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Carbohydrate–protein interactions at interfaces: synthesis of thiolactosyl glycolipids and design of a working model for surface plasmon resonance

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Received 29th October 2002, Accepted 7th February 2003
First published as an Advance Article on the web 24th February 2003

Thiolactosyl lipids designed for carbohydrate-protein binding studies have been synthesised. One representative was selected for binding studies with a plant lectin RCA_{120}, the agglutinin from *Ricinus communis*. The interactions were measured quantitatively in real time using a BIAcore surface plasmon resonance instrument. Removal of much of the galactose from the thiolactosyl lipid *in situ* with β-galactosidase showed that the lectin binding was highly specific. A dissociation constant $K_D = 8.77 \times 10^{-8}$ M was measured for 1-{2-[2-(2-[β-D-galactopyranosyl-(1 → 4)-1-thio-β-D-glucopyranosyl]ethoxy)ethoxy]ethoxy}octadecane **30** which is four orders of magnitude greater than that determined for binding to lactose in solution. A concentration of lactose of >80 mM was required to block the lectin binding to thiolactosyl lipid in a neomembrane.

Introduction

A primary event in many biological processes involved in cell–cell recognition/adhesion is the specific attachment of biological polymers (normally proteins) to glycolipids or glycoproteins frequently located in cell membranes.[1,2]

The specific recognition of saccharides by lectins has been the subject of many studies despite the fact that the function of many lectins (particularly those from plants) is still unknown.[3,4]

Many factors determine whether specific molecular recognition can occur at an interface, and whether the amount of binding is sufficient to trigger a relevant biological cascade such as mast cell degranulation, cell division, or differentiation.

The binding of saccharides to lectins in solution in a 1:1 complex, although specific, reveals fairly low binding constants of less than 10^4 M^{-1}.[2] However, when the same saccharides are present in liposomes or when they are immobilised at an interface in a way that makes possible significant interaction, binding constants of 10^6 M^{-1} or higher are observed.[1,5] Part of the explanation for this difference in the observed binding constant is the fact that at an interface it is possible to form clusters of ligands, provided that the ligand molecules are not rigidly bound by very short covalent linkers to the surface. Such clustering makes possible multivalent associations with proteins that have more than one binding site. The strength of binding is thereby increased significantly.[6]

Cooperative effects can, in principle, occur in a polyvalent interaction because the free energy of the interaction between a ligand and a lectin may be positive, neutral or negative compared with the free energy of monovalent interaction between a ligand and a lectin. The term cooperativity was introduced into the biochemical literature to explain the behaviour of multi-subunit or multi-chain enzyme assemblies, particularly allosteric enzymes. Alteration in the conformation of one chain resulting from the binding of substrate, allosteric activator or inhibitor may change the conformation of a neighbouring chain and so influence the binding of a second molecule of substrate. The cooperativity effect can be seen as a sigmoidal plot of rate against substrate concentration for positive cooperativity and a reduced hyperbolic plot for negative cooperativity. Such plots compare with the standard hyperbolic plot to V_{max} expected from the Michaelis–Menten equation. Whilst the concept of cooperativity has been applied to a number of specific molecular interactions in chemistry it is difficult to quantitate for polyvalent systems unless a quantitative comparison of polyvalent and monovalent interactions can be made. In most polyvalent systems, the number of ligand–receptor interactions is unknown and therefore the magnitude of the cooperativity factor α cannot be calculated.[5] The cooperativity term modifies the valency term of the equation quantifying the interaction. However, examples of such cooperative effects are rare.[5]

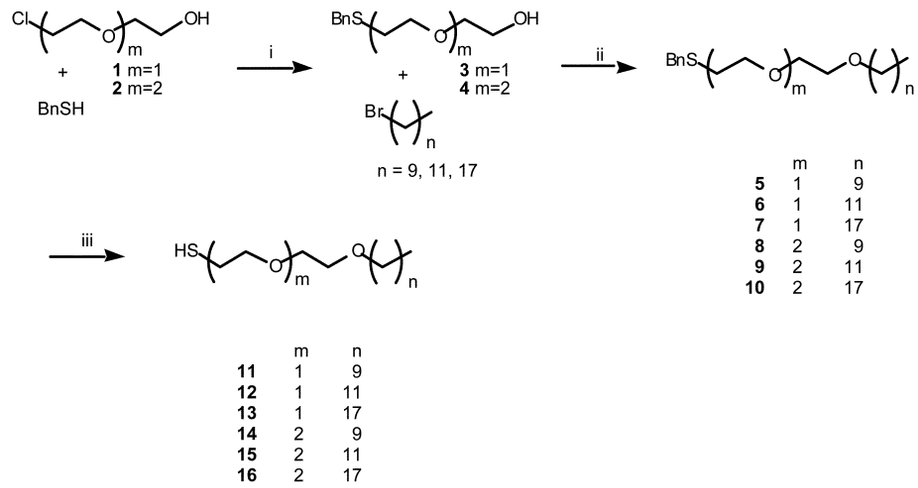
A further complication when examining binding events at interfaces is that non-specific binding of proteins occurs at apolar sites.[7] Recent work has shown that the introduction of polyethyleneglycol (PEG) derivatives or other polar molecules at an interface can significantly reduce the non-specific binding and promote specific interactions.[8]

The presentation of a ligand held at an interface can influence the strength of interaction with an appropriate lectin on a scale from 0–100%.[9] Rigid conformations are likely to militate against ligand–lectin interactions and result in enthalpically diminished binding. While the concept is easy to describe it is difficult to quantitate. Examples of antibody binding to antigens are usually monovalent although potentially the two arms of the antibody have potential for bivalent binding. Entropies of translation, rotation and solvation all play a role. Ligands held at surfaces have already sacrificed some degrees of freedom that would have contributed to the free energy of interaction in solution. At an interface one is dealing essentially with two-dimensional chemistry and the entropic cost of association is less than for the interaction of the same species diffusing in three dimensions.

There are many unexplained examples in the biological literature of the effect of different lipid structures, that are either part of a glycolipid or are part of the phospholipid microenvironment, changing the degree of interaction between the glycoside (bound to the lipid) and a lectin[10,11] or enzyme.[12]

Described below are syntheses of model compounds designed for use in experiments to investigate some of the factors that may control the specific molecular interactions between carbohydrates immobilised at a neomembrane interface and proteins in solution. Surface plasmon resonance (SPR) was used to follow binding events at a synthetic membrane using a BIAcore 2000 instrument. A recent review describes many advantages of using SPR to measure the interaction between saccharide ligands presented in a membrane environment and protein analytes.[13] The experiments described below focused on neoglycolipid **30** (see Scheme 2 later). Experiments with the other glycolipids prepared will be described elsewhere.

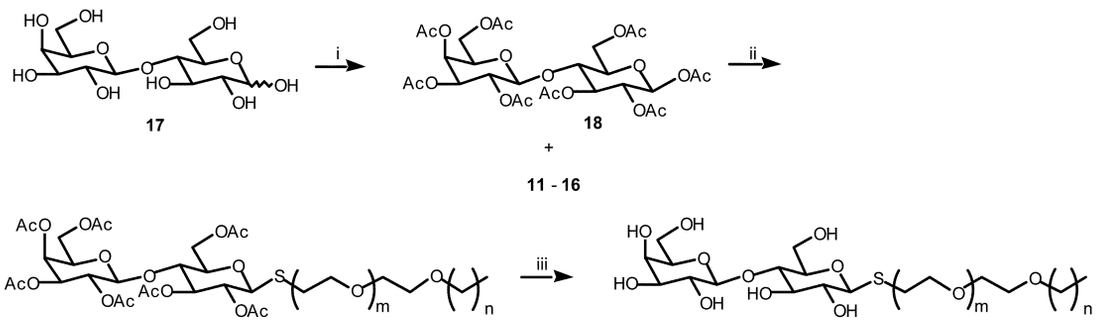
DOI: 10.1039/b210672h



	m	n
5	1	9
6	1	11
7	1	17
8	2	9
9	2	11
10	2	17

	m	n
11	1	9
12	1	11
13	1	17
14	2	9
15	2	11
16	2	17

Scheme 1 Reagents and conditions: i, MeONa, MeOH; ii, NaOH, Bu_4NHSO_4, NaI; iii, Na–NH_3.



	m	n
19	1	9
20	1	11
21	1	17
22	2	9
23	2	11
24	2	17

	m	n
25	1	9
26	1	11
27	1	17
28	2	9
29	2	11
30	2	17

Scheme 2 Reagents and conditions: i, NaOAc, Ac_2O; ii, BF_3OEt_2; iii, NaOMe, MeOH.

Results and discussion

A series of novel thiolactosyl glycolipids were synthesised with PEG linkers between the disaccharide and an alkyl chain. Alkyl chains of various lengths were incorporated in order to determine the effect of chain length on the behaviour of the neoglycolipid in artificial membranes. The PEG linkers were introduced to study the effect of PEG chain length on recognition of the carbohydrate by lectins. It was expected that the nature of the PEG linker would affect the way in which the carbohydrate was presented on the surface of the membrane and hence the interaction with lectins. Lactose was chosen because it is readily available, it is a common core of glycolipids, and it binds readily to lectins such as the 120 kDa agglutinin from *Ricinus communis* that recognizes β-linked galactosyl residues. It was intended to use enzymatic reactions to change the ligand on the surface of the chip. The thiolactosyl linkage was selected because it is more resistant to hydrolysis by glycosidases than O-linked glycoside and therefore resistant to adventitious glycosidases in the enzyme preparation to be used to modify the chip surface. A number of thiolactosyl derivatives were synthesised with different degrees of hydrophilic character in the spacer group between the thiolactosyl residue and the hydrocarbon chain. A similar series of compounds have been synthesized with a normal O-glycosidic linkage.[14]

Chemical synthesis

The synthesis of the linkers was carried out as shown in Scheme 1. In the first stage α,ω-bifunctional PEG dimer **1** and trimer **2** with a monochloro end-group were converted into the corresponding benzyl thiol derivatives **3** and **4** respectively.[15] Further reaction with C_{10}, C_{12} and C_{18} 1-bromoalkanes in the presence alkali, sodium iodide and a phase transfer catalyst gave the corresponding alkylated compounds **5**–**7** and **8**–**10** respectively.[16] Debenzylation with sodium and liquid ammonia liberated the thiol group for condensation with octaacetyllactose **18** prepared from lactose **17** by a standard procedure (Scheme 2).[17] The condensation reaction was promoted by boron trifluoride etherate in dichloromethane to yield the protected glycolipids **19**–**24**.[18] Treatment of each of the peracetylated compounds with sodium methoxide/methanol gave the neoglycolipids **25**–**30** which were purified separately by flash chromatography on silica (Scheme 2).

Biological results

It was planned to attach the thioglycolipid on the surface of the SPR chip to avoid the potential difficulty of aggregation of the glycolipid in solution which would have made the interpretation of data (that requires an accurate concentration term for the analyte) very difficult. It is also known that the best results with the BIAcore system are obtained with analytes of more than 10 kDa molecular weight. Earlier work showed that glycopeptides could be attached to a CM5 sensor chip (that has a surface of oxidised dextran) by amide links[19] and also to the surface of a chip coated with streptavidin by forming a biotinylated-hydrazide of the saccharides that then bound to the streptavidin.[20] Both of these methods fix the ligands tightly at the surface of a sensor chip preventing diffusion and therefore

multivalent binding. In the planned experiments, lateral diffusion within the lipid layer would be possible which would make possible the formation of lipid rafts. Such phase separation would be encouraged by using alkyl chain lengths in the neoglycolipid different from those of the components of the membrane.[21,22] In the experiments reported here this condition was approached by incorporating the neoglycolipid in a dipalmitoylphosphatidylcholine neomembrane. A similar approach was used to follow the binding of glycopeptide antibiotics such as vancomycin to synthetic lipopeptides held on an HPA chip surface.[23]

The absorption of phospholipid and phospholipid/glycolipid mixtures onto the alkane thiol surface of an HPA sensor chip was accomplished by flowing a suspension of lipid, homogenized into buffer at 40 °C, slowly over the chip which was held at 35 °C. The surface can be divided into four separate tracks separated by micropneumatically-controlled valves within the BIAcore apparatus. Previous reports have described the formation of liposomes or small unilamellar vesicles that were then exposed to the chip surface.[23] It was found that the formation of carefully sized liposomal or vesicular structures was unnecessary. Deposition followed a stepped curve with periods of association followed by a sudden loss of signal as assemblies of lipids peeled away from the surface. An uptake corresponding to 3,000–4,000 response units (RU) over a period of three hours was common. At the end of each phase of deposition the flow rate was increased to 100 μl min^{-1} to remove loosely adherent lipid. This was followed by a pulse of 20 mM sodium hydroxide to stabilize the neomembrane formed. Neomembranes could be used for several weeks with repeated regenerations with sodium hydroxide to remove any bound lectin. Moreover, when the response on a surface finally decayed to a non-reproducible level, a new neomembrane of the same or a different glycolipid/phospholipid could be deposited on the thioalkyl layer of the chip surface by raising the temperature to 35 °C and following the procedure described in the experimental section to deposit a new neomembrane. It was observed that 15 mol% of glycolipid in a 0.5 mM suspension of dipalmitoylphosphatidylcholine in buffer presented a suitable concentration of glycolipid and that a 12.5 μM solution of RCA_{120} in the same buffer gave highly reproducible results, although under carefully controlled conditions a 10 mol% ligand concentration and a much lower concentrations of RCA_{120} were used for kinetic studies. Attempts to use distearoylphosphatidylcholine or dioleoloylphosphatidylcholine as the carrier failed to give good deposition/response behaviour of the glycolipid analyte.

Albumin binds well to a hydrophobic surface such as that presented by the alkylthiol chains on an HPA sensor chip with no neomembrane present. There was no significant uptake of albumin onto the chip, proving that there was good coverage of the chip surface. Non-specific binding was not a problem and therefore the association measured was specific. Equally, binding of RCA_{120} to the diphosphatidylcholine alone (the negative control) was negligible but was in any case subtracted from all test runs. There was no evidence of mass transfer problems above a flow rate of 10 μl min^{-1} so all measurements of binding were done at 10 or 20 μl min^{-1}.

A typical binding experiment is illustrated in Fig. 1. The upper curve A shows the response obtained with a layer of 15% neoglycolipid **30** (Scheme 2) in a dipalmitoylphosphatidylcholine neomembrane. Evidence for the specificity of the interaction was obtained by exposing the sensor chip surface to the β-galactosidase from *Bacillus circulans*. Measurement of the binding of RCA_{120} both before and after exposure to an 8.6 μM solution of enzyme for 45 min showed that there was a 57% loss in binding after exposure to enzyme (curve B, Fig. 1) confirming that the RCA_{120} was binding to the β-galactosyl unit of the thiolactosyl glycolipid. A similar experiment with asialofetuin peptides on a CM5 sensor chip has been reported

Table 1 Relative rates of association[a] of β-galactosidase with neoglycolipid **30**

Concentration of enzyme/nM	Relative rate of association
25	0.022
33	0.019
50	0.018
75	0.017

[a] The relative rates of association calculated from the linear fit would normally rise with concentration. The decrease is due to the loss of ligand through enzyme attack.

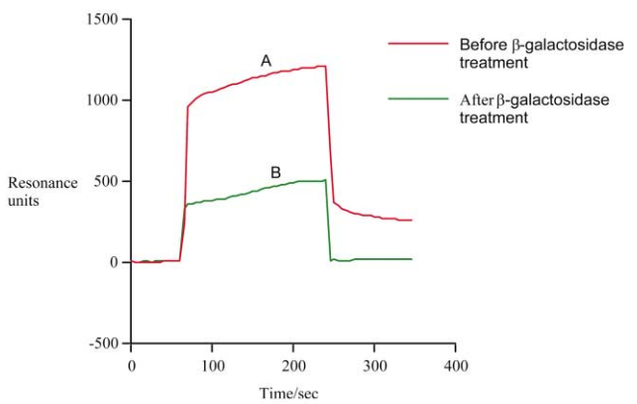


Fig. 1 Sensorgram showing the binding of *Ricinus communus* agglutinin (RCA_{120}) to a neomembrane of 15 mol% of compound **30** in dipalmitoylphosphatidylcholine. The upper curve (A) represents the binding to RCA_{120} before treatment with β-galactosidase and the lower curve (B) shows the binding after exposure of the ligand to the enzyme (7.7 μM) for 45 min at a flow rate of 5 μL min^{-1}.

but the enzymatic digestion with β-galactosidase from jack bean was carried out in solution and then starting material and the enzyme-digested asialofetuin glycopeptide were immobilised on the chip to measure the RCA_{120} binding.[20]

An attempt was made to follow the binding of the enzyme to the surface at very low concentrations (25–75 nM) where the hydrolysis reaction would be slow. The sensorgrams showed that binding did occur but it was reduced as the concentration of enzyme increased confirming the rapid hydrolytic reaction (Table 1). The observation that all of the curves gave a lower baseline and a lower response to the binding of RCA_{120} after exposure to enzyme confirmed the loss of ligand and made a full kinetic analysis difficult because the dissociation part of the curve represented loss of the two components namely the enzyme, by reversible dissociation, and enzymatically-cleaved ligand. However, the potential of this approach to the study of enzyme–substrate interactions is clear, provided only that non-reacting substrate analogues are attached to the sensor chip.

Kinetic measurements were carried out with 10 mol% glycolipid **30** and dilute solutions (25–150 nM) of RCA_{120} to minimise bulk effects attributable to changes in the refractive index of the solution flowing over the chip. The set of sensorgrams obtained (Fig. 2) showed clear rates of binding and of dissociation. The equations used to calculate the equilibrium association and dissociation constants have been described in detail[24–26] and are based on 1:1 Langmuir binding. In brief, glycolipid **30** binds to the RCA_{120} lectin and the rate of binding for components A and B binding to give complex AB can be described by the equation

$$\frac{d[AB]}{dt} = k_a[A][B] - k_d[AB]$$

where k_a and k_d are respectively the rate constants for association and dissociation. In the BIAcore apparatus the glycolipid

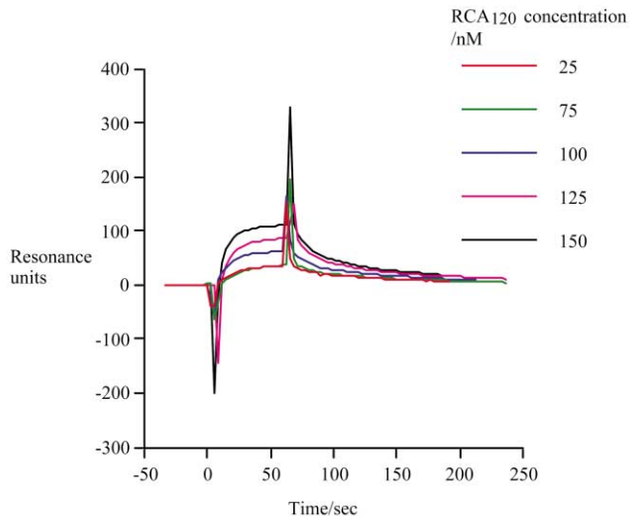


Fig. 2 Sensorgram showing the binding of RCA_{120} agglutinin to a neomembrane of 10 mol% of compound **30** in dipalmitoylphosphatidylcholine with a range of concentrations of RCA_{120}. NB The lower two concentration curves are nearly coincident.

B is immobilised on the surface of the sensor chip and so the concentration of the complex AB is identical to that of bound lectin A. The concentration of bound lectin gives a proportional response R_t.at time t. If $[B]_0$ is the total concentration of ligand then

$$[B] = [B]_0 - [AB]$$

The maximum response (at saturation) R_{max} is proportional to the total ligand concentration and $(R_{max} - R_t)$ is proportional to the free ligand concentration. The flow of lectin is constant during the association phase and therefore the concentration of free analyte is equal to the constant concentration of analyte passing over the sensor chip. If the concentrations of complex and free glycolipid are expressed in terms of sensor response the equation can be rewritten as

$$\frac{dR_t}{dt} = k_a C(R_{max} - R_t) - k_d R_t \quad (1)$$

where C is the concentration of injected lectin. Eqn. (1) can be rearranged to give eqn. (2):

$$\frac{dR_t}{dt} = k_a C R_{max} - (k_a C + k_d) R_t \quad (2)$$

This can be integrated to give eqn. (3):

$$\ln\left\{1 - R_t \frac{(k_a C + k_d)}{k_a C R_{max}}\right\} = -(k_a C + k_d)t \quad (3)$$

Eqn. (3) can be rewritten as:

$$R_t = \frac{k_a C R_{max}}{k_a C + k_d}(1 - e^{-(k_a C + k_d)t}) \quad (4)$$

At $t = 0$, $e^{-(k_a C + k_d)t} = 1$ and $R_t = 0$. Thus from eqn. (2), the initial slope is

$$\frac{dR_t}{dt} = k_a C R_{max}$$

Also, from eqn. (2), $-(k_a C + k_d$ is the observed rate constant for the association step. Eqn. (4) can be rewritten as eqn. (5)

$$R_t = \frac{r_0}{k_{obs}}(1 - e^{-k_{obs}t}) \quad (5)$$

where $r_0 = k_a C R_{max}$ and

$$k_{obs} = k_a C + k_d \quad (6)$$

The BIAcore software evaluates the initial rate r_0 and the observed rate constant for association k_{obs} by numerical fitting of the R_t *versus* t plot, against eqn. (5). The true rate constant for association, k_a, is then obtained from a plot of k_{obs} *versus* C, the analyte (lectin in the present case) concentration (eqn. (6)). The dissociation constant, k_d, is given by the intercept on the k_{obs} axis by extrapolation to $C = 0$. In practice, the accuracies of values of k_d obtained in this way are not very good, particularly for low values. Accordingly, as in the present work, k_d is best evaluated from the dissociation phase of the sensorgram after the analyte solution has been replaced by the solution (buffer) minus the analyte. Under these conditions, the dissociation curve is given by

$$\frac{dR_t}{dt} = -k_d R_t$$

which on integration gives

$$\ln\frac{R_0}{R_t} = k_d t \quad (7)$$

where R_0 is the sensor response at the beginning of the dissociation phase and R_t is the response after time t. This equation can be rewritten as

$$R_t = R_0 e^{-k_d t}$$

From this equation, the BIAcore software evaluates k_d by numerical fitting of the dissociation curve. With the values of k_a and k_d to hand, the equilibrium constant K is directly evaluated.

All of these calculations assume a 1:1 interaction between lectin and glycolipid. While there is a possibility that RCA_{120} agglutinin might bind to two adjacent glycolipid molecules no evidence for this could be found. Thus plots of

$$\ln\left(\frac{dR}{dt}\right)$$

versus R and

$$\ln\frac{R_0}{R}$$

versus t (eqn. (7)) corresponding to association and dissociation phases respectively were linear.

The observed equilibrium constant for association, K_A, is the mean of values obtained from ten data sets and has a value of 1.14 (± 0.12) × 10^7 M^{-1}. This value is almost an order of magnitude lower than that of that for the binding of RCA_{120} to asialofetuin[23] (K_A=1.62 × 10^8 M^{-1}) but almost four orders of magnitude higher than the binding constant for free lactose to RCA_{120} where K_A=3.8 × 10^3 M^{-1}.[1]

Inhibition experiments were carried out by adding lactose (0–100 mM) to the RCA_{120} solution before it was passed over the ligand on the chip surface. Maximum binding was observed at zero concentration of free lactose. Binding was completely inhibited at 80 mM lactose (Fig. 3). That such a high concentration of lactose (80 mM) was required to inhibit the binding of RCA_{120} to the thiolactosyl glycolipid **30** confirmed the tight binding of the RCA_{120} to the thiolactosyl glycolipid observed in the kinetic experiments.

The experiments described above provide a further example

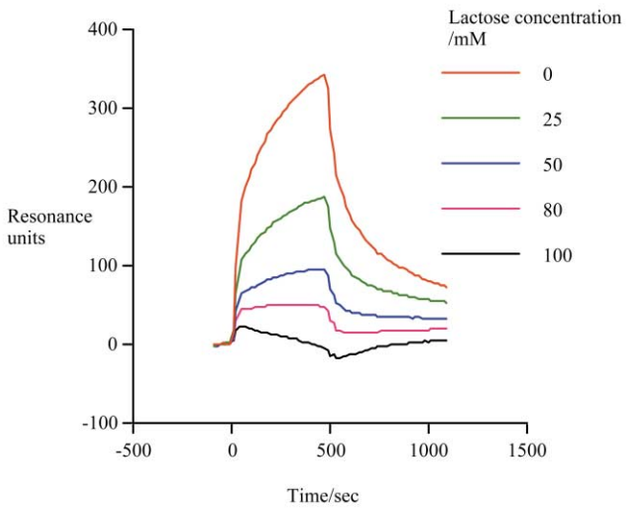


Fig. 3 Sensorgram showing the inhibition of binding of RCA_{120} to neoglycolipid **30**. The curves show binding at various concentrations of lactose.

of the greatly increased strength of interaction between a carbohydrate-binding protein (lectin) and its carbohydrate receptor on a membrane surface compared with the corresponding interaction in solution. As discussed earlier, other factors also are probably important, included amongst which are thermodynamic factors associated with entropy changes and in torsional entropy changes in particular,[27] and differences in water activity between the bulk solution and the interface which would affect the solvation of the interacting species.

By determination of K_A at 5 °C temperature intervals between 5 °C and 30 °C the standard enthalpy of association, ΔH, was determined from the van't Hoff equation:

$$\ln K = \frac{\Delta H}{R}\left(\frac{1}{T}\right)$$

The gradient of a plot of ln *K versus* (1/*T*) based on fourteen observations taken at 5 °C intervals was used to calculate ΔH. The corresponding entropy of association was then determined from the relationship:$\Delta G = \Delta H - T\Delta S$. At 290 K, $\Delta G = -39.8$ kJ mol^{-1}, $\Delta H = -33.6$ kJ mol^{-1} and $\Delta S = 21$ J mol^{-1} K^{-1}. These figures are similar to those found by other workers for lectin to carbohydrate binding using titration microcalorimetry.[28,29]

Experimental

NMR spectra were obtained using a Bruker 400 MHz spectrometer. Chemical shifts are given as parts per million (δ). Coupling constants are quoted in Hz. MS spectra were determined using a Waters Micromass AutoSpec mass spectrometer. Optical rotations were measured using an Optical Activity Ltd. Model AA1000 polarimeter at 589 nM with a path length of 2 dm. Concentrations (*c*) are quoted in g 1 cm^{-1}. TLC analysis was carried out on aluminium backed plates (Merck Kieselgel 60 F254). Spots were visualised by spraying with with 5% H_2SO_4 and heating. Column chromatography was carried out using Kieselgel 60F254 (Merck). RCA_{120} and dipalmitoylphosphatidylcholine were obtained from Sigma. Binding studies were monitored, in real time, by surface plasmon resonance using an HPA chip in a BIAcore 2000 automatic instrument.

2-(2-Benzylsulfanylethoxy)ethanol 3

Benzyl mercaptan (31.4 cm^3, 267.8 mmol) under an atmosphere of nitrogen was added to a mixture of sodium methoxide (24 g, 446.3 mmol) in anhydrous MeOH (500 cm^3). To this mixture, 2-(2-chloroethoxy)ethanol **1** (50 g, 403 mmol) was added dropwise. The reaction mixture was heated under reflux overnight. The reaction was followed by TLC (CH_2Cl_2–MeOH, 9:1 v/v, R_f 0.71). After 19 h, the reaction mixture was poured into a saturated aqueous solution of NaCl (500 cm^3) and product was extracted with CH_2Cl_2 (3 × 1 dm^3). The organic phase was dried ($MgSO_4$), filtered and evaporated under reduced pressure. The crude oil was purified by chromatography on silica (CH_2Cl_2 ⟶ CH_2Cl_2–MeOH, 15:1 v/v) to give the sulfide **3** (75.36 g, 88%) as a clear oil; δ_H(300 MHz; $CDCl_3$) 2.47 (1 H, t, *J* 6.2, OH), 2.64 (2 H, t, *J* 6.6, 2-CH_2), 3.50–3.56 (2 H, m, 4-CH_2), 3.60 (2 H, t, *J* 6.6, 3-CH_2), 3.69–3.73 (2 H, m, 5-CH_2), 3.77 (2 H, s, 1-CH_2), 7.23–7.37 (5 H, m, Ph); δ_C(75 MHz; $CDCl_3$) 30.7 (2-C), 36.5 (1-C), 61.6 (5-C), 70.1 (4-C), 71.9 (3-C), 127.0 (9-C), 128.4, 128.8 (7, 7′, 8, 8′-C), 138.1 (6-C); *m*/*z*(CI) 230.1215 (M + NH_4^+. $C_{11}H_{20}NO_2S$ requires 230.1212), 151 (100%), 91 (17.2) and 35 (6.0).

The same synthetic pathway was used to prepare the two series of compounds described below and therefore the detailed synthetic method is only described for the first member of each group of compounds. A complete NMR spectrum and mass spectral analysis is given for each compound of the two related series (*i.e.* those derived from compound **3** (series A) and those derived from compound **4** (series B).

Series A

[2-(2-Decyloxyethoxy)ethylsulfanylmethyl]benzene 5. Aqueous NaOH [37.7 g, 943 mmol, in 38 g H_2O (50% w/w)], tetrabutylammonium hydrogensulfate (1.28 g, 3.78 mmol) and sodium iodide (0.71 g, 4.72 mmol) were added to a mixture of 2-(2-benzylsulfanylethoxy)ethanol **3** (10 g, 47.15 mmol) and 1-bromodecane (12.7 cm^3, 61.29 mmol). The two-phase mixture was stirred vigorously overnight at 90 °C under an atmosphere of nitrogen. The reaction was followed by TLC (hexane–EtOAc, 3:7 v/v, R_f 0.69). After stirring overnight, the reaction mixture was poured into water (500 cm^3) and the product was extracted with CH_2Cl_2 (3 × 500 cm^3). The organic extracts were combined, dried ($MgSO_4$), filtered and evaporated under reduced pressure to give a crude oil which was purified by silica chromatography (hexane–EtOAc, 17:1 v/v) to give compound **5** (15.08 g, 91%) as clear oil; δ_H(300 MHz; $CDCl_3$) 0.82 (3 H, t, *J* 6.7, CH_3), 1.20–1.35 (14 H, m, 8, 9, 10, 11, 12, 13, 14-CH_2), 1.46–1.57 (2 H, m, 7-CH_2), 2.56 (2 H, t, *J* 6.8, 2-CH_2), 3.39 (2 H, t, *J* 6.8, 6-CH_2), 3.47–3.58 (6 H, m, 3, 4, 5-CH_2), 3.70 (2 H, s, 1-CH_2), 7.13–7.27(5 H, m, Ph); δ_C(75 MHz; $CDCl_3$) 14.1 (15-C), 22.6 (14-C), 26.0 (8-C), 29.3, 29.4, 29.5, 29.5, 29.6 (7, 9, 10, 11, 12-C), 31.8 (13-C), 30.4 (2-C), 36.5 (1-C), 70.0, 70.3, 70.8, 71.5 (3, 4, 5, 6-C), 126.8 (19-C), 128.4, 128.8 (17, 17′, 18, 18′-C), 138.4 (16-C); *m*/*z*(CI) 370.2780 (M + NH_4^+. $C_{21}H_{40}NO_2S$ requires 370.2780), 280 (9%), 220 (13.1), 151 (100.0), 108 (12.3) and 91 (8.5%).

2-(2-Decyloxyethoxy)ethanethiol 11. Liquid ammonia (150 cm^3) was condensed into a flask at −78 °C. Small pieces of cleaned and dried sodium (1.2 g, 52.2 mmol, were added to obtain a permanent blue colouration. The mixture was stirred at −78 °C for 10 min. A solution of [2-(2-decyloxyethoxy)-ethylsulfanylmethyl]benzene **5** (3 g, 8.52 mmol) in anhydrous THF (25 cm^3) was added dropwise and the reaction mixture was stirred at −78 °C for 1 h. The temperature was slowly increased to −30 °C (1.5 h) and the reaction refluxed for 30 min. The reaction mixture was cooled to −78 °C and quenched with wet THF (75% THF in H_2O, 25 cm^3) until the blue colouration disappeared. The reaction flask was warmed to room temperature under nitrogen to facilitate the evaporation of ammonia. The resulting white suspension was slowly diluted with water (200 cm^3) and the product extracted with CH_2Cl_2 (3 × 150 cm^3). The combined organic phase was dried ($MgSO_4$), filtered and evaporated under reduced pressure to give a crude yellow oil which was purified by chromatography on silica (CH_2Cl_2) to

give compound **11** (1.94 g, 87%) as a clear oil; TLC (CH_2Cl_2, R_f 0.50); δ_H(300 MHz; $CDCl_3$) 0.85 (3 H, t, *J* 6.0, 7.0, CH_3), 1.15–1.36 (14 H, m, 7, 8, 9, 10, 11, 12, 13-CH_2), 1.50–1.61 (3 H, m, S*H* (t, *J* 8.3), 6-CH_2), 2.67 (2 H, dt, *J* 6.4, 6.6, 1-CH_2), 3.43 (2 H, t, *J* 6.8, 5-CH_2), 3.52–3.63 (6 H, m, 2, 3, 4-CH_2); δ_C(75 MHz; $CDCl_3$) 14.1 (14-C), 22.6 (13-C), 24.2 (1-C), 26.0 (7-C), 29.3, 29.4, 29.5, 29.6, 29.6 (6, 8, 9, 10, 11-C), 31.8 (12-C), 69.9, 70.2, 72.8 (2, 3, 4-C), 71.5 (5-C); *m*/*z*(TOF MS ES+) 285.1862 (M + Na. $C_{14}H_{30}O_2NaS$ requires 285.1864).

Acetyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1⟶4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside 18. A mixture of D-lactose **17** (50 g, 146 mmol), anhydrous sodium acetate (48 g, 584 mmol) and acetic anhydride (124 cm^3, 1.31 mol) was heated (95–100 °C) on an oil-bath under an atmosphere of nitrogen until a clear solution was obtained. The mixture was then boiled under reflux for 2 h. The reaction was followed by TLC (toluene–EtOAc, 1:1 v/v, R_f 0.47). The reaction mixture was poured into crushed ice (1 L) and stirred for 1 h. The precipitate formed was filtered, washed with ice-cold water and dried under reduced pressure over P_2O_5 The white/off-brown solid was recrystallized from EtOH to give the peracetate **18** (78.3 g, 79%) as a white crystalline solid; mp 90–92 °C (from EtOH) (lit.[30] 91–93 °C, lit.,[31] 90–91 °C) $[\alpha]_D^{23}$; −4.13 (*c* 1 in $CHCl_3$) (lit.,[32] $[\alpha]_D^{22}$ −4.4 (*c* 1 in $CHCl_3$)); δ_H(300 MHz; $CDCl_3$) 1.92 (3 H, s, CH_3), 2.00 (3 H, s, CH_3), 2.01 (9 H, s, CH_3), 2.02 (3 H, s, CH_3), 2.03 (3 H, s, CH_3), 2.06 (3 H, s, CH_3), 2.08 (3 H, s, CH_3), 2.12 (3 H, s, CH_3), 3.74 (1 H, m, 5-H), 3.80 (1 H, m, 4-H), 3.85 (1H, m, 5′-H), 3.98–4.15 (3 H, m, 6a-H, 6a′-H, 6b′-H), 4.42 (1 H, dd, *J* 1.7, 12.1, 6b-H), 4.44 (1 H, d, *J* 7.9, 1′-H), 4.92 (1 H, dd, *J* 3.4, 10.5, 3′-H), 5.01 (1 H, dd, *J* 8.3, 9.4, 2-H), 5.07 (1 H, dd, *J* 7.9, 10.5, 2′-H), 5.21 (1 H, dd, *J* 8.7, 9.4, 3-H), 5.33 (1 H, dd, *J* 0.9, 3.4, 4′-H), 5.64 (1 H, d, *J* 8.3, 1-H); δ_C(75 MHz; $CDCl_3$) 20.4 (CH_3), 20.5 (CH_3 × 2), 20.6 (CH_3 × 2), 20.7 (CH_3 × 2), 20.8 (CH_3), 60.8 (6′-C), 61.6 (6-C), 66.5 (4′-C), 68.9 (2′-C), 70.4 (2-C), 70.6 (3′-C), 70.9 (3-C), 73.4 (5-C), 75.6 (4-C), 91.4 (1-C), 100.9 (1′-C), 168.8 (C=O), 169.0 (C=O), 169.5 (C=O), 169.6 (C=O), 170.0 (C=O), 170.1 (C=O), 170.3 (C=O), 170.3 (C=O); *m*/*z*(FAB, NBA) 701.1909 (M + Na^+. $C_{28}H_{38}Na\ O_{19}$ requires 701.1905), 619 (78%), 559 (12.5), 331 (100), 289 (11.3), 229 (6.9) and 137 (42.5).

1-{2-[2-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1⟶4)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucopyranosyl)ethoxy]ethoxy}-decane 19. BF_3OEt_2 (1.0 cm^3, 8.13 mmol) was added dropwise over a period of 15 min to a solution of acetyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1⟶4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside **18** (3.67 g, 5.42 mmol) and [2-(2-decyloxy)-ethoxy]ethanethiol **11** (1.85 g, 7.05 mmol) in anhydrous CH_2Cl_2 (40 cm^3) at room temperature under an atmosphere of nitrogen. The reaction was followed by TLC (hexane–EtOAc, 1:1 v/v, R_f 0.36). After 3 h, the reaction mixture was diluted with CH_2Cl_2(200 cm^3) and washed with 1 M HCl(aq) (3 × 140 cm^3) and brine (3 × 140 cm^3). The organic layer was dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residue was purified by chromatography on silica (hexane–EtOAc, 1:1 v/v) to give compound **19** (3.48 g, 73%) as a white foamy solid/syrup; $[\alpha]_D^{26}$ −17.9 (*c* 0.44 in $CHCl_3$), δ_H(300 MHz; $CDCl_3$) 0.85 (3 H, t, *J* 6.4, 7.0, CH_3), 1.17–1.36 (14 H, m, 13, 14, 15, 16, 17, 18, 19-CH_2), 1.50–1.61 (2 H, m, 12-CH_2), 1.93 (3 H, s, CH_3), 2.01 (3 H, s, CH_3), 2.02 (3 H, s, CH_3), 2.02 (3 H, s, CH_3), 2.03 (3 H, s, CH_3), 2.09 (3 H, s, CH_3), 2.12 (3 H, s, CH_3), 2.66–2.78 (1 H, m, 7-CH_{2a}), 2.83–2.95 (1 H, m, 7-CH_{2b}), 3.41 (2 H, t, *J* 6.8, 11-CH_2), 3.50–3.70 (7 H, m, 5-H, 8, 9, 10-CH_2), 3.74 (1 H, dd, *J* 9.2, 9.4, 4-H), 3.84 (1 H, b dd, *J* 6.8, 6.8, 5′-H), 4.00–4.15 (3 H, m, 6a-H, 6a′-H, 6b′-H), 4.40–4.48 (2 H, m, 1′-H (d, *J* 7.7), 6b-H), 4.53 (1 H, d, *J* 10.2, 1-H), 4.88 (1 H, dd, *J* 9.4, 10.2, 2-H), 4.92 (1 H, dd, *J* 3.4, 10.6, 3′-H), 5.07 (1 H, dd, *J* 7.9, 10.4, 2′-H), 5.17 (1 H, dd, *J* 9.0, 9.2, 3-H), 5.35 (1 H, dd, *J* 0.8, 3.4, 4′-H) δ_C(75 MHz; $CDCl_3$) 14.1 (20-C), 20.5 (CH_3), 20.6 (CH_3 × 3), 20.7 (CH_3), 20.7 (CH_3), 20.8 (CH_3), 22.6 (19-C), 26.0 (13-C), 29.3, 29.4, 29.6 (× 4) (7, 12, 14, 15, 16, 17-C), 31.8 (18-C), 60.7 (6′-C), 62.1 (6-C), 66.5 (4′-C), 69.0 (2′-C), 70.4 (2-C), 70.6 (5′-C), 70.9 (3′-C), 69.9, 70.3, 71.1, 71.6 (8, 9, 10, 11-C), 73.7 (3-C), 76.2 (4-C), 76.7 (5-C), 83.5 (1-C), 101.1 (1′-C), 169.0 (C=O), 169.6 (C=O), 169.6 (C=O), 170.0 (C=O), 170.1 (C=O), 170.3 (C=O × 2); *m*/*z*(TOF MS ES+) 903.3671 (M + Na. $C_{40}H_{64}O_{19}NaS$ requires 903.3660).

1-{2-[2-(β-D-Galactopyranosyl-(1⟶4)-1-thio-β-D-glucopyranosyl)ethoxy]ethoxy}decane 25. Anhydrous MeOH (40 cm^3) was added to a flask containing 1-{2-[2-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1⟶4)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucopyranosyl)ethoxy]ethoxy}decane **19** (3.42 g, 3.88 mmol) and sodium methoxide (21 mg, 0.39 mmol) at room temperature under an atmosphere of nitrogen. The reaction mixture was stirred at room temperature and reaction was followed by TLC (CH_2Cl_2–MeOH, 9:1 v/v, R_f 0.06). A precipitate started to form after about 1 h. The reaction mixture was left to stir overnight at room temperature. After 15 h it was diluted with CH_2Cl_2 (30 cm^3) and MeOH (20 cm^3) to dissolve the precipitate. The solution was neutralised with Amberlyst 15 (pH 7.0, pH paper), filtered and evaporated under reduced pressure to give compound **25** (2.19 g, 96%) as a white crystalline solid; mp 160–163 °C, −16.8 (*c* 0.44 in MeOH); δ_H(300 MHz; MeOH-d^4) 0.80 (3 H, m, CH_3), 1.13–1.32 (14 H, m, 13, 14, 15, 16, 17, 18, 19-CH_2), 1.40–1.53 (2 H, m, 12-CH_2), 2.66–2.77 (1 H, m, 7-CH_{2a}), 2.78–2.91 (1 H, m, 7-CH_{2b}), 3.17 (1 H, dd, *J* 8.1, 8.7, 2-H), 3.31–3.87 (19 H, m, 2′, 3, 3′, 4, 4′, 5, 5′, 6a, 6a′, 6b, 6b′-H, 8, 9, 10, 11-CH_2), 4.26 (1 H, d, *J* 7.4, 1′-H), 4.35 (1 H, d, *J* 9.8, 1-H); δ_C(75 MHz; MeOH-d^4) 14.5 (CH_3), 23.7 (19-C), 26.9 (13-C), 30.1, 30.2, 30.4, 30.4, 30.5, 30.5 (7, 12, 14, 15, 16, 17-C), 33.0 (18-C), 62.0 (6′-C), 62.4 (6-C), 71.1, 71.2, 72.2, 72.4 (8, 9, 10, 11-C), 70.3, 72.5, 74.1, 74.8, 77.1, 77.8, 80.4, 80.5 (2, 3, 4, 5, 2′, 3′, 4′, 5′-C), 87.0 (1-C), 105.0 (1′-C); *m*/*z*-(TOF MS ES+) 609.2926 (M + Na. $C_{26}H_{50}O_{12}NaS$ requires 609.2921).

[2-(2-Dodecyloxyethoxy)ethylsulfanylmethyl]benzene 6. An aqueous solution containing NaOH [37.7 g, 943 mmol, in 38 g H_2O (50% w/w)], tetrabutylammonium hydrogensulfate (1.28 g, 3.78 mmol) and sodium iodide (0.71 g, 4.72 mmol) was added to a mixture of. 2-(2-benzylsulfanylethoxy)ethanol **2** (10 g, 47.15 mmol) and 1-bromododecane (14.7 cm^3, 61.29 mmol), The reaction conditions and isolation of the crude oil were the same as described for compound **5**. The reaction was followed by TLC (hexane–EtOAc, 10:1 v/v, R_f 0.33). The oil was purified by chromatography on silica (hexane–EtOAc, 12:1 v/v) to give compound **6** (16.17 g, 90%) as clear oil; δ_H(300 MHz; $CDCl_3$) 0.82 (3 H, t, *J* 6.7, CH_3), 1.15–1.35 (18 H, m, 8, 9, 10, 11, 12, 13, 14, 15, 16-CH_2), 1.45–1.54 (2 H, m, 7-CH_2), 2.56 (2 H, t, *J* 6.8, 2-CH_2), 3.39 (2 H, t, *J* 6.8, 6-CH_2), 3.47–3.58 (6 H, m, 3, 4, 5-CH_2), 3.70 (2 H, s, 1-CH_2), 7.13–7.22 (5 H, m, Ph); δ_C(75 MHz; $CDCl_3$) 14.1 (17-C), 22.6 (16-C), 26.0 (8-C), 29.3, 29.5, 29.6, 29.6 (× 4) (7, 9, 10, 11, 12, 13, 14-C), 30.4 (2-C), 31.8 (15-C), 36.5 (1-C), 70.0, 70.3, 70.8, 71.5 (3, 4, 5, 6-C), 126.8 (21-C), 128.4, 128.8 (19, 19′, 20, 20′-C), 138.4 (18-C); *m*/*z*(CI) 398.3096 (M + NH_4^+. $C_{23}H_{44}NO_2S$ requires 398.3093), 308 (5%), 248 (6.5), 151 (43.2), 108 (8.5), 91 (8.5) and 44 (100.0).

2-(2-Dodecyloxyethoxy)ethanethiol 12. Liquid ammonia (150 cm^3) was condensed into a flask at −78 °C. Small pieces of cleaned and dried sodium (1.2 g, 52.2 mmol) were added until a permanent blue colouration was obtained. The mixture was stirred at −78 °C for 10 min. A solution of [2-(2-dodecyloxyethoxy)ethylsulfanylmethyl]benzene **6** (3 g, 7.89 mmol) in anhydrous THF (25 cm^3) was added dropwise and the reaction mixture was stirred at −78 °C for 1 h. The reaction conditions and purification were the same as described for compound **11**

and gave a crude yellow oil which was purified by chromatography on silica (CH_2Cl_2) to give compound **12** (1.72 g, 75%) as a clear oil; TLC (CH_2Cl_2, R_f 0.52); δ_H(300 MHz; $CDCl_3$) 0.85 (3 H, t, *J* 6.0, 7.0, CH_3), 1.15–1.38 (18 H, m, 7, 8, 9, 10, 11, 12, 13, 14, 15-CH_2), 1.50–1.61 (3 H, m, S*H* (t, *J* 8.3), 6-CH_2), 2.67 (2 H, dt, *J* 6.4, 6.6, 1-CH_2), 3.43 (2 H, t, *J* 6.8, 5-CH_2), 3.52–3.63 (6 H, m, 2, 3, 4-CH_2); δ_C(75 MHz; $CDCl_3$) 14.1 (16-C), 22.6 (15-C), 24.2 (1-C), 26.0 (7-C), 29.3, 29.4, 29.6 (× 5) (6, 8, 9, 10, 11, 12, 13-C), 31.8 (14-C), 69.9, 70.2, 72.8 (2, 3, 4-C), 71.5 (5-C); *m*/*z*(TOF MS ES+) 313.2182 (M + Na. $C_{16}H_{34}O_2NaS$ requires 313.2177).

1-{2-[2-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1⟶4)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucopyranosyl)ethoxy]ethoxy}-dodecane 20. BF_3OEt_2 (0.90 cm^3, 7.24 mmol) was added dropwise over a period of 15 min to a solution of acetyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1⟶4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside **18** (3.27 g, 4.82 mmol) and [2-(2-dodecyloxy)ethoxy]ethanethiol **12** (1.68 g, 5.79 mmol) in anhydrous CH_2Cl_2 (40 cm^3) at room temperature under an atmosphere of nitrogen. The reaction conditions and purification were the same as described for compound **19**. Compound **20** (3.48 g, 73%) was obtained as a white foamy solid/syrup; $[\alpha]_D^{26}$ −17.9 (*c* 0.44 in $CHCl_3$); δ_H(300 MHz; $CDCl_3$) 0.85 (3 H, t, *J* 6.4, 7.0, CH_3), 1.15–1.346 (18 H, m, 13, 14, 15, 16, 17, 18, 19. 20, 21-CH_2), 1.50–1.61 (2 H, m, 12-CH_2), 1.93 (3 H, s, CH_3), 2.01 (3 H, s, CH_3), 2.02 (3 H, s, CH_3), 2.02 (3 H, s, CH_3), 2.03 (3 H, s, CH_3), 2.09 (3 H, s, CH_3), 2.12 (3 H, s, CH_3), 2.66–2.78 (1 H, m, 7-CH_{2a}), 2.83–2.95 (1 H, m, 7-CH_{2b}), 3.41 (2 H, t, *J* 6.8, 11-CH_2), 3.50–3.70 (7 H, m, 5-H, 8, 9, 10-CH_2), 3.74 (1 H, dd, *J* 9.2, 9.4, 4-H), 3.84 (1 H, b dd, *J* 6.8, 6.8, 5′-H), 4.00–4.15 (3 H, m, 6a-H, 6a′-H, 6b′-H), 4.40–4.48 (2 H, m, 1′-H (d, *J* 7.7), 6b-H), 4.53 (1 H, d, *J* 10.2, 1-H), 4.88 (1 H, dd, *J* 9.4, 10.2, 2-H), 4.92 (1 H, dd, *J* 3.4, 10.6, 3′-H), 5.07 (1 H, dd, *J* 7.9, 10.4, 2′-H), 5.17 (1 H, dd, *J* 9.0, 9.2, 3-H), 5.35 (1 H, dd, *J* 0.8, 3.4, 4′-H) δ_C(75 MHz; $CDCl_3$) 14.1 (22-C), 20.5 (CH_3), 20.6 (CH_3 × 3), 20.7 (CH_3), 20.7 (CH_3), 20.8 (CH_3), 22.6 (21-C), 26.0 (13-C), 29.3, 29.4, 29.6 (× 4) (7, 12, 14, 15, 16, 17, 18, 19-C), 31.8 (20-C), 60.7 (6′-C), 62.1 (6-C), 66.5 (4′-C), 69.0 (2′-C), 70.4 (2-C), 70.6 (5′-C), 70.9 (3′-C), 69.9, 70.3, 71.1, 71.6 (8, 9, 10, 11-C), 73.7 (3-C), 76.2 (4-C), 76.7 (5-C), 83.5 (1-C), 101.1 (1′-C), 169.0 (C=O), 169.6 (C=O), 1696 (C=O), 170.0 (C=O), 170.1 (C=O), 170.3 (C=O × 2); *m*/*z*(TOF MS ES+) 931.3981 (M + Na. $C_{42}H_{68}O_{19}NaS$ requires 931.3973).

1-{2-[2-(β-D-Galactopyranosyl-(1⟶4)-1-thio-β-D-glucopyranosyl)ethoxy]ethoxy}dodecane 26. Anhydrous MeOH (50 cm^3) was added to a flask containing 1-{2-[2-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1⟶4)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucopyranosyl)ethoxy]ethoxy}dodecane **20** (3.1 g, 3.41 mmol) and sodium methoxide (19 mg, 0.34 mmol) at room temperature under an atmosphere of nitrogen. The reaction mixture was stirred at room temperature for 18 h. The reaction was followed by TLC (CH_2Cl_2–MeOH, 9:1 v/v, R_f 0.10). The reaction conditions and purification were the same as those described for compound **25**. Compound **26** (2.08 g, 99%) was obtained as a white amorphous solid; mp 158–160 °C; $[\alpha]_D^{26}$ −19.0 (*c* 0.42 in pyridine); δ_H(300 MHz; MeOH-d^4-$CDCl_3$, 2:1 v/v) 0.81 (3 H, t, *J* 6.4, 7.0, CH_3), 1.13–1.32 (18 H, m, 13, 14, 15, 16, 17, 18, 19, 20, 21-CH_2), 1.40–1.53 (2 H, m, 12-CH_2), 2.66–2.77 (1 H, m, 7-CH_{2a}), 2.78–2.91 (1 H, m, 7-CH_{2b}), 3.17 (1 H, dd, *J* 8.1, 8.7, 2-H), 3.31–3.87 (19 H, m, 2′, 3, 3′, 4, 4′, 5, 5′, 6a, 6a′, 6b, 6b′-H, 8, 9, 10, 11-CH_2), 4.26 (1 H, d, *J* 7.4, 1′-H), 4.35 (1 H, d, *J* 9.8, 1-H); δ_C(75 MHz; MeOH-d^4-$CDCl_3$, 2:1 v/v) 14.5 (22-C), 23.7 (21-C), 26.9 (13-C), 30.0, 30.1, 30.3, 30.3, 30.4 (× 4) (7, 12, 14, 15, 16, 17, 18, 19-C), 33.0 (20-C), 62.0 (6′-C), 62.4 (6-C), 71.1, 71.2, 72.2, 72.4 (8, 9, 10, 11-C), 70.3, 72.5, 74.1, 74.8, 77.1, 77.8, 80.4, 80.5 (2, 3, 4, 5, 2′, 3′, 4′, 5′-C), 87.0 (1-C), 105.0 (1′-C); *m*/*z*(TOF MS ES+) 637.3220 (M + Na. $C_{28}H_{54}O_{12}NaS$ requires 637.3234).

[2-(2-Octadecyloxyethoxy)ethylsulfanylmethyl]benzene 7. An aqueous solution containing NaOH [37.7 g, 943 mmol, in 38 g H_2O (50% w/w)], tetrabutylammonium hydrogensulfate (1.28 g, 3.78 mmol) and sodium iodide (0.71 g, 4.72 mmol) was added to a mixture of 2-(2-benzylsulfanylethoxy)ethanol **3** (10 g, 47.15 mmol) and 1-bromooctadecane (20.9 cm^3, 61.29 mmol). The reaction conditions and purification were the same as those described for compound **6**. Compound **7** (18.79 g, 86%) was obtained as a clear oil; δ_H(300 MHz; $CDCl_3$) 0.82 (3 H, t, *J* 6.7, CH_3), 1.15–1.35 (30 H, m, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22-CH_2), 1.45–1.54 (2 H, m, 7-CH_2), 2.56 (2 H, t, *J* 6.8, 2-CH_2), 3.39 (2 H, t, *J* 6.8, 6-CH_2), 3.47–3.58 (6 H, m, 3, 4, 5-CH_2), 3.70 (2 H, s, 1-CH_2), 7.13–7.22 (5 H, m, Ph); δ_C(75 MHz; $CDCl_3$) 14.1 (23-C), 22.6 (22-C), 26.0 (8-C), 29.3, 29.5, 29.6, 29.7 (× 9) (7, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20-C), 30.4 (2-C), 31.8 (21-C), 36.5 (1-C), 70.0, 70.3, 70.8, 71.5 (3, 4, 5, 6-C), 126.8 (27-C), 128.4, 128.8 (25, 25′, 26, 26′-C), 138.4 (24-C); *m*/*z*(CI) 482.4034 (M + NH_4^+. $C_{29}H_{56}NO_2S$ requires 482.4032), 342 (38%), 217 (7.3), 151 (100.0), 108 (10.5) and 91 (10.0).

[2-(2-Octadecyloxy)ethoxy]ethanethiol 13. Liquid ammonia (150 cm^3) was condensed into a flask at −78 °C. Small pieces of cleaned dried sodium (1.2 g, 52.2 mmol) were added until a permanent blue colouration was obtained. The mixture was stirred at −78 °C for 10 min. A solution of [2-(2-octadecyloxyethoxy)ethylsulfanylmethyl]benzene **7** (3 g, 6.46 mmol) in anhydrous THF (25 cm^3) was added dropwise and the reaction mixture was stirred at −78 °C for 1 h. The reaction conditions and purification were the same as those described for compound **11**. Compound, **13**, (1.80 g, 74%) was obtained as a white waxy solid; TLC (CH_2Cl_2, R_f 0.58); δ_H(300 MHz; $CDCl_3$) 0.85 (3 H, t, *J* 6.0, 7.0, CH_3), 1.15–1.38 (30 H, m, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21-CH_2), 1.50–1.61 (3 H, m, S*H* (t, *J* 8.3), 6-CH_2), 2.67 (2 H, dt, *J* 6.4, 6.6, 1-CH_2), 3.43 (2 H, t, *J* 6.8, 5-CH_2), 3.52–3.63 (6 H, m, 2, 3, 4-CH_2); δ_C(75 MHz; $CDCl_3$) 14.1 (22-C), 22.6 (21-C), 24.2 (1-C), 26.0 (7-C), 29.3, 29.5, 29.6, 29.7 (× 10) (6, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19-C), 31.8 (20-C), 69.9, 70.2, 72.8 (2, 3, 4-C), 71.5 (5-C); *m*/*z*(TOF MS ES+) 397.3118 (M + Na. $C_{22}H_{46}O_2NaS$ requires 397.3116).

1-{2-[2-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1⟶4)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucopyranosyl)ethoxy]ethoxy}-octadecane 21. BF_3OEt_2 (0.70 cm^3, 5.68 mmol) was added dropwise over a period of 15 min to a solution of acetyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1⟶4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside **18** (2.57 g, 3.78 mmol) and [2-(2-octadecyloxy)ethoxy]ethanethiol **13** (1.7 g, 4.54 mmol) in anhydrous CH_2Cl_2 (40 cm^3) at room temperature under an atmosphere of nitrogen. The reaction was followed by TLC (hexane–EtOAc, 1:1 v/v, R_f 0.40). Reaction conditions and purification were the same as described for compound **19**. Compound **21** (2.95 g, 78%) was obtained as a white foamy solid; $[\alpha]_D^{26}$ −17.9 (*c* 0.44 in $CHCl_3$); δ_H(300 MHz; $CDCl_3$) 0.85 (3 H, t, *J* 6.4, 7.0, CH_3), 1.15–1.34 (30 H, m, 13, 14, 15, 16, 17, 18, 19. 20, 21, 22, 23, 24, 25, 26, 27-CH_2), 1.50–1.61 (2 H, m, 12-CH_2), 1.93 (3 H, s, CH_3), 2.01 (3 H, s, CH_3), 2.02 (3 H, s, CH_3), 2.02 (3 H, s, CH_3), 2.03 (3 H, s, CH_3), 2.09 (3 H, s, CH_3), 2.12 (3 H, s, CH_3), 2.66–2.78 (1 H, m, 7-CH_{2a}), 2.83–2.95 (1 H, m, 7-CH_{2b}), 3.41 (2 H, t, *J* 6.8, 11-CH_2), 3.50–3.70 (7 H, m, 5-H, 8, 9, 10-CH_2), 3.74 (1 H, dd, *J* 9.2, 9.4, 4-H), 3.84 (1 H, b dd, *J* 6.8, 6.8, 5′-H), 4.00–4.15 (3 H, m, 6a-H, 6a′-H, 6b′-H), 4.40–4.48 (2 H, m, 1′-H (d, *J* 7.7), 6b-H), 4.53 (1 H, d, *J* 10.2, 1-H), 4.88 (1 H, dd, *J* 9.4, 10.2, 2-H), 4.92 (1 H, dd, *J* 3.4, 10.6, 3′-H), 5.07 (1 H, dd, *J* 7.9, 10.4, 2′-H), 5.17 (1 H, dd, *J* 9.0, 9.2, 3-H), 5.35 (1 H, dd, *J* 0.8, 3.4, 4′-H) δ_C(75 MHz; $CDCl_3$) 14.1 (28-C), 20.5 (CH_3), 20.6 (CH_3 × 3), 20.7 (CH_3), 20.7 (CH_3), 20.8 (CH_3), 22.6 (27-C), 26.0 (13-C), 29.3, 29.5, 29.6, 29.7 (× 11) (7, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25-C), 31.8 (26-C), 60.7 (6′-C), 62.1 (6-C),

66.5 (4′-C), 69.0 (2′-C), 70.4 (2-C), 70.6 (5′-C), 70.9 (3′-C), 69.9, 70.3, 71.1, 71.6 (8, 9, 10, 11-C), 73.7 (3-C), 76.2 (4-C), 76.7 (5-C), 83.5 (1-C), 101.1 (1′-C), 169.0 (C=O), 169.6 (C=O), 169.6 (C=O), 170.0 (C=O), 170.1 (C=O), 170.3 (C=O × 2); *m*/*z*(TOF MS ES+) 1015.4904 (M + Na. $C_{48}H_{80}O_{19}NaS$ requires 1015.4912).

1-{2-[2-(β-D-Galactopyranosyl-(1→4)-1-thio-β-D-glucopyranosyl)ethoxy]ethoxy}octadecane 27. Anhydrous MeOH (60 cm^3) was added with stirring to a mixture of 1-{2-[2-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucopyranosyl)ethoxy]ethoxy}octadecane **21** (2.9 g, 2.92 mmol) and sodium methoxide (16 mg, 0.29 mmol) at room temperature under an atmosphere of nitrogen. Reaction conditions and purification was the same as that described for compound **25**. The reaction was followed by TLC (CH_2Cl_2–MeOH, 9:1 v/v, R_f 0.14). Compound **27** (1.91 g, 94%) was obtained as a white amorphous solid; mp 162–165 °C; $[\alpha]_D^{26}$ −16.0 (*c* 0.42 in pyridine); δ_H(300 MHz; MeOH-d^4-$CDCl_3$, 2:1 v/v) 0.81 (3 H, t, *J* 6.4, 7.0, CH_3), 1.13–1.32 (30 H, m, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27-CH_2), 1.40–1.53 (2 H, m, 12-CH_2), 2.66–2.77 (1 H, m, 7-CH_{2a}), 2.78–2.91 (1 H, m, 7-CH_{2b}), 3.17 (1 H, dd, *J* 8.1, 8.7, 2-H), 3.31–3.87 (19 H, m, 2′, 3, 3′, 4, 4′, 5, 5′, 6a, 6a′, 6b, 6b′-H, 8, 9, 10, 11-CH_2), 4.26 (1 H, d, *J* 7.4, 1′-H), 4.35 (1 H, d, *J* 9.8, 1-H); δ_C(75 MHz; MeOH-d^4-$CDCl_3$, 2:1 v/v) 14.5 (28-C), 23.7 (27-C), 26.9 (13-C), 30.0, 30.1, 30.3, 30.3, 30.4 (× 4) (7, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25-C), 33.0 (26-C), 62.0 (6′-C), 62.4 (6-C), 71.1, 71.2, 72.2, 72.4 (8, 9, 10, 11-C), 70.3, 72.5, 74.1, 74.8, 77.1, 77.8, 80.4, 80.5 (2, 3, 4, 5, 2′, 3′, 4′, 5′-C), 87.0 (1-C), 105.0 (1′-C); *m*/*z*(TOF MS ES+) 721.4164 (M + Na. $C_{34}H_{66}O_{12}NaS$ requires 721.4173).

Series B

2-[2-(2-Benzylsulfanylethoxy)ethoxy]ethanol 4. 2-[2-(2-Chloroethoxy)ethoxy]ethanol **2** (50 g, 298 mmol) was added dropwise under nitrogen to a mixture of benzyl mercaptan (31.4 cm^3, 362.8 mmol) and sodium methoxide (24 g, 268 mmol) in anhydrous MeOH (500 cm^3). The reaction mixture was boiled under reflux overnight. The reaction was followed by TLC (CH_2Cl_2–MeOH, 15:1 v/v, R_f 0.62). After 20 h, the reaction mixture was poured into a saturated aqueous solution of NaCl (500 cm^3) and the product was extracted with CH_2Cl_2 (3 × 1 dm^3). The organic phase was dried ($MgSO_4$), filtered and evaporated under reduced pressure. The crude oil was purified by chromatography on silica (CH_2Cl_2 → CH_2Cl_2–MeOH, 15:1 v/v) to give compound **4** (68.68 g, 90%) as a clear oil; δ_H(300 MHz; $CDCl_3$) 2.51–2.59 (3 H, m, 2-CH_2,-OH), 3.50–3.61 (8 H, m, 3, 4, 5, 6-CH_2), 3.62–3.68 (2 H, m, 7-CH_2), 3.70 (2 H, s, 1-CH_2), 7.23–7.37 (5 H, m, Ph); δ_C(75 MHz; $CDCl_3$) 30.5 (2-C), 36.5 (1-C), 61.6 (7-C), 70.2, 70.2, 70.6 (3, 4, 5-C), 72.4 (6-CH_2), 127.0 (11-C), 128.4, 128.8 (9, 9′, 10, 10′-C), 138.2 (8-C); *m*/*z* 274.1478 (M + NH_4^+. $C_{13}H_{24}NO_3S$ requires 274.1477), 184 (34.0%), 168 (41.5), 152 (93.0), 124 (73.5) and 91 (89.0).

{2-[2-(2-Decyloxyethoxy)ethoxy]ethylsulfanylmethyl}benzene 8. Aqueous NaOH [31.2 g, 781 mmol, in 32 g H_2O (50% w/w)],tetrabutylammonium hydrogensulfate (1.06 g, 3.12 mmol) and sodium iodide (0.59 g, 3.91 mmol) were added to a mixture of 2-[2-(2-benzylsulfanylethoxy)ethoxy]ethanol **4** (10 g, 39.05 mmol) and 1-bromodecane (10.6 cm^3, 50.76 mmol). Reaction conditions and purification were the same as described for compound **5**. The reaction was followed by TLC (hexane–EtOAc, 5:1 v/v, R_f 0.35). The crude oil obtained was purified by chromatography on silica (hexane–EtOAc, 5:1 v/v) to give compound **8** (12.03 g, 78%) as clear oil; δ_H(300 MHz; $CDCl_3$) 0.80 (3 H, t, *J* 6.8, CH_3), 1.10–1.32 (14 H, m, 10, 11, 12, 13, 14, 15, 16-CH_2), 1.46–1.55 (2 H, m, 9-CH_2), 2.56 (2 H, t, *J* 6.8, 2-CH_2), 3.36 (2 H, t, *J* 6.8, 8-CH_2), 3.47–3.58 (10 H, m, 3, 4, 5, 6, 7-CH_2), 3.69 (2 H, s, 1-CH_2), 7.13–7.27 (5 H, m, Ph); δ_C(75 MHz; $CDCl_3$) 14.0 (17-C), 22.6 (16-C), 26.0 (10-C), 29.3, 29.4, 29.5, 29.6 (× 2) (9, 11, 12,13, 14-C), 30.5 (2-C), 31.8 (15-C), 36.5 (1-C), 70.0, 70.2, 70.5, 70.6, 70.8, 71.5 (3, 4, 5, 6, 7, 8-C), 126.9 (21-C), 128.4, 128.9 (19, 19′, 20, 20′-C), 138.3 (18-C); *m*/*z*(CI) 414.3046 (M + NH_4^+. $C_{23}H_{44}NO_3S$ requires 414.3042), 386 (13.0%), 326 (100.0), 292 (20.0), 264 (64.5), 236 (35.0), 151 (50.5), 108 (34.0) and 91 (7.0).

2-[2-(2-Decyloxyethoxy)ethoxy]ethanethiol 14

Liquid ammonia (150 cm^3) was condensed into a flask at −78 °C. Small pieces of cleaned and dried sodium (1.2 g, 52.2 mmol) were added until a permanent blue colouration was obtained. The mixture was stirred at −78 °C for 10 min. A solution of {2-[2-(2-decyloxyethoxy)ethoxy]ethylsulfanylmethyl}benzene **8** (3 g, 7.57 mmol) in anhydrous THF (25 cm^3) was added *via* a syringe and the reaction mixture was stirred at −78 °C for 1 h. The reaction conditions and purification were the same as those described for compound **11**. The crude yellow oil was purified by chromatography on silica (CH_2Cl_2–EtOAc, 30:1 v/v) to give compound **14** (1.71 g, 74%) as a clear oil; TLC (CH_2Cl_2–EtOAc, 30:1 v/v, R_f 0.35); δ_H(300 MHz; $CDCl_3$) 0.85 (3 H, t, *J* 6.4, 7.0, CH_3), 1.15–1.38 (14 H, m, 9, 10, 11, 12, 13, 14, 15-CH_2), 1.50–1.61 (3 H, m, SH(t, *J* 8.3), 8-CH_2), 2.67 (2 H, dt, *J* 6.4, 6.6, 1-CH_2), 3.43 (2 H, t, *J* 6.8, 7-CH_2), 3.50–3.65 (10 H, m, 2, 3, 4, 5, 6-CH_2); δ_C(75 MHz; $CDCl_3$) 14.1 (16-C), 22.6 (15-C), 24.2 (1-C), 26.0 (9-C), 29.3, 29.5, 29.5, 29.6 (× 2) (8, 10, 11, 12, 13-C), 31.8 (14-C), 70.0, 70.2, 70.5, 70.6, 71.5, 72.8 (2, 3, 4, 5, 6, 7-C); *m*/*z*(TOF MS ES+) 329.2132 (M + Na. $C_{16}H_{34}O_3NaS$ requires 329.2126).

1-{2-[2-(2-[2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucopyranosyl]ethoxy)-ethoxy]ethoxy}decane 22. BF_3OEt_2 (1.0 cm^3, 8.08 mmol) was added dropwise over a period of 15 min to a solution of acetyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside **18** (3.66 g, 5.39 mmol) and 2-[2-(2-decyloxyethoxy)ethoxy]ethanethiol **14** (1.7 g, 5.56 mmol) in anhydrous CH_2Cl_2 (40 cm^3) at room temperature under an atmosphere of nitrogen. The reaction was followed by TLC (CH_2Cl_2-acetone, 10:1 v/v, R_f 0.18). The reaction conditions and purification were the same as used for compound **19**. The residue was deacetylated without further purification.

1-{2-[2-(2-[β-D-Galactopyranosyl-(1→4)-1-thio-β-D-glucopyranosyl]ethoxy)ethoxy]ethoxy}decane 28. Anhydrous MeOH (60 cm^3) was added to a mixture of 1-{2-[2-(2-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucopyranosyl]ethoxy)ethoxy]ethoxy}decane **22** (4.51 g, 6.65 mmol) and sodium methoxide (36 mg, 0.67 mmol) at room temperature under an atmosphere of nitrogen. The reaction mixture was stirred at room temperature and the progress of the reaction was followed by TLC (CH_2Cl_2–MeOH, 4:1 v/v, R_f 0.37). A precipitate started to form after about 1.5 h. The reaction was left to stir overnight at room temperature. After 20 h the reaction mixture was diluted with CH_2Cl_2 (100 cm^3) and MeOH (150 cm^3) to dissolve the precipitate. The solution was neutralised with Amberlyst 15, filtered and evaporated under reduced pressure to give a crude white solid which was purified by chromatography on silica (CH_2Cl_2–MeOH, 4:1 v/v) to give compound **28** (1.85 g, 63% over two steps) as a white crystalline solid; mp 155–157 °C; $[\alpha]_D^{26}$ −17.5 (*c* 0.41 in MeOH); δ_H(300 MHz; MeOH-d^4) 0.80 (3 H, m, CH_3), 1.12–1.35 (14 H, m, 15, 16, 17, 18, 19, 20, 21-CH_2), 1.40–1.53 (2 H, m, 14-CH_2), 2.66–2.7 (1 H, m, 7-CH_{2a}), 2.78–2.91 (1 H, m, 7-CH_{2b}), 3.17 (1 H, dd, *J* 8.1, 8.7, 2-H), 3.26–3.84 (23 H, m, 2′, 3, 3′, 4, 4′, 5, 5′, 6a, 6a′, 6b, 6b′-H, 8, 9, 10, 11, 12, 13-CH_2), 4.25 (1 H, d, *J* 7.3, 1′-H), 4.34 (1 H, d, *J* 9.8, 1-H); δ_C(75 MHz; MeOH-d^4)

14.5 (22-C), 23.7 (21-C), 27.2 (15-C), 30.2, 30.5, 30.6, 30.7 (× 2), 30.8 (7, 14, 16, 17, 18, 19-C), 33.0 (20-C), 62.0 (6′-C), 62.4 (6-C), 71.1, 71.3, 72.5 (× 2), 72.3, 72.4 (8, 9, 10, 11, 12, 13-C), 70.3, 72.5, 74.1, 74.8, 77.1, 77.8, 80.4, 80.5 (2, 3, 4, 5, 2′, 3′, 4′, 5′-C), 87.0 (1-C), 105.0 (1′-C); *m*/*z*(TOF MS ES+) 653.3188 (M + Na. $C_{28}H_{54}O_{13}NaS$ requires 653.3183).

{2-[2-(2-Dodecyloxyethoxy)ethoxy]ethylsulfanylmethyl}-benzene 9. Aqueous NaOH [31.2 g, 781 mmol, in 32 g H_2O (50% w/w)], tetrabutylammonium hydrogensulfate (1.06 g, 3.12 mmol) and sodium iodide (0.59 g, 3.91 mmol) were added to a mixture of 2-[2-(2-benzylsulfanylethoxy)ethoxy]ethanol **4** (10 g, 39.05 mmol) and 1-bromododecane (12.7 cm^3, 50.76 mmol). The reaction conditions and purification were the same as described for compound **5**. The reaction was followed by TLC (hexane–EtOAc, 9:2 v/v, R_f 0.41). The crude oil was purified by chromatography on silica (hexane–EtOAc, 6:1 v/v) to give compound **9** (13.15 g, 80%) as a clear oil; δ_H(300 MHz; $CDCl_3$) 0.80 (3 H, t, *J* 6.8, CH_3), 1.10–1.32 (18 H, m, 10, 11, 12, 13, 14, 15, 16, 17, 18-CH_2), 1.46–1.55 (2 H, m, 9-CH_2), 2.56 (2 H, t, *J* 6.8, 2-CH_2), 3.36 (2 H, t, *J* 6.8, 8-CH_2), 3.47–3.58 (10 H, m, 3, 4, 5, 6, 7-CH_2), 3.69 (2 H, s, 1-CH_2), 7.13–7.27 (5 H, m, Ph); δ_C(75 MHz; $CDCl_3$) 14.0 (19-C), 22.6 (18-C), 26.0 (10-C), 29.3, 29.4, 29.5 (× 4), 29.6 (9, 11, 12, 13, 14, 15, 16-C), 30.5 (2-C), 31.8 (17-C), 36.5 (1-C), 70.0, 70.2, 70.5, 70.6, 70.8, 71.5 (3, 4, 5, 6, 7, 8-C), 126.9 (21-C), 128.4, 128.9 (19, 19′, 20, 20′-C), 138.3 (18-C); *m*/*z*(CI) 442.3354 (M + NH_4^+. $C_{25}H_{48}NO_3S$ requires 442.3355), 320 (6.5), 292 (26.0), 184 (6.5), 151 (100.0), 108 (19.0) and 91 (12.5).

2-[2-(2-Dodecyloxyethoxy)ethoxy]ethanethiol 15. Liquid ammonia (150 cm^3) was condensed into a flask at −78 °C under an atmosphere of nitrogen. Small pieces of clean dried sodium (1.2 g, 52.2 mmol) were added until a permanent blue colouration was obtained. The mixture was stirred at −78 °C for 10 min. A solution of {2-[2-(2-dodecyloxyethoxy)ethoxy]-ethylsulfanylmethyl}benzene **9** (3 g, 7.07 mmol) in anhydrous THF (25 cm^3) was added *via* a syringe and the reaction mixture was stirred at −78 °C for 1 h. Reaction conditions and purification were the same as those described for compound **11**. The yellow oil was purified by chromatography on silica (CH_2Cl_2–EtOAc, 30:1 v/v) to give compound **15** (1.03 g, 44%) as a clear oil; TLC (CH_2Cl_2–EtOAc, 30:1 v/v, R_f 0.44); δ_H(300 MHz; $CDCl_3$) 0.85 (3 H, t, *J* 6.4, 7.0, CH_3), 1.15–1.38 (18 H, m, 9, 10, 11, 12, 13, 14, 15, 16, 17-CH_2), 1.50–1.61 (3 H, m, S*H*(t, *J* 8.3), 8-CH_2), 2.67 (2 H, dt, *J* 6.4, 6.6, 1-CH_2), 3.43 (2 H, t, *J* 6.8, 7-CH_2), 3.50–3.65 (10 H, m, 2, 3, 4, 5, 6-CH_2); δ_C(75 MHz; $CDCl_3$) 14.1 (18-C), 22.6 (17-C), 24.2 (1-C), 26.0 (9-C), 29.3, 29.5, 29.6 (× 4), 29.7 (8, 10, 11, 12, 13, 14, 15-C), 31.8 (16-C), 70.0, 70.2, 70.5, 70.6, 71.5, 72.8 (2, 3, 4, 5, 6, 7-C); *m*/*z*(TOF MS ES+) 357.2432 (M + Na. $C_{18}H_{38}O_3NaS$ requires 357.2439).

1-{2-[2-(2-[2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1⟶4)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucopyranosyl]ethoxy)-ethoxy]ethoxy}dodecane 23. BF_3OEt_2 (0.46 cm^3, 3.78 mmol) was added dropwise over a period of 15 min to a solution of acetyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1⟶4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside **8** (1.71 g, 2.52 mmol) and 2-[2-(2-dodecyloxyethoxy)ethoxy]ethanethiol **15** (1.01 g, 3.02 mmol) in anhydrous CH_2Cl_2 (30 cm^3) at room temperature under an atmosphere of nitrogen. Reaction conditions and purification were the same as described for compound **22**.

1-{2-[2-(2-[β-D-Galactopyranosyl-(1⟶4)-1-thio-β-D-gluco-pyranosyl]ethoxy)ethoxy]ethoxy}dodecane 29. Anhydrous MeOH (60 cm^3) was added to a mixture of crude 1-{2-[2-(2-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1⟶4)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucopyranosyl]ethoxy)ethoxy]ethoxy}do-decane **23** (2.61 g, 3.85 mmol) and sodium methoxide (21 mg, 0.39 mmol) at room temperature under an atmosphere of nitrogen. Reaction conditions and purification were the same as described for compound **28**. Compound **29** (1.12 g, 67% over two steps) was obtained as a white amorphous solid; mp 147–150 °C; $[\alpha]_D^{26}$ −19.1 (*c* 0.45 in pyridine); δ_H(300 MHz; MeOH-d^4) 0.85 (3 H, m, CH_3), 1.20–1.45 (18 H, m, 15, 16, 17, 18, 19, 20, 21, 22, 23-CH_2), 1.40–1.60 (2 H, m, 14-CH_2), 2.66–2.77 (1 H, m, 7-CH_{2a}), 2.78–2.91 (1 H, m, 7-CH_{2b}), 3.17 (1 H, dd, *J* 8.1, 8.7, 2-H), 3.26–3.84 (23 H, m, 2′, 3, 3′, 4, 4′, 5, 5′, 6a, 6a′, 6b, 6b′-H, 8, 9, 10, 11, 12, 13-CH_2), 4.25 (1 H, d, *J* 7.3, 1′-H), 4.34 (1 H, d, *J* 9.8, 1-H); δ_C(75 MHz; MeOH-d^4) 14.5 (24-C), 23.7 (23-C), 27.2 (15-C), 29.9, 30.0, 30.1, 30.2, 30.3 (× 4) (7, 14, 16, 17, 18, 19, 20, 21-C), 33.0 (22-C), 62.0 (6′-C), 62.4 (6-C), 71.1, 71.3, 72.5 (× 2), 72.3, 72.4 (8, 9, 10, 11, 12, 13-C), 70.3, 72.5, 74.1, 74.8, 77.1, 77.8, 80.4, 80.5 (2, 3, 4, 5, 2′, 3′, 4′, 5′-C), 87.0 (1-C), 105.0 (1′-C); *m*/*z*(TOF MS ES+) 681.3494 (M + Na. $C_{30}H_{58}O_{13}NaS$ requires 681.3496).

{2-[2-(2-Octadecyloxyethoxy)ethoxy]ethylsulfanylmethyl}-benzene 10. Aqueous NaOH [31.2 g, 781 mmol, in 32 g H_2O (50% w/w)], tetrabutylammonium hydrogensulfate (1.06 g, 3.12 mmol) and sodium iodide (0.59 g, 3.91 mmol) were added to a mixture of 2-[2-(2-benzylsulfanylethoxy)ethoxy]ethanol **4** (10 g, 39.05 mmol) and 1-bromooctadecane (17.3 cm^3, 50.76 mmol). Reaction conditions and purification were the same as described for compound **5**. The reaction was followed by TLC (hexane–EtOAc, 9:2 v/v, R_f 0.47). The crude oil obtained was purified by chromatography on silica (hexane–EtOAc, 6:1 v/v) to give compound **10** (14.2 g, 72%) as a white waxy solid; δ_H(300 MHz; $CDCl_3$) 0.81 (3 H, t, *J* 6.8, CH_3), 1.10–1.32 (30 H, m, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24-CH_2), 1.46–1.55 (2 H, m, 9-CH_2), 2.56 (2 H, t, *J* 6.8, 2-CH_2), 3.36 (2 H, t, *J* 6.8, 8-CH_2), 3.47–3.58 (10 H, m, 3, 4, 5, 6, 7-CH_2), 3.69 (2 H, s, 1-CH_2), 7.13–7.27 (5 H, m, Ph); δ_C(75 MHz; $CDCl_3$) 14.0 (25-C), 22.6 (24-C), 26.0 (10-C), 29.3, 29.4, 29.6, 29.7 (× 10) (9, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22-C), 30.5 (2-C), 31.8 (23-C), 36.5 (1-C), 70.0, 70.2, 70.5, 70.6, 70.8, 71.5 (3, 4, 5, 6, 7, 8-C), 126.9 (29-C), 128.4, 128.9 (27, 27′, 28, 28′-C), 138.3 (26-C); *m*/*z*(CI) 526.4298 (M + NH_4^+. $C_{25}H_{48}NO_3S$ requires 526.4294), 436 (7.0%), 404 (8.0), 376 (19.0), 332 (7.5), 268 (10.2), 151 (100.0), 108 (44.0) and 91 (27.0).

2-[2-(2-Octadecyloxyethoxy)ethoxy]ethanethiol 16. Liquid ammonia (150 cm^3) was condensed into a flask at −78 °C under nitrogen. Small pieces of clean dried sodium (1.2 g, 52.2 mmol) were added until a permanent blue colouration was obtained. The mixture was stirred at −78 °C for 10 min. A solution of {2-[2-(2-octadecyloxyethoxy)ethoxy]ethylsulfanylmethyl}benzene **10** (3 g, 5.90 mmol) in anhydrous THF (25 cm^3) was added *via* a syringe and the reaction mixture was stirred at −78 °C for 1 h. Reaction conditions and purification were the same as described for compound **15**. Compound **16** (1.57 g, 67%) was obtained as a white waxy solid; TLC (CH_2Cl_2–EtOAc, 30:1 v/v, R_f 47); δ_H(300 MHz; $CDCl_3$) 0.85 (3 H, t, *J* 6.4, 7.0, CH_3), 1.20–1.38 (30 H, m, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19,20, 21, 22, 23-CH_2), 1.50–1.61 (3 H, m, S*H*(t, *J* 8.3), 8-CH_2), 2.67 (2 H, dt, *J* 6.4, 6.6, 1-CH_2), 3.43 (2 H, t, *J* 6.8, 7-CH_2), 3.50–3.65 (10 H, m, 2, 3, 4, 5, 6-CH_2); δ_C(75 MHz; $CDCl_3$) 14.1 (24-C), 22.6 (23-C), 24.2 (1-C), 26.0 (9-C), 29.4, 29.5, 29.6, 29.7 (× 10) (8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21-C), 31.8 (22-C), 70.0, 70.2, 70.5, 70.6, 71.5, 72.8 (2, 3, 4, 5, 6, 7-C); *m*/*z*(TOF MS ES+) 441.3376 (M + Na. $C_{24}H_{50}O_3NaS$ requires 441.3378).

1-{2-[2-(2-[2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1⟶4)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucopyranosyl]ethoxy)-ethoxy]ethoxy}octadecane 24. BF_3OEt_2 (0.46 cm^3, 3.78 mmol) was added dropwise over a period of 15 min to a solution of acetyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1⟶4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside **18** (2.21 g, 3.26 mmol) and 2-[2-(2-octadecyloxyethoxy)ethoxy]ethanethiol **15** (1.5 g,

3.59 mmol) in anhydrous CH_2Cl_2 (30 cm^3) at room temperature under an atmosphere of nitrogen. The reaction was followed by TLC (CH_2Cl_2–acetone, 10:1 v/v, R_f 0.47). Reaction conditions and purification were the same as those described for compound **22**.

1-{2-[2-(2-[β-D-Galactopyranosyl-(1⟶4)-1-thio-β-D-glucopyranosyl]ethoxy)ethoxy]ethoxy}octadecane 30. Anhydrous MeOH (70 cm^3) was added to a mixture of crude 1-{2-[2-(2-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1⟶4)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucopyranosyl]ethoxy)ethoxy]ethoxy}octadecane **24** (3.47 g, 5.12 mmol) and sodium methoxide (28 mg, 0.51 mmol) with stirring under an atmosphere of nitrogen. Reaction conditions and purification was the same as described for compound **28**. The reaction was followed by TLC (CH_2Cl_2–MeOH, 4:1 v/v, R_f 0.40). Compound **30** (1.47 g, 61% over two steps) was obtained as a white amorphous solid; mp 141–144 °C; $[a]_D^{26}$ −16.9 (*c* 0.46 in pyridine); δ_H(300 MHz; MeOH-d^4) 0.85 (3 H, m, CH_3), 1.20–1.45 (30 H, m, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29-CH_2), 1.40–1.60 (2 H, m, 14-CH_2), 2.66–2.77 (1 H, m, 7-CH_{2a}), 2.78–2.91 (1 H, m, 7-CH_{2b}), 3.17 (1 H, dd, *J* 8.1, 8.7, 2-H), 3.26–3.84 (23 H, m, 2′, 3, 3′, 4, 4′, 5, 5′, 6a, 6a′, 6b, 6b′-H, 8, 9, 10, 11, 12, 13-CH_2), 4.25 (1 H, d, *J* 7.3, 1′-H), 4.34 (1 H, d, *J* 9.8, 1-H); δ_C(75 MHz; MeOH-d^4) 14.5 (30-C), 23.7 (29-C), 27.2 (15-C), 29.8, 29.9, 30.0, 30.0, 30.1, 30.2 (× 9) (7, 14, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27-C), 33.0 (28-C), 62.0 (6′-C), 62.4 (6-C), 71.1, 71.3, 72.5 (× 2), 72.3, 72.4 (8, 9, 10, 11, 12, 13-C), 70.3, 72.5, 74.1, 74.8, 77.1, 77.8, 80.4, 80.5 (2, 3, 4, 5, 2′, 3′, 4′, 5′-C), 87.0 (1-C), 105.0 (1′-C); *m/z*(TOF MS ES+) 765.4431 (M + Na. $C_{36}H_{70}O_{13}NaS$ requires 765.4435).

BIAcore experiments

Preparation of the sensor chip. An HPA sensor chip was washed overnight at 25 °C with degassed, ultrafiltered 20 mM HEPES buffer pH 7.0 (containing 90 mM Na Cl) at a flow rate of 2 μl min^{-1}. The chip was then washed with 40 mM octyl glucoside dissolved in buffer for 7 min at a flow rate of 5 μl min^{-1} immediately prior to deposition of the selected lipids. Dipalmitoyl phosphatidylcholine dissolved in chloroform:methanol 1:1 v/v was mixed with varying amounts of the selected thiolactosyl lipid in the same solvent to give solutions of 10, 15 and 25 mol% of thioglycolipid. A control sample of pure dipalmitoylphosphatidylcholine was used. The solutions were evaporated under reduced pressure, dried for several hours over P_2O_5 in a vacuum desiccator, and then suspended in HEPES buffer by warming to 30 °C and vigorously mixed (vortex mixer) to give a final concentration of phospholipid of 0.5 mM. Each of the four channels of the sensor chip was exposed for 3 h to a different concentration of thioglycolipid from 0 to 25 mol% at a flow rate of 2 μl min^{-1} and 35 °C, to allow the lipids to impregnate the existing thioalkyl layer attached to the gold surface by the manufacturers.

The chip surface was washed with a fast flow of buffer (100 μl min^{-1} for 5 min) to remove loosely adherent lipid, followed by two washes with 20 mM NaOH (5 μl min^{-1} for 5 min each) and then with buffer to stabilize the phospholipid monolayer.

Determination of the optimum glycolipid and analyte concentrations. A solution of *Ricinus communis* agglutinin (RCA_{120}) (12.5 μM) was passed over each ligand channel (5 μl min^{-1} for 5 min), followed by buffer to establish association and dissociation rates. All binding studies were measured at 25 °C and a solution of 50 mM NaOH (2 × 5 μl min^{-} for 3 min) followed by buffer was used to regenerate the ligand surface.

Channel 3 (with 15 mol% thioglycolipid) gave the best response and was exposed to varying concentrations of RCA_{120} (5, 12.5, 25, 50 μM) at a flow rate of 5 μl min^{-1} for 5 min.

Tests for non-specific binding, a negative control, and mass transfer effects. To test for non-specific binding channel 1 (with phospholipid ligand only) was exposed to the analyte RCA_{120} (12.5 μM, the optimum concentration) at 5 μl min^{-1} for 5 min.

Channel 4 (with the highest concentration of thioglycolipid) was exposed to a solution of albumin (25 μM) at 5 μl min^{-1} for 6 min as a negative control.

Binding experiments of analyte $RCA_{120}$to 15 mol% thioglycolipid ligand were performed at a range of flow rates from 5 to 50 μl min^{-1} to check whether mass transfer was a problem.

Enzymatic modification of the galactopyranoside ligands. Channel 4 was exposed to a solution of RCA_{120} (12.5 μM) for 5 min to establish a positive response level before exposure to β-galactosidase in buffer (1 mg ml^{-1}) (*Bacillus circulans*) at a flow rate of 5 μl min^{-1} for 45 min. A further injection of RCA_{120} for 5 min showed that the binding was reduced by 57%.

Kinetic measurements (binding constants and inhibition by free lactose). The concentration of RCA_{120} analyte was reduced to 25, 75, 100, 125 and 150 nM to minimize bulk refractive index changes and possible mass transport effects. Each solution was injected into both channels and channel 1 (the negative control of phospholipid) was subtracted from channel 2 (10 mol% thioglycolipid) to remove any bulk refractive index changes or possible non-specific interactions. The main thioglycolipid used in these studies was compound **30**.

Inhibition of binding of the analyte RCA_{120} was achieved by adding free lactose to the analyte before passing the solution over the thioglycolipid ligand. Solutions of 100 nm RCA_{120} contained 0, 25, 50, 80 and 100 μM lactose.

Acknowledgements

We thank Dr Jose A. Ribeiro Martins and Robert Jenkins for advice on synthetic techniques and the BBSRC and the Erasmus Organisation for financial support.

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