N-Methylsansalvamide A Peptide Analogues. Potent New Antitumor Agents

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Sansalvamide A, a cyclic depsipeptide isolated from a marine fungus of the genus *Fusarium*, is composed of four hydrophobic amino acids (Phe, two Leu, Val) and one hydroxy acid ((*S*)-2-hydroxy-4-methylpentanoic acid; *O*-Leu) with five stereogenic centers all having *S*-stereochemistry. We have recently synthesized the corresponding cyclic peptide (Gu, W.; Liu, S.; Silverman, R. B. *Organic Lett.* **2002**, *4*, 4171−4174) and found that it too has antitumor activity. *N*-Methylation can enhance potency and selectivity for peptides. Consequently, here we synthesize 12 different *N*-methylated sansalvamide A peptide analogues and show that for several different tumor cell lines three of these analogues are more potent than the natural product; in pancreatic cells, sansalvamide A shows little activity, but the *N*-methylsansalvamide peptides are potent cytotoxic agents.

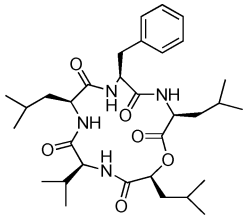
Introduction

In 2003, 221 000 men in the United States were diagnosed with prostate cancer and over 29 000 died of this disease.[1] Prostate cancer remains the most prevalent form of malignancy and is now the fourth most frequent cause of cancer-related death in men. However, to date, there is no effective cure for advanced and aggressive forms of prostate cancer. Breast cancer is the second most common cancer-related cause of death in women with about 40 000 women succumbing last year out of the 213 000 new cases.[2] Although breast cancer mortality has declined over the past decade, breast cancer continues to represent a major threat to the lives and productivity of women. Last year there were 30 700 new cases of pancreatic cancer in the United States and 30 000 deaths; 54 200 new cases of melanoma were detected last year with 7600 deaths. Patients diagnosed with pancreatic cancer have little hope for cure, because no effective therapies are currently available. Most patients with pancreatic cancer die within six months of diagnosis, and its mortality and incidence are almost equal.

New antitumor agents are needed, and the best source of diverse compounds with antitumor activity is Nature.[3] However, Nature supplies a limited number of compounds, so it is imperative to modify compounds with known antitumor activity to increase potency and minimize toxicity. Despite the many known bioactive cyclic peptides and cyclic depsipeptides derived from natural sources,[4] the usual toxicological, pharmacological, or pharmokinetic problems prevent them from proceeding further into development. However, these bioactive compounds constitute a wide array of lead compounds ripe for lead modification and improved therapeutic indices by incorporation of nonnatural amino acids.

Cyclic peptides comprise a potent class of naturally occurring bioactive molecules. Cyclic peptides have several advantages as drug candidates. These compounds do not have charges at the peptide amino and carboxyl termini and lack zwitterionic character; therefore, they are more lipophilic and membrane permeable. Oral bioavailability is increased by faster membrane absorption in the digestive tract,[5] and cyclic peptides have much greater half-lives in vivo than linear peptides.[6] Because of the cyclic nature of these compounds, bond rotation is restricted, creating more rigid three-dimensional structures. This conformational constraint can result in greater binding affinity and selectivity for protein ligands. Even slight changes in the ring structure of molecules such as cyclosporin and didemnin B greatly affect their biological activities, which emphasize their specificity of binding.[7]

Sansalvamide A (**1**) is a cyclic depsipeptide produced by a marine fungus of the genus *Fusarium* found on Little San Salvador Island, Bahamas.[8] This natural product is a highly lipophilic compound that was found to have significant cancer cell cytotoxicity with a mean IC_{50} value of 27.4 μg/mL against the National Cancer Institute's 60-cell-line panel. A study of the mechanism of action of this natural product in the poxvirus molluscum contagiosum virus (MCV)[9] showed that it is an inhibitor of topoisomerase I. We have synthesized the corresponding cyclic peptide of sansalvamide A (**2**) and found that it also has antitumor activity.[10]



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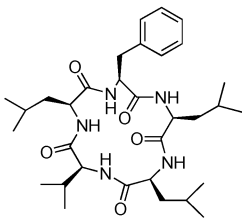

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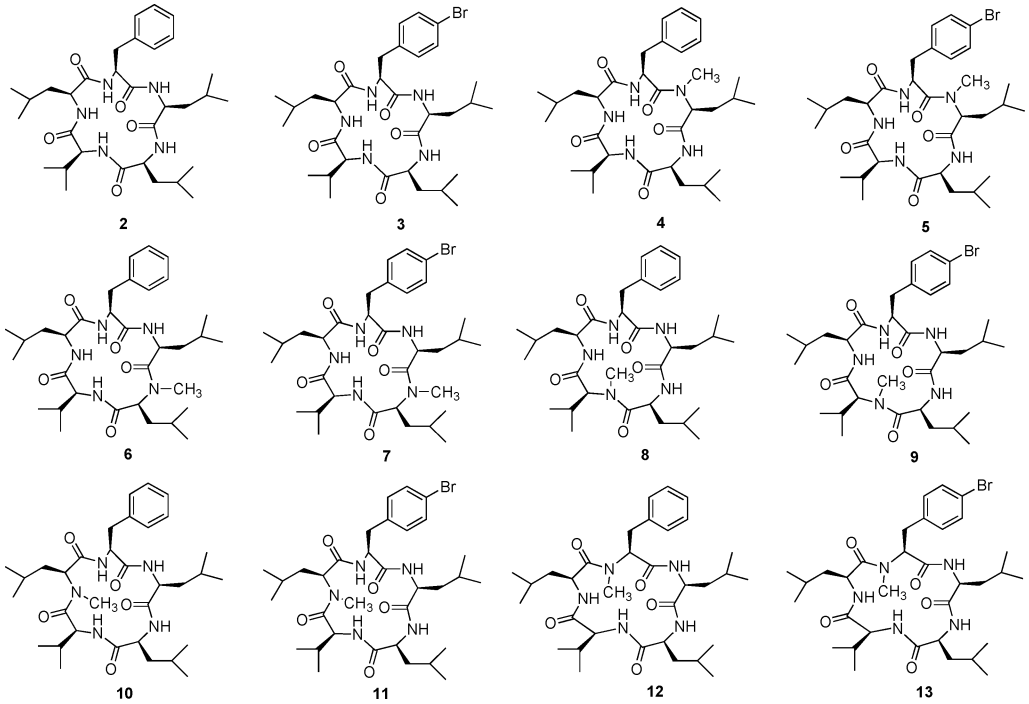


Figure 1. Structures of sansalvamide A (**1**) and sansalvamide A cyclic peptide analogues synthesized and tested.

We wanted to see how effective modifications of sansalvamide A peptide would be in enhancing its antitumor activity. Many natural product cyclic peptides and depsipeptides with biological activity contain at least one *N*-methylated amino acid.[11] *N*-Methylation has often been used to increase the potency or selectivity of a peptide.[12] This modification restricts the affected residue and the amino acid preceding it to an extended conformation,[13] and it blocks potential intramolecular hydrogen-bonding sites and proteolytic enzyme cleavage sites, thus potentially enhancing the pharmacokinetic properties of a peptide. Consequently, we synthesized a library of *N*-methylsansalvamide A peptide analogues to determine the effect on activity of both *N*-methylation as well as para-bromination of the phenylalanine residue in sansalvamide A peptide (Figure 1). Here we show that several of these nonnatural analogues are more potent than the natural product sansalvamide A in certain cell lines.

Chemistry

The general synthetic approach for the resin-bound *N*-methylated sansalvamide A peptides is similar to that used for the parent cyclic peptide[10] and is shown for one example in Scheme 1.

The resin-bound cyclic peptide was cleaved with neat TFA to give the free cyclic *N*-methylpeptide or with bromine to give the corresponding *p*-bromophenylalanine-containing cyclic *N*-methylpeptide (Scheme 2).

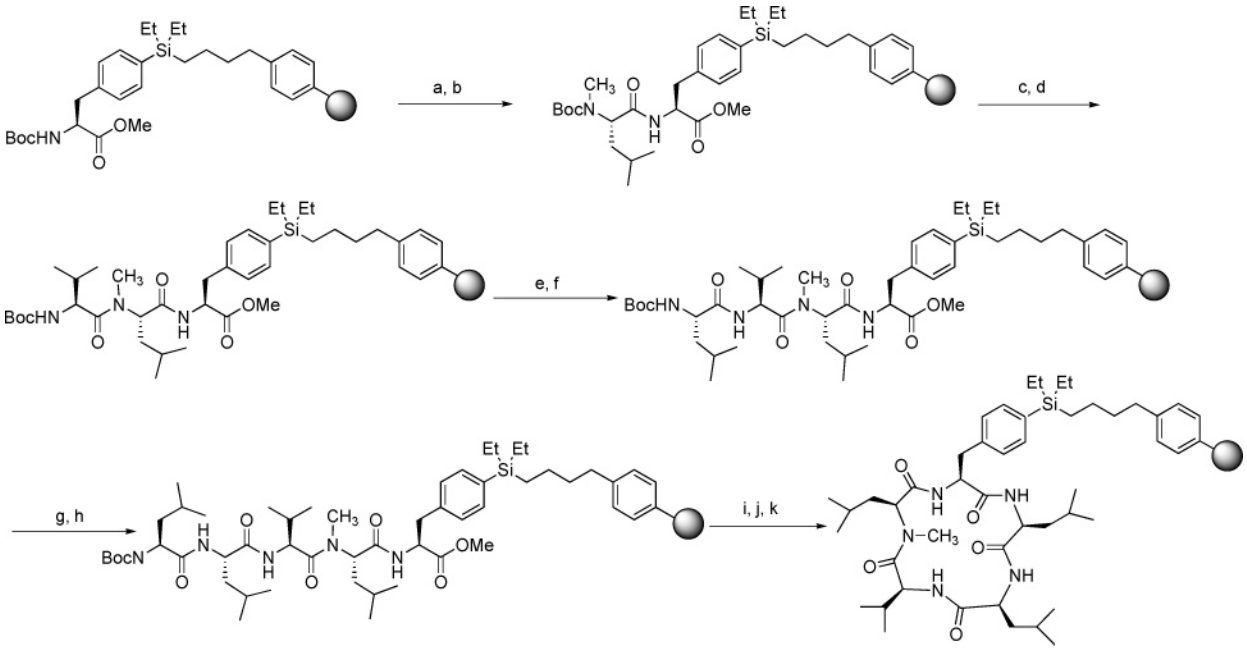
Incorporation of the *N*-methyl group into each cyclic peptide (except **12** and **13**) was accomplished by initial synthesis of the corresponding *N*-methyl amino acid. Typically the synthesis of *N*-methyl amino acids uses methyl iodide as the methylating agent.[14] Trifluoroacetylation activates the amino group for deprotonation with mild bases and allows selective methylation to occur.[15] For analogues **12** and **13**, *N*-methylated polymer-bound phenylalanine (**19**) was synthesized from *p*-iodophenylalanine as shown in Scheme 3.

Results and Discussion

Activity against PC-3 Human Prostate Cancer, MDA-MB231 Human Breast Cancer, WM-115 Human Melanoma Cells, and S2-013 and AsPC-1 Human Pancreatic Cancer Cells. Sansalvamide A (**1**) and all of the *N*-methylsansalvamide A peptide analogues (**2**−**13**) were screened for their activity against five different human cancer cell lines: prostate, breast, melanoma (all Figure 2), and two pancreatic cancers (Figure 3). The compounds that generally demonstrated good activity (relative to sansalvamide A) were **5**, **11**, and **13** (**13** not so enhanced with PC-3 or MDA-MB231 cells; **7** had high activity with MDA-MB231 cells). At 10 μM concentration, compounds **5** and **11** were as potent or more potent than sansalvamide A against both PC-3 (human prostate) and MDA-MB231 (human breast) cancer cells (Figure 2). Compounds **8**−**10** exhibited moderate activity against MDA-MB231 cells. Although compounds **11** and **13** showed very good activity against WM-115 human melanoma cells at 10 μM concentration, they were not as potent as sansalvamide A (**5** also showed moderate activity). Whereas **11** was more potent than sansalvamide A at 10 μM concentration against AsPC-1 human pancreatic cancer (poorly differentiated cells), **2**, **5**, and **13** were about comparable to the activity of sansalvamide A (Figure 3A). With the well-differentiated S2-013 human pancreatic cancer cell line, **5**, **11**, and **13** were all much more potent than sansalvamide A, and **2** was somewhat more potent than sansalvamide A (Figure 3B). It is apparent that for PC-3, MDA-MB231, and WM-115 cells, the cyclic depsipeptide sansalvamide A is much more favorable than the corresponding cyclic peptide **2**, but this change makes little difference for both of the human pancreatic cancer cell lines.

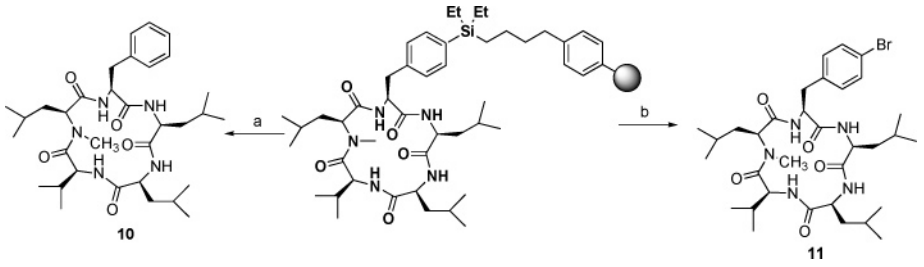
The effect of *N*-methylation depends on the cell line used. Compounds **2** and **3** are cyclic peptides that do not contain the methyl group. With PC-3 cells (Figure

Scheme 1. Solid-Phase Synthesis of the Cyclic Peptides[a]



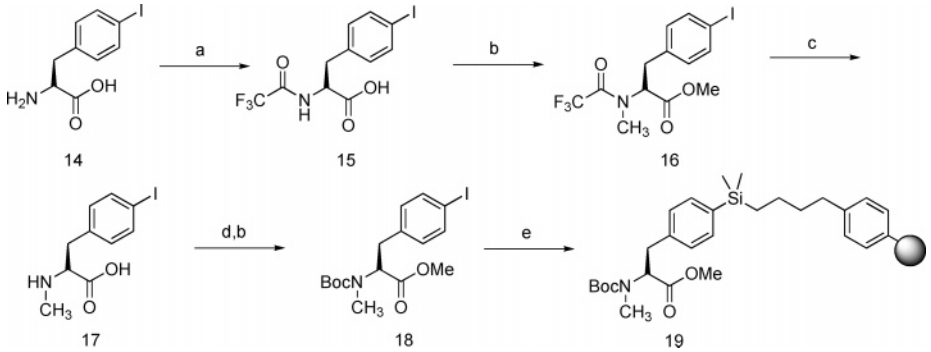
[a] Reagents and conditions: (a) 50% TFA in CH_2Cl_2, rt, 15 min; (b) Boc-MeLeu-OH (5 equiv), HATU (5 equiv), DIPEA (15 equiv), NMP, 6 h; (c) 50% TFA in CH_2Cl_2, rt, 15 min; (d) Boc-Val-OH (5 equiv), HATU (5 equiv), DIPEA (15 equiv), NMP, 6 h; (e) 50% TFA in CH_2Cl_2, rt, 15 min; (f) Boc-Leu-OH (5 equiv), HATU (5 equiv), DIPEA (15 equiv), NMP, 6 h; (g) 50% TFA in CH_2Cl_2, rt, 15 min; (h) Boc-Leu-OH (5 equiv), HATU (5 equiv), DIPEA (15 equiv), NMP, 6 h; (i) LiOH (5 equiv), THF/H_2O (7:1); (j) 50% TFA in CH_2Cl_2, rt, 15 min; (k) PyBOP (5 equiv), DIPEA (15 equiv), NMP, 24 h.

Scheme 2. Cleavage of the Cyclic Peptides from the Solid Support[a]



[a] Reagents and conditions: (a) neat TFA, 24 h; (b) Br_2, CH_2Cl_2, 15 min.

Scheme 3. *N*-Methylation of Phenylalanine Analogues



Reagents and conditions: (a) $(F_3CCO)_2O$, TEA, acetone, rt; (b) MeI, K_2CO_3, acetone, room temperature, (a and b, 87%); (c) K_2CO_3, acetone/H_2O, 45 °C, 93%; (d) Boc_2O, NaOH, then step b, 89%; (e) ref 10.

2A), **2** has the lowest activity of all of the compounds tested (although most of the compounds are not very active), and **3** is only more potent than **2**, **4**, **7**, and **12**. In the case of MDA-MB231 cells (Figure 2B), the effect of *N*-methylation is more striking; only **4**, **6**, **7**, and **12** are less potent than **2**, and **2**, **4**, **6**, **7**, and **12** are less potent than **3**. Compound **2** is about average in its potency against WM-115 cells (Figure 2C), but only **4** and **8** are less potent than **3**. This is in sharp contrast with the effect of the nonmethylated analogue **2** in AsPC-1 human pancreatic cancer cells (Figure 3A), where **2** is comparable to some of the most potent compounds, namely, **5**, **13**, and sansalvamide A (**11** is a little more potent than **2**).

In general, bromination appears to be favorable; the combination of *N*-methylation and bromination is especially beneficial. Except for **6** > **7** with PC-3 cells (Figure 2A), **2** > **3** with WM-115 cells (Figure 2C), **2** >

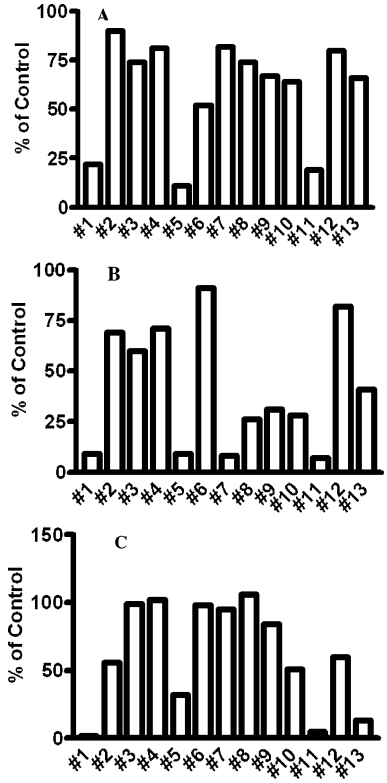


Figure 2. Effect of compounds on viable (A) PC-3, (B) MDA-MB231, and (C) WM-115 cells. Five thousand cells were plated per well in replicate wells of a 24-well plate. All cells were grown in DMEM (Invitrogen, Carlsbad, CA) supplemented with 10% heat-inactivated calf serum (US Bio-Technologies, Parkerford, PA) and 100 μg/mL Pen-Strep (Invitrogen) and amphotericin B (Biologos, Montgomery, IL). Cells were maintained at 37 °C in humidified 5% CO_2. The compounds were dissolved in DMSO and diluted to a final concentration of 10 μM in DMEM with supplements as specified. All cells were cultured for 72 h, and cell numbers were counted. All test conditions were assayed in duplicate wells, and the results of the two wells were averaged. The cell number was expressed as a percentage of the untreated control cells.

3 with AsPC-1 cells (Figure 3A), and **2** > **3**, **6** > **7**, and **8** > **9** with S2-013 cells (Figure 3B), the brominated analogues were more potent than the corresponding nonbrominated compounds. In fact, some of the brominated analogues were *much* more potent than their nonbrominated counterparts: with PC-3 cells (Figure 2A) **5** ≫ **4** and **11** ≫ **10**; with MDA-MB231 cells (Figure 2B) **5** ≫ **4**; with AsPC-1 cells (Figure 3A) **11** ≫ **10**; and **5** ≫ **4**, **11** ≫ **10**, and **13** ≫ **12** for both WM-115 cells (Figure 2C) and S2-013 cells (Figure 3B).

Concentration Dependence of the Antitumor Activity for Selected Compounds. All of the compounds tested showed concentration dependence. As a representative example, compound **5** was a concentration-dependent inhibitor of PC-3 cell growth from 1 to 10 μM concentration after a 24 h exposure (Figure 4). Compound **13** was found to show concentration dependence on the inhibition of growth of AsPC-1 cells from 0.1 to 100 μM concentration (Figure 5). Similar results were obtained with **5** and **11** (data not shown). Compounds **5**, **11**, and **13** also showed a concentration dependence with S2-013 cells (data not shown).

Time-Dependent Antitumor Activity of Selected Compounds. Compound **11** exhibited time-dependent inhibition of PC-3 cell growth from 12 to 72 h exposure

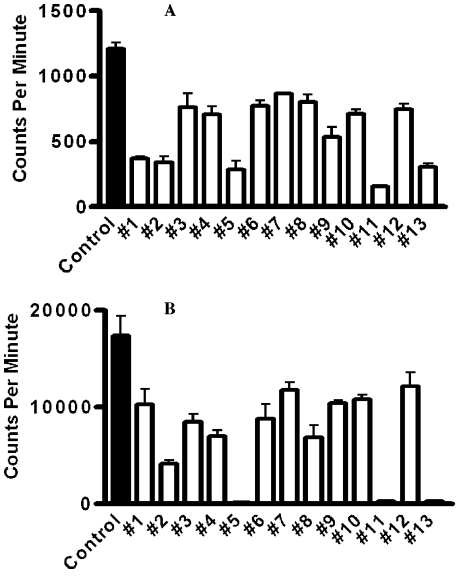


Figure 3. Effect of compounds on viable (A) AsPC-1 and (B) S2-013 cells. Cells were plated in 24-well plates at a concentration of 50 000 cells/well. After reaching 50% confluence, they were incubated in serum-free medium for 24 h, which was then replaced with fresh serum-free medium with or without treatment with 10 μM of each cyclic peptide. After the required period of culture, cellular DNA synthesis was assayed by adding 0.5 Ci [*methyl*-^{3}H]thymidine per well and incubating cells for another 6 h. The cells were then washed twice with PBS, fixed with 10% trichloroacetic acid, and solubilized by adding 250 μL of 0.4 M NaOH to each well. Radioactivity, indicating incorporation of [*methyl*-^{3}H]thymidine into DNA, was measured by adding scintillation cocktail and counting with a scintillation counter.

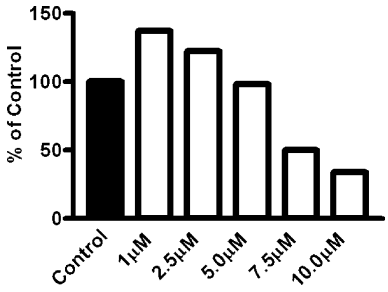


Figure 4. Dose-dependent activity of compound **5** on viable PC-3 cells. Compound **5** was added to cells in a range of concentrations, from 1 to 10 μM. The EC_{50} was approximately 7.5 μM for compound **5**. Each data point is a mean of duplicate wells. All results are expressed as cell number in test conditions as a percentage of untreated controls.

with half-maximal cell growth inhibition at about 20 h; half-maximal inhibition of cell proliferation with WM-115 cells was about 8 h (Figure 6). It was revealed that even a short exposure (12−24 h) resulted in a greater than 50% reduction in cell number. Thus the compounds do not need to remain present for the antiproliferative effect to be evident. Compounds **11** and **13** exhibited time-dependent inhibition of both AsPC-1 (Figure 7A,B, respectively) and S2-013 (Figure 7C,D, respectively) cells from 24 to 72 h. Using 10 μM of cyclic peptides, a decrease in cell number was seen in cells treated with peptide, while those treated with vehicle (DMSO) alone continued to grow. Each peptide analyzed caused a significant ($p < 0.05$) decrease in the number of cells at 72 h. Treatment of AsPC-1 and S2-013 cells with the cyclic peptides resulted in cell death through apoptosis,

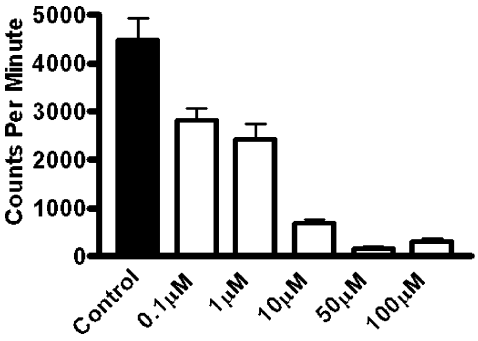


Figure 5. Dose-dependent activity of compound **13** on viable AsPC-1 cells. Compound **13** was added to cells in a range of concentrations, from 0.1 to 100 μM. See Figure 3 legend for procedure.

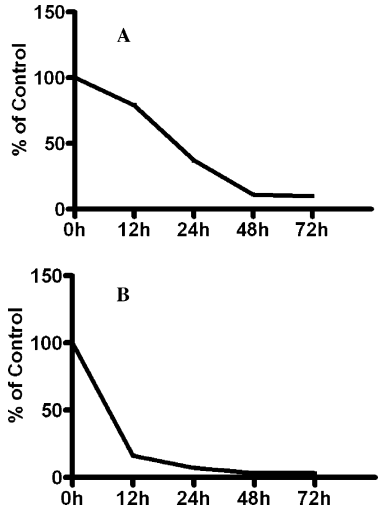


Figure 6. Inhibitory activity does not require continuous exposure. To determine if a continuous exposure of the cells to the test compounds was required for suppression of proliferation, compound **11** was added to duplicate wells of (A) PC-3 or (B) WM 115 cells. At various time points from 12 to 72 h the test agent was removed and the cells were washed and incubated with standard DMEM without **11**. Cells were maintained and counted at 72 h. Untreated control cells (with diluted DMSO alone) were maintained in parallel as control. Each data point is a mean of duplicate wells. All results are expressed as viable cell number in test conditions as a percentage of untreated controls.

as indicated by the rounding up of the cells that were detaching from the plates. Even though the total cell number decreased dramatically with treatment, the percentage of cells that remained viable was unchanged, indicating that the compounds are not causing cytotoxicity.

Summary and Conclusions

Cyclic peptide analogues (**2**−**13**) of the natural product cyclic depsipeptide sansalvamide A (**1**) were synthesized and tested for their antitumor activity against PC-3 human prostate cancer cells, MDA-MB231 human breast cancer cells, WM-115 human melanoma cells, and AsPC-1 and S2-013 human pancreatic cancer cells. For the prostate, breast, and melanoma cell lines, the cyclic depsipeptide sansalvamide A was more potent than the corresponding cyclic peptide (**2**), but for the pancreatic cell lines the cyclic peptide was comparable to (AsPC-1 cells) or more potent than (S2-013 cells) sansalvamide A. The effects of *N*-methylation depend

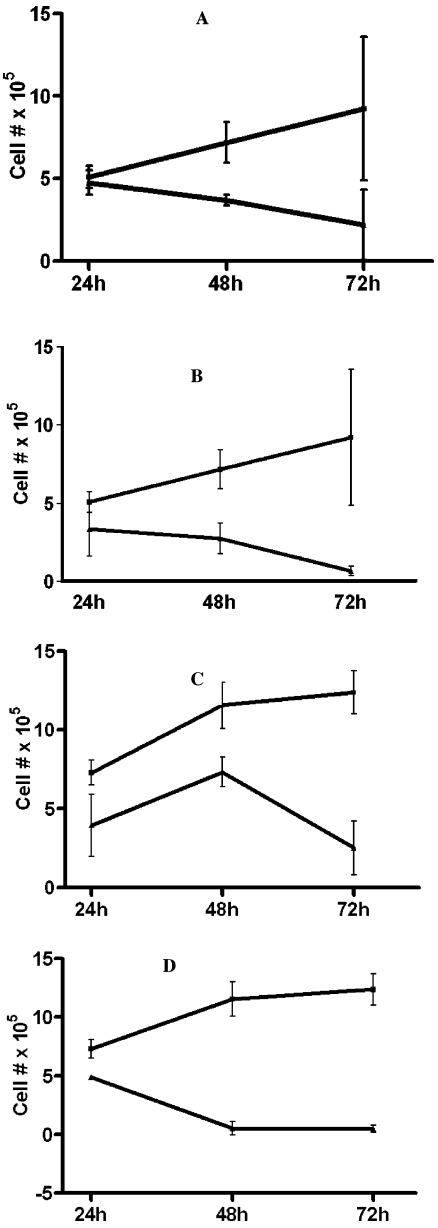


Figure 7. Time-dependent effect of (A) compound **11** and (B) compound **13** on AsPC1 cell growth and (C) compound **11** and (D) compound **13** on S2-013 cell growth. The upper lines in each graph represent the control data with no compound added, and the bottom lines are the results with compound added. Cells were regularly seeded into three 6-well plates and incubated at 37 °C for 24 h. Cells were then cultured in serum-free medium for another 24 h and treated in fresh serum-free medium with or without cyclic peptides (10 μM) for 24, 48, and 72 h. At the end of each time period the cells were trypsinized to produce a single cell suspension, and the cell number in each well was determined using the Guava Technologies ViaCount Assay (Guava Technologies Inc, Hayward, CA).

on the cell line used; the greatest effect was with MDA-MB231 cells. The increase in potency by *N*-methylation generally did not enhance the potency above that for sansalvamide A, except for S2-013 cells, where several of the compounds were considerably more potent than sansalvamide A. Generally, para-bromination of the phenylalanine of the cyclic peptide was favorable relative to the nonbrominated cyclic peptide. The combination of *N*-methylation and para-bromination produced analogues **5** and **11**, which are more potent than

sansalvamide A against PC-3 and MDA-MB231 cells and produced **5**, **11**, and **13**, which are more potent than sansalvamide A against both pancreatic cell lines tested (considerably more potent for the S2-013 cell line).

It is likely that *N*-methylation and para-bromination of the natural product sansalvamide A could lead to even more potent analogues than the modification of sansalvamide A peptide as was reported here. Given the differences in potencies of the various analogues relative to sansalvamide A, it appears that different targets may be involved in their activities; the known inhibition of topoisomerase I in the poxvirus molluscum contagiosum virus (MCV) by sansalvamide A[9] may not be relevant to the activity of these compounds in cancer cells.

Experimental Section

General Methods. Conventional organic solvents were purchased from Fisher. All of the reagents were purchased from Aldrich Chemical Co and were used without further purification unless stated otherwise. Butyl diethylsilane polystyrene (PE-DES−SiH, 1.45 mmol/g) was purchased from Argonaut Technologies Inc. Methylene chloride was distilled under N_2 from calcium hydride. Flash chromatography was performed with Merck silica gel (230−400 mesh). TLC plates (silica gel 60-F254) were purchased from VWR Scientific. All ^{1}H NMR spectra were recorded on Varian Gemini 300 MHz, Mercury 400 MHz, or Inova 500 MHz spectrometers (75, 100, or 125 MHz for ^{13}C NMR spectra). Chemical shifts (δ) are reported downfield from tetramethylsilane (Me_4Si) in parts per million (ppm). Compounds were visualized with a ninhydrin spray reagent or a UV/vis lamp. Mass spectra were recorded either on a VG Instrument VG70-250SE high-resolution mass spectrometer (ESI) or on a Micromass Quattro II spectrometer (APCI).

General Solid-Phase Synthesis of Sansalvamide A Peptide and Its *N*-Methyl Peptides. A suspension of resin **19** (2 g, 0.09 mmol/g) was treated with 50% TFA in CH_2Cl_2 (20 mL) for 15 min and then washed with CH_2Cl_2, 0.1 N HCl/THF, MeOH, and DMF (dimethylformamide). The washed resin was suspended in NMP (*N*-methylpyrrolidinone) (20 mL), treated with Boc-R_1Leu-OH (5 eqiuv; R_1 = H or CH_3), HATU (*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate) or HCTU (5 equiv), and DIPEA (diisopropylethylamine) (15 equiv) for 6 h. After being washed with DMF, 0.1 N HCl/THF, MeOH, and DMF, the resin was cycled through the same set of conditions for deprotection, washing, coupling, and washing as above using Boc-R_2Val-OH (5 equiv; R_2 = H or CH_3), Boc-R_3Leu-OH (5 equiv; R_3 = H or CH_3), and Boc-R_4Leu-OH (5 equiv; R_4 = H or CH_3) successively in the peptide elongation. After being washed with DMF, 0.1 N HCl/THF, MeOH, and DMF, the deprotected linear peptide bound to the resin was shaken with LiOH (5 equiv) in THF/H_2O (7:1, 20 mL) at room temperature for 12 h. After being washed with DMF, 0.1 N HCl/THF, MeOH, and DMF, the resin was treated with 50% TFA in CH_2Cl_2 (20 mL) for 15 min and then washed with CH_2Cl_2, 0.1 N HCl/THF, MeOH, and DMF. Cyclization was carried out by treatment of the resin in NMP (20 mL) with PyBOP (benzotriazol-1-yl-oxytrispyrrolidinylphosphonium hexafluorophosphate) (5 equiv), and DIPEA (15 equiv) for 24 h followed by washing with DMF, 0.1 N HCl/THF, MeOH, and CH_2Cl_2. The resin was then treated with neat TFA for 24 h or with a solution of Br_2 in CH_2Cl_2 at room temperature to release the cyclic peptide or its bromide. The cleavage solution was filtered, and the resin was rinsed with CH_2Cl_2 (20 mL). Concentration of the combined filtrates gave the crude product, which was filtered through a short silica gel plug with ethyl acetate to afford the corresponding cyclic peptides. All of the cyclic peptides were >98% pure by two different HPLC analyses (see the Supporting Information).

Cyclic Peptide (2) (7.0 mg, 66% yield from 200 mg of resin **19**): ^{1}H NMR (500 MHz, CD_3OD) δ 0.82 (d, J = 6.5 Hz, 3 H), 0.85 (d, J = 6.5 Hz, 3 H), 0.90 (d, J = 6.5 Hz, 3 H), 0.96 (d, J = 6.5 Hz, 9 H), 1.00 (d, J = 6.0 Hz, 6 H), 1.38−1.41 (m, 1 H), 1.44−1.50 (m, 2 H), 1.63−1.66 (m, 2 H), 1.72−1.75 (m, 2 H), 1.81−2.00 (m, 3 H), 2.04−2.09 (m, 1 H), 3.07 (dd, J = 14.0 Hz, 11.0 Hz, 1 H), 4.54 (dd, J = 11.0 Hz, 4.5 Hz, 1 H), 3.71 (brs, 1 H), 4.54 (dd, J = 11.0 Hz, 4.5 Hz, 1 H), 4.71 (dd, J = 9.0 Hz, 5.0 Hz, 1 H), 7.25 (m, 5 H); ^{13}C NMR (125 MHz, CD_3OD) δ 18.6, 20.3, 22.1, 22.2, 22.3, 23.5, 23.6, 23.8, 26.0, 26.3, 26.9, 32.2, 38.0, 39.5, 41.6, 41.9, 52.6, 56.4, 58.2, 60.5, 62.2, 128.0, 129.6, 129.7, 130.3, 138.8, 171.5, 173.0, 173.7, 174.0, 174.1; HRMS (APCI, M + 1) calcd for $C_{32}H_{52}N_5O_5$ 586.3969, found 586.3958.

Cyclic Peptide (3) (8.1 mg, 68% yield from 200 mg of resin **19**): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.83 (d, J = 7.0 Hz, 3 H), 0.86 (d, J = 6.5 Hz, 3 H), 0.89 (d, J = 7.0 Hz, 3 H), 0.96 (d, J = 7/5 Hz, 9 H), 0.99 (d, J = 7.0 Hz, 6 H), 1.37−1.43 (m, 1 H), 1.48−1.50 (m, 2 H), 1.56−1.59 (m, 2 H), 1.70−1.72 (m, 2 H), 1.81−2.00 (m, 3 H), 2.36 (m, 1 H), 3.08 (dd, J = 13.0 Hz, 5.5 Hz, 1 H), 3.22 (t, J = 7.5 Hz, 1 H), 3.66 (brs, 1 H), 4.03 (t, J = 7.5 Hz, 1 H), 4.35 (dd, J = 10.5 Hz, 6 Hz, 1 H), 4.42 (t, J = 8.0 Hz, 1 H), 4.78 (dd, J = 9.0 Hz, 3.0 Hz, 1 H), 7.25 (m, 5 H); ^{13}C NMR (125 MHz, CD_3OD) δ 19.2, 20.8, 22.5, 22.7, 23.0, 23.5, 23.8, 24.0, 26.1, 26.5, 26.9, 32.2, 38.2, 39.4, 41.8, 42.2, 52.4, 56.6, 58.3, 60.5, 62.8, 128.4, 130.8 (2 C), 132.2 (2 C), 135.6, 173.0, 173.7, 174.3, 174.5, 174.8; HRMS (ESI, M + 1) calcd for $C_{32}H_{51}BrN_5O_5$ 664.3074 and 666.3054, found 664.3065 and 666.3061.

Cyclic Peptide (4) (7.5 mg, 70% yield from 200 mg of resin **19**): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.77 (d, J = 15.5 Hz, 6 H), 0.92−0.97 (m, 18 H), 1.42−1.51 (m, 1 H), 1.53−1.57 (m, 2 H), 1.63−1.67 (m, 2 H), 1.72−1.79 (m, 2 H), 2.18 (brs, 1 H), 2.81 (s, 3 H), 3.02 (dd, J = 15.0 Hz, 5.5 Hz, 1 H), 3.14 (t, J = 15.0 Hz, 1 H), 3.49 (brs, 1 H), 4.23 (d, J = 8.5 Hz, 1 H), 4.43 (d, J = 7.0 Hz, 1 H), 4.81 (dd, J = 13.0 Hz, 5.5 Hz, 1 H), 5.2 (t, J = 15.5 Hz, 1 H), 6.89 (brs, 1 H), 7.17−7.24 (m, 5 H), 7.91 (brs, 1 H), 7.93 (brs, 1 H), 8.08 (d, J = 9.0 Hz, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.7, 19.8, 21.7, 22.5 (2 C), 22.8, 23.1, 23.4, 24.7, 25.2, 25.3, 29.2, 30.9, 37.3, 38.8, 40.5, 40.8, 51.4, 52.4, 54.6, 56.7, 60.3, 127.2, 128.8 (2 C), 129.3 (2 C), 136.4, 171.8, 171.9, 173.2, 173.9, 174.1; HRMS (EI, M^+) calcd for $C_{33}H_{53}N_5O_5$ 599.4047, found 599.4048.

Cyclic Peptide (5) (8.5 mg, 70% yield from 200 mg of resin **19**): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.79 (d, J = 15.0 Hz, 3 H), 0.85 (d, J = 14.5 Hz, 3 H), 0.97 (dd, J = 12.0 Hz, 6.0 Hz, 18 H), 1.46−1.56 (m, 2 H), 1.58−1.64 (m, 2 H), 1.66−1.70 (m, 2 H), 1.88−1.91 (m, 1 H), 2.03 (brs, 1 H), 2.87 (s, 3 H), 2.97 (dd, J = 12.5 Hz, 5 Hz, 1 H), 3.15 (t, J = 12.0 Hz, 1 H), 3.59 (brs, 1 H), 4.32 (d, J = 6.0 Hz, 1 H), 4.38 (brs, 1 H), 4.88 (dd, J = 12.5 Hz, 5.0 Hz, 1 H), 5.75 (brs, 1 H), 6.93 (brs, 1 H), 7.10 (d, J = 8.0 Hz, 2 H), 7.40 (d, J = 8.0 Hz, 2 H), 7.62 (brs, 1 H), 7.82 (d, J = 8.5 Hz, 1 H). 7.94 (brs, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.7 (2 C), 21.7, 22.4 (2 C), 22.9, 23.1, 23.4, 24.9, 25.2, 25.4, 29.2, 29.9, 30.9, 37.0, 37.8, 40.2, 40.5, 51.2, 52.3, 56.6, 65.2, 121.2, 131.1 (2 C), 131.9 (2 C), 135.4, 171.6, 172.0, 173.3, 173.4, 174.1; HRMS (EI, M^+) calcd for $C_{33}H_{52}BrN_5O_5$ 677.3152 and 679.3131, found 677.3148 and 679.3140.

Cyclic Peptide (6) (7.8 mg, 72% yield from 200 mg of resin **19**): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.86 (d, J = 6.0 Hz, 3 H), 0.88 (d, J = 7.0 Hz, 3 H), 0.90 (d, J = 5.5 Hz, 3 H), 0.94 (dd, J = 11.0 Hz, 6.5 Hz, 12 H), 0.99 (t, J = 6.5 Hz, 3 H), 1.45−1.50 (m, 2 H), 1.56−1.61 (m, 2 H), 1.74 (brs, 2 H), 1.86−1.92 (m, 1 H), 2.20 (m, 1 H), 3.10 (s, 3 H), 3.22 (dd, J = 16.0 Hz, 8 Hz, 1 H), 3.34 (dd, J = 16.0 Hz, 6.5 Hz, 1 H), 3.47 (t, J = 7.5 Hz, 1 H), 3.68−3.37 (m, 1 H), 3.90 (brs, 1 H), 4.06 (t, J = 7.0 Hz, 1 H), 4.33 (brs, 1 H), 4.86 (dd, J = 13.5 Hz, 7.0 Hz, 1 H), 6.32 (brs, 1 H), 7.19 (d, J = 7.0 Hz, 2 H), 7.28−7.33 (m, 3 H), 7.51 (brs, 1 H), 7.57 (brs, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.7, 19.7, 22.2, 22.3, 22.6, 22.9 (2 C), 23.1 (2 C), 24.9, 25.0, 26.1, 30.0, 31.8, 36.9, 38.5, 40.0, 41.2, 49.1, 54.3, 59.8, 60.6, 127.5, 129.1 (2 C), 129.3 (2 C), 136.6, 171.8, 172.1, 172.2, 173.0, 173.4; HRMS (ESI, M + 1) calcd for $C_{33}H_{54}N_5O_5$ 600.4125, found 600.4117.

Cyclic Peptide (7) (8.8 mg, 72% yield from 200 mg of resin **19**): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.86 (d, J = 4.0 Hz, 6 H), 0.89 (d, J = 6.5 Hz, 3 H), 0.91−0.96 (m, 12 H), 0.98 (t, J = 6.0

Hz, 3 H), 1.40−1.50 (m, 2 H), 1.55−1.62 (m, 2 H), 1.72, (t, J = 6.5 Hz, 1 H), 1.89−1.95 (m, 2 H), 2.05−2.08 (m, 2 H), 3.00 (s, 3 H), 3.19 (dd, J = 14 Hz, 5 Hz, 1 H), 3.34 (dd, J = 14.0 Hz, 9 Hz, 1 H), 3.44 (t, J = 7.5 Hz, 1 H), 3.97 (d, J = 6.0 Hz, 1 H), 4.09 (t, J = 8.0 Hz, 1 H), 4.25−4.31 (m, 1 H), 4.60 (brs, 1 H), 4.84 (t, J = 12.0 Hz, 1 H), 6.54 (brs, 1 H), 7.08 (d, J = 8.0 Hz, 2 H), 7.42 (d, J = 8.0 Hz, 2 H), 7.68 (d, J = 8.0 Hz, 1 H), 7.85 (brs, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.6, 19.6, 22.3 (2 C), 22.5, 22.8, 23.1 (2 C), 24.9, 25.1, 29.9, 30.0, 36.3, 38.4, 38.5, 40.0, 41.2, 49.2, 54.1, 57.6, 59.8, 69.3, 121.4, 131.0 (2 C), 132.1 (2 C), 135.8, 171.0, 171.9, 172.1, 172.9, 173.3; HRMS (EI, M^+) calcd for $C_{33}H_{52}BrN_5O_5$ 677.3152 and 679.3131, found 677.3172 and 679.3155.

Cyclic Peptide (8) (7.8 mg, 70% yield from 200 mg of resin **19**): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.78 (d, J = 6.5 Hz, 3 H), 0.88 (t, J = 5.0 Hz, 12 H), 0.92 (d, J = 6.0 Hz, 3 H), 0.97 (t, J = 7.0 Hz, 6 H), 1.47−1.51 (m, 1 H), 1.53−1.58 (m, 2 H), 1.65−1.71 (m, 4 H), 1.88 (t, J = 6.5 Hz, 1 H), 2.92−2.98 (m, 1 H), 3.16−3.21 (dd, J = 14.0 Hz, 8 Hz, 1 H), 3.29 (dd, J = 14.0 Hz, 9 Hz, 1 H), 4.02−4.07 (m, 1 H), 4.20 (dd, J = 14.5 Hz, 9 Hz, 1 H), 4.41 (dd, J = 15.0 Hz, 6 Hz, 1 H), 4.87 (dd, J = 15.5 Hz, 8 Hz, 1 H), 6.35 (d, J = 6.5 Hz, 1 H), 7.20 (d, J = 7.5 Hz, 2 H), 7.25−7.32 (m, 3 H), 7.33 (d, J = 6.0 Hz, 1 H), 7.59 (d, J = 6.0 Hz, 1 H), 7.69 (d, J = 9.5 Hz, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.5, 20.3, 21.3, 22.2, 22.8, 22.9 (2 C), 23.4, 25.0 (2 C), 25.1, 26.6, 27.6, 30.0, 35.4, 40.2, 40.5, 41.3, 46.6 (2 C), 48.8 (2 C), 52.2, 55.2, 57.4, 127.3, 129.0 (2 C), 129.1 (2 C), 136.8, 170.9, 171.3, 171.8, 172.9, 174.1; HRMS (EI, M^+) calcd for $C_{33}H_{53}N_5O_5$ 599.4047, found 599.4042.

Cyclic Peptide (9) (8.5 mg, 70% yield from 200 mg of resin **19**): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.82 (d, J = 6.0 Hz, 3 H), 0.88−0.95 (m, 15 H), 0.97 (dd, J = 10.5 Hz, 6.5 Hz, 6 H), 1.49−1.52 (m, 1 H), 1.55−1.58 (m. 2 H), 1.61−1.75 (m, 4 H), 1.87−1.89 (m, 1 H), 2.90 (dd, J = 12.5 Hz, 6.5 Hz, 1 H), 3.08 (dd, J = 13 Hz, 7 Hz, 1 H), 3.22−3.26 (m, 1 H), 3.99 (dd, J = 15 Hz, 6.5 Hz, 1 H), 4.18 (dd, J = 14.5 Hz, 6.0 Hz, 1 H), 4.44 (brs, 1 H), 4.88 (dd, J = 13.5 Hz, 8.0 Hz, 1 H), 6.3 (brs, 1 H), 7.09 (d, J = 8.0 Hz, 2 H), 7.39 (brs, 1 H), 7.42 (d, J = 8.0 Hz, 2 H), 7.56 (d, J = 9.0 Hz, 1 H), 7.64 (brs, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.5, 20.3, 21.4, 22.0, 22.7, 23.0 (2 C), 23.3, 24.0, 25.2 (2 C), 27.5, 29.9, 35.2, 40.5, 40.7, 41.4, 48.2, 51.3, 52.4, 55.8, 61.8, 121.1, 131.1 (2 C), 132.0 (2 C), 135.9, 170.8, 171.1, 171.8, 173.1, 173.8; HRMS (EI, M^+) calcd for $C_{33}H_{52}BrN_5O_5$ 677.3152 and 679.3131, found 677.3132 and 679.3109.

Cyclic Peptide (10) (7.4 mg, 69% yield from 200 mg of resin **19**): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.81 (d, J = 6.5 Hz, 3 H), 0.84 (dd, J = 6.5 Hz, 2 Hz, 12 H), 0.89 (t, J = 7.0 Hz, 6 H), 0.92 (d, J = 7.0 Hz, 3 H), 1.34−1.40 (m, 2 H), 1.44−1.49 (m, 2 H), 1.51−1.55 (m, 2 H), 1.71−1.76 (m, 1 H), 2.02−2.07 (m, 1 H), 3.07 (dd, J = 14.0 Hz, 5 Hz, 1 H), 3.37 (dd, J = 14.0 Hz, 6 Hz, 1 H), 3.24 (s, 3 H), 3.44 (t, J = 5.5 Hz, 1 H), 4.40 (dd, J = 15.5 Hz, 7.5 Hz, 1 H), 4.5 (T, J = 10 Hz, 1 H), 4.80−4.84 (m, 1 H), 4.91 (dd, J = 15.5 Hz, 9 Hz, 1 H), 5.90 (d, J = 8.9 Hz, 1 H), 6.25 (d, J = 7.5 Hz, 1 H), 7.17 (d, J = 6.3 Hz, 2 H), 7.25−7.29 (m, 3 H), 7.81 (d, J = 8.0 Hz, 1 H), 8.68 (d, J = 9.5 Hz, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.1, 19.5, 22.2, 22.6, 22.7 (3 C), 23.2, 23.5, 24.5, 25.1, 25.4, 29.9, 30.6, 37.4, 41.5, 42.2, 51.7, 51.9, 53.5, 55.1, 65.7, 127.3, 128.9 (2 C), 129.6 (2 C), 136.7, 169.6, 170.6, 171.6, 172.3, 172.5; HRMS (ESI, M + 1) calcd for $C_{33}H_{54}N_5O_5$ 600.4125, found 600.4115.

Cyclic Peptide (11) (8.6 mg, 71% yield from 200 mg of resin **19**): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.89 (d, J = 9.5 Hz, 6 H), 0.92 (d, J = 7.0 Hz, 12 H), 0.96 (t, J = 7.5 Hz, 6 H), 1.40−1.46 (m, 1 H), 1.58−1.66 (m. 2 H), 1.86−1.93 (m, 4 H), 2.01−2.05 (m, 1 H), 3.08 (brs, 1 H), 3.22 (s, 3 H), 3.43 (brs, 1 H), 3.68−3.70 (m, 1 H), 3.87 (brs, 1 H), 4.21 (brs, 1 H), 4.50 (t, J = 8.5 Hz, 1 H), 4.63 (d, J = 8 Hz, 1 H), 6.64 (brs, 1 H), 6.84 (brs, 1 H), 7.09 (d, J = 7.0 Hz, 2 H), 7.43 (d, J = 7.0 Hz, 2 H), 7.48 (brs, 1 H), 7.53 (brs, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.5, 19.8, 21.9, 22.3 (2 C), 23.0 (2 C), 23.1 (2 C), 24.9, 25.5, 25.6, 29.9, 30.7, 37.8, 39.6, 40.6, 53.5 (2 C), 54.8, 55.6, 67.2, 121.5, 131.1 (2 C), 132.1 (2 C), 135.4, 171.2, 172.5, 173.4, 173.8; HRMS (ESI, M + Na) calcd for $C_{33}H_{52}BrN_5O_5Na$ 700.3052 and 702.3032, found 700.3038 and 702.3022.

Cyclic Peptide (12) (7.8 mg, 72% yield from 200 mg of resin **19**): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.81 (d, J = 6.5 Hz, 3 H), 0.85 (d, J = 6.5 Hz, 6 H), 0.89 (t, J = 3.0 Hz, 6 H), 0.90 (t, J = 6.5 Hz, 6 H), 0.94 (dd, J = 6.5 Hz, 4.5 Hz, 3 H), 1.42−1.45 (m, 1 H), 1.49−1.56 (m, 2 H), 1.59−1.64 (m, 2 H), 1.68−1.72 (m, 2 H), 2.08 (brs, 1 H), 2.76 (s, 3 H), 3.17 (dd, J = 13.0 Hz, 5.5 Hz, 1 H), 3.32 (t, J = 7.5 Hz, 1 H), 3.50 (m, 1 H), 3.66 (dd, J = 10.5 Hz, 6 Hz, 1 H), 4.28 (m, 2 H), 4.75 (m, 1 H), 6.64 (brs, 1 H), 6.97 (brs, 1 H), 7.15 (d, J = 6.5 Hz, 2 H), 7.23−7.30 (m, 3 H), 7.62 (brs, 1 H), 7.83 (d, J = 9.0 Hz, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.5, 191.9, 21.7, 22.0, 22.5, 22.9, 23.2, 23.3, 25.1, 25.2, 25.3, 27.9, 34.2, 39.7, 40.0, 40.8, 41.0, 48.4, 53.4, 53.5, 60.5, 69.5, 127.4, 129.0 (2 C), 129.1 (2 C), 136.9, 170.4, 172.6 (2 C), 172.7, 174.1; HRMS (EI, M^+) calcd for $C_{33}H_{53}N_5O_5$ 599.4047, found 599.4046.

Cyclic Peptide (13) (8.7 mg, 71% yield from 200 mg of resin **19**): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85 (d, J = 6.5 Hz, 3 H), 0.89 (d, J = 6.5 Hz, 3 H), 0.92 (d, J = 7.0 Hz, 3 H), 0.94 (d, J = 6.0 Hz, 12 H), 0.97 (d, J = 6.5 Hz, 3 H), 1.38−1.42 (m, 1 H), 1.48−1.53 (m, 2 H), 1.58−1.61 (m, 2 H), 1.71 (d, J = 5.0 Hz, 1 H), 2.38 (brs, 1 H), 2.83 (s, 3 H), 3.18 (dd, J = 13.5 Hz, 5.5 Hz, 1 H), 3.46 (t, J = 6.5 Hz, 1 H), 3.51−3.56 (m, 1 H), 3.66 (dd, J = 9.5 Hz, 5.0 Hz, 1 H), 4.24 (d, J = 7.0 Hz, 1 H), 4.32 (dd, J = 14.0 Hz, 5.5 Hz, 1 H), 4.79 (m, 1 H), 6.88 (brs, 1 H), 7.06 (d, J = 8.5 Hz, 2 H), 7.44 (d, J = 8.5 Hz, 2 H), 7.67 (brs, 1 H), 7.77 (d, J = 9.0 Hz, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.2, 19.9, 21.8, 22.2, 22.6, 22.7, 23.1, 23.2, 25.1, 25.3 (2 C), 28.3, 33.9, 39.3, 40.2, 40.8, 41.1, 48.5, 53.4, 53.7, 66.1, 69.9, 121.3, 130.8 (2 C), 132.1 (2 C), 136.0, 170.3, 172.2, 172.5, 172.9, 174.0; HRMS (EI, M^+) calcd for $C_{33}H_{52}BrN_5O_5$ 677.3152 and 679.3131, found 677.3150 and 679.3138.

***N*-Trifluoroacetyl-4-iodophenylalanine (15).** To a solution of 4-iodophenylalanine (**14**, 2 mmol, 0.58 g) and triethylamine (16 mmol, 4.6 mL) in acetone (20 mL) was added slowly trifluoroacetic anhydride (16 mmol, 2.3 mL) with stirring at room temperature. The mixture was stirred overnight and then concentrated. The residue was dissolved in 1 N HCl (20 mL) and then extracted with ethyl acetate (3 × 20 mL), and the organic layer was washed with brine. The solution was dried over anhydrous Na_2SO_4 and concentrated to give the crude product (0.73 g), which was taken on without further purification.

***N*-Methyl-*N*-trifluoroacetyl-4-iodophenylalanine Methyl Ester (16).** To a solution of **15** (0.73 g) in 50 mL of anhydrous acetone was added anhydrous K_2CO_3 (18 mmol, 2.48 g) and iodomethane (36 mmol, 2.25 mL), and the reaction mixture stirred at room temperature for 16 h. The reaction was monitored by TLC. The suspension was filtered off, the solvent was evaporated, and the residue was taken up in 100 mL of ethyl acetate. The solution was washed with water, 0.5 N HCl, and brine, respectively, dried over anhydrous Na_2SO_4, filtered, and concentrated to give an oily residue. The crude product was purified by flash chromatography on silica gel to afford a light yellow oil product (0.7 g, 87%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.90 (s, 3 H), 3.03 (dd, J = 14.8 Hz, 5.6 Hz, 1 H), 3.32 (d, J = 14.8 Hz, 11.2 Hz, 1 H), 3.70 (s, 3 H), 4.91 (m, 1 H), 6.91 (d, J = 7.6 Hz, 2 H); ^{13}C NMR (10 MHz, $CDCl_3$) δ 33.8, 52.9, 60.8, 61.1, 92.8, 114.8, 130.9, 136.0 (2 C), 137.9 (2 C), 157.3, 169.2; HRMS (ESI, M + 1) calcd for $C_{13}H_{14}F_3INO_3$ 415.9971, found 415.9980.

***N*-Methyl-4-iodophenylalanine (17).** To a solution of **16** (0.7 g, 1.69 mmol) in 50 mL of acetone and water (1:1) was added 0.37 g (2.7 mmol) of anhydrous K_2CO_3. The mixture was heated to 45 °C and was stirred overnight. After the starting material had disappeared (TLC monitoring), the reaction solution was concentrated to about half volume and diluted with brine, and the pH of the solution was adjusted to 3. The precipitate was collected by filtration and washed with water and THF to give 0.48 g (93%) of a white solid, which was used for the next reaction without further purification.

***N*-Boc-*N*-methyl-4-iodophenylalanine Methyl Ester (18).** To a solution of NaOH (63 mg, 1.58 mmol) in 25 mL of water was added **17** (0.48 g) and then the mixture stirred until the solid dissolved. A solution of Boc_2O (6.32 mmol, 1.38 g) in 20

mL of alcohol was added and the mixture was stirred at room temperature for 40 min. After being quenched with 1 N HCl (50 mL), the reaction was extracted with ethyl acetate (3 × 20 mL), the organic phase was combined, washed with water and brine, respectively, dried over Na_2SO_4, and concentrated under vacuum to yield crude *N*-Boc-*N*-methyl-4-iodophenylalanine. This product was dissolved in a solution of 40 mL of acetone and iodomethane (12.64 mmol, 0.79 mL), and anhydrous K_2CO_3 (0.87 g, 6.32 mmol) was added. The resultant mixture was stirred for 14 h at room temperature. After this time, the product was filtered, and the solvent was evaporated under reduced pressure. The organic layer was separated, dried over Na_2SO_4, and concentrated under vacuum to yield 0.64 g of a yellow oil. The crude product was purified by flash chromatography (1:5 ethyl acetate:hexanes) to afford a light yellow oil (0.59 g, 89%): ^{1}H NMR (500 MHz, DMSO, 100 °C) δ 1.32 (s, 9 H), 2.66 (s, 3 H), 2.99 (dd, J = 14 Hz, 11 Hz, 1 H), 3.16 (dd, J = 14 Hz, 5 Hz, 1 H), 3.69 (s, 3 H), 4.59 (m, 1 H), 7.04 (d, J = 8 Hz, 2 H), 7.63 (d, J = 8 Hz, 2 H); ^{13}C NMR (125 Hz, $CDCl_3$) δ 28.3 (3 C), 32.1, 34.7, 52.4, 59.4, 61.6, 92.0, 131.2 (2 C), 137.3, 137.6 (2 C), 171.6, 171.8; HRMS (EI, M) calcd for $C_{16}H_{22}INO_4$ 419.0588, found 419.0586.

Procedure for Attachment of *N*-Boc-*N*-methyl-4-iodophenylalanine Methyl Ester to and Cleavage from the Silane Linker Resin (19). To a solution of *N*-Boc-*N*-methyl-4-iodophenylalanine methyl ester (800 mg, 2 mmol) in NMP (20 mL) was added butyl diethylsilane polystyrene (PE-DES-SiH, 1 g, 1.45 mmol/g) and KOAc (300 mg, 0.3 mmol). The reaction mixture was deaerated by passing a slow stream of argon through it for 15 min. After the addition of $Pd_2(dba)_3$·$CHCl_3$ (110 mg, 0.1 mmol), the reaction flask and reflux condenser were wrapped with aluminum foil, and the mixture was stirred at 110 °C for 24 h. After being cooled to room temperature and washed with CH_2Cl_2, DMF, 1 N HCl/THF (1:7, 30 min), MeOH, and CH_2Cl_2, an aliquot of the resin (200 mg) was treated with a solution of Br_2 (15 μL) in CH_2Cl_2 (10 mL) for 20 min. The cleavage solution was filtered, and the resin was rinsed with CH_2Cl_2 (5 mL). Concentration of the combined filtrates gave 7.5 mg of *N*-Boc-4-bromophenylalanine methyl ester, which indicated that the loading level was 0.1 mmol/g (this represents both *s*-cis and *s*-trans isomers): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.32 and 1.37 (2s, 2 × 9 H), 2.68 and 2.71(2 s, 2 × 3 H), 2.96 (m, 2 × 1 H), 3.23 (m, 2 × 1 H), 3.72 and 3.74 (2 s, 2 × 3 H), 4.46 (m, 2 × 1 H), 4.87 and 4.89 (2 d, J = 5.6, 2 × 1 H), 7.06 (m, 2 × 2 H), 7.39 (m, 2 × 2 H); ^{13}C NMR (125 Hz, $CDCl_3$) δ 28.3 and 28.4 (2 × 3 C), 32.1 and 32.9 (2 × 1 C), 34.6 and 35.1 (2 × 1 C), 52.4 and 52.5 (2 × 1 C), 59.5 and 61.6 (2 × 1 C), 80.3 and 80.6 (2 × 1 C), 120.6 and 120.7 (2 × 1 C), 130.9 and 131.0 (2 × 2 C), 136.6 and 136.9 (2 × 1 C), 155.0 and 155.9 (2 × 1 C), 171.4 and 171.7 (2 × 1 C); HRMS (EI, M) calcd for $C_{16}H_{22}BrNO_4$ 372.0804 and 374.0784, found 372.0803 and 374.0780.

Cell Proliferation and Cytotoxicity Assays for PC-3, MDA-MB231, and WM-115 Cells. The ability of the compounds to inhibit cell proliferation or induce cytotoxicity was tested on three human cancer lines: PC-3 (prostate cancer), MDA-MB231 (breast cancer), and WM-115 (melanoma). The procedures were adapted from Hanford et al.[16] Five thousand cells were plated per well in replicate wells of a 24-well plate. All cells were grown in DMEM (Invitrogen, Carlsbad, CA) supplemented with 10% heat-inactivated calf serum (US Bio-Technologies, Parkerford, PA) and 100 μg/mL Pen-Strep (Invitrogen) and amphotericin B (Biologos, Montgomery, IL). Cells were maintained at 37 °C in humidified 5% CO_2. The compounds were dissolved in DMSO and diluted to a final concentration of 10 μM (or lower as specified) in DMEM with supplements as specified. All cells were cultured for 72 h, and cell numbers were counted. All test conditions were assayed in duplicate wells, and the results of the two wells were averaged. The cell number was expressed as a percentage of the untreated control cells.

Pancreatic Cancer Cell Lines. Two cancer cell lines, S2-013 (well-differentiated) and AsPC-1 (poorly differentiated), were purchased from American Type Culture Collection (Manassas, VA). Both cell lines were grown in DMEM and plated as monolayers in the medium supplemented with 10% fetal bovine serum in a humidified atmosphere of 95% O_2 and 5% CO_2 at 37 °C. The cells were regularly seeded into 75 cm^2 flasks with media changes every second or third day. For experiments, cells were grown to 70% confluence, digested with trypsin-EDTA, and plated in either 6-, 24-, or 48-well plates.

DNA Synthesis in Pancreatic Cells by [*methyl*-^{3}H]-Thymidine Incorporation. Cells were plated in 24-well plates at a concentration of 50 000 cells/well. After reaching 50% confluence, they were incubated in serum-free medium for 24 h, which was then replaced with fresh serum-free medium with or without treatment with 10 μM of each cyclic peptide. After the required period of culture, cellular DNA synthesis was assayed by adding 0.5 Ci [*methyl*-^{3}H]thymidine per well and incubating the cells for another 6 h. The cells were then washed twice with PBS, fixed with 10% trichloroacetic acid, and solubilized by adding 250 μL of 0.4 M NaOH to each well. Radioactivity, indicating incorporation of [*methyl*-^{3}H]thymidine into DNA, was measured by adding scintillation cocktail and counting with a scintillation counter (LKB Rack-Beta; Wallac, Turku, Finland).

Cell Proliferation Assay for Pancreatic Cells. Cells were regularly seeded into three 6-well plates and incubated at 37 °C for 24 h. Cells were then cultured in serum-free medium for another 24 h and treated in fresh serum-free medium with or without cyclic peptides (0.1−100 μM) for 24, 48, and 72 h. At the end of each time period, the cells were trypsinized to produce a single cell suspension, and the cell number in each well was determined using Guava Technologies ViaCount Assay (Guava Technologies Inc, Hayward, CA).

Statistical Analysis. Data were analyzed by ANOVA with Dunnett's or Bonferoni's corrections for multiple comparisons, as appropriate. This analysis was performed with the Prism software package (GraphPad, San Diego, CA). Data were expressed as mean ± SEM.

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Supporting Information Available: HRMS and HPLC data for compounds **1**−**13**. This material is available free of charge via the Internet at http://pubs.acs.org.

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