Self-Assembly of Benzothiadiazole-Functionalized Dinuclear Platinum Acetylide Bolaamphiphile for Bioimaging Application

Entai Shi,a Zhao Gao,a Ming Yuan,a Xiaoyu Wang,*b and Feng Wang*a

a Key Laboratory of Soft Matter Chemistry, Chinese Academy of Sciences, Department of Polymer Science and Engineering, University of Science and Technology of China, Hefei, Anhui 230026 (P. R. China)
Fax: (+86) 551 3606 095; E-mail: drfwang@ustc.edu.cn
b Qiushi Academy for Advanced Studies, Zhejiang University, Hangzhou, Zhejiang 310027 (P. R. China)
E-mail: xy_wang@zju.edu.cn.

Supporting Information

1. Materials and methods
2. Synthetic route to the desired bolaamphiphile 1
   2.1 Synthesis of compound 5
   2.2 Synthesis of compound 7
   2.3 Synthesis of bolaamphiphile 1
   2.4 Synthesis of bolaamphiphile 1
   2.5 Synthesis of bolaamphiphile 1
3. UV-Vis measurements for bolaamphiphiles 1–2
4. Emission lifetime measurements for bolaamphiphile 1
5. DLS measurements for bolaamphiphile 1
6. Nile Red encapsulation experiments for 2–3
7. Concentration dependent 1H NMR spectra of 1 in d-chloroform
8. Concentration dependent 1H NMR spectra of 1 in d4-methanol
9. CLSM image for bolaamphiphile 1
10. ITC measurement for bolaamphiphile 2
1. Materials and methods

N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC·HCl), 4-dimethylamino pyridine (DMAP), copper iodide were reagent grade and used as received. 3,4,5-Tris[2-[2-(2-methoxyethoxy)ethoxy]ethoxy] benzoic acid was synthesized according to a previously reported procedure (S. Ghosh, M. R. Molla and A. Das, Chem. Commun. 2011, 47, 8934–8936). Other reagents and solvents were employed as purchased.

NMR spectra were collected on a Varian Unity INOVA-300 spectrometer with TMS as the internal standard. Electrospray ionization mass spectra (ESI-MS) were obtained on a Bruker Esquire 3000 plus mass spectrometer (Bruker–Franzen Analytik GmbH Breman, Germany) equipped with an ESI interface and an ion trap analyzer. UV/Vis spectra were recorded on a UV-1800 Shimadzu spectrometer. FL spectra were recorded on a FluoroMax-4 Spectrofluorometer. All fluorescence lifetimes were obtained using the JY-Horiba FluoroHub single photon counting module. ITC experiments were carried out using a Microcal VP-ITC apparatus.

To determine the cell viability, A549 cells were seeded at a density of 5×10^4 cells/well in 96 well plate at 37°C in 5% CO₂. 1 with 2-fold dilution ranging from 3.125×10^-6 to 2×10^-4 M was incubated with cells in 10% DMEM. After 72 h, 10 μl of resazurin solution (Molecular Probes) was added to each well. After 1 h of incubation at 37°C, the absorbance of each well was read on a microplate reader at 570 nm. The relative cell viability (%) related to control wells without treatment was calculated by OD_test/OD_control×100, for which the OD_test is the absorbance of 1 and OD_control is the absorbance of control cell. The cellular uptake of 1 was explored by confocal laser scanning microscopic (CLSM) experiments. The confluent A549 cells grown on glass slide were incubated with 1 at 1.0 × 10^-4 M in DMEM culture medium supplemented with 10% FBS. After 24 h, 48 h, and 72 h of incubation, the coverslip were washed three times with phosphate-buffered saline (PBS) and then examined using a CLSM (Leica).
2. Synthetic route to the desired bolaamphiphile 1

Scheme S1. Synthetic route to the bolaamphiphile 1.

Scheme S2. Synthetic route to the bolaamphiphiles 2–3.

2.1 Synthesis of compound 5

4,7-Diethynyl-2,1,3-benzothiadiazole 4 (80.0 mg, 0.44 mmol), trans-[Pt(PEt₃)₂I₂] (720 mg, 1.46 mmol), CuI (8.00 mg, 0.04 mmol) in THF/E₂NH (50 mL, 4:1, v/v) were stirred at...
room temperature for 12 h. The solvents were removed with a rotary evaporator and the residue was extracted with H₂O/CH₂Cl₂. The organic extracts were combined and concentrated to provide a yellow oil, which was further purified by flash column chromatography (petroleum ether/dichloromethane, 1:1 v/v as the eluent) to provide 5 as a yellow solid (253 mg, 44%). The ¹H NMR spectrum of compound 5 is shown in Figure S1. ¹H NMR (300 MHz, CDCl₃, room temperature) δ (ppm): 7.35 (s, 2H), 2.43–2.20 (m, 24H), 1.28–1.06 (m, 36H).

**Figure S1.** ¹H NMR spectrum (300 MHz, CDCl₃, room temperature) of compound 5.

### 2.2 Synthesis of compound 7

Benzoic acid 6 (1.28 g, 2.05 mmol), 4-aminophenylacetylene (200 mg, 1.71 mmol), EDC•HCl (457 mg, 2.38 mmol) and DMAP (208 mg, 1.71 mmol) were dissolved in CH₂Cl₂ and stirred at room temperature for 24 h. The reaction mixture was then extracted with H₂O/CH₂Cl₂, washed with brine and the solvent was removed with a rotary evaporator. The
residue was purified by flash column chromatograph (CH$_2$Cl$_2$/CH$_3$OH, 10:1, v/v as the eluent) to afford compound 7 as a pale yellow liquid (0.96 g, 78%). The $^1$H NMR spectrum of compound 7 is shown in Figure S2. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ (ppm): 8.75 (s, 1H), 7.68 (d, $J = 8.7$ Hz, 2H), 7.46 (d, $J = 8.6$ Hz, 2H), 7.22 (s, 2H), 4.20 (m, 6H), 3.85–3.75 (m, 6H), 3.70 (m, 6H), 3.67–3.58 (m, 12H), 3.56–3.47 (m, 6H), 3.30–3.35 (s, 9H), 3.05 (s, 1H). The $^{13}$C NMR spectrum of compound 7 is shown in Figure S3. $^{13}$C NMR (75 MHz, CDCl$_3$, room temperature) $\delta$ (ppm): 165.80, 152.27, 141.48, 139.23, 132.64, 129.77, 120.24, 117.32, 107.51, 83.57, 72.28, 71.81, 70.44, 69.67, 68.84, 58.86. ESI–MS m/z: [M + Na]$^+$, calculated 730.34; found 730.62.

Figure S2. $^1$H NMR spectrum (300 MHz, CDCl$_3$, room temperature) of compound 7.
Figure S3. $^{13}$C NMR spectrum (75 MHz, CDCl$_3$, room temperature) of monomer 7.

Figure S4. Electrospray ionization spectrum of monomer 7.
2.3 Synthesis of bolaamphiphile 1

Compound 5 (100 mg, 0.077 mmol), 7 (188 mg, 0.19 mmol), CuI (4.00 mg, 0.02 mmol) in THF/Et₂NH (25 mL, 4 : 1, \(v/v\)) were stirred at room temperature for 20 h. The solvents were then removed and the resulting mixture was extracted with H₂O/CH₂Cl₂. The solvent for the combined organic extracts was removed with a rotary evaporator. The residue was purified by flash column chromatography (dichloromethane/acetonitrile, 1:1 \(v/v\) as the eluent) to afford bolaamphiphile 1 as a red solid (104 mg, 55%). The \(^1\)H NMR spectrum of 1 is shown in Figure S5. \(^1\)H NMR (300 MHz, CDCl₃, room temperature) \(\delta\) (ppm): 8.16 (s, 2H), 7.51 (d, \(J = 8.6\) Hz, 4H), 7.32 (s, 2H), 7.29 (d, \(J = 8.6\) Hz, 4H), 7.21 (s, 4H), 4.24 (m, 12H), 3.90–3.77 (m, 12H), 3.69–3.57 (m, 24H), 3.57–3.49 (m, 12H), 3.36 (s, 18H), 2.44–2.07 (m, 24H), 1.32–1.16 (m, 36H). The \(^{13}\)C NMR spectrum of 1 is shown in Figure S6. \(^{13}\)C NMR (75 MHz, CDCl₃, room temperature) \(\delta\)(ppm): 165.11, 152.53, 141.79, 131.41, 130.39, 119.93, 107.77, 77.24, 72.37, 71.90, 70.56, 69.79, 69.21, 58.98, 16.36, 8.43. The \(^{31}\)P NMR spectrum of 1 is shown in Figure S7. \(^{31}\)P NMR (162 MHz, CDCl₃, room temperature) \(\delta\)(ppm): 11.22; ESI–MS m/z: [M + H]^+, calculated 2457.98; found 2458.10.
Figure S5. $^1$H NMR spectrum (300 MHz, CDCl$_3$, room temperature) of 1.

Figure S6. $^{13}$C NMR spectrum (75 MHz, CDCl$_3$, room temperature) of 1.
Figure S7. $^{31}\text{P}$ NMR spectrum (162 MHz, CDCl$_3$, room temperature) of 1.

Figure S8. Electrospray ionization spectrum of 1.
2.4 Synthesis of bolaamiphile 2

A mixture of compounds 8 (130 mg, 0.11 mmol), 8 (188 mg, 0.26 mmol), CuI (4.00 mg, 0.02 mmol) in THF/Et₂NH (25 mL, 4 : 1, v/v) was stirred at room temperature for 20 h. The solvents were then removed and the resulting mixture was extracted with H₂O/CH₂Cl₂. The combined organic extracts were dried over anhydrous Na₂SO₄ and removed with a rotary evaporator. The crude product was purified by flash column chromatography (dichloromethane/acetonitrile, 1 : 1 v/v as the eluent) to afford bolaamphiophile 2 as a yellow solid (126 mg, 48%). The ¹H NMR spectrum of 2 is shown in Figure S9. ¹H NMR (300 MHz, CDCl₃, room temperature) δ (ppm): 8.12 (s, 2H), 7.52 (d, J = 7.23 Hz, 4H), 7.22 (d, J = 10.6 Hz, 8H), 7.13 (s, 4H), 4.30–4.17 (m, 12H), 3.81 (m, 6H), 3.72 (m, 12H), 3.69–3.58 (m, 24H), 3.53 (m, 12H), 3.36 (d, J = 12.4 Hz, 18H), 2.28–1.97 (m, 24H), 1.28–1.15 (m, 36H). The ¹³C NMR spectrum of 2 is shown in Figure S10. ¹³C NMR (75 MHz, CDCl₃, room temperature) δ (ppm): 164.06, 151.48, 140.69, 130.36, 129.35, 118.95, 106.74, 76.22, 71.33, 70.87, 69.53, 68.75, 68.17, 57.97, 15.31, 7.34. ESI–MS m/z: [M + 2 Na]²⁺, calculated 1222.50; found 1222.96.
Figure S9. $^1$H NMR spectrum (300 MHz, CDCl$_3$, room temperature) of 2.

Figure S10. $^{13}$C NMR spectrum (75 MHz, CDCl$_3$, room temperature) of 2.
2.5 Synthesis of bolaamphiphile 3

A mixture of compound 8 (83.0 mg, 0.067 mmol), 9 (107 mg, 0.15 mmol), CuI (4.00 mg, 0.02 mmol) in THF/Et₂NH (25 mL, 4 : 1, v/v) was stirred at room temperature for 22 h. The solvents were removed and the resulting mixture was extracted with H₂O/CH₂Cl₂. The combined organic extracts were dried over anhydrous Na₂SO₄ and removed with a rotary evaporator. The crude product was purified by flash column chromatography.
(dichloromethane/acetonitrile, 1:1 v/v as the eluent) to afford 3 as a yellow solid (68.0 mg, 42%). The $^1$H NMR spectrum of compound 3 is shown in Figure S12. $^1$H NMR (300 MHz, CDCl$_3$, room temperature) $\delta$ (ppm): 7.43 (s, 4H), 7.30 (d, $J = 8.6$ Hz, 4H), 7.13 (s, 4H), 7.01 (d, $J = 8.5$ Hz, 4H), 4.30-4.18 (m, 12H), 3.92–3.78 (m, 12H), 3.74 (m, 12H), 3.70–3.59 (m, 24H), 3.54 (m, 12H), 3.37 (d, $J = 3.6$ Hz, 18H), 2.28–2.06 (m, 24H), 1.23 (m, 36H). The $^{13}$C NMR spectrum of 3 is shown in Figure S13. $^{13}$C NMR (75 MHz, CDCl$_3$, room temperature) $\delta$ (ppm): 163.70, 151.36, 147.15, 130.76, 129.41, 120.17, 108.53, 76.21, 71.17, 69.70, 68.59, 67.89, 58.02, 15.28, 7.33. ESI–MS m/z: [M + 2 Na]$^{2+}$, calculated 1223.48; found 1223.97.

![Figure S12. $^1$H NMR spectrum (300 MHz, CDCl$_3$, room temperature) of 3.](image-url)
Figure S13. $^{13}$C NMR spectrum (75 MHz, CDCl$_3$, room temperature) of 3.

Figure S14. Electrospray ionization spectrum of 3.
3. UV-Vis measurements for bolaamphiphiles 1–2

Figure S15. (a) UV-Vis spectra for bolaamphiphile 1 (red) and 2 (black) in THF (T = 20 °C, c = 1 × 10^{-4} M). The low-energy optical transition observed in 1 (λ_{max} = 490 nm, assigned to be MLCT peak) is not manifest for 2, underlying the importance of electron-deficient ligand to induce such a low-energy optical transition. (b) Normalized UV-Vis spectra of 1 (T = 20 °C, c = 1 × 10^{-4} M) in various aprotic and protic solvents.
4. Emission lifetime measurements for bolaamphiphile 1

Figure S16. Emission lifetime measurements for bolaamphiphile 1 in (a) THF; (b) n-butanol; (c) 3% THF in aqueous solution. For all of the three solvents the emission lifetimes reach to nano-second range, indicating that only fluorescent emission could be observed for 1. Briefly, with the utilization of time correlated single photon counting technique, the emission decay profile of 1 in THF is mono-exponential with a 4.1 ns lifetime, whilst in polar n-butanol and aqueous solutions the profiles are tri-exponential with 1.03 ns and 1.53 ns lifetimes, respectively.
5. DLS measurements for bolaamphiphile 1

Figure S17. DLS measurements for 1 in n-butanol (black) and THF (red) and aqueous solutions (blue) (T = 20 °C, c = 1 × 10^{-4} M).
6. Nile Red encapsulation experiments for 2–3

![Graph a](image1)

![Graph b](image2)

**Figure S18.** Fluorescence intensity at 635 nm of Nile Red versus the logarithm of concentration of (a) 2 and (b) 3. The CAC values for 2 and 3 are determined to be $2.69 \times 10^{-5}$ and $3.16 \times 10^{-5}$ M, respectively. The comparable values for bolaamphiphiles 1–3 demonstrate that either modification of \( \pi \)-conjugated moiety or removal of hydrogen bonding unit exerts few effect on their self-assembly behaviors, supporting the entropy-driven self-assembly process in aqueous solution.
7. Concentration-dependent $^1$H NMR spectra of 1 in $d$-chloroform

Figure S19. Concentration-dependent $^1$H NMR spectra of 1 in $d$-chloroform at different monomer concentrations: (a) 50.0 mM; (b) 42.0 mM; (c) 33.0 mM; (d) 25.0 mM; (e) 18.0 mM; (f) 13.0 mM; (g) 6.30 mM; (h) 2.10 mM; (i) 0.70 mM. Upon increasing the concentration of bolaamphiphile 1, significant downfield shifts are observed for the amide protons, indicating the formation of intermolecular hydrogen bonding arrays between the amide groups. On the other hand, no obvious chemical shifts occur for the aromatic peaks, implying that the triethylphosphine units on 1 impart severe steric hindrance and thereby hamper the intermolecular $\pi$–$\pi$ stacking interactions.
8. Concentration dependent $^1$H NMR spectra of 1 in $d_4$-methanol

Figure S20. Concentration-dependent $^1$H NMR spectra of 1 in $d_4$-methanol at different concentrations: (a) 3.00 mM; (b) 1.00 mM; (c) 0.60 mM; (d) 0.40 mM; (e) 0.30 mM. The aromatic peaks exhibit distinct signals, meanwhile no obvious shifts occur for the aromatic peaks when varying the monomer concentration suggesting that $\pi-\pi$ stacking interactions between the organoplatinum chromophores make minor contributions for the aggregation process.
9. CLSM image for bolaamphiphile 1

**Figure S21.** Magnified CLSM image for cellular uptake of 1. The fluorescent clusters located in the plasma of A549 cells after treatment with 1.0 × 10⁻⁴ M of 1 for 72 h are indicated by the white arrows, suggesting the internalization of the aggregates in the cell plasma with high efficiency.

**Figure S22.** *left,* Fluorescent microscopy images of dose-dependent bolaamphiphile 1 in A549 cells; *middle,* nuclear staining image with DAPI; and *right,* merged image of 1 and DAPI for 48 h post incubation.
10. ITC measurement for bolaamphiphile 2

**Figure S23.** Heat effect for the ITC experiments by injecting aliquots of solution of 2 in aqueous solution \((5 \times 10^{-5} \text{ M})\) into the pure solvent.