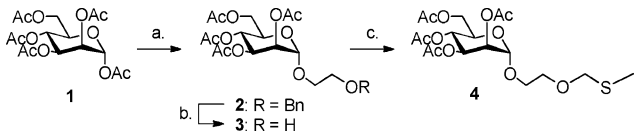


Fig. 2 The structure and synthetic plan of an oxabutane glycomimic of an oligomannoside.

Results and discussion

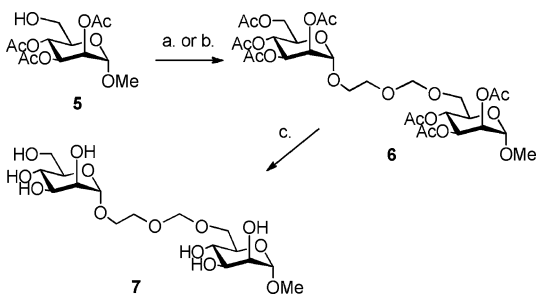
We chose concanavalin A (ConA) as a representative mannose-binding lectin to assess the effectiveness of OB-glycomimics as a substitute for natural oligomannoside ligands, since ConA is best studied among commercially available lectins, being abundant with the available binding constants toward oligosaccharide ligands.[15] The affinity of typical small oligomannosides for ConA decreases in the order: Manα1,6(Manα1,3)Man > Manα1,2Manα1,2Man > Manα1,2Man > Manα1,4Man ≈ Manα1,3Man > Manα1,6Man ≈ MeMan. Among the above small oligomannosides, the linear trimannoside, Manα1,2Manα1,2Man, has a structure mimickable by OB-glycomimics: the internal mannose unit can be substituted with OB unit to build a dumbbell-shaped mimic, Manα1-OB-2Man. The OB-glycomimics would be more effectively applied for larger oligosaccharides, because the time, cost, and effort required for their synthesis would become exponentially greater as the size of oligosaccharides increases. Although the trisaccharide, Manα1,6(Manα1,3)Man, have no internal mannose units to meet the concept of OB-glycomimics, we were interested in the effcts of inserting OB unit in the glycosidic linkages of this oligosaccharide. We thus synthesized Manα1-OB-6(Manα1-OB-3)Man. We also synthesized Manα1-OB-6Man, Manα1-OB-3(Manα1-OB-2)Man and Manα1-OB-6(Manα1-OB-2)Man for comparison.

We synthesized an OB glycomimic donor **4** in three steps from per-*O*-acetyl mannose **1** (Scheme 1). The reaction of compound **1** with mono-benzyl protected ethylene glycol in the presence of $SnCl_4$ gave 2-benzyloxyethyl glycoside **2**, which was subsequently debenzylated to give 2-hydroxyethyl glycoside **3** in 49%. Direct mannosylation of ethylene glycol yielded **3** in 15%,[16] accompanied by a divalent mannoside in 11% yield. We thus took the former method to synthesize compound **4** in large scale. Compound **4** was synthesized by Pummerer-type methylthiomethylation[17] of the hydroxyl group of **3** in 58%.



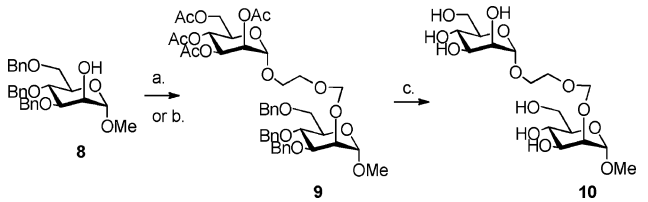
Scheme 1 a) $HO(CH_2)_2OBn$, $SnCl_4/CH_2Cl_2$; b) H_2, Pd–C/CH_3OH (49%); c) DMSO-Ac_2O–AcOH 7 : 5 : 1 (58%).

Compound **4** was coupled to a 6-free acceptor **5**[18] in the usual glycosidation conditions for thioglycosides promoted with methyl triflate[19] to give the coupled product **6** in 33% (Scheme 2). The low yield was probably due to decomposition of MTM group, which is much more reactive than thioglycosides. Therefore, we needed to find milder yet effective conditions to improve the yield. The frozen conditions for glycosidations developed by Ito *et al.*[20] matched our demand and the coupling reaction conducted in frozen *p*-xylene afforded 56% yield. Deacetylation of compound **6** gave Manα1-OB-6Man (**7**) in 70%.



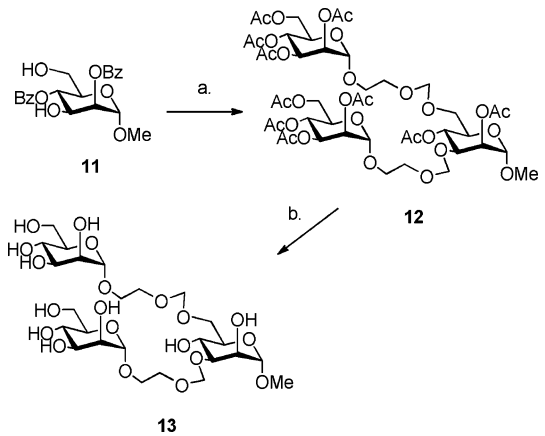
Scheme 2 a) **4**, CH_3OTf/CH_2Cl_2, –60 °C (33%); b) **4**, CH_3OTf/2,4,6-tri-*tert*-butylpyridine, *p*-xylene, –78 °C → 4 °C (55%); c) CH_3ONa/CH_3OH (70%).

The coupling of compound **4** to a 2-free acceptor **8**[21] was also conducted by two methods, *i.e.*, the normal and frozen conditions (Scheme 3). The results were similar to those of the previous 1,6-mimic and the frozen conditions improved the yield of compound **9** from 27% for the normal conditions to 54%. We used only the frozen conditions for the other coupling reactions hereafter. Debenzylation and deacetylation of **9** gave Manα1-OB-2Man (**10**) in 61%.

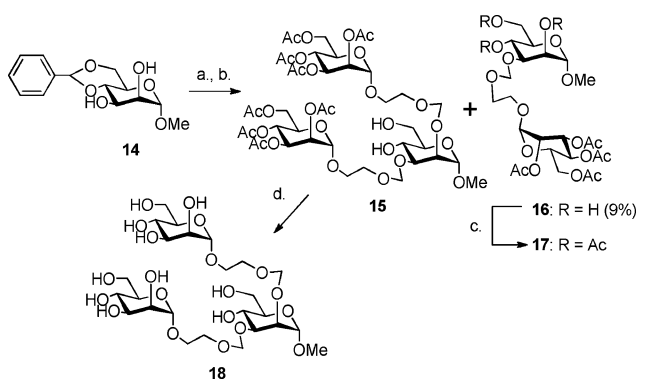


Scheme 3 a) **4**, CH_3OTf/CH_2Cl_2, –60 °C (27%); b) **4**, CH_3OTf/2,4,6-tri-*tert*-butylpyridine, *p*-xylene, –78 °C → 4 °C (54%); c) H_2, Pd–C/CH_3OH then CH_3ONa/CH_3OH (61%).

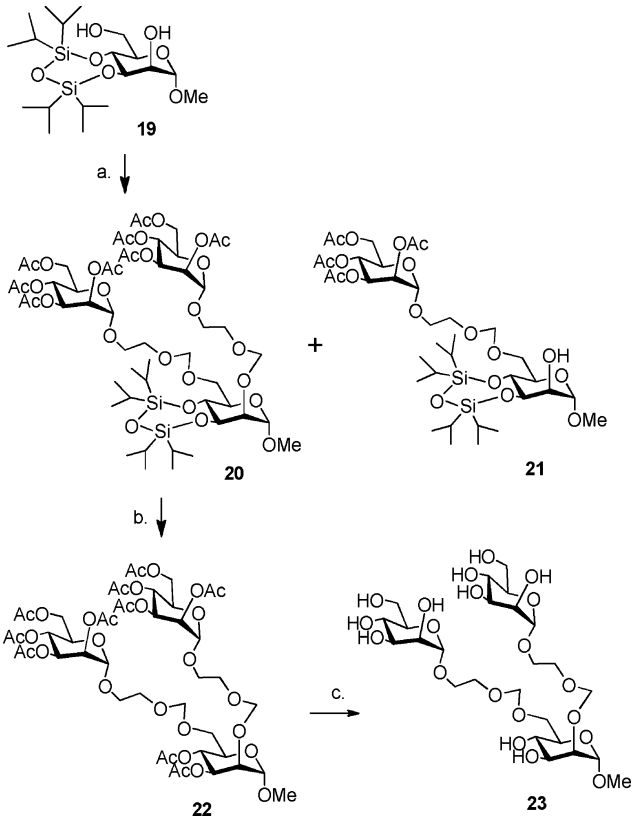
A branched OB-glycomimic, Manα1-OB-6(Manα1-OB-3)Man (**13**), was synthesized by coupling of compound **4** to a 3,6-free acceptor **11**[22] (Scheme 4). The coupling intermediate was acetylated for structure elucidation to give the fully-acetylated mimic **12**, which was deacetylated to give **13** in 39% yield. Another branched OB-glycomimic, Manα1-OB-2(Manα1-OB-3)Man (**18**), was synthesized in a similar manner from a 2,3-free acceptor **14**[23] in 19% total yield (Scheme 5). In the coupling reaction, mono-functionalized mannoside **16** was accompanied in 9% yield, whose structure was elucidated by ^{1}H NMR analysis of the per-acetylated derivative **17**. A third branched OB-glycomimic, Manα1-OB-6(Manα1-OB-2)Man (**23**), was synthesized from a 2,6-free acceptor **19**.[24] The coupling of compounds **4** and **19** then deprotection gave compound **23** (Scheme 6). In the coupling reaction, the mono-functionalized derivative **21** was also produced. Compound **21** was derivatizable to compound **6** by desilylation and deacetylation. The branched OB-glycomimics **13**, **18**, and **23** are pentasaccharide mimics if we regard the OB linker as a substitute for a mannose unit, and the synthesis of these mimics was extremely simple compared to that of the original pentasaccharides. It should be noted here that the 2-oxabutane linker contains a very acid sensitive acyclic acetal linker. It can be expected that the use of acid labile protecting groups is prohibited during the synthesis of this type of glycomimetics.



Scheme 4 a) **4**, CH_3OTf/2,4,6-tri-*tert*-butylpyridine, *p*-xylene, –78 °C → 4 °C; CH_3ONa/CH_3OH; Ac_2O–Py (44%); b) CH_3ONa/CH_3OH (89%).



Scheme 5 a) **4**, CH_3OTf/2,4,6-tri-*tert*-butylpyridine, *p*-xylene, –78 °C → 4 °C; b) 80% AcOH, 45 °C (20% + 9% of **16**); c) Ac_2O–Py; d) CH_3ONa/CH_3OH (94%).



Scheme 6 a) **4**, CH_3OTf/2,4,6-tri-*tert*-butylpyridine, *p*-xylene, –78 °C → 4 °C (52% + 39% of **21**); b) tetra-butylammonium fluoride (TBAF), THF; Ac_2O–Py (83%); c) CH_3ONa/CH_3OH (84%).

The affinity of the synthesized glycomimics toward ConA was evaluated by latex agglutination lectin assay (LALA)[25] and enzyme-linked lectin assay (ELLA)[26] in IC_{50} and by equilibrium dialysis assay (EDA)[27] in K_d (Table 1). In LALA, aggregation

Table 1 Affinity of oxabutane (OB) glycomimics toward ConA

	IC_{50}/μM[a]		EDA	
Ligand	LALA	ELLA	K_d/μM[a]	*n*
MeMan	165 (1)	529 (1)	205 (1)	0.96
Manα1,2Man	94 (3)	148 (4)	57 (4)	0.98
7	74 (2)	506 (1)	62 (3)	0.97
10	37 (5)	125 (4)	26 (8)	0.95
13	96 (2)	183 (3)	54 (4)	0.99
18	48 (3)	127 (4)	57 (4)	0.96
23	ND	ND	83 (3)	1.00

[a] Numbers in parentheses are relative potency.

of the polystyrene beads coated with mannosylated bovine serum albumin in the presence of ConA was observed. IC_{50} is the concentration of a compound to inhibit the aggregation by 50%. In ELLA, the horseradish peroxidase-labeled ConA bound on yeast mannan-coated wells of a microtiter plate after several washings was quantified by absorption at 415 nm derived from the peroxidase-catalyzed oxidation of 2,2′-azino diethylbenzothiazoline sulfonic acid. IC_{50} is the concentration of a compound to inhibit the binding by 50%. In EDA, a ligand solution in buffer was divided by dialysis membrane into two compartments and ConA was added into one of two compartments so that ConA was restricted in one compartment and the ligand could freely pass through the membrane. The free ligand concentration after equilibrium was determined by phenol-H_2SO_4 quantification of the sugar in the compartment containing only the ligand. The unbound ligand concentrations were determined for several runs with varied initial ligand concentrations and the K_d values and n, the number of bound ligands per subsite, were deduced by Scatchard plots. There are limited numbers of methods with which n of binding can be deduced, especially when there are no labels on either lectin or sugar to evaluate the concentration. EDA is a classical method but still a convenient and the most inexpensive method to determine both K_d and n.

The affinity of the ligands toward ConA was consistent between LALA and EDA, the relative binding potency being in the order: Manα1-OB-2Man (**10**) > the others > MeMan, the affinity of **10** being distinctive from others. ELLA showed a slightly different tendency: the others > Manα1-OB-6Man (**7**) = MeMan. The difference might be due to the low sensitivity of ELLA, which produces more reliable data for multivalent ligand bindings.[28]

ConA is tetramer above pH 7 and the monomer has a subsite primarily recognizing the core trisaccharide, Manα1,6(Manα1,3)Man.[1] On the other hand, a trisaccharide Manα1,2Manα1,2Man has the same magnitude of affinity for ConA as that of the core trisaccharide.[15] The affinity of the disaccharide Manα1,2Man to ConA, though 2.7-fold less than that of the trisaccharide Manα1,2Manα1,2Man, has shown to be at least 4-fold higher than those of the other disaccharides with different glycosidic linkages. These distinctive affinities of α1,2-linked oligomannosides toward ConA were found to be mainly due to enhanced entropy effect, which was ascribed to increased number of binding modes due to sliding of the mannose residues with strongly binding 3-, 4-, 6- hydroxyl groups at the monosaccharide-binding site. In our case, the trisaccharide mimic Manα1-OB-2Man (**10**) increased the affinity to ConA by 2-fold over the dimannoside Manα1,2Man, a similar enhancement as has been observed for the trimannoside Manα1,2Manα1,2Man (2.7-fold). However, the enhanced affinity of **10** is unlikely due to the increased number of binding modes, because the analog **10** has the same number of hydroxyl groups as that of the dimannoside. Probably, two mannose residues of Manα1-OB-2Man (**10**) are appropriately separated so as to fit the two mannose binding sites supposedly occupied by two nonreducing mannose residues of the high affinity core trisaccharide Manα1,6(Manα1,3)Man (Fig. 3). This binding model is supported by the fact that addition of a further branch on the OB glycomimic **10**; *i.e.*, Manα1-OB-2(Manα1-OB-3)Man (**18**) and Manα1-OB-2(Manα1-OB-6)Man (**23**), resulted in diminished affinity to ConA. The extended core trimannoside mimic, Manα1-OB-6(Manα1-OB-3)Man (**13**),

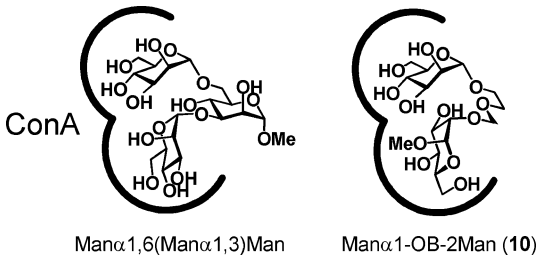


Fig. 3 Proposed binding modes of a natural trisaccharide Manα1,6(Manα1,3)Man and Manα1-OB-2Man (**10**) toward ConA.

showed almost the same affinity as those of the disaccharide Manα1,2Man and OB glycomimic **18**. The almost unity n, the number of bound ligands per subsite, suggested that the better affinities of these OB glycomimics than that of MeMan are not because of multivalent binding of mannose residues to different subsites.

Conclusions

OB-glycomimics, the mimics with the oxabutane linkers imitating internal monosaccharide units of oligosaccharides, were synthesized much more straightforwardly than the original oligomannosides are supposed to be. They showed good affinities for ConA comparable to those of the original oligomannosides. These good affinities are likely due to occupation of two mannose-binding sites in a ConA subsite by the mannose units of OB glycomimics. Among the synthesized OB-glycomimics, Manα1-OB-2Man (**10**) showed the best affinity probably owing to the appropriate spacing between two mannose units. These OB-glycomimics could be further developed for the antagonists of biologically important lectins and for the tags of a drug delivery system. It should be noted that the flexibility of the OB linker as opposed to pyranosides may be sometimes detrimental to the overall affinity of this glycomimetic.

Experimental

General methods

All solvents and reagents were reagent-grade, and in cases in which further purification was required, standard procedures were followed.[29] Reactions were monitored by TLC (thin-layer chromatography). TLC was performed on pre-coated silica gel Merck 60-F254 plates (Art 5715) and visualized by the quenching of fluorescence and/or by charring after spraying with 1% $CeSO_4$-1.5% $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$-10% H_2SO_4. Flash column chromatography was performed on Merck Kieselgel 60 (Art 7734), Wako gel C-300 or Kanto Silica gel 60 N (spherical, neutral) with the solvent systems specified. Optical rotations were determined with a Horiba SEPA-200 polarimeter using a 1 dm length cell. ^{1}H NMR spectra were recorded at 600 (Bruker AV-600), 400 (Varian Unity-400) or 270 MHz (JEOL EX-270). Internal tetramethylsilane (δ 0 ppm) in $CDCl_3$ or a DOH peak (δ 4.8 ppm) in D_2O was used as a standard. Chemical shifts were expressed in ppm referenced to the standard. The multiplicity of signals was abbreviated as follows: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, dt = doublet of triplets, ddd = doublet of doublets of doublets, br = broad signal, and m = multiplet. Protons on the sugars at non-reducing ends

were designated as H-1′, H-1″, *etc.* ^{13}C NMR spectra were recorded at 67.8 MHz (JEOL JNM-EX-270) or 100.6 MHz (Varian Unity-500), and a solvent peak (δ 77.0 ppm) in $CDCl_3$ or internal acetone (δ 30.8 ppm) in D_2O was used as a standard. High-resolution mass spectra (HRMS) were recorded on a Mariner Biospectrometry Workstation ESI-TOF mass spectrometer. The reagents for lectin assay, *i.e.* latex beads (10% suspension, 10 mL diameter), ConA (C2010, powder), were obtained from Sigma. DVS was purchased from Aldrich. LALA was carried out using the method of Yuasa and coworkers.[24] ELLA was carried out using the method of Pagé and coworkers.[25] Optical absorbance at 405 nm in ELLA was measured on a Model 550 Microplate Reader (Bio-Rad).

Methylthiomethoxyethyl 2,3,4,6-tetra-*O*-aectyl-α-D-mannopyranoside (4). 2′-Hydroxyethyl 2,3,4, 6-tetra-*O*-acetyl-α-D-mannopyranoside (**3**)[16] (153 mg, 390 μmol) was dissolved in DMSO-Ac_2O–AcOH (7 : 5 : 1, 6.5 mL) at RT. After 24 h, the solution was diluted with EtOAc and washed with sat. $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtered, and evaporated. The residue was chromatographed on siliga gel (hexane–EtOAc 2 : 1) to give **4** (103 mg, 58%). R_f 0.64 (Hexane–EtOAc 1 : 2); $[\alpha]_D^{25}$ +41.5 (*c* 1.00 in $CHCl_3$); δ_H (600 MHz, $CDCl_3$, 25 °C, Me_4Si) 5.37 (1H, dd, $J_{2,3}$ 3.4, $J_{3,4}$ 9.9 Hz, H-3), 5.29 (1H, t, $J_{3,4} = J_{4,5}$ 9.9 Hz, H-4), 5.28 (1H, dd, $J_{1,2}$ 1.7, $J_{2,3}$ 3.4 Hz, H-2), 4.89 (1H, d, $J_{1,2}$ 1.7 Hz, H-1), 4.69 (1H, d, *J* 11.6 Hz, OC*H*HS), 4.66 (1H, d, *J* 11.6 Hz, OCH*H*S), 4.29 (1H, dd, $J_{5,6a}$ 5.2, $J_{6a,6b}$ 12.0 Hz, H-6a), 4.12 (1H, dd, $J_{5,6b}$ 2.4, $J_{6a,6b}$ 12.2 Hz, H-6b), 4.07 (1H, ddd, $J_{5,6b}$ 2.4, $J_{5,6a}$ 5.2, $J_{4,5}$ 9.9 Hz, H-5), 3.87–3.69 (4H, m, OCH_2CH_2O), 2.16, 2.11, 2.05, 2.00 (15H, each s, COCH_3 × 4, SCH_3); δ_C (67.8 MHz, $CDCl_3$, 25 °C, Me_4Si) 170.5, 169.9, 169.7, 169.6, 97.5, 75.3, 69.4, 68.9, 68.3, 66.9, 66.3, 66.0, 62.3, 20.8, 20.7, 20.59, 20.57, 13.6; HRMS (ESI): *m*/*z* calcd for $C_{18}H_{28}O_{11}S$+Na, 475.1251; found, 475.1246.

Methyl 6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannnopyranosyl-oxyethoxymethyl)-2,3,4-tri-*O*-acetyl-α-D-mannopyranoside (6). A mixture of methyl 2,3,4-tri-*O*-acetyl-α-D-mannopyranoside (**5**)[18] (50 mg, 156 μmol), **4** (92 mg, 203 μmol), 2,4,6-tri-*tert*-butylpyridine (TTBP) (116 mg, 467 μmol) and activated molecular sieves 4A (100 mg) in *p*-xylene (2.6 mL) was stirred under N_2 at RT for 30 min. Immediately after the addition of MeOTf (51 μL, 451 μmol), the mixture was rapidly mixed and frozen over dry ice in acetone. The mixture was stored in refrigerator at 4 °C for 48 h and defrosted at RT. Et_3N (60 μL) was added to deactivate MeOTf and the mixture was diluted with EtOAc and filtered through Celite. The filtrate was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (hexane–EtOAc 4 : 3 → 1 : 2) to give **6** (60 mg, 53%). R_f 0.34 (hexane–EtOAc 1 : 2). $[\alpha]_D^{25}$ +55.3 (*c* 0.40 in $CHCl_3$); δ_H (600 MHz, $CDCl_3$, 25 °C, Me_4Si) 5.35 (1H, dd, $J_{2',3'}$ 3.4, $J_{3',4'}$ 9.9 Hz, H-3′), 5.34–5.31 (2H, m, H-3, H-4), 5.29 (1H, t, $J_{3',4'} = J_{4',5'}$ 9.9 Hz, H-4′), 5.27 (1H, dd, $J_{1',2'}$ 1.6, $J_{2',3'}$ 3.4 Hz, H-2′), 5.24 (1H, dd, $J_{1,2}$ 1.6, $J_{2,3}$ 3.0 Hz, H-2), 4.88 (1H, d, $J_{1',2'}$ 1.6, H-1′), 4.75 (1H, d, *J* 6.8 Hz, OC*H*HO), 4.73 (1H, d, *J* 6.8 Hz, OCH*H*O), 4.72 (1H, d, $J_{1,2}$ 1.6 Hz, H-1), 4.29 (1H, dd, $J_{5',6'a}$ 5.1, $J_{6'a,6'b}$ 12.3 Hz, H-6′a), 4.11 (1H, dd, $J_{5',6'b}$ 2.3, $J_{6'a,6'b}$ 12.3 Hz, H-6′b), 4.05 (1H, ddd, $J_{4',5'}$ 9.9, $J_{5',6'b}$ 2.3, $J_{5',6'a}$ 5.1 Hz, H-5′), 3.92 (1H, ddd, $J_{5,6b}$ 2.6, $J_{5,6a}$ 4.8, $J_{4,5}$ 9.6 Hz, H-5), 3.84–3.66 (4H, m, OCH_2CH_2O), 3.70 (1H, dd, $J_{5,6a}$ 4.8, $J_{6a,6b}$ 11.3 Hz, H-6a), 3.64 (1H, dd, $J_{5,6b}$ 2.6, $J_{6a,6b}$ 11.3 Hz, H-6b), 3.41 (3H, s, OCH_3), 2.16 (6H, s, COCH_3), 2.11 (3H, s, COCH_3), 2.05 (6H, s, COCH_3), 1.99 (6H, s, COCH_3); δ_C (67.8 MHz, $CDCl_3$, 25 °C) 170.6, 170.2, 169.99, 169.95, 169.8, 169.8, 169.7, 98.5, 97.7, 95.7, 76.5, 69.5, 69.3, 68.9, 68.4, 67.2, 66.5, 66.3, 66.0, 62.3, 55.2, 20.92, 20.86, 20.8, 20.71, 20.68, 20.64; HRMS (ESI): *m*/*z* calcd for $C_{30}H_{44}O_{20}$+Na, 747.2325; found, 747.2297.

Methyl 6-*O*-(α-D-mannnopyranosyl-oxyethoxymethyl)-α-D-mannopyranoside (7). To a stirred solution of **6** (60 mg, 83 μmol) in dry MeOH (3 mL) was added 0.1 M NaOMe (100 μL). After 30 min, the solution was neutralized with Dowex50 (H^+) and evaporated to give **7** (25 mg, 70%) as syrup. R_f 0.32 (*i*PrOH-H_2O–NH_3 8 : 3 : 1). $[\alpha]_D^{25}$ +95.5 (*c* 1.0 in H_2O); δ_H (600 MHz, D_2O, 60 °C) 4.88 (1H, d, $J_{1',2'}$ 1.6 Hz, H-1′), 4.87–4.78 (2H, m, OCH_2O), 4.73 (1H, d, $J_{1,2}$ 1.5 Hz, H-1), 3.95 (1H, dd, $J_{1',2'}$ 1.6, $J_{2',3'}$ 3.4 Hz, H-2′), 3.92–3.62 (16H, m, H-2, H-3, H-3′, H-4, H-4′, H-5, H-5′, H-6a, H-6′a, H-6b, H-6′b, OCH_2CH_2O), 3.39 (3H, s, OCH_3); δ_C (67.8 MHz, D_2O, 25 °C, acetone) 101.6, 100.5, 95.8, 73.4, 71.9, 71.1, 70.6, 70.5, 67.6, 67.4, 67.3, 67.1, 61.6, 55.5; HRMS (ESI): *m*/*z* calcd for $C_{16}H_{30}O_{13}$+Na, 453.1585; found, 453.1557.

Methyl 2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannnopyranosyl-oxyethoxymethyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (9). A mixture methyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**8**)[21] (54 mg, 116 μmol), compound **4** (81 mg, 179 μmol), TTBP (45 mg, 182 μmol) and activated molecular sieves 4A (100 mg) in *p*-xylene (2.6 mL) was stirred under N_2 at RT for 30 min. Immediately after the addition of MeOTf (40 μL, 353 μmol), the mixture was rapidly mixed and frozen over dry ice in acetone. The mixture was stored in refrigerator at 4 °C for 48 h and defrosted at RT. Et_3N (50 μL) was added to deactivate MeOTf and the mixture was diluted with EtOAc and filtered through Celite. The filtrate was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (hexane–EtOAc 4 : 3→1:2) to give **9** (55 mg, 54%). R_f 0.20 (hexane–EtOAc 1 : 1); $[\alpha]_D^{25}$ +58.0 (*c* 0.66 in $CHCl_3$); δ_H (600 MHz, $CDCl_3$, 25 °C, Me_4Si) 7.36–7.15 (15H, m, Ph), 5.35 (1H, dd, $J_{2',3'}$ 3.5, $J_{3',4'}$ 10.0 Hz, H-3′), 5.29–5.26 (2H, m, H-2′, H-4′), 4.88 (1H, d, *J* 10.8 Hz, PhC*H*H), 4.84 (3H, m, OCH_2O, H-1′), 4.80 (1H, d, $J_{1,2}$ 1.7 Hz, H-1), 4.71 (1H, d, *J* 11.7 Hz, PhC*H*H), 4.68 (1H, d, *J* 11.7 Hz, PhCH*H*), 4.64 (1H, d, *J* 12.1 Hz, PhC*H*H), 4.54 (1H, d, *J* 12.1 Hz, PhCH*H*), 4.50 (1H, d, *J* 10.8 Hz, PhCH*H*), 4.28 (1H, dd, $J_{5',6'a}$ 5.1, $J_{6'a,6'b}$ 12.3 Hz, H-6′a), 4.08 (1H, dd, $J_{5',6'b}$ 2.3, $J_{6'a,6'b}$ 12.3 Hz, H-6′b), 4.01 (1H, ddd, $J_{5',6'b}$ 2.3, $J_{5',6'a}$ 5.1, $J_{4',5'}$ 10.0 Hz, H-5′), 3.99 (1H, dd, $J_{1,2}$ 1.7, $J_{2,3}$ 2.7 Hz, H-2), 3.93–3.55 (9H, m, H-3, H-4, H-5, H-6a, H-6b, OCH_2CH_2O), 3.35 (3H, s, OCH_3), 2.15 (3H, s, COCH_3), 2.09 (3H, s, COCH_3), 2.03 (3H, s, COCH_3), 1.99 (3H, s, COCH_3); δ_C (67.8 MHz, $CDCl_3$) 170.6, 170.0, 169.8, 169.7, 138.4, 138.2, 128.4, 128.3, 127.8, 127.7, 127.6, 127.55, 127.48, 99.6, 97.6, 95.7, 79.6, 76.5, 75.1, 74.7, 73.9, 73.3, 72.2, 71. 4, 69.4, 69.0, 68.3, 67.0, 66.7, 66.1, 62.4, 54.8, 20.9, 20.72, 20.66, 20.64; HRMS (ESI): *m*/*z* calcd for $C_{45}H_{56}O_{17}$+Na, 891.3417; found, 891.3420.

Methyl 2-*O*-(α-D-mannnopyranosyl-oxyethoxymethyl)-α-D-mannopyranoside (10). A mixture of **9** (74 mg, 85 μmol) and 10% Pd–C (18 mg) in dry MeOH (1 mL) was stirred under 1 atm H_2 for 1 day at RT. The catalyst was filtered off and washed throughly with MeOH. After evaporation of the filtrate, the residue was dissolved in dry MeOH (3 mL) and 0.5 M NaOMe (100 μL) was added to the solution. After 30 min, the solution was neutralized

with Dowex50(H^+) and evaporated to give **10** (16.5 mg, 45%). R_f 0.36 (*i*PrOH-H_2O–NH_3 8 : 3 : 1); $[\alpha]_D^{25}$ +67.9 (*c* 0.28 in H_2O); δ_H (600 MHz, D_2O, 60 °C) 4.89 (1H, d, $J_{1,2}$ 1.7 Hz, H-1), 4.88 (1H, d, *J* 7.5 Hz, OC*H*HO), 4.86 (1H, d, *J* 7.5 Hz, OCH*H*O), 4.85 (1H, d, $J_{1,2}$ 1.6 Hz, H-1′), 3.96 (1H, dd, $J_{1,2}$ 1.7, $J_{2,3}$ 3.5 Hz, H-2), 3.92 (1H, dd, $J_{1',2'}$ 1.6, $J_{2',3'}$ 3.5 Hz, H-2′), 3.91–3.58 (14H, m, H-3, H-3′, H-4, H-4′, H-5, H-5′, H-6a, H-6′a, H-6b, H-6′b, OCH_2CH_2O), 3.40 (3H, s, OCH_3); δ_C (150.92 MHz, D_2O, 25 °C, acetone) 100.0, 99.6, 96.2, 77.3, 72.9, 72.7, 70.7, 70.3, 70.2, 67.3, 67.2, 67.0, 66.5, 61.14, 61.12, 55.0; HRMS (ESI): *m*/*z* calcd for $C_{16}H_{30}O_{13}$+Na, 453.1585; found, 453.1548.

Methyl 3,6-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannnopyranosyl-oxyethoxymethyl)- 2,4-di-*O*-acetyl-α-D-mannopyranoside (12). A mixture of methyl 2,4-di-*O*-benzoyl-α-D-mannopyranoside (**11**)[22] (50 mg, 124 μmol), **4** (225 mg, 497 μmol), TTBP (123 mg, 496 μmol), and activated molecular sieves 4A (160 mg) in *p*-xylene (4 mL) was stirred under N_2 at RT for 30 min. Immediately after the addition of MeOTf (110 μL, 972 μmol), the mixture was rapidly mixed and frozen over dry ice in acetone. The mixture was stored in refrigerator at 4 °C for 48 h and defrosted at RT. Et_3N (140 μL) was added to deactivate MeOTf and the mixture was diluted with EtOAc and filtered through Celite. The filtrate was washed with brine, dried over $MgSO_4$, and evaporated. The residue was dissolved in 3 mL MeOH and to the solution was added 0.5 M NaOMe (100 μL). After 30 min, the solution was neutralized with Dowex50 (H^+) and evaporated. The residue was dissolved in pyridine–acetic anhydride (1 : 1, 2 mL). After 3 h, the solution was evaporated and the residue was chromatographed on silica gel (hexane–EtOAc 1 : 3) to give **12** (27 mg, 20%). R_f 0.26 (hexane–EtOAc 2 : 5); $[\alpha]_D^{25}$ +49.2 (*c* 0.90 in $CHCl_3$); δ_H (600 MHz, $CDCl_3$, 25 °C, Me_4Si) 5.35 (1H, dd, $J_{2'',3''}$ 3.4, $J_{3'',4''}$ 10.1 Hz, H-3″), 5.33 (1H, dd, $J_{2',3'}$ 3.2, $J_{3',4'}$ 10.4 Hz, H-3′), 5.31–5.23 (3H, m, H-4, H-4′, H-4″), 5.29 (1H, dd, $J_{1'',2''}$ 1.4, $J_{2'',3''}$ 3.4 Hz, H-2″), 5.27 (1H, dd, $J_{1',2'}$ 1.5, $J_{2',3'}$ 3.2 Hz, H-2′), 5.24 (1H, dd, $J_{1,2}$ 1.5, $J_{2,3}$ 3.4 Hz, H-2), 4.90 (1H, d, $J_{1'',2''}$ 1.4 Hz, H-1″), 4.88 (1H, d, $J_{1',2'}$ 1.5 Hz, H-1′), 4.75 (1H, d, *J* 7.3 Hz, OC*H*HO), 4.74 (2H, s, OCH_2O), 4.70 (1H, d, $J_{1,2}$ 1.5 Hz, H-1), 4.64 (1H, d, *J* 7.3 Hz, OCH*H*O), 4.30 (1H,dd, $J_{5'',6''a}$ 5.0, $J_{6''a,6''b}$ 12.2 Hz, H-6″a), 4.29 (1H, dd, $J_{5',6'a}$ 5.1, $J_{6'a,6'b}$ 12.2 Hz, H-6′a), 4.13-4.08 (3H, m, H-3, H-6′b, H-6″b), 4.05 (1H, ddd, $J_{4'',5''}$ 9.8, $J_{5'',6''a}$ 5.0, $J_{5'',6''b}$ 2.3 Hz, H-5″), 4.02 (1H, ddd, $J_{4',5'}$ 9.8, $J_{5',6'a}$ 5.1, $J_{5',6'b}$ 2.4 Hz, H-5′), 3.86–3.60 (11H, m, H-5, H-6a, $OCH_2CH_2O \times 2$), 3.61 (1H, dd, $J_{5,6b}$ 2.7, $J_{6a,6b}$ 11.3 Hz, H-6b), 3.39 (3H, s, OCH_3), 2.15 (9H, s, $COCH_3 \times 3$), 2.11 (9H, s, $COCH_3 \times 3$), 2.00 (6H, s, $COCH_3 \times 2$), 1.99 (6H, s, $COCH_3 \times 2$); δ_C (67.8 MHz, $CDCl_3$, 25 °C) 170.70, 170.68, 170.4, 170.03, 169.96, 169.82, 169.80, 169.7, 98.6, 97.8, 97.7, 95.8, 94.6, 76.5, 72.8, 69.7, 69.55, 69.49, 69.4, 69.05, 69.00, 68.4, 67.7, 67.3, 66.9, 66.8, 66.5, 66.1, 62.4, 60.4, 55.1, 21.1, 21.0, 20.9, 20.8, 20.76, 20.71, 20.68, 14.2; HRMS (ESI): *m*/*z* calcd for $C_{45}H_{66}O_{30}$+Na, 1109.3539; found, 1109.3559.

Methyl 3,6-di-*O*-(α-D-mannnopyranosyl-oxyethoxymethyl)-α-D-mannopyranoside (13). To a stirred solution of **12** (27 mg, 25 μmole) in dry MeOH (3 mL) was added 0.5 M NaOMe (100 μL). After 30 min, the solution was neutralized with Dowex50 (H^+) and evaporated to give **13** (15 mg, 91%). R_f 0.22 (*i*PrOH-H_2O–NH_3 8 : 3 : 1); $[\alpha]_D^{25}$ +67.9 (*c* 0.72 in H_2O); δ_H (600 MHz, D_2O, 60 °C) 5.19 (1H, d, *J* 11.6 Hz, OC*H*HO), 5.19 (1H, d, $J_{1,2}$ 1.7 Hz, H-1″), 5.17 (1H, d, *J* 11.6 Hz, OCH*H*O), 5.17 (1H, d, $J_{1,2}$ 1.7 Hz, H-1′), 5.11 (1H, d, *J* 6.9 Hz, OC*H*HO), 5.09 (1H, d, *J* 6.9 Hz, OCH*H*O), 5.04 (1H, d, $J_{1,2}$ 1.6 Hz, H-1), 4.36 (1H, dd, $J_{1,2}$ 1.6, $J_{2,3}$ 3.0 Hz, H-2), 4.25 (1H, dd, $J_{1'',2''}$ 1.6, $J_{2'',3''}$ 3.0 Hz, H-2″), 4.24 (1H, dd, $J_{1',2'}$ 1.6, $J_{2',3'}$ 3.0 Hz, H-2′), 4.22–3.93 (23H, m, H-3, H-3′, H-3″, H-4, H-4′, H-4″, H-5, H-5′, H-5″, H-6a, H-6′a, H-6″a, H-6b, H-6′b, H-6″b, $OCH_2CH_2O \times 2$), 3.70 (3H, s, OCH_3); δ_C (67.8 MHz, D_2O, 25 °C, acetone) 101.4, 100.5, 95.7, 95.3, 78.0, 73.3, 71.8, 71.1, 70.6, 69.0, 67.33, 67.26, 67.03, 66.96, 66.2, 61.5, 55.5; HRMS (ESI): *m*/*z* calcd for $C_{25}H_{46}O_{20}$+Na, 689.2482; found, 689.2481.

Methyl 2,3-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannnopyranosyl-oxyethoxymethyl)- α-D-mannopyranoside (15). A mixture of methyl 4,6-*O*-benzylidene-α-D-mannopyranos-ide (**14**)[23] (50 mg, 180 μmol), **4** (172 mg, 380 μmol), TTBP (220 mg, 889 μmol), and activated molecular sieves 4A (180 mg) in *p*-xylene (4.5 mL) was stirred under N_2 at RT for 30 min. Immediately after the addition of MeOTf (60 μL, 530 μmol), the mixture was rapidly mixed and frozen over dry ice in acetone. The mixture was stored in refrigerator at 4 °C for 48 h and defrosted at RT. Et_3N (80 μL) was added to the mixture to deactivate MeOTf. The mixture was diluted with EtOAc and filtered through Celite. The filtrate was washed with brine, dried over $MgSO_4$, and evaporated. The residue was dissolved in 80% AcOH (3 mL) at 40 °C. After 6 h, the solution was evaporated and chromatographed on silica gel to give **15** (23 mg, 13%) and methyl 3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannnopyranosyl-oxyethoxy-methyl)-α-D-mannopyranoside (**16**) (10 mg, 9%). Compound **16** was acetylated in pyridine-acetic anhydride (1 : 1, 2 mL) to give methyl 3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannnopyranosyl-oxyethoxymethyl)-2,4,6-tri-*O*-acetyl-α-D-mannopyranoside (**17**).

Compound **15**: R_f 0.31($CHCl_3$–MeOH 20 : 1); $[\alpha]_D^{25}$ +51.0 (*c* 0.96 in $CHCl_3$); δ_H (600 MHz, $CDCl_3$, 25 °C, Me_4Se) 5.38 (1H, dd, $J_{2',3'}$ 3.4, $J_{3',4'}$ 10.0 Hz, H-3′), 5.35 (1H, dd, $J_{2'',3''}$ 3.4, $J_{3'',4''}$ 10.0 Hz, H-3″), 5.34 (1H, d, $J_{2',3'}$ 3.4 Hz, H-2′), 5.29 (2H, t, $J_{3',4'} = J_{4',5'}$ 10.0 Hz, H-4′, H-4″), 5.28 (1H, d, $J_{2'',3''}$ 3.4 Hz, H-2″), 4.89 (1H, s, H-1″), 4.87 (1H, d, *J* 7.1 Hz, OC*H*HO), 4.86 (1H, s, H-1′), 4.85 (1H, d, *J* 7.1 Hz, OC*H*HO), 4.82 (2H, d, *J* 7.1 Hz, OCH*H*O × 2), 4.80 (1H, s, H-1), 4.30 (1H, dd, $J_{5'',6''a}$ 5.2, $J_{6''a,6''b}$ 12.2 Hz, H-6″a), 4.28 (1H, dd, $J_{5',6'a}$ 5.1, $J_{6'a,6'b}$ 12.1 Hz, H-6′a), 4.12 (1H, dd, $J_{5'',6''b}$ 1.7, $J_{6''a,6''b}$ 12.2 Hz, H-6″b), 4.10 (1H, dd, $J_{5',6'b}$ 1.9, $J_{6'a,6'b}$ 12.1 Hz, H-6′b), 4.05–4.02 (2H, m, H-5′, H-5″), 3.97–3.81 (6H, m, H-2, H-4, $OCH_2CH_2O \times 2$), 3.78 (1H, dd, $J_{2,3}$ 3.1, $J_{3,4}$ 9.3 Hz, H-3), 3.73–3.66 (4H, m, $OCH_2CH_2O \times 2$), 3.62 (1H, d, $J_{4,4OH}$ 1.4 Hz, 4OH), 3.57 (1H, dt, $J_{4,5}$ 9.5, $J_{5,6a} = J_{5,6b}$ 4.2 Hz, H-5), 3.37 (3H, s, OCH_3), 2.53 (1H, t, $J_{6a,6OH} = J_{6b,6OH}$ 6.4 Hz, 6OH), 2.16 (6H, s, $COCH_3 \times 2$), 2.10 (6H, s, $COCH_3 \times 2$), 2.06 (3H, s, $COCH_3$), 2.05 (3H, s, $COCH_3$), 1.997 (3H, s, $COCH_3$), 1.990 (3H, s, $COCH_3$); δ_C (67.8 MHz, $CDCl_3$, 25 °C) 170.7, 170.1, 170.05, 170.00, 169.84, 169.77, 169.7, 99.7, 97.9, 97.6, 96.2, 81.1, 77.2, 76.0, 72.4, 70.1, 69.5, 69.3, 69.03, 68.98, 68.5, 68.4, 67.2, 67.1, 66.9, 66.8, 66.1, 66.0, 62.6, 62.4, 54.8, 20.9, 20.73, 20.68; HRMS (ESI): *m*/*z* calcd for $C_{41}H_{62}O_{28}$+Na, 1025.3327; found, 1025.3376.

Compound **17**: R_f 0.14 (CH_3Cl–MeOH 20 : 1); $[\alpha]_D^{25}$ +58.0 (*c* 0.05 in CH_3Cl); δ_H (600 MHz, $CDCl_3$, 25 °C, Me_4Si) 5.33 (1H, dd, $J_{2',3'}$ 3.1, $J_{3',4'}$ 10.1 Hz, H-3′), 5.30 (1H, d, $J_{2',3'}$ 3.1 Hz, H-2′), 5.29 (1H, t, $J_{3',4'} = J_{4',5'}$ 10.1 Hz, H-4′), 5.25 (1H, d, $J_{2,3}$ 3.1 Hz, H-2), 5.23 (1H, t, $J_{3,4} = J_{4,5}$ 10.1 Hz, H-4), 4.90 (1H, s, H-1′), 4.75 (1H, d, *J* 7.2 Hz, OC*H*HO), 4.71(1H, s, H-1), 4.64 (1H, d, *J* 7.2 Hz,

OCH*H*O), 4.30 (1H, dd, $J_{5',6'a}$ 5.0, $J_{6a',6'b}$ 12.4 Hz, H-6′a), 4.27 (1H, dd, $J_{5,6a}$ 5.5, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.13–4.09 (3H, m, H-3, H-6b, H-6′b), 4.02 (1H, ddd, $J_{5',6'a}$ 5.0, $J_{5',6'b}$ 2.2, $J_{4',5'}$ 10.1 Hz, H-5′), 3.92 (1H, ddd, $J_{5,6a}$ 5.5, $J_{5,6b}$ 2.3, $J_{4,5}$ 10.1 Hz, H-5), 3.82–3.65 (4H, m, OCH_2CH_2O), 3.39 (3H, s, OCH_3), 2.18 (6H, m, COCH_3 × 2), 2.09 (9H, m, COCH_3 × 3), 2.03 (3H, s, COCH_3), 1.98 (s, 3H, COCH_3); δ_C (150.92 MHz, $CDCl_3$, 25 °C) 170.7, 170.6, 170.2, 169.9, 169.73, 169.71, 169.7, 98.6, 97.8, 94.4, 72.4, 69.6, 69.3, 69.0, 68.5, 68.4, 67.3, 66.9, 66.7, 66.1, 62.7, 62.4, 55.1, 21.0, 20.84, 20.81, 20.72, 20.69, 20.66, 20.61; HRMS (ESI): *m*/*z* calcd for $C_{30}H_{44}O_{20}$+Na, 747.2325; found, 747.2344.

Methyl 2,3-di-*O*-(α-D-mannnopyranosyl-oxyethoxymethyl)-α-D-mannopyranoside (18). To a stirred solution of **15** (30 mg, 30 μmol) in dry MeOH (2 mL) was added 0.5 M NaOMe (100 μL). After 30 min, the solution was neutralized with Dowex50 (H^+) and evaporated to give **18** (19 mg, 95%). R_f 0.21 (*i*PrOH-H_2O–NH_3 8 : 3 : 1); $[\alpha]_D^{25}$ +86.3 (*c* 0.40 in H_2O); δ_H (600 MHz, D_2O, 70 °C) 5.39–5.33 (7H, m, H-1, H-1′, H-1″, OCH_2O × 2), 4.50 (1H, dd, $J_{1,2}$ 1.8, $J_{2,3}$ 3.0 Hz, H-2), 4.43 (2H, dd, $J_{1',2'}$ 1.8, $J_{2',3'}$ 4.4 Hz, H-2′, H-2″), 4.38–4.08 (23H, m, H-3, H-3′, H-3″, H-4, H-4′, H-4″, H-5, H-5′, H-5″, H-6a, H-6′a, H-6″a, H-6b, H-6′b, H-6″b, OCH_2CH_2O × 2), 3.70 (3H, s, OCH_3); δ_C (67.8 MHz, D_2O, 25 °C, acetone) 100.6, 100.5, 99.8, 96.3, 95.4, 77.1, 75.9, 73.4, 73.4, 73.2, 71.2, 70.6, 67.7, 67.5, 67.4, 67.0, 66.9, 66.7, 61.6, 55.5; HRMS (ESI): *m*/*z* calcd for $C_{25}H_{46}O_{20}$+Na, 689.2482; found, 689.2447.

Methyl 2,6-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannnopyranosyl-oxyethoxymethyl)-3, 4-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxane-1,3-diyl)-α-D-mannopyranoside (20). A mixture of methyl 3, 4-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxane-1,3-diyl)-α-D-mannopyranoside (**19**)[24] (27 mg, 62 μmol), **4** (64 mg, 141 μmol), TTBP (78 mg, 315 μmol), and activated molecular sieves 4A (100 mg) in *p*-xylene (2.5 mL) was stirred under N_2 at RT for 30 min. Immediately after the addition of MeOTf (21 μL, 186 μmol), the mixture was rapidly mixed and frozen over dry ice in acetone. The mixture was stored in refrigerator at 4 °C for 48 h and defrosted at RT. Et_3N (30 μL) was added to the mixture to deactivate MeOTf. The mixture was diluted with EtOAc and filtered through Celite. The filtrate was washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (Hexane–EtOAc 4 : 3→1:3) to give **20** (41 mg, 53%) and methyl 6- *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannnopyranosyl-oxyethoxymethyl)-3,4-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxane-1,3-diyl)-α-D-mannopyranoside (**21**) (21 mg, 40%).

Compound **20**: R_f 0.32 (hexane–EtOAc 1 : 2); $[\alpha]_D^{25}$ +53.8 (*c* 0.47 in $CHCl_3$); δ_H (600 MHz, $CDCl_3$, 25 °C, Me_4Si) 5.36 (1H, dd, $J_{2'',3''}$ 3.6, $J_{3'',4''}$ 10.0 Hz, H-3″), 5.35 (1H, dd, $J_{2',3'}$ 3.6, $J_{3',4'}$ 10.0 Hz, H-3′), 5.29 (1H, t, $J_{3'',4''} = J_{4'',5''}$ 10.0 Hz, H-4″), 5.285 (1H, d, $J_{2'',3''}$ 3.6 Hz, H-2″), 5.28 (1H, t, $J_{3',4'} = J_{4',5'}$ 10.0 Hz, H-4′), 5.275 (1H, d, $J_{2',3'}$ 3.6 Hz, H-2′), 4.97 (1H, d, *J* 7.0 Hz, OC*H*HO), 4.88 (2H, s, H-1′, H-1″), 4.78 (2H, d × 2, *J* 7.0, 6.7 Hz, OCH*H*O × 2), 4.75 (1H, d, *J* 6.7 Hz, OC*H*HO), 4.67 (1H, s, H-1), 4.30 (1H, dd, $J_{5'',6''a}$ 5.3, $J_{6''a,6''b}$ 12.0 Hz, H-6″a), 4.29 (1H, dd, $J_{5',6'a}$ 5.3, $J_{6'a,6'b}$ 12.0 Hz, H-6′a), 4.09 (2H, dd, $J_{5',6'b}$ 2.2, $J_{6''a,6''b}$ 12.0 Hz, H-6′b, H-6″b), 4.05 (1H, ddd, $J_{4'',5''}$ 10.0, $J_{5'',6''a}$ 5.3, $J_{5'',6''b}$ 2.2 Hz, H-5′), 4.045 (1H, ddd, $J_{4',5'}$ 10.0, $J_{5',6'a}$ 5.3, $J_{5',6'b}$ 2.2 Hz, H-5′), 3.98 (1H, dd, $J_{2,3}$ 3.3, $J_{3,4}$ 8.9 Hz, H-3), 3.91–3.86 (3H, m, H-2, H-4, H-6a), 3.84-3.62 (10H, m, H-5, H-6b, OCH_2H_2O × 2), 3.38 (3H, s, OCH_3), 2.160 (3H, s, COCH_3), 2.156 (3H, s, COCH_3), 2.11 (3H, s, COCH_3), 2.09 (3H, s, COCH_3), 2.04 (6H, s, COCH_3 × 2), 1.99 (6H, s, COCH_3 × 2), 1.25–0.96 (28H, m, C*H*(CH_3)$_2$); δ_C (150.92 MHz, $CDCl_3$, 25 °C): 170.6, 170.0, 169.8, 169.7, 100.2, 97.8, 97.7, 96.2, 95.8, 75.9, 74.4, 72.1, 70.7, 69.54, 69.48, 69.0, 68.4, 67.24, 67.21, 67.16, 66.6, 66.2, 66.1, 62.4, 54.8, 20.9, 20.74, 20.67, 17.5, 17.4, 17.3, 17.2, 12.9, 12.7, 12.2, 12.1; HRMS (ESI): *m*/*z* calcd for $C_{53}H_{88}O_{29}Si_2$+Na, 1267.4851; found, 1267.4869.

Methyl 2,6-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannnopyranosyl-oxyethoxy-methyl)-3,4-di-*O*-acetyl-α-D-mannopyranoside (22). To a stirred solution of **20** (28 mg, 22.5 μmol) in THF (0.5 mL) was added 1 M TBAF in THF (50 μL, 50 μmol). After 12 h at RT, the solution was evaporated and the residue was chromatographed on silica gel ($CHCl_3$–MeOH 10 : 1). The product was acetylated with pyridine–Ac_2O (1 : 1, 1 mL) for 6 h. The reaction was quenched by adding MeOH at 0 °C and the solution was evaporated. The residue was chromatographed on siliga gel to give **22** (20 mg, 82%). R_f 0.23 (hexane–EtOAc 1 : 3); $[\alpha]_D^{25}$ +52.8 (*c* 0.29 in $CHCl_3$); δ_H (600 MHz, $CDCl_3$, 25 °C, Me_4Si) 5.37–5.32 (3H, m, H-3′, H-3″, H-4), 5.30–5.26 (5H, m, H-2′, H-2″, H-3, H-4′, H-4″), 4.87 (1H, s, H-1′, H-1″), 4.80–4.77 (3H, m, -OCH_2O-, H-1), 4.73 (1H, d, *J* 6.8 Hz, -OC*H*HO-), 4.73 (1H, d, *J* 7.0 Hz, -OC*H*HO-), 4.30–4.26 (2H, m, H-6′a, H-6″a), 4.12–4.10 (2H, m, H-6′b, H-6″b), 4.06–4.02 (2H, m, H-5′, H-5″), 4.00 (1H, t, $J_{1,2} = J_{2,3}$ 1.7 Hz, H-2), 3.86 (1H, ddd, $J_{5,6a}$ 5.2, $J_{5,6b}$ 2.4, $J_{4,5}$ 9.9 Hz, H-5), 3.83–3.64 (9H, m, H-6a, -OCH_2CH_2O- × 2), 3.61 (1H, dd, $J_{5,6b}$ 2.3, $J_{6a,6b}$ 11.2 Hz, H-6b), 3.40 (3H, s, OCH_3), 2.16 (6H, s, COCH_3 × 2), 2.11 (6H, s, COCH_3 × 2), 2.06–2.03 (12H, m, COCH_3 × 4), 1.99 (6H, s, COCH_3 × 2); δ_C (150.9 MHz, $CDCl_3$, 25 °C) 170.6, 170.1, 170.0, 169.8, 99.5, 97.7, 95.73, 95.68, 74.4, 71.0, 69.5, 69.4, 69.0, 68.4, 67.2, 67.0, 66.8, 66.5, 66.3, 66.1, 62.42, 62.36, 55.1, 20.9, 20.8, 20.72, 20.67, 20.64; HRMS (ESI): *m*/*z* calcd for $C_{45}H_{66}O_{30}$+Na, 1109.3539; found, 1109.3556.

Methyl 2,6-di-*O*-(α-D-mannnopyranosyl-oxyethoxymethyl)-α-D-mannopyranoside (23). To a stirred solution of **22** (20 mg, 19 μmole) in dry MeOH (2 mL) was added 0.5 M NaOMe (100 μL). After 30 min, the solution was neutralized with Dowex50 (H^+) and evaporated to give **23** (11 mg, 86%). R_f 0.18 (*i*PrOH-H_2O–NH_3 8 : 3 : 1); $[\alpha]_D^{25}$ +66.1 (*c* 0.40 in H_2O); δ_H (D_2O, 600 MHz, 40 °C) 5.09–4.99 (7H, m, H-1, H-1′, H-1″, -OCH_2O- × 2), 4.16–3.84 (26H, m, H-2, H-2′, H-2″, H-3, H-3′, H-3″, H-4, H-4′, H-4″, H-5, H-5′, H-5″, H-6a, H-6a′, H-6a″, H-6b, H-6b′, H-6b″, -OCH_2CH_2O- × 2), 3.60 (3H, s, OCH_3); δ_C (150.92 MHz, D_2O, 25 °C, acetone) 100.5, 100.1, 96.6, 95.8, 77.7, 73.4, 71.2, 70.6, 67.6, 67.4, 67.1, 67.0, 61.6, 55.6; HRMS (ESI): *m*/*z* calcd for $C_{25}H_{46}O_{20}$+Na, 689.2482; found, 689.2478.

Equlibrium dialysis assay. We used a clear acrylic multi-sample dialysis cell (Sanplatec Corp. EB-0), which consists of a pair of bars (L 300 × W 35 × H 15 mm) each having 8 cylinders (ø 12 × D 7 mm) in a row. By tightly sandwiching a dialysis membrane sheet (L 300 × W 35 mm) on the bored surface of the bars using nine screws in a row, one can make eight chambers with a 1 mL volume bisected by the dialysis membrane. Each chamber has two needle holes for the access to two rooms with a syringe. An assay solution (1 mL), which is bisected by a dialysis membrane, includes a sugar ligand with an appropriate concentration, NaCl (0.15 M), $CaCl_2$ (5 mM), $MnCl_2$ (5 mM), and $MgCl_2$ (5 mM) in 0.01 M phosphate-buffered saline (pH 7.3). One of the two bisected

solutions is prepared so as to include 62.5 μM ConA. The ConA concentration was determined by the Lowry method. The dialysis cell was slowly shaken on a bath thermostated at 30 °C. After 7 h, the 100 μL solution free of ConA was diluted 2-fold with water, which was then subjected to phenol-sulfuric acid assay for sugar concentration. The calibration curves were made for all the synthesized sugar ligands. The free sugar ligand concentrations thus obtained were used to determine the K_d and n values using Scatchard plots.

Acknowledgements

This work was supported by a grant from the Global COE Program and a Grant-in-Aid for Scientific Research (No. 19655056) from the Japanese Ministry of Education, Culture, Sports, Science and Technology. We thank Mr Satoshi Ganaha for his help in the large scale synthesis of compound **4**.

References

1 (*a*) N. Sharon, H. Lis, *Lectins 2nd Ed.*, Springer, The Netherlands, 2003; (*b*) N. Sharon and H. Lis, *Glycobiology*, 2004, **14**, 53R–62R; (*c*) M. Ambrosi, N. R. Cameron and B. G. Davis, *Org. Biomol. Chem.*, 2005, **3**, 1593–1608; (*d*) H. Ghazarian, B. Idoni and S. B. Oppenheimer, *Acta Histochem.*, 2011, **113**, 236–247.
2 (*a*) N. Sharon, *Biochim. Biophys. Acta, Gen. Subj.*, 2006, **1760**, 527–537; (*b*) J. A. Willment and G. D. Brown, *Trends Microbiol.*, 2008, **16**, 27–32.
3 (*a*) S. Y. C. Wong, G. Arsequell, ed., *Immunobiology of Carbohydrates*, Kluwer Academic/Plenum Publishers, New York, 2003; (*b*) B. Ernst and J. L. Magnani, *Nat. Rev. Drug Discovery*, 2009, **8**, 661–677; (*c*) A. Gupta, R. K. Gupta and G. S. Gupta, *J. Sci. Indust. Res.*, 2009, **68**, 465–483.
4 (*a*) R. D. Cummings, R. P. McEver, in *Essentials of Glycobiology second ed.* (ed.: A. Varki, R. D. Cummings, J. D. Esko, H. H. Freeze, P. Stanley, C. R. Bertozzi, G. W. Hart, M. E. Etzler), Cold Spring Harbor Laboratory Press, New York, 2009, pp. 439–457; (*b*) M. B. Lawrence, *Curr. Opin. Chem. Biol.*, 1999, **3**, 659–665; (*c*) M. Schön, *Therapeutics and Clinical Risk Management*, 2005, **1**, 201–208.
5 (*a*) R. Kannagi, M. Izawa, T. Koike, K. Miyazaki and N. Kimura, *Cancer Sci.*, 2004, **95**, 377–384; (*b*) I. P. Witz, *Cancer Metastasis Rev.*, 2008, **27**, 19–30.
6 (*a*) J. W. Pollard, *Nat. Rev. Cancer*, 2004, **4**, 71–78; (*b*) A. Sica, T. Schioppa, A. Mantovani and P. Allavena, *Eur. J. Cancer*, 2006, **42**, 717–727; (*c*) C. E. Lewis and J. W. Pollard, *Cancer Res.*, 2006, **66**, 605–612; (*d*) G. Solinas, G. Germano, A. Mantovani and P. Allavena, *J. Leukocyte Biol.*, 2009, **86**, 1065–1073.
7 (*a*) L. Juillerat-Jeanneret and F. Schmitt, *Med. Res. Rev.*, 2007, **27**, 574–590; (*b*) P. Raia, S. Mallidia, X. Zhenga, R. Rahmanzadeha, Y. Mira, S. Elringtona, A. Khurshida and T. Hasana, *Adv. Drug Delivery Rev.*, 2010, **62**, 1094–1124.
8 (*a*) N. Yamazaki, S. Kojima, N. V. Bovin, S. André, S. Gabius and H.-J. Gabius, *Adv. Drug Delivery Rev.*, 2000, **43**, 225–244; (*b*) Y. Ikehara, T. Niwa, L. Biao, S. K. Ikehara, N. Ohashi, T. Kobayashi, Y. Shimizu, N. Kojima and H. Nakanishi, *Cancer Res.*, 2006, **66**, 8740–8748; (*c*) H. Zhang, Y. Ma and X.-L. Sun, *Med. Res. Rev.*, 2009, **30**, 270–289.
9 (*a*) K. M. Halkes, P. M. St. Hilaire, P. R. Crocker and M. Meldal, *J. Comb. Chem.*, 2003, **5**, 18–27; (*b*) S. A. W. Gruner, E. Locardi, E. Lohof and H. Kessler, *Chem. Rev.*, 2002, **102**, 491–514; (*c*) E. E. Simanek, G. J. McGarvey, J. A. Jablonowski and C.-H. Wong, *Chem. Rev.*, 1998, **98**, 833–862; (*d*) R. Bänteli, P. Herold, C. Bruns, J. T. Patton, J. L. Magnani and G. Thoma, *Helv. Chim. Acta*, 2000, **83**, 2893–2907.
10 (*a*) S. Dedola, S. A. Nepogodiev and R. A. Field, *Org. Biomol. Chem.*, 2007, **5**, 1006–1017; (*b*) J. L. Jiménez Blanco, F. Ortega-Caballero, C. Ortiz Mellet and J. M. García Fernández, *Beilstein J. Org. Chem.*, 2010, **6**(No. 20); (*c*) V. Subramanian, M. Moumé-Pymbock, T. Hu and D. Crich, *J. Org. Chem.*, 2011, **76**, 3691–3709.
11 (*a*) A. Imberty, E. P. Mitchell and M. Wimmerová, *Curr. Opin. Struct. Biol.*, 2005, **15**, 525–534; (*b*) S. Rabbani, X. Jiang, O. Schwardt and B. Ernst, *Anal. Biochem.*, 2010, **407**, 188–195.
12 (*a*) S. A. Linehan, L. Martínez-Pomares and S. Gordon, *Microbes Infect.*, 2000, **2**, 279–288; (*b*) S. Zamze, L. Martinez-Pomares, H. Jones, P. R. Taylor, R. J. Stillion, S. Gordon and S. Y. Wong, *J. Biol. Chem.*, 2002, **277**, 41613–41623.
13 (*a*) T. B. H. Geijtenbeek, S. J. van Vliet, G. C. F. van Duijnhoven, C. G. Figdor and Y. van Kooyk, *Placenta*, 2001, **22**, S19–S23; (*b*) C. G. Figdor, Y. van Kooyk and G. J. Adema, *Nat. Rev. Immunol.*, 2002, **2**, 77–84.
14 (*a*) D. L. Jack, N. J. Klein and M. W. Turner, *Immunol. Rev.*, 2001, **180**, 86–99; (*b*) M. Gadjeva, S. Thiel and J. C. Jensenius, *Curr. Opin. Immunol.*, 2001, **13**, 74–78; (*c*) D. L. Worthley, P. G. Bardy and C. G. Mullighan, *Int. Med. J.*, 2005, **35**, 548–555; (*d*) J. M. Ruskamp, M. O. Hoekstra, M. M. Rovers, A. G. M. Schilder and E. A. M. Sanders, *Arch. Otolaryngol., Head Neck Surg.*, 2006, **132**, 482–486.
15 (*a*) D. K. Mandal, N. Kishore and C. F. Brewer, *Biochemistry*, 1994, **33**, 1149–1156; (*b*) M. C. Chervenak and E. J. Toone, *Biochemistry*, 1995, **34**, 5685–5695; (*c*) D. Gupta, T. K. Dam, S. Oscarson and C. F. Brewer, *J. Biol. Chem.*, 1997, **272**, 6388–6392; (*d*) T. K. Dam and C. F. Brewer, *Chem. Rev.*, 2002, **102**, 387–429.
16 W. Hayes, H. M. I. Osborn, S. D. Osborne, R. A. Rastall and B. Romagnoli, *Tetrahedron*, 2003, **59**, 7983–7996.
17 P. M. Pojer and S. J. Angyal, *Aust. J. Chem.*, 1978, **31**, 1031–1040.
18 A. J. Watters, R. C. Hockett and C. S. Hudson, *J. Am. Chem. Soc.*, 1939, **61**, 1528–1530.
19 H. Hashimoto, T. Endo and Y. Kajihara, *J. Org. Chem.*, 1997, **62**, 1914–1915.
20 M. Takatani, J. Nakano, M. A. Arai, A. Ishiwata, H. Ohta and Y. Ito, *Tetrahedron Lett.*, 2004, **45**, 3929–3932.
21 T. Peters, *Liebigs Ann. Chem.*, 1991, 135–141.
22 S. Oscarson and P. Svahnberg, *Carbohydr. Res.*, 1998, **309**, 207–212.
23 V. Kumar, H. M. Gauniyal and A. K. Shaw, *Tetrahedron: Asymmetry*, 2007, **18**, 2069–2078.
24 J. Thiem, V. Duckstein, A. Prahst and M. Matzke, *Liebigs Ann. Chem.*, 1987, 289–295.
25 H. Yuasa, T. Haraguchi and T. Itagaki, *J. Carbohydr. Chem.*, 2009, **28**, 78–93.
26 D. Pagé, D. Zanini and R. Roy, *Bioorg. Med. Chem.*, 1996, **4**, 1949–1961.
27 B. Honoré, in *Protein–Ligand Interactions: hydrodynamics and calorimetry* (Eds: S. E. Harding, B. Z. Chowdhry), Oxford University Press, Oxford, 2001, pp. 19–46.
28 M. Izumi, S. Okumura, H. Yuasa and H. Hashimoto, *J. Carbohydr. Chem.*, 2003, **22**, 317–329.
29 D. D. Perrin, W. L. Armarego, D. R. Perrin, *Purification of Laboratory Compounds 2nd ed.*Pergamon Press: London, 1980.

