Supporting Information

Lee et al. 10.1073/pnas.1221442110

SI Materials and Methods

General Synthetic Procedures. All reactions were carried out under an argon atmosphere with dry solvents under anhydrous conditions unless otherwise noted. Reaction yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials unless otherwise stated. Reagents were used without further purification unless otherwise stated. Silica gel 60 (particle size = 0.040–0.063 mm; Merck) was used for flash column chromatography. NMR spectra were recorded on Bruker DRX-500 or AMX-400 or Varian Inova-400 instruments and calibrated using residual undeuterated solvent as an internal reference. The following abbreviations were used to explain multiplicities: br, broad; d, doublet; m, multiplet; q, quartet; quin, quintuplet; s, singlet; sep, septet; sext, sextet; t, triplet. Infrared (IR) spectra were recorded on a Perkin-Elmer 1600 series FT-IR Spectrometer. Electrospray ionization MS (ESI-MS) experiments were performed on an API 100 Perkin-Elmer SCIEX Single Quadrupole Mass Spectrometer at 4,000 V emitter voltage. High-resolution mass spectra (HRMS) were recorded on a VG ZAB-ZSE mass spectrometer using ESI. Reactions for the synthesis of facial amphiphiles (FAs) intermediates were drawn in Scheme S1.

3α,7α,12α-Trihydroxycholane (1). To MeOH (900 mL) and acetic acid (100 mL) solution at room temperature, cholic acid (50 g) and NaOMe (20 g) in portions were added. Kolbe electrolysis was carried out using 4.5 V (*ca.* 1.5 A) direct current with two platinum meshes (2 × 2 cm) spaced ∼2–3 mm apart. The reaction was run for ∼40 h, and then, 900 mL water were added to the mixture within 10 min. The mixture was stirred for 0.5 h and then filtered to give a white solid as the crude product (∼85% purity). Recrystallization in MeOH-H_2O gave **1** (17 g, 35%) with >95% purity.

^{1}H NMR (500 MHz, $CDCl_3$) δ = 3.99 (brs, 1H), 3.84 (brs, 1H), 3.45–3.41 (m, 1H), 3.18 (m, 1H), 2.71 (brs, 1H), 2.63 (m, 1H), 2.20–2.15 (m, 2H), 1.95–1.03 (m, 22H), 0.97–0.85 (m, 9H), 0.73 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 73.6, 72.3, 68.9, 47.9, 46.8, 42.1, 41.9, 40.0, 38.6, 35.8, 35.8, 35.1, 35.0, 30.7, 28.4, 28.0, 26.8, 23.6, 22.9, 19.7, 18.1, 14.9, 12.9.

3α,7α,12α-Tri(2-Propenyloxy)-Cholane (2) (General Procedure for Allylation). To a solution of **1** (17 g, 45 mmol) in dry tetrahydrofuran (THF, 200 mL), NaH (60% in mineral oil, 8 g, 0.2 mol) at 0 °C was added. The mixture was slowly warmed to room temperature and stirred for 1 h, and then, allyl iodide was added (33 g, 0.2 mol). After overnight reaction, saturated NH_4Cl solution was added. The organic phase was extracted with EtOAc, washed with brine, and dried over Na_2SO_4. After filtration and removal of solvent in vacuo, the residue was purified by silica column chromatography to give **2** (19 g, 85%).

^{1}H NMR (500 MHz, $CDCl_3$) δ = 6.05–5.95 (m, 3H), 5.36–5.28 (m, 3H), 5.22–5.14 (m, 3H), 4.16–4.14 (m, 2H), 4.08–4.07 (m, 2H), 3.88–3.79 (m, 2H), 3.62 (brs, 1H), 3.40 (brs, 1H), 3.24–3.20 (m, 1H), 2.35–1.01 (m, 24H), 0.98–0.92 (m, 9H), 0.73 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 136.5, 136.3, 116.6, 115.8, 115.8, 81.2, 79.5, 75.3, 69.8, 69.7, 69.1, 47.0, 46.7, 42.9, 42.4, 40.2, 38.7, 36.0, 35.8, 35.4, 29.3, 28.4, 28.1, 27.9, 23.7, 23.4, 19.7, 18.2, 15.0, 13.0.

3α,7α,12α-Tri(2-Hydroxyethoxy)-Cholane (3) (General Procedure for Ozonolysis and Reduction). At −78 °C, ozone was bubbled into a solution of **2** (19 g, 38 mmol) in CH_2Cl_2 (100 mL) and MeOH (100 mL) until blue color persisted. Excess ozone was removed with a stream of oxygen until the blue color disappeared. Me_2S (20 mL, 0.27 mol) was then added, and the reaction was stirred for 0.5 h. After adding $NaBH_4$ (10 g, 0.26 mol) in 5% NaOH solution (100 mL), the reaction was slowly warmed to room temperature and stirred overnight. The reaction was carefully quenched with 2 N HCl solution to pH 5–6 followed by removal of organic solvent in vacuo. The remaining aqueous solution was extracted with EtOAc, and the organic layer was washed with brine and dried over Na_2SO_4. After filtration and removal of solvent in vacuo, the residue was purified by column chromatography to give **3** (15 g, 77%).

^{1}H NMR (500 MHz, $CDCl_3$) δ = 3.82–3.70 (m, 8H), 3.66–3.61 (m, 3H), 3.43–3.39 (m, 2H), 3.28 (brs, 1H), 3.22 (brs, 1H), 3.05 (brs, 3H), 2.25–1.05 (m, 24H), 0.98–0.93 (m, 9H), 0.75 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 81.3, 80.0, 76.8, 70.5, 69.5, 62.6, 62.5, 62.4, 47.1, 46.7, 42.1, 38.7, 35.6, 35.2, 28.3, 27.6, 23.2, 18.4, 14.9, 12.9.

3α-Benzyloxy-7α,12α-Dihydroxycholane 4. To a solution of **1** (17 g, 45 mmol) in dry THF (200 mL) held at 0 °C, NaH (60% in mineral oil, 6 g, 0.15 mol) in portions and Bu_4NI (1 g, 3 mmol) were added. The reaction was warmed to room temperature and stirred for 1 h, and then, BnBr (7 mL, 60 mmol) was added by syringe pump over 10 h. After proceeding overnight, the reaction was carefully quenched with water. The solution was extracted with EtOAc. The organic layer was washed sequentially with 2N HCl, saturated $NaHCO_3$ solution and brine, and dried over Na_2SO_4. After filtration and removal of solvent in vacuo, the residue was purified by column chromatography to give **4** (15 g, 70%).

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.43–7.33 (m, 5H), 4.66–4.60 (m, 2H), 4.06 (brs, 1H), 3.90 (brs, 1H), 3.32–3.30 (m, 1H), 2.34–2.29 (m, 2H), 1.95–1.12 (m, 24H), 1.05–0.97 (m, 9H), 0.76 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 139.6, 128.7, 128.0, 127.7, 79.1, 73.4, 70.0, 68.7, 48.0, 46.9, 42.4, 35.8, 35.6, 35.4, 35.0, 28.0, 27.2, 23.0, 18.1, 14.9, 13.0.

3α-Benzyloxy-7α,12α-di-(2-propenyloxy)-cholane (**5**) was synthesized from **4** according to the general allylation procedure above. Yield: 89%; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.44–7.34 (m, 5H), 6.05–5.98 (m, 2H), 5.41–5.31 (m, 2H), 5.21–5.16 (m, 2H), 4.66–4.63 (m, 2H), 4.16–4.14 (m, 2H), 3.91–3.77 (m, 2H), 3.63 (s, 1H), 3.42 (d, J = 3.0 Hz, 1H), 3.32–3.27 (m, 1H), 2.42–1.05 (m, 26 H), 1.02–0.97 (m, 9H), 0.75 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 139.7, 136.2, 136.2, 128.4, 127.6, 127.4, 115.6, 115.6, 81.0, 79.3, 75.0, 69.7, 69.5, 69.4, 46.7, 46.5, 42.7, 42.2, 40.0, 38.5, 35.7, 35.6, 35.2, 29.0, 28.2, 27.8, 27.7, 23.4, 23.4, 23.2, 19.5, 18.0, 14.8, 12.7.

3α-Benzyloxy-7α,12α-di-(2-hydroxyethoxy)-cholane (**6**) was synthesized from **5** according to the general ozonolysis/reduction procedure. Yield: 78%; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.33–7.24 (m, 5H), 4.54 (s, 2H), 3.79–3.65 (m, 6H), 3.56 (s, 1H), 3.27–3.13 (m, 4H), 2.21–1.02 (m, 26H), 0.94–0.84 (m, 9H), 0.68 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 139.2, 128.3, 127.5, 127.3, 81.0, 78.8, 76.3, 69.9, 69.8, 69.3, 62.3, 62.2, 46.6, 46.4, 41.8, 38.2, 35.2, 34.9, 27.9, 27.4, 22.8, 18.0, 14.5, 12.5.

7α,12α-Di-(2-propenyloxy)-cholane (**7**) was synthesized from 7α,12α-dihydroxycholane (1) according to the general allylation procedure. Yield: 92%; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 5.97–5.88 (m, 2H), 5.33–5.22 (m, 2H), 5.11–5.06 (m, 2H), 4.08–4.04 (m, 2H), 3.81–3.67 (m, 2H), 3.55 (brs, 1H), 3.31 (brs, 1H), 2.20–1.04 (m, 24H), 0.91–0.86 (m, 9H), 0.66 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 136.5, 115.6, 81.4, 75.8, 69.6, 69.5, 47.0, 46.7, 43.1, 38.7, 36.0, 35.8, 28.5, 28.1, 23.6, 22.0, 18.2, 15.0, 13.0.

7α,12α-Di-(2-hydroxyethoxy)-cholane (**8**) was synthesized from **7** according to the general ozonolysis/reduction procedure. Yield: 87%; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 3.76–3.65 (m, 6H), 3.58 (brs, 1H), 3.35–3.33 (m, 23H), 3.24–3.21 (m, 1H), 2.27 (brs, 2H), 2.14–1.02 (m, 26H), 0.92–0.86 (m, 9H), 0.69 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 81.0, 75.8, 69.6, 69.0, 62.3, 62.3, 46.7, 46.3, 42.7, 39.6, 35.4, 35.4, 27.9, 27.6, 22.9, 18.0, 14.5, 12.5.

7α,12α-Di-((O-β-D-Maltopyranosyl)Ethyloxy)-Cholane (FA-2; General Procedure for Glycosylation). A suspension of **8** (1.0 g, 2.2 mmol), 1-thio-ethyl-hepta-O-benzoyl-β-D-maltoside (9.0 g, 8.0 mmol), and 4 Å molecular sieves (2 g) in anhydrous CH_2Cl_2 (100 mL) were stirred for 30 min at room temperature. After cooling the solution to −30 °C, crystallized *N*-iodosuccinimide (1.8 g, 8.0 mmol) and silver trifluorosulfonate (563 mg, 2.2 mmol) were added. The reaction was slowly warmed to room temperature and stirred overnight. After filtration through a pad of celite, the reaction mixture was quenched with saturated $Na_2S_2O_3$ solution and extracted with CH_2Cl_2. The organic layer was washed with brine and dried over Na_2SO_4. After filtration and removal of solvent in vacuo, the residue was passed through a short silica gel column to give crude syrup, which was used directly without additional purification.

To a solution of the above residue in dry CH_2Cl_2 (50 mL) and MeOH (50 mL), NaOMe (500 mg) was added. The reaction mixture was stirred overnight and then neutralized with Dowex 50WX2 ion exchange resin. After filtration and removal of solvent in vacuo, the residue was purified by column chromatography to give a white solid FA-2 (1.78 g, 72% over two steps).

^{1}H NMR (500 MHz, CD_3OD) δ = 5.14 (d, *J* = 5.0 Hz, 1H), 5.13 (d, *J* = 5.0 Hz, 1H), 4.38 (d, *J* = 9.5 Hz, 2H) 4.00–3.15 (m, 34H), 2.20–0.85 (m, 35H), 0.67 (s, 3H); ^{13}C NMR (500 MHz, CD_3OD) δ = 104.0, 102.8, 81.2, 81.2, 77.7, 77.6, 76.4, 74.7, 74.6, 74.0, 62.6, 48.5, 47.8, 47.4, 45.0, 40.8, 36.8, 36.4, 24.2, 20.3, 18.6, 15.0, 13.0; HRMS: calculated for $C_{52}H_{90}O_{24}Na^+$ [M + Na^+]: 1,121.5714, found 1,121.5696; IR = 3,374, 2,934, 1,107 cm^{-1}.

3α-Hydroxyl-7α,12α-di-((O-β-D-Maltopyranosyl)Ethyloxy)-Cholane (FA-3). FA-3 was synthesized from **6** according to a procedure similar to the synthesis of FA-2, except that an additional hydrogenation step was conducted to remove the benzyl protective groups. Hydrogenation was conducted in MeOH in the presence of 10% Pd/C. FA-3 was purified by column chromatography as a white solid (56% over three steps).

^{1}H NMR (500 MHz, CD_3OD) δ = 5.16 (d, *J* = 4.0 Hz, 1H), 5.15 (d, *J* = 3.5 Hz, 1H), 4.49 (d, *J* = 7.5 Hz, 1H), 4.40 (d, *J* = 7.5 Hz, 1H), 4.00–3.15 (m, 35H), 2.20–0.88 (m, 33H), 0.71 (s, 3H); ^{13}C NMR (500 MHz, CD_3OD) δ = 104.1, 103.9, 103.0, 102.9, 83.1, 81.4, 77.7, 76.5, 75.1, 74.8, 74.2, 73.0, 71.5, 69.9, 69.5, 69.4, 68.8, 62.8, 62.2, 48.4, 48.0, 47.4, 44.2, 43.4, 41.0, 39.6, 36.8, 35.6, 31.5, 29.8, 29.5, 28.7, 24.2, 23.4, 20.4, 18.6, 15.0, 13.0; HRMS: calculated for $C_{52}H_{91}O_2^+$ [M + H^+]: 1,115.5771, found 1,115.5834; IR = 3,371, 2,928, 2,866, 2,359, 1,372, 1,075, 1,035 cm^{-1}.

3α,7α,12α-Tri((O-β-D-maltopyranosyl)ethyloxy)-cholane (FA-4) was synthesized from **3** using a procedure similar to the synthesis of FA-2. Yield: 67% over two steps; white solid.

^{1}H NMR (500 MHz, CD_3OD) δ = 5.21–5.18 (m, 3H), 4.40 (d, *J* = 8.0 Hz, 1H), 4.39 (d, *J* = 8.0 Hz, 1H), 4.36 (d, *J* = 8.0 Hz, 1H), 4.10–3.20 (m, 51H), 2.31–0.88 (m, 33H), 0.70 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ = 103.3, 103.2, 101.9, 101.82, 101.8, 81.9, 80.7, 80.3, 80.2, 80.1, 76.9, 76.8, 76.7, 76.5, 75.6, 75.5, 75.4, 74.1, 74.0, 73.8, 73.7, 73.6, 73.1, 70.6, 70.5, 70.4, 69.1, 68.9, 68.1, 67.8, 67.3, 61.8, 61.4, 61.3, 61.2, 46.9, 46.4, 43.1, 42.3, 39.9, 38.6, 35.9, 35.3, 35.1, 34.9, 28.6, 28.5, 27.7, 27.4, 23.4, 23.2, 22.3, 19.3, 17.0, 14.0, 12.0; HRMS: calculated for $C_{66}H_{114}O_{36}Na^+$ [M + Na^+]: 1,505.6982, found 1,505.6971; IR = 3,351, 2,930, 2,496, 2,238, 2,072, 1,368, 1,119, 1,035, 983 cm^{-1}.

3α,7α,12α-Tri((O-β-D-glucopyranosyl)ethyloxy)-cholane (FA-5) was synthesized according to the general glycosylation procedure from the reaction of **3** and 1-thio-ethyl-tetra-(O-benzoyl)-β-D-glucoside. Yield: 66% over two steps; white solid.

^{1}H NMR (500 MHz, CD_3OD) δ = 4.41 (d, *J* = 8.0 Hz, 1H), 4.37 (d, *J* = 8.0 Hz, 1H), 4.34 (d, *J* = 8.0 Hz, 1H), 4.10–3.15 (m, 33H), 2.31–0.95 (m, 30H), 0.90 (t, *J* = 7.0 Hz, 3H), 0.72 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): 104.5, 104.2, 83.0, 81.7, 78.1, 78.1, 78.0, 77.9, 77.8, 77.6, 75.2, 75.2, 75.1, 71.7, 71.6, 70.1, 69.9, 69.7, 69.1, 68.9, 68.3, 62.8, 63.0, 47.5, 36.9, 35.9, 23.4, 18.6, 15.0, 13.0 ppm; HRMS: calculated for $C_{48}H_{84}O_{21}Na^+$ [M + Na^+]: 1,019.5397, found 1,019.5400; IR = 3,361, 2,929, 2,868, 2,490, 2,072, 1,458, 1,368, 1,163, 1,076, 983, 618 cm^{-1}.

7α,12α-Di-(((2-(Trimethylamino)Ethyl)Phosphoryl)Ethyloxy)-Cholane (FA-6). To a solution of **8** (1.0 g, 2.2 mmol) and Et_3N (0.72 mL, 5.0 mmol) in dry toluene (50 mL), ethylene chlorophosphate (0.42 mL, 4.6 mmol) at 0 °C was added. The reaction was then warmed to room temperature and stirred for 1 h. Precipitated salt was filtered out and washed with dry toluene (50 mL). The filtrate was concentrated in vacuo, and the crude residue was used directly without additional purification.

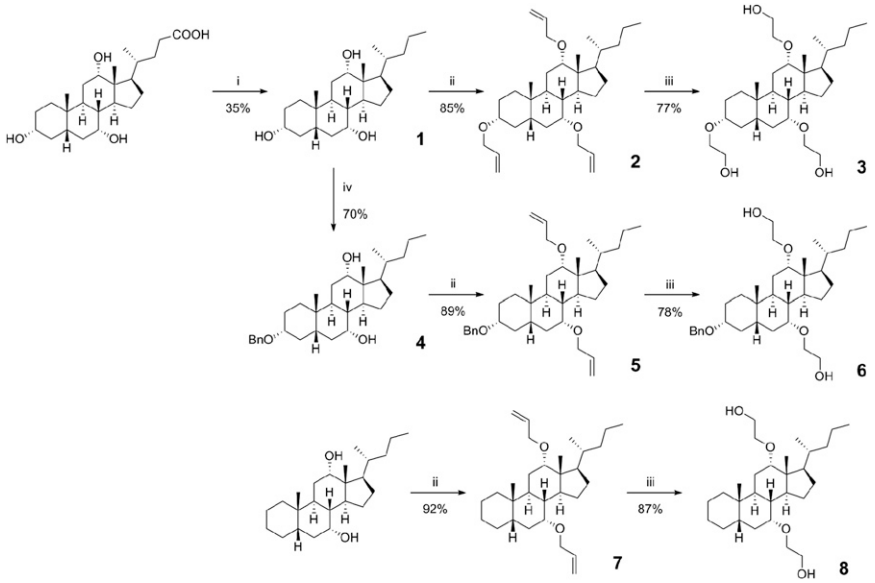
A solution of the above residue in dry acetonitrile (100 mL) in a sealed tube was cooled with a dry ice/acetone bath during addition of Me_3N (10 mL). The reaction was then maintained at 70 °C for 24 h. After removal of solvent in vacuo, the residue was purified by column chromatography to give FA-6 as a white solid (1.14 g, 64% over two steps).

^{1}H NMR (400 MHz, CD_3OD) δ = 4.36 (brs, 4 H), 4.05–4.00 (m, 4H), 3.80–3.69 (m, 6H), 3.58 (s, 1H), 3.42–3.36 (m, 3H), 3.25 (s, 18H), 2.20–1.02 (m, 23H), 0.97 (d, *J* = 6.8 Hz, 3H), 0.93 (s, 3H), 0.88 (t, *J* = 6.8 Hz, 3H), 0.72 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD): δ = 82.0, 76.9, 68.3, 66.4, 66.3, 66.2, 65.6, 65.3, 65.1, 59.5, 53.8, 53.7, 53.6, 46.6, 44.1, 43.3, 40.0, 38.8, 37.7, 36.5, 35.5, 29.3, 29.0, 28.5, 27.9, 27.8, 23.3, 21.8, 19.7, 17.6, 14.0, 12.1; HRMS: calculated for $C_{38}H_{74}N_2O_{10}P_2Na^+$ [M + Na^+]: 803.4711, found 803.4730; IR = 3,356, 2,927, 2,866, 1,473, 1,074, 1,033, 670 cm^{-1}.

3α-Hydroxyl-7α,12α-di-(((2-(Trimethylamino)Ethyl)Phosphoryl)Ethyloxy)-Cholane (FA-7). FA-7 was synthesized from **6** according to a procedure similar to the synthesis of FA-6, except that an additional hydrogenation step was conducted to remove the benzyl protective groups. Hydrogenation was conducted in MeOH in the presence of 10% Pd/C. FA-7 was purified by column chromatography as a white solid (65% over three steps).

^{1}H NMR (400 MHz, CD_3OD) δ = 4.25 (brs, 3 H), 4.05–3.95 (m, 4H), 3.76–3.53 (m, 5H), 3.53 (s, 1H), 3.41–3.38 (m, 1H), 3.31 (s, 2H), 3.25 (s, 1H), 3.04 (s, 18H), 2.20–1.02 (m, 23H), 0.92 (d, *J* = 6.0 Hz, 3H), 0.87 (s, 3H), 0.83 (t, *J* = 7.0 Hz, 3H), 0.66 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD): δ = 82.6, 77.6, 72.9, 69.2, 69.1, 68.8, 67.4, 67.4, 67.4, 67.1, 67.0, 66.9, 66.8, 60.9, 60.9, 54.9, 54.8, 44.2, 43.4, 40.9, 39.7, 39.6, 36.9, 36.5, 35.6, 31.5, 29.4, 28.8, 24.1, 23.3, 20.6, 18.6, 14.9, 13.0; HRMS: calculated for $C_{38}H_{75}N_2O_{11}P_2^+$ [M + H^+]: 797.4840, found 797.4860; IR = 3,322, 2,930, 2,867, 2,481, 2,221, 2,071, 1,688, 1,471, 1,377, 1,230, 1,085, 1,059 cm^{-1}.

1. Zhang Q, et al. (2007) Designing facial amphiphiles for the stabilization of integral membrane proteins. *Angew Chem Int Ed Engl* 46(37):7023–7025.



Scheme S1. Synthesis of key intermediates for facial amphiphiles. (*i*) Kolbe electrolysis, HOAc, NaOMe, MeOH; (*ii*) allyl iodide, NaH, THF; (*iii*) O_3, then $NaBH_4$, CH_2Cl_2, MeOH; and (*iv*) BnBr, NaH, Bu_4NI, THF.

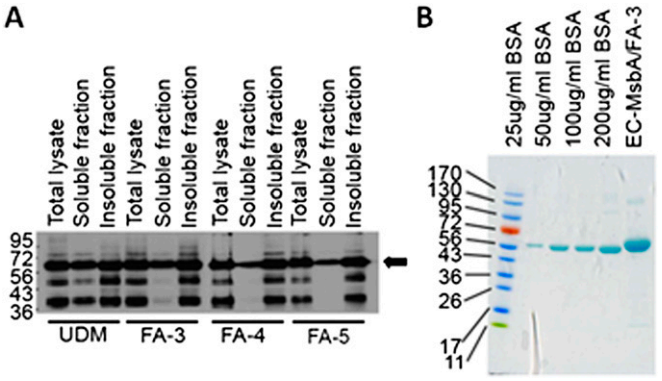


Fig. S1. MsbA extraction and purification using FAs. (*A*) Extraction of MsbA (arrow; 67 kDa monomer) from *Escherichia coli* whole cells using FAs. The proteins were separated by SDS/PAGE and stained by immunoblot using an anti-His antibody. (*B*) SDS/PAGE analysis of MsbA purified in FA-3 along with the protein standard BSA stained by Coomassie Brilliant Blue. For both *A* and *B*, sizes of molecular mass markers in the leftmost lane (kDa) are shown.

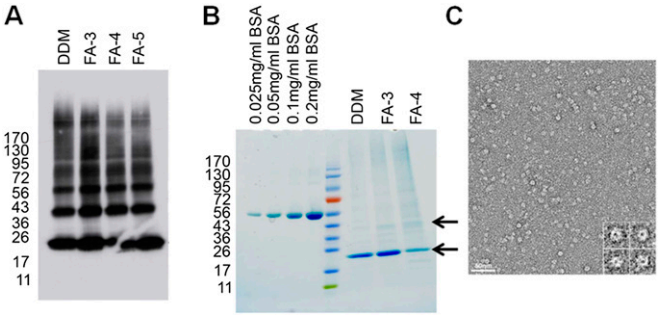


Fig. S2. Connexin 26 (Cx26) extraction and purification using FAs. (*A*) Extraction of Cx26 from *Sf9* insect cell membranes using FAs. Proteins were separated by SDS/PAGE and stained by immunoblot using an anti-His antibody. (*B*) Purification of Cx26 to homogeneity using dodecyl-β-D-maltoside (DDM), FA-3, or FA-4 throughout chromatography steps. Protein was analyzed by SDS/PAGE and stained using Coomassie Brilliant Blue. For both *A* and *B*, sizes of molecular mass markers in the leftmost lane (kDa) are shown. Protein bands in *A* represent Cx26 monomers, dimers, trimers, and higher oligomers from low to high, respectively. In *B*, the arrows indicate the Cx26 monomer band and faint dimer band. (*C*) Negative stain EM of Cx26 in FA-3 shows a field of relatively uniform particles with the characteristic doughnut appearance with a pool of stain in the pore excluded from the protein composing the channel (*Inset*).

Table S1. X-ray data collection statistics for Cx26/DDM, FA-3, and FA-5 complexes

	Cx26/FA-3	Cx26/FA-5	Cx26/DDM
Space group	*R*32	*P*213	*R*32
Unit cell dimension (Å)	$a = 156.1$, $b = 156.1$, $c = 160.1$; $\alpha = \beta = 90°$, $\gamma = 120°$	$a = 154.0$, $b = 154.0$, $c = 154.0$; $\alpha = \beta = \gamma = 90°$	$a = 193.9$, $b = 193.9$, $c = 365.5$; $\alpha = \beta = 90°$, $\gamma = 120°$
Resolution (Å)	40.0–3.3	50.0–3.1	50.0–4.2
Total observations	252,815	657,821	2,013,758
Unique observations	9,762	22,329	19,949
Redundancy	5.5 (2.1)	5.3 (5.2)	18.1 (12.2)
Completeness (%)	97.0 (70.0)	98.5 (99.9)	100.0 (100.0)
Mean $I/\sigma(I)$	25.1 (0.8)	9.8 (3.7)	18.6 (5)
R_{merge} (%)	8.0 (75.0)	16.1 (42.6)	22.5 (62.8)

Table S2. X-ray data collection statistics for EC-MsbA/FA-3 and EC-MsbA/FA-5 complex

	EC-MsbA/FA-3	EC-MsbA/FA-5
Space group	$P2_12_12_1$	$P2_12_12_1$
Unit cell dimension (Å)	$a = 115.44$, $b = 250.29$, $c = 284.95$; $\alpha = \beta = \gamma = 90°$	$a = 115.50$, $b = 248.19$, $c = 279.70$; $\alpha = \beta = \gamma = 90°$
Resolution (Å)	125.14–6.40	121.51–5.59
Total observations	38,455	70,903
Unique observations	15,504	24,239
Redundancy	2.5	2.9
Completeness (%)	88.7 (92.2)	95.4 (89.2)
$I/\sigma(I)$	6.7 (2.4)	8.7 (2.8)
R_{merge} (%)	8.9 (42.9)	6.5 (35.2)

Table S3. X-ray data collection statistics for bacteriorhodopsin (BR) crystallized in dimyristoylphosphatidylcholine (DMPC) : FA bicelles

	BR/FA-4:DMPC (Protein Data Bank ID code 4HYX)	BR/FA-7:DMPC (Protein Data Bank ID code 4HWL)
Data collection		
Space group	*P*21	*P*21
Unit cell dimension		
a, *b*, *c* (Å)	45.89, 112.92, 56.29	46.16, 113.29, 56.19
β (°)	113.83	114.27
Resolution (Å)	56.46 (2.00)	46.67 (2.00)
R_{merge}	7.4 (39.9)	7.4 (55.0)
I/σ (I)	5.3 (1.1)	6.5 (1.7)
Completeness (%)	92.6 (86.0)	96.6 (95.1)
Redundancy	2.1 (2.1)	2.1 (2.1)
Refinement		
Resolution (Å)	46.85–1.99	42.08–2.00
Number of reflections	32,391	33,257
R_{work}/R_{free}	0.181/0.218	0.159/0.172
Number of atoms		
Protein	3,560	3,512
Ligand/ion	172	91
Water	33	32
rmsds		
Bond lengths (Å)	0.022	0.021
Bond angles (°)	2.003	1.881

Table S4. Crystallization statistics for P450s in various FAs

	FA-3	FA-4	FA-6	FA-7	Without FA
CYP24A1 (1)	2.5 Å (3K9Y)	N.T.	3.4 Å	N.T.	>3.0 Å
CYP2B4 (2)	N.T.	1.76 Å (3MVR)	N.T.	Crystals*	Unsuccessful
CYP2B4-ticlopidine (3)	Crystals*	2.67 Å (3KW4)	N.T.	Crystals*	N.T.
CYP2B4-clopidogrel (3)	Crystals*	No crystal	N.T.	3.1 Å (3ME6)	N.T.
CYP2B4-tBPA (inactivator) (4)	N.T.	3.5/3.0 Å (3R1A and 3R1B)	N.T.	N.T.	Unsuccessful
CYP2B4-amlodipine (5)	N.T.	No crystal	N.T.	2.25 Å (3TMZ)	N.T.
CYP2B4-4-CPI (6)	N.T.	No crystal	2.80 Å (3TK3)	No crystal	N.T.
CYP2B6-4-CPI (7)	Crystals*	2.0 Å (3IBD)	N.T.	Crystals*	Crystals†
CYP2B6-4-BP/4-NBP (8)	N.T.	Crystals*	N.T	2.1/2.8 Å (3QOA and 3QU8)	Unsuccessful
CYP2B6-amlodipine (5)	N.T.	No crystal	N.T.	2.8 Å (3UA5)	N.T.

The four-digit Protein Data Bank ID codes were included in parenthesis with the resolution data for reported structures. N.T., not tested.
*Poor diffraction (>6 Å) in the preliminary screening relative to other FA candidates.
†Needle crystals, no diffraction.

1. Annalora AJ, et al. (2010) Crystal structure of CYP24A1, a mitochondrial cytochrome P450 involved in vitamin D metabolism. *J Mol Biol* 396(2):441–451.
2. Wilderman PR, et al. (2010) Plasticity of cytochrome P450 2B4 as investigated by hydrogen-deuterium exchange mass spectrometry and X-ray crystallography. *J Biol Chem* 285(49): 38602–38611.
3. Gay SC, et al. (2010) Structures of cytochrome P450 2B4 complexed with the antiplatelet drugs ticlopidine and clopidogrel. *Biochemistry* 49(40):8709–8720.
4. Gay SC, et al. (2011) Structural analysis of mammalian cytochrome P450 2B4 covalently bound to the mechanism-based inactivator tert-butylphenylacetylene: Insight into partial enzymatic activity. *Biochemistry* 50(22):4903–4911.
5. Shah MB, et al. (2012) Conformational adaptation of human cytochrome P450 2B6 and rabbit cytochrome P450 2B4 revealed upon binding multiple amlodipine molecules. *Biochemistry* 51(37):7225–7238.
6. Wilderman PR, et al. (2012) Investigation by site-directed mutagenesis of the role of cytochrome P450 2B4 non-active-site residues in protein-ligand interactions based on crystal structures of the ligand-bound enzyme. *FEBS J* 279(9):1607–1620.
7. Gay SC, et al. (2010) Crystal structure of a cytochrome P450 2B6 genetic variant in complex with the inhibitor 4-(4-chlorophenyl)imidazole at 2.0-A resolution. *Mol Pharmacol* 77(4): 529–538.
8. Shah MB, Pascual J, Zhang Q, Stout CD, Halpert JR (2011) Structures of cytochrome P450 2B6 bound to 4-benzylpyridine and 4-(4-nitrobenzyl)pyridine: Insight into inhibitor binding and rearrangement of active site side chains. *Mol Pharmacol* 80(6):1047–1055.