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Photochemical transformation of lipoic acid-based ligands: probing the effects of solvent, ligand structure, oxygen and pH[†]

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We have combined optical absorption with the Ellman's test to identify the parameters that affect the transformation of the 5-membered dithiolanes to thiols in lipoic acid (LA) and its derivatives during UV-irradiation. We found that the nature and polarity of the solvent, the structure of the ligands, acidity of the medium and oxygen can drastically affect the amount of photogenerated thiols. These findings are highly relevant to the understanding of the photochemical transformation of this biologically relevant compound, and would benefit the increasing use of LA-based ligands for the surface functionalization of various nanomaterials.

α -Lipoic acid (LA) is a natural antioxidant present in living organisms with several implications in health and medicine.^{1,2} Pioneering work by Calvin and co-workers showed that lipoic acid was responsible for the oxidative decarboxylation of pyruvic acid to give rise to acetyl-coenzyme A (acetyl-coA).³ In live cells, the oxidized (LA) and reduced (dihydrolipoic acid, DHLA) forms exist in equilibrium, creating a potent redox couple with a reported standard redox potential of -0.32 V.⁴ Lipoic acid and its derivatives exhibit direct free radical scavenging properties and studies have provided evidence that lipoic acid as a supplement decreases oxidative stress *in vivo*.^{5,6} LA or DHLA is also known to react with reactive oxygen species such as superoxide radicals, peroxy radicals, and singlet oxygen.⁵⁻⁷ The biosynthesis of lipoic acid is not well understood, but the compound is commercially available as a racemic mixture, DL- α -lipoic acid.⁸

Due to their strong coordination interaction with metal ions lipoic acid and its reduced form, dihydrolipoic acid, have recently attracted much attention as capping ligands for inorganic

nanocrystals made of metal (Au and Ag) or semi-conducting quantum dot (QD) cores.⁹⁻¹³ Indeed it has been shown that the bidentate DHLA groups exhibit stronger coordination interactions with luminescent quantum dot and Au nanoparticle (AuNP) surfaces compared to their monothiol counterparts.¹⁴ A series of LA-modified ligands have been designed over the past decade, including polyethylene glycol (PEG)- and zwitterion-appended molecules (Fig. 1A), in order to provide nanocrystals that are hydrophilic and stable in a wide range of biological conditions.^{12,15,16} LA-based ligands have also been used by our group and others to prepare plasmonic nanoparticles as well as luminescent nanoclusters made of gold and silver cores *via* the reduction of metal-ligand complexes by sodium borohydride.¹⁷⁻²² In this method, the particle size is controlled by selecting the metal precursor-to-ligand molar ratio used. For instance, the growth reaction yields plasmonic gold and silver nanoparticles when the metal-to-ligand molar ratio is larger than one, while the formation of fluorescent nanoclusters (AuNCs and AgNCs) emitting in the red and near-IR region of the optical spectrum is favoured for molar ratios smaller than one.^{18,20,21}

Capping luminescent QDs (*e.g.*, ZnS-overcoated CdSe nanocrystals) with LA-based ligands, carried out *via* ligand exchange of the native hydrophobic cap, requires the use of DHLA or DHLA-derivatives, which are routinely prepared *via* sodium borohydride reduction,^{9,23} ligation of the QDs with the oxidized form (LA-based) of the ligands could not be implemented.^{9,24} Though successful, this approach is tedious but more importantly it can be deleterious to certain functional groups with great utility in biology, such as aldehyde ($-CHO$) and azide ($-N_3$). We have recently shown that cap-exchange on QDs can be performed starting with the oxidized LA-based ligands using UV photoirradiation ($\lambda < 400$ nm) of hydrophobic QDs mixed with LA ligands, or by simply pre-irradiating the oxidized ligands followed by phase transfer.^{25,26} Aldehyde- and azide-functionalized QDs prepared using this photoligation strategy were successfully tested in biorthogonal coupling reactions, including hydrazone ligation and CLICK coupling.²⁷ We have also found that AuNCs

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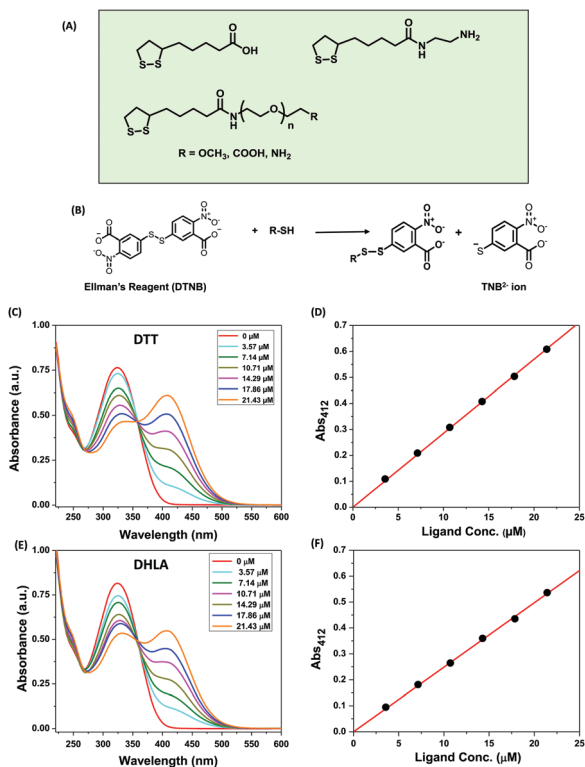


Fig. 1 (A) Chemical structures of a few representative lipionic acid-based ligands (the oxidized form) are shown on top: LA, amine-modified LA and PEG-modified LA (LA-PEG-R). (B) Schematic representation of the chemical reaction involved in the Ellman's assay. (C) UV-Vis absorption spectra collected from solutions of DTNB mixed with DTT (dithiothreitol), and (D) the corresponding Beer-Lambert plot of Abs_{412} vs. ligand concentration. (E) UV-Vis absorption data collected from solutions of DTNB mixed with DHLA (chemically reduced), and (F) the corresponding Beer-Lambert plot.

grown using photo-irradiated LA-PEG ligands (referred to as LA-PEG*) and in the absence of chemical reducing agents yielded nanoclusters with blue, yellow and red fluorescence.²⁸ Additionally, the photoemission from these nanoclusters was affected by the nature of the terminal group in the LA-PEG ligands. For instance, growth of nanoclusters using methoxy-appended LA-PEG (LA-PEG-OCH₃*) yielded red-emitting clusters, while blue emitting materials were grown in the presence of amine-terminated LA-PEG (LA-PEG-NH₂*) ligand.

The photosensitive nature of lipionic acid resides in the strained 5-membered ring around the S-S bond, resulting in the appearance of a well-defined absorption feature centered at 335 nm. When a solution of lipionic acid is exposed to UV irradiation, it undergoes a photochemical transformation that yields among others a mixture of dithiol-appended molecules (e.g., DHLA), along with several byproducts including oligomeric polysulfide and sulfoxide compounds; only a few of those have been clearly identified.^{3,26,29-31} There is no unanimous view about the mechanism of this photochemical transformation or the exact nature of the final by-products, which tend to be affected by parameters such as irradiation time, the energy of the UV photons and nature of the solvent.²⁹⁻³¹

Herein, we investigate the nature of the photochemical transformation of the dithiolane ring (in LA-based ligands) by focusing on one important aspect: what governs the photochemical transformation of LA and LA-PEG ligands to thiol containing compounds under UV-irradiation? Parameters that will be probed include the effects of varying the solvent used, the exact structure of the LA-based molecules, nature of the terminal group (inert or reactive), importance of dissolved oxygen, humidity and the presence of mineral or organic acids in the medium. For this, we rely on the Ellman's assay as a simple analytical technique to estimate the percentage of thiols generated during irradiation. It involves the rapid reaction of the Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid), DTNB) with the generated thiols in neutral and alkaline media, producing 2-nitro-5-thiobenzoate (TNB²⁻) ions which have a well-defined absorption contribution at 412 nm (Fig. 1B).³² In addition to being rapid the reaction is stoichiometric and the concentration of TNB²⁻ can be quantified spectrophotometrically using the extinction (or the molar absorption) coefficient at 412 nm: $\epsilon_{412}(\text{TNB}^{2-}) = 14\,150\text{ M}^{-1}\text{ cm}^{-1}$.³³

To ensure the reliability of the Ellman's assay, we first performed measurements using solutions of dithiothreitol (DTT) and the chemically reduced form of lipionic acid, *i.e.* dihydrolipoic acid; these will serve as reference samples. DHLA is the smallest and simplest version of the DHLA-based molecules, which can be prepared by NaBH₄-reduction following the protocol detailed in ref. 9, 15, 16 and 23. Several solutions of DTT (in water) or DHLA (in methanol) with concentrations ranging from 1 to 6 mM were prepared. Then, an aliquot of each solution was mixed with the Ellman's reagent pre-diluted in pH 8 buffer and reacted for 10 min (see Experimental section). The absorption spectra collected from those solutions (shown in Fig. 1) were used to assemble a Beer-Lambert plot of the absorbance at 412 nm vs. ligand concentration. The absorption data from both compounds were readily fitted to a straight line, with a slope $\cong 28\,490\text{ M}^{-1}\text{ cm}^{-1}$ for DTT and $\cong 25\,700\text{ M}^{-1}\text{ cm}^{-1}$ for NaBH₄-reduced lipionic acid (DHLA). Comparing this value to the molar absorption coefficient of the TNB²⁻ reported in the literature ($14\,150\text{ M}^{-1}\text{ cm}^{-1}$ for monothiol compounds), leads us to conclude that the presence of two thiols per molecule (in DTT) essentially doubles the concentration of generated TNB²⁻ in the solutions. We have also performed the Ellman's assay of cysteine (a monothiol compound) using the same buffer conditions and the absorption data yielded a slope of $13\,890\text{ M}^{-1}\text{ cm}^{-1}$ which is very close to the literature value (see ESI,† Fig. S1). The slope extracted from DHLA data ($25\,700\text{ M}^{-1}\text{ cm}^{-1}$) is ~ 1.8 times larger than that measured for cysteine, which implies the near complete reduction of the dithiolane ring using NaBH₄, essentially yielding two thiols per DHLA molecule.³³ It also indicates that reduced DHLA molecules have a tendency to reoxidize back to LA because intramolecular oxidation in solution conditions is spontaneously promoted for this compound (*i.e.*, substantially faster than for DTT). Below, we describe results of the Ellman's test applied to UV-irradiation of the LA-based ligands. The data collected from solutions of photo-irradiated ligands in various solutions will be used to extract an

estimate for the concentration of thiols produced in each solvent, by comparing the molar absorption coefficient extracted from fitting the Beer–Lambert plot (for each ligand–solvent system) to the value of TNB^{2-} , $\epsilon_{412}(\text{TNB}^{2-}) = 14\,150\text{ M}^{-1}\text{ cm}^{-1}$. To provide data that take into account the fact that in an ideal case each lipoic acid molecule would yield two thiol groups, we introduce a factor 2 in the conversion, as described in the experimental section (see below).

The utility of LA and DHLA in nanoparticle coating is limited, due to a lack of clearly defined hydrophilic block, which has led to the development of a series of PEG- and zwitterion-modified ligands to expand the range of solubility conditions.^{27,34,35} We centre the investigation on the photochemical transformation of the LA-PEG family of ligands. LA-PEG ligands are soluble in both aqueous and non-aqueous solvents. In addition, they preserve the characteristic absorption of the dithiolane group at 335 nm. We first carried out the UV-irradiation of LA-PEG-OCH₃ in an array of organic solvents ranging from polar protic (*e.g.*, methanol) to nonpolar aprotic (*e.g.*, CCl₄), as well as water, using an excitation band centred at 350 nm. Fig. 2 summarizes the changes in the absorption spectra of the ligand dispersed in those solvents as a function of the irradiation time (see ESI,† Fig. S2, for additional spectra). Clearly, there is a progressive decrease in the absorbance peak at 335 nm with increasing irradiation time, but the overall evolution of the spectra varied from one solvent to another. In polar protic solvents like water and methanol, the spectra show a steady change with an isosbestic point around

290 nm. Similar behaviour was observed for solutions in acetonitrile, which is aprotic with moderate polarity. In comparison, UV-irradiation of LA-PEG-OCH₃ in weakly polar and non-polar solvents (*e.g.*, DCM, ethyl acetate and carbon-tetrachloride), yielded spectra with no isosbestic point where the decrease in the absorption peak at 335 nm is coupled with a rise in the absorbance at $\lambda < 320\text{ nm}$. This trend is indicative of the higher rates of photooxidation and polymerization in these solvents.³ All spectra showed saturation after 25 to 30 min irradiation time regardless of the solvent used (see Fig. 2).

The Ellman's assay was then applied to the above solutions, once the UV-irradiation was complete, to extract a measure for the relative thiol yields for LA-PEG-OCH₃* compared to the nominal value for cysteine and DTT, or that anticipated for fully reduced lipoic acid (DHLA, see Table 1). At least 5 to 6 solutions were prepared from each of the UV irradiated ligand samples (by dilution) and multiple measurements were carried out. Briefly, an aliquot of each irradiated solution was mixed with the Ellman's reagent diluted in pH 8 buffer and reacted for 10 min before collecting an absorption spectrum (see Experimental section for additional details). The absorption spectra and Beer–Lambert plots for LA* and LA-PEG-OCH₃* (irradiated ligands) are shown in ESI,† Fig. S3. The average percentage of thiols obtained from these measurements are listed in Table 1. Data show that except for solutions in water there is a clear correlation between the percent thiols measured and the combined effects of polarity and proticity of the solvent, including data for acetonitrile and DMSO solutions. The percentage yield of thiols was $\sim 29\%$ for ligand irradiated in DI water. The percent thiol was the lowest in carbon tetrachloride (27%) but significantly higher in alcohols, with the highest value measured for methanol and ethanol solutions ($\sim 60\text{--}62\%$). We should note that the trend shown in Table 1 is consistent with the absorption data in Fig. 2, where rather complex profiles were measured in non-polar and aprotic solvents.

We assessed the effects of dissolved oxygen in the medium on the percent thiols measured at saturation (*i.e.*, after 30 min irradiation) in water and methanol solutions. For this the UV-irradiation of LA-PEG-OCH₃ was also carried out in solutions of argon-bubbled water and methanol. The obtained thiol yields were compared to those measured under atmospheric conditions, see Table 2. While the change in thiol yield was marginal in methanol (from 62% to 65%), there was a significant increase from 29% to 55% in argon bubbled water. Clearly, the effects of removing oxygen on the transformation are more

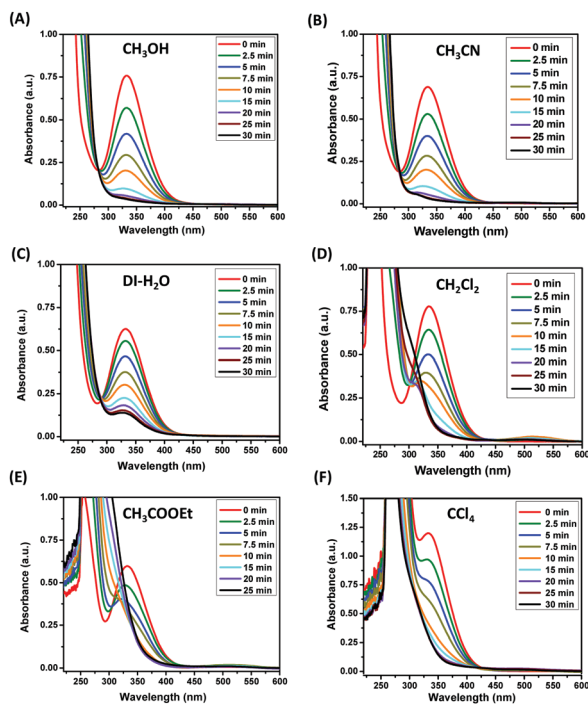


Fig. 2 Time-dependent UV-vis absorption spectra of LA-PEG₇₅₀-OCH₃* irradiated in: (A) methanol, (B) acetonitrile, (C) water, (D) dichloromethane, (E) ethyl acetate and (F) carbon tetrachloride. Spectra were collected using a 10 mm optical path cuvette. The ligand concentration is 6.0 mM. Note the additional strong absorbance at 300–320 nm for CH₃COOEt and CCl₄ solutions.

Table 1 Percent thiol extracted from the Ellman's assay for LA-PEG₇₅₀-OCH₃ ligand UV-irradiated in different solvents. The provided polarity values are from ref. 36

Solvent	Polarity	Thiol (%)	Solvent	Polarity	Thiol (%)
Water	10.2	29	DMSO	7.2	42
Methanol	5.1	62	Acetonitrile	5.8	34
Ethanol	4.3	60	DCM	3.1	33
1-Propanol	4.0	50	Ethyl acetate	4.4	28
1-Butanol	3.9	52	THF	4.0	34
2-Propanol	3.9	40	CCl ₄	1.6	27

Table 2 Percent thiol measured using the Ellman's assay for LA-PEG₇₅₀-OCH₃* ligand in a few representative solvents, with and without nitrogen or argon bubbling. This allowed us to test the effects of air moisture and dissolved oxygen

Solvent	Thiol (%)
Methanol (HPLC grade)	62
Methanol (Ar-bubbled)	65
Anhydrous methanol	56
Anhydrous methanol + 0.1% H ₂ O	63
DI-water	29
DI-water (Ar-bubbled)	55

pronounced in water solutions. This indicates that oxidation is highly favourable in an aqueous medium and is triggered even by trace amount of dissolved oxygen, whereas in methanol the photochemical transformation occurs through other pathways which are not dependent on oxygen (see discussion below). This is also evidenced by the relatively higher yield of thiols measured for ligands irradiated in alcohol solutions (Table 1). Additionally, we applied the Ellman's test to a ligand solution in anhydrous methanol. We found that the thiol percentage was slightly lower (56%) than that measured for HPLC grade methanol. This led us to think that moisture present in HPLC methanol contributes towards thiol formation. To test this hypothesis, we applied the Ellman's test to solution in anhydrous methanol containing 0.1% DI water (catalytic amount), and found that indeed the measured thiol percentage increased to 63%, essentially the value measured for HPLC grade solvent. However, adding a larger fraction of water (*e.g.*, 5%) to HPLC grade methanol did not increase the measured thiol percentage (Table 2).

We then complemented the above data by probing the effects of altering the nature of the terminal group in PEG-free compounds (*e.g.*, LA vs. LA-modified with ethylene diamine, LA-NH₂), or inserting a terminal reactive group at the lateral end of LA-PEG ligands, on the % thiol for ligands UV-irradiated in methanol. We also probed the effects of adding acid or base to the ligand solution prior to UV-irradiation. We tracked the absorption spectra and applied the Ellman's test to LA*, LA-NH₂*, and LA-PEG-R* presenting reactive terminal groups (R = -NH₂, -COOH). Note the slight difference in the PEG size used between the various LA-PEG ligands: LA-PEG₇₅₀-OCH₃ vs. LA-PEG₆₀₀-COOH/NH₂. Fig. 3 shows the time-dependent UV-Vis absorption spectra of the various ligands collected in methanol during UV-irradiation. The spectra show that there is a clear difference between amine-appended ligands and those presenting COOH groups. While the decrease to essentially baseline in the characteristic absorption band at 335 nm was complete after 30 min for LA* and LA-PEG₆₀₀-COOH*, the trend was different for amine-appended ligands. For these ligands, the reduction in the peak at 335 nm was partial (~50% reduction), and there was an additional weak absorbance centered at ~300–310 nm. These features were especially prominent in the case of solutions of LA-PEG-NH₂*. The values of thiol yield obtained from the Ellman's test applied to solutions of these ligands are listed in Table 3. The yield is lower for LA-PEG-NH₂* in water and in

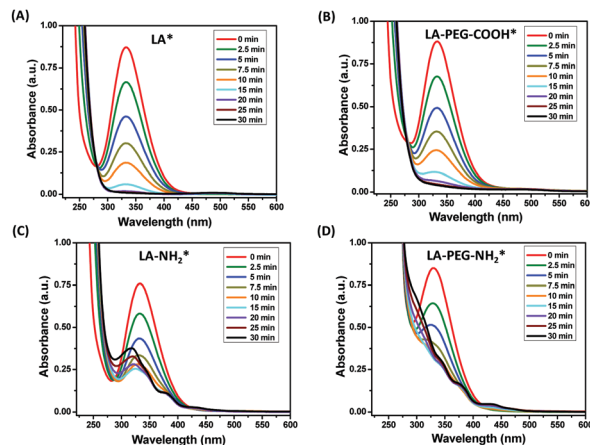


Fig. 3 UV-visible absorbance spectra of (A) LA*, (B) LA-PEG₇₅₀-COOH* (C) LA-NH₂* and (D) LA-PEG₆₀₀-NH₂* in methanol at different time intervals during UV-irradiation. The concentration is 6.0 mM and the cuvette path length is 10.0 mm.

Table 3 Thiol yield estimated for ligands with different terminal groups. The value estimated for PEGylated ligands in the presence of added acid are also listed

Ligand	Thiol (%) in water	Thiol (%) in methanol
LA-PEG ₇₅₀ -OCH ₃ *	29	62
LA-PEG ₆₀₀ -COOH*	55	74
^a LA-PEG ₆₀₀ -NH ₂ *	36	45
LA*	—	83
LA-NH ₂ *	—	42
LA* + PEG ₇₅₀ (~1 mol. eq.)	—	84
LA* + HCl (2 mol. eq.)	—	81
LA-PEG ₇₅₀ -OCH ₃ * + HCl (~2 mol. eq.)	67	73
LA-PEG ₇₅₀ -COOH* + HCl (2 mol. eq.)	—	75
LA-PEG ₆₀₀ -NH ₂ * + HCl (~6 mol. eq.)	—	72
^b DHLA-PEG ₆₀₀ -NH ₂	48	—

^a The water solution becomes slightly turbid during the photoirradiation. ^b The compound was prepared by reducing LA-PEG-NH₂ in ethanol and water mixture (3 : 1), followed by the Ellman's test in pH 8 buffer.

methanol compared to LA-PEG₆₀₀-COOH*, which supports the absorption data in Fig. 3. Note that chemically reduced DHLA-PEG-NH₂ (using NaBH₄) also gave lower thiol percentage (~48%), see Table 3. These observations led us to infer that the terminal amine group interferes with the degree of transformation of disulphide rings to thiols. These results contrast with the data collected for ligands with a terminal COOH, where higher % thiols were measured.

We performed a few control experiments to verify that the substantial increase in thiol yield measured for LA-PEG-COOH* (compared to LA-PEG-OCH₃*) was due to the presence of carboxyl groups. We found that UV-irradiation of LA in methanol solutions yielded a similar thiol percentage (~83%), and the addition of equimolar amount of free PEG to the solution did not affect that value (Table 3). This implies that the presence of tethered carboxyl groups in the ligands has increased the conversion of intermediate radical to thiols during irradiation. Such result can possibly be attributed to the fact that the carboxylic

acid groups inhibit the polymerization of the dithiyl radicals formed during UV-irradiation, as proposed by Calvin and co-workers.³ We could not directly test this hypothesis for LA in water, due to its poor solubility. Instead, dissolution in water was facilitated by the addition of sodium hydroxide to convert LA to LA-salt. The yield of thiols extracted from the Ellman's test following UV-irradiation of the LA-salt in basic (water) conditions was 30%; this value is comparable to that measured for LA-PEG-OCH₃* in water (see Table 1), or reported in ref. 29.

Finally, the importance of introducing "free" carboxyl groups (*i.e.*, untethered to the ligands) was tested by adding organic or mineral acids to the ligand solution prior to UV-irradiation and Ellman's test. Indeed, we found that when an acid (*e.g.*, HCl, ascorbic acid or hexanoic acid) was added to the solution before UV-irradiation, the thiol yields increased from 62% to 73% for LA-PEG-OCH₃* in methanol (see Table 3). The same behaviour was observed for LA-PEG-NH₂*, where the yield in methanol increased from 45% to 72% when excess HCl was used. The presence of acid has also improved the progression of the absorption profiles (*e.g.*, see ESI,[†] Fig. S4). This is attributed to the fact that acid protonates the amine group, thus preventing the occurrence of potential nucleophilic side-reactions. Addition of base was found to reduce the % thiol measured for LA-PEG-OCH₃*. Data for other acids and NaOH are shown in Table S1 (ESI[†]).

We would like to briefly discuss possible mechanisms for the abstraction of the hydrogen necessary for the production of thiol groups, following UV irradiation of the ligand solution. Upon absorbing a UV photon, the dithiolane group in the LA derivative forms a transient species (a diradical) which then undergoes several potential transformations as schematically summarized in Fig. 4. Thiol formation, which is one of the

transformation products, requires hydrogen abstraction. Our data indicate that such process is strongly affected by the nature of the solvent (*e.g.*, polarity). We focus our discussion on transformations occurring in water and alcohols (such as methanol). In water, the splitting of O–H bond leads to the formation of sulfenic acid which produces, *via* disproportionation (dismutation), dithiol and bis(hydroxyl) compounds. The latter can lose a water molecule to form a sulfoxide product (Fig. 4, path 1).³ Conversely, in methanol solutions, such reaction (hydrogen abstraction from the OH group) would lead to the formation of sulfenic ester, which upon decomposition produces several products including sulfone, dithioic acid, sulfinic acid, thioaldehyde and hydrogen sulfide (see Fig. 4, path 2), as described by Calvin and co-workers.³ Another source of the required hydrogen is abstraction from the methyl group by the sulfur radicals, leading to the formation of thiols (–SH) along with CH₂OH radicals; the latter can lose the hydrogen from the O–H group yielding formaldehyde (Fig. 4, paths 3 and 4). Irradiation of the ligands dissolved in other alcohols such as ethanol, 1-propanol or 2-propanol would generate acetaldehyde, or propionaldehyde or acetone, respectively, though the hydrogen transfer rate will vary from one alcohol to another. The involvement of O–H hydrogen in the photochemical transformation of the dithiolane rings to thiolated species was previously verified using transient absorption measurements in solutions of deuterated methanol and water. In particular, the authors measured slower quenching of the triplet excited state of LA in D₂O and CH₃O–D; the rates were ~1.5 times slower than those measured in H₂O and CH₃O–H.²⁹ In non-polar solvents, where C–H bonds are the only likely hydrogen source, abstraction and thiol formation compete with other processes including oligomer formation, oxidation and intramolecular rearrangement. These competing processes naturally reduce the thiol yield in these media. We should note that oxidation and oligomer formation are not limited to non-polar solvents. They can also occur in aqueous and alcohol media, even though paths 1 to 4 would dominate in those systems (Fig. 4, paths 5 and 6).^{26,29–31}

In conclusion, we have investigated the photochemical transformation of lipoic acid-based ligands promoted by UV-irradiation, by focusing on changes in the absorption spectra and the thiol yield using the Ellman's test. We explored the effects of varying the structure of the ligand including the nature of the terminal group, nature and polarity of the solvent, along with the presence of oxygen and added acid on the measured thiol yields during UV excitation. We found that the presence of terminal carboxyl group and the use of polar protic solvents provided the highest yields of thiol compounds. Conversely, aprotic solvents and amine-type terminal groups drastically altered the absorption properties and lowered the yields of generated thiols in the medium. Overall, our results agree with the widely accepted mechanism that the photochemical transformation involves a disulphide to diradical transformation followed by subsequent hydrogen atom abstraction from a suitable donor molecule (*e.g.*, protic solvent, COOH) as schematically summarized in Fig. 4.^{26,30} These findings

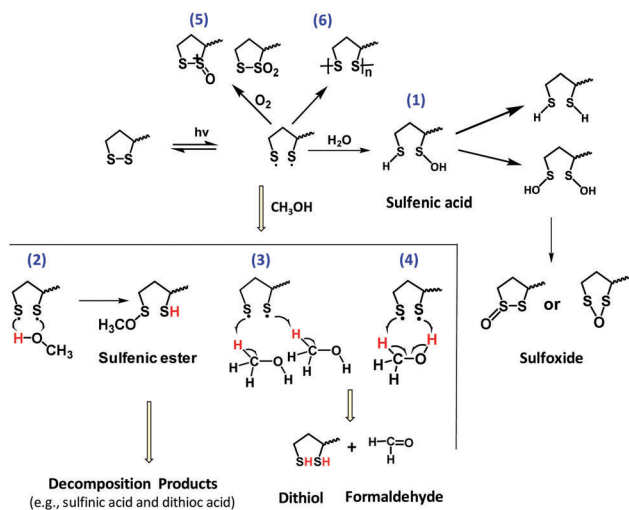


Fig. 4 Schematic description of the mechanistic pathways yielding thiol-appended ligands for various photochemical transformations of LA-based ligands under UV-irradiation. Ring dissociation leads to the formation of a diradical, which can undergo oxidation and polymerization. Possible modes of hydrogen atom abstraction by the diradical from solvent leading to the formation of thiolated and other byproducts in water and in methanol are shown.

could further our understanding of the importance of LA in various biological processes. They could also impact the use of lipoic acid and its derivatives to surface functionalize metal and semiconducting nanocrystals. The interactions of these ligands with QD surfaces under UV irradiation and/or during the growth of fluorescent nanoclusters merits further investigation.²⁵

Experimental section

Materials

Lipoic acid (99%), polyethylene glycol ($M_w = 600$ Da) and polyethylene glycol methyl ether ($M_w = 750$ Da) along with the phosphate salts were purchased from Sigma Aldrich (St Louis, MO). All the organic solvents including DCM, ethyl acetate, carbon tetrachloride, acetonitrile, DMSO, methanol, ethanol and propanol were also purchased from Sigma Aldrich. The Ellman's reagent (5,5'-dithio-bis(2-nitrobenzoic acid), $\geq 98\%$), dithiothreitol and EDTA were purchased from Sigma Aldrich and Mallinckrodt Chemicals (St Louis, MS), respectively.

Ligand synthesis

Synthesis of lipoic acid-appended with polyethylene glycol (LA-PEG) ligands with $-\text{OCH}_3$, $-\text{NH}_2$ and $-\text{COOH}$ terminal groups was carried out using the protocols detailed in our previous work.^{15,16} When needed chemical reduction of the ligand was carried out in a mixture of ethanol and water (3 : 1) using NaBH_4 . Oxidized LA and its derivatives were used for the photoirradiation experiments. A typical ligand solution used for UV-irradiation was prepared in the desired solvent using 10 or 20 mL scintillation vials. A magnetic bar was introduced and the solution was then exposed to UV-light under continuous stirring.

Photo-irradiation experiments

The UV-irradiation experiments were carried using a UV photo-reactor (Model LZC-4 V, Luzchem Research, Inc., Ottawa, Canada) containing 14 lamps, six installed on top and four on each side (see picture provided in the ESI,† Fig. S6). The system provides a signal with maximum peak at 350 nm and a power of 4.5 mW cm^{-2} at the sample position (see emission spectrum in ESI,† Fig. S5). The sample was periodically retrieved and an absorption spectrum was collected using a UV-Vis spectrophotometer (model UV 2450 from Shimadzu) until saturation (*i.e.*, when there are no more changes in the absorption spectrum). Six solutions with concentrations varying from 1 mM to 6 mM were prepared by diluting the above UV irradiated solution in the starting solvent. When the UV-irradiation was carried out using solvent that was not miscible with buffer, the compound was dried first under vacuum then dissolved either in methanol then mixed with buffer, or directly in the buffer. The buffer solutions were prepared using DI water (nanopure, 18 M Ω).

Ellman's assay

A stock solution of 5 mM Ellman's reagents was prepared by dissolving 4 mg of DTNB in 2 mL of phosphate buffer pH 8 (100 mM) containing 1 mM EDTA (ethylenediamine tetracetate acid).

25 μL of this stock solution was further diluted with 2.765 mL of the same buffer. Then, 10 μL of the diluted ligand solution (described above) was added and the reaction was allowed to proceed for ~ 10 minutes. The final concentrations of the ligand in the solutions used in the Ellman's test were varied between 3.57 μM and 21.4 μM , while the concentration of the Ellman's reagent was maintained at $\sim 45 \mu\text{M}$. The absorbance spectra were collected using a 10 mm optical path quartz cell (FUV from Spectrocell, Orelan, PA). The absorbance at 412 nm was plotted against the ligand concentration, fitted to a linear function and a measure of the percent thiol (% thiol) was extracted from the slope using the equation:

$$\text{Thiol (\%)} = \frac{\text{slope}}{2\varepsilon} \times 100 \quad (1)$$

where ε is the molar extinction/absorption coefficient of TNB^{2-} ion at 412 nm. The factor 2 accounts for the fact that each dithiolane ring should ideally yield two thiol groups.

We should note that because the collected absorption spectra have high signal to noise ratio, the error in estimating the absorbance value at each concentration is low ($\sim 2\text{--}3\%$). Additionally, the line fit to the Beer-Lambert plots as shown in Fig. 1 and additional plots in the ESI,† yield very small uncertainties ($R^2 > 0.99$). Thus, values for the % thiol are reported with overall error that does not exceed 5%.

Conflicts of interest

The authors declare no conflicts of interests.

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