Diethylene glycol ether-linked 3,4,5-trihydroxybenzamides as triply branched dendritic anchors to CdSe/ZnS core/shell type nanoparticles: potential hydrophilic fluorescent probes†

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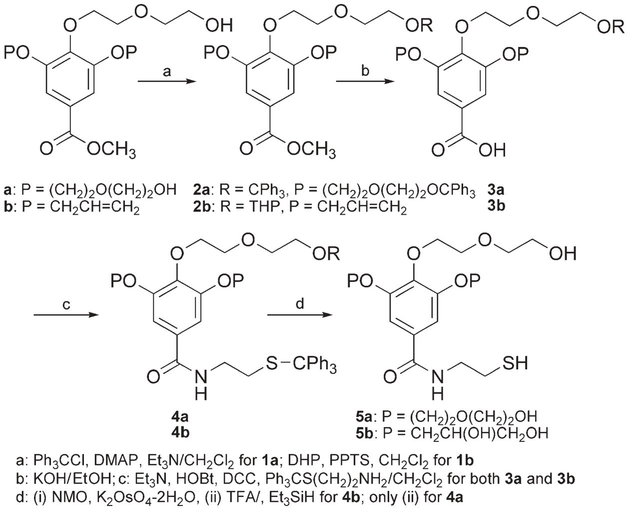
Functionalized *N*-2-mercaptoethyl-gallamides bearing three or five hydroxyl units that are tethered with diethylene glycol ether(s) allow for transferring hydrophobically pyridine-capped CdSe/ZnS core/shell nanoparticles from an organic to an aqueous layer with intact fluorescent profiles.

During the past decade, semiconductor nanocrystals (quantum dots) have attracted scientists' attention in view of their unique size dependent chemical and physical properties.[1–3] These highly fluorescent nanocrystals have shown great potential in light-emitting[4] and nonlinear optical[5] devices, solar cells, and especially as biological labels.[6] For biological applications, robust, water soluble functionalized nanohybrids are required. To achieve this goal, synthetic strategies like surface modification with water soluble ligands,[6b] silanization,[7] and encapsulation with block-copolymer micelles[8] have been used.

Herein we report a new type of hydrophilic nanohybrid where tripodal, dendritic gallamides-**5** bearing a thiol anchoring group with attached ethylene glycol unit(s) toward the periphery are employed to overcoat CdSe/ZnS core/shell nanoparticles. The unique feature of the nanohybrids is that they allow for further functional group transformation of the terminal hydroxyl groups to carboxylic acid, pyruvate, phosphate, carbohydrate, and bio-active building blocks.[9] In addition, they may serve as template systems in extending single-site to tri- or penta-site multivalent probes.[10] Particularly in site-to-site carbohydrate–protein interactions, moderate affinity has been identified. Wrapping such a type of functionalized gallamides around nanocrystals may further enhance polyvalent interactions with biomolecules.[11]

The requisite dendritic gallamides, **5a** and **5b**, were prepared from methyl 3,4,5-tris-[2-(2-hydroxy-ethoxy)-ethoxy]-benzoate (**1a**) and 3,5-bis-allyloxy-4-[2-(2-hydroxy-ethoxy)-ethoxy]-benzoate (**1b**), respectively. They are readily available from methyl gallate by treatment with straight 2-chloroethoxyethanol (3 equiv) or 2-chloroethoxyethanol (1 equiv) followed by allyl bromide (2 equiv) and K_2CO_3 in refluxed 2-butanone.† The three terminal hydroxyl groups in **1a** were protected quantitatively as trityl ethers-**2a** (3.5 mol% *N*,*N*-dimethylaminopyridine (DMAP), Ph_3CCl, and Et_3N in CH_2Cl_2), Scheme 1. On the other hand, the single hydroxyl unit in **1b** was converted to tetrahydropyranyl (THP) ether-**2b** in 95% yield. Both compounds were subjected to basic hydrolysis by using KOH in warm ethanol (60 °C) for 4 h to afford the corresponding acids **3a** and **3b** in 75 and 95% yields, respectively. Direct amidations were carried out (55 and 80% yields, respectively) on both acids **3a,b** by coupling with 2-tritylsulfanyl-ethylamine in the presence of Et_3N, dicyclohexylcarbodiimide (DCC), and 1-hydroxybenzotriazole (HOBT) in DMF. Finally, the trityl groups in **4a** were unmasked by Et_3SiH in trifluoroacetic acid (TFA) at ambient temperature in 30 min to furnish the ligand **5a** in 95% yield. Dihydroxylation of the allyl groups in **4b** in aqueous acetone led to the corresponding tetraol-**4b′** (95% yield). Subsequent deprotection of both THP and trityl groups in the tetraol-**4b′** by Et_3SiH in TFA led to **5b**. Both readily oxidized thiols were isolated in reasonably pure form by filtration of the reaction mixture through a short plug of Celite.



Scheme 1 Synthesis of dendrons **5a** and **5b**.

Direct heating of TOPO-capped CdSe/ZnS core/shell nanoparticles[12] in warm pyridine (70 °C) for 12 h and then concentration *in vacuo* allows one to fully replace TOPO on the particle surface with intact size and photophysical attributes.[13] Similar to TOPO, pyridine is weakly bound[14] to the surface of core/shell type nanocrystals and therefore acts as an excellent passivating agent. The pyridine-capped nanocrystals allow for more efficient access to surface modification. Pyridine was replaced by ligands **5a,b** from the surface of CdSe/ZnS by partitioning in a biphasic system, Scheme 2. The pyridine-capped nanocrystals were placed in toluene and a solution of the ligand **5** in 10% aqueous MeOH with 0.5 equiv of $NaBH_4$ (to avoid oxidation of the thiols) was

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† Electronic supplementary information (ESI) available: experimental procedures, spectral data, ^1H and ^{13}C NMR spectra for **1–5a,b**, and ^1H NMR spectra and HR–TEM images for **6a,b**. See http://www.rsc.org/suppdata/cc/b4/b418806c/

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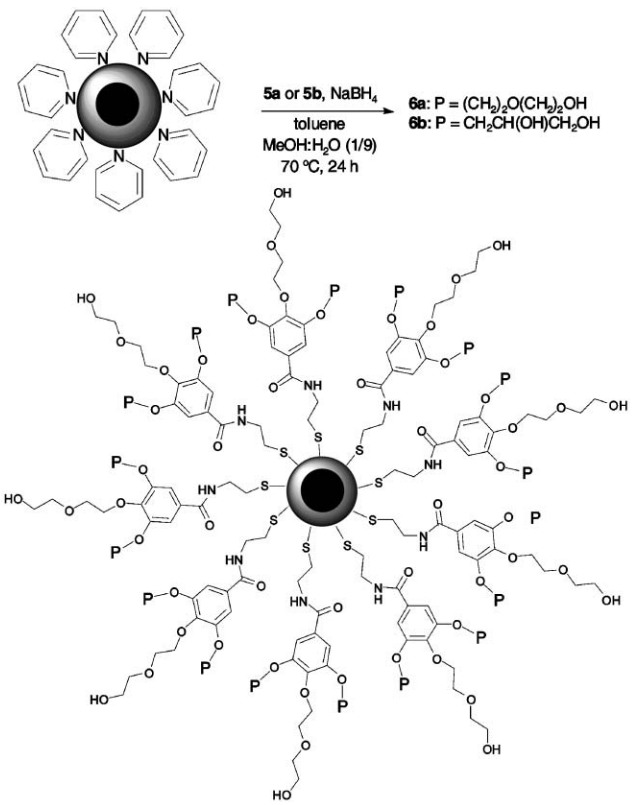

Scheme 2 Anchoring of **5a** and **5b** to CdSe/ZnS nanoparticles.

added. The biphasic system was heated at 70–80 °C for 12–24 h to provide the desired water soluble nanohybrids-**6a** and **6b**. Heating and vigorous stirring of the biphasic system are essential to effect emulsion formation that facilitates the phase transfer with concomitant coordination-to-bonding exchange action to bring the nanocrystals in a nanohybrid form into the aqueous layer. The organic phase, initially red in color, changed to colorless. And, the originally colorless aqueous phase turned to red, indicative of complete nanoparticle transfer.

This phase-transfer extraction process is irreversible in nature. On the CdSe/ZnS nanocrystal surface, the coordinating pyridine was supplanted by the thiolate dendrons (**5a** and **5b**) in a covalent bond fashion. In addition, the exchange process is further facilitated at elevated temperature (*i.e.* 70–80 °C) in view of the volatility[15] of pyridine. Therefore, once the organic soluble, pyridine-capped nanocrystals are phase-transferred to the resultant aqueous soluble, dendron-capped nanocrystals, they cannot be transferred back to the toluene layer.[16] When the temperature of the partitioning process is lowered, the rate of oxidation of thiol is faster than the rate of ligand exchange. This will slow or even shut down the exchange process. To avoid this, NaBH$_4$ was thus added and the biphasic ligand-exchange process was operated at 70–80 °C. After completion of surface modification, free pyridine was removed by extensive extraction of the aqueous layer with toluene. The aqueous layer was then separated and the nanohybrids-**6a,b** were selectively precipitated by adding THF. The pure dendron-capped nanohybrids can thus be collected by centrifugation. The washing process with THF was repeated 3–4 times to remove all freely dissociated residual thiolate ligands.

For the characterization of the nanohybrids-**6a** and **6b**, their absorption and photoluminescence spectra (in deionised H$_2$O) were examined and compared with those (in toluene) of pyridine-capped CdSe/ZnS core/shell nanoparticles, Fig. 1. Both absorption spectra show first exciton absorption peaks at *ca.* 569 and 550 nm with 50% and 25% decrease in intensity, respectively, relative to those of the original pyridine-capped nanocrystals.[17] On the other hand, the emission maxima for **6a** and **6b** appear at *ca.* 587 and

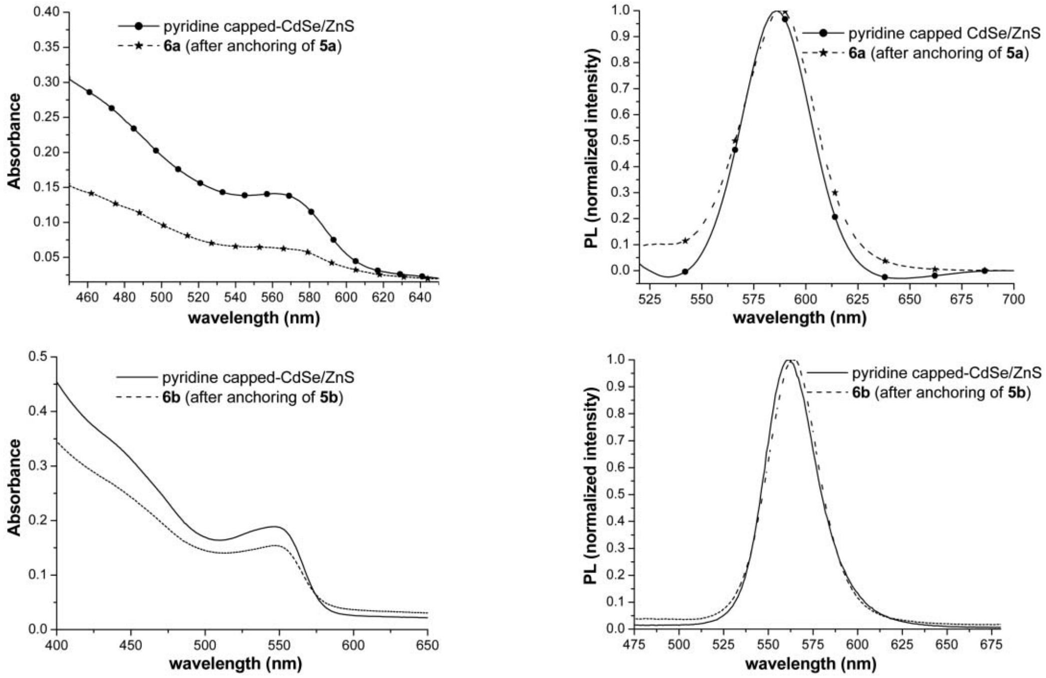

Fig. 1 Absorption and photoluminescence spectra of (top) pyridine-capped CdSe/ZnS (toluene) and **6a** (H$_2$O); (bottom) pyridine-capped CdSe/ZnS (toluene) and **6b** (H$_2$O).

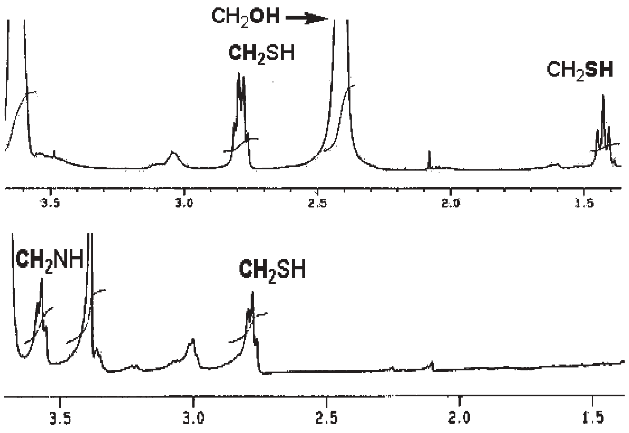

Fig. 2 Partial ^{1}H NMR spectra of **5a** (top, $CDCl_3$) and **6a** (bottom, D_2O).

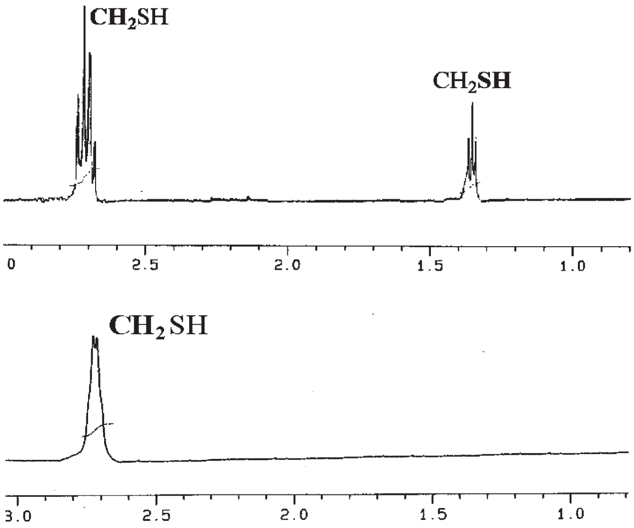

Fig. 3 Partial ^{1}H NMR spectra of **5b** (top, $CDCl_3$) and **6b** (bottom, D_2O).

564 nm, respectively. They are slightly red-shifted by 2 nm as compared to those of pyridine-capped nanocrystals, presumably due to slight increases in the overall size of the hybrids. The evidence supporting the efficient attachment of thiols **5a** and **5b** to the CdSe/ZnS surface comes from characteristic signal changes of the $–CH_2CH_2SH$ fragment in their (in $CDCl_3$) and in the nanohybrids' (in D_2O) ^{1}H NMR spectra, Figs. 2 and 3. The quartet at 2.78 and 2.72 ppm corresponds to $–CH_2CH_2SH$ in **5a** and **5b**, respectively. Peak broadening (usually observed after attachment) and slight chemical shift changes (by 0.03 ppm) of the methylene unit along with disappearance of the thiol proton signals were observed for the corresponding nanohybrids.

The averaged extent of coverage was found to be greater than 82% with minor pyridine attachment as judged by the integration values of the aromatic signals in their ^{1}H NMR spectra in D_2O.† In addition, the nanoparticle size distributions for **6a** and **6b** range from 4.0–4.8 and 3.1–3.8 nm, respectively, as evidenced by high resolution transmission electron microscopy (HR–TEM) measurements. The TEM images clearly indicate the crystallinity of the nanocrystals in these nanohybrids.† The nanohybrids **6a** and **6b** show excellent stability for at least 6 months.

In summary, we have documented the synthesis of a new type of gallamide-based dendritic thiols possessing diethylene glycol ether tether(s) with three or five diverted hydroxyl terminals. We have also demonstrated a simple biphasic system for ligand exchange on the surface of CdSe/ZnS nanocrystals. After covering the surfaces of nanocrystals with these thiols, the resultant nanohybrids become highly water soluble and retain the morphological and photophysical properties of the original nanocrystals. Further extension of this system to the synthesis of other metal- or metal oxide-centered nanohybrids of biological interest is underway.

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