Supporting Information

Coating of carboxymethyl dextran on liposomal curcumin to improve the anticancer activity

Qiyu Huang, Lili Zhang, Xiaoyi Sun, Ke Zeng, Juan Li and You-Nian Liu**

College of Chemistry and Chemical Engineering, Central South University, Changsha, Hunan, 410083, China.

E-mail: juanli@csu.edu.cn; *liuyounian@csu.edu.cn*

Tel.(Fax): 86-731-88879616

Synthesis of mPEG-OA

To be specific, 10.0 g of mPEG2000, 60 mg of KBr and 4 mg of TEMPO (200:20:1 in molar ratio) were weighed out and dissolved in 70 mL deionized water. NaClO of 15 mmol (11 mL) was cooled down to 0^{o}C in ice bath and had pH adjusted to 10 by HCl. The above NaClO was then quickly added into mPEG mixture and stirred in ice bath for 5 h. NaOH (0.5 M) was used to maintain the pH around 10 during the reaction. After the oxidation was complete, 10 mL of ethanol was added and the pH was adjusted to 3 by adding 3 M HCl. The oxidized product mPEG-COOH was extracted twice by dichloromethane followed by drying with anhydrous Na_2SO_4. Light yellow sticky liquid was obtained after the removing the solvents by rotary evaporation. It was then washed twice by diethyl ether and turned into white solid after cooling.

The solid mPEG-COOH was dissolved in dichloromethane and followed by the addition of 6 mL $SOCl_2$. The mixture was refluxed for 1 h at 45^{o}C and the residual $SOCl_2$ was removed by rotary evaporation. The obtained white solid was again dissolved in dichloromethane and 1.5 g (5.6 mmol) oleylamine was added. The mixture was stirred for 2 h at 0^{o}C and appeared as light yellow liquid. After removing the organic solvent, washing by diethyl ether several times and drying under vacuum, the final product of mPEG-OA was obtained in white powder.

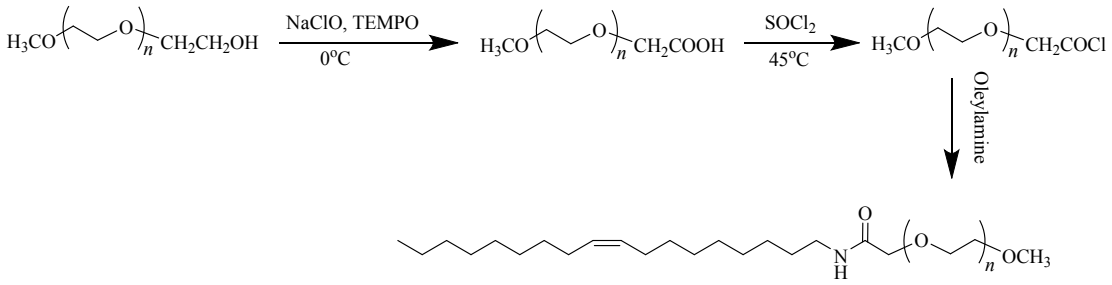

Figure S1. Synthesis of mPEG-OA

Synthesis of PEG-OA

The synthesis of PEG-OA was similar to that of mPEG-OA except for that the amount of KBr, TEMPO and NaClO used was doubled to oxidize the extra -OH on PEG.

Synthesis of CMD-OA

The synthesis of CMD was as follows. Dextran T-10 (1.0 g, equal to 6 mmol glucopyranose units) was dissolved in 30.0 mL of 2.0 M NaOH solution containing 0.1 M bromoacetic acid (0.41 g, 3 mmol). After stirring at room temperature for 12 h, the mixtures were dialyzed against deionized water for 24 h, then against 0.1 M HCl for 24 h and finally against deionized water for 24 h. The white powder of CMD was obtained after lyophilization and used for the synthesis of CMD-OA thereafter. CMD (0.3 g, equal to 0.5 mmol COOH) was dissolved in 20 mL DMF with small amount of LiCl and heated at 80°C for 10 min. After being cooled down to room temperature, CMD was activated with a 0.1 M NHS/0.4 M EDC mixture (10 mL) for 10 min in an ice bath. Oleylamine (0.2 mmol) in DMF was dropped into activated CMD and the solution was stirred at room temperature for 24 h. The crude product was precipitated into absolute alcohol twice, and then dialyzed against deionized water for 72 h. The final product of hydrophobized CMD was obtained after lyophilization.

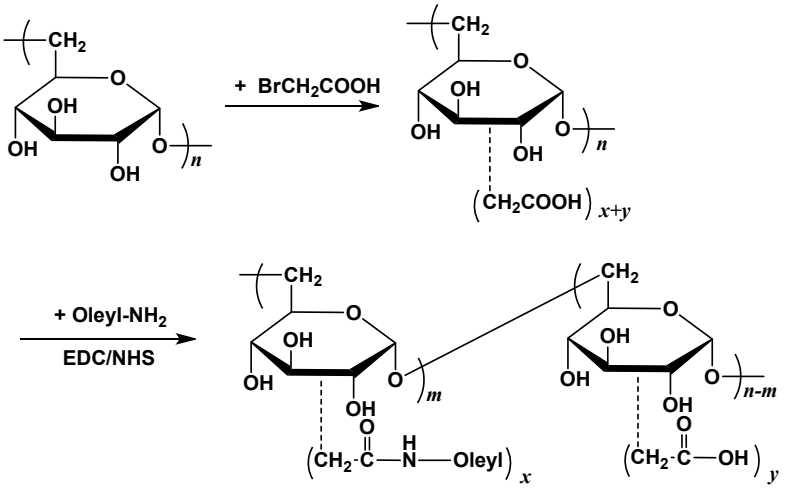

Figure S2. Synthesis of CMD-OA

Titration

For CMD and CMD-OA, to be specific, 10 mL of deionized water and 2.5 mL of 0.1 M NaOH were added into 0.05 g of the tested samples and heated under stirring. Two to three drops of indicator phenolphthalein was then added into each solution. After stirring for 30 min, titration was performed by adding 0.1 M HCl dropwise. When the solution turned colorless, the solution was subjected to stirring for another 30 s. The above steps were repeated until no further red color appeared. The content of carboxymethyl groups (the degree of substitution, D_s) was calculated by the following equation:

$$D_s=0.162A/(1-0.058A),\ A=(V_1C_1-V_2C_2)/m$$

In the equation, V_1 (mL) stands for the volume of NaOH used and V_2 (mL) means the volume of HCl consumed to neutralize the NaOH; C_1 and C_2 are the molecular concentrations of NaOH and HCl, respectively, and m stands for the weight of the sample. So A is the amount of NaOH needed to neutralize 1 g of CMD, which corresponds to the amount of —COOH groups in 1 g of CMD.

In this method, the D_{s1} is defined as the molar fraction of glucopyranose units carrying a CH_2COOH group, where it is assumed that only one hydroxyl group can be substituted by carboxymethyl group per unit of dextran. Furthermore, the D_{s2} of carboxymethyl groups on CMD-OA substituted by oleylamine groups was also determined.

The titration results showed that 1.30 mL of HCl was consumed for CMD. When

put in the equation, it generated a D_{s1} value of 45.1%, suggesting that 45.1% of -OH groups on the glucopyranose units were substituted with CH_2COOH groups during the carboxymethylation process. Also, the HCl consumption of 2.25 mL for CMD-OA led to a molar amount of residual [COOH] groups of 0.025 mmol per 0.05 g of CMD-OA, which was 0.50 mmol[COOH]/g. This generated the D_{s2} value of 31.7%, indicating 31.7% per glucopyranose units were substituted by oleylamine groups, with the residual [COOH] remaining 13.4% per glucopyranose units approachable for further modifications.

For PEG-OA, the method was similar and 2.37 mL of HCl was consumed to neutralize the NaOH, indicating the molar amount of [COOH] groups to be (2.5 mL × 0.1 M - 2.37 mL × 0.1 M) = 0.013 mmol per 0.05 g of PEG-OA, which was 0.26 mmol[COOH]/g.

NMR

Nuclear magnetic resonance (AVANCEIII 500M) was performed to confirm the synthesis of CMD-OA, mPEG-OA and PEG-OA, TMS was used as internal reference. About 2 mg sample was weighed out and dissolved in deuterium DMSO (CMD-OA) and deuterochloroform (mPEG-OA and PEG-OA).

For CMD-OA, δ (DMSO-d_6): 0.91 (oleyl methyl protons), 1.30 (oleyl methylene protons), 2.04 (oleyl CH=CHC<u>H</u>$_2$), 3.15–3.85 (dextran glucosidic protons), 4.03 (CMD C<u>H</u>$_2$CONH), 4.40–5.15 (hydroxyl and anomeric protons of dextran), 5.39 (oleyl C<u>H</u>=C<u>H</u>).

For mPEG-OA, δ ($CDCl_3$): 0.88 (oleyl methyl protons), 1.26 (oleyl methylene protons), 2.01 (oleyl CH=CHC<u>H</u>$_2$), 2.95 (oleyl CONHC<u>H</u>$_2$), 3.26 (mPEG methoxy end protons), 3.45–3.85 (PEG methylene protons), 4.15 (PEG C<u>H</u>$_2$CONH), 5.35 (oleyl C<u>H</u>=C<u>H</u>).

For PEG-OA, δ ($CDCl_3$): 0.89 (oleyl methyl protons), 1.28 (oleyl methylene protons), 2.02 (oleyl CH=CHC<u>H</u>$_2$), 3.12 (oleyl CONHC<u>H</u>$_2$), 3.45–3.85(PEG methylene protons), 4.17 (PEG C<u>H</u>$_2$CONH), 5.35 (oleyl C<u>H</u>=C<u>H</u>).

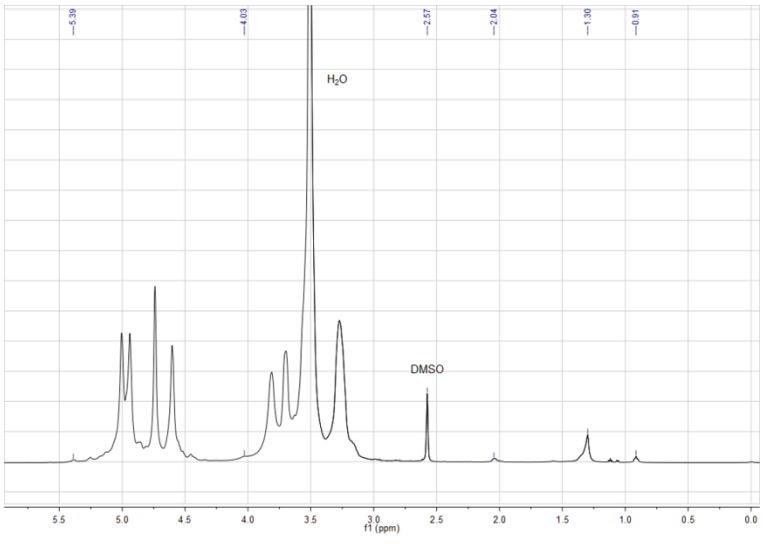

Figure S3. ^{1}H NMR spectra of CMD-OA