

Electronic supplementary information (ESI)

Sensitive Naked-Eye Detection of Hg²⁺ based on the Aggregation and Filtration of Thymine Functionalized Vesicles Caused by Selective Interaction between Thymine and Hg²⁺

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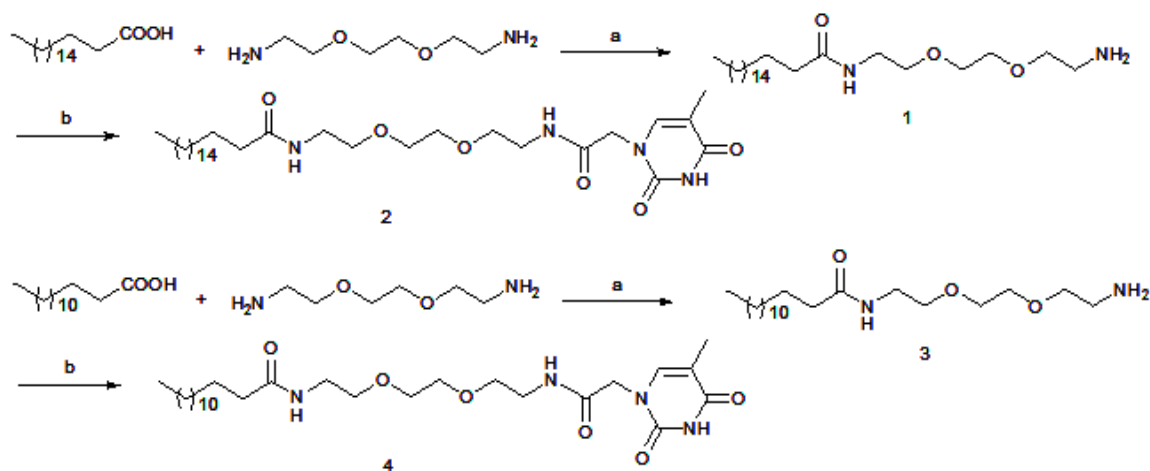
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1. Materials and Method

10,12-pentacosadiynoic acid (PCDA) was purchased from Sigma-Aldrich Chemicals and was further purified by dissolving in chloroform and then filtered to remove unpolymerized monomers before use. $\text{Hg}(\text{NO}_3)_2$ and other metal ions were purchased from Beijing Chemical Reagents Company (Beijing, China). N-hydroxysuccinimide(NHS) and 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide(EDCI) 1,2-bis(2-aminoethoxy) ethane(EDEA) Thymine-1-acetic(T) were purchased from Sigma-Aldrich. Size distribution of vesicles were measured by using a Zetasizer, Nano ZS Malvern Instruments Ltd. Elemental analyses (C, H, and N) were determined on an Elementar Vario EL analyzer. The ^1H NMR spectra were recorded on Bruker 300AV spectrometer. Chemical shifts for protons are quoted in parts per million downfield from tetramethylsilane and are referenced to the solvent peak (for CDCl_3 , ^1H NMR: 7.26 ppm); Abbreviations are used in the description of NMR data as follows: chemical shift (δ , ppm), multiplicity (s=singlet, d=doublet, t =triplet, q=quartet, m=multiplet), coupling constant (J , Hz). The mass spectra for MALDI-TOF were recorded on Bruker Daltonics Inc. BIFLEX III.

2. General Route for Synthesis



(a) N-hydroxysuccinimide, N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride, methylene chloride, 0°C, 24 hr. (b) N-hydroxysuccinimide, N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride, thymine acetic acid, methylene chloride, 0°C, 24 hr.

Fig.S1 General route for synthesis.

2.1 Synthesis of Compound 1

N-(2-(2-(2-aminoethoxy)ethoxy)ethyl) octadecyl amide (C18-EDEA)

Stearic acid 1.419g(5mmol) EDCl 1.107g(5mmol) NHS 0.687g(5mmol) and EDEA 5ml(6mmol) was added in a 100ml flask with 20ml of methylene dichloride and stirred for 24 hours in ice-salt baths. The reaction processes were detected by thin layer chromatography techniques(TLC) in organic synthesis reaction. After the reaction was completed, then the solution was transport to a beaker with saturated NaCl solution. Later the organic phase was evaporated and then purified by silica gel column chromatography using methylene dichloride and methanol(10:1~5:1) and could be re-crystallized by using ethanol to

obtain white powder(1.052g),with a yield of 57%. Elemental Analyses: Theory for $C_{24}H_{50}N_2O_3$ (414.2): C, 69.52; H, 12.15; N, 6.76; Practice for $C_{24}H_{50}N_2O_3$: C, 69.48; H, 12.13; N, 6.74;

1H NMR($CDCl_3$,400MHz) δ (ppm): 3.60-3.43(m,12H), 2.17(t,J=7.5, 2H), 1.61(s,12H),1.27-1.24(m,28H),0.87(t,J=6.6, 3H)

MALDI-TOF-MS: 415.2[M + H] $^+$ 。

2.2 Synthesis of Compound 2

Synthesis of C18-EDEA-T

Thymine-1-acetic acid 0.185g(1mmol), EDCI 0.230g(1mmol) NHS 0.138g(1mmol) was added in a 100ml flask with 20ml of methylene chloride and stirred for 1 hour. C18-EDEA(0.432g, 1.05mmol) were dissolve in methylene dichloride(10ml). Then the solution was slowly dripped into C18-EDEA to stir for 24 hours in ice-salt baths. The reaction processes were detected by thin layer chromatography techniques(TLC) in organic synthesis reaction. After the reaction was completed, then the solution was transport to a beaker with saturated NaCl solution. Later the organic phase was evaporated and then purified by silica gel column chromatography using methylene dichloride and methanol(20:1~15:1). Pure product 0.157g was obtained, with a yield of 31%. Elemental Analyses: Theory for $C_{31}H_{56}N_4O_6$ (580.8): C, 64.12; H, 9.72; N, 9.65; Practice for $C_{31}H_{56}N_4O_6$: C,64.09; H,9.71; N,9.64.

^1H NMR(CDCl_3 ,400MHz) δ (ppm): 9.05(s,1H), 6.93(s,1H), 4.44(s,2H), 3.80-3.44(m,12H),2.19(t,J=7.4,2H),1.92(s,3H),1.63-1.59(m,2H),1.25(m,28H),0.89(t,J=6.5,3H).

MALDI-TOF-MS: 603.5 $[\text{M} + \text{Na}]^+$

2.3 Synthesis of Compound 3

N-(2-(2-(2-aminoethoxy)ethoxy)ethyl) tetradecyl amide(C14-EDEA)

Myristic acid 1.142g(5mmol) EDCI 1.104g(5mmol) NHS 0.690g(5mmol) and EDEA 5ml(6mmol) was added in a 100ml flask with 20ml of methylene dichloride and stirred for 24 hours in ice-salt baths. The reaction processes were detected by thin layer chromatography techniques(TLC) in organic synthesis reaction. After the reaction was completed, the solution was transport to a beaker with saturated NaCl solution. Later the organic phase was evaporated and then purified by silica gel column chromatography using methylene dichloride and methanol(10:1~5:1) and could be re-crystallized by using ethanol to obtain white power(1.272g), with a yield of 67%.Elemental Analyses: Theory for $\text{C}_{20}\text{H}_{42}\text{N}_2\text{O}_3$ (358.2): C, 67.00; H, 11.80; N, 7.81; Practice for $\text{C}_{20}\text{H}_{42}\text{N}_2\text{O}_3$: C, 67.01; H, 11.78; N, 7.80;

^1H NMR(CDCl_3 ,400MHz) δ (ppm): 3.61-3.44(m,12H), 2.18(t,J=7.5, 2H), 1.62(s,12H),1.28-1.25(m,20H),0.88(t,J=6.6,3H)

MALDI-TOF-MS: 359.2 $[\text{M} + \text{H}]^+$

2.4 Synthesis of Compound 4

Synthesis of C14-EDEA-T 2

Thymine-1-acetic acid 0.182g(1mmol), EDCI 0.227g(1mmol) NHS 0.132g(1mmol) was added in a 100ml flask with 20ml of methylene chloride and stirred for 1 hour. C14-EDEA(0.377g, 1.05mmol) were dissolve in methylene dichloride(10ml). Then the solution was dripped into C14-EDEA to stir for 24 hours in ice-salt baths. The reaction processes were detected by thin layer chromatography techniques(TLC) in organic synthesis reaction. After the reaction was completed, then the solution was transport to a beaker with saturated NaCl solution. Later the organic phase was evaporated and then purified by silica gel column chromatography using methylene dichloride and methanol(20:1~15:1). Pure product 0.149g was obtained, with a yield of 27%.

Elemental Analyses: Theory for $C_{27}H_{48}N_4O_6$ (524.3): C, 61.81; H, 9.22; N, 10.68; Practice for $C_{27}H_{48}N_4O_6$: C.61.82; H.9.20; N.10.66.

$^1\text{HNMR}(\text{CDCl}_3, 400\text{MHz})\delta(\text{ppm})$: 8.94(s,1H), 6.95(s,1H), 4.45(s,2H), 3.80-3.44(m,12H), 2.18(t, J=7.4, 2H), 1.94(s,3H), 1.63-1.59(m,2H), 1.25(m,20H), 0.88(t, J=6.5, 3H)

MALDI-TOF-MS: 547.3 $[\text{M} + \text{Na}]^+$

3. Preparation of PCDA mixed Vesicles

The PCDA vesicles were prepared according to the protocols in the literature^[10b]. The typical procedure for the preparation of mixed vesicles is as follows. The components (PCDA and T-probe) were dissolved in CH₃OH at various molecular ratios. The solvent was evaporated under reduced pressure, and deionized water was added to obtain the desired concentration. The resulting suspension was sonicated for 20min at approximately 80°C. After the sonication, the solution was filtered to remove dispersed lipid aggregates using a 0.45μm filter. The solution was then cooled to room temperature and stored overnight at 4°C to induce the crystallization of the lipid membranes. Polymerization was carried out under irradiation at 254 nm for 30s.

4. The principle of thymine-R₁(T-probe) coordinate with Hg²⁺.

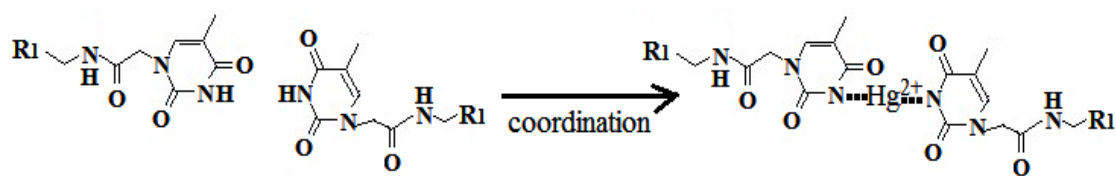


Fig.S2 the principle of thymine-R₁(T-probe) coordinate with Hg²⁺.

5. Reference

(10b) J. Deng, Z. Sheng, K. Zhou, M. Duan, C. Yu and L. Jiang,
Bioconjugate Chem. 2009, **20**, 533-537.