Total Synthesis of Motuporin and 5-[L-Ala]-Motuporin

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Total synthesis of the cyclic peptide hepatotoxin motuporin is described, including an efficient synthesis of the constituent amino acid Adda. Three strategies to motuporin are outlined with their relative strengths and weaknesses. Cyclization of the linear peptide precursor was found to proceed moderately well for peptides containing the *N*-methyldehydrobutyrine residue masked as a threonine, but significant C-terminal epimerization occurred in the presence of the dehydroamino acid. Replacement of the *N*-methyldehydrobutyrine residue by L-alanine was explored to assess the contribution of this dehydroamino acid to the biochemical activity of motuporin. Some epimerization also was observed during cyclization of the alanine-containing peptide. Synthetic motuporin and both isomers of 5-[L-Ala]-motuporin inhibit the activity of protein phosphatase-1 (PP1) in rat adipocyte lysates with comparable IC_{50} values. These results indicate that the *N*-methyldehydrobutyrine residue is not essential for PP1 inhibition.

Introduction

Motuporin (**1**, Figure 1) is a cyclic pentapeptide isolated from the marine sponge *Theonella swinhoei* (Gray).[1] It is cytotoxic toward a variety of human cancer cells in vitro and inhibits protein phosphatase type 1 (PP1) with K_d < 1 nM. The structure of motuporin was determined using NMR spectroscopy and mass spectrometry. It bears remarkable resemblance to the cyanobacterial natural products known as nodularins[2,3] and is related to the heptapeptide microcystins (e.g., compounds **2**−**4**, Figure 1).[4−7] Specifically, these cyclic peptides all contain the unusual amino acid (2*S*,3*S*,8*S*,9*S*,4*E*,6*E*)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (Adda),[2] an α,β-unsaturated amino acid such as *N*-methyldehydroalanine (mdha) or *N*-methyldehydrobutyrine (mdhb), an *iso*-linked D-glutamate, and an *iso*-linked D-aspartate or β-methyl D-aspartate residue. Nodularins and microcystins are potent, competitive inhibitors of protein phosphatases PP1 and PP2A, displaying a slight selectivity for the latter.[8−10] In animals, they are hepatotoxic as a consequence of their accumulation in the liver, possibly through bile acid transporters.[11] This activity is believed to account for the death of livestock that drink from water contaminated with cyanobacterial blooms,[12] and recently the use of contaminated water in dialysis led to the death of 50 human patients.[13] Moroever, increased incidence of cancer in regions where cyanobacteria proliferate may be related to the presence of these toxins, since microcystin-LR has been shown to promote tumor formation in mice.[14] The antineoplastic activity of motuporin[1] and the tumor-promoting activity of microcystin-LR[15,16] and nodularin[17] represent an interesting paradox in the biochemical profiles of these peptides and suggest that the biological outcome of phosphatase inhibition is critically dependent upon cellular localization and the specific phosphatase that is targeted. The structural differences between microcystin-LR and motuporin may contribute to a difference in localization of these compounds in tissues or cells. Thus, interest in these natural products centers on their use as biochemical tools for studying the functions of phosphatases in cells and their role as lead

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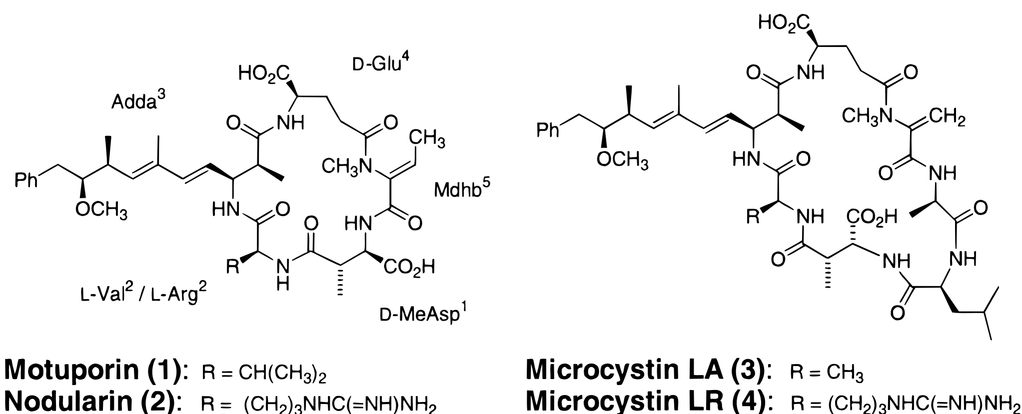

Figure 1.

compounds for the development of subclass specific phosphatase inhibitors.

A crystal structure of microcystin-LR bound to PP1 was published in 1995.[18] The Adda residue lodges in a hydrophobic groove on the surface of the protein, and the glutamate α-carboxyl group and the adjacent amide carbonyl make hydrogen bonds to two metal-coordinated water molecules. A covalent bond is formed between the inhibitor and the protein through conjugate addition of the thiol from cysteine-273 to the dehydroalanine in the cyclic peptide. Microcystin undergoes very little conformational adjustment upon binding to PP1, suggesting that the peptide is preorganized for binding to this enzyme. Motuporin contains the essential features of microcystin that appear to be required for binding to PP1, and it adopts a similar three-dimensional structure.[19−22] Molecular modeling studies suggest that motuporin binds to PP1 in a manner similar to microcystin-LR, although the mdhb *β*-carbon in PP1-bound motuporin is predicted to rest approximately 7 Å from the cysteine-273 thiol, accounting for the observation that motuporin does not covalently modify the protein. Structural differences such as this may be significant with respect to binding affinity and phosphatase selectivity, and synthetic approaches to the microcystin and nodularin peptides are expected to facilitate further investigations of their structural requirements for phosphatase inhibition and selectivity.

We initiated a program to synthesize motuporin with the intention of developing a convergent route that could be adapted to the preparation of other nodularin and microcystin natural products, as well as related analogues. Our general strategy has been described previously.[23] Prior to, and during the course of our work, several other groups have published the results of their studies in this area, including syntheses of Adda,[24−30] the first total synthesis of motuporin,[31] and a total synthesis of microcystin-LA.[32] We have published a synthetic route to *N*-Boc-Adda methyl ester[33] and the structurally related *β*-methyl-D-aspartate.[23] Herein, we describe the optimization of this chemistry and elaboration of these fragments to provide synthetic motuporin and a related analogue, 5-[L-Ala]-motuporin.

Synthetic Strategy. At the outset of this work, we proposed to prepare motuporin in a convergent manner through the assembly and cyclization of linear pentapeptide dimethyl ester **5**. Installation of the dehydrobutyrine double bond was anticipated to be performed late in the synthesis, although we recognized that the synthetic route could be adapted to incorporate this double bond earlier. We chose to cyclize the linear precursor at the C-terminus of the valine residue to achieve maximum convergency. This approach would allow incorporation of the Adda residue as the last residue of the pentapeptide to achieve the shortest linear synthesis. Moreover, we reasoned that none of the peptide bonds in motuporin or its precursors displayed any special advantage over the others with regard to the chances of α-epimerization during cyclization and that closure at an unsaturated acid, if we chose to introduce the dehydrobutyrine double bond early, could present a significant hurdle. Our retrosynthetic analysis is summarized in Figure 2.

Synthesis of *N*-Boc-Adda Methyl Ester (9). The Adda residue is both unique to microcystins and nodularins and characteristic of these natural products. As such, the synthesis of this residue constitutes the foundation of any total synthesis of molecules in this class. For convergency, we chose to synthesize *N*-Boc-Adda methyl ester (**9**) via a Wittig or related olefination reaction between a nucleophile derived from bromide **10** and aldehyde **11** as shown in Figure 3. Ample literature precedent lends support to this approach and indicates

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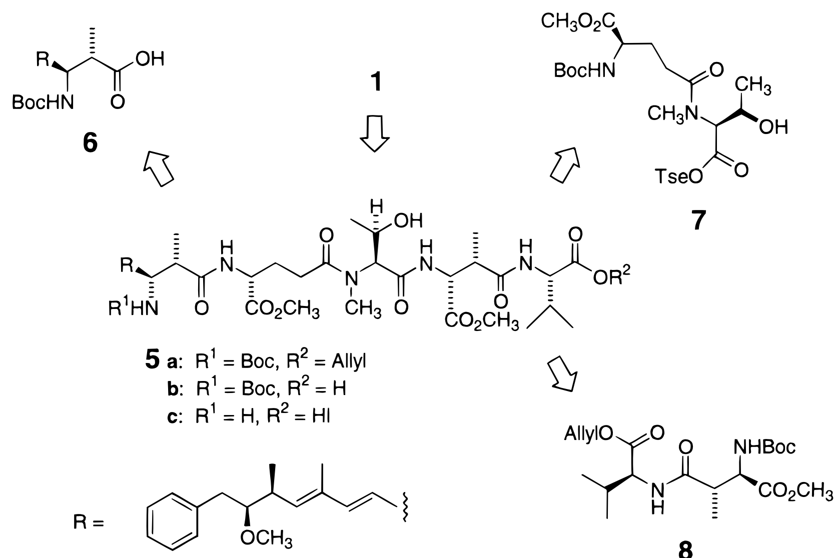


Figure 2.

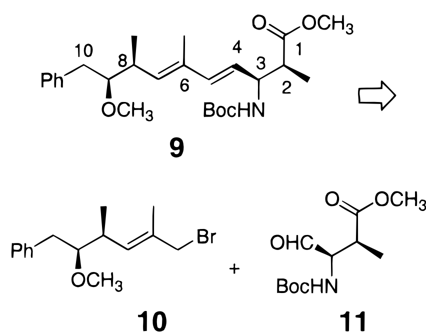


Figure 3.

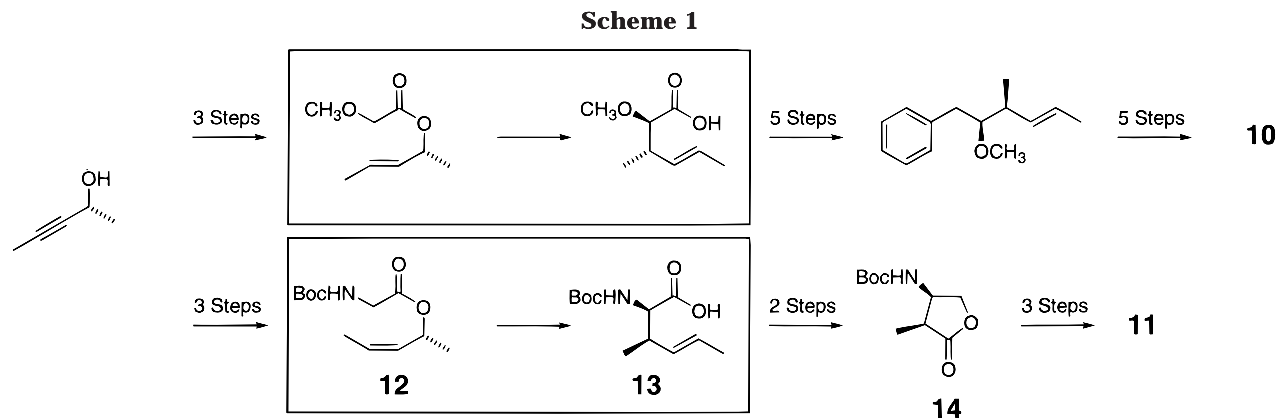
that we might be able to improve upon the yields and selectivities observed by previous workers.[24-26]

In a previously published approach to Adda,[33] we made extensive use of Ireland–Claisen rearrangement chemistry to produce both fragments of Adda from a common precursor, namely, (*R*)-3-pentyn-2-ol (Scheme 1). This approach contains several attractive elements, such as the derivation of both components of Adda from a common precursor and a high level of efficiency in the introduction of all four asymmetric carbons. However, overall this route was considered to be too long for large scale synthesis. Also, we encountered some difficulties in reproducing the hydrolysis of lactone **14** without epimerization of the adjacent C_2 chiral center. Therefore, we investigated ways of improving existing chemistry from our group and others to produce an efficient and convergent route to **9** that retained many of the strongest aspects of the previously published chemistry but avoided some of the pitfalls.

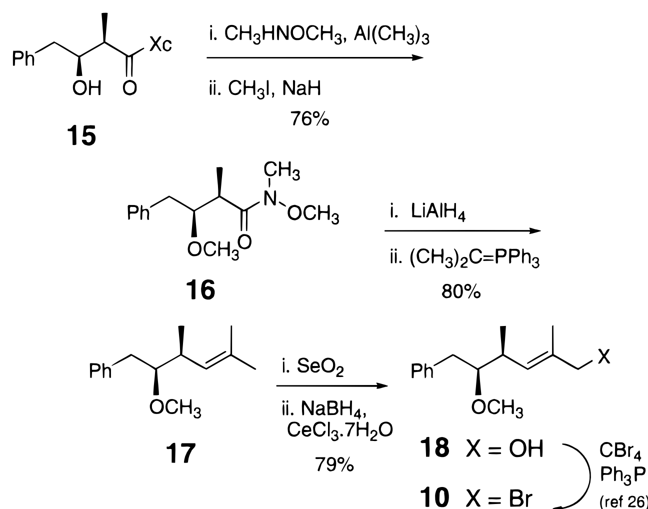
Upon reinvestigation of work by Rinehart[24] and Beatty,[26] we decided to retain the Evans aldol chemistry employed by these workers for the synthesis of the C_5–C_{10} fragment of Adda. Thus, aldol **15** was prepared in 69% yield and 95% de from phenylacetaldehyde, then transaminated with *N,O*-dimethylhydroxylamine in the presence of $Al(CH_3)_3$ (78% yield; Scheme 2), and methylated (NaH, MeI, 95%) to give compound **16**. Reduction of the Weinreb amide with $LiAlH_4$ gave an aldehyde that was homologated under standard Wittig conditions with isopropylidenetriphenylphosphorane to provide the alkene **17** in 80% yield for the two steps. As reported previously,[33] we then were able to employ the completely regioselective oxidation mediated by SeO_2 to convert this alkene to the *E*-allylic alcohol **18**. Some overoxidation occurs in the SeO_2 reaction, and consequently the crude reaction mixture was treated with $CeCl_3·7H_2O$ and $NaBH_4$ to reduce the enal back to the allylic alcohol prior to workup. Alcohol **18** is a known compound[26] and may be converted to the corresponding bromide **10** by treatment with triphenylphosphine and carbon tetrabromide.

The C_1–C_4 fragment of Adda was prepared from (*R*)-3-pentyn-2-ol as reported previously,[23] with some modi-

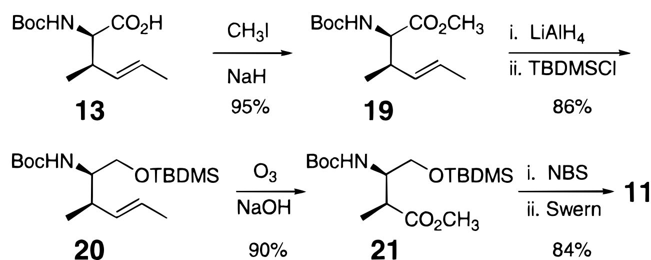
Scheme 1



Scheme 2



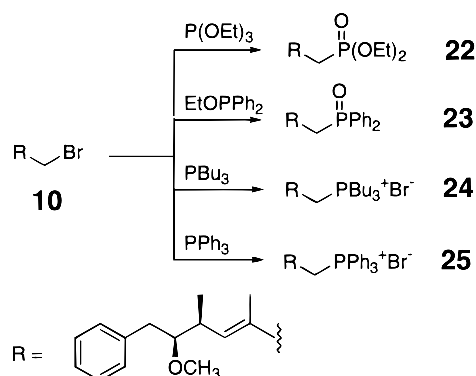
Scheme 3



fications. Acylation of the alkynol with *N*-Boc-L-glycine under standard dicyclohexylcarbodiimide conditions, followed by hydrogenation over Lindlar's catalyst, provided *cis*-olefin **12** in 88% overall yield (Scheme 1). Then, following the precedents established by Kazmaier,[34] compound **12** was treated with LDA at −78 °C, in the presence of zinc chloride, to promote a highly stereoselective Claisen rearrangement to acid **13**, which was isolated in 75% yield. This acid was converted to its corresponding methyl ester **19** in 95% yield using sodium bicarbonate and methyl iodide in DMF (Scheme 3).[35] Reduction of ester **19** with $LiAlH_4$, followed by protection of the product alcohol with TBDMSCl, gave silyl ether **20** (86% over two steps). Then, the alkene was converted directly to a methyl ester under the conditions described by Marshall[36] to provide compound **21**. Careful monitoring of this reaction was essential to avoid epimerization at the center adjacent to the newly formed ester carbonyl, and workup was performed immediately upon observation of the characteristic blue color of the ozone-saturated solvent without allowing the reaction mixture to warm to room temperature (Scheme 3).

Removal of the silyl group at this juncture was prone to butyrolactone formation under either acidic or basic conditions, and prior experience had taught us that saponification of this lactone was difficult to achieve without epimerization. Thus, extremely mild deprotection conditions were essential. The method of Taylor,[37] employing NBS in aqueous DMSO, proved eminently suitable for this task, and treatment of silyl ether **21** under

Scheme 4



these conditions cleanly provided the corresponding alcohol in a pleasing 84% yield, with no apparent lactone formation. Immediate oxidation of the seco-ester to the desired aldehyde **11** proceeded quantitatively under standard Swern conditions. No attempt was made to purify this sensitive aldehyde, which was used immediately in olefination reactions to form *N*-Boc-Adda methyl ester.

At the outset of our work, all previous syntheses of Adda had employed Wittig chemistry to install the disubstituted 4,5-carbon−carbon double bond.[24−26] A shortcoming of this approach is the relatively poor selectivity for *E*-double bond geometry that is obtained using standard Wittig reagents. Subsequently, several groups have employed palladium-mediated cross-coupling reactions to introduce the 4,5-double bond with excellent stereocontrol.[27,29,32] However, we were motivated to attempt improving upon the conventional olefination reaction by use of modified ylid chemistry. A survey of the literature indicated that we might achieve better *E*-selectivity by replacing the standard triphenylphosphorane with either a phosphine oxide or a trialkylphosphorane. The use of tributylphosporane looked particularly promising on the basis of previous work by Vedejs.[38−40]

Bromide **10** was transformed to phosphonate **22**, phosphine oxide **23**, and tributylphosphorane **24** (Scheme 4), and each of these reagents was explored as a potential coupling partner for aldehyde **11** with the following results. Phosphonate **22** could be deprotonated with ^{n}BuLi to give a characteristic red anion but was insufficiently reactive to couple with aldehyde **11**. The anion formed from phosphine oxide **23** and either ^{n}BuLi or ^{t}BuLi reacted with aldehyde **11** to provide a product, which was tentatively identified as the result of anion acylation by the ester function in compound **11**. Finally, we were disappointed to find that tributylphosphorane **24** failed to react with aldehyde **11**, even upon warming, following deprotonation with ^{n}BuLi, or ^{t}BuOK and 18-crown-6. Under most of these reaction conditions, aldehyde **11** was destroyed during the reaction, and the instability of this compound may be responsible for the failure of the attempted olefination reactions.

Faced with these results, we eventually resorted to attempting to optimize the known Wittig reaction between triphenylphosphorane **25** and aldehyde **11**. Un-

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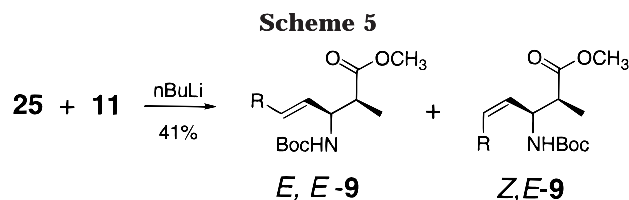
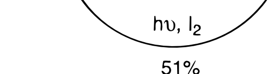
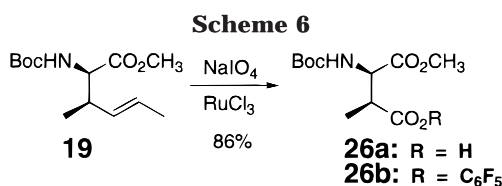
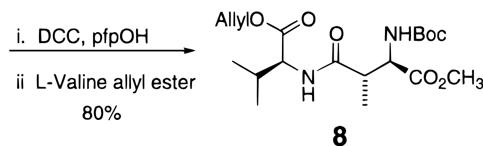
Scheme 5



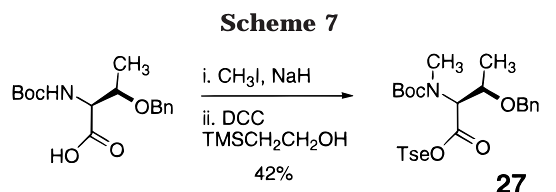


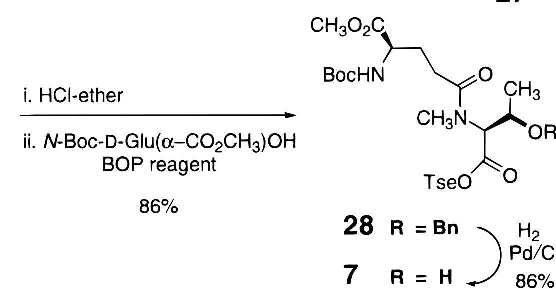
Scheme 6

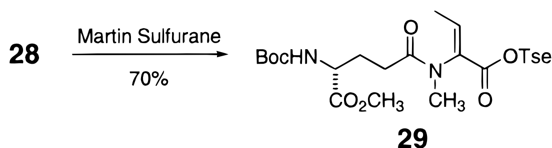




Scheme 7







fortunately, no significant improvement in *E*/*Z*-selectivity could be achieved either by using "salt-free" conditions or by performing the reaction at higher temperatures, suggesting that the product mixture may represent the result of kinetic control. Under our best conditions, a 2:1 mixture of *E* and *Z* isomers of *N*-Boc-Adda methyl ester was obtained and after careful chromatographic separation, the desired *E*-isomer (*E*,*E*-**9**) was isolated in a relatively poor 41% yield (Scheme 5).

Unwilling to accept this low yield at such an early stage in our synthesis we determined to explore conditions for isomerizing the side product *Z*,*E*-isomer of *N*-Boc-Adda methyl ester (*Z*,*E*-**9**) to the corresponding *E*,*E*-isomer.[41] These investigations proved fruitful when it was found that tungsten lamp irradiation of a solution of the diene in CH_2Cl_2 containing a catalytic amount of iodine promoted a clean isomerization of the *Z*,*E*-diene to the desired *E*,*E*-diene (Scheme 5). This reaction appears to reach an equilibrium in which the *E*,*E*-diene is preferred, and on a preparative scale the *E*,*E*-diene was isolated in an acceptable 51% yield, increasing the overall conversion of alcohol **10** to *N*-Boc-Adda methyl ester (**9**) to 50%.

Synthesis of the *iso*-D-β-Methylaspartyl-L-valine Dipeptide Fragment. As reported previously, the β-methylaspartate residue that occurs in microcystins and nodularins, including motuporin, may be obtained from an intermediate that is common to our synthesis of Adda. Thus, alkene **19** was transformed to carboxylic acid **26** by treatment with $NaIO_4$ and $RuCl_3$,[42] then converted to its pentafluorophenyl ester, and coupled with L-valine allyl ester to provide dipeptide **8** in 80% yield (Scheme 6).

Synthesis of *iso*-D-Glutamyl-Containing Dipeptides. In our original synthetic plans, we envisaged incorporating the mdhb residue required for motuporin in the form of an L-threonine residue that could be dehydrated in the penultimate step of the synthesis. Late introduction of this double bond would avoid any potential complications due to conjugate addition to this center. A similar approach was initiated by Valentekovich and Schreiber, who derived this residue from D-threonine, although these workers eventually achieved dehydration concomitantly with ester saponification to provide the product in one step following cyclization of the peptide.[31,43] In the event, we found that dehydration of the threonine residue late in the synthesis presented a number of problems, and consequently we simultaneously examined incorporation of the mdhb residue early in the synthesis as a component of the glutamyl-containing fragment used for our assembly of the macrocycle (see below).

Commercially available *N*-Boc-*O*-benzyl-L-threonine was converted to *N*-Boc-*N*-Me-*O*-benzyl-L-threonine in 75% yield under the conditions developed by Benoiton,[44] and the carboxyl group was protected as its trimethylsilyl ethyl (Tse) ester **27** (Scheme 7). Following removal of the Boc group using HCl-saturated ether, this residue was condensed with *N*-Boc-glutamate α-methyl ester[45] in the presence of the BOP reagent to provide dipeptide **28** in 86% yield. The benzyl group in this dipeptide was removed by hydrogenolysis over 10% Pd/C in ethanol to give the desired alcohol **7** in 86% yield. This fragment was either deprotected at the C-terminus by treatment with TBAF and used in the synthesis as a glutamyl-*N*-methylthreonine dipeptide or dehydrated prior to removal of the Tse group and incorporated as a glutamyl-*N*-methyldehydrobutyrine dipeptide. Initial attempts to dehydrate compound **28** using Burgess salt,[46] or Ph_3P and DEAD,[47] gave no reaction, even upon warming; however, dehydration was finally achieved using the Martin sulfurane.[48,49] Thus, on treating dipeptide **28** with Martin

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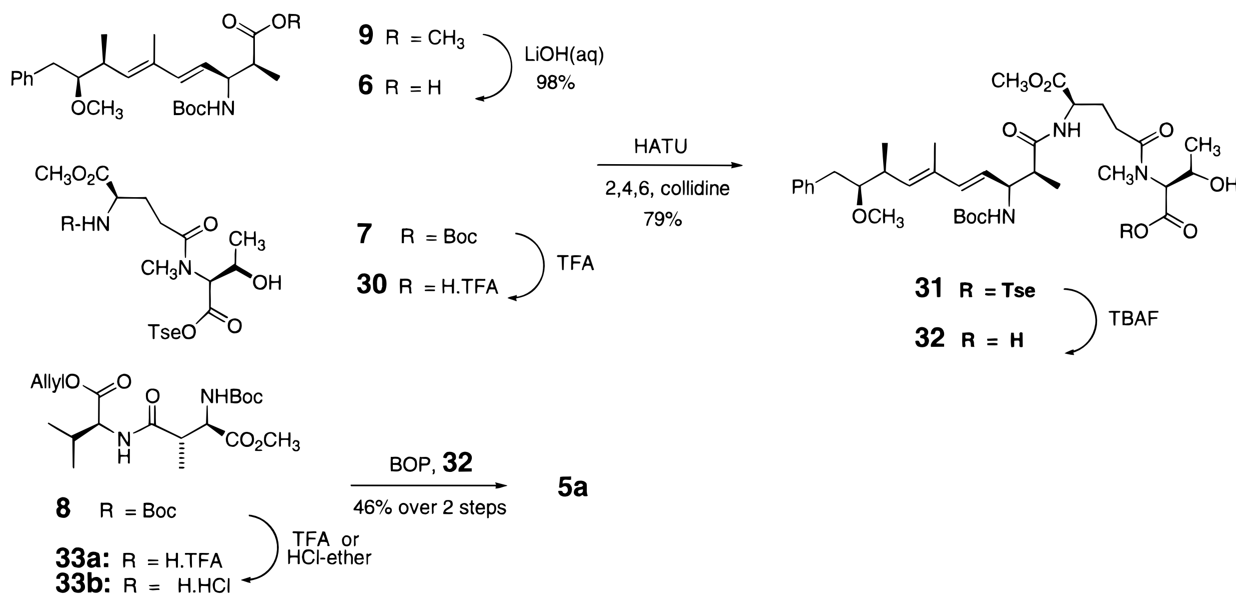
Scheme 8



sulfurane in DMF at 120 °C, clean formation of a dehydrobutyrine-containing peptide was observed, giving **29** in 70% yield. Martin sulfurane is known to display a strong preference for a *trans*-coplanar disposition of the leaving groups in the dehydration of secondary alcohols, and therefore we anticipated that the product of this dehydration should contain the desired (*Z*)-methyldehydrobutyrine. That this was indeed the case was established by comparison of the chemical shift of the vinylic proton with similar examples in the literature and by analysis of the ^{1}H and ^{13}C NMR spectra of compound **29** in comparison with those of motuporin[1] and other known *N*-acyl crotonates.[50] Compound **29** exhibits a vinylic proton chemical shift of 7.00 ppm (quartet) and a vinylic methyl chemical shift of 1.81 ppm. These values are consistent with *Z*-olefin geometry and closely match the correponding chemical shifts for motuporin (7.02 and 1.72 ppm, respectively). As further evidence for the *Z*-double bond geometry in **29**, the $^{1}H-^{13}C$ coupling constant between the dehydrobutyrine carbonyl and vinylic proton is 2.7 Hz. A coupling constant of ~10 Hz would be expected for the *E*-isomer.[51,52]

Assembly of the Linear Pentapeptide. We have explored several strategies for the assembly of linear pentapeptide precursors to the motuporin macrocycle. These strategies differ in the order of fragment assembly and in the timing of the dehydration step to install the mdhb double bond. Because we initially experienced some difficulty in forming an amide bond between the C-terminus of *N*-Boc-Adda and an appropriate tetrapeptide, we were led to explore the coupling of *N*-Boc-Adda with dipeptide fragment **7** (Scheme 8). Saponification of *N*-Boc-Adda methyl ester (**9**) using aqueous lithium hydroxide provided the necessary carboxylic acid **6**. Meanwhile, treatment of dipeptide **7** with TFA in CH_2Cl_2 gave the complementary coupling partner **30**. It is worth noting that attempts to deprotect the amino terminus of **7** using HCl-saturated ether were unsuccessful, possibly as a result of the operation of an intramolecular *N,O*-transacylation reaction under these conditions. The coupling of compounds **6** and **30** was achieved using HATU[53,54] to provide tripeptide **31** in 79% yield, whereas the use of diphenylphosphinic chloride[55] in this step resulted in a mixture of similar products that was presumed to arise from epimerization of the chiral center at C-2 in the Adda residue. Subsequent removal of the trimethylsilylethyl group using TBAF and condensation with fragment **33a** using BOP[56] gave a 41% yield of the target pentapeptide **5a**. HATU proved to be slightly superior in this coupling step, providing 50% of the desired product. In contrast, reaction of the C-terminal pentafluorophenyl ester was slow and did not give a clean product.[57]

Although the above route was generally satisfactory, to attain a higher level of convergency in our synthesis, we returned to the strategy of assembling the pentapeptide from a tetrapeptide plus *N*-Boc-Adda as originally intended. Thus, the glutamyl-*N*-methylthreonine dipeptide ester **7** was deprotected with TBAF and condensed with **33** using HATU to form tetrapeptide **34** in 59% overall yield as shown in Scheme 9. Following removal of the Boc group using TFA,[58] *N*-Boc-Adda pentafluorophenyl ester (**35**) was attached in the presence of Hünig's base to produce pentapeptide **5a** in 69% yield (Scheme 10). Although the yields via this route are slightly lower, the opportunity to introduce the complex Adda residue later in the synthesis represents a significant advantage in terms of material throughput.

A third strategy for synthesis of the pentapeptide was developed to simplify the final steps of the synthesis when it was found that dehydration of the threonine

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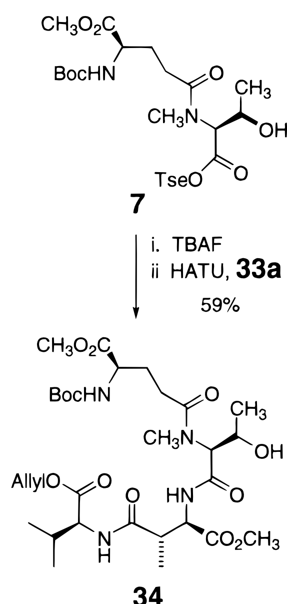
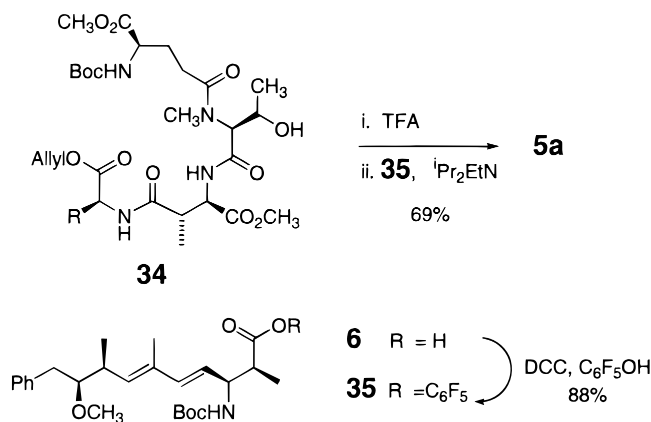
(58) The use of HCl/ether in this deprotection resulted in problems similar to the ones observed for compound **7**, most likely as a result of *N,O*-transacylation.

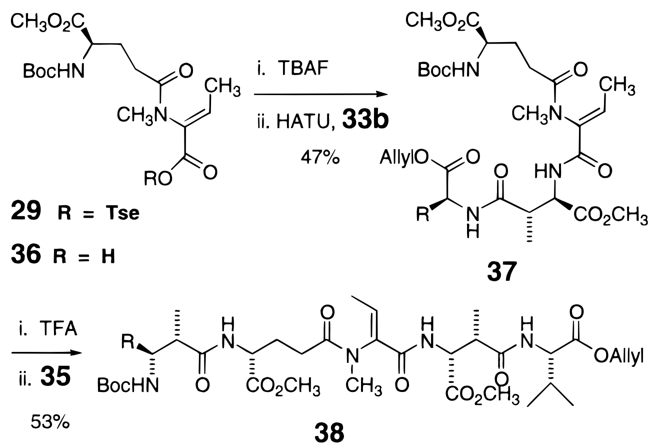
Scheme 9



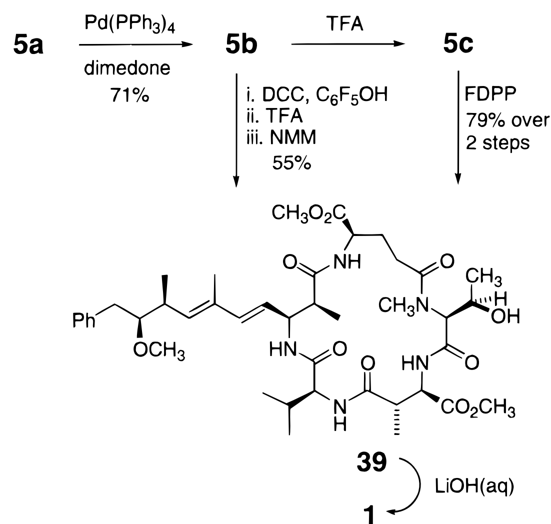
Scheme 10



Scheme 11



Scheme 12



residue late in the synthesis presented some difficulties and appeared to contribute to a loss of purity due to side reactions (see below). Thus, deprotection of the C-terminus of dipeptide **29** using TBAF, followed by condensation with fragment **33** using HATU, provided tetrapeptide **37** in a modest 47% yield. The low yield for this step is not surprising given the known difficulty of coupling to α,β-unsaturated acids.[59] Following removal of the Boc group in TFA/CH_2Cl_2, the tetrapeptide was coupled with *N*-Boc-Adda pentafluorophenyl ester **35** in the presence of diisopropylethylamine to form the linear pentapeptide **38** in 53% yield (Scheme 11).

Macrocyclization Studies. Cyclization of pentapeptide **5** was investigated using a variety of reagents to ascertain the best conditions for formation of the macrocycle (Scheme 12). Cleavage of the allyl ester in **5a** was achieved in 93% yield using $Pd(PPh_3)_4$ and dimedone to give **5b**, and acidolytic removal of the Boc group followed using TFA/CH_2Cl_2 to provide **5c**. Cyclization in the presence of pentafluorophenyl diphenylphosphinate (FDPP)[60,61] proceeded smoothly and consistently to provide the macrocycle in a pleasing 79% yield. In contrast, use of diphenylphosphoryl azide[62] instead of FDPP only proceeded to a meager 26% yield. Alternatively, treatment of **5b** with pentafluorophenol and DCC converted the C-terminus to its pentafluorophenyl ester. When this ester was treated with TFA/CH_2Cl_2 to remove the Boc group, then dissolved in CH_2Cl_2, and added slowly to a solution of NMM in the same solvent, macrocycle **39** was obtained in approximately 55% yield over the three steps. This approach provided a sample of the macrocycle that was slightly cleaner than the one obtained using FDPP on a single occasion, but unfortunately this result was not reproducible.

Macrocyclization of the mdhb-containing pentapeptide **38** was anticipated to proceed more readily than cyclization of the threonine-containing peptide because of the additional conformational constraint imposed by the presence of the mdhb double bond. In the event, cyclization was achieved under high dilution conditions (1 mM) via the pentafluorophenyl ester as shown in Scheme 13, but a 4:1 mixture of two similar products was obtained. Chromatographic separation of this mixture, followed by NMR and MS analysis, resulted in assignment of the minor component of this mixture as motuporin dimethyl ester (**40**) on the basis of its spectral similarity to material

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Scheme 13

38 → i. Pd(PPh$_3$)$_4$, dimedone; ii. DCC, C$_6$F$_5$OH; iii. TFA; iv. NMM; 10% → 40 → LiOH(aq) → 1

R = Ph, OCH$_3$

prepared by Anderson from the natural product.[1] The major component was tentatively assigned as a diastereomer of **40** resulting from epimerization at the valine α-carbon.

In summary, both FDPP and the pentafluorophenyl ester route provide good yields of the threonine-containing macrocycle **39**, with the latter approach giving a cleaner product. In contrast, cyclization of the dehydrobutyrine-containing peptide **38** proceeds primarily with epimerization and gives the desired macrocycle **40** only as a minor product.

Saponification of the Methyl Esters: Synthesis of Motuporin. To prepare motuporin from macrocycle **39**, it was necessary to dehydrate the threonine residue and saponify the two methyl esters. Initially, we proposed to perform the dehydration in a separate step prior to saponification, because we expected that this route would permit milder conditions and a shorter reaction time to be employed in the final step, possibly minimizing the formation of side products. However, model studies with compound **28** indicated that this dehydration would be difficult to achieve, and an attempt to dehydrate macrocycle **39** using the Martin sulfurane was unsuccessful.[63] Consequently, because we only had small quantities of material available, we elected to exploit the chemistry of Valentekovich and Schreiber and perform the dehydration concomitantly with the saponification.[31] Thus, dimethyl ester **39** was treated LiOH in aqueous methanol,[32,64] and the product was purified by HPLC using a mixture of methanol and 0.7% Na_2SO_4(aq). These conditions provided a sample of motuporin disodium salt that was ~95% pure by HPLC and that coeluted with an authentic sample of motuporin provided by Prof. Raymond Anderson. The FABMS of this sample matches that expected for motuporin disodium salt, and the ^{1}H NMR spectrum is consistent with previously published data but is contaminated by small amounts of aliphatic impurities.

The final step in this synthesis is difficult to follow. Saponification of the two methyl esters appears to proceed rapidly; however, the dehydration reaction is slower, and previous workers have indicated that the double bond equilibrates under the reaction conditions, requiring a longer reaction time to obtain a single double bond isomer. We reasoned that leaving the saponified cyclic peptide under basic conditions for longer than necessary to cleave the ester functionalities was leading to the production of side products, lowering the overall yield of motuporin and making the final purification difficult. Therefore, we decided to adopt an alternative strategy for the synthesis of motuporin in which the mdhb double bond was introduced prior to the macrocylization step. We anticipated that the shorter reaction time that would be necessary for the final step in this synthesis would lead to a cleaner final product.

These considerations led to the preparation of mdhb-containing macrocycle **40**. Unfortunately, saponification of this compound gave rise to even more side products than before, making purification by RP-HPLC, preparative TLC, or ion-exchange chromatography especially difficult. Deprotection of macrocycle **40** by S_N2-type dealkylation[65,66] of the two methyl esters using either NaI or KOTMS[67] gave a crude mixture that appeared to contain a product of the correct mass but was unlikely to readily yield pure motuporin.

Some comments on the purification of motuporin are warranted. As a diacid, this cyclic peptide is particularly difficult to purify using standard methods. Reverse-phase HPLC using methanol−aqeuous TFA mixtures provided samples that eluted as a single peak, but invariably these samples were found to contain multiple components when analyzed under different solvent conditions. We have found that the most reliable and informative conditions for analyzing the purity of motuporin samples are 60:40 MeOH/0.7% Na_2SO_4(aq). Unfortunately, the large amount of Na_2SO_4 that is obtained following evaporation of product-containing fractions when these conditions are used for preparative purposes makes extraction of the pure peptide cumbersome. Other techniques such as ion-exchange chromatography and preparative TLC were found not to possess sufficient resolution to separate the similar components (probably diastereomers) of our product mixture.

In summary, several routes to motuporin have been explored, but none of these routes is entirely satisfactory. The dimethyl ester of motuporin was prepared cleanly and in good overall yield, but the final ester cleavage step gave complex product mixtures from which we were unable to extract pure motuporin. The best sample of motuporin that was obtained displays general spectroscopic similarity to authentic motuporin by NMR, but it is clearly not homogeneous and exhibits an optical rotation that is significantly lower than that observed for the natural product.

Synthesis of 5-[L-Ala]-Motuporin. As part of our studies of motuporin, we were interested in developing a convergent approach that would be amenable to the preparation of nodularin and microcystin analogues[68−73] for studying the structure−function relationships for inhibition of protein phosphatases by these molecules.

(63) Previous workers reported that they failed to achieve the selective dehydration of a D-threonine residue at the same position, see ref 43.

(64) Kaestle, K. L.; Anwer, M. K.; Audhya, T. K.; Goldstein, G. *Tetrahedron Lett.* **1991**, *32*, 327−330.

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(73) Maude, A. B.; Mehrota, A. P.; Gani, D. *J. Chem. Soc., Perkin. Trans. 1* **1997**, 2513−2526.

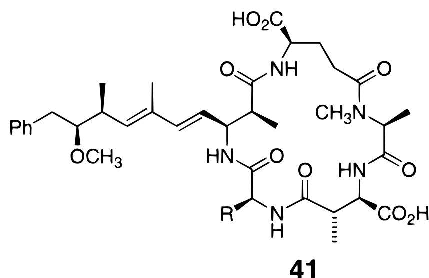

Figure 4.

It has been shown that, in microcystins, Adda and the acidic residues β-methylaspartate and glutamate participate in noncovalent bonding interactions with PP1,[18,74] and that the mdha residue is required for secondary covalent attachment to the protein.[75] In contrast, the mdhb residue in motuporin does not bind covalently to PP1.[20,76] Structural studies have indicated that the mdhb side chain in motuporin is turned downward and shifted away from the critical cysteine-273 residue, whereas the mdha side chain in microcystins binds in close proximity to the thiol and undergoes covalent attachment via a conjugate addition reaction.[20] As may be expected by comparison to motuporin and nodularin, the ability to covalently modify PP1 is not essential for inhibition of phosphatase activity by microcystins or for microcystin toxicity.[73,77] Sodium borohydride mediated reduction of the mdha residue in microcystin-LR gave a mixture of diastereomeric dihydromicrocystins, each of which is a nanomolar inhibitor of PP1.[73] These reports suggest that modified nodularins that lack the dehydrobutyrine double bond also should be potent PP1 inhibitors. Further comparison of the mdha-containing microcystins and mdhb-containing nodularins indicates that the vinylic methyl group in nodularins also may be unnecessary, although the location of this carbon in the complex with PP1 is known to differ between the two ring systems. To investigate these ideas, we decided to prepare 5-[L-Ala]-motuporin (**41**, Figure 4), in which the mdhb residue is replaced by L-alanine. This particular analogue was selected because L-alanine is readily available and incorporates the two modifications to the nodularin structure that were of interest, namely reduction of the double bond and removal of the methyl group that differs between the nodularin and microcystin ring systems. 5-[L-Ala]-Motuporin is only available by total synthesis.

Our approach to the total synthesis of 5-[L-Ala]-motuporin followed that for motuporin, differing only in the replacement of glutamyl-*N*-methylthreonine fragment **7** by a glutamyl-*N*-methylalanine-containing dipeptide. Thus, commercially available *N*-Boc-L-alanine was converted to *N*-Boc-*N*-Me-L-alanine in 90% yield under the conditions of Benoiton.[44] The carboxyl group was protected as a trimethylsilyl ethyl ester to give *N*-Boc-*N*-Me-L-alanine trimethylsilyl ethyl ester **42** in 89% yield (Scheme 14). Removal of the Boc group with TFA gave the corresponding amine TFA salt, which was coupled

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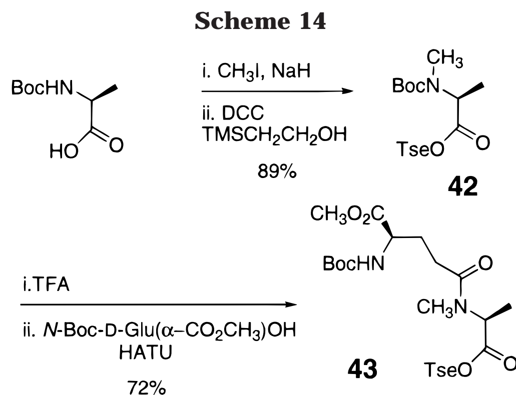
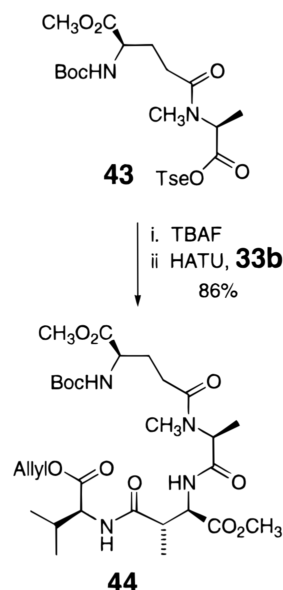
(77) Namikoshi, M.; Wook, B.; Sun, F.; Rinehart, K. L.; Evans, W. R.; Carmicheal, W. W. *Chem. Res. Toxicol.* **1993**, *6*, 151–158.

Scheme 14



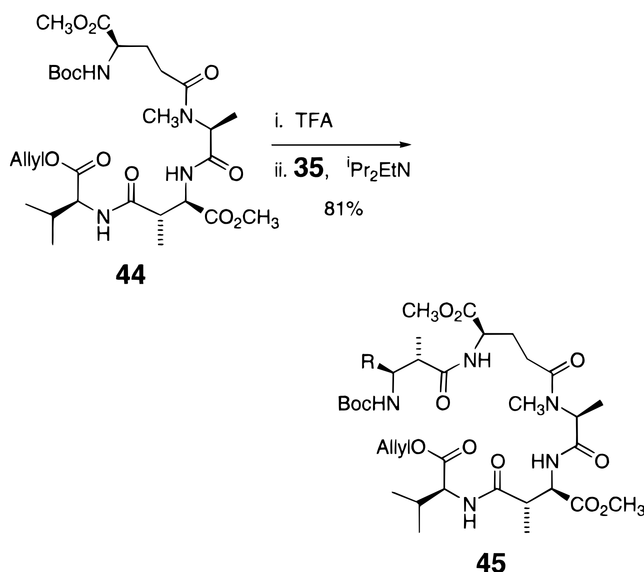
Scheme 15

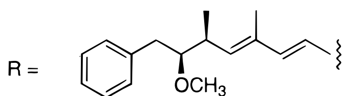


with *N*-Boc-D-glutamic acid-α-methyl ester using HATU to give dipeptide **43** in 72% yield. Removal of the Tse ester using TBAF and condensation of the resulting acid with fragment **33** in the presence of HATU gave tetrapeptide **44** in 86% yield (Scheme 15). Then, removal of the Boc group with TFA followed by treatment with *N*-Boc-Adda pentafluorophenyl ester (**35**) in the presence of diisopropyl ethylamine provided the pentapeptide **45** in 81% yield (Scheme 16). As for motuporin, deprotection of the linear peptide and macrocyclization was achieved via the C-terminal pentafluorophenyl ester under high dilution conditions to form the macrocycle **46** in a combined 27% yield for the four steps (Scheme 17). A minor cyclization product (18%) also was isolated and tentatively assigned as the valine α-carbon epimer (**47**). The structural assignments for these two molecules were made on the basis of their relative yields and spectral similarities between compound **46** and motuporin dimethyl ester **39**. Saponification of **46**, followed by HLPC purification, gave a product that displays NMR and mass spectral data consistent with 5-[L-Ala]-motuporin (**41**). For comparison, saponification of compound **47** was performed in a similar manner, to provide a product tentatively assigned as structure **48**.

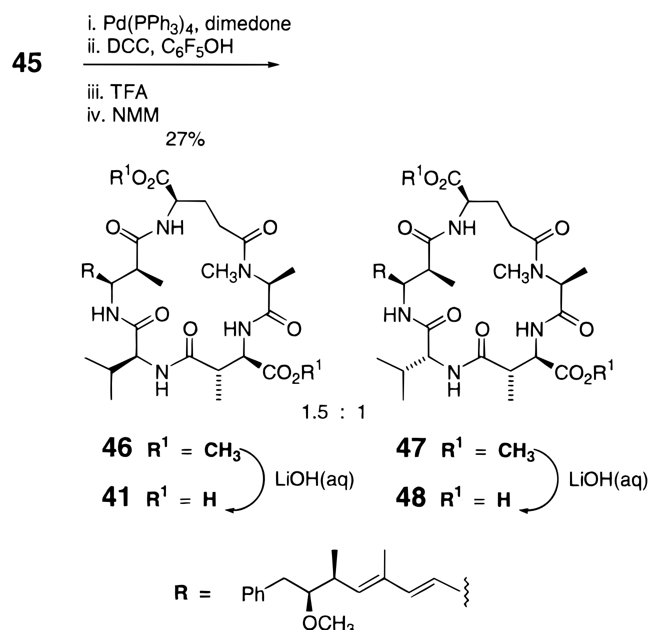
Enzyme Assays. Synthetic motuporin (**1**) and the two 5-[L-Ala]-motuporin isomers (**41 and 48**) were evaluated as inhibitors of protein phosphatase-1 (PP1) activity in rat adipocyte lysates[78] using microcystin-LR as a reference. Under the assay conditions employed, microcystin-

Scheme 16





Scheme 17



LR inhibits PP1 activity with $IC_{50} = 0.25$ nM. Synthetic motuporin displays an $IC_{50} = 4.0$ nM, compared to literature values of $IC_{50} < 1$ nM vs PP1 for the natural product[1] and $IC_{50} = 1.1$ nM vs PP2A for synthetic motuporin prepared by Valentkovich and Schreiber.[31] The small discrepancy in these values probably reflects differences in enzyme source and the assay conditions employed. Whereas rat adipocyte lysates were used in our experiments, previous workers have employed the purified PP1 catalytic subunit isolated from rabbit skeletal muscle.[79]

5-[L-Ala]-Motuporin is a slightly weaker inhibitor of PP1 activity than motuporin itself, with $IC_{50} = 18.4$ nM; nonetheless, the high level of activity shown by 5-[L-Ala]-motuporin indicates that the *N*-methyldehydrobutyrine residue in motuporin is not essential for the inhibition of PP1. Compound **48** is a marginally weaker inhibitor of PP1 than 5-[L-Ala]-motuporin (**41**), with $IC_{50} = 37.8$ nM. Because compounds **41** and **48** differ in potency by only a factor of 2, these data do not provide any further insight into the structures of these molecules. However, the observation that both **41** and **48** are potent inhibitors of PP1 bolsters our conclusion regarding the contribution of the *N*-methyldehydrobutyrine residue to PP1 inhibition and indicates that diastereomers of motuporin (and motuporin analogues) also may be effective PP1 inhibitors.

Conclusions

In conclusion, none of the above approaches to motuporin are entirely satisfactory. In each case, a major hurdle is presented by the purification of the final product following saponification of the methyl esters, which appears to occur with multiple side reactions. Small samples of motuporin have been obtained, although none with greater than approximately 95% purity. Notably, saponification of macrocycle **39** containing an L-threonine residue appears to be significantly less satisfactory than saponification of the equivalent macrocycle containing a D-threonine residue as prepared by Valentkovich and Schreiber. 5-[L-Ala]-Motuporin has been prepared from its dimethyl ester, and the yields for all except the final two steps are generally better than for motuporin. However, the cyclization gives a mixture of two products, and the final saponification is not a clean reaction, resulting in a difficult purification. Synthetic 5-[L-Ala]-motuporin is a potent inhibitor of PP1, indicating that the replacement of *N*-methyldehydrobutyrine by *N*-methylalanine is not severely detrimental to the activity of nodularin and motuporin cyclic peptides. We suggest that future approaches to motuporin and nodularin cyclic peptides should avoid synthesis of the methyl esters and instead rely on acid-labile carboxyl protecting groups such as *tert*-butyl esters. Moreover, our results indicate that motuporin analogues lacking the dehydroamino acid should retain inhibitory activity against protein phosphatases.

Experimental Section

Microcystin-LR was obtained from Calbiochem. All other reagents used were of the highest purity available from Aldrich. Solvents were HPLC grade. Anhydrous THF was distilled from sodium/benzophenone. Dichloromethane and acetonitrile were distilled from calcium hydride. Triethylamine and diisopropylethylamine were distilled and stored over 4 Å molecular sieves. NMR spectra are reported for solutions in $CDCl_3$ unless otherwise indicated. Residual chloroform was used as a reference and set at 7.26 ppm. Optical rotations were recorded at ambient temperature (23 °C).

(2*R*,3*S*)-3-Hydroxy-2-methyl-4-phenylbutan-(*N*-methyl-*O*-methyl)-hydroxamide. To the hydrochloride salt of *N,O*-dimethyl hydroxylamine (1.3 g, 13.34 mmol, 2.2 equiv) in CH_2Cl_2 (10 mL) under N_2 at −20 °C was added trimethylaluminum (2 M in CH_2Cl_2, 6.67 mL, 13.34 mmol, 2.2 equiv). After 5 min, a solution of **15** (2.14 g, 6.06 mmol) in CH_2Cl_2 (20 mL + 2 × 4 mL rinses) precooled to −60 °C was added via a cannula. The mixture was allowed to warm to 0−4 °C and

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stirred for 3 h. Upon completion of the reaction, the CH_2Cl_2 solution was poured into a mixture of ice and saturated sodium bicarbonate solution, and the aqueous layer was extracted with copious CH_2Cl_2. The combined organic extracts were washed with H_2O and then with brine and dried over anhydrous $MgSO_4$. Chromatography on SiO_2 (30−50% ethyl acetate in hexanes) provided the product as a yellow oil (1.12 g, 4.72 mmol, 78%): R_f = 0.25 (1:1, ethyl acetate/hexanes); $[\alpha]_D$ −14.1° (*c* 1, EtOH); IR (film) 3419, 2938, 1653, 1636, 1495, 1456, cm^{-1}; ^1H NMR (360 MHz) δ 7.21−7.39 (5H, m, aromatic), 4.13 (1H, ddd, *J* = 4, 7, 10 Hz, H-3), 3.7 (1H, br s, OH), 3.49 (3H, s, OCH_3), 3.17 (3H, s, NCH_3), 2.91 (1H, dd, *J* = 8, 14 Hz, H-4), 2.90 (1H, br s, H-2), 2.74 (1H, dd, *J* = 7, 14 Hz, H-4), 1.27 (3H, d, *J* = 7 Hz, CH_3); ^{13}C NMR (90 MHz) δ 178, 138.3, 129.1, 128.3, 126.2, 72.82, 61.1, 40.0, 37.3, 31.7, 10.0; MS *m/z* (rel int) 238 (100, M + 1); HRMS (CI) calcd for $C_{13}H_{20}NO_3$ (M + H) 238.1443, found 238.1426. Anal. Calcd for $C_{13}H_{19}NO_3$: C, 65.80; H, 8.07; N, 5.89. Found: C, 64.79; H, 8.06; N, 5.75. The chiral auxiliary (4*R*,5*S*)-(−)-4-methyl-5-phenyl-2-oxazolidinone also was recovered from this reaction; it elutes from the silica gel column after the hydroxamide product.

(2*R*,3*S*)-3-Methoxy-2-methyl-4-phenylbutan-(*N*-methyl-*O*-methyl)-hydroxamide (16). To a solution of 3-hydroxy-2-methyl-4-phenylbutan-(*N*-methyl-*O*-methyl)-hydroxamide (1.1 g, 4.64 mmol) in dry THF (20 mL) at 0 °C was added sodium hydride (60% in oil, 186 mg, 4.64 mmol, 1 equiv) in one portion. Methyl iodide (2.9 mL, 46.4 mmol, 10 equiv) was filtered into the reaction mixture through a pad of sodium carbonate. After 48 h of stirring at room temperature, the reaction mixture was poured into ice-cold saturated NH_4Cl solution, and the organic components were extracted into ethyl acetate. The combined organic extracts were washed with brine and dried over anhydrous $MgSO_4$. Column chromatography on SiO_2 (40% ethyl acetate in hexanes) provided the title compound as a yellow oil (890 mg, 3.55 mmol, 76%): R_f = 0.38 (1:1, ethyl acetate/hexanes); $[\alpha]_D$ −5.5° (*c* 1.0, $CHCl_3$); IR (film) 2974, 2937, 1660, 1455 cm^{-1}; ^1H NMR (300 MHz) δ 7.17−7.30 (5H, m, aromatic), 3.61, (1H, ddd, *J* = 3, 9, 9 Hz, H-3), 3.46 (3H, s, OCH_3), 3.25 (3H, s, OCH_3), 3.15, (3H, s, NCH_3), 2.85−2.91 (2H, m, H-4, H-2), 2.74 (1H, dd, *J* = 8, 15 Hz, H-4), 1.22 (3H, d, *J* = 9 Hz, CH_3); ^{13}C NMR (75 MHz) δ 139.1, 129.6, 128.1, 126.0, 84.2, 61.1, 58.9, 40.2, 39.0, 32.5, 13.6; MS *m/z* (rel int) 252 (26, M + H), 223 (24), 222 (100), 190 (20). Anal. Calcd for $C_{14}H_{21}NO_3$: C, 66.91; H, 8.42; N, 5.57. Found: C, 66.75; H, 8.46; N, 5.56.

(2*E*,2*S*,3*S*)-5-Methoxy-2,3-dimethyl-6-phenylhex-2-ene (17). To a solution of (2*R*,3*S*)-3-methoxy-2-methyl-4-phenylbutan-(*N*-methyl-*O*-methyl)-hydroxamide (1 g, 3.98 mmol) in dry THF (40 mL) at 0 °C under nitrogen was added lithium aluminun hydride (151 mg, 3.98 mmol, 1 equiv) in portions. This mixture was stirred at room temperature for 1 h and then recooled to 0 °C. Acetone (5 mL) was added and the stirring continued for 10 min. The whole mixture was then poured into an ice-cold mixture of 1:1 0.1 M citric acid (aqueous) and hexanes and stirred vigorously at 0 °C for 30 min. The aqueous and organic layers were separated, and the organic layer was washed with brine and then dried over anhydrous sodium sulfate. Filtration and evaporation gave **(2*R*,3*S*)-3-methoxy-2-methyl-4-phenylbutanal** as a colorless oil: R_f = 0.67 (2:3, ethyl acetate/hexanes); ^1H NMR (300 MHz) δ 9.65 (1H, d, *J* = 1 Hz, CHO), 7.17−7.29 (5H, m, aromatic), 3.86 (1H, ddd, *J* = 3, 6, 6 Hz, H-3), 3.27 (3H, s, OCH_3), 2.96 (1H, dd, *J* = 6, 14 Hz, H-4), 2.69 (1H, dd, *J* = 8, 14 Hz, H-4), 2.13−2.39 (1H, m, H-2), 1.18 (3H, d, *J* = 9 Hz, CH_3). This aldehyde was used without further purification. In a separate flask, isopropyltriphenylphosphonium iodide (1.72 g, 1 equiv) was suspended in dry THF (15 mL) under nitrogen and treated with ^{n}BuLi (2.49 mL, 1.6 M, 1 equiv) at −10 °C. After 30 min, a red-brown solution was obtained. This solution was cooled to −78 °C and added dropwise via a cannula to a solution of the aldehyde in dry THF (15 mL) under nitrogen at −78 °C. The color of the ylid initially dissipated upon addition to the aldehyde but eventually was retained. The resulting mixture was stirred at −78 °C for 1 h, removed to a −10 °C cold bath, and allowed to warm slowly to room temperature overnight. The reaction was quenched at 0 °C by the addition of 10% EtOH in THF (10 mL). The solution was concentrated to half volume, then supplemented with hexanes, and filtered to remove the triphenyl phosphine oxide. The filtrate was washed with water and then brine and dried over anhydrous magnesium sulfate. Column chromatography on SiO_2 (2−10% ethyl acetate in hexanes) provided the product as a yellow oil (690 mg, 3.12 mmol, 80%): R_f = 0.39 (1:19, ethyl acetate/hexanes); $[\alpha]_D$ −2.6° (*c* 2.0, $CHCl_3$); IR (film) 3028, 2968, 2928, 2871, 2825, 1496, 1453, cm^{-1}; ^1H NMR (360 MHz) δ 7.18−7.32 (5H, m, aromatic), 5.07 (1H, d, *J* = 8 Hz, H-3), 3.25 (3H, s, OCH_3), 3.14−3.19 (1H, m, H-5), 2.86 (1H, dd, *J* = 4, 14 Hz, H-6), 2.68 (1H, dd, *J* = 11, 14 Hz, H-6), 2.48−2.54 (1H, m, H-4), 1.73 (3H, s, vinylic CH_3), 1.56 (3H, s, vinylic CH_3), 1.02 (3H, d, *J* = 7 Hz, CH_3); ^{13}C NMR (90 MHz) δ 139.8, 131.3, 129.4, 128.0, 127.7, 125.8, 87.4, 58.5, 38.2, 36.6, 25.9, 17.9, 16.7; MS *m/z* (rel int) 218 (0.6, M^+), 135 (100); HRMS (EI) calcd for $C_{15}H_{22}O$ 218.1671, found 218.1668.

(2*E*,4*S*,5*S*)-5-Methoxy-2,4-dimethyl-6-phenylhex-2-en-1-ol (18). A solution of (2*E*,2*S*,3*S*)-5-methoxy-2,3-dimethyl-6-phenylhex-2-ene (690 mg, 3.17 mmol) and SeO_2 (702 mg, 6.33 mmol, 2 equiv) in EtOH (12 mL) was heated under reflux for 2 h. Additional SeO_2 (350 mg, 3.17 mmol, 1 equiv) was added, and heating was continued for an additional 5 h. The mixture was allowed to cool and then filtered through a glass wool plug to remove the black precipitate that had formed. The filtrate was cooled to 0 °C and $CeCl_3 \cdot 7H_2O$ (1.18 g, 3.17 mmol, 1 equiv) was added, creating a red solution. $NaBH_4$ (360 mg, 9.51 mmol, 3 equiv) was added portionwise, causing vigorous effervescence. This mixture was stirred for 30 min at 0 °C, diluted with EtOAc, and washed with saturated aqueous $NaHCO_3$ solution and then brine. The organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated to give a yellow oil (520 mg, 2.22 mmol, 70%): R_f = 0.21 (1:1, ethyl acetate: hexanes); $[\alpha]_D$ −3.8° (*c* 1.0, EtOH); IR (film) 3425, 2827, 1496 cm^{-1}; ^1H NMR (360 MHz) δ 7.14−7.30 (5H, m, aromatic), 5.32 (1H, d, *J* = 11 Hz, H-3), 3.99 (2H, s, H-1), 3.25 (3H, s, OCH_3), 3.17−3.29 (1H, m, H-5), 2.80 (1H, dd, *J* = 4, 14 Hz, H-6), 2.70 (1H, dd, *J* = 7, 14 Hz, H-6), 2.50−2.61 (1H, m, H-4), 1.57 (3H, s, vinylic CH_3), 1.02 (3H, d, *J* = 7 Hz, CH_3); ^{13}C NMR (90 MHz) δ 139.6, 134.7, 129.4, 128.6, 128.1, 125.9, 87.0, 68.8, 58.5, 38.1, 36.0, 16.4, 13.8; MS *m/z* (rel int) 252 (17, M + NH_4^+), 185 (100), 135 (92), 120 (20), 91 (23); HRMS (CI) calcd for $C_{15}H_{26}NO_2$ 252.1963, found 252.1968.

(2*R*)-3-Pentyn-2-yl *N*-Boc-Glycinate. To a solution of (2*R*)-3-pentyn-2-ol (1.5 g, 17.9 mmol) in CH_2Cl_2 (80 mL) was added *N*-Boc glycine (2.84 g, 16.2 mmol), DCC (3.69 g, 17.9 mmol), and DMAP (199 mg, 1.62 mmol), and the reaction mixture was stirred at room temperature for 3 h. The solid dicyclohexylurea was removed by filtration and washed with CH_2Cl_2 (2 × 50 mL). The filtrate was washed with 1 M HCl, saturated aqueous sodium bicarbonate, and brine. The organic layer was dried ($MgSO_4$), concentrated, and purified by chromatography on SiO_2 (8:1, hexane/ethyl acetate) to provide the title compound as a colorless oil (3.57 g, 91%): R_f = 0.26 (4:1, hexane/ethyl acetate); $[\alpha]_D$ +69.1° (*c* 3.49, $CHCl_3$); IR (film) 3348, 2980, 2938, 2255, 1757, 1515 cm^{-1}; ^1H NMR (300 MHz) δ 1.40 (9H, s), 1.49 (3H, s), 1.84 (3H, d, *J* = 2.0 Hz), 3.93 (2H, d, *J* = 4.8 Hz), 5.00 (1H, br s), 5.46−5.50 (1H, m); ^{13}C NMR (75 MHz) δ 21.5, 28.2 (3C), 42.6, 61.7, 76.6, 77.2, 79.7, 81.3, 155.3, 169.0; HRMS calcd for $C_{12}H_{19}NO_4$ [M + H]$^+$ 242.1392, found 242.1394. Anal. Calcd for $C_{12}H_{19}O_4N$: C, 59.74; H, 7.94; N, 5.81. Found: C, 59.45; H, 7.87; N, 5.87.

***Z*-(2*R*)-3-Penten-2-yl *N*-Boc-Glycinate (12).** To a solution of (2*R*)-3-pentyn-2-yl *N*-Boc-glycinate (3.48 g, 14.4 mmol) in ethyl acetate (100 mL) was added $Pd/BaSO_4$ (348 mg) and quinoline (348 mg), and the reaction mixture was hydrogenated at atmospheric pressure for 1.5 h. The catalyst was removed by filtration through a pad of Celite and rinsed with ethyl acetate (2 × 50 mL). The filtrates were concentrated and purified by chromatography on SiO_2 (8:1, hexane/ethyl acetate) to give **12** as a colorless oil (3.42 g, 97%): R_f = 0.33 (4:1, hexane/ethyl acetate); $[\alpha]_D$ −13.6° (*c* 1.94, $CHCl_3$); IR (film) 3388, 2981, 2931, 1754, 1715, 1511 cm^{-1}; ^1H NMR (300 MHz) δ 1.24 (3H, d, *J* = 5.9 Hz), 1.40 (9H, s), 1.73 (3H, d, *J* = 1.3

Hz), 3.88 (2H, t, $J = 5.2$ Hz), 5.00 (1H, br s), 5.34–5.41 (1H, m), 5.57–5.63 (1H, m), 5.71–5.76 (1H, m); ^{13}C NMR (75 MHz) δ 13.0, 20.3, 28.1 (3C), 42.5, 67.9, 79.4, 127.5, 129.6, 155.4, 169.2; HRMS calcd for $C_{12}H_{21}O_4N$ $[M + H]^+$ 244.1549, found 244.1541. Anal. Calcd for $C_{12}H_{21}O_4N$: C, 59.24; H, 8.70. Found: C, 59.20; H, 8.52; N, 5.80.

***E*-(2*S*,3*R*)-2-(*N*-Boc-amino)-3-methyl-4-hexenoic Acid (13).** ^{n}BuLi (19.20 mL, 30.7 mmol, 1.6 M in hexane) was added to a solution of diisopropylamine (5.10 mL, 36.3 mmol) in THF (30 mL) at −78 °C under N_2 and stirred at this temperature for 20 min. Compound **12** (3.40 g, 13.9 mmol) in THF (20 mL) was added via a cannula. After 5 min, a solution of $ZnCl_2$ (2.28 g, 16.7 mmol) in THF (20 mL) was added, and the reaction mixture was allowed to warm slowly to room temperature overnight. The yellow reaction mixture was treated with 1 M HCl (5 mL) and concentrated under vacuum to give an oily residue, which was dissolved in ether and washed with 1 M HCl. The ether layer was extracted with 1 M NaOH. The combined aqueous extracts were acidified with 1 M HCl and then back-extracted with ether. The ether extracts were dried ($MgSO_4$) and concentrated to provide **13** as a colorless oil (3.04 g, 89%): $[\alpha]_D$ +5.8° (*c* 0.6, $CHCl_3$); IR (film) 3423, 2981, 2931, 1715, 1504, cm^{-1}; ^{1}H NMR (300 MHz) δ 1.11 (3H, d, $J = 6.9$ Hz), 1.46 (9H, s), 1.69 (3H, d, $J = 6.3$ Hz), 2.68–2.78 (1H, m), 4.23–4.28 (1H, m), 4.91 (1H, d, $J = 8.3$ Hz), 5.28–5.35 (1H, m), 5.54–5.61 (1H, m); ^{13}C NMR (75 MHz) δ 16.9, 17.9, 28.3 (3C), 39.0, 58.1, 80.2, 127.6, 130.2, 155.8, 176.8; HRMS calcd for $C_{12}H_{21}O_4N$ $[M + H]^+$ 244.1549, found 244.1542.

Methyl *E*-(2*S*,3*R*)-2-(*N*-Boc-Amino)-3-methyl-4-hexenoate (19). To a solution of acid **13** (1.80 g, 7.4 mmol) in DMF (40 mL) was added solid $NaHCO_3$ (1.25 g, 14.8 mmol) and methyl iodide (2.30 mL, 37.0 mmol), and the mixture was stirred at room temperature for 24 h. Following dilution with water, the crude product was extracted with ethyl acetate, and the organic extracts were dried ($MgSO_4$), concentrated, and purified by chromatography on SiO_2 (8:1, hexane/ethyl acetate) to give the title compound as a colorless oil (1.82 g, 95%): R_f = 0.36 (4:1, hexane/ethyl acetate); $[\alpha]_D$ −6.0° (*c* 1.74, $CHCl_3$); IR (film) 3381, 2963, 2925, 1750, 1500 cm^{-1}; ^{1}H NMR (300 MHz) δ 1.07 (3H, d, $J = 6.6$ Hz), 1.45 (9H, s), 1.67 (3H, d, $J = 5.9$ Hz), 2.67–2.69 (1H, m), 3.74 (3H, s), 4.21–4.23 (1H, m), 4.91 (1H, d, $J = 6.7$ Hz), 5.22–5.30 (1H, m), 5.48–5.52 (1H, m); ^{13}C NMR (75 MHz) δ 16.8, 17.8, 28.2 (3C), 39.3, 51.7, 58.1, 79.5, 127.1, 130.4, 155.4, 172.2; HRMS calcd for $C_{13}H_{23}O_4N$ $[M + H]^+$ 258.1705, found 258.1698. Anal. Calcd for $C_{13}H_{23}O_4N$: C, 60.68; H, 9.01; N, 5.44. Found: C, 60.63; H, 8.88; N, 5.29.

α-Methyl (2*R*,3*S*)-*N*-Boc-3-Methylaspartate (26a). To a solution of alkene **19** (3.0 g, 11.66 mmol) in CCl_4 (25 mL), CH_3CN (25 mL), and H_2O (35 mL) was added sodium periodate (10.0 g, 46.6 mmol) and ruthenium(III) chloride (54 mg, 2.2 mol %). This mixture was stirred at room temperature overnight. Upon completion of the reaction, the mixture was diluted with CH_2Cl_2 (100 mL), and the phases were separated. The aqueous phase was extracted with CH_2Cl_2 (2 × 50 mL), dried ($MgSO_4$), concentrated, and purified by chromatography on SiO_2 (10% methanol in dichloromethane) to yield **26a** as a colorless oil (2.61 g, 86%): $[\alpha]_D$ −8.9° (*c* 1.82, $CHCl_3$); IR (film) 3438, 2095, 1652, 1504 cm^{-1}; ^{1}H NMR (300 MHz) δ 1.29 (3H, d, $J = 7.2$ Hz), 1.46 (9H, s), 3.32 (1H, m), 3.75 (3H, s), 4.54 (1H, dd, $J = 3.2, 9.2$ Hz), 5.45 (1H, d, $J = 9.5$ Hz); ^{13}C NMR (75 MHz) δ 13.5, 28.3 (3C), 41.4, 52.4, 55.2, 80.2, 155.9, 171.2, 178.1; HRMS calcd. for $C_{11}H_{19}O_6N$ $[M + H]^+$ 262.1290, found 262.1280.

α-Methyl β-Pentafluorophenyl (2*R*,3*S*)-*N*-Boc-3-Methylaspartate (26b). To a solution of acid **26a** (840 mg, 3.21 mmol) in CH_2Cl_2 (75 mL) was added DCC (662 mg, 3.21 mmol), DMAP (79 mg, 0.642 mmol), and pentafluorophenol (1.18 g, 6.42 mmol), and the mixture was stirred at room temperature overnight. The solid dicyclohexylurea was removed by filtration and washed with CH_2Cl_2 (2 × 50 mL). The filtrate was washed with 1 M HCl, saturated aqueous $NaHCO_3$, 1 M NaOH, and brine. The organic extracts were dried ($MgSO_4$), concentrated, and purified by chromatography on SiO_2 (8:1, hexane/ethyl acetate) to give **26b** as a white solid (1.16 g, 85%): R_f = 0.35 (4:1, hexane/ethyl acetate); $[\alpha]_D$ −19.5° (*c* 0.74, $CHCl_3$); IR (film) 3416 (br), 2095, 1778, 1645, 1518 cm^{-1}; ^{1}H NMR (360 MHz) δ 1.37 (3H, d, $J = 7.1$ Hz), 1.46 (9H, s), 3.56–3.60 (1H, m), 3.76 (3H, s), 4.79 (1H, dd, $J = 3.4, 8.3$ Hz), 5.40 (1H, d, $J = 8.3$ Hz); ^{13}C NMR (90 MHz) δ 12.7, 28.2 (3C), 41.5, 52.6, 55.0, 80.4, 136.4, 138.1, 139.2, 139.5, 140.9, 142.3, 155.6, 169.8, 170.5; HRMS calcd for $C_{17}H_{18}O_6NF_5$ $[M + H]^+$ 428.1135, found 428.1141.

Dipeptide (8). To a solution of the activated ester **26b** (427 mg, 1 mmol) in CH_2Cl_2 (20 mL) containing DMAP (140 mg, 1.14 mmol) was added a solution containing the TFA salt of L-valine allyl ester (310 mg, 1.14 mmol) and diisopropylethylamine (0.55 mL, 3.12 mmol) in CH_2Cl_2 (5 mL). After 48 h of stirring at room temperature, the reaction mixture was diluted with CH_2Cl_2 and washed with 10% aqueous citric acid solution, saturated aqueous sodium bicarbonate, and brine. The organic extracts were dried ($MgSO_4$), concentrated, and purified by chromatography on SiO_2 (2:1, hexane/ethyl acetate) to give **8** as a white solid (320 mg, 80%): R_f = 0.40 (1:1, hexane/ethyl acetate); $[\alpha]_D$ −5.5° (*c* 0.64, $CHCl_3$); IR (film) 3533, 2971, 2933, 1748, 1716, 1658, 1499 cm^{-1}; ^{1}H NMR (300 MHz) δ 0.83 (3H, d, $J = 6.9$ Hz), 0.86 (3H, d, $J = 6.9$ Hz), 1.22 (3H, d, $J = 7.2$ Hz), 1.36 (9H, s), 2.07–2.14 (1H, m), 3.08–3.12 (1H, m), 3.64 (3H, s), 4.33 (1H, dd, $J = 3.7, 9.6$ Hz), 4.43 (1H, dd, $J = 4.9, 8.5$ Hz), 4.54 (2H, t, $J = 7.4$ Hz), 5.18 (1H, d, $J = 10.4$ Hz), 5.25 (1H, d, $J = 13.4$ Hz), 5.76–5.89 (2H, m), 6.45 (1H, d, $J = 8.6$ Hz); ^{13}C NMR (75 MHz) δ 15.0, 17.6, 18.8, 28.2 (3C), 31.0, 41.6, 52.2, 56.1, 57.0, 65.6, 79.5, 118.6, 131.4, 155.9, 171.1, 171.6, 173.4; HRMS calcd for $C_{19}H_{32}O_7N_2$ $[M + H]^+$ 401.2288, found 401.2284. Anal. Calcd for $C_{19}H_{32}O_7N_2$; C, 56.99; H, 8.05; N, 6.99. Found: C, 57.24; H, 7.98; N, 6.84.

(2*R*,3*R*)-2-(*N*-Boc-Amino)-3-methylhex-4-enol. Lithium aluminum hydride (412 mg, 10.88 mmol) was added portionwise to a solution of ester **19** (1.40 g, 5.44 mmol) in ether (75 mL) at 0 °C, and this reaction mixture was allowed to stir at room temperature for 1.5 h. After recooling to 0 °C, the reaction was quenched by addition of water (0.5 mL), 1 M NaOH (0.5 mL), and water (0.5 mL). Excess $MgSO_4$ was added to the reaction mixture, and stirring was continued for 1 h. The solids were removed by filtration and washed several times with ether. The combined filtrates were concentrated to obtain the alcohol as a colorless oil (1.12 g, 90%): R_f = 0.34 (1:1, hexane/ethyl acetate); $[\alpha]_D$ −0.8° (*c* 1.3, $CHCl_3$); IR (film) 3416 (br), 2130, 1634 cm^{-1}; ^{1}H NMR (300 MHz) δ 0.98 (3H, d, $J = 6.8$ Hz), 1.39 (9H, s), 1.62 (3H, d, $J = 6.2$ Hz), 2.34–2.38 (1H, m), 3.45–3.60 (3H, m), 4.74 (1H, d, $J = 8.3$ Hz), 5.25–5.33 (1H, m), 5.41–5.49 (1H, m); ^{13}C NMR (75 MHz) δ 17.4, 17.8, 28.3 (3C), 37.9, 57.0, 63.9, 79.3, 126.2, 132.2, 156.5; HRMS calcd for $C_{12}H_{23}O_4N$ $[M + H]^+$ 230.1756, found 230.1748.

(2*R*,3*R*)-2-(*N*-Boc-Amino)-3-methylhex-4-enol *tert*-Butyldimethylsilyl ether (20). To a solution of (2*R*,3*R*)-2-(*N*-Boc-amino)-3-methylhex-4-enol (950 mg, 4.36 mmol) in DMF (15 mL) was added imidazole (445 mg, 6.54 mmol) and TBDMSCl (723 mg, 4.80 mmol). The mixture was allowed to stir at room temperature for 15 h, diluted with (1:1) benzene/ethyl acetate (75 mL), and washed with 10% aqueous citric acid solution and brine. The organic extracts were dried ($MgSO_4$), concentrated, and purified by chromatography on SiO_2 (3:1, ether/hexane) to obtain the product as a colorless oil (1.42 g, 96%): R_f = 0.58 (4:1, hexane/ethyl acetate); $[\alpha]_D$ +13.3° (*c* 1.28, $CHCl_3$); IR (film) 3416 (br), 2951, 2836, 1708, 1497 cm^{-1}; ^{1}H NMR (300 MHz) δ 0.42 (6H, s), 0.91 (9H, s), 1.00 (3H, d, $J = 6.9$ Hz), 1.43 (9H, s), 1.66 (3H, d, $J = 5.6$ Hz), 2.41–2.48 (1H, m), 3.46–3.64 (3H, m), 4.53 (1H, d, $J = 8.1$ Hz), 5.31–5.49 (2H, m); ^{13}C NMR (75 MHz) δ −5.4 (2C), 17.4, 17.9, 18.3, 25.9 (3C), 28.3 (3C), 37.4, 55.9, 63.2, 78.9, 125.7, 132.6, 155.7; HRMS calcd for $C_{18}H_{37}O_3NSi$ $[M + H]^+$ 344.2621, found 344.2608.

Methyl (2*S*,3*R*)-3-(*N*-Boc-Amino)-4-(*tert*-butyldimethylsilyloxy)-2-methyl-butanoate (21). Ozone was bubbled through a solution of alkene **20** (1.52 g, 4.42 mmol) in CH_2Cl_2 (80 mL) and 2.5 M methanolic NaOH (8.8 mL) at −78 °C. After 135 min, the yellow reaction mixture acquired the characteristic blue color of ozone. The reaction mixture was diluted with ether and water and then extracted with CH_2Cl_2 (Note:

allowing the reaction mixture to warm to room temperature results in epimerization at C-2). The organic extracts were washed with brine, dried ($MgSO_4$), and concentrated to obtain the product ester as a colorless oil (1.44 g, 90%): $R_f = 0.52$ (4:1, hexane/ethyl acetate); $[\alpha]_D$ +13.6° (*c* 1.16, $CHCl_3$); IR (film) 3416.4 (br), 2924, 2854, 1715, 1645, 1490 cm^{-1}; ^{1}H NMR (300 Hz) δ 0.04 (6H, s), 0.88 (9H, s), 1.23 (3H, d, $J = 6.6$ Hz), 1.45 (9H, s), 2.85−2.88 (1H, m), 3.52−3.58 (2H, m), 3.68 (3H, s), 3.77−3.79 (1H, m), 5.34 (1H, d, $J = 9.5$ Hz); ^{13}C NMR (75 MHz) δ −5.6 (2C), 14.9, 18.2, 25.8 (3C), 28.4 (3C), 39.5, 51.5, 54.1, 63.5, 79.1, 155.6, 175.6; HRMS calcd for $C_{17}H_{35}O_5NSi$ $[M + H]^+$ 362.2363, found 362.2359.

Methyl (2*S*,3*R*)-3-(*N*-Boc-Amino)-4-oxo-2-methylbutanoate (11). NBS (730 mg, 4.10 mmol) was added to compound **20** (1.35 g, 3.73 mmol) in DMSO (10 mL) and water (0.4 mL) at 0 °C. The mixture was allowed to stir at 10 °C for 2 h, then diluted with ether, and washed with brine. After drying ($MgSO_4$), the solution was concentrated and purified by chromatography on SiO_2 (4:1, ether/hexane) to provide a white solid (770 mg, 84%): $R_f = 0.22$ (1:1, hexane/ethyl acetate); $[\alpha]_D$ +2.8° (*c* 1.62, $CHCl_3$); IR (film) 3416.4 (br), 2369, 1771.1, 1680 cm^{-1}; ^{1}H NMR (360 MHz) δ 1.26 (3H, d, $J = 7.2$ Hz), 1.45 (9H, s), 2.86−2.89 (1H, m), 3.67 (2H, d, $J = 5.1$ Hz), 3.70 (3H, s), 5.39 (1H, br s); ^{13}C NMR (75 MHz) δ 14.7, 28.3 (3C), 39.8, 51.7, 54.8, 63.9, 79.6, 156.2, 175.6; HRMS calcd for $C_{11}H_{21}O_5N$ $[M + H]^+$ 248.1498, found 248.1508.

DMSO (107 μL, 1.5 mmol) was added to a solution of oxalyl chloride (375 μL, 0.75 mmol) in CH_2Cl_2 (2 mL) at −78 °C. After 15 min, the alcohol prepared above (124 mg, 0.5 mmol) in CH_2Cl_2 (5 mL) was added. The resultant cloudy mixture was allowed to stir at −78 °C for 1 h, triethylamine (279 μL, 2 mmol) was added, and the reaction mixture was allowed to stir for 45 min at −78 °C. Following dilution with CH_2Cl_2, the solution was washed with saturated aqueous NH_4Cl, saturated aqueous $NaHCO_3$, and brine and dried ($MgSO_4$). Evaporation of the solvent provided aldehyde **11** (123 mg), which was used without further purification: $R_f = 0.48$ (1:1, hexane/ethyl acetate); ^{1}H NMR (300 MHz) δ 1.26 (3H, d, $J = 7.2$ Hz), 1.44 (9H, s), 3.26−3.30 (1H, m), 3.66 (3H, s), 4.32 (1H, dd, $J = 3.4$ and 9.4 Hz), 5.59 (1H, d, $J = 9.1$ Hz), 9.63 (1H, s).

***N*-Boc-Adda Methyl Ester (*E*,*E*-9).** To phosphonium salt **25** (420 mg, 0.75 mmol) in THF (5 mL) at −78 °C was added *n*-butyllithium (470 μL, 0.75 mmol, 1.6 M in hexane), and this mixture was stirred at −78 °C for 15 min and then at 0 °C for 30 min. Aldehyde **11** (184 mg, 0.75 mmol) in THF (5 mL) was added, and the reaction mixture was allowed to warm to room temperature over 20 h. The reaction was quenched by the addition of saturated aqueous ammonium chloride and then extracted with ether. The combined organic extracts were washed with brine, dried ($MgSO_4$), concentrated, and purified by chromatography on SiO_2 (8:1, hexane/ethyl acetate) to give the product as a colorless oil (138 mg, 41%): $R_f = 0.38$ (4:1, hexane/ethyl acetate); $[\alpha]_D$ −13.3° (*c* 0.73, $CHCl_3$); IR (film) 3416 (br), 2973, 1708, 1650, 1497 cm^{-1}; ^{1}H NMR (300 MHz) δ 1.03 (3H, d, $J = 6.7$ Hz), 1.22 (3H, d, $J = 7.2$ Hz), 1.45 (9H, s), 1.60 (3H, s), 2.57−2.79 (4H, m), 3.17−3.21 (1H, m), 3.23 (3H, s), 3.66 (3H, s), 4.37 (1H, br s), 5.29 (1H, br s), 5.37−5.47 (2H, m), 6.19 (1H, d, $J = 15.6$ Hz), 7.12−7.26 (5H, m); ^{13}C NMR (75 MHz) δ 12.7, 14.3, 16.2, 28.4 (3C), 36.8, 38.4, 44.1, 51.5, 54.7, 58.5, 79.3, 86.9, 125.1, 125.8, 128.1, 129.3, 132.4, 135.9, 136.0, 136.2, 139.4, 155.4, 175.1; HRMS calcd for $C_{26}H_{39}O_5N$ $[M + H]^+$ 446.2906, found 446.2887. The isomeric product *Z*,*E*-**9** was isolated in 20% yield: $R_f = 0.40$ (4:1, hexane/ethyl acetate); ^{1}H NMR (360 MHz) δ 1.03 (3H, d, $J = 6.7$ Hz), 1.20 (3H, d, $J = 7.2$ Hz), 1.44 (9H, s), 1.73 (3H, s), 2.53−2.74 (3H, m), 2.86−2.90 (1H, dd, $J = 3.5$, 14.1 Hz), 3.23 (3H, s), 3.23−3.25 (1H, m), 3.67 (3H, s), 4.83 (1H, br s), 5.19−5.25 (2H, m), 5.35 (1H, d, $J = 9.6$ Hz), 5.93 (1H, d, $J = 1.8$ Hz), 7.15−7.28 (5H, m).

Adda Isomerization. To *Z*,*E*-**9** (85 mg, 0.19 mmol) in CH_2Cl_2 (38 mL) at −78 °C was added I_2 (10 mg, 0.038 mmol), and the mixture was photolyzed using a tungsten lamp for 110 min. The reaction mixture was diluted with CH_2Cl_2 (30 mL), washed with 10% aqueous $Na_2S_2O_3$ solution (2.25 mL) followed by brine (15 mL), and then dried ($MgSO_4$). Purification by silica gel chromatography (8:1, hexane/ethyl acetate) gave *E*,*E*-**9** (43.5 mg, 51%).

***N*-Boc-Adda (6).** To *N*-Boc-Adda methyl ester (102 mg, 0.23 mmol) in THF (6 mL) was added 1 M LiOH (1.15 mL), and the reaction mixture was stirred at room temperature for 68 h, acidified with 1 M HCl, and extracted with ether. The organic extracts were dried ($MgSO_4$) and concentrated to obtain the product acid (97 mg, 98%), which was used without further purification: $[\alpha]_D$ −14.2° (*c* 1.84, $CHCl_3$); IR (film) 3416 (br), 2129, 1645 cm^{-1}; ^{1}H NMR (300 MHz) δ 1.02 (3H, d, $J = 6.5$ Hz), 1.25 (3H, d, $J = 5.8$ Hz), 1.44 (9H, s), 1.60 (3H, s), 2.55−2.83 (4H, m), 3.12−3.20 (1H, m), 3.23 (3H, s), 4.37 (1H, br s), 5.27−5.50 (3H, m), 6.20 (1H, d, $J = 15.6$ Hz), 7.12−7.26 (5H, m); ^{13}C NMR (75 MHz) δ 12.7, 14.5, 16.2, 28.4 (3C), 36.7, 38.3, 44.1, 54.4, 58.6, 79.5, 87.0, 124.9, 125.9, 128.1, 129.3, 132.4, 135.9, 136.4, 139.3, 155.6, 179.1; HRMS calcd for $C_{25}H_{37}O_5N$ $[M + H]^+$ 432.2750, found 432.2728.

***N*-Boc-Adda Pentafluorophenyl Ester (35).** To acid **6** (33 mg, 0.0765 mmol) in CH_2Cl_2 (3 mL) was added pentafluorophenol (43 mg, 0.230 mmol), DCC (24 mg, 0.115 mmol), and DMAP (2 mg), and the mixture was stirred at room temperature for 2 h. After dilution with CH_2Cl_2, the solution was washed with 1 M HCl, saturated aqueous sodium bicarbonate, and brine, dried ($MgSO_4$), and concentrated. Chromatography on SiO_2 (8:1, hexane/ethyl acetate) gave the pentafluorophenyl ester as a colorless oil (40 mg, 88%): $R_f = 0.55$ (4:1, hexane/ethyl acetate); $[\alpha]_D$ −11.0° (*c* 1.16, $CHCl_3$); IR (film) 3416 (br), 2095, 1645 cm^{-1}; ^{1}H NMR (360 MHz) δ 1.03 (3H, d, $J = 6.7$ Hz), 1.38 (3H, d, $J = 7.1$ Hz), 1.46 (9H, s), 1.62 (3H, s), 2.56−2.83 (4H, m), 3.16−3.20 (1H, m), 3.24 (3H, s), 4.56 (1H, br s), 5.05 (1H, br s), 5.42−5.52 (2H, m), 6.27 (1H, d, $J = 15.6$ Hz), 7.18−7.28 (5H, m); ^{13}C NMR (90 MHz) δ 12.6, 13.8, 13.9, 16.1, 28.3 (3C), 36.7, 38.2, 43.8, 43.9, 54.3, 58.6, 79.8, 86.8, 123.4, 129.5, 132.2, 136.3, 136.4, 147.5, 139.3, 139.6, 139.8, 142.3, 155.2, 170.8; HRMS calcd for $C_{31}H_{36}O_5NF_5$ $[M + H]^+$ 598.2592, found 598.2604.

(2*S*,3*R*)-*N*-Boc-*N*-methyl-*O*-benzyl-L-Threonine Trimethylsilylethyl Ester (27). *N*-Boc-*N*-methyl-*O*-benzyl-L-threonine (1.18 g, 3.65 mmol) and 4-(dimethylamino)pyridine (75 mg, 0.37 mmol, 0.15 equiv) were dissolved in dry THF and cooled to 0 °C under nitrogen. Trimethylsilylethanol (1.05 mL, 7.31 mmol, 2 equiv) was added dropwise, followed by 1,1′-dicyclohexylcarbodiimide (580 mg, 4.75 mmol, 1.3 equiv) in one portion. The solution was allowed to warm to room temperature and stirred for 36 h, during which time a white precipitate formed. This mixture was filtered; the filtrate was concentrated and redissolved in ethyl acetate then washed with saturated aqueous $NaHCO_3$, saturated aqueous NH_4Cl, and brine; and dried over anhydrous $MgSO_4$. Column chromatography on SiO_2 (10% ethyl acetate in hexanes) provided the product as a colorless oil (0.86 g, 2.03 mmol, 56%): $R_f = 0.6$ (1:2, ethyl acetate/hexanes); $[\alpha]_D$ +10.5° (c 1.0, $CHCl_3$); IR (film) 2957, 2933, 2902, 2874, 2864, 1733, 1693 cm^{-1}; ^{1}H NMR (300 MHz, 3:1 mixture of rotamers) δ 7.20−7.40 (5H, m, arom), 4.9 (0.6H, d, $J = 5$ Hz, H-2), 4.58−4.63 (1.3H, m, CH_2, H-2 min.), 4.38−4.44 (1H, m, CH_2), 4.09−4.31 (3H, m, CH_2O, H-3), 2.99 (0.6H, s, NCH_3), 3.00 (0.3H, s, NCH_3), 1.49 (6H, s, Boc), 1.45 (3H, s, Boc), 1.21−1.28 (3H, m, H-4), 0.90−1.01 (2H, m, CH_2Si), 0.40 (3H, s, $(CH_3)_3Si$), 0.30 (6H, s, $(CH_3)_3Si$); ^{13}C NMR (75 MHz) δ 128.1, 127.2, 79.7, 75.3, 74.8, 71.5, 71.0, 63.5, 63.1, 33.1, 28.4, 17.6, 16.4, −1.5; MS *m*/*z* (rel int) 424 (19, M+H), 368 (35), 324 (50), 296 (100), 282 (31), 160 (35), 91 (38). Anal. Calcd for $C_{22}H_{37}NO_5Si$: C, 62.38; H, 8.81; N, 3.31. Found: C, 62.42; H, 8.64; N, 3.24.

***N*-Boc-D-γ-Glutamyl-(α-methyl ester)-*N*-methyl-*O*-benzyl-L-threonine Trimethylsilylethyl Ester (28).** To *N*-Boc-*N*-Me-*O*-Bn-L-threonine trimethylsilyl ethyl ester (119 mg, 0.28 mmol) in CH_2Cl_2 (5 mL) was added HCl-saturated ether (5 mL), and the solution was stirred at room temperature for 90 min and then concentrated 3 times from CH_2Cl_2 to provide the hydrochloride salt. This salt and NMM (154 μL, 1.4 mmol) were dissolved in CH_2Cl_2 (5 mL) and added to a solution of *N*-Boc-D-glutamate α-methyl ester (74 mg, 0.28 mmol) in CH_2Cl_2 (3 mL) at 0 °C. BOP (137 mg, 0.308 mmol) was added, and the reaction mixture was allowed to warm to

room temperature overnight. Following dilution with CH_2Cl_2 (30 mL), the mixture was washed with 1 M HCl (5 mL), saturated aqueous sodium bicarbonate, and brine and then dried ($MgSO_4$). Removal of the solvents in vacuo and chromatography on SiO_2 (1:1, hexane/ethyl acetate) gave the title compound as a colorless oil (136 mg, 86%): R_f = 0.46 (1:1, hexane/ethyl acetate); $[\alpha]_D$ +8.1° (*c* 0.96, $CHCl_3$); IR (film) 3424 (br), 2102, 1650, 1455 cm^{-1}; ^{1}H NMR (300 MHz) δ 0.03 (9H, s), 0.94 (2H, t, *J* = 8.3 Hz), 1.18 (3H, d, *J* = 6.2 Hz), 1.26 (2H, t, *J* = 7.1 Hz), 1.43 (9H, s), 2.15−2.19 (2H, m), 2.44−2.55 (2H, m), 3.13 (3H, s), 3.72 (3H, s), 4.40 (1H, d, *J* = 11.6 Hz), 4.61 (1H, d, *J* = 11.6 Hz), 5.31 (1H, br s), 5.45 (1H, d, *J* = 3.9 Hz), 7.27−7.33 (5H, m); ^{13}C NMR (75 MHz, mixture of rotamers) δ −1.6 (3C), 16.2, 17.5, 27.9, 28.3 (3C), 29.4, 34.1, 52.0, 53.6, 60.5, 63.2, 71.5, 75.3, 79.6, 127.1, 127.3, 127.4, 127.5, 128.1, 128.2, 138.3, 155.2, 169.7, 172.6, 172.6, 173.3; HRMS calcd for $C_{28}H_{46}O_8N_2Si$ [M + H]$^+$ 567.3102, found 567.3082.

***N*-Boc-D-γ-Glutamyl-(α-methyl ester)-*N*-methyl-L-threonine Trimethylsilylethyl Ester (7).** To dipeptide **28** (480 mg, 0.85 mmol) was added 10% Pd/C (100 mg) and ethanol (25 mL). The suspension was placed under hydrogen at atmospheric pressure for 5 h and then filtered through a pad of Celite, rinsing several times with ethyl acetate. The combined filtrates were concentrated, and the product was purified by chromatography on SiO_2 (10% MeOH in CH_2Cl_2) to give **7** as a colorless oil (350 mg, 86%): R_f = 0.49 (10% MeOH in CH_2Cl_2); $[\alpha]_D$ −26.3° (*c* 1.08, $CHCl_3$); IR (film) 3416 (br), 2095, 1645 cm^{-1}; ^{1}H NMR (360 MHz) δ 0.04 (9H, s), 1.02 (2H, t, *J* = 8.9 Hz), 1.25 (3H, d, *J* = 6.1 Hz), 1.44 (9H, s), 1.88−1.93 (1H, m), 2.27−2.57 (3H, m), 3.11 (3H, s), 3.75 (3H, s), 4.21−4.28 (4H, m), 4.31−4.36 (1H, m), 4.52 (1H, t, *J* = 6.3 Hz), 4.60 (1H, br s); ^{13}C NMR (90 MHz, mixture of rotamers) δ −1.7 (3C), 17.3, 19.9, 27.6, 28.1 (3C), 29.2, 35.6, 52.2, 52.8, 63.5, 64.1, 65.5, 79.8, 155.5, 169.9, 172.9, 173.1; HRMS calcd for $C_{21}H_{40}O_8N_2Si$ [M + H]$^+$ 477.2632, found 477.2623.

Dipeptide 30. Dipeptide **7** (40 mg, 100 μmol) was dissolved in 1:1 TFA/CH_2Cl_2 (0.5 mL) and stirred at room temperature for 15 min. Following evaporation of the solvents, the resulting salt was dissolved in saturated aqueous sodium bicarbonate solution (10 mL) and extracted twice with ethyl acetate. The combined extracts were washed with brine and then concentrated to provide the crude product (26 mg, 87%), which was used without further purification.

Tripeptide 31. To dipeptide **30** (102 mg, 0.27 mmol) was added *N*-Boc-Adda (**6**) (97 mg, 0.225 mmol) in CH_2Cl_2 (2 mL). The solvent was evaporated and replaced with dry DMF (2 mL). Collidine (89 μL, 0.765 mmol) was added dropwise, and the solution was cooled to 0 °C. HATU (103 mg, 0.27 mmol) was added, and the reaction mixture was allowed to stir for 14.5 h at room temperature. Following dilution of the reaction mixture with 1:1 benzene/ethyl acetate, it was washed with 10% citric acid (aqueous), saturated aqueous sodium bicarbonate, and brine and dried over anhydrous magnesium sulfate. Removal of the drying agent and evaporation of the solvent followed by chromatography on SiO_2 (95:5, $CHCl_3/CH_3OH$) provided the tripeptide (140 mg, 79%) as a colorless oil: R_f = 0.32 (1:1, ethyl acetate/hexanes); $[\alpha]_D$ −26.0° (*c* 0.5, $CHCl_3$); ^{1}H NMR (300 MHz) δ(mixture of conformers) 0.01−0.10 (10H, m), 1.00 (6H, d, *J* = 6 Hz), 1.12−1.33 (10H, m) 1.40−1.50 (11H, m), 1.60 (3.5H, s), 1.78−2.09 (1H, m), 21.-2.27 (1H, m), 2.30−2.45 (1H, m), 2.39 (2H, s), 2.49−2.74 (4H, m), 2.80 (1H, dd, *J* = 5, 14 Hz), 3.08 (1.5H, d, *J* = 7 Hz), 3.11−3.25 (1H, m), 3.23 and 3.24 (4.5H two singlets), 3.7−3.75 (3H, m), 4.13−4.30 (3H, m), 4.4−4.78 (2H, m), 5.19 (1H, dd, *J* = 5, 7 Hz), 5.39 (1H, d, *J* = 11 Hz), 5.42−5.43 (1H, m), 6.19 (1H, dd, *J* = 7, 16 Hz), 7.15−7.30 (8H, m); ^{13}C NMR (90 MHz, mixture of rotamers) δ 12.73, 14,13, 15.31, 16.96, 17.10, 19.56, 26.65 (br), 28.86, 30.29 (br), 35.68 (br), 36.53, 38.18, 44.53 (br), 51.15, 52.37, 52.47, 55.27 (br), 58.55, 58.55, 60.32, 63.39, 63.68, 63.90, 64.39, 65.89, 79.07, 86.95, 125.76, 125.9, 128.11, 129.35, 132.43, 132.62, 135.75, 139.36, 155.87, 169.79, 172.41, 173.47, 174.94; MS *m/z* (rel int) 790 (14), 135 (100); HRMS calcd for $C_{41}H_{67}N_3O_{10}Si$ [M]$^+$ 790.4674, found 790.4626.

Pentapeptide 5a from 31. Tripeptide **31** (76 mg, 96 μmol) was dissolved in THF (5 mL) and cooled to 0 °C. TBAF·H_2O (33 mg, 125 μmol) was added, and the solution was stirred for 2 h at 0 °C. Additional TBAF·H_2O (5 mg) was added, and the mixture was stirred for 5.5 h at room temperature. After dilution with ethyl acetate, the solution was washed with 0.1 M HCl and brine and dried (Na_2SO_4). The drying agent was removed, and the solvents were evaporated to give the crude acid, which was used without further purification. Dipeptide **8** (46 mg, 115 μmol) was dissolved in 1:2 TFA/CH_2Cl_2 (3 mL) and stirred at room temperature for 30 min. The solvents were evaporated to provide amine salt **33**. This salt was redissolved in CH_2Cl_2 (1 mL) and NMM (42 μL, 384 μmol) was added. The resulting solution was added to the crude acid prepared above dissolved in CH_2Cl_2 (2 mL), and the mixture was cooled to 0 °C under nitrogen. BOP reagent (47 mg, 106 μmol) was added in one portion, and the reaction mixture was allowed to warm to room temperature and stirred overnight. Following the addition of more CH_2Cl_2, the solution was washed with 10% citric acid (aqueous), saturated aqueous $NaHCO_3$, and brine and dried (Mg_2SO_4). Following removal of the drying agent and evaporation of the solvents, chromatography on SiO_2 provided **5a** (43 mg, 46%) as a colorless solid: R_f = 0.38 (ethyl acetate); ^{1}H NMR (360 MHz) δ 0.83 (3H, d, *J* = 7 Hz), 0.88 (3H, d, *J* = 7 Hz), 1.13 (3H, d, *J* = 7 Hz), 1.23 (3H, d, *J* = 7 Hz), 1.38 (9H, s), 1.62 (3H, s), 1.74−1.88 (1H, m), 1.92−2.03 (1H, m), 2.10−2.21 (1H, m), 2.31−2.44 (2H, m), 2.50−2.72 (4H, m), 2.80 (1H, dd, *J* = 4, 1 Hz), 2.88 (0.5H, s), 2.97 (0.5H, s), 3.05 (1H, s), 3.10−3.28 (1H, m), 3.22 (2H, s), 3.46−3.53 (0.5H, m), 3.89−4.00 (1H, m), 3.73 and 3.74 (5H, two singlets), 4.36−4.50 (2H, m), 4.52−4.70 3H, m), 4.70−4.85 (1H, m), 5.23 (1H, dd, *J* = 1, 11 Hz), 5.29 (3H, s), 5.33−5.36 (1H, m), 5.60−5.70 (1H, m), 5.84−5.96 (1H, m), 6.04−6.21 (1H, m), 6.59−6.70 (1H, m), 6.71−6.81 (1H, m), 7.16−7.26 (5H, m), 7.38 (1H, d, *J* = 9 Hz), 7.53 (0.5H, d, *J* = 5 Hz); ^{13}C NMR (90 MHz) δ 12.75, 13.49, 14.98, 15.71, 16.15, 17.63, 18.73, 18.91, 19.56, 23.75, 27.47, 28.39, 30.71, 33.57, 36.58, 38.18, 41.12, 51.28, 52.40, 52.55, 53.38, 54.81, 56.70, 57.06, 57.35, 58.53, 65.81, 66.00, 86.83, 118.77, 124.91, 125.94, 128.14, 129.34, 131.68, 132.54, 135.95, 137.04, 139.32, 155.67, 171.10, 172.61, 173.29.

Tetrapeptide (34). To dipeptide **8** (80 mg, 0.20 mmol) in CH_2Cl_2 (3 mL) was added HCl-saturated ether (3 mL), and the solution was stirred at room temperature for 1 h. The solvents were removed under reduced pressure and reconcentrated repeatedly from ether to remove excess HCl and provide the amine salt **33b**. Dipeptide **7** (95.3 mg, 0.20 mmol) was dissolved in THF (3 mL) and cooled to 0 °C. TBAF·H_2O (78.4 mg, 0.30 mmol) was added, and the mixture was allowed to stir at room temperature overnight. Following acidification with 1 M HCl, the mixture was extracted with ethyl acetate and the combined organic extracts were washed with brine and dried ($MgSO_4$). Removal of the drying agent and evaporation of the solvent gave the free carboxylic acid, which was used without further purification. To this acid was added **33a** in DMF (2.5 mL), and the mixture was cooled to 0 °C. Collidine (80 μL, 0.60 mmol) was added, followed by HATU (91 mg, 0.24 mmol). The reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 20 h. Following dilution with 1:1 benzene/ethyl acetate (20 mL), the solution was washed with 1 M HCl, saturated aqueous $NaHCO_3$, and brine and dried ($MgSO_4$). Concentration followed by chromatography on SiO_2 (5% hexane in ethyl acetate) gave tetrapeptide **34** (77 mg, 59%) as a colorless oil: R_f = 0.29 (5% hexane in ethyl acetate); $[\alpha]_D$ −13.8° (*c* 0.73, $CHCl_3$); IR (film) 3416, 2088, 1736, 1645 cm^{-1}; ^{1}H NMR (360 MHz, mixture of rotamers) δ 0.88 (6H, dd, *J* = 6.6 and 12.6 Hz), 1.15 (3H, d, *J* = 6.2 Hz), 1.22 (3H, d, *J* = 7.1 Hz), 1.42 (9H, s), 1.55−1.59 (1H, m), 2.14−2.19 (2H, m), 2.48−2.57 (2H, m), 3.12 (3H, s), 3.69 (3H, s), 3.72 (3H, s), 4.29−4.47 (3H, m), 4.56−4.72 (3H, m), 5.07 (1H, d), 5.24 (1H, d, *J* = 10.4 Hz), 5.32 (1H, d, *J* = 15.3 Hz), 5.48 (1H, d, *J* = 7.9 Hz), 5.64 (1H, d, *J* = 7.5 Hz), 5.82−5.94 (1H, m), 6.44 (1H, d, *J* = 7.8 Hz), 7.40 (1H, d, *J* = 8.7 Hz); ^{13}C NMR (90 MHz, mixture of rotamers), δ 13.4, 15.4, 17.5, 18.9, 19.6, 23.6, 27.3, 28.2 (3C), 29.2, 30.9, 34.1, 40.1, 52.6, 54.5, 57.0, 61.4, 63.1, 66.0, 66.5, 80.0, 119.0, 131.5, 155.6, 155.8, 171.1, 171.5, 172.9, 173.3, 173.7, 173.9, 174.0; HRMS calcd for $C_{30}H_{50}O_{12}N_4$ [M + H]$^+$ 659.3503, found 659.3406.

Pentapeptide 5a. Tetrapeptide **34** (51 mg, 0.0744 mmol) was dissolved in 1:1 TFA/CH_2Cl_2 (4 mL) and stirred at room temperature for 1 h. The solvents were removed under reduced pressure, and the residue was redissolved in CH_2Cl_2 and reconcentrated repeatedly to remove excess TFA. The remaining salt was combined with DIPEA (37 μL, 0.21 mmol) and DMAP (1 mg, 0.00744 mmol) in CH_3CN (3 mL) and added to a solution of *N*-Boc-Adda pentafluorophenyl ester (42 mg, 0.070 mmol) in CH_3CN (3 mL). The reaction mixture was stirred at room temperature for 94 h, diluted with CH_2Cl_2 (30 mL), and washed with 1 M HCl, then brine, and dried ($MgSO_4$). Removal of the solvent in vacuo followed by chromatography on SiO_2 (5% MeOH in ethyl acetate) gave pentapeptide **5a** (47 mg, 69%) as a colorless oil: R_f = 0.54 (10% MeOH in CH_2Cl_2); $[\alpha]_D$ −19.6° (*c* 0.5, $CHCl_3$); IR (film) 3416, 2073, 1645, 1436 cm^{-1}; ^1H NMR (360 MHz, CD_3OD, mixture of rotamers) δ 0.95 (6H, d, J = 6.9 Hz), 1.03 (3H, d, J = 6.8 Hz), 1.14 (3H, d, J = 7.0 Hz), 1.18 (3H, d, J = 7.1 Hz), 1.22 (3H, d, J = 7.2 Hz), 1.46 (9H, s), 1.64 (3H, d, J = 6.8 Hz), 1.98−2.01 (2H, m), 2.13−2.17 (2H, m), 2.48−2.69 (6H, m), 2.94 (2H, d, J = 10.5 Hz), 3.10 (3H, s), 3.23 (3H, s), 3.69 (3H, s), 3.72 (3H, s), 4.21−2.33 (2H, m), 4.62 (2H, d, J = 4.6 Hz), 5.23 (1H, d, J = 10.4 Hz), 5.35 (1H, d, J = 17.1 Hz), 5.40 (1H, d, J = 9.5 Hz), 5.41−5.49 (1H, m), 5.92−5.99 (1H, m), 6.21 (1H, d, J = 15.1 Hz), 7.15−7.26 (5H, m); ^{13}C NMR (90 MHz, CD_3OD, mixture of rotamers) δ 13.1, 13.2, 15.6, 16.3, 16.7, 18.6, 19.7, 20.9, 21.1, 21.3, 27.8, 28.9, 30.5, 30.7, 31.9, 33.5, 34.1, 37.8, 39.2, 41.8, 41.9, 45.5, 45.6, 52.9, 53.2, 55.7, 57.1, 58.9, 59.1, 63.2, 64.1, 64.3, 66.1, 66.9, 67.2, 80.5, 88.6, 119.1, 127.2, 129.4, 130.7, 133,6, 134.1, 134.2, 136.8, 137.1, 137.6, 140.8, 157.8, 171.9, 172.5, 172.6, 173.7, 173.8, 175.4, 175.8, 176.4, 177.3, 177.5; HRMS calcd for $C_{50}H_{77}N_5O_{14}$ $[M]^+$ 972.5545, found 972.5535.

***N*-Boc-D-γ-Glutamyl-(α-methyl ester)-*Z*-*N*-methyldehydrobut-2-enoic Acid Trimethylsilylethyl Ester (29).** To the dipeptide alcohol **28** (130 mg, 0.273 mmol) in DMF (10 mL) was added a solution of Martin sulfurane (367 mg, 0.546 mmol) in DMF (5 mL). This mixture was stirred at 120 °C overnight, cooled to room temperature, diluted with 1:1 benzene/ethyl acetate (30 mL), and washed with water followed by 10% aqueous NaOH solution. The organic layer was dried ($MgSO_4$), concentrated, and purified by chromatography on SiO_2 (2:1, hexane/ethyl acetate) to give **29** (88 mg, 70%) as a colorless oil: R_f = 0.36 (1:1, hexane/ethyl acetate); $[\alpha]_D$ −7.7° (*c* 0.75, $CHCl_3$); IR (film) 3431, 2362, 2341, 1645 cm^{-1}; ^1H NMR (360 MHz, 50 °C) δ 0.07 (9H, s), 1.05 (2H, t, J = 8.3 Hz), 1.81 (3H, d, J = 7.0 Hz), 1.90−2.18 (4H, m), 2.98 (3H, s), 3.72 (3H, s), 4.22−4.31 (3H, m), 5.18 (1H, br s), 7.00 (1H, q, J = 7.0 Hz); 13C NMR (90 MHz, mixture of rotamers), δ −1.5 (3C), 13.5, 17.4, 27.5, 27.8, 28.3 (3C), 29.1, 29.4, 34.7, 52.2, 53.0, 63.4, 63.9, 79.7, 134.6, 136.7, 139.0, 139.3, 155.4, 163.9, 172.1, 172.8; HRMS calcd for $C_{21}H_{38}N_2O_7Si$ $[M + H]^+$ 459.2527, found 459.2531.

***N*-Boc-D-γ-glutamyl-(α-methyl ester)-*Z*-*N*-methyldehydrobut-2-enoic acid (36).** To dipeptide **29** (26 mg, 0.057 mmol) in THF (3 mL) at 0 °C was added TBAF·H_2O (22 mg, 0.086 mmol). The mixture was allowed to warm to room temperature overnight. Acidification with 1 M HCl was followed by extraction with ethyl acetate, then the organic extracts were washed with brine, dried ($MgSO_4$) and concentrated to give acid **36** (20 mg, 100%) as a colorless oil, which was used without further purification. $[\alpha]_D$ −4.1° (*c* 1.1, $CHCl_3$); IR (film) 3445, 2081, 1694, 1638, 1518 cm^{-1}; ^1H NMR (360 MHz, 50 °C) δ 1.43 (9H, s), 1.83−1.86 (3H, m), 1.90−2.03 (2H, m), 2.15−2.21 (2H, m), 3.02 (3H, s), 3,72 (3H, s), 4.16−4.22 (1H, m), 5.32−5.34 (1H, m), 7.13 (1H, q, J = 7.0 Hz); HRMS calcd for $C_{16}H_{26}N_2O_7$ $[M + H]^+$ 359.1818, found 359.1821.

Tetrapeptide 37. Dipeptide **8** (52 mg, 0.13 mmol) was dissolved in 1:3 TFA/CH_2Cl_2 (4 mL) and stirred at room temperature for 20 min. The solvents were removed under reduced pressure, and the residue was redissolved in CH_2Cl_2 and reconcentrated repeatedly to remove excess TFA. The dry sample was stored under high vacuum overnight to give the TFA salt (54 mg), which was used without further purification. This salt was combined with dipeptide acid **36** (46.5 mg, 0.13 mmol) in DMF (2 mL) at 0 °C, and collidine (52 μL, 0.42 mmol) was added, followed by HATU (54 mg, 0.154 mmol). The reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 22 h. Following dilution with 1:1 benzene/ethyl acetate (20 mL), the solution was washed with 1 M HCl, saturated aqueous $NaHCO_3$, and brine and dried ($MgSO_4$). Concentration followed by chromatography on SiO_2 (5% hexane in ethyl acetate) gave tetrapeptide **37** (39 mg, 47%) as a colorless oil: R_f = 0.31 (5% MeOH in CH_2Cl_2); $[\alpha]_D$ −15.3° (*c* 1.1, $CHCl_3$); IR (film) 3457, 2101, 1734, 1642, 1555 cm^{-1}; ^1H NMR (360 MHz, 50 °C) δ 0.88 (3H, d, J = 7.1 Hz), 0.91−0.97 (3H, m), 1.25 (3H, d, J = 7.2 Hz), 1.41 (9H, s), 1.75 (3H, d, J = 7.0 Hz), 2.01−2.21 (3H, m), 2.37−2.42 (1H, m), 3.03 (3H, s), 3.26−3.28 (1H, m), 3.70 (3H, s), 3.73 (3H, s), 4.41−4.46 (1H, m), 4.67 (2H, d, J = 6.9 Hz), 5.25 (1H, d, J = 10.4 Hz), 5.34 (1H, d, J = 17.2 Hz), 5.86−5.96 (1H, m), 6.16 (1H, d, J = 7.3 Hz), 6.31 (1H, br s), 6.99 (1H, q, J = 6.7 Hz), 7.19 (1H, d, J = 9.1 Hz), 7.35 (1H, d, J = 8.1 Hz); ^{13}C NMR (90 MHz, mixture of rotamers), δ 13.1, 13.2, 14.5, 15.3, 17.0, 17.4, 18.9, 19.3, 26.8, 26.9, 28.3 (3C), 29.2, 29.5, 30.5, 30.6, 34.1, 34.6, 40.6, 52.2, 52.3, 52.9, 53.0, 53.4, 53.6, 54.7, 54.9, 57.0, 57.5, 66.0, 66.4, 19.4, 118.7, 118.8, 131.5, 131.7, 135.6, 135.9, 136.2, 136.6, 156.2, 163.4, 171.3, 172.2, 172.3, 173.4, 173.5; HRMS calcd for $C_{30}H_{48}N_4O_{11}$ $[M + H]^+$ 641.3398, found 641.3387.

Pentapeptide 38. Tetrapeptide **37** (38 mg, 0.0593 mmol) was dissolved in 1:3 TFA/CH_2Cl_2 (4 mL) and stirred at room temperature for 35 min. The solvents were removed under reduced pressure, and the residue was redissolved in CH_2Cl_2 and reconcentrated repeatedly to remove excess TFA. The dry sample was stored under high vacuum overnight to provide the TFA salt (39 mg, 100%), which was used without further purification. This salt was combined with DIPEA (31 μL, 0.178 mmol) and DMAP (1.5 mg, 0.0119 mmol) in CH_3CN (2 mL) and added to a solution of *N*-Boc-Adda pentafluorophenyl ester (**35**) (35 mg, 0.0593 mmol) in CH_3CN (2 mL). The reaction mixture was stirred at room temperature for 96 h, diluted with CH_2Cl_2 (25 mL), washed with 1 M HCl and then brine, and dried ($MgSO_4$). Removal of the solvent in vacuo followed by chromatography on SiO_2 (5% MeOH in ethyl acetate) gave pentapeptide **38** (30 mg, 53%) as a colorless oil: R_f = 0.32 (5% MeOH in CH_2Cl_2); $[\alpha]_D$ −18.4° (*c* 0.56, $CHCl_3$); IR (film) 3339, 2966, 2938, 1743, 1666, 1497 cm^{-1}; ^1H NMR (500 MHz, CD_3OD, 50 °C) δ 0.94 (3H, d, J = 7.0 Hz), 0.96 (3H, d, J = 7.0 Hz), 1.03 (3H, d, J = 7.0 Hz), 1.13 (3H, d, J = 7.5 Hz), 1.24 (3H, d, J = 6.0 Hz), 1.44 (9H, s), 1.62 (3H, s), 1.78 (3H, d, J = 7.5 Hz), 1.99−2.02 (2H, m), 2.10−2.17 (2H, m), 2.58−2.70 (6H, m), 2.81 (2H, dd, J = 5.0 and 13.5 Hz), 3.06 (3H, s), 3.21 (3H, s), 3.68 (3H, s), 3.72 (3H, s), 4.16−4.19 (1H, m), 4.32 (1H, d, J = 6.0 Hz), 4.62 (2H, d, J = 6.0 Hz), 5.22 (1H, d, J = 10.4 Hz), 5.32 (1H, d, J = 16.5 Hz), 5.39 (1H, t, J = 9.0 Hz), 5.48−5.54 (1H, m), 5.90−5.97 (1H, m), 6.18 (1H, d, J = 15.5 Hz), 6.90 (1H, q, J = 6.5 Hz), 7.14−7.26 (5H, m); ^{13}C NMR (90 MHz, CD_3OD) δ 13.0, 13.6, 15.4, 16.6, 16.5, 18.4, 19.5, 27.6, 27.8, 28.0, 28.8 (3C), 30.6, 30.7, 31.8, 35.3, 37.7, 39.1, 41.3, 45.2, 52.8, 53.1, 53.3, 56.4, 56.5, 58.8, 58.9, 66.7, 80.4, 88.5, 118.9, 127.1, 129.2, 130.6, 133.4, 133.9, 134.1, 136.7, 136.9, 137.1, 137.6, 140.6, 157.7, 172.5, 173.5, 174.6, 176.5, 177.1; HRMS calcd for $C_{50}H_{75}N_5O_{13}$ $[M]^+$ 954.5439, found 954.5346.

Compound 39 via Macrocylization Using FDPP. To the pentapeptide **5a** (47 mg, 0.0484 mmol) in THF (4.7 mL) was added dimedone (20.3 mg, 0.1452 mmol) and $Pd(PPh_3)_4$ (5.6 mg, 4.8 μmol), and the mixture was stirred at room temperature for 45 h. Following removal of the solvent in vacuo, the residue was purified by chromatography on SiO_2 (ethyl acetate and then methanol) to obtain the pentapeptide acid **5b** (32 mg, 71%). This acid was dissolved in 1:1 TFA/CH_2Cl_2 (3 mL) and stirred at room temperature for 1 h. Evaporation of the solvents was followed by repeated reconcentration from CH_2Cl_2 to remove the excess TFA. The oily residue was stored under high vacuum overnight to obtain the pentapeptide amine as its TFA salt (**5c**). To this amine salt (32.5 mg, 0.0343 mmol) in DMF (34.3 mL) was added NMM (38 μL, 0.343 mmol) and FDPP (26.4 mg, 0.0686 mmol), and the solution was stirred at room temperature for 24 h. Concentration and

purification by chromatography on SiO_2 (5% MeOH in ethyl acetate) provided macrocycle **39** as a colorless oil (22 mg, 79%).

Compound 40. To the pentapeptide **38** (50 mg, 0.0524 mmol) in THF (6 mL) was added dimedone (22 mg, 0.157 mmol) and $Pd(PPh_3)_4$ (6 mg, 5.24×10^{-3} mmol), and the mixture was stirred at room temperature for 19 h. Following removal of the solvent under reduced pressure, chromatography on SiO_2 (ethyl acetate/methanol) gave the pentapeptide acid (48 mg, 100%). To this acid (48 mg, 0.0524 mmol) in ethyl acetate (3 mL) was added pentafluorophenol (29 mg, 0.157 mmol), DCC (16.2 mg, 0.0786 mmol), and DMAP (1.3 mg, 0.0105 mmol), and the mixture was stirred at room temperature overnight. Removal of the solvent under reduced pressure followed by chromatography on SiO_2 (5% MeOH in ethyl acetate) gave the activated ester (44 mg, 78%). This material was dissolved in CH_2Cl_2 (5 mL), and TFA (2 mL) was added. After stirring at room temperature for 1 h, the reaction mixture was concentrated, redissolved in CH_2Cl_2, and reconcentrated repeatedly to remove the excess TFA. The residue was kept under high vacuum overnight. To the dry sample (45 mg, 0.041 mmol) was added CH_3CN (41 mL) followed by DIPEA (143 μL, 0.82 mmol) and DMAP (1.0 mg, 0.008 mmol). The dilute solution was stirred at room temperature for 5 d and then concentrated under reduced pressure. Chromatography on SiO_2 (5% MeOH in ethyl acetate) provided the macrocycle **40** (4.1 mg, 13%): $R_f = 0.34$ (5% MeOH in CH_2Cl_2); $[\alpha]_D$ −16.2° (*c* 1.2, $CHCl_3$); IR (film) 3423, 2095, 1638 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD), δ(50 °C) 0.83 (3H, d, $J = 6.5$ Hz), 0.87 (3H, d, $J = 7.0$ Hz), 1.00 (3H, d, $J = 6.5$ Hz), 1.10 (3H, d, $J = 7.0$ Hz), 1.30 (3H, d, $J = 7.0$ Hz), 1.60 (3H, s), 1.76 (3H, d, $J = 7.5$ Hz), 2.01−2.14 (2H, m), 2.36−2.39 (2H, m), 2.56−2.62 (2H, m), 2.68 (2H, dd, $J = 7.5$, 14.0 Hz), 2.75−2.82 (1H, m), 3.01−3.07 (2H, m), 3.09 (3H, s), 3.23 (3H, s), 3.24−3.27 (2H, m), 3.70 (3H, s), 3.70−3.76 (1H, m), 3.87 (3H, s), 4.32 (1H, br s), 4.38 (2H, t, $J = 3.0$ Hz), 4.48−4.50 (1H, m), 4.69 (1H, dd, $J = 4.0$, 6.5 Hz), 5.43 (1H, d, $J = 9.5$ Hz), 5.57 (1H, dd, $J = 8.5$, 16.0 Hz), 6.25 (1H, d, $J = 16.0$ Hz), 6.98 (1H, q, $J = 7.0$ Hz), 7.15−7.21 (5H, m); ^{13}C NMR (90 MHz, CD_3OD), δ 12.8, 13.3, 16.4, 16.5, 16.7, 17.0, 19.6, 19.8, 26.0, 27.6, 29.5, 29.9, 34.7, 35.1, 37.7, 38.9, 39.8, 45.3, 52.4, 52.7, 53.1, 53.8, 55.8, 57.5, 58.1, 58.7, 88.3, 126.1, 127.1, 129.2, 130.5, 133.7, 137.0, 137.4, 138.2, 139.1, 140.5, 165.6, 171.2, 173.6, 174.3, 175.2, 176.3, 176.6; HRMS calcd for $C_{42}H_{61}N_5O_{10}$ $[M + H]^+$ 796.4497, found 796.4491.

Motuporin (1). To macrocycle dimethyl ester **40** (4.1 mg, 5.15 μmol) in THF (0.5 mL) at 0 °C was added 1 M LiOH (26 μL, 0.026 mmol), and the solution was stirred for 1 h. The reaction mixture was acidified with Amberlyte IR-120 ion-exchange resin, diluted with ethyl acetate, filtered through a plug of glass wool, and then concentrated. The crude product (4 mg) was purified by reverse-phase HPLC (C-18, 70:30 $MeOH/H_2O$, 0.1% TFA) to obtain motuporin **1** as a white solid: $[\alpha]_D$ (disodium salt) = −12.3° (*c* 0.4, CH_3OH), lit. $[\alpha]_D$ −73° (*c* 0.00718, CH_3OH); IR (film) 3431, 2917, 2854, 2369, 2334, 1645 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ 0.77 (3H, d, $J = 7.0$ Hz), 0.85 (3H, d, $J = 7.0$ Hz), 0.92 (3H, d, $J = 6.5$ Hz), 1.00 (3H, d, $J = 6.5$ Hz), 1.22 (3H, d, $J = 7.0$ Hz), 1.63 (3H, s), 1.74 (3H, d, $J = 7.0$ Hz), 2.18−2.30 (2H, m), 2.46−2.55 (2H, m), 2.57−2.61 (2H, m), 2.67 (1H, dd, $J = 7.5$ and 14.0 Hz), 2.82 (1H, dd, $J = 4.5$ and 14.0 Hz), 3.02−3.07 (2H, m), 3.07 (3H, s), 3.23 (3H, s), 3.23−3.27 (2H, m), 4.24−4.28 (2H, m) 4.42 (2H, $J = 3.5$ Hz), 5.39 (1H, d, $J = 10.0$ Hz), 5.62 (1H, dd, $J = 9.0$ and 16.0 Hz), 6.22 (1H, d, $J = 16.0$ Hz), 6.94 (1H, q, $J = 7.0$ Hz), 7.14−7.25 (5H, m); HRMS calcd for $C_{40}H_{57}N_5O_{10}$ $[M + H]^+$ 768.4184, found 768.4220.

***N*-Boc-*N*-Methyl-L-alanine Trimethylsilylethyl Ester (42).** To a solution of *N*-Boc-*N*-Me-L-alanine (560 mg, 2.76 mmol) in CH_2Cl_2 (10 mL) at 0 °C was added 2-trimethylsilyl ethanol (0.436 mL, 3.04 mmol), DCC (627 mg, 3.04 mmol), and DMAP (68 mg, 0.552 mmol). The reaction mixture was allowed to warm to room temperature overnight. The solid dicylohexylurea was removed by filtration and washed with CH_2Cl_2 (2 × 25 mL). The combined filtrates were washed with 10% aqueous citric acid, saturated aqueous sodium bicarbonate, and brine, dried ($MgSO_4$), and concentrated. Chromatography on SiO_2 (8:1, hexane/ethyl acetate) gave **42** (750 mg, 89%) as a colorless oil: $R_f = 0.43$ (4:1, hexane/ethyl acetate); $[\alpha]_D$ −26.2° (*c* 1.1, $CHCl_3$); IR (film) 2952, 2362, 1743, 1701, 1462 cm^{-1}; ^{1}H NMR (500 MHz) (mixture of rotamers) δ 0.05 (9H, s), 1.00 (2H, t, $J = 8.0$ Hz) 1.39 (3H, d, $J = 5.5$ Hz), 1.45 (9H, s), 1.47 (9H, s), 2.80 (3H, s), 2.86 (3H, s), 4.22 (2H, t, $J = 8.5$ Hz), 4.42 (1H, q, $J = 6.5$ Hz), 4.82 (1H, $J = 7.0$ Hz); ^{13}C NMR (90 MHz, 50 °C) δ −1.8 (3C), 14.6, 17.2, 28.1 (3C), 30.4, 53.5, 62.7, 79.5, 171.2; HRMS calcd for $C_{14}H_{29}NO_4Si$ $[M + H]^+$ 304.1944, found 304.1959. Note: the ^{1}H NMR spectrum at room temperature exhibits two sets of signals due to rotamers. These signals coalesce into one set of signals at 323 K, as is clearly evident from signals due to the *N*-Boc and *N*-Me groups appearing as singlets at 1.47 and 2.83 ppm, respectively. The α-proton is not clearly visible at 323 K, although on expanding the spectrum one observes two broad singlets at 4.45 and 4.81 ppm, respectively.

***N*-Boc-D-Glutamyl-(α-methyl ester)-*N*-methyl-L-alanine Trimethylsilylethyl Ester (43).** *N*-Boc-*N*-methyl-L-alanine trimethylsilylethyl ester (167 mg, 0.55 mmol) was dissolved in 1:2 TFA/CH_2Cl_2 (3 mL) and stirred at room temperature for 30 min. The reaction mixture was concentrated, and the residue was dissolved in CH_2Cl_2 and reconcentrated repeatedly to remove the excess TFA. The dry sample was stored under high vacuum overnight to give the product TFA salt (175 mg, 100%), which was used without purification. *N*-Boc-D-Glutamic acid-α-methyl ester (131 mg, 0.50 mmol) and the TFA salt were dissolved in DMF (7 mL) at 0 °C and collidine (198 μL, 1.50 mmol) was added, followed by HATU (209 mg, 0.55 mmol). The reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 19 h. After dilution with 1:1 benzene/ethyl acetate (25 mL), the mixture was washed with 1 M HCl, saturated aqueous $NaHCO_3$, and then brine and dried ($MgSO_4$). Chromatography on SiO_2 (1:1, hexane/ethyl acetate) gave the title compound as a colorless oil (160 mg, 72%): $R_f = 0.42$ (1:1, hexane/ethyl acetate); $[\alpha]_D$ −34.7° (*c* 0.53, $CHCl_3$); IR (film) 3423, 2362, 2334, 1722, 1645 cm^{-1}; ^{1}H NMR (360 MHz, 50 °C) δ 0.01 (9H, s), 0.96 (2H, t, $J = 8.5$ Hz), 1.34 (3H, d, $J = 7.3$ Hz), 1.41 (9H, s), 1.94−2.04 (1H, m), 2.12−2.21 (1H, m), 2.89 (3H, s), 3.70 (3H, s), 4.17 (2H, t, $J = 8.0$ Hz), 4.21−4.25 (1H, m), 5.17 (1H, q, $J = 7.3$ Hz); ^{13}C NMR (90 MHz 50 °C) (major rotamer) δ −1.7 (3C), 14.3, 17.4, 27.6, 28.2 (3C), 29.6, 31.3, 52.0, 52.3, 55.1, 63.3, 79.7, 155.4, 171.8, 172.0, 172.7; HRMS calcd for $C_{20}H_{38}N_2O_7Si$ $[M + H]^+$ 447.2527, found 447.2544.

Tetrapeptide 44. Dipeptide **43** (155 mg, 0.35 mmol) was dissolved in THF (5 mL) at 0 °C, and TBAF·H_2O (139 mg, 0.525 mmol) was added. The reaction mixture was allowed to warm to room temperature overnight, acidified with 1 M HCl, and extracted with ethyl acetate. The organic extracts were washed with brine, dried ($MgSO_4$), and concentrated to give the acid (121 mg, 100%) as a colorless oil, which was used in the next step without further purification: $[\alpha]_D$ −33.7° (*c* 1.3, $CHCl_3$); IR (film) 3452, 2095, 1645 cm^{-1}; ^{1}H NMR (360 MHz, 50 °C) δ 1.37−1.40 (3H, d, hidden by Boc group), 1.40 (9H, s), 1.93−1.97 (1H, m), 2.17−2.22 (1H, m), 2.36−2.52 (2H, m), 2.92 (3H, s), 3.71 (3H, s), 4.25−4.29 (1H, m), 5.18−5.22 (1H, m), 5.41 (1H, d, $J = 7.9$ Hz); HRMS calcd for $C_{15}H_{26}N_2O_7$ $[M + H]^+$ 347.1818, found 347.1819. A sample of the acid (49 mg, 0.14 mmol) was combined with **33b** (0.14 mmol) in DMF (2 mL). The solution was cooled to 0 °C, and collidine (56 μL, 0.42 mmol) was added, followed by HATU (59 mg, 0.154 mmol). The reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 22 h. After dilution with 1:1 benzene/ethyl acetate (20 mL), the solution was washed with 1 M HCl, saturated aqueous sodium bicarbonate, and brine and then dried ($MgSO_4$). Chromatography on SiO_2 (5% hexane in ethyl acetate) gave the tetrapeptide (76 mg, 86%) as a colorless oil: $R_f = 0.32$ (5% MeOH in CH_2Cl_2); $[\alpha]_D$ −31.0° (*c* 0.9, $CHCl_3$); IR (film) 3445, 2095, 1736, 1645, 1511 cm^{-1}; ^{1}H NMR (360 MHz, 50 °C) δ 0.89 (3H, d, $J = 6.9$ Hz), 0.93 (3H, d, $J = 6.9$ Hz), 1.24 (3H, d, $J = 7.2$ Hz), 1.35 (3H, d, $J = 7.1$ Hz), 1.45 (9H, s), 2.05−2.07 (1H, m), 2.19−2.27 (2H, m), 2.38−2.44 (1H, m), 2.55−2.61 (1H, m), 2.94 (3H, s), 3.11−3.13 (1H, m), 3.71 (3H, s), 3.75 (3H, s), 4.29−4.31 (1H, m), 4.50−4.52 (1H, m),

4.65 (2H, d, J = 6.9 Hz), 5.26−5.43 (4H, m), 5.86−5.96 (1H, m), 6.19 (1H, d, J = 6.7 Hz); ^{13}C NMR (90 MHz) δ 13.2, 15.4, 17.5, 18.3, 27.1, 28.2 (3C), 29.5, 30.5, 31.1, 38.6, 41.0, 51.8, 52.2, 52.5, 53.1, 54.3, 56.7, 65.9, 79.7, 118.4, 131.4, 155.6, 171.2, 171.3, 171.8, 172.7, 172.8, 173.7; HRMS calcd for $C_{29}H_{48}N_4O_{11}$ [M + H]$^+$ 629.3397, found 629.3427.

Pentapeptide 45. Tetrapeptide **44** (138 mg, 0.22 mmol) was dissolved in 1:2.5 TFA/CH_2Cl_2 (7 mL) and stirred at room temperature for 45 min. After removal of the solvents under reduced pressure, the residue was redissolved in CH_2Cl_2 and reconcentrated repeatedly to remove the excess TFA. The dry sample was stored under high vacuum overnght to give the TFA salt (141.3 mg, 100%), which was used without further purification. This salt was combined with DMAP (5.4 mg, 0.044 mmol) and DIPEA (115 μL, 0.66 mmol) in CH_3CN (5 mL) and added to a solution of *N*-Boc-Adda pentafluorophenyl ester (**35**) (131.5 mg, 0.22 mmol) in CH_3CN (5 mL). This mixture was stirred at room temperature for 90 h, diluted with CH_2Cl_2 (25 mL), washed with 1 M HCl and then brine, and dried ($MgSO_4$). Concentration followed by purification by chromatography on SiO_2 (5% MeOH in ethyl acetate) gave unreacted *N*-Boc-Adda pentafluorophenyl ester (14.2 mg) and pentapeptide **45** (150 mg, 81% based on recovered starting material) as a colorless oil: R_f = 0.33 (5% MeOH in CH_2Cl_2); $[\alpha]_D$ −31.6° (*c* 0.9, $CHCl_3$); IR (film) 3445, 2060, 1736, 1645, 1504 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD, 50 °C) δ 0.93−0.96 (6H, m), 1.02 (3H, d, J = 6.7 Hz), 1.16 (3H, d, J = 6.9 Hz), 1.21 (3H, d, J = 6.9 Hz), 1.31 (3H, d, J = 7.1 Hz), 1.44 (9H, s), 1.62 (3H, s), 1.96−1.99 (2H, m), 2.12−2.24 (2H, m), 2.46−2.49 (3H, m), 2.57−2.69 (3H, m), 2.80 (2H, dd, J = 6.5, 14.0 Hz), 2.94 (3H, s), 3.21 (3H, s), 3.68 (3H, s), 3.72 (3H, s), 4.19 (1H, d, J = 5.0 Hz), 4.31 (1H, d, J = 6.5 Hz), 4.47 (1H, dd, J = 5.0. 9.0 Hz), 4.53 (1H, d, J = 5.0 Hz), 4.62 (2H, d, J = 5.7 Hz), 5.22 (1H, dd, J = 1.2, 10.4 Hz), 5.33 (1H, d, J = 1.5, 17.2 Hz), 5.39 (1H, d, J = 9.4 Hz), 5.53 (1H, dd, J = 6.5, 15.5 Hz), 5.89−5.97 (1H, m), 6.19 (1H, d, J = 15.6 Hz), 7.13−7.25 (5H, m); ^{13}C NMR (90 MHz, CD_3OD) δ 13.1, 13.6, 15.4, 16.2, 16.5, 18.5, 19.5, 27.8, 28.8 (3C), 30.8, 31.6, 31.9, 37.6, 38.9, 41.4, 45.4, 52.8, 53.0, 53.6, 55.9, 58.7, 58.9, 66.6, 80.2, 88.3, 118.9, 126.8, 127.0, 129.2, 130.5, 133.4, 133.7, 136.9, 137.4, 140.5, 157.6, 172.4, 173.5, 173.6, 174.8, 176.4, 177.2; HRMS calcd for $C_{49}H_{75}N_5O_{13}$ [M]$^+$ 942.5439, found 942.5413.

Compound 46. Macrocylization via the Pentafluorophenyl Ester. Pentapeptide **45** (148 mg, 0.157 mmol) was dissolved in THF (16 mL), and dimedone (66 mg, 0.471 mmol) and $Pd(PPh_3)_4$ (18 mg, 0.0157 mmol) were added. The solution was stirred at room temperature for 21 h. After removal of the solvents under reduced pressure, chromatography on SiO_2 (ethyl acetate/methanol) provided the pentapeptide acid (117 mg, 82%). To a portion of this acid (31.5 mg, 0.035 mmol) in ethyl acetate (3 mL) was added pentafluorophenol (19.3 mg, 0.105 mmol), DCC (11.0 mg, 0.053 mmol), and DMAP (0.7 mg, 0.007 mmol), and the solution was stirred at room temperature overnight. Concentration and chromatography on SiO_2 (5% MeOH in ethyl acetate) gave the activated ester (30 mg, 80%). This ester was dissolved in CH_2Cl_2 (5 mL), and TFA (2 mL) was added. After stirring at room temperature for 1 h, the reaction mixture was concentrated, redissolved in CH_2Cl_2, and re-evaporated repeatedly to remove all of the excess TFA. This sample was kept under high vacuum overnight. To the dry cyclization precursor was added CH_3CN (28 mL) followed by DIPEA (98 μL, 0.56 mmol) and DMAP (0.7 mg, 5.6×10^{-3} mmol). This dilute solution was stirred at room temperature for 7 d. Evaporation of the solvent followed by chromatography on SiO_2 (5% MeOH in ethyl acetate) gave **46** (9.3 mg, 42%): R_f = 0.3 (5% MeOH in CH_2Cl_2); ^{1}H NMR (500 MHz, CD_3OD, 50 °C) δ 0.95 (3H, d, J = 7.5 Hz), 1.01 (3H, d, J = 6.5 Hz), 1.12 (3H, d, J = 6.5 Hz), 1.15 (3H, d, J = 7.0 Hz), 1.23 (3H, d, J = 7.0 Hz), 1.40 (3H, d, J = 6.5 Hz), 1.62 (3H, s), 1.90−1.92 (1H, m), 2.25−2.40 (2H, m), 2.59−2.69 (4H, m), 2.78−2.82 (2H, dd, J = 4.5 and 14.0 Hz), 3.13−3.19 (2H, m), 3.03 (3H, s), 3.21−3.24 (2H, m), 3.23 (3H, s), 3.66−3.72 (1H, m), 3.72 (3H, s), 3.74 (3H, s), 3.77 (1H, d, J = 6.5 Hz), 4.47−4.50 (2H, m), 4.55 (1H, d, J = 3.0 Hz), 5.17−5.19 (1H, m), 5.40 (1H, d, J = 9.5 Hz), 5.50 (1H, dd, J = 7.5 and 15.0 Hz), 6.25 (1H, d, J = 16.0 Hz), 7.14−7.26 (5H, m); HRMS calcd for $C_{41}H_{61}N_5O_{10}$ [M + H]$^+$ 784.4497, found 784.4490 and **47** (5.9 mg, 27%): R_f = 0.32 (5% MeOH in CH_2Cl_2); $[\alpha]_D$ −56.0° (*c* 0.6, $CHCl_3$); IR (film): 3438, 2102, 1638 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD, 50 °C) δ 0.93 (3H, d, J = 6.5 Hz), 0.95 (3H, d, J = 7.0 Hz), 1.02 (3H, d, J = 7.0 Hz), 1.09 (3H, d, J = 7.0 Hz), 1.24 (3H, d, J = 7.0 Hz), 1.31 (3H, d, J = 7.0 Hz), 1.66 (3H, s), 1.90−1.93 (1H, m), 2.27−2.43 (2H, m), 2.59−2.70 (2H, m), 2.74−2.77 (2H, m), 2.78−2.83 (2H, dd, J = 5.0 and 14.0 Hz), 2.92 (3H, s), 3.17−3.22 (2H, m), 3.23 (3H, s), 3.23−3.27 (2H, m), 3.68−3.73 (1H, m), 3.73 (3H, s), 3.75 (3H, s), 3.82 (1H, d, J = 7.0 Hz), 4.17 (2H, t, J = 7.0 Hz), 4.56 (1H, d, J = 3.0 Hz), 5.33−5.38 (1H, m), 5.43 (1H, d, J = 10 Hz), 5.78 (1H, dd, J = 1.0 and 15.5 Hz), 6.25 (1H, d, J = 15.5 Hz), 7.15−7.26 (5H, m); ^{13}C NMR (90 MHz, CD_3OD) δ 13.0, 13.3, 15.3, 16.5, 17.5, 18.6, 19.8, 27.7, 29.6, 30.0, 30.7, 31.4, 37.6, 39.0, 40.7, 45.2, 52.8, 53.1, 53.2, 56.1, 57.4, 58.8, 62.1, 88.4, 126.5, 127.1, 129.2, 130.5, 133.0, 133.1, 133.7, 134.0, 137.1, 138.2, 140.6, 172.6, 173.1, 173.9, 174.9, 176.7, 177.3; HRMS calcd for $C_{41}H_{61}N_5O_{10}$ [M + H]$^+$ 784.4497, found 784.4490.

5-[L-Ala]-Motuporin (41). To macrocycle dimethyl ester **46** (27 mg, 0.0344 mmol) in THF (2 mL) at 0 °C was added 1 M LiOH (172 μL, 0.172 mmol). The mixture was stirred for 2 h and then acidified with Amberlyte IR-120 ion-exchange resin. The suspension was filtered through a plug of glass wool and then concentrated and purified by reverse-phase HPLC (C-18, 70:30 MeOH/H_2O, 0.1% TFA) to obtain 5-[L-Ala]-motuporin **41** as a white solid: $[\alpha]_D$ −29° (*c* 0.1, CH_3OH); IR (film) 3438, 2847, 2362, 2341, 1645 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ 0.95 (3H, d, J = 6.0 Hz), 1.01 (6H, d, J = 7.0 Hz), 1.14 (3H, d, J = 7.0 Hz), 1.20 (3H, d, J = 7.5 Hz), 1.37−1.40 (3H, m), 1.62 (3H, s), 1.91−1.94 (1H, m), 2.25−2.40 (2H, m), 2.57−2.69 (2H, m), 2.78−2.83 (2H, m), 2.89−2.94 (2H, m), 3.03 (3H, s), 3.18−3.23 (2H, m), 3.23 (3H, s), 3.23−3.26 (2H, m), 3.23−3.26 (2H, m), 3.82 (1H, t, J = 7.5 Hz), 4.38−4.53 (3H, m), 5.21−5.24 (1H, m), 5.39 (1H, d, J = 9.5 Hz), 5.52 (1H, dd, J = 7.0 and 16.0 Hz), 6.24 (1H, d, J = 15.5 Hz), 7.14−7.26 (5H, m); ^{13}C NMR (90 MHz, CD_3OD), δ 12.9, 13.9, 16.1, 16.5, 19.5, 19.7, 27.5, 30.4, 30.6, 31.2, 37.7, 38.9, 40.6, 46.2, 54.3, 55.4, 56.2, 58.8, 61.4, 88.4, 126.3, 127.1, 129.2, 130.5, 133.8, 136.9, 138.1, 140.5, 172.9, 173.5, 174.2, 175.5, 175.6, 176.8; HRMS calcd for $C_{39}H_{57}N_5O_{10}$ [M + H]$^+$ 756.4184, found 756.4187.

Protein Phosphatase Assay. Protein phosphatase assays were performed in reaction mixtures containing 0.5 μL 3T3-L1 cell lysate,[78] 4 μL PP1 buffer (50 mM Hepes, pH 7.4, 2 mM EDTA, 2 mg mL^{-1} glycogen, 0.2% 2-mercaptoethanol, 0.1 mM PMSF, 1 mM benzamidine, and 10 μg mL^{-1} aprotinen), 15 μL PP1 buffer containing 6 nM okadaic acid, and 0.5 μL of inhibitor solution or an appropriate control. These mixtures were incubated at 37 °C for 1 min before the addition of 10 μL ^{32}P-labeled phosphorylase (15 μM in 50 mM Hepes, pH 7.4, 15 mM caffeine, 0.1 mM EDTA, 0.2% 2-mercaptoethanol; specific activity 2000−3000 cpm pmol^{-1}) to initiate the reaction. After 6 min, the reactions were terminated by the addition of 90 μL ice-cold 20% trichloroacetic acid, and 5 μL 2% BSA in H_2O was added to aid protein precipitation. The samples were allowed to stand on ice for 10 min, and then the proteins were pelleted by centrifugation at 15 000g for 2 min. The amount of radioactive phosphate released into the supernatant was measured by scintillation counting. All measurements were performed in duplicate in at least two separate assays.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra for compounds **7**–**9**, **29**, **39**, **40**, **43**, and **47**; ^{1}H NMR spectrum and HPLC trace for compound **1**; ^{1}H NMR spectra for compounds **41** and **46**; and ^{1}H NMR and mass spectrum for compound **48**. This material is available free of charge via the Internet at http://pubs.acs.org.

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