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Novel Bio-friendly and Non-toxic Thiocarbohydrate Stabilizers of Gold Nanoparticles[#]

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Several bio-friendly carbohydrate disulfides and thiocarbohydrates have been synthesized *via* the reaction of D-(+)-gluconic acid δ-lactone with aminoalkylthiols, leading to *n*-gluconamidoalkyldisulfides {di-(2-gluconamidoethyl)disulfide **(L1)**, di(3-gluconamidopropyl)-disulfide **(L2)**, di(4-gluconamidobutyl) disulfide **(L3)** and (2-gluconamidoethyl)thiol **(L4)**}. Acetylation of hydroxy groups in **L1-L3** and subsequent reduction produced the following disulfides and thiols: acetylated di(2-gluconamidoethyl)disulfide **(L5)**, acetylated di(3-gluconamidopropyl)disulfide **(L6)**, acetylated di(4-gluconamidobutyl)disulfide **(L7)**, acetylated di(2-gluconamidoethyl)thiol **(L8)**, acetylated di(3-gluconamidopropyl)thiol **(L9)** and acetylated di(4-gluconamidobutyl)thiol **(L10)**. Compounds **L1-L10** were characterized by combination of NMR and infrared spectroscopy, microanalysis, mass spectrometry and in a selected case X-ray crystallographic data. These thiocarbohydrate compounds were used to stabilize gold nanoparticles to gold glyconanoparticles (AuNPs) of sizes in the range of *ca.* 2-9 nm. The thiocrabohydrates are non-toxic toward both cancer and normal cell lines and have IC_{50} values generally ≥ 200 μM.

[#]Dedicated to Professor Cedric Holzapfel on the occasion of his 80th birthday on October 3, 2015

1. Introduction

A large number of gold compounds have been studied as anticancer and anti-HIV agents[1-10] and as drug delivery vehicles in the form of gold nanoparticles in therapeutics because of their bio-inertness and cellular imaging ability.[11] However, development of gold compounds and gold nanoparticles as therapeutics[12] are hampered by their unstable nature and lack of biocompatibility.[11,13] It has, therefore, become necessary to find both non-toxic and biocompatible ligands in order to produce gold compounds that would help overcome the above shortcomings. For general biomedical applications, surface-functionalization of gold nanoparticles (AuNPs) is essential in order to target specific diseased cells selectively.[14] AuNPs can be stabilized by monolayers protected clusters (MPCs) that have thiol and thiolate functional groups.[15-18] However, MPCs are usually insoluble in water and toxic to normal cells, a property which hampers their use for biomedical application. This restriction to biomedical application is in reality quite severe and have only been addressed by very few research groups.[19-22] For instances, stable and water soluble gold MPCs, protected by poly(ethyleneglycol)thiol (PEG-SH),[19-20] tiopronin(*N*-2-mercaptopropionylglycine),[21] and oligo(ethyleneglycol)thiol[22] have been developed. These gold MPCs have shown enhanced chemical and thermal stability. The use of PEG as stabilizer for biomedical application (gene delivery vehicles) for example, is either facilitated by external forces or associated with significantly low transfection efficiencies due to the possible entrapment of PEG-modified vehicles inside the endosomes.[23-24] These particles are susceptible to electrostatic aggregation following changes in pH or ionic strength of the medium.[22] Polyethyleneimine and chitosan are two other ligand systems which have been widely used for drug delivery, but their use have been limited by severe toxicity and low

transfection efficiency, respectively.[25] Recently glycol-AuNPs have been found to be interesting delivery vehicles due to their PEG-like biocompatibility and their cell-mediated interactions.[26-28]

In view of the unique characteristics and increasing understanding of the biological recognition phenomena involving carbohydrates and proteins, several glycogold nanoparticles have been developed.[29-48] In 2005, Halks *et al*.[49] validated the interaction of AuNPs with concanavalin A in a processes where AuNPs were prepared from cysteamine-extended saccharides, *via* the reductive amination of saccharides with trityl-protected cysteamine, followed by detritylation. We have developed facile synthetic processes for a number of novel thiocarbohydrates without protecting any group; a facile one-step synthesis of their corresponding gold nanoparticles and demonstrated that these thiocarbohydrates offer alternative to MPCs as non-toxic careers which could be useful in drug delivery application.

2. EXPERIMENTAL

2.1 Materials

Toluene, dichloromethane and methanol used as solvents were dried using a SPS-1 stand-alone solvent purifier distillation over standard reagents under N_2 prior to use. D-(+)-gluconic acid δ-lactone, 4-(dimethylamino)pyridine (DMAP), *tetra*-octylammonium bromide (TOABr), 2-aminoethanethiol and potassium hydroxide were purchased from Sigma-Aldrich; while 3-aminopropane-1-thiol, 4-aminobutane-1-thiol, 5-aminopentane-1-thiol and 6-aminohexane-1-thiol were purchased from Ivy fine chemicals (USA) and used as received. All glassware were thoroughly cleaned with aqua regia and rinsed with double distilled water and dried prior to use.

All solutions for AuNP synthesis were prepared using water purified with a Millipak (Millipore Q-POD) system 40 (0.22 pm) filtration system.

2.2 Instrumentation

All NMR spectra were recorded in D_2O or $CDCl_3$ on a Bruker Ultra shield (400 MHz) at room temperature. ^1H and $^{13}C\{^1H\}$ chemical shifts were referenced to the residual signals of the protons or carbons of the NMR solvents and are quoted in ppm: D_2O at 4.65 ppm for ^1H NMR spectrum and $CDCl_3$ at 7.24 and 77.50 ppm for ^1H and $^{13}C\{^1H\}$ spectra respectively. Infrared spectra were recorded on a Bruker Tensor 27 fitted with an ATP-IR probe and Perkin Elmer BX FT-IR spectrometer. Raman spectra were acquired using a PerkinElmer Raman Station 400 benchtop Raman spectrometer. The source was a near-infrared 785 nm laser (100 mW) with a spot size of 100 μm. A spectral range of 200-3200 cm^{-1} was employed. The detector was a temperature controlled Charged Coupled Device (CCD) detector (-50 ^oC) incorporating a 1024 × 256 pixel sensor. The spectra were acquired using Spectrum software and images were acquired using Spectrum IMAGE software, both supplied by PerkinElmer (Bucks, UK). Elemental analyses were performed on a Vario Elementar IIImicrocube CHNS analyzer at the Rhodes University, South Africa. ESI-MS spectra were recorded on a Waters API Quattro Micro spectrophotometer at the University of Stellenbosch, South Africa. Melting points were determined using a Q600 Series™ Thermal Analyzer. The UV-vis spectra were recorded on a Varian Spectrophotometer with 1 cm cuvettes in the 200-1000 nm range at room temperature. The size and morphology of the gold nanoparticles were analyzed using a JOEL-JEM 1010 transmission electron microscope operating at 120 kV. Sample preparation involved placing approximately 10 μL of the colloidal samples, after centrifugation, on carbon coated copper grids that were allowed to dry for a few minutes. About 100-150 particle diameters were acquired to

calculate mean diameter using macbiophotonic imagej software. Energy Dispersive X-ray (EDX) analysis data were collected on an Oxford X max scanning electron microscope mega 3L MH, coupled with a Tescan Vega3, at 20keV and working distance of 15 mm.

2.3 Synthesis of gluconamidoalkylthiol compounds

2.3.1 General procedure for synthesis of compounds L1-L3

The syntheses of **L1-L3** were performed according to the literature[50] with slight modifications. D-(+)-Gluconic acid δ-lactone was dissolved in methanol (30 mL) at 50 ^{o}C and was cooled to room temperature before the addition of the appropriate *n*-aminoalkylthiol and triethylamine. The mixture was then stirred overnight at room temperature and the white precipitates obtained were filtered, washed with isopropanol (3 x 10 mL) and acetone (3 x 10 mL) to afford flaky white solids of compounds **L1-L3**. Full spectroscopy and micro analysis characterization of these compounds can be found at the supporting information.

2.3.2 Synthesis of (2-glucoamidoethyl)thiol (L4)

The compound were synthesized by reduction of di(*n*-gluconamidoethyl)disulfide according to literature procedures.[51,52] Compound **L1** (0.50 g, 0.98 mmol) was dissolved in water (10 mL) at 40 ^{o}C followed by addition of 5% acetic acid (0.5 mL) and zinc dust (1.0 g, 15.3 mmol) and stirred for a maximum of 2 h at room temperature. The mixture was filtered and the filtrate was reduced to half the volume, and acetone was then added to give a white solid. The product was filtered, washed several times with methanol to remove excess acetic acid and dried under high

vacuum to obtain pure **L4**. Yield = 98%. ^{1}H NMR (D_2O, 400 MHz): δ 4.15 (d, *J* = 3.04 Hz, 1H, H-2), 3.91 (s, 1H, H-3), 3.65 (s, 1H, H-3), 3.57 (s, 1H, H-5), 3.46 (q, *J* = 4.8 Hz, 2H, H-6), 3.28 (q, *J* = 6.8 Hz, 2H, -NCH_2-), 2.64 (t, *J* = 6.8 Hz, 2H, -CH_2SH); $^{13}C\{^{1}H\}$ NMR (D_2O, 100 MHz): δ 179.8 (C-1), 72.2 (C-2), 70.2 (C-3), 69.6 (C-4), 68.6 (C-5), 61.0 (C-6), 21.4 (-NCH_2-), 21.4 (-CH_2S-). FT-IR (neat, cm^{-1}): 1649 (C=O), 1561 (N-H), 1099 (C-N), 1220 (O-C), 3301 (O-H broad), 1438 (CH_2 str). Anal. Calcd for $C_8H_{17}O_6NS$: C, 29.95; H, 5.30; N, 4.37; S, 10.02%. Found: C, 28.90; H, 5.30; N, 4.25; S, 10.58%. HRMS (ESI): *m/z* $[M+H]^+$ Calcd 256.0855; found: 256.0857.

2.3.3 General procedure for synthesis of L5-L7

In a typical experiment a solution of di(*n*-gluconamidoalkyl)disulfide (**L1**), acetic anhydride (1 mL) (1:1 ratio to hydroxyl group) and catalytic amount of DMAP in pyridine (1 mL) was stirred at room temperature for 18 h. The mixture was then diluted with dichloromethane (20 mL), washed with 1 M $HCl_{(aq)}$ (5 x 30 mL) and the organic layer dried over anhydrous $MgSO_4$ and the solvent evaporated to give spongy white solids. The spectroscopy and micro analysis characterization of these compounds can be found at the supporting information.

2.3.4 General synthesis of compounds L8-L10

In a typical reaction a mixture of disulfide **L5**, 5% acetic acid (10 mL) and zinc dust in acetonitrile (20 mL) was stirred for 2 h. The mixture was filtered and the filtrate evaporated to dryness. The product was dissolved in dichloromethane and washed in a separatory funnel with

saturated aqueous $NaHCO_3$ (2x 5 mL). The organic layer was dried with anhydrous $MgSO_4$ and concentrated using rotary evaporator to afford a spongy white solid of **L8**. Full spectroscopy and micro analysis characterization of these compounds can also be found at the supporting information.

2.4 Synthesis of gluconamidoalkylthiol gold nanoparticles (AuNPs1-3)

Freshly prepared **L1**, **L2** and **L3** were each dissolved in water and freshly prepared aqueous 25 mM solution of $H[AuCl_4]$ was added to them. The resulting solution was vigorously stirred and freshly prepared aqueous 2.93 mmol of a 1 M solution of $NaBH_4$ was added drop wise.[53,54] Hydrogen gas evolved and a dark-brown suspension formed instantly. Stirring continued for an additional 4 h at ambient temperature, after which the mixture was centrifuged to give a brown to purple solid products depending on concentration of the stabilizer used. The nanoparticles were purified by washing with methanol. The product was re-dissolved in distilled water and kept in fridge for storage. Various concentrations of stabilizers were used as follows: **AuNPs-1a-c, L1**: (18 mmol), (25 mmol) and (30 mmol); **AuNPs-2a-c, L2**: (18 mmol), (25 mmol) and (30 mmol) and **AuNPs-3a-c, L3**: (20 mmol), (25 mmol) and (30 mmol).

2.5 Synthesis of acetylated gluconamidoalkylthiol (L8-L10) gold nanoparticles (AuNPs 4-6)

A literature method by Brust *et al*.[55] was used to prepare **AuNPs 4-6**. An aqueous solution of $H[AuCl_4]$ (30 mmol) in 100 mL conical flask was dissolved in a mixture of tetraoctylammonium bromide (TOABr) (50 mmol) in toluene (20 mL). The two-phase mixture was vigorously stirred until all the tetrachloroaurate was transferred into the organic layer and compound **L8**, **L9** or **L10**

was added to the organic phase of each specific reaction. A freshly prepared aqueous solution of sodium borohydride (8.33 mL, 0.4 mmol) was added drop wise with vigorous stirring for 3 h. The organic layer was separated, diluted with hexane (100 mL) and kept for 24 h at -18 ^{o}C. The dark brown precipitate formed was centrifuged to give a dark brown product. Three libraries of AuNPs with each stabilizer were synthesized by varying the stabilizer to gold ratio. In each of AuNPs preparation, the final product was dissolved in small amounts of toluene for storage purpose. The concentrations of stabilizers used are as follows: **AuNPs-4a-c, L8:** (6 mmol), (8 mmol), (12 mmol), **AuNPs-5a-c, L9:** (10.0 mmol), (12.5 mmol), (15.0 mmol) and **AuNPs-6a-c, L10:** (10.0 mmol), (12.5 mmol), (15.0 mmol).

2.6 X-ray structural determination

Single-crystal X-ray diffraction data for compound **1b** were collected on a Bruker APEXII diffractometer with Mo Kα (λ = 0.71073 Å) radiation and diffractometer to crystal distance of 5.00 cm. The initial cell matrix was obtained from three series of scans at different starting angles. Each series consisted of 12 frames collected at intervals of 0.5º in a 6º range about with an exposure time of 10 s per frame. The reflections were successfully indexed by an automated indexing routine built in the APEXII program suite. The data were collected using the full sphere data collection routine to survey the reciprocal space to the extent of a full sphere to a resolution of 0.75 Å. Data were harvested by collecting 2982 frames at intervals of 0.5º scans in ω and φ with exposure times of 10 s per frame.[56] A successful solution by the direct methods of SHELXS97 provided all non-hydrogen atoms from the *E*-map. All non-hydrogen atoms were refined with anisotropic displacement coefficients. All hydrogen atoms were included in the

structure factor calculation at idealized positions and were allowed to ride on the neighbouring atoms with relative isotropic displacement coefficients.[57]

2.7 Totoxicity evaluation

Toxicity tests of the glycomonomers were performed using MTS assay. The assay was performed as previously reported.[58] Primary human hepatocytes (PM1) cells were placed in 96-well microtiter plates at 1x106 cells/mL and allowed to stabilize for 1 h at 37 ºC and 5% CO_2. Thereafter, two-fold serially diluted compounds (test compounds and Raltegravir used as a control) were added to the plates to allow for 8 final compound concentrations ranging from 200 µM to 1.5625 µM in a total volume of 200 µL/well. Auranofin, serially-diluted from 12.5 µM to 0.0977 µM was used as a second control compound. The cells and compounds were then incubated for 96 h at 37 ºC and 5% CO_2. To each well, 20 µL of CellTiter Aqueous One solution (Promega, USA) was added; the plates were incubated for 4 h and absorbance was read at 490 nm on a multiplate reader (xMark, Biorad). The IC_{50} values were determined as the concentration of the compound required to reduce the cell viability by 50% and were calculated using Origin 6.1 software (OriginLab Corporation, USA). The values obtained were averages of at least three separate experiments (n = 6).

2.8 In vitro anticancer screening of gold glyconanoparticles

The growth inhibitory effects of the glyco AuNPs were tested in the 3-cell line panel consisting of MCF7 (breast cancer), HCT116 (colon cancer) and PC3 (prostate cancer) by Sulforhodamine

B (SRB) assay. For screening experiment, the cells (3-19 passages) were inoculated in a 96-well microtiter plates at plating densities of 7-10 000 cells/well and were incubated for 24 h. After 24 h one plate was fixed with TCA to represent a measurement of the cell population for each cell line at the time of drug addition (t_0).The other plates with cells were treated with the experimental drugs which were previously dissolved in DMSO and diluted in medium to produce 5 concentrations (6.25-100 μg/mL or 0.01-100 μM). Cells without drug addition served as control. The blank contains complete medium without cells. Parthenolide was used as a reference standard. The plates were incubated for 48 h after addition of the compounds. Viable cells were fixed to the bottom of each well with cold 50% trichloroacetic acid, washed, dried and dyed by SRB. Unbound dye was removed and protein-bound dye was extracted with 10 mM Tris base for optical density determination at the wavelength 540 nm using a multiwell spectrophotometer. Optical density measurements were used to calculate the net percentage cell growth. The IC_{50} values were determined as the concentration of the compound required to reduce the cell viability by 50% and were calculated using Origin 6.1 software (OriginLab Corporation, USA). The values obtained were averages of at least three separate experiments.

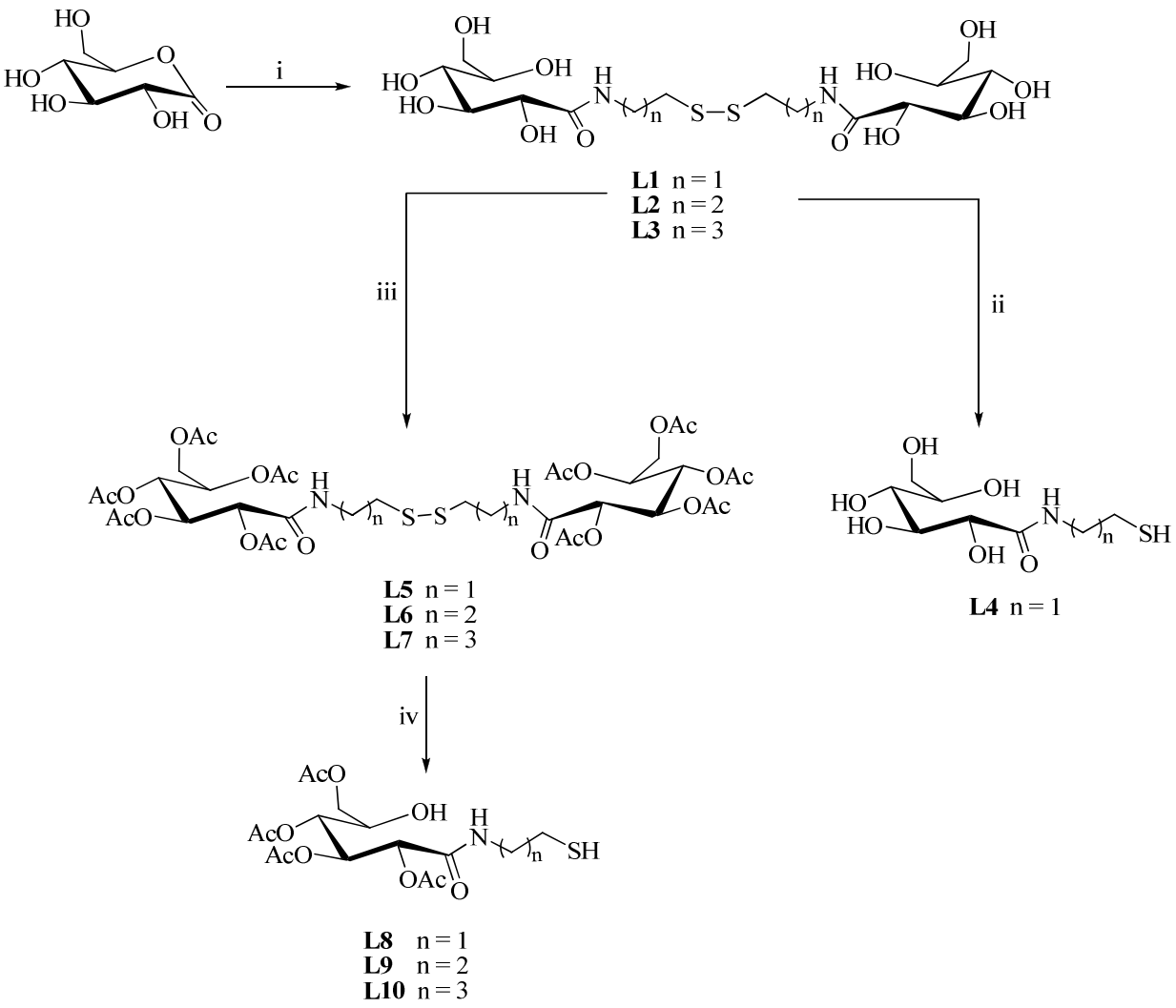
3. RESULTS AND DISCUSSION

3.1. Synthesis of gluconamidoalkyl disulfides and thiols

On the basis of the results by Gu *et al*.[59] which demonstrated the importance of having a carbohydrate head group at an appropriate distance from the surface of immobilized bilayers of nanoparticles, we synthesized various gluconamidoalkyl disulfides and thiols (Scheme 1). These compounds were synthesized *via* the ring opening of D-(+) gluconic acid δ-lactone with the

appropriate aminoalkylthiol hydrochloride in the presence of triethylamine, leading to the formation of **L1-L3** (Scheme 1). The formation of the disulfides takes place *via* deprotonation of the sulfhydryl groups.[59] The Raman spectrum of **L1** (Figure S1), shows a peak at 510 cm^{-1}, evidence for the presence of S-S linkage in the disulfides. Confirmation of the S-S bond formation was further provided by the single crystal X-ray structure of **L2** (Figure 1). To further confirm the formation of compounds **L1-L3**, they were per-acetylated to give **L5-L7**.

The ^{1}H NMR spectra of each of three acetylated products showed the presence of five singlets around 2.0 ppm assigned to the five acetate groups in **L5-L7** (Figure S2). $^{13}C\{^{1}H\}$ NMR spectra were used to further support presence of acetyl groups, as well as carbonyl and amides groups in **L5-L7**; a typical spectrum is Figure S3.



Scheme 1. Schematic representation of thiocarbohydrate synthesis. Conditions: *(i)* MeOH, aminoalkylthiol, Et_3N, rt, 24 h, 42-92%; (ii) Zn dust, 5% CH_3COOH, H_2O, rt, 2 h, 98%; (iii) Ac_2O, pyridine, DMAP, rt, 24 h, 83-84%**;** (iv) Zn dust, MeCN, 5% CH_3COOH, rt, 3 h, 90-96%.

Microanalysis and high resolution mass spectral data for **L1-L3** and **L5-L7** at m/z = 509.1010, 537.1791, 565.2101, 929.2538, 957.2842 and 986.3179 respectively were further evidence that pure products were isolated. Since one of our aims was to synthesize carbohydrate thiols as stabilizers, the disulfide L**1** was reduced with zinc dust in acetic acid to afford compound **L4** in high yields**.** Acetylated analogue, **L8-10**, were prepared by reducing **L5-L7** respectively. To

further confirm the successful syntheses of **L4** and **L8-L10**, these compounds were acetylated and their ^{1}H NMR spectra showed the presence of six singlets around 2.34-2.02 ppm that were assigned to the six acetate groups. High resolution mass spectrometry and microanalysis were used to confirm the nature and purity respectively of **L4** and **L8-L10.**

3.2 *Molecular structure of L2*

Single crystals suitable for X-ray analysis of compound **L2** were grown by slow diffusion of methanol into a water solution of compound **L2** at -4 ^{o}C. Crystallographic data and structural refinement parameters are given in Table 1, whereas molecular geometry and selected bond lengths and angles are presented in Figure 1. Compound **L2** crystalized with two molecules in a cell unit. The two molecules are the same but not mirror image of each other. Hydrogen bonding is observed in compound **L2** and the strength is described as medium with angles between 130^{o} and 173^{o}. The hydrogen bonding observed is intra-atomic. The bond length between N1 and C4 carbonyl of the amide is 1.323 Å, which is within the range reported for similar functional groups.[60] Furthermore, the bond length for S1-C9 and S1-S2 are 1.823(5) Å and 2.029(2) Å, similar to those reported for disulfide derivatives.[61] Compound **L2** is a long chain symmetrical molecule with bond angle about S1 or S2 of 104^{o}.

Table 1: Crystal data and structure refinement for compound **L2**

	L2
Empirical formula	$C_{18}H_{36}N_2O_{12}S_2$
Formula weight	536.61
Temperature/K	99.85
Wavelength	0.71073
Crystal system	monoclinic
Space group	$P2_1$
a/Å	11.0828(16)
b/Å	8.3612(12)
c/Å	25.823(4)
α/°	90
β/°	92.896(3)
γ/°	90
Volume/Å^3	2389.8(6)
Z	4
Density (Mg/m^3)	1.491
Reflections collected	28761
Goodness-of-fit on F^2	1.013
Independent reflections	10392 [R_{int} = 0.0486, R_{sigma} = 0.0600]
Final R indexes [I>=2σ (I)]	R_1 = 0.0498, wR_2 = 0.1113
Final R indexes [all data]	R_1 = 0.0838, wR_2 = 0.1343

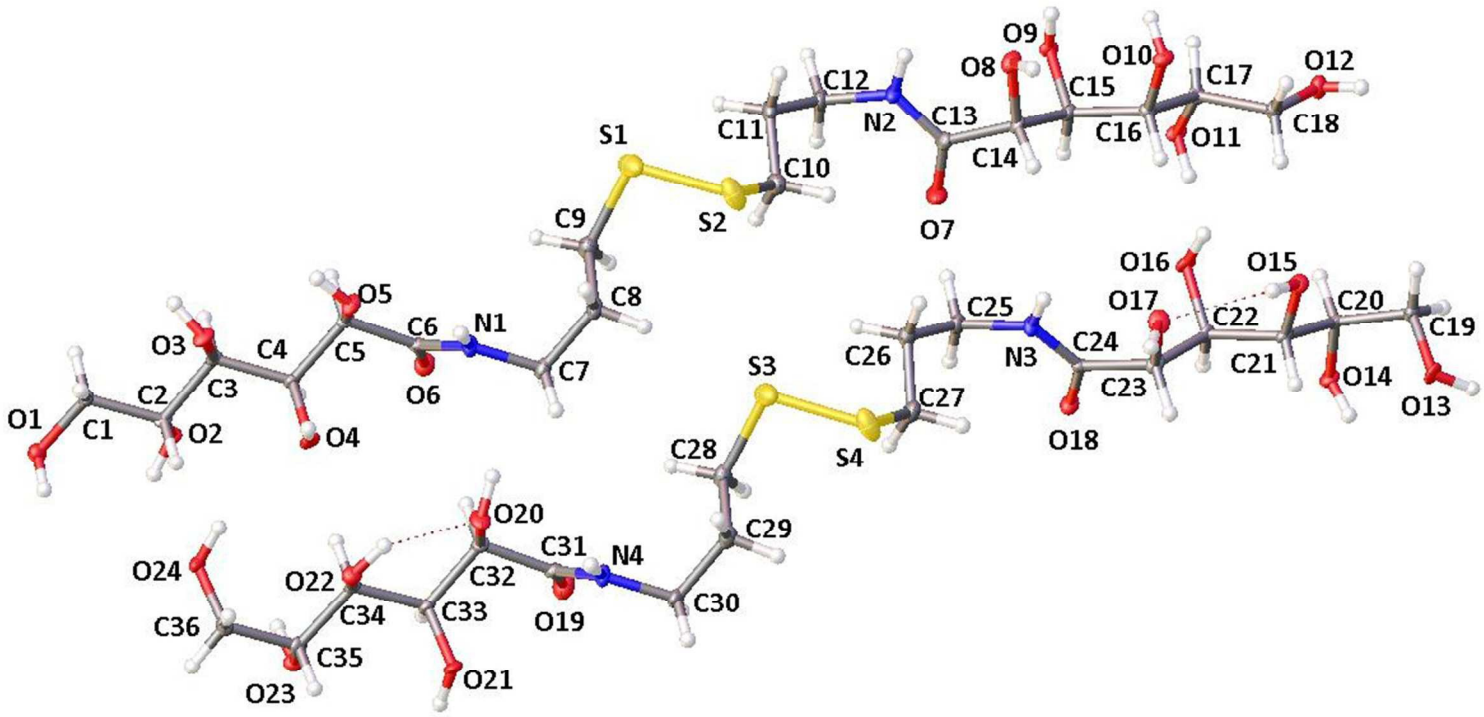
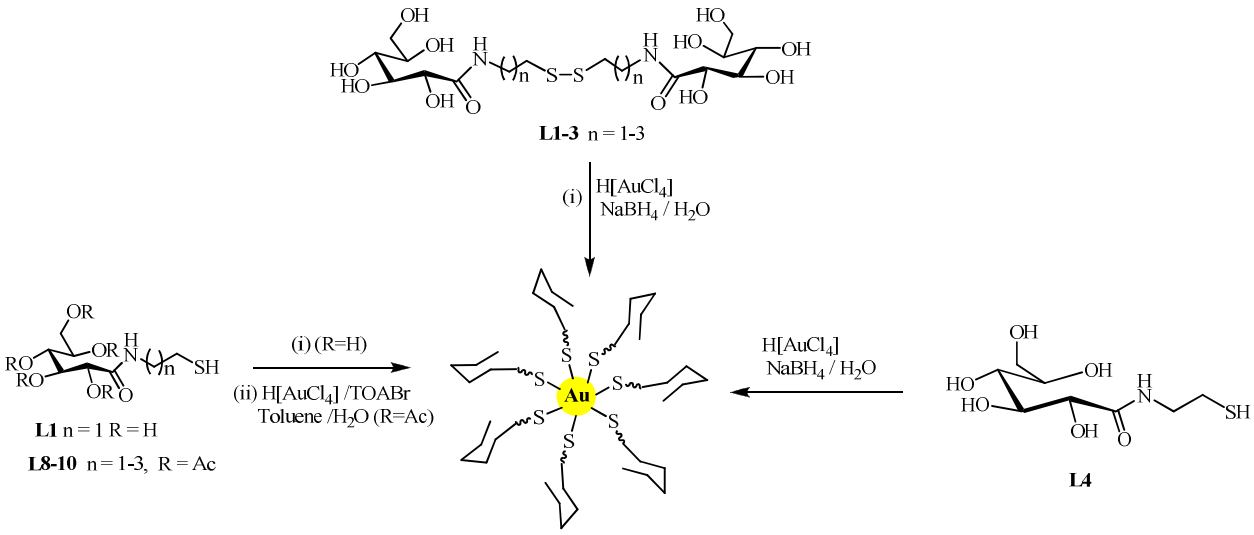


Figure 1. Molecular structure of **L2** drawn with 50% probability ellipsoids. Selected bond lengths [Å] and bond angles [°]: S1-S2, 2.028(18); S1-C9, 1.821(4); S2-C10, 1.815(4); O1-C1, 1.431(4); O6-C6, 1.241(5); N1-C6, 1.326(5); N1-C7, 1.465(5); C9-S1-S2, 104.38(15); C1-S2-S1, 104.17(15); C6-N1-C7, 123.80(4); O6-C6-N1, 123.60(4).

3.3 Synthesis of gluco gold nanoparticles

Compounds **L1-L3** and **L4** were used to stabilize gold nanoparticles (AuNPs); using the direct method described in the literature.[53,54,62-65] Aqueous solutions of **L1-L3** and **L4** with $H[AuCl_4]$ were each treated with excess of $NaBH_4$ (Scheme 2). Three different concentrations of **L1-L3** and **L4** were used to prepare these gold nanoparticles (Table 2). The AuPNs obtained were red, purple or brown depending on the alkyl chain length and concentration of the stabilizer. Formation of AuNPs by **L1-L3** and **L4** was established by UV-vis spectroscopy, which showed typical surface plasmon absorption bands *ca*. 500-539 nm (Figures S4) of self-assembled AuNPs.[62-65] The AuNPs obtained with **L1-L3** and **L4** were similar. This could be due to both disulfides and thiols being converted to thiolates on addition of $NaBH_4$ before the formation of the AuNPs. Such thiolate formation has been reported by several authors.[66-68] The λ_{max} of the absorption bands increased marginally as the alkyl chain length of ligands increased; a situation that can be atributed to aggregation of particles. Aggregation has been linked to decrease in inter-particle distance less than the diameter of the particles, which causes coupling interactions.[69] The coupling interactions lead to broadening of peaks and shifts to longer wavelenghts as shown in Figures S4.



Scheme 2. Synthesis of thiocarbohydrate AuNPs

Table 2: Summary of λ_{max} and particle sizes of thiocarbohydrate AuNPs **1-3** as amount of stabilizer is varied

AuNPs	Stabilizer (mmol)	λ_{max} (nm)	Particle size (nm)[a] ±STD_{DEV}
1a	18.0	518	4.1±0.8
1b	25.0	519	1.6±0.5
1c	30.0	519	2.1±0.8
2a	18.0	520	4.5±0.8
2b	25.0	519	3.2±0.7
2c	30.0	518	2.9±0.7
[b]**3a**	20.0	522	4.9±1.0
3b	25.0	521	3.1±2.0
3c	30.0	519	3.1±1.5

[a]Particle diameter was calculated from100-150 indiviual particles using macbiophotonic imagej software. [b]18 mmol of **L3** failed as stabilizer for **3a**, hence 20 mmol was used.

The morphology and size distribution of AuNPs **1-3** were determined using TEM. Most of the AuNPs were well-distributed spherical nanocrystals (Figure 2), with diameters between 1.6±0.5 and 4.9±1.0 nm (Table 2). The size of the AuNPs increased marginally as the alkyl chain length increased but decreased with increasing concentration of the stabilizer used. This is attributed to aggregation of particles, possibly enhanced by long chain effect, which was demonstrated by broadening of UV-vis spectra (*vide supra*) (Figure S4). The slight increase in size as chain length increases could be due to enforced intermolecular van der Waal's interactions between ligand strands.[47-49] Also ligands with longer alkyl chain required higher minimum ligand concentration to stabilized AuNPs compared to shorter chain, e.g. **3a** required at least 20 mmol whilst **L9** required 10 mmol. This is probably due to insufficient full-coverage of the stabilizer molecules on the surface of the AuNPs similar to observation.[70-72] For compounds **L8-L10**, which are not soluble in water, the Brust-Schifrin method[55] was used to synthesize AuNPs **4-6**, with $NaBH_4$ and tetraoctylammonium bromide (TOABr) as reducing and phase transfer catalyst[55] respectively (Scheme 2). The resultant AuPNs similarly showed surface plasmon resonace peaks at *ca*. 502-535 nm; signifying smaller and well-dispersed particles (Table 3 and Figures S5).

The particles sizes based on TEM micrograph were in the range of 3.8-9.3 nm (Table 3). Higher concentrations of **L9** and **L10** were required to form monodispersed gold nanoparticles due to extension of alkyl chain as explained above.[70] When the alkyl chain length was increased to n = 3 (i.e. AuNP-**6a-c)** it was at 10 mmol of **L10** did we observed the formation of well-dispersed particles, with mean diameter 6.6±3.2 nm (Figure 3). At higher concentrations of **L10** (*ca*. 12.5 and 15 mmol) the AuPNs aggregated as confirmed by broad UV-vis spectrometry and TEM image (Figure 3 and Figure S5) probably due to stronger van der Waal's interactions as a result of increase in ligand chain-length and bulky acetyl group.[70] What was peculiar about the AuNPs

after washing with hexane and methanol is the fact that the EDX of all the AuNPs **4**a showed Br peaks; suggesting TOABr used as phase transfer agent[55] persisted possibly due to adsorption of Br- on the surface of the AuNPs (Figure 4). It is predicted that self-assembly of AuNPs may be the result of strong dative bond of sulfur to gold atoms (40-50 kcal mol^{-1}) with a strength that is close to the well-documented gold-gold interactions.[73,74]

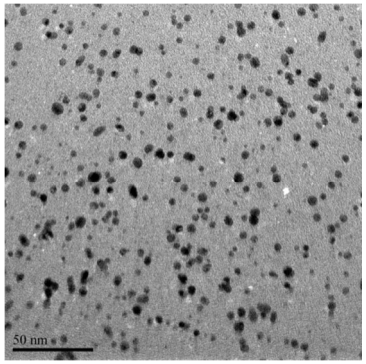
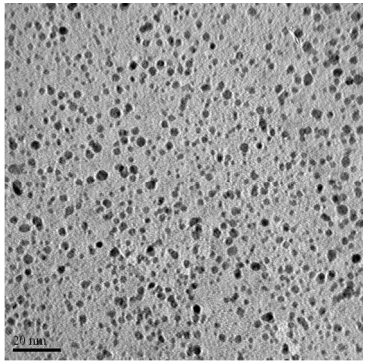
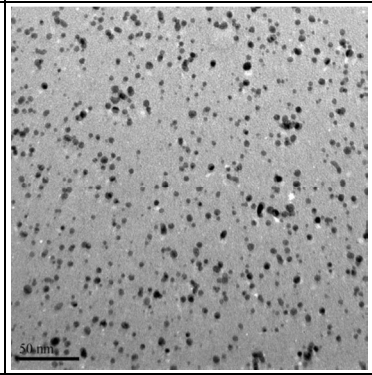
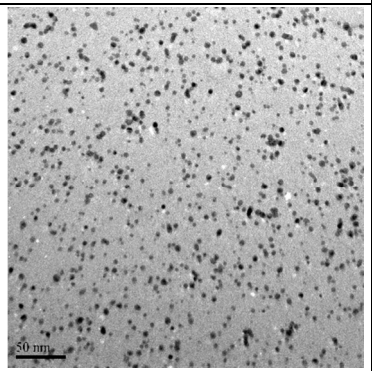
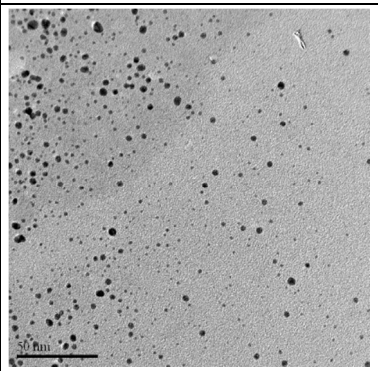
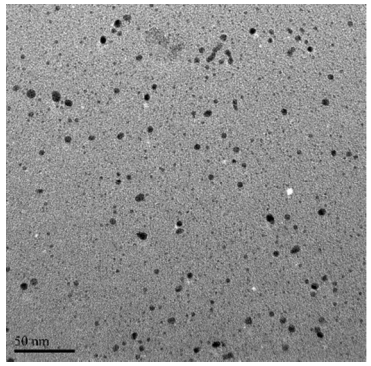
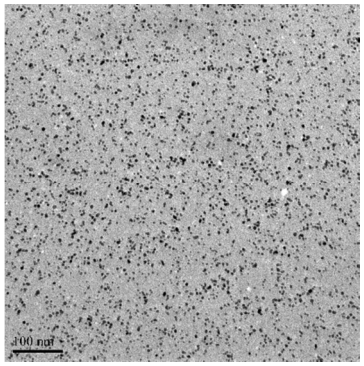
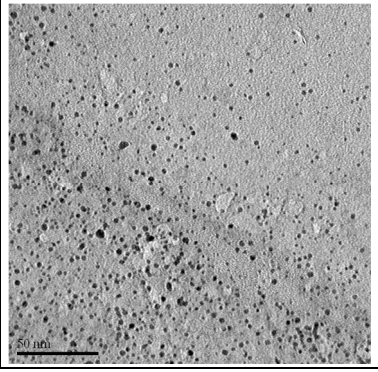
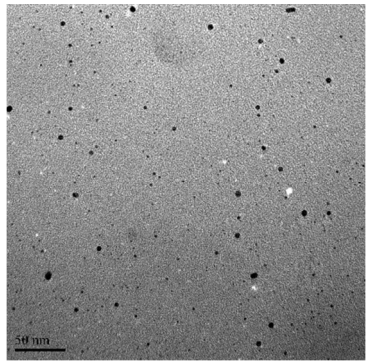
Compounds (mmol)	**AuNP 1**	**AuNP 2**	**AuNP 3**
18.0			
25.0			
[a]30.0			

Figure 2: TEM micrograph of AuNPs **1-3** showing varying amounts of stabilizers **L1-L3**. (a) The scale for all the micrograph is 50 nm except for AuNP**1** (30.0 mmol) which is 100 nm.

Table 3: Summary of λ_{max} and particle sizes of gluco AuNPs **4-6** as amount of stabilizer is varied

AuNP	Stabilizer (mmol)	λ_{max} (nm)	Particle size (nm) $\pm STD_{DEV}$
4a	6.0	520	8.8±2.1
4b	8.0	529	5.9±1.9
4c	12.0	520	6.3±1.0
5a	10.0	518	3.8± 1.1
5b	12.5	517	5.8±1.9
5c	15.0	535	5.9±2.0
6a	10.0	502	6.6±3.2
6b	12.5	508	agr
6c	15.0	-	agr

agr = aggregated AuNPs

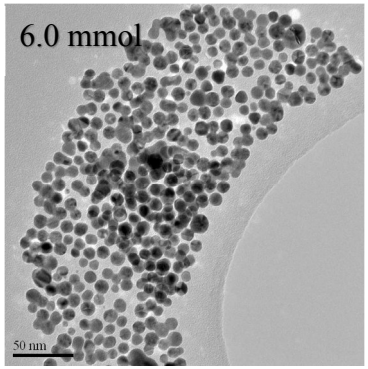
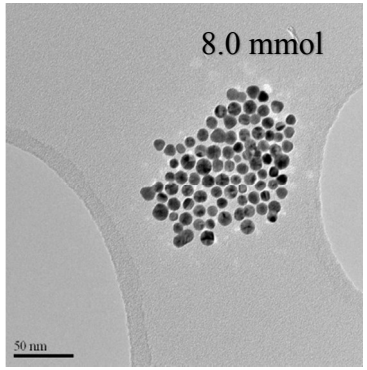
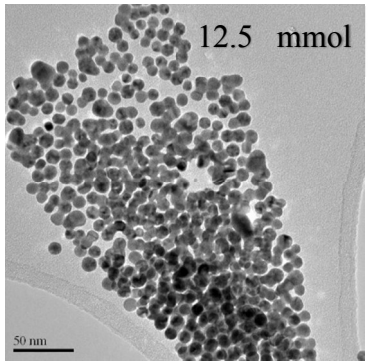
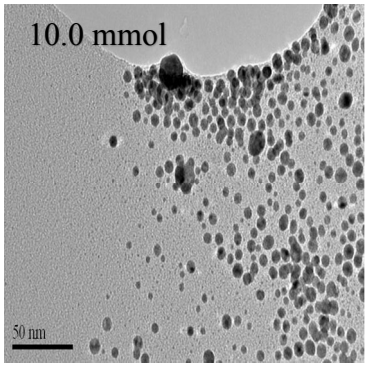
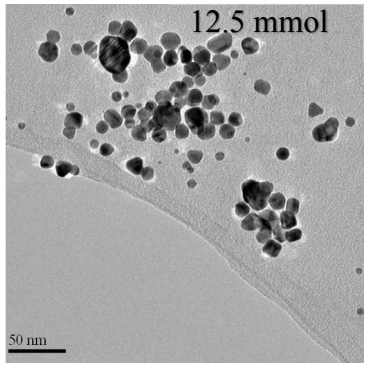
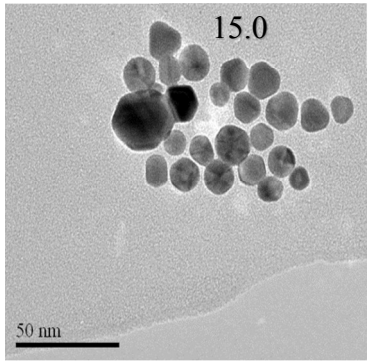
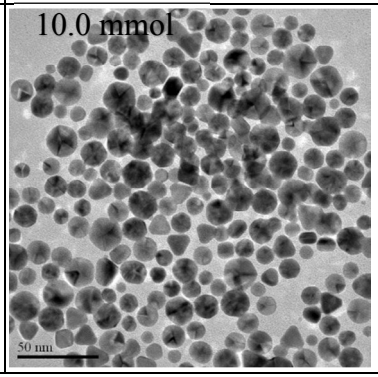
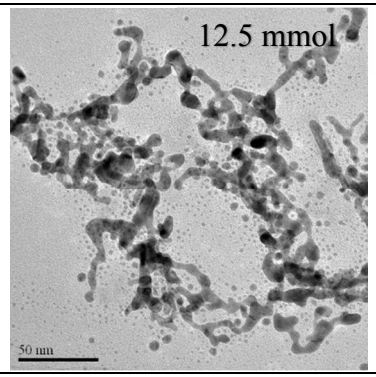
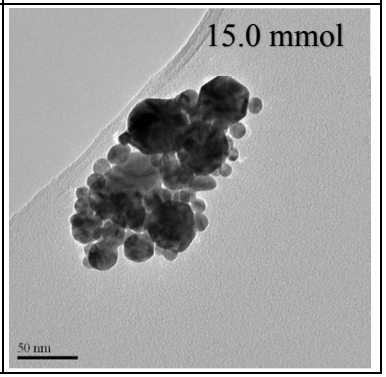
L / AuNPs	TEM micrograph as amount of stabilizer is varied (mmol) at scale bar 50 nm		
L8 **AuNP 4**			
L9 **AuNP 5**			
L10 **AuNP 6**			

Figure 3: TEM micrograph of **AuNPs 4-6** showing effects of varying amount of stabilizers on nature of AuNPs

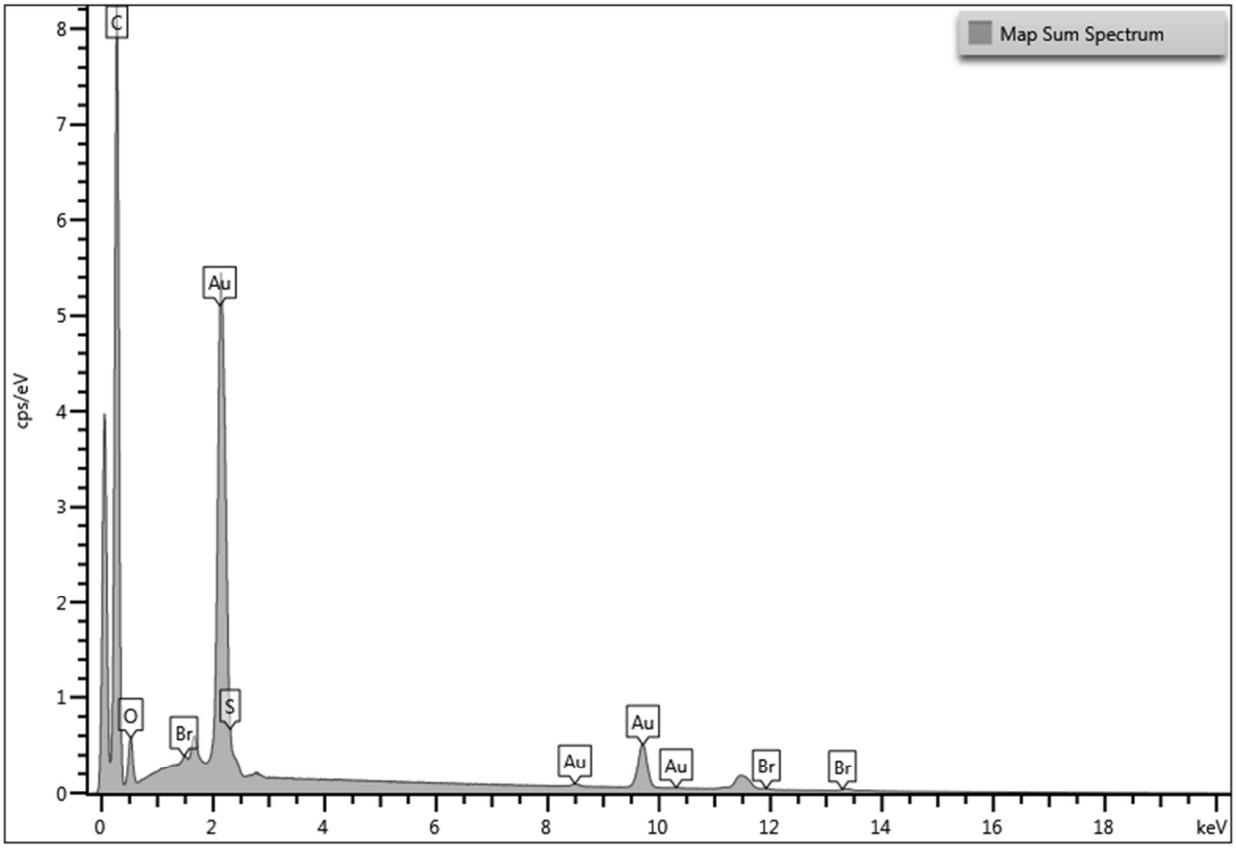


Figure 4. EDX of **AuNPs-4a** showing the presence of bromide in the nanoparticles

3.4 Stability of the gold nanoparticles

Stability of gold nanoparticles were studied at critical coagulation concentration in high NaCl concentrations and monitored by UV-vis spectroscopy. The UV-vis spectrum showed no significant red shift after the addition of 200 μL of 10% NaCl (1.7 M). Even after addition of 400 μL of 1.7 M NaCl solution, no visible colour change was observed and λ_{max} shifted only by 4 nm (Figure S6); signifying that the AuNPs were stable at high concentration of NaCl. These observations confirmed that glucoamidoalkyl disulfides and thiols can be used as stabilizers for AuNPs even at high concentrations of NaCl and that these AuNPs could therefore be used in

drug delivery applications.[75] Also after storing the AuNPs at 4 °C for 4-5 months, their UV-vis spectra (Figure 5) remained essentially the same and EDX analysis (Figure 6) still showed the presence of gold in the sample.[76-78] This confirms that these carbohydrate-sulfur compounds are excellent stabilizers for AuNPs. In comparison to nanoparticles protected by ionic and polymeric stabilizers, [79-80] self-assembled monolayers, by taking advantage of strong thiol-gold interactions and van der Waals attractions of neighboring molecules, exhibit superior stability,[81]a prerequisite for practical applications. However, susceptibility to ligand exchange with thiols in cells are expected to be minimal.[81]

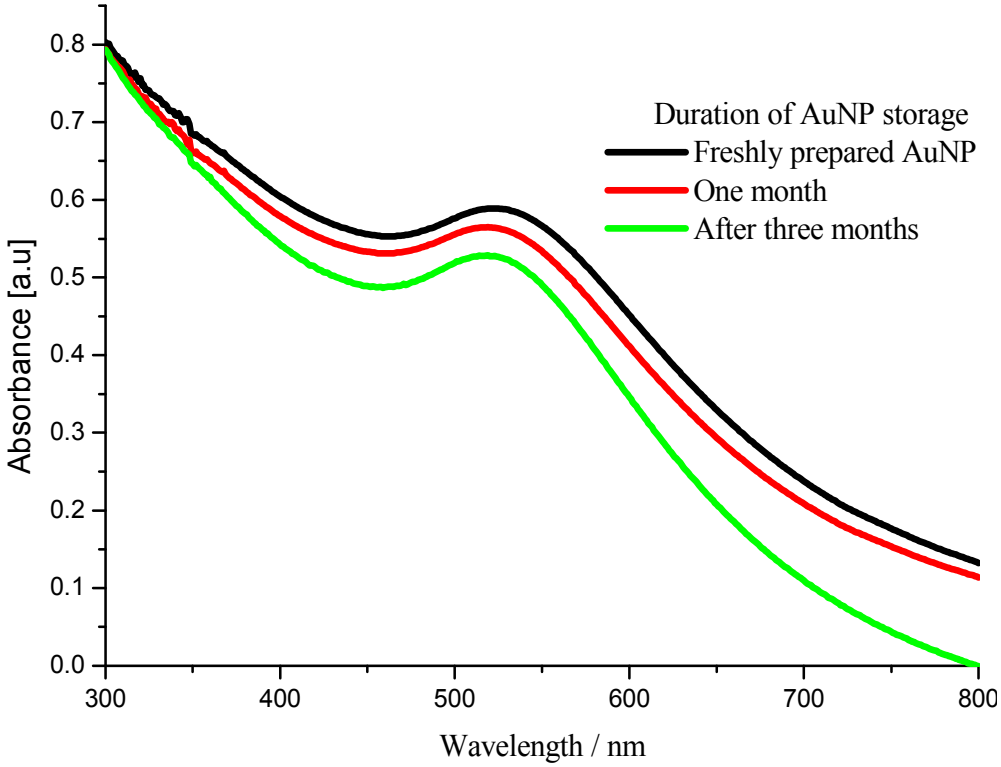


Figure 5: Stability studies of AuNPs over time as shown by UV-vis spectra of **AuNPs-1a** over a 3 month period.

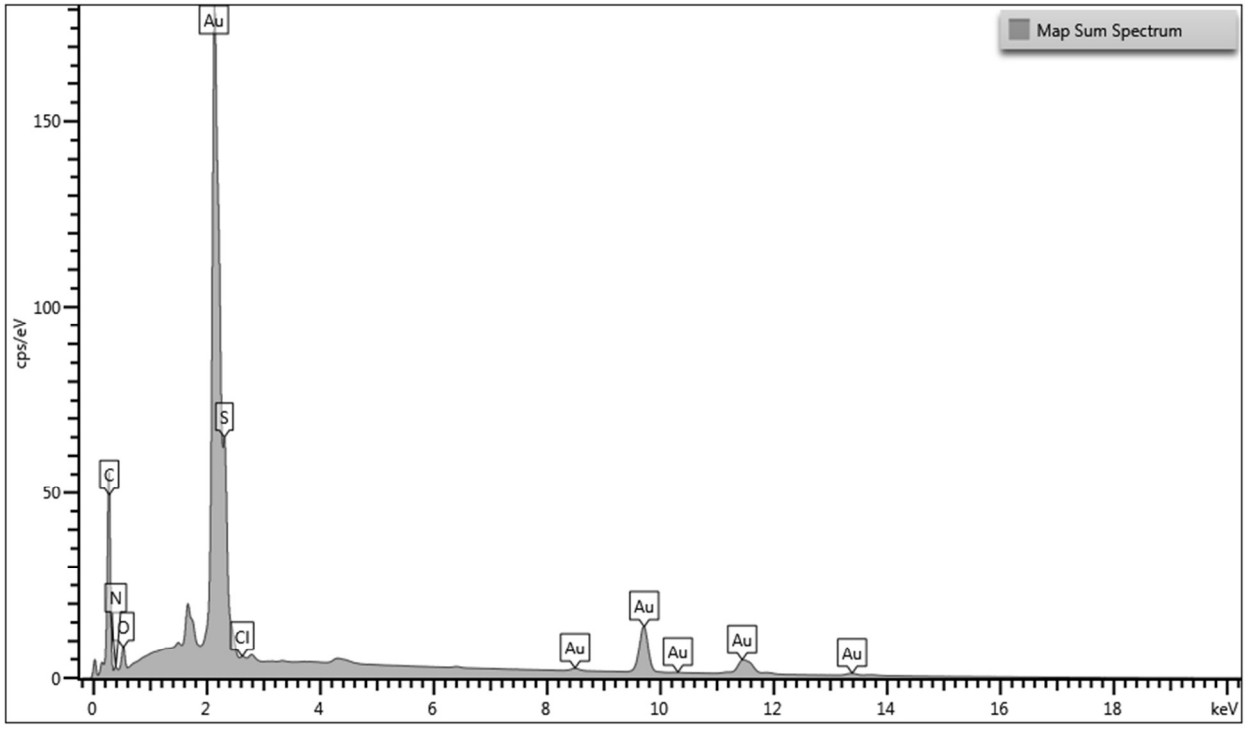


Figure 6: EDX spectrum of **AuNPs-1**a showing the absence of bromide in the nanoparticles

3.5 Toxicity and cytotoxicity of compounds L1-4 and L8-L10

Toxicity of compounds **L1-L10** towards PM1 cell line was evaluated; with Raltegravir (an integrase inhibitor), and Auranofin as controls. These two drugs were chosen due to their activities towards HIV[82] and cancer cells[83-84] respectively, since our main aim is to develop non-toxic stabilizers as delivery vehicles for anticancer and HIV drugs. The results showed that the compounds are non-toxic towards PM1 cells lines, with IC_{50} value $\geq$ 200 μM except for compounds **L6** and **L7** which had IC_{50} of 44.63±7.10 μM and 42.08±7.47 μM, respectively (Table 5). Compounds **L6** and **L7** are thus considered moderately toxic. Having establish that the stabilizers used to prepare AuNPs are non-toxic, the stabilizers **L1-L3** and their AuNPs were further tested against three cancer cell lines, namely MCF7 (breast), HCT116 (colon) and PC3

(prostate). **AuNPs 4-6** were not tested against these cancer cell lines due to the persistance of TOABr (*vide infra*) which is known to be highly toxic to normal cells. Compounds **L1-L5** and **L8-L10** were inactive against the three cancer cell lines with $IC_{50} \geq 100$ μM (Table 5 and Figure S7). **AuNPs 1-3** (18.0 mmol) of size ranging between *ca*. 4-5 nm, showed total inhibition growth (TGI) values above 100 μg/mL for the three cell lines and were thus classified as inactive. However, **AuNPs-1** and **AuNPs-2** showed slight improvement in activities against MCF7 with IC_{50} values of 88.6 and 91.4 μM respectively (Table S1 and Figure S8), but can largely also be considered inactive.

Table 5: Toxicity of **L1-L10** toward PM1 cells lines.

Compounds	**L1-5**	**L6**	**L7**	**L8-10**	Control	
					Raltegravir	Auranofin
$IC_{50}\pm STD_{DEV}$ (μM)	≥ 200	44.63±7.10	42.07±7.50	≥ 200	≥ 200	0.667±0.21

4. Conclusions

Novel gluco- disulfides and thiols were prepared from D-(+) gluconic acid δ-lactone *via* amidation ring opening of the cyclic carbohydrate by aminoalkylthiols. The ring opening of the cyclic carbohydrate led to disulfides which were successfully reduced by zinc dust to the corresponding thiols. Toxicity studies of these compounds on PM1 cell line reveal that they are non-toxic and can be used to stabilize gold nanoparticles. With compounds **L1-L3** and **L8-L10** gold nanoparticles of sizes in the range of *ca*. 2-5 and 4-9 nm respectively could be prepared. Compounds **L1-L3** and the all the gold nanoparticles are inactive against all the three cancer cell lines except gold nanoparticles **1a** and gold nanoparticles **2a**, which show weak activity against

MCF7. The nanoparticles are very stable in high concentrated NaCl medium and several months at -18 ^{o}C and therefore could be useful in drug delivery applications.

Supporting Information

Electronic Supporting Information: ^{1}H, ^{13}C{H} NMR and UV-vis spectra of compounds and AuNPs. Crystallographic data has been deposited with the Cambridge Crystallographic Data Centre with CCDC 1005537. Copies of this information may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44-1223-336063; e-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

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References

1. S. Ahmad, *Coord. Chem. Rev*. 2004, **248**, 231.

2. C. F. Shaw III. *Biochemistry and Technology*, H. Schmidbaur, ed. (Chichester, United Kingdom: John Wiley & Sons), 1999, 259.

3. M. J. McKeage, L. Maharaj and S. J. Berners-Price, *Coord. Chem. Rev*. 2002, **232**, 127.

4. E. R. T. Tiekink, *Crit. Rev. Oncol. Hematol*. 2002, **42**, 225.

5. L. Messori, and G. Marcon, eds. A. Sigel, and H. Sigel, (Basel, Switzerland: FontisMedia S. A. and Marcel Dekker Inc.), 2004, 385.

6. I. Kostova, *Anticancer Agents Med. Chem.* 2006, **6**, 19.

7. C. G. Hartinger, A. A. Nazarov, S. M. Ashraf, P. J. Dyson and B. K. Keppler, *Cur. Med. Chem.* 2008, **15**, 2574-2591

8. E. R. T. Tiekink, *Gold Bull.* 2003, **36**, 117.

9. M. Viotte, B. Gautheron, M. M. Kubicki, I. E. Nifant'ev and S. P. Fricker, *Met.-Based Drugs* 1995, **2**, 311.

10. T. Okada, B. K. Patterson, S. Q. Ye and M. E. Gurney, *Virology* 1993, **192**, 631.

11. S. K. Libutti, G. F. Paciotti, L. Myer, R. Haynes, W. Gannon and M. Walker, *Nanomedicine* 2009, **27**, 3586.

12. C. R. Patra, R. Bhattacharya, D. Mukhopadhyay and P. Mukherjee, *Adv. Drug Deliv. Rev.* 2010, **62**, 346.

13. S. J. Berners-Price, G. R. Girard, D. T. Hill, B. M. Sutton, P. S. Jarrett, L. F. Faucete, R. K. Johnson, C. K. Mirabelli and P. Sadler, *J. Med. Chem.* 1990, **33,** 1386.

14. R. K. DeLong, C. M. Reynolds, Y. Malcolm, A. Schaeffer, T. Severs and A. Wanekaya *Nanotech, Sci. App.* 2010, **3**, 53.

15. M. Brust, M. Walker, D. Bethell, D. J. Schiffrin and R. Whyman, *J. Chem. Soc. Chem. Commun.*, 1994, 801.

16. R. H. Terrill, T. A. Postlethwaite, C.-H. Chen, C. D. Poon, A. Terzis, A. Chen, J. E. Hutchison, M. R. Clark, G. Wignall, J. D. Londono, D. Superfine, M. Falvo, C. S. Johnson, E. T. Samulski and R. W. Murray, *J. Am. Chem. Soc.*, 1995, **117**, 12537.

17. M. J. Hostetler, J. E. Wingate, C.-J. Zhong, J. E. Harris, R. W. Vachet, M. R. Clark, J. D. Londono, S. J. Green, J. J. Stokes, G. D. Wignall, G. L. Glish, M. D. Porter and R. W. Murray, *Langmuir*, 1998, **14**, 17.

18. A. C. Templeton, W. P. Wuelfing and R. W. Murray, *Acc. Chem. Res.*, 2000, **33**, 27.

19. W. P. Wuelfing, S. M. Gross, D. T. Miles and R. W. Murray, *J. Am. Chem. Soc*., 1998, **120**, 12696.

20. E. E. Foos, A. W. Snow, M. E. Twigg and M. G. Ancona, Chem. Mater., 2002, **14**, 2401.

21. A. C. Templeton, S. W. Chen, S. M. Gross and R. W. Murray, *Langmuir*, 1999, **15**, 66.

22. A. G. Kanaras, F. S. Kamounah, K. Schaumburg, C. J. Kiely and M. Brust, *Chem. Commun*., 2002, **20**, 2294.

23. K. K. Snadhu, M. C. McIntosh, M. J. Simard, W. S. Smith, and M. V. Rotello, *Bioconjugate Chem*. 2002, **13**, 3.

24. H. Wang, Y. Chen, X.-Y. Li, and Y. Liu, *Mol. Pharmaceutics* 2007, **4**, 189.

25. A. M. Mintzer and E. E. Simanek, *Chem. Rev*. 2009, **109**, 259.

26. M. J. De la Fuente, G. A. Barrientos, C. T. Rojas, J. Rojo, J. Canada, A. Fernandez and S. Penades, *Angew. Chem.* 2001, **113**, 2318.

27. G. A. Barrientos, M. J. de la Fuente, T. C. Rojas, A. Fernandez, and S. Penades, *Chem. Eur. J.* 2003, **9**, 1909.

28. M. J. de la Fuente, D. Alcantara and S. Penades, *IEE Trans. Nanobiosci.* 2007, **6**, 275.

29. C. R. Patra, R. Bhattacharya, D. Mukhopadhyay and P. Mukherjee, *Adv. Drug Deliv. Rev.* 2010, **62**, 346.

30. W. Wets and K. Drickamer, *Annu. Re*v. *Biochem.* 1996, **65**, 44.

31. A. Varki, *Glycobiology* 1993, **3**, 97.

32. R. A. Dwek, *Chem. Re*v. 1996, **96**, 683.

33. A. Housni, H. Cai, S. Liu, S. H. Pun and R. Narain, *Langmuir* 2007, **23**, 5056.

34. J. M. de la Fuente, A. G. Barrientos, T. C. Rojas, J. Rojo, J. Canada, A. Fernandez and S. Penadés, *Angew. Chem., Int. Ed.* 2001, **40**, 2257.

35 A. G. Barrientos, J. M. de la Fuente, T. C. Rojas, A. Fermandez and S. Penades, *Chem. Eur. J.* 2003, **9**, 1909.

36. J. L. de Paz, R.Ojeda, A. G. Barrientos, S. Penades and M. Martin-Lomas, *Tetrahedron: Asymmetry* 2005, **16**, 149.

37. P. Crespo, R. Litran, T. C. Rojas, M. Multigner, J. M. de la Fuente, J. C. Sanchez-Lopez, M. A. Garcia, A. Hernando, S. Penades and A. Fernandez, *Phys. Rev. Lett.* 2004, **93**, 87204.

38. H. Otsuka, Y. Akiyama, Y. Nagasaki and K. Kataoka, *J. Am. Chem. Soc.* 2001, **123**, 8226.

39. C. S. Tsai, T. B. Yu and C. T. Chen, *Chem. Commun.* 2005, 4273.

40. A. G. Barrientos, J. M. de la Fuente, T. C. Rojas, A. Fernandez and S. Penadés, *Chem. Eur. J.* 2003, **9**, 1909.

41. M. Zheng and X. Huang, *J. Am. Chem. Soc.* 2004, **126**, 12047.

42. J. Rojo, V. Dỳaz, J. M. de la Fuente, I. Segura, A. G. Barrientos, H.-H. Riese, A. Bernad and S. Penadés, *ChemBioChem* 2004, **5**, 291.

43. R. Ojeda, L. J. de Paz, G. A. Barrientos, M. Martyn-Lomas and S. Penades, *Carbohydr. Res.* 2007, **342**, 448.

44. C. L. Schofield, R. A. Field and D. A. Russell, *Anal. Chem.* 2007, **79**, 1356.

45. C. L. Schofield, A. H. Haines, R. A. Field and D. A. Russell, *Langmuir* 2006, **22**, 6707.

46. A. J. Reynolds, A. H. Haines and D. A. Russell, *Langmuir* 2006, **22**, 1156.

47. J. M. de la Fuente, D. Alcántara, P. Eaton, P. Crespo, T. C. Rojas, A. Fernández, A. Hernando and S. Penadés, *J. Phys. Chem. B* 2006, **110**, 13021.

48. C. López-Cartes, T. C. Rojas, R. Litrán, D. Martinez, de la J. M. Fuente, S. Penadés, and A. Fernández, *J. Phys. Chem. B* 2005, **109**, 8761.

49. K. M. Halkes, A. C. de Souza, C. E. P. Maljaars, G. J. Gerwig, and J. P. Kamerling, *Eur. J. Org. Chem.* 2005, 3650.

50. X. Jiang, M. Ahmed, Z.Deng and R.Narain, *Bioconjugate Chem*. 2009, **20**, 994.

51. M. Erlandsson and M. Hällbrink, *Int.l J. Pep. Res. and Ther*. 2005, **11**, 261.

52. J. L. Wardell, in S. Patai (ed.). The Chemistry of the Thiol Group, Part 1, *Wiley*, NY, 1974, 163.

53. J. M. De la Fuente, A. G. Barrientos, T. C. Rojas, J. Rojo, J. Cańada, A. Fernández And S. Penadés, *Angew. Chem. Int.* Ed. 2001, **40**, 2257; (b54) B. Nolting, J-J. Yu, G-yu. Liu, S-J. Cho, S. Kauzlarich and J. Gervay-Hague, *Langmuir,* 2003, **19***,* 6465.

55. M. Brust, J. Fink, D. Bethell, D. J. Schiffrin and C. Kiely, *J. Chem. Soc. Chem. Commun.* 1995, 1655.

56. Bruker-AXS. 2009 APEX2, SADABS, and SAINT Software Reference Manuals. Bruker-AXS, Madison, Wisconsin, USA.

57. G. M. Sheldrick; SHELXL. *Acta Cryst.* 2008, **A64**, 112.

58. T. L. Riss and R. A. Moravec, *Mol. Biol. Cell (Suppl.)* 1992, **3**, 184a.

59. Y. Gu, R. Y. LaBell, D. F. O'Brien and S. S. Saavedra, *Angew. Chem. Int. Ed*. 2001, **40**, 2320.

60. F. H. Allen, O. Kennard, D. G. Watson, L. Brammer and A. G. Orpen, *J. Chem. Soc. Perkin Trans. II* 1987, S1.

61. O. Knop, R. J. Boyd and S. C. Choi, *J. Am. Chem. Soc.* 1988, **110**, 7299.

62. H. A. Biebuyck, C. D. Bain and G. M. Whitesides, *Langmuir* 1994, **10**, 1825.

63. C. C. Lin, Y. C. Yeh, C. Y. Yang, G. F. Chen, Y. C. Chen, Y. C. Wu and C. C. Chen, *Chem. Commun*. 2003, 2920.

64. H. Otsuka, Y. Akiyama, Y. Nagasaki and K. Kataoka, *J. Am. Chem. Soc***.** 2001, **123**, 8226.

65. S. A. Svarovsky, Z. Szekely and J. J. Barchi Jr, Tetrahedron*: Asymmetry* 2005, **16**, 587.

66. P. Fenter, A. Eberhardt, and P. Eisenberger, *Science* 1994, **266**, 1216.

67. Zubragel, C. Deuper, F. Schneider, M. Neumann, M. Grunze, A. Schertel and C. WÖll, *Chem. Phys. Lett.* 1995, **238**, 308.

68. M. S. Yeganah, S. M. Dougal, R. S. Polizzotti and P. Rabinowitz, *Phys. Rev. Lett.* 1995, **74**, 1811.

69. Y-J. Chuang, X. Zhou, Z. Pan and C.Turchi, *Biochem. Biophys Res. Commun*., 2009, **389**, 1.

70. H. Hinterwirth, S. Kappel,T. Waitz,T. Prohaska,W. Lindner and M. Lämmerhofe, *ACS Nano* 2013, **7**, 1129

71. A. E. Lanterna, E. A. Coronado and A. M. Granados, *J. Phys. Chem. C* 2012, **116**, 6520.

72. S. D. Techane, L. J. Gambleand and D. G. Castner, *J. Phys. Chem. C* 2011, **115**, 9432.

73. E. Pensa, E. Cortes, G. Corthey, P. Carro, C. Vericat, M. H. Fonticelli, G. Benitez, A. A. Rubert and R. C. Salvarezza, *Acc. Chem. Res*. 2012, **45**, 1183.

74. A. Ulman, *Chem. Rev*. 1996, **96**, 1533.

75. M. Ahmed, Z. Deng, S. Liu, R. Lafrenie, A. Kumar and R. Narain, *Bioconjugate Chem*. 2009, **20**, 2169.

76. K. Mollazadeh-Moghaddam, B. Varasteh Moradi, R. Dolatabadi-Bazaz, M. Shakibae and A. R. Shahverdi, *Avicenna J. Med. Biotech.* 2011, **3**, 195.

77. G. Singaravelu, J. S. Arockiamary, V. G. Kumarb and K. Govindaraju, *Colloid Surf. B Biointerfaces* 2007, **57**, 97.

78. A. Sobczak-Kupiec, D. Malina, M. Zimowska and Z. Wzorek, Dig. *J. Nanomat. Biostruct*. 2011, **6**, 803.

79. R. Hunter, J. *Foundations of Colloid Science*; Oxford University Press: New York, 1987; Vol. 1.

80. D. H. Napper, *Polymeric Stabilization of Colloidal Dispersions*; Academic Press: London, 1983.

81. S-Y. Lin, Y-T. Tsai, C-C. Chen, C-M. Lin, C. Chen, *J. Phys. Chem. B* 2004**, 108**, 2134.

82. A. Savarino *Expert Opin Investig Drugs* 2006, **15**, 1507.

83. C. K. Mirabelli, B. D. Jensen, M. R. Mattern, C. M. Sung, S. M. Mong, D. T. Hill, S. W. Dean, P. S. Schein, R. K. Johnson, S. T. Crooke, *Anti-Cancer Drug Des*. 1986, **1**, 223.

84. P. Fonteh, D. Meyer, *Metallomics* 2009, **1**, 427.