

Supplementary Material

Recognition of lectin with high S/N ratio: carbohydrate- / tri(ethylene glycol)alkanethiol co-adsorbed monolayer

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Experimental

1. Materials

1.1 Synthesis of 1-Mercapto-11-hydroxy-3,6,9-trioxaundecane (EGC2SH)

Tetraethylene glycol monotosylate: Tetraethylene glycol (9.70 g, 50 mmol), triethyl amine (2.02 g, 20 mmol), and THF (150 ml) were placed in a three-necked flask and stirred at 0°C. A THF solution (20 ml) of p-toluenesulfonyl chloride (1.91 g, 10 mmol) was added dropwise to the mixture, and the reaction mixture was stirred for 4 days at room temperature. The reaction mixture was poured into aqueous HCl (5 wt%), and the product was extracted with chloroform. The crude product obtained by solvent evaporation was purified by gel permeation chromatography (GPC) (colorless liquid, 82% yield). The product was characterized by ¹H NMR. C₁₅H₂₄O₇S; MW=348.37

¹H-NMR (CDCl₃, 500MHz) δ 2.44 (3H, s), 3.58-3.74 (14H, m), 4.16 (3H, t, J=4.80 Hz), 7.34 (2H, d, J=8.70 Hz), 7.80 (2H, d, J=8.25 Hz)

Tetraethylene glycol monothioacetate: Tetraethylene glycol monotosylate (3.48 g, 10 mmol) and DMF (100 mL) were placed in a three-necked flask in a nitrogen atmosphere and stirred at room temperature. S-potassium thioacetate (3.43 g, 30 mmol) was added to the mixture, and the reaction mixture was stirred for 12 hours at room temperature. The reaction mixture was poured into aqueous HCl (5 wt%), and the product was extracted with chloroform. The crude product (colorless liquid, quantitative yield) obtained by solvent evaporation and drying in a vacuum was used for the subsequent reaction without purification. C₁₀H₂₀O₅S; MW=252.33

¹H-NMR (CDCl₃, 500MHz) δ 2.33 (3H, s), 3.09 (2H, t, J=6.40 Hz), 3.56-3.75 (14H, m)

1-Mercapto-11-hydroxy-3,6,9-trioxaundecane: Tetraethylene glycol monothioacetate (2.52 g, 10 mmol) and EtOH (200 ml) were placed in a three-necked flask in a nitrogen atmosphere and

stirred at room temperature. Potassium t-butoxide (1.12 g, 10 mmol) was added to the mixture, and the reaction mixture was stirred for 3 hours at room temperature. The reaction mixture was poured into aqueous HCl (5 wt%), and the product was extracted with chloroform. The crude product obtained by solvent evaporation was purified by silica-gel column chromatography (chloroform/MeOH=100/1 v.v., colorless liquid, 59% yield). $C_8H_{18}O_4S$; MW=210.29

1H -NMR ($CDCl_3$, 500MHz) δ 1.63 (1H, t, J=8.25 Hz), 2.70 (2H, t, J=7.33 Hz), 3.59-3.76 (14H, m)

Anal.Calcd for $C_8H_{18}O_4S$: C, 45.69; H, 8.560, S, 15.24. Found: C,45.29; H, 8.579, S, 14.89.

1.2 1-Mercapto-13-hydroxy-5,8,11-trioxatridecane (EGC4SH)

1-Bromo-13-hydroxy-5, 8,11-trioxatridecane: 1,4-dibromobutane (8.64 g, 40 mol), triathlon glycol (60.1 g, 400 mol), and THF (800 ml) were placed in a three-necked flask, and stirred at room temperature. Potassium t-but oxide (4.48 g, 40 mol) was added to the mixture, and the reaction mixture was refluxed for 6 hours. The solvent was evaporated, the reaction mixture was poured into aqueous Hal (5 wt%), and the product was extracted with chloroform. The crude product obtained by solvent evaporation was purified by silica-gel column chromatography (chloroform/MeOH=100/0~0.5 v.v., colorless liquid, 28% yield). $C_{10}H_{21}O_4Br$; MW=285.18

1H -NMR ($CDCl_3$, 500MHz) δ 1.69-1.77 (2H, m), 1.91-1.98 (2H, m), 3.43 (2H, t, J=6.63 Hz), 3.49 (2H, t, J=6.43 Hz), 3.56-3.75 (12H, m)

1-Bromo-13-benzoyloxy-5,8,11-trioxatridecane: 1-bromo-13-hydroxy-5,8,11-trioxatridecane (2.85 g, 10 mmol), triethyl amine (2.52 g, 25 mmol), and THF (100 mL) were placed in a three-necked flask, and stirred at 0°C. A THF solution (20 mL) of benzoyl chloride (2.81 g, 20 mmol) was added dropwise to the mixture, and the reaction mixture was stirred for 24 hours at room temperature. The reaction mixture was poured into aqueous HCl (5 wt%), and the product was extracted with chloroform. The crude product obtained by solvent evaporation was purified by GPC (colorless liquid, 87% yield). $C_{17}H_{25}O_4Br$; MW=389.29

1H -NMR ($CDCl_3$, 500MHz) δ 1.62-1.78 (2H, m), 1.90-1.99 (2H, m), 3.40-3.78 (12H, m), 3.84 (3H, t, J=4.83 Hz), 4.49 (2H, t, J=5.05 Hz), 7.44 (2H, t, J=7.78 Hz), 7.56 (1H, t, J=7.33 Hz), 8.06 (2H, d, J=7.30 Hz)

1-Acetylthio-13-benzoyloxy-5,8,11-trioxatridecane:

1-Bromo-13-benzoyloxy-5,8,11-trioxatridecane (779 mg, 2 mmol) and DMF (50 mL) were

placed in a three-necked flask in a nitrogen atmosphere and stirred at room temperature. S-potassium thioacetate (684 mg, 6 mmol) was added to the mixture, and the reaction mixture was stirred for 4 hours at room temperature. The reaction mixture was poured into aqueous HCl (5 wt%), and the product was extracted with chloroform. The crude product obtained by solvent evaporation was purified by GPC (colorless liquid, 72% yield). $C_{19}H_{28}O_6S$; MW=384.49

1H -NMR ($CDCl_3$, 500MHz) δ 2.31 (3H, s), 2.85-2.91 (2H, m), 3.42-3.47 (2H, m), 3.54-3.57 (2H, m), 3.62-3.74 (6H, m), 3.84 (3H, t, J=5.05 Hz), 4.48 (2H, t, J=4.80 Hz), 7.43 (2H, t, J=7.80 Hz), 7.56 (1H, t, J=7.33 Hz), 8.05 (2H, d, J=8.20Hz)

1-Mercapto-13-hydroxy-5,8,11-trioxatridecane:

1-Acetylthio-13-benzoyloxy-5,8,11-trioxatridecane (769 mg, 2 mmol) and EtOH (80 mL) were placed in a three-necked flask in a nitrogen atmosphere and stirred at room temperature. Potassium t-butoxide (224 mg, 2 mmol) was added to the mixture, and the reaction mixture was stirred for 3 hours at room temperature. The reaction mixture was poured into aqueous HCl (5 wt%), and the product was extracted with chloroform. The crude product obtained by solvent evaporation was purified by silica-gel column chromatography (chloroform/MeOH=100/0~0.5 v.v., colorless liquid, 83% yield). $C_{10}H_{22}O_4S$; MW=238.34

1H -NMR ($CDCl_3$, 500MHz) δ 1.35 (1H, t, J=7.80 Hz), 1.64-1.74 (4H, m), 2.51-2.60 (2H, m), 3.44-3.53 (2H, m), 3.56-3.77 (12H, m)

Anal.Calcd for $C_{10}H_{22}O_4S$: C, 50.39; H, 9.230, S, 13.45. Found: C,49.67; H, 9.382, S, 12.74.

1.3 Synthesis of 1-Mercapto-15-hydroxy-7,10,13-trioxatridecane (EGC6SH) and 1-Mercapto-17-hydroxy-9,12,13-trioxatridecane (EGC8SH): EGC6SH and EGC8SH were made in a similar way to EGC4SH.

EGC6SH: colorless liquid, 90% yield, $C_{12}H_{26}O_4S$; MW=266.40.

1H -NMR ($CDCl_3$, 500MHz) δ 1.31-1.45 (5H, m), 1.55-1.64 (4H, m), 2.52 (2H, q, J=7.47 Hz), 3.45 (2H, t, J=6.88 Hz), 3.56-3.75 (12H, m)

Anal.Calcd for $C_{12}H_{26}O_4S$: C, 54.10; H, 9.760, S, 12.03. Found: C,53.39; H, 9.680, S, 11.30.

EGC8SH: colorless liquid, 91% yield, $C_{14}H_{30}O_4S$; MW=294.45.

1H -NMR ($CDCl_3$, 500MHz) δ 1.24-1.42 (9H, m), 1.53-1.64 (4H, m), 2.51 (2H, q, J=7.32 Hz), 3.44 (2H, t, J=6.65 Hz), 3.55-3.75 (12H, m)

Anal.Calcd for $C_{14}H_{30}O_4S$: C, 57.11; H, 10.19, S, 10.89. Found: C,56.11; H, 10.24, S, 10.42.

1.4 Others

Proteins (Con A (Vector Laboratories) and BSA (sigma)) and peptides (Angiotensin, bradykinin, and RGDS (Peptide Institute, Inc.)) were used as received. MethoxyPEG-thiol (PEG 2000, SUNBRIGHT(ME-020SH, MW 2000)) was purchased from NOF Corporation. Other chemicals used in this experiment were special grade. All aqueous solutions were prepared using ultrapure water obtained from a Millipore system.

2 Modification of gold surface with the EGCnSH or MalC12SH- EGCnSH monolayers.

Self- assembled EGCnSH or MalC12SH- EGCnSH hybrid monolayers were formed on an unmodified gold chip surface (BIACORE SIA Kit Au, BIACORE, Uppsala, Sweden) by using 10 μ M EGCnSH (or MalC12SH- EGCnSH (1:9)) in 20% ethanol – 10 mM PBS buffer (0.1 M NaCl, pH 7.2). The modification time was 30 min (10 μ l/min). The modified chip surface was sequentially washed with PBS buffer and HBS-EP buffer (10 mM HEPES buffer, 0.15 M NaCl, 3 mM EDTA, 0.05% Tween 20, pH 7.4) . The total amount of adsorbed molecules was evaluated in terms of the Resonance Unit (1RU= 1 pg/mm²) changes. The electrochemical reductive desorption method was also adopted to evaluate the packing density of each monolayer. Electrochemical measurement was carried out in 0.5 M KOH solution at room temperature after the solution had been deaerated by flowing Ar gas through it. The sweep rate was 50 mV/sec.

3 Evaluation of the amount of adsorbed proteins (Con A, BSA) and peptides (Angiotensin, Bradykinin, and RGDS) on the EGCnSH or MalC12SH- EGCnSH hybrid monolayers

Protein and peptide adsorption was evaluated by SPR measurement (BIACORE 2000 system). In this experiment, 10 μ M Con A was applied to each monolayer for 20 min (10 μ l/min) and followed by the running buffer (HBS-EP, 10 μ l/min). The following condition was adopted for other proteins and peptides; sample concentrations: BSA (10 μ M), angiotensin (0.1 mg/ml), bradykinin (0.1 mg/ml), and RGDS (0.1 mg/ml); other conditions were the same as with Con A. NaOH solutions (10-20 mM) were used as a regeneration reagent (for 30 sec, 60 μ l/min).

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