Supporting Information

Mass spectrometric analysis of acid-assisted photochemical release of the trimethyl lock system on the monolayers on gold

Geunhyeok Yu and Woon-Seok Yeo*

[*] G. Yu and Prof. Dr. W. –S. Yeo

Department of Bioscience and Biotechnology, Bio/Molecular Informatics Center, Konkuk University, Seoul 143-701 (Korea)

1. Experimental

1.1 Materials and methods

Gold-coated wafers were prepared by sequential vacuum deposition of titanium (10 nm) and gold (50 nm) onto silicon wafers. Gold nanoparticles (AuNPs, 40 nm diameter) were prepared using the method previously reported by Schwartzberg et al..[1] Tri(ethylene glycol)-terminated alkanethiol, carboxylic acid-terminated alkanethiol, and *N*-(2-aminoethyl)maleimide were prepared using the method reported by Houseman et al..[2] 2,4,6-Trihydroxyacetophenone monohydrate (THAP), *N*-hydroxysuccinimide (NHS), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC·HCl), dimethyl sulfoxide (DMSO), dimethylformamide (DMF), ethyl acetate (EA), methanol (MeOH), tetrahydrofuran (THF), dichloromethane (DCM), trifluoroacetic acid (TFA), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), 3,3′-dithiodipropionic acid, sodium borohydride ($NaBH_4$), 4-dimethylaminopyridine (DMAP), sulfuric acid, 3,3-dimethylacrylic acid, methanesulfonic acid, rriethylsilane, 1-hexanol, and benzylamine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cysteamine hydrochloride, 2,5-dimethyl-1,4-benzoquinone, and

triphenylmethanol were purchased from Tokyo Chemical Industry (Tokyo, Japan). Sodium Chloride (NaCl), magnesium sulfate ($MgSO_4$), bromine (Br_2), acetonitrile (ACN), and hydrogen peroxide were purchased from Junsei Chemical Co., Ltd. (Tokyo, Japan). Sodium carbonate (Na_2CO_3), sodium sulfate (Na_2SO_4), sodium bicarbonate ($NaHCO_3$), potassium carbonate (K_2CO_3), hexanes, and hydrochloric acid (HCl) were purchased from DAEJUNG Co., Ltd. (Seoul, Korea). Diethyl ether, acetic acid, and ammonium chloride (NH_4Cl) were purchased from Samchun Chemical Co., Ltd. (Seoul, Korea). Amine-modified microspheres (0.2 μm, ex.580/em.605) were purchased from Invitrogen (Carlsbad, CA, USA). Absolute ethanol was purchased from Merck (Darmstadt, Germany). Chloroform-D was purchased from Cambridge Isotope Laboratories (Andover, MA, USA).

Photoreduction in solution

Q-PTL-ester or amide (0.1 μmol) in MeOH or EtOH (100 μL) was irradiated at 395 nm (3 W) for 5 min, incubated for additional 5 min at room temperature, and analyzed by MALDI-TOF MS with AuNPs (1.1 nM) as a matrix. For photoreduction under acidic conditions, HCl (5 μmol) was added to the solution. The solutions were then analyzed by MALDI-TOF MS with AuNPs as a matrix.

Preparation of Q-PTL-presenting SAMs

Gold chips were cleaned with a piranha solution (sulfuric acid/hydrogen peroxide (30%) = 7:3) before use. A gold chip was immersed in a mixed solution (1 mM in ethanol) of tri(ethylene glycol)-terminated alkanethiol and carboxylic acid-terminated alkanethiol in a ratio of 3:7 for 12 hr. Following incubation, the gold chip was washed with ethanol and dried under a stream of nitrogen. The resulting carboxylic acid-presenting monolayers were activated with EDC (100 mM in DMSO) and NHS (120 mM in DMSO) for 2 hr, washed with DMSO and ethanol,

and dried under a stream of nitrogen. The monolayers were then treated with *N*-(2-aminoethyl)maleimide (400 mM in DMSO) for 2 hr, washed with DMSO and ethanol, and dried under a stream of nitrogen. The maleimide-presenting monolayers were immersed into Q-PTL-ester or amide solution (1 mM in MeOH) for 2hr in the dark, washed with methanol, and dried under a stream of nitrogen.

Photoreduction on the surface

The Q-PTL-presenting SAMs were immersed in a 24-well plate containing MeOH (3 mL) and irradiated at 395 nm for 5 min. The SAMs were transferred to a new well filled with MeOH, incubated for additional 5 min at room temperature, rinsed with MeOH, and dried under a stream of nitrogen. The SAMs were then analyzed by MALDI-TOF MS with THAP as a matrix. For photoreduction under acidic conditions, HCl (3 μmol or 15 μmol) was added to MeOH (3 mL) in the 24-well plate.

MALDI-TOF MS analysis

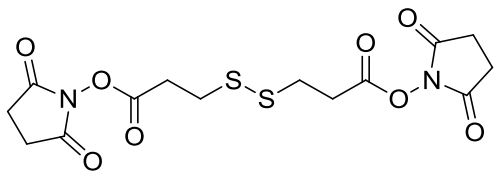
Mass analysis was performed with an AutoFlex III MALDI-TOF mass spectrometer (Bruker Daltonics, Billerica, MA, USA) using a Smart Beam laser as an ionization source. All spectra were acquired at an accelerating voltage of 19 kV, with a 50 Hz repetition rate, and in positive mode with an average of 1000 shots using AuNPs (1.1 nM in ethanol) and THAP (6 mg/mL in acetonitrile) as a matrix.

Fluorescence imaging

The prepared maleimide-presenting monolayers were immersed into Q-PTL-NHS solution (1 mM in DCM) for 2 hr in the dark, washed with DCM, and dried under a stream of nitrogen. Then, the monolayers were treated with a mixed solution (50 mM in water) of DIEA and amine-

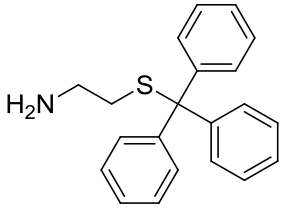
modified red fluorescent microspheres for 1 hr in the dark, washed with water and ethanol, and dried under a stream of nitrogen. The resulting Q-PTL-microsphere-presenting monolayers were immersed in a 24-well plate containing MeOH (3 mL) with HCl (15 μmol). Half of the monolayers were covered with a photomask. The monolayers were irradiated at 395 nm for 5 min, transferred to a new well filled with MeOH, incubated for additional 5 min, rinsed with MeOH, and dried under a stream of nitrogen. They were analyzed by fluorescent microscopy through a Texas Red filter.

1.2 Synthesis



Bis(2,5-dioxopyrrolidin-1-yl) 3,3'-disulfanediyldipropanoate (1)

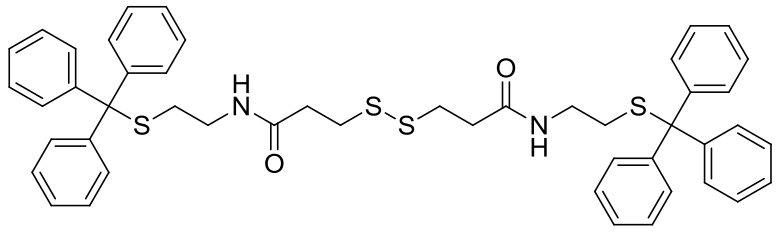
Dithiodipropionic acid (500 mg, 2.38 mmol) and NHS (600 mg, 5.21 mmol) were dissolved in THF (6 mL). EDCI·HCl (1 g, 5.22 mmol) in DCM (7 mL) was added dropwise, and the resulting solution was stirred for 2 hr under nitrogen. After solvent evaporation, the residue was dissolved in DCM, washed with brine, and dried by $MgSO_4$. After filtration, the filtrate was concentrated and triturated with EtOH to give **1** (578 mg, 60% yield) as a white solid. ^{1}H NMR (500 MHz, Chloroform-d) δ 3.11-3.06 (m, 8H), 2.87 (s, 8H).



2-(Tritylthio)ethanamine (2)

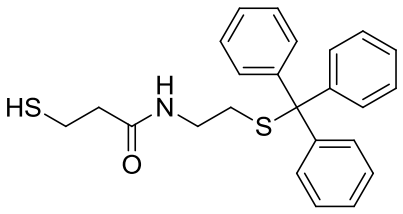
Triphenylmethanol (2 g, 7.68 mmol) and cysteamine hydrochloride (1.31 g, 11.53 mmol) were

dissolved in DCM (25 mL), and TFA (2 mL) was added dropwise. The resulting solution was stirred for 2 hr under nitrogen. After solvent evaporation, the residue was dissolved in EA, washed with 10% Na_2CO_3 solution and dried by Na_2SO_4. After filtration, the filtrate was concentrated to give **2** (2.0 g, 81% yield) as a white solid. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.47-7.27 (m, 15H), 2.60 (t, J = 7.5 Hz, 2H), 2.50 (t, J = 7.5 Hz, 2H).



3,3'-Disulfanediylbis(N-(2-(tritylthio)ethyl)propanamide) (3)

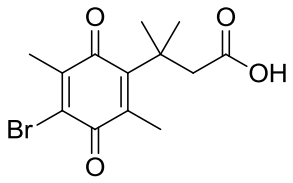
To the solution of **1** in DMF (404 mg, 1.00 mmol), was added **2** (1.68g, 5.26 mmol). The resulting solution was stirred for 2 hr under nitrogen and 50 mL of DCM was added. The organic layer was washed with brine and dried by $MgSO_4$. After filtration, the filtrate was concentrated and triturated with MeOH to give **3** (584 mg, 72% yield) as a white solid. ^{1}H NMR (500MHz, Chloroform-d) δ 7.44-7.23 (m, 30H), 3.09 (q, J = 6 Hz, 4H), 2.93 (t, J = 7 Hz, 4H), 2.48 (t, J = 7 Hz, 4H), 2.43 (t, J = 6 Hz, 4H).



3-Mercapto-N-(2-(tritylthio)ethyl)propanamide (4)

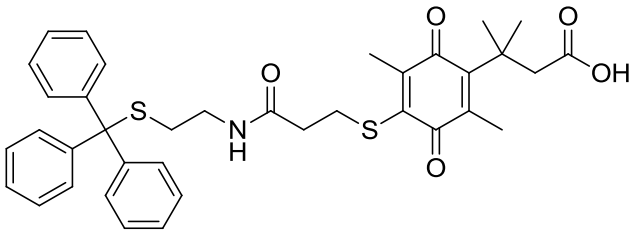
Compound **3** (300 mg, 0.37 mmol) was dissolved in THF (25 mL) at 50°C, and tris(2-carboxyethyl)phosphine hydrochloride (342 mg, 1.19 mmol in water (5 mL) was added**.** The mixture was stirred for 1 hr under nitrogen, then allowed to cool to room temperature, and stirred overnight. After solvent evaporation, the residue was dissolved in DCM, washed with brine, and dried by $MgSO_4$. After filtration, the filtrate was concentrated and triturated with

hexanes to give **4** (240 mg, 80% yield) as a white solid. ^{1}H NMR (500MHz, Chloroform-d) δ 7.43-7.23 (m, 15H), 3.13 (q, J = 6 Hz, 2H), 2.78 (q, J = 8 Hz, 2H), 2.45 (t, J = 6 Hz, 2H), 2.42 (t, J = 7 Hz, 2H).



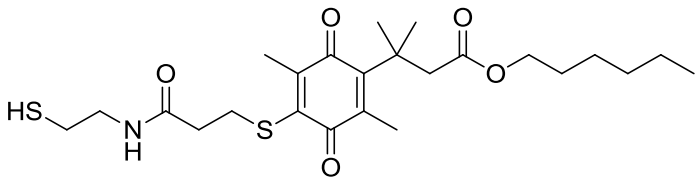
3-(4-Bromo-2,5-dimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)-3-methylbutanoic acid (5)

The synthesis of **5** has been reported previously.[3] Briefly, 2,5-dimethyl-1,4- benzoquinone (1.00 g, 7.38 mmol) was dissolved in the mixture of MeOH (10 mL), water (40 mL) and ether (20 mL). $NaBH_4$ (1.42 g, 37.58 mmol) was added and then the yellow solution became colorless quickly. After 20 min, the solution was extracted by ether (3 x 50 mL), and the combined organic layer washed with brine and dried by $MgSO_4$. The solvent evaporation gave 2,5-dimethyl-1,4- hydroquinone (0.97g, 7.02 mmol, 95%) as a white solid. The resulting product and 3,3-dimethylacrylic acid (0.84 g, 8.42 mmol) were dissolved in methanesulfonic acid (30 mL) and heated to 70°C and stirred overnight under nitrogen. The mixture was poured into ice and extracted with EA (4 x 50mL). The combined organic layer was washed with brine and dried by $MgSO_4$. The recrystallization in hexanes gave the lactone (1.24g, 5.63 mmol, 80%) as a white solid. The above product was dissolved in acetic acid (55 mL) and Br_2 (0.637 mL) in acetic acid (7.41 mL) was added dropwise. The resulting red solution was exposed to the air and stirred overnight in the dark. The solution was poured into water and extracted with DCM until the solution became colorless. The combined organic layer was extracted with saturated $NaHCO_3$. The combined aqueous layer was acidified with conc. HCl slowly, and then extracted with ethyl acetate. The combined organic layer was dried by $MgSO_4$ and concentrated to give **5** (1.06 g, 60%) as a yellow solid. ^{1}H NMR (500 MHz, Chloroform-d) δ 3.03 (s, 2H), 2.23 (s, 3H), 2.16 (s, 3H), 1.47 (s, 6H).



3-(2,5-Dimethyl-3,6-dioxo-4-((3-oxo-3-((2-(tritylthio)ethyl)amino)propyl)thio)cyclohexa-1,4-dien-1-yl)-3-methylbutanoic acid (6)

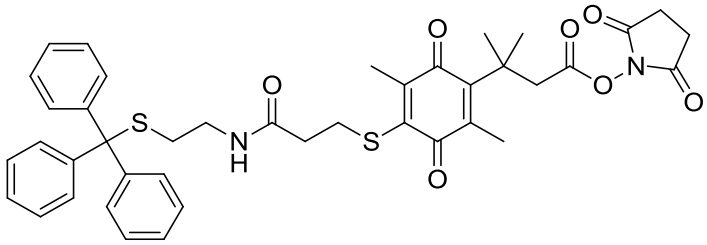
To a solution of **4** (77 mg, 0.19 mmol) in MeOH (10 mL) was added **5** (50 mg, 0.16 mmol), followed by K_2CO_3 (24 mg, 0.17 mmol). The resulting solution was stirred for 3 hr under nitrogen in the dark, evaporated, and purified by silica gel column chromatography. (DCM : EA = 1:2 → EA → MeOH : EA = 20:1) to give **6** (30 mg, 29%) as a yellow oil. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.39-7.22 (m, 15H), 3.12-3.07 (m, 4H), 2.95 (s, 2H), 2.43 (t, J = 6 Hz, 2H), 2.32 (t, J = 6.5 Hz, 2H), 2.15 (s, 3H), 2.07 (s, 3H), 1.45 (s, 6H).



Hexyl 3-(4-((3-((2-mercaptoethyl)amino)-3-oxopropyl)thio)-2,5-dimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)-3-methylbutanoate (7)

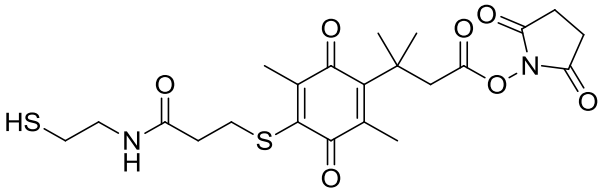
To a solution of **6** (23 mg, 0.036 mmol) in DCM (1 mL) were added 1-hexanol (14.6 mg, 0.14 mmol) in DCM (500 μL), EDCI·HCl (13.8 mg, 0.072 mmol) in DCM (1 mL) and DMAP (8.8 mg, 0.072 mmol) in DCM (500 μL). The resulting solution was stirred for 2 hr under nitrogen in the dark. The solvent was removed, and the residue was purified by silica gel column chromatography. (DCM : EA = 10:1). The resulting product (7.6 mg, 0.01 mmol) in DCM (3 mL) was then treated with triethylsilane (100 μL) and trifluoroacetic acid (100 μL) to remove the trityl group for 30 min under nitrogen in the dark. The mixture was washed with saturated NH_4Cl, dried by $MgSO_4$, and filtered. The filtrate was concentrated and the residue was

purified by silica gel column chromatography (DCM : EA = 10:1) to give **7** (3 mg, 59%) as a yellow oil. ^{1}H NMR (500 MHz, Chloroform-d) δ 3.98 (t, J = 6.5 Hz, 2H), 3.47 (q, J = 6.5 Hz, 2H), 3.21 (t, J = 7 Hz, 2H), 2.96 (s, 2H), 2.68 (q, J = 6.5 Hz, 2H), 2.46 (t, J = 7 Hz, 2H), 2.19 (s, 3H), 2.17 (s, 3H), 1.45 (s, 6H), 1.30-1.27 (m, 8H), 0.90 (t, J = 7 Hz, 3H). ^{13}C NMR (125 MHz, Chloroform-d) δ 188.37, 183.40, 173.27, 170.80, 154.00, 149.67, 139.44, 139.03, 64.62, 47.58, 42.66, 38.08, 36.94, 31.40, 29.53, 28.51, 28.46, 25.57, 24.50, 22.53, 15.09, 14.56, 14.01.



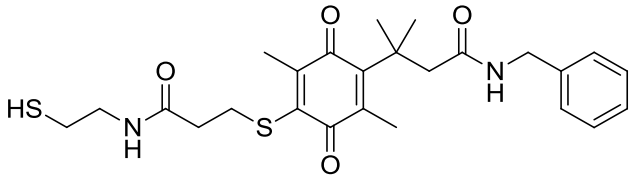
2,5-Dioxopyrrolidin-1-yl 3-(2,5-dimethyl-3,6-dioxo-4-((3-oxo-3 ((2(tritylthio)ethyl) amino)propyl)thio)cyclohexa-1,4-dien-1-yl)-3-methylbutanoate (8)

To a solution of **6** (60 mg, 0.09 mmol) in DCM (3 mL) was added NHS (13 mg, 0.11 mmol) in THF (1 mL). EDCI·HCl (21.5 mg, 0.11 mmol) in DCM (1 mL) was added dropwise, and the mixture was stirred for 2 hr under nitrogen in the dark. The solvent was removed, and the residue was purified by silica gel column chromatography (DCM : EA = 8:1) to give **8** (22,4 mg, 32%). ^{1}H NMR (500 MHz, Chloroform-d) δ 7.40-7.22 (m, 15H), 3.25 (s, 2H), 3.19 (t, J = 7 Hz, 2H), 3.08 (q, J = 6 Hz, 2H), 2.72 (s, 4H), 2.44 (t, J = 6 Hz, 2H), 2.37 (t, J = 7 Hz, 2H), 2.21 (s, 3H), 2.13 (s, 3H), 1.57 (s, 6H).



2,5-Dioxopyrrolidin-1-yl 3-(4-((3-((2-mercaptoethyl)amino)-3-oxopropyl)thio)-2,5-dimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)-3-methylbutanoate (9)

To a solution of **8** (12.4 mg, 0.017 mmol) in DCM (4 mL) was added triethylsilane (200 μL) and trifluoroacetic acid (200 μL). The mixture was stirred for 30 min under nitrogen in the dark, washed with saturated NH_4Cl, dried by $MgSO_4$, and filtered. The solvent was removed, and the residue was triturated with hexanes to give **9** (5 mg, 60%) as a yellow solid. ^{1}H NMR (500 MHz, Chloroform-d) δ 3.46 (q, J = 6 Hz, 2H), 3.26 (s, 2H), 3.23 (t, J = 7 Hz, 2H), 2.83 (s, 4H), 2.70 (q, J = 6.5 Hz, 2H), 2.47 (t, J = 6.5 Hz, 2H), 2.24 (s, 3H), 2.17 (s, 3H), 1.57 (s, 6H).



***N*-Benzyl-3-(4-((3-((2-mercaptoethyl)amino)-3-oxopropyl)thio)-2,5-dimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)-3-methylbutanamide (10)**

To a solution of **8** (17 mg, 0.023 mmol) in DCM (2 mL) was added benzyl amine (9.86 mg, 0.092 mmol) in DCM (1 mL), followed by DIEA (5.94 mg, 0.046 mmol) in DCM (200 μL). The mixture was stirred for 30 min under nitrogen in the dark, washed with saturated brine, dried by $MgSO_4$, and filtered. The solvent was removed, and the residue was triturated with hexanes. The resulting product (14.3 mg, 0.0195 mmol) in DCM (3 mL) was then treated with triethylsilane (100 μL) and trifluoroacetic acid (100 μL) to remove the trityl group for 30 min under nitrogen in the dark. The mixture washed with saturated NH_4Cl, dried by $MgSO_4$, and filtered. The solvent was removed, and the residue was triturated with hexanes to give 10 (6.8 mg, 71%) as a yellow solid. ^{1}H NMR (500 MHz, Chloroform-d) δ 7.37-7.30 (m, 3H), 7.20 (d, J = 7 Hz, 2H), 4.37 (d, J = 5.5 Hz, 2H), 3.35 (q, J = 6.5 Hz, 2H), 3.17 (t, J = 6.0 Hz, 2H), 2.86 (s, 2H), 2.60 (q, J = 7 Hz, 2H), 2.32 (t, J = 6.5 Hz, 2H), 2.19 (s, 3H), 2.16 (s, 3H), 1.49 (s, 6H). ^{13}C NMR (125 MHz, Chloroform-d) δ 188.30, 183.05, 172.02, 170.92, 155.11, 150.62, 138.78, 137.59, 137.40, 128.87, 127.78, 127.30, 49.52, 43.49, 43.01, 38.46, 36.50, 29.29, 28.56, 24.27, 15.18, 14.33.

2. Supplementary figures and tables

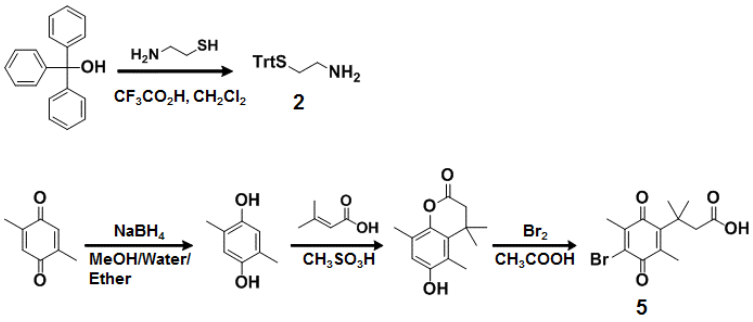


Fig. S1 Synthesis of the intermediate **2** and **5**.

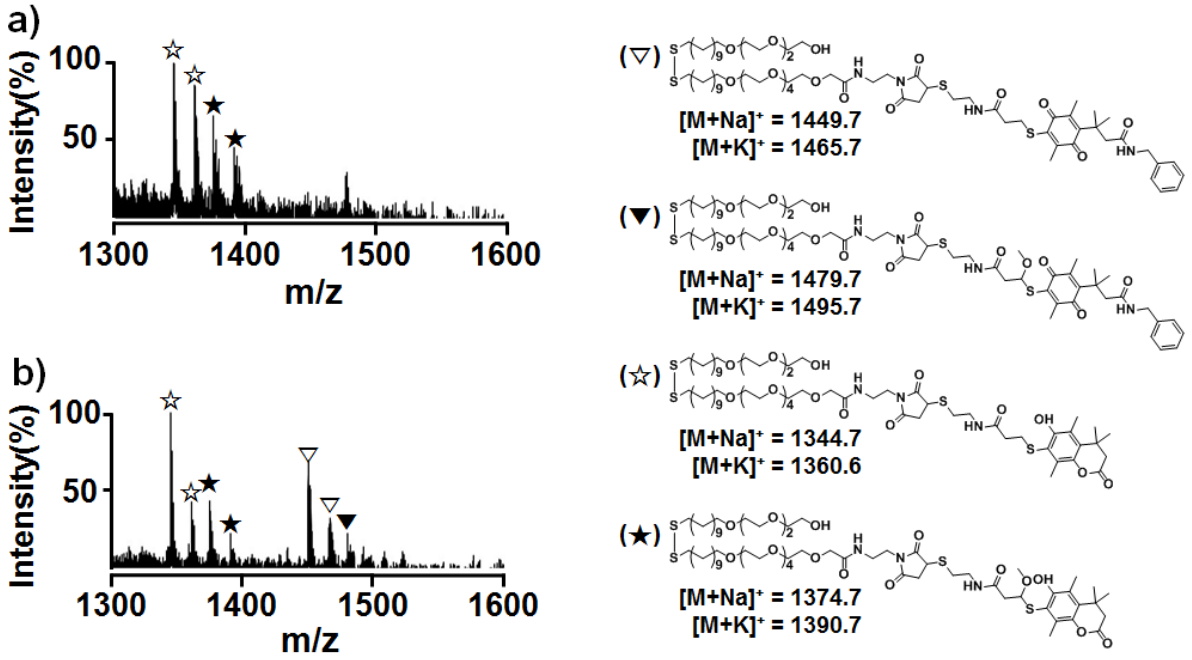


Fig. S2 MALDI-TOF MS analysis of the Q-PTL-presenting monolayers treated with 1 mM HCl revealed a higher yield of photochemical lactonized products (☆, ★) compared to untreated surfaces.

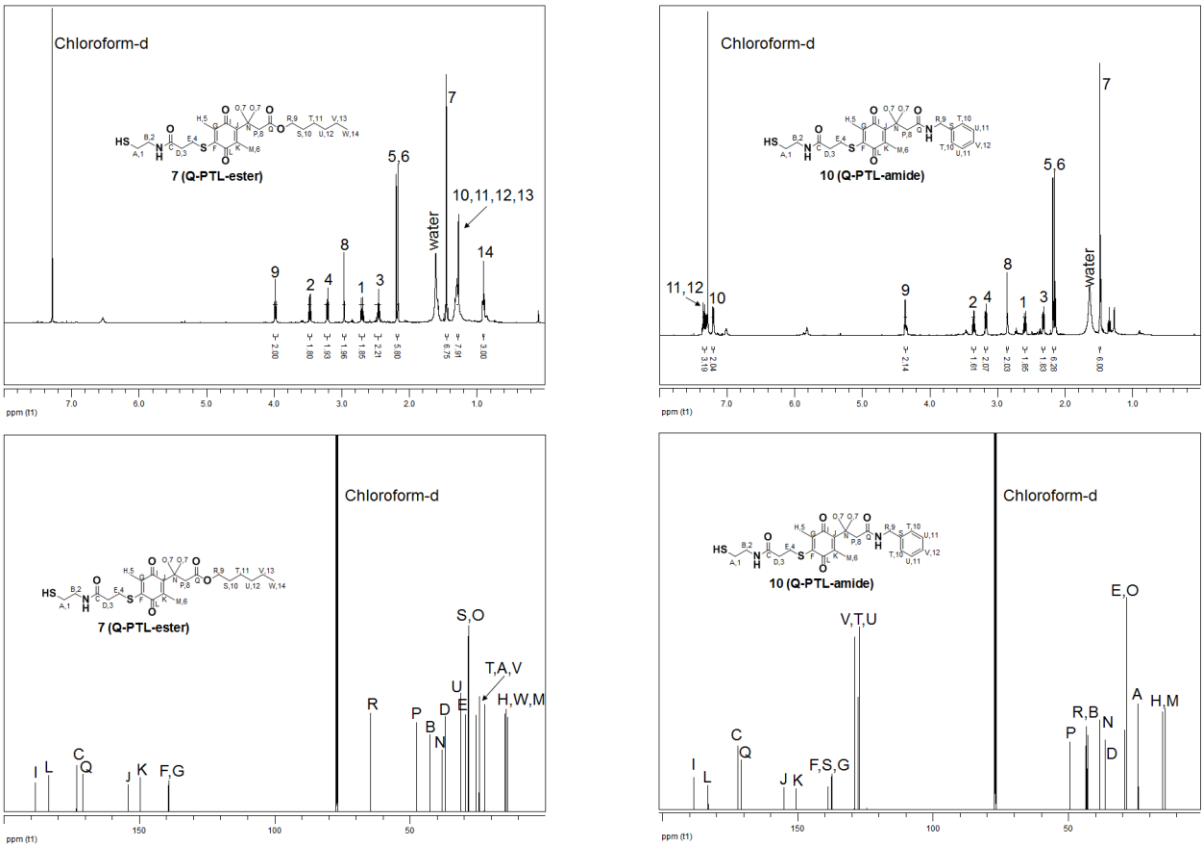


Fig. S3 ^{1}H NMR and ^{13}C NMR spectra of compounds **7** and **10**

References

[1] Schwartzberg AM, Olson TY, Talley CE, Zhang JZ. Synthesis, characterization, and tunable optical properties of hollow gold nanospheres. *J. Phys. Chem. B,* **2006**, *110*, 19935-44.

[2] Houseman BT, Gawalt ES, Mrksich M. Maleimide-functionalized self-assembled monolayers for the preparation of peptide and carbohydrate biochips. *Langmuir*, **2003**, *19*, 1522-31.

[3] Walton DP, Dougherty DA. *J. Am. Chem. Soc.*, **2017**, *139*, 4655.