Phosphonioalkylthiosulfate zwitterions—new masked thiol ligands for the formation of cationic functionalised gold nanoparticles

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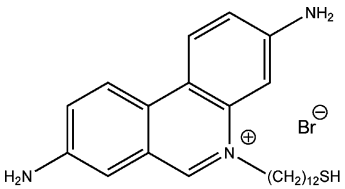
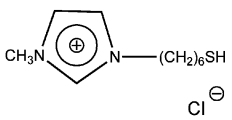
We report the synthesis and structural characterisation of a new family of stable phosphonioalkylthiosulfate zwitterions, $R_3P^+(CH_2)_nS_2O_3^-$ (R = Ph or Bu, n = 3,4,6, 8 or 10) which behave as cationic masked thiolate ligands with applications in the functionalisation of gold nanoparticles, having potential as new diagnostic biorecognition systems. The ligands were prepared by treatment of ω-bromoalkylphosphonium salts with sodium thiosulfate. The crystal and molecular structures of the zwitterions (R = Ph, n = 3) and (R = Bu, n = 3) were determined. A series of phosphonioalkanethiolate-capped gold nanoparticles dispersed in water was prepared by borohydride reduction of potassium tetrachloroaurate in the presence of the zwitterions in a dichloromethane–water system. UV-visible spectroscopy and scanning transmission electron-microscopy indicated that capped nanoparticles of *ca.* 5 nm diameter were present.

Introduction

There is much current interest in the development of receptor-functionalised metal and semiconductor nanoparticles which are able to recognise and interact with specific biomolecules. Such systems are expected to form the basis of new diagnostic biosensor technologies and novel therapeutic agents. The most widely studied systems are based on gold. The modification of the surface of gold nanoparticles with various functionalised thiols containing reporter groups makes them versatile systems and opens a range of possibilities for the specific recognition of other molecules, and in the design of optical sensor systems based on the consequent modifications to the surface plasmon resonance properties of the gold nanoparticle.[1,2] However, comparatively few such reported thiols involve a cationic head group which offers the ability to bind negatively charged analytes. The fluorescent ethidium thiolate (**1**) has been employed in the design of a gold cluster system used to bind DNA.[3] Gold nanoparticles bearing the imidazoliumalkane thiol (**2**) have been shown to act as simple colourimetric anion sensors.[4] Gold nanoparticles modified with simple alkylammonium alkanethiols have also been shown to bind to DNA.[5,6] Rotello and co-workers[7] synthesised gold nanoparticles functionalised with a mixture of octanethiol and 11-trimethylammonium-undecanethiol, so-called mixed monolayer protected gold clusters (MMPCs), and showed that these MMPCs with trimethylammonium cationic head groups on the surface can interact electrostatically and bind with the negatively charged phosphate backbone of 37mer duplex DNA. They also studied the ability of these MMPCs to inhibit DNA transcription *in vitro*, their utilisation in gene delivery into cells and as transfection vectors.[8]



(**1**)



(**2**)

Our own interest in the chemistry of phosphonium systems has led us to design and synthesise a family of novel phosphonioalkylthiosulfate ligands which can be used to functionalise the surface of gold nanoparticles. The phosphonium moiety offers a number of advantages including biocompatibility and the relative ease of preparing a wide range of derivatives, *e.g.*, phosphonium analogues having a fluorescent reporter head group or phosphine oxide derivatives having the ability to form hydrogen-bonded complexes with biomolecules. The cationic phosphonioalkanethiol (**3**) has been shown to have a remarkable ability to pass through mitochondrial membranes and to accumulate inside the mitochondrion, with consequent influence on the chemistry of the host cell.[9] Thiobutyltriphenylphosphonium bromide was used successfully by Murphy *et al.*[10] to identify changes in the redox state of mitocondrial thiol compounds during oxidative stress. They demonstrated that the lipophilic triphenylphosphonium cation interacts with the negatively charged mitochondrial matrix causing its accumulation inside the isolated mitochondria and in mitochondria from living cells.[11] These workers also detected the formation of disulfide bonds due to reaction of the thiol groups in the matrix with the mitochondrial protein and low-molecular weight thiols during the oxidative stress. This showed that such salts containing lipophilic cations can be used to determine mitochondrial dysfunction.[10–12] Thiol (**3**) has been prepared from the hydrolysis of the related thioacetate salt (**4**) and shown to undergo gradual oxidation in air to form the disulfide-bridged

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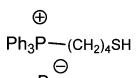
system (**5**).[13] As gold-containing drugs, such as auranofin and triethylphosphine gold(I) chloride, have demonstrated their antitumour effects,[14] there is also an interest in the possibility of studying the extent to which phosphonioalkanethiol-capped gold nanoparticles might be incorporated into cells and exert new biological effects. Here we report our studies of the synthesis and characterisation of a family of phosphonioalkylthiosulfate zwitterions and their *in situ* reaction with reduced gold salts to form cationic-functionalised gold nanoparticles.



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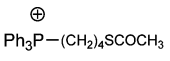

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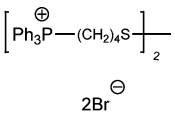

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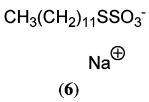


(**6**)

Results and discussion

Synthesis of phosphonioalkylthiosulfate zwitterions

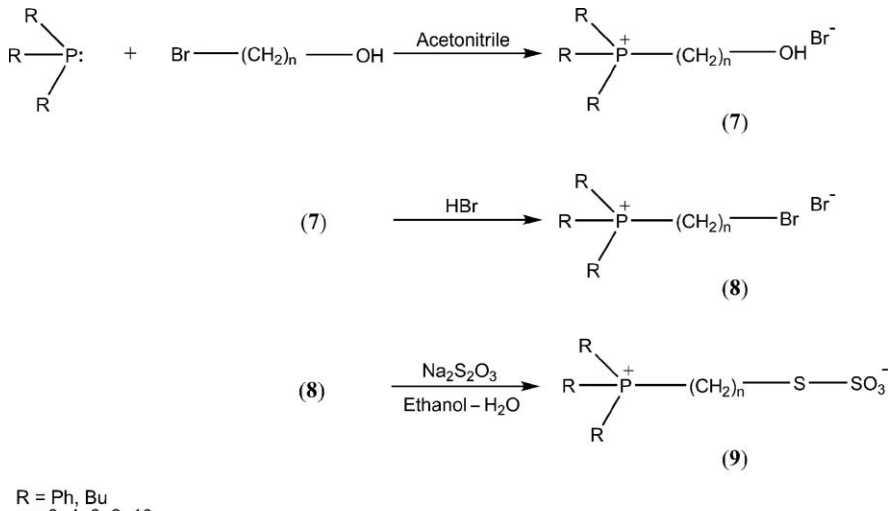
The synthesis of the series of triphenyl- and tributyl-phosphonioalkylthiosulfate zwitterions is shown in Scheme 1. The design of our ligands has been influenced by the work of Lukkari *et al.*[15] and Murray *et al.*[16] who described the generation of alkanethiolate-protected gold surfaces and clusters from Bunte salts such as the sodium *S*-dodecylthiosulfate (**6**), which were identical to those obtained directly from the use of the free thiols. The sulfur–sulfur bond of the Bunte salt undergoes cleavage with loss of sulfite ion as a result of the interaction with the gold surface. Such compounds are easily prepared by treatment of a bromoalkane with sodium thiosulfate in aqueous ethanol. Adapting this approach, we have converted readily accessible ω-bromoalkylphosphonium salts (**8**) into a series of phosphonioalkylthiosulfate zwitterions (**9A–9E**, R = phenyl, n = 3, 4, 6, 8, 10; **9F**, R = butyl, n = 3), which act as cationic masked thiols, being sources of the phosphonioalkanethiolates in the synthesis of the functionalised gold nanoparticles.

The shorter alkyl chain compounds are crystalline solids and are soluble in polar organic solvents. In addition, the tributylphosphoniopropylthiosulfate (**9F**, R = Bu, n = 3) is also water-soluble, making it attractive for studies in biological media.

Analytical data and electrospray mass spectrometry support the formulation of these compounds as phosphonioalkylthiosulfate zwitterions. When studied by ESMS in negative ion mode, all compounds exhibited a well-defined molecular ion, whereas in positive ion mode, an ion corresponding to M + Na was observed. The synthesis of a trimethylphosphonium analogue of the triphenylphosphoniopropylthiosulfate zwitterion was also attempted by following the same synthetic route. However, the step involving treatment of the intermediate 3-bromoalkylphosphonium salt with sodium thiosulfate resulted in the cleavage of the trimethylphosphonium group.

Structural characterisation of phosphonioalkylthiosulfate zwitterions

We have carried out an X-ray structural study of the triphenylphosphoniopropylthiosulfate (**9A**, R = Ph, n = 3) which confirms the expected structure (Fig. 1). Crystal data and structure refinement details are presented in Table 1. Selected bond lengths and bond angles are presented in Table 2(a). There are no intramolecular interactions between the cationic head group and the thiosulfato group which could lead to the formation of a quasi-7-membered cyclic structure from the electrostatic attraction of P^+ with O^-. Similarly, intermolecular electrostatic effects do not appear to dominate the way in which the dipolar units pack in the crystal, which is rather unusual. Along the [101] direction the individual units pack in a head to head manner in which 'supramolecular edge to face' interactions[17] between the triphenylphosphonio units and possible weak C–H · · · O hydrogen bonds (*ca.* 2.5 Å) seem to dominate. Along the [10−1] direction an intermolecular 'head to tail' arrangement is found, whereas along the [010] direction, the individual units associate as 'head to tail' dimers.



(**7**)

(**8**)

(**9**)

Scheme 1

Table 1 Crystal data and structure refinement for 3-triphenylphosphoniopropylthiosulfate and 3-tributylphosphoniopropylthiosulfate

Compound	**9A**	**9F**
Empirical formula	$C_{21}H_{21}O_3PS_2$	$C_{15}H_{33}O_3PS_2$
Formula weight	416.47	356.50
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/n$	$P2_1/n$
a/Å	11.6475(3)	9.0500(3)
b/Å	14.2692(3)	14.3012(4)
c/Å	12.0730(3)	15.0732(6)
β/°	106.561(1)	96.326(2)
Volume/Å^3	1923.30(8)	1938.98(11)
Z	4	4
D_{calc}/Mg m^{-3})	1.438	1.221
Absorption coefficient/mm^{-1}	0.380	0.364
F(000)	872	776
Crystal	Colourless block	Colourless block
Crystal size/mm^3	0.15 × 0.08 × 0.08	0.10 × 0.10 × 0.03
θ range for data collection/°	3.39–25.02	3.07–25.02
Reflections collected	6267	23499
Independent reflections	3381 [$R_{int} = 0.0434$]	3406 [$R_{int} = 0.0797$]
Completeness to $\theta = 25.02°$	99.7%	99.7%
Max. and min. transmission	0.9924 and 0.9630	0.9892 and 0.9645
Data/restraints/parameters	3381/0/244	3406/39/194
Goodness-of-fit on F^2	1.043	1.042
Final R indices [$F^2 > 2\sigma(F^2)$]	$R1 = 0.0416$, $wR2 = 0.0994$	$R1 = 0.0594$, $wR2 = 0.1487$
R indices (all data)	$R1 = 0.0565$, $wR2 = 0.1068$	$R1 = 0.0775$, $wR2 = 0.1589$
Largest diff. peak and hole	0.311 and −0.444 e Å^{-3}	1.078 and −0.633 e Å^{-3}

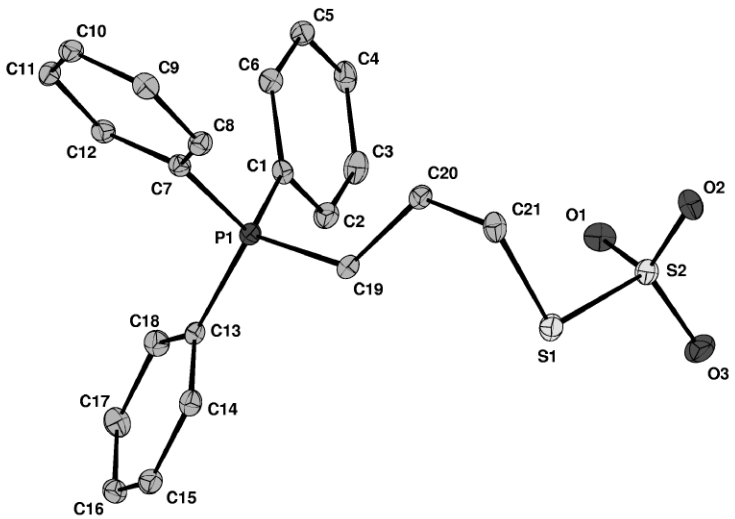


Fig. 1 Molecular structure of 3-triphenylphosphoniopropylthiosulfate (**9A**)-an ORTEP drawing with 30% probability ellipsoids.

Similarly, an X-ray structural study of the 3-tributylphosphoniopropylthiosulfate (**9F**, R = Bu, n = 3) confirmed the expected structure (Fig. 2). Crystal data and structure refinement details are presented in Table 1. Selected bond lengths and bond angles are presented in Table 2(b). Fig. 3 shows possible hydrogen bonding between C–H bonds α to the phosphonium centre and the thiosulfato oxygen atoms which influence the packing of the molecules in the unit cell. The structural parameters for these hydrogen bond interactions are presented in Table 3.

Synthesis of phosphonium-monolayer protected gold clusters

Previous studies have indicated that gold nanoparticles and colloidal gold solutions have the ability to cleave the S–S bonds present in organic disulfides and alkylthiosulfates (**6**), yielding organic thiolates which then bind to the surface of the particle.[1,15,16] Consequently, we were confident that the S–S bond of the phosphonioalkylthiosulfate zwitterions would be cleaved under the reductive conditions used for the synthesis of the capped nanoparticles, to form the related phosphonioalkylthiolates, *e.g.*, (**10**), (Scheme 2). This was confirmed by sodium borohydride reduction of the salt (**9A**), the resulting phosphonioalkylthiolate being trapped with iodomethane to form the salt (**11**). NMR spectroscopy and electrospray mass spectrometry supported the formulation of this compound. When studied by MALDI TOFMS in positive ion mode, (accurate mass analysis), an ion corresponding to the 3-methylthiopropylphosphonium cation was observed.

Table 2 Selected bond lengths [Å] and angles [°] in 3-phosphoniopropylthiosulfates

(a) 3-Triphenylphosphoniopropylthiosulfate **9A**			
C1–P1	1.795(2)	O1–S2	1.4507(19)
C7–P1	1.792(2)	O2–S2	1.4442(18)
C13–P1	1.794(2)	O3–S2	1.4484(19)
C19–P1	1.805(2)	S1–S2	2.1117(9)
C21–S1	1.820(3)		
C20–C19–P1	115.39(17)	C21–S1–S2	99.77(9)
C20–C21–S1	114.77(18)	O2–S2–O3	115.11(12)
C7–P1–C1	107.97(11)	O2–S2–O1	113.38(11)
C7–P1–C13	111.12(11)	O3–S2–O1	113.15(12)
C1–P1–C13	110.13(11)	O2–S2–S1	105.60(7)
C7–P1–C19	111.92(11)	O3–S2–S1	101.20(8)
C1–P1–C19	108.79(11)	O1–S2–S1	106.97(8)
C13–P1–C19	106.90(11)		

(b) 3-Tributylphosphoniopropylthiosulfate **9F**			
P1–C9	1.796(4)	S1–S2	2.1030(14)
P1–C5	1.798(3)	S2–O2	1.439(3)
P1–C13	1.799(4)	S2–O3	1.440(3)
P1–C1	1.805(3)	S2–O1	1.441(3)
S1–C15	1.805(4)		
C9–P1–C5	107.56(17)	O3–S2–O1	113.90(18)
C9–P1–C13	108.13(18)	O2–S2–S1	104.75(14)
C5–P1–C13	110.89(17)	O3–S2–S1	102.34(13)
C9–P1–C1	110.16(16)	O1–S2–S1	106.67(14)
C5–P1–C1	110.32(18)		
C13–P1–C1	109.73(18)		
C15–S1–S2	99.78(14)		
O2–S2–O3	114.45(18)		
O2–S2–O1	113.3(2)		

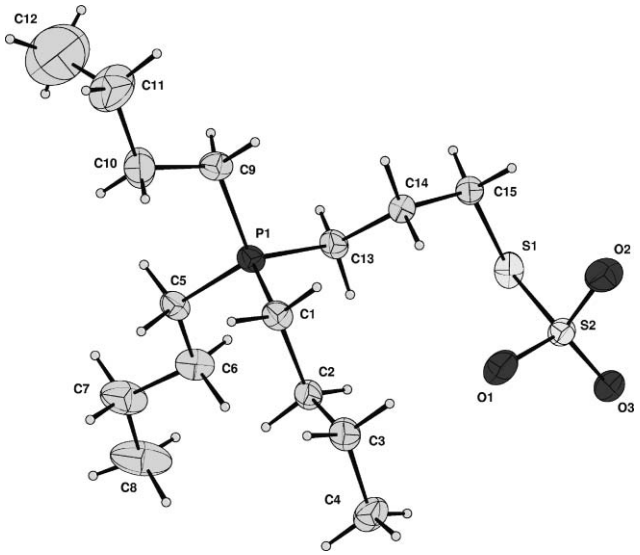


Fig. 2 Molecular structure of 3-tributylphosphoniopropylthiosulfate (**9F**).

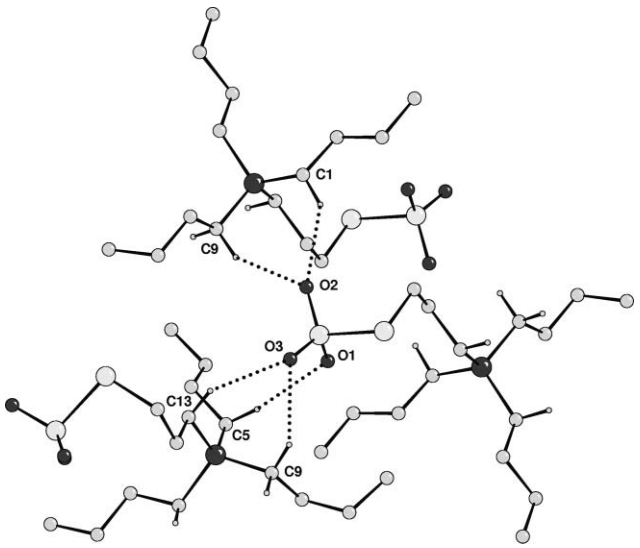


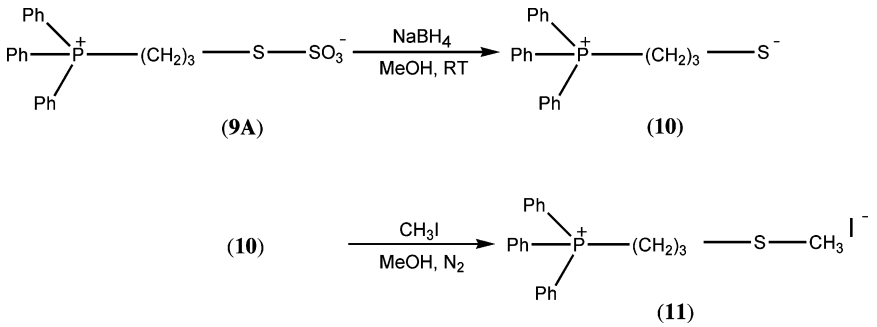
Fig. 3 Intermolecular contacts showing possible hydrogen bonding between C–H bonds α to the phosphonium centre and thiosulfato oxygen atoms in 3-tributylphosphoniopropylthiosulfate.

The phosphonioalkanethiolate-capped gold nanoparticles were synthesised in a two phase liquid–liquid system, following the methods developed by Brust[18] and Murray,[16] *via* reduction of potassium tetrachloroaurate in biphasic media with an excess of sodium borohydride, with two variations based on the use of dichloromethane–water instead of toluene–water as the biphasic system, and without the use of tetraoctylammonium bromide to aid transfer of the $AuCl_4^-$ ions to the organic solvent, as it was felt that this role would be fulfilled by the phosphoniothiosulfate zwitterion. The preparation technique was the same for 3-triphenylphosphoniopropylthiosulfate (**9A**), 6-triphenylphosphoniohexylthiosulfate (**9C**), 8-triphenylphosphoniooctylthiosulfate (**9D**), 10-triphenylphosphoniodecylthiosulfate (**9E**), and tributylphosphoniopropylthiosulfate (**9F**) zwitterions and was as follows. A solution of the zwitterion was prepared in dichloromethane (DCM) and solid potassium tetrachloroaurate (0.5 mol equiv.) was then added to the solution. This was vigorously stirred for 10 minutes until the gold salt was totally dissolved. The reduction was carried out by adding dropwise a freshly prepared aqueous solution of sodium borohydride with

Table 3 Intermolecular contacts for hydrogen bonding between C–H bonds α to the phosphonium centre and thiosulfato oxygen atoms in 3-tributylphosphoniopropylthiosulfate **9F**

Donor	H	Acceptor	Symm	D–H	H···A	D···A	D–H···A
C(1)	H(1A)	O(2)	$1	0.99	2.44	3.2657	140
C(5)	H(5B)	O(1)	$2	0.99	2.44	3.4173	171
C(9)	H(9A)	O(2)	$1	0.99	2.39	3.2529	146
C(9)	H(9B)	O(3)	$2	0.99	2.45	3.2929	142
C(13)	H(13B	O(3)	$2	0.99	2.54	3.3672	141

$1 = 1 − x, −y, 1 − z, $2 = −1/2 + x, 1/2 − y, −1/2 + z, P1···O2 = 3.744(13) $1, P1···O3 = 3.541(12) $2, P1···O1 = 4.730(16).



Scheme 2

vigorous stirring. After 24 hours, the stirring was stopped, the aqueous layer separated and purified by three DCM extractions so as to remove the excess of capping agent. All the extracts were monitored by TLC, using methanol (10%): dichloromethane (90%) as mobile phase. A total removal of the excess organic ligand was observed by TLC after the third DCM extraction. When the tetrachloroaurate was added to the solution of the zwitterion in DCM, the stirring organic solution turned yellow, and then became dark purple–blue after the $NaBH_4$ addition, indicating that the reduction had taken place. In the case of the triphenylphosphonioalkylthiosulfate ($n = 3$, 6, and 8), when the stirring was stopped after 24 hours, the DCM layer was colourless and the aqueous phase was dark purple–blue, indicating that phosphonioalkylthiolate-capped nanoparticles were present in the aqueous phase (Scheme 3).

When 10-triphenylphosphoniodecylthiosulfate and 3-tributylphosphoniopropylthiosulfate were used, dark blue particles of aggregated colloidal gold were observed at the interface between the aqueous and organic phases, showing no affinity for either the dichloromethane or water. It would appear that as the carbon chain length increases to more than 8 in the triphenylphosphonium ligands, the formation of phosphonium monolayer-protected gold colloids stable in an aqueous medium becomes more difficult. Replacing the triphenylphosphonium cationic group by tributylphosphonium group, also destabilises the nanoparticles.

Evidence for the formation of gold nanoparticles was provided by UV-visible spectroscopy. A broad band centred at 520 nm was observed in the aqueous phase in the dark purple–blue solutions containing gold nanoparticles functionalised with **9A**, **9C** and **9D** (Fig. 4), indicating that the particle size is between 5–10 nm in diameter, according to the values for other thiolate-capped gold nanoparticles reported in the literature.[19] The solution of the gold nanoparticles functionalised with triphenylphosphoniopropylthiosulfate zwitterion was also investigated by scanning transmission electron microscopy (STEM). Highly uniform spherical nanoparticles of *ca.* 5 nm can be observed in the STEM image (Fig. 5), supporting the information given by the UV-visible spectrum. The colloidal solutions presented good stability over 4 months at room temperature. The solutions were monitored by UV-visible spectroscopy and no changes were observed in their spectra during this period.

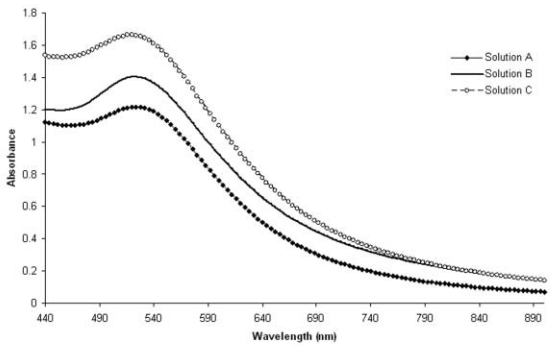
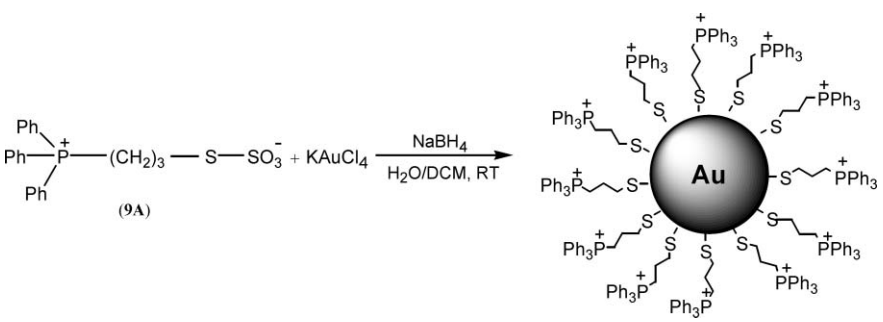


Fig. 4 UV-visible absorption spectra of the aqueous phases obtained in the syntheses of phosphonium-MPCs in a two-phase liquid–liquid system in presence of the triphenylphosphoniopropylthiosulfate (Solution A), triphenylphosphoniohexylthiosulfate (Solution B), and triphenylphosphoniooctylthiosulfate (Solution C) zwitterions as the precursors of the protecting ligands.



Scheme 3

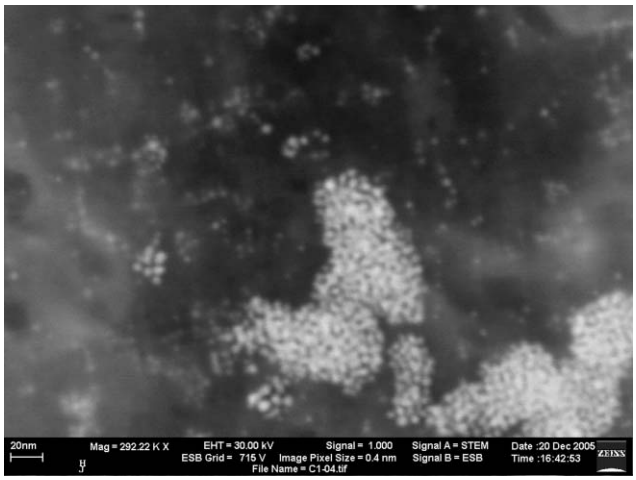


Fig. 5 STEM micrograph of the gold nanoparticles functionalised with triphenylphosphoniopropylthiosulfate zwitterion.

Conclusion

Functionalisation of the surface of metal nanoparticles is essential for the development of these species for use in biomolecular recognition or as novel therapeutic agents. In addition to providing specific receptor sites, the functionalising ligand should also impart the characteristics of organic molecules to the metal nanoparticle; *i.e.* solubility in various solvents, which allows them to be studied using conventional spectroscopic techniques.

Cationic-functionalised nanoparticles have demonstrated versatility in a number of biomedical applications, including transfection vectors and DNA surface recognition. To the best of our knowledge, all the cationic-functionalising ligands currently reported in the literature are based on ammonium species. Here we have reported the synthesis and characterisation of the first alternative system, based on phosphonioalkylthiosulfate zwitterions. Phosphonium groups offer a number of advantages, including biocompatibility and the ease of synthesis of a wide range of derivatives with different recognition capabilities. We have also demonstrated that our phosphonioalkylthiosulfate zwitterions readily disproportionate into phosphonioalkylthiolates *in situ* during the synthesis of gold nanoparticles produced by the borohydride reduction of gold(III) salts. UV spectroscopic and electron microscopic studies have shown that the phosphoniothiolates bind to the surface of the gold nanoparticles which are typically 5 nm in diameter. The resulting cationic-functionalised gold nanoparticles are dispersable in aqueous solution which augurs well for their future use in biological applications.

We are currently investigating the synthesis of related ligand systems and investigating the potential of phosphonioalkylthiolate-functionalised gold nanoparticles and surfaces as biorecognition systems using surface plasmon resonance techniques.

Experimental

General

^{1}H and ^{31}P NMR spectra were obtained in $CDCl_3$ and in $CDCl_3$–DMSO mixture on a Bruker DMX 250 (250 MHz) spectrometer. Electrospray mass spectra were recorded using an Applied Biosystems "QStar-Pulsar-i" hybrid quadrupole time of flight LCMS-MS instrument. UV-visible spectra of aqueous colloidal solutions were obtained on an ATI UNICAM UV2 spectrometer. Analytical thin layer chromatography (TLC) was performed on Merck silica gel $60F_{254}$ plates using 90 : 10 dichloromethane : methanol as eluent system. Elemental analyses were carried out by MEDAC Ltd.

Synthesis of the series of triphenyl- and tributyl-phosphonioalkylthiosulfate zwitterions

The synthesis of the series of triphenyl- and tributyl-phosphonioalkylthiosulfate zwitterions is shown in Scheme 1. All the compounds were generated by the reaction of triphenylphosphine or tributylphosphine (3.8 mmol) with the appropriate bromo-alcohol (15 mmol) in acetonitrile under reflux for four hours, to obtain the corresponding hydroxyalkylphosphonium salts (**7**). The salts (**7**) were dissolved in HBr (48%) and heated under reflux for five hours to obtain the bromoalkylphosphonium salts (**8**). Finally, a series of phosphonioalkylthiosulfate zwitterions (**9**) was prepared by treatment of the salts (**8**) (1 mol) with sodium thiosulfate (1.5 mol) in aqueous ethanol under reflux for five hours. Progress of the reactions was monitored by TLC, using 20% methanol : 80% dichloromethane as mobile phase. All the compounds (**8**) and (**9**) were obtained by dichloromethane extraction of the reaction mixtures and initially purified by trituration with dry diethyl ether. Only the reaction of tributylphosphine with the corresponding bromo-alcohols was performed under nitrogen. The 4-triphenylphosphoniobutylthiosulfate (**9B**) was prepared from the commercial (4-bromobutyl)triphenylphosphonium bromide.

3-Triphenylphosphoniopropylthiosulfate (**9A**). Colourless crystals, mp 240–243 °C. Found: C, 60.53; H, 5.11; $C_{21}H_{21}O_3PS_2$ requires: C, 60.56; H, 5.08%. ESMS 417 [M + H$^+$], 439 [M + Na$^+$], 855 [2M + Na$^+$]. δ ^{31}P NMR ($CDCl_3$–DMSO) = 23.2 ppm; δ ^{1}H NMR ($CDCl_3$–DMSO) = 1.6 (2H, m), 2.6 (2H, m), 3.1 (2H, m), 7.2–7.3 (15H, m) ppm.

4-Triphenylphosphoniobutylthiosulfate (**9B**). Colourless crystals, mp 256–260 °C. Found: C, 61.24; H, 5.55; $C_{22}H_{23}O_3PS_2$ requires: C, 61.38; H, 5.38%. ESMS 431 [M + H$^+$], 453 [M + Na$^+$], 883 [2M + Na$^+$]. δ ^{31}P NMR ($CDCl_3$–DMSO) = 23.6 ppm; δ ^{1}H NMR ($CDCl_3$–DMSO) = 1.6 (2H, m), 2.2 (2H, m), 2.7 (2H, t), 3.05 (2H, m), 7.3–7.5 (15H, m) ppm.

6-Triphenylphosphoniohexylthiosulfate (**9C**). Colourless crystals, mp 63–65 °C. Found: C, 62.12; H 5.95; $C_{24}H_{27}O_3PS_2$ requires: C, 62.86; H, 5.93%. ESMS 459 [M + H$^+$], 481 [M + Na$^+$], 939 [2M + Na$^+$]. δ ^{31}P NMR ($CDCl_3$) = 24.06 ppm; δ ^{1}H NMR ($CDCl_3$) = 1.5 (2H, m), 1.6 (4H, m), 1.7 (2H, m), 3.05 (2H, t), 3.4 (2H, m), 7.6–7.8 (15H, m) ppm.

8-Triphenylphosphoniooctylthiosulfate (**9D**). Pale cream powder, mp 40–45 °C. Found: C, 62.14; H, 6.37; $C_{26}H_{31}O_3PS_2{\cdot}H_2O$ requires C, 61.88; H, 6.59%. ESMS 485 [M$^+$], 510 [M + Na$^+$]. δ ^{31}P NMR ($CDCl_3$) = 23.8 ppm; δ ^{1}H NMR ($CDCl_3$) = 1.2 (5H, m), 1.5 (7H, m), 2.4 (1H, m), 2.9 (1H, t), 3.3 (2H, m), 7.6–7.7 (15H, m) ppm.

10-Triphenylphosphoniodecylthiosulfate (**9E**). Pale cream powder, mp 60–65 °C. Found: C, 63.69; H, 6.75; $C_{28}H_{35}O_3PS_2{\cdot}H_2O$ requires C, 63.13; H, 7.00%. ESMS 513 [M$^+$], 538 [M + Na$^+$]. δ ^{31}P NMR ($CDCl_3$) = 23.6 ppm; δ ^{1}H NMR ($CDCl_3$) = 1.0 (8H, m), 1.4 (6H, m), 3.2 (2H, m), 3.9 (4H, m), 7.5–7.7 (15H, m) ppm.

3-Tributylphosphoniopropylthiosulfate (**9F**). Colourless crystals, mp 133–136 °C. Found: C, 50.27; H, 9.69; $C_{15}H_{33}O_3PS_2$ requires C, 50.53; H, 9.33%. ESMS 357 [M + H$^+$], 379 [M + Na$^+$], 735 [2M + Na$^+$], 1091 [3M + Na$^+$]. δ ^{31}P NMR ($CDCl_3$) = 33.7 ppm; δ ^{1}H NMR ($CDCl_3$) = 0.9 (9H, t), 1.5 (12H, m), 2.1 (8H, m), 2.5 (2H, m), 3.1 (2H, t) ppm.

Synthesis of 3-(methylthio)propyl-triphenylphosphonium iodide (11)

The alkylation of (**10**) was carried out using the following method: triphenylphosphoniopropylthiosulfate (0.5 mmol) was dissolved in 3 mL of methanol. A freshly prepared aqueous solution of sodium borohydride (5 mmol) was then added drop by drop to the reaction flask, in order to allow formation of the zwitterion $Ph_3P^+(CH_2)_3S^-$. The mixture was stirred for 3 hour at room temperature. The formation of 3-(methylthio)propyl-triphenylphosphonium iodide was achieved by the reaction of (**10**) and iodomethane (5 mmol) under nitrogen and the mixture was stirred overnight at room temperature. Progress of the reaction was monitored by TLC, using 10% methanol : 90% dichloromethane as a mobile phase. The resulting mixture was extracted with dichloromethane, the organic phase was collected and after removing the solvent, the resulting compound (**11**) was initially purified by trituration with dry diethyl ether.

3-(Methylthio)propyl-triphenylphosphonium iodide (**11**). Pale cream solid, 68% yield; mp 136–138 °C. Accurate MALDI TOFMS analysis: found 351.1307 [M $^+$]; $C_{22}H_{24}PS$ requires 351.1336 [M$^+$]; δ ^{31}P NMR ($CDCl_3$) = 24.3 ppm, δ ^{1}H NMR ($CDCl_3$) = 1.9 (3H, s), 2.8 (2H, t), 3.3 (2H, m), 3.8 (2H, m), 7.6–7.8 (15H, m) ppm.

X-Ray crystallography study of the triphenylphosphoniopropylthiosulfate and tributylphosphoniopropylthiosulfate zwitterions

All single crystal X-ray diffraction data were collected on a Bruker Nonius KappaCCD mounted at the window of a Mo FR591 rotating anode (λ = 0.71073 Å). The crystals were kept at a temperature of 120 K, and the data were corrected for absorption by the empirical multi-scan method employed in SORTAV.[20] The structures were solved *via* direct methods and refined by full matrix least squares on F^2 (SHELX97).[21] Non-hydrogen atoms were refined anisotropically and hydrogen atoms were treated using a riding model.†

Synthesis of phosphonium-monolayer protected gold clusters

A solution of the zwitterions corresponding to the protecting ligand was prepared in DCM (0.25 mmol, 14 mmol L^{-1}) and potassium tetrachloroaurate (0.12 mmol, 7 mmol L^{-1}) was then added to the solution. This was vigorously stirred for 10 minutes until the gold salt was totally dissolved. The reduction was carried out by adding dropwise a freshly prepared aqueous solution of sodium borohydride (3 mL, 400 mmol L^{-1}) with vigorous stirring, and 15 mL of deionised water was then added to the mixture. After 24 hours, the stirring was stopped. And three DCM extractions were then carried out for the purification of the aqueous phase.

TEM

One drop of a dispersion of the phosphoniopropylthiolate-capped gold nanoparticle sample in methanol was placed onto a 300-mesh copper grid, the solvent allowed to evaporate, and then the grid was carbon coated. A Carl Zeiss STM SUPRA™ 40VP GEMINI™ FE-SEM with a Multi-Mode STEM (30.00 kV) detection system was used to determine the average particle size for the sample studied.

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