Supporting Information

The amphiphilic oligomer-based polymeric micelles as cisplatin nanocarriers for cancer therapy

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Materials and methods

Materials

Styrene (St), 9H-carbazole were all purchased from Shanghai Chemical Reagent Co., Ltd., as analytical reagents and used without further purification. 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), 1-hydroxybenzotriazole (HOBt), diethyl 2,2’-azanediyl diacetate, Azobisisobutyronitrile (AIBN) (chemically pure, Shanghai Chemical Reagent Co., Ltd.) was recrystallized from anhydrous methanol. Methacryloyl chloride was produced by Haimen Best Fine Chemical Industry Co. Ltd. and used after distillation. Tetrahydrofuran (THF) and cyclohexanone were purified by reduced pressure distillation. All other reagents were commercially available and used as received.

Synthesis of polystyrene-N(CH$_2$COOH)$_2$ \((PS(COOH)_{2})\)

Synthesis of 4-(((9H-carbazole-9-carbonothioyl)thio)methyl)benzoic acid (TMBA). 9H-carbazole (8 g, 0.048 mol) was dissolved in anhydrous DMSO, and then potassium hydroxide (3.36 g, 0.06 mol) were added. The mixture was stirred at room temperature for 4~5 hours until completely dissolved and was subsequently cooled to 0 °C. Then CS$_2$ (4.57 g, 0.06 mol) was added dropwise. The reaction mixture was stirred for another 4~5 h, followed by addition of 4-(chloromethyl) benzoic acid (10.23 g, 0.06 mol) and stirring overnight. The crude product was isolated by precipitation from massive water and recrystallized from anhydrous ethanol, then light yellow crystal was obtained (yield: 65%). $^1$H NMR (400MHz, CDCl$_3$, δ, ppm): 4.79(s, 2H, CH$_2$), 7.37(t, 2H, C$_6$H$_4$ in carbazole ring), 7.43(t, 2H, C$_6$H$_4$ in carbazole ring), 7.58(d, 2H, C$_6$H$_4$ in benzene ring), 7.98(d, 2H, C$_6$H$_4$ in benzene ring), 8.11(d, 2H, C$_6$H$_4$ in carbazole ring), 8.44(d, 2H, C$_6$H$_4$ in carbazole ring). ESI-MS (+): calcd=378.0617 for [M + H]$^+$, found = 378.0618.

Synthesis of diethyl 2,2’-((4-(((9H-carbazole-9-carbonothioyl)thio)methyl)benzoyl)azanediyl)diacetate (TMBA). TMBA (0.33 g, 0.87 mmol), diethyl
2,2’-azanediyldiacetate (0.15 g, 0.79 mmol), EDC (0.183 g, 0.95 mmol), HOBt (0.129 g, 0.95 mmol) were added in anhydrous THF. The reaction mixture was stirred for 24 h at room temperature. After the solvent was evaporated under reduced pressure, the crude product was column chromatographed on silica-gel (elution with EtOAc/petroleum = 1:2) to give bright yellow crystal (yield: 82\%). \(^1\)H NMR (400 MHz, CDCl\(_3\), \(\delta\), ppm): 1.24 (t, 6H, \(\text{CH}_3\)), 4.08 (q, 4H, \(\text{CH}_2\text{CH}_3\)), 4.20 (dd, 4H, N\(\text{CH}_2\text{COO}\)), 4.72 (s, 2H, \(\text{CH}_2\)), 7.36 (t, 2H, \(\text{C}_6\text{H}_4\) in carbazole ring), 7.43 (t, 2H, \(\text{C}_6\text{H}_4\) in carbazole ring), 7.51 (d, 2H, \(\text{C}_6\text{H}_4\) in benzene ring), 7.55 (d, 2H, \(\text{C}_6\text{H}_4\) in carbazole ring), 7.98 (d, 2H, \(\text{C}_6\text{H}_4\) in benzene ring), 8.43 (d, 2H, \(\text{C}_6\text{H}_4\) in carbazole ring).

**Preparation of PS(COOH)\(_2\).** To a solution of monomer St (520.7 mg, 5 mmol) and RAFT agent TMBA (548.1 mg, 1 mmol) in cyclohexanone (2.0 mL), AIBN (82.1 mg, 0.5 mmol) was added. The resulting solution was degassed by three freeze-evacuate-thaw cycles and heated at 70 °C for 4 h under argon. The mixture was added into a large amount of cooled anhydrous ethanol (100 mL). The precipitate was filtered and dissolved in hot ethanol (250 mL), followed by adding aqueous solution of NaOH (20 mL, 10\%). Under stirring, the reaction mixture was refluxed for 3 h and cooled to room temperature, followed by adding hydrochloric acid until the pH of the solution reaching 3. The precipitate was filtered and dried under vacuum at ambient temperature for 24 h to obtain the polymer (PS-N(CH\(_2\)COOH)\(_2\)) (yield: 31\%). \(^1\)H NMR (400 MHz, CDCl\(_3\), \(\delta\), ppm): 1.5-2.5 (\(\text{CH}_2\text{CHC}_6\text{H}_5\)), 4.0-4.5 (N(CH\(_2\)COOH)\(_2\)), 6.5-7.5 (-C\(_6\)H\(_5\)), 8.0-8.2 (NCOC(CH\(_2\)CH)\(_2\)C).

**Synthesis of poly(St-co-MAADA) (PSM) and polystyrene-COOH (PS-COOH) for comparison**

**Synthesis of diethyl 2,2’-(methacryloylazanediyl)diacetate (DMAAD).** Diethyl 2,2’-azanediylacetate (1.9 g, 10 mmol) and triethylamine (1.0 g, 10 mmol) were dissolved in 20 mL anhydrous THF and cooled to 0 °C in a water-ice bath. Methacryloyl chloride (1.0 g, 10 mmol) was added dropwise, and the mixture was left to react at room temperature for 12 h. The reaction mixture was filtered, concentrated...
under reduced pressure, the crude product was column chromatographed on silica-gel (elution with EtOAc/petroleum=1:4) to give light yellow liquid (yield: 79%). \(^1\)H NMR(400MHz, CDCl\(_3\), \(\delta\), ppm): 1.23 (t, 6H, CH\(_2\)CH\(_3\)), 1.93 (s, 3H, CH\(_2\)=CCH\(_3\)), 4.08 (q, 4H, CH\(_2\)CH\(_3\)), 4.20 (dd, 4H, CH\(_2\)), 5.10 (s, 1H, =CH\(_2\)), 5.21 (s, 1H, =CH\(_2\)).

TOF-MS (EI): calcd for C\(_{12}\)H\(_{19}\)NO\(_5\): 257.1263, found: 257.1265.

**Synthesis of PSM.** To a solution of monomer DMAAD (257.3 mg, 1 mmol) and RAFT agent TMBA (75.5 mg, 0.2 mmol) in cyclohexanone (2.0 mL), AIBN (17 mg, 0.1 mmol) was added. The resulting solution was degassed by three freeze-evacuate-thaw cycles and heated at 70 °C for 4 h under argon. After St (208.3 mg, 2 mmol) was injected into the flask, the reaction mixture was stirred for another 12 h. The mixture was added into a large amount of cooled anhydrous ethanol (100 mL). The precipitate was filtered and dissolved in hot ethanol (250 mL), followed by adding aqueous solution of NaOH (20 mL, 10%). Under stirring, the reaction mixture was refluxed for 3 h and cooled to room temperature, followed by adding hydrochloric acid until the pH of the solution reached 3. The precipitate was filtered and dried under vacuum at ambient temperature for 24 h to obtain the PSM (yield: 45%). \(^1\)H NMR (400MHz, CDCl\(_3\), \(\delta\), ppm): 1.5-2.5 (CH\(_3\)CCON), 1.5-2.5 (CH\(_2\)CHC\(_6\)H\(_5\)), 4.0-4.5 (N(CH\(_2\)COOH)\(_2\)), 6.5-7.5 (-C\(_6\)H\(_5\)), 8.0-8.2 (NCOC(CH\(_2\)CH\(_2\))C).

**Synthesis of PS-COOH.** To a solution of monomer St (208 mg, 2 mmol) and RAFT agent TMBA (75.5 mg, 0.2 mmol) in cyclohexanone (2.0 mL), AIBN (17 mg, 0.1 mmol) was added. The resulting solution was degassed by three freeze-evacuate-thaw cycles and heated at 70 °C for 4 h under argon. The mixture was added into a large amount of cooled anhydrous ethanol (100 mL). The precipitate was filtered and dissolved in THF (2 mL), then the obtained solution was added into aqueous solution of NaBH\(_4\) (0.5 g/100ml). The reaction mixture was stirred overnight, and the precipitate was filtered and dried under vacuum at ambient temperature for 24 h to obtain the polymer (PS-COOH) (yield: 37%). \(^1\)H NMR (400MHz, CDCl\(_3\), \(\delta\), ppm): 1.5-2.5 (CH\(_3\)CCON), 1.5-2.5 (CH\(_2\)CHC\(_6\)H\(_5\)), 6.5-7.5 (-C\(_6\)H\(_5\)), 8.0-8.2
(NCOC(CHCH)$_2$C).

Scheme S1 Synthetic route of different carboxyl-containing oligomers

**Preparation of the Pt-coordinated complex based on different oligomers**

The micelle solutions were prepared by dropwise addition of 4 mL deionized water into the solution of each oligomer in 2 mL THF (2 mg/mL) at room temperature and then stirring overnight to completely evaporate the THF solvent. Predetermined amount of cisplatin solution (water, 1 mg/ml) was added to the micelle solution. After stirred for overnight in dark, the solution was followed dialysis against water for 12 h at room temperature with a molecular weight cutoff (MWCO) of 1000. The dialysis medium was refreshed four or five times. The Pt-coordinated complex based on
different oligomers were collected through centrifugation (8000 rpm) and dried under vacuum at ambient temperature for 24 h. Drug loading efficiency were calculated according to the following formula:

Drug loading efficiency (%) = (weight of loaded drug/weight of drug in feed) × 100%

**Pt Release under different conditions**

Predetermined amount of PS(COOH)$_2$-Pt in 2 mL PBS were put into dialysis bag (MWCO=1000), which was in 30 mL PBS bath at 37 °C. At certain time point, 1 mL PBS was sampled. The platinum concentration was determined by ICP-AES.

**Cell culture and preparation**

Sk-BR3 cell lines (purchased from Shanghai Cell Institute Country Cell Bank, China) were cultured as monolayer in RPMI-1640 medium supplemented with 10 % heat-inactivated fetal bovine serum at 37 °C in a humidified incubator (5 % CO$_2$ in air, v/v).

**Preparation of Nile Red-encapsulated Pt-coordinated complex based on different oligomers**

To investigate cellular uptake of Pt-coordinated complexes based on oligomers, Nile Red was used as a fluorescence probe. Nile Red was loaded into micelles by adding an acetone solution of Nile Red (20 μL, 1 mM) to the polymer solution in THF (0.4 mL, 5 mg/mL), followed by dropwise addition of water, and then stirring overnight. In order to completely remove THF, acetone and free Nile Red, the solution was followed dialysis against water for 16 h at room temperature with a molecular weight cutoff (MWCO) of 1000. Predetermined amount of cisplatin solution (water, 1 mg/ml) was added to the micelle solution. After stirred for overnight in dark, the solution was followed dialysis against phosphate buffer (10 mM, pH 7.4) for 16 h at room temperature with a molecular weight cutoff (MWCO) of 1000. Nile Red-encapsulated Pt-coordinated complex based on different oligomers were collected through centrifugation (8000 rpm) and dried under vacuum at ambient temperature for 24 h. The amount of platin was determined by ICP-AES.

**Cellular Uptake of Nile Red-encapsulated Pt-coordinated complex based on**
different oligomers

The cellular uptake and intracellular release behavior of Nile Red-loaded NPs (prepared in the similar way as mentioned above) were followed using fluorescence microscopy (OLYMPUS IX 51) with Sk-Br3 cell lines. After the Sk-Br3 cells were cultured in a disc to ~70% confluence (~8×10^4 cells/disc), 100 μL physiological saline of Nile Red-loaded micelles (containing 2 μg Nile Red per disc) was added. The discs were incubated for 30 min, the culture medium was removed, and then the cells were rinsed five times with physiological saline prior to the fluorescence measurement.

**Cell Viability Test**

**Table S1** Concentrations of cisplatin or Pt-coordinated complexes and corresponding Pt concentrations in the medium

<table>
<thead>
<tr>
<th>Run</th>
<th>Cisplatin</th>
<th>PS(COOH)_2-Pt</th>
<th>PSM-Pt</th>
<th>PS-COOH-Pt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample Pt</td>
<td>Concentration(μg/mL)</td>
<td>Concentration(μg/mL)</td>
<td>Concentration(μg/mL)</td>
</tr>
<tr>
<td>1</td>
<td>28.2</td>
<td>18.3</td>
<td>41.099</td>
<td>7.48</td>
</tr>
<tr>
<td>3</td>
<td>7.04</td>
<td>4.58</td>
<td>10.275</td>
<td>1.87</td>
</tr>
<tr>
<td>4</td>
<td>3.52</td>
<td>2.29</td>
<td>5.143</td>
<td>0.936</td>
</tr>
<tr>
<td>5</td>
<td>1.76</td>
<td>1.14</td>
<td>2.571</td>
<td>0.468</td>
</tr>
<tr>
<td>6</td>
<td>0.88</td>
<td>0.572</td>
<td>1.286</td>
<td>0.234</td>
</tr>
<tr>
<td>7</td>
<td>0.44</td>
<td>0.286</td>
<td>0.643</td>
<td>0.117</td>
</tr>
<tr>
<td>8</td>
<td>0.22</td>
<td>0.143</td>
<td>0.321</td>
<td>0.058</td>
</tr>
</tbody>
</table>

Sulforhodamine B (SRB) is used as an assay for assessing the effects of drug carriers in various concentrations. In brief, Sk-Br3 cells was placed in 96-well plates (1.3×10^4 cells per well) and four duplicate wells were set up in each sample. The culture medium was replaced with the medium containing of cisplatin or Pt-coordinated complexes of different concentrations (as shown in Table S1) and cultured at 37 °C in a humidified incubator (5 % CO₂ in air, v/v). After cultured for 72 h, the medium was poured away and 10 % (w/v) trichloroacetic acid in Hank’s balanced salt solution (100 μL) was added and stored at 4 °C for 1 h. Then, the stationary liquid was discarded, the cells were washed with deionized water for five times before air drying and stained with 0.4 % (w/v) SRB solution (100 μL per well)
for 30 min at room temperature. Following the remove of SRB, the cells were washed with 0.1% acetic acid solution for five times. Bound SRB dye was solubilized with 10 mmol/L Tris-base solution (150 μL, pH = 10.5). The test optical density (OD) value was calculated by the absorbance at 531 nm of each individual well measured with a spectrophotometer.

**Tumor model**

$2 \times 10^6$ Sk-Br3 cells suspended in 60 μL phosphate buffered saline (PBS) were subcutaneously injected into the flank region of each nude female mouse. The experiment was not carried out until the volume of the tumor grew to approximately 60 mm$^3$. All of animal experimental procedures were approved by Soochow University Laboratory Animal Center.

**In vivo therapy**

Sk-Br3 tumor-bearing nude female mice were randomized into 3 treatment groups (5 mice/group): saline treated, cisplatin treated (0.5 mg/kg), PS(COOH)$_2$-Pt treated (with an equivalent cisplatin dosage of 0.5 mg/kg). The specimens (saline, cisplatin, PS(COOH)$_2$-Pt) were intratumorally injected from day 0 at 4-day intervals. The therapeutic efficiency was evaluated according to the tumor size, which was calculated as $(\text{tumor length} \times \text{tumor width})^2/2$.

**In vivo systematic toxicity**

In order to evaluate the safety of PS(COOH)$_2$ in vivo, the normal female Balb/c mice without tumors were randomly divided into two groups (3 mice/group): saline, PS(COOH)$_2$ (20mg/kg). 24 h after intraperitoneal injection, almost 0.5 mL of blood from each mouse was collected for hematology analysis.

To evaluate the safety of PS(COOH)$_2$-Pt in vivo, the normal female Balb/c mice without tumors were randomly divided into two groups (3 mice/group): saline, PS(COOH)$_2$-Pt (with an equivalent cisplatin dosage of 1 mg/kg). 24 hours after intraperitoneal injection, all mice were sacrificed, and organs including heart, liver, spleen, lung and kidney were harvested for histopathological examination with Haematoxylin & Eosin (H&E) staining.
Characterization

$^1$H NMR spectra were measured by a Bruker 400 MHz NMR spectrometer using CDCl$_3$ as the solvent and tetramethylsilane (TMS) as the internal reference at ambient temperature. Molecular weight ($M_n$) and polydispersity ($M_w/M_n$) relative to PS were measured on a gel permeation chromatography using a Waters 1515 pump and differential refractometer. THF was used as the mobile phase at a flow rate of 1.0 mL/min. The samples for transmission electron microscopy (TEM) observations were prepared by placing a drop of the micelle solution (0.2 mg/mL) on copper grids, which were coated with thin films of Formvar and then carbon. TEM images were obtained using a TecnaiG220 electron microscope at an acceleration voltage of 200 kV. Before measurements samples were stained with 0.1 wt% phosphotungstic acid, the size and zeta potential of the micelles were determined using dynamic light scattering (DLS), which was carried out at 25 °C using the Zetasizer Nana-ZS from Malvern Instruments equipped with a 633 nm He-Ne laser by back-scattering detection. Steady-state fluorescence spectra were recorded using a FLS920 spectrofluorometer (Edinburgh Co., UK) with an excitation wavelength of 335 nm with a pyrene probe. The intensity ratio of the third band (383 nm) to the first band (372 nm) of the pyrene emission spectrum ($I_3/I_1$) was used to determine the polarity of the pyrene environment. The energy disperse spectroscopy (EDS) was obtained using a LEO 1530-vp scanning electron microscope (SEM) equipped with the Oxford EDS system. The element analysis of Fe and Pt in the samples was carried out by a JY2000 Ultrace inductively coupled plasma atomic emission spectrometer (ICP-AES) after aqua regia digest.
**Table S2** Characteristic data of the oligomers

<table>
<thead>
<tr>
<th>Oligomer</th>
<th>Conversion (%)</th>
<th>Mn (NMR)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mn (GPC)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>PDI</th>
<th>CMC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS(COOH)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>31</td>
<td>820</td>
<td>900</td>
<td>1.08</td>
<td>27</td>
</tr>
<tr>
<td>PSM</td>
<td>45</td>
<td>1100&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1200</td>
<td>1.27</td>
<td>19</td>
</tr>
<tr>
<td>PS-COOH</td>
<td>37</td>
<td>700</td>
<td>800</td>
<td>1.15</td>
<td>32</td>
</tr>
</tbody>
</table>

<sup>a</sup> Molecular weight determined by NMR analysis.  
<sup>b</sup> Molecular weight and polydispersity estimated by GPC.  
<sup>c</sup> The number of hydrophilic monomer determined by the number ratio of the protons of carbazole ring (δ = 8.0-8.2) to those of -(CH<sub>2</sub>COOH)<sub>2</sub> (δ = 4.0-4.5).  

Electronic Supplementary Material (ESI) for Nanoscale  
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**Fig. S1** Size distribution of the micelles based on PS(COOH)$_2$, PSM and PS-COOH before (A) and after (B) complexing with cisplatin.
Fig. S2 Fluorescence microscopy images of Sk-BR3 cells treated with Nile Red-loaded PS(COOH)$_2$-Pt (A) and Nile Red-loaded PSM-Pt for 30 min (B)
Fig. S3 pH-dependent Pt release from PSM-Pt (A) and PS-COOH-Pt (B) at 37 °C (200 μg of Pt in 2 mL of PBS in a dialysis bag of MWCO=1000)