Design and Synthesis of Macrocyclic Peptomers as Mimics of a Quorum Sensing Signal from *Staphylococcus aureus*

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Supporting Information.

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General experimental information.

Analytical and preparative HPLC were performed using a Shimadzu system equipped with a UV/vis detector (SCL-10Avp controller, LC-6AD pumps, SPD-10Avp UV/vis detector). Purities were determined by integration of peaks at 220 nm. A C18-silica reverse-phase analytical column (Shimadzu Premier; 5 μm, 250 x 4.6 mm) was used for routine analysis (conditions: flow rate = 1.0 mL/min; mobile phase A = 0.1% TFA in water; mobile phase B = 0.1% TFA in acetonitrile). A C18-silica reverse-phase preparative column (Vydac; 10 μm, 250 x 25 mm) was used for preparative work (conditions: flow rate = 9.0 mL/min; mobile phase A = 0.1% TFA in water; mobile phase B = 0.1% TFA in acetonitrile).

LC-MS data were obtained using a Shimadzu LCMS-2010 equipped with two pumps (LC-10ADvp), controller (SCL-10Avp), autoinjector (SIL-10ADvp), UV diode array detector (SPD-M10Avp), and single quadrupole analyzer (by electrospray ionization (ESI)). A C18-silica wide-pore reverse-phase analytical column (Supelco; 5 μm, 150 × 2.1 mm) was used for all LC-MS work (conditions: flow rate = 200 μL/min; mobile phase A = 0.4% formic acid in water; mobile phase B = 0.2% formic acid in acetonitrile).

Matrix-assisted laser desorption/ionization (MALDI) MS data were obtained using a Bruker REFLEX II equipped with a 337 nm laser, a reflectron, and delayed extraction. In positive ion mode, the acceleration voltage was 25 kV.

All chemical reagents and solvents were purchased from commercial sources (Aldrich, Advanced ChemTech, Acros, J. T. Baker, and Novabiochem) and used as is, with the exception of dichloromethane (CH_2Cl_2), which was distilled over calcium hydride immediately prior to use.

Wang-linker derivatized polystyrene resin (100–200 mesh, loading = 1.0 mmol/g; Novabiochem) was used for all solid-phase syntheses. When necessary, solvent was removed from samples post-resin cleavage using a Thermosavant SC250 Express SpeedVac equipped with an OFP-400 vacuum pump and a RVT4104 refrigerated vapor trap.

Microwave instrumentation and methods.

Microwave (μW)-assisted reactions were performed in either a Milestone MicroSYNTH Labstation multimodal μW synthesis reactor (1000 W max)[1] or a CEM Focused μW Discover monomodal synthesis system (300 W max).[2] All μW-assisted solid-phase reactions in the Milestone reactor were performed under temperature control as described previously.[3] All μW-assisted solid-phase reactions in the CEM reactor were performed under temperature control with stirring in CEM 10 mL glass reaction vessels fitted with CEM septa.

Computational modeling protocol.

Molecular modeling experiments to generate visual overlays (shown in Figure 4 in the text) and fit scores were performed using the MOE software suite (v. 2006.08; Chemical Computing Group of Canada).[4] A Flexible Alignment conformational search was performed that compared peptide **2** to the proposed peptomer **3** or **4** (using the default parameters for Flexible Alignment in MOE). The same calculation was performed for peptide **2** onto itself, and this fit score value was set as 100% alignment. The reported fit values for peptide **2** onto peptomer **3** (95.4%) and **2** onto peptomer **4** (97.3%) are relative to the fit value of peptide **2** onto itself.

Peptomer synthesis methods.

Representative procedure for macrocyclic peptomer synthesis. Wang resin (300 mg, loading = 1.0 mmol/g) was swelled in $CHCl_3$ (2 mL). A solution of 0.28 g Fmoc-L-Ala-OH (0.90 mmol, 3 equiv.), 0.16 g 1-hydroxybenzotriazole (HOBt, 1.20 mmol, 4 equiv.), 0.23 mL diisopropylcarbodiimide (DIC, 1.50 mmol, 5 equiv.), 0.26 mL diisopropylethylamine (DIPEA, 1.50 mmol, 5 equiv.), and 0.001 g 4-(dimethylamino)pyridine (DMAP, 0.008 mmol, 0.027 equiv.) in dimethylformamide (DMF, 1 mL) was added to the pre-swelled resin in $CHCl_3$. The reaction mixture was heated with stirring in the CEM μW reactor at 60 °C for 10 min. The reaction mixture was filtered, and the resin was washed with DMF (3 x 1 mL), EtOH (3 x 1 mL), CH_2Cl_2 (3 x 1 mL), and $CHCl_3$ (1 x 1 mL), and drained. The resin was swelled again in $CHCl_3$ (2 mL), and this Fmoc-L-Ala-OH coupling procedure was repeated. Following an analogous washing procedure, the resin was dried overnight under high vacuum. UV Fmoc quantification (as determined by basic deprotection of a small portion of beads) revealed that the resin loading of Fmoc-Ala-OH was 0.65 mmol/g.[5]

The Fmoc-L-Ala-loaded resin (0.65 mmol/g) was deprotected by soaking the resin in a 4% 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)/DMF solution at rt (2 x 1 mL, 10 min each), after which the resin was washed with DMF (5 x 1 mL) and drained. Two peptoid residues were coupled to the L-Ala-loaded resin (**5**) using our previously reported, μW-assisted peptoid submonomer synthesis method.[3] To 100 mg resin was added a solution of 0.18 g bromoacetic acid (1.36 mmol, 20 equiv.) and 0.20 mL DIC (1.36 mmol, 20 equiv.) in DMF (1 mL). The reaction mixture was heated with stirring in the Milestone μW reactor to 35 °C in 30 s. The reaction mixture was filtered, and the resin was washed with DMF (5 x 1 mL) and drained. A solution of *sec*-butylamine ("sb", 1.36 mmol, 20 equiv.) in DMF (1 mL) was added to the resin, and the mixture was heated with stirring in the Milestone μW reactor to 95 °C in 90 s. The reaction mixture was filtered again, and the resin was washed with DMF (5 x 1 mL), and drained. This sequence was repeated for the coupling of the second peptoid residue (to incorporate benzyl amine, "pm").

Next, two β-peptide residues were coupled to the resin-bound trimer. The resin was swelled in $CHCl_3$ (1 mL). A solution of 0.06 g Fmoc-β-Ala-OH (0.20 mmol, 3 equiv.) and 0.05 mL DIC (0.34 mmol, 5 equiv.) in DMF (1 mL) were added to the pre-swelled resin in $CHCl_3$. The reaction mixture was heated with stirring in the Milestone μW at 60 °C for 10 min (with a 30 sec ramp). The resin was washed with DMF (2 x 1 mL) and $CHCl_3$ (1 x 1 mL), and drained. This

Fmoc-β-Ala-OH coupling procedure was repeated. Thereafter, the reaction mixture was filtered, and the resin was washed with DMF (3 x 1 mL) and CH_2Cl_2 (3 x 1 mL), and drained.

At this point in the synthesis (*i.e.*, tetramer **6**), it was possible to determine the efficiency of the two peptoid couplings and the one Fmoc-β-Ala-OH coupling using UV Fmoc quantification;[5] this was performed in test cases and was found to be ~75% efficient. To cap any unprotected amines (*i.e.*, truncation sequences in which Fmoc-β-Ala-OH had failed to couple), the resin was acetylated by addition of a solution of 0.12 mL acetic anhydride (1.36 mmol, 20 equiv.) and 0.11 mL DIPEA (0.68 mmol, 10 equiv.) in CH_2Cl_2 (1 mL). The reaction mixture was allowed to stir for 20 min at rt. The reaction mixture was filtered, and the resin was washed with DMF (3 x 1 mL) and CH_2Cl_2 (3 x 1 mL), and drained.

Following this "capping" procedure, Fmoc deprotection of the resin-bound tetramer (**6**) was performed by soaking the resin in a 4% DBU/DMF solution at room temperature (2 x 1 mL, 10 min each). The reaction mixture was filtered, and the resin was washed with DMF (3 x 1 mL), CH_2Cl_2 (3 x 1 mL), and $CHCl_3$ (1 x 1 mL), and drained. The second Fmoc-β-Ala-OH residue was coupled to the resin using the same coupling procedure, followed by a second capping step, to yield resin-bound pentamer **7**. UV Fmoc quantification of test reactions showed 85% efficiency for the coupling of this second Fmoc-β-Ala-OH residue.[5]

Cyclization-cleavage of peptomer **7** was performed by adding 0.008 mL piperidine (0.08 mmol, 2 equiv.) to 100 mg of resin (0.44 mmol/g) in DMF (1 mL). The reaction mixture was allowed to stir for 24 h at rt. The beads were filtered, and the filtrate was isolated. The resin was washed with DMF (1 x 0.25 mL), H_2O (1 x 0.25 mL), CH_2Cl_2 (1 x 0.25 mL), and CH_3CN (1 x 0.25 mL). The filtrate and resin washes were combined, and the solutions were concentrated *in vacuo* using a SpeedVac at 40 °C to yield crude macrocyclic peptomer **4** as a pale yellow solid.

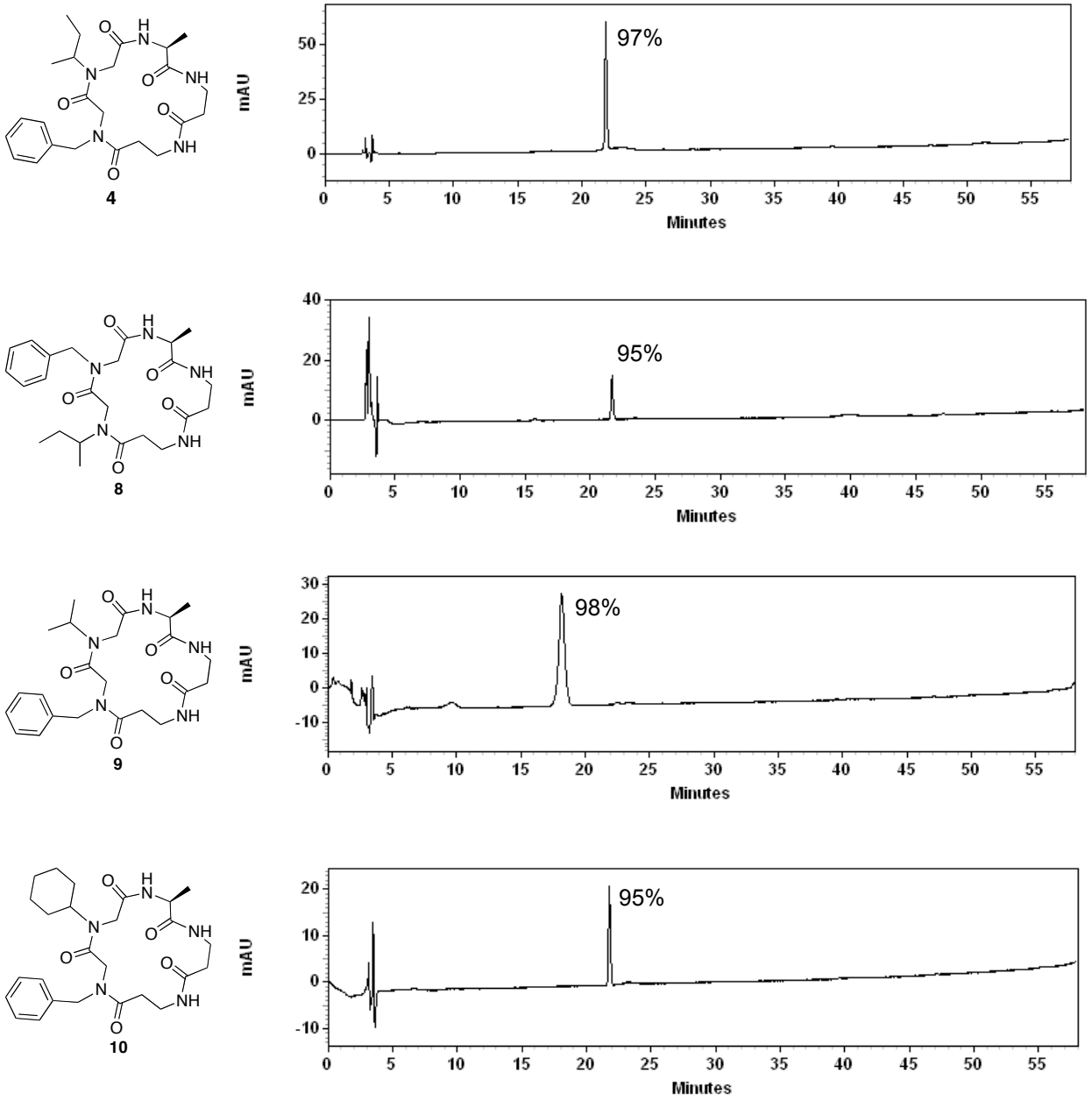
LC-MS analyses were performed on peptomer samples throughout the synthesis optimization process to gauge the extent of reaction and product purity (gradient elution: 10–60% solvent B in solvent A over 25 min).

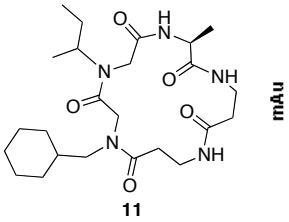
Peptomer library synthesis. Peptomer library synthesis was performed in parallel on a 30 mg resin scale (per compound) according to the optimized solid-phase synthesis conditions described above. The resulting macrocyclic peptomers were purified to homogeneity by preparative HPLC (gradient elution: 25–55% solvent B in solvent A over 15 min). Peptomers synthesized from racemic *sec*-butylamine "sb" (**4**, **8**, **11**, **12**, **13**, **16**, and **17**) were isolated as diastereomeric mixtures, except **16** in which the diastereomers were separated (**16a** and **16b**).

Library purity analyses. After preparative HPLC purification, samples were re-analyzed by analytical HPLC (gradient elution: 10–90% solvent B in solvent A over 50 min). The analytical HPLC traces for peptomers **4** and **8–17** are included below. Fractions containing the peptomers were collected and analyzed by MALDI MS. These MS data for peptomers **4** and **8–17** are included in Table S-1 below.

Peptomer HPLC data.

UV detection at 220 nm.





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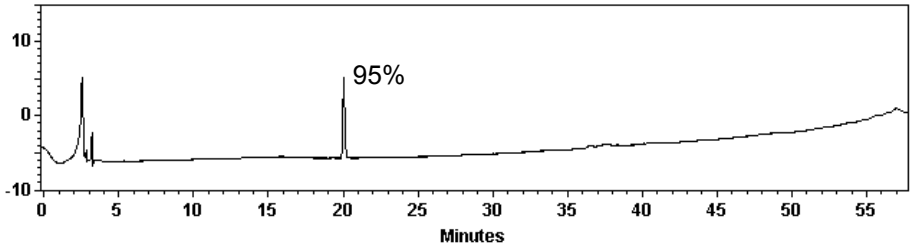
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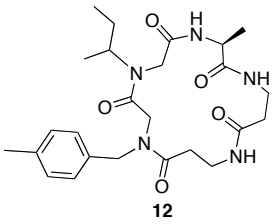

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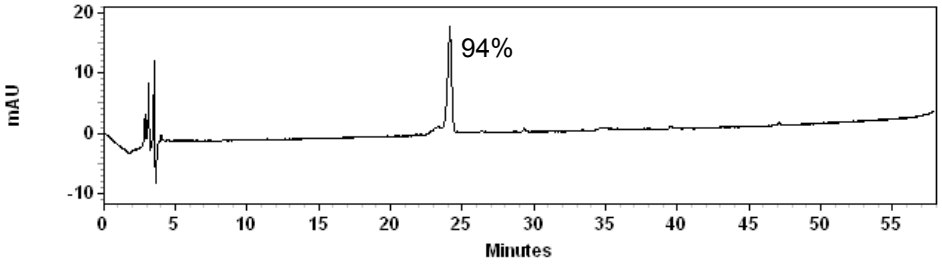
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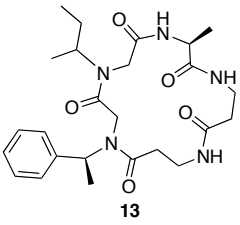

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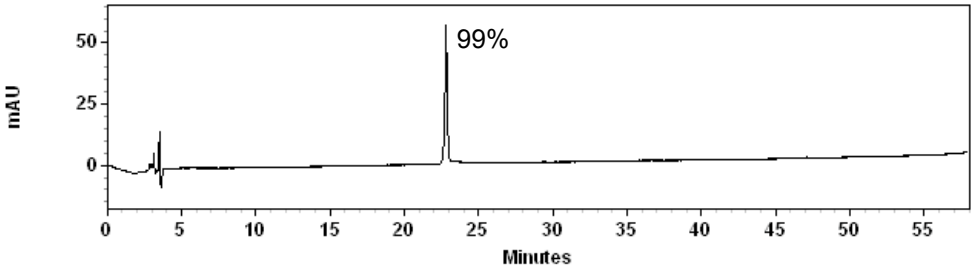
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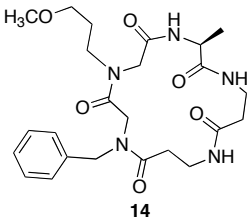

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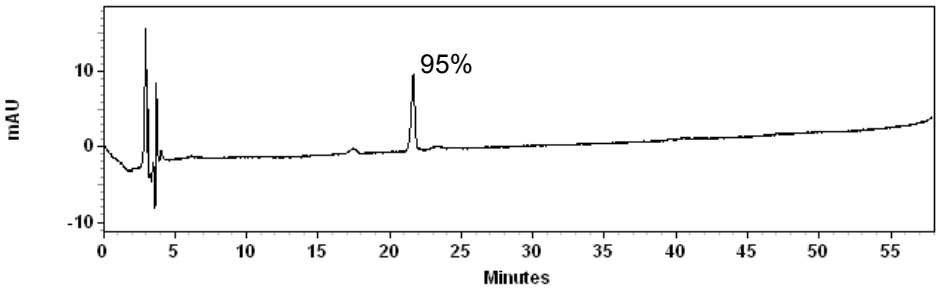
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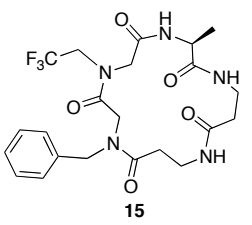

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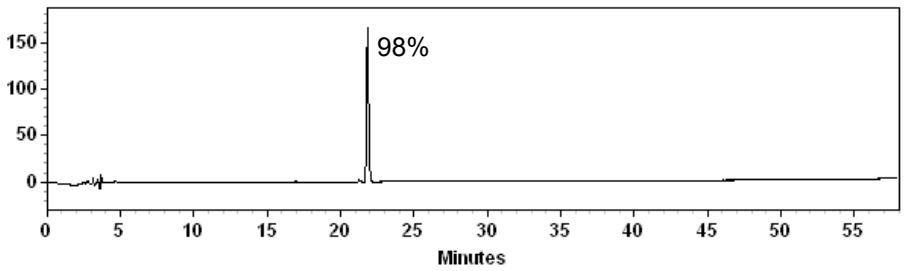
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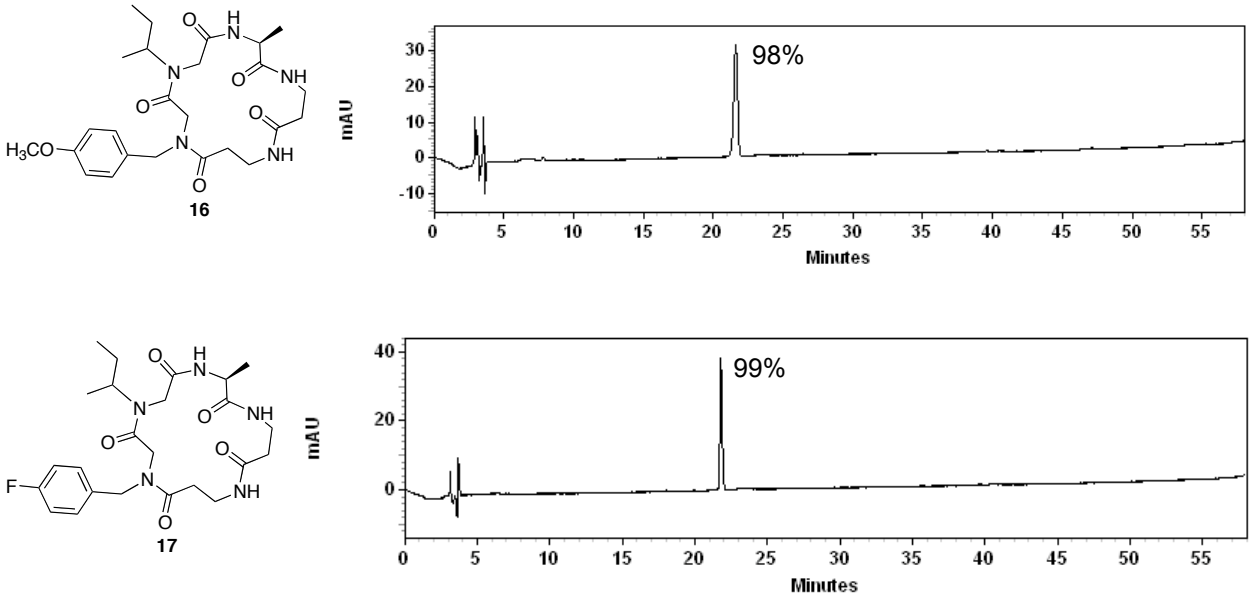

Peptomer MS data.

Table S-1. MALDI MS data for peptomer library members

peptomer	mass calc.	mass obs.
4	473.2	496.1 $[M+Na^+]^+$
8	473.2	496.1 $[M+Na^+]^+$
9	459.2	482.0 $[M+Na^+]^+$
10	499.2	522.1 $[M+Na^+]^+$
11	479.3	502.0 $[M+Na^+]^+$
12	487.2	510.0 $[M+Na^+]^+$
13	487.2	510.0 $[M+Na^+]^+$
14	489.2	512.0 $[M+Na^+]^+$
15	499.2	522.1 $[M+Na^+]^+$
16	503.2	526.1 $[M+Na^+]^+$
17	491.2	514.1 $[M+Na^+]^+$

Bacterial assay protocols and data.

Bacterial strain, compound handling, and reagents. The wild type group I *Staphylococcus aureus* strain RN6390b was used in all bacteriological assays.[6] Stock solutions of synthetic compounds (1 mM) were prepared in DMSO. Assays were performed in flat-bottom, polystyrene 96-well microtiter plates (Corning Costar, cat. no. 3370). All assays were performed in triplicate on multiple days. Biological reagents were purchased from Fisher Scientific and used according to enclosed instructions. Tryptic soy broth (TSB) medium was prepared according to packaging (30 g/L). Crystal violet was purchased from Acros. A sample of the *Staphylococcus epidermidis* AIP (shown in Figure S-1) was generously donated by Dr. Michael Otto (NIH) for use as a control.[7]

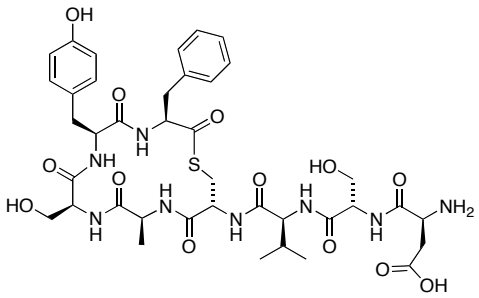

Figure S-1. Structure of the *S. epidermidis* AIP, a known antagonist of AgrC-I ($IC_{50} \approx 300$ nM).[7b]

Instrumentation. Absorbance measurements were obtained using a PerkinElmer Wallac 2100 EnVision™ multilabel plate reader using Wallac Manager v1.03 software. A 595 nm filter was used for measuring bacterial cell density (OD_{600}) and crystal violet absorbance (Abs_{595}).

S. aureus **biofilm assay protocol.** A modified variant of the polystyrene adherence (biofilm formation) assay described by Christensen *et al.*[8] was used in this study.[9,10] In brief, a 2 μL aliquot of peptomer or *S. epidermidis* AIP stock solution in DMSO (1 mM peptomers **4** and **8**–**17**; 0.001—10 mM AIP), to give a final concentration of 10 μM or 0.01—100 μM, respectively, was added to wells in a 96-well microtiter plate. TSB media (200 μL) without compound was added to the perimeter wells of the plate (to avoid concentration changes due to media evaporation), and six wells were reserved as negative controls containing 2 μL DMSO (see Figure S-2 below). An overnight culture of *S. aureus* RN6390b was diluted 1:100 with TSB medium containing 1% w/w D-glucose. The diluted culture was added to the inner 60 wells of the plate (200 μL per well). Plates were grown at 37 °C without shaking for 24 h, after which OD_{600} was measured.

The bacterial culture was removed from the plate by "dumping" (*i.e.*, the plate was turned upside down over a Pyrex dish and shaken to remove culture from the wells). The wells were washed with phosphate buffered saline (pH 7.5; 250 μL per well; 3X, with dumping of washes). The adhered *S. aureus* cells were fixed by drying the plate at 55 °C for 1 h in an oven. The fixed cells were stained by the addition of a crystal violet solution (0.1% solution in H_2O; 200 μL per well). The plate was allowed to sit at rt for 5 min, after which the stain solution was removed by

dumping, and the wells were washed with H_2O (200 μL per well; 2X, with dumping of washes). Stain was solubilized from the cells by the addition of AcOH (30% solution in H_2O; 200 μL per well) and shaking the plate for 15 min at rt on an orbital shaker. Absorbance at 595 nm was measured and normalized to cell density (OD_{600}) per well. The normalized absorbance of each well was then divided by the absorbance value for the negative control (DMSO without compound), making the normalized absorbance of the negative control = 1. The primary assay data is shown below in Figure S-3.

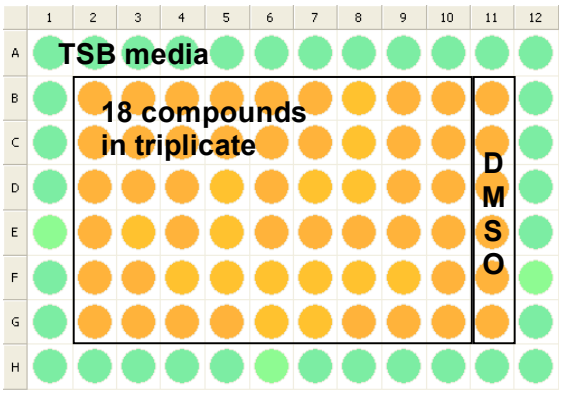

Figure S-2. Schematic of biofilm assay set-up in a 96-well microtiter plate.

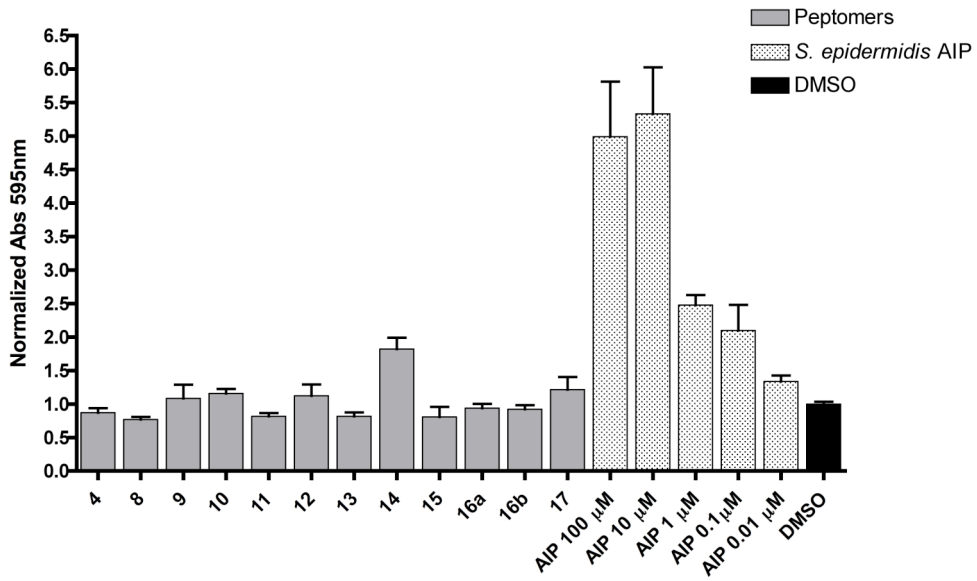

Figure S-3. *S. aureus* RN6390b biofilm assay data. Peptomers **4** and **8**–**17** were tested at 10 μM; *S. epidermidis* AIP was tested at five concentrations (0.01—100 μM) to confirm a previous report.[7a] Diastereomers of **16** (*i.e.*, **16a** and **16b**) were tested separately.

Effect of peptomers on *S. aureus* growth. The optical density (OD_{600}) of *S. aureus* RN6390b was measured after 24 h at 37 °C in the presence of peptomers **4** and **8**–**17** or DMSO. No appreciable effects on bacterial growth were observed at peptomer concentrations ranging from 1 nM – 10 μM (see Figure S-4).

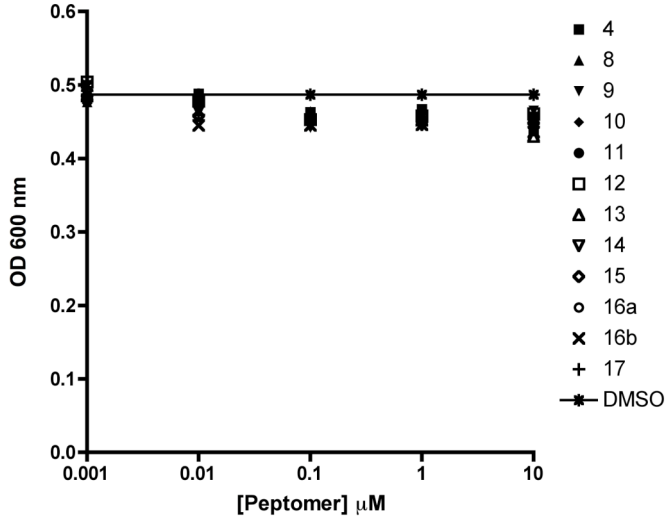

Figure S-4. Optical density of *S. aureus* RN6390b in the presence of peptomers **4** and **8**–**17** (1 nM – 10 μM). Control is 1% DMSO in bacterial culture.

References and notes.

1. For additional information about this μW synthesis reactor, see: http://www.milestonesci.com/

2. For additional more information about this μW synthesis reactor, see: http://www.cem.com/

3. Gorske, B. C.; Jewell, S. A.; Guerard, E. J.; Blackwell, H. E. *Org. Lett.* **2005**, *7*, 1521-1524.

4. For additional information on MOE software, including a comprehensive list of publications citing MOE for related modeling studies, see: http://www.chemcomp.com/index.htm

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10. We acknowledge Prof. Warren Rose (UW–Madison School of Pharmacy) for helpful discussions on *S. aureus* biofilm assays.