Supplementary materials for Analytical Chemistry

Fabrication of Versatile Cyclodextrin-functionalized Upconversion Luminescence Nanoplatform for Biomedical Imaging

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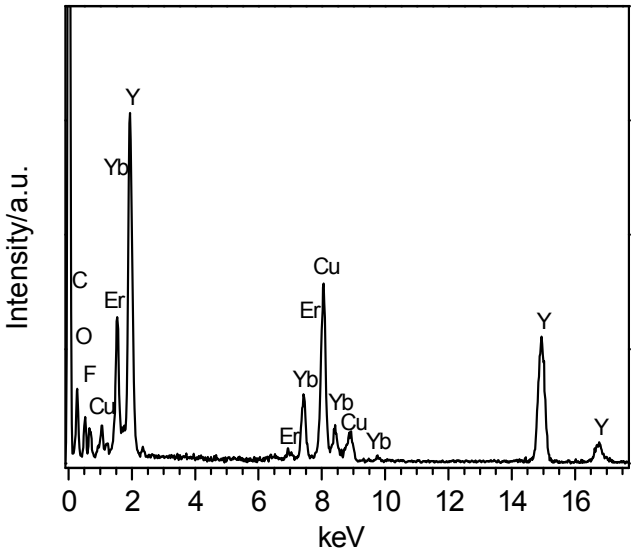


Figure S1. EDXA spectrum of the as prepared $NaYF_4$: Yb,Er samples. The presence of Cu elemental results from copper grid during TEM measurements.

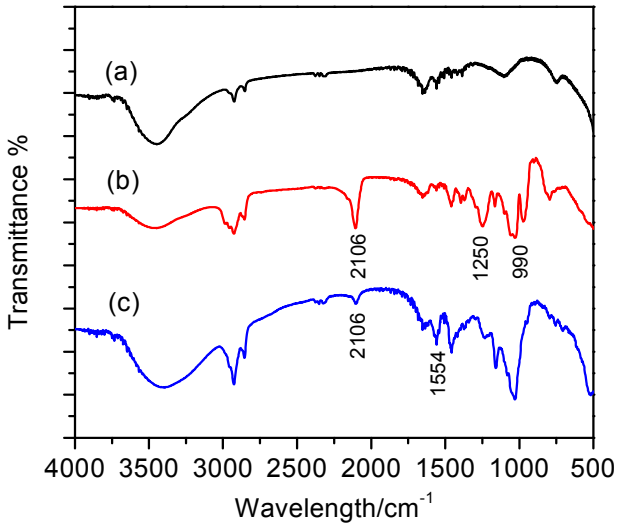


Figure S2. The FTIR spectra of (a) OA/UCNPs, (b) azide-functionalized UCNPs and (c) *β*PCD/UCNPs.

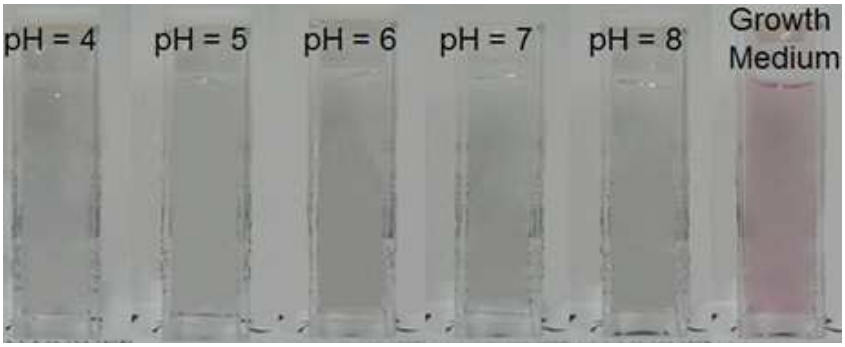


Figure S3. The photographs of *β*PCD/UCNPs in 20 mM phosphate-buffered saline at different pH from 4.0-8.0 and serum supplemented cell growth medium.

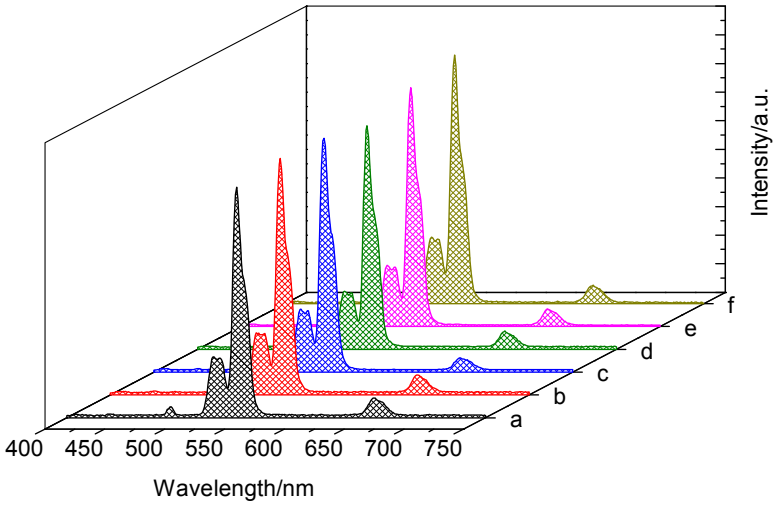


Figure S4. Upconversion luminescence spectra of *β*PCD/UCNPs upon 980 nm excitation in 20 mM phosphate-buffered saline at different pH from 4.0-8.0 (a-e) and serum supplemented cell growth medium (f). The dispersions were 48 h old by the time the spectra were measured.

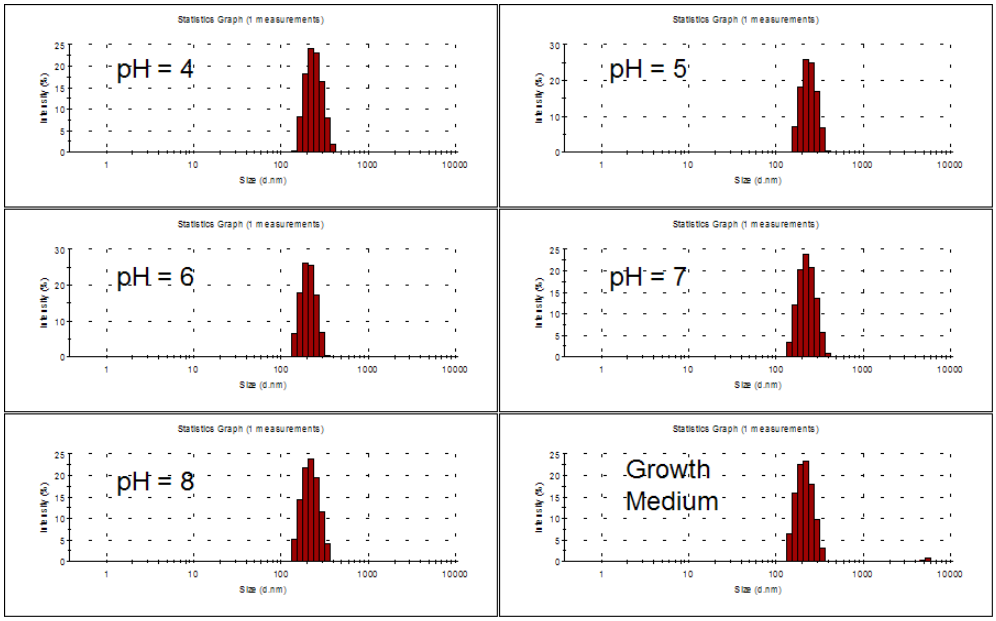


Figure S5. The dynamic light scattering of *β*PCD/UCNPs in 20 mM phosphate-buffered saline at different pH from 4.0-8.0 and serum supplemented cell growth medium.

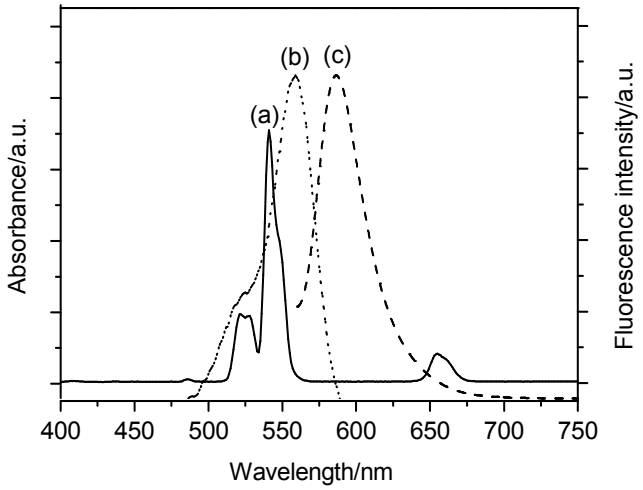


Figure S6. (a) UCL spectrum (λ_{ex} = 980 nm) of *β*PCD/UCNPs in water. (b) the absorption spectrum and (c) fluorescence emission spectrum (λ_{ex} = 550 nm) of Ad-RB in water.

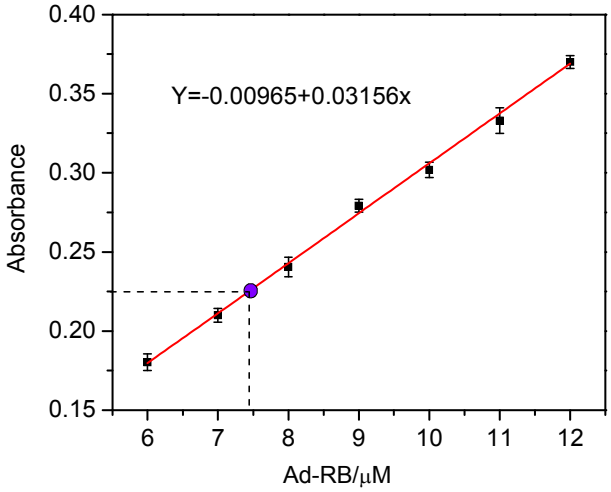


Figure S7. Absorbance at 555 nm of the free Ad-RB in aqueous solution as a function of its concentration. The density of *β*PCDs on the surface of UCNPs is calculated using the detailed titration spectra. According to the obtained titration equation (Y = -0.00965 + 0.03156x) and the absorbance (the violet dot on the line) of Ad-RB/*β*PCD/UCNPs (0.33 mg·mL^{-1}, 600 μL), the *β*PCDs content of *β*PCD/UCNPs is determined as 390 *β*PCDs per *β*PCD/UCNPs.

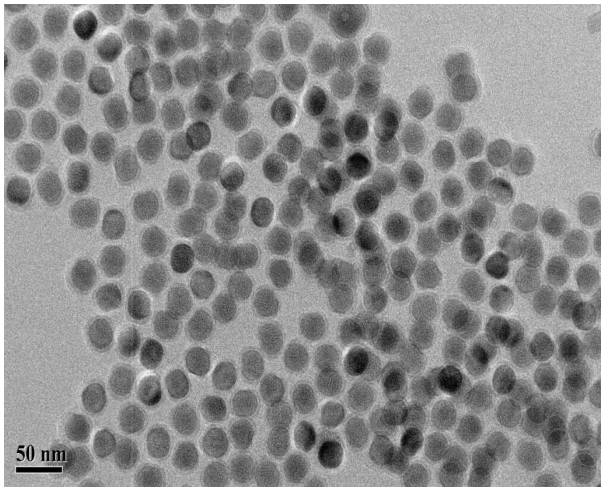


Figure S8. The TEM image of the SiO_2/UCNPs dispersed in water.

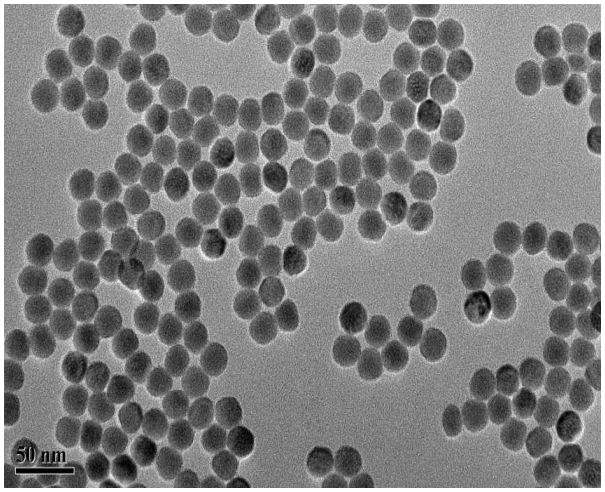


Figure S9. The TEM image of the PAA/UNPS dispersed in water.

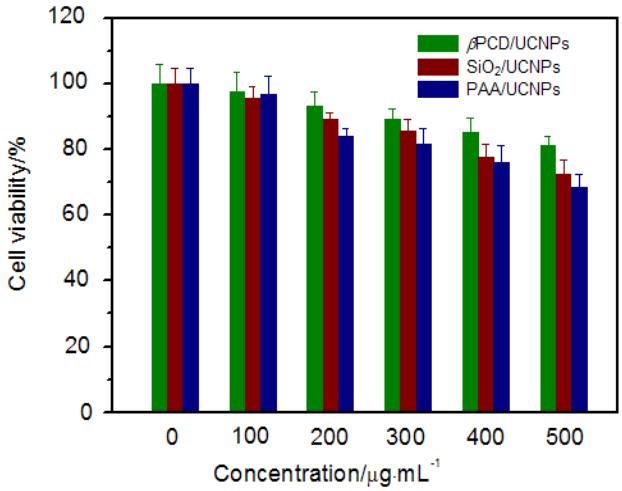


Figure S10. Cell viability values (%) estimated by MTT proliferation. HeLa cells were incubated with 0−0.5 mg·mL^{-1} βPCD/UCNPs (green), SiO_2/UCNPs (red) and PAA/UCNPs (blue) at 37 ^{o}C for 48 h. Cells without added nanoparticles were taken as the control experiment, and the viability was set as 100%. The final reported data were expressed as a percentage of the control (mean standard deviation). Three independent experiments containing duplicates were performed.

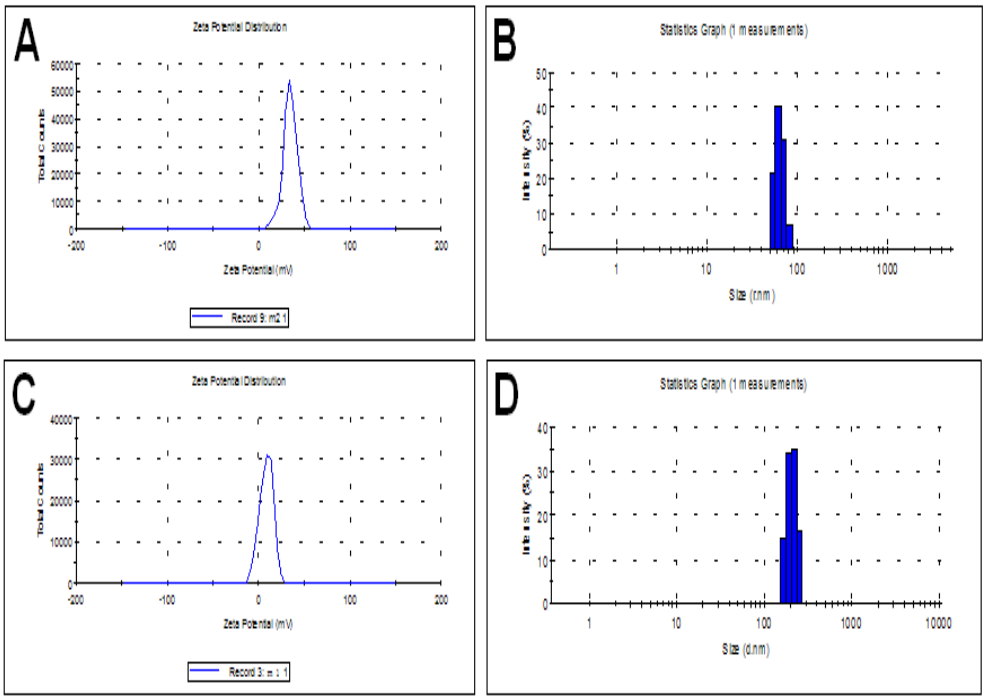
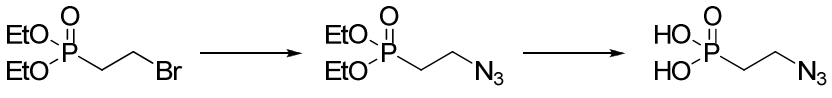


Figure S11. The zeta potential of (A) *β*PCD/UCNPs and (C) Ad-RGD/*β*PCD/UCNPs in water. The dynamic light scattering of (B) *β*PCD/UCNPs and (D) Ad-RGD/*β*PCD/UCNPs in water.

Synthesis of 2-azidoethylphosphonic acid (compound 3):[1]



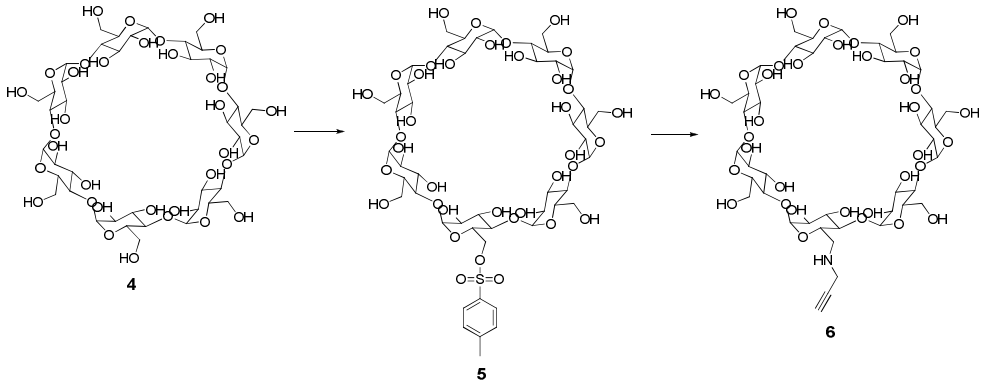
1 **2** **3**

Diethyl (2-bromoethyl) phosphonate (compound **1**) (2.0 g, 8.16 mmol), dry sodium azide (746.6 mg, 12.3 mmol) was dissolved in anhydrous acetone (15 mL). The solution was refluxed under argon for 24 h. The reaction mixture was filtered over a pad of celite and the celite washed several times with dry acetone. The organic liquid was removed under reduced pressure and purification by column

chromatography using silica gel (hexane: EtOAc = 1:1) yielded compound **2** as a clear/light brown oil (1.4 g, 85.0% yield). ^{1}H NMR (400 Hz, $CDCl_3$): δ = 1.33 (t, J = 6.8 Hz, 6H), 2.13 (dt, J = 18.6, 8.0 Hz, 2H), 3.67 (dt, J = 12.4, 7.6 Hz, 2H), 4.09 (m, 4H). ESI-MS m/z 208.4 $[M+H]^+$.

A solution of compound **2** (1.2 g, 5.80 mmol) in anhydrous acetonitrile (25 mL) was cooled to 0 ^oC and then treated with trimethylbromosilane (3.5 g, 23.2 mmol) drop wise over 15 min under an atmosphere of argon and vigorous stirring. The resulting solution was stirred for an additional 24 h at room temperature. The solvent was removed under reduced pressure and then a 5:5 mixture of CH_3OH/H_2O (10 mL) was added. The resulting solution was stirred at room temperature for an additional 24 h. The solvent was removed under reduced pressure then coevaporated with toluene (3 × 10 mL) and then fully dried in a vacuum desicator overnight to yield 2-azidoethylphosphonic acid (compound **3**) as a yellow gummy/oil product (0.86 g, 98.0% yield). ^{1}H NMR (400 Hz, D_2O): δ = 1.87 (dt, J = 18.8, 7.3 Hz, 2H), 3.48 (dt, J =15.5, 7.3 Hz, 2H). ESI-MS m/z 450.1 $[3M-H]^-$.

Synthesis of Mono-6^A-N-propargylamino-6A-deoxy-β-cyclodextrin (compound 6):[2]

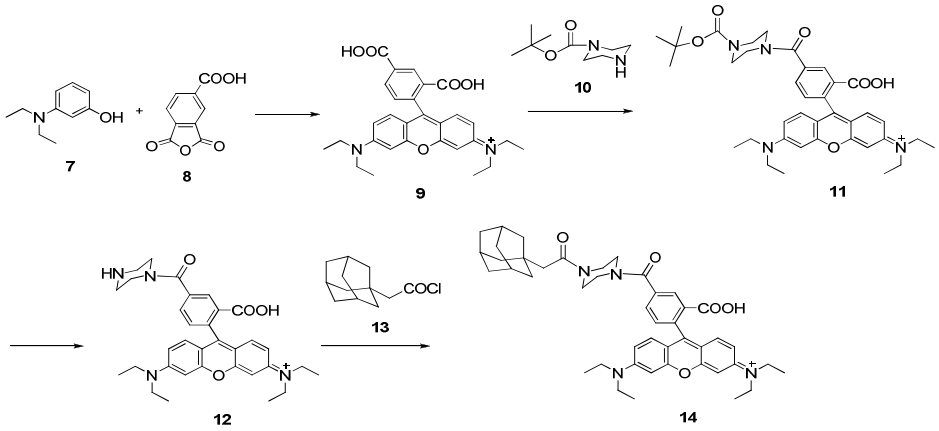


β-cyclodextrin (compound **4**) (15.0 g, 13.2 mmol) was suspended in a NaOH solution (8.0 g of NaOH in water 300 mL) at 0 ^oC. Then p-toluenesulfonyl chloride (TsCl, 6.0 g) was added into the solution with vigorous stirring. After stirring for 3 h, another portion of TsCl (9.0 g) was added. After stirring for 5 h, the unreacted TsCl was then filtered over a pad of celite. The filtrate was cooled to 0 ^oC and 100 mL of 10% HCl was added, affording a white solid product as a precipitate that was

collected after cooling at 4 ^{o}C overnight. The white solid was recrystallized in water to yield compound **5** (6.0 g, 34.2% yield). ^{1}H NMR (400 Hz, DMSO-d_6): δ = 2.69 (3H), 3.09-3.91 (42H), 4.75-4.86 (6H), 5.60-5.80 (14H), 7.44 (2H), 7.75 (2H). ESI-MS m/z 1311.7 $[M+Na]^{+}$.

A total of compound **5** (4.2 g, 3.26 mmol) dissolved in propargylamine (8 mL). Under a argon atmosphere, The mixture was stirring at 45 ^{o}C for 24 h. Then the mixture was cooled to room temperature and precipitated into 200 mL of acetonitrile. The precipitate was obtained and purified by dissolving in 50 mL of a water–methanol mixture and reprecipitating in 50 mL acetonitrile several times to remove the unreacted propargyl amine. After fully dried in a vacuum desicator overnight, Mono-6^{A}-N-propargylamino-6A-deoxy-β-cyclodextrin (compound **6**) were obtained as a white solid product (2.86 g, 74.3% yield). ^{1}H NMR (400 Hz, DMSO-d_6): δ = 2.07 (2H), 3.35 (1H), 3.25-3.53 (14H), 3.62-3.88 (28H), 4.89 (7H). ESI-MS m/z 1172.6 $[M]^{+}$.

Synthesis of rhodamine B conjugated adamantane (compound 14):[3]



*Synthesis of (compound **9**):* Powdered N,N-Diethyl-3-aminophenol (compound **7**) (1.87 g, 11.30 mmol) and 1,2,4-benzenetricarboxylic anhydride (compound **8**) (2.46 g, 12.8 mmol) were added to a 100 mL three-neck round-bottom flask. The mixture was stirred magnetically and heated to 200 ^{o}C under nitrogen atmosphere for 6 h. Then another portion of compound **7** (1.40 g, 8.50 mmol) and 85% H_3PO_4 (7.0 mL) were added, and the resulting mixture was heated for an additional 6 h at 200 ^{o}C

under nitrogen atmosphere. After the dark purple reaction mixture was cooled to ambient temperature, methanol (120 mL) and water (80 mL) were added. Then the aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic solutions were dried, and the solvent was removed in vacuo to yield a dark purple solid. Purification by column chromatography using silica gel (CH_2Cl_2 : CH_3OH = 3 : 1) yielded pure compound **9**: ^{1}H NMR (400 Hz, d_4-CD_3OD): δ = 1.30-1.64 (t, J = 6.8 Hz, 12H), 3.49-3.54 (q, 8H), 6.81 (d, J = 2.0 Hz, 2H), 6.85-6.88 (dd, J = 9.2, 2.0 Hz, 2H), 7.01-7.03 (d, J = 9.2 Hz, 2H), 7.28-7.30 (d, J = 8.0 Hz, 1H), 8.16-8.18 (d, J = 8.0, 1.2 Hz, 1H), 8.70 (d, J = 1.2 Hz,1H); ESI-MS m/z 487.3 $[M]^+$.

*Synthesis of (compound **11**):* A 100 mL round bottom flask was charged with the mixture of compound **9** (1.7 mmol, 889.0 mg), dicyclohexylcarbodiimide (1.7 mmol, 361.3 mg), 4-dimethylaminopyridine (0.16 mmol, 24.2 mg) and acetone (20 mL). After the suspended compounds were completely dissolved, mono-t-Boc-piperazine (compound **10**) (1.7 mmol, 559.5 mg) was slowly introduced to the solution with vigorous stirring, and the solution was further stirred at room temperature for 2 h. The solvent was concentrated under reduced pressure to yield a dark purple solid. Purification by column chromatography using silica gel (CH_2Cl_2 : CH_3OH = 4 : 1) yielded pure compound **11**: ^{1}H NMR (400 Hz, $CDCl_3$): δ = 1.27-1.30 (t, J = 7.2 Hz, 12H), 1.49 (s, 9H), 3.52-3.80 (16 H), 6.93 (d, J = 2.4 Hz, 2H), 6.98-7.01 (dd, J = 7.2, 2.4 Hz, 2H), 7.25-7.27 (d, J = 7.2 Hz, 2H), 7.36-7.38 (d, J = 7.6 Hz, 1H), 7.68-7.70 (dd, J = 7.2, 1.6 Hz, 1H), 8.14 (d, J = 1.6 Hz, 1H). ESI-MS m/z 655.3 $[M+H]^+$.

*Synthesis of (compound **12**):* Concentrated HCl (0.5 mL) was added dropwise to a solution of compound **11** (340.0 mg, 0.52 mmol) in acetone (10 mL) with vigorous stirring at room temperature. After stirring for 2 h, the solvent was removed under reduced pressure. The resulting residue was redissolved in acetone (10 mL), and triethylamine (1.5 mL) was added. After stirring for an additional 2 h, the solvent was removed under reduced pressure. Purification by column chromatography using silica gel (CH_2Cl_2 : CH_3OH = 2 : 1) yielded compound **12** as a dark purple powder (210.3 mg, 73.0% yield). ^{1}H NMR (400 Hz, $CDCl_3$): δ = 1.14 (t, J = 6.5 Hz, 12H), 3.24-3.96

(16H), 6.95 (d, J = 2.4 Hz, 2H), 6.99-7.02 (dd, J = 7.2, 2.4 Hz, 2H), 7.23-7.27 (d, J = 7.2 Hz, 2H), 7.38-7.40 (d, J = 7.6 Hz, 1H), 7.69-7.71 (dd, J = 7.2, 1.6 Hz, 1H), 8.36 (d, J = 1.6 Hz 1H). ESI-MS m/z 556.3 $[M+H]^+$.

*Synthesis of (compound **13**):* Oxalyl chloride (135 mg, 1.07 mmol) was added into a stirred solution of adamantane-1-acetic acid (200 mg, 1.03 mmol) in dry CH_2Cl_2 (10 mL) under a nitrogen atmosphere at room temperature. When the evolution of gas had ceased, dimethylformamide (0.5 mL) was added dropwise in order to make the mixture homogeneous. After stirring for an additional 2 h, the solvent was removed under reduced pressure. Then CH_2Cl_2 (10 mL) was added and the resulting mixture was concentrated again in order to remove all residual oxalyl chloride. In this way 218.0 mg of crude intermediate compound **13** was isolated.

*Synthesis of (compound **14**):* A solution of the above-described compound **13** (61.8 mg, 0.29 mmol) and compound **12** (160.1 mg, 0.29 mmol) were dissolved in CH_2Cl_2 (8 mL), and then triethylamine (0.2 mL) was added. After stirring at room temperature overnight, the solvent was removed under reduced pressure. Purification by column chromatography using silica gel (CH_2Cl_2 : CH_3OH = 10 : 1) yielded rhodamine B conjugated adamantane (compound **14**) as a dark purple powder (180.3 mg, 85.0% yield). ^1H NMR (400 Hz, $CDCl_3$): δ = 1.17 (t, J = 6.5 Hz, 12H), 1.42 (15H), 2.24 (s, 2H), 3.26-3.92 (16H), 6.85 (d, J = 2.4 Hz, 2H), 7.11 (dd, J = 7.4, 2.4 Hz, 2H), 7.25 (d, J = 7.4 Hz, 2H), 7.38 (d, J = 7.6 Hz, 1H), 7.71 (dd, J = 7.4, 1.8 Hz, 1H), 8.54 (d, J = 1.5 Hz 1H). ESI-MS m/z 732.0 $[M]^+$.

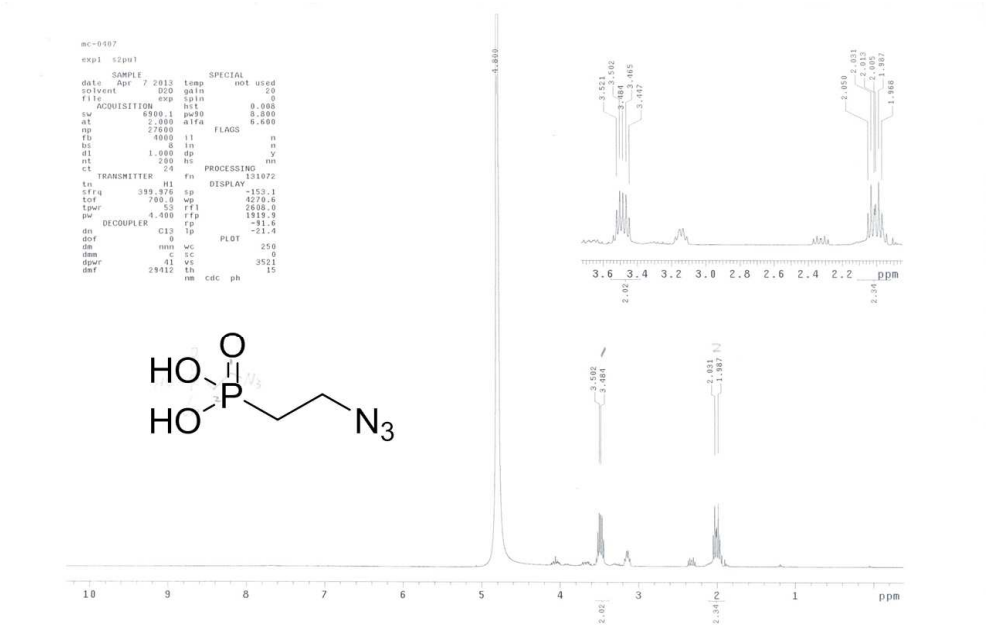
Reference

[1] S. H. Yang, D. J. Lee, M. A. Brimble, *Org. Lett.* **2011**, *13*, 5604.

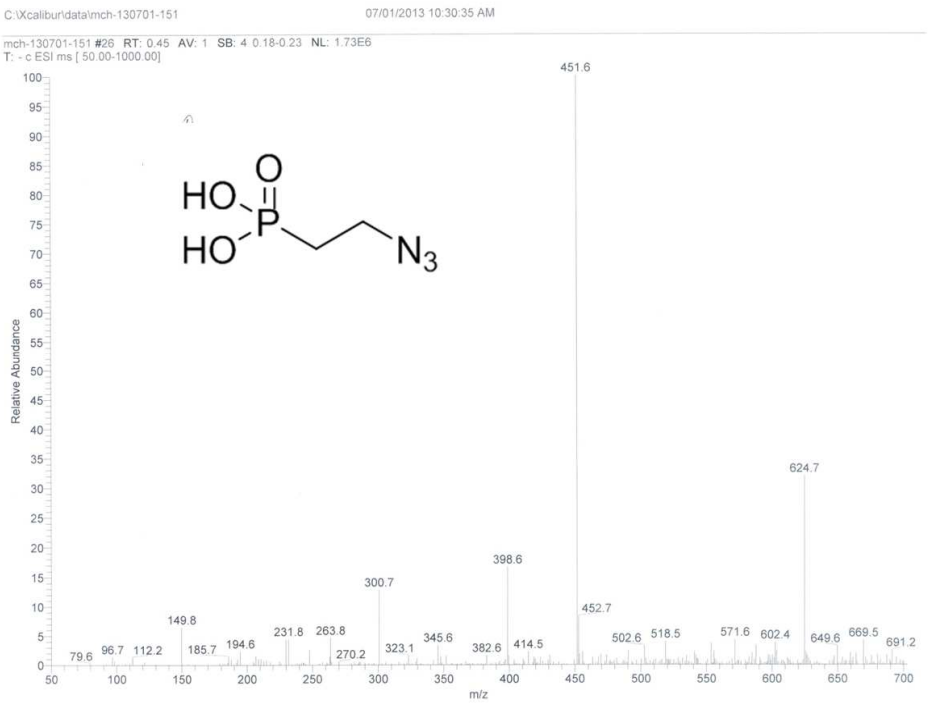
[2] Z. Guo, Y. Jin, T. Liang, Y. Liu, Q. Xu, X. Liang, A. Lei, *J. Chromatogr. A* **2009**, *1216*, 257.

[3] L. Yuan, W. Lin, Y. Xie, B. Chen, S. Zhu, *J. Am. Chem. Soc.* **2012**, *134*, 1305.

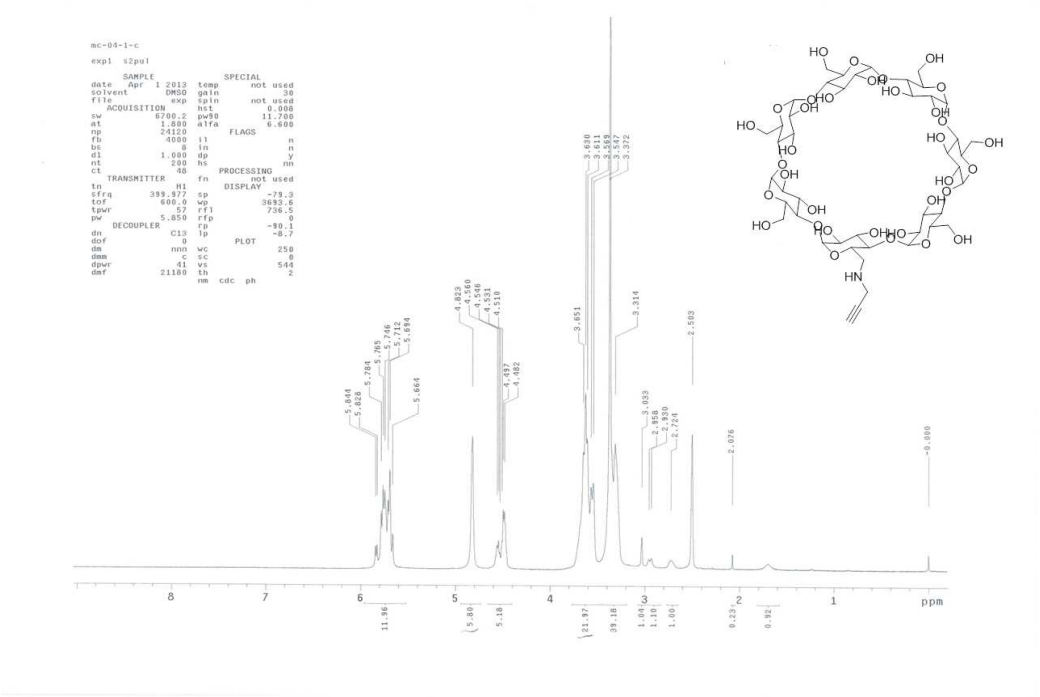
^{1}H NMR of 2-azidoethylphosphonic acid *(compound **3**)* in D_2O



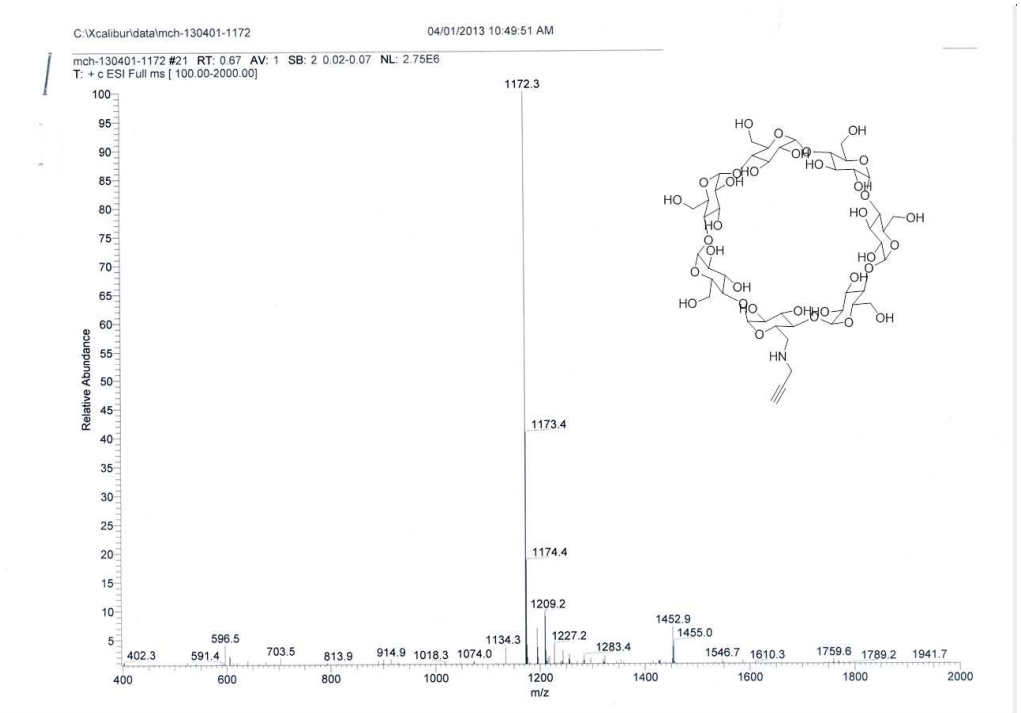
ESI-MS of 2-azidoethylphosphonic acid *(compound **3**)*



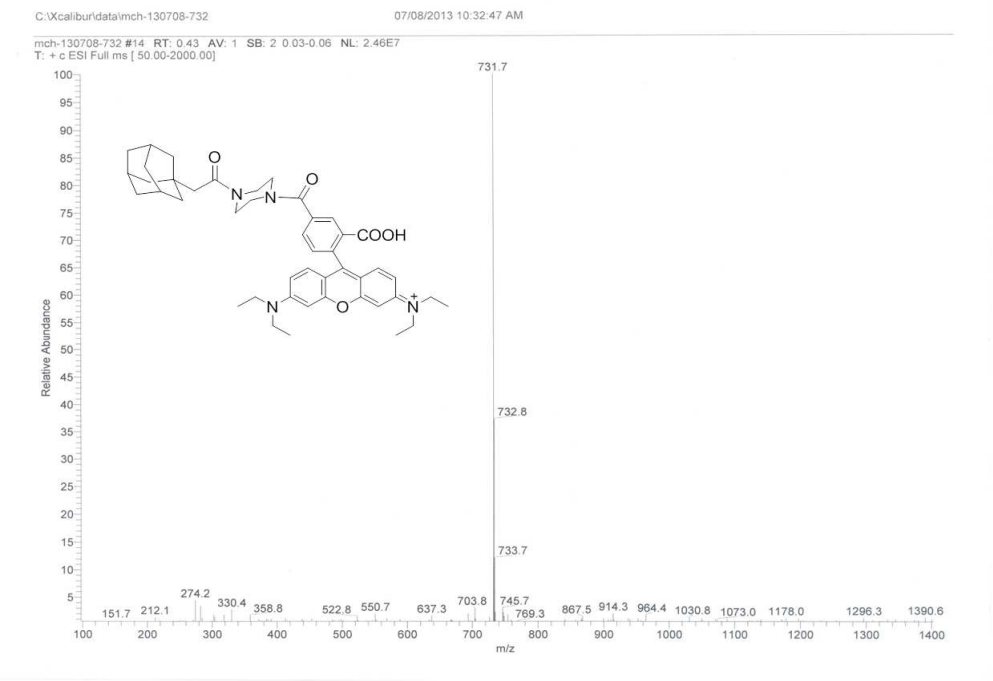
^{1}H NMR of Mono-6^{A}-N-propargylamino-6A-deoxy-β-cyclodextrin *(compound **6**)* in DMSO-d_6



ESI-MS of Mono-6^{A}-N-propargylamino-6A-deoxy-β-cyclodextrin *(compound **6**)*



^{1}H NMR of *rhodamine B conjugated adamantane (compound **14**)* in $CDCl_3$



ESI-MS of *rhodamine B conjugated adamantine (compound **14**)*