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Synthesis and Cytotoxicity of Novel Sansalvamide A Derivatives

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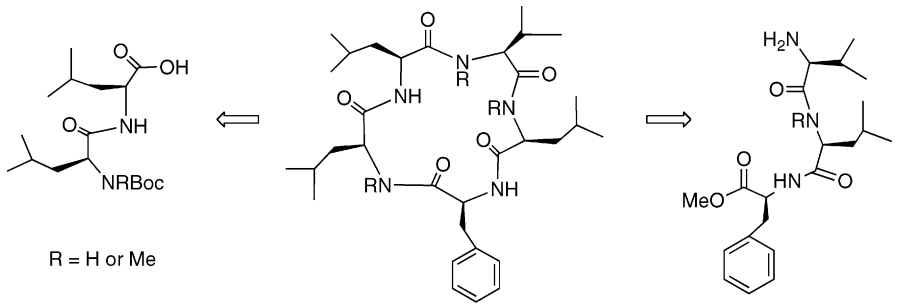
ABSTRACT



Described are the syntheses of 14 derivatives of the natural product Sansalvamide A, where two are more active against HCT 116 colon cancer cell lines than the natural product. These derivatives were synthesized using a combinatorial-type strategy that permits elucidation of the amino acid role in the cytotoxicity, and they lay the groundwork for development of new anticancer agents.

Sansalvamide A is a depsipeptide exhibiting antitumor activity.[1−3] It was discovered by Fenical and co-workers from a marine fungus of the genus *Fusarium*[3]. It has been characterized as a cytotoxin and antiviral agent. It demonstrates a mean IC_{50} of 27.4 μg/mL in the NCI's 60 cell line panel[3] and a potency of 3.5 μg/mL against colon cancer cell line COLO205. It is more potent than a drug currently on the market (mitomycin C), which exhibits an IC_{50} value of 5.3 μg/mL against this same cell line.[3] It is also known to inhibit topoisomerase activity or, more specifically, topoisomerase-catalyzed DNA relaxation.[2] Topoisomerase activity is important for biological processes such as DNA replication, repair, and transcription.

Sansalvamide A is composed of four hydrophobic amino acids and one hydrophobic hydroxy acid. An elegant solid-phase synthesis of Sansalvamide A[4] and the Sansalvamide A peptide[5] has been completed by Silverman et al, and the peptide demonstrates 10 times greater potency against the human colon carcinoma than the natural product depsipeptide (cell line HCT-116). However, little is known about the mechanism of action of Sansalvamide A or the structure−activity relationship (SAR) between active compounds and the biological target. It is important to establish the SAR and determine if further development of this class as antitumor and antiviral agents would be useful. Additionally, it is valuable to examine the biological activity of not only derivatives relating to the natural product with the L-amino acids but also the opposite enantiomers of these derivatives. As a result of our experience working in solution phase and the hydrophobicity of the residues, we developed a solution phase route for the synthesis of Sansalvamide A derivatives. Figure 1 depicts the two fragments involved in our synthesis route.

† Contributed equally to the work reported.

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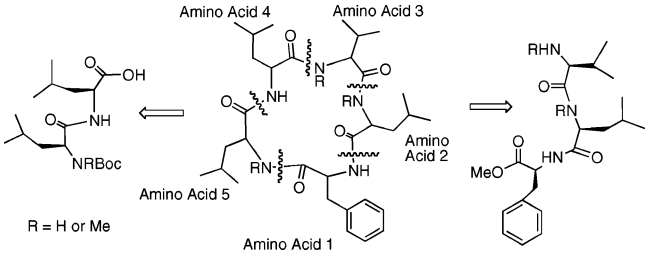


Figure 1. Retrosynthetic approach.

Herein we describe the synthesis of 14 novel Sansalvamide A derivatives using the amino acids shown (Figure 2). The significant aspects of our work include the consecutive

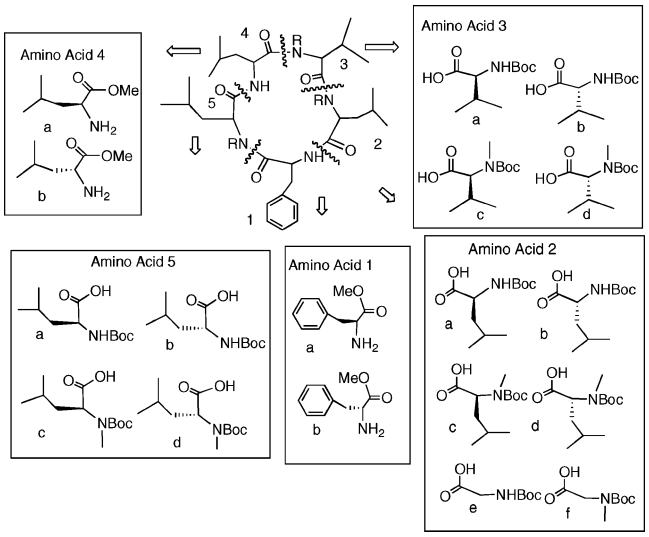
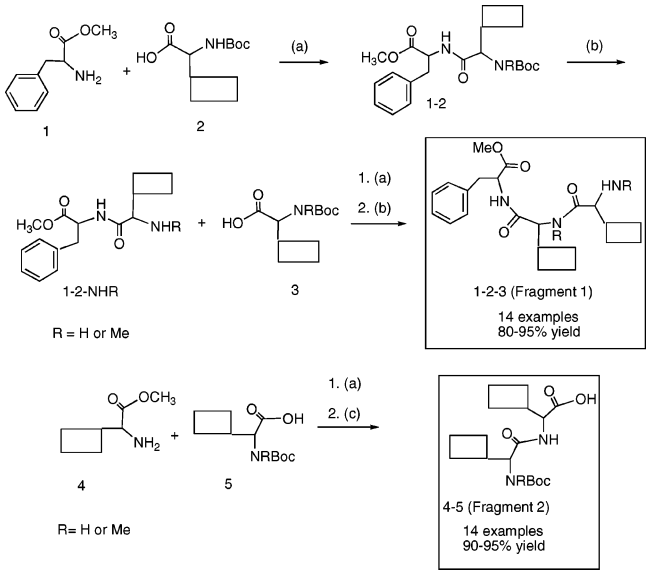


Figure 2. Monomers used in the synthesis of derivatives.

placement of *N*-methyl amino acids within the macrocycles and comparison of their cytotoxicity to those containing NH amino acids. This resulted in the discovery of two derivatives that exhibited cytotoxicity against colon cancer cell lines (HCT-116). In addition, we synthesized derivatives utilizing all L-amino acids and all D-amino acids. Furthermore, the synthesis of the peptide derivatives (as opposed to depsipeptide derivatives) provided macrocycles biologically more stable than those containing a labile lactone. The peptide derivatives demonstrate higher potency in the colon cancer assays (presumably due to their greater stability in cells).[5]

Our synthesis utilized a convergent approach (Figure 1). Using 2(1-*H*-benzotriazole-1-yl)-1,1,3-tetramethyl-uronium tetrafluoroborate (TBTU) as a coupling reagent and diisopropylethylamine (DIPEA), acid-protected residue **1a,b** and *N*-Boc-protected residue **2a**−**f** (Scheme 1) were coupled to give the dipeptide 1-2-Boc (80−94% yield). Deprotection of the amine on residue **2** using TFA gave the free amine **1-2** (∼quantitative yields). Coupling of this dipeptide to

Scheme 1. Synthesis of Sansalvamide A Fragments[a]



[a] Conditions: (a) coupling agent [TBTU and/or HATU (1.25 equiv ea)], DIPEA (3 equiv), CH_3CN; (b)TFA, anisole (2 equiv), CH_2Cl_2; (c)LiOH (4 equiv), MeOH.

monomer **3a**−**d** gave the desired tripeptide (Fragment 1) in good yields (80−95%).[6]

The synthesis of Fragment 2 was completed by coupling residue **4a,b** to residue **5a**−**d** to give the dipeptide 4-5-Boc (90−95% yield) (Scheme 1). The amine was deprotected in Fragment 1 using TFA, and the acid was deprotected in Fragment 2 using lithium hydroxide. Fragments 1 and 2 were coupled using multiple coupling agents,[7] yielding 14 examples of linear pentapeptides (50−74% yield) (Scheme 2).[6]

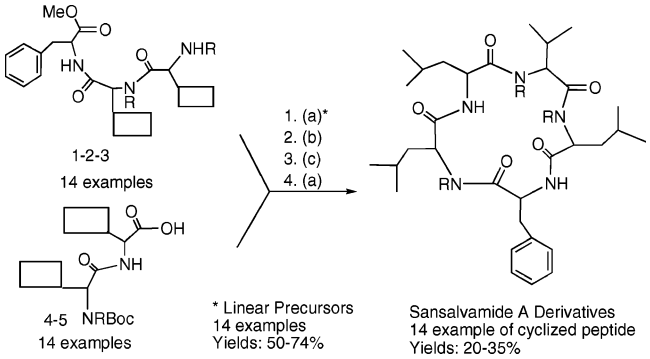
The linear pentapeptides were amine deprotected using HCl (pH < 2). Upon completion, the reaction was concentrated in vacuo, and the acid was deprotected by neutralizing the reaction with lithium hydroxide and then adding an additional 4 equiv of lithium hydroxide in methanol to give pH = ∼11.[8] Following acid deprotection, the reaction was concentrated in vacuo and subjected to HATU, TBTU, PyAOP, PyBrop, and/or DEPBT coupling reagents (∼0.5

(6) All dipeptide and tripeptide structures were confirmed using ^{1}H NMR. All linear hexameric peptides were confirmed using LCMS and ^{1}H NMR, and cyclized peptides were all confirmed using LCMS. (Note: ^{1}H NMR was also used for cyclized peptides, but because of their complexity, they were not seen as the primary confirmation for cyclized compounds).

(7) (a) 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. (b) Robinson, J. L.; Taylor, R. E.; Liotta, L. A.; Bolla, M. L.; Azevedo, E. V.; Medina, I.; McAlpine, S. R. *Tetrahedron Lett.* **2004**, *45*, 2147 (c) Liotta, L. A.; Medina, I.; Robinson, J. L.; Carroll, C. L.; Pan, P.-S.; Corral, R.; Johnston, J. V. C.; Cook, K. M.; Curtis, F. A.; Sharples, G. J.; McAlpine, S. R. *Tetrahedron Lett.* **2004**, *45*, 8447 (d) Ring-closing reactions are slow and typically low yielding. Unpublished results from the Guy lab at UCSF and recently our lab have found that the use of several coupling reagents facilitates ring-closing reactions by providing a choice of reagents for the specific substrate.

(8) All linear protected pentamers were amine deprotected and then confirmed via LCMS. Upon confirmation that the linear amine deprotections were completed, the acid deprotections were undertaken, and confirmation of the fully amine and acid deprotected pentamers were determined via LCMS.

Scheme 2. Cyclization of Sansalvamide Derivatives[a]



[a] Conditions: (a) coupling agent [for linear peptides, typically HATU and TBTU (~0.75 equiv ea); for cyclizations, HATU, DEPBT, PyAOP, PyBROP, and/or TBTU (~0.5 equiv ea)], DIPEA (3 equiv), CH_3CN; (b) HCl, anisole (2 equiv), CH_2Cl_2; (c) LiOH (4 equiv), MeOH.

equiv each) and DIPEA (~6 equiv until pH = 8).[7] The final macrocyclizations took approximately 4 days because of the low concentration (0.005−0.01 M) required to maximize the yield. The one-pot ring-closing yields varied from 20% to 35%. The final compounds were then purified using reverse phase HPLC and confirmed via LCMS.[9] The final isolated compounds are shown in Figure 3.

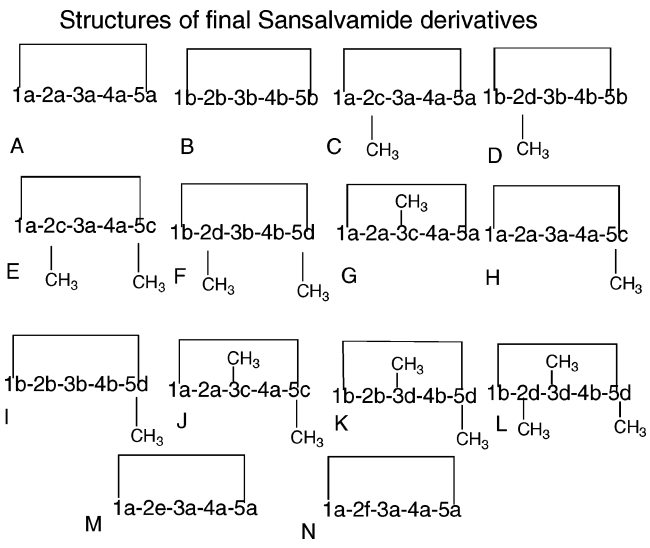


Figure 3. Structures of Sansalvamide derivatives

(9) The 14 macrocyclic peptides in Scheme 2 have LCMS data and yields given in Supporting Information. One representative example of a macrocyclic pentapeptide is as follows (note: MS data is given as major peaks with +23 [Na^+] and +1 being those peaks):

Macrocyclic Pentapeptide
1b-2b-3b-4b-5d (MW=599) MS: 599.9, 621/622
Yield: 35%

These 14 macrocyclic compounds were tested for their cytotoxicity against HCT 116 colon cancer cells.[10] Described in Table 1 are the compounds and their IC_{50} against this cell line.

Table 1. Cytotoxicity Assay Results

compound	structure	IC_{50}[a] (μg/mL)
San A		3.5
A	1a-2a-3a-4a-5a	1.5
B	1b-2b-3b-4b-5b	NSA
C	1a-2c-3a-4a-5a	NSA
D	1b-2d-3b-4b-5b	NSA
E	1a-2c-3a-4a-5c	NSA
F	1b-2d-3b-4b-5d	NSA
G	1a-2c-3c-4a-5a	2.6
H	1a-2a-3a-4a-5c	N/A
I	1b-2b-3b-4b-5d	N/A
J	1a-2e-3a-4b-5a	NSA
K	1a-2e-3a-4b-5a	N/A
L	1a-2e-3a-4b-5a	N/A
M	1a-2e-3a-4b-5a	N/A
N	1a-2e-3a-4b-5a	N/A

[a] NSA, ≥78 μg/mL; N/A, not available.

The two active compounds (Figure 4) include the nonmethylated peptide derivative of Sansalvamide A (A), and the

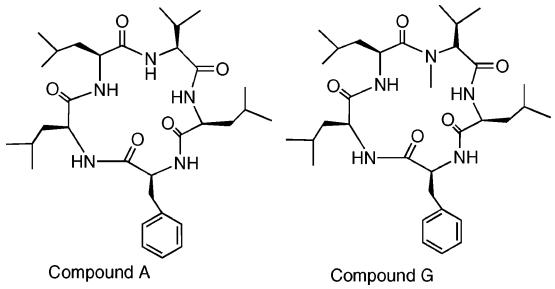


Figure 4. Structures of two active compounds.

mono-*N*-methylated derivative on the three residue (G). The error limits of the assay (0.1 μg/mL) are such that the mono-*N*-methylated derivative (G) is clearly more active than Sansalvamide A, and Sansalvamide A peptide (A) is twice as active as the natural product. The 3-*N*-methyl derivative (G) is a new compound, and interestingly enough, the 2-*N*-methyl depsipeptide derivative (i.e., the natural product termed *N*-methyl Sansalvamide) is not nearly as active as compound G. Thus, our result suggests an interesting structure−activity relationship (SAR) between the compound and its biological target. This novel derivative and others are being tested in additional assays, and new methylated derivatives are currently being synthesized in order to explore this SAR.

(10) The protocol for the HCT 116 colon cancer assay is described in the Supporting Information.

In summary, the synthesis of 14 Sansalvamide A derivatives have provided two compounds that exhibited potency greater than that of the natural product. Compounds A and G demonstrated improved cytotoxicity against HCT-116 colon adeno carcinoma cancer cell lines over that seen with Sansalvamide A. Compound G was a new structure, one that had not previously been synthesized and tested for antitumor activity. The two active derivatives are being assessed for incorporation of photoaffinity tags so that the cellular target can be isolated. Additional derivatives are currently being synthesized. These derivatives, along with those described in this paper, will be tested against other colon cancer cell lines, as well as against four other types of cancer cell lines. The results of these assays will be published in due course.

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Note Added after ASAP Publication. There was an error in the listing of contributing authors and a missing author name in the version published ASAP July 8, 2005; the corrected version was published ASAP July 15, 2005.

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

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