Supporting Information

Chemical Selective Surface Glyco-Functionalization of Liposome through Staudinger Ligation

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Experimental

Materials. All solvents and reagents were purchased from commercial sources and were used as received, unless otherwise noted. Deionized water with a resistivity of 18Ω was used as a solvent in all polymerization reactions.

Method. Thin-layer chromatography (TLC) was performed on Whatman silica gel aluminum backed plates of 250 μm thickness on which spots were visualized with UV light or by charring the plate after dipping in 10% H_2SO_4 in methanol. ^{1}H NMR spectra were recorded with a Varian INOVA 300 MHz spectrometer. In all cases, the sample concentration was 10 mg/mL, and the appropriate deuterated solvent was used as an internal standard. Dynamic Light Scattering was recorded with 90Plus particle size analyzer (BIC). Fluorescent spectrum was measured with FluoroMax-2 (ISA).

1-Succinimidyl 3-diphenylphosphino-4-methoxycarbonylbenzoate (4) was synthesized as literature method.[1]

DPPE-Triphosphine 5: DPPE (0.26 g, 0.376 mmol) was dissolved in 40 mL of CH_2Cl_2, and 0.8 mL Et_3N was added. After stirring for 30 min at room temperature, a solution of succinimidyl 3-diphenylphosphino-4-methoxycarbonylbenzoate (**4**) (0.23 g, 0.500 mmol) in 50 mL CH_2Cl_2 was added. The reaction mixture was stirred at room temperature for 24 h and then concentrated. The crude product was purified by silica gel chromatography using chloroform/methanol (4:1) as elute to afford **5** (0.283 g, 73%). ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{31}P NMR ($CDCl_3$, 121 MHz) spectra are attached.

Oxidation of 2: A solution of H_2O_2 (3%, 25 mL) was added to a solution of **2** (150 mg) in 3 mL $CHCl_3$ at room temperature, then the mixture was stirred at 45℃ for 6 h. The insoluble materials were collected by filtration. And the chloroform layer was separated and concentrated in vacuum. The two parts were combined to afford phosphine oxide 1-Methyl-2-Diphenylphosphinoterephthalate oxide **3** (154 mg, 98%).

Reduction of 3: A mixture of **3** (0.172 g, 0.45 mmol), trichlorosilane (0.3 mL, 3 mmol), triethylamine (0.4 mL, 3mmol) was dissolved in toluene (10 mL) and stirred at reflux for 6 h under nitrogen. Then the mixture was cooled to room temperature and excess of trichlorosilane was evaporated in vacuo. Next saturated $NaHCO_3$ aqueous solution (1 mL) was added. The mixture was stirred for 5 min at room temperature; the organic layer was separated, dried over $MgSO_4$ and evaporated to afford **2** (0.158 g, 97%).

Preparation of Liposome

DPPC (30 mg, 40.87 μmol), cholesterol (8 mg, 20.4 μmol), DPPE-Triphosphine **5** (3.5 mg, 3.2 μmol) (2:1:0.5% molar ratio) were dissolved in 3.0 mL chloroform. The

solvent was gently removed on an evaporator under reduced pressure to form a thin lipid film on the flask wall and kept in a vacuum chamber overnight. Then, the lipid film was swelled in the dark with 2.5 mL PBS buffer (pH 7.4) form the multilamellar vesicle suspension. Followed, subjected 10 freeze-thaw cycles involving quenching in liquid N_2 and then immersion in a 50℃ water-bath. The crude lipid suspension thus formed, followed by extruded through polycarbonate membranes (pore size 2000 nm, 600 nm, 200 nm, 100 nm gradually) to obtain the liposome.

Carboxyfluorescein-encapsulated liposome was prepared in the same condition above by containing 85 mM Carboxyfluorescein (CF). Separation of the CF encapsulated liposome from non-encapsulated CF was achieved by gel chromatography, which involved passage through a 1.5 × 20 cm Column of Sephadex G-50.

Conjugation of lactose onto liposome surface

azido-lactose (4 mg, 0.01 mmol) in 0.2 mL PBS buffer (Argon bubble before use) was added into 2 mL of liposome obtained above. The Staudinger ligation was conducted at room temperature for 6 h under an argon atmosphere, then unreacted azido-lactose was removed by gel filtration (1.5 × 20 cm Column of Sephadex G-50). The size of liposomes during the Staudinger ligation was monitored over time by using 90Plus particle analysizer. Control experiment was conducted in the absence of azide-lactose.

Fluorescence Assay

The stability of liposome was also monitored by measurement of fluorescent leakage using FluoroMax-2 (ISA). 20 μL reaction solution and 1980 μL PBS were mixed, and then diluted into 10 times. The fluorescent intensity was measured by using FluoroMax-2 (ISA). Control experiment was conducted in the absence of azide-lactose.

Determination of concentration of lactose on the liposome surface

Standard curve was prepared as described by Saha *et al.*[2] with free lactose solution. To 0.5 mL of liposome with lactose, 0.5 mL of 0.5% phenol solution was added and mixed. Then 2.5 mL concentrated H_2SO_4 was added directly into the solution. The mixture was then vortexed, and allowed to stand for 30 min at room temperature. The optical eadings were taken at 490 nm. The amount of lactose was calculated from calibration curve.

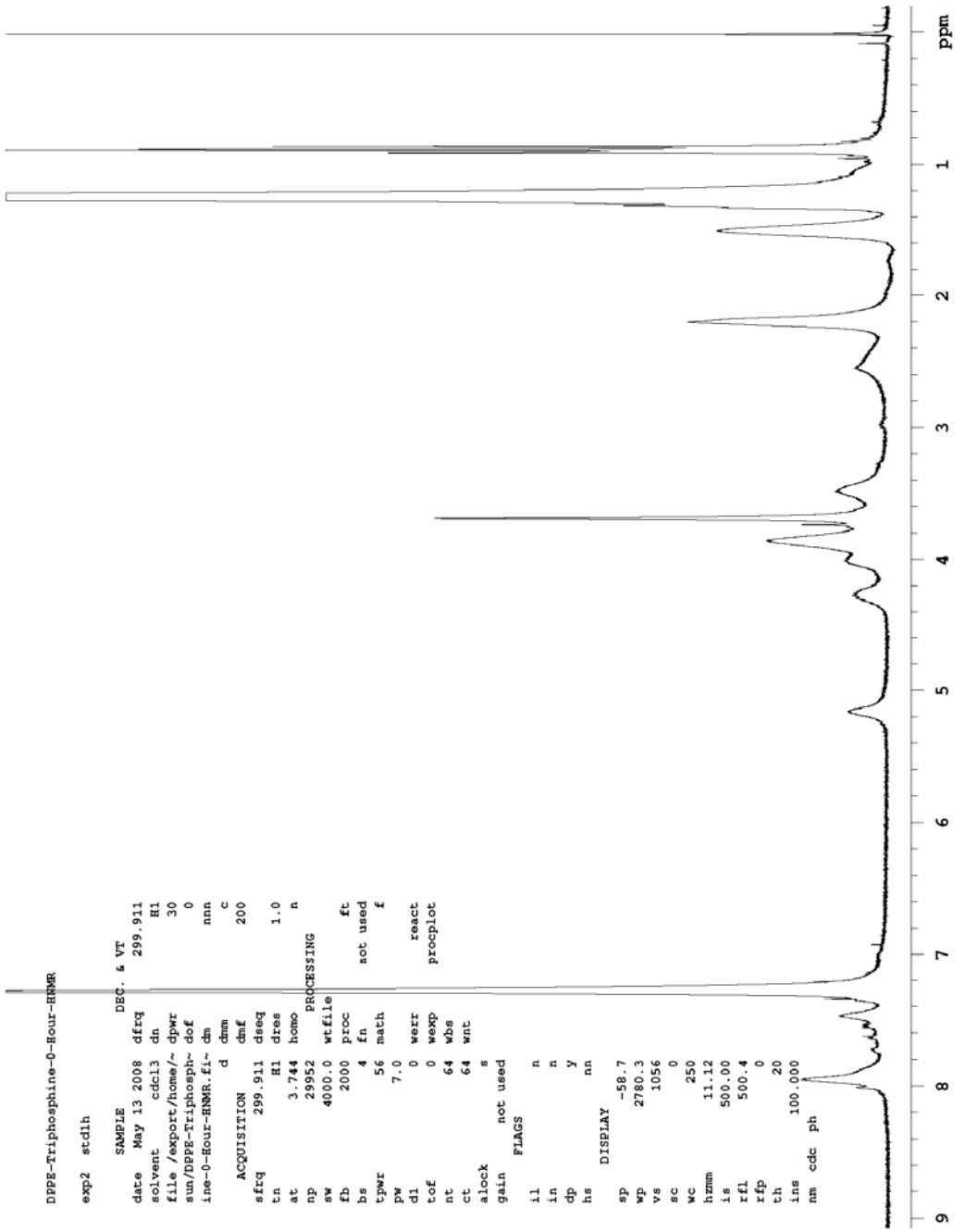
Characterization of specific binding between lactose on the liposome surface and lectin

To access the accessibility of lactose grafted onto the liposome, Lectin (*β*-galactose binding lectin, *Arachis hypogae,* 120 kDa, SIGMA) PBS solution (0.5 mg/0.5 mL, pH 7.4) was added into 100 μL PBS solution of lactose conjugated liposome. The size of liposomes and stability of liposome were monitored with DLS over time. Control was conducted with liposome without lactose.

Reference:

1. C. C.-Y. Wang, T. S. Seo, Z. Li, H. Ruparel, J. Ju, *Bioconjugate Chem*. 2003, **14**, 697.
2. S. K. Saha, and C.F. Brewer, *Carbohydr. Res.*, **1994**, *254*, 157–167.

^{1}H NMR spectrum of DPPE-Triphosphine conjugate **5** (in $CDCl_3$)



^{31}P NMR spectrum of DPPE-Triphosphine conjugate **5** (in $CDCl_3$)