Electronic Supplementary Material (ESI) for Chemical Science.

Polymyxins Facilitate Entry into Mammalian Cells

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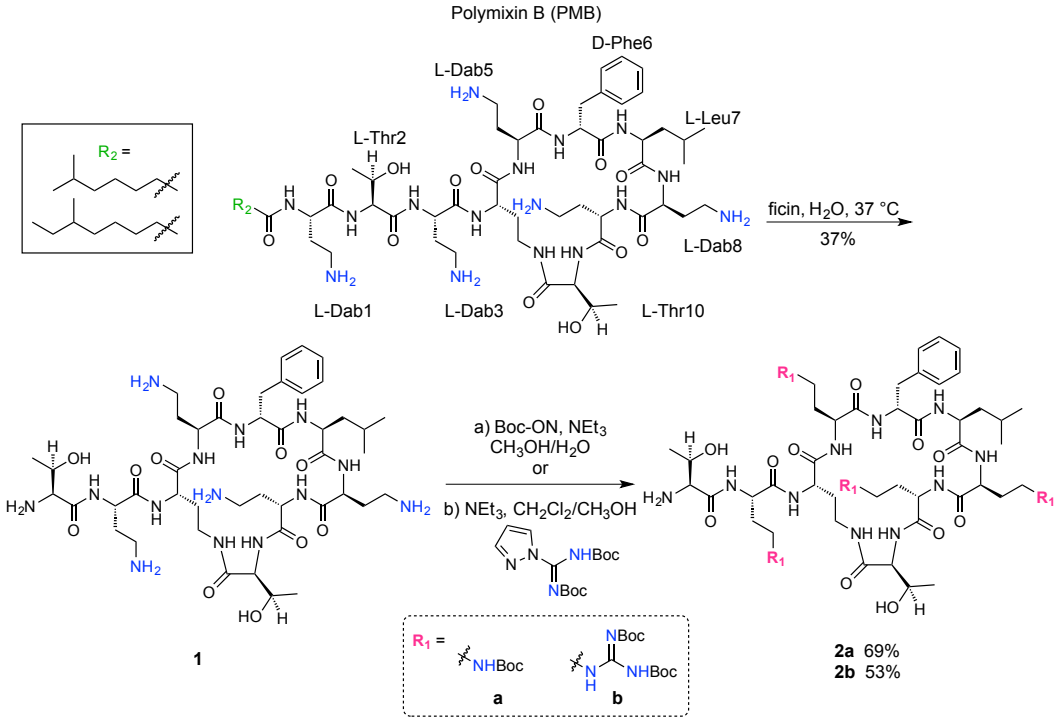
Materials

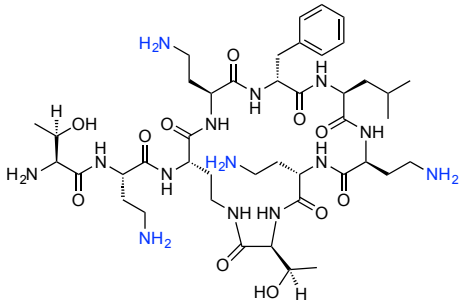
Materials obtained from commercial suppliers were used without further purification. Polymyxin B and tobramycin were purchased from TCI America. H-Dab(Boc)-OMe was purchased from Chem Impex International. Ficin was purchased from MP Biomedicals. Deuterated NMR solvents were purchased from Cambridge Isotope Laboratories. Amiloride, sucrose, and genistein were purchased from Sigma-Aldrich. Nystatin and EDTA/Trypsin were purchased from VWR and chlorpromazine was purchased from Fisher. Steptavidin-PE-Cy5 was purchased from Biolegend. PBS, F-12 media, Versene, streptavidin Cy-5, LysoTracker Green, and Hoescht dye were purchased from Life Technologies. Streptavidin saporin was purchased from Advanced Targeting Systems. 35 mm glass bottom culture dishes were purchased from MatTek. CellTiter-Blue was purchased from Promega. DOPC (1,2-dioleoyl-snglycero-3-phosphocholine), DOPE (1,2-dioleoyl-sn-glycero-3-phospho-ethanolamine), and cholesterol were purchased from Avanti Polar Lipids.

Instrumentation

NMR spectra were recorded on a Varian VX 500 MHz spectrometer or a Varian 400 MHz spectrometer. Mass spectra were recorded at UCSD Chemistry and Biochemistry Mass Spectrometry Facility utilizing an Agilent 6230 HR-ESI-TOF mass spectrometer. Reverse-phase HPLC purification (CLIPEUS, C18, 5μm, 10x250 mm, Higgins analytical) and analysis (Eclipse, XDB-C18, 5μm, 4.6x150 mm) were carried out on an Agilent 1200 series instrument or Beckman Coulter System Gold 127P Solvent Module. Flow cytometry studies were performed on a BD FACSCalibur. Confocal laser scanning microscopy was performed using a Nikon A1R inverted fluorescence microscope with z-stepping motor. Particle size, polydispersity, and surface charge of the lipid vesicles were measured by dynamic light scattering on a Zetasizer Nano ZS (model ZEN3600 from Malvern Instruments).



Scheme S1. Synthesis of Boc protected PMBN and Boc guanidinylated PMBN.

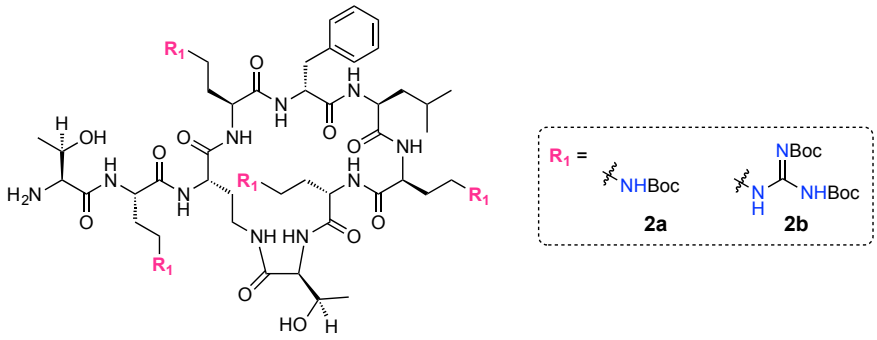


1

PMBN (1)

PMB (2.0 g, 1.4 mmol) was dissolved in 50 mL of H_2O. Then 42 mg of dithiothreitrol and 450 mg of ficin (320-500 milk clotting units/mg) were added and the reaction was heated to 37 °C and stirred overnight. When all PMB was consumed and only PMBN was detected by HPLC, the reaction was heated to reflux to denature the enzyme. After cooling, the precipitate was filtered off and the mother liqueur was evaporated under reduced pressure. The product was purified by automated flash chromatography Teledyne Isco Redisep Rf C18 30g gold column using a gradient of 0 – 15% ACN (0.1 % TFA) in H_2O (0.1% TFA), resulting in the TFA salt of **1** as a beige solid (1.5 g, 1.0 mmol, 72% yield). ^{1}H NMR (400 MHz, D_2O) δ 7.41 – 7.28 (m, 3H), 7.24 (d, *J* = 7.6 Hz, 2H), 4.58 – 4.45 (m, 3H), 4.32 – 4.12 (m, 7H), 3.95 (d, *J* = 5.5 Hz, 1H), 3.38 – 3.25 (m, 1H), 3.21 – 2.96 (m, 9H), 2.93 – 2.74 (m, 2H), 2.29 – 1.77 (m, 10H), 1.50 – 1.23 (m, 5H), 1.17 (d, *J* = 6.3 Hz, 3H), 0.77 – 0.59 (m, 7H). ^{13}C NMR (126 MHz, D_2O) δ 174.99, 173.33, 173.30, 172.72, 172.68, 171.94, 171.59, 171.39, 168.13, 163.29, 163.01, 162.73, 162.44, 135.41, 128.93, 127.37, 119.73, 117.41, 115.09, 112.77, 66.18, 66.01, 59.44, 58.16, 55.76, 52.85, 51.84, 51.72, 51.67, 51.19, 50.38, 39.04, 36.78, 36.38, 36.33, 36.08, 35.85, 30.51, 29.65, 28.61,

28.13, 27.76, 23.37, 22.28, 20.21, 19.05, 18.49. HR-ESI-MS calculated for $C_{43}H_{74}N_{14}O_{11}$ $[M+H]^+$ 963.5733, found 963.5734.



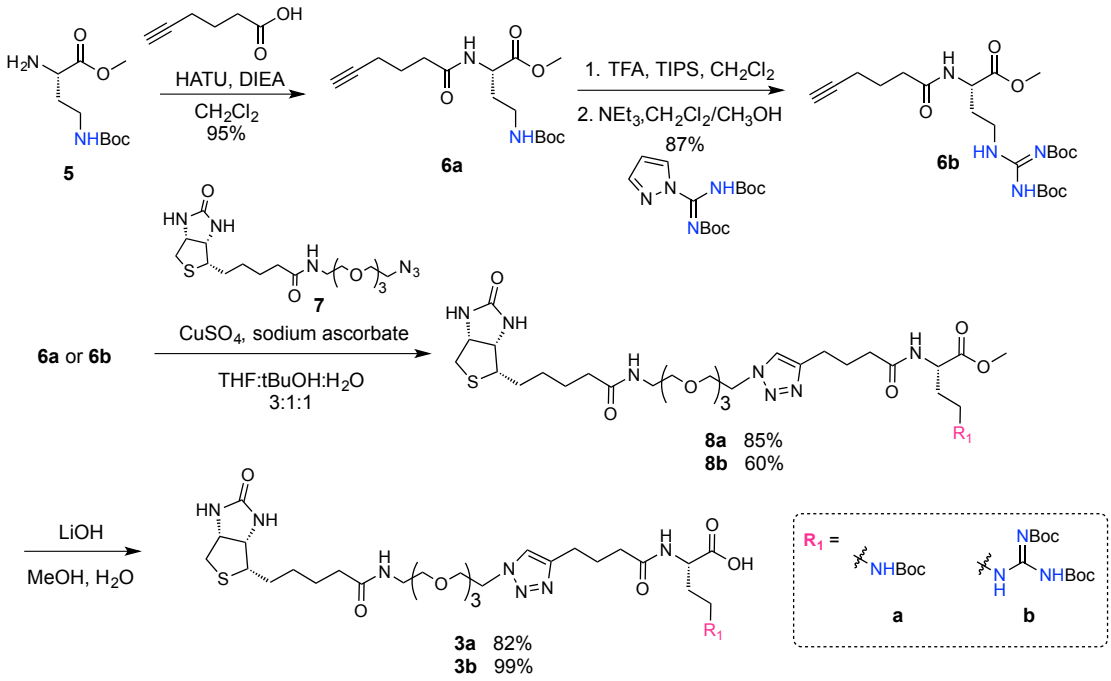
Compound 2a

In a 10 mL flask was added the TFA salt of PMBN (**1**, 104 mg, 0.0678 mmol), 2 mL MeOH, 1 mL H_2O, and NEt_3 (110 mg, 1.02 mmol, 140 µL). Then Boc-ON (67 mg, 0.27 mmol) was added and the reaction was stirred for 24 hours. The reaction was evaporated under reduced pressure and CH_2Cl_2 was added and washed with saturated $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The product was isolated by automated flash chromatography (0 - 20% MeOH in CH_2Cl_2 over 18 mins) to afford the product **2a** as a white solid (63.8 mg, 0.039 mmol, 69% yield). ^{1}H NMR (500 MHz, CD_3OD) δ 7.34 – 7.20 (m, 5H), 4.49 – 4.37 (m, 2H), 4.36 – 4.21 (m, 4H), 4.15 – 4.04 (m, 3H), 4.01 (d, *J* = 3.7 Hz, 1H), 3.60 – 3.49 (m, 1H), 3.25 – 2.90 (m, 11H), 2.25 – 2.14 (m, 1H), 2.08 – 1.71 (m, 10H), 1.50 – 1.15 (m, 44H), 0.93 – 0.83 (m, 1H), 0.71 (s, 3H), 0.65 (s, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 175.41, 175.20, 174.17, 174.05, 173.74, 173.35, 172.71, 158.74, 158.39, 137.42, 130.40, 129.72, 128.06, 80.37, 80.14, 69.45, 67.28, 61.50, 61.09, 58.29, 54.82, 54.08, 52.91, 52.19, 52.07, 51.80, 40.49, 38.03, 37.79, 37.53, 36.75, 34.18, 33.24, 32.81, 32.28, 31.55, 30.81, 28.82, 28.40, 24.78, 23.80, 21.38, 20.82, 19.70. HR-ESI-MS calculated for $C_{63}H_{106}N_{14}O_{19}$ $[M+Na]^+$ 1385.7651, found 1385.7640.

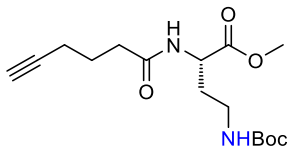
Compound 2b

In a 10 mL flask was added the TFA salt of PMBN (**1**, 112 mg, 0.0728 mmol), 2 mL MeOH, 2 mL CH_2Cl_2, and NEt_3 (110 mg, 1.09 mmol, 152 µL). Then *N,N′*-Di-Boc-1*H*-pyrazole-1-carboxamidine (88 mg, 0.284 mmol) was added and the reaction was stirred for 24 hours. The reaction was evaporated under reduced pressure and CH_2Cl_2 was added and washed with saturated $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The product was isolated by automated flash chromatography (0 - 10% MeOH in CH_2Cl_2 over 22 mins) to afford the product **2b** as a white solid (75 mg, 0.039 mmol, 53% yield). ^{1}H NMR (500 MHz, CD_3OD) δ 7.34 – 7.13 (m, 5H), 4.60 – 4.43 (m, 2H), 4.40 (t, *J* = 8.1 Hz, 1H), 4.31 – 4.16 (m, 5H), 4.14 – 4.01 (m, 1H), 3.95 (d, *J* = 4.8 Hz, 1H), 3.72 – 3.35 (m, 9H), 3.27 – 2.94 (m, 4H), 2.29 – 1.74 (m, 10H), 1.61 – 1.15 (m, 80H), 0.94 – 0.42 (m, 1H), 0.73 (dd, *J* = 15.8, 5.8 Hz, 6H). ^{13}C NMR (126 MHz, CD_3OD) δ 175.02, 173.95, 173.65, 173.50, 172.52, 164.59, 164.47, 157.85, 157.62, 153.99, 153.90, 150.97, 137.71, 130.29, 129.69, 128.03, 84.42, 84.35, 84.28, 84.22, 80.45, 80.41, 69.52, 67.23, 61.94, 61.16, 58.18, 57.47, 54.12, 53.62, 52.82,

52.37, 52.29, 51.91, 40.45, 38.86, 38.61, 38.57, 37.82, 37.70, 36.79, 33.39, 32.90, 32.43, 32.35, 31.98, 31.13, 30.78, 30.75, 28.74, 28.71, 28.69, 28.65, 28.35, 25.00, 23.81, 21.52, 20.93, 19.78. HR-ESI-MS calculated for $C_{87}H_{146}N_{22}O_{27}$ $[M+Na]^+$ 1954.0620, found 1954.0622.



Scheme S2. Synthesis of biotinylated linker.

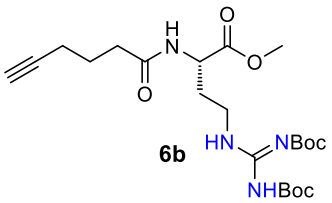


6a

Compound 6a

5-hexynoic acid (257 µL, 262 mg, 2.33 mmol), 1.1 mL of DIEA (804 mg, 6.22 mmol), and 8.6 mL DMF (filtered through silica), and HATU (887 mg, 2.33 mmol) were added to a 50 mL round bottom flask and allowed to stir for 10 min to give a yellow solution. Next, H-Dab(Boc)-OMe·HCl (418 mg, 1.56 mmol) was added and the reaction was stirred overnight. To the reaction was added CH_2Cl_2, which was washed with 2% citric acid and then sat. $NaHCO_3$. The organic layer was dried, filtered, and evaporated under reduced pressure. The product was isolated by automated flash chromatography (20 - 90% EtOAc in hexanes over 15 mins) to afford the product as a viscous oil (467 mg, 1.43 mmol, 92% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.48 (d, *J* = 7.4 Hz, 1NH), 5.19 – 5.13 (m, 1NH), 4.67 (td, *J* = 8.5, 4.5 Hz, 1H), 3.48 – 3.34 (m, 1H), 3.00 – 2.85 (m, *J* = 13.4, 9.2, 4.9 Hz, 1H),

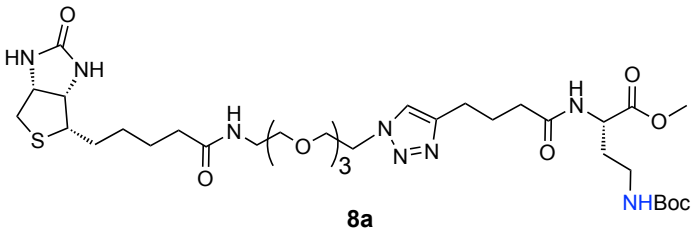
2.40 (t, *J* = 7.4 Hz, 2H), 2.26 (td, *J* = 6.9, 2.6 Hz, 2H), 2.08 – 2.00 (m, 1H), 1.98 (t, *J* = 2.7 Hz, 1H), 1.90 – 1.83 (m, 2H), 1.80 – 1.71 (m, 1H), 1.42 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.05, 172.80, 156.23, 83.42, 79.56, 77.41, 77.16, 76.91, 69.45, 52.77, 49.67, 36.51, 34.96, 33.17, 28.52, 24.12, 17.92. HR-ESI-MS calculated for $C_{16}H_{26}N_2O_5$ $[M+Na]^+$ 349.1734, found 349.1735.



6b

Compound 6b

In a 25 mL flask was added BDabyne-OMe (**6a**, 212 mg, 0.629 mmol), CH_2Cl_2, triisopropylsilane (57 µL) and trifluoroacetic acid (1 mL). The solution was stirred for one hour and evaporated under reduced pressure. The residue was redissolved in CH_2Cl_2 (2 mL) and NEt_3 (1 mL). Then *N,N'*-Di-Boc-1*H*-pyrazole-1-carboxamidine (403 mg, 1.30 mmol) was added and the reaction was stirred overnight. The reaction was diluted with CH_2Cl_2 and washed with saturated $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The product was isolated by automated flash chromatography (20 - 60% EtOAc in hexanes over 19 mins) to afford the product **6b** as an oil. ^{1}H NMR (400 MHz, CD_3OD) δ4.49 (dd, *J* = 8.5, 5.1 Hz, 1H), 3.71 (s, 3H), 3.54 (m, 1H), 3.38 (m, 1H), 2.41 (t, *J* = 7.4 Hz, 2H), 2.28 – 2.21 (m, 3H), 2.12 – 1.95 (m, 2H), 1.83 (p, *J* = 7.3 Hz, 2H), 1.53 (s, 9H), 1.49 (s, 9H). ^{13}C NMR (126 MHz, CD_3OD) δ 175.53, 173.71, 164.47, 157.75, 154.01, 84.49, 84.16, 80.50, 70.25, 52.84, 51.54, 38.22, 35.59, 31.73, 28.61, 28.23, 25.77, 18.60. HR-ESI-MS calculated for $C_{22}H_{37}N_4O_7$ $[M+H]^+$ 469.2657, found 469.2658.

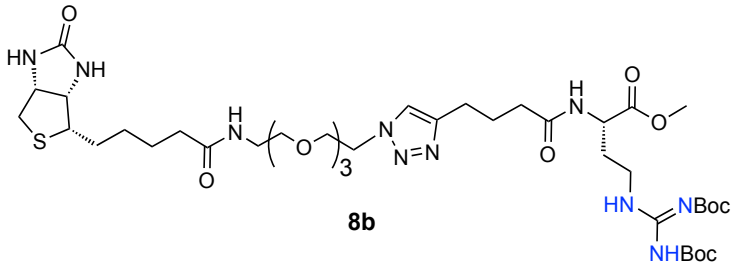


8a

Compound 8a

BDabyneOMe (**6a**, 28.5 mg, 0.064 mmol) and Biotin-PEG-N_3 (**7**, 30.0 mg, .064 mmol) with a 1 mL 3:1:1 mixture of THF, t-BuOH, and H_2O was were added to a 10 mL round bottom flask and purged with argon for 10 min. Next, 35 µL of a freshly prepared 1M of a sodium ascorbate and then a 28 µL of 7.5% solution of $CuSO_4 \cdot 5H_2O$, both prepared in degassed water, were added. The reaction was stirred overnight. The reaction was evaporated under reduced pressure. The product was isolated by automated flash chromatography (2 - 17% MeOH in CH_2Cl_2 over 18 mins) to afford the product as a white solid (49.8 mg, 0.080 mmol, 85% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.57 (s, 1H), 7.43 (d, *J* = 7.9 Hz, 1NH), 6.94 (brs, 1NH), 6.27 (brd, *J* = 22.5 Hz, 1NH), 5.40 (brd, *J* = 23.1 Hz, 1NH),

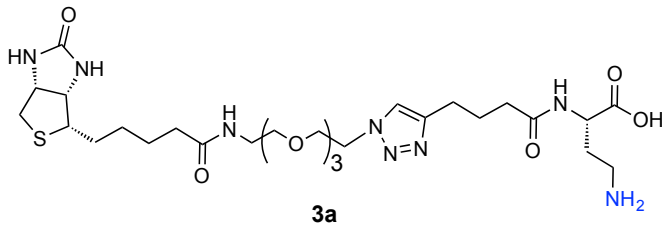
5.24 (brs, 1NH), 4.62 (td, *J* = 8.5, 4.5 Hz, 1H), 4.54 – 4.48 (m, 3H), 4.35 – 4.30 (m, 1H), 3.88 (t, *J* = 5.1 Hz, 2H), 3.72 (s, 3H), 3.64 – 3.56 (m, 8H), 3.54 (t, *J* = 5.1 Hz, 2H), 3.48 – 3.31 (m, 3H), 3.14 (td, *J* = 7.3, 4.8 Hz, 1H), 3.00 (dt, *J* = 13.5, 5.7 Hz, 1H), 2.90 (dd, *J* = 12.8, 4.9 Hz, 1H), 2.77 (t, *J* = 7.4 Hz, 2H), 2.73 (d, *J* = 12.8 Hz, 1H), 2.33 (t, *J* = 7.4 Hz, 2H), 2.24 – 2.13 (m, 2H), 2.08 – 1.96 (m, 4H), 1.86 – 1.57 (m, 5H), 1.42 (s, 10H). ^{13}C NMR (126 MHz, $CDCl_3$) δ173.65, 173.52, 173.50, 163.87, 156.21, 147.29, 122.54, 79.49, 70.61, 70.52, 70.49, 70.15, 70.06, 69.64, 61.96, 60.21, 55.73, 52.68, 50.22, 49.86, 40.70, 39.24, 36.78, 35.82, 35.36, 32.54, 28.56, 28.25, 28.17, 25.69, 25.49, 24.86. HR-ESI-MS calculated for $C_{34}H_{58}N_8O_{10}S$ $[M+Na]^+$ 793.3889, found 793.3885.



8b

Compound 8b

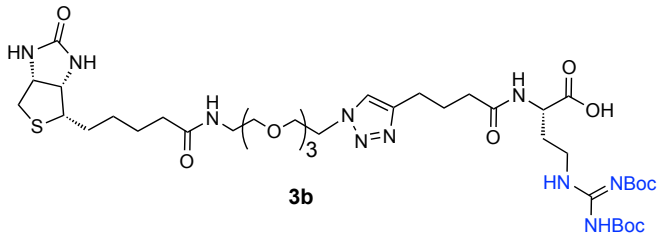
BGuanDabyneOMe (**6b**, 28.5 mg, 0.064 mmol) and Biotin-PEG-N_3 (30.0 mg, 0.064 mmol) with a 1 mL 3:1:1 mixture of THF, t-BuOH, and H_2O was added to a 10 mL round bottom flask and purged with argon for 10 min. Next, 35 µL of a freshly prepared 1M of a sodium ascorbate and then a 28 µL of 7.5% solution of $CuSO_4 \cdot 5H_2O$, both prepared in degassed water, were added. The reaction was stirred overnight. The reaction was evaporated under reduced pressure. The product was isolated by automated flash chromatography (5 - 12% MeOH in CH_2Cl_2 over 19 mins) to afford the product as a white solid (49.8 mg, 0.080 mmol, 85% yield). ^{1}H NMR (500 MHz, CD_3OD): δ 7.85 (s, 1H), 4.55 (t, *J* = 5.1 Hz, 2H), 4.52 – 4.47 (m, 2H), 4.31 (dd, J_1 = 8, 4.5 Hz, 1H), 3.89 (t, *J* = 5 Hz, 2H), 3.72 (s, 3H), 3.61 – 3.52 (m, 11H), 3.39 (q, *J* = 7 Hz, 1H), 3.35 (q, *J* = 5.5 Hz, 2H), 3.22 – 3.18 (m, 1H), 2.93 (dd, J_1 = 12.5, 5 Hz, 1H), 2.75 (dd, J_1 = 8.5, 7.3 Hz, 2H), 2.70 (d, *J* = 13 Hz, 1H), 2.37 – 2.34 (m, 2H), 2.21 (t, *J* = 7.3 Hz, 2H), 2.10 – 2.05 (m, 1H), 2.03 – 1.96 (m, 3H), 1.77– 1.39 (m, 26H); ^{13}C NMR (126 MHz, CD_3OD): δ 176.17, 176.08, 175.63, 173.75, 166.09, 164.47, 157.74, 153.99, 124.26, 84.49, 80.49, 71.56, 71.49, 71.43, 71.26, 70.57, 70.41, 63.35, 61.60, 57.03, 52.89, 51.54, 51.34, 41.08, 40.47, 40.34, 38.24, 36.77, 36.72, 35.99, 31.79, 29.78, 29.50, 28.61, 28.23, 26.87, 26.57, 25.68. HR-ESI-MS calculated for $C_{40}H_{68}N_{10}O_{12}S$ $[M+H]^+$ 913.4812, found 913.4811.



3a

Compound 3a

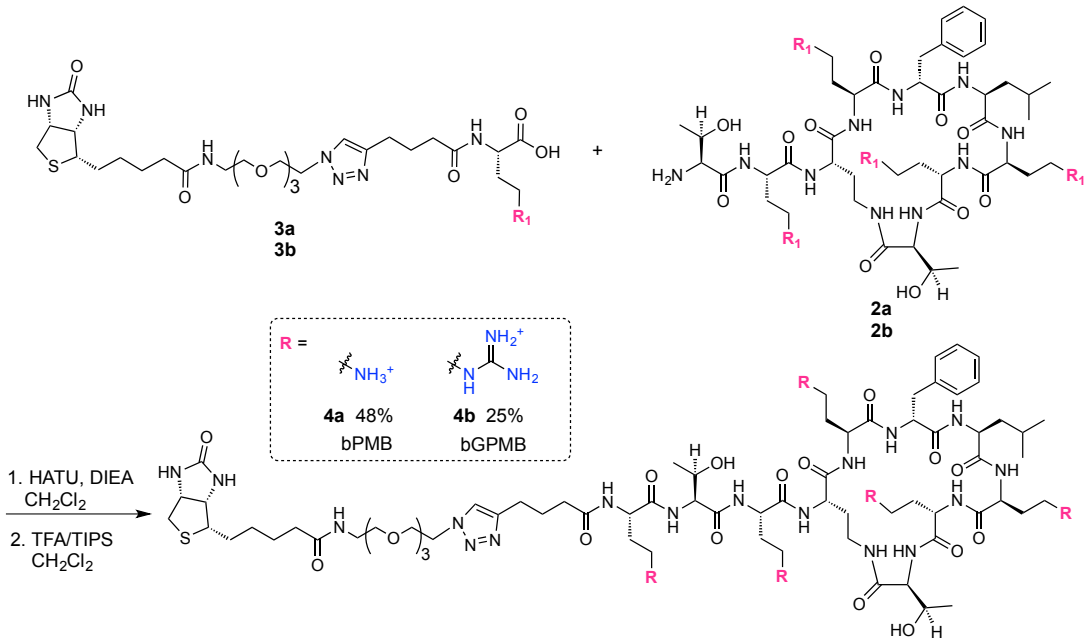
BDabOMeBiotin (**8a**, 27 mg, 0.035 mmol), 1.25 mL of MeOH, and 0.42 mL of 0.1 M LiOH solution in water (1 mg, .042 mmol) were added to a 10 mL round bottom flask and stirred overnight. The compound was desalted on a C-18 sep-pak (waters) to provide the product **3a** as a white solid (22 mg, 0.029 mmol, 82% yield). ^{1}H NMR (500 MHz, CD_3OD) δ 7.87 (s, 1H), 4.57 – 4.53 (m, 2H), 4.49 (ddd, *J* = 7.9, 4.9, 0.7 Hz, 1H), 4.33 – 4.27 (m, 2H), 3.91 – 3.87 (m, 2H), 3.63 – 3.56 (m, 10H), 3.53 (t, *J* = 5.5 Hz, 2H), 3.35 (t, *J* = 5.5 Hz, 2H), 3.19 (tt, *J* = 3.6, 3.2 Hz, 2H), 3.06 – 2.98 (m, 1H), 2.92 (dd, *J* = 12.7, 5.0 Hz, 1H), 2.75 (t, *J* = 7.6 Hz, 2H), 2.70 (d, *J* = 12.7 Hz, 1H), 2.32 (t, *J* = 7.5 Hz, 2H), 2.21 (t, *J* = 7.4 Hz, 2H), 2.07 – 1.94 (m, 4H), 1.78 – 1.54 (m, 6H), 1.45 – 1.40 (m, 11H).; ^{13}C NMR (126 MHz, CD_3OD): δ 176.17, 176.08, 175.63, 173.75, 166.09, 164.47, 157.74, 153.99, 124.26, 84.49, 80.49, 71.56, 71.49, 71.43, 71.26, 70.57, 70.41, 63.35, 61.60, 57.03, 52.89, 51.54, 51.34, 41.08, 40.47, 40.34, 38.24, 36.77, 36.72, 35.99, 31.79, 29.78, 29.50, 28.61, 28.23, 26.87, 26.57, 25.68; HR-ESI-MS calculated for $C_{33}H_{56}N_8O_{10}S$ $[M+Na]^+$ 779.3737, found 779.3732.



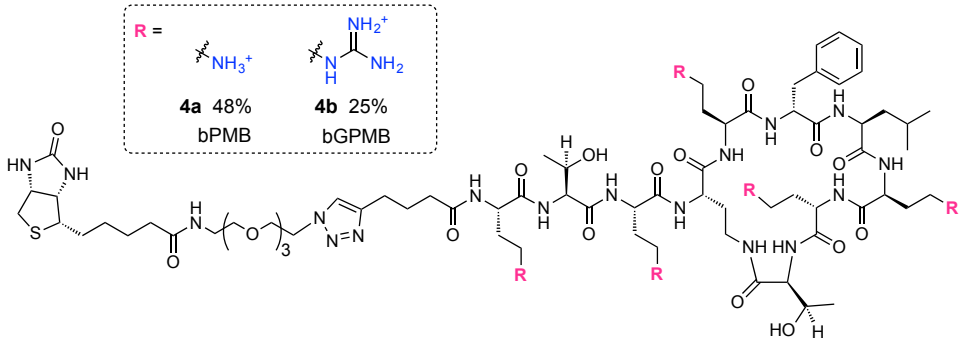
3b

Compound 3b

BGDabOMeBiotin (**8b**, 27 mg, 0.035 mmol), 1.25 mL of MeOH, and 0.42 mL of 0.1 M LiOH solution in water (1 mg, .042 mmol) were added to a 10 mL round bottom flask and stirred overnight. The compound was desalted on a C-18 sep-pak (waters) to provide the product **3b** as a white solid (22 mg, 0.029 mmol, 82% yield). ^{1}H NMR (500 MHz, CD_3OD) δ 7.87 (s, 1H), 4.55 (t, *J* = 5.0 Hz, 2H), 4.49 (dd, *J* = 7.8, 4.8 Hz, 1H), 4.33 – 4.27 (m, 2H), 3.89 (t, *J* = 5.1 Hz, 2H), 3.62 – 3.54 (m, 8H), 3.53 (t, *J* = 5.5 Hz, 2H), 3.35 (t, *J* = 5.4 Hz, 2H), 3.30 – 3.24 (m, 1H), 3.22 – 3.17 (m, 1H), 2.92 (dd, *J* = 12.7, 5.0 Hz, 1H), 2.75 (t, *J* = 7.6 Hz, 2H), 2.70 (d, *J* = 12.7 Hz, 1H), 2.35 (t, *J* = 7.3 Hz, 2H), 2.21 (t, *J* = 7.4 Hz, 2H), 2.17 – 2.09 (m, 1H), 2.04 – 1.96 (m, 2H), 1.88 – 1.78 (m, 1H), 1.77 – 1.55 (m, 5H), 1.52 (s, 9H), 1.48 – 1.39 (m, 11H).; ^{13}C NMR (126 MHz, CD_3OD) δ 178.59, 176.14, 174.91, 166.13, 158.21, 149.44, 148.27, 124.30, 79.84, 71.57, 71.50, 71.45, 71.27, 70.55, 70.42, 63.36, 61.61, 57.03, 53.91, 51.31, 41.07, 40.36, 38.43, 36.73, 36.36, 34.47, 29.78, 29.51, 28.80, 26.87, 26.71, 25.80. HR-ESI-MS calculated for $C_{39}H_{66}N_{10}O_{12}S$ $[M+H]^+$ 899.4655, found 899.4653.



Scheme S3. Synthesis of polymyxin and guanidinopolymyxin transporters.



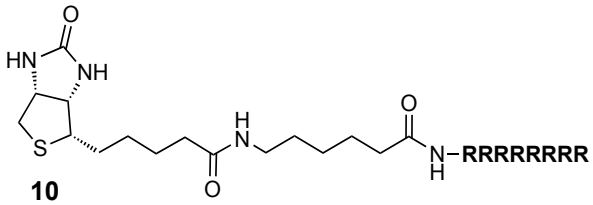
bPMB (10a)

BiotinBDabOH (**3a**, 20.6 mg, 0.0258 mmol), DIEA (8.6 mg, 0.067 mmol, 11.6 µL), HATU (10.2 mg, 0.027 mmol) and DMF (1 mL) were added to a 10 mL flask and stirred for 10 min. Then Boc-PMBN (**2a**, 30.4 mg, 0.022 mmol) was added to the reaction and stirred overnight. The reaction was diluted with CH_2Cl_2 and washed with 5% citric acid and then saturated $NaHCO_3$. The organic layer was then dried using $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was then taken up in CH_2Cl_2/TFA (1:1, 1 mL) containing triisopropylsilane (10 µL) and stirred for 2 hours. The reaction was evaporated under reduced pressure the product was isolated by automated reverse phase flash chromatography using a Teledyne Isco Redisep Rf C18 5.5 g Gold column [5 - 30% ACN (0.1% TFA) in H_2O (0.1% TFA) over 14 mins]. The fractions containing the desired product were lyophilized to provide **4a** as a white solid as the TFA salt (19.9 mg, 0.0092 mmol, 48% yield). ^{1}H NMR (500 MHz, D_2O) δ 7.89 (dd, *J* = 10.2, 1.9 Hz, 1H), 7.34 – 7.22 (m, 3H), 7.17 (d, *J* = 5.2 Hz, 2H), 4.58 – 4.34 (m, 8H), 4.34 – 4.29 (m, 1H), 4.29 – 4.25 (m, 1H), 4.24 – 4.08 (m, 7H), 3.93 – 3.87 (m, 2H), 3.61 – 3.48 (m, 9H), 3.33 – 3.18 (m, 4H),

3.12 – 2.93 (m, 10H), 2.92 – 2.85 (m, 1H), 2.84 – 2.75 (m, 1H), 2.75 – 2.63 (m, 4H), 2.33 – 2.26 (m, 2H), 2.23 – 1.74 (m, 17H), 1.68 – 1.24 (m, 10H), 1.16 – 1.07 (m, 5H), 0.64 (d, *J* = 37.2 Hz, 7H). ^{13}C NMR (126 MHz, D_2O) δ 176.73, 176.24, 174.90, 173.25, 173.18, 173.09, 172.89, 172.67, 172.62, 172.54, 172.47, 172.27, 172.21, 171.86, 171.61, 171.47, 171.28, 171.24, 165.17, 163.24, 162.82 (TFA, q, *J* = 35.7 Hz), 146.46, 135.32, 128.83, 127.27, 124.28, 124.15, 116.23 (TFA, q, *J* = 292.1 Hz), 69.43, 69.29, 69.26, 68.67, 68.43, 68.39, 66.89, 66.66, 66.08, 61.92, 60.08, 59.32, 58.93, 58.79, 55.62, 55.21, 52.66, 52.57, 51.74, 51.64, 51.55, 51.39, 51.25, 51.05, 50.92, 50.36, 50.24, 39.54, 38.94, 38.74, 36.69, 36.27, 36.19, 36.10, 35.94, 35.80, 35.70, 35.27, 34.22, 30.43, 30.31, 29.59, 29.54, 28.67, 28.48, 28.34, 28.09, 27.71, 27.60, 27.54, 25.00, 24.50, 24.44, 23.52, 23.42, 23.30, 22.18, 20.11, 18.95, 18.73, 18.58, 16.13. HR-ESI-MS calculated for $C_{71}H_{120}N_{22}O_{18}S$ $[M+Na]^+$ 1623.8764, found 1623.8766.

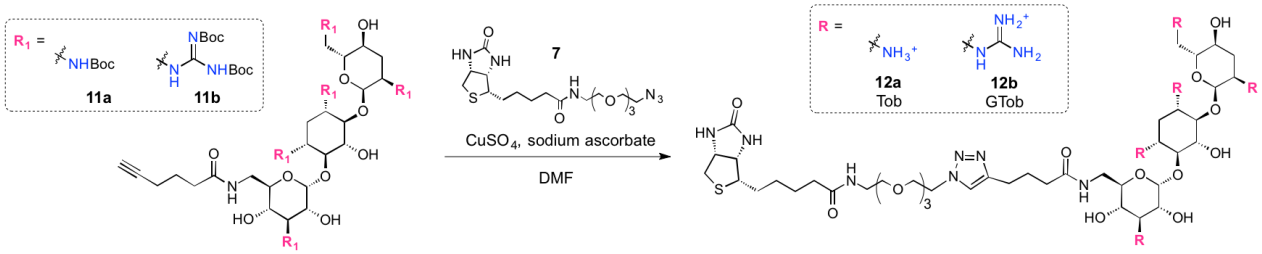
bGPMB (4b)

BiotinBGDabOH (**3b**, 20.2 mg, 0.0223 mmol), DIEA (12.02 mg, 0.093 mmol, 16.2 µL), PyBrop (10.4 mg, 0.0223 mmol) and DMF (1 mL) were added to a 10 mL flask and stirred for 10 min. Then BocGuan-PMBN (**2b**, 36.0 mg, 0.0186 mmol) was added to the reaction and stirred overnight. The reaction was diluted with CH_2Cl_2 and washed with 5% citric acid and then saturated $NaHCO_3$. The organic layer was then dried using $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was then taken up in CH_2Cl_2/TFA (1:1, 1 mL) containing triisopropylsilane (10 µL) and stirred for 2 hours. The reaction was evaporated under reduced pressure the product was isolated by automated reverse phase flash chromatography using a C18 5.5 g Gold column [15 - 35% ACN (0.1% TFA) in H_2O (0.1% TFA) over 15 mins]. The fractions were lyophilized to provide **4b** as a white solid as the TFA salt (19.9 mg, 0.0092 mmol, 25% yield). ^{1}H NMR (500 MHz, D_2O) δ 7.89 – 7.86 (m, 1H), 7.44 – 7.33 (m, 3H), 7.30 – 7.26 (m, 2H), 4.65 – 4.54 (m, 4H), 4.51 – 4.39 (m, 5H), 4.36 – 4.18 (m, 9H), 4.02 – 3.97 (m, 2H), 3.71 – 3.59 (m, 11H), 3.42 – 3.25 (m, 13H), 3.23 – 2.96 (m, 7H), 2.81 – 2.71 (m, 3H), 2.44 – 2.35 (m, 2H), 2.27 (td, *J* = 7.3, 1.8 Hz, 3H), 2.24 – 2.10 (m, 6H), 2.23 – 1.30 (m, 28H), 1.27 – 1.18 (m, 6H), 0.89 – 0.70 (m, 7H). ^{13}C NMR (126 MHz, D_2O) δ 75, 176.59, 176.38, 175.00, 174.28, 173.96, 173.87, 173.83, 173.38, 173.32, 172.93, 172.63, 172.25, 172.25, 172.00, 171.67, 171.48, 171.43, 165.24, 163.35, 163.07, 162.79, 162.50 (TFA, q, *J* = 35.7 Hz), 156.77, 156.68, 156.64, 156.54, 147.26, 135.40, 128.91, 127.37, 123.55, 117.43, 115.11 (TFA, q, *J* = 291.5 Hz), 69.57, 69.52, 69.39, 68.78, 68.72, 66.85, 66.54, 66.06, 62.01, 60.19, 59.48, 59.29, 59.04, 55.86, 55.30, 52.59, 51.95, 51.70, 51.50, 51.18, 50.53, 49.84, 39.64, 39.04, 38.85, 37.83, 37.76, 37.39, 36.77, 35.39, 34.47, 34.38, 30.80, 29.76, 29.19, 27.84, 27.66, 25.10, 24.82, 23.95, 23.47, 22.29, 20.21, 19.09, 18.86, 18.72. HR-ESI-MS calculated for $C_{76}H_{130}N_{32}O_{18}S$ $[M+3H]^{3+}$ 604.6727, found 604.6722.



Octaarginine (bArg8)

bArg8 was synthesized using standard solid phase peptide synthesis protocols (Rink amide resin). An aminohexanoic acid (Ahx) spacer was introduced in the N-terminus and biotin-NHS (4 eq) was coupled to the peptide's N-terminus over 1 h at rt in DMF containing DIEA (8 eq). The peptide was cleaved from the resin using TFA/TIS/water (95:2.5:2.5) at rt for 3h. The resin was filtered off and the peptide precipitated by the addition of cold ether and further standing at 4 degrees overnight. The crude was purified by HPLC using a semiprep RP-C18 column [5 – 60% ACN (0.1% TFA) in H_2O (0.1% TFA) over 9 min]. HRMS of the isolated peack confirms the identity of the biotinylated peptide. Purity was confirmed by analytical HPLC. HR-ESI-MS calculated for $C_{64}H_{124}N_{36}O_{11}S$ $[M+2H]^{2+}$ 926.4376, found 926.4374.



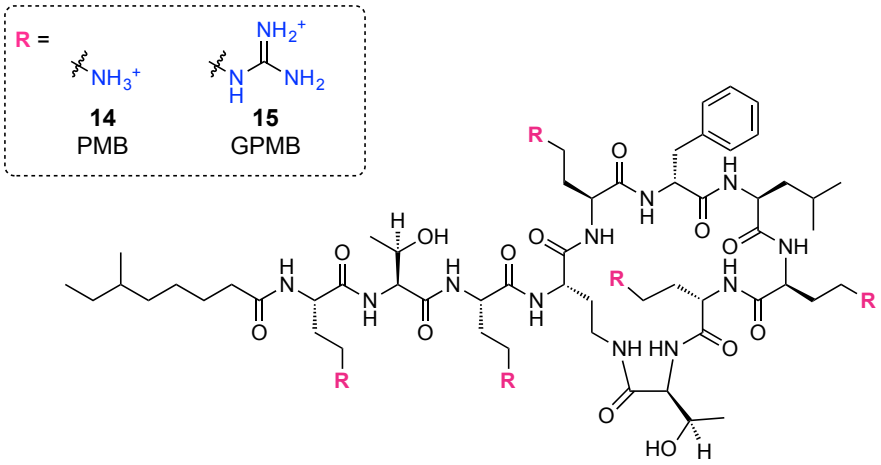
Scheme S4. Synthesis of bGTob and bTob.

Synthesis of alkyne-boc-tobramycin (**11a**), alkyne-boc-guan-tobramycin (**11b**) and GTob-biotin (**12b)** were prepared according to literature procedures.[1]

bTob (12a)

Alkyne-Boc-Tob (**11a**, 25mg, 0.024 mmol) and biotin-PEG-N_3 (16 mg, 0.035 mmol) were dissolved in DMF (500 uL) and treated with 0.2M solution of sodium ascorbate in H_2O (25 µL) and 0.2M solution of $CuSO_4 \cdot 5H_2O$ (25 µl). The reaction was stirred overnight at room temperature under argon. The reaction was evaporated under reduced pressure. The crude product was dissolved in CH_2Cl_2 and washed with aqueous KCN solution, EDTA (0.3 M, pH 8) and brine. The organic layer was then dried using $MgSO_4$, filtered and evaporated under reduced pressure. The residue was then dissolved in CH_2Cl_2/TFA (1:1, 1 mL) containing triisopropylsilane (10 µL) and stirred for 2 hours. The reaction was evaporated under reduced pressure and the product was purified by HPLC using a semiprep RP-C18 column [5 – 30% ACN (0.1% TFA) in H_2O (0.1% TFA) over 12 min]. The fractions containing the desired product were lyophilized to provide the product as a white solid, (20 mg, 0.013 mmol, 54% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.80 (s, 1H), 5.70 (s,

1H), 4.96 (s, 1H), 4.50 (m, 3H), 4.30 (m, 1H), 3.91-3.80 (m, 6H), 3.73 (m, 1H), 3.66 (m, 2H), 3.60-3.40 (m, 16H), 3.38-3.27 (m, 3H), 3.26 (s, 2H), 3.19 (m, 2H), 2.88 (m, 1H), 2.65 (m, 3H), 2.45 (d, 1H, *J*=12.3 Hz), 2.22 (m, 3H), 2.13 (m, 2H), 1.96-1.83 (m, 4H), 1.62-1.40 (m, 4H), 1.28 (s, 2H). ^{13}C NMR (126 MHz, D_2O): δ 176.82, 165.28, 163.30, 163.02, 162.74, 162.45, 146.91, 123.97, 119.77, 117.44, 115.12, 112.80, 100.86, 94.10, 83.56, 77.32, 74.12, 70.92, 70.31, 69.58, 69.53, 69.40, 69.38, 68.78, 68.60, 68.00, 66.48, 64.32, 62.02, 60.20, 55.30, 54.62, 50.15, 49.72, 48.31, 47.70, 39.74, 39.63, 38.94, 38.86, 35.38, 34.86, 29.25, 27.81, 27.71, 27.64, 25.09, 24.93, 23.78. HR-ESI-MS calculated for $C_{42}H_{77}N_{12}O_{14}S$ $[M+H]^+$ 1005.5397, found 1005.5400.



PMB (14)

PMB was isolated from the mixture of isomers by HPLC using a RP-C18 column [5 – 50% ACN (0.1% TFA) in H_2O (0.1% TFA) over 40 mins]. Purity was confirmed by analytical HPLC. HR-ESI-MS calculated for $C_{56}H_{98}N_{16}O_{13}$ $[M+Na]^+$ 1225.7397, found 1225.7395.

GPMB (15)

MeOH (15 mL) and NEt_3 (239 mg, 2.36 mmol, 329 µL) were added to **14** (226 mg, 0.157 mmol) followed by *N,N'*-Di-Boc-1*H*-pyrazole-1-carboxamidine (195 mg, 0.142 mmol) and stirred overnight. The reaction was evaporated under reduced pressure and CH_2Cl_2 was added and washed with saturated $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was then dissolved in CH_2Cl_2/TFA (1:1, 4 mL) containing triisopropylsilane (44 µL) and stirred for 2 hours. The reaction was diluted with 5 mL of CH_2Cl_2 and extracted with 10 mL of H_2O. The water was evaporated under reduced pressure and the product was isolated by HPLC using a RP-C18 column [5-50% ACN (0.1% TFA) in H_2O (0.1% TFA) over 40 mins]. The fractions containing the desired product were lyophilized to provide the TFA salt of GPMB as a white solid, (74.7 mg, 0.0378 mmol, 24% yield). ^{1}H NMR (400 MHz, D_2O): δ 7.42 – 7.29 (m, 3H), 7.26 (d, *J* = 6.9 Hz, 2H), 4.56 – 4.50 (m, 1H), 4.45 (ddd, *J* = 17.2, 8.7, 5.3 Hz, 3H), 4.35 – 4.21 (m, 6H), 4.20 – 4.14 (m, 2H), 3.43 – 2.98 (m, 14H), 2.33 (t, *J* = 7.2 Hz,

2H), 2.22 – 1.74 (m, 12H), 1.67 – 1.03 (m, 17H), 0.82 (t, *J* = 6.8 Hz, 6H), 0.75 (s, 3H), 0.68 (s, 3H). HR-ESI-MS calculated for $C_{61}H_{108}N_{26}O_{13}$ $[M+2H]^{2+}$ 707.4367, found 707.4351.

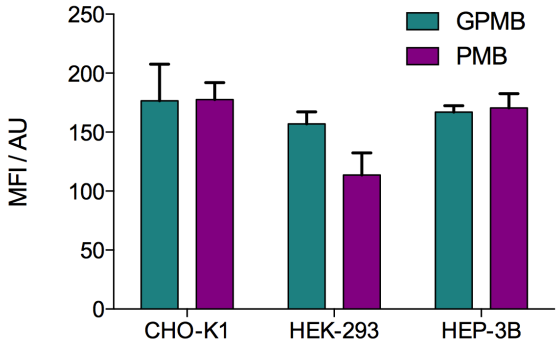


Figure S1. Cellular uptake in various cell lines. Cellular uptake of GPMB and PMB conjugated to ST-PE-Cy5. CHO-K1, HEK-293, and HEP-3B cells were incubated with conjugate (5nM) at 37 °C for 1 h. Mean fluorescence intensity was measured by flow cytometry. The background signal from untreated cells was subtracted.

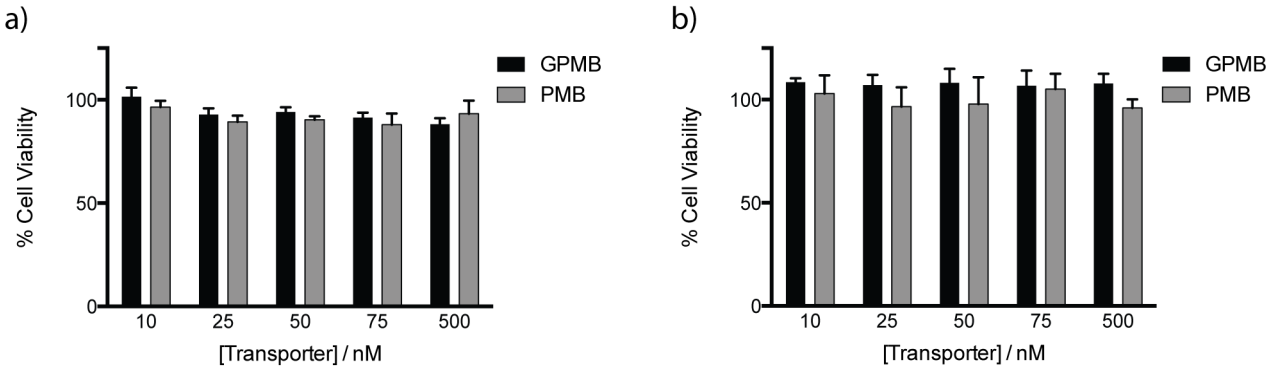


Figure S2. Cell viability. CHO-K1 cells (a) and HEK-293 cells (b) were incubated with various concentrations of GPMB-biotin or PMB-biotin in complete media for 72 hours in a 96-well plate. Cell titer blue was added and incubated an additional 4 hours. Cell viability was calculated by measuring the fluorescence intensity at 560/590.

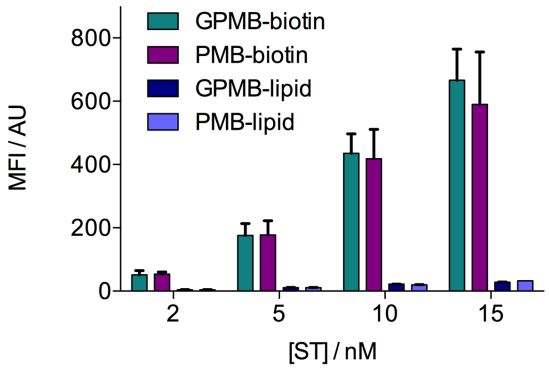


Figure S3. Cellular uptake control. ST-PE-Cy5 was incubated with bPMB, bGPMB, PMB, or GPMB, then diluted to the desired final ST-PE-Cy5 concentrations. The mixtures were then added to CHO-K1 cells and incubated at 37 °C for 1 h. Mean fluorescence intensity was measured by flow cytometry and the background signal from untreated cells was subtracted.

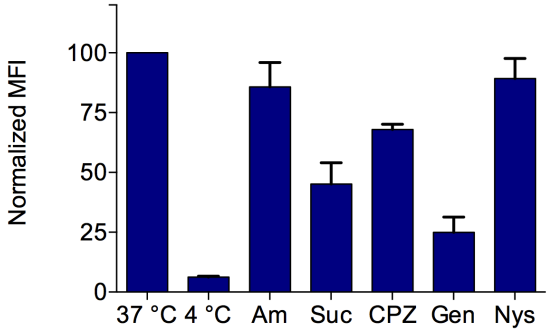


Figure S4. Mechanisms of GTob uptake. CHO-K1 cells were incubated with GTob conjugated to streptavidin-PE-Cy5 (5 nM) for 1 h at 37 °C or 4 °C. For inhibition experiments, cells were pretreated with amiloride (Am, 10 min, 5mM), sucrose (Suc, 30 min, 400mM), chlorpromazine (CPZ, 30 min, 20 μM), genistein (Gen, 30 min, 200 μM), or nystatin (Nys, 30 min, 5 μM) at 37 °C prior to incubation with the conjugates (5 nM) for 1h at 37 °C in the presence of inhibitor (except Am). The background signal from untreated cells was subtracted and the MFI was normalized.

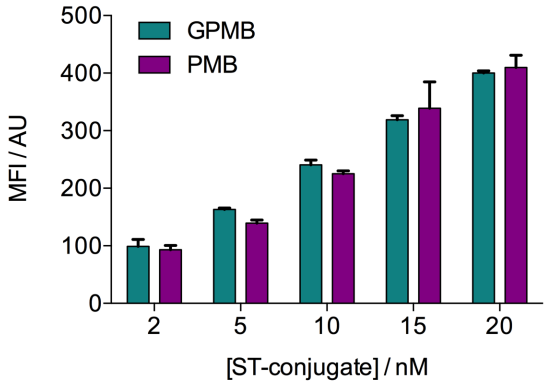


Figure S5. Cellular uptake of ST-Cy5. CHO-K1 cells were incubated with PMB or GPMB conjugated to streptavidin-PE-Cy5 at various concentrations for 1 h at 37 °C. Mean fluorescence intensity was measured and the background signal from untreated cells was subtracted.

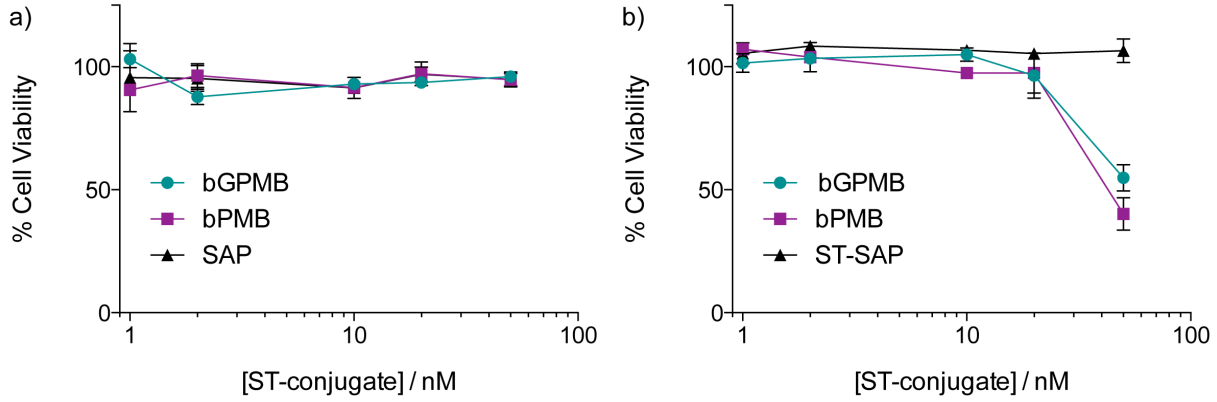


Figure S6. Cellular uptake of saporin. a) Saporin (no streptavidin) was incubated with bPMB or bGPMB for 20 min then dliuted to final saporin concentrations and added to CHO-K1 cells. After four days, the number of viable cells was determined using CellTiter-Blue assay and measuring the flourescence intensity at 560/590. b) pgsA cells were incubated with transporter-streptavidin-saporin conjugates at 37 °C. After four days, the number of viable cells was determined using CellTiter-Blue and measuring fluorescence intensity at 560/590

	Z-average (± SD) / nm	PDI (± SD)	Z-potential (± SD) / mV
Plain liposomes	143.9 (4.0)	0.122 (.039)	3.78 (0.09)
GPMB liposomes	138.2 (1.9)	0.166 (0.021)	26.9 (0.70)
PMB liposomes	139.7 (1.7)	0.164 (0.056)	22.0 (0.70)

Table S1. Physicochemical characterization of liposomes. Size, polydispersity, and zeta-potential of evaluated liposomes. Plain liposomes were compared to liposomes mixed with 10 mol% GPMB or 10 mol% PMB.

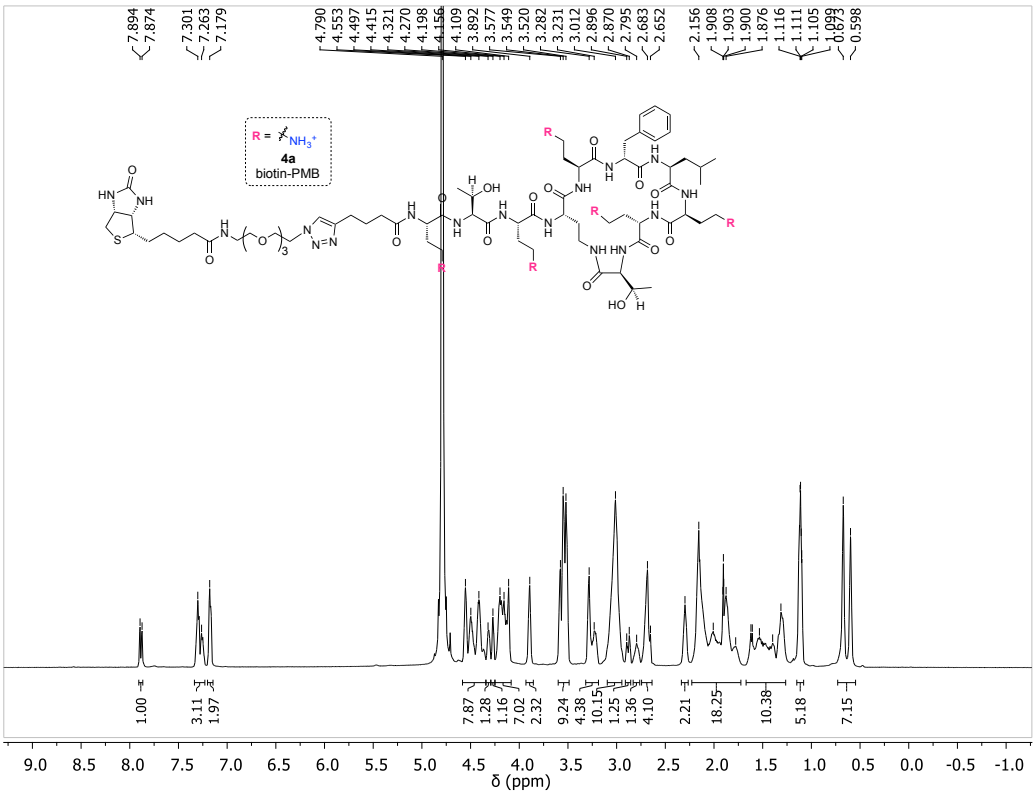


Figure S7. ^{1}H NMR of PMB-biotin (**4a,** D_2O, 500 MHz).

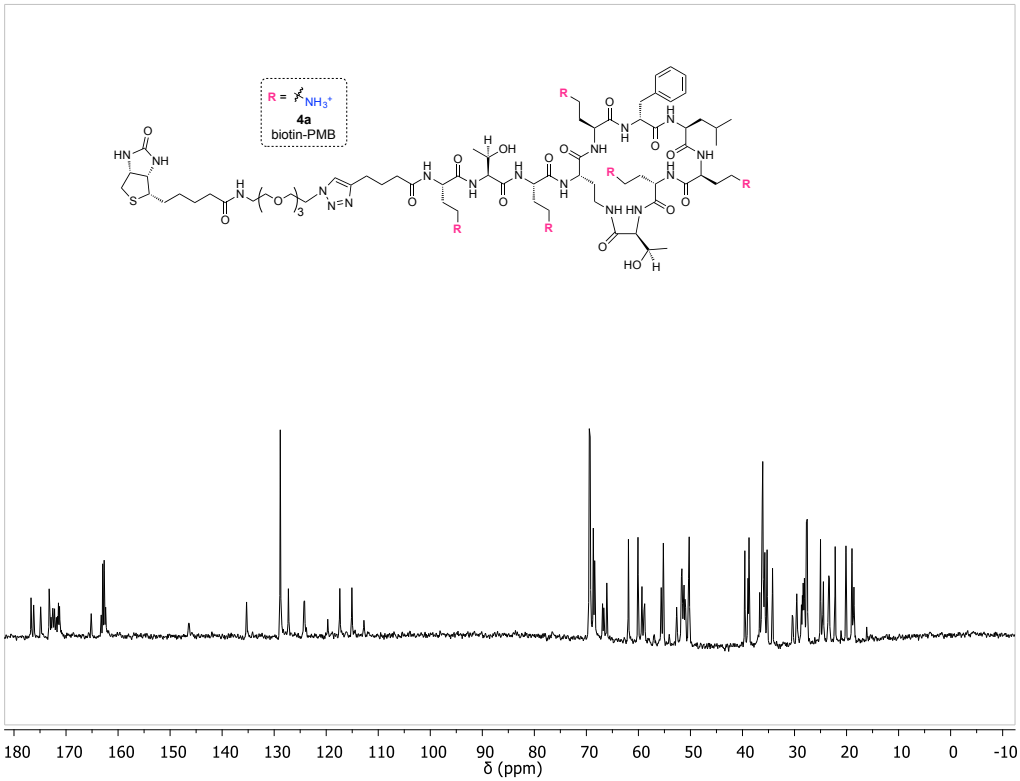


Figure S8. ^{13}C NMR of PMB-biotin (**4a,** D_2O, 126 MHz).

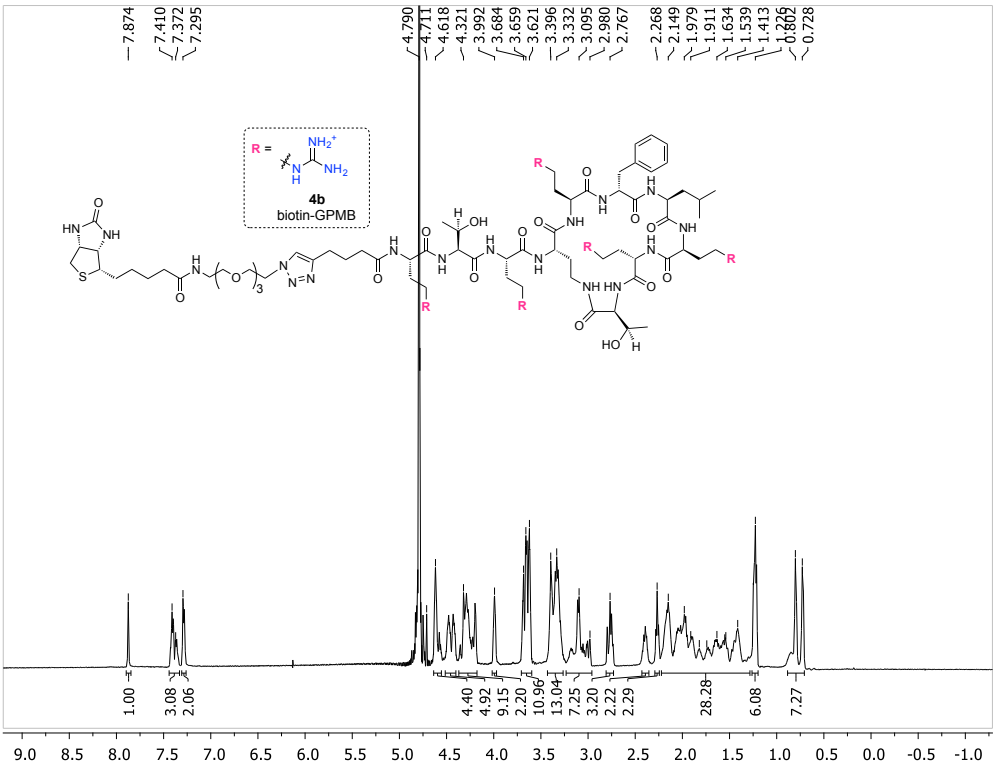


Figure S9. ^{1}H NMR of GPMB-biotin (**4b,** D_2O, 500 MHz).

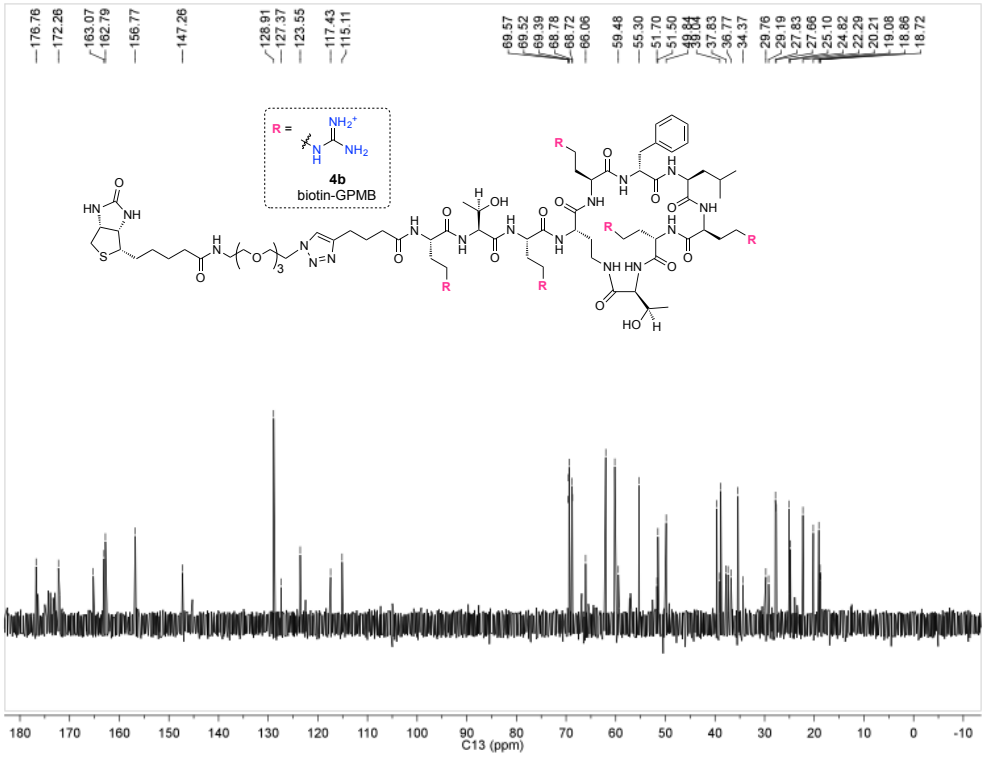


Figure S10. ^{13}C NMR of GPMB-biotin (**4b,** D_2O, 126 MHz)

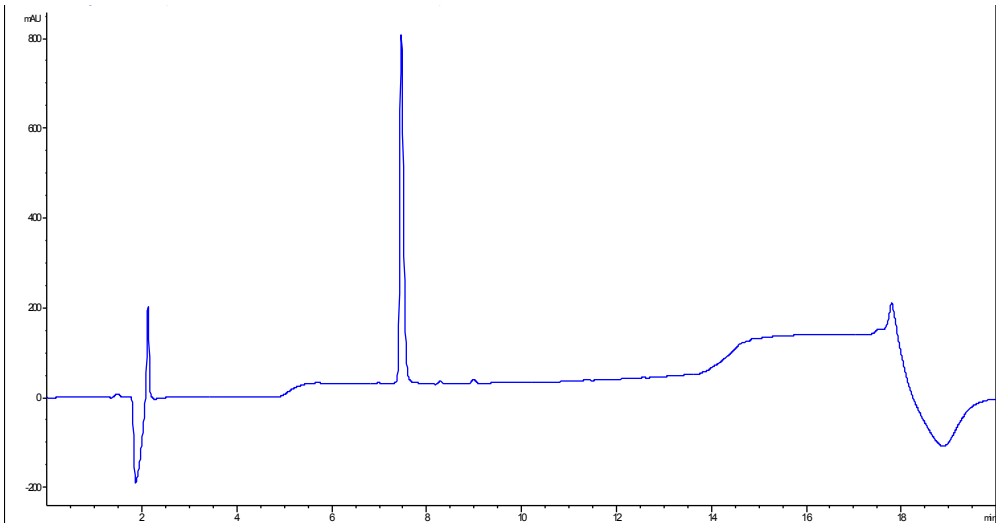


Figure S11. Analytical HPLC trace for bArg8. [RP-C18, 5 – 60% ACN (0.1% TFA) in H_2O (0.1% TFA) over 9 min]

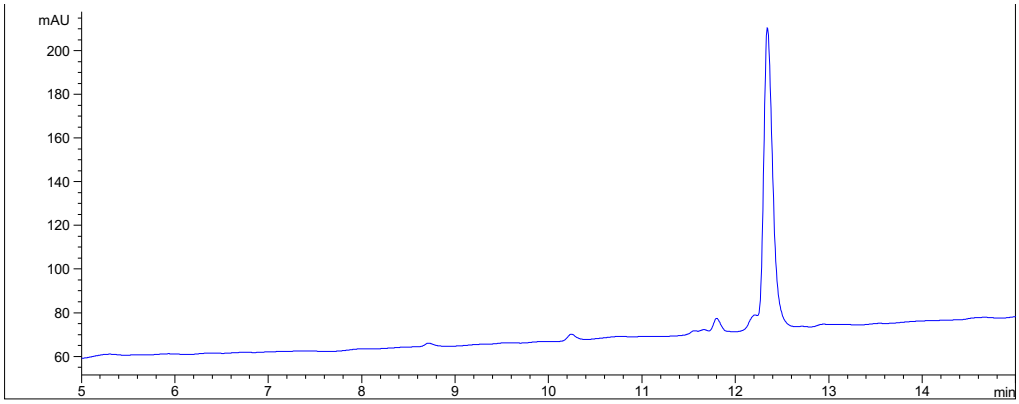


Figure S12. Analytical HPLC trace for PMB [RP-C18 column, 5 – 50% ACN (0.1% TFA) in H_2O (0.1% TFA) over 15 mins].

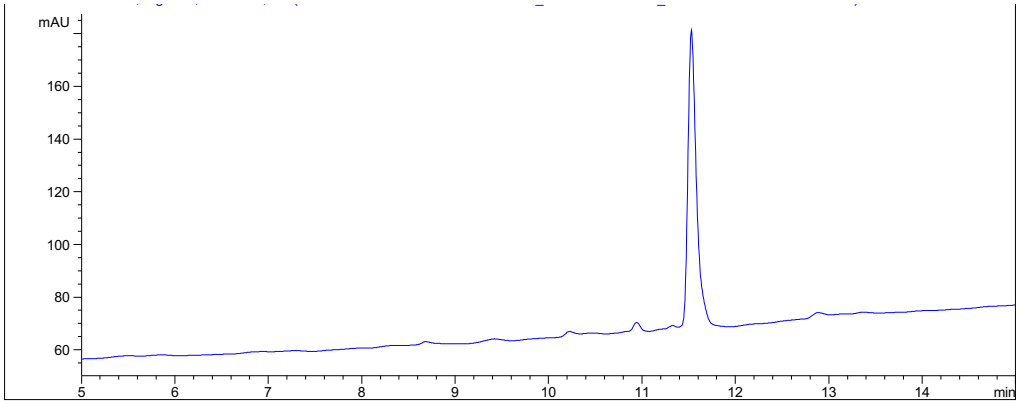


Figure S13. Analytical HPLC trace for GPMB [RP-C18 column, 5 – 50% ACN (0.1% TFA) in H_2O (0.1% TFA) over 15 mins].

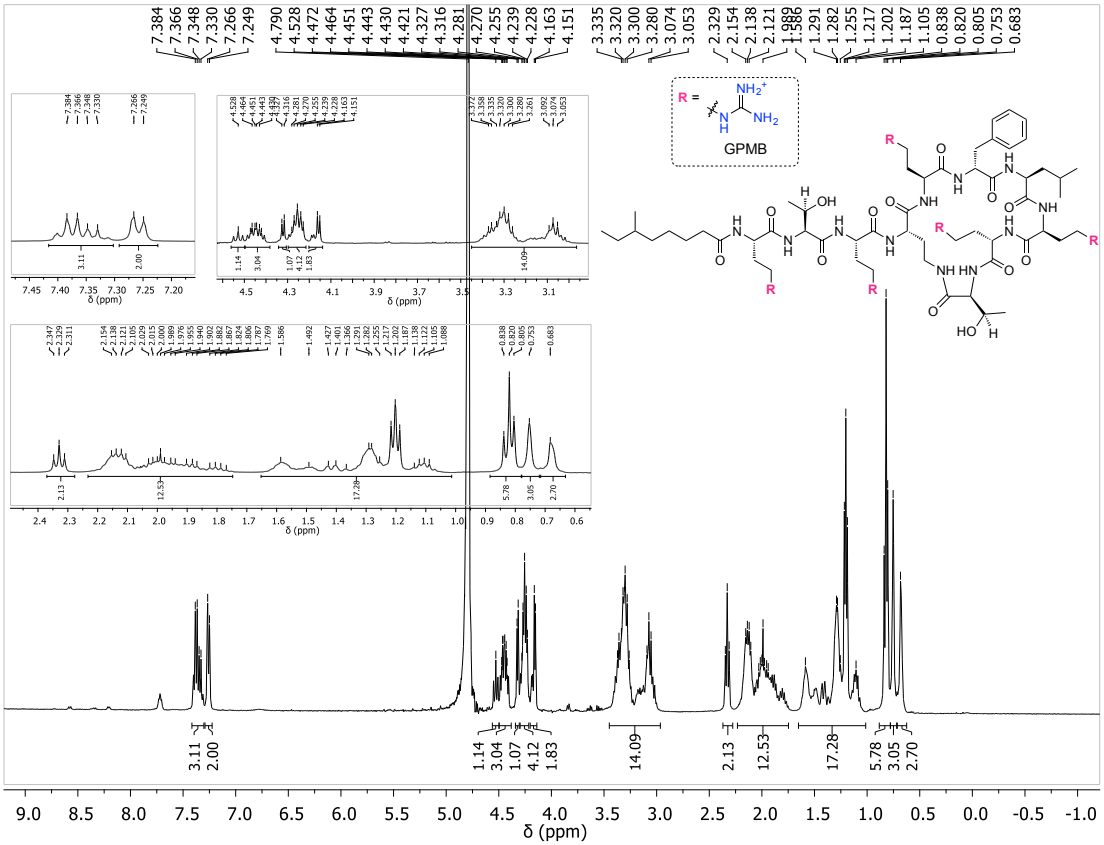


Figure S14. ^{1}H NMR of GPMB (D_2O, 500 MHz).

Supporting References

(1) Dix, A. V.; Fischer, L.; Sarrazin, S.; Redgate, C. P. H.; Esko, J. D.; Tor, Y. *Chembiochem* **2010**, *11*, 2302.