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Total Synthesis of *trans*, *trans*-Sanguinamide B and Conformational Isomers

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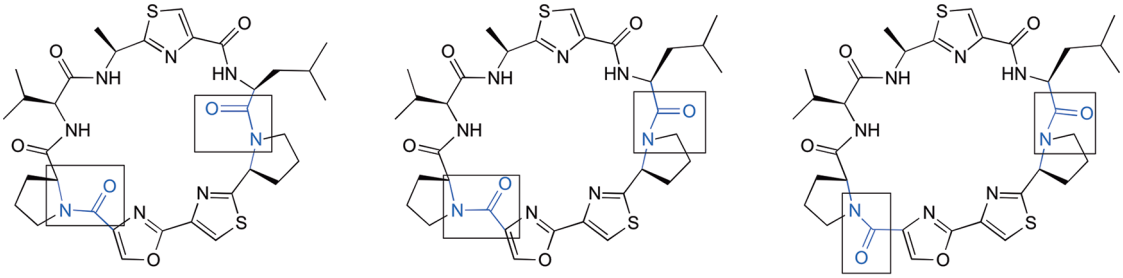
ABSTRACT



The first total synthesis of Sanguinamide B is reported, prepared via an efficient synthetic strategy. The natural product, *trans,trans*-Sanguinamide B (1), was generated in a thermodynamic ratio with *trans,cis*-Sanguinamide B (2) and *cis,cis*-Sanguinamide B (3). Complete conversion of the *cis,cis*-Sanguinamide B conformer (3) to the natural product (1) and the *trans,cis*- conformer (2) was achieved by heating to 170 °C. Biological evaluation indicated that the Sanguinamide B conformers disrupted the activity of a virulence determinant in *P. aeruginosa*.

Cyclic peptides isolated from natural products show tremendous promise as lead structures in the development of novel drug candidates. To date there are 720 cyclic peptides used clinically as drug candidates: 38% of these candidates are in clinical trials, 56% are in advanced preclinical phases, and 5% are on the market.[1] These peptide drugs are used to treat a wide range of diseases, including prostate and breast cancer, HIV, osteoporosis, and multiple autoimmune diseases.[2]

Many cyclic peptide lead structures have been isolated from natural products, whereupon these molecules are synthesized and evaluated for their biological activity. Molinski et al.[3] discovered a new macrocycle, Sanguinamide B (San B), which was isolated from a single species of nudibranch, *Hexabranchus sanguineaus*. Structural analysis of one product isolated from the sponge was determined to be *trans*,*trans*-Sanguinamide B (**1**, Scheme 1), where the descriptor *trans*,*trans* refers to the conformation about the two prolyl amide bonds in the molecule. Macrolides isolated from this sponge have shown antifungal, antibacterial, and cytotoxic properties, which are thought to be the result of these compounds disrupting actin filament formation in the

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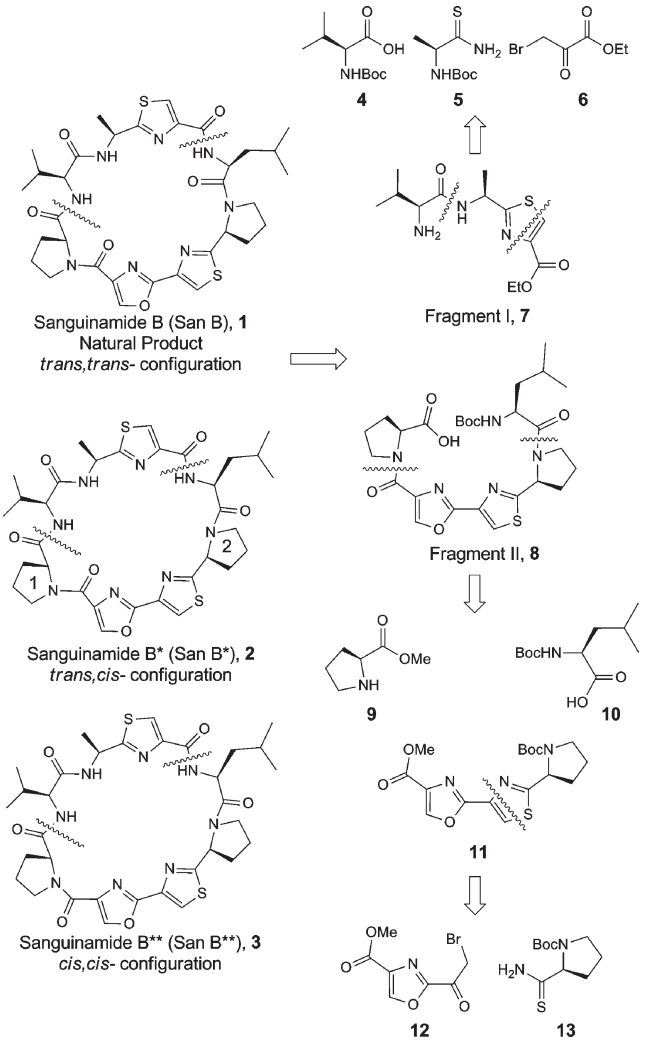
cells.[4] The San B natural product contains two thiazoles and one oxazole, and is a modified octapeptide macrocycle. Unlike other natural products isolated from this sponge, San B contains two proline residues that are presumed to control the conformation of the macrocycle[5] and thus influence its biological activity.[6,7] It has also been established that although the inclusion of proline residues within a macrocycle increases the number of conformational states by decreasing the energy difference between the *trans*- and *cis*-isomers, it also limits the number of available low energy conformations because of allylic 1,3-interactions. As noted by Taunton and Deng in their synthesis of *trans*,*trans*- and *cis*,*cis*-Ceratospongamide,[5] two different conformations were stable and did not interconvert. Gerwick et al. showed that these two isomers also had distinct biological activity; indeed, *trans*,*trans*-Ceratospongamide inhibits transcriptional activation of IL-1β (IC_{50} = 32 nM), whereas the *cis*, *cis*- rotamer is inactive.[8]

The potent cytotoxic and antibiotic properties of other macrolides isolated from the nudibranch *H. sanguineus* sponge, and the small microgram quantities of the compound that are available from the natural source, mean that alternative methods for evaluating biological activity are required. Herein we report the first total synthesis of the natural product, San B (**1**), which exists as the *trans*, *trans*- configuration about each proline residue and confirm its structure. In addition, we report the synthesis of two other San B conformers: *trans*,*cis*-Sanguinamide B (**2**, San B*, Scheme 1), which maintains *trans*- configuration about Pro-1 and is *cis*- about Pro-2, and *cis*,*cis*-Sanguinamide B (**3**, San B**, Scheme 1), which adopts the *cis*-configuration about both proline residues.

The San B conformers (**1**−**3**) were synthesized via the coupling of two fragments; Fragment I and Fragment II (Scheme 1). Fragment I was derived from a Hantzsch thiazole reaction between Ala thioamide derivative **5** and (α)-bromoketone **6**, followed by *N*-terminal extension with Val. Fragment II was also obtained via a Hantzsch reaction between oxazole (α)-bromoketone **12** and Pro thioamide derivative **13** to form oxazole-thiazole moiety **11**. Oxazole (α)-bromoketone **12** was obtained from the cyclization and oxidation of a Ser residue. Subsequent extension of the core oxazole-thiazole moiety **11** via peptide coupling to Pro and Leu furnished Fragment II.

The synthesis of Fragment I began with the protection of commercially available Boc-Ala-OH **14** using (trimethylsilyl) diazomethane (TMSD) in methanol, converting the acid to an ester (Scheme 2). The ester was transformed into an amide using ammonium hydroxide, which was subsequently converted to the desired thioamide **5** using Lawesson's Reagent. The thioamide was subjected to modified Hantzsch thiazole synthesis conditions that

Scheme 1. Retrosynthetic Strategy for San B Conformers



preserved stereochemical integrity at Cα of the Ala residue.[9] Specifically, thioamide **5** was treated with ethyl bromopyruvate **6** and potassium bicarbonate to generate a hydroxyl thiazoline intermediate. This intermediate was subsequently dehydrated to thiazole **15** with trifluoroacetic anhydride (TFAA), pyridine, and triethylamine (TEA). Removal of the Boc protecting group with trifluoroacetic acid (TFA), followed by peptide coupling to Boc-Val-OH **4** with *O*-(Benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) and *N*,*N*-diisopropylethylamine (DIPEA), and subsequent amine deprotection with TFA furnished the desired Fragment I (**7**).

Fragment II, comprised of two consecutive heterocycles and three amino acids, was synthesized by constructing the heterocycles first in order to optimize the overall yield for this fragment.[10] Using standard peptide coupling conditions, H_2N-Ser(Bzl)-OMe **16** was coupled to dimethoxy acetal bromopyruvic acid **17**, and the benzyl protecting

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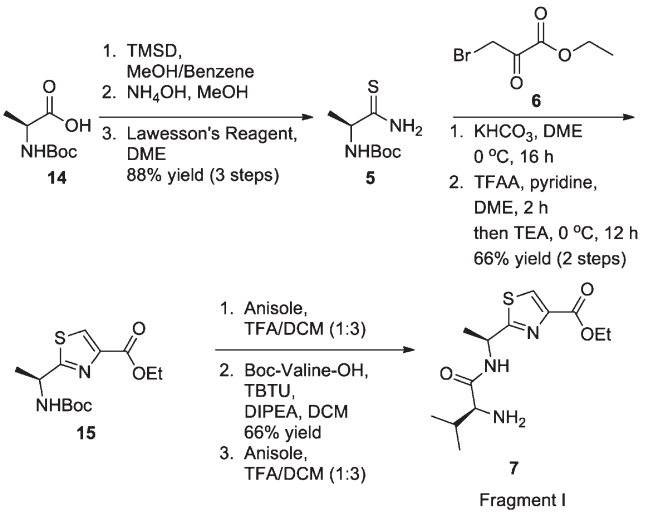
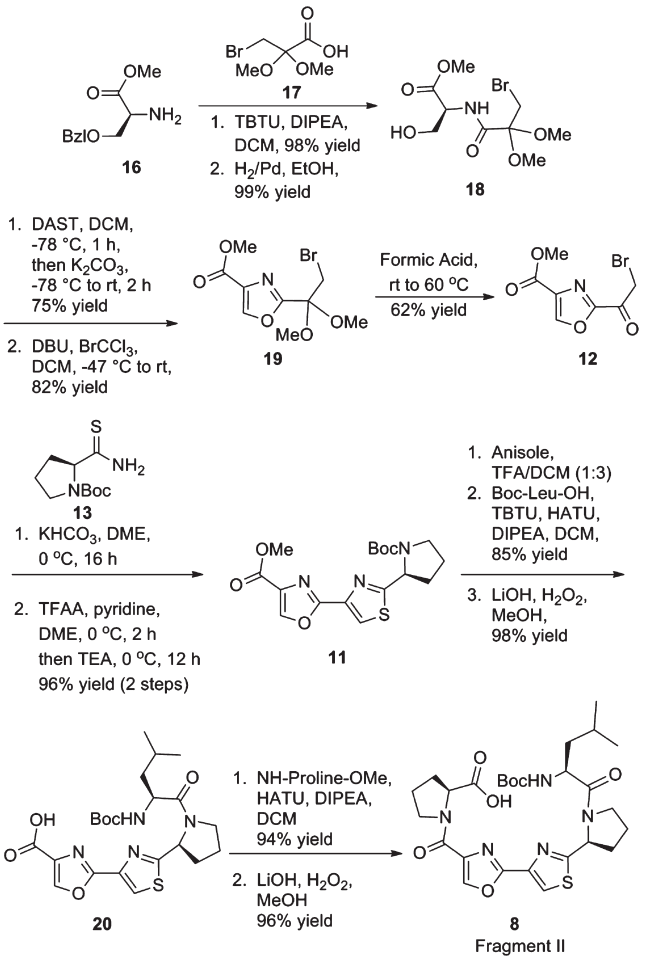
Scheme 2. Synthesis of Fragment I (**7**)



Scheme 3. Synthesis of Fragment II (**8**)



group on Ser was removed via hydrogenolysis, furnishing **18** (Scheme 3). Formation of the oxazole was accomplished via fluorination of the serine hydroxyl with diethylaminosulfur trifluoride (DAST), cyclization with potassium carbonate to yield an oxazoline intermediate, and finally oxidation with bromotrichloromethane and 1,8-Diazabicyclo-[5.4.0]undec-7-ene (DBU) to generate the oxazole **19**.[11] To prepare **19** for installation of the thiazole, the ketone was deprotected with formic acid to furnish an oxazole-bromoketone moiety **12**. Using a modified Hantzsch thiazole synthesis procedure, thioamide **13** was treated with oxazole-bromoketone **12** and potassium bicarbonate to generate a hydroxyl thiazoline intermediate. The desired thiazole **11** was generated via dehydration of the hydroxyl thiazoline with TFAA and pyridine. With both heterocycles installed, the Boc group was removed from **11** and the amine was coupled to Boc-Leu-OH **10** with 2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU), TBTU, and DIPEA. Hydrolysis of the methyl ester[12] furnished **20**, subsequent coupling with NH-Pro-OMe **9**, and then methyl ester hydrolysis furnished Fragment II (**8**).

Peptide coupling of free amine Fragment I (**7**) and free acid Fragment II (**8**) produced a protected linear peptide (Scheme 4). Deprotection of the acid, followed by subsequent deprotection of the amine resulted in linear precursor **21**, which was ready for cyclization. Macrocyclization of **21** was performed under highly dilute conditions (0.007 M) and afforded the naturally occurring *trans*,*trans*-conformer (San B, **1**), *trans*,*cis*-Sanguinamide B (San B*, **2**) as well as *cis*, *cis*-Sanguinamide B (San B**, **3**).

Conformers were synthesized in a 1:1:30 ratio[13] of San B: San B*:San B**, whereby the *cis*,*cis*-Sanguinamide B (San B**, **3**) was favored over the naturally occurring compound *trans*,*trans*-Sanguinamide B (San B, **1**) or its conformer (San B*, **2**). It is well established that prolyl amide bonds adopting *cis*/*trans* conformation alter the difference in chemical shifts between β and γ carbons of proline residues ($\Delta_{\beta\gamma}$).[14] A proline that adopts *cis*- conformation about its amide bond characteristically has larger $\Delta_{\beta\gamma}$ than a proline in the *trans*- conformation. The $\Delta_{\beta\gamma}$ for the natural product, **1** of Pro-1 and Pro-2 are 4.2 and 2.4 ppm, respectively. These values and the spectroscopic data for *trans*,*trans*-San B **1** were in good agreement with data for the natural product published by Molinski et al (4.7 and 2.1 ppm, respectively).[3] The $\Delta_{\beta\gamma}$ for San B* **2** are 3.8 and 13.5 ppm, respectively, indicative of the *trans*,*cis*- conformation, and the $\Delta_{\beta\gamma}$ for San B** **3** are 9.4 and 15.5 ppm, respectively, which corresponds to the *cis*,*cis*- conformer.

Our data indicate that the kinetic product is the *cis*,*cis*- conformer, and that the synthesis of the naturally

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(13) SanB/SanB* inseparable mixture was synthesized in 1:15 ratio to SanB** (determined by mass). Integration of the ^{1}H NMR absorptions showed that SanB and SanB* were present in 1:1 ratio.

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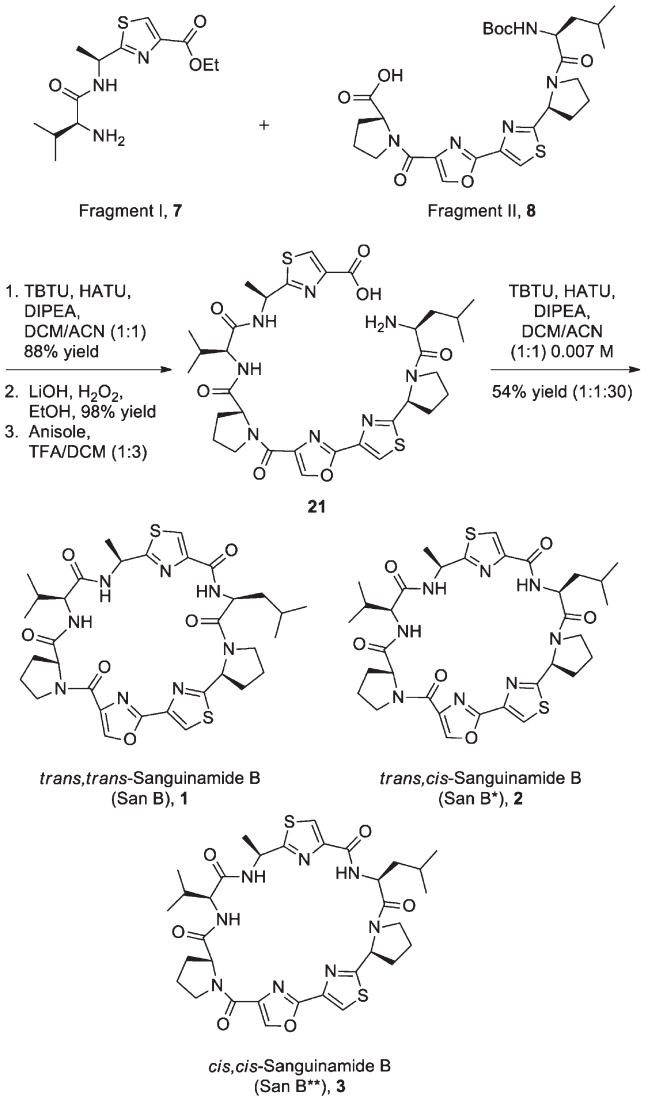
Scheme 4. Macrocyclization Generates Both the Natural Product *trans*,*trans*-Sanguinamide B (**1**) and *trans*,*cis*-Sanguinamide B (**2**), and *cis*,*cis*-Sanguinamide B (**3**)



occurring *trans*,*trans*-conformer is under thermodynamic control. In order to investigate this, we explored the thermodynamic stability of *cis*,*cis*-Sanguinamide B **3**. We found that heating **3** at 170 °C in DMSO for a period of 20 h produced a complete conversion to a 1:1 mixture of *trans*,*trans*-Sanguinamide B **1** and *trans*,*cis*-Sanguinamide B **2** (see Supporting Information). This transformation was monitored by LC−MS and ^{1}H NMR (it was also observed that **3** is stable up to 120 °C). Since no *cis*,*cis*-conformer was isolated from the natural product, it is likely that synthesis is thermodynamically controlled.

To explore the difference in biological activity of the different San B conformers, we tested the macrocycles against *Pseudomonas aeruginosa* bacteria. Macrocycles have been shown to exhibit antibacterial activity against respiratory pathogens, and the use of macrolide antibiotics in the treatment of cystic fibrosis (CF) patients has produced promising results,[15] as this disease predisposes patients to bacterial lung infections.[16] Through extension and retraction of the fimbriae at the cell poles, bacteria are able to move along a solid surface in a form of locomotion known as twitching motility.[17] We examined the effects of the San B conformers on twitching motility of a type IV fimbriae-positive strain of *P. aeruginosa* using a twitching motility assay. The area of twitching motility was reduced in size by 24% when cells were treated with the San B (**1**) and San B* (**2**) mixture at 1.4 μM, however, treatment with San B** (**3**) at 1.4 μM showed no significant decrease in twitching motility area (see Supporting Information). Our preliminary data indicate that the San B and San B* mixture affects the activity of an important virulence determinant in *P. aeruginosa*. Further studies are being conducted to determine if the observed San B mediated reduction in twitching motility is due to affecting pilin gene expression or fimbrial formation and assembly.

In summary, we have described the first total synthesis of the natural product *trans*,*trans*-Sanguinamide B (**1**), verified the structure that was proposed by Molinski et al.[3] and characterized *trans*,*cis*-Sanguinamide B (**2**) in the presence of the natural product. Additionally, we identified and characterized the *cis*,*cis*-Sanguinamide B (**3**) conformer and explored its thermodynamic stability. These results underscore the important role played by the proline residues in dictating the thermodynamic product.

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Supporting Information Available. General experimental procedures, NMR and mass spectral data for compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.