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Biaryl-Bridged Macrocyclic Peptides: Conformational Constraint via Carbogenic Fusion of Natural Amino Acid Side Chains

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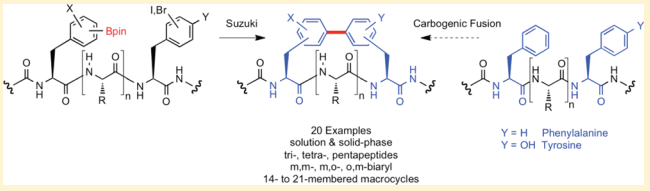
Supporting Information

ABSTRACT: A general method for constraining peptide conformations via linkage of aromatic sidechains has been developed. Macrocyclization of suitably functionalized tri-, tetra- and pentapeptides via Suzuki–Miyaura cross-coupling has been used to generate side chain to side chain, biaryl-bridged 14- to 21-membered macrocyclic peptides. Biaryl bridges possessing three different configurations, meta–meta, meta–ortho, and ortho–meta, were systematically explored through regiochemical variation of the aryl halide and aryl boronate coupling partners, allowing fine-tuning of the resultant macrocycle conformation. Suzuki–Miyaura macrocyclizations were successfully achieved both in solution and on solid phase for all three sizes of peptide. This approach constitutes a means of constraining peptide conformation via direct carbogenic fusion of side chains of naturally occurring amino acids such as phenylalanine and tyrosine, and so is complementary to strategies involving non-natural, for example, hydrocarbon, bridges.



INTRODUCTION

Peptide hormones play a key role in mammalian regulatory processes, and so in principle represent attractive points of therapeutic intervention in dysregulated biological systems.[1] However, their poor pharmacokinetic properties usually limit their direct utility as therapeutic agents.[2] Consequently, strategies for stabilizing the bioactive conformation of therapeutically important peptides, while limiting their metabolic clearance, are of considerable interest.[3] It has been known for many years that macrocyclic peptides can exhibit improved pharmacological and pharmacokinetic properties over their acyclic counterparts.[4] These advantages stem from the conformational preorganization imposed by the macrocyclic framework, which can be exploited in stabilizing the bioactive peptide conformation and reducing susceptibility to protease cleavage. Multiple opportunities for the macrocyclization of linear peptides can be envisaged,[5] involving linkages between N- and C-termini, between termini and side chains, or between side chains. The latter approach has the advantage of not disrupting potential interactions between the N- or C-termini and the target receptor. These side chain to side chain macrocyclization strategies have been widely explored and can be used to stabilize specific conformational motifs such as α-helices.[6]

Common synthetic strategies for generating macrocyclic peptides via side chain to side chain linkages have included: ring closing olefin metathesis (RCM) reactions between side chains bearing terminal alkene groups;[6a] amide-coupling reactions, for example between lysine and aspartic acid;[6b] and copper-catalyzed azide–alkyne cycloaddition (CuAAC) reactions between alkyne- and azide-substituted side chains.[6c] In these cases, it is typically not envisaged that the newly created bridge is part of the bioactive peptide pharmacophore, but rather a means of forming the macrocyclic ring, thereby influencing the conformation of a peptidic region elsewhere in the macrocycle.

As a result of our interest in bioactive peptides such as glucagon-like peptide 1 (GLP-1),[7] somatostatin,[8] and the enkephalins,[9] all of which feature noncontiguous aromatic amino acids which are potentially proximal in space, as illustrated in **1**, we envisaged a complementary peptide macrocyclization strategy, whereby side chain to side chain bridges comprised of naturally occurring amino acids were an integral component of the bioactive pharmacophore. Thus, the resultant biaryl-bridged macrocyclic peptides, such as **2**, would possess both a constrained peptide backbone and a preorganized lipophilic, aromatic region for potential interaction with the relevant receptor. Intriguingly, biaryl peptide motifs such as these are found widely in biologically active natural products,[10] such as the biphenomycins (e.g., **3**),[11] arylomycins (e.g., **4**),[12] and RP 66453 (**5**),[13] supporting our hypothesis that the profile of biologically active peptides could be modulated through this type of 'natural side-chain bridging' strategy. We therefore chose to develop a flexible synthetic approach to the construction of biaryl-bridged peptide macrocycles, which would in due course allow systematic exploration of this approach to constraint of bioactive peptides.

Since, in principle, all three of the unsubstituted positions on the aromatic ring of a phenylalanine side chain (*o*-, *m*-, and *p*-),

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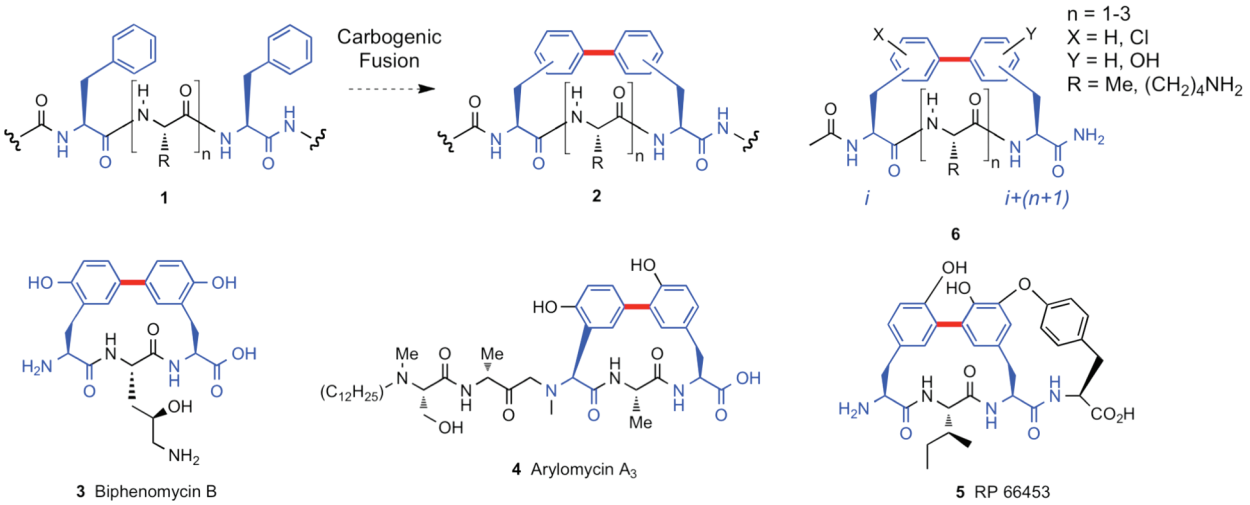

and either of the unsubstituted positions on the aromatic ring of a tyrosine side chain (*o*- and *m*-) could be linked to a second aromatic amino acid side chain, there are a number of possible configurations of such a biaryl-bridge. All three natural product classes referred to above feature a *m*,*m*-biaryl bridge on a tripeptide backbone. Since modeling studies suggested that changes in configuration of the biaryl bridge (and hence macrocycle ring size) would have a marked effect on peptide conformation, we saw a benefit in extending the bridge permutations beyond the *m*,*m*-systems found in these natural products to include also *m*,*o*- and *o*,*m*-bridged systems. In addition, since pairs of aromatic amino acid residues are present in the peptide hormones of interest at $i/i + 2$, $i/i + 3$, and $i/i + 4$ positions on the peptide chain, we sought approaches to constructing macrocycles of each bridge configuration for tri-, tetra-, and pentapeptide backbones (**6**) employing alanines as intervening amino acid units for simplicity.

It is known that introduction of additional substituents at the α-position of amino acids can bias conformation by restricting access to regions of the Ramachandran Φ/Ψ dihedral surface.[14] We therefore wanted to ensure that any synthetic methodology we developed would be compatible with such substitution patterns. Consequently, we incorporated into our program selected examples of α-methylated, that is, quaternary, amino acids. By virtue of their $i/i + 4$ biaryl-bridges, we recognized the possibility that the proposed pentapeptide systems had the potential for helical conformations, by analogy with a number of reported helix stabilization approaches.[6] Finally, we also wanted to demonstrate that substituents could be incorporated successfully into either of the bridging aromatic rings, for example, *p*-hydroxy-substituents to mimic tyrosine residues.

We envisaged that closure of the macrocyclic ring via Suzuki–Miyaura cross-coupling reaction between a borylated phenylalanine and an appropriately placed halogenated phenylalanine residue would provide the most flexible strategy for constructing libraries of biaryl-bridged systems.[15] The requisite macrocyclization precursors could thus be constructed either in solution or on solid phase by standard peptide coupling approaches. Key to our strategy is our recently reported methodology using iridium-catalyzed borylation chemistry on substituted phenylalanines to form the corresponding arylboronates.[16] We therefore anticipated having ready access to the necessary regiochemical variants of borylated and halogenated phenylalanine derivatives, either from our methodology, from Miyaura borylation of halogenated phenylalanine derivatives, or from commercial sources. Herein, we report the realization of this strategy and the successful construction of a diverse set of biaryl-bridged macrocyclic peptides. During the course of our program, a related and complementary study was reported.[17]

RESULTS AND DISCUSSION

1. Solution-Phase Synthesis of *m*,*m*-Bridged Biaryl Macrocyclic Peptides. We elected to explore the synthesis of *meta*–*meta*-bridged systems initially via a solution-phase synthesis, in order that we could determine the optimum conditions for the key Suzuki–Miyaura cross-coupling, without the reaction-monitoring complications associated with resin-bound substrates and products. Furthermore, we chose to construct macrocyclic tri-, tetra-, and pentapeptides which represented a carbogenic fusion of a phenylalanine at the *i* position with a tyrosine at the $i + 2$, $i + 3$, and $i + 4$ positions, respectively, since these coupling reactions entailed use of an *o*-substituted aryl halide; we presumed that optimized conditions for these systems would then be generally applicable to unhindered systems. For consistency, we decided to incorporate an N-terminal acetyl and a C-terminal primary amide in all the macrocyclic peptides we synthesized.

The synthesis of the *m*,*m*-bridged tripeptide macrocycle is outlined in Scheme 1a. The *m*-borylated phenylalanine derivative **7** can be prepared regioselectively on a 20 g scale using our previously described methodology.[16] This could be hydrolyzed selectively to yield the carboxylic acid **8**, for coupling with the appropriate peptide fragment. Iodotyrosine **9** was protected as its *O*-benzyl ether methyl ester **10**, and then coupled to Boc-(L)-alanine to yield the dipeptide **11**. Transformation of this key intermediate to the macrocyclization substrate was accomplished by deprotection to yield hydrochloride **12**, followed by coupling with **8** to yield tripeptide **13**. This material proved unstable toward chromatography, and so was submitted directly to the Suzuki–Miyaura macrocyclization. A screen of conditions for this reaction demonstrated that use of $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$ as catalyst, together with CsF as base in degassed dioxane, yielded optimum results. Thus, after heating at 90 °C for 18 h, at a concentration of 0.02 M, the macrocycle **14** was isolated in 60% yield for the three steps from **11**. Macrocycle **14** was converted to the corresponding acetamide **15**, which yielded the desired product **16** in a one-pot procedure involving hydrogenolytic cleavage of the chloro-substituent and benzyl protecting group, hydrolysis of the methyl ester, and amide formation.

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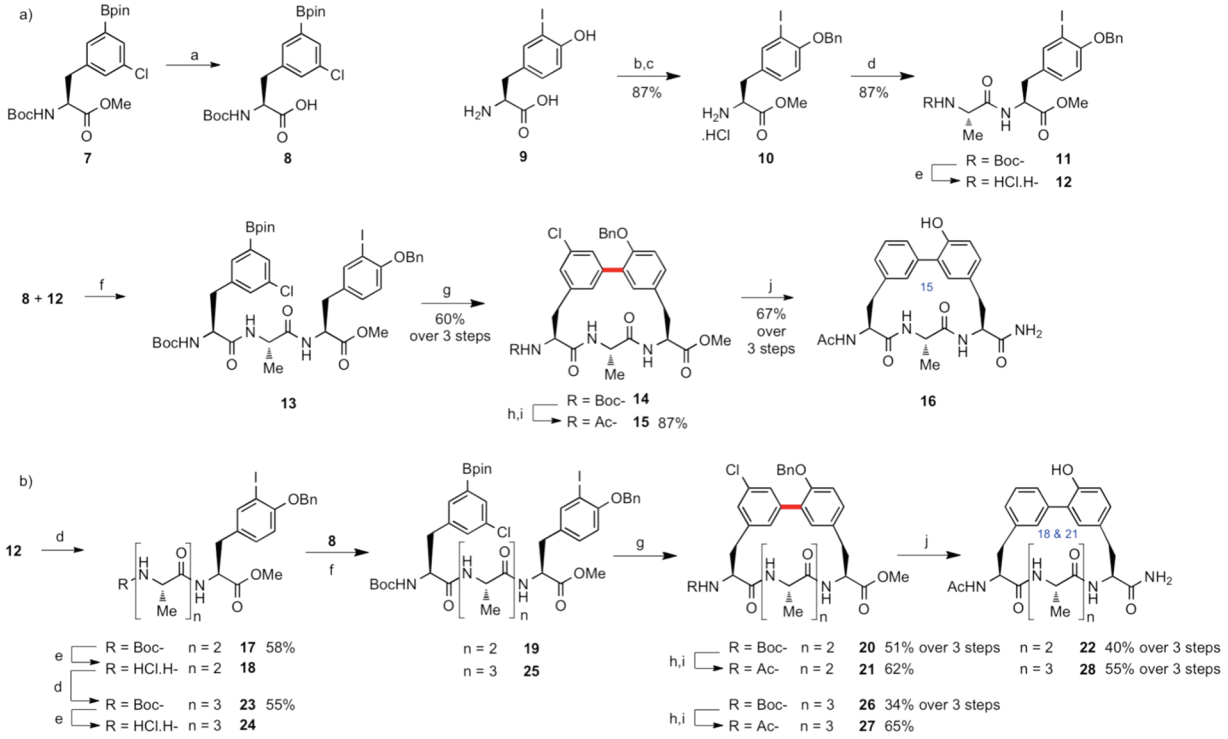
Scheme 1. Solution-Phase Synthesis of (a) *m,m*-Bridged Biaryl Tripeptide Macrocycles and (b) *m,m*-Bridged Biaryl Tetra- and Pentapeptide Macrocycles[a]



[a]Reaction conditions: (a) $LiOH{\cdot}H_2O$ (aq), MeOH, rt, 40 min (aq); (b) NaOH (aq), $CuSo_4$ (aq), MeOH (aq), 60 °C, 10 min, BnBr, 12 h; (c) $SOCl_2$, MeOH, rt, 2 h; (d) Boc-(L)-alanine, PyBOP, NEt_3, rt, 3 h; (e) HCl, dioxane, rt, 5 h; (f) PyBOP, DIPEA, DMF, rt, 12 h; (g) $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$, CsF (aq), dioxane 90 °C, 18 h; (h) HCl, dioxane, rt, 5 h; (i) Ac_2O, DIPEA, DMF, rt, 12 h; (j) $Pd(OH)_2/C$, NH_4OH (aq) H_2, 40 °C, 12 h, then $LiOH{\cdot}H_2O$ (aq), MeOH, rt, 5 h, then PyBOP, NH_3, DMF, DCM, rt, 5 h.

Several aspects of this synthetic sequence are noteworthy. The chloro-substituent carried through the synthesis had served in effect as a regiochemical directing group, ensuring selective borylation to yield the requisite 3,5-disubstituted phenylalanine **7**, but not appearing in the product **16**. However, it also proved possible to conduct a selective hydrogenolysis of the benzyl protecting group in macrocycle **14**, thereby retaining the chloro-substituent as a point of future diversification (results not shown). Additionally, although the product of the macrocyclization, **14**, was converted to the simple *N*-acetyl, primary amide derivative **16**, its protection regime renders it suitable for embedding within larger peptide sequences as a conformational constraint element.

The corresponding biaryl-bridged tetra- and pentapeptide macrocycles were prepared using an analogous synthetic sequence, as depicted in Scheme 1b. Coupling of amine hydrochloride **12** to a further Boc-(L)-alanine fragment yielded tripeptide **17**, which, following deprotection to yield the amine hydrochloride **18**, could be coupled with **8** to yield the tetrapeptide macrocyclization precursor **19**. This was again subjected directly to the optimized Suzuki–Miyaura conditions at 0.02 M concentration to yield macrocycle **20** in 51% yield over the three steps from **17**. Conversion to the macrocycle product was achieved via formation of the N-terminal acetamide **21**, and then one-pot deprotection/amidation to yield **22**. This compound was observed to exist as a 1.0:0.4 mixture of conformers by NMR, reflecting the conformational restraint imposed by the macrocyclic ring. Similarly, the tripeptide hydrochloride **18** could be intercepted and converted to the tetrapeptide **23**, which yielded the pentapeptide macrocycle precursor **25** following deprotection to **24** and coupling to **12**. In this case, macrocyclization yielded the 21-membered system **26** in 34% overall yield from **23**, which could be converted to the N-terminal acetamide **27** and then to the C-terminal primary amide **28**. In this case, the molecule existed as a 1.0:0.2 mixture of conformers by ^1H NMR.

Although this solution-phase approach proved successful and allowed straightforward optimization of the macrocyclization step, the synthesis of these macrocyclic peptides proved challenging from a practical standpoint. Thus, the routes involved multiple purification steps, and the yields of the final steps were variable, primarily because of the lower solubility of these larger systems and the attendant difficulties in purifying them chromatographically. Consequently, for the other members of the library, we decided to adopt a solid-phase synthesis strategy, whereby the Suzuki–Miyaura macrocyclization step would be conducted on a resin-bound substrate.

2. Solid-Phase Synthesis of *m,m*-Bridged Biaryl Macrocyclic Pentapeptides. The synthesis of a prototypical *m,m*-biaryl bridged pentapeptide is shown in Scheme 2. MBHA resin was used together with a Boc-protection strategy, so that release of the macrocyclic product from the resin would yield a C-terminal primary amide directly. The *m*-borylated phenylalanine derivative **8** was incorporated into the peptide chain and the resultant pentapeptide capped with an acetyl group to yield the solid phase-supported (SPS) substrate **29**. This was subjected to Suzuki–Miyaura macrocyclization under essentially the same conditions as the analogous solution phase-reaction. In the absence of a straightforward means of monitoring reaction progress, the formation of resin-bound

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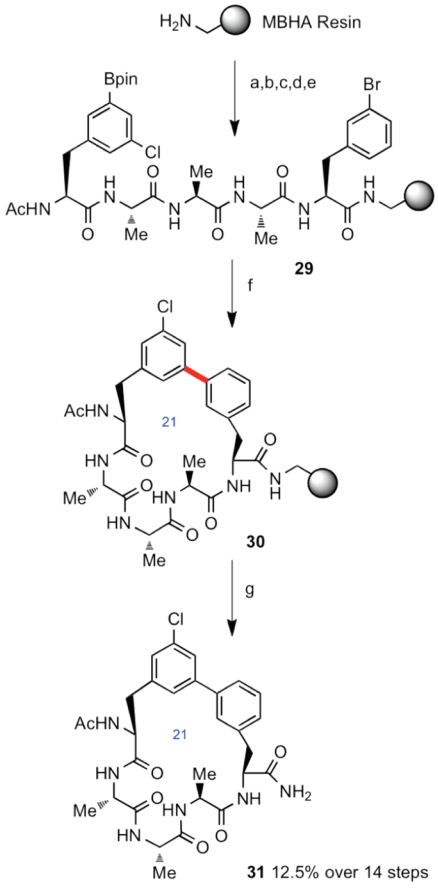
Scheme 2. Solid-Phase Synthesis of *m,m*-Bridged Biaryl Macrocyclic Peptides[a]



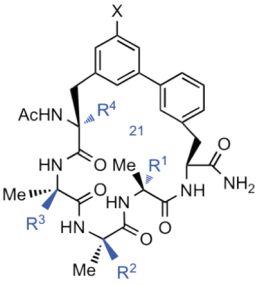
[a]Reaction conditions: (a) *N*-BOC-(L)-3-bromophenylalanine, PyBOP, HOAt, DIPEA, DMF, rt, 90 min; (b) TFA, DCM, 1 × 5 min, 1 × rt, 20 min; (c) *N*-BOC-(L)-alanine, PyBOP, HOAt, DIPEA, DMF, rt, 90 min; (d) repeated deprotection and amino acid coupling; (e) Ac_2O, DIPEA, DMF; (f) $Pd(OAc)_2$, dppf, dioxane, CsF, H_2O, 90 °C, 16 h; (g) pentamethylbenzene, TFA, HBr, rt, 2 h.

macrocycle **30** was presumed to be complete in a comparable reaction time. The product was cleaved from the resin using a TFA/HBr mixture and purified by HPLC to yield the desired macrocycle **31** in 12.5% overall yield, based upon the theoretical maximum resin loading.

We were pleased to confirm that the macrocyclization reaction could be accomplished on an SPS-substrate, as was demonstrated by Planas and colleagues.[17] As illustrated, bromoaryl as well as iodoaryl systems also underwent macrocyclization. Consequently, having confirmed that the approach was viable, we adopted SPS-synthesis as our standard strategy for constructing these biaryl-peptide macrocycles. Since we had previously demonstrated the ability to remove the chloro-substituent by hydrogenolysis, no further chemistry was conducted on the macrocycle product **31**. To demonstrate that this SPS−Suzuki−Miyaura macrocyclization strategy was compatible with other conformational constraint elements, we prepared a series of macrocyclic peptides, **32**−**35**, bearing additional methyl substituents at *i* + 4, *i* + 3, *i* + 2, and *i* + 1 positions, respectively, as shown in Table 1.

Macrocycles **32**−**34** were accessible via introduction of Boc-protected aminoisobutyric acid (Aib) units at the appropriate point in the sequence depicted in Scheme 2. The syntheses of

Table 1. Examples of *m,m*-Bridged Biaryl Macrocyclic Pentapeptides



	X	R^1	R^2	R^3	R^4
32	Cl	Me	H	H	H
33	Cl	H	Me	H	H
34	Cl	H	H	Me	H
35	H	H	H	H	Me

macrocyclic peptides **33** and **34** containing an additional α-methyl group at the *i* + 2 and *i* + 1 positions, respectively, yielded two isomeric products in each case, which were separable by HPLC. To determine whether these isomer pairs were diastereoisomers (resulting from epimerization of a stereocenter during the synthesis) or atropisomers (resulting from conformational constriction and therefore inability to undergo conformational exchange at room temperature), each product was subjected to a variable temperature NMR study. Thus, NMR spectra were obtained for each isomer in both pairs (**33a** and **33b**, and **34a** and **34b**) in d_6-DMSO at 400 MHz, over the temperature range from 30 to 110 °C, in 20 °C increments. A final spectrum was obtained after the temperature had returned to 30 °C. In all cases, the final spectrum at 30 °C, after heating, was identical to the original spectrum at 30 °C, before heating, indicating that there had been no interconversion between isomers within each pair. Since it seems unlikely that atropisomers would be resistant to interconversion at 110 °C, we concluded that the isomer pairs are diastereoisomers, resulting from an epimerization during one of the coupling steps in the solid-phase synthesis.

To prepare macrocycle **35**, featuring a novel, quaternary amino acid which placed an additional methyl group at the α-carbon of the *i* position in the peptide, it was necessary to generate the novel boronic acid **36**, which was prepared via the route shown in Scheme 3. This sequence is based upon an established method for constructing homochiral, quaternary amino acids,[18] which utilized a suitably protected (*S*)-alanine derivative **37**, from which the homochiral oxazolidinone **38** can be prepared. We adapted this approach by alkylating **38** to yield the 3-iodobenzyl substituted system **39**, which could be cleaved with potassium trimethylsilanolate,[19] to yield the quaternary amino acid **40**, with the correct stereochemical configuration. Amino acid **40** was then converted to amido methyl ester **41**, which was subjected to a Miyaura borylation to yield **42**. Hydrolysis of **42** delivered the requisite boronic acid **36**, which was used directly in the peptide synthesis.

We did not prepare the final potential member of the series, which would possess an α-methyl group at the *i* + 4 position of the peptide, but based upon the results with other members of the series, we are confident that this would be accessible if required.

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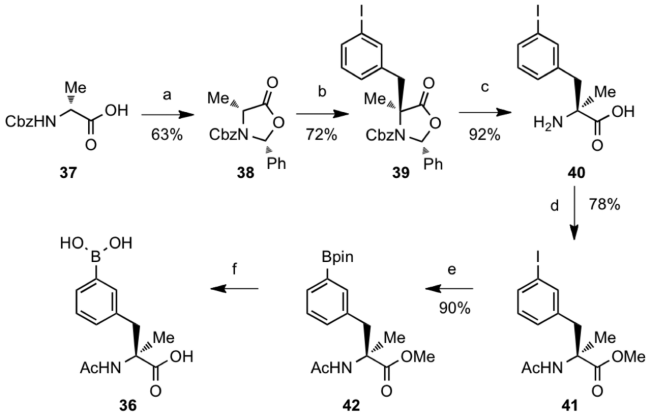
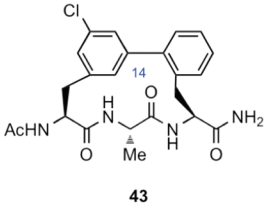
Scheme 3. Synthesis of Quaternary Amino Acid 36[a]

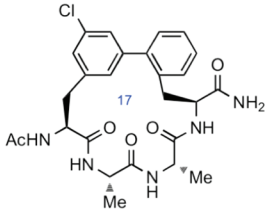


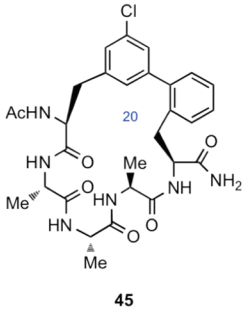
[a]Reaction conditions: (a) (dimethoxymethyl)benzene, $ZnCl_2$, $SOCl_2$, THF, 0 °C, 4 h; (b) 3-iodo-benzyl bromide, LiHMDS, THF, −30 °C, 1 h; (c) $KOSiMe_3$, THF, 75 °C, 2.5 h; (d) $SOCl_2$, MeOH, 0−25 °C, 2.5 h, then Ac_2O, DIPEA, DMAP, DMF, 0−25 °C, 12 h; MeONa, MeOH, reflux, 3 h; (e) B_2pin_2, $Pd(dppf)Cl_2·CH_2Cl_2$, KOAc, degassed DMSO, 85 °C, 6 h; (f) $LiOH·H_2O$ (aq), MeOH, rt, 12 h; product used directly in next step.

3. Solid-Phase Synthesis of *m,o*-Biaryl-Bridged Macrocyclic Pentapeptides Using a Boc-Protection Strategy.

An analogous synthetic strategy was initially adopted for synthesis of the second series of macrocyclic peptides containing a *meta,ortho*-configuration at the biaryl bridge. The synthetic approach described in Scheme 2 was modified accordingly, by loading the resin with *N*-Boc-(L)-2-iodophenylalanine. This permitted the synthesis of the biaryl bridged macrocyclic tri-, tetra-, and pentapeptides **43**, **44**, and **45**, respectively. By virtue of the *m,o*-configuration, these systems possess a macrocyclic ring which is one atom smaller than the *m,m*-series, which represents a significant increase in strain for the 14-membered macrocyclic tripeptide **43**. Furthermore, the macrocyclization reaction entails a more sterically hindered coupling reaction. Nevertheless, it is still apparently possible to close these macrocycles using this SPS−Suzuki−Miyaura methodology. Compound **44** was not isolated, but the C-terminal carboxylic acid was isolated instead, presumably due to an unexpected hydrolysis during the cleavage step or during the acetylation procedure.





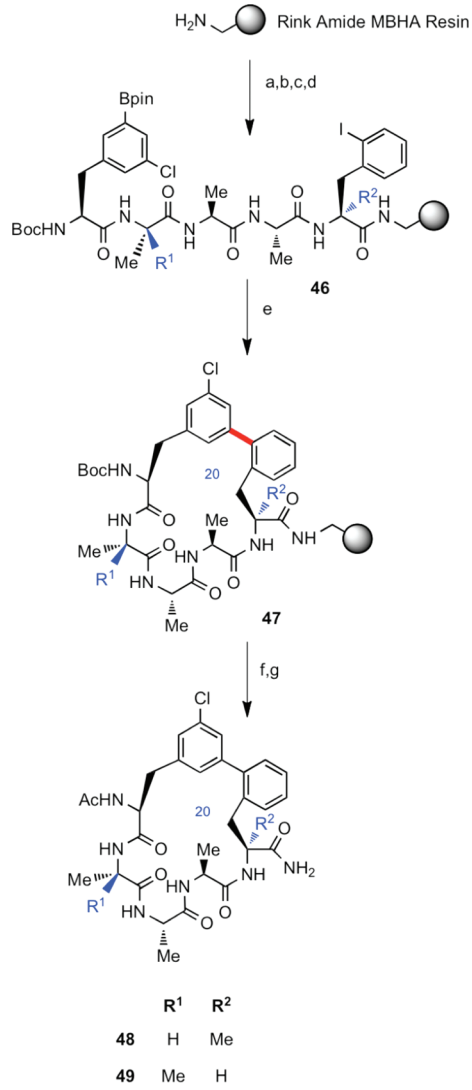


As with the *m,m*-bridged series, we wanted to establish whether it was also possible to incorporate substituents at the α-position of selected amino acid units, in order to further constrain peptide conformation. However, this route failed to deliver any products when α-methyl substituents were incorporated at the *i* and *i* + 4 positions. At this juncture, we were uncertain whether the principal issue was an inability to close the macrocyclic ring, or failure to cleave the product from the resin under the harsh conditions employed. To better understand this issue, we decided to adopt an Fmoc-based SPS-strategy instead, since this offered a much milder resin cleavage regime.

4. Solid-Phase Synthesis of *m,o*-Biaryl-Bridged Macrocyclic Pentapeptides Using an Fmoc-Protection Strategy.

The revised synthetic sequence is illustrated in Scheme 4.

Scheme 4. Solid-Phase Synthesis of *m,o*-Bridged Biaryl Macrocyclic Peptides Using an Fmoc-Protection Strategy[a]



[a]Reaction conditions: (a) *N*-Fmoc-(L)-2-iodophenylalanine, PyBOP, HOAt, DIPEA, DMF, rt, 90 min; (b) piperidine, DMF, rt, 2 × 10 min; (c) *N*-Fmoc-(L)-alanine, PyBOP, HOAt, DIPEA, DMF, rt, 90 min; (d) repeated deprotection and amino acid coupling; (e) $Pd(OAc)_2$, dppf, dioxane, CsF, H_2O, 90 °C, 16 h; (f) TFA/H_2O (95:5), rt, 3 h; (g) AcOH, PyBOP, HOAt, DIPEA, DMF, rt, 3 h.

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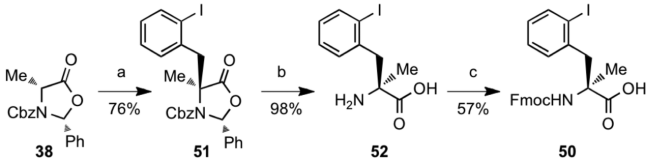
Rink amide MBHA resin was selected once again to provide the C-terminal amide directly upon final peptide cleavage. The resin was loaded with *N*-Fmoc-(L)-2-iodophenylalanine and the peptide chain built using repetitive deprotection/peptide coupling steps. The Boc-protected amino acid **8** was used as an N-terminal residue since it was readily available. However, we recognized that this complicated the closing stages of each synthesis because it was no longer possible to selectively deprotect the peptide N-terminus in order to add an acetyl group while the peptide was still bound to the resin. Therefore, following Suzuki–Miyaura macrocyclization of the precursor **46** to yield the resin-bound macrocycle **47**, cleavage was effected with TFA to yield a product with a free N-terminus which was then acetylated in solution to yield the target macrocycles. Using this approach, it was possible to generate both desired macrocycles, **48** and **49**, bearing an α-methyl substituent at the $i + 4$ and $i + 1$ positions, respectively, which were purified by HPLC.

To prepare macrocycle **48**, featuring a novel quaternary amino acid which places an additional methyl group at the α-carbon of the $(i + 5)$-position in the peptide, it was necessary to generate the novel Fmoc-protected boronic acid derivative **50**, which was prepared via the route shown in Scheme 5. Thus,

Scheme 5. Synthesis of Quaternary Amino Acid 50[a]



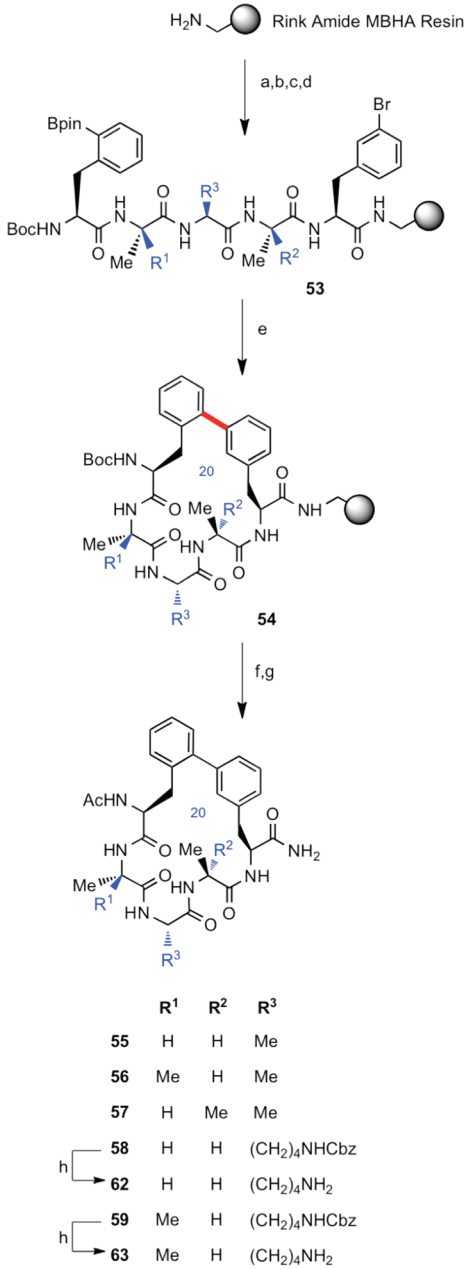
[a]Reaction conditions: (a) 2-iodo-benzyl bromide, LiHMDS, THF, −30 °C, 1 h, then rt, 3 h; (b) $KOSiMe_3$, THF, 75 °C, 2.5 h; (c) TMSCl, DCM, 60 °C, 6 h, then FmocCl, DIPEA, 0–25 °C, 30 h.

using the previously described homochiral oxazolidinone **38**,[18] alkylation with 2-iodobenzyl bromide to afford quaternary substituted oxazolidinone **51**, followed by hydrolysis,[19] yielded the parent amino acid **52**. Fmoc protection of **52** then provided the requisite quaternary amino acid **50** for incorporation into the solid-phase synthesis.

Thus, across these two series of *m,m*-bridged and *o,m*-bridged systems, we have shown it is possible to incorporate additional α-substituents at every position along the macrocyclic peptide chain.

5. Solid-Phase Synthesis of *o,m*-Biaryl-Bridged Macrocyclic Pentapeptides Using an Fmoc-Protection Strategy. Having determined that an Fmoc-protection SPS-strategy offered the most effective approach to construction of these biaryl-bridged macrocyclic peptides, we adopted this approach for the final series of *o,m*-bridged systems we had designed. The synthesis is outlined in Scheme 6 and differs from earlier series in the use of an *o*-borylated phenylalanine derivative at the N-terminal position of the chain. It was found that a further improvement could be made in the synthesis by conducting the intramolecular Suzuki–Miyaura coupling of the resin-bound peptide **53** under microwave conditions to yield resin-bound macrocycle **54**. The benefits of conducting the Suzuki–Miyaura coupling under microwave conditions was also highlighted by Planas and colleagues.[17] Macrocycle **54** could be cleaved from the resin and acetylated to yield the desired macrocyclic product **55**, which was purified by HPLC. This general strategy

Scheme 6. Solid-Phase Synthesis of *o,m*-Bridged Biaryl Macrocyclic Peptides Using an Fmoc-Protection Strategy[a]



[a]Reaction conditions: (a) *N*-Fmoc-(L)-3-bromophenylalanine, PyBOP, HOAt, DIPEA, DMF, rt, 90 min; (b) piperidine, DMF, rt, 2 × 10 min; (c) *N*-Fmoc-amino acid, PyBOP, HOAt, DIPEA, DMF, rt, 90 min; (d) repeated deprotection and amino acid coupling; (e) $Pd(PPh_3)_4$, K_2CO_3 (aq), DME, 140 °C, 20 min; (f) TFA/H_2O (95:5), rt, 3 h; (g) Ac_2O, DIPEA, DMF, rt, 3 h; (h) Pd/C (10 mol%), DMF, rt, 24 h.

could be used to prepare the related pentapeptides **56–59**, tripeptide **60**, and tetrapeptide **61.** As shown in Scheme 6, the Cbz-protected pentapeptides **58** and **59** could be further deprotected via hydrogenolysis to yield the lysine-containing pentapeptides **62** and **63**.

The key *o*-borylated phenylalanine derivative **64** could be prepared as shown in Scheme 7. Thus, esterification of *o*-bromophenylalanine **65** yielded fully protected system **66**, which was subjected to a Miyaura-borylation,[20] to yield the

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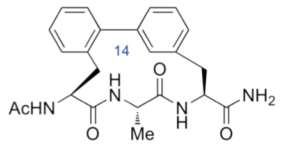
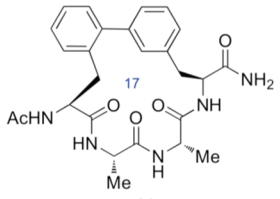
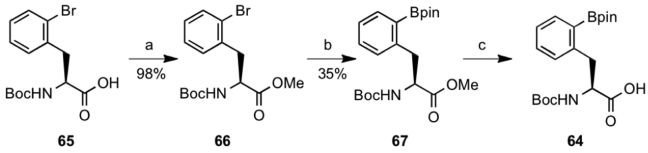




borylated derivative **67**. Ester hydrolysis yielded derivative **64**, which was used directly in the solid-phase peptide synthesis.

Scheme 7. Synthesis of *o*-Borylated Amino Acid 64[a]



[a]Reaction conditions: (a) MeI, $NaHCO_3$, DMF, rt, 12 h; (b) $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$, B_2pin_2, KOAc, degassed dioxane, 85 °C, 3 h; (c) $LiOH{\cdot}H_2O$ (aq), MeOH, rt, 50 min; product used directly in next step.

The borylation step was slow and required recharging several times with additional aliquots of catalyst in order to drive the reaction to completion, presumably because of the hindrance from the adjacent *ortho*-substituent.

6. Spectroscopic Analysis of Macrocyclic Peptides. Although our principal objective was to be able to constrain peptides via side chain to side chain bridges that were an integral component of the bioactive pharmacophore, rather than to investigate the stabilization of specific secondary structural motifs, we examined the macrocyclic systems described above for any evidence of any secondary structure. We looked initially by circular dichroism (CD). Measurements were taken in buffered aqueous solution at approximately 100 μM concentration, and in most cases, the resultant spectra were unlike those expected for turn, sheet, or helical conformations.[21] However, NMR experiments performed later suggested the presence of aggregated forms of the peptides, which can interfere with CD measurements. In the cases of peptides **56** and **57**, although the spectra did not match an ideal helical profile, they did possess maxima and minima in the appropriate regions of the spectra. We therefore examined their conformations more closely by ^{1}H NMR. This, together with their physical form in aqueous buffer, further supported the presence of aggregated species. More soluble analogues of *o*,*m*-bridged pentapeptides, **62** and **63**, containing a lysine residue at the i + 3 position were therefore prepared (Scheme 6). These peptides did indeed show enhanced solubility, but again appeared to aggregate. This phenomenon could be a general property of these amphiphilic macrocyclic biaryl-bridged systems, which possess both a polar, peptidic face and a lipophilic biaryl face. CD measurements in aqueous buffer represent a stringent test for the presence of secondary structural motifs, such as helices. It is possible that measurements in nonaqueous systems would increase the likelihood of observing secondary structure. This will be examined in future studies.

■ CONCLUSION

Our studies demonstrate that biaryl-bridged macrocyclic peptides can be generated with a range of biaryl configurations and macrocyclic ring sizes, via both solution-phase and solid-phase approaches, using a Suzuki–Miyaura cross-coupling methodology. In addition to constructing biaryl-bridged macrocycles with the *m*,*m*-configuration commonly found in natural products, we have shown that *m*,*o*- and *o*,*m*-systems are accessible via this approach. These complement the *p*,*p*- and *m*,*p*-systems described recently by Planas et al.,[17] and suggest that the remaining biaryl configurations are likely to be accessible also, providing that ring strain in the product is not excessive. We have also shown that it is possible to construct biaryl-bridged macrocyclic peptides that incorporate additional elements of steric constraint, such as α-methyl-substituted amino acids. We have provided examples where such substituents are featured at each of the possible positions in a pentapeptide chain.

Although we explored both solution-phase and solid-phase approaches (with two different protection regimens), we eventually concluded that a solid-phase approach, using an Fmoc-protection strategy, represented the most practical method of constructing these macrocyclic peptides. However, our initial studies of the key Suzuki–Miyaura macrocyclization in solution provided a straightforward means for us to directly monitor reaction outcome across a panel of diverse reaction conditions. It therefore constitutes a good initial strategy for future studies of this type, where reaction optimization is likely to be necessary but direct monitoring methods for solid-phase supported substrates/products are limited. The stepwise solution-phase approach also allowed us to determine that the combined yields for the three steps up to and including the key Suzuki–Miyaura macrocyclization in the *m*,*m*-biaryl series were in the 40–67% range. The best overall yields we were able to achieve with the solid-phase approach were with the lysine-derived pentapeptides **62** (48%) and **63** (50%) in the *o*,*m*-biaryl series. These represent averages of 95% per step over the 15-step sequence. Assuming ~99% efficiency for the 14 other steps, this would also imply a yield for the solid-phase Suzuki–Miyaura cross-coupling of ~58%, which is consistent with the solution-phase studies. Most of the other examples gave much lower isolated yields, even though crude HPLC traces indicated a single major product. We attribute this difference to the more challenging physical properties of these nonbasic systems, resulting in material loss during HPLC purification through, for example, adherence to surfaces. This is also consistent with the increased solubility of the lysine-derived macrocyclic peptides in aqueous buffer in comparison with the low solubility and tendency to aggregation observed in many other examples.

It appears from CD analysis that the biaryl-bridged macrocyclic peptides adopt distinct conformations in solution, rather than behaving as a random coil. However, it was not possible to recognize specific secondary structural motifs such as turns or helices. It did appear that these systems were prone to aggregation in aqueous solution, which might be a consequence of their amphiphilic nature. This tendency complicated interpretation of CD and NMR. Nevertheless, good solubility in aqueous buffer could be achieved via introduction of lysine residues.

The methodology we have established offers the prospect of constraining the conformations of biologically active peptides, which possess phenylalanine or tyrosine side chains within 1–3 residues of each other, via direct carbogenic fusion of their aromatic rings. Future studies will examine the structures and activities of such systems, created by embedding these macrocyclic motifs at relevant points within the biologically active peptide sequence.

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EXPERIMENTAL SECTION

General Procedures. All reactions were carried out under an argon atmosphere with dry solvent under anhydrous conditions, unless otherwise noted.

Solvents. Dry toluene, diethyl ether (Et_2O), and methylene chloride (CH_2Cl_2) were obtained by passing commercially available predried, oxygen-free formulations through activated alumina columns. Tetrahydrofuran was distilled from sodium. Anhydrous *N*,*N*-dimethyl formamide (DMF) and methanol (MeOH) were purchased in anhydrous form. Hexanes (HPLC grade), water (HPLC grade), *n*-heptane (HPLC grade), methanol (HPLC grade), SDA3A denatured ethanol (HPLC grade), formic acid 96.0%+ (reagent grade), and ammonium hydroxide (reagent grade) were used as supplied.

Chromatography. Column chromatography was performed using an automated flash chromatography system. Preparative thin layer chromatography was performed on precoated glass-backed plates (Whatman Partisil PK6F Silica Gel 60 Å 1000 μm) and visualized by ultraviolet radiation (λ = 254 nm). Analytical thin layer chromatography was performed on precoated glass-backed plates (Merck Kieselgel 60 F_{254}) and visualized by ultraviolet radiation (λ = 254 nm) or acidic potassium permanganate solutions as appropriate. Solvents for chromatography were used as supplied.

CD Measurements. Peptides were dissolved in a buffer of 25 mM Na_2HPO_4, pH 7, to a concentration of approximately 100 μM. Peptide and buffer blank solutions were placed in a 2 mm cell, and CD spectra were acquired over a range of 260−190 nm, with a 0.5 nm step size and a 3 s averaging time, and each spectrum is an average over 3 scans.

Peptide NMR Studies. NMR samples were prepared by dissolving peptides in 25 mM Na_2HPO_4, pH 5 (90% H_2O/10% D_2O). A small amount of DSS was added as an internal reference. Experiments were performed on a 500 MHz spectrometer at 298 K. For all peptides, a 1D proton spectrum was recorded with 4096 complex points over a sweep-width of 9 ppm and 128 scans. Selected peptides were further characterized by recording 2D TOCSY and ROESY spectra. TOCSY spectra were acquired with 4096 × 128 points, 16 scans per increment, and a 50 ms mixing time. ROESY spectra were acquired with 2048 × 128 points, 64 scans per increment, and 300 ms mixing time. Spectra were processed with NMRPipe,[21] or MestReNova. A 90° phased-shifted sine bell or squared sine bell window functions were applied in both dimensions, followed by zero-filling to twice the original size and Fourier transformation. Chemical shifts were referenced to the internal DSS standard at 0.00 ppm.

General Procedure A for Boc SPPS Chemistry. Peptides were prepared on 0.20 mmol scale by manual stepwise solid-phase peptide synthesis using PyBOP/HOAt/DIPEA activation on MBHA resin. Amino acid (4 equiv), PyBOP (4 equiv), HOAt (4 equiv), and DIPEA (8 equiv) in DMF (4 mL) were employed in each coupling step (90 min). Boc deprotections were achieved with TFA/CH_2Cl_2 (1:1, 4 mL) for 5 and 20 min. The peptide-resin was neutralized with TEA/CH_2Cl_2 (1:9, 4 mL) for 2 × 10 min. Capping of the resin was performed using Ac_2O (50 equiv) and DIPEA (50 equiv) in DMF (5 mL). Coupling yields were monitored by quantitative ninhydrin assay.

General Procedure B for Fmoc SPPS Chemistry. Peptides were typically prepared on 0.20 mmol scale by manual stepwise solid-phase peptide synthesis using PyBOP/HOAt/DIPEA activation on Rink Amide MBHA resin. Amino acid (4 equiv), PyBOP (4 equiv), HOAt (4 equiv), and DIPEA (5 equiv) in 4 mL of DMF were employed in each coupling step (90 min). For couplings using synthesized or expensive amino acids, only 1.5−2 eqiv of these reagents were used, with a correspondingly longer reaction time (4−16 h). Fmoc deprotections were achieved with piperidine/DMF (1:4, 4 mL) for 2 × 10 min. Coupling yields were monitored by quantitative ninhydrin assay.

General Procedure C1 for Suzuki Coupling. A vial fitted with a magnetic stirring bar was charged with $Pd(OAc)_2$ (4.5 mg, 0.02 mmol), dppf (33 mg, 0.06 mmol), and degassed dioxane (2 mL). The suspension was heated to 60 °C for 10 min and then transferred to a 10 mL microwave tube containing the peptide-resin (0.20 mmol), CsF (3 M in H_2O, 0.20 mL, 0.60 mmol). and degassed dioxane (10 mL). The sealed microwave tube was stirred at 90 °C for 16 h. After the reaction, the resin was filtered, washed (3 × 5 mL *i*-PrOH, 5 × 5 mL DMF, 5 × 5 mL CH_2Cl_2). and dried.

General Procedure C2 for Suzuki Coupling. A microwave vial fitted with a magnetic stirrer bar was charged with the peptide resin (0.1−0.25 mmol), degassed DME (2 mL), degassed 2 M K_2CO_3 (0.5 mL), and $Pd(PPh_3)_4$ (5 mol %). The suspension was heated in a microwave to 140 °C for 10 min. A further 5 mol % $Pd(PPh_3)_4$ was added and the suspension heated to the same temperature for a further 10 min. The resin was filtered, washed (3 × 5 mL H_2O, 3 × 5 mL CH_2Cl_2), and dried.

General Procedure D for Cleavage from the Resin (Boc). The peptide-resin was placed in a round-bottom flask with a stirring bar. A solution of pentamethylbenzene (593 mg, 4.00 mmol), TFA (6.3 mL), and HBr (30% in AcOH, 0.37 mL) was added to the peptide-resin and stirred for 2 h at rt. The resin was removed by filtration and rinsed with TFA (2 × 2 mL). The filtrate was concentrated to about 0.5 mL and then added to cold MTBE (10 mL). The precipitated resin was centrifuged. The residue was washed with MTBE (10 mL) and centrifuged two more times. The crude peptide was submitted to HPLC purification.

General Procedure E for Cleavage from the Resin (Fmoc). The peptide-resin was placed in a round-bottom flask with a stirring bar. A solution of TFA/H_2O (95:5, 10.0 mL) was added to the peptide-resin and stirred for 3 h at rt. The resin was removed by filtration and rinsed with TFA (2 × 2 mL). The filtrate was concentrated to about 0.5 mL and then added to cold MTBE (10 mL). The precipitated resin was centrifuged. The residue was washed with MTBE (10 mL) and centrifuged two more times.

General Procedure F1 for Acetylation. The final peptides amino group was capped with AcOH (1.1 equiv), PyBOP (1.1 equiv), HOAt (1.1 equiv), and DIPEA (3 equiv) in DMF (6 mL). The crude reaction mixture was concentrated in vacuo and submitted to HPLC purification.

General Procedure F2 for Acetylation. The precipitated peptide was dissolved in DMF (1−2 mL) before addition of Ac_2O (1.5−3 equiv) and DIPEA (3−6 equiv). The reaction mixture was stirred at rt for 1−3 h before the reaction mixture was concentrated. In some cases, the resulting acetylated peptide could be partially purified by precipitation from cold Et_2O. The residue or precipitate was then submitted to HPLC purification.

General Procedures G1−6 for HPLC Purification. Compounds were screened against a standard HPLC screening panel which includes reverse phase and normal phase HPLC columns and then purified using DAD monitoring at 210−360 nm and mass spectrometer detection in APCI mode positive scanning from 175 to 900 Da, using one of the following methods.

*Method **G1***. Reverse phase conditions on a 150 mm × 21.2 mm 5 μm column with a gradient of 5−100% B over 8.5 min with a flow rate of 28 mL/min. Mobile phase A was 0.1% formic acid in water, and mobile phase B was 0.1% formic acid in methanol.

*Method **G2***. Normal phase conditions on a 250 mm × 21.2 mm 5 μm column with a gradient of 5−100% B over 8.5 min with a flow rate of 28 mL/min. Mobile phase A was *n*-heptane, and mobile phase B was ethanol.

*Method **G3***. Normal phase conditions on a 250 mm × 21.2 mm 5 μm silica column with a gradient of 5−100% B over 8.5 min with a flow rate of 28 mL/min. Mobile phase A was *n*-heptane, and mobile phase B was ethanol.

*Method **G4***. Normal phase conditions on a 21.2 mm × 250 mm 5 μm cellulose column with a gradient of 5−100% B over 8.5 min with a flow rate of 28 mL/min. Mobile phase A was *n*-heptane, and mobile phase B was ethanol.

*Method **G5:*** Reverse phase conditions on a 21.2 mm × 150 mm 5 μm pentafluorophenyl column with a gradient of 5−100% B over 8.5 min with a flow rate of 28 mL/min. Mobile phase A was 0.1% formic acid in water, and mobile phase B was 0.1% formic acid in methanol.

*Method **G6***. Reverse phase conditions on a 21.2 mm × 150 mm 5 μm C18 column with a gradient of 5−100% B over 8.5 min with a flow rate of 28 mL/min. Mobile phase A was 0.1% ammonium hydroxide

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in water, and mobile phase B was 0.1% ammonium hydroxide in methanol.

(*S*)-Methyl 2-((*tert*-Butoxycarbonyl)amino)-3-(3-chloro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-propanoate (7). A 500 mL round-bottomed flask fitted with a reflux condenser and magnetic stirring bar was charged with (*S*)-methyl 2-((*tert*-butoxycarbonyl)amino)-3-(3-chlorophenyl)propanoate (10.3 g, 32.7 mmol), bis(pinacolato)diboron (12.5 g, 49.0 mmol), $[Ir(OMe)COD]_2$ (0.217 g, 0.327 mmol), and 4,4′-di-*tert*-butyl-2,2′-bipyridine (dtbpy) (0.176 g, 0.654 mmol). Hexanes (163 mL) were added and the reaction was heated to reflux for 16 h. Subsequent removal of residual solvent in vacuo, the residue was purified by an automated system (FLASH 65iTM column; hexanes/EtOAc 95:5 to hexanes/EtOAc 80:20) leading to 3,5-isomer **7** (12.8 g, 29.1 mmol, 89%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.63 (s, 1H), 7.42 (s, 1H), 7.18 (s, 1H), 5.02 (d, J = 7.5 Hz, 1H), 4.58−4.59 (m, 1H), 3.71 (s, 3H), 3.12 (dd, J = 13.5, 5.3 Hz, 1H), 2.98 (dd, J = 13.5, 6.2 Hz, 1H), 1.41 (s, 9H), 1.32 (s, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.9, 154.8, 137.5, 133.9, 133.7, 133.0, 131.9, 84.0, 79.9, 54.3, 52.2, 37.7, 28.2, 24.8; HRMS (ESI) calcd for $C_{21}H_{32}BClNO_6$ 440.2006; found, 440.1998.

(*S*)-2-((*tert*-Butoxycarbonyl)amino)-3-(3-chloro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanoic Acid (8). To a stirred solution of (*S*)-methyl 2-((*tert*-butoxycarbonyl)-amino)-3-(3-chloro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-phenyl)propanoate (**7**) (141 mg, 0.3 mmol) in MeOH (3 mL) was added $LiOH·H_2O$ (84 mg, 2.00 mmol) in H_2O (2 mL) at rt. The mixture was stirred at the same temperature for 40 min. The reaction mixture was acidified with 1 M aqueous HCl to pH ∼ 2. The aqueous layer was extracted with EtOAc (3 × 50 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo providing crude acid **8** (138 mg). Because of the instability of this material, it was carried forward without further purification.

(*S*)-Methyl 2-Amino-3-(4-(benzyloxy)-3-iodophenyl)-propanoate Hydrochloride (10). 3-Iodo-L-tyrosine **9** (2.5 g, 8.14 mmol) was dissolved in water (2.5 mL) and 2 M NaOH (9 mL). $CuSO_4$ (1.02 g) was added and the resulting solution was warmed to 60 °C for 10 min. The reaction changed from blue to green during that time. The solution was cooled to rt and charged with MeOH (35 mL) followed by BnBr (1.16 mL, 9.77 mmol). The reaction was stirred for 12 h during which time the product precipitated as a white solid. The solid was filtered and washed sequentially with water (50 mL) and 1 M HCl (50 mL) then dried in vacuo, resulting in a tan powder (2.9 g, 6.71 mmol, 82%). This material was carried forward without further purification. To a cooled solution of MeOH (30 mL) was added dropwise $SOCl_2$ (4.63 mL, 63.4 mmol) followed by the addition of the HCl salt of H_2NTyr(3-I)(Bn)−OH (2.75 g, 6.34 mmol). The reaction mixture was warmed to rt and stirred for 2 h. The reaction mixture was concentrated in vacuo and washed with cold Et_2O (2 × 10 mL) providing the methyl ester **10** as a pure yellow powder (2.46 g, 5.50 mmol, 87%): ^{1}H NMR (500 MHz, CD_3OD) δ 7.71 (s, 1H), 7.49 (d, J = 7.5 Hz, 2H), 7.37 (t, J = 7.5 Hz, 2H), 7.30 (t, J = 7.2 Hz, 1H), 7.20 (d, J = 7.5 Hz, 1H), 6.99 (d, J = 8.3 Hz, 1H), 5.17 (s, 2H), 4.29−4.22 (m, 1H), 3.80 (s, 3H), 3.17 (dd, J = 14.4, 5.8 Hz, 1H), 3.07 (dd, J = 14.4, 7.3 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 170.1, 158.2, 141.3, 137.8, 131.8, 129.4, 128.8, 128.2, 114.3, 87.6, 72.0, 55.2, 54.0, 36.0; HRMS calcd for $C_{17}H_{19}INO_3$ 412.0404; found, 412.0396.

(*S*)-Methyl 3-(4-(Benzyloxy)-3-iodophenyl)-2-((*S*)-2-((*tert*-butoxycarbonyl)amino)propanamido)propanoate (11). (*S*)-Methyl 2-amino-3-(4-(benzyloxy)-3-iodophenyl)propanoate hydrochloride (**10**) (322 mg, 0.78 mmol) was suspended in CH_2Cl_2 (10 mL). To this suspension, PyBOP (530 mg, 1.02 mmol), NEt_3 (0.142 mL, 1.02 mmol), and Boc-Ala-OH (178 mg, 0.94 mmol) were added and the reaction mixture was stirred at rt for 3 h. The reaction mixture was poured into water (15 mL) and the aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo, the residue was purified by an automated system (Flash 40+S column; hexanes/EtOAc 91:9 to hexanes/EtOAc 0:100) leading to dipeptide **11** (396 mg, 0.68 mmol, 87%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53 (d, J = 2.1 Hz, 1H), 7.48 (d, J = 7.0 Hz, 2H), 7.42−7.37 (m, 2H), 7.32 (t, J = 7.3 Hz, 1H), 7.02 (dd, J = 8.3, 2.1 Hz, 1H), 6.76 (d, J = 8.3 Hz, 1H), 6.59 (br s, 1H), 5.12 (s, 2H), 4.91 (br s, 1H), 4.77 (dd, J = 13.1, 5.8 Hz, 1H), 4.18−4.09 (m, 1H), 3.72 (s, 3H), 3.08 (dd, J = 14.0, 5.8 Hz, 1H), 2.98 (dd, J = 14.0, 5.7 Hz, 1H), 1.44 (s, 9H), 1.33 (d, J = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.2, 171.4, 156.3, 140.3, 140.2, 136.4, 130.2, 130.1, 128.5, 127.8, 126.9, 112.5, 86.7, 70.8, 53.2, 52.3, 36.4, 28.2, 18.1; HRMS (ESI) calcd for $C_{25}H_{32}IN_2O_6$ 583.1300; found, 583.1300.

Boc-(Cyclo-*m*,*m*)-[(3-Cl)FAY]-CO_2Me (14). (*S*)-Methyl 3-(4-(benzyloxy)-3-iodophenyl)-2-((*S*)-2-((*tert*-butoxycarbonyl)amino)-propanamido)propanoate (**11**) (146 mg, 0.250 mmol) was dissolved in 4 M HCl in dioxane (8 mL, 32.0 mmol) and stirred at rt for 5 h. The suspension was concentrated in vacuo. The resultant hydrochloride salt (**12**) of the dipeptide was suspended in CH_2Cl_2 (12.0 mL). To this suspension, DIPEA (0.131 mL, 0.75 mmol), PyBOP (169 mg, 0.325 mmol), and (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-chloro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-propanoic acid (**8**) (138 mg, 0.325 mmol) were added and the reaction mixture was stirred at rt for 12 h. The reaction mixture was diluted with EtOAc (80 mL) and washed with 1 M aqueous HCl (2 × 30 mL), saturated aqueous $NaHCO_3$ (25 mL), and brine (40 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo to give tripeptide **13**. Because of the instability of this material upon purification, the crude product was submitted to the next reaction. In a 100 mL flask were the tripeptide **13**, Pd(dppf)Cl_2·CH_2Cl_2 (10.2 mg, 0.013 mmol), and CsF (1 M in H_2O, 1.5 mL, 1.5 mmol) in degassed dioxane (62.5 mL). The flask was sealed and heated to 90 °C for 18 h. The reaction mixture was diluted with EtOAc (150 mL) and washed with water (2 × 25 mL) and brine (2 × 25 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo, the residue was purified by an automated system (KP-Sil 25 g column; hexanes/EtOAc 80:20 to hexanes/EtOAc 0:100) leading to cyclic peptide **14** (96 mg, 0.151 mmol, 60%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.45 (s, 1H), 7.38−7.29 (m, 3H), 7.28−7.24 (m, 3H), 7.00 (s, 2H), 6.90 (s, 1H), 6.87 (d, J = 8.3 Hz, 1H), 6.78 (s, 1H), 6.69 (d, J = 8.3 Hz, 1H), 5.55 (d, J = 8.0 Hz, 1H), 4.93−4.82 (m, 3H), 4.76−4.73 (m, 1H), 4.52−4.48 (m, 1H), 3.80 (s, 3H), 3.19 (dd, J = 14.3, 7.2 Hz, 1H), 2.91 (d, J = 13.2 Hz, 1H), 2.80 (d, J = 13.2 Hz, 1H), 2.49 (dd, J = 14.3, 8.4 Hz, 1H), 1.48 (s, 9H), 1.36 (d, J = 6.9 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.8, 171.7, 170.3, 155.2, 154.0, 139.8, 137.7, 136.9, 132.7, 131.3, 129.7, 129.3, 129.1, 128.5, 128.4, 128.2, 128.0, 127.5, 126.6, 112.3, 79.8, 70.0, 54.5, 53.4, 52.6, 49.0, 37.6, 36.5, 28.3, 19.0; HRMS (ESI) calcd for $C_{34}H_{39}ClN_3O_7$ 636.2471; found, 636.2459.

Ac-(Cyclo-*m*,*m*)-[(3-Cl)FAY]-CO_2Me (15). (*m*,*m*)-Cyclo Boc-F(3-Cl)AY-CO_2Me **14** (50 mg, 0.079 mmol) was dissolved in 4 M HCl in dioxane (2 mL, 8.0 mmol) and stirred at rt for 3 h. The suspension was concentrated in vacuo. The hydrochloride salt of the tripeptide was suspended in DMF (2.0 mL). To this suspension, DIPEA (0.138 mL, 0.79 mmol) and Ac_2O (0.075 mL, 0.790 mmol) were added and the reaction mixture was stirred at rt for 12 h. The reaction mixture was diluted with EtOAc (50 mL)and washed with 1 M aqueous HCl (2 × 20 mL), saturated aqueous $NaHCO_3$ (2 × 20 mL), and brine (20 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was recrystallized leading to cyclic peptide **15** (38 mg, 0.066 mmol, 83%): ^{1}H NMR (600 MHz, d_6-DMSO) δ 9.01 (d, J = 9.3 Hz, 1H), 8.76 (d, J = 8.5 Hz, 1H), 7.63−7.60 (m, 2H), 7.43−7.34 (m, 5H), 7.30 (t, J = 7.0 Hz, 1H), 7.22 (s, 1H), 7.19 (d, J = 8.4 Hz, 1H), 7.09 (d, J = 8.4 Hz, 1H), 6.99 (s, 1H), 6.88 (s, 1H), 5.16 (d, J = 12.1 Hz, 1H), 5.08 (d, J = 12.1 Hz, 1H), 4.75−4.65 (m, 2H), 4.62 (t, J = 9.9 Hz, 1H), 3.71 (s, 3H), 3.09 (d, J = 14.8 Hz, 1H), 2.99 (d, J = 4.2 Hz, 2H), 2.91 (dd, J = 14.8, 10.8 Hz, 1H), 1.89 (s, 3H), 1.24 (d, J = 7.0 Hz, 3H); ^{13}C NMR (150 MHz, d_6-DMSO) δ 172.3, 171.5, 168.8, 153.6, 139.1, 138.9, 136.9, 131.3, 131.3, 129.9, 129.4, 129.2, 128.2, 128.0, 127.9, 127.5, 127.1, 127.0, 112.5, 69.4, 52.3, 52.3, 52.2, 47.4, 37.4, 35.1, 22.4, 18.6; HRMS (ESI) calcd for $C_{31}H_{33}ClN_3O_6$ 578.2052; found, 578.2060.

Ac-(Cyclo-*m*,*m*)-[FAY]-NH_2 (16). To a suspension of palladium hydroxide on carbon (16.8 mg, 20 wt %, 0.024 mmol) in MeOH (4 mL), (*m*,*m*)-cyclo Ac-F(3-Cl)AY-CO_2Me **15** (69 mg, 0.119 mmol) and NH_4OH (30% in H_2O, 0.310 mL, 2.39 mmol) were added. The reaction mixture was flushed with hydrogen gas and the reaction

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mixture was stirred under hydrogen for 12 h at 40 °C. The crude reaction mixture was filtered through a short plug of Celite (MeOH) and concentrated in vacuo. Carrying this material forward without further purification, the newly formed intermediate was dissolved in THF (1.8 mL), MeOH (0.37 mL), and H_2O (0.18 mL). LiOH (57 mg, 2.38 mmol) was added and the reaction mixture was stirred at rt for 5 h. The reaction mixture was acidified with 1 M aqueous HCl to pH ∼ 2. The aqueous layer was extracted with EtOAc (5 × 15 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo. Carrying this material forward without further purification, the newly formed intermediate was dissolved in DMF (1 mL) and CH_2Cl_2 (5 mL). To this solution, PyBOP (93 mg, 0.179 mmol) was added. After ammonia gas was bubbled through the solution for 5 min, the reaction mixture was stirred at rt for 5 h. The reaction mixture was concentrated in vacuo and the residue was purified by an automated system (KP-Sil 10 g column; CH_2Cl_2/MeOH 95:5 to CH_2Cl_2/MeOH 80:20) leading to cyclic peptide **16** (35 mg, 0.08 mmol, 67%): ^{1}H NMR (600 MHz, d_6-DMSO) δ 9.35 (s, 1H), 8.66 (d, J = 9.0 Hz, 1H), 8.63 (d, J = 9.0 Hz, 1H), 7.56 (d, J = 7.6 Hz, 1H), 7.48 (d, J = 7.6 Hz, 1H), 7.39 (s, 1H), 7.21 (t, J = 7.6 Hz, 1H), 7.14−7.09 (m, 2H), 6.96−6.90 (m, 3H), 6.79 (d, J = 8.2 Hz, 1H), 4.78−4.71 (m, 1H), 4.69−4.64 (m, 1H), 4.47 (dt, J = 8.9, 3.5 Hz, 1H), 3.03 (dd, J = 13.7, 6.3 Hz, 1H), 2.96 (dd, J = 13.7, 2.7 Hz, 1H), 2.89−2.81 (m, 2H), 1.88 (s, 3H), 1.22 (d, J = 7.0 Hz, 3H); ^{13}C NMR (150 MHz, d_6-DMSO) δ 173.1, 171.8, 168.9, 168.6, 152.6, 138.2, 136.5, 130.0, 129.9, 129.3, 129.0, 127.7, 127.2, 127.1, 126.7, 115.1, 53.4, 52.6, 47.4, 37.6, 36.5, 22.5, 19.0; HRMS (ESI) calcd for $C_{23}H_{27}N_4O_5$ 439.1976; found, 439.1994.

(6*S*,9*S*,12*S*)-Methyl 12-(4-(Benzyloxy)-3-iodobenzyl)-2,2,6,9-tetramethyl-4,7,10-trioxo-3-oxa-5,8,11-triazatridecan-13-oate (17). A solution of (*S*)-methyl 3-(4-(benzyloxy)-3-iodophenyl)-2-((*S*)-2-((*tert*-butoxycarbonyl)amino)propanamido)propa-noate (**11**) (14.0 g, 24.0 mmol) in ethyl acetate (100 mL) at 0 °C was treated with 4 M HCl in ethyl acetate (100 mL, 400 mmol) and stirred at rt for 5 h. The suspension was concentrated in vacuo to yield the hydrochloride salt **12** of the dipeptide. To a solution of Boc-Ala-OH (5.46 g, 28.9 mmol) and DIPEA (12.6 mL, 72.2 mmol) in DMF (70 mL) at 0 °C was added EDCI (6.90 g, 36.0 mmol) and HOBt (4.87 g, 36.0 mmol). The mixture was then stirred at 0 °C for 1 h, whereupon the hydrochloride salt in DMF (30 mL) was added. The mixture was warmed to rt and stirred for 16 h. The mixture was concentrated under reduced pressure to give the crude product which was purified via flash chromatography (silica gel, petroleum ether/EtOAc (83:17 to 50:50)) leading to tripeptide **17** (9.0 g, 14 mmol, 58%): ^{1}H NMR (600 MHz, CD_3OD) δ 7.62 (d, J = 1.7 Hz, 1H), 7.49 (d, J = 7.3 Hz, 2H), 7.36 (t, J = 7.6 Hz, 2H), 7.29 (t, J = 7.3 Hz, 1H), 7.17−7.12 (m, 1H), 6.91 (d, J = 8.4 Hz, 1H), 5.12 (s, 2H), 4.58 (dd, J = 8.0, 6.1 Hz, 1H), 4.37−4.30 (m, 1H), 4.11−4.01 (m, 1H), 3.67 (s, 3H), 3.05 (dd, J = 14.0, 6.1 Hz, 1H), 2.92 (dd, J = 14.0, 8.0 Hz, 1H), 1.43 (s, 9H), 1.31 (d, J = 7.1 Hz, 3H), 1.27 (d, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, CD_3OD) δ 175.4, 174.7, 173.0, 157.7, 141.2, 138.2, 132.4, 131.5, 129.5, 128.8, 128.2, 113.8, 87.1, 80.6, 71.8, 55.2, 52.7, 51.4, 50.1, 36.9, 28.7, 18.3; HRMS (ESI) calcd for $C_{28}H_{37}IN_3O_7$ 654.1671; found, 654.1680.

Boc-(Cyclo-*m*,*m*)-[(3-Cl)FAAY]-CO_2Me (20). (6*S*,9*S*,12*S*)-Methyl 12-(4-(benzyloxy)-3-iodobenzyl)-2,2,6,9-tetramethyl-4,7,10-trioxo-3-oxa-5,8,11-triazatridecan-13-oate (**17**) (163 mg, 0.250 mmol) was dissolved in 4 M HCl in dioxane (8 mL, 32.0 mmol) and stirred at rt for 5 h. The suspension was concentrated in vacuo. The hydrochloride salt (**18**) of the tripeptide was suspended in DMF (12.0 mL). To this suspension, DIPEA (0.131 mL, 0.75 mmol), PyBOP (169 mg, 0.325 mmol), and (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-chloro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanoic acid (**8**) (138 mg, 0.325 mmol) were added and the reaction mixture was stirred at rt for 12 h. The reaction mixture was diluted with EtOAc (80 mL) and washed with 1 M aqueous HCl (2 × 30 mL), saturated aqueous $NaHCO_3$ (25 mL), and brine (40 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo to give tetrapeptide **19**. Because of the instability of this material upon purification, the crude product was submitted to the next reaction. In a 100 mL flask were the tetrapeptide **19**, Pd(dppf)Cl_2·CH_2Cl_2 (10.2 mg, 0.013 mmol), and CsF (1 M in H_2O, 1.5 mL, 1.5 mmol) in degassed dioxane (62.5 mL). The flask was sealed and heated to 90 °C for 18 h. The reaction mixture was diluted with EtOAc (150 mL) and washed with water (2 × 25 mL) and brine (2 × 25 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo, the residue was purified by an automated system (KP-C-18-HS 12 g column; H_2O/MeCN 100:0 to H_2O/MeCN 0:100) leading to cyclic peptide **20** (91 mg, 0.129 mmol, 51%): ^{1}H NMR (600 MHz, CD_3CN) δ 7.60 (s, 1H), 7.41−7.35 (m, 4H), 7.35−7.29 (m, 3H), 7.16 (s, 1H), 7.10−7.01 (m, 3H), 6.78 (d, J = 3.8 Hz, 2H), 5.52 (d, J = 6.1 Hz, 1H), 5.08 (d, J = 11.6 Hz, 1H), 5.05 (d, J = 11.6 Hz, 1H), 4.96−4.88 (m, 1H), 4.34−4.28 (m, 1H), 4.25−4.18 (m, 1H), 4.15−4.08 (m, 1H), 3.72 (s, 3H), 3.19−3.09 (m, 2H), 3.03−2.92 (m, 2H), 1.46 (s, 9H), 1.23 (d, J = 7.2 Hz, 3H), 1.18 (d, J = 7.0 Hz, 3H); ^{13}C NMR (150 MHz, CD_3CN) δ 173.0, 172.6, 171.3, 155.7, 155.1, 140.8, 139.8, 138.0, 133.1, 132.2, 131.1, 130.6, 129.9, 129.6, 129.3, 129.2, 129.1, 128.7, 128.5, 128.3, 113.8, 80.0, 70.9, 55.3, 52.8, 52.7, 50.0, 49.6, 38.9, 36.9, 28.5, 18.0, 17.5; HRMS (ESI) calcd for $C_{37}H_{44}ClN_4O_8$ 707.2842; found, 707.2833.

Ac-(Cyclo-*m*,*m*)-[(3-Cl)FAAY]-CO_2Me (21). (*m*,*m*)-Cyclo Boc-F(3-Cl)AAY-CO_2Me (**20**) (194 mg, 0.274 mmol) was dissolved in 4 M HCl in dioxane (4 mL, 16.0 mmol) and stirred at rt for 3 h. The suspension was concentrated in vacuo. The hydrochloride salt of the tetrapeptide was suspended in DMF (5 mL). To this suspension, DIPEA (0.479 mL, 2.74 mmol) and Ac_2O (0.259 mL, 2.74 mmol) were added and the reaction mixture was stirred at rt for 12 h. The reaction mixture was diluted with EtOAc (100 mL), and washed with 1 M aqueous HCl (2 × 20 mL), saturated aqueous $NaHCO_3$ (2 × 20 mL), and brine (20 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo and the residue was purified by an automated system (KP-Sil 10 g column; CH_2Cl_2/MeOH 97:3 to CH_2Cl_2/MeOH 90:10) leading to cyclic peptide **21** (111 mg, 0.171 mmol, 62%). ^{1}H NMR (600 MHz, CD_3OD) δ 7.51 (s, 1H), 7.43 (d, J = 2.0 Hz, 1H), 7.41 (s, 1H), 7.32−7.26 (m, 4H), 7.25−7.21 (m, 1H), 7.13 (s, 1H), 7.04 (dd, J = 8.4, 2.0 Hz, 1H), 7.00 (d, J = 8.4 Hz, 1H), 5.02 (d, J = 11.9 Hz, 1H), 4.99 (d, J = 11.9 Hz, 1H), 4.91 (dd, J = 9.1, 3.8 Hz, 1H), 4.61 (dd, J = 8.5, 2.5 Hz, 1H), 4.17 (q, J = 7.3 Hz, 1H), 4.10 (q, J = 7.0 Hz, 1H), 3.74 (s, 3H), 3.20−3.12 (m, 2H), 3.02−2.92 (m, 2H), 1.99 (s, 3H), 1.24 (d, J = 7.3 Hz, 3H), 1.22 (d, J = 7.0 Hz, 3H); ^{13}C NMR (150 MHz, CD_3OD) δ 174.4, 174.0, 173.1, 172.7, 172.4, 155.8, 141.5, 139.7, 138.5, 133.8, 132.8, 131.1, 130.9, 130.7, 130.4, 129.8, 129.4, 129.2, 128.7, 128.3, 114.5, 71.6, 55.3, 53.6, 52.8, 50.6, 50.4, 39.2, 37.2, 22.5, 17.8, 17.6; HRMS (ESI) calcd for $C_{34}H_{38}ClN_4O_7$ 649.2423; found, 649.2435.

Ac-(Cyclo-*m*,*m*)-[FAAY]-NH_2 (22). To a suspension of palladium hydroxide on carbon (19.4 mg, 20 wt %, 0.027 mmol) in MeOH (2.7 mL), (*m*,*m*)-cyclo Ac-F(3-Cl)AAY-CO_2Me (**21**) (88 mg, 0.136 mmol) and NH_4OH (30% in H_2O, 0.352 mL, 2.71 mmol) were added. The reaction mixture was flushed with hydrogen gas and the reaction mixture was stirred under hydrogen for 12 h at 40 °C. The crude reaction mixture was filtered through a short plug of Celite (MeOH) and concentrated in vacuo. Carrying this material forward without further purification, the newly formed intermediate was dissolved in THF (1.3 mL), MeOH (0.26 mL), and H_2O (0.13 mL). LiOH (40 mg, 1.68 mmol) was added and the reaction mixture was stirred at rt for 5 h. The reaction mixture was acidified with 1 M aqueous HCl to pH ∼ 2. The aqueous layer was extracted with EtOAc (5 × 15 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo. Carrying this material forward without further purification, the newly formed intermediate was dissolved in DMF (0.7 mL) and CH_2Cl_2 (3.5 mL). To this solution, PyBOP (66 mg, 0.126 mmol) was added. After ammonia gas was bubbled through the solution for 5 min, the reaction mixture was stirred at rt for 5 h. The reaction mixture was concentrated in vacuo and the residue was purified by an automated system (KP-Sil 10 g column; CH_2Cl_2/MeOH 95:5 to CH_2Cl_2/MeOH 80:20) leading to cyclic peptide **22** (28 mg, 0.08 mmol, 40%). *Note concerning the NMR data of the following compound: Due to a mixture of conformers, the proton assignment of ^{1}H NMR data was carried out for the two major compounds (1:0.4 ratio) in this mixture. The ^{13}C NMR data represents a mixture of all conformers.* ^{1}H NMR (600 MHz, d_6-DMSO) δ 9.31 (s, 1H),

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9.24 (s, 0.4H), 8.55 (d, J = 7.9 Hz, 1H), 8.22 (d, J = 9.5 Hz, 1H), 8.10 (t, J = 7.4 Hz, 0.8H), 7.98 (d, J = 8.6 Hz, 0.4H), 7.64 (d, J = 7.6 Hz, 1H), 7.50−7.45 (m, 2.4H), 7.38 (d, J = 6.9 Hz, 1H), 7.32−7.21 (m, 2.2H), 7.16−7.09 (m, 3.8H), 7.08−7.03 (m, 0.8H), 6.97−6.93 (m, 1.4H), 6.92−6.88 (m, 1H), 6.82 (d, J = 8.3 Hz, 0.4H), 6.80 (d, J = 8.2 Hz, 1H), 4.90−4.83 (m, 1H), 4.65 (dt, J = 7.5, 1.8 Hz, 1H), 4.49−4.33 (m, 0.8H), 4.24−4.10 (m, 2.8H), 3.15−3.11 (m, 1H), 3.05 (dd, J = 13.1, 3.2 Hz, 0.4H), 2.98−2.89 (m, 2.4H), 2.85−2.77 (m, 1.4H), 2.73 (dd, J = 13.6, 10.6 Hz, 0.4H), 1.92 (s, 3H), 1.91 (s, 1.2H), 1.19 (d, J = 7.5 Hz, 3H), 1.14 (d, J = 6.8 Hz, 3H), 1.05 (d, J = 6.8 Hz, 1.2H), 0.95 (d, J = 6.9 Hz, 1.2H); ^{13}C NMR (150 MHz, d_6-DMSO) δ 173.1, 171.1, 170.7, 169.7, 169.4, 168.9, 168.8, 154.2, 152.4, 138.6, 138.4, 136.2, 136.1, 130.9, 130.1, 129.7, 129.4, 129.0, 128.9, 128.5, 128.0, 127.7, 127.6, 127.5, 127.5, 127.4, 127.2, 127.1, 126.7, 115.8, 115.7, 115.7, 62.7, 54.9, 54.1, 52.8, 50.5, 48.5, 48.4, 47.7, 47.5, 47.4, 38.4, 37.9, 36.9, 36.5, 22.6, 22.4, 18.8, 18.7, 18.2, 18.0; HRMS (ESI) calcd for $C_{26}H_{32}N_5O_6$ 510.2347; found, 510.2350.

(6*S*,9*S*,12*S*,15*S*)-Methyl 15-(4-(Benzyloxy)-3-iodobenzyl)-2,2,6,9,12-pentamethyl-4,7,10,13-tetraoxo-3-oxa-5,8,11,14-tetraazahexadecan-16-oate (23). (6*S*,9*S*,12*S*)-Methyl 12-(4-(benzyloxy)-3-iodobenzyl)-2,2,6,9-tetramethyl-4,7,10-trioxo-3-oxa-5,8,11-triazatridecan-13-oate (**17**) (9.0 g, 14 mmol) in ethyl acetate (35 mL) at 0 °C was treated with 4 M HCl in ethyl acetate (35 mL, 140 mmol) and stirred at rt for 5 h. The suspension was concentrated in vacuo to yield the hydrochloride salt **18** of the tripeptide. To a solution of Boc-Ala-OH (3.10 g, 16.4 mmol) and DIPEA (7.2 mL, 41.4 mmol) in DMF (70 mL) at 0 °C was added EDCI (3.96 g, 20.7 mmol) and HOBt (2.79 g, 21.5 mmol). The mixture was then stirred at 0 °C for 1 h, whereupon the hydrochloride salt in DMF (30 mL) was added. The mixture was warmed to rt and stirred for 16 h. The mixture was concentrated under reduced pressure to give the crude product which was purified via preparatory HPLC (250 × 50 mm, 10 μm column, mobile phase H_2O/CH_3CN (65:35 to 35:65) containing 0.1% ammonia, flow rate 80 mL/min, UV detection at 220 nm) leading to tetrapeptide **23** (5.5 g, 7.6 mmol, 55%): ^{1}H NMR (600 MHz, d_6-DMSO) δ 8.21 (d, J = 7.3 Hz, 1H), 7.90 (d, J = 7.5 Hz, 1H), 7.84 (d, J = 7.3 Hz, 1H), 7.63 (d, J = 1.9 Hz, 1H), 7.48 (d, J = 7.4 Hz, 2H), 7.40 (t, J = 7.6 Hz, 2H), 7.32 (t, J = 7.3 Hz, 1H), 7.19 (dd, J = 8.4, 1.9 Hz, 1H), 6.98 (dd, J = 7.7, 4.7 Hz, 2H), 5.15 (s, 2H), 4.42−4−38 (m, 1H), 4.31−4.21 (m, 2H), 3.98−3.89 (m, 1H), 3.57 (s, 3H), 2.94 (dd, J = 13.9, 5.7 Hz, 1H), 2.85 (dd, J = 13.9, 8.8 Hz, 1H), 1.37 (s, 9H), 1.17−1.14 (m, 9H); ^{13}C NMR (150 MHz, d_6-DMSO) δ 172.3, 172.1, 171.5, 171.5, 155.4, 155.0, 139.3, 136.6, 131.4, 130.2, 128.3, 127.6, 127.0, 112.6, 86.4, 77.9, 69.9, 53.5, 51.7, 49.5, 47.7, 47.7, 34.9, 28.1, 18.2, 18.1, 17.9; HRMS (ESI) calcd for $C_{31}H_{42}IN_4O_8$ 725.2042; found, 725.2022.

Boc-(Cyclo-*m*,*m*)-[(3-Cl)FAAAY]-CO_2Me (26). (6*S*,9*S*,12*S*,15*S*)-Methyl 15-(4-(benzyloxy)-3-iodobenzyl)-2,2,6,9,12-pentamethyl-4,7,10,13-tetraoxo-3-oxa-5,8,11,14-tetraazahexadecan-16-oate (**23**) (181 mg, 0.250 mmol) was dissolved in 4 M HCl in dioxane (4 mL, 16.0 mmol) and stirred at rt for 5 h. The suspension was concentrated in vacuo. The hydrochloride salt (**24**) of the tetrapeptide was suspended in DMF (12.0 mL). To this suspension, DIPEA (0.131 mL, 0.75 mmol), PyBOP (169 mg, 0.325 mmol), and (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-chloro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanoic acid (**8**) (138 mg, 0.325 mmol) were added and the reaction mixture was stirred at rt for 12 h. The reaction mixture was diluted with EtOAc (80 mL), and washed with 1 M aqueous HCl (2 × 30 mL), saturated aqueous $NaHCO_3$ (25 mL), and brine (40 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo to give tetrapeptide (**25**). Because of the instability of this material upon purification, the crude product was submitted to the next reaction. In a 100 mL flask were the pentapeptide (**25**), Pd(dppf)Cl_2·CH_2Cl_2 (10.2 mg, 0.013 mmol), and CsF (1 M in H_2O, 1.5 mL, 1.5 mmol) in degassed dioxane (62.5 mL). The flask was sealed and heated to 90 °C for 18 h. The reaction mixture was diluted with EtOAc (150 mL) and washed with water (2 × 25 mL) and brine (2 × 25 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo, the residue was purified by an automated system (KP-C-18-HS 12 g column; H_2O/MeCN 0:100 to H_2O/MeCN 0:100) leading to cyclic peptide **26** (66 mg, 0.129 mmol, 34%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.72 (d, J = 7.2 Hz, 1H), 7.66 (s, 1H), 7.38−7.31 (m, 5H), 7.31−7.27 (m, 2H), 7.17 (dd, J = 8.4, 1.9 Hz, 1H), 7.15−7.10 (m, 2H), 6.95 (s, 1H), 6.93 (d, J = 8.4 Hz, 1H), 6.84 (br s, 1H), 5.59 (br s, 1H), 5.10 (d, J = 12.0 Hz, 1H), 5.05 (d, J = 12.0 Hz, 1H), 4.64 (t, J = 9.6 Hz, 1H), 4.47−4.39 (m, 1H), 4.26−4.19 (m, 1H), 4.17−4.11 (m, 1H), 3.98−3.94 (m, 1H), 3.71 (s, 3H), 3.19 (d, J = 12.9 Hz, 1H), 3.09−3.03 (m, 1H), 3.02−2.83 (m, 2H), 1.48 (s, 9H), 1.44 (d, J = 7.3 Hz, 3H), 1.38 (d, J = 7.1 Hz, 3H), 1.09 (d, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.3, 173.1, 172.6, 172.3, 172.0, 156.2, 154.1, 139.3, 136.8, 136.2, 133.0, 132.1, 131.0, 130.1, 129.9, 129.0, 128.4, 127.7, 126.9, 126.8, 113.1, 81.6, 70.3, 58.4, 54.0, 52.4, 51.1, 49.0, 37.9, 37.2, 29.6, 28.2, 24.8, 17.5, 16.9; HRMS (ESI) calcd for $C_{40}H_{49}ClN_5O_9$ 778.3213; found, 778.3212.

Ac-(Cyclo-*m*,*m*)-[(3-Cl)FAAAY]-CO_2Me (27). (*m*,*m*)-Cyclo Boc-F(3-Cl)AAAY-CO_2Me (**26**) (120 mg, 0.154 mmol) was dissolved in 4 M HCl in dioxane (4 mL, 16.0 mmol) and stirred at rt for 3 h. The suspension was concentrated in vacuo. The hydrochloride salt of the pentapeptide was suspended in DMF (5 mL). To this suspension, DIPEA (0.269 mL, 1.54 mmol) and Ac_2O (0.145 mL, 1.54 mmol) were added and the reaction mixture was stirred at rt for 12 h. The reaction mixture was diluted with EtOAc (100 mL), and washed with 1 M aqueous HCl (2 × 20 mL), saturated aqueous $NaHCO_3$ (2 × 20 mL), and brine (20 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo and the residue was purified by an automated system (KP-Sil 10 g column; CH_2Cl_2/MeOH 97:3 to CH_2Cl_2/MeOH 80:20) leading to cyclic peptide **27** (72 mg, 0.100 mmol, 65%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.07 (d, J = 5.9 Hz, 1H), 7.82 (br s, 1H), 7.71 (br s, 1H), 7.55 (s, 1H), 7.47 (d, J = 8.7 Hz, 1H), 7.28−7.23 (m, 5H), 7.22−7.18 (m, 2H), 7.08−7.04 (m, 2H), 6.87 (s, 1H), 6.84 (d, J = 8.5 Hz, 1H), 5.02 (d, J = 12.2 Hz, 1H), 4.95 (d, J = 12.2 Hz, 1H), 4.47 (t, J = 10.1 Hz, 1H), 4.25−4.18 (m, 1H), 4.16−4.10 (m, 1H), 4.10−4.05 (m, 1H), 3.86 (d, J = 10.9 Hz, 1H), 3.59 (s, 3H), 3.11 (d, J = 13.3 Hz, 1H), 3.03−2.95 (m, 2H), 2.95−2.88 (m, 1H), 1.95 (s, 3H), 1.37 (d, J = 7.3 Hz, 3H), 1.35 (d, J = 7.2 Hz, 3H), 1.00 (d, J = 7.2 Hz, 3H),; ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.4, 174.1, 173.8, 173.6, 172.7, 172.0, 154.2, 138.7, 137.1, 136.7, 132.8, 132.1, 130.7, 129.8, 129.8, 129.4, 128.5, 128.4, 127.6, 127.1, 126.8, 113.1, 70.2, 58.4, 54.4, 52.7, 52.5, 51.3, 49.8, 37.4, 37.0, 29.6, 22.8, 16.8, 16.7; HRMS (ESI) calcd for $C_{37}H_{43}ClN_5O_8$ 720.2795; found, 720.2812.

Ac-(Cyclo-*m*,*m*)-[FAAAY]-NH_2 (28). To a suspension of palladium hydroxide on carbon (11.1 mg, 20 wt %, 0.016 mmol) in MeOH (2.9 mL), (*m*,*m*)-cyclo Ac-F(3-Cl)AAAY-CO_2Me (**27**) (57 mg, 0.079 mmol) and NH_4OH (30% in H_2O, 0.205 mL, 1.58 mmol) were added. The reaction mixture was flushed with hydrogen gas and the reaction mixture was stirred under hydrogen for 12 h at 40 °C. The crude reaction mixture was filtered through a short plug of Celite (MeOH) and concentrated in vacuo. Carrying this material forward without further purification, the newly formed intermediate was dissolved in THF (1.2 mL), MeOH (0.24 mL) and H_2O (0.12 mL). LiOH (38 mg, 1.58 mmol) was added and the reaction mixture was stirred at rt for 5 h. The reaction mixture was acidified with 1 M aqueous HCl to pH ∼ 2. The aqueous layer was extracted with EtOAc (5 × 15 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo. Carrying this material forward without further purification, the newly formed intermediate was dissolved in DMF (0.7 mL) and CH_2Cl_2 (3.3 mL). To this solution, PyBOP (62 mg, 0.119 mmol) was added. After ammonia gas was bubbled through the solution for 5 min, the reaction mixture was stirred at rt for 5 h. The reaction mixture was concentrated in vacuo and the residue was purified by an automated system (KP-Sil 10 g column; CH_2Cl_2/MeOH 95:5 to CH_2Cl_2/MeOH 70:30) leading to cyclic peptide **28** (25 mg, 0.043 mmol, 55%). *Note concerning the NMR data of the following compound: Due to a mixture of conformers, the proton assignment of ^{1}H NMR data was carried out for the two major compounds (1:0.2 ratio) in this mixture. The ^{13}C NMR data represents a mixture of all conformers.* ^{1}H NMR (600 MHz, d_6-DMSO) δ 9.32 (s, 1H), 9.29 (s, 0.2H), 8.55 (d, J = 7.6 Hz, 1H), 8.50 (d, J = 7.8 Hz, 0.2H), 8.48 (d, J = 7.7 Hz, 1H), 8.36 (d, J = 7.9 Hz, 0.2H), 8.30 (d, J = 7.3 Hz, 0.2H), 8.24 (d, J = 6.5 Hz, 1H), 7.98 (d, J = 8.0 Hz, 0.2H),

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7.85 (d, J = 8.2 Hz, 1H), 7.62 (d, J = 6.7 Hz, 0.2H), 7.58 (s, 1H), 7.49 (d, J = 7.7 Hz, 1H), 7.42 (d, J = 7.8 Hz, 0.2H), 7.41−7.35 (m, 1.4H), 7.31−7.26 (m, 2H), 7.26−7.20 (m, 1.2H), 7.20−7.17 (m, 1.2H), 7.15 (d, J = 7.7 Hz, 0.2H), 7.07 (s, 1.2H), 7.00−6.95 (m, 1.2H), 6.85−6.79 (m, J = 8.2 Hz, 1.2H), 4.42−4.36 (m, 1.2H), 4.27−4.16 (m, 4.4H), 4.16−4.08 (m, 1.4H), 3.18−3.04 (m, 1.2H), 2.93−2.85 (m, 1.6H), 2.85−2.79 (m, 2H), 1.87 (s, 3H), 1.85 (s, 0.6H), 1.22 (d, J = 7.4 Hz, 3H), 1.22−1.13 (m, 8.4H); ^{13}C NMR (150 MHz, d_6-DMSO) δ 172.9, 172.8, 172.4, 172.2, 171.9, 171.7, 171.5, 171.5, 170.9, 169.2, 169.1, 152.8, 138.6, 138.4, 131.6, 131.0, 130.2, 130.0, 129.3, 129.2, 128.4, 127.5, 127.5, 127.3, 127.2, 126.8, 126.7, 126.6, 125.5, 115.5, 54.5, 54.2, 54.2, 49.1, 48.5, 48.3, 48.2, 47.7, 47.4, 36.9, 36.5, 22.4, 18.8, 18.0, 17.3, 17.0, 16.9; HRMS (ESI) calcd for $C_{29}H_{37}N_6O_7$ 581.2781; found, 581.2708.

Ac-(Cyclo-*m*,*m*)-[(3-Cl)FAAAF]-NH$_2$ (31). The pentapeptide macrocyclization precursor was synthesized on solid support using general procedure A. It was subjected to Suzuki−Miyaura macrocyclization general procedure C1. The macrocyclic product was cleaved from the resin using general procedure D and acetylated using general procedure F1. The resultant peptide was purified by HPLC using general procedure G4 to yield the solid **31** (15.0 mg, 12.5% yield) as a single peak (purity >98%; see Supporting Information for pdf of HPLC trace): ^{1}H NMR (700 MHz, d_6-DMSO) δ 8.59 (d, J = 8.0 Hz, 1H), 8.50 (d, J = 8.0 Hz, 1H), 8.30 (d, J = 6.4 Hz, 1H), 7.93 (d, J = 8.0 Hz, 1H), 7.88 (s, 1H), 7.70 (s, 1H), 7.58−7.54 (m, 2H), 7.49−7.44 (m, 2H), 7.34 (t, J = 7.7 Hz, 1H), 7.29 (s, 1H), 7.20 (d, J = 7.5 Hz, 1H), 7.15 (s, 1H), 4.47−4.42 (m, 2H), 4.31−4.24 (m, 2H), 4.17−4.12 (m, 1H), 3.06 (d, J = 15.0 Hz, 1H), 3.04−3.00 (m, 1H), 2.97−2.93 (m, 1H), 2.89 (dd, J = 14.7, 10.0 Hz, 1H), 1.87 (s, 3H), 1.22 (d, J = 7.5 Hz, 3H), 1.20 (d, J = 6.9 Hz, 3H), 1.17 (d, J = 7.3 Hz, 3H); HRMS (ESI) calcd for $C_{29}H_{35}ClN_6O_6$ 599.2379; found, 599.2392.

Ac-(Cyclo-*m*,*m*)-[(3-Cl)FAA(Aib)F]-NH$_2$ (32). The pentapeptide macrocyclization precursor was synthesized on solid support using general procedure A. It was subjected to Suzuki−Miyaura macrocyclization general procedure C1. The macrocyclic product was cleaved from the resin using general procedure D and acetylated using general procedure F1. The resultant peptide was purified by HPLC using general procedure G2 to yield the solid **32** (9.2 mg, 6%) as a single peak (purity >98%; see Supporting Information for pdf of HPLC trace): ^{1}H NMR (700 MHz, d_6-DMSO) δ 8.47−8.44 (m, 1H), 8.02−7.97 (m, 1H), 7.94 (d, J = 8.4 Hz, 1H), 7.63−7.57 (m, 1H), 7.54 (d, J = 6.2 Hz, 1H), 7.52 (s, 1H), 7.44 (d, J = 8.0 Hz, 2H), 7.40 (s, 1H), 7.29 (d, J = 7.3 Hz, 2H), 7.25 (s, 1H), 7.18 (s, 2H), 4.61−4.56 (m, 1H), 4.56−4.52 (m, 1H), 4.20−4.15 (m, 1H), 3.88−3.82 (m, 1H), 3.15 (d, J = 13.7 Hz, 1H), 3.06 (d, J = 12.8 Hz, 1H), 2.93−2.84 (m, 2H), 1.85 (s, 3H), 1.42 (s, 3H), 1.28 (s, 3H), 1.11 (d, J = 6.7 Hz, 3H), 0.88 (d, J = 6.8 Hz, 3H); HRMS (ESI) calcd for $C_{30}H_{37}ClN_6O_6$ 613.2536; found, 613.2557.

Ac-(Cyclo-*m*,*m*)-[(3-Cl)FA(Aib)AF]-NH$_2$ (33). The pentapeptide macrocyclization precursor was synthesized on solid support using general procedure A. It was subjected to Suzuki−Miyaura macrocyclization general procedure C1. The macrocyclic product was cleaved from the resin using general procedure D and acetylated using general procedure F1. The resultant peptide was isolated as two isomers, which were separated and purified by HPLC using general procedure G1 to yield the solid **33a** (5.0 mg, 4%) as a single peak (purity >98%; see Supporting Information for pdf of HPLC trace): ^{1}H NMR (700 MHz, d_6-DMSO) δ 8.30 (d, J = 9.1 Hz, 1H), 8.10 (d, J = 7.7 Hz, 1H), 8.04 (s, 1H), 7.70 (s, 1H), 7.64 (d, J = 5.3 Hz, 1H), 7.56 (s, 1H), 7.49−7.44 (m, 3H), 7.30 (d, J = 7.7 Hz, 2H), 7.22−7.16 (m, 3H), 4.56−4.53 (m, 1H), 4.45−4.40 (m, 1H), 4.30−4.22 (m, 1H), 4.16−4.10 (m, 1H), 3.09 (d, J = 12.9 Hz, 1H), 2.96−2.88 (m, 2H), 2.78 (t, J = 12.6 Hz, 1H), 1.89 (s, 3H), 1.35 (s, 3H), 1.18−1.13 (m, 6H), 0.91 (d, J = 6.7 Hz, 3H); HRMS (ESI) calcd for $C_{30}H_{37}ClN_6O_6$ 613.2536; found, 613.2532; and the solid **33b** (3 mg, 2%) as a single peak (purity >98%; see Supporting Information for pdf of HPLC trace): ^{1}H NMR (700 MHz, d_6-DMSO) δ 8.30 (s, 1H), 8.25 (d, J = 9.0 Hz, 1H), 8.15−8.09 (m, 1H), 7.74 (s, 1H), 7.68−7.63 (m, 1H), 7.51 (s, 1H), 7.49−7.44 (m, 2H), 7.32−7.25 (m, 4H), 7.23 (s, 1H), 7.18 (s, 1H), 4.66−4.60 (m, 1H), 4.60−4.55 (m, 1H), 4.14−4.07 (m, 2H), 3.23−3.19 (m, 1H), 3.11 (d, J = 13.7 Hz, 1H), 3.02−2.95 (m, 1H), 2.78−2.70 (m, 1H), 1.91−1.86 (m, 3H), 1.41 (s, 3H), 1.25 (s, 3H), 1.04 (d, J = 6.2 Hz, 3H), 0.96 (d, J = 6.3 Hz, 3H); HRMS (ESI) calcd for $C_{30}H_{37}ClN_6O_6$ 613.2536; found, 613.2532.

Ac-(Cyclo-*m*,*m*)-[(3-Cl)F(Aib)AAF]-NH$_2$ (34). The pentapeptide macrocyclization precursor was synthesized on solid support using general procedure A. It was subjected to Suzuki−Miyaura macrocyclization general procedure C1. The macrocyclic product was cleaved from the resin using general procedure D and acetylated using general procedure F1. The resultant peptide was isolated as two isomers, which were separated and purified by HPLC using general procedure G1 to yield the solid **34a** (3.0 mg, 2%) as a single peak (purity >98%; see Supporting Information for pdf of HPLC trace): ^{1}H NMR (700 MHz, d_6-DMSO) δ 8.41 (d, J = 9.5 Hz, 1H), 8.16 (d, J = 7.6 Hz, 1H), 7.74 (d, J = 7.8 Hz, 1H), 7.68−7.66 (m, 2H), 7.64 (s, 1H), 7.54−7.50 (m, 3H), 7.32 (d, J = 7.9 Hz, 2H), 7.28 (s, 1H), 7.19 (s, 1H), 7.17 (d, J = 7.5 Hz, 1H), 4.62−4.55 (m, 1H), 4.40−4.34 (m, 1H), 4.34−4.28 (m, 1H), 4.13−4.06 (m, 1H), 3.09 (dd, J = 13.7, 2.9 Hz, 1H), 3.07−3.02 (m, 1H), 2.96 (dd, J = 13.7, 9.3 Hz, 1H), 2.73 (t, J = 12.9 Hz, 1H), 1.92 (s, 3H), 1.47 (s, 3H), 1.16 (d, J = 6.8 Hz, 3H), 1.11 (d, J = 7.3 Hz, 3H), 1.05 (s, 3H); HRMS (ESI) calcd for $C_{30}H_{37}ClN_6O_6$ 613.2536; found, 613.2532; and the solid **34b** (6.0 mg, 4%) as a single peak (purity >98%; see Supporting Information for pdf of HPLC trace): ^{1}H NMR (700 MHz, d_6-DMSO) δ 8.52 (s, 1H), 8.15 (d, J = 7.2 Hz, 1H), 8.13 (d, J = 6.9 Hz, 1H), 8.12−8.05 (m, 1H), 7.93−7.89 (m, 1H), 7.86 (s, 1H), 7.74−7.68 (m, 1H), 7.56−7.50 (m, 3H), 7.35−7.30 (m, 2H), 7.23 (s, 1H), 7.19 (s, 1H), 4.50−4.44 (m, 2H), 4.36 (t, J = 7.2 Hz, 1H), 4.18−4.14 (m, 1H), 3.16−3.10 (m, 2H), 3.02−2.96 (m, 1H), 2.85 (t, J = 12.3 Hz, 1H), 1.95−1.92 (m, 3H), 1.51 (s, 3H), 1.33 (d, 3H), 1.28 (d, J = 6.4 Hz, 3H), 0.90−0.86 (m, 3H); HRMS (ESI) calcd for $C_{30}H_{37}ClN_6O_6$ 613.2536; found, 613.2536.

Ac-(Cyclo-*m*,*m*)-[(α-Me)FAAAF]-NH$_2$ (35). The pentapeptide macrocyclization precursor was synthesized on solid support using general procedure A. It was subjected to Suzuki−Miyaura macrocyclization general procedure C1. The macrocyclic product was cleaved from the resin using general procedure D and acetylated using general procedure F1. The resultant peptide was purified by HPLC using general procedure G3 to yield the solid **35** (1.5 mg, 2%) as a single peak (purity >98%; see Supporting Information for pdf of HPLC trace): ^{1}H NMR (700 MHz, d_6-DMSO) δ 9.20 (s, 1H), 8.84 (s, 1H), 8.25−8.15 (m, 2H), 8.00−7.95 (m, 1H), 7.57−7.50 (m, 2H), 7.45 (d, J = 12.4 Hz, 1H), 7.42−7.36 (m, 2H), 7.33 (t, J = 7.5 Hz, 1H), 7.25 (d, J = 7.3 Hz, 1H), 7.17 (d, J = 6.2 Hz, 1H), 6.98 (s, 1H), 6.58−6.50 (m, 1H), 4.21−4.17 (m, 1H), 4.00−3.93 (m, 2H), 3.93−3.86 (m, 1H), 3.23 (d, J = 12.1 Hz, 1H), 3.18−3.12 (m, 1H), 3.08−3.01 (m, 1H), 2.96 (d, J = 13.4 Hz, 1H), 1.92 (s, 3H), 1.45 (d, J = 7.1 Hz, 3H) 1.35 (d, J = 7.0 Hz, 3H), 0.95 (s, 3H), 0.89−0.85 (m, 3H); HRMS (ESI) calcd for $C_{30}H_{38}N_6O_6Na$ 601.2745; found, 601.2749.

(2*R*,4*R*)-Benzyl 4-Methyl-5-oxo-2-phenyloxazolidine-3-carboxylate (38). To a solution of (*R*)-2-(((benzyloxy)carbonyl)-amino)propanoic acid (**37**) (10 g, 44.8 mmol) and (dimethoxymethyl) benzene (6.82 g, 44.8 mmol) in THF (75 mL) at 0 °C was added $SOCl_2$ (3.27 mL, 44.8 mmol). After stirring the reaction mixture for 5 min, $ZnCl_2$ (6.11 g, 44.8 mmol) was added and the reaction mixture was stirred for 3 h at 0 °C. At his stage, another portion of $SOCl_2$ (0.654 mL, 8.96 mmol) and $ZnCl_2$ (1.22 g, 8.96 mmol) was added, and the reaction mixture was stirred for an additional 1 h. The reaction mixture was quenched by dropwise addition of water so that the reaction temperature did not exceed 10 °C. It was extracted with Et_2O (200 mL). The organic phase was washed with water until almost neutral, with saturated aqueous $NaHCO_3$ (2 × 40 mL) and water (40 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo, and the residue was purified by an automated system (FLASH 65i column; hexanes/EtOAc 92:8 to hexanes/EtOAc 83:17) leading to oxazolidine **38** (8.8 g, 28.3 mmol, 63%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.52−7.12 (m, 10H), δ 6.64 (br s, 1H), 5.23−5.12 (m, 2H), 4.52−4.46 (m, 1H), 1.59 (d, J = 4.9 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.3, 136.8, 135.2, 129.6, 128.7, 128.6, 128.5, 128.3, 127.9, 126.4,

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126.1, 88.9, 67.8, 52.0; HRMS (ESI) calcd for $C_{18}H_{18}NO_4$ 312.1230; found, 312.1228.

(2*R*,4*S*)-Benzyl 4-(3-Iodobenzyl)-4-methyl-5-oxo-2-phenyloxazolidine-3-carboxylate (39). A solution of (2*R*,4*R*)-benzyl 4-methyl-5-oxo-2-phenyloxazolidine-3-carboxylate (**38**) (6.5 g, 20.9 mmol) and 3-iodo-benzyl bromide (6.2 g, 20.88) in THF (42 mL) was added dropwise at −30 °C to a solution of LiHMDS (1 M in THF, 22.1 mL, 22.1 mmol) diluted in THF (167 mL). The reaction mixture was stirred at this temperature for 1 h and then allowed to warm to rt and stirred for 3 h. Saturated aqueous $NaHCO_3$ (100 mL) was added and the mixture was extracted with Et_2O (2 × 200 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo, and the residue was purified by an automated system (FLASH 65i column; hexanes/EtOAc 95:5 to hexanes/EtOAc 81:19) leading to oxazolidine **39** (7.9 g, 14.98 mmol, 72%). *Note concerning the NMR data of the following compound: Due to a mixture of rotamers, the proton assignment of ^{1}H NMR data was carried out for the two compounds in this mixture (3:1 ratio). The ^{13}C NMR data represents a mixture of the two rotamers.* ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.64 (d, J = 7.8 Hz, 1H), 7.61 (s, 1H), 7.49 (d, J = 7.1 Hz, 0.6H), 7.44 (t, J = 7.2 Hz, 0.6H), 7.42−7.33 (m, 2.6H), 7.33−7.27 (m, 2.9H), 7.27−7.24 (m, 0.9H), 7.21 (t, J = 7.3 Hz, 2H), 7.17 (d, J = 7.2 Hz, 2H), 7.12 (d, J = 7.6 Hz, 1H), 7.00−6.97 (t, J = 7.8 Hz, 1.3H), 6.89−6.84 (m, 2.3H), 5.52 (s, 0.3H), 5.38 (d, J = 12.0 Hz, 0.3H), 5.36 (s, 1H), 5.13 (d, J = 12.0 Hz, 0.3H), 5.07 (d, J = 12.2 Hz, 1H), 5.00 (d, J = 12.2 Hz, 1H), 3.72 (d, J = 13.5 Hz, 1H), 3.33 (d, J = 13.7 Hz, 0.3H), 3.07 (d, J = 13.5 Hz, 1H), 3.02 (d, J = 13.7 Hz, 0.3H), 1.95 (s, 3H), 1.87 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.0, 173.8, 152.2, 151.9, 138.6, 138.3, 137.5, 136.9, 136.7, 136.6, 136.5, 136.0, 134.9, 134.9, 130.4, 129.8, 129.7, 128.9, 128.9, 128.8, 128.6, 128.5, 128.4, 128.2, 128.1, 127.9, 126.7, 126.7, 94.6, 94.5, 89.4, 89.2, 68.0, 67.5, 64.5, 64.0, 41.9, 40.2, 24.9, 23.9; HRMS (ESI) calcd for $C_{25}H_{23}INO_4$ 528.0666; found, 528.0675.

(*S*)-2-Amino-3-(3-iodophenyl)-2-methylpropanoic Acid (40). A mixture of (2*R*,4*S*)-benzyl 4-(3-iodobenzyl)-4-methyl-5-oxo-2-phenyloxazolidine-3-carboxylate (**39**) (1.35 g, 2.56 mmol) and $KOSiMe_3$ (90% pure, 1.10 g, 7.68 mmol) was suspended in THF (45 mL) and heated to 75 °C for 2.5 h. MeOH (75 mL) was added and the reaction mixture was concentrated in vacuo. The residue was redissolved in MeOH and applied to a 20 g SCX-2 ion exchange cartridge (0.59 mmol/g loading) eluting with MeOH and then with Et_3N (0.2 M in MeOH). The Et_3N/MeOH fraction was concentrated in vacuo leading to amino acid **40** (0.72 g, 2.36 mmol, 92%): ^{1}H NMR (600 MHz, CD_3OD) δ 7.69 (s, 1H), 7.65 (d, J = 7.7 Hz, 1H), 7.29 (d, J = 7.7 Hz, 1H), 7.10 (t, J = 7.7 Hz, 1H), 3.23 (d, J = 14.1 Hz, 1H), 2.86 (d, J = 14.1 Hz, 1H), 1.49 (s, 3H); ^{13}C NMR (150 MHz, CD_3OD) δ 175.6, 140.3, 138.7, 137.8, 131.5, 130.7, 95.3, 62.8, 43.7, 23.6; HRMS (ESI) calcd for $C_{10}H_{13}INO_2$ 305.9986; found, 305.9984.

(*S*)-Methyl 2-Acetamido-3-(3-iodophenyl)-2-methylpropanoate (41). To MeOH (46 mL), $SOCl_2$ (1.91 mL, 26.2 mmol) was added dropwise at 0 °C. (*S*)-2-amino-3-(3-iodophenyl)-2-methylpropanoic acid (**40**) (0.72 g, 2.36 mmol) in MeOH (46 mL) was added, and after stirring for 30 min at 0 °C, the reaction mixture was allowed to warm to rt After 2 h, the reaction mixture was concentrated in vacuo. Carrying this material forward without further purification, the newly formed intermediate was suspended in CH_2Cl_2 (131 mL). To this suspension, DIPEA (2.86 mL, 16.4 mmol), Ac_2O (1.24 mL, 13.1 mmol), and DMAP (16 mg, 0.13 mmol) were added at 0 °C. After stirring for 12 h at rt, the reaction mixture was concentrated in vacuo. The residue was redissolved in MeONa (0.2 M in MeOH, 100 mL, 20.0 mmol) and heated to reflux for 3 h. The reaction mixture was concentrated in vacuo and the residue was taken up in EtOAc (150 mL) and washed with water/brine (1:1, 2 × 80 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo and the residue was purified by an automated system (Flash 40+M column; hexanes/EtOAc 90:10 to hexanes/EtOAc 40:60) yielding amino acid **41** (1.84 g, 5.09 mmol, 78%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.57−7.53 (m, 1H), 7.40 (s, 1H), 7.02−6.96 (m, 2H), 6.08 (br s, 1H), 3.79 (s, 3H), 3.53 (d, J = 13.5 Hz, 1H), 3.13 (d, J = 13.5 Hz, 1H), 1.98 (s, 3H), 1.64 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.1, 169.6, 138.9, 138.8, 135.8, 129.8, 128.9, 94.1, 61.1, 52.7, 40.1, 23.9, 23.3; HRMS (ESI) calcd for $C_{13}H_{17}INO_3$ 362.0248; found, 362.0250.

(*S*)-Methyl 2-Acetamido-2-methyl-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanoate (42). In a 100 mL flask was (*S*)-methyl 2-acetamido-3-(3-iodophenyl)-2-methylpropanoate (**41**) (1.8 g, 4.98 mmol), Pd(dppf)Cl_2·CH_2Cl_2 (182 mg, 0.249 mmol) B_2pin_2 (2.53 g, 9.97 mmol) and KOAc (1.96 g, 19.9 mmol) in degassed DMSO (36 mL). The flask was sealed and heated to 85 °C for 6 h. The reaction mixture was poured into brine/water (1:1, 40 mL) and extracted with EtOAc (2 × 80 mL). The combined organic layers were washed with brine (3 × 40 mL), dried (Na_2SO_4), and concentrated in vacuo, the residue was purified by an automated system (Flash 40+M column; hexanes/EtOAc 65:35 to hexanes/EtOAc 30:70) yielding boronic ester **42** (1.63 g, 4.51 mmol, 90%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 (d, J = 7.4 Hz, 1H), 7.48 (s, 1H), 7.26 (t, J = 7.4 Hz, 1H), 7.13 (d, J = 7.4 Hz, 1H), 6.00 (br s, 1H), 3.77 (s, 3H), 3.53 (d, J = 13.5 Hz, 1H), 3.19 (d, J = 13.5 Hz, 1H), 1.97 (s, 3H), 1.65 (s, 3H), 1.32 (s, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.3, 169.6, 136.2, 135.6, 133.1, 132.6, 127.6, 83.7, 61.1, 52.5, 40.7, 24.9, 24.8, 23.9, 23.1; HRMS (ESI) calcd for $C_{19}H_{29}BNO_5$ 362.2133; found, 362.2138.

(*S*)-2-Acetamido-3-(3-boronophenyl)-2-methylpropanoic Acid (36). To a stirred solution of (*S*)-methyl 2-acetamido-2-methyl-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanoate (**42**) (361 mg, 1.0 mmol) in MeOH (4 mL) was added LiOH·H_2O (210 mg, 5.0 mmol) in H_2O (4 mL) at rt. The mixture was stirred at the same temperature for 12 h. The reaction mixture was acidified with 1 M aqueous HCl to pH ∼ 2. The aqueous layer was extracted with EtOAc (4 × 50 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo providing crude acid **36**. Because of the instability of this material, it was carried forward without further purification.

Ac-(Cyclo-*m*,*o*)-[(3-Cl)FAF]-NH_2 (43). The tripeptide macrocyclization precursor was synthesized on solid support using general procedure A. It was subjected to Suzuki−Miyaura macrocyclization general procedure C1. The macrocyclic product was cleaved from the resin using general procedure D and acetylated using general procedure F1. The resultant peptide was purified by HPLC using general procedure G2 to yield the solid **43** (23.0 mg, 25%) as a single peak (purity >98%; see Supporting Information for pdf of HPLC trace): ^{1}H NMR (700 MHz, d_6-DMSO) δ 8.18 (d, J = 7.1 Hz, 1H), 7.60 (s, 1H), 7.49−7.35 (m, 3H), 7.32 (t, J = 7.2 Hz, 1H), 7.27 (t, J = 7.4 Hz, 1H), 7.25 (s, 1H), 7.23 (s, 1H), 7.16−7.12 (m, 2H), 6.98 (br s, 1H), 4.61−4.44 (m, 1H), 4.38−4.31 (m, 1H), 4.31−4.26 (m, 1H), 3.28−3.16 (m, 1H), 3.06−2.90 (m, 2H), 2.80 (br s, 1H), 1.88 (s, 3H), 0.99 (d, J = 7.1 Hz, 3H). HRMS (ESI) calcd for $C_{23}H_{25}ClN_4O_4$ 457.1637; found, 457.1646.

Ac-(Cyclo-*m*,*o*)-[(3-Cl)FAAF]-OH (44). The tetrapeptide macrocyclization precursor was synthesized on solid support using general procedure A. It was subjected to Suzuki−Miyaura macrocyclization general procedure C1. The macrocyclic product was cleaved from the resin using general procedure D and acetylated using general procedure F1. The resultant peptide was purified by HPLC using general procedure G3 to yield the solid **44** (2.0 mg, 2%) as a single peak (purity >98%; see Supporting Information for pdf of HPLC trace): ^{1}H NMR (700 MHz, d_6-DMSO) δ 8.35 (d, J = 6.1 Hz, 1H), 8.15 (s, 1H), 7.66 (d, J = 6.8 Hz, 1H), 7.33−7.29 (m, 3H), 7.28−7.24 (m, 2H), 7.21 (d, J = 7.1 Hz, 1H), 7.18 (s, 1H), 7.15−7.11 (m, 1H), 7.07 (s, 1H), 7.05 (s, 1H), 4.80−4.76 (m, 1H), 4.38−4.33 (m, 1H), 4.01−3.96 (m, 2H), 3.18−3.12 (m, 1H), 3.06−3.01 (m, 1H), 2.98 (d, J = 12.3 Hz, 1H), 2.96−2.91 (m, 1H), 1.92 (s, 3H), 1.22 (d, J = 7.4 Hz, 3H), 0.97 (d, J = 7.1 Hz, 3H); HRMS (ESI) calcd for $C_{26}H_{29}ClN_4O_6$ 529.1854 ; found, 529.1866.

Ac-(Cyclo-*m*,*o*)-[(3-Cl)FAAAF]-NH_2 (45). The pentapeptide macrocyclization precursor was synthesized on solid support using general procedure A. It was subjected to Suzuki−Miyaura macrocyclization general procedure C1. The macrocyclic product was cleaved from the resin using general procedure D and acetylated using general procedure F1. The resultant peptide was purified by HPLC using general procedure G1 to yield the solid **45** (1.5 mg, 1%) as a

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single peak (purity >98%; see Supporting Information for pdf of HPLC trace): ^{1}H NMR (700 MHz, d_6-DMSO) δ 8.36 (d, J = 7.7 Hz, 1H), 8.20−8.13 (m, 2H), 8.11−8.06 (m, 1H), 7.44 (d, J = 8.3 Hz, 1H), 7.32 (s, 1H), 7.30 (d, J = 9.5 Hz, 1H), 7.28−7.26 (m, 2H), 7.25 (s, 1H), 7.19 (s, 1H), 7.17 (d, J = 7.3 Hz, 1H), 7.15 (s, 1H), 7.08−7.04 (m, 1H), 4.46 (d, J = 6.9 Hz, 1H), 4.28 (d, J = 8.6 Hz, 1H), 4.11 (t, J = 6.6 Hz, 1H), 4.03 (dd, J = 11.3, 3.8 Hz, 1H), 3.97 (t, J = 6.5 Hz, 1H), 3.09−3.03 (m, 1H), 3.01−2.96 (m, 1H), 2.96−2.92 (m, 2H), 1.87 (s, 3H), 1.13 (d, J = 6.6 Hz, 3H), 1.12−1.10 (m, 3H), 1.08 (d, J = 6.6 Hz, 3H); HRMS (ESI) calcd for $C_{29}H_{35}ClN_6O_6$ 599.2379; found, 599.2380.

Ac-(Cyclo-*m,o*)-[(3-Cl)FAAA(α-Me)F]-NH$_2$ (48). The pentapeptide macrocyclization precursor was synthesized on solid support using general procedure B. It was subjected to Suzuki−Miyaura macrocyclization general procedure C2. The macrocyclic product was cleaved from the resin using general procedure E and acetylated using general procedure F2. The resultant peptide was purified by HPLC using general procedure G6 to yield the solid **48** (2 mg, 2%) as a single peak (purity >98%; see Supporting Information for pdf of HPLC trace): ^{1}H NMR (700 MHz, d_6-DMSO) δ 8.50−8.44 (m, 1H), 8.29−8.23 (m, 1H), 8.22−8.14 (m, 1H), 8.01 (d, J = 6.9 Hz, 1H), 7.63 (s, 1H), 7.40−7.34 (m, 1H), 7.30−7.27 (m, 1H), 7.26−7.20 (m, 3H), 7.20−7.16 (m, 1H), 7.13 (s, 1H), 7.05 (d, J = 7.3 Hz, 2H), 4.51−4.46 (m, 1H), 4.28−4.21 (m, 2H), 4.08−4.03 (m, 1H), 3.31 (d, J = 13.8 Hz, 1H), 3.26−3.20 (m, 1H), 3.01 (d, J = 13.4 Hz, 1H), 2.71 (t, J = 13.0 Hz, 1H), 1.75 (s, 3H), 1.33 (s, 3H), 1.25−1.17 (m, 9H); HRMS (ESI) calcd for $C_{30}H_{38}ClN_6O_6$ 613.2536; found, 613.2531.

Ac-(Cyclo-*m,o*)-[(3-Cl)F(Aib)AAF]-NH$_2$ (49). The pentapeptide macrocyclization precursor was synthesized on solid support using general procedure B. It was subjected to Suzuki−Miyaura macrocyclization general procedure C2. The macrocyclic product was cleaved from the resin using general procedure E and acetylated using general procedure F2. The resultant peptide was purified by HPLC using general procedure G3 to yield the solid **49** (5 mg, 4%) as a single peak (purity >98%; see Supporting Information for pdf of HPLC trace): ^{1}H NMR (700 MHz, d_6-DMSO) δ 8.84 (s, 1H), 8.68 (s, 1H), 7.53 (d, J = 7.6 Hz, 1H), 7.44 (d, J = 7.1 Hz, 1H), 7.37 (d, J = 7.8 Hz, 1H), 7.32 (t, J = 7.5 Hz, 1H), 7.26 (t, J = 7.4 Hz, 1H), 7.22 (s, 1H), 7.20−7.17 (m, 3H), 7.13 (s, 1H), 7.10 (s, 1H), 6.92 (s, 1H), 4.58−4.51 (m, 1H), 4.32−4.26 (m, 1H), 4.03 (p, J = 7.2 Hz, 1H), 3.92 (p, J = 7.3 Hz, 1H), 3.27 (dd, J = 13.6, 6.3 Hz, 1H), 3.04 (dd, J = 14.9, 6.3 Hz, 1H), 2.92 (dd, J = 13.5, 10.6 Hz, 1H), 2.59 (dd, J = 14.8, 8.5 Hz, 1H), 1.98 (s, 3H), 1.27 (s, 3H), 1.24 (d, J = 7.4 Hz, 3H), 1.08 (s, 3H), 0.95 (d, J = 7.1 Hz, 3H); HRMS (ESI) calcd for $C_{30}H_{38}ClN_6O_6$ 613.2536; found, 613.2538.

(2*R*,4*S*)-Benzyl 4-(2-Iodobenzyl)-4-methyl-5-oxo-2-phenyloxazolidine-3-carboxylate (51). A solution of (2*R*,4*R*)-benzyl 4-methyl-5-oxo-2-phenyloxazolidine-3-carboxylate (**38**) (8.82 g, 28.3 mmol) and 2-iodo-benzyl bromide (8.41 g, 28.3 mmol) in THF (38 mL) was added dropwise at −30 °C to a solution of LiHMDS (1 M in THF, 31.2 mL, 31.2 mmol) diluted in THF (151 mL). The reaction mixture was stirred at this temperature for 1 h and then allowed to warm to rt and stirred for 3 h. Saturated aqueous $NaHCO_3$ (100 mL) was added and the mixture was extracted with Et_2O (2 × 200 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo, and the residue was purified by an automated system (KP-Sil 340 g column; hexanes/EtOAc 95:5 to hexanes/EtOAc 82:18) leading to oxazolidine **51** (11.38 g, 21.58 mmol, 76%). *Note concerning the NMR data of the following compound: Due to a mixture of rotamers, the proton assignment of ^{1}H NMR data was carried out for the two compounds in this mixture (2:1 ratio). The ^{13}C NMR data represents a mixture of the two rotamers.* ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.90−7.87 (m, 1.5H), 7.41−7.28 (m, 8H), 7.28−7.12 (m, 7.5H), 7.09−7.03 (m, 0.5H), 6.98−6.91 (m, 1.5H), 6.84−6.78 (m, 2H), 5.89 (br s, 0.5H), 5.72 (s, 1H), 5.27−5.14 (m, 1H), 4.98 (d, J = 12.2 Hz, 1H), 4.95 (d, J = 12.2 Hz, 1H), 3.90−3.80 (m, 1H), 3.63 (d, J = 13.4 Hz, 0.5H), 3.43−3.38 (m, 1.5H), 2.04 (s, 3H), 1.92 (s, 1.5H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.2, 173.2, 173.1, 173.1, 173.0, 152.3, 152.2, 151.6, 140.5, 138.4, 138.0, 137.9, 136.8, 136.2, 136.2, 135.1, 130.5, 130.1, 129.8, 129.2, 129.2, 129.1, 128.6, 128.4, 128.3, 128.2, 128.2, 128.1, 127.9, 126.7, 101.5, 101.5, 101.4, 101.4, 89.3, 68.0, 67.3, 64.0, 63.6, 45.8, 44.5, 25.4, 25.4, 24.4; HRMS (ESI) calcd for $C_{25}H_{22}INO_4$ 528.0666; found, 528.0669.

(*S*)-2-Amino-3-(2-iodophenyl)-2-methylpropanoic Acid (52). A mixture of (2*R*,4*S*)-benzyl 4-(2-iodobenzyl)-4-methyl-5-oxo-2-phenyloxazolidine-3-carboxylate (**51**) (2.0 g, 3.79 mmol) and $KOSiMe_3$ (90% pure, 1.62 g, 11.4 mmol) was suspended in THF (63 mL) and heated to 75 °C for 2.5 h. MeOH (100 mL) was added and the reaction mixture was concentrated in vacuo. The residue was redissolved in MeOH and applied to a 40 g SCX-2 ion exchange cartridge (0.59 mmol/g loading) eluting with MeOH and then with Et_3N (0.2 M in MeOH). The Et_3N/MeOH fraction was concentrated in vacuo leading to amino acid **52** (1.13 g, 3.7 mmol, 98%): ^{1}H NMR (600 MHz, CD_3OD) δ 7.90 (d, J = 7.6 Hz, 1H), 7.43 (d, J = 7.6 Hz, 1H), 7.34 (t, J = 7.6 Hz, 1H), 6.99 (t, J = 7.6 Hz, 1H), 3.40 (d, J = 14.5 Hz, 1H), 3.36 (d, J = 14.5 Hz, 1H), 1.52 (s, 3H); ^{13}C NMR (150 MHz, CD_3OD) δ 176.0, 141.3, 139.7, 132.2, 130.2, 129.7, 103.3, 63.5, 47.5, 23.2; HRMS (ESI) calcd for $C_{10}H_{13}INO_2$ 305.9986; found, 305.9987.

(*S*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(2-iodophenyl)-2-methylpropanoic Acid (50). A mixture of (*S*)-2-amino-3-(2-iodophenyl)-2-methylpropanoic acid (**52**) (575 mg, 1.89 mmol) and TMSCl (0.48 mL, 3.77 mmol) was suspended in CH_2Cl_2 (20 mL) and heated to reflux for 6 h. DIPEA (0.69 mL, 3.96 mmol) and FmocCl (0.54 g, 2.07 mmol) were added to the reaction mixture at 0 °C. The reaction mixture was allowed to warm to rt and stirred for 30 h. The reaction mixture was concentrated in vacuo and residue was redissolved in EtOAc (100 mL). The organic layer was washed with 1 M HCl (2 × 30 mL) and brine (30 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo, and the residue was purified by an automated system (KP-Sil 25 g column; CH_2Cl_2/MeOH 95:5 to CH_2Cl_2/MeOH 80:20) leading to Fmoc-carbamate **50** (570 mg, 1.08 mmol, 57%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.84 (d, J = 7.1 Hz, 1H), 7.77 (d, J = 6.8 Hz, 2H), 7.60 (t, J = 7.7 Hz, 2H), 7.40 (t, J = 7.0 Hz, 2H), 7.31 (t, J = 7.1 Hz, 2H), 7.19 (t, J = 6.4 Hz, 1H), 7.04 (br s, 1H), 6.91 (t, J = 6.3 Hz, 1H), 5.29 (s, 1H), 4.58−4.48 (m, 1H), 4.48−4.36 (m, 1H), 4.23 (t, J = 6.3 Hz, 1H), 3.62−3.43 (m, 2H), 1.57 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.8, 155.1, 143.7, 141.3, 139.9, 139.0, 131.1, 128.7, 128.1, 127.7, 127.0, 125.0, 119.9, 102.7, 66.6, 60.2, 47.2, 44.3, 23.2; HRMS (ESI) calcd for $C_{25}H_{23}INO_4$ 528.0676; found, 528.0666.

Ac-(Cyclo-*o,m*)-[FAAAF]-NH$_2$ (55). The pentapeptide macrocyclization precursor was synthesized on solid support using general procedure B. It was subjected to Suzuki−Miyaura macrocyclization general procedure C2. The macrocyclic product was cleaved from the resin using general procedure E and acetylated using general procedure F2. The resultant peptide was purified by HPLC using general procedure G3 to yield the solid **55** (22 mg, 8%) as a single peak (purity >98%; see Supporting Information for pdf of HPLC trace): ^{1}H NMR (700 MHz, d_6-DMSO) δ 8.37 (d, J = 6.4 Hz, 1H), 8.31 (d, J = 8.6 Hz, 1H), 7.77 (br s, 1H), 7.67 (br s, 1H), 7.41 (d, J = 7.6 Hz, 1H), 7.38−7.23 (m, 4H), 7.23−7.18 (m, 2H), 7.18−7.08 (m, 3H), 4.67−4.63 (m, 1H), 4.32 (br s, 1H), 4.05 (p, J = 6.9 Hz, 1H), 3.99 (p, J = 6.5 Hz, 1H), 3.89−3.83 (m, 1H), 3.18 (dd, J = 14.7, 4.0 Hz, 1H), 3.13−3.05 (m, 2H), 2.78−2.73 (m, 1H), 1.85 (s, 3H), 1.25 (d, J = 7.4 Hz, 3H), 1.19 (d, J = 7.1 Hz, 3H), 1.13 (d, J = 7.0 Hz, 3H); HRMS (ESI) calcd for $C_{29}H_{36}N_6O_6$ 565.2769; found, 565.2773.

Ac-(Cyclo-*o,m*)-[F(Aib)AAF]-NH$_2$ (56). The pentapeptide macrocyclization precursor was synthesized on solid support using general procedure B. It was subjected to Suzuki−Miyaura macrocyclization general procedure C2. The macrocyclic product was cleaved from the resin using general procedure E and acetylated using general procedure F2. The resultant peptide was purified by HPLC using general procedure G3 to yield the solid **56** (7.6 mg, 7%) as a single peak (purity >98%; see Supporting Information for pdf of HPLC trace): ^{1}H NMR (700 MHz, d_6-DMSO) δ 8.52 (s, 1H), 8.32 (s, 1H), 7.83 (d, J = 6.8 Hz, 1H), 7.59 (d, J = 7.1 Hz, 1H), 7.51 (d, J = 8.2 Hz, 1H), 7.42 (d, J = 7.5 Hz, 1H), 7.33−7.21 (m, 5H), 7.14−7.08 (m, 4H), 4.52 (s, 1H), 4.32−4.24 (m, 1H), 4.03 (t, J = 7.2 Hz, 1H), 3.88 (t, J = 7.0 Hz, 1H), 3.17 (dd, J = 14.5, 4.0 Hz, 1H), 3.13 (dd, J = 14.3, 3.9 Hz, 1H), 3.09−3.02 (m, 1H), 2.92−2.85 (m, 1H), 1.83 (s, 3H), 1.29 (d, J = 3.8 Hz,

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6H), 1.20 (d, J = 7.1 Hz, 3H), 1.03 (d, J = 7.3 Hz, 3H); HRMS (ESI) calcd for $C_{30}H_{38}N_6O_6$ 579.2925; found, 579.2923.

Ac-(Cyclo-*o,m*)-[FAA(Aib)F]-NH$_2$ (57). The pentapeptide macrocyclization precursor was synthesized on solid support using general procedure B. It was subjected to Suzuki–Miyaura macrocyclization general procedure C2. The macrocyclic product was cleaved from the resin using general procedure E and acetylated using general procedure F2. The resultant peptide was purified by HPLC using general procedure G3 to yield the solid **57** (7.8 mg, 7%) as a single peak (purity >98%; see Supporting Information for pdf of HPLC trace): ^{1}H NMR (700 MHz, d_6-DMSO) δ 8.58 (s, 1H), 8.46 (s, 1H), 8.21 (s, 1H), 8.02 (s, 1H), 7.44–7.35 (m, 1H), 7.34–7.31 (m, 1H), 7.31–7.27 (m, 2H), 7.27–7.23 (m, 2H), 7.22–7.17 (m, 1H), 7.09–7.05 (m, 1H), 7.04–6.99 (m, 1H), 6.92 (s, 1H), 6.85–6.79 (m, 1H), 4.75–4.68 (m, 1H), 4.29–4.10 (m, 1H), 4.03–3.95 (m, 1H), 3.73 (s, 1H), 3.32–3.21 (m, 2H), 3.21–3.15 (m, 1H), 3.10–2.97 (m, 1H), 1.84–1.74 (m, 3H), 1.38–1.31 (m, 3H), 1.27–1.20 (m, 6H), 1.17–1.12 (m, 3H); HRMS (ESI) calcd for $C_{30}H_{38}N_6O_6$ 579.2925; found, 579.2924.

Ac-(Cyclo-*o,m*)-[FAF]-NH$_2$ (60). The tripeptide macrocyclization precursor was synthesized on solid support using general procedure B. It was subjected to Suzuki–Miyaura macrocyclization general procedure C2. The macrocyclic product was cleaved from the resin using general procedure E and acetylated using general procedure F2. The resultant peptide was purified by HPLC using general procedure G1 to yield the solid **60** (3.6 mg, 4%) as a single peak (purity >98%; see Supporting Information for pdf of HPLC trace): ^{1}H NMR (500 MHz, d_6-DMSO) δ 8.11 (br s, 1H, NH), 7.71 (br s, 1H, NH), 7.52 (s, 1H, NH), 7.46–7.43 (m, 1H, Ar–H), 7.41–7.26 (m, 5H, Ar–H), 7.25 (s, 2H, NH_2), 7.14 (dd, J = 7.5 Hz, 1.5, 1H, Ar–H), 7.08 (dt, J = 7.5, 1.5 Hz, 1H, Ar–H), 4.62 (td, J = 9.0, 4.4 Hz, 1H, CHN), 4.36–4.30 (m, 2H, CHN), 3.28 (d, J = 14.8 Hz, 1H, CH_2Ar), 3.20–3.13 (m, 1H, CH_2Ar), 2.98–2.89 (m, 1H, CH_2Ar), 2.84 (dd, J = 14.7, 6.9 Hz, 1H, CH_2Ar), 1.95 (s, 3H, Ac), 1.09 (d, J = 6.8 Hz, 3H, $CHCH_3$). HRMS (ESI) calcd for $C_{23}H_{27}N_4O_4$ 423.2027; found, 423.2035.

Ac-(Cyclo-*o,m*)-[FAAF]-NH$_2$ (61). The tetrapeptide macrocyclization precursor was synthesized on solid support using general procedure B. It was subjected to Suzuki–Miyaura macrocyclization general procedure C2. The macrocyclic product was cleaved from the resin using general procedure E and acetylated using general procedure F2. The resultant peptide was purified by HPLC using general procedure G1 to yield the solid **61** (3.9 mg, 4%) as a single peak (purity >98%; see Supporting Information for pdf of HPLC trace): ^{1}H NMR (500 MHz d_6-DMSO) δ 8.58 (d, J = 7.4 Hz, 1H, NH), 8.34 (s, 2H, NH_2), 8.25 (d, J = 9.5 Hz, 1H, NH), 7.98 (d, J = 8.3 Hz, 1H, NH), 7.47 (s, 1H, NH), 7.28–7.15 (m, 4H, Ar–H), 7.10–6.99 (m, 4H, Ar–H), 4.93 (m, 1H, CHN), 4.68 (m, 1H, CHN), 3.98–3.87 (m, 2H, CHN), 3.12 (d, J = 13.2 Hz, 2H, CH_2Ar), 2.75 (dd, J = 15.2 Hz, 11.9, 1H, CH_2Ar), 2.61–2.54 (m, 1H, CH_2Ar), 1.68 (s, 3H, Ac), 1.14 (d, J = 7.4 Hz, 3H, $CHCH_3$), 1.02 (d, J = 6.6 Hz, 3H, $CHCH_3$). HRMS (ESI) calcd for $C_{26}H_{32}N_5O_5$ 494.2398; found, 494.2400.

Ac-(Cyclo-*o,m*)-[FAKAF]-NH$_2$ (62). The pentapeptide macrocyclization precursor was synthesized on solid support using general procedure B. It was subjected to Suzuki–Miyaura macrocyclization general procedure C2. The macrocyclic product was cleaved from the resin using general procedure E and acetylated using general procedure F2. The resultant peptide (**58**) was subjected to hydrogenation using 10 mol % Pd/C in DMF for 24 h to yield the crude product, which was purified by HPLC using general procedure G5 to yield the solid **62** (75 mg, 48%) as a single peak (purity >98%; see Supporting Information for pdf of HPLC trace): ^{1}H NMR (700 MHz, d_6-DMSO) δ 8.58–7.25 (m, 7H), 7.25–6.73 (m, 10H), 4.39–3.50 (m, obscured by H_2O peak, baseline correction shows 5H), 2.97–2.90 (m, 2H), 2.77 (q, J = 7.8 Hz, 2H), 2.33–2.20 (m, 1H), 1.62–1.48 (m, 3H), 1.45–1.22 (m, 4H), 1.16–0.74 (m, 9H); HRMS (ESI) calcd for $C_{32}H_{43}N_7O_6$ 622.3347; found, 622.3342.

Ac-(Cyclo-*o,m*)-[F(Aib)KAF]-NH$_2$ (63). The pentapeptide macrocyclization precursor was synthesized on solid support using general procedure B. It was subjected to Suzuki–Miyaura macrocyclization general procedure C2. The macrocyclic product was cleaved from the resin using general procedure E and acetylated using general procedure F2. The resultant peptide (**59**) was subjected to hydrogenation using 10 mol % Pd/C in DMF for 24 h to yield the crude product, which was purified by HPLC using general procedure G5 to yield the solid **63** (79 mg, 50%) as a single peak (purity >98%; see Supporting Information for pdf of HPLC trace): ^{1}H NMR (700 MHz, d_6-DMSO) δ 9.08–8.40 (m, 3H), 8.01–7.38 (m, 4H), 7.38–6.92 (m, 9H), 4.39–3.50 (m, obscured by H_2O peak, baseline correction shows 5H), 3.01–2.79 (m, 2H), 2.79–2.65 (m, 1H), 2.65–2.53 (m, 1H), 2.48–2.40 (m, 1H), 2.25–2.17 (m, 1H), 2.12–1.93 (m, 1H), 1.62–1.48 (m, 3H), 1.48–1.37 (m, 1H), 1.36–1.20 (m, 2H), 1.08–0.85 (m, 8H), 0.84–0.63 (m, 3H); HRMS (ESI) calcd for $C_{33}H_{45}N_7O_6$ 636.3504; found, 636.3501.

(*S*)-Methyl 3-(2-Bromophenyl)-2-((*tert*-butoxycarbonyl)-amino)propanoate (66). To a suspension of (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(2-bromophenyl)propanoic acid (**65**) (4.0 g, 11.6 mmol) and $NaHCO_3$ (1.95 g, 23.2 mmol) in DMF (39 mL), methyl iodide (3.63 mL, 58.1 mmol) was added and stirred at room temperature for 12 h. The reaction mixture was poured into water (100 mL) and extracted with EtOAc (2 × 150 mL). The combined organic phases were washed with brine (100 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by an automated system (Flash 40+M column; hexanes/EtOAc 100:0 to hexanes/EtOAc 85:15) to give ester **66** (4.06 g, 11.33 mmol, 98% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54 (d, J = 7.9 Hz, 1H), 7.26–7.18 (m, 2H), 7.10 (t, J = 6.8 Hz, 1H), 5.07 (d, J = 6.7 Hz, 1H), 4.64 (dd, J = 13.7, 7.3 Hz, 1H), 3.71 (s, 3H), 3.30 (dd, J = 13.6, 5.8 Hz, 1H), 3.10 (dd, J = 13.6, 8.4 Hz, 1H), 1.37 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.3, 154.9, 136.0, 132.8, 131.2, 128.5, 127.4, 125.0, 79.8, 53.5, 52.3, 38.6, 28.2; HRMS (ESI) calcd for $C_{15}H_{21}BrNO_4$ 358.0654; found, 358.0649.

(*S*)-Methyl 2-((*tert*-Butoxycarbonyl)amino)-3-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanoate (67). In a 250 mL flask was (*S*)-methyl 3-(2-bromophenyl)-2-((*tert*-butoxycarbonyl)amino)propanoate (**65**) (4.06 g, 11.33 mmol), $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$ (415 mg, 0.567 mmol), B_2pin_2 (4.32 g, 17.0 mmol), and KOAc (4.45 g, 45.3 mmol) in degassed dioxane (113 mL). The flask was sealed and heated to 85 °C for 3 h. The reaction mixture was poured into brine/water (1:1, 80 mL) and extracted with EtOAc (2 × 100 mL). The combined organic layers were washed with brine (80 mL), dried (Na_2SO_4), and concentrated in vacuo; the residue was purified by a Biotage system (Flash 40+M column; hexanes/EtOAc 91:9 to hexanes/EtOAc 80:20) yielding boronic ester **67** and Boc-Phe-CO_2Me (2.84 g). This mixture was submitted to HPLC purification yielding boronic ester **67** (1.62 g, 4.0 mmol, 35%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.81 (d, J = 7.2 Hz, 1H), 7.40 (dt, J = 7.6, 1.2 Hz, 1H), 7.29–7.21 (m, 3H), 5.95 (d, J = 8.1 Hz, 1H), 4.37 (ddd, J = 10.7, 8.1, 4.2 Hz, 1H), 3.75 (s, 3H), 3.29–3.23 (m, 1H), 3.20 (dd, J = 13.3, 4.2 Hz, 1H), 1.39 (s, 6H), 1.38 (s, 6H), 1.32 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.3, 155.5, 143.5, 136.1, 131.4, 130.0, 126.1, 84.0, 79.2, 56.2, 52.0, 37.1, 28.2, 24.9, 24.6; HRMS (ESI) calcd for $C_{21}H_{33}BNO_6$ 406.2395; found, 406.2402.

(*S*)-2-((*tert*-Butoxycarbonyl)amino)-3-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanoic Acid (64). To a stirred solution of (*S*)-methyl 2-((*tert*-butoxycarbonyl)amino)-3-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanoate (**67**) (405 mg, 1.0 mmol) in MeOH (4 mL) was added $LiOH{\cdot}H_2O$ (121 mg, 3.0 mmol) in H_2O (4 mL) at rt. The mixture was stirred at the same temperature for 50 min. The reaction mixture was acidified with 1 M aqueous HCl to pH ~ 2. The aqueous layer was extracted with EtOAc (3 × 50 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo providing crude acid **64**. Because of the instability of this material, it was carried forward without further purification.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR data for compounds **7**–**28**, **38**–**42**, **50**–**52**, and **66**, and ^{1}H NMR and HPLC data for compounds **31**–**35**, **43**–**49** and **55**–**63**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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