## **Supporting Information**

## Janus Particles Self-Assembled by Small Organic Molecules Based on Atypical Asymmetric Gemini Surfactant Design

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## Claim:

The animal experiments were performed in accordance with the guide for the care and use of medical laboratory animals (Ministry of Health, China). All animal procedures in this work were approved by the Institutional Animal Experimental Ethics Committee of Sichuan University (Chengdu, China, 13 Approval file No. 2015014A).

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SI.1 Synthesis and structure information



## Synthesis of 2-(2,6-dimethylphenylamino)-N,N-diethyl-N-(2-hydroxyethyl)-2oxo- ethanaminium bromide (13)

2-bromoethanol 53.2g (426mmol) and lidocaine 100g (426mmol) were added in a flask, sealed. The mixture was heated at 100 °C for 8 hours. The reaction residual was cooled into room temperature, and mixed with ethyl acetate 100 ml with vigorous stirring. The white solid in the suspension was filtered, and washed with ethyl acetate (100mL x 3). The white solid was dried under reduced pressure, and obtained 124.4g product **13** as white solid, yield 81.2%. Melt point: 185.5~186.4°C. <sup>1</sup>H NMR (400MHz, CD<sub>3</sub>OD)  $\delta$  : 7.11~7.16 (m, 3H), 4.50~4.51 (m, 2H), 4.05~4.07 (m, 2H), 3.75~3.87 (m, 6H), 2.26 (s, 6H), 1.43 (t, *J* = 7.2Hz, 6H). <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD)  $\delta$  : 8.28, 18.65, 56.81, 56.93, 58.48, 61.63, 128.92, 129.31, 134.19, 136.80, 164.15. HRMS: [C<sub>16</sub>H<sub>27</sub>N<sub>2</sub>O<sub>2</sub>]<sup>+</sup>, 279.2079, found 279.2075.



## General method on synthesis of product 3~6

To the mixture of **13** (6.0g, 16.7mmol), triphosgene (2.47g, 8.34mmol) and 1,2dichloroethane (100ml), pyridine (1.4g, 17.6mmol) in 1,2-dichloroethane (100ml) were added dropwise at room temperature for 20min. After addition, this mixture was allowed to stirring at room temperature for 3h. Then the fatty alcohol (16.7mmol) in 1,2-dichloroethane (30ml) was added. This solution was stirred for another 16h at  $50^{\circ}$ C, and cooled to room temperature.

Saturated brine (200ml) was poured into the reaction flask. The organic layer was washed with saturated brine (200mL x 6). The organic solvent was removed in vacuo. The residue was further purified by chromatography with  $CH_2Cl_2-CH_3OH$  as eluent to give product as white powder.

### Characterization data of compounds 3



2-(2,6-dimethylphenylamino)-N,N-diethyl-N-(2-(heptyloxycarbonyloxy)ethyl)-2-

oxoethanaminium chloride (3). Obtained as white

powder, yield: 43%. Melting Point:  $137.7 \sim 138.3^{\circ}$ C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  : 10.34 (s, 1H), 7. 00~7.08 (m, 3H), 5.00 (m, 2H), 4.64 (br, 2H), 4.16 (t, *J* = 6.8 Hz, 2H), 4.04 (m, 2H), 3.66~3.76 (m, 4H), 2.24 (s, 6H), 1.60~1.63 (m, 2H), 1.54~1.56 (m, 8H), 0.86 (t, *J* = 7.2 Hz, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  : 8.33, 14.06, 14.19, 18.82, 21.06, 22.54, 25.53, 26.89, 28.48, 28.81, 31.64, 56.40, 57.56, 57.82, 60.39, 60.59, 69.33, 76.79, 127.42, 128.12, 133.11, 135.04, 154.31, 161.77. HRMS: [C<sub>24</sub>H<sub>41</sub>N<sub>2</sub>O<sub>4</sub>]<sup>+</sup>: 421.3107, found: 421.3070. Content of Cl<sup>-</sup> by IC: 99.1%. Content by HPLC (UV detector, 220nm, Zorbex 300SB-C3, 20mmol/L NH<sub>4</sub>Ac-H<sub>2</sub>O : CH<sub>3</sub>CN, 90% for 0~10min and 10% for 10~30min, 1.5ml/min): 100%. (See Fig.S1).







Figure S1: <sup>1</sup>H NMR, <sup>13</sup>C NMR, HRMS and HPLC of compound 3

## Characterization data of compounds 4



2-(2,6-dimethylphenylamino)-N,N-diethyl-2-oxo-N-(2-(pentyloxycarbonyloxy)ethyl) ethanaminium chloride (4). Obtained as white powder, yield: 39%.

Melting Point: 88.1~90.2°C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  : 11.01 (s, 1H), 7. 02~7.09 (m, 3H), 5.00 (br, 2H), 4.66 (br, 2H), 4.14 (t, J = 6.8 Hz, 2H), 4.05 (m, 2H), 3.68~3.74 (m, 4H), 2.26 (s, 6H), 1.65 (t, J = 6.8 Hz, 2H), 1.49 (t, J = 7.2 Hz, 6H), 1.34~1.32 (m, 4H), 0.91 (t, J = 7.2 Hz, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  : 8.33, 13.90, 18.80, 22.21, 25.53, 27.68, 28.16,56.40, 57.55, 57.94, 60.60, 69.16, 127.41, 128.11, 133.11, 135.06, 154.32, 161.80. HRMS:  $[C_{22}H_{41}N_2O_4]^+$ : 393.2753, found: 393.2646. Content of Cl<sup>-</sup>: 98.7%. Content by HPLC (UV detector, 220nm, Zorbex 300SB-C3, 20mmol/L NH<sub>4</sub>Ac-H<sub>2</sub>O : CH<sub>3</sub>CN, 90% for 0~10min and 10% for 10~30min, 1.5ml/min): 97.4%. (See Fig.S4).









Figure S2: <sup>1</sup>H NMR, <sup>13</sup>C NMR of compound 4

#### Characterization data of compounds 5



2-(2,6-dimethylphenylamino)-N,N-diethyl-N-(2-(nonyloxycarbonyloxy)ethyl)-2-oxoethanaminium chloridemp (5). Obtained as white powder, yield:

41%. Melting Point: 92.7~94.3°C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  : 10.47 (br, 1H), 7. 03~7.11 (m, 3H), 5.08 (s, 2H), 4.66~4.69 (m, 2H), 4.15 (t, J = 6.7 Hz, 2H), 4.04~4.11 (m, 2H), 3.67~3.83 (m, 4H), 2.28 (br, 6H), 1.99 (br, 2H), 1.63-1.70 (m, 2H), 1.54 (t, J = 7.2 Hz, 6H), 1.30~1.37 (m, 4H), 0.90 (t, J = 4.0 Hz, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  : 8.50, 13.92, 18.92, 18.94, 22.24, 27.69, 28.18, 56.57, 57.85, 60.54, 69.30, 127.62, 128.19, 132.80, 135.11, 154.30, 161.75. HRMS: [C<sub>26</sub>H<sub>45</sub>N<sub>2</sub>O<sub>4</sub>]<sup>+</sup>, 449.3379, found: 449.3387. Content of Cl<sup>-</sup> by IC: 99.3%. Content by HPLC (UV detector, 220nm, Zorbex 300SB-C3, 20mmol/L NH<sub>4</sub>Ac-H<sub>2</sub>O : CH<sub>3</sub>CN, 90% for 0~10min and 10% for 10~30min, 1.5ml/min): 100% (See Fig.S3).





Figure S3: <sup>1</sup>H NMR, <sup>13</sup>C NMR, HRMS and HPLC of compound 4

## Characterization data of compounds 6



powder, yield: 45%. Melting Point: 102.2~103.0°C. 1H NMR (400 MHz,

CDCl<sub>3</sub>)  $\delta$ : 10.52 (br, 1H), 7. 03~7.11 (m, 3H), 5.08 (s, 2H), 4.67 (t, J = 4.6 Hz, 2H), 4.14 (t, J = 6.8 Hz, 2H), 4.04~4.06 (m, 4H), 2.28 (s, 6H), 2.03 (br, 2H), 1.67 (t, J = 6.8 Hz, 2H), 1.53 (t, J = 7.2 Hz, 6H), 1.26~1.30 (m, 18H), 0.88 (t, J = 6.8 Hz, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$ : 8.49, 14.14, 18.92, 22.70, 28.50, 29.19, 29.35, 29.49, 29.58, 29.64, 31.92, 56.57, 57.82, 57.87, 60.54, 69.32, 127.60, 128.18, 132.83, 135.11, 154.30, 161.76. HRMS:  $[C_{29}H_{51}N_2O_4]^+$ , 491.3638, found: 491.3642. Content of Cl<sup>-</sup> by IC: 99.1 %. Content by HPLC (UV detector, 220nm, Zorbex 300SB-C3, 20mmol/L NH<sub>4</sub>Ac-H<sub>2</sub>O: CH<sub>3</sub>CN, 90% for 0~10min and 10% for 10~30min, 1.5ml/min): 100%. (See Fig.S4).







Figure S4: <sup>1</sup>H NMR, <sup>13</sup>C NMR, HRMS and HPLC of compound 6

Synthesis of N-(2-(2,6-dimethylphenylamino)-2-oxoethyl)-N,N-diethyl-3-hydroxy propan-1-aminium chloride (14)



3-bromopropan-1-ol 5.9g (42.7mmol) and lidocaine 10g (42.7mmol) were added in a flask. The mixture was heated at 110°C for 8 hours. The reaction residual was cooled into room temperature, and mixed with ethyl acetate 100 ml with vigorous stirring. The white solid in the suspension was filtered, and washed with ethyl acetate (100mL x 3). The white solid was dried under reduced pressure, and obtained 13.8g product **14** as white solid, yield 80.3%. Melting Point: 182.0~183.2°C. <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD)  $\delta$  : 7. 12~7.19 (m, 3H), 4.44 (s, 2H), 3.67~3.74 (m, 8H), 2.27 (s, 6H), 2.07~2.00 (m, 2H), 1.44 (t, *J* = 7.2 Hz, 6H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  : 8.32, 18.65, 18.74, 26.35, 56.54, 57.72, 58.37, 59.34, 129.01, 129.36, 134.16, 136.75, 136.70. HRMS: [C<sub>17</sub>H<sub>29</sub>N<sub>2</sub>O<sub>2</sub>]<sup>+</sup>: 293.2224, found: 293.2224.



Figure S5: <sup>1</sup>H NMR and <sup>13</sup>C NMR of compound 12

## Synthesis of N-(2-(2,6-dimethylphenylamino)-2-oxoethyl)-N,N-diethyl-3-(heptyl oxycarbonyloxy)propan-1-aminium chloride (7)



To the mixture of heptan-1-ol 1.94g (16.7mmol) triphosgene (2.47g, 8.34mmol) and 1,2-dichloroethane (30ml), pyridine (1.4g, 17.6mmol) in 1,2-dichloroethane (20ml) were added dropwise at room temperature for 20min. After addition, this mixture was allowed to stirring at 50 °C for 3h. Then the mixture was added dropwise to **12** (5.5g, 16.7mmol) in 1,2-dichloroethane (200ml). This solution was stirred for another 16h at 50 °C, and cooled to room temperature.

Saturated brine (200ml) was poured into the reaction flask. The organic layer was washed with saturated brine (200mL x 6). The organic solvent was removed in vacuo. The residue was further purified by chromatography with CH<sub>2</sub>Cl<sub>2</sub>-CH<sub>3</sub>OH as eluent to give product (7) as white syrupy solid, yield: 26%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  : 11.03 (s, 1H), 7. 03~7.10 (m, 3H), 4.95 (s, 2H), 4.26 (t, *J* = 5.6 Hz, 2H), 4.13 (t, *J* = 6.8 Hz, 2H), 3.64~3.77 (m, 6H), 2.30~2.36 (m, 2H), 2.27 (s, 6H), 1.62~1.67 (m, 2H), 1.50 (t, *J* = 7.2 Hz, 6H), 1.25~1.34 (m, 8H), 0.89 (t, *J* = 6.8 Hz, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  : 8.12, 14.04 18.78, 22.27, 22.54, 25.58, 28.55, 28.83, 31.64, 55.43, 56.70, 57.04, 64.07, 70.21, 127.39, 128.11, 133.12, 135.03, 154.77, 161.80. HRMS: [C<sub>25</sub>H<sub>43</sub>N<sub>2</sub>O<sub>4</sub>]<sup>+</sup>: 435.3217, found: 435.3218. Content of Cl<sup>-</sup> by IC: 99.1%.





Figure S6: <sup>1</sup>H NMR, <sup>13</sup>C NMR and HRMS of compound 7



Synthesis of 2-(2,6-dimethylphenylamino)-N,N-diethyl-N-(2-(nonanoyloxy)ethyl)-2-oxoethanaminium chloride (8)

Nonanoic acid 2.6g (16.7mmol) and CH<sub>2</sub>Cl<sub>2</sub> 10mL were added in a dry flask. The solution of oxalyl chloride 1.3g (10.2mmol) in CH<sub>2</sub>Cl<sub>2</sub> 10mL were added dropwise at room temprature during 10min. The mixture was stirred for 20min, and added dropwise into a mixture of **13** (6g, 16.7mmol), pyridine (2.0g, 25.0mmol) and CH<sub>2</sub>Cl<sub>2</sub> 30mL in a new flask at room temprature. After the reaction mixture was stirred at 40°C for 12 hours, saturated brine (200ml) was poured into the flask. The organic layer was washed with saturated brine (200mL x 6). The organic solvent was removed in vacuo. The residue was further purified by chromatography (CH<sub>2</sub>Cl<sub>2</sub> : CH<sub>3</sub>OH = 20 : 1) to obtained product **8** (2.8g) as white powder, yield: 37%. Melting Point: 121.5~123.5°C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  : 11.02 (s, 1H), 7. 02~7.10 (m, 3H), 5.04 (s, 2H), 4.62 (t, *J* = 4.8 Hz, 2H), 3.99 (t, *J* = 4.8 Hz, 2H), 3.71~3.77 (m, 2H), 2.33 (t, *J* = 7.6 Hz, 2H), 2.27 (s, 6H), 1.58~1.61 (m, 2H), 1.51 (t, *J* = 6.8 Hz, 6H),

1.30 (br, 10H), 0.88 (t, J = 6.8 Hz, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  : 8.35, 14.09, 18.82, 22.62, 24.61, 29.07, 29.17, 31.77, 33.93, 56.30, 57.16, 57.48, 57.65, 127.44, 128.14, 133.06, 135.10, 161.85, 172.92. HRMS:  $[C_{25}H_{43}N_2O_3]^+$ : 419.3268, found: 419.3268. Content of Cl<sup>-</sup>: 99.2%.



#### Figure S7: <sup>1</sup>H NMR, <sup>13</sup>C NMR of compound 8

## Synthesis of N-(2-(2,6-dimethylphenylamino)-2-oxoethyl)-N,N-diethyldodecan-1aminium chloride (9)

Lidocaine 5g (21.3mmol) and 1-bromododecane 10.6g (42.6mmol) were added in a flask. The mixture was heated at 100 °C for 24 hours. The reaction residual was cooled into room temperature, and mixed with ethyl acetate 100 ml with vigorous stirring. The white solid in the suspension was filtered, and washed with ethyl acetate (100mL x 3). The white solid was dried under reduced pressure, and obtained product **9** (4.0g) as white powder, yield: 47%. Melting Point: 112.5~113.8°C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  : 11.14 (s, 1H), 7. 02~7.09 (m, 3H), 4.86 (s, 2H), 3.66 (t, *J* = 6.8 Hz, 4H), 3.58~3.64 (m, 2H), 3.64~3.77 (m, 6H), 2.27 (s, 6H), 1.80 (br, 2H), 1.47 (t, *J* = 7.2 Hz, 6H), 1.25~1.34 (m, 18H), 0.88 (t, *J* = 7.2 Hz, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  : 8.25, 14.11 18.83, 22.21, 22.66, 26.41, 29.02, 29.29, 29.38, 29.54, 29.55, 31.87, 55.24, 56.99, 59.46, 127.35, 128.10, 133.20, 135.00, 161.84. HRMS: [C<sub>26</sub>H<sub>47</sub>N<sub>2</sub>O]<sup>+</sup>: 403.3683, found: 403.3682. Content of Cl<sup>-</sup> by IC: 98.3%.





Figure S8: <sup>1</sup>H NMR, <sup>13</sup>C NMR and HRMS of compound 9

## Synthesis of 2-(2,6-dimethylphenylamino)-N,N-diethyl-N-(2-(heptyloxy)ethyl)-2oxoethanaminium chloride (10)

Heptan-1-ol 3.7g (32.0mmol), 2-bromoethanol 4.0g (32.0mmol) and sulfuric acid (98%) 3.2g (32.0mmol) were added in a flask, and heated at 150°C for 2 hours. After cooled into room temperature, the mixture was poured into cooled water 200mL, and extracted with  $CH_2Cl_2$  (30mL x 3).

The organic layer dried with anhydrous sodium sulfate, and added into another flask together with lidocaine 5g (21.3mmol). After the solvent was removed in vacuo, the residual was stirred at 110 °C for 8 hours. Saturated brine (200ml) was poured into the flask. The organic layer was washed with saturated brine (200mL x 6). The organic solvent was removed in vacuo. The residue was further purified by chromatography (CH<sub>2</sub>Cl<sub>2</sub> : CH<sub>3</sub>OH = 20 : 1) to obtained product **10** (0.89g) as colourless syrupy, yield: 10.1%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub> : CF<sub>3</sub>COOD = 50 : 1)  $\delta$  : 7.13~7.16 (m, 1H), 7. 06~7.08 (m, 2H), 4.70 (s, 1.5H), 4.58 (s, 0.5H), 3.63~3.88 (m, 7H), 3.48 (t, *J* = 3.2 Hz, 2H), 2.19 (s, 6H), 1.62~1.67 (m, 2H), 1.58 (t, *J* = 6.8 Hz, 2H), 1.42~1.47 (m, 6H), 1.26~1.28 (m, 8H), 0.88 (t, *J* = 7.2 Hz, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  : 8.40, 14.06, 18.76, 22.41, 26.04, 29.02, 29.42, 31.73,

55.08, 56.28, 57.22, 58.79, 64.26, 71.98, 127.40, 128.11, 133.12, 135.12, 162.17. HRMS: [C<sub>23</sub>H<sub>41</sub>N<sub>2</sub>O<sub>2</sub>]<sup>+</sup>: 377.3163, found: 377.3157. Content of Cl<sup>-</sup> by IC: 99.3%.





Figure S9: <sup>1</sup>H NMR, <sup>13</sup>C NMR and HRMS of compound 10

Synthesis of 2-(diethylamino)-N-(2,6-dimethylphenyl)-N-methylacetamide hydro chloride (15)



To a dry flask, lidocaine 10g (42.7mmol) and anhydrous THF 50mL were added under argon. NaH (60%) 1.7g (42.7mmol) was then added at  $-5^{\circ}$ C during 5min. Iodomethane 6.4g (45mmol) in anhydrous THF 20mL was added dropwise during 20min, and stirred at  $-5^{\circ}$ C for 2 hours.

Hydrochloric acid (0.1N) 300mL was added into the reaction solution, and extracted with CH<sub>2</sub>Cl<sub>2</sub> (50mL x 3). The organic layer was washed with saturated brine (200mL) and removed in vacuo. The residue was further purified by chromatography (CH<sub>2</sub>Cl<sub>2</sub> : CH<sub>3</sub>OH = 50 : 1) to obtained product **15** (11.9g) light yellow oil, yield: 61%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  : 7. 07~7.19 (m, 3H), 3.13 (s, 3H), 2.91 (s, 2H), 2.68 (q, *J* =7.2 Hz, 4H), 0.98 (t, *J* = 7.2 Hz, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  : 11.66, 17.61, 29.66, 34.40, 47.56, 53.26, 128.33, 129.07, 135.56, 140.20, 169.88. HRMS: [C<sub>15</sub>H<sub>25</sub>N<sub>2</sub>O]<sup>+</sup>: 249.1961, found: 249.1961.





Figure S10: <sup>1</sup>H NMR and <sup>13</sup>C NMR of compound 15

# Synthesis of 2-((2,6-dimethylphenyl)(methyl)amino)-N,N-diethyl-N-(2-(heptyloxy carbonyloxy)ethyl)-2-oxoethanaminium chloride (11)



Compound **15** 10.0g (37.1mmol) was dissolved in  $CH_2Cl_2$  100mL, washed with solution of  $Na_2CO_3$  (1N, 50mL x 2). The organic layer was dried by anhydrous sodium sulfate, filtered, and removed in vacuo.

The residue and 2-bromoethanol 4.6g (37.1mmol) were added in a flask. The mixture was heated at  $100^{\circ}$ C for 8 hours. The reaction residual was cooled into room temperature, and mixed with ethyl acetate 100 ml with vigorous stirring. The white solid in the suspension was filtered, and washed with ethyl acetate (100mL x 3). The white solid was dried under reduced pressure.

The white solid was then mixed with triphosgene (2.47g, 8.34mmol) and 1,2dichloroethane (100ml) in a flask. Pyridine (1.4g, 17.6mmol) in 1,2-dichloroethane (100ml) were added dropwise at room temperature for 20min. After addition, this mixture was allowed to stirring at room temperature for 3h. Then the heptan-1-ol 1.94g (16.7mmol) in 1,2-dichloroethane (30ml) was added. This solution was stirred for another 16h at 50 $^{\circ}$ C, and cooled to room temperature.

Saturated brine (200ml) was poured into the reaction flask. The organic layer was washed with saturated brine (200mL x 6). The organic solvent was removed in vacuo. The residue was further purified by chromatography with CH<sub>2</sub>Cl<sub>2</sub>-CH<sub>3</sub>OH as eluent to give product (**11**) 5.8g as colourless syrupy, yield: 33%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>: CF<sub>3</sub>COOD = 50 : 1)  $\delta$  : 7. 29~7.11 (m, 3H), 5.37 (s, 0.5H), 4.57 (H<sub>a</sub>, 2H), 4.18 (t, *J* = 6.8 Hz, 2H), 4.07 (H<sub>b</sub>, 2H), 3.66~3.76 (m, 4.5H), 3.36 (s, 0.5H), 3.12 (s, 2H), 2.25 (s, 5H), 2.16 (s, 1H), 1.71~1.64 (m, 2H), 1.27~1.44 (m, 14H), 0.88 (t, *J* = 4.4 Hz, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub> : CF<sub>3</sub>COOD = 50 : 1)  $\delta$  : 7.54, 13.92, 17.24, 22.48, 28.40, 28.76, 31.59, 35.62, 37.03, 55.85, 56.36, 57.61, 60.50, 69.74, 129.20, 130.12, 130.16, 134.19, 134.78, 136.76, 154.60(d, *J* = 9.9 Hz), 163.60, 164.24. HRMS: [C<sub>25</sub>H<sub>43</sub>N<sub>2</sub>O<sub>4</sub>]<sup>+</sup>: 435.3217, found: 435.3220. Content of Cl<sup>-</sup> by IC: 98.8%.





Figure S11: <sup>1</sup>H NMR, <sup>13</sup>C NMR and HRMS of compound 11

Synthesis of 2-bromo-N-cyclohexylacetamide (16)



Cyclohexanamine 5g (50mmol) and triethylamine 6.1g (60mmol) were dissolved by  $CH_2Cl_2$  100mL in a dry flask. 2-bromoacetyl bromide 10.1g (50mmol) in  $CH_2Cl_2$ 50mL was added dropwise at room temperature for 20min. After addition, this mixture was allowed to stirring at room temperature for 3h.

The reaction solution was poured into solution of Na<sub>2</sub>CO<sub>3</sub> (1N) 300mL. The organic layer was separated, dried by anhydrous sodium sulfate, filtered. The organic solvent was removed in vacuo, and the residue was recrystallized (cyclohexane : ethyl acetate = 5 : 1) to give product (**16**) 16.7g as white powder, yield: 77%. Melting Point: 105.8~107.5°C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  : 6.45 (br, 1H), 4.03 (s, 2H), 3.74~3.84 (m, 1H), 1.91~1.95 (m, 2H), 1.71~1.96 (m, 2H), 1.61~1.66 (m, 1H), 1.40~1.44 (m, 2H), 1.25~ 1.39 (m, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  : 24.71, 25.40, 32.78, 42.73, 48.65, 164.84. HRMS: [C<sub>8</sub>H<sub>15</sub><sup>35</sup>ClNO]<sup>+</sup>: 176.0837, found: 176.0834.



Figure S12: <sup>1</sup>H NMR and <sup>13</sup>C NMR of compound 14

## Synthesis of 2-(cyclohexylamino)-N,N-diethyl-N-(2-(heptyloxycarbonyloxy)ethyl) -2-oxoethanaminium chloride (12)



2-bromo-N-cyclohexylacetamide (16) 10g (45.4mmol) and 2-(diethylamino) ethanol 5.3g (45.4mmol) were added in a flask, and heated at  $110^{\circ}$ C for 8h.

After cooling to room temperature, the residue was mixed with triphosgene (6.7g, 22.7mmol) and 1,2-dichloroethane (100ml) in a flask. Pyridine (3.6g, 45.4mmol) in 1,2-dichloroethane (100ml) were added dropwise at room temperature for 20min. After addition, this mixture was allowed to stirring at room temperature for 3h. Then the heptan-1-ol 1.94g (16.7mmol) in 1,2-dichloroethane (30ml) was added. This solution was stirred for another 24h at 50°C, and cooled to room temperature.

Saturated brine (200ml) was poured into the reaction flask. The organic layer was washed with saturated brine (200mL x 6). The organic solvent was removed in vacuo. The residue was further purified by chromatography with CH<sub>2</sub>Cl<sub>2</sub>-CH<sub>3</sub>OH as eluent to give product (**12**) 7.5g as colourless syrupy, yield: 38%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  : 9.58 (d, *J* = 7.6 Hz , 1H), 4.66 (t, *J* = 4.8 Hz, 2H), 4.62 (br, 2H), 4.16 (t, *J* = 6.8 Hz, 2H), 3.96~3.98 (m, 2H), 3.59~3.75 (m, 5H), 1.84~1.88 (m, 2H), 1.75~1.79 (m, 2H), 1.63~1.68 (m, 2H), 1.58~1.59 (m, 1H), 1.48 (t, *J* = 7.2 Hz, 6H), 1.20~1.45 (m, 13H), 0.88 (t, *J* = 7.2 Hz, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  : 8.35, 14.05 22.55, 24.79, 25.27, 25.55, 28.50, 28.82, 31.64, 32.09, 49.24, 56.61, 57.90, 58.11, 60.54, 69.28, 154.32, 161.70. HRMS: [C<sub>22</sub>H<sub>43</sub>N<sub>2</sub>O<sub>4</sub>]<sup>+</sup>: 399.3217, found: 3993.219. Content of Cl<sup>-</sup> by IC: 99.3%.





Figure S13: <sup>1</sup>H NMR, <sup>13</sup>C NMR and HRMS of compound 12

SI.2 CMC determination of compound 3, 8 and 9

Table S1 Surface tension determination of compound 3

Concentration	Surface tension (mN/m)					
(mmol/L)	Run 1	Run 2	Run 3	Run4	Run5	Mean
56	30.1	30	30.1	30.2	30.1	30.1
28	33.2	33.4	33.4	33.3	33.2	33.3
14	32.7	32.7	32.8	32.6	32.8	32.7
7	31.6	31.5	31.8	31.5	31.7	31.6
3.5	32.5	31.7	32.0	31.9	32.3	32.1
1.8	34.3	33.9	35.0	33.8	35.1	34.4
0.88	38.2	37.7	38.6	38.5	38.0	38.2
0.44	43.6	42.2	40.6	43.3	40.8	42.1







Figure S14 CMC of compound 3 calculated by surface tension

Concentration	Surface tension (mN/m)					
(mmol/L)	Run 1	Run 2	Run 3	Run4	Run5	Mean
56	28.5	28.4	28.2	27.9	30.0	28.2
28	24.3	23.8	23.9	24.9	23.5	24.1
14	23.3	23.5	23.2	22.9	22.9	23.2
7	21.0	21.2	20.4	20.4	20.5	20.7
3.5	21.5	21.5	22.0	21.9	22.4	21.9
1.8	23.7	24.2	23.0	22.3	22.1	23.1
0.88	22.4	23.0	23.0	23.3	23.2	23.0
0.44	31.0	30.8	30.0	30.3	29.6	30.3
0.22	35.6	36.6	34.3	34.2	33.4	34.8

Table S2 Surface tension determination of compound 8

0.11	39.4	41.6	41.4	41.8	41.1	41.1
0.056	44.2	44.2	44.4	43.8	43.7	44.1
0.028	44.4	48.3	48.2	47.2	48.2	47.3





Figure S15 CMC of compound 6 calculated by surface tension

Table S3 Surface tension determination of compound 9

Concentration	Surface tension (mN/m)					
(mmol/L)	Run 1	Run 2	Run 3	Run4	Run5	Mean
28	23.7	26.1	26.2	26.2	26.2	26.2
14	24.3	25.8	25.6	25.4	24.8	25.4
7	24.6	26.0	25.7	25.6	25.1	25.4
3.5	24.8	25.7	25.1	25.0	25.1	25.1
1.8	25.3	24.6	24.0	24.1	23.7	24.5
0.88	23.0	19.6	20.0	20.6	23.9	23.4
0.44	32.7	33.9	35.3	33.6	32.0	33.0
0.22	39.0	38.9	36.8	37.3	38.2	37.4





Figure S16 CMC of compound 7 calculated by surface tension

The surface tension of compound **3**, **8** and **9** in different concentrations at  $25^{\circ}$ C in pure water was detected respectively. The CMC was calculated by that data.<sup>S1</sup>

## SI.3 EC<sub>50</sub> determination of compound 3 and 8

 $EC_{50}$  of compound **3** and **8** was measured by "up-and-down" method.<sup>S2</sup> Effective sciatic nerve blockade that lasted for more than 30min was defined as positive response, otherwise negative response. When a positive response was developed after a given dose, the next concentration that was 1.2 times lower than the last one was administrated. If a negative response was resulted, the next concentration with 20% increment was given. The experiment stopped when change in response nature (from positive to negative response, or otherwise) accumulated at five times.  $EC_{50}$  and 95% confidence interval was calculated. <sup>S3</sup>

## SI.4 TEM observations with phosphotungstic acid

## **General method**

The compound was dissolved in pure water with vigorous magnetic stirring (1000 rpm) at 30°C for 12 hours. The solution for TEM observation were prepared by depositing a drop of solution mentioned above onto a 400 mesh copper grid covered with thin amorphous carbon. A drop of phosphotungstic acid (2%) was added for negative staining, which lasted for 5min. Then the sample would be observed by a Hitachi H-600 TEM (120KV) instrument. <sup>S4</sup>

Each sample was observed for at least twice. For each compound, we began observation from a relative lower concentration (7mmol/L). If we could not find JPs at this concentration, we would observe this compound at a higher concentration (28mmol/L except compound 8 and 10). Because nothing could be observed when the sample of compound 8 and 10 was at concentration of 28mmol/L (the electron beam cannot be transmitted for the sample was too thick), we began observation of these compounds from 14mmol/L. For each compound, if no JPs was found at two different concentration for twice, we thought this compound could not self-assemble into JPs in this condition, and record the typical images of micelles we observed.



7mmol/L

Figure S17 TEM for Compound 4



28mmol/L

## Figure S18 TEM for Compound 5



7mmol/L

14mmol/L

## Figure S19 TEM for Compound 6



28mmol/L

Figure S20 TEM for Compound 7



7mmol/L

28mmol/L

Figure S21 TEM for Compound 8







7mmol/L

14mmol/L





28mmol/L





Figure S25 TEM for Compound 12

## SI.5 TEM observations with other stain agents

## **General method**

Compound **3** was dissolved in pure water with vigorous magnetic stirring (1000 rpm) at  $30^{\circ}$ C for 12 hours. The solution (7mmol/L) for TEM observation were prepared by depositing a drop of solution mentioned above onto a 400 mesh copper grid covered with thin amorphous carbon. A drop of phosphomolybdic acid (2%) or uranyl acetate (2%) was added for negative staining, which lasted for 5min. Then the sample would be observed by a Hitachi H-600 TEM (120KV) instrument.



Figure S26 TEM for Compound 3 stained by phosphomolybdic acid



Figure S27 TEM for Compound 3 stained by uranyl acetate

#### **SI.6 DLS measurements**

Dynamic light scattering (DLS) measurements were performed with Malvern Zetasizer Nano-ZS90. He-Ne laser with a wavelength of 633nm was used. The temperature was set to 25°C and the scattering angle was fixed at 90°. The results were calculated by number mean. <sup>S4</sup>

## Table S4 DLS measurements of compound 3

Entry	Conc. (mmol/L)	T(℃)	Number Mean(d.nm)	PdI
1	7	25	48.70	0.223
2	14	25	32.60	0.263
3	25	25	39.12	0.334
4	28	25	51.45	0.368
5	42	25	77.65	0.390



7mmol/L



14mmol/L



## 25mmol/L



28mmol/L



42mmol/L

Figure S28 Particle size distribution by number mean

## **SI.7 Cryo-TEM observation**

The Cryo-TEM samples were prepared in a controlled environment vitrification system (CEVS). The sample solution  $(4\mu L)$  was coated onto a TEM copper grid. The grid was blotted with two pieces of filter paper for about 2 seconds, leading to the

formation of a solution thin film. Then the grid was quickly plunged into a reservoir of liquid ethane (-165°C, cooled by liquid nitrogen) and kept in liquid nitrogen until the observation. After transferring the grid to a cryogenic sample holder (Gatan 626) and putting the holder into a JEOL JEM-1400 Plus TEM (120KV) instrument at about -174, we could observe the nanostructure of the sample. <sup>S6</sup>

Without the operation of negative staining by phosphotungstic acid, photographs of spherical micelles were unclear on the concentration of 3.5mmol/L on these experiments. So we began these observations on the concentration of 7mmol/L.

#### SI.8 Determination of onset time

The in vivo effect of **3** and **8** was investigated on a rat sciatic nerve block model in a blind, randomized and controlled fashion.



\*  $n = 6 \sim 8$  (n = 6 at 3.5mM; n = 8 at 7mM). In comparison between **3** and **8** at 3.5mmol/L, p = 0.069.

Figure S29 Determination of onset time on rat sciatic nerve block model

All animal experimental procedures were approved by Committee of Scientific Research and Institutional Animal Experimental Ethics, West China Hospital, Sichuan University (No. 2015014A). Sprague Dowley rats (Dossy Experimental Animal Company, Chengdu, China) weighted 200~400g were housed at room temperature and moiety of 40%~60%, in 12h light/12h dark cycle with free access to food and water. Animals were acclimated to experimental environments before tests.

During acclimation, baseline of sensory and motor function was measured every day for three consecutive days, averaged, and recorded. Animals with normal baselines were used, and were randomized into groups ( $n = 6 \sim 8$  for each group).

Rats received sciatic nerve block under sedation with inhaled 1.5%~2.0% isoflurane (v/v %) mixing with 100% oxygen. 0.2 ml of test solution was injected through a 27-Gauge syringe that was inserted at the mid-point between trochanter and ischial tuberosity.<sup>S7,S8</sup> In preliminary experiments, 100% successful rate was obtained with standard peri-sciatic nerve injection procedure common local anesthetics, besides no signs of nerve injury was observed for injection.

Sensation of the injected limb was evaluated after sciatic nerve block by revised hot plate test at 10min, 30min, hourly in 4 hours, every 2 hours within 12 hours, and every 12h thereafter. <sup>S9</sup> In revised hot plate test, rats were gently held, the paw of injected limb was placed on a 52°C metal plate. The paw withdrawal latency (PWL) induced by heat stimulation represents the degree of sensation block. The baseline of PWL lasted no longer than 3s, and the cutoff time of PWL was set at 12s to avoid tissue injury. PWL > 7s was considered effective nerve blockade. The time for PWL to exceed 7s was the onset time of sensory block; PWL returned below 7s was the offset of sensory block; the interval between onset and offset time was the duration of nerve blockade.

Systemic adverse effect was inspected during the injection and 5min after injection. To exclude false positive results, only the efficacy lasting for more than 5min was recorded to be onset. Any signs of sedation, convulsion, seizure, ataxia, excitation, or death were recorded. So did local adverse effects including necrosis, sustain paralysis, muscle spasm, self-mutilation, and so forth.

## References

- S1 Feng, D.; Zhang, Y.; Chen, Q.; Wang, J.; Li; B. Feng, Y. J. Surfact. Deterg. 2012, 15, 657–661.
- S2 Cheng, H.M.; Lee, S.M.; Macleod, B.A.; Ries, C.R; Schwarz, S.K.W. Can. J. Anesth. 2011, 58, 443-450.

- S3 Dixon, W.J.; Mood, A.M. J. Am. Statist. Ass. 1948, 43, 109.S1 Cheng, H.M.; Lee,
  S.M.; Macleod, B.A.; Ries, C.R; Schwarz, S.K.W. Can. J. Anesth. 2011, 58, 443-450.
- S4 Li, X.; Yang, H.; Xu, L.; Fu, X.; Guo, H.; Zhang, X. Macromol. Chem. Phys. 2010, 211, 297–302.
- S5 Schacher, F.; Betthausen, E.; Walther, A.; Schmalz, H.; Pergushov, D.V.; Müller, A.H.E. *ACS Nano* **2009**, *3*, 2095–2102.
- S6 Erhardt, R.; Zhang, M.; Böker, A.; Zettl, H.; Abetz, C.; Frederik, P.; Krausch, G.; Abetz, V.; Müller, A.H.E. J. Am. Chem. Soc. 2003, 125, 3260–3267.
- S7 Binshtok, A.M.; Gerner P.; Bae, S. Anesthesiology 2009, 111, 127-137.
- S8 Thahammer, J.R.; Vladimirova, M.; Bershadsky, B.; Strchartz, G.R. Anesthesiology 1995, 82, 1013-1025.
- S9 Brummett, C.M.; Norat, M.A.; Palmisano, J.M.; Lydic, R. Anesthesiology 2008, 109, 502-511.