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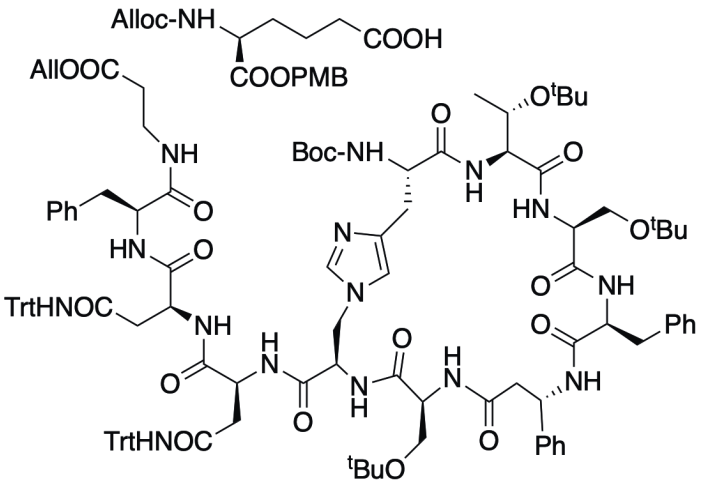

Progress Toward the Assembly of the Bicyclic Theonellamide Skeleton

Jyoti P. Mukherjee, Joyeeta Roy, Chyree S. Batton, Saroj Yadav, Douglas Wong and Carol M. Taylor*

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Abstract. An orthogonally protected τ-histidinoalanine residue was synthesized via regioselective alkylation of optically pure Boc-L-His-OTCE (TCE = trichloroethyl) with a sulfamidate electrophile derived from Fmoc-D-Ser-OBn. The goal was the synthesis of a non-natural theonellamide, invoking readily accessible variants of the other 10 amino acids. Peptide fragments corresponding to the east and west rings of "theonellamide X" were synthesized in solution. Each ring was formed independently, providing insights into protecting group limitations, side reactions, and the optimal order of events to approach the formation of the bicyclic system. Ultimately, an undecapeptide was prepared, with the eastern ring formed. The twelfth amino acid, an L-α-aminoadipic acid building block, was prepared and preliminary investigations into its attachment to the undecapeptide are reported.

1. Introduction

Theonellamides (TNMs) A-E,[1] F (**1**),[2] G[3] and I,[4] along with theonegramide[5] and theopalauamide,[6] are bicyclic dodecapeptides that were isolated from sponges of the *Theonella* genus and shown to inhibit the growth of prototypical fungi and cancer cell lines. In the case of theopalauamide, it was established unequivocally that the peptide was produced by symbiotic filamentous bacteria.[7,8] The peptides are characterized by a

bridging τ-histidinoalanine residue and a high degree of post-translationally modified residues. Since the disclosure of the structure of theonellamide F (**1**, Figure 1) in 1989,[2] many studies have been reported seeking to uncover the biological activity of these architecturally novel dodecapeptides.

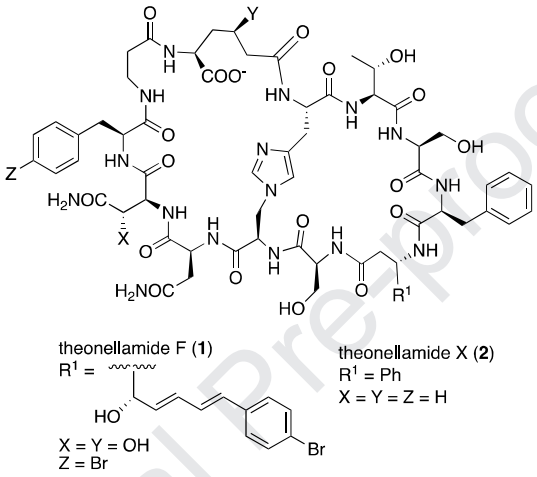


Figure 1

Theonellamides were the first low molecular weight compounds reported to induce the formation of extraordinarily large vacuoles, *i.e.*, fluid-filled compartments in the cytoplasm. Watabe and coworkers made this observation in 3Y1 rat embryonic fibroblasts, where TNMs did not kill the cells, giving the first indication of potential as good molecular probes to investigate intracellular membrane structures.[9,10] Vacuole formation is a well-known marker of microbial activity, that often precedes cell lysis. Through affinity chromatography, Wada et al. established that theonellamides A and F bind to two enzymes from rabbit liver: 17β-hydroxysteroid dehydrogenase IV and glutamate dehydrogenase.[11] This 2000 discovery of affinity for a steroid dehydrogenase was prophetic.

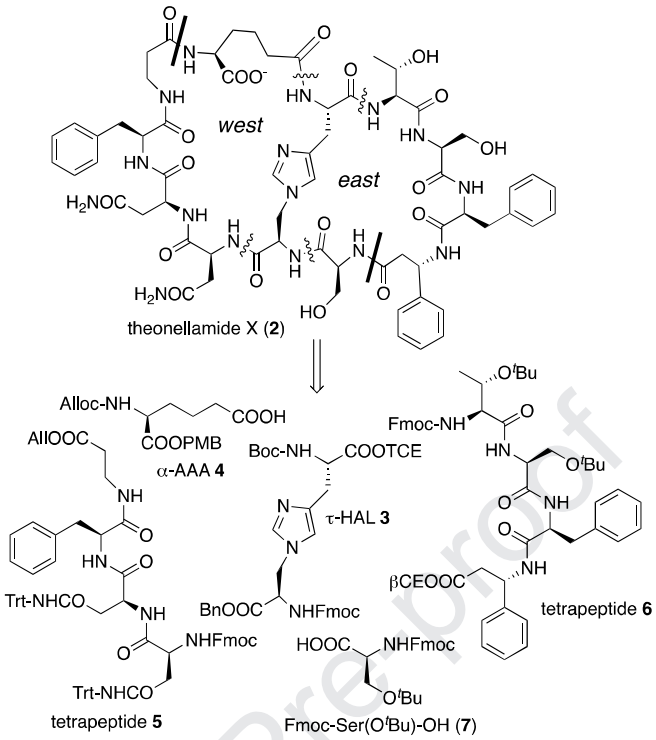
About 10 years ago, breakthrough chemical genetics studies revealed theopalauamide to be a nanomolar antifungal agent that disrupts the cell membrane via binding to ergosterol and up-regulates β-D-glucan synthesis.[12,13] In 2013, Espiritu et al. confirmed that only 3β-hydroxy-functionalized steroids bind to TNM.[14] They used solid-state deuterium NMR, and surface plasmon resonance experiments, to demonstrate that the steroid enhances the affinity of the TNM for lipid bilayers. The same year, Nishimura et al. produced fluorescent conjugates of TNMs that served as probes for sterols in liposomes.[15] In 2016, Espiritu et al. used ^{31}P NMR and dynamic light scattering to demonstrate sterol concentration-dependent disruption of phosphatidylcholine membranes by TNM A.[16] Cornelio et al. recently reported NMR titration experiments to determine the binding constant of TNM A and 25-hydroxycholesterol in aqueous DMSO. They concluded that the steroid binds in the hydrophobic cavity of the TNM, reducing the polarity of the latter, and thereby facilitating interactions with lipid membranes.[17] The vast majority of these highly collaborative studies have been conducted using TNM A, from natural sources.

The chemical synthesis of theonellamides has the potential to produce useful amounts of material and to probe the role of the various residues via structure-activity studies. In the early 1990s, Tohdo, Hamada and Shioiri reported progress toward the synthesis of theonellamide F (**1**), in the form of conference proceedings and communications.[18-24] They formed each of the two rings independently but no plan was enunciated for the formation of the bicycle. Cyclizations were performed between the *p*-bromophenylalanine and β-alanine residues in the western ring and between the phenylalanine and (3*S*,4*S*,5*E*,7*E*)-3-amino-4-hydroxy-6-methyl-8-bromophenyl)-5,7-

octadienoic acid (Aboa) residues in the eastern ring using diphenylphosphoryl azide at high dilution in yields of 21 and 24% respectively.

We have recently reported the synthesis of some of the modified residues, namely *erythro*-hydroxyasparagine,[25] τ-histidinoalanine,[26] and (2*S*,4*R*)-2-amino-4-hydroxy-adipic acid.[27] We describe herein our strategy for the assembly of the *bis*-macrocycle and its application to "theonellamide X" (**2**), a non-natural congener. With the exception of τ-histidinoalanine, compound **2** invokes amino acids that are commercially available, *viz*. the simplest representation of residues that occur in the natural products, or surrogates thereof. Noteably, the Aboa/Apoa residue was replaced by β-phenylalanine. This retains the ring size of the eastern ring, the configuration at C3 of the residue, and π-electron density in the truncated side chain.

Construction of the bicyclic structure requires a carefully choreographed amalgamation of the fragments and building blocks (Scheme 1). Since the major element of conformational restraint in the molecule is the τ-HAL residue, we set out to construct this cornerstone and build on the two rings sequentially. We chose cyclization sites that would involve non-epimerizable activation sites, viz. those of β-aminoacids. Moreover, the linear precursors, with the τ-HAL residue embedded between peptide "arms" ought to be able to achieve conformations amenable to cyclization. Our protecting group strategy embraced Hirschmann's concept of "coordinated orthogonal deprotection,"[28] viz., Alloc/allyl ester in the western ring and Fmoc/β-cyanoethyl (βCE) ester in the eastern ring. In each ring, the terminal protecting groups ought to be removed chemoselectively and simultaneously. "Permanent" protection of the side chain functional groups with traditional acid-labile groups, suffices for the current model system.



Scheme 1. Retrosynthetic analysis of theonellamide X (**2**) (solid/bold line indicates site of cyclization; wavy line is a regular disconnection).

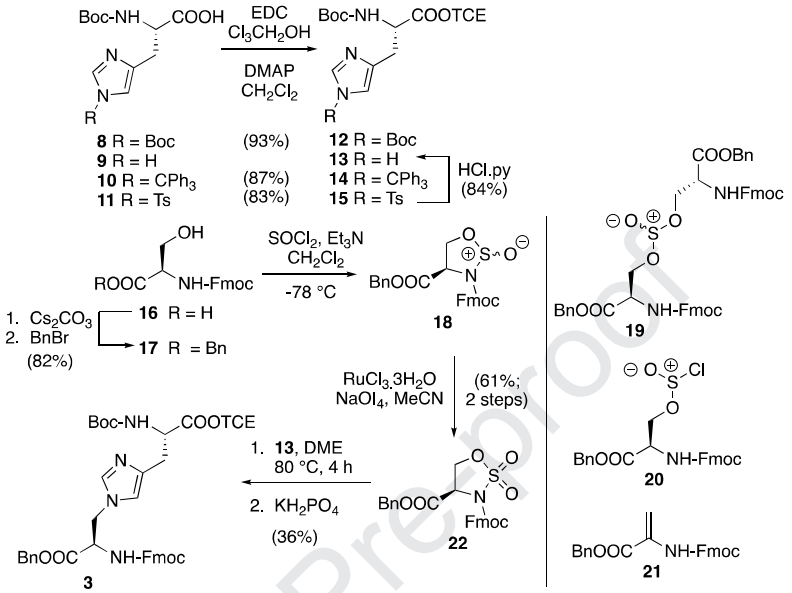
2. Results and Discussion

Pivotal to the successful synthesis of a theonellamide is an efficient synthesis of the τ-histidinoalanine (τ-HAL) building block.[29] We sought maximum flexibility vis-à-vis protecting groups. The vast majority of precedent for protection of *bis*-amino acids comes from the lantibiotic literature.[30] There is much fanfare these days about protecting group free synthesis. Wuts cautions, "There are, however, many classes of molecules where our chemical technology is still not completely adequate to completely avoid the use of protecting groups." He identities peptides as one of the three classes of compounds.[31] We are not aware of any system for *bis*-amino acids that conforms to Barany and Merrifield's rigorous definition of an orthogonal system as "a set of

completely independent classes of protecting groups. In a system of this kind, each class of groups can be removed in any order and in the presence of all other classes."[32] Following considerable thought and experimentation, we arrived at building block **3**. For the α-amino groups, the Fmoc[33] and Boc groups were selected. The Fmoc group is removed via β-elimination and the Boc group via acidolysis. Benzyl and trichloroethyl (TCE)[34] esters were employed for the α-carboxylates. While both can be removed by reduction, the former involves a hydrogenolytic process and the latter a two-electron mechanism. The TCE ester was approached with well-founded caution (vide infra), but a methyl ester – perhaps the more obvious choice – could not be selectively removed, even with tin(II) hydroxide.[35] Moreover, Shioiri and co-workers had reported that alkaline hydrolysis of such an ester was accompanied by "slight epimerization."[21]

Aware of the propensity for histidine to racemize on activation of the carboxylate,[36] we sought to protect the τ-nitrogen of the imidazole, initially with Boc (Scheme 2). Compound **12** was thus prepared in short order, but we were unable to remove the *Nτ*-Boc group to give **13**, without accompanying side reactions of the TCE ester. Treatment with sodium methoxide[37] led to transesterification. Exposure to a weaker, non-nucleophilic base (Cs_2CO_3, imidazole, MeCN),[38] led to cleavage of the TCE ester and formation an insoluble material that was reminiscient of that described by Sheehan *et al*.[39] While TCE ester **13** could be prepared in good chemical yield, direct from **9**, it was indeed found to be a ~60:40 mixture of enantiomers by chiral HPLC; τ-trityl protected **14** was key to that analysis. Ultimately, **13** was prepared from Boc-His(*Nτ*-Ts)-OH (**11**)[40] via carbodiimide-mediated esterification,[41] followed by cleavage of the tosyl amide with pyridinium hydrochloride, according to Beyerman et al.[42]

Unfortunately, in our hands, unwanted formylation was observed with DMF as solvent. Dimethylsulfoxide afforded the requisite solubility without this side reaction.

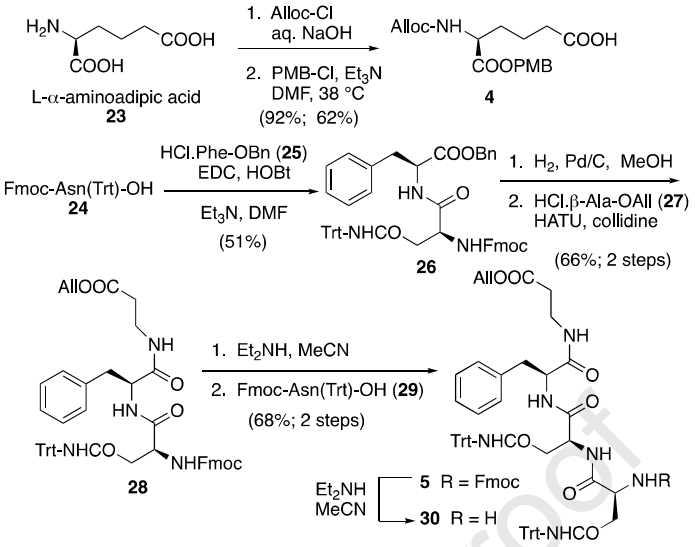


Scheme 2. Synthesis of orthogonally protected τ-histidinoalanine **3**.

Sulfamidate **22** was prepared from Fmoc-D-Ser-OBn (**17**) by analogy to literature procedures.[43] Thus, treatment of **17** with thionyl chloride led to the formation of sulfimidite **18**, requiring base. Variation in the nature of the base and the temperature of the reaction afforded little optimization; disappearance of starting material was not complete before competing side reactions led to a complex mixture. Careful chromatography of **18** and oxidation to sulfamidate **22** was satisfactory; but when crude mixtures of **18** were subjected to oxidation, it was difficult to obtain useful amounts of pure **22**. Ultimately, it was discovered that disulfite ester **19** was a major side product of the first step, isolable in ~25% yield. This side reaction was previously recognized by Shiokawa et al.[44] and could be minimized by the gradual addition of a solution of **17** to a

dilute solution of thionyl chloride and triethylamine. Chlorosulfite ester **20** and dehydroalanine **21** were also observed in the NMR and mass spectra of product mixtures. Alkylation of Boc-His-OTCE (**13**) with high purity sulfamidate **22**, followed by hydrolysis of the sulfamic acid, afforded **τ-3**. As observed previously,[43a] none of the π-regioisomer was observed. Despite considerable investigation and attempted optimization of the coupling reaction, low yields prevailed. A contributing factor is the instability of the sulfamidate. The reaction of **22** with **13** struggles to complete with the degradation of **22** to an intractable mixture, probably involving a dehydroalanine intermediate. The low affinity of an imidazole for a sulfamidate electrophile is corroborated by De Luca and coworkers who reported that the thiol of the cysteine residue in a pentapeptide (Ac-Cys-Gly-His-Val-Ala-NH_2) reacts with the sulfamidate derived from Boc-Ser-OMe to form a lanthionine,[45] with the unprotected histidine residue providing no competition. Preliminary experiments indicated that each protecting group of τ-HAL **3** could be removed chemoselectively.[46]

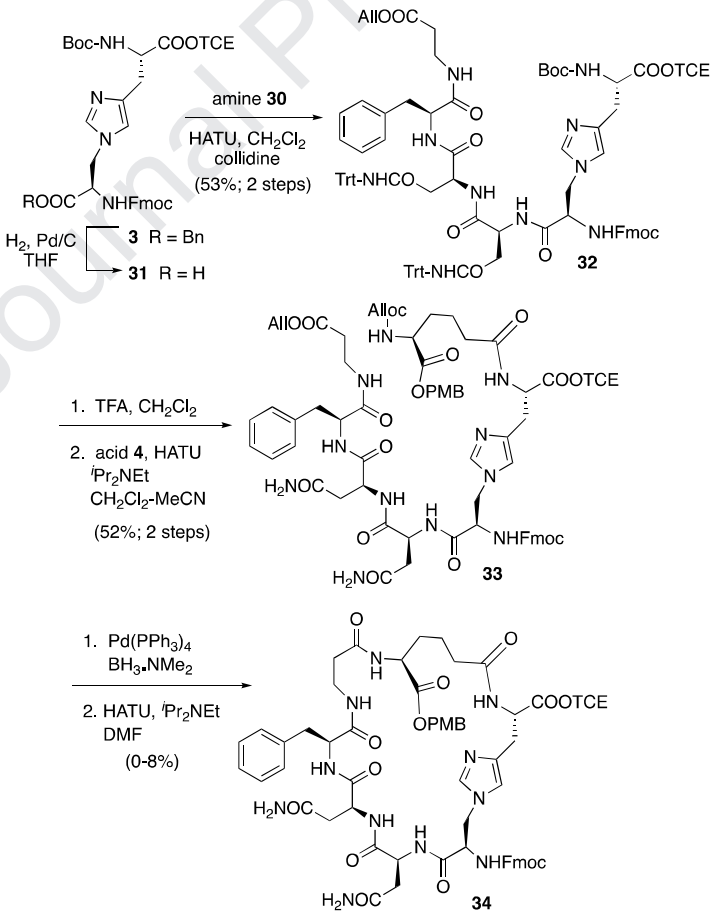
We next describe the assembly of the western hemisphere. L-α-Amino-adipic acid building block **4** was prepared by analogy to our previous report.[27] Tetrapeptide Fmoc-Asn(Trt)-Asn(Trt)-Phe-β-Ala-OAll (**5**) was prepared according to standard solution phase protocols. Asparagine building block **24** was coupled with phenylalanine benzyl ester (**25**) to give dipeptide **26**. Hydrogenolysis of **26** afforded the corresponding acid that was coupled with the hydrochloride salt of the allyl ester of β-alanine (**27**), to afford tripeptide **28**. The Fmoc-group was removed from the N-terminus of **28** and a second asparagine appended to give compound **5**. The N-terminus was deprotected immediately prior to coupling with τ-HAL, to give amine **30**.



Scheme 3. Assembly of the Western fragments of theonellamide X (**2**).

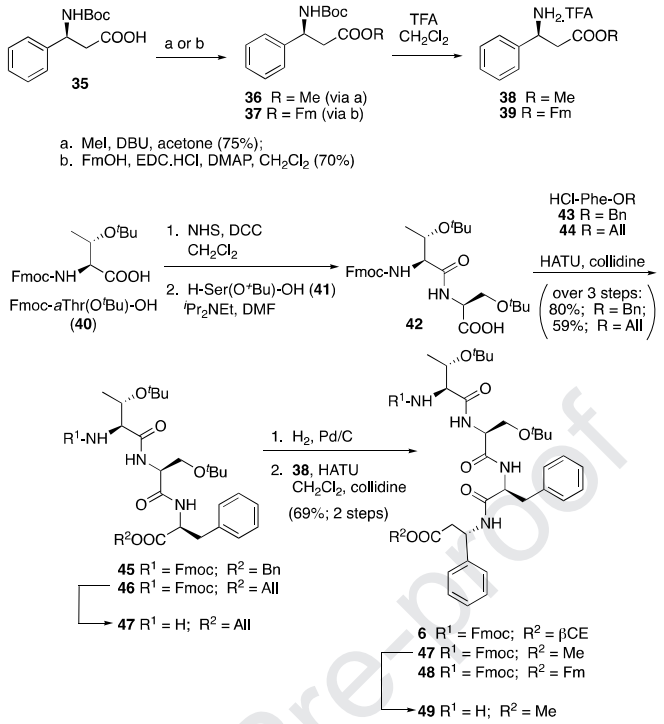
In parallel, the benzyl ester of τ-HAL **3** was removed hydrogenolytically. Tetrahydrofuran was the solvent of choice for this reaction with complex reaction mixtures arising when ethyl acetate or alcohols were used as solvent. It is likely that reductive dehalogenation of the TCE group was the problem here. The resulting amine **30** and acid **31** were coupled using HATU and collidine[47] to give **32**. Treatment of hexapeptide **32** with TFA led to removal of the Boc-group of the τ-HAL residue and cleavage of the trityl amides. Condensation of freshly prepared acid **4** with the amine derived from **32** afforded linear heptapeptide **33**. We were aware of four previous examples of simultaneous removal of two allyl-based protecting groups as a prelude to macrocyclization: Yokokawa and Shiori's synthesis of antillatoxin,[48] Luesch and coworkers' synthesis of lyngbyastatin,[49] the synthesis of somamide A by Yokokawa and Shioiri[50] and the assembly of an α-helical mimetic by Alewood and coworkers.[51] A range of allyl acceptors were trialed and the reaction was found to be highly dependent on the source and quality of $Pd(PPh_3)_4$, with only traces of the macrocycle **34** isolated,

following cyclization. Over time, we came to realize that the TCE ester was not surviving the Pd-catalyzed deallylation reaction conditions. This protecting group gives rise to distinctive NMR signals, e.g., for linear heptapeptide **33** in DMSO-d_6: δ 4.84 and 4.91 ppm (^2J = 12.2 Hz) for the diastereotopic CH_2 protons in the ^{1}H spectrum and δ 73.8 (CH_2) and 95.5 (CCl_3) ppm in the ^{13}C spectrum. The molecular ion cluster of TCE-containing species is also characteristic. These "signatures" were typically missing from the NMR and mass spectra of crude product mixtures. A small scale trial, replacing the TCE ester with a methyl ester, led to successful deallylation and cyclization, confirming the incompatibility of the TCE protecting group with the conditions required to remove the Alloc and allyl ester protecting groups.



Scheme 4. Assembly of the Western ring of theonellamide X.

Independent investigation of the assembly of the Eastern hemisphere is depicted in Schemes 5 and 6. Our original retrosynthetic analysis (Scheme 1) involved a β-cyanoethyl (βCE) ester[52] at the C-terminus, *viz.* fragment **6**. Early reports had shown that the βCE group could be removed by piperidine.[53] Tetrapeptide **6** was synthesized, in early studies, but upon coupling to the τ-HAL residue, gave a mixture of two peptides that were inseparable and did not perform as expected in subsequent attempts to deprotect and cyclize. Indeed, while this ester protecting group was advocated for use in peptide synthesis as early as 1989,[52] there have been no reports of its application in complex molecule synthesis. Since our philosophy was for this ester to be removed under the same conditions as an Fmoc group, two other esters of Nα-Boc-β-phenylalanine (**35**) were prepared, viz. methyl ester **36** and fluorenylmethyl[54] ester **37** (Scheme 5). The Boc group was removed from each to give the corresponding trifluoroacetate salts, **38** and **39**.

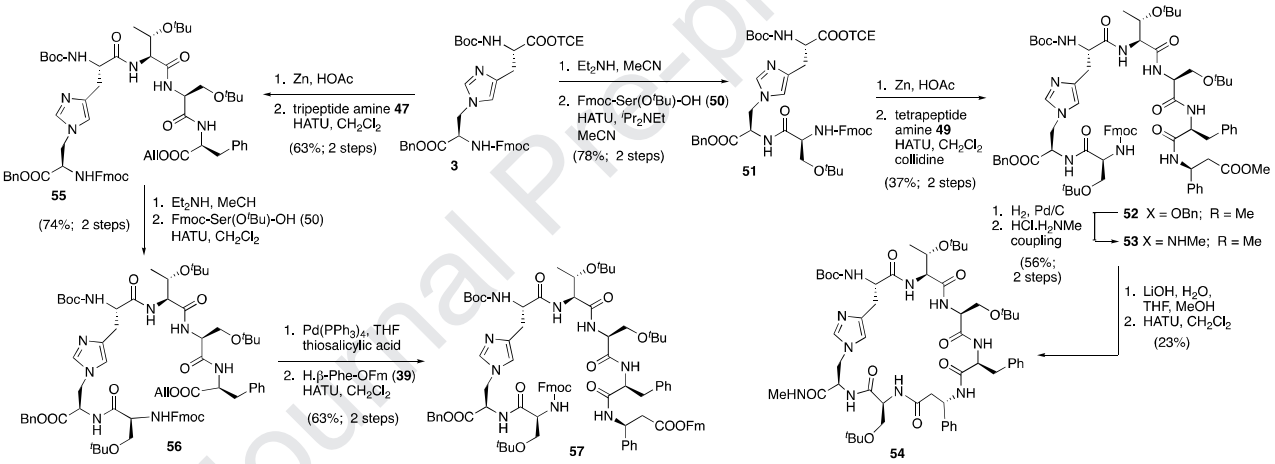


Scheme 5. Assembly of the Eastern fragment of theonellamide X (**2**).

Commercially available *allo*-threonine derivative **40** was activated as its NHS ester and coupled with side-chain protected serine **41** to give dipeptide acid **42**. Elongation in the C-terminal direction with phenylalanine esters **43** (benzyl) or **44** (allyl) gave the corresponding tripeptides **45** and **46**. Hydrogenolysis of **45** and coupling with methyl ester **38** gave tetrapeptide **47**. Tetrapeptide **48**, with the fluorenylmethyl ester at the C-terminus, could be prepared by analogy. However, the Fmoc group could not be removed selectively from the N-terminus.[55] Thus, tripeptide **46** was employed and the β-phenylalanine residue appended at a later stage.

Selective removal of the Fmoc group from τ-HAL **3** (Scheme 6, center) gave a primary amine that was acylated with a commercially available serine derivative **50** (Scheme 6, clockwise from **3**). Reductive cleavage of the trichloroethyl ester from

compound **51** gave a free acid that was coupled with tetrapeptide amine **49**. Removal of the Fmoc group from compound **52** was accompanied by cyclization to form a diketopiperazine,[56] with concomitant loss of the benzyl ester, as suggested by mass spectrometry. Thus, to continue independent studies of the eastern ring, we converted benzyl ester **52** to methyl amide **53**. The methyl ester and the Fmoc carbamate could be removed from **53** under alkaline hydrolysis. Cyclization was induced by HATU to give the eastern macrocycle **54**; this reaction was not optimized.



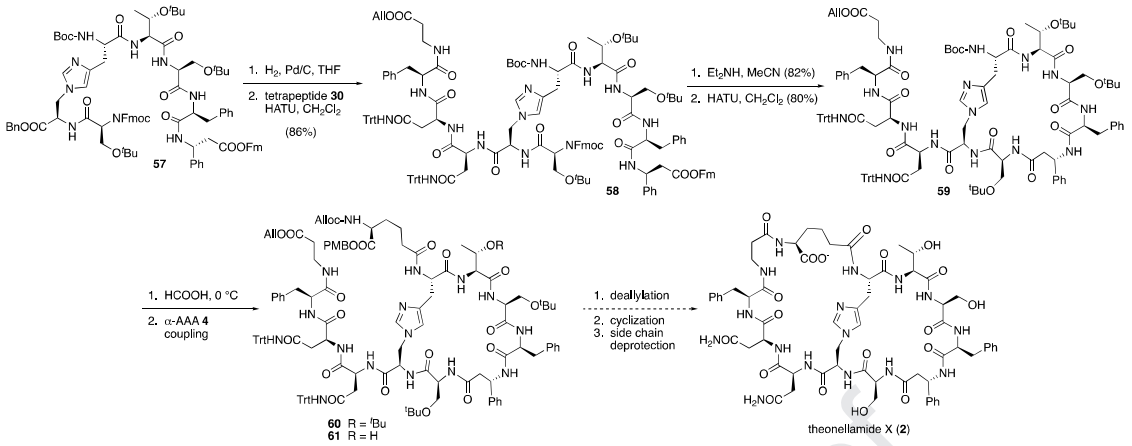
Scheme 6. Assembly of the Eastern ring of theonellamide X.

While the formation of **54** demonstrated the feasibility of cyclization between the β-Phe and Ser residues in the eastern ring, compound **54** does not lend itself to further elaboration. The necessary order of events, for assembly of the bicycle,[57] was emerging. Rather than introducing a methyl amide, we ought to introduce a relevant peptide, prior to Fmoc removal. With the long-term view, favoring the fluorenylmethyl ester for the β-Phe residue, pentapeptide **55** was produced, in good yield from τ-HAL **3**. Addition of the

Ser residue gave rise to hexapeptide **56**. Deallylation and incorporation of the β-Phe residue led to heptapeptide **57**.

Hydrogenolysis of the benzyl ester of the τ-HAL residue in compound **57** gave the corresponding acid. While histidine epimerization was difficult to quantify, the appearance of a second singlet (typically slightly downfield of that attributable the Boc group in the desired epimer), in peptides derived from **3**, alerted us to the likelihood of epimerization at Cα of the histidine of τ-HAL. It is not widely appreciated that histidine residues can epimerize during palladium-catalyzed reactions. Méry and Calas proposed that Pd^0 behaves as a Lewis acid, activating the His C=O, thereby increasing the acidity of Hα, which is in close proximity to the Nπ that can serve as an internal base.[58] The inclusion of small volumes of acetic acid suppressed this side reaction. These concerns about epimerization led to the realization that the basic Nπ could serve as an internal base for the purposes of peptide couplings, averting the need to add an external base. Thus, in the left-hand side of Scheme 6, and through Scheme 7, amide bond formation was achieved without the addition of collidine or diisopropylethylamine.

The acid derived from **57** was coupled with tetrapeptide amine **30** (Scheme 3), to give the branched undecapeptide **58** (Scheme 7). Gratifyingly, the Fmoc and Fm ester protecting groups were removed, within 2 hours, under standard conditions in 84% yield. Cyclization, with HATU was proceeded in an impressive 80% yield. This compares favorably with the 24% yield reported by Shioiri and coworkers for cyclization of the east ring between the Phe and Aboa residues.[21]



Scheme 7. Construction of the bicycle and deprotection.

Removal of the Boc group was attempted under a range of conditions, following literature precedents that gave us hope that this could be done chemoselectively. Given the solubility issues experienced with compounds **53** and **54**, we particularly sought conditions that would leave the trityl amides intact. Jiang et al.[59] had shown that bromocatechol borane[60] could removal a Boc group, leaving trityl amides intact. Sakaitani and Ohfune first reported the combination of TBSOTf/lutidine to remove Boc groups via a silyl carbamate.[61] Rich and coworkers achieved this in the presence of a trityl amide.[62] Bastiaans et al. used the protocol in their cyclotheonamide B synthesis, leaving a *tert*-butyl (aryl) ether unharmed.[63] The most promising conditions we identified were those described by Tan and Ma in their salinamide A synthesis,[64] viz. neat formic acid. However, the major product of this reaction arose from loss of one *tert*-butyl ether, as well as the Boc carbamate. Coupling with α-AAA building block **4** gave a mixture that included decapeptides **60** and **61**, as confirmed by mass spectrometry.

What remains, to complete the bicyclic structure of the theonellamides, is removal of the two allyl-based protecting groups, cyclization of the western ring and global

deprotection of the side chain functional groups. It would be highly advantageous to optimize the Boc-deprotection to produce a single compound prior to advancing these goals.

3. Conclusions

We have described the synthesis of an orthogonally protected τ-HAL building block that has served well in the assembly of the 12 amino acids of the theonellamide skeleton. The trichloroethyl (TCE) ester has provided some complications in that it suffers from nucleophilic attack and reductive cleavage. As Davies has said, "In peptide synthetic circles dioxopiperazines are usually unwelcome;"[65] that was certainly the case during efforts to elaborate **52** to the eastern macrocycle. Fortunately, conversion of benzyl ester **52** to undecapeptide **58**, laid the foundation for cleavage of the Fmoc and Fm ester protecting groups, followed by formation of the east ring. Preliminary studies are described to append the last amino acid, α-amino-adipic acid. While this is the end of the road for Taylor and coworkers, we hope that the lessons learned in our laboratory over the past decade or so will inspire others to pursue the chemical synthesis of the theonellamides, and study the structure-activity relationships of these beautiful and valuable molecules.

4. Experimental Section

4.1 General

Reactions were performed under a nitrogen atmosphere unless otherwise noted. Reagents were obtained from commercial sources and used directly; exceptions are

noted. Diethylamine, diisopropylethylamine, 2,4,6-collidine and triethylamine were dried and distilled from CaH_2 and stored over KOH pellets. Compounds were visualized by UV fluorescence or by staining with ninhydrin. Flash chromatography was performed using silica gel from a range of sources, over the years, including Agela Technologies (40-60μ). HPLC was performed on a Waters 600 system, interfaced with Empower™ software, and equipped with a Waters 2487 UV detector, monitoring absorption at 218 and 254 nm. Semi-preparative RP work was conducted on a 10 mm diameter Altima® C18 10μ column with a flow rate of 3 mL min^{-1}; eluants are detailed in each procedure. NMR spectra were recorded on Bruker AV-400 or AV-500 liquid spectrometers. Proton NMR data is reported in ppm downfield from TMS as an internal standard or calibrated on solvent peaks. High resolution mass spectra were recorded using electrospray ionization on an Agilent 6230 ESI TOF instrument. Rotations were measured on a Jasco P2000 polarimeter.

4.2 Synthesis of Trichloroethyl Esters

2,2,2-Trichloroethanol (1.5 equiv.) was added dropwise to a solution of Boc-His(X)-OH (1.0 equiv.) in CH_2Cl_2 (0.1 mM) under N_2 at 0 °C. EDC hydrochloride (1.2 equiv.) was added in portions, the mixture warmed to rt and stirred for 16 h.

Boc-His(Boc)-OTCE (12). On a 0.23 mmol scale, starting from Boc-His(Boc)-OH (**8**). Dichloromethane (20 mL) and water (15 mL) were added. The aqueous layer was further extracted with CH_2Cl_2 (2 x 15 mL). The combined organic layers were washed with sat'd aq. $NaHCO_3$ (15 mL), sat'd aq. NH_4Cl (15 mL) and brine (15 mL), dried over $MgSO_4$, filtered and concentrated. The yellow residue was subjected to flash

chromatography, eluting with 0.5-2.0% MeOH in CH_2Cl_2 to give Boc-His(Boc)-OTCE (**12**) as a viscous oil (105 mg, 93%). R_f 0.68 (95:5 CH_2Cl_2-MeOH). $[\alpha]^{25}_D$ +1.06 (*c* 0.8, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.45 (s, 9H), 1.61 (s, 9H), 3.09 (dd, *J* = 14.8 4.7 Hz, 1H), 3.18 (dd, *J* = 14.8, 5.5Hz, 1H), 4.67 (d, *J* = 12.0 Hz, 1H), 4.67-4.74 (m, 1H), 4.83 (d, *J* = 12.0 Hz, 1H), 6.03 (d, *J* = 8.2 Hz, 1H), 7.19 (s, 1H), 8.02 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 27.9, 28.3, 29.6, 53.2, 74.6, 80.0, 85.8, 94.6, 114.9, 137.0, 138.1, 146.7, 155.5, 170.5. HRMS (ESI+) calcd for $C_{18}H_{27}Cl_3N_3O_6$ $(M+H)^+$ 486.0965, obsd 486.0975.

Boc-His(Trt)-OTCE (14). On a 0.20 mmol scale, starting from Boc-His(Trt)-OH (**10**). Dichloromethane (20 mL) and water (15 mL) were added. The aqueous layer was further extracted with CH_2Cl_2 (2 x 15 mL). The combined organic layers were washed with sat'd aq. $NaHCO_3$ (15 mL), sat'd aq. NH_4Cl (15 mL) and brine (15 mL), dried over $MgSO_4$, filtered and concentrated. The yellow residue was subjected to flash chromatography, eluting with 0.5-1.5% MeOH in CH_2Cl_2 to give Boc-His(Trt)-OTCE (**14**) as a colorless solid (110 mg, 87%). R_f 0.55 (95:5 CH_2Cl_2-MeOH). $[\alpha]^{25}_D$ -10.3 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.43 (s, 9H), 3.07 (dd, *J* = 14.5, 4.1 Hz, 1H), 3.17 (dd, *J* = 14.5, 4.8 Hz, 1H), 4.55 (d, *J* = 11.8 Hz, 1H), 4.60 (d, *J* = 11.8 Hz, 1H), 4.63-4.71 (m, 1H), 6.34 (d, *J* = 7.8 Hz, 1H), 6.58 (s, 1H), 7.05-7.14 (m, 6H), 7.28-7.36 (m, 9H), 7.43 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 28.3, 29.6, 74.5, 75.3, 79.6, 94.5, 119.7, 128.1 (2C), 129.7, 136.1, 138.8, 142.2, 155.6, 170.4. HRMS (ESI+) calcd for $C_{32}H_{33}Cl_3N_3O_4$ $(M+H)^+$ 628.1537, obsd 628.1541.

Boc-His(Ts)-OTCE (15). On a 3.66 mmol scale, starting from Boc-His(Ts)-OTCE (**11**). Dichloromethane (40 mL) and water (30 mL) was added and the aqueous

layer further extracted with CH_2Cl_2 (2 x 20 mL). The combined organic layers were washed with sat'd aq. $NaHCO_3$ (30 mL), sat'd aq. NH_4Cl (30 mL) and brine (30 mL), dried over $MgSO_4$, filtered and concentrated. The yellow residue was subjected to flash chromatography, eluting with 0.5-2.0% MeOH in CH_2Cl_2 to give Boc-His(Ts)-OTCE (**15**) as a colorless solid (1.10 g, 83%). R_f 0.65 (95:5 CH_2Cl_2-MeOH). $[\alpha]_D^{25}$ +5.3° (*c* 0.6, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.42 (s, 9H), 2.44 (s, 3H), 3.05 (dd, *J* = 15.0, 4.6 Hz, 1H), 3.14 (dd, *J* = 15.0, 4.6 Hz, 1H), 4.55 (d, *J* = 11.9 Hz, 1H), 4.68-4.73 (m, 1H), 4.69 (d, *J* = 11.9 Hz, 1H), 5.91 (d, *J* = 8.3 Hz, 1H), 7.08 (s, 1H), 7.36 (d, *J* = 8.3 Hz, 2H), 7.79 (d, *J* = 8.3 Hz, 2H), 7.92 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.7, 28.3, 29.7, 52.9, 74.5, 80.1, 94.4, 114.9, 127.4, 130.5, 134,7, 136.5, 139.8, 146.5, 155.4, 170.2. HRMS (ESI+) calcd for $C_{20}H_{25}{}^{35}Cl_3N_3O_6S$ (M+H) 540.0524; obsd 542.0540.

Boc-His-OTCE (13). Pyridinium hydrochloride (3.0 g, 25.9 mmol, 7.0 equiv) was added to a stirred solution of **15** (2.0 g, 3.70 mmol, 1.0 equiv.) in DMSO (3 mL) at rt under N_2. The mixture was stirred for another 2.5 h, poured onto H_2O (30 mL) and extracted with EtOAc (3 x 60 mL). The combined organic layers were washed sequentially with sat'd aq. $NaHCO_3$ (30 mL), H_2O (30 mL), brine (30 mL), then dried over $MgSO_4$, filtered, and concentrated to obtain a yellow residue that was purified by flash chromatography, eluting with a gradient of 1-5% MeOH in CH_2Cl_2, to give Boc-His-TCE (**13**) as colorless foam (1.2 g, 84%). R_f 0.35 (95:5 CH_2Cl_2-MeOH). $[\alpha]^{25}{}_D$ -13.33 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.38 (s, 9H), 3.02-3.18 (m, 2H), 4.49-4.64 (m, 2H), 4.72 (d, *J* = 11.8 Hz, 1H), 6.07 (d, *J* = 7.5 Hz, 1H), 6.83 (s, 1H), 7.54 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 28.2, 29.2, 53.6, 74.4, 80.0, 94.4, 115.6, 133.9,

135.3, 155.6, 170.6. HRMS (ESI+) calcd for $C_{13}H_{19}Cl_3N_3O_4$ $(M+H)^+$ 386.0441, obsd 386.0443.

*4.3 Synthesis of τ-Histidinoalanine Building Block **3***

Fmoc-D-Ser-OBn (17). Cesium carbonate (1.39 g, 4.27 mmol, 0.50 equiv.) was added to a solution of Fmoc-D-Ser-OH (**16**) (2.8 g, 8.54 mmol, 1.00 equiv.) in dry MeOH (35 mL). The mixture was stirred at rt for 2 h, concentrated, and the residue dissolved in dry DMF (19 mL) under N_2. Benzyl bromide (1.2 mL, 875 mg, 10.29 mmol, 1.20 equiv.) was added dropwise and the mixture stirred overnight. The mixture was partitioned between EtOAc (70 mL) and H_2O (70 mL). The aqueous layer was further extracted with EtOAc (70 mL), and the combined organic extracts washed with sat'd aq. $NaHCO_3$ (3 x 20 mL) to remove Fmoc-D-Ser-OH. The organic layer was washed with brine (40 mL), dried over $MgSO_4$, filtered and concentrated. The pale yellow oil was purified by flash chromatography on silica gel, eluting with 2:1 hexanes-EtOAc, to give Fmoc-D-Ser-OBn (**17**) as a clear oil that gave a colorless solid, following refrigeration overnight (2.90 g, 82%). R_f 0.38 (1:1 EtOAc-hex). $[\alpha]^{25}_D$ -3.4 (c 1.0, MeOH). ^{1}H NMR (400 MHz,$CDCl_3$) δ 2.22 (br s, 1H), 3.91 (d, *J* = 9.8 Hz, 1H), 4.01 (d, *J* = 9.8 Hz, 1H), 4.20 (t, *J* = 6.5, 1H), 4.36-4.48 (m, 3H), 5.21 (s, 2H), 5.77 (d, *J* = 7.5 Hz, 1H), 7.28-7.33 (m, 7H), 7.39 (t, *J* = 7.4 Hz, 2H), 7.58 (d, *J* = 7.1 Hz, 2H), 7.75 (d, *J* = 7.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 47.1, 56.1, 63.3, 67.2, 67.5, 120.0, 125.1, 127.0, 127.1, 127.7, 128.2, 128.5, 128.6, 135.0, 141.3 (2C), 143.6, 143.8, 156.2, 170.3. HRMS (ESI) calcd for $C_{25}H_{24}NO_5$ $(M+H)^+$ 418.1654, obsd 418.1670.

Sulfamidate 22. A solution of triethylamine (1.42 mL, 1.02 g, 10.1 mmol, 3.0 equiv) in CH_2Cl_2 (28 mL) was stirred at -40 °C (MeCN/dry ice bath) under N_2 in a two-necked flask, fitted with a dropping funnel (vertical) and rubber septum (side). Distilled thionyl chloride (500 μL, 800 mg, 6.7 mmol, 2.0 equiv) was added dropwise over 5 min via the septum and stirring continued for 5 min. A solution of Fmoc-D-Ser-OBn (**17**) (1.42 g, 3.4 mmol, 1.0 equiv) in CH_2Cl_2 (55 mL) was added dropwise from the dropping funnel over a period of 2 h. Following completion of the addition, the mixture was maintained at -40 °C and stirred for another 2 h, then gradually warmed to rt over 2 h (ice bath left in place, but no more dry ice added). Pyridine (1.00 mL, 1.06 g, 13.4 mmol, 4.0 equiv) was added dropwise and stirring continued for another 14 h. Water (70 mL) was added and the mixture was extracted with CH_2Cl_2 (55 mL). The organic layer was washed with 5% citric acid (28 mL), H_2O (70 mL) and brine (30 mL), dried over $MgSO_4$, filtered and concentrated. The crude diastereomeric sulfimidites (**18**) were obtained as an orange foam and used as such for the next step. R_f 0.47 (minor), 0.40 (major) (2:1 hexanes-EtOAc).

The residue was dissolved in MeCN (14 mL) and cooled to 0 °C. Sodium periodate (790 mg, 3.6 mmol, 1.1 equiv) was added followed by ruthenium (III) chloride (70 mg, 0.34 mmol, 0.1 equiv). Water (14 mL) was added dropwise to the solution and the mixture was left to stir vigorously for 1 h at 0 °C. The mixture was warmed to rt, stirred an additional 1 h, and then water (28 mL) and Et_2O (55 mL) were added. The two layers separated and the aqueous layer extracted further with Et_2O (50 mL). The combined organic layers were washed with sat'd aq. $NaHCO_3$ (80 mL), H_2O (80 mL) and brine (80 mL), dried over $MgSO_4$, filtered and concentrated. The yellow residue was

purified by flash chromatography on silica gel, eluting with 5:1 hexanes-EtOAc, followed by 3:1 hexanes-EtOAc, to give sulfamidate **22** as a colorless solid (980 mg, 61 % over two steps). R_f 0.37 (2:1 hexanes-EtOAc). $[\alpha]^{25}_D$ +11.1 (*c* 0.6, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.22-4.32 (m, 1H), 4.44 (app. t, *J* = 8.8 Hz, 1H), 4.56 (appt. t, *J* = 8.8 Hz, 1H), 4.72 (d, *J* = 8.7 Hz, 1H), 4.78-4.93 (m, 1H), 4.83 (d, *J* = 8.7 Hz, 1H), 5.18-5.27 (m, 2H), 7.30 –7.37 (m, 7H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.67 (br s, 2H), 7.76 (d, *J* = 7.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 46.4, 57.9, 68.0, 68.6, 70.6, 120.0, 125.2, 125.3, 127.3, 128.0, 128.2, 128.7, 128.8, 134.2, 141.2 (2C), 142.7, 142.9, 149.4, 166.4. HRMS (ESI) calcd for $C_{25}H_{20}NO_7S$ $(M-H)^-$ 478.0966, obsd 478.0969.

τ-HAL derivative 3. Boc-L-His-OTCE **(13)** (945mg, 2.46 mmol, 1.2 equiv.) was added to a solution of sulfamidate **22** (980 mg, 2.05 mmol, 1.0 equiv.) in 1,2-DME (46 mL). This solution was heated at 80 °C under N_2 and stirred for 5 h. A solution of potassium dihydrogen phosphate (1 M, 30 mL) was added to the mixture which was stirred at rt for 5 min. The mixture was partitioned between EtOAc (60 mL) and H_2O (60 mL) to dissolve the precipitate. The two layers were separated, and the aqueous layer was extracted further with EtOAc (60 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated. The clear oil was subjected to flash chromatography on silica gel, eluting with 2:1 hexanes-EtOAc, to give τ-HAL **3** as a colorless oil (590 mg, 36%). R_f 0.34 (95:5 CH_2Cl_2-MeOH). $[\alpha]^{25}_D$ -3.9 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.42 (s, 9H), 3.03 (dd, *J* = 14.8, 4.5 Hz, 1H), 3.11 (dd, *J* = 14.8, 4.7 Hz, 1H), 4.20 (t, *J* = 6.4 Hz, 1H), 4.32 (br s, 2H), 4.40 (appt. t, *J* = 8.6 Hz, 1H), 4.52 (appt. t, *J* = 8.6 Hz, 1H), 4.60–4.64 (m, 2H), 4.68 (d, *J* = 12.1 Hz, 1H), 4.82 (d, *J* = 12.1 Hz, 1H), 5.17 (d, *J* = 11.9 Hz, 1H), 5.28 (d, *J* = 11.8 Hz, 1H), 5.60 (br s, 1H) 6.18 (d, *J* = 7.8 Hz,

1H), 6.36 (s, 1H), 7.27-7.42 (m, 9H), 7.58 (d, *J* = 7.3 Hz, 2H), 7.79 (d, *J* = 7.2 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 28.3, 29.6, 47.0, 47.7, 53.5, 54.7, 67.2, 68.2, 74.5, 79.8, 94.7, 117.1, 120.0, 124.9, 125.0, 127.1, 127.2, 127.8, 128.9, 129.0, 129.1, 134.2, 137.6, 141.3, 143.4, 143.5, 155.5, 155.6, 168.7, 170.6. HRMS (ESI) calcd for $C_{38}H_{40}Cl_3N_4O_8$ $(M+H)^+$ 785.1906, obsd 785.1923.

*4.4 Synthesis of L-α-Aminoadipic Acid Building Block **4***

Alloc-L-α-AAA-OH. L-α-Aminoadipic acid (**23**) (250 mg, 1.55 mmol, 1.0 equiv.) was added to a solution of sodium hydroxide (223 mg, 5.60 mmol, 3.6 equiv.) in water (12.5 mL) at 0 °C. Allyl chloroformate (247 μL, 281 mg, 2.35 mmol, 1.5 equiv.) was added dropwise over 30 min and stirring continued for 4 h in an ice bath. The mixture was washed with diethyl ether (20 mL). The aqueous layer was acidified to pH 2 by the addition of conc. HCl and extracted with EtOAc (4 x 20 mL). The combined extracts were dried over $MgSO_4$, filtered and concentrated to give 2-(allyloxycarbonylamino)-adipic acid (350 mg, 92%). R_f 0.29 (6:4:1 $CHCl_3$-MeOH-H_2O).

Alloc-L-α-AAA-OPMB (4). Triethylamine (139 μL, 106 mg, 1.05 mmol, 1.1 equiv) and *p*-methoxybenzyl chloride (128 μL, 162 mg, 0.95 mmol, 1.0 equiv.) were added to a solution of Alloc-α-AAA-OH (255 mg, 0.95 mmol, 1.0 equiv.) in DMF (6 mL) at 38 °C and stirring continued at 38 °C overnight. Ethyl acetate (5 mL) was added and the solution was filtered to remove the precipitate of triethylammonium hydrochloride. The filtrate was diluted with ethyl acetate (20 mL), washed with water (10 mL), brine (10 mL), dried over $MgSO_4$, filtered and concentrated. The pale yellow oil was purified by flash chromatography on silica gel, eluting with 98:2 (CH_2Cl_2-MeOH) to

afford the desired ester **4** as colorless oil (235 mg, 62%). R_f 0.32 (95:5 CH_2Cl_2-MeOH). $[\alpha]^{25}_D$ -4.09 (*c* 1.0, $CHCl_3$), ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.65-1.74 (m, 3H), 1.80-1.96 (m, 1H), 2.28-2.42 (m, 2H), 3.82 (s, 3H), 4.33-4.45 (m, 1H), 4.57 (d, *J* = 5.4 Hz, 2H), 5.13 (q, *J* = 11.8 Hz, 2H), 5.22 (d, *J* = 10.4 Hz, 1H), 5.31 (d, *J* = 17.2 Hz, 1H), 5.47 (d, *J* = 8.0 Hz, 1H), 5.86-5.94 (m, 1H), 6.89 (d, *J* = 8.6 Hz, 2H), 7.29 (d, *J* = 8.6 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.3, 31.8, 53.6, 55.3, 65.9, 67.2, 114.0, 117.9, 127.3, 130.2, 132.6, 155.9, 159.8, 172.2. HRMS (ESI+) calcd for $C_{18}H_{24}NO_7$ $(M+H)^+$ 366.1547, obsd 366.1537.

4.5 Synthesis of Tetrapeptide **5**

Fmoc-Asn(Trt)-Phe-OBn (26). Phenylalanine benzyl ester hydrochloride (**25**) (200 mg, 0.68 mmol, 1.00 equiv.) was added to a solution of Fmoc-Asn(Trt)-OH (**24**) (160 mg, 0.68 mmol, 1.00 equiv.) in CH_2Cl_2 (2 mL) at 0 °C under N_2. Triethylamine (189 µL, 137 mg, 1.36 mmol, 2.00 equiv.), EDC (138 mg, 0.7 mmol, 1.05 equiv.) and HOBt (136 mg, 1.0 mmol, 1.50 equiv.) were added sequentially. After 20 min, the ice bath was removed, the mixture warmed to rt and stirred for another 12 h. The mixture was concentrated and applied to a flash column in a minimum volume of CH_2Cl_2, eluting with 98:2 CH_2Cl_2: MeOH to give dipeptide **26** (295 mg; 51%). R_f 0.50 (95:5 CH_2Cl_2-MeOH). $[\alpha]^{25}_D$ -1.2 (*c* 0.8, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.53-1.74 (m, 1H), 2.63 (dd, *J* = 15.0, 4.6 Hz, 1H), 2.94-3.03 (m, 2H), 3.07 (d, *J* = 15.0 Hz, 1H), 4.19 (app t, *J* = 7.1 Hz, 1H), 4.28-4.37 (m, 2H), 4.50-4.60 (m, 1H), 4.78 (q, *J* = 6.6 Hz, 1H), 5.07 (d, *J* = 12.0 Hz, 1H), 5.15 (d, *J* = 12.0 Hz, 1H), 6.42 (br s, 1H), 6.92 (br s, 1H), 6.99 (br s, 1H), 7.12-7.18 (m, 3H), 7.22-7.30 (m, 23H), 7.42 (t, *J* = 7.1 Hz, 2H), 7.57 (d, *J* = 7.1 Hz,

2H), 7.79 (d, *J* = 7.4 Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 37.7, 38.1, 47.1, 51.3, 54.0, 67.2, 67.4, 70.9, 120.1, 125.3, 127.0, 127.2, 127.8, 128.1, 128.5, 128.6 (2C), 128.7, 129.3, 135.2, 135.7, 141.3, 143.8, 143.9, 144.4, 156.4, 170.6, 170.8, 170.9. HRMS (ESI+) calcd for $C_{54}H_{48}N_3O_6$ $(M+H)^+$ 834.3538, obsd 834.3526.

HCl.*β*-Ala-OAll (27). β-Alanine (502 mg, 5.6 mmol, 1.0 equiv.), allyl alcohol (3.4 mL, 2.9 g, 27.0 mmol, 4.8 equiv.) and chlorotrimethylsilane (1.2 mL, 1.03 g, 9.4 mmol, 1.6 equiv.) were heated at 100 °C for 4 h. The mixture was cooled to rt, poured onto diethyl ether (115 mL) and stirred at 0 °C for 24 h. The solid was collected by filtration to give the hydrochloride salt **27** (800 mg, 86%). R_f 0.38 (9:1 CH_2Cl_2-MeOH). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.96 (t, *J* = 6.7 Hz, 2H), 3.38 (t, *J* = 6.7 Hz, 2H), 4.63 (d, *J* = 5.8 Hz, 2H), 5.24 (dd, *J*= 10.4, 1.1 Hz, 1H), 5.33 (dd, *J* = 17.2, 1.4 Hz, 1H), 5.86-5.96 (m, 1H), 8.20 (br s, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 31.3, 35.7, 65.9, 118.8, 131.7, 170.9. HRMS (ESI+) calcd for $C_6H_{11}NO_2$ $(M+H)^+$ 130.0868, obsd 130.0863.

Fmoc-Asn(Trt)-Phe-*β*-Ala-OAll (28). 10% Pd-C (18 mg, 0.34 mmol, 2.0 equiv.) was added to a solution of dipeptide **26** (140 mg, 0.17 mmol, 1.0 equiv) in dry MeOH (2 mL). The vessel was evacuated and then opened up to an atmosphere of H_2 and the mixture stirred at rt for 4 h. The mixture was filtered through a plug of Celite® and the filtrate concentrated to give the carboxylic acid as a colorless oil (100 mg, 0.14 mmol) that was used directly in the next step without further purification. R_f 0.25 (9:1 CH_2Cl_2-MeOH).

Fmoc-Asn(Trt)-Phe-OH (100 mg, 0.14 mmol, 1.05 equiv.) was dissolved in CH_2Cl_2 (2 mL) and cooled to 0 °C under N_2. β-Alanine allyl ester hydrochloride (**27**) (22 mg, 0.13 mmol, 1.00 equiv.) was added, as a solid, followed by 2,4,6-collidine (35 µL, 32

mg, 0.26 mmol, 2.00 equiv.) and HATU (52 mg, 0.14 mmol, 1.05 equiv.). After 20 min, the mixture was warmed to rt and stirred for 12 h. The mixture was concentrated and applied to a flash column in a minimum volume of CH_2Cl_2. Elution with a gradient of 2-5% MeOH in CH_2Cl_2 yielded tripeptide **28** as a colorless foam (95 mg, 66% over 2 steps). R_f 0.49 (9:1 CH_2Cl_2-MeOH). $[\alpha]_D^{25}$ -5.2 (*c* 0.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.25-2.38 (m, 2H), 2.75-2.81 (m, 1H), 2.94-2.98 (m, 2H), 3.05-3.08 (m, 1H), 3.26-3.31 (m, 2H), 4.18 (t, *J* = 7.0 Hz, 1H), 4.28-4.39 (m, 2H), 4.42-4.46 (m, 1H), 4.51 (d, *J* = 5.6 Hz, 2H), 4.55-4.57 (m, 1H), 5.24 (d, *J* = 10.4 Hz, 1H), 5.30 (d, *J* = 16.2 Hz, 1H), 5.83-5.93 (m, 1H), 6.22 (d, *J* = 6.7 Hz, 1H), 6.55 (t, *J* = 5.5 Hz, 1H), 6.85 (d, *J* = 7.0 Hz, 1H), 7.00 (br s, 1H), 7.13-7.35 (m, 25H), 7.42 (d, *J* = 7.4 Hz, 2H), 7.58 (d, *J* = 7.2 Hz, 2H), 7.78 (d, *J* = 6.7 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 34.8, 37.5, 38.1, 47.0, 51.8, 54.6, 65.2, 67.4, 70.8, 118.4, 120.0, 125.1, 127.0, 127.1, 127.2, 127.8, 128.0, 128.7, 129.3, 132.0, 136.5, 141.3, 143.7 (2C), 144.2, 156.3, 170.0, 170.2, 170.6, 171.5. HRMS (ESI+) calcd for $C_{53}H_{51}N_4O_7$ $(M+H)^+$ 855.3752, obsd 855.3759.

Fmoc-Asn(Trt)-Asn(Trt)-Phe-*β*-Ala-OAll (5). Diethylamine (2 mL) was added to a solution of tripeptide **28** (160 mg, 0.18 mmol, 1.00 equiv.) in acetonitrile (2 mL). The mixture was stirred for 1 h at rt, after which time, TLC indicated that the starting material had been consumed. The solution was concentrated, and then concentrated twice more from CH_3CN (2 x 6 mL) to remove the excess Et_2NH. The residue was applied to a flash column in a minimum volume of CH_2Cl_2, eluting with 9:1 CH_2Cl_2-MeOH to give the amine, H-Asn(Trt)-Phe-β-Ala-OAll (118 mg, 0.18 mmol). R_f 0.42 (9:1 CH_2Cl_2-MeOH).

HATU (74 mg, 0.19 mmol, 1.05 equiv.) and 2,4,6-collidine (54 µL, 50 mg, 0.40 mmol, 2.2 equiv.) were added to a solution of the intermediate amine (118 mg, 0.18

mmol, 1.00 equiv.) and Fmoc-Asn(Trt)-OH (**29**) (116 mg, 0.19 mmol, 1.05 equiv.) in CH_2Cl_2 (4 mL) at 0 °C under N_2. After 20 min, the mixture was warmed to rt and stirred 14 h. The mixture was concentrated and applied to a flash column in a minimum volume of CH_2Cl_2. Elution with 98:2 CH_2Cl_2-MeOH gave tetrapeptide **5** as a colorless foam (148 mg, 68% over two steps). R_f 0.52 (9:1 CH_2Cl_2-MeOH). $[\alpha]^{25}_D$ -18.8 (*c* 0.5, $CHCl_3$). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.20 (t, *J* = 7.3 Hz, 1H), 2.40 (d, *J* = 11.7 Hz, 1H), 2.57-2.77 (m, 5H), 2.95-2.97 (m, 1H), 3.03 (d, *J* = 10.9 Hz, 1H), 3.08-3.10 (m, 1H), 4.21 (t, *J* = 6.6 Hz, 1H), 4.25-4.38 (m, 3H), 4.42-4.45 (m, 1H), 4.48 (m, 1H), 4.53 (d, *J* = 5.0 Hz, 2H), 5.22 (d, *J* = 10.4 Hz, 1H), 5.30 (d, *J* = 17.1 Hz, 1H), 5.89-5.94 (m, 1H), 7.06 (t, *J* = 7.1 Hz, 1H), 7.11-7.27 (m, 39H), 7.25-7.29 (m, 1H), 7.39-7.46 (m, 1H), 7.66 (d, *J* = 8.2 Hz, 1H), 7.71-7.77 (m, 3H), 7.91 (d, *J* = 7.4 Hz, 2H), 8.10 (d, *J* = 8.0 Hz, 1H), 8.26 (d, *J* = 7.4 Hz, 1H), 8.57 (s, 1H), 8.82 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 33.7, 35.1, 37.1, 38.5, 38.7, 47.1, 50.4, 52.5, 54.7, 64.9, 66.4, 69.9, 118.3, 120.6, 121.4, 125.7 (2C), 126.5, 126.8, 126.9, 127.6, 127.9, 128.1, 128.5, 129.0, 129.4, 133.5, 138.6, 141.2, 144.2, 144.3, 145.1, 145.2, 156.2, 169.3, 170.1, 170.9, 171.0 (2C), 171.7. HRMS (ESI+) calcd for $C_{76}H_{71}N_6O_9$ $(M+H)^+$ 1211.5277, obsd 1211.5271.

4.6 Construction of the Western Macrocycle

Hexapeptide 32. 10% Pd-C (90 mg, 30% w/w) was added to a solution of *τ*-histidinoalanine **3** (300 mg, 0.4 mmol, 1.0 equiv.) in anhydrous THF (8 mL). The vessel was evacuated and then opened to an atmosphere of H_2 and the suspension stirred at rt for 3h. The reaction mixture was filtered through a plug of Celite® and the filtrate was concentrated. The residue was purified by flash chromatography eluting with 20% EtOH

in CH_2Cl_2 to give the free acid **31** as a colorless oil (225 mg, 84%). R_f 0.20 (9:1 CH_2Cl_2-MeOH). $[\alpha]^{25}_D$ -2.98 (*c* 1.0, $CHCl_3$). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.35 (s, 9H), 2.82-2.90 (m, 2H), 4.11-4.24 (m, 4H), 4.29 (t, *J* = 10.9 Hz, 2H), 4.35-4.40 (m, 1H), 4.82 (d, *J* = 12.3 Hz, 1H), 4.91 (d, *J* = 12.3 Hz, 1H), 6.87 (s, 1H), 7.28 (d, *J* = 7.8 Hz, 1H), 7.33 (t, *J* = 7.4 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.45 (s, 1H), 7.65 (t, *J* = 12.0 Hz, 2H), 7.89 (d, *J* = 7.5 Hz, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) 28.6, 29.8, 47.1, 47.3, 54.1, 55.4, 66.1, 74.0, 79.0, 95.5, 118.0, 120.6, 125.6, 125.7, 127.6, 128.1, 136.9, 138.1, 141.2, 144.3, 144.4, 155.7, 156.2, 171.3, 171.6; HRMS (ESI+) calcd for $C_{31}H_{34}Cl_3N_4O_8$ $(M+H)^+$ 695.1437, obsd 695.1442.

Diethylamine (3 mL) was added to a solution of tetrapeptide Fmoc-Asn(Trt)-Asn(Trt)-Phe-*β*-Ala-OAll (**5**) (480 mg, 0.38 mmol, 1.00 equiv.) in dry acetonitrile (3 mL). The mixture was stirred at rt for 2 h, concentrated, and concentrated twice more from CH_3CN (2 x 6 mL) to remove the excess Et_2NH. The residue was then applied to a flash column in a minimum volume of CH_2Cl_2, eluting with 9:1 CH_2Cl_2-MeOH to give compound **30** (330 mg). R_f 0.30 (9:1 CH_2Cl_2-MeOH).

A solution of acid **31** (225 mg, 0.32 mmol, 1.00 equiv.) in CH_2Cl_2 (6 mL), collidine (92 µL, 85 mg, 0.70 mmol, 2.20 equiv.) and HATU (138 mg, 0.36 mmol, 1.10 equiv.) were added sequentially to a solution of amine H-Asn(Trt)-Asn(Trt)-Phe-*β*-Ala-OAll (**30**) (330 mg, 0.32 mmol, 1.00 equiv.) in CH_2Cl_2 (8 mL) at 0 °C under N_2. After 20 min, the mixture was warmed to rt and stirred overnight. The mixture was concentrated and the residue applied to a flash column in a minimum volume of CH_2Cl_2. Subsequent elution with a gradient of 5-10% of MeOH in CH_2Cl_2 gave hexapeptide **32** as a light brown solid (282 mg, 53% over two steps from τ-HAL **3**). R_f 0.35 (9:1 CH_2Cl_2-MeOH).

$[\alpha]^{25}_{D}$ -8.15 (*c* 0.9, MeOH).^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.33 (s, 9H), 2.19 (t, *J* = 7.4 Hz, 2H), 2.57-2.64 (m, 3H), 2.77 (dd, *J* = 15.5, 7.4 Hz, 1H), 2.84-3.12 (m, 5H), 3.81 (app t, *J* = 13.9 Hz, 1H), 3.99 (d, *J* = 13.9 Hz, 1H), 4.06-4.20 (m, 3H), 4.31 (td, *J* = 8.6, 4.8 Hz, 1H), 4.38-4.43 (m, 1H), 4.48-4.50 (m, 2H), 4.52 (d, *J* = 5.4 Hz, 2H), 4.73 (dd, *J* = 14.5, 8.3 Hz, 1H), 4.81 (d, *J* = 12.2 Hz, 1H), 4.90 (d, *J* = 12.2 Hz, 1H), 5.21 (dd, *J* = 10.5, 1.3 Hz, 1H), 5.29 (dd, *J* = 17.3, 1.3 Hz, 1H), 5.87-5.95 (m, 1H), 6.98-7.35 (m, 40H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.50 (br s, 1H), 7.61-7.72 (m, 3H), 7.87 (d, *J* = 7.5 Hz, 2H), 8.03 (d, *J* = 8.0 Hz, 1H), 8.23 (d, *J* = 7.3 Hz, 1H), 8.47 (d, *J* = 7.9 Hz, 1H), 8.64 (s, 1H), 8.74 (s, 1H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 28.3, 28.6 29.8, 31.1, 33.7, 33.8, 35.0, 37.2, 38.5, 38.7, 47.0, 48.0, 50.1, 50.4, 54.3, 54.7, 55.4, 56.1, 64.9, 66.4, 69.8, 69.9, 70.1, 73.9, 78.9, 95.6, 118.2, 118.3, 120.6, 121.3, 125.7, 125.8, 126.5, 126.8, 127.5, 127.9, 128.1, 128.5, 128.9, 129.5, 133.1, 136.9, 138.2, 138.5, 141.2, 144.1, 144.2, 145.0, 145.2, 155.7, 156.3, 169.1, 169.2, 169.9, 170.9, 171.1, 171.4; HRMS (ESI+) calcd for $C_{92}H_{91}Cl_3N_{10}O_{14}$ $(M+H)^+$ 1665.5860, obsd 1665.5852.

Heptapeptide 33. Trifluoroacetic acid (2 mL) was added dropwise to a solution of hexapeptide **32** (160 mg, 0.09 mmol, 1.00 equiv.) in dry CH_2Cl_2 (2 mL) at 0 °C under N_2. The mixture was stirred for 4 h, then concentrated three times from toluene to give the free amine as a colorless oil (109 mg). R_f 0.10 (9:1 $CHCl_3$-MeOH).

A solution of acid **4** (35 mg, 0.09 mmol, 1.0 equiv.) in MeCN (3 mL), $^{i}Pr_2NEt$ (43 μL, 32 mg, 0.25 mmol, 2.5 equiv.) and HATU (38 mg, 0.1 mmol, 1.05 equiv.) were added sequentially to a solution of the amine (109 mg, 0.1 mmol, 1.05 equiv.) in CH_2Cl_2 (3 mL) at 0 °C under N_2. After 20 min, the mixture was warmed to rt and stirred for 16 h. The mixture was concentrated and applied to a flash column in a minimum volume of

CH_2Cl_2. Elution with a gradient of 5-10% of MeOH in CH_2Cl_2 gave heptapeptide **33** as a colorless solid (71 mg, 52%). R_f 0.40 (8:2 $CHCl_3$-MeOH). $[\alpha]^{25}_D$ -10.7 (*c* 0.25, DMSO). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.49-1.59 (m, 3H), 1.67-1.73 (m, 1H), 2.05-2.13 (m, 2H), 2.34-2.45 (m, 2H), 2.45 (td, *J* = 6.2, 1.4 Hz, 2H), 2.56 (dd, *J* = 15.3, 5.6 Hz, 1H), 2.83 (dd, *J* = 13.8, 9.3 Hz, 1H), 2.95-3.05 (m, 1H), 3.03 (dd, *J* = 13.8, 4.5 Hz, 1H), 3.13 (dd, *J* = 16.8, 7.2 Hz, 1H), 3.24-3.30 (m, 3H), 3.74 (s, 3H), 3.97-4.02 (m, 1H), 4.18 (dd, *J* = 13.9, 7.2 Hz, 2H), 4.22-4.24 (m, 2H), 4.32-4.36 (dt, *J* = 13.5, 4.8 Hz, 1H), 4.43-4.51 (m, 4H), 4.54 (dt, *J* = 5.5, 1.8 Hz, 2H), 4.58-4.66 (m, 2H), 4.72 (dd, *J* = 14.8, 7.8 Hz, 1H), 4.83 (d, *J* = 12.2 Hz, 1H), 4.90 (d, *J* = 12.2 Hz, 1H), 5.03 (s, 2H), 5.17 (dd, *J* = 13.0, 1.8 Hz, 1H), 5.21 (ddd, *J* = 15.0, 4.0, 2.0 Hz, 1H), 5.28 (d, *J* = 21.5, 1.9 Hz, 1H), 5.29 (ddd, *J* = 23.5, 4.5, 2.2 Hz, 1H), 5.84-5.95 (m, 2H), 6.91 (d, *J* = 8.7 Hz, 2H), 6.95 (br s, 1H), 7.00 (br s, 1H), 7.15-7.20 (m, 3H), 7.24 (d, *J* = 7.4 Hz, 2H), 7.27 (d, *J* = 8.6 Hz, 2H), 7.32 (t, *J* = 7.5 Hz, 2H), 7.40-7.42 (m, 3H), 7.43 (br s, 1H), 7.47 (br s, 1H), 7.63-7.68 (m, 1H), 7.65 (d, *J* = 7.4 Hz, 1H), 7.67 (d, *J* = 7.4 Hz, 1H), 7.78 (d, *J* = 8.5 Hz, 1H), 7.89 (d, *J* = 7.6 Hz, 2H), 8.06 (d, *J* = 8.2 Hz, 1H), 8.32 (d, *J* = 7.3 Hz, 1H), 8.46 (d, *J* = 7.3 Hz, 1H), 8.51 (d, *J* = 7.8 Hz, 1H), 8.79 (br s, 1H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 22.1, 30.1, 30.5, 33.9, 34.7, 35.3, 37.1, 37.3, 38.1, 47.0, 47.8, 50.2, 50.6, 52.7, 54.3, 54.9, 55.6, 56.1, 65.0, 66.2, 66.4, 73.9, 95.5, 114.3, 117.6, 117.8, 118.3, 119.2, 120.5, 125.7, 125.8, 126.7, 127.6 (2C), 128.1, 128.3, 128.6, 129.5, 130.2, 133.1, 133.9, 141.1, 144.1, 144.2, 156.4, 156.5, 159.6, 169.3, 170.9, 171.1, 171.2, 171.3 (2C), 172.1, 172.2, 172.5, 172.7; HRMS (ESI+) calcd for $C_{67}H_{76}Cl_3N_{11}O_{18}$ $(M+H)^+$ 1428.4514, obsd 1428.4529.

Western Cyclic Heptapeptide 34. Borane dimethylamine complex (7 mg, 0.12 mmol) and $Pd(PPh_3)_4$ (9 mg, 0.01 mmol) were added sequentially to a stirred solution of

linear heptapeptide **33** (30 mg, 0.02 mmol) in THF (2 mL) at room temperature under N_2. The flask was wrapped in aluminum foil to protect the reaction from light and the mixture was stirred for 3 h, then filtered through a pad of Celite® in a Pasteur pipette. The filtrate was concentrated and used directly in the next reaction.

HATU (20 mg, 0.05 mmol, 2.5 equiv.) and $^{i}Pr_2NEt$ (17 μL, 12 mg, 0.1 mmol, 5.0 equiv) were added to a solution of the deprotected, linear heptapeptide in DMF (10 mL) at rt and mixture was stirred for 4 h. Another batch of HATU (20 mg, 0.05 mmol, 2.5 equiv.) and $^{i}Pr_2NEt$ (17 μL, 12 mg, 0.1 mmol, 5.0 equiv) were added and stirring was continued for another 16 h. The mixture was concentrated and the residue purified by reversed-phase HPLC, eluting with a gradient of 25-50% MeCN in water over 25 min, to give the cyclic heptapeptide **34** (R_T 16.5 min) as a colorless, amorphous solid (2 mg, 8%). R_f 0.45 (8:2 $CHCl_3$-MeOH). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.41-1.53 (m, 3H), 1.58-1.67 (m, 1H), 2.03-2.16 (m, 2H), 2.32 (dd, *J* = 16.0, 9.1 Hz, 1H), 2.41 (t, *J* = 7.0 Hz, 2H), 2.70 (dd, *J* = 15.5, 6.8 Hz, 1H), 2.85-2.93 (m, 1H), 2.98-3.04 (m, 1H), 3.12 (dd, *J* = 14.7, 3.6 Hz, 1H), 3.23 (dd, *J* = 13.9, 6.6 Hz, 1H), signals for 4H buried under the H_2O peak, 3.75 (s, 3H), 4.11-4.50 (m, 9H), 4.17 (t, *J* = 6.8 Hz, 1H), 4.50 (app. q, *J* = 6.6 Hz, 1H), 4.58-4.68 (m, 1H), 4.70-4.79 (m, 1H), 4.89 (d, J = 12.2 Hz, 1H), 4.98-5.04 (m, 4H), 6.91 (d, *J* = 8.5 Hz, 2H), 6.94 (br, 1H), 7.14 (br, 1H), 7.17-7.29 (m, 9H), 7.31 (t, *J* = 7.5 Hz, 2H), 7.29-7.35 (m, 1H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.38-7.46 (m, 1H), 7.64 (t, *J* = 8.0 Hz, 1H), 7.71 (br, 1H), 7.78 (d, *J* = 8.6 Hz, 1H), 7.89 (d, *J* = 7.5 Hz, 2H), 8.22 (d, *J* = 7.2 Hz, 1H), 8.24 (d, *J* = 8.2 Hz, 1H), 8.38-8.44 (m, 1H), 8.44 (d, *J* = 7.9 Hz, 1H), 8.58 (d, *J* = 5.8 Hz, 1H). HRMS (ESI+) calcd for $C_{60}H_{67}Cl_3N_{11}O_{15}$ $(M+H)^+$ 1286.3884, obsd 1286.3848.

*4.7 Synthesis of β-Phenylalanine Building Blocks **36** and **37***

Boc-β-Phe-OMe (36). DBU (163 µL, 167 mg, 1.1 mmol, 1.5 equiv.) and methyl iodide (233 µL, 532 mg, 3.8 mmol, 5.0 equiv.) were added sequentially to a stirred solution of Boc-*β*-Phe-OH (**35**) (200 mg, 0.75 mmol, 1.0 equiv.) in acetone (5 mL) at rt under N_2. The mixture was stirred for 3 h and then the acetone was removed by evaporation. The residue was diluted with EtOAc (10 mL) and washed water (10 mL). The aqueous layer was further extracted with EtOAc (15 mL) and the combined organic layers were washed with H_2O (10 mL), dried over $MgSO_4$, filtered and concentrated to give Boc-*β*-Phe-OMe (**36**) (157 mg; 69%). R_f 0.65 (95:5 CH_2Cl_2-MeOH). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.42 (s, 9H), 2.81 (dd, J = 15.0, 5.6 Hz, 1H), 2.85-2.92 (m, 1H), 3.61 (s, 3H), 5.10-5.20 (m, 1H), 5.48 (br s, 1H), 7.23-7.35 (m, 5H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 28.3, 40.8, 51.2, 51.7, 79.7, 126.1, 127.5, 128.6, 141.1, 155.0, 171.3. HRMS (ESI+) calcd for $C_{15}H_{21}NO_4Na$ $(M+Na)^+$ 302.1368, obsd 302.1353.

Boc-*β*-Phe-OFm (37). Fluorenylmethyl alcohol (220 mg, 1.13 mmol, 1.2 equiv.) was added to a solution of Boc-*β*-Phe-OH (**35**) (250 mg, 0.94 mmol, 1.0 equiv.) in CH_2Cl_2 (5 mL) under N_2. DMAP (60 mg, 0.47 mmol, 0.5 equiv.) was added in portions, followed by portion wise addition of EDC.HCl (220 mg, 1.13 mmol, 1.2 equiv.) at 0 °C. The mixture was warmed to rt and stirred for 16 h. The solution was partitioned between H_2O (30 mL) and CH_2Cl_2 (30 mL). The layers were separated, and the aqueous layer extracted further with CH_2Cl_2 (2 × 15 mL). The combined organic layers were washed sequentially with sat′d aq $NaHCO_3$ (30 mL), sat′d aq. NH_4Cl (30 mL) and brine (30 mL), dried over $MgSO_4$, filtered and concentrated. The yellow residue was purified by flash

chromatography, eluting with 10-20% EtOAc in hexanes to give Boc-*β*-Phe-OFm (**37**) as a colorless solid (320 mg, 70%). R_f 0.50 (4:1 hexanes-EtOAc). $[\alpha]^{20}_D$ -10.1 (*c* 0.8, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (s, 9H), 2.86 (dd, *J* = 14.4, 4.4 Hz, 1H), 2.97 (br d, *J* = 14.4 Hz, 1H), 4.07 (t, *J* = 7.1 Hz, 1H), 4.28 (d, *J* = 7.1 Hz, 2H), 5.07-5.21 (br s, 1H), 5.55 (br s, 1H), 7.19-7.30 (m, 7H), 7.35 (t, *J* = 7.4 Hz, 2H), 7.43 (dd, *J* = 7.2, 2.4 Hz, 2H), 7.71 (d, *J* = 7.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.2, 40.7, 46.5, 51.0, 66.5, 79.6, 119.9, 124.8, 126.0, 127.0, 127.4, 127.6, 128.5, 141.1, 143.5, 155.0, 170.8. HRMS (ESI) calcd for $C_{28}H_{29}NNaO_4$ $(M+Na)^+$ 466.1994, obsd 466.1998.

*4.8 Synthesis of Tripeptides **45** and **46***

Fmoc-*a*Thr-Ser-OH (42). *N*-Hydroxysuccinimide (81 mg, 0.70 mmol, 1.0 equiv.) and DCC (146 mg, 0.70 mmol, 1.0 equiv.), were added sequentially to a solution of Fmoc-*allo*-Thr(O^{t}Bu)-OH (**40**) (280 mg, 0.70 mmol, 1.0 equiv.) in anhydrous CH_2Cl_2 (10.0 mL) at 0 °C under N_2. The mixture was warmed to rt and stirred for 4 h, then filtered to remove the dicyclohexylurea (washing well with CH_2Cl_2) and concentrated to ~5 mL. After standing in the freezer for 2 h, the mixture was filtered again (washing twice with cold CH_2Cl_2). The filtrate was concentrated and the intermediate NHS ester dissolved in dry DMF (1.0 mL) and cooled to 0 °C under N_2. After 10 min, H-Ser(O^{t}Bu)-OH (**41**) (115 mg, 0.70 mmol, 1.0 equiv.) was added to the reaction mixture, followed by the dropwise addition of ^{i}Pr$_2$NEt (140 μL, 109 mg, 0.84 mmol, 1.2 equiv.). The mixture was warmed to rt and stirred for 16 h under N_2. The mixture was diluted with EtOAc (25 mL) and washed with 2 M HCl (40 mL). The aqueous layer was further extracted with EtOAc (25 mL) and the combined organic layers were washed with H_2O

(40 mL), dried over $MgSO_4$, filtered and concentrated to give Fmoc-*allo*-Thr(O^{t}Bu)-Ser(O^{t}Bu)-OH (**42**) (303 mg, 80% yield) that was used directly in the next step without further purification.

Fmoc-*a*Thr(O^{t}Bu)-Ser(O^{t}Bu)-Phe-OBn (45). Fmoc-*allo*-Thr(O^{t}Bu)-Ser(O^{t}Bu)-OH (**42**) (303 mg, 0.4 mmol, 1.0 equiv.) was dissolved in anhydrous CH_2Cl_2 (5.0 mL) and cooled to 0 °C under N_2. After 10 min, HCl-Phe-OBn (**43**) (106 mg, 0.4 mmol, 1.0 equiv.) was added to the reaction mixture, followed by the dropwise addition of 2,4,6-collidine (106 μL, 96 mg, 0.80 mmol, 2.0 equiv.). After the addition of HATU (152 mg, 0.4 mmol, 1.1 equiv.), the mixture was warmed to rt and stirred for 15 h under N_2, concentrated and the residue purified via column chromatography, eluting with 33-50% hexanes in EtOAc to isolate Fmoc-*allo*-Thr(O^{t}Bu)-Ser(O^{t}Bu)-Phe-OBn (**45**) as a colorless foam (272 mg; 50% over 2 steps). R_f 0.18 (2:1 Hex-EtOAc). $[\alpha]^{25}_D$ +18.2 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.11 (s, 9H), 1.16 (s, 9H), 3.06 (dd, *J* = 13.9, 6.2 Hz, 1H), 3.12 (dd, *J* = 13.9, 5.8 Hz, 1H), 3.29 (app. t, *J* = 8.3 Hz, 1H), 3.79 (dd, *J* = 8.5, 3.8 Hz, 1H), 3.91-4.02 (m, 1H), 4.11-4.19 (m, 1H), 4.22 (t, *J* = 6.8 Hz, 1H), 4.24-4.33 (m, 1H), 4.36-4.44 (m, 1H), 4.46 (dd, *J* = 10.3, 6.8 Hz, 1H), 4.90 (dd, *J* = 13.6 Hz, 6.1 Hz, 1H), 5.12 (d, *J* = 12.2 Hz, 1H), 5.16 (d, *J* = 12.2 Hz, 1H), 5.48 (br s, 1H), 6.99-7.05 (m, 3H), 7.10 (d, *J* = 6.2 Hz, 1H), 7.13-7.19 (m, 3H), 7.23-7.34 (m, 7H), 7.39 (t, *J* = 5.4 Hz, 3H), 7.59 (dd, *J* = 7.3 Hz, 1.9 Hz, 2H), 7.76 (d, *J* = 7.5 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.1, 27.2, 28.2, 37.8, 47.1, 52.7, 53.5, 60.7, 60.9, 67.1, 67.2, 67.5, 74.2, 74.7, 120.0, 125.0, 125.1, 126.9, 127.0, 127.7, 128.4, 128.4 (2C), 128.5, 129.2, 135.2, 135.8, 141.3, 143.8, 156.7, 169.7, 170.0, 170.8. HRMS (ESI) calcd for $C_{46}H_{56}N_3O_8$ $(M+H)^+$ 778.4062, obsd 778.4070.

Fmoc-Phe-OAllyl.[66] Allyl alcohol (3.5 mL, 51.6 mmol, 10.0 equiv.) was added dropwise to a solution of Fmoc-Phe-OH (2.00 g, 5.16 mmol, 1.0 equiv.) in CH_2Cl_2 (30 mL) under N_2. DMAP (315 mg, 2.58 mmol, 0.5 equiv.) was then added in portions followed by portion-wise addition of EDC.HCl (1.2 g, 6.2 mmol, 1.2 equiv.) at 0 °C. The mixture was warmed to rt and stirred for 16 h. The solution was partitioned between H_2O (50 mL) and CH_2Cl_2 (70 mL). The layers were separated, and the aqueous layer extracted further with CH_2Cl_2 (2 x 20 mL). The combined organic layers were washed sequentially with sat′d aq. $NaHCO_3$ (40 mL), sat′d aq. NH_4Cl (40 mL) and brine (40 mL), then dried over $MgSO_4$, filtered and concentrated. The yellow residue was purified by flash chromatography, eluting with 10-20% EtOAc in hexanes to give Fmoc-Phe-OAll as a colorless solid (1.85 g, 84%). R_f 0.25 (9:1 hexanes-EtOAc). $[\alpha]^{20}_D$ +15.9 (*c* 0.8, $CHCl_3$); Lit.[66a] $[\alpha]^{27}_D$ + 15.2 (*c* 0.9, $CHCl_3$); Lit.[66b] $[\alpha]^{20}_D$ + 15.9 (*c* 0.8, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.05 (dd, *J* = 14.0, 6.4 Hz, 1H), 3.13 (dd, *J* = 14.0, 5.6 Hz, 1H), 4.15 (t, *J* = 7.2 Hz, 1H), 4.29 (dd, *J* = 10.4, 7.2 Hz, 1H), 4.40 (dd, *J* = 10.4, 7.2 Hz, 1H), 4.57 (d, *J* = 6.0 Hz, 2H), 4.68 (dd, *J* = 14.0, 6.0 Hz, 1H), 5.19 (d, *J* = 10.4 Hz, 1H), 5.25 (d, *J* = 17.2 Hz, 1H), 5.40 (d, *J* = 8.0 Hz, 1H), 5.77-5.85 (m, 1H), 7.09 (d, *J* = 6.8 Hz, 2H), 7.16-7.28 (m, 5H), 7.35 (t, *J* = 7.2 Hz, 2H), 7.52 (t, *J* = 7.2 Hz, 2H), 7.71 (d, *J* = 7.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 38.0, 47.0, 54.7, 65.9, 66.8, 118.8, 119.8, 124.9, 125.0, 126.9 (2C), 127.5, 128.4, 129.2, 131.3, 135.6, 141.1, 143.6, 143.7, 155.4, 171.1. HRMS (ESI) calcd for $C_{27}H_{26}NO_4$ $(M+H)^+$ 428.1856, obsd 428.1876.

Fmoc-*a*Thr(O^{t}Bu)-Ser(O^{t}Bu)-Phe-OAllyl (46). Fmoc-*a*Thr(O^{t}Bu)-Ser(O^{t}Bu)-OH (**42**) was prepared, as described above, on a scale of 2.57 mmol, to afford 1.26 g of dipeptide acid (90% yield).

Diethylamine (3 mL) was added to a solution of Fmoc-Phe-OAll (1.06 g, 2.57 mmol) in anhydrous CH_3CN (5 mL). The mixture was stirred at rt for 2 h, concentrated, and concentrated twice more from CH_3CN (2 x 3 mL). The residue was applied to a flash column in a minimum volume of CH_2Cl_2, eluting with 9:1 CH_2Cl_2-MeOH to give amine **44** (490 mg). R_f 0.60 (95:5 CH_2Cl_2:MeOH).

Fmoc-*allo*-Thr(O^{t}Bu)-Ser(O^{t}Bu)-OH (**42**) (1.26 g, 2.33 mmol, 1.0 equiv.) was dissolved in anhydrous CH_2Cl_2 (10 mL) and cooled to 0 °C under N_2. After 10 min, a solution of H-Phe-OAll (**44**) (490 mg, 2.4 mmol, 1.0 equiv.) in CH_2Cl_2 (5 mL) was added dropwise to the reaction mixture, followed by the sequential addition of ^{i}Pr$_2$NEt (815 μL, 4.66 mmol, 2.0 equiv.) and HATU (975 mg, 2.56 mmol, 1.1 equiv.) under the same conditions. The mixture was warmed to rt and stirred for 15 h under N_2. The solution was partitioned between H_2O (40 mL) and CH_2Cl_2 (40 mL). The layers were separated, and the aqueous layer extracted further with CH_2Cl_2 (2 x 25 mL). The combined organic layers were washed sequentially with sat′d aq. $NaHCO_3$ (40 mL), sat′d aq. NH_4Cl (40 mL) and brine (40 mL), then dried over $MgSO_4$, filtered and concentrated. The yellow residue was purified by flash chromatography, eluting with 1-4% MeOH in CH_2Cl_2 to isolate Fmoc-*allo*-Thr(O^{t}Bu)-Ser(O^{t}Bu)-Phe-OAll (**46**) as a colorless foam (1.1 g, 59% over 2 steps). R_f 0.33 (95:5 CH_2Cl_2-MeOH). $[\alpha]^{20}_D$ +13.4 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.13 (s, 9H), 1.16 (s, 9H), 1.16-1.18 (m, 3H), 3.07 (dd, *J* = 14.0, 6.3 Hz, 1H), 3.14 (dd, *J* = 14.0, 5.6 Hz, 1H), 3.31 (t, *J* = 8.3 Hz, 1H), 3.80 (dd, *J* = 8.5, 3.8 Hz, 1H), 3.96 (br s, 1H), 4.14 (br s, 1H), 4.21 (t, *J* = 6.8 Hz, 1H), 4.24-4.33 (m, 1H), 4.37-4.43 (m, 1H), 4.45 (dd, *J* = 10.4, 6.9 Hz, 1H), 4.58 (d, *J* = 5.8 Hz, 2H), 4.88 (dd, *J* = 13.7, 6.1 Hz, 1H), 5.21 (dd, *J* = 10.4, 1.1 Hz, 1H), 5.27 (dd, *J* = 17.2, 1.4 Hz, 1H), 5.49

(br s, 1H), 5.83 (ddt, *J* = 17.2, 10.4, 5.8 Hz, 1H), 7.08-7.13 (m, 3H), 7.14-7.24 (m, 3H), 7.30 (t, *J* = 7.4 Hz, 2H), 7.39 (td, *J* = 7.0, 2.4 Hz, 3H), 7.58 (d, *J* = 7.4 Hz, 2H), 7.75 (d, *J* = 7.6 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.1, 27.3, 28.2, 37.9, 47.1, 52.8, 53.4, 60.7, 60.9, 65.9, 67.2, 67.4, 74.2, 74.7, 118.8, 119.9, 125.0, 125.1, 127.0 (2C), 127.7 (2C), 128.4, 129.2, 131.5, 135.9, 141.3, 143.8, 156.6, 169.7, 169.9, 170.7. HRMS (ESI) calcd for $C_{42}H_{54}N_3O_8$ $(M+H)^+$ 728.3911, obsd 728.3921.

*4.9 Synthesis of Tetrapeptide **47***

Fmoc-*a*Thr(O^{t}Bu)-Ser(O^{t}Bu)-Phe-OH. 10% Pd-C (80 mg) was added to a solution of tripeptide **45** (320 mg, 0.42 mmol) in dry EtOAc (20 mL). The suspension was stirred at rt for 3 h under an atmosphere of H_2. The mixture was filtered through a Celite ® pad and the filtrate concentrated and applied to a flash column in a minimum volume of CH_2Cl_2. Elution with 95:5 CH_2Cl_2-MeOH yielded the tripeptide acid as a colorless solid (270 mg). R_f 0.15 (9:1 CH_2Cl_2-MeOH).

TFA.H-*β*-Phe-OMe (38). Trifluoroacetic acid (600 µL) was added to a solution of Boc-*β*-Phe-OMe (157 mg, 0.56 mmol) in CH_2Cl_2 (5 mL) and the mixture stirred for 12 h at rt under N_2. The mixture was concentrated and concentrated twice more from toluene. Compound **38** was obtained as a crystalline solid (188 mg, 85% over two steps). R_f 0.10 (2:1 Hex-EtOAc). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.91 (dd, *J* = 17.5 Hz, 4.7 Hz, 1H), 3.17 (dd, *J* = 17.5, 9.0 Hz, 1H), 3.67 (s, 3H), 4.62 (dd, *J* = 9.0, 4.7 Hz, 1H), 7.35-7.38 (m, 5H), 8.24 (br s, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 37.3, 52.3, 52.4, 127.0, 129.3, 129.7, 134.8, 171.6. HRMS (ESI) calcd for $C_{10}H_{14}NO_2$ $(M^{+)}$: 180.1019; obsd 180.1025.

Fmoc-*a*Thr(O^{t}Bu)-Ser(O^{t}Bu)-Phe-β-Phe-OMe (47). Solid TFA.H-*β*-Phe-OMe (**38**) (115 mg, 0.39 mmol, 1.0 equiv.), 2,4,6-collidine (130 µL, 118 mg, 0.97 mmol, 2.5 equiv.) and HATU (148 mg, 0.39 mmol, 1.0 equiv.) were added sequentially to a solution of the intermediate carboxylic acid (270 mg, 0.39 mmol, 1.0 equiv.) in CH_2Cl_2 (10 mL) at 0 °C under N_2. After 20 min, the mixture was warmed to rt and stirred for 15 h. the mixture was concentrated and applied to a flash column in a minimum volume of CH_2Cl_2. Elution with a gradient of 2-5% MeOH in CH_2Cl_2 yielded tetrapeptide **47** as a colorless foam (254 mg, 69% over two steps). R_f 0.55 (95:5 CH_2Cl_2-MeOH). $[\alpha]^{25}_D$ -15.8 (*c* 0.5, MeOH). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.95 (s, 9H), 1.14 (d, *J* = 6.1 Hz, 3H), 1.23 (s, 9H), 2.80 (dd, *J* = 15.6 Hz, 5.5 Hz, 1H), 2.89-2.92 (m, 1H), 2.97 (dd, *J* = 15.3 Hz, 8.5 Hz, 1H), 3.38-3.44 (m, 2H), 3.57 (s, 3H), 3.59 (d, *J* = 9.1 Hz, 1H), 4.13-4.26 (m, 4H), 4.29 (d, *J* = 3.9 Hz, 1H), 4.56 (dd, *J* = 9.0, 5.5 Hz, 1H), 4.70-4.77 (m, 1H), 5.44 (app. q, *J* = 7.2 Hz 1H), 5.62 (br s, 1H), 7.04-7.07 (m, 3H), 7.09 (d, *J* = 5.8 Hz, 1H), 7.14-7.17 (m, 2H), 7.20-7.23 (m, 1H), 7.28-7.33 (m, 7H), 7.40 (d, *J* = 7.4 Hz, 1H), 7.44 (d, *J* = 7.4 Hz, 1H), 7.56 (d, *J* = 7.0 Hz, 1H), 7.57 (d, *J* = 6.9 Hz, 1H), 7.78 (d, *J* = 7.0 Hz, 1H), 7.79 (d, *J* = 7.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 18.5, 27.1, 28.2, 37.2, 41.0, 46.9, 50.1, 51.7, 54.5, 60.2, 61.9, 66.1, 67.8, 73.8, 75.3, 120.2, 124.9, 125.0, 126.4, 126.5, 127.1, 127.2, 127.4, 127.9, 128.0, 128.4, 128.6, 128.8, 137.8, 140.9, 141.3, 143.5, 158.0, 170.0, 170.2, 170.8, 171.0. HRMS (ESI+) calcd for $C_{49}H_{61}N_4O_9$ $(M+H)^+$ 849.4433, obsd 849.4431.

*4.10 Construction of the Eastern Macrocycle **54***

Tripeptide 51. Diethylamine (2 mL) was added to a solution of *τ*-histidinoalanine **3** (348 mg, 0.54 mmol) in dry CH_3CN (3 mL). The mixture was stirred at rt for 1h, concentrated, and then concentrated twice more from CH_3CN (2 x 3 mL). The residue was then applied to a flash column in a minimum volume of CH_2Cl_2, eluting with 9:1 CH_2Cl_2: MeOH to give the free amine (200 mg). R_f 0.52 (9:1 CH_2Cl_2-MeOH).

A solution of Fmoc-Ser(O^tBu)-OH (**50**) (140 mg, 0.28 mmol, 1.0 equiv.) in CH_3CN (3 mL), $^iPr_2NEt$ (156 µL, 394 mg, 0.9 mmol, 2.5 equiv.) and HATU (150 mg, 0.39 mmol, 1.05 equiv.) were added sequentially to a solution of the intermediate amine (200 mg, 0.32 mmol, 1.0 equiv.) in CH_2Cl_2 (4 mL) at 0 °C under N_2. After 20 min, the mixture was warmed to rt and stirred for 12 h, concentrated and applied to a flash column in a minimum volume of CH_2Cl_2 eluting with 2% MeOH in CH_2Cl_2 to give **51** (324 mg, 78%). R_f 0.50 (95:5 CH_2Cl_2-MeOH). $[\alpha]^{25}_D$ 1.0 (*c* 1.0, MeOH). ^{1}H NMR (CD_3OD-d_4, 500 MHz) δ 1.16 (s, 9H), 1.40 (s, 9H), 2.89 (dd, *J* = 14.5, 9.2 Hz, 1H), 3.04 (dd, *J* = 15.1, 4.2 Hz, 1H), 4.20-4.28 (m, 2H), 4.35 (d, *J* = 9.8 Hz, 1H), 4.38 (d, *J* = 9.8 Hz, 1H), 4.45-4.49 (m, 3H), 4.74-4.82 (m, 1H), 4.75 (d, *J* = 12.1 Hz, 1H), 5.19 (d, *J* = 12.1 Hz, 1H), 5.25 (d, *J* = 12.1 Hz, 1H), 6.82 (br d, *J* = 9.3 Hz, 1H), 7.30-7.42 (m, 9H), 7.51 (br s, 1H), 7.68 (d, *J* = 6.9 Hz, 2H), 7.81 (d, *J* = 7.5 Hz, 2H); ^{13}C NMR (CD_3OD-d_4, 125 MHz) δ 27.8, 28.8, 30.8, 54.8, 54.9, 55.3, 62.9, 68.3, 68.8 (2C), 75.1, 75.5, 80.8, 96.3, 121.0, 126.3, 126.4, 128.3, 128.9, 129.7, 129.8, 129.9, 136.7, 139.1, 142.7, 145.2, 145.3 (2C), 157.8, 158.5, 170.2, 172.1, 173.1. HRMS (ESI+) calcd for $C_{45}H_{53}Cl_3N_5O_{10}$ $(M+H)^+$ 928.2853, obsd 928.2861.

Eastern linear heptapeptide benzyl ester (52). Zinc dust (1.2 g, 18.5 mmol, 50.0 equiv.) was added to a solution of tripeptide **51** (340 mg, 0.37 mmol, 1.0 equiv.) in

glacial acetic acid (7 mL). The mixture was stirred at rt for 16 h. The zinc dust and colorless precipitate were removed by vacuum filtration through a Celite® pad, washing well with EtOAc. The filtrate was concentrated and the residue dissolved in EtOAc (5 mL), washed with H_2O (5 mL), brine (5 mL), dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography eluting with 9:1 CH_2Cl_2: MeOH to give the free acid as a colorless solid (170 mg; 58%). R_f 0.25 (9:1 CH_2Cl_2: MeOH).

Diethylamine (3 mL) was added to a solution of tetrapeptide Fmoc-*allo*-Thr(O^{t}Bu)-Ser(O^{t}Bu)-Phe-*β*-Phe-OMe (**47**) (250 mg, 0.29 mmol, 1.00 equiv.) in dry acetonitrile (3 mL). The mixture was stirred at rt for 2 h, concentrated, and concentrated twice more from CH_3CN (2 x 6 mL) to remove the excess Et_2NH. The residue was applied to a flash column in a minimum volume of CH_2Cl_2, eluting with 9:1 CH_2Cl_2: MeOH to give H-*allo*-Thr(O^{t}Bu)-Ser(O^{t}Bu)-Phe-*β*-Phe-OMe (**49**) (132 mg). R_f 0.30 (9:1 CH_2Cl_2-MeOH).

The solid amine **49** (132 mg, 0.21 mmol, 1.00 equiv.), collidine (70 µL, 64 mg, 0.53 mmol, 2.00 equiv.) and HATU (84 mg, 0.22 mmol, 1.05 equiv.) were added sequentially to a solution of the intermediate acid (170 mg, 0.21 mmol, 1.00 equiv.) in CH_2Cl_2 (10 mL) at 0 °C under N_2. After 20 min, the mixture was warmed to rt and stirred for 18 h, concentrated and applied to a flash column in a minimum volume of CH_2Cl_2. Elution with a gradient of 2-5% MeOH in CH_2Cl_2 yielded heptapeptide **52** as a colorless foam (192 mg, 37 % over 2 steps). R_f 0.48 (9:1 CH_2Cl_2: MeOH). $[\alpha]^{25}_D$ –13.1 (*c* 0.4, MeOH). ^{1}H NMR (DMSO-d_6, 500 MHz, 333K) δ 1.02-1.03 (m, 3H), 1.04 (s, 9H), 1.09 (s, 18H), 1.36 (s, 9H), 2.74-2.84 (m, 5H), 2.99 (dd, J = 13.9, 5.7 Hz, 1H), 3.39-3.45 (m,

4H), 3.53 (s, 3H), 3.88-3.93 (m, 1H), 4.17 (dd, *J* = 13.4, 6.4 Hz, 2H), 4.23-4.30 (m, 6H), 4.35 (dd, *J* = 14.3, 5.6 Hz, 2H), 4.54 (dd, *J* = 13.9, 8.0 Hz, 1H), 4.71 (dd, *J* = 12.6, 7.1 Hz, 1H), 5.12-5.16 (m, 2H), 5.22 (q, *J* = 7.7 Hz, 1H), 6.88 (br s, 1H), 7.08-7.16 (m, 6H), 7.24 (t, *J* = 7.6 Hz, 3H), 7.27-7.35 (m, 9H), 7.42 (t, *J* = 7.5 Hz, 2H), 7.59 (dd, *J* = 13.8, 7.2 Hz, 1H), 7.72 (d, *J* = 4.9 Hz, 3H), 7.88 (d, *J* = 7.6 Hz, 2H), 8.19 (d, *J* = 7.6 Hz, 1H), 8.34 (d, *J* = 7.5 Hz, 1H), 8.42 (d, *J* = 7.6 Hz, 1H). ^{13}C NMR (DMSO-d_6, 125 MHz, 333K) δ 19.3, 27.6, 27.7, 28.5, 28.6, 38.2, 46.9, 47.2, 50.0, 51.7, 53.7, 54.5, 55.9, 59.4, 61.8, 62.2, 63.4, 66.4, 67.0, 67.5, 73.4, 73.5, 74.1, 78.9, 109.8, 120.4, 120.5, 121.8, 125.7, 126.6, 126.9, 127.5, 127.7, 128.1, 128.3 (2C), 128.4, 128.5, 128.7, 128.8, 129.5, 135.9, 136.0, 137.8 (2C), 140.0, 141.2, 142.1, 144.3, 144.4, 155.6, 156.3, 169.6 (2C), 170.0, 170.5, 170.5, 170.8, 172.8; HRMS (ESI+) calcd for $C_{77}H_{100}N_9O_{16}$ $(M+H)^+$ 1406.7288, obsd 1406.7265.

Eastern Linear Heptapeptide Methyl Amide (53). 10% Pd-C (32 mg, 0.60 mmol) was added to a solution of heptapeptide **52** (120 mg, 0.08 mmol) in dry THF (8 mL). The vessel was evacuated, opened up to an atmosphere of H_2 and then stirred at rt for 4 h. The reaction mixture was filtered through a plug of Celite® and the filtrate concentrated to give the carboxylic acid as a colorless oil (80 mg, 0.06 mmol) that was used directly in the next step without further purification. R_f 0.15 (9:1 CH_2Cl_2-MeOH).

Recrystallized methylamine hydrochloride (4 mg, 0.06 mmol, 1.00 equiv.), triethylamine (20 µL, 15 mg, 0.15 mmol, 2.50 equiv.) and BOP (28 mg, 0.06 mmol, 1.00 equiv.) were added sequentially to a solution of the intermediate acid (80 mg, 0.06 mmol, 1.00 equiv.) in CH_2Cl_2 (10 mL) at 0 °C under N_2. After 20 min, the mixture was warmed to rt and stirred for 18 h, concentrated and applied to a flash column in a minimum

volume of CH_2Cl_2. Elution with a gradient of 2-5% MeOH in CH_2Cl_2 yielded heptapeptide **53** as colorless foam (64 mg, 56% over 2 steps). R_f 0.42 (9:1 CH_2Cl_2-MeOH). $[\alpha]^{25}_D$ –18.4 (*c* 0.4, MeOH). ^{1}H NMR* (CD_3OD, 500 MHz) δ 1.06 (s, 9H), 1.14 (d, *J* = 6.5 Hz, 3H), 1.16 (s, 9H), 1.18 (s, 9H), 1.42 (s, 9H), 2.70 (s, 3H), 2.78 (dd, *J* = 15.6, 6.4 Hz, 1H), 2.87-2.96 (m, 3H), 3.01 (dd, *J* = 14.5, 5.5 Hz, 1H), 3.13-3.17 (m, 1H), 3.53-3.64 (m, 4H), 3.58 (s, 3H), 4.05-4.08 (m, 1H), 4.15 (app t, *J* = 5.9 Hz, 1H), 4.23 (t, *J* = 6.0 Hz, 2H), 4.31-4.51 (m, 6H), 4.61 (dd, *J* = 8.4, 5.9 Hz, 1H), 4.76 (dd, *J* = 7.8, 5.3 Hz, 1H), 5.33 (dd, *J* = 8.5, 6.5 Hz, 1H), 7.07 (br s, 1H), 7.13-7.14 (m, 5H), 7.21-7.31 (m, 7H), 7.38 (t, *J* = 7.4 Hz, 2H), 7.66 (d, *J* = 7.3 Hz, 2H), 7.79 (d, *J* = 7.5 Hz, 2H), 7.89 (br s, 1H). ^{13}C NMR (CD_3OD, 125 MHz) δ 19.5, 26.6, 27.7 (2C), 28.7, 28.8, 38.9, 41.7, 51.6, 52.4, 54.9, 56.0, 56.4, 56.5, 57.3, 61.7, 62.2, 62.7, 62.9, 67.9, 68.2, 75.0, 75.9, 76.0, 81.0, 121.0, 126.2, 126.3, 127.7 (2C), 128.2, 128.3, 128.5, 128.9, 129.5, 129.6, 130.3, 138.4, 142.2, 142.6, 142.7, 145.2, 157.6, 158.6, 170.7, 172.1, 172.3, 172.5, 172.8, 173.4, 174.8. HRMS (ESI+) calcd for $C_{71}H_{97}N_{10}O_{15}$ $(M+H)^+$ 1329.7129, obsd 1329.7116. *data reported for the major species. At the time this work was conducted, we assumed these were different conformations of the heptapeptide but now recognize that they might be diastereomers arising from epimerization of the τ-HAL residue.

Eastern Cyclic Heptapeptide 54. A solution of LiOH (0.6 mg, 0.023 mmol, 2.0 equiv.) in H_2O (150 µL) was added to a stirred solution of linear heptapeptide **53** (15 mg, 0.011 mmol, 1.0 equiv.) in 3:1 THF/MeOH (600 µL) at 0 °C under N_2. After 20 min, the mixture was warmed to rt and stirred for 14 h. A second portion of LiOH (0.6 mg, 0.023 mmol, 2.0 equiv.) in H_2O (150 µL) was added and stirring continued for another 6 h to produce the deprotected linear heptapeptide. Volatiles were evaporated under reduced

pressure; the residue was diluted with water (5 mL) and extracted with 5% MeOH/DCM (2 x 15 mL). The combined organic layers were dried over $MgSO_4$, filtered, concentrated and triturated with Et_2O to obtain the deprotected linear heptapeptide as a yellow solid (9.0 mg, 36%).

HATU (12 mg, 0.032 mmol, 4.0 equiv.) was added to a stirred solution of the deprotected linear heptapeptide (9.0 mg 0.008 mmol, 1.0 equiv.) in CH_2Cl_2 (9 mL) and stirred at rt for 36 h. The mixture was concentrated, and the residue purified by flash chromatography, eluting with 1-3% MeOH in CH_2Cl_2 to give the east ring **54** as a colorless solid (2 mg, 23%). R_f 0.40 (97:3 CH_2Cl_2-MeOH). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.02 (s, 9H), 1.06 (d, *J* = 6.4 Hz, 3H), 1.20 (s, 9H), 1.37 (s, 9H), 1.53 (s, 9H),2.67 (d *J* = 9.3 Hz, 2H), 2.81-3.15 (m, 3H), 2.95 (d, *J* = 4.7 Hz, 3H), 3.24 (dd, *J* =14.5, 3.7 Hz, 1H), 3.46 (dd, *J* = 9.2, 3.6 Hz, 1H), 3.54 (dd, *J* = 14.2, 2.0 Hz, 1H), 3.67 (dd, *J* = 9.2, 4.2 Hz), 3.73 (dd, *J* = 9.9, 4.9 Hz, 1H), 3.76 (dd, *J* = 5.9, 5.0 Hz, 1H), 3.79 (d, *J* = 10.1 Hz, 1H), 3.82 (dd, *J* = 11.0, 7.4 Hz, 1H), 4.00 (app. t, *J* = 3.3 Hz, 1H), 4.21 (app. q, *J* = 4.2 Hz, 1H), 4.50 (dt, *J* = 7.6, 3.8 Hz, 1H), 4.57 (ddd, *J* = 12.0, 7.6, 2.5 Hz, 1H), 4.60-4.67 (m, 1H), 4.77 (app. t, *J* = 9.7 Hz, 1H), 5.01 (d, *J* = 15 Hz, 1H), 5.40 (app. t, *J* = 10.2 Hz, 1H), 6.76 (d, *J* = 4.4 Hz, 1H), 7.04 (d, *J* = 8.7 Hz, 1H), 7.19-7.24 (m, 5H), 7.26-7.39 (m, 9H), 7.50 (s, 1H), 7.67 (d, *J* = 9.0 Hz, 1H). HRMS (ESI+) calcd for $C_{55}H_{83}N_{10}O_{12}$ $(M+H)^+$ 1075.6186, obsd 1075.6195.

*4.11 Synthesis of Alternate Eastern Heptapeptide **57***

Pentapeptide 55. Zinc dust (1.5 g, 22.94 mmol, 50.0 equiv.) was added to a solution of orthogonally protected *τ*-HAL **3** (360 mg, 0.45 mmol, 1.0 equiv.) in glacial

acetic acid (2.5 mL). The mixture was stirred at rt for 16 h. Residual zinc dust and the colorless precipitate of $ZnCl_2$ were removed by vacuum filtration through a Celite® pad, washing well with EtOAc (3 x 15 mL). The filtrate was washed with H_2O (15 mL), brine (15 mL), dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography eluting with 9:1 CH_2Cl_2-MeOH to give the free acid (255 mg, 86%) as a colorless solid. R_f 0.20 (9:1 CH_2Cl_2- MeOH).

Diethylamine (2.0 mL) was added to a solution of Fmoc-*allo*-Thr(O^{t}Bu)-Ser(O^{t}Bu)-Phe-OAllyl (**46**) (300 mg, 0.42 mmol, 1.0 equiv.) in dry MeCN (4.0 mL). The mixture was stirred at rt for 2 h, concentrated, and concentrated twice more from CH_3CN (2 x 10 mL). The residue was applied to a flash column in a minimum volume of CH_2Cl_2 and eluted with 19:1 CH_2Cl_2-MeOH to give H-*allo*-Thr(O^{t}Bu)-Ser(O^{t}Bu)-Phe-OAll (**47**) (195 mg, 92%) as colorless, viscous liquid. R_f 0.40 (9:1 CH_2Cl_2-MeOH).

A solution of amine, H-*allo*-Thr(O^{t}Bu)-Ser(O^{t}Bu)-Phe-OAllyl (**47**) (195 mg, 0.39 mmol, 1.0 equiv.) in CH_2Cl_2 (3 mL), and HATU (180 mg, 0.468 mmol, 1.2 equiv.) were added sequentially to a solution of the intermediate acid (255 mg, 0.39 mmol, 1.0 equiv.) in CH_2Cl_2 (10 mL) at 0 °C under N_2. After 20 min, the mixture was warmed to rt and stirred for 16 h. The reaction mixture was concentrated, and the residue purified by flash chromatography, eluting with a gradient of 1-3% MeOH in CH_2Cl_2 to give pentapeptide **55** (345 mg, 78%) as colorless solid. R_f 0.50 (95:5 CH_2Cl_2-MeOH). $[\alpha]^{25}_D$ +9.4 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.05 (d, *J* = 6.2 Hz, 3H), 1.09 (s, 9H), 1.13 (s, 9H), 1.45 (s, 9H), 2.84 (dd, *J* = 14.5, 6.5 Hz, 1H), 2.96 (dd, *J* = 14.5, 4.7 Hz, 1H), 3.06-3.18 (m, 1H), 3.12 (app. t, *J* = 6.0 Hz, 2H), 3.43-3.52 (m, 1H), 3.64 (dd, *J* = 8.8, 5.7 Hz, 1H), 3.96-4.07 (m, 1H), 4.20 (t, *J* = 6.5 Hz, 1H), 4.24-4.35 (m, 3H), 4.41 (dd, *J* = 10.6,

6.3 Hz, 1H), 4.44-4.62 (m, 3H), 4.56 (d, *J* = 5.7 Hz, 1H), 4.84 (q, *J* = 7.0 Hz, 1H), 5.14-5.28 (m, 4H), 5.74-5.88 (m, 2H), 6.20 (br s, 1H), 6.44 (br , 1H), 7.12-7.43 (m, 16H), 7.53-7.60 (m, 3H), 7.66 (br, 1H), 7.77 (d, *J* = 7.1 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.7, 27.3, 28.2, 28.3, 30.2, 37.6, 47.1, 47.8, 53.5, 54.8, 55.2, 59.6, 61.0, 65.7, 66.9, 67.1, 68.1, 73.8, 74.6, 79.8, 117.6, 118.4, 1120.0, 124.8, 125.0, 126.7, 127.0, 127.1, 127.8, 128.3, 128.7, 128.8, 129.0, 129.2, 131.6, 134.4, 136.5, 137.1, 141.3, 143,5, 155.6, 168.6, 169.8, 169.9, 170.9, 172.2. HRMS (ESI+) calcd for $C_{63}H_{80}N_7O_{13}$ $(M+H)^+$ 1142.5809, obsd 1142.5848.

Hexapeptide 56. Diethylamine (2.0 mL) was added to a solution of the pentapeptide **55** (325 mg, 0.285 mmol, 1.0 equiv.) in dry MeCN (4.0 mL). The mixture was stirred at rt for 2 h, concentrated, and concentrated twice more from CH_3CN (2 x 6 mL). The residue was applied to a flash column in a minimum volume of CH_2Cl_2 and eluted with 19:1 CH_2Cl_2-MeOH to give the amine (255 mg, 95%) as a colorless, viscous liquid. R_f 0.45 (9:1 CH_2Cl_2-MeOH).

A solution of the intermediate amine (250 mg, 0.272 mmol, 1.0 equiv.) in CH_2Cl_2 (3 mL), and HATU (125 mg, 0.326 mmol, 1.2 equiv.) were added sequentially to a solution of Fmoc-Ser(O^{t}Bu)-OH (**50**) (105 mg, 0.272 mmol, 1.0 equiv.) in CH_2Cl_2 (6 mL) at 0 °C under N_2. After 20 min, the mixture was warmed to rt and stirred for 18 h. The mixture was concentrated, and the residue purified by flash chromatography, eluting with a gradient of 1-2% MeOH in CH_2Cl_2 to give hexapeptide **56** (300 mg, 86%) as a colorless solid. R_f 0.50 (95:5 CH_2Cl_2-MeOH). $[\alpha]^{25}_D$ +9.0 (*c* 1.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.05 (d, *J* = 6.3 Hz, 3H), 1.09 (s, 9H), 1.12 (s, 9H), 1.18 (s, 9H), 1.44 (s, 9H), 2.83 (dd, *J* = 14.6, 6.3 Hz, 1H), 2.91 (dd, *J* = 14.6, 5.7 Hz, 1H), 3.08 (dd, *J* =

14.0, 7.0 Hz, 1H), 3.14 (dd, *J* = 14.0, 6.0 Hz, 1H), 3.43 (app. t, *J* = 8.1 Hz, 1H), 3.42-3.52 (m, 1H), 3.64 (dd, *J* = 8.8, 5.8 Hz, 1H), 3.74-3.83 (m, 1H), 4.20-4.47 (m, 8H), 4.43-4.52 (m, 1H), 4.53 (d, *J* = 12.6 Hz, 2H), 4.76-4.82 (m, 1H), 4.84 (q, *J* = 7.0 Hz, 1H), 5.16-5.28 (m, 4H), 5.73-5.88 (m, 2H), 6.24 (br s, 1H), 6.54 (s, 1H), 7.13-7.43 (m, 28H), 7.49-7.64 (m, 3H), 7.60 (d, *J* = 7.4 Hz, 2H), 7.75 (d, *J* = 7.4 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.7, 27.3, 28.2, 23.3, 30.2, 37.6, 47.1, 47.8, 53.5, 54.8, 55.2, 59.6, 61.0, 65.7, 67.1, 68.1, 73.8, 74.6, 79.8, 117.6, 118.4, 120.0, 124.8, 125.0, 126.7, 127.0, 127.1, 127.8, 128.3, 128.8 (2C), 120.0, 129.2, 131.6, 134.4, 136.5, 137.1, 141.3, 143.5, 155.6 (2C), 168.6, 169.8, 169.9, 170.9, 172.2. HRMS (ESI+) calcd for $C_{70}H_{93}N_8O_{15}$ $(M+H)^+$ 1285.6760, obsd 1285.6775.

Heptapeptide 57. $Pd(PPh_3)_4$ (16 mg, 0.014 mmol, 0.2 equiv.) was added to a stirred solution of linear hexapeptide **56** (90 mg, 0.07 mmol, 1.0 equiv.) and $BH_3.NHMe_2$ (8 mg, 0.14 mmol, 2.0 equiv.) in THF (3.0 mL) containing AcOH (5 µL) at rt under N_2. The resulting mixture was allowed to stir for 6 h at rt, during which time it became black. The mixture was concentrated, and the residue purified by flash chromatography eluting with 95:5 CH_2Cl_2-MeOH to give the free acid (65 mg, 75%) as a colorless solid. R_f 0.40 (95:5 CH_2Cl_2-MeOH).

Trifluoroacetic acid (0.5 mL) was dropwise added to a stirred solution of Boc-β-Phe-OFm (**37**) (40 mg, 0.09 mmol, 1.0 equiv.) in CH_2Cl_2 (2.0 mL) at 0 °C under N_2. The mixture was stirred for 2 h, volatiles were evaporated, diluted with CH_2Cl_2 (20 mL), washed consecutively with sat′d aq. $NaHCO_3$ (15 mL), H_2O (15 mL), brine (15 mL) and dried over $MgSO_4$, filtered, concentrated to give amine **39** (29 mg, 85%) as a colorless,

viscous liquid. It was used as such for the next step without further purification. R_f 0.50 (95:5 CH_2Cl_2-MeOH).

A solution of the intermediate hexapeptide acid (65 mg, 0.052 mmol, 1.0 equiv.) in CH_2Cl_2 (2 mL), and HATU (20 mg, 0.052 mmol, 1.2 equiv.), were added sequentially to a stirred solution of H-β-Phe-OFm (**39**) (18 mg, 0.052 mmol, 1.0 equiv.) in CH_2Cl_2 (4 mL) at 0 °C under N_2. After 20 min, the mixture was warmed to rt and stirred for 16 h. The reaction mixture was concentrated, and the residue purified by flash chromatography eluting with a gradient of 1-3% MeOH in CH_2Cl_2 to give heptapeptide **57** (65 mg, 84%) as a colorless solid. R_f 0.50 (95:5 CH_2Cl_2-MeOH). $[\alpha]^{25}_D$ -8.5 (*c* 1.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.00 (s, 9H), 1.08 (d, *J* = 6.7 Hz, 3H), 1.12 (s, 9H), 1.13 (s, 9H), 1.45 (s, 9H), 2.73-2.86 (m, 2H), 2.95-3.06 (m, 2H), 3.11 (dd, *J* = 14.9, 6.0 Hz, 1H), 3.41 (t, *J* = 7.5 Hz, 1H), 3.43-3.53 (m, 2H), 3.73-3.81 (m, 1H), 4.07-4.18 (m, 4H), 4.18-4.28 (m, 4H), 4.32-4.44 (m, 5H), 4.68-4.77 (m, 2H), 5.16 (d, *J* = 11.9 Hz, 1H), 5.23 (d, *J* = 11.9 Hz, 1H), 5.52 (br, 1H), 5.70 (br, 1H), 6.35 (br, 1H), 6.47 (br, 1H), 7.10-7.55 (m, 31H), 7.58 (dd *J* = 7.5, 5.0 Hz, 2H), 7.71 (d, *J* = 7.5 Hz, 2H), 7.75 (d, *J* = 7.5 Hz, 2H), 8.15 (s, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 18.4, 27.2, 27.3, 28.1, 28.2, 28.3 (2C), 37.1, 41.2, 46.7, 47.1, 50.3, 53.6, 54.6, 54.8, 60.4, 61.3, 65.9, 66.7, 67.2, 68.2, 73.7, 74.4, 74.9, 77.3, 80.2, 119.8, 119.9, 120.0, 125.0, 125.3, 126.2, 126.7, 127.0, 127.1 (2C), 127.4, 127.6, 127.7 (2C), 128.2, 128.6, 128.8, 129.0, 129.1, 129.2, 134.3, 137.4, 141.0, 141.1, 141.2, 141.3 (2C), 143.6, 143.7, 143.8, 144.0, 155.9, 156.2, 168.5, 169.9, 170.4, 170.5 (2C), 171.2, 173.4. HRMS (ESI+) calcd for $C_{90}H_{108}N_9O_{16}$ $(M+H)^+$ 1570.7914, obsd 1570.7963.

*4.12 Synthesis of Undecapeptides **58** and **59***

Linear undecapeptide 58. Pd/C (80 mg, 10% w/w) was added portionwise to a solution of linear heptapeptide **57** (80 mg, 0.05 mmol, 1.0 equiv.) in THF (2 mL) containing AcOH (5 µL) and stirred for 2.5 h under a hydrogen atmosphere. The reaction mixture was filtered through a Celite® pad, washing well with EtOAc (2 x10 mL). The filtrate was concentrated, and the residue purified by flash chromatography eluting with 95:5 CH_2Cl_2-MeOH to give the free acid (58 mg, 77%) as a colorless solid. R_f 0.30 (95:5 CH_2Cl_2- MeOH).

A solution of the intermediate heptapeptide acid (58 mg, 0.04 mmol, 1.0 equiv.) in CH_2Cl_2 (2 mL), and HATU (18 mg, 0.047 mmol, 1.2 equiv.) were added sequentially to a stirred solution of tetrapeptide amine **30** (as prepared from **5** in §4.6, 40 mg, 0.04 mmol, 1.0 equiv.) in CH_2Cl_2 (4 mL) at 0 °C under N_2. After 20 min, the mixture was warmed to rt and stirred for 16 h. The reaction mixture was concentrated, and the residue purified by flash chromatography eluting with a gradient of 1-3% MeOH in CH_2Cl_2 to give the undecapeptide **58** as colorless solid (82 mg, 86%). R_f 0.40 (97:3 CH_2Cl_2-MeOH). $[\alpha]^{25}_D$ -6.5 (*c* 1.25, $CHCl_3$). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 0.98 (d, *J* = 11.3 Hz, 3H), 0.99 (s, 9H), 1.02 (s, 9H), 1.04 (s, 9H), 1.31 (s, 9H), 2.16 (t, *J* = 7.4 Hz, 2H), 2.55-2.67 (m, 5H), 2.69-2.81 (m, 5H), 2.84 (dd, *J* = 6.5, 5.5 Hz, 1H), 2.84-3.09 (m, 6H), 3.20-3.30 (m, 1H), 3.30-3.42 (m, 2H), 3.70-3.80 (m, 1H), 3.76-3.87 (m, 1H), 3.93-4.04 (m, 1H), 4.13-4.36 (m, 11H), 4.48 (dd, *J* = 14.8, 7.3 Hz, 1H), 4.51 (d, *J* = 5.5 Hz, 2H), 4.55 (dd, *J* = 14.3, 7.6 Hz), 4.71 (dd, *J* = 14.3.7.8 Hz, 1H), 4.72-4.80 (m, 1H), 5.16-5.24 (m, 3H), 5.28 (dd, *J* = 17.2, 1.6 Hz, 1H), 5.85-5.3 (m, 1H), 6.81 (br, 1H), 6.95 (br, 1H), 6.93-7.42 (m, 56H), 7.55 (t, *J* = 8.4 Hz, 2H), 7.56-7.74 (m, 2H), 7.68 (d, *J* = 7.7 Hz), 7.70 (d, *J* =

7.8 Hz, 1H), 7.86 (d, *J* = 7.5 Hz (4H), 7.99 (d, *J* = 7.8 Hz, 1H), 8.05 (d, *J* = 8.0 Hz, 1H), 8.22 (d, *J* = 6.2 Hz, 1H), 8.27 (d, *J* = 7.0 Hz), 8.29 (s, 1H), 8.39 (d, *J* = 7.8 Hz, 1H), 8.47 (br d, *J* = 5.9 Hz, 1H), 8.61 (br, 1H), 8.74 (br, 1H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 19.5, 27.5, 27.6, 28.4, 28.6, 33.7, 35.1 37.2, 38.2, 38.6, 33.7, 35.1, 37.2, 38.2, 38.6, 40.1, 40.1, 40.4, 40.6, 40.9, 46.6, 47.1, 49.9, 50.2, 50.5, 54.3, 54.7, 55.9, 58.9, 61.9, 62.1, 64.9, 66.2, 66.4, 67.7, 69.9. 70.0, 73.4, 74.0, 78.9, 79.6, 118.3, 120.5, 120.6, 125.5, 125.6, 125.8, 126.5, 126.7, 126.8, 127.0, 127.5, 127.6 (2C), 127.9, 128.1, 128.2, 128.4, 128.5, 128.8, 129.0, 129.5 (2C), 133.1, 137.6, 1385, 141.1, 141.2, 141.8, 144.0 (2C), 144.2, 144.4, 145.0, 145.1, 145.2, 155.7, 156,5, 169.2, 169.5, 169.9, 170.1, 170.4, 170.9, 171.1, 171.3. HRMS (ESI+) calcd for $C_{144}H_{160}N_{15}O_{22}$ $(M+H)^+$ 2452.1896, obsd 2452.1865.

Undecapeptide Macrocycle 59. Diethylamine (2.0 mL) was added to a solution of linear undecapeptide **58** (110 mg, 0.045 mmol, 1.0 equiv.) in dry MeCN (4 mL). The mixture was stirred at rt for 2 h, concentrated, and concentrated twice more from CH_3CN (2 x 15 mL). The residue was triturated with Et_2O (15 mL) and hexane (15 mL) to give the to give the deprotected undecapeptide (76 mg, 82%) as colorless solid. R_f 0.30 (9:1 CH_2Cl_2-MeOH).

HATU (28 mg, 0.074 mmol, 2.0 equiv.) was added to a stirred solution of the deprotected undecapeptide (76 mg, 0.037 mmol, 1.0 equiv.) in CH_2Cl_2 (55 mL) and stirred at rt for 36 h. The reaction mixture was concentrated, and the residue purified by flash chromatography eluting with a gradient of 0.1-2.0% MeOH in CH_2Cl_2 to give the cyclic undecapeptide **59** as colorless solid (60 mg, 80%). R_f 0.45 (97:3 CH_2Cl_2-MeOH). $[\alpha]^{25}_D$ -4.3 (*c* 0.75, $CHCl_3$). ^{1}H NMR (DMSO-d_6, 500 MHz, 60 °C) δ 1.04 (s, 9H), 1.12 (d, *J* = 6.4 Hz, 3H), 1.17 (s, 18H), 1.42 (s, 9H), 2.25 (t, *J* = 7.4 Hz, 2H), 2.44 (dd, *J* =

13.7, 3.3 Hz, 1H), 2.58 (dd, *J* = 15.5, 6.9 Hz, 1H), 2.64-2.80 (m, 4H), 2.78 (dd, *J* = 15.5 Hz, 6.9 Hz, 1H), 2.98 (dd, *J* = 13.8, 4.8 Hz, 1H), 2.86-3.26 (m, 6H), 3.35-3.54 (m, 4H), 3.95-4.08 (m, 1H), 4.12-4.53 (m, 11H), 4.19 (app. q, *J* = 5.0 Hz, 1H), 4.53 (dd, *J* = 5.5, 1.3 Hz, 2H), 4.71 (td, *J* = 8.3, 5.5 Hz, 1H), 4.82 (td, *J* = 8.0, 3.3 Hz, 1H), 5.18-5.26 (m, 1H), 5.21 (dd, *J* = 10.6, 1.3 Hz, 1H), 5.29 (dd, *J* = 17.2, 1.5 Hz, 1H), 5.91 (ddt, *J* = 17.3, 10.6, 5.4 Hz, 1H), 7.03-7.29 (m, 52H), 7.64-7.80 (m, 2H), 7.82 (d, *J* = 8.1 Hz, 1H), 8.19 (d, *J* = 7.8 Hz, 1H), 8.27 (s, 1H), 8.48 (d, *J* = 7.5 Hz, 1H), 8.52 (s, 1H); ^{13}C NMR (DMSO-d_6, 125 MHz, 60 °C) δ 18.6, 26.7, 26.8, 26.9, 27.8, 27.9, 33.2, 34.5, 36.9, 37.9, 39.4, 39.6, 39.8, 39.9, 40.1, 49.9, 50.4, 53.9, 59.4, 60.7, 64.2, 65.8, 69.3, 69.4, 72.8, 73.8, 78.7, 78.9, 117.5, 125.8, 126.0, 126.1, 126.6, 127.1, 127.2, 127.8, 127.9, 128.0, 128.3 (2C), 128.6, 128.8, 132.4, 137.6, 142.2, 144.4, 144.5, 154.8, 167.1, 168.6, 168.8, 169.1, 169.3, 169.6, 170.1, 170.2, 170.4, 170.5. HRMS (ESI+) calcd for $C_{115}H_{138}N_{15}O_{19}$ $(M+H)^+$ 2033.0293, obsd 2033.0341.

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Progress Toward the Assembly of the Bicyclic Theonellamide Skeleton

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HIGHLIGHTS

- synthesis of orthogonally protected τ-histidinoalanine
- use of trichloroethyl and fluorenylmethyl esters in peptide synthesis
- strategy and progress toward the theonellamide bicyclic skeleton
- attention to racemization of histidine and histidinoalanine
- efficient solution-phase synthesis of peptide fragments

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: