Supplementary Information for:

Colorimetric Detection of *Ricinus communis* Agglutinin 120 using Optimally Presented Carbohydrate Stabilised Gold Nanoparticles

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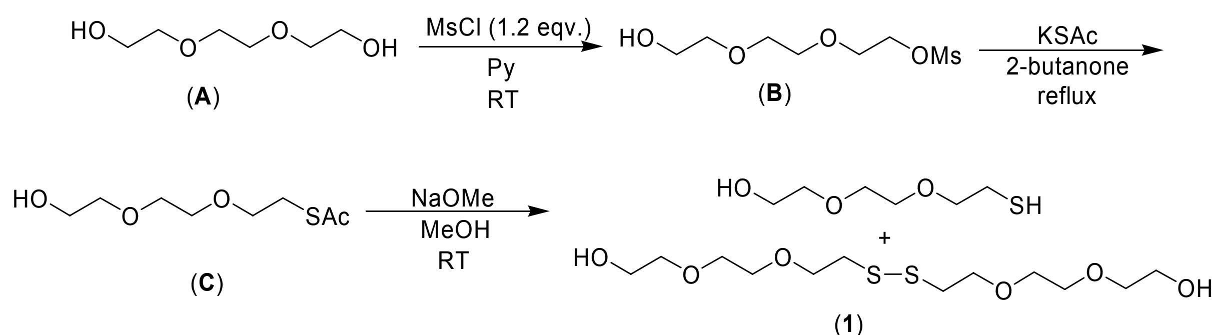
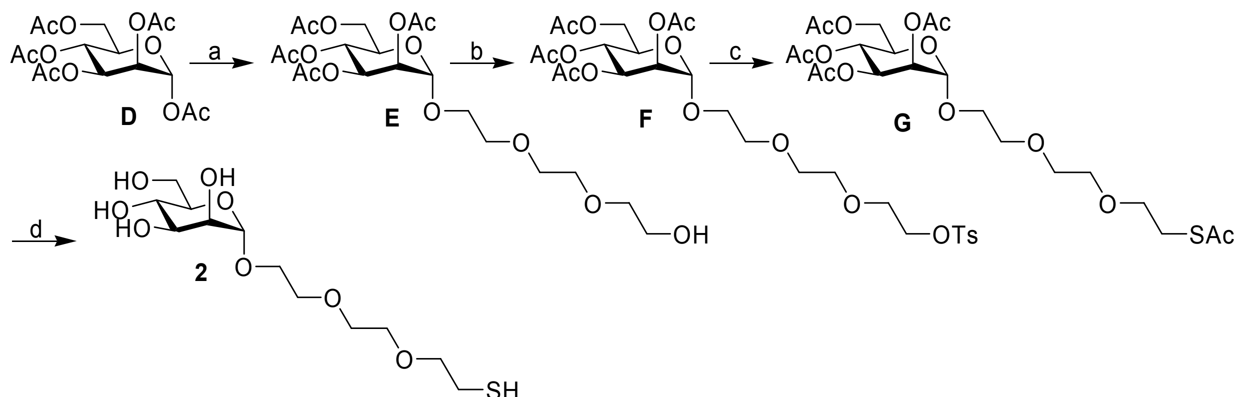
Synthesis of Thiolated Triethylene Glycol (1):



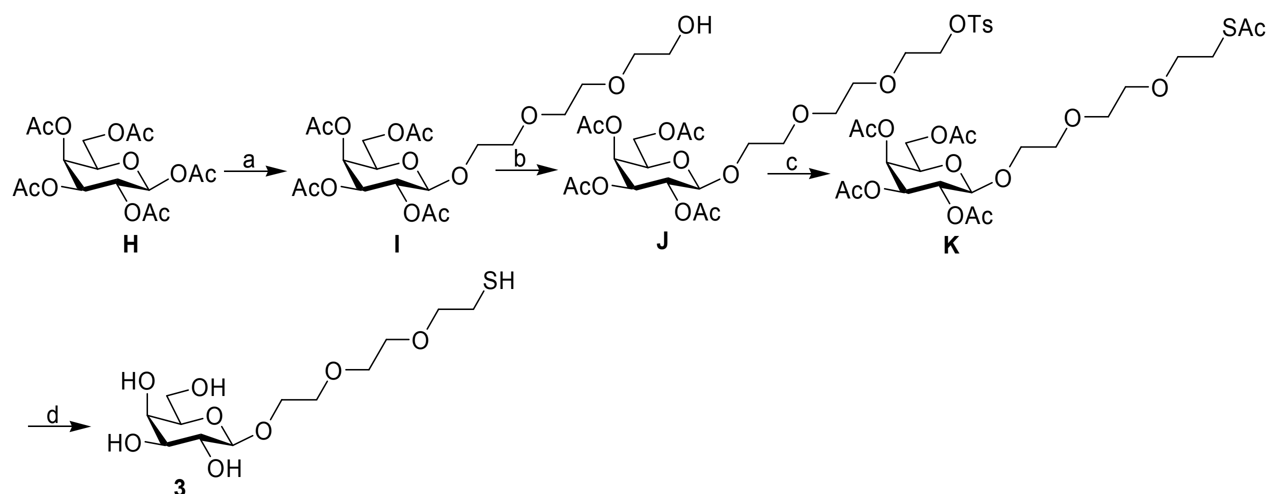
A mono-mesylate of triethylene glycol was synthesised followed by a nucleophilic substitution with potassium thioacetate. Deacetylation was carried out to obtain the desired trigol thiol linker. At the last stage, a mixture of thiol and disulfide was obtained. However, as both of these products react with the gold nanoparticles in a similar fashion there is no need for separation.

Synthesis of the carbohydrate derivatives:



Known per-*O*-acetylated α-D-mannopyranoside (**D**) was converted to the corresponding triethylene glycoside (**E**) by the reaction with triethylene glycol in the presence of BF_3Et_2O in 68% isolated yield. The terminal OH group was then tosylated by usual method using tosyl chloride in pyridine to afford the tosyl derivative **F**. Next, substitution of the tosyl group with thioacetate was achieved by refluxing compound **F** in 2-butanone in the presence of potassium thioacetate to furnish compound **G** in 82% overall yield. The resulting thioacetate derivative (**G**) was de-*O*-acetylated using NaOMe in methanol to afford the target compound **2** in 88% yield.

The corresponding galactose derivative (**3**) was prepared by following exactly the same experimental protocol.



Experimental section:

General Methods

All reagents and solvents were dried prior to use according to standard methods.[1] Commercial reagents were used without further purification unless otherwise stated. Analytical TLC was performed on silica gel 60-F_{254} (Merck or Whatman) with detection by fluorescence and/or by charring following immersion in a 10% ethanolic solution of sulfuric acid. Flash chromatography was performed with silica gel 230-400 mesh (Qualigens, India). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance spectrometer at 300 and 75 MHz, respectively, using Me_4Si as internal standards, as appropriate. NMR characterisation data are represented using peak-multiplicity abbreviations as s-singlet, d-doublet, t-triplet, q-quartet and m-multiplet. Coupling constants are given in Hertz (Hz). Low-resolution MS and HRMS were obtained using ESI ionisation.

(1) Perrin, D. D.; Amarego, W. L.; Perrin, D. R. *Purification of Laboratory Chemicals*; Pergamon: London, 1996.

Compound E. To a solution of compound **D** (3.0 g, 7.7 mmol) and triethylene glycol (1.1 mL, 8.5 mmol) in dry CH_2Cl_2 (30 mL) was added BF_3Et_2O (1.9 mL, 15.4 mmol) and the resulting solution was allowed to stir at room temperature for 5 hours when the starting material was completely consumed (TLC). The solution was diluted with CH_2Cl_2 (20 mL) and washed successively with H_2O (2×50 mL), $NaHCO_3$ (2×50 mL) and brine (50 mL). The organic layer was separated, dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by flash chromatography using 1:1 *n*-hexane-EtOAc as eluent to afford pure compound **E** (2.5 g, 68%) as a colourless syrup. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.33 (dd, 1H, *J* 6.6 Hz, 9.9 Hz), 5.25 (m, 2H), 4.86 (d, 1H, *J* 1.5 Hz), 4.26 (dd, 1H, *J* 5.1 Hz, 12.3 Hz), 4.09 (dd, 1H, *J* 2.4 Hz, 12.3 Hz), 4.05 (m, 1H), 3.80 (m, 1H), 3.72-3.57 (m, 10H), 2.14 (s, 3H), 2.08 (s, 3H), 2.03 (s, 3H), 1.97 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 170.6, 170.0, 169.9, 169.6, 97.6, 72.5, 70.6, 70.2, 69.9, 69.4, 69.0, 68.3, 67.2, 66.0, 62.3, 61.6, 20.8, 20.6, 20.5(2). HRMS calcd. for $C_{20}H_{36}O_{13}N$ $[M+NH_4]^+$: 498.2187, found 498.2183.

Compound G. To a solution of compound **E** (2 g, 4.2 mmol) in pyridine (15 mL) was added *p*-TsCl (955 mg, 5 mmol) and the mixture was allowed to stir at room temperature for 3 hours when TLC showed complete conversion of the starting material. The solvents were evaporated and co-evaporated with toluene. The resulting syrup (**F**) was dissolved in 2-butanone (40 mL) followed by addition of KSAc (960 mg, 8.4 mmol) and the mixture was stirred under reflux for 2 hours. The solvents were evaporated, the residue was dissolved in CH_2Cl_2 (30 mL) and washed with H_2O (2×50 mL) and brine (50 mL). The organic layer was separated, dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by flash chromatography using 2:1 *n*-hexane-EtOAc as eluent to afford pure compound **G** (1.8 g, 82%) as a yellow syrup. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.26 (dd, 1H, *J* 6.9 Hz, 9.9 Hz), 5.20 (m, 2H), 4.81 (d, 1H, *J* 1.2 Hz), 4.23 (dd, 1H, *J* 5.1 Hz, 12.3 Hz), 4.06-

3.98 (m, 2H), 3.74 (m, 1H), 3.65-3.52 (m, 8H), 3.03 (t, 2H, *J* 6.3 Hz), 2.27 (s, 3H), 2.09 (s, 3H), 2.04 (s, 3H), 1.98 (s, 3H), 1.93 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 195.2, 170.4, 169.8, 169.7, 169.5, 97.5, 70.4, 70.1, 69.8, 69.5, 69.3, 68.9, 68.2, 67.2, 65.9, 62.2, 30.3, 28.6, 20.7, 20.5, 20.4(2). HRMS calcd. for $C_{22}H_{38}O_{13}NS$ $[M+NH_4]^+$: 556.2064, found 556.2061.

Compound 2. To a solution of compound **G** (1.5 g, 2.8 mmol) in MeOH (15 mL), NaOMe (0.5M in MeOH, 1 mL) was added and the solution was stirred at room temperature for 2 hours. The solution was neutralized with DOWEX 50W H^+ resin, filtered and evaporated *in vacuo* to afford the target compound **2** (800 mg, 88%) as a white amorphous solid. ^{1}H NMR (300 MHz, D_2O) δ: 4.83 (d, 1H, *J* 1.5 Hz), 3.91 (dd, 1H, *J* 1.5 Hz, 3.0 Hz), 3.85-3.76 (m, 3H), 3.75-3.60 (m, 10H), 2.91 (t, 1H, *J* 6.0 Hz), 2.69 (t, 1H, *J* 6.0 Hz). ^{13}C NMR (D_2O, 75 MHz) δ: 101.2, 74.0, 73.5, 71.8, 71.3, 70.9, 70.8, 70.7, 70.5, 69.7, 68.0, 67.7, 62.2, 38.6, 24.4. HRMS calcd. for $C_{12}H_{24}O_8SNa$ $[M+Na]^+$: 351.1090, found 351.1087.

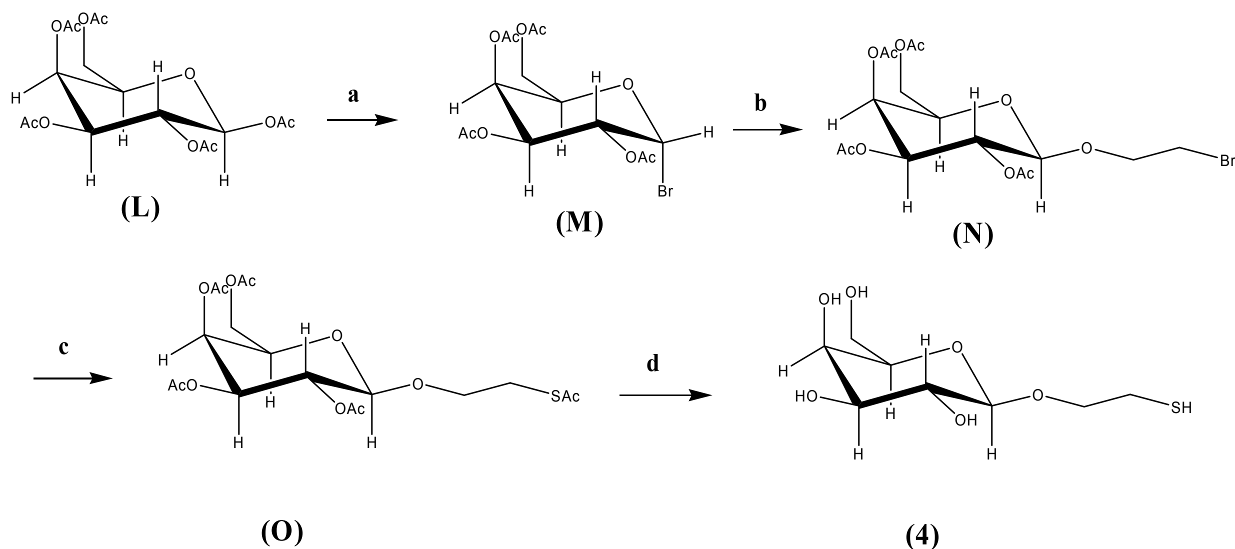
For galactose derivatives (**H** to **K** to **3**), the experimental protocol was the same as above.

Compound I. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.38 (m, 1H), 5.19 (dd, 1H, *J* 7.8 Hz, 8.7 Hz), 5.06 (dd, 1H, 2.1 Hz, 8.7 Hz), 4.62 (d, 1H, *J* 7.8 Hz), 4.17 (m, 1H), 3.98 (m, 2H), 3.79 (m, 1H), 3.69-3.53 (m, 10H), 2.11 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 1.98 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 170.5, 170.3, 170.1, 169.8, 101.4, 70.9, 70.6, 70.4, 70.2, 70.0, 69.8, 68.7, 68.5, 67.1, 66.1, 62.3, 61.5, 20.8, 20.6, 20.4(2). HRMS calcd. for $C_{20}H_{36}O_{13}N$ $[M+NH_4]^+$: 498.2187, found 498.2182.

Compound J. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.36 (m, 1H), 5.17 (dd, 1H, *J* 7.5 Hz, 8.4 Hz), 5.01 (dd, 1H, 2.1 Hz, 8.4 Hz), 4.56 (d, 1H, *J* 7.5 Hz), 4.13 (m, 1H), 3.92 (m, 2H), 3.74 (m, 1H), 3.64-3.56 (m, 8H), 3.07 (t, 1H, *J* 6.6 Hz), 2.32 (s, 3H), 2.13 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 1.96 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 195.4, 170.3, 170.2, 170.1, 169.4, 101.2, 70.8, 70.5(2), 70.3, 70.2, 69.7, 68.9, 68.7, 67.0, 61.2, 30.5, 28.7, 20.7, 20.6, 20.5(2). HRMS calcd. for $C_{22}H_{38}O_{13}NS$ $[M+NH_4]^+$: 556.2064, found 556.2060.

Compound 3. ^{1}H NMR (300 MHz, D_2O) δ: 4.37 (d, 1H, *J* 7.8 Hz), 4.02 (m, 2H), 3.85 (m, 1H), 3.78 (m, 1H), 3.76-3.55 (m, 10H), 3.51 (dd, 1H, *J* 7.8 Hz, 8.4 Hz), 2.68 (t, 1H, *J* 6.0 Hz). ^{13}C NMR (D_2O, 75 MHz) δ: 101.6, 73.9, 71.5, 70.9, 69.5, 68.5, 68.3, 67.9, 67.4, 59.7, 21.8. HRMS calcd. for $C_{12}H_{24}O_8SNa$ $[M+Na]^+$: 351.1090, found 351.1086.

Synthesis of 2-mercaptoethyl-β-D-galactopyranoside (4)



Reagents **a** CH_3CO_2H, HBr/CH_3CO_2H, **b** $Hg(CN)_2/HgBr_2$, $HO\text{-}CH_2\text{-}CH_2\text{-}Br$, **c** K^+SAc, **d** NaOMe/MeOH, Dowex H^+.

Synthesis of Bromo 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (M)

A solution of β-D-galactose pentaacetate **(L)** (3.01 g, 7.71 mmol) dissolved in acetic acid (15 mL) with stirring, was placed in an ice bath to reduce fuming whilst a HBr/acetic acid solution (15 mL) was added drop wise. The ice bath was removed after 5 min and the resulting yellow mixture was stirred for 30 min. Dichloromethane (100 mL) was added to the mixture, the organic layer was separated, washed initially with ice water (3 x 30 mL) and then washed a further three times with a saturated aqueous solution of sodium hydrogen carbonate. The mixture was then dried over anhydrous sodium sulphate, filtered and evaporated *in vacuo* until the title compound was obtained as a pale yellow foam (2.02 g, 64 %0). M.p. 115 °C. $[\alpha]^{25}$ D = +1 (*c* 1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 6.67 (1H, d, $J_{1,2}$ 3.9 Hz, H-1), 5.48 (1H, bd, $J_{3,4}$ 3.1 Hz, H-4), 5.36 (1H, dd, $J_{1,2}$ 10.6 Hz, $J_{3,4}$, H-3), 5.01 (1H, dd, $J_{1,2}$, $J_{2,3}$, H-2), 4.44 (1H, t, J 6.6 Hz, H-5), 4.15 (1H, dd, $J_{5,6a}$ 6.3 Hz, $J_{6a,6b}$ 11.3 Hz, H-6a), 4.07 (1H, dd, $J_{6a,6b}$, $J_{5,6b}$ 6.8 Hz, H-6b) 2.12, 2.07, 2.02, 1.97 (12H, 4xs, $4COCH_3$); δ_C (75 MHz, $CDCl_3$) 170.4, 170.2, 170.0, 169.8 ($4\underline{C}OCH_3$), 88.1 (C_1), 71.0, 67.9, 67.7, 66.9 (4CH), 60.8 (C_6), 20.6, 20.5, 20.4, 20.3 ($4COCH_3$).

Synthesis of 2-bromoethyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (N)

The following manipulation was carried out under a nitrogen atmosphere. Bromo 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside **(M)** (2.02 g, 4.91 mmol) was dissolved upon the addition of dichloromethane (15 mL). This mixture was transferred *via* cannulae to a round-bottomed flask containing oven-dried 4 Å molecular sieves (1.5 g). The glassware that had contained **(M)** was rinsed thoroughly with dichloromethane (5 mL), the washing was also transferred to the solution containing the molecular sieves and the mixture was left to stir. 2-bromoethanol (0.68 mL, 9.90 μmol) was added to the mixture *via* a syringe through the suba seal. $Hg(CN)_2$ (1.36 g, 5.38 mmol) and $HgBr_2$ (0.18 g, 4.99 mmol) were quickly added to the mixture. The nitrogen source was removed and the white reaction mixture was left stirring overnight. Potassium bromide (24.21 g) was dissolved with stirring in distilled water (200 mL) to make a 1 M solution. Dichloromethane (100 mL) was added to the bromide mixture; the organic layer was separated, washed with the

aqueous potassium bromide solution (3 x 30 mL) and washed again with a saturated aqueous solution of sodium hydrogen carbonate (3 x 30 mL). The organic phase was dried over magnesium sulphate, filtered and evaporated *in vacuo*. Purification by column chromatography over silica gel (eluting with 1:1 hexane/ethyl acetate) followed by evaporation and crystallization with hexane gave the title compound **(N)** (1.34 g, 60 %) as white crystals. M.p. 98 °C (ethyl acetate), $[\alpha]^{25}$ D = +1 (*c* 1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 5.32 (1H, bd, $J_{3,4}$ 3.2 Hz, H-4), 5.14 (1H, dd, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 10.4 Hz, H-2), 4.95 (1H, dd, $J_{2,3}$, $J_{3,4}$, H-3), 4.47 (1H, d, $J_{1,2}$, H-1), 4.09 (4H, m, O-C$\underline{H}_2$-CH_2.Br, H-5, H-6a), 3.86 (1H,t, H-6b), 3.76 (1H, m, O-CH_2-C$\underline{H}_2$-Br), 2.08, 2.01, 1.98, 1.91 (12H, 4xs, 4$COCH_3$); δ_C (75 MHz, $CDCl_3$) 170.5, 170.4, 170.3, 169.7 (4$\underline{C}OCH_3$), 101.6 (C-1), 70.9, 70.8, 69.9, 68.7, 68.2, 67.1, 66.9, 61.4, 30.2 (O-$\underline{C}H_2$-CH_2-Br), 21.0, 20.9, 20.8, 20.7 (4CO$\underline{C}H_3$).

Synthesis of 2-acetylthioethyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (O)

A mixture of compound **(N)** (0.92 g, 2.0 mmol), butanone (10 mL) and potassium thioacetate (1.15 g, 10.0 mmol) was refluxed for 2 h and underwent a colour change from orange to red/brown. On completion of the reaction the mixture was left to cool, filtered, washed with acetone and evaporated *in vacuo* which required heating. Purification by column chromatography over silica gel (eluting with 7:3 ethyl acetate/hexane) and further evaporation *in vacuo* yielded the title compound **(O)** (0.88 g, 98 %) as a pale orange coloured foam. M.p. 113 °C (dichloromethane). $[\alpha]^{25}$ D = +5 (*c* 1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 5.22 (1H bd, $J_{3,4}$ 3.6 Hz, H-4), 5.01 (1H, dd, $J_{1,2}$ 8.4 Hz, $J_{2,3}$ 10.4 Hz, H-2), 4.86 (1h, dd, $J_{2,3}$, $J_{3,4}$, H-3), 4.37 (1H, d, $J_{1,2}$, H-1), 3.98 (2H, m, H-6a, H-6b), 3.85 (2H, m, H-5, O-C$\underline{H}_2$-CH_2-SAc), 2.18 (3H, s, SCOC$\underline{H}_3$), 1.99, 1.93, 1.88, 1.82 (12H, 4xs, 4COC$\underline{H}_3$); δ_C (75 MHz, $CDCl_3$) 195.1 (S$\underline{C}OCH_3$), 170.3, 170.2, 170.0, 169.4 (4$\underline{C}OCH_3$), 101.2 (C-1), 70.6, 70.5, 68.5, 68.4, 66.9 (C-6), 61.1 (O-CH_2-$\underline{C}H_2$-SAc), 30.3 (O-$\underline{C}H_2$-CH_2-SAc), 28.6 (SCO$\underline{C}H_3$), 20.5, 20.4 (x2), 20.3 (4CO$\underline{C}H_3$); *m/z* (ES) HRMS: Found: 468.1533. $C_{18}H_{26}O_{11}S$ ($M+NH_4^+$) Requires 468.1534.

Synthesis of 2-mercaptoethyl-β-D-galactopyranoside (4)

Compound **(O)** (0.23 g, 0.51 mmol) was dissolved in dry methanol (2.3 mL). Sodium methanolate (0.5 mL, 0.2 M in MeOH) was added and the mixture stirred at room temperature until the reaction was complete as verified by thin layer chromatography. The mixture was initially dark red and on completion of the reaction was pale orange in colour. Dowex H^+ (ion exchange resin), dried over a Buchner funnel using methanol, was added to the basic mixture until the pH was approximately 7 as verified by Universal Indicator paper. The mixture was filtered and evaporated *in vacuo* giving the title compound **(4)** as a cream coloured foam, obtained as the disulfide. $[\alpha]^{25}{}_D = -7$ (*c* 1, $CHCl_3$); υ_{MAX} (KBr disc)/cm^{-1} 3386 (O-H stretching), 2882 (CH_2 stretch), 1297 (CH_2 twisting) 1079 (C-O); δ_H (400 MHz, D_2O) 4.43 (1H, d, $J_{1,2}$ 7.6 Hz, H-1), 4.17 (1H, m O-C$\underline{H}_2$-CH_2-SH) , 3.96 (1H, m, O-C$\underline{H}_2$-CH_2-SH), 3.91 (1H, bd, $J_{3,4}$ 3.6 Hz, H-4), 3.81-3.72 (2H, m, H-6a, H-6b), 3.68 (1H, dd, $J_{2,3}$ 9.6 Hz, $J_{3,4}$, H-3), 3.51 (1H, dd, $J_{1,2}$, $J_{2,3}$, H-2), 3.01 (2H, m, O-CH_2-C$\underline{H}_2$-SH); δ_C (75 MHz, D_2O) 102.5 (C-1), 74.6, 71.9, 69.9, 67.8, 67.5 (O-CH_2-$\underline{C}H_2$-SH), 60.1 (C-6), 36.4 (O-$\underline{C}H_2$-CH_2-SH); *m/z* (ES) HRMS; Found: 501.1068. $C_{16}H_{30}O_{12}S_2$ ($M+Na^+$) Requires 501.1071.

Addition of Proteins to the "Protein Resistant" Monolayer Stabilised Nanoparticles.

Results obtained from the addition of RCA_{120} (0.02 – 0.8 μM) to the triethylene glycol (**1**) - stabilised gold nanoparticles can be seen in Figure 1. Although there is a slight increase in absorbance on addition of the lectin, this result can be attributed to the turbidity of the RCA_{120} solution. The surface plasmon absorption band does not shift or broaden, signifying that RCA_{120} does not cause non-specific aggregation of the particles.

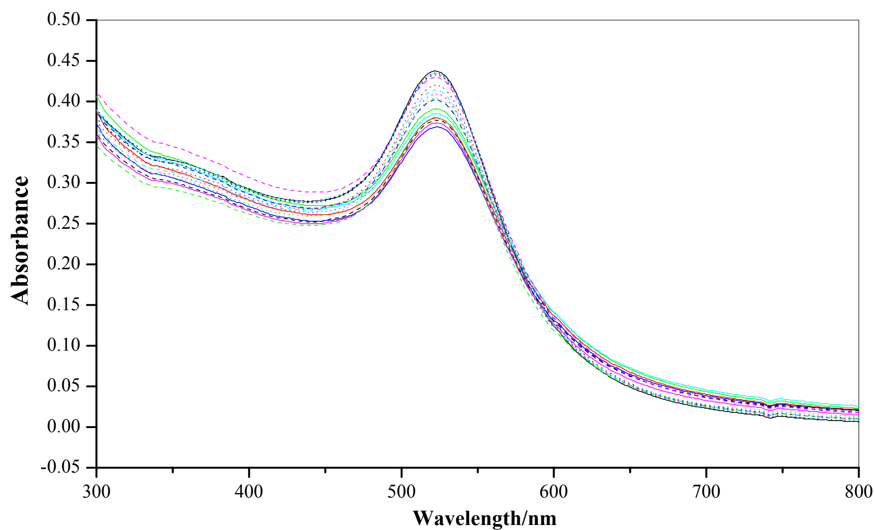


Figure 1 Addition of RCA_{120} to triethylene glycol stabilised gold nanoparticles: (—) 0 μM; (—) 0.8 μM; (—) 0.7 μM; (—) 0.6 μM; (—) 0.5 μM; (—) 0.4 μM; (---) 0.3 μM; (---) 0.25 μM; (---) 0.2 μM; (---) 0.15 μM; (---) 0.1 μM; (---) 0.09 μM; (·····) 0.08 μM; (·····) 0.07 μM; (·····) 0.06 μM; (·····) 0.03 μM; (·····) 0.02 μM; (·····) 0.01 μM.

Addition of Con A (0.7 – 2.4 μM) to the PEGylated nanoparticles can be seen in Figure 2. Again no significant red-shift in the surface plasmon absorption band was observed.

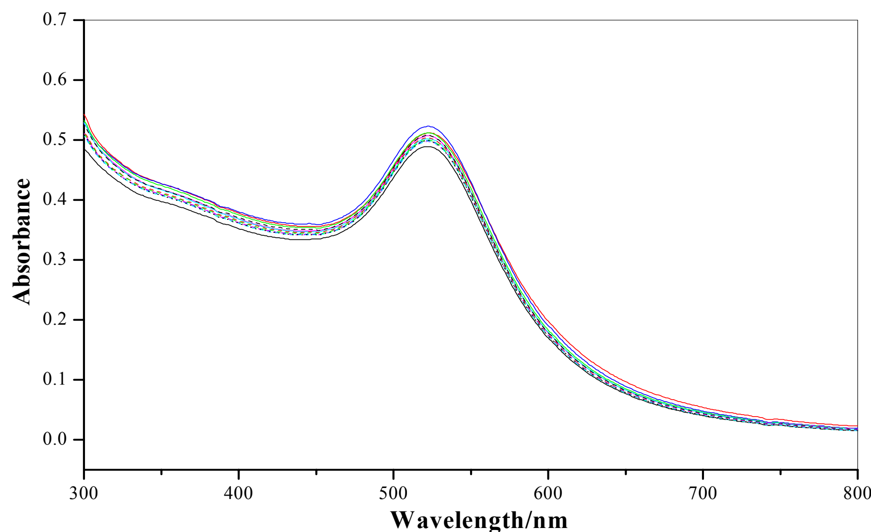


Figure 2 Addition of Con A to triethylene glycol stabilised gold nanoparticles: (—) 0 μM; (—) 2.4 μM; (—) 2.2 μM; (—) 2.0 μM; (—) 1.8 μM; (—) 1.6 μM; (---) 1.3 μM; (---) 1.2 μM; (---) 1.1 μM; (---) 1.0 μM; (---) 0.9 μM; (---) 0.8 μM; (·····) 0.7 μM.

Figure 3 shows the UV-visible absorption spectra obtained upon addition of varying concentrations of BSA (0.7 – 2.4 μM) to the pegylated nanoparticles. It can be seen that there is no significant change in absorbance, indicating that nanoparticle aggregation has not occurred and suggesting that non-specific protein adsorption does not occur with the BSA protein.

It can be concluded that the thiolated triethylene glycol derivate renders the gold nanoparticles inert to non-specific adsorption of protein. This result is important for the ligand density experiments. Using triethylene glycol to dilute the carbohydrate on the nanoparticles ensures that aggregation only occurs due to the specific recognition between the sugar and its cognate lectin.

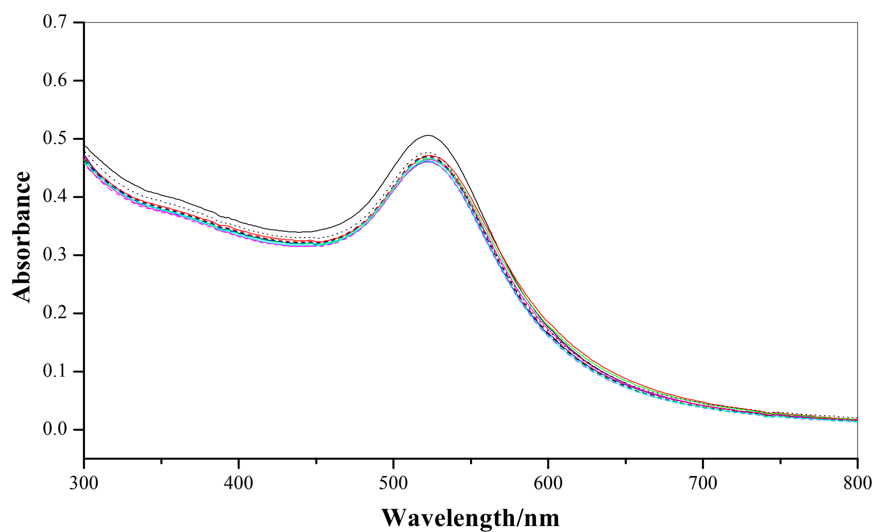


Figure 3. Addition of BSA to triethylene glycol stabilised gold nanoparticles: (—) 0 μM; (—) 2.4 μM; (—) 2.2 μM; (—) 2.0 μM; (—) 1.8 μM; (—) 1.6 μM; (---) 1.3 μM; (---) 1.2 μM; (---) 1.1 μM; (---) 1.0 μM; (---) 0.9 μM; (---) 0.8 μM; (·····) 0.7 μM.