Role of Amphiphilicity in the Design of Synthetic Mimics of Antimicrobial Peptides with Gram-negative Activity

Hitesh D. Thaker,[†] Alper Cankaya,[†] Richard W. Scott,[#] and Gregory N. Tew[*,†]

[†]Department of Polymer Science & Engineering, University of Massachusetts, Amherst, MA 01003;
[#]PolyMedix Inc., 170 North Radnor-Chester Road, Suite 300, Radnor, PA 19087.

tew@mail.pse.umass.edu

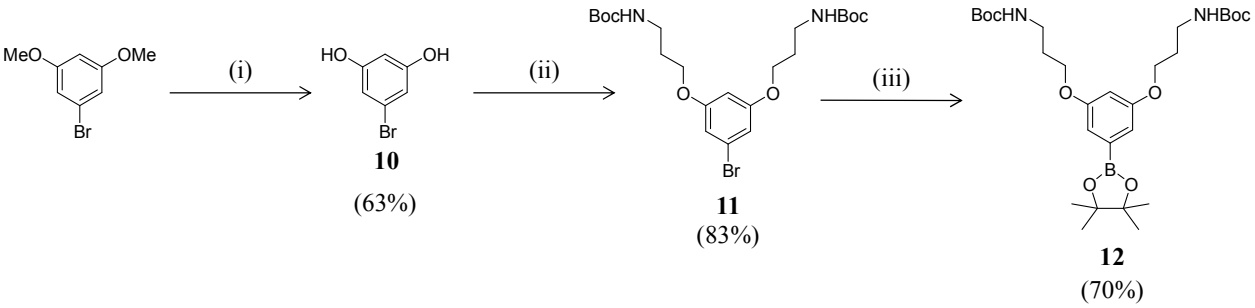
Supporting Information

I. Materials and Instrumentation

Materials: 1-bromo-3,5-dimethoxy-benzene and bispinacolatodiboron were purchased from Sigma-Aldrich. Boron tribromide, dibromobenzene, and trifluoroacetic acid were obtained as reagent grade from VWR and used as received. 3-(Boc-amino)propyl bromide was purchased from Ace Synthesis, LLC. The starting boronic esters were purchased from Boron Molecular. The catalyst 1,1'-bis(diphenylphosphino) ferrocene palladium (II) chloride and anhydrous dimethyl sulfoxide (DMSO) were purchased from Alfa Aesar. The HPLC grade solvents *N*,*N*-dimethylformamide (DMF), toluene, ethyl acetate, water, acetonitrile, pentane and hexanes were purchased from Aldrich, Fisher Scientific or Acros and used as received. Dichloromethane (DCM) (HPLC grade, Fisher Scientific) was distilled from CaH_2 under nitrogen.

Instrumentation: ^{1}H and ^{13}C NMR spectra were recorded at 300 MHz and 75 MHz, respectively, using a Bruker DPX-300 NMR spectrometer. Chemical shifts (δ) are reported in ppm and coupling constants (*J*) in Hz. The abbreviations for splitting patterns are: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; m, multiplet. Mass spectral data were obtained at the University of Massachusetts, Mass Spectrometry Facility from a JEOL JMS 700 instrument (JEOL, Peabody, MA). Analytical HPLC was carried out on a Waters 2695 Separation Module HPLC system using an Agilent Zorbax SB-C_8, 80 Å, 4.6 x 150 mm ID (5 μm) column, eluted by water and acetonitrile, both containing 0.1% of TFA, and detected by a UV detector at a wavelength of 254 nm. The elution was performed by gradually increasing the ratio of acetonitrile in water by 1% per minute, starting with 100% water, with a flow rate of 1 ml/min.

II. Synthetic Procedures for SMAMPs



Scheme S1. Synthesis of compound **12**. i) BBr_3, DCM, 0 °C to RT, overnight; ii) 3-(Boc-amino)propyl bromide, K_2CO_3, DMF, water, 45 °C, overnight; iii) Bispinacolatodiboron, $PdCl_2(dppf)\cdot CH_2Cl_2$, KOAc, DMSO, 80 °C, overnight.

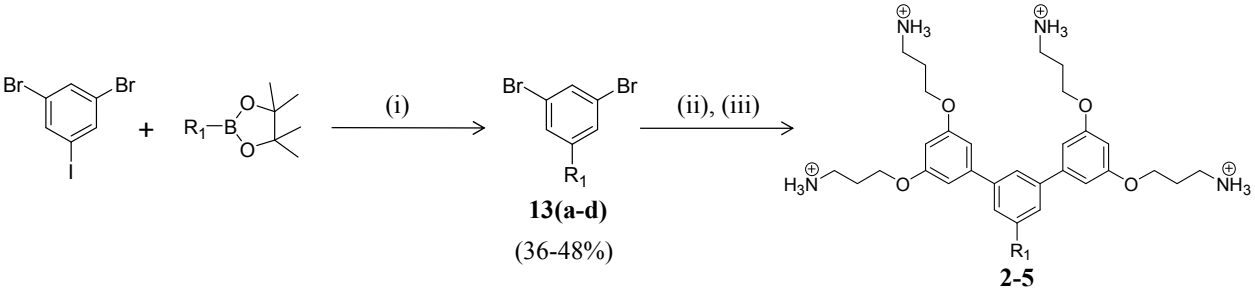
Compound **12** has been synthesized according to the previously described literature procedure using Scheme S1, but the synthesis is described here for convenience.[1]

Synthesis of 5-bromobenzene-1,3-diol (10). 1-bromo-3,5-dimethoxybenzene (8.00 g, 36.9 mmol) was added to 300 ml of dry DCM in an oven dried round bottom flask. The solution was cooled down to 0 °C in an ice-bath followed by dropwise addition of BBr_3 (25.0 g, 100 mmol). After 2 h the mixture was allowed to warm to room temperature and stirred overnight. The reaction was terminated by dropwise addition of methanol (10 ml), and the mixture was poured into water and stirred for 2 h. Then a saturated sodium bicarbonate solution (100 ml) was added and extracted with ethyl acetate (50 ml x 3). The organic layer was washed with saturated sodium bicarbonate, brine and then dried over Na_2SO_4. The residue, after concentration, was purified using column chromatography using ethyl acetate/hexanes (1:4 v/v) as the eluent. Yield = 4.39 g (63%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.66 (t, J = 3.6 Hz, 2H), 6.37 (t, J = 2.0 Hz, 2H), 6.18 (q, J = 2.0 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.47, 121.89, 109.46, 101.83. FAB-MS *m/z*: calculated 189.0, found 189.1.

Synthesis of Compound 11. In a Schlenk flask, Compound **10** (2.7 g, 14.3 mmol) and potassium carbonate (9.8 g, 71.4 mmol) were stirred in DMF (25 ml) and water (2.5 ml) at room temperature for 20 min and then heated to 45 °C. 3-(Boc-amino)propyl bromide (10.89 g, 45.7 mmol) was then added, and the resulting mixture was stirred at 45 °C overnight. The mixture

was cooled down to room temperature and extracted using ethyl acetate and water (50 ml each). The organic layer was washed with brine and dried over Na_2SO_4. The residue, after concentration, was purified using column chromatography using ethyl acetate/hexanes (1:4 v/v) as the eluent. Yield = 6.13 g (83%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.89 (s, 2H), 6.68 (d, *J* = 2.1 Hz, 2H), 6.46 (t, *J* = 2.1 Hz, 1H), 3.94 (t, *J* = 6.2 Hz, 4H), 3.04 (q, *J* = 6.6 Hz, 4H), 1.78 (t, *J* = 6.50 Hz, 4H), 1.36 (s, 18H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 160.32, 155.50, 122.13, 109.81, 100.59, 77.39, 65.64, 36.69, 28.93, 28.12. FAB-MS *m/z*: calculated 503.4, found 503.2.

Synthesis of Compound 12. Compound **11** (3.0 g, 5.96 mmol), bispinacolatodiboron (1.68 g, 6.56 mmol) and potassium acetate (2.92 g, 29.8 mmol) were stirred in DMSO (30 ml) at room temperature under N_2 protection in an oven-dried round bottom flask. Then $PdCl_2(dppf)\cdot CH_2Cl_2$ (0.289 g, 0.357 mmol) was added to the mixture followed by stirring at 80 °C overnight. After cooling down to room temperature, the mixture was filtered through celite and extracted using ethyl acetate and water (100 ml each). The organic layer was washed with water, brine, and then dried over Na_2SO_4. The residue after concentration was purified using column chromatography using ethyl acetate/hexanes (3:7 v/v) as the eluent. Yield = 2.27 g (70%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.93 (d, *J* = 2.4 Hz, 2H), 6.54 (t, *J* = 2.4 Hz, 1H), 4.76 (s, 2H), 4.02 (t, *J* = 5.9 Hz, 4H), 3.31 (q, *J* = 6.4 Hz, 4H), 1.95 (m, 4H), 1.44 (s, 18H), 1.33 (s, 12H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.68, 156.12, 112.57, 105.38, 84.04, 79.33, 65.95, 38.13, 29.55, 28.55, 24.97. FAB-MS *m/z*: calculated 550.5, found 550.4.



Scheme S2. Synthesis of aryl SMAMPs **2-5**. i) $PdCl_2(dppf)\cdot CH_2Cl_2$, toluene, water, K_3PO_4, 100 °C, 20 hours; (ii) Compound **12**, $PdCl_2(dppf)\cdot CH_2Cl_2$, toluene, water, K_3PO_4, 100 °C, 20 hours; iii) TFA, DCM, 3 hours

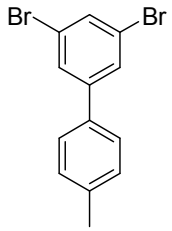
Note: The synthesis of SMAMP **1** has already been reported in the previous literature.[1]

General procedure for Suzuki coupling.

In a dry Schlenk tube, dibromoarene (1.05 mmol, 1 eq.) was added to the boronic ester compound (2.41 mmol, 2.3 eq.), K_3PO_4 (4.19 mmol, 4 eq.) and $PdCl_2(dppf)\cdot CH_2Cl_2$ (0.052 mmol, 0.05 eq.) along with 9 ml toluene and 0.9 ml water. The mixture was stirred for five minutes, and then the Schlenk tube was degassed by three freeze-pump-thaw cycles followed by purging with nitrogen. The mixture was then stirred at 100 °C for 20 h. The reaction mixture, cooled to room temperature, was quenched with water (25 ml) and extracted with ethyl acetate (30 ml x 3). The combined organic layers were washed with a saturated aqueous solution of $NaHCO_3$ (3 times) and brine, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was purified using column chromatography using an ethyl acetate/ hexane mixture.

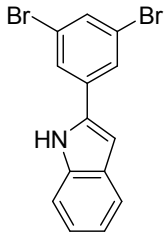
General procedure for the deprotection of the Boc groups.

The compound (150 mg) was stirred in a mixture of TFA and DCM (1:1 v/v) for 3 h. The solution was concentrated using a rotary evaporation and dissolved in methanol. The procedure was repeated two more times and then the concentrate was dried overnight under vacuum. The solid was then dissolved in a minimal amount of methanol and precipitated using a hexane/ether mixture (1:1 v/v). The mixture was centrifuged for 1 min and the supernatant liquid was removed. This process was repeated twice. The residue was then dried under vacuum to remove any residual solvent to give the final pure product in its TFA salt form. The purity of the product was checked with HPLC and found to be >95%.



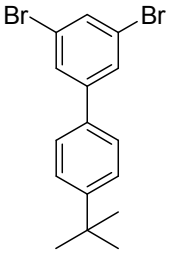
13a

Synthesis of Compound 13a. 1,3-dibromo-5-iodobenzene (1.29 g, 3.57 mmol) and 4,4,5,5-tetramethyl-2-(4-methylphenyl)-1,3,2-dioxaborolane (0.6 g, 2.75 mmol) were reacted according to the Suzuki coupling procedure and purified using column chromatography using pure hexane to give compound **13a**. Yield = 0.41 g (46%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.82 (d, *J* = 1.7 Hz, 2H), 7.75 (t, *J* = 1.7 Hz, 2H), 7.58 (m, 2H), 7.25 (d, *J* = 8.0Hz, 2H), 2.32 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 144.00, 138.14, 134.05, 131.70, 129.56, 128.13, 126.74, 122.98, 20.59. HR-MS *m/z*: calculated 323.9149, found 323.9147.



13b

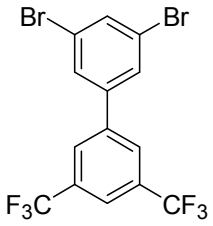
Synthesis of Compound 13b. 1,3-dibromo-5-iodobenzene (1.93 g, 5.34 mmol) and 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indole (1 g, 4.11 mmol) were reacted according to the Suzuki coupling procedure and purified by column chromatography using pure hexane. The product was then recrystallized using hexanes to give compound **13b**. Yield = 0.52 g (36%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.28 (s, 1H), 7.71 (d, *J* = 1.7 Hz, 2H), 7.63 (m, 1H), 7.58 (t, *J* = 1.7 Hz, 1H), 7.39 (dd, *J* = 1.0, 8.1 Hz, 1H), 7.22 (dd, *J* = 1.2, 8.1 Hz, 1H), 7.14 (ddd, *J* = 1.1, 7.0, 8.0 Hz, 1H), 6.84 (dd, *J* = 1.0, 2.2 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 136.53, 135.23, 134.08, 132.14, 128.28, 126.12, 123.00, 122.81, 120.54, 120.17, 110.56, 101.39. HR-MS *m/z*: calculated 348.9102, found 348.9063.



13c

Synthesis of Compound 13c. 1,3-dibromo-5-iodobenzene (1.81 g, 5 mmol) and 2-(4-tert-butylphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (1 g, 3.84 mmol) were reacted according

to the Suzuki coupling procedure and purified by column chromatography using pure hexane to give compound **13c**. Yield = 0.6 g (43%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65 (d, J = 1.7 Hz, 2H), 7.62 (t, J = 1.7 Hz, 1H), 7.47 (s, 4H), 1.37 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.80, 144.76, 135.51, 132.38, 128.93, 126.87, 126.12, 123.32, 34.77, 31.42. HR-MS *m/z*: calculated 367.9599, found 367.9601.



13d

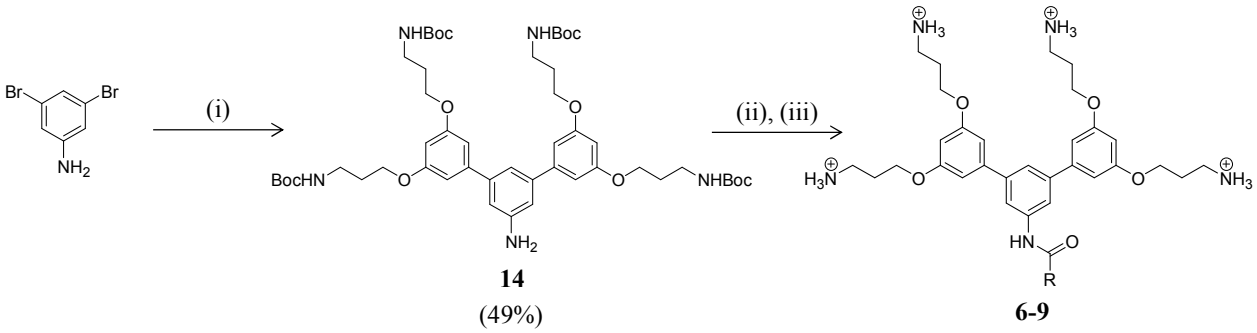
Synthesis of Compound 13d. 1,3-dibromo-5-iodobenzene (2.07 g, 5.73 mmol) and 2-[3,5-bis(trifluoromethyl)phenyl]-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (1.5 g, 4.41 mmol) were reacted according to the Suzuki coupling procedure and purified by column chromatography using pure hexane to give compound **13d**. Yield = 0.95 g (48%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (d, J = 1.7 Hz, 2H), 7.92 (m, 1H), 7.76 (t, J = 1.7 Hz, 1H), 7.67 (d, J = 1.7 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 141.01, 139.86, 133.77, 131.96 (q, J_{CF} = 33 Hz), 128.54, 126.71 (d, J_{CF} = 3.8 Hz), 123.27, 122.53 (q, J_{CF} = 271 Hz), 121.62 (m) HR-MS *m/z*: calculated 447.8720, found 447.8730.

Synthesis of SMAMP 2. Compound **13a** (0.2 g, 0.61 mmol) was reacted with compound **12** (0.78 g, 1.41 mmol) using the Suzuki Coupling procedure and purified by column chromatography using an ethyl acetate/hexane mixture (2:3 v/v) followed by the deprotection procedure to give compound **2**. The overall yield of the two steps was 78% and the purity of the final product was >95%. ^{1}H NMR (300 MHz, MeOD) δ 7.75 (d, J = 1.6 Hz, 2H), 7.70 (d, J = 1.8 Hz, 1H), 7.61 (dd, J = 1.8, 8.2 Hz, 2H), 7.31 (m, 2H), 6.90 (t, J = 2.1 Hz, 4H), 6.61 (d, J = 2.1 Hz, 2H), 4.19 (t, J = 5.8 Hz, 8H), 3.19 (t, J = 7.2 Hz, 8H), 2.40 (s, 3H), 2.18 (m, 8H). ^{13}C NMR (75 MHz, MeOD) δ 161.54, 144.76, 143.63, 143.52, 139.24, 138.70, 130.60, 128.08, 126.06, 125.58, 107.53, 101.52, 66.42, 38.56, 28.39, 21.13. HR-MS *m/z*: calcd 613.3754, found 613.3739

Synthesis of SMAMP 3. Compound **13b** (0.25 g, 0.71 mmol) was reacted with compound **12** (0.9 g, 1.64 mmol) using the Suzuki Coupling procedure and purified by column chromatography using an ethyl acetate/hexane mixture (2:3 v/v) followed by the deprotection procedure to give compound **3**. The overall yield of the two steps was 80% and the purity of the final product was >95%. ^{1}H NMR (300 MHz, MeOD) δ 8.00 (d, *J* = 1.5 Hz, 2H), 7.67 (d, *J* = 1.7 Hz, 1H), 7.55 (d, *J* = 7.8 Hz, 1H), 7.42 (d, *J* = 8.1 Hz, 1H), 7.12 (ddd, *J* = 1.2, 7.0, 8.2 Hz, 1H), 7.02 (m, 1H), 6.94 (m, 5H), 6.62 (t, *J* = 2.2 Hz, 2H), 4.21 (t, *J* = 5.8 Hz, 8H), 3.19 (t, *J* = 7.3 Hz, 8H), 2.19 (m, 8H). ^{13}C NMR (75 MHz, MeOD) δ 161.59, 144.61, 143.61, 139.00, 138.82, 135.28, 130.50, 125.65, 124.24, 123.03, 121.29, 120.66, 112.15, 107.48, 101.70, 100.42, 66.45, 38.59, 28.45. HR-MS *m/z*: calcd 638.3706, found 638.3696

Synthesis of SMAMP 4. Compound **13c** (0.3 g, 0.82 mmol) was reacted with compound **12** (1.04 g, 1.89 mmol) using the Suzuki Coupling procedure and purified by column chromatography using an ethyl acetate/hexane mixture (2:3 v/v) followed by the deprotection procedure to give compound **4**. The overall yield of the two steps was 32% (loss due to column) and the purity of the final product was >95%. ^{1}H NMR (300 MHz, MeOD) δ 7.77 (d, *J* = 1.6 Hz, 2H), 7.68 (m, 3H), 7.53 (m, 2H), 6.91 (d, *J* = 2.2 Hz, 4H), 6.61 (t, *J* = 2.2 Hz, 2H), 4.19 (t, *J* = 5.9 Hz, 8H), 3.19 (t, *J* = 7.3 Hz, 8H), 2.18 (m, 8H), 1.38 (s, 9H). ^{13}C NMR (75 MHz, MeOD) δ 161.57, 151.96, 144.81, 143.59, 143.56, 139.22, 127.91, 126.90, 126.14, 125.62, 107.55, 101.55, 66.45, 38.59, 35.42, 31.77, 30.69, 28.45. HR-MS *m/z*: calcd 655.4223, found 655.4218

Synthesis of SMAMP 5. Compound **13d** (0.35 g, 0.78 mmol) was reacted with compound **12** (0.99 g, 1.79 mmol) using the Suzuki Coupling procedure (column chromatography using an ethyl acetate/hexane mixture 2:3 v/v) followed by the deprotection procedure to give compound **5**. The overall yield of the two steps was 61% and the purity of the final product was >95%. ^{1}H NMR (300 MHz, MeOD) δ 8.29 (s, 2H), 8.02 (s, 1H), 7.85 (m, 3H), 6.95 (d, *J* = 2.2 Hz, 4H), 6.63 (t, *J* = 2.2 Hz, 2H), 4.20 (t, *J* = 5.8 Hz, 8H), 3.19 (t, *J* = 7.3 Hz, 8H), 2.18 (m, 8H). ^{13}C NMR (75 MHz, MeOD) δ 161.62, 144.96, 144.15 (d, *J* = 4.5 Hz), 140.72, 133.57, 133.13, 128.89, 127.55, 126.68, 126.57, 118.20 (q, *J* = 291 Hz), 107.65, 101.78, 66.46, 38.57, 28.41. HR-MS *m/z*: calcd 735.3345, found 735.3344



Scheme S3. Synthesis of aryl SMAMPs **6-9**. i) Compound **12**, $PdCl_2(dppf)\cdot CH_2Cl_2$, toluene, water, K_3PO_4, 100 °C, 20 hours; (ii) EDC, HOBt, DCM, R.T., 2 days; iii) TFA, DCM, 3 hours

Synthesis of Compound 14. 3,5-dibromoaniline (0.7 g, 2.8 mmol) was reacted with compound **12** (3.53 g, 6.41 mmol) according to the Suzuki coupling procedure and purified by column chromatography using an ethyl acetate/hexanes mixture (3:2 v/v) followed by precipitation in a hexane/ether mixture to give compound **14**. Yield = 1.32 g (49%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.11 (s, 1H), 6.85 (d, *J* = 1.4 Hz, 2H), 6.72 (d, *J* = 2.1 Hz, 4H), 6.44 (t, *J* = 2.1 Hz, 2H), 4.77 (s, 4H), 4.05 (t, *J* = 5.9 Hz, 8H), 3.82 (s, 2H), 3.33 (q, *J* = 6.2 Hz 8H), 1.99 (m, 8H), 1.43 (s, 37H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.21, 156.09, 147.05, 143.69, 142.86, 116.92, 113.31, 106.26, 100.48, 79.32, 65.95, 38.06, 29.62, 28.49. HR-MS *m/z*: calculated 937.5412, found 937.5400.

General procedure for the amide EDC/HOBt Coupling.

In a round bottom flask under a nitrogen atmosphere, compound **14** (0.32 mmol, 1 eq.), aryl carboxylic acid (0.42 mmol, 1.3 eq.) and HOBT (0.42 mmol, 1.3 eq.) were dissolved in dry DCM (5 ml). The mixture was cooled to 0 °C and EDC (0.42 mmol, 1.3 eq.) was added. The reaction mixture was stirred at room temperature for 2 days to ensure complete conversion, and then the mixture quenched with water (10 ml) and extracted with ethyl acetate (20ml x 3). The combined organic layer was washed with a saturated aqueous solution of $NaHCO_3$ (20 ml) and brine (20 ml), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The crude product was purified using column chromatography using an ethyl acetate/hexane mixture.

Synthesis of SMAMP 6. Compound **14** (0.19 g, 0.2 mmol) was reacted with benzoic acid (0.035 g, 0.28 mmol) according to the amide EDC/HOBt coupling procedure (column chromatography

using an ethyl acetate/hexane mixture 3:2 v/v) followed by the deprotection procedure to give compound **6**. The overall yield of the two steps was 78% and the purity of the final product was >95%. ^{1}H NMR (300 MHz, MeOD) δ 7.97 (m, 4H), 7.59 (m, 2H), 7.52 (m, 2H), 6.87 (d, *J* = 2.2 Hz, 4H), 6.59 (t, *J* = 2.1 Hz, 2H), 4.17 (t, *J* = 5.8 Hz, 8H), 3.17 (t, *J* = 7.3 Hz, 8H), 2.16 (m, 8H).^{13}C NMR (75 MHz, MeOD) δ 169.09, 161.55, 144.48, 143.57, 140.74, 136.06, 133.15, 129.74, 128.67, 122.89, 120.24, 107.43, 101.68, 66.42, 38.57, 28.43. HR-MS *m/z*: calculated 642.3655, found 646.3652.

Synthesis of SMAMP 7. Compound **14** (0.4 g, 0.43 mmol) was reacted with indole acetic acid (0.098 g, 0.55 mmol) according to the amide EDC/HOBt coupling procedure (column chromatography using an ethyl acetate/hexane mixture 1:1 v/v) followed by the deprotection procedure to give compound **7**. The overall yield of the two steps was 62% and the purity of the final product was 88%. ^{1}H NMR (300 MHz, MeOD) δ 7.81 (s, 2H), 7.62 (t, *J* = 11.0 Hz, 1H), 7.49 (s, 1H), 7.37 (d, *J* = 7.6 Hz, 1H), 7.26 (s, 1H), 7.11 (t, *J* = 7.5 Hz, 1H), 7.03 (t, *J* = 7.4 Hz, 1H), 6.83 (d, *J* = 1.8 Hz, 4H), 6.57 (d, *J* = 1.8 Hz, 2H), 4.16 (t, *J* = 5.5 Hz, 8H), 3.89 (s, 2H), 3.16 (t, *J* = 7.2 Hz, 8H), 2.16 (m, 8H). ^{13}C NMR (75 MHz, MeOD) δ 173.61, 164.25, 161.50, 144.49, 143.57, 140.80, 138.17, 128.66, 124.92, 122.57, 119.97, 119.47, 119.21, 112.38, 109.40, 107.39, 101.67, 66.39, 49.85, 49.57, 49.28, 49.00, 48.72, 48.43, 48.15, 38.56, 35.09, 28.40. HR-MS *m/z*: calculated 695.3921, found 695.3924.

Synthesis of SMAMP 8. Compound **14** (0.3 g, 0.32 mmol) was reacted with 2-naphthoic acid (0.071 g, 0.42 mmol) according to the amide EDC/HOBt coupling procedure (column chromatography using an ethyl acetate/hexane mixture 1:1 v/v) followed by the deprotection procedure to give compound **8**. The overall yield of the two steps was 74% and the purity of the final product was >95%. ^{1}H NMR (300 MHz, MeOD) δ 8.57 (s, 1H), 8.01 (m, 6H), 7.61 (m, 3H), 6.90 (d, *J* = 2.1 Hz, 4H), 6.61 (t, *J* = 2.1 Hz, 2H), 4.19 (t, *J* = 5.7 Hz, 8H), 3.18 (t, *J* = 7.3 Hz, 8H), 2.17 (m, 8H). ^{13}C NMR (75 MHz, MeOD) δ 169.11, 161.57, 144.52, 143.61, 140.86, 136.49, 134.06, 133.24, 130.13, 129.55, 129.29, 129.19, 128.88, 128.06, 125.10, 122.88, 120.26, 107.44, 101.69, 66.42, 38.57, 28.44. HR-MS *m/z*: calculated 692.3812, found 692.3770.

Synthesis of SMAMP 9. Compound **14** (0.3 g, 0.32 mmol) was reacted with 4-phenylbenzoic acid (0.083 g, 0.42 mmol) according to the amide EDC/HOBt coupling procedure (column chromatography using an ethyl acetate/hexane mixture 1:1 v/v) followed by the deprotection

procedure to give compound **9**. The overall yield of the two steps was 20% (major loss was attributed to the column) and the purity of the final product was >95%. ^{1}H NMR (300 MHz, MeOD) δ 8.09 (d, *J* = 8.5 Hz, 2H), 7.99 (d, *J* = 1.4 Hz, 2H), 7.81 (m, 2H), 7.71 (m, 2H), 7.56 (s, 1H), 7.49 (dd, *J* = 8.1, 6.6 Hz, 2H), 7.41 (m, 1H), 6.90 (d, *J* = 2.2 Hz, 4H), 6.61 (t, *J* = 2.1 Hz, 2H), 4.19 (t, *J* = 5.8 Hz, 8H), 3.18 (t, *J* = 7.3 Hz, 8H), 2.17 (m, 8H). ^{13}C NMR (75 MHz, MeOD) δ 168.88, 161.56, 146.19, 144.50, 143.59, 141.11, 140.75, 134.60, 130.13, 129.34, 128.20, 128.15, 120.34, 116.28, 107.48, 101.74, 66.48, 38.60, 28.43. HR-MS *m/z*: calculated 718.3968, found 718.3908.

III. Antimicrobial and Hemolytic Assays

Antimicrobial Activity. All of the biological testing was conducted by Polymedix, Inc. (Philadelphia, PA) using a modified microbroth dilution assay recommended by the Clinical and Laboratory Standards Institute (CLSI) that has been developed for determining in-vitro antimicrobial activities of cationic agents.[2, 3] Modifications were made to minimize loss of the antimicrobial agent due to both adsorption onto glass or plastic surfaces and the precipitation at high concentrations. The bacterial strains were grown in Mueller-Hinton broth (MH broth) at 37 °C overnight, and the bacterial growth was measured by turbidity as the optical density at λ = 600 nm (OD_{600}) using an Eppendorf BioPhotometer. The bacterial strain was diluted to a working solution of 10^6 colony forming units per ml (OD_{600}= 0.001). The SMAMPs were first dissolved in DMSO to form a stock solution of 10 mg/ml and then the Hancock Solution (0.01% acetic acid, 0.2% Bovine Serum Albumin) was used to a make 2-fold dilution stock series. 10 µl of the dilutions were added to each corresponding well of a 96-well round bottom polypropylene plate along with 90 µl of the diluted bacterial strain to the respective wells in duplicate. Minimum Inhibitory Concentrations (MICs) were obtained by measuring cell growth at OD_{600} after incubation with the compounds for 18 h at 37 °C. Each compound was tested as the TFA salt against ATCC bacterial strains (*E. coli* 25922, *S. aureus* 27660, *E. faecalis* 29212 and *K. pneumoniae* 13883).

Hemolytic Activity. The HC_{50} was determined by measuring the quantity of hemoglobin released from red blood cells (RBCs) after their lysis. RBCs were collected by centrifugation from human whole blood and diluted in a TBS solution (150 mM NaCl, 10 mM Tris pH 7.4) to obtain a 0.22% RBC stock suspension. In a 96-well plate, serial 1:2 dilutions of each compound in water were added to the RBC solution (final concentrations tested: ≤ 1000 µg/ml) and the plate was incubated in a shaker at 37 ^oC for 1 h. After centrifugation at 3000 rpm for 5 min, 30 µl of supernatant was removed and added to 100 µl of H_2O in a sterile polystyrene 96-well flat bottom plate. The hemoglobin concentration in the supernatant was read at OD_{405}. Melittin was used as a positive control, and the most concentrated sample (200 µg/ml) was used as a reference for 100% hemolysis. A control solution without any compound was used as a reference for 0% hemolysis.

Cytotoxicity against other cells. Cytotoxicity was evaluated in a colorimetric assay using a transformed human liver cell line (HepG2 cells) and an embryonic mouse cell line (3T3 cells). This assay measures the bio-reduction of a novel tetrazolium compound to a soluble formazan product by viable cells. Cells were incubated for one hour in the presence of a SMAMP in serum-free medium before viability determinations. Cytotoxicity values are reported as EC_{50} against 3T3 cells and HepG2 cells (Table S1 and S2).

IV. Supplementary Figures and Tables

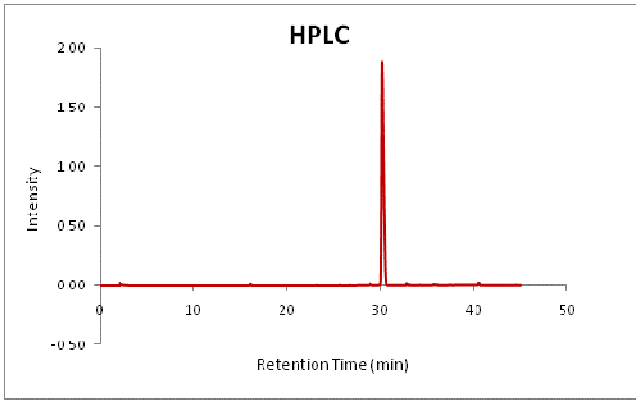


Figure S1. HPLC trace of SMAMP **2**.

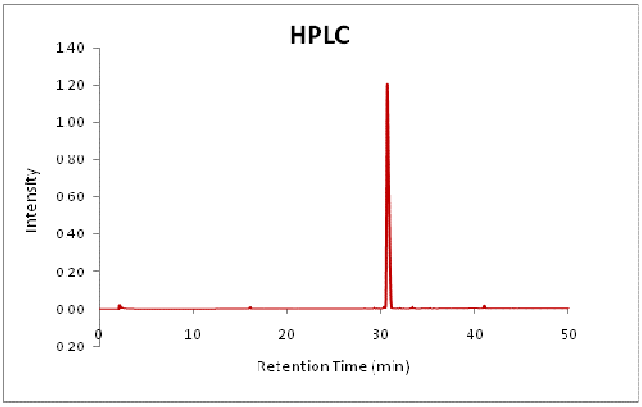


Figure S2. HPLC trace of SMAMP **3**.

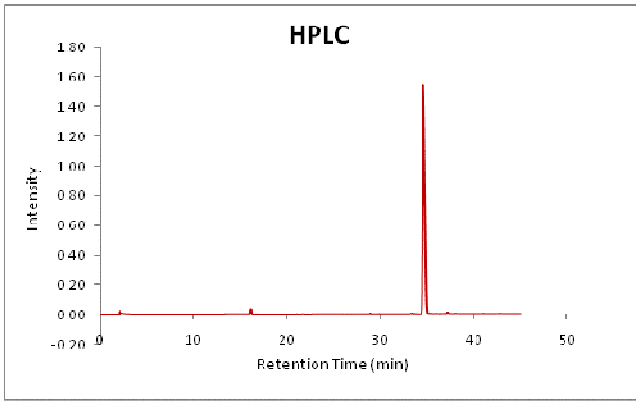


Figure S3. HPLC trace of SMAMP **4**.

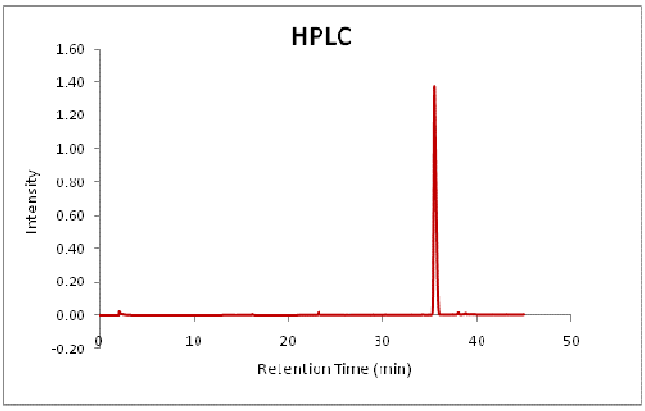


Figure S4. HPLC trace of SMAMP **5**.

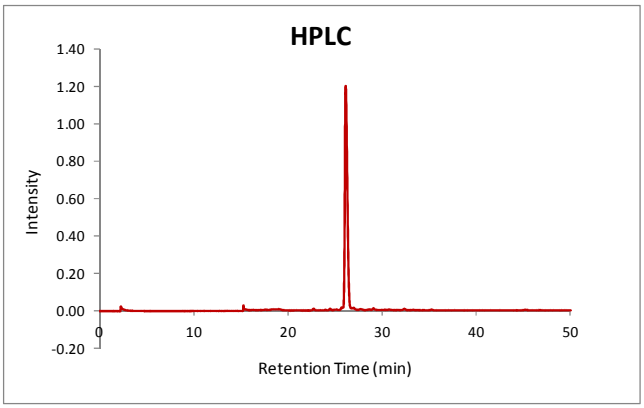


Figure S5. HPLC trace of SMAMP **6**.

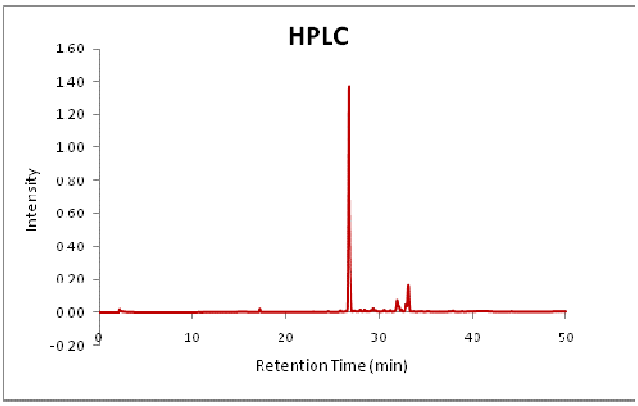


Figure S6. HPLC trace of SMAMP **7**.

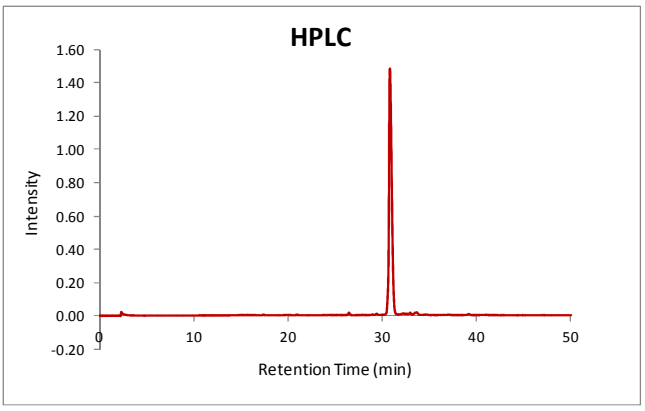


Figure S7. HPLC trace of SMAMP **8**.

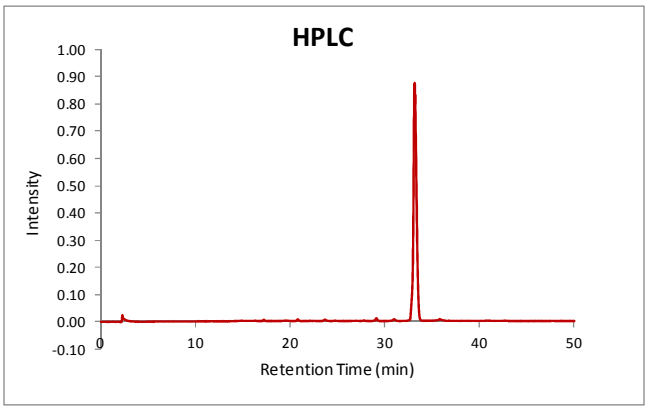


Figure S8. HPLC trace of SMAMP **9**.

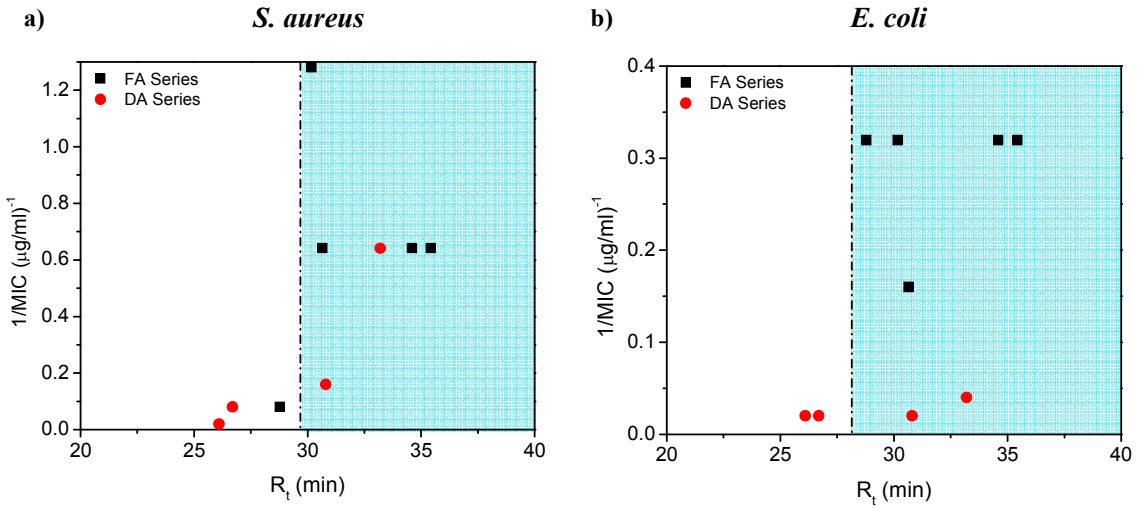
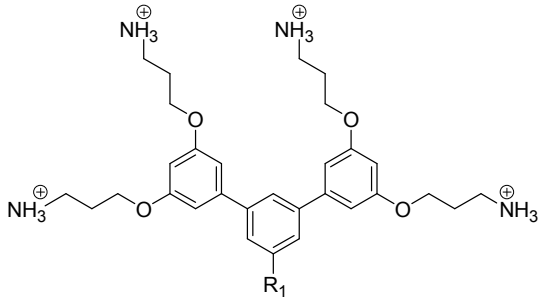
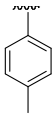
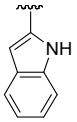
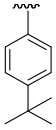
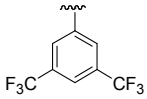


Figure S9. Plots of the antimicrobial potency vs. HPLC retention time (R_t) for a) *S. aureus* and b) *E. coli* for the DA series and FA series SMAMPS. The blue region represents the active window for antimicrobial activity.

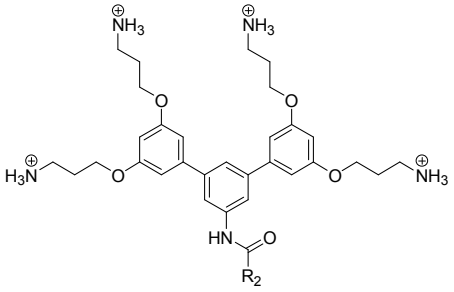
Table S1. Broad spectrum antibacterial activity and cytotoxicity of SMAMPs with a facially amphiphilic topology

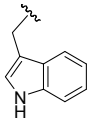
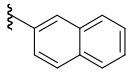
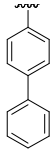


SMAMP	R_1	R_t[a] (min)	MIC (μg/ml)				3T3[b] (μg/ml)	HepG2[b] (μg/ml)	HC_{50} (μg/ml)
			SA	*EC*	*EF*	*KP*			
1		28.8	12.5	3.13	1.56	12.5	241	426	537
2		30.2	0.78	3.13	1.56	6.25	129	190	279
3		30.6	1.56	6.25	12.5	>50	57	77	634
4		34.6	1.56	3.13	1.56	3.13	100	173	41
5		35.4	1.56	3.13	1.56	3.13	69	171	34

[a] Measured by HPLC using a C8 column with a gradient of 1% acetonitrile / min starting with 100% water. [b] Cytotoxicity reported as EC_{50} values. The abbreviations used for bacterial strains are as follows: *SA*, *Staphylococcus aureus*; *EC*, *Escherichia coli*; *EF*, *Enterococcus faecalis* and *KP*, *Klebsiella pneumoniae*. n.d., not determined.

Table S2. Broad spectrum antibacterial activity and cytotoxicity of SMAMPs with disrupted amphiphilic topology



SMAMP	R_2	R_t[a] (min)	MIC (µg/ml)				3T3[b] (µg/ml)	HepG2[b] (µg/ml)	HC_{50} (µg/ml)
			SA	*EC*	*EF*	*KP*			
6		26.1	50	>50	>50	>50	122	257	>1000
7		26.7	12.5	>50	50	>50	394	911	519.2
8		30.8	6.25	>50	12.5	>50	123	255	422.5
9		33.2	1.56	25	6.25	>50	30	55	112.8

[a] measured by HPLC using C8 column with a gradient of 1% acetonitrile / min starting with 100% water. [b]Cytotoxicity reported as EC_{50} values. The abbreviations used for bacterial strains are as follows: *SA*, *Staphylococcus aureus*; *EC*, *Escherichia coli*; *EF*, *Enterococcus faecalis* and *KP*, *Klebsiella pneumoniae*.

Table S3. The integy moment (IW) values of the SMAMPs in the FA and the DA series calculated using VolSurf software (grid spacing of 0.2).

SMAMP	IW	SMAMP	IW
1	0.3078	**6**	0.2135
2	0.2955	**7**	0.236
3	0.2416	**8**	0.2195
4	0.3214	**9**	0.2174
5	0.4089		

V. References

1. Thaker, H. D.; Som, A.; Ayaz, F.; Lui, D.; Pan, W.; Scott, R. W.; Anguita, J.; Tew, G. N. Synthetic mimics of antimicrobial peptides with immunomodulatory responses. *J. Am. Chem. Soc.* **2012,** *134*, 11088-11091.

2. Steinberg, D. A.; Hurst, M. A.; Fujii, C. A.; Kung, A. H.; Ho, J. F.; Cheng, F. C.; Loury, D. J.; Fiddes, J. C. Protegrin-1: a broad-spectrum, rapidly microbicidal peptide with in vivo activity. *Antimicrob. Agents Chemother.* **1997,** *41*, 1738-1742.

3. Yan, H.; Hancock, R. E. Synergistic interactions between mammalian antimicrobial defense peptides. *Antimicrob. Agents Chemother.* **2001,** *45*, 1558-1560.