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

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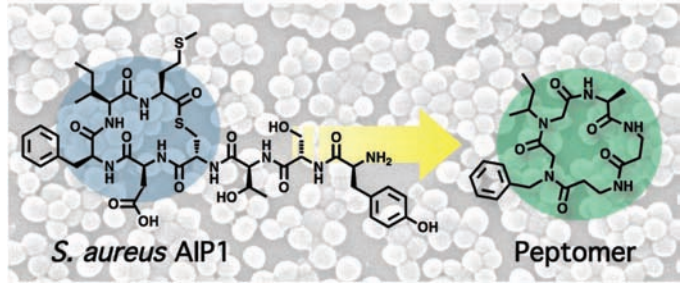
ABSTRACT



We report the design and synthesis of macrocyclic peptide-peptoid hybrids (peptomers) as analogs of autoinducing peptide I (AIP-I) from *Staphylococcus aureus*. Our solid-phase synthetic route includes efficient microwave-assisted reactions and a tandem macrocyclization-cleavage step, and we demonstrate its compatibility with parallel synthesis through the generation of a focused peptomer library. One of the peptomers was capable of stimulating biofilm formation in *S. aureus*, a phenotype linked to AIP-I receptor (AgrC-I) inhibition.

There is an urgent, global need for new antibacterial therapies, and quorum sensing (QS) has emerged as an attractive target for intervention.[1] Many bacterial pathogens have evolved to overcome traditional antibiotics that target growth.[2] However, as inhibition of QS pathways should block virulence, not bacterial growth, resistant mutants are anticipated to arise at considerably slower rates (if at all).[3] Gram-positive bacteria use small peptides as QS signals,[1] and the macrocyclic autoinducing peptides (AIPs) utilized by *Staphylococcus aureus* for QS have been well studied due to the enormous clinical relevance of this pathogen.[4] Non-native ligands that intercept QS pathways in *S. aureus* would hold significant value as chemical tools to study the fundamental mechanisms of QS and their role in pathogenesis.[1b,c,5,6] In this context, peptidomimetics that

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(5) Gorske, B. C.; Blackwell, H. E. *Org. Biomol. Chem.* **2006**, *4*, 1441–1445.

(6) For a related approach using antibodies, see: (a) Park, J.; Jagasia, R.; Kaufmann, G. F.; Mathison, J. C.; Ruiz, D. I.; Moss, J. A.; Meijler, M. M.; Ulevitch, R. J.; Janda, K. D. *Chem. Biol.* **2007**, *14*, 1119–1127.

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(8) For a review of peptoids, see: (a) Patch, J. A.; Kirshenbaum, K.; Seurynck, S. L.; Zuckermann, R. N.; Barron, A. E. In *Pseudopeptides in Drug Development*; Nielsen, P. E., Ed. Wiley-VCH: Weinheim, Germany, 2004.

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display enhanced biostability relative to native QS peptides represent an important ligand class; such mimetics remain largely unexplored as QS modulators in Gram-positive bacteria, however.[7] We hypothesized that *N*-substituted glycine oligomers, or peptoids, would be a versatile structure class from which to design such ligands due to their high biostability and ease of synthesis.[8,9] Furthermore, peptoids have been successfully developed as mimics of peptides and proteins to probe numerous other biological phenomena.[10] Here, we report our initial investigations toward the design and solid-phase synthesis of macrocyclic peptide-peptoid hybrids (or peptomers)[11] as analogs of AIP-I from *S. aureus* (Figure 1). We demonstrate the utility of our synthetic route

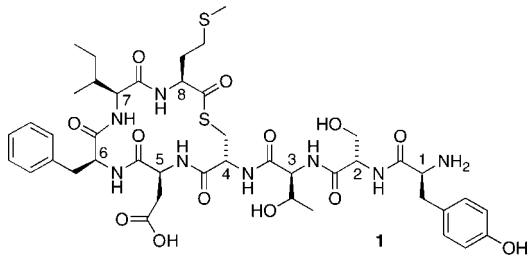


Figure 1. *S. aureus* group I autoinducing peptide (AIP-I, **1**).

through the construction of a focused library of peptomers and report the initial biological testing of these compounds. One of the peptomers was found to stimulate a QS response in *S. aureus* (i.e., biofilm formation) and represents to our knowledge the first peptomer modulator of a QS phenotype in bacteria.

S. aureus uses AIP ligands and their cognate transmembrane receptors, AgrC proteins, for QS.[1,4] AIP-AgrC binding activates a two-component intracellular signaling system that ultimately triggers the virulence response. *S. aureus* has evolutionarily diverged into four distinct groups (I−IV), each with its own unique AIP ligand and AgrC receptor. Researchers have seized the opportunity to delineate a set of structure-activity relationships (SARs) in these four peptidic systems to better understand AIP-mediated QS.[4] The majority of these SAR data were derived for AIP-I (**1**) from group I *S. aureus* (Figure 1). We built on these general SARs in the present study for the design of peptoid analogs of AIP-I (**1**). Acyclic AIP-I fails to activate AgrC receptors, indicating that the macrocycle enforces an active conformation.[4] We reasoned that replacing the thioester with a more hydrolytically stable linkage could provide AIP-I analogs with enhanced agonistic, or antagonistic, activity. We note, however, that thioester replacement with lactones or lactams in certain α-peptide AIP analogs reduced their agonistic activities but had virtually no effect on antagonistic activities.[4,7] These prior findings suggested that such substitutions should be made with care in the design of new analogs.

In AIP-I (**1**), the presence of Phe-6 and Ile-7 are essential for agonistic activity (e.g., replacement with Ala decreases its EC_{50} by ∼500-fold).[12] Additionally, truncated analogs of AIP-I that lack the exocyclic Tyr-1-Ser-2-Thr-3 tail also display low agonistic activity; interestingly, these peptides exhibit strong *antagonistic* activity against AgrC receptors instead. Recently, Williams and co-workers found that replacement of Asp-5 with Ala in these truncated cyclic peptides yielded one of the most potent AgrC-I antagonists reported (**2**, IC_{50} = 5 nM; Figure 2).[4c] Together, these SAR

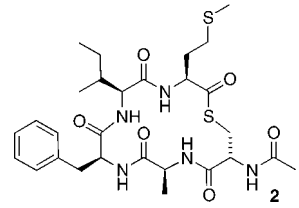


Figure 2. Truncated AIP-I analog (**2**) with antagonistic activity against AgrC-I.

data suggested that peptoid mimics of peptide **2** that lack a thioester linkage could be modulators (most likely antagonists) of AgrC-I.

Our initial ligand design involved several incremental changes to the structure of peptide **2**; each step was guided by computational studies to gauge the impact of the perturbations on the overall conformation of **2**.[13] First, we sought to determine whether the two key hydrophobic residues in **2**, Phe-6 and Ile-7, could be converted into their analogous peptoid residues (*N*pm and *N*ssb, respectively).[8] For ease of synthesis in this first generation ligand design, we selected to convert only these two residues into peptoid units. Thus, we would generate peptide-peptoid hybrid products (or peptomers).[11,14] Second, we chose to replace the Cys-4-derived thioester with a more hydrolytically stable amide linkage. We selected L-2,3-diaminopropionic acid as a Cys-4 replacement at the outset. However, modeling studies revealed that the 16-membered ring of this peptomer analog failed to overlay well with the Williams inhibitor **2**; in fact, the *N*-substituted side chains were ∼60° out of plane relative to the peptidic side chains (data not shown). Further computational work revealed that by expanding the ring by one atom through the replacement of Ala-5 with (*S*)-3-aminobutanoic acid (to give peptomer **3**; Figure 3), the

(9) (a) Gorske, B. C.; Jewell, S. A.; Guerard, E. J.; Blackwell, H. E. *Org. Lett.* **2005**, *7*, 1521–1524. (b) Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moos, W. H. *J. Am. Chem. Soc.* **1992**, *114*, 10646–10647.

(10) For selected recent examples, see: (a) Xiao, X. S.; Yu, P.; Lim, H. S.; Sikder, D.; Kodadek, T. *Angew. Chem., Int. Ed.* **2007**, *46*, 2865–2868. (b) Hara, T.; Durell, S. R.; Myers, M. C.; Appella, D. H. *J. Am. Chem. Soc.* **2006**, *128*, 1995–2004. (c) Seurynck, S. L.; Patch, J. A.; Barron, A. E. *Chem. Biol.* **2005**, *12*, 77–88.

(11) Ostergaard, S.; Holm, A. *Mol. Divers.* **1997**, *3*, 17–27.

(12) McDowell, P.; Affas, Z.; Reynolds, C.; Holden, M. T. G.; Wood, S. J.; Saint, S.; Cockayne, A.; Hill, P. J.; Dodd, C. E. R.; Bycroft, B. W.; Chan, W. C.; Williams, P. *Mol. Microbiol.* **2001**, *41*, 503–512.

(13) Using molecular mechanics in MOE (v. 2006. 08). See Supporting Information for details.

(14) Peptomers were modeled with *N*ssb units (derived from *S*-sec-butyl amine) but replaced with racemic (*N*sb) units for synthesis.

(15) (a) Blackwell, H. E. *Org. Biomol. Chem.* **2003**, *1*, 1251–1255. (b) Kappe, C. O. *Angew. Chem., Int. Ed.* **2004**, *43*, 6250–6284.

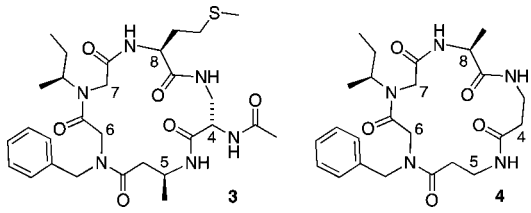


Figure 3. (Left) Initial cyclic peptomer (**3**) designed to mimic peptide **2**. (Right) Streamlined cyclic peptomer **4**. *N*pm and *N*ssb peptoid units replace residues 6 and 7 in peptide **2**, respectively.

peptide (**2**) and peptomer structures occupied virtually the same space (95.4% fit value; Figure 4 left).[13] Third, in this

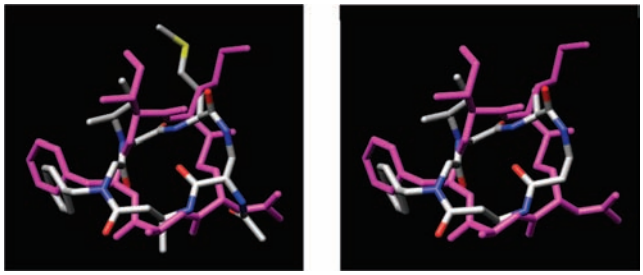


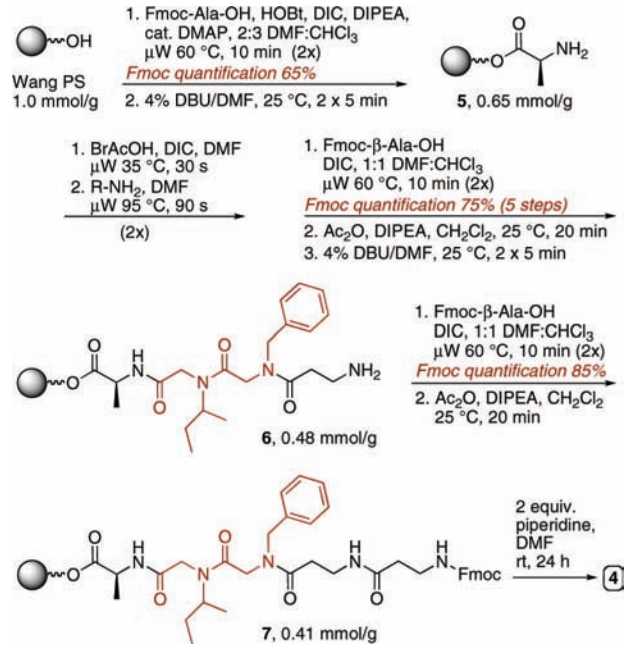
Figure 4. Overlaid computed models. (Left) AIP-I analog **2** (magenta) with peptomer **3** (colored by atom type). (Right) AIP-I analog **2** (magenta) with peptomer **4** (colored by atom type).

initial study of AIP-I mimics, we sought a highly streamlined peptomer target to expedite synthesis. Additional modeling of **3** with further structural replacements revealed that peptomer **4**, containing an L-Ala-(β-Ala)$_2$ linkage (Figure 3), was an excellent mimic of peptide **2** (97.3% fit value; Figure 4 right).[13] We therefore selected peptomer **4** as our target scaffold for this study.

We designed a solid-phase synthetic route to macrocyclic peptomers based on scaffold **4**, as solid-phase methods for both α-peptide and peptoid syntheses are robust and well established (Scheme 1).[9] For ease of synthesis, this route made use of only commercially available *N*-Fmoc-amino acids, amines, and resin. In addition, we incorporated microwave (μW)-assisted reactions throughout the route,[15] as these transformations can dramatically expedite the rates of solid-phase syntheses.[9a] We selected an ester-linker derived resin (Wang),[16] as we sought to explore a tandem cyclization-cleavage procedure to generate the macrocyclic peptomers in the final step.

Our synthesis began with the loading of Fmoc-L-Ala-OH onto Wang-linker derived polystyrene beads using a carbodiimide (DIC) coupling reaction (Scheme 1). Acceptable

Scheme 1. Solid-Phase Synthesis of Peptomer Mimic **4**



ester loading (~70%) was achieved in a short reaction time (20 min) using μW heating (as determined by UV Fmoc quantitation).[17] DBU (1,8-diaza-bicyclo[5.4.0] undec-7-ene) was used for subsequent Fmoc deprotection.[18] We next coupled the two peptoid residues (*N*sb and *N*pm) to resin **5** using our recently reported, μW-assisted peptoid synthesis method.[9a,14] Thereafter, we performed DIC coupling reactions under μW heating and a basic Fmoc deprotection to couple two units of Fmoc-βAla-OH to the resin-bound peptomer. The resin was acetylated after each peptide coupling reaction to block any unreacted amines from subsequent reactions. This procedure generated resin-bound, acyclic peptomer **7** with a loading of 0.41 mmol/g.[17]

We reasoned that subjecting resin **7** to base would facilitate both Fmoc-deprotection and a tandem macrocyclization-cleavage to release peptomer **4**. We were pleased to observe clean formation of the 17-membered macrocycle **4** upon treatment of **7** with 2 equiv of piperidine at room temperature (rt; 25% conversion from **7**). The crude peptomer was separated from the Fmoc piperidine-fulvene adduct by preparative reverse-phase HPLC to give peptomer **4** in >95% purity and 13% overall yield (calculated from Ala-resin **5**). Longer reaction times, either at room temperature or elevated temperatures (e.g., 40−80 °C in a μW reactor), failed to increase the macrocyclization-cleavage yield (data not shown). We therefore utilized the room temperature, macrocyclization-cleavage conditions for the rest of this study.

We further examined our peptomer synthesis method through the construction of a small (11-member) peptomer library. Each library member differed in the composition of its two peptoid units (Figure 5). Eleven amine building blocks

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(17) Carpino, L. A.; Han, G. Y. *J. Org. Chem.* **1972**, *37*, 3404–3409.
(18) We used DBU, as opposed to piperidine, for Fmoc deprotection throughout this synthesis to eliminate premature compound cleavage via amide formation.

(19) Vuong, C.; Saenz, H. L.; Gotz, F.; Otto, M. *J. Infect. Dis.* **2000**, *182*, 1688–1693.

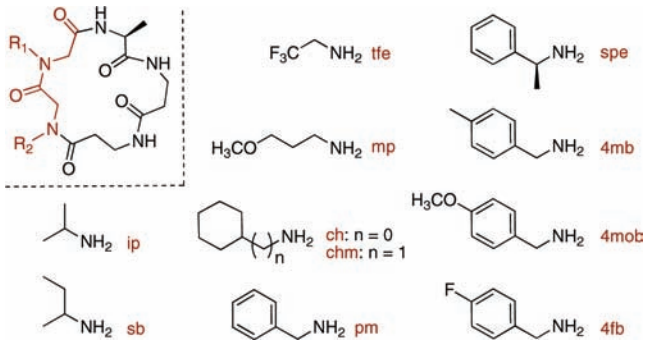

Figure 5. Generic structure of peptomer library (left corner) and amines (with abbreviations) used in construction of the library.[14]

were selected for library synthesis that differed in their overall steric bulk, hydrophobicity, and electronics. These macrocyclic peptomers were designed not only to study the scope of our method but also to eventually probe if differing peptoid structure in the key Phe-6-Ile-7 positions affected peptomer activity against AgrC-I.

We prepared the peptomer library (**4** and **8**−**17**) in parallel using our optimized synthetic method (Table 1), and isolated each compound in ≥94% purity by preparative reverse-phase HPLC. Overall yields were 11−22% for this multiple-step solid-phase synthesis, and the yields of the tandem macrocyclization-cleavage step ranged from 15−36%. Consistent with our previous report,[9a] we found that the μW-assisted peptoid synthesis procedure allowed for the efficient incorporation of both sterically hindered and electronically deactivated amines into the macrocyclic peptomers (e.g., spe and 4fb, respectively; Figure 5).

Table 1. Structures and Yields for Peptomer Library Members

peptomer[a]	R_1	R_2	cyclization yield (%)[b,c]	overall yield (%)[c,d]
4	*N*sb	*N*pm	22	13
8	*N*pm	*N*sb	31	15
9	*N*ip	*N*pm	33	20
10	*N*ch	*N*pm	25	13
11	*N*sb	*N*chm	15	10
12	*N*sb	*N*4mb	23	11
13	*N*sb	*N*spe	36	20
14	*N*mp	*N*pm	32	15
15	*N*tfe	*N*pm	16	11
16	*N*sb	*N*4mob	36	22
17	*N*sb	*N*4fb	21	13

[a] See Figure 5 for full peptomer structures. [b] Isolated yield of cyclization-cleavage step after purification to ≥94% purity by HPLC. [c] Peptomers containing *N*sb were isolated as mixtures of diastereomers; yields report both diastereomers. [d] Overall yield of macrocyclic peptomer based on loading of Ala-resin **5** (0.65 mmol/g).

We sought to determine if the peptomers were capable of modulating the activity of AgrC-I in *S. aureus*. A previous report by Otto and co-workers showed that inhibition of AgrC-I (either by genetic knockout or with a known AgrC-I inhibitor, the *S. epidermidis* AIP) can stimulate *S. aureus* biofilm formation in a static biofilm assay.[19] The precise role of AgrC proteins in *S. aureus* biofilm formation is still being elucidated and appears to be environmentally sensitive;[20] however, we reasoned that this biofilm assay would allow us to quickly assess if the peptomers could modulate AgrC-I. We examined peptomers **4** and **8**−**17** and the *S. epidermidis* AIP at 10 μM in static biofilm assays (using *S. aureus* RN6390b)[19] and observed that one peptomer (**14**) was capable of stimulating biofilm formation by 2-fold versus the untreated control and to 34% the level of the *S. epidermidis* AIP (see Figure S-3; Supporting Information). Additional experiments are required to elucidate the mechanism by which peptomer **14** promotes biofilm formation in *S. aureus*. Nevertheless, as **14** was identified from a relatively small group of compounds (11), this result serves to underscore the potential value of peptidomimetic tools to study QS in Gram-positive bacteria.

In summary, we have designed peptomer analogs of AIP-I from *S. aureus* and developed a new solid-phase synthetic route that provides access to these compounds. This straightforward route incorporates a series of μW-assisted reactions and a tandem macrocyclization-cleavage step. We applied this method for the parallel synthesis of a collection of macrocyclic peptomer mimics of a truncated AIP-I analog (**2**). One of the peptomers (**14**) was found to stimulate biofilm formation in *S. aureus*, a phenotype linked to AgrC-I inhibition. Ongoing studies are focused on applying our synthetic route to peptomers of heightened structural complexity (i.e., peptomer **3**) and further evaluating the activities of peptomer mimetics in QS assays in *S. aureus*.

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Supporting Information Available: Full details of peptomer synthesis and characterization, modeling studies, and biological testing and data. This material is available free of charge via the Internet at http://pubs.acs.org.

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