Supporting Information

Release of Liposome Contents by Cell-Secreted Matrix Metalloproteinase-9

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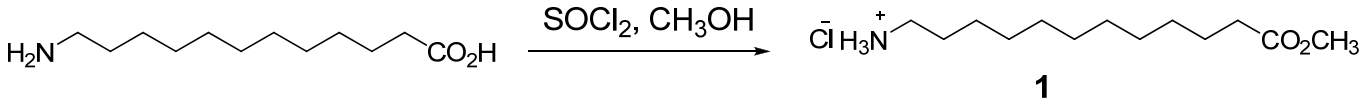
Synthetic details for PyGPO4:

Reagents were purchased from Alfa Aesar or TCI America. EDCI.HCl was purchased from ChemPep, Inc. All organic solvents used were distilled. Melting points were determined using a micro melting point apparatus. ^{1}H and ^{13}C NMR spectra were recorded on 300 or 400 MHz spectrometer. NMR spectra was recorded either using $CDCl_3$ or DMSO-d_6 as solvents with TMS as the internal standard. TLC was performed with Adsorbil plus IP, 20 x 20 cm plate, 250 μm (Altech Associates, Inc). TLC plates were visualized under UV light or in an iodine chamber. Flash column chromatography was carried out with Merck Silica gel 60.



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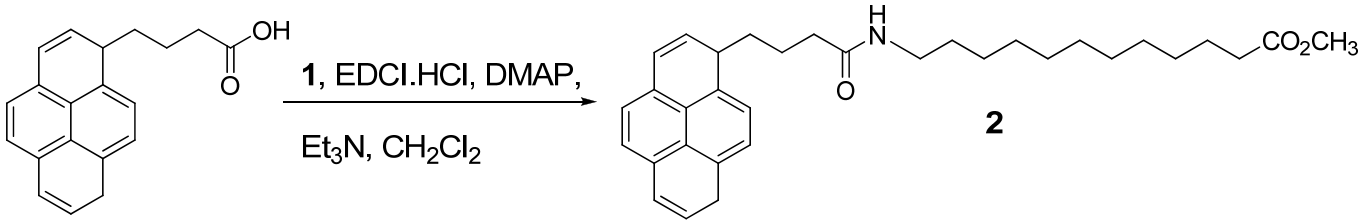
Compound 1: To a suspension of 12-aminolauric acid (5.0 g, 23.2 mmol, 1 equiv) in methanol (50 mL) under nitrogen, thionyl chloride (2.7 mL, 1.6 equiv) was added at 0 ^{o}C. The reaction mixture was stirred under a nitrogen atmosphere at room temperature for 2 h and then heated to reflux for 2 h. After cooling the reaction to room temperature, the organic solvents were evaporated in a rotary evaporator giving a white solid. The white solid was dried under vacuum and used for the next step without further purification. Yield: 5.30 g (99%), mp: 157-160 ^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 1.22-1.44 (m, 14H) 1.55-1.66 (m, 2H) 1.76 (quin, J = 7.5 Hz, 2H) 2.30 (t, J = 7.5 Hz, 2H) 2.98 (t, J = 7.7 Hz, 2H) 3.67 (s, 3H) 8.31 (br, s, 3H).



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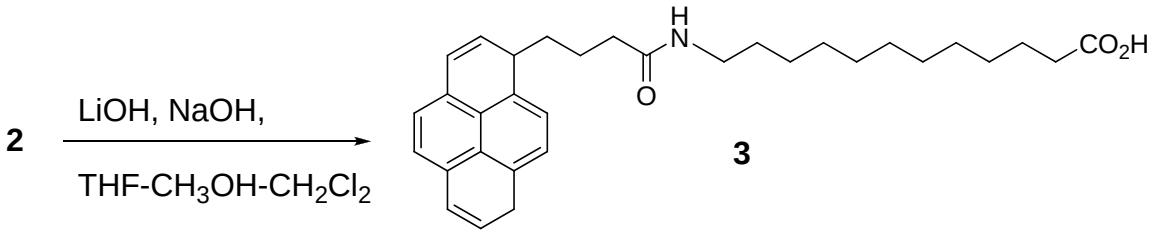
Compound 2: To a suspension of pyrenebutyric acid (2.0 g, 6.93 mmol) in CH_2Cl_2 (180 mL) under nitorgen triethylamine (1.32 mL, 9.47 mmol) was added. The resultant mixture was stirred for 5 min, cooled to 0 ^{o}C and compound **1** (1.446 g, 6.30 mmol), 4-(N,N-dimethylamino)pyridine (DMAP; 0.924 g, 7.56 mmol) and EDCI.HCl (1.450 g, 7.56 mmol) were added sequentially. The reaction mixture was stirred at room temperature under nitrogen overnight. The reaction was quenched with water and the organic layer was washed with 1 N HCl, 1 N NaOH and brine respectively. The organic layer was then

dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by silica gel flash chromatography (eluant: 1:1 ethylacetate in hexane, $R_f = 0.3$) to obtain the pure product as yellowish brown solid. Yield: 2.08 g (60%), mp: 95-97 ^oC; ^1H NMR ($CDCl_3$, 300 MHz): δ ppm 1.24 (br, s, 14H) 1.45 (br, s, 2H) 1.51-1.67 (m, 3H) 2.13-2.33 (m, 6H) 3.17-3.29 (m, 2H) 3.40 (t, J = 7.0 Hz, 2H) 3.66 (s, 3H) 5.34 (br, s, 1H) 7.87 (d, J = 7.7 Hz, 1H) 7.95-8.06 (m, 3H) 8.08-8.20 (m, 4H) 8.31 (d, J = 9.3 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ ppm 25.05, 27.07, 28.31, 29.07, 29.28, 29.40, 29.50, 29.55, 29.63, 29.82, 32.94, 33.89, 35.76, 39.06, 51.81 124.14, 124.83, 125.43, 125.61, 126.80, 127.18, 127.89, 128.10, 128.85, 129.96, 129.99, 131.11, 131.56, 137.24, 172.31, 174.00.



Compound 3: The ester **2** (1.264 g, 2.52 mmol) was hydrolyzed with LiOH (1.057 g, 25.1 mmol) in MeOH-THF-CH_2Cl_2 (20-10-5 mL). A few drops of 1 N NaOH was also added. The reaction mixture was stirred overnight when a white residue formed. On acidification with 2 N HCl, the white solid dissolved. After evaporation of the solvents, the white solid obtained was washed with water, filtered and lyophilized. Yield: 1.162 g (94%), mp: 129-132 ^oC; ^1H NMR (DMSO-d_6, 300 MHz) δ ppm 1.19 (m, 14H) 1.33-1.48 (m, 4H) 2.02 (d, J = 6.8 Hz, 2H) 2.14 (t, J = 7.0 Hz, 2H) 2.22 (d, J = 6.4 Hz, 2H) 3.06 (d, J = 6.0 Hz, 2H) 3.21-3.32 (m, 2H) 7.83 (br, s, 1H) 7.94 (d, J = 7.7 Hz, 1H) 8.07 (d, J = 7.7 Hz, 1H) 8.14 (s, 2H) 8.19-8.31 (m, 4H) 8.38 (d, J = 9.3 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ ppm 24.43, 26.39, 27.61, 28.49, 28.68, 28.72, 28.86, 28.90, 28.96, 29.14, 32.24, 33.62, 35.05, 38.38, 38.89, 123.45,124.22, 124.75, 124.89, 126.10, 126.46, 127.16, 127.42, 127.46, 128.13, 129.28, 130.40, 130.87, 136.55, 171.61, 174.45.

Synthesis of pyrene conjuagted lipo-peptide PyGPO4:

The synthesized pyrene conjugated fatty acid (compound **3**) was used in 5 fold excess for coupling with the N-terminus of the peptide under microwave condition, following the same protocol as discussed in the experimental procedure. The purification procedure was the same as described for **GPO4** in the manuscript and characterized by MALDI-ToF mass spectrum. M^+ calculated: 2535.43; observed: 2534.54.

Liposome preparation using PyGPO4:

A stock solution of the lipo-peptide was prepared by dissolving the compound in methanol:chloroform (1:5) mixture to give a final concentration of 1 mg/mL. A lipid thin film was prepared by adding 1.4 mL of this stock solution to 250 µL of POPC solution in $CHCl_3$ and rotary evaporating the organic solvents at 40 ^{0}C. The thin film was dried overnight in vacuum. The dry lipid film was next hydrated with 25 mM HEPES buffer (pH = 8.0) containing 100 mM NaCl, 10 mM $CaCl_2$ and 10 µM $ZnCl_2$ by slow rotation at 60 ^{0}C for 1 h. After storing at 4 ^{0}C for 3 h, the hydrated solution was sonicated by probe sonicator for 1 h followed by 11 cycles of extrusion through a 100 nm polycarbonate membrane. The liposomes were diluted 20 times before recording the fluorescent spectra.

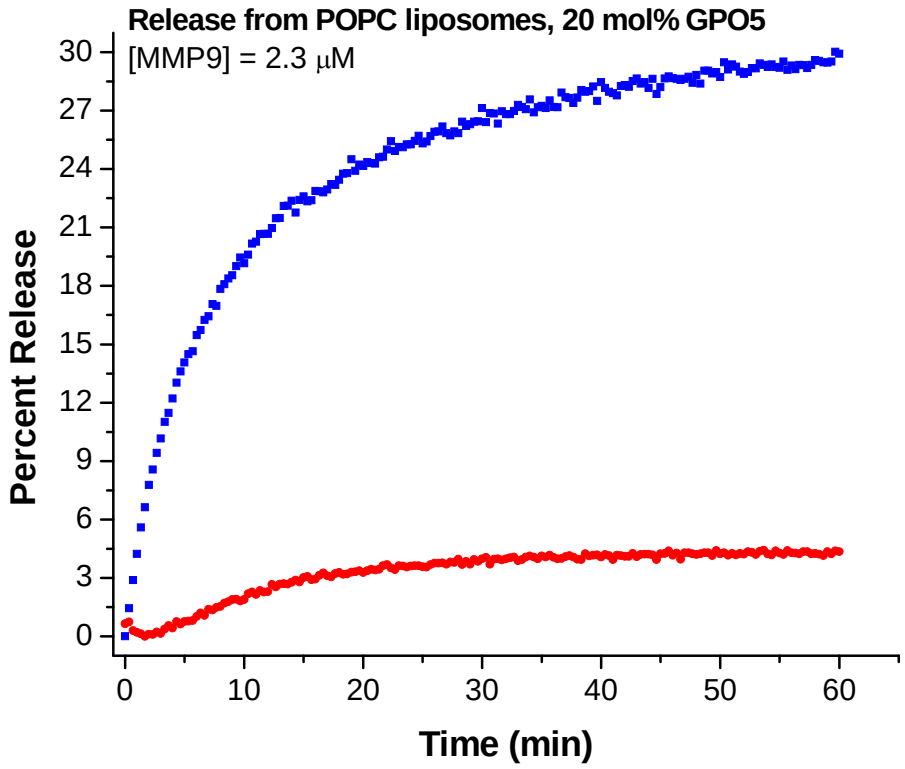


Figure S1. The carboxyfluorescein release profiles from POPC liposome containing 20 mol% of the lipopeptide **GPO5** (λ_{ex} = 480 nm; λ_{em} = 518 nm) in the presence and absence of 2.3 µM MMP-9 in 25 mM HEPES buffer, pH = 8.0 containing 100 mM NaCl, 10 mM $CaCl_2$ and 10 mM $ZnCl_2$. The enzyme released 30% of the dye in 1 h (blue squares). In the absence of MMP-9, <5% of the dye was released in 1 h (red circles).

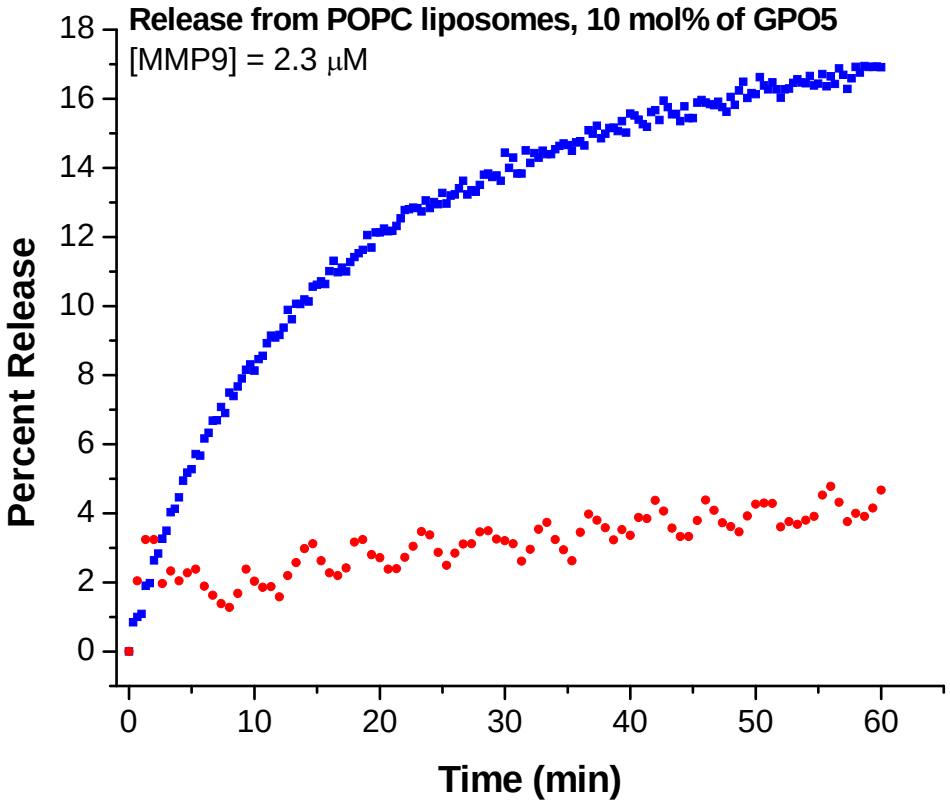


Figure S2. The carboxyfluorescein release profiles from POPC liposome containing 10 mol% of the lipopeptide **GPO5** (λ_{ex} = 480 nm; λ_{em} = 518 nm) in the presence and absence of 2.3 μM MMP-9 in 25 mM HEPES buffer, pH = 8.0 containing 100 mM NaCl, 10 mM $CaCl_2$ and 10 mM $ZnCl_2$. The enzyme released 18% of the dye in 1 h (blue squares). In the absence of MMP-9, <5% of the dye was released in 1 h (red circles).

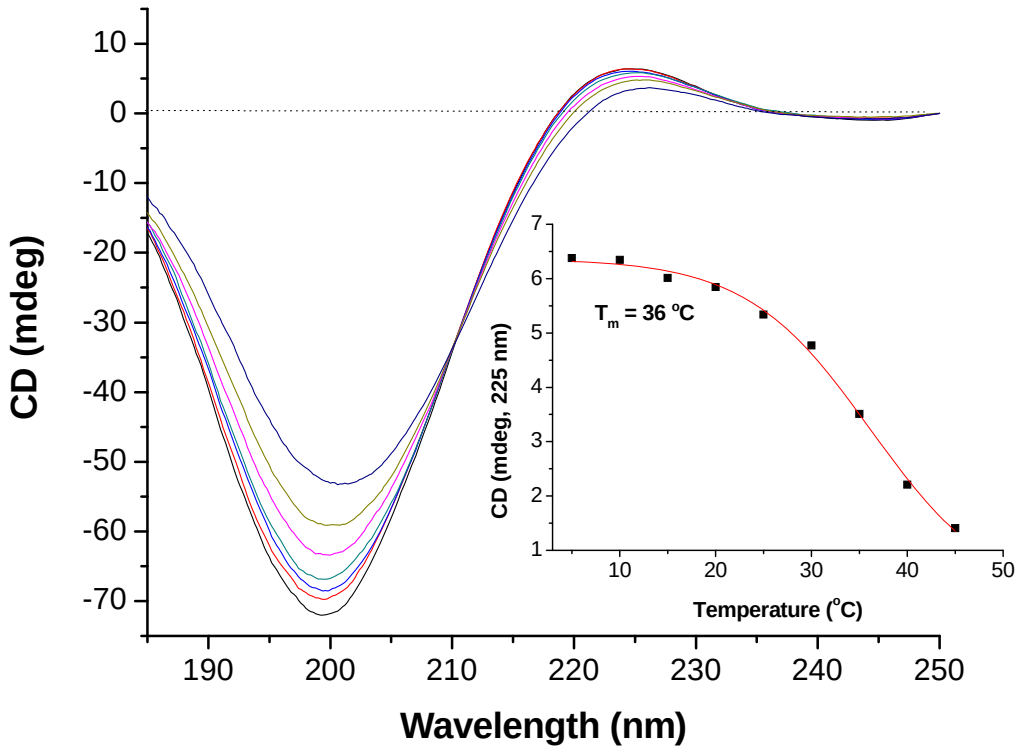


Figure S4. The temperature dependent CD spectra (5 – 45 °C) of the lipopeptide **PyGPO4** in aqueous solution. The presence of the positive peak at 225 nm and the large negative peak at 198 nm indicate triple helical structure of this lipopeptide in aqueous solution. The inset shows the melting curve for **GPO5** monitored at 225 nm. The melting temperature was calculated to be 36 °C.