Electronic supplementary information (ESI)

Sensitive Naked-Eye Detection of Hg^{2+} based on the Aggregation and Filtration of Thymine Functionalized Vesicles Caused by Selective Interaction between Thymine and Hg^{2+}

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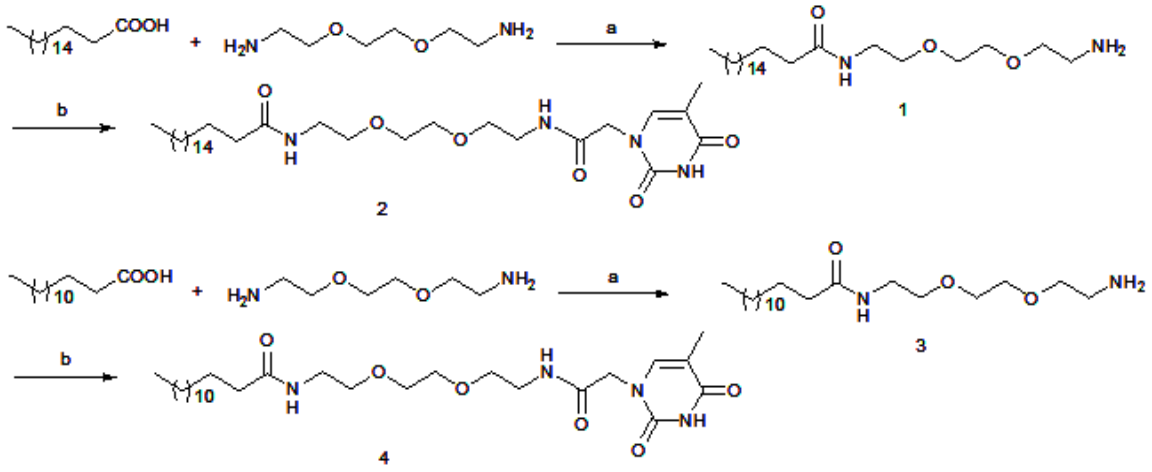
1. Materials and Method

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. $Hg(NO_3)_2$ 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 1HNMR 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 power(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;

^{1}HNMR($CDCl_3$,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), EDCl 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.

^{1}HNMR($CDCl_3$,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 $[M + Na]^+$

2.3 Synthesis of Compound 3

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

Myristic acid 1.142g(5mmol) EDCl 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 $C_{20}H_{42}N_2O_3$ (358.2): C, 67.00; H, 11.80; N, 7.81; Practice for $C_{20}H_{42}N_2O_3$: C, 67.01; H, 11.78; N, 7.80;

^{1}HNMR($CDCl_3$,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 $[M + H]^+$

2.4 Synthesis of Compound 4

Synthesis of C14-EDEA-T 2

Thymine-1-acetic acid 0.182g(1mmol), EDCl 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}HNMR($CDCl_3$,400MHz)δ(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 $[M + Na]^+$

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 CH_3OH 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-R_1(T-probe) coordinate with Hg^{2+}.

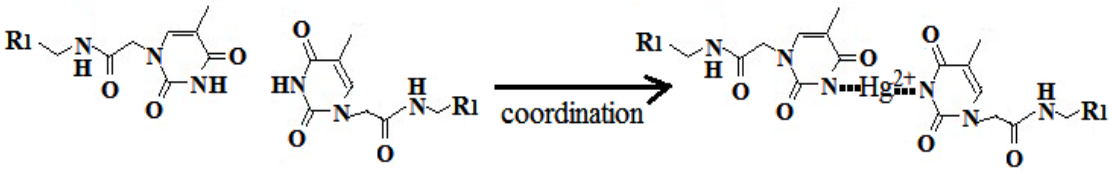


Fig.S2 the principle of thymine-R_1(T-probe) coordinate with Hg^{2+}.

5. Reference

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