Supplementary Information

Serum proteins potentiate therapeutic efficacy of lysocin E against *S. aureus*

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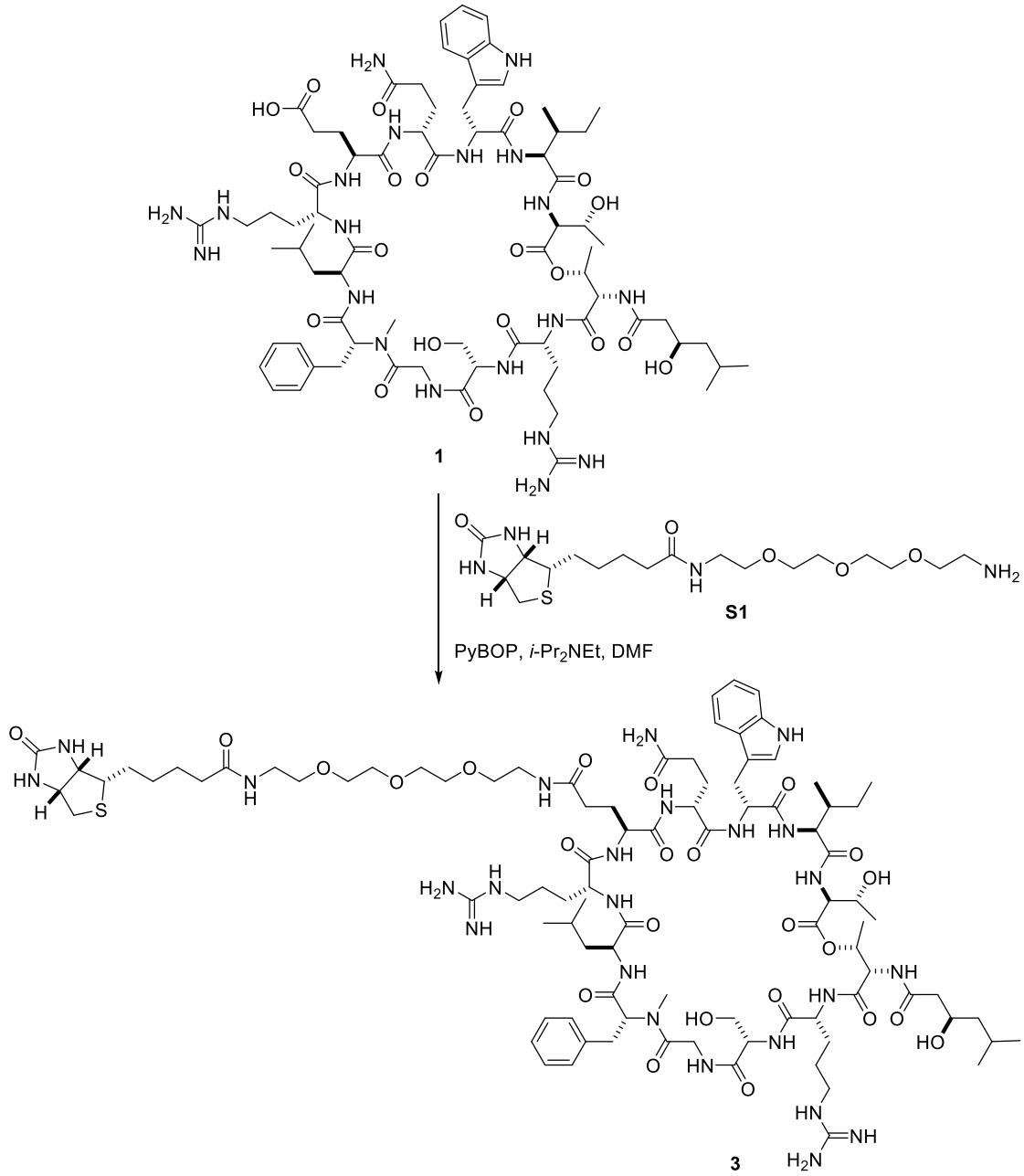
Supplementary information included in this file:

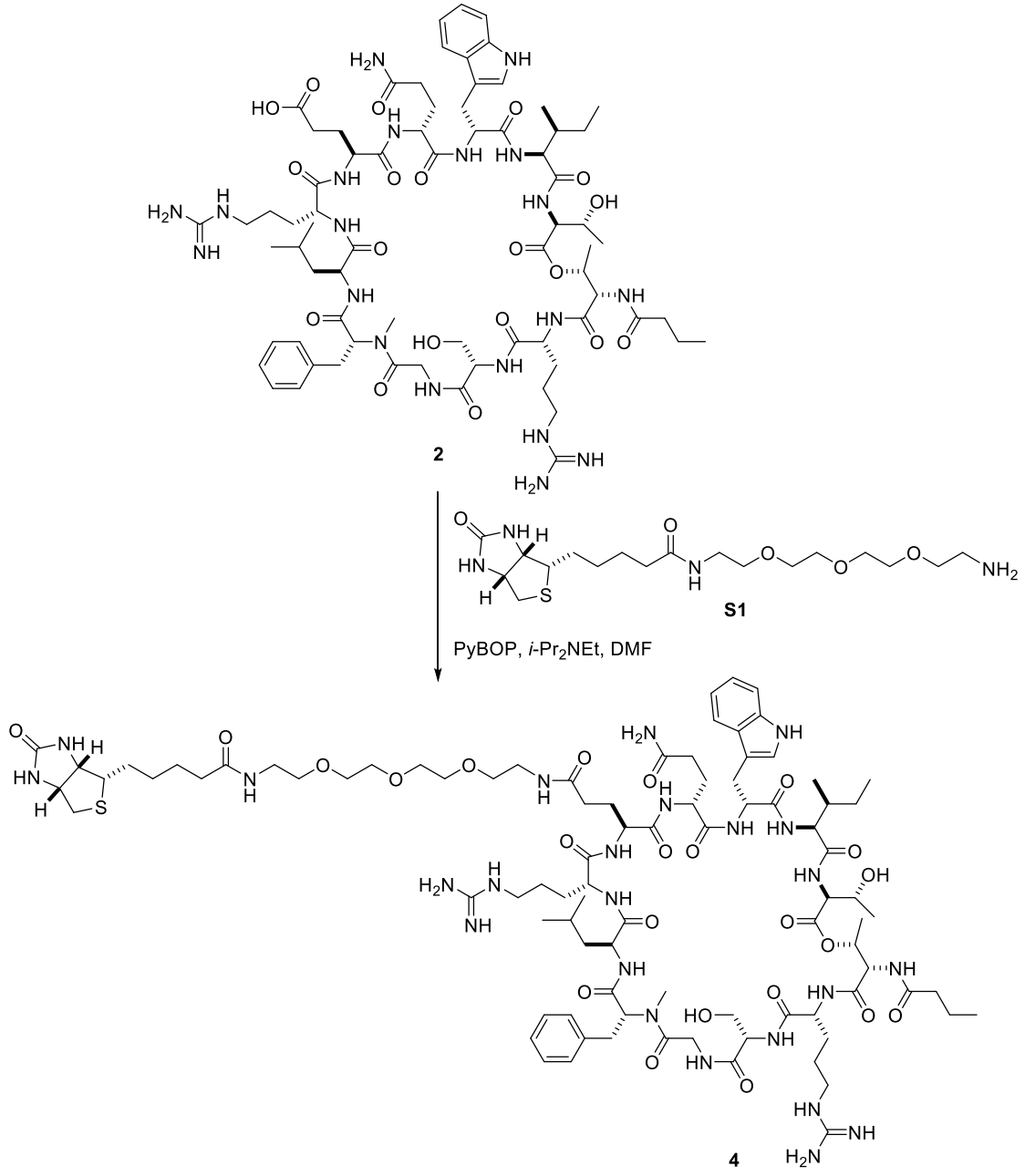
Supplementary Methods

General Remarks. All reactions sensitive to air or moisture were performed under argon (Ar) atmosphere in dry solvents, unless otherwise noted. CH_2Cl_2 and DMF were purified by a Glass Contour Solvent Dispensing System (Nikko Hansen & Co., Osaka, Japan). All other reagents were used as supplied unless otherwise stated. Analytical thin-layer chromatography was performed using E. Merck Silica gel 60 F254 pre-coated plates. High performance liquid chromatography (HPLC) experiments were performed on an HPLC system equipped with a PU-4180 RHPLC pump (JASCO Products Co., Oklahoma City, OK, USA), an 1100 HPLC system (Agilent Technologies, Santa Clara, CA, USA), or a 1200 HPLC system (Agilent). ^{1}H and $^{13}C\{^{1}H\}$ nuclear magnetic resonance (NMR) spectra were recorded on an ECX 500 (500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR) spectrometer (JEOL Ltd., Tokyo, Japan). Chemical shifts are denoted in δ (ppm) relative to residual solvent peaks as an internal standard ($CDCl_3$, ^{1}H δ 7.26, ^{13}C δ 77.0; DMSO-d_6, ^{1}H δ 2.50). Infrared spectra were recorded on an FT/IR-4100 spectrometer (JASCO). HRMS spectra were recorded on a MicrOTOFII (Bruker Daltonics, Billerica, MA, USA) electrospray ionisation time of flight (TOF) mass spectrometer. Optical rotations were recorded on a P-2200 polarimeter (JASCO).



3 (Biotinylated lysocin E). A solution of PyBOP (3.12 mg, 6.00 μmol) in DMF (150 μL) and *i*-Pr_2NEt (1.95 μL, 12.0 μmol) was added to a solution of **1** (Lysocin E) (0.993 mg, 0.600 μmol) and amine **S1** (7.53 mg, 18.0 μmol) in DMF (150 μL) at 24 °C. The resultant mixture was stirred at 24 °C for 3 h. MeOH/H_2O (58.0/42.0) containing 0.05% TFA (400 μL) was added to the reaction mixture at 0 °C. The resultant solution was concentrated. The residue was purified by a first HPLC purification (column: Inertsil ODS-4 4.6 × 250 mm, eluent A: MeOH + 0.05% TFA, eluent B: H_2O + 0.05% TFA, A/B = 62.0/38.0, flow rate: 0.80 mL/min, temperature: 40 °C, detection: UV 280 nm), second HPLC purification (column: TSKgel Amide-80 7.8 × 300 mm, eluent A: MeCN + 0.05% TFA, eluent B: H_2O + 0.05% TFA, A/B = 88.0/12.0, flow rate: 2.0 mL/min, temperature: 40 °C, UV 280 nm), and third HPLC purification (column: Inertsil ODS-4 4.6 × 250 mm, eluent A: MeOH + 0.05% TFA, eluent B: H_2O + 0.05% TFA, linear gradient A/B = 53.0/47.0 to 78.0/22.0 over 50 min,

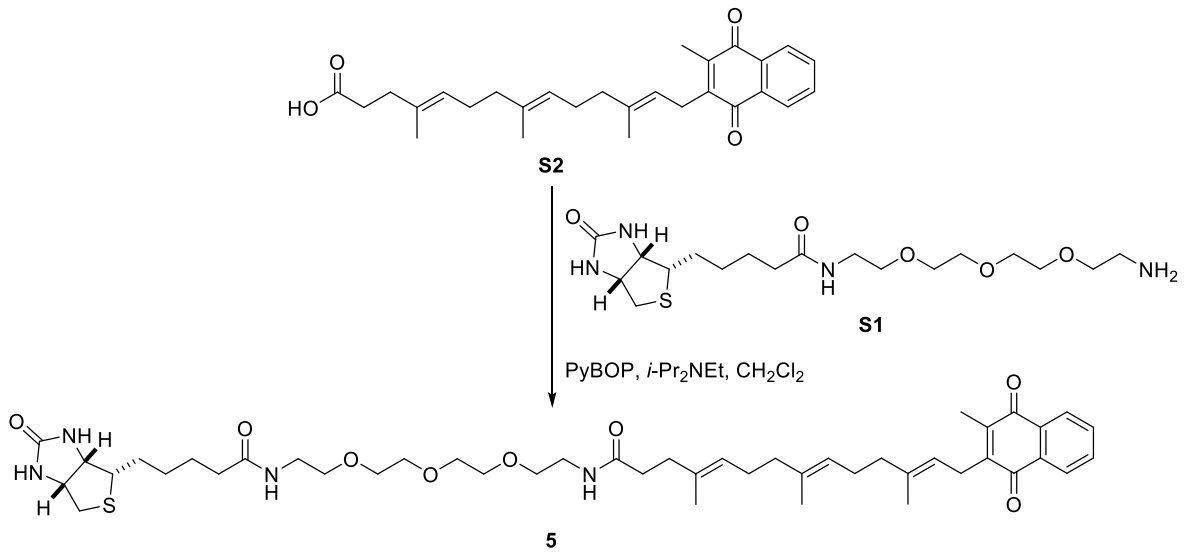
flow rate: 2.0 mL/min, temperature: 40 °C, detection: UV 280 nm) to give **3** (0.583 mg, 0.258 μmol, 43%): white solid; HRMS (ESI-TOF) calcd for $C_{93}H_{150}N_{24}O_{24}S$ $[M+2H]^{2+}$ 1010.0499, found 1010.0511.



4 (Biotinylated lysocin E Bu-type). Lysocin E Bu-type (**2**) was prepared according to the main references[13].The HRMS and HPLC data of **2** were identical to those of the reported data [CAS 2027547-29-9].

A solution of PyBOP (3.73 mg, 7.16 μmol) in DMF (179 μL) and *i*-Pr_2NEt (2.32 μL, 14.3 μmol) was added to a solution of **2** (Lysocin E Bu-type) (1.20 mg, 0.716 μmol) and amine **S1** (9.00 mg, 21.5 μmol) in DMF (179 μL) at 29 °C. The resultant mixture was stirred at 29 °C for 3 h. H_2O containing 0.05% TFA (1.80 mL) was added to the reaction mixture at 0 °C. The resultant solution was lyophilised. The residue was dissolved in MeCN/H_2O (25.0/75.0) containing 0.05% TFA (5.00 mL). The solution was loaded onto an InertSep Slim C18-

B column (360 mg). The column was washed with MeCN/H_2O (25.0/75.0) containing 0.05% TFA (5.00 mL). The crude peptide was eluted with MeCN/H_2O (60.0/40.0) containing 0.05% TFA (10.0 mL). The eluate was lyophilised. The residue was purified by a first HPLC purification (column: TSKgel Amide-80 7.8 × 300 mm, eluent A: MeCN + 0.05% TFA, eluent B: H_2O + 0.05% TFA, A/B = 88.0/12.0, flow rate: 2.0 mL/min, temperature: 40 °C, detection: UV 280 nm) and second HPLC purification (column: Inertsil ODS-4 10 × 250 mm, eluent A: MeOH + 0.05% TFA, eluent B: H_2O + 0.05% TFA, A/B = 65.0/35.0, flow rate: 2.0 mL/min, temperature: 40 °C, detection: UV 280 nm) to give **4** (0.723 mg, 0.329 μmol, 46%): white solid; HRMS (ESI-TOF) calcd for $C_{90}H_{143}N_{24}O_{23}S$ $[M+H]^+$ 1960.0474, found 1960.0446.

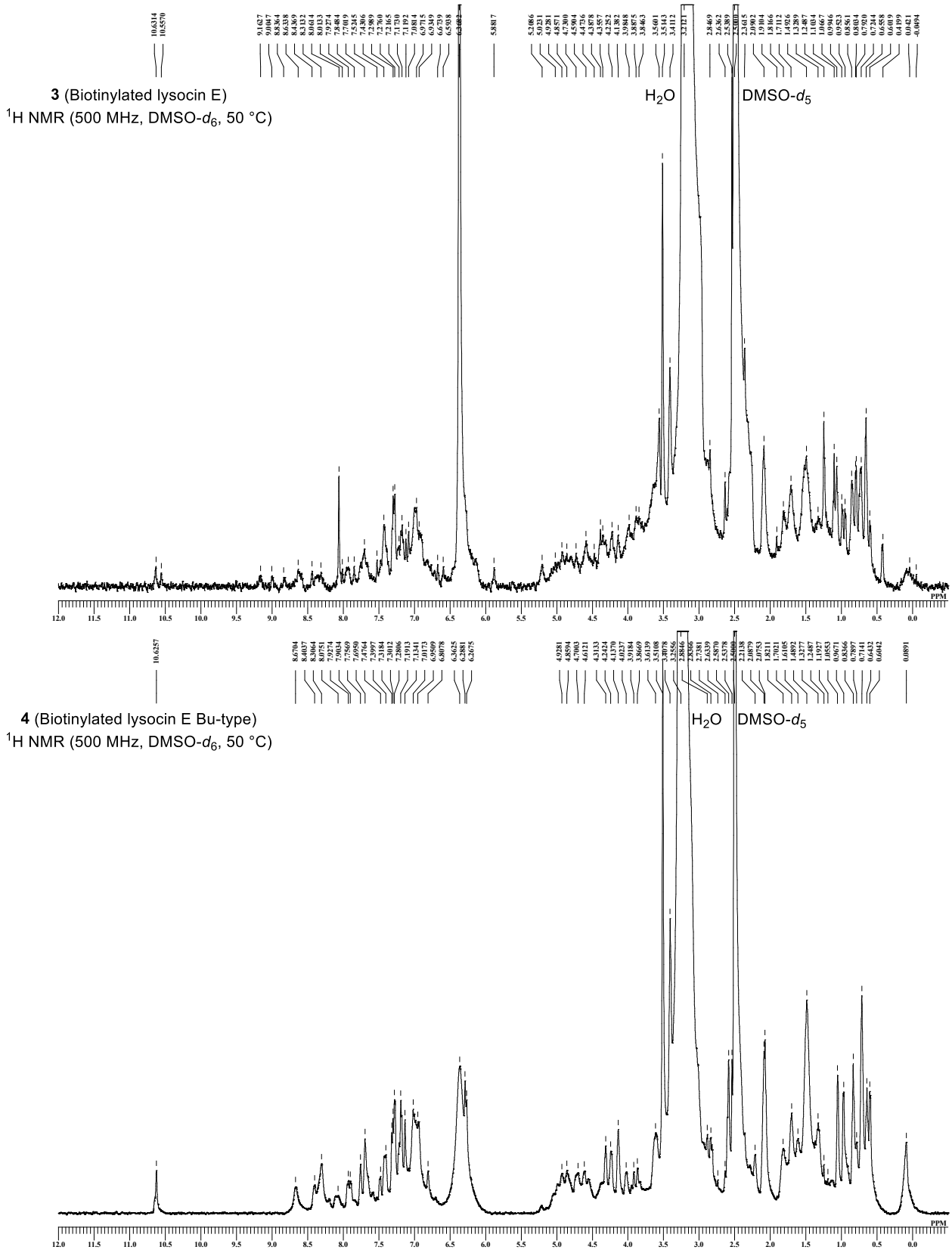


5 (Biotinylated menaquinone). Carboxylic acid **S2** was synthesised according to the literature[S1]. The ^{1}H NMR spectrum of **S2** was identical to that of the reported data [CAS 85216-29-1] [S1].

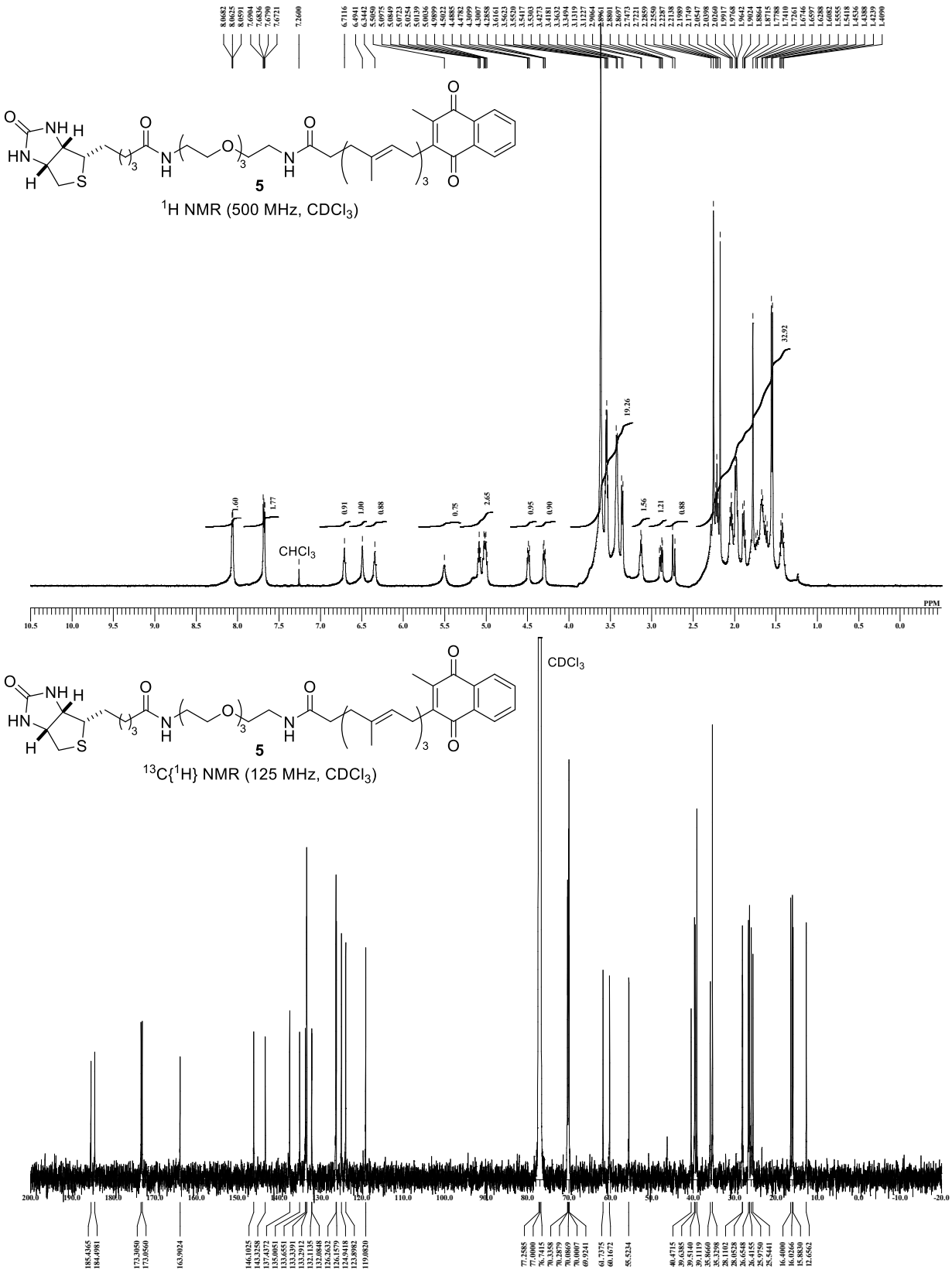
A solution of PyBOP (6.57 mg, 12.6 μmol) in CH_2Cl_2 (56.2 μL) and a solution of *i*-Pr_2NEt (2.20 μL, 12.6 μmol) in CH_2Cl_2 (56.2 μL) were added to a solution of the above carboxylic acid **S2** (3.66 mg, 8.42 μmol) and amine **S1** (5.29 mg, 12.6 μmol) in CH_2Cl_2 (56.2 μL) at 0 °C. The reaction mixture was stirred at room temperature for 2 h, and then concentrated. The residue was purified by preparative thin-layer chromatography ($CHCl_3$/MeOH 10/1) to give biotinylated menaquinone **5** (5.14 mg, 6.15 μmol, 73%): yellow film; $[\alpha]_D^{23}$ +17.5 (*c* 0.257, $CHCl_3$); IR (film) 3287, 3081, 2922, 1696, 1655, 1549, 1451, 1293, 1100 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.41−2.29 (34H, m), 2.73 (1H, d, J = 12.6 Hz), 2.89 (1H, dd, J = 13.2, 5.2 Hz), 3.13 (1H, m), 3.35−3.62 (16H, m), 4.30 (1H, dd, J = 7.5, 4.6 Hz), 4.49 (1H, dd, J = 6.9, 5.2 Hz), 4.99−5.20 (3H, m), 5.51 (1H, s), 6.34 (1H, t, J = 5.2 Hz), 6.49 (1H, s), 6.71 (1H, t, J = 5.2 Hz), 7.68 (2H, dd, J = 5.7, 3.4 Hz), 8.06 (2H, m); ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$) δ 12.7, 15.9, 16.0, 16.4, 25.5, 26.0, 26.4, 26.7, 28.1, 35.3, 35.9, 39.1, 39.5, 39.6, 40.5, 55.5, 60.2, 61.7, 69.9−70.3 (8C), 119.1, 123.9, 124.9, 126.2, 126.3 (2C), 132.1, 133.29, 133.34 (2C), 133.7, 135.0, 137.4, 143.3, 146.1, 163.9, 173.1, 173.3, 184.5, 185.4; HRMS (ESI-TOF) calcd for $C_{46}H_{66}N_4O_8SNa$ $[M+Na]^+$ 857.4494, found 857.4472.

Supplementary Reference

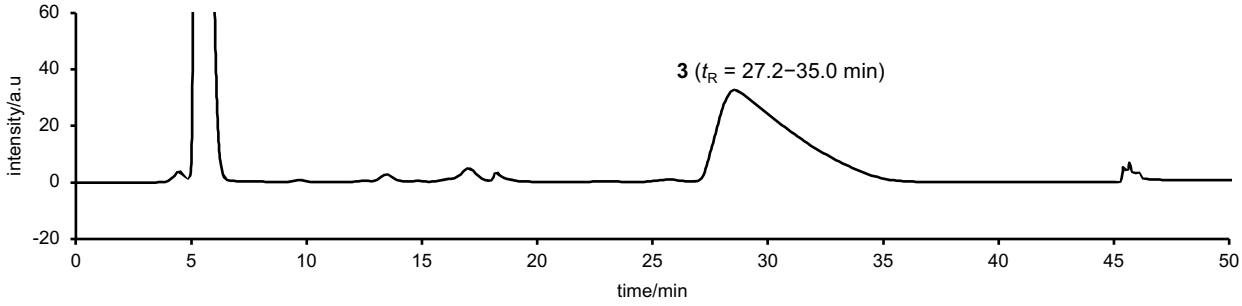
S1 Fujii, S. *et al*. Systematic synthesis and anti-inflammatory activity of omega-carboxylated menaquinone derivatives--Investigations on identified and putative vitamin K(2) metabolites. *Bioorg*. *Med*. *Chem*. **23**, 2344-2352, doi:10.1016/j.bmc.2015.03.070 (2015).



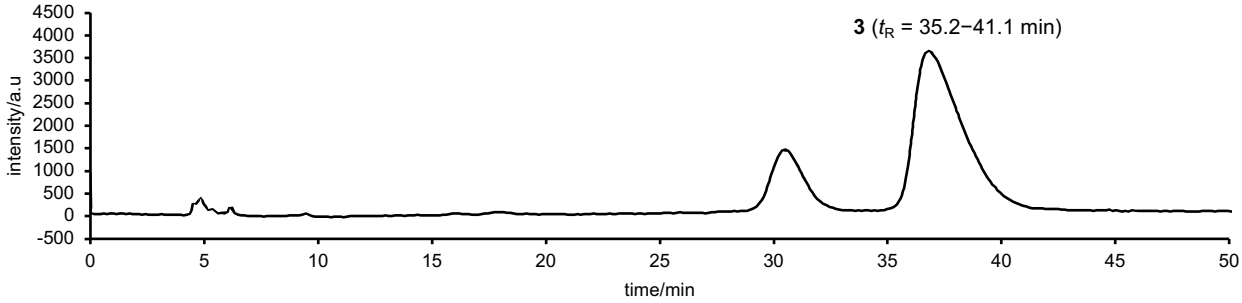
Supplementary Figure 1. ^{1}H NMR spectra of **3** and **4**.



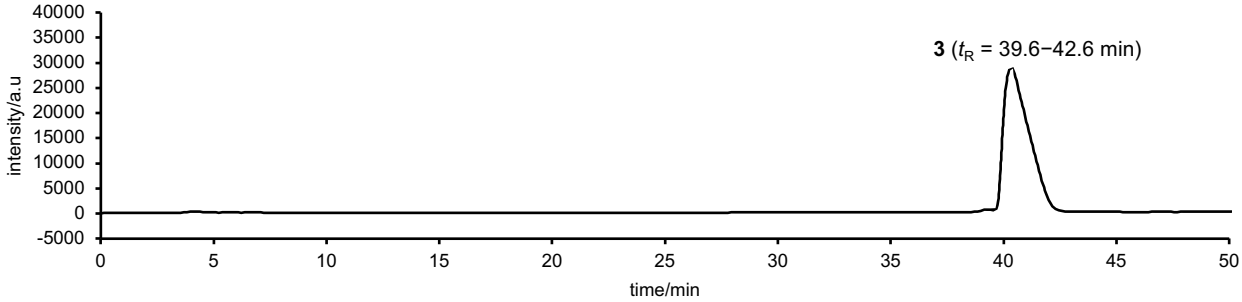
Supplementary Figure 2. ^{1}H and $^{13}C\{^1H\}$ NMR spectra of **5** (Biotinylated menaquinone).



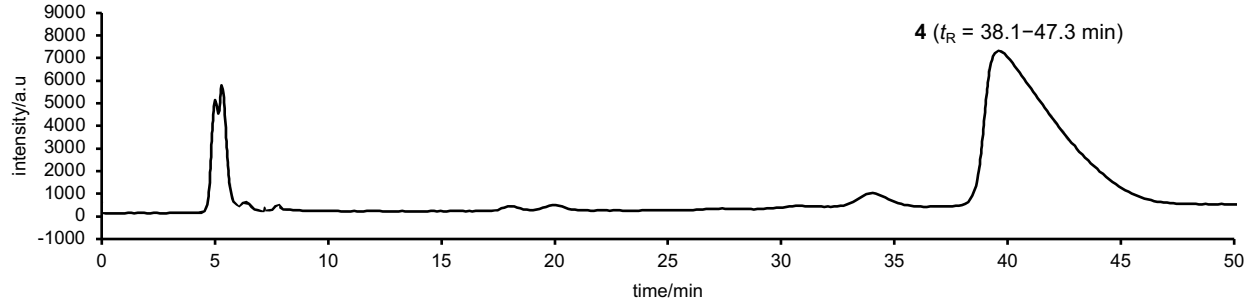
Supplementary Figure 3. HPLC chromatogram for first HPLC purification of **3** (Biotinylated lysocin E). Column: Inertsil ODS-4 4.6 × 250 mm, eluent A: MeOH + 0.05% TFA, eluent B: H_2O + 0.05% TFA, A/B = 62.0/38.0, flow rate: 0.80 mL/min, temperature: 40 °C, detection: UV 280 nm.



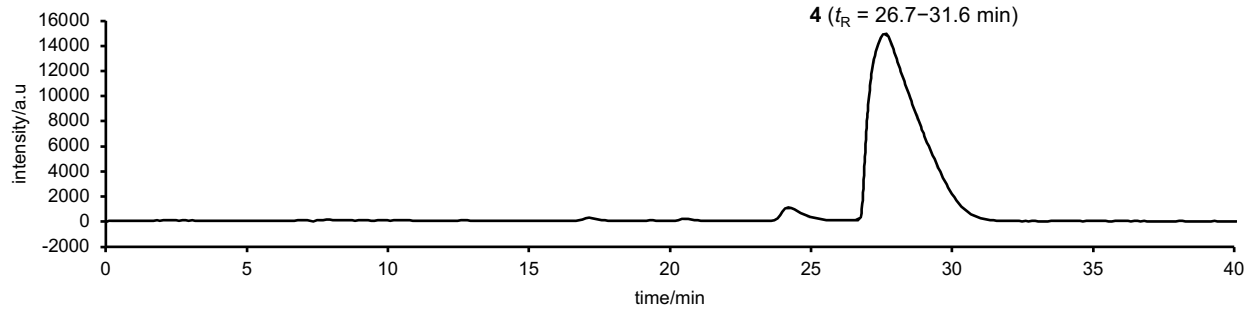
Supplementary Figure 4. HPLC chromatogram for second HPLC purification of **3** (Biotinylated lysocin E). Column: TSKgel Amide-80 7.8 × 300 mm, eluent A: MeCN + 0.05% TFA, eluent B: H_2O + 0.05% TFA, A/B = 88.0/12.0, flow rate: 2.0 mL/min, temperature: 40 °C, UV 280 nm.



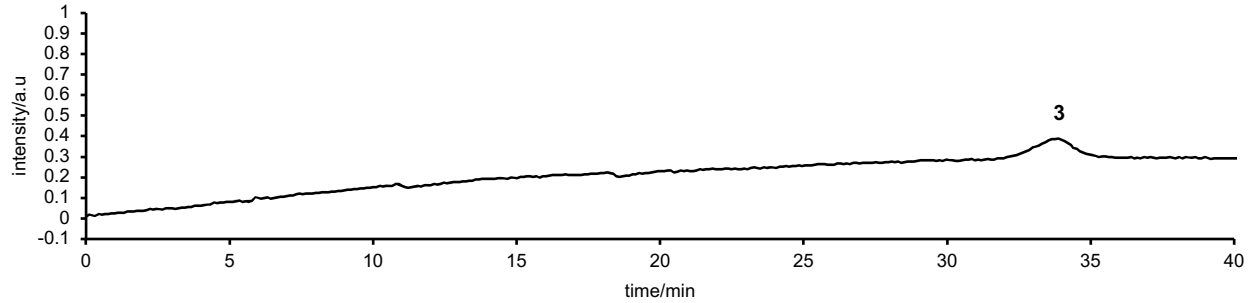
Supplementary Figure 5. HPLC chromatogram for third HPLC purification of **3** (Biotinylated lysocin E). Column: Inertsil ODS-4 4.6 × 250 mm, eluent A: MeOH + 0.05% TFA, eluent B: H_2O + 0.05% TFA, linear gradient A/B = 53.0/47.0 to 78.0/22.0 over 50 min, flow rate: 2.0 mL/min, temperature: 40 °C, detection: UV 280 nm.



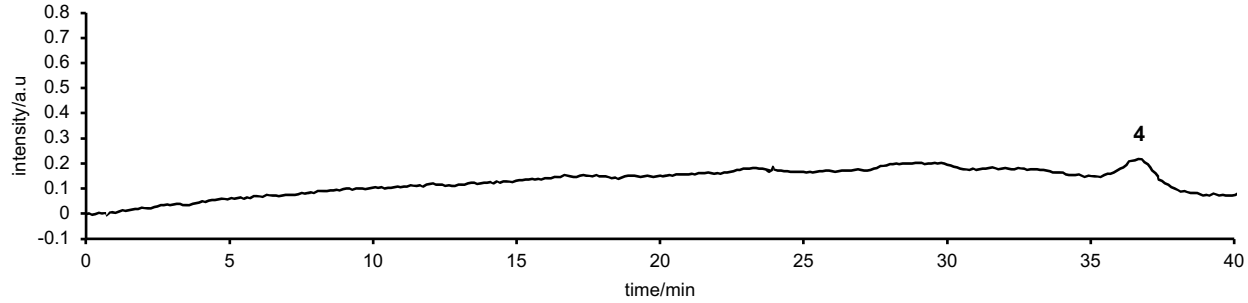
Supplementary Figure 6. HPLC chromatogram for first HPLC purification of **4** (Biotinylated lysocin E Bu-type). Column: TSKgel Amide-80 7.8 × 300 mm, eluent A: MeCN + 0.05% TFA, eluent B: H_2O + 0.05% TFA, A/B = 88.0/12.0, flow rate: 2.0 mL/min, temperature: 40 °C, UV 280 nm.



Supplementary Figure 7. HPLC chromatogram for second HPLC purification of **4** (Biotinylated lysocin E Bu-type). Column: Inertsil ODS-4 10 × 250 mm, eluent A: MeOH + 0.05% TFA, eluent B: H_2O + 0.05% TFA, A/B = 65.0/35.0, flow rate: 2.0 mL/min, temperature: 40 °C, detection: UV 280 nm.



Supplementary Figure 8. HPLC chromatogram of purified **3** (Biotinylated lysocin E). Column: Inertsil ODS-4 4.6 × 250 mm, eluent A: MeOH + 0.05% TFA, eluent B: H_2O + 0.05% TFA, A/B = 64.0/36.0, flow rate: 0.50 mL/min, temperature: 40 °C, detection: UV 280 nm.



Supplementary Figure 9. HPLC chromatogram hart of purified **4** (Biotinylated lysocin E Bu-type). Column: Inertsil ODS-4 4.6 × 250 mm, eluent A: MeOH + 0.05% TFA, eluent B: H_2O + 0.05% TFA, A/B = 62.0/38.0, flow rate: 0.50 mL/min, temperature: 40 °C, detection: UV 280 nm.