Supporting Information

Facile Synthetic Method of Alkanethiol Spacer for Bio-interface

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General

Materials

2-Chlorotrityl chloride (CTC) resin, N-hydroxybenzotriazole (HOBt) and Fmoc-OSu was obtained from BeadTech Inc. (Korea). N-Hydroxysuccinimide (NHS) and succinic anhydride were purchased from Fluka (Japan). 11-Mercapto-1-triethyleneglycol-undecanoic acid (HSC_{11}-EG_{3}-COOH) was purchased from ProChimia Surfaces, Sopot (Poland). All other reagents were purchased from Sigma-Aldrich (USA). The solvents were reagent grade from Daejung Chemicals Co. (Korea) and used without purification.

Instruments

For the resin characterization and analysis, FT-IR (Bomem, FTLA2000) and elemental analysis (Leco, CHNS-932) were used. For the fabrication of the gold spot, an e-beam evaporator (Anelva, VN-43) was used. In order to analyze the compound’s structure, 1H-NMR (Japan Electronic Optics Laboratory, JNM-LA300) was used. For the surface characterization and analysis, X-ray photoelectron spectroscopy (XPS) (SIGMA PROBE, ThermoVG) and static water contact angle measurement (SEO, Phoenix 300) were used. For the fluorescence analysis, fluorescence scanner (Pekin Elmer, ScanArray® Lite) was used. Probe DNA (5’-NH₂-TCTATAACAAT-3’), complementary target DNA (5’-Cy₃-ATTGTTATTAGGA-3’) and non-complementary control DNA solution (5’-Cy₃-GTACCTATTGCCCT-3’) were purchased from Bioneer (Daejeon, South Korea). Tris-EDTA buffer solution was also purchased from Bioneer (Daejeon, South Korea).

Experimental Section

1. Synthesis of Fmoc-TEG-COOH

4,7,10-Trioxa-1,13-tridecanediamine (NH₂-TEG-NH₂, 2.2 g, 10 mmol) was dissolved in 50 mL acetonitrile. Succinic anhydride (1 g, 10 mmol) dissolved in 25 mL acetonitrile was added dropwise to the mixture which was vigorously stirred for 2 h. Then, the waxy product was formed, which was washed with acetonitrile 2–3 times. It was redissolved in 50 mL 50 %
acetonitrile/water and cooled in an ice bath. After adding N,N-diisopropylethylamine (DIPEA, 2.26 mL, 13 mmol), Fmoc-OSu (4.4 g, 13 mmol) dissolved in 25 mL acetonitrile was added dropwise for 1 h. The reaction mixture was vigorously stirred for 2 h at room temperature. The solvent was evaporated and the residue was dissolved in basic solution (NaHCO₃). This mixture was washed with ethylacetate 2~3 times. Then aqueous solution was acidified using 1N HCl and extracted with ethylacetate. The final organic solution was dried out by anhydrous MgSO₄ and evaporated in vacuo. The oily product was obtained in 97% (5.26 g) yield. 1HNMR (300 MHz, CDCl₃, 25 ºC, TMS): δ (ppm) = 1.74 (m, 4H), 2.47 (t, 2H), 2.64 (t, 2H), 3.30 (m, 4H), 3.45-3.60 (m, 12H), 4.19 (t, 1H), 4.39 (d, 2H), 7.29 (t, 2H), 7.38 (t, 2H), 7.59 (d, 2H), 7.74 (d, 2H).

2. Anchoring of Fmoc-TEG-COOH on CTC resin

Compound 1 (407 mg, 0.75 mmol) and DIPEA (392 μL, 2.25 mmol) were dissolved in distilled CH₂Cl₂ (15 mL). This mixture was added to CTC resin (1.0 g, 1.3 mmol/g) and stirred on a shaker at room temperature. After 2 h, 2 mL DIPEA : MeOH (1 : 10 mixture) was added to cap residual chloride group on CTC resin. The resin was filtered and washed with CH₂Cl₂, MeOH (3 times, each). The amount of compound 1 loaded on resin was found to be 0.43 mmol/g by Fmoc-titration and elemental analysis. To remove Fmoc group, the resin was treated with 10 mL 20 % piperidine/DMF solution for 1 h. The resulting resin (resin 2) was washed with DMF, CH₂Cl₂, and MeOH (3 times, each).

3. Synthesis of 11,11′-dithiodiundecanoic acid

11-Mercaptoundecanoic acid (MUA) (1 g, 4.58 mmol) was dissolved in 20 mL THF, and iodine (23 mg, 0.18 mmol) was dissolved in dimethyl sulfoxide (DMSO, 2 mL). The iodine solution was added to the MUA solution and the mixture was stirred at 45 ℃ until the color change from pale yellow to red. After evaporating the solvent, the residue was dissolved in ether and washed with water 3 times. After evaporating the solvent in vacuo, the disulfide compound was obtained as a white solid in 91% (0.91 g) yield. 1H NMR (300 MHz, DMSO, 25 ℃, TMS): δ (ppm) = 1.25 (m, 24H), 1.65 (q, 8H), 2.36 (t, 4H), 2.70 (t, 4H).

4. Coupling of 11, 11′-dithiodiundecanoic acid on resin 2
Compound 3 (2 eq), N,N’-diisopropylcarbodiimide (DIC, 4 eq), 1-hydroxybenzotriazole hydrate (HOBT, 4eq), and DIPEA (4eq) were dissolved in NMP to activate the carboxylic group of compound 3. The mixture was added to resin 2, and allowed to react overnight at 40 °C. After 12 h, the resin was filtered and washed with DMF, CH₂Cl₂, and MeOH (3 times, each). The progress of coupling was monitored by checking the free amine group on resin using the Kaiser test.

5. Cleavage of alkanethiol spacer from the resin

Before cleavage of alkanethiol spacer from CTC resin, resin 4 was treated with dithiothreitol (DTT, 5 eq) in NMP for 12 h. The resulting alkanethiol spacer (compound 5) was cleaved with cleavage cocktail composed of 1% trifluoroacetic acid and 1% triisopropylsilane in CH₂Cl₂ for 10 min (×3). The residue was concentrated by evaporating the solvent (yield : 87%). 1H NMR (300 MHz, DMSO, 25 ºC, TMS): δ (ppm) = 1.23 (m, 12H), 1.47 (q, 4H), 1.60 (q, 4H), 2.02 (t, 2H), 2.28 (t, 2H), 2.40 (t, 2H), 2.81 (t, 2H), 3.06 (q, 4H), 3.36-3.35 (m, 12H); Mass analysis of alkanethiol spacer (calcd 520.72 for C₂₅H₄₈N₂O₇S) [M-H]-, found 519.5)

6. Self-assembled monolayer (SAM) on gold-coated silicon chip by alkenethiol spacer

SAMs by alkanethiol spacers were built on the gold-coated silicon chip as follows. The gold-coated silicon chips were immersed in 1 mM spacer solutions (MUA or TEG-alkanethiol spacer in EtOH) respectively. After 6 h, the gold spots were rinsed by EtOH (×3), deionized water (×3) and EtOH (×3), and dried under N₂ blowing. These chips were analyzed by XPS and water contact angle.

7. Probe DNA conjugation on the gold spots

To carry out DNA hybridization assay on SAM surface, the gold spots on the silicon wafer were freshly prepared by deposition method. SAMs of alkanethiol spacers (MUA, TEG-alkanethiol HS-C₁₁-EG₃-COOH) on gold spot was achieved by the same procedure as above. To activate the terminal carboxyl group of spacers, the gold spots, which contains each SAM
layer were immersed in 1 mM EDC (1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride) and 2 mM NHS (N-hydroxysuccinimide)/ MES (2-(N-morpholino)ethanesulfonic acid) buffer solution (pH 6.0) for 1 h at room temperature. The specific concentration of probe DNA (5’-NH₂-T CCTAATAACAAT-3’) was added to the solution containing the gold spots, thus the final concentrations of probe DNA became 10 μM. After 2 h, the gold spots with probe DNA were rinsed by deionized water (×3) and EtOH (×3) subsequently, and then dried under N₂ blowing. The gold spots with each SAM layer were kept in a desiccator before use.

8. DNA hybridization assay

The general DNA hybridization assay procedure is described as follows. The probe DNA immobilized gold spots were incubated in 1 mM or 10 mM of the target DNA (5’-Cy3-ATTGTTATTAGGA-3’), and 10 mM of non-complementary control DNA (5’-Cy3-GTACCTATTGCCT-3’) in Tris-EDTA buffer solution (TE, 100 mM Tris buffer containing 1 mM EDTA) for 2 h at room temperature. Thereafter, the gold spots were rinsed with TE buffer (×3) and deionized water, and then dried under N₂ blowing. The fluorescence on the gold spots was analyzed by fluorescence scanner.
S. Figure 1. FT-IR spectra of (a) CTC resin, (b) TEG loaded resin (2) and (c) alkanethiol spacer loaded resin (4).
S. Figure 2. Mass data of alkanethiol spacer (calcd 520.72 for C$_{25}$H$_{48}$N$_2$O$_7$S) [M-H]$^-$, found 519.5)