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SYNTHESIS OF DIVALENT α-D-MANNOPYRANOSYLATED CLUSTERS HAVING ENHANCED BINDING AFFINITIES TOWARDS CONCANAVALIN A AND PEA LECTINS

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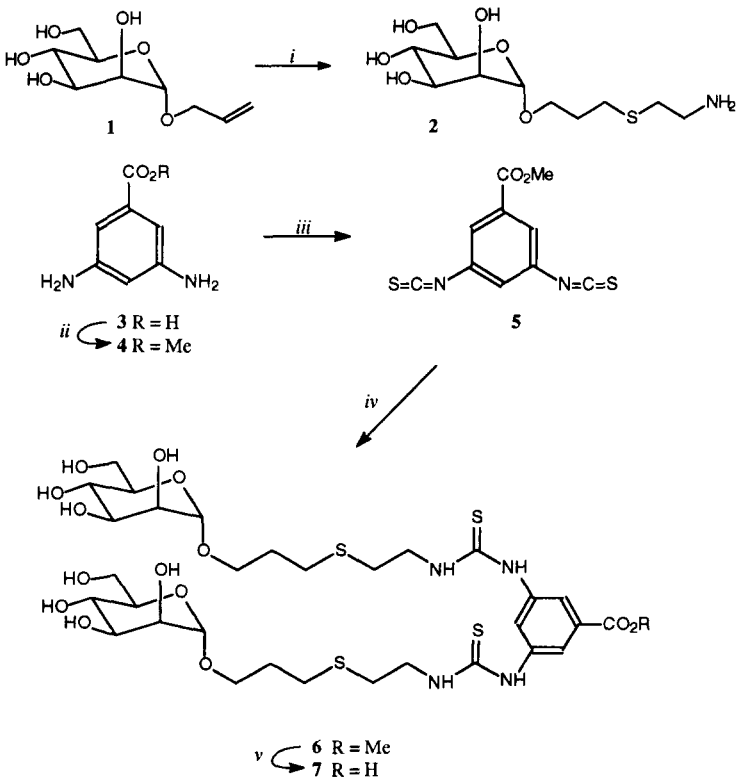
Abstract: Two different α-D-mannopyranosides having aminated heteroaliphatic (**2**) and *p*-isothiocyanatophenylated (**10**) aglycones were prepared and used as key precursors for the conjugation to a 3,5-diaminobenzoic acid core (**3**) using thiourea and amide coupling chemistry. The resulting divalent mannopyranoside clusters **6**, **7**, and **12** were shown to inhibit binding of Concanavalin A and pea lectins to yeast mannan more efficiently than their corresponding monosaccharide derivatives.

Cell surface multiantennary glycoproteins ending with mannopyranoside residues have been shown to act as high affinity receptors for bacterial and viral attachment to host tissues.[1] Similarly, circulating serum mannose binding proteins (MBP) are responsible for "immune" protection against a number of fimbriated pathogens through mannose protein binding interactions.[2] Macrophages are also known to posses mannose-binding proteins (lectins) that help clear mannosylated pathogens.[3] It is therefore of interest to understand the fundamental mannose binding interactions occurring at the molecular level. The potential outcome of a precise understanding of the above carbohydrate-protein interactions may result in the design of potent inhibitors of pathogenic infections and cell-specific targeting devices. One such application may consist in the synthesis of mannoside clusters conjugated to oligonucleotides for their efficient delivery to HIV infected macrophages.[4]

As most carbohydrate-protein interactions are of low affinity,[5] glycobiologists have made extensive use of multivalent neoglycoconjugates to improve the overall binding avidity of synthetic glycoconjugates.[6] Previous studies from our group have involved neoglycoproteins,[7] telomers,[8] glycopolymers,[9] and more recently, glycodendrimers.[10] This latter group of ligands, having well organized and well characterized multivalency, have generally proved to have powerful inhibitory properties. In a recent model study,[11] we demonstrated that properly designed mannosylated dendrimers using L-lysine core showed a many fold increase of inhibitory properties in the binding of Concanavalin A and pea lectins to yeast mannan. As the design of smaller molecules with high inhibitory properties would be of value, we report herein the synthesis of two divalent mannosylated ligands bearing two types of aglycones for comparison purposes, along with their inhibitory properties using the two plant lectins described above.

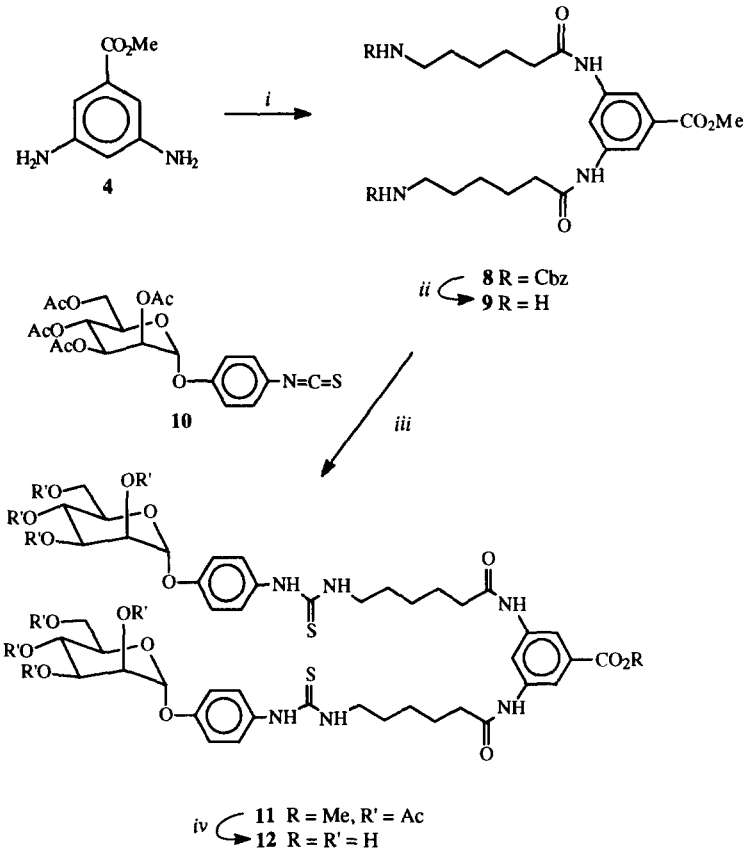
Both divalent target α-D-mannopyranoside ligands have been designed to share common structural similarities by using an aromatic core to which two different spacer arms in the *meta*-positions were connected to two terminal mannopyranosyl residues. The main difference between the two clusters resides in the nature of the aglycones (heteroaliphatic or aryl). These basic structural features have been shown to influence the affinity of mannopyranoside ligands towards Concanavalin A and pea lectins, with the aromatic aglycone favoring the interactions.[12]

The first divalent α-D-mannopyranoside, having an heteroaliphatic spacer, was synthesized according to Scheme 1. Radical addition of cysteamine hydrochloride to known allyl α-D-mannopyranoside **1**[13] (H_2O, 254 nm, 35° C, 3 h) provided 3-(2-aminoethylthio)propyl mannopyranoside **2** in 85% yield.[14] The tethering aromatic core was prepared by esterification of 3,5-diaminobenzoic acid **3** (MeOH, H_2SO_4, reflux, 2 days) to afford methyl ester **4** in 65% yield. Transformation of both aromatic amines into bis-isothiocyanate was accomplished with thiophosgene ($C(S)Cl_2$, CH_2Cl_2, DIPEA, 25 °C, 45 min) to provide **5** in 96% yield. Coupling of mannopyranosylated amine **2** to **5** (2.2 equiv, DMSO, DIPEA) via thioureylene bond formation provided divalent α-D-mannopyranosylated ester ligand **6** in 94% yield. Hydrolysis of the methyl ester (0.05M NaOH, 1.5 h) afforded divalent acid **7** in 93% yield.



Scheme 1. *(i)* $HSCH_2CH_2NH_2$, H_2O, 254 nm, 35 °C, 3 h, 85%; *(ii)* H_2SO_4, MeOH, reflux, 48 h, 65%; *iii)* $C(S)Cl_2$ (5 equiv), DIPEA, CH_2Cl_2, 25 °C, 45 min, 96%; *(iv)* **2** (2.2 equiv), DIPEA, DMSO, 25 °C, 30 min, 94%; *(v)* 0.05M NaOH, 25 °C, 1.5 h, 93%.

The second divalent mannopyranoside having an aromatic aglycone (**12**) was prepared using a similar approach, but the extension of the spacer arm was conducted on the aromatic core **4** rather than on the glycosyl moiety (Scheme 2). Coupling of methyl 3,5-diaminobenzoate **4** with 6-(carbobenzyloxyamino)-hexanoyl chloride (obtained by refluxing the acid in $SOCl_2$ for 3 h) provided the bis-Cbz-protected amino acid **8** in 76% yield. Removal of the terminal Cbz protecting groups (H_2, 10% Pd-C, MeOH) gave diamine **9**, quantitatively. Coupling of **9** with *p*-isothiocyanatophenyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (**10**)[15] (2.2 equiv, DMF, DIPEA, 25 °C, 1 h) afforded α-D-mannopyranosylated dimer **11** in 65% yield. Zemplén de-*O*-acetylation under standard conditions (1 M NaOMe, MeOH, 1.5 h) and methyl ester hydrolysis (0.05 M NaOH, 1.5 h) afforded **12** in 91% yield after neutralization and lyophilization.



Scheme 2. *(i)* $CbzHN(CH_2)_5C(O)Cl$ (2.5 equiv), DIPEA, CH_2Cl_2, 0 °C, 15 min, 76%; *(ii)* H_2, 10% Pd-C, MeOH, 25 °C, 4 h, quant; *(iii)* **10** (2.2 equiv), DIPEA, DMF, 25 °C, 1 h, 65%; *(iv)* a: 1 M NaOMe, MeOH, 25 °C, 1.5 h; b: 0.05 M NaOH, 25 °C, 1.5 h, 91%.

Preliminary inhibition of binding experiments of Concanavalin A and *Pisum sativum* (pea) lectins to yeast mannan by divalent mannosylated ligands **6**, **7**, and **12** were effected using peroxidase-labeled lectin assays (ELLA) as previously described.[11] Methyl and allyl (**1**) α-D-mannopyranoside and the corresponding monomer precursor of **10** (*p*-nitrophenyl α-D-mannopyranoside) were used as reference standards. As expected,[12] *p*-nitrophenyl α-D-mannopyranoside was found to inhibit Con A binding to yeast mannan 8.7-fold better (IC_{50} 106 μM) than the corresponding methyl α-D-mannopyranoside (IC_{50} 924 μM), while inhibition of pea lectin was only 2.6 times better (IC_{50} ≥1500 μM). Divalent ligand **6**, deprived of aromatic aglycone, inhibited the binding of Con A to yeast mannan with an IC_{50} of 6.7 μM, while the IC_{50} value for pea lectin was only 129 μM (Figure 1, Table 1). Surprisingly, the more water soluble acid **7** was 4.6-fold *less* efficient than the ester **6** for Con A. Divalent ligand **12**, with an aromatic aglycone, proved to be less efficient, with IC_{50}'s of 36.8 and ≈575 μM for Con A and pea lectins, respectively. These values represent large improvements when compared to their relative monosaccharides and methyl α-D-mannopyranoside (Table 1), showing once again the importance of valency effect in protein-carbohydrate interactions. The huge difference of the binding affinities observed between the two lectins might reside in the fact that, at physiological pH, Con A exists as tetramers that facilitate the formation of stable cross-linked lattice with the mannosylated ligands,[16] whereas the dimeric character of the pea lectin does not promote such stable lattice.

Alternatively, divalent ester **6** and acid **7** were 5.5- and 1.2-fold more potent to inhibit the binding of Con A to yeast mannan than compound **12**. The same phenomenon was also observed for pea lectin inhibitions where ligands **6** and **7** demonstrated IC_{50}' s about 4.5 and 3.7 times lower than that of compound **12**. Analogous ester **11** was not tested because of its poor water solubility. These results are surprising since it has been previously demonstrated that the presence of aromatic aglycones enhanced the binding properties

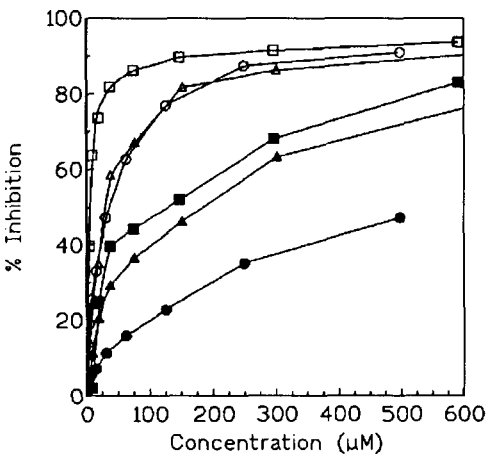


Figure 1. Inhibition of binding of Con A and pea lectins to Yeast mannan by divalent mannosylated ligands **6** (Con A (□), pea (■)), **7** (Con A (Δ), pea (▲)) and **12** (Con A (○), pea (●)).

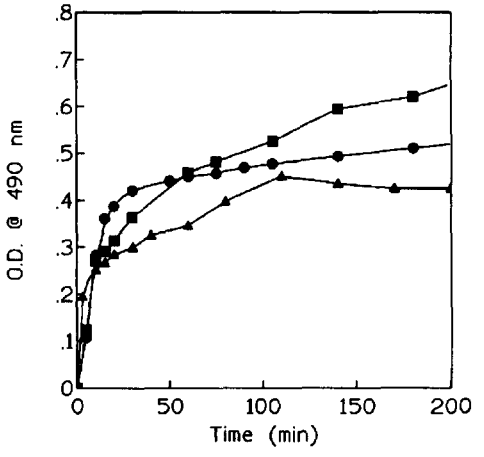


Figure 2. Turbidimetric analysis of Con A (0.9 mg/mL) with divalent mannosylated ligands (0.1 mg/mL) **6** (■), **7** (▲) and **12** (●).

Table 1. Inhibition of Binding of Concanavalin A and Pea Lectins to Yeast Mannan by α-D- Mannopyranosides and Divalent Mannosylated Ligands.

Compound	Con A IC_{50} (μM)	Relative Potency[a]	Pea Lectin IC_{50} (μM)	Relative Potency
Methyl α-D-Man	924	1.0	3850	1.0
pNO_2-Ph α-D-Man	106	8.7	≥ 1500	2.6
Allyl α-D-Man (**1**)	261	3.5	940	4.1
6	6.7	138 (69)	129	29.8 (14.9)
7	30.5	30.3 (15.2)	185	20.8 (10.4)
12	36.8	25 (12.5)	575[b]	6.7 (3.4)

[a] Value in parentheses are based on a per-mannoside residue.

[b] Extrapolated from Figure 1

of mannopyranosides for these two lectins.[12] These observations, therefore, not only underline the importance of the valency effect but also the geometry of the ligands that enables the formation of stable cross-linked lattice.

The mechanism by which the divalent ligands can efficiently inhibit the binding of both lectins to the mannan polysaccharide can be best explained by the relative stability of the lectin-carbohydrate binding interactions. The capacity of the divalent inhibitors to form insoluble cross-linked lattices with the lectins was unequivocally demonstrated by turbidimetric assays (Fig. 2). Clearly visible insoluble complexes could be readily seen after a few minutes of contact.

In conclusion, two types of divalent α-D-mannopyranosylated ligands bearing an heteroaliphatic or an aryl spacer arm anchored to an aromatic core were synthesized. Both divalent clusters exhibited greatly improved affinities for the plant lectins, with the effect being more pronounced for the tetravalent lectin (Con A) over the divalent pea lectin. Even when calculated on the molar basis of their mannopyranoside contents, the cluster effect was present (3.4 to 69). The possibility of varying the aglycone character and length offers interesting insights for the design of other glycoconjugates of biological relevance. Work is now in progress to further improve the binding abilities of mannosylated ligands.

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14. All compounds showed satisfactory NMR spectra (Brücker AMX 500 MHz) and mass spectral data. Compound **2**: $[\alpha]_D$ = + 55.5° (c 1.00, MeOH); ^{1}H NMR (D_2O) δ 4.68 (d, 1H, *J* = 1.7 Hz, H-1); ^{13}C NMR (D_2O) δ 100.5 (C-1); CI calcd for $C_{11}H_{23}NO_6S$: 297.1; found 298.0 (M + 1, 100% base peak); Compound **4**: mp 119-120 °C; ^{1}H NMR (DMSO-d_6) δ 3.73 (s, 3H, CO_2Me); ^{13}C NMR (DMSO-d_6) δ 51.6 (CH_3); Compound **5**: mp 95-96 °C; ^{13}C NMR ($CDCl_3$) δ 133.3 (N=C=S); Compound **6**: $[\alpha]_D$ = +23.6° (c 0.50, MeOH); ^{1}H NMR (D_2O) δ 3.85 (s, 3H, CO_2Me), 4.91 (d, 2H, *J* = 1.6 Hz, H-1); ^{13}C NMR (D_2O) δ 99.3 (C-1), 52.6 (CO_2Me), 179.1 (C=S); FAB-MS (pos.) calcd for $C_{32}H_{52}N_4O_{14}S_4$ 844.2; found 845.3 (M + 1, 0.5% base peak); Compound **7**: ^{1}H NMR (D_2O) same chemical shifts as **6** but disappearance of methyl ester singlet at δ 3.85 ppm; Compound **8**: mp 102-104 °C; ^{1}H NMR ($CDCl_3$) δ 1.22 (m, 4H, NHC(O)$CH_2CH_2CH_2$), 2.22 (t, 4H, *J* = 7.3 Hz, NHC(O)CH_2), 3.73 (s, 3H, CO_2Me), 7.25 (s, 10H, Ar (Cbz)); FAB-MS (pos.) calcd for $C_{36}H_{44}N_4O_8$: 660.3; found 661.3 (M + 1, 2.1% base peak); Compound **9**: CI calcd for $C_{20}H_{32}N_4O_4$: 392.2; found 393.0 (M + 1, 24.9% base peak); Compound **11**: $[\alpha]_D$ = +44.3° (c 1.50, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.45 (d, 2H, *J* = 1.8 Hz, H-1), 7.02 (d, 4H, *J* = 8.7 Hz, H-ortho (aglycone)); ^{13}C NMR ($CDCl_3$) δ 96.1 (C-1), 181.1 (C=S); FAB-MS calcd for $C_{62}H_{78}N_6O_{24}S_2$: 1354.5; found 1355.4 (M + 1, 0.3% base peak); Compound **12**: $[\alpha]_D$ = +47.0° (c 0.03, MeOH); ^{1}H NMR (DMSO-d_6) δ 5.29 (d, 2H, *J* = 1.8 Hz, H-1), 4.20-5.10 (4m, 8H, OH's), 9.94 (s, 1H, CO_2H); ^{13}C NMR (DMSO-d_6) δ 99.4 (C-1); FAB-MS calcd for $C_{45}H_{60}N_6O_{16}S_2$: 1004.4; found 1005.4 (0.4% base peak).
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