Synthetic Mimics of Antimicrobial Peptides with Immunomodulatory Responses

Hitesh D. Thaker,[†] Abhigyan Som,[†,§] Furkan Ayaz,[§] Richard W. Scott,[#] Juan Anguita,[*,§] and Gregory N. Tew[*,†,§]

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

janguita@vasci.umass.edu; *tew@mail.pse.umass.edu*

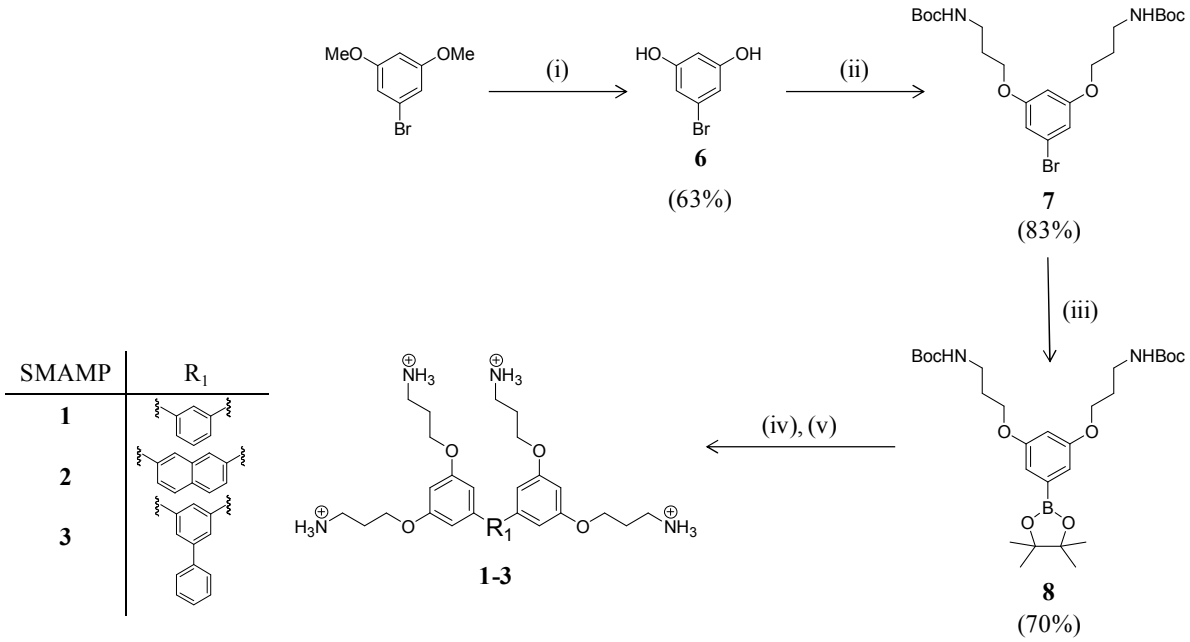
Supporting Information

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. Dibromonaphthalene was obtained from TCI America and 3,5-dibromo-1-phenyl benzene was purchased from ChemPacific corporation. 3-(Boc-amino)propyl bromide was purchased from Ace Synthesis, LLC. 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 aryl SMAMPs **1-3**. 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, $PdCl_2(dppf)\cdot CH_2Cl_2$, DMSO, KOAc, 80 °C, overnight; iv) Dibromoarene, $PdCl_2(dppf)\cdot CH_2Cl_2$, toluene, water, K_3PO_4, 100 ^oC, 20 h; v) TFA, DCM, RT, 3 h.

Synthesis of 5-bromobenzene-1,3-diol (6). In an oven dried round bottom flask, 1-bromo-3,5-dimethoxybenzene (8.00 g, 36.9 mmol) was added to 300 ml of dry DCM. The solution was cooled down to 0 °C in an ice-bath and BBr_3 (25.0 g, 100 mmol) was added dropwise. After 2 h the mixture was allowed to warm to room temperature and stirred overnight. Methanol (10 ml) was added dropwise to terminate the reaction. The mixture was poured into water and stirred for 2 h. Then 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%). ^1H 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 7. Compound **6** (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. The resulting mixture was stirred at 45 °C overnight. The mixture was cooled down to room temperature and poured into a mixture of 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-d6) 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 8. In an oven-dried round bottom flask, compound **7** (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. Then $PdCl_2(dppf)\cdot CH_2Cl_2$ (0.289 g, 0.357 mmol) was added. The resulting mixture was stirred at 80 °C overnight. The reaction was then cooled down to room temperature and the mixture was filtered through celite and extracted using ethyl acetate (100 ml). 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, *J* = 6.3 Hz, 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.

General procedure for Suzuki coupling.

In a 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 Schlenk tube was degassed by three freeze-pump-thaw cycles, purged with nitrogen, and then the mixture was 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.

Note: In some cases, the Suzuki intermediate obtained after the column contained an impurity that could be attributed to the boronic ester side product formed during the reaction (^{1}H NMR peak at 1.23 ppm in $CDCl_3$). This impurity was removed during an additional precipitation step in the deprotection of the Boc groups. The overall yield of the two steps combined is reported.

General procedure for the deprotection of the Boc groups.

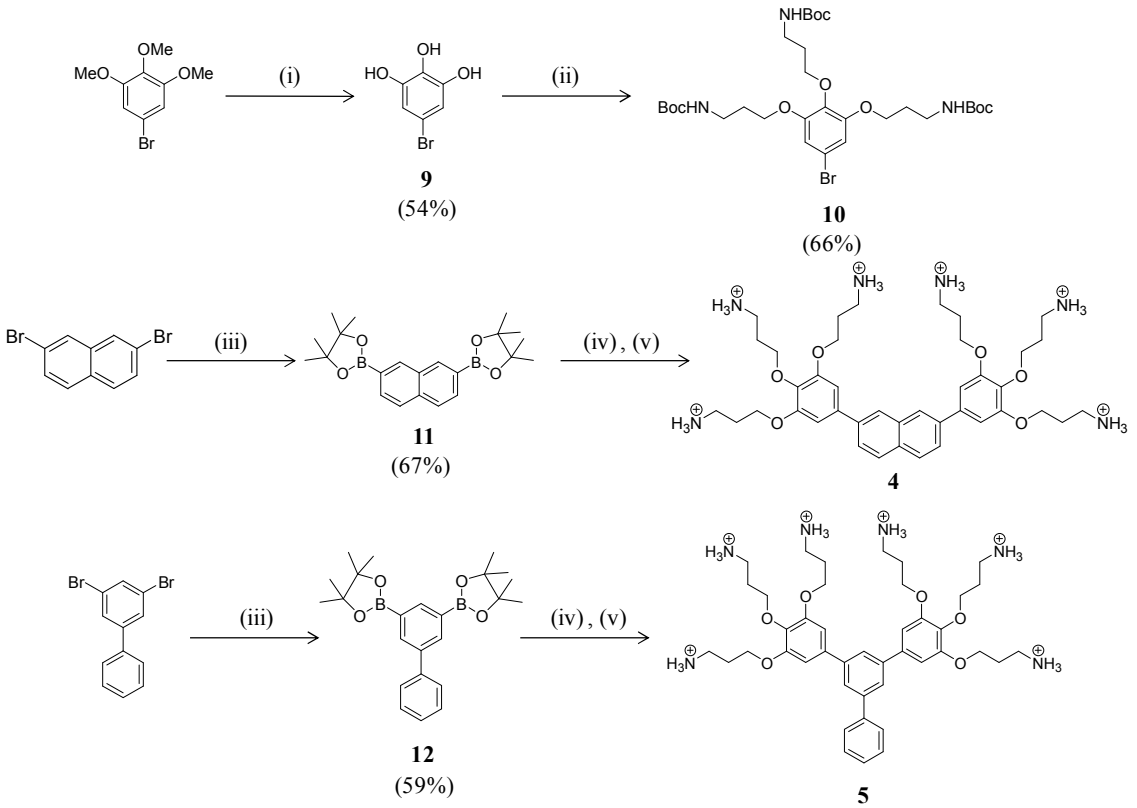
The Suzuki coupling intermediate (150 mg) was stirred in a mixture of TFA and DCM (1:1 v/v) for 3 h. The solution was concentrated and dried overnight under vacuum. The solid was then dissolved in a minimal amount of methanol and precipitated using a hexane/ether mixture (1:1 v/v). The mixture was centrifuged 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%.

Synthesis of Compound 1. Dibromobenzene (0.2 g, 0.84 mmol) was reacted with compound **8** (1.07 g, 1.95 mmol) according to the Suzuki coupling procedure (column chromatography using an ethyl acetate/hexanes mixture 2:3 v/v) followed by the deprotection procedure to give compound **1**. The overall yield of the two steps was 72% and the purity of the final product was >95%. ^{1}H NMR (300 MHz, CD_3OD) δ 7.74 (t, J = 1.8 Hz, 1H), 7.57 (m, 2H), 7.50 (m, 1H), 6.84 (d, J = 2.2 Hz, 4H), 6.59 (t, J = 2.2 Hz, 2H), 4.18 (t, J = 5.8 Hz, 8H), 3.18 (t, J = 7.3 Hz, 8H), 2.18 (m, 8H). ^{13}C NMR (75 MHz, CD_3OD) δ 161.52, 144.77, 142.93, 130.31, 127.55, 126.75, 107.42, 101.42, 38.57, 28.41. HR-MS *m/z*: calculated 523.3284, found 523.3227.

Synthesis of Compound 2. Dibromonapthalene (0.3 g, 1.05 mmol) was reacted with compound **8** (1.32g, 2.41 mmol) according to the Suzuki coupling procedure (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 67% and the purity of the final product was >95%. %. ^{1}H NMR (300 MHz, CD_3OD) δ 8.16 (d, J = 1.8 Hz, 2H), 7.96 (d, J = 8.6 Hz, 2H),

7.77 (dd, *J* = 1.8, 8.6 Hz, 1H), 6.98 (d, *J* = 2.1 Hz, 4H), 6.61 (t, *J* = 2.1 Hz, 2H), 4.21 (t, *J* = 5.8 Hz, 8H), 3.20 (t, *J* = 7.4 Hz, 8H), 2.19 (m, 8H). ^{13}C NMR (75 MHz, CD_3OD) δ 161.59, 144.62, 140.09, 135.30, 133.71, 129.26, 127.10, 126.69, 107.48, 101.59, 66.42, 38.61, 28.44. HR-MS *m/z*: calculated 573.3441, found 573.3405.

Synthesis of Compound 3. 1,3-dibromo-5-phenylbenzene (0.2 g, 0.64 mmol) was reacted with compound **8** (0.81 g, 1.47 mmol) according to the Suzuki coupling procedure (column chromatography using an ethyl acetate/hexanes mixture 2:3 v/v) followed by the deprotection procedure to give compound **3**. The overall yield of the two steps was 43% and the purity of the final product was >95%. ^{1}H NMR (300 MHz, CD_3OD) δ 7.77 (d, *J* = 1.6 Hz, 2H), 7.72 (m, 3H), 7.50 (m, 2H), 7.40 (m, 1H), 6.91 (d, *J* = 2.2 Hz, 4H), 6.62 (t, *J* = 2.2 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, CD_3OD) δ 161.45, 144.58, 143.65, 143.49, 142.07, 129.88, 128.65, 128.15, 126.18, 125.75, 107.41, 101.44, 66.31, 38.45, 28.29. HR-MS *m/z*: calculated 599.3597, found 599.3636.



Scheme S2. Synthesis of aryl SMAMPs **4** and **5**. i) BBr_3, DCM, -78 °C to RT, overnight; ii) 3-(Boc-amino)propyl bromide, K_2CO_3, DMF, 60 °C, overnight; iii) Bispinacolatodiboron,

$PdCl_2(dppf)\cdot CH_2Cl_2$, DMSO, KOAc, 80 ^oC, overnight; iv) Compound **10**, $PdCl_2(dppf)\cdot CH_2Cl_2$, Toluene, water, K_3PO_4, 100 ^oC, 20 h; v) TFA, DCM, RT, 3 h.

Synthesis of Compound 9. Compound **9** was synthesized according to a literature procedure.[1] 5-bromo-1,2,3-trimethoxybenzene (4.94 g, 20 mmol) was dissolved in CH_2Cl_2 or DCM (40 ml), and a CH_2Cl_2 solution of BBr_3 (1 M, 60 ml) was slowly added at –78 °C under nitrogen. The mixture was allowed to warm to 25 °C. After overnight stirring, the reaction mixture was poured into ice/water (200 mL) and extracted with ethyl acetate (100 ml x 3). The combined organic extract was washed with water, dried over anhydrous Na_2SO_4, and filtered off from an insoluble fraction. The filtrate was evaporated under a reduced pressure and purified using column chromatography using ethyl acetate/ DCM mixture (1:9 v/v) as the eluent. Yield = 2.21g (54%). ^1H NMR (300 MHz, DMSO-d_6) δ 9.26 (s, 2H), 8.34 (s, 1H), 6.40 (d, J = 0.7 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 147.19, 132.58, 109.71, 108.98. FAB-MS *m/z*: calculated 203.94, found 203.9

Synthesis of Compound 10. Compound **9** (1.6 g, 7.8 mmol) and potassium carbonate (8.83 g, 64 mmol) were stirred in DMF (30 ml) and water (3 ml) at room temperature for 30 min and then heated to 60 °C. 3-(Boc-amino) propyl bromide (8.34 g, 35.1 mmol) was added. The resulting mixture was stirred at 60 °C overnight. The mixture was cooled down to room temperature and poured into a mixture of ethyl acetate and water. The organic layer was washed with brine and dried over Na_2SO_4. The residue after concentration was purified with column chromatography using ethyl acetate/hexane (3:7 v/v) as the eluent. Yield = 3.5g (66%). ^1H NMR (300 MHz, $CDCl_3$) δ 6.70 (s, 2H), 5.34 (s, 1H), 5.12 (s, 2H), 4.01 (q, J = 5.4 Hz, 6H), 3.35 (m, 6H), 1.96 (m, 6H), 1.44 (s, 27H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.55, 155.52, 152.64, 135.86, 115.60, 109.29, 78.63, 78.52, 71.07, 66.63, 37.89, 37.46, 29.33, 29.02, 27.92, 27.87, 27.79. FAB-MS *m/z*: calculated 676.3, found 676.2.

Synthesis of Compound 11. In an oven-dried round bottom flask, dibromonapthalene (3 g, 10.49 mmol), bispinacolatodiboron (5.90 g, 23.08 mmol) and potassium acetate (5.14 g, 52.45 mmol) were stirred in DMSO (30 ml) at room temperature under N_2 protection. Then $PdCl_2(dppf)\cdot CH_2Cl_2$ (0.424 g, 0.525 mmol) was added. The resulting mixture was stirred at 80 °C overnight. Then the reaction was cooled down to room temperature. The mixture was filtered through celite and extracted with ethyl acetate (150 ml). The organic layer was washed with

water, brine, and then dried over Na_2SO_4 and purified using column chromatography using DCM/hexanes (1:9 v/v) as the eluent. Yield = 2.65 g (67%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.40 (d, *J* = 1.1 Hz, 2H), 7.85 (dd, *J* = 1.1, 8.2 Hz, 2H), 7.79 (m, 2H), 1.37 (s, 24H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 136.56, 136.02, 131.63, 130.97, 126.25, 83.32, 24.37. FAB-MS *m/z*: calculated 380.23, found 380.2.

Synthesis of Compound 12. In an oven-dried round bottom flask, 1,3-dibromo-5-phenylbenzene (1 g, 3.2 mmol), 4,4,5,5-tetramethyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (1.80 g, 7.05 mmol) and potassium acetate (1.57 g, 16 mmol) were stirred in DMSO (30 ml) at room temperature under N_2 protection. Then $PdCl_2(dppf)\cdot CH_2Cl_2$ (0.13 g, 0.16 mmol) was added. The resulting mixture was stirred at 80 °C overnight. Then the reaction was cooled down to room temperature. The mixture was filtered through celite and extracted with ethyl acetate (100 ml). The organic layer was washed with water, brine, and then dried over Na_2SO_4. The residue was purified using column chromatography using DCM/hexanes (1:4 v/v) as the eluent. Yield = 0.72 g (59%) ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.27 (m, 1H), 8.15 (q, *J* = 1.2 Hz, 2H), 7.67 (m, 2H), 7.42 (ddd, *J* = 1.3, 6.5, 7.5 Hz, 2H), 7.32 (m, 1H), 1.36 (s, 24H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 141.12, 140.27, 139.90, 136.47, 128.63, 127.45, 127.20, 83.92, 24.99. FAB-MS *m/z*: calculated 406.1, found 406.2.

Synthesis of Compound 4. Compound **11** (0.3 g, 0.79 mmol) and compound **10** (1.23 g, 1.815 mmol) were reacted according to the Suzuki coupling procedure (purified by column chromatography using ethyl acetate/hexane/DCM mixture - 1:4:4 v/v/v) followed by the deprotection of the Boc groups to give compound **4**. The overall yield of the two steps was 75% and the purity of the compound was >95%. ^{1}H NMR (300 MHz, CD_3OD) δ 8.17 (d, *J* = 1.7 Hz, 2H), 7.96 (d, *J* = 8.5 Hz, 2H), 7.78 (dd, *J* = 1.7, 8.5 Hz, 2H), 7.11 (s, 4H), 4.30 (t, *J* = 6.0 Hz, 8H), 4.18 (t, *J* = 5.7 Hz, 4H), 3.24 (m, 12H), 2.19 (m, 12H). ^{13}C NMR (75 MHz, CD_3OD) δ 153.84, 139.90, 138.92, 137.78, 135.33, 133.41, 129.26, 126.93, 126.58, 107.21, 72.35, 67.36, 39.31, 38.40, 29.04, 28.50. HR-MS *m/z*: calculated 719.4496, found 719.4512.

Synthesis of Compound 5. Compound **12** (0.25 g, 0.62 mmol) and compound **10** (0.96 g, 1.42 mmol) were reacted according to the Suzuki coupling procedure (purified by column chromatography using ethyl acetate/hexanes mixture - 2:3 v/v) followed by deprotection procedure to give compound **5**. The overall yield of the two steps was 76% and the purity of the

compound was >95%. ^{1}H NMR (300 MHz, CD_3OD) δ 7.72 (m, 5H), 7.49 (m, 2H), 7.41 (m, 1H), 7.03 (s, 4H), 4.26 (m, 8H), 4.16 (t, J = 5.6 Hz, 4H), 3.23 (m, 12H), 2.17 (m, 12H). ^{13}C NMR (75 MHz, CD_3OD) δ 153.61, 143.61, 143.43, 142.01, 138.99, 137.68, 129.76, 128.57, 128.13, 125.97, 125.80, 107.21, 72.08, 67.18, 39.05, 38.18, 28.84, 28.29. HR-MS *m/z*: calculated 745.4731, found 745.4625.

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. Minimal 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 (RBC) after their lysis. RBC 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 ^{o}C 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. Cell Culture and Immunomodulatory Assays

General: Ultra-purified LPS from *Salmonella enterica* subsp. *enterica* serovar Minnesota R595 (*S. minnesota*) was obtained from List Biological Laboratories, Inc. (Campbell, CA). Polymyxin B was purchased from SIGMA (Sigma Aldrich Inc., St. Louis, MO). RAW 264.7 cells (*Mus musculus*, RAW 264.7 gamma NO (-), CRL 2278) were obtained from ATCC (Manassas, VA). Recombinant mouse IL-10 was purchased from BD Biosciences (San Diego, CA). A 10 mg/ml stock solution of SMAMPs was prepared in DMSO (SIGMA) and diluted in Hank's Balanced Salt Solutions (HBSS, LONZA, Walkersville, MD) as necessary for the immunomodulatory assays.

Cell Culture: RAW 264.7 cells were cultured in complete RPMI 1640 media (ATCC) containing 10% FBS and 1% penicillin/streptomycin. Mouse bone marrow derived macrophages (BMDMs) were obtained following standard procedure.[4] In brief, mouse bone marrow cells were collected from the femoral shafts by flushing three times with 1 ml of cold complete RPMI 1640 supplemented with 20% L929-conditioned RPMI 1640. The cell suspensions were cultured in 100 × 15-mm petri dishes (Fisher Scientific, Pittsburgh, PA) in 20% L929-conditioned RPMI 1640 for 8 days at 37 °C with 5% CO_2. Following incubation, non-adherent cells were eliminated and adherent macrophages scraped, counted, and resuspended in complete RPMI 1640 media.

Immunomodulatory Assays: RAW 264.7 cells (1×10^6 cells/ml) or BMDMs (0.5×10^6 cells/ml) were exposed to a SMAMP solution(1.0 μg/ml or 5.0 μg/ml) or DMSO control (0.01% DMSO or 0.05% DMSO) for 1 h, and then challenged with LPS (100 ng/mL) or HBSS for 15-18 h. Tissue culture supernatants were assessed by ELISA (BD Biosciences, Mouse TNF ELISA Set II, Mouse IL-6 ELISA, Mouse IL-10 ELISA; R&D Systems, Mouse CXCL1/KC) as per the manufacturer's instructions.

Recombinant mouse IL-10 pre-treated assay. RAW 264.7 cells (1×10^6 cells/ml) were pre-treated with or without recombinant mouse IL-10 (50 ng/ml), then with SMAMPs (5.0 μg/ml) or DMSO control (0.05% DMSO) for 1 h, and stimulated with LPS (100 ng/ml) or HBSS for 18 h. The stimulation supernatants were analyzed for TNF by ELISA (BD Biosciences; Mouse TNF ELISA Set II) as per the manufacturer's instructions.

V. Supplementary Figures and Tables

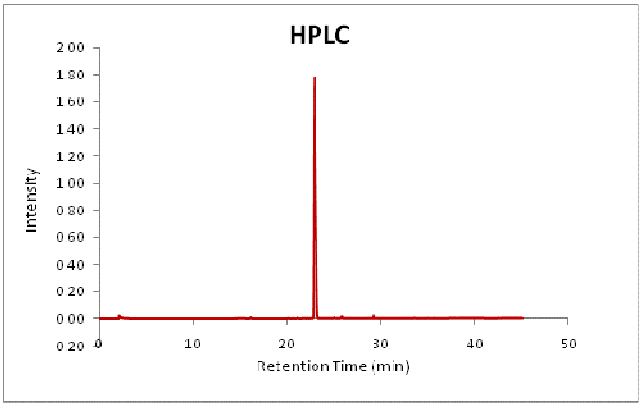


Figure S1. HPLC trace of SMAMP **1**.

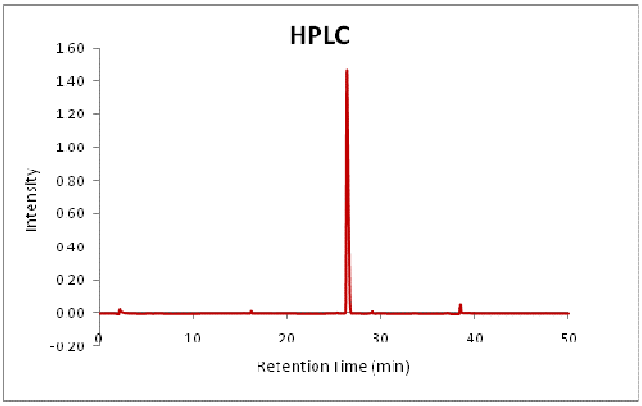


Figure S2. HPLC trace of SMAMP **2**.

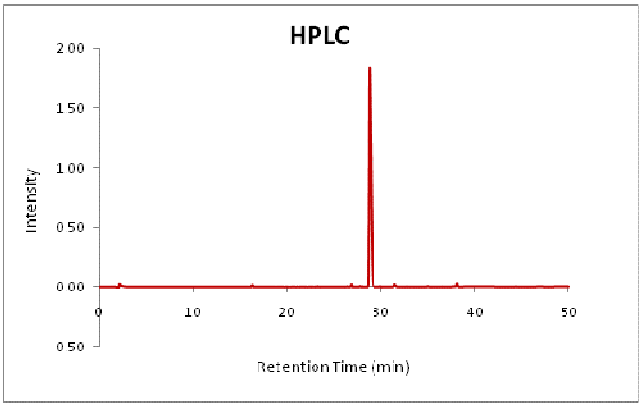


Figure S3. HPLC trace of SMAMP **3**.

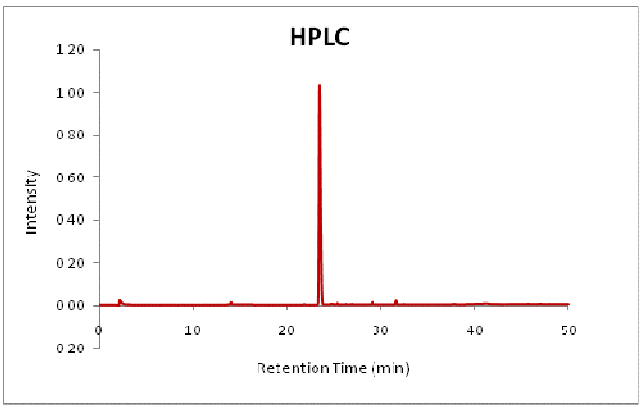


Figure S4. HPLC trace of SMAMP **4**.

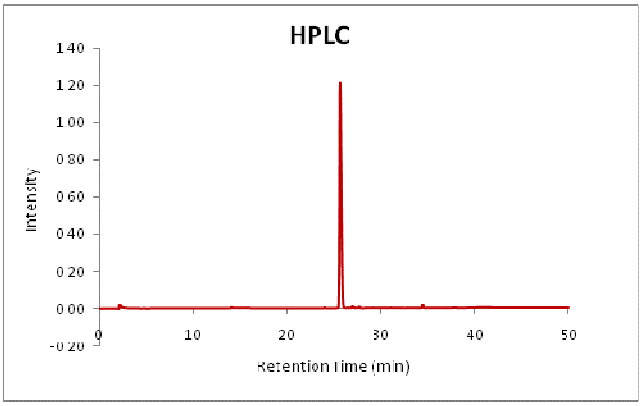


Figure S5. HPLC trace of SMAMP **5**.

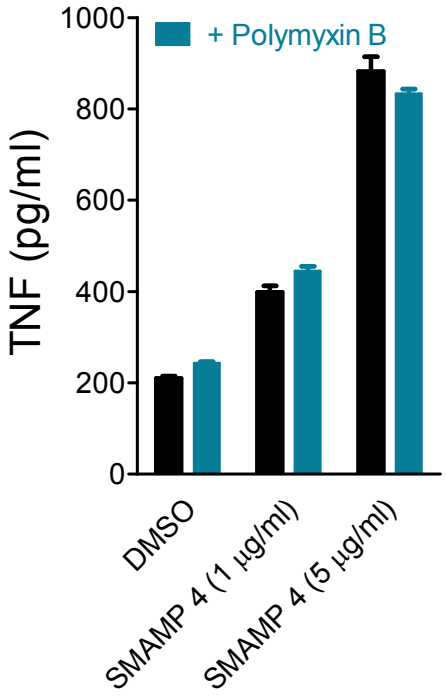


Figure S6. RAW 264.7 cells (1×10^6 cells/ml) were treated with or without 10 µg/ml Polymyxin B followed by incubation with SMAMP **4** (1.0 µg/ml or 5.0 µg/ml) or 0.05% DMSO for 18 h. The stimulation supernatants were analyzed for TNF production by ELISA. The data are presented as the average ± s.e.m. of triplicate samples.

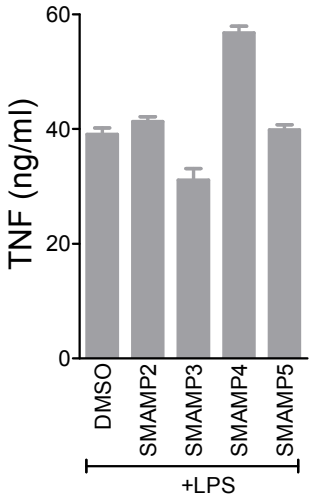
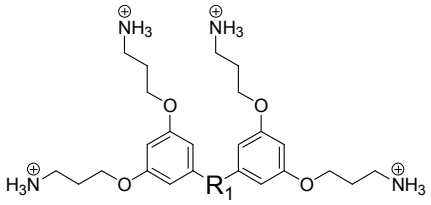
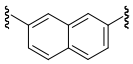
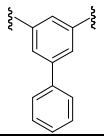


Figure S7. RAW 264.7 cells (1×10^6 cells/ml) were pre-incubated with SMAMPs (5.0 μg/ml) or 0.01% DMSO (denoted as DMSO in Figure) for 1 h and stimulated with LPS (100 ng/ml) for 18 h. The stimulation supernatants were analyzed for TNF. The data are presented as the average ± s.e.m. of triplicate samples.

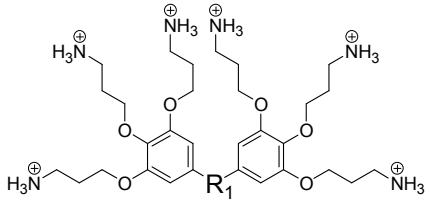
Table S1. Broad spectrum antibacterial activity and cytotoxicity of SMAMPs with 4 charges

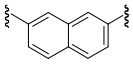
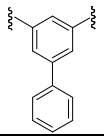


SMAMP	R_1	R_t[a] (min)	MIC (μg/ml)				3T3[b] (μg/ml)	HepG2[b] (μg/ml)	HC_{50} (μg/ml)
			SA	*EC*	*EF*	*KP*			
1		22.9	12.5	50	50	>50	814.3	>1000	>1000
2		26.4	12.5	25	6.25	50	n.d.	n.d.	195
3		28.8	12.5	3.13	1.56	12.5	240.6	426.1	537

[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*. n.d., not determined.

Table S2. Broad spectrum antibacterial activity and cytotoxicity of SMAMPs with 6 charges



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*			
4		23.5	3.13	3.13	12.5	25	165.4	291.8	656
5		25.7	6.25	6.25	>50	12.5	109.5	224.4	>1000

[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*.

VI. References

1. Li, W. S.; Yamamoto, Y.; Fukushima, T.; Saeki, A.; Seki, S.; Tagawa, S.; Masunaga, H.; Sasaki, S.; Takata, M.; Aida, T. *J. Am. Chem. Soc.* **2008,** *130*, 8886-8887.

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4. Zhang, X.; Goncalves, R.; Mosser, D. M. *Curr. Protoc. Immunol.* **2008,** *Chapter 14*, Unit 14 11.