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Optimizing lectin-carbohydrate interactions: improved binding of divalent α-mannosylated ligands towards Concanavalin A

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The synthesis and binding properties to Jack bean phytohaemagglutinin in (Concanavalin A, Con A) of a new family of divalent α-D-mannopyranoside ligands are described. The synthesis of these ligands is based on the coupling of commercially available diamines to *p*-isothiocyanatophenyl 2,3,4,6 tetra-*O*-acetyl-α-D-mannopyranoside (4). The resulting dimers 6, 15 to 22 and 30 were tested for their relative inhibitory potency by solid-phase enzyme-linked lectin assays (ELLA) using methyl α-D-mannopyranoside as standard. Divalent mannosylated ligand 35 bearing a non-aromatic aglycon was also tested for comparison purposes. Concentrations necessary for 50% inhibition (IC_{50}s) of binding of yeast mannan to Jack bean phytohaemagglutinin (Con A) were determined. The inhibitions showed dimers to be approximately 10- to 90-fold more potent than methyl α-D-mannopyranoside. Variations in the intra-mannosyl distance proved to be an important factor for optimum binding.

***Keywords*: mannose, divalent ligands, lectin, Concanavalin A, glycodendrimer**

Introduction

Carbohydrate-protein interactions have been recognized to mediate critical processes in cellular events, one of which involves bacterial and viral homing to host tissues [1]. More specifically, cell surface multiantennary glycoproteins bearing terminal mannoside residues have been shown to act as high affinity ligands for different fimbriated pathogens [2]. These latter ones are usually cleared from the blood circulation by the intervention of mannose binding proteins (MBP) [3] and/or macrophages [4] which also rely on mannose protein binding interactions.

It has been demonstrated that the low binding affinities of single carbohydrate ligands for their receptors can be efficiently compensated through multivalent interactions ('cluster effect') [5]. This strategy has involved the use of small clusters [5], neoglycoproteins [6], telomers [7], glycopolymers [8], and glycodendrimers [9] as multivalent neoglycoconjugate carriers. The latter class of ligands demonstrated powerful inhibitory properties against their specific lectin(s). However, quantitative measurements of biophysical interactions with neoglycoproteins and glycopolymers were hampered by their heterogeneity. Moreover, their therapeutic utility is limited by their high immunogenicity. In a recent model study [10], mannosylated dendrimers with L-lysine cores of valencies of 2, 4, 8 and 16 showed the most dramatic increase in Concanavalin A (Con A) binding to occur between dimeric and tetrameric forms (5.3-fold increase), whereas only two-fold increase was observed between the octavalent and hexadecavalent forms. This study demonstrated that small carbohydrate ligands can be effective inhibitors. Consequently shapes and geometry optimizations through variations of bond angles and intra-molecular glycosyl distance can provide further improvements. As the design of such molecules can shed some light on the geometric factors affecting multivalent carbohydrate-protein interactions in solution, we describe herein the synthesis of a number of divalent α-D-mannopyranoside ligands along with their inhibitory properties using the plant lectin Con A as a model.

Experimental procedures

General methods

Melting points were determined on a Gallenkamp apparatus and are uncorrected. The ^1H and ^{13}C NMR spectra were obtained on a Brücker 500 MHz AMX NMR spectrometer. The proton chemical shifts (δ) are given to internal chloroform (7.24 ppm) for $CDCl_3$ solutions, to internal DMSO (2.49 ppm) for DMSO-d_6 solutions, and to internal HOD (4.65 ppm) for D_2O solutions. The carbon chemical shifts are given relative to deuterochloroform (77.0 ppm), to DMSO-d_6 (39.5 ppm), and to internal

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acetone (2.21 ppm) for D_2O solutions. The assignments were based on COSY, DEPT, and HMQC experiments. Optical rotations were measured on a Perkin-Elmer 241 polarimeter and were run at 23 °C. Mass spectra were recorded on a VG 7070-E spectrometer (CI ether) and Kratos Concept IIH for FABMS using glycerol matrix. Thin layer chromatography (TLC) was performed using silica gel 60 F-254 and column chromatography on silica gel 60. Optical densities (OD) for the ELLA tests and turbidimetric measurements were performed on a Dynatech MR 600 Microplate Reader. Methyl α-D-mannopyranoside and diamines were purchased from Aldrich (WI). The lectins from *Canavalia ensiformis* Con A and Con A-peroxidase labelled, along with yeast mannan from *Saccharomyces cerevisiae* were purchased from Sigma (cat. no. 2631, L 6397 and M 7504 respectively).

p-Nitrophenyl α-D-mannopyranoside (**2**)

Compound **2** was obtained from the de-*O*-acetylation of *p*-nitrophenyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside [11] under standard Zemplén conditions in 97% yield. The product was recrystallized from water; mp 178–179 °C; $[\alpha]_D + 141.0^\circ$ (c = 0.20, H_2O) [lit. [11] mp 183–184 °C; $[\alpha]_D + 145^\circ$ (c = 0.20, H_2O)].

p-Isothiocyanatophenyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (**4**)

p-Aminophenyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside **3** (the synthesis of **3** from compound **2** has been reported elsewhere [10]) (93 mg, 2.12 mmol) was dissolved into dichloromethane (35 ml) containing diisopropylethylamine (DIPEA) (900 µl, 2.5 eq.). Thiophosgene (400 µl, 2.5 eq.) was added and the solution was stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography using hexanes/EtOAc (1:1, by vol) as eluent. The product was recrystallized from ethanol giving pure **4** (72 mg) in 72% yield; mp 135–136 °C; $[\alpha]_D + 101.0^\circ$ (c = 1.00, $CHCl_3$); IR ($CHCl_3$) $\nu = 2094.8$ (N=C=S, b); ^{1}H NMR ($CDCl_3$): δ 2.01, 2.03 (2X), 2.17 (4s, 12 H, Ac), 4.02 (ddd, 1 H, $J_{4,5} = 10.0$ Hz, $J_{5,6} = 5.4$ Hz, $J_{5,6'} = 2.4$ Hz, H-5), 4.05 (dd, 1 H, $J_{6,6'} = 12.1$ Hz, H-6′), 4.24 (dd, 1 H, H-6), 5.33 (dd, 1 H, $J_{3,4} = 10.1$ Hz, H-4), 5.40 (dd, 1 H, $J_{1,2} = 1.8$ Hz, $J_{2,3} = 3.5$ Hz, H-2), 5.47 (d, 1 H, H-1), 5.50 (dd, 1 H, H-3), 7.04 (d, 2 H, $J_{o,m} = 9.1$ Hz, H-ortho), 7.15 (d, 2 H, H-meta); ^{13}C NMR ($CDCl_3$): δ 20.6 (3C) (Ac), 20.8 (Ac), 62.1 (C-6), 65.8 (C-4), 68.7 (C-3), 69.2 (C-2), 69.5 (C-5), 95.9 (C-1), 117.5 (C-ortho), 126.2 (N=*C*=S), 127.0 (C-meta), 135.3 (C-para), 154.3 (C-ipso), 169.6–170.4 (C=Os); mass spectrum (CI) (rel. intensity) *m*/*z* 421.9 (M^+–isothiocyanate, 22%), 330.8 (M^+–aglycon, 100%). Anal. Calcd for $C_{21}H_{23}NO_{10}S$: C, 52.39; H, 4.81; N, 2.91. Found: C, 52.14; H, 4.83; N, 2.90.

Synthesis of peracetylated divalent mannopyranosyl ligand (**5**)

Compound **3** (35 mg, 80 µmol) was dissolved in CH_3CN (2 ml) containing a catalytic amount of DIPEA (pH > 8.0). Compound **4** (46 mg, 1.2 eq.) was then added and the solution was refluxed for 72 h. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography using EtOAc/$CHCl_3$ (7:3 by vol) as eluent, giving pure compound **5** (40 mg) in 55% yield. The product failed recrystallization; mp: sintered at 95–98 °C; $[\alpha]_D + 88.8^\circ$ (c = 0.50, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 2.00, 2.01, 2.02, 2.16 (4s, 24 H, Ac), 4.02–4.06 (m, 4 H, H-5 and H-6′), 4.24 (dd, 2 H, $J_{5,6} = 5.6$ Hz, $J_{6,6'} = 12.6$ Hz, H-6), 5.34, (dd, 2 H, $J_{3,4} = 10.1$ Hz, $J_{4,5} = 10.0$ Hz, H-4), 5.39 (dd, 2 H, $J_{1,2} = 1.8$ Hz, $J_{2,3} = 3.5$ Hz, H-2), 5.48 (dd, 2 H, H-1), 5.50 (dd, 2 H, H-3), 7.09 (d, 4 H, $J_{O,M} = 8.9$ Hz, H-ortho), 7.28 (d, 4 H, H-meta), 7.73 (bs, 2 H, NH); ^{13}C NMR ($CDCl_3$) δ 20.6 (3C), 20.8 (Ac), 62.0 (C-6), 65.7 (C-4), 68.6 (C-3), 69.2 (2C) (C-2 and C-5), 95.8 (C-1), 117.3 (C-ortho), 127.3 (C-meta), 131.9 (C-para), 154.4 (C-ipso), 169.6, 169.9 (4C), 170.4 (C=Os), 180.5 (C=S); mass spectrum (pos FAB) (relative intensity) *m*/*z* 921.3 ($M^+ + 1$, 2.9%), 331.2 (M^+–aglycon, 16.4%). Anal. Calcd for $C_{41}H_{48}N_2O_{20}S$: C, 53.48; H, 5.25; N, 3.04. Found: C, 53.80; H, 5.43; N, 3.25.

General procedure for the synthesis of peracetylated divalent mannopyranosyl ligands (**7–14**)

To a solution of diamine (1 eq.) in dichloromethane (1 ml per 5 mg of diamine) containing a catalytic amount of DIPEA (pH > 8.0) was added compound **4** (2.2 eq) and the solution was stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography using EtOAc:$CHCl_3$ (7:3, by vol) and/or $CHCl_3$:MeOH (9:1, by vol) as eluent to provide the corresponding acetylated mannosyl dimers. All products failed recrystallization.

Spectroscopic and analytical data for peracetylated divalent mannopyranosyl ligands (**7–14**)

Compound **7**: yield: 87%; mp: sintered at 110–114 °C; $[\alpha]_D + 60.7^\circ$ (c = 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.00, 2.01, 2.02, 2.17 (4s, 24 H, Ac), 3.79 (m, 4 H, α-CH_2), 4.09 (m, 2 H, $J_{4,5} = 10.0$ Hz, $J_{5,6} = 4.7$ Hz, H-5), 4.12 (dd, 2 H, $J_{6,6'} = 12.4$ Hz, H-6′), 4.24 (dd, 2 H, H-6), 5.36 (dd, 2 H, $J_{3,4} = 10.1$ Hz, H-4), 5.41 (dd, 2 H, $J_{1,2} = 1.8$ Hz, $J_{2,3} = 3.4$ Hz, H-2), 5.50 (dd, 2 H, H-3), 5.51 (d, 2 H, H-1), 6.80 (bs, 2 H, –CH_2–N*H*–), 7.10 (d, 4 H, $J_{o,m} = 8.6$ Hz, H-ortho), 7.17 (d, 4 H, H-meta); 7.80 (bs, 2 H, aromatic-N*H*); ^{13}C NMR ($CDCl_3$) δ 20.63 (2C), 20.70, 20.8 (Ac), 44.9 (α-C), 62.1 (C-6), 65.9 (C-4), 68.8 (C-3), 69.2 (2C) (C-2 and C-5), 95.9 (C-1), 117.9 (C-ortho), 127.8 (C-meta), 130.7 (C-para), 154.8 (C-ipso), 169.6, 169.9, 170.0, 170.6 (C=Os), 180.1 (C=S); mass spectrum (pos FAB) (rel. intensity), *m*/*z* 1023.3

($M^+ + 1$, 5.6%), 331.1 (M^+ – aglycon, 11.8%). Anal. Calcd for $C_{44}H_{54}N_4O_{20}S_2$: C, 51.66; H, 5.32; N, 5.48. Found: C, 51.44; H, 5.28; N, 5.34.

Compound **8**: yield: 92%; mp: sintered at 98–102 °C; $[\alpha]_D + 59.6°$ (c = 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.00, 2.01 (2X), 2.16 (4s, 24 H, Ac), 1.57 (m, 4 H, β-CH_2), 3.52 (m, 4 H, α-CH_2), 4.06 (ddd, 2 H, $J_{4,5} = 10.0$ Hz, $J_{5,6} = 5.1$ Hz, $J_{5,6'} = 2.4$ Hz, H-5), 4.09 (dd, 2 H, $J_{6,6'} = 12.2$ Hz, H-6′), 4.23 (dd, 2 H, H-6), 5.35 (dd, 2 H, $J_{3,4} = 10.1$ Hz, H-4), 5.38 (dd, 2 H, $J_{1,2} = 1.8$ Hz, $J_{2,3} = 3.5$ Hz, H-2), 5.50 (dd, 2 H, H-3), 5.51 (d, 2 H, H-1), 6.12 (bs, 2 H, –CH_2–N*H*–), 7.10 (d, 4 H, $J_{o,m} = 9.0$ Hz, H-ortho), 7.14 (d, 4 H, H-meta); 7.80 (bs, 2 H, aromatic-N*H*); ^{13}C NMR ($CDCl_3$) δ 20.6 (2C), 20.7, 20.8 (Ac), 25.8 (β-C), 44.2 (α-C), 62.1 (C-6), 65.9 (C-4), 68.7 (C-3), 69.2 (C-2), 69.3 (C-5), 96.0 (C-1), 118.0 (C-ortho), 127.4 (C-meta), 130.9 (C-para), 154.8 (C-ipso), 169.6, 169.9, 170.0, 170.5 (C=Os), 181.2 (C=S); mass spectrum (pos FAB) (rel. intensity), *m/z* 1051.4 ($M^+ + 1$, 9.3%), 331.1 (M^+ – aglycon, 14.9%). Anal. Calcd for $C_{46}H_{58}N_4O_{20}S_2$: C, 52.56; H, 5.56; N, 5.33. Found: C, 52.23; H, 5.71; N, 5.00.

Compound **9**: yield: 99%; mp: sintered at 90–94 °C; $[\alpha]_D + 59.9°$ (c = 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.99 (2X), 2.01, 2.16 (4s, 24 H, Ac), 1.20 (m, 4 H, γ-CH_2), 1.51 (m, 4 H, β-CH_2), 3.54 (m, 4 H, α-CH_2), 4.04 (ddd, 2 H, $J_{4,5} = 10.1$ Hz, $J_{5,6} = 5.3$ Hz, $J_{5,6'} = 2.3$ Hz, H-5), 4.05 (dd, 2 H, $J_{6,6'} = 12.3$ Hz, H-6′), 4.22 (dd, 2 H, H-6), 5.33 (dd, 2 H, $J_{3,4} = 10.1$ Hz, H-4), 5.38 (dd, 2 H, $J_{1,2} = 1.9$ Hz, $J_{2,3} = 3.5$ Hz, H-2), 5.48 (dd, 2 H, H-3), 5.49 (d, 2 H, H-1), 5.88 (bs, 2 H, –CH_2–N*H*–), 7.10 (d, 4 H, $J_{o,m} = 9.1$ Hz, H-ortho), 7.13 (d, 4 H, H-meta), 7.82 (bs, 2 H, aromatic-N*H*); ^{13}C NMR ($CDCl_3$) δ 20.6 (2C), 20.7, 20.8 (Ac), 26.1 (γ-C), 28.8 (β-C), 45.1 (α-C), 62.1 (C-6), 65.9 (C-4), 68.7 (C-3), 69.3 (2C) (C-2 and C-5), 96.0 (C-1), 118.0 (C-ortho), 127.4 (C-meta), 131.0 (C-para), 154.7 (C-ipso), 169.6, 169.9, 170.0, 170.5 (C=Os), 181.0 (C=S); mass spectrum (pos. FAB) (rel. intensity) *m/z* 1079.4 ($M^+ + 1$, 10.7%), 331.1 (M^+ – aglycon, 17.4%). Anal. Calcd for $C_{48}H_{62}N_4O_{20}S_2$: C, 53.42; H, 5.79; N, 5.19. Found: C, 53.31; H, 5.90; N, 5.17.

Compound **10**: yield: 99%; mp: sintered at 73–76 °C; $[\alpha]_D + 54.5°$ (c = 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.21 (m, 12 H, ε-CH_2, δ-CH_2, γ-CH_2), 1.51 (t, 4 H, β-CH_2), 2.00 (2X), 2.02, 2.17 (4s, 24 H, Ac), 3.54 (m, 4 H, α-CH_2), 4.05 (m, 4 H, $J_{5,6} = 5.6$ Hz, $J_{5,6'} = 2.3$ Hz, $J_{6,6'} = 12.3$ Hz, H-5 and H-6′), 4.23 (dd, 2 H, H-6), 5.33 (dd, 2 H, $J_{3,4} = 10.1$ Hz, $J_{4,5} = 10.0$ Hz, H-4), 5.37 (dd, 2 H, $J_{2,3} = 3.3$ Hz, H-3), 5.42 (dd, 2 H, $J_{1,2} = 1.8$ Hz, H-2), 5.48 (d, 2 H, H-1), 5.88 (bs, 2 H, –CH_2–N*H*–), 7.12 (m, 8 H, H-ortho and H-meta), 7.98 (bs, 2 H, aromatic-N*H*); ^{13}C NMR ($CDCl_3$) δ 20.7 (3C), 20.8 (Ac), 26.7 (ε-C), 28.9 (δ-C), 29.0 (γ-C), 29.2 (β-C), 45.4 (α-C), 62.0 (C-6), 65.7 (C-4), 68.6 (C-3), 69.1 (C-2), 69.2 (C-5), 117.9 (C-ortho), 127.3 (C-meta), 130.9 (C-para), 154.5 (C-ipso), 169.6, 169.8, 169.9, 170.4 (C=Os), 180.7 (C=S); mass spectrum (pos. FAB) (rel. intensity) *m/z* 1135.4 ($M^+ + 1$, 8.4%), 331.1 (M^+ – aglycon, 17.9%). Anal. Calcd for $C_{52}H_{70}N_4O_{20}S_2$: C, 55.02; H, 6.21; N, 4.94. Found: C, 55.06; H, 6.07; N, 4.66.

Compound **11**: yield: 97%; mp: sintered at 82–86 °C; $[\alpha]_D + 58.0°$ (c = 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.01 (2X), 2.03, 2.17 (4s, 24 H, Ac), 3.55 (m, 4 H, γ-CH_2), 3.60 (m, 4 H, β-CH_2), 3.74 (m, 4 H, α-CH_2), 4.07 (m, 4 H, $J_{4,5} = 10.1$ Hz, $J_{5,6} = 5.2$ Hz, $J_{6,6'} = 12.4$ Hz, H-5 and H-6′), 4.24 (dd, 2 H, H-6), 5.35 (dd, 2 H, $J_{3,4} = 10.0$ Hz, H-4), 5.40 (dd, 2 H, $J_{1,2} = 1.8$ Hz, $J_{2,3} = 3.4$ Hz, H-2), 5.50 (d, 2 H, H-1), 5.51 (dd, 2 H, H-3), 7.08 (d, 4 H, $J_{o,m} = 8.6$ Hz, H-ortho), 7.20 (d, 4 H, H-meta); ^{13}C NMR ($CDCl_3$) δ 20.7 (2C), 20.8 (2C) (Ac), 45.0 (α-C), 62.1 (C-6), 65.9 (C-4), 68.8 (C-3), 69.3 (C-2), 69.4 (2C) (C-5 and β-C), 70.2 (γ-C), 96.0 (C-1), 117.7 (C-ortho), 126.8 (C-meta), 131.4 (C-para), 154.3 (C-ipso), 169.6, 169.9, 170.0, 170.5 (C=Os), 181.8 (C=S); mass spectrum (pos. FAB) (rel. intensity) *m/z* 1111.4 ($M^+ + 1$, 4.5%), 331.1 (M^+ – aglycon, 8.4%). Anal. Calcd for $C_{48}H_{62}N_4O_{22}S_2$: C, 51.89; H, 5.62; N, 5.04. Found: C, 51.58; H, 5.65; N, 4.86.

Compound **12**: yield; 65%; mp: sintered at 71–74 °C; $[\alpha]_D + 57.0°$ (c = 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.31 (dt, 2 H, NHC(O)$CH_2CH_2CH_2$), 1.59 (m, 4 H, NHC(O)CH_2CH_2 and NHC(O)$CH_2CH_2CH_2CH_2$) 2.01, 2.03 (2X), 2.18 (4s, 24 H, Ac), 2.17 (t, 2 H, NHC(O)CH_2), 3.40 (dd, 2 H, NHCH_2CH_2NHC(O)), 3.59 (dd, 2 H, $CH_2CH_2CH_2$NHC(S)), 3.77 (dd, 2 H, NHCH_2CH_2NHC(O)), 4.06 (m, 4 H, $J_{4,5} = 10.0$ Hz, $J_{5,6} = 5.3$ Hz, $J_{5,6'} = 2.4$ Hz, $J_{6,6'} = 12.6$ Hz, H-5 and H-6′), 4.25 (dd, 2 H, H-6), 5.36 (dd, 2 H, $J_{3,4} = 10.0$ Hz, H-4), 5.40 (dd, 2 H, $J_{1,2} = 1.9$ Hz, $J_{2,3} = 3.4$ Hz, H-2), 5.50 (d, 2 H, H-1), 5.51 (dd, 2 H, H-3), 6.44 (t, 2 H, CH_2N*H*C(S)), 6.56 (m, 1 H, CH_2N*H*C(O)), 7.12 (d, 4 H, $J_{o,m} = 8.8$ Hz, H-ortho), 7.18 (d, 4 H, H-meta), 7.70 (m, 2 H, aromatic-N*H*); ^{13}C NMR ($CDCl_3$) δ 20.7 (3C), 20.9 (Ac), 24.9 (NHC(O)CH_2CH_2), 26.0 (NHC(O)$CH_2CH_2CH_2$), 28.5 (NHC(O)$CH_2CH_2CH_2CH_2$), 36.2 (NHC(O)CH_2), 39.7 (NHCH_2CH_2NHC(O)), 42.2 (NHCH_2CH_2NHC(O)), 45.0 ($CH_2CH_2CH_2$NHC(S)), 62.1 (C-6), 65.9 (C-4), 68.7 (C-3), 69.3 (2C) (C-2 and C-5), 96.0 (C-1), 117.9 (C-ortho), 127.4 (C-meta), 131.1 (C-para), 154.7 (C-ipso), 169.7, 170.0 (2C), 170.5 (C=Os), 174.0 (NH*C*(O)), 181.1 (C=S); mass spectrum (pos. FAB) (rel. intensity) *m/z* 1136.5 ($M^+ + 1$, 0.6%), 331.1 (M^+ – aglycon, 4.4%).

Compound **13**: yield: 64%; $[\alpha]_D + 43.3°$ (c = 0.80, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.27 (dt, 4 H, γ-CH_2), 1.57 (dt, 4 H, β-CH_2), 1.62 (dt, 4 H, δ-CH_2), 2.01, 2.02, 2.03, 2.17 (4s, 24 H, Ac), 2.17 (t, 4 H, ε-CH_2), 3.35 (m, 4 H, CH_2NHC(O)), 3.59 (dd, 4 H, α-CH_2), 4.06 (m, 4 H, $J_{4,5} = 10.0$ Hz, $J_{5,6} = 5.2$ Hz, $J_{5,6'} = 2.4$ Hz, $J_{6,6'} = 12.6$ Hz, H-5 and H-6′), 4.24 (dd, 2 H, H-6), 5.35 (dd, 2 H, $J_{3,4} = 10.1$ Hz, H-4), 5.39 (dd, 2 H, $J_{1,2} = 1.9$ Hz, $J_{2,3} = 3.4$ Hz, H-2), 5.49 (d, 2 H, H-1), 5.51 (dd, 2 H, H-3), 6.15 (m, 2 H, N*H*C(O)), 6.40 (m, 2 H, CH_2N*H*C(S)), 7.10 (d, 4 H, $J_{o,m} = 8.9$ Hz, H-ortho), 7.18 (d, 4 H, H-meta), 7.85 (m, 2 H, aromatic-N*H*); ^{13}C NMR ($CDCl_3$) δ 20.6 (3C), 20.8 (Ac), 25.0 (δ-C), 26.0

(γ-C), 28.5 (β-C), 36.2 (ε-C), 40.1 ($CH_2NHC(O)$), 44.9 (α-C), 62.1 (C-6), 65.9 (C-4), 68.8 (C-3), 69.3 (2C) (C-2 and C-5), 96.0 (C-1), 117.8 (C-ortho), 127.3 (C-meta), 131.3 (C-para), 154.5 (C-ipso), 169.6, 170.0 (2C), 170.5 (C=Os), 174.1 (NH*C*(O)), 181.2 (C=S); mass spectrum (pos. FAB) (rel. intensity) *m/z* 1250.1 ($M^+ + 1$, 4.1%), 331.1 (M^+–aglycon, 3.9%).

Compound **14**: yield: 85%; mp: sintered at 111–114 °C; $[\alpha]_D + 55.8^\circ$ (c = 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.97, 2.00, 2.01, 2.16 (4s, 24 H, Ac), 4.01 (ddd, 2 H, $J_{4,5} = 10.0$ Hz, $J_{5,6} = 5.3$ Hz, $J_{5,6'} = 2.5$ Hz, H-5), 4.03 (dd, 2 H, $J_{6,6'} = 12.4$ Hz, H-6′), 4.21 (dd, 2 H, H-6), 4.78 (m, 4 H, α-CH_2), 5.33 (dd, 2 H, $J_{3,4} = 10.1$ Hz, H-4), 5.37 (dd, 2 H, $J_{1,2} = 1.8$ Hz, $J_{2,3} = 3.5$ Hz, H-2), 5.46 (d, 2 H, H-1), 5.48 (dd, 2 H, H-3), 6.14 (m, 2 H, CH_2NH), 7.09 (d, 4 H, $J_{o,m} = 8.9$ Hz, H-ortho), 7.13 (s, 4 H, spacer aromatic-Hs), 7.14 (d, 4 H, H-meta), 7.86 (m, 2 H, aromatic-NH); ^{13}C NMR ($CDCl_3$) δ 20.6 (3C), 20.8 (Ac), 48.8 (α-C), 62.1 (C-6), 65.8 (C-4), 68.7 (C-3), 69.2 (C-2), 69.3 (C-5), 95.9 (C-1), 118.0 (C-ortho), 127.5 (C-meta), 127.9 (spacer C-ortho and C-meta), 130.7 (spacer C-para), 136.9 (C-para), 154.8 (C-ipso), 169.6, 169.9 (2C), 170.4 (C=Os), 181.5 (C=S); mass spectrum (pos. FAB) (rel. intensity) *m/z* 1099.3 ($M^+ + 1$, 2.2%), 331.1 (M^+–aglycon, 2.4%). Anal. Calcd for $C_{50}H_{58}N_4O_{20}S_2$: C, 54.64; H, 5.32; N, 5.10. Found: C, 54.65; H, 5.40; N, 5.00.

Synthesis of peracetylated divalent mannopyranosyl ligand (**29**)

Compound **28** [10] (75 mg, 0.132 mmol) was dissolved in DMF (1 ml) containing hydrazinium acetate (200 µl of 2.5M stock solution) and the solution was stirred at open atmosphere overnight at room temperature. The reaction mixture was diluted in EtOAc and washed successively with equal volumes of saturated $NaHCO_3$ solution, water and saturated NaCl solution. The organic phase was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using EtOAc:$CHCl_3$ (7:3, by vol) as eluent giving pure **29** in 65% yield (45 mg); mp: sintered at 63–65 °C; $[\alpha]_D + 60.6^\circ$ (c = 1.50, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.00 (2X), 2.02, 2.16 (Ac), 2.71 (t, 4 H, J = 6.4 Hz, β-CH_2), 3.01 (t, 4 H, α-CH_2), 4.07 (m, 4 H, $J_{4,5} = 10.0$ Hz, $J_{5,6} = 5.3$ Hz, $J_{5,6'} = 2.4$ Hz, $J_{6,6'} = 12.4$ Hz, H-5 and H-6′), 4.24 (dd, 2 H, H-6), 5.32 (dd, 2 H, $J_{3,4} = 10.1$ Hz, H-4), 5.38 (dd, 2 H, $J_{1,2} = 1.8$ Hz, $J_{2,3} = 3.3$ Hz, H-2), 5.43 (d, 2 H, H-1), 5.50 (dd, 2 H, H-3), 6.99 (d, 4 H, $J_{o,m} = 9.0$ Hz, H-ortho), 7.44 (d, 4 H, H-meta), 8.12 (m, 2 H, NH); ^{13}C NMR ($CDCl_3$) δ 20.7 (2C), 20.9 (2C) (Ac), 34.2 (β-C), 36.5 (α-C), 62.1 (C-6), 65.9 (C-4), 68.9 (C-3), 69.0 (C-5), 69.4 (C-2), 96.1 (C-1), 117.0 (C-ortho), 121.7 (C-meta), 132.5 (C-para), 152.7 (C-ipso), 169.5, 169.7, 170.1, 170.6 (C=Os); mass spectrum (pos. FAB) (rel. intensity) *m/z* 1053.3 ($M^+ + 1$, 19.0%), 331.1 (M^+–aglycon, 59.2%). Anal. Calcd for $C_{46}H_{56}N_2O_{22}S_2$: C, 52.47; H, 5.36; N, 2.66. Found: C, 52.61; H, 5.28; N, 2.28.

General de-*O*-acetylation procedure of peracetylated mannopyranosyl ligands

The acetylated dimer was dissolved in MeOH (1 ml per 5 mg dimer) containing 1 M NaOMe (pH $\geqslant$ 8.5). The solution was stirred at room temperature for 2 h. The solution was neutralized with Amberlite IR-120(H^+) ion exchange resin, filtered through cotton wool and evaporated under reduced pressure. The methanolic residue was then dissolved in water and lyophilized, giving the corresponding de-*O*-acetylated product in quantitative yield as an amorphous white solid. All products failed recrystallization.

Spectroscopic and analytical data for de-*O*-acetylated divalent mannopyranosyl ligands (**6**, **15–22**, **30**)

Compound **6**: $[\alpha]_D + 104.0^\circ$ (c = 0.20, H_2O); ^{1}H NMR (D_2O) δ 3.75–3.95 (m, 8 H, H-4, H-5, H-6, H-6′), 4.11 (dd, 2 H, $J_{2,3} = 3.3$ Hz, $J_{3,4} = 9.5$ Hz, H-3), 4.23 (dd, 2 H, $J_{1,2} = 1.7$ Hz, H-2), 5.67 (d, 2 H, H-1), 7.23 (d, 4 H, $J_{o,m} = 8.9$ Hz, H-ortho), 7.32 (d, 4 H, H-meta); ^{13}C NMR (D_2O) δ 61.6 (C-6), 67.4 (C-4), 70.7 (C-2), 71.3 (C-3), 74.3 (C-5), 99.1 (C-1), 118.6 (C-ortho), 129.0 (C-meta), 132.2 (C-para), 155.3 (C-ipso), 179.9 (C=S); mass spectrum (pos. FAB) (rel. intensity) *m/z* 585.2 ($M^+ + 1$, 17.8%), 152.1 (M^+–aglycon, 10.5%).

Compound **15**: $[\alpha]_D + 111.7^\circ$ (c = 1.00, MeOH); ^{1}H NMR (D_2O) δ 3.73–3.81 (m, 10 H, H-5, H-6, H-6′, α-CH_2), 3.83 (dd, 2 H, $J_{3,4} = 9.7$ Hz, $J_{4,5} = 9.8$ Hz, H-4), 4.10 (dd, 2 H, $J_{2,3} = 3.3$ Hz, H-3), 4.22 (dd, 2 H, $J_{1,2} = 1.8$ Hz, H-2), 5.65 (d, 2 H, H-1); ^{13}C NMR (D_2O) δ 43.6 (α-C), 60.2 (C-6), 66.0 (C-4), 69.4 (C-2), 70.0 (C-3), 72.9 (C-5), 97.8 (C-1), 117.5 (C-ortho), 127.5 (C-meta), 130.3 (C-para), 154.1 (C-ipso), 179.5 (C=S); mass spectrum (pos. FAB) (rel. intensity) *m/z* 687.4 ($M^+ + 1$, 12.6%), 152.1 (M^+–aglycon, 11.6%).

Compound **16**: $[\alpha]_D + 92.4^\circ$ (c = 1.00, MeOH); ^{1}H NMR (D_2O) δ 1.58 (m, 4 H, β-CH_2), 3.53 (m, 4 H, α-CH_2), 3.71 (m, 2 H, $J_{4,5} = 9.7$ Hz, H-5), 3.79 (m, 4 H, H-6 and H-6′), 3.83 (dd, 2 H, $J_{3,4} = 9.6$ Hz, H-4), 4.08 (dd, 2 H, $J_{2,3} = 3.2$ Hz, H-3), 4.18 (dd, 2 H, $J_{1,2} = 1.8$ Hz, H-2), 5.61 (d, 2 H, H-1), 7.18 (d, 8 H, $J_{o,m} = 7.7$ Hz, H-ortho and H-meta); ^{13}C NMR (D_2O) δ 25.4 (β-C), 43.8 (α-C), 60.2 (C-6), 66.0 (C-4), 69.4 (C-2), 70.0 (C-3), 72.9 (C-5), 97.8 (C-1), 117.5 (C-ortho), 127.0 (C-meta), 131.5 (C-para), 153.8 (C-ipso), 178.8 (C=S); mass spectrum (pos. FAB) (rel. intensity) *m/z* 715.4 ($M^+ + 1$, 5.4%), 152.1 (M^+–aglycon, 13.2%).

Compound **17**: $[\alpha]_D + 102.9^\circ$ (c = 1.00, MeOH); ^{1}H NMR (D_2O) δ 1.34 (m, 4 H, γ-CH_2), 1.58 (m, 4 H, β-CH_2), 3.52 (m, 4 H, α-CH_2), 3.72 (m, 2 H, $J_{4,5} = 9.7$ Hz, H-5), 3.77 (m, 4 H, H-6 and H-6′), 3.83 (dd, 2 H, $J_{3,4} = 9.8$ Hz, H-4), 4.07 (dd, 2 H, $J_{2,3} = 3.3$ Hz, H-3), 4.17 (dd, 2 H, $J_{1,2} = 1.7$ Hz, H-2), 5.59 (d, 2 H, H-1), 7.17 (d, 8 H, $J_{o,m} = 9.7$ Hz, H-ortho and H-meta); ^{13}C NMR (D_2O) δ 25.5 (γ-C), 28.1 (β-C), 44.3 (α-C), 60.1 (C-6), 66.0 (C-4), 69.5 (C-2), 70.0 (C-3), 72.9 (C-5), 97.9 (C-1), 117.4 (C-ortho), 126.4

(C-meta), 130.1 (C-para), 153.7 (C-ipso), 179.4 (C=S); mass spectrum (pos. FAB) (rel. intensity) *m/z* 743.4 ($M^+ + 1$, 7.6%), 152.1 (M^+–aglycon, 17.1%).

Compound **18**: $[\alpha]_D + 90.6°$ (c = 0.50, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.26 (m, 10 H, γ-CH_2, δ-CH_2, ε-CH_2), 1.50 (bd, 4 H, β-CH_2), 3.39–3.51 (m, 10 H, H-4, H-5, H-6′, α-CH_2), 3.59 (dd, 2 H, $J_{5,6} = 1.7$ Hz, $J_{6,6'} = 11.4$ Hz, H-6), 3.65 (dd, 2 H, $J_{2,3} = 3.3$ Hz, $J_{3,4} = 9.1$ Hz, H-3), 3.80 (dd, 2 H, $J_{1,2} = 1.6$ Hz, H-2), 4.4–5.0 (m, 8 H, OHs), 5.29 (d, 2 H, H-1), 7.02 (d, 4 H, $J_{o,m} = 8.9$ Hz, H-ortho), 7.23 (d, 4 H, H-meta), 7.53 (m, 2 H, CH_2NH), 9.23 (m, 2 H, aromatic-NH); ^{13}C NMR (DMSO-d_6) δ 26.6 (ε-C), 28.6 (δ-C), 28.8 (γ-C), 29.0 (β-C), 43.9 (α-C), 61.4 (C-6), 66.9 (C-4), 70.1 (C-2), 70.7 (C-3), 74.9 (C-5), 99.3 (C-1), 116.7 (C-ortho), 125.2 (C-meta), 133.3 (C-para), 153.4 (C-ipso), 180.4 (C=S); mass spectrum (pos. FAB) (rel. intensity) *m/z* 799.4 ($M^+ + 1$, 22.3%), 152.1 (M^+–aglycon, 13.5%).

Compound **19**: $[\alpha]_D + 96.4°$ (c = 1.00, MeOH); ^{1}H NMR (D_2O) δ 3.70–3.81 (m, 18 H, H-5, H-6, H-6′, α-CH_2, β-CH_2, γ-CH_2), 3.82 (dd, 2 H, $J_{3,4} = 9.4$ Hz, $J_{4,5} = 10.0$ Hz, H-4), 4.10 (dd, 2 H, $J_{2,3} = 3.2$ Hz, H-3), 4.22 (d, 2 H, $J_{1,2} = 1.5$ Hz, H-2), 5.66 (d, 2 H, H-1), 7.23 (bs, 8 H, H-ortho and H-meta); ^{13}C NMR (D_2O) δ 43.7 (α-C), 60.2 (C-6), 66.1 (C-4), 68.3 (β-C), 69.2 (γ-C), 69.4 (C-2), 70.0 (C-3), 73.0 (C-5), 117.5 (C-ortho), 127.0 (C-meta), 131.8 (C-para), 153.9 (C-ipso), 179.1 (C=S); mass spectrum (pos. FAB) (rel. intensity) *m/z* 775.4 ($M^+ + 1$, 4.5%), 152.1 (M^+–aglycon, 3.3%).

Compound **20**: $[\alpha]_D + 63.0°$ (c = 0.50, H_2O); ^{1}H NMR (D_2O) δ 1.37 (m, 2 H, NHC(O)$CH_2CH_2CH_2$), 1.66 (m, 2 H, NHC(O)$CH_2CH_2CH_2CH_2$) 1.67 (m, 2 H, NHC(O)CH_2CH_2), 2.31 (m, 2 H, NHC(O)CH_2), 3.48 (m, 2 H, $NHCH_2CH_2$ NHC(O)), 3.57 (m, 2 H, $CH_2CH_2CH_2$NHC(S)), 3.76–3.88 (m, 6 H, $NHCH_2CH_2$NHC(O), H-4, H-5, H-6, H-6′), 4.13 (dd, 2 H, $J_{2,3} = 3.2$ Hz, $J_{3,4} = 9.4$ Hz, H-3), 4.25 (dd, 2 H, $J_{1,2} = 2.0$ Hz, H-2), 5.70 (d, 2 H, H-1), 7.27 (d, 4 H, $J_{o,m} =$ 7.9 Hz, H-ortho), 7.28 (d, 4 H, H-meta); ^{13}C NMR (D_2O) δ 24.5 (NHC(O)CH_2CH_2), 24.9 (NHC(O)$CH_2CH_2CH_2$), 27.6 (NHC(O)$CH_2CH_2CH_2CH_2$), 35.2 (NHC(O)CH_2), 38.3 ($NHCH_2CH_2$NHC(O)), 43.6 ($NHCH_2CH_2$NHC(O)), 44.2 ($CH_2CH_2CH_2$NHC(S)), 60.2 (C-6), 66.1 (C-4), 69.4 (C-2), 69.9 (C-3), 73.0 (C-5), 97.8 (C-1), 117.6 (C-ortho), 127.3 (C-meta), 131.9 (C-para), 153.9 (C-ipso), 176.6 (NHC(O)), 179.8 (C=S); mass spectrum (pos. FAB) (rel. intensity) *m/z* 799.9 ($M^+ + 1$, 24.7%), 152.1 (M^+–aglycon, 46.7%).

Compound **21**: $[\alpha]_D + 98.3°$ (c = 0.30, MeOH); ^{1}H NMR (D_2O) δ 1.39 (m, 4 H, γ-CH_2), 1.68 (m, 8 H, β-CH_2 and δ-CH_2), 2.31 (t, 4 H, ε-CH_2), 3.39 (m, 4 H, CH_2NHC(O)), 3.56 (dd, 4 H, α-CH_2), 3.71–3.87 (m, 8 H, H-4, H-5, H-6, H-6′) 4.11 (dd, 2 H, $J_{2,3} = 3.3$ Hz, $J_{3,4} = 9.4$ Hz, H-3), 4.24 (dd, 2 H, $J_{1,2} = 1.4$ Hz, H-2), 5.66 (d, 2 H, H-1), 7.21 (d, 4 H, $J_{o,m} = 9.0$ Hz, H-ortho), 7.25 (d, 4 H, H-meta); ^{13}C NMR (D_2O) δ 24.6 (δ-C), 24.7 (γ-C), 25.0 (β-C), 35.3 (ε-C), 38.1 (CH_2NHC(O)), 44.5 (α-C), 60.2 (C-6), 66.1 (C-4), 69.4 (C-2), 69.9 (C-3), 73.0 (C-5), 97.8 (C-1), 116.7 (C-ortho), 128.7 (C-meta), 131.4 (C-para), 153.9 (C-ipso), 176.5 (NHC(O)), 180.2 (C=S); mass spectrum (pos. FAB) (rel. intensity) *m/z* 913.3 ($M^+ + 1$, 0.5%), 152.1 (M^+–aglycon, 23.0%).

Compound **22**: $[\alpha]_D + 84.8°$ (c = 1.00, DMSO); ^{1}H NMR (DMSO-d_6) δ 3.39–3.51 (m, 6 H, H-4, H-5, H-6′), 3.59 (m, 2 H, H-6), 3.65 (dd, 2 H, $J_{2,3} = 3.2$ Hz, $J_{3,4} = 9.1$ Hz, H-3), 3.80 (dd, 2 H, $J_{1,2} = 1.5$ Hz, H-2), 4.25–4.75 (m, 8 H, OHs), 4.68 (s, 4 H, α-CH_2), 5.30 (d, 2 H, H-1), 7.03 (d, 4 H, $J_{o,m} = 8.8$ Hz, H-ortho), 7.27 (m, 8 H, H-meta and spacer aromatic-Hs); ^{13}C NMR (DMSO-d_6), δ 47.0 (α-C), 61.1 (C-6), 66.7 (C-4), 70.1 (C-3), 70.7 (C-2), 74.9 (C-5), 99.3 (C-1), 117.0 (C-ortho), 125.6 (spacer C-ortho and C-meta), 127.3 (C-meta), 133.2 (spacer C-para), 137.7 (C-para), 153.6 (C-ipso), 180.9 (C=S); mass spectrum (pos. FAB) (rel. intensity) *m/z* 763.0 ($M^+ + 1$, 2.4%), 152.1 (M^+–aglycon, 3.9%).

Compound **30**: $[\alpha]_D + 90.0°$ (c = 0.50, MeOH); ^{1}H NMR (DMSO-d_6) δ 2.70 (t, 4 H, J = 7.0 Hz, β-CH_2), 2.99 (t, 4 H, α-CH_2), 3.38–3.50 (m, 6 H, H-4, H-5, H-6′), 3.58 (m, 2 H, H-6), 3.64 (m, 2 H, H-3), 3.80 (m, 2 H, $J_{1,2} = 1.5$ Hz, H-2), 4.40 (t, 2 H, J = 5.9 Hz, OH-6), 4.68 (d, 2 H, J = 6.0 Hz, OH-3), 4.77 (d, 2 H, J = 5.6 Hz, OH-4), 4.94 (d, 2 H, J = 4.4 Hz, OH-2), 5.26 (d, 2 H, H-1), 7.00 (d, 4 H, $J_{o,m} = 9.0$ Hz, H-ortho), 7.47 (d, 4 H, H-meta), 9.90 (s, 2 H, NH); ^{13}C NMR (DMSO-d_6) δ 33.6 (β-C), 35.9 (α-C), 61.0 (C-6), 66.7 (C-4), 70.1 (C-2), 70.7 (C-3), 74.9 (C-5), 99.3 (C-1), 117.1 (C-ortho), 120.4 (C-meta), 133.5 (C-para), 152.2 (C-ipso), 168.6 (C=O); mass spectrum (pos. FAB) (rel. intensity) *m/z* 717.2 ($M^+ + 1$, 7.8%), 152.1 (M^+–aglycon, 29.5%).

N-(2-aminoethyl)-6-carbobenzyloxyamino-hexanamide (**24**)

6-(Carbobenzyloxyamino)caproic acid **23** (40 mg, 0.151 mmol), was dissolved in thionyl chloride (3 ml) and refluxed under nitrogen for 3 hours. The solvent was evaporated and co-evaporated with toluene under reduced pressure. The residue was dissolved in dry CH_2Cl_2 (1 ml) and added dropwise to a stirring solution of ethylene diamine (50 µl, 5 eq.) in CH_2Cl_2 (1 ml) containing DIPEA (75 µl) at 0 °C. After a stirring period of 30 min at 0 °C, a white precipitate formed in solution. This precipitate was filtered, dissolved in water and lyophilized, giving pure **24** in 60% yield (28 mg); ^{1}H NMR ($CDCl_3$) δ 1.37 (m, 2 H, NHC(O)$CH_2CH_2CH_2$), 1.57 (m, 4 H, NHC(O)CH_2CH_2 and NHC(O)CH_2CH_2 CH_2CH_2), 2.17 (t, 2 H, J = 7.2 Hz, NHC(O)CH_2), 2.79 (t, 2 H, J = 5.6 Hz, CH_2NH_2), 3.12–3.30 (m, 4 H, $NHCH_2CH_2$NHC(O) and $H_2NCH_2CH_2$), 4.96 (bm, 1 H, NHC(O)), 5.07 (s, 2 H, CH_2Ph), 6.05 (bm, 1 H, NHC(O)), 7.33 (s, 5 H, Ph); mass spectrum (pos. FAB) (rel. intensity) *m/z* 308.2 ($M^+ + 1$, 41.9%).

1,2-bis(6-carbobenzyloxyaminohexanamido)ethane (**26**)

Compound **26** was synthesized following the same procedure described above, except that only 0.4 eq. of ethylene diamine was used this time, giving pure **26** in 72% yield: ^{1}H NMR ($CDCl_3$) δ 1.30 (m, 4 H, NHC(O)$CH_2CH_2CH_2$),

1.56 (m, 8 H, NHC(O)CH_2CH_2 and NHC(O)$CH_2CH_2CH_2$ CH_2), 2.11 (t, 4 H, NHC(O)CH_2,), 3.15 (m, 4 H, C(O)NH CH_2CH_2NHC(O)), 3.33 (m, 4 H, $CH_2CH_2CH_2$NHC(O)), 4.90 (bm, 2 H, NHC(O)), 5.06 (s, 4 H, CH_2Ph), 6.10 (bm, 2 H, NHC(O)), 7.32 (s, 10 H, Ph); mass spectrum (pos. FAB) (rel. intensity) *m/z* 555.3 ($M^+ + 1$, 36.9%).

N-(2-aminoethyl)6-aminohexanamide (**25**) and 1,2-bis(6-aminohexanamido)ethane (**27**)

Compound **24** (25 mg, 81.3 μmol) was dissolved in a solution of MeOH (5 ml) containing 10% Pd-C (3 mg). Hydrogen was bubbled for 2 h at room temperature and the completeness of the reaction was estimated from the ^{1}H NMR spectrum by the disappearance of the aromatic protecting group at δ 7.32 ppm. The solution was then filtered through Celite, rinsed with CH_2Cl_2 and evaporated under reduced pressure giving the resulting diamine in 90% yield (13 mg) which was immediately used for the next step without further characterization. Compound **27** was synthesized from compound **26** in 95% yield using the same procedure, except that the hydrogenolysis was done in a mixture EtOH : THF, 1 : 1 (v : v) due to the lack of solubility of compound **26** in MeOH.

Allyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (**32**)

Allyl α-D-mannopyranoside **31** [12] (1.60 g, 7.30 mmol) was dissolved in a mixture of acetic anhydride (50 ml) and pyridine (250 ml) and stirred at room temperature overnight. The solvent was evaporated and coevaporated with toluene under reduced pressure. Crystallization of the crude product from ethanol/ether gave pure **32** in 92% yield (2.61 g); mp 53–54 °C; $[\alpha]_D + 49.2°$ (c = 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.95, 2.00, 2.07, 2.11 (4s, 12 H, Ac), 3.97 (ddd, 1 H, $J_{4,5} = 10.1$ Hz, $J_{5,6} = 5.3$ Hz, $J_{5,6'} = 2.5$ Hz, H-5), 3.99 (dd, 1 H, $J_{\alpha,\alpha'} = 12.8$ Hz, $J_{\alpha',\beta} = 6.3$ Hz, H-α′), 4.07 (dd, 1 H, $J_{6,6'} = 12.2$ Hz, H-6′), 4.15 (dd, 1 H, $J_{\alpha,\beta} = 5.3$ Hz, H-α), 4.24 (dd, 1 H, H-6), 4.83 (d, 1 H, $J_{1,2} = 1.8$ Hz, H-1), 5.20 (dd, 1 H, $J_{cis} = 10.4$ Hz, $J_{gem} = 1.5$ Hz, H-cis), 5.22 (dd, 1 H, $J_{2,3} = 3.4$ Hz, H-2), 5.25 (dd, 1 H, $J_{3,4} = 10.1$ Hz, H-4), 5.27 (dd, 1 H, $J_{trans} = 17.2$ Hz, H-trans), 5.33 (dd, 1 H, H-3), 5.86 (m, 1 H, C*H*=CH_2); ^{13}C NMR ($CDCl_3$) δ 20.6 (2C), 20.7, 20.8 (Ac), 62.5 (C-6), 66.2 (C-4), 68.6 (2C) (C-5 and C-α), 69.1 (C-3), 69.6 (C-2), 96.6 (C-1), 118.4 (CH=*C*H_2), 132.9 (*C*H=CH_2), 169.7, 169.8, 170.0, 170.6 (C=Os); mass spectrum (CI) (rel. intensity) 388.9 ($M^+ + 1$, 0.2%), 330.8 (M^+–aglycon, 100%). Anal. Calcd for $C_{17}H_{24}O_{10}$: C, 52.58; H, 6.23. Found: C, 52.66; H, 6.41.

3-Thia-heptanoic acid 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (**33**)

Compound **32** (230 mg, 0.59 mmol), was dissolved in deoxygenated CH_3CN (20 ml) (obtained by bubbling N_2) containing mercaptopropionic acid (180 μl, 3.5 eq.). The reaction mixture was irradiated with UVG-11 Mineralight at 254 nm at 30 °C for 2 h. The solvent was evaporated under reduced pressure and the residual oil was dissolved in EtOAc and successively washed with equal volumes of water and saturated NaCl solution. The organic phase was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The product was purified by silica gel column chromatography using $CHCl_3$: MeOH (5 : 1, by vol) as eluent giving pure **33** as a colourless oil in 84% yield (245 mg); $[\alpha]_D + 46.5°$ (c = 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.87 (m, 2 H, OCH_2CH_2), 1.97, 2.02, 2.08, 2.13 (4s, 12 H, Ac), 2.62 (m, 4 H, $OCH_2CH_2CH_2$S and SCH_2CH_2COOH), 2.76 (t, 2 H, J = 2.6 Hz, SCH_2CH_2COOH), 3.51 (m, 1 H, O*CH*′CH_2), 3.78 (m, 1 H, O*CH*CH_2), 3.97 (ddd, 1 H, $J_{4,5} = 10.0$ Hz, $J_{5,6} = 5.2$ Hz, $J_{5,6'} = 2.4$ Hz, H-5), 4.10 (dd, 1 H, $J_{6,6'}$ = H-6′), 4.25 (dd, 1 H, H-6), 4.79 (d, 1 H, $J_{1,2} = 1.7$ Hz, H-1), 5.21 (dd, 1 H, $J_{2,3} = 3.4$ Hz, H-2), 5.25 (dd, 1 H, $J_{3,4} = 10.0$ Hz, H-4), 5.31 (dd, 1 H, H-3); ^{13}C NMR ($CDCl_3$) δ 20.7 (2C), 20.8, 20.9 (Ac), 26.8 (SCH_2CH_2COOH), 28.5 (OCH_2CH_2), 29.0 (SCH_2CH_2COOH), 35.1 (OCH_2 CH_2CH_2S), 62.5 (C-6), 66.2 (C-4), 66.4 (OCH_2CH_2), 68.5 (C-5), 69.2 (C-3), 69.6 (C-2), 97.5 (C-1), 165.8 (COOH), 169.8, 170.2 (2C), 170.8 (C=Os); mass spectrum (CI) (rel. intensity) *m/z* 477.0 (M^+–OH, 1.8 %), 435.0 (M^+–Ac, 5.7%), 330.8 (M^+–aglycon, 100%).

Synthesis of peracetylated divalent mannopyranosyl ligand (**34**)

Compound **33** (30 mg, 61 μmol) was dissolved in dichloromethane (2 ml) containing hexamethylenediamine (3 mg, 25 μmol) and a catalytic amount of DIPEA (pH > 8.0). 1, 3-Diisopropylcarbodiimide (DIC) (10 μl, 61 μmol) and HOBt (10 mg, 61 μmol) were added and the solution was stirred at room temperature for 3 h. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography using $CHCl_3$: MeOH (9 : 1, by vol) as eluent, giving pure **34** in 73% yield (20 mg); $[\alpha]_D + 29.0°$ (c = 0.90, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.32 (m, 4 H, $CH_2CH_2CH_2$NHC(O)), 1.48 (m, 4 H, CH_2CH_2NHC(O)), 1.87 (m, 4 H, OCH_2CH_2), 1.97, 2.02, 2.08, 2.13 (4s, 24 H, Ac), 2.44 (m, 4 H, SCH_2CH_2C(O)NH), 2.61 (m, 4 H, $OCH_2CH_2CH_2$S), 2.79 (m, 4 H, SCH_2CH_2C(O)NH), 3.22 (m, 4 H, CH_2NHC(O)), 3.51 (m, 2 H, O*CH*′CH_2), 3.78 (m, 2 H, O*CH*CH_2), 3.97 (ddd, 2 H, $J_{4,5} = 9.7$ Hz, $J_{5,6} = 5.3$ Hz, $J_{5,6'} = 2.4$ Hz, H-5), 4.10 (dd, 2 H, $J_{6,6'} = 12.2$ Hz, H-6′), 4.24 (dd, 2 H, H-6), 4.79 (d, 2 H, $J_{1,2} = 1.8$ Hz, H-1), 5.20 (dd, 2 H, $J_{2,3} = 3.3$ Hz, H-2), 5.25 (dd, 2 H, $J_{3,4} = 10.1$ Hz, H-4), 5.30 (dd, 2 H, H-3), 6.00 (m, 2 H, NH); ^{13}C NMR ($CDCl_3$) δ 20.7 (3C), 20.9 (Ac), 26.0 ($CH_2CH_2CH_2$NHC(O)), 27.5 (SCH_2CH_2C(O)NH), 28.5 ($OCH_2CH_2CH_2$S), 28.8 (OCH_2CH_2), 29.4 (CH_2CH_2 NHC(O)), 36.7 (SCH_2CH_2C(O)NH), 39.1 (CH_2NHC(O)), 62.5 (C-6), 66.2 (C-4), 66.4 (OCH_2CH_2), 68.6 (C-5), 69.2 (C-3), 69.6 (C-2), 97.5 (C-1), 169.7, 170.1 (2C), 170.6, 171.1

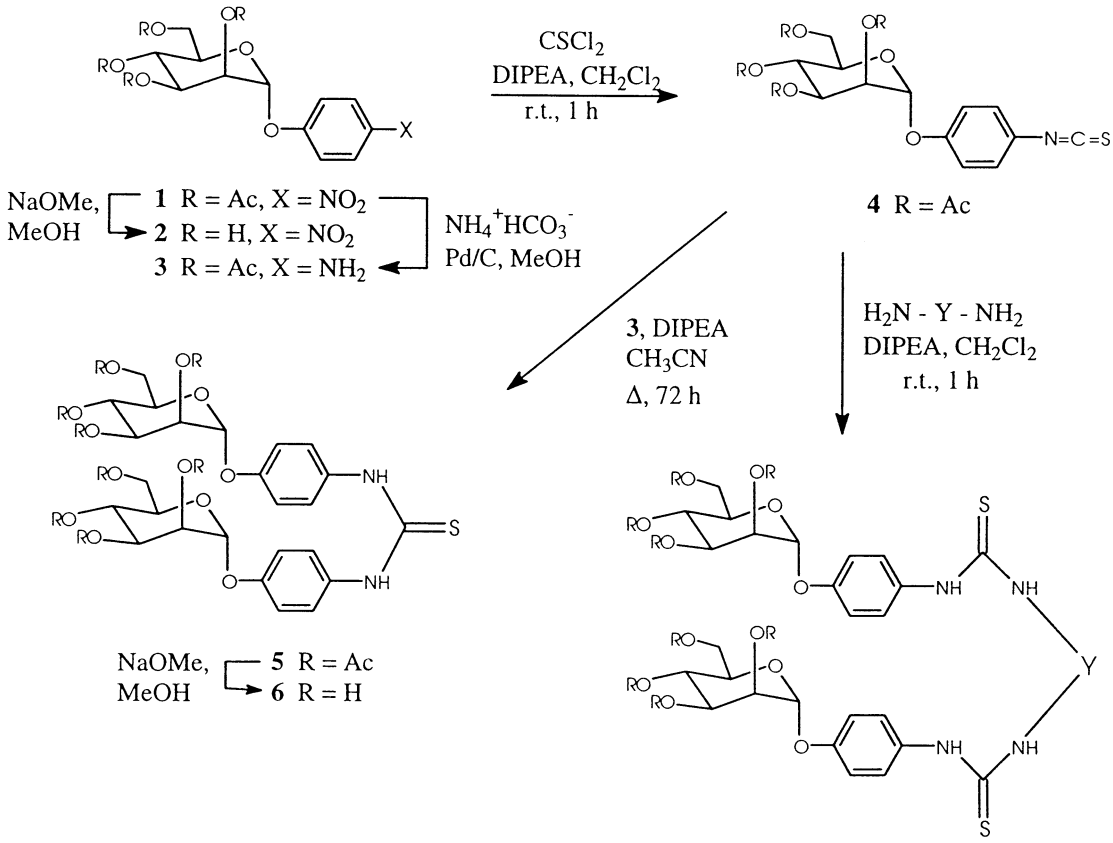
(C=Os); mass spectrum (pos. FAB) (rel. intensity) *m/z* 1250.1 ($M^+ + 1$, 4.1 %), 331.1 (M^+–aglycon, 3.9%).

De-*O*-acetylated divalent mannopyranosyl ligand (**35**)

Compound **34** was de-*O*-acetylated in quantitative yield using the general procedure described above; $[\alpha]_D + 25.8^\circ$ (c = 0.40, H_2O); ^{1}H NMR (D_2O) δ 1.36 (bs, 4 H, $CH_2CH_2CH_2NHC(O)$), 1.42 (m, 4 H, $CH_2CH_2NHC(O)$), 1.99 (tt, 4 H, J = 6.8 Hz, OCH_2CH_2), 2.61 (t, 4 H, J = 6.8 Hz, $SCH_2CH_2C(O)NH$), 2.74 (m, 4 H, $OCH_2CH_2CH_2S$), 2.90 (t, 4 H, J = 6.8 Hz, $SCH_2CH_2C(O)NH$), 3.27 (t, 4 H, J = 6.8 Hz, $CH_2NHC(O)$), 3.65–3.75 (m, 6 H, H-3, H-5, $OCH'CH_2$), 3.79–3.91 (m, 6 H, H-4, H-6′, $OCHCH_2$), 3.96 (dd, 2 H, $J_{5,6}$ = 1.5 Hz, $J_{6,6'}$ = 12.5 Hz, H-6), 4.02 (dd, 2 H, $J_{1,2}$ = 1.5 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 4.94 (d, 2 H, H-1); ^{13}C NMR (D_2O) δ 25.1 ($CH_2CH_2CH_2NHC(O)$), 26.9 ($SCH_2CH_2C(O)NH$), 27.5 ($OCH_2CH_2CH_2S$), 27.7 (OCH_2CH_2), 28.1 ($CH_2CH_2NHC(O)$), 35.3 ($SCH_2CH_2C(O)NH$), 38.8 ($CH_2NHC(O)$), 60.5 (C-6), 65.7 (OCH_2CH_2), 66.3 (C-4), 69.6 (C-2), 70.2 (C-3), 72.3 (C-5), 99.3 (C-1), 173.8 (C=O); mass spectrum (pos. FAB) (rel. intensity) *m/z* 755.3 (M^+ + sodium, 22.9%), 265.0 (M^+ – spacer, 5.0%), 152.1 (M^+ – aglycon, 2.0%).

Enzyme Linked Lectin Assay (ELLA)

ELLA tests were performed on Linbro (Titertek) microtitration plates using a procedure described elsewhere [10]. Briefly, the wells were coated with 100 µl of 10 µg ml^{-1} mannan solution and the following compounds were tested as stock solutions of 1 mg ml^{-1} in 0.01 M PBS (pH 7.3) for



7, 15 Y = -CH_2-CH_2-
8, 16 Y = -CH_2-$(CH_2)_2$-CH_2-
9, 17 Y = -CH_2-$(CH_2)_4$-CH_2-
10, 18 Y = -CH_2-$(CH_2)_8$-CH_2-
11, 19 Y = -$(CH_2$-CH_2-$O)_2$-CH_2-CH_2-
12, 20 Y = -CH_2-CH_2-NHC(O)-$(CH_2)_4$-CH_2-
13, 21 Y = -CH_2-$(CH_2)_4$-C(O)NH-$(CH_2)_2$-NHC(O)-$(CH_2)_4$-CH_2-
14, 22 Y = -CH_2-C_6H_4-CH_2-

Scheme 1.

the inhibition of binding of peroxidase-labelled Con A to mannan: mannose, methyl α-D-mannopyranoside, allyl α-D-mannopyranoside (**31**), *p*-nitrophenyl α-D-mannopyranoside (**2**) as reference monovalent compound, and divalent mannosyl ligands **6**, **15** to **22**, **33** and **35**. The data were plotted and analysed using Graphpad Inplot Software, v. 4.03. The percentage inhibition were calculated as follows:

$$\% \text{ Inhibition} = (A_{(\text{no inhibitor})} - A_{(\text{with inhibitor})})/A_{(\text{no inhibitor})} \times 100$$

IC_{50}s were reported as the concentration required for 50% inhibition of the coating antigen (mannan). All tests were done in triplicate.

Turbidimetric analysis

Turbidimetric experiments were performed as described elsewhere [10]. In brief, 75 µl of Con A (2 mg ml^{-1} PBS) was mixed with 25 µl of compound **17** (0.25 mg ml^{-1} PBS) on a Linbro (Titertek) microtitration plate and the optical density (hν = 490 nm) was monitored for 2 h room temperature. Each test was done in triplicate.

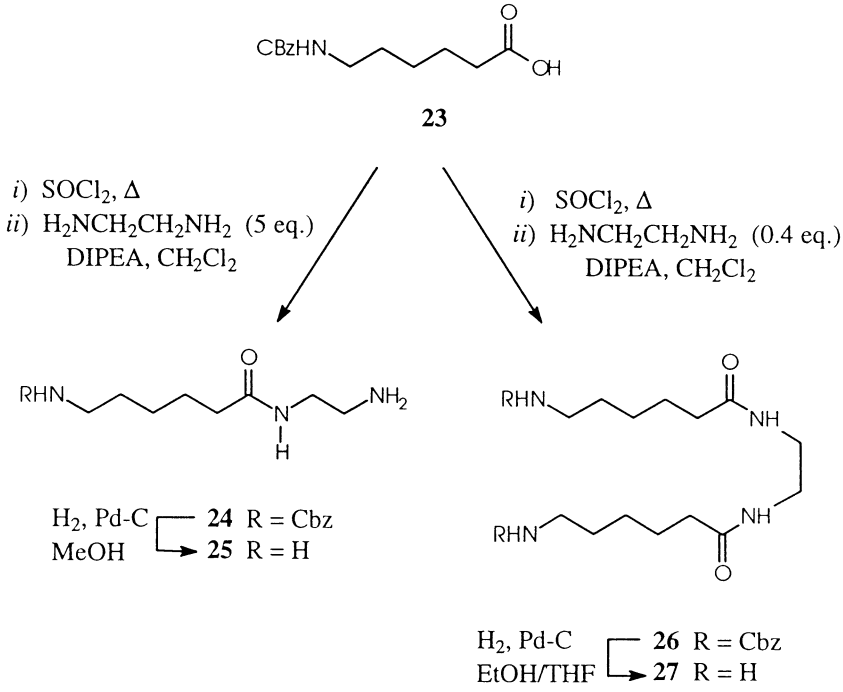
Results and discussion

Con A is unequivocally the most thoroughly studied phytohaemagglutinin [13]. The energetics of its binding interactions [14] and its X-ray data [15] with mannose derivatives are readily available. This lectin has been shown to preferentially bind α-substituted D-mannopyranoside over D-mannose [16]. Furthermore, α-aromatic aglycons contribute significantly to the binding interactions [17]. We have already reported the binding properties of high molecular weight synthetic aryl mannoside dendrimers and glycopolymers in inhibition experiments with *Saccharomyces cerevisiae* yeast mannan [10]. In the present work, similar binding studies are done for a family of smaller divalent ligands.

Synthesis

The synthesis of divalent mannosylated ligands with aromatic aglycons was based upon the coupling of commercially available or synthetic diamines with *p*-isothiocyanatophenyl α-D-mannopyranoside derivative *via* thiourea bonds. Sugar isothiocyanates are useful precursors with sufficient versatility to be used in the preparation of a variety of carbohydrate derivatives and can be synthesized *via* different routes [18].

Thus, reduction of peracetylated *p*-nitrophenyl α-D-mannopyranoside (**1**) [11] by catalytic transfer hydrogenation (10% Pd-C, NH_4HCO_3) (Scheme 1) gave *p*-aminophenyl glycoside **3** in 94% yield [10] which was treated with thiophosgene to provide *p*-isothiocyanatophenyl monomer **4** in 72% yield. This stable intermediate was then self-coupled with its amine precursor **3** to provide divalent ligand **5** in 55% yield. Alternatively, **4** was coupled with different diamines under basic conditions (DIPEA, CH_2Cl_2, pH $\geqslant$ 8.0) to give divalent ligands **7** to **14** in 64 to 99% yields. Standard Zemplén de-*O*-acetylation (NaOMe,

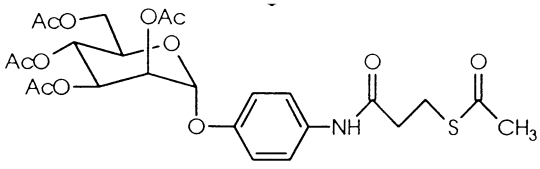


Scheme 2.

MeOH, pH $\geqslant$ 8.5) afforded the corresponding unprotected mannosylated derivatives **6** and **15** to **22** in quantitative yields.

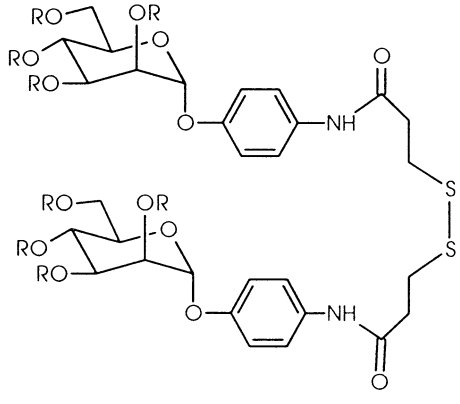
Diamine spacers **25** and **27** (Scheme 2) used for the synthesis of divalent mannosyl ligands **20** and **21** were not commercially available and were thus synthesized. They were prepared to increase the intermannoside distances and the water solubility of the final products relative to the ligands bearing simple alkyl chains as spacer. Formation of 6-(carbobenzyloxyamino)hexanoyl chloride by refluxing the acid in $SOCl_2$, followed by immediate coupling with ethylenediamine in CH_2Cl_2 gave protected spacers **24** and **26** in 60 and 72% yields respectively. Removal of the carbobenzyloxy protecting groups by catalytic hydrogenation (10% Pd-C, H_2) gave compounds **25** and **27** in 90 and 95% yields respectively.

A similar type of ligand with aryl aglycon was also synthesized from the previously reported thioacetate derivative **28** [10] (Scheme 3). Chemoselective de-S-acetylation [19] of **28** was achieved using hydrazinium acetate in DMF to provide a thiol that was readily oxidized to disulfide **29** in 65% yield when let to stir overnight at open atmosphere. De-*O*-acetylated product **30** was also obtained in quantitative yield using the Zemplén conditions described above.



28

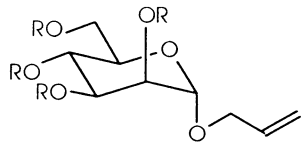
H_2NNH_2 HOAc
DMF, O_2
r.t., overnight



NaOMe, MeOH → **29** R = Ac; **30** R = H

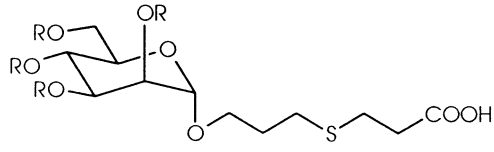
Scheme 3.

The choice for an aromatic aglycon was justified by the fact that the inhibitory property of the known *p*-nitrophenyl α-D-mannopyranoside (2) towards Con A was almost twice that of methyl α-D-mannopyranoside [17]. Therefore, divalent mannosylated ligand **35** bearing a non-aromatic aglycon was also included for comparison purposes. Allyl α-D-mannopyranoside **31** [12] was peracetylated to give **32** in 92% yield using standard procedures (Scheme 4), followed by anti-Markovnikov addition of mercaptopropionic acid



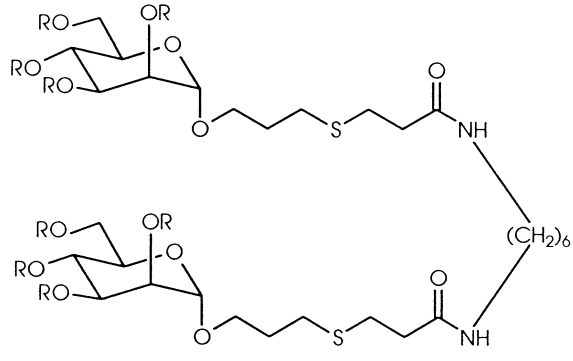
Ac_2O, pyridine → **31** R = H; **32** R = Ac

$HSCH_2CH_2COOH$
CH_3CN, hv, 2 h



33 R = Ac

H_2N-$(CH_2)_6$-NH_2
DIC, HOBt
CH_2Cl_2, r.t., 3 h



NaOMe, MeOH → **34** R = Ac; **35** R = H

Scheme 4.

by photolysis ($h\nu = 254$ nm) to give thiopropanoic acid derivative **33** in 84% yield. Compound **33** was coupled with hexamethylenediamine using standard peptide coupling strategy (DIC, HOBt, CH_2Cl_2) to afford divalent ligand **34** in 73% yield. De-*O*-acetyalated **35** was obtained in quantitative yield using the Zemplén procedure.

Inhibition experiments

The efficiency of each divalent mannosylated ligand to inhibit the binding of yeast mannan to Con A was measured by ELLA tests. Because Con A is known to bind yeast mannan, this naturally occurring polysaccharide was used as coating antigen in microtitre plates and horseradish peroxidase-labelled Con A was used for detection. The concentration of the coating antigen adsorbed on the plate relative to the one of the peroxidase-labelled lectin was adjusted to approximately 80% of the total binding capacity based on standard dilution determinations. Divalent mannosylated ligands **6**, **15** to **22**, **30** and **35** were then added to the lectin-antigen complexes and IC_{50}s were determined.

The results from the inhibition with mannopyranose monomers (D-mannose, methyl α-D-mannopyranoside, **2** and **31**) clearly confirmed that an increase in the hydrophobicity of the aglycon also increased the binding character of the mannoside residue towards the lectin (Table 1). As reported before, the enhanced binding character of *p*-nitrophenyl α-D-mannopyranoside **3** over the other monomers was the direct result of a network of pi-pi interactions between the aryl aglycon and two tyrosine residues found in the binding site region of Con A [20].

The inhibition tests showed that divalent mannosylated ligands were approximately 10- to 90-fold more potent than methyl α-D-mannopyranoside (Table 1). The best divalent mannoside inhibitor was shown to be compound **17** (Figure 1) with an IC_{50} of 10 μM, being almost 90 times more potent then methyl α-D-mannopyranoside (IC_{50} 924 μM) and 10 times more potent than its corresponding aryl mannoside monomer **2** (IC_{50} 106 μM). It also inhibited the binding of Con A to mannan two to five times better than any other tested divalent ligands (Table 1). By taking into consideration that each ligand bears two mannoside residues, the relative inhibitory effect accounts for 44.5-fold increased inhibitory potential relative to that of the methyl α-D-mannoside and 5.3-fold increase compared to *p*-nitrophenyl α-D-mannopyranoside **2**. Surprisingly, this is also 1.3- and 1.2-fold higher than the value that we have previously reported for two glycodendrimers bearing 8 and 16 analogous mannoside residues respectively [10]. These values clearly underline the importance of the geometry and the intramolecular mannoside distance since the glycosyl-aglycon moiety remained constant for the tested compounds.

Con A is a tetramer at physiological pH and possesses one carbohydrate binding site per subunit [13]. This tetrameric arrangement was shown to favour the cross-linking of the lectin with divalent and complex carbohydrate ligands [21]. Unequivocal evidence for this cross-linking phenomena was obtained by turbidimetric analysis of Con A with compound **17**. A drastic increase in optical density accompanied by a distinct precipitate were observed within a few minutes after mixing compound **17** with a solution of

Table 1. Inhibition of binding of yeast mannan to Concanavalin A by divalent α-mannopyranoside ligands.

Compound	*IC_{50} (μM)*	*Relative potency*[a] *to Me α-D-Man*	*Relative potency*[a] *to pNO_2-Ph α-D-Man*
D-Mannose (α, β)	> 2500	–	–
Me α-D-Man	924	1.0	0.1
Allyl α-D-Man (**31**)	261	3.5	0.4
pNO_2-Ph α-D-Man **(2)**	106	8.8	1.0
6	132	7.0 (3.5)	0.8 (0.4)
15	38	24.3 (12.2)	2.8 (1.4)
16	28	33.2 (16.6)	3.8 (1.9)
17	10	88.9 (44.5)	10.6 (5.3)
18	28[b]	33.6 (16.8)	3.8 (1.9)
19	47	19.7 (9.9)	2.3 (1.2)
20	41	22.5 (11.3)	2.6 (1.3)
21	45	20.7 (10.4)	2.4 (1.2)
22	49	18.8 (9.4)	2.2 (1.1)
30	76[b]	12.1 (6.1)	1.4 (0.7)
35	156	5.9 (3.0)	0.7 (0.4)

[a] Values in parentheses are based on a per-mannoside residues.
[b] Compound dissolved in 2% DMSO solution.

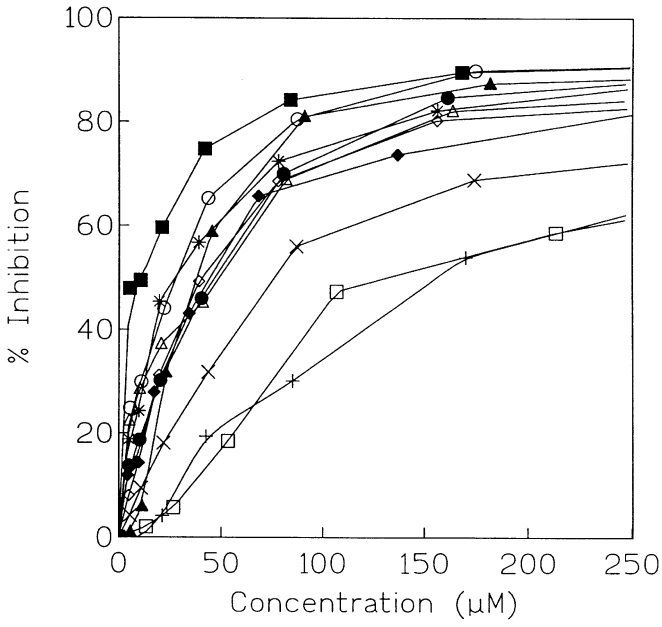


Figure 1. Inhibition of binding of Con A to Yeast Mannan by divalent mannosylated ligands **6** (□), **15** (▲), **16** (*), **17** (■), **18** (○), **19** (●), **20** (◇), **21** (◆), **22** (△), **30** (X), and **35** (+).

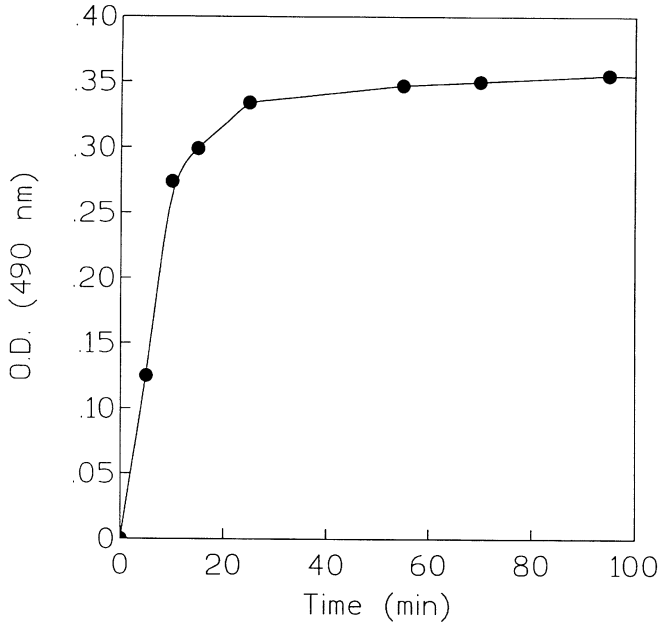


Figure 2. Turbidimetric analysis (micro-quantitative precipitation) of Con A (1.5 mg ml^{-1}) with compound **17** (0.063 mg ml^{-1}). The measurements were done in PBS using an ELISA plate reader ($\lambda = 490$ nm) at 25 °C.

the lectin (Figure 2). The inhibition tests demonstrated that a spacer length of 6 carbon units (or 6 atoms) between the two thiourea bonds gave optimum inhibition (Figure 3). However, the exact structure of the isomer responsible for

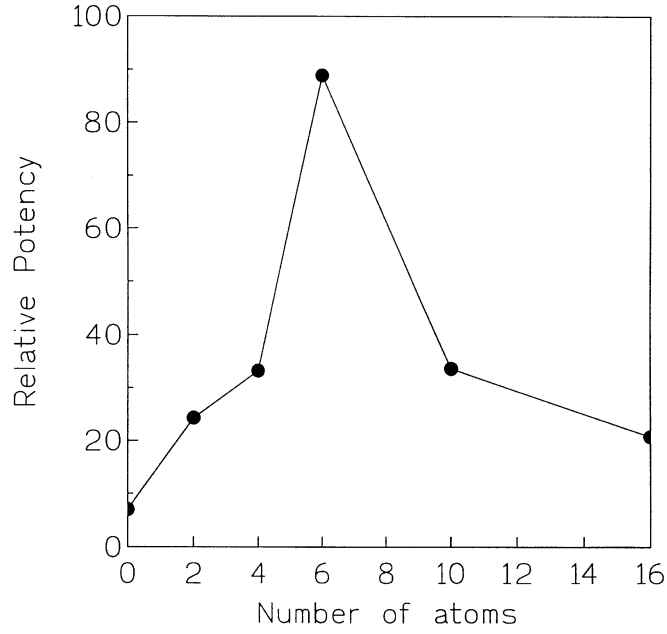


Figure 3. Effect of spacer length on the relative inhibition potency (relative to methyl α-D-mannopyranoside) of divalent mannosylated ligands.

the cross-linking is not yet established since four possible conformations can be adopted at the thiourea bond (E/Z, E/E, Z/Z and Z/E).

Any other divalent ligand carrying a shorter spacer were less potent inhibitors (Figure 3) probably due to steric repulsion between neighbouring lectin molecules. Therefore compound **6**, with the aromatic aglycons only distant by a thiourea bond, is probably only acting as a monomer (IC_{50} 132 μM). But on the opposite, a too long spacer might be too flexible to enable the formation of a stable lattice, reducing the cross-linking efficiency of the ligand. Compound **19** bearing a triethyleneglycol (PEG) spacer was also not a very good inhibitor (IC_{50} 47 μM), probably because of internal hydrogen bonding that would fold the spacer on itself and reduce its actual length. Furthermore, the presence of a spacer that restricts the flexibility of the molecule too much, as observed for compound **22**, seemed to decrease the binding ability of the ligand (IC_{50} 49 μM).

From all the dimers tested bearing aromatic aglycons, compound **30** was the one that demonstrated the lowest potency (IC_{50} 76 μM). The fact that this compound was dissolved in a 2% DMSO solution (due to its lack of solubility) did not seem to be a determining factor in the binding assays since the same procedure was also followed for compound **18** which had an IC_{50} value of 27 μM, the second best after compound **17**. This low value might rather be a reflection of a different conformation adopted in solution due of the presence of a disulfide bond in its spacer instead of a carbon-carbon bond observed in the other ligands.

As observed in the monomer inhibition tests, mannosylated dimer **35**, which does not have an aromatic aglycon, demonstrated low inhibition (IC_{50} 156 μM) compared to any other divalent ligands tested. Monomer **2** was even 1.5-fold more potent then **35**, showing once again the enhancing binding character of aryl aglycons.

Conclusion

A new family of divalent mannosylated ligands with aromatic aglycon were synthesized in relatively good yields from the coupling of readily available diamines with peracetylated *p*-isothiocyanatophenyl α-D-mannopyranoside. Removal of the acetates gave compounds that showed 2.3- to 10.6-fold increases in binding capacities compared to *p*-nitrophenyl α-D-mannopyranoside, which is still high (up to 5.3-fold more potent) when expressed on a molar basis of mannoside residues. The intra-mannoside distance seems to play a dominant role for the efficient binding of these molecules, along with the presence of aromatic aglycons. These small, low-molecular weight molecules might have potential applications spanning from inhibition of bacterial adherence to inhibition of inflammation processes. Preliminary results have also demonstrated strong binding interactions originating from α-D-Man*p*-(1 → 3)-[α-D-Man*p* (1 → 6)]-α-D-Man*p*-OR trisaccharide suggesting that incorporation of this structure to these dimers would create extremely potent ligands. Work is on going in order to reach these goals.

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