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Synthesis and Cytotoxicity of a New Class of Potent Decapeptide Macrocycles

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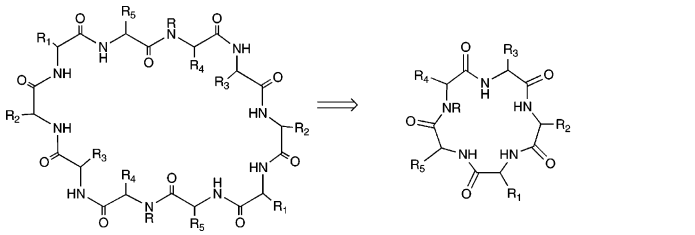
ABSTRACT



Decapeptide Derivatives **Sansalvamide A Derivatives**

Described are the syntheses of five decapeptides that are *C*-2-symmetrical derivatives of the natural product pentapeptide sansalvamide A. Derivatives were made using a succinct convergent synthesis. These analogues share no structural homology to current cancer drugs, are cytotoxic at levels on par with existing drugs treating cancers, and demonstrate selectivity for drug-resistant pancreatic cancer cell lines over noncancerous cell lines. These molecules are excellent chemotherapeutic leads in the search for new anticancer agents.

Pancreatic cancer is the fifth most deadly cancer in the U.S. Only 10% of patients are eligible for surgery,[1] and less than 20% of pancreatic cancers respond to the drug of choice, gemcitabine.[2] The 5-year survival rate for patients with pancreatic cancers is less than 5%.[2,3] With such a low response rate to current chemotherapeutic treatments, there is an immediate need for new drugs that provide options to pancreatic cancer patients. Recent work describing the synthesis and biological activity of pentapeptide derivatives based on the natural product sansalvamide A (San A) as potential therapies for pancreatic cancers has brought attention to this new compound class.[4] San A is a pentadepsipeptide that exhibits antitumor activity. It was discovered by Fenical and co-workers from a marine fungus of the genus *Fusarium*.[15] San A derivatives have shown potent

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cytotoxicity against pancreatic,[4b,5] colon,[4c,e,f,6] breast, prostate, and melanoma cancers,[4d] clearly indicating the potential of this compound class as a platform useful in targeting multiple cancers.

Synthesis of macrocyclic decapeptides is difficult due to cyclizations that typically generate compounds in low yields. There have been a number of recent examples in the synthesis of large macrocyclic peptides that have utilized either solution-phase, solid-phase, chemoenzymatic, or template-directed synthesis.[7] Further, there are a significant number of large macrocycles, ranging from 4 to 12 amino acids that have been successfully used as antitumor, antibiotic, and immunosupression agents.[7,8] In this paper, we describe a succinct and convergent approach of the synthesis of five San A-based decapeptides. One of these five compounds displays extraordinary potency against pancreatic cancers and has sub-nanomolar IC_{50} values for two pancreatic cancer cell lines. In addition, this compound demonstrates a 33-fold differential selectivity for cancer cells over normal cells and is 43-fold more potent against pancreatic cancer cell lines than the current drug of choice, gemcitabine (Gem). Thus, this is the first of its structural class to be synthesized, and this new class demonstrates extraordinary potency against drug-resistant pancreatic cancer cell lines. Indeed, it shows greater than 1000-fold more potency than its structural "monomer": macrocyclic pentapeptide San A. Further, these decapeptides molecules share no homology to known pancreatic cancer drugs.

Our succinct and convergent synthesis utilizes the amino acids shown **(**Figure 1) and the synthetic strategy described in Scheme 1. Our solution-phase approach, involving a single linear pentapeptide, is amenable to inserting L- and D-amino acids systematically within the di-San A derivative. This route was also designed to facilitate large-scale synthesis for extensive biological studies. Syntheses of five di-San A derivatives were completed using amino acids shown (Figure 1) via the synthetic route outlined (Scheme 1). Using 2(*1*H-benzotriazole-1-yl)-1,1,3-tetramethyluronium tetrafluoroborate (TBTU) and diisopropylethylamine (DIPEA), acid-protected residue 1(a−b) and N-Boc protected residue 2(a−

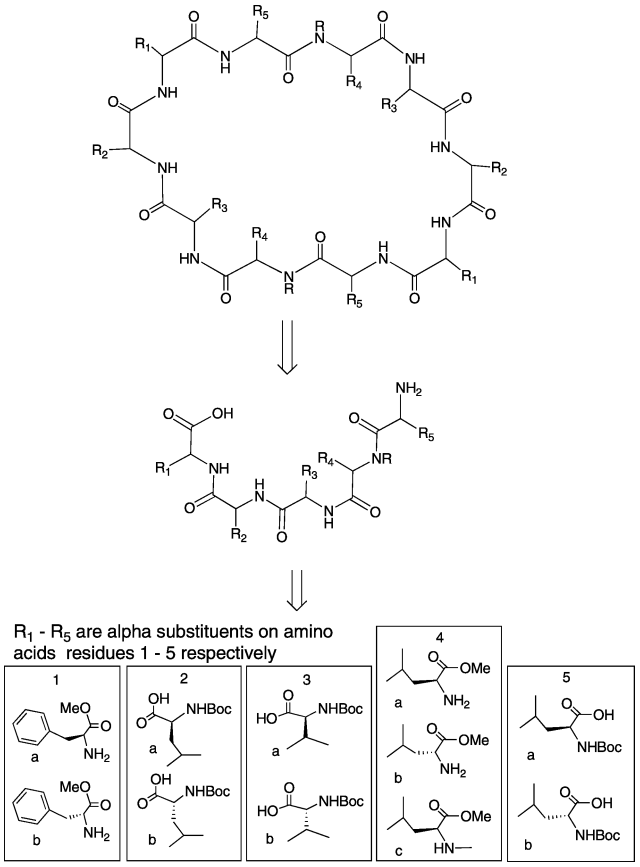
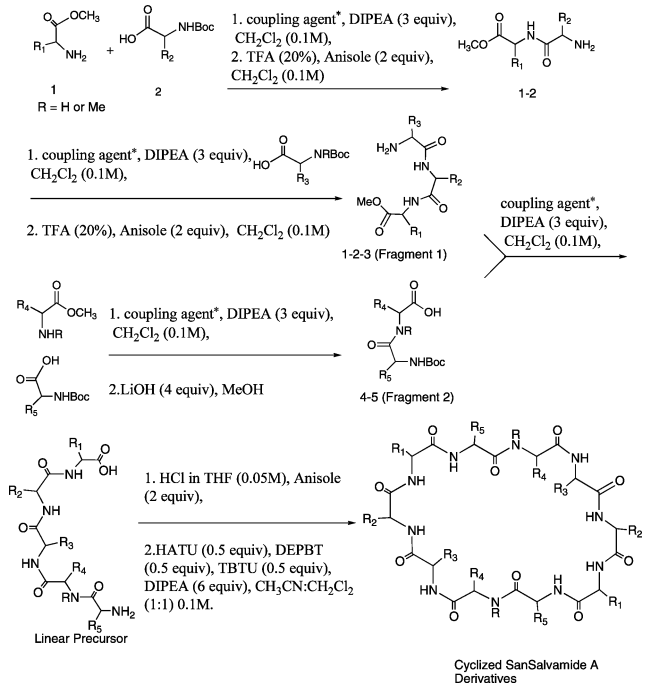


Figure 1. Retrosynthetic approach for di-SanA.

Scheme 1. Synthesis of Decapeptides Derivatives



*TBTU (1.2 equiv) and/or HATU (0.75 equiv).[19]

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b) (Figure 1) were coupled to give the dipeptides 1−2-Boc (90−95% yield). Deprotection of the amine on residue 2 using TFA gave the free amines 1−2 (∼quantitative yields).

Coupling of this dipeptide to monomer 3(a−b) gave the desired tripeptides (fragment 1) in good yields (80%-95%).[9] The synthesis of fragment 2 was completed by coupling residue 4(a−c) to residue 5 (a−b) to give the dipeptide 4−5-Boc (90−95% yield). The amine was deprotected on 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,[4c,e,g,10] yielding 31 examples of linear pentapeptides (70−90% yield).

Cyclizing large macrocycles is usually very challenging, and typically, the yields are low. The recent discovery of high-yielding conditions[11] provided the final decapeptide macrocycles in good yields. Dissolving the linear pentapeptide in THF (0.05 M), addition of 2 equiv of anisole, and approximately 8 drops of concentrated HCl per 0.3 mmol of linear pentapeptide led to partially deprotected amine within 24 h. Four drops of HCl per 0.3 mmol of peptide were added. The reaction was allowed to stir at room temperature and then checked after 24 h by LCMS. Typically, deprotection of the acid and amine were complete within 2 days.[12] Upon completion, the reaction is concentrated in vacuo and dried on the high vac. The dried, crude, free-amine free-acid linear pentapeptide was dissolved in a 1:1 ratio of CH_3CN/CH_2Cl_2 (0.1 M). Addition of DIPEA (6 equiv) and three coupling agents (HATU, DEPBT, and TBTU 0.5 equiv each) to reaction gave a clear solution. Reactions were usually complete in 2−4 h.[13] Workup with methylene chloride and ammonium chloride, concentration in vacuo, and purification via flash chromotagraphy and subsequently LCMS provided the final products (yields ranged from 30% to 90% depending on the substrate) (Figure 2).

Testing the chemotherapeutic activity of these derivatives against two pancreatic cancer cell lines, PL-45 and BxPC3, showed that compound **5** is extremely cytotoxic against both pancreatic cancer cell lines (Figure 3). We have also determined the IC_{50} values for compound **5** and found that they are sub-nanomolar for both cancer cell lines (Figure 4)! Further, **5** is 33-fold less potent against normal skin fibroblasts than against cancer cell lines, thus demonstrating differential selectivity. In addition, **5** displayed up to 43-fold improved cytotoxicity against PL-45 than the drug of choice, gemcitabine. Finally, compound **5** demonstrates potency against drug-resistant colon cancer cell lines (HCT-116), exhibiting an IC_{50} of 14-fold differential selectivity for colon cancer cells over normal cells and 400-fold greater potency against HCT-116 than gemcitabine. This suggests that **5** is acting via a mechanism of action that involves a target common to drug-resistant cell lines from multiple tumorgenic tissues.

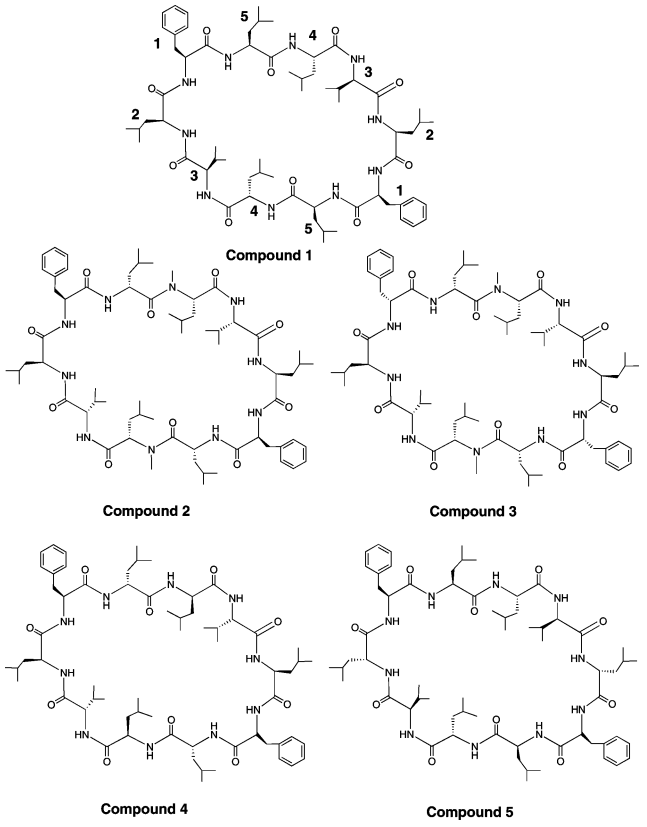

Figure 2. Structures of five decapeptide derivatives.

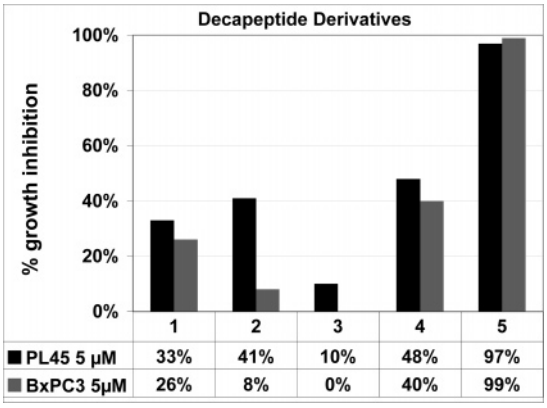

Figure 3. Cytotoxicity assays of five decapaptides. Each data point is an average of four wells from three assays at 5 μM. Error = ±5%; DMSO was used as a control (= 100% growth).

(9) Dipeptide and tripeptide structures were confirmed using ^{1}H NMR. All linear pentapeptides were confirmed using LCMS and ^{1}H NMR. (Note: ^{1}H NMR were taken for cyclized peptides, but due to their complexity, they were not seen as the primary confirmation for cyclized compounds). See the Supporting Information for spectra.

(10) Unpublished results from the Guy laboratory at the Department of Chemical Biology and Therapeutics, St Jude Children's Research Hospital, Memphis, TN 38103, and published results from our laboratory show that the use of several coupling reagents facilitates formation of the peptide bond in high yields.

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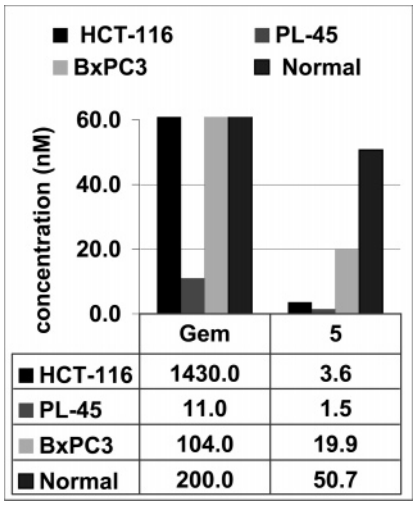
(12) For details on the reaction conditions, see the Supporting Information.

(13) It was straightforward to follow the reactions via LCMS as the starting material double-deprotected linear precursor would appear at 5.0−5.5 min and the cyclized product would appear between 6.1 and 7.0 min.



	Gem	5
HCT-116	1430.0	3.6
PL-45	11.0	1.5
BxPC3	104.0	19.9
Normal	200.0	50.7

Figure 4. IC_{50} assay of most potent decapeptide run in **HCT-116** (colon cancer cell lines), **PL-45**, **BxPC3**, and **WS-1** (WS-1 = normal cells, skin fibroblasts). Data represents results from a concentration curve taken from four concentrations, where each concentration data point is from four separate experiments performed in quadruplicate. Margin of error = ±5%. Concentrations are nM.

The structural differences between compounds **1**−**4** and compound **5** are subtle. Compound **5** has four D-amino acids in positions 2 and 3, respectively. By comparison, compound **1** has two D-amino acids in position 3, compound **2** also has two D-amino acids in position 5, compound **3** has four D-amino acids at positions 1 and 5, and compound **4** has four D-amino acids at positions 4 and 5. Based on the fact that compound **5** is so potent (i.e., greater than 1000-fold more cytotoxic than the other four compounds), while the other compounds exhibit modest cytotoxicity, we believe that **5** is reaching a key biological target inside the cell or on the cell surface. Presumably, the specific conformation of **5** plays a significant role in binding to this biological target, which is why only **5** is active, while the other decapeptides derivatives show very limited potency.

In summary, we have outlined the synthesis of five compounds that are related to a potent class of cytotoxic agents, sansalvamide A. One compound, **5**, demonstrated extraordinary potency against the drug-resistant pancreatic cancer cell lines PL-45 and BxPC3. Future derivatives that incorporate additional D-amino acids at positions 2 and 3 will be synthesized and tested. Their potency will be reported in due course.

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Supporting Information Available: General experimental procedures, cytotoxicity assay protocol, and 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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