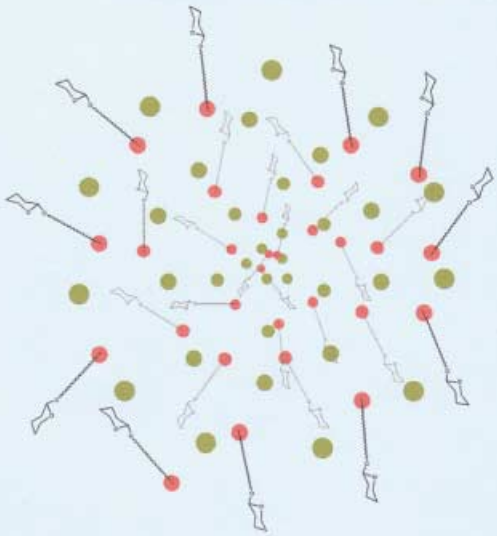
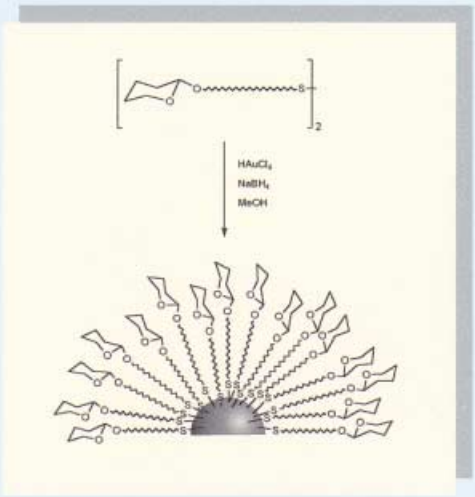
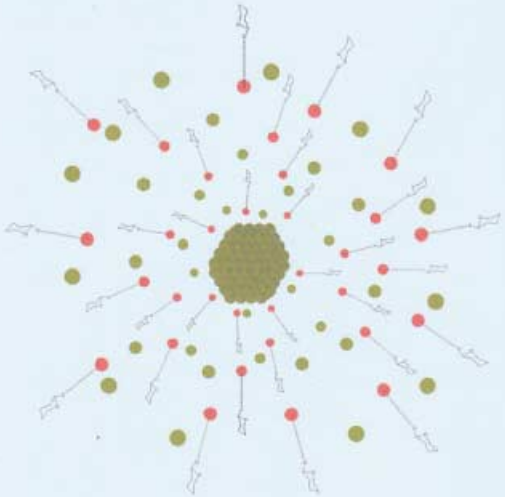
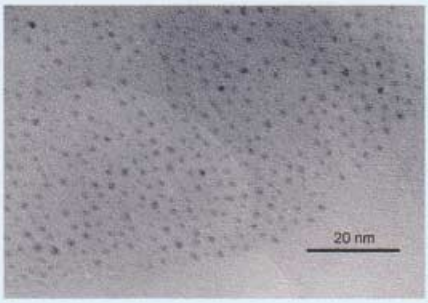
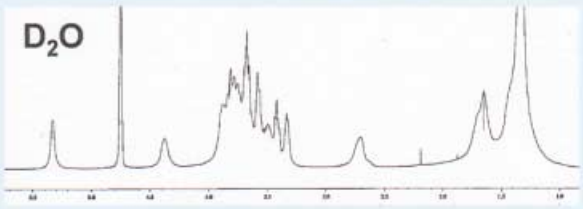
Gold Glyconanoparticles: Synthetic Polyvalent Ligands Mimicking Glycocalyx-Like Surfaces as Tools for Glycobiological Studies

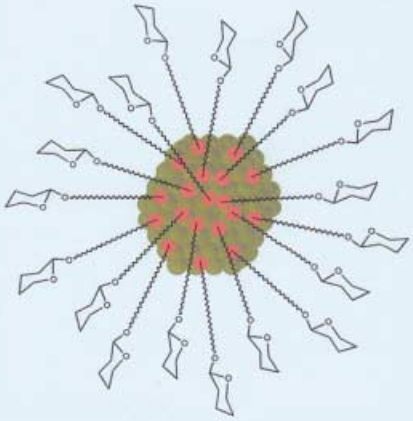












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Gold Glyconanoparticles: Synthetic Polyvalent Ligands Mimicking Glycocalyx-Like Surfaces as Tools for Glycobiological Studies

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Abstract: A simple and versatile methodology is described for tailoring sugar-functionalised gold nanoclusters (*glyconanoparticles*) that have 3D polyvalent carbohydrate display and globular shapes. This methodology allows the preparation of *glyconanoparticles* with biologically significant oligosaccharides as well as with differing carbohydrate density. Fluorescent *glyconanoparticles* have been also prepared for labelling cells in biological tests. The materials are water soluble, stable under physiological conditions and present an exceptional small core size. All of them have been characterised by ^{1}H NMR, UV and IR spectroscopy, TEM and elemental analysis. Their highly polyvalent network can mimic glycosphingolipid clustering and interactions at the plasma membrane, providing an controlled system for glycobiological studies. Furthermore, they are useful building blocks for the design of nanomaterials.

Keywords: carbohydrates · gold · multivalency · nanoparticles · nanotechnology

Introduction

The surface of most types of cells is covered with a dense coating of glycoconjugates (glycoproteins and glycolipids), the so-called glycocalyx. Repulsive forces to prevent non-specific adhesion of cells have been attributed to the glycocalyx.[1] In some cell configurations, however, this repulsive barrier is counterbalanced by the formation of cell–cell contacts through attractive forces. Cell-surface oligosaccharides contribute to these specific contacts, mainly by interactions with proteins (lectins).[2] In addition, there is now also evidence that cells use attractive forces between surface oligosaccharides as a mechanism for cell adhesion and recognition.[3]

The existence of specific interactions between carbohydrates has been demonstrated in the species-specific cell aggregation of marine sponges,[4] in morula compaction in mouse embryos and in other cellular proliferation processes.[5] Characteristic features of this interaction are a strong dependency on divalent cations, high specificity and low affinity. Although the existence of this interaction has now been accepted, its mechanism has not yet been clarified, due to the difficulty in analysing weak affinity interactions. The first thermodynamic data on carbohydrate–carbohydrate interactions between simple monosaccharides were obtained in our laboratory with a model system composed of synthetic receptors, named glycophanes, and a series of 4-nitrophenyl glycosides.[6, 7]

The main challenge facing attempts to understand and control interactions between carbohydrates is the low affinity of the interactions. Nature overcomes this problem by a polyvalent presentation of ligands and receptors at the cell surface.[8] Attempts to identify and quantify carbohydrate–carbohydrate interactions in solution with monomeric ligands have been unsuccessful.[9, 10] Multivalent carbohydrate presentation with liposomes,[11] polymers[12] and neoglycoproteins[13] has recently been used to obtain quantitative information on this interaction. Polyvalence seems to be mandatory if an increase in the strength of this interaction is to be achieved.

Our laboratory has recently developed an integrated approach to obtain chemical tools for studying Ca^{2+}-mediated carbohydrate self-recognition.[14] The elusive nature of these interactions requires data input from different methodologies to give a complete picture of the mechanism involved. Our integrated approach is based on self-assembled monolayers (SAM), by which neoglycoconjugates of important naturally

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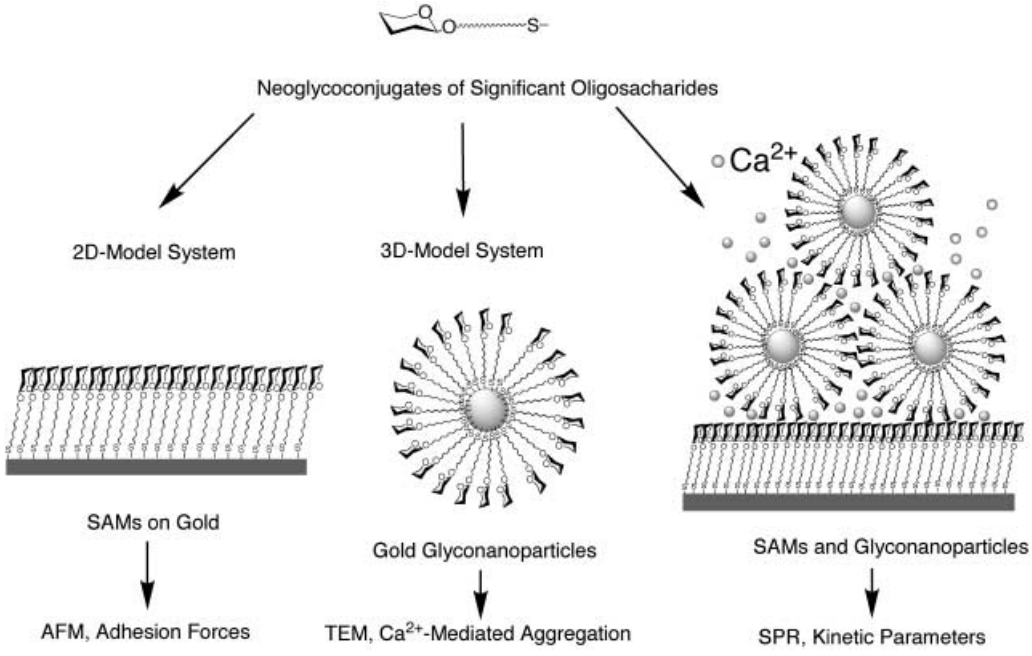
occurring oligosaccharides are attached to two- or three-dimensional (2D or 3D) gold surfaces. In this way, highly polyvalent arrays of carbohydrates, with well-defined chemical compositions, are created (Scheme 1). The 2D self-assembly of monolayers on gold is a strategy that has already been used by different groups to study carbohydrate–protein interactions.[15]

Our 3D polyvalent model system is based on gold nanoclusters functionalised with carbohydrate antigens (glyconanoparticles concept).[14] These glyconanoparticles are water-soluble polyvalent model systems that mimic glycosphingolipid (GSL) clusters. With these nanoparticles we have already demonstrated, by means of transmission electron microscopy (TEM), the selective ability of the Le^X determinant for self-recognition in calcium-containing aqueous solutions.[14] With well-defined 2D SAMs of Le^X antigen we have also determined, by means of atomic force microscopy (AFM), the adhesion forces between individual Le^X molecules.[16] Furthermore, the combination of SAMs of alkanothiolates on gold with carbohydrate epitopes as the substrate, gold glyconanoparticles as the analyte and detection by surface plasmon resonance (SPR) has allowed us to quantify the kinetics of the putative Ca^{2+}-mediated carbohydrate self-interactions (Scheme 1).[17]

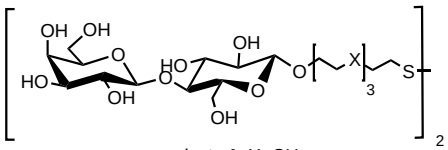
In this paper we describe in detail the methodology for tailoring these sugar-functionalised gold nanoclusters with a 3D polyvalent carbohydrate network, globular shapes and well-defined chemical composition. With this strategy we have prepared and characterised water-soluble gold glyconanoparticles, functionalised with mono-, di- and trisaccharides, as new polyvalent tools for studying carbohydrate interactions and for interfering in cell–cell adhesion processes. By varying the number and/or the nature of the neoglycoconjugates, a great structural diversity of nanoparticles can be created. Our first goal was to prepare available chemical tools that mimic GSL clustering at the cell membrane.[18] The glyconanoparticle approach also offers the possibility of preparing SAMs with different densities and spacers that resemble oligosaccharide groups in glycoproteins. Hybrid nanoparticles composed of carbohydrates and other ligands (fluorescence probes, peptides or other types of molecules) can also be prepared. The introduction of additional ligands can be used to guide the assembly of the gold clusters, thus creating a wealth of different nanostructures.[19] Therefore, these gold glyconanoparticles can be considered new and versatile polyvalent tools for basic studies in glycobiology, and also as appropriate building blocks for design of nanomaterials.

We illustrate the usefulness of this approach by the preparation and characterisation of gold glyconanoparticles functionalised with the monosaccharide glucose (*gluco*: Glc), the disaccharides lactose (*lacto*: Galβ1-4Glc) and maltose (*malto:* Glcα1-4Glc) or the trisaccharide Lewis X (Le^X: Galβ1-4[Fucα1-3]GlcNAc) antigen. This antigen mediates, through a homotypic carbohydrate–carbohydrate interaction, morula compaction and aggregation of F9 teratocarcinoma cells,[9] while lactoceramide is involved in melanoma lung metastasis in mice.[20] In addition, lactose is the primer disaccharide in the biosynthesis of all glycosphingolipids.[5a] Glucose and maltose nanoparticles have been also synthesised as reference systems.

Neoglycoconjugates **1**–**6** have been prepared and attached to gold nanoclusters. Linkers of different length and nature, **7**–**14**, have been used in the synthesis of the neoglycoconjugates. The preparation of gold glyconanoparticles protected with the neoglycoconjugates, hybrid *lacto*-nanoparticles with differing ligand density, and *lacto*- and Le^X-nanoparticles tagged with a fluorescence probe demonstrates the versatility of this approach.

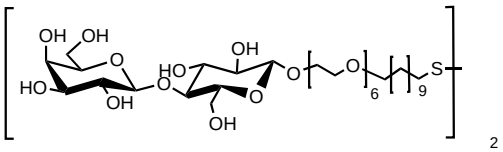


Scheme 1. Strategy for studying carbohydrate–carbohydrate interactions based on 2D and 3D models that mimic carbohydrate presentation at the cell surface.

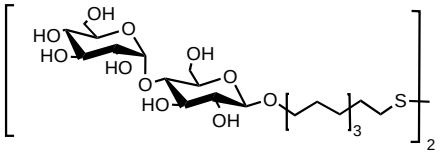


lacto **1,** X=CH_2

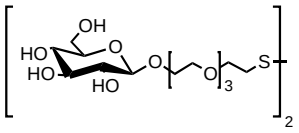
lacto **2,** X=O



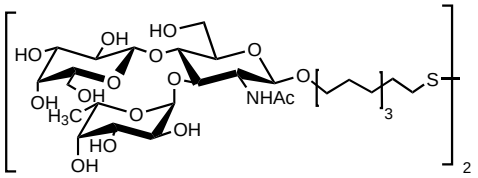
lacto **3**



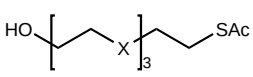
malto **4**



gluco **5**

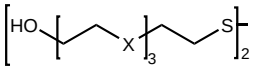


Le^x **6**



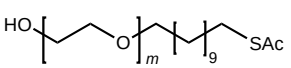
7, X=CH_2

8, X=O



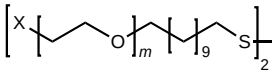
9, X=CH_2 ($C_{11}S$)

10, X=O (EG_4S)



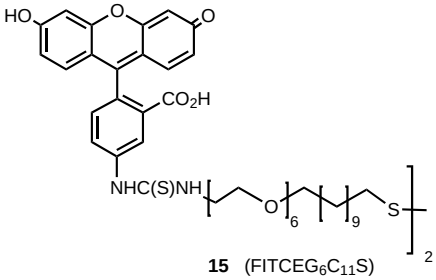
11, *m*=3

12, *m*=6



13, X=OH, *m*=3 ($EG_3C_{11}S$)

14, X=NH_2,*m*=6 ($EG_6C_{11}S$)



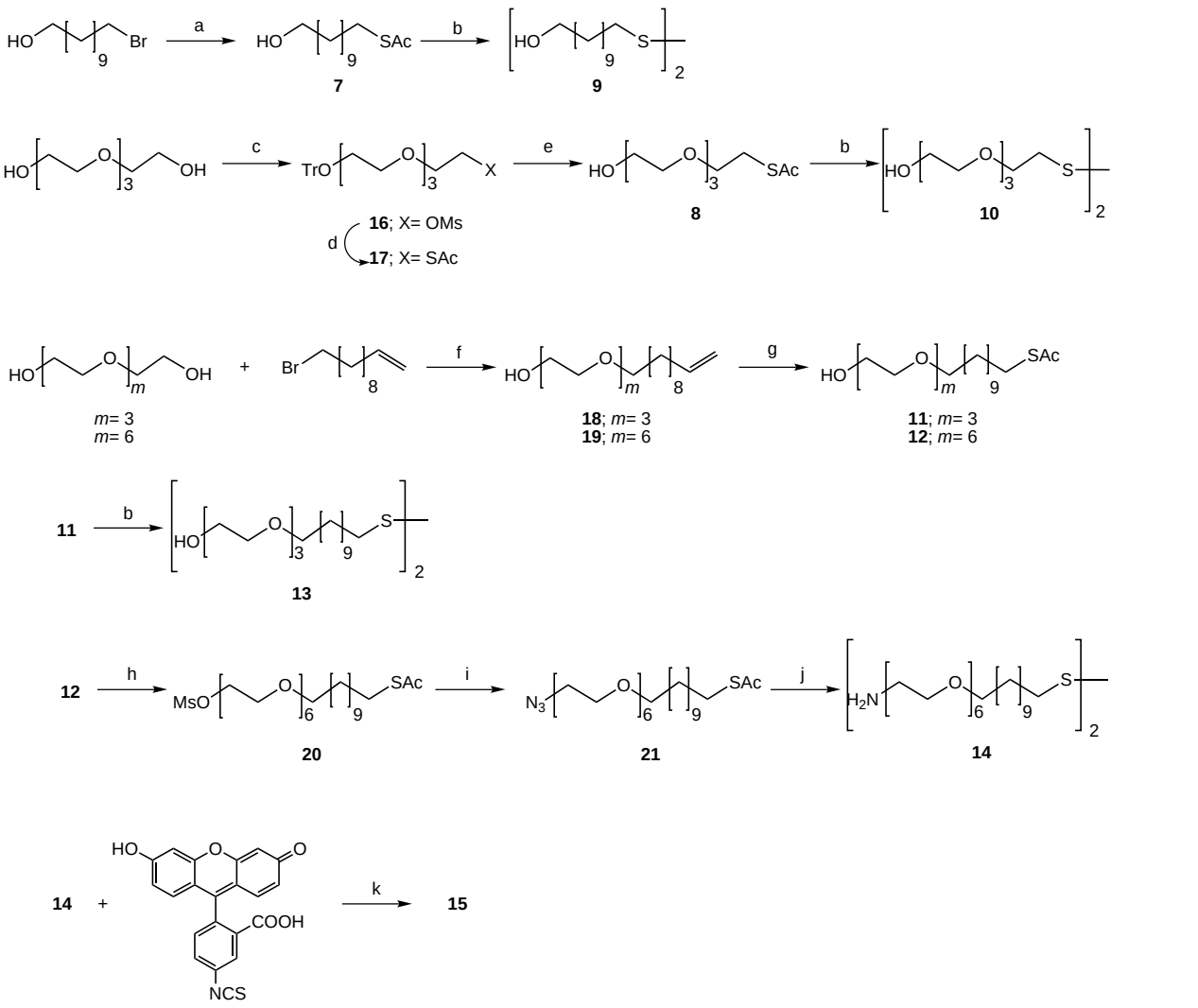
15 ($FITCEG_6C_{11}S$)

Results and Discussion

Synthesis of neoglycoconjugates 1–5: Thiol-derivatised neoglycoconjugates of lactose (**1**–**3**), maltose (**4**) and glucose (**5**) have been prepared for attachment to gold surfaces. The synthesis of neoglycoconjugate **6** has already been reported.[21] Neoglycoconjugates **1**, **2** and **6** are common molecules used in self-recognition studies of lactose and Le^X molecules by AFM,[16] biosensors,[17] and TEM.[14]

Synthesis of the disulfides **1**–**6** involves glycosidation of conveniently protected oligosaccharide derivatives with 11-thioacetate undecanol **7** (for **1**, **4** and **6**) or 11-thioacetate-3,6,9,-trioxaundecanol **8** (for **2** and **5**), by means of the trichloroacetimidate method.[22] These linkers have been used to test the influence of their nature in the formation and properties of SAMs on gold. The 11-thioundecanol provides well-packed SAMs, while less ordered SAMs have to be expected from the 11-thio-3,6,9-trioxaundecanol.[23] The larger linker **12**, which contains both the 11-thio-undecanol and hexa(ethylene glycol) moieties, was also synthesised for preparation of the neoglycoconjugate **3**, while linkers **9**, **10**, **13** and **14** were prepared for the synthesis of hybrid nanoparticles, as was the fluorescence probe **15**.

The synthesis of spacers **7**–**14** was carried out by conventional reaction sequences (Scheme 2). Linker **7** was prepared from the commercially available 11-bromoundecanol by nucleophilic displacement of bromide with potassium thioacetate, in 90% yield. Deprotection of **7** with NaOMe/MeOH gave the disulfide **9**, in near quantitative yield, after complete oxidation of the free thiol. For spacer **8**, tetra(ethylene glycol) was treated with trityl chloride and the resulting monotritylated derivative was mesylated to give **16**. Substitution of the mesylated group by thioacetate gave **17**, and subsequent cleavage of the trityl group with acetic acid in water, gave the 11-thioacetate-3,6,9-trioxa-undecanol **8** in 20% overall yield (four steps). Deprotection of the thioacetate group of **8**, as for

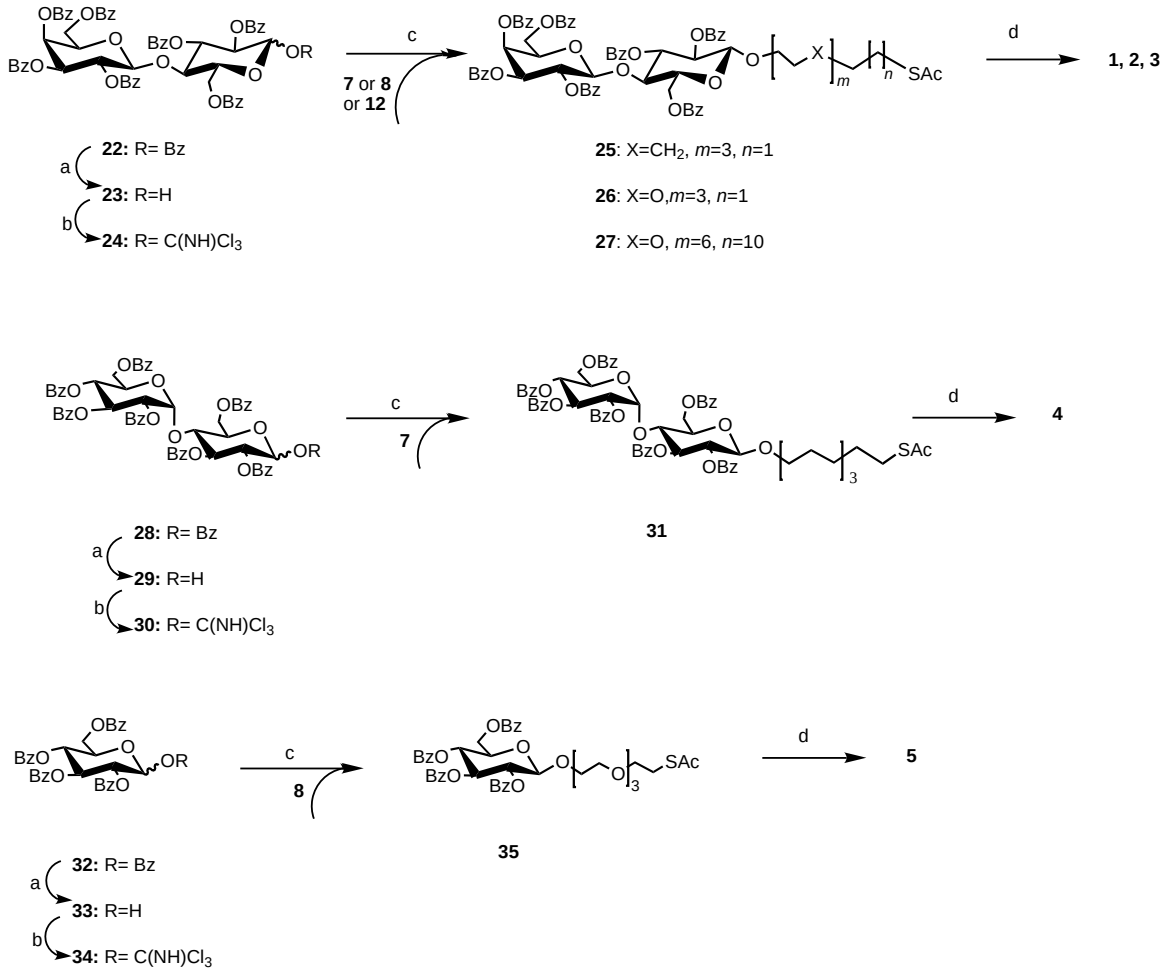


Scheme 2. a) KSAc, DMF; b) MeONa/MeOH; c) TrCl, py, 0 °C, ClMs; d) KSAc, butanone, reflux; e) AcOH/H_2O, 70 °C; f) NaOH aq (50 %), 100 °C; g) AcSH, AIBN, THF, *hν*, reflux; h) MsCl, py, RT; i) NaN_3, EtOH, $N(Bu)_4I$; j) HLiAl, THF, 0 °C; k) MeOH/THF 1:1, NaOH 1M.

7 above, gave the disulfide **10** in good yield. Linkers **11**–**14** were synthesised by previously described methods.[23] The reaction of the commercially available tri- or hexa(ethylene glycol) and 11-bromideundec-1-ene in aqueous NaOH solution gave **18** ($m = 3$) or **19** ($m = 6$), respectively, in 63 % yield. Addition of thioacetic acid in THF to the olefins **18** or **19**, a reaction catalysed by azobisisobutyronitrile (AIBN), afforded **11** ($m = 3$) or **12** ($m = 6$), respectively, in good yield. Compound **11** was deprotected to give the disulfide **13**, which was later used in the preparation of hybrid nanoparticles. After transformation of compound **12** to the mesyl derivative **20**, nucleophilic substitution with NaN_3 in ethanol afforded **21** in 70 % yield. Reduction of **21** at 0 °C with $LiAlH_4$ in THF gave a quantitative yield of the disulfide **14**. Conjugate **15** was obtained in 76 % yield by treatment of fluorescein isothiocyanate (FITC) with **14**, under basic conditions, in MeOH/THF.

For the synthesis of the *lacto*-, *malto*- and *gluco*-neoglycoconjugates **1**–**5** (Scheme 3), the perbenzoylated derivatives **22**, **28** and **32** were prepared. The glycosyl donors **24**, **30** and **34** were readily obtained from these compounds, in good yield (72 %, two steps), by selective de-O-benzoylation at the anomeric centre (to give **23**, **29** and **33**, respectively) and subsequent treatment with trichloroacetonitrile and 1,8-diazobicyclo[5.4.0]undec-7-ene (DBU) in CH_2Cl_2. The glycosyl acceptors **7**, **8** and **12** are bifunctional spacers with a hydroxyl group, which is free to establish the glycosidic linkage with the glycosyl donors **24**, **30** and **34**, and a thiol function, which allows later coupling to the gold surfaces.

Glycosidation of spacers **7**, **8** and **12**, with the benzoylated lactose derivative **24** and with trimethylsilyl triflate (TMSOTf) as the promoter, gave the neoglycoconjugates **25**, **26** and **27** in 70 %, 85 % and 70 % yield, respectively. Initial attempts at glycosidation with an acetylated lactose derivative as donor afforded mainly the corresponding *ortho*-ester.[24] The β-anomeric configuration of the benzoylated neoglycoconjugates was confirmed by the ^1H and ^{13}C NMR spectra. Zemplén deprotection[25] of **25**, **26** and **27** gave the fully deprotected *lacto*-neoglycoconjugates **1**, **2** and **3**, respectively, in good yields. Compounds **1**–**3** were isolated in disulfide form, as confirmed by NMR spectroscopy, and were used in this form for the preparation of the gold-protected surfaces. In



Scheme 3. a) $AcO^-N_2H_5^+$, DMF, 60 °C; b) Cl_3CCN, DBU, CH_2Cl_2; c) TMSOTf, CH_2Cl_2, RT; d) MeONa/MeOH

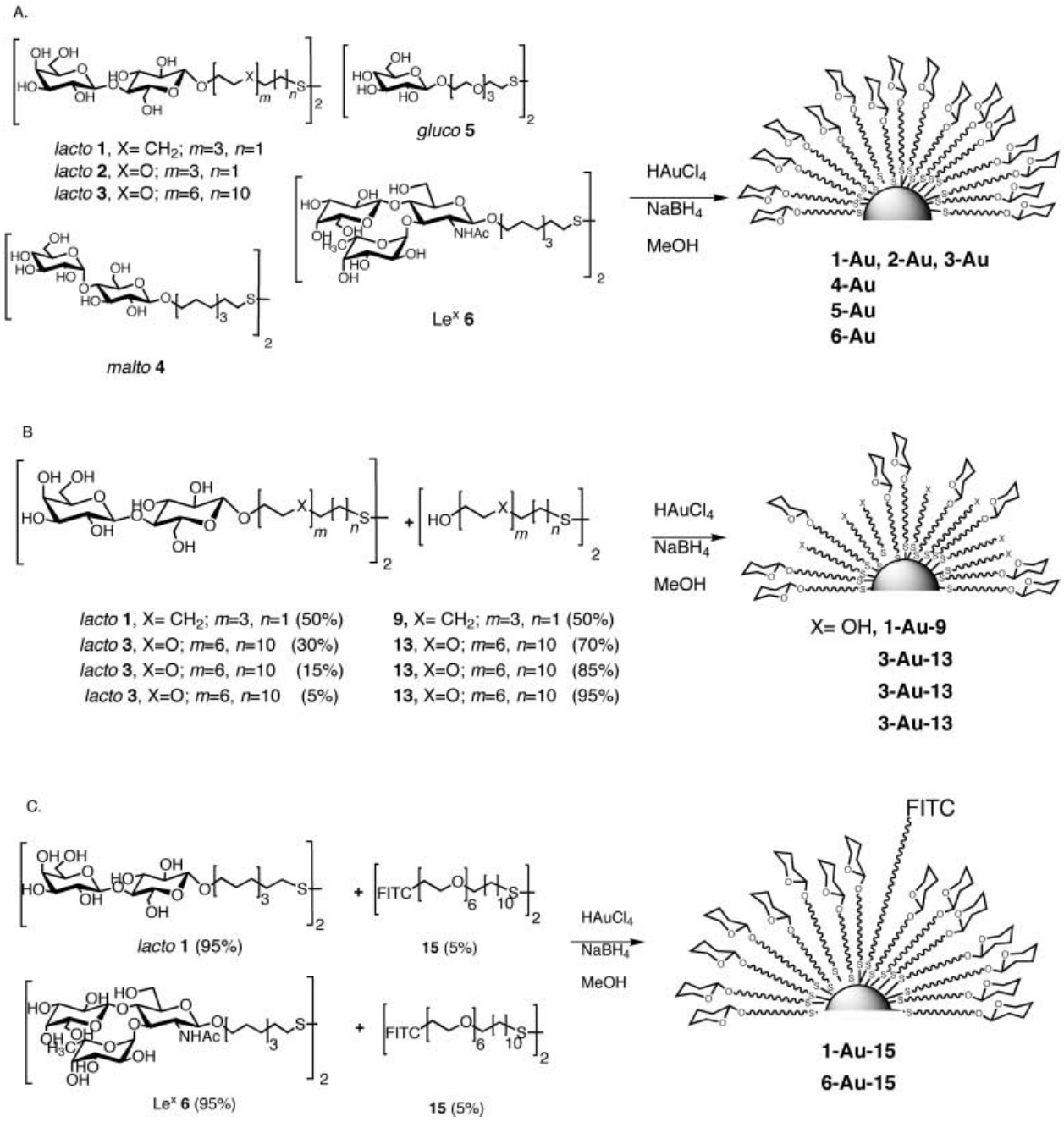
the same way, glycosidation of linker **7** with the benzoylated maltose derivative **30** afforded **31** in 70% yield. After deprotection, this compound gave the *malto*-neoglycoconjugate **4** as the disulfide derivative. A similar reaction sequence gave the *gluco*-neoglycoconjugate **5** in good yield (Scheme 3). Le^X-neoglycoconjugate **6** was obtained by a previously described method.[21]

Neoglycoconjugates **1**–**6** have been coupled in situ to gold nanoclusters to obtain the water-soluble glyconanoparticles. The same molecules have also been attached to 2D gold surfaces for evaluation of the intermolecular forces involved in carbohydrate–carbohydrate interactions by atomic force microscopy (AFM)[16] and surface plasmon resonance (SPR) spectroscopy.[17] Scheme 4 depicts the preparation of three different types of water-soluble gold glyconanoparticles that constitute our 3D-polyvalent models for studies of glycobiology.

Preparation of gold glyconanoparticles: The *lacto*-, *malto*-, *gluco*- and Le^X-protected glyconanoparticles (**1-Au** to **6-Au**, Scheme 4A) provide a glycocalyx-like surface with a well-defined synthetic matrix and globular shape. They are appropriate models for mimicking glycosphingolipid (GSL) clustering at the plasma membrane and to investigate cell adhesion through carbohydrate–carbohydrate interactions in solution. Glyconanoparticles (**1-Au-9** and **3-Au-13**, Scheme 4B), hybrids of the neoglycoconjugates **1** and **3** and the linkers **9** and **13**, have also been prepared to study the influence of oligosaccharide density in the molecular recognition of carbohydrates. Finally, for the application of the glyconanoparticles in in vitro and in vivo studies of cell adhesion processes, fluorescence tagged *lacto* and Le^X nanoparticles (**1-Au-15** and **6-Au-15**, Scheme 4C) were prepared with the fluorescein derivative **15**, as a fluorescence probe.

The preparation of glyconanoparticles was carried out by following a modified procedure of that described by Brust et al.[26] for the synthesis of monolayer-protected gold nanoclusters. Monolayer-protected gold nanoparticles with different organic molecules have recently been prepared for different purposes, but few of them are water-soluble.[27, 28] However, our approach provides, for the first time, water-soluble gold nanoclusters protected with SAMs of certain carbohydrates.

The glyconanoparticles **1-Au** to **6-Au** were obtained by adding a methanolic solution of the corresponding neoglycoconjugate to an aqueous solution of tetrachloroauric acid ($HAuCl_4$). By reduction of the resulting mixture with $NaBH_4$, a yellow to dark suspension was immediately formed. The suspension was shaken for about two hours, then the solvent was removed. They were purified by dialysis and/or centrifu-



Scheme 4. Preparation of: A) glyconanoparticles; B) hybrid glyconanoparticles; C) fluorescence glyconanoparticles.

gal filtering and characterised by ^{1}H NMR, UV-visible, and FT-infrared spectroscopy, transmission electron microscopy (TEM) and elemental analysis. The glyconanoparticles prepared are water-soluble, stable and can be manipulated as a water-soluble macromolecule.

Figure 1 shows the ^{1}H NMR spectra in D_2O and [D_6]DMSO and the corresponding TEM images for the *lacto*-**3-Au** and the *malto*-**4-Au** nanoparticles. A mean diameter of 1.8 nm and 1.0 nm was found for the gold core for the **3-Au** and **4-Au** samples, respectively. For TEM images of **1-Au** and **6-Au** see reference [14]. The gold particles stabilised with lactose (**1-Au**) show a narrower and more homogeneous particle size distribution than the particles stabilised with the Le^X-conjugate (**6-Au**). A mean diameter of 1.8 nm was found in both samples for the gold core of the funcionalised nanoparticles. Such a particle size corresponds, according to previous work,[29] to an average number of 201 gold atoms per particle. Elemental analysis for **1-Au**, **2-Au**, **3-Au** and **6-Au** confirms a ratio of 70 (**1-Au**), 63 (**2-Au**), 90 (**3-Au**) and 97 (**6-Au**) molecules per 201 gold atoms. These analyses correspond to molecular weights of 76 kD, 73 kD, 110 kD and 109 kD respectively. It is worth mentioning that the maltose-protected nanoclusters **4-Au** present an average core particle size of less than 1.3 nm. This size corresponds to the smallest soluble protected gold nanoclusters described to date and should correlate with an average number of gold atoms less than 79.[29] Elemental analysis of **4-Au** confirms a 1:1 ratio of maltose to gold atoms.

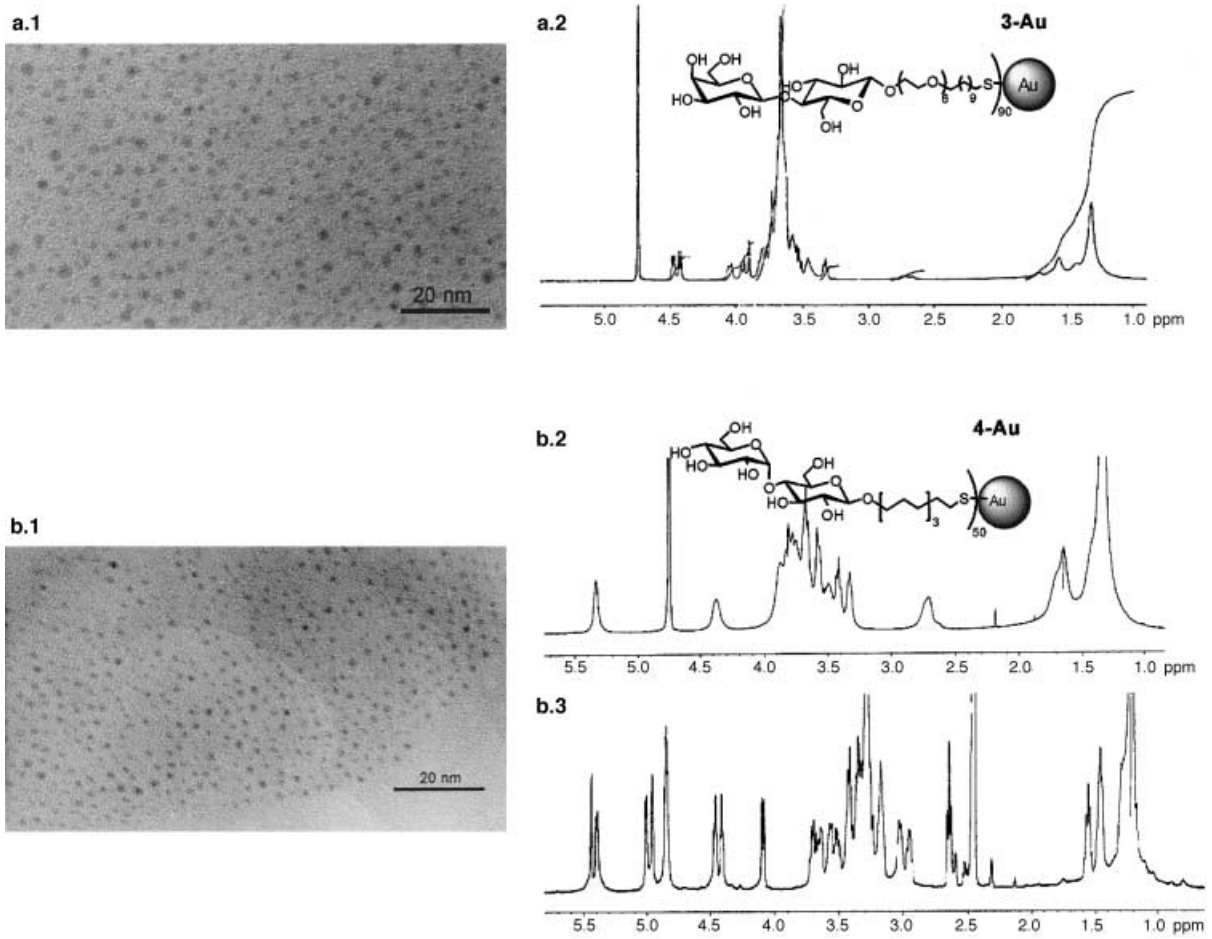


Figure 1. TEM micrographs and ^{1}H NMR spectra: a.1) TEM of glyconanoparticles **3-Au** in H_2O; a.2) ^{1}H NMR of glyconanoparticles **3-Au** in D_2O; b.1) TEM of glyconanoparticles **4-Au** in H_2O; b.2) ^{1}H NMR of glyconanoparticles **4-Au** in D_2O; b.3) ^{1}H NMR of glyconanoparticles **4-Au** in [D_6]DMSO.

The glucose-protected nanoparticles **5-Au** as well as nanoclusters protected with the linker **10** (**10-Au**) were also prepared as control models. The *gluco*-nanocluster (**5-Au**) also shows an average core diameter of 2 nm. The **10-Au** nanoclusters are highly soluble in water and methanol. The TEM images (data not shown) clearly show differences related to the sugar-protected clusters. The core size shows a bimodal distribution with average particle sizes of 2.0 nm and 6.0 nm. In some regions the largest particles form aggregates up to 100 nm in size. All nanoparticles prepared were stable in aqueous solutions for months and no agglomeration was detected on TEM examination.

The solubility of the nanoparticles differs from that of the corresponding neoglycoconjugates. For example, *lacto*-**1**, which is soluble in methanol but poorly soluble in water, gives nanoparticles that are insoluble in methanol but have good solubility in water. The Le^X derivative **6** is soluble in methanol and water; its nanoparticle, however, is insoluble in methanol but highly soluble in water. In contrast, both the maltose neoglycoconjugate **4** and the corresponding nanoparticles are highly water-soluble. The differences in solubility have been used to purify the glyconanoparticles from the unreacted disulfides by washing them with methanol.

Hybrid glyconanoparticles with neoglycoconjugates **1** and **3** and linkers **9** and **13** (**1-Au-9** and **3-Au-13**), presented here, were prepared to demonstrate the influence of the carbohydrate density in the recognition of specific carbohydrate receptors (enzymes, lectins and toxins).[30, 31] Hybrid *lacto*-nanoclusters of differing density were prepared from solutions containing different ratios of neoglycoconjugates **1** or **3** and linkers **9** or **13** in methanol (Scheme 4B). The mole fraction of the two disulfides was varied, while the total concentration of disulfide in the solution was kept constant. Depending on the neoglycoconjugate and the nature of the linker used, the composition of the resulting nanoclusters, and of the solution from which they were prepared, were the same or different. The hybrid nanoparticles prepared from a 1:1 molar ratio solution of the neoglycolipid **1** and the aliphatic linker **9** contain the same number of both molecules, as indicated by integration of the ^{1}H NMR spectrum. However, attempts to prepare glyconanoparticles from a mixture of **1** and the tetra (ethylene glycol) linker **10** resulted in pure glyconanoparticles **1-Au**, even at a 1:10 ratio of **1** to **10**, indicating that chemisorption of tetra(ethyleneglycol) on gold is less favourable than chemisorption of the aliphatic 11-thio-undecanol.[23]

The *lacto*-nanoparticles composed of **3** and linker **13** were also prepared from solutions of **13** and 5%, 15% or 30% of **3** in methanol (Scheme 4B), resulting in nanoparticles **3-Au-13** with increasing amounts of the lactose derivative. Figure 2 shows the ^{1}H NMR and corresponding TEM micrographs of

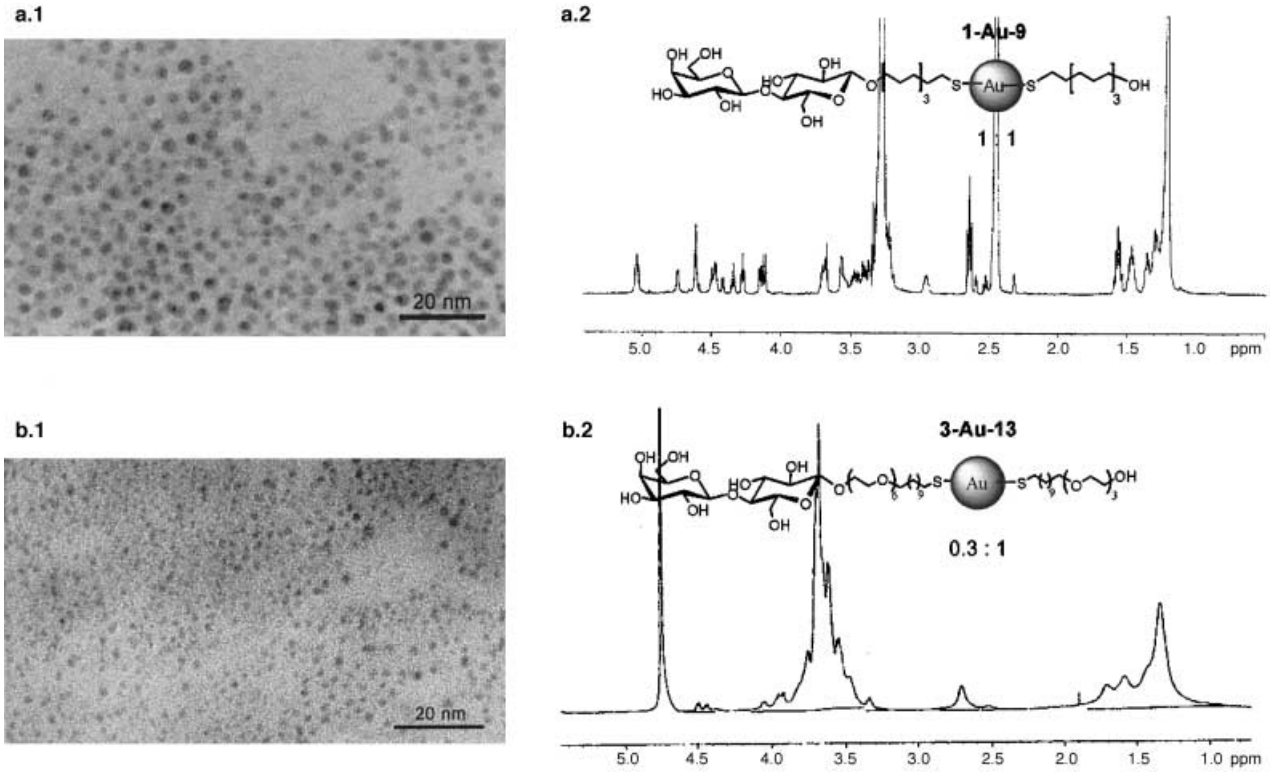


Figure 2. TEM micrographs and ^{1}H NMR spectra: a.1) TEM of glyconanoparticles **1-Au-9** in DMSO; a.2) ^{1}H NMR of glyconanoparticles **1-Au-9** in [D_6]DMSO; b.1) TEM of glyconanoparticles **3-Au-13** (30%) in H_2O; b.2) ^{1}H NMR of glyconanoparticles **3-Au-13** (30%) in D_2O.

1-Au-9 and **3-Au-13**. Mean diameters of 2.0 nm and 1.5 nm were found, respectively, for the gold core of the corresponding glyconanoparticles. Homogenous size distribution was found for both nanoclusters.

An interesting issue is the presentation of the carbohydrate molecules at the nanoparticle surface. Significant changes are observed in the ^{1}H NMR spectra of the neoglycolipids in free form and when coupled to the nanoclusters. The spectra of the *lacto* nanoparticles **1-Au** and **2-Au** in D_2O differ strongly from those of the *lacto*-disulfides **1** and **2**,[14] showing the line broadening of slowly rotating macromolecules in solution. The signals of the methylene protons closest to the thiolate/Au interface nearly disappear, as it is usual in alkanethiol monolayer-protected nanoclusters. This difference is also observed in the case of the **6-Au** nanoparticle, although the spectrum of the free neoglycolipid **6** shows similar broadening for all signals, indicating that some intramolecular association is already present in the Le^X disulfide in water.[21]

The most significant fact, however, is that the changes observed in the ^{1}H NMR spectra of the nanoparticles depend on the solvent. The glyconanoparticles **1-Au**, **2-Au**, **4-Au** and **1-Au-9** are soluble in DMSO. In this solvent, signal broadening in the ^{1}H NMR spectra of **1-Au** (data not shown), **4-Au** and **1-Au-9** was not observed (Figure 1, b.3, and Figure 2, a.2) in contrast to the broadening observed in D_2O for all glyconanoparticles except for **3-Au**. In this case the length and flexibility of the linker can explain the well-resolved signals in the spectrum in D_2O (Figure 1, a.2). The well-resolved signals in the spectra in DMSO have enabled accurate integration to determine the ratio between different ligands in the case of the hybrid *glyconanoparticles* (see Experimental Section).

An important point for the application of our model to the investigation of in vivo cell-adhesion processes is the stability to enzymatic degradation. The steric crowding of the carbohydrate moiety at the nanoparticle surface may inhibit its recognition and degradation by enzymes.[30] Preliminary results of the enzymatic hydrolysis of the neoglycoconjugates **1** and **2**, and their corresponding nanoclusters **1-Au** and **2-Au**, by β-galactosidase of *E. Coli*, resulted in a barely detectable hydrolysis of **1-Au** and **2-Au**.[14] However, low-density glyconanoparticles **3-Au-13** are processed by the enzyme, though to a lesser extent than the neoglycoconjugate **3** (unpublished results). It is clear that the accessibility of the ligand will influence the ability of a protein to bind it. A high density of ligands may show biological properties that are different from those of the same ligand presented at low density.

Fluorescent-tagged nanoparticles **1-Au-15** and **6-Au-15** (Scheme 4C) were also prepared from solutions of **1** and **6** that contained 5% of the fluorescein derivative **15** in methanol, with the aim of having a fluorescence probe for labelling the nanoparticles for biological tests. Fluorescein derivative **15** has a longer linker that the *lacto* and Le^X neoglycoconjugates **1** and **6**. The molar ratio of fluorescein to neoglycoconjugates in the prepared nanoparticles is around 1:20. The fluorescence nanoparticles are water-soluble and have a fluorescence emission band at 514 nm at a concentration of 30 $\mu g\,mL^{-1}$. The introduction of a fluorescein molecule into the *lacto* and Le^X nanoparticles confers on them the possibility of establishing additional aromatic interactions. This is clearly reflected in the two-dimensional

hexagonal structure obtained by TEM. This structure may be attributed to hydrophobic and $\pi-\pi$ interaction of the fluorescein with the carbon grid.[19]

In conclusion, we have presented a methodology for preparing, in a simple way, a wealth of polyvalent tools that mimic glycosphingolipids at the cell surface. *Glyconanoparticles* with differing carbohydrate density or incorporating other organic molecules can also be prepared by this methodology. They are composed of an exceptionally small metallic core to which antigenic glycoconjugates are covalently linked. They are soluble under physiological conditions, are stable against enzymatic degradation and provide a glycocalyx-like surface with a globular shape and a well-defined structure. These features convert the glyconanoparticles into a promising new tool for biological and biotechnological applications.[32] Furthermore, the nanoparticle methodology allows us to the control of size and pattern arrangement of the metallic cluster, providing useful building blocks for design of nanomaterials.[19] The application of this methodology to the preparation of glyconanoparticles with other biologically significant oligosaccharides, as well as to preparation of glyconanoparticles with semiconductor and magnetic properties, is already under way.

Experimental Section

General procedures: TLC analysis was performed on silica gel 60 F_{254} precoated on aluminium plates (Merck); the compounds were detected by staining with sulphuric acid/ethanol (1:9, v/v) followed by heating at over 200 °C. Column chromatography was carried out on silica gel 60 (0.2–0.5 mm; 0.2–0.063 mm; 0.040–0.015 mm; Merck). Optical rotations were determined with a Perkin–Elmer 341 polarimeter. ^{1}H and ^{13}C NMR spectra were acquired on Bruker DPX-300, DRX-400, and DRX-500 spectrometers and chemical shifts are given in ppm (δ) relative to the residual signal of the solvent used. Elemental analyses were performed with a Leco CHNS-932 apparatus, after drying analytical samples over phosphorous pentoxide for 24 hours. MALDI-TOF mass spectra were recorded with a MALDI-TOF GSG system spectrometer. Samples of the products were dissolved in MeOH at mM concentration and 2,5-dihidroxybenzoic acid was used as a matrix. UV spectra were carried out with a UV/Vis Perkin–Elmer Lambda 12 spectrometer. Fluorescence spectra were carried out in a Aminco–Bowman Series 2 Spectronic Instruments luminiscence spectrometer in miliQ water.

For TEM examinations, a single drop (20 μL) of the aqueous solution (ca. 0.1 mg mL^{-1}) of the gold glyconanoparticles was placed onto a copper grid coated with a carbon film. The grid was left to dry in air for several hours at room temperature. TEM analysis was carried out in a Philips CM200 microscope working at 200 kV. The particle size distribution of the gold nanoparticles was evaluated from several micrographs by means of an automatic image analyser.

Preparation of the starting materials **7**, **9**, **16**, **17**, **8**, **10**, **18**, **19**, **11**, **12**, **13**, **20**, **21**, **14**, **22**, **28**, **32**, **23**, **29**, **33**, **24**, **30** and **34** are reported in the Supporting Information.

29,29′-Dithiobis(*N*-fluorescein,*N*′-3,6,9,12,15,18-hexaoxanonacosanylthiourea(15): Fluorescein isothiocyanate (50 mg, 0.13 mmol, 6 equiv) and 1M aqueous sodium hydroxide (65 μL) were added to compound **14** (20 mg, 0.02 mmol, 1 equiv) in a mixture of methanol and THF (1:1, 5 mL). The reaction mixture was then concentrated to dryness under reduced pressure. The crude residue was purified by silica gel column chromatography with CH_2Cl_2/methanol/AcOH (4:1:0.05) as eluent to give the fluorescein compound **15** (14 mg, 0.0082 mmol, 76%). ^{1}H NMR (300 MHz, CD_3OD): $\delta = 7.20-6.50$ (m, 9H; FITC), 4.09 (s, 3H), 3.80–3.30 (m, 21H), 3.12 (t, 2H; CH_2O), 2.65 (m, 2H; CH_2S), 1.60–1.00 ppm (m, 18H; CH_2CH_2); MALDI-TOF: m/z: 860.7 [$M/2-H_2O$+Na]$^+$, 876.7 [$M/2-H_2O$+K]$^+$.

General procedure for imidate glycosylations

Synthesis of compounds 25, 26, 27, 31 and 35: A solution of the tricholoroacetimidate **24**, **30** or **34** (1 equiv) and the acceptor **7**, **8** or **12** (1.5 equiv) in dry CH_2Cl_2 (20 mL) was treated with TMSOTf (0.04 equiv) at room temperature under an argon atmosphere. After 15 min, the reaction mixture was concentrated to dryness and purified using a flash column.

11-Thioacetylundecanyl-2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (25): Reaction of trichloroacetimidate **24** (3.5 g, 2.91 mmol) and the acceptor **7** (0.75 g, 3.5 mmol) afforded **25**. Column chromatography (hexane/AcOEt 3:1) gave **25** as an amorphous solid (2.64 g, 2.03 mmol, 70%). $[\alpha]_D^{20} = +49.2$ ($c = 1$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.20-7.10$ (m, 35H; 7Bz), 5.78 (t, $J =$ 9.5 Hz, 1H; H-3), 5.70(m, 2H; H-2′, H-4′), 5.43 (dd, $J = 1.0$, 8.0 Hz, 1H; H-2), 5.36 (dd, $J = 10.5$, 3.5 Hz, 1H; H-3′), 4.85 (d, $J = 8.0$ Hz, 1H; H-1), 4.66 (d, $J = 8.0$ Hz, 1H; H-1′), 4.58 (dd, $J = 10.5$, 2.0 Hz, 1H; H-6a), 4.47 (dd, $J = 12.3$, 4.3 Hz, 1H; H-6b), 4.23 (t, $J = 9.5$ Hz, 1H; H-4), 3.88–3.78 (m, 3H; H-5, H-5′, OCH_2-), 3.72 (dd, $J = 11.0$, 6.5 Hz, 1H; H-6′a), 3.66 (dd, $J = 11.2$, 6.7 Hz, 1H; H-6′b), 3.41 (m, 1H; CH_2O), 2.83 (t, $J = 7.5$ Hz, 2H; CH_2S), 2.30 (s, 3H; SAc), 1.50–1.07 ppm (m, 18H; 9 CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 196.1$ (COS) 165.8, 165.5, 165.3, 165.1, 164.7 (7COO), 133.4, 133.3, 133.1, 129.9, 129.7, 129.6, 129.3, 128.7, 128.4, 128.2, 101.1, 100.8 (C-1, C-1′), 76.0, 72.9, 71.3, 70.2, 69.8, 62.4 (C-6), 61.0 (C-6′), 31.8 (CH_3COS), 30.6, 29.6, 29.4, 29.1, 29.0, 28.7 ppm; MALDI-TOF: m/z: 1320 [M+Na]$^+$; elemental analysis calcd (%) for $C_{74}H_{72}O_{19}S$ (1297): C 68.4, H 5.6; found: C 68.3 H 5.6.

11-Thioacetyl-3,6,9-trioxaundecanyl-2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (26): Reaction of trichloroacetimidate **24** (3.5 g, 2.91 mmol) and the acceptor **8** (0.86 g, 3.49 mmol) gave **26**. Column chromatography (hexane/AcOEt 3:2) gave **26** as an amorphous solid (3.4 g, 2.63 mmol, 80%). $[\alpha]_D^{20} = +32.0°$ ($c = 1$, in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.07-7.19$(m, 35H; 7Bz), 5.76 (t, $J = 9.5$ Hz, 1H; H-3), 5.70–5.66 (m, 2H; H-2′, H-4′), 5.42 (dd, $J = 9.5$, 8.0 Hz, 1H; H-2), 5.33 (dd, $J = 10.5$, 3.5 Hz, 1H; H-3′), 4.84 (d, $J = 7.8$ Hz, 1H; H-1), 4.81 (d, $J = 8.1$ Hz, 1H; H-1′), 4.60 (m, 1H; H-6), 4.48 (m, 1H; H-6), 4.25 (t, $J = 9.6$ Hz, 1H; H-4), 3.93–3.81 (m, 4H; H-5, H-5′, H-6′, H-6′), 3.76–3.33 (m, 12H; CH_2O), 3.06 (t, $J = 6.3$ Hz, 1H; CH_2S), 2.34 ppm (s, 3H; SAc); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 171.1$, 165.8, 165.4, 165.1, 165.2, 164.8, 133.5, 133.4, 133.1, 130.0, 129.7, 129.6, 128.5, 128.3, 128.2, 101.2, 101.0 (C-1, C-1′), 76.1, 73.0, 71.8, 71.4, 70.6, 70.5, 70.4, 69.9, 69.7, 69.3, 67.5, 62.4, 61.1 (C-6, C-6′), 60.4, 29.7 (CH_2S), 28.8 ppm (SAc); MALDI-TOF: m/z: 1327 [M+Na]$^+$; elemental analysis calcd (%) for $C_{71}H_{68}O_{22}S \cdot \frac{1}{2} H_2O$ (1313): C 64.6, H 5.3; found: C 64.6, H 5.3.

29-Thioacetyl-3,6,9,12,15,18-hexaoxanonacosyl-2,3,4,6-tetra-*O*-benzoyl-β-D-galactopiranosyl(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopiranoside (27): Reaction of trichloroacetimidate **24** (1.74 g, 1.45 mmol) and the acceptor **12** (1 g, 1.74 mmol) gave **27**. Column chromatography (hexane/AcOEt 3:2) gave **27** as an amorphous solid (1.6 g, 1.01 mmol, 70%). $[\alpha]_D^{20} = +20.0°$ ($c =$ 1, in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.08-7.20$(m, 35H; 7Bz), 5.77(t, $J = 10.0$ Hz, 1H; H-3), 5.70–5.67 (m, 2H; H-2′, H-4′), 5.43 (t, 1H, $J = 8.5$ Hz, H-2), 5.34 (dd, $J = 10.5$, 3.0 Hz, 1H; H-3′), 4.84 (d, $J = 7.5$ Hz, 1H; H-1), 4.67 (d, $J = 8.1$ Hz, 1H; H-1′), 4.57 (d, $J = 12.0$ Hz, 1H; H-6), 4.45 (dd, $J = 12.0$, 4.5 Hz, 1H; H-6), 4.22 (t, $J = 9.5$ Hz, 1H; H-4), 3.90–3.78 (m,2H), 3.70–3.30 (m, 28H), 2.83 (t, $J = 7.0$ Hz, 2H; CH_2S), 2.29 (s, 3H; SAc), 1.54–1.50 (m, 4H), 1.23 ppm (s, 16H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 1.97$ (COS), 165.9, 165.6, 165.4, 165.3, 165.2, 164.8 (CH=CH), 133.4, 133.2 (CH=CH), 130.0, 129.8, 129.7, 128.6, 128.4, 128.2, 101.3, 101.0 (C-1, C-1′), 76.1, 73.0, 71.8, 71.5, 71.4, 70.6, 69.9, 69.4, 67.5, 62.4 61.1 (C-6, C-6′), 30.6, 29.5, 28.8, 26.1 ppm; MALDI-TOF: m/z: 1587 [M+Na]$^+$; elemental analysis calcd (%) for $C_{86}H_{98}O_{25}S$ (1562): C 66.0, H 6.3; found: C 65.5, H 6.5.

11-Thioacetylundecanyl-2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (31): Reaction of trichloroacetimidate **30** (3.5 g, 2.91 mmol) and the acceptor **7** (0.75 g, 3.5 mmol) afforded **31**. Column chromatography (hexane/AcOEt 3:1) afforded **31** as an amorphous solid (2.60 g, 2.03 mmol, 70%). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.20-7.17$ (m, 35H; 7Bz), 6.06 (t, $J = 10.0$ Hz, 1H; H-3′), 5.73 (t, $J =$ 9.0 Hz, 1H; H-3), 5.71 (d, $J = 3.5$ Hz, 1H; H-1′), 5.62 (t, $J = 10.0$ Hz, 1H; H-4′), 5.28 (dd, $J = 10.0$, 7.5 Hz, 1H; H-2), 5.23 (dd, $J = 10.5$, 9.5 Hz, 1H; H-2′), 4.89 (dd, $J = 12.0$, 2.5 Hz, 1H; H-6′), 4.75 (d, $J = 7.5$ Hz, 1H; H-1), 4.72 (dd, $J = 12.5$, 4.5 Hz, 1H; H-6), 4.48 (t, $J = 9.0$ Hz, 1H; H-4), 4.44 (m,

1H; H-5′), 4.37 (dd, J = 12.0, 3.0 Hz, 1H; H-6), 4.24 (dd, J = 12.0, 4.5 Hz, 1H; H-6), 4.11–4.06 (m, 2H; H-6, H-5), 3.85 (m, 1H; CH_2O), 3.46 (m, 1H; CH_2O), 2.81 (t, J = 7.5 Hz, 2H; CH_2S), 2.32 (t, 3H; SAc), 1.70–0.92 ppm (m, 18H; 9 CH_2); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 196.0 (COS), 165.6, 165.0 (Bz), 133.3, 133.2, 133.0, 129.9, 129.8, 129.7, 129.5, 128.9, 128.5, 128.3, 128.1, 128.0 (CH=CH) 100.7, 96.3, (C-1, C-1′), 75.0, 73.2, 72.3, 70.8, 70.1, 69.9, 69.0, 63.5, 62.5 (C-6, C-6′), 30.6 (CH_2O), 29.3, 29.1, 29.0, 28.7 ppm (CH_2CH_2); MALDI-TOF: m/z: 1320 $[M+Na]^+$

11-Thioacetyl-3,6,9-trioxaundecanyl-2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside (35): Reaction of trichloroacetimidate **34** (1.3 g, 1.8 mmol) and the acceptor **8** (0.33 g, 1.3 mmol) gave **34**. Column chromatography (hexane/AcOEt 3:2) gave **34** as an amorphous solid (0.8 g, 1 mmol, 75%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.10–7.80 (m, 8H; Bz), 7.60–7.20 (m, 12H; Bz), 5.90 (t, J = 9.6 Hz, 1H; H-3), 5.68 (t, J = 9.9 Hz, 1H), 5.52 (t, J = 8.7 Hz, 1H), 4.97 (d, J = 7.8 Hz, 1H; H-1), 4.64 (dd, J = 12.3, 3.3 Hz, 1H), 4.49 (dd J = 12.0, 5.1 Hz, 1H), 4.20–4.18 (m, 3H), 4.05–3.95 (m, 1H; H-6a) 3.90–3.75 (m, 1H; H6b), 3.70–3.35 (m, 12H; OCH_2CH_2O), 3.08 (t, J = 6.5 Hz, 2H; CH_2S), 2.32 ppm (s, 3H; SAc).

General procedure for debenzoylations

Synthesis of compounds 1, 2, 3, 4 and 5: A solution of **25, 26**, **27, 31** or **35** (1 equiv) in dry MeOH (25 mL) was treated with 1N methanolic solution of NaOMe. The reaction was stirred under an argon atmosphere. The reaction mixture was left for 24 h, without the argon atmosphere, to complete the oxidation of the thiol group. After 24 h, TLC (CH_2Cl_2/MeOH 35%) showed the appearance of a new product (R_f = 0.5). The reaction was neutralised with Amberlist IR-120 resin, filtered and evaporated to dryness. The residue was washed with diethyl ether (15 mL) to remove methyl benzoate.

11,11′-Dithiobis[undecanyl(β-D-galactopyranosyl)(1→4)-β-D-glucopyranoside] (1): This compound was prepared from **25** (3 g, 2.31 mmol). The product precipitated as a white solid that, after washing with cold ethanol, gave the disulfide **1** (0.97 g, 1.84 mmol, 80%). $[\alpha]_D^{20} = -5.3^\circ$ (c = 0.9 in MeOH); ^{1}H NMR (500 MHz, MeOD): δ = 4.35 (d, J = 7.5 Hz, 1H; H-1), 4.27 (d, J = 8.0 Hz, 1H; H-1′), 3.90–3.86 (m, 2H), 3.83 (dd, 1H), 3.80 (dd, J = 3.5, 0.5 Hz, 1H; H-4′), 3.76 (dd, J = 11.5, 8.0 Hz, 1H; H-6), 3.68 (dd, J = 11.5, 5.0 Hz, 1H; H-6′), 3.38 (m, 1H; H-5), 3.22 (dd, J = 8.9, 8.0 Hz, 1H; H-4), 2.57 (t, J = 7.5 Hz, 2H; CH_2S), 1.64–1.28 ppm (m, 19H; CH_2); ^{13}C NMR (125 MHz, MeOD): δ = 105.9, 105.1 (C-1, C-1′), 81.5, 77.9, 77.3, 75.6, 73.4, 71.8, 71.2, 63.4, 62.9, 56.6, 32.7, 31.9, 31.5, 31.1, 30.7, 28.0 ppm; MALDI-TOF: m/z: 1096 $[M+K]^+$, 597.3; elemental analysis calcd (%) for $(C_{23}H_{43}O_{11}S)_2$ (1054): C 52.0, H 8.3; found: C 51.7, H 8.3.

11,11′-Dithiobis[3,6,9-trioxaundecanyl(β-D-galactopyranosyl)(1→4)-β-D-glucopyranoside] (2): This compound was prepared from **26** (3 g, 2.68 mmol). The product precipitated as a white solid that, after washing with cold ethanol, gave the disulfide **2** (1.14 g, 2.14 mmol, 80%). $[\alpha]_D^{20} = -4.0^\circ$ (c = 0.7 in MeOH); ^{1}H NMR (300 MHz, D_2O): δ = 4.51 (d, J = 8.1 Hz, 1H; H-1′), 4.45 (d, J = 7.8 Hz, 1H; H-1), 4.04–4.00 (m, 1H; H-5), 3.98 (m, 1H; H-6), 3.92 (d, J = 3.0 Hz, 1H; H-4), 3.38–3.20 (m, 1H; H-5′), 2.98–2.71 ppm (m, 2H; CH_2S); ^{13}C NMR (125 MHz, MeOD): δ = 105.9, 105.1 (C-1, C-1′), 81.5 (C-4), 77.9, 77.3, 77.1, 75.7, 75.6, 73.4 (C-2′), 72.8, 72.4, 72.1, 70.6 (CH_2O), 63.3, 62.8 ppm (C-6′, C-6); MALDI-TOF: m/z: 1089 $[M+Na]^+$, 601; elemental analysis calcd (%) for $(C_{20}H_{38}O_{14}S)_2 \cdot H_2O$ (1084): C 44.2 H: 7.2; found: C 44.0 H 7.7

29,29′-Dithiobis[3,6,9,12,15,18-hexaoxaundecanyl(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside] (3): This compound was prepared from **27** (0.8 g, 0.5 mmol). The product precipitated as a white solid that, after washing with cold ethanol, gave the disulfide **3** (0.34 g, 0.42 mmol, 85%). $[\alpha]_D^{20} = -6.4^\circ$ (c = 0.7 in MeOH); ^{1}H NMR (500 MHz, D_2O): δ = 4.47(d, J = 8.0 Hz, 1H; H-1′), 4.41 (d, J = 7.5 Hz, 1H; H-1), 4.03 (m, 1H), 3.94 (m, 1H; H-6), 3.90 (d, J = 3.0 Hz, 1H; H-4′), 3.82–3.62 (m, 30H), 3.57–3.50 (m, 4H), 3.46–3.43 (m, 2H), 3.32 (t, J = 8.5 Hz, 1H; H-4), 2.60–2.55 (m, 2H; CH_2S), 1.60–1.55 (m, 4H), 1.38–1.30 ppm (m, 14H); ^{13}C NMR (125 MHz, D_2O): δ = 102.6, 101.8 (C-1, C-1′), 78.1, 75.0, 74.9, 74.4, 74.0, 72.4, 72.2, 70.7, 70.6, 69.6, 69.5, 69.4, 69.3, 68.3, 68.2, 60.6, 59.8 (C-6, C-6′), 30.5, 29.5, 29.3, 29.2, 29.0, 28.9, 28.5, 25.8, 25.7 ppm; MALDI-TOF: m/z: 1620 $[M+K]^+$, 860; elemental analysis calcd (%) for $(C_{35}H_{67}O_{17}S)_2 \cdot H_2O$ (1600): C 51.9 H: 8.5; found: C 51.8 H 8.9.

11,11′-Dithiobis[undecanyl(α-D-glucopyranosyl)(1→4)-β-D-glucopyranoside] (4): This compound was prepared from **31** (3 g, 2.31 mmol). The product precipitated as a white solid that, after washing with cold ethanol, gave the disulfide **4** (0.97 g, 1.84 mmol, 80%). $[\alpha]_D^{20} = +57.3$ (c = 1 in MeOH); ^{1}H NMR (500 MHz, MeOD): δ = 5.13 (d, J = 3.5 Hz, 1H; H-1′), 4.24 (d, J = 8.0 Hz, 1H; H-1), 3.41 (dd, J = 9.5, 3.5 Hz, 1H; H-2′), 3.20 (t, J = 9.2 Hz, 1H; H-2), 2.65 (t, J = 7.5 Hz, 2H; CH_2S), 1.66–1.57 (m, 4H; 2CH_2), 1.38–1.29 ppm (m, 14H; 7CH_2); ^{13}C NMR (125 MHz, MeOD): δ = 101.0, 99.6 (C-1, C-1′), 78.0, 74.5, 73.3, 71.4, 70.8, 68.2, 67.7, 59.4, 58.9 (C-6, C-6′), 27.5, 27.3, 27.0, 26.2, 26.1, 23.8 ppm; MALDI-TOF: m/z: 1077 $[M+Na]^+$, 595; elemental analysis calcd (%) for $(C_{23}H_{43}O_{11}S)_2 \cdot 5H_2O$ (1144): C 48.4, H 8.3; found: C 48.4, H 7.8.

11,11′-Dithiobis(3,6,9-trioxaundecanyl-β-D-glucopyranoside) (5): This compound was prepared from **35** (0.8 g, 1 mmol). The product precipitated as a white solid that, after washing with cold ethanol, gave the disulfide **5** (0.37 g, 2.14 mmol, quantitative). $[\alpha]_D^{20} = -4.0$ (c = 0.7 in MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ = 4.28 (d, J = 7.5 Hz, 1H; H-1), 4.04–4.00 (m, 1H; H-5), 3.84 (d, J = 13.5 Hz, 1H), 3.75–3.60 (m, 14H; OCH_2CH_2), 3.37–3.20 (m, 6H), 3.17 (t, J = 8.7 Hz, 1H), 2.89 ppm (t, J = 6.5 Hz, 2H; CH_2S); ^{13}C NMR (125 MHz, CD_3OD): δ = 102.7 (C-1), 76.2, 76.1, 73.3, 69.8, 69.7, 69.7, 69.5, 68.7, 67.9, 61.0, 37.9 ppm; MALDI-TOF: m/z: 765.1 $[M+Na]^+$, 781.8 $[M+K]^+$, 395.1 $[M/2+Na]^+$.

General procedure for preparation of glyconanoparticles

Synthesis of compounds 1-Au, 2-Au, 3-Au, 4-Au, 5-Au, 6-Au and 10-Au: A solution of disulfide (0.012 M, 5.5 equiv) in MeOH was added to a solution of tetrachloroauric acid (0.025 M, 1 equiv) in water. $NaBH_4$ (1M, 22 equiv) in water was then added in small portions with rapid shaking. The black suspension formed was shaken for an additional 2 h and the solvent was then removed. The residue (20–50 mg of crude product) was dissolved in 10 mL of NANOPURE water and was purified by centrifugal filtering (CENTRIPLUS M_r = 30000, 1 h, 3000 xg). The process was repeated until the nanoparticles were free of salts and starting material (absence of signals due to disulfide and Na^+ in ^{1}H NMR and ^{23}Na NMR spectra). The residue in the centriplus filter was dissolved in water (2 mL) and lyophilised. The molecular formula of the nanoparticles was calculated, based on the average diameter obtained by TEM[32], and confirmed by elemental analysis.

***lacto*-C_{11}SAu (1-Au)**: Reaction of disulfide **1** (200 mg, 0.19 mmol) and $HAuCl_4$ (25 mg, 0.073 mmol) gave nanoparticle **1-Au** (17 mg, 2.22×10^{-4} mmol) as a dark brown powder. Average diameter and number of gold atoms: 1.8 nm, 201; ^{1}H NMR (500 MHz, D_2O): δ = 4.50–4.42 (m, 2H; H-1, H-1′), 4.10–3.30 (m, 11H), 2.00–1.10 ppm (m, 12H); ^{1}H NMR (500 MHz, [D_6]DMSO): δ = 5.04 (m, 2H), 4.73 (d, J = 4.5 Hz, 1H), 4.60 (m, 2H), 4.50–4.46 (m, 2H), 4.15 (d, J = 6.5 Hz, 1H; H-1), 4.12 (d, J = 8.0 Hz, 1H; H-1′), 3.70 (m, 2H), 3.56 (m, 2H), 3.49–3.26 (m, 5H), 2.95 (m, 1H; H-5), 2.64 (t, J = 7.0 Hz, 1.2H), 2.52 (t, J = 7.0 Hz, 0.7H; CH_2S), 1.58–1.45 (m, 4H, CH_2CH_2), 1.25 ppm (m, 16H; CH_2CH_2); UV/Vis (H_2O): λ = 520 nm (surface plasmon band); IR (KBr): $\tilde{\nu}$ = 3414, 2921, 2850, 2360, 1639, 1425, 1065 cm^{-1}; elemental analysis calcd (%) for $(C_{23}H_{44}O_{11}S)_{70}Au_{201}$ (76594): C 25.2, H 4.05; found C 25.1 H 4.40

***lacto*-EG_4SAu (2-Au)**: Reaction of the disulfide **2** (100 mg, 0.09 mmol) and $HAuCl_4$ (13.6 mg, 0.04 mmol) gave **2-Au** (8 mg, 1.09×10^{-4} mmol) as a dark brown powder. Average diameter and number of gold atoms: 1.8 nm, 201. ^{1}H NMR (300 MHz, D_2O): δ = 4.50–4.30 (br m, 2H; H-1, H-1′), 4.10–3.35 (br m, 21H), 3.26 ppm (br m, 2H); ^{1}H NMR (500 MHz, [D_6]DMSO): δ = 5.06 (br m, 2H), 4.75 (br m, 1H), 4.65 (m, 2H), 4.18 (br m, 2H; H-1, H-1′), 3.86 (m, 2H), 3.58–3.37 (m, 18H), 3.01 (br m, 1H), 2.61 ppm (t, J = 4.5 Hz; CH_2S); UV (H_2O): λ = 520 nm (surface plasmon band); elemental analysis calcd (%) for $(C_{20}H_{37}O_{14}S)_{63}Au_{201}$ (73203): C 20.6, H 3.2; found C 20.7 H 3.6

***lacto*-EG_6C_{11}SAu (3-Au)**: Reaction of the disulfide **3** (70 mg, 0.088 mmol) and $HAuCl_4$ (5.42 mg, 0.016 mmol) gave **3-Au** (9 mg, 8.11×10^{-5} mmol) as a black powder. Average diameter and number of gold atoms: 2.2 nm, 201. ^{1}H NMR (500 MHz, D_2O): δ = 4.47 (d, J = 7.5 Hz, 1H; H-1), 4.42 (d, J = 7.5 Hz, 1H; H-1′), 4.05–3.89 (m, 3H), 3.82–3.46 (m, 35H), 3.34–3.31 (m, 1H), 2.73–2.67 (m, 0.8H), 1.72–1.04 ppm (m, 16H); UV (H_2O): λ = 520 nm (surface plasmon band); elemental analysis calcd (%) for $(C_{35}H_{67}O_{17}S)_{90}Au_{202}$ (110984): C 34.0, H 5.43; found C 33.40 H 5.6.

***malto*-C_{11}SAu (4-Au)**: Reaction of the disulfide **4** (50 mg, 0.1 mmol) and $HAuCl_4$ (6.16 mg, 0.018 mmol) gave **4-Au** (12 mg, 2.09×10^{-4} mmol) as a brown powder. Average diameter and number of gold atoms: $<$1.3 nm, $<$79. ^{1}H NMR(500 MHz, D_2O): δ = 5.33 (br s, 1H; H-1′), 4.37(br s, 1H; H-1), 3.87–3.33 (m, 15H); 2.70 (br s, 2H; CH_2S), 1.65–1.33 ppm (br m, 19H); ^{1}H NMR (500 MHz, [D_6]DMSO): δ = 5.44 (s, 1H), 4.39 (d, J =

5.5 Hz, 1H), 5.00 (d, $J = 4.5$ Hz, 1H), 4.96 (s, 1H), 4.85 (m, 2H), 4.47–4.40 (m, 2H), 4.09 (d, $J = 8.0$ Hz, 1H; H-1), 3.72–3.64 (m, 2H), 3.57–3.50 (m, 3H), 3.02–2.92 (m, 3H), 2.64 (t, $J = 7.0$ Hz, 2H; CH_2S), 1.57–1.44 (m, 4H; CH_2CH_2), 1.10 ppm (m, 16H; CH_2CH_2); UV (H_2O): $\lambda = 520$ nm (surface plasmon band); elemental analysis calcd (%) for $(C_{23}H_{44}O_{11}S)_nAu_n$ ($n < 79, n \cdot 724$): C 39.0. H 6.07; found C 39.5, H 6.07.

***gluco*-EG$_4$S-Au (5-Au)**: Reaction of **5** (40 mg, 0.055 mmol) and $HAuCl_4$ (6.8 mg, 0.02 mmol) gave **5-Au** (2 mg) as a brown powder. ^{1}H NMR (500 MHz, D_2O): $\delta = 4.50-4.40$ (d, H-1), 4.15–3.20 (m), 2.93 ppm (t, CH_2S); UV (H_2O): $\lambda = 510$ nm (surface plasmon band)

Le^X-C$_{11}$S-Au (6-Au): Reaction of **6** (20 mg, 0.015 mmol) and $HAuCl_4$ (1.85 mg, 0.005 mmol) gave **6-Au** (4 mg, 4.5×10^{-5} mmol) as a brown powder. Average diameter and number of gold atoms: 1.8 nm, 201. ^{1}H NMR (500 MHz, D_2O): $\delta = 5.15$ (br m, 1H), 4.50 (br m, 2H), 4.10–3.30 (br m, 18H), 2.00 (br m, 2H), 1.70–1.00 ppm (br m, 20H); IR (KBr): $\tilde{\nu} = 3552$, 3414, 2923, 2029, 1638, 1617, 1075, 622 cm^{-1}; UV (H_2O): $\lambda = 520$ nm (surface plasmon band); elemental analysis calcd (%) for $(C_{31}H_{56}NO_{15}S)_{97}Au_{201}$ (108928.3): C 33.2, H 5.0, N 1.3; found: C 33.2, H 5.3, N 1.4.

***EG$_4$*S-Au (10-Au)**: Reaction of **10** (50 mg, 0.21 mmol) and $HAuCl_4$ (21.7 mg, 0.06 mmol) gave **10-Au** (16 mg) as a blue violet powder. Average diameter: 7 nm and 2 nm. ^{1}H NMR (500 MHz, D_2O): $\delta = 4.00-3.00$ ppm (br m, 14H); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 4.50$ (br m, 1H), 4.00–3.00 ppm (br m, 14H); UV (H_2O): $\lambda = 515$ nm (surface plasmon band)

Preparation of glyconanoparticles of different density

Synthesis of compounds 1-Au-9, 3-Au-13 (5%, 15%, 30% in lactose), 1-Au-15 and 6-Au-15: A mixture of disulfides in a different ratio (5.5 equiv) was dissolved in MeOH and added to the solution of tetrachloroauric acid (1 equiv). An aqueous solution of $NaBH_4$ (1M, 22 equiv) was then added in several portions, with rapid shaking. The reaction was shaken for a further 2 h, then the solvent was removed. The residue (20–50 mg of crude product) was dissolved in 10 mL of NANOPURE water and purified by centrifugal filtering (CENTRIPLUS $M_r > 10000$, 1 h, 3000 xg). The process was repeated until the nanoparticles were free of salts and starting material (absence of signals due to disulfide and Na^+ in ^{1}H NMR and ^{23}Na NMR spectra). The residue in the centriplus filter was dissolved in water (2 mL) and lyophilised. The molecular formula of the nanoparticles was calculated, based on the average diameter obtained by TEM, and confirmed by elemental analysis. The molar ratio between two ligands in the nanoparticle was calculated by integration of the signal corresponding to the anomeric protons of the sugar and that corresponding to the closest methylene protons to gold.

***lacto*-C$_{11}$S-Au-SC$_{11}$OH (1-Au-9)**: Reaction of a 1:1 mixture of **1** (53 mg, 0.1 mmol) and **9** (17 mg, 0.1 mmol) with $HAuCl_4$ (13.6 mg, 0.04 mmol) gave **1-Au-9** (11.2 mg). Average diameter and number of gold atoms: 2 nm, 201–225. The ratio of compound **1** to **9** in the product was 1:1.2, by integration of the signal corresponding to the anomeric protons of the lactose and the triplet corresponding to the closest methylene protons to gold. This nanoparticle was insoluble in water but soluble in DMSO. ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 5.03$ (m, 2H), 4.59 (d, $J = 4.5$ Hz; 1H), 4.61 (m, 2H), 4.51–4.47 (m, 2H), 4.36–4.33 (m, 1H), 4.15 (d, $J = 7.0$ Hz, 1H; H-1), 4.12 (d, $J = 7.0$ Hz, 1H; H-1′), 3.71–3.68 (m, 2H), 3.50–3.42 (m, 8H), 2.96 (m, 1H), 2.65 (t, $J = 7.0$ Hz, 4H; CH_2S), 1.59–1.54 (m, 4H), 1.48–1.45 (m, 4H), 1.35–1.21 ppm (m, 37H); UV (DMSO): $\lambda =$ 524 nm (surface plasmon band)

***lacto*-EG$_6$C$_{11}$S-Au-SC$_{11}$EG$_3$OH (3-Au-13)**

3-Au-13 (5% *lacto*): Reaction of a 5:95 mixture of **3** (12 mg, 0.015 mmol) and **13** (100 mg, 0.300 mmol) with $HAuCl_4$ (19.55 mg, 0.057 mmol) gave **3-Au-13** (5 mg) as a beige powder containing 5% of lactoside. The nanoparticles were soluble in H_2O:MeOH. Average diameter and number of gold atoms: 1.7 nm, 140. ^{1}H NMR (500 MHz, D_2O): $\delta = 4.05-3.54$ (m), 3.34 (m, 3H), 1.89–1.34 ppm (br m)

3-Au-13 (15% *lacto*): Reaction of a 15:85 mixture of **3** (12 mg, 0.015 mmol) and **13** (33 mg, 0.100 mmol) with $HAuCl_4$ (7.05 mg, 0.021 mmol) gave **3-Au-13** (5 mg) as a beige solid, keeping the original ratio of 15% lactoside. Their water solutions were colourless. Average diameter and number of gold atoms: 1.5 nm, 116. ^{1}H NMR (500 MHz, D_2O): $\delta = 4.50-4.43$ (2d, 2H; H-1, H-1′), 4.05–3.54 (m, 100H), 3.34 (m, 3H), 1.89–1.34 ppm (br m); UV (H_2O): $\lambda = 400$ nm (surface plasmon band).

3-Au-13 (30% *lacto*): Reaction of a 30:70 mixture of **3** (10.7 mg, 0.014 mmol) and **13** (15 mg, 0.045 mmol) with $HAuCl_4$ (7.54 mg, 0.016 mmol) gave **3-Au-13** (12.6 mg) as a beige solid containing 30% lactoside. Their water solutions were colourless. Average diameter and number of gold atoms: 1.5 nm, 116. ^{1}H NMR (500 MHz, D_2O): $\delta = 4.50-4.43$ (2d, 2H; H-1, H-1′), 4.05–3.54 (m, 168H), 3.34 (m, 3H), 2.70 (br s, 9H; CH_2S), 1.89–1.34 ppm (br m, 94H); UV (H_2O): $\lambda = 400$ nm (surface plasmon band)

***lacto*-C$_{11}$ S-Au-SC$_{11}$EG$_6$FITC (1-Au-15)**: Reaction of a 5:95 mixture of **1** (20 mg, 0,019 mmol) and **15** (1.7 mg, 0.001 mmol)) with $HAuCl_4$ (2.4 mg, 0.007 mmol) gave **1-Au-15** as a dark brown powder (4.2 mg). Average diameter and number of gold atoms: 2 nm, 201. ^{1}H NMR (500 MHz, D_2O): $\delta = 4.50-4.42$ (m, 2H; H-1, H-1′), 4.1–3.3 (m, 11H), 2.68 (br s, CH_2S), 2.0–1.1 ppm (m, 12H); UV (H_2O): $\lambda = 520$ nm (surface plasmon band); fluorescence (H_2O): $\lambda_{ex} = 480$ nm; $\lambda_{em} = 514$ nm.

Le^X-C$_{11}$S-Au-SC$_{11}$EG$_6$FITC (6-Au-15): Reaction of a 5:95 mixture of **6** (20 mg, 0.014 mmol) and **15** (1.2 mg, 0,0007 mmol) with $HAuCl_4$ (1.7 mg, 0.005 mmol) gave **6-Au-15** (4 mg) as a dark brown powder. Average diameter and number of gold atoms: 2 nm, 201. ^{1}H NMR (500 MHz, D_2O): $\delta = 5.15$ (br m, 1H), 4.50 (br m, 2H), 4.10–3.30 (br m, 18H), 2.00 (br m, 2H), 1.70–1.00 ppm (br m, 20H); UV (H_2O): $\lambda = 520$ nm; fluorescence (H_2O): $\lambda_{ex} = 480$ nm; $\lambda_{em} = 514$ nm.

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