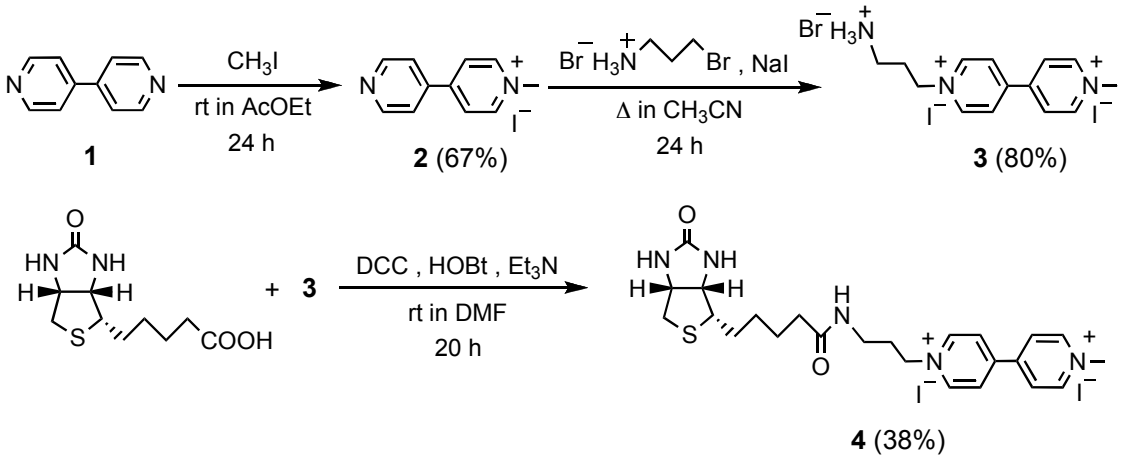
SUPPLEMENTAL METHODS

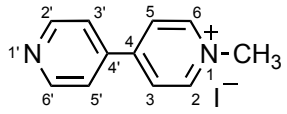
Synthesis of biotinylated PQ- 1) General scheme: The synthesis of biotinylated PQ **4** was carried out according to Scheme 1. Thus, precursor **3** was prepared by the successive *N*-alkylation of 4,4'-bipyridine **1** with iodomethane and 3-bromopropylamine hydrobromide. The condensation reaction of (+)-biotin and precursor **3** was performed by using dicyclohexylcarbodiimide (DCC) in the presence of 1-hydoroxybenzotriazole (HOBt) and triethylamine to yield the target biotinylated PQ **4** in a moderate yield.



Scheme 1

2) General Methods: All melting points were measured using a Yanagimoto Micro Melting Point apparatus and are uncorrected. IR spectra were recorded on a JASCO FT/IR-7000 spectrophotometer. ^{1}H NMR spectra were recorded on JEOL JNM-EX270 spectrometers at 270 MHz and JEOL JNM-ECS400 spectrometer at 400 MHz using tetramethylsilane (TMS) as an internal standard and measured in MHz using TMS as an internal standard and samples were measured in hexadeuteriodimethyl sulfoxide (DMSO-d_6). Chemical shifts (δ) are expressed in parts per million downfield from TMS. All reagents were used directly as obtained commercially unless otherwise noted. *N*,*N*-Dimethylformamide (DMF) was distilled from CaH_2 just prior to use.

3) Synthesis of 1-methyl-4-(4'-pyridyl)pyridinium iodide (**2**): Compound **2** was prepared as described in the literature (1). A solution of 4,4'-bipyridine **1** (3.12 g, 20.0 mmol) and iodomethane (14.91 g, 105.0 mmol) in ethyl acetate (100 ml) was stirred in a sealed tube at room temperature for 24 h. The precipitate was filtered off, dried in *vacuo*, and recrystallized from ethanol to give **2** (3.97 g, 67%) as yellow needles.



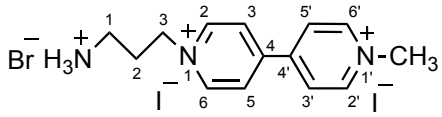
2

2: Yellow needles (EtOH); mp 258 ℃ (dec.). (lit. (9) 253-254 ℃).

IR (KBr) 3028, 2940, 1655, 1603, 1549, 812, 714 cm^{-1}.

^1H NMR (DMSO-d_6) δ 4.40 (3H, s, CH_3), 8.05 (2H, dd, J=4.4, 1.7 Hz, 3', 5'-H),
8.64 (2H, d, J=7.0 Hz, 3, 5-H), 8.88 (2H, dd, J=4.4, 1.7 Hz, 2', 6'-H),
9.16 (2H, d, J=7.0 Hz, 2, 6-H).

4) Synthesis of 3-(1'-methyl-4,4'-bipyridinium)propylammonium bromide diiodide (**3**): After a solution of 3-bromopropylamine hydrobromide (1.31 g, 6.0 mmol) and NaI (1.08 g, 7.2 mmol) in acetonitril (30 ml) was stirred at room temperature for 10 min under argon, **2** (0.89 g, 3.0 mmol) was added to the mixture and refluxed for 24 h. The precipitate was filtered off, and recrystallized from methanol to give **3** (1.36g, 80%) as orange needles.



3

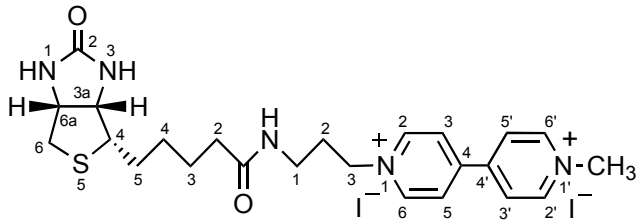
3: Orange needles (MeOH); mp 285 ℃ (dec.).

IR (KBr) 3440 (NH), 3040, 3000, 2830, 1647, 1568, 841, 710 cm^{-1}.

^1H NMR (DMSO-d_6) δ 2.34 (2H, tt, J=7.0, 7.0 Hz, 2-CH_2), 2.94 (2H, t, J=7.0 Hz, 1-CH_2),
4.48 (3H, s, CH_3), 4.87 (2H, t, J=7.0 Hz, 3-CH_2), 8.01 (3H, br s, NH_3Br),
8.84 (2H, d, J=7.0 Hz, 3', 5'-H), 8.91 (2H, d, J=7.0 Hz, 3, 5-H),
9.36 (2H, d, J=7.0 Hz, 2', 6'-H), 9.51 (2H, d, J=7.0 Hz, 2, 6-H).

5) Synthesis of N-[3-(1'-methyl-4,4'-bipyridinium)propyl]-5-[(3aS,4S,6aR)-2-oxohexahydrothieno [3,4-d]imidazol- 4-yl]pentanamide diiodide (**4**): After a solution of HOBt (0.297 g, 2.2 mmol) and DCC (0.454 g, 2.2 mmol) in dry DMF (40 ml) was stirred at room temperature for 10 min under argon, (+)-biotin (0.489 g, 2.0 mmol) was added to the mixture, which was then stirred for 10 min. After the addition of viologen **3** (1.354 g, 2.4 mmol) and triethylamine (0.344 g, 3.4 mmol), the mixture was stirred for 20 h under the same conditions. The reaction mixture was cooled in an ice bath and the precipitate was filtrated off, washed with successively with acetonitrile, hot chloroform, and hot acetonitrile under

irradiation with ultrasonic waves to give a dark brown solid **4** (0.533 g, 38%). Further purification was performed by treattment with activated charcoal.



4

4: Dark brown solid (hygroscopic).

IR (KBr) 3426, 3310 (NH), 3040, 2932, 1688 (C=O), 1640, 1562, 828, 760 cm^{-1}.

^1H NMR (DMSO-d_6) δ 1.20–1.70 (6H, m, $C\underline{H_2}C\underline{H_2}C\underline{H_2}CH_2CO$), 2.00–2.20 (4H, m, CH_2CO, $NCH_2C\underline{H_2}CH_2N^+$),

2.57 (1H, d, J=12.4 Hz, 6-H in thieno-imid), 2.82 (1H, dd, J=12.4, 5.1 Hz, 6-H in thieno-imid),

3.04–3.20 (3H, m, 4-H in thieno-imid, $NC\underline{H_2}CH_2CH_2N^+$),

4.10–4.20 (1H, m, 3a-H), 4.25–4.35 (1H, m, 6a-H), 4.45 (3H, s, CH_3),

4.71 (2H, t, J=6.7 Hz, $NCH_2CH_2C\underline{H_2}N^+$), 6.39, 6.41 (each 1H, s, 1, 3-NH in thieno-imid),

8.01 (1H, br s, NH), 8.79 (2H, d, J=6.8 Hz, 3', 5'-H in bipy),

8.83 (2H, d, J=7.0 Hz, 3, 5-H in bipy), 9.31 (2H, d, J=6.8 Hz, 2', 6'-H in bipy),

9.42 (2H, d, J=7.0 Hz, 2, 6-H in bipy).

SUPPLEMENTAL REFERENCE

1. Takenaka, S., Shigemoto, N., and Kondo, H. (1998) *Supramol Chem* **9**(1), 47 - 56