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Synthesis of new macromolecular, functionalized carboxylic-acid–PEG–DHLA surface ligands

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ABSTRACT

An efficient method for the synthesis of new macromolecular surface ligands for quantum dots functionalization has been developed. The new ligands contain a dihydrolipoic acid unit which is connected to either a mono- or a diacid terminal function by a PEG chain.

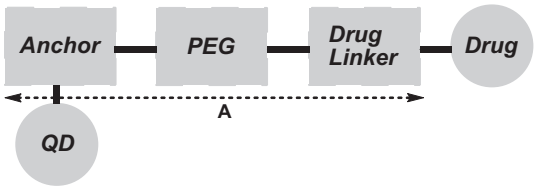
Semiconductor and metallic nanoparticle core shell architectures are of considerable current interest. Nanotechnologies have recently afforded new tools for biomedical applications such as medical diagnostics, site-specific delivery of drugs, and imaging.[1] Mainly peptides, proteins, oligonucleotides, and DNA have been linked to nanoparticles such as CdSe/ZnS quantum dots (QDs)[2] or metallic nanoparticles such as gold or silver.[3]

Several strategies have been described previously to functionalize the surface of water-soluble QDs. Small size, biocompatible, and water-soluble QDs have been developed based on anchors such as mercaptoacetic acid (MAA),[4] dihydrolipoic acid (DHLA),[5] dithiothreitol (DTT),[6] thiolated oligonucleotides,[7] mercaptosilanes,[8] or chiral penicillamine.[9] These fragments are coupled to the QD surface by means of thiol/dithiol mono/bidentate ligands. To these chelators are linked poly-ethylene glycol (PEG) chains to generate hydrophylic and biocompatible nanoparticles.[10] For instance, suitable preparation of biocompatible functionalized PEG–QDs has been recently described by Bawendi[11] and Mattoussi[12] using lipoic acid derivatives as coordinating units.

During our search for original methods to prepare biocompatible QDs for traceable biomolecule site-specific delivery and/or in vitro and in vivo imaging purposes, we needed to develop easy and efficient methods for the synthesis of Anchor–PEG–Drug linker assemblies **A**, as outlined in Scheme 1. Each fragment, Anchor, PEG and Drug linker, plays a specific role as QD chelating moiety, solubilizing motif and drug linker unit, respectively.

In this study, we report an efficient synthetic route to the original diacid derivatives **1** and **2** to mono-acid **3** (Scheme 2) as capping compounds. In these compounds, the anchor function is a chelating bis-thiol unit generated from lipoic acid **6**. The lipoic acid fragment and the PEG chains are linked together by two different functional groups, that is either an amide (**1**) or a urea function (**2** and **3**).

One of the expected advantages of using the dicarboxylic acid derivatives **1** and **2** is the presence of a free carboxylic acid residue which will promote solvation of the nanoparticles. The dithiol ligands have been selected due to their strong chelating properties toward the surface of CdSe–ZnS core–shell QDs.[5] Based on previous works,[1d] we decided to increase the water solubility and the biocompatibility of these QDs—in addition to the deprotonation of the carboxylic acid moieties—by the introduction of a polyethylene glycol segment allowing hydrophilic interactions with polar



Scheme 1. Schematic representation of capping QDs with three-component units **A**.

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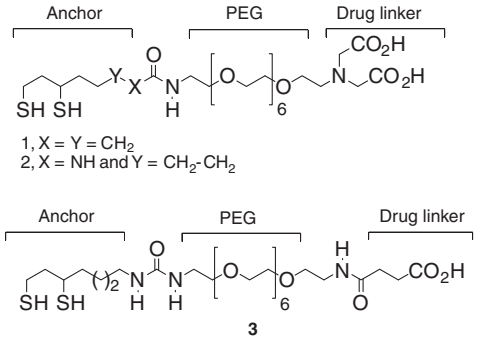
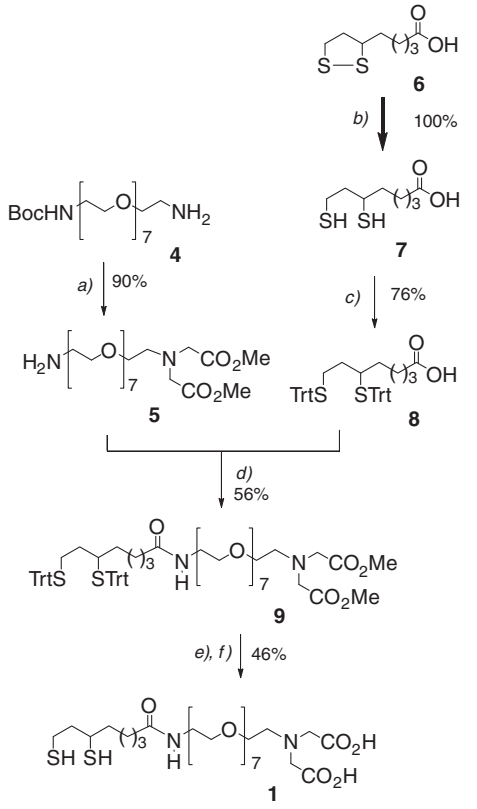


Scheme 2. Chemical structure of **1**, **2**, and **3**.

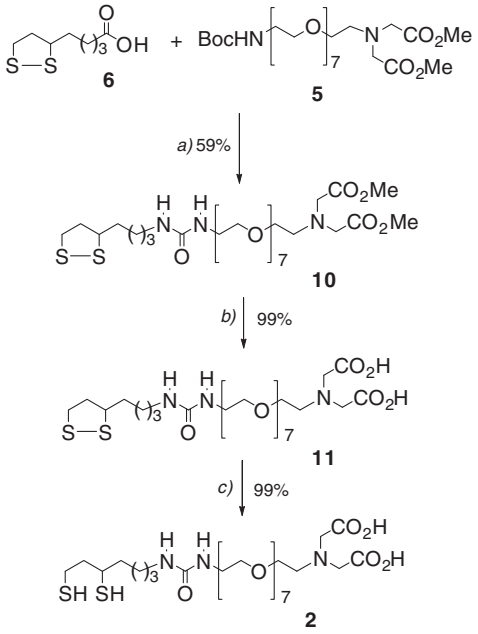
solvents such as water.[11] The optimum length of the PEG chain ($n = 6$) has been evaluated in order to obtain QDs with a maximum water solubility.[12]

As described in Schemes 3–6, ligands **1**, **2**, and **3** have been prepared in three/four steps from commercially available (+/−)-lipoic acid **6**. Compound **1** has been prepared by a simple amidification reaction of **6**, while **2** and **3** have been obtained using a Curtius rearrangement[13] as the key step.

The synthesis of the target compound **1** started from lipoic acid **6**, as shown in Scheme 3. Reduction of **6** with $NaBH_4$ at room temperature afforded **7** in quantitative yield. S-Trilylation of **7** by trityl



Scheme 3. Synthetic approach to the surface ligand **1**. Reagents and conditions: (a) (1) MeCN, $BrCH_2CO_2Me$, K_2CO_3, 12 h, rt; (2) H_2O; (3) HCl·diethyl ether, 4 h, rt. (b) (1) 0.25 M $NaHCO_3$, $NaBH_4$, H_2O, 3 h, rt; (2) 6 M HCl (until pH ~1). (c) (1) TrtCl, CH_2Cl_2, TFA, 3 h, rt; (2) 1 M NaOH; (3) column chromatography (silica gel, eluent: 96:4 CH_2Cl_2–MeOH mixture). (d) (1) MeCN, EDC, DMAP, 12 h, rt; (2) column chromatography (silica gel, eluent: 96:4 CH_2Cl_2–MeOH mixture). (e) 3:1 MeOH–water mixture, LiOH, 3 h, rt. (f) (1) CH_2Cl_2, TFA, Et_3SiH, 48 h, rt; (2) column chromatography (silica gel, eluent: 96:4 ethyl acetate–MeOH mixture then 5:4:1 acetone–water–MeOH mixture).



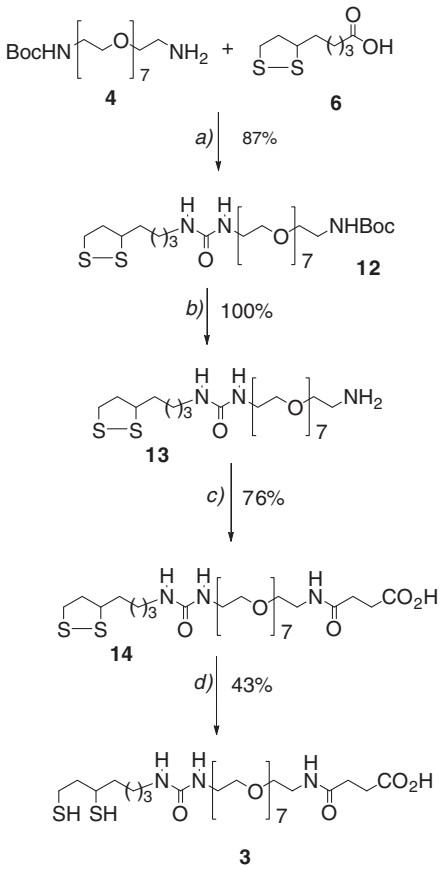
Scheme 4. Synthetic approach to the surface ligand **2**. Reagents and conditions: (a) (1) DMF, Et_3N, DPPA, 80 °C until the end of nitrogen production (~15 min); (2) DMF, **5**, 12 h, 80 °C; (3) $NaHCO_3$, 12 h, rt; (4) column chromatography (silica gel, eluent: 90:10 CH_2Cl_2–MeOH mixture. (b) (1) 2:1 MeOH–water mixture, LiOH, 3 h, rt; (2) preparative HPLC.[19] (c) (1) 1:1 EtOH–water mixture, TCEP·HCl, pH ~4 (0.1 M NaOH), 2 h, rt; (2) preparative HPLC.[19]

chloride (TrtCl) in the presence of trifluoroacetic acid afforded the corresponding di-tritylated lipoic acid **8** in 76% yield. This fragment was combined then with the PEG derived, *N*-Boc-protected amine **5**. The reaction was performed in acetonitrile in the presence of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) as the coupling reagent, with a catalytic amount of 4-(dimethylamino)-pyridine (DMPA). The reaction gave **9** in 56% yield. Finally, hydrolysis of **9** with LiOH in a methanol–water mixture followed by a de-tritylation reaction using triethylsilane[14] provided the pure bis-thiol/diacid derivative **1** in 46% overall yield.[16a]

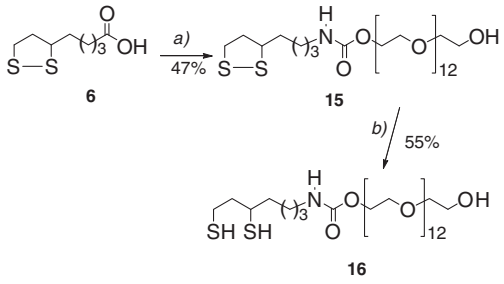
The key step for the preparation of **2** was the Curtius rearrangement of the azide obtained from lipoic acid **6** and diphenylphosphoryl azide (DPPA). The rearrangement was performed in the presence of *N*-Boc-amine **5** and triethylamine at 80 °C (step *a* in Scheme 4). The desired urea **10** was obtained in 59% overall yield.[14] Then, the treatment of **10** with, successively, LiOH and tris-(2-carboxyethyl)phosphine hydrochloride (TCEP·HCl[15]) as the reductant, in a 1:1 ethanol–water mixture at room temperature, led to pure **2** in 39% overall yield.[16b]

Our established methodology based on the Curtius rearrangement was used then to prepare monoacid **3** (Scheme 5). The reaction of **6** with amine **4** in the presence of DPPA afforded the desired urea **12** in 87% yield. The *N*-Boc deprotection of **12** was performed with hydrochloric acid, giving **13** quantitatively. The N-acylation reaction was performed with succinic anhydride leading to **14** in 76% yield. Finally, by combining **14** with TCEP·HCl at pH ~4 (0.1 M NaOH), compound **3** was obtained in 43% yield.[16c]

Our strategy to prepare the desired soluble QDs is to cap the ZnS core–shell of QDs[17] with a mixture of **1** (bearing the potentially active molecule) and the bis-thiol/alcohol **16** as the 'inert' ligand, in a 1:9 ratio.[11a] According to Scheme 6, ligand **16**[18] was prepared in two steps from **6** in 24% overall yield, via a Curtius rearrangement using DPPA in the presence of dodecaethyleneglycol and trietylamine at 80 °C giving the intermediate carbamate **15**. To our knowledge, only a few publications describe the preparation of



Scheme 5. Synthetic approach to the surface ligand **3**. Reagents and conditions: (a) (1) DMF, Et_3N, DPPA, 80 °C until the end of nitrogen production (~15 min); (2) DMF, **4**, 12 h, 80 °C; (3) $NaHCO_3$, 12 h, rt; (4) column chromatography (silica gel, eluent: 90:10 CH_2Cl_2–MeOH mixture). (b) MeOH, HCl·diethyl ether, 4 h, rt. (c) (1) pyridine, succinic anhydride, 48 h, rt; (2) column chromatography (silica gel, eluent: 90:10 CH_2Cl_2–MeOH mixture). (c) (1) 1:1 EtOH–water mixture, TCEP·HCl, pH ~4 (0.1 M NaOH), 2 h, rt; (2) preparative HPLC.[19]



Scheme 6. Synthetic route to the ligand **16**. Reagents and conditions: (a) (1) dodecaethyleneglycol, DPPA, Et_3N, 12 h, 80 °C; (2) column chromatography (silica gel, eluent: 80:20 CH_2Cl_2–MeOH mixture). (b) (1) 4:1 MeOH–water mixture, $NaBH_4$, 2.5 h, rt; (2) column chromatography (silica gel, eluent: 90:10 CH_2Cl_2–MeOH mixture).

carbamates from **6**.[13b] Reduction of the 1,2-dithiolane ring of **15** by $NaBH_4$ afforded the desired compound **16**.

In conclusion, we have prepared the macromolecular, functionalized carboxylic-acid–PEG–DHLA ligands **1**, **2**, and **3** as capping molecules. These compounds were easily synthesized from the commercially available (+/−)-lipoic acid **6** through an amidification reaction or a Curtius rearrangement. Further studies on the use of compounds **1**–**3** as capping ligands are in progress.

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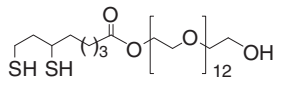
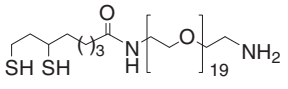
12. Two other ligands **17** and **18** have been prepared. They showed good water-solubility but they have obtained in poor yields due to purification difficulties.





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16. (a) Compound **1**: pale yellow oil; ^{1}H NMR (D_2O, 400 MHz): δ 1.17–1.88 (m, 8H); 2.15 (t, J = 7.2 Hz, 2H); 2.44–2.64 (m, 2H); 2.88 (m, 1H); 3.27 (t, J = 5.3 Hz, 2H); 3.40–3.45 (m, 2H); 3.51 (t, J = 5.3 Hz, 2H); 3.53 (m, 24H); 3.74–3.79 (M, 2H); 3.83 (s, 4H); IR (CH_2Cl_2): 3441, 2911, 1734, 1668, 1518, 1349, 1105 and 951 cm^{-1}; MS: $(M+H)^+$: m/z = 675; $(M-H)^-$: m/z = 673.
(b) Compound **2**: colorless oil; colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.37–2.00 (m, 8H); 2.61–2.79 (m, 2H); 2.92 (m, 1H); 3.18 (t, J = 6.0 Hz, 2H); 3.37 (t, J = 5.3 Hz, 2H); 3.57–3.73 (m, 28H); 3.92 (m, 2H); 4.33 (br s, 4H); IR (CH_2Cl_2): 3369, 2913, 1740, 1669, 1584, 1351, 1194, 1142 and 951 cm^{-1} ; MS: $(M+H)^+$: m/z = 690; $(M-H)^-$: m/z = 688.
(c) Compound **3**: colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.39–1.98 (m, 8H); 2.52–2.60 (m, 2H); 2.63–2.81 (m, 4H); 2.92 (m, 1H); 3.18 (t, J = 6.4 Hz, 2H); 3.38 (t, J = 5.3 Hz, 2H); 3.42–3.49 (m, 2H); 3.53–3.69 (m, 28H), 7.18 (br m, 1H); IR (CH_2Cl_2): 3437, 3365, 2913, 1731, 1671, 1558, 1349, 1104 and 951 cm^{-1}; MS: $(M+H)^+$: m/z = 674; $(M-H)^-$: m/z = 672.
17. CdSe/ZnS (core/shell) quantum dots (powder, hydrophobic, emission at 530 or 630 nm, Ref: PL-QD-O-530 and PL-QD-O-630) provided by PlasmaChem GmbH, Berlin, Germany info@plasmachem.com.
18. Compound **16**: colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.40–1.97 (m, 8H); 2.08 (br m, 3H); 2.62–2.80 (m, 2H); 2.91 (m, 1H); 3.18 (m, 2H); 3.57–3.77 (m, 46H), 4.22 (m, 2H); 4.88 (br m, 1H); IR (CH_2Cl_2): 3684, 3599, 3445, 2891, 1721, 1515, 1349, 1103 and 954 cm^{-1} ; MS: $(M+H)^+$: m/z = 752; $(M-H)^-$: m/z = 750.
19. Preparative HPLC: Macherey-Nagel 250 $\times$ 40 mm C18 Nucleodur 10 μ. Eluent: MeCN 0.07%TFA/H_2O 0.07%TFA. 10% MeCN during 3 min, gradient to 95% MeCN in 37 min, then 95% MeCN during 8 min. Fractions collection following UV absorption at 254 nm.