(1 H, d, J = 3.0 Hz, CH), 2.50 (3 H, s, NCH_3), 2.45 (1 H, dd, J = 9.5, 4.0 Hz, CH), 2.36 (1 H, app pentet, J = 8.7 Hz, CH), 2.12 (1 H, m, CH), 2.05 (1 H, m, CH_2), 2.02 (1 H, m, CH), 1.85 (1 H, m, CH_2), 1.76 (1 H, septet, J = 6.5 Hz, CH), 1.55 (1 H, m, CH_2), 1.38 (3 H, s, CH_3), 0.97 (3 H, d, J = 6.5 Hz, CH_3), 0.96 (3 H, d, J = 6.5 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 178.99 (s), 79.31 (d), 67.05 (d), 61.95 (t), 53.88 (d), 52.49 (s), 51.64 (d), 44.03 (d), 43.11 (d), 36.60 (q), 32.85 (t), 32.78 (q), 30.75 (t), 24.52 (d), 21.10 (q), 20.42 (q); IR ($CDCl_3$) 2970, 2920, 2860, 1765, 1435, 1420, 1365, 1125, 975 cm^{-1}; mass spectrum (EI) 263, 220, 206, 178, 136, 108, 40. High-resolution mass spectrum calcd for $C_{16}H_{25}NO_2$: 263.1885. Found: 263.1887.

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Emerimicins III and IV and Their Ethylalanine[12] Epimers. Facilitated Chemical-Enzymatic Synthesis and a Qualitative Evaluation of Their Solution Structures

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Abstract: The peptaibol antibiotics, emerimicin III and IV (Ac-Phe[1]-MeA[2]-MeA[3]-MeA[4]-Val[5]-Gly[6]-Leu[7]-MeA[8]-MeA[9]-Hyp[10]-Gln[11]-*R*-EtA[12]-Hyp[13]-Xxx[14]-Phol[15], where Xxx = Ala for emerimicin III and Xxx = MeA for emerimicin IV) and their EtA[12] epimers have been synthesized using a combined approach involving solution-phase fragment condensation with a final papain-mediated coupling of the 1-6 and 7-15 fragments. The yield of this final step, ranging from 62 to 80% for the four peptides, was a dramatic improvement over efforts to couple these fragments chemically using DCC/HOBt. A qualitative evaluation of the solution structures of these peptides in DMSO is consistent with a right-handed, predominantly 3_{10} helical conformation throughout the length of the sequence. The antibacterial activity of synthetic emerimicins III and IV was found to be comparable to the native material. The absolute stereochemistry at position 12 has minimal effect on either the biological activity or the solution conformation of the emerimicins.

Introduction

The emerimicins, produced by *Emericellopsis microspora* in the presence of *trans*-4-propyl-L-proline,[2] belong to the class of peptaibol antibiotics commonly found in filamentous fungi.[3] Structurally, these compounds are characterized by several residues of α,α-dialkyl amino acids such as α-methylalanine (MeA or Aib, aminoisobutyric acid[4]) and α-ethylalanine (EtA, or Iva, isovaline[4]), the presence of a C-terminal amino alcohol, and an N-terminal acetyl group. The main interest in peptaibols stems from their ability to form voltage-dependent ion-conducting pores in lipid bilayer membranes, and alamethicin, a 20-residue peptaibol discovered in 1967,[5] is the most intensively studied model for voltage-gated channels. Employing mainly various gas chromatography-mass spectrometry techniques, Rinehart et al.[6,7] determined the sequences of the emerimicins to be Ac-Phe[1]-MeA[2]-MeA[3]-MeA[4]-Val[5]-Gly[6]-Leu[7]-MeA[8]-MeA[9]-Hyp[10]-Gln[11]-*S*-EtA[12]-Hyp[13]-Xxx[14]-Phol[15] where Xxx = MeA for emerimicin IV,[7] the principal component, and Xxx = Ala for emerimicin III, the minor component. The configuration of EtA[12], originally assigned as *S*, was subsequently revised to *R*, based on chiral gas chromatography[8] and X-ray analysis.[9] As part of our long-standing interest in the conformational attributes of α,α-dialkyl amino acids and the molecular mechanisms by which peptaibols

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(4) In the literature, the names aminoisobutyric acid (Aib) and isovaline (Iva) are prevalent. The abbreviation Iva is confusing because it is used also to denote the isovaleryl residue. In the first systematic synthetic studies on α,α-disubstituted amino acids by Kenner's group (refs 32 and 34), the names α-methylalanine and α-ethylalanine were introduced. We support this self-explanatory nomenclature and we have proposed abbreviations (ref 10) consisting of Me or Et to designate an α-alkyl substituent and the one letter code used for the amino acid i.e., MeA or EtA. The abbreviations of other amino acids correspond to IUPAC-IUB rules (*Eur. J. Biochem.* **1984**, *138*, 9-37). Other abbreviations: Phol, L-phenylalaninol; DCC, *N,N'*-dicyclohexylcarbodiimide; HOBt, 1-hydroxybenzotriazole; DMF, dimethylformamide; DABS, 4-(dimethylamino)azobenzene-4'-sulfonyl group; Piv, pivaloyl; Ox, oxazolone residue; Bzl, benzyl; Z, benzyloxycarbonyl; Boc, (*tert*-butyloxy)carbonyl.

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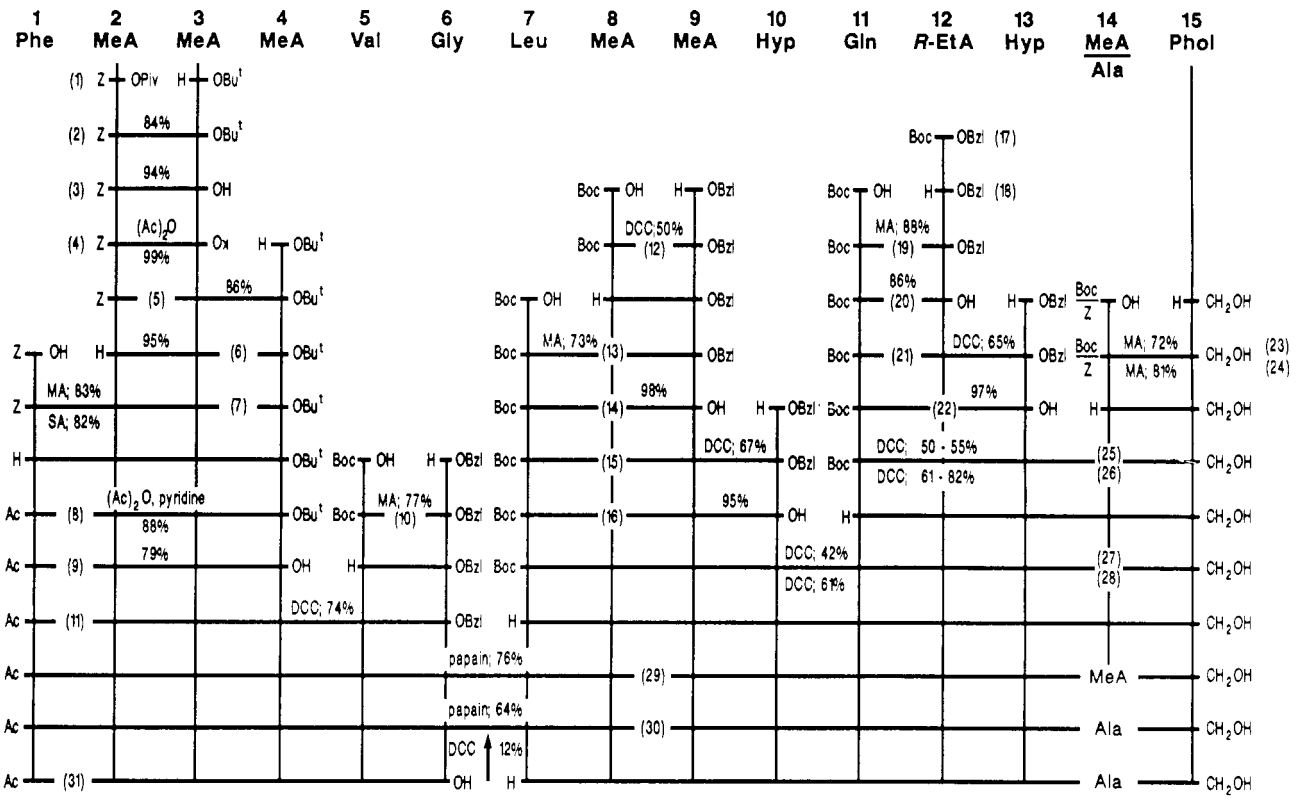

Figure 1. Chemical–enzymatic synthesis of emerimicin IV (**29**) and III (**30**). The synthesis of *S*-EtA[12] epimers **29a** and **30a** followed the same strategy, which is detailed in the Experimental Section. Abbreviations: OPiv = pivaloyl mixed anhydride; MA = mixed anhydride with isobutyl chloroformate; SA = symmetrical anhydride; Ox = oxazolone; DCC = dicyclohexylcarbodiimide and 1-hydroxybenzotriazole as additive.

function, we engaged in a synthetic effort to prepare emerimicins III and IV.

All of the synthetic challenges previously encountered in other work on the peptaibols are exemplified in the sequences of the emerimicins. They are rich in sterically hindered α,α-dialkyl amino acids whose reactivity is much lower than typical α-amino acids. Consequently, a solid-phase approach employing standard stepwise synthesis protocols is not suitable for their synthesis, due to incomplete couplings and accumulation of deletion sequences as side products, especially for those peptaibols containing homosequences such as -MeA-MeA-MeA-. Emerimicins contain acid-labile MeA-Hyp and EtA-Hyp bonds; hence synthetic procedures requiring treatment of the intermediate peptides with acids can lead to the cleavage of these bonds. The two hydroxyproline (Hyp) residues in the sequences pose a problem due to the presence of the reactive side chain OH group. In addition, there is little experience with incorporating chiral α-ethylalanine (EtA) into peptaibol chains.[10] The known increase in risk of racemization[11,12] for C-terminal and penultimate α-amino acids involved in the synthesis of peptides containing α,α-disubstituted amino acids is another concern. Although new coupling methods may make solid-phase incorporation of these unusual amino acids into peptides[13] feasible, classical solution synthesis is still the preferred route to the peptaibols[14-19] because coupling at each step can be optimized and the intermediates fully characterized.

Working from this perspective, we initiated a synthetic strategy for the emerimicins which required a final chemical coupling of the 1–9 and 10–15 fragments. Although synthetic methods could be readily optimized to produce the needed fragments in good yield, the final reaction was inefficient, and a revised strategy involving chemical coupling of the 1–6 and 7–15 fragments gave only slightly higher yields of the desired product. The failure of two different synthetic strategies at the crucial final step led us to investigate an enzymatic approach. The advantages of protease-catalyzed peptide synthesis, such as mild reaction conditions, absence of racemization, and minimal protection and activation requirements are well-recognized.[20] Nevertheless, enzymatic coupling of longer peptide sequences has yet to reach the versatility of well-established chemical methods. Using the same 1–6 and 7–15 fragments, we were able to complete the final coupling by means of papain in a 1-h reaction with nearly pure emerimicin precipitating from solution in high yield.

The α,α-dialkyl amino acids found in the emerimicins and other peptaibols are interesting from not only a synthetic point of view but also the conformational impact these unusual residues have on a peptide. For MeA, theoretical analyses first suggested a reduction in accessible φ and ψ values relative to alanine and other α-monoalkyl amino acids.[21] While in theory both extended and helical conformations are accessible to MeA, numerous experimental observations in both solution and solid state have revealed

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the propensity of MeA to induce helical conformations.[22] Other α,α-dialkyl amino acids have not been characterized as extensively, by either computational or experimental approaches. One issue is whether or not these residues have the same helix-inducing behavior as MeA, or if they are more likely to adopt extended conformations.[23] In the case of chiral α,α-dialkyl amino acids, the additional issue of what impact their chirality might have on helical screw sense has also been addressed.[24] One theoretical study has suggested an enhanced likelihood of extended conformations for EtA when compared to MeA.[25] The few studies of EtA in the crystalline state show both an extended structure for the isolated residue[9] and a helical conformation when incorporated into a tetrapeptide.[10]

The ability to prepare pure quantities of the emerimicins has afforded us the opportunity to investigate the conformational properties of these peptides and to prepare analogues directed at probing the conformational properties of EtA. Here we report our combined chemical–enzymatic synthesis of the emerimicins III and IV as well as their EtA[12] epimers, their biological activities, and a qualitative characterization of their solution structures in DMSO.

Results and Discussion

Development of the Synthetic Scheme (Figure 1). Efforts toward the synthesis of the emerimicins were initiated by our communication[26] on the synthesis of the N-terminal nonapeptide Ac-Phe[1]-MeA[2]-MeA[3]-MeA[4]-Val[5]-Gly[6]-Leu[7]-MeA[8]-MeA[9]-OBzl. Subsequently, 15 further segments derived from the N-terminal 10 amino acids were synthesized by other groups and their conformational preferences studied.[27-30] We revised and improved our previous synthesis of the above nonapeptide with the aim of using it both for structural studies[31] and for a final coupling involving the 1–9 and 10–15 fragments (a 9 + 6 coupling). Although a segment having a sterically hindered C-terminal α-methylalanine (MeA[9]) would seem to be a questionable choice for the final condensation, several facts prompted us to explore this possibility. The N-terminal nonapeptide contains two MeA residues at its C-terminus; therefore, the problem of preserving chiral integrity is absent. In common coupling reactions, steric hindrance at the carbonyl group can be expected to be less severe than at the amino group in derivatives of α-methylalanine.[32] In a test condensation of Boc-Leu[7]-MeA[8]-MeA[9]-OH + H-Hyp[10]-OBzl using DCC/HOBt, the expected emerimicin tetrapeptide was obtained in reasonable yield. Furthermore, the reported alamethicin syntheses[14-17] showed that segments with two C-terminal MeA residues can be successfully combined with peptides containing typical α-amino acids at their N-termini.

Two routes were explored for the synthesis of the emerimicin C-terminal hexapeptide Hyp[10]-Gln[11]-*R*-EtA[12]-Hyp[13]-Xxx[14]-Phol[15] needed for a 9 + 6 coupling. At first, stepwise elongation from the carboxy terminus using urethane protection to suppress racemization was attempted. However, this approach had to be discontinued because the intermediate tetrapeptide[10] Boc-*R*-EtA[12]-Hyp[13](Bzl)-Ala[14]-Phol[15] could not be lengthened at its N-terminus. This failure may be attributed to spontaneous cleavage at the Hyp-Ala linkage after N-deprotection, in analogy to the known case of H-MeA-Pro-Trp-OH.[33] In a successful alternate approach, the key segment was Boc-Gln[11]-*R*-EtA[12]-Hyp[13]-OBzl, prepared from Boc-Gln[11]-*R*-EtA[12]-OH and Hyp-OBzl (OH unprotected). C-Terminal elongation (+Ala[14]-Phol[15]) followed by N-terminal elongation (+Z-Hyp, OH unprotected) using DCC/HOBt furnished the needed hexapeptide Z-Hyp[10]-Gln[11]-*R*-EtA[12]-Hyp[13]-Ala[14]-Phol[15] in moderate yield. This result was ultimately significant in the development of an alternative synthetic plan because it indicated that the dipeptide Gln[11]-*R*-EtA[12] would be a crucial intermediate in other approaches to C-terminal segments of emerimicin.

The final 9 + 6 coupling using DCC/HOBt was inefficient, furnishing a complex mixture from which pure emerimicin III could be isolated in 5% yield only after extensive purification. Although the initial strategy proved to be a failure, a great deal of constructive information applicable to further synthetic efforts was generated. First, N-terminal segments of emerimicins were found to be accessible by solution synthesis, and their yields could be readily optimized. Second, C-terminal fragments could be synthesized without protection in both Hyp residues, and the important role of the previously mentioned dipeptide intermediate Gln-*R*-EtA had to be considered in any alternative strategy. Third, the final coupling is a critical issue, and the 6 + 9 condensation presented itself as an obvious alternative to the 9 + 6 strategy, since the 1–6 and 7–15 segments contain Gly and Leu at the C- and N-terminus, respectively, which usually can be combined efficiently by chemical means.

Our effort to chemically couple the 1–6 and 7–15 segments (Figure 1) was only slightly more successful than the 9 + 6 coupling, yielding emerimicin III in low (12%) yield, again only after extensive purification. The low yields of the final 9 + 6 and 6 + 9 couplings could be explained by combined effects of the presence of unprotected reactive hydroxyl groups and steric hindrance due to multiple α,α-dialkyl amino acids which makes normally slower side reactions of unprotected functional groups dominate. This explanation prompted a trial enzymatic coupling of the same 1–6 and 7–15 segments because the inherent regioselectivity of proteolytic enzymes for α-carboxyl and α-amino groups would preclude the formation of side products.

Synthesis of Fragments. N-Terminal Hexapeptide, Ac-Phe-MeA-MeA-MeA-Val-Gly-OBzl (11). The key segment in this hexapeptide is the sterically hindered sequence, MeA-MeA-MeA, which was synthesized using the pivalic mixed anhydride and oxazolone methods successively. For joining the first two units of MeA, the mixed anhydride method with pivaloyl chloride was used.[32] The mixed anhydride of benzyloxycarbonyl-α-methylalanine and pivalic acid (Z-MeA-OPiv, **1**) is an activated intermediate in which the adverse steric effect of the α-methylalanyl residue is counterbalanced, i.e., reaction of an amine at the pivaloyl carbonyl carbon would be at least as strongly sterically retarded as the desired peptide-forming reaction. Subsequent C-elongation of the dipeptide Z-MeA-MeA-OH (**3**) by MeA-OBu^t using the oxazolone method afforded the homotripeptide **5** (Z-MeA-MeA-MeA-OBu^t) in an overall 66% yield.[32,34] To avoid racemization, the N-terminal Ac-Phe was introduced in an indirect fashion by first acylating the N-deprotected homotripeptide **6** using Z-Phe in a mixed anhydride and then replacing the Z-protecting group by an acetyl group as shown in Figure 1. For carboxy-terminal elongation of the peptide **9**, better yields were obtained when the

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activation step (DCC/HOBt) was performed at a slightly elevated temperature (30–40 °C) and the aminolysis was allowed to proceed for 3 days. Although 1-ethyl-3-(3-(dimethylamino)propyl)-carbodiimide hydrochloride (EDAC·HCl) has been reported as an efficient reagent for coupling to C-terminal MeA,[18] when applied to the 1–4 + 5–6 coupling, the product yield was low with almost all of the unreacted starting acetyltetrapeptide recoverable in the reaction workup.

In general, synthesis of the N-terminal hexapeptide **11** proceeded very cleanly. All intermediates as well as the final product could be easily crystallized as pure compounds and not one chromatographic purification was needed.

C-Terminal Nonapeptides, Boc-Leu-MeA-MeA-Hyp-Gln-EtA-Hyp-Xxx-Phol (27, 28, 27a, 28a). While N-terminal hexapeptide **11** is common to both emerimicins III and IV and their *S*-EtA[12] epimers, four C-terminal nonapeptides were needed for assembly of the final pentadecapeptides. As summarized in Figure 1, the required nonapeptides (**27**, EtA = *R*-EtA, Xxx = MeA; **28**, EtA = *R*-EtA, Xxx = Ala; **27a**, EtA = *S*-EtA, Xxx = MeA; **28a**, EtA = *S*-EtA, Xxx = Ala) were all prepared in an analogous manner by condensation of the 7–10 and 11–15 segments using DCC/HOBt activation. This strategy was dictated by the fact that the 7–10 segment is common to all four nonapeptides, and by the opportunity to use a relatively racemization-resistant residue, hydroxyproline, at the carboxy terminus. In the synthesis of the 7–10 fragment, the difficult coupling of two MeA residues was done using DCC/HOBt and was repeated several times under different conditions with the aim of optimizing the method. In all experiments, crude dipeptide (Boc-MeA-MeA-OBzl, **12**) required repeated purification, and the reproducible yield (50%) was far below that (84%) obtained via the pivaloyl mixed anhydride method used in the synthesis of the N-terminal emerimicin segment **2**.

The 11–15 segments (**25**, **26**, **25a**, **26a**), each having an N-terminal glutamine, were chosen because our initial experience in the synthesis of C-terminal emerimicin sequences demonstrated the need for a route avoiding N-deprotection of N-terminal α-ethylalanine. This route required Boc-Gln-EtA as a starting dipeptide for the synthesis of the 11–15 segments. We used a Boc/OBzl protection and left the hydroxyproline OH unprotected throughout the preparation of all intermediates comprising residues 7–15. The usefulness of MeA benzyl esters has been questioned by other authors[17,18] due to difficulties encountered in catalytic removal of the benzyl group. In contrast to the reported 50% yield upon hydrogenation of Boc-Val-MeA-MeA-OBzl,[17] we found the hydrogenolytic cleavage of Boc-Leu-MeA-MeA-OBzl (**13**) to proceed with a yield of 98%. The C-terminal nonapeptides contain two acid-labile bonds, MeA[9]-Hyp[10] and EtA[12]-Hyp[13]. To avoid degradation of the peptide chain, cleavage of the Boc-protecting group was performed under carefully controlled conditions using 1:1 TFA/CH_2Cl_2 for 15–18 min.

In general, synthesis of the C-terminal nonapeptides was much more difficult than the preparation of the N-terminal hexapeptide **11**. Presumably the unprotected OH of hydroxyproline led to the formation of side products, particularly in the coupling of the 7–10 and 11–15 segments, and the resulting mixtures required extensive purification.

Enzymatic Coupling. Papain was chosen for the final coupling because of its relatively low sequence specificity. Its primary specificity is for hydrophobic residues in the S_2-subsite which is compatible with the hydrophobic side chain of Val in position P_2.[35] Of concern was the potential impact of the conformationally constrained amino acid, MeA, on recognition and binding of the peptide substrates to papain. Many proteolytic enzymes recognize an extended conformation of the peptide chains which optimizes hydrogen bonding between the active site and the substrate. The presence of three contiguous MeA residues in the hexapeptide substrate with their preference[31] for a helical conformation might well have precluded enzyme binding. A preliminary experiment which showed that the benzyl ester of the N-terminal hexapeptide (Ac-Phe-MeA-MeA-MeA-Val-Gly-OBzl, **11**) was hydrolyzed by papain alleviated this concern and may indicate that only two residues adjacent to the cleavage site need to be in the extended conformation for papain hydrolysis. The obvious potential side reaction in enzymatic coupling, cleavage of existing peptide bonds, is essentially eliminated by the presence of hydroxyprolines in the C-terminal fragment and MeA, or EtA, in both the N-terminal and C-terminal fragments. With the exception of one bond in each fragment (Val[5]-Gly[6] and Hyp[10]-Gln[11]), all amide linkages contain hydroxyproline as the amine component or are flanked by α,α-dialkyl amino acids, both known to enhance resistance to most proteolytic enzymes. Nevertheless, the coupling reaction was performed in methanol/phosphate buffer at pH 9.0 to suppress the peptidase activity of the enzyme.[36] The final coupling of the 1–6 and 7–15 fragments was efficient and the easiest step in the overall synthesis, affording as precipitates the nearly pure emerimicins IV (**29**) and III (**30**) in yields of 76% and 64%, respectively. In a typical experiment, TFA·H-Leu-MeA-MeA-Hyp-Gln-*R*-EtA-Hyp-MeA-Phol (80 μmol) was added to a stirred suspension of Ac-Phe-MeA-MeA-MeA-Val-Gly-OBzl (**11**, 120 μmol) in methanol containing phosphate buffer. The pH was adjusted to 9 prior to the addition of papain and dithioerythritol, and methanol and phosphate buffer were added as needed to facilitate stirring. After 1 h, the starting hexapeptide **11** was no longer detectable, and the product, emerimicin IV (**29**), had precipitated from solution (109 mg, 69 μmol, 88% pure by HPLC for a yield of 76%). The *S*-EtA[12] epimers of both emerimicins were obtained similarly in yields of 80% (**29a**) and 62% (**30a**). Neither scale up of the synthesis nor the use of impure nucleophile affected the yield.

The facile synthesis afforded by papain coupling of the two emerimicin fragments stands in marked contrast to the difficulties seen when traditional chemical methods were attempted. Minimal protection is one of the dominant strategies chosen for the synthesis of larger peptides due to side reactions in removal of side chain protecting groups. However, as indicated previously, the balance between the desired coupling and reactions at these unprotected sites may be shifted to the later due to the steric hindrance introduced by multiple α,α-dialkyl amino acids. Alternatively, a maximal protection strategy requires harsh conditions for deprotection which also enhances the likelihood of side products. Selective coupling through the use of the enzymatic approach offers clear advantages[20] in that it is compatible with minimal protection and preserves the chiral integrity of the substrates. The enzymatic condensation of the 1–6 and 7–15 emerimicin fragments, with coupling at a Gly-Leu sequence often found in other peptaibols, suggests that a hybrid chemical–enzymatic approach may be generally applicable to the total syntheses of other peptaibol antibiotics. Our preliminary results with the total synthesis of alamethicin[37] are consistent with this prediction.

Solution Structure of the Emerimicins and Their EtA[12] Epimers. For the four peptides studied, sequential assignment methods[38] based on P. COSY[39] and phase-sensitive NOESY[40] experiments were used to identify the ^{1}H resonances (Table I) of residues other than MeA and EtA, which lack the α proton required to establish scalar connectivity between the amide and side chain β protons. In a typical P. COSY experiment (Figure 2a), the 7 intraresidue NH_i–αH_i connectivities expected for Phe[1], Val[5], Gly[6], Leu[7], Gln[11], Ala[14], and Phol[15] of emerimicin III were observed. The spin systems of each of these residues as well as those of the two hydroxyprolines could be identified based on their characteristic

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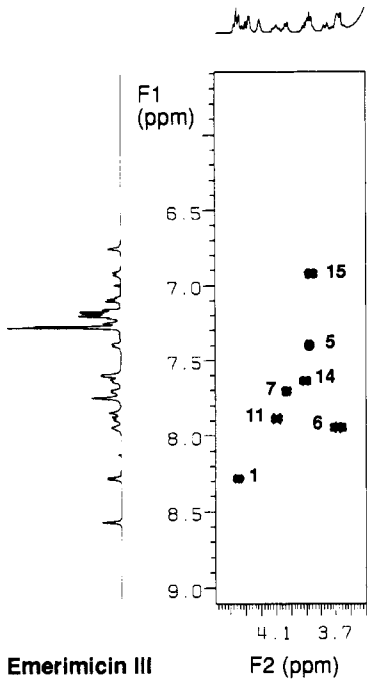


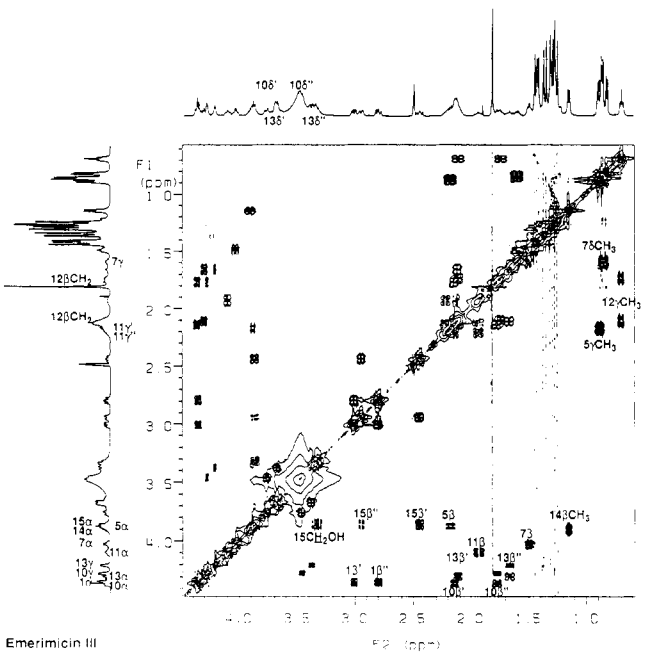
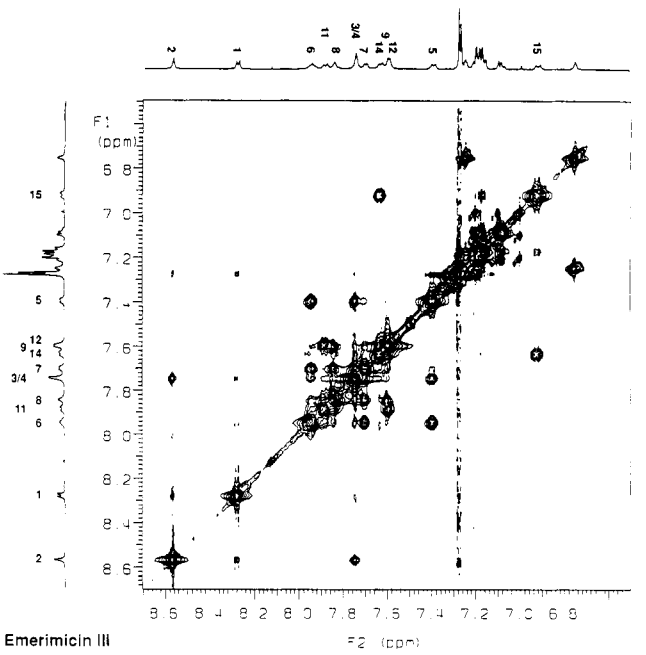


Figure 2. Expanded contour plots of ^{1}H 500-MHz P. COSY spectrum of synthetic emerimicin III in DMSO-d_6 (20 °C). (a, top) Fingerprint region. The seven expected NH_i–αH_i connectivities are labeled by residue number. (b, bottom) α/aliphatic region. Labels above and below cross-peaks and along the F_2 spectrum indicate frequencies in F_2. Labels along the F_1 spectrum identify other resonances necessary for tracing connectivities. J-connectivities for Phe[1], Val[5], Leu[7], Hyp[10], Hyp[13], and Phol[15] can be seen.



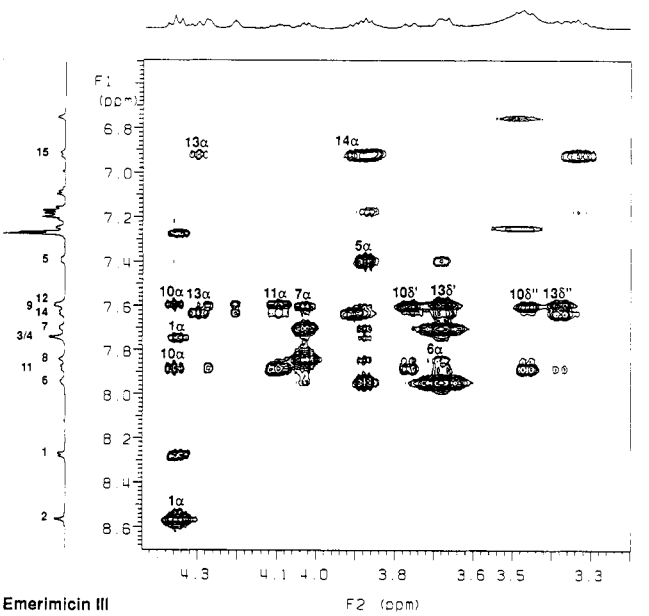


Figure 3. Expanded contour plots of ^{1}H 500-MHz NOESY spectrum of synthetic emerimicin III in DMSO-d_6 (20 °C). (a, top) Amide region showing NN(i,i+1) and NN(i,i+2) connectivities. Amide proton resonances are labeled by residue number along F_1 and F_2. (b, bottom) Amide/α region showing αN(i,i+1), αN(i,i+2), and αN(i,i+3) connectivities. Labels above cross-peaks indicate frequencies in F_2; amide proton resonances are labeled by residue number on the one-dimensional spectrum along F_1.

connectivity patterns[38] (Figure 2b and supplementary material). Phe[1] and Phol[15] were readily differentiable by the additional αH_i–CH_2OH_i connectivity of Phol[15]. Resonances in the ethyl side chain of EtA[12] were assigned based on βCH_2–γCH_3 connectivites (In the case of emerimicin III shown in Figure 2b, βCH_2 = 1.731 and 2.091 ppm and γCH_3 = 0.689 ppm). Sequential assignment of the amide protons of MeA residues was possible from NH_i–NH_{i+1} connectivities observed in the NOESY spectra (see Figure 3a for emerimicin III and supplementary material). In each case, these connectivities are broken at residue 3, because the amide protons of MeA[3] and MeA[4] are coincident for all four peptides, and at the hydroxyproline residues (positions 10 and 13). With the amide proton assignments complete, MeA methyl resonances were assigned based on NOE connectivities of the methyl protons to the amide proton in the same residue (NH_i–βH_i) and to the amide proton in the following residue (βH_i–NH_{i+1}, data not shown). Sequential assignment of Hyp[10] and Hyp[13] was possible from NH_i–δH_{i+1}, δH_i–NH_{i+1}, and α_i–NH_{i+1} connectivities (see Figure 3b and supplementary material). Notably, only a single set of resonances was observed for each peptide, suggesting no cis–trans isomerization of the amide bonds preceding each of the Hyp residues.

A comparison of the four peptides (Table I) reveals that for the first 10 residues, differences in proton chemical shift between *R*- and *S*-EtA[12] epimers, and between Ala[14] and MeA[14] analogues are insignificant (<0.06 ppm). The minor chemical shift dif-

Table I. ^{1}H Resonance Assignments for Synthetic Emerimicins IV and III and Their *S*-EtA^{12} Epimers at 20 °C in DMSO-d_6[a]

residue	resonance	emerimicin IV (**29**)	emerimicin III (**30**)	[*S*-EtA^{12}] emerimicin IV (**29a**)	[*S*-EtA^{12}] emerimicin III (**30a**)
Ac	CH_3	1.809	1.810	1.807	1.808
Phe^1	NH	8.274	8.279	8.282	8.272
	α	4.347	4.346	4.344	4.346
	β	3.004	3.004	3.005	3.003
		2.793	2.792	2.790	2.789
	arom[b] 2,6	7.275	7.276	7.280	7.282
	3,5	7.197	7.198	7.197	7.197
MeA^2	NH	8.562	8.567	8.576	8.561
	CH_3	1.267	1.266	1.269 (both)	1.267 (both)
		1.270	1.270		
MeA^3	NH[c]	7.741	7.745	7.738	7.736
	CH_3	1.246	1.245	1.244	1.243
		1.287	1.286	1.284	1.284
MeA^4	NH[c]	7.741	7.745	7.738	7.736
	CH_3	1.343	1.342	1.341	1.340
		1.366	1.365	1.364	1.363
Val^5	NH	7.396	7.396	7.387	7.384
	α	3.874	3.872	3.897	3.889
	β	2.171	2.169	2.176	2.173
	γ	0.854	0.854	0.849	0.849
		0.888	0.886	0.883	0.884
Gly^6	NH	7.942	7.944	7.939	7.939
	α	3.652	3.650	3.648	3.646
		3.709	3.706	3.704	3.706
Leu^7	NH	7.700	7.701	7.702	7.700
	α	4.031	4.027	4.036	4.024
	β	1.493	1.487	1.482	1.474
	γ	1.598	1.587	1.592	1.585
	δ	0.823	0.815	0.815	0.811
		0.864	0.857	0.859	0.852
MeA^8	NH	7.849	7.843	7.906	7.892
	CH_3	1.321	1.304	1.315	1.291
		1.428	1.423	1.431	1.420
MeA^9	NH	7.598	7.601	7.562	7.555
	CH_3	1.299	1.309	1.264	1.267
		1.439	1.442	1.431	1.433
Hyp^{10}	α	4.352	4.356	4.348	4.350
	β	1.757	1.769	1.749	1.764
		2.136	2.141	2.126	2.126
	γ CH	4.263	4.271	4.273	4.275
	OH	~5.1 (br)	~5.1 (br)	5.109	5.105
	δ	3.447	3.457	3.435	3.439
		3.754	3.763	3.742	3.739
Gln^{11}	NH	7.872	7.884	7.814	7.820
	α	4.084	4.094	4.142	4.157
	β	1.901	1.924	1.885	1.901
	γ	2.120	2.211	2.098	2.109
		2.092	2.123	2.187	2.272
	ϵ	6.742	6.754	6.735	6.739
		7.235	7.252	7.232	7.233
EtA^{12}	NH	7.487	7.592	7.657	7.756
	CH_2	1.737	1.731	1.841	1.825
		2.106	2.091	2.009	2.074
	γCH_3	0.710	0.689	0.785	0.783
	βCH_3	1.385	1.408	1.324	1.310
Hyp^{13}	α	4.173	4.291	4.165	4.289
	β	1.645	1.656	1.666	1.680
		2.073	2.111	2.051	2.095
	γ CH	4.202	4.202	4.219	4.208
	OH	~5.1 (br)	~5.1 (br)	5.059	5.087
	δ	3.367	3.376	3.351	3.339
		3.625	3.675	3.671	3.703
MeA/Ala^{14}	NH	7.499	7.634	7.457	7.665
	α	NA[d]	3.899	NA[d]	3.901
	CH_3	1.137	1.146	1.163	1.157
		1.299		1.298	
Phol^{15}	NH	6.966	6.921	6.986	6.954
	α	3.850	3.857	3.811	3.837
	βCH_2	2.509	2.438	2.506	2.442
		2.926	2.942	2.912	2.936
	CH_2OH	3.281	3.324 (both)	3.291	3.337 (both)
		3.364		3.361	
	arom[b] 2,6	7.168	7.172	7.183	7.189
	3,5	7.109	7.091	7.092	7.080

[a] In ppm, referenced to DMSO-d_6 residual proton signal. [b] Assignment of the 4-aromatic proton for residues 1 and 15 was not possible due to signal overlap and strong coupling effects. [c] In all four peptides, MeA^3 NH and MeA^4 NH are coincident. [d] NA, not applicable.

ferences that exist are localized to the region of the modified residues. Substitution of Ala for MeA^{14} results in ~0.10 ppm upfield shift in the EtA^{12} NH resonance and ~0.12 ppm upfield shift for the Hyp^{13} α hydrogen. Changing the chirality of EtA^{12}

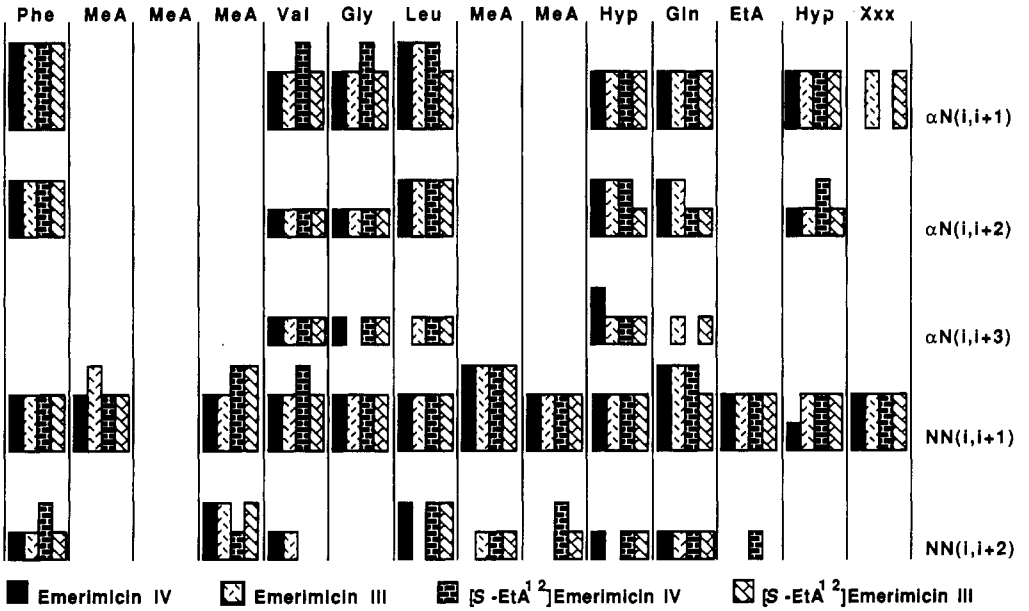


Figure 4. Summary of sequential backbone NOESY connectivities observed for synthetic emerimicin IV, emerimicin III, [*S*-EtA^{12}]emerimicin IV, and [*S*-EtA^{12}]emerimicin III in DMSO-d_6 at 20 °C. For every residue in the sequence four columns are shown. Each column represents the relative intensity of the indicated NOE cross-peak type for one of the synthetic peptides. Cross-peak intensities are approximated as strong, medium, and weak, and are indicated by bar heights. Xxx = Ala for emerimicin III and [*S*-EtA^{12}]emerimicin III; MeA for emerimicin IV and [*S*-EtA^{12}]emerimicin IV.

from *R* to *S* results in similar slight changes in the Gln^{11} γHs and the EtA resonances. The largest difference is seen for the amide proton of EtA^{12} in emerimicin IV, which shifts downfield by 0.17 ppm. The chemical shift data suggest little conformational difference among the four peptides.

MeA-containing peptides are known to adopt helical conformations in the crystalline as well as the solution state,[22] and the observation of NOE connectivities characteristic of helices[38] was anticipated. Interresidue backbone NOE connectivities typical of helices are tabulated in Figure 4 for emerimicin III and IV and their EtA^{12} epimers. All NOESY cross-peaks were of the same phase as the diagonal peaks. In Figure 4, Hyp δH is treated as equivalent to an amide proton, and the relative strength of the NOE connectivities (strong, medium, or weak) is indicated by column height. As mentioned previously, all possible NH$_i$–NH$_{i+1}$, δH$_i$–NH$_{i+1}$, and NH$_i$–δH$_{i+1}$ connectivities are observed in the four peptides, with the sole exception being that of MeA^3-MeA^4. In addition, all possible αN(*i*,*i*+1), αN(*i*,*i*+2), and αN(*i*,*i*+3) connectivities are observed (Figures 3b and 4 and supplementary material), with the exception of αN(*i*,*i*+3) for Phe^1, which may reflect some fraying of the helix at its N terminus. The NN(*i*,*i*+2) connectivities observed are also consistent with a helical conformation. Any qualitative difference in the pattern of observed NOE connectivities between the four peptides is a consequence of signal overlap which precluded positive identification of a given NOE. The approximate quantitation of cross-peak intensities based on peak height (shown in Figure 4) reveals little to suggest that emerimicin III and IV and their *S*-EtA^{12} epimers differ dramatically from one another in conformation. The upfield position of the *R*-EtA^{12} γCH$_3$ resonance (0.710/0.689 ppm) relative to that of the *S*-epimer (0.783/0.785 ppm) is consistent with a helical conformation, in that the ethyl side chain would be directed toward Phol^{15} on the same side of the helix. Furthermore, the similarity in intensity of the αN(*i*,*i*+2) and αN(*i*,*i*+3) cross-peak intensities is more consistent with a 3_{10} than an α helical conformation.[38]

The intramolecular hydrogen bonding pattern of the four peptides is summarized in Table II and is also consistent with a helical conformation for each. For most of the residues in the peptides, temperature coefficients of the amide protons (Δδ/Δ*T*) are less than the absolute value of 3 ppb/K which is diagnostic of intramolecular hydrogen bonding. In every case the temperature dependence was linear across the range examined. The large coefficients for Phe^1 and MeA^2 are consistent with a 3_{10} helical conformation at the N-terminus of all four peptides. The only other solvent-exposed amide protons in the sequence are Leu^7 and MeA^8, suggesting a defect in the helix at that point. Our previous studies[41] have suggested that this type of discontinuity in the sequence of hydrogen-bonded amide protons may be consistent

Table II. Temperature Dependence of Amide Proton Chemical Shifts (Δδ/Δ*T* in ppb/K) for Synthetic Emerimicins III and IV and Their *S*-EtA^{12} Epimers in DMSO-d_6

	emerimicin IV (**29**)	emerimicin III (**30**)	[*S*-EtA^{12}] emerimicin IV (**29a**)	[*S*-EtA^{12}] emerimicin III (**30a**)
Phe^1	−4.6	−4.8	−4.6	−4.7
MeA^2	−6.3	−6.5	−6.3	−6.4
MeA^3 [a]	−2.2	−2.3	−2.4	−2.1
MeA^4 [a]	−2.5	−2.5	−2.2	−2.2
Val^5	−0.4	−0.5	−0.4	−0.4
Gly^6	−1.8	−1.9	−1.8	−1.9
Leu^7	−3.4	−3.6	−3.6	−3.7
MeA^8	−5.4	−5.4	−5.9	−6.0
MeA^9	−0.8	−0.8	−0.4	−0.4
Gln^{11}	−1.5	−1.6	−1.5	−1.6
EtA^{12}	−0.7	−1.1	−1.5	−1.6
MeA/Ala^{14}	−1.0	−0.8	−1.0	−1.2
Phol^{15}	−0.9	−0.2	−0.8	−0.4

[a] Coefficients for MeA^3 and MeA^4 may be interchanged as these resonances are degenerate at lower temperatures

Table III. Summary of Coupling Constants ($^3J_{NH-\alpha H}$ in Hz) for Synthetic Emerimicins III and IV and Their *S*-EtA^{12} Epimers in DMSO-d_6 at 20 °C

	emerimicin IV (**29**)	emerimicin III (**30**)	[*S*-EtA^{12}] emerimicin IV (**29a**)	[*S*-EtA^{12}] emerimicin III (**30**)
Phe^1	6.3	6.2	6.4	6.2
Val^5	7.6	7.4	7.7	7.3
Gly^6	5.6, 5.8	4.5, 5.1	4.1, 5.3	4.5, 5.2
Leu^7	5.2	5.5	4.7	6.0
Gln^{11}	8.8	8.6	8.3	8.7
Ala^{14}	NA[a]	7.3	NA	8.1
Phol^{15}	9.0	8.9	8.9	8.9

[a] NA, not applicable.

(41) Beusen, D. D.; Hutton, W. C.; Kotyk, J. J.; Zabrocki, J.; Leplawy, M. T.; Marshall, G. R. In *Peptides 1990. Proceedings of the 21st European Peptide Symposium*; Giralt, E., Andreu, D., Eds.; ESCOM Publishers: Leiden, 1991; pp 545–547.

Table IV. Antimicrobial Activity of Native Emerimicin IV Triacetate and Synthetic Emerimicins[a]

	growth inhibition zones (diameter in mm) for						
	emerimicin IV triacetate		[S-EtA[12]] emerimicin IV triacetate	emerimicin IV	[S-EtA[12]] emerimicin IV	emerimicin III	[S-EtA[12]] emerimicin III
microorganism	native[b]	synthetic (32)	(32a)	(29)	(29a)	(30)	(30a)
B. subtilis	+	+	–	++	+	+	+
S. lutea	+	+	+	++	++	+	+
S. lutea sensitive	+	+	+	++	++	++	++
E. coli	–	–	–	–	–	–	–
S. aureus	–	trace	–	+	+	+	+
S. pyogenes	+	+	++	++	++	+	++
M. avium	+	+	++	++	++	+	++

[a] Each sample was dissolved in methanol to give a concentration of 1 mg/mL. The disks (6.35 mm, $^1/_4$ in.) containing 20 μL of solution (20 μg of sample) were dried and spotted onto agar plates, each seeded with a microorganism. Inhibition zones were measured after 16 h of incubation at 37 °C. Key: –, no inhibition; +, inhibition zone less than 15 mm; ++, inhibition zone 15 mm or greater; trace, inhibition zone 8 mm or less (nearly equivalent to the 7 mm zone obscured by the disk). [b] This is a derivative of native emerimicin IV supplied by Professor K. L. Rinehart, Jr., and the tests were performed in 1983. The authentic sample is no longer available for current comparative studies.

with a mixed $\alpha/3_{10}$ helix. Within the estimated experimental error of 0.5 ppb/K, the temperature coefficients are remarkably invariant across the series of analogues.

Backbone coupling constants for the peptides of this study are summarized in Table III. Generally, $^3J_{NH-\alpha H}$ for helical structures ($\Phi \cong \pm 60°$) is anticipated to be less than 5 Hz[38] and only the values for Gly[6] and Leu[7] fall within this range (4–6 Hz). The slope of the calibration curve relating $^3J_{NH-\alpha H}$ to Φ is steep in this region,[42] however, so slight increases in Φ above the canonical helical value could significantly increase the observed $^3J_{NH-\alpha H}$, while maintaining the 1–4 or 1–5 hydrogen bonding pattern of α and 3_{10} helices. Structural studies of emerimicin fragments have revealed 3_{10} hydrogen bonding patterns with very large values of Φ (for example, $\Phi = -109$ for Val of Z-MeA$_3$-Val-Gly-OMe[22a]), suggesting that the large coupling constant of Gln[11] (8.3–8.8 Hz) does not preclude a helical conformation for that residue. Differences in $^3J_{NH-\alpha H}$ for a given residue across the series are generally less than 0.5 Hz, and none are large enough to suggest a dramatic change in conformation with substitution at position 12 or 14.

In summary, the ^{1}H NMR data in DMSO consisting of chemical shifts, short-range backbone NOE connectivities, $^3J_{NH-\alpha H}$, and amide proton temperature coefficients are consistent with a helical conformation for emerimicins III and IV and their EtA[12] epimers that extends throughout the length of the sequence. The two closely spaced hydroxyproline residues at positions 10 and 13, as well as the EtA residue at position 12, do not appear to break the helix. Inverting the chirality of EtA appears to have minimal effect on the conformation of the emerimicins in DMSO. Since hydroxyproline can only be accommodated in a right-handed helix, and the uninterrupted helical connectivities over the entire sequence are inconsistent with a switch in helix sense, the qualitative structural assessment suggests that all four peptides adopt a right-handed screw sense. Furthermore, the hydrogen bonding pattern at the N-terminus, and the estimates of backbone NOE intensities are consistent with a helix which is predominantly 3_{10} in character. Quantitative evaluation of the solution structures of emerimicin III and IV and their *S*-EtA[12] epimers will be reported separately.

Antibacterial Activity. A comparison of the antibacterial activity of the synthetic material with natural emerimicins was complicated by some delay between the original measurements on the natural product (emerimicin IV) and the synthetic material, and the absence of additional natural product for side-by-side comparison. Nonetheless, the antibiotic activity of the synthetic emerimicin IV triacetate derivative (Table IV) is nearly identical to that of the native triacetate derivative, which is restricted to Gram-positive bacteria. The only apparent discrepancy occurs for *S. aureus* where the native material is inactive and the synthetic sample has trace activity. Synthetic emerimicin IV and [*S*-EtA[12]]emerimicin IV generally exhibited higher activity than their corresponding triacetate derivatives, with the exception of *E. coli*, where none of the compounds showed activity. The configuration of EtA at position 12 appears to have little effect on antimicrobial activity, in that emerimicins III and IV and their EtA[12] epimers were all active against the entire range of Gram-positive bacteria tested.

(42) Pardi, A.; Billeter, M.; Wüthrich, K. *J. Mol. Biol.* **1984**, *180*, 741–751.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Optical rotations were measured in a 1-dm cell (1 mL) on a Perkin-Elmer polarimeter (Model 241) at 589 nm (Na D line). Fast atom bombardment (FAB) mass spectra were recorded on a Finnigan 3300 spectrometer equipped with a capillaritron gas gun from Phrasor Scientific (Duarte, CA). For thin-layer chromatography (TLC) 250-nm silica gel GF precoated uniplates (Analtech) were used with the solvent systems indicated. The chromatograms were developed with chlorine followed by starch/KI or ninhydrin spray. For flash chromatography, columns packed with silica gel 60 (Merck) were used (2 cm × 15 cm, 4 cm × 15 cm, 5.5 cm × 15 cm). Analytical high-performance liquid chromatography (HPLC) was performed on a Spectra-Physics instrument with an SP8800 ternary pump, using a Vydac C_{18} column, 0.46 × 25 cm, particle size 5 μm at a flow rate of 1.0 mL/min, UV detection at 220 nm, and solvents (A) 0.05% trifluoroacetic acid in H_2O and (B) 0.038% trifluoroacetic acid in acetonitrile/H_2O (90:10). Preparative HPLC was done on a Vydac C_{18} column, 10 mm × 25 cm, particle size 5 μm, at a flow rate 4 mL/min using the same solvents. Amino acid analysis done on a Beckman System Gold Chromatography instrument used DABS–amino acid derivatives; DABS–MeA, –EtA, –Hyp, and –Phol were calibrated using test solutions. For MeA homosequences, the recommended 24-h vapor-phase hydrolysis in constant boiling HCl had to be extended to 48 h, with incomplete hydrolysis of MeA-MeA bonds even under these conditions. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. MeA-OBu^t[32] was obtained as reported. The preparations of Z-MeA,[32] Z-MeA-OPiv[32] (**1**), *R*-EtA,[10] Boc-MeA-Phol[10] (**23**), and Boc-Ala-Phol[10] (**24**) have been described elsewhere. Boc-*S*-EtA ($[\alpha]^{23}_D$ +8.5°, *c* 0.5, EtOH) was prepared in analogy to Boc-*R*-EtA.[10] All other amino acid derivatives and reagents are commercially available.

Z-MeA-MeA-OBu^t (2). This dipeptide was obtained according to the procedure reported for Z-MeA-MeA-OMe[32] except that instead of an equimolar amount of amino acid ester, 2 equiv of MeA-OBu^t (obtained from Z-MeA-OBu^t by hydrogenation[29]) and 1 equiv of Z-MeA-OPiv (**1**) were used. Yields ranged 70–84%, with the higher yield obtained when distilled pure MeA-OBu^t was used. For further synthetic steps the crude crystalline product was used. Crystallized from ether/light petroleum, mp 133–135 °C. Anal. Calcd for $C_{20}H_{30}N_2O_5$ (378.5): C, 63.46; H, 7.99; N, 7.40. Found: C, 63.31; H, 7.71; N, 7.28.

Z-MeA-MeA-OH (3). A solution of Z-MeA-MeA-OBu^t (**2**) (15.14 g, 40 mmol) in trifluoroacetic acid (100 mL) was kept at room temperature for 1 h and then evaporated. Toluene was added and evaporated to remove traces of trifluoroacetic acid. Isolation of the acidic fraction in the usual manner afforded 12.12 g (94%) of benzyloxycarbonyl dipeptide, mp 156–158 °C, raised by recrystallization from aqueous methanol to 161 °C. Anal. Calcd for $C_{16}H_{22}O_5N_2$ (322.36): C, 59.61; H, 6.88; N, 8.69. Found: C, 59.52; H, 6.69; N, 8.54. The preparation of this N-protected dipeptide by a different route (Z-Cl + H-MeA-MeA-ONa) has been reported.[31]

Z-MeA-MeAOx. 2-(1′-((Benzyloxycarbonyl)amino)-1′-methylethyl)-4,4-dimethyloxazolone (4). The preparation of this compound

involved the brief heating of Z-MeA-MeA-OH with acetic anhydride.[32] In several runs crystalline product was obtained in 95–99% yield, mp 121–123 °C. No purification was needed except removal of traces of acetic anhydride by the addition of toluene and evaporation.

Z-MeA-MeA-MeA-OBu^t (5). Prepared by heating **4** with distilled MeA-OBu^t (1.5 equiv) in acetonitrile solution,[34] yield 86%.

H-MeA-MeA-MeA-OBu^t (6). Hydrogen was bubbled through a stirred solution of the benzyloxycarbonyl derivative **5** (9.27 g, 20 mmol) in methanol (200 mL) containing 5% palladium–charcoal (300 mg) until carbon dioxide evolution ceased (4 h). TLC revealed no remaining starting material. Evaporation of the filtered solution resulted in an oily residue which crystallized slowly from a mixture of hexane and a small amount of ethyl acetate: yield 6.26 g (95%); mp 87–92 °C (analytical sample 91–93 °C); R_f 0.5 (CH_2Cl_2/MeOH, 10:1). Anal. Calcd for $C_{16}H_{31}N_3O_4$ (329.43): C, 58.33; H, 9.48; N, 12.75. Found: C, 58.09; H, 9.51; N, 12.48.

Z-Phe-MeA-MeA-MeA-OBu^t (7). To a stirred solution of Z-Phe-OH (5.99 g, 20 mmol) in tetrahydrofuran (16 mL, dried), *N*-methylmorpholine (2.32 mL, 20 mmol) was added. At −15 °C, isobutyl chloroformate (2.76 mL, 20 mmol) was added in portions and after 10 min a cold solution of H-MeA-MeA-MeA-OBu^t (**6**) (6.0 g, 18.2 mmol) in tetrahydrofuran (16 mL) was added dropwise below −12 °C. Stirring was continued at −12 °C for 1 h and then at room temperature for 8 h. After evaporation, the solid residue was dissolved in ethyl acetate, the solution was washed 2 times each with 5% citric acid and 5% Na_2CO_3 and then dried over Na_2SO_4, and the liquid was evaporated. Crystallization from ethyl acetate yielded 9.35 g (83.5%) of tetrapeptide **7**: mp 187–191 °C (analytical sample mp 190–191 °C); R_f 0.57 (CH_2Cl_2/MeOH (10:1)); $[\alpha]^{23}_D$ −10.4° (*c* 2, MeOH). Anal. Calcd for $C_{33}H_{46}N_4O_7$ (610.73): C, 64.89; H, 7.59; N, 9.17. Found: C, 64.30; H, 7.52; N, 9.17. FAB-MS *m/z* 611 (MH^+), 633 (MNa^+). ^{13}C NMR ($CDCl_3$) δ 24.37, 24.49, 24.72, 24.81, 24.90, 25.72 (MeA CH_3), 27.76, 27.86, 27.96 (Bu^t CH_3), 36.94 (Phe βC), 56.21, 56.72, 57.08, 57.49 (MeA, Phe αC), 67.40 (Z CH_2), 80.32 (Bu^t quat. C), 127.31, 128.25, 128.47, 128.62, 128.91, 129.95, 135.89, 136.15 (Phe, Z arom. C), 156.49 (Z, C=O), 171.05, 172.40, 173.44, 173.92 (C=O). This compound could be obtained in similar yield using a symmetrical anhydride route from (Z-Phe)$_2$O.[43]

Ac-Phe-MeA-MeA-MeA-OBu^t (8). Benzylcxycarbonyl tetrapeptide **7** (6.1 g, 10 mmol) was hydrogenated and the product isolated as in the preparation of **6**. After 4 h, TLC revealed no remaining starting material. The crude oily product dissolved in toluene (200 mL) was treated with pyridine (6.7 mL) followed by acetic anhydride (6.7 mL). Stirring was continued for 4 h and ethyl acetate was added to dissolve precipitated product. The solution was washed as usual and the product was isolated as a neutral fraction and crystallized from ethyl acetate/hexane: yield 4.56 g (88%); mp 179–180 °C; $[\alpha]^{23}_D$ +22.8° (*c* 1, MeOH); R_f 0.78 (CH_2Cl_2/MeOH (1:1)), R_f 0.17 ($CHCl_3$/MeOH (9:1)). Anal. Calcd for $C_{27}H_{42}N_4O_6 \cdot {}^1/_2H_2O$ (527.64): C, 61.46; H, 8.20; N, 10.61. Found: C, 61.26; H, 8.12; N, 10.23. FAB-MS *m/z* 519 (MH^+), 541 (MNa^+). ^{13}C NMR (DMSO-d_6) δ 22.14 (Ac CH_3), 23.27, 23.44, 23.99, 25.23, 26.26, 26.54 (MeA CH_3), 27.53 (Bu^t CH_3), 36.38 (Phe βC), 55.05, 55.27, 55.60, 55.94 (Phe, MeA αC), 78.92 (Bu^t quat. C), 126.41, 128.09, 129.24, 137.33 (Phe arom. C), 170.35, 172.60, 172.89, 173.10, 173.14 (C=O).

Ac-Phe-MeA-MeA-MeA-OH (9). The *tert*-butyl ester **8** (3 g, 5.78 mmol) was treated with trifluoroacetic acid (25 mL), and after 1 h the solution was evaporated. The residual solid was dissolved in 5% Na_2CO_3 (80 mL), the solution was washed with ethyl acetate, and the aqueous phase was acidified with 1 N HCl and extracted with ethyl acetate (4 × 50 mL). Crude crystalline product obtained after evaporation was recrystallized from a mixture of ethyl acetate/hexane containing small amounts of methanol, yield 1.51 g of TLC pure acid **9**. Additional amounts of **9** (0.6 g) were obtained from the acidic aqueous phase after saturation with NaCl and repeated extraction with ethyl acetate: yield 2.11 g (79%); mp 205–206 °C; $[\alpha]^{23}_D$ +33.4° (*c* 1, MeOH); R_f 0.62 (CH_2Cl_2/MeOH (1:1)). Anal. Calcd for $C_{23}H_{34}N_4O_6$ (462.53): C, 59.71; H, 7.41; N, 12.11. Found: C, 59.57; H, 7.41; N, 11.92. FAB-MS *m/z* 463 (MH^+).

Boc-Val-Gly-OBzl (10). To a cooled (−20 °C) solution of Boc-Val-OH (4.35 g, 20 mmol) in 15 mL of DMF/CH_2Cl_2 (1:1) was added *N*-methylmorpholine (2.32 mL, 20 mmol) followed by dropwise addition of isobutyl chloroformate (2.76 mL, 20 mmol). After 10 min, GlyOBzl in 20 mL of DMF/CH_2Cl_2 (1:1), prepared from its *p*-toluenesulfonate salt (6.74 g, 20 mmol) by treating with *N*-methylmorpholine (2.32 g, 20 mmol), was added dropwise at −20 °C. Stirring was continued for 1 h at −10 °C and then the solution was allowed to reach room temperature and left for 1 h. Isolation of the product followed the usual procedure for Boc-peptide esters. The oily dipeptide crystallized from ethyl acetate/hexane: yield 5.59 g (77%); mp 77–79 °C; $[\alpha]^{23}_D$ −27.8° (*c* 1, MeOH); R_f 0.68 (CH_2Cl_2/MeOH (10:1)). Anal. Calcd for $C_{19}H_{28}N_2O_5$ (364.43): C, 62.61; H, 7.74; N, 7.68. Found: C, 62.61; H, 7.69; N, 7.64.

Ac-Phe-MeA-MeA-MeA-Val-Gly-OBzl (11). Boc-Val-Gly-OBzl (**10**) (5.0 g, 13.7 mmol) was deprotected by treating with saturated EtOAc/HCl (4 N) solution (55 mL) for 45 min and then filtered and evaporated. The oily residue was dissolved in ethyl acetate (20 mL) and treated with diethyl ether (80 mL) to precipitate semisolid HCl·H-Val-Gly-OBzl which was washed with diethyl ether yielding 4.1 g (99%) of TLC pure HCl·H-Val-Gly-OBzl used for the condensation step, R_f 0.7 (CH_2Cl_2/MeOH (10:1)). Ac-Phe-MeA-MeA-MeA-OH (**9**) (1.80 g, 3.9 mmol) and HOBt hydrate (657 mg, 3.9 mmol) in DMF (7 mL) were activated with DCC (805 mg, 3.9 mmol) at 25–40 °C for 1 h. At room temperature a solution of HCl·H-Val-Gly-OBzl (1.18 g, 3.9 mmol) and *N*-methylmorpholine (0.45 mL, 3.9 mmol) in DMF (7 mL) was added. After 96 h of stirring at room temperature, the solution was cooled (0 °C), the precipitated DCU filtered off, and the solvent evaporated. The oily residue was dissolved in ethyl acetate (200 mL), washed as usual for neutral peptides, and evaporated, yielding 2.7 g of crude product. After the product was dissolved in 100 mL of boiling ethyl acetate, the solution was filtered and treated with hexane (50 mL). After cooling (room temperature, then freezer) three crops of crystalline product were obtained: 1.53 g (mp 198–200 °C), 0.41 g (mp 196–199 °C), 0.1 g (mp 199–201 °C). Overall yield was 2.04 g (74%). Starting acetyl tetrapeptide (140 mg, 7.8%) was recovered from the $NaHCO_3$ solution used in the usual acidic fractionation protocol. All fractions contained traces of dicyclohexylurea; however, they were sufficiently pure for the final condensation. Analytical sample, mp 205–207 °C; $[\alpha]^{23}_D$ +13.6° (*c* 0.5, MeOH); R_f 0.72 (CH_2Cl_2/MeOH (1:1)), R_f 0.37 (CH_2Cl_2/MeOH (10:1)). Anal. Calcd for $C_{37}H_{52}N_6O_8$ (708.84): C, 62.70; H, 7.40; N, 11.85. Found: C, 62.63; H, 7.43; N, 11.86. FAB-MS *m/z* 709 (MH^+), 731 (MNa^+), 747 (MK^+). Amino acid analysis: observed (calcd) Phe 0.89 (1), MeA 2.65 (3), Val 0.87 (1), Gly 1.00 (1). ^{13}C NMR (CD_3OD) δ 19.97, 20.13 (Val CH_3), 21.52 (Ac CH_3), 26.42, 26.67, 26.90, 27.13, 28.04, 28.22 (MeA CH_3), 32.01, 33.18 (Val, Phe βC), 43.67 (Gly αC), 58.82, 59.25, 59.36, 59.76, 62.59 (Phe, MeA, Val αC), 69.32 (Bzl CH_2), 130.09, 130.29, 130.45, 131.47, 132.55, 132.97, 138.80, 139.66 (Phe, Bzl arom. C) 172.23, 175.24, 175.42, 176.13, 177.96, 178.75, 179.48 (C=O).

This coupling was repeated several times with yields ranging 70–75%. When in a similar experiment, 1-ethyl-3-(3-(dimethylamino)propyl)-carbodiimide hydrochloride (EDAC·HCl) in the presence of HOBt hydrate was used in the condensation step, TLC pure product was obtained directly after the washing procedure; however the yield was below 9% and almost all of the unreacted starting acetyltetrapeptide was recovered from the $NaHCO_3$ solution.

Boc-MeA-MeA-OBzl (12). Boc-MeA-OH (4.06 g, 20 mmol) was activated using HOBt hydrate (3.24 g, 20 mmol) and DCC (4.12 g 20 mmol) as described for **11**. MeA-OBzl used for coupling was prepared by treating TosOH·MeA-OBzl (7.3 g, 20 mmol) in DMF (30 mL) with *N*-methylmorpholine (2.2 mL, 20 mmol). The coupling reaction was continued for 70 h and the crude oily product (4.8 g) was isolated according to the procedure for neutral peptides described for **11**. Crystallization from a mixture of ethyl acetate/light petroleum afforded the following: first crop 2.93 g, mp 119–121 °C, TLC pure; second crop 0.48 g, mp 110–117 °C, TLC revealed traces of impurities. The filtrate was evaporated and the residual oily product purified by flash chromatography (CH_2Cl_2/acetone (7:3)) yielding an additional amount (0.35 g) of TLC pure **12**. The combined fractions (3.81 g, 50.4%) were used for the next step. This preparation was repeated several times affording **12** in a yield of 49–56%: analytical sample recrystallized from ethyl acetate/light petroleum, mp 121–122 °C; R_f 0.7 (CH_2Cl_2/MeOH (10:1)). Anal. Calcd for $C_{20}H_{30}N_2O_5$ (378.46): C, 63.47; H, 7.99; N, 7.40. Found: C, 63.32, H, 7.85; N, 7.28.

Boc-Leu-MeA-MeA-OBzl (13). Boc-MeA-MeA-OBzl (**12**) (5.67 g, 15 mmol) was deprotected using a 4 N dioxane/HCl solution (30 mL) as already described for **11**, affording HCl·H-MeA-MeA-OBzl (4.12 g, 87.3%) as a glassy solid dried under oil pump vacuum. Boc-Leu-OH·H_2O (2.50 g, 10 mmol) in CH_2Cl_2 (15 mL) and DMF (15 mL) was coupled to HCl·MeA-MeA-OBzl (3.15 g, 10 mmol) in DMF (10 mL) via the mixed anhydride procedure described for **7**. Crude solid product yield was 4.3 g. After crystallization from ethyl acetate/hexane the yield was 3.6 g (73%), mp 131–133 °C. Analytical sample (crystallized from the same solvent), mp 137 °C; $[\alpha]^{23}_D$ −7.7° (*c* 1, MeOH); R_f 0.82 (butanol/AcOH/water (4:1:1)), R_f 0.78 (CH_2Cl_2/MeOH (10:1)), R_f 0.1 (CH_2Cl_2/acetone (15:1)); FAB-MS *m/z* 492 (MH^+), 514 (MNa^+). Anal. Calcd for $C_{26}H_{41}N_3O_6$ (491.6): C, 63.52; N, 8.41; N, 8.55. Found: C, 63.30; H, 8.16; N, 8.45.

Boc-Leu-MeA-MeA-OH (14). Boc-Leu-MeA-MeA-OBzl (**13**) (3.44 g, 7 mmol) in methanol (35 mL) was hydrogenated in the presence of

(43) Wünsch, E.; Wendlberger, G. *Chem. Ber.* **1967**, *100*, 160–172.

10% Pd/C (500 mg) at a pressure of 4–5 kg/cm^2 on a Parr apparatus for 10 h. After evaporation of the filtered solution, the residual solid was washed several times with hexane: yield 2.74 g (98%); mp 211–214 °C (dec), raised by crystallization from ethyl acetate/hexane to 215–216 °C; $[\alpha]^{23}_D$ −17.9 (*c* 1, MeOH); FAB-MS *m/z* 402 (MH^+), 424 (MNa^+), 440 (MK^+). Anal. Calcd for $C_{19}H_{35}N_3O_6$ (401.49): C, 56.93; H, 8.78; N, 10.46. Found: C, 56.61; H, 8.62; N, 10.23.

Boc-Leu-MeA-MeA-Hyp-OBzl (15). Boc-Leu-MeA-MeA-OH (**14**) (2 g, 5 mmol) and HOBt hydrate (765 mg, 5 mmol) were activated with DCC (1.02 g, 5 mmol) and coupled to HCl·Hyp-OBzl (1.29 g, 5 mmol) as described for **11**. The coupling reaction was continued for 48 h. After evaporation, the oily residue was dissolved in butanol and washed as usual for neutral peptides. TLC of the crude oily product revealed a complex mixture which required extensive purification by flash chromatography using CH_2Cl_2/MeOH (38:1) followed by CH_2Cl_2/MeOH (19:1). Yield 2.03 g (67%); mp 157–159 °C (dec); $[\alpha]^{23}_D$ −51.8° (*c* 0.5, MeOH); R_f 0.49 (CH_2Cl_2/MeOH (8:1)), R_f 0.35 (CH_2Cl_2/MeOH (18:1)); FAB-MS *m/z* 605 (MH^+), 627 (NMa^+). Anal. Calcd for $C_{31}H_{48}N_4O_8$ (604.72): C, 61.56; H, 8.00; N, 9.26. Found: C, 61.58; H, 7.84, N, 9.08. ^{13}C NMR (DMSO-d_6) δ 21.60, 22.92 (Leu CH_3), 24.09, 24.19, 24.52, 24.68, 25.04 (MeA CH_3, Leu γC), 28.17 (Boc CH_3), 35.65 (Hyp βC), 39.96 (Leu βC), 53.32 (Leu αC), 54.56, 55.57, 56.02 (MeA αC, Hyp δC), 59.17 (Hyp αC), 65.55 (Bzl CH_2), 69.17 (Hyp γC), 78.18 (Boc C), 127.75, 127.94, 128.35, 136.11 (Bzl arom. C), 155.73 (Boc C=O), 171.59, 172.02, 172.34, 173.00 (C=O).

Boc-Leu-MeA-MeA-Hyp-OH (16). Boc-Leu-MeA-MeA-Hyp-OBzl (**15**) (1.81 g, 3 mmol) was hydrogenated as described for **14**, yielding 1.54 g (100%) of **16** as a glassy hygroscopic solid decomposing at 135–160 °C: HPLC purity 95%, t_R 7.21 min (gradient 30–60% B in 25 min); $[\alpha]^{23}_D$ −76.7° (*c* 1, MeOH). FAB-MS *m/z* 515 (MH^+). Calcd for $C_{24}H_{42}N_4O_8$ 514. Amino acid analysis: observed (calcd) Leu 0.77 (1), MeA 1.98 (2), Hyp 1.00 (1).

Boc-*R*-EtA-OBzl (17) and Its *S*-Enantiomer (17a). To Boc-*R*-EtA-OH[10] (10.86 g, 50 mmol) in DMF (40 mL) was added Et_3N (7 mL, 50 mmol) followed by benzyl bromide (9.35 g, 55 mmol). Stirring was continued for 14 h. After filtration, evaporation, and washing as usual for neutral Boc-derivatives, oily **17** was obtained which crystallized slowly after the addition of a few drops of methanol: yield 13.51 g (88%); mp 67–69 °C; $[\alpha]^{23}_D$ +8.9° (*c* 1, EtOH), $[\alpha]^{23}_D$ +10.6° (*c* 1, MeOH). Anal. Calcd for $C_{17}H_{25}O_4N$ (307.37): C, 66.42; H, 8.19; N, 4.56. Found: C, 66.66; H, 8.38; N, 4.51. ^{13}C NMR ($CDCl_3$) δ 8.30 (EtA γCH_3), 22.94 (EtA βCH_3), 28.40 (Boc CH_3), 30.30 (EtA βCH_2), 60.01 (EtA αC), 67.10 (Bzl CH_2), 79.50 (Boc quat. C), 128.09, 128.18, 128.42, 135.58 (Bzl arom. C), 154.20 (Boc C=O), 174.16 (EtA C=O). Boc-*S*-EtA-OBzl (**17a**) was prepared in an analogous manner: yield 82%; mp 66.5–67.5 °C; $[\alpha]^{23}_D$ −10.6° (*c* 1, MeOH); R_f 0.48 (hexane/ethyl acetate (4:1)), R_f 0.79 (CH_2Cl_2/acetone (30:1)).

HCl·H-*R*-EtA-OBzl (18) and Its *S*-Enantiomer (18a). Boc-*R*-EtA-OBzl (**17**) (9.22 g, 30 mmol) was deprotected as described in the prepartion of **11** and diluted with diethyl ether. Crystallization was initiated by the addition of small amounts of solid obtained by evaporation of a few drops of the mother solution: yield 5.93 g (94%); mp 139–140 °C; $[\alpha]^{23}_D$ +3.9° (*c* 1, EtOH). Anal. Calcd for $C_{12}H_{18}NO_2Cl$ (243.60): C, 59.13; H, 7.42; N, 5.74. Found: C, 58.95; H, 7.31; N, 5.52. ^{13}C NMR ($CDCl_3$) δ 8.35 (EtA γC), 22.27 (EtA βCH_3), 30.93 (EtA βCH_2), 61.39 (EtA αC), 67.97 (Bzl CH_2), 128.26, 128.45, 128.51, 134.69 (Bzl arom. C), 170.42 (EtA C=O). HCl·H-*S*-EtA-OBzl (**18a**): yield 92%; mp 138–139 °C: $[\alpha]^{23}_D$ −3.6° (*c* 1, MeOH); R_f 0.8 ($CHCl_3$/MeOH/AcOH (40:3:1)).

Boc-Gln-*R*-EtA-OBzl (19) and Its *S*-EtA Epimer (19a). Boc-Gln-OH (4.92 g, 20 mmol) in DMF/CH_2Cl_2 (20 mL, 1:1) was coupled to HCl·H-*R*-EtA-OBzl (**18**) in DMF (5 mL) via the mixed anhydride procedure described for **10**. Stirring was continued for 12 h. The crude oily product (9 g) was purified by flash chromatography using CH_2Cl_2/acetone (3:1): yield 7.66 g (88%); mp 96–102 °C: $[\alpha]^{23}_D$ −17.9° (*c* 1, MeOH); FAB-MS *m/z* 436 (MH^+). Anal. Calcd for $C_{22}H_{33}N_3O_6$ (435.50): C, 60.67; H, 7.63; N, 9.64. Found: C, 60.72; H, 7.63; N, 9.60. ^{13}C NMR ($CDCl_3$) δ 8.05 (EtA γC), 22.20 (EtA βCH_3), 28.26 (Boc CH_3), 28.94, 29.61 (EtA βCH_2, Gln βC), 31.81 (Gln γC), 53.70 (Gln αC), 60.28 (EtA αC), 67.18 (Bzl CH_2), 79.99 (Boc C), 128.22, 128.31, 128.53, 135.59 (Bzl arom. C), 155.93 (Boc C=O), 170.75, 173.86, 175.34 (C=O). Boc-Gln-*S*-EtA-OBzl (**19a**) was prepared in an analogous manner; however, it could not be crystallized: yield 73%; $[\alpha]^{23}_D$ −22.3° (*c* 1, MeOH); R_f 0.23 (CH_2Cl_2/acetone (2:1)); R_f 0.61 (CH_2Cl_2/acetone (1:1)).

Boc-Gln-*R*-EtA-OH (20) and Its *S*-EtA Epimer (20a). Boc-Gln-*R*-EtA-OBzl (**19**) (6.53 g, 15 mmol) in methanol was hydrogenated in the presence of 10% Pd/C (750 mg) at a pressure of 3–4 kg/cm^2 as described for **14**. The oily product crystallized from ethyl acetate/hexane: yield 4.45 g (86%); mp 165–167 °C (dec); $[\alpha]^{23}_D$ −29.1° (*c* 1, MeOH); FAB-MS *m/z* 346 (MH^+). Anal. Calcd for $C_{15}H_{27}O_6N_3 \cdot {}^1/_4H_2O$ (349.86): C, 51.48; H, 7.94; N, 12.00. Found: C, 51.79; H, 7.97; N, 12.03. ^{13}C NMR (DMSO-d_6) δ 8.07 (EtA γCH_3), 22.08 (EtA βCH_3), 27.69 (Gln βC or EtA βCH_2), 28.22 (Boc CH_3), 28.79 (Gln βC or EtA βCH_2), 31.56 (Gln γC), 54.25 (Gln αC), 58.78 (EtA αC), 78.15 (Boc quat. C), 155.13 (Boc C=O), 170.79, 173.70, 175.06 (C=O). Boc-Gln-*S*-EtA-OH (**20a**): yield 90%; mp 200–201 °C (dec); $[\alpha]^{23}_D$ −18.5° (*c* 1, MeOH); R_f 0.80 (MeOH/ethyl acetate/AcOH (15:5:1)); R_f 0.18 ($CHCl_3$/MeOH/AcOH (85:10:5)).

Boc-Gln-*R*-EtA-Hyp-OBzl (21) and Its *S*-EtA Epimer (21a). Boc-Gln-*R*-EtA-OH (**20**) (4.14 g, 12 mmol) and HOBt hydrate (1.84 g, 12 mmol), were activated with DCC (2.25 g, 12 mmol) and coupled to HCl·Hyp-OBzl (3.09 g, 12 mmol) as described for **11**. Stirring was continued for 48 h. After evaporation the oily residue was taken into butanol and washed as usual for neutral peptides. The crude oily product was purified by flash chromatography using 1:1 CH_2Cl_2/acetone: yield 4.53 g (69%); mp 167–170 °C (dec); $[\alpha]^{23}_D$ −70.4° (*c* 1, MeOH); R_f 0.43 (CH_2Cl_2/MeOH (10:1)), R_f 0.34 (ethyl acetate/MeOH (7:1)); FAB-MS *m/z* 549 (MH^+), 571 (MNa^+). Analytical sample crystallized from CH_3OH/H_2O had mp 175–177 °C. Anal. Calcd for $C_{27}H_{40}N_4O_8$ (548.62): C, 59.11; H, 7.35; N, 10.21. Found: C, 58.79; H, 7.40; N, 10.15. Amino acid analysis: observed (calcd) Glx 1.00 (1), EtA 0.71 (1), Hyp 0.96 (1). ^{13}C NMR ($CDCl_3$) δ 7.63 (EtA γC), 21.82 (EtA βCH_3) 27.73 (Gln βC or EtA βCH_2), 28.37 (Boc CH_3), 29.66 (EtA βCH_2 or Gln βC), 31.79 (Gln γC), 35.94 (Hyp βC), 53.97 (Gln αC), 55.48 (Hyp δC), 59.75, 60.17 (Hyp αC, EtA αC), 66.66 (Bzl CH_2), 70.56 (Hyp γC), 80.24 (Boc quat. C), 128.16, 128.21, 128.49, 135.67 (Bzl arom. C), 156.60 (Boc C=O), 171.25, 171.38, 172.67, 175.84 (C=O). For crude Boc-Gln-*S*-EtA-Hyp-OBzl (**21a**) prepared in an analogous manner, TLC revealed a complex mixture. The most efficient purification involved repeated flash chromatography using 10:1 CH_2Cl_2/MeOH and then 15:1:0.1 CH_2Cl_2/MeOH/AcOH: yield 62% of a white amorphous product; $[\alpha]^{23}_D$ −56.3° (*c* 1.07, MeOH); R_f 0.28 and 0.19, respectively, for the solvent systems given above.

Boc-Gln-*R*-EtA-Hyp-OH (22) and Its *S*-EtA Epimer (22a). Hydrogenation of Boc-Gln-*R*-EtA-Hyp-OBzl (**21**) (3.29 g, 6 mmol) was performed at a pressure of 3–4 kg/cm^2 as described for **14**. The hygroscopic product was washed with hexane and dried over P_2O_5 under vacuum: yield 2.66 g (97%); mp 163–165 °C (dec); HPLC purity 99%, t_R 9.70 min (gradient 10–30% B in 25 min); $[\alpha]^{23}_D$ −60.1° (*c* 1, MeOH). FAB-MS *m/z* 459 (MH^+), 481 (MNa^+). Anal. Calcd for $C_{20}H_{34}N_4O_8$ (458.5): C, 52.37; H, 7.47; N, 12.21. Found: C, 51.95; H, 7.50; N, 11.91. Boc-Gln-*S*-EtA-Hyp-OH (**22a**), prepared in an analogous manner (yield 100%), was used without purification.

Boc-Gln-*R*-EtA-Hyp-MeA-Phol (25) and Its *S*-EtA Epimer (25a). Boc-Gln-*R*-EtA-Hyp-OH (**22**) (2.29 g, 5 mmol) and HOBt hydrate (765 mg, 5 mmol) were activated with DCC (1.03 g, 5 mmol) in DMF (10 mL) solution at room temperature and then reacted for 48 h with MeA-Phol (1.19 g, 5 mmol), freshly prepared from **23** by treatment with 4 N HCl in dioxane. The crude product (3.31 g, glassy solid) was isolated as a neutral fraction by the usual partition procedure using butanol (80 mL) as an organic solvent. TLC revealed a complex mixture which was purified twice by flash chromatography using 5:1 CH_2Cl_2/MeOH: yield 1.7 g (50.5%); hygroscopic. The preparation was repeated several times on a smaller scale with yields ranging 50–55%: decomposes at 140–160 °C; HPLC purity 96%, t_R 17.5 min (gradient 20–40% B in 25 min), t_R 12.16 min (isocratic, 27% B); $[\alpha]^{23}_D$ −4.0° (*c* 1, MeOH); FAB-MS *m/z* 677 (MH^+), 699 (MNa^+). Calcd for $C_{33}H_{52}N_6O_9$ (676). Amino acid analysis: observed (calcd) Glx 0.93 (1), EtA 0.97 (1), Hyp 1.00 (1), MeA 1.00 (1), Phol 1.00 (1). ^{13}C NMR (DMSO-d_6) δ 7.38 (EtA γCH_3), 20.24 (EtA βCH_3), 23.70, 26.77 (MeA CH_3), 27.12 (EtA βCH_2 or Gln βC), 28.11 (Boc CH_3), 28.44 (Gln βC or EtA βCH_2), 31.78 (Gln γC), 36.34, 36.55 (Hyp βC, Phol βC), 52.48 (Phol αC), 54.49 (Gln αC), 56.10, 56.24 (MeA αC, Hyp δC), 58.33, 61.73 (EtA αC, Hyp αC), 63.18 (Phol CH_2OH), 69.06 (Hyp γC), 78.34 (Boc quat. C), 125.69, 127.86, 129.10, 139.21 (Phol arom. C), 155.48 (Boc C=O), 171.51, 172.25, 173.52, 173.62, 173.70 (C=O).

Boc-Gln-*S*-EtA-Hyp-MeA-Phol (**25a**) was prepared in an analogous manner except that the solvent system CH_2Cl_2/MeOH/AcOH (70:10:1) was used in the second flash chromatography: yield 50%; $[\alpha]^{23}_D$ −19.1° (*c* 0.55, MeOH); HPLC purity 95.5%, t_R 18.91 min (gradient 20–40% B in 25 min); R_f 0.40 CH_2Cl_2/MeOH (5:1); R_f 0.22 CH_2Cl_2/MeOH/AcOH (70:10:1); FAB-MS *m/z* 677 (MH^+). Calcd for $C_{33}H_{52}N_6O_5$ 676. Amino acid analysis: observed (calcd) Glx 0.88 (1), EtA 1.09 (1), Hyp 0.94 (1), MeA 0.95 (1), Phol 1.00 (1).

Boc-Gln-*R*-EtA-Hyp-Ala-Phol (26) and Its *S*-EtA Epimer (26a). The reaction was performed (16 h) on a 3 mmol scale as described for **25** using equimolar amounts of **22** and Ala-Phol freshly prepared from **24** by hydrogenation with 10% Pd/C in methanol. No washing was needed to isolate the product. When DMF was evaporated, the residual oil was

dissolved in ethyl acetate (20 mL). After several hours crystalline **26** precipitated, yield 1.21 g (61%). In repeated experiments the best yield was 80%: mp 237–238 °C; HPLC purity 100%, t_R 15.55 min (gradient 20–40% B in 25 min), t_R 9.06 min (isocratic 27% B); $[\alpha]^{23}_D$ −9.2° (*c* 1, MeOH); R_f 0.58 (butanol/AcOH/water (4:1:1)), R_f 0.52 ($CHCl_3$/MeOH (4:1)). FAB-MS *m/z* 663 (MH^+). Anal. Calcd for $C_{32}H_{50}N_6O_9$ (662.78): C, 57.98; H, 7.60; N, 12.68. Found: C, 57.71; H, 7.71; N, 12.57. Amino acid analysis: observed (calcd) Glx 0.98 (1), EtA 1.02 (1), Hyp 0.98 (1), Ala 1.00 (1), Phol 1.02 (1). ^{13}C NMR (DMSO-d_6) δ 7.27 (EtA γCH_3), 17.00 (Ala CH_3), 19.87 (EtA βCH_3), 27.05 (Gln βC or EtA βCH_2, 1 missing), 28.13 (Boc CH_3), 31.73 (Gln γC), 36.53, 36.82 (Hyp βC, Phol βC), 49.30 (Ala αC), 52.33 (Phol αC), 54.37 (Gln αC), 56.22 (Hyp δC), 58.26, 60.88 (EtA αC, Hyp αC), 62.97 (Phol CH_2OH), 68.95 (Hyp γC), 78.32 (Boc quat. C), 125.52, 127.87, 129.14, 138.80 (Phol arom. C), 155.23 (Boc C═O), 171.12, 171.53, 172.50, 173.42, 173.60 (C═O).

Boc-Gln-*S*-EtA-Hyp-Ala-Phol (**26a**) was prepared in an analogous manner except that crude product was purified twice by flash chromatography using 7:1 CH_2Cl_2/MeOH followed by repeated purification using 3:1 ethyl acetate/MeOH: yield 60%, white amorphous product; HPLC purity 92%, t_R 17.28 min (gradient 20–40% B in 25 min); R_f 0.31 CH_2Cl_2/MeOH (5:1); R_f 0.25 ethyl acetate/MeOH (3:1); $[\alpha]^{23}_D$ −25.9° (*c* 0.3, MeOH); FAB-MS *m/z* 663 (MH^+), calcd for $C_{32}H_{50}N_6O_9$ 662. Amino acid analysis: observed (calcd) Glx 0.94 (1), EtA 1.06 (1), Hyp 0.92 (1), Ala 1.00 (1), Phol 0.96 (1).

Boc-Leu-MeA-MeA-Hyp-Gln-*R*-EtA-Hyp-MeA-Phol (27) and Its *S*-EtA Epimer (27a). Boc-Leu-MeA-MeA-Hyp-OH (**16**) (360 mg, 0.7 mmol) and HOBt hydrate (108 mg, 0.7 mmol) were activated with DCC (145 mg, 7 mmol) in DMF (2 mL) at room temperature for 30 min. Boc-Gln-*R*-EtA-Hyp-MeA-Phol (**25**) (473 mg, 0.7 mmol) was deprotected by treatment with CF_3COOH/CH_2Cl_2 (1:1) (7 mL). TLC revealed that removal of the Boc group was complete after 15 min. Dimethyl ether was added, the precipitated solid was filtered off, washed with dimethyl ether, and dried over KOH under oil pump vacuum. The resulting 478 mg (99%) of CF_3COOH-Gln-*R*-EtA-Hyp-MeA-Phol was treated with *N*-methylmorpholine (0.08 mL, 0.7 mmol) in DMF (2 mL) and coupled (14 h) to activated **16** described above. The crude product (615 mg) was isolated as a neutral fraction by the usual partition procedure using butanol (30 mL) as an organic solvent. TLC revealed a complex mixture which was purified twice by flash chromatography using 7:1 CH_2Cl_2/MeOH followed by repeated purification using 5:1 CH_2Cl_2/MeOH: yield 315 mg (42%); decomposes 185–190 °C; HPLC purity 94%, t_R 14.56 min (gradient 30–60% B in 25 min), t_R 9.36 min (isocratic 38% B); R_f 0.75 (BuOH/AcOH/ethyl acetate/H_2O (1:1:1:1)); $[\alpha]^{23}_D$ +9.0° (*c* 0.5, MeOH); FAB-MS *m/z* 1073 (MH^+), 1095 (MNa^+). Calcd for $C_{52}H_{84}N_{10}O_{14}$, 1072. Amino acid analysis: observed (calcd) Leu 0.98 (1), MeA 2.71 (3), Hyp 1.84 (2), Glx 1.05 (1), EtA 0.92 (1), Phol 1.00 (1). ^{13}C NMR (DMSO-d_6) δ 7.07 (EtA γCH_3), 19.85 (EtA βCH_3), 21.68, 22.93 (Leu CH_3), 23.19, 23.44, 24.14, 24.70, 25.45, 25.62, 26.72, 26.88 (MeA CH_3, Leu γC, 1 extra), 28.03 (Gln βC or EtA βCH_2), 28.23 (Boc CH_3), 28.99 (EtA βCH_2 or Gln βC), 31.76 (Gln γC), 36.56, 36.60, 36.66 (Hyp βC, Phol βC), 39.91 (Leu βC), 52.42, 52.68 (Phol αC, Gln αC), 53.36 (Leu αC), 56.02, 56.03, 56.08, 56.21, 56.84 (Hyp δC, MeA αC), 58.28, 60.97, 61.88 (Hyp αC, EtA αC), 63.29 (Phol CH_2OH), 69.00, 69.03 (Hyp γC), 78.28 (Boc quat. C), 125.74, 127.85, 129.08, 139.23 (Phol arom. C), 155.96 (Boc C═O), 171.48, 171.85, 172.05, 172.37, 173.28, 173.53, 173.73, 173.80, 175.89 (C═O).

Boc-Leu-MeA-MeA-Hyp-Gln-*S*-EtA-Hyp-MeA-Phol (**27a**) was prepared in an analogous manner except that the solvent system CH_2Cl_2/MeOH/AcOH (70:10:1) was used for flash chromatography: yield 50%; white amorphous product; HPLC purity 98%, t_R 13.18 min (gradient 30–60% B in 25 min), R_f 0.17 (CH_2Cl_2/MeOH/AcOH (70:10:1)); $[\alpha]^{23}_D$ +1.19° (*c* 0.5, MeOH); FAB-MS *m/z* 1073 (MH^+), 1095 (MNa^+). Calcd for $C_{52}H_{84}N_{10}O_{14}$, 1072. Amino acid analysis: observed (calcd) Leu 0.99 (1), MeA 2.74 (3), Hyp 1.88 (2), Glx 0.91 (1), EtA 1.06 (1), Phol 1.00 (1). ^{13}C NMR (DMSO-d_6) δ 8.17 (EtA γCH_3), 21.55, 22.68, 22.97, 23.35, 23.52, 24.15, 24.32, 25.43, 25.80, 26.25, 26.71 (Leu γC, Leu CH_3, MeA CH_3, EtA βCH_3, 1 extra), 28.22 (Boc CH_3), 28.77, 28.98, 31.64 (Gln βC, Gln γC, EtA βCH_2), 36.55, 36.69 (Leu, Hyp, Phol βC, 2 missing), 52.25, 52.59, 53.30 (Leu, Gln, Phol αC), 55.99, 56.05, 56.15, 56.20 (MeA αC, Hyp δC, 1 missing), 59.51, 61.06, 61.82 (Hyp, EtA αC), 63.27 (Phol CH_2OH), 69.00, 69.02 (Hyp γC), 78.17 (Boc quat. C), 125.71, 127.83, 129.17, 139.29 (Phol arom. C), 155.78 (Boc C═O), 171.67, 171.80, 171.85, 172.45, 173.38, 173.60, 173.68, 175.82 (C═O, 1 missing).

Boc-Leu-MeA-MeA-Hyp-Gln-*R*-EtA-Hyp-Ala-Phol (28) and Its *S*-EtA Epimer (28a). The procedure described for **27** was used. From **16** (257 mg, 0.5 mmol) and CF_3COOH-Gln-*R*-EtA-Hyp-Ala-Phol (338 mg, 0.5 mmol), 504 mg of crude **28** was obtained after the usual partitioning procedure using butanol as an organic phase. It was purified by flash chromatography using 5:1 CH_2Cl_2/MeOH followed by repeated purification with 19:1 CH_2Cl_2/MeOH: yield 322 mg (61%); HPLC purity 95%, t_R 13.46 min (gradient 30–60% B in 25 min), t_R 7.38 min (isocratic 38% B); R_f 0.57 (BuOH/AcOH/ethyl acetate/H_2O (1:1:1:1)). $[\alpha]^{23}_D$ +11.7 (*c* 1, MeOH). Amino acid analysis: observed (calcd) Leu 0.96 (1), MeA 1.78 (2), Hyp 1.86 (2), Glx 0.94 (1), EtA 1.03 (1), Ala 1.00 (1), Phol 1.03 (1); FAB-MS *m/z* 1059 (MH^+), 1081 (MNa^+). Calcd for $C_{51}H_{82}N_{10}O_{14}$, 1058. ^{13}C NMR (CD_3OD) 7.89 (EtA γCH_3), 17.25 (Ala CH_3), 20.36, 20.41, 21.99 (Leu CH_3, EtA βCH_3), 23.41, 24.09, 25.45, 25.83, 26.23, 27.00 (MeA CH_3 and Leu γC, 1 extra signal), 28.32 (Gln βC or EtA βCH_2), 28.83 (Boc CH_3), 29.72 (EtA βCH_2 or Gln βC), 31.13 (Gln γC), 33.18 (extra), 37.80, 37.96, 38.10 (Hyp βC, Phol βC), 41.46 (Leu βC), 51.55 (Ala αC), 54.30, 54.82, 54.92, 55.54, 57.80, 57.98, 58.12, 58.48 (Phol, MeA, Gln, Leu αC, Hyp δC, 1 extra), 60.33, 60.42, 62.83, 63.01 (Hyp, EtA αC, 1 extra), 63.71 (Phol CH_2OH), 71.22 (Hyp γC, overlapped), 80.91 (Boc quat. C), 127.26, 129.27, 130.49, 139.65 (Phol arom. C), 158.41 (Boc C═O), 174.06, 174.14, 174.96, 175.17, 175.21, 175.29, 175.42, 175.88, 176.49, 177.00, 177.66 (C═O, 2 extra).

Boc-Leu-MeA-MeA-Hyp-Gln-*S*-EtA-Hyp-Ala-Phol (**28a**) was prepared in an analogous manner except that CH_2Cl_2/MeOH/AcOH (50:10:0.5) was used for flash chromatography: HPLC purity 96%, t_R 12.32 min (gradient 30–60% B in 25 min); R_f 0.19 (CH_2Cl_2/MeOH/AcOH (50:10:0.5)); $[\alpha]^{23}_D$ +1.70 (*c* 0.5, MeOH); FAB-MS *m/z* 1059 (MH^+), 1081 (MNa^+), calcd for $C_{51}H_{82}N_{10}O_{14}$, 1058. Amino acid analysis: observed (calcd) Leu 0.99 (1), MeA 1.84 (2), Hyp 1.91 (2), Glx 0.89 (1), EtA 1.10 (1), Ala 1.00 (1), Phol 1.02 (1); ^{13}C NMR (DMSO-d_6) δ 8.16 (EtA γCH_3), 16.87 (Ala CH_3), 21.51, 22.88, 22.97, 23.30, 24.15, 24.29, 24.80, 25.45, 25.86, 26.21 (Leu γC, Leu CH_3, MeA CH_3, EtA βCH_3, 2 extra), 28.22 (Boc CH_3), 28.68, 31.29, 31.60 (Gln βC, Gln γC, EtA βCH_2), 36.54, 36.72, 36.99 (Hyp βC, Phol βC), 40.06 (Leu βC), 49.22, 49.27 (Ala αC, 1 extra), 52.26, 52.37, 53.31 (Leu, Gln, Phol αC), 55.97, 56.03, 56.10 (MeA αC, Hyp δC, 1 missing), 59.66, 61.04, 61.08 (Hyp, EtA αC), 63.18 (Phol CH_2OH), 68.83, 69.00 (Hyp γC), 78.12 (Boc quat. C), 125.70, 127.85, 129.33, 138.99 (Phol arom. C), 155.76 (Boc C═O), 171.45, 171.67, 171.89, 171.95, 172.37, 172.52, 173.31, 173.71, 175.55, 175.91 (C═O, 1 extra).

Emerimicin IV (29) and [*S*-EtA[12]]Emerimicin IV (29a). Boc-Leu-MeA-MeA-Hyp-Gln-*R*-EtA-Hyp-MeA-Phol (**27**) (214 mg, 0.2 mmol) was deprotected by treating with TFA/CH_2Cl_2 (5 mL, 1:1) in the presence of anisole (0.3 mL). Removal of the Boc group was complete after 18 min (R_f of **27** 0.75, R_f of deprotected **27** 0.56, BuOH/AcOH/ethyl acetate/H_2O (1:1:1:1)). Diethyl ether was added to precipitate the solid product which was filtered off, washed with diethyl ether, and dried over KOH under oil pump vacuum to produce the trifluoroacetic acid salt (195 mg, 90%) of the C-terminal nonapeptide of emerimicin IV. This salt (87 mg, 0.08 mmol) was added to a stirred suspension of Ac-Phe-MeA-MeA-MeA-Val-Gly-OBzl (**11**) (85 mg, 0.12 mmol) in methanol (300 μL) containing phosphate buffer (700 μL, pH 9) and the pH was adjusted to 9 using 3 N NaOH. Papain (5 mg, Sigma No. P-4762) and dithioerythritol (2 mg) were added, and the suspension was stirred at room temperature. After 20 min the suspension became more dense due to product precipitation. MeOH (100 μL) and phosphate buffer (400 μL) were added to make stirring possible. The reaction continued for 1 h (HPLC revealed no presence of **11**) and the solid was filtered off and washed with water, phosphate buffer, 1 N $NaHSO_4$ and again with water. The resulting 109 mg of emerimicin IV (**29**) had a purity of 88% by HPLC, corresponding to a yield of 76%. This was purified by preparative HPLC, t_R 11.5 min (gradient 50–65% B in 45 min). From 55 mg, 45 mg of material of purity >99% resulted: mp 243–245 °C; $[\alpha]^{23}_D$ +16.8° (*c* 0.25, MeOH). FAB-MS *m/z* 1573 (MH^+), 1595 (MNa^+), 1611 (MK^+), calcd for $C_{77}H_{120}N_{16}O_{19}$ 1572. Amino acid analysis: observed (calcd) Phe 1.01 (1), MeA 5.90 (6), Val 0.94 (1), Gly 1.00 (1), Leu 1.00 (1), Hyp 1.89 (2), Glx 1.10 (1), EtA 0.94 (1), Phol 1.03 (1). ^{13}C NMR (DMSO-d_6) δ 7.00 (EtA γCH_3), 18.44, 19.13, 19.81 (Val CH_3, EtA βCH_3), 21.74, 22.31, 22.59, 23.19, 23.40, 23.50, 23.76, 24.05, 24.32, 24.64, 24.95, 25.23, 25.49, 26.08, 26.68, 26.83, 28.03, 28.85, 31.60 (Ac CH_3, MeA CH_3, Val βC, Leu γC, Leu CH_3, Gln βC, Gln γC, EtA βCH_2, 1 missing), 36.29, 36.54, 36.58, 36.79 (Phe, Leu, Hyp, Phol βC, 1 missing), 42.90 (Gly αC), 52.38, 52.59, 52.63, 54.95, 55.82, 55.85, 55.93, 55.98, 56.04, 56.06, 56.24, 56.77, 58.24, 59.78, 60.93, 61.85 (Phe, MeA, Val, Leu, Hyp, Gln, EtA, Phol αC, and Hyp δC), 63.26 (Phol CH_2OH), 68.99 (Hyp γC, overlapped), 125.68, 126.32, 127.79, 128.05, 129.04, 129.09, 137.47, 139.17 (Phe, Phol arom. C), 169.92, 170.26, 171.46, 171.65, 171.88, 172.08, 172.18, 172.37, 173.13, 173.62, 173.68, 173.76, 174.83, 174.93, 175.60, 175.91 (C═O).

[*S*-EtA[12]]Emerimicin IV (**29a**) was obtained in an analogous manner from deprotected **27a** (67 mg, 0.06 mmol) and **11** (64 mg, 0.09 mmol). The resulting 88 mg of **29a** had a purity of 88% (by HPLC) corresponding to a yield of 80%. This was purified by preparative HPLC, t_R

10.80 min (gradient 50–65% B in 45 min): purity >99%, $[\alpha]^{23}_D$ +6.8° (*c* 1, MeOH). FAB-MS *m/z* 1573 (MH^+), 1595 (MNa^+), 1611 (MK^+), calcd for $C_{77}H_{120}N_{16}O_{19}$ 1572. Amino acid analysis: observed (calcd) Phe 0.98 (1), MeA 5.75 (6), Val 0.96 (1), Gly 1.06 (1), Leu 1.00 (1), Hyp 1.88 (2), Glx 0.96 (1), EtA 0.93 (1), Phol 1.02 (1). ^{13}C NMR (DMSO-d_6) δ 8.17 (EtA γCH_3), 18.40, 19.18 (Val CH_3), 21.77, 22.34, 22.64, 23.29, 23.51, 23.80, 24.07, 24.33, 24.68, 24.78, 25.26, 25.33, 25.51, 26.12, 26.27, 26.74, 28.73, 28.87, 28.97, 31.30, 31.52 (Ac CH_3, Leu γC, Leu CH_3, MeA CH_3, EtA βCH_3, Val βC, Gln βC, Gln γC, EtA βCH_2), 36.32, 36.56, 36.70 (Phe, Leu, Hyp, Phol βC, 2 missing), 42.84 (Gly αC), 52.16, 52.52, 52.62, 55.00, 55.84, 55.86, 55.96, 56.06, 56.18, 56.19, 56.25 (Phe αC, MeA αC, Leu αC, Hyp δC, Gln αC, Phol αC, 1 missing) 59.50, 59.67, 61.06, 61.84 (Val, Hyp, EtA αC), 63.31 (Phol CH_2OH), 69.02, 69.08 (Hyp γC), 125.72, 126.36, 127.82, 128.08, 129.13, 129.20, 137.51, 139.28 (Phe, Phol arom. C), 169.88, 170.28, 171.68, 171.70, 171.84, 171.86, 171.93, 172.22, 172.33, 173.25, 173.66, 173.68, 174.86, 174.96, 175.57, 175.90 (C═O).

Emerimicin III (30) and [*S*-EtA^{12}]Emerimicin III (30a). Emerimicin III (**30**) was obtained from **11** and deprotected **28** in a manner similar to that described for emerimicin IV (**29**). The purity of the crude product was 87%, corresponding to a yield of 64%. HPLC purification under the same conditions as above afforded 38 mg (purity >99%) from 45 mg of crude product: t_R 8.1 min (gradient 50–65% B in 45 min); mp 234–236 °C; $[\alpha]^{23}_D$ +14.8° (*c* 0.5, MeOH). FAB-MS *m/z* 1559 (MH^+), 1581 (MNa^+), 1598 (MK^+), calcd for $C_{76}H_{118}N_{16}O_{19}$ 1558. Amino acid analysis: observed (calcd) Phe 1.01 (1), MeA 5.05 (5), Val 0.92 (1), Gly 1.07 (1), Leu 1.00 (1), Hyp 1.97 (2), Ala 1.04 (1), Glx 1.11 (1), EtA 0.98 (1), Phol 0.99 (1). ^{13}C NMR (DMSO-d_6) δ 6.82 (EtA γCH_3), 16.81 (Ala CH_3), 18.43, 19.12, 19.46 (Val CH_3, EtA βCH_3), 21.72, 22.30, 22.57, 23.15, 23.47, 23.73, 24.04, 24.31, 24.62, 24.94, 25.20, 25.23, 25.47, 26.07, 26.63, 27.97, 28.83, 31.63 (Ac CH_3, Leu γC, Leu CH_3, MeA CH_3, Gln βC, EtA βCH_2, Val βC, Gln γC), 36.28, 36.76, 36.77, 36.85 (Phe, Hyp, Phol, Leu βC, 1 missing), 42.90 (Gly αC), 49.26 (Ala αC), 52.10, 52.64, 52.78, 54.94, 55.81, 55.83, 55.92, 55.98, 56.05, 56.23, 56.63 (Phe, MeA, Leu, Gln, Phol αC and Hyp δC), 58.19, 59.78, 60.96, 61.03 (Val, Hyp, EtA αC), 63.14 (Phol CH_2OH), 68.90, 68.97 (Hyp γC), 125.65, 126.31, 127.80, 128.04, 129.08, 129.21, 137.46, 138.90 (Phe, Phol arom. C), 169.92, 170.24, 171.31, 171.64, 171.93, 172.17, 172.36, 172.59, 173.06, 173.68, 173.94, 174.83, 174.93, 175.60, 175.92 (C═O, 1 missing).

[*S*-EtA^{12}]Emerimicin III (**30a**) was obtained from **11** and deprotected **28a** in a manner analogous to that described for emerimicin IV (**29**). The purity of crude precipitated **30a** was 89% corresponding to a yield of 62%. *HPLC purification under the same conditions as above afforded* **30a** of purity >99%: t_R 7.4 min (gradient 50–65% B in 45 min); $[\alpha]^{23}_D$ +7.2° (*c* 1, MeOH). FAB-MS *m/z* 1559 (MH^+), 1581 (MNa^+), calcd for $C_{76}H_{118}N_{16}O_{19}$ 1558. Amino acid analysis: observed (calcd) Phe 0.96 (1), MeA 4.85 (5), Val 0.94 (1), Gly 1.04 (1), Leu 1.00 (1), Hyp 1.88 (2), Ala 1.03 (1), Glx 0.96 (1), EtA 0.95 (1), Phol 0.94 (1). ^{13}C NMR (DMSO-d_6) δ 8.17 (EtA γCH_3), 16.87 (Ala CH_3), 18.41, 19.18 (Val CH_3), 21.78, 22.35, 22.63, 22.88, 23.25, 23.54, 23.80, 24.06, 24.32, 24.70, 24.76, 25.25, 25.32, 25.51, 26.12, 26.24 (Ac CH_3, Leu γC, Leu CH_3, MeA CH_3, EtA βCH_3, 1 extra), 28.68, 28.87, 28.98, 31.30, 31.54, (Val βC, Gln βC, Gln γC, EtA βCH_2, 1 extra), 36.32, 36.69, 36.71, 36.72, 37.00 (Phe, Hyp, Phol, Leu βC), 42.84 (Gly αC), 49.24 (Ala αC), 52.25, 52.28, 52.56, 54.98, 55.84, 55.87, 55.95, 56.06, 56.13, 56.25 (Phe αC, MeA αC, Leu αC, Hyp δC, Gln αC, Phol αC, 1 missing), 59.67, 61.05, 61.10 (Val, Hyp, EtA αC, 1 missing), 63.24 (Phol CH_2OH), 68.87, 69.08 (Hyp γC), 125.71, 126.36, 127.85, 128.09, 129.14, 129.35, 137.51, 139.01 (Phe, Phol arom. C), 169.91, 170.29, 171.39, 171.62, 171.70, 171.89, 171.95, 172.22, 172.33, 172.40, 173.20, 173.74, 174.86, 174.95, 175.57, 175.93 (C═O).

Emerimicin III (30) by Chemical Coupling. Ac-Phe-MeA-MeA-MeA-Val-Gly-OH (**31**) (23 mg, 0.037 mmol) dissolved in DMF (0.5 mL) and HOBt (5 mg, 0.037 mmol) was activated with DCC (7.7 mg, 0.037 mmol) at room temperature for 1 h. Boc-Leu-MeA-MeA-Hyp-Gln-*R*-EtA-Hyp-Ala-Phol (**28**) (43 mg, 0.040 mmol) was deprotected by treatment with CF_3COOH/CH_2Cl_2 (1:1, 1.2 mL) in the presence of anisole (0.05 mL). TLC revealed that removal of the Boc group was complete after 16 min (R_f of protected **28**, 0.57; R_f of deprotected **28**, 0.43; BuOH/AcOH/ethyl acetate/H_2O (1:1:1:1)). Diethyl ether was added to precipitate the solid product which was filtered off, washed with diethyl ether, and dried over KOH under oil pump vacuum. The resulting TFA-salt of the emerimicin III C-terminal nonapeptide (39.5 mg, 92%) was treated with *N*-methylmorpholine (0.005 mL) in DMF (0.2 mL) and coupled (30 h) to activated **31**. The crude dried reaction mixture (75 mg) was dissolved in methanol (5 mL) and chromatographed on Sephadex LH-20 (2.8 cm × 80 cm) using anhydrous methanol as the eluent (4 mL/h). Fractions between 140 and 160 mL were evaporated and chromatographed again on Sephadex LH-20 in anhydrous methanol to yield 7 mg (12%) of emerimicin III (purity >96% by HPLC) which was identical with the product from enzymatic coupling described above.

Ac-Phe-MeA-MeA-MeA-Val-Gly-OH (31). Ac-Phe-MeA-MeA-MeA-Val-Gly-OBzl (**11**) (1.88 g, 2.65 mmol) in methanol was hydrogenated at 2–3 kg/cm^2 in the presence of 10% Pd/C (200 mg). After 3 h, TLC revealed no evidence of benzyl ester **11**. The crude product (poorly soluble in ethyl acetate), mp 235–240 °C, was purified by fractional crystallization from methanol/ethyl acetate: yield 1.49 g (91%); mp 244–245 °C; $[\alpha]^{23}_D$ +16.0° (*c* 0.5, MeOH). FAB-MS *m/z* 619 (MH^+), 641 (MNa^+), 657 (MK^+). Anal. Calcd for $C_{30}H_{46}N_6O_8$ (618.72): C, 58.23; H, 7.49; N, 13.58. Found: C, 58.47; H, 7.53; N, 13.52. ^{13}C NMR (CD_3OD) δ 20.01, 20.20 (Val CH_3), 21.58 (Ac CH_3), 26.56, 26.87, 26.94, 27.08, 27.21, 28.05 (MeA CH_3), 32.01 (Val βC), 33.18 (Phe βC), 43.33 (Gly αC), 58.49, 59.26, 59.39, 59.85, 62.59 (Phe, MeA, Val αC), 129.4–132.4 (multiple lines, Phe arom. C), 139.64 (Phe arom. C), 174.02, 175.25, 175.44, 176.0, 177.97, 178.77, 179.50 (C═O).

Emerimicin IV Triacetate (32) and [*S*-EtA^{12}]Emerimicin IV Triacetate (32a). To a stirred solution of emerimicin IV (**29**) (10 mg, 0.0063 mmol) in DMF (1 mL) was added pyridine (10 μL, 0.123 mmol), acetic anhydride (10 μL, 0.106 mmol) and 4-(dimethylamino)pyridine (1 mg, 0.008 mmol) to catalyze the acetylation reaction. After 2 h at room temperature, HPLC revealed that free emerimicin was no longer present. The mixture was evaporated (oil pump), dissolved in *tert*-butyl alcohol and lyophilized. The crude product (11 mg) was purified by preparative HPLC, t_R 17.0 min (gradient 60–80% B in 40 min). Analytical HPLC revealed material of 96% purity, t_R 15.31 (isocratic 70% B). FAB-MS *m/z* 1699 (MH^+), 1721 (MNa^+). Calcd for $C_{83}H_{126}N_{16}O_{22}$ 1698. [*S*-EtA^{12}]Emerimicin IV triacetate (**32a**) was obtained in an analogous manner. Preparative HPLC, t_R 16.4 min (gradient as above). Analytical HPLC revealed material of 97% purity, t_R 14.85 (isocratic 70% B). FAB-MS *m/z*, same as above.

NMR Analyses. Carbon-13 (75, 125, and 150 MHz) nuclear magnetic resonance spectra were obtained on Varian XI-300, Unity 500, or Unity 600 spectrometers. Structural studies were performed on a Varian Unity 500 spectrometer using 16 mM DMSO-d_6 solutions. Proton resonance assignments, with the exception of MeA and EtA protons, were done using P. COSY[39] aquisitions at 20 °C (4096 points, 1024 t_1 increments, 8 transients, sweep width 5000 Hz). Data sets were zero-filled to 2K × 8K using a 90°-shifted sine bell squared filter in t_1, for a final digital resolution of 2.5 Hz/pt in F_1 and 0.625 Hz/pt in F_2. Phase-sensitive NOESY[40] analyses at 20 °C consisted of 2048 ([*S*-EtA^{12}]emerimicin IV and emerimicin IV) or 4096 ([*S*-EtA^{12}]emerimicin III and emerimicin III) points; 512 increments; 16 transients; sweep width 5000 Hz; and τ_{mix} = 0.3 sec. Data sets were zero-filled to 4K × 4K ([*S*-EtA^{12}]emerimicin IV and emerimicin IV) or 2K × 8K ([*S*-EtA^{12}]emerimicin III and emerimicin III) with a 90° shifted sine bell filter in both dimensions. Amide proton $\Delta\delta/\Delta T$ were determined in a series of 1-D experiments ranging from 20 to 60 °C in 10 degree increments. Coupling constants were evaluated from one-dimensional experiments. Values for overlapped resonances were confirmed using curve fitting software written in our laboratory.

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Supplementary Material Available: Plots of ^1H 500-MHz NMR spectra in DMSO-d_6 at 20 °C for emerimicins III and IV and their *S*-EtA^{12} epimers (one-dimensional spectra), contour plots of the fingerprint and aliphatic regions of the P. COSY spectra for emerimicin IV, [*S*-EtA^{12}]emerimicin III, and [*S*-EtA^{12}]emerimicin IV, contour plots of the amide and α/amide regions of NOESY spectra for emerimicin IV, [*S*-EtA^{12}]emerimicin III, and [*S*-EtA^{12}]emerimicin IV, and contour plots of the α/Hyp-δ region of NOESY spectra for emerimicins III and IV and their *S*-EtA^{12} epimers (20 pages). Ordering information is given on any current masthead page.