Expedient Synthesis of SMAMPs via Click Chemistry

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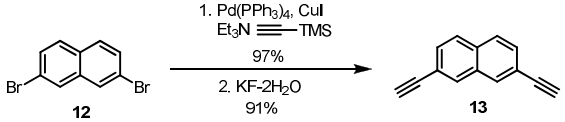
Supporting Information

I. General Methods

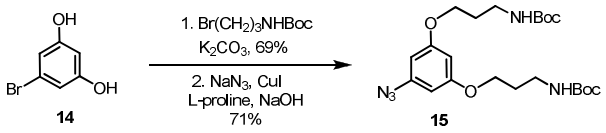
All the chemicals (reagent grade) were purchased from Aldrich, VWR, Acros, or Fisher and used as received unless otherwise indicated. CuI was purified according to literature procedure.[1] Dichloromethane (CH_2Cl_2)and triethylamine (TEA) were distilled over CaH_2 under nitrogen prior to use. Tetrahydrofuran (THF) was distilled from sodium/benzophenone. All reactions involving air or moisture sensitive reagents or intermediates were performed under an inert atmosphere of nitrogen in glassware that was flame or oven-dried. Reaction temperatures refer to the temperature of the cooling or heating bath. Volatile solvents were removed under reduced pressure using a Büchi rotary evaporator at 25–30 °C (bath temperature). Column chromatography was performed using pressurized air and the indicated solvent system on 230-400 mesh silica gel (Silicycle flash F60) according to the method of Still,[2] unless otherwise noted. ^{1}H NMR spectra were obtained at 300 MHz using a Bruker DPX-300 and ^{13}C NMR spectra were obtained at 100 MHz using a Avance-400 NMR spectrometer. Chemical shifts (δ) are reported in ppm and coupling constants (J) in Hz. The abbreviations for splitting patterns are the following: s, singlet; br s, broad singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; m, multiplet. Mass spectral data including results from high resolution mass spectrometry (HRMS) were obtained at the University of Massachusetts, Mass Spectrometry Facility. Analytical HPLC was carried out on a Waters system using an Agilent Zorbax SB-C_8, 80 Å, 4.6 mm x 150 mm ID (5 μm) column, eluted by water and acetonitrile, both containing 0.1% of TFA. Detection was by UV detector at 254 nm wavelength. The elution was performed by gradually increasing the ratio of acetonitrile in water by 1%/min, starting with 100% water, with a flow rate of 1 mL/min. The purity of the final compounds, as determined by analytical HPLC, were all greater than 95%.

II. Representative Synthetic Procedure (SMAMP **7**)

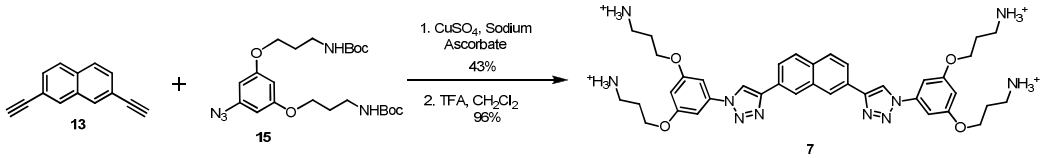


2,7-Diethynylnaphthalene (13). Trimethylsilylacetylene (2.7 g, 3.9 mL, 28 mmol), $Pd(PPh_3)_4$ (0.40 g, 0.35 mmol), and CuI (66 mg, 0.35 mmol) were added sequentially to a solution of dibromide **12** (1.0 g, 3.5 mmol) and triethylamine (12 mL) in THF (40 mL) at room temperature.

The reaction mixture was wrapped in aluminum foil and heated at 65 ºC for 24 h. After cooling to room temperature, the solvent was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (100 mL) and washed with 1 M citric acid (10 mL), saturated aqueous $NaHCO_3$ (10 mL), and brine (10 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The resulting residue was purified by column chromatography, eluting with hexanes to give 1.1 g (97%) of the intermediate silane as a white solid. KF-$2H_2O$ (0.75 g, 8.0 mmol) was added to a solution of the intermediate silane (0.51 g, 1.6 mmol) in THF/MeOH (1:1, 10 mL) at room temperature, and the solution was stirred for 2 h. After removal of the solvent, the residue was dissolved in ethyl acetate and washed with water. The organic layer was dried ($MgSO_4$) and evaporated under reduced pressure to give 0.31 g of **13** (91%) as a reddish solid.



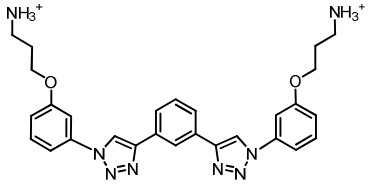
***tert*-Butyl 3,3'-(5-azido-1,3-phenylene)bis(oxy)bis(propane-3,1-diyl)dicarbamate (15)**. K_2CO_3 (7.4 g, 53 mmol) was added in one portion to a solution of **14** (2.0 g, 11 mmol) in DMF (19 mL) and water (2.0 mL) at room temperature. The suspension was stirred for 20 min, and then gradually heated to 45 ºC over 20 min. 3-(Boc-amino)propyl bromide (8.1 g, 34 mmol) was added in one portion and the resulting mixture was stirred for 29 h. After cooling to room temperature, water (40 mL) was added and then extracted with Et_2O (3 x 40 mL). The organic layers were washed with water (5 x 40 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by column chromatography, eluting with ethyl acetate/hexanes (1/4→1/2) to give 3.7 g (69%) of the intermediate bromide as a white foam, which turned into a white solid upon standing at room temperature overnight. The intermediate bromide (0.5 g, 1.0 mmol) was added to a suspension of NaN_3 (0.13 g, 2.0 mmol), CuI (19 mg, 0.10 mmol), L-proline (34 mg, 0.29 mmol), and NaOH (11 mg, 0.29 mmol) in ethanol/water (7:3, 2.0 mL) at room temperature. The suspension was heated at 95 ºC for 30 h and cooled to room temperature. After the addition of water (3 mL), the mixture was extracted with CH_2Cl_2 (3 x 12 mL). The organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified with column chromatography, eluting with ethyl acetate/hexanes (1/3→1/1) to give 0.33 g (71%) of **15** as a light yellow oil.



SMAMP 7. $CuSO_4$-$5H_2O$ (40 mg, 0.16 mmol) was added to a vigorously stirred suspension of **13** (93 mg, 0.53 mmol), **15** (0.51 g, 1.1 mmol), and sodium ascorbate (32 mg, 0.16 mmol) in *t*-BuOH (8 mL) and water (2 mL) at room temperature. The mixture was stirred for 16 h. Water (10 mL) was added and the mixture was extracted with ethyl acetate (3 x 30 mL). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography, eluting with ethyl acetate/hexanes (1/3→1/1) to give 250 mg (43%) of **16** and 140 mg (41%) of a product in which **13** had only reacted with one equivalent of **15.**

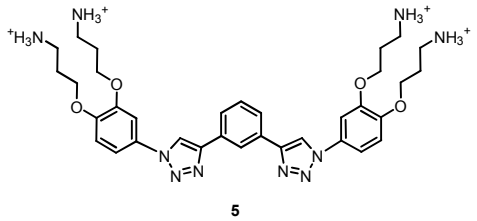
Trifluoroacetic acid (2.5 mL) was added to a solution of **16** (230 mg, 0.20 mmol) in CH_2Cl_2 (8.0 mL) at room temperature, and the resulting solution was stirred for 3 h. The solvent was evaporated under reduced pressure. The residue was then dissolved in a minimal amount of methanol and precipitated 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 136 mg (96%) of **7** as its TFA salt.

III. SMAMP Characterization



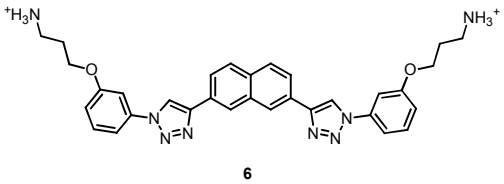
4

^{1}H NMR (300 MHz, DMSO-d_6) δ 9.45 (s, 2 H), 8.61 (s, 1 H), 7.95 (dd, J = 7.7, 1.3 Hz, 2 H), 7.87 (br s, 6 H), 7.69–7.51 (m, 7 H), 7.15–7.07 (m, 2 H), 4.21 (t, J = 6.0 Hz, 4 H), 3.02 (d, J = 6.0 Hz, 4 H), 2.13 – 2.01 (m, 4 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.4, 147.2, 137.8, 131.1, 131.1, 130.0, 125.4, 122.3, 120.2, 115.1, 112. 3, 106.4, 65.3, 36.5, 26.9. HRMS *m/z*: calculated 511.2570, found 511.2578.



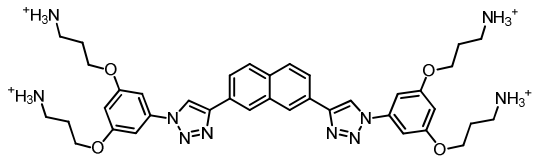
5

^{1}H NMR (300 MHz, CD_3OD) δ 9.03 (s, 2 H), 8.57 (s, 1 H), 7.95 (dd, J = 7.8, 1.7 Hz, 2 H), 7.62 (t, J = 7.8 Hz, 1 H), 7.21 (d, J = 2.2 Hz, 4 H), 6.72 (t, J = 2.1 Hz, 2 H), 4.24 (t, J = 5.8 Hz, 8 H), 3.20 (t, J = 7.3 Hz, 8 H), 2.29–2.13 (m, 8 H). ^{13}C NMR (101 MHz, CD_3OD) δ 161.8, 148.9, 139.5, 131.9, 130.7, 126.7, 123.7, 120.4, 102.5, 100.4, 66.7, 38.4, 28.2. HRMS *m/z*: calculated 657.3625, found 657.3596.



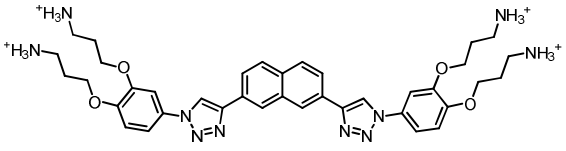
6

^{1}H NMR (300 MHz, CD_3OD) δ 9.09 (s, 2 H), 8.54 (s, 2 H), 8.10 – 8.00 (m, 4 H), 7.63 (s, 2 H), 7.57 (d, J = 5.2 Hz, 4 H), 7.19 – 7.10 (m, 2 H), 4.28 (t, J = 5.8 Hz, 4 H), 3.23 (t, J = 7.2 Hz, 4 H), 2.24 (dt, J = 12.8, 6.3 Hz, 4 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.5, 147.4, 137.8, 132.6, 131.1, 128.8, 128.6, 124.2, 120.2, 115.0, 112.3, 106.3, 65.4, 36.6, 27.0. HRMS *m/z*: calculated 561.2726, found 561.2696.



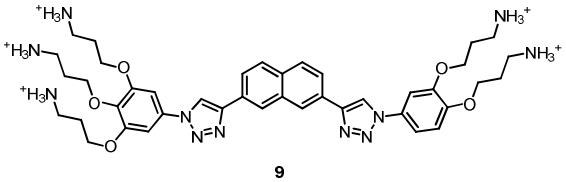
7

^{1}H NMR (300 MHz, CD_3OD) δ 9.11 (s, 2 H), 8.56 (s, 2 H), 8.11 – 8.00 (m, 4 H), 7.23 (d, *J* = 2.1 Hz, 4 H), 6.72 (t, *J* = 2.1 Hz, 2 H), 4.25 (t, *J* = 5.8 Hz, 8 H), 3.21 (t, *J* = 7.3 Hz, 8 H), 2.27 – 2.15 (m, 8 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.4, 147.2, 138.2, 133.4, 132.5, 128.8, 128.5, 124.1, 124.0, 120.3, 101.4, 99.0, 65.4, 36.3, 36.3, 26.8, 26.8. HRMS *m/z*: calculated 707.3782, found 707.3748.



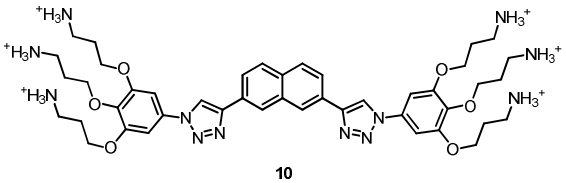
8

^{1}H NMR (300 MHz, CD_3OD) δ 8.99 (s, 2 H), 8.42 (s, 2 H), 7.95 (q, *J* = 8.5 Hz, 4 H), 7.13 (s, 4 H), 6.61 (s, 2 H), 4.18 – 4.13 (m, 8 H), 3.18 – 3.12 (m, 8 H), 2.24 – 2.06 (m, 8 H). ^{13}C NMR (101 MHz, CD_3OD) δ 162.0, 149.3, 139.8, 135.1, 134.6, 129.8, 129.5, 125.7, 125.3, 120.8, 102.7, 100.7, 66.8, 38.5, 28.3. HRMS *m/z*: calculated 707.3782, found 707.3773.



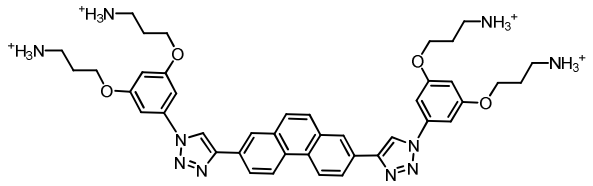
9

^{1}H NMR (300 MHz, CD_3OD) δ 8.95 (d, *J* = 8.8 Hz, 2 H), 8.34 (s, 2 H), 7.89 (dd, *J* = 15.6, 8.3 Hz, 4 H), 7.20 (s, 2 H), 7.08 (s, 2 H), 6.56 (s, 1 H), 4.22 – 4.08 (m, 10 H), 3.24 – 3.12 (m, 10 H), 2.36 – 2.07 (m, 10 H). ^{13}C NMR (101 MHz, CD_3OD) δ 161.9, 154.2, 149.3, 139.7, 138.3, 135.0, 134.4, 131.5, 129.7, 129.4, 125.6, 125.2, 120.8, 102.6, 100.5, 72.3, 67.6, 66.7, 39.1, 38.4, 38.3, 29.1, 28.4, 28.3. HRMS *m/z*: calculated 780.4309, found 780.4351.



10

^{1}H NMR (300 MHz, CD_3OD) δ 9.11 (s, 2 H), 8.54 (s, 2 H), 8.04 (s, 4 H), 7.35 (s, 4 H), 4.31–4.15 (m, 14 H), 3.24–3.17 (m, 8 H), 2.37–2.03 (m, 14 H). ^{13}C NMR (101 MHz, CD_3OD) δ 154.2, 149.3, 138.3, 135.1, 134.5, 129.8, 129.5, 125.7, 125.3, 121.0, 119.6, 116.7, 100.6, 72.3, 67.6, 39.1, 38.3, 29.1, 28.4. HRMS *m/z*: calculated 852.475, found 852.415.



11

^{1}H NMR (300 MHz, CD_3OD) δ 8.93 (s, 2 H), 8.71 (d, *J* = 8.7 Hz, 2 H), 8.31 (s, 2 H), 8.09 (d, *J* = 8.1 Hz, 2 H), 7.79 (s, 2 H), 7.11 (s, 4 H), 6.59 (s, 2 H), 4.20 – 4.15 (m, 8 H), 3.21 – 3.15 (m, 8 H), 2.22 – 2.15 (m, 8 H). ^{13}C NMR (101 MHz, CD_3OD) δ 161.5, 148.6, 139.2, 133.2, 130.7, 128.6, 128.3, 125.5, 124.5, 124.2, 119.5, 101.8, 99.7, 66.6, 38.4, 28.3. HRMS *m/z*: calculated 756.348, found 756.386.

IV. HPLC Chromatograms of SMAMPs **4-11**

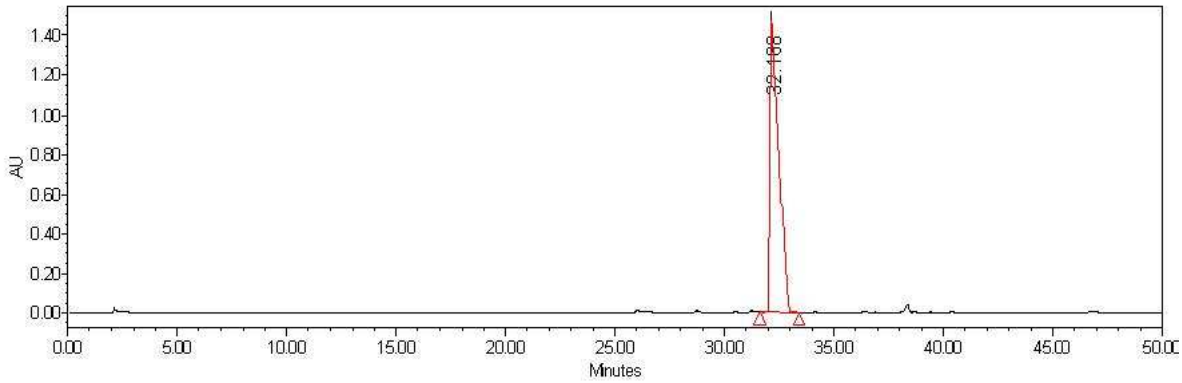


Figure S1 HPLC Chromatogram of SMAMP **4**

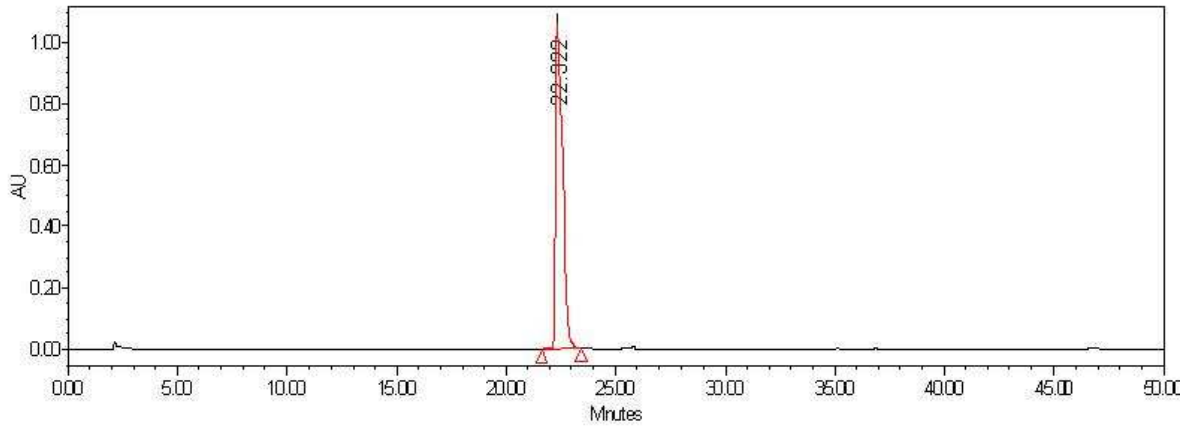


Figure S2 HPLC Chromatogram of SMAMP **5**

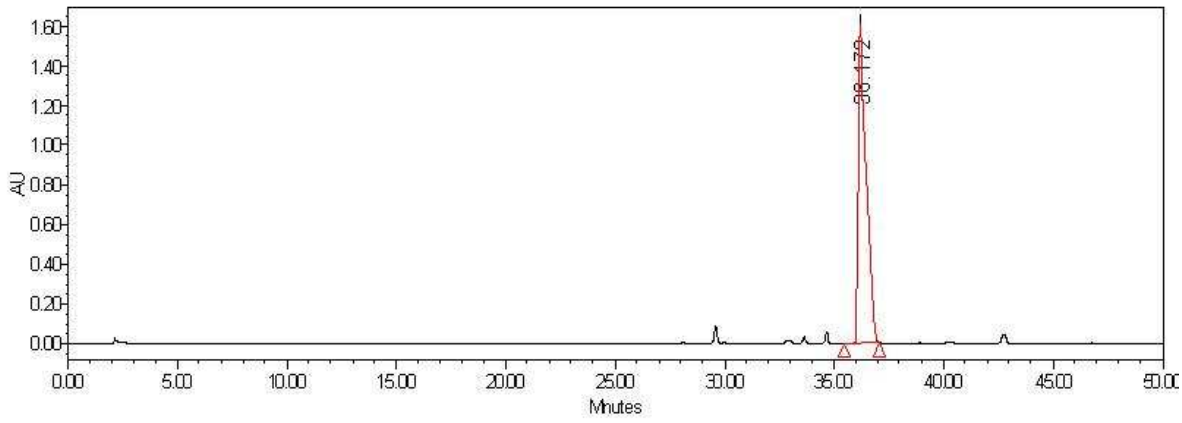


Figure S3 HPLC Chromatogram of SMAMP **6**

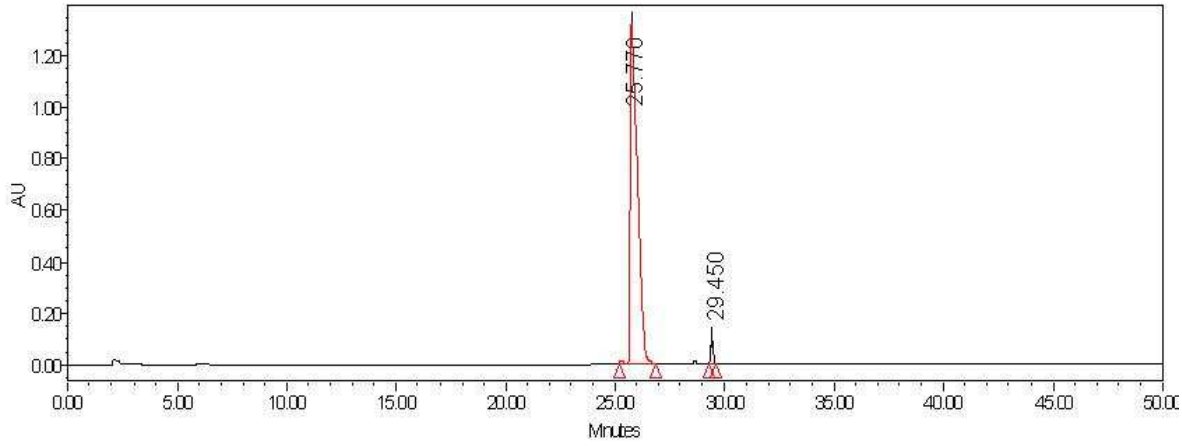


Figure S4 HPLC Chromatogram of SMAMP **7**

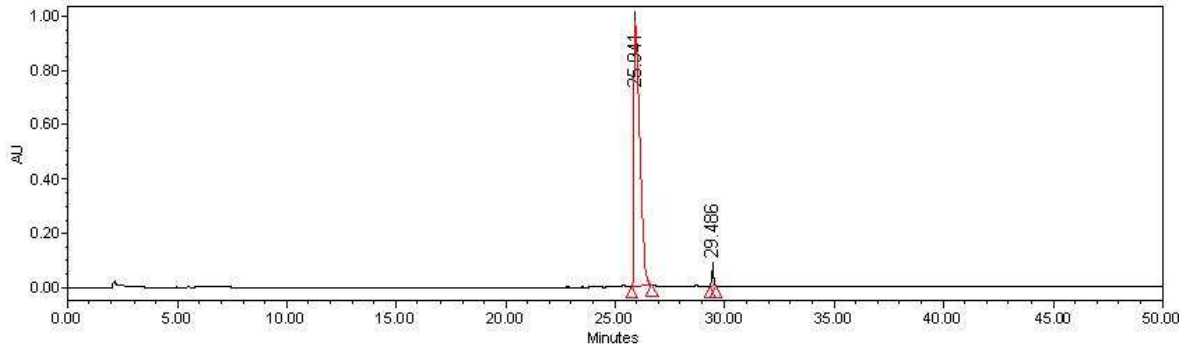


Figure S5 HPLC Chromatogram of SMAMP **8**

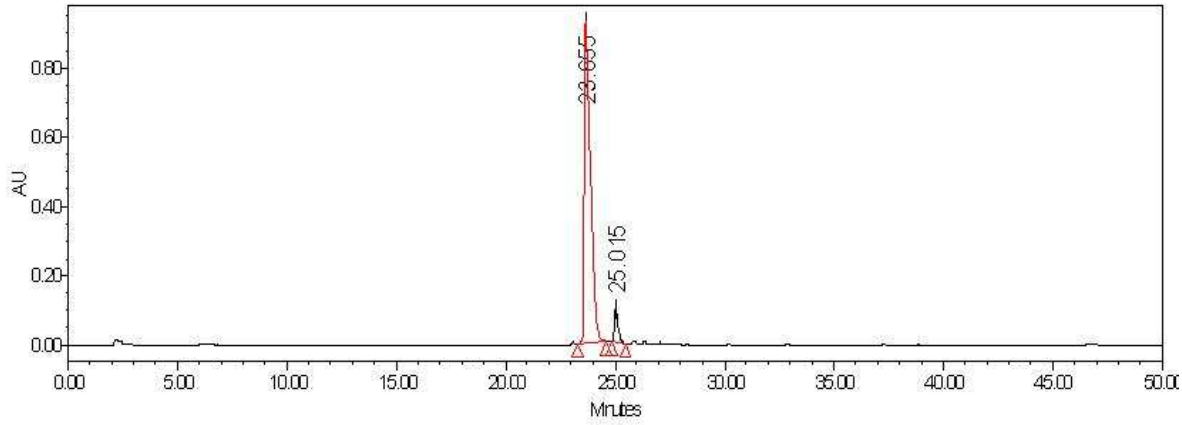


Figure S6 HPLC Chromatogram of SMAMP **9**

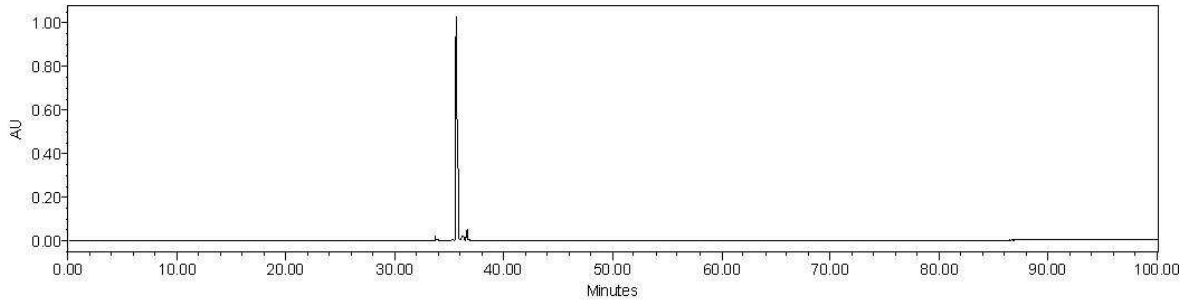


Figure S7 HPLC Chromatogram of SMAMP **10**

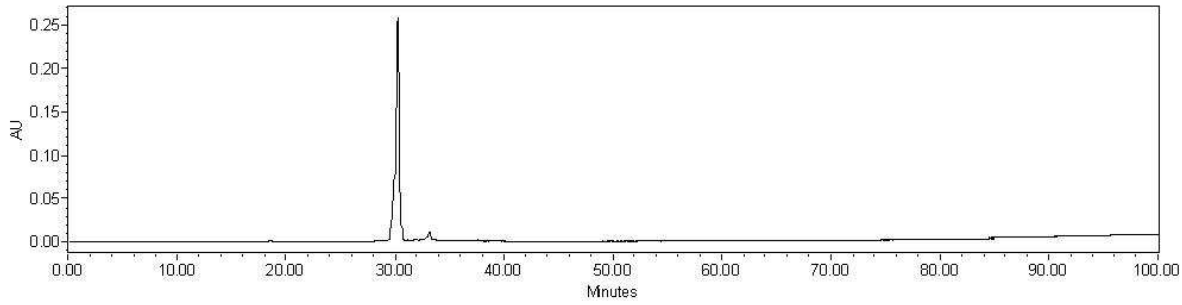


Figure S8 HPLC Chromatogram of SMAMP **11**

V. 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.[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 ºC

overnight, and the bacterial growth was measured by turbidity as the optical density at $\lambda = 600$ nm (OD) using an Eppendorf BioPhotometer. The bacterial strain was diluted to a working solution of 10^6 colony forming units per ml ($OD_{600} = 0.001$ for studies without serum, $OD_{600} = 0.002$ for studies in the presence of serum). 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.

For measuring antimicrobial activity in the absence of serum:
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 ºC.

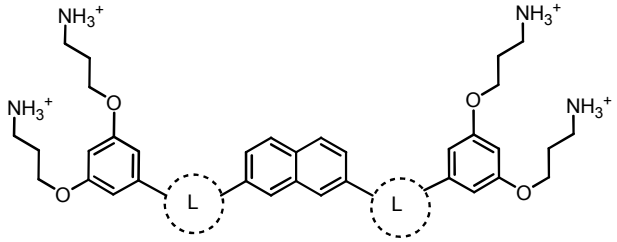
For measuring antimicrobial activity in the presence of serum:
10 μL of the dilutions were added to each corresponding well of a 96-well round bottom polypropylene plate along with 40 μL of mouse serum (non-medicated, non-immunized, unfiltered, female CD-1) to the respective wells in duplicate. After incubation for 30 min at 37 ºC, 50 μL of the diluted bacterial strain was added. The plate was incubated for 18 h at 37 ºC and MICs were obtained by measuring cell growth at OD_{600}.

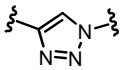
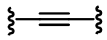
Each compound was tested as the TFA salt against ATCC bacterial strains (E. coli 25922, S. aureus 27660, E. faecalis 29212, P. aeruginosa 10145, and K. pneumoniae 13883).

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 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.

VI. Supplementary Tables

Table S1 Antimicrobial and HemolyticActivity of SMAMPs **7, 17, 18**



SMAMP	L	MIC(μg/mL)				HC_{50} (μg/mL)
		SA[a]	*SA+40%MS*[b]	*EC*[c]	*EC+40%MS*[d]	
7		12.5	0.39	>50	6.25	693.4
17	None	12.5	1.56-3.13	25	25	194.9
18		3.13	3.13	3.13	12.5	3.4

[a] *S. aureus (ATCC 27660)*

[b] *S. aureus (ATCC 27660)* in the presence of 40% mouse serum

[c] *E. coli (ATCC 25922)*

[d] *E. coli (ATCC 25922)* in the presence of 40% mouse serum

VII. References

1. Dieter, R. K.; Silks, L. A.; Fishpaugh, J. R.; Kastner, M. E. *J. Am. Chem. Soc.* **1985,** *107,* 4679-4692.
2. Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923-2925.
3. (a) Steinberg, D. A.; Hurst, M. A.; Fujii, C. A.; Kung, A. H.; Ho, J. F.; Cheng, F. C.; Loury, D. J.; Fiddes, J. C. *Antimicrob. Agents Chemother.* **1997**, *41*, 1738-1742. (b) Yan, H.; Hancock, R. E. *Antimicrob. Agents Chemother.* **2001**, *45*, 1558-1560.