Supporting Information

Protein Patterning Based on Electrochemical Activation of Bioinactive Surfaces with Hydroquinone-Caged Biotin

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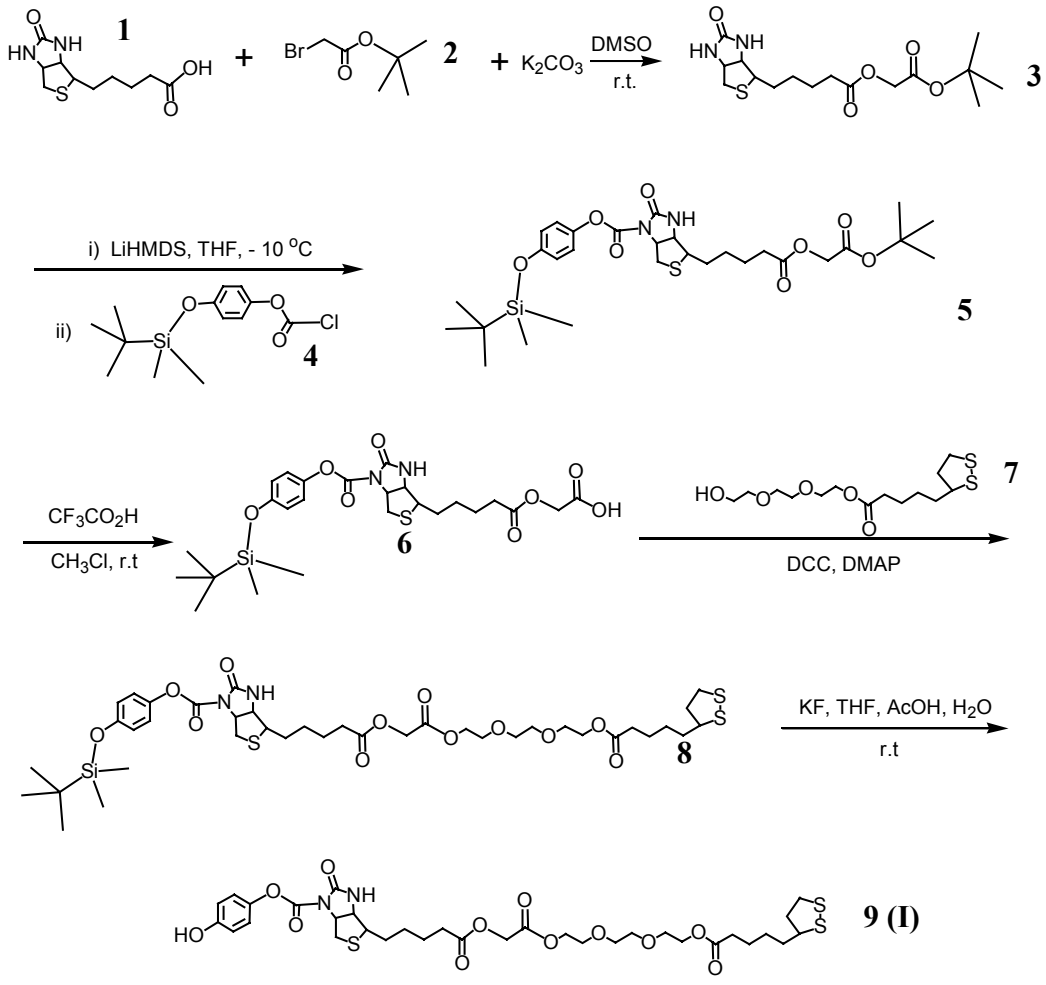
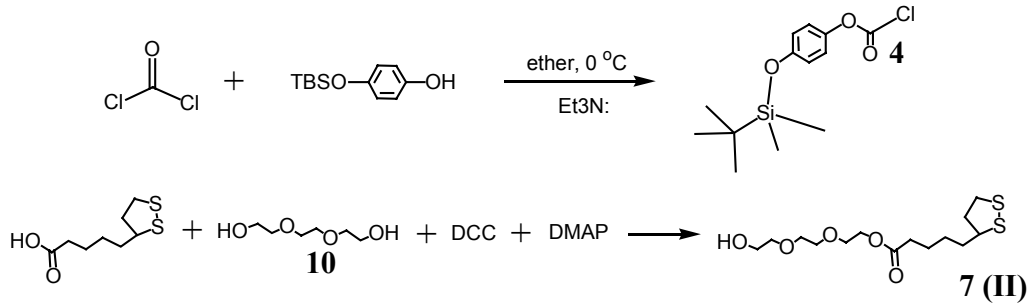
Synthesis of compound I and II





3: A mixture of d-biotin **1** (1 eq), *tert*-butyl bromoacetate **2** (1.2 eq), and potassium carbonate (1.3 eq) in DMSO (1.5mL per 1mmol of biotin) was stirred for 5 hours at room temperature. After the reaction was completed, work-up was done by chloroform/water. Additional washing of chloroform layer was done with water or brine to remove DMSO. The organic layer was collected, dried over $MgSO_4$, and concentrated under reduced pressure. To this residue was poured ether and the resulting suspension was gently heated. White solid was formed while cooling and the solid was collected by filtration in 89% yield. The resulting biotin ester **3** showed good purity, and no further purification was needed. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.69 (s, 1H, -NH), 5.05 (s, 1H, -NH), 4.49-4.56 (m, 3H), 4.34-4.36 (m, 1H, -CH), 3.07-3.20 (m, 1H, -CH-), 2.92-2.95 (m, 1H), 2.7502.78 (d, 1H, J=12.8 Hz), 2.45-2.49 (m, 2H, -CH_2CO-), 1.69-1.78 (m, 6H), 1.50 (s, 9H, t-Bu); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.4, 167.5, 163.6, 82.9, 62.2, 61.5, 60.5, 55.8, 40.9, 33.8, 28.5(6), 28.5(1), 28.4(6), 25.1.

5: Biotin ester **3** was dissolved in THF (5mL per 1mmol of biotin ester) and the solution cooled to -10 °C. To this solution was added LiHMDS (1 M in THF, 1eq) over 5 min, after which the mixture was stirred for additional 5 min, followed by addition of 4-(*tert*-butyldimethylsilyloxy)phenoxycarbonyl chloride **4** solution (1.2 eq) in THF. After 1 hr the reaction mixture was concentrated and purified using silica-gel chromatography (THF/hexane = 1/1 V/V) to give **5** in 55% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.05-7.07 (d, 2H, J=8.63 Hz), 6.83-6.84 (d, 2H, J=8.61 Hz), 4.95-4.96 (m, 1H), 4.48-4.60 (dd, 2H), 4.20-4.30 (m, 1H), 3.24-3.27 (m, 2H), 3.07-3.11 (m, 1H), 2.45-2.49 (m, 2H), 1.76-1.89 (m, 5H), 1.46 (s, 9H), 1.00 (s, 9H), 0.21 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.8, 167.0, 155.4, 153.4, 144.3, 135.7, 122.2, 120.4, 82.4, .62.9, 61.0, 57.5, 55.2, 38.6, 33.3, 31.5, 28.2, 28.0, 27.8, 25.6, 24.5, -4.6.

6: Carbamate **5** was dissolved in dichloromethane/trifluoroacetic acid (3/1 V/V) and the solution was stirred at room temperature. After 15 h, the reaction mixture was concentrated and the residual yellow oil was poured into chloroform/water mixture. Organic layer was collected, dried with $MgSO_4$ and concentrated. The residue was solidified in ether/hexane (1/5 V/V) to form white solid, which is collected by filtration to give **6** in quantitative yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.23 (s, 1H), 7.04-7.05 (d, 1H, J=8.83 Hz), 6.83-6.85 (d, 1H,J=8.84 Hz), 4.95-4.97 (m, 1H), 4.81-4.84 (d, 1H, J=16.1 Hz), 4.53-4.56 (d, 1H, J=16.1 Hz), 4.32-4.34 (m, 1H), 3.22-3.24 (m, 2H), 3.07-3.11 (m, 1H), 2.50-2.55 (m, 1H), 2.30-2.40 (m, 1H), 1.74-1.85 (m, 4H), 1.40-1.55 (m, 2H), 1.00 (s, 9H), 0.21 (s, 6H).

9: Compound **6** (1 eq) and **7** (1.5 eq) were coupled using DCC (1.1 eq) in the presence of catalytic DMAP (0.2 eq) in chloroform at room temperature for 24 hours to afford **8** in 64% yield after purification using silica-gel chromatography (THF/hexane = 1/1 V/V). Compound **8** (1 eq) was dissolved in THF/AcOH (1/1 V/V) and a few drops of water was added to this solution to dissolve KF (5 eq) completely. After stirring for 12 h at room temperature solvent was evaporated and then the

residue was dissolved in THF. Precipitated salts were removed by filtration and the filtrate was concentrated to load on silica-gel chromatography (THF/hexane 2/1 V/V). TBS-deprotected product **9** was obtained in 90% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.99 (s, 1H), 6.95-6.97 (d, 2H, J=8.76 Hz), 6.78-6.80 (m, 3H), 4.82-4.91 (m, 1H), 4.62-4.66 (d, 1H), 4.54-4.56 (d, 1H), 4.32-4.36 (m, 1H), 4.20-4.26 (m, 3H), 3.67-3.75 (m, 8H), 3.51-3.56 (m, 1H), 3.01-3.21 (m, 5H), 2.34-2.37 (m, 3H), 2.26-2.29 (t, 2H), 1.82-1.86 (m, 1H), 1.66-1.74 (m, 8H), 1.43-1.50 (m, 3H), 1.29 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.1, 173.5, 168.7, 156.2, 154.9, 143.6, 136.2, 125.9, 122.8, 70.9, 69.6, 69.3, 68.4, 65.0, 63.9, 63.4, 60.9, 58.1, 56.8, 55.6, 40.6, 38.9, 35.0, 34.6, 34.3, 33.8, 30.7, 29.1, 28.4, 28.3, 25.0. MALDI-MS: calcd. for $C_{33}H_{46}N_2O_{12}S_3$: 781.2111 $[M+Na]^+$, found: 781.5184 $[M+Na]^+$.

7: Thioctic acid (1 eq) and compound **10** (5 eq) were coupled using DCC (1.2 eq) in the presence of catalytic DMAP (0.1 eq) in chloroform at room temperature for 24 hours to afford ester **7**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.24-4.28 (t, 1H, J=2.22 Hz), 3.59-3.75 (m, 9H), 3.51-3.61 (m, 1H), 3.15-3.19 (m, 2H), 2.42-2.60 (m, 1H), 2.37-2.40 (t, 2H, J=3.71 Hz), 1.92-1.95 (m, 1H), 1.67-1.74 (m, 4H), 1.49-1.55 (m, 2H).

4: Phosgene (stock solution in toluene) was diluted in ether. To this solution were dropped mono-*tert*-butyldimethylsilyl (TBS)-protected hydroquinone and triethylamine (both in ether) at 0 °C for 2 hrs. Then solvent in the mixture was evaporated. To this residue was added anhydrous ether to adjust the concentration to ~0.5 M. Precipitated ammonium salt was removed by filtration and the solution was stored in refrigerator.