Bioorganic & Medicinal Chemistry 17 (2009) 5806–5825

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

A comprehensive study of Sansalvamide A derivatives: The structure–activity relationships of 78 derivatives in two pancreatic cancer cell lines

Po-Shen Pan, Robert C. Vasko, Stephanie A. Lapera, Victoria A. Johnson, Robert P. Sellers, Chun-Chieh Lin, Chung-Mao Pan, Melinda R. Davis, Veronica C. Ardi, Shelli R. McAlpine *

Department of Chemistry and Biochemistry, San Diego State University, 5500 Campanile Drive, San Diego, CA 92182-1030, United States

ARTICLE INFO

Article history:
Received 2 June 2009
Revised 9 July 2009
Accepted 10 July 2009
Available online 15 July 2009

Keywords:
Sansalvamide A
Pancreatic cancer
Macrocycle
Cyclic peptides
Natural products
Structure–activity relationship
Cytotoxicity

ABSTRACT

We report an extensive structure–activity relationship (SAR) of 78 compounds active against two pancreatic cancer cell lines. Our comprehensive evaluation of these compounds utilizes SAR that allow us to evaluate which features of potent compounds play a key role in their cytotoxicity. This is the first report of 19 new second-generation structures, where these new compounds were designed from the first generation of 59 compounds. These 78 structures were tested for their cytotoxicity and this is the first report of their activity against two pancreatic cancer cell lines. Our results show that out of 78 compounds, three compounds are worth pursuing as leads, as they show potency of $\geqslant$55% in both cancer cell lines. These three compounds all have a common structural motif, two consecutive D-amino acids and an *N*-methyl moiety. Further, of these three compounds, two are second-generation structures, indicating that we can incorporate and utilize data from the first generation to design potency into the second generation. Finally, one analog is in the mid nanomolar range, and has the lowest IC_{50} of any reported San A derivative. These analogs share no structural homology to current pancreatic cancer drugs, and are cytotoxic at levels on par with existing drugs treating other cancers. Thus, we have established Sansalvamide A as an excellent lead for killing multiple pancreatic cancer cell lines.

1. Introduction

Natural products provide an excellent source of lead structures for new drugs. These novel structures are critical for development of new therapeutic small molecules that target unique biological pathways. Sansalvamide A (San A) is one such natural product (Fig. 1). San A, which was isolated from a marine fungus (*Fusarium* ssp.), exhibits antitumor activity on multiple cancer cell lines.[1–3] The natural product is a depsipeptide (Fig. 1) and, therefore, prone to ring opening at the ester bond by esterases. Silverman and co-workers found that the natural product peptide (where the hydroxy acid is converted to an amino acid) was 10-fold more active than the natural product depsipeptide.[4,5] Thus, to avoid deactivation via ring-opening all 78 derivatives reported by our laboratory[6,7] were synthesized as the San A peptide derivatives. To date, the synthesis of 89 analogs have been reported, 78 by us and 11 by Silverman et al.[4] All 89 derivatives are referred to as 'San A-amide' derivatives. The cytotoxicity of San A-amide derivatives against pancreatic,[4,5,8] colon,[3,6,9,10] breast, prostate, and melanoma cancers[4] clearly indicates San A-amide's excellent potential as a new therapeutic agent for the treatment of various cancers and supports further exploration of this class of compounds. All 11 of the San A-amide derivates prepared by Silverman and co-workers contain only L-amino acids[4,5,11,12] and these demonstrate reasonable potency against the colon cancer cell line HCT-116. They attribute potency against this cell line to the placement of multiple *N*-methyl moieties on the macrocycle. However, our extensive studies on HCT-116[13,14] and our results reported here for pancreatic cancer cell lines PL45 and BxPC-3 indicate that placement of multiple *N*-methyl moieties does not generate a potent molecule

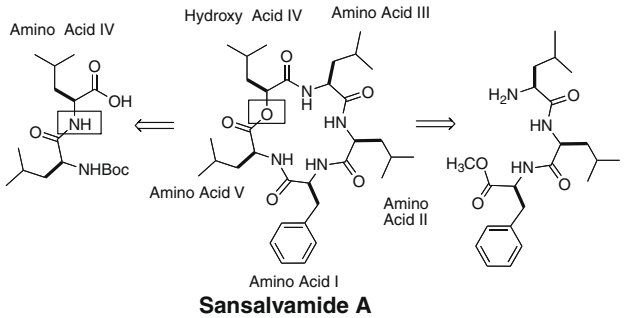


Figure 1. Retrosynthetic approach.

* Corresponding author.
E-mail address: mcalpine@sciences.sdsu.edu (S.R. McAlpine).

0968-0896/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2009.07.017

against these two cell lines and that there are other factors that contribute to the molecule's potency.

We report here an extensive structure–activity relationship (SAR) of 78 compounds tested for their cytotoxicity against two pancreatic cancer cell lines. This report includes the synthesis and activity of 19 new compounds (second generation) that were designed from the first generation of 59 compounds. These data provide a global view of the San A series and their complex SAR against PL45 and BxPC-3. Two of the three most potent compounds' structures are second-generation compounds and, thus, their potency is reported here for the first time. These three structures clearly point to a common modality, where one *N*-methyl and two consecutively placed D-amino acids are important for potency. Further, one compound, 63, has cytotoxicity in the mid nanomolar region, which is the lowest reported IC_{50} of any San A derivative.

Peptides are sometimes considered poor drugs for two reasons: solubility and rapid degradation within cells. In order for linear peptides to achieve 3D structures that will bind appropriately to their protein targets, they are often composed of extended sequences of amino acids, making them insoluble. Cyclic peptides, like San A, often perform better as drugs than linear peptides because a small number of amino acids define a rigid 3D structure. San A-amide analogs also have the advantage that they are lipophilic and therefore have rapid membrane absorption.[15] Further, cyclic peptides tend to have greater binding affinity for protein targets than their linear counterparts or other small molecules because they have restricted bond rotation and conformational restraint, which may lock a molecule into an ideal binding mode.[16] In addition, cyclic peptides degrade much slower than linear peptides because proteases have difficulty cleaving amide bonds located within a macrocycle.[15] Cyclic peptides also have commercially available chemical diversity (i.e., amino acids), are efficiently synthesized, have defined 3D structures (which is typically required for good binding affinity for protein targets), are effective at penetrating cell membranes, and are stable within cells. To date, there are 720 clinically used peptide drugs or drug candidates: 38% of these are in clinical trials, 56% are in advanced preclinical phases, and 5% are on the market.[17–19] These peptide drugs are used as prostate and breast cancer antitumor agents, HIV protease inhibitors, osteoporosis-treating drugs, and immunosuppressants.[19–21] Thus, there is outstanding precedence for treating diseases with peptides.

Pancreatic cancer is the fifth most deadly cancer in the US. Only 10% of patients are eligible for surgery[22] and less than 20% of pancreatic cancers respond to the current drugs of choice, one of which is Gemcitabine (2,2-difluorodeoxycytidine).[23] Although progress has been made on improving survival rates, current therapy is far from acceptable. Indeed, the five-year survival rate for patients with pancreatic cancers is still less than 5%.[24] With such a poor rate of response to current chemotherapeutic methods, there is an immediate need for new and effective pancreatic cancer treatments. This work reports the SAR of 78 San A-amide derivatives against two drug-resistant pancreatic cancer cell lines and establishes a structural template that can be used to design new compounds.

2. Results and discussion

2.1. Synthesis of all compounds

All 78 derivatives described here were constructed as the peptide analogs (Fig. 1, where the hydroxy acid was exchanged for an amino

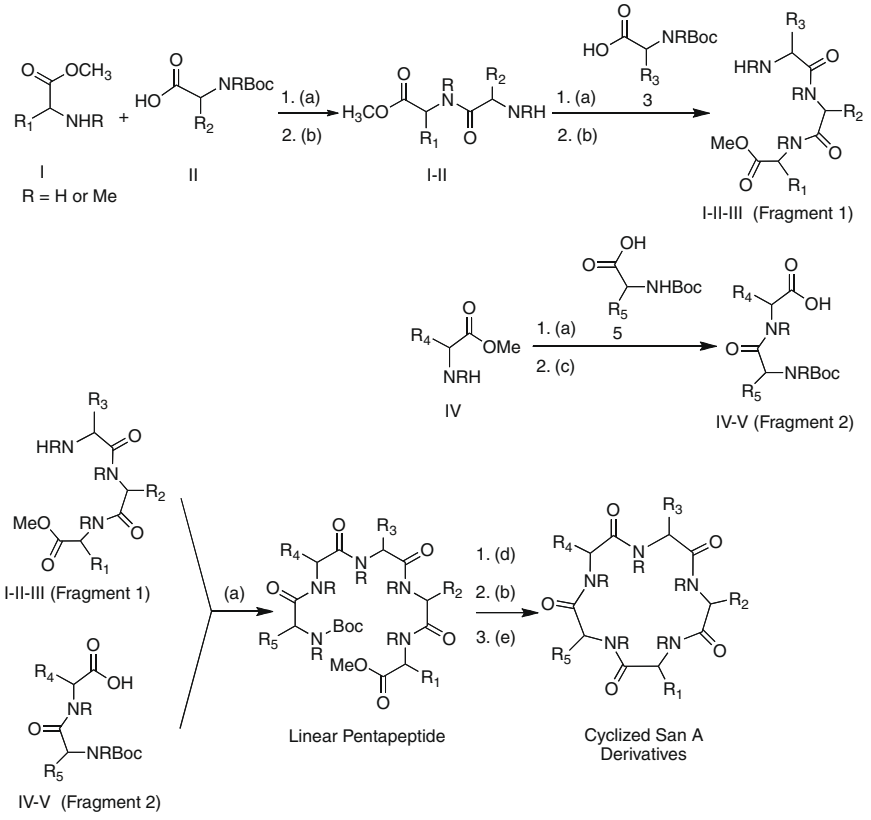


Figure 2. Synthesis of macrocycles. Reagents and conditions: (a) coupling agent (TBTU (1.2 equiv), and/or HATU (0.75 equiv)), DIPEA (4 equiv), DCM (0.1 M); (b) TFA (20%), anisole (2 equiv), DCM; (c) LiOH (4 equiv), MeOH; (d) LiOH (10 equiv), MeOH; (e) HATU (0.7 equiv), DEPBT (0.7 equiv), TBTU (0.7 equiv), DIPEA (6 equiv), DMF:DCM:CH_3CN (2:2:1) 0.01 M.

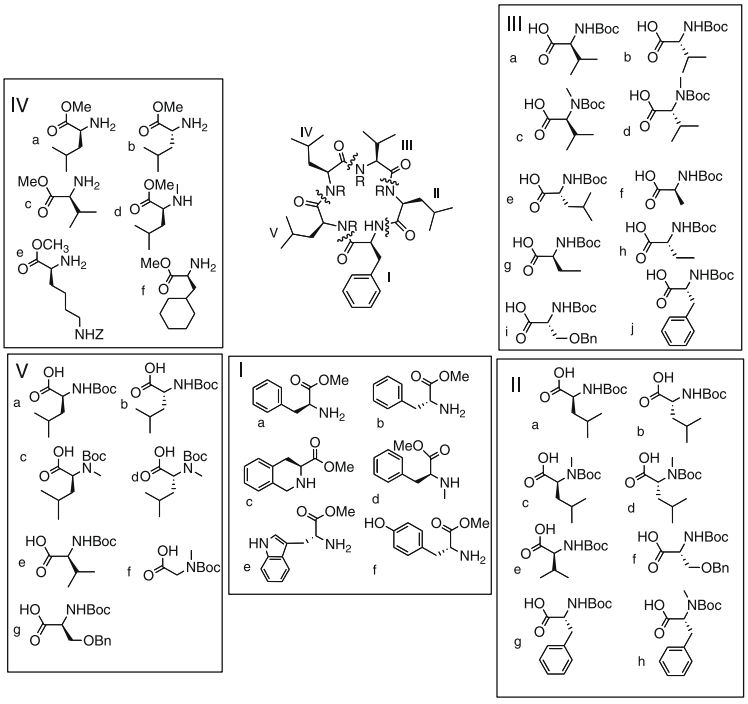


Figure 3. Amino acids used in the synthesis of 78 derivatives.

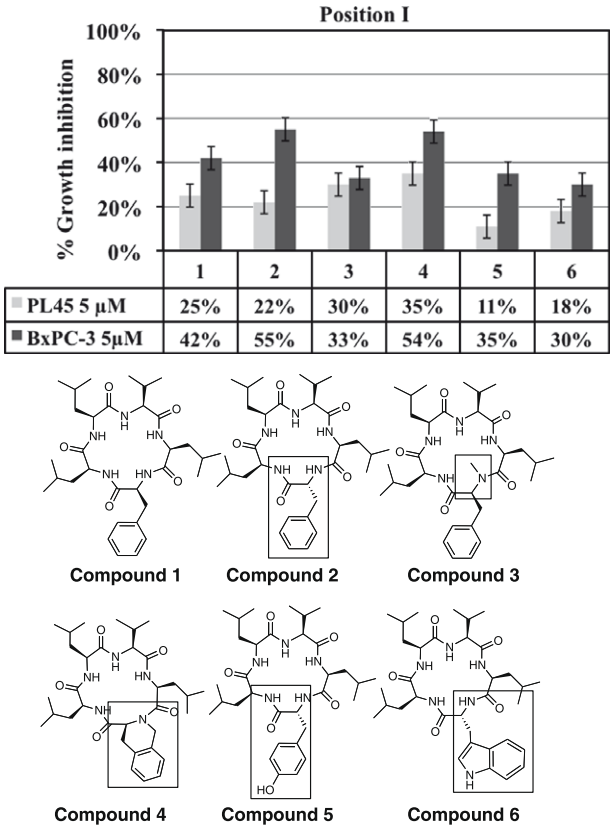


Figure 4. Compounds with alterations at position I. Data is represented as % growth inhibition relative to 1% DMSO control. Each data point is an average of four wells run in three assays. All assays were run for 72 h at 5 µM compound concentration.

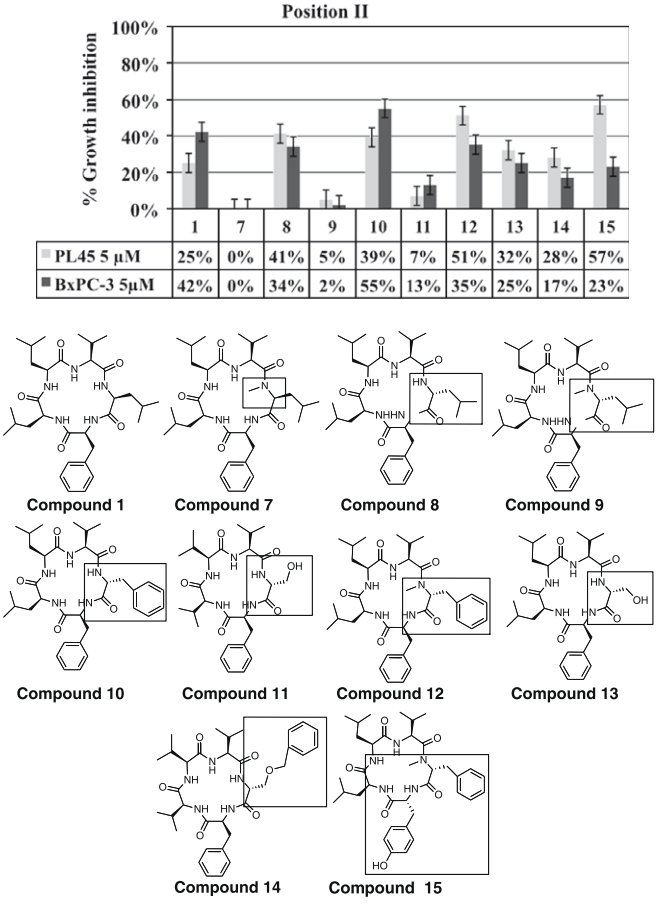


Figure 5. Compounds with alterations at positions II and/or I. Data is represented as % growth inhibition relative to 1% DMSO control. Each data point is an average of four wells run in three assays. All assays were run for 72 h at 5 µM compound concentration.

acid at position IV). A succinct synthetic protocol has been developed for the creation of these molecules.[6,25] Our compounds have been designed to explore how the placement of *N*-methyl moieties, D-amino acids, aromatic, and hydrophilic amino acids impact the cytotoxicity of the molecule. These derivatives have log *P* values between 0.18 and 3.35 (see Supplementary data for a table), thus meeting Lipinski's rules for solubility and effective diffusion through cellular membranes.[26,27] Our synthetic approach utilized a convergent solution-phase strategy in order to establish a reliable and inexpensive route for the large-scale production of compound needed for additional biological studies (Fig. 2). Our outlined route provides rapid and high-yielding synthesis of these compounds.

The synthesis of the San A-amide derivatives, were completed using the amino acids shown in Figure 3 via the synthetic strategy shown (Fig. 2). Using these amino acids, we were able to significantly vary the San A-amide structures in order to explore the role of stereochemistry, *N*-methyl moieties and aromatic or hydrophilic amino acids in cytotoxicity.

The 78 structures and their cytotoxicity against the two pancreatic cancer cell lines are discussed in order of position. That is, structures are arranged in order of amino acids that are altered from the original San A peptide, where alterations to residues in positions I–V are described in Figures 4–8, respectively.

Using 2(1-*H*-benzotriazole-1-yl)-1,1,3-tetramethyl-uronium tetrafluoroborate (TBTU), and diisopropylethylamine (DIPEA), acid protected residue **I** (**a**–**f**) and *N*-Boc protected residue **II** (**a**–**h**) were coupled to give the dipeptide **I–II-Boc** (80–94% yield). Deprotection of the amine on residue **II** using TFA gave the free amine, **I–II** (~quantitative yields). Coupling of the dipeptide to monomer **III** (**a–j**) gave the desired tripeptide (*Fragment 1*) in good yields (80–95%).[28] The synthesis of *Fragment 2* was completed by coupling residue **IV** (**a**–**f**) to residue **V(a–g**) to give dipeptide **IV–V-Boc** (90–95% yield). The amine was deprotected on *Fragment 1* using TFA and the acid was revealed in *Fragment 2* using lithium hydroxide. *Fragment 1* and *Fragment 2* were coupled using multiple coupling agents,[7,25,29,30] yielding 78 examples of linear pentapeptides (66–90% yield).[28]

In the case of the di- and tripeptide construction, acid/base workup removed excess reagents and side products, and the NMR indicated compounds did not usually require further purification. Typically, it was only in the case of pentapeptides and macrocycles that we found it necessary to purify compounds via silica chromatography, making the synthesis efficient. The purity of all compounds was verified via NMR and/or LC–MS. The linear peptides were then cyclized using similar conditions developed in our laboratory.[25] Upon cyclization, the final compounds were purified via flash chromatography and/or HPLC. When appropriate, the side chains were deprotected (serines, lysines, and tyrosines). The purity of all compounds was verified by NMR and LC–MS.[25]

2.2. Structure–activity relationships (SAR)

Although major efforts have been made, few truly novel classes of compounds have shown activity against drug-resistant pancreatic cancer tumors.[23,24] With this in mind, our work elucidates an

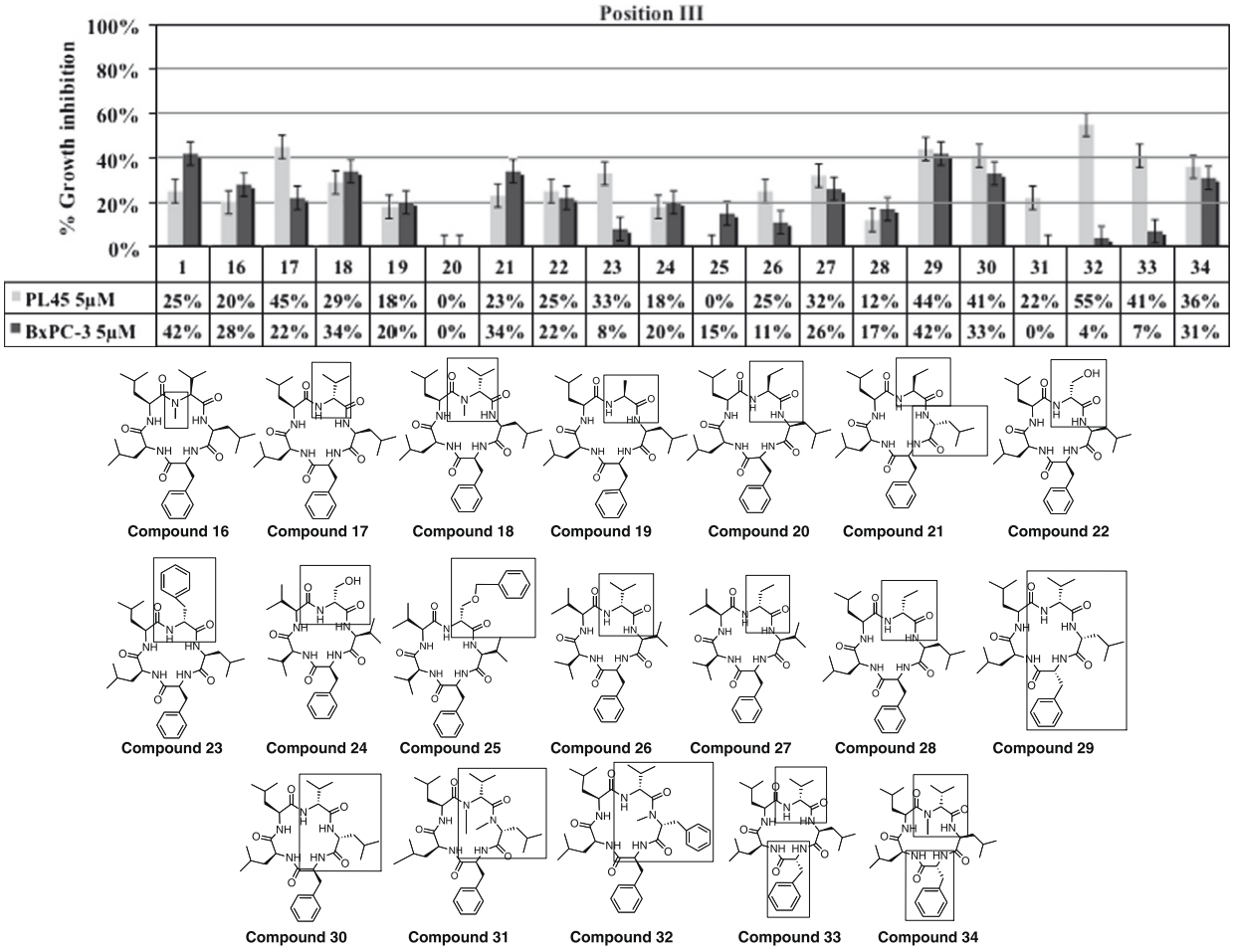
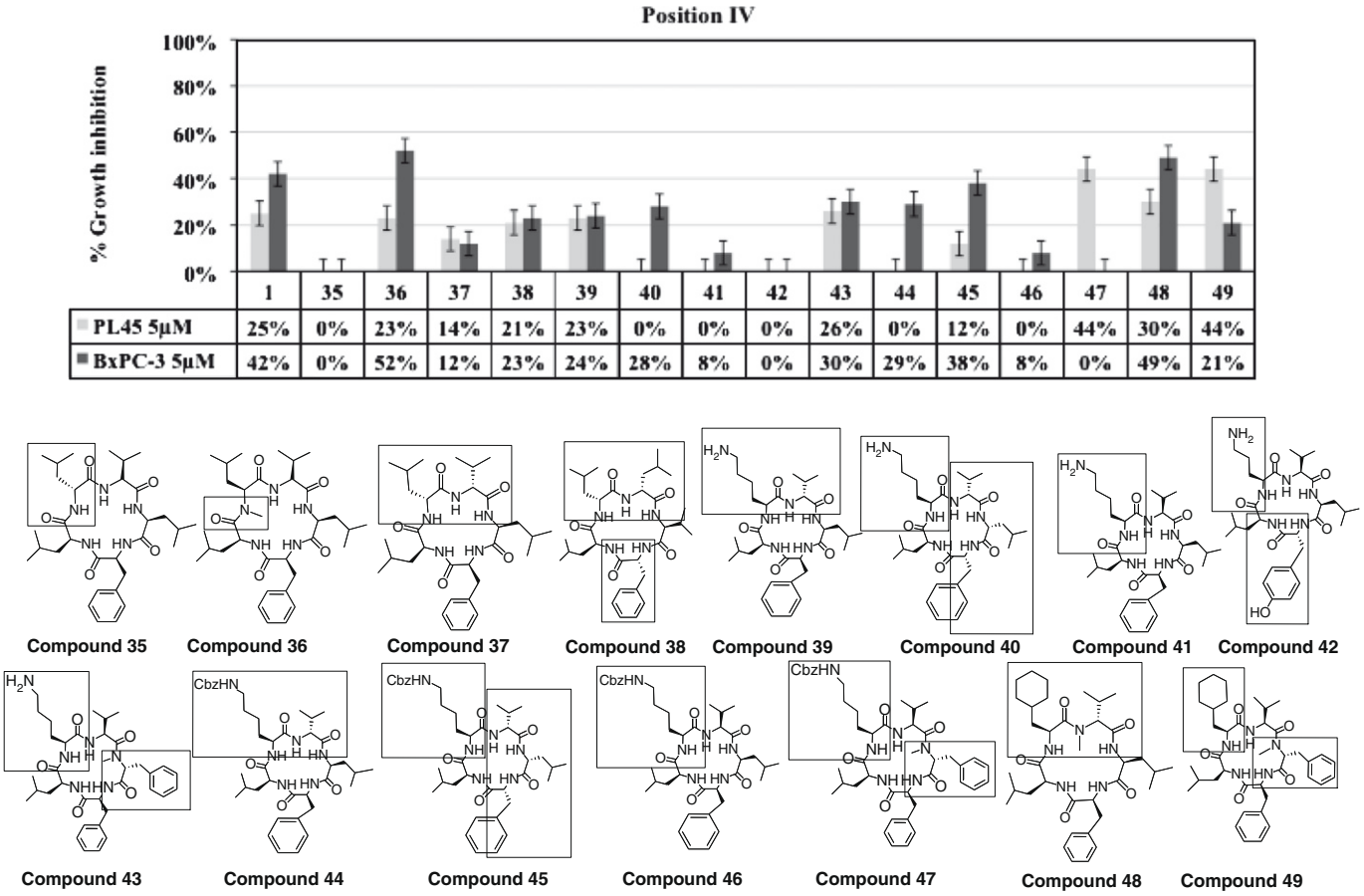


Figure 6. Compounds with alterations in positions III and/or I/II. Data is represented as % growth inhibition relative to 1% DMSO control. Each data point is an average of four wells run in three assays. All assays were run for 72 h at 5 μM compound concentration.



	1	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
PL45 5μM	25%	0%	23%	14%	21%	23%	0%	0%	0%	26%	0%	12%	0%	44%	30%	44%
BxPC-3 5μM	42%	0%	52%	12%	23%	24%	28%	8%	0%	30%	29%	38%	8%	0%	49%	21%

Figure 7. Compounds with alterations at positions IV and/or I–III. Data is represented as % growth inhibition relative to 1% DMSO control. Each data point is an average of four wells run in three assays. All assays were run for 72 h at 5 μM compound concentration.

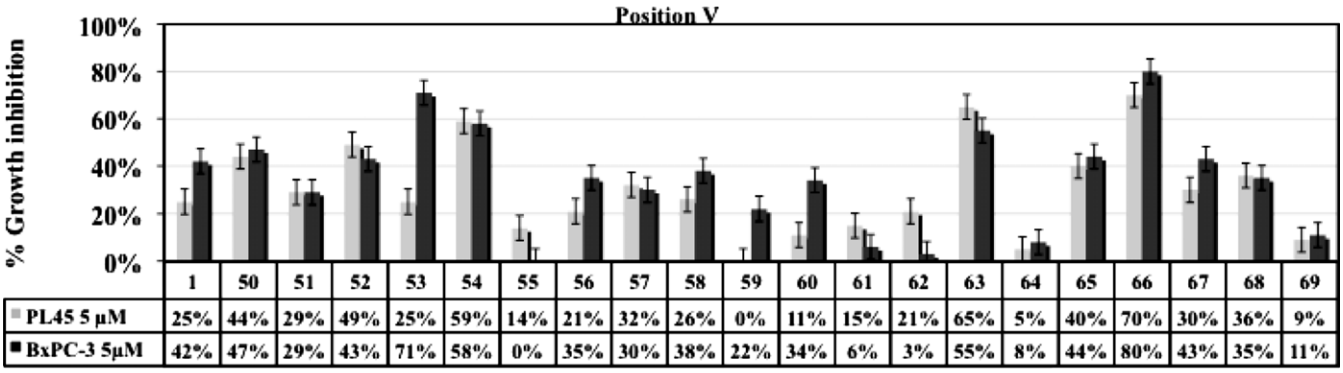
understanding of the structure–activity relationship (SAR) of 78 San A derivatives against two drug-resistant pancreatic cancer cell lines (PL45, BxPC-3).[31] This SAR study describes an overview of the cytotoxicity in cell-based assays for the San A series of molecules and establishes a relationship between the structures and stereochemistry of the active compounds' amino acid side chains and their growth inhibition against these two cell lines. We also highlight three compounds, two of which are second generation, and are potent (⩾55% cytotoxicity) in both cell lines. All three compounds have similar structural motifs: an *N*-methyl incorporated in two consecutive D-amino acids, where one of these residues is a D-Phe. Given that there is an incomplete understanding of the structure–activity relationship (SAR) of San A, we used a medicinal chemistry approach to evaluate which structural features are key to potency. This approach involves synthesizing analogs that have both single and multiple changes relative to the San A natural product peptide and testing them for potency. The core peptide structure (Fig. 3) serves as a scaffold and compounds' potency is organized by position (I–V), highlighting amino acids altered from the original San A-amide pentapeptide macrocycle (Figs. 4–8). Cell proliferation was monitored by measuring how much ^{3}H-thymidine was incorporated into a cell's DNA. This data can be analyzed to determine a particular compound's activity. ^{3}H-thymidine uptake assays were run using two distinguishable pancreatic cancer cell lines: PL45 and BxPC-3. Data shown gives percent growth inhibition at 5 μM concentrations. Only growth inhibition values greater than or equal to 55% are considered 'potent' and only those compounds that surpass this threshold in both cell lines are deemed lead structures. The use of two cell lines ensures that our compounds are consistently inhibiting drug-resistant pancreatic cancers and clearly establishes SAR. Potency exhibited by the San A peptide **1** is shown so that comparisons can be made between the natural product peptide and our synthetic analogs.

2.3. SAR position I

The histogram in Figure 4 shows the percent growth inhibition produced by the addition of compounds (5 μM concentration) that have changes to position I against two pancreatic cancer cell lines. Compared to **1**, two compounds show a moderate increased potency against PL45 and BxPC-3: compound **2**, which incorporated a D-phenylalanine, and **4** places a constrained aromatic moiety, an L-tetrahydroisoquinoline amino acid (aa), at position I. However, both compounds are only potent against one cell line, and thus they are not leads. Compounds **5** and **6** show that a H-bonding element (tyrosine and tryptophan, respectively) does not improve the cytotoxicity of the molecules over that of San A-amide. In summary, no molecules with a change to position I are considered leads.

2.4. SAR position II

The histogram in Figure 5 shows the percent growth inhibition for compounds with changes made to position II, where **15** is a second-generation structure that also incorporates a change at position I. Compound **7**, which incorporates an *N*-methyl-L-leucine at position II, shows a significant decrease in growth inhibition over that of compound **1**. Replacing the L-leucine with a D-leucine at II (compound **8**) slightly increased



	1	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69
PL45 5 μM	25%	44%	29%	49%	25%	59%	14%	21%	32%	26%	0%	11%	15%	21%	65%	5%	40%	70%	30%	36%	9%
BxPC-3 5μM	42%	47%	29%	43%	71%	58%	0%	35%	30%	38%	22%	34%	6%	3%	55%	8%	44%	80%	43%	35%	11%

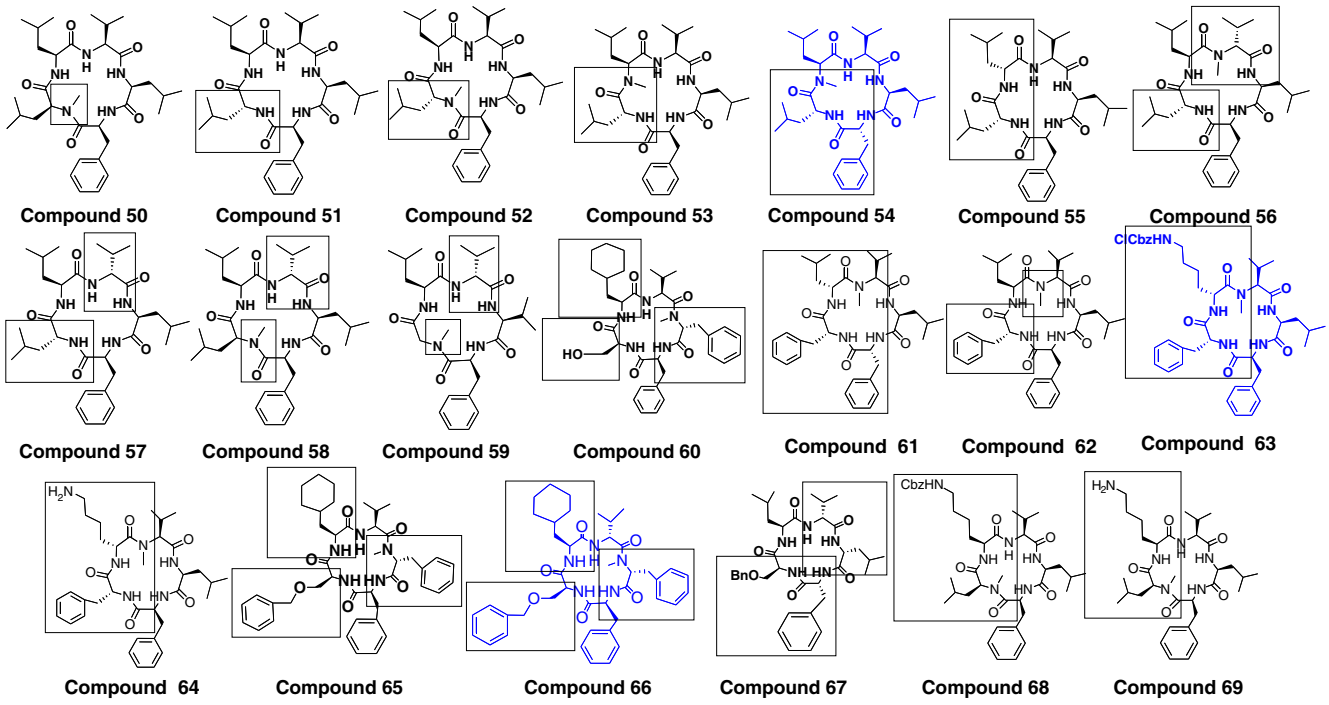
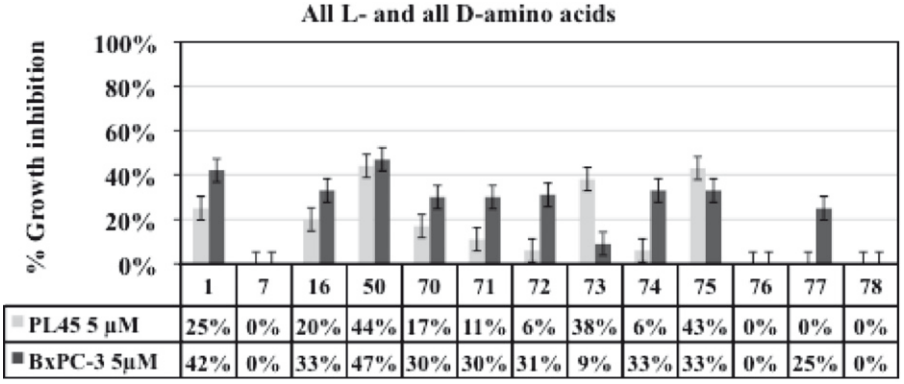


Figure 8. Compounds with alterations at position V and/or I–IV. Data is represented as % growth inhibition relative to 1% DMSO control. Each data point is an average of four wells run in three assays. All assays were run for 72 h at 5 μM compound concentration.

potency against PL45 but decreased potency against BxPC-3. However, incorporation of an *N*-methyl-D-leucine (compound **9**) dramatically decreased the potency against both cell lines. Inclusion of an aromatic moiety (compounds **10, 12**, and **14**), versus a hydrophilic moiety (compounds **11** and **13**) indicates that aromatic residues improve cytotoxicity relative to compounds with polar residues at II (e.g., compare **10**/**12** to **13**, and **11** to **14**). A new second-generation compound, **15**, contains two aromatic moieties. This molecule is cytotoxic against one cell line, showing $\geqslant$55% cytotoxicity against one cell line, but it is poor against a second cell line, as such it is not considered a valuable lead structure.

2.5. SAR position III

The histogram in Figure 6 shows the percent inhibition of growth by compounds with changes at position III (some of which also contain changes at positions I and/or II), where compounds **32**–**34** are all second-generation structures. Compound **16** incorporates an *N*-methyl-valine, compound **17** a D-valine, and compound **18**, a *N*-methyl-D-valine at position III. None show greater than 55% growth inhibition against one cell line. In contrast to **1**, which contains an isopropyl moiety at position III, compounds containing a methyl **19** or an ethyl **20** moiety were tested and showed significantly reduced potency against both cell lines. Compound **21** contains an ethyl moiety at position III along with a D-leucine at position II and shows lower cytotoxicity than **8**, which is its 'parent' compound. Interestingly, placing either a hydrophilic element or an aromatic element at position III, **22**/**24** or **23**/**25**, respectively, does not lead to increased potency against either cell line. Compounds **26** and **27** share an almost identical core structure, with the same residues in positions I, II, IV, and V, but **26** has an D-valine and **27** has D-ethyl glycine at III. Both compounds are below 55% against both cell lines. Similarly **28** is also not potent. Compound **29**, which incorporates a D-Phe at I, a D-Leu at II, and a D-valine at III, shows some potency against both cell lines, but under the 55% required to make this molecule a lead. Compounds **30** and **31** involved alterations at both positions II and III, and both have potency below 55% in each cell line. However, a new second-generation compound, **32**, exhibited improved cytotoxicity against PL45 relative to both its starting molecules, **12** and **17**, but not against both cell lines. Comparing **32** to **33** and **34** show



	1	7	16	50	70	71	72	73	74	75	76	77	78
PL45 5 μM	25%	0%	20%	44%	17%	11%	6%	38%	6%	43%	0%	0%	0%
BxPC-3 5μM	42%	0%	33%	47%	30%	30%	31%	9%	33%	33%	0%	25%	0%

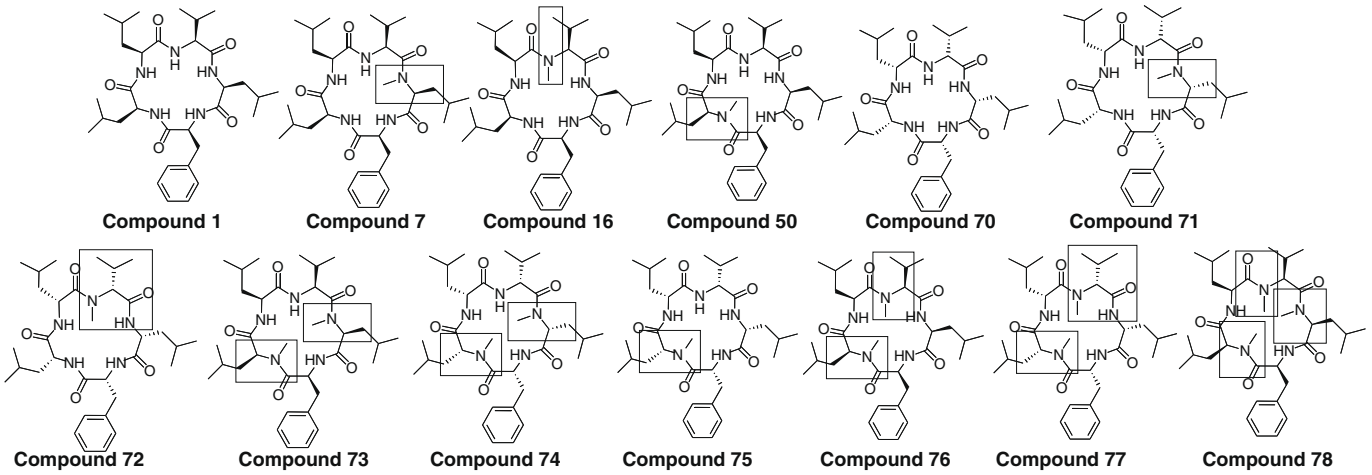


Figure 9. Compounds with *N*-methyl moieties and their enantiomers. Data is represented as % growth inhibition relative to 1% DMSO control. Each data point is an average of four wells run in three assays. Error = ±5%. All assays were run for 72 h at 5 μM compound concentration.

that the incorporation of an *N*-methyl and two non-consecutively placed D-amino acids is not enough to produce compounds with ⩾55% cytotoxicity in PL45.

2.6. SAR position IV

The histogram in Figure 7 shows the percent inhibition of growth by compounds with changes to position IV (where some also include changes to position(s) I, II and/or III). Compounds **37, 42, 43, 47**, and **49** are all second-generation structures. In contrast to potent compound **17**, which has a D-aa at III, **35** (D-aa at position IV), shows 0% inhibition against both cell lines. Further, the derivative with the *N*-methyl at III is non-potent, whereas compound **36**, which incorporates an *N*-methyl moiety at IV, showed reasonable inhibition against both cell lines. Compound **37**, which has 2 D-amino acids consecutively placed at III and IV, was not potent; neither was **38**, which has D-aa substitutions at positions I, III, and IV. Compounds **39**–**43** all contain a lysine at position IV, as well as other changes at various other positions. All five compounds are uniformly poor growth inhibitors against both cell lines. Not surprisingly, the hydrophobic compounds that are structurally related to these polar compounds, **44**–**47**, respectively, are also relatively non-toxic and only show a low level of potency against these cell lines. Further, the incorporation of an *N*-methyl D-valine at III, and a cyclohexyl moiety at position IV produced compound **48**, which showed improved cytotoxicity against both cell lines relative to its parent (**18**). Finally, second-generation compound **49**, exhibited underwhelming cytotoxicity against both cell lines.

2.7. SAR position V

The histogram in Figure 8 shows the percent inhibition of growth by compounds with changes at position V (where some also include changes at position(s) I, II, III and/or IV), where compounds **60–69** are all second-generation compounds. Compound **50** incorporates an *N*-methy-L-leucine at position 5, compound **51** a D-leucine, and compound **52** an *N*-methyl D-leucine. Both **50** and **52** exhibit good growth inhibition against both cell lines but are not ⩾55%. Interestingly **53**, which contains an *N*-methyl-L-leucine at position IV and D-leucine at position V, showed some promise in one cell line. Similar to previous observations, the inclusion of a D-Phe (at I) made **54** a promising lead structure. Incorporating a D-leucine at positions IV and V gave non-toxic **55**. Not surprisingly compounds **56** and **57**, which contain a D-leucine at position V and are structurally similar to non-potent compound **51**, showed poor growth inhibition against both cell lines. Interestingly, compounds **57** and **58** have reduced potency compared to their parent compounds **1**, **17**, **50**, or **51**. Compound **60** has a polar residue, and as noted earlier, this property makes the molecule not effective. **61** and **62** are both relatively non-toxic; yet **63** shows reasonable potency in both cell lines, with cytotoxicity values of ⩾55%. Similar to **54**, compound **63**, has two consecutive D-amino acids and an *N*-methyl moiety. Consistent with previous observations, polar derivative **64** is not toxic, nor are derivatives **68** and **69**. Comparison of compound **65** and **66** indicates that two consecutively placed D-amino acids play an important role in the potency of **66**, where **66** is also considered a lead. Compound **67** is based on **29** (Fig. 6), yet it does not perform as well as **29**. In summary, three

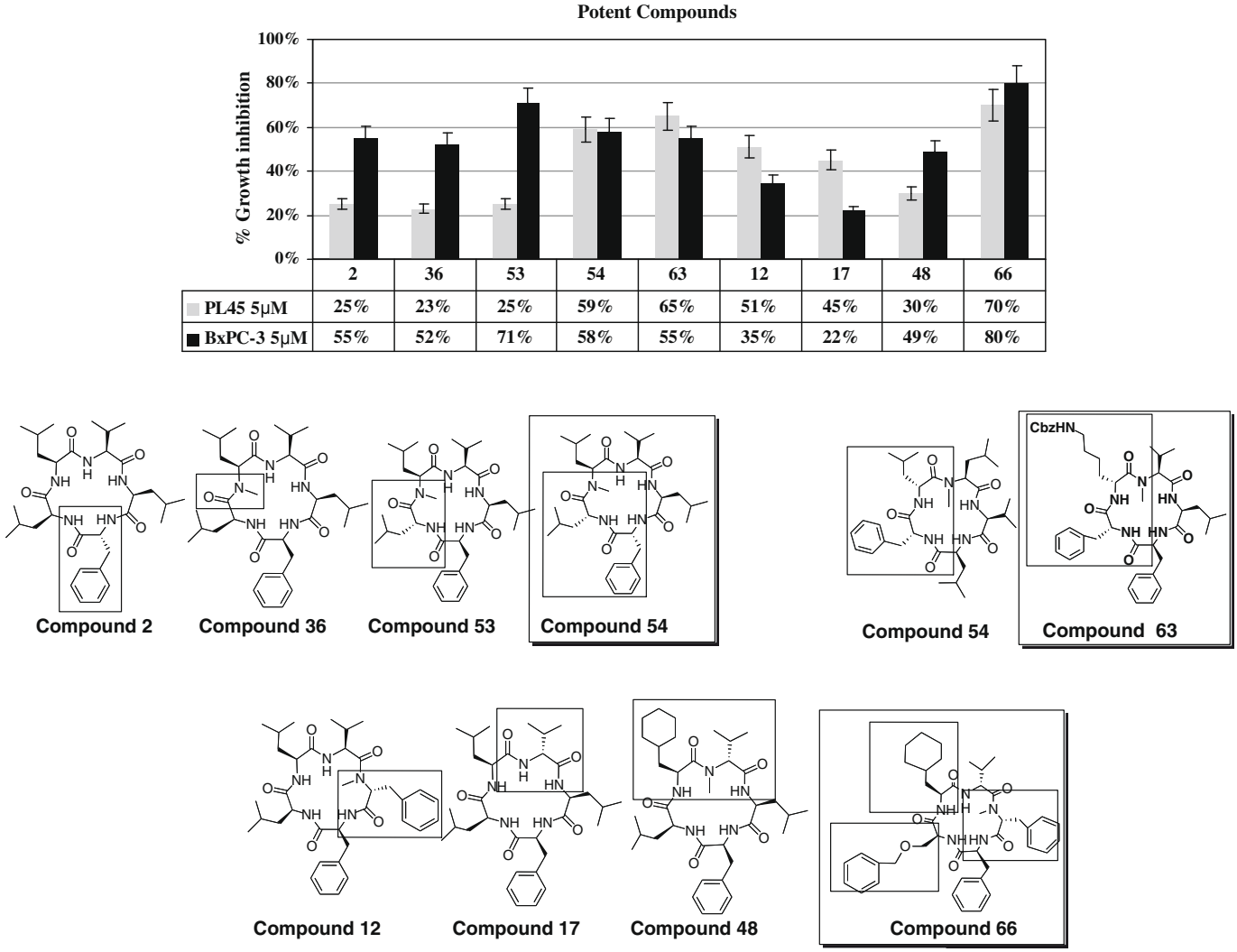


Figure 10. Most potent compounds and the compounds from which they were designed. Data is represented as % growth inhibition relative to 1% DMSO control. Each data point is an average of four wells run in three assays. All assays were run for 72 h at 5 μM compound concentration.

compounds, two being second generation, show greater than 55% in each cell line: **54**, **63**, and **66**.

2.8. SAR all L and D-amino amino acids

The histogram in Figure 9 shows the percent inhibition of growth by compounds that contain all L or all D-amino acids. Compound **70** is the enantiomer of **1**, and its cytotoxicity is lower than that of **1**. Compound **71** is the enantiomer of **7** and, in addition to all D-amino acids, it contains an *N*-methyl at position II. Although it is more potent than **7**, it is not as potent as **1**. Compound **16** and **72** are enantiomers with an *N*-methyl at position III, and both are non-potent. Compound **50** with an *N*-methyl at V is potent and interestingly its enantiomer **75** maintains relatively similar potency. Enantiomers **73** and **74**, which contain 2-*N*-methyl moieties (positions II and V), are also not active. The same is true for enantiomers **76** and **77**, which also contain 2-*N*-methyl moieties (positions III and V). Finally compound **78** with three *N*-methyls is not active. Thus, this series of molecules has no compounds with $\geqslant$55% cytotoxicity in any cell line.

2.9. Design of potent compounds

Shown in Figure 10 are the three most potent compounds: **54**, **63**, and **66** and the compounds from which they were designed. Two of these three compounds are reported here for the first time. It is important to note that all three of these compounds have an *N*-methyl associated with two consecutive D-amino acids, where one of these is a D-phenylalanine. In every case one can see that the final compound showed an average improved potency in both cell lines over the compounds used to rationally design them. Compound **54** was designed from the combination of several compounds: **2**, **36**, and **53**, and **63** was designed from compound **54**. Finally **66** was designed from **12, 17**, and **48**.

Compound **54**, which contains a *N*-methyl at IV and D-amino acids at positions I and V, was designed from compounds **2**, **36**, and **53**, where **2** contained a D-amino acid at I, **36** an *N*-methyl at IV, and **53** had both a D-amino acid at V and an *N*-methyl at IV. Thus, although these three compounds, **2**, **36**, and **53** showed some potential, **54** shows increased potency for both cell lines over that shown by the three initial compounds from which they were designed. Designed from **54** (this can be perceived by rotating **54** clockwise by one position), **63** maintained the motif of an *N*-methyl followed by two consecutive D-amino acids. Its improved cytotoxicity over that of **54** may result from a large hydrophobic moiety placed at position IV or the addition of another aromatic moiety at position 1. Compound **66** was designed from compounds 12, 17, and **48** where it is comprised of an *N*-methyl D-Phe at II (from **12**), a D-Val at III (from **17**), a cyclohexyl moiety at IV (from **48**), and the addition of a benzyl protected serine ether at V. Thus, **66** has three aromatic moieties placed around its macrocyclic back-

bone and it is significantly more potent than its precursors. It is interesting to note that several other derivatives, specifically **15** and **32**, also contain two consecutive D-amino acids and an *N*-methyl moiety, yet these two compounds are only cytotoxic against PL45, and in fact show very poor cytotoxicity against BxPC-3. **15** has a tyrosine at I, and thus, this may impact its cytotoxicity. **32** is structurally similar to **66**, but it lacks the hydrophobic moieties at IV and V, which, as noted with **63**, appear to play an important role in the generating a cytotoxic molecule.

In summary, we report here for the first time three potent compounds out of 78 derivatives, where potency was designed into our compounds using known structural features that increase their cytotoxicity, that is, *N*-methyls and D-amino acids. We successfully generated compounds that show consistency across both pancreatic cancer cell lines, and have devised a general approach to generating improved derivatives.

2.10. IC_{50} determination

Inhibition constants were calculated for the three most potent compounds by plotting five concentrations (50, 10, 0.5, and 0.1 μM) and extracting data from the curves (Supplementary data). All relationships are logarithmic in nature. We have identified two compounds, **54** and **66**, that show low micromolar cytotoxicity and one compound, **63**, that shows mid nanomolar cytotoxicity against pancreatic cancer cell lines. The IC_{50} values for the most potent compounds are shown in Figure 11, where these compounds show up to ~40-fold greater cytotoxicity than the parent natural product peptide **1**. It is interesting to note that although **63** does not display the highest cytotoxicity values against the two cell lines at 5 μM, it does show by far the best IC_{50} value, 500 nM in PL45. This appears to result from the 5 μM data point residing (consistently) lower than the curve. Thus, overall we have found a single compound (**63**) with a nanomolar IC_{50} value against these drug-resistant cancer cell lines, as well as determined that **66** shows potential as a lead. Both compounds are new derivatives, reported here for the first time.

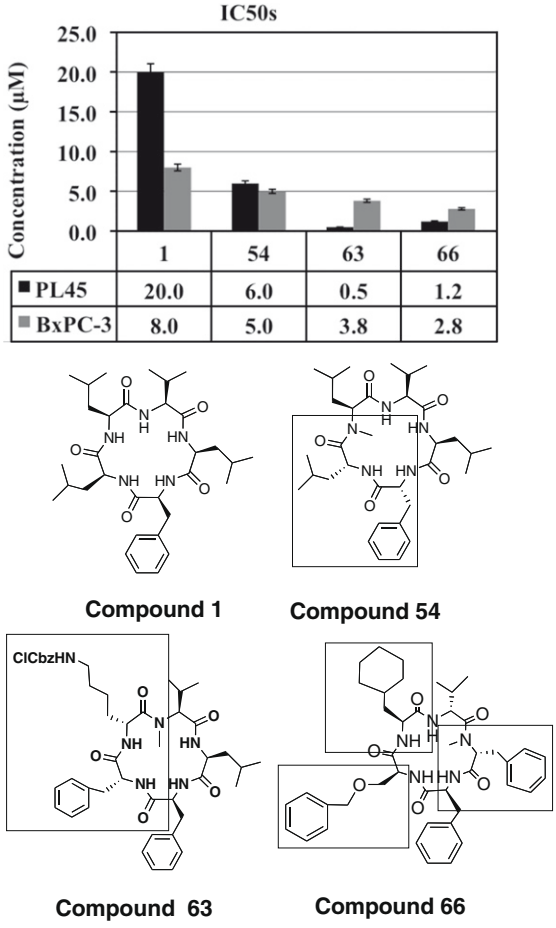


Figure 11. IC_{50s} of potent compounds. Each data point is an average of four wells run in three assays at 50, 10, 0.5, and 0.1 μM.

2.11. Summary of SAR results

The most important feature to emerge from this SAR study is the observation that molecules that are potent contain two consecutive D-amino acids and an *N*-methyl located next to the D-amino acids. Indeed, all potent compounds contain two consecutive D-amino acids, one of which is a D-phenylalanine, and an *N*-methyl on or next to the D-amino acids. It is easy to recognize this pattern by comparing the potency of **2**, **36**, and **53** to **54** and that of **12**, **17**, and **48** to **66**. Neither of the parent compounds are as cytotoxic as **54** and **66**, respectively.

Further, comparison of the potency of **65** to **66** clearly indicates that two D-amino acids next to each other significantly improve the compound's cytotoxicity. Further validating our hypothesis is comparison of compounds **30** and **31**, where **30** contains two D-amino acids sequentially placed, and **31** contains two *N*-methyl D-amino acids successively placed. Both have low cytotoxicity relative to our lead compounds. Thus, it is not enough to have two sequential D-amino acids, nor two *N*-methyl D-amino acids to generate a lead structure. Rather, as observed by others in this pentapeptide system, D-amino acids play an important role in achieving a favorable conformation.[32–34]

Finally, it is important to note that structurally similar compound **56** contains 2 D-amino acids and an *N*-methyl but the D-amino acids are not consecutive, and its potency is poor relative to that of **54** or **66**. Also note that **34** is not nearly as potent as other compounds with consecutive D-amino acids. Finally, more than one *N*-methyl and/or more than two consecutively placed D-amino acids are not potent (i.e., **61**, **76**, **77**, and **78**).

In summary, all compounds found to exhibit cytotoxicity that was equal to or greater than 55% in each pancreatic cancer cell line contained a single *N*-methyl and two sequentially placed D-amino acids. As such, we surmise that two factors are important for potency: a single *N*-methyl in conjunction with two consecutively placed D-amino acids, where one is a D-phenylalanine. This theory is validated by several current examples described in the recent literature, where cyclic peptides, specifically pentapeptides, with D-amino acids lock the macrocycle into a specific conformation.[32–34] Further, it is well established that these cyclic pentapeptides mimic beta and gamma turns and serve as templates for the appropriate positioning of suitable binding motifs for proteins.[35,36] Thus, we believe that the inclusion of two successive D-amino acids coupled with a single *N*-methyl locks the macrocycle into a suitable position for binding to its biological target.

3. Conclusion

We report here for the first time a comprehensive SAR evaluation using two generations of compounds, where 19 new structures and 59 previously published structures were described and examined for their cytotoxicity against two drug-resistant pancreatic cancer cell lines. This global assessment included the determination that placement of a single *N*-methyl and two consecutively placed D-amino acids are required for compounds to exhibit ⩾55% cytotoxicity in both cell lines, where one compound exhibited a 500 nM IC_{50}. Two of the three most potent compounds were second-generation compounds,

and these two compounds had improved IC_{50}s over the first-generation structure. Our three most potent small molecules target drug-resistant pancreatic cancer cell lines, and share no homology with other classes of chemotherapeutic agents and have reasonable *C* log *P* values[37] and molecular weights (600–738). Studies on third-generation compounds are on going and will be reported in due course.

4. Experimental

4.1. General remarks

All coupling reactions were performed under argon atmosphere with the exclusion of moisture. All reagents were used as received. Anhydrous methylene chloride Dri Solv (EM), anhydrous tetrahydrofuran, anhydrous dimethylformamide, and anhydrous acetonitrile Dri Solv (EM) were obtained from VWR, and were packed under nitrogen with a septum cap. Diisopropylethylamine (DIPEA) was purchased from Aldrich, packaged under nitrogen in a sure seal bottle. The coupling agent HATU came from: Applied Biosystems at 850 Lincoln Center Dr. Foster City, CA 94404, USA. Tel.: +1 800 327 3002 and the coupling agents TBTU NovaBiochem. DEPBT [3(diethoxyphosphoryloxy)-1,2,3-benzotriazine-4(3H)] was purchased from Aldrich (order number 49596-4). The ^{1}H NMR spectra were recorded on the Varian at 200 MHz or 400 MHz or 500 MHz. LC–MS was performed at San Diego State University using HP1100 Finnigan LCQ. Flash column chromatography used 230–400 mesh 32–74 lm 60 Å silica gel from Bodman Industries.

4.2. Thymidine incorporation/growth inhibition assay

Proliferation of the PL-45 and BxPC-3 pancreatic cancer cells was tested in the presence and absence of the compounds using ^{3}H-thymidine uptake assays. Cells treated with the compounds were compared to DMSO controls for their ability to proliferate as indicated by the incorporation of ^{3}H-thymidine into their DNA. Cells were cultured in 96-well plates at a concentration of 2000–2500 cells/well in DMEM (Gibco) supplemented with L-glutamine, 10% fetal bovine serum and 1% penicillin–streptomycin antibiotic. After incubation for 6 h, the compounds were added. The compounds were dissolved in DMSO at a final concentration of 1% and tested at the concentrations indicated in the manuscript. The DMSO control was also at 1%. After the cells had been incubated with the compounds for 56 h, 1 mCi ^{3}H-thymidine per well was added and the cells were cultured for an additional 16 h (for the cells to have a total of 72 h treatment), at which time the cells were harvested using a PHD cell harvester (Cambridge Technology Inc.). The samples were then counted (CPM) in a scintillation counter for 5 m. Decreases in ^{3}H-thymidine incorporation, as compared to DMSO controls, are an indication that the cells are no longer progressing through the cell cycle or synthesizing DNA, as is shown in the studies presented. Mean growth inhibition (n = 8–12) is the 1 minus CPM of compound-treated cells over DMSO-treated cells. IC_{50} were determined using 0, 0.1, 0.5, 5, 10, and 50 μM of compound. All calculations including mean, SEM, and IC_{50} were performed on Excel.

4.3. Synthesis

For synthesis details of the first-generation compounds see Ref. 6. For second-generation compounds see Ref. 7. Potent compounds' final characterization data are listed below.

4.3.1. General peptide synthesis

All peptide coupling reactions were carried out under argon with dry solvent, using methylene chloride for dipeptide and tripeptide couplings and a mixture of dimethylformamide, methylene chloride, and acetonitrile for pentapeptide couplings. The amine (1.1 equiv) and acid (1 equiv) were weighed into a dry flask along with 4–12 equiv of DIPEA and 1.1 equiv of TBTU.[†] Anhydrous methylene chloride was added to generate a 0.1 M solution. The solution was stirred at room temperature and reactions were monitored by TLC. Reactions were run for 1 h before checking via TLC. If reaction was not complete additional 0.25 equiv were of HATU and TBTU were added. If reaction was complete then workup was done by washing with aqueous HCl (pH 1) and saturated sodium bicarbonate. (Note: if acetonitrile was used for the reaction, methylene chloride was added to reaction upon workup and the resulting solution was washed with the aqueous solvents.) After back extraction of aqueous layers with methylene chloride, organic layers were combined, dried over sodium sulfate, filtered and concentrated. Flash chromatography using a gradient of ethyl acetate–hexane gave our desired peptide.

4.3.2. General amine deprotection

Amines were deprotected using 20% TFA in methylene chloride (0.1 M) with 2 equiv of anisole. The reactions were monitored by TLC, where the TLC sample was first worked up in a mini-workup using DI water and methylene chloride to remove TFA. Reactions were allowed to run for 1–2 h and then concentrated in vacuo.

4.3.3. General acid deprotection

Acids were deprotected using ~4 equiv of lithium hydroxide (or until pH ~11) in methanol (0.1 M). The peptide was placed in a flask, along with lithium hydroxide and methanol and stirred overnight. Within 12 h the acid was usually deprotected. Workup of reactions involved the acidification of reaction solution using HCl to pH 1. The aqueous solution was extracted three times with methylene chloride, and the combined organic layer was dried, filtered, and concentrated in vacuo.

4.3.4. Macrocyclization procedure (in situ)

All pentapeptides were acid and amine deprotected using concentrated HCl (eight drops per 0.3mmol of linear pentapeptide) in THF (0.05 M). Anisole (2 equiv) was added to the reaction and the reaction was stirred at room temperature. The reaction typically took 4 days, but TLC and LC–MS were used to monitor the reaction every 12 h. LC–MS data typically indicated the reaction was ~50% complete after the first day. Addition of four drops of concentrated HCl per 0.3 mmol of pentapeptide, stirring at RT overnight and checking the reaction via LC–MS usually showed ~75% completion. On the fourth day verification of the presence of the free amine and free acid and disappearance of the starting linear protected pentapeptide permitted workup. The reaction was concentrated in vacuo and the crude, dry, double deprotected peptide (free acid and free amine) was dissolved in a minimum solution of DMF: methylene chloride: acetonitrile (2:2:1 ratio). Three coupling agents (DEPBT, HATU, and TBTU) were used at ~0.5–0.75 equiv each. These coupling agents were dissolved in a calculated volume of dry 40% DMF, 40% methylene chloride, and 20% acetonitrile that would give a 0.01 M overall solution when included in the volume used for the deprotected peptide. The coupling agents were then added to the deprotected peptide solution. DIPEA (6 equiv or more in order to neutralize the pH) were then added to the reaction. The coupling agents are typically not very soluble in acetonitrile, which is why a combination of solvents is used.

After 1 h, TLC and LC–MS (where the LC–MS sample was worked up prior to injection) indicated that a product spot was developing.

[†] Some coupling reactions would not go to completion using only TBTU and therefore ~0.25 equiv of HATU, and/or DEPBT were used. In a few cases up to 1.7 equiv of all three coupling reagents were used.

The comparison R_f value in the product spot on TLC was the protected linear pentapeptide. The reactions were always complete after 3 h, and monitoring the starting material deprotected pentapeptide via LC–MS was the easiest method of determining completion. Upon completion, the reaction was worked up by washing with aqueous HCl (pH 1) and saturated sodium bicarbonate. After back extraction of aqueous layers with large quantities of methylene chloride, the organic layers were combined, dried, filtered and concentrated. All macrocycles were purified by initially running a crude plug of compound using an ethyl acetate/hexane gradient on silica gel, then running a column on the isolated product. Finally, when necessary reverse phase-HPLC was used for additional purification using a gradient of acetonitrile and DI water with 0.1% TFA.

4.3.5. Synthesis of compound 15

4.3.5.1. Dipeptide MeO-Val-Leu-NHBoc. Dipeptide MeO-Val-Leu-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 370 mg (2.2 mmol, 1.1 equiv) of amine OMe-Val-NH_2, 500 mg (2.0 mmol, 1.0 equiv) of acid HO-Leu-NHBoc, 1.4 mL (8 equiv) of DIPEA, 515.2 mg (1.6 mmol, 0.8 equiv) of TBTU, 305 mg (0.8 mmol, 0.4 equiv) The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the dipeptide (625 mg, 90% yield).

R_f: 0.5 (EtOAc:Hex 1:2).

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.9–1.0 (m, 12H), 1.4 (s, 9H), 1.6–1.8 (m, 2H), 2.1–2.3 (m, 1H), 3.7 (s, 3H), 4.0–4.1 (m, αH), 4.4–4.5 (m, αH), 4.8–4.9 (br, 1H), 6.6 (d, 1H).

4.3.5.2. Dipeptide MeO-Val-Leu-NH_2. Dipeptide MeO-Val-Leu-NH_2 was synthesized following the 'General amine deprotection'. This dipeptide was taken on to the next reaction without further purification or characterization. (446 mg, 100% yield).

4.3.5.3. Tripeptide MeO-Val-Leu-Leu-NHBoc. Tripeptide MeO-Val-Leu-Leu-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 689 mg (2.82 mmol, 1.0 equiv) of amine MeO-Val-Leu-NH_2, 774 mg (3.11 mmol, 1.0 equiv) of acid HO-Leu-NHBoc, 1.9 mL (4 equiv) of DIPEA, 544 mg (1.69 mmol, 0.6 equiv) of TBTU, 644 mg (1.69 mmol, 0.6 equiv) of HATU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the tripeptide (887 mg, 60% yield).

R_f: 0.5 (EtOAc:Hex 1:1).

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.8–1.1 (m, 12H), 1.4 (s, 9H), 2.8 (s, 3H), 3.7 (s, 3H), 4.0–4.2 (m, αH), 4.4–4.6 (m, 2αH), 4.9 (d, 1H), 6.5–6.7 (d, 2H).

4.3.5.4. Dipeptide MeO-D-Tyr-*N*-Me-D-Phe-NBoc. Dipeptide MeO-D-Tyr-*N*-Me-D-Phe-NBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 318 mg (1.4 mmol, 1.1 equiv) of amine MeO-D-Tyr-NH_2, 576 mg (1.3 mmol, 1.0 equiv) of acid HO-*N*-Me-D-Phe-NBoc, 1.4 mL (8 equiv) of DIPEA, 515.2 mg (1.6 mmol, 0.8 equiv) of TBTU, 380 mg (0.8 mmol, 0.6 equiv) of HATU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the dipeptide (625 mg, 90% yield).

R_f: 0.5 (EtOAc:Hex 1:2).

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.4 (m, 9H), 2.8 (s, 3H), 2.9–3.4 (m, 4H), 3.7 (s, 3H), 4.7–4.9 (m, 2αH), 6.0 (br, 1H), 6.2 (br, 1H), 6.7–7.0 (dd, 4H), 7.1–7.3 (m, 5H).

4.3.5.5. Dipeptide MeO-D-Tyr-*N*-Me-D-Phe-NH. Dipeptide MeO-D-Tyr-*N*-Me-D-Phe-NH was synthesized following the 'General amine deprotection'. This dipeptide was taken on to the next reaction without further purification or characterization. (625 mg, 100% yield).

4.3.5.6. Dipeptide HO-Val-Leu-Leu-NHBoc. Dipeptide HO-Val-Leu-Leu-NHBoc was synthesized following the 'General acid deprotection'. This tripeptide was taken on to the next reaction without further purification or characterization. (661 mg, 88% yield).

4.3.5.7. Pentapeptide MeO-D-Tyr-*N*-Me-D-Phe-Val-Leu-Leu-NHBoc. Pentapeptide MeO-D-Tyr-*N*-Me-D-Phe-Val-Val-Leu-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 503.4 mg (1.41 mmol, 1.0 equiv) of amine Meo-D-Tyr-D-MePhe-NH, 689 mg (1.55 mmol, 1.0 equiv) of acid HO-Val-Leu-Leu-NHBoc, 0.86 mL (3.5 equiv) of DIPEA, 91 mg (0.28 mmol, 0.2 equiv) of TBTU, and 322 mg (0.85 mmol, 0.6 equiv) HATU,169 mg (0.56 mmol, 0.4 equiv) of DEPBT. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the pentapeptide (727 mg, 60% yield).

R_f: 0.5 (EtOAc:Hex 2:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.6–0.8 (m, 6H), 0.8–1.0 (m, 12H), 1.5–1.8 (m, 5H), 2.6 (m, 2H), 2.8–3.4 (m, 4H), 3.7 (s, 3H), 4.2 (m, αH), 4.4–4.5 (m, αH), 4.5–4.6 (m, αH), 4.6–4.7 (m, αH), 4.7–4.8 (m, αH), 5.0 (d, 1H), 6.6–6.8 (br, 2H), 6.7–7.4 (m, 9H).

4.3.5.8. Macrocycle D-Tyr-*N*-Me-D-Phe-Val-Leu-Leu. Macrocycle D-Tyr-*N*-Me-D-Phe-Val-Leu-Leu was synthesized following the 'Macrocyclization procedure'. Utilizing 182.4 mg (0.27 mmol, 1.0 equiv) of linear pentapeptide, 0.19 mL (4 equiv) of DIPEA, 52.6 mg (0.16 mmol, 0.6 equiv) of TBTU, 41.5 mg (0.11 mmol, 0.4 equiv) HATU, and 16.3 mg (0.11 mmol, 0.4 equiv) of DEPBT. The crude reaction was purified by reverse phase-HPLC to yield the macrocycle (18 mg, 10% yield).

R_f: 0.5 (EtOAc:Hex 4:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.6–1.0 (m, 18H), 1.6–1.8 (m, 4H), 1.8–2.0 (m, 1H), 2.9 (s, 3H), 2.6–3.2 (m, 4H), 4.1 (m, αH), 4.2 (m, αH), 4.3–4.5 (m, αH), 4.6–4.7 (m, αH), 5.5 (m, αH), 6.7–7.0 (m, 4H), 7.1–7.4 (m, 5H).

LC–MS: m/z calcd for $C_{33}H_{51}N_5O_5$ (M+1) = 598.39, found 598.3.

4.3.6. Synthesis of compound 32

4.3.6.1. Dipeptide MeO-Phe-*N*-Me-D-Phe-NBoc. Dipeptide MeO-Phe-*N*-Me-D-Phe-NBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 509 mg (1.51 mmol, 1.1 equiv) of amine MeO-Phe-NH_2, 600 mg (1.37 mmol, 1.0 equiv) of acid HO-*N*-Me-D-Phe-NBoc, 1.5 mL (4 equiv) of DIPEA, 828 mg (2.58 mmol, 1.2 equiv) of TBTU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the dipeptide (520.1 mg, 86% yield).

R_f: 0.7 (EtOAc:Hex 1:1).

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.4 (s, 9H), 2.7 (s, 3H), 3.1–3.2 (m, 4H), 3.7 (s, 3H), 4.8–5.0 (m, 2αH), 7.1–7.3 (dd, 10H).

4.3.6.2. Dipeptide MeO-Phe-*N*-Me-D-Phe-NH. Dipeptide MeO-Phe-*N*-Me-D-Phe-NH was synthesized following the 'General amine deprotection'. This dipeptide was taken on to the next reaction without further purification or characterization. (521 mg, 100% yield).

4.3.6.3. Tripeptide MeO-Phe-*N*-Me-D-Phe-D-Val-NHBoc. Tripeptide MeO-Phe-*N*-Me-D-Phe-D-Val-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 521 mg (1.5 mmol, 1.1 equiv) of amine MeO-Phe-*N*-Me-D-Phe-NH, 302.3 mg (1.39 mmol, 1.0 equiv) of acid HO-D-Val-NHBoc, 0.97 mL (4 equiv) of DIPEA, 268 mg (0.83 mmol, 0.6 equiv) of TBTU, and 317 mg (0.83 mmol, 0.6 equiv) of HATU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the tripeptide (316 mg, 45% yield).

R_f: 0.3 (EtOAc:Hex 3:7).

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.5 (d, 6H), 1.4 (s, 9H), 2.9 (s, 3H), 3.1 (t, 4H), 3.7 (s, 3H), 4.1 (m, αH), 4.8 (m, αH), 5.0 (d, 1H), 5.5 (m, αH), 7.2-7.3 (dd, 10H).

4.3.6.4. Tripeptide MeO-Phe-D-MePhe-D-Val-NH_2. Tripeptide MeO-Phe-*N*-Me-D-Phe-D-Val-NH_2 was synthesized following the 'General amine deprotection'. This tripeptide was taken on to the next reaction without further purification or characterization. (316 mg, 100% yield).

4.3.6.5. Dipeptide MeO-Leu-Leu-NHBoc. Dipeptide MeO-Leu-Leu-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 401 mg (2.2 mmol, 1.1 equiv) of amine MeO-Leu-NH_2, 500 mg (2.0 mmol, 1.0 equiv) of acid HO-Leu-NHBoc, 1.4 mL (4 equiv) of DIPEA, 774 mg (1.2 mmol, 1.0 equiv) of TBTU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the dipeptide (703 mg, 98% yield).

R_f: 0.35 (EtOAc:Hex 2.5:7.5).

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.9–1.0 (m, 12H), 1.4 (s, 9H), 1.6–1.8 (m, 6H), 3.7 (s, 3H), 4.0–4.1 (m, αH), 4.5–4.7 (m, αH), 4.8–4.9 (br, 1H), 6.4 (d, 1H).

4.3.6.6. Dipeptide HO-Leu-Leu-NHBoc. Dipeptide HO-Leu-Leu-NHBoc was synthesized following the 'General acid deprotection'. This dipeptide was taken on to the next reaction without further purification or characterization. (349 mg, 58% yield).

4.3.6.7. Pentapeptide MeO-Phe-*N*-Me-D-Phe-D-Val-Leu-Leu-NHBoc. Pentapeptide MeO-Phe-*N*-Me-D-Phe-D-Val-Leu-Leu-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 316 mg (0.72 mmol, 1.1 equiv) of amine MeO-Phe-*N*-Me-D-Phe-D-Val-NH_2, 240 mg (0.65 mmol, 1.0 equiv) of acid HO-Leu-Leu-NHBoc, 0.92 mL (8 equiv) of DIPEA, 126 mg (0.39 mmol, 0.6 equiv) of TBTU, 174 mg (0.45 mmol, 0.7 equiv) HATU, and 78 mg (0.26 mmol, 0.4 equiv) of DEPBT. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the pentapeptide (172 mg, 34% yield).

R_f: 0.3 (EtOAc:Hex 1:1).

^{1}H NMR (400 MHz, CD_3OD): δ 0.7-0.9 (m, 18H), 1.4 (s, 9H), 1.4–1.6 (m, 2H), 2.0–2.2 (m, 4H), 2.8 (m, 1H), 3.0 (m, 1H), 3.0–3.2 (m, 4H), 3.3 (s, 3H), 3.7 (s, 3H), 4.0–4.1 (m, αH), 4.3–4.4 (m, αH), 4.6–4.7 (m, αH), 5.1 (m, αH), 5.3 (m, αH), 7.1–7.3 (m, 10H).

4.3.6.8. Macrocycle Phe-*N*-Me-D-Phe-D-Val-Leu-Leu. Macrocycle Phe-*N*-Me-D-Phe-D-Val-Leu-Leu was synthesized following the 'Macrocyclization procedure'. Utilizing 139 mg (0.21 mmol, 1.0 equiv) of linear pentapeptide, 0.3 mL (8 equiv) of DIPEA, 41 mg (0.13 mmol, 0.6 equiv) of TBTU, 65 mg (0.17 mmol, 0.8 equiv) HATU, and 13 mg (0.04 mmol, 0.2 equiv) of DEPBT. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) yield the macrocycle (2.3 mg, 0.07% yield).

R_f: 0.3 (EtOAc:Hex 1:1).

^{1}H NMR (400 MHz, CD_3OD): δ 0.8–1.0 (m, 18H), 1.3 (m, 2H), 4.4–1.6 (m, 4H), 2.6 (m, 1H), 3.2–3.4 (m, 7H), 4.2 (m, αH), 4.6 (m, αH), 5.0 (m, 3αH), 7.2 (dd, 10H).

LC–MS: *m*/*z* calcd for $C_{36}H_{51}N_5O_5$ (M+1) = 634.82, found 634.5.

4.3.7. Synthesis of compound 33

4.3.7.1. Dipeptide MeO-D-Phe-Leu-NHBoc. Dipeptide MeO-D-Phe-Leu-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 951 mg (4.4 mmol, 1.1 equiv) of amine MeO-D-Phe-NH_2, 1000 mg (4.0 mmol, 1.0 equiv) of acid HO-Leu-NHBoc, 2.8 mL (4 equiv) of DIPEA, 1545 mg (4.8 mmol, 1.2 equiv) of TBTU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the dipeptide (1381 mg, 88% yield).

R_f: 0.3 (EtOAc:Hex 3:7).

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.95 (d, 6H), 1.4 (s, 9H), 1.6–1.8 (m, 3H), 3.2 (m, 2H), 3.7 (s, 3H), 4.0–4.1 (m, αH), 4.8–5.0 (m, αH), 6.4–6.6 (d, 1H), 7.1–7.4 (m, 5H).

4.3.7.2. Dipeptide MeO-D-Phe-Leu-NH_2. Dipeptide MeO-D-Phe-Leu-NH_2 was synthesized following the 'General amine deprotection'. This dipeptide was taken on to the next reaction without further purification or characterization. (1029 mg, 100% yield).

4.3.7.3. Tripeptide MeO-D-Phe-Leu-D-Val-NHBoc. Tripeptide MeO-D-Phe-Leu-D-Val-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 343 mg (1.17 mmol, 1.1 equiv) of amine MeO-D-Phe-Leu-NH_2, 230.4 mg (1.06 mmol, 1.0 equiv) of acid HO-D-Val-NHBoc, 1.49 mL (8 equiv) of DIPEA, 408 mg (1.25 mmol, 1.2 equiv) of TBTU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the tripeptide (443.8 mg, 85% yield).

R_f: 0.7 (EtOAc:Hex 1:1).

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.9–1.0 (m, 12H), 1.5 (s, 12H), 1.6–1.8 (m, 3H), 2.2 (m, 2H), 3.1 (m, 2H), 3.7 (s, 3H), 3.9–4.0 (dd, 1H), 4.4 (m, αH), 4.9 (dd, αH), 5.0 (m, αH), 6.3 (d, 1H), 6.6 (m, 1H), 7.0–7.4 (m, 5H).

4.3.7.4. Tripeptide MeO-D-Phe-Leu-D-Val-NH_2. Tripeptide MeO-D-Phe-Leu-D-Val-NH_2 was synthesized following the 'General amine deprotection'. This tripeptide was taken on to the next reaction without further purification or characterization. (353.5 mg, 100% yield).

4.3.7.5. Dipeptide MeO-Leu-Leu-NHBoc. Dipeptide MeO-Leu-Leu-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 401 mg (2.2 mmol, 1.1 equiv) of amine MeO-Leu-NH_2, 500 mg (2.0 mmol, 1.0 equiv) of acid HO-Leu-NHBoc, 1.4 mL (4 equiv) of DIPEA, 774 mg (1.2 mmol, 1.0 equiv) of TBTU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the dipeptide (627.4 mg, 87.2% yield).

R_f: 0.35 (EtOAc:Hex 2.5:7.5).

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.9–1.0 (m, 12H), 1.4 (s, 9H), 1.6–1.8 (m, 6H), 3.7 (s, 3H), 4.0–4.1 (br, αH), 4.5–4.7 (m, αH), 4.8–4.9 (br, 1H), 6.4 (d, 1H).

4.3.7.6. Dipeptide HO-Leu-Leu-NHBoc. Dipeptide HO-Leu-Leu-NHBoc was synthesized following the 'General acid deprotection'. This dipeptide was taken on to the next reaction without further purification or characterization. (571.8 mg, 91% yield).

4.3.7.7. Pentapeptide MeO-D-Phe-Leu-D-Val-Leu-Leu-NHBoc. Pentapeptide MeO-D-Phe-Leu-D-Val-Leu-Leu-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 443.8 mg (1.1 mmol, 1.1 equiv) of amine MeO-D-Phe-Leu-D-Val-NH_2, 354 mg (1. mmol, 1.0 equiv) of acid HO-Leu-Leu-NHBoc, 1.44 mL (8 equiv) of DIPEA, 198 mg (0.62 mmol, 0.6 equiv) of TBTU, and 235 mg (0.62 mmol, 0.6 equiv) HATU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the pentapeptide (545 mg, 73% yield).

R_f: 0.6 (EtOAc:Hex 1:1).

^{1}H NMR (400 MHz, CD_3OD): δ 0.7–0.9 (m, 24H), 1.4 (s, 9H), 1.5–1.7 (m, 6H), 2.2 (m, 2H), 3.0 (m, H), 3.1 (m, H), 3.7 (s, 3H), 4.0–4.2 (m, 2αH), 4.3–4.5 (m, 2αH), 4.6–4.7 (m, H), 7.0–7.4 (m, 5H).

4.3.7.8. Macrocycle MeO-D-Phe-Leu-D-Val-Leu-Leu-NHBoc. Macrocycle D-Phe-Leu-D-Val-Leu-Leu was synthesized following the 'Macrocyclization procedure'. Utilizing 226 mg (0.37 mmol, 1.0 equiv) of linear pentapeptide, 0.67 mL (10 equiv) of DIPEA,

96.04 mg (0.3 mmol, 0.8 equiv) of TBTU, 113.7 mg (0.3 mmol, 0.8 equiv) HATU, and 44.8 mg (0.15 mmol, 0.4 equiv) of DEPBT. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) yield the macrocycle (1 mg, 0.5 % yield).

R_f: 0.3 (100% EtOAc).

^{1}H NMR (500 MHz, CD_3OD): δ 0.7–0.9 (m, 24H), 1.2–1.7 (m, 9H), 2.0–2.1 (m, H), 2.2 (m, 1H), 3.0 (m, H), 3.1 (m, H), 3.9 (d, αH), 4.0 (dd, αH), 4.4–4.6 (m, 2αH), 5.2 (t, H), 7.0–7.4 (m, 5H).

LC–MS: m/z calcd for $C_{32}H_{51}N_5O_5$ (M+1) = 586.78, found 586.6.

4.3.8. Synthesis of compound 34

4.3.8.1. Dipeptide MeO-D-Phe-Leu-NHBoc. Dipeptide MeO-D-Phe-Leu-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 480 mg (2.2 mmol, 1.1 equiv) of amine MeO-D-Phe-NH_2, 505 mg (2.0 mmol, 1.0 equiv) of acid HO-Leu-NHBoc, 1.4 mL (4 equiv) of DIPEA, 772 mg (2.4 mmol, 1.2 equiv) of TBTU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the dipeptide (786 mg, 94% yield).

R_f: 0.9 (EtOAc:Hex 1:1).

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.8–1.0 (m, 6H), 1.4 (s, 9H), 1.6 (m, 2H), 1.8–2.0 (m, 1H), 3.0–3.2 (m, 2H), 3.7 (s, 3H), 4.0–4.2 (br, 1H), 4.8 (br, αH), 4.8–5.0 (q, αH), 6.5 (m, 1H), 7.1–7.4 (m, 5H).

4.3.8.2. Dipeptide HO-D-Phe-Leu-NHBoc. Dipeptide HO-D-Phe-Leu-NHBoc was synthesized following the 'General acid deprotection'. This dipeptide was taken on to the next reaction without further purification or characterization. (632 mg, 89% yield).

4.3.8.3. Dipeptide MeO-Leu-Leu-NHBoc. Dipeptide MeO-Leu-Leu-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 801 mg (4.4 mmol, 1.1 equiv) of amine MeO-Leu-NH_2, 1.0 g (4.0 mmol, 1.0 equiv) of acid HO-Leu-NHBoc, 2.8 mL (4 equiv) of DIPEA, 1.5 g (4.8 mmol, 1.2 equiv) of TBTU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the dipeptide (1.4 g, 99% yield).

R_f: 0.9 (EtOAc:Hex 1:1).

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.9–1.0 (m, 12H), 1.4 (s, 9H), 1.6–1.8 (t, 6H), 3.7 (s, 3H), 4.0–4.1 (dd, αH), 4.5–4.7 (m, αH), 4.8–4.9 (br, 1H), 6.4 (d, 1H).

4.3.8.4. Dipeptide HO-Leu-Leu-NHBoc. Dipeptide HO-Leu-Leu-NHBoc was synthesized following the 'General acid deprotection'. This dipeptide was taken on to the next reaction without further purification or characterization. (1.2 g, 82% yield).

4.3.8.5. Tripeptide MeO-*N*-Me-D-Val-Leu-Leu-NHBoc. Tripeptide MeO-*N*-Me-D-Val-Leu-Leu-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 258 mg (2.3 mmol, 1.1 equiv) of amine MeO-*N*-Me-D-Val-NH, 563 mg (1.6 mmol, 1.0 equiv) of acid HO-Leu-Leu-NHBoc, 1.1 mL (4 equiv) of DIPEA, 620 mg (1.6 mmol, 1.0 equiv) of HATU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the tripeptide (341 mg, 40% yield).

R_f: 0.5 (EtOAc:Hex 1:1).

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.9–1.1 (m, 18H), 1.5 (s, 9H), 1.6–1.8 (m, 1H), 2.1 (m, 2H), 2.8 (s, 1H), 3.0 (d, 3H), 3.7 (s, 3H), 4.0–4.2 (br, 2H), 4.2 (m, αH), 5.0 (s, αH), 5.1 (s, αH), 5.2–5.4 (br, αH), 5.5 (m, 1H), 6.8 (d, 1H).

4.3.8.6. Tripeptide MeO-*N*-Me-D-Val-Leu-Leu-NH_2. Tripeptide MeO-*N*-Me-D-Val-Leu-Leu-NH_2 was synthesized following the 'General amine deprotection'. This dipeptide was taken on to the next reaction without further purification or characterization. (278 mg, 100% yield).

4.3.8.7. Pentapeptide MeO-D-Phe-Leu-D-MeVal-Leu-Leu-NHBoc. Pentapeptide MeO-D-Phe-Leu-*N*-Me-D-Val-Leu-Leu-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 278 mg (0.63 mmol, 1.1 equiv) of amine MeO-*N*-Me-D-Val-Leu-Leu-NH_2, 217 mg (0.57 mmol, 1.0 equiv) of acid HO-D-Phe-Leu-NHBoc, 0.40 mL (5 equiv) of DIPEA, 172 mg (0.57 mmol, 1.0 equiv) of DEPBT, and 45 mg (0.11 mmol, 0.2 equiv) HATU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the pentapeptide (229 mg, 55% yield).

R_f: 0.35 (EtOAc:Hex 1:1).

^{1}H NMR (400 MHz, CD_3OD): δ 0.7–0.9 (m, 24H), 1.4 (s, 9H), 1.5–1.7 (m, 6H), 3.1 (s, 3H), 3.2–3.4 (m, 2H), 3.5 (s, 3H), 4.1 (d, αH), 4.2 (d, αH), 4.4 (t, αH), 4.5–4.6 (m, 2αH), 4.6–4.7 (m, 2H), 5.0 (d, 1H), 6.7 (d, 1H), 6.8 (m, 1H), 7.0 (d, 1H), 7.1–7.3 (m, 5H).

4.3.8.8. Macrocycle D-Phe-Leu-*N*-Me-D-Val-Leu-Leu. Macrocycle D-Phe-Leu-*N*-Me-D-Val-Leu-Leu was synthesized following the 'Macrocyclization procedure'. Utilizing 193.2 mg (0.31 mmol, 1.0 equiv) of linear pentapeptide, 0.6 mL (11 equiv) of DIPEA, 50.3 mg (0.16 mmol, 0.5 equiv) of TBTU, 83.3 mg (0.22 mmol, 0.7 equiv) HATU, and 46.8 mg (0.16 mmol, 0.5 equiv) of DEPBT. The crude reaction was purified by reverse phase-HPLC to yield the macrocycle (15 mg, 8% yield).

R_f: 0.25 (EtOAc:Hex 1:1).

^{1}H NMR (400 MHz, CD_3OD): δ 0.8–1.0 (m, 24H), 1.3–1.8 (m, 9H), 2.2 (m, 1H), 2.8 (s, 3H), 2.9–3.1 (m, 2H), 4.0 (m, αH), 4.3 (m, αH), 4.5 (m, 2αH), 4.8 (m, αH), 7.0 (d, 1H), 7.2–7.3 (m, 5H), 7.6 (d, 1H), 8.3 (m, 1H), 8.7 (s, 1H).

LC–MS: m/z calcd for $C_{33}H_{53}N_5O_5$ (M+1) = 600.4, found 600.7.

4.3.9. Synthesis of compound 37

4.3.9.1. Dipeptide MeO-Phe-Leu-NHBoc. Dipeptide MeO-Phe-Leu-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 476 mg (2.2 mmol, 1.1 equiv) of amine MeO-Phe-NH_2, 500 mg (2.0 mmol, 1.0 equiv) of acid HO-Leu-NHBoc, 1.4 mL (4 equiv) of DIPEA, 708 mg (2.2 mmol, 1.1 equiv) of TBTU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the dipeptide (682 mg, 87% yield).

R_f: 0.9 (EtOAc:Hex 1:1).

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.8–1.0 (m, 6H), 1.4 (s, 9H), 1.6 (m, 3H), 3.0–3.2 (m, 2H), 3.7 (s, 3H), 4.0–4.2 (br, 1H), 4.8 (br, αH), 4.8–5.0 (q, αH), 6.5 (m, 1H), 7.1–7.4 (m, 5H).

4.3.9.2. Dipeptide MeO-Phe-Leu-NH_2. Dipeptide MeO-Phe-Leu-NH_2 was synthesized following the 'General amine deprotection'. This dipeptide was taken on to the next reaction without further purification or characterization. (508 mg, 100% yield).

4.3.9.3. Tripeptide MeO-Phe-Leu-D-Val-NHBoc. Tripeptide MeO-Phe-Leu-D-Val-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 960 mg (2.5 mmol, 1.1 equiv) of amine MeO-Phe-Leu-NH_2, 485 mg (2.2 mmol, 1.0 equiv) of acid HO-D-Val-NHBoc, 1.6 mL (4 equiv) of DIPEA, 788 mg (2.5 mmol, 1.1 equiv) of TBTU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the tripeptide (951 mg, 87% yield).

R_f: 0.7 (EtOAc:Hex 1:1).

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.9–1.1 (m, 12H), 1.5 (s, 9H), 1.6–1.7 (m, 2H), 1.8 (s, 1H), 2.1 (m, 1H), 3.1 (m, 2H), 3.7 (s 3H), 3.9 (dd, 1H), 4.4 (br, αH), 4.8 (dd, αH), 5.0 (d, αH), 6.3 (s, 1H), 6.6 (d, 1H), 7.1–7.3 (m, 5H).

4.3.9.4. Tripeptide MeO-Phe-Leu-D-Val-NH_2. Tripeptide MeO-Phe-Leu-D-Val-NH_2 was synthesized following the 'General amine deprotection'. This dipeptide was taken on to the next reaction without further purification or characterization. (758 mg, 100% yield).

4.3.9.5. Dipeptide MeO-D-Leu-Leu-NHBoc. Dipeptide MeO-D-Leu-Leu-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 801 mg (4.4 mmol, 1.1 equiv) of amine MeO-D-Leu-NH_2, 1.0 g (4.0 mmol, 1.0 equiv) of acid HO-Leu-NHBoc, 2.8 mL (4 equiv) of DIPEA, 1.5 g (4.8 mmol, 1.2 equiv) of TBTU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the dipeptide (1.4 g, 99% yield).

R_f: 0.9 (EtOAc:Hex 1:1).

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.9–1.0 (m, 12H), 1.4 (s, 9H), 1.6–1.8 (t, 6H), 3.7 (s, 3H), 4.0–4.1 (dd, αH), 4.5–4.7 (m, αH), 4.8–4.9 (br, 1H), 6.4 (d, 1H).

4.3.9.6. Dipeptide HO-D-Leu-Leu-NHBoc. Dipeptide HO-D-Leu-Leu-NHBoc was synthesized following the 'General acid deprotection'. This dipeptide was taken on to the next reaction without further purification or characterization. (1.2 g, 82% yield).

4.3.9.7. Pentapeptide MeO-Phe-Leu-D-Val-D-Leu-Leu-NHBoc. Pentapeptide MeO-Phe-Leu-D-Val-D-Leu-Leu-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 758 mg (1.9 mmol, 1.0 equiv) of amine MeO-Phe-Leu-D-Val-NH_2, 667 mg (1.9 mmol, 1.0 equiv) of acid HO-D-Leu-Leu-NHBoc, 3.6 mL (11 equiv) of DIPEA, 373 mg (1.2 mmol, 0.6 equiv) of TBTU, 116 mg (0.38 mmol, 0.2 equiv) of DEPBT, and 441 mg (1.1 mmol, 0.6 equiv) HATU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the pentapeptide (379 mg, 27% yield).

R_f: 0.4 (EtOAc:Hex 1:1).

^{1}H NMR (400 MHz, CD_3OD): δ 0.8–1.0 (m, 24H), 1.4 (s, 9H), 1.5–1.7 (m, 6H), 2.8 (s, 3H), 3.0–3.2 (m, 1H), 3.3 (d, 1H), 3.6 (s, 3H), 4.1 (d, αH), 4.2 (d, αH), 4.4 (t, αH), 4.5–4.6 (m, 2αH), 4.6–4.7 (m, 2H), 6.1 (d, 1H), 7.1–7.3 (m, 5H), 7.4 (m, 2H), 7.6 (d, 1H).

4.3.9.8. Macrocycle Phe-Leu-D-Val-D-Leu-Leu. Macrocycle Phe-Leu-D-Val-D-Leu-Leu was synthesized following the 'Macrocyclization procedure'. Utilizing 257 mg (0.43 mmol, 1.0 equiv) of linear pentapeptide, 0.9 mL (12 equiv) of DIPEA, 68.3 mg (0.21 mmol, 0.5 equiv) of TBTU, 113 mg (0.30 mmol, 0.7 equiv) HATU, and 63.7 mg (0.21 mmol, 0.5 equiv) of DEPBT. The crude reaction was purified by reverse phase-HPLC to yield the macrocycle (1.4 mg, 1% yield).

R_f: 0.25 (EtOAc:Hex 1:1).

^{1}H NMR (400 MHz, CD_3OD): δ 0.7–1.0 (m, 24H), 1.3–1.8 (m, 9H), 2.0 (m, 1H), 2.9–3.1 (m, 2H), 3.6 (m, αH), 3.8 (m, αH), 4.2 (m, αH), 4.5 (m, αH), 4.6 (m, αH), 7.1–7.3 (m, 5H),7.2 (d, 1H), 7.6 (d, 1H), 8.2 (d, 1H), 8.6 (d, 1H), 8.7 (s, 1H).

LC–MS: m/z calcd for $C_{32}H_{51}N_5O_5$ (M+1) = 586.4, found 587.5.

4.3.10. Synthesis of compound 49

4.3.10.1. Dipeptide MeO-Phe-D-MePhe-NBoc. Dipeptide MeO-Phe-*N*-Me-D-Phe-NBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 515.5 mg (2.4 mmol, 1.1 equiv) of amine OMe-Phe-NH_2, 606 mg (2.2.0 mmol, 1.0 equiv) of acid HO-*N*-Me-D-Phe-NBoc, 1.52 mL (4 equiv) of DIPEA, 767 mg (2.4 mmol, 1.1 equiv) of TBTU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the dipeptide (436.7 mg, 55% yield).

R_f: 0.5 (EtOAc:Hex 1:1).

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.4 (s, 9H), 1.6 (m, 2H), 2.6–2.8 (d, 3H), 3.2–3.2 (m, 4H), 3.7 (s, 3H), 4.6–5.0 (m, 2αH), 6.9–7.1 (s, 1H), 7.2–7.4 (m, 10H), 8.1 (m, 1H).

4.3.10.2. Dipeptide MeO-Phe-*N*-Me-D-Phe-NH. Dipeptide MeO-Phe-*N*-Me-D-Phe-NH was synthesized following the 'General amine deprotection'. This dipeptide was taken on to the next reaction without further purification or characterization. (337.1 mg, 100% yield).

4.3.10.3. Tripeptide MeO-Phe-*N*-Me-D-Phe-Val-NHBoc. Tripeptide MeO-Phe-*N*-Me-D-Phe-Val-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 337 mg (1.0 mmol, 1.1 equiv) of amine MeO-Phe-*N*-Me-D-Phe-NH, 196 mg (0.9 mmol, 1.0 equiv) of acid HO-Val-NHBoc, 0.7 mL (4 equiv) of DIPEA, 116 mg (0.36 mmol, 0.4 equiv) of TBTU, and 240 mg (0.63mmol, 0.7 equiv) The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the tripeptide (324 mg, 63% yield).

R_f: 0.5 (EtOAc:Hex 1:1).

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.5–0.7 (m, 6H), 1.4 (s, 9H), 1.5 (m, 1H), 2.9 (s, 3H), 3.0–3.4 (m, 4H), 3.7 (s, 3H), 4.2 (m, αH), 4.8 (m, αH), 5.1 (d, 1H), 5.4 (m, αH), 6.8 (d, 1H), 7.0–7.4 (m, 10H).

4.3.10.4. Tripeptide MeO-Phe-*N*-Me-D-Phe-Val-NH_2. Tripeptide MeO-Phe-*N*-Me-D-Phe-Val-NH_2 was synthesized following the 'General amine deprotection'. This dipeptide was taken on to the next reaction without further purification or characterization. (264 mg, 100% yield).

4.3.10.5. Dipeptide MeO-Cha-Leu-NHBoc. Dipeptide MeO-Cha-Leu-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 489.1 mg (2.2 mmol, 1.1 equiva.) of amine MeO-Cha-NH_2, 500 mg (2.0 mmol, 1.0 equiv) of acid HO-Leu-NHBoc, 1.4 mL (8 equiv) of DIPEA, 708 mg (2.2 mmol, 1.0 equiv) of TBTU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the dipeptide (710 mg, 88% yield).

R_f: 0.5 (EtOAc:Hex 1:2).

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.8–1.0 (m, 6H), 1.1–1.3 (m, 4H), 1.4 (s, 9H), 1.6–1.8 (m, 9H), 3.7 (s, 3H), 4.0–4.2 (dd, αH), 4.5–4.7 (m, αH), 4.8–4.9 (br, 1H), 6.2 (d, 1H).

4.3.10.6. Dipeptide HO-Cha-Leu-NHBoc. Dipeptide HO-Cha-Leu-NHBoc was synthesized following the 'General acid deprotection'. This dipeptide was taken on to the next reaction without further purification or characterization. (674 mg, 99% yield).

4.3.10.7. Pentapeptide MeO-Phe-*N*-Me-D-Phe-Val-Cha-Leu-NHBoc. Pentapeptide MeO-Phe-*N*-Me-D-Phe-Val-Cha-Leu-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 264 mg (0.6 mmol, 1.1 equiv) of amine MeO-Phe-*N*-Me-D-Phe-Val-NH_2, 217.4 mg (0.54 mmol, 1.0 equiv) of acid HO-Cha-Leu-NHBoc, 0.4 mL (4 equiv) of DIPEA, 70 mg (0.22 mmol, 0.4 equiv) of TBTU, and 165 mg (0.43 mmol, 0.8 equiv) HATU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the pentapeptide (210 mg, 60% yield).

R_f: 0.5 (EtOAc:Hex 2:1).

^{1}H NMR (400 MHz, CD_3OD): δ 0.4–0.6 (dd, 3H), 0.6–0.8 (dd, 3H), 0.8–1.0 (m, 6H), 1.0–1.2 (m, 4H), 1.4 (s, 9H), 1.6–1.8 (m, 9H), 3.0 (s, 3H), 2.8–3.1 (m, 2H), 3.1–3.4 (m, 2H), 3.7 (s, 3H), 4.1 (m, αH), 4.3–4.4 (m, αH), 4.4–4.6 (m, αH), 4.7–4.9 (m, αH), 5.0 (br, 1H), 5.4–5.5 (m, αH), 6.4 (d, 1H), 6.6 (d, 1H), 6.8 (d, 1H), 7.0–7.4 (m, 10H).

4.3.10.8. Macrocycle Phe-*N*-Me-D-Phe-Val-Cha-Leu. Macrocycle Phe-*N*-Me-D-Phe-Val-Cha-Leu was synthesized following the 'Macrocyclization procedure'. Utilizing 210 mg (0.25 mmol, 1.0 equiv) of linear pentapeptide, 0.2 mL (4 equiv) of DIPEA, 57 mg (0.18 mmol, 0.7 equiv) of TBTU, 67 mg (0.18 mmol, 0.7 equiv) HATU, and 53 mg (0.18 mmol, 0.7 equiv) of DEPBT. The crude reaction was purified by reverse phase-HPLC to yield the macrocycle (28 mg, 14% yield).

R_f: 0.5 (EtOAc:Hex 3:1).

^{1}H NMR (400 MHz, CD_3OD): δ 0.4–0.5 (dd, 3H), 0.6–0.7 (dd, 3H), 0.8–1.0 (m, 6H), 1.0–1.2 (m, 4H), 1.6–1.8 (m, 9H), 3.0 (s, 3H), 2.8–

3.1 (m, 4H), 4.1 (m, αH), 4.2–4.3 (m, αH), 4.4–4.7 (m, 2αH), 5.1–5.2 (m, αH), 7.0–7.4 (m, 10H).

LC–MS: m/z calcd for $C_{33}H_{51}N_5O_5$ (M+1) = 598.39, found 598.3.

4.3.11. Synthesis of compound 60

4.3.11.1. Dipeptide MeO-Phe-*N*-Me-D-Phe-NBoc. Dipeptide MeO-Phe-*N*-Me-D-Phe-NBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 258 mg (1.20 mmol, 1.1 equiv) of amine MeO-Phe-NH_2, 500 mg (1.09 mmol, 1.0 equiv) of acid HO-*N*-Me-D-Phe-NBoc, 0.95 mL (5 equiv) of DIPEA, and 385 mg (1.20 mmol, 1.1 equiv) of TBTU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the dipeptide (480 mg, 99% yield).

R_f: 0.5 (EtOAc:Hex 2:3); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.4 (s, 9H), 2.7 (s, 3H), 3.1–3.2 (m, 4H), 3.7 (s, 3H), 4.1–4.2 (m, 1H), 4.7 (m, 2αH), 7.1–7.3 (dd, 10H).

4.3.11.2. Dipeptide MeO-Phe-*N*-Me-D-Phe-NH. Dipeptide MeO-Phe-*N*-Me-D-Phe-NH was synthesized following the 'General amine deprotection'. This dipeptide was taken on to the next reaction without further purification or characterization. (371 mg, 100% yield).

4.3.11.3. Tripeptide MeO-Phe-*N*-Me-D-Phe-Val-NHBoc. Tripeptide MeO-Phe-*N*-Me-D-Phe-Val-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 371 mg (1.09 mmol, 1.1 equiv) of amine MeO-Phe-*N*-Me-D-Phe-NH, 215 mg (0.99 mmol, 1.0 equiv) of acid HO-Val-NHBoc, 0.95 mL (5 equiv) of DIPEA, 349 mg (1.09 mmol, 1.1 equiv) of TBTU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the tripeptide (491 mg, 92% yield). R_f: 0.5 (EtOAc:Hex 2:3); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.0 (d, 6H), 1.4 (s, 9H), 2.9 (s, 3H), 3.1 (t, 4H), 3.7 (s, 3H), 4.1–4.3 (m, 2αH), 4.7–4.8 (m, 2αH), 5.0–5.1 (br, 1H), 5.5 (m, 1H), 7.2–7.3 (dd, 10H).

4.3.11.4. Tripeptide MeO-Phe-*N*-Me-D-Phe-Val-NH_2. Tripeptide MeO-Phe-*N*-Me-D-Phe-Val-NH_2 was synthesized following the 'General amine deprotection'. This dipeptide was taken on to the next reaction without further purification or characterization. (400 mg, 100% yield).

4.3.11.5. Dipeptide MeO-Cha-Ser(Bn)-NHBoc. Dipeptide MeO-Cha-Ser(Bn)-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 412 mg (1.86 mmol, 1.1 equiv) of amine MeO-Cha-NH_2, 500 mg (1.69 mmol, 1.0 equiv) of acid HO-Ser(Bn)-NHBoc, 1.4 mL (5 equiv) of DIPEA, 597 mg (1.86 mmol, 1.1 equiv) of TBTU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the dipeptide (781 mg, 99% yield). R_f: 0.5 (EtOAc:Hex 2:3); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.4 (s, 20H), 1.6–1.7 (m, 2H), 3.7 (s, 3H), 3.8 (d, 2H), 4.1 (m, 2H), 4.4 (s, 2H), 4.5 (d, 2αH), 7.3–7.4 (dd, 5H).

4.3.11.6. Dipeptide HO-Cha-Ser(Bn)-NHBoc. Dipeptide HO-Cha-Ser(Bn)-NHBoc was synthesized following the 'General acid deprotection'. This dipeptide was taken on to the next reaction without further purification or characterization. (715 mg, 94% yield).

4.3.11.7. Pentapeptide MeO-Phe-*N*-Me-D-Phe-Val-Cha-Ser(Bn)-NHBoc. Pentapeptide MeO-Phe-*N*-Me-D-Phe-Val-Cha-Ser(Bn)-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 400 mg (0.91 mmol, 1.1 equiv) of amine MeO-Phe-*N*-Me-D-Phe-Val-NH_2, 370 mg (0.82 mmol, 1.0 equiv) of acid HO-Cha-Ser(Bn)-NHBoc, 0.57 mL (4 equiv) of DIPEA, 132 mg (0.41 mmol, 0.5 equiv) of TBTU, and 156 mg (0.41 mmol, 0.5 equiv) HATU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the pentapeptide (279 mg, 39% yield). R_f: 0.5 (EtOAc:Hex 1:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.1–1.2 (br, 6H), 1.4 (s, 20H), 2.0 (s, 3H), 3.0–3.1 (m, 2H), 3.1–3.2 (m, 2H), 3.4 (dd, 1H), 3.6 (m, 1H), 3.7 (s, 3H), 3.9 (m, 2H), 4.3 (br, αH), 4.4 (m, αH), 4.5 (m, αH), 4.5 (s, 2H), 4.6 (m, αH), 4.7–4.8 (m, αH), 5.5 (m, 2H), 6.7 (m, 1H), 7.0 (d, 1H), 7.2–7.4 (m, 15H).

4.3.11.8. Macrocycle Phe-D-MePhe-Val-Cha-Ser(Bn). Macrocycle Phe-D-MePhe-Val-Cha-Ser(Bn) was synthesized following the 'Macrocyclization procedure'. Utilizing 242 mg (0.32 mmol, 1.0 equiv) of linear pentapeptide, 0.22 mL (4 equiv) of DIPEA, 51 mg (0.16 mmol, 0.5 equiv) of TBTU, 61 mg (0.16 mmol, 0.5 equiv) HATU, and 48 mg (0.16 mmol, 0.5 equiv) of DEPBT. The crude reaction was purified by reverse phase-HPLC to yield the macrocycle (7.2 mg, 3.5% yield). R_f: 0.5 (EtOAc:Hex 9:1); ^{1}H NMR (400 MHz, CD_3OD): 0.9–1.0 (br, 6H), 1.5–1.7 (m, 11H), 1.9 (m, 2H), 3.0–3.1 (m, 4H), 3.6–3.7 (m, 2H), 4.1 (m, αH), 4.2 (m, αH), 4.3 (m, 2αH), 4.4 (m, αH), 4.5 (m, 1H), 5.1 (m, 1H), 5.3 (m, 1H), 5.4 (m, 1H), 7.1–7.3 (dd, 10H); LC–MS: m/z calcd for $C_{39}H_{49}N_5O6$ (M+1) = 648, found 650.2.

4.3.12. Synthesis of compound 61

4.3.12.1. Macrocycle D-Phe-Leu-*N*-Me-Val-D-Leu-D-Phe. Macrocycle D-Phe-Leu-*N*-Me-Val-D-Leu-D-Phe was synthesized following the 'Macrocyclization procedure'. Utilizing 151 mg (0.23 mmol, 1.0 equiv) of linear pentapeptide, 0.61 mL (8 equiv) of DIPEA, 45.8 mg (0.14 mmol, 0.6 equiv) of TBTU, 52.5 mg (0.13 mmol, 0.6 equiv) HATU, and 41 mg (0.14 mmol, 0.6 equiv) of DEPBT. The crude reaction was purified by reverse phase-HPLC to yield the macrocycle (42 mg, 28% yield).

R_f: 0.5 (EtOAc:Hex 4:1).

^{1}H NMR (400 MHz, CD_3OD): δ 0.8–1.0 (m, 18H), 1.3 (s, 1H), 1.5 (t, 4H), 1.7 (m, 1H), 2.8 (m, 1H), 3.0 (d, 2H), 3.2 (m, 2H), 3.2 (s, 3H), 4.2 (m, 3αH), 4.5 (m, 2αH), 7.1–7.3 (m, 10H), 7.7 (s, 1H), 7.9 (s, 1H), 8.4 (d, 1H), 8.5 (s, 1H).

LC–MS: m/z calcd for $C_{36}H_{51}N_5O_5$ (M+1) = 633.82, found 634.7.

4.3.13. Synthesis of compound 62

4.3.13.1. Macrocycle Phe-Leu-*N*-Me-Val-Leu-D-Phe. Macrocycle Phe-Leu-*N*-Me-Val-Leu-D-Phe was synthesized following the 'Macrocyclization procedure'. Utilizing 287 mg (0.44 mmol, 1.0 equiv) of linear pentapeptide, 0.61 mL (8 equiv) of DIPEA, 84 mg (0.26 mmol, 0.6 equiv) of TBTU, 100 mg (0.26 mmol, 0.6 equiv) HATU, and 78 mg (0.26 mmol, 0.6 equiv) of DEPBT. The crude reaction was purified by reverse phase-HPLC to yield the macrocycle (75 mg, 26% yield).

R_f: 0.5 (EtOAc:Hex 4:1).

^{1}H NMR (400 MHz, CD_3OD): δ 0.8–1.0 (m, 18H), 1.3–1.8 (m, 6H), 2.7 (m, 1H), 2.9 (m, 2H), 3.1 (m, 2H), 3.2 (s, 3H), 4.4 (m, 3αH), 4.7 (m, 2αH), 7.1–7.3 (m, 10H), 7.7 (s, 1H), 8.0 (s, 1H), 8.2 (d, 1H), 8.7 (s, 1H).

LC–MS: m/z calcd for $C_{36}H_{51}N_5O_5$ (M+1) = 633.82, found 635.1.

4.3.14. Synthesis of compound 63

4.3.14.1. Dipeptide MeO-Phe-Leu-NHBoc. Dipeptide MeO-Phe-Leu-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 951 mg (4.4 mmol, 1.1 equiv) of amine MeO-Phe-NH_2, 1000 mg (4.0 mmol, 1.0 equiv) of acid HO-Leu-NHBoc, 2.8 mL (4 equiv) of DIPEA, 1546 mg (4.8 mmol, 1.2 equiv) of TBTU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the dipeptide (1564 mg, 99% yield).

R_f: 0.8 (EtOAc:Hex 1:1).

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.8–1.0 (m, 6H), 1.4 (s, 9H), 1.6 (m, 2H), 3.0–3.1 (m, 2H), 3.7 (s, 3H), 4.8–5.1 (s, 2H), 4.1 (m, αH), 4,8 (m, αH), 6.5 (d, 1H), 7.0–7.3 (m, 5H).

4.3.14.2. Dipeptide MeO-Phe-Leu-NH_2. Dipeptide MeO-Phe-Leu-NH_2 was synthesized following the 'General amine deprotection'. This dipeptide was taken on to the next reaction without further purification or characterization. (1165 mg, 100% yield).

4.3.14.3. Tripeptide MeO-Phe-Leu-*N*-Me-Val-NBoc. Tripeptide MeO-Phe-Leu-*N*-Me-Val-NBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 510 mg (1.74 mmol, 1.1 equiv) of amine MeO-Phe-Leu-NH_2, 367 mg (1.58 mmol, 1.0 equiv) of acid HO-*N*-Me-Val-NBoc, 1.1 mL (4 equiv) of DIPEA, 723 mg (1.9 mmol, 1.2 equiv) of HATU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the tripeptide (585 mg, 73% yield).

R_f: 0.6 (EtOAc:Hex 1:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.9–1.1 (m, 12H), 1.5 (s, 9H), 1.6–1.8 (m, 1H), 2.3 (m, 2H), 2.8 (s, 3H), 3.1 (m, 2H), 3.7 (s, 3H), 4.0 (d, αH), 4.4 (m, αH), 4.8 (m, αH), 6.3 (d, 1H), 6.5 (d, 1H), 7.1–7.3 (m, 5H).

4.3.14.4. Tripeptide MeO-Phe-Leu-*N*-Me-Val-NH. Tripeptide MeO-Phe-Leu-*N*-Me-Val-NH was synthesized following the 'General amine deprotection'. This tripeptide was taken on to the next reaction without further purification or characterization. (468 mg, 100% yield).

4.3.14.5. Tetrapeptide MeO-Phe-Leu-*N*-Me-Val-D-Lys(2-Cl-Cbz)-NHBoc. Tetrapeptide MeO-Phe-Leu-*N*-Me-Val-D-Lys(2-Cl-Cbz)-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 468 mg (1.1 mmol, 1.1 equiv) of amine MeO-Phe-Leu-*N*-Me-Val-NH, 436 mg (1.0 mmol, 1.0 equiv) of acid HO-Lys(2-Cl-Cbz)-NHBoc, 1.27 mL (7 equiv) of DIPEA, 135 mg (0.4 mmol, 0.4 equiv) of TBTU, and 320 mg (0.8 mmol, 0.8 equiv) of HATU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the dipeptide (675 mg, 80% yield).

R_f: 0.35 (EtOAc:Hex 1:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.9–1.0 (m, 12H), 1.4 (s, 9H), 1.4–1.8 (m, 9H), 2.3 (m, 2H), 3.0 (s, 3H), 3.1–3.2 (m, 2H), 3.7 (s, 3H), 4.3 (m, αH), 4.4 (d, 1H), 4.6 (m, αH), 4.8 (m, αH), 4.9 (m, αH), 5.2 (s, 2H), 5.3 (d, 1H), 6.4 (d, 1H), 6.5 (d, 1H), 7.1–7.4 (m, 9H).

4.3.14.6. Tetrapeptide MeO-Phe-Leu-*N*-Me-Val-D-Lys(2-Cl-Cbz)-NH_2. Tetrapeptide MeO-Phe-Leu-*N*-Me-Val-D-Lys(2-Cl-Cbz)-NH_2 was synthesized following the 'General amine deprotection'. This tetrapeptide was taken on to the next reaction without further purification or characterization. (590 mg, 100% yield).

4.3.14.7. Pentapeptide MeO-Phe-Leu-*N*-Me-Val-D-Lys(2-Cl-Cbz)-D-Phe-NHBoc. Pentapeptide MeO-Phe-Leu-*N*-Me-Val-D-Lys(2-Cl-Cbz)-D-Phe-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 570 mg (0.8 mmol, 1.1 equiv) of amine MeO-Phe-Leu-*N*-Me-Val-D-Lys(2-Cl-Cbz)-NH_2, 195 mg (0.7 mmol, 1.0 equiv) of acid HO-D-Phe-NHBoc, 0.6 mL (5 equiv) of DIPEA, 95 mg (0.3 mmol, 0.4 equiv) of TBTU, and 224 mg (0.6 mmol, 0.8 equiv) HATU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the pentapeptide (549 mg, 79% yield).

R_f: 0.3 (EtOAc:Hex 1:1).

^{1}H NMR (400 MHz, CD_3OD): δ 0.7–0.9 (m, 12H), 1.4 (s, 9H), 1.3–1.7 (m, 9H), 2.3 (m, 2H), 3.0 (s, 3H), 3.1–3.2 (m, 4H), 3.7 (s, 3H), (d, αH), 4.3–4.4 (m, 2αH), 4.8–4.9 (m, 2αH), 5.0 (br, αH), 5.2 (s, 2H), 6.4–6.5 (dd, 2H), 6.9 (d, 1H), 7.1–7.5 (m, 14H).

4.3.14.8. Macrocycle Phe-Leu-*N*-Me-Val-D-Lys(2-Cl-Cbz)-D-Phe. Macrocycle Phe-Leu-*N*-Me-Val-D-Lys(2-Cl-Cbz)-D-Phe was synthesized following the 'Macrocyclization procedure'. Utilizing 228 mg (0.27 mmol, 1.0 equiv) of linear pentapeptide, 0.38 mL (8 equiv) of DIPEA, 43.8 mg (0.14 mmol, 0.5 equiv) of TBTU, 62.3 mg (0.16 mmol, 0.6 equiv) HATU, and 49 mg (0.16 mmol, 0.6 equiv) of DEPBT. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) yield the macrocycle (37.5 mg, 16.8% yield).

R_f: 0.5 (EtOAc:Hex 4:1).

^{1}H NMR (400 MHz, CD_3OD): δ 0.8–1.0 (m, 12H), 1.2–1.6 (m, 8H), 1.7–1.9 (m, 1H), 2.1–2.3 (m, 2H), 2.9 (s, 3H), 2.7–3.1 (m, 4H), 4.1–4.3 (m, 3αH), 4.5 (m, αH), 4.7 (m, αH), 5.1 (d, 1H), 5.2 (s, 2H), 7.0–7.4 (m, 15H).

LC–MS: m/z calcd for $C_{44}H_{57}ClN_6O_7$ (M+1) = 818.41, found 818.2.

4.3.15. Removal of carbobenzyloxy groups via acid to yield compound 64

4.3.15.1. Macrocycle Phe-Leu-*N*-Me-D-Val-D-Lys-D-Phe. Utilizing 26 mg (0.03 mmol, 1.0 equiv) of compound 140 and 0.32 mL of HBr to remove the protecting group on the Lysine at Position D-Lys(2-Cl-Cbz). The crude reaction was taken on to the next reaction without further purification or characterization (20.8 mg, 100% yield).

^{1}H NMR (400 MHz, CD_3OD): δ 0.8–1.0 (m, 12H), 1.2–1.7 (m, 8H), 1.7–1.9 (m, 1H), 2.1–2.3 (m, 2H), 2.9 (s, 3H) 2.8–2.9 (m, 4H), 4.2 (m, αH), 4.3 (m, 2αH), 4.5 (m, αH), 4.7 (m, αH), 5.0 (d, 1H), 7.0–7.3 (m, 9H)

LC–MS: m/z calcd for $C_{36}H_{52}N_6O_5$ (M+1) = 649.84, found 649.6

4.3.16. Synthesis of compound 65

4.3.16.1. Dipeptide MeO-Phe-*N*-Me-D-Phe-NBoc. Dipeptide MeO-Phe-*N*-Me-D-Phe-NBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 258 mg (1.20 mmol, 1.1 equiv) of amine MeO-Phe-NH_2, 500 mg (1.09 mmol, 1.0 equiv) of acid HO-*N*-Me-D-Phe-NBoc, 0.95 mL (5 equiv) of DIPEA, and 385 mg (1.20 mmol, 1.1 equiv) of TBTU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the dipeptide (480 mg, 99% yield).

R_f 0.5 (EtOAc:Hex 2:3); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.4 (s, 9H), 2.7 (s, 3H), 3.1–3.2 (m, 4H), 3.7 (s, 3H), 4.1–4.2 (m, 1H), 4.7 (m, 2αH), 7.1–7.3 (dd, 10H).

4.3.16.2. Dipeptide MeO-Phe-*N*-Me-D-Phe-NH. Dipeptide MeO-Phe-*N*-Me-D-Phe-NH was synthesized following the 'General amine deprotection'. This dipeptide was taken on to the next reaction without further purification or characterization. (371 mg, 100% yield).

4.3.16.3. Tripeptide MeO-Phe-*N*-Me-D-Phe-Val-NHBoc. Tripeptide MeO-Phe-*N*-Me-D-Phe-Val-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 371 mg (1.09 mmol, 1.1 equiv) of amine MeO-Phe-*N*-Me-D-Phe-NH, 215 mg (0.99 mmol, 1.0 equiv) of acid HO-Val-NHBoc, 0.95 mL (5 equiv) of DIPEA, 349 mg (1.09 mmol, 1.1 equiv) of TBTU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the tripeptide (491 mg, 92% yield). R_f: 0.5 (EtOAc:Hex 2:3); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.0 (d, 6H), 1.4 (s, 9H), 2.9 (s, 3H), 3.1 (t, 4H), 3.7 (s, 3H), 4.1–4.3 (m, 2αH), 4.7-4.8 (m, 2αH), 5.0–5.1 (br, 1H), 5.5 (m, 1H), 7.2–7.3 (dd, 10H).

4.3.16.4. Tripeptide MeO-Phe-*N*-Me-D-Phe-Val-NH_2. Tripeptide MeO-Phe-*N*-Me-D-Phe-Val-NH_2 was synthesized following the 'General amine deprotection'. This dipeptide was taken on to the next reaction without further purification or characterization. (400 mg, 100% yield).

4.3.16.5. Dipeptide MeO-Cha-Ser(Bn)-NHBoc. Dipeptide MeO-Cha-Ser(Bn)-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 412 mg (1.86 mmol, 1.1 equiv)

of amine MeO-Cha-NH_2, 500 mg (1.69 mmol, 1.0 equiv) of acid HO-Ser(Bn)-NHBoc, 1.4 mL (5 equiv) of DIPEA, 597 mg (1.86 mmol, 1.1 equiv) of TBTU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the dipeptide (781 mg, 99% yield). R_f: 0.5 (EtOAc:Hex 2:3); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.4 (s, 20H), 1.6–1.7 (m, 2H), 3.7 (s, 3H), 3.8 (d, 2H), 4.1 (m, 2H), 4.4 (s, 2H), 4.5 (d, 2αH), 7.3–7.4 (dd, 5H).

4.3.16.6. Dipeptide HO-Cha-Ser(Bn)-NHBoc. Dipeptide HO-Cha-Ser(Bn)-NHBoc was synthesized following the 'General acid deprotection'. This dipeptide was taken on to the next reaction without further purification or characterization. (715 mg, 94% yield).

4.3.16.7. Pentapeptide MeO-Phe-*N*-Me-D-Phe-Val-Cha-Ser(Bn)-NHBoc. Pentapeptide MeO-Phe-*N*-Me-D-Phe-Val-Cha-Ser(Bn)-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 400 mg (0.91 mmol, 1.1 equiv) of amine MeO-Phe-*N*-Me-D-Phe-NH, 370 mg (0.82 mmol, 1.0 equiv) of acid HO-Cha-Ser(Bn)-NHBoc, 0.57 mL (4 equiv) of DIPEA, 132 mg (0.41 mmol, 0.5 equiv) of TBTU, and 156 mg (0.41 mmol, 0.5 equiv) HATU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the pentapeptide (279 mg, 39% yield). R_f: 0.5 (EtOAc:Hex 1:1); 1H NMR (500 MHz, CDCl3): δ 1.1–1.2 (br, 6H), 1.4 (s, 20H), 2.0 (s, 3H), 3.0–3.1 (m, 2H), 3.1–3.2 (m, 2H), 3.4 (dd, 1H), 3.6 (m, 1H), 3.7 (s, 3H), 3.9 (m, 2H), 4.3 (br, αH), 4.4 (m, αH), 4.5 (m, αH), 4.5 (s, 2H), 4.6 (m, αH), 4.7–4.8 (m, αH), 5.5 (m, 2H), 6.7 (m, 1H), 7.0 (d, 1H), 7.2–7.4 (m, 15H).

4.3.16.8. Macrocycle Phe-*N*-Me-D-Phe-Val-Cha-Ser(Bn). Macrocycle Phe-*N*-Me-D-Phe-Val-Cha-Ser(Bn) was synthesized following the 'Macrocyclization procedure'. Utilizing 242 mg (0.32 mmol, 1.0 equiv) of linear pentapeptide, 0.22 mL (4 equiv) of DIPEA, 51 mg (0.16 mmol, 0.5 equiv) of TBTU, 61 mg (0.16 mmol, 0.5 equiv) HATU, and 48 mg (0.16 mmol, 0.5 equiv) of DEPBT. The crude reaction was purified by reverse phase-HPLC to yield the macrocycle (7.2 mg, 2.0% yield). R_f: 0.5 (EtOAc:Hex 9:1); ^{1}H NMR (400 MHz, CD_3OD): δ 0.9–1.0 (br, 6H), 1.4–1.6 (m, 11H), 1.6–1.7 (m, 2H), 2.2 (br, 1H), 3.0–3.1 (m, 4H), 3.6–3.8 (m, 2H), 4.4–4.5 (m, 2αH), 4.5–4.6 (br, 2αH), 5.1 (m, 2H), 7.1 (m, 2H), 7.1–7.3 (dd, 15H)LC–MS: m/z calcd for $C_{43}H_{55}N_5O6$ (M+1) = 738, found 738.36.

4.3.17. Synthesis of compound 66

4.3.17.1. Dipeptide MeO-Phe-*N*-Me-D-Phe-NBoc. Dipeptide MeO-Phe-*N*-Me-D-Phe-NBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 258 mg (1.20 mmol, 1.1 equiv) of amine MeO-Phe-NH_2, 500 mg (1.09 mmol, 1.0 equiv) of acid HO-*N*-Me-D-Phe-NBoc, 1.0 mL (5 equiv) of DIPEA, and 385 mg (1.20 mmol, 1.1 equiv) of TBTU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the dipeptide (480 mg, 99% yield).

R_f: 0.5 (EtOAc:Hex 2:3); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.4 (s, 9H), 2.7–2.8 (s, 3H), 3.1–3.2 (s, 4H), 3.7 (s, 3H), 4.1–4.2 (m, 1H), 4.9–5.0 (br, 2αH), 7.2–7.4 (dd, 10H).

4.3.17.2. Dipeptide MeO-Phe-*N*-Me-D-Phe-NH. Dipeptide MeO-Phe-*N*-Me-D-Phe-NH was synthesized following the 'General amine deprotection'. This dipeptide was taken on to the next reaction without further purification or characterization. (371 mg, 100% yield).

4.3.17.3. Tripeptide MeO-Phe-*N*-Me-D-Phe-D-Val-NHBoc. Tripeptide MeO-Phe-*N*-Me-D-Phe-D-Val-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 371 mg (1.09 mmol, 1.1 equiv) of amine MeO-Phe-*N*-Me-D-Phe-NH, 215 mg (0.99 mmol, 1.0 equiv) of acid HO-D-Val-NHBoc, 0.95 mL (5 equiv) of DIPEA, 349 mg (1.09 mmol, 1.1 equiv) of TBTU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the tripeptide (512 mg, 96% yield). R_f: 0.5 (EtOAc:Hex 2:3); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.0–1.1 (m, 6H), 1.4 (s, 9H), 2.9 (m, 1H), 3.0 (s, 3H), 3.4–3.5 (br, 4H), 3.7 (s, 3H), 4.1–4.2 (m, 2αH), 4.8 (m, αH), 5.0 (br, 1H), 5.4 (br, 1H), 7.2–7.3 (m, 10H).

4.3.17.4. Tripeptide MeO-Phe-*N*-Me-D-Phe-D-Val-NH_2. Tripeptide MeO-Phe-*N*-Me-D-Phe-D-Val-NH_2 was synthesized following the 'General amine deprotection'. This dipeptide was taken on to the next reaction without further purification or characterization. (417 mg, 100% yield).

4.3.17.5. Dipeptide MeO-Cha-Ser(Bn)-NHBoc. Dipeptide MeO-Cha-Ser(Bn)-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 412 mg (1.86 mmol, 1.1 equiv) of amine MeO-Cha-NH_2, 500 mg (1.69 mmol, 1.0 equiv) of acid HO-Ser(Bn)-NHBoc, 1.4 mL (5 equiv) of DIPEA, 597 mg (1.86 mmol, 1.1 equiv) of TBTU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the dipeptide (780 mg, 99% yield). R_f: 0.5 (EtOAc:Hex 2:3); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.4 (s, 20H), 1.5–1.6 (m, 2H), 3.7 (s, 3H), 3.9 (dd, 2H), 4.1 (br, 2H), 4.5 (s, 2H), 4.6 (m, 2αH), 7.2–7.3 (s, 5H).

4.3.17.6. Dipeptide HO-Cha-Ser(Bn)-NHBoc. Dipeptide HO-Cha-Ser(Bn)-NHBoc was synthesized following the 'General acid deprotection'. This dipeptide was taken on to the next reaction without further purification or characterization. (766 mg, 99% yield).

4.3.17.7. Pentapeptide MeO-Phe-*N*-Me-D-Phe-D-Val-Cha-Ser(Bn)-NHBoc. Pentapeptide MeO-Phe-*N*-Me-D-Phe-D-Val-Cha-Ser(Bn)-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 417 mg (0.94 mmol, 1.1 equiv) of amine MeO-Phe-*N*-Me-D-Phe-D-Val-NH_2, 386 mg (0.86 mmol, 1.0 equiv) of acid-Cha-Ser(Bn)-NHBoc, 0.60 mL (4 equiv) of DIPEA, 138 mg (0.43 mmol, 0.5 equiv) of TBTU, and 164 mg (0.43 mmol, 0.5 equiv) HATU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the pentapeptide (210 mg, 28% yield). R_f: 0.5 (EtOAc:Hex 3:2); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.9–1.0 (m, 6H), 1.4 (s, 9H), 1.5–1.6 (s, 11H), 1.8 (m, 2H), 2.8 (s, 3H), 3.0 (m, 2H), 3.2 (m, 2H), 3.6 (s, 3H), 3.8 (m, 1H), 4.2 (m, 2αH), 4.4 (m, 2αH), 4.5 (m, 2H), 4.7 (m, αH), 5.0 (br, 1H), 6.2 (d, 1H), 6.4 (d, 1H), 6.5 (d, 1H), 7.1–7.3 (dd, 15H).

4.3.17.8. Macrocycle Phe-*N* -Me-D-Phe-D-Val-Cha-Ser(Bn). Macrocycle Phe-*N*-Me-D-Phe-D-Val-Cha-Ser(Bn) was synthesized following the 'Macrocyclization procedure'. Utilizing 182 mg (0.24 mmol, 1.0 equiv) of linear pentapeptide, 0.16 mL (4 equiv) of DIPEA, 39 mg (0.12 mmol, 0.5 equiv) of TBTU, 46 mg (0.12 mmol, 0.5 equiv) HATU, and 36 mg (0.12 mmol, 0.5 equiv) of DEPBT. The crude reaction was purified by reverse phase-HPLC to yield the macrocycle (1.0 mg, 1.0% yield). R_f: 0.5 (EtOAc:Hex 3:1); ^{1}H NMR (400 MHz, CD_3OD): δ 0.9–1.0 (dd, 6H), 1.2–1.3 (m, 4H), 1.3–1.4 (m, 6H), 1.7–1.8 (m, 1H), 1.9 (br, 2H), 2.9 (s, 3H), 3.0–3.1 (m, 3H), 3.1–3.2 (m, 3H), 3.7 (m, 2H), 3.8 (m, 2H), 3.9 (m, αH), 4.2 (m, 2αH), 4.4 (m, 2αH, 4.5 (s, 2H), 7.1–7.3 (dd, 15H); LC–MS: m/z calcd for $C_{43}H_{55}N_5O_6$ (M+1) = 738, found 738.5.

4.3.18. Synthesis of compound 67

4.3.18.1. Dipeptide MeO-D-Phe-D-Val-NHBoc. Dipeptide MeO-D-Phe-D-Val-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 476 mg (2.2 mmol, 1.1 equiv) of amine MeO-D-Phe-NH_2, 500 mg (2.0 mmol, 1.0 equiv) of acid HO-D-Val-NHBoc, 1.4 mL (4 equiv) of DIPEA, and 773 mg (2.4 mmol, 1.2 equiv) of TBTU. The crude reaction was washed 2 × 100 mL pH 1 water and 10 × 100 mL saturated $NaHCO_3$ to yield the dipeptide (788 mg, 95% yield). R_f: 0.5 (EtOAc:Hex 35:65); ^{1}H NMR

(200 MHz, $CDCl_3$): δ 0.95 (d, 6H), 1.5 (s, 9H), 1.6–1.8 (m, 3H), 3.2 (m, 2H), 3.7 (s, 3H), 4.0–4.1 (m, αH), 4.8–5.0 (m, αH), 6.4–6.6 (d, 1H), 7.1–7.4 (m, 5H).

4.3.18.2. Dipeptide MeO-D-Phe-D-Val-NH_2. Dipeptide MeO-D-Phe-D-Val-NH_2 was synthesized following the 'General amine deprotection'. This dipeptide was taken on to the next reaction without further purification or characterization. (596 mg, 100% yield).

4.3.18.3. Tripeptide MeO-D-Phe-D-Leu-D-Val-NHBoc. Tripeptide MeO-D-Phe-D-Leu-D-Val-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 596 mg (1.9 mmol, 1.1 equiv) of amine MeO-D-Phe-D-Leu-NH_2, 379 mg (1.7 mmol, 1.0 equiv) of acid HO-D-Val-NHBoc, 1.2 mL (5 equiv) of DIPEA, 672 mg (0.85 mmol, 2.0 equiv) of TBTU. The crude reaction was washed 3 × 100 mL saturated NaCl. It was then purified by column chromatography (silica gel, EtOAc/Hex) to yield the tripeptide (857 mg, 94% yield). R_f: 0.5 (EtOAc:Hex 1:1); ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.9–1.0 (m, 12H), 1.5 (s, 12H), 1.6–1.8 (m, 3H), 2.1 (m, 1H), 3.1 (d, 2H), 3.7 (s, 3H), 3.9–4.0 (dd, 1H), 4.4 (m, αH), 4.9 (m, αH), 5.0 (d, αH), 6.3 (d, 1H), 6.5 (d, 1H), 7.0–7.4 (m, 5H).

4.3.18.4. Tripeptide MeO-D-Phe-D-Leu-D-Val-NH_2. Tripeptide MeO-D-Phe-D-Leu-D-Val-NH_2 was synthesized following the 'General amine deprotection'. This dipeptide was taken on to the next reaction without further purification or characterization. (502 mg, 100% yield).

4.3.18.5. Dipeptide MeO-Leu-Ser(Bn)-NHBoc. Dipeptide MeO-Leu-Ser(Bn)-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 339 mg (1.9 mmol, 1.1 equiv) of amine MeO-Leu-NH_2, 500 mg (1.7 mmol, 1.0 equiv) of acid HO-Ser(Bn)-NHBoc, 1.2 mL (4 equiv) of DIPEA, 652 mg (2.0 mmol, 1.2 equiv) of TBTU. The crude reaction was washed 2 × 100 mL with pH 1 water and 10 × 100 mL with saturated $NaHCO_3$ to yield the dipeptide (636 mg, 89% yield). R_f: 0.5 (EtOAc:Hex 35:65); ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.9–1.0 (m, 6H), 1.4 (s, 9H), 1.6–1.8 (m, 5H), 3.5 (dd, 2H), 3.7 (s, 3H), 3.9–4.0 (dd, 1H), 4.2 (m, αH), 4.5 (s, 2H), 4.6 (m, αH), 5.3 (d, 1H), 6.9 (d, 1p), 7.3 (m, 5H).

4.3.18.6. Dipeptide HO-Leu-Ser(Bn)-NHBoc. Dipeptide HO-Leu-Ser(Bn)-NHBoc was synthesized following the 'General acid deprotection'. This dipeptide was taken on to the next reaction without further purification or characterization. (631 mg, 98% yield).

4.3.18.7. Pentapeptide MeO-D-Phe-D-Leu-D-Val-Leu-Ser(Bn)-NHBoc. Pentapeptide MeO-D-Phe-D-Leu-D-Val-Leu-Ser(Bn)-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 488 mg (1.2 mmol, 1.1 equiv) of amine MeO-D-Phe-D-Leu-D-Val-NH_2, 463 mg (1.1 mmol, 1.0 equiv) of acid HO-Leu-Ser(Bn)-NHBoc, 1.2 mL (6 equiv) of DIPEA, and 436 mg (1.4 mmol, 1.2 equiv) of TBTU. The crude reaction was washed 2 × 100 mL with pH 1 water and 10 × 100 mL with saturated $NaHCO_3$. It was then purified by column chromatography (silica gel, EtOAc/Hex) to yield the pentapeptide (435 mg, 52% yield). R_f: 0.5 (EtOAc:Hex 65:35); ^{1}H NMR (400 MHz, CD_3OD): δ 0.8–0.9 (m, 18H), 1.3 (s, 1H), 1.4 (s, 9H), 1.6–1.8 (m, 7H), 2.1 (m, 1H), 3.0–3.2 (m, 2H), 3.6–3.8 (m, 4H), 4.2 (m, αH), 4.3 (m, αH), 4.4 (m, 2αH), 4.5–4.7 (d, 3H), 7.1–7.3 (m, 10H), 7.8 (d, 1H), 8.0 (d, 1H), 8.1 (d, 1H).

4.3.18.8. Pentapeptide HO-D-Phe-D-Leu-D-Val-Leu-Ser(Bn)-NHBoc. Pentapeptide HO-D-Phe-D-Leu-D-Val-Leu-Ser(Bn)-NHBoc was synthesized following the 'General acid deprotection'. This pentapeptide was taken on to the next reaction without further purification or characterization. (252 mg, 60% yield).

4.3.18.9. Pentapeptide HO-D-Phe-D-Leu-D-Val-Leu-Ser(Bn)-NH_2. Pentapeptide HO-D-Phe-D-Leu-D-Val-Leu-Ser(Bn)-NH_2 was synthesized following the 'General amine deprotection'. This pentapeptide was taken on to the next reaction without further purification. (191 mg, 100% yield). LC–MS: m/z calcd for $C_{36}H_{53}N_5O_7$ (M+1) = 668.8, found 669.0.

4.3.18.10. Macrocycle D-Phe-D-Leu-D-Val-Leu-Ser(Bn). Macrocycle D-Phe-D-Leu-D-Val-Leu-Ser(Bn) was synthesized following the 'Macrocyclization procedure'. Utilizing 220 mg (0.32 mmol, 1.0 equiv) of linear pentapeptide, 0.34 mL (8 equiv) of DIPEA, 53.0 mg (0.17 mmol, 0.5 equiv) of TBTU, 62.7 mg (0.17 mmol, 0.5 equiv) HATU, and 49.4 mg (0.17 mmol, 0.5 equiv) of DEPBT. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the macrocycle (36.5 mg, 17% yield). R_f: 0.5 (EtOAc:Hex 70:30); ^{1}H NMR (500 MHz, CD_3OD): δ 0.8–1.1 (m, 18H), 1.4 (s, 3H), 1.6–1.8 (m, 7H), 2.1 (d, 1H), 2.2 (m, 1H), 3.2–3.4 (m, 1H), 3.8 (m, 2H), 4.2 (m, 3αH), 4.5 (m, αH), 4.7 (m, 2αH), 6.9 (d, 1H), 7.2–7.5 (m, 10H), 7.9 (d, 1H), 8.2 (d, 1H), 8.4 (d, 1H), 8.7 (d, 1H); LC–MS: m/z calcd for $C_{36}H_{51}N_5O_6$ (M+1) = 650.8, found 651.4.

4.3.19. Synthesis of compound 68

4.3.19.1. Dipeptide MeO-Phe-Leu-NHBoc. Dipeptide MeO-Phe-Leu-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 477 mg (2.2 mmol, 1.1 equiv) of amine MeO-Phe-NH_2, 500 mg (2.0 mmol, 1.0 equiv) of acid HO-Leu-NHBoc, 1.4 mL (4 equiv) of DIPEA, 773 mg (2.4 mmol, 1.2 equiv) of TBTU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the dipeptide (785 mg, 99% yield). 0.5 (EtOAc:Hex 1:4).

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.9 (m, 6H), 1.4–1.5 (s, 9H), 1.6–1.7 (br, 3H), 3.1–3.2 (t, 2H), 3.7–3.8 (s, 3H), 4.0–4.2 (m, αH), 4.7–4.9 (m, αH, 1H), 6.5 (d, 1H), 7.0–7.2 (br, 2H), 7.3–7.4 (s, 3H).

4.3.19.2. Dipeptide MeO-Phe-Leu-NH_2. Dipeptide MeO-Phe-Leu-NH_2 was synthesized following the 'General amine deprotection'. This dipeptide was taken on to the next reaction without further purification or characterization. (584 mg, 100% yield).

4.3.19.3. Tripeptide MeO-Phe-Leu-Val-NHBoc. Tripeptide MeO-Phe-Leu-Val-NHBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 578 mg (1.98 mmol, 1.0 equiv) of amine MeO-Phe-Leu-NH_2, 433 mg (1.94 mmol, 1.02 equiv) of acid HO-Val-NHBoc, 1.90 mL (6 equiv) of DIPEA, 466 mg (1.44 mmol, 0.7 equiv) of TBTU, 275 mg (0.72 mmol, 0.4 equiv) of HATU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the tripeptide (886 mg, 91% yield).

R_f: 0.5 (EtOAc:Hex 2:3).

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.8–1.0 (m, 12H), 1.4–1.5 (s, 9H), 1.6–1.7 (m, 3H), 2.0–2.2 (m, 1H), 3.1 (d, 2H), 3.7 (s, 3H), 3.8–3.9 (q, αH), 4.4–4.5 (m, αH), 4.8–4.9 (q, αH), 5.0 (d, 1H), 6.3 (d, 1H), 6.4–6.5 (d, 1H), 7.1 (m, 2H), 7.2–7.4 (m, 3H).

4.3.19.4. Tripeptide MeO-Phe-Leu-Val-NH_2. Tripeptide MeO-Phe-Leu-Val-NH_2 was synthesized following the 'General amine deprotection'. This dipeptide was taken on to the next reaction without further purification or characterization. (705 mg, 100% yield).

4.3.19.5. Dipeptide MeO-Lys(Cbz)-*N*-Me-Leu-NBoc. Dipeptide MeO-Lys(Cbz)-*N*-Me-Leu-NBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 742 mg (2.2 mmol, 1.1 equiv) of amine MeO-Lys(Cbz)-NH_2, 500 mg (2.0 mmol, 1.0 equiv) of acid HO-*N*-Me-Leu-NBoc, 1.4 mL (4 equiv) of DIPEA, 458 mg (1.4 mmol, 0.7 equiv) of TBTU. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the dipeptide (1.05 g, 91% yield).

R_f: 0.5 (EtOAc:Hex 2:3).

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.9–1.0 (t, 6H), 1.2–1.4 (m, 2H), 1.5 (s, 9H), 1.6–1.7 (m, 6H), 1.8–1.9 (m, 1H), 2.7–2.8 (s, 3H), 3.1–3.2 (q, 2H), 3.7 (s, 3H), 4.5–4.7 (br, αH), 4.8–4.9 (br, αH), 5.1 (s, 2H), 6.4–6.5 (br, 1H), 6.5 (br, 1H), 7.3 (s, 1H), 7.4 (s, 4H).

4.3.19.6. Dipeptide HO-Lys(Cbz)-*N*-Me-Leu-NBoc. Dipeptide HO-Lys(Cbz)-*N*-Me-Leu-NBoc was synthesized following the 'General acid deprotection'. This dipeptide was taken on to the next reaction without further purification or characterization. (947 mg, 93% yield).

4.3.19.7. Pentapeptide MeO-Phe-Leu-Val-Lys(Cbz)-*N*-Me-Leu-NBoc. Pentapeptide MeO-Phe-Leu-Val-Lys(Cbz)-*N*-Me-Leu-NBoc was synthesized following the 'General peptide synthesis' procedure. Utilizing 705 mg (1.80 mmol, 1.1 equiv) of amine MeO-Phe-Leu-Val-NH_2, 940 mg (1.72 mmol, 1.0 equiv) of acid HO-Lys(Cbz)-*N*-Me-Leu-NBoc, 2.4 mL (8 equiv) of DIPEA, 347 mg (1.08 mmol, 0.6 equiv) of TBTU, 342 mg (0.90 mmol, 0.50 equiv) HATU, and 216 mg (0.72 mmol, 0.4 equiv) DEPBT. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the pentapeptide (573 mg, 37% yield).

R_f: 0.5 (EtOAc:Hex 7:3).

^{1}H NMR (400 MHz, $CDCL_3$): δ 0.8–1.0 (m, 18H), 1.3–1.4 (m, 2H), 1.5 (m, 12H), 1.6–1.7 (m, 5H), 1.7–1.8 (s, 1H), 1.8–1.9 (br, 1H), 2.2 (br, 1H), 3.7–3.8 (d, 3H), 3.0–3.2 (m, 4H), 3.7 (s, 3H), 4.2 (m, αH), 4.3–4.4 (br, αH), 4.5 (br, αH), 4.6–4.7 (br, αH), 4.8 (m, αH), 4.9 (br, 1H), 5.1 (s, 2H), 6.6–6.8 (br, 2H), 6.9 (br, 1H) 7.1–7.2 (d, 2H), 7.2–7.3 (m, 5H), 7.3–7.4 (m, 4H).

4.3.19.8. Macrocycle Phe-Leu-Val-Lys(Cbz)-*N*-Me-Leu. Macrocycle Phe-Leu-Val-Lys(Cbz)-*N*-Me-Leu was synthesized following the 'Macrocyclization procedure'. Utilizing 478 mg (0.61 mmol, 1.0 equiv) of linear pentapeptide, 0.63 mL (6 equiv) of DIPEA, 97.3 mg (0.30 mmol, 0.50 equiv) of TBTU, 115 mg (0.30 mmol, 0.50 equiv) HATU, and 90.7 mg (0.30 mmol, 0.50 equiv) of DEPBT. The crude reaction was purified by column chromatography (silica gel, EtOAc/Hex) to yield the macrocycle (47.6 mg, 10.5% yield).

R_f: 0.5 (EtOAc:Hex 4:1).

^{1}H NMR (400 MHz, $CDCL_3$): δ 0.7–1.1 (m, 18H), 1.2 (m, 1H), 1.4–1.6 (m, 6H), 1.6–1.8 (m, 3H), 1.9 (s, 2H), 1 2.7 (s, 3H), 2.9–3.0 (m, 1H), 3.1–3.3 (m, 3H) 3.9 (t, αH), 4.3 (m, αH), 4.4 (br, αH), 4.4–4.6 (br, αH), 5.0 (m, αH), 5.1 (s, 2H, 1αH), 6.7 (br, 1H), 7.1–7.3 (m, 7H), 7.3–7.4 (m, 6H), 7.7–7.8 (s, 1H).

LC–MS: m/z calcd for $C_{41}H_{60}N_6O_7$ (M+1) = 748.45, found 749.5.

4.3.20. Removal of carbobenzyloxy groups via acid to yield compound 69

Utilizing 47.6 mg (0.06 mmol, 1 equiv) of pentapeptide Phe-Leu-Val-Lys(Cbz)-*N*-Me-Leu, the carbobenzyloxy group was removed by dissolving the compound in 33% Hydrogen bromine (HBr) in glacial acetic acid (0.1 M). The crude reaction was concentrated down in vacuo and purified via reversed-phase HPLC. (17.2 mg, 84% yield).

R_f: 0.5 (EtOAc:Hex 5:1).

^{1}H NMR (400 MHz, CD_3OD): δ 0.7–1.1 (m, 18H), 1.2 (m, 1H), 1.4–1.6 (m, 6H), 1.6–1.8 (m, 3H), 1.9 (s, 2H), 2.7 (s, 3H), 2.9–3.0 (m, 1H), 3.1–3.3 (m, 3H), 3.9 (t, αH), 4.3 (m, αH), 4.4 (br, αH), 4.4–4.6 (br, αH), 5.0 (m, αH), 5.1 (s, 2H, 1 αH), 6.7 (br, 1H), 7.3–7.4 (m, 6H), 8.4 (s, 1H).

LC–MS: m/z calcd for $C_{33}H_{54}N_6O_5$ (M+1) = 614.82, found 615.5.

Acknowledgments

We thank San Diego State University, the Frasch foundation (658-HF07), and CSUPERB for financial support. We thank the Howell Foundation for support of R.C.V. We thank NIW T90DK07015 for support of R.C.V. We thank NIH 1U54CA132379-01A1 for support of SRM and VA.

Supplementary data

Supplementary data (^{1}H NMR data and LC–MS data for compounds and their intermediates, *C* log *P* values, % inhibition, and a list of compounds with their structural variation by position) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.07.017.

References and notes

1. Cueto, M.; Jensen, P. R.; Fenical, W. *Phytochemistry* **2000**, *55*, 223.
2. Hwang, Y.; Rowley, D.; Rhodes, D.; Gertsch, J.; Fenical, W.; Bushman, F. *Mol. Pharmacol.* **1999**, *55*, 1049.
3. Belofsky, G. N.; Jensen, P. R.; Fenical, W. *Tetrahedron Lett.* **1999**, *40*, 2913.
4. Liu, S.; Gu, W. D. L.; Ding, X.-Z.; Ujiki, M.; Adrian, T. E.; Soff, G. A.; Silverman, R. B. *J. Med. Chem.* **2005**, *48*, 3630.
5. Ujiki, M.; Milam, B.; Ding, X.-Z.; Roginsky, A. B.; Salabat, M. R.; Talamonti, M. S.; Bell, R. H.; Gu, W.; Silverman, R. B.; Adrian, T. E. *Biochem. Biophys. Res. Commun.* **2006**, *340*, 1224.
6. Styers, T. J.; Kekec, A.; Rodriguez, R. A.; Brown, J. D.; Cajica, J.; Pan, P.-S.; Parry, E.; Carroll, C. L.; Medina, I.; Corral, R.; Lapera, S.; Otrubova, K.; Pan, C.-M.; Mcguire, K. L.; Mcalpine, S. R. *Bioorg. Med. Chem.* **2006**, *14*, 5625.
7. Rodriguez, R. A.; Pan, P.-S.; Pan, C.-M.; Ravula, S.; Lapera, S. A.; Singh, E. K.; Styers, T. J.; Brown, J. D.; Cajica, J.; Parry, E.; Otrubova, K.; Mcalpine, S. R. *J. Org. Chem.* **2007**, *72*, 1980.
8. Pan, P. S.; Curtis, F. A.; Carroll, C. L.; Medina, I.; Liotta, L. A.; Sharples, G.; Mcalpine, S. R. *Bioorg. Med. Chem.* **2006**, *14*, 4731.
9. Otrubova, K.; Styers, T. J.; Pan, P.-S.; Rodriguez, R.; Mcguire, K. L.; Mcalpine, S. R. *Chem. Commun.* **2006**, 1033.
10. Carroll, C. L.; Johnston, J. V. C.; Kekec, A.; Brown, J. D.; Parry, E.; Cajica, J.; Medina, I.; Cook, K. M.; Corral, R.; Pan, P.-S.; Mcalpine, S. R. *Org. Lett.* **2005**, *7*, 3481.
11. Lee, Y.; Silverman, R. B. *Org. Lett.* **2000**, *2*, 3743.
12. Gu, W.; Liu, S.; Silverman, R. B. *Org. Lett.* **2002**, *4*, 4171.
13. Otrubova, K.; Lushington, G. H.; Vander Velde, D.; Mcguire, K. L.; Mcalpine, S. R. *J. Med. Chem.* **2008**, *51*, 530.
14. Pan, P. S.; Mcguire, K.; Mcalpine, S. R. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5072.
15. Amidon, G. L.; Lee, H. J. *Annu. Rev. Pharmacol. Toxicol.* **1994**, *34*, 321.
16. Wenger, R. M. *Prog. Allergy* **1986**, *38*, 46.
17. Jarvis, L. M. *Chem. Eng. News* **2006**.
18. Marx, V. *Chem. Eng. News* **2005**, *83*, 17. http://pubs.acs.org/cen/business/83/i11/8311bus1.html.
19. The most recent peptide drugs include Byetta from Amylin: <http://www.drugs.com/pro/byetta.html>http://www.drugs.com/pro/byetta.html<http://www.drugs.com/pro/byetta.html><http://www.drugs.com/pro/byetta.html>, and from Aileron comes stapled peptides: http://pubs.acs.org/cen/coverstory/86/8622cover.html.
20. Singh, E. K.; Sellers, R. P.; Alexander, L. D.; Mcalpine, S. R. *Curr. Opin. Drug Disc. Dev.* **2008**, *11*, 544.
21. Loffet, A. *Eur. Peptide Soc.* **2002**, *8*, 1.
22. Murr, M. M.; Sarr, M. G.; Oishi, A. J.; Heerden, J. A. *CA Cancer J. Clin.* **1994**, *44*, 304.
23. Burris, H. A.; Moore, M. J.; Andersen, J.; Greem, M. R.; Rothenberg, M. I.; Modiano, M. R.; Cripps, M. C.; Portenoy, R. K.; Sotorniolo, A. M.; Tarassaoff, P.; Nelson, R.; Dorr, F. A.; Stephens, C. D.; Vonhoff, D. *J. Clin. Oncol.* **1997**, *15*, 2403.
24. Sener, S. F.; Fremgen, A.; Menck, H. R.; Winchester, D. P. *J. Am. Coll. Surg.* **1999**, *189*, 1.
25. Styers, T. J.; Rodriguez, R. A.; Pan, P.-S.; Mcalpine, S. R. *Tetrahedron Lett.* **2006**, *47*, 515.
26. The *C* log *P* values were calculated using an algorithm. The log *P* value of a compound, which is the logarithm of its partition coefficient between *n*-octanol and water $\log(c_{octanol}/c_{water})$, is a well established measure of the compound's hydrophilicity. Low hydrophilicities and therefore high log *P* values cause poor absorption or permeation. It has been shown for compounds to have a reasonable probability of being well absorbt their log *P* value must not be greater than 5.0. The distribution of calculated log *P* values of more than 3000 drugs on the market underlines this fact.
27. Lipinskis rules state that the ideal drug contains no more than five H-doners, no more than 10 H-acceptors, molecular weights of less than 500, and log *P* values $\leqslant$5.0.
28. Dipeptide and tripeptide structures were confirmed using ^{1}H NMR. All linear pentapeptides were confirmed using LC–MS 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 Supplementary data for spectra.
29. Geistlinger, T. R.; Mcreynolds, A. C.; Guy, R. K. *Chem. Biol.* **2004**, *11*, 273. Multiple coupling agents reference.
30. 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.
31. Two cell lines were chosen to provide evidence that our compounds were targeting a type of cancer, that is, pancreatic. PL-45 is a primary pancreatic

ductial adenocarcinoma and BxPC3 is the cell line of choice for xenograft mouse model studies.
32. Chatterjee, J.; Mierke, D. F.; Kessler, H. *J. Am. Chem. Soc.* **2006**, *128*, 15164.
33. Heller, M.; Sukopp, M.; Tsomaia, N.; John, M.; Mierke, D. F.; Reif, B.; Kessler, H. *J. Am. Chem. Soc.* **2006**, *128*, 13806.
34. Zhang, X.; Nikiforovich, G. V.; Marshall, G. R. *J. Med. Chem.* **2007**, *50*, 2921.
35. Tyndall, J. D.; Pfieiffer, B.; Abbenante, G.; Fairlie, D. P. *Chem. Rev.* **2005**, *105*, 793.
36. Viles, J. H.; Mitchell, J. B.; Gough, S. L.; Doyle, P. M.; Harris, C. J.; Sadler, P. J.; Thornton, J. M. *Eur. J. Biochem.* **1996**, *242*, 352.
37. See Supplementary data for *C* log *P* values.