

A Bis(phosphine)-Modified Peptide Ligand for Stable and Luminescent Quantum Dots in Aqueous Media

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Received: 01.05.2013; Accepted after revision: 06.06.2013

Abstract: We describe a new class of ligands for semiconductor nanoparticles (quantum dots = QDs), which bind well and allow for their facile dissolution in aqueous solution. As a proof of principle, we have designed and synthesized a novel bis(phosphine)-modified peptide (BPMP) and shown that it has the ability to solubilize quantum dots in aqueous media. We further showed that the corresponding phosphine oxide derivatives of these new ligands are less good at solubilizing the quantum dots. These new bis(phosphine)-modified peptide ligands are easy to prepare and may well replace thiol-containing binding sequences in functionalized peptides for quantum dot coating, potentially resulting in quantum dots with higher quantum yields.

Key words: bis(phosphine)-modified peptides, nanoparticles, quantum dots, quantum dot stabilization, ligands

Semiconductor nanocrystals (quantum dots = QDs) exhibit unique optical properties that are desirable for applications in biological imaging.¹ Quantum dots are highly photostable and show broad absorption spectra. Their narrow emission bands can be tuned by altering the particle size. Various synthetic routes provide access to monodispersed quantum dots with very good control over their size dispersion. These quantum dots are typically coated with trioctylphosphine oxide (TOPO) and are highly lipophilic.² The challenge that remains for applications in biological systems is the choice of appropriate ligands that can either replace or interact with the trioctylphosphine oxide coating to generate water-soluble quantum dots, that are still monodispersed and show comparable high photochemical quantum yields. Several approaches to solubilize quantum dots in aqueous media have been reported in the literature.³ The traditional approaches use mono- or polyfunctional ligands containing thiol or phosphine groups to replace the trioctylphosphine oxide coating on the surface of quantum dots.⁴ The Weiss group has developed peptides containing a specially designed binding sequence with several cysteine residues.⁵ These polypeptide-based ligands can be easily conjugated, for example, onto dyes, and the resulting quantum dots can be used for biological imaging or for the generation of singlet oxygen in living cells.⁶ The drawback of this approach is the lower quantum yield of the peptide-coated quantum dots which use thiol ligands compared to quantum dots with a trio-

ctylphosphine oxide coating. This effect of the thiol groups has also been described by Huang and Tomalia⁷ during their studies of thiol-modified dendrons as ligands for quantum dots. However, when they used phosphine-modified dendrons as ligands, the resulting quantum dots again showed the desired high quantum yields. Kim and Bawendi⁸ used oligomeric phosphine ligands and also observed good quantum yields. Prompted by these results, we decided to design and synthesize a novel bis(phosphine)-modified peptide (BPMP) and study its ability to solubilize quantum dots in aqueous media. Bis(phosphine)-modified peptides could replace thiol-containing binding sequences in functionalized peptides for quantum dot coating, potentially resulting in quantum dots with higher quantum yields.

Several phosphine-containing amino acids and peptides have been prepared and studied, many by the Gilbertson group.⁹ We initially focused our attention on the bis(phosphine) modified tetrapeptide **1** which would contain what we hoped would be the necessary segments to insure both good binding to the quantum dots and solubility (Scheme 1). Thus we proposed to couple the free acid functionality of the doubly protected tetrapeptide **2** (PG¹NH-Gly-Gly-Glu-Gly-OPG²) with the known bis[2-(diphenylphosphino)ethyl]amine (**3**)¹⁰ to give via a simple amide formation, after deprotection, the bidentate phosphine-modified tetrapeptide. This compound would have the required quantum dot binding domain and a solubilizing hydrophilic domain linked by a small spacer unit ligand. We report herein the synthesis and quantum dot binding and solubilizing properties of the modified peptide **1**.

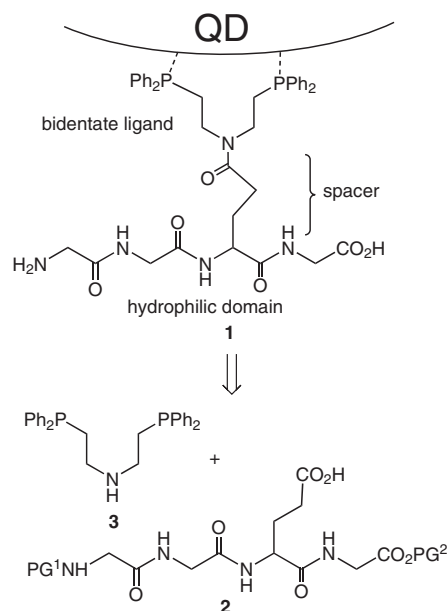
The protected peptide **2** could arise by simple peptide coupling reactions of the following three fragments: the N-protected diglycine **4**, glutamic acid 5-benzyl ester **5**, and the simple glycine ester **6** (Scheme 2). This approach would allow one to easily choose the two protecting groups PG¹ and PG² to facilitate further couplings of the phosphine-modified peptide onto functionalized peptides for future applications. In this initial study we equipped both terminal ends of the peptide **9** with acid-labile protecting groups (Boc for the amine and a *tert*-butyl ester for the carboxylic acid) to allow simultaneous deprotection after coupling to the bis(phosphine)amine **3** without the need of an additional purification step for the phosphine-modified peptide.

SYNTHESIS 2013, 45, 2426–2430

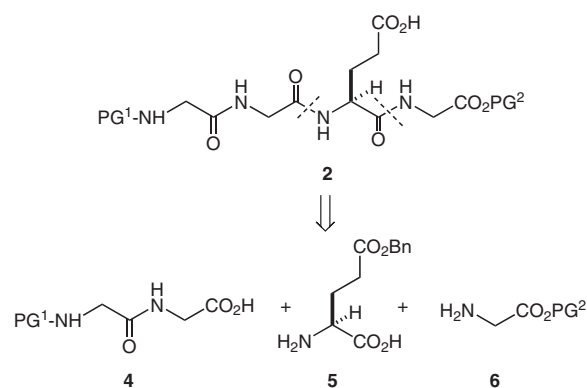
Advanced online publication: 17.07.2013

DOI: 10.1055/s-0033-1339340; Art ID: SS-2013-M0320-OP

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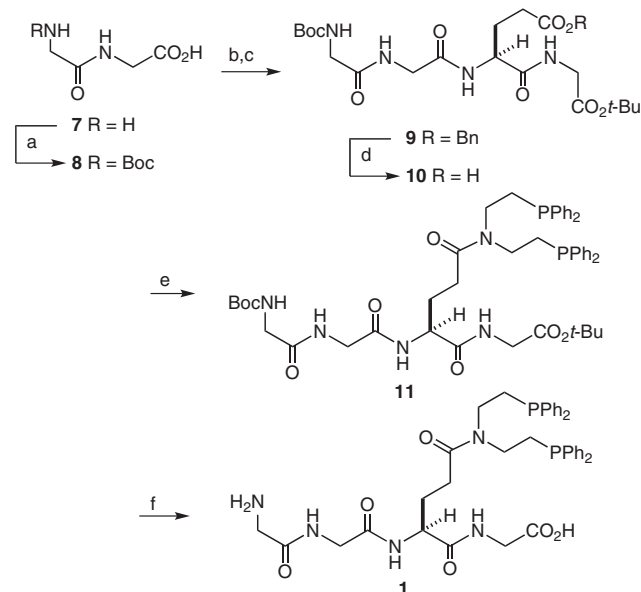
Scheme 1 Design of bis(phosphine)-modified peptide 1



Scheme 2 Retrosynthesis of acid 2

Commercially available diglycine **7** (glycylglycine) was protected as the *N*-Boc carbamate **8**¹¹ (Boc-Gly-Gly) in 88% yield by treatment with di-*tert*-butyl dicarbonate (Boc₂O) and triethylamine (Scheme 3). Reaction of **8** with ethyl chloroformate in the presence of *N,N*-diisopropylethylamine followed by addition of glutamic acid 5-benzyl ester **5** afforded the expected amide, which was used directly without extensive purification. Reaction of this acid with 1*H*-1,2,3-benzotriazol-1-ol (HOBt) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide in *N,N*-dimethylformamide using *N,N*-diisopropylethylamine and then addition of glycine *tert*-butyl ester furnished the desired fully protected tripeptide **9**¹² in 73% yield over the two steps. Hydrogenolysis of the benzyl ester of **9** using palladium on carbon in ethyl acetate gave the acid **10** in 88% yield. Reaction of this acid **10** with 1*H*-1,2,3-benzotriazol-1-ol and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide in dichloromethane followed by addition of bis[2-(diphenylphosphino)ethyl]amine (**3**) followed by workup

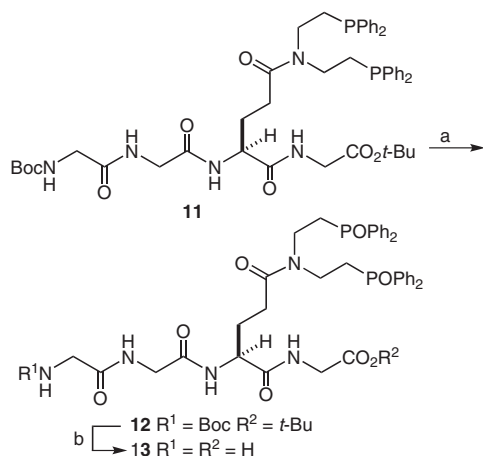
afforded the desired amide **11** in 77% yield. Finally, deprotection of both the *N*-Boc group and the *tert*-butyl ester occurred in quantitative crude yield by treatment of **11** with trifluoroacetic acid in dichloromethane to give the modified tetrapeptide **1**. This bis(phosphine)-modified peptide seems to be similar to triphenylphosphine in its propensity toward oxidation and thus is fairly stable to oxidation if normal precautions are taken.



Scheme 3 Reagents and conditions: (a) Boc₂O, Et₃N, dioxane, 88%; (b) 1. ClCO₂Et, DIPEA, THF, 0 °C; 2. **5**, DIPEA; (c) 1. HOBt, EDC·HCl, DMF; 2. DIPEA, NH₂CH₂CO₂*t*-Bu, 73% (2 steps, from **8**); (d) H₂, Pd/C, EtOAc, 88%; (e) 1. HOBt, EDC·HCl, CH₂Cl₂; 2. **3**, 77%; (f) TFA, CH₂Cl₂, 96%.

To compare the efficiency of binding of these phosphine-modified peptides to the well-known phosphine oxide-modified systems (e.g., TOPO), we decided to prepare the bis(phosphine oxide) of the modified tetrapeptide. The protected bis(phosphine) **11** was treated with molecular oxygen in the presence of low-level ultraviolet light at room temperature for three hours to give the corresponding bis(phosphine oxide) **12** in quantitative yield (Scheme 4). Removal of the *N*-Boc and *tert*-butyl ester protecting groups with trifluoroacetic acid gave the desired bis(phosphine oxide) **13** in quantitative yield.

Tributylphosphine (TBP)/trioctylphosphine oxide coated quantum dots could be transferred from organic solvents to water with the bidentate bis(phosphine)-modified peptide **1** described above by using methods similar to those developed by Chan et al., who solubilized CdSe/ZnS quantum dots with monothiol ligands.¹³ While the bis(phosphine)-modified peptides could solubilize the CdSe/ZnS quantum dots in water and buffer, the phosphine oxide peptide **13** could not under similar conditions (see experimental section), suggesting that the phosphine oxide peptides did not effectively replace the bound trioctylphosphine oxide/tributylphosphine ligands. It is important to note that the bis(phosphine)-modified peptide



Scheme 4 Reagents and conditions: (a) O₂, UV (unfiltered Hg lamp), 23 °C, 3 h, quant.; (b) TFA, CH₂Cl₂, 95%.

coating yielded smaller-sized quantum dots than commercially available quantum dots and phycochelatins-related peptide-coated quantum dots. The bis(phosphine)-modified peptide-coated quantum dots were stable for more than three months. Extraction of the diffusion constant of the bis(phosphine)-modified peptide quantum dots from the analysis of the fluorescence correlation spectroscopy (FCS) data (not shown) indicated a final hydrodynamic diameter of ~8 nm.¹⁴ Meanwhile, little aggregation was detected, making these probes potentially useful for single-molecule tracking studies. Due to the smaller sizes of the bis(phosphine)-modified peptide quantum dots, they may also be useful in certain applications for biological imaging (e.g., in vivo studies and cellular entry). A quantum yield decrease of ~40% occurred due to the exchange, suggesting that the ZnS surfaces of the quantum dots may be incompletely passivated by the bis(phosphine)-modified peptides. It should be pointed out that the original quantum dots before the bis(phosphine) coating had a quantum yield of ~20%. Peptides with additional phosphines for multidentate ligands may increase the quantum yields of the quantum dots and are currently being investigated.

We have prepared the bis(phosphine)-modified tetrapeptide **1** as a model bis(phosphine)-modified peptide and shown that it readily solubilizes quantum dots to give a final particle with a hydrodynamic diameter of ~8 nm. These are smaller-sized quantum dots than commercially available ones. We have also shown that the corresponding bis(phosphine oxide) tetrapeptide **13** did not solubilize quantum dots as well.¹⁵ Further work on the preparation of novel bis(phosphine)-modified peptides and their use with quantum dots is under way.

All reactions were carried out under an argon atmosphere unless otherwise specified. THF and Et₂O were distilled from benzoquinone ketyl radical under an argon atmosphere. CH₂Cl₂, toluene, benzene, pyridine, Et₃N, and DIPEA were distilled from CaH₂ under an argon atmosphere. DMSO was distilled over CaH₂ and stored

over 4 Å MS. *i*-Pr₂NH was distilled from NaOH and MeOH was distilled from Mg turnings under an argon atmosphere. Commercial *t*-BuOOH was dried over 4 Å powdered MS for 30 min prior to use. All other solvents or reagents were purified according to literature procedures. ¹H NMR and ¹³C NMR spectra were obtained on ARX-400, ARX-500, or Avance-500 spectrometers. IR spectra were recorded on Nicolet 501 or Nicolet AVATAR 370 instruments using liquid films (neat) or in CDCl₃ soln on NaCl plates, and only the significant absorption bands are recorded (in cm⁻¹). MS was carried out on a Waters LCT Premier HRMS using ESI-TOF. TLC was carried out using precoated silica gel sheets (Merck 60 F254). Visual detection was performed with UV light, *p*-anisaldehyde stain, KMnO₄ stain or I₂. Flash chromatography was performed using SilicaFlash™ P60 (60 Å, 40–63 mm) silica gel from SiliCycle Inc. with compressed air. The QDs were purchased from Quantum Dot Corp. (now a subsidiary of Life Technologies) and consisted of CdSe/ZnS core/shell and a proprietary amphiphilic polymer coat. The coat protects the QDs from environmental influences and provides stability in H₂O. These QDs were further modified by conjugation to streptavidin (QDot 605 streptavidin conjugate, 1000-1).¹⁶ The buffer is proprietary.

2-[2-(*tert*-Butoxycarbonylamino)acetamido]acetic Acid (**8**)¹¹

Et₃N (3.16 mL, 22.71 mmol) and Boc₂O (3.63 g, 16.65 mmol) were added to a suspension of diglycine (**7**; 2.00 g, 15.14 mmol) in a mixture of dioxane (60 mL) and H₂O (10 mL) at 0 °C. The mixture was stirred at 23 °C for 16 h, diluted with H₂O (250 mL), and acidified to pH 3 by addition of solid KHSO₄. The mixture was extracted with EtOAc (5 × 50 mL), the combined organic phases were dried (Na₂SO₄), and all solvents were removed under reduced pressure; this gave **8** as a white solid, which was used without further purification.

Yield: 3.10 g (13.35 mmol, 88%).

¹H NMR (400 MHz, DMSO): δ = 12.55 (br s, 1 H), 8.03 (t, *J* = 5.5 Hz, 1 H), 6.96 (t, *J* = 5.7 Hz, 1 H), 3.75 (d, *J* = 5.7 Hz, 2 H), 3.56 (d, *J* = 5.8 Hz, 2 H), 1.37 (s, 9 H).

N-*tert*-Butylcarbamoylglycylglycyl Glutamate 5-Benzyl Ester (**9**)

ClCO₂Et (0.96 mL, 10.08 mmol) was added dropwise to a soln of **8** (1.80 g, 7.75 mmol) and DIPEA (1.73 mL, 10.85 mmol) in THF (50 mL) at 0 °C. The mixture was stirred for 1 h while warming up to 10 °C. The mixture was cooled to –15 °C and a precooled (–15 °C) suspension of **5** (3.31 g, 13.95 mmol) in a mixture of THF (50 mL) and DIPEA (2.31 mL, 13.95 mmol) was added via a Teflon cannula. The mixture was stirred for 2 h while warming to 23 °C and then for a further 14 h at 23 °C. H₂O (300 mL) was added and the mixture was acidified to pH 5 by the addition of solid KHSO₄. The mixture was extracted with EtOAc (5 × 50 mL), the combined organic phases were washed with sat. aq NaCl (2 × 50 mL) and dried (Na₂SO₄) and all solvents were removed under reduced pressure. The crude residue was dissolved in DMF (40 mL); then HOBT (1.57 g, 11.60 mmol) and EDC·HCl (2.37 g, 12.38 mmol) were added and the mixture was stirred for 1 h at 23 °C. DIPEA (2.11 mL, 12.76 mmol) and NH₂CH₂CO₂*t*-Bu·HCl (2.08 g, 12.38 mmol) were added and the mixture was stirred for 16 h at 23 °C. H₂O (200 mL) and EtOAc (200 mL) were added and the layers were separated. The aqueous layer was extracted with EtOAc (2 × 100 mL), the combined organic layers were washed with sat. aq NaCl (5 × 60 mL) and dried (Na₂SO₄), and all solvents were removed under reduced pressure. The residue was purified by flash column chromatography (silica gel, EtOAc–acetone, 8:2); this gave **9**¹² as a semi-solid gum.

Yield: 3.21 g (5.68 mmol, 73% over 2 steps).

¹H NMR (400 MHz, CDCl₃): δ = 7.89 (br d, *J* = 7.6 Hz, 1 H), 7.80 (br s, 1 H), 7.71 (br s, 1 H), 7.36–7.30 (m, 5 H), 5.99 (br s, 1 H), 5.10 (s, 2 H), 4.77–4.69 (m, 1 H), 4.08–3.84 (m, 6 H), 2.55–2.45 (m, 2 H), 2.25–2.00 (m, 2 H), 1.45 (s, 9 H), 1.43 (s, 9 H).

^{13}C NMR (100 MHz, CDCl_3): δ = 172.6, 171.1, 170.0, 168.9, 168.3, 155.9, 135.5, 128.1, 127.85, 127.8, 81.4, 79.4, 66.0, 52.0, 43.6, 42.7, 41.6, 30.0, 28.0, 27.6, 27.2.

HRMS (ESI-TOF): m/z $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{27}\text{H}_{40}\text{N}_4\text{O}_9$: 587.2687; found: 587.2704.

12-[(2-*tert*-Butoxy-2-oxoethyl)amino]carbonyl-2,2-dimethyl-4,7,10-trioxo-3-oxa-5,8,11-triazapentadecan-15-oic Acid (10)
Pd/C (5%, 250 mg) was added to a soln of **9** (2.50 g, 4.42 mmol) in EtOAc (150 mL) and H_2 was slowly bubbled through the stirred soln at 23 °C for 48 h. THF (60 mL) was added to dissolve the precipitate formed and the mixture was filtered through Celite. After evaporation of all solvents under reduced pressure, Et₂O (200 mL) was added and the product was isolated by filtration to give **10** as a white solid.

Yield: 1.84 g (3.87 mmol, 88%).

^1H NMR (400 MHz, DMSO): δ = 12.08 (br s, 1 H), 8.23 (br s, 1 H), 8.01 (br d, J = 7.9 Hz, 1 H), 7.96 (br s, 1 H), 6.99 (br s, 1 H), 4.35–4.24 (m, 1 H), 3.81–3.51 (m, 6 H), 2.30–2.20 (m, 2 H), 1.98–1.88 (m, 1 H), 1.80–1.69 (m, 1 H), 1.40 (s, 9 H), 1.37 (s, 9 H).

^{13}C NMR (100 MHz, DMSO): δ = 174.3, 171.8, 170.1, 169.1, 169.1, 156.2, 81.0, 78.5, 52.0, 43.7, 42.3, 41.8, 30.4, 28.6, 28.1, 27.8.

HRMS (ESI-TOF): m/z $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{20}\text{H}_{34}\text{N}_4\text{O}_9$: 497.2218; found: 497.2236.

***tert*-Butyl 12-(3-{Bis[2-(diphenylphosphino)ethyl]amino}-3-oxopropyl)-2,2-dimethyl-4,7,10,13-tetraoxo-3-oxa-5,8,11,14-tetraazahexadecan-16-oate (11)**
HOBT (0.213 g, 1.58 mmol) and EDC-HCl (0.302 g, 1.58 mmol) were added to a soln of **9** (0.750 g, 1.58 mmol) in CH_2Cl_2 (30 mL) and the mixture was stirred at 23 °C for 30 min. A soln of **3** (0.696 g, 1.58 mmol) in CH_2Cl_2 (5 mL) was added and the mixture was stirred for a further 16 h at 23 °C. Sat. aq NaCl (100 mL), H₂O (100 mL), and EtOAc (200 mL) were added and the aqueous phase was extracted with EtOAc (100 mL). The combined organic layers were washed with sat. aq NaCl (6 × 50 mL) and dried (Na_2SO_4), and all solvents were removed under reduced pressure. The residue was purified by flash column chromatography (silica gel, EtOAc–acetone, 8:2); this gave **11** as a semi-solid gum.

Yield: 1.09 g (1.21 mmol, 77%).

^1H NMR (400 MHz, CDCl_3): δ = 8.19 (br d, J = 6.6 Hz, 1 H), 7.41–7.28 (m, 21 H), 7.04 (br s, 1 H), 7.96 (br s, 1 H), 5.66 (br s, 1 H), 4.39–4.34 (m, 1 H), 3.95–3.76 (m, 6 H), 3.52–3.32 (m, 2 H), 3.27–3.15 (m, 2 H), 2.40–2.06 (m, 6 H), 1.99–1.90 (m, 2 H), 1.42 (s, 9 H), 1.39 (s, 9 H).

^{13}C NMR (100 MHz, CDCl_3): δ = 173.0, 171.3, 170.5, 169.0, 168.6, 155.9, 137.0 (d, J = 11.9 Hz), 136.8 (d, J = 11.9 Hz), 132.6 (d, J = 18.6 Hz), 132.5 (d, J = 18.6 Hz), 129.0 (d, J = 5.1 Hz), 128.8 (d, J = 5.1 Hz), 128.6 (d, J = 6.8 Hz), 128.5 (d, J = 6.8 Hz), 81.7, 80.0, 53.0, 45.4 (d, J = 25.4 Hz), 44.2, 43.6 (d, J = 22.9 Hz), 43.0, 41.8, 29.3, 28.3, 27.9, 27.5 (d, J = 15.3 Hz), 26.8, 26.3 (d, J = 13.6 Hz).

^{31}P NMR (162 MHz, CDCl_3): δ = –20.52, –21.64.

2-[(2-{2-[(2-Aminoacetyl)amino]acetyl}amino)-5-{bis[2-(diphenylphosphino)ethyl]amino}-5-oxopentanoyl]amino]acetic Acid (1)

TFA (5 mL) was added to a soln of **11** (500 mg, 0.56 mmol) in CH_2Cl_2 (20 mL) and the mixture was stirred for 3 h at 23 °C. Toluene (20 mL) was added and the mixture was evaporated under reduced pressure; this gave **1** (partial TFA salt) as a semi-solid gum.

Yield: 460 mg (0.54 mmol, 96%).

^{31}P NMR (162 MHz, CDCl_3): δ = –18.99, –21.69.

HRMS (ESI-TOF): m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{39}\text{H}_{45}\text{N}_5\text{O}_6\text{P}_2$: 742.2918; found: 742.2937.

***tert*-Butyl 12-(3-{Bis[2-(diphenylphosphoryl)ethyl]amino}-3-oxopropyl)-2,2-dimethyl-4,7,10,13-tetraoxo-3-oxa-5,8,11,14-tetraazahexadecan-16-oate (12)**

O_2 was bubbled for 5 h through a soln of **11** (300 mg, 0.334 mmol) in THF (30 mL) while the mixture was irradiated with UV light (Hg lamp). The solvent was removed under reduced pressure; this gave **12**.

Yield: 311 mg (0.334 mmol, quant).

^1H NMR (400 MHz, CDCl_3): δ = 8.05 (br s, 1 H), 7.87 (br d, J = 6.4 Hz, 1 H), 7.69–7.63 (m, 8 H), 7.50–7.32 (m, 13 H), 5.96 (br s, 1 H), 4.39 (ddd, J = 8.1, 7.9, 3.9 Hz, 1 H), 4.24 (dd, J = 6.4, 6.4 Hz, 1 H), 4.08 (dd, J = 17.0, 5.9 Hz, 1 H), 3.86–3.71 (m, 3 H), 3.64 (dd, J = 6.2, 6.2 Hz, 1 H), 3.55–3.35 (m, 4 H), 2.61–2.49 (m, 4 H), 2.32–2.12 (m, 2 H), 2.07–1.02 (m, 2 H), 1.35 (s, 9 H), 1.34 (s, 9 H).

^{31}P NMR (162 MHz, CDCl_3): δ = 30.41, 30.22.

HRMS (ESI-TOF): m/z $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{48}\text{H}_{61}\text{N}_5\text{O}_{10}\text{P}_2$: 952.3785; found: 952.3802.

2-[(2-{2-[(2-Aminoacetyl)amino]acetyl}amino)-5-{bis[2-(diphenylphosphoryl)ethyl]amino}-5-oxopentanoyl]amino]acetic Acid (13)

TFA (4 mL) was added to a soln of **12** (311 mg, 0.334 mmol) in CH_2Cl_2 (15 mL) and the mixture was stirred for 3 h at 23 °C. Toluene (20 mL) was added and the mixture was evaporated under reduced pressure; this gave **13** (partial TFA salt) as a semi-solid gum.

Yield: 282 mg (0.318 mmol, 95%).

^{31}P NMR (162 MHz, CDCl_3): δ = 35.51, 33.86.

HRMS (ESI-TOF): m/z $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{39}\text{H}_{45}\text{N}_5\text{O}_8\text{P}_2$: 796.2636; found: 796.2607.

Solubilization of Quantum Dots

CdSe/ZnS QDs (613 nm-emitting) in TBP/TOPO were precipitated with MeOH and air-dried (~5–10 mg). Phosphine-containing peptide **1** (~200 mg) was dissolved in CHCl_3 (1.5 mL) and this soln was added to the precipitated QDs for 2 h. Then 18.2 M H_2O (4.5 mL) was added to the CHCl_3 soln, and the mixture was stirred at 60 °C for >3 h. KOH (~100 mg) was added to the soln to deprotonate the carboxylic acid of phosphine-containing peptide. The CHCl_3 layer was extracted from the soln and discarded. The H_2O layer containing the phosphine peptide-QDs was centrifuged to separate the QDs from the solid white precipitate, and the QDs were then syringe-filtered (0.2 μm filter). The phosphine peptide-QDs were then run through a NAP column (Amersham Biosciences) for purification. The hydrodynamic radius of the QDs was measured by fluorescence correlation spectroscopy (FCS) as stated in the manuscript. These measurements showed definitively that the phosphine-coated QDs had a smaller hydrodynamic radius (8 nm) than the commercially available product QD605 (Invitrogen QDs), the hydrodynamic radius of which is 30.3 ± 3.2 nm. Since FCS is a sensitive tool for measuring hydrodynamic radius, TEM and DLS after the phosphine coating were not performed. FCS does not rely on the fluorescence spectrum to give values for size; rather it relies on the residence/diffusion of the fluorescent species within a defined excitation volume. Details of FCS and the hydrodynamic radius of peptide-coated QDs and QDC quantum dots have been described elsewhere.¹⁴

Acknowledgment

We thank the National Science Foundation (CHE 0614591) and a Jonsson Comprehensive Cancer Center multidisciplinary grant for generous support of this work.

Supporting Information for this article is available online at <http://www.thieme-connect.com/ejournals/toc/synthesis>.

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