

**Supporting Information for**

**Dynamic Glycovesicle Systems for Amplified QCM Detection of  
Carbohydrate-Lectin Multivalent Biorecognition**

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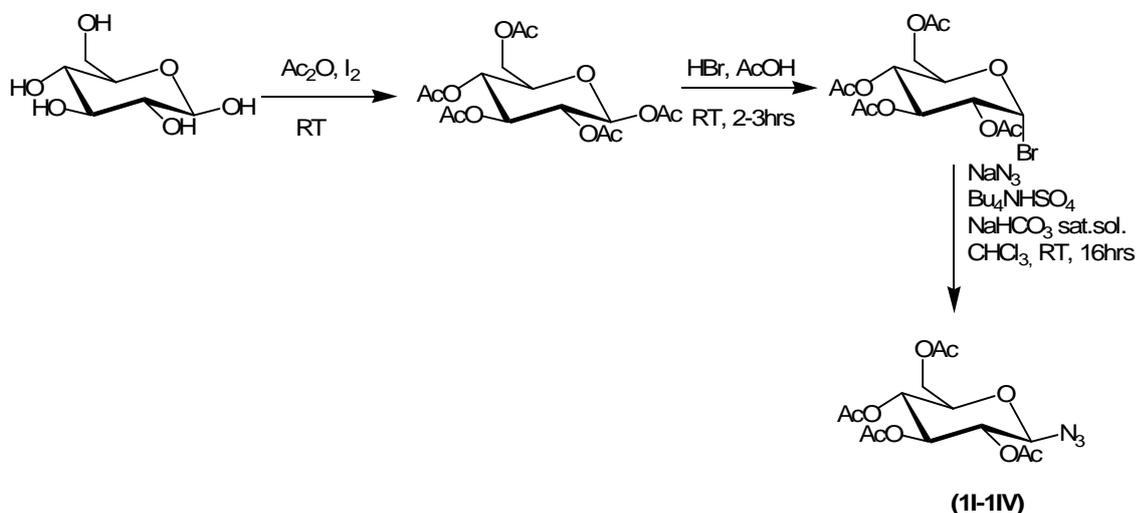
**Materials and Methods:** All of the materials used were obtained from commercial sources from either Sigma-Aldrich or Fluka and used without further purification. Analytical TLC was performed on silica gel 60-F<sub>254</sub> (Whatman) with detection by immersion in 5% methanolic H<sub>2</sub>SO<sub>4</sub> followed by charring or by fluorescence. Column chromatography was performed with silica gel 60 A C.C 70-200  $\mu$ m.

<sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on an ARX 300 MHz Bruker spectrometer in CDCl<sub>3</sub> and CD<sub>3</sub>CN with the use of the residual solvent peak as reference. Mass spectrometric studies were performed in the positive ion mode using a quadrupole mass spectrometer (Micromass, Platform II). Samples were continuously introduced into the mass spectrometer through a Waters 616HPLC pump. The temperature (60°C) and the extraction cone voltage (V<sub>c</sub>=5-10V) were usually set to avoid fragmentations.

QCM measurements were performed using the A100 instrument from Attana Biosensors. The experiments were run in the continuous-flow QCM system at a flow rate of 20  $\mu$ L/min. The experiments were performed in a Tris buffer at pH 7.4.

**Glycoligands Preparation:** Alkyl glycosides were prepared to act as synthetic glycolipids which would then be partitioned in phospholipid vesicles. QCM was then used to investigate the lectin recognition properties of these biomimetic assemblies. The alkyl glycosides were prepared using “click chemistry” i.e. a Huisgen-type azide-alkyne cycloaddition to give a 1,4-di-substituted 1,2,3-triazole unit<sup>1-4S</sup>.

**General Preparation Procedure for Glycosyl Azides:** The glycosyl azides were prepared from the glycosyl bromides in a two-phase reaction and characterised by NMR showing agreement with literature.<sup>5S</sup>

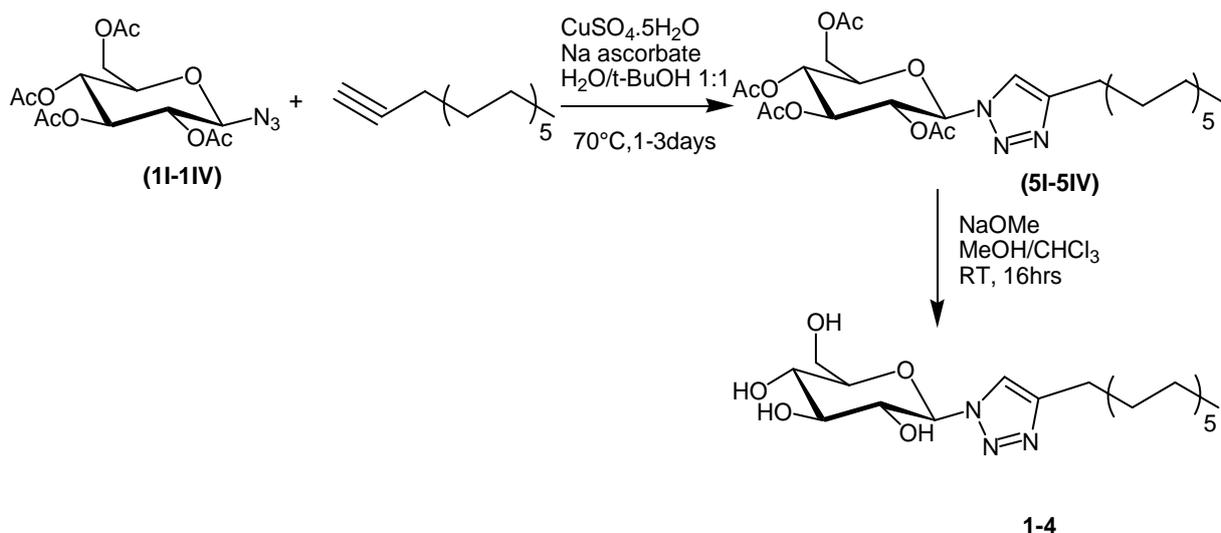


**Fig 1. 1-Azido sugar peracetate synthesis.**

The glycosyl bromides were prepared as follows:

To 2 gr of sugar peracetate in glacial acetic acid (10 ml) stirring under inert conditions was added HBr (33% in acetic acid) (5 ml) dropwise. The mixture was stirred at room temperature for another 3 hours before being diluted with dichloromethane (40 ml) and poured into ice-water. The layers were separated and further washing with sat. NaHCO<sub>3</sub> (3x20 ml) was followed by drying over sodium sulphate and evaporation of the solvent to yield yellow syrup. This syrup was used directly in the next step, phase transfer azidation. The crude glycosyl bromides were weighed before being dissolved in chloroform (20 ml). A saturated NaHCO<sub>3</sub> solution (20 ml) was then added to the stirring chloroformic solution followed by NaN<sub>3</sub> (5 eq.) and the phase transfer catalyst tetrabutylammonium hydrogen sulphate (1 eq.). This two phase mixture was stirred at room temperature overnight. The work-up was by washing of the chloroformic phase with water x 1, sat. NaHCO<sub>3</sub> x1, followed by a brine wash. After drying over sodium sulphate the solvent was then evaporated of to yield white solids which could be recrystallised from methanol to yield anomerically pure azides which were characterised with reference to the literature.

**Coupling and Deprotection to give Alkylglycosides:** Similar to a reported procedure<sup>6S</sup> a suspension of CuSO<sub>4</sub>.5H<sub>2</sub>O (0.2 eq.) and Na ascorbate (0.4 eq.) dissolved in H<sub>2</sub>O (5ml) was added to a stirring suspension of glycosyl azide (1 eq. 500 mg) and 1-Tetradecyne (1 eq.) in t-butanol (5 ml). The cloudy mixture was heated to 70°C and stirred at this temperature for 24-72 hours. On cooling slowly to 4°C the product precipitated from solution and could be filtered off.



**Fig. 2** Alkyl glycoside by “click chemistry”.

The peracetylated products were then deprotected using the classic sodium methoxide in MeOH method (1 eq.) stirring at room temperature for 16 hours. Chloroform was used as a co-solvent where necessary for complete solvation. Acidic ion-exchange resin (Amberlite IR-120) was then added and at neutralisation was filtered off and washed with MeOH. The solvent was then evaporated off to afford the products **1-4**. They were further purified by crystallisation from CHCl<sub>3</sub>/MeOH.

*1-Azido-1-deoxy-β-D-glucopyranoside tetraacetate (1I)*

<sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>, 25°C): δ=1.99 (s, 3H), 2.022 (s, 3H), 2.068 (s, 3H), 2.092 (s, 3H), 3.75 (m, 1H), 4.15 (dd, 1H, *J*=12.5, 2.4 Hz), 4.26 (dd, 1H, *J*=12.5, 4.8 Hz), 4.63 (d, 1H, *J*=8.7 Hz), 4.95 (t, 1H, *J*=9.6 Hz), 5.093 (t, 1H, *J*=9.6 Hz), 5.212 (t, 1H, *J*=9.6 Hz). <sup>13</sup>C NMR (300MHz, CDCl<sub>3</sub>, 25°C): δ=20.5, 20.5, 20.5, 20.7, 61.6, 67.8, 70.6, 72.5, 73.6, 87.9, 169.2, 169.3, 170.1, 170.6.

*1-Azido-1-deoxy-α-D-mannopyranoside tetraacetate (1II).*

<sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>, 25°C): δ= 1.988 (s, 3H), 2.052 (s, 3H), 2.107 (s, 3H), 2.202 (s, 3H), 3.75 (m, 1H), 4.19 (dd, 1H, *J*=12.3, 2.7 Hz), 4.28 (dd, 1H, *J*=12.3, 5.4 Hz), 4.73 (d, 1H, *J*=1.2 Hz), 5.03 (dd, 1H, *J*=9.9, 3.3 Hz), 5.26 (t, 1H, *J*=9.9), 5.4 (dd, *J*=3.3, 1.2 Hz). <sup>13</sup>C NMR (300MHz, CDCl<sub>3</sub>, 25°C): δ=20.47, 20.55, 20.58, 20.68, 62.11, 65.62, 68.20, 69.13, 70.62, 87.41, 169.52, 169.61, 169.72, 170.45.

*1-Azido-1-deoxy-β-D-galactopyranoside tetraacetate (1III)*

$^1\text{H}$  NMR (300MHz,  $\text{CDCl}_3$ ,  $25^\circ\text{C}$ )  $\delta$  = 1.98 (s, 3H), 2.05 (s, 3H); 2.08(s, 3H), 2.16 (s, 3H,  $\text{CH}_3$ ), 4.00 (t, 1H,  $J=6.6$  Hz); 4.14-4.17 (m, 2H,) 4.59 (d, 1H,  $J = 8.7$  Hz ), 5.02 (dd, 1H,  $J =10.3, 3.3$  Hz), 5.15 (dd, 1H,  $J = 10.4, 8.7$  Hz), 5.41 (d,  $J = 3.3$  Hz, 1H).  $^{13}\text{C}$  NMR (300MHz,  $\text{CDCl}_3$ ,  $25^\circ\text{C}$ )  $\delta$  =20.65, 20.74, 20.78, 20.80, 61.3, 66.9, 68.1, 70.8, 72.9 , 88.4, 169.5,170.1, 170.3, 170.5.

*1-Azido-1-deoxy- $\beta$ -D-Maltopyranoside heptaacetate (1IV)*

$^1\text{H}$ NMR (300MHz,  $\text{CDCl}_3$ ,  $25^\circ\text{C}$ ):  $\delta$ =1.99(s, 3H), 2.009(s, 3H), 2.024(s, 3H), 2.039(s, 3H), 2.048(s, 3H), 2.1(s, 3H), 2.152(t, 3H), 3.78(m, 1H), 4.0(m, 2H), 4.25 (m, 2H), 4.5 (dd, 1H,  $J=12.3, 2.4$  Hz) 4.7 (d, 1H,  $J=8.4$  Hz), 4.78 (t, 1H,  $J=8.6$  Hz), 4.85 (dd, 1H,  $J=9.9, 3.6$  Hz), 5.05 (t, 1H,  $J=9.9$  Hz), 5.25 (t, 1H,  $J=8.6$  Hz), 5.29 (s, 1H), 5.35 (t, 1H,  $J=10.2$  Hz), 5.4 (d, 1H,  $J=3.9$  Hz).  $^{13}\text{C}$  NMR (300MHz,  $\text{CDCl}_3$ ,  $25^\circ\text{C}$ )  $\delta$ =20.5, 20.5, 20.6, 20.6, 20.7, 20.8, 20.8, 60.4, 62.5, 67.9, 68.6, 69.2, 70.0, 71.4, 72.3, 74.2, 75.1, 87.5, 95.7, 169.4, 169.5, 169.9, 170.1, 170.4, 170.5, 170.5.

*1H-1,2,3-Triazole,4-dodecyl-1-(2,3,4,6-tetra-O-acetyl- $\beta$ -D-glucopyranosyl)-(5I)*

$^1\text{H}$  NMR (300MHz,  $\text{CDCl}_3$ ,  $25^\circ\text{C}$ ):  $\delta$ =0.87(t, 3H,  $J=6.6\text{Hz}$ ), 1.255(m, 18H), 1.67 (t, 2H,  $J=7.2\text{Hz}$ ), 1.877 (s, 3H), 2.007 (s, 3H), 2.043 (s, 3H), 2.219 (s, 3H), 2.714 (t, 2H,  $J=7.8\text{Hz}$ ), 4.2 (m, 3H), 5.22 (dd, 1H,  $J =10.3, 3.3$  Hz), 5.55 (m, 2H), 5.8 (d, 1H,  $J = 9.3$  Hz ), 7.55 (s,1H).

*1H-1,2,3-Triazole,4-dodecyl-1-(2,3,4,6-tetra-O-acetyl- $\alpha$ -D-mannopyranosyl)-(5II)*

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz):  $\delta$ =0.87 (t, 1H,  $J=6.3\text{Hz}$ ), 1.25 (m 22H), 2.0 (s, 3H), 2.09 (m, 12H), 3.95 (m, 1H), 4.19 (dd, 1H,  $J=12.6, 2.4$  Hz), 4.33 (dd, 1H,  $J=12.6, 6$  Hz), 5.26 (dd, 1H,  $J=9.9, 3.3$  Hz), 5.35 (t, 1H,  $J=9.9\text{Hz}$ ), 5.7 (dd,  $J=3.3, 1.5$  Hz). 6.1 (d, 1H,  $J=1.2$  Hz) 7.48 (s, 1H).

*1H-1,2,3-Triazole,4-dodecyl-1-(2,3,4,6-tetra-O-acetyl- $\beta$ -D-galactopyranosyl)-(5III)*

$^1\text{H}$  NMR (300MHz,  $\text{CDCl}_3$ ,  $25^\circ\text{C}$ ):  $\delta$ =0.87(t, 3H,  $J=6.6\text{Hz}$ ), 1.255(m, 18H), 1.67 (t, 2H,  $J=7.2\text{Hz}$ ), 1.877 (s, 3H), 2.007 (s, 3H), 2.043 (s, 3H), 2.219 (s, 3H), 2.714 (t, 2H,  $J=7.8\text{Hz}$ ), 4.2 (m, 3H), 5.22 (dd, 1H,  $J=10.3, 3.3$  Hz), 5.55 (m, 2H), 5.8 (d, 1H,  $J= 9.3$  Hz ), 7.55 (s,1H).

*1H-1,2,3-Triazole,4-dodecyl-1-(2,3,4,6,8,9,10,12-hepta-O-acetyl-β-D-maltopyranosyl)*  
**(5IV)**

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, 25°C) □: δ=7.423 (s, 1H), 5.845 (d, 1H, *J*=9.3 Hz), 5.45–5.40 (m, 2 H) 5.34 (d, 1 H), 5.05 (dd, 1H, *J*= 10.3, 7.8 Hz), 4.87 (dd, 1 H, *J*=10.4, 3.4 Hz,) 4.47 (dd, 1 H, *J*=12.4, 2.4 Hz), 4.25 (m, 2 H), 4.0–3.93 (m, 3H), 2.67 (t, 2H, *J*=7.8 Hz), 2.11 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 2.02 (s, 3H), 2.014 (s, 3H), 1.99 (s, 3H), 1.82 (s, 3H), 1.63 (t, 2H, *J*=7.4 Hz), 1.24 (m, 18H), 0.86 (t, 3H, *J*=6.6 Hz).

*1H-1,2,3-Triazole- 4-dodecyl-1-β-D-glucopyranosyl-(1)*

<sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD) □: δ=0.81 (t, 3H, *J*=6.7 Hz), 1.2 (m, 18H), 1.57 (t, 2H, *J*=7.2 Hz), 2.63 (t, 2H, *J*=7.8 Hz), 3.48 (dd, 1H, *J*=9, 9 Hz), 3.56 (m, 2H), 3.71 (dd, 1H, *J*=12.0, 5.0 Hz), 3.85 (m, 1H), 5.244 (s, 1H), 5.37 (d, 1H, *J*=9.0 Hz), 7.53 (s, 1H). <sup>13</sup>C NMR (300MHz, CD<sub>3</sub>OD, 25°C) δ=14.4, 23.7, 149.26, 26.31, 30.3, 30.45, 30.48, 30.5, 30.66, 30.73, 30.75, 33.05, 62.42, 70.94, 73.99, 78.6, 81.12, 89.5, 122.34. ES-MS calcd. for C<sub>20</sub>H<sub>36</sub>N<sub>3</sub>O<sub>5</sub>Na [M+Na]<sup>+</sup>: 421.513, found 421.4.

*1H-1,2,3-Triazole- 4-dodecyl-1-α-D-mannopyranosyl-(2)*

<sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD) □: δ=0.82 (t, 3H, *J*=6.7 Hz), 1.22 (m, 18H), 1.6 (t, 2H, *J*=7.2 Hz), 2.65 (t, 2H, *J*=7.8 Hz), 3.32 (t, 1H, *J*=1.5 Hz), 3.45 (m, 1H), 3.65 (dd, 1H, *J*=9.6, 2.1 Hz), 3.73-3.9 (m, 2H), 4.17 (d, 1H, *J*=2.1 Hz), 5.76 (s, 1H), 7.85 (s, 1H). <sup>13</sup>C NMR (300MHz, CDCl<sub>3</sub>, 25°C) δ=14.5, 23.4, 26.1, 30.1, 30.14, 30.18, 30.4, 32.7, 62.0, 67.2, 71.7, 74.4, 80.7, 87.6, 122.6. ES-MS calcd. for C<sub>20</sub>H<sub>36</sub>N<sub>3</sub>O<sub>5</sub>Na [M+Na]<sup>+</sup>: 421.513, found 421.3.

*1H-1,2,3-Triazole- 4-dodecyl-1-β-D-galactopyranosyl-(3)*

<sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD) □: δ=0.89 (t, 3H, *J*=6.7 Hz), 1.3 (m, 18H), 1.68 (t, 2H, *J*=7.2 Hz), 2.7 (t, 2H, *J*=7.8 Hz), 3.68 (dd, 1H, *J*=9.6, 3.3 Hz), 3.74-3.9 (m, 3H), 3.98 (d, 1H, *J*=3.3 Hz), 4.12 (t, 1H, *J*=9.3 Hz), 5.5 (d, 1H, *J*=9.0 Hz), 7.96 (s, 1H). <sup>13</sup>C NMR (300MHz, CD<sub>3</sub>OD, 25°C) δ=14.448 (CH<sub>3</sub>), 23.755 (all CH<sub>2</sub>), 26.392, 30.310, 30.494, 30.540, 30.708, 30.776, 30.803, 33.098, 49.050, 62.518 (C-6), 70.485, 71.487, 75.443, 79.979, 90.224 (C-1), 121.859, 149.448. ES-MS calcd. for C<sub>20</sub>H<sub>36</sub>N<sub>3</sub>O<sub>5</sub>Na [M+Na]<sup>+</sup>: 421.513, found 421.2.

*1H-1,2,3-Triazole- 4-dodecyl-1-β-D-maltopyranosyl-(4)*

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, 25°C): δ=0.82 (t, 3H, J=6.6 Hz), 1.22 (m, 18H), 1.68 (t, 2H, J=7.2 Hz), 2.71 (t, 2H, J=7.2 Hz) 3.3 (t, 1H, J=9.3 Hz), 3.5 (dd, 1H, J=9.3 Hz), 3.7(m, 4H), 3.85 (m, 3H), 3.95 (t, 1H, J=9Hz), 5.25 (d, 1H, J=9 Hz), 5.6 (d, 1H, J=3.6 Hz), 7.95(s, 1H).  
<sup>13</sup>C NMR (300MHz, CD<sub>3</sub>OD, 25°C) δ=14.39, 23.38, 25.92, 29.80, 29.98, 30.02, 30.06, 30.20, 30.27, 32.63, 62.09, 70.90, 73.36, 73.48, 74.31, 74.53, 77.93, 78.76, 79.02, 88.74, 101.87, 122.73, 149.56. ES-MS calcd. for C<sub>26</sub>H<sub>46</sub>N<sub>3</sub>O<sub>10</sub>Na [M+Na]<sup>+</sup>: 583.655, found 583.6

### **In Situ preparation of Concanavalin A layer on QCM electrode**

Gold coated quartz crystals were rendered hydrophobic by immersion in piranha solution (H<sub>2</sub>SO<sub>4</sub>/H<sub>2</sub>O<sub>2</sub> 7:3, V:V, 30 s, r.t.), then extensive rinsing with deionized water and ethanol was followed by immersion in ethanolic solutions of octadecanethiol (**ODT**) (1 mM, 16 hrs followed by rinsing with hexane and ethanol). The crystals were then blown dry with a stream of N<sub>2</sub> and placed in the QCM chip setup. Polysaccharide Mannan adsorbed on polystyrene was demonstrated as an effective Con A immobilisation procedure in experiments by Pei et al.<sup>8S</sup> In an extension of this procedure an octadecanethiol (ODT) monolayer was saturated with the polysaccharide giving a stable film through repeated injections at a low concentration of Mannan (50 µg/ml). Following this Mannan-ODT film deposition, passing of buffered Con A solutions at 1 µM concentration resulted in the formation of dense lectin layers which left to stabilise with time (20-30 minutes). The Mannan-ODT film could be regenerated with multiple injections of buffer at pH 1.5.

### **Equilibrium Affinity Constant Calculation**

The affinity was calculated following the Langmuir Isotherm.<sup>9S</sup>

$$\theta = \frac{K_{eq}[C]}{1 + K_{eq}[C]}$$

In terms of QCM measurements  $\theta$  the fractional occupancy can be approximated by  $\Delta F / \Delta F_{max}$  giving

$$\frac{\Delta F}{\Delta F_{max}} = \frac{K_{eq}[C]}{1 + K_{eq}[C]}$$

rearranged :

$$\frac{1}{\Delta F} = \frac{1}{\Delta F_{\max} K_{eq} [C]} + \frac{1}{\Delta F_{\max}}$$

A plot of  $1/\Delta F$  against  $1/\Delta C$  can thus give access to the equilibrium association constant by fitting of a straight line.

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