Mannose-substituted PPEs detect lectins: A model for Ricin sensing

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Electronic Supplementary Information (ESI)

**Compound 2**: 1,4-Dihydroxy-2,5-diiodobenzene (5.05 g, 14.0 mmol) and 2-[2-(2-chloroethoxy)ethoxy]ethanol (9.44 g, 56.1 mmol) were dissolved in DMF (30 mL). Potassium carbonate (55 g, 0.4 mol) was added to the solution. The reaction mixture was stirred under nitrogen at reflux temperature for 3 d. After the solution was cooled, the solid was filtered off. The solvent was evaporated and the reaction mixture was extracted with chloroform and washed with water. The organic layers were dried over MgSO₄ and evaporated to dryness. The solid was crystallized from acetonitrile and further purified by chromatography on silica gel (ethyl acetate/methanol 97:3). A colorless solid (2) was obtained in 34% yield (2.93 g).

MP: 77-78°C. IR (KBr, cm⁻¹): 3424, 2943, 2886, 1487, 1467, 1355, 1326, 1265, 1240, 1218, 1126, 1117, 1086, 1063, 1031, 884, 858, 835, 798. ¹H NMR (500 MHz, CDCl₃):
δ7.22 (s, 2H, Ar-H), 4.09 (t, 4H, J_H,H = 4.55 Hz), 3.86 (t, 4H, J_H,H = 4.55 Hz), 3.77 (t, 4H, J_H,H = 4.55 Hz), 3.71 (t, 4H, J_H,H = 4.55 Hz), 3.68 (t, 4H, J_H,H = 4.55 Hz), 3.60 (t, 4H, J_H,H = 4.55 Hz).  

13C NMR (300 MHz, CDCl3): δ 152.76, 123.18, 86.28, 72.43, 71.07, 70.38, 70.11, 69.49, 61.64.

**Compound 3**: Compound 2 (6.26 g, 10.0 mmol) and (trimethylsilyl)acetylene (2.45 g, 25.0 mmol) were reacted in diisopropylamine / THF (1:4 v/v) (100 mL) in the presence of CuI (24 mg, 0.13 mmol), Pd(OAc)2 (22 mg, 0.10 mmol) and PPh3 (131 mg, 0.50 mmol). The solution was stirred overnight at room temperature. The mixture was filtered to remove the ammonium salts and the solvent was evaporated in vacuo. The solid residue was dissolved in THF (50 mL) and 1M solution of tetrabutylammonium fluoride in THF (16 mL, 16 mmol) was added. The reaction mixture was stirred for 10 min at room temperature. The solvent was removed in vacuo and the product was isolated on a silica gel column (ethyl acetate/hexane/methanol 20:20:1). A pale yellow solid (3) was obtained in 31% yield (1.30 g).

IR (KBr, cm⁻¹): 3405, 3241, 2944, 2861, 2103, 1716, 1501, 1494, 1455, 1401, 1350, 1273, 1223, 1197, 1134, 1059, 1032, 941, 862.  

1H NMR (300 MHz, CDCl3): δ6.94 (s, 2H, Ar-H), 4.09 (t, 4H, J_H,H = 4.8 Hz), 3.81 (t, 4H, J_H,H = 4.8 Hz), 3.71 (t, 4H, J_H,H = 4.8 Hz), 3.62 (t, 4H, J_H,H = 4.8 Hz), 3.54 (t, 4H, J_H,H = 4.8 Hz), 3.32 (s, 2H).  

13C NMR (300 MHz, CDCl3): δ 153.66, 117.98, 113.28, 82.85, 79.38, 72.40, 70.92, 70.28, 69.43, 69.27, 61.56.  


Found:  C, 60.15; H, 6.85.

**Compound 4**: α-D-Mannose pentaacetate (2.34 g, 6.00 mmol) and compound 2 (1.87 g, 3.00 mmol) were dissolved in anhydrous CH2Cl2 (10 mL). Then BF3-Et2O (2.3 mL, 18 mmol) was added slowly. The reaction mixture was stirred at room temperature for 24 h and then poured into 5% aqueous NaHCO3 (20 mL). The organic layer was separated, washed with 5% aqueous NaHCO3 and water. The organic phase was dried over MgSO4.
and evaporated to dryness. The product was isolated by chromatography on silica gel (ethyl acetate/hexane/methanol 10:10:1) and 1.80 g of the product was obtained as an oil (47 % yield).

IR (cm\(^{-1}\)): 2933, 2877, 1748, 1481, 1368, 1346, 1224, 1135, 1084, 1048, 979, 935. 
\(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\)7.19 (s, 2H, Ar-H), 5.31 (q, 2H), 5.26-5.21(m, 4H), 4.83(s, 2H), 4.24(q, 2H), 4.06(m, 8H), 3.84(t, 4H), 3.73(t, 6H), 3.64(t, 10H), 2.10(s, 6H), 2.05(s, 6H), 1.98(s, 6H), 1.94(s, 6H). \(^{13}\)C NMR (400 MHz, CDCl\(_3\)): \(\delta\)170.53, 169.87, 169.74, 169.58, 152.92, 123.22, 97.56, 86.23, 71.01, 70.72, 70.12, 69.91, 69.48, 69.43, 68.93, 68.23, 67.27, 65.99, 62.26, 20.79, 20.66, 20.61, 20.59. Anal. Calcd for C\(_{46}\)H\(_{64}\)I\(_2\)O\(_{26}\): C, 42.94; H, 5.01. Found: C, 42.94; H, 4.96.

**Polymerization of Mannose-PPE (5):** Compound 4 (286 mg, 0.320 mmol) and compound 3 (141 mg, 0.335 mmol) were dissolved in a mixture of piperidine (0.5 mL), THF (0.5 mL), and methanol (0.5 mL) in a Schlenk flask with a flow of nitrogen and with magnetic stirring. (Ph\(_3\)P)\(_2\)PdCl\(_2\) (5 mg, 7 µmol) and CuI (1 mg, 6 µmol) were added to the flask. The reaction mixture was stirred under nitrogen at 40 °C for 16 h. The solution was slowly added to acetone (300 mL). The precipitate was washed with cold water. An orange solid (5) was obtained in 95 % yield (340 mg). The number average molecular weight was measured to be 61,640 with a polydispersity (M\(_w\)/M\(_n\)) of 1.50 (eluent : DMF).
IR (KBr, cm\(^{-1}\)): 3446, 2937, 2871, 2204, 1732, 1510, 1421, 1221, 1054, 883. \(^1\)H NMR (500 MHz, DMSO-d\(_6\)): \(\delta\) 7.30, 7.19, 4.71, 4.70, 4.63, 4.56, 4.43, 4.23, 3.83, 3.68, 3.55, 3.47, 3.41, 3.19. \(^{13}\)C NMR (400 MHz, DMSO-d\(_6\)): \(\delta\) 152.99, 117.31, 99.95, 73.89, 72.36, 70.92, 70.26, 70.17, 70.09, 69.85, 69.51, 69.01, 66.92, 65.66, 61.23, 60.20. Anal. Calcd for (C\(_{52}\)H\(_{76}\)O\(_{26}\))\(_n\): C, 55.91; H, 6.86. Found: C, 55.95; H, 6.71.

**Compounds 6**: Compound 4 (447 mg, 0.500 mmol) and \(p\)-methoxyphenylacetylene (145 mg, 1.10 mmol) were dissolved in a mixture of piperidine (2 mL), THF (2 mL), and methanol (2 mL) in a Schlenk flask with a flow of nitrogen and with magnetic stirring. (Ph\(_3\)P)\(_2\)PdCl\(_2\) (7 mg, 10 \(\mu\)mol) and Cul (2 mg, 10 \(\mu\)mol) was added to the flask. The reaction mixture was stirred under nitrogen at 40 °C for 16 h and the solvent was removed in vacuo. Water (20 mL) was added to the flask. The precipitate was collected, washed with water and an ethyl acetate – hexane mixture (1:3). A pale yellow oil (6) was obtained in 65 % yield (310 mg).

IR (cm\(^{-1}\))): 3369, 2928, 1604, 1515, 1410, 1247, 1218, 1132, 1058, 1026, 832. \(^1\)H NMR (400 MHz, DMSO-d\(_6\)): \(\delta\) 7.46 (d, 4H, Ar-H), 7.13 (s, 2H, Ar-H), 6.99 (d, 4H, Ar-H), 4.70 (m, 4H), 4.61 (s, 2H), 4.55 (d, 2H), 4.21 (t, 2H), 4.16 (t, 4H), 3.80 (m, 10H), 3.68 – 3.36. \(^{13}\)C NMR (400 MHz, DMSO-d\(_6\)): \(\delta\) 159.61, 152.91, 132.82, 116.58, 114.50, 114.46, 113.23, 99.98, 94.97, 84.85, 73.92, 70.96, 70.29, 70.22, 69.94, 69.53, 69.10, 69.00, 66.96, 65.69, 61.27, 55.32, 54.92.
Optical Data for the Reaction of 5 with ConA.

Mannosylated PPE with Con A

Figure 1. Quenching of 5 with ConA at pH = 7.2 (0.01M phosphate buffer), 0.1mM CaCl₂, and 0.1mM MnCl₂

Figure 2. Stern Volmer plot of the experiment shown in Figure 1.
Figure 3. Quenching of 5 with bovine serum albumine (BSA). Only a very slight deaggregation effect is visible.

Figure 4. Stern Volmer plot of the experiment shown in Figure 3.
Figure 5. Quenching of 5 with biotin-labeled mannose and addition of streptavidin-coated polystyrene spheres.
Figure 6. Quenching of 5 with Lectin from *Artocarpus integrifolia* (Jacalin).

Figure 7. Stern Volmer plots of the experiments shown in Figure 1 and Figure 6.
Preparation of spherical aggregates of Con A with 5:

A 10 mL of stock solution was prepared with 10 mg/mL of Con A in phosphate buffer with pH of 7.2. Serial dilutions were made according to the chart below where each sequential dilution of Con A was a factor of 10 less than the previous dilution. The volume of each solution was 10 mL. 10 mg of polymer 5 was dissolved in 10 mL of DMSO. 100 µL of the solution of polymer 5 was added to each dilution to yield a polymer concentration of ~10 µg/mL. The solutions were allowed to incubate for 48 h at ambient temperature in capped vials after which the solutions were examined for aggregates or agglutination. Solutions 1 and 2 were found to form fluorescent precipitates which had settled to the bottom. Solution 2 was utilized as the aggregates were less dense and more easily prepared for imaging. Using a pipette, the 1 mL was drawn from the bottom of the solutions. A few drops were placed onto parafilm which provided a hydrophobic working surface for TEM grid preparation. TEM grids (SPI Supplies, Formvar Carbon, 400 mesh Cu grid) were dragged through the droplets and allowed to air dry while protected from dust and air-born particulate. The TEM grids were viewed utilizing a Hitachi H-8000 TEM. Images were acquired digitally and are unaltered.

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<th>Solution 2</th>
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