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ORIGINAL ARTICLE

Synthesis and Characterization of Novel Cationic Lipids Derived from Thio Galactose

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Abstract Two double chain cationic lipids QAS C_n-2-S ($n = 12$, 14) derived from thio galactose and carbamate-linkage tertiary amine were synthesized and their structures were confirmed by MS, TOF-MS, ^{1}H NMR and ^{13}C NMR. The QAS C_{12}-2-S revealed superior surface activity compared with QAS C_{14}-2-S with lower CMC and γ_{CMC}. Though Lipo C_{12}-2-S displayed large average particle-size with high polydispersity, positive charged Lipo C_n-2-S can be combined with the negative charged DNA, also negatively stained TEM images confirmed the formation of vesicles. All the above prove that the Lipo C_n-2-S is helpful for gene transfection.

Keywords Synthesis · Thio galactose · Cationic lipids · Vesicle

Introduction

As one of the significant means of cancer treatment, gene therapy has received much attention for decades. It is crucial to find a carrier to enhance transfection efficiency with the in-depth research of this technology.

Cationic liposome–DNA complexes are attracting considerable attention as gene vectors due to their safety and other inherent advantages over viral delivery methods [1–3]. These advantages include ease and variability of preparation, lack of immunogenicity, and a capacity for DNA of unlimited size, allowing for delivery of artificial human chromosomes [4]. However, cationic liposomes have low transfection efficiency in comparison with vital carriers. The transfection efficiency could be enhanced owing to the specific binding of ligands and receptors. As we all know, there is rich asialoglycoprotein on the surface of the liver organ, which as a receptor can specially recognize the compounds whose terminal part has non-reductive galactose and GalNAc [5]. It has been confirmed that the galactose receptor-mediated pathway is the best for the hepatocytes in the all receptor-mediated pathways [6]. The liver targeting and transfection efficiency would be significantly improved by the galactose-modified drug [7] or polymer carriers [8] in this way.

In 1998, Kawakami et al. [8] reported three novel galactosylated cholesterol derivatives, cholesten-5-yloxy-*N*-(4-((1-imino-c-β-D-thiogalactosyl-ethyl) amino) alkyl) formamide (Gal-C2-Chol, Gal-C4-Chol, Gal-C6-Chol). Liposome/DNA complexes prepared with these lipids showed low cytotoxicity in human hepatoma HepG2 cells. Gal-C4-Chol (in Fig. 1)/DC-Chol/DOPE (3:3:4) liposomes showed higher transfection activity. In 2007, Shigeta et al. [9] proposed a novel pH-sensitive histidine-modified galactosylated cholesterol derivative (Gal-His-C4-Chol, shown in Fig. 1), for a more efficient gene delivery to hepatocytes. These researches show that thiogalactosyl group may have a good influence on gene transfection efficiency. Additionally, carbamate as a linker has chemical stability in a certain degree, but it can be decomposed by variation of pH. So the transfection efficiency of the cationic galactolipid could be enhanced and the cytotoxicity could be reduced in theory.

In this paper, we synthesized two novel double chains cationic lipids C_n-2-S derived from thio galactose and carbamate-linkage tertiary amine and investigated their physico-chemical properties such as critical micelle

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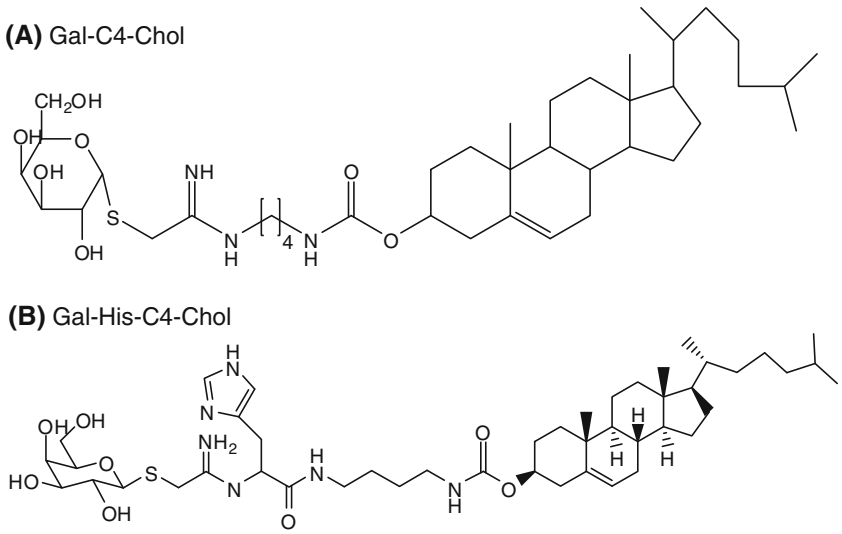


Fig. 1 Chemical structures of Gal-C4-Chol **a** and Gal-His-C4-Chol **b**

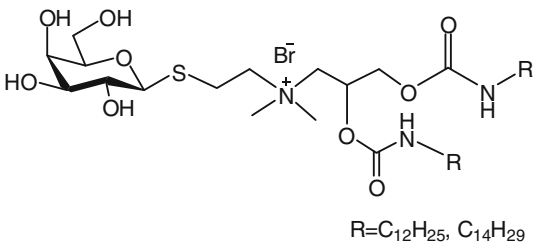


Fig. 2 General structure of the cationic thio galactolipid

concentrations (CMC), surface tension at CMC (γ_{CMC}), critical packing parameter (*P*). An ingenious combination of thio galactose and carbamate was represented on the structure of this new galactolipid (Fig. 2). The vesicle formation was determined by transmission electron microscopy (TEM) as well as particle-size and zeta-potential.

Experimental Procedures

General

D-Galactose was purchased from the Sinopharm Chemical Reagent Co. Ltd., China. Thiourea and Potassium metabisulfite were purchased from Guangdong Shantou Xilong Chemical Factory, China. 1,2-Dibromoethane was obtained from Chengdu Kelong Chemical Reagent Factory, China. Potassium carbonate, sodium sulfate and sodium methoxide were purchased from Tianjin Fuchen Chemical Plant, China. D113 cation exchange resin was purchased from Shenyang Xinxing Reagent Factory, China. 1,2-Dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) was purchased from Sigma-Aldrich (USA). Dichloromethane, chloroform, methanol, ethanol, hexane, ethyl acetate, and acetone were of analytical grade. Deionized water was used in all measurements.

The mass spectrum was obtained with Liquid chromatography/quadrupole time of flight tandem mass spectrometry (Micromass Britain, LC/Q-TOF-MS) and High Performance Liquid Chromatography/Mass selective Detector (HP, America, Model HP 1100LC/MSD). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker 400-MHz instrument (Bruker, Switzerland, Model AVANCE II 400 MHz). Melting points were tested with a micro-melting point apparatus (Ningbo Yongxin Optical Co., Ltd., China, Model X-4). The surface tension was determined with a processor tensiometer (Bowing Industry Corporation, America, Model TX-500C) by the spinning drop principle. Particle sizes and Zeta potentials were measured by nano particle size and zeta potential measurement (Zetasizer nano series Nano-ZS90, Malvern, UK). Vesicles were observed with a transmission electron microscope (JEOL, Japan, Model JEM-1200EX).

Synthesis

The thiogalactopyranoside was synthesized in four steps, as shown in Scheme 1. The cationic galactolipid was obtained during the quarterisation process. The general procedure is described in Scheme 2.

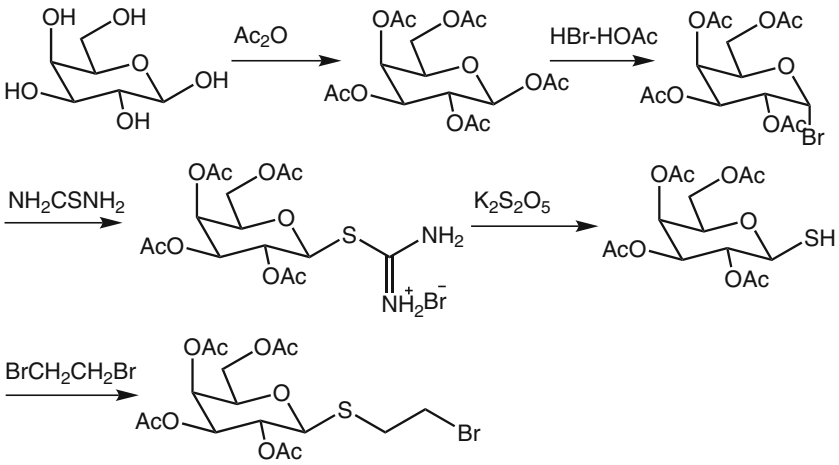
*Preparation of 2,3,4,6-Tetra-O-acetyl-α-D-galactopyranosyl bromide (**1**)*

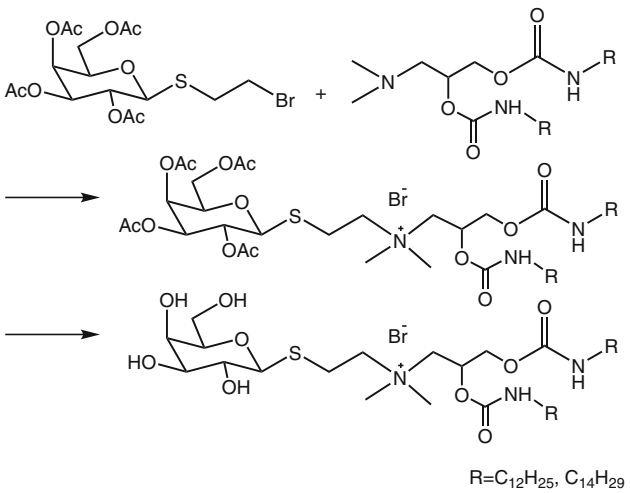
2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl bromide was prepared according to Ref. [10].

*Preparation of 2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosylthiol (**2**)*

Compound **1** (6.00 g, 14.63 mmol) was dissolved in anhydrous acetone (7 mL), thiourea (1.17 g, 14.63 mmol)

Scheme 1 Synthesis route of thiogalactopyranoside





Scheme 2 Synthesis route of cationic galactolipid

was added and the mixture was refluxed for 1 h. After a rapid removal of acetone, the residue was dissolved in water (10 mL) and CH_2Cl_2 (10 mL). Potassium metabisulfite (2.59 g, 11.88 mmol) was now added and the mixture was refluxed for another 15 h. The reaction was monitored by thin layer chromatography (hexane: ethyl acetate = 5:2, v/v). The solution was then cooled down to r.t., and the mixture was extracted with CH_2Cl_2 twice. The dichloromethane extracts were combined, dried over anhydrous sodium sulfate, evaporated in vacuo and the resulting residue was subjected to column chromatography (hexane: ethyl acetate, 5:2, v/v) to obtain the white crystals m.p. 81.6–84.5 °C, in a 49.6 % yield.

^{1}H NMR (400 MHz, MeOD, δ ppm): 1.99,2.07,2.08,2.17 (12H, 4 × s, 4 × CH_3COO (Gal)), 2.36,2.39 (1H, d, J = 12.0, Gal-*SH*), 3.94–3.97 (1H, m, H-5(Gal)), 4.12–4.14 (2H, d, J = 6.8, H-6,6′(Gal)), 4.52–4.56 (1H, t, H-1(Gal)), 5.01–5.04 (1H, dd, J_1 = 10.0, J_2 = 3.2, H-2(Gal)), 5.16–5.21 (1H, t, H-3(Gal)), 5.43–5.44 (1H, dd, J_1 = 3.2, J_2 = 0.8, H-4(Gal)), 7.28 (s, $CDCl_3$);

^{13}C NMR (400 MHz, MeOD, δ ppm):169.84,169.97, 170.16,170.38 (C=O(Gal)), 79.19 (C-1(Gal)), 76.73–77.37 ($CDCl_3$), 67.24,70.84,71.57,74.95 (C-2,3,4,5(Gal)), 61.47 (C-6(Gal)), 20.56,20.67,20.69,20.83 (CH_3COO(Gal));

MS (positive): m/z 387.1420 $[M + Na]^+$.

*Preparation of 2-Bromoethyl-2,3,4,6-tetra-O-acetyl-β-D-thiogalactopyranoside (**3**)*

A mixture of compound **2** (2.00 g, 5.49 mmol) and potassium carbonate (800 mg, 5.80 mmol) in acetone–water (4:3, 14 mL) was stirred under nitrogen. Then, 1,2-dibromoethane (3.6 mL, 7.85 mmol) was added and the reaction was stirred at room temperature. The reaction was monitored by thin layer chromatography with hexane: ethyl acetate (2:1, v/v) as eluent. The mixture was extracted twice with CH_2Cl_2 (50 mL). The combined organic layer was dried with anhydrous sodium sulfate and concentrated to give a yellow oil. The residue was purified by silica gel chromatography using hexane and ethyl acetate (2:1, v/v) as the eluent to give the bromide intermediate as white crystals m.p. 71.8–74.5 °C, in a 44.6 % yield.

^{1}H NMR (400 MHz, MeOD, δ ppm): 1.99,2.05,2.09, 2.17 (12H, 4 × s, 4 × CH_3COO(Gal)), 2.97–3.23 (2H, m, S-CH_2, in which 2.97–3.05 (1H, m), 3.17–3.25 (1H, m)), 3.52–3.61 (2H, m, CH_2Br), 3.94–3.97(1H, t, H-5(Gal)), 4.08–4.17 (2H, m, H-6,6′(Gal)), 4.54–4.56 (1H, d, J = 8.0, H-1(Gal)), 5.03–5.07 (1H, dd, J_1 = 12.0, J_2 = 4.0, H-2 (Gal)), 5.22–5.27 (1H, t, H-3(Gal)), 5.44–5.45 (1H, d, J = 2.4, H-4(Gal)), 7.28 (s, $CDCl_3$);

^{13}C NMR (400 MHz, MeOD, δ ppm): 169.56,169.98, 170.13,170.41 (C=O(Gal)), 84.51 (C-1(Gal)), 76.73–77.36 ($CDCl_3$), 67.00,67.24,71.70,74.72 (C-2,3,4,5(Gal)), 61.69 (C-6(Gal)), 32.81(CH_2Br), 30.64(S-CH_2), 20.57,20.67,20. 70,20.77 (CH_3COO(Gal));

MS (positive): m/z $[M + Na]^+$ 495.0378 (1Br) $[2\ M + Na]^+$ 967.0661.

*Preparation of 3-(Dimethylamino)-propane-1, 2-di-alkylcarbamate (**4**)*

Tertiary amines with carbamate as the important intermediates were synthesized according to the literature procedures [11].

*Preparation of 2,3-Bis(dodecylcarbamoyloxy)-N,N-dimethyl-N-[2-(2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranosyl) Ethyl] Propanyl Ammonium Bromide (**5**)*

Anhydrous compound **3** (1.25 g, 2.66 mmol) and 3-(dimethylamino) propanyl-1,2-didodecylcarbamate (1.5 g, 2.67 mmol) were dissolved in anhydrous ethanol (15 mL). The mixture was refluxed for 48 h under a stream of nitrogen. The solvent was evaporated in vacuo and the resulting residue was subjected to silica gel column chromatography ($[CHCl_3/NH_3 = 5/1]/CH_3OH = 10/1$, v/v). Compound 5 was obtained as a light yellow viscous fluid with a 30 % yield.

^{1}H NMR (400 MHz, MeOD, δ ppm): 0.88,0.90,0.92 (6H, t, 2 × CH_2CH_3), 1.29 (36H, s, 2 × $(CH_2)_9$), 1.49,1.51 (4H, 2 × s, 2 × $NHCH_2CH_2$), 1.96,2.05,2.06,2.16 (12H, 4 × s, 4 × CH_3COO(Gal)), 3.08–3.26 (12H, m, 2 × $NHCH_2$, SCH_2(Et-Linker), 2 × N^+CH_3), 3.31 (s, MeOD), 3.69–3.79 (4H, m, 2 × N^+CH_2), 4.10–4.42 (5H, m, H-5,6,6′(Gal), $NHCOOCH_2$), 4.88 (s, H_2O), 4.92–4.94 (1H, d, $J = 8.0$, H-1(Gal)), 5.15–5.23 (2H, m, H-2,3(Gal)), 5.48–5.52 (2H, m, H-4(Gal), NHCOO*CH*);

^{13}C NMR (400 MHz, MeOD, δ ppm): 171.28,171.53, 171.80,172.22 (C=O(Gal)), 156.84,157.87 (NHC=O), 83.96,84.86 (C-1, α/β(Gal)), 68.37,69.17,72.95,76.43 (C-2,3,4,5(Gal)), 67.58 (NHCOOCH), 65.21,66.45 ($N^+(CH_2)_2$), 64.93 ($NHCOOCH_2$), 62.86 (C-6(Gal)), 52.61,52.87 ($N^+(CH_3)_2$), 49.00 (MeOD), 41.99,42.11 ($NHCH_2$), 22.95–33.08 (($CH_2)_{10}$, SCH_2(Et-Linker)), 20.48,20.57,20.69,20.80 (CH_3COO(Gal)), 14.44 (CH_3CH_2);

MS (positive): *m*/*z* 932.4318 $[M-Br]^+$.

*Preparation of 2,3-Bis(dodecylcarbamoyloxy)-N,N-dimethyl-N-[2-(1-thio-β-D-galactopyranosyl) Ethyl] Propanyl Ammonium Bromide (**6**, denoted as QAS C_{12}-2-S)*

Compound **5** (0.83 g, 0.82 mmol) was dissolved in anhydrous ethanol (15 mL). Sodium methoxide (0.31 g, 5.74 mmol) was added and the mixture was stirred for 1 h. D113 cation exchange resins were added when TLC ($CHCl_3/CH_3OH = 4/1$, v/v) showed the above reaction was completed. Until the pH of the solution had changed to neutral, chloroform (5 mL) was added to distill the mixture. The solvent was removed by rotary evaporation after filtration. Compound **6** was obtained as a light yellow solid with a 100 % yield.

^{1}H NMR (400 MHz, MeOD, δ ppm): 0.88,0.90,0.92 (6H, t, 2 × CH_2CH_3), 1.29 (36H, s, 2 × $(CH_2)_9$), 1.49,1.51 (4H, 2 × s, 2 × $NHCH_2CH_2$), 3.02–3.26 (12H, m, 2 × $NHCH_2$ 2 × N^+CH_3, OCH_2 (Et-Linker)), 3.31 (s, MeOD), 3.46–3.87 (10H, m, 2 × N^+CH_2, H-2,3,4,5,6,6′(Gal)), 4.06–4.11 (2H, m, $NHCOOCH_2$) 4.42–4.46 (1H, dd, $J_1 = 12.0$, $J_2 = 4.0$, H-1(Gal)), 4.89 (s, H_2O), 5.47–5.57 (1H, m, NHCOO*CH*), 8.55 (6H, s, 2 × NH, 6 × OH);

^{13}C NMR (400 MHz, MeOD, δ ppm): 155.44,156.58 (NHC=O), 85.66,86.36 (C-1, α/β(Gal)), 69.38,69.84,74.66, 79.60 (C-2,3,4,5(Gal)), 66.30 (NHCOO*CH*), 65.51,65.75 (N^+CH_2), 63.72 ($NHCOOCH_2$), 61.83 (C-6(Gal)), 50.89,51.13 ($N^+(CH_3)_2$), 49.00 (MeOD), 40.55,40.67 ($NHCH_2$), 22.37–31.70 (($CH_2)_{10}$, SCH_2(Et-Linker)), 13.10 (CH_3CH_2);

MS (positive): *m*/*z* 764.7 $[M-Br]^+$.

*Preparation of 2,3-Bis(tetradecylcarbamoyloxy)-N,N-dimethyl-N-[2-(2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranosyl) Ethyl] Propanyl Ammonium Bromide (**7**)*

Compound **7** was synthesized as the same procedure as compound **5** with a 33.3 % yield.

^{1}H NMR (400 MHz, MeOD, δ ppm): 0.88,0.90,0.92 (6H, t, 2 × CH_2CH_3), 1.29 (44H, s, 2 × $(CH_2)_{11}$), 1.49,1.51 (4H, 2 × s, 2 × $NHCH_2CH_2$), 1.96,2.05,2.06,2.16 (12H, 4 × s, 4 × CH_3COO(Gal)), 3.08–3.26 (12H, m, 2 × $NHCH_2$, SCH_2(Et-Linker), 2 × N^+CH_3), 3.30 (s, MeOD), 3.67–3.81 (4H, m, 2 × N^+CH_2), 4.06–4.41 (5H, m, H-5,6,6′(Gal), $NHCOOCH_2$), 4.88 (s, H_2O), 4.91–4.94 (1H, d, $J = 8.8$, H-1(Gal)), 5.15–5.23 (2H, m, H-2,3(Gal)), 5.48 (1H, s, H-4(Gal)), 5.51–5.52 (1H, d, $J = 4.8$, NHCOO*CH*), 7.90 (s, $CHCl_3$);

^{13}C NMR (400 MHz, MeOD, δ ppm): 171.27,171.44, 171.52,171.79 (C=O(Gal)), 157.95,156.84 (NHC=O), 83.94,84.86 (C-1, α/β(Gal)), 79.47 ($CHCl_3$) 68.35,69.15,72.94,76.42 (C-2,3,4,5(Gal)), 67.56 (NHCOOCH), 65.21,66.43 ($N^+(CH_2)_2$), 64.87 ($NHCOOCH_2$), 62.86 (C-6(Gal)), 52.59,52.87 ($N^+(CH_3)_2$), 49.00 (MeOD), 41.98,42.10 ($NHCH_2$), 22.94-33.08 (($CH_2)_{12}$, SCH_2(Et-Linker)), 20.57, 20.70,20.72,20.81 (CH_3COO(Gal)), 14.45 (CH_3CH_2);

MS (positive): *m*/*z* 988.7 $[M-Br]^+$.

*Preparation of 2,3-Bis(tetradecylcarbamoyloxy)-N,N-dimethyl-N-[2-(1-thio-β-D-galactopyranosyl) Ethyl] Propanyl Ammonium Bromide (**8**, denoted as QAS C_{14}-2-S)*

Compound **8** was synthesized as the same procedure as compound **6** with a 100 % yield.

^{1}H NMR (400 MHz, MeOD, δ ppm): 0.88,0.89,0.91 (6H, t, 2 × CH_2CH_3), 1.28 (44H, s, 2 × $(CH_2)_{11}$), 1.49, 1.50 (4H, 2 × s, 2 × $NHCH_2CH_2$), 1.96,2.15 (2 × s, OH), 3.04–3.17 (6H, m, 2 × $NHCH_2$, SCH_2(Et-Linker)), 3.20 (6H, s, $N^+(CH_3)_2$) 3.30 (s, MeOD), 3.47–3.88 (10H, m, 2 × N^+CH_2, H-2,3,4,5,6,6′(Gal)), 4.07–4.11,4.25–4.27 (2H, m, $NHCOOCH_2$) 4.45–4.48 (1H, dd, $J_1 = 9.6$, $J_2 = 5.6$, H-1(Gal)), 4.84 (s, H_2O), 5.48–5.56 (1H, m, NHCOO*CH*), 7.89 (s, $CHCl_3$), 8.48 (s, NH);

^{13}C NMR (400 MHz, MeOD, δ ppm): 156.84,157.96 (NHC=O), 87.01,87.69 (C-1, α/β(Gal)), 81.03($CHCl_3$), 67.71,70.76,71.28,76.09 (C-2,3,4,5(Gal)), 67.22 (NHCOO*CH*), 65.16,66.98 (N^+CH_2), 63.25 ($NHCOOCH_2$), 63.18 (C-6(Gal)), 52.29,52.53 ($N^+(CH_3)_2$), 49.00 (MeOD), 41.95,42.08 ($NHCH_2$), 23.29–33.07 (($CH_2)_{12}$, SCH_2 (Et-Linker)), 14.45 (CH_3CH_2);

MS (positive): m/z 820.4453 $[M-Br]^+$.

Equilibrium Surface Tension

The surface tensions of the galactolipid solutions of different concentrations were measured twice using a tensiometer at 25 ± 0.1 °C until the experimental error was within 0.2 mN·m^{-1}. The curves of surface tension versus logarithm of surfactant concentrations (γ-log C) were plotted and the critical micelle concentration (CMC) values were determined from the break points. The surface tensions at CMC (γ_{CMC}) were also obtained from the curves of γ-log C. The maximum Gibbs surface excess, Γ_{max}, at the air–water was calculated by Gibbs adsorption isotherm equation (Eq. 1), where the value of n (a constant determined by the number of species constituting the surfactant and adsorbed at the interface) is taken as 1, R is the gas constant (8.314 J mol^{-1}K^{-1}), T is the absolute temperature (K), and dγ/dlog C is determined by the slope of the curve of dγ-log C when the concentration is near the CMC.

$$\Gamma_{max} = \frac{-1}{2.303\ nRT}\left[\frac{d\gamma}{d\log C}\right]_T \text{ mol} \times \text{cm}^{-2} \quad (1)$$

The minimum average area occupied per surfactant molecule A_{min} (nm^2) at the air/water interface when the surface adsorption was saturated was calculated by Eq. 2, where N is Avogadro's number.

$$A_{min} = \frac{10^{14}}{N_A \Gamma_{max}} \text{ nm}^2 \quad (2)$$

The critical packing parameter, P, which is widely used to elaborate the formation and transformation of the system of the surfactant congeries was calculated by Eq. 3, where V is the volume of hydrophobic portion of surfactant, and a_0 is the area of the molecular head or the hydrophilic part. L_c is the maximum extended length of the hydrophobic chain. Their values were estimated from Eqs. 4–6.

$$P = \frac{V}{a_0 L_c} \quad (3)$$

$$V = 54.30 \times C_3 + 27.05 \times C_2 \text{ Å}^3 \quad (4)$$

$$L_c = 1.50 + 1.265 \times C_1 \text{ Å}^3 \quad (5)$$

$$a_0 = A_{min} \quad (6)$$

where, C_1, C_2 and C_3 are the amount of carbon, methylene and methyl, respectively.

Size and Zeta Potential Measurements

In the formulation of cationic liposomes containing DOPE as a helper lipid, cationic lipid/DOPE at a molar ratio of 1:1 was prepared by conventional thin film evaporation and an ultrasonic method [12, 13]. The aqueous solutions of the cationic liposomes were sonicated in the ultrasonic bath. The liposomes were obtained by filtration through the 200 nm membrane, final concentration of which was 1 mg/mL. Particle sizes and zeta potentials were measured at r.t. by Nanoparticle Size and Zeta Potential Measurement.

Transmission Electron Microscopy

Observation by transmission electron microscope (TEM) was performed with a negative staining method. A drop of solution was placed on a Formvar covered TEM grid (copper grid, 3.02 mm, 200 mesh) and stained with a drop of 2 wt% phosphotungstic acid aqueous solution. The excess solution was removed by blotting with a filter paper. TEM, operating at 80.5 kV, was used to investigate the samples.

Results and Discussion

CMC, γ_{CMC}, Γ_m, A_m and P

The surface tensions of aqueous solutions of QAS C_n-2-S were measured at 25 ± 0.1 °C. The results of surface tension measurements are shown in Fig. 2. The surface tension decreases with increasing concentration and then reaches a clear break point, which is taken as the CMC. CMC as one of the main parameters for surfactants is the concentration at which micelles are formed initially, which will be useful for the formation of molecular self-organized assemblies. The values of CMC, γ_{CMC}, Γ_m, A_m and P are given in Tables 1 and 2.

It is well known that the CMCs of conventional ionic surfactants decrease with the increase of the carbon number of the hydrophobic chain up to 16 [14]. In this research, CMCs of QAS C_n-2-S increased from 4.5 × 10^{-6} to

Table 1 Physicochemical properties of the surfactants QAS C_n-2-S(O)

Surfactants	γ_{CMC}/(mN·m^{-1})	CMC/(mol·L^{-1})	Γ_{max}/(mol·cm^{-2})	A_{min}/(nm^2)
QAS C_{12}-2-O	26.1	2.4×10^{-4}	7.07×10^{-10}	0.235
QAS C_{12}-2-S	25.7	4.5×10^{-6}	8.28×10^{-10}	0.201
QAS C_{14}-2-S	51.0	4.0×10^{-5}	5.21×10^{-10}	0.319

Note: Data of QAS C_{12}-2-O is from Ref. [11]

Table 2 Critical packing parameter of the surfactants QAS C_n-2-S

Surfactants	V/Å^3	L_c/Å	P
QAS C_{12}-2-S	1,001.65	50.835	0.98
QAS C_{14}-2-S	1,109.31	55.755	0.62

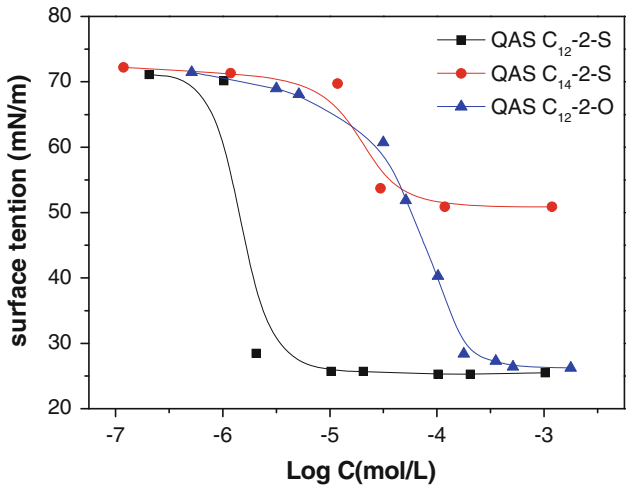


Fig. 3 Surface tension (γ) versus log C of QAS C_n-2-S in H_2O at 25 ± 0.1 °C

Table 3 Size and Zeta-potential of liposomes

Liposomes	Average size/nm	PDI	Zeta potential/mV
Lipo C_{12}-2-S	613.8	0.607	44.3
Lipo C_{14}-2-S	377.7	0.410	39.9

4.0×10^{-5} mol·L^{-1} with increasing the hydrophobic chain from 12 to 14, which is not consistent with the tendency of the common homologous. Generally, hydrocarbon chain of the surfactant demonstrates weak affinity with water molecules due to its hydrophobicity, thus making the interfacial free energy between the hydrophobic hydrocarbon chain and water much higher. In order to reduce this high interfacial free energy, the hydrophobic hydrocarbon chain often remains in a coiled state. When the length of the hydrophobic hydrocarbon chain exceeds a certain value, the coiled and disorder state of the hydrophobic chain will be further strengthened, so that it hinders the self-assembly process of the surfactant molecules and the formation of micelles, thus giving higher CMC [15].

As expected, the surfactants QAS C_n-2-S exhibited remarkable physicochemical properties with low CMC and great efficiency at lowering surface tension. As shown in Fig. 3, it is clearly seen that QAS C_{12}-2-S displayed smaller CMC than that of the conventional surfactant QAS C_{12}-2-O [11]. Of note, the CMC of the surfactant was smaller by approximately two orders of magnitude, while γ_{CMC} decreased by about 0.5 mN m^{-1}. This indicates that the surfactant QAS C_{12}-2-S with sulfur bond absorbs strongly at the air–water interface compared to QAS C_{12}-2-O, orienting itself to cause superior surface activity [16]. The water solubility of QAS C_n-2-S at 25 °C was markedly dependent on the chain length, which affected the regularity of the equilibrium surface tension of QAS C_{12}-2-S. The critical surface tension (γ_{CMC}) of QAS C_n-2-S was thought to decrease with the increase of the chain length. However, the γ_{CMC} of QAS C_{12}-2-S increased from 25.7 to 51.04 mN·m^{-1} while n ranged from 12 to 14, which may be ascribed to the low water solubility of QAS C_{14}-2-S. Furthermore, considering the small steric hindrance among the long hydrocarbon chains of QAS C_n-2-S, the alkyl chains can rotate freely and have the possibility to cover each other. This may result in a decrease in methyl groups and an increase in methylene groups at the outer surface. As the surface energy of the methylene group is larger than that of the methyl group, the more methylene groups at the outer surface, the larger the value of γ_{CMC} of QAS C_n-2-S. The surface energy of the methylene group may also contribute to the fact that the γ_{CMC} of QAS C_{14}-2-S was larger than that of QAS C_{12}-2-S.

It is of vital importance whether vesicles could be formed or not for QAS C_n-2-S as a gene delivery vehicle in future. Tanford [17] and Israelachvili [18] raised that the critical packing parameter P predicts the formation of various surfactant. Different P values are compatible with different geometric shapes of the aggregates. When P is less than 1/3, spherical micelles are the preferred form of aggregation, as the ratio of surfactant head group area is large in comparison to the hydrophobic part. Cylindrical micelles form when P is between 1/3 and 1/2. When P is greater than 1/2, first formed are highly curved bilayer vesicles and then flat bilayers as P goes to 1. The structure

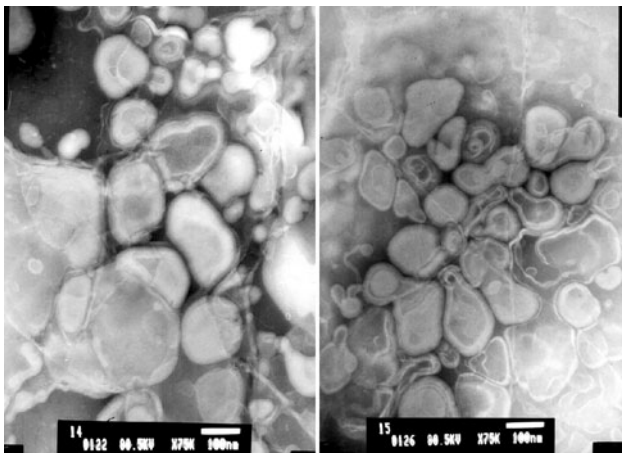


Fig. 4 Negatively stained (phosphotungstic acid) TEM image of the vesicles: *Left* is Lipo C_{12}-2-S, *Right* is C_{14}-2-S

of QAS C_n-2-S is single head and two hydrophobic carbon chains. As for QAS C_{12}-2-S, P is 0.98, which indicates flat bilayers are formed. In addition, the P value of QAS C_{14}-2-S is 0.62, which shows curved bilayer vesicles are energetically favored.

Particle Size and Zeta-Potential

Particle-size refers to the equivalent diameter of the particles of the cationic liposomes. Zeta-potential is the potential of the shear plane, which is an important indicator to characterize the stability of colloidal dispersions. Positively charged cationic liposomes can be combined with the negatively charged DNA to achieve the purpose of gene transfection. As a result, the particle size and zeta potential of cationic liposomes directly affect their transfection efficiency.

The results on particle sizes and zeta potentials of the prepared liposomes are given in Table 3. From the recorded data it is seen that the cationic liposome with a shorter hydrophobic chain exhibits a larger size with a high polydispersity and higher zeta-potential. A PDI of the liposome over 0.5 could put its gene delivery at a disadvantage. As a result, large size and high PDI of Lipo C_{12}-2-S might have a poor influence on gene transfection efficiency. However, Lipo C_n-2-S shows a positive charge, which is expected to compound with negatively charged plasmid DNA and to obtain a satisfactory gene transfection efficiency. Whether the results of transfection efficiency are good or not should be determined with actual biological experiments in the future.

Formation of Vesicles

To further confirm the formation of vesicles, a TEM measurement is employed. A typical negatively stained TEM image of vesicles is formed in C_n-2-S/DOPE aqueous system, as shown in Fig. 4. The bilayer structure and existence of inner aqueous phase can be clearly seen, indicating the QAS C_n-2-S is capable of vesicle formation.

The left presented the different sizes of C_{12}-2-S/DOPE liposome, mainly large particle-size vesicles. In addition, the right illustrated the vesicle formation of C_{14}-2-S/DOPE liposome. Evidently, Lipo C_{14}-2-S displayed ones of smaller size and better polydispersity compared to Lipo C_{12}-2-S, which was consistent with the results of size measurement. According to the critical packing parameter P, QAS C_{14}-2-S was inclined to form vesicles and QAS C_{12}-2-S favored flat bilayers, which lead to the size of Lipo C_{14}-2-S much smaller. In the aqueous system, the average particle size of liposomes showed comparatively large due to vesicles aggregation.

Conclusion

Two double chain cationic lipids C_n-2-S derived from thio galactose were synthesized and confirmed by MS, TOF-MS, ^{1}H NMR and ^{13}C NMR. QAS C_{12}-2-S appears lower CMC value and γ_{CMC} than QAS C_{14}-2-S counterpart. Vesicles are formed in aqueous solution and it is consistent with the result of the critical packing parameter. Moreover, negatively stained TEM images confirmed the formation of vesicles.

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