Supporting Information

for

Chemoenzymatic synthesis of water-soluble lipid I fluorescent probes

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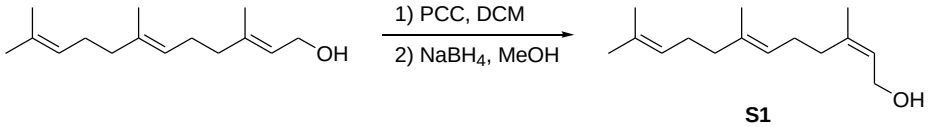
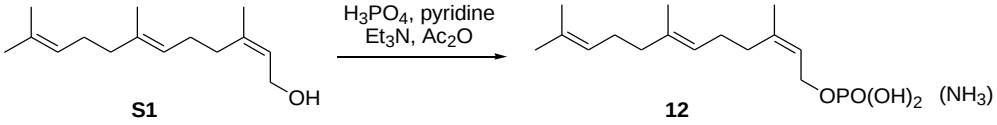
General

All chemicals were purchased from commercial sources and used without further purification unless otherwise noted. Tetrahydrofuran (THF) was distilled from potassium benzophenone ketyl under an argon atmosphere prior to use. Methylene Chloride (CH_2Cl_2), was distilled from calcium hydride under an Argon atmosphere. All stirring was performed with an internal magnetic stirrer. Reactions were monitored by thin-layer chromatography (TLC) performed with 0.25 mm coated commercial silica gel plates (EMD, Silica Gel $60F_{254}$) using UV light for visualization at 254 nm, or developed with ceric ammonium molybdate or anisaldehyde or copper sulfate or ninhydrin solutions by heating on a hot plate. Reactions were also monitored by using SHIMADZU LCMS-2020 with solvents: A: 0.1% formic acid in water, B: acetonitrile. And reactions were also monitored by SHIMADZU prominence HPLC using Phenomenex Kinetex 1.7 μ XB-C18 100A column (150 x 2.10 mm) and monitoring at 220, 254 nm with solvents: A: 0.05 M ammonium bicarbonate in water, B: acetonitrile. Flash chromatography was performed with Whatman silica gel (Purasil 60 Å, 230-400 Mesh). Proton magnetic resonance (^{1}H-NMR) spectral data were recorded on 400, and 500 MHz instruments. Carbon magnetic resonance (^{13}C-NMR) spectral data were recorded on 100 and 125 MHz instruments. Phosphorus magnetic resonance (^{31}P-NMR) spectral data were recorded on 162 MHz instruments. For all NMR spectra, chemical shifts (δH, δC) were quoted in parts per million (ppm), and J values were quoted in Hz. ^{1}H and ^{13}C NMR spectra were calibrated with residual undeuterated solvent ($CDCl_3$: δH =7.26 ppm, δC =77.16ppm; CD_3CN: δH=1.94ppm, δC =1.32ppm; CD_3OD: δH =3.31ppm, δC =49.00 ppm; DMSO-d_6: δH=2.50ppm, δC =39.5ppm; D_2O: δH=4.79 ppm) as an internal reference. The following abbreviations were used to designate the multiplicities: s=singlet, d=doublet, dd=double doublets, t=triplet, q=quartet, quin=quintet, hept=heptet, m=multiplet, br=broad. Infrared (IR) spectra were recorded on a Perkin-Elmer FT1600 spectrometer.



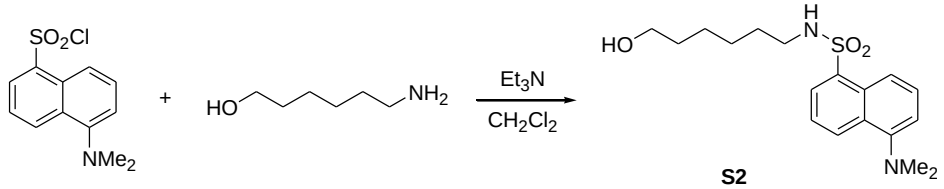
S1

To a suspended solution of PCC (0.33 g, 1.5 mmol) in DCM (30 mL) was added farnesol (0.25 mL, 1.0 mmol). After being stirred for 1 h at rt, the reaction was diluted with ether (30 mL) and filtered through silica gel, and the filtrate was concentrated. To a solution of the residue in MeOH (10 mL) was added $NaBH_4$ (0.08 g, 2.0 mmol) at 0 ^oC. After being stirred for 30 min, the reaction was quenched with sat. aq. NH_4Cl solution, and MeOH was removed under reduced pressure. The aqueous layer was extracted with hexanes and the combined organic layer was dried over anhydrous Na_2SO_4. Concentration under reduced pressure followed by purification by silica gel column chromatography (hexanes/ethyl acetate = 95/5) afforded **S1** (0.16 g, 36%). ^1H NMR (400 MHz, Chloroform-*d*) δ 5.44 (td, *J* = 7.1, 1.7 Hz, 1H), 5.14 – 5.06 (m, 2H), 4.10 (dd, *J* = 7.2, 1.0 Hz, 2H), 2.15 – 1.95 (m, 8H), 1.75 (s, 3H), 1.68 (s, 3H), 1.60 (s, 6H); LRMS (EI) calcd for $C_{15}H_{27}O$ ($[M + H]^+$): 223.21, found: 223.18.



S1 **12**

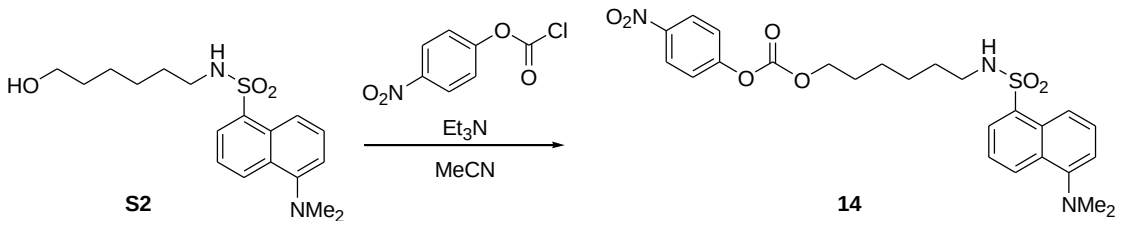
To a solution of phosphoric acid (7.0 mg, 0.072 mmol), pyridine (29 μL, 0.36 mmol), and **S1** (80 mg, 0.36 mmol) was added Et_3N (20 μL, 0.14 mmol). After being stirred for 30 min, acetic anhydride (14 μL, 0.14 mmol) was added to the reaction mixture. The reaction mixture was stirred at 80 ^oC for 24 h, and the reaction was cooled to room temperature. The reaction was quenched with water (1 ml) and stirred for 1 h at 80 ^oC. The reaction mixture was cooled to room temperature, and the aqueous phase was extracted with ether (1 ml x 3). Lyophilization of the aqueous phase gave the crude product. Purification by DOWEX 50WX8 afforded (2*Z*,6*E*)-farnesyl phosphate **12** (21 mg, 73%) as a white solid. ^1H NMR (400 MHz, Deuterium Oxide) δ 5.40 (t, *J* = 7.3 Hz, 1H), 5.20 – 5.12 (m, 2H), 4.35 (t, *J* = 7.0 Hz, 2H), 2.15 – 2.00 (m, 8H), 1.73 (s, 3H), 1.66 (s, 3H), 1.59 (s, 6H); LRMS (EI) calcd for $C_{15}H_{28}O_4P$ ($[M + H]^+$): 303.17, found: 303.19.



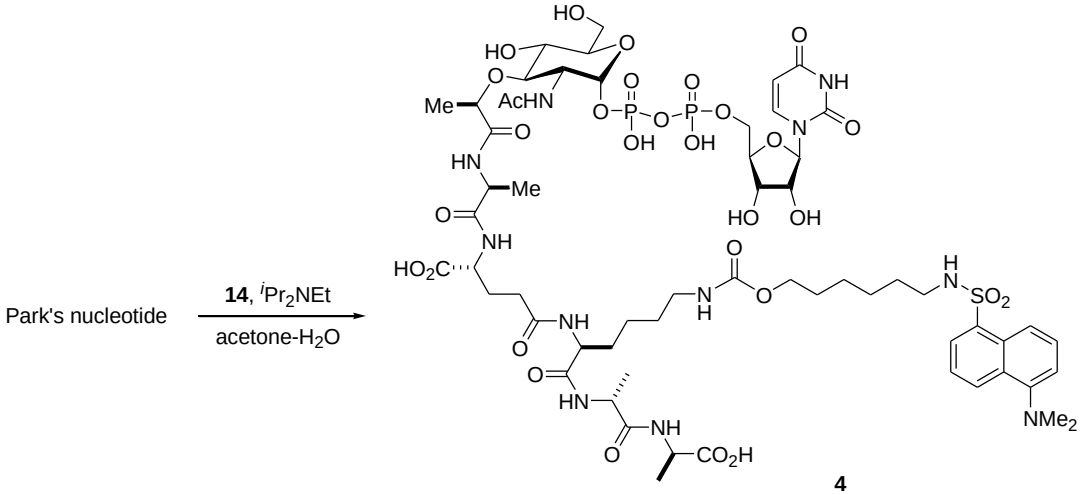
S2

To a solution of 6-amino-1-hexanol (19 mg, 0.16 mmol) and dansyl chloride (36 mg, 0.13 mmol) in DCM/DMF (4 : 1, 1.0 mL) was added Et_3N (28 μL, 0.20 mmol). After being stirred for 7 h at rt, the reaction was quenched with sat. aq. $NaHCO_3$ solution. The aqueous layer was extracted with AcOEt and the combined organic layer was dried over Na_2SO_4. Concentration under reduced pressure followed by purification by silica gel column chromatography (AcOEt) afforded **S2** (44 mg, 94%). ^1H NMR (400 MHz, Chloroform-*d*) δ 8.59 (brs, 1H), 8.32 (d, *J* = 8.6 Hz, 1H), 8.26 (dd, *J* = 7.3, 1.3 Hz, 1H),

7.61 – 7.51 (m, 2H), 7.23 (d, *J* = 7.4 Hz, 1H), 4.63 (t, *J* = 6.2 Hz, 1H), 3.50 (t, *J* = 6.6 Hz, 2H), 2.93 (s, 6H), 2.95 – 2.85 (m, 2H), 1.40 – 1.32 (m, 4H), 1.15 (h, *J* = 3.9, 2.9 Hz, 4H); LRMS (EI) calcd for $C_{18}H_{27}N_2O_3S$ ($[M + H]^+$): 351.17, found: 351.21.



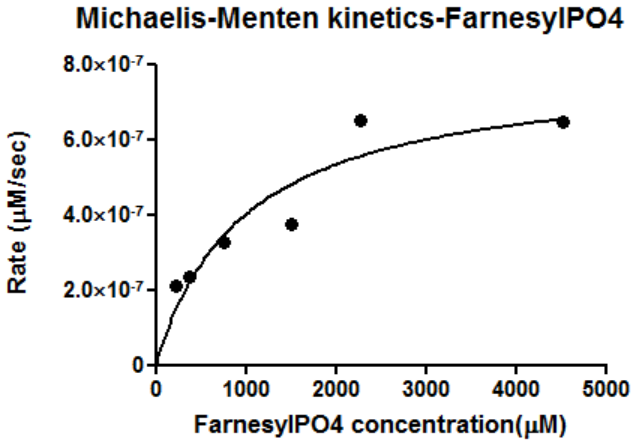
To a solution of **S2** (20 mg, 0.057 mmol) and Et_3N (16 μL, 0.12 mmol) in MeCN (0.2 mL) was added 4-nitrophenyl chloroformate (18 mg, 0.088 mmol). After being stirred for 20 h at rt, the reaction was quenched with sat. aq. $NaHCO_3$ solution. The aqueous layer was extracted with AcOEt and the combined organic layer was dried over Na_2SO_4. Concentration under reduced pressure followed by purification by silica gel column chromatography (hexanes/AcOEt = 8/2 – 6/4) afforded fluorescent linker **14** (28 mg, 98%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.61 (d, *J* = 8.5 Hz, 1H), 8.33 (d, *J* = 7.4 Hz, 1H), 8.30-8.24 (m, 1H), 8.27 (d, *J* = 9.2 Hz, 2H), 7.57 (q, *J* = 7.9 Hz, 2H), 7.37 (d, *J* = 9.2 Hz, 2H), 7.25 (brs, 1H), 4.74 (t, *J* = 6.2 Hz, 1H), 4.18 (t, *J* = 6.6 Hz, 2H), 2.95 (s, 6H), 2.97 – 2.85 (m, 2H), 1.65 – 1.56 (m, 2H), 1.47 – 1.38 (m, 2H), 1.29 – 1.21 (m, 4H); LRMS (EI) calcd for $C_{25}H_{30}N_3O_7S$ ($[M + H]^+$): 516.18, found: 516.26.



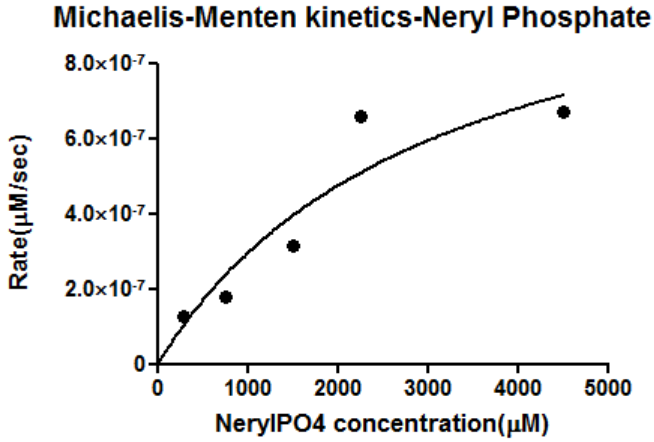
To a solution of park's nucleotide (2.6 mg, 2.2 μmol) and Hünig's base (1.6 μL, 8.9 μmol) in Acetone/H_2O (3 : 1, 0.4 mL) was added **14** (2.3 mg, 4.5 μmol). After being stirred for 3 h at rt, the reaction mixture was filtered and was concentrated under reduced pressure. The crude product was purified by reverse phase HPLC [column: HYPERSIL GOLD™ (175 Å, 12 μm, 250 x 10 mm), solvents: a gradient elution of 0 : 100 to 30 : 70 CH_3CN : 0.05 M aq. NH_4HCO_3 over 30 min, flow rate: 2.0 mL/min, UV: 254nm] to afford park's nucleotide-N^{ε}-C6-dansyl **(4)** (3.3 mg, 96%, the retention time: 22.7 min). ^{1}H NMR (400 MHz, Deuterium Oxide) δ 8.46 (d, *J* = 8.6 Hz, 1H), 8.27 (d, *J* = 8.7 Hz, 1H),

8.23 (d, *J* = 7.3 Hz, 1H), 7.89 (d, *J* = 8.1 Hz, 1H), 7.66 (q, *J* = 7.4 Hz, 2H), 7.38 (d, *J* = 7.6 Hz, 1H), 5.91 (d, *J* = 3.7 Hz, 1H), 5.89 (d, *J* = 7.9 Hz, 1H), 5.41 (dd, *J* = 7.3, 3.2 Hz, 1H), 4.32 – 4.26 (m, 2H), 4.25 – 4.02 (m, 10H), 3.90 (dt, *J* = 9.7, 3.0 Hz, 1H), 3.82 – 3.70 (m, 3H), 3.66 – 3.54 (m, 3H), 3.04 (t, *J* = 6.3 Hz, 2H), 2.92 (t, *J* = 6.5 Hz, 2H), 2.84 (s, 6H), 2.26 – 2.19 (m, 2H), 2.13 – 2.02 (m, 1H), 1.95 (s, 3H), 1.87 – 1.78 (m, 1H), 1.74 – 1.62 (m, 2H), 1.47 – 1.32 (m, 4H), 1.37 (d, *J* = 7.2 Hz, 3H), 1.34 (d, *J* = 6.7 Hz, 3H), 1.29 (d, *J* = 7.7 Hz, 3H), 1.27 (d, *J* = 7.6 Hz, 3H), 1.13 – 1.06 (m, 2H), 1.04 – 0.97 (m, 2H), 0.85 – 0.68 (m, 4H); LRMS (EI) calcd for $C_{59}H_{90}N_{11}O_{30}P_2S$ ($[M + H]^+$): 1526.51, found: 1526.40.

(A)



(B)



The kinetic parameter evaluation via MraY activity assay. (A)The correlation between the concentration of substrate (farnesyl phosphate; *x* axis) and rate (V; *y* axis). (B) The correlation between the concentration of substrate (neryl phosphate; *x* axis) and rate (V; *y* axis). The kinetic assays were carried out at room temperature in 50mM Tris-HCl pH 8.0, Triton X-100 (0.5%, 11.25 μL), 0.5 M $MgCl_2$ (10 μL), 2 M KCl (10 μL), 2 mM park's nucleotide-N^{ϵ}-C6-dansyl (3.75 μL), 20 mM farnesyl phosphate or 10 mM neryl phosphate. To a stirred reaction mixture, 1 mg/μL P-60 membrane fractions (15 μL) was added (total volume of reaction mixture: 100 μL). The reaction mixture was quenched with $CHCl_3$ (200 μL). Two phases were mixed via vortex and centrifuged at 25,000g for 10 min. The upper aqueous phase was assayed via reverse-phase HPLC. The water phase (10 μL) was injected into HPLC (column: Kinetex 5u C8 100Å, 150x4.60 mm; solvent: acetonitrile : 0.05 M aq. NH_4HCO_3 = 25 : 75; UV: 350 nm; flow rate: 0.5 mL/min) and the area of the peak for lipid I derivative was quantified. The figures and kinetic parameters values (K_M and V_{max}) were obtained by nonlinear regression analysis using the Michaelis–Menten equation with the GraphPad Prism program(GraphPad Software, San Diego, CA).

Purification of *Hy*MraY

MraY was cloned from *Hydrogenivirga spp*. into a pBAD (Invitrogen, Carlsbad, CA) derived vector. Expression was performed in the Lemo21(DE3) strain (NEB, Ipswich, MA). Cells were lysed and the membrane fraction was solubilized in decyl-maltoside (Anatrace, Maumee, OH). The protein was purified by affinity chromatography using an N-terminal His-tag followed by size-exclusion chromatography.