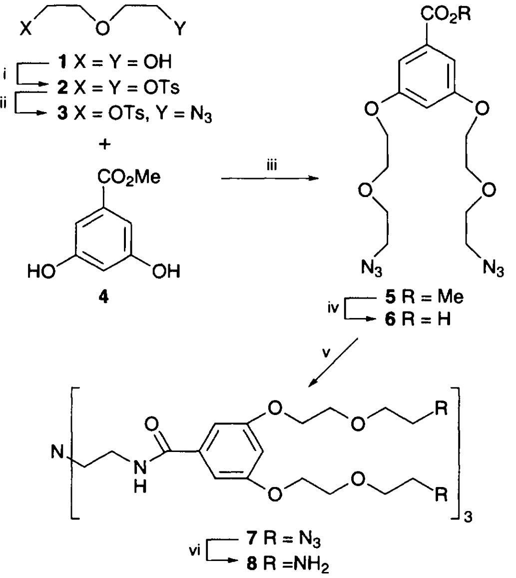
Synthesis and lectin binding properties of dendritic mannopyranoside

Daniel Pagé, Sivasubramanian Aravind and René Roy*†

Department of Chemistry, University of Ottawa, Ottawa, Ontario, Canada K1N 6N5

A hexavalent spheroid dendrimer ending with α-D-mannopyranoside residues was constructed by a convergent approach using *para*-isothiocyanatophenyl α-D-mannopyranoside and dendritic amine as key conjugation reaction.

It is now well established that carbohydrates play significant roles in biological systems, spreading from cellular recognition and adhesion to cell growth and differentiation.[1] Wide interest



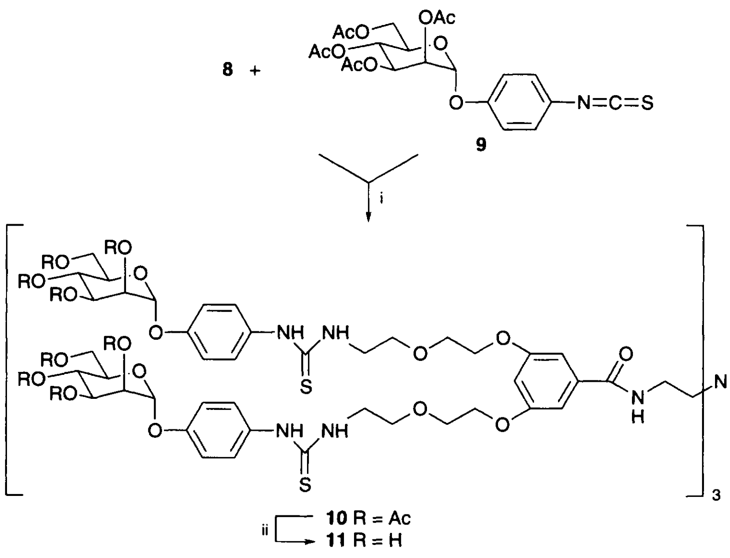
Scheme 1 *Reagents and conditions*: i, TsCl, Et_3N, CH_2Cl_2, room temp., 8 h, 85%; ii, NaN_3, 95% EtOH, reflux, 48 h, 43%; iii, K_2CO_3, MeCN, reflux, 24 h, 83%; iv, 1 mol dm^{-3} KOH, EtOH, reflux, 12 h, 93%; v, EDC, HOBt, DIPEA, CH_2Cl_2, room temp., 8 h, 82%; vi, H_2, 10% Pd–C, MeOH, room temp., 8 h, 93%

has been devoted to the study of the interplay of cell-surface receptors with their corresponding binding carbohydrate moieties. More specifically, terminal mannoside residues have been found to interact with receptors found on macrophages,[2] hepatic sinusoidal cells[3] and different invading pathogens.[4] Therefore, the development of synthetic glycoconjugate analogues that mimic natural oligosaccharides could provide inhibitors of pathogenic infections and targeting devices.

Unfortunately, most carbohydrate–receptor interactions are weak[5] and in order to compensate for their low binding affinity, different strategies based on multivalent interactions ('cluster effect')[6] have been designed, including carbohydrate clusters,[7] telomers,[8] neoglycoproteins[6] and, more recently, glycopolymers.[9] However, the use of poorly defined heterogeneous mixtures of ligands precludes unambiguous interpretations of quantitative biophysical studies on the role of multivalency in ligand binding. Moreover, the potential immunogenicity of these macromolecules makes them inappropriate candidates for some therapeutic uses.

To address these issues, our group recently developed a new family of potent bi-directional dendritic carbohydrate inhibitors having well organized and well characterized multivalency.[10] As an extension of our previous work, we report herein the synthesis of a new spherical dendrimer having six terminal α-D-mannopyranoside residues, along with its inhibitory properties using two plant lectins as models.

The dendrimer core was synthesized following a blockwise convergent procedure. The hydrophilic diethylene glycol spacer arm was first synthesized according to Scheme 1. The azidotoluene-*p*-sulfonate spacer **3** was prepared by tosylation of diethylene glycol **1** (TsCl, Et_3N, CH_2Cl_2) to provide crystalline **2** (mp 87–89 °C) in 85% yield, followed by treatment with NaN_3 in 95% ethanol under reflux to afford compound **3** in 43% yield.‡ Compound **3** (2.4 equiv.) was subsequently coupled with methyl 3,5-dihydroxybenzoate **4** (K_2CO_3, MeCN) to give azido ester **5** in 83% yield (Scheme 1). Saponification of **5** (1 mol dm^{-3} KOH, EtOH, reflux, 12 h) provided acid **6** (93%)



Scheme 2 *Reagents and conditions*: i, DIPEA, DMF, room temp., 2 h, 65%; ii, 1 mol dm^{-3} NaOMe, MeOH, room temp., 2 h, quantitive

which constituted the key building block of the dendrimer. Synthesis of the dendritic cluster **7** was performed in 82% yield by first coupling acid **6** (3.6 equiv.) with tris(2-ethylamino)amine using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) and 1-hydroxybenzotriazole (HOBt). The terminal azides of **7** were then reduced into amine groups by catalytic hydrogenation (H_2, 10% Pd–C, MeOH) to provide hexaamine **8** in 93% yield. The completeness of the reaction was estimated from the IR spectrum of the dendrimer which showed the absence of characteristic peak from residual azide stretching at 2109 cm^{-1}. The dendritic polyamine **8** was then coupled with *p*-isothiocyanatophenyl 2,3,4,6-tetra-*O*-acetyl α-D-mannopyranoside **9**[11] (6.6 equiv., DIPEA, DMF) giving α-D-mannosylated dendrimer **10** in 65% yield, which was subsequently de-*O*-acetylated under standard Zemplén conditions (1 M NaOMe, MeOH) to afford **11** in quantitative yield (Scheme 2).

Preliminary biological testing included double immunodiffusion assay using the lectin Concanavalin A where dendrimer **11** exhibited a sharp precipitin band. Dendrimer **11** was further tested in enzyme-linked lectin assays (ELLA) using peroxidase-labelled Concanavalin A and *Pisum sativum* (pea) lectins. Dendrimer **11** inhibited the binding of Con A to yeast mannan with an IC_{50} of 10.3 μmol dm^{-3} (61.8 μmol dm^{-3} on a per-mannoside basis) while the inhibition of pea lectin was only 16% at the same concentration (338 μmol dm^{-3} for 32% inhibition). These values represent slight improvement (1.7 fold for Con A) when compared to their monosaccharide counterpart, *p*-nitrophenyl α-D-mannopyranoside (IC_{50}'s 105.6 and 2489 μmol dm^{-3} for Con A and pea lectin respectively) and illustrate once again the potency of glycodendrimers in carbohydrate–protein interactions. Although Con A is known to bind methyl α-D-Man approximately four times better ($K_a \sim 11$ mM^{-1}) than pea lectin ($K_a \sim 2.7$ mM^{-1}),[12] the huge difference in the inhibitory values observed between the two lectins might also reside in the fact that, at physiological pH, Con A exists as tetramers while pea lectin is dimeric. This might facilitate the formation of more stable cross-linked lattice with the mannosylated dendrimer.[13]

We thank the Natural Sciences and Engineering Research Council of Canada (NSERC) for financial support and for a postgraduate Scholarship to D. P.

Footnotes

† E-Mail: rroy@oreo.chem.uottawa.ca

‡All compounds showed satisfactory NMR spectra (Brücker AMX 500 MHz) and, where possible, mass spectral data. Compound **5**: [CI: Calc. for $C_{16}H_{22}N_6O_6$, 394.1. Found, 395.0 (M + 1, 12.6% base peak)]. Compound **7**: [FAB-MS (positive): Calc. for $C_{51}H_{72}N_{22}O_{15}$, 1232.6. Found, 1233.7 (M + 1, 2.7% base peak)]; ^1H NMR ($CDCl_3$) δ 2.70 (m, 6 H, NCH_2CH_2), 3.35 (t, 12 H, *J* 5.0 Hz, $CH_2CH_2N_3$), 3.49 (m, 6 H, NCH_2CH_2), 3.68 (m, 24 H, CH_2OCH_2), 4.00 (t, 12 H, *J* 4.5 Hz, Ar-O-CH_2), 6.55 (d, 3 H, *J* 2.1 Hz, H-para), 6.97 (d, 6 H, H-*ortho*), 7.25 (brs, 3 H, NHCO); ^{13}C NMR ($CDCl_3$) δ 50.6 (CH_2N_3), 55.9 (NCH_2CH_2), 67.6 ($CH_2CH_2N_3$), 70.1 (Ar-O-CH_2CH_2), 105.4 (C-*para*), 105.7 (C-*ortho*), 136.0 (C-*ipso*), 156.8 (C-*meta*), 167.4 (C=O). Compound **8**: [FAB-MS (pos.): Calc. for $C_{51}H_{84}N_{10}O_{15}$, 1076.6. Found, 1077.6 (M + 1, 14.4% base peak)]. Compound **10**: ^1H NMR ($CDCl_3$) same signals for dendrimer core as in **7**, except signal at δ 3.35 shifted to δ 3.75 (m, 12 H), 5.44 (d, 6 H, *J* 1.8 Hz, H-1); ^{13}C NMR ($CDCl_3$) δ 96.0 (C-1). Compound **11**: ^1H NMR [$(CD_3)_2SO$] δ 5.29 (s, 6 H, H-1).

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Received, 29th April 1996; Com. 6/03016E