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Synthesis of Structurally Diverse and Defined Bivalent Mannosides on Saccharide Scaffolding

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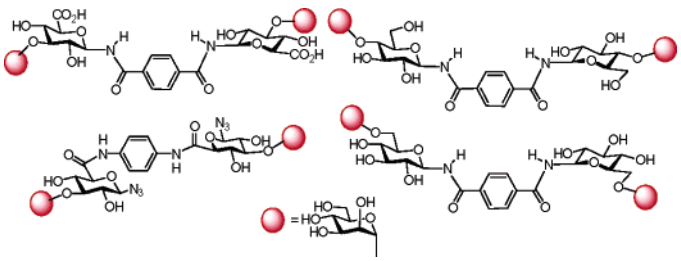
ABSTRACT



The synthesis of bivalent mannosides by the grafting of α-D-mannopyranoside onto monosaccharide acceptors and conjugation to terephthalic acid or phenylenediamine is described. Computational methods were used to predict accessible orientations and distances between the mannose units.

Cells communicate through complex interaction of carbohydrate polymers with their receptors.[1] Such biopolymers constitute a high-density coding system. Multivalent carbohydrates[2,3] form part of this polymer class and are important in generating high-affinity binding, mediating cell−cell recognition, adhesion, and modulation of signal transduction. Synthetic multivalent ligands have proven to be useful in defining new biological mechanisms.[4] Mechanisms of binding of such ligands to receptors are diverse; for example, cross-linking of lectins by multivalent ligands[5] as well as chelate effects operate. Opportunities exist for the development of therapeutics[6] and vaccines.[7] Small glycoclusters can exhibit interesting properties. For example a synthetic compound can be identified, from a series of rigidified multivalent ligands, each exposing the same headgroup (lactose), that exhibits selective blocking of one galectin when evaluated against a panel of galectins.[8] This suggests more generally that detailed three-dimensional structure−activity relationships of multivalent ligands will be interesting. These have not been explored to date, although crystal structures of ligand−receptor complexes are known and relationships

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between architecture and biological activity have been described (e.g., small clusters versus dendrimers versus polymers). For many glycocluster structures, it may be difficult to define the bioactive conformations that the ligands adopt if flexible scaffolding is used for display of the recognition component. Herein we describe synthesis of ligands with potential to cross-link mannose receptors and exploration of their conformation by computational methods.

Scaffolds[9,10] based on glycosylamides **1** (Figure 1) have been synthesized and their structure investigated.[11,12] We

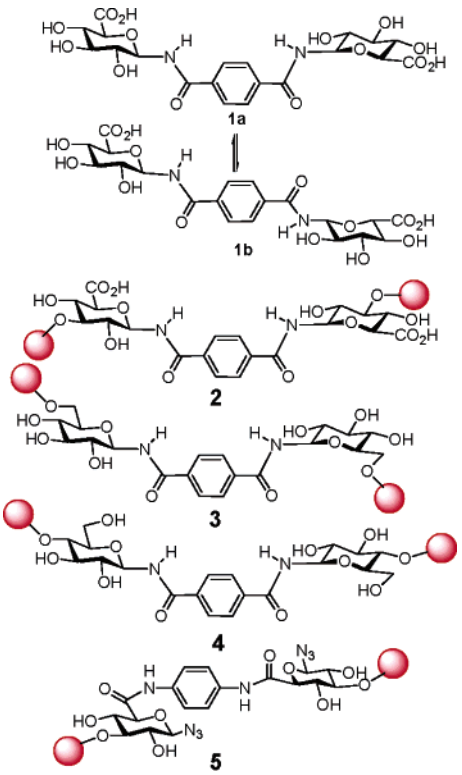

Figure 1. Two-dimensional structures of **1**−**5**. Red sphere = α-D-mannopyranoside.

envisaged grafting the α-D-mannopyranoside headgroup onto hydroxyl groups of the core structure **1** or its related glucose analogue to generate **2**−**4** (Figure 2) with potential to cross-link mannose receptors. Because of a limited number of degrees of conformational freedom, it is possible to predict or determine the spatial relationships between their mannose residues. In addition, we designed **5**, a dimer built on a phenylenediamine unit.[13] A prediction of the preferred conformations of **2**−**4** was first undertaken by computational

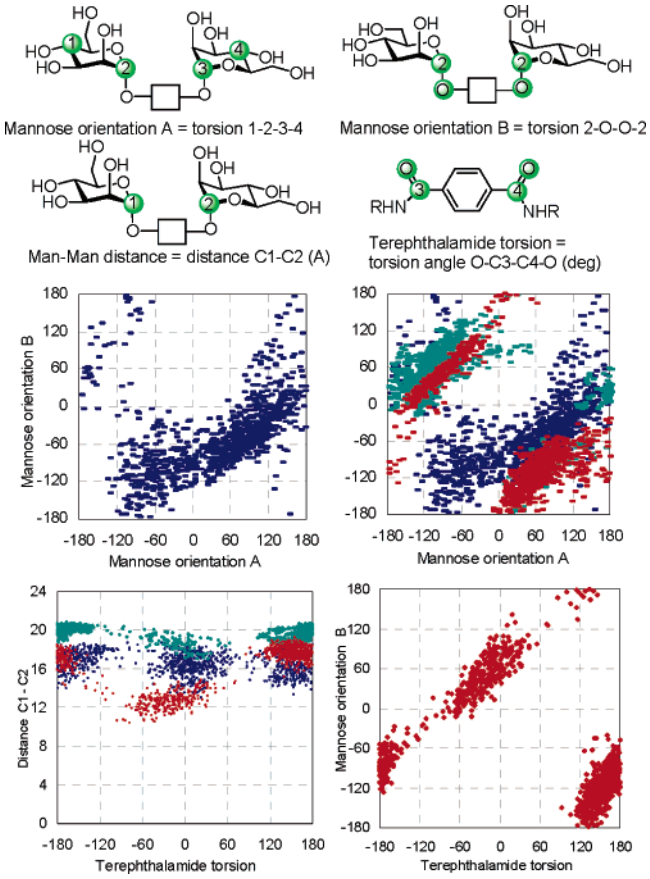

Figure 2. Top graphs show mannose orientation A plotted vs mannose orientation B for 1000 conformers of **2**−**4** sampled during stochastic dynamics simulations. The profile for **2** is blue, that for **3** is green, and that for **4** is red. The distance between mannose residues (Å) as a function of terephthalamide torsion is shown bottom left. The mannose orientation B as a function of terephthalamide torsion for **4** is shown at the bottom right. The Man−Man distance for **3** is 16−20 Å and that for **4** is 10−14 Å, when similar orientations of mannose are found for **3** and **4** (i.e., when terephthalamide torsion is 0 ± 60°).

methods (Macromodel 8.5)[14] before synthesis of the compounds. All calculations were carried out using the GB/SA solvation model[15] for water and the OPLS-AA force field.[16] First, the favored angles for the dihedrals Φ and Ψ[17] for the glycosidic linkage between the mannose and glucose/glucuronic acid residues were calculated by systematic exploration of Φ and Ψ space and also by conformational searching techniques, of model disaccharides.[18,19] The glycosidic torsion angles for the lowest energy structures agreed with related disaccharides calculated previously.[20]

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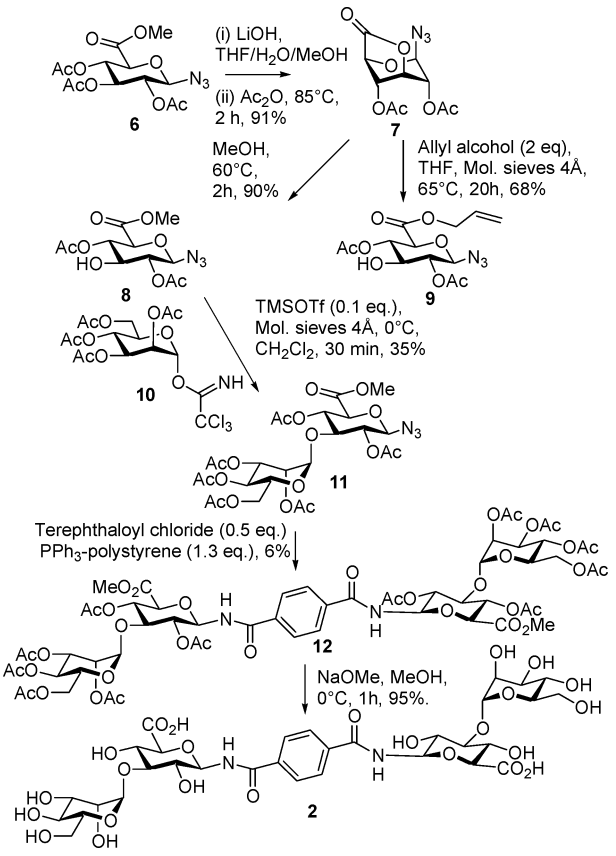
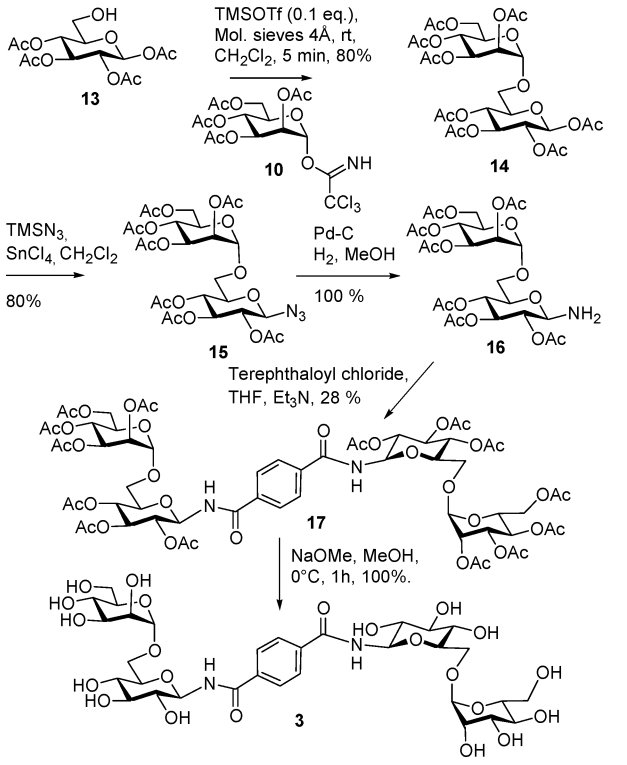
(17) Glycosidic torsion Φ is defined as $H_1-C_1-Ox_c-Cx$, and Ψ is defined as $C_1-Ox-Cx-Hx^{\bullet}$.

Scheme 1



Scheme 2



Experimental evidence suggests that the (*Z*)-anti conformation is preferred for the amide of type **1**,[21] and a presumption was made that this would also be the case for **2**−**4**.[22] In addition, we assumed that two arrangements, similar to **1a** and **1b**, where the carbonyl groups are coplanar (or close to coplanar) with the aromatic ring would be accessible to **2**−**4**.[23] Models were thus built for the divalent compounds and stochastic dynamics simulations carried out to explore conformational space accessible to **2**−**4**.[24] Structures (1000) were sampled during the course of simulations and used for the analysis. What is of interest is the relative spatial orientation of the two mannose residues and the distance between these units. These were profiled as outlined in Figure 2. The plots predict that presentations of mannose residues, in terms of orientation and distance, are diverse for each molecule. It is apparent that, even if **2**−**4** can access conformations where the mannose orientations are similar, the distance between the mannose residues still differs. For example, the profiles for **3** (green) and **4** (red) have regions of overlap when the mannose orientation A is between −180 and 0° and when mannose orientation B = 0−120°; the plot that shows the relationship between mannose orientation B and the terephthalamide torsion indicates that this arrangement occurs when the terephthalamide torsion is between −60 and 60°. However, in this case the distance between mannose residues for **3** is 16−20 Å, whereas for **4** it is 10−14 Å.

The synthesis of novel bivalent structures was undertaken having established that they would have distinct mannose presentations. We have assumed that the presentation of mannosides on glucuronic acid or glucose-based scaffolds would be equivalent and chose our targets on the basis of the envisaged ease of synthesis. The synthesis of **2** was achieved from **6** (Scheme 1).[25] Saponification of **6** followed

(18) Both dihedral drive and conformational search SUMM methods in Macromodel 8.5 were used. Structures of model disaccharides are provided in Supporting Information.

(19) For a review on theoretical approaches to prediction of oligosaccharide structure, see: Imberty, A.; Pérez, S. *Chem. Rev.* **2000**, *100*, 4567.

(20) Previous computational work on related disaccharides was carried out using the HSEA program. See: Jansson, P.-E.; Kenne, L.; Persson, K.; Widmalm, G. *J. Chem. Soc., Perkin Trans. 1* **1990**, 591.

(21) Anti conformation is defined as a conformational isomer with the dihedral angle H1−C1−N−H = 180 ± 90°. Z refers to the amide configuration.

(22) In addition to previous studies, glycosylamides prepared in our laboratory, have (*Z*)-anti conformation in X-ray crystal structures. Rawe, S.; Murphy, P. V. Private communication.

(23) Both arrangements of carbonyl groups are generated during conformational searching protocols to locate low-energy structures.

(24) **Stochastic Dynamics Procedures.** A temperature of 300 K, a time step of 1.5 fs, an equilibration time of 1.0 ps, and a simulation time of 5 ns were used, and 500 structures were sampled over the duration of a 5 ns simulation. Two simulations were carried out for each compound; one was started from a low-energy structure where the carbonyl groups were cis (O=C−C=O torsion = 0°, similar to **1a**) and one from a structure where the carbonyl groups were trans (O=C−C=O torsion = 180°, similar to **1b**). The results of both simulations were combined for the analysis shown in Figure 2. The glycosylamide torsions (H_1-C_1-N-H) were constrained to 180 ± 5°, and a force constant of 2500 was applied. Profiles of the glycosidic torsion angles and glycosylamide torsion and terephthalamide torsion for the sampled structures were consistent with expected preferred conformers for **2**−**4**.

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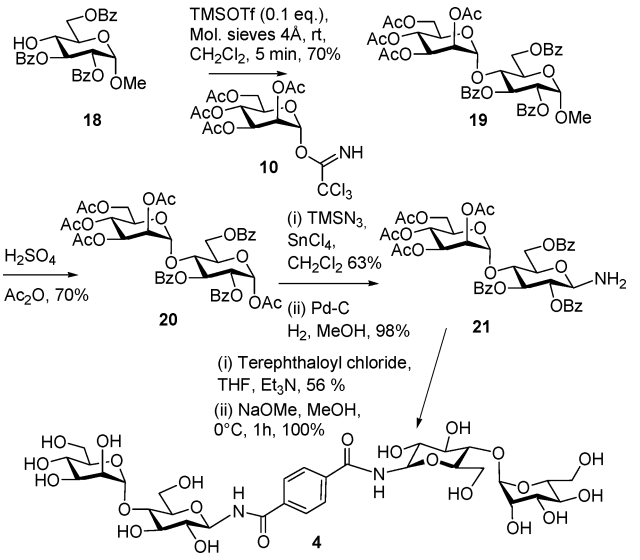
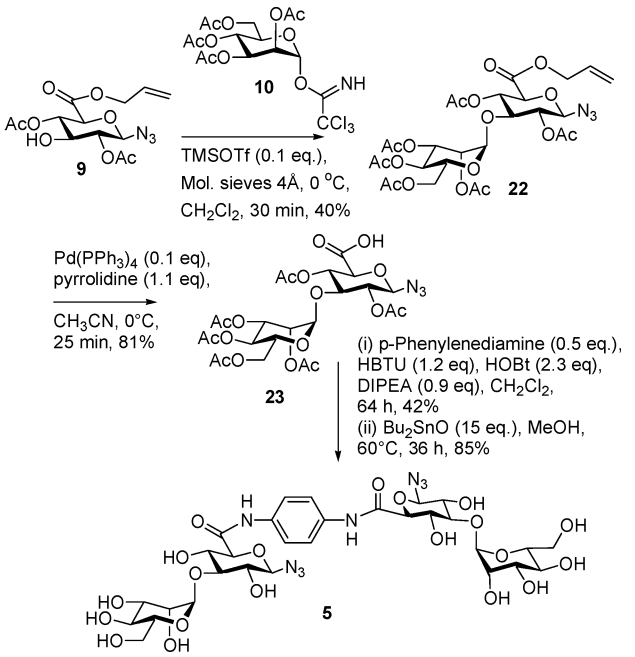
Scheme 3



Scheme 4



by reaction with acetic anhydride gave the 6,3-lactone **7**. This lactone reacted readily with alcohols to give **8** or **9**. The Schmidt glycosidation of **8** with imidate **10**[26] gave **11**, which was subsequently converted, in very low yield,[27] to the dimer **12** on reaction with terephthaloyl chloride promoted by a polymer-supported aryl phosphine. Removal of the protecting groups from **12** gave **2**.

The tetraacetate **13**[28] was reacted with **10** as above to give **14**. This was converted to azide **15** using $SnCl_4$ and azidotrimethylsilane. The amine **16** was prepared from **15** by catalytic hydrogenation, and its subsequent reaction with terephthaloyl chloride in the presence of triethylamine in THF gave the dimer **17** (28%). This was converted to **3** by Zemplen deacetylation (Scheme 2). The synthesis of **4** was achieved via the tribenzoate **18**,[29] which on glycosidation gave disaccharide **19**. Acetolysis gave **20**, and this product was converted to amide **21** via a glycosyl azide. Reaction of **21** with terephthaloyl chloride in THF in the presence of triethylamine followed by removal of the protecting groups gave **4** (Scheme 3).

The divalent mannoside **5** was prepared as shown in Scheme 4.[30] The Schmidt glycoside coupling reaction of **9** with **10** gave **22**. The allyl ester protecting group was removed by palladium catalysis[31] to give acid **23**. Coupling of **23** with *p*-phenylenediamine promoted by HBTU/HOBt gave, after prolonged reaction time, the protected divalent compound. Removal of the acetate protecting groups by the standard methods (NaOMe/MeOH, LiOH, hydrazine) was unexpectedly problematic and gave a mixture containing unidentified products, but the use of dibutyltin oxide[32] in methanol proved to be satisfactory and gave **5**.[33]

In summary, we described synthesis of structurally diverse bivalent mannosides on saccharide scaffolding. Mannose−mannose orientations and distances were determined by location on the scaffold and preferred glycosidic and terephthalamide torsions. Each compound showed a distinct three-dimensional structural profile. A comparison of the biological properties of **2**−**5** will be reported in due course.

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Supporting Information Available: Analytical data for **2**−**5** and ^{1}H and ^{13}C-NMR spectra for all new compounds and selected additional conformational analysis on **2**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(27) Better yields (~30%) are obtained if PPh_3 is used, but purification is more difficult due to competing formation of a soluble iminophosphorane. The iminophosporane is covalently linked to polymer-based supporting reagent, which is removed by filtration.

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(30) Conformational profile of **5** will be discussed in a subsequent paper.

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(33) Compounds **2**−**5** were purified by reverse-phase HPLC.