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ARTICLE

Synthesis of proton caged disulphide compounds for gold nanoparticles functionalization

Cite this: DOI: 10.1039/xoxxoooooox

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Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/xoxxoooooox

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The pH plays a fundamental role in many biological systems, and it is important to be capable of monitoring and manipulating it. A method for intracellular pH control has been recently developed based on a proton caged compound (PCC), which releases protons upon irradiation with light of proper wavelength. Intracellular modulation of PCCs uptake can be achieved by gold nanosized vectors. This, however, is conditioned by the possibility of conjugating PCCs and vectors. Here, we present the synthesis of purposely designed proton caged disulphide sulphonyl urethanes, which bind gold nanoparticles through the disulphide bridge and display photoreactivity through an o-nitrophenyl moiety. The new compounds have been characterized by FTIR, ^{1}HNMR, TEM and TGA and their photoreactivity in the UV range has been probed, after functionalizing them with gold nanoparticles.

Introduction

The pH has a fundamental role in many biological processes. It is diagnostic of the phase of the cell growth cycle[1]; it influences the protein folding[2] and aggregation[3] as well as the protein receptors affinity[4], the cellular osmotic response[5], the ion transport[6], the membrane polarization[7] and the nutrient transport[8]. The onset of cells apoptosis arises with simultaneous cytosolic decrease of pH[9].

Intracellular pH measurements are usually based on fluorescent probes and sensors[10], Positron Emission Tomography (PET) radiotracers (mostly in case of tumour cells)[11] and microelectrodes[12]. Furthermore, methods based on Surface Enhanced Raman Scattering (SERS) of exogenous functionalized gold nanorods were also developed[13].

Cellular pH manipulation and monitoring is important for the purpose of studying biochemical processes in vivo, such as enzyme reaction rates, macromolecule conformation, and membrane fluidity.

A method for intracellular pH manipulation and monitoring was recently developed be employing a purposely synthesized Proton Caged Compound (PCC), the 1-(2-nitrophenyl)-ethylhexadecyl sulphonate ester (HDNS)[14]. PCCs and orto-nitrobenzyl derivatives in particular[15], are compounds capable of imposing net acidification, by releasing protons upon irradiation in the proper wavelength range. When dosed to a test cell line, the NIH-3T3 fibroblasts, the designed HDNS was able to induce intracellular acidification on command, *i.e.* after irradiation in the UV range.

A way of achieving further control over intracellular acidification is by regulating the PCCs uptake through the employment of nanosized vectors. Gold NanoParticles (AuNPs) have been extensively used as intracellular vectors aiming at active biosensing, enhanced imaging contrast, drug delivery, and tumour targeting. In particular, the delivery of drugs with AuNPs can result in higher concentrations than with normal drug delivery schemes, hence increasing the overall drug efficiency[16]. A way to achieve spatiotemporal control of drugs release is to cage them, couple the caged drugs to gold nanoparticles and release them upon UV-A radiation[17]. This system can be envisaged to target tumor cells through enhanced permeation and retention.

The use of AuNPs as PCCs vectors is conditioned by the possibilities of binding them together. This implies designing and synthesizing a properly functionalized PCC. In this paper, we present the synthesis and characterization of DiSulphided Proton Caged Compounds (SS-PPCs). In particular, we synthesized two photosensitive di-sulphided sulphonyl urethane compounds, the disulfanediyldiesane-6,1-diyl bis{[1-(2-nitrophenyl)ethoxy]sulfonyl}carbamate (NE-SS-hepta) and the disulfanediyldinonane-9,1-diylbis{[1-(2-nitrophenyl)ethoxy]sulfonyl}carbamate (NE-SS-deca). In the procedure, a few test compounds were obtained and their synthesis is reported too. In the design of the SS-PCCs, the o-nitrobenzyl group is introduced as light sensitive moiety[18], whereas the disulphide bridge is introduced for the selective binding with the AuNPs.

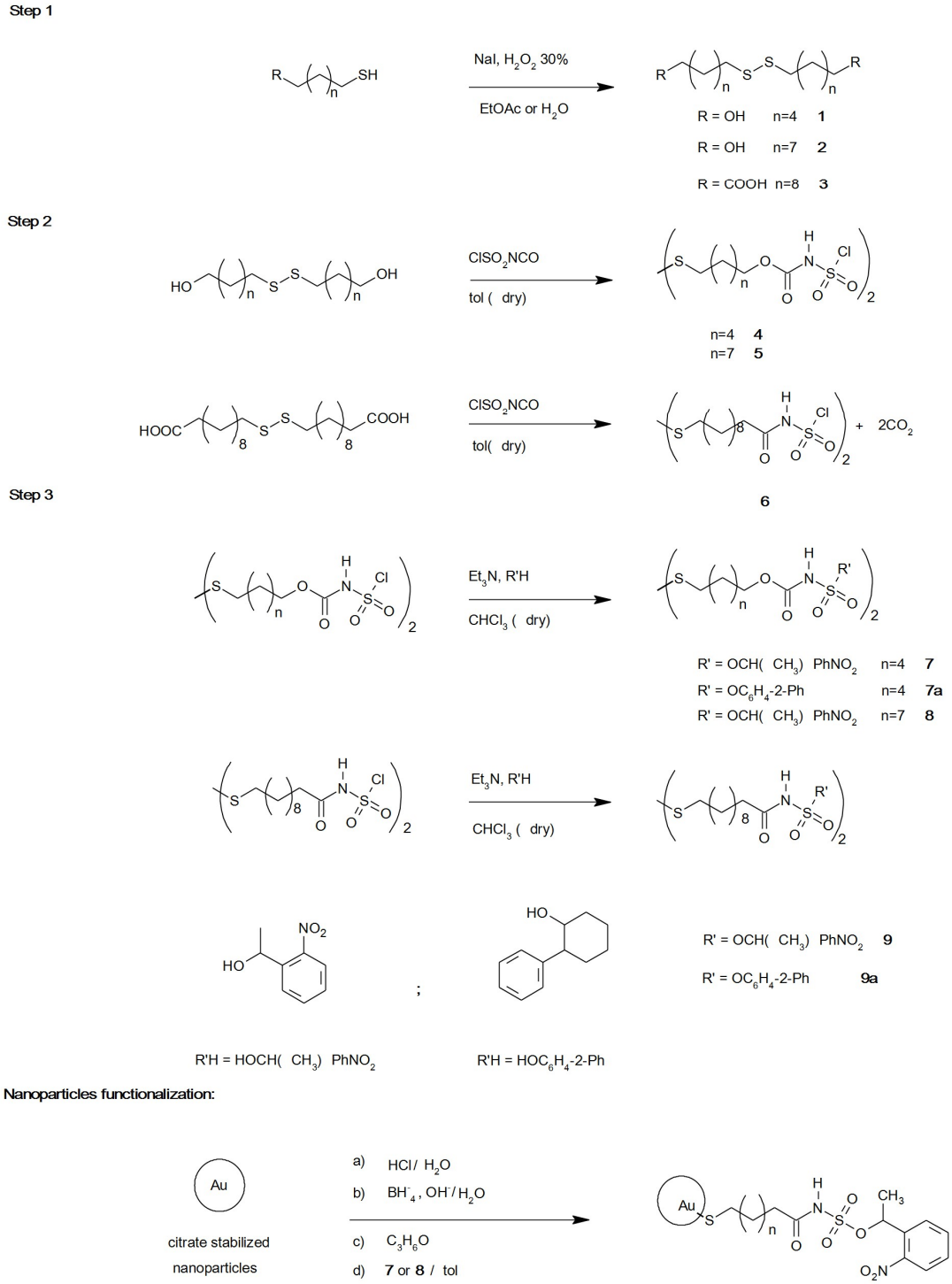
Upon synthesis, both NE-SS-hepta and NE-SS-deca were used for the functionalization of 22 nm diameter AuNPs and the photosensitive properties of the resulting functionalized nanoparticles were probed.

Results and discussion

Synthesis Procedure

The synthesis strategy of NE-SS-hepta and -deca is based on the conversion of primary alcohol into urethanes proposed by Burgess et al.[19]. In particular, the core of our strategy corresponds to the first step of the alcohol conversion, i.e. the reaction of chlorosulphonyl isocyanate ($ClSO_2N=C=O$) with an alcohol to yield an alkyl (chlorosulphonyl) carbamate ($ClSO_2NHCO_2$-Alkyl). Then, the chlorosulphonyl group $ClSO_2$-R may react again with a second alcohol, the 1-(2-

nitrophenyl) ethanol to yield the desired sulphonyl urethanes with photosensitive proton caged o-nitrophenyl moieties. We introduce the disulphide bridge in the very beginning of our reaction pathway, through the selective oxidation of a thiolated alcohol (HS-CH_n-OH) which yields a disulphide glycol (OH-CH_n-S-S-CH_nOH). In terms of reactivity with AuNPs, thiols, sulphides and disulphides show similar behaviours[20]. The choice of introducing the disulphide bridge, rather than any of the two other groups is determined by the necessity of protecting the -SH group in the reactions, where also alcohols are present. The overall synthesis is carried out according to the three-stepped reaction reported in **Scheme 1**.



Scheme 1 Synthetic route of the three steps procedure and nanoparticles functionalization.

In the first step of the reaction, i.e. the thiol oxidation, the starting 6-mercapto-1-hexanol (R=OH, n=4), or the 9-mercapto-1-nonanol (R=OH, n=6) undergoes a mild oxidation to yield the corresponding disulphides (compounds **1-2** in **Scheme 1**). The gentle oxidation is carried out with NaI and H_2O_2 30% and ensures the formation of the disulphide bridge without over-oxidation of the hydroxyl group[21].

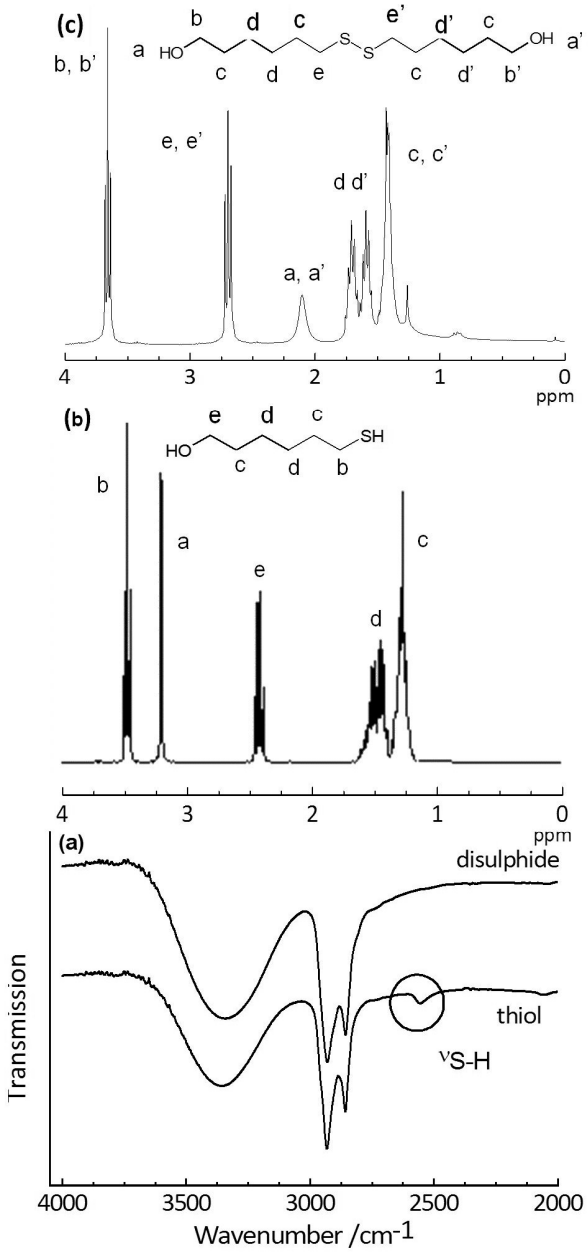


Fig. 1 (a) FTIR spectrum of the starting thiol 6-mercapto 1-hexanol (bottom line) and of compound **1** (top line). In the spectrum of the thiol the ν_{SH} stretching vibration at 2555 cm^{-1} is clearly present. The curves are arbitrarily shifted. **(b)** ^{1}H-NMR spectrum of the 6-mercapto-1-hexanol. **(c)** ^{1}H-NMR spectrum of compound **1**.

The reaction was monitored by TLC (diethyl ether: hexane, 8:2) and FTIR. The products were isolated and characterized by FTIR and ^{1}H-NMR spectroscopy (**Fig.s 1**). A test reaction was carried out also on the 11-mercaptododecanoic acid (R=COOH, n=8) to check the reactivity of a carboxylic acid derivate.

In **Fig.1(a)** the infrared spectrum of one of the starting thiols, the 6-mercapto 1-hexanol, is reported (bottom line). Four peaks can be observed, at 2555 cm^{-1}, 2857 cm^{-1}, 2932 cm^{-1} and 3350 cm^{-1} and are attributed to the ν_{S-H}, alkyl chain and ν_{O-H} stretching vibrations, respectively. The selective formation of the disulphide bridge upon oxidation is evident from the infrared spectrum of compound **1** (6,6'-disulfanediyldihexan-1-ol) which is also reported in **Fig. 1(a)** (top line). Here, the ν_{S-H} stretching vibration at 2555 cm^{-1} disappears, due to the breakage of the S-H and subsequent S-S bond formation. The ν_{O-H} stretching vibration at 3350 cm^{-1} is still present, indicating that the –OH has not reacted. The FTIR spectra of compounds **2** and **3** (not shown) have similar characteristics. The ^{1}HNMR spectra of the starting 6-mercapto1-hexanol and of compound **1** are reported in **Fig.s 1 (b)** and **(c)**, respectively. These spectra confirm the selective formation of the disulphide bridge upon thiol oxidation. In particular, the quartet at 2.50 ppm has been assigned to the protons of the methylene bonded to –SH (CH_2-SH). Upon oxidation, the S-S bridge forms and the proton of the thiol group is lost. This induces a chemical shift of the methylene peak to 2.69 ppm and a reduction of the multiplicity from a quartet to a triplet.

The second step of the reaction, i.e. the cholorination, consists in the condensation of the chlorosulphonyl isocyanate with compounds **1-3**[22] to yield the chlorosulphonyl urethane compounds **4** and **5** and the chlorosulphonyl amide **6**. The chlorosulphonyl isocyanate displays a strongly exothermic reaction in water; therefore, care must be taken to work in anhydrous conditions, in nitrogen atmosphere. The condensation reactions were carried out at 25-30°C for 1 h and yielded a brown dense oil from the compound **1-2** or a yellow oil from compound **3**, respectively.

The reactions were monitored by FTIR spectroscopy.

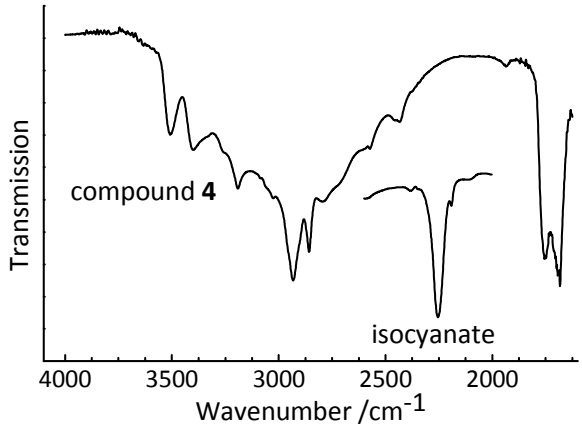


Fig.2 FTIR spectrum of compound **4**. The completion of reaction was deduced from the disappearance of the C=N stretching (2250 cm^{-1}) characteristic of the isocyanate

The development of the reactions could be followed through the disappearance of the $\nu_{C=N}$ stretching vibration of the isocyanate at 2250 cm^{-1} and the appearance of the stretching vibrations of ν_{NH} and $\nu_{C=O}$ belonging to the urethane groups (RCO_2NHSO_2Cl). The frequency of the ν_{NH} and $\nu_{C=O}$ vibrations are 3191 cm^{-1} and 1685 cm^{-1} for compounds **4** and **5** and 3188 and 1694 cm^{-1} for compound **6**, respectively. Furthermore, in the case of compound **6** the decarboxylation can also be

monitored through the release of CO_2 [23] (**Scheme 1**). The FTIR spectrum of compound **4** is reported in **Fig. 2**. No further purifications were made of compounds **4-6**.

The final step of the synthesis, the alcohol condensation, is the reaction of the chlorosulphonyl urethane compounds **4-5** with 1-(2-nitrophenyl) ethanol to yield the final SS-PCCs **7** and **8**. This is a variant of the tosylation procedure[24], where the chlorosulphonyl group is on the alkyl chain and the hydroxyl group is a substituent of the aromatic compound. Also these preparations require an anhydrous environment in nitrogen atmosphere.

Since this is the step where the photosensitive group is introduced, care must be paid, to avoid accidental exposure to light. Furthermore, a test reaction was performed on forehand on compound **4** with a non-light sensitive alcohol, the trans-2-phenyl-1-cyclohexanol, to make sure that the procedure would yield the desired product, without having to deal with light sensitive materials. This resulted in the non-light sensitive disulphided sulphonyl urethane **7a**. Finally, the variant of the tosylation with both light sensitive and light non-sensitive alcohols was performed on the amide **6**, to yield compounds **9** and **9a**, respectively.

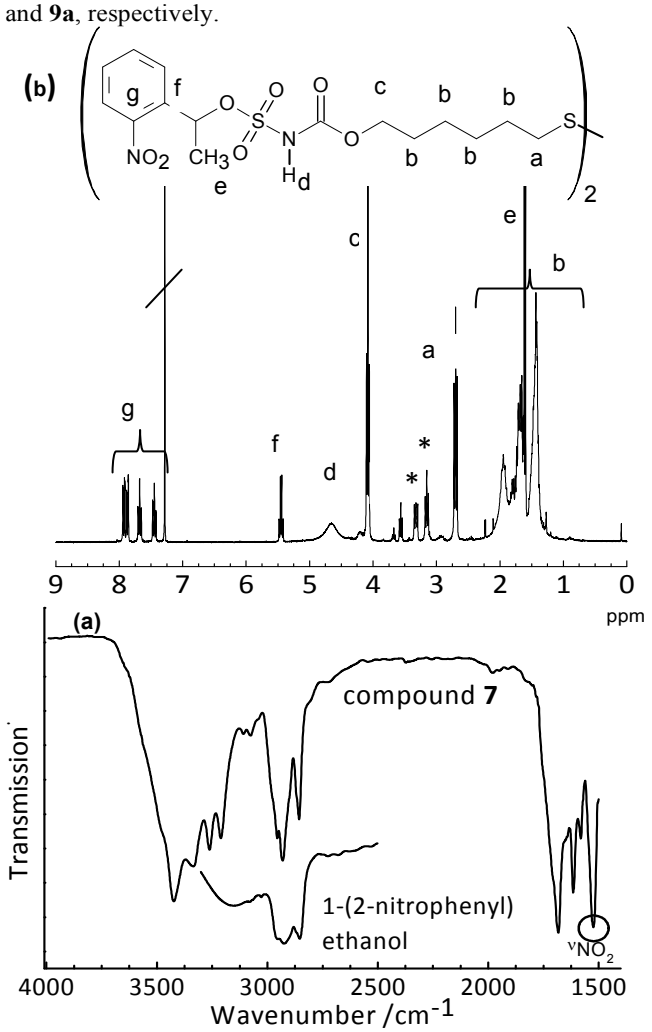


Fig.3 (a) FTIR and **(b)** ^{1}H-NMR of NE-SS-hepta (compound **7**). In panel **(a)** the characteristic bands of the 1-(2 nitrophenyl) ethanol are also reported for comparison. In panel **(b)** the characteristic signals the compound **7** are identified. The peaks marked with* belong to impurities.

In this step, the alcohol was dissolved in dry tri-ethylamine and added drop-wise to a solution of the compounds **4, 5** or **6** in dry dichloromethane. The formation of a white smock indicated the onset of the reaction[25], which, then, was carried on for 24 h under constant stirring. Subsequent work-up yielded yellow solid compounds (**7** through **9a** in **Scheme 1**).

Both FTIR and ^{1}HNMR spectra of the final compounds show the simultaneous presence of the aromatic ring as well as of the sulphonylamide, thus indicating that the tosylation reaction occurred. In **Fig.s 3 (a)** and **(b)** the FTIR and ^{1}HNMR spectra of NE-SS-hepta (compound **7**) are reported. The FTIR spectrum shows the ν_{CH} stretching of the aromatic ring between 3000 and 3100 cm^{-1} as well as the ν_{N-H} above 3300 $cm^{-1}$and the $\nu_{C=O}$ stretching of the carbonyl group at 1684 cm^{-1}. The typical ν_{O-H} stretching of the alcohol at 3150 cm^{-1} (inset) is, instead, removed. It is also worth noticing the peak at 1527 cm^{-1}, which can be attributed to the symmetric NO_2 stretching vibration. The ^{1}HNMR spectrum exhibits both the aromatic (Ph 7.95-7.40 ppm, CHPh 5.45 ppm) and the dithio-carbamate or dithio-sulphonamide signals (CH_2 1.95-1.40 ppm, CH_2S 2.69 ppm, CH_2OCO 4.08 ppm or $CH_2C=O$ 2.15 ppm).

Functionalization of the gold nanoparticles Photoactivity Properties

The synthesis of NE-SS-hepta and –deca aims at the achievement of compounds which can used for AuNPs functionalization and possess photoacidity properties. In order to verify both capabilities, 22(±2) nm AuNPs stabilized with citrate[26] were purposely synthesized and probed by surface plasma resonance (**Fig.1s** in the Supplementary Information). Afterwards, they were functionalized with the SS-PCCs and their photoreactivity probed in the UV – range.

Functionalization procedure

The functionalization of the AuNPs was performed according to the two phases method proposed by Martin *et al*[27]. The reaction is carried out in two steps, formally a reduction step (step **b)** in **Scheme 1**) and a transfer/functionalization (step **c)** and **d)**). In this procedure HCl (step **a)**) acts as control of the ionic strength and acetone as the transferring agent. We preferred it over the Brust method[28], because the latter employs a tetraoctyl ammonium bromide salt as transferring agent. Though this is supposed to be removed in the preparation procedure, its presence as accidental residue is toxic for *in vitro* experiments. It must be mentioned that Martin et al. report that their preparation method is not effective on 3 to 5 nm AuNPs stabilized with citrate, but only on $HAuCl_4$. However, when using citrate-AuNPs, they skipped the reduction phase and probed the transfer/functionalization phase only, clearly because gold is already reduced in the citrate-AuNPs. When following the whole procedure on 22 nm AuNPs, we performed also the reduction step and the whole functionalization reaction went to completion. We think that the reagents of the reduction step actually create the correct ionic strength for the transfer to occur. Anyway, when performed without $NaBH_4$, also in our case we did not observe nanoparticles transfer. The functionalized AuNPs were repeatedly filtered with a 2-3 nm pores polycarbonate membrane, till the solution became colourless.

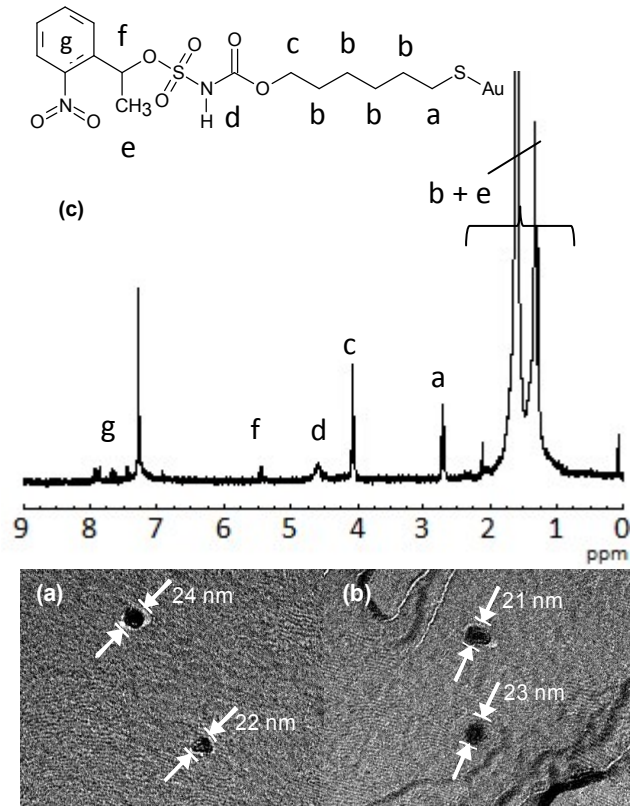


Fig.4 (a) TEM image of AuNPs stabilized with citrate. Magnification 50Kx. **(b)** TEM image of AuNPs after functionalization with NE-SS-hepta. Magnification 50 K x. **(c)** ^{1}H-NMR of AuNPs – NE-SS-hepta. The signals of NE-SS-hepta are still present. The peaks marked with * in **Fig. 3(b)**, instead, have disappeared upon functionalization

Afterwards, TEM images of the AuNPs were collected before and after the functionalization with NE-SS-hepta and are reported in **Fig. 4 (a)** and **(b)**. On average 22 nm AuNPs are observed in both cases, though some occasional 44 nm aggregates are also present. The ^{1}H-NMR spectra of NE-SS-hepta and –deca upon functionalization with AuNPs, show the same characteristic peaks as the free compounds, though remarkably without impurities. This implies that the impurities do not have a thiol or a sulphide group to bind to gold, and can easily be removed in the functionalization step. The ^{1}H-NMR spectrum of the functionalized NE-SS-hepta is reported in **Fig. 4 (c)**. A small broadening of the peaks can be attributed to the binding of the molecule to the AuNPs[29]. Furthermore, a thermogravimetric analysys was performed on NE-SS-deca/AuNPs. We found, on average, 2969 ligands per nanoparticle (**Fig. 2S** in the Supplementary Information).

Photoactivity Measurements

The photoreactivity properties of NE-SS-hepta and –deca/AuNPs, were probed by recording FTIR spectra before and after irradiation in the UV range. The spectra were collected on the films and the samples were irradiated for 1 minute with light in the range 275-375 nm. The photolysis reaction occurs according to the scheme reported in **Fig. 5b**. The reaction may proceed via the conjugated acid (1), but it quickly equilibrates to the cyclic intermediate (2) which dissociates **(3)** releasing a proton[15]. The efficiency of the photolysis depends on the strength of RO^-.

The difference FTIR spectrum before and after irradiation of NE-SS-hepta/AuNPs is reported in **Fig. 5a**. in the energy range 1500 cm^{-1} -1200 cm^{-1} which is characteristic for the nitro- and nitroso- groups stretching vibrations. The peaks are broadened partially due to the conjugation with the nanoparticles, but characteristic features can be recognized. In particular, the positive peaks at 1423 cm^{-1}, 1380 cm^{-1} and 1270 cm^{-1} and the negative one at 1347 cm^{-1} and 1527 cm^{-1} are observed. The positive peaks are assigned to the nitrosoketone (3), in *cis* conformation (1423 cm^{-1} and 1380 cm^{-1}) and in *trans* conformation (1270 cm^{-1}). The negative peaks are due to the disappearance of the nitro group.

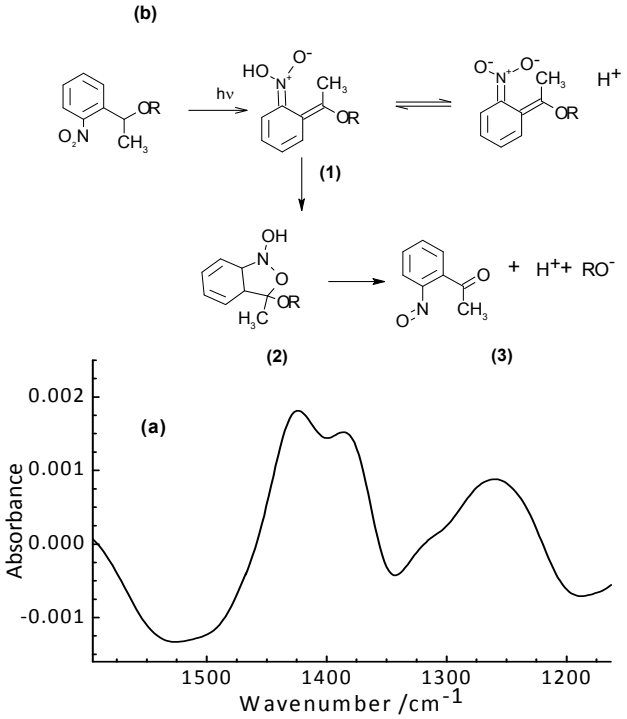


Fig.5 (a) FTIR difference spectrum of NE-SS-hepta/AuNPs, before and after irradiation with UV light. **(b)** Photolysis reaction Scheme, -OR is $-OSO_2\,NHCOO-(CH_6)_2$-S-Au

Conclusions

Triggering and controlling the intracellular pH is of primary importance in several biological processes and has been achieved, among other methods, through purposely designed proton caged compounds. In order to modulate their intracellular uptake, new proton caged compounds were designed which can be conjugated to gold nanoparticles. In this paper we reported the synthesis, characterization, functionalization with gold nanoparticles and the photoreactivity of two di-sulphided sulphonyl urethanes: the disulfanediyldiesane-6,1-diyl bis{[1-(2-nitrophenyl)ethoxy]sulfonyl}carbamate and the disulfanediyldinonane-9,1-diylbis{[1-(2-nitrophenyl)ethoxy]sulfonyl}carbamate, named NE-SS-hepta and NE-SS-deca, respectively.

The synthesis of the disulphided caged compounds was carried out in three steps. In the first one a thiolated alcohol is gently oxidized to obtain the corresponding disulphide-bridged glycol. This way the thiol group is protected and the sulphur-based functionality for the interaction with the gold nanoparticles is

introduced. The two following steps are reactions of a cholorosulphonyl isocyanate, first with the disulphide-bridged glycol, and then with a second alcohol carrying the photosensitive moiety. The resulting proton caged compounds were, then, functionalized with gold nanoparticles and the capability of releasing protons was probed by infrared spectroscopy upon irradiation in the UV-range. The degree of gold nanoparticles functionalization was determined by thermogravimetric analysis.

Experimental

General details

The reaction steps **2** and **3** were performed under nitrogen atmosphere. Prior to use, the solvents were freshly distilled under an inert atmosphere over sodium–benzophenone (toluene) and calcium hydride ($CHCl_3$). Et_3N was distilled on P_2O_5. NaI, $Na_2O_3S_2$ (Chemika) HCl, H_2O_2 30% (Carlo Erba), 6-Mercapto-1-hexanol, 9-Mercapto-1-nonanol, 11-Mercaptoundecanoicacid, EtOAc, clorosulphonylisocyanate, $NaHCO_3$, trans-2-Phenyl-1-cyclohexanol (Aldrich), 1-(2-nitrophenyl) ethanol (Enamine) were used as received.

FT IR spectra were routinely recorded as thin films on a Perkin Elmer SpectrumOne spectrometer.

^{1}H NMR spectra were recorded on an Avance 300 instrument operating at 300.13 MHz.

Energy filtered-transmission electron micrographs were observed in a Zeiss EM902 Transmission Electron Microscope (TEM), operating at 80 keV and equipped with an "in-column" electron energy filter. The filter was settled to collect the elastic electrons (DE = 0), with the result to enhance image contrast and resolution due to the elimination in the image formation of the inelastic electrons (reduction of the chromatic aberration). Briefly, a droplet of solution (1 mg/mL) was deposited onto 400 mesh copper grid covered with a very thin (less than 20 nm) amorphous carbon film. The excess of liquid was removed by placing the grid onto a piece of filter paper. Finally, the grid was dried at RT.

The photoreactivity measurements were performed on a Bruker IFS66/VS interferometer equipped with deuterium discharge lamp (Acton Research Corporation) and a 275-375 nm band-pass FGUV11 filter.

In the following section the IUPAC nomenclature of the compounds is adopted and the correspondence with the compound numbering of **Scheme 1** is indicated.

Syntheses of compounds

General procedure for the thiols oxidation

6,6'-disulfanediyldihexan-1-ol - compound **1**: The 6-mercapto-1-hexanol (5 mmol; 684 **µ**l) was dissolved in 10 ml of EtOAc. Then, H_2O_2 at 30% (5 mmol; 0.55 mL) and the catalyst NaI (0.05 mmol; 7.5 mg) were added, under stirring at RT the reaction was followed by TLC. After 0.5h a saturate aqueous solution of $Na_2O_3S_2$ (15 mL) was added to the mixture under continuous stirring in order to remove the excess of iodine. This turned the solution colour from yellow to white.

The organic phase was extracted with EtOAc (3x15 mL), washed with brine and dried over anhydrous Na_2SO_4. Afterwards, the solvent was removed under reduced pressure, and the crude product (650 mg, 97 %) was column chromatographed on silica gel yielding colorless oil, which solidifies overnight. The solid was, then, used for the following steps. ν_{max}/cm^{-1} (thin film) 3333 (OH); 2938, 2862 (CH_2). $\delta_H(CDCl_3)$ 3.66 (4H, t, OCH_2); 2.69 (4H, t, S_2CH_2); 1.83 (2H, br, OH); 1.75-1.20 (16H, m, CH_2).

9,9'-disulfanediyldinonan-1-ol - compound **2**: The reaction between the 9-mercapto-1-nonanol (2 mmol, 375.5 mL), H_2O_2 30% (0.22 mL, 2 mmol) and NaI (3 mg; 0.02 mmol) in EtOAc (5 mL) was performed in similar conditions as described for compound **1**. The compound **2** was obtained as a white solid (274 mg, 78). ν_{max}/cm^{-1} (thin film) 3352 (OH); 2932, 2957 (CH_2). δ_H ($CDCl_3$) 3.65 (4H, t, OCH_2); 2.69 (4H, t, S_2CH_2); 1.77 (2H, br, OH); 1.68-1.20 (28H, m, CH_2).

11,11'-disulfandiyldiundecanoic acid - compound **3**: The reaction between 11-mercaptoundecanoicacid (0.5 mmol; 110 mg), H_2O_2 30% (0.055 mL, 0.5 mmol) and NaI (0.75 mg; 0.005 mmol) in H_2O was performed in similar conditions as described for **1,** except for the solvent. The compound **3** was obtained as a white solid (84 mg, 77%). ν_{max}/cm^{-1} (thin film) 2916, 2947 (CH_2), 1695 (CO); δ_H ($CDCl_3$) 2.70 (4H, t, SCH_2); 2.37 (4H, d, $OCCH_2$); 1.69-1.26 (32H, m, CH_2).

General procedure for the chlorination

disulfanediyldiesane-6,1-diyl bis[(chlorosulfonyl)carbamate] – Compound **4**. The chlorosulphonyl isocyanate (5.64 mmol, 491 µL) was dissolved in 3 mL dry toluene and placed in a 100 mL round-bottomed-flask connected to a dropping funnel under nitrogen atmosphere. The compound **1** (2.45 mmol; 654 mg; in a ratio 1:2.30 mmol with the isocyanate) was also dissolved in 3 mL dry toluene and added to the isocyanate through the dropping funnel, in 20 min, under stirring at RT. Then, the moisture was kept under stirring for further 45 min, paying attention to protect it from direct irradiation. The reaction was controlled with TLC (9:1 diethyl ether/petroleum) and FT-IR. The excess solvent was removed at reduce pressure and yielded a brown dense oil, which was stored in the dark. ν_{max}/cm^{-1} (thin film) 2928, 2858 (CH_2); 1682 (C=O); 1165 (COC).

disulfanediyldinonane-9,1-diyl bis[(chlorosulfonyl)carbamate] - Compound **5**: The reaction between the chlorosulphonyl isocyanate (1.80 mmol; 156 µL) and the crude compound **2** (274 mg; 0.78 mmol) in dry toluene (1.5 mL) was performed in similar conditions as described for **4**. The compound **5** was obtained as a brown dense oil.

ν_{max}/cm^{-1} (thin film) 2930, 2855 (CH_2); 1692 (C=O), 1160 (COC).

[disulfanediylbis(1-oxoundecane-11,1-diyl)]disulfamyl chloride - Compound **6:** The reaction between the chlorosulphonyl isocyanate (0.81 mmol; 70.47 µL) and the compound **3** (0.35 mmol; 150 mg) in dry toluene (1 mL) was performed in similar conditions as described for compound **4**. Then, compound **6** was obtained as yellow oil. ν_{max}/cm^{-1} (thin film) 2932, 2858 (CH_2); 1736 (CO)

General procedure for alcohol condensation

disulfanediyldiesane-6,1-diyl bis{[1-(2-nitrophenyl)ethoxy]sulfonyl}carbamate - Compound **7**: The compound **4** (630 mg) was dissolved in $CHCl_3$ (3 mL) and placed in a 50 mL round-bottomed-flask connected to a dropping funnel in nitrogen atmosphere. A solution of Et_3N (1.53 mmol;212 µL) and the 1-(2-nitrophenyl)ethanol (1.6 mmol; 256 mg), was added through the dropping funnel in 15 min to the compound **4**, kept under stirring at RT. After 24 hours, the crude product was treated with a mixture of water (1 mL) and ether (5 mL). The organic phase was extracted and successively washed with a 2M HCl 5% $NaHCO_3$ water and then dried with Na_2SO_4. The solvent was removed to yield a yellow-green sludge (222 mg; 25%). ν_{max}/cm^{-1} (thin film) 2932,

2857 (CH_2); 1685 (CO); 1523s,1344m.(N=O); δH($CDCl_3$) 7.94–7.42 (10H, m, C_6H_5); 5.47-541 (2H, q, $OCHCH_3$); 4.76 (2H, br, NH); 4.07(4H, t, $COOCH_2$); 2.70 (4H, t, SCH_2); 1.69-1.26 (16H, m, CH_2); 1.61 (6H, d, CH_3).

disulfanediyldiesane-6,1-diyl bis[(2-phenylcyclohexy)sulfonyl]carbamate - Compound **7a**: The reaction between the trans-2-phenyl-1-cyclohexanol (1.53mmol; 216 mg) Et_3N (1.53 mmol; 212 μL) and the crude compound **4** (631 mg) in dry $CHCl_3$ (3 ml) was performed under similar conditions as described for **7**. The compound **8** was obtained as a yellow-green sludge (595 mg, 58%). ν_{max}/cm^{-1} (thin film) 2931, 2857 (CH_2); 1731 (CO). δ_H($CDCl_3$) 7.35–7.26 (10H, m, C_6H_5); 4.66 (2H, br, NH); 3.69 (2H, dt, OCH); 4.09 (4H, t, $COOCH_2$); 2.71 (4H, t, SCH_2); 1.72-1.40 (16H, m, CH_2).

disulfanediyldinonane-9,1-diyl bis{[1-(2-nitrophenyl)ethoxy]sulfonyl}carbamate - Compound **8**: The reaction between the 1-(2-nitrophenyl)ethanol (0.52 mmol, 86.84 mg) Et_3N (0.52 mmol; 72.77 μL) and the crude compound **5** (633 mg) in dry $CHCl_3$ (1 ml) was performed under similar conditions as described for **4** . The compound **9** was obtained as a yellow-green sludge (589 mg, 66%). ν_{max}/cm^{-1} (thin film): 2920, 2851 (CH_2); 1682 (CO); 1524, 1350 (N=O). δ_H($CDCl_3$) 7.94–7.42 (10H, m, C_6H_5); 5.44 (3H, q, $OCHCH_3$); 4.76 (2H, br, NH); 4.07(4H, t, $COOCH_2$); 2.70 (4H, t, SCH_2); 1.77-1.25 (28H, m, CH_2); 1.62 (6H, d, CH_3).

di-1-(2-nitrophenyl)ethyl [disulfanediylbis(1-oxoundecane-11,1-diyl)]bissulfamate - Compound **9**:
The reaction between the 1-(2-nitrophenyl)ethanol (0.22 mmol, 35 mg), Et_3N (0.22 mmol, 30 μL) and the crude compound **6** (130 mg) in dry $CHCl_3$ (2 mL) was performed in similar conditions as described for compound **7**. The compound **11** was obtained as a yellow sludge (164 mg, 56%). ν_{max}/cm^{-1} (thin film): 2930, 2850 (CH_2), 1739 (CO), 1525, 1348 (N=O). δ_H($CDCl_3$) 7.90-7.43 (10H, m, C_6H_5); 5.44 (2H, q, $OCHCH_3$); 2.66 (4H, t, SCH_2); 2,30 (4H, m, CH_2CO), 1.72-1.25 (32H, m, CH_2); 1.61 (6H, d, CH_3).

di-2-phenylcyclohexyl[disulfanediylbis(1-oxoundecane-11,1-diyl)]bissulfamate - Compound **9a**: The reaction between the trans-2-phenyl-1-cyclohexanol (0.3 mmol, 28.5 mg) Et_3N (0.20 mmol, 28 μL) and crude compound **6** (184 mg) in dry $CHCl_3$ (2 mL) was performed in similar conditions as described for compound **7a**. The compound **9a** was obtained as a yellow solid (74 mg, 30%). ν_{max}/cm^{-1} (thin film): 2925, 2852 (CH_2); 1708 (CO). δ_H($CDCl_3$) 7.35–7.26 (10 H, m, C_6H_5); 4.76 (2H, br, NH); 3.67 (2H, dt, OCH); 2.70 (4H, t, SCH_2); 2.36 (4H, m, CH_2CO); 1.68-1.42 (32H, m, CH_2).

AuNPs Synthesis - 20 mL of an aqueous solution containing $KAuCl_4$ at 0.25 mM was heated to the boiling point, and then, 1 mL of an aqueous solution of sodium citrate at 34 mM (1 wt. %) was added under vigorous stirring. This solution was heated for about ten minutes. The solution turns to red attesting the formation of Au nanoparticles. The nanoparticles exhibit a plasmon band at 520 nm.

AuNPs functionalization. 15μL of a 2N HCl aqueous solution were added to 1.5 mL of a 30 mM citrate-AuNPs aqueous solution, under stirring. A change of colour from red to purple was, then, observed. Afterwards, 2.2 mL of a 26.5 M $NaBH_4$ and 26.5 M NaOH aqueous solution and 2.2 mL of acetone were added, always under stirring. Then, 50 mg of NE-SS-hepta or of NE-SS-deca were dissolved in 3 mL toluene and added to the aqueous solution. The mixture was kept 1 day under constant stirring at RT. After the work-up to separate the organic phase, the toluene volume was reduced to 0.5 mL and 2 mL ethanol were added. The solution was, then, stored 1 day at -15°C. A precipitate was obtained and collected by repeatedly filtration with a polycarbonate membrane filter (cut-off 12000 MW, corresponding to 2-3 nm pores).

Acknowledgements

The authors wish to thank Hynd Remita (Université Paris-Sud, Orsay France) for providing the gold nanoparticles.

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