Supplementary Material (ESI) for Chemical Communications

Conjugated Polymer Nanoparticles for Biochemical Protein Kinase Assay

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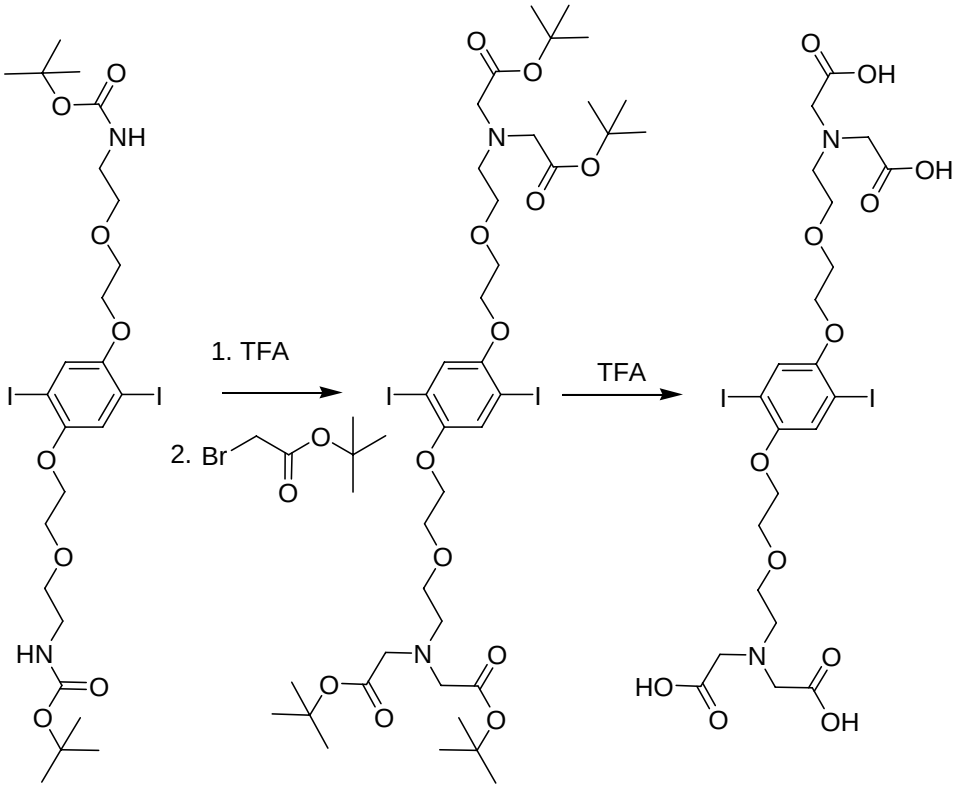
General

Chemicals, including solvents, were purchased from Aldrich and used as received. Palladium(0) catalyst was purchased from Strem. Rhodamine labeled Kemptides were purchased from Anaspec. Recombinant PKA enzyme was purchased from Upstate. NMR spectra were obtained by using Bruker AVANCE-400 NMR Spectrometer with a SpectroSpin superconducting magnet. UV-vis spectra were recorded using a Hewlett Packard 8453 Diode array spectrophotometer. Fluorescence spectra were obtained using a FLS920 fluorescence spectrometer (Edinburgh Instruments). For determination of quantum yield (QY), 9,10-bis(phenylethynyl)anthracene (QY=1.0) in cyclohexane was used as a fluorescence standard. Melting point was measured by using a Mel-Temp apparatus (Barnstead). Molecular weights of monomers were determined by high resolution mass spectroscopy (HRMS, Bruker Daltonics APEXIV 4.7 Tesla Fourier Transform Ion Cyclotron Resonance Mass Spectrometer). Dialysis and solvent exchange of CPN were conducted by using an Ultrafiltration Stirred Cell (Millipore) with membrane filters (Ultracel Ultrafiltration Disc, molecular weight cut-off (MWCO): 10,000 and 3,000). Transmission electron microscopic study was carried out using JEOL JEM 100 CX II Scanning Transmission Electron Microscope. TEM samples were prepared by placing a drop of the particle solution on one side of a Cu grid. After a few minutes, excess solution was removed by wicking it away with filter paper. The grid was introduced into the instrument after ~ 1 hour aging under ambient conditions.

Synthesis.



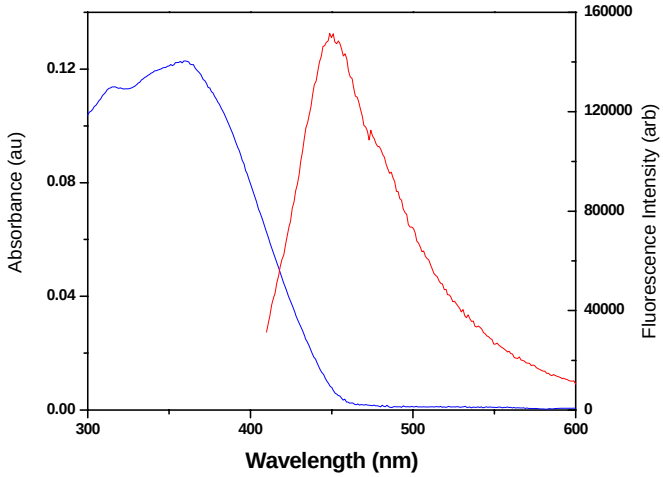
3 **2** **1**

Synthesis of **2**. 15.5mL of trifluoroacetic acid (TFA) was added into a solution of 14.8g (20mmol) of **3** in 200mL of dichloromethane. The reaction mixture was stirred for overnight at room temperature. The solution was evaporated under reduced pressure yielding brown oil. The oil was further dried under vacuum. 3.03g (20mmol) NaI, 19.0 g (88.7mmol) 1,8-Bis(dimethylamino)naphthalene, 11.2g (81mmol) K_2CO_3, and 200mL acetonitrile (anhydrous) were added into the oil. The solution was purged with argon and refluxed at 85°C for one hour. 12.5mL (84.7mmol) t-butyl bromoacetate was added and the solution was left to reflux overnight. The solid was filtered off and the filtrate was evaporated under reduced pressure. The crude material was then dissolved in ethyl acetate (EA), while insoluble in EA was filtered out. The EA solution was washed with 1M HCl, 1M KOH, brine, and then water, respectively. The organic layer was dried over magnesium sulfate and the concentrated material was further purified via column chromatography (Hexane:Etheyl acetate=3:1, v/v). The product was dried under vacuum and 7.88g (yield: 39%) of a viscous yellow oil was recovered. 1H NMR (in CDCl3): 7.23(s, 2H); 4.08(t, 4H); 3.84(t, 4H); 3.75(t, 4H); 3.53(s, 8H); 3.00(t, 4H); 1.45(s, 25H). 13C NMR (in CDCl3): 170.92, 153.24, 123.58, 86.61, 81.05, 71.03, 70.35, 69.42, 56.83, 53.58. High resolution mass (HRMass): calculated 992.2392, found 993.2465.

Synthesis of **1**. 1.03g(1mmol) **2** was dissolved into 5ml dichloromethane then reacted with 5mL(65mmol) TFA for overnight. The solution was evaporated yielding brown oil which was dissolved into 5mL of 1M KOH then the solution was concentrated under reduced pressure. The concentrate was then mixed with 30mL of acetone and stored at -20°C for overnight. White powders were collected via centrifugation, and the pallet was washed with water, and then dried under vacuum. (Yield: 72%). 1H NMR (in DMSO-d6): 12.2(bs, 1.7H); 7.354(s, 2H); 4.070(t, 3.9H); 3.688(t, 4.1H); 3.595(t, 4.1H); 3.480(s, 7.9H); 2.858(t, 4H). 13C NMR(in DMSO-d6):172.48, 152.44, 122.74, 86.83, 69.55, 68.65, 55.21, 53.16. Melting point: 121-122°C. HRMass: calculated 767.9899, found 768.9943

Synthesis of PPE. 13.0 mmol (100 mg) of 1, 13.3 mmol (63.6mg) of pentiptycene diacetylene, 13.3 mmol (63.6 mg), 5mL DMSO, 3.32mL morpholine, 173.5 μL of 52.5mM CuI in morpholine (9 μmol, 1.7mg), and 1.5mL of 4.3 mM $Pd(PPh_3)_4$ in morpholine (6 μmol, 7.5mg) were reacted under nitrogen at 80°C for 56 hours. The clear yellow slightly viscous solution was precipitated into 60mL cold acetone. A yellow palate was collected via centrifugation. The palate was dissolved into 2mL DMSO then precipitated into 25mL cold acetone. The solution was centrifuged and a yellow gel collected. The gel was dried under vacuum, weighed, and then dissolved in DMSO-d6 for NMR and spectroscopic characterization. Yield=86%. 1H NMR (in DMSO-d6): 7.5 (br), 7.0 (br), 6.1 (br), 5.9, 4.7, 4.4, 4.3, 4.1, 4.0, 3.8, 3.6, 3.5, 3.0, 2.9. UV λmax: 378 nm (water); emission max: 450 nm (water).

Figure 1. Spectra of PPE in water



Fabrication of particle.

1 mL of 1mg/mL (994 μM per repeating unit) PPE in water was diluted into 198.5 mL of a buffer containing 5 mM MES (pH 5.5) and 0.5 % Tween20 to yield a final CPN concentration of 5 μM. Gallium chloride was then added (0.5mL of 0.1 M $GaCl_3$) to yield a final concentration of 250 μM. The solution was mixed overnight and filtered through a sterile 0.2μm membrane and stored at 4°C in a sterile container (Particle solution).

Titration of peptide substrate.

1 μM Rhodamine labeled kemptide (LRRASLG) and rhodamine labeled phosphokemptide (LRRApSLG) were made in a buffer (50 mM Tris, pH 7.5, 10 mM $MgCl_2$, and 0.2% NaN_3). The absorbance at 570nm was measured to insure the correct concentration of the peptide substrates. The peptide solutions were combined to produce 1μM calibrators with 0%, 5%, 10%, 20%, 50%, and 100% phosphokemptide, respectively. 25 μL of these solutions were mixed with 75 μL of the particle solution (above) and incubated for 30 minutes. The fluorescence was measured at both 455 nm (quenching) and 585 nm (FRET) by excitation at 400 nm.

IC50 experiment.

Enzymatic reactions were set up to measure the effect of staurosporin inhibitor on PKA activity using the CPN based kinase assay. Reaction volumes of 25 μl were prepared by combining 10 μl of 2.5X enzyme/substrate mix (2.5 μM Rhodamine-kemptide / 0.15 U/mL PKA enzyme in assay buffer [50 mM Tris, pH 7.5, 10 mM $MgCl_2$, 0.2% NaN_3]) with 5 μl of 5X inhibitor solution (varying concentrations of staurosporin), and the reaction initiated by the addition of 10 μl of 2.5X ATP mix (25 μM in assay buffer). Final reaction conditions contained 0.03 U/mL PKA enzyme, 0.5 μM Rhodamine kemptide substrate, 10 μM ATP in assay buffer with varying concentrations of staurosporin. 0.5 μM Phosphokemptide calibrators were prepared in parallel as described previously. Five replicates of each sample/calibrator were prepared in 96-well ½-area plates, incubated for 60min at room temperature, spiked with 75 μl of CPN particle solution (above), incubated for 30 minutes at room temperature and analyzed using the Spectramax plate reader from Molecular Devices, Inc. (Ex. 400nm, Em. 455nm and 585nm). Fluorescent outputs were used to calculate emission ratio (Em. 455 / Em. 585) for each sample and percent phosphorylation was determined through non-linear regression curves (2^{nd} order exponential decay) generated with the calibrator set. IC50 values were determined with sigmoid curve fitting as shown in figure 4.

Figure 2. Dynamic light scattering (DLS) results. Polydispersity index is a parameter calculated from a cumulants analysis of the DLS measured intensity autocorrelation function.

Name:	particle in water		
SOP:	particle in water-3ml.sop		
Filename:	mm7136ph202.dts	**Dispersant:**	Water
Record:	3	**Dispersant RI:**	1.33
Sample RI:	1.59	**Dispersant visc. (cP):**	0.8905
Absorption Index:	0.01	**Date and Time:**	Monday, May 21, 2007 2:15:3...

Temperature (C):	25.0	**Cell type:**	Square glass cuvette with roun...
Count rate (Kcps):	392.1	**Cell position (mm):**	2.00
Run duration (s):	60	**Attenuation index:**	10

Z-Average size (nm):	27.58	**Peak 1 Mean:**	29.98	**% (Intensity):**	88.0	**Width:**	12.17
Polydispersity index:	0.254	**Peak 2 Mean:**	243.9	**% (Intensity):**	11.0	**Width:**	98.68
		Peak 3 Mean:	0	**% (Intensity):**	0.0	**Width:**	0