Total Synthesis of the Serine-Threonine Phosphatase Inhibitor Microcystin-LA

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Received May 20, 1996[⊗]

Abstract: Reversible protein phosphorylation, which is mediated by kinases and phosphatases, is a major control element of the cell. There is a diverse group of toxic natural products that inhibit certain phosphatases, thereby disrupting normal biochemical pathways. These toxins can be useful for dissecting the individual biochemical pathways associated with each of these enzymes. This Article describes the first total synthesis of one such toxin, the cyclic heptapeptide microcystin-LA. The synthesis features a convergent route that is amenable to analog preparation in the search for selective phosphatase inhibitors. A new route to the unusual amino acid Adda is described, which incorporates an efficient diastereoselective aspartate alkylation and diene synthesis via a Suzuki coupling reaction. This work also features an efficient preparation of an *N*-methylalanine containing peptide via a Horner−Emmons condensation and several difficult amino acid coupling reactions that relied heavily on Carpino's remarkable HATU reagent.

Introduction

There is considerable interest in the elucidation of signaling pathways[1] in the central nervous system, both between neurons, via neurotransmitters, and within them. The elements responsible for transmitting signals from membrane bound receptors to other destinations within the neuron comprise a complex system that is only partially understood. One important aspect of this system is the reversible phosphorylation of proteins, which is mediated by kinases (phosphorylation catalysts)[2] and phosphatases (dephosphorylation catalysts)[3] that are indirectly linked, through modulation by second messengers such as cAMP or Ca^{2+}, to the activation of dopamine and *N*-methyl-D-aspartate (NMDA) receptors.[4] Reversible protein phosphorylation is a major control element of neurons and other cells, moderating such diverse functions as neurotransmission, muscle contraction, glycogen synthesis, T-cell activation, and cell proliferation.[5−9] Excessive protein phosphorylation, whether through the activation of kinases or through the inhibition of phosphatases, can lead to uncontrolled cellular proliferation, suggesting an active role for the phosphatases in tumor suppression.[5]

There is a diverse group of structurally interesting microbial defense toxins that act by inhibiting certain phosphatases, thereby disrupting the normal biochemical pathways. Examples include okadaic acid,[10] tautomycin,[11] calyculin,[12] and the microcystins,[13] all of which are potent competitive inhibitors of two major classes of phosphatases, protein phosphatase 1 (PP1), and protein phosphatase 2A (PP2A).[14] Several endogenous phosphoproteins, e.g., phospho-DARP 32, are substrates of PP2A but are selective inhibitors of PP1.[3,4] In contrast, the toxins inhibit both PP1 and PP2A, generally with little selectivity. The exceptions are cantharidin,[15] thyrsiferyl 23-acetate,[16] and okadaic acid,[9] which are PP2A selective. As a result of this selectivity, these toxins can be useful for dissecting the individual biochemical pathways associated with each of these enzymes, thereby providing a clearer picture of phosphatase control in some systems.[9] Further information could be gained through the similar use of a PP1-selective inhibitor, but compounds suitable for studies of this type are not yet known. The key to discovering such a PP1 selective inhibitor may lie in the chemical modification of existing nonselective inhibitors, and as the first step toward this goal we report the first total synthesis of a microcystin.[17,18]

[⊗] Abstract published in *Advance ACS Abstracts,* November 15, 1996.

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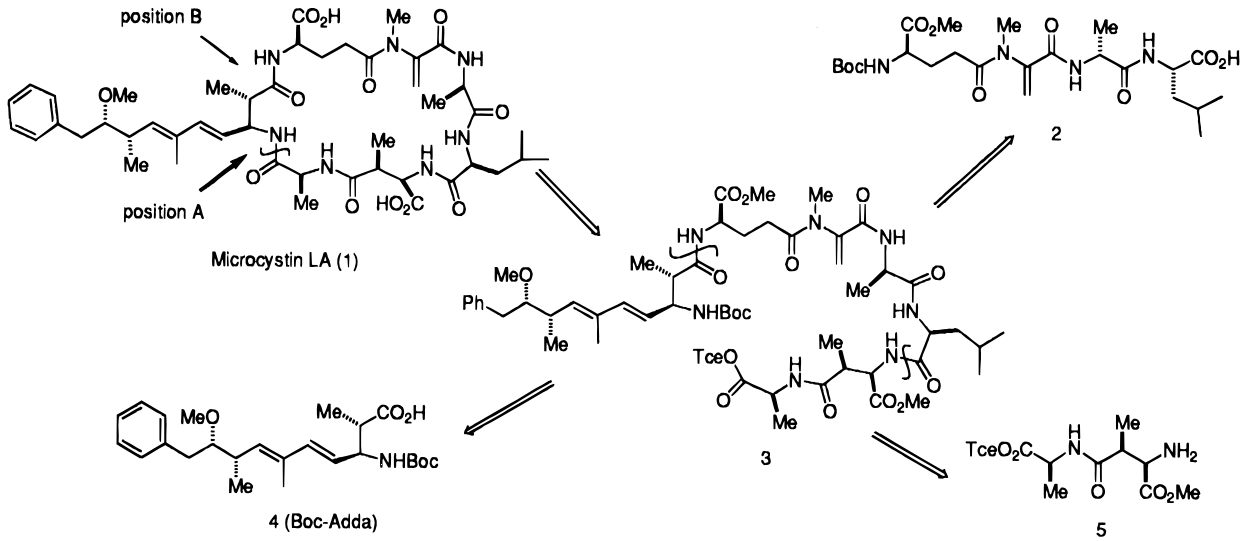
Scheme 1



The microcystins are produced by microcystis,[18] a genus of cyanobacteria (blue-green algae) that populates freshwater in many parts of the world and is responsible for the poisoning deaths of animals that consume or inhabit heavily contaminated waters.[13] The microcystins comprise a series of more than 40[19] hepatotoxic and tumor-promoting cyclic heptapeptides with the general structure *cyclo*-(D-Ala−X−(D)-*erythro*-*β*-methyl-*iso*-Asp−Y−Adda−(D)-*iso*-Glu−*N*-methyldehyhdro-Ala−), where the residues are numbered sequentially from (D)-Ala (1) to *N*-methyldehydroalanine (7), and the letters X and Y represent variable positions that are occupied by the only "natural" (L) amino acids in the molecule. For example, with microcystin-LA (**1**),[20] leucine (L) fills the "X" site and alanine (A) occupies the "Y" site. These toxins exhibit a number of synthetically challenging structural features, including the unusual amino acid Adda (cf. **4**), *iso*-(D)-glutamic and *iso*-(D)-*β*-methyl aspartic acid residues and *N*-methyl dehydroalanine (Mdha). Many of these features are conserved from one toxin to the next, but the variable regions X and Y can tolerate a fair amount of structural diversity without complete loss of toxicity.[21] The cyclic pentapeptides nodularin[22] and motuporin[23] share some of these features, and the latter has been synthesized by Schreiber and Valentekovich.[24] Recent X-ray diffraction studies of microcystin-bound PP1,[25] along with previous kinetic studies with synthetically modified microcystin and nodularin derivatives, have indicated that the structural features Adda, iso-Glu, and Mdha play important roles in phosphatase recognition.[26]

Results and Discussion

Synthetic Plan. Our approach to the synthesis of microcystin-LA begins with a division of the most challenging of these structural components into the three fragments shown in Scheme 1: (*i*) the Mdha-containing fragment **2**, (*ii*) the Adda fragment **4**, and (*iii*) the iso-*erythro*-*β*-methyl aspartate fragment **5**. According to this strategy, the difficult *N*-methyl acylation leading to fragment **2** would be completed early in the synthesis, the fragments **2** and **5** would be prepared and joined in a 4 + 2 segment coupling to form the hexapeptide, and the Adda component would be introduced near the end of the synthesis, just prior to macrocyclization. Epimerization at the acid terminal of fragment **2** during the 4 + 2 segment coupling was to be minimized by employing the new segment coupling techniques of Carpino,[27a] and epimerization of the *β*-amido Adda derivative **4** during its incorporation was not expected to pose a problem.[24] Orthogonal protection of the advanced synthetic intermediates would be accomplished with the Boc group and with methyl and trichloroethyl esters, as shown in Scheme 1.

Our decision to close the macrocycle at Adda-Ala (position A, Scheme 1) rather than at Adda-Glu (position B, Scheme 1) was based on conformational preferences and stereoelectronics, including the location of the Mdha residue within the cyclization precursor. Specifically, tertiary amides exhibit little or no preference for the trans conformations assumed by secondary amides and can even prefer the cis forms if supported by other favorable interactions such as intramolecular H-bonds.[28] Cis amides can promote cyclization in peptides by orienting the reactive functionalities in closer proximity, thereby reducing the entropy penalty that is usually incurred upon cyclization. Furthermore, full advantage of this type of turn induction can be achieved when the turn inducing structure lies midway along the cyclization precursor.[29ab]

The conformation of the cyclization precursor is only one of two major issues that demand consideration in the cyclization step. The second is that of steric hindrance in the coupling

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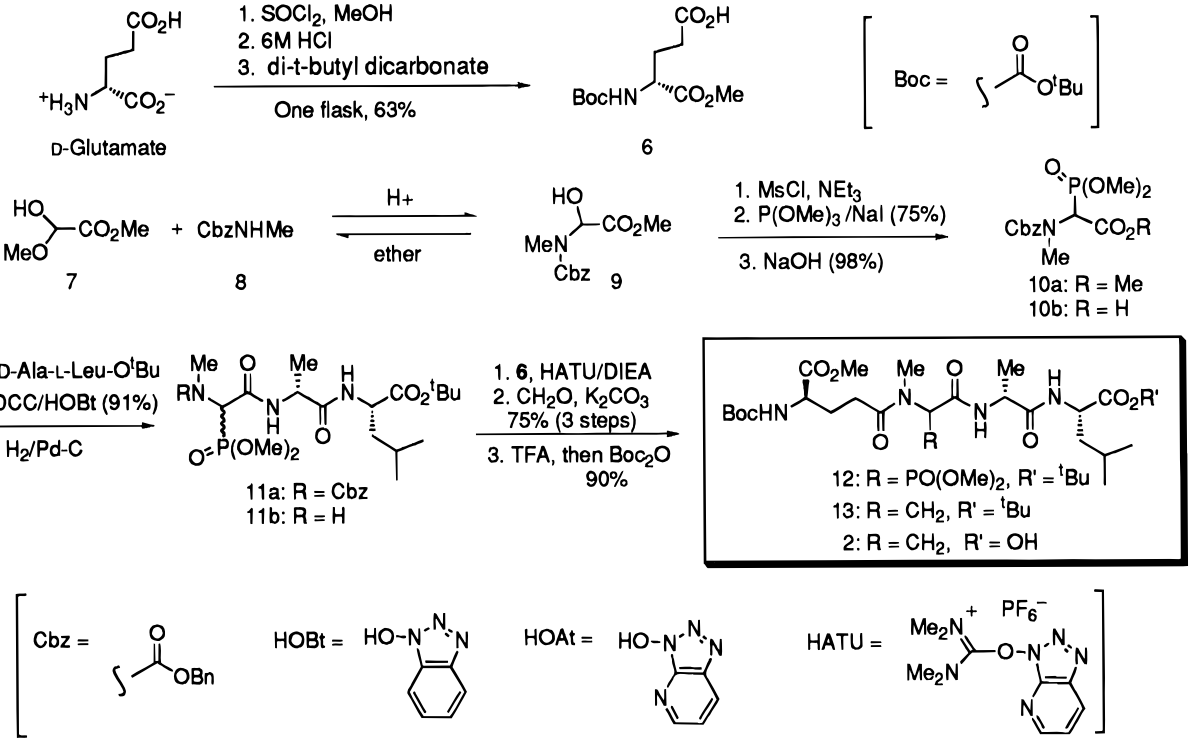
Scheme 2



residues, which can lead to some of the more frequently observed side reactions during cyclization, such as dimerization (or oligomerization) or epimerization of the C-terminal residue. In general, cyclization attempts involving hindered amines (i.e., *N*-methyl residues, Val or Ile residues) are ill-advised,[29b] but cyclization attempts between two (*S*)-amino acid residues can also retard the cyclization reaction.[29b,c] In fact, for penta- and hexapeptides consisting entirely of L residues, the cyclization reaction often does not proceed until the acid terminal residue epimerizes to the D configuration.[29d] Based on these observations, the Adda-Ala bond (position A) was chosen as the most attractive site for ring closure since it involves a union between (*S*) and (*R*) residues, and because the amine component is relatively unencumbered with a sterically nondemanding α-vinyl group.

Preparation of Fragment 2. Having established this general strategy, our synthesis of microcystin-LA begins with the largest subunit, the Mdha containing fragment **2**. Of the many available routes to dehydro amino acids and peptides,[30,31] relatively few have been applied to the preparation of *N*-alkylated versions of this functionality. In a recent example, Schreiber and Valentekovich introduced the dehydro functionality of motuporin in the final step by the hydroxide-catalyzed elimination of the free hydroxyl group of an *N*-methyl threonine residue.[24] A similar strategy involves the elimination of sulfonium salts[31a] or sulfoxides[31b] derived from cysteine or related residues. In a different approach, Rich et al. demonstrated the propensity of dehydro derivatives to N-alkylate selectively over saturated residues, but good yields can be difficult to obtain, and some substrates do not react.[28,31c] Our reluctance to rely on selective N-alkylation and our desire for a flexible route that would allow the incorporation of a variety of dehydro R groups for future analog preparation prompted us to explore other possibilities that would present a more direct route to this functionality. Based on these considerations, modification of Schmidt's method[31d,e] seemed best suited for our purposes.

Our synthesis of Segment **2** (Scheme 2) thus begins with the acid catalyzed condensation of methyl glyoxylate hemiacetal **7**[32] and *N*-methyl benzyl carbamate **8**[33] in ether, which gives a ca. 65:35 equilibrium mixture of **8** and **9**, respectively. We made no attempt to drive the equilibrium, because the starting materials are readily available in large quantity and the purification is simple: water soluble **7** is easily removed during aqueous workup, and *N*-methyl benzyl carbamate is recycled after silica gel chromatography to give the desired condensation product **9** in 27−37% yield (ca. 90% based on recovered carbamate). Although **9** rapidly equilibrates with its precursors **7** and **8** in the presence of strong acid, pure samples are stable for over 1 year at −15 °C. Conversion of **9** into the phosphonylsarcosine derivative **10a**[34] was achieved in 75% yield by activation with methanesulfonyl chloride followed by in situ treatment with sodium iodide and trimethyl phosphite. After saponification, the resultant acid **10b** was appended to D-Ala-L-Leu-O^{t}Bu[35] (DCC/HOBt), to supply the tripeptide **11a** in high yield as a 1:1 mixture of diastereomers.

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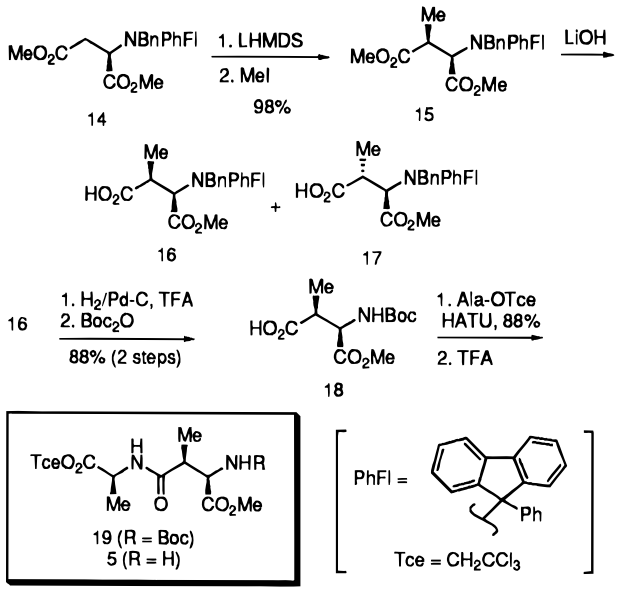
The next coupling reaction required the free amine **11b** and Boc-D-Glu-OMe (**6**). We initially used Fmoc protection for this purpose, anticipating later deprotection with an amine base prior to macrocyclization. However, attempts to couple the secondary amine **11b** with Fmoc-Glu-OMe (HATU/Hünig's base; HATU = *O*-(7-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, vide infra) were thwarted by competing Fmoc deprotection. Concurrent model studies also implicated base catalyzed imide formation at the aspartate residue as a potential side reaction during Fmoc deprotection later in the synthesis.[36ac] Since Cbz deprotection conditions were likewise incompatible with microcystin functionality, we instead chose the Boc protecting group. Our initial concerns over the stability of the dehydroalanine residue to the acidic Boc deprotection conditions were allayed by model studies demonstrating that the Mdha residue survived prolonged exposure to trifluoroacetic acid.

In order to more conveniently prepare the known selectively protected D-glutamate **6**[37] required to proceed through the sequence, we developed a one-pot synthesis from D-glutamic acid. The procedure (Scheme 2) combines quantitative thionyl chloride-induced esterification[38] with selective γ-ester hydrolysis[36b] and *N*-*tert*-butyloxycarbonylation and gives multigram quantities of the protected amino acid **6** as its dicyclohexylamine salt in 63% overall yield.[39] Regeneration of the free acid was accomplished by acidification with aqueous $NaHSO_4$ prior to coupling with the *N*-methylamine **11b**.

Although the reactivity of secondary amines such as **11b** is comparable with primary amines in S_N2 reactions, such is not the case with peptide coupling reactions, where low coupling rates often lead to undesired side reactions and extensive racemization. In these difficult coupling cases, the acid component of a coupling pair needs more powerful carboxyl activation, but this also raises concerns over racemization. Furthermore, the traditional HOBt (1-hydroxybenzotriazole, Scheme 2) based reagents (DCC/HOBt, BOP, etc.) and other methods such as *N*-carboxyanhydride activation tend to give suboptimal or inconsistent results.[40] That the unsuccessful cases involving HOBt often give benzotriazole active esters as major products[41] underscores the low reactivity of the benzotriazole ester toward hindered secondary amines.[42,43,27b−e]

These difficulties with hindered amino acid couplings have been addressed by several groups, and a number of specialized reagents are now available that facilitate the union of sterically hindered *N*-methyl amino acid derivatives.[41b,43] The HATU reagent developed by Carpino has proven particularly effective for these difficult couplings. With HATU, acid activation proceeds through the active ester derived from hydroxyazabenzotriazole (HOAt, Scheme 2), which is proposed to assist coupling via intramolecular general base catalysis that results in good reactivity toward *N*-methyl amines.[27b−e] Thus, the HATU mediated coupling of the secondary amine **11b** with (D)-Boc-Glu-OMe (**6**) in DMF provided the phosphonate diastereomers **12** in good yield. Without purification beyond an aqueous workup, the diastereomers were treated with aqueous formaldehyde and potassium carbonate, which afforded the desired dehydropeptide **13**, now a single isomer, in 75% yield over three steps from **11a**. Conversion into the acid **2** was accomplished in high yield by TFA/thioanisole induced deprotection of the Boc and *tert*-butyl ester groups, followed by in situ reprotection of the amine group with di-*tert*-butyldicarbonate.

Scheme 3



Preparation of Fragment 5. Having secured segment **2**, we next turned to the preparation of the D-*erythro*-β-methylaspartate containing dipeptide **5**. This effort begins with the benzyl/phenylfluorenyl protected aspartate **14** (Scheme 3), obtained in three steps and 78% overall yield from D-aspartic acid as described.[44a] We have previously shown that **14** can be alkylated (LHMDS/MeI) regio- and stereoselectively to give the crystalline, *syn*-β-methyl diastereomer **15** exclusively and in near quantitative yield.[44] This method provides >30 g of the requisite aspartate derivative in a high yielding route that requires no chromatography. Conversion of **15** into the β-acid **16** was accomplished with excellent regioselectivity by treatment with lithium hydroxide at 60 °C, although this reaction was also accompanied by 30% epimerization at carbon 3 to give **17** (Scheme 3). Attempts to suppress this side reaction by conducting the reaction at low temperature (0 °C to room temperature) failed due to the inherent low reactivity of compound **15**,[45] but the epimers could be easily separated by selective crystallization of **16**, which gave the desired acid in 70% yield after chromatography of the mother liquors (see

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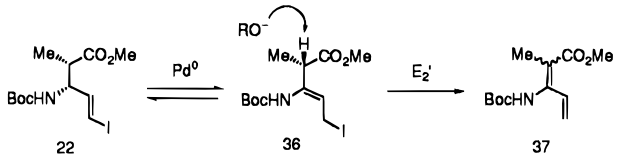
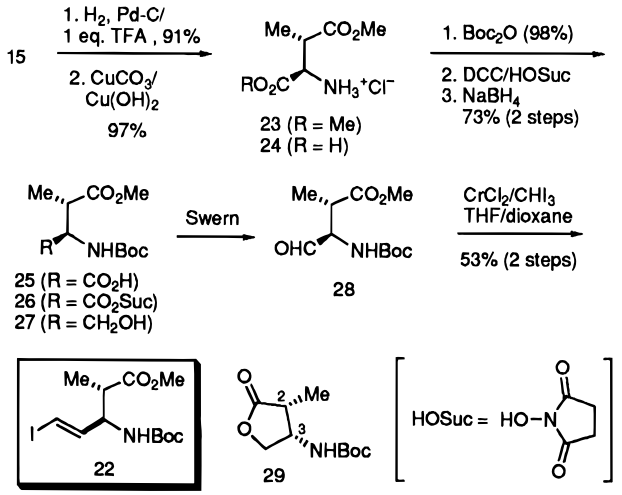


Figure 1.

Scheme 4

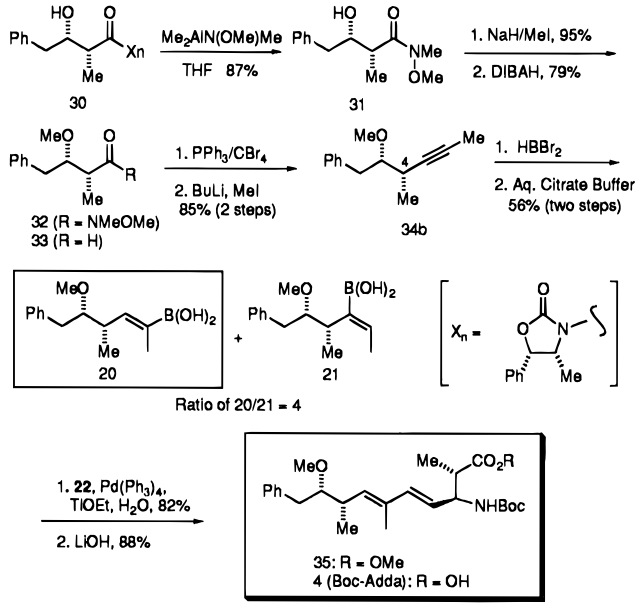


Experimental section). The stereochemistry of **16** was confirmed by re-esterification (K_2CO_3/MeI/DMF) and comparison with **15**.

Although **16** can be coupled with amino esters in high yield with HATU, the conditions required for the subsequent removal of the benzyl/phenylfluorenyl N-protecting groups (H_2/Pd-C/TFA) were not compatible with preservation of the trichloroethyl ester group we had chosen for the acid terminal of fragment **5**. So instead of coupling **16** and alanine trichloroethyl ester directly, we first replaced the *N*-benzyl-*N*-phenylfluorenyl groups with the Boc group (H_2/Pd-C/TFA, then Boc_2O) which could later be removed with TFA without affecting the trichloroethyl ester. The HATU/DIEA mediated union of the Boc derivative **18** and alanine trichloroethyl ester[46] gave better yields of the dipeptide **19** (88%) than DCC/DMAP (71%), although neither method showed any epimerization according to ^{1}H NMR analysis. The Boc dipeptide **19** was subsequently N-deprotected with TFA to give **5** immediately prior to the 4 + 2 segment coupling with fragment **2** (vida infra).

Synthesis of Fragment 4. With the tetrapeptide **2** and the dipeptide **5** in hand, we next set about synthesizing the protected Adda derivative **4**. Several syntheses of Adda have appeared in recent years,[24,47] but most of these routes suffered from a lack of selectivity during diene formation via the Wittig condensation approach. We developed a new route that would not only diverge from the methylated aspartate derivative **15**, which we had already used for fragment **5**, but that would also enable the mild and selective introduction of the Adda side chain

Scheme 5



by formation of the diene σ bond in a Suzuki cross coupling reaction.[48] This protocol is mild, chemoselective, and has worked remarkably well in highly functionalized substrates. With this plan in mind, our synthesis of Adda originates with the preparation of the backbone fragment, vinyl iodide **22** (Scheme 4), which was obtained from the β-methyl-D-aspartate derivative **15** as described below.

Whereas the route to **5** (Scheme 4) required regioselective hydrolysis of the distal methyl ester of **15**, Adda called for the complementary selective hydrolysis of the α-ester. One excellent method for effecting this type of deprotection is the copper(II) assisted hydrolysis of the diester ammonium salt.[38] This transformation (Scheme 4) requires N-deprotection of the benzylphenylfluorenyl aspartate **15** (H_2/Pd-C/TFA), which produces the hydrochloride salt **23** after an appropriate workup (see Experimental Section). Treatment of this salt with cupric carbonate in water/ethanol at 50−60 °C for several hours, followed by reductive workup with H_2S, gave the requisite acid **24** with high selectivity and in nearly quantitative yield. Boc protection was followed by active ester formation (DCC/*N*-hydroxysuccinimide),[49] and in situ reduction with sodium borohydride (73%).[50] Formation of the lactone **29**, which occurs spontaneously under basic conditions, is avoided by a final acid quench to give the amino alcohol **27**. The rate of lactonization is reduced by the 2,3-*cis* carbamate and methyl substituents of **29** (Scheme 4),[51] but chromatographed samples of **27** still slowly lactonized at room temperature. When stored at −15 °C, however, the alcohol solidified, and was stable for months when kept in this manner. Swern oxidation[52] of **27** gave the epimerization-prone aldehyde **28** in excellent yield, which was used immediately in the next step without purification. Transformation of **28** into the requisite iodoalkene **22** was thus carried out with $CrCl_2/CHI_3$ (53% yield) according to Takai's procedure[53] to give exclusively the *trans* alkene isomer.

(45) The distal ester of this compound is surprisingly unreactive, presumably due to steric hinderance from the *N*-phenylfluorenyl and α-methyl groups. The compound does not react with hydrazine in refluxing methanol. The failure of ester hydrazinolysis with hindered substrates has been noted before: Davies, J. S.; Mohammed, A. K. *J. Chem. Soc., Perkin Trans.1* **1981**, 2982.

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(50) Nikawa, J.; Shiba, T. *Chem. Lett.* **1979**, 981.

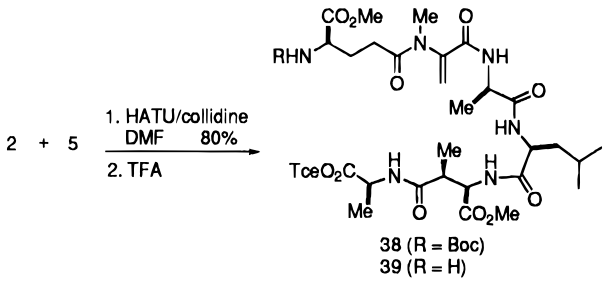
(51) Similar substrates without 2,3-syn substituents appear to lactonize spontaneously. See Baldwin, J. E.; North, M.; Flinn, A. *Tetrahedron Lett.* **1987**, *28*, 3167, and references therein.

(52) (a) Mancuso, A. J.; Swern, D. *Synthesis* **1981**, 165. (b) Tidwel, T. T. *Synthesis* **1990**, 857.

The synthesis of the second Suzuki coupling partner, the boronic acid **20**, begins with the known Evans aldol product **30** (Scheme 5).[47a,54] Transformation into the Weinreb amide **31** (87%)[55] was followed by methyl ether formation (NaH/MeI, 95%) and DIBAH reduction (79%)[12] to the aldehyde **33**. This aldehyde was readily converted into the methyl alkyne **34b** (via the dibromoalkene **34a**, not shown) in 85% yield via the Corey−Fuchs protocol,[56] but the subsequent transformation of **34b** into the requisite boronic acid **20** proved troublesome. Hydroborations of internal alkynes are known to proceed with moderate to poor regioselectivity, but branching α to the alkyne (i.e., position 4 of **34b**) reportedly raises this regioselectivity to acceptable levels.[57] Recent modifications, such as the employment of pinacolborane with[58] or without[59] catalysts such as Cp_2-ZrHCl, reportedly give significant improvements in hydroboration regioselectivities and efficiencies for a variety of terminal and internal alkynes, but attempts to hydroborate alkyne **34b** according to these methods resulted only in its slow conversion into complex product mixtures. When **34b** was subjected to the Suzuki−Miyaura protocol for hydroboration and dealkylative boronic ester formation,[60] the alkyne failed to react. Conversely, catecholborane[57] gave fairly efficient hydroboration when carried out in a nonchelating solvent such as benzene but was essentially nonselective (1.5:1 ratio of **20**:**21**). Of the reagents we investigated, dibromoborane dimethyl sulfide[61] gave the best results for the transformation of **34b** to **20**, but when the reaction progressed past 50% conversion, products from methyl ether cleavage and elimination began to appear. These side reactions were suppressed in the presence of Ag_2CO_3 but not completely. Nonetheless, $Br_2BH{\cdot}SMe_2$ hydroboration of **34b** followed by hydrolysis of the resultant dihaloborane gave 56% (74% based on recovered **34b**) of the boronic acids **20** and **21**, in a 4:1 ratio respectively, which were readily separated by chromatography. The undesired isomer **21** was stable for weeks at −15 °C, but the desired isomer **20** was unstable and was immediately subjected to the next step.

With the Suzuki coupling partners **20** and **22** in hand, we attempted the palladium catalyzed Suzuki cross-coupling reaction.[48] Our initial attempts with 1 M KOH as the base for this coupling resulted in the formation of small amounts of the product **35**, but the bulk of the vinyliodide was converted into a single isomer of the undesired diene **37**, as verified by HRMS and ^{1}H NMR (no attempt was made to assign the stereochemistry of **37**). We suspect that this diene forms via the pathway shown in Figure 1, whereby the palladium catalyzed alkene isomerization of **22** to **36** is followed by E_2' elimination to give **37**.[62] This side reaction was avoided by employing thallium ethoxide[63a] in the presence of water,[63b] and under these new conditions the palladium catalyzed Suzuki cross-coupling of vinyl iodide **22** and the boronic acid **20** provided the fully protected ADDA fragment **35** in 82% yield after HPLC purification. Saponification of the methyl ester (LiOH/THF/water) then gave the requisite Boc-ADDA-OH **4** in 88% yield after silica gel chromatography.

Scheme 6



Preparation of Fragment 3 and Macrocyclization. Having prepared the components **2**, **4**, and **5**, we were ready to assemble the cyclization precursor **3** and began this process with the 4 + 2 segment coupling of fragments **2** and **5** (Scheme 6). Segment couplings can be troublesome, because racemization generally occurs faster during the activation of *N*-acyl α-amino acids than it does during activation of the urethane-protected amino acids used in conventional stepwise coupling protocols.[27a] This problem has often led to the rejection of segment coupling strategies in favor of stepwise coupling methods, at the unfortunate sacrifice of convergence. However, through the development of new reagents and improved reaction conditions, low-racemization segment couplings are now possible in many cases. For example, Carpino et al. have shown that acid activation by HATU in the presence of collidine (rather than the more commonly used Hünig's base) gives little epimerization even in difficult cases.[27a] Using this method, the segment acylation of **5** with **2** proceeded well, supplying the hexapeptide **38** in 80% yield with no observable epimerization. Deprotection of the Boc group was accomplished with TFA immediately prior to coupling with the Adda fragment **4**.

For the final coupling reaction, which gives the cyclization precursor **3**, we relied again on the remarkable efficiency of HATU.[27c,e] The heptapeptide **3** was thus obtained in satisfactory yield and with no epimerization from the HATU/collidine mediated coupling of the hexapeptide **39** and the Adda fragment **4** (Scheme 7). Deprotection of the trichloroethyl ester of **3** proceeded well (Zn/HOAc,[46] 84%, Scheme 7), and the resultant acid **40** was activated for macrocyclization by treatment with pentafluorophenol and DCC, followed by *N*-Boc deprotection (TFA) of the resultant active ester **41**.[64a] The original literature protocols for peptide cyclization via the pentafluorophenyl ester call for dropwise addition of the amine TFA salt to a mixture of saturated aqueous $NaHCO_3$ or Na_2CO_3 and chloroform,[64] but we were concerned about the potential for carbonation of the amine group under these conditions. We therefore attempted this reaction under both the literature conditions and by substituting pH 9.5 phosphate buffer for carbonate. This change resulted in a 10−20% improvement in yield, suggesting that bicarbonate buffer may indeed not be optimal for this type of cyclization. Macrolactamization was induced by the dropwise addition of a 1 mM solution of the pentafluorophenyl ester TFA salt to a two phase mixture of chloroform and pH 9.5 phosphate buffer, which gave the cyclized diester in 56% yield from the active ester **41** after reversed phase HPLC purification. Slow addition was not necessary for the cyclization, since addition over 10 min gave the same result as did syringe pump addition over several hours. Our synthesis concludes with the lithium hydroxide induced saponification of the methyl esters (LiOH/

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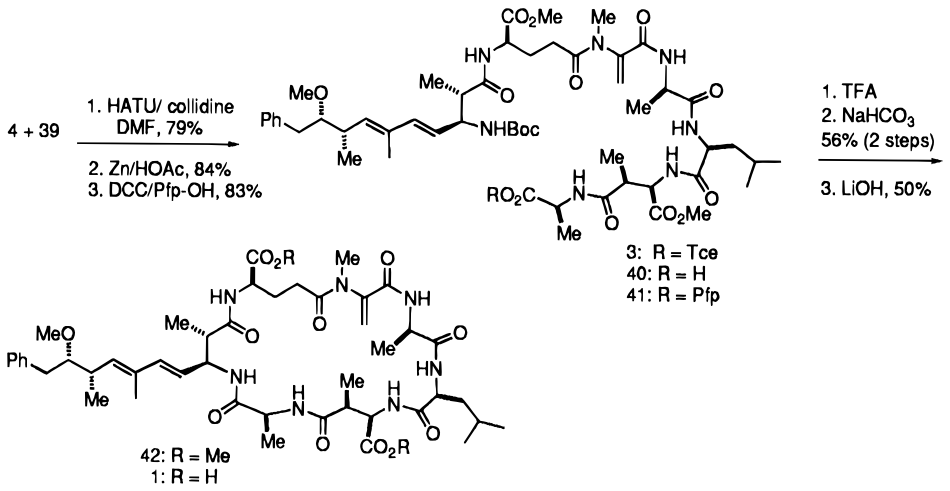
Scheme 7



THF/H_2O, 0 °C), which gives a 50% yield of microcystin-LA (**1**).[65,66] The synthetic microcystin-LA co-eluted with authentic material by reversed phase analytical HPLC (80:20 MeOH:0.2% aqueous TFA), and the ^{1}H NMR spectra of the samples in D_2O were identical.[67,68]

Conclusion

In summary, the first total synthesis of a microcystin described herein features a convergent route that is amenable to analog preparation in the search for selective phosphatase inhibitors. It also features an efficient aspartate alkylation method that provides large quantities of the alkylated aspartate **15**, a key intermediate en route to both the *erythro*-β-methyl aspartate and Adda amino acid units. To our knowledge, this work marks the first application of Schmidt's phosphonoglycine method to the preparation of *N*-alkyl dehydroamino acid containing peptides and demonstrates the compatibility of the *N*-methyldehydro residue with Boc and *tert*-butyl ester protection schemes. Finally, the success of this route is due in no small part to Carpino's remarkable HATU reagent in several difficult couplings of sterically hindered or racemization-prone amino acid residues.

(65) The remainder of the material is isomeric with the desired product (HRMS and ^{1}H NMR). Assuming it to result from base catalyzed epimerization competing with saponification, we attempted a number of alternative saponification methods but with no success. Cesium carbonate (ref 66a) gave levels of isomerization comparable to those observed with lithium hydroxide, and barium hydroxide gave more isomerization. Lithium hydroperoxide (ref 66b−d) generated only small amounts of the desired product along with multiple side products. The level of isomerization during this step is consistent with levels observed by Schreiber and Valentekovich for the analogous saponification step in their synthesis of motuporin: Dr. Robert J. Valentekovich, The University of California at Irvine, personal communication, 1996.

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(67) The final product **1** was only slightly soluble in D_2O, which complicated characterization by ^{1}H NMR analysis due to the large HDO solvent peak. The water supression NMR method developed by Shaka eliminated this problem. See: Hwang, T. L.; Shaka, A. J. *J. Magn. Reson. Series A* **1995**, *112*, 275.

(68) Whereas the proton NMR of microcystin-LA in D_2O exhibited a single set of sharp resonances, but such was not the case with most of the peptide intermediates. The ^{1}H spectra of these compounds exhibited rotamer-induced peak broadening and doubling at room temperature in $CDCl_3$ or DMSO-d_6. Coalescence of the rotamers generally occurred at 70−90 °C in DMSO-d_6, which facilitated peak assignment and characterization. The cyclized diester **42** exhibited severe peak broadening and doubling in DMSO-d_6 at room temperature, but, when heated to 70−90 °C, imide formation occurred as evidenced by the absence of a methyl ester resonance in the ^{1}H NMR spectrum and by the loss of one unit of methanol according to HRMS. The spectrum of **42** in $CDCl_3$ exhibited the presence of one major rotamer and <10% of a minor one, which began to coalesce at 45 °C.

Experimental Section

General Methods. ^{1}H and ^{13}C NMR spectra were obtained on an Omega 500 (500 MHz) or a General Electric GN-500 (500 MHz) spectrometer. For spectra measured in organic solvents, data are reported in ppm from internal tetramethylsilane for ^{1}H NMR and in ppm from the solvent for ^{13}C NMR. For ^{1}H spectra taken in D_2O, data are reported in ppm relative to HDO (3.80 ppm). Data are reported as follows: chemical shift, multiplicity (app = apparent, par obsc = partially obscured, ovrlp = overlapping, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, abq = ab quartet), coupling constant, and integration. Infrared (IR) spectra were taken with a Perkin-Elmer Model 1600 series FTIR spectrophotometer. Optical rotations were obtained with a JASCO DIP-360 digital polarimeter. Melting points (mp) were obtained from a Laboratory Devices Mel-Temp melting-point apparatus and are reported uncorrected. Elemental analyses were performed by Desert Analytics, Tucson, AZ. Thin-layer chromatography (TLC) was performed on 0.25 mm Merck precoated silica gel plates (60 F-254), and silica gel chromatography was performed using ICN 200−400 mesh silica gel. Normal phase preparative HPLC was conducted with a 25 × 100 mm Waters prep Nova-Pak HR silica cartridge (WAT038511), and reversed phase preparative HPLC was conducted with a 25 × 100 mm Waters prep Nova-Pak HR C_{18} cartridge (WAT038510). Reversed phase analytical HPLC was conducted with a 4.6 × 250 mm Rainin Microsorb-MV C_{18} column (86-200-C5). Solution pH measurements were made with Whatman type CF pH 0−14 indicator paper strips. Inert atmosphere operations were conducted under nitrogen passed through a Drierite drying tube in oven or flame-dried glassware. Anhydrous tetrahydrofuran (THF) was distilled first from calcium hydride and then from potassium; anhydrous ether was distilled from potassium. Triethylamine and methylene chloride were purified by distillation from calcium hydride. Diisopropylethylamine (DIEA) and DMF were dried over sieves. LHMDS (1.0 M in THF) was purchased from Aldrich Chemical Company. HATU was purchased from PerSeptive Biosystems Inc. D-Boc-Glu(OH)-OMe was prepared by the one-pot procedure described in the text, and full experimental details will be published elsewhere. Alanine trichloroethyl ester was prepared through a higher yielding modification of the literature protocol,[46a] as described below. *N*-Methyl benzylcarbamate was prepared from benzylchloroformate and aqueous methylamine as suggested in the literature.[33] Methylglyoxylate hemiacetal was prepared according to the literature procedure.[32] All other reagents were used as purchased from Aldrich, Sigma-Aldrich, or Acros unless otherwise stated.

***N*-Carbobenzyloxy-α-hydroxysarcosine Methyl Ester (9).** To a stirred solution of 35.3 g (214 mmol) of *N*-methylbenzylcarbamate (**8**)[33]

and 25.7 g (214 mmol) of methylglyoxylate hemiacetal **7**[32] in 214 mL of anhydrous ether was added 5.0 g (21 mmol) of camphor sulfonic acid at room temperature. After 30 h, the solution was shaken with 200 mL of 50% saturated aqueous $NaHCO_3$. The water layer was extracted with an additional 200 mL of ether, and the combined ether portions were washed with brine, dried with $MgSO_4$, filtered, and concentrated to give 40 g of a clear oil containing mainly **9** and **8** (in a 1:2 ratio, respectively) and a trace of **7** by ^{1}H NMR analysis. The material was chromatographed (3:7 EtOAc:hexanes) to give 14.9 g (27%) of the title compound **9** and 24.5 g of recovered **8**. Spectral data for **9** are as follows: R_f 0.35 (1:1 EtOAc:hexanes); IR (thin film): 3408, 2954, 1750 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6, 70 °C): δ 2.77 (s, 3H), 3.66 (s, 3H), 5.12 (s, 2H), 5.77 (br s, 1H); 6.61 (br s, 1H); 7.31−7.39 (m, 5H); MS *m/e* calcd for $C_{12}H_{16}NO_5^+$ (M + H$^+$): 254.1028. Found 254.1022.

***N*-Carbobenzyloxy-α-dimethylphosphonylsarcosine Methyl Ester (10a).**[34] To 13.3 g (52.6 mmol) of **9** and 16.0 g (158 mmol) of triethylamine in 100 mL of dry CH_2Cl_2 at −20 °C was added dropwise 6.63 g (57.9 mmol) of freshly distilled methanesulfonyl chloride. The mixture was warmed to room temperature after 2 h and stirred for an additional 15 h. The solvent was then removed under vacuum and replaced with 50 mL of dry acetonitrile. Powdered potassium iodide 8.73 g (52.6 mmol) was added followed by 7.83 g (63.1 mmol) of trimethylphosphite. After vigorous stirring for 22 h, the solvent was removed under vacuum, and the residue was partitioned between EtOAc and water. The aqueous layer was extracted with an additional portion of EtOAc, and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated to give an orange oil. Chromatography (9:1 EtOAc:hexanes) gave 13.6 g (75%) of **10a** as a pale yellow oil: R_f 0.35 (EtOAc) IR (thin film) 2957, 1750, 1704 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$), two rotamers: δ 3.09 (s, 3H, minor rotamer), 3.12 (s, 3H, major rotamer), 3.73, 3.75, 3.777, 3.781, 3.798, 3.803, 3.829, 3.833, 3.86 (9S, 9H from each rotamer,[69] assigned to the methyl esters of the phosphonate and the carboxylate groups), 5.11 (d, J = 12.3, 1H, minor rotamer), 5.15−5.22 (obsc d, 1H, minor rotamer), 5.19 (app dd, 2H, J = 11.7, major rotamer), 5.32 (d, J = 26.5, 1H, minor rotamer), 5.56 (d, J = 26.0, 1H, major rotamer), 7.32−7.38 (m, 5H from each rotamer); MS *m/e* calcd for $C_{14}H_{21}NO_7P$ (M + H$^+$): 345.0972, found 345.0963.

***N*-Carbobenzyloxy-α-dimethylphosphonylsarcosine (10b).**[34] To a solution of 10.9 g (31.6 mmol) of **10a** in 30 mL of THF at room temperature was added a solution of 1.52 g (36.3 mmol) of lithium hydroxide in 20 mL of water. After stirring vigorously for 3 h, the bulk of the THF was removed in vacuo and the remaining solution was washed with one 25 mL portion of ether. The aqueous portion was then acidified with 5 mL of 6 M HCl and extracted with three 20 mL portions of EtOAc. The combined organic layers were washed with brine, dried with $MgSO_4$, and concentrated to give 9.8 g (98%) of a colorless oil that solidified upon standing and was pure according to ^{1}H NMR. Crystallization from EtOAc/hexanes gave 8.8 g (88%) of **10b** as a white solid: mp 94−96 °C; R_f 0.60 (80:20:15:1 CH_2Cl_2:MeOH:HOAc:$H_2$0); IR (thin film) 3433−2578 br, 2956, 1739, 1700 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$, major rotamer): δ 3.10 (s, 3H); 3.76 (d, J = 11.2, 3H), 3.89 (d, J = 11.2, 3H), 5.14 (d, J = 12.4, 1H), 5.21 (d, J = 12.4, 1H), 5.60 (d, J = 26.7, 1H), 7.30−7.38 (m, 5H), 10.20 (br s, 1H); MS *m/e* calcd for $C_{13}H_{19}NO_7P^+$ (M + H$^+$): 332.0894, found 332.0991. analysis calcd for $C_{13}H_{18}NO_7P$: C, 47.13; H, 5.49; N, 4.23. Found: C, 46.92; H, 5.67; N, 4.40.

***N*-Carbobenzyloxy- α-dimethylphosphonylsarcosyl-(D)-alanyl-(L)-leucine-*tert*-butyl Ester (11a).** To 7.79 g (19.8 mmol) of *N*-Cbz-(D)-Ala-(L)-Leu-O^{t}Bu in 40 mL of methanol was added 1.2 g of 10% palladium on carbon. The mixture was hydrogenated at 1 atm for 2.5 h, then filtered through Celite, and concentrated. The residue was reconcentrated from toluene and then from CH_2Cl_2/hexanes to remove residual methanol. The residue was then dissolved in CH_2Cl_2, and 7.20 g (21.8 mmol) of **10b** was added, followed by 2.94 g (21.8 mmol) of HOBt and, at 0° C, 4.50 g (21.8 mmol) of DCC. The mixture was stirred for 2 h at 0° C and for 12 h at room temperature. After filtering through Celite and concentrating, the resultant oil was dissolved in EtOAc and washed succcessively with saturated citric acid, saturated $NaHCO_3$, and brine. The solution was dried over $MgSO_4$, filtered, concentrated, and chromatographed (EtOAc) to give 10.3 g (91%) of **11a** as a white foam consisting of a 1:1 mixture of two diastereomers: R_f 0.24 and 0.38 (EtOAc); IR: 3289, 2957, 1734, 1697, 1667, 1247, 1150, 1035; FAB MS *m/e* calcd for $C_{26}H_{43}N_3O_9P^+$ (M + H$^+$): 572.2737, found 572.2743. The high R_f diastereomer could be selectively crystallized in low yield from ether/hexanes at −15°C over 1−2 weeks: mp 54−63 °C (dec); ^{1}H NMR (500 MHz, DMSO-d_6, 110 °C) δ 0.85 (d, J = 6.1, 3H), 0.90 (d, J = 6.4, 3H), 1.26 (d, J = 7.1, 3H), 1.41 (s, 9H), 1.53 (app t, J = 7.1, 2H), 1.64 (app dq, J = 7.1, 7.1, 1H), 3.02 (s, 3H), 3.68, 3.69 (two ovrlp d, J = 10.6 and 10.7, 6H), 4.17 (br app q, J = 7.2, 1H), 4.38 (br app dq, J = 7.4, 1H), 5.14 (s, 2H), 5.32 (d, J = 23.9, 1H), 7.32 (m, 1H), 7.36 (app d, J = 4.2, 4H), 7.83 (br d, J = 7.7, 1H), 7.99 (br d, J = 6.8, 1H). ^{1}H NMR data for the other isomer was obtained from a proton spectrum of the diastereomeric mixture, and are as follows: ^{1}H NMR (500 MHz, DMSO-d_6, 110 °C) δ 0.86 (par obsc d, J = 5.9, 3H), 0.91 (par obsc d, J = 5.9, 3H), 1.26 (d, J = 6.9, 3H), 1.41 (s, 9H), 1.53 (app t, J = 7.1, 2H), 1.64 (app dq, J = 7.1, 7.1, 1H), 3.00 (s, 3H), 3.69 (d, J = 10.8, 6H), 4.17 (app q, J = 7.2, 1H), 4.38 (br dq, J = 7.4, 1H), 5.14 (s, 2H), 5.30 (par obsc d, J = 23.9, 1H), 7.32 (m, 1H), 7.36 (app d, J = 4.2, 4H), 7.86 (par obsc br m, 2H).

Boc-D-*iso*-Glu(OMe)-MeΔAla-(D)-Ala-(L)-Leu-O^{t}Bu (13). 11a (5.0 g, 8.75 mmol) was hydrogenated in 16 mL of methanol over 500 mg of 10% palladium on carbon for 2.5 h at 1 atm, then filtered, and concentrated. The residue was reconcentrated twice from 5 mL of toluene to remove residual methanol, and the remaining oil was dissolved in 15 mL of DMF. Boc-Glu-OMe (**6**) (3.43 g, 13.1 mmol) was then added, followed by 3.39 g (26.3 mmol) of DIEA, and, after cooling to 0 °C, 4.99 g (13.1 mmol) of HATU. After stirring for 1 h at 0 °C and 17 h at room temperature, the resultant orange solution was partitioned between 200 mL of 50% saturated citric acid and 200 mL of ether. The organic portion was washed with 150 mL of 50% saturated $NaHCO_3$, and the aqueous portions were back extracted twice with 150 mL each of ether. The combined organic portions were then washed with brine, dried with $MgSO_4$, filtered, and concentrated to give an off-white foam consisting of the two diastereomers of **12**, which could be resolved by TLC, but were inseparable by silica gel chromatography. R_f 0.26 and 0.36 (EtOAc); IR (thin film) 3326, 2962, 1728, 1712, 1697, 1666 cm^{-1}; FAB MS calcd for M + Na$^+$: 703.3295; found: 703.3291. The NMR spectra for this 1:1 mixture of diastereomers was further complicated by the presence of rotamers and spin coupling to phosphorus. For this reason, and to obtain a higher overall yield for the sequence, further purification and ^{1}H NMR characterization were delayed until after the Horner−Emmons reaction, which gives a single stereoisomer: to the crude mixture of diastereomers was added 40 mL of THF, 20 mL of water, 4.8 g (35 mmol) of K_2CO_3, and 2.1 mL (ca. 10 equivalents) of 37% aqueous formaldehyde. The mixture was stirred vigorously for 8 h at room temperature, then poured into 100 mL of water, and extracted twice with 100 mL of ether. The organic extracts were washed with 100 mL of 50% saturated citric acid, and the aqueous wash was back extracted with 100 mL of ether. Combined organic portions were washed with brine, dried over $MgSO_4$, filtered, and concentrated to give 4.95 g of an off-white foam. Chromatography (EtOAc) gave 3.80 g (75%) of **13** as a foam that exibited no distinct melting point. R_f 0.38 (EtOAc); IR (thin film) 3320, 2971, 1722, 1658 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6, 90 °C) δ 0.85 (d, J = 6.8, 3H), 0.89 (d, J = 6.5, 3H), 1.29 (d, J = 7.6, 3H), 1.37 (s, 9H), 1.40 (s, 9H), 1.53 (app t, J = 7.6, 2H), 1.63 (dq, J = 6.8, 6.8, 1H), 1.82 (app dq, J = 8.3, 8.3, 1H), 1.95 (app dq, J = 7.2, 7.2, 1H), 2.32 (br app q, J = 7.2, 1H), 3.01 (br s, 3H), 3.62 (s, 3H), 3.98 (br app q, J = 6.0, 1H), 4.17 (app q, J = 7.2, 1H), 4.39 (dq, J = 7.6, 7.6, 1H), 5.52 (br s, 1H), 5.96 (br s, 1H), 6.79 (br s, 1H), 7.74 (br s, 1H), 7.86 (br d, J = 7.6, 1H); MS calcd for $C_{28}H_{49}N_4O_9^+$ (M + H$^+$): 585.3499; found: 585.3489.

Boc-D-*iso*-Glu(OMe)-MeΔAla-(D)-Ala-(L)-Leu-OH (2). To 1.00 g (1.71 mmol) of **13** in 6 mL of TFA was added 212 mg (1.71 mmol) of thioanisole. After 30 min, the solution was concentrated and then reconcentrated once from CH_2Cl_2/hexanes and three times from 20 mL portions of EtOAc. The remaining residue was diluted with 4 mL of water, and 1.1 g (10.3 mmol) of Na_2CO_3 was added, followed by, after cooling to 0 °C, 410 mg (1.88 mmol) of di-*tert*-butyldicarbonate in 4 mL of dioxane. The mixture was warmed to room temperature, stirred for 12 h, then poured into a separatory funnel, diluted with 30 mL of water, and washed twice with ether. The aqueous portion was acidified to pH 2−3 with saturated citric acid and extracted twice with EtOAc.

The combined extracts were washed with brine, dried with $MgSO_4$, filtered, and concentrated to an oil. Chromatography (1:10:90 HOAc:MeOH:CH_2Cl_2) gave 817 mg (90%) of **2** as a white foam. A sample precipitated as a white powder from ether/hexanes at −78 °C exhibited no distinct melting point: R_f 0.40 (1:10:90 HOAc:MeOH:CH_2Cl_2); IR (thin film) 3318, 2959, 1722, 1653 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6, 90 °C) δ 0.85 (d, J = 6.4, 3H), 0.88 (d, J = 6.8, 3H), 1.37 (s, 9H), 1.55 (t, J = 6.8, 2H), 1.62 (app dq, J-13.5, 7.2, 1H), 1.80 (app dq, J = 14.7, 6.8, 1H), 1.95 (app dq, J = 12.7, 6.0, 1H), 2.31 (m, 3H), 3.00 (s, 3H), 3.61 (s, 3H), 3.98 (br app q, J = 5.2, 1H), 4.26 (app q, J = 7.6, 1H), 4.40 (app dq, J = 7.2, 7.2, 1H), 5.52 (br s, 1H), 5.98 (br s, 1H), 6.89 (br s, 1H), 7.79 (br s, 1H) 7.92 (br s, 1H); FAB MS *m/e* calcd for $C_{24}H_{41}N_4O_9^+$ ($M + H^+$): 529.2873. Found: 529.2895.

D-*erythro*-*N*-(9-Phenylfluoren-9-yl)-*N*-benzyl-β-methylaspartic Acid α-Methyl Ester (16). To 4.19 g (8.28 mmol) of **15** in 150 mL of THF was added 45 mL of MeOH, and the resultant solution was heated to 60° C. Lithium hydroxide monohydrate (3.47 g, 82.8 mmol) in 90 mL of H_2O was then added over 10 min, and the resultant cloudy mixture was stirred at 60 °C for 24 h. After cooling to room temperature, the mixture was washed twice with ether, and the combined organic phases were back-extracted twice with water. The combined aqueous phases were acidified with saturated citric acid and extracted twice with EtOAc. The combined EtOAc phases were washed once each with water and brine, dried over $MgSO_4$, filtered, concentrated to approximately 1/2 the original volume, and diluted with hexanes. The desired epimer (1.64 g) was obtained as a crystalline solid, and the mother liquors were chromatographed (65 g silica/g sample, 25:75 EtOAc:hexanes) to provide an additional 1.12 g (69% combined yield) of the title compound **16**: R_f 0.63 (1:1 EtOAc:hexanes); IR (thin film) 3055 br, 2933, 1700 $cm^{-1;}$ ^1H NMR (500 MHz, $CDCl_3$) δ 0.66 (d, J = 7 Hz, 3H), 2.43 (dq, J = 7, 11.5 Hz, 1H), 3.00 (s, 3H), 3.57 (d, J = 12 Hz, 1H), 4.51 (d, J = 13.5 Hz, 1H), 4.85 (d, J = 13.5 Hz, 1H), 7.25−7.84 (m, 20H); HRMS *m/e* calcd for $(M+H)^+$:492.2176. Found 492.2174. Anal. Calcd for $C_{32}H_{29}NO_4$:C, 78.16; H, 5.96; N, 2.85. Found: C, 78.14; H, 6.01; N, 2.87. The undesired epimer **17** was obtained as 1.18 g (29%) of a white solid: R_f 0.33 (1:1 EtOAc:hexanes); IR (thin film) 3055 br, 2933, 1716 $cm^{-1;}$ ^1H NMR (300 MHz, $CDCl_3$) δ 1.10 (d, J = 7.1 Hz, 3H). 2.18 (dq, J = 11.3, 7.2 Hz, 1H). 2.88 (s, 3H), 3.41 (d, J = 11.3 Hz, 1H), 4.05 (d, J = 13.4 Hz, 1H), 4.35 (d, J = 13.4 Hz, 1H), 7.18-7.75 (m, 20H); MS *m/e* calcd for $(M+H)^+$: 492.2176, found 492.2168.

Boc-D-*erythro*-β-methyl Aspartic Acid α-Methyl Ester (18). A Par bottle was charged with 2.71 g (5.51 mmol) of **16**, 51 mL of EtOAc, and 9 mL of CH_2Cl_2. After the solids had dissolved, 0.85 mL (1.10 mmol) of TFA was added, carefully followed by 405 mg of 10% Pd/C. The mixture was hydrogenated at 50 psi for 14 hours, then filtered through Celite, and concentrated. The resultant white crust was partitioned between 50 mL of ether and 24 mL of water. The aqueous layer was washed once with ether, and the combined organic portions were extracted once with 20 mL of water. The combined aqueous phases were then cooled to 0° C, and 2.92 g (27.1 mmol) of Na_2CO_3 was added, followed by 1.40 mL (6.06 mmol) of di-*tert*-butyldicarbonate in 33 mL of dioxane, and the resultant slurry was warmed to room temperature, stirred vigorously for 25 h, and then partitioned between ether and water. The aqueous layer was washed once with ether, and the combined organic phases were back-extracted once with water. The combined aqueous phases were then acidified with 1 M $NaHSO_4$ and extracted twice with EtOAc. The combined EtOAc phases were washed twice with water and once with brine, dried over $MgSO_4$, filtered, and concentrated to a clear oil. Chromatography (30 g silica/g sample, 1:49:50 HOAc:EtOAc:hexanes) gave 1.27 g (88%) of **18** as a colorless oil: R_f 0.35 (1:10 MeOH:CH_2Cl_2); IR (thin film) 3355 br, 2977, 2611, 1711 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ (major rotamer) 1.04 (d, J = 7.5 Hz, 3H), 1.37 (s, 9H), 2.81 (dq, J = 6.5,7 hz, 1H), 3.60 (s, 3H), 4.37 (dd, J = 6, 8.5 Hz, 1H), 6.95 (d, J = 8.5 Hz, 1H); MS *m/e* calcd for $(M+H)^+$: 262.1290, found 262.1289. analysis calcd for $C_{11}H_{19}NO_6$: C, 50.56; H, 7.34; N, 5.36. Found: C, 50.28; H, 7.19; N, 5.07.

Alanine Trichloroethylester, *p*-Toluene Sulfonate.[46a] To 6.78 g (76 mmol) of L-alanine in 150 mL of toluene in a round bottom flask equipped with a Dean-Stark trap was added 68 mL of trichloroethanol and 21.6 g (114 mmol) of *p*-toluenesulfonic acid. The mixture was refluxed with azeotropic water removal for 15 h, then cooled to room temperature, diluted with 440 mL of fresh ether, and cooled in an ice bath for 2 h. The resultant white precipitate was collected by filtration and recrystallized from MeOH/Et_2O to give 22.3 g (81%) of alanine trichlorethyl ester *p*-toluene sulfonate as a white solid. The physical properties of the product matched the literature values.[46a] The free amine was generated as follows: the tosylate salt (998 mg 2.76 mmol) was shaken with 30 mL of 50% saturated $NaHCO_3$ and 30 mL of ether. The aqueous portion was extracted with an additional 30 mL of ether, and the combined organic extracts were dried with K_2CO_3, filtered, and concentrated to give 531 mg (100%) of alanine trichloroethyl ester, which was used directly in the next step.

Boc-D-*erythro*-β-Me-*iso*-Asp(OMe)-Ala-OTce (19). The acid **18** (360 mg 1.38 mmol) and 531 mg (2.76 mmol) of alanine trichloroethyl ester were concentrated together from 5 mL of toluene, and the residue was dissolved in 4 mL of DMF and cooled in an ice bath. To the solution was added 535 mg (4.14 mmol) of DIEA, followed by 577 mg (1.52 mmol) of HATU. The solution was warmed to room temperature after 1 h and stirred for an additional 8 h. The mixture was worked up as for compound **13** to give 644 mg of an off-white solid. Chromatography (1:1 EtOAc:hexanes, 36 g of SiO_2) gave 563 mg (88%) of **19**. Crystals were obtained from EtOAc/hexanes at −15 °C: mp 161−162 °C; R_f 0.44 (1:1 EtOAc:hexanes); IR (thin film) 3330, 2979, 1750 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.28 (d, J = 7.6, 3H), 1.45 (s, 9H), 1.50 (d, J = 7.2, 3H), 3.11 (m, 1H), 3.74 (s, 3H), 4.44 (dd, J = 4.0, 9.9, 1H), 4.63 (d, J = 11.9, 1H), 4.64 (par obsc dq, J = 7.2, 7.2, 1H), 4.96 (d, J = 11.9, 1`H), 5.72 (d, J = 8.7, 1H), 6.20 (d, J = 6.4, 1H) HRMS *m/e* calcd for $C_{16}H_{26}N_2O_7Cl_3^+$ ($M + H^+$): 463.0805. Found: 463.0805.

D-*erythro*-β-Methyl Dimethylaspartate Hydrochloride (23). To 19.0 g (37.6 mmol) of **15** in 20 mL of CH_2Cl_2 and 40 mL of MeOH was added 8.57 g (75.2 mmol) of TFA. The resultant solution was hydrogenated over 3.8 g of 10% Pd-C at 52 psi for 8 h. The mixture was then carefully filtered through Celite, and the filter pad was rinsed with copious CH_2Cl_2 and MeOH. The CH_2Cl_2 was removed under vacuum, and the resultant methanolic slurry was filtered through Celite to remove the white solids. The filtrate was concentrated to an oil, which was then partitioned between 20 mL of water and 40 mL of ether. Three milliliters of concentrated HCl was added, and the mixture was shaken. The aqueous portion was removed, cooled with ice, and basified to pH 10.0 with cold concentrated ammonium hydroxide. The mixture was then extracted twice with EtOAc, and the combined EtOAc portions were washed with brine, dried with K_2CO_3, and concentrated to give a colorless oil that was pure according to ^1H NMR analysis. The oil was immediately dissolved in dry ether, and a methanolic solution of 37.6 mmol of HCl (prepared by carefully dissolving 2.7 mL (37.6 mmol) of acetyl chloride in 10 mL of MeOH at 0 °C) was added to generate the HCl salt. The solvent was removed under vacuum to give a white residue, which was then dissolved in CH_2Cl_2, diluted with hexanes, and reconcentrated to give 7.25 g (91%) of **23** as a white solid that was pure according to ^1H NMR. Crystallization from MeOH/EtOAc gave white needles: mp 124−126 °C; IR (thin film) 3413 br, 2956, 1745, 1234 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 1.33 (d, J = 7.5, 3H), 3.49 (dq, J = 4.1, 7.5, 1H), 3.76 (s, 3H), 3.85 (s, 3H), 4.51 (d, J = 4.1, 1H); MS *m/e* calcd for $(M - Cl^-)^+$: 176.0919. Found 176.0917. Anal. Calcd for $C_7H_{14}NO_4Cl$: C, 39.72; H, 6.68; N, 6.62. Found: C, 39.72; H, 6.72; N, 6.50.

D-*erythro*-β-methylaspartic Acid β-Methyl Ester Hydrochloride (24). To 2.00 g (9.45 mmol) of **23** in 32 mL of water and 8 mL of EtOH was added 2.52 g (11.4 mmol) of basic cupric carbonate ($CuCO_3$·$Cu(OH)_2$), and the mixture was stirred vigorously at 70 °C for 2.5 h. The mixture was then filtered through Celite, and the filter pad was rinsed with copious water. The resultant blue solution was diluted with more water to bring the final volume to 60 mL. Hydrogen sulfide was then bubbled through the solution until an aliquot filtered through Celite was colorless. After filtering the remaining black mixture through Celite, the resultant clear, colorless solution was concentrated under vacuum at 50 °C to constant mass, which provided 1.83 g (98%) of **24** as a white solid that was pure according to ^1H NMR analysis: mp 270−280 °C (dec); IR (KBr) 3425−2367 br, 1719, 1619 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 1.27 (d, J = 7.3, 3H), 3.33 (dq, J = 4.0, 11.1, 1H), 3.71 (s, 3H), 4.25 (d, J = 3.74, 1H); MS *m/e* calcd for $C_6H_{12}NO_4$ $(M - Cl^-)^+$: 162.0763. Found 162.0768. Anal.

Calcd for $C_8H_{13}NO_4$: C, 36.46; H, 6.13; N, 7.09. Found: C, 36.16; H, 6.33; N, 6.97.

Boc-D-*erythro*-*β*-methylaspartate *β*-Methyl Ester (25). To 5.29 g (26.8 mmol) of **24** in 53 mL of 1 M pH 12.5 phosphate buffer (resultant pH = 8.0) was added 53 mL of dioxane, and the resultant mixture was cooled in an ice bath, and 1.5 g (26.7 mmol) of KOH was added. When the base had dissolved, 7.01 g (32.2 mmol) of di-*tert*-butyldicarbonate was added in one portion, and 10 min later the mixture was allowed to warm to room temperature where stirring was continued for 1.5 h (resultant pH = 7.0). At this point, an additional 1.5 g (26.7 mmol) of KOH was added and stirring was continued for 8 h (final pH = 9.0). The mixture was washed with one portion of ether, and to the remaining aqueous layer was added a solution of 6.6 mL of concentrated HCl in 50 mL of 1 M pH 3.1 citrate buffer (final pH = 3.5). The mixture was then extracted twice with ether, and the combined organic extracts were washed with brine, dried over $MgSO_4$, filtered, and concentrated to give a colorless oil. Crystallization from EtOAc/hexanes at −20 °C gave 6.43 g of a white solid that was contaminated by a trace of ninhydrin-positive baseline material according to TLC analysis (4:1:95 MeOH:HOAc:$CHCl_3$). Recrystallization (EtOAc/hexanes, −20 °C) gave 5.20 g of pure white crystals. The mother liquors were combined and chromatographed to supply an additional 1.79 g for a total of 6.99 g, 99% of **25**: mp 72−73 °C; IR (thin film) 3300−2600 br, 1724, cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, major rotamer of a 10:1 mixture) δ 1.27 (d, J = 7.4 Hz, 3H), 1.44 (s, 9H), 3.27 (dq, J = 3.7, 7.2, 1H), 3.70 (s, 3H), 4.53 (dd, J = 3.7, 9.4, 1H), 5.51 (d, J = 9.4, 1H); MS *m/e* calcd for $(M+H)^+$: 262.1290. Found 262.1298. Anal. Calcd for $C_{11}H_{19}NO_6$: C, 50.56; H, 7.34; N, 5.36. Found: C, 50.77; H, 7.42; N, 5.06.

(2*S*,3*R*)-*N*-*tert*-Butoxycarbonyl-2-methyl-3-amino-4-hydroxy Methyl Butanoate (27). To 4.78 g (18.3 mmol) of **25** in 19 mL of dry Glyme was added 2.21 g (19.2 mmol) of *N*-hydroxysuccinimide. After cooling in an ice bath, 3.96 g (19.2 mmol) of DCC was added. The mixture was stirred for 1 h at 0 °C and for 2.5 h at room temperature, then diluted with 20 mL of dry ether, filtered through Celite, and concentrated to give the active ester **26** as white solid. The active ester was crystallized from ether/hexanes for characterization, but, since crystallization or chromatography resulted in lower overall yields, it was generally used crude in the next step. Spectral data for **26**: IR (thin film) 3353, 2980, 1740, 1710 $cm^{-1;}$ ^1H NMR (500 MHz, $CDCl_3$) δ 1.36 (d, J = 7.3 Hz, 1H), 1.46 (s, 9H), 2.83 (br s, 4H), 3.35 (dq, J = 3.9, 7.3 Hz, 1H), 3.75 (s, 3H), 4.89 (dd, J = 3.9, 10.0 Hz, 1H), 5.60 (d, 10.0H); MS *m/e* calcd for $(M+H)^+$: 359.1448, found 359.1455. Reduction: the crude active ester **26** was dissolved in 17 mL of dry THF and cooled in an ice bath, and then 1.73 g (45.7 mmol) of $NaBH_4$ was added. After 2 h, the mixture was warmed to room temperature and stirred vigorously for an additional 15 h. The mixture was then added dropwise to 400 mL of an ice cold, vigorously stirred solution of 1 M pH 3.1 citric acid buffer. The resultant mixture was extracted twice with ether, and the combined extracts were washed once with water, once with brine, dried over $MgSO_4$, filtered, and concentrated to give an oil. Chromatography (1:1 EtOAc:hexanes) gave 3.30 g (73%) of the alcohol **27** as a white solid: R_f 0.27 (1:1 EtOAc:hexanes); IR (thin film) 3420 br, 2978, 1718 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.23 (d, J = 7 Hz, 3H), 1.43 (s, 9H), 2.87 (dq, J = 5.5, 6.5 Hz, 1H), 3.61 (dd, J = 5.5, 11 Hz, 1H), 3.65 (dd, J = 5.5, 11 Hz, 1H), 3.68 (s, 3H), 3.76 (overlap app dq, J = 5.5, 8.5 Hz, 1H), 5.42 (d, J = 8.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.9, 28.3, 39.7, 51.9, 54.8, 63.9, 79.7, 156.4, 175.9; MS *m/e* calcd for $(M+H)^+$: 248.1498. Found 248.1500.

(2*R*,3*S*)-*N*-*tert*-Butoxycarbonyl-2-amino-3-carbomethoxybutanal (28). To 193 mg (1.52 mmol) of freshly distilled oxalyl chloride in 2.5 mL of CH_2Cl_2 at −50° C was added 173 mg (2.03 mmol) of dry DMSO. After 15 min, 250 mg (1.02 mmol) of **27** in 7 mL of CH_2Cl_2 was added via cannula over 10 min. The resultant cloudy mixture was stirred for 1 h, at which point 416 mg (4.07 mmol) of dry triethylamine in 1.5 mL of CH_2Cl_2 was added dropwise. The reaction was quenched after 40 min by the addition of 0.5 mL of water and was then diluted with 20 mL of hexanes. The mixture was washed twice with 5 mL of ice cold 1 M $NaHSO_4$, and the combined aqueous phases were back extracted once with ether. The combined organic material was then washed once with 10 mL of saturated $NaHCO_3$, twice with 10 mL of water, and once with 10 mL of brine, dried over $MgSO_4$, filtered, and concentrated under vacuum to constant mass to give 248 mg (100%) of **28** as a colorless oil: R_f 0.65 (40:60 EtOAc:hexanes); IR (thin film) 3377, 2967, 2833, 2722, 1722, cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.28 (d, J = 7.5 Hz, 3H), 1.47 (s, 9H), 3.31 (dq, J = 3.5, 7.5 Hz, 1H), 3.68 (s, 3H), 4.34 (dd, J = 3.5, 9.5 Hz), 5.60 (d, J = 9.0 Hz), 9.65 (s, 1H); MS *m/e* calcd for $(M+H)^+$: 246.1342, found 246.1338. The material was used immediately in the next step without further purification.

***trans*-(3*R*,4*S*)-*N*-*tert*-Butoxycarbonyl-1-iodo-3-amino-4-carbomethoxy-1-pentene (22).** The crude aldehyde **28** (248 mg 1.02 mmol) in 7.5 mL of dioxane was cannulated into a flask equipped with a stirbar and charged with a slurry of 750 mg (6.09 mmol) of flame-dried chromium(II) chloride in 1.3 mL of THF. To the resultant green slurry was added 1.04 g (3.04 mmol) of iodoform, and the resultant dark brown mixture was stirred vigorously in the dark for 19 h. The mixture was then partitioned between ether and 50% saturated brine, and the phases were separated. The aqueous phase was then saturated with NaCl and extracted twice with ether. The combined organic material was then washed twice with brine, dried over $MgSO_4$, filtered, and concentrated to give a yellow oil. The oil was chromatographed (60 g silica/g sample, 1:9 EtOAc:hexanes) to give 197 mg (53%) of **22** as a colorless oil that solidified over time: R_f 0.63 (30:70 EtOAc:hexanes); IR (thin film) 3422, 3044, 2978, 2300, 1711 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.21 (d, 7.5 Hz, 3H), 1.43 (s, 9H), 2.70 (m, 1H), 3.68 (s, 3H), 4.27 (m, 1H), 6.32 (dd, J = 1, 14 Hz, 1H), 6.45 (dd, J = 6.5, 14 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.4, 28.3, 42.8, 51.9, 56.9, 78.4, 79.8, 144.0, 155.3, 174.8; MS *m/e* calcd for $(M+H)^+$: 370.0516. Found 370.0516. Anal. Calcd for $C_{12}H_{20}INO_4$: C, 39.03; H, 5.47; N, 3.79. Found: C, 39.32; H, 5.47; N, 3.74.

(2*R*,3*S*)-*N*-Methyl-*N*-methoxy-2-methyl-3-hydroxy-4-phenylbutanamide (Weinreb Amide 31). To a slurry of 7.90 g (81.0 mmol) of *N*,*O*-dimethyl hydroxylamine hydrochloride and 35 mL of THF in a flame dried flask at 0 °C was added dropwise over 20 min 40.5 mL (81.0 mmol) of 2 M trimethylaluminum in hexanes (caution: vigorous effervescence), and the resultant solution was stirred for 30 min. A solution of 10.0 g (29.2 mmol) of **30** in 20 mL of dry THF was then added over 15 min, and, after stirring for 1 h, the solution was cannulated into 350 mL of rapidly stirred, ice-cold 1 M HCl (caution: vigorous effervescence). The mixture was then extracted twice with 400 mL of EtOAc, and the combined organic phases were washed once each with water and brine, dried over $MgSO_4$, filtered, and concentrated under vacuum to give a clear oil. Chromatography (100 g silica/g sample, 4:6 EtOAc:hexanes) gave 6.06 g (87%) of **31** as a clear oil: R_f 0.55 (50:50 EtOAc:hexanes); IR (thin film) 3424, 3036, 2931, 1637 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.23 (d, J = 7.0 Hz, 3H), 2.71 (dd, J = 7.0, 13.5 Hz, 1H), 2.85 (par obsc, 1H), 2.89 (dd, J = 7.5, 13.5 Hz, 1H), 3.46 (br s, 3 H), 3.65 (br s, 1H), 4.11 (dt, J = 3, 7.0 Hz, 1H), 7.21−7.30 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 10.03, 31.75, 37.21, 40.01, 61.20, 72.86, 126.3, 128.4, 129.1, 138.3, 177.9; MS *m/e* calcd for $(M+H)^+$: 238.1443. Found 238.1447. Anal. Calcd for $C_{13}H_{19}NO_3$: C, 65.78; H, 8.09; N, 5.90. Found: C, 65.78; H, 8.04; N, 5.81.

(2*R*,3*S*)-*N*-Methyl-*N*-methoxy-2-methyl-3-methoxy-4-phenylbutanamide (Weinreb Amide 32). To 3.81 g (16.1 mmol) of **31** in 56 mL of dry THF and 24 mL of dry DMF was added 10.0 mL (160 mmol) of iodomethane. The resultant solution was cooled to 0 °C, and 1.93 g (48.1 mmol) of 60% sodium hydride in oil was added. After stirring for 1.5 h, the solution was poored into 300 mL of ice cold 1 M pH 7 phosphate buffer, and the mixture was extracted twice with 250 mL of EtOAc. The combined organic phases were washed once each with water and brine, dried over $MgSO_4$, filtered, and concentrated under vacuum to give an oil. Chromatography (25 g silica/g sample, 25:75 EtOAc:hexanes) gave 3.84 g (95%) of **32** as a clear oil: R_f 0.45 (40:60 EtOAc:hexanes); IR (thin film) 3060, 2931, 2813, 1660 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.21 (d, J = 7.0 Hz, 3H), 2.74 (dd, J = 7.0, 14 Hz, 1H), 2.86 (dd, J = 3.5, 14 Hz, 1H), 2.90 (obsc dq, 1H), 3.15 (s, 3H), 3.24 (s, 3H), 3.44 (br s, 3H), 3.61 (app dt, J = 4, 7 Hz, 1H), 7.17−7.23 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.43, 32.01, 38.48, 39.62, 58.84, 60.95, 83.75, 125.97, 128.04, 129.51, 138.77, 175.95; MS *m/e* calcd for $(M+H)^+$: 252.1600. Found 252.1597. Anal. Calcd for $C_{14}H_{21}NO_3$: C, 66.89; H, 8.44; N, 5.57. Found: C, 66.79; H, 8.35; N, 5.53.

(2*R*,3*S*)-2-Methyl-3-methoxy-4-phenylbutanal (33). To 1.94 g (7.72 mmol) of **32** in 53 mL of dry CH_2Cl_2 at −100 °C was added over 10 min 10.3 mL (15.4 mmol) of 1.5 M diisobutylaluminum hydride in toluene. After 1.5 h, 2 mL of dry methanol was added, and the solution was warmed to 0 °C. To the resultant solution was added 50 mL of ice-cold 1 M tartaric acid, and the mixture was stirred vigorously for 20 min at 0 °C. The phases were separated, and the aqueous phase was extracted twice with CH_2Cl_2. The combined organic phases were dried over $MgSO_4$, filtered through Celite, and concentrated under vacuum to a clear oil. Chromatography (100 g silica/g sample, 6:94 EtOAc:hexanes) gave 1.17 g (79%) of **33** as a clear oil: R_f 0.36 (10:90 EtOAc:hexanes); IR (thin film) 3055, 2933, 2822, 2711, 1722 br, 1600 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.18 (d, J = 7 Hz, 3H), 2.39 (dq, J = 3.5, 7 Hz, 1H), 2.72 (dd, J = 7, 13.5 Hz, 1H), 3.00 (dd, J = 6.5, 13.5 Hz, 1H), 3.26 (br s, 3H), 3.90 (ddd, J = 3.5, 6.5, 6.5 Hz, 1H), 7.21−7.32 (m, 5H), 9.69 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 7.5, 37.4, 48.9, 58.0, 81.6, 126.5, 128.6, 129.2, 138.0, 204.3; MS *m/e* calcd for $(M+NH_4)^+$: 210.1495. Found 210.1503.

Dibromoalkene 34a. To 7.59 g (22.9 mmol) of carbon tetrabromide in 35 mL of dry CH_2Cl_2 at −20° C was added over 5 min 11.1 g (42.3 mmol) of triphenylphosphine in 12 mL of dry CH_2Cl_2. After 15 min the resultant orange solution was cooled to −78 °C, and 2.20 g (11.4 mmol) of **33** in 11 mL of dry CH_2Cl_2 was added by cannula over 10 min. The solution faded to a light yellow after 10 min, at which point it was concentrated under vacuum to a brown crust. Chromatography (80 g silica/g sample, 1:99 EtOAc:hexanes) gave 3.80 g (95%) of **34a** as a colorless oil: R_f 0.39 (5:95 EtOAc:hexanes); IR (thin film) 3055, 3022, 2922, 2822, 1605 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.07 (d, J = 7 Hz, 3H), 2.59 (ddq, J = 5, 7, 9.5 Hz, 1H), 2.73 (dd, J = 5.5, 14 Hz, 1H), 2.81 (dd, J = 7, 14 Hz, 1H), 3.25 (s, 3H), 3.30 (ddd, J = 5.5, 5.5, 7.5 Hz, 1H), 6.36 (d, J = 9.5 Hz, 1H), 7.20−7.31 (m 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.7, 37.9, 41.6, 58.6, 85.0, 88.7, 126.2, 128.4, 129.3, 138.7, 141.2; MS *m/e* calcd for $(M+NH_4)^+$: 363.9912. Found 363.9902.

(4*R*,5*S*)-4-Methyl-5-methoxy-6-phenyl-2-hexyne (34b). To 2.55 g (7.33 mmol) of **34a** in 100 mL of dry THF at −78 °C was added dropwise over 10 min 13.7 mL (25.6 mmol) of 1.87 M nBuLi in hexanes, and the solution was stirred for 1 h. To the resultant purple solution was added 4.6 mL (73.4 mmol) of iodomethane, and the solution was then warmed to ambient temperature and stirred for 2 h. The solution was then partitioned between ether and 5% $NaSO_3$, and the phases were separated. The organic phase was washed once each with water and brine, dried over $MgSO_4$, filtered, and concentrated under vacuum to an oil. Purification via MPLC (1:99 EtOAc:hexanes) gave 1.31 g (89%) of **34b** as a colorless oil: R_f 0.24 (1:99 EtOAc:hexanes); IR (thin film) 3066, 2922, 1944, 1600, cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.19 (d, J = 7 Hz, 3H), 1.83 (d, J = 2.5 Hz, 3H), 2.55 (ddq, J = 2.5, 6.5, 7 Hz, 1H), 2.85 (dd, J = 7.5, 14 Hz, 1H), 2.98 (dd, J = 4, 14 Hz, 1H), 3.24 (ddd, J = 4, 6.5, 7 Hz, 1H), 3.27 (s, 3H), 7.20−7.30 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 3.7, 17.4, 30.1, 37.9, 58.5, 77.5, 81.1, 85.9, 126.0, 128.1, 129.6, 139.2; MS *m/e* calcd for $(M+NH_4)^+$: 220.1702. Found 220.1700. Anal. Calcd for $C_{14}H_{18}O$: C, 83.11; H, 8.99. Found: C, 83.42; H, 9.07.

Boronic Acid 20. To 400 mg (1.98 mmol) of **34b** in 1 mL of dry CH_2Cl_2 was added 545 mg (1.98 mmol) of Ag_2CO_3, and the mixture was cooled to 0 C. To the mixture was then added 6.0 mL (6.0 mmol) of 1 M $Br_2BH{\cdot}SMe_2$ in CH_2Cl_2, and the resultant mixture was stirred vigorously in the dark for 11 h at 0 °C. The mixture was then poured into 40 mL of rapidly stirring ice cold 0.5 M pH 3.1 citrate buffer and extracted twice with EtOAc. The combined organic phases were washed once with water and twice with brine, dried over $MgSO_4$, filtered, and concentrated under vacuum to an oil. Rapid chromatography (100 g silica/g oil, 25:75 EtOAc:hexanes) gave 220 mg (45%) of **20** as a colorless oil, which required immediate use to avoid significant decomposition: R_f 0.20 (30:70 EtOAc:hexanes); IR (thin film) 3031, 2940, 2858, 1628, cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.07 (d, J = 6.5 Hz, 3H), 1.70 (d, J = 1.5 Hz, 3H), 2.73 (dd, J = 8, 14 Hz, 1H), 2.78 (par obsc mult., 1H), 2.85 (dd, J = 4.5, 14 Hz, 1H), 3.27 (obsc. mult., 1H), 3.27 (s, 3H), 6.64 (dd, J = 1.5, 9.5 Hz, 1H), 7.19−7.29 (m, 5H). Also isolated during the chromatography was 54 mg (11%) of the undesired isomer **21** as a clear oil: R_f 0.32 (30:70 EtOAc:hexanes); ^1H NMR (500 MHz, $CDCl_3$) δ 1.08 (d, J = 7 Hz, 3H), 1.22 (d, J = 7 Hz, 3H), 2.66 (dd, J = 10, 13.5 Hz, 1H), 2.68 (par. obsc. mult., 1H), 3.12 (dd, J = 4.5, 13.5 Hz, 1H), 3.37 (ddd, J = 2, 4.5, 10 Hz, 1H), 3.42 (s, 3H), 6.21 (q, J = 7 Hz, 1H), 7.20−7.33 (m, 5H). Chromatography fractions containing unreacted starting material were purified via HPLC (0.5:99.5 EtOAc:hexanes) to give 69 mg (18%) of recovered **34b**.

Boc-Adda-OMe (35). To 184 mg (0.74 mmol) of **20** was added 222 mg (0.60 mmol) of **22**, 5 mL of THF, and 43 mg (2.4 mmol) of water. The colorless solution was cooled to 0 °C, and 49 mg (0.07 mmol) of $Pd[PPh_3]_4$ in 1.5 mL of dry THF was added via cannula. After 5 min, 377 mg (1.51 mmol) of TlOEt was added to the orange solution over a 10 min period. After 2 h, the light brown suspension was partitioned between EtOAc and water, and the aqueous phase was extracted twice with EtOAc. The combined organic phases were washed twice with water and once with brine, dried over $MgSO_4$, filtered, and concentrated under vacuum to give a dark residue. The residue was filtered through silica (10:90 EtOAc:hexanes) to give an orange oil, which was then purified by HPLC (4:96 EtOAc:hexanes) to give 219 mg (82%) of **35** as a yellow oil: R_f 0.39 (20:80 EtOAc:hexanes); IR (thin film) 3424, 3025, 2931, 1713 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.02 (d, J = 6.5 Hz, 3H), 1.22 (d, J = 7 Hz, 3H), 1.45 (s, 9H), 1.59 (d, J = 1 Hz, 3H), 2.58 (ddq, J = 6.5, 7, 9.5 Hz, 1H), 2.67 (dd, J = 7.5, 14 Hz, 1H), 2.72−2.77 (m, 1H), 2.80 (dd, J = 4.5, 13.5 Hz, 1H), 3.18 (ddd, J = 4.5, 6.5, 7.5 Hz, 1H), 3.23 (s, 3H), 3.66 (s, 3H), 4.36 (br, 1H), 5.27−5.30 (m, 1H), 5.38 (d, J = 9.5 Hz, 1H), 5.43 (dd, J = 6.5, 15.5 Hz, 1H), 6.18 (d, J = 15.5 Hz, 1H), 7.17−7.28 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 12.6, 14.3, 16.1, 28.3, 36.6, 38.2, 44.0, 51.6, 54.4, 58.6, 79.2, 86.9, 125.1, 125.9, 128.1, 129.4, 132.4, 135.9, 136.1, 139.3, 155.5, 175.3; MS *m/e* calcd for $(M+H)^+$: 446.2906. Found 446.2899.

Boc-Adda (4). To 80 mg (0.18 mmol) of **35** was added 4.7 mL of THF and 0.91 mL (0.90 mmol) of 1 M LiOH. The resultant mixture was stirred vigorously for 50 h at ambient temperature and then was partitioned between ether and water. The aqueous phase was washed with ether, and the combined ether phases were back-extracted once with water. The combined aqueous phases were then acidified with 1 M $NaHSO_4$ and extracted twice with EtOAc. The combined EtOAc phases were washed twice with water and once with brine, dried over $MgSO_4$, filtered, and concentrated to an oil. The sample was filtered through silica (20:79:1 EtOAc/hexanes/HOAc) to give 68 mg (88%) of **4** as an oil: R_f 0.44 (25:74:01 EtOAc:hexanes:HOAc); IR (thin film) 3055, 2966, 2866, 1727 br cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 0.94 (d, J = 7 Hz, 3H), 0.96 (d, J = 7 Hz, 3H), 1.35 (s, 9H), 1.51 (br s, 3H), 2.52 (par obsc m, 2H), 2.63 (dd, J = 7, 14 Hz, 1H), 2.72 (dd, J = 5, 14 Hz, 1H), 3.16 (s, 3H), 3.23 (ddd, J = 5, 5.5, 7 Hz, 1H), 4.22 (app q, J = 8 Hz, 1H), 5.37 (par obsc dd, J = 7.5, 15.5 Hz, 1H), 5.39 (d, J = 10.5 Hz, 1H), 6.08 (d, J = 15.5 Hz, 1H), 6.91 (d, J = 8.5 Hz, 1H), 7.15−7.27 (m, 5H); MS *m/e* calcd for $(M+H)^+$: 432.2751. Found 432.2761.

Boc-D-*iso*-Glu(OMe)-MeΔAla-(D)-Ala-(L)-Leu-(D)-*erythro*-β-Me-*iso*-Asp(OMe)-(L)-Ala-OTce (38). Dipeptide **19** (314 mg, 0.68 mmol) was mixed with 2 mL of TFA for 30 min and concentrated, and the residue was partitioned between EtOAc and saturated $NaHCO_3$. The aqueous layer was extracted with two additional portions of EtOAc, and the combined organic material was washed with brine, dried with K_2CO_3, filtered, and concentrated. To the residue was added 431 mg (0.82 mmol) of the tetrapeptide **2** and 4 mL of toluene. The mixture was then concentrated, and the residue was dissolved in 2 mL of DMF. To the solution was added 247 mg (2.04 mmol) of collidine and, after cooling to 0 °C, 310 mg (0.82 mmol) of HATU. The solution was stirred for 1 h at 0 °C, and for 19 h at room temperature. The mixture was worked up as for compound **13**, substituting 1:1 Ether:EtOAc for the extractions, to give 729 mg of crude product. Chromatography (5:95 isopropyl alcohol:CH_2Cl_2 to elute the nonpolar impurities, then 10:90 isopropyl alcohol:CH_2Cl_2 to elute the product) gave 476 mg (80%) of **38**: R_f 0.49 (1:9 isopropyl alcohol:EtOAc) IR (thin film) 3301, 2957, 1747, 1651 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6, 90 °C) δ 0.83 (d, J = 6.4, 3H), 0.87 (d, J = 6.8, 3H), 1.06 (d, J = 7.2, 3H), 1.30 (d, J = 7.2, 3H), 1.36 (obsc d, 3H), 1.37 (s, 9H), 4.69 (par obsc t, J = 7.2, 2H), 1.60 (m, 1H), 1.82 (m, 1H), 1.96 (m, 1H), 2.32 (m, 2H), 2.96 (par obsc m, 1H), 3.00 (br s, 3H), 3.60 (s, 3H), 3.61 (s, 3H), 3.97 (br app q, J = 5.6, 1H), 4.28 (app q, J = 7.2, 1H), 4.37 (par obsc m, 1H), 4.38 (par obsc m, 1H), 4.52 (dd, J = 5.6, 8.4, 1H),4.80 (d, J = 12.3, 1H), 4.92 (d, J = 12.3, 1H), 5.52 (br s, 1H), 5.96 (br s, 1H), 6.80 (br

s, 1H), 7.74 (br d, $J = 8.0$, 1H), 7.88 (br m, 2H), 8.29 (br d, $J = 6.8$, 1H); FAB MS calcd for $C_{35}H_{56}Cl_3N_6O_{13}$ $(M+H)^+$: 873.2970. Found: 873.2969.

Boc-Adda-(D)-*iso*-Glu(OMe)-MeΔAla-(D)-Ala-(L)-Leu-(D)-*erythro*-*β*-Me-*iso*-Asp(OMe)-(L)-Ala-OTce (3). Compound **38** (94 mg 0.11 mmol) was mixed with 1 mL of TFA for 30 min. The solution was then concentrated, and the resultant oil was partitioned between EtOAc and saturated aqueous $NaHCO_3$. The aqueous portion was extracted with two additional portions of EtOAc, and the combined organic extracts were then washed with brine, dried with K_2CO_3, filtered, and concentrated. To the resultant residue was added 40 mg (0.090 mmol) of Boc-Adda-OH as a solution in CH_2Cl_2. The solution was again concentrated and then reconcentrated from 2 mL of toluene. To the resultant residue was added 0.5 mL of DMF and 33 mg (0.27 mmol) of collidine. After cooling the solution in an ice bath, 41 mg (0.11 mmol) of HATU was added. After stirring the solution for 1 h at 0 °C and 14 h at room temperature, 1 mL of saturated $NaHCO_3$ was added. The mixture was stirred for 10 min and then was partitioned between ether and water. The aqueous layer was extracted with two additional portions of ether, and the combined organic layers were washed with brine, dried with $MgSO_4$, and concentrated. Chromatography (EtOAc to elute nonpolar material, then 1:10 isopropyl alcohol:EtOAc to elute the product) gave 74 mg (69%) of **3**: R_f 0.39 (10:5 EtOAc:isopropyl alcohol); IR (thin film) 3299, 2957, 1747 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6, 70 °C) δ 0.82 (d, $J = 6.4$, 3H), 0.86 (d, $J = 6.8$, 3H), 0.96 (d, $J = 6.8$, 3H),1.00 (d, $J = 7.2$, 3H), 1.06 (d, $J = 7.2$, 3H), 1.29 (d, $J = 7.2$, 3H), 1.36 (par obsc d, 3H), 1.37 (s, 9H), 1.50 (app t, $J = 6.8$, 2H), 1.55 (d, $J = 0.8$, 3H), 1.59 (m, 1H), 1.82 (m, 1H), 1.96 (m, 1H), 2.21−2.35 (m, 2H), 2.55 (m, 2H), 2.65 (dd, $J = 7.6$, 14.0, 1H), 2.74 (dd, $J = 4.8$, 14.0, 1H), 2.74 (dd, $J = 4.8$, 14.0, 1H), 2.97 (par obsc m, 1H), 2.98 (br s, 3H), 3.17 (s, 3H), 3.24 (ovrlp dt, $J = 5.2$, 7.2, 1H), 3.588 (s, 3H), 3.593 (s, 3H), 4.05 (br app q, $J = 6.0$, 1H), 4.22 (br app q, $J = 6.8$, 1H), 4.27 (app q, $J = 7.6$, 1H), 4.33−4.38 (m, 2H), 4.51 (dd, $J = 5.6$, 8.8, 1H), 4.79 (d, $J = 11.9$, 1H), 4.93 (d, $J = 11.9$, 1H), 5.38 (d, $J = 9.5$, 1H), 5.44 (dd, $J = 3.2$, 15.9, 1H), 5.48 (br s, 1H), 5.98 (br s, 1H), 6.07 (d, $J = 15.9$, 1H), 6.32 (br s, 1H), 7.15−7.19 (m, 3H), 7.24−7.27 (m, 2H), 7.79 (d, $J = 9.2$, 1H), 7.92 (br ovrlp d, 2H), 8.01 (br d, $J = 7.2$, 1H), 8.35 (d, $J = 7.2$, 1H). FAB MS calcd for $C_{55}H_{83}N_7O_{15}Cl_3^+$ $(M+H)^+$: 1186. Found: 1186. FAB MS calcd for the molecular envelope of $C_{55}H_{82}N_7O_{15}Cl_3Na^+$ $(M + Na)^+$: 1208.5 (83.3%), 1209.5 (54.6%), 1210.5 (100.0%), 1211.5 (57.8%), 1212.5 (46.0%), 1213.5 (22.1%), 1214.5 (10.0%). Found: 1208.5 (83.2%), 1209.5 (57.0%), 1210.5 (100.0%), 1211.5 (57.8%), 1212.5 (44.0%), 1213.5 (20.0%), 1214.5 (6.4%).

Boc-Adda-D-*iso*-Glu(OMe)-MeΔAla-(D)-Ala-(L)-Leu-(D)-*erythro*-*β*-Me-*iso*-Asp(OMe)-(L)-Ala-OH (40). To 70 mg (0.0589 mmol) of **3** in 2 mL of HOAc was added 0.5 g of zinc powder. The mixture was stirred vigorously for 4 h and then filtered through Celite. The filter cake was rinsed with copious EtOAc, and the filtrate was concentrated. Chromatography of the residue (10:1:0.1 CH_2Cl_2:isopropyl alcohol:HOAc to elute the nonpolar material, then 10:2:0.1 to elute the product) gave 52 mg (84%) of **40**: R_f 0.31 (10:2:0.1 CH_2Cl_2:isopropyl alcohol:HOAc); IR (thin film) 3301, 2958, 1743, 1650 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6, 90°C) δ 0.82 (d, $J = 6.4$, 3H), 0.86 (d, $J = 6.4$, 3H), 0.97 (d, $J = 6.8$, 3H), 1.01 (d, $J = 7.2$, 3H), 1.04 (d, $J = 7.2$, 3H), 1.25 (d $J = 7.2$, 3H), 1.29 (d, $J = 7.2$, 3H), 1.37 (s, 9H), 1.51 (app t $J = 7.6$, 2H), 1.55 (s, 3H), 1.60 (par obsc app dq, $J = 13.5$, 6.8, 1H), 1.84 (app dq, $J = 14.3$, 6.4, 1H), 1.96 (app dq, $J = 13.1$, 6.4, 1H), 2.29 (m, 2H), 2.57 (m, 2H), 2.66 (dd, $J = 13.9$, 7.2, 1H), 2.74 (dd, $J = 13.9$, 4.8, 1H), 2.95 (par obsc m, 1H), 2.98 (br s, 3H), 3.18 (s, 3H), 3.24 (ovrlp t, $J = 5.2$, 7.2, 1H), 3.59 (s, 3H), 3.60 (s, 3H), 4.06 (br app q, $J = 7.6$, 1H) 4.14−4.28 (three ovrlp m, 3H), 4.37 (app dq, $J = 7.6$, 1H), 4.48 (dd, $J = 5.6$, 8.8, 1H), 5.38 (d, $J = 9.6$, 1H), 5.45 (par obsc dd, $J = 6.8$, 15.9, 1H), 5.46 (par obsc br s, 1H), 5.98 (br s, 1H), 6.07 (d, $J = 15.5$, 1H), 6.25 (br s, 1H), 7.18 (m, 3H), 7.25 (app t, $J = 7.6$, 2H), 7.77 (br d, $J = 8.8$, 1H), 7.82−7.86 (br m, 2H), 7.92 (br d, $J = 6.8$, 1H), 7.96 (br d, $J = 6.4$, 1H); FAB MS calcd for $C_{53}H_{81}N_7O_{15}$ $(M+H)^+$: 1056.6. Found: 1056.6. FAB MS calcd for the molecular envelope of $C_{53}H_{80}NaN_7O_{15}$ $(M + Na)^+$: 1078.6 (100.0%), 1079.6 (63.4%), 1080.6 (22.7%), 1081.6 (5.9%). Found: 1078.6 (100.0%), 1079.6 (58.8%), 1080.6 (20.8%), 1081.6 (5.0%).

Microcystin-LA Dimethyl Ester (42). To 52 mg (0.049 mmol) of **40** was added 24 mg (0.13 mmol) of pentafluorophenol in 1 mL of EtOAc. The solution was cooled in an ice bath, and 13 mg (0.064 mmol) of DCC was added. The mixture was stirred for 2 h at 0 °C and for 22 h at room temperature, then filtered through Celite, concentrated, and passed through a plug of dry silica gel (10:1 EtOAc: isopropyl alcohol) to give 50 mg (83%) of **41**: R_f 0.38 (9:1 EtOAc: acetone); FAB MS calcd for $C_{53}H_{81}N_7O_{15}^+$ (loss of C_6F_5): 1056. Found: 1056. The active ester **41** was used in the next step without further purification: to 33 mg (0.027 mmol) of **41** was added 1 mL of freshly distilled TFA. After 50 min, the mixture was concentrated and then reconcentrated twice from $CHCl_3$. The residue was then dissolved in 27 mL of $CHCl_3$ and added dropwise over 40 min to a vigorously stirred mixture of 27 mL of $CHCl_3$ and 27 mL of 0.5 M, pH 9.5 phosphate buffer. After stirring the mixture for an additional hour, the layers were separated. The chloroform layer was passed through a plug of cotton, and the aqueous portion was extracted twice with EtOAc. The combined EtOAc layers were washed with brine and added to the $CHCl_3$ portion. The solution was then dried with $MgSO_4$ and concentrated. Reversed phase HPLC (52:48 CH_3CN:10 mM HOAc) provided 14 mg (56%) of **42**. R_f 0.35 (2:8 isopropyl alcohol:CH_2Cl_2); IR (thin film) 3296, 2927, 1741, 1649 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.844 (d, $J = 6.4$, 3H), 0.900 (d, $J = 6.4$, 3H), 1.02 (d, $J = 6.8$, 3H), 1.12, 1.13 (two ovrlp d, $J = 6.8$, 7.2, 6H), 1.29 (d, $J = 7.2$, 3H), 1.37 (d, $J = 7.2$, 3H), 1.56 (obsc m, 2H), 1.60 (s, 3H), 1.66 (m, 1H), 1.75 (m, 2H), 2.21 (m, 1H), 2.45 (m, 1H), 2.53−2.64 (m, 2H), 2.68 (dd, $J = 7.6$, 13.9, 1H), 2.80 (obsc dd, 1H), 2.81 (obsc m, 1H), 3.20 (m, 1H), 3.24 (s, 3H), 3.31 (s, 3H), 3.69 (s, 3H), 3.94 (s, 3H), 4.18 (dq, $J = 6.0$, 6.0, 1H), 4.38 (dq, $J = 8.0$, 8.0, 1H), 4.56 (dd, $J = 2.0$, 8.4, 1H), 4.65 (app q, $J = 9.1$, 1H), 4.72 (m, 1H), 4.86 (dq, $J = 4.0$, 1H), 5.28 (dd, $J = 8.8$, 15.5, 1H), 5.30 (s, 1H), 5.41 (d, $J = 9.9$, 1H), 5.99 (s, 1H), 6.25 (d, $J = 15.5$, 1H), 6.73 (d, $J = 9.1$, 1H), 6.81 (d, $J = 8.4$, 1H), 6.89 (d, $J = 9.6$, 1H), 7.19 (app t, $J = 7.6$, 3H), 7.25−7.28 (obsc m, 2H), 7.90 (d, $J = 6.0$, 1H), 8.28 (br d, $J = 8.4$, 1H) FAB MS calcd for $C_{48}H_{72}N_7O_{12}^+$ $(M+H)^+$: 938.5238. Found: 938.5222.

Microcystin-LA (1). To 6.0 mg (0.0064 mmol) of **42** in 1 mL of THF at 0 °C was added 1 mL of cold 0.1 M LiOH. After stirring vigorously for 5 h at 0 °C, the mixture was acidified with 1 M $NaHSO_4$ (ca. 4 drops) and extracted three times with EtOAc. The combined organic portions were washed with brine, dried with $MgSO_4$, filtered, and concentrated. Preparative reversed phase HPLC (72:28 MeOH: 0.2% aqueous TFA) gave 3 mg (50%) of an unidentified isomer (FAB MS calcd for $C_{46}H_{68}N_7O_{12}^+$ $(M+H)^+$ 910.4925. Found 910.4920) and 3 mg (50%) of microcystin-LA **(1)**: IR (thin film) 3307, 2926, 1640, 1504, 1257 cm^{-1}; ^1H NMR (500 MHz, D_2O; some resonances are obscured by the solvent peak) δ 0.860 (d, $J = 6.7$, 3H), 0.900 (d, $J = 6.7$, 3H), 1.02 (two overlp d, $J = 7.3$, 6H), 1.05 (d, $J = 7.3$, 3H), 1.28 ($J = 7.3$, 3H), 1.39 (d, $J = 7.3$, 3H), 1.67 (m, 2H), 1.70 (s, 3H), 1.90−2.05 (m, 3H), 2.60 (m, 1H), 2.69−2.80 (three ovrlp m, 3H), 2.92 (dd, $J = 5$, 14, 1H), 3.01 (dd, $J = 7.9$, 7.9, 1H), 3.16 (m, 1H), 3.27 (s, 3H), 3.39 (s, 3H), 3.47 (m, 1H), 3.96 (app t, $J = 7.9$, 1H), 4.30 (m, 1H), 4.39 (m, 1H), 5.54 (s, 1H), 5.51−5.64 (par obsc m, 2H), 5.90 (s, 1H), 6.31 (d, $J = 17.1$, 1H), 7.28 (app d, $J = 6.7$, 3H), 7.36 (app t, $J = 6.7$, 2H); FAB MS calcd for $C_{46}H_{68}N_7O_{12}$ $(M+H)^+$ 910.4925. Found 910.4942. The ^1H NMR spectrum of the synthetic sample was identical to the ^1H NMR spectrum of authentic microcystin-LA, and the synthetic sample coeluted with authentic microcystin-LA by analytical reversed phase HPLC (80:20 MeOH:0.2% aqueous TFA).

Acknowledgment. We are indebted to Professor Richard E. Moore for an authentic sample of microcystin-LA. We are grateful to Dr. Catherine J. Leblanc for early synthetic studies of Adda and to Professor. A. J. Shaka and Gilbert Mackin for assistance in NMR characterization of the final product. We also thank Professor Peter Wipf, Dr. Michael F. Cristofaro, and Dr. Robert J. Valentekovich for helpful discussions. Partial support by the National Institutes of Health (NS-27600) is gratefully acknowledged.

JA961683E