Combinatorial Synthesis through Disulfide Exchange: Discovery of Potent Psammaplin A Type Antibacterial Agents Active against Methicillin-Resistant *Staphylococcus aureus* (MRSA)

K. C. Nicolaou,* Robert Hughes, Jeffrey A. Pfefferkorn, Sofia Barluenga, and A. J. Roecker[a]

Abstract: Psammaplin A is a symmetrical bromotyrosine-derived disulfide natural product isolated from the *Psammaplysilla* sponge, which exhibits in vitro antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA). Inspired by the structure of this marine natural product, a combinatorial scrambling strategy for the construction of heterodimeric disulfide analogues was developed and applied to the construction of a 3828-membered library starting from 88 homodimeric disulfides. These psammaplin A analogues were screened directly against various gram positive bacterial strains leading to the discovery of a series of potent antibacterial agents active against methicillin-resistant *Staphylococcus aureus* (MRSA). Among the most active leads derived from these studies are compounds **104**, **105**, **113**, **115**, **123**, and **128**. The present, catalytically-induced, disulfide exchange strategy may be extendable to other types of building blocks bearing thiol groups facilitating the construction of diverse discovery-oriented combinatorial libraries.

Keywords: antibiotics · chemical biology · combinatorial synthesis · psammaplin A · total synthesis

Introduction

Combinatorial chemistry has become an important tool for both the drug discovery process and chemical biology studies.[1] Particularly interesting has been the application of combinatorial techniques to the synthesis and structure–activity optimization of biologically active natural products.[2] Typically, the structural complexities of these molecules pose unique challenges which necessitate the development of novel solution or solid phase combinatorial strategies.[3] In many cases, the methodologies developed in this way can later be applied to the synthesis of combinatorial libraries with more general applications. With such considerations in mind, we undertook the solution phase combinatorial synthesis and biological evaluation of a novel heterodimeric disulfide library inspired by the antibacterial marine natural product psammaplin A (**1**, Figure 1).

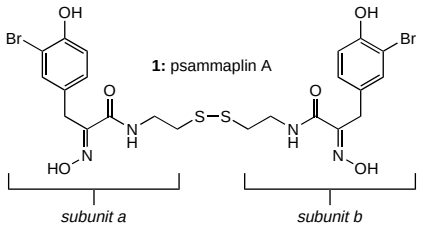

Figure 1. Structure of psammaplin A, an antibacterial marine natural product isolated from *Psammaplysilla* sp.

Psammaplin A (**1**, Figure 1) is a symmetrical bromotyrosine-derived disulfide dimer that was originally isolated in 1987 from the *Psammaplysilla* sponge.[4] Early studies revealed that psammaplin A had general antibacterial and antitumor properties. Later studies demonstrated that psammaplin A (**1**) exhibited significant in vitro antibacterial activity against both *Staphylococcus aureus* (SA) and methicillin-resistant *Staphylococcus aureus* (MRSA)[5] Given the increasingly rapid emergence of multi-drug resistant bacterial strains and the corresponding threat to public health, there is signifigant interest in the development of structurally novel antibacterial agents such as psammaplin A.[6, 7] Additionally,

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psammaplin A has been reported to exhibit weak inhibition of a number of enzymes including DNA gyrase,[5] topoisomerase II,[8] farnesyl protein transferase,[9] and leucine aminopeptidase.[9] Despite these reports of biological activity, however, no investigations into the structure–activity relationships of psammaplin A have been disclosed, and its chemical mechanism of action remains unknown. In light of this, we undertook the synthesis of a combinatorial library of disulfide containing molecules in order to find potent and structurally simplified analogues which could be used as tools for studying psammaplin A's mechanism of action and its potential therapeutic value.

From the viewpoint of combinatorial chemistry, psammaplin A (**1**, Figure 1) is rather interesting owing to its two identical domains (subunits a and b) which are linked through a disulfide bridge. At the outset of this work it was unknown whether one or both of these subunits were necessary for antibacterial activity nor was it clear whether the disulfide moiety played a mechanistic or structural role in the agent's biological action. Combinatorially, we envisioned utilizing the disulfide motif as a readily exchangeable linkage[10] which would allow rapid construction of a heterodimeric analogue library suitable for defining structure–activity relationships.[11] It is well precedented that disulfide bonds will readily undergo facile exchange reactions with other disulfides in high yield under mild conditions.[12] For example, and as shown in Figure 2, if two homodimeric disulfides, A-SS-A and B-SS-B, are mixed under basic conditions in the presence of a suitable catalyst they will undergo rapid exchange reactions to afford a statistical mixture of three disulfides: A-SS-A, A-SS-B, and B-SS-B in a ratio of 1:2:1, respectively, resulting from the degeneracy of the A-SS-B and B-SS-A products.

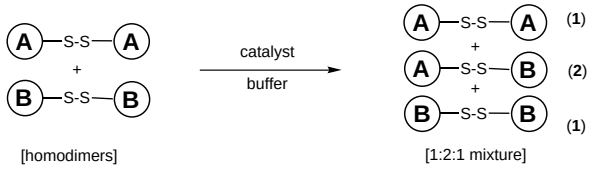


Figure 2. General principle for construction of novel heterodimeric disulfides through scrambling and equilibration of homodimeric disulfides.

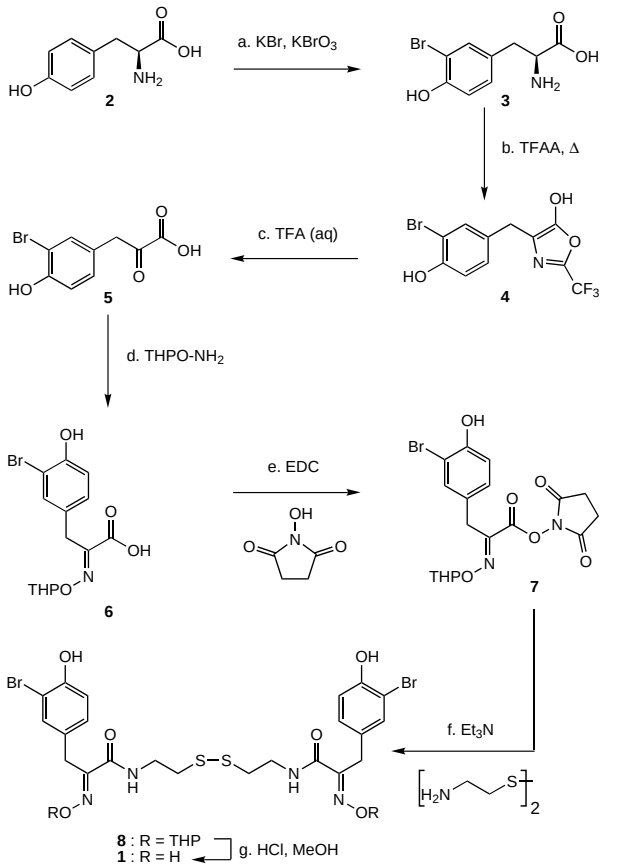
It was anticipated that a combinatorial strategy of this type would provide an opportunity to simultaneously evaluate modifications at remote sites of the psammaplin A architecture for potential synergistic effects which might be difficult to uncover through a traditional SAR approach. This program evolved through several stages as described in this and the following article.[13] Initially, psammaplin A (**1**) and a number of designed homodimeric analogues were individually synthesized. These synthetic homodimers, along with other commercially available homodimeric disulfides, were then systematically scrambled to create a large (> 3800-membered) library of heterodimeric analogues which was screened for antibacterial activity to identify structurally novel lead compounds that were then re-synthesized in homogenous form to confirm their antibacterial activities. Subsequently, (see, following article)[13] the antibacterial properties of these novel heterodimeric leads were further refined through the synthesis and biological evaluation of several promising series of simplified analogues. Finally, a collection of analogues with structural modifications aimed at elucidating the molecular mechanism of action of these psammaplin-type compounds were constructed and employed in order to better understand how this unique class of antibacterial agents function.

Results and Discussion

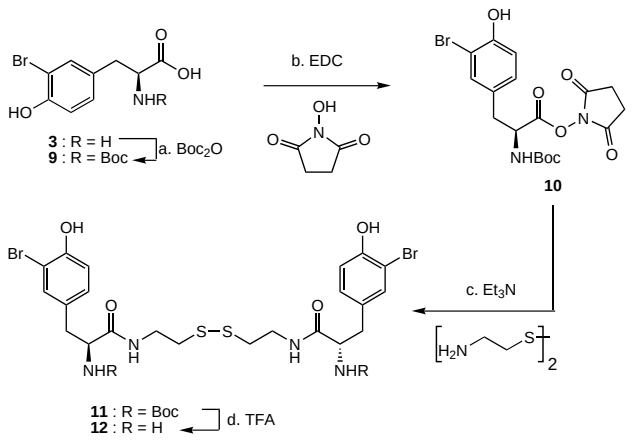
Synthesis of psammaplin A and homodimeric analogues: Prior to commencing the combinatorial aspects of this work, we first required synthetic access to psammaplin A (**1**, Figure 1) and 44 designed homodimeric analogues (see Figure 3) to be used as symmetrical disulfides in the projected construction of the combinatorial library. To this end, Hoshino's synthesis[14] of **1** was modified as shown in Scheme 1 to accommodate our requirements for a general approach to a variety of psammaplin-type structures. As shown, L-tyrosine (**2**) was mono-brominated by treatment with potassium bromate/potassium bromide to afford 3-bromotyrosine (**3**) in 81% yield.[15] Amino acid **3** was then refluxed in TFAA (for abbreviations of reagents and protecting groups, see legends in schemes) to form trifluoromethyloxazolone **4**, which was distilled from the reaction vessel and hydrolyzed with aqueous TFA to provide α-keto acid **5** in 60% yield over two steps.[16] This α-keto acid was subsequently condensed with *O*-(tetrahydro-2*H*-pyran-2-yl)-hydroxylamine, affording oxime **6**. After considerable optimization, it was found that crude carboxylic acid **6** could be reliably coupled with cystamine [$(H_2NCH_2CH_2S)_2$] through the intermediacy of the succinate ester. Hence, acid **6** was treated with *N*-hydroxysuccinimide in the presence of EDC to give succinate ester **7**, which was isolated and immediately treated with cystamine and Et_3N to provide, through a bis-coupling reaction, amide **8**. Finally, the THP protecting groups on the oximes were removed (HCl, MeOH, 60 °C) to afford psammaplin A (**1**) in 36% yield over four steps. With the psammaplin A synthesis completed, the sequence illustrated in Scheme 1 was repeated for the construction of homodimeric analogues **13**–**36** (see Figure 3) using nine different

Abstract in Greek:

Abstract in Greek: Η ψαμμαπλίνη Α είναι ένα φυσικό προϊόν, δισουλφιδικό και συμμετρικό παράγωγο της βρωμοτυροσίνης, που απομονώθηκε από τον σπόγγο *Psammaplysilla* και το οποίο παρουσιάζει *in vitro* αντιβακτηριακή δράση έναντι του ανθεκτικού στη μεθισιλλίνη σταφυλόκοκκου του χρυσίζοντα [Methicillin-resistant *Staphylococcus aureous* (MSRA)]. Εμπνευσμένοι από τη δομή του φυσικού αυτού προϊόντος αναπτύξαμε μια αναμικτική μεθοδολογία συνδυαστικής χημείας για τη σύνθεση ετεροδιμερών δισουλφιδικών παραγώγων που εφαρμόστηκε στη σύνθεση μιας βιβλιοθήκης 3828 ενώσεων από 88 ομοδιμερή δισουλφίδια.Τα συνθετικά αυτά ανάλογα της ψαμμαπλίνης Α ελέγχθηκαν άμεσα για βιολογική δράση έναντι μιας ποικιλίας gram θετικών στελεχών βακτηρίων και οδήγησαν στην ανακάλυψη μιας σειράς ισχυρών αντιβιοτικών παραγόντων δραστικών έναντι του ανθεκτικού στη μεθισιλλίνη σταφυλόκοκκου του χρυσίζοντα (MSRA). Ανάμεσα στις πιο δραστικές ενώσεις οδηγούς που ανακαλύφθηκαν από αυτές τις μελέτες είναι οι ενώσεις **104**, **105**, **113**, **115**, **123** και **128**. Η παρούσα στρατηγική με ανταλλαγή δισουλφιδίων μέσω κατάλυσης είναι δυνατόν να επεκταθεί και σε άλλους τύπους ενώσεων που περιλαμβάνουν θειόλη στο μόριό τους, παρέχοντας έτσι τη δυνατότητα για τη σύνθεση και ανακάλυψη ποικιλίας βιβλιοθηκών με συνδυαστική χημεία.



Scheme 1. Total synthesis of psammaplin A (**1**) from L-tyrosine (**2**). a) $KBrO_3$ (0.3 equiv), KBr (2.0 equiv), H_2O (0.25 M HCl), 23 °C, 12 h, 81 %; b) TFAA (excess), 80 °C, 12 h; c) 70 % TFA (aq), 23 °C, 24 h, 60 % over two steps; d) THP-ONH_2 (1.5 equiv), EtOH, 23 °C, 12 h; e) NHS (1.9 equiv), EDC (1.7 equiv), 1,4-dioxane, 23 °C, 2 h; f) Et_3N (2.0 equiv), cystamine · 2HCl (0.5 equiv), 1,4-dioxane/MeOH 1:1, 23 °C, 12 h; g) HCl (4.0 equiv), CH_2Cl_2/MeOH (20:1), 60 °C, 2 h, 36 % over five steps. EDC = 1-[3-(dimethylamino)propyl)]-3-ethylcarbodiimide; NHS = *N*-hydroxysuccinimide; TFA = trifluoroacetic acid; TFAA = trifluoroacetic anhydride; THP-ONH_2 = *O*-(tetrahydro-2*H*-pyran-2-yl)-hydroxylamine.



Scheme 2. Synthesis of diamino-psammaplin A (**12**). a) di-*tert*-Butyl dicarbonate (2.0 equiv), $NaHCO_3$ (5.0 equiv), THF/H_2O 1:1, 23 °C, 12 h; b) NHS (1.9 equiv), EDC (1.7 equiv), 1,4-dioxane, 23 °C, 2 h; c) Et_3N (2.0 equiv), cystamine · 2HCl (0.5 equiv), 1,4-dioxane/MeOH 1:1, 23 °C, 12 h, 46 % over three steps; d) TFA (10.0 equiv), CH_2Cl_2, 23 °C, 2 h, 95 %.

amino acids (phenylalanine, 4-fluorophenylalanine, 4-chlorophenylalanine, tyrosine, 3-chlorotyrosine, 3-bromotyrosine, 3,5-dibromotyrosine, phenylglycine, and 3-methoxytyrosine) and three differently substituted hydroxylamines [*O*-(tetrahydro-2*H*-pyran-2-yl)-hydroxylamine, *O*-methyl-hydroxylamine, and *O*-benzyl-hydroxylamine].

With these oxime-containing analogues in hand, several additional homodimeric analogues were prepared in an effort to determine the biological importance of other structural features of the psammaplin A skeleton. These analogues included amino acid derivatives **11**, **12**, and **38**–**48** (Figure 3) which were synthesized as illustrated in Scheme 2 for the particular di-amino disulfide analogue **12**. Hence, 3-bromotyrosine **3** was N-protected by treatment with di-*tert*-butyldicarbonate in the presence of $NaHCO_3$ to provide *N*-Boc-3-bromotyrosine (**9**). Crude compound **9** was then treated with *N*-hydroxysuccinimide and EDC to afford succinate ester **10**, which was coupled with cystamine as described above to provide di-amide **11** in 46 % yield over three steps. Compound **11** was fully deprotected (TFA, CH_2Cl_2, 23 °C) to provide the free amino analogue **12** in 95 % yield. In addition, sulfonamide analogues **49**–**53** (Figure 3) and amide analogues (**54** and **55**, Figure 3) were synthesized by standard techniques. Finally, additional homodimeric disulfide building blocks containing various substituted alkyl, aromatic, and heteroaromatic skeletons (see compounds **56**–**99**, Figure 3) were obtained from commercial sources in order to enhance the library's structural diversity.

Preliminary disulfide exchange experiments: With the requisite symmetrical building blocks in hand, we next sought to determine the optimal disulfide exchange conditions paying particular attention to address the following two concerns: a) the reaction conditions ought to afford approximately statistical ratios of scrambled products in high yields and purities; and b) the required reagents and their by-products ought to be sufficiently innocuous toward bacteria so that the disulfide products could be transferred directly from the reaction mixture to the screening assays for antibacterial activity without the need for any work-up or purification procedures.

Based on ample literature precedent regarding disulfide exchange chemistry, suitable reaction conditions for this process were rapidly developed as illustrated for the representative example shown in Scheme 3. Hence, the two homodimeric disulfides **14** and **29** were mixed in a DMSO/H_2O (3:1) solvent system buffered to pH 8.3 with a standard phosphate buffer. Subsequently, a catalytic amount (0.15 equiv) of dithiothreitol (**100**) was added and the reaction mixture was stirred for 12 h at 23 °C. Reaction progress was monitored by HPLC analysis as illustrated in the side panels of Scheme 3. Thus, prior to addition of reagent (Scheme 3, panel a) only the two parent homodimers were present in the HPLC trace, whereas upon addition of dithiothreitol, a third product, heterodimer **101**, was detected as indicated by the corresponding HPLC trace (Scheme 3, insert panel b, 12 h, equilibrium).[17] After normalization, integration of the three disulfide peaks in this trace revealed their respective concentrations to be in a ratio of 1.0:2.1:1.0.[18] Repetition of this

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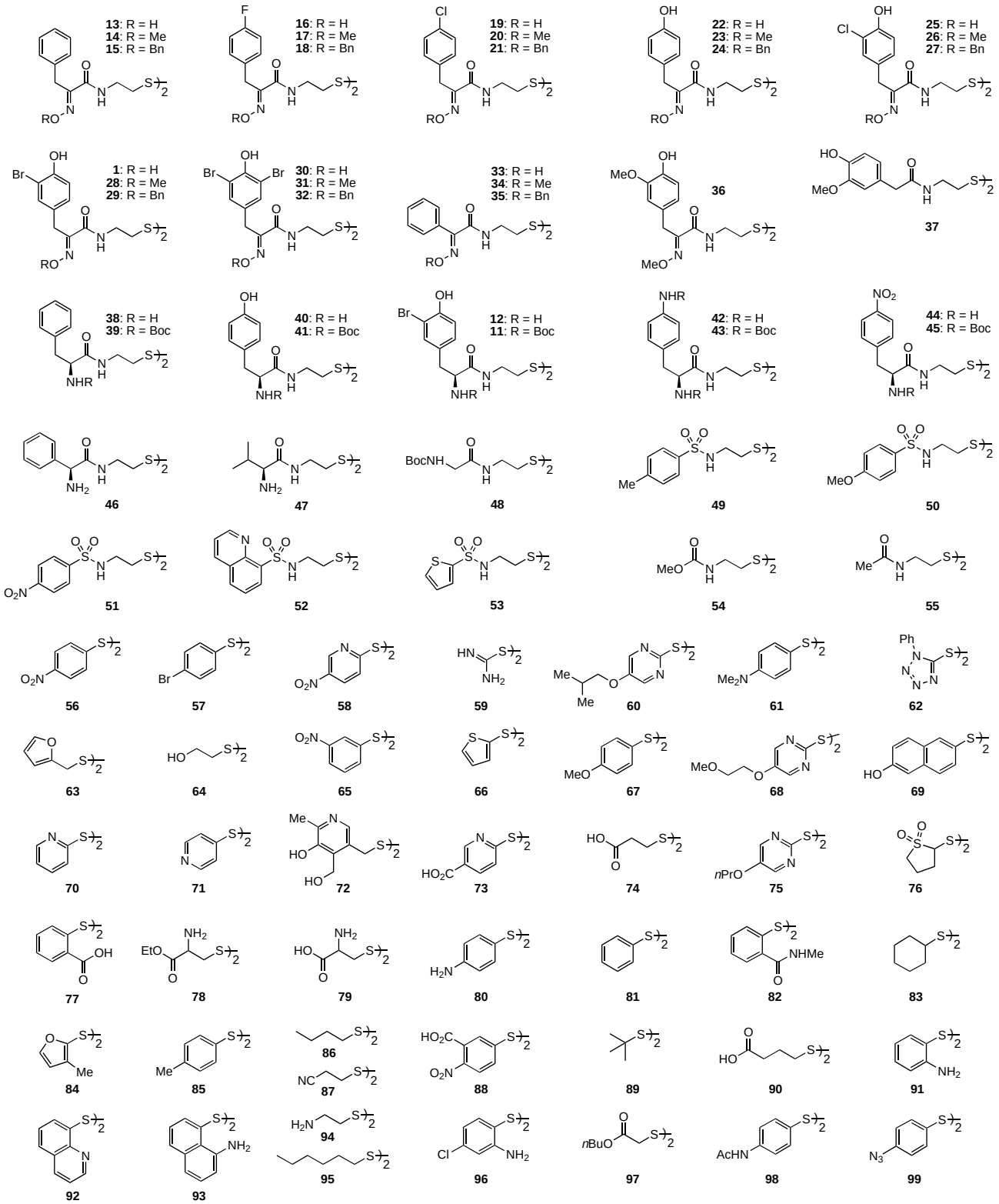
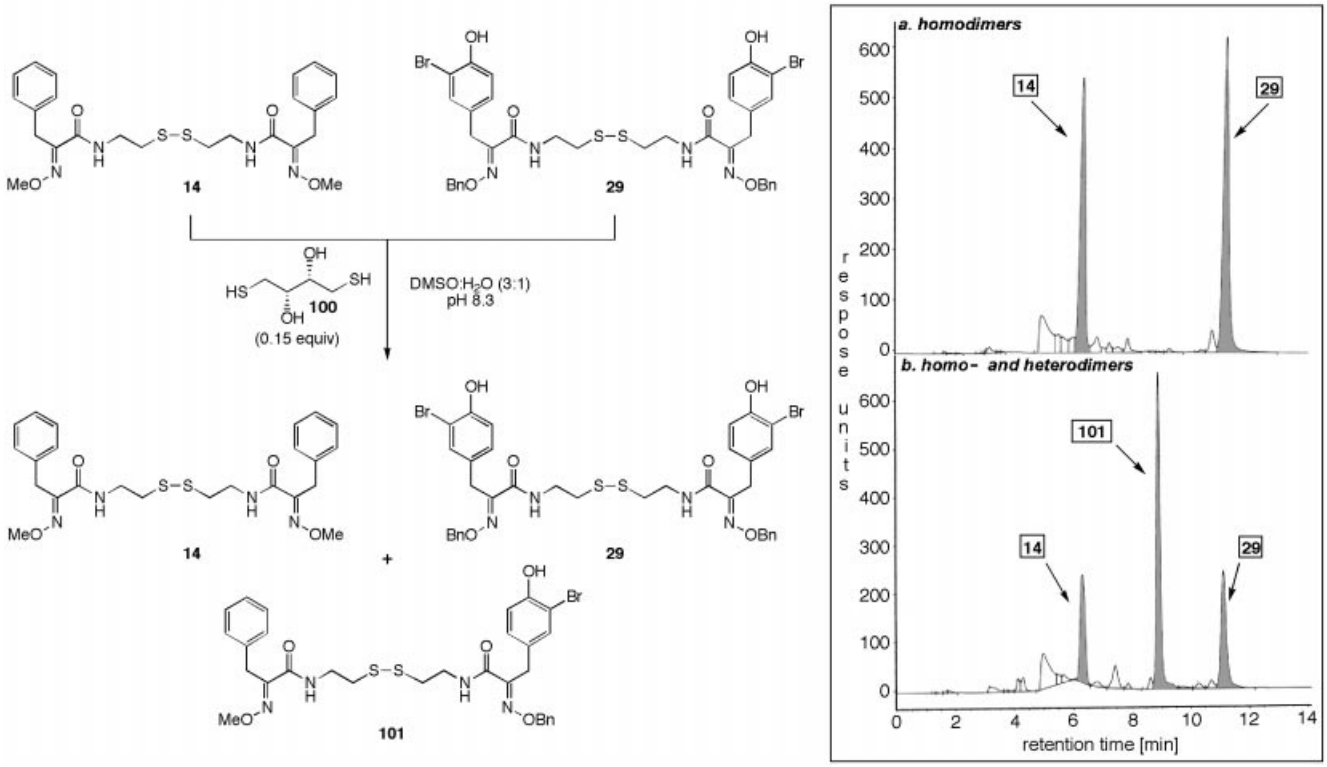


Figure 3. Structures of homodimeric disulfides utilized in library synthesis. Compounds **1**, **11**, **12**, **13**–**48** were synthesized as illustrated in Schemes 2 and 3. Compounds **49**–**55** were synthesized by standard techniques. Compounds **56**–**99** were purchased from commercial sources.

experiment with other, structurally dissimilar, building blocks from Figure 3 were conducted in a similar manner in order to ensure that this reaction time and catalyst loading were sufficient to allow for complete equilibration with no free thiol species remaining. As discussed later, post-synthetic analysis of representative library members did, in fact, confirm that these reaction conditions lead generally and reproducibly to statistical mixtures.

With the scrambling conditions defined, we next proceeded to determine whether the disulfide products could be



Scheme 3. Example of disulfide exchange reaction: An equimolar (1.0 mM in each homodimer) solution of homodimers (**14** and **29**) in DMSO:H_2O (3:1) (insert panel a) was treated with 0.15 equiv dithiothreitol (**100**) and stirred at 23 °C for 12 h to yield homodimers **14** and **29** and heterodimer **101** (insert panel b).

screened directly from the reaction mixture. Specifically, this required evaluating whether or not the catalyst, dithiothreitol, and the resulting by-product *trans*-1,2-dithiane-4,5-diol were biologically inert toward bacteria at the concentration library members were to be screened. This was confirmed by screening both dithiothreitol and *trans*-1,2-dithiane-4,5-diol against SA 6538 and MRSA 700 698 strains. Encouragingly, at 50 $\mu g\,mL^{-1}$, which is 50-fold greater than the concentration at which either of these compounds would be present in the screening mixture, neither of them exhibited any detectable antibacterial activity. These results suggested that the presence of these compounds in trace amounts would not interfere with the biological results of the intended assays, and consequently, we anticipated screening library members directly from the reaction mixtures. Similarly, it was confirmed that the DMSO solvent and phosphate buffer medium used in the scrambling reaction would be sufficiently diluted in the screening process so as to avoid any undesirable effects.

Solution phase combinatorial synthesis of psammaplin A analogue library: With the requisite symmetrical building blocks prepared and the reaction conditions for their exchange defined, construction of the combinatorial psammaplin A analogue library proceeded smoothly. The synthesis was conducted in 96-well plates as illustrated in Figure 4. In order to accommodate the 88 homodimeric starting materials (Figure 3), a total of 88 96-well plates were arranged in an 8×11 array. All wells were filled initially with a DMSO/buffer (3:1) solution. The homodimers were then dispensed so that each homodimer occupied one entire column and one entire

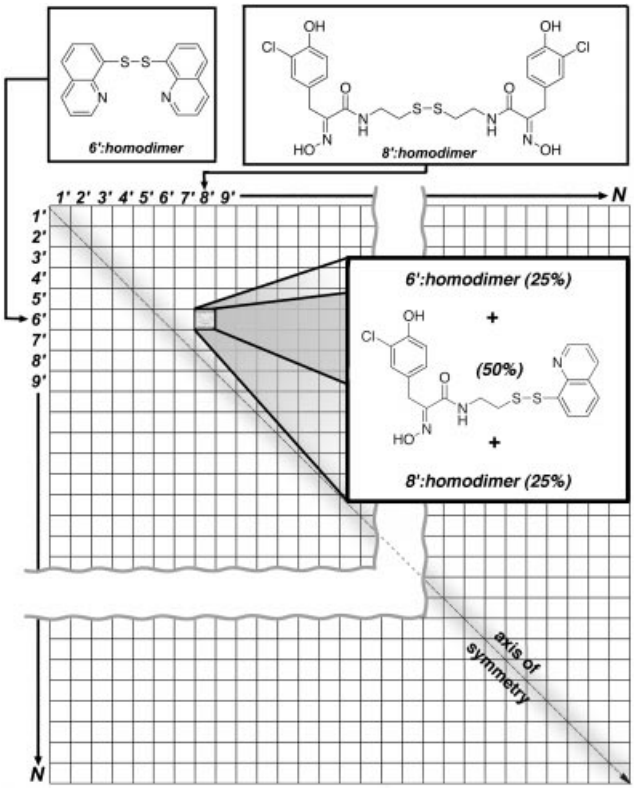


Figure 4. Schematic representation of library format.

row with 0.4 μmol of each homodimer deposited per well. For example, homodimer **8′** (Figure 4) was dispensed to each well in the **8′** row and the **8′** column. Once complete, each well

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contained two different homodimers with the exception of wells on the matrix diagonal (see below for further discussion of symmetry properties of this matrix). Next, a solution of dithiothreitol in DMSO was dispensed into each well (0.15 equiv). Prior to scrambling, the final reaction concentration was 1.0 mM in each homodimer. The plates were individually agitated and then allowed to stand for 12 h at ambient temperature. These conditions promoted scrambling of the two disulfides in each well to afford the corresponding heterodimer, a new compound as illustrated in Figure 4 wherein homodimers **6′** and **8′** are exchanged to afford a heterodimer present at approximately twice the concentration of either of the two original homodimers.

Notably, formatting the library in this manner resulted in the formation of two symmetrical copies of the library. In other words, this library is reflected across the diagonal (see Figure 4) so that the products in well (**6′**, **8′**) are identical to those in well (**8′**, **6′**). While necessitating additional manipulations, this duplicity simplified library construction. More importantly, it was anticipated that screening two independently synthesized copies of the library for biological activity would produce duplicate data whose accuracy would be enhanced.

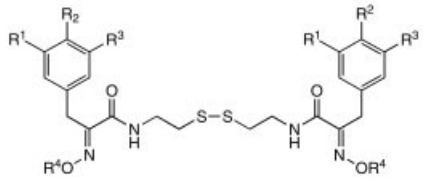
After the synthesis, it was necessary to determine the purity and distribution of reaction products in the library by analyzing 100 representative reaction wells. This task was accomplished through direct HPLC analysis of the reaction mixtures in these wells (see Supporting Information for tabulated data). In summary, 95% of the reaction wells sampled contained $\geq 50\%$ of heterodimer suggesting that, in general, the reactions proceeded smoothly and completely (as a 50% yield of heterodimer would be expected if the components reached a precise equilibrium).

Antibacterial screening of psammaplin A library: Once the library was complete, high throughput antibacterial screening was undertaken to identify heterodimeric analogues of psammaplin A exhibiting biological activity. This screening posed an interesting challenge since each well contained a mixture of three compounds in the approximate ratio of 1:2:1 [homodimer (A-SS-A):heterodimer (A-SS-B):homodimer (B-SS-B)], and it was, therefore, expected that the total activity of any given well would be a composite[18] of the activities of the three compounds in the well. While we do not generally favor mixture-based screening, in this case the synthetic efficiency attained by synthesizing the heterodimeric analogues as mixtures outweighed the necessary, but trivial, deconvolution efforts. Indeed, such deconvolution of active compounds from this library was quite straightforward and fell into two scenarios. In the first, and simpler case, for a well in which both homodimeric components were biologically inactive, any detectable activity for that well could be attributed to the heterodimeric product. The second case was more complicated and occurred if one or both of the homodimeric starting materials in a given well were to be active (for example, when heterodimers were constructed from psammaplin A itself). Thus, at assay concentrations higher than the MIC (minimum inhibitory concentration) value of the active homodimer, every well containing that homodimer displayed activity and, consequently, no useful information regarding the biological activity of the heterodimer could be obtained. To overcome this effect, the assay concentration needed to be incrementally lowered to a point below which the homodimeric component(s) was no longer active (i.e., screening concentration $<$ MIC value of homodimer). If at this lower concentration the mixture in the well retained activity, it was assumed that the heterodimer was worthy of further investigation. Importantly, since the heterodimer is present at *twice* the concentration of each homodimer (due to degeneracy), it was anticipated that this deconvolution through a concentration gradient would be relatively straightforward. To assist in this process, all homodimeric starting materials were initially screened for antibacterial activity, and the results are presented in Table 1. Notably, from the synthetic homodimer subset, only compounds **1** (psammaplin A), **13**, **16**, **22**, **25**, and **30** (see Figure 3 for structures) exhibited significant antibacterial activity. All of the active synthetic homodimers contained a free α-oxime and most were also halogenated (either bromo or chloro) on the tyrosine. Several of the commercial homodimeric building blocks also displayed antibacterial activities as illustrated in Table 1 including compounds **56**, **65**, **66**, **69** and **82** (see Figure 3 for structures). The remaining homodimers (i.e., compounds not listed in Table 1) were found to have no appreciable antibacterial properties at 50 $\mu g\,mL^{-1}$.

Experimentally, evaluation of the library was accomplished by screening the entire, symmetrically reflected library at three concentrations (10.0, 5.0, and 2.5 μM in heterodimer) against two bacterial strains (SA 6538 and MRSA 700698). To accomplish this goal, aliquots were transferred from the reaction plates to shallow well dilution plates containing DMSO, retaining the original library format. These plates were then screened for antibacterial activity. For each strain and concentration, the wells of the plates were optically read for turbidity and a graphical representation of the biological activity in each plate was generated. The graphical representations for the individual plates were then assembled to form the arrays illustrated in Figure 5 for the SA 6538 strain at the three concentrations indicated.

Closer examination of the arrays in Figure 5 illustrates the two types of screening scenarios described above. At the highest concentration (10.0 μM) there was a significant number of rows and columns in which almost every well was active, indicating that at least one of the homodimers in that position was active and overwhelming the activity of the other compound(s) in those wells. As the concentration was halved (i.e., 5.0 μM), the numbers of completely active rows diminished, and at the lowest concentration (i.e., 2.5 μM) any residual effect of the homodimer(s) was lost. An identical trend was observed for screening in the MRSA 700698 strain (data not shown). In these arrays, white squares denote wells with highly active compounds while black squares represent inactive compounds. The various shades of blue denote compounds of intermediate activity. In general, the most active compounds were selected for further evaluation. Active compounds were identified by visual assessment of the arrays as shown in Figure 5. Priority was given to compounds which exhibited activity at the lower concentra-

Table 1. Antibacterial activity (MIC $\mu g\,mL^{-1}$) of psammaplin A (**1**) and other biologically active homodimeric compounds.



	R^1	R^2	R^3	R^4	SA[a] 6538	SA[b] 13709	SA[c] 29213	SA[d] 25923	MRSA[e] 700698	MRSA[f] 43300	MRSA[g] 700787	MRSA[h] 700788	MRSA[i] 700789	AVG[j] SA	AVG[k] MRSA
1	Br	OH	H	H	6.25	3.12	6.25	6.25	6.25	3.12	3.12	3.12	6.25	5.47	3.90
13	H	H	H	H	12.5	3.12	6.25	12.5	25	3.12	6.25	3.12	12.5	8.59	8.85
16	H	F	H	H	6.25	3.12	6.25	6.25	6.25	3.12	6.25	6.25	6.25	5.47	4.95
22	H	OH	H	H	>50	>50	>50	>50	>50	50	>50	50	50	>50	>50
25	Cl	OH	H	H	6.25	3.12	6.25	6.25	6.25	3.12	3.12	3.12	6.25	4.68	3.40
30	Br	OH	Br	H	6.25	1.56	6.25	12.5	12.5	3.12	6.25	3.12	6.25	6.64	5.47
56	- see Figure 3 -				3.12	0.09	3.12	3.12	3.12	1.56	3.12	6.25	3.12	2.36	2.34
65	- see Figure 3 -				3.12	0.09	3.12	3.12	3.12	1.56	3.12	3.12	3.12	2.36	2.47
66	- see Figure 3 -				6.25	0.78	12.5	12.5	6.25	3.12	6.25	12.5	12.5	8.01	8.85
69	- see Figure 3 -				0.39	0.05	0.78	0.39	0.78	0.39	0.78	0.78	0.78	0.40	1.10
82	- see Figure 3 -				1.56	0.78	1.56	0.78	0.78	1.56	1.56	1.56	1.56	1.17	1.30

[a] *Staphylococcus aureus* ATCC 6538. [b] *Staphylococcus aureus* ATCC 13709. [c] *Staphylococcus aureus* ATCC 29213. [d] *Staphylococcus aureus* ATCC 25923. [e] *Staphylococcus aureus* ATCC 700698, resistant to methicillin and heterogeneous susceptibility to vancomycin. [f] *Staphylococcus aureus* ATCC 43300, resistant to methicillin. [g] *Staphylococcus aureus* ATCC 700787, resistant to methicillin and intermediate susceptibility to vancomycin. [h] *Staphylococcus aureus* ATCC 700788, resistant to methicillin and intermediate susceptibility to vancomycin. [i] *Staphylococcus aureus* ATCC 700789, resistant to methicillin and intermediate susceptibility to vancomycin. [j] Average MIC calculated from four strains of *Staphylococcus aureus* (6538, 13709, 29213, and 25923). [k] Average MIC calculated from five strains of methicillin-resistant *Staphylococcus aureus* (700698, 43300, 700787, 700788, and 700798).

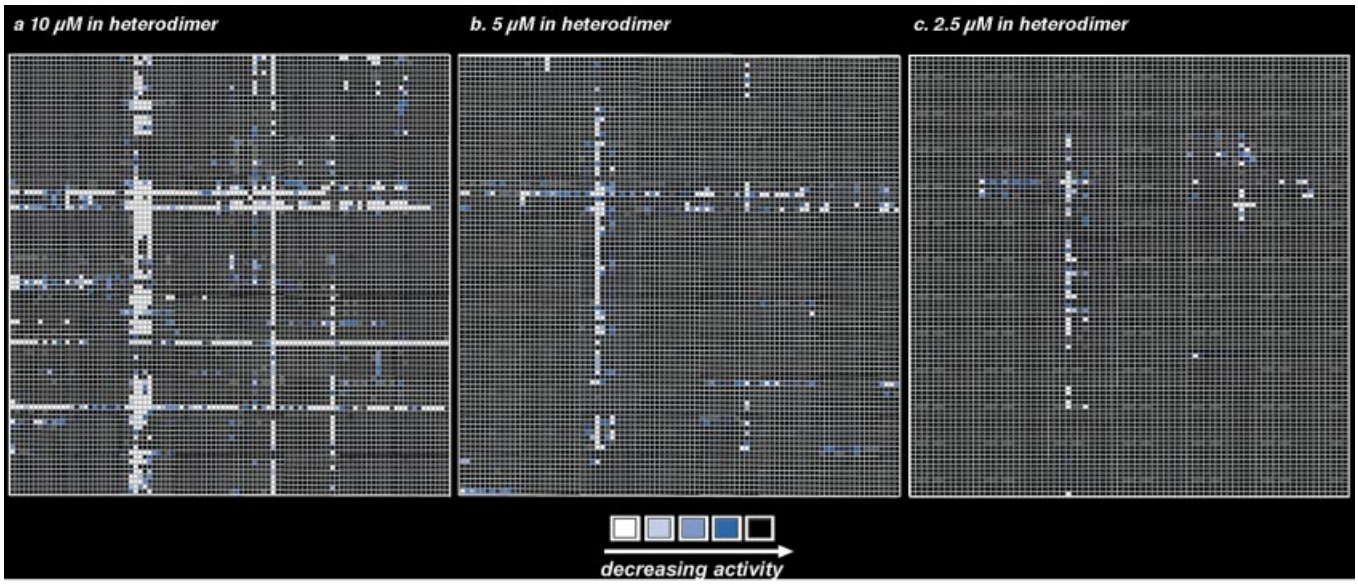


Figure 5. Biological profile of combinatorial disulfide library in *Staphylococcus aureus* ATCC 6538 at the indicated concentrations. See Figure 4 and text for a description of the library format.

tions and to compounds which were active in both the SA and MRSA (data not shown) bacterial strains.

Using this method, a collection of heterodimeric disulfides with presumed antibacterial activity was identified. These compounds fell into three general categories with representative examples shown in Figure 6. Type **A** heterodimers were comprised of two similar psammaplin-like components (see structure **102**). Type **B** heterodimers consisted of one psammaplin-like unit conjugated to an aryl or alkyl component (see structure **103**). Type **C** heterodimers bear no resemblance to the original natural product (see structure **104**). Compounds of type **A** were expected to be active, particularly in cases where both homodimeric components were biologically active (see Table 1 for antibacterial activity of homodimers). However, since the goal of this study was to identify structurally simpler and more potent analogues of psammaplin A, it was decided that compounds of this type would not be pursued further. Instead, efforts were focused on the

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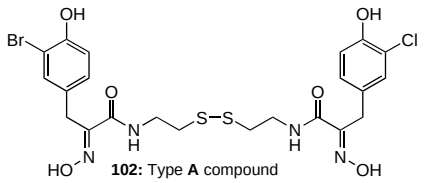
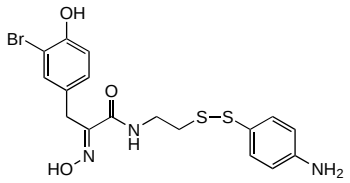
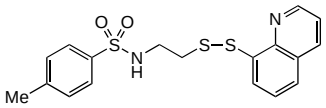


102: Type **A** compound



103: Type **B** compound



104: Type **C** compound

Figure 6. Representative examples from the three types (**A**–**C**) of biologically active heterodimers identified through library screening. See text for a description of the three types.

equally potent compound types **B** and **C** which, owing to their significantly lower molecular weights, might be better candidates for further optimization as compared to the natural product, psammaplin A (**1**).

Table 2 lists the compounds of type **B** identified through this screening method. Notably, most of the compounds of this type retained the halogenated tyrosine moiety of psammaplin A,with chloro- and bromo-aryl groups occurring interchangeably (for example, see **107** and **108**). Besides the halogenated tyrosine compounds, several phenylglycine derivatives (**123**–**125**) were also found to be active. After identification, the compounds of Table 2 were individually resynthesized by scrambling of the two homodimeric constituents using a procedure identical to that employed for the library construction with the exception that THF was used instead of DMSO to simplify product isolation. The desired heterodimer was separated from the two homodimeric starting materials by preparative TLC (thin-layer chromatography) to afford the product as a spectroscopically and chromatographically homogenous material. It should be noted, there was some concern as to the long-term stability of these purified heterodimers owing to their propensity towards slow re-equilibration to a homo/heterodimer mixture. To minimize this potential complication, all new heterodimers were screened immediately after purification, and subsequently stored at low temperature ($\leq 0\,^{\circ}C$) as DMSO solutions.

In order to avoid duplicity, in cases where both the bromo- and chloro-containing compounds (for example, see **107** and **108**) exhibited biological activity, only the chloro analogue was re-synthesized for these initial rescreening studies. Given that psammaplin A (**1**) and the corresponding chloro-analogue (**25**) exhibited identical antibacterial activities (see data, Table 1), this omission was not expected to result in a void of structure–activity information. Once identified and re-synthesized, these compounds were assayed in a panel of methicillin-susceptible and methicillin-resistant strains of *Staphylococcus aureus* and the results are shown in Table 2. In general, heterodimers containing aromatic or heteroaromatic moieties were more potent than those containing alkyl groups as illustrated by the fact that the three most active compounds, **105**, **113**, and **115**, included 4-aminophenyl, 2-quinyl, and 2-furyl groups, respectively. The most potent heterodimer, compound **105**, contained a 4-aminophenyl subunit and was approximately four-fold more active than psammaplin A (**1**) as an antibacterial agent.

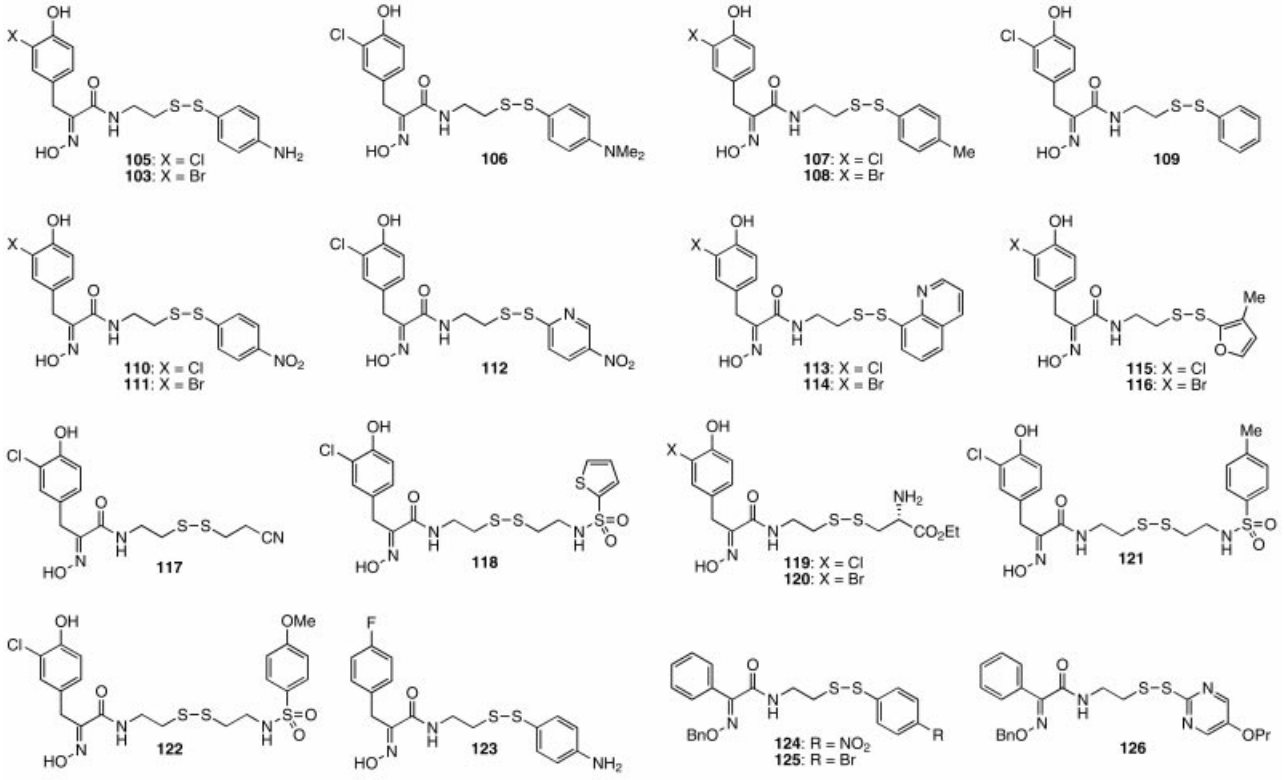
Interestingly, a qualitative assessment of the validity of the library screening method could be obtained by comparing the concentration at which a compound's activity was visibly detectable (i.e., Figure 5) with its average MIC values against SA and MRSA which were determined after re-synthesis. In general, there was a good correlation with the most potent analogues identified at the lowest concentration level (2.5 μM) and the least potent ones identified at the highest level (10.0 μM), as illustrated in Figure 5.

Active compounds identified as type **C** are shown in Table 3. The compounds contained an aryl sulfonamide motif linked through the disulfide bridge to an aromatic or heteroaromatic moiety. These compounds were usually less potent than those of type **B**. Like those of the previous category (type **C**), however, the MIC values obtained for these compounds compared favorably with the concentrations at which they were identified from screening of the initial library. Given the structural dissimilarity between these compounds and psammaplin A, it is currently not known whether or not they act at the same site or through the same mechanism as the natural product. However, the structural simplicity of these compounds coupled with the fact that several of them (for example sulfonamides **104** and **128**) are more potent that psammaplin A, suggests that their further investigation is warranted. Significantly, the identification of compounds such as **104** illustrates an important feature of this combinatorial approach which allows for the identification of novel, biologically active heterodimeric products formed from inactive homodimeric components (i.e., compounds **49** and **92**, see Figure 3). Indeed, it would have been highly unlikely that such a heterodimer (i.e., **104**) could have been discovered through a traditional SAR study.

Conclusion

In this article we have described a novel strategy for the efficient construction of a large combinatorial library of compounds based on the structure of psammaplin A (**1**), a marine-derived antibacterial agent. The disulfide exchange chemistry described (termed combinatorial exchange synthesis) provided a matrix in which each well contained two symmetrical disulfides and produced a third, heterodimeric, compound; the antibacterial profile of which was determined through direct screening. This rapid protocol for the construction and screening of a combinatorial library led to the identification of several structurally distinct psammaplin A analogues, including compounds **104**, **105**, **113**, **115**, **123**, and **128** (Table 4) all of which demonstrated higher antibacterial

Table 2. Type B heterodimers and corresponding MIC [μg mL^{-1}] values.



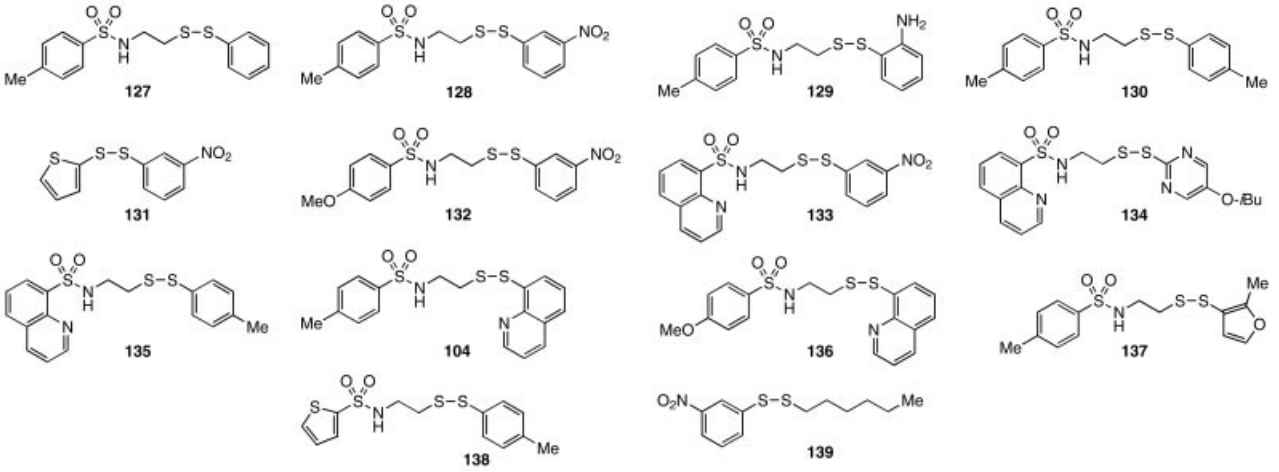
	SA[a] 6538	SA[b] 13709	SA[c] 29213	SA[d] 25923	MRSA[e] 700698	MRSA[f] 43300	MRSA[g] 700787	MRSA[h] 700788	MRSA[i] 700789	AVG[j] SA	AVG[k] MRSA	ID[l] LEVEL
1	6.25	3.12	6.25	6.25	6.25	3.12	3.12	6.25	3.12	5.47	4.37	-
25	6.25	3.12	6.25	6.25	6.25	3.12	3.12	3.12	6.25	4.68	4.37	-
105	1.56	0.19	1.56	1.56	3.12	0.78	0.78	0.78	0.78	1.22	1.25	2.5 μM
106	3.12	0.78	6.25	6.25	6.25	1.56	3.12	6.25	3.12	4.10	4.06	2.5 μM
107	3.12	0.39	1.56	1.56	12.5	1.56	1.56	1.56	1.56	1.65	3.74	5.0 μM
109	6.25	0.39	3.12	3.12	12.5	1.56	3.12	1.56	3.12	4.00	4.37	5.0 μM
110	6.25	0.09	6.25	6.25	12.5	3.12	3.12	3.12	3.12	4.71	5.00	10.0 μM
112	12.5	0.39	6.25	3.12	25	6.25	6.25	3.12	12.5	5.57	10.6	5.0 μM
113	3.12	0.39	3.12	3.12	3.12	0.39	1.56	3.12	3.12	2.43	2.26	5.0 μM
115	3.12	0.18	1.56	1.56	6.25	0.78	1.56	1.56	3.12	1.61	2.65	5.0 μM
117	6.25	6.25	6.25	6.25	12.5	3.12	3.12	6.25	6.25	6.25	6.25	5.0 μM
118	12.5	6.25	50	50	25	6.25	6.25	12.5	12.5	23.4	12.5	10.0 μM
119	12.5	1.56	6.25	6.25	50	3.12	12.5	12.5	12.5	5.14	18.1	10.0 μM
121	12.5	3.12	25	25	25	6.25	6.25	12.5	12.5	13.3	12.5	5.0 μM
122	25	6.25	25	25	25	12.5	12.5	12.5	12.5	20.3	15	5.0 μM
123	3.12	3.12	3.12	6.25	6.25	3.12	3.12	3.12	3.12	3.90	3.74	5.0 μM
124	25	0.78	6.25	6.25	12.5	12.5	3.12	12.5	12.5	12.1	10.6	10.0 μM
125	>50	25	>50	>50	>50	>50	>50	>50	>50	>50	>50	10.0 μM
126	25	1.56	25	25	25	25	12.5	50	25	19.1	27.5	10.0 μM

See Table 1, footnotes ([a]–[k]) for bacterial strain information. [l] ID level denotes the screening concentration at which the compound was identified as biologically active.

activity than the natural product. Interestingly, several of these analogues (especially compound **105**) have in vitro antibacterial properties similar to several clinically used antibiotics, including ciprofloxacin and vancomycin. More importantly, these newly discovered agents demonstrate consistent antibacterial activity, even in strains resistant to other antibiotics. In the following paper, we describe our efforts toward the optimization of several of these heterodimeric lead compounds examining issues such as potency, toxicity and non-specific protein binding as well as probing, through molecular design and chemical synthesis, the mechanism of action of this class of antibacterial agents.[13] In general, the simplicity, generality, and efficiency of the described combinatorial chemistry suggests its application in other situations. Several such extensions are currently under consideration, including construction of other focused com-

0947-6539/01/0719-4288 $ 17.50+.50/0 *Chem. Eur. J.* **2001**, *7*, No. 19

Table 3. Type C heterodimers and corresponding MIC [μg mL^{-1}] values.



	SA[a] 6538	SA[b] 13709	SA[c] 29213	SA[d] 25923	MRSA[e] 700698	MRSA[f] 43300	MRSA[g] 700787	MRSA[h] 700788	MRSA[i] 700789	AVG[j] SA	AVG[k] MRSA	ID[l] LEVEL
127	12.5	0.38	12.5	12.5	25	6.25	6.25	6.25	3.12	9.5	8.3	10.0 μM
128	6.25	0.04	3.12	3.12	6.25	1.56	1.56	3.12	3.12	3.14	2.63	10.0 μM
129	50	>50	25	12.5	25	12.5	6.25	12.5	12.5	29.1	13.8	10.0 μM
130	12.5	>50	25	6.25	25	12.5	6.25	12.5	12.5	14.6	13.8	5.0 μM
131	25	12.5	50	50	6.25	6.25	6.25	12.5	12.5	34.4	8.33	10.0 μM
132	>50	3.12	>50	>50	>50	>50	25	>50	>50	>50	>50	10.0 μM
133	12.5	0.39	12.5	12.5	12.5	6.25	3.12	6.25	6.25	9.47	5.99	10.0 μM
134	25	12.5	25	>50	25	50	12.5	50	25	20.8	28.1	10.0 μM
135	12.5	3.12	>50	>50	12.5	>50	3.12	12.5	6.25	>50	28.1	10.0 μM
104	12.5	0.78	6.25	3.12	6.25	3.12	3.12	3.12	3.12	4.1	2.75	5.0 μM
136	>50	>50	25	12.5	25	12.5	12.5	12.5	12.5	>50	13.1	5.0 μM
137	25	0.78	12.5	12.5	25	6.25	6.25	12.5	12.5	12.7	9.90	10.0 μM
138	25	0.39	6.25	12.5	25	6.25	6.25	12.5	6.25	7.91	9.90	10.0 μM
139	25	0.78	6.25	3.12	3.12	3.12	1.56	1.56	3.12	8.78	2.21	10.0 μM

See Table 1, footnotes ([a]–[k]) for bacterial strain information. [l] ID level denotes the screening concentration at which the compound was identified as biologically active.

binatorial libraries based on additional natural product scaffolds. Although rare, several other disulfide-containing natural products have been reported. Selected examples include the endothelin-converting enzyme inhibitor B-90063[20] (**143**, Figure 7), the alkaloid polycarpine[21] (**144**, Figure 7) an antitumor agent active against human colon cancer cells, the marine natural product citorellamine[22] (**145**, Figure 7) and the antibacterial agent cortamidine oxide[23] (**146**, Figure 7).

In a more general sense, the present combinatorial strategy may also be applied to the synthesis of other discovery-oriented (i.e., non-targeted) libraries useful in both drug discovery and chemical biology efforts. In fact, given the mildness and tolerance of the disulfide exchange reaction, one could envision its application to sets of highly diverse and structurally complex building blocks selected from the myriad of commercially available disulfides and thiols. Arguably, this dimerization strategy should enable the construction of more structurally diverse discovery libraries as compared to a conventional combinatorial strategy wherein a single central scaffold is derivatized. Moreover, one could envision that after identification of lead structures by this combinatorial strategy, the disulfide linkage might be excised and replaced with other more desirable structural units during the normal lead optimization process.

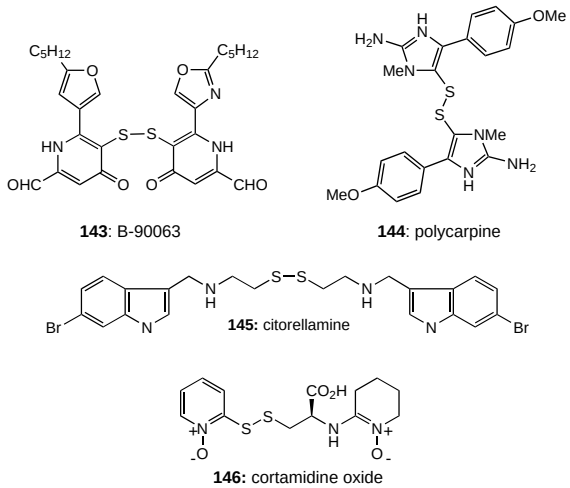
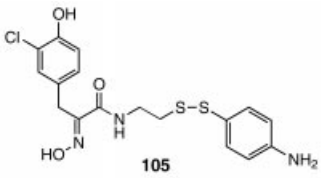
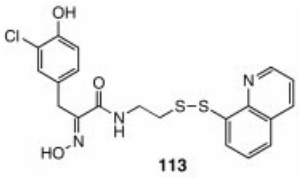


Figure 7. Structures of other selected dimeric natural products linked through a disulfide moiety.

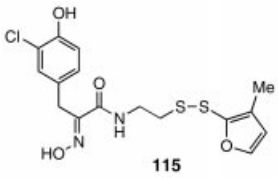
Table 4. Antibacterial activities (MIC μg mL^{-1}) of lead heterodimers identified from combinatorial library.



105



113



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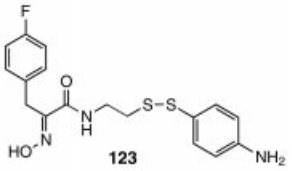

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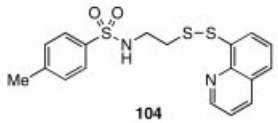
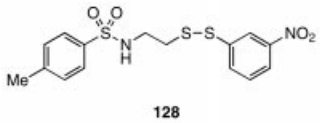


104



128

	SA[a] 6538	SA[b] 13709	SA[c] 29213	SA[d] 25923	MRSA[e] 700698	MRSA[f] 43300	MRSA[g] 700787	MRSA[h] 700788	MRSA[i] 700789	AVG[j] SA	AVG[k] MRSA
1: psammaplin A	6.25	3.12	6.25	6.25	6.25	3.12	3.12	6.25	3.12	5.47	3.96
105	1.56	0.19	1.56	1.56	3.12	0.78	0.78	0.78	0.78	1.22	1.11
113	3.12	0.39	3.12	3.12	3.12	0.39	1.56	3.12	3.12	2.43	1.40
115	3.12	0.19	1.56	1.56	6.25	0.78	1.56	1.56	3.12	1.61	2.24
123	3.12	3.12	3.12	6.25	6.25	3.12	3.12	3.12	3.12	3.90	3.12
104	12.5	0.78	6.25	3.12	6.25	3.12	3.12	3.12	3.12	4.10	2.45
128	6.25	0.04	3.12	3.12	6.25	1.56	1.56	3.12	3.12	3.14	2.63
140: ciprofloxacin	3.12	0.04	0.19	0.19	25	0.19	25	25	25	0.89	16.7
141: vancomycin	0.78	0.19	0.78	1.56	1.56	0.78	12.5	1.56	3.12	0.83	5.34

See Table 1, footnotes ([a]–[k]) for bacterial strain information.

Experimental Section

General: All reactions were conducted under an argon atmosphere using anhydrous solvents, unless otherwise noted. Anhydrous THF, diethyl ether (ether), and methylene chloride were obtained by passing them through commercially available alumina columns. All reagents were purchased at the highest commercial quality and used without further purification. Reactions were monitored by thin-layer chromatography carried out on 0.25 mm E. Merck silica glass plates (60F-254) using UV light or *p*-anisaldehyde solution as visualizing agents. Flash chromatography was performed on E. Merck silica gel (60, particle size 0.0400–0.063 mm). Preparative thin-layer chromatography was conducted on 0.25 E. Merck silica gel plates (60F-254). All solution phase combinatorial reactions were conducted in 2 mL, 96-well Whatman Uniplates (7701–5200). All reported yields refer to chromatographically and spectroscopically (^{1}H NMR) homogenous materials. NMR spectra were recorded on Bruker AMX-500 or AMX-400 instruments and calibrated using residual undeuterated solvent as an internal reference. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Mass spectra were recorded on a VG ZAB ZSE mass spectrometer under MALDI-FTMS conditions using DHB as matrix.

Synthesis of psammaplin A (1): A solution of 3-bromotyrosine[14] (**3**) (10.0 g, 1.0 equiv, 40.7 mmol) in trifluoroacetic anhydride (70 mL) was heated to 40 °C for 12 h. The excess trifluoroacetic anhydride was then removed under reduced pressure in order to provide trifluromethyloxazolone **4**. This unstable intermediate was dissolved in 70% aqueous TFA (50 mL) and allowed to stand for 6 h during which time a white precipitate formed. This solid was isolated by vacuum filtration and washed with cold water (2 × 15 mL) to provide α-keto acid **5** (6.34 g, 60%) as previously reported.[13] To a solution of α-keto acid **5** (1.0 g, 1.0 equiv, 3.86 mmol) in ethanol (15 mL) was added *O*-(tetrahydro-2*H*-pyran-2-yl)-hydroxylamine (903 mg, 2.0 equiv, 0.72 mmol) and the reaction mixture was stirred at 23 °C for 12 h. The reaction mixture was then concentrated under reduced pressure and the resulting oil (**9**) was dissolved in EtOAc (50 mL) and washed with 1.0 N HCl (2 × 50 mL) and brine (1 × 50 mL). The organic layer was then dried over $MgSO_4$ and concentrated to afford acid **6** which was utilized without further purification. This crude acid **6** was dissolved in dioxane (10 mL) and to this solution was added *N*-hydroxylsuccinimide (843 mg, 1.9 equiv, 7.33 mmol) and EDC (1.29 g, 1.7 equiv, 6.56 mmol) and the reaction was stirred at 23 °C for 2 h after which time it was concentrated and the resulting oil dissolved in EtOAc (50 mL) and washed with saturated $NaHCO_3$ (2 × 50 mL), 1.0 N HCl (2 × 50 mL) and brine (1 × 50 mL). The organic layer was then dried over $MgSO_4$ and concentrated to affort succinate ester **7** which was used without further purification. This crude ester **7** was dissolved in dioxane (10 mL) and to this solution was added a second solution of cystamine (313 mg, 0.5 equiv, 1.39 mmol) and Et_3N (0.7 mL, 2.0 equiv, 5.5 mmol) in MeOH (10 mL). The resulting reaction mixture was stirred at 23 °C for 12 h after which point it was concentrated and the crude amide produce was passed over a short silica gel column (50% EtOAc in hexanes) to remove any unacylated or monoacylated cystamine. Without further purification, the resulting coupling product **8** was dissolved in CH_2Cl_2/MeOH 20:1 (10 mL), placed in a pressure tube, and anhydrous HCl (10 mL of a 1.0 M ether solution) was added. The solution was then heated to 60 °C for 2 h, and after cooling, was concentrated under reduced pressure to give a crude yellow oil which was purified by column chromatography (silica gel, 10 → 100% EtOAc in hexanes) to afford psammaplin A (**1**) (918 mg, 36% over four steps). R_f = 0.10 (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max}$ = 3379, 1651, 1513, 1427, 1360, 1279, 1212, 1040, 1007 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD): δ = 7.25 (d, J = 1.8 Hz, 2H), 6.96 (dd, J = 8.5, 2.0 Hz, 1H), 6.65 (d, J = 8.2 Hz, 1H), 3.68 (s, 2H), 3.40 (t, J = 6.8 Hz, 2H), 2.68 (t, J = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ = 166.0, 153.8, 153.3, 134.6, 130.7, 130.6, 117.2, 110.6, 39.7, 38.6, 28.8; HRMS (MALDI-FTMS): calcd for $C_{22}H_{24}Br_2N_4O_6S_2$ $[M+Na]^+$: 684.9396, found: 684.9426.

Homodimers **13**–**36** were synthesized in an identical fashion to that described for psammaplin A and selected physical data for representative members is given below.

Compound 13: R_f = 0.25 (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max}$ = 3350, 2917, 1652, 1528, 1451, 1428, 1203, 1103, 1051 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.14 (d, J = 8.1 Hz, 4H), 7.09 (t, J = 7.9 Hz, 4H), 7.01 (t, J = 7.9 Hz, 2H), 3.80 (s, 4H), 3.38 (t, J = 6.7 Hz, 4H), 2.66 (t, J = 6.7 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.1, 153.3, 138.2, 130.2, 129.5, 127.4, 39.7, 38.6, 30.2; HRMS (MALDI-FTMS): calcd for $C_{22}H_{26}N_4O_4S_2$ $[M+Na]^+$: 497.1288, found: 497.1284.

Compound 14: R_f = 0.71 (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max}$ = 3335, 2935, 1667, 1520, 1452, 1213, 1045 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$):

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$\delta = 7.30-7.22$ (m, 8H), 7.17 (t, $J = 6.2$ Hz, 2H), 7.08 (t, $J = 5.6$ Hz, 2H), 3.99 (s, 6H), 3.91 (s, 4H), 3.60 (q, $J = 6.4$ Hz, 4H), 2.80 (t, $J = 6.4$ Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 162.7$, 151.7, 136.1, 129.2, 126.4, 126.3, 62.9, 38.2, 37.5, 29.7; HRMS (MALDI-FTMS): calcd for $C_{24}H_{30}N_4O_4S_2$ $[M+Na]^+$: 525.1601, found: 525.1585.

Compound 15: $R_f = 0.74$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 1666, 1519, 1020, 737, 690 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.36-7.18$ (m, 20H), 7.08 (m, 2H), 5.24 (s, 4H), 3.99 (s, 4H), 3.60 (m, 4H), 2.76 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 162.9$, 153.2, 137.3, 136.2, 130.1, 128.5, 128.4, 128.1, 128.0, 126.4, 39.2, 38.1, 30.0; HRMS (MALDI-FTMS): calcd for $C_{36}H_{38}N_4O_4S_2$ $[M+Na]^+$: 677.227, found: 677.2250.

Compound 16: $R_f = 0.05$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3307, 2920, 1660, 1508, 1438, 1221, 1018, 670 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.30$ (dd, $J = 8.8$, 5.3 Hz, 4H), 7.16 (m, 2H), 6.93 (t, $J = 8.8$ Hz, 4H), 3.93 (s, 4H), 3.62 (q, $J = 6.2$ H, 4H), 2.82 (t, $J = 6.2$ Hz, 4H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 164.2$, 161.8, 153.2, 134.2, 132.0, 116.1, 39.7, 38.6, 29.3; HRMS (MALDI-FTMS): calcd for $C_{22}H_{24}F_2N_4O_4S_2$ $[M+Na]^+$: 533.1099, found: 533.1080.

Compound 17: $R_f = 0.25$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3409, 2939, 1670, 1514, 1431, 1222, 1046, 914, 823, 732 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.25$ (m, 4H), 7.08 (m, 2H), 6.91 (t, $J = 8.8$ Hz, 4H), 3.98 (s, 6H), 3.86 (s, 4H), 3.61 (q, $J = 6.5$ Hz, 4H), 2.80 (t, $J = 6.4$ Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 162.7$, 160.3, 151.6, 131.8, 130.8, 115.0, 63.0, 38.2, 37.6, 28.9; HRMS (MALDI-FTMS): calcd for $C_{24}H_{28}F_2N_4O_4S_2$ $[M+Na]^+$: 561.1412, found: 561.1423.

Compound 18: $R_f = 0.73$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 1669, 1604, 1508, 1427, 1219, 1014, 821 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.37-7.21$ (m, 14H), 7.05 (t, $J = 5.8$ Hz, 2H), 6.90 (t, $J = 8.5$ Hz, 4H), 5.21 (s, 4H), 3.90 (s, 4H), 3.59 (q, $J = 6.2$ Hz, 4H), 2.78 (t, $J = 6.2$ Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 162.5$, 152.1, 136.6, 131.7, 130.9, 130.8, 128.5, 128.3, 128.1, 115.2, 114.9, 38.2, 37.5, 29.2; HRMS (MALDI-FTMS): calcd for $C_{36}H_{36}F_2N_4O_4S_2$ $[M+H]^+$: 691.2219, found: 691.2237.

Compound 20: $R_f = 0.75$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3404, 2937, 1669, 1520, 1212, 1091, 1046, 915, 804, 730 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.23-7.18$ (br s, 8H), 7.07 (m, 2H), 3.98 (s, 6H), 3.86 (s, 4H), 3.61 (q, $J = 6.4$ Hz, 4H), 2.80 (t, $J = 6.4$ Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 162.5$, 151.3, 134.6, 132.2, 130.6, 128.5, 63.1, 38.2, 37.6, 29.1.

Compound 21: $R_f = 0.74$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 1669, 1518, 1486, 1436, 1360, 1208, 1015 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.38-7.33$ (m, 6H), 7.28 (d, $J = 7.3$ Hz, 4H), 7.21 (m, 8H), 7.10 (t, $J =$ 5.9 Hz, 2H), 5.22 (s, 4H), 3.92 (s, 4H), 3.60 (q, $J = 6.2$ Hz, 4H), 2.79 (t, $J =$ 6.2 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 162.9$, 152.0, 136.5, 134.5, 132.2, 130.5, 128.7, 128.6, 128.4, 128.2, 77.6, 34.1, 33.3, 29.2; HRMS (MALDI-FTMS): calcd for $C_{36}H_{36}Cl_2N_4O_4S_2$ $[M+Na]^+$: 745.1447, found: 745.1478.

Compound 22: $R_f = 0.20$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3369, 2920, 1739, 1677, 1648, 1510, 1458, 1372, 1205, 1128, 1090 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD): $\delta = 6.95$ (d, $J = 8.5$ Hz, 4H), 6.52 (d, $J =$ 8.8 Hz, 4H), 3.68 (s, 4H), 3.38 (t, $J = 6.8$ Hz, 4H), 2.66 (t, $J = 6.8$ Hz, 4H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 163.6$, 156.9, 132.1, 131.3, 128.9, 116.2, 39.7, 38.6, 29.2; HRMS (MALDI-FTMS): calcd for $C_{22}H_{26}N_4O_6S_2$ $[M+Na]^+$: 529.1186, found: 529.1189.

Compound 23: $R_f = 0.30$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3413, 1651, 1513, 1437, 1355, 1222, 1040, 1012, 950 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.17$ (t, $J = 5.9$ Hz, 2H), 7.07 (br d, $J = 8.2$ Hz, 4H), 6.67 (br d, $J = 8.5$ Hz, 4H), 3.97 (s, 6H), 3.80 (s, 4H), 3.53 (q, $J =$ 6.2 Hz, 4H), 2.71 (t, $J = 6.2$ Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 163.2, 154.7, 152.1, 130.3, 127.4, 115.5, 63.0, 38.4, 37.5, 28.8; HRMS (MALDI-FTMS): calcd for $C_{24}H_{30}N_4O_6S_2$ $[M+Na]^+$: 557.1499, found: 577.1502.

Compound 24: $R_f = 0.05$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3347, 1659, 1513, 1225, 1015, 732 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta =$ 7.35–7.28 (m, 10H), 7.17 (m, 2H), 7.08 (d, $J = 8.5$ Hz, 4H), 6.68 (d, $J =$ 8.6 Hz, 4H), 5.21 (s, 4H), 3.87 (s, 4H), 3.52 (m, 4H), 2.65 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 163.2$, 154.2, 153.1, 135.9, 131.2, 128.7, 128.5, 128.3, 115.3, 38.7, 37.5, 29.1; HRMS (MALDI-FTMS): calcd for $C_{36}H_{38}N_4O_6S_2$ $[M+H]^+$: 687.2305, found: 687.2293.

Compound 25: $R_f = 0.11$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3409, 1655, 1512, 1427, 1287, 1215, 1013, 950 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.10$ (d, $J = 1.8$ Hz, 2H), 6.93 (dd, $J = 8.2$, 2.0 Hz, 2H), 6.67 (d, $J = 8.2$ Hz, 2H), 3.69 (s, 4H), 3.42 (t, $J = 7.0$ Hz, 4H), 2.69 (t, $J = 6.8$ Hz, 4H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 166.0$, 153.2, 152.7, 131.6, 130.4, 129.8, 121.4, 117.5, 102.6, 39.7, 38.6, 28.9; HRMS (MALDI-FTMS): calcd for $C_{22}H_{24}Cl_2N_4O_6S_2$ $[M+Na]^+$: 597.0406, found: 597.0406.

Compound 26: $R_f = 0.14$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3388, 2938, 2360, 1660, 1525, 1427, 1047, 909, 732 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.21$ (d, $J = 2.0$ Hz, 2H), 7.12 (t, $J = 5.8$ Hz, 2H), 7.05 (dd, $J =$ 8.2, 1.8 Hz, 2H), 6.83 (d, $J = 8.5$ Hz, 2H), 6.19 (br s, 2H), 3.99 (s, 6H), 3.79 (s, 4H), 3.59 (q, $J = 6.2$ Hz, 4H), 2.78 (t, $J = 6.5$ Hz 4H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 162.7$, 151.4, 150.2, 129.7, 129.2, 129.0, 119.8, 116.2, 63.1, 38.3, 37.5, 28.6; HRMS (MALDI-FTMS): calcd for $C_{24}H_{28}Cl_2N_4O_6S_2$ $[M+H]^+$: 603.0900, found: 603.0892.

Compound 27: $R_f = 0.03$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3384, 1660, 1502, 1437, 1365, 1287, 1208, 1012 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.35-7.30$ (m, 10H), 7.23 (m, 2H), 7.07–7.05 (m, 4H), 6.83 (dd, $J = 8.2$, 3.2 Hz, 2H), 5.21 (d, $J = 2.9$ Hz, 2H), 3.82 (br s, 4H), 3.58 (m, 4H), 2.75 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 162.6$, 151.9, 150.1, 136.5, 129.8, 129.4, 129.1, 128.6, 128.3, 128.2, 119.7, 126.1, 38.3, 37.4, 28.8; HRMS (MALDI-FTMS): calcd for $C_{36}H_{36}Cl_2N_4O_6S_2$ $[M+Na]^+$: 777.1345, found: 777.1349.

Compound 28: $R_f = 0.05$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3381, 1659, 1527, 1424, 1215, 1045, 910, 731, 671 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.37$ (d, $J = 2.0$ Hz, 2H), 7.12 (dd, $J = 8.2$, 2.0 Hz, 2H), 7.10 (t, $J = 7.64$ Hz, 2H), 6.86 (d, $J = 8.2$ Hz, 2H), 4.00 (s, 6H), 3.80 (s, 4H), 3.60 (q, $J = 6.2$ Hz, 4H), 2.79 (t, $J = 6.4$ Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 162.6, 151.4, 150.9, 132.6, 130.1, 129.6, 115.9, 109.9, 63.1, 38.3, 37.6, 28.5; HRMS (MALDI-FTMS): calcd for $C_{24}H_{28}Br_2N_4O_6S_2$ $[M+Na]^+$: 712.9709, found: 712.9687.

Compound 29: $R_f = 0.13$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3375, 2360, 1660, 1526, 1423, 1016 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta =$ 7.39–7.30 (m, 8H), 7.14 (t, $J = 5.8$ Hz, 2H), 7.09 (dd, $J = 8.2$, 1.8 Hz, 2H), 6.82 (d, $J = 8.0$ Hz, 2H), 5.22 (s, 4H), 3.83 (s, 4H), 3.58 (q, $J = 6.5$ Hz, 4H), 2.74 (t, $J = 6.2$ Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 162.7$, 152.0, 151.5, 136.5, 132.9, 130.1, 129.7, 128.6, 128.3, 128.1, 116.8, 110.6, 38.6, 37.6, 28.5; HRMS (MALDI-FTMS): calcd for $C_{36}H_{36}Br_2N_4O_6S_2$ $[M+Na]^+$: 865.0335, found: 865.0327.

Compound 30: $R_f = 0.20$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3397, 1734, 1639, 1458, 1381, 1229 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD): $\delta =$ 7.28 (s, 4H), 3.68 (s, 4H), 3.42 (t, $J = 6.8$ Hz, 2H), 2.70 (q, $J = 6.7$ Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 165.8$, 152.7, 150.8, 134.1, 132.4, 112.1, 39.8, 38.7, 28.6; HRMS (MALDI-FTMS): calcd for $C_{22}H_{22}Br_4N_4O_6S_2$ $[M+Na]^+$: 840.7606, found: 840.7606.

Compound 31: $R_f = 0.21$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3394, 1665, 1526, 1474, 1046, 910, 734 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.35$ (s, 4H), 7.09 (t, $J = 5.8$ Hz, 2H), 3.99 (s, 6H), 3.76 (s, 4H), 3.61 (q, $J = 6.2$ Hz, 4H), 2.81 (t, $J = 6.4$ Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 162.4, 150.8, 148.1, 132.7, 130.6, 109.7, 63.2, 38.3, 37.5, 28.2; HRMS (MALDI-FTMS): calcd for $C_{24}H_{26}Br_4N_4O_6S_2$ $[M+Na]^+$: 868.7919, found: 868.7882.

Compound 32: $R_f = 0.24$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3387, 2358, 1665, 1524, 1474, 1016 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta =$ 7.38–7.28 (m, 7H), 7.07 (t, $J = 5.8$ Hz, 2H), 5.90 (br s, 2H), 5.21 (s, 4H), 3.80 (s, 4H), 3.61 (q, $J = 6.2$ Hz, 4H), 2.79 (t, $J = 6.2$ Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 162.3$, 151.3, 148.0, 136.3, 132.8, 130.6, 128.7, 128.5, 128.4, 128.3, 128.2, 109.6, 77.6, 38.3, 37.5, 28.4; HRMS (MALDI-FTMS): calcd for $C_{36}H_{34}Br_4N_4O_6S_2$ $[M+Na]^+$: 1020.8545, found: 1020.8589.

Compound 34: $R_f = 0.34$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3277, 2936, 1652, 1530, 1443, 1257, 1051, 911, 731, 691, 648 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.61-7.58$ (m, 4H), 7.39–7.33 (m, 6H), 3.97 (s, 6H), 6.75 (m, 2H), 3.70 (q, $J = 6.2$ Hz, 4H), 2.88 (t, $J = 6.4$ Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 163.4$, 152.5, 131.2, 130.1, 128.6, 126.7, 62.8, 38.2, 37.3; HRMS (MALDI-FTMS): calcd for $C_{22}H_{26}N_4O_4S_2$ $[M+Na]^+$: 497.1288, found: 497.1283.

Compound 35: $R_f = 0.40$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3283, 1653, 1525, 1443, 1360, 1231, 1016 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.61$ (d, $J = 6.4$ Hz, 4H), 7.38–7.28 (m, 20H), 6.64 (t, $J = 5.9$ Hz, 2H), 5.21 (s, 4H), 3.66 (m, 4H), 2.78 (t, $J = 6.4$ Hz, 4H); ^{13}C NMR (100 MHz,

$CDCl_3$): $\delta = 163.4, 152.9, 137.1, 130.1, 129.6, 128.6, 128.5, 128.3, 128.1, 77.6, 38.1, 37.2$; HRMS (MALDI-FTMS): calcd for $C_{34}H_{34}N_4O_4S_2$ $[M+Na]^+$: 649.1914, found: 649.1907.

Compound 36: $R_f = 0.45$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3338, 1659, 1514, 1444, 1227, 1046, 731 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.10$ (m, 2H), 6.85 (brs, 2H), 6.78 (brs, 4H), 5.53 (s, 2H), 3.99 (s, 6H), 3.84 (s, 6H), 3.82 (s, 4H), 3.69 (q, $J = 6.2$ Hz, 4H), 2.80 (t, $J = 6.5$ Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 162.8, 151.2, 145.1, 144.2, 127.8, 122.0, 114.2, 112.0, 62.9, 55.8, 38.2, 37.6, 29.3$; HRMS (MALDI-FTMS): calcd for $C_{26}H_{34}N_4O_8S_2$ $[M+H]^+$: 595.1891, found: 595.1905.

Synthesis of di-amino psammaplin A analogues 11 and 12: Di-*tert*-butyldicarbonate (974 mg, 1.1 equiv, 4.47 mmol) was added to a solution of 3-bromotyrosine (1.0 g, 1.0 equiv, 4.06 mmol) and $NaHCO_3$ (1.71 g, 5.0 equiv, 20.3 mmol) in THF/H_2O 1:1 (10 mL). The reaction mixture was stirred at 23 °C for 12 h, and the THF was then removed under reduced pressure. To the residual reaction contents was added EtOAc (20 mL) and the solution was then acidified to pH 4.5 using 1.0 N HCl. The organic layer was washed with brine (1×20 mL), dried over $MgSO_4$, and concentrated to afford *N*-Boc-3-bromotyrosine (**9**) which was utilized without further purification. This product was added dissolved in dioxane (15 mL) and *N*-hydroxysuccinimide (887, 1.9 equiv, 7.71 mmol) and EDC (1.49 g, 1.7 equiv, 7.60 mmol) were added. The reaction mixture was then stirred at 23 °C for 2 h after which time it was concentrated under reduced pressure and the resulting oil was taken up into EtOAc (50 mL). This solution was washed with 0.5 N HCl (1×50 mL), saturated $NaHCO_3$ (2×50 mL), and brine (1×50 mL) before being dried over $MgSO_4$ and concentrated to provide succinamate ester **10** which was utilized without further purification. This activated ester was immediately dissolved in dioxane (10 mL) and to this solution was added a second solution of cystamine (502 mg, 0.5 equiv, 2.23 mmol) and Et_3N (1.25 mL, 2.0 equiv, 8.92 mmol) in MeOH (10 mL). The resulting reaction mixture was stirred at 23 °C for 12 h and then concentrated and purified by flash chromatography (silica gel, 25% EtOAc in hexanes) to afford di-*N*-Boc-amino-psammaplin A (**11**) (1.55 g, 46% over three steps). $R_f = 0.44$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} = 3309$, 1655, 1366, 1249, 1165, 1045, 909, 733 cm^{-1}; ^1H NMR (400 MHz, CD_3OD): $\delta = 7.32$ (m, 1H), 7.01 (m, 1H), 6.85 (d, $J = 7.6$ Hz, 1H), 5.77 (m, 1H), 4.45 (m, 1H), 3.46–3.39 (m, 2H), 2.91 (brs, 2H), 2.70–2.45 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 172.2, 155.9, 151.7, 133.2, 129.8, 116.2, 110.0, 80.3, 60.4, 55.9$; HRMS (MALDI-FTMS): calcd for $C_{32}H_{44}Br_2N_4O_8S_2$ $[M+Na]^+$: 857.0859, found: 857.0881.

A portion of compound **11** (700 mg, 1.0 equiv, 0.84 mmol) was dissolved in CH_2Cl_2 (25 mL). TFA (5.0 mL) was then added and the reaction mixture was stirred at 23 °C for 2 h before being concentrated under reduced pressure to afford a crude yellow oil which was purified by flash chromatography (silica gel, 10 → 100% EtOAc in hexanes) to provide di-amino-psammaplin A **12** (505 mg, 95%). $R_f = 0.12$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} = 3393$, 1642, 1236, 1045 cm^{-1}; ^1H NMR (400 MHz, CD_3OD): $\delta = 7.11$ (d, $J = 2.0$ Hz, 1H), 6.80 (dd, $J = 8.2$ Hz, 1H), 6.61 (d, $J = 8.2$ Hz, 1H), 3.73 (t, $J = 7.0$ Hz, 1H), 3.30 (m, 1H), 3.12 (m, 1H), 2.75 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 172.2, 157.6, 137.6, 133.3, 130.4, 120.1, 113.7, 58.3, 42.0, 40.3, 39.9, 30.2$; HRMS (MALDI-FTMS): calcd for $C_{22}H_{28}Br_2N_4O_4S_2$ $[M+Na]^+$: 656.9811, found: 656.9804. Homodimers **38**–**48** were synthesized in an identical fashion to that described for compounds **11** and **12** and selected physical data for representative members is given below.

Compound 38: $R_f = 0.43$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 2945, 1670, 1480, 1199, 1137 cm^{-1}; ^1H NMR (600 MHz, CD_3OD): $\delta = 5.76–5.69$ (m, 10H), 2.48–2.39 (m, 4H), 1.93 (m, 2H), 1.80 (m, 2H), 1.57 (m, 2H), 1.51 (m, 2H), 1.10–1.06 (m, 4H); ^{13}C NMR (100 MHz, CD_3OD): $\delta =$ 169.7, 135.6, 130.5, 130.1, 128.8, 55.8, 39.5, 38.7, 37.8; HRMS (MALDI-FTMS): calcd for $C_{22}H_{30}N_4O_2S_2$ $[M+H]^+$: 447.1883, found: 447.1899.

Compound 39: $R_f = 0.45$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3299, 2975, 1657, 1528, 1366, 1247, 1169, 733 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): $\delta = 7.24–7.20$ (m, 10H), 7.00 (brs, 2H), 5.60 (brs, 2H), 4.49 (m, 2H), 3.68 (m, 2H), 3.02 (m, 4H), 2.63 (m, 2H), 2.53 (m, 2H), 1.37 (s, 18H); ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 172.1, 155.6, 136.8, 129.4, 128.4, 126.7, 79.9, 55.8, 38.9, 38.1, 37.6, 28.3$; HRMS (MALDI-FTMS): calcd for $C_{32}H_{46}N_4O_6S_2$ $[M+Na]^+$: 669.2751, found: 669.2769.

Compound 42: $R_f = 0.05$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3412, 2360, 1675, 1201, 1143, 1018, 952 cm^{-1}; ^1H NMR (400 MHz, CD_3OD): $\delta = 6.89$ (q, $J = 10.2$ Hz, 8H), 3.61 (t, $J = 7.3$ Hz, 2H), 2.97–2.88 (m, 4H), 2.69–2.64 (m, 4H), 2.18–2.14 (m, 4H); ^{13}C NMR (100 MHz, CD_3OD): $\delta =$ 169.7, 137.1, 132.5, 131.9, 124.9, 55.6, 39.6, 38.1, 37.9; HRMS (MALDI-FTMS): calcd for $C_{22}H_{32}N_6O_2S_2$ $[M+H]^+$: 477.2101, found: 477.2101.

Compound 44: $R_f = 0.15$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 2930, 2354, 1667, 1518, 1349, 1197, 1136 cm^{-1}; ^1H NMR (600 MHz, CD_3OD): $\delta = 6.64$ (d, $J = 8.8$ Hz, 4H), 5.95 (d, $J = 8.8$ Hz, 4H), 2.58 (t, $J = 7.0$ Hz, 2H), 1.95 (m, 2H), 1.82 (m, 2H), 1.74–1.65 (m, 4H), 1.11 (m, 4H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 169.2, 148.9, 143.4, 131.8, 124.9, 55.1, 39.3, 38.1, 37.7$.

Compound 45: $R_f = 0.18$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3312, 1679, 1652, 1519, 1347, 1166, 910, 733 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 8.10$ (d, $J = 8.5$ Hz, 4H), 7.40 (d, $J = 8.2$ Hz, 4H), 5.79 (d, $J =$ 8.2 Hz, 2H), 3.54–3.38 (m, 4H), 3.17 (dd, $J = 13.5$, 6.5 Hz, 2H), 3.08 (dd, $J = 13.5$, 8.2 Hz, 2H), 2.72–2.69 (m, 1H), 2.59–2.56 (m, 1H), 1.35 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 171.8, 155.8, 146.9, 144.8, 130.3, 123.5, 80.3, 55.2, 38.7, 38.2, 37.7, 28.3$; HRMS (MALDI-FTMS): calcd for $C_{32}H_{44}N_6O_{10}S_2$ $[M+Na]^+$: 759.2452, found: 759.2451.

Compound 47: $R_f = 0.11$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 2970, 2408, 1667, 1475, 1198, 837, 800, 722 cm^{-1}; ^1H NMR (600 MHz, CD_3OD): $\delta = 3.63–3.61$ (m, 4H), 3.47 (m, 2H), 2.84 (m, 4H), 2.15 (m, 2H), 1.02 (t, $J = 6.1$ Hz, 12H); ^{13}C NMR (150 MHz, CD_3OD): $\delta = 169.7, 59.8, 39.7, 38.2, 31.7, 19.0, 18.0$.

Synthesis of the combinatorial library (Figure 5): Prior to synthesis, eighty-eight 2 mL, 96-well plates were positioned in an 8×11 array. To each well was added 334 μL of a DMSO/buffer 3:1 (pH 8.3) solution. Subsequently, 8 μL of a 50 mM stock solution of each homodimer was added to every well in the row and column designated for that compound (see Figure 4 for further clarification). Once all homodimers were dispensed, 50 μL of a 2.4 mM solution of dithiothrietol was added to every well. The plates were individually agitated and then allowed to stand at 23 °C for 12 h. Once the synthesis was complete, the products from 100 representative wells were sampled and analyzed by HPLC for overall purity and product distribution. See Supporting Information for further details and tabulated data.

Antibacterial screening of combinatorial library: Initially, three series of dilution plates were prepared from the original synthesis plates at concentrations of 1000 μM, 500 μM, and 250 μM in DMSO. The library was then screened from these three sets of dilution plates in SA 6538 (grown in Tryhcase Soy Broth, BBL 11043) and MRSA 700698 (grown in Brain Heart Infusion broth, DIFCO 237400). In each strain and for each concentration, the compounds were transferred to 96-well plates containing inoculated broth so as to give final compound concentrations of 10.0 μM, 5.0 μM, and 2.5 μM. After addition of the compounds, the plates were incubated at 37 °C for 24 h and then read for turbidity at 600 nm using a Molecular Devices Spectra MAX instrument. Graphical representations of this data were then generated and assembled as shown in Figure 5.

Re-synthesis of biologically active heterodimers: Heterodimers identified as active through screening of the combinatorial were re-synthesized and purified. These compounds were synthesized in an identical fashion to the combinatorial reactions with the exception that THF was substituted for DMSO to facilitate work up procedures. All compounds were purified by preparative thin-layer chromatography and spectroscopically characterized. Selected physical data for representative members is given below.

Compound 103: $R_f = 0.54$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3401, 1661, 1514, 1430, 1290, 1223, 1019, 826, 707 cm^{-1}; ^1H NMR (400 MHz, CD_3OD): $\delta = 7.44$ (brs, 1H), 7.31 (d, $J = 5.4$ Hz, 2H), 7.14 (dd, $J = 6.5$, 1.3 Hz, 1H), 6.83 (d, $J = 6.7$ Hz, 1H), 6.70 (d, $J = 5.2$ Hz, 2H), 3.86 (s, 2H), 3.63 (t, $J = 5.3$ Hz, 2H), 2.88 (t, $J = 5.2$ Hz, 2H); ^{13}C NMR (125 MHz, CD_3OD): $\delta = 165.8, 153.7, 143.9, 142.2, 134.2, 126.6, 119.3, 117.0, 110.1, 40.9, 38.6, 28.5$; HRMS (MALDI-FTMS): calcd for $C_{17}H_{18}BrN_3O_3S_2$ $[M]^+$: 454.9967, found: 454.9967.

Compound 104: $R_f = 0.52$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3318, 1443, 1325, 1155, 1090, 820, 667 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 8.93$ (m, 1H), 8.18 (d, $J = 8.2$ Hz, 1H), 8.07 (d, $J = 7.4$ Hz, 1H), 7.68 (m, 3H), 7.57 (t, $J = 7.3$ Hz, 1H), 7.47 (m, 1H), 7.22 (d, $J = 7.36$ Hz, 2H), 5.10 (t, $J = 5.6$ Hz, 1H), 3.32 (q, $J = 6.2$ Hz, 2H), 2.80 (t, $J = 5.8$ Hz, 2H), 2.39 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 149.6, 136.8, 136.4, 129.7, 126.9, 126.8, 125.8, 125.3, 121.9, 41.9, 37.0, 21.5$; HRMS (MALDI-FTMS): calcd for $C_{18}H_{18}N_2O_2S_3$ $[M+H]^+$: 391.0603, found: 391.0596.

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Compound 105: $R_f = 0.18$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3410, 1671, 1524, 1433, 1290, 1225, 1020, 831, 707 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.21$ (d, $J = 8.5$ Hz, 2H), 7.11 (d, $J = 2.0$ Hz, 1H), 6.94 (dd, $J =$ 8.2, 2.0 Hz, 1H), 6.68 (d, $J = 8.2$ Hz, 1H), 6.54 (d, $J = 8.5$ Hz, 2H), 3.69 (s, 2H), 3.46 (t, $J = 6.8$ Hz, 2H), 2.71 (d, $J = 7.3$ Hz, 2H); ^{13}C NMR (125 MHz, CD_3OD): $\delta = 163.5$, 152.1, 151.6, 150.4, 134.7, 130.2, 128.8, 116.7, 114.6, 109.0, 108.1, 38.4, 36.5, 28.1; HRMS (MALDI-FTMS): calcd for $C_{17}H_{18}ClN_3O_3S_2$ $[M]^+$: 411.0473, found: 411.0469.

Compound 106: $R_f = 0.15$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3381, 1654, 1591, 1503, 1431, 1360, 1196, 670 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.43$ (d, $J = 8.5$ Hz, 2H), 7.29 (d, $J = 2.0$ Hz, 1H), 7.13 (dd, $J =$ 8.2, 2.0 Hz, 1H), 6.88 (d, $J = 8.2$ Hz, 1H), 6.79 (m, 1H), 6.68 (d, $J = 8.8$ Hz, 2H), 3.82 (s, 2H), 3.64 (d, $J = 6.2$ Hz, 2H), 2.97 (s, 6H), 2.85 (t, $J = 6.2$ Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 133.7$, 129.6, 129.5, 129.3, 116.0, 113.5, 40.58, 37.47, 37.35, 28.0; HRMS (MALDI-FTMS): calcd for $C_{19}H_{22}ClN_3O_3S_2$ $[M+H]^+$: 440.0864, found: 440.0854.

Compound 107: $R_f = 0.16$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3381, 1656, 1531, 1426, 1209, 1015, 907, 804, 730 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.30$ (s, 1H), 7.41 (d, $J = 8.2$ Hz, 2H), 7.30 (d, $J = 1.7$ Hz, 1H), 7.13–7.10 (m, 4H), 7.03 (m, 1H), 6.88 (d, $J = 8.5$ Hz, 1H), 5.57 (s, 1H), 3.86 (s, 2H), 3.61 (q, $J = 6.2$ Hz, 2H), 2.82 (t, $J = 6.2$ Hz, 2H), 2.32 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 162.9$, 152.9, 149.9, 137.6, 133.2, 129.9, 129.7, 129.5, 129.2, 128.9, 116.1, 37.7, 37.4, 28.1, 21.0; HRMS (MALDI-FTMS): calcd for $C_{18}H_{19}ClN_2O_3S_2$ $[M+H]^+$: 411.0598, found: 411.0592.

Compound 109: $R_f = 0.17$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3381, 1654, 1532, 1429, 1208, 1017, 737, 685 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.34$ (s, 1H), 7.51 (dm, $J = 8.5$ Hz, 2H), 7.32–7.28 (m, 3H), 7.24 (tm, $J = 9.2$ Hz, 1H), 7.14 (dm, $J = 7.3$ Hz, 1H), 7.20 (m, 1H), 6.87 (dd, $J =$ 8.2, 0.88 Hz, 1H), 3.86 (s, 2H), 3.61 (q, $J = 6.2$ Hz, 2H), 2.84 (t, $J = 5.6$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 162.9$, 152.9, 149.9, 136.7, 129.7, 129.5, 129.2, 129.1, 127.9, 127.2, 119.6, 116.1, 37.7, 37.5, 28.1; HRMS (MALDI-FTMS): calcd for $C_{17}H_{17}ClN_2O_3S_2$ $[M+H]^+$: 397.0442, found: 397.0436.

Compound 110: $R_f = 0.13$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3384, 1657, 1512, 1338, 1208, 907, 847, 733 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.15$ (d, $J = 4.7$ Hz, 2H), 7.62 (d, $J = 6.8$ Hz, 2H), 7.29 (d, $J =$ 2.1 Hz, 1H), 7.14 (dd, $J = 8.2$, 2.1 Hz, 2H), 6.96 (m, 1H), 6.87 (d, $J = 8.2$ Hz, 1H), 5.57 (brs, 1H), 3.85 (s, 2H), 3.59 (q, $J = 6.2$ Hz, 2H), 2.90 (t, $J =$ 6.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 163.5$, 152.9, 150.0, 129.7, 129.5, 129.1, 128.0, 126.1, 124.1, 119.6, 116.1, 38.0, 37.7, 28.0, 14.2; MS (ESI): calcd for $C_{17}H_{16}ClN_3O_5S_2$ $[M+H]^+$: 442, found: 442.

Compound 112: $R_f = 0.45$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3349, 1595, 1489, 1429, 1294, 1218, 1021, 825 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD): $\delta = 9.12$ (s, 1H), 8.32 (dd, $J = 8.8$, 2.6 Hz, 1H), 7.81 (d, $J = 9.1$ Hz, 1H), 7.09 (d, $J = 2.0$ Hz, 1H), 6.93 (dd, $J = 8.5$, 2.0 Hz, 1H), 6.65 (d, $J =$ 8.2 Hz, 1H), 3.69 (s, 2H), 3.45 (t, $J = 6.4$ Hz, 2H), 2.92 (t, $J = 6.2$ Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 169.2$, 164.1, 153.3, 152.8, 146.2, 144.0, 133.3, 131.5, 130.3, 129.8, 121.1, 117.5, 39.5, 39.2, 28.9; HRMS (MALDI-FTMS): calcd for $C_{16}H_{15}ClN_4O_5S_2$ $[M+H]^+$: 443.0245, found: 443.0246.

Compound 113: $R_f = 0.10$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3356, 1656, 1495, 1040, 729 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.89$ (d, $J = 4.4$ Hz, 1H), 8.17 (d, $J = 8.2$ Hz, 1H), 8.10 (d, $J = 7.3$ Hz, 1H), 7.64 (d, $J = 8.2$ Hz, 1H), 7.54 (dd, $J = 7.6$ Hz, 1H), 7.46 (dd, $J = 8.5$, 4.4 Hz, 1H), 7.29 (d, $J = 1.7$ Hz, 1H), 7.16 (m, 1H), 7.12 (dd, $J = 8.5$, 1.7 Hz, 1H), 6.86 (q, $J =$ 8.2 Hz, 1H), 3.83 (s, 2H), 3.61 (q, $J = 6.2$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 164.1$, 153.2, 150.0, 149.5, 136.7, 136.2, 129.7, 129.3, 129.4, 128.7, 128.5, 126.8, 125.6, 125.3, 121.8, 119.7, 116.1, 38.1, 36.7, 28.1; HRMS (MALDI-FTMS): calcd for $C_{16}H_{18}ClN_3O_3S_2$ $[M+H]^+$: 448.0551, found: 448.0542.

Compound 114: $R_f = 0.50$ (silica gel, EtOAc/hexanes 1:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 9.03$ (m, 1H), 8.28 (dd, $J = 6.4$, 1.2 Hz, 1H), 8.21 (dd, $J = 5.8$, 0.8 Hz, 1H), 8.02 (brs, 1H), 7.75 (d, $J = 5.8$ Hz, 1H), 7.65 (d, $J = 6.2$ Hz, 1H), 7.57–7.54 (m, 1H), 7.51 (s, 2H), 3.89 (s, 2H), 3.74 (q, $J =$ 5.0 Hz, 2H), 3.00 (t, $J = 4.9$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 163.2, 151.3, 149.6, 149.0, 141.6, 136.6, 136.5, 132.3, 131.1, 128.1, 128.0, 127.0, 126.9, 125.8, 125.3, 123.9, 123.5, 122.3, 111.7, 38.1, 36.4, 27.5.

Compound 115: $R_f = 0.43$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3383, 1658, 1531, 1428, 1219, 1017, 732 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.31$ (brs, 1H), 7.26 (brs, 1H), 7.16 (dd, $J = 8.5$, 1.5 Hz, 1H), 7.01 (m, 1H), 6.89 (d, $J = 8.5$ Hz, 1H), 6.40 (brs, 1H), 3.87 (s, 2H), 3.68 (q, $J =$ 6.2 Hz, 1H), 2.84 (t, $J = 5.8$ Hz, 1H), 2.36 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 164.2$, 153.1, 148.1, 141.0, 129.7, 129.5, 129.2, 116.0, 114.3, 37.7, 37.4, 28.1, 11.9; HRMS (MALDI-FTMS): calcd for $C_{16}H_{17}ClN_2O_4S_2$ $[M+H]^+$: 401.0391, found: 401.0376.

Compound 117: $R_f = 0.18$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3383, 1664, 1513, 1429, 1290, 1222, 1020, 951, 706 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.11$ (d, $J = 2.0$ Hz, 1H), 6.95 (dd, $J = 7.0$, 2.1 Hz, 1H), 6.69 (d, $J = 8.2$ Hz, 1H), 3.71 (s, 2H), 3.46 (t, $J = 6.8$ Hz, 2H), 2.83–2.71 (m, 6H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 166.1$, 153.3, 153.2, 131.5, 130.4, 129.9, 121.4, 119.8, 117.5, 39.6, 38.8, 34.4, 28.9, 18.2; HRMS (MALDI-FTMS): calcd for $C_{14}H_{16}ClN_3O_3S_2$ $[M+H]^+$: 396.0214, found: 396.0205.

Compound 118: $R_f = 0.30$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3415, 2916, 1661, 1513, 1433, 1319, 1226, 1152, 1023, 951, 706 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.65$ (dd, $J = 4.4$, 0.6 Hz, 1H), 7.51 (dd, $J = 3.5$, 1.2 Hz, 1H), 7.11 (d, $J = 2.3$ Hz, 1H), 7.03 (dd, $J = 4.4$, 3.8 Hz, 1H), 6.95 (dd, $J = 8.2$, 2.0 Hz, 1H), 6.68 (d, $J = 8.5$ Hz, 1H), 3.71 (s, 2H), 3.42 (t, $J =$ 6.7 Hz, 2H), 3.15 (t, $J = 6.4$ Hz, 2H), 2.67 (m, 4H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 166.0$, 154.8, 153.1, 146.1, 133.3, 133.2, 131.6, 129.9, 128.7, 120.3, 117.5, 105.5, 43.4, 39.7, 38.9, 30.1; HRMS (MALDI-FTMS): calcd for $C_{17}H_{20}ClN_3O_5S_4$ $[M+Na]^+$: 531.9866, found: 531.9871.

Compound 119: $R_f = 0.22$ (silica gel, EtOAc/hexanes 1:1); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.11$ (d, $J = 2.0$ Hz, 1H), 6.94 (dd, $J = 7.0$, 2.3 Hz, 1H), 6.68 (d, $J = 8.2$ Hz, 1H), 4.10 (q, $J = 7.0$ Hz, 2H), 3.70 (m, 3H), 3.46 (td, $J = 7.1$, 2.1 Hz, 2H), 3.00 (dd, $J = 7.2$, 5.3 Hz, 1H), 2.86 (dd, $J = 7.0$, 7.0 Hz, 1H), 2.76 (t, $J = 6.7$ Hz, 2H); HRMS (MALDI-FTMS): calcd for $C_{16}H_{22}ClN_3O_5S_2$ $[M+H]^+$: 436.0762, found: 436.0774.

Compound 121: ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.64$ (d, $J = 8.2$ Hz, 1H), 7.26 (d, $J = 7.9$ Hz, 1H), 7.11 (d, $J = 1.7$ Hz, 1H), 6.94 (dd, $J = 8.5$, 2.1 Hz, 1H), 6.68 (d, $J = 8.5$ Hz, 1H), 3.70 (s, 3H), 3.39 (t, $J = 7.0$ Hz, 1H), 3.30 (t, $J = 7.0$ Hz, 2H), 3.05 (t, $J = 7.0$ Hz, 1H), 2.66–2.58 (m, 2H), 2.50 (t, $J =$ 7.0 Hz, 2H).

Compound 122: $R_f = 0.23$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3384, 2919, 1660, 1502, 1431, 1321, 1152, 1020 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.69$ (d, $J = 7.0$ Hz, 2H), 7.11 (d, $J = 2.0$ Hz, 1H), 6.96–6.93 (m, 3H), 6.68 (d, $J = 8.2$ Hz, 1H), 3.75 (s, 3H), 3.70 (s, 2H), 3.40 (t, $J =$ 6.5 Hz, 2H), 3.04 (t, $J = 6.5$ Hz, 2H), 2.66–2.59 (m, 4H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 166.0$, 164.6, 153.2, 152.8, 133.4, 131.5, 130.4, 130.3, 129.8, 121.4, 117.5, 115.5, 56.3, 43.1, 39.8, 39.0, 38.4, 28.9; MS (EIS): calcd for $C_{20}H_{24}ClN_3O_6S_3$ $[M+Na]^+$: 556, found: 556.

Compound 123: $R_f = 0.26$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3283, 1660, 1514, 1336, 1015, 848, 739, 692 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.15$ (d, $J = 9.1$ Hz, 2H), 7.61 (d, $J = 9.1$ Hz, 4H), 7.42–7.32 (m, 8H), 5.25 (s, 2H), 3.71 (q, $J = 6.2$ Hz, 2H), 2.92 (t, $J = 6.4$ Hz, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 163.6$, 161.0, 159.0, 152.1, 151.5, 132.2, 131.4, 130.2, 128.7, 125.6, 119.4, 116.7, 116.4, 116.2, 40.4, 38.8, 37.3, 28.1; HRMS (MALDI-FTMS): calcd for $C_{23}H_{21}N_3O_4S_2$ $[M+Na]^+$: 490.0866, found: 490.0866.

Compound 124: $R_f = 0.23$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3400, 1657, 1534, 1465, 1441, 1254, 1013, 811, 693 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.61–7.59$ (m, 2H), 7.41–7.33 (m, 12H), 5.23 (s, 2H), 3.70 (q, $J = 7.7$ Hz, 2H), 2.86 (t, $J = 7.7$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 163.1, 152.8, 132.2, 131.1, 130.2, 129.4, 128.6, 128.5, 128.4, 128.1, 126.8, 37.6, 37.5; HRMS (MALDI-FTMS): calcd for $C_{23}H_{21}BrN_2O_2S_2$ $[M+H]^+$: 501.0301, found: 501.0287.

Compound 125: $R_f = 0.63$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 2932, 1665, 1541, 1409, 1276, 1005, 911, 736, 693 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.42$ (m, 1H), 7.90 (s, 2H), 7.63 (dd, $J = 7.6$, 1.2 Hz, 2H), 7.39–7.32 (m, 6H), 7.29–7.22 (m, 2H), 5.23 (s, 2H), 3.76 (q, $J = 5.5$ Hz, 2H), 3.66 (t, $J = 6.4$ Hz, 2H), 3.09 (dd, $J = 5.8$, 4.1 Hz, 2H), 1.74 (q, $J = 6.7$ Hz, 2H), 1.01 (t, $J = 7.3$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 163.1$, 153.7, 151.2, 144.6, 137.4, 131.1, 130.1, 129.6, 128.6, 128.3, 128.2, 128.1, 127.8, 126.5, 76.8, 70.4, 40.6, 36.9, 22.3, 10.3.

Compound 127: $R_f = 0.76$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3281, 2920, 1708, 1647, 1596, 1438, 1325, 1225, 1156, 1022, 952, 744 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.56$ (d, $J = 8.2$ Hz, 2H), 7.38 (d, $J =$ 7.0 Hz, 2H), 7.24–7.15 (m, 5H), 3.0 (dd, $J = 7.0$, 7.0 Hz, 2H), 2.62 (dd, $J =$ 7.0, 7.0 Hz, 2H), 2.30 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 144.8$, 139.0, 138.4, 130.9, 130.4, 129.0, 128.4, 128.1, 43.3, 38.9, 21.6; HRMS (MALDI-FTMS): calcd for $C_{15}H_{17}NO_2S_3$ $[M]^+$: 339.0415, found: 339.0416.

Compound 128: $R_f = 0.52$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3282, 1525, 1348, 1156, 1090, 810, 733, 665, 551 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 8.32$ (br s, 1H), 8.05 (d, $J = 8.2$ Hz, 1H), 7.78 (d, $J = 7.9$ Hz, 1H), 7.70 (d, $J = 7.9$ Hz, 2H), 7.49 (t, $J = 8.2$ Hz, 1H), 7.29 – 7.26 (m, 2H), 4.93 (t, $J = 6.2$ Hz, 1H), 3.26 (q, $J = 6.2$ Hz, 2H), 2.82 (t, $J = 6.4$ Hz, 2H), 2.43 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 148.7$, 143.8, 139.6, 136.7, 132.7, 129.9, 129.8, 127.0, 121.8, 121.6, 41.5, 38.0, 21.5; HRMS (MALDI-FTMS): calcd for $C_{15}H_{16}N_2O_4S_3$ $[M+Na]^+$: 407.0164, found: 407.0158.

Compound 129: $R_f = 0.22$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3368, 1606, 1441, 1320, 1164, 1085 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta =$ 7.71 (d, $J = 8.2$ Hz, 2H), 7.31 – 7.29 (m, 3H), 7.16 (td, $J = 8.2$, 1.8 Hz, 1H), 6.72 (dd, $J = 7.9$, 1.2 Hz, 1H), 6.65 (td, $J = 7.3$, 1.2 Hz, 1H), 4.85 (t, $J =$ 5.8 Hz, 1H), 4.36 (br s, 2H), 3.33 (q, $J = 6.2$ Hz, 2H), 2.77 (t, $J = 6.4$ Hz, 2H), 2.43 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 148.0$, 143.6, 136.9, 135.5, 131.5, 129.7, 127.0, 118.6, 117.7, 115.8, 41.2, 37.4, 21.5; HRMS (MALDI-FTMS): calcd for $C_{15}H_{18}N_2O_2S_3$ $[M+H]^+$: 355.0592, found: 355.0592.

Compound 130: $R_f = 0.72$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3418, 1647, 1489, 1440, 1325, 1156, 1022, 809, 662 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.76$ (d, $J = 6.4$ Hz, 2H), 7.44 (d, $J = 6.4$ Hz, 2H), 7.36 (d, $J =$ 6.4 Hz, 2H), 7.20 (d, $J = 6.5$ Hz, 2H), 4.81 (m, 1H), 3.35 (q, $J = 5.0$ Hz, 2H), 2.82 (t, $J = 5.0$ Hz, 2H), 2.51 (s, 3H), 2.42 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 143.9$, 138.3, 137.3, 133.5, 130.4, 130.2, 129.6, 127.5, 41.6, 38.1, 21.9, 21.5; HRMS (MALDI-FTMS): calcd for $C_{16}H_{19}NO_2S_3$ $[M+H]^+$: 353.0572, found: 353.0577.

Compound 132: $R_f = 0.25$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 1595, 1525, 1347, 1259, 1152, 1096, 1025 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 8.31$ (s, 1H), 8.06 (d, $J = 5.3$ Hz, 1H), 7.76 (m, 3H), 7.50 (t, $J = 5.3$ Hz, 1H), 7.25 (s, 1H), 6.94 (d, $J = 5.6$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 163.1$, 140.3, 132.7, 131.2, 129.9, 129.2, 121.8, 121.6, 114.3, 55.6, 41.4, 38.0; HRMS (MALDI-FTMS): calcd for $C_{15}H_{16}N_2O_5S_3$ $[M+Na]^+$: 423.0114, found: 423.0130.

Compound 133: $R_f = 0.25$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3279, 1524, 1386, 1163, 1071, 835, 791, 731 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 9.04$ (dd, $J = 4.4$, 1.7 Hz, 1H), 8.41 (dd, $J = 7.3$, 1.5 Hz, 1H), 8.31 (dd, $J = 8.2$, 1.5 Hz, 1H), 8.21 (t, $J = 2.0$ Hz, 1H), 8.09 (dd, $J = 8.2$, 1.2 Hz, 1H), 8.01 (dm, $J = 7.9$ Hz, 1H), 7.71 (d, $J = 7.9$ Hz, 1H), 7.66 (t, $J =$ 7.9 Hz, 1H), 7.59 (dd, $J = 8.5$, 4.4 Hz, 1H), 7.45 (t, $J = 7.9$ Hz, 1H), 3.21 (q, $J = 6.5$ Hz, 2H), 2.80 (t, $J = 6.2$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 151.4, 143.1, 139.5, 137.1, 135.6, 133.5, 132.6, 131.1, 129.9, 128.8, 125.7, 122.4, 121.7, 121.5, 41.7, 37.8; HRMS (MALDI-FTMS): calcd for $C_{17}H_{15}N_3O_4S_3$ $[M+H]^+$: 422.0303, found: 422.0303.

Compound 134: $R_f = 0.38$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 2961, 1542, 1406, 1327, 1277, 1163, 1010, 910, 834 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 8.99$ (dd, $J = 4.4$, 1.8 Hz, 1H), 8.44 (dd, $J = 7.4$, 1.5 Hz, 1H), 8.32 (s, 2H), 8.28 (dd, $J = 8.5$, 1.8 Hz, 1H), 8.06 (dd, $J = 8.2$, 1.5 Hz, 1H), 7.65 (dd, $J = 8.2$, 7.4 Hz, 1H), 7.55 (dd, $J = 8.2$, 4.1 Hz, 1H), 7.49 (m, 1H), 3.78 (d, $J = 6.4$ Hz, 2H), 3.30 (q, $J = 5.6$ Hz, 2H), 2.84 (t, $J = 5.8$ Hz, 2H), 2.12 – 2.04 (m, 1H), 1.02 (d, $J = 6.8$ Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 160.9, 151.6, 151.3, 144.9, 143.3, 136.8, 136.6, 133.3, 130.9, 125.6, 122.3, 120.0, 75.4, 41.5, 38.6, 28.2, 18.9; HRMS (MALDI-FTMS): calcd for $C_{19}H_{22}N_4O_3S_3$ $[M+H]^+$: 451.0927, found: 451.0938.

Compound 135: $R_f = 0.28$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3280, 1490, 1423, 1328, 1163, 1073, 835, 791, 685, 584 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 9.05$ (dd, $J = 4.4$, 1.5 Hz, 1H), 8.43 (dd, $J = 7.3$, 1.5 Hz, 1H), 8.30 (dd, $J = 8.5$, 1.8 Hz, 1H), 8.08 (dd, $J = 8.2$, 1.2 Hz, 1H), 7.66 (t, $J = 7.9$ Hz, 1H), 7.59 (dd, $J = 8.2$, 4.1 Hz, 1H), 7.25 (d, $J = 8.5$ Hz, 2H), 7.05 (d, $J = 8.2$ Hz, 2H), 6.78 (t, $J = 5.8$ Hz, 1H), 3.23 (q, $J = 6.2$ Hz, 2H), 2.72 (t, $J = 6.2$ Hz, 2H), 2.30 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 151.4$, 143.2, 137.7, 136.9, 135.8, 133.4, 132.7, 131.1, 129.8, 129.1, 128.7, 125.7, 122.3, 41.5, 37.4, 21.0; HRMS (MALDI-FTMS): calcd for $C_{18}H_{18}N_2O_3S_3$ $[M+H]^+$: 391.0612, found: 391.0612.

Compound 136: $R_f = 0.23$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3424, 1590, 1484, 1443, 1319, 1255, 1149, 1084 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 8.93$ (d, $J = 2.6$ Hz, 1H), 8.18 (d, $J = 8.2$ Hz, 1H), 8.07 (d, $J =$ 7.3 Hz, 1H), 7.73 (d, $J = 8.8$ Hz, 1H), 7.68 (d, $J = 7.9$ Hz, 1H), 7.57 (t, $J =$ 7.9 Hz, 1H), 7.47 (dd, $J = 8.2$, 4.4 Hz, 1H), 6.89 (d, $J = 8.8$ Hz, 2H), 3.85 (s, 3H), 3.32 (q, $J = 6.2$ Hz, 2H), 2.82 (t, $J = 6.4$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 149.6$, 136.4, 136.0, 131.5, 129.2, 129.1, 128.5, 126.8, 125.8, 125.3, 121.9, 120.0, 114.3, 55.6, 41.8, 37.0; HRMS (MALDI-FTMS): calcd for $C_{18}H_{18}N_2O_3S_3$ $[M+H]^+$: 407.0552, found: 407.0558.

Compound 137: $R_f = 0.63$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 1443, 1319, 1225, 1149, 1048 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.73$ (d, $J = 8.2$ Hz, 2H), 7.32 (d, $J = 8.2$ Hz, 2H), 7.26 (s, 1H), 6.35 (d, $J = 1.2$ Hz, 1H), 3.31 (q, $J = 6.5$ Hz, 2H), 2.77 (t, $J = 6.2$ Hz, 2H), 2.44 (s, 3H), 2.30 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 155.8$, 143.6, 141.2, 136.9, 129.8, 127.1, 114.1, 112.5, 41.1, 37.8, 21.5, 11.9.

Compound 138: $R_f = 0.74$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 3096, 2864, 1487, 1406, 1331, 1226, 1154, 1020, 951, 853, 806, 722, 586 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.59 – 7.55$ (m, 2H), 7.38 (d, $J = 8.2$ Hz, 2H), 7.13 (d, $J = 8.2$ Hz, 2H), 7.07 (dd, $J = 5.0$, 3.8 Hz, 1H), 4.93 (br t, $J =$ 5.9 Hz, 1H), 3.36 (q, $J = 6.2$ Hz, 2H), 2.79 (t, $J = 6.2$ Hz, 2H), 2.34 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 137.9$, 132.9, 132.2, 132.0, 130.0, 129.2, 127.4, 41.4, 37.5, 21.1; HRMS (MALDI-FTMS): calcd for $C_{13}H_{15}NO_2S_4$ $[M]^+$: 344.9980, found: 344.9977.

Compound 139: $R_f = 0.87$ (silica gel, EtOAc/hexanes 1:1); IR (film): $\tilde{\nu}_{max} =$ 2926, 2858, 1528, 1461, 1347, 1269, 1121, 1065, 876, 801, 734 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 8.42$ (t, $J = 2.0$ Hz, 1H), 8.04 (ddd, $J = 8.2$, 2.1, 0.8 Hz, 1H), 7.81 (d, $J = 7.9$ Hz, 1H), 7.49 (t, $J = 7.9$ Hz, 1H), 2.77 (t, $J =$ 7.4 Hz, 2H), 1.66 (quint, $J = 7.6$ Hz, 2H), 1.39 – 1.23 (m, 7H), 0.86 (t, $J =$ 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 148.7$, 140.9, 132.1, 129.6, 121.2, 121.1, 39.1, 31.3, 28.8, 28.1, 22.5, 13.9; MS (ESI): calcd for $C_{12}H_{17}NO_2S_2$ $[M-H]^+$: 270, found: 270.

Determination of MIC values for individual compounds: After re-synthesis, the minimum inhibitory concentration (MIC) values of chromatographically and spectroscopically homogenous compounds were determined in a panel of bacterial strains using the two-fold serial dilution technique in 96-well plates. The concentration of the initial dilution was 50 $\mu g\,mL^{-1}$ and a total of 11 two-fold dilutions were performed. The MIC value was taken to be the lowest concentration that visible inhibited growth of the microorganism as determined by visual inspection. SA 6538, SA 29313, MRSA 700787, MRSA 700788 and MRSA 700789 were grown in Tryhcase Soy Broth (BBL 11043). SA 700698 was grown in Brain Heart Infusion broth (Difco 237400). SA 13709 and MRSA 33591 were grown in Nutrient broth (Difco 233000). SA 25923 and MRSA 43300 were grown in Mueller Hinton broth (Difco 0757).

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