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A progressive synthetic strategy for class B synergimycins

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Abstract—Described are the syntheses of four macrocyclic peptides that are the core structure of class B synergimycins, and the synthesis of a final class B derivative. Our synthetic route to these synergimycin derivatives allows the incorporation of amino acid substitutions at all points in the macrocycle, leading to structurally diverse class B analogs.

Antibiotic resistance is an extreme public health concern[1] and previously treatable infectious diseases are becoming life-threatening infections. In order to treat these diseases a common strategy involves resurrecting older classes of antibiotics via derivatization. A number of potent antibiotics are large macrocyclic peptides.[2] The synthesis of new antibiotics in these classes involves constructing peptide derivatives of these macrocycles. In an effort to design new antibiotics, we synthesized class B synergimycin derivatives based on virginiamycin S_1 (VS_1) (Fig. 1).[3] Our synthetic approach was intended to simplify the exchange of amino acids in order to probe the structure–activity relationship of each residue.

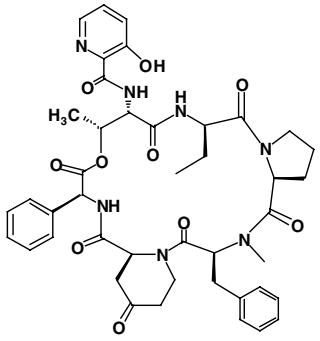

Figure 1. Virginiamycin S_1 (VS_1).

Herein we describe a synthesis strategy that provides four core class B macrocycles and one initial class B synergimycin derivative. Class A and class B synergimycins are known to work synergistically to inhibit protein synthesis in bacteria.[4] The target site for the two classes is the peptidyltransferase center of the *50S* ribosomal subunit.[5] Renewed antibacterial activity is known to occur upon structural changes to residues within the macrocycle.[6]

The work described here utilizes a strategy where substitution of amino acids in any position is relatively straightforward. In addition, the success of the recent antibiotic synercid,[6] which contains a class B derivative, suggests the potential for making new antibiotics using our strategy.

The specific binding site for class B on the ribosome is well established.[7] The first three residues (**1**, **2**, and **3**) remain the same for this family of the synergimycins. It is known that picolinic residue **1** is responsible for binding to the ribosome and it is also thought that residues **2** and **3** make critical contacts with the ribosome.[8] Conformational studies of class B natural products show these compounds all have a *cis* peptide configuration between the **4** and **5** residue, a type VI β-turn reinforced by an intramolecular hydrogen bond between **6** and **3**, and a biased rotational state where the aromatic ring of **5** is oriented toward residue **4**.[9] Due to the structural importance of residues **1**, **2**, and **3** for binding to the ribosome, only one small change was made to residue **3**.[8] In order to simplify the synthesis of our initial derivatives, the phenylglycine (**3**) in the natural product was exchanged for a phenylalanine. It is not clear what effect this will have on antibiotic activity.

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It is known that residue **4** is embedded into a lipophilic region of the bacterial ribosome and that the alteration of residue **4** to a more hydrophobic residue leads to increased synergistic activity.[10] In addition, studies indicate the importance of π-stacking between the ketone on residue **4** and the aromatic group of residue **5**.[9] Therefore, we chose four substitutions at position **4** (**4a**–**d**) in an effort to elucidate the influence of this residue on π-stacking with residue **5**. Finally, we chose two substitutions in position **5**. (L)-Tyrosine (**5a**) was chosen because it resembled the amino acid in the natural product (VS_1), and (L)-tryptophan (**5b**) was chosen to promote a π-stacking interaction between **4** and **5**.[11] In this preliminary study, we chose to keep residue **6** the same as that in the natural product, (L)-proline, because it forces a β-turn element into the macrocyclic architecture and it is responsible for hydrogen bonding to residue **3**. Residue **7** appears to play a small role in the conformation and biological activity of the macrocycle.[9] Based on these previous studies, we chose (L)-leucine as residue **7**.

The three disconnections shown in our initial convergent synthesis strategy (Fig. 2) allow us to readily exchange amino acid residues. Macrocyclization was designed to occur between the primary amine on residue **7** and the free acid on residue **2**. Previous successful syntheses of the natural product have shown that ring closing can occur between residues **3** and **4**,[12] and between the secondary amine **4** and **5**.[13]

Our efforts started with the construction of fragment 1. Using *O*-(7-azabenzatrizol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) and Hunig's base, commercially available L-threonine methyl ester (**2**) and 3-hydroxy picolinic acid (**1**) were coupled in methylene chloride (Fig. 3) to give fragment 1 (86% yield). For fragment 2 synthesis we coupled L-phenylalanine methyl ester (**3**) with the *N*-Boc protected residue of **4** [(*S*)-(*N*)-Boc-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (**4a**), (*N*)-Boc-glycine (**4b**), (*R*)-(*N*)-Boc-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (**4c**), and (*S*)-(−)-Boc-2-piperidine carboxylic acid (**4d**)]. This gave four examples of dipeptide **3-4X-Boc** (80–94% yield).[14] Deprotection of the amine on residue **4** using 20% TFA and 2 equiv of anisole in methylene chloride gave the free amine **3-4X** (∼quantitative yields). Coupling of the dipeptides to *N*-(α)–Boc-L-tyrosine (**5a**) or *N*-(α)–Boc-L-tryptophan (**5b**) using HATU gave the eight desired tripeptides in high yields (65–94%).[14] Deprotection of the acid using barium hydroxide in methanol gave eight fragment 2 compounds in quantitative yields. Fragment 3 was synthesized using HATU as the activating agent and coupling the free amine of L- proline methyl ester (**6**) with *N*-(α)-Boc-leucine (**7**).

In our initial strategy, we coupled fragment 1 with fragment 2 (Fig. 4), yielding five pentapeptides in yields ranging from 45% to 90%.[14] These pentapeptides were then coupled to fragment 3 to give five linear heptapeptide precursors (yields 26–63%).[14] Attempted cyclization of the five linear heptapeptides failed using conditions that had worked in a similar class of mac-

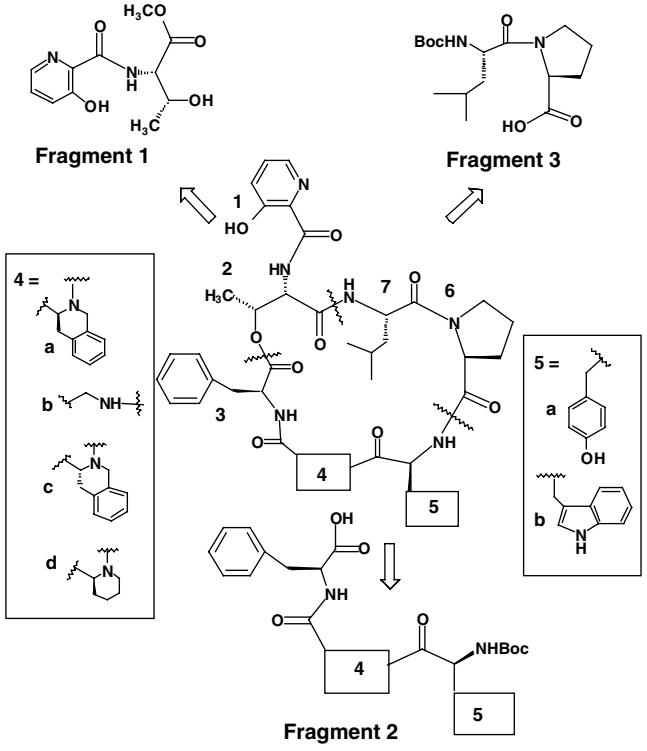

Figure 2. Amino acids used in class B derivative synthesis.

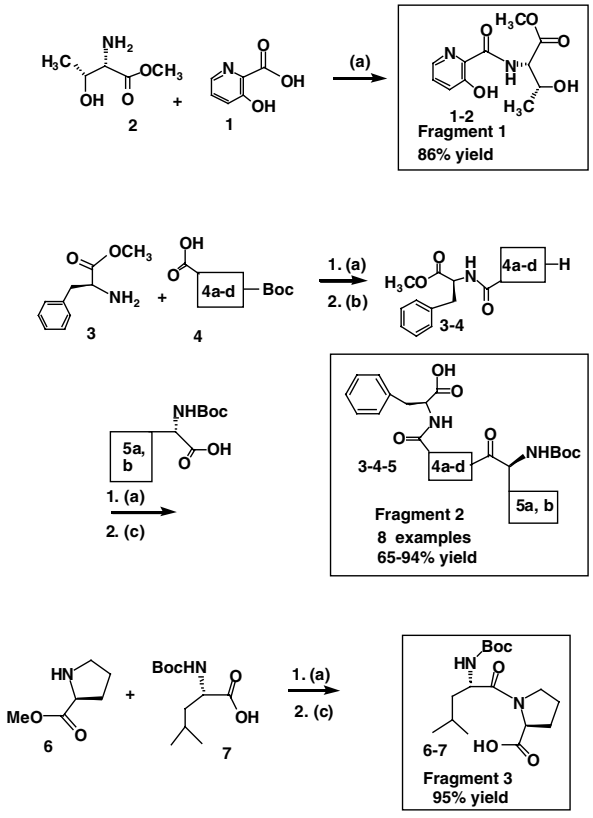

Figure 3. Initial synthesis of fragments for class B derivatives. Reagents and conditions: (a) HATU (1.2 equiv), Hunig's base (3 equiv), CH_3CN; (b) TFA (20%), CH_2Cl_2, anisole (2 equiv); (c) $Ba(OH)_2$ (4 equiv), MeOH.

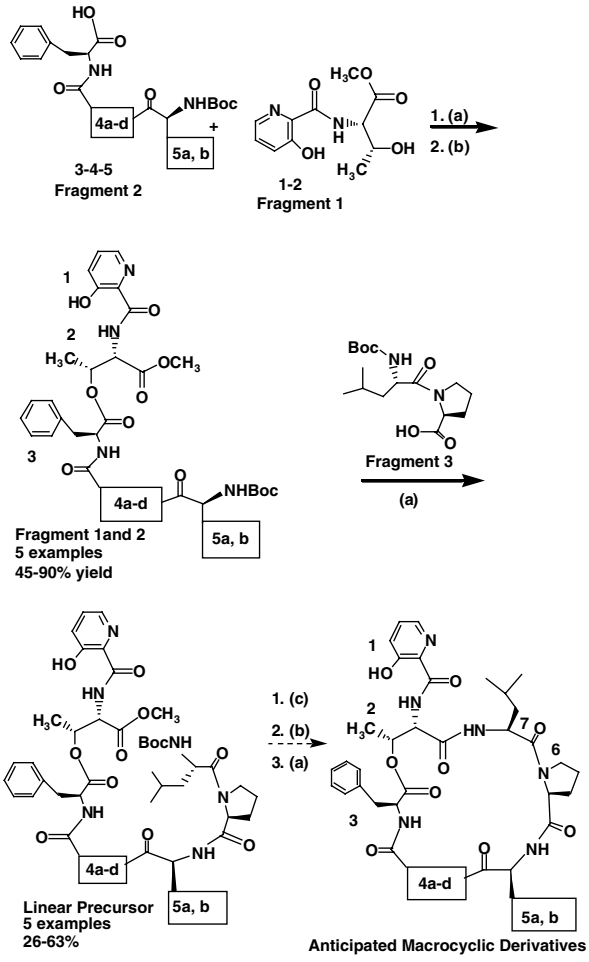

Figure 4. Initial synthesis of macrocyclic derivatives. Reagents and conditions: (a) HATU (1.2 equiv), TBTU, PyBop, and/or DEPBT used as coupling reagents (1.2 equiv), Hunig's base (3 equiv), CH_3CN; (b) TFA (20%), CH_3CN, anisole (2 equiv); (c) $Ba(OH)_2$ (4 equiv), MeOH.

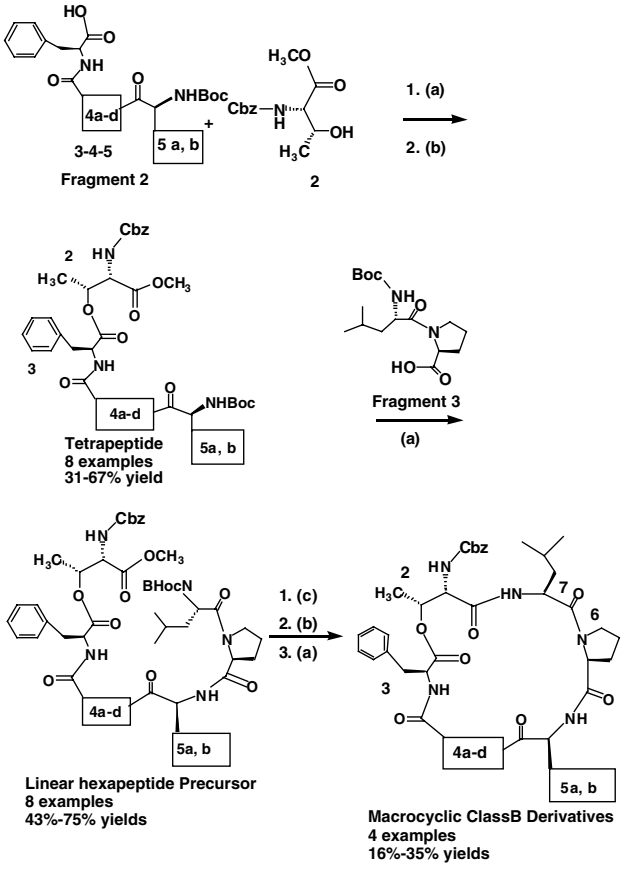

Figure 5. Modified synthesis of macrocyclic derivatives. Reagents and conditions: (a) HATU (1.2 equiv), TBTU, PyBop, and/or DEPBT used as coupling reagents (1.2 equiv), Hunig's base (3 equiv), CH_3CN; (b) TFA (20%), CH_3CN, anisole (2 equiv); (c) $Ba(OH)_2$ (4 equiv), MeOH.

rocyclic peptides.[15] These conditions involved deprotecting the acid using 4 equiv of barium hydroxide, and quenching with TFA in acetonitrile with 2 equiv of anisole. Upon deprotection of the amine we subjected the crude, dry product to HATU, TBTU, and DEPBT coupling reagents (1.2 equiv each), with Hunig's base (3 equiv) in acetonitrile.[16] The final macrocyclizations ran over approximately 4 days at low concentration (0.005–0.01 M) in order to maximize the yields. However, upon LCMS analysis at each stage of this in situ deprotection and macrocyclization, it appeared that residue **1** was base sensitive and was labile under the barium hydroxide deprotection conditions. Although other conditions were explored for this deprotection, only basic conditions successfully removed the methyl ester, which also removed residue **1**. In addition, a protection strategy was investigated for the hydroxyl on residue **1**. However, these conditions were not straightforward[13] and another modified synthetic route was developed.

We modified our strategy and used a Cbz-protected residue **2** in place of fragment 1 (Fig. 5). Coupling of residue **2** to fragment 2 makes the eight tetrapeptides in 31–67% yields.[14] Then, the amine on residue **5** was deprotected and was coupled to fragment 3 (43–75% yields for eight examples of hexapeptides). These hexapeptides were then subjected to the macrocyclization conditions used in the previous strategy and four macrocycles were obtained in reasonable yields (16–35%).[14,17]

A class B derivative was achieved by removing the Cbz protecting group using hydrogenation and Pd/C and then coupling residue **1** to the core macrocycle (Fig. 6),[18] thus successfully validating our revised synthetic strategy.

In summary, we have developed a useful synthetic route to class B synergimycin derivatives that will allow the incorporation of amino acid substitutions at all points in the macrocycle.[19] This will lead to structurally diverse class B synergimycins that have the potential to be antibiotics against resistant strains of bacteria. The successful synthesis of an initial class B derivative using this route establishes the viability of our approach. We are currently carrying out the synthesis of other derivatives and upon completion, antibacterial assays will be run on these compounds in order to establish a structure–activity relationship.

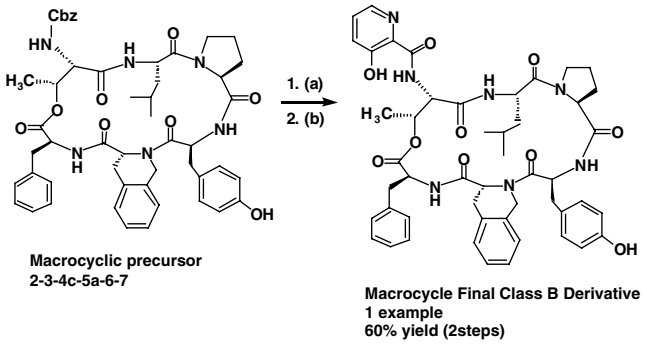

Figure 6. Modified synthesis of macrocyclic derivatives. Reagents and conditions: (a) H_2, Pd/C, CH_3CN; (b) HATU (1.2 equiv), Hunig's base (3 equiv), CH_3CN.

Acknowledgements

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14. Yield ranges reflect those for each individual amino acid monomer that is used in that particular step of the synthesis, that is, yields vary depending on the unique amino acid used in the reaction at that stage of the synthesis.
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16. Bolla, M. L.; Azevedo, E. V.; Smith, J. M.; Taylor, R. E.; Ranjit, D. K.; Segall, A. M.; McAlpine, S. R. *Org. Lett.* **2003**, *5*, 109–112, Ring-closing reactions are slow and typically low yielding. Unpublished results from the Guy Laboratory at UCSF, and our laboratory have found that the use of several coupling reagents facilitates ring-closing reactions by providing a choice of reagents for the specific substrate. This is in lieu of optimizing each individual reaction for each individual coupling agent.
17. The four macrocyclic peptides in Figure 5 are listed as numerical structures, MS data, and yields as follows (note: MS data is given as major peaks with $+45[2\times Na^+ - 1]$, +23 $[Na^+]$, and +1 being those peaks):

    (a) 2-3-4a-5a-6-7 (MW = 914) MS: 960.40, 939.45, 936.50, 915.5. Yield: 16% Purity: ~80%
    (b) 2-3-4b-5a-6-7 (MW = 812) MS: 857.7, 835.8, 813.9. Yield: 35% Purity: ~85%
    (c) 2-3-4c-5a-6-7 (MW = 914) MS: 956.4, 933.2, 915.1. Yield: 20% Purity: ~75%
    (d) 2-3-4d-5b-6-7 (MW = 890) MS: 937.7, 913.6, 891.5. Yield: 17% Purity: ~75%.
18. The final macrocyclic class B derivative has the following data: (MW = 901) MS: 946.5. 924.5, 924.4. Yield: 60% for two steps Purity: ~80%.
19. All compounds were characterized using NMR and LCMS.