IMPROVED SYNTHESIS OF TETRAETHYLENE GLYCOL-DERIVED THIOL FOR MONOLAYER-PROTECTED GOLD NANOPARTICLES

By

Andrzej Balinski

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Approved:

Professor David Cliffel

Professor David W. Wright

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CHAPTER I

INTRODUCTION

1.1 Monolayer-Protected Gold Nanoparticles

Monolayer-protected particles originate from studies involving self-assembled monolayers (SAMs) generated by the spontaneous assembly of organic molecules on two-dimensional (2-D) substrates. In 1983 Nuzzo et al. reported adsorption of disulfides on zerovalent gold substrates in well-ordered, regularly oriented array.[2] Later on, studies involving SAMs on flat substrates were adapted to form three-dimensional (3-D) SAM-coated structures with a gold core, resulting in monolayer-protected gold nanoparticles (AuNPs) which can be handled as isolable species and further functionalized with a variety of ligands, such as thiols, disulfides, dialkyl sulfides, thiosulfates, xanthates, carbamates, phosphines, phosphine oxides, amines, carboxylates, selenides, and isocyanides.[3,4] The unique properties of thiolate monolayer-protected AuNPs make them excellent candidates for use in a variety of advanced technologies. Among many applications such as electronics,[5] nonlinear optics,[6] catalysis,[3,7] vapor sensing[8] monolayer-protected AuNPs are strong candidates for bioanalytical applications.[9]

Brust and co-workers were able to increase the stability of colloidal gold in solutions by developing a novel synthesis of alkanethiol-stabilized gold nanoparticles, opening a new field of 3D-SAMs.[10] This synthetic route allows the control of size, chemical behavior, and stability. AuNPs developed by Brust et al. are air-stable, functionalized and easy to handle while having new intriguing properties that would place them in between molecules and bulk material.[11]

In the Brust synthesis, tetrachloroauric acid ($HAuCl_4$) and a phase transfer agent, tetraoctylammonium bromide (TOABr), are dissolved in water and then vigorously stirred with solution of dodecanothiol ($C_{12}H_{25}SH$) in toluene. $AuCl_4^-$ is transferred from aqueous to organic solution by a TOA^+ counterion and Au^{3+} is reduced to Au^{1+} by thiols, yielding a $(AuC_{12}H_{25}SH)_n$ polymer.[12,13] Aqueous $NaBH_4$ is added to reduce the gold, and the $Au_x(C_{12}H_{25}S)_y$ nanoparticles are formed. (Figure 1.1)

$$HAu^{III}Cl_4 + C_{12}H_{25}SH \xrightarrow[C_6H_5Me]{N(C_8H_{17})_4,\ H_2O} (Au^{I}C_{12}H_{25}S)_n \xrightarrow[H_2O]{NaBH_4} Au_x(C_{12}H_{25}S)_y$$

Figure 1.1 Two-phase Brust reaction scheme

While this method is simple and straightforward, persistent contamination of isolated nanoparticles with residual TOABr is a significant drawback.[14] Such contamination can influence potential applications of AuNPs, such as vapor-sensors employing thin nanoparticle films on interdigital electrodes that rely on changes in electron tunneling currents arising from reversible vapor sorption.[15] Another important issue involves analysis of AuNPs by mass spectrometry (MS), because TOABr is a quaternary amine with permanent positive charge and causes analyte ion suppression.[16] Multiple methods of purification have been developed and proved to be costly and time consuming. [17]

While the Brust synthesis was a major advance, alkanethiolate protected nanoparticles are not soluble in water[10,18] and therefore are incompatible with biomolecular systems. Many widely-studied water-soluble nanoparticles utilize charged ligands such as carboxylic acids.

Unfortunately, charged ligands promote non-specific binding with biological molecules through electrostatic interactions.[19]

1.2 Gold Monolayer-Protected Clusters for biological systems

Among bioanalytical applications, emphasis has been directed to new approaches to eliminate nonspecific interactions between nanoparticles and proteins. In order for monolayer-protected nanoparticle to be optimized for biological systems, it must meet certain basic criteria: (i) solubility in aqueous solution, (ii) it has to be able to undergo ligand exchange in order to provide specific function for targeted biomolecule, (iii) it should minimize any nonspecific interactions with the biological system that it will be introduced to, (iv) it has to have certain size and shape as it can have implications in toxicity,[20] and (v) it has to be stable and free from aggregation and agglomeration in high ionic strength solutions.[19,20] Most of the reported water-soluble AuNPs are stabilized by an alkanethiol that is terminated with carboxylic acid or some other strongly ionic group.[21-23] The solubility of these NPs depends often on changes in pH or ionic strength of the solution. Hydrogen bonding is yet another issue that can lead to particle agglomeration.[24]

The first charge-neutral, water-soluble, thiolated poly(ethylene glycol) (PEG-SH) monolayer-protected gold nanoparticles were reported by Wuelfing et al., who synthesized their AuNPs using α-methoxy-ω-mercapto-poly(ethylene glycol) (PEG-SH, MW 5000).[25] While these particles showed good chemical and thermal stability, further functionalization of such nanoparticles *via* ligand exchange proved to be impossible due to extreme size of the attached

ligand. PEG-SH chains form random coils on the nanoparticle surface, preventing introduced exchanging ligand from reaching that surface of the gold core.[25-28]

AuNPs can be synthesized with much shorter thiolated ethylene glycol chains, making them amenable to ligand exchange reactions for further functionalization.[19,26,27,29] Small ethyelene glycols are also soluble in water, charge neutral and have good protein binding resistance.[19] AuNPs featuring such ligands were first reported by Foos et al.,[26] who used $CH_3(OCH_2CH_2)_nSH$ with n = 2, 3, and 4. But that method was based on a two-phase solution synthesis[10] developed by Brust, $CH_3(CH_2)_5S$ ligands were place exchanged with $CH_3(OCH_2CH_2)_nSH$ (n = 2, 3, and 4). While this approach is quite simple and efficient, TOABr will be apparent in the final product.

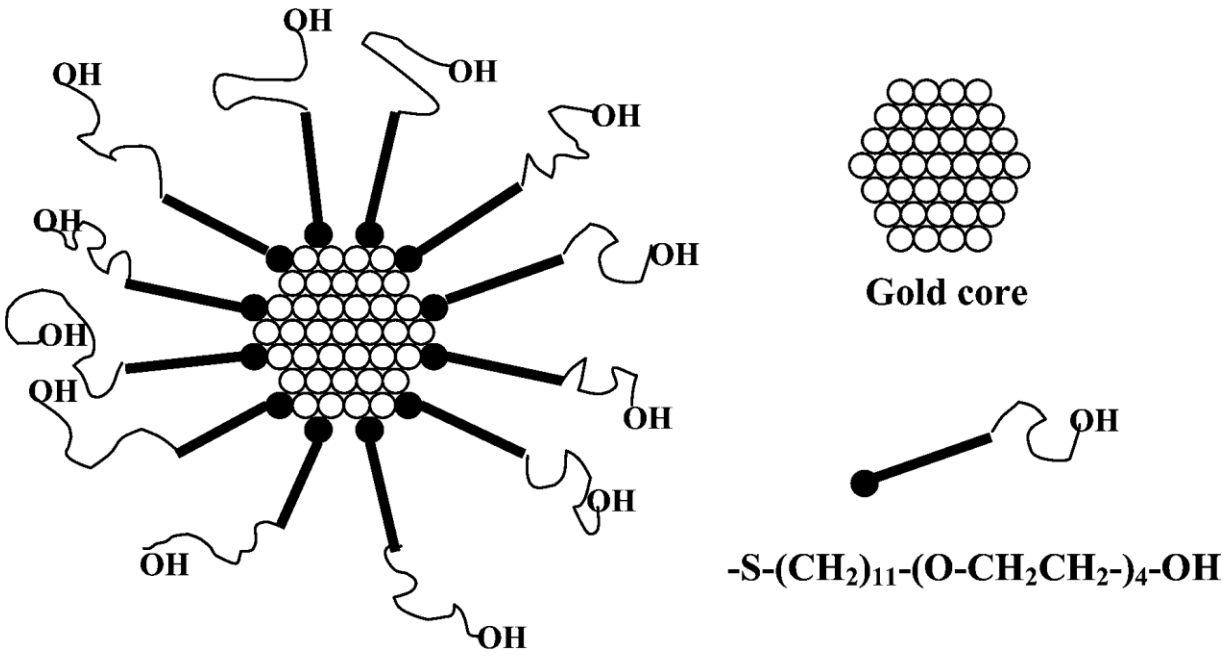

Figure 1.2. Schematic representation of a gold nanoparticle protected by a monolayer of monohydroxy (1-mercaptoundec-11-yl) tetraethylene glycol. The hydrophobic C_{11}-chain confers extreme stability to the cluster, while the hydrophilic tetraethylene glycol unit ensures solubility in water. (Reprinted from Ref. 28. Copyright 2002 Chemical Communications (Cambridge, United Kingdom))

Kanaras et al.[29] reported direct synthesis of a thioalkylated ethylene glycol-protected AuNP from alcoholic solution. This ligand is much longer ($HS(CH_2)_{11}(OCH_2CH_2)_4OH$) and composed of two parts: a thioalkylated hydrophobic block that is proven to provide very good stability for the NP, and a tetraethylene glycol terminus that provides a highly hydrophilic, yet uncharged, shell (Figure. 1.2).

The first direct synthesis of short oligo(ethylene glycol)-protected AuNPs was reported by Huang and co-workers.[19,27] This research group used $CH_3(OCH_2CH_2)_nSH$ with (n = 2, 3 and 4) as a ligand - same set of ligands as Foos's research group. Synthesis was carried out in methanol with 9-18% water content, yielding 40-50% AuNP product. Ion-exchange chromatography and gel electrophoresis experiments proved that these AuNPs are completely resistant to nonspecific interactions with DNA, RNA, and proteins, providing good starting material for targeted biological applications.

CHAPTER II

SYNTHESIS OF MERCAPTOTETRAETHYLENE GLYCOL

2.1. Introduction

Among many polymers, poly(ethylene glycol) (PEG) is one of the most widely used materials in various fields such as drug delivery, gene delivery, lithium polymer electricity storage systems, plastics industry and so on. PEG is a very important biocompatible polymer that facilitates solubilization and long-term circulation of proteins, viruses and other biological macromolecules.[30] It can provide charge-neutral synthetic coatings on nanoparticles that dictate its solubility and interactions with macromolecules and cell surfaces.

(5) **(6)**

Figure 2.1 **.** Meracoptotetraethylene glycol MTEG (**5**) and dimercaptotetraethylene glycol Di-MTEG (**6**)

Tetraethylene glycol (HO-$(C_2H_4O)_4$-H) can be functionalized by substituting one or both terminal hydroxyl groups with the thiol group (SH). The resulting mono-functionalized molecule, mercaptotetraethylene glycol (MTEG), can be used as a capping ligand for the synthesis of monolayer-protected AuNPs, while a bi-functionalized molecule (di-thiol) can be used as very important substrate for synthesis of biodegradable disulfide polymers[31] as well as linking agent in between multiple AuNPs.[3] There have been several synthetic strategies described for derivatizing PEG to thiol. As a first step they all require conversion of PEG to a *p*-

toluenesulfonate (tosylate) ester by reaction with *p*-toluenesulfonyl chloride (triethylamine or another tertiary amine is necessary to remove HCl byproduct), followed by the separation of mono-tosylate and di-tosylate by silica gel chromatography. There are three general methodologies for further derivatization. The first is the Bunte salt method,[32] which requires conversion of tosylate to halide. The halide reacts with $Na_2S_2O_3$, and then is refluxed with 1M HCl (Figure 2.2).

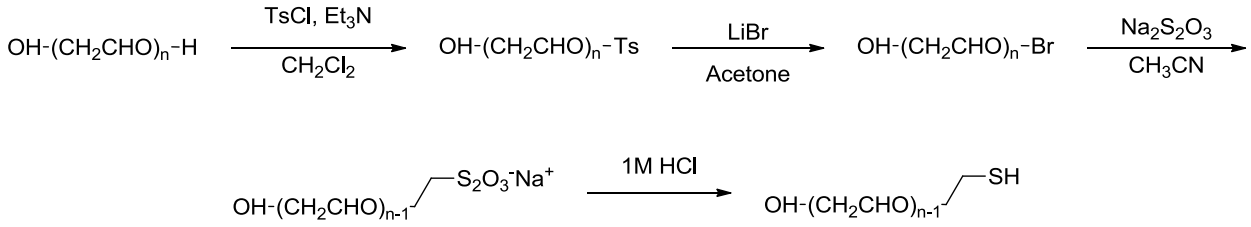

Figure 2.2. Synthesis of mercaptopolyethylene glycol based on Bunte salt method

The second, isothiouronium salt method,[33] is accomplished by reacting PEG tosylate with thiourea in order to obtain the isothiouronium salt, followed by hydrolysis with 2M NaOH (Figure 2.3).

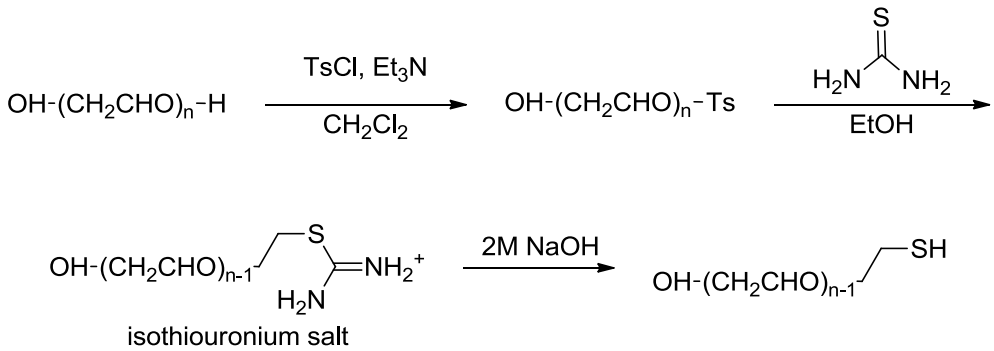

Figure 2.3. Synthesis of mercaptotetraethylene glycol based on isothiouronium salt method

The third is the thioacetate method[28,34] which is accomplished by conversion of tosylate to the thiol through thioacetate-protected PEG. This is easily hydrolysed by 1M HCl to form the final product (Figure 2.4).

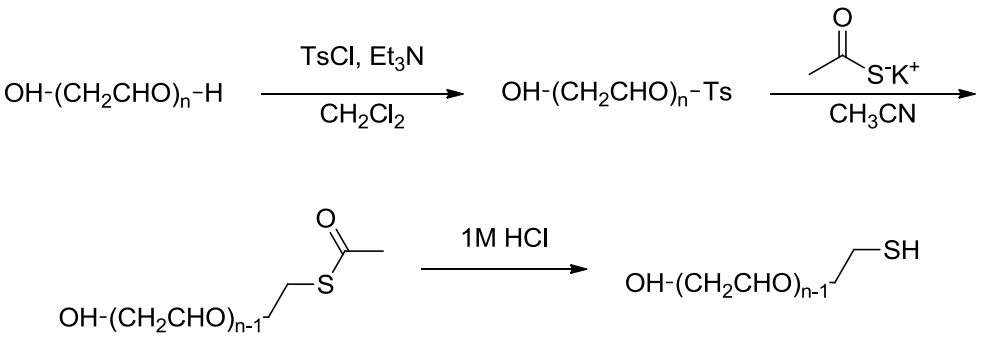

Figure 2.4. Synthesis of mercaptotetraethylene glycol using potassium thioacetate.

In this synthesis, the formation of disulfide byproducts can be easily prevented by isolating the protected mercaptan (**4**) (Figure 2.5), which can be stored and quickly de-protected when needed. Since thiols are oxidized by air to disulfides, this ability makes the thioacetate method is ideal for this work.

2.2 Experimental Procedures

2.2.1 Reagents and Chemicals

All chemicals were used as received from the manufacturer without further purification. Triethylamine, p-toluenesulfonyl chloride, tetraethylene glycol, thioacetic acid, sodium hydride, petroleum ether, dichloromethane, ethyl acetate, acetonitrile and hydrochloric acid were purchased from Acros, Sigma-Aldrich or EMD. Sodium thioacetate was synthesized in house immediately prior to use by reacting thioacetic acid with sodium hydride under argon. $KMnO_4$ staining solution was prepared in house.

*2.2.2 Synthesis of tetraethylene glycol tosylate (**2**) and tetraethylene glycol ditosylate(**3**)*

P-toluenesulfonyl chloride (0.05 mol, 9.82g) in 80 ml of methylene chloride was added dropwise to a mixture of 0.05 mol (9.71g) of tetraethylene glycol (**1**) and 0.05 mol (5.06g, 3.67ml) of triethylamine over 1 hour at 0°C. The mixture was then stirred overnight at room temperature. A white triethylamine hydrochloride precipitate was filtered off and washed with 50 ml of methylene chloride. The methylene chloride was removed under reduced pressure to leave pale yellow oil, which was purified by flash chromatography on silica using dichloromethane and acetonitrile (3:1, v/v) → (1:1, v/v) → (0:1, v/v). Tetraethylene glycol ditosylate (**3**) eluted first followed by tetraethylene glycol tosylate (**2**). Solvents were evaporated to give: 3.76g (15% yield) for (**3**) and 11.32g (65% yield) for (**2**), colorless oil in both cases. ^{1}H NMR (**2**) ($CDCl_3$) δ 7.81-7.79 (d, 2 H, aromatic), 7.35-7.33 (d, 2 H, aromatic), 4.17-4.15 (t, 2 H, $O_2SOC\mathit{H}_2$), 3.72-3.59 (m, 14 H, $OC\mathit{H}_2$), 2.45 (s, 3 H, $-C\mathit{H}_3$). ^{1}H NMR (**3**) ($CDCl_3$) δ.81-7.79 (d, 4 H, aromatic), 7.35-7.33 (d, 4 H, aromatic), 4.17-4.15 (t, 2 H, $O_2SOC\mathit{H}_2$), 3.72-3.59 (m, 14 H, $OC\mathit{H}_2$), 2.45 (s, 6 H, $-C\mathit{H}_3$).

*2.2.3 Synthesis of MTEG (**5**)*

A 0.03 mol portion of (**2**) (10.45g) in dry acetonitrile (20 ml) was added dropwise over 15 minutes to 0.036 mol of freshly prepared sodium thioacetate in dry acetonitrile under argon. The mixture was stirred for 3 hours at room temperature. The white sodium tosylate precipitate was filtered off and the solvent evaporated under reduced pressure. The residue was dissolved in 25 mL of a 1 M solution of hydrochloric acid and heated under reflux for 2 hours. The solvent was evaporated and the residue purified by flash chromatography on silica using dichloromethane/acetonitrile with 0.1% triethylamine (3:1, v/v) → (2:1, v/v) → (1:1, v/v),

Solvents were evaporated to give 5.44g of pale yellow oil (86% yield). ^{1}H NMR ($CDCl_3$) δ 3.76-3.61 (m, 14 H, OC*H*$_2$), 2.73-2.68 (m, 2 H, -C*H*$_2$S), 1.62 (t, 1 H, S*H*).

*2.2.4 Synthesis of Di-MTEG (**6**)*

A 0.01 mol portion of (**3**) (5.03g) in dry acetonitrile (20 ml) was added dropwise over 15 minutes to 0.022 mol of freshly prepared sodium thioacetate in dry acetonitrile under argon. The mixture was stirred for 3 hours at room temperature. The white disodium ditosylate precipitate was filtered off and the solvent evaporated under reduced pressure. The residue was dissolved in 20 mL of a 1 M solution of hydrochloric acid and heated under reflux for 2 hours, dithioacetate is not soluble in aqueous hydrochloric acid, therefore reaction takes place on the phase border. The solvent was evaporated and the residue purified by flash chromatography on silica using dichloromethane with 0.1% triethylamine initially, then dichloromethane/acetonitrile with 0.1% triethylamine (10:1, v/v) → (5:1, v/v). Solvents were evaporated to give 2.01g of pale yellow oil (88.9% yield). ^{1}H NMR ($CDCl_3$) δ 3.76-3.61 (m, 12 H, OC*H*$_2$), 2.73-2.68 (m, 4 H, -C*H*$_2$S), 1.62-1.56 (t, 2 H, S*H*).

2.2.5 NMR

Approximately 3-5 mg of sample was weighed into an NMR tube and dissolved in ~ 0.7 mL of deuterated chloroform. Spectra were obtained on a 400 MHz Bruker AV-I FT NMR collecting 16 scans with a d1 delay of 1 second.

2.2.6 GC-MS

Approximately 100 ug of sample was dissolved in dichloromethane. Traces were obtained on a Varian Saturn 2100T GC/MS/MS with HP-5MS column (J&W Scientific, 30 meters, 0.25 mm ID). Injector set to 280°C with split of 50. Temperature program as follows:

start at 150°C and hold for 4 minutes, then ramped to 280°C at 15°C/min and hold for 3 minutes. Column was at constant flow rate of 1.0 ml/min.

2.3 Results and discussion

The main objective was to develop a simple and cost efficient route to obtain MTEG. The synthesis described here, depicted in Figure 5, is based on modified thioacetate reaction briefly noted in the introduction (Figure 2.4). Tetraethylene glycol (**1**) was converted to tetraethylene glycol tosylate by reaction with *p*-toluenesulfonyl chloride. Since tetraethylene glycol has two terminal OH groups, tosylation occurs on both ends, but adding 1 mol equiv of *p*-toluenesulfonyl chloride to tetraethylene glycol produced 65% monotosylate (**2**) and 15% ditosylate (**3**), in good agreement with reported yields for these compounds.[28,32] Separation is accomplished by column chromatography on silica gel using mixture of dichloromethane and acetonitrile. Steinem et al. reported higher yields with a petroleum ether/ethyl acetate solvent system for separation but these results could not be reproduced.[35]

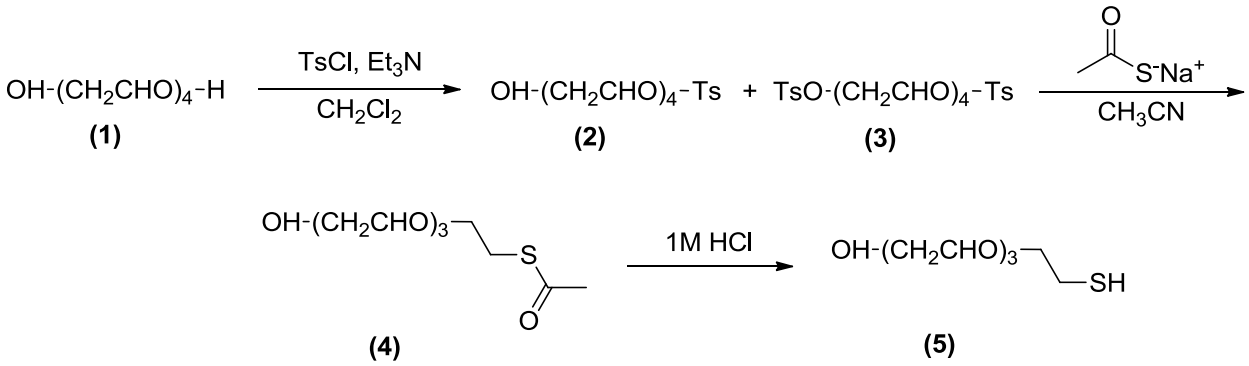

Figure 2.5. Synthesis of MTEG

While it is fairly simple to separate mono (**2**) and ditosylate derivatives (**3**), unreacted tetraethylene glycol (**1**) trails monotosylate derivative very closely. In order to obtain pure

MTEG, separation of monotosylate from unreacted tetraethylene glycol is very important. Thin liquid chromatography (TLC) plates stained with $KMnO_4$ solution were used to monitor separation progress and proved to be qualitatively accurate.

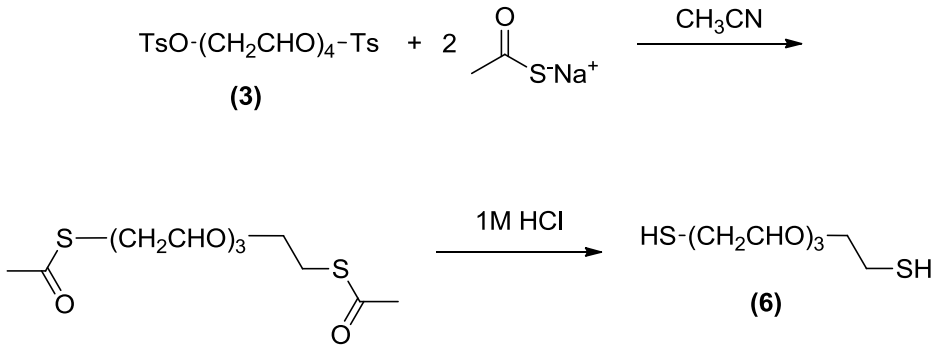

Figure 2.6. Synthesis of Di-MTEG

Tosylate is well known to be a very good leaving group, and its PEG derivatives undergo nucleophilic substitutions at much higher rates than PEG halides, yet Feldheim et al.[28] reacted their PEG tosylate with LiBr to obtain monobromo PEG derivative. This unnecessary extra step appears to result in the lower yield of the final product. Therefore, a monobromo derivative was not pursued in this work.

The next step is the reaction with sodium thioacetate. Thioacetate is a reagent of choice because the reaction occurs under mild conditions and leads to a protected mercaptan (**4**), which can be a convenient form of storage for the thiol. The thiol can be released under acidic aqueous conditions compatible with MTEG (**5**). In this work the mercaptan (**4**) was not isolated, but rather was used as a raw material for the next step, in which it was refluxed in 1M HCl for 2 hours, then separated by flash chromatography. MTEG was found to be soluble in all solvents tested (water, methanol, DMSO, THF, acetone, acetonitrile, dichloromethane, chloroform, DMF and diethyl ether), therefore purification by extraction is not suitable in this case and would result

in very low yields. Vacuum distillation also would not be expected to provide a purification route since boiling points of MTEG, tetraethylene glycol and tosylate are close to each other. The purity of final product was confirmed by NMR and GC-MS.

2.4 Conclusions

MTEG was successfully synthesized using a modified thioacetate method. This approach requires only three steps, compared to four steps for the Bunte salt method[32,36] and Feldheim's modified thioacetate method.[28] Moreover, the overall yield for this method (56%) is much higher than the Bunte salt (13%) and Feldheim's thioacetate method (16%). The published isothiouronium method has a similar yield (57%) and number of steps, but this yield is inflated by the mass of silica contaminant. This contaminant is present in the product because ethanol, known to partially dissolve silica, is used as one the eluents in the flash chromatography of the final product. The use of an acetonitrile/dichloromethane solvent system eliminated this contaminant and reduced the amount of solvents for purification. Furthermore, both mono- and di-thiol forms can be easily obtained and stored in the mercaptan form, preventing the formation of disulfides.

CHAPTER III

DIRECT SYNTHESIS OF MERCAPTOTETRAETHYLENE GLYCOL MONOLAYER-PROTECTED CLUSTERS

3.1. Introduction

PEG-protected nanoparticles have been widely studied and used as a biocompatible material that exhibits good resistance to nonspecific binding with biological molecules. Rationally designed poly-functionalized, water-soluble AuNPs have potential for performing multiple functions within the space of a single cell.

The synthesis of water soluble monolayer-protected AuNPs can be accomplished by using a thiolated PEG protecting ligand in a modified Brust reaction.[10,19,25,27] In the modified Brust reaction, thiolated PEG capping ligand is added to a solution of tetrachloroauric acid, where Au^{3+} is reduced to Au^{1+}, yielding a mixture of gold-PEG-S polymer.[12,13] Addition of $NaBH_4$ leads to further reduction of Au^{I} to Au^{0} and formation of PEG-S protected AuNPs.[37]

$$HAu^{III}Cl_4 + PEGSH \xrightarrow[20\%\ CH_3COOH]{80\%\ MeOH} (Au^{I}PEGS)_n \xrightarrow[MeOH]{NaBH_4} Au_x(PEGS)_y$$

Figure 3.1. Modified one-phase Brust reaction scheme for PEG ligands

The first one-phase synthesis of PEG-S monolayer-protected AuNPs was reported by Wuelfing et al., who synthesized their nanoparticles in traditional two phase reaction setup using α-methoxy-ω-mercapto-poly(ethylene glycol) (PEG-SH, MW 5000).[25] Unfortunately further functionalization of such nanoparticles *via* ligand exchange proved to be impossible due to extreme size of the attached ligand. Short ethylene glycol monolayer-protected AuNPs were

initially reported by Foos et al., but the final product was a result of place exchange reaction from previously synthesized alkanethiolate AuNPS.[26] Huang and co-workers reported the direct synthesis of ethylene glycol monolayer-protected AuNPs utilizing *α*-methoxy-*ω*-mercapto derivatives (chain lengths of n = 2, 3 and 4).[19] This synthesis was accomplished in a mixed solvent environment, in which 8-19% water in methanol proved to be the optimal range for obtaining stable, water soluble nanoparticles.

A-methoxy-*ω*-mercapto-tetraethylene glycol proved to be an excellent protecting ligand for multiple reasons, such as: (i) it has well-defined length of 1.6 nm, as opposed to long PEG chains that are prone to stretching and retracting depending on solvent polarity,[19] (ii) forms a densely packed monolayer while PEG forms loose random structure on the surface, (iii) mixed monolayer for further applications can be easily prepared while PEG forms coils and prevents functional ligand from reaching the surface of the core.

MTEG is much easier and cheaper to prepare than *α*-methoxy-*ω*-mercapto-tetraethylene glycol and can provide AuNPs with the same properties at lower cost and shorter preparation times. Synthesis of MTEG AuNPs can be accomplished by adapting PEG-SH synthesis depicted in Figure 9.

$$HAu^{III}Cl_4 + MTEG \xrightarrow{MeOH} (Au^{I}MTEG)_n \xrightarrow[MeOH]{NaBH_4} Au_x(MTEG)_y$$

Figure 3.2. Modified Brust reaction scheme for MTEG ligands.

3.2 Experimental Procedures

3.2.1 Reagents and Chemicals

$HAuCl_4 \cdot 3H_2O$ was synthesized in-house as described elsewhere.[38] MTEG was synthesized in-house according to the method described in Chapter 2. Sodium borohydride ($NaBH_4$, 98+%) and methanol were purchased from Sigma-Aldrich. Water was purified by Barnstead NANOpure system($\geq$ 18 MΩ).

3.2.2 MTEG AuNP Synthesis

Two syntheses were performed. In the first, 0.54 mmol of $HAuCl_4 \cdot 3H_2O$ (0.212 g) was dissolved in 60 ml of methanol/acetic acid mixture (6:1 ratio), 1.08 mmol of MTEG (0.227g) was added and the reaction mixture stirred for 30 minutes before 5.4 mmol (0.203g) of $NaBH_4$ in 10 ml of methanol was added rapidly.

In the second synthesis 0.54 mmol of $HAuCl_4 \cdot 3H_2O$ (0.212 g) was dissolved in 60 ml of methanol, 1.08 mmol of MTEG (0.227g) was added and the reaction mixture stirred for 30 minutes before 5.4 mmol (0.203g) of $NaBH_4$ in 10 ml of methanol was added dropwise. In both cases the solution was allowed to stir for 4 hours before the solvent was evaporated. Black residue was dissolved in water and transferred in to dialysis tubing (Thermo, SnakeSkin® Plated Dialysis Tubing, 10,000 MWCO) for 4 days, changing the water three times a day.

3.2.3 NMR

Approximately 3-5 mg of sample was weighed into an NMR tube and dissolved in ~ 0.7 mL of deuterated chloroform. Spectra were obtained on a 400 MHz Bruker AV-I FT NMR collecting 16 scans with a d1 delay of 1 second.

3.2.4 IM-MS

A saturated sample of MTEG AuNPs in 1 μL of deionized H_2O was combined with 10 μL of saturated CHCA and 1% NaCl in MeOH. A 1 μL portion of this was deposited on a stainless steel plate using the dried droplet method. All MALDI-IM-MS analyses were performed using a Synapt HDMS (Waters Corp., Manchester, UK), equipped with a frequency-tripled Nd:YAG (355 nm) laser operated at a pulse repetition frequency of 200 Hz. All spectra were acquired in the positive ion mode at laser energy settings approximately 10% above threshold values. Gold-containing ion signals were extracted and identified using the MassLynx 4.1 (Waters Corp.) software package.

3.2.5 UV-VIS

UV−vis spectra were obtained on a Cary 100 Bio UV−vis spectrophotometer in the range of 350−800 nm.

3.2.6 TEM

Samples were prepared by placing 1 drop of 1 mg/ml solution of AuNPs in DI water onto 400 mesh ultrathin carbon film/holey carbon grids (Ted Pella, Redding, CA, Product # 01824) and dried under vacuum overnight. TEM images were obtained on a Phillips CM20 electron microscope operating at 200 kV at magnifications of 200Kx. Cluster diameters were measured along the major elliptical axis using ImageJ version 1.41 (available at http://rsbweb.nih.gov/ij/).

3.2.7 Thermal gravimetric analysis

The organic composition was determined using TGA (ISI TGA 1000, Instrument Specialists Inc. Twin Lakes, WI). Prior to analysis, samples were dried under vacuum overnight to remove moisture. Typical experiments consisted of 5-10 mg of dry MPCs in a platinum pan under a N_2 flow of ~60 mL/min. Data was recorded between 20 – 800 °C at a rate of 20 °C/min.

3.3 Results and discussion

The objective of this work was to attempt the synthesis of AuNPs protected by MTEG (synthesis described in Chapter II). Initial synthesis was based on the reported on well-described tiopronin AuNP synthesis,[23] 6:1 ratio of methanol to acetic acid was used with 3:1 ratio of MTEG to $HAuCl_4$ and rapid addition of 10:1 excess of $NaBH_4$ to $HAuCl_4$. While this approach produced AuNPs, the observed yield (34%) was lower than reported for α-methoxy-ω-mercapto-tetraethylene glycol AuNPs.[19] The AuNP product appeared to be completely soluble in methanol and water, but only partially soluble in dichloromethane. Extra MTEG ligand was present, as evidenced by a large organic fraction observed by TGA (49%). The majority of the product was soluble in dichloromethane and dark brown in color, having an average core diameter of 1.2 ± 0.3 nm (Figure 3.3). The product which was insoluble in dichloromethane had an average core diameter of 3.6 ± 1.4 nm, and contained a gray precipitate. This precipitate may have been remaining gold-MTEG complexes that were not reduced or a product of AuNP decomposition.

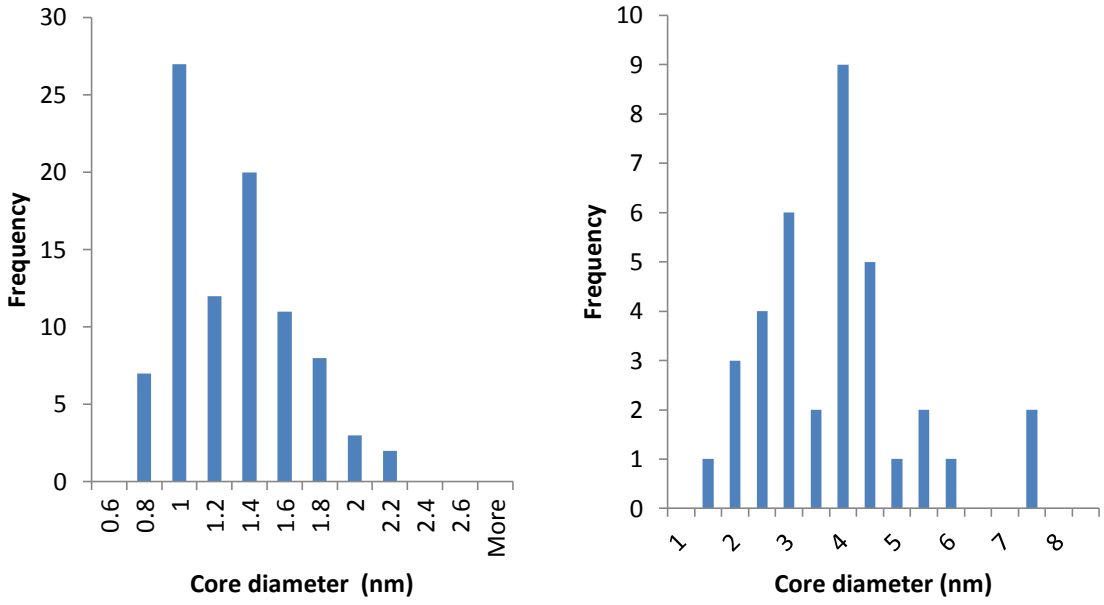

Figure 3.3. Histograms of dichloromethane soluble and insoluble fractions

In second synthesis the acetic acid was not added, the thiol-to-gold ratio was decreased and $NaBH_4$ (in 5 ml methanol) was added dropwise over 2 minutes. The purpose of these modifications was to achieve larger AuNPs in a simpler solvent system. The product of this synthesis was soluble in methanol and water, and completely insoluble in dichloromethane. The AuNPs were larger than the first batch synthesized, as evidenced by the larger surface plasmon band. The stability of these AuNPs is superior, having resisted aggregation to date (6 weeks). The properties of these AuNPs are optimized for biological applications due to their enhanced hydrophilicity and strong stability.

3.4 Conclusions

A simple synthetic method has been designed that produces MTEG-protected AuNPs in moderate yields using easily available materials. These AuNPs exhibit strong hydrophilicity, resistance to aggregation, and are expected to possess bioresistant properties. The relatively short MTEG ligand provides an easy route for further functionalization with biologically active molecules. Therefore, these particles are optimal for use under physiological conditions and meet the requirements for bio-analytical applications.

APPENDIX

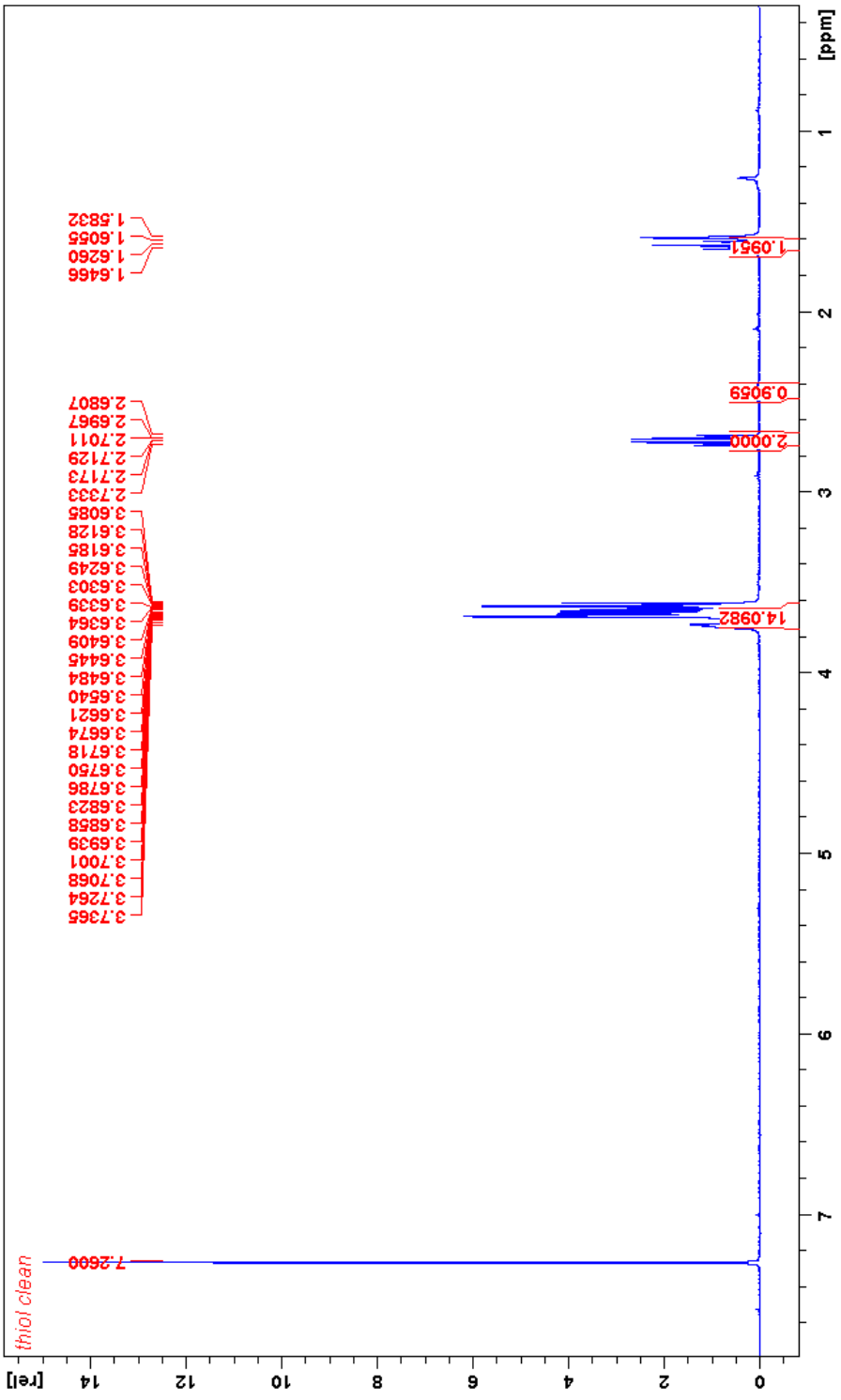

Figure A1. ^{1}H NMR of MTEG

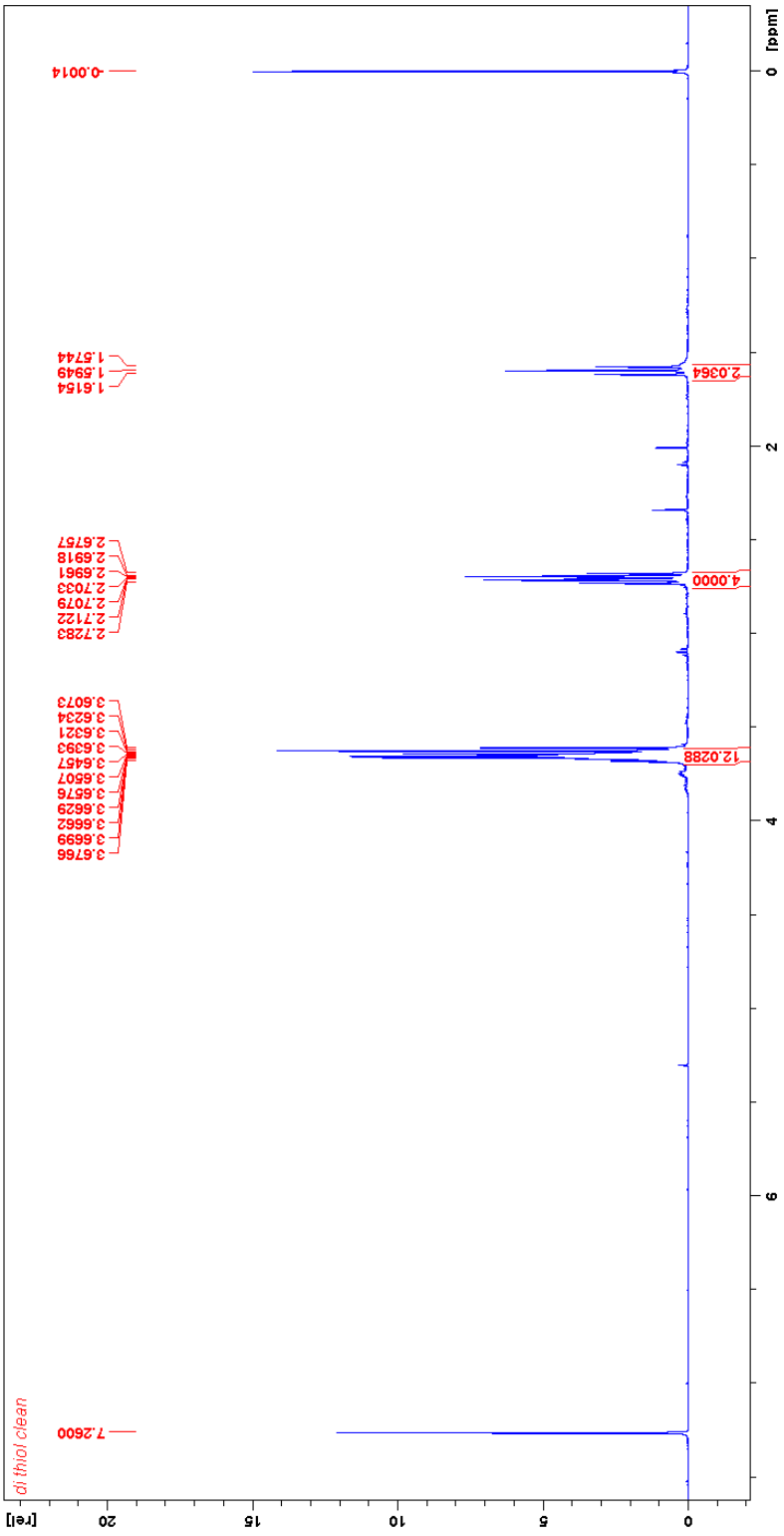

Figure A2. ^{1}H NMR of Di-MTEG

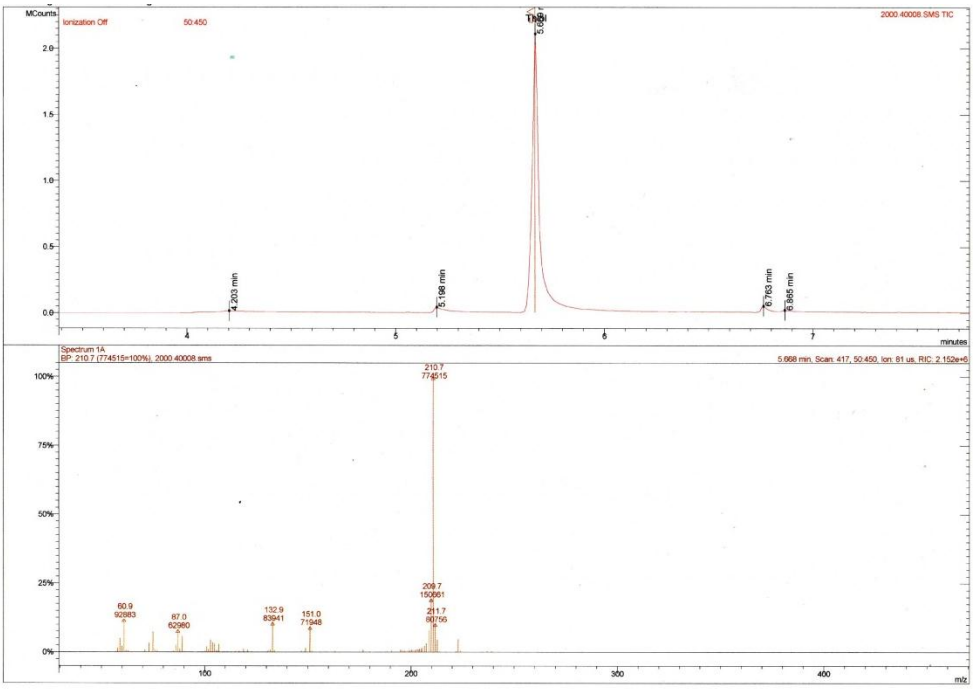

Figure A3. GC-MS of MTEG

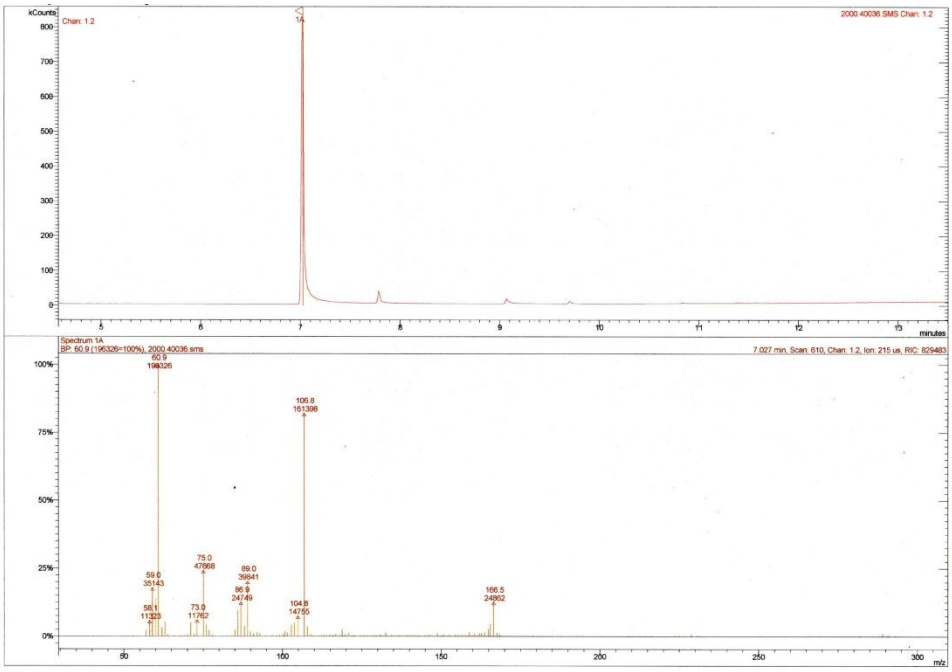

Figure A4. GC-MS of Di-MTEG

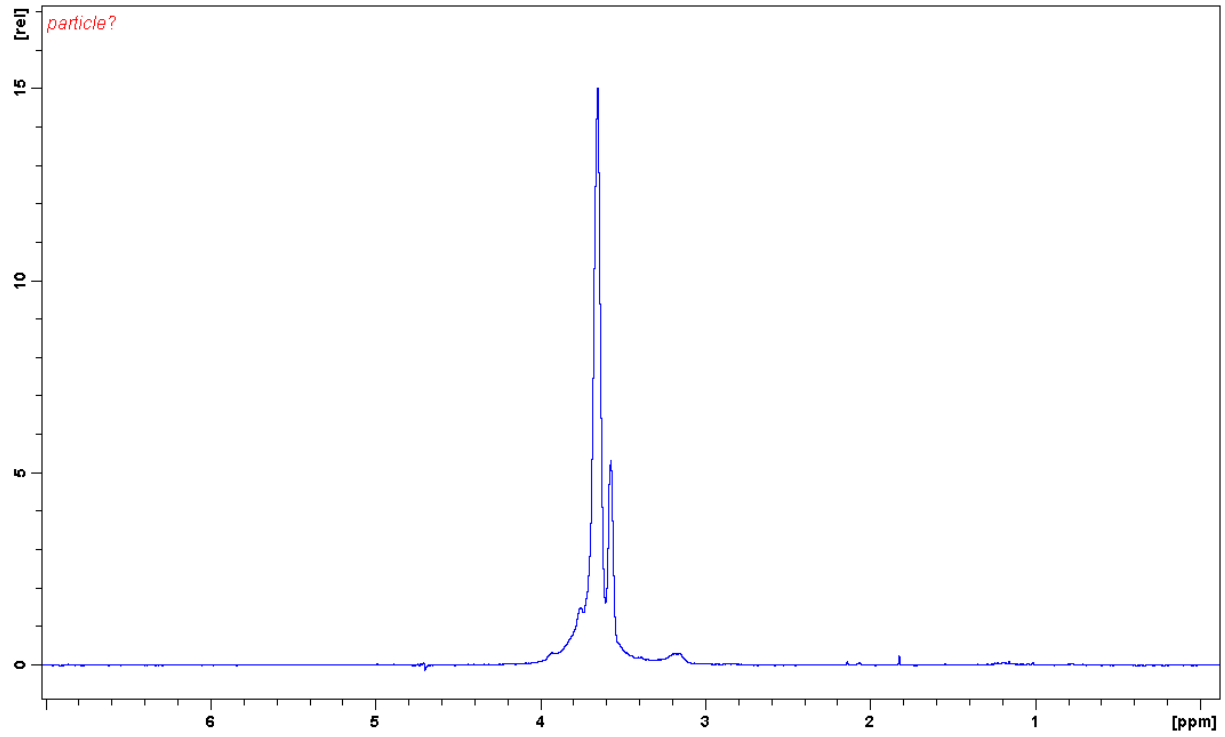

Figure A5. ^{1}H NMR of MTEG AuNP

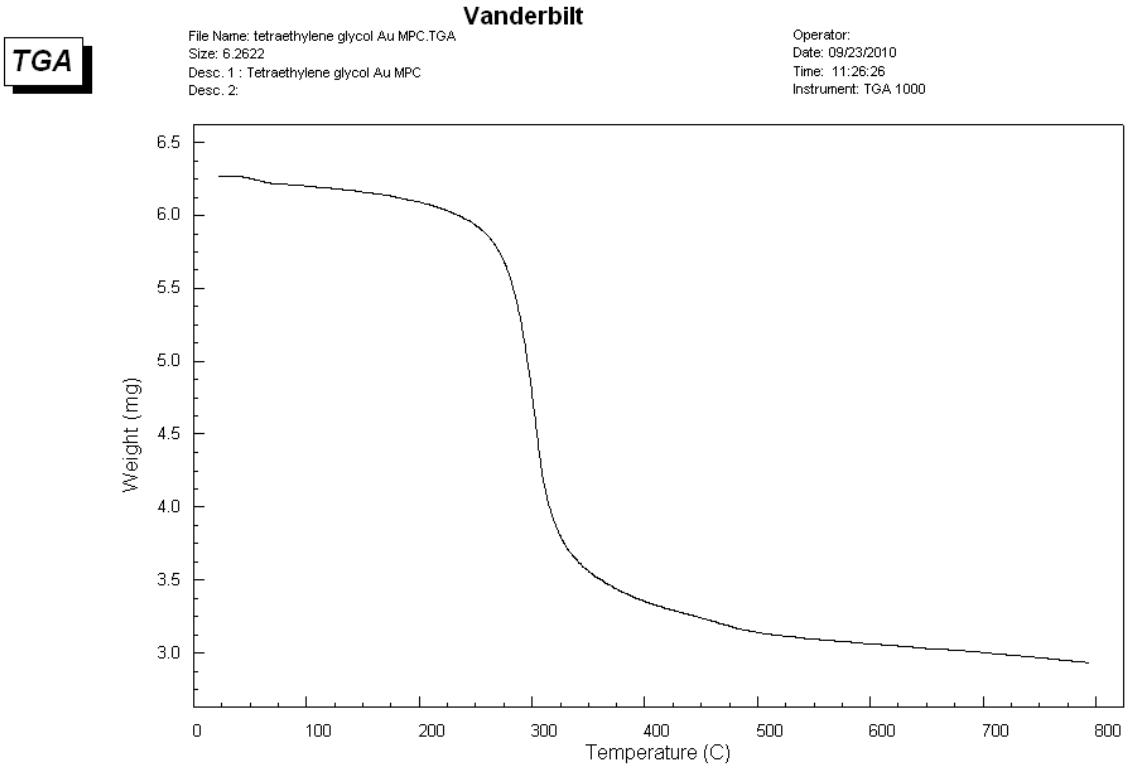

Figure A6. TGA of MTEG AuNP – first synthesis

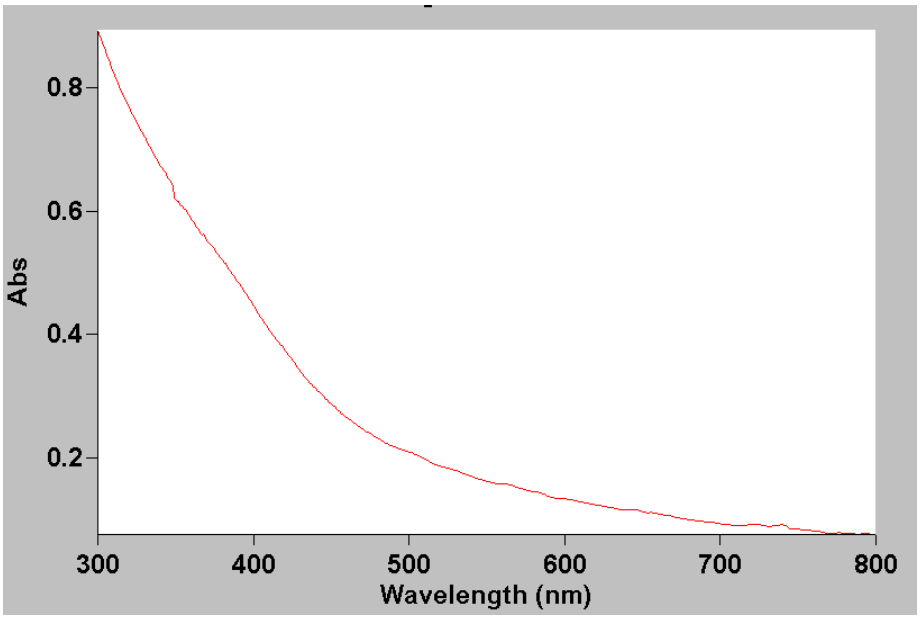

Figure A7. UV-VIS of MTEG AuNP – fraction soluble in dichloromethane

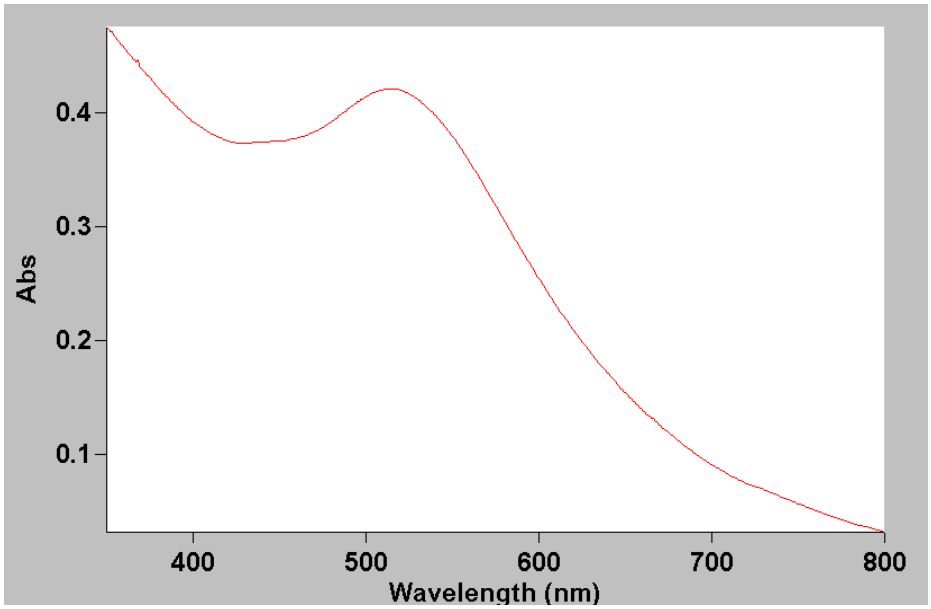

Figure A8. UV-VIS of MTEG AuNP – fraction insoluble in dichloromethane

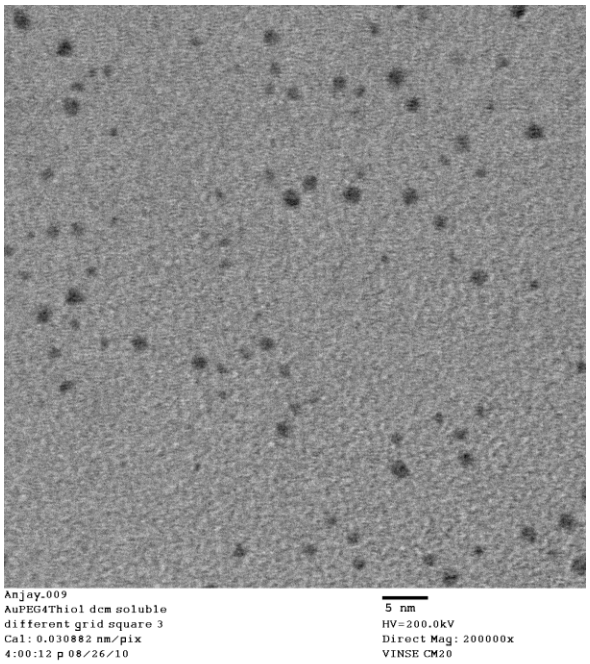

Figure A9. TEM of MTEG AuNP – fraction soluble in dichloromethane

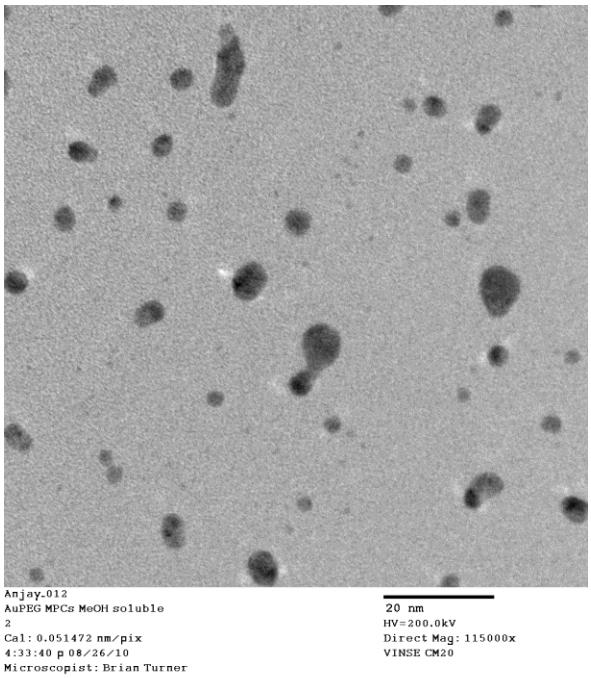

Figure A10. TEM of MTEG AuNP – fraction insoluble in dichloromethane

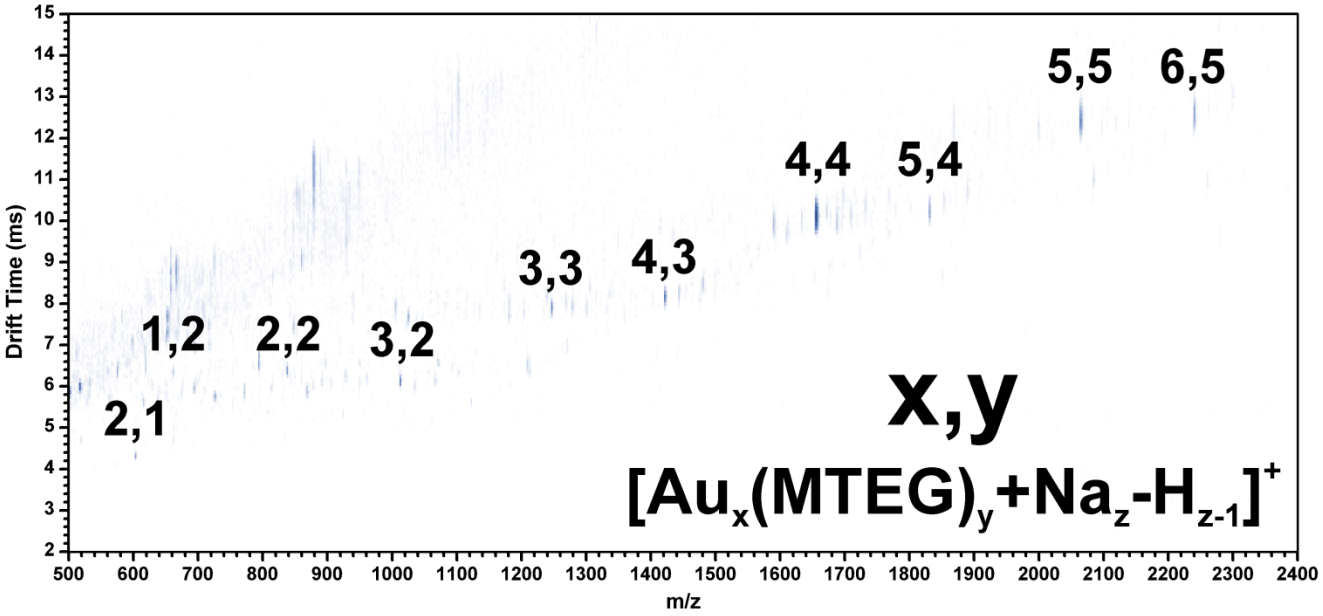

Figure A11. Ion mobility-mass spectrum of MTEG AuNPs. Peaks are annotated x,y for $[Au_x(MTEG)_y+Na_z-H_{z-1}]^+$. Most abundant gold-thiolate ions are tetrameric (4,4) and pentameric (5,5) complexes, stoichiometrically similar to gold-tiopronin complex ions.[1]

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