JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

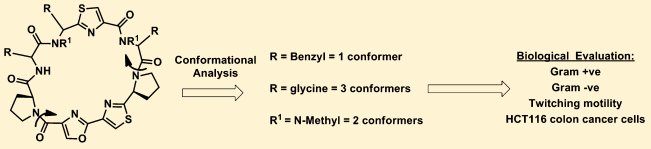
Synthesis, Structure–Activity Analysis, and Biological Evaluation of Sanguinamide B Analogues

Hendra Wahyudi, Worawan Tantisantisom, Xuechao Liu, Deborah M. Ramsey, Erinprit K. Singh, and Shelli R. McAlpine*

School of Chemistry, Gate 2 High Street, Dalton 219, University of New South Wales, Sydney, NSW 2052, Australia

Supporting Information

ABSTRACT: We report the first synthesis of sanguinamide B analogues. Substituting N-methylated (*N*-Me) amino acids, glycine (Gly), and L- or D-phenylalanine (Phe) into the backbone of sanguinamide B showed that only L- and D-Phe residues controlled the macrocycle conformation. The N-methylated and glycine analogues all had multiple conformations, whereas the L- and D-Phe derivatives only had a single conformation. Testing of all conformer analogues showed that inclusion of an L- or D-Phe was a superior design element than incorporating the *N*-Me moiety that is often utilized to control macrocyclic conformation. Finally, we show that there is an ideal Phe residue (in this case L-Phe) for generating compounds that have the greatest inhibitory effect on bacterial motility. Our data support the hypothesis that the macrocyclic conformation is dictated by the benzyl moiety requiring a "pseudoequatorial" position, and all other energy considerations are secondary.



INTRODUCTION

A proven source of new antibiotics are natural products,[1] and screening compounds isolated from natural sources has produced effective antimicrobial agents including penicillin, vancomycin, and erythromycin.[2] We recently reported the synthesis of the natural product sanguinamide B (San B) (Figure 1, **1**).[3] San B (**1**) was isolated from a nudibranch collected in the Yasawa Island chain in Figi, *Hexabranchus sanguineus,* meaning "six-gilled blood-colored" sea slug with a spongiverous diet.[4,5] *H. sanguineus* and its egg masses contain bioactive polyketide macrolides including kabiramides[6] and ulapualides[7] (Figure 1). In 2009, Molinski and co-workers determined that the structure of **1** was a modified macrocyclic octapeptide containing five L-configuration amino acids, two thiazoles (Th), and one oxazole (Ox).[4]

STRUCUTURAL FEATURES OF SANGUINAMIDE B

Compound **1** falls into a unique class of natural products, as it bears directly linked azoles: a 4,2-oxazole-thiazole moiety. Over the last two decades there has been a surge in the discovery of natural product compounds that contain directly linked azoles, and many of these compounds are now candidates for drug development.[8−13] Mixed 4,2-bisheterocycle tandem pairs, however, are extremely rare in peptides, and only two natural products have been reported to date to contain an oxazole-thiazole subunit:[8] leucamide A[14] and microcin B17 (Figure 1).[15] Investigation of the two oxazole-thiazole units present in microcin B17 showed that altering or removing the two thiazole-oxazole pairs significantly reduced antibiotic activity.[15] Given the importance of the mixed tandem bisheterocycle moiety in multiple natural products, evaluating the structure–activity relationship of **1** analogues was appealing.

The two prolines (Pro) present in **1** also make this natural product very intriguing. Prolines, such as *N*-methyl (*N*-Me) amino acids, are common structural motifs in bioactive peptides that dramatically alter the 3D conformation of macrocycles.[16] Specifically, the rotation about prolyl amide bonds is restricted, and the proline residue may adopt either a *cis* or *trans* configuration.[4] A *cis*-amide bond generates a different 3D shape of the macrocycle than a *trans*-amide bond, where one may induce a bioactive conformation and the other an inactive conformation. This phenomena was exhibited with the isolation and biological evaluation of two conformational isomers, *cis,cis*- and *trans,trans*-ceratospongamide (Figure 2), which were isolated from a symbiotic sponge (*Sigmadocia symbiotica*). Ceratospongamide's structure consists of a modified heptapeptide with a thiazole, methyloxazoline, and two prolines.[17] When tested for anti-inflammatory activity, only the *trans,trans* conformer exhibited potent inhibition of $sPLA_2$ expression (ED_{50} 32 nM), and the *cis,cis* isomer was inactive.[17]

RESULTS AND DISCUSSION

Design of Sanguinamide B Analogues. One common modification made when exploring the SAR of cyclic peptides is the incorporation of an *N*-methyl moiety within the backbone of the peptide. N-Methylating peptides control the macrocyclic confirmation in a manner that is similar to a proline residue. Nonmethylated peptide bonds sit in a *trans* orientation about the amide. Addition of *N*-Me on the amide nitrogen increases the propensity for producing the *cis*-peptide bond about the amide.[18,19] Thus, methylation has a significant effect on the backbone conformation of cyclic peptides, and the presence of

Received: August 20, 2012
Published: October 10, 2012

dx.doi.org/10.1021/jo3017499 | J. Org. Chem. 2012, 77, 10596−10616

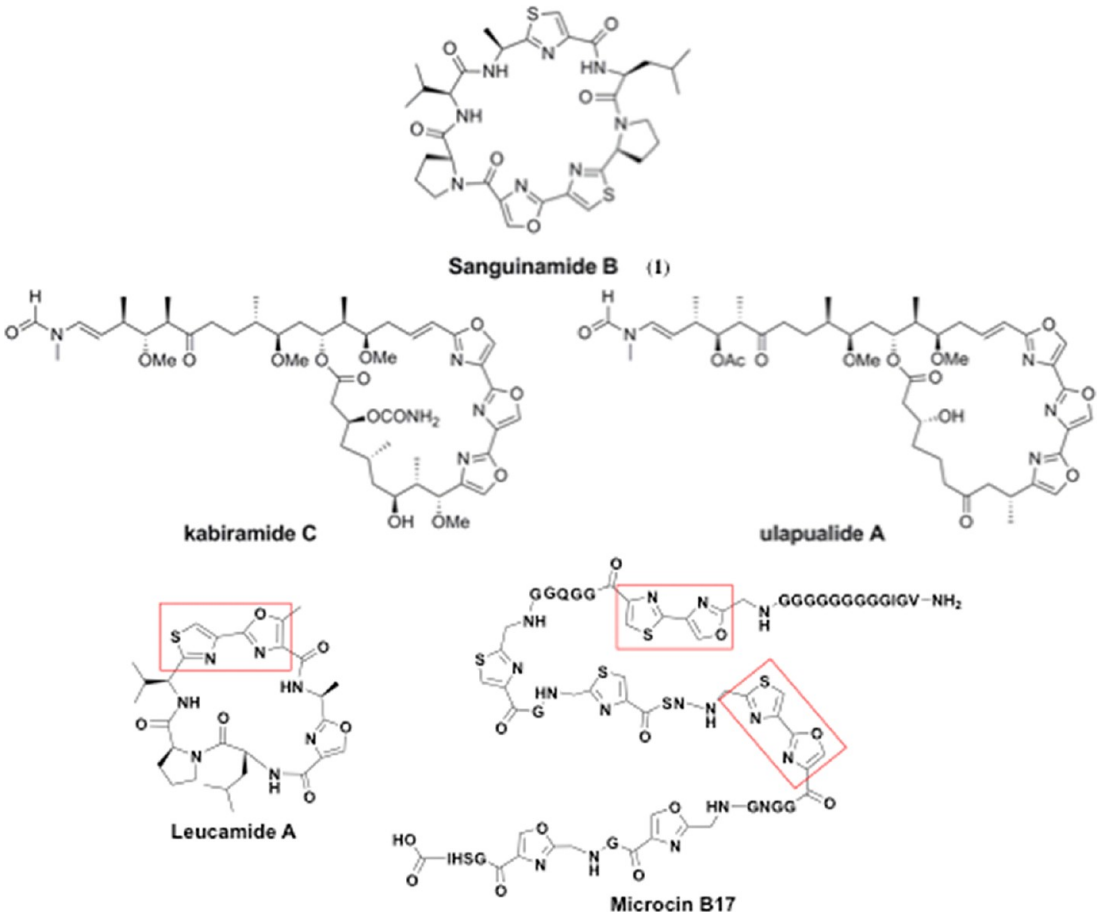


Figure 1. Examples of bioactive polyketide macrolides isolated from *H. sanguineus* and natural products with tandem bisheterocycles pairs.

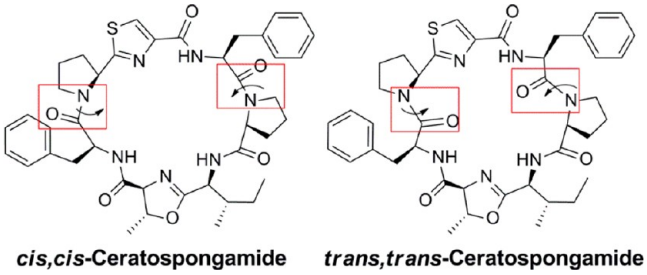


Figure 2. Conformational isomers *cis,cis*- and *trans,trans*-ceratospongamide.

a single *N*-Me minimizes the number of conformations that a macrocycle can adopt, thereby enhancing receptor selectivity by potentially locking the peptide into a single bioactive conformation.[20−25] Furthermore, incorporating *N*-Me amino acids into peptides can result in analogues with improved biological activity and pharmacological properties for several reasons, including improvement in the molecule's membrane permeability and oral availability, a key property for drug development.[26] Thus, the *N*-Me moiety is an important tool in optimizing potency of cyclic peptides. There are many natural product cyclic peptides that contain *N*-Me amino acids with notable pharmacological profiles, IB-01212,[27] koshikamides,[28] and cyclosporine A,[29] being the most prominent. Cyclosporine A, a well-known immunosuppressant, is a cyclic undecapeptide with seven *N*-methyls,[30] and its metabolic stability is partly due to the presence of *N*-Me groups.[31,32]

We performed an *N*-Me scan on **1** to evaluate the impact of this moiety on the biological activity. The *N*-Me substituent is applied at two different positions within the macrocycle (Figure 3), *N*-Me Ala (**2**), and *N*-Me Leu (**3**). The third possible analogue of **1**, introduction of the *N*-methylvaline, was not synthesized because coupling the *N*-methylvaline residue to the proline gave very poor yields.

Contrary to substituting an *N*-Me into **1**, introduction of a Gly provides flexibility to the macrocycle conformation (Figure 3). A glycine residue scan produced macrocycles whereby glycine was placed at each of the three amino acids within **1**. Glycine analogues, where Gly replaces Val, Ala, or Leu (compounds **4**, **5**, and **6**, respectively) are shown in Figure 3.

Another common modification made to peptides when exploring their SAR is exchanging L- for D-amino acids. The conformation of macrocyclic peptides with small ring sizes (e.g., four to eight amino acid residues) is dictated by the amino acid stereochemistry in the peptide sequence.[33,34] A single D-amino acid (D-AA) substitution within a macrocycle results in a γ-turn conformation of the D-amino acid and the two amino acids on either side. This γ-turn is in equilibrium with a βII′-turn.[35] Thus, inserting a D-amino acid into the macrocyclic backbone significantly impacts the overall 3-D macrocyclic conformation. Additionally, including D-AAs in a peptide sequence stabilizes the analogue against enzymatic degradation.[33] Interestingly, generating the enantiomer of a peptide sequence has typically led to compounds losing their biological activity. Thus, a productive approach to determining the ideal substitution site

dx.doi.org/10.1021/jo3017499 | J. Org. Chem. 2012, 77, 10596−10616

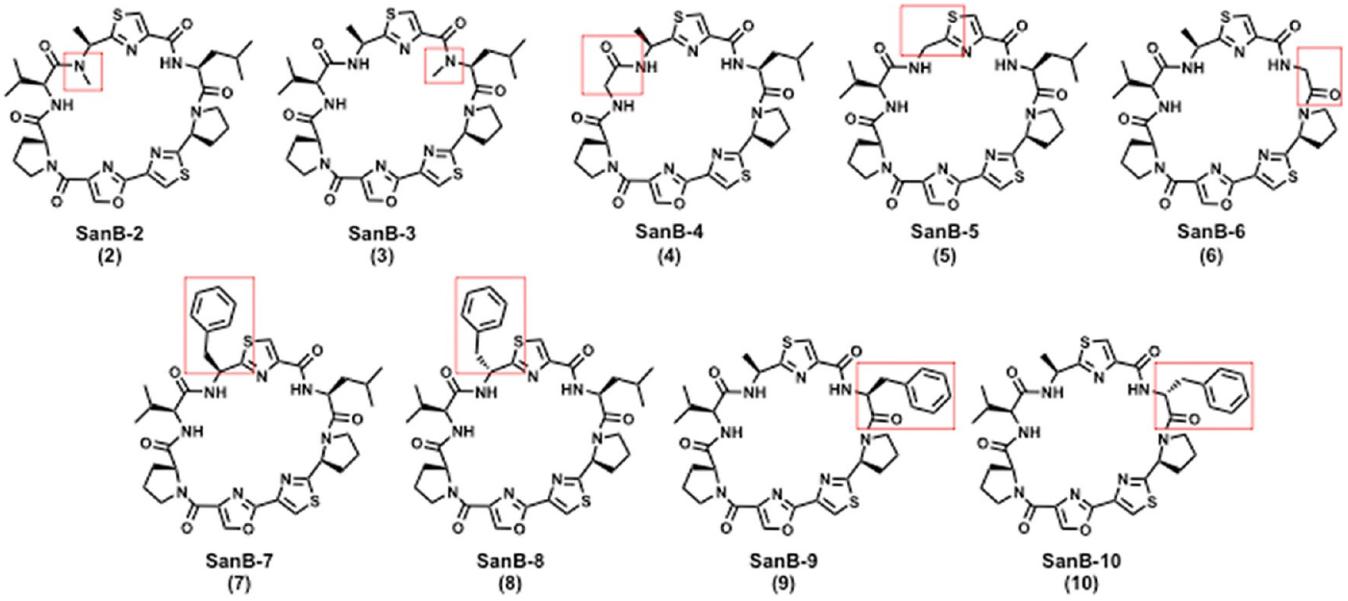


Figure 3. *N*-Me series (2,3), glycine series (4−6), aromatic series (7−10) of San B analogues.

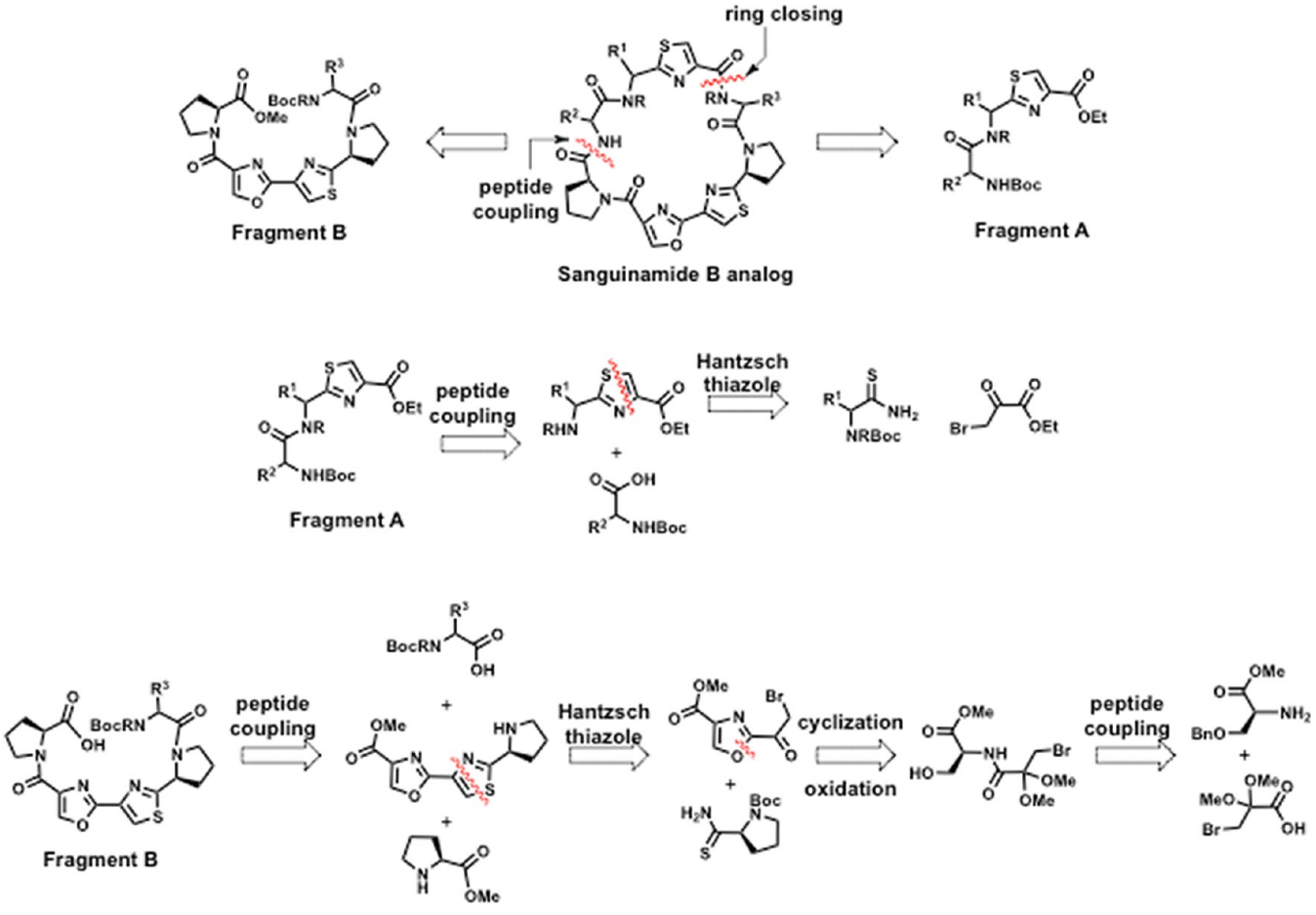


Figure 4. Retrosynthetic strategies of San B fragments A and B.

for a D-AA involves a D-scan.[36] This particular approach was used to generate the drug cilengitide.[37]

Utilizing an L-Phe scan and D-Phe scan (Figure 3) allowed us to evaluate the impact of bulky substituent as well as the effects of an L-AA on the molecule conformation. Substitution of both Phe stereoisomers provides compounds whereby we can evaluate the impact of the residues' stereochemistry. It is anticipated that one Phe enantiomer will generate a pseudoaxial moiety, and the other a pseudoequatorial moiety, thus dictating the overall macrocyclic conformation.

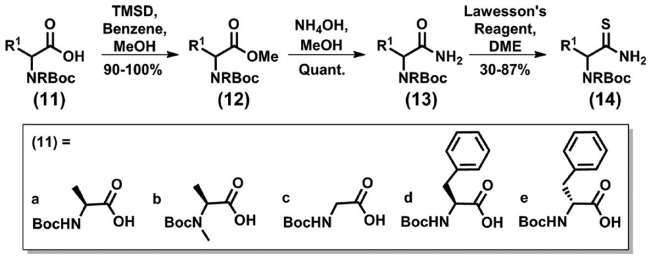
Synthesis of San B Analogues. San B analogues (Figure 4) were synthesized via cyclization of a linear octapeptide

dx.doi.org/10.1021/jo3017499 | J. Org. Chem. 2012, 77, 10596−10616

precursor, which was generated using a convergent solution phase approach. The linear octapeptide was obtained by coupling two fragments: fragment A (a tripeptide containing a thiazole) and fragment B (a pentapeptide containing the tandem oxazole-thiazole unit).

Synthesis of fragment A (Figure 4) involved first forming the thiazole moiety using a modified Hantzsch thiazole synthesis between a thioamide (where R^1 varied depending on the analogue) and an α-bromo ketone species. The thioamide was made in three steps via conversion from the acid precursor (Scheme 1) to the amide and subsequently the thioamide. N-terminal elongation of the thiazole with another amino acid carrying an R^2 substituent generated fragment A.

Scheme 1. Synthesis of Thioamide



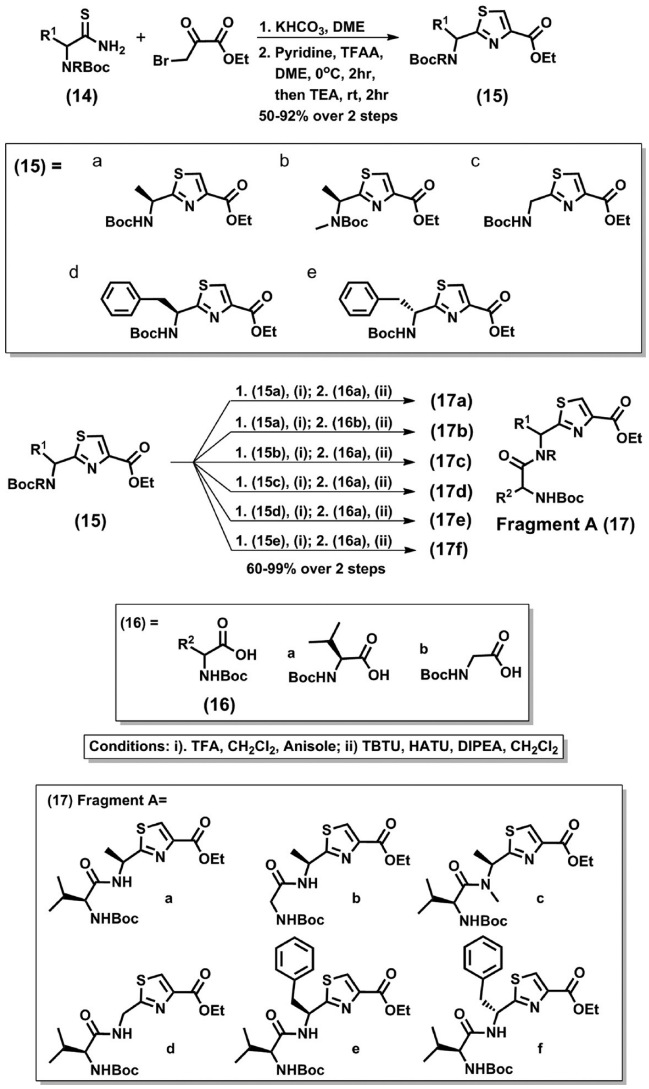
Synthesis of fragment B (Figure 4) involved forming the tandem oxazole-thiazole moiety using the same modified Hantzsch thiazole method between a proline thioamide and an α-bromo ketone oxazole. Subsequent coupling of the core oxazole-thiazole moiety with another proline and an amino acid containing an R^3 substituent generated fragment B. The α-bromo ketone oxazole was made by performing a cyclization and dehydration step starting from the serine precursor. The serine-bromoketal was synthesized by coupling a bromo-ketal acid and a benzyl-protected serine. Subsequent hydrogenolysis to remove the benzyl protecting group generated the desired serine precursor. The proline thioamide was achieved by converting the acid precursor to the thioamide via the amide (Scheme 1).

Synthesis of the thioamide started with conversion of the acid to the methyl ester (**11**, Scheme 1) using (trimethylsilyl)-diazomethane (TMSD) in a solvent mixture of benzene and methanol. The ester **12** was then converted to amide **13** using ammonium hydroxide in methanol. Utilizing Lawesson's reagent on the amide afforded the thioamide **14**.

Condensing the thioamide **14** with an ethyl bromopyruvate in the presence of $KHCO_3$ in DME formed a thiazoline (Scheme 2). The thiazoline was then subjected to dehydration conditions using trifluoroacetic anhydride (TFAA) and pyridine in DME to afford the thiazole **15**. Amine deprotection of the thiazole moiety using trifluoroacetic acid (TFA) gave a free amine species, which was then coupled to an amino acid carrying R^2 substituents (**16**) in the presence of *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU), 2-(7-Aaza-1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU), and *N*,*N*-diisopropylethylamine (DIPEA) in CH_2Cl_2 to give fragment A (**17**).

Synthesis of fragment B involved coupling a dimethoxyacetal bromopyruvic acid (**18**, Scheme 3) with a benzyl-protected serine **19** in the presence of TBTU, HATU, and DIPEA in CH_2Cl_2 to form the pseudodipeptide fragment **20**. Hydro-

Scheme 2. Synthesis of San B Fragment A

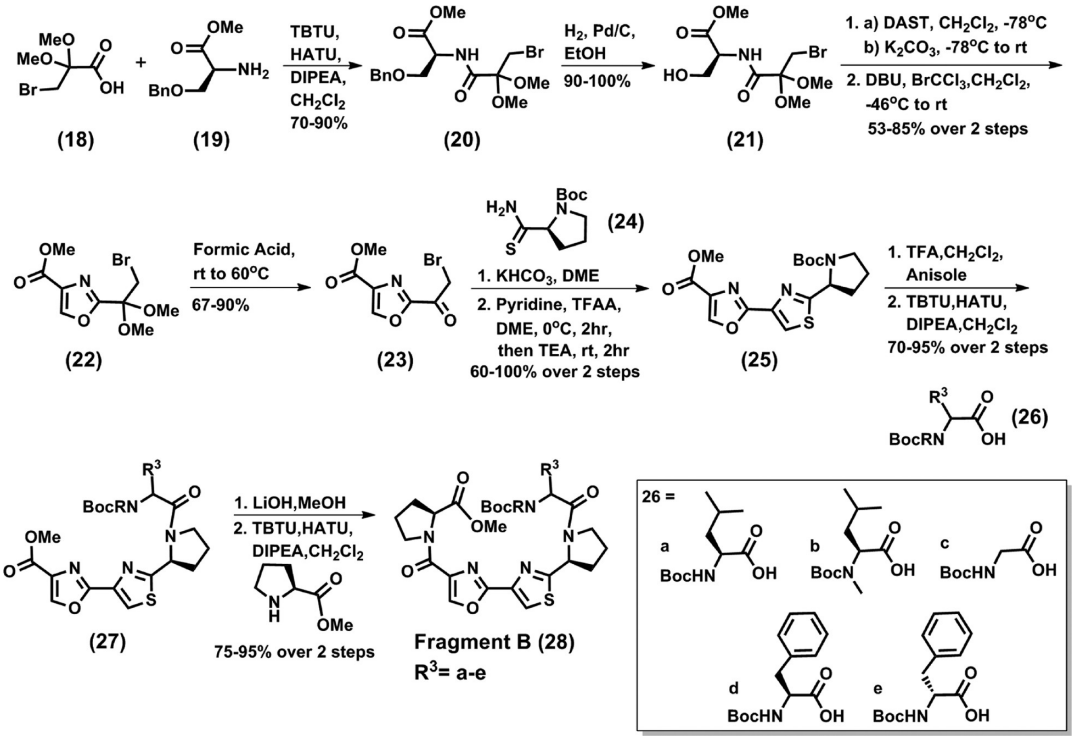


genolysis of this fragment generated the free serine **21**. This fragment was then subjected to the diethylaminosulfur trifluoride (DAST) fluorinating agent in CH_2Cl_2, followed by addition of K_2CO_3, which induced cyclization, generating the oxazoline intermediate. Oxidation of the oxazoline using $BrCCl_3$ with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in CH_2Cl_2 afforded the oxazole product **22**. Deprotection of ketone using formic acid at reflux prepared the α-bromo ketone oxazole **23** for thiazole formation. The α-bromo ketone oxazole was then introduced to the same modified Hantzsch thiazole synthesis with the premade proline thioamide **24** to form the tandem oxazole-thiazole moiety **25**. Removal of the Boc group using TFA to this moiety facilitated the coupling of the next amino acid carrying the R^3 substituent, **26**, in the presence of TBTU, HATU, and DIPEA in CH_2Cl_2 to afford the pseudotetrapeptide fragment **27**. Ester hydrolysis of this fragment using LiOH in methanol afforded the free acid species, which was coupled to the second proline residue to form fragment B **28**.

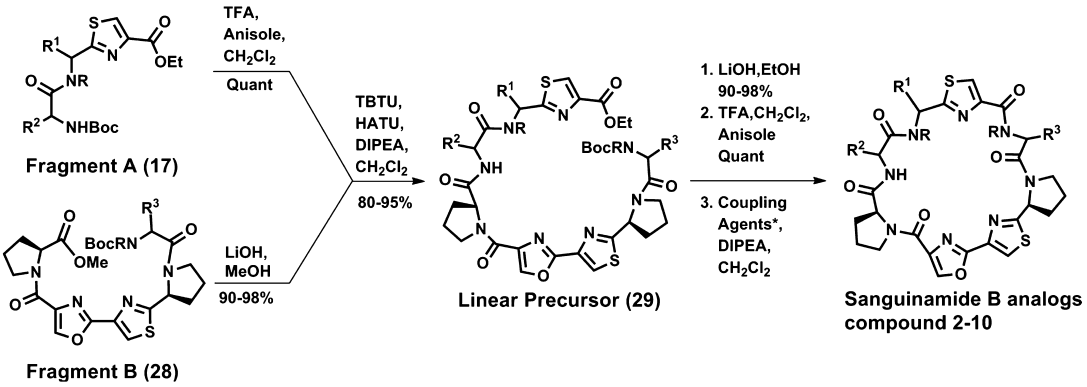
With fragment A (**17**, Scheme 4) and fragment B, **28**, in hand, coupling of these two fragments in the presence of TBTU, HATU, and DIPEA in CH_2Cl_2 afforded the protected linear precursor, **29**. Subsequent acid deprotection using LiOH

dx.doi.org/10.1021/jo3017499 | J. Org. Chem. 2012, 77, 10596−10616

Scheme 3. Synthesis of San B Fragment B



Scheme 4. Synthesis of Linear Precursor of San B and Cyclization[a]



[a]See Supporting Information for specific coupling agents and conditions.

in ethanol followed by amine deprotection using TFA in CH_2Cl_2 yielded the double deprotected linear precursor. Using a cocktail of coupling agents and DIPEA in dilute condition in CH_2Cl_2, cyclization of the double deprotected linear precursor was taken to form the San B analogues **2**–**10** (Figures 4–5).[38]

Structural Analysis of San B Analogues. The cyclic peptide derivatives were purified using HPLC and LCMS to identify unique conformers for each analogue. As previously noted in the synthesis of **1**, we identified two conformers of **1** upon synthesis.[10] The final structure and purities of the nine San B analogues and their conformers were established via ^1H and ^{13}C NMR and 2D NMR experiments (^1H–^1H COSY, ^1H–^{13}C HSQC, ^1H–^{13}C HMBC), as well as HPLC, LC/MS, and HRMS (see Supporting Information). To evaluate the structure of each analogue, we ran proton NMR temperature dependence plots to assess which temperature would be ideal for gathering the 2-D data. Given the potential interconversion about the prolyl or *N*-methyl amide bonds, gathering 2-D data would be best achieved using a temperature where the structure was primarily in a single conformation (indicated by sharp proton NMR peaks). Shown in Figure 5 are the temperature dependence plots of ^1H NMR for biologically active **7** and **8** analogues. All temperature-dependence plots of ^1H NMR for all other derivatives are available in the Supporting Information. In contrast to **1**, where 2-D data was collected at 263 K, 2-D data was collected at 308 K for **7**, while 2-D NMR data for **8** was collected at 318 K.[10]

Evaluation of the ideal temperature that generated defined peaks in each region for each analogue afforded the ideal temperature to run the HSQC, HMBC, and COSY NMR on each derivative. Identification of the configuration of each San B isomer about the proline bonds involved examining the chemical shifts of their respective Pro β and γ carbons. A Pro amide bond that adopts *cis* orientation has larger $\Delta\beta\gamma$ than a Pro with an amide bond in the *trans* orientation.[18] Analysis using the proline β and γ carbons of each analogue defined its

dx.doi.org/10.1021/jo3017499 | J. Org. Chem. 2012, 77, 10596–10616

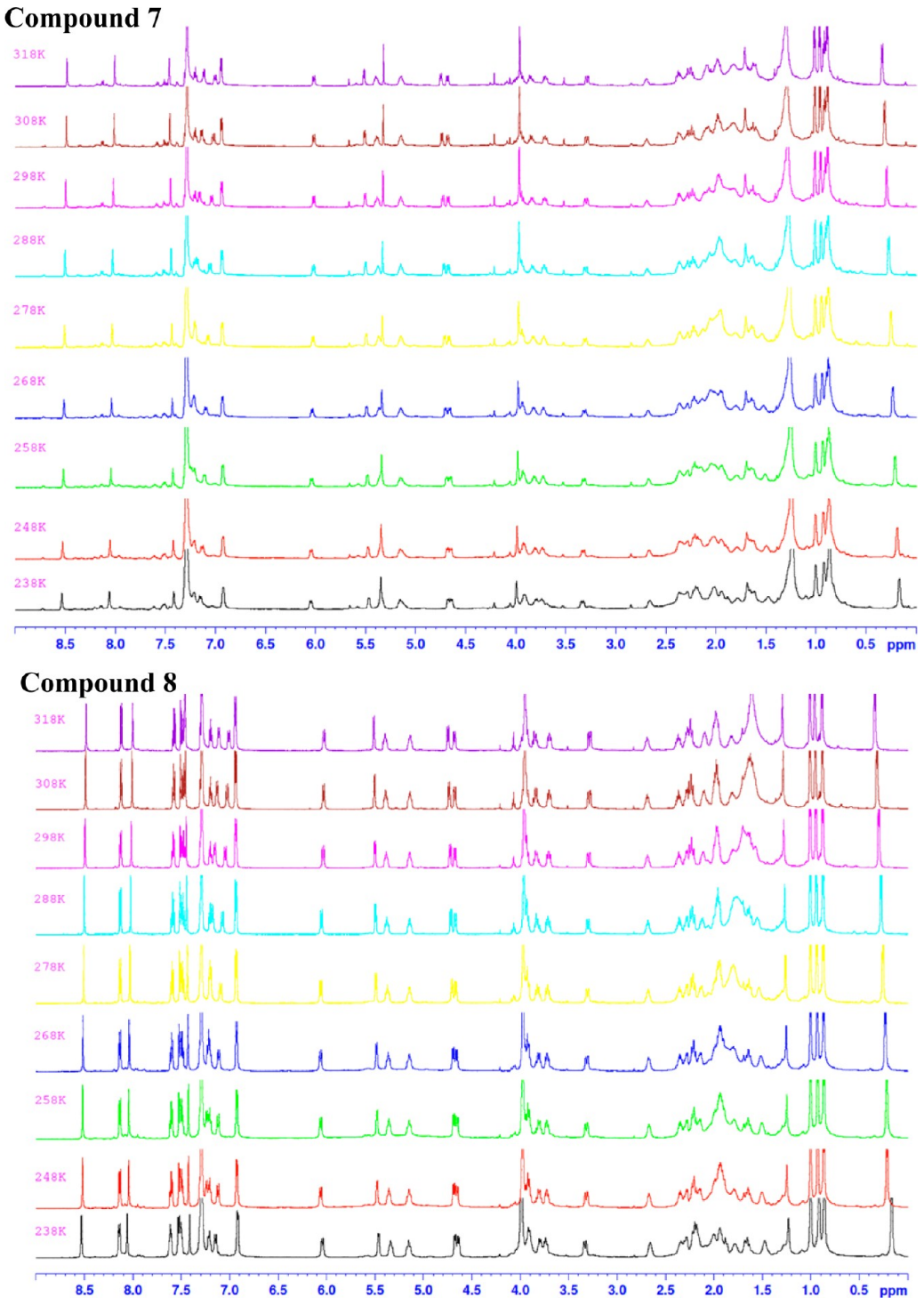


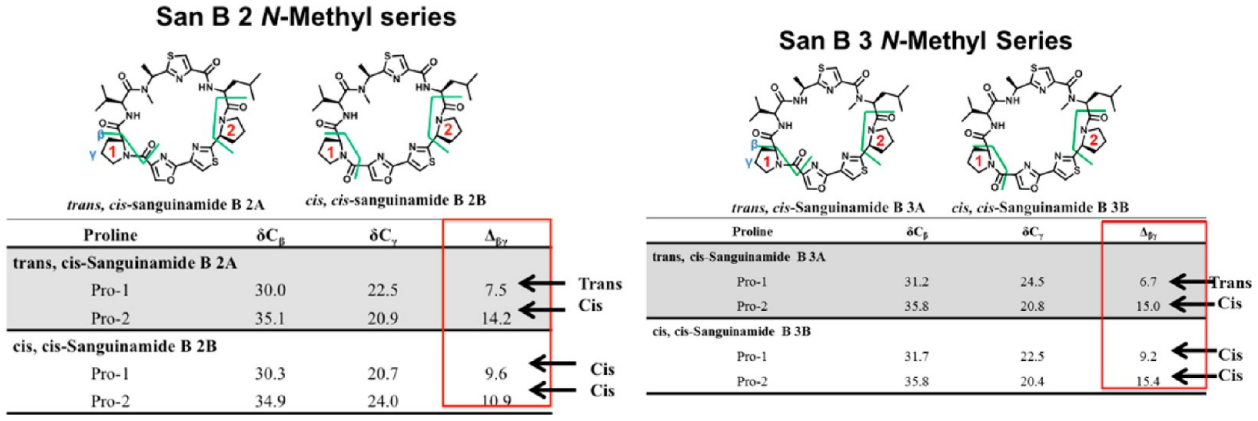
Figure 5. ^{1}H NMR temperature dependence plots of **7** and **8** ($CDCl_3$, 600 MHz).

conformation. A table of the β and γ carbon shifts for each conformer are shown (Figures 6, 7, and 8).

Given that population of a specific conformation that is appropriately oriented to bind to a protein or DNA target dictates the degree of desired biological activity, knowing the configuration about the prolyl residue is critical. As described earlier, *N*-Me moieties rigidify macrocycles, making them less flexible and allowing fewer conformers than the same compound without an *N*-Me. In our San B series, we observed that only proline 1 was able to interconvert between *cis* and *trans* configuration (Figure 6). Proline 2 remained as *cis* in both conformers of **2** and **3**. This observation is likely a result of the *N*-Me moiety that is placed within the macrocyclic backbone, whereby the *N*-Me requires a *cis* configuration about the prolyl bond.

In contrast to *N*-Me moieties, it is anticipated that incorporating glycine analogues will provide greater flexibility to the backbone compared to the inclusion of *N*-Me amino acids. This is observed by the number of conformers isolated for each compound with a glycine analogue. We observed three conformers when the glycine was substituted next to the proline 1 residue. The prolyl amide configuration of the two

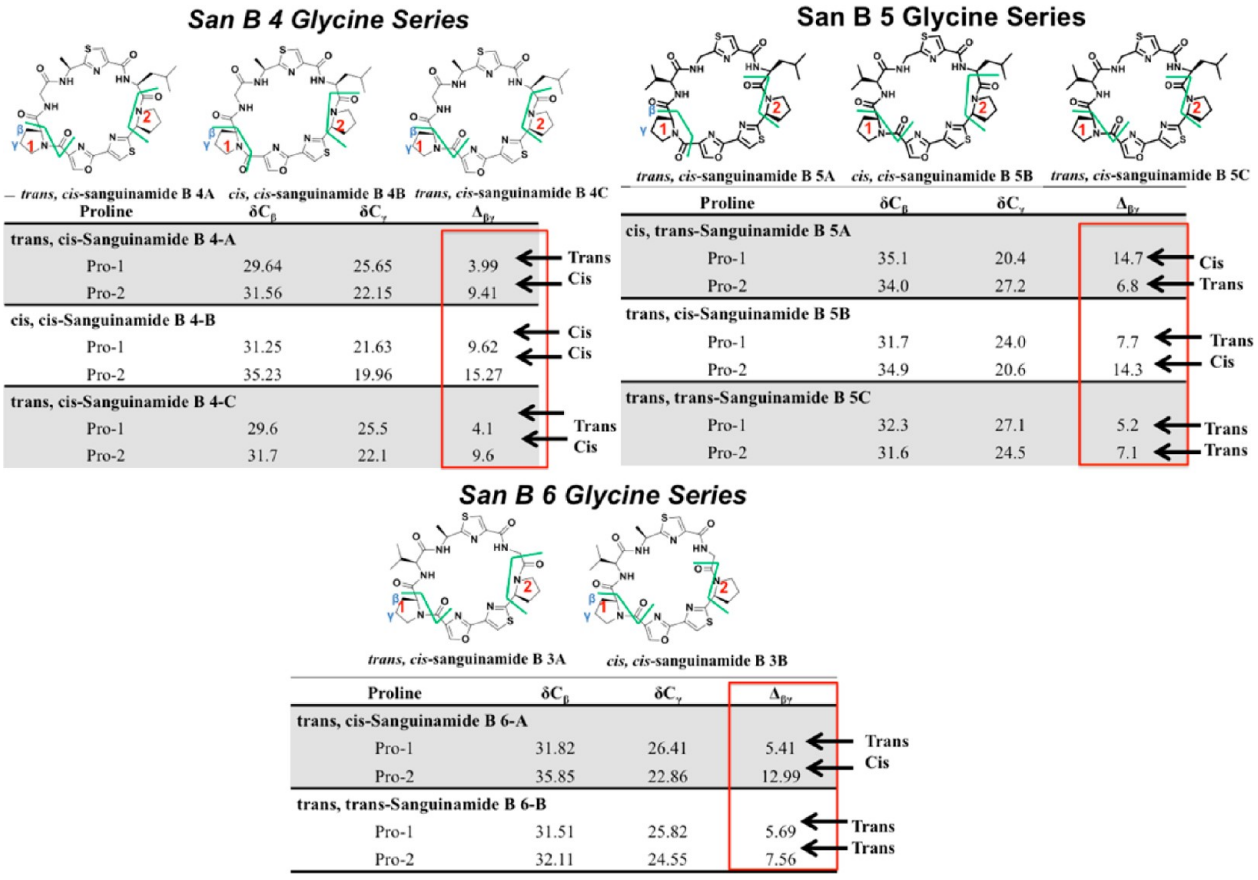
dx.doi.org/10.1021/jo3017499 | J. Org. Chem. 2012, 77, 10596−10616



Proline	δC_β	δC_γ	$\Delta_{\beta\gamma}$	
trans, cis-Sanguinamide B 2A				
Pro-1	30.0	22.5	7.5	Trans
Pro-2	35.1	20.9	14.2	Cis
cis, cis-Sanguinamide B 2B				
Pro-1	30.3	20.7	9.6	Cis
Pro-2	34.9	24.0	10.9	Cis

Proline	δC_β	δC_γ	$\Delta_{\beta\gamma}$	
trans, cis-Sanguinamide B 3A				
Pro-1	31.2	24.5	6.7	Trans
Pro-2	35.8	20.8	15.0	Cis
cis, cis-Sanguinamide B 3B				
Pro-1	31.7	22.5	9.2	Cis
Pro-2	35.8	20.4	15.4	Cis

Figure 6. Chemical shift of C_β and C_γ prolines of the *N*-methyl conformers.



Proline	δC_β	δC_γ	$\Delta_{\beta\gamma}$	
trans, cis-Sanguinamide B 4-A				
Pro-1	29.64	25.65	3.99	Trans
Pro-2	31.56	22.15	9.41	Cis
cis, cis-Sanguinamide B 4-B				
Pro-1	31.25	21.63	9.62	Cis
Pro-2	35.23	19.96	15.27	Cis
trans, cis-Sanguinamide B 4-C				
Pro-1	29.6	25.5	4.1	Trans
Pro-2	31.7	22.1	9.6	Cis

Proline	δC_β	δC_γ	$\Delta_{\beta\gamma}$	
cis, trans-Sanguinamide B 5A				
Pro-1	35.1	20.4	14.7	Cis
Pro-2	34.0	27.2	6.8	Trans
trans, cis-Sanguinamide B 5B				
Pro-1	31.7	24.0	7.7	Trans
Pro-2	34.9	20.6	14.3	Cis
trans, trans-Sanguinamide B 5C				
Pro-1	32.3	27.1	5.2	Trans
Pro-2	31.6	24.5	7.1	Trans

Proline	δC_β	δC_γ	$\Delta_{\beta\gamma}$	
trans, cis-Sanguinamide B 6-A				
Pro-1	31.82	26.41	5.41	Trans
Pro-2	35.85	22.86	12.99	Cis
trans, trans-Sanguinamide B 6-B				
Pro-1	31.51	25.82	5.69	Trans
Pro-2	32.11	24.55	7.56	Trans

Figure 7. Chemical shift of C_β and C_γ prolines of the glycine conformers.

conformers were *trans* at proline 1 and *cis* at proline 2 (**4A** and **4C**, Figure 7). These conformers have almost identical shifts; however, they have different retention times on the HPLC. Their identical proline orientation but unique retention times indicate that they are conformational analogues, each showing a unique 3-D conformation. The third conformer (**4B**, Figure 7) prefers a *cis,cis* configuration about both proline residues, suggesting that the glycine residue provides enough flexibility to allow proline 1 to reorient from *trans* to *cis*-.

Analogue **5** also has three conformers, but all three have distinct configurations about the prolyl amide bond. The glycine allows the macrocyclic backbone to be flexible enough for proline 1 and 2 to flip between *cis* and *trans* orientation (**5A** and **5B**, Figure 7). In contrast to compound **4**, this glycine analogue prefers the *trans,trans* configuration over the *cis,cis* (**5C**, Figure 7). The third glycine analogue only has two conformations: *trans,cis* (**6A**, Figure 7) and *trans,trans* (**6B**, Figure 7). These data support the hypothesis that modification of a single residue significantly alters the 3-D conformation. Further, the data support the hypothesis that each conformation is driven by unpredictable factors: replacing an amino acid at different locations within a macrocycle does not cause a single uniform effect. Finally, the data strongly reinforce the concept that the *only* valid method of determining the effect of a single modification on a macrocycle is to synthesize it and evaluate its final conformation.

Because glycines do not have side chains, they do not have energy requirements for positioning these side chains. This

dx.doi.org/10.1021/jo3017499 | J. Org. Chem. 2012, 77, 10596−10616

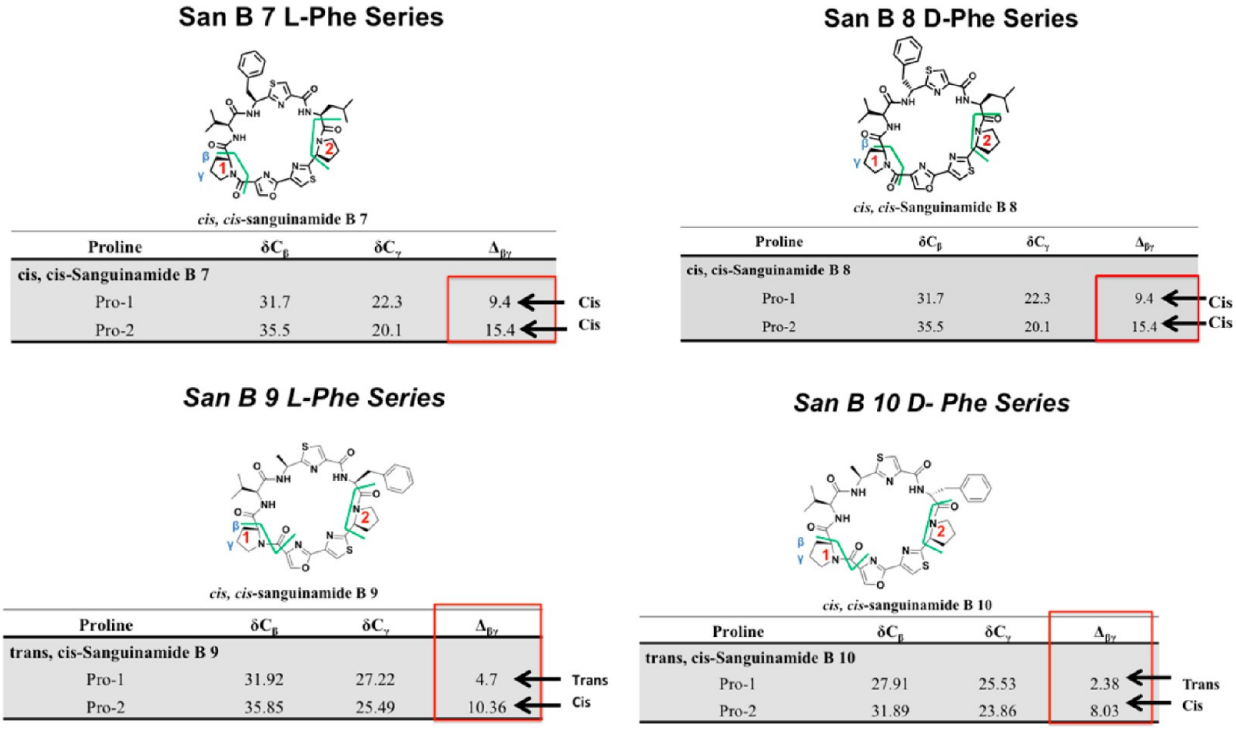


Figure 8. Chemical shift of C_β and C_γ prolines of the L- and D-Phe conformers.

means that glycines allow the macrocycle to respond to other energy related factors that drive the conformation, which produced multiple conformations for each glycine analogue. In contrast, L- and D-Phe have large side chains, whereby positioning of these side chains becomes a factor in determining the lowest energy conformation for each macrocycle. Exchanging the alanine for an L-phenylalanine (L-Phe)-generated compound **7** (Figure 8). Given that the benzyl side chain was anticipated to play a role in dictating the conformation, it is not surprising that only a single conformation was generated for each derivative. The absence of other conformations in the presence of an L-Phe substitution strongly supports our hypothesis that a single modification alters the 3-D conformation, and that the lowest energy conformation must be determined by experiment. Further, these data strongly support the hypothesis that the L-Phe analogue has locked the macrocycle into a single conformation by requiring it be placed in a "pseudoequatorial" position.

Insertion of a D-Phe residue at the same position as the L-Phe also generated only a single low energy conformation: *cis,cis* about the proline orientation (**7** and **8**, Figure 8). Comparison of the two Phe series to the corresponding glycine analogues (Figure 8) confirms the hypothesis that glycine allows the macrocyclic backbone freedom, while the phenylalanine analogues do not. Further, it should be noted that the Phe residues induce a single conformation, whereas the *N*-Me moieties induced two conformations. Thus, Phe residues are potential design elements, providing a superior approach for locking the molecule into a single conformation than utilizing the standard *N*-Me moiety. Assuming that a desirable conformation (i.e., one that is most effective at binding to the biological target) is achieved, improvements in the biological activity will then be observed.

Insertion of an L- and D-Phe into the backbone between proline 2 and the thiazole generated compounds **9** and **10** (Figure 8). As observed with compounds **7** and **8**, compounds **9** and **10** each had a single conformation. Although the L- and D-Phe in compounds **9** and **10** induce a *trans,cis* configuration about the two proline residues, as opposed to *cis,cis* seen in compounds **7** and **8**, formation of a single conformation reinforces the concept that Phe is an extremely useful design element.

In summary, placement of an *N*-methyl moiety within the macrocyclic backbone has the reported impact of inducing only two of the three possible configurations about the prolyl residue. In contrast to the *N*-methyl analogues, glycine-substituted analogues have three conformations, consistent with glycine providing flexibility within the macrocyclic backbone. Finally, we demonstrated that an L- or D-Phe locks the macrocycle into a single conformation, thereby constituting an ideal design element, superior to *N*-Me. We hypothesize that this is due to a "pseudoequatorial" requirement by the benzyl side chain. Although L- and D-Phe's have been substituted into other macrocycles, this is the first report demonstrating that they are more effective than *N*-Me moieties at locking a macrocycle into a single conformation.

■ BIOLOGICAL ACTIVITY

To measure the antimicrobial efficacy of San B conformers against Gram-positive and Gram-negative bacteria, we measured the cell viability of *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) following treatment with 50 μM of compound. The San B conformers did not reduce cell viability below 50% for either bacterial strain, indicating that the analogues did not exhibit antimicrobial activity (Supporting Information Figure S1).

Numerous bacterial pathogens require motility to establish infections and migrate from the initial site of colonization, and inhibitors of bacterial motility can dramatically impact other disease-promoting actions such as biofilm formation.[39]

dx.doi.org/10.1021/jo3017499 | J. Org. Chem. 2012, 77, 10596−10616

Pseudomonas aeruginosa (*P. aeruginosa*) are rod-shaped, Gram-negative bacteria with two motility organelles: a single unsheathed polar flagellum and type IV pili. The polar flagellum is a long, whip-like structure used in swarming and swimming motilities. Type IV pili are polar appendages responsible for adhesion to epithelium, biofilm formation, and swarming and twitching motilities. We examined the effects of all San B conformers on twitching motility using a type IV fimbriae-positive strain of *P. aeruginosa* (PAO1).

P. aeruginosa PAO1 (expressing type IV pili) or *S. aureus* (negative control, nonmodal) were stab-inoculated through a thin agar plate, containing either no compound (controls) or with 50 μM of San B analogues. The zone of motility was measured, and the twitching motility area was expressed as a percent of the untreated PAO1 control.

The most effective compound was the phenylalanine analogue **9** (*trans*,*cis* conformer), where the area of twitching motility was reduced in size by 74% when cells were treated with 50 μM of compound (Figure 9). Only one analogue

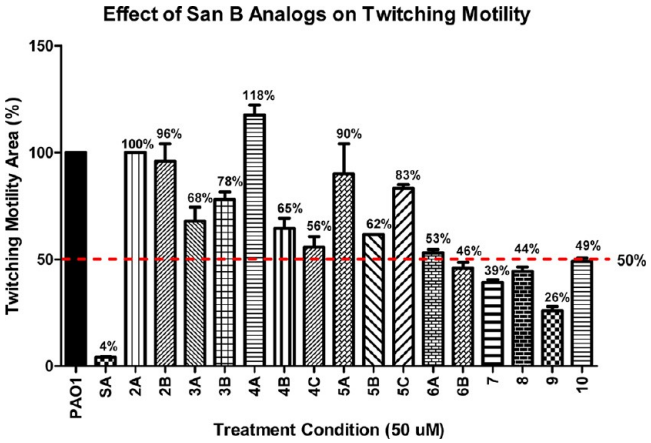


Figure 9. Twitching motility area (expressed as a percent of the untreated control) for untreated *P. aeruginosa* (PAO1; positive control), nonmodal *S. aureus* (SA; negative control) and *P. aeruginosa* PAO1 treated with 50 μM San B analogues (2A-10). The zone of twitching motility for untreated PAO1 was set at 100%.

compared favorably to the Phe derivatives, and it was compound **6B** (*trans*,*trans*). Consistent with our theory, all analogues containing Phe (**7**−**10**, Figure 8) have greater than 50% reduction in twitching motility. These data support the hypothesis that the Phe residues facilitate biological activity. Further, in both cases the L-Phe analogues are more effective than the D-Phe analogues in reducing twitching motility, highlighting that the modifications are not just assisting bioactivity but are related to an induced conformation from the Phe residue.

CONCLUSION

This is the first report describing the synthesis, conformational analysis, and structure−activity relationship of sanguinamide B analogues. Furthermore, it is the first proof that the exchange of an amino acid residue for an L- or D-Phe is a key design element. Our data support the fact that not only do both L- and D-Phe lock the macrocycle into a single conformation, performing better than if one installed the traditional *N*-Me moiety, these residues may also enhance the binding activity of the macrocycle to their molecular target(s). N-Methylation inhibits the peptide's ability to hydrogen bond, whereas Phe substitution does not, which could explain the observed reduction in the pilicidal activity compared to Phe-containing analogues. Not unexpectedly, there is a preferred stereochemistry of the Phe, in this case L-Phe, which is likely related to the "pseudoequatorial" position into which it is placed within the macrocycle. Finally, our data show that Phe-containing San B analogues reduce twitching motility by 50%, suggesting these compounds act as pilicides. Motility inhibitors are an emerging trend in antimicrobial development because they target novel systems essential for pathogenesis, and these small molecules are important candidates for further structure−activity studies.

EXPERIMENTAL SECTION

Experimental Procedures: Synthesis. *General Remarks.* All reactions were carried out under N_2 atmosphere with dry solvent under anhydrous conditions, unless indicated otherwise. Reagents were commercially obtained without further purification, unless otherwise stated. Reaction was monitored via thin-layer chromatography (TLC) carried out on silica gel plates using UV light at λ = 254 nm for visualization, and potassium permanganate in water with heat and developing agents. Silica gel was used for flash chromatography. NMR spectra were obtained at 298 K, while variable temperature (VT) NMR spectra were taken from 318 K to 238 K. LC/MS was recorded on an LCMS system connected to a trap running in positive electrospray ionization (ESI+) mode. The mobile phase was composed of DDI water with 0.1% (v/v) formic acid (solvent A), and HPLC grade acetonitrile with 0.1% (v/v) formic acid (solvent B). The gradient elution was conducted as follows: flow rate 0.5 mL/min; initial 70% solvent A, 30% solvent B; at 4 min 100% solvent B; at 12 min 70% solvent A, 30% solvent B. Semipreparative reversed-phase HPLC was carried out on an LCMS system. The mobile phase was composed of DDI water with 0.1% (v/v) formic acid (solvent A), and HPLC grade acetonitrile with 0.1% (v/v) formic acid (solvent B). The gradient elution was conducted as follows: flow rate 2 mL/min; initial 70% solvent A, 30% solvent B hold for 35 min; at 35 min 100% solvent B hold for 13 min; at 48 min 70% solvent A, 30% solvent B hold for 2 min.

General Peptide Synthesis. All peptide coupling reactions were carried out under N_2 atmosphere with dry solvent, using methylene chloride and/or acetonitrile for peptide couplings. The amine (1.1−1.5 equiv) and acid (1.0 equiv) were weighed into a dry flask along with 4−8 equiv of DIPEA and 1.1 equiv of TBTU. (Some coupling reactions would not go to completion using only TBTU and therefore 0.8−1.0 equiv of HATU, and/or 3-(diethoxyphosphoryloxy)-3*H*-benzo[*d*][1,2,3]triazin-4-one (DEPBT) or 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) were used. In a few cases, up to 0.8 equiv of all three coupling reagents were used.) Anhydrous methylene chloride and/or acetonitrile were added to generate a 0.1 M solution. The solution was stirred at room temperature for 1 h and reactions were monitored by TLC upon completion. The crude reaction was extracted with pH 1 hydrochloric acid solution, saturated sodium bicarbonate solution, and finally brine. The organic layers were combined, dried over sodium sulfate, filtered, and concentrated *in vacuo*. The crude product underwent purification via flash column chromatography on silica gel using a gradient of ethyl acetate−hexane to yield the desired peptide.

General Acid Protection. The acid was purged with N_2 and dissolved in a solvent mixture of benzene/methanol (3:2) to a concentration of 0.1 M. A solution of (trimethylsilyl)diazomethane (2.0 M in diethyl ether) was added dropwise to the reaction until the solution turned lightly yellow, and the mixture was stirred for 30 min. The reaction was monitored by TLC upon completion, and then the methyl ester was concentrated *in vacuo* and taken on to the next reaction without further purification.

General Amine Deprotection. Amines (0.1 M) were deprotected using 20% trifluoroacetic acid in methylene chloride with 2.0 equiv of anisole. Reactions were carried out for 1−2 h and were monitored by

dx.doi.org/10.1021/jo3017499 | J. Org. Chem. 2012, 77, 10596−10616

TLC upon completion. The amines were concentrated *in vacuo* and taken on to the next reaction without further purification.

General Acid Deprotection. Acids (0.1 M) were deprotected using 2 equiv of lithium hydroxide with 3.4 equiv of hydrogen peroxide in methanol or ethanol. The peptide was dissolved in methanol or ethanol and cooled to 0 °C. Hydrogen peroxide was added followed by lithium hydroxide. The reaction was monitored by TLC and usually done in 1−2 h. Upon completion, sodium thiosulfate (3.8 equiv) was added to neutralize the peroxide, and 5% hydrochloric acid was added till the solution pH was 1. The aqueous solution was extracted five times with methylene chloride, and the combined organic layer was dried, filtered, and concentrated *in vacuo* and taken on to the reaction without further purification.

Macrocyclization Procedure (with Syringe Pump). Three coupling agents (DEPBT or DMTMM, HATU, and TBTU) were used at ∼0.5 to 0.8 equiv each. These coupling agents were dissolved in 3/4 of a calculated volume of dry methylene chloride that would give a 0.001 to 0.0007 M overall concentration when included in the volume used for the deprotected peptide. The crude, dry, double-deprotected peptide (free acid and free amine) was dissolved in the other 1/4 solvent volume of methylene chloride. DIPEA (8 equiv) was then added to the solution containing coupling reagents dissolved in methylene chloride. The double-deprotected peptide was then added to the bulk solution dropwise using a syringe pump at a rate of 30 mL/h. The reaction was monitored via LCMS and generally complete in 1−2 h. Upon completion, the reaction was worked up by washing with aqueous hydrochloric acid (pH 1) and saturated sodium bicarbonate. After back extraction of aqueous layers with large quantities of methylene chloride, the organic layers were combined, dried, filtered, and concentrated. All macrocycles were first purified by flash column chromatography using an ethyl acetate/hexane gradient on silica gel. Finally, when necessary, reversed-phase HPLC was used for additional purification using a gradient of acetonitrile and deionized water with 0.1% formic acid.

General Amide Formation. Boc-protected amino ester (1.0 equiv) was dissolved in 50% ammonium hydroxide and 50% methanol to a concentration of 0.1 M. The reaction mixture was stirred overnight and monitored by TLC upon completion. Upon completion, the solvent was concentrated *in vacuo*, and the amides were taken on to the next reaction without further purification.

General Thioamide Formation. Boc-protected amide (1.0 equiv) was converted into Boc-protected thioamide using Lawesson's Reagent (0.8 equiv) in 0.4 M 1,2-dimethoxyethane at room temperature under N_2. The mixture was stirred overnight and monitored by TLC upon completion. Upon completion, the solvent was concentrated *in vacuo*. Boc-protected thioamide was purified by flash column chromatography using an ethyl acetate/methylene chloride or ethyl acetate/hexane gradient on silica gel.

General Thiazole Synthesis (Modified Hantzsch). Thiazole synthesis reaction was carried out under N_2 with anhydrous 1,2-dimethoxyethane. $KHCO_3$ (8.0 equiv) was added to the dry flask containing peptidyl thioamide (1.0 equiv). Anhydrous 1,2-dimethoxyethane (0.1 M) was added to the reaction, and it was stirred at room temperature for 15 min. α-Bromo ketone residue (3.0 equiv) was added (0.1 mL/min), and the reaction mixture was stirred overnight. Upon reaction completion, confirmed by TLC, the desired thiazoline intermediate was concentrated in vacuo, redissolved in ethyl acetate, extracted with brine, dried over sodium sulfate, filtered, and concentrated in vacuo. The crude thiazoline intermediate was redissolved in 1,2-dimethoxyethane and stirred at 0 °C for 15 min. Next, pyridine (9.0 equiv) was added (0.1 mL/min) to a solution of thiazoline in 1,2-dimethoxyethane (0.05 M) at 0 °C under N_2 for the dehydration of the thiazoline to yield the desired thiazole. The reaction was stirred for 15 min and then TFAA (4.0 equiv) was added to the reaction mixture (0.1 mL/min). After 3 h, triethylamine (TEA) (2.0 equiv) was added to the reaction mixture (0.1 mL/min), and the reaction was stirred at room temperature for an additional 2−3 h or until complete as determined by TLC. Upon completion, the crude reaction was washed with pH 1 hydrochloric acid solution (100 mL × 2), saturated sodium bicarbonate solution (100 mL × 10), and finally brine (100 mL × 2). The organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo*. The washed reaction underwent a purification with flash column chromatography using a gradient of ethyl acetate/methylene chloride or ethyl acetate/hexane to give the desired peptidyl-thiazole. Finally, when necessary, reversed-phase HPLC was used for additional purification using a gradient of acetonitrile and deionized water with 0.1% formic acid.

*Compound **2**. MeO-Ala-NMeBoc (**12b**).* MeO-Ala-NMeBoc was synthesized following the General Acid Protection procedure, utilizing 1.51 g (7.45 mmol, 1.0 equiv) of HO-Ala-NMeBoc (**11b**) and 5.30 mL of (trimethylsilyl)diazomethane in 29.8 mL of methanol and 44.7 mL of benzene. The methyl ester was concentrated *in vacuo* and taken on to the next reaction without further purification (1.62 g, quantitative yield) as clear crystals. R_f: 0.89 (hexanes/ethyl acetate 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (t, 3H, $CHCH_3$); 1.50 (s, 9H, $C(CH_3)_3$); 2.81−2.92 (m, 3H, NCH_3); 3.75 (s, 3H, OCH_3); 4.37−4.49 (br, 1αH); 4.82−4.93 (br, 1H, N*H*).

*NH_2-Ala-NMeBoc (**13b**).* NH_2-Ala-NMeBoc was synthesized following the General Amide Formation procedure, utilizing 1.62 g (7.45 mmol, 1.0 equiv) of MeO-Ala-NMeBoc (**12b**) dissolved in 37.3 mL of ammonium hydroxide (25% in water) and 37.3 mL of methanol. The resulting amide was taken on to the next reaction without further purification (1.50 g, quantitative yield) as a white powder (rotamers 2:1). R_f: 0.15 (hexanes/ethyl acetate 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.36 (d, J = 7.04 Hz, 3H, $CHCH_3$); 1.50 (s, 9H, $C(CH_3)_3$); 2.83 (s, 3H, NCH_3); 4.27−4.95 (br, 2H, αH and N*H*); 5.78−6.34 (br, 2H, NH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.8, 28.4, 30.9, 80.2, 81.0, 156.4, 174.7.

*Thioamide-Ala-NMeBoc (**14b**).* Thioamide-Ala-NMeBoc was synthesized following the General Thioamide Formation procedure, utilizing 1.50 g (7.45 mmol, 1.0 equiv) of NH_2-Ala-NMeBoc (**13b**) and 2.26 g (5.59 mmol, 0.75 equiv) of Lawesson's Reagent dissolved in 74.5 mL of 1,2-dimethoxyethane. The thioamide was purified via column chromatography on silica gel (hexanes/ethyl acetate 1:1 to 3:7) to afford the desired thioamide (797 mg, 49% yield) as white crystals. R_f: 0.6 (hexanes/ethyl acetate 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43 (s, 9H, $C(CH_3)_3$), 1.48 (d, J = 7.27 Hz, 3H, $CHCH_3$), 2.80 (s, 3H, NCH_3); 4.83−4.95 (m, 1H, αH), 7.86−8.07 (br, 1H, NH_aH_b), 8.07−8.28 (br, 1H, NH_aH_b). ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.4, 28.4, 30.1, 58.5, 81.0, 156.7, 208.8.

*EtO-Thiazole-Ala-NMeBoc (**15b**).* EtO-Thiazole-Ala-NMeBoc was synthesized following the General Thiazole Synthesis procedure, utilizing 797 mg (3.65 mmol, 1.0 equiv) of thioamide-Ala-NMeBoc (**14b**) and 2.92 g (29.2 mmol, 8.0 equiv) of potassium bicarbonate dissolved in 36.5 mL of 1,2-dimethoxyethane. A 1.38 mL amount of ethyl bromopyruvate (11.0 mmol, 3.0 equiv) was added dropwise (0.1 mL/min) to yield the thiazoline intermediate. The thiazoline was dehydrated using 2.66 mL (32.9 mmol, 9.0 equiv) of pyridine, 2.03 mL (14.6 mmol, 4.0 equiv) of trifluoroacetic anhydride, and 1.02 mL (7.30 mmol, 2.0 equiv) of triethylamine in 36.5 mL of 1,2-dimethoxyethane to afford the desired thiazole (826 mg, 72% yield) as a light yellow oil. R_f: 0.78 (hexanes/ethyl acetate 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.37−1.44 (t, 3H, OCH_2CH_3); 1.49 (s, 9H, $C(CH_3)_3$); 1.67−1.73 (d, J = 7.2 Hz, 3H, $CHCH_3$); 2.79 (s, 3H, NCH_3); 4.36−4.46 (q, 2H, OCH_2CH_3); 5.40−5.80 (m, 1H, 1αH); 8.13 (s, 1H, SC*H*). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.7, 17.1, 28.6, 29.4, 61.5, 80.9, 128.2, 147.2, 155.5, 161.5, 173.8. HRMS (ESI-TOF): M + Na^+, found 337.1187 $C_{14}H_{22}N_2O_4S$ requires 337.1198.

*EtO-Thiazole-Ala-NMeH (**15b-1**).* EtO-Thiazole-Ala-NMeH was synthesized following the General Amine Deprotection procedure. The amine was taken on to the next reaction without further purification or characterization. (571 mg, quantitative yield) as a light brown oil.

*EtO-Thiazole-Ala-NMe-Val-NHBoc (**17c**).* EtO-Thiazole-Ala-NMe-Val-NHBoc was synthesized following the General Peptide Synthesis procedure, utilizing 512 mg (2.39 mmol, 1.0 equiv) of amine EtO-Thiazole-Ala-NMeH (**15b-1**), 571 mg (2.63 mmol, 1.1 equiv) of acid HO-Val-NHBoc (**16a**), 844 mg (2.63 mmol, 1.1 equiv) of TBTU, and 1.67 mL (9.56 mmol, 4 equiv) of DIPEA dissolved in 23.9 mL of methylene chloride under N_2. The crude product underwent a final

dx.doi.org/10.1021/jo3017499 | *J. Org. Chem.* 2012, 77, 10596−10616

purification via column chromatography on silica gel (hexanes/ethyl acetate 19:1 to 3:1) to afford the desired peptide (562 mg, 57% yield) as a yellow oil. R_f: 0.55 (hexanes/ethyl acetate 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.95−1.00 (d, J = 6.7 Hz, 3H, CHCH(CH_3)$_2$); 1.00−1.06 (d, J = 6.7 Hz, 3H, CHCH(CH_3)$_2$); 1.39−1.46 (t, 3H, OCH$_2$$CH_3$); 1.48 (s, 9H, C($CH_3$)$_3$); 1.70−1.76 (d, J = 7.3 Hz, 3H, CHCH_3); 1.95−2.12 (m, 1H, CHCH(CH$_3$)$_2$); 2.99 (s, 1H, NCH_3); 4.38−4.48 (q, 2H, OCH_2CH$_3$); 5.28−5.40 (m, 1H, NH); 6.15−6.24 (m, 1H, 1αH); 8.15 (s, 1H, SCH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.4, 16.0, 17.2, 19.7, 28.4, 30.3, 31.2, 50.8, 55.4, 61.5, 79.7, 128.2, 147.0, 156.0, 161.3, 171.4, 172.7.

EtO-Thiazole-Ala-NMe-Val-NH$_2$ (17c-1), Fragment A. EtO-Thiazole-Ala-NMe-Val-NH$_2$ was synthesized following the General Amine Deprotection procedure. The amine was taken on to the next reaction without further purification or characterization (426 mg, quantitative yield) as a light brown oil.

Experimental procedures and compound characterizations for **18**−**28a-1** were performed as described in ref 3.

EtO-Thiazole-Ala-NMe-Val-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc (29-2). EtO-Thiazole-Ala-NMe-Val-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc was synthesized following the General Peptide Synthesis procedure, utilizing 506 mg (0.88 mmol, 1.0 equiv) of acid HO-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc (**28a-1**), 304 mg (0.97 mmol, 1.1 equiv) of amine EtO-Thiazole-Ala-NMe-Val-NH$_2$ (**17c-1**), 225 mg (0.70 mmol, 0.8 equiv) of TBTU, 266 mg (0.70 mmol, 0.8 equiv) of HATU, and 1.22 mL (7.00 mmol, 8.0 equiv) of DIPEA dissolved in 9.00 mL of methylene chloride. The crude product underwent a final purification via column chromatography on silica gel (ethyl acetate/methanol 99:1) to afford the desired peptide (294 mg, 38% yield). R_f: 0.30 (ethyl acetate/methanol 99:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.64−0.74 (m, 3H, CHCH(CH_3)$_2$); 0.74−0.82 (m, 3H, CHCH-(CH_3)$_2$); 0.85−0.91 (d, J = 6.7 Hz, 3H, CH(CH_3)$_2$); 0.91−0.96 (d, J = 6.7 Hz, 3H, CH(CH_3)$_2$); 1.26−1.34 (t, 3H, OCH$_2$$CH_3$); 1.35 (s, 9H, C($CH_3$)$_3$); 1.39−1.49 (m, 2H, CHC$H_2$CH(CH$_3$)$_2$); 1.57−1.64 (d, J = 8.7 Hz, 3H, CHCH_3); 1.65−1.78 (m, 1H, CHCH(CH$_3$)$_2$); 1.84−1.96 (m, 2H, CHCH$_2$CH_2CH$_2$); 1.87−2.05 (m, 1H, CHCH(CH$_3$)$_2$); 1.96−2.00 and 2.09−2.16 (m, 2H, CHCH_2CH$_2$CH$_2$); 2.00−2.09 (m, 2H, CHCH$_2$CH_2CH$_2$); 2.16−2.43 (m, 2H, CHCH_2CH$_2$CH$_2$); 2.79 and 2.91 (s, 1H, NCH_3); 3.59−3.85 and 3.98−4.16 (m, 4H, CHCH$_2$CH$_2$CH_2); 4.24−4.36 (q, 2H, OCH_2CH$_3$); 4.41−4.53 (m, 1H, 1αH); 4.62−4.76 (m, 1H, 1αH); 5.23−5.36 (m, 1H, 1αH); 5.43−5.52 (m, 1H, 1αH); 5.84−6.14 (m, 1H, 1αH); 7.31−7.43 (m, 1H, NH); 7.86 and 8.07 (s, 1H, SCH); 8.04 (s, 1H, SCH); 8.21 and 8.26 (s, 1H, OCH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.4, 16.0, 17.5, 19.8, 21.8, 22.0, 23.5, 24.5, 24.6, 25.4, 27.8, 28.4, 30.3, 31.7, 42.1, 47.1, 49.1, 50.4, 50.8, 54.3, 58.8, 61.0, 61.4, 61.7, 79.6, 120.7, 121.4, 128.3, 137.9, 142.8, 143.3, 146.9, 155.7, 156.4,161.1, 170.8, 171.4, 172.0, 172.5, 172.8, 174.3. LC/MS (ESI): m/z calcd for $C_{41}H_{58}N_8O_9S_2$ (M + H$^+$) = 893.37, found 893.00. HRMS (ESI-TOF): M + H$^+$, found 871.3846 $C_{41}H_{58}N_8O_9S_2$ requires 871.3840.

HO-Thiazole-Ala-NMe-Val-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc (29-2a). HO-Thiazole-Ala-NMe-Val-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc was synthesized following the General Acid Deprotection procedure, utilizing 294 mg (0.338 mmol, 1.0 equiv) of EtO-Thiazole-Ala-NMe-Val-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc (**29-2**) and 113 mg (2.70 mmol, 8 equiv) of LiOH·H$_2$O in 4.00 mL of ethanol. The acid was taken on to the next reaction without further purification (222 mg, 78% yield). LC/MS (ESI): m/z calcd for $C_{39}H_{54}N_8O_9S_2$ (M + H$^+$) = 843.35, found 843.00.

HO-Thiazole-Ala-NMe-Val-Pro-Oxazole-Thiazole-Pro-Leu-NH$_2$ (29-2b). HO-Thiazole-Ala-NMe-Val-Pro-Oxazole-Thiazole-Pro-Leu-NH$_2$ was synthesized following the General Amine Deprotection procedure, utilizing 222 mg (0.264 mmol, 1.0 equiv) of HO-Thiazole-Ala-NMe-Val-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc (**29-2a**), 57.4 μL (0.53 mmol, 2.0 equiv) of anisole, and 0.54 mL of trifluoroacetic acid in 2.10 mL of methylene chloride. HO-Thiazole-Ala-NMe-Val-Pro-Oxazole-Thiazole-Pro-Leu-NH$_2$ was taken on to the next reaction without further purification or characterization (196 mg, quantitative yield). LC/MS (ESI): m/z calcd for $C_{34}H_{46}N_8O_7S_2$ (M + H$^+$) = 743.29, found 743.00.

•*Compound 2.* Compound **2** was synthesized following the Macrocyclization Procedure, utilizing 196 mg (0.264 mmol, 1.0 equiv) of HO-Thiazole-Ala-NMe-Val-Pro-Oxazole-Thiazole-Pro-Leu-NH$_2$ (**29-2b**), 68.0 mg (0.210 mmol, 0.8 equiv) of TBTU, 80.0 mg (0.210 mmol, 0.8 equiv) of HATU, 63.0 mg (0.210 mmol, 0.8 equiv), and 370 μL (2.11 mmol, 8 equiv) of DIPEA in 377 mL of methylene chloride. The crude product underwent an initial purification via column chromatography on silica gel (ethyl acetate/methanol 9:1), and the resulting semipure residue was subjected to reversed-phase HPLC purification to afford a 3.6:1 ratio of compound **2-A** (8.9 mg) and compound **2-B** (7.6 mg) in a 37.6% yield. LC/MS (ESI): m/z calcd for $C_{34}H_{44}N_8O_6S_2$ (M + H$^+$) = 724.28, found 725.00. HRMS (ESI-TOF): M + Na$^+$, found 747.2723 $C_{34}H_{44}N_8O_6S_2$ requires 747.2706.

•*Compound 2-A.* ^{1}H NMR (308 K, 600 MHz, $CDCl_3$): δ 0.97−1.06 (m, 6H, CHCH(CH_3)$_2$); 0.96−1.04 (m, 6H, CH(CH_3)$_2$); 1.58−1.62 (br, 3H, CHCH_3); 1.61−1.68 (m, 1H, CHCH$_2$CH(CH$_3$)$_2$); 1.72−1.78 and 1.84−1.94 (m, 2H, CHCH_2CH(CH$_3$)$_2$); 1.80 and 1.97−2.03 (m, 2H, C$H_2\gamma$ Pro2); 2.01−2.06 (m, 2H, C$H_2\gamma$ Pro1); 2.03−2.05 and 2.48−2.50 (m, 2H, C$H_2\beta$ Pro1); 2.19−2.25 (m, 1H, CHCH(CH$_3$)$_2$); 2.31−2.39 (m, 2H, C$H_2\beta$ Pro2); 3.27 (s, 1H, NCH_3); 3.69−3.83 (m, 4H, C$H_2\delta$ Pro); 4.71−4.75 (m, 1H, 1αH); 4.75−4.81 (m, 1H, 1αH); 5.02−5.09 (m, 1H, 1αH); 5.47−5.51 (m, 1H, 1αH); 5.89−5.94 (m, 1H, 1αH); 7.11−7.20 (m, 1H, NH); 7.48−7.54 (m, 1H, NH); 7.55 (s, 1H, SCH); 8.04 (s, 1H, SCH); 8.08 (s, 1H, OCH). ^{13}C NMR (150 MHz, $CDCl_3$): δ 17.8, 18.1, 19.3, 20.9 (C$H_2\gamma$ Pro2), 22.5 (C$H_2\gamma$ Pro1), 22.4, 23.4, 24.3, 29.5, 30.0 (C$H_2\beta$ Pro1), 30.5, 30.6, 35.1 (C$H_2\beta$ Pro2), 41.3, 46.7, 49.1, 51.3, 55.0, 59.8, 62.5, 122.2, 122.4, 137.9, 140.9, 142.7, 148.9, 154.0, 161.1, 170.8, 171.4, 172.3, 172.9, 173.8, 174.3. $\triangle\beta\gamma$ = (7.5. ppm; 14.2 ppm).

•*Compound 2-B.* ^{1}H NMR (318 K, 600 MHz, $CDCl_3$): δ 0.67−0.94 (m, 6H, CHCH(CH_3)$_2$); 0.94−1.11 (m, 6H, CH(CH_3)$_2$); 1.62−1.69 (m, 1H, CHCH$_2$CH(CH$_3$)$_2$); 1.64−1.69 and 1.79−1.86 (m, 3H, CHCH_3); 1.65−1.72 and 1.93−1.96 (m, 2H, CHCH_2CH(CH$_3$)$_2$); 1.76−1.85 and 1.94−2.03 (m, 2H, C$H_2\gamma$ Pro1); 2.07−2.14 (m, 2H, C$H_2\gamma$ Pro2); 2.12−2.19 and 2.28−2.42 (m, 2H, C$H_2\beta$ Pro2); 2.18−2.20 and 2.33−2.39 (m, 2H, C$H_2\beta$ Pro1); 2.41−2.46 (m, 1H, CHCH(CH$_3$)$_2$); 3.24 (s, 1H, NCH_3); 3.58−67 and 4.18−4.22 (m, 2H, C$H_2\delta$ Pro1); 3.67−3.85 (m, 2H, C$H_2\delta$ Pro2); 4.76−4.80 and 4.88−4.92 (m, 1H, 1αH); 4.83−4.90 (m, buried, 1H, 1αH); 5.03−5.16 (m, 1H, 1αH); 5.37−5.42 and 5.52−5.59 (m, 1H, 1αH); 5.78−5.84 (m, 1H, 1αH); 7.00−7.06 (m, 1H, NH); 7.41−7.45 (m, 1H, NH); 7.60 (s, 1H, SCH); 7.89 (s, 1H, SCH); 8.31 (s, 1H, OCH). ^{13}C NMR (150 MHz, $CDCl_3$): δ 16.7, 18.1, 18.2, 19.6, 20.1, 20.7 (C$H_2\gamma$ Pro1), 22.0, 23.4, 24.0 (C$H_2\gamma$ Pro2), 24.3, 30.3 (C$H_2\beta$ Pro1), 30.7, 34.9 (C$H_2\beta$ Pro2), 46.8, 47.3, 48.8, 51.4, 55.0, 59.6, 59.9, 123.7, 124.3, 125.7, 137.9, 142.7, 148.9, 154.0, 161.1, 170.8, 171.4, 172.3, 172.9, 173.8, 174.3. $\Delta\beta\gamma$ (Pro1, Pro2) = (9.6 ppm; 10.9 ppm).

Compound 3. Experimental procedures and compound characterizations for **12a**−**17a-1** were performed as described in ref 3.

MeO-Oxazole-Thiazole-Pro-NMe-Leu-NBoc (27b). MeO-Oxazole-Thiazole-Pro-NMe-Leu-NBoc was synthesized following the General Peptide Synthesis procedure, utilizing 114 mg (0.41 mmol, 1.0 equiv) of amine MeO-Oxazole-Thiazole-Pro-NH (**25-1**), 110 mg (0.45 mmol, 1.1 equiv) of acid HO-NMe-Leu-NBoc (**26b**), 106 mg (0.33 mmol, 0.8 equiv) of TBTU, 125 mg (0.33 mmol, 0.8 equiv) of HATU, and 0.43 mL (2.46 mmol, 6 equiv) of DIPEA dissolved in 4.5 mL of methylene chloride. The crude product underwent a final purification via column chromatography on silica gel (hexanes/ethyl acetate 17:3 to 1:1) to afford the desired peptide (213 mg, 97% yield) as white flakes. R_f: 0.3 (hexanes/ethyl acetate 3:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.80−0.93 (m, 6H, CHCH$_2$CH(CH$_3$)$_2$); 1.38 and 1.41 (s, 9H, C(CH_3)$_3$); 1.39−1.54 (m, 1H, CHCH$_2$CH(CH$_3$)$_2$); 1.43−1.67 (m, 1H, CHCH_2CH(CH$_3$)$_2$); 1.93−2.22 (m, 2H, CHCH$_2$CH_2CH$_2$); 2.14−2.45 (m, 2H, CHCH_2CH$_2$CH$_2$); 2.76 and 2.78 (s, 3H, NCH_3); 3.49−3.76 (m, 2H, CHCH$_2$CH$_2$CH_2); 3.85 (s, 3H, OCH_3); 4.73−4.82 and 4.97−5.07 (m, 1H, 1αH); 5.40−5.47 (dd, J = 7.46, 2.44 Hz, 1H, 1αH); 7.99 (s, 1H, SCH); 8.23 (s, 1H, OCH). ^{13}C NMR (75 MHz,$CDCl_3$): δ 22.0, 23.0, 24.5, 24.8, 28.4, 29.8, 31.7, 37.8, 47.1, 52.2, 53.5, 55.0, 58.7, 79.9, 121.5, 134.2, 142.1, 143.7, 156.1, 157.4, 161.4,

dx.doi.org/10.1021/jo3017499 | J. Org. Chem. 2012, 77, 10596−10616

171.5, 174.5. HRMS (ESI-TOF): M + Na^+, found 529.2082 $C_{24}H_{34}N_4O_6S$ requires 529.2097.

*HO-Oxazole-Thiazole-Pro-NMe-Leu-NBoc (**27b-1**).* HO-Oxazole-Thiazole-NMe-Leu-NBoc was synthesized following the General Acid Deprotection procedure, utilizing 213 mg (0.42 mmol, 1.0 equiv) of MeO-Oxazole-Thiazole-Pro-NMe-Leu-NBoc (**27b**) and 52.9 mg (1.26 mmol, 3.0 equiv) of $LiOH·H_2O$ in 4.2 mL of methanol. The acid was taken on to the next reaction without further purification (207 mg, quantitative yield) as a clear oil.

*MeO-Pro-Oxazole-Thiazole-Pro-NMe-Leu-NBoc (**28b**).* MeO-Pro-Oxazole-Thiazole-Pro-NMe-Leu-NBoc was synthesized following the General Peptide Synthesis procedure, utilizing 207 mg (0.42 mmol, 1.0 equiv) of acid HO-Oxazole-Thiazole-Pro-NMe-Leu-NBoc (**27b-1**), 59.5 mg (0.46 mmol, 1.1 equiv) of amine MeO-Pro-NH, 109 mg (0.34 mmol, 0.8 equiv) of TBTU, 129 mg (0.34 mmol, 0.8 equiv) of HATU, and 0.44 mL (2.52 mmol, 6.0 equiv) of DIPEA dissolved in 4.2 mL of methylene chloride. The crude product underwent a final purification via column chromatography on silica gel (hexanes/ethyl acetate 4:1 to 0:1) to afford the desired peptide (265 mg, 96% yield). R_f: 0.45 (hexanes/ethyl acetate 0:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.82−0.92 (m, 6H, $CHCH_2CH(CH_3)_2$); 1.38 and 1.41 (s, 9H, $C(CH_3)_3$); 1.42−1.50 (m, 1H, $CHCH_2CH(CH_3)_2$); 1.44−1.64 (m, 2H, $CHCH_2CH$); 1.77−1.89 (m, 2H, $CHCH_2CH_2CH_2$); 1.91−2.12 (m, 2H, $CHCH_2CH_2CH_2$); 1.89−1.96 and 2.13−2.18 (m, 2H, $CHCH_2CH_2CH_2$); 2.10−2.39 (m, 2H, $CHCH_2CH_2CH_2$); 2.75 and 2.77 (s, 3H, NCH_3); 3.60−3.78 and 4.01−4.09 (m, 4H, $CHCH_2CH_2CH_2$); 3.62 and 3.64 (s, 3H, OCH_3); 4.50−4.59 and 5.19−5.28 (m, 1H, 1αH); 4.72−4.82 and 4.95−5.06 (m, 1H, 1αH); 5.37−5.46 (m, 1H, 1αH); 7.78 and 7.88 (s, 1H, SCH); 8.17 and 8.19 (s, 1H, OCH). ^{13}C NMR (75 MHz,$CDCl_3$): δ 21.8, 22.0, 22.9, 24.4, 27.9, 28.3, 29.1, 29.7, 31.6, 37.8, 46.7, 47.1, 47.6, 48.7, 52.3, 53.5, 54.9, 58.6, 59.9, 60.3, 60.7, 79.9, 120.6, 137.8, 142.8, 143.1, 155.1, 156.1, 160.2, 171.0, 173.4. HRMS (ESI-TOF): M + Na^+, found 626.2606 $C_{29}H_{41}N_5O_7S$ requires 626.2625

*HO-Pro-Oxazole-Thiazole-Pro-NMe-Leu-NBoc (**28b-1**), Fragment B.* HO-Pro-Oxazole-Thiazole-Pro-NMe-Leu-NBoc was synthesized following the General Acid Deprotection procedure, utilizing 265 mg (0.44 mmol, 1.0 equiv) of MeO-Pro-Oxazole-Thiazole-Pro-NMe-Leu-NBoc (**28b**), 55 mg (1.32 mmol, 3.0 equiv) of $LiOH·H_2O$ in 4.40 mL of methanol. The acid was taken on to the next reaction without further purification (259 mg, quantitative yield) as clear flakes.

*EtO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-NMe-Leu-NHBoc (**29-3**).* EtO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-NMe-Leu-NHBoc was synthesized following the General Peptide Synthesis procedure, utilizing 259 mg (0.44 mmol, 1.0 equiv) of acid HO-Pro-Oxazole-Thiazole-Pro-NMe-Leu-NBoc (**28b-1**), 137 mg (0.49 mmol, 1.1 equiv) of amine EtO-Thiazole-Ala-Val-NH_2 (**17a-1**), 112 mg (0.35 mmol, 0.8 equiv) of TBTU, 133 mg (0.35 mmol, 0.8 equiv) of HATU, and 0.46 mL (2.64 mmol, 6.0 equiv) of DIPEA dissolved in 4.40 mL of methylene chloride. The crude product underwent a final purification via column chromatography on silica gel (ethyl acetate/methanol 99:1) to afford the desired peptide (400 mg, 94% yield). R_f: 0.30 (ethyl acetate/methanol 99:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.74−0.81 and 0.84−0.92 (dd, J = 21.8, 6.3 Hz, 3H, $CHCH(CH_3)_2$); 0.82−0.97 (dd, J = 14.4, 6.3 Hz, 3H, $CHCH(CH_3)_2$); 0.92−1.02 (dd, J = 11.9, 6.3 Hz, 6H, $CHCH_2CH(CH_3)_2$); 1.34−1.44 (t, 3H, OCH_2CH_3); 1.49 (s, 9H, $C(CH_3)_3$); 1.49−1.60 (m, 1H, $CHCH_2CH(CH_3)_2$); 1.52−1.73 (m, 2H, $CHCH_2CH(CH_3)_2$); 1.62−1.72 (d, J = 7.0 Hz, 3H, $CHCH_3$); 1.98−2.05 (m, 2H, $CHCH_2CH_2CH_2$); 2.00−2.22 (m, 2H, $CHCH_2CH_2CH_2$); 2.07−2.19 and 2.24−2.31 (m, 2H, $CHCH_2CH_2CH_2$); 2.25−2.49 (m, 2H, $CHCH_2CH_2CH_2$); 2.28−2.38 (m, 1H, $CHCH(CH_3)_2$); 2.80−2.89 (m, 3H, NCH_3); 3.61−3.68 and 3.73−3.87 (m, 2H, $CHCH_2CH_2CH_2$); 4.08−4.29 (m, 2H, $CHCH_2CH_2CH_2$); 4.29−4.39 (m, 1H, 1αH); 4.34−4.46 (q, 2H, OCH_2CH_3); 4.72−4.82 and 5.30−5.37 (m, 1H, 1αH); 4.82−4.91 and 5.06−5.16 (m, 1H, 1αH); 5.14−5.24 and 5.35−5.48 (m, 1H, 1αH); 5.46−5.57 (m, 1H, 1αH); 7.09−7.18 (d, J =8.6 Hz, 1H, NH); 7.36−7.42 (d, J =7.3 Hz, 1H, NH); 7.94 and 8.05 (s, 1H, SCH); 8.04 and 8.08 (s, 1H, SCH); 8.29 (s, 1H, OCH). ^{13}C NMR (75 MHz,$CDCl_3$): δ 14.3, 17.5, 17.8, 19.5, 21.0, 22.1, 22.2, 23.0, 24.5, 24.7, 25.5, 27.8, 28.3, 29.5, 31.8, 37.8, 46.7, 47.1, 47.5, 49.5, 53.5, 55.0, 58.6, 58.8, 61.4, 61.6, 80.1, 121.0, 121.7, 127.3, 127.5, 137.5, 142.5, 143.5,146.9, 155.2, 156.2, 156.8, 164.3, 170.4, 170.9, 171.6, 174.1, 175.0. LC/MS (ESI): m/z calcd for $C_{41}H_{58}N_8O_9S_2$ (M + H^+) = 871.38, found 871.00. HRMS (ESI-TOF): M + Na^+, found 893.3653 $C_{41}H_{58}N_8O_9S_2$ requires 893.3666

*HO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-NMe-Leu-NHBoc (**29-3a**).* HO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-NMe-Leu-NHBoc was synthesized following the General Acid Deprotection procedure, utilizing 400 mg (0.470 mmol, 1.0 equiv) of EtO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-NMe-Leu-NHBoc (**29-3**) and 80 mg (1.877 mmol, 4 equiv) of $LiOH·H_2O$ in 5.00 mL of ethanol. The acid was taken on to the next reaction without further purification (274 mg, 71% yield). LC/MS (ESI): m/z calcd for $C_{39}H_{55}N_8O_9S_2$ (M + H^+) = 843.35, found 843.00

*HO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-NMe-Leu-NH_2 (**29-3b**).* HO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-NMe-Leu-NH_2 was synthesized following the General Amine Deprotection procedure, utilizing 274 mg (0.334 mmol, 1.0 equiv) of HO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-NMe-Leu-NHBoc (**29−3a**), 70.0 μL (0.67 mmol, 2.0 equiv) of anisole, and 0.70 mL of trifluoroacetic acid in 2.60 mL of methylene chloride. HO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-NMe-Leu-NH_2 was taken on to the next reaction without further purification or characterization (241 mg, quantitative yield). LC/MS (ESI): m/z calcd for $C_{34}H_{46}N_8O_7S_2$ (M + H^+) = 743.30, found 743.00.

•*Compound **3**.* Compound **3** was synthesized following the Macrocyclization Procedure, utilizing 241 mg (0.334 mmol, 1.0 equiv) of HO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-NMe-Leu-NH_2 (**29-3b**), 87.0 mg (0.270 mmol, 0.8 equiv) of TBTU, 103.0 mg (0.270 mmol, 0.8 equiv) of HATU, 75.0 mg (0.270 mmol, 0.8 equiv) of DMTMM, and 350 μL (2.00 mmol, 6 equiv) of DIPEA in 334 mL of methylene chloride. The crude product was directly subjected to reversed-phase HPLC purification to afford a 1:1.1 of compound **3-A** (66.5 mg), and compound **3-B** (103.9 mg) in a 25.2% overall yield. HRMS (ESI-TOF): M + Na^+, found 747.2711 $C_{34}H_{44}N_8O_6S_2$ requires 747.2723.

•*Compound **3-A**.* ^{1}H NMR (318 K, 600 MHz, $CDCl_3$): δ 0.92−0.96 and 0.99−1.03 (m, 3H, $CHCH(CH_3)_2$); 0.99−1.02 and 1.04−1.08 (m, 3H, $CHCH(CH_3)_2$); 0.91−1.05 (m, 6H, $CHCH_2CH(CH_3)_2$); 1.44−1.57 and 1.60−1.69 (m, 1H, $CHCH_2CH(CH_3)_2$); 1.53−1.58 and 1.64−1.68 and 1.76−1.81 (m, 3H, $CHCH_3$); 1.68−1.74 and 1.81−1.88 (m, 2H, $CHCH_2CH(CH_3)_2$); 1.74−2.09 (m, 2H, $CHCH_2CH_2CH_2$); 2.00−2.18 (m, 2H, $CHCH_2CH_2CH_2$); 2.08−2.14 and 2.24−2.41 and 2.43−2.49 (m, 2H, $CHCH_2CH_2CH_2$); 2.13−2.20 and 2.22−2.52 (m, 2H, $CHCH_2CH_2CH_2$); 2.35−2.56 (m, 1H, $CHCH(CH_3)_2$); 3.09−3.16 and 3.21−3.34 (m, 3H, NCH_3); 3.61−4.00 (m, 2H, $CHCH_2CH_2CH_2$); 3.84−3.97 and 4.08−4.13 (m, 2H, $CHCH_2CH_2CH_2$); 4.31−4.38 and 4.54−4.59 (m, 1H, 1αH); 4.76−4.84 and 5.16−5.24 (m, 1H, 1αH); 5.32−5.47 (m, 1H, 1αH); 5.47−5.75 (m, 1H, 1αH); 5.67−5.81 (m, 1H, 1αH); 6.80−6.85 and 6.91−6.96 and 7.00−7.07 (m, 1H, NH); 7.11−7.15 and 7.21−7.27 and 7.34−7.39 (m, 1H, NH); 7.65 and 7.74 and 7.92 (s, 1H, SCH); 7.49 and 7.73 and 8.01 (s, 1H, SCH); 7.95 and 8.20 and 8.33 (s, 1H, OCH). ^{13}C NMR (318 K, 150 MHz,$CDCl_3$): δ 16.8, 17.5, 19.5, 19.7, 20.8 ($CH_2\gamma$ Pro2), 21.1, 21.4, 21.8, 22.6, 23.0, 24.5, 24.6, 25.0 ($CH_2\gamma$ Pro1), 25.8, 26.5, 29.2, 29.5, 29.8, 30.0, 31.2 ($CH_2\beta$ Pro1), 31.7, 32.3, 32.8, 33.8, 34.6, 34.9, 35.3, 35.8 ($CH_2\beta$ Pro2), 37.5, 46.2, 46.8, 47.1, 47.4, 47.9, 48.9, 49.2, 53.6, 54.3, 55.9, 58.2, 59.0, 59.1, 59.4, 60.9, 63.0, 63.3, 121.0, 121.2, 122.3, 127.6, 127.9, 128.6, 129.0, 142.0, 142.1, 144.2,146.9, 155.2, 156.2, 156.8, 162.4, 164.3, 170.4, 170.9, 171.6, 174.1, 175.0. $\triangle\beta\gamma$(Pro1, Pro2) = (6.7 ppm; 15.0 ppm).

•*Compound **3-B**.* ^{1}H NMR (318 K, 600 MHz, $CDCl_3$): δ 0.82−0.87 (d, J = 6.61 Hz, 3H, $CHCH(CH_3)_2$); 0.94−0.99 (d, J = 6.61 Hz, 3H, $CHCH(CH_3)_2$); 0.90−1.00 (m, 6H, $CHCH_2CH(CH_3)_2$); 0.95−1.01 (m, 3H, $CHCH_3$); 1.45−1.52 (m, 1H, $CHCH_2CH(CH_3)_2$); 1.57−1.63 and 1.81−1.87 (m, 2H, $CHCH_2CH(CH_3)_2$); 1.76−1.82 and 1.89−1.94 (m, 2H, $CHCH_2CH_2CH_2$); 1.85−1.91 and 2.25−2.32 (m, 2H, $CHCH_2CH_2CH_2$); 1.96−2.02 and 2.06−2.12 (m, 2H, $CHCH_2CH_2CH_2$); 2.22−2.29 (m, 2H, $CHCH_2CH_2CH_2$); 2.78−2.85

dx.doi.org/10.1021/jo3017499 | J. Org. Chem. 2012, 77, 10596−10616

(m, 1H, CHC*H*(CH_3)$_2$); 3.09−3.14 (s, 3H, NCH_3); 3.77−3.84 (m, 2H, CHCH_2CH_2CH_2); 3.77−3.83 and 3.84−3.91 (m, 2H, CHCH_2CH_2CH_2); 4.65−4.71 (m, 1H, 1αH); 4.72−4.78 (m, 1H, 1αH); 5.07−5.14 (m, 1H, 1αH); 5.49−5.54 (m, 1H, 1αH); 5.63−5.69 (m, 1H, 1αH); 6.23−6.33 (m, 1H, N*H*); 7.15−7.24 (m, 1H, N*H*); 7.46 (s, 1H, SC*H*); 8.00 (s, 1H, SC*H*); 8.35 (s, 1H, OC*H*). ^{13}C NMR (318 K, 150 MHz,$CDCl_3$): δ 16.4, 16.6, 19.5, 20.4 (C$H_2\gamma$ Pro2), 22.5 (C$H_2\gamma$ Pro1), 22.7, 23.0, 24.5, 28.4, 31.7 (C$H_2\beta$ Pro1), 35.8 (C$H_2\beta$ Pro2), 37.4, 45.4, 46.2, 47.8, 53.9, 56.6, 58.7, 64.2, 123.0, 129.5, 136.7, 140.5, 143.1, 147.9, 157.8, 161.0, 167.7, 168.5, 170.3, 171.3, 171.5, 173.3, 173.6. $\Delta\beta\gamma$ (Pro1, Pro2) = (9.2 ppm; 15.4 ppm).

Compound ***4****. EtO-Thiazole-Ala-Gly-NHBoc (****17b****).* EtO-Thiazole-Ala-Gly-NHBoc was synthesized following the General Peptide Synthesis procedure, utilizing 200 mg (0.995 mmol, 1.0 equiv) of amine EtO-Thiazole-Ala-NH_2 (**15a-1**), 192 mg (1.10 mmol, 1.1 equiv) of acid HO-Gly-NHBoc (**16c**), 320 mg (0.995 mmol, 1.0 equiv) of TBTU, and 0.70 mL (3.99 mmol, 4 equiv) of DIPEA dissolved in 10 mL of methylene chloride under N_2. The reaction mixture was stirred for 3 h and was monitored by TLC upon completion. The crude product underwent a final purification via column chromatography on silica gel (hexanes/ethyl acetate 1:1) to afford the desired peptide (220 mg, 62% yield) as a yellow oil. R_f: 0.325 (hexanes/ethyl acetate 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.33−1.38 (t, 3H, OCH$_2$CH_3); 1.39 (s, 9H, C(CH_3)$_3$); 1.59−1.62 (d, 3H, CHCH_3); 3.84−3.86 (s, 2H, CH_2); 4.32−4.39 (m, 2H, OCH_2CH$_3$); 5.34−5.44 (m, 1H, 1αH), 8.05 (s, 1H, SC*H*). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.09, 21.00, 28.51, 44.19, 47.40, 61.94, 80.08, 126.39, 127.91, 147.03, 156.16, 161.31, 169.92, 173.44. HRMS (ESI-TOF): M + Na^+, found 380.1247 $C_{15}H_{23}N_3O_5S$ requires 380.1256.

EtO-Thiazole-Ala-Gly-NH_2 *(****17b-1****), Fragment A.* EtO-Thiazole-Ala-Gly-NH_2 was synthesized following the General Amine Deprotection procedure, utilizing 220 mg (0.616 mmol, 1.0 equiv) of EtO-Thiazole-Ala-Gly-NHBoc (**17b**), 0.14 mL (1.23 mmol, 2.0 equiv) of anisole, and 1.5 mL of trifluoroacetic acid in 4.5 mL of methylene chloride. EtO-Thiazole-Ala-Gly-NH_2 was taken on to the next reaction without further purification or characterization (201 mg, quantitative yield) as a light brown oil.

*EtO-Thiazole-Ala-Gly-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc (****29-4****).* EtO-Thiazole-Ala-Gly-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc was synthesized following the General Peptide Synthesis procedure, utilizing 300 mg (0.521 mmol, 1.0 equiv) of acid HO-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc (**28a-1**), 201 mg (0.782 mmol, 1.5 equiv) of amine EtO-Thiazole-Ala-Gly-NH_2 (**17b-1**), 167 mg (0.521 mmol, 1.0 equiv) of TBTU, 198 mg (0.521 mmol, 1.0 equiv) of HATU, and 0.73 mL (4.17 mmol, 8.0 equiv) of DIPEA dissolved in 5.2 mL of methylene chloride under N_2. The reaction mixture was stirred for 3 h and was monitored by TLC and LCMS upon completion. The crude product underwent a final purification via column chromatography on silica gel (ethyl acetate/methanol 19:1) to afford the desired peptide (390 mg, 94% yield) as yellow flakes. R_f: 0.25 (ethyl acetate/methanol 9:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86−0.99 (m, 6H, CH_3CHCH_3); 1.37 (s, 9H, C(CH_3)$_3$); 1.48−1.56 (t, 2H, OCH$_2$CH_3); 1.56−1.65 (m, 1H, CH$_3$C*H*CH$_3$); 1.65−1.82 (d, 3H, CHCH_3); 1.99−2.09 (m, 2H, CHCH$_2$CH_2CH$_2$); 2.09−2.19 (m, 2H, CHCH$_2$ CH_2CH$_2$); 2.21−2.32 (m, 2H, CHCH_2 CH$_2$CH$_2$); 2.33−2.46 (m, 2H, CHCH_2 CH$_2$CH$_2$); 3.63−3.73 (m, 2H, CHCH$_2$ CH$_2$CH_2); 3.78−3.87 (m, 2H, CHCH$_2$ CH$_2$CH_2); 3.86−3.94 (d, 2H, CH$_2$); 4.07−4.19 (m, 3H, OCH$_2$CH$_3$); 4.42−4.52 (m, 1H, 1αH); 4.51−4.59 (m, 1H, 1αH); 5.28−5.34 (m, 1H, 1αH); 5.45−5.54 (m, 1H, 1αH); 7.86 (s, 1H, SC*H*); 7.99 (s, 1H, SC*H*); 8.32 (s, 1H, OC*H*). ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.26, 18.42, 20.52, 21.83, 23.34, 24.59, 25.60, 28.29, 28.65, 31.68, 42.04, 47.06, 47.42, 49.50, 50.38, 58.80, 61.35, 61.98, 79.60, 120.90, 127.43, 127.56, 137.35, 143.49, 146.46, 155.68, 156.51, 160.84, 169.42, 171.38, 172.81, 174.36. HRMS (ESI-TOF): M + Na^+, found 837.3029 $C_{37}H_{50}N_8O_9S_2$ requires 837.3040.

*HO-Thiazole-Ala-Gly-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc (****29-4a****).* HO-Thiazole-Ala-Gly-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc was synthesized following the General Acid Deprotection procedure, utilizing 390 mg (0.478 mmol, 1.0 equiv) of EtO-Thiazole-Ala-Gly-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc (**29-4**) and 161 mg (3.83 mmol, 8 equiv) of LiOH·H_2O in 4.78 mL of ethanol. The acid was taken on to the next reaction without further purification (354 mg, 94% yield). LC/MS (ESI): m/z calcd for $C_{35}H_{46}N_8O_9S_2$ (M + H^+) = 786.28, found 787.00

HO-Thiazole-Ala-Gly-Pro-Oxazole-Thiazole-Pro-Leu-NH_2 *(****29-4b****).* HO-Thiazole-Ala-Gly-Pro-Oxazole-Thiazole-Pro-Leu-NH_2 was synthesized following the General Amine Deprotection procedure, utilizing 354 mg (0.449 mmol, 1.0 equiv) of HO-Thiazole-Ala-Gly-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc (**29-4a**), 97 μL (0.897 mmol, 2.0 equiv) of anisole, and 1.25 mL of trifluoroacetic acid in 3.75 mL of methylene chloride. HO-Thiazole-Ala-Gly-Pro-Oxazole-Thiazole-Pro-Leu-NH_2 was taken on to the next reaction without further purification or characterization (353 mg, quantitative yield). LC/MS (ESI): m/z calcd for $C_{30}H_{38}N_8O_7S_2$ (M + H^+) = 686.80, found 687.00

•*Compound* ***4****.* Compound **4** was synthesized following the Macrocyclization Procedure, utilizing 353 mg (0.514 mmol, 1.0 equiv) of HO-Thiazole-Ala-Gly-Pro-Oxazole-Thiazole-Pro-Leu-NH_2 (**29-4b**), 165 mg (0.514 mmol, 1.0 equiv) of TBTU, 235 mg (0.617 mmol, 1.0 equiv) of HATU, and 0.72 mL (4.11 mmol, 8 equiv) of DIPEA in 147 mL of methylene chloride under N_2. The reaction was monitored by LCMS upon completion. The crude product underwent an initial purification via column chromatography on silica gel (ethyl acetate/methanol 9:1), and the resulting semipure residue was subjected to reversed-phase HPLC purification to afford a 1:0.1:4.48 of compound **4-A** (14.1 mg), compound **4-B** (3.9 mg), and compound **4-C** (52.3 mg) in a 66.6% overall yield. HRMS (ESI-TOF): M + Na^+, found 691.2094 $C_{30}H_{36}N_8O_6S_2$ requires 691.2097.

•*Compound* ***4-A****.* ^{1}H NMR (298 K, 600 MHz, $CDCl_3$): δ 0.89−0.99 (m, 6H, CH(CH_3)$_2$); 0.92−1.01 (d, 3H, CHCH_3); 1.53−1.59 (m, 1H, CHCH$_2$C*H*(CH_3)$_2$); 1.48−1.59 (m, 2H, CHCH_2CH); 1.84−2.08 (m, 2H, C$H_2\gamma$ Pro1); 1.96−2.06 (m, 2H, C$H_2\gamma$ Pro2); 2.02−2.45 (m, 2H, C$H_2\beta$ Pro1); 2.24−2.28 (m, 2H, C$H_2\beta$ Pro2); 3.35−4.21 (m, 2H, C$H_2\delta$ Pro2); 3.83−3.88 (m, 2H, C$H_2\delta$ Pro1); 4.49−4.52 (m, 1H, 1αH); 4.75−4.81 (m, 1H, 1αH); 5.09−5.13 (m, 2H, CH$_2$); 5.54−5.46 (m, 1H, C*H*CH$_3$); 5.55−5.58 (m, 1H, C*H*CH$_2$CH); 7.96 (s, 1H, SCH); 8.24 (s, 1H, SCH); 8.38 (s, 1H, OCH).^{13}C NMR (150 MHz,$CDCl_3$): δ 16.0, 22.1, 22.15 (C$H_2\gamma$ Pro2), 24.4, 25.65 (C$H_2\gamma$ Pro1), 29.64 (C$H_2\beta$ Pro1), 31.56 (C$H_2\beta$ Pro2), 41.4, 45.51,48.04, 49.76, 58.8, 60.45, 62.37, 63.74, 121.4, 122.5,125.63, 127.83, 139.17, 143.4, 148.47. $\Delta\beta\gamma$ = (3.99 ppm; 9.41 ppm)

•*Compound* ***4-B****.* ^{1}H NMR (278 K, 600 MHz, CD_3CN): δ 0.87−0.91 (d, 3H, CHCH_3); 0.91−0.97 (m, 6H, CH(CH_3)$_2$); 1.44−1.51 (m, 1H, CHCH$_2$C*H*(CH_3)$_2$); 1.60−1.77 (m, 2H, CHCH_2CH); 1.85−1.97 (m, 2H, C$H_2\gamma$ Pro1); 1.90−1.96 (m, 2H, C$H_2\gamma$ Pro2); 2.08−2.18 (m, 2H, C$H_2\beta$ Pro1); 1.90−2.33 (m, 2H, C$H_2\beta$ Pro2); 3.56−3.65 (m, 2H, C$H_2\delta$ Pro2); 3.65−3.76 (m, 2H, C$H_2\delta$ Pro1); 4.34−4.39 (m, 1H, 1αH); 4.74−4.76 (m, 1H, 1αH); 5.07−5.09 (m, 2H, CH$_2$); 5.34−5.38 (m, 1H, C*H*CH$_3$); 5.47−5.55 (m, 1H, C*H*CH$_2$CH); 7.94 (s, 1H, SCH); 8.01 (s, 1H, SCH); 8.47 (s, 1H, OCH). ^{13}C NMR (278 K, 150 MHz, CD_3CN): δ 19.96 (C$H_2\gamma$ Pro2), 21.28, 21.63 (C$H_2\gamma$ Pro1), 22.74, 24.27, 31.25 (C$H_2\beta$ Pro1), 35.23 (C$H_2\beta$ Pro2), 40.87, 45.28, 46.71, 47.39, 58.73, 59.41, 62.03, 63.3, 122.13, 124.43, 125.54, 128.47, 140.58, 142.67, 143.3. $\Delta\beta\gamma$ = (9.62 ppm; 15.27 ppm).

•*Compound* ***4-C****.* ^{1}H NMR (298 K, 600 MHz, CD_3CN): δ 0.87−0.91 (d, 3H, CHCH_3); 0.89−97 (m, 6H, CH(C_3)$_2$); 1.49−1.57 (m, 1H, CHCH$_2$C*H*(CH_3)$_2$); 1.57−1.87 (m, 2H, CHCH_2CH); 1.81−2.04 (m, 2H, C$H_2\gamma$ Pro1); 1.94−2.02 (m, 2H, C$H_2\gamma$ Pro2); 1.97−2.41 (m, 2H, C$H_2\beta$ Pro1); 2.13−2.33 (m, 2H, C$H_2\beta$ Pro2); 3.77−3.84 (m, 2H, C$H_2\delta$ Pro2); 3.78−3.91 (m, 2H, C$H_2\delta$ Pro1); 4.44−4.50(m, 1H, 1αH); 4.71−4.79 (m, 1H, 1αH); 5.04−5.14 (m, 2H, CH$_2$); 5.39−5.43 (m, 1H, C*H*CH$_3$); 5.49−5.54 (m, 1H, C*H*CH$_2$CH); 7.51 (s, 1H, SCH); 7.92 (s, 1H, SCH); 7.72 (s, 1H, OCH). ^{13}C NMR (298 K, 150 MHz, CD_3CN): δ 22.1 (C$H_2\gamma$ Pro2), 22.21, 22.97, 23.45, 24.4, 25.5 (C$H_2\gamma$ Pro1), 29.6 (C$H_2\beta$ Pro1), 31.7 (C$H_2\beta$ Pro2), 41.27, 45.51, 46.67, 48.11, 58.8, 60.38, 62.3, 63.67, 121.68, 122.63, 125.69, 139.3, 143.45. $\Delta\beta\gamma$ = (4.1 ppm; 9.6 ppm).

Compound ***5****.* Experimental procedures and compound characterizations for **12c−15c** were performed as described.[40]

EtO-Thiazole-Gly-NH_2 *(****15c-1****).* EtO-Thiazole-Gly-NH_2 was synthesized following the General Amine Deprotection procedure. The

dx.doi.org/10.1021/jo3017499 | J. Org. Chem. 2012, 77, 10596−10616

amine was taken on to the next reaction without further purification or characterization (66.1 mg, quantitative yield) as a light brown oil.

*EtO-Thiazole-Gly-Val-NHBoc (**17d**).* EtO-Thiazole-Gly-Val-NHBoc was synthesized following the General Peptide Synthesis procedure, utilizing 66.1 mg (0.35 mmol, 1.0 equiv) of amine EtO-Thiazole-Gly-NH_2 (**15c-1**), 83.6 mg (0.39 mmol, 1.1 equiv) of acid HO-Val-NHBoc (**16a**), 125 mg (0.39 mmol, 1.1 equiv) of TBTU, and 244 μL (1.40 mmol, 4 equiv) of DIPEA dissolved in 35.0 mL of methylene chloride under N_2. The crude product underwent a final purification via column chromatography on silica gel (hexanes/ethyl acetate 19:1 to 3:1) to afford the desired peptide (135 mg, 99% yield) as yellow oil. R_f: 0.33 (hexanes/ethyl acetate 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.91−0.97 (d, J = 7.0 Hz, 3H, CHCH(CH_3)$_2$); 0.97−1.03 (d, J = 7.0 Hz, 3H, CHCH(CH_3)$_2$); 1.43 (t, 3H, OCH$_2$$CH_3$); 1.45 (s, 9H, C($CH_3$)$_3$); 2.16−2.33 (m, 1H, CHC$H$(CH$_3$)$_2$); 3.98−4.07 (m, 1H, α$H$); 4.39−4.49 (q, 2H, OC$H_2CH_3$); 4.79−4.86 (d, J = 6.82 Hz, 2H, αH); 4.98−5.16 (m, 1H, NH); 7.00−7.19 (m, 1H, NH); 8.15 (s, 1H, SCH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.4, 18.1, 19.6, 28.4, 31.3, 41.2, 59.9, 61.4, 79.5, 128.3, 146.5, 156.4, 161.4, 165.8, 169.9, 173.1. HRMS (ESI-TOF): M + H$^+$, found 386.1738 $C_{17}H_{27}N_3O_5S$ requires 386.1778.

*EtO-Thiazole-Gly-Val-NH_2 (**17c-1**), Fragment A.* EtO-Thiazole-Gly-Val-NH_2 was synthesized following the General Amine Deprotection procedure. The amine was taken on to the next reaction without further purification or characterization (101 mg, quantitative yield) as a light brown oil.

*EtO-Thiazole-Gly-Val-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc (**29-5**).* EtO-Thiazole-Gly-Val-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc was synthesized following the General Peptide Synthesis procedure, utilizing 184 mg (0.32 mmol, 1.0 equiv) of acid HO-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc (**28a-1**), 101 mg (0.35 mmol, 1.1 equiv) of amine EtO-Thiazole-Gly-Val-NH_2 (**17c-1**), 83.5 mg (0.26 mmol, 0.8 equiv) of TBTU, 98.9 mg (0.26 mmol, 0.8 equiv) of HATU, and 0.45 mL (2.56 mmol, 8.0 equiv) of DIPEA dissolved in 3.20 mL of methylene chloride. The crude product underwent a final purification via column chromatography on silica gel (ethyl acetate/methanol 99:1) to afford the desired peptide (224 mg, 84% yield). R_f: 0.30 (ethyl acetate/methanol 99:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.68−0.80 (d, J = 20.2, 6.2 Hz, 6H, CHCH(CH_3)$_2$); 0.81−0.96 (ddd, J = 29.1, 12.8, 6.2 Hz, 6H, CHCH$_2$CH(CH_3)$_2$); 1.26−1.34 (t, 3H, OCH$_2$$CH_3$); 1.36 (s, 9H, C($CH_3$)$_3$); 1.34−1.53 (m, 2H, CHC$H_2$CH(CH$_3$)$_2$); 1.63−1.79 (m, 1H, CHC$H$(CH$_3$)$_2$); 1.86−1.97 (m, buried, 2H, CHCH$_2$CH_2CH$_2$); 1.99−2.13 (m, 2H, CHCH$_2$CH_2CH$_2$); 1.93−2.03 and 2.07−2.15 (m, 2H, CHCH_2CH$_2$CH$_2$); 2.08−2.24 (m, 1H, CH(CH$_3$)$_2$); 2.16−2.43 (m, 2H, CHCH_2CH$_2$CH$_2$); 3.58−3.86 and 3.99−4.14 (m, 4H, CHCH$_2$CH$_2$CH_2); 4.26−4.36 (q, 2H, OCH_2CH$_3$); 4.33−4.47 (m, 1H, 1αH); 4.41−4.53 (m, 1H, 1αH); 4.58−4.77 (m, 2H, 2αH); 4.63−4.73 and 5.10−5.19 (m, 1H, 1αH); 5.39−5.53 (m, 1H, 1αH); 6.94−7.02 (d, J = 8.6 Hz, 1H, NH); 7.35−7.43 (d, J = 8.6 Hz, 1H, NH); 7.87 and 7.96 (s, 1H, SCH); 7.96 and 8.01 (s, 1H, SCH); 8.03−8.10 (m, 1H, NH); 8.18−8.24 (m, 1H, OCH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.3, 17.8, 18.2, 19.4, 21.0, 21.7, 23.4, 24.5, 24.6, 25.5, 27.9, 28.3, 30.4, 31.7, 41.1, 42.0, 47.1, 49.4, 50.4, 58.5, 58.8, 60.3, 61.4, 62.1, 79.5, 120.9, 128.2, 137.6, 142.6, 143.4, 146.5, 155.8, 156.6,161.2, 169.4, 170.7, 171.7, 172.4, 172.9, 174.4. LC/MS (ESI): m/z calcd for $C_{39}H_{54}N_8O_9S_2$ (M + H$^+$) = 843.35, found 843.00. HRMS (ESI-TOF): M + Na$^+$, found 865.3433 $C_{39}H_{54}N_8O_9S_2$ requires 865.3353

*HO-Thiazole-Gly-Val-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc (**29-5a**).* HO-Thiazole-Gly-Val-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc was synthesized following the General Acid Deprotection procedure, utilizing 224 mg (0.266 mmol, 1.0 equiv) of EtO-Thiazole-Gly-Val-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc (**29-5**) and 33.6 mg (0.80 mmol, 3 equiv) of LiOH·H_2O in 2.70 mL of ethanol. The acid was taken on to the next reaction without further purification (89.2 mg, 41% yield). LC/MS (ESI): m/z calcd for $C_{37}H_{50}N_8O_9S_2$ (M + H$^+$) = 815.27, found 815.00.

*HO-Thiazole-Gly-Val-Pro-Oxazole-Thiazole-Pro-Leu-NH_2 (**29-5b**).* HO-Thiazole-Gly-Val-Pro-Oxazole-Thiazole-Pro-Leu-NH_2 was synthesized following the General Amine Deprotection procedure, utilizing 89.2 mg (0.109 mmol, 1.0 equiv) of HO-Thiazole-Gly-Val-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc (**29-5a**), 240 μL (0.218 mmol, 2.0 equiv) of anisole, and 0.20 mL of trifluoroacetic acid in 0.80 mL of methylene chloride. HO-Thiazole-Gly-Val-Pro-Oxazole-Thiazole-Pro-Leu-NH_2 was taken on to the next reaction without further purification or characterization (78 mg, quantitative yield). LC/MS (ESI): m/z calcd for $C_{32}H_{43}N_8O_7S_2$ (M + H$^+$) = 715.27, found 715.00.

•*Compound **5**.* Compound **5** was synthesized following the Macrocyclization Procedure, utilizing 78.0 mg (0.109 mmol, 1.0 equiv) of HO-Thiazole-Gly-Val-Pro-Oxazole-Thiazole-Pro-Leu-NH_2 (**29-5b**), 42.0 mg (0.131 mmol, 1.2 equiv) of TBTU, 50.0 mg (0.131 mmol, 1.2 equiv) of HATU, and 120 μL (0.66 mmol, 6 equiv) of DIPEA in 110 mL of methylene chloride. The crude product was directly subjected to reversed-phase HPLC purification to afford a 2.4:1:2.9 of compound **5-A** (3.1 mg), compound **5-B** (4.7 mg), and compound **5-C** (4.2 mg) in a 28.6% overall yield. LC/MS (ESI): m/z calcd for $C_{34}H_{46}N_8O_7S_2$ (M + Na$^+$ + 2H$^+$) = 721.27, found 721.00. HRMS (ESI-TOF): M + 2Na$^+$ + H$^+$, found 743.2390 $C_{32}H_{40}N_8O_6S_2$ requires 743.2386.

•*Compound **5-A**.* ^{1}H NMR (298 K, 600 MHz, $CDCl_3$): δ 0.87−0.95 (m, 6H, CHCH(CH_3)$_2$); 0.82−0.90 and 0.94−1.00 (m, 6H, CHCH$_2$CH(CH_3)$_2$); 1.63−1.67 (m, 1H, CHCH(CH$_3$)$_2$); 1.76−1.81 (m, 1H, CHCH$_2$CH(CH$_3$)$_2$); 1.80−1.88 (m, 2H, CHCH_2CH(CH$_3$)$_2$); 1.97−2.06 (m, 2H, CHCH$_2$CH_2CH$_2$); 2.01−2.08 (m, 2H, CHCH$_2$CH_2CH$_2$); 2.21−2.25 and 2.36−2.41 (m, 2H, CHCH_2CH$_2$CH$_2$); 2.30−2.38 (m, 2H, CHCH_2CH$_2$CH$_2$); 3.79−3.86 (m, 4H, CHCH$_2$CH$_2$CH_2); 3.98−4.03 (m, 2H, 2αH); 4.70−4.75 (m, 1H, 1αH); 5.10−5.14 (m, 1H, 1αH); 5.47−5.49 and 5.57−5.60 (m, 1H, 1αH); 5.52−5.56 and 5.63−5.69 (m, 1H, 1αH); 6.81−6.86 (m, 1H, NH); 7.13−7.18 (m, 1H, NH); 7.48 and 7.61 (s, 1H, SCH); 7.55 and 7.73 (s, 1H, SCH); 7.88 and 8.03 (s, 1H, OCH). ^{13}C NMR (298 K, 150 MHz, $CDCl_3$): δ 10.9, 14.1, 19.6, 20.4 (CH_2γ Pro1), 21.2, 21.4, 22.1, 22.7, 23.3, 25.3, 27.2 (CH_2γ Pro2), 31.1, 34.0 (CH_2β Pro2), 35.1 (CH_2β Pro1), 39.4, 48.0, 48.7, 55.5, 59.4, 66.8, 119.6, 120.3, 128.8, 130.8, 138.4, 140.8, 141.0, 143.5, 146.9, 152.1, 155.9, 169.4, 171.3, 171.7, 173.0, 174.4, 175.1. Δβγ (Pro1, Pro2) = (14.7 ppm; 6.8 ppm).

•*Compound **5-B**.* ^{1}H NMR (308 K, 600 MHz, $CDCl_3$): δ 0.84−0.90 (m, 6H, CHCH(CH_3)$_2$); 0.92−1.04 (m, 6H, CHCH$_2$CH(CH_3)$_2$); 1.53−1.63 (m, 1H, CHCH$_2$CH(CH$_3$)$_2$); 1.64−1.66 (m, 1H, CHCH(CH$_3$)$_2$); 1.64−1.69 and 1.97−2.04 (m, 2H, CHCH_2CH(CH$_3$)$_2$); 1.90−2.10 (m, 2H, CHCH$_2$CH_2CH$_2$); 2.07−2.12 and 2.47−2.52 (m, 2H, CHCH$_2$CH_2CH$_2$); 2.06−2.10 and 2.32−2.38 (m, 2H, CHCH_2CH$_2$CH$_2$); 2.13−2.22 and 2.38−2.48 (m, 2H, CHCH_2CH$_2$CH$_2$); 3.65−3.69 (m, 2H, 2αH); 3.72−3.99 (m, 4H, CHCH$_2$CH$_2$CH_2); 4.97−5.04 (m, 1H, 1αH); 5.08−5.18 (m, 1H, 1αH); 5.39−5.55 (m, 1H, 1αH); 5.40−5.45 and 5.56−5.60 (m, 1H, 1αH); 6.81−6.86 (m, 1H, NH); 7.12−7.17 (m, 1H, NH); 7.66 and 7.79 (s, 1H, SCH); 7.69 (s, 1H, SCH); 8.00 (s, 1H, OCH). ^{13}C NMR (308 K, 150 MHz, $CDCl_3$): δ 19.0, 20.6 (CH_2γ Pro2), 22.1, 22.6, 22.7, 23.3, 24.0 (CH_2γ Pro1), 24.4, 31.0, 31.7 (CH_2γ Pro1), 34.9 (CH_2β Pro2), 41.4, 47.3, 48.7, 49.4, 57.6, 59.3, 59.4, 70.2, 119.7, 120.2, 135.9, 140.7, 142.2, 143.5, 152.1, 155.6, 170.3, 171.9, 172.1, 175.5, 175.6, 176.3. Δβγ (Pro1, Pro2) = (14.3 ppm; 7.7 ppm).

•*Compound **5-C**.* ^{1}H NMR (308 K, 600 MHz, $CDCl_3$): δ 0.82−0.90 (m, 6H, CHCH(CH_3)$_2$); 0.94−1.13 (m, 6H, CHCH$_2$CH(CH_3)$_2$); 1.62−1.81 (m, 2H, CHCH_2CH(CH$_3$)$_2$); 1.63−1.66 (m, 1H, CHCH(CH$_3$)$_2$); 1.70−1.87 (m, 1H, CHCH$_2$CH(CH$_3$)$_2$); 2.02−2.06 (m, 2H, CHCH$_2$CH_2CH$_2$); 2.11−2.26 (m, 2H, CHCH$_2$CH_2CH$_2$); 2.32−2.44 and 2.51−2.59 (m, 2H, CHCH_2CH$_2$CH$_2$); 2.32−2.38 and 2.40−2.45 (m, 2H, CHCH_2CH$_2$CH$_2$); 3.74−4.00 (m, 4H, CHCH$_2$CH$_2$CH_2); 3.99−4.03 (m, 2H, 2αH); 4.87−4.93 (m, 1H, 1αH); 5.05−5.17 (m, 1H, 1αH); 5.46−5.54 (m, 1H, 1αH); 5.52−5.64 (m, 1H, 1αH); 6.81−6.86 (m, 1H, NH); 7.12−7.17 (m, 1H, NH); 7.94 (s, 1H, SCH); 7.96 (s, 1H, SCH); 8.23 and 8.24 (s, 1H, OCH). ^{13}C NMR (308 K, 150 MHz, $CDCl_3$): δ 19.7, 20.5, 21.2, 21.8, 22.1, 23.2, 24.5 (CH_2γ Pro2), 24.7, 27.1 (CH_2γ Pro1), 31.0, 31.6 (CH_2γ Pro2), 32.3 (CH_2β Pro2), 42.0, 47.3, 48.7, 50.1, 58.6, 58.8, 59.3, 59.5, 66.8, 120.5, 121.1, 136.9, 140.8, 142.5, 142.6, 143.6, 156.9, 165.2, 171.9, 172.1, 174.1, 174.9, 175.0. Δβγ (Pro1, Pro2) = (5.2 ppm; 7.1 ppm).

dx.doi.org/10.1021/jo3017499 | J. Org. Chem. 2012, 77, 10596−10616

Compound **6**. *MeO-Oxazole-Thiazole-Pro-Gly-NHBoc (**27c**).* MeO-Oxazole-Thiazole-Pro-Gly-NHBoc was synthesized following the General Peptide Synthesis procedure, utilizing 110 mg (0.393 mmol, 1.0 equiv) of amine OMe-Oxazole-Thiazole-Pro-NH (**25-1**), 103 mg (0.59 mmol, 1.5 equiv) of acid HO-Gly-NHBoc (**26c**), 126 mg (0.393 mmol, 1.0 equiv) of TBTU, 149 mg (0.393 mmol, 1.0 equiv) of HATU, and 0.40 mL (1.57 mmol, 4 equiv) of DIPEA dissolved in 4 mL of methylene chloride under N_2. The reaction mixture was stirred for 3 h and was monitored by TLC upon completion. The crude product underwent a final purification via column chromatography on silica gel (hexanes/ethyl acetate 1:1 to 1:3) to afford the desired peptide (151 mg, 88% yield) as yellow flakes. R_f: 0.65 (hexanes/ethyl acetate 1:3). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.29 (s, 9H, C(CH_3)$_3$); 1.97−2.13 (m, 2H, CHCH$_2$ CH_2CH$_2$); 2.16−2.29 (m, 2H, CHCH_2 CH$_2$CH$_2$); 3.41−3.56 (m, 2H, CHCH$_2$ CH$_2$CH_2); 3.57−3.72 (t, 2H, CH$_2$); 3.80 (s, 3H, OCH$_3$); 5.28−5.49 (m, 1H, 1αH); 7.95 (s, 1H, SC*H*), 8.22 (s, 1H, OC*H*). ^{13}C NMR (75 MHz, $CDCl_3$): 24.20, 28.01, 29.54, 31.72, 34.77, 43.12, 46.17, 46.81, 52.32, 58.78, 58.92, 79.67, 121.82, 122.03, 133.71, 141.69, 142.65, 143.87, 143.97, 155.98, 157.44, 157.75, 161.62, 168.77, 174.42. HRMS (ESI-TOF): M + H$^+$, found 437.1482 $C_{19}H_{24}N_4O_6S$ requires 437.1450.

*HO-Oxazole-Thiazole-Pro-Gly-NHBoc (**27c-1**).* HO-Oxazole-Thiazole-Pro-Gly-NHBoc was synthesized following the General Acid Deprotection procedure, utilizing 151 mg (0.345 mmol, 1.0 equiv) of MeO-Oxazole-Thiazole-Pro-Gly-NHBoc (**27c**) and 116 mg (2.76 mmol, 8.0 equiv) of LiOH·H_2O in 3.5 mL of methanol. Upon completion, the reaction was diluted with methylene chloride. The acid was taken on to the next reaction without further purification (137 mg, 94% yield) as yellow flakes.

*MeO-Pro-Oxazole-Thiazole-Pro-Gly-NHBoc (**28c**).* MeO-Pro-Oxazole-Thiazole-Pro-Gly-NHBoc was synthesized following the General Peptide Synthesis procedure, utilizing 137 mg (0.324 mmol, 1.0 equiv) of acid HO-Oxazole-Thiazole-Pro-Gly-NHBoc (**27c-1**), 63 mg (0.486 mmol, 1.5 equiv) of the amine MeO-Pro-NH, 123 mg (0.324 mmol, 1.0 equiv) HATU, and 0.45 mL (2.59 mmol, 4.0 equiv) of DIPEA dissolved in 4 mL of methylene chloride under N_2. The reaction mixture was stirred for 3 h and was monitored by TLC upon completion. The crude product underwent a final purification via column chromatography on silica gel (hexanes/ethyl acetate 1:3) to afford the desired peptide (157 mg, 91% yield) as yellow flakes. R_f: 0.45 (hexanes/ethyl acetate 0:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.30 (s, 9H, C(CH_3)$_3$); 1.83−1.98 (m, 2H, CHCH$_2$ CH_2CH$_2$); 2.01−2.12 (m, 2H, CHCH$_2$ CH_2CH$_2$); 2.12−2.23 (m, 2H, CHCH_2 CH$_2$CH$_2$); 2.23−2.34 (m, 2H, CHCH_2 CH$_2$CH$_2$); 3.06−3.19 (m, 2H, CHCH$_2$ CH$_2$CH_2); 3.44−3.58 (m, 2H, CH$_2$); 3.64 (s, 3H, OCH$_3$); 3.88−3.95 (m, 2H, CHCH$_2$ CH$_2$CH_2); 4.48−4,58 (m, 1H, 1αH); 5.18−5.30 (m, 1H, 1αH); 7.84 (s, 1H, SC*H*), 8.18 (s, 1H, OC*H*). ^{13}C NMR (75 MHz, $CDCl_3$): 24.13, 25.09, 28.23, 29.54, 31.79, 43.34, 52.20, 52.31, 60.10, 60.79, 79.60, 120.90, 136.69, 142.93, 155.78, 156.21, 156.34, 156.64, 168.40, 168.50, 172.25, 173.31. HRMS (ESI-TOF): M + H$^+$, found 534.2015 $C_{24}H_{31}N_5O_7S$ requires 534.1978.

*HO-Pro-Oxazole-Thiazole-Pro-Gly-NHBoc (**28c-1**), Fragment B.* HO-Pro-Oxazole-Thiazole-Pro-Gly-NHBoc was synthesized following General Acid Deprotection procedure, utilizing 157 mg (0.295 mmol, 1.0 equiv) of MeO-Pro-Oxazole-Thiazole-Pro-Gly-NHBoc (**28c**) and 151 mg (2.36 mmol, 8.0 equiv) of LiOH·H_2O in 2.95 mL of methanol. Upon completion, the reaction was diluted with methylene chloride. The acid was taken on to the next reaction without further purification (150 mg, 98% yield) as yellow flakes.

*EtO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-Gly-NHBoc (**29-6**).* EtO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-Gly-NHBoc was synthesized following the General Peptide Synthesis procedure, utilizing 150 mg (0.288 mmol, 1.0 equiv) of acid HO-Pro-Oxazole-Thiazole-Pro-Gly-NHBoc (**28c-1**), 130 mg (0.433 mmol, 1.5 equiv) of amine EtO-Thiazole-Ala-Val-NH$_2$ (**17a-1**), 90 mg (0.288 mmol, 1.0 equiv) of TBTU, 109 mg (0.288 mmol, 1.0 equiv) of HATU, 79 mg (0.288 mmol, 1.0 equiv) of DMTMM, and 0.4 mL (2.31 mmol, 8.0 equiv) of DIPEA dissolved in 5.8 mL of methylene chloride under N_2. The reaction mixture was stirred for 3 h and was monitored by TLC and LCMS upon completion. The crude product underwent a final purification via column chromatography on silica gel (ethyl acetate/methanol 19:1) to afford the desired peptide (170 mg, 74% yield) as yellow flakes. R_f: 0.325 (ethyl acetate/methanol 9:1). ^{1}H NMR (300 MHz, CD_3OD): δ 0.94−1.74 (m, 6H, CH_3CHCH_3); 1.46 (s, 9H, C(CH_3)$_3$); 1.48−1.57 (t, 2H,CH$_2$); 1.61−1.68 (dd, 3H,CHCH_3); 1.45−1.7 (m, 2H, CHCH$_2$ CH_2CH$_2$); 1.92−2.02 (m, 2H, CHCH$_2$ CH_2CH$_2$); 2.03−2.11 (m, 2H, CHCH$_2$ CH_2CH$_2$); 2.21−2.33 (m, 2H, CHCH_2 CH$_2$CH$_2$); 2.33−2.49 (m, 2H, CHCH_2 CH$_2$CH$_2$); 3.81−3.93 (m, 2H, CHCH$_2$ CH$_2$CH_2); 4.04−4.16 (m, 2H, CHCH$_2$ CH$_2$CH_2); 4.16−4.24 (t, 3H, OCH$_2$CH_3); 4.37−4.47 (m, 2H, OCH_2CH$_3$); 4.70−4.79 (m, 1H, 1αH); 5.28−5.42 (dd, 1H, 1αH); 5.54−5.69 (m, 1H, 1αH); 8.24 (s, 1H, SC*H*); 8.33 (s, 1H, SC*H*); 8.44 (s, 1H, OC*H*). ^{13}C NMR (75 MHz, CD_3OD): 13.26, 18.57, 18.72, 19.68, 24.90, 27.38, 30.89, 31.79, 32.06, 42.53, 48.59, 49.17, 58.58, 58.81, 59.09, 61.26, 79.26, 121.75, 127.80, 128.33, 137.50, 142.71, 143.31, 146.20, 150.32, 157.01, 160.94, 161.48, 169.67, 171.81, 172.06, 172.31. LC/MS (ESI): *m*/*z* calcd for $C_{36}H_{48}N_8O_9S_2$ (M + H$^+$) = 800.94, found 801.00. HRMS (ESI-TOF): M + Na$^+$, found 823.2874 $C_{36}H_{48}N_8O_9S_2$ requires 823.2884.

*HO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-Gly-NHBoc (**29-6a**).* HO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-Gly-NHBoc was synthesized following the General Acid Deprotection procedure, utilizing 170 mg (0.212 mmol, 1.0 equiv) of EtO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-Gly-NHBoc (**29-6**) and 71 mg (1.69 mmol, 8 equiv) of LiOH·H_2O in 2.20 mL of ethanol. Upon completion, the reaction was diluted with methylene chloride. The acid was taken on to the next reaction without further purification (160 mg, 97.5% yield). LC/MS (ESI): *m*/*z* calcd for $C_{34}H_{44}N_8O_9S_2$ (M + H$^+$) = 772.89, found 773.00.

*HO-Thiazole-Ala-NMe-Val-Pro-Oxazole-Thiazole-Pro-Leu-NH$_2$ (**29-6b**).* HO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-Gly-NH$_2$ was synthesized following the General Amine Deprotection procedure, utilizing 160 mg (0.207 mmol, 1.0 equiv) of HO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-Gly-NHBoc (**29-6a**), 0.05 mL (0.42 mmol, 2.0 equiv) of anisole, and 0.50 mL of trifluoroacetic acid in 2.50 mL of methylene chloride. The reaction was completed in 1 h; the reaction was concentrated in vacuo. HO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-Gly-NH$_2$ was taken on to the next reaction without further purification or characterization (196 mg, quantitative yield). LC/MS (ESI): *m*/*z* calcd for $C_{30}H_{38}N_8O_6S_2$ (M + H$^+$) = 670.80, found 671.00.

•*Compound* **6**. Compound **6** was synthesized following the Macrocyclization Procedure, utilizing 140 mg (0.208 mmol, 1.0 equiv) of HO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-Gly-NH$_2$ (**29-6b**), 67.0 mg (0.208 mmol, 1.0 equiv) of TBTU, 79.0 mg (0.208 mmol, 1.0 equiv) of HATU, 57.4 mg (0.208 mmol, 1.0 equiv) of DMTMM, and 0.29 mL (1.66 mmol, 8 equiv) of DIPEA in 297 mL of methylene chloride under N_2. The reaction was monitored by LCMS upon completion. The crude product underwent an initial purification via column chromatography on silica gel (ethyl acetate/methanol 9:1), and the resulting semipure residue was subjected to reversed-phase HPLC purification to afford a 5.5:1 of compound **6-A** (18.4 mg) and compound **6-B** (6.3 mg) in a 15% overall yield. LC/MS (ESI): *m*/*z* calcd for $C_{29}H_{34}N_8O_6S_2$ (M + H$^+$) = 654.76, found 655.00 HRMS (ESI-TOF): M + H$^+$ found 655.2110 $C_{29}H_{34}N_8O_6S_2$ requires 655.2121.

•*Compound* **6-A**. ^{1}H NMR (318 K, 600 MHz, $CDCl_3$): δ 0.86−1.01 (m, 6H, CH(CH_3)$_2$); 1.7−1.78 (d, 3H, CHCH_3); 1.4−1.47 (m, 2H, CHC*H*); 1.97−2.07 (m, 2H, C$H_2\gamma$ Pro1); 2.02−2.13 (m, 2H, C$H_2\gamma$ Pro2); 1.98−2.35 (m, 2H, C$H_2\beta$ Pro2); 2.07−2.3 (m, 2H, C$H_2\beta$ Pro1); 3.83−3.92 (m, 2H, C$H_2\delta$ Pro2); 3.84−3.87 (m, 2H, C$H_2\delta$ Pro1); 4.17−4.24 (m, 2H, CH$_2$); 4.72−4.78 (m, 1H, 1αH); 4.78−4.99 (m, 1H, 1αH); 5.37−5.56 (m, 1H, CHCH$_3$); 5.48−5.5 (m, 1H, 1αH); 7.52 (s, 1H, SCH); 7.94 (s, 1H, SCH); 8.38 (s, 1H, OCH). ^{13}C NMR (318 K, 150 MHz, $CDCl_3$): δ 17.61, 19.58, 22.86C$H_2\gamma$ Pro2), 23.34, 26.41 (C$H_2\gamma$ Pro1), 31.82 (C$H_2\beta$ Pro1), 35.85 (C$H_2\beta$ Pro2), 42.07, 47.8, 49.01, 59.39, 60.27, 63.86, 64.1, 120.17, 121.89, 122.67, 125.49, 127.6, 142.94, 143.3, 149.4. $\Delta\beta\gamma$ = (5.41 ppm; 12.99 ppm).

•*Compound* **6-B**. ^{1}H NMR (318 K, 600 MHz, CD_3OD): δ 0.95−1.04 (m, 6H, CH(CH_3)$_2$); 1.44−1.47 (d, 3H, CHCH_3); 1.35−1.39

dx.doi.org/10.1021/jo3017499 | J. Org. Chem. 2012, 77, 10596−10616

(m, 2H, CHC*H*); 1.87−2.0 (m, 2H, C$H_2\gamma$ Pro1); 1.9−2.24 (m, 2H, C$H_2\gamma$ Pro2); 1.89−2.15 (m, 2H, C$H_2\beta$ Pro1); 2.32−2.54 (m, 2H, C$H_2\beta$ Pro2); 3.7−3.79 (m, 2H, C$H_2\delta$ Pro2); 3.72−3.88 (m, 2H, C$H_2\delta$ Pro1); 4.85−4.87 (m, 1H, 1αH); 5.07−5.37 (m, 2H, CH_2); 5.43−5.5 (m, 1H, C*H*CH_3); 5.48−5.50 (m, 1H, 1αH); 5.93−6.00 (m, 1H, 1αH); 8.1 (s, 1H, SCH); 8.44 (s, 1H, SCH); 8.74 (s, 1H, OCH). ^{13}C NMR (318 K, 150 MHz, CD_3OD): δ 17.53, 18.33, 19.22, 24.55 (C$H_2\gamma$ Pro2), 25.82 (C$H_2\gamma$ Pro1), 31.51 (C$H_2\beta$ Pro1), 32.11 (C$H_2\beta$ Pro2), 42.3, 46.42, 46.81, 47.63, 59.22, 60.24, 60.56, 63.62, 120.87, 122.89, 129.05, 150.85. $\Delta\beta\gamma$ = (5.69 ppm; 7.56 ppm).

Compound 7. Compound **12d** is commercially available (CAS no. 51987-73-6).

NH_2-Phe-NHBoc (13d). NH_2-Phe-NHBoc was synthesized following the General Amide Formation procedure, utilizing 285 mg (1.02 mmol, 1.0 equiv) of MeO-Phe-NHBoc (**12d**) dissolved in 5.0 mL of ammonium hydroxide (25% in water) and 5.0 mL of methanol. The resulting amide was taken on to the next reaction without further purification (270 mg, quantitative yield) as white crystals. R_f: 0.15 (hexanes/ethyl acetate 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43 (s, 9H, C(CH_3)$_3$); 2.88−3.25 (m, 2H, CHCH_2Ph); 4.29−4.52 (m, 1αH); 5.09−5.32 (m, 1H, N*H*); 5.72−5.97 (br, 1H, NH_2); 5.97−6.17 (br, 1H, NH_2); 7.13−7.46 (m, 5H, Ph). ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.7, 38.6, 55.8, 80.7, 127.2, 128.9, 129.5, 136.8, 155.8, 174.3.

Thioamide-Phe-NHBoc (14d). Thioamide-Phe-NHBoc was synthesized following the General Thioamide Formation procedure, utilizing 270 mg (1.02 mmol, 1.0 equiv) of NH_2-Phe-NHBoc (**13d**) and 311 mg (0.77 mmol, 0.75 equiv) of Lawesson's Reagent dissolved in 10.2 mL of 1,2-dimethoxyethane. The thioamide was purified via column chromatography on silica gel (hexanes/ethyl acetate 19:1 to 9:1) to afford the desired thioamide (171 mg, 60% yield) as white crystals. R_f: 0.6 (hexanes/ethyl acetate 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.39 (s, 9H, C(CH_3)$_3$); 2.98−3.25 (m, 2H, CHCH_2Ph); 4.66−4.82 (m, 1αH); 5.46−5.62 (m, 1H, N*H*); 7.17−7.37 (m, 5H, Ph); 7.67−7.87 (br, 1H, NH_2); 7.87−8.09 (br, 1H, NH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.7, 42.4, 61.4, 81.0, 127.2, 128.9, 129.5, 136.8, 155.8, 208.7.

EtO-Thiazole-Phe-NHBoc (15d). EtO-Thiazole-Phe-NHBoc was synthesized following the General Thiazole Synthesis procedure, utilizing 171 mg (0.61 mmol, 1.0 equiv) of Thioamide-Phe-NHBoc (**14d**) and 489 mg (4.88 mmol, 8.0 equiv) of potassium bicarbonate dissolved in 6.1 mL of 1,2-dimethoxyethane. A 0.23 mL amount of ethyl bromopyruvate (1.83 mmol, 3.0 equiv) was added dropwise (0.1 mL/min) to yield the thiazoline intermediate. The thiazoline was dehydrated using 0.44 mL (5.49 mmol, 9.0 equiv) of pyridine, 0.34 mL (2.44 mmol, 4.0 equiv) of trifluoroacetic anhydride, and 0.17 mL (1.22 mmol, 2.0 equiv) of triethylamine in 6.1 mL of 1,2-dimethoxyethane to afford the desired thiazole (210 mg, 92% yield) as a clear oil. R_f: 0.55 (hexanes/ethyl acetate 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.31 (s, 9H, C(CH_3)$_3$); 1.33 (t, 3H, OCH$_2$CH_3); 3.02−3.41 (m, 2H, CHCH_2Ph); 4.25−4.42 (q, 2H, OCH_2CH$_3$); 5.09−5.31 (m, 1H, 1αH); 5.44−5.64 (m, 1H, N*H*); 6.99−7.24 (m, 5H, Ph); 7.99 (s, 1H, SC*H*). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.6, 28.4, 41.5, 54.1, 61.7, 80.4, 126.9, 127.2, 128.9, 129.5, 136.5, 147.4, 155.2, 161.7, 173.7. HRMS (ESI-TOF): M + H^+, found 377.1526 $C_{19}H_{24}N_2O_4S$ requires 377.1535.

EtO-Thiazole-Phe-NH_2 (15d-1). EtO-Thiazole-Phe-NH_2 was synthesized following the General Amine Deprotection procedure. The amine was taken on to the next reaction without further purification or characterization (154 mg, quantitative yield) as a clear oil.

EtO-Thiazole-Phe-Val-NHBoc (17e). EtO-Thiazole-Phe-Val-NHBoc was synthesized following the General Peptide Synthesis procedure, utilizing 154 mg (0.56 mmol, 1.0 equiv) of amine EtO-Thiazole-Phe-NH_2 (**15d-1**), 133 mg (0.61 mmol, 1.1 equiv) of acid HO-Val-NHBoc (**16a**), 144 mg (0.45 mmol, 0.8 equiv) of TBTU, 171 mg (0.45 mmol, 0.8 equiv) of HATU, and 0.59 mL (3.36 mmol, 6 equiv) of DIPEA dissolved in 5.6 mL of methylene chloride under N_2. The crude product underwent a final purification via column chromatography on silica gel (hexanes/ethyl acetate 1:0 to 7:3) to afford the desired peptide (190 mg, 72% yield) as a clear oil. R_f: 0.50 (hexanes/ethyl acetate 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.65−0.80 (dd, J = 23.0, 6.9 Hz, 3H, CHCH(CH_3)$_2$); 0.65−0.71 and 0.77−0.85 (dd, J = 38.3, 6.9 Hz, 3H, CHCH(CH_3)$_2$); 1.35 (s, 9H, C(CH_3)$_3$); 1.35 (t, 3H, OCH$_2$CH_3); 1.97−2.14 (m, 1H, CHC*H*(CH_3)$_2$); 3.11−3.49 (m, 2H, CHCH_2Ph); 3.82−4.00 (m, 1H, 1αH); 4.30−4.42 (q, 2H, OCH_2CH$_3$); 5.22−5.38 (m, 1H, N*H*); 5.51−5.64 (m, 1H, 1αH); 7.00−7.24 (m, 5H, Ph); 7.28−7.41 (m, 1H, N*H*); 8.00 (s, 1H, SC*H*). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.6, 17.8, 19.6, 28.7, 30.7, 38.9, 41.2, 52.6, 60.5, 62.0, 80.1, 127.2, 127.8, 128.9, 129.5, 136.5, 147.4, 156.1, 161.7, 171.9. HRMS (ESI-TOF): M + H^+, found 476.2207 $C_{24}H_{33}N_3O_5S$ requires 476.2219.

EtO-Thiazole-Phe-Val-NH_2 (17e-1), Fragment A. EtO-Thiazole-Phe-Val-NH_2 was synthesized following the General Amine Deprotection procedure. The amine was taken on to the next reaction without further purification or characterization (150 mg, quantitative yield) as a clear oil.

EtO-Thiazole-Phe-Val-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc (29-7). EtO-Thiazole-Phe-Val-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc was synthesized following the General Peptide Synthesis procedure, utilizing 212 mg (0.36 mmol, 1.0 equiv) of acid HO-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc (**28a-1**), 150 mg (0.40 mmol, 1.1 equiv) of amine EtO-Thiazole-Phe-Val-NH_2 (**17e-1**), 93.1 mg (0.29 mmol, 0.8 equiv) of TBTU, 110 mg (0.29 mmol, 0.8 equiv) of HATU, and 0.38 mL (2.16 mmol, 6.0 equiv) of DIPEA dissolved in 3.60 mL of methylene chloride. The crude product underwent a final purification via column chromatography on silica gel (ethyl acetate/methanol 99:1) to afford the desired peptide (253 mg, 74% yield). R_f: 0.30 (ethyl acetate/methanol 99:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.57−0.65 (d, J = 6.91 Hz, 3H, CHCH(CH_3)$_2$); 0.73−0.84 (d, J = 6.91 Hz, 3H, CHCH(CH_3)$_2$); 0.94−1.01 (d, J = 6.91 Hz, 3H, CHCH$_2$CH(CH_3)$_2$); 0.99−1.06 (d, J = 6.91 Hz, 3H, CHCH$_2$CH(CH_3)$_2$); 1.36−1.45 (t, 3H, OCH$_2$CH_3); 1.45 (s, 9H, C(CH_3)$_3$); 1.44−1.58 (m, 2H, CHCH_2CH-(CH_3)$_2$); 1.73−1.87 (m, 1H, CHCH$_2$C*H*(CH_3)$_2$); 1.95−2.09 (m, 2H, CHCH$_2$CH_2CH$_2$); 2.00−2.20 (m, 2H, CHCH_2CH$_2$CH$_2$); 2.03−2.14 and 2.22−2.32 (m, 2H, CHCH$_2$CH_2CH$_2$); 2.17−2.28 (m, 1H, CHC*H*(CH_3)$_2$); 2.12−2.17 and 2.25−2.53 (m, 2H, CHCH_2CH$_2$CH$_2$); 3.18−3.37 and 3.41−3.59 (m, 2H, CHCH_2Ph); 3.64−3.93 (m, 2H, CHCH$_2$CH$_2$CH_2); 4.06−4.29 (m, 2H, CHCH$_2$CH$_2$CH_2); 4.21−4.33 (m, 1H, 1αH); 4.36−4.49 (q, 2H, OCH_2CH$_3$); 4.48−4.62 (m, 1H, 1αH); 4.69−4.79 and 5.21−5.27 (m, 1H, 1αH); 5.43−5.72 (m, 1H, 1αH); 5.48−5.62 (m, 1H, 1αH); 6.97−7.30 (m, 5H, Ph); 7.35−7.44 (m, 1H, N*H*); 7.95 (s, 1H, SC*H*); 7.96−7.98 (m, 1H, N*H*); 8.00 and 8.06 (s, 1H, SC*H*); 8.24 and 8.28 (s, 1H, OC*H*); 8.29−8.31 (m, 1H, N*H*). ^{13}C NMR (75 MHz,$CDCl_3$): δ 14.6, 17.2, 19.6, 21.9, 23.7, 24.9, 25.7, 28.7, 29.3, 29.7, 31.8, 40.7, 42.3, 47.1, 49.6, 50.4, 52.5, 58.9, 61.5, 79.8, 121.1, 126.9, 127.6, 128.6, 129.3, 136.7, 137.6, 143.5, 147.2, 155.8, 156.9,161.5, 162.1, 171.0, 171.3, 172.8, 174.7. LC/MS (ESI): m/z calcd for $C_{46}H_{60}N_8O_9S_2$ (M + Na^+) = 955.38, found 955.00. HRMS (ESI-TOF): M + Na^+, found 955.3806 $C_{46}H_{60}N_8O_9S_2$ requires 955.3823.

HO-Thiazole-Phe-Val-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc (29-7a). HO-Thiazole-Phe-Val-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc was synthesized following the General Acid Deprotection procedure, utilizing 253 mg (0.267 mmol, 1.0 equiv) of EtO-Thiazole-Phe-Val-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc (**29-7**), and 89.6 mg (2.14 mmol, 8 equiv) of LiOH·H_2O in 2.67 mL ethanol. The acid was taken on to the next reaction without further purification (208 mg, 86% yield). LC/MS (ESI): m/z calcd for $C_{39}H_{54}N_8O_9S_2$ (M + H^+) = 905.36, found 905.00.

HO-Thiazole-Phe-Val-Pro-Oxazole-Thiazole-Pro-Leu-NH_2 (29-7b). HO-Thiazole-Phe-Val-Pro-Oxazole-Thiazole-Pro-Leu-NH_2 was synthesized following the General Amine Deprotection procedure, utilizing 208 mg (0.23 mmol, 1.0 equiv) of HO-Thiazole-Phe-Val-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc (**29-7a**), 50.0 μL (0.46 mmol, 2.0 equiv) of anisole, and 0.46 mL of trifluoroacetic acid in 1.84 mL of methylene chloride. HO-Thiazole-Phe-Val-Pro-Oxazole-Thiazole-Pro-Leu-NH_2 was taken on to the next reaction without further purification or characterization (185 mg, quantitative yield). LC/MS (ESI): m/z calcd for $C_{39}H_{48}N_8O_7S_2$ (M + H^+) = 805.31, found 805.00.

●*Compound 7.* Compound 7 was synthesized following the Macrocyclization Procedure, utilizing 185 mg (0.230 mmol, 1.0

dx.doi.org/10.1021/jo3017499 | J. Org. Chem. 2012, 77, 10596−10616

equiv) of HO-Thiazole-Phe-Val-Pro-Oxazole-Thiazole-Pro-Leu-NH_2 (**29-7b**), 59.0 mg (0.184 mmol, 0.8 equiv) of TBTU, 70.0 mg (0.184 mmol, 0.8 equiv) of HATU, 51.0 mg (0.184 mmol, 0.8 equiv) of DMTMM, and 240 μL (1.38 mmol, 6 equiv) of DIPEA in 230 mL of methylene chloride. The crude product was directly subjected to reversed-phase HPLC purification to afford compound **7** (12.9 mg) in a 14.2% overall yield. LC/MS (ESI): *m*/*z* calcd for $C_{39}H_{46}N_8O_6S_2$ (M + H^+) = 787.31, found 787.00. HRMS (ESI-TOF): M + Na^+, found 809.2864 $C_{39}H_{46}N_8O_6S_2$ requires 809.2880.

•*Compound* ***7***. ^{1}H NMR (308 K, 600 MHz, $CDCl_3$): δ 0.28−0.34 (d, *J* = 5.9 Hz, 3H, CHCH(CH_3)$_2$); 0.83−0.91 (d, *J* = 5.9 Hz, 3H, CHCH(CH_3)$_2$); 0.91−0.97 (d, *J* = 5.9 Hz, 3H, $CHCH_2CH(CH_3)_2$); 0.97−1.04 (d, *J* = 5.9 Hz, 3H, $CHCH_2CH(CH_3)_2$); 1.54−1.62 (m, 1H, CH $CH_2CH(CH_3)_2$); 1.57−1.66 and 1.95−2.03 (m, 2H, $CHCH_2CH(CH_3)_2$); 1.77−1.85 and 1.94−2.02 (m, 2H, $CHCH_2CH_2CH_2$); 1.91−2.00 and 2.07−2.15 (m, 2H, $CHCH_2CH_2CH_2$); 1.92−1.99 and 2.32−2.41 (m, 2H, $CHCH_2CH_2CH_2$); 2.19−2.32 (m, 2H, $CHCH_2CH_2CH_2$); 2.19−2.27 and 3.25−3.32 (m, 2H, $CHCH_2Ph$); 2.64−2.73 (m, 1H, CH(CH_3)$_2$); 3.66−3.74 and 3.93−4.01 (m, 2H, $CHCH_2CH_2CH_2$); 3.80−3.88 and 3.90−3.97 (m, 2H, $CHCH_2CH_2CH_2$); 4.63−4.70 (m, 1H, 1αH); 4.69−4.76 (m, 1H, 1αH); 5.09−5.17 (m, 1H, 1αH); 5.34−5.43 (m, 1H, 1αH); 5.47−5.53 (m, 1H, 1αH); 5.99−6.06 (d, *J* = 11.0 Hz, 1H, NH); 6.89−6.97 and 7.16−7.33 (m, 5H, Ph); 6.99−7.05 (d, *J* = 11.0 Hz, 1H, NH); 7.10−7.17 (d, *J* = 8.8 Hz, 1H, NH); 7.46 (s, 1H, SCH); 8.02 (s, 1H, SCH); 8.49 (s, 1H, OCH). ^{13}C NMR (150 MHz, $CDCl_3$): δ 15.1, 19.5, 20.1 ($CH_2\gamma$ Pro2), 22.0, 22.3 ($CH_2\gamma$ Pro1), 23.3, 24.2, 28.5, 31.7 ($CH_2\beta$ Pro1), 35.4 ($CH_2\beta$ Pro2), 36.8, 41.3, 46.6, 47.5, 48.9, 51.4, 57.0, 58.8, 64.1, 122.3, 125.8, 126.9, 128.6, 128.7, 137.2, 140.4, 143.4, 146.8, 157.4, 159.9, 162.0, 169.0, 170.4, 170.9, 172.7, 173.3 Δ$\beta\gamma$ (Pro1, Pro2) = (9.4 ppm; 15.3 ppm).

Compound ***8***. Compound **12e** is commercially available (CAS no. 77119-84-7).

*NH_2-D-Phe-NHBoc (**13e**)*. NH_2-D-Phe-NHBoc was synthesized following the General Amide Formation procedure, utilizing 360 mg (1.29 mmol, 1.0 equiv) of MeO-D-Phe-NHBoc (**12e**) dissolved in 6.5 mL of ammonium hydroxide (25% in water) and 6.5 mL of methanol. The resulting amide was taken on to the next reaction without further purification (341 mg, quantitative yield) as a white powder (rotamers 2:1). R_f: 0.15 (hexanes/ethyl acetate 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43 (s, 9H, C(CH_3)$_3$); 2.88−3.25 (m, 2H, $CHCH_2Ph$); 4.27−4.56 (m, 1αH); 5.11−5.35 (m, 1H, NH); 5.74−5.99 (br, 1H, NH_2); 5.99−6.19 (br, 1H, NH_2); 7.11−7.43 (m, 5H, Ph). ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.7, 38.9, 55.8, 80.7, 127.2, 128.9, 129.5, 136.8, 155.8, 174.3.

*Thioamide-D-Phe-NHBoc (**14e**)*. Thioamide-D-Phe-NHBoc was synthesized following the General Thioamide Formation procedure, utilizing 341 mg (1.29 mmol, 1.0 equiv) of NH_2-D-Phe-NHBoc (**13e**) and 392 mg (0.97 mmol, 0.75 equiv) of Lawesson's Reagent dissolved in 12.9 mL of 1,2-dimethoxyethane. The thioamide was purified via column chromatography on silica gel (hexanes/ethyl acetate 19:1 to 9:1) to afford the desired thioamide (225 mg, 62% yield) as white crystals. R_f: 0.6 (hexanes/ethyl acetate 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.39 (s, 9H, C(CH_3)$_3$); 2.98−3.25 (m, 2H, $CHCH_2Ph$); 4.66−4.82 (m, 1αH); 5.46−5.62 (m, 1H, NH); 7.17−7.37 (m, 5H, Ph); 7.75−7.95 (br, 1H, NH_2); 7.95−8.18 (br, 1H, NH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.7, 42.4, 61.4, 81.0, 127.2, 128.9, 129.5, 136.8, 155.8, 208.7.

*EtO-Thiazole-D-Phe-NHBoc (**15e**)*. EtO-Thiazole-D-Phe-NHBoc was synthesized following the General Thiazole Synthesis procedure, utilizing 225 mg (0.80 mmol, 1.0 equiv) of Thioamide-D-Phe-NHBoc (**14e**) and 644 mg (6.43 mmol, 8.0 equiv) of potassium bicarbonate dissolved in 8.0 mL of 1,2-dimethoxyethane. A 0.30 mL amount of ethyl bromopyruvate (2.40 mmol, 3.0 equiv) was added dropwise (0.1 mL/min) to yield the thiazoline intermediate. The thiazoline was dehydrated using 0.58 mL (7.20 mmol, 9.0 equiv) of pyridine, 0.44 mL (3.20 mmol, 4.0 equiv) of trifluoroacetic anhydride, and 0.22 mL (1.60 mmol, 2.0 equiv) of triethylamine in 8.0 mL of 1,2-dimethoxyethane to afford the desired thiazole (169 mg, 56% yield) as a clear oil. R_f: 0.55 (hexanes/ethyl acetate 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.38 (s, 9H, C(CH_3)$_3$); 1.40 (t, 3H, OCH_2CH_3); 3.13−3.41 (m, 2H, $CHCH_2Ph$); 4.31−4.50 (q, 2H, OCH_2CH_3); 5.19−5.36 (m, 1H, 1αH); 5.36−5.54 (m, 1H, NH); 7.03−7.32 (m, 5H, Ph); 8.05 (s, 1H, SCH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.6, 28.4, 41.8, 54.1, 61.7, 80.4, 126.9, 127.2, 128.9, 129.5, 136.5, 147.4, 155.2, 161.7, 173.7. HRMS (ESI-TOF): M + H^+, found 377.1525 $C_{19}H_{24}N_2O_4S$ requires 377.1535.

*EtO-Thiazole-D-Phe-NH_2 (**15e-1**)*. EtO-Thiazole-D-Phe-NH_2 was synthesized following the General Amine Deprotection procedure. The amine was taken on to the next reaction without further purification or characterization (124 mg, quantitative yield) as a clear oil.

*EtO-Thiazole-D-Phe-Val-NHBoc (**17f**)*. EtO-Thiazole-D-Phe-Val-NHBoc was synthesized following the General Peptide Synthesis procedure, utilizing 124 mg (0.45 mmol, 1.0 equiv) of amine EtO-Thiazole-D-Phe-NH_2 (**15e-1**), 109 mg (0.50 mmol, 1.1 equiv) of acid HO-Val-NHBoc (**16a**), 116 mg (0.36 mmol, 0.8 equiv) of TBTU, 137 mg (0.36 mmol, 0.8 equiv) of HATU, and 0.47 mL (2.70 mmol, 6 equiv) of DIPEA dissolved in 4.5 mL of methylene chloride under N_2. The crude product underwent a final purification via column chromatography on silica gel (hexanes/ethyl acetate 19:1 to 2:1) to afford the desired peptide (157 mg, 73% yield) as a clear oil. R_f: 0.48 (hexanes/ethyl acetate 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.66−0.80 (dd, *J* = 18.6, 6.5 Hz, 3H, CHCH(CH_3)$_2$); 0.66−0.74 and 0.79−0.86 (dd, *J* = 31.5, 6.9 Hz, 3H, CHCH(CH_3)$_2$); 1.38 (s, 9H, C(CH_3)$_3$); 1.37 (t, 3H, OCH_2CH_3); 1.86−2.14 (m, 1H, $CHCH(CH_3)_2$); 3.13−3.49 (m, 2H, $CHCH_2Ph$); 3.84−3.98 (m, 1H, 1αH); 4.32−4.43 (q, 2H, OCH_2CH_3); 5.11−5.21 (d, *J* = 9.0 Hz, 1H, NH); 5.22−5.32 (d, *J* = 9.0 Hz, 1H, NH); 5.53−5.64 (m, 1H, 1αH); 7.04−7.28 (m, 5H, Ph); 8.00 (s, 1H, SCH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.6, 17.8, 19.6, 28.7, 30.7, 38.9, 41.2, 52.6, 60.2, 61.7, 80.1, 127.2, 127.8, 129.2, 129.5, 136.5, 147.4, 156.1, 161.4, 166.1, 171.9. HRMS (ESI-TOF): M + Na^+, found 498.2024 $C_{24}H_{33}N_3O_5S$ requires 498.2039.

*EtO-Thiazole-D-Phe-Val-NH_2 (**17f-1**), Fragment 1*. EtO-Thiazole-D-Phe-Val-NH_2 was synthesized following the General Amine Deprotection procedure. The amine was taken on to the next reaction without further purification or characterization (124 mg, quantitative yield) as a light brown oil.

*EtO-Thiazole-D-Phe-Val-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc (**29-8**)*. EtO-Thiazole-D-Phe-Val-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc was synthesized following the General Peptide Synthesis procedure, utilizing 177 mg (0.30 mmol, 1.0 equiv) of acid HO-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc (**28a-1**), 124 mg (0.33 mmol, 1.1 equiv) of amine EtO-Thiazole-D-Phe-Val-NH_2 (**17f-1**), 77.0 mg (0.24 mmol, 0.8 equiv) of TBTU, 91.2 mg (0.24 mmol, 0.8 equiv) of HATU, and 0.42 mL (2.40 mmol, 8.0 equiv) of DIPEA dissolved in 3.00 mL of methylene chloride. The crude product underwent a final purification via column chromatography on silica gel (ethyl acetate/methanol 99:1) to afford the desired peptide (208 mg, 73% yield). R_f: 0.30 (ethyl acetate/methanol 99:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.57−0.63 and 0.68−0.76 (d, *J* = 6.91 Hz, 3H, CHCH(CH_3)$_2$); 0.51−0.64 and 0.75−0.80 (m, 3H, CHCH(CH_3)$_2$); 0.91−0.99 (d, *J* = 6.91 Hz, 3H, $CHCH_2CH(CH_3)_2$); 0.96−1.04 (3H, $CHCH_2CH(CH_3)_2$); 1.34−1.43 (t, 3H, OCH_2CH_3); 1.42 (s, 9H, C(CH_3)$_3$); 1.41−1.57 (m, 2H, $CHCH_2CH(CH_3)_2$); 1.71−1.84 (m, 1H, $CHCH_2CH(CH_3)_2$); 1.92−2.07 (m, 2H, $CHCH_2CH_2CH_2$); 1.97−2.19 (m, 2H, $CHCH_2CH_2CH_2$); 2.02−2.12 and 2.18−2.28 (m, 2H, $CHCH_2CH_2CH_2$); 2.12−2.25 (m, 1H, CHCH(CH_3)$_2$); 2.10−2.15 and 2.20−2.51 (m, 2H, $CHCH_2CH_2CH_2$); 3.18−3.35 and 3.40−3.57 (m, 2H, $CHCH_2Ph$); 3.64−3.91 (m, 2H, $CHCH_2CH_2CH_2$); 4.07−4.25 (m, 2H, $CHCH_2CH_2CH_2$); 4.22−4.34 (m, 1H, 1αH); 4.33−4.46 (q, 2H, OCH_2CH_3); 4.47−4.60 (m, 1H, 1αH); 4.67−4.77 and 5.21−5.33 (m, 1H, 1αH); 5.42−5.69 (m, 1H, 1αH); 5.45−5.60 (m, 1H, 1αH); 6.97−7.30 (m, 5H, Ph); 7.35−7.44 (m, 1H, NH); 7.91 and 7.93 (s, 1H, SCH); 7.96−7.98 (m, 1H, NH); 7.97 and 8.03 (s, 1H, SCH); 8.22 and 8.28 (s, 1H, OCH); 8.29−8.31 (m, 1H, NH). ^{13}C NMR (75 MHz,$CDCl_3$): δ 14.4, 17.1, 19.4, 21.7, 23.4, 24.6, 25.6, 28.3, 29.7, 31.8, 40.7, 42.2, 47.1, 49.5, 50.4, 52.5, 52.8, 58.2, 58.8, 61.4, 79.7, 120.9, 127.0, 127.6, 128.6, 129.3, 136.7, 137.8, 142.7, 143.6, 147.0, 155.8,

dx.doi.org/10.1021/jo3017499 | J. Org. Chem. 2012, 77, 10596−10616

156.6,161.4, 171.0, 171.3, 172.5, 172.8, 174.4. LC/MS (ESI): m/z calcd for $C_{46}H_{60}N_8O_9S_2$ (M + Na^+) = 955.38, found 955.00. HRMS (ESI-TOF): M + Na^+, found 955.3811 $C_{46}H_{60}N_8O_9S_2$ requires 955.3823.

*HO-Thiazole-D-Phe-Val-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc (**29-8a**).* HO-Thiazole-D-Phe-Val-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc was synthesized following the General Acid Deprotection procedure, utilizing 208 mg (0.22 mmol, 1.0 equiv) of EtO-Thiazole-D-Phe-Val-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc (**29-8**) and 73.8 mg (1.76 mmol, 8 equiv) of LiOH·H_2O in 2.20 mL of ethanol. The acid was taken on to the next reaction without further purification (199 mg, quantitative yield). LC/MS (ESI): m/z calcd for $C_{44}H_{56}N_8O_9S_2$ (M + H^+) = 905.36, found 905.00.

*HO-Thiazole-D-Phe-Val-Pro-Oxazole-Thiazole-Pro-Leu-NH_2 (**29-8b**).* HO-Thiazole-D-Phe-Val-Pro-Oxazole-Thiazole-Pro-Leu-NH_2 was synthesized following the General Amine Deprotection procedure, utilizing 199 mg (0.22 mmol, 1.0 equiv) of HO-Thiazole-D-Phe-Val-Pro-Oxazole-Thiazole-Pro-Leu-NHBoc (**29-8a**), 47.8 μL (0.44 mmol, 2.0 equiv) of anisole, and 0.44 mL of trifluoroacetic acid in 1.76 mL of methylene chloride. HO-Thiazole-D-Phe-Val-Pro-Oxazole-Thiazole-Pro-Leu-NH_2 was taken on to the next reaction without further purification or characterization (177 mg, quantitative yield). LC/MS (ESI): m/z calcd for $C_{39}H_{48}N_8O_7S_2$ (M + H^+) = 805.31, found 805.00.

•*Compound **8**.* Compound **8** was synthesized following the Macrocyclization Procedure, utilizing 177 mg (0.220 mmol, 1.0 equiv) of HO-Thiazole-D-Phe-Val-Pro-Oxazole-Thiazole-Pro-Leu-NH_2 (**29-8b**), 58.0 mg (0.180 mmol, 0.8 equiv) of TBTU, 69.0 mg (0.180 mmol, 0.8 equiv) of HATU, 50.0 mg (0.180 mmol, 0.8 equiv) of DMTMM, and 230 μL (1.32 mmol, 8 equiv) of DIPEA in 220 mL of methylene chloride. The crude product was directly subjected to reversed-phase HPLC purification to afford a compound **8** (22.1 mg) in a 19.8% yield. HRMS (ESI-TOF): M + Na^+, found 809.2865 $C_{39}H_{46}N_8O_6S_2$ requires 809.2880.

•*Compound **8**.* ^{1}H NMR (318 K, 600 MHz, $CDCl_3$): δ 0.31−0.37 (d, J = 8.2 Hz, 3H, CHCH(CH_3)$_2$); 0.86−0.91 (d, J = 8.2 Hz, 3H, CHCH(CH_3)$_2$); 0.93−0.99 (d, J = 8.2 Hz, 3H, $CHCH_2CH(CH_3)_2$); 0.99−1.04 (d, J = 8.2 Hz, 3H, $CHCH_2CH(CH_3)_2$); 1.57−1.63 (m, 1H, CH $CH_2CH(CH_3)_2$); 1.58−1.65 and 1.97−2.04 (m, 2H, $CHCH_2CH(CH_3)_2$); 1.79−1.85 and 1.95−2.02 (m, 2H, $CHCH_2CH_2CH_2$); 1.93−2.00 and 2.07−2.13 (m, 2H, $CHCH_2CH_2CH_2$); 1.94−2.00 and 2.33−2.41 (m, 2H, $CHCH_2CH_2CH_2$); 2.21−2.32 (m, 2H, $CHCH_2CH_2CH_2$); 2.21−2.28 and 3.25−3.31 (m, 2H, $CHCH_2Ph$); 2.66−2.72 (m, 1H, CH(CH$_3$)$_2$); 3.67−3.73 and 3.95−4.02 (m, 2H, $CHCH_2CH_2CH_2$); 3.82−3.88 and 3.90−3.97 (m, 2H, $CHCH_2CH_2CH_2$); 4.65−4.71 (m, 1H, 1αH); 4.72−4.77 (m, 1H, 1αH); 5.11−5.17 (m, 1H, 1αH); 5.36−5.43 (m, 1H, 1αH); 5.48−5.54 (m, 1H, 1αH); 5.99−6.06 (d, J = 10.6 Hz, 1H, NH); 6.91−6.97 and 7.16−7.23 and 7.26−7.33 (m, 5H, Ph); 6.99−7.04 (d, J = 10.6 Hz, 1H, NH); 7.09−7.15 (d, J = 10.6 Hz, 1H, NH); 7.47 (s, 1H, SCH); 8.00 (s, 1H, SCH); 8.49 (s, 1H, OCH). ^{13}C NMR (318 K, 150 MHz, $CDCl_3$): δ 15.1, 19.5, 20.1 ($CH_2\gamma$ Pro2), 22.0, 22.3 ($CH_2\gamma$ Pro1), 23.3, 24.2, 28.5, 31.7 ($CH_2\beta$ Pro1), 35.5 ($CH_2\beta$ Pro2), 36.8, 41.3, 46.6, 47.5, 48.9, 51.4, 57.0, 58.8, 64.1, 122.3, 125.8, 126.9, 128.6, 128.7, 137.2, 140.4, 143.4, 146.8, 157.4, 159.9, 162.0, 169.0, 170.4, 170.9, 172.7, 173.3. $\Delta\beta\gamma$ (Pro1, Pro2) = (9.4 ppm; 15.4 ppm).

*Compound **9**. MeO-Oxazole-Thiazole-Pro-Phe-NHBoc (**27d**).* MeO-Oxazole-Thiazole-Pro-L-Phe-NHBoc was synthesized following the General Peptide Synthesis procedure, utilizing 125 mg (0.45 mmol, 1.0 equiv) of amine OMe-Oxazole-Thiazole-Pro-NH (**25-1**), 179 mg (0.675 mmol, 1.5 equiv) of acid HO-L-Phe-NHBoc (**26d**), 144 mg (0.45 mmol, 1.0 equiv) of TBTU, 171 mg (0.45 mmol, 1.0 equiv) of HATU, and 0.63 mL (3.6 mmol, 4 equiv) of DIPEA dissolved in 4.5 mL of methylene chloride under N_2. The reaction mixture was stirred for 3 h and was monitored by TLC upon completion. The crude product underwent a final purification via column chromatography on silica gel (hexanes/ethyl acetate 1:1 to 1:3) to afford the desired peptide (199 mg, 84% yield) as yellow flakes. R_f: 0.58 (hexanes/ethyl acetate 1:3). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.35 (s, 9H, C(CH_3)$_3$); 2.15−2.52 (m, 2H, $CHCH_2CH_2CH_2$); 2.87−2.99 (m, 2H, $CHCH_2CH_2CH_2$); 3.15−3.25 (m, 2H, $CHCH_2C$); 3.28−3.48 (m, 2H, $CHCH_2CH_2CH_2$); 3.93 (s, 3H, OCH_3); 4.69−4.84 (m, 1H, 1αH); 5.10−5.23 (m, 1H, 1αH); 7.29−7.39 (m, 2H, CHCHCH); 7.39−7.49 (m, 3H, CHCHCH); 8.08 (s, 1H, SCH), 8.29 (s, 1H, OCH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.53, 28.30, 34.15, 37.96, 47.35, 52.32, 59.56, 60.50, 79.97, 115.63, 122.07, 127.39, 128.82, 128.89, 129.33, 129.47, 136.45, 141.82, 143.75, 155.39, 155.49, 157.91, 161.48. HRMS (ESI-TOF): M + H^+, found 527.1949 $C_{26}H_{30}N_4O_6S$ requires 527.1920.

*HO-Oxazole-Thiazole-Pro-D-Phe-NHBoc (**27d-1**).* HO-Oxazole-Thiazole-Pro-D-Phe-NHBoc was synthesized following the General Acid Deprotection procedure, utilizing 199 mg (0.378 mmol, 1.0 equiv) of MeO-Oxazole-Thiazole-Pro-L-Phe-NHBoc (**27d**) and 127 mg (3.03 mmol, 8.0 equiv) of LiOH·H_2O in 5 mL of methanol. The acid was taken on to the next reaction without further purification (188 mg, 97% yield) as yellow flakes.

*MeO-Pro-Oxazole-Thiazole-Pro-L-Phe-NHBoc (**28d**).* MeO-Pro-Oxazole-Thiazole-Pro-D-Phe-NHBoc was synthesized following the General Peptide Synthesis procedure, utilizing 188 mg (0.368 mmol, 1.0 equiv) of acid HO-Oxazole-Thiazole-Pro-L-Phe-NHBoc (**27d-1**), 71 mg (0.552 mmol, 1.5 equiv) of the amine MeO-Pro-NH, 140 mg (0.368 mmol, 1.0 equiv) of HATU, and 0.51 mL (2.94 mmol, 8.0 equiv) of DIPEA dissolved in 4 mL of methylene chloride under N_2. The reaction mixture was stirred for 3 h and was monitored by TLC upon completion. The crude product underwent a final purification via column chromatography on silica gel (hexanes/ethyl acetate 1:3) to afford the desired peptide (220 mg, 96% yield) as yellow flakes. R_f: 0.3 (hexanes/ethyl acetate 0:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22 (s, 9H, C(CH_3)$_3$); 1.71−1.80 (m, 2H, $CHCH_2CH_2CH_2$); 1.91−2.07 (m, 2H, $CHCH_2CH_2CH_2$); 2.13−2.26 (m, 2H, $CHCH_2CH_2CH_2$); 2.81−2.94 (m, 2H, $CHCH_2CH_2CH_2$); 3.35−3.45 (t, 2H, $CHCH_2C$); 3.57−3.69 (m, 2H, $CHCH_2CH_2CH_2$); 3.54 (s, 3H, OCH_3); 3.87−3.9 (m, 2H, $CHCH_2CH_2CH_2$); 4.39−4.51 (m, 1H, 1αH); 5.10−5.19 (m, 1H, 1αH); 5.43−5.54 (m, 1H, 1αH); 7.03−7.09 (m, 2H, CHCHCH); 7.09−7.17 (m, 3H, CHCHCH); 7.71 (s, 1H, SCH); 7.81 (o, 1H, OCH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.35, 25.21, 29.12, 34.50, 38.71, 48.59, 48.68, 51.94, 61.11, 60.60, 79.31, 126.99, 128.33, 128.57, 129.27, 129.34, 136.23, 142.97, 143.47, 155.06, 156.54, 165.39, 171.00, 173.22, 173.48. HRMS (ESI-TOF): M + H^+ found 624.2477 $C_{31}H_{37}N_5O_7S$ requires 624.2447.

*HO-Pro-Oxazole-Thiazole-Pro-L-Phe-NHBoc (**28d-1**), Fragment B.* HO-Pro-Oxazole-Thiazole-Pro-D-Phe-NHBoc was synthesized following General Acid Deprotection procedure, utilizing 220 mg (0.353 mmol, 1.0 equiv) of MeO-Pro-Oxazole-Thiazole-Pro-L-Phe-NHBoc (**28d**) and 118 mg (2.82 mmol, 8.0 equiv) of LiOH·H_2O in 4 mL of methanol. The acid was taken on to the next reaction without further purification (204 mg, 95% yield) as yellow flakes.

*EtO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-L-Phe-NHBoc (**29-9**).* EtO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-D-Phe-NHBoc was synthesized following the General Peptide Synthesis procedure, utilizing 204 mg (0.335 mmol, 1.0 equiv) of acid HO-Pro-Oxazole-Thiazole-Pro-L-Phe-NHBoc (**28d-1**), 150 mg (0.503 mmol, 1.5 equiv) of amine EtO-Thiazole-Ala-Val-NH_2 (**17a-1**), 86 mg (0.268 mmol, 0.8 equiv) of TBTU, 102 mg (0.268 mmol, 0.8 equiv) of HATU, 0.47 mL (2.68 mmol, 8.0 equiv) of DIPEA dissolved in 4 mL of methylene chloride under N_2. The reaction mixture was stirred for 3 h and was monitored by TLC upon completion. The crude product underwent a final purification via column chromatography on silica gel (ethyl acetate/methanol 5:1) to afford the desired peptide (266 mg, 89% yield) as yellow flakes. R_f: 0.3 (ethyl acetate/methanol 5:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.76−0.95 (dd, 6H, CH_3CHCH_3); 1.37 (s, 9H, C(CH_3)$_3$); 1.20−1.27 (dd, 3H,$CHCH_3$); 1.53−1.69 (t, 2H,OCH_2CH_3); 1.99−2.05 (m, 2H, $CHCH_2CH_2CH_2$); 2.05−2.14 (m, 2H, $CHCH_2CH_2CH_2$); 2.14−2.27 (m, 2H, $CHCH_2CH_2CH_2$); 2.23−2.48 (m, 2H, $CHCH_2CH_2CH_2$); 2.54−2.78 (m, 2H, CHCH(CH$_3$)$_2$); 2.79−3.15 (m, 2H, $CHCH_2CH_2CH_2$); 3.22−3.44 (m, 2H, $CHCH_2C$); 3.58−3.92 (m, 2H, $CHCH_2CH_2CH_2$); 4.09−4.22 (m, 1H, 1αH); 4.29−4.43 (t, 3H, OCH_2CH_3); 4.6−4.84 (m, 1H, 1αH); 5.17−5.35 (m, 1H, 1αH); 5.39−5.44 (m, 1H, 1αH); 5.44−5.54 (m, 1H, 1αH); 7.04−7.19 (m, 2H, CHCHCH); 7.19−7.29 (m, 3H, CHCHCH); 7.92 (s, 1H, SCH); 8.05 (s, 1H, SCH); 8.28 (s, 1H,

dx.doi.org/10.1021/jo3017499 | J. Org. Chem. 2012, 77, 10596−10616

OCH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.18, 19.51, 21.54, 24.53, 24.92, 28.29, 29.64, 31.51, 39.21, 47.49, 49.41, 53.86, 58.30, 58.59, 60.36, 61.69, 126.82, 127.46, 128.53, 128.77, 129.35, 129.43, 136.24, 143.53, 146.90, 154.35, 155.18, 156.72, 161.33, 161.55, 170.88, 172.60, 173.45. LC/MS (ESI): m/z calcd for $C_{43}H_{54}N_8O_9S_2$ (M + Na^+) = 913.00, found 913.00. HRMS (ESI-TOF): M + H^+ found 891.3527 $C_{43}H_{54}N_8O_9S_2$ requires 891.3533.

*HO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-L-Phe-NHBoc (**29-9a**).* HO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-D-Phe-NHBoc was synthesized following the General Acid Deprotection procedure, utilizing 266 mg (0.299 mmol, 1.0 equiv) of EtO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-L-Phe-NHBoc (**29-9**) and 100 mg (2.39 mmol, 8 equiv) of LiOH·H_2O in 3 mL of ethanol. Upon completion, the reaction was diluted with methylene chloride. The acid was taken on to the next reaction without further purification (240 mg, 93% yield). LC/MS (ESI): m/z calcd for $C_{41}H_{50}N_8O_9S_2$ (M + H^+) = 863.01, found 863.00.

*HO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-L-Phe-NH$_2$ (**29-9b**).* HO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-D-Phe-NH_2 was synthesized following the General Amine Deprotection procedure, utilizing 240 mg (0.278 mmol, 1.0 equiv) of HO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-L-Phe-NHBoc (**29-9a**), 0.06 mL (0.556 mmol, 2.0 equiv) of anisole, and 0.75 mL of trifluoroacetic acid in 2.25 mL of methylene chloride. The reaction was completed in 1 h; the reaction was concentrated in vacuo. HO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-L-Phe-NH_2 was taken on to the next reaction without further purification or characterization (221 mg, quantitative yield). LC/MS (ESI): m/z calcd for $C_{36}H_{42}N_8O_7S_2$ (M + H^+) = 762.90, found 762.00.

•*Compound **9**.* Compound **9** was synthesized following the Macrocyclization Procedure, utilizing 221 mg (0.29 mmol, 1.0 equiv) of HO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-L-Phe-NH_2 (**29-9b**), 75.0 mg (0.232 mmol, 0.8 equiv) of TBTU, 89 mg (0.232 mmol, 0.8 equiv) of HATU, 69 mg (0.184 mmol, 1.0 equiv) of DEPBT, and 1.21 mL (6.96 mmol, 24 equiv) of DIPEA in 434 mL of methylene chloride under N_2. The reaction was monitored by TLC and LCMS upon completion. The crude product underwent an initial purification via column chromatography on silica gel (ethyl acetate/methanol 9:1), and the resulting semipure residue was subjected to reversed-phase HPLC purification to afford compound **9** (1.1 mg) in 35.9% yield. LC/MS (ESI): m/z calcd for $C_{36}H_{40}N_8O_6S_2$ (M + H^+) = 744.88, found 745.00. HRMS (ESI-TOF): M + Na^+, found 767.2398 $C_{36}H_{40}N_8O_6S_2$ requires 767.2410.

•*Compound **9**.* ^{1}H NMR (288 K, 600 MHz, $CDCl_3$): δ 0.85−0.99 (m, 6H, $CH(CH_3)_2$); 1.22−1.29 (m, 2H, CHC*H*); 1.25−1.36 (d, 3H, $CHCH_3$); 1.59−1.64 (m, 2H, $CH_2\gamma$ Pro2); 2.02−2.15 (m, 2H, $CH_2\gamma$ Pro1); 1.88−2.26 (m, 2H, $CH_2\beta$ Pro2); 2.31−2.38 (m, 2H, $CH_2\beta$ Pro1); 3.01−3.12 (m, 2H, $CHCH_2C$); 3.73−3.81 (m, 2H, $CH_2\delta$ Pro2); 3.8−3.9 (m, 2H, $CH_2\delta$ Pro1); 4.70−4.76 (m, 1H, 1αH); 4.76−4.82 (m, 1H, 1αH); 5.08−5.10 (m, 1H, $CHCH_3$); 5.22−5.28 (m, 1H, 1αH); 5.30−5.36 (m, 1H, 1αH); 7.18−7.32 (m, 3H, CHCHCH); 7.42−7.44 (m, 2H, CHCCH); 7.79 (s, 1H, SCH); 7.91 (s, 1H, SCH); 8.39 (s, 1H, OCH). ^{13}C NMR (288 K, 150 MHz, $CDCl_3$): δ 19.8, 22.82, 25.49 ($CH_2\gamma$ Pro2), 27.33 ($CH_2\gamma$ Pro1), 31.42 ($CH_2\beta$ Pro1), 35.85 ($CH_2\beta$ Pro2), 37.24, 42.67, 45.78, 46.6, 48.01, 52.48, 56.77, 58.82, 64.16, 114.69, 116.46, 125.62, 126.47, 126.58, 126.96, 128.24, 129.95, 143.4. $\Delta\beta\gamma$ = (4.7 ppm; 10.36 ppm).

*Compound **10**. MeO-Oxazole-Thiazole-Pro-D-Phe-NHBoc (**27e**).* MeO-Oxazole-Thiazole-Pro-D-Phe-NHBoc was synthesized following the General Peptide Synthesis procedure, utilizing 75 mg (0.267 mmol, 1.0 equiv) of amine OMe-Oxazole-Thiazole-Pro-NH (**25-1**), 106 mg (0.401 mmol, 1.5 equiv) of acid HO-D-Phe-NHBoc (**26e**), 86 mg (0.267 mmol, 1.0 equiv) of TBTU, 102 mg (0.267 mmol, 1.0 equiv) of HATU, and 0.37 mL (2.14 mmol, 8.0 equiv) of DIPEA dissolved in 2.7 mL of methylene chloride under N_2. The reaction mixture was stirred for 3 h and was monitored by TLC upon completion. The crude product underwent a final purification via column chromatography on silica gel (hexanes/ethyl acetate 1:1 to 1:3) to afford the desired peptide (132 mg, 94% yield) as yellow flakes. R_f: 0.58 (hexanes/ethyl acetate 1:3). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.4 (s, 9H, $C(CH_3)_3$); 1.8−2.14 (m, 2H, $CHCH_2$ CH_2CH_2); 2.18−2.48 (m, 2H, $CHCH_2$ CH_2CH_2); 2.85−3.13 (m, 2H, $CHCH_2C$);3.26−3.81 (m, 2H, $CHCH_2$ CH_2CH_2); 3.57−3.72 (t, 2H, CH_2); 3.82 (s, 3H, OCH_3); 4.35−4.81 (m, 1H, 1αH); 5.37−5.59 (m, 1H, 1αH); 7.09−7.19 (m, 2H, CHCHCH); 7.19−7.29 (m, 3H, CHCHCH); 8.07 (s, 1H, SCH), 8.29 (s, 1H, OCH). ^{13}C NMR (75 MHz, $CDCl_3$): 23.80, 28.28, 31.60, 39.32, 46.72, 53.13, 53.89, 58.78, 79.88, 127.13, 128.55, 128.79, 129.32, 129.42, 134.18, 136.19, 141.94, 143.71, 155.35, 157.85, 161.52, 171.59, 173.16, 174.16. HRMS (ESI-TOF): M + H^+ found 527.1959 $C_{26}H_{30}N_4O_6S$ requires 527.1920.

*HO-Oxazole-Thiazole-Pro-D-Phe-NHBoc (**27e-1**).* HO-Oxazole-Thiazole-Pro-D-Phe-NHBoc was synthesized following the General Acid Deprotection procedure, utilizing 132 mg (0.251 mmol, 1.0 equiv) of MeO-Oxazole-Thiazole-Pro-Gly-NHBoc (**27e**) and 84 mg (2.01 mmol, 8.0 equiv) of LiOH·H_2O in 2.5 mL of methanol. The acid was taken on to the next reaction without further purification (122 mg, 95% yield) as yellow flakes.

*MeO-Pro-Oxazole-Thiazole-Pro-D-Phe-NHBoc (**28e**).* MeO-Pro-Oxazole-Thiazole-Pro-D-Phe-NHBoc was synthesized following the General Peptide Synthesis procedure, utilizing 122 mg (0.238 mmol, 1.0 equiv) of acid HO-Oxazole-Thiazole-Pro-D-Phe-NHBoc (**27e-1**), 46 mg (0.357 mmol, 1.5 equiv) of the amine MeO-Pro-NH, 91 mg (0.238 mmol, 1.0 equiv) of HATU, and 0.33 mL (1.91 mmol, 8.0 equiv) of DIPEA dissolved in 3 mL of methylene chloride under N_2. The reaction mixture was stirred for 3 h and was monitored by TLC upon completion. The crude product underwent a final purification via column chromatography on silica gel (hexanes/ethyl acetate 1:3) to afford the desired peptide (121 mg, 82% yield) as yellow flakes. R_f: 0.3 (hexanes/ethyl acetate 0:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.37 (s, 9H, $C(CH_3)_3$); 1.45−1.7 (m, 2H, $CHCH_2$ CH_2CH_2); 1.76−2.08 (m, 2H, $CHCH_2$ CH_2CH_2); 2.18−2.43 (m, 2H, $CHCH_2$ CH_2CH_2); 2.54−2.7 (m, 2H, $CHCH_2$ CH_2CH_2); 2.82−3.08 (m, 2H, $CHCH_2$ CH_2CH_2); 3.19−3.44 (m, 2H, $CHCH_2$ CH_2CH_2); 3.69 (s, 3H, OCH_3); 4.49−4.76 (m, 1H, 1αH); 5.17−5.35 (m, 1H, 1αH); 5.36−5.62 (m, 1H, 1αH); 6.97−7.14 (m, 2H, CHCHCH); 7.14−7.27 (m, 3H, CHCHCH); 7.83 (s, 1H, SCH), 8.24 (s, 1H, OCH). ^{13}C NMR (150 MHz, $CDCl_3$): 24.53, 25.23, 28.61, 29.61, 31.65, 39.27, 47.66, 48.78, 52.24, 58.72, 59.86, 60.77, 79.74, 120.92, 127.66, 128.49, 128.74, 129.32, 129.40, 142.52, 143.28, 155.32, 156.49, 160.31, 171.03, 171.58, 173.93. HRMS (ESI-TOF): M + H^+ found 624.2486 $C_{31}H_{37}N_5O_7S$ requires 624.2447.

*HO-Pro-Oxazole-Thiazole-Pro-D-Phe-NHBoc (**28e-1**), Fragment B.* HO-Pro-Oxazole-Thiazole-Pro-D-Phe-NHBoc was synthesized following General Acid Deprotection procedure, utilizing 121 mg (0.195 mmol, 1.0 equiv) of MeO-Pro-Oxazole-Thiazole-Pro-D-Phe-NHBoc (**28e**) and 65 mg (1.56 mmol, 8.0 equiv) of LiOH·H_2O in 2 mL of methanol. The acid was taken on to the next reaction without further purification (113 mg, 95% yield) as yellow flakes.

*EtO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-D-Phe-NHBoc (**29-10**).* EtO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-D-Phe-NHBoc was synthesized following the General Peptide Synthesis procedure, utilizing 113 mg (0.185 mmol, 1.0 equiv) of acid HO-Pro-Oxazole-Thiazole-Pro-D-Phe-NHBoc (**28e-1**), 83 mg (0.278 mmol, 1.5 equiv) of amine EtO-Thiazole-Ala-Val-NH_2 (**17a-1**), 59 mg (0.185 mmol, 1.0 equiv) of TBTU, 70 mg (0.185 mmol, 1.0 equiv) of HATU, 51 mg (0.185 mmol, 1.0 equiv) of DMTMM, and 0.26 mL (1.48 mmol, 8.0 equiv) of DIPEA dissolved in 2 mL of methylene chloride under N_2. The reaction mixture was stirred for 3 h and was monitored by TLC upon completion. The crude product underwent a final purification via column chromatography on silica gel (ethyl acetate/methanol 19:1) to afford the desired peptide (158 mg, 96% yield) as yellow flakes. R_f: 0.325 (ethyl acetate/methanol 9:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.84−1.02 (dd, 6H, CH_3CHCH_3); 1.19−1.27 (t, 2H,OCH_2CH_3); 1.38 (s, 9H, $C(CH_3)_3$); 1.64−1.68 (dd, 3H,$CHCH_3$); 1.85−2.01 (m, 2H, $CHCH_2$ CH_2CH_2); 2.12−2.24 (m, 2H, $CHCH_2$ CH_2CH_2); 2.25−2.36 (m, 2H, $CHCH_2$ CH_2CH_2); 2.83−3.09 (m, 2H, $CHCH_2CH_2CH_2$); 3.11−3.24 (m, 2H, $CHCH_2CH$); 3.59−3.76 (m, 2H, $CHCH_2$ CH_2CH_2); 4.03−4.14 (m, 2H, $CHCH_2$ CH_2CH_2); 4.27−4.42 (t, 3H, OCH_2CH_3); 4.43−4.6 (m, 1H, 1αH); 4.62−4.79 (m, 1H, 1αH); 5.06−5.29 (dd, 1H, 1αH); 5.31−5.54 (m, 1H, 1αH); 7.06−7.18 (m, 2H, CHCHCH); 7.18−7.28 (m, 3H, CHCHCH); 7.91 (s,

dx.doi.org/10.1021/jo3017499 | J. Org. Chem. 2012, 77, 10596−10616

1H, SC*H*); 8.05 (s, 1H, SC*H*); 8.25 (s, 1H, OC*H*). ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.00, 17.50, 18.49, 19.49, 21.03, 23.76, 29.64, 31.58, 32.17, 43.41, 46.74, 47.69, 49.48, 58.65, 60.38, 61.38, 61.68, 79.83, 115.58, 116.93, 127.17, 127.55, 128.56, 128.79, 129.40, 129.48, 136.18, 146.79, 155.30, 161.37, 171.06, 171.66, 173.16, 174.45. LC/MS (ESI): *m*/*z* calcd for $C_{43}H_{54}N_8O_9S_2$ (M + H^+) = 891.07, found 891.00. HRMS (ESI-TOF): M + H^+ found 891.3527 $C_{43}H_{54}N_8O_9S_2$ requires 891.3533.

*HO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-D-Phe-NHBoc (**29-10a**).* HO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-D-Phe-NHBoc was synthesized following the General Acid Deprotection procedure, utilizing 158 mg (0.177 mmol, 1.0 equiv) of EtO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-D-Phe-NHBoc (**29-10**) and 59 mg (1.42 mmol, 8 equiv) of LiOH·H_2O in 2.00 mL ethanol. Upon completion, the reaction was diluted with methylene chloride. The acid was taken on to the next reaction without further purification (150 mg, 98% yield). LC/MS (ESI): *m*/*z* calcd for $C_{41}H_{50}N_8O_9S_2$ (M + H^+) = 863.01, found 863.00.

*HO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-D-Phe-NH_2 (**29-10b**).* HO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-D-Phe-NH_2 was synthesized following the General Amine Deprotection procedure, utilizing 150 mg (0.174 mmol, 1.0 equiv) of HO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-D-Phe-NHBoc (**29-10a**), 0.04 mL (0.347 mmol, 2.0 equiv) of anisole, and 0.50 mL of trifluoroacetic acid in 2.50 mL of methylene chloride. The reaction was completed in 1 h and concentrated in vacuo. HO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-D-Phe-NH_2 was taken on to the next reaction without further purification or characterization (140 mg, quantitative yield). LC/MS (ESI): *m*/*z* calcd for $C_{36}H_{42}N_8O_7S_2$ (M + H^+) = 762.90, found 763.00.

•*Compound **10**.* Compound **10** was synthesized following the Macrocyclization Procedure, utilizing 140 mg (0.184 mmol, 1.0 equiv) of HO-Thiazole-Ala-Val-Pro-Oxazole-Thiazole-Pro-D-Phe-NH_2 (**29-10b**), 59.0 mg (0.184 mmol, 1.0 equiv) of TBTU, 69.9 mg (0.184 mmol, 1.0 equiv) of HATU, 50.8 mg (0.184 mmol, 1.0 equiv) of DMTMM, and 0.26 mL (1.47 mmol, 8 equiv) of DIPEA in 263 mL of methylene chloride under N_2. The reaction was monitored by TLC and LCMS upon completion. The crude product underwent an initial purification via column chromatography on silica gel (ethyl acetate/methanol 9:1), and the resulting semipure residue was subjected to reversed-phase HPLC purification to afford a compound **10** (33.3 mg) in a 18.3% yield. LC/MS (ESI): *m*/*z* calcd for $C_{36}H_{40}N_8O_6S_2$ (M + H^+) = 744.88, found 745.00. HRMS (ESI-TOF): MH^+ found 745.2590 $C_{36}H_{40}N_8O_6S_2$ requires 745.2590.

•*Compound **10**.* ^{1}H NMR (288 K, 600 MHz, $CDCl_3$): δ 0.77−0.92 (m, 6H, $CH(CH_3)_2$); 1.32−1.35 (d, 3H, $CHCH_3$); 1.32−1.45 (m, 2H, CHC*H*); 1.90−1.98 (m, 2H, $CH_2\gamma$ Pro2); 2.05−2.13 (m, 2H, $CH_2\gamma$ Pro1); 2.13−2.29 (m, 2H, $CH_2\beta$ Pro1); 2.22−2.29 (m, 2H, $CH_2\beta$ Pro2); 3.02−3.13 (m, 2H, $CHCH_2C$); 3.77−3.81 (m, 2H, $CH_2\delta$ Pro2); 4.13−4.23 (m, 2H, $CH_2\delta$ Pro1); 4.36−4.42 (m, 1H, 1αH); 4.39−4.77 (m, 1H, 1αH); 5.31−5.36 (m, 1H, $CHCH_3$); 5.21−5.33 (m, 1H, 1αH); 5.33−5.39 (m, 1H, 1αH); 7.11−7.25 (m, 2H, CHCCH); 7.26−7.31 (m, 3H, CHCHCH); 7.73 (s, 1H, SCH); 8.08 (s, 1H, SCH); 8.31 (s, 1H, OCH). ^{13}C NMR (288 K, 150 MHz, $CDCl_3$): δ 19.39, 20.4, 23.86 ($CH_2\gamma$ Pro2), 25.53 ($CH_2\gamma$ Pro1), 27.01 ($CH_2\beta$ Pro1), 28.93, 31.89 ($CH_2\beta$ Pro2), 39.24, 46.52, 47.48, 58.65, 58.81, 59.29, 61.54, 62.35, 121.3, 127.22, 127.4, 128.65, 129.42, 129.5, 143.5. $\Delta\beta\gamma$ = (2.38 ppm; 8.03 ppm).

Experimental Procedures: Biological Methods. *Strains, Media, Antibiotics, and Cell Culture.* *Pseudomonas aeruginosa* strain PAO1 (ATCC 15692), as well as clinical isolates of enterotoxigenic *Escherichia coli* (Type O88) and penicillin-resistant *Staphylococcus aureus* (SA), were kind gifts from Dr. Suhelen Egan at the School of Biotechnology and Biomolecular Sciences, University of New South Wales. *E. coli* was propagated on Luria−Bertani (LB) medium with 1.5% agar (10 g/L tryptone; 5 g/L yeast extract; 10 g/L sodium chloride; 15 g/L agar). *P. aeruginosa* was propagated on LB medium lacking sodium chloride (LANS; 10 g/L tryptone; 5 g/L yeast extract; 15 g/L agar). *S. aureus* was propagated on Mueller−Hinton (MH) medium (Carl Roth GmbH, Karlsruhe, Germany) containing 1.5% agar.

Twitching Motility Assays. Subsurface stab twitching motility assays were performed as previously described.[3] Briefly, *P. aeruginosa* PAO1 was stab-inoculated through a 1% agar LANS plate containing no compound (untreated control) or the indicated San B analogues (50 μM). *S. aureus* was inoculated through a 1% agar MH plate and served as a negative control for the assay. Plates were incubated for 48 h at 37 °C. The zone of motility was measured and recorded (in mm^2).

In Vitro Antimicrobial Assays. For bacterial cytotoxicity assays, overnight cultures of *E. coli* or *S. aureus* were grown in MH broth at 37 °C with shaking (250 rpm). On the next day, cultures were diluted 1:100 in fresh MH broth, and bacteria were grown statically in the presence of 50 μM San B analogues for 5 h at 37 °C. Either gentamicin or vancomycin-treated cells (50 μM; Sigma-Aldrich) were the positive controls for the assays, and DMSO-treated cells (1% v/v; Sigma-Aldrich) were the negative controls. Microbial viability was assessed using the BacTiter-Glo microbial assay (Promega, Madison, WI) following the manufacturer's instructions. Relative fluorescence units were measured using a MPL4 Orion microplate luminometer (Berthold Technologies).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR, mass spectral, and bacterial cytotoxicity data for compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +61-4-1672-8896; fax:+61-4-9385-6141; e-mail: s.mcalpine@unsw.edu.au.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the University of New South Wales Sydney for support of H.W., D.R.M., and S.R.M., and the Frasch foundation (658-HF07).

■ REFERENCES

(1) Peláez, F. *Biochem. Pharmacol.* **2006**, *71*, 981−990.
(2) Butler, M. S.; Buss, A. D. *Biochem. Pharmacol.* **2006**, *71*, 919−929.
(3) Singh, E.; Ramsey, D. M.; McAlpine, S. R. *Org. Lett.* **2012**, *14*, 1198−1201.
(4) Dalisay, D. S.; Rogers, W. W.; Edison, A. S.; Molinski, T. F. *J. Nat. Prod.* **2009**, *72*, 732−738.
(5) Ángel, V. *Molluscan Res.* **2002**, *22*, 289−301.
(6) Matsunaga, S.; Fusetani, N.; Hashimoto, K.; Koseki, K.; Noma, M. *J. Am. Chem. Soc.* **1986**, *108*, 847−849.
(7) Maddock, J.; Pattenden, G.; Wight, P. G. *J. Comput.-Aided Mol. Des.* **1993**, *7*, 573−586.
(8) Riego, E.; Hernández, D.; Albericio, F.; Álvarez, M. *Synthesis* **2005**, *1907*, 1922.
(9) Baumann, M.; Bazendale, I. R.; Ley, S. V.; Nikbin, N. *Beilstein J. Org. Chem.* **2011**, *7*, 422−495.
(10) Duchler, M. *J. Drug Target.* **2012**, *20*, 389−400.
(11) Boger, D. L.; Lee, J. K. *J. Org. Chem.* **2000**, *65*, 5996−6000.
(12) Dang, Q.; Yan, L.; Cashion, D. K.; Kasibhatla, S. R.; Jiang, T.; Taplin, F.; Jacintho, J. D.; Li, H.; Sun, Z.; Fan, Y.; DaRe, J.; Tian, F.; Li, W.; Gibson, T.; Lemus, R.; van Poelje, P. D.; Potter, S. C.; Erion, M. D. *J. Med. Chem.* **2011**, *54*, 153−165.
(13) Rzuczek, S. G.; Pilch, D. S.; Liu, A.; LaVoie, E. J.; Rice, J. E. *J. Med. Chem.* **2010**, *53*, 3632−3644.
(14) Kehraus, S.; König, G. M.; Wright, A. D.; Woerheide, G. *J. Org. Chem.* **2002**, *67*, 4989−4992.
(15) Roy, R. S.; Kelleher, N. L.; Milne, J. C.; Walsh, C. T. *Chem. Biol.* **1999**, *6*, 305−318.

(16) Takeuchi, Y.; Marshall, G. R. *J. Am. Chem. Soc.* **1998**, *120*, 5363−5372.
(17) Tan, L. T.; Williamson, R. T.; Gerwick, W. H.; Watts, K. S.; McGough, K.; Jacobs, R. *J. Org. Chem.* **2000**, *65*, 419−425.
(18) Siemion, I. Z.; Wieland, T.; Pook, K.-H. *Angew. Chem., Int. Ed.* **1975**, *14*, 702−703.
(19) Deng, S.; Taunton, J. *J. Am. Chem. Soc.* **2002**, *124*, 916−917.
(20) Pan, P. S.; Vasko, R. C.; Lapera, S. A.; Johnson, V. A.; Sellers, R. P.; Lin, C.-C.; Pan, C.-M.; Davis, M. R.; Ardi, V. C.; McAlpine, S. R. *Bio. Org. Med. Chem.* **2009**, *17*, 5806−5825.
(21) Otrubova, K.; Lushington, G. H.; Vander Velde, D.; McGuire, K. L.; McAlpine, S. R. *J. Med. Chem.* **2008**, *51*, 530−544.
(22) Otrubova, K.; McGuire, K. L.; McAlpine, S. R. *J. Med. Chem.* **2007**, *50*, 1999−2002.
(23) Rodriguez, R. A.; Pan, P.-S.; Pan, C.-M.; Ravula, S.; Lapera, S. A.; Singh, E. K.; Styers, T. J.; Brown, J. D.; Cajica, J.; Parry, E.; Otrubova, K.; McAlpine, S. R. *J. Org. Chem.* **2007**, *72*, 1980−2002.
(24) Heller, M.; Sukopp, M.; Tsomaia, N.; John, M.; Mierke, D. F.; Reif, B.; Kessler, H. *J. Am. Chem. Soc.* **2006**, *128*, 13806−13814.
(25) Chatterjee, J.; Mierke, D. F.; Kessler, H. *J. Am. Chem. Soc.* **2006**, *128*, 15164−15172.
(26) Davis, M. R.; Singh, E. K.; Wahyudi, H.; Alexander, L. D.; Kunicki, J.; Nazarova, L. A.; Fairweather, K. A.; Giltrap, A. M.; Jolliffe, K. A.; McAlpine, S. R. *Tetrahedron* **2012**, *68*, 1029−1051.
(27) Shaw, P. E. *EMBO Rep.* **2002**, *3*, 521−526.
(28) Rajakumar, P.; Selvam, S.; Shanmugaiah, V.; Mathivanan, N. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5270−5273.
(29) Cai, Y.; Chai, D.; Wang, R.; Bai, N.; Liang, B.-B.; Liu, Y. *J. Antimicrob. Chemother.* **2011**, *66*, 968−978.
(30) Svarstad, H.; Bugge, H. C.; Dhillion, S. S. *Biodivers. Conserv.* **2000**, *9*, 1521−1541.
(31) Sagan, S.; Karoyan, P.; Lequin, O.; Chassaing, G.; Lavielle, S. *Curr. Med. Chem.* **2004**, *11*, 2799−2822.
(32) Baynham, P. J.; Ramsey, D. M.; Gvozdyev, B. V.; Cordonnier, E. M.; Wozniak, D. J. *J. Bacteriol.* **2006**, *188*, 132−140.
(33) Tugyi, R.; Uray, K.; Iván, D.; Fellinger, E.; Perkins, A.; Hudecz, F. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 413−418.
(34) Chatterjee, J.; Gilon, C.; Hoffman, A.; Kessler, H. *Acc. Chem. Res.* **2008**, *41*, 1331−1342.
(35) Haubner, R.; Gratias, R.; Diefenbach, B.; Goodman, S. L.; Jonczyk, A.; Kessler, H. *J. Am. Chem. Soc.* **1996**, *118*, 7461−7472.
(36) Aumailley, M.; Gurrath, M.; Müller, G.; Calvete, J.; Timpl, R.; Kessler, H. *FEBS Lett.* **1991**, *291*, 50−54.
(37) Mas-Moruno, C.; Rechenmacher, F.; Kessler, H. *Anti-Cancer Agents Med. Chem.* **2010**, *10*, 753.
(38) Styers, T. J.; Rodriguez, R. A.; Pan, P.-S.; McAlpine, S. R. *Tetrahedron Lett.* **2006**, *47*, 515−517.
(39) Rasmussen, L.; White, E. L.; Pathak, A.; Ayala, J. C.; Wang, H.; Wu, J.-H.; Benitez, J. A.; Silva, A. *J. Antimicrob. Agents Chemother.* **2011**, *55*, 4134−4143.
(40) Moody, C. J.; Bagley, M. C. *J. Chem. Soc., Perkin Trans. 1* **1998**, 601−608.