Synthesis and Siderophore Activity of Albomycin-like Peptides Derived from N^5-Acetyl-N^5-hydroxy-L-ornithine

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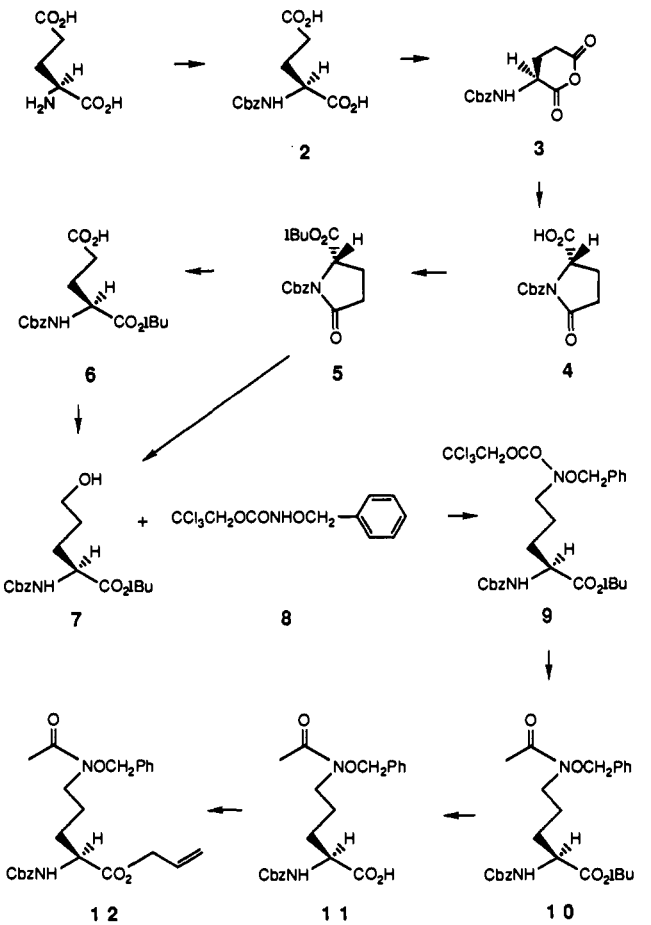
N^5-Acetyl-N^5-hydroxy-L-ornithine (1), the key constituent of several microbial siderophores, has been synthesized in 23% yield overall from *N*-Cbz-L-glutamic acid 1-*tert*-butyl ester (6) derived from L-glutamic acid. Reduction of 6 to 7 and treatment with *N*-[(trichloroethoxy)carbonyl]-*O*-benzylhydroxylamine (8), and diethyl azodicarboxylate and triphenylphosphine followed by deprotection produced the protected N^5-acetyl-N^5-hydroxy-L-ornithine derivatives 11 and 12 in large quantities (10–20 g). Following α-amino and α-carboxyl deprotections of 11 and 12, EEDQ [2-ethoxy-*N*-(ethoxycarbonyl)-1,2-dihydroquinoline] mediated peptide coupling and final deprotection provided amino acid 1 and six albomycin-like peptides (20, 23, 25, 28, 35, and 36). The growth-promoting ability of each was evaluated with the siderophore biosynthesis mutant *Shigella flexneri* SA240 (SA 100 *iuc*D::Tn5). These results indicate that substantial modification of the framework of peptide-based siderophores can be tolerated by microbial iron-transport systems.

Siderophores are low molecular weight iron(III)-transport compounds which are excreted by several microorganisms. In order to sequester and solubilize biologically essential ferric ion from the environment, most siderophores contain hydroxamic acid or catechol functional groups as common structural components.[1] Under conditions of low iron concentration, microorganisms grow slowly and produce copious amounts of both siderophores and siderophore receptor transport proteins.[2] Members of the ferrichrome siderophore family[3] shown in Figure 1 contain three residues of N^5-acetyl-N^5-hydroxy-L-ornithine (1). In particular, the albomycins[4] are natural antibiotics[4f] first isolated from *Streptomyces subtropicus* which contain this linear tripeptide as their hexadentate, octahedral ligand for ferric ion and utilize[5] the ferrichrome iron transport system[6] for intracellular transport. As part of our continuing interest in siderophores,[7] we sought to design a convenient synthesis and to study the iron-transporting ability of various peptide derivatives of

Scheme I



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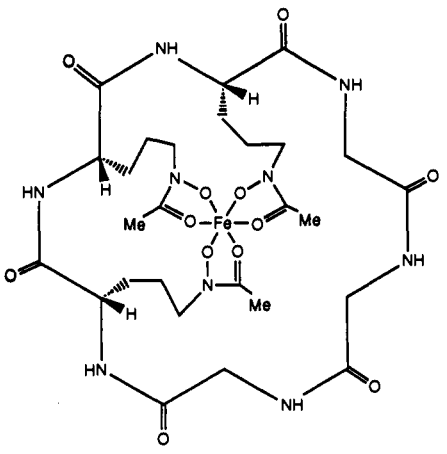
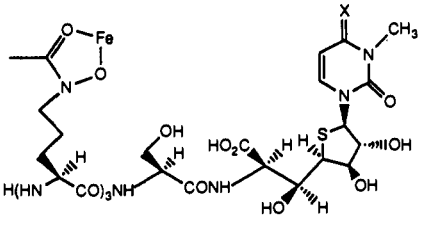


FERRICHROME



THE ALBOMYCINS

X = $NCONH_2$ or O or NH

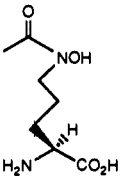


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Figure 1.

N^5-acetyl-N^5-hydroxy-L-ornithine (1). The ability to promote the growth of *S. flexneri* SA240 (SA 100 *iuc*D::Tn5), a siderophore biosynthesis deficient mutant, provides a convenient means of measuring iron transport. This investigation was conducted to determine the amino acid residues of albomycin which were necessary for iron transport by the ferrichrome iron transport system. The degree of structural modification that could be tolerated without destroying the ability to behave as a siderophore is also explored. The potential use of such peptide systems as drug-delivery agents[8] is reported in the following paper.

The important structural component of the ferrichrome and albomycin siderophores, N^5-acetyl-N^5-hydroxy-L-ornithine (1) or structurally similar versions, has been previously synthesized by several groups,[9] the most effective route for producing reasonable quantities of material being that of Benz.[9h] In considering alternate routes in which the desired amino acid 1 or its protected version could be produced in 10–20-g quantities, several factors needed to be considered with regard to protecting groups. These included selective manipulation, introduction–deprotection in high yield, and minimization of racemization. However, a most important factor was the use of protecting groups which produced volatile byproducts upon removal so that extensive purification could be avoided. Therefore, the carbobenzyloxy (Cbz), carbotrichloroethoxy (troc), and benzyl groups, together with the *tert*-butyl and allyl esters, were anticipated to be most useful. In order for this study to be successful, large amounts of 11 and 12 were needed so that sufficient amounts of final products would be available for biological evaluation. L-Glutamic acid was chosen as a starting material, since it is an inexpensive, commercially available chiral compound with the correct number of carbon atoms.[9n] This paper describes the large-scale synthesis of protected versions of N^5-acetyl-N^5-hydroxy-L-ornithine (11 and 12) and their manipulation to provide various peptide derivatives.

Results and Discussion

Amino Acid and Peptide Synthesis. The successful synthesis of hydroxamates 11 and 12 is shown in Scheme I. L-Glutamic acid was converted to Cbz-L-glutamic acid (2), followed by treatment with dicyclohexylcarbodiimide (DCC) in THF at 0 °C to give anhydride 3. Filtration of the precipitated dicyclohexylurea and treatment of the filtrate with dicyclohexylamine (DCHA) gave lactam 4 as a solid DCHA salt. Acidification produced *N*-Cbz-L-pyroglutamic acid (4). In order to facilitate purification, 4 was esterified with catalytic perchloric acid and *tert*-butyl acetate to provide *tert*-butyl ester 5. However, following flash chromatography, the optical rotation of 5 was nonreproducible, giving rotation values ranging from −20° to −32° (chloroform) from reaction to reaction (25–75-g scale) utilizing the same lot of starting L-glutamic acid. Apparently, racemization occurred during the DCC-mediated cyclization, despite maintenance of a reaction temperature of 0 °C. At temperatures of −10 °C or less the reaction rate appeared to be retarded since dicyclohexylurea only began to precipitate from solution as the reaction temperature approached −5 to 0 °C. After removal of the dicyclohexylurea by filtration, the rearrangement of 3 to acid 4 was induced by refluxing the reaction mixture in either THF or *tert*-butyl alcohol without added DCHA. Despite earlier precedent[10] which indicated that treatment of anhydride 3 with alcohols such as methanol produced the α-methyl ester exclusively, treatment with anhydrous *tert*-butyl alcohol produced only the rearrangement product 4, as expected. Esterification of the material obtained thermally with *tert*-butyl acetate and catalytic acid afforded 5, which, following chromatography, also gave low optical rotations. Therefore, it was concluded that racemization had not occurred during the DCHA-induced rearrangement to provide 4, but during the DCC-mediated anhydride formation. The problem of low optical purity was easily remedied by repeated crystallization. *tert*-Butyl ester 5 was dissolved in ethyl acetate followed by addition of hexanes resulting in precipitation of a near racemic solid.[11] Evaporation of the filtrate and repeating the sequence provided material of near absolute optical purity. Despite this inconvenience, this process was routinely performed on a 100-250-g scale.

(8) For a preliminary attempt at utilizing ferrichrome siderophore sulfonamides in drug delivery, see: Zahner, H.; Diddens, H.; Keller-Schierlein, W.; Nageli, H. *Jpn. J. Antibiot.* **1977**, *XXX Suppl.*, S-201.

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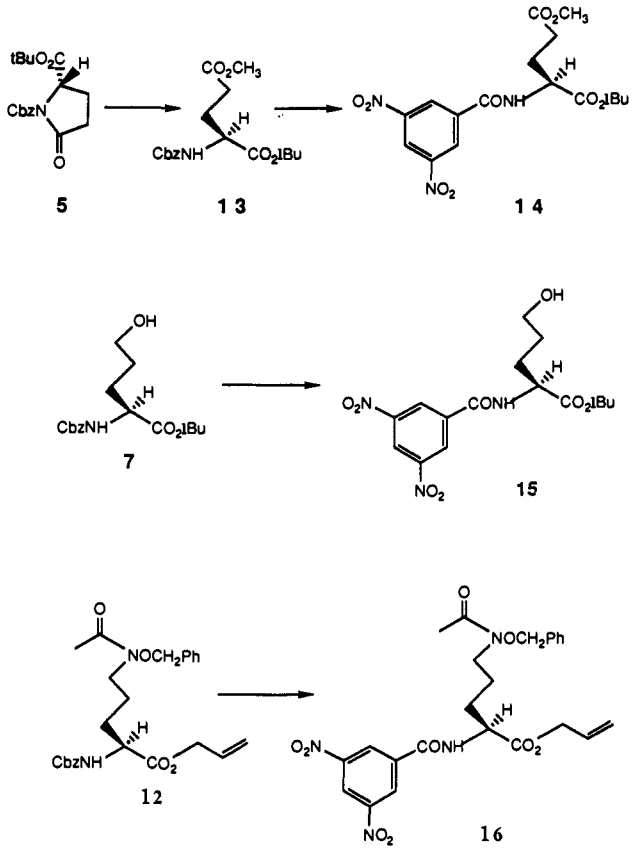
(10) (a) Sheehan, J. C.; Bolhofer, W. A. *J. Am. Chem. Soc.* **1950**, *72*, 2469. (b) Bergmann, M.; Zervas, L.; Salzmann, L. *Ber. Dtsch. Chem. Ges.* **1933**, *66*, 1288.

(11) For a recent example of this phenomenon see: O'Donnell, M. J.; Bennett, W. D.; Wu, S. *J. Am. Chem. Soc.* **1989**, *111*, 2353.

Scheme II



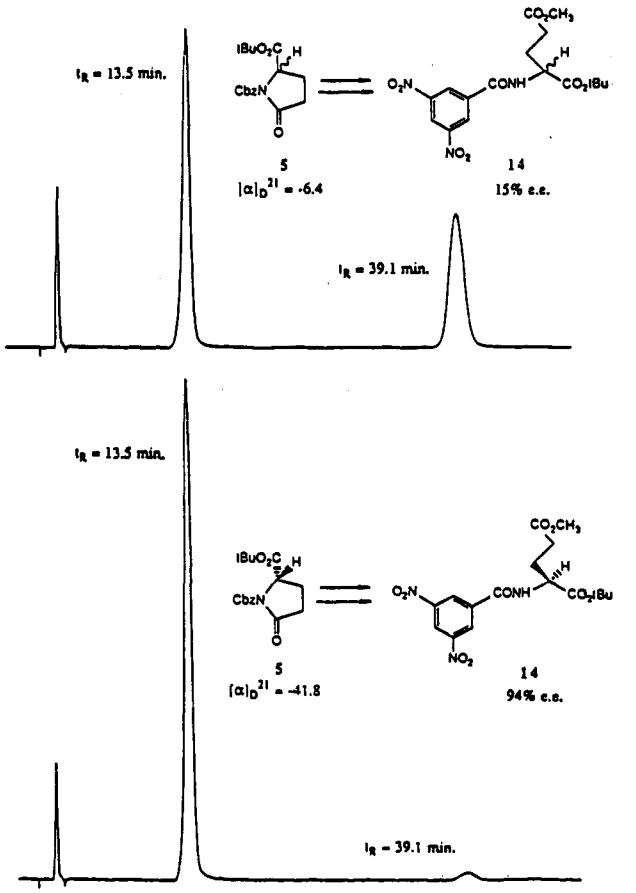


Figure 2. Column, Pirkle covalent D-naphthylalanine (serial #100001, 25 cm × 4.6 mm i.d.); UV detection, 254 nm; solvent, 20% 2-propanol in hexanes; flow rate, 2.0 mL/min; chart speed, 0.2 in./min.

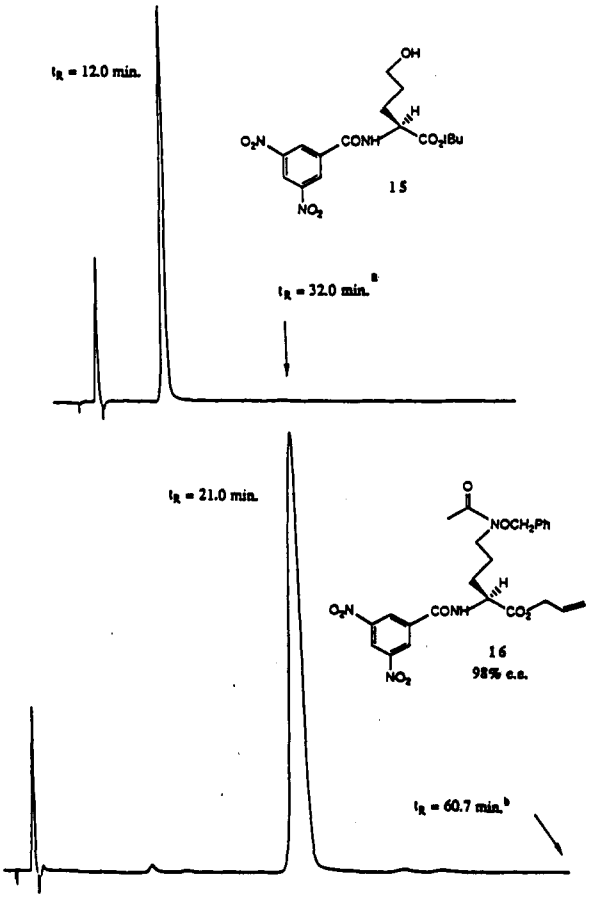


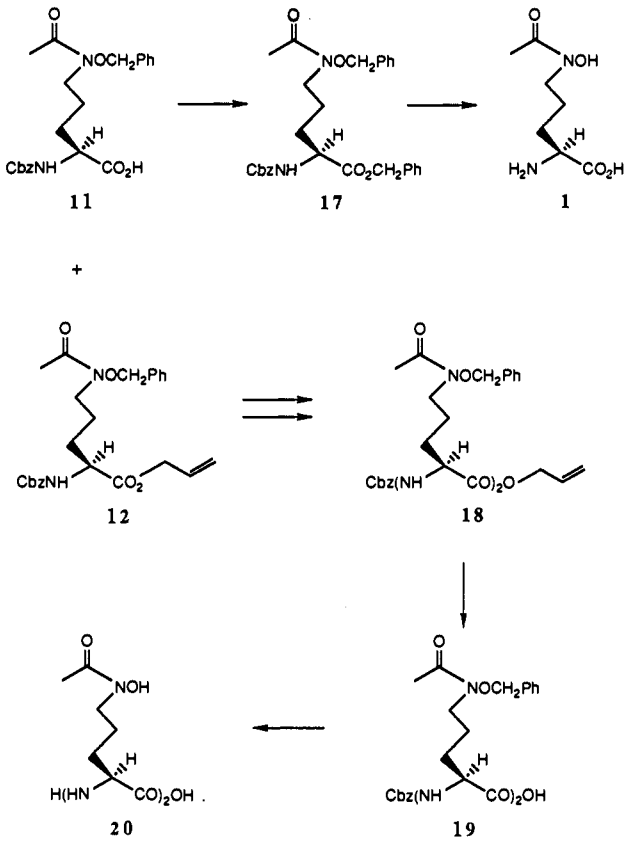
Figure 3. Column, Pirkle covalent D-naphthylalanine (serial #100001, 25 cm × 4.6 mm i.d.); UV detection, 254 nm; solvent, 20% 2-propanol in hexanes; flow rate, 2.0 mL/min; chart speed, 0.2 in./min. [a]Peak visible when near racemic mixture used. [b]Peak visible when optically impure material used.

The optical purity of **5** was confirmed by derivatization to give **14** (Scheme II), which was suited for analysis by the chiral Pirkle covalent D-naphthylalanine HPLC method.[12] Treatment of lactam **5** with catalytic potassium carbonate in anhydrous methanol produced methyl ester **13**. This crude ester was hydrogenated in the presence of acetic acid to remove the Cbz group. Subsequent acylation with 3,5-dinitrobenzoyl chloride afforded **14**. Analysis by HPLC (Figure 2) and comparison with a similar derivative obtained from near racemic **5** revealed an optical purity of approximately 94% ee. The yield of optically pure **5** after repeated crystallization was approximately 20% overall from L-glutamic acid.

Hydrolysis of **5** with 1 equiv of aqueous KOH in ice-cold methanol produced acid **6** in 100% yield. Conversion of **6** to norvaline derivative **7** was accomplished in a stepwise fashion. Formation of a mixed anhydride with ethyl chloroformate and triethylamine in THF, filtration, and subsequent reduction with an excess of sodium borohydride in water and THF gave alcohol **7** in 68% yield. Alternatively, direct treatment of **5** with sodium borohydride in THF and water provided **7** in 55% yield. In order to determine the effect of these reaction sequences on the optical integrity of **7**, it was derivatized as shown in Scheme II. Hydrogenation in the presence of acetic acid in methanol followed by acylation with 3,5-dinitrobenzoyl

(12) (a) Pirkle, W. H.; Mahler, G.; Hyun, M. H. *J. Liq. Chromatogr.* **1986**, *9*, 443. (b) Pirkle, W. H.; Pochapsky, T. C.; Mahler, G. S.; Corey, D. E.; Reno, D. S.; Alessi, D. M. *J. Org. Chem.* **1986**, *51*, 4991.

Scheme III

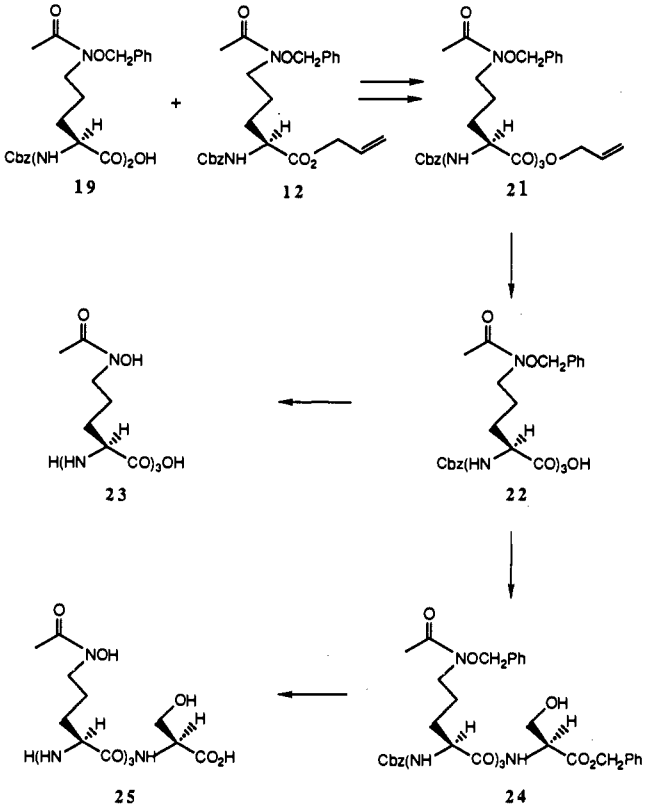


chloride afforded **15** in 79% yield after chromatography. As shown in Figure 3, analysis by the Pirkle HPLC method revealed no evidence of racemization.

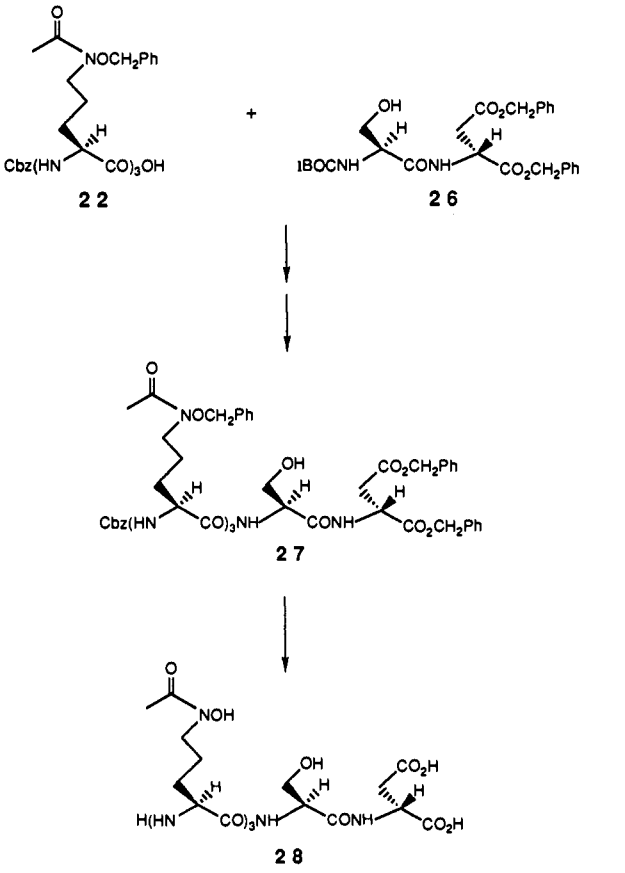
Reaction of alcohol **7** and *N*-troc-*O*-benzylhydroxylamine (**8**) with diethyl azodicarboxylate and triphenylphosphine under Mitsunobu[13] conditions provided fully protected hydroxamate **9** in 71% isolated yield (Scheme I). The trichloroethyl carbamate group was easily replaced by an acetyl group to produce **10** in 81% yield by treatment with zinc dust[14] and acetic anhydride in acetic acid with ultrasonication.[15] The *tert*-butyl ester group of **10** was removed in near quantitative yield by brief treatment with trifluoroacetic acid to give **11**. Esterification to the allyl ester **12** was accomplished in 54% yield by heating **11** in acetonitrile containing *O*-allyldiisopropylisourea.[16] In order to ensure the optical purity of **12**, this material was converted to **16** by treatment with HBr in acetic acid to remove the Cbz group and immediate reaction with 3,5-dinitrobenzoyl chloride (Scheme II). Pirkle HPLC analysis of **16** (Figure 3) indicated that no severe racemization had occurred.

With large quantities (10–20 g of each) of the protected derivatives of N^5-acetyl-N^5-hydroxy-L-ornithine (**11** and **12**), the key siderophore components, in hand, attention was turned to the synthesis of a number of albomycin-like peptide analogues. Ideally, we wished to synthesize each of the consecutive peptide components of albomycin (Figure 1). This required preparation of the amino acid N^5-acetyl-N^5-hydroxy-L-ornithine (**1**), the corresponding dipeptide **20**, and tripeptide **23** (Schemes III and IV).

Scheme IV



Scheme V



Coupling of an L-serine residue to the tripeptide would produce a tris(N^5-acetyl-N^5-hydroxy-L-ornithine)-L-serine peptide **25**. Coupling of a seryl aspartate dipeptide to the protected tripeptide of N^5-acetyl-N^5-hydroxy-L-ornithine

(13) Mitsunobu, O. *Synthesis* **1981**, 1.
(14) Just, G.; Grozinger, K. *Synthesis* **1976**, 457.
(15) Abdulla, R. F. *Aldrichimica Acta* **1988**, *21*, 31.
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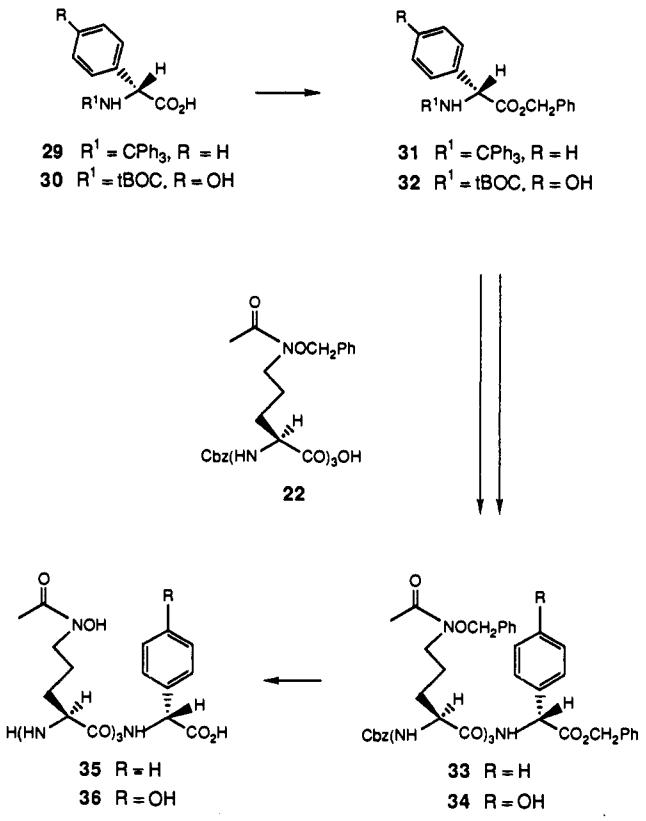
Scheme VI



would ultimately give a pentapeptide **28** (Scheme V) similar in structure to the pentapeptide component of albomycin, with a second carboxyl group available for the future syntheses of other analogues and conjugates which might be susceptible to peptidases that are responsible for release of the toxic thioribosyl moiety from albomycin.[17] Finally, although peptides containing D-amino acids are poor substrates for both permeases and peptidases,[18] β-lactam antibiotics including Eli Lilly's carbacephem LY163892[19] (Loracarbef), which contains a D-phenylglycine side chain, were considered potential candidates for use in a possible siderophore-mediated β-lactam drug delivery system.[20] Therefore, both D-phenylglycyl and *p*-hydroxy-D-phenylglycyl amino acid residues were chosen as components for investigation and were incorporated into the siderophore to produce the peptides **35** and **36** (Scheme VI).

As described later, deprotection of acid **11** to produce **1** was anticipated to be straightforward. However, the purity of the final product was unacceptable as measured by a poor combustion analysis. This seemed to be linked to coelution of a trace impurity, possibly a chelated metal ion, during purification of **11** by chromatography. The final deprotected **1** was amber, but was expected to be colorless without metal chelation. In order to avoid this problem, acid **11** was converted to benzyl ester **17** in 67% yield by treatment with *O*-benzyldiisopropylisourea[16] (Scheme III). Final deprotection, as described subsequently, produced pure **1** as an off-white solid.

The syntheses of the desired peptide analogues were completed with the 2-ethoxy-*N*-(ethoxycarbonyl)-1,2-dihydroquinoline[21] (EEDQ) coupling approach shown in Scheme III. Treatment of allyl ester **12** with HBr in acetic acid and dichloromethane removed the Cbz group. Evaporation, neutralization, and extraction produced the corresponding free amine, which was quickly added to a mixture of acid **11** and EEDQ in dichloromethane to give dipeptide **18** in 55% isolated yield. ^{13}C NMR analysis of this dipeptide revealed two sharp singlets located at 51.68 and 52.64 ppm for the stereogenic center methine carbons, supporting the formation of a single diastereomer. Removal of the allyl protecting group with catalytic tetrakis(triphenylphosphine)palladium(0)[22] and *O*-benzylhydroxylamine as an allyl cation scavenger produced dipeptide acid **19** as a white, amorphous solid in 67% purified yield. Again, ^{13}C NMR analysis revealed only two sharp singlets at 53.10 and 55.66 ppm for the stereogenic methine carbons. Coupling to provide tripeptide allyl ester **21** was accomplished in a similar fashion. Allyl ester **12** (Scheme IV) was treated with HBr and acetic acid in dichloromethane, evaporated, and neutralized, and the corresponding free amine was isolated by extraction. Addition of EEDQ and dipeptide acid **19** produced **21** in 78% yield after chromatography. ^{13}C NMR revealed three signals at 51.24, 51.87, and 53.25 ppm assigned to the stereogenic methine carbons with absolutely no signal doubling. Removal of the allyl group, as before, gave the crystalline tripeptide acid **22** in 74% isolated yield. ^{13}C NMR analysis of **22** indicated three signals at 53.10, 53.76, and 55.71 ppm for the stereogenic methine carbons, indicating that only one diastereomer was present within the limits of detection by ^{13}C NMR.

The synthesis of C-terminal seryl tetrapeptide **25** is shown in Scheme IV. Coupling of tripeptide acid **22** with the free amine of L-serine benzyl ester in the presence of EEDQ produced the solid, protected tetrapeptide **24** in 87% yield. However, ^{13}C NMR analysis of this protected peptide revealed doubling of all four signals assigned to the stereogenic methine carbon atoms (51.78, 51.89, 52.25, 52.37, 53.70, 53.80, 54.53, and 54.61 ppm). Although we initially suspected that either racemization occurred during the reaction or that the serine benzyl ester used was not enantiomerically pure, these were ruled out. ^{13}C NMR analysis of the deprotected peptide, described later, revealed only sharp singlets for the stereogenic methine carbons, suggesting that the previous signal doubling was apparently an artifact produced by different conformational forms of the protected version.

Pentapeptide **28** was prepared as shown in Scheme V. Coupling of *tert*-(butyloxycarbonyl)-L-serine and the free amine of dibenzyl-D-aspartate was accomplished with EEDQ to provide dipeptide **26**. Removal of the *tert*-butyloxycarbonyl protecting group with trifluoroacetic acid

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in dichloromethane followed by neutralization and extraction afforded the free amine. EEDQ-induced coupling of the amine and tripeptide acid **22** produced the protected pentapeptide **27** in 67% yield. ^{13}C NMR analysis of this pentapeptide revealed four sharp singlets assigned to the stereogenic methine carbons at 51.48, 52.50, 53.34, and 55.46 ppm, indicating the formation of a single diastereomer. Fortuitous overlap of two carbons for the signal at 53.34 ppm was apparent since the intensity of this signal was a factor of two larger than the other signals.

With the syntheses of the protected albomycin-like peptide analogues complete, our next goal was to prepare the siderophore–phenylglycine peptides (Scheme VI). *N*-(Triphenylmethyl)-D-phenylglycine (**29**) was converted to benzyl ester **31** in 60% yield by reaction with benzyl bromide and potassium carbonate in DMF. The triphenylmethyl group was removed by very brief treatment with trifluoroacetic acid followed by neutralization and extractive isolation which gave a crude free amine. This amine was immediately coupled with tripeptide acid **22** by reaction with EEDQ to give protected tetrapeptide **33** in 85% yield. ^{13}C NMR analysis revealed four sharp singlets assigned to the stereogenic methine carbons at 51.72, 52.01, 53.50 and 56.33 ppm, indicating the presence of only one diastereomer.

p-Hydroxy-D-phenylglycine-derived tetrapeptide **36** was synthesized as shown in Scheme VI. *N*-(*tert*-(Butoxycarbonyl)-D-*p*-hydroxyphenylglycine **30** was converted to the benzyl ester **32** in 58% yield with benzyl bromide and potassium bicarbonate in DMF. The *tert*-butoxycarbonyl group was removed by treatment with trifluoroacetic acid. Neutralization and extractive isolation gave the free amine which was immediately treated with EEDQ and tripeptide acid **22** to provide protected tetrapeptide **34** in 64% purified yield. ^{13}C NMR analysis revealed four sharp singlets assigned to the stereogenic methine carbons at 52.13, 52.30, 53.69, and 56.14 ppm. Again, apparently, only one diastereomer was produced.

Deprotection of protected amino acid **17** and protected peptides **19**, **22**, **24**, **27**, **33**, and **34** was accomplished by hydrogenation using 10% palladium on carbon in a mixture of methanol and distilled deionized water under exposure to hydrogen for 3–4 h at atmospheric pressure. Following filtration of the catalyst and lyophilization, the deprotected amino acid **1** and peptides **20**, **23**, **25**, **28**, **35**, and **36** were isolated as off-white to light tan solids in yields ranging from 83 to 100%. All produced a positive ferric chloride test for hydroxamic acids as indicated by the appearance of a reddish-purple color. However, upon inspection by nuclear magnetic resonance, both ^{1}H and ^{13}C, several interesting observations were made. It is known[23] that hydroxamic acids and hydroxamates can exist in a number of tautomeric forms, with possibilities of geometrical isomerism and internal hydrogen bonding. This phenomenon has been studied[24] by ^{1}H, ^{13}C, ^{14}N quadrupolar, and ^{17}O NMR, as well as by infrared spectroscopy. However, we would like to describe briefly the observations noted in our amino acid and peptide systems.[25] The ^{13}C NMR spectra for each free peptide revealed no signal doubling for the *stereogenic* methine carbons, which would seem to indicate that only one diastereomer was present within the limits of detection by ^{13}C NMR in deuterium oxide at 20–25 °C with 1,4-dioxane as a internal reference.

NMR Considerations

For simplicity sake, the amino acid N^5-acetyl-N^5-hydroxy-L-ornithine (**1**) will be used for this discussion. It should be emphasized that the observations reported for this compound are consistent throughout the entire series of deprotected peptides **20**, **23**, **25**, **28**, **35**, and **36**. The ^{1}H NMR spectrum of the relatively simple amino acid **1** revealed apparent evidence of the various forms of hydroxamates. Ideally, one should expect the methyl signal of the hydroxamate *N*-acetyl group at 1.94 ppm to be a simple singlet. However, a small shoulder was evident to the upfield side of this signal. Apparently, this represents one or more of the other forms being either tautomeric or geometric in nature. This became very evident in the broadband proton decoupled ^{13}C NMR spectrum of compound **1**. The methyl and methylene signals each displayed doubled signals consisting of one large signal with a nearby smaller signal. This was especially noticeable for the signal of the methylene carbon α to the nitrogen of the hydroxamate. The main signal appeared at 47.23 ppm with a smaller signal at 50.87 ppm. Evidence supporting this was found in the 135° DEPT ^{13}C NMR spectra[26] (methylene carbons appear as negative signals), in which the signal at 50.87 ppm was assigned as that of a methylene carbon. Surprisingly, the signal for the stereogenic methine carbon at 54.35 ppm appeared as only one sharp signal. In fact, the ^{1}H and ^{13}C NMR spectrum of a sample of N^6-acetyl-N^6-hydroxy-DL-lysine synthesized by Maurer and Miller[7g] displayed similar spectral signal doubling. It could be argued that these signals could be evidence of hydrogenolysis of the N–O bond resulting in the formation of the amine in very small amounts. However, this was discounted for two reasons. An elegant comparative study by Fujii and Hatanaka[27] demonstrated that essentially no hydrogenolysis occurs during the removal of a benzyl protecting group from a hydroxamic acid. This was supported by the acceptable elemental combustion analyses obtained for those compounds that exhibited the unusual ^{1}H and ^{13}C NMR spectral behavior. Although not conclusive, we were not surprised to find that the ^{13}C NMR data reported by Benz[4e] during the isolation of albomycin δ_2 included an unassigned signal at 50.8 ppm. The possibility that these spectral observations may be the result of trace metal contamination was ruled out by an extensive series of NMR studies of gallium chelates of **1** and the corresponding peptides.[25]

Biological Studies

The main objective of this work was to determine the amino acid residues of albomycin (Figure 1) which were necessary for iron transport by the ferrichrome iron-transport system. With this in mind, deprotected amino acid **1** and peptides **20**, **23**, **25**, **28**, **35**, and **36** were evaluated for their ability to transport iron. The bioassay for siderophore activity of these hydroxamates was measured by the relative ability of a particular compound to promote the growth of *S. flexneri* SA 240 (SA 100 *iuc*D:Tn5), a

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Table I. Biological Activity of the Albomycin-like Peptide Components

	zone of stimulation,[a] mm						
concn	1	2	23	25	28	35	36
5 mM	NA[b]	NA[b]	40	18	NA[b,c]	45	45
1 mM			37	12		41	40
500 μM			30	12		28	25
100 μM			13	NA[b]		17	15
50 μM			NA[b]			NA[b]	NA[b]
10 μM							
1 μM							

[a] *S. flexneri* SA240 overnight culture, 100 mL of 10^{-3} dilution into 100 mL L-EDDA (250 μg/mL) agar. Plates were spotted with 10 μL samples of siderophores at dilutions indicated. Plates were incubated at 37 °C for 18 h and examined for zones of stimulation around compounds. [b] No activity. [c] No activity with this particular assay.

strain lacking the ability to synthesize siderophores. As shown in Table I, the biological activity of each peptide is shown at various concentrations as measured by a zone of growth stimulation. Several conclusions can be drawn from this data. The first is that the amino acid, N^5-acetyl-N^5-hydroxy-L-ornithine (**1**), the corresponding dipeptide **20**, and pentapeptide **28** showed no zone of growth stimulation and, therefore, no apparent siderophore activity. However, tripeptide **23** and tetrapeptides **25**, **35**, and **36** display siderophore activity by clearly promoting bacterial growth. These results suggest that the trihydroxamate unit in the peptides **23**, **25**, **35**, and **36** are recognized by the receptor proteins of the ferrichrome iron-transport system. Interestingly, despite its structural similarity to the albomycin peptide fragment, pentapeptide **28** revealed no siderophore activity in this particular assay. However, in a growth curve assay utilizing another microorganism, *Escherichia coli* X580, **28** revealed slight growth promoting activity. A complete detailed report of the siderophore activity of these compounds with *E. coli* X580 will be reported elsewhere.[28]

The C-terminus of the iron-binding trihydroxamate portion of tripeptide **23** apparently can tolerate some degree of structural modification, as indicated by the siderophore activity of peptides **35** and **36**. This assay was also completed with two isogenic *E. coli* strains that differ only in the presence and absence of the *fhu*A gene. The RW193 strain is an *E. coli* K12 *ent*A, *fhu*A positive organism and the AN193 strain is an *ent*A, *fhu*A negative mutant which is deficient in the ferrichrome hydroxamate receptor. For the RW193 strain, results were comparable to the results observed with the *S. flexneri* SA 240 (SA 100 *iuc*D:Tn5) mutant strain. For the *E. coli* mutant AN193, *no growth stimulation* was observed for any of the compounds tested, suggesting that indeed the active compounds are being transported by the ferrichrome iron-transport system.[28] With this evidence in mind, it may be possible to utilize the ferrichrome iron-transport system as a possible drug-delivery route to smuggle toxic substances selectively into pathogenic enterobacteria. Work in connection with the concept of iron-transport-mediated drug delivery of β-lactam antibiotics is reported in the following paper in this journal.

Experimental Section

General Methods. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra (IR) were recorded on a Perkin-Elmer 1240 spectrophotometer. TF refers to thin film and KBr refers to a potassium bromide disk for infrared spectra. Proton spectra were obtained on a Magnachem A-200 or a General Electric GN-300 spectrometer. Carbon-13 NMR were obtained on a General Electric GN-300 spectrometer. Chemical shifts are reported in ppm relative to tetramethylsilane (deuterochloroform or deuteromethanol solvents) or 1,4-dioxane (3.55 ppm in deuterium oxide) for proton NMR. For ^{13}C NMR, references were the center peak of deuterochloroform (77.0 ppm), the center peak of deuteromethanol (49.0 ppm), or in deuterium oxide the signal for 1,4-dioxane (66.5 ppm). Peak assignments for ^{13}C NMR were made with the assistance of the Distortionless Enhanced Polarization Transfer pulse program.[26] Electron impact mass spectra, chemical ionization mass spectra, and fast atom bombardment mass spectra were recorded on an AEI Scientific Apparatus MS 902, Du Pont DP 102, and Finnigan MAT Model 8430 spectrometers. High-pressure liquid chromatography was carried out with a Beckman/Altex Model 332 instrument and a Pirkle covalent D-naphthylalanine (serial #100001, 25 cm × 4.6 mm i.d.) column. Optical rotations were obtained with a Rudolf Research Autopol III polarimeter with spectral-grade solvents. Analytical TLC was conducted with commercially available aluminum-backed 0.2 mm silica gel 60 F-254 plates. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. Radial preparative chromatography was performed with a Harrison Research Chromatotron Model 7924 and flash silica gel column chromatography was conducted with Merck silica gel 60. Solvents used were dried and purified by standard methods.[29] The term "dried" refers to the drying of an organic solution over anhydrous magnesium sulfate.

N^2-(Benzyloxycarbonyl)-L-glutamic acid (2) was prepared by the procedure of Goldschmidt and Jutz.[30]

N^2-(Benzyloxycarbonyl)-L-pyroglutamic Acid (4).[31] To a solution of *N*-Cbz-glutamic acid (**2**; 104.55 g, 372 mmol) in THF at 0 °C was added slowly a solution of DCC (78.99 g, 383 mmol)[32] in 400 mL of THF. The mixture was stirred vigorously overnight. The dicyclohexylurea was filtered off and washed with THF. The filtrate was directly treated with DCHA (68.74 g, 379 mmol) in THF at 0 °C. The reaction mixture was vigorously stirred overnight, evaporated at ambient temperature under vacuum, and suspended in water and ethyl acetate. To this suspension was added 1.0 M $NaHSO_4$ (410 mL, 390 mmol) with vigorous stirring followed by filtration. The organic phase was immediately separated and the aqueous layer was extracted with ethyl acetate. The organic phase was washed with distilled water, brine, dried, filtered, and evaporated to provide 86.16 g (88%) of pyroglutamic acid **4** as an oil which slowly crystallized on standing: TLC (acetic acid–ethyl acetate, 1:20) R_f = 0.54; IR (TF) 3440–2500 (br), 1790, 1750, 1720 cm^{-1}; $[\alpha]^{25}_D$ = −23.1° (*c* = 2.47, $CHCl_3$) [lit.[33] $[\alpha]^{25}_D$ = −29.1° (*c* = 1.01, MeOH)]; ^1H NMR ($CDCl_3$, 200 MHz) δ 2.10–2.80 (m, 4 H, CH_2), 4.71 (dd, 1 H, *J* = 4 and 8 Hz, NC*H*CO), 5.27 (s, 2 H, benzylic H), 7.20–7.50 (m, 5 H, aromatic H); MS (CI, isobutane) *m/z* 264 (M + 1).

N^2-(Benzyloxycarbonyl)-L-pyroglutamic Acid 1-*tert*-Butyl Ester (5).[33] A solution of the acid **4** (86.16 g, 327 mmol) in 500 mL of *tert*-butyl acetate was stirred with catalytic 70% perchloric acid[34] (1.0 mL) overnight at room temperature in a sealed flask. After venting, the *tert*-butyl acetate solution was washed with water, saturated $NaHCO_3$, and brine. The organic phase was dried, filtered, and evaporated to give crude *tert*-butyl ester **5**. Crude **5** was purified by column chromatography eluting with ethyl acetate–hexanes (1:1). Repeated recrystallization from ethyl acetate–hexanes provided 21.47 g (18.1% from L-glutamic acid) of optically pure **5** as white needles: TLC (ethyl acetate–hexanes, 1:1) R_f = 0.62; mp 53–55 °C (lit.[33] mp 58–59 °C); IR (TF) 1795, 1740, 1720 cm^{-1}; $[\alpha]^{21}_D$ = −41.8° (*c* = 6.71, $CHCl_3$), $[\alpha]^{21}_D$ = −39.6° (*c* = 5.00, DMF) [lit.[33] $[\alpha]^{25}_D$ = −41.9° (*c* = 1.0, DMF)]; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.39 (s, 9 H, $C(CH_3)_3$), 1.95–2.75

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(m, 4 H, CH_2), 4.55 (dd, 1 H, J = 8 Hz, NC*H*CO), 5.28 (AB q, 2 H, J = 16 Hz, benzylic H), 7.28–8.00 (m, 5 H, aromatic H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 21.01, 26.99, 30.17, 58.59, 67.21, 81.56, 127.31, 127.57, 127.72, 134.41, 150.06, 169.40, 172.38; MS (CI, isobutane) m/z 320 (M + 1).

1-*tert*-Butyl-N^2-(3,5-dinitrobenzoyl)-L-glutamic Acid 5-Methyl Ester (14). To a solution of **5** (0.660 g, 2.07 mmol) in 5 mL of anhydrous methanol was added a catalytic amount of K_2CO_3 (0.057 g, 0.413 mmol). TLC analysis after stirring under nitrogen for 0.5 h revealed the absence of **5**. The solvent was evaporated. The residue was diluted with ethyl acetate, washed with water and brine, and dried. Filtration and evaporation provided 0.437 g of a clear oil (**13**) which was homogeneous by TLC (R_f = 0.39, 33% ethyl acetate–67% hexanes). To this oil was added 5 mL of methanol, 72 μL (1.24 mmol, 1.0 equiv) of glacial acetic acid, and 10% Pd/C. This mixture was exposed to hydrogen and monitored by TLC until no starting material was present (1 h). The Pd/C was removed by filtration through Celite and the methanol was evaporated to give an oil. To this oil was added 5 mL of anhydrous dichloromethane, 3,5-dinitrobenzoyl chloride (0.287 g, 1.24 mmol), and triethylamine (0.347 mL, 2.49 mmol). The resulting mixture was stirred overnight under nitrogen. The dichloromethane was evaporated and the residue was diluted with ethyl acetate and water. The organic phase was washed with brine, dried, filtered, and evaporated to provide an oil. Repeated radial chromatography eluting with ethyl acetate–hexanes (1:4) gave 0.143 g (17%) of pure **14** as an oil which slowly solidified to give a pale yellow solid: TLC (ethyl acetate–hexanes, 1:2) R_f = 0.74; $[\alpha]^{26}_D$ = +13.2° (c = 1.43, $CHCl_3$); IR (TF) 3340, 3100, 2980, 1735, 1670, 1540 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.51 (s, 9 H, $C(CH_3)_3$), 2.10–2.40 (m, 2 H, CH_2), 2.45–2.55 (m, 2 H, CH_2), 3.72 (s, 3 H, OCH_3), 4.66–4.78 (m, 1 H, NC*H*CO), 8.05 (d, 1 H, J = 8 Hz, N*H*), 8.97–9.03 (m, 2 H, aromatic H), 9.12–9.18 (m, 1 H, aromatic H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 26.58, 27.83, 30.13, 52.05, 53.38, 83.14, 121.06, 127.25, 137.12, 148.51, 162.36, 170.50, 173.99; MS (EI with self chemical ionization) m/z 413 (M + 1).

N^2-(Benzyloxycarbonyl)-L-glutamic Acid 1-*tert*-Butyl Ester (6).[35] To a solution of **5** (37.1 g, 116.1 mmol) in 100 mL of ice-cold methanol was added 117 mL of 1 M KOH. The reaction was monitored by TLC and when complete the methanol was evaporated. The residue was dissolved in water and ethyl acetate. The organic phase was separated and discarded, and the aqueous phase acidified with 1 M HCl and extracted with ethyl acetate. The combined organic phases were washed with brine, dried, filtered, and evaporated to provide 39.15 g (100%) of **6** as a colorless oil: TLC (methylene chloride–ethyl acetate 1:2) R_f = 0.50; IR (TF) 3430–2870 (br), 1718 (br) cm^{-1}; $[\alpha]^{21}_D$ = +3.9° (c = 14.0, $CHCl_3$), $[\alpha]^{21}_D$ = −19.4° (c = 2.20, MeOH) [lit.[35] $[\alpha]^{25}_D$ = −26.2° (c = 1.0, MeOH)]; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.46 (s, 9 H, $C(CH_3)_3$), 1.87–2.55 (m, 4 H, CH_2), 4.25–4.35 (m, 1 H, NC*H*CO), 5.10 (s, 2 H, benzylic H), 5.53 (d, 1 H, J = 8 Hz, N*H*), 7.30 (s, 5 H, aromatic H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 27.69, 27.86, 29.89, 53.64, 66.97, 82.47, 128.01, 128.10, 128.44, 136.10, 155.99, 170.92, 177.89; MS (CI, isobutane) m/z 338 (M + 1).

N^2-(Benzyloxycarbonyl)-5-hydroxy-L-norvaline 1-*tert*-Butyl Ester (7).[36] To a solution of acid **6** (77.3 g, 229 mmol) in 500 mL of THF at 0 °C was added triethylamine (23.19 g, 229 mmol) and ethyl chloroformate (27.36 g, 252 mmol). The mixture was stirred at 0 °C for 1 h and then warmed to room temperature. Triethylamine hydrochloride was removed by filtration and to the filtrate at 0 °C was added a solution of sodium borohydride (19.07 g, 504 mmol) in 100 mL of water. The reaction mixture was stirred at room temperature for 4 h and acidified with 2 M HCl. Ethyl acetate was added, the organic phase was separated, and the aqueous phase was extracted with ethyl acetate. The combined organic phases were washed with 2 M KOH and brine, dried, filtered, and evaporated to provide an oil. The oil was purified by flash column chromatography eluting with ethyl acetate–hexanes (1:1) to give 50.36 g (68%) of alcohol **7** as a colorless oil: TLC (ethyl acetate–hexanes 1:2) R_f = 0.55; IR (TF) 3430, 1710 (br) cm^{-1}; $[\alpha]^{21}_D$ = +3.9° (c = 14.0, $CHCl_3$) [lit.[36] $[\alpha]^{25}_D$ = +6.7° (c = 3.4, $CHCl_3$)]; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.46 (s, 9 H, $C(CH_3)_3$), 1.55–2.00 (m, 4 H, CH_2), 2.28 (br s, 1 H, CH_2OH), 3.69 (t, 2 H, J = 6 Hz, CH_2OH), 4.25–4.38 (m, 1 H, NC*H*CO), 5.13 (s, 2 H, benzylic H), 5.50 (d, 1 H, J = 12 Hz, N*H*), 7.38 (s, 5 H, aromatic H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 27.37, 27.68, 28.58, 53.73, 61.12, 66.20, 81.35, 127.46, 127.89, 135.85, 155.67, 171.23; MS (CI, isobutane) m/z 324 (M^+).

N^2-(3,5-Dinitrobenzoyl)-5-hydroxy-L-norvaline 1-*tert*-Butyl Ester (15). To a solution of **7** (0.350 g, 1.08 mmol) in 5 mL of methanol was added 63 μL (1.08 mmol) of glacial acetic acid and 0.100 g of 10% Pd/C. Hydrogen was bubbled through the solution for 1 h. The Pd/C was removed by filtration through Celite and the methanol was evaporated to give an oil. To this oil was added 2 mL of anhydrous methylene chloride, triethylamine (150 μL, 1.08 mmol), and 3,5-dinitrobenzoyl chloride (0.250 g, 1.08 mmol) followed quickly by another 150 μL of triethylamine. This mixture was stirred overnight under nitrogen and then evaporated. The residue was dissolved in ethyl acetate and water. The organic phase was washed with brine, dried, filtered, and evaporated to provide an oil. This oil was purified by radial chromatography eluting with ethyl acetate–hexanes (1:1) to give 0.327 g (79%) of an oil which slowly solidified: mp 108–110 °C; TLC (ethyl acetate–hexanes 1:2) R_f = 0.58; IR (TF) 3600–3200 (br), 2980, 1735, 1655, 1545 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.49 (s, 9 H, $C(CH_3)_3$), 1.68–1.78 (m, 2 H, CH_2), 1.98–2.09 (m, 2 H, CH_2), 3.22 (br s, 1 H, CH_2OH), 3.62–3.81 (m, 2 H, CH_2OH), 4.78 (AB q, 1 H, J = 13 and 14 Hz, NC*H*CO), 8.38 (br d, 1 H, J = 8 Hz, N*H*), 9.20 (d, 2 H, J = 2 Hz, aromatic H), 9.10 (t, 1 H, J = 2 Hz, aromatic H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 27.80, 28.84, 53.40, 61.94, 79.30, 82.72, 120.93, 127.35, 137.23, 148.40, 162.49, 171.21; exact mass (EI with self ionization occurring) calcd for $C_{16}H_{22}N_3O_8$ (M + 1) 384.1407, found 384.1408 (M + 1).

N^2-(Benzyloxycarbonyl)-N^5-(benzyloxy)-N^5-[(2,2,2-trichloroethoxy)carbonyl]-L-ornithine 1-*tert*-Butyl Ester (9).[36] To a solution of alcohol **7** (44.05 g, 137 mmol) in 400 mL of THF was added *N*-[(2,2,2-trichloroethoxy)carbonyl]-*O*-benzylhydroxylamine (**8**; 34.11 g, 114 mmol) and solid triphenylphosphine (89.90 g, 343 mmol). Diisopropyl azodicarboxylate (69.16 g, 343 mmol) was added dropwise to the reaction mixture under nitrogen and stirred at room temperature overnight. After evaporation, the crude oil was purified by flash chromatography eluting with ethyl acetate–hexanes (1:4) to provide 48.99 g (71%) of **9** as an oil: TLC (ethyl acetate–hexanes 1:3) R_f = 0.66; IR (TF) 3450, 1720 (br) cm^{-1}; $[\alpha]^{21}_D$ = +8.6° (c = 0.7, $CHCl_3$) [lit.[36] $[\alpha]^{26}_D$ = +9.7° (c = 2.2, $CHCl_3$)]; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.42 (s, 9 H, $C(CH_3)_3$), 1.58–1.92 (m, 4 H, CH_2), 3.53 (t, 2 H, J = 6 Hz, CH_2N), 4.20–4.32 (m, 1 H, NC*H*CO), 4.82 (s, 2 H, CCl_3CH_2), 4.91 (s, 2 H, benzylic H), 5.09 (s, 2 H, benzylic H), 5.32 (d, 1 H, J = 8 Hz, N*H*), 7.25–7.45 (m, 5 H, aromatic H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 22.70, 27.90, 29.96, 49.17, 53.91, 66.86, 75.10, 77.19, 82.24, 95.23, 128.05, 128.47, 128.79, 129.47, 134.77, 136.25, 155.02, 155.77, 171.13; MS (positive ion FAB, *m*-nitrobenzyl alcohol matrix) m/z 605 (M + 2), 603 (M^+), 549 (M − $C(CH_3)_3$ + 2), 547 (M − $C(CH_3)_3$). Anal. ($C_{27}H_{33}N_2O_7Cl_3$) C, H, N, Cl.

N^5-Acetyl-N^5-(benzyloxy)-N^2-(benzyloxycarbonyl)-L-ornithine 1-*tert*-Butyl Ester (10). Zinc dust was activated by washing consecutively with 6 M HCl, 1 M HCl, acetone, and methanol followed by drying under vacuum. A solution of the protected hydroxamate **9** (48.99 g, 81.39 mmol) and acetic anhydride (31 mL, 325.5 mmol) in 200 mL of glacial acetic acid was treated with the freshly activated zinc dust (79.81 g, 1.221 mol) under ultrasonication. After 3 h, the zinc dust was removed by filtration and washed with ethyl acetate. The filtrate was evaporated to an oil which was dissolved in ethyl acetate and washed with a solution of saturated sodium carbonate. The organic phase was washed with brine, dried, filtered, and evaporated to provide an oil. Purification by flash chromatography eluting with ethyl acetate–hexanes (1:1) gave 30.77 g (81%) of hydroxamate **10** as an oil: TLC (ethyl acetate–hexanes 1:1) R_f = 0.33; IR (TF) 3395, 1705 (br), 1645 cm^{-1}; $[\alpha]^{21}_D$ = +10.4° (c = 4.10, $CHCl_3$), $[\alpha]^{21}_D$ = −0.9° (c = 4.10, EtOAc) [lit.[36] $[\alpha]^{26}_D$ = −2.9° (c = 3.05, EtOAc)]; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.41 (s, 9 H, $C(CH_3)_3$), 1.50–2.00 (m, 4 H, CH_2), 2.06 (s, 3 H, CH_3CON), 3.40–3.77 (m, 2 H, CH_2N), 4.15–4.38 (m, 1 H, NC*H*CO), 4.76 (s,

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2 H, benzylic H), 5.07 (s, 2 H, benzylic H), 5.61 (d, 1 H, J = 8 Hz, NH), 7.34 (s, 5 H, aromatic H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.13, 22.44, 27.60, 29.56, 44.49, 53.75, 66.43, 75.94, 81.67, 127.72, 128.14, 128.37, 128.61, 128.86, 134.13, 136.12, 155.63, 170.99, 171.90; MS (CI, isobutane) m/z 471 (M + 1). Anal. ($C_{26}H_{34}N_2O_6$) C, H, N.

N^5-Acetyl-N^5-(benzyloxy)-N^2-(benzyloxycarbonyl)-L-ornithine (11).[7h] A solution of *tert*-butyl ester **10** (30.67 g, 65.49 mmol) in 100 mL of trifluoroacetic acid[37] was stirred at room temperature with monitoring by TLC. The trifluoroacetic acid was evaporated and the residue was chromatographed by eluting with 1:20:15 acetic acid–ethyl acetate–hexanes. After evaporation of the solvent, the residual oil was dissolved in ethyl acetate and washed with water to remove traces of acetic acid. The organic phase was washed with brine, dried, filtered, and evaporated to provide 27.0 g (100%) of **11** as a colorless oil: TLC (acetic acid–ethyl acetate–hexanes 1:20:15) R_f = 0.20; IR (TF) 3440–2500 (br), 1715 (br), 1615 (br) cm^{-1}; $[\alpha]^{26}_D$ = +6.4° (c = 14.90, $CHCl_3$) [lit.[7h] (for the DCHA salt), $[\alpha]^{25}_D$ = +8.4° (c = 2.8, MeOH)]; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.60–2.00 (m, 4 H, CH_2), 2.10 (s, 3 H, CH_3CON), 3.64 (t, 2 H, J = 7 Hz, CH_2N), 4.40–4.50 (m, 1 H, NCHCO), 4.81 (s, 2 H, benzylic H), 5.10 (s, 2 H, benzylic H), 5.63 (d, 1 H, J = 8 Hz, NH), 7.29–7.50 (m, 5 H, aromatic H), 9.40 (br s, 1 H, CO_2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 19.88, 22.67, 29.22, 44.63, 53.16, 66.67, 76.08, 127.73, 127.80, 128.18, 128.48, 128.78, 128.96, 133.89, 136.03, 156.04, 172.85, 174.41; MS (CI, isobutane) m/z 415 (M + 1).

Benzyl N^5-Acetyl-N^5-(benzyloxy)-N^2-(benzyloxycarbonyl)-L-ornithinate (17). To *tert*-butyl ester **10** (0.718 g, 1.53 mmol) was added 5 mL of trifluoroacetic acid. After stirring for 0.5 h, the solution was evaporated, diluted with ethyl acetate, washed repeatedly with water and brine, then dried, filtered, and evaporated to provide crude **11** as an oil. To this oil was added *O*-benzyldiisopropylisourea (0.450 g, 1.92 mmol) in 5 mL of anhydrous THF. This solution was stirred under nitrogen at room temperature for 22 h then cooled to −78 °C, filtered to remove diisopropylurea, and evaporated to give an oil. Radial chromatography, eluting with ethyl acetate–hexanes (1:1), gave 0.522 g (67%) of **17** as an oil: TLC (ethyl acetate–hexanes 1:1) R_f = 0.39; IR (TF) 3310, 3040, 2950, 1740, 1720, 1660 cm^{-1}; $[\alpha]^{25}_D$ = +3.6° (c = 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.55–1.90 (m, 4 H, CH_2), 2.04 (s, 3 H, CH_3CON), 3.50–3.70 (m, 2 H, CH_2N), 4.35–4.50 (m, 1 H, NCHCO), 4.73 (s, 2 H, benzylic H), 5.00–5.20 (m, 4 H, benzylic H), 5.58 (d, 1 H, J = 8 Hz, NH), 7.20–7.40 (m, 15 H, aromatic H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.27, 22.79, 29.50, 44.0–45.0 (br m), 53.58, 66.78, 66.96, 76.17, 127.87, 127.94, 128.04, 128.24, 128.33, 128.42, 128.55, 128.80, 129.01, 134.26, 135.12, 136.13, 155.82, 171.87; exact mass (EI) calcd for $C_{29}H_{32}N_2O_6$ (M^+) 504.2260, found 504.2266.

N^5-Acetyl-N^5-hydroxy-L-ornithine (1). To a solution of benzyl ester **17** (0.370 g, 0.733 mmol) in 10 mL of methanol and 5 mL of deionized distilled water was added 0.074 g of 10% Pd/C. After hydrogen was bubbled through the solution for 4 h, the catalyst was removed by filtration and the methanol was evaporated. The residual aqueous solution was extracted with ethyl acetate and the aqueous phase was lyophilized to give 0.122 g (87%) of **1** as an off-white solid: $FeCl_3$ positive (red-purple); mp 189–190 °C (lit.[4c] 204 °C); paper chromatography (Whatman No. 1, water–2-propanol 1:2) R_f = 0.68; IR (KBr) 3600–2500 (br), 1580 (br); $[\alpha]^{25}_D$ = −0.6° (c = 0.954, deionized distilled water), $[\alpha]^{25}_D$ = +26.0° (c = 0.100, 1 M HCl made from deionized, distilled water) [lit.[4c] $[\alpha]^{20}_D$ = +21.3° (c = 0.500, 1 N HCl)]; ^{1}H NMR (D_2O, 300 MHz) δ 1.40–1.80 (m, 4 H, CH_2), 1.94 (s with a small sh, 3 H, CH_3CON), 3.40–3.60 (m, 3 H, CH_2N and NCHCO); ^{13}C NMR (D_2O, 75 MHz, all signals observed at 20 °C reported) δ 19.28, 19.58 sh (methyl signal), 21.83, 22.42, 27.35, 27.55, 47.23, 50.87 (methylene signals), 54.35 (methine signal), 169.29 (small), 173.75, 174.29 (carbonyl signals); MS (positive ion FAB, glycerol) m/z 191 (M + 1). Anal. ($C_7H_{14}N_2O_4$) C, H, N.

Allyl N^5-Acetyl-N^5-(benzyloxy)-N^2-(benzyloxycarbonyl)-L-ornithinate (12). A solution of *O*-allyldiisopropylisourea (18.70 g, 101 mmol) and acid **11** (20.95 g, 50.8 mmol) in acetonitrile was stirred at 60 °C overnight under nitrogen. The diisopropylurea was removed by filtration and the filtrate was evaporated to give an oil. The oil was purified by flash chromatography eluting first with ethyl acetate–hexanes (1:1 then 2:1) to provide 12.45 g (54%) of **12** as a colorless oil: TLC (ethyl acetate–hexanes 1:1) R_f = 0.59; IR (TF) 3300, 1740, 1718, 1650 cm^{-1}; $[\alpha]^{26}_D$ = +6.0° (c = 4.20, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.80–2.00 (m, 4 H, CH_2), 2.10 (s, 3 H, CH_3CON), 3.50 (m, 2 H, CH_2N), 4.35–4.50 (m, 1 H, NCHCO), 4.60 (d, 2 H, J = 6 Hz, allylic H), 4.78 (s, 2 H, benzylic H), 5.10 (s, 2 H, benzylic H), 5.27 (dd, 1 H, J = 11 and 17 Hz, olefinic H), 5.50 (d, 1 H, J = 11 Hz, NH), 5.75–5.90 (m, 1 H, olefinic H), 7.15–7.35 (m, 5 H, aromatic H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.25, 22.78, 29.45, 44.78 (br), 53.51, 65.72, 66.71, 76.12, 118.62, 127.84, 127.90, 128.27, 128.50, 128.76, 128.97, 131.31, 134.20, 136.10, 155.80, 171.69, 172.15 (br); MS (CI, isobutane) m/z 455 (M + 1). Anal. ($C_{25}H_{30}N_2O_6$) C, H, N.

Allyl N^2-(3,5-Dinitrobenzoyl)-N^5-acetyl-N^5-(benzyloxy)-L-ornithinate (16). To a solution of allyl ester **12** (0.258 g, 0.570 mmol) in 2 mL of dichloromethane was added 1.0 mL of 30% HBr in acetic acid under nitrogen. The mixture was stirred for 10 min and evaporated. Dichloromethane (2.0 mL) was added followed by triethylamine (159 μL, 1.141 mmol) and 3,5-dinitrobenzoyl chloride (0.132 g, 0.570 mmol). The mixture was stirred overnight and diluted with ethyl acetate and water, and the organic phase was washed with brine, then dried, filtered, and evaporated to give an oil which was purified by radial chromatography, eluting with ethyl acetate–hexanes (1:1). Fractions which were homogeneous by TLC were pooled and evaporated to give 0.043 g of pure **16** as an oil: TLC (ethyl acetate–hexanes 1:1) R_f = 0.59; IR (TF) 3300, 1745, 1670, 1665, 1635 (br); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.50–2.10 (m, 4 H, CH_2), 2.16 (s, 3 H, CH_3CON), 3.60–3.90 (m, 2 H, CH_2N), 4.64–4.68 (m, 2 H, allylic H), 4.70–4.80 (m, 1 H, NCHCO), 4.83 (s, 2 H, benzylic H), 5.22–5.40 (m, 2 H, olefinic H), 5.82–5.98 (m, 1 H, olefinic H), 7.30–7.42 (m, 5 H, aromatic H), 8.57 (d, 1 H, J = 6 Hz, NH), 9.10–9.20 (m, 3 H, aromatic H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.33, 24.03, 27.31, 44.37, 53.95, 66.16, 76.43, 119.01, 121.05, 127.87, 128.77, 129.14, 131.39, 133.87, 137.20, 148.52, 162.99, 171.26, 173.15; exact MS (EI) calcd for $C_{24}H_{26}N_4O_9$ (M^+) 514.1700, found 514.1697.

Allyl N^5-acetyl-N^5-(benzyloxy)-N^2-(benzyloxycarbonyl)-L-ornithyl-N^5-acetyl-N^5-(benzyloxy)-L-ornithinate (18). To a solution of **12** (6.04 g, 13.37 mmol) in 20 mL of dichloromethane was added 20 mL of 30% HBr in acetic acid and the resulting mixture was stirred for 10 min under nitrogen. The solution was evaporated and the residue was dissolved in ethyl acetate and water. The organic phase was discarded. The aqueous phase was treated in the presence of ethyl acetate with a solution of saturated sodium carbonate (pH of ca. 8–9). The layers were separated, and the aqueous phase was reextracted twice with ethyl acetate. The organic layers were pooled, washed with brine, dried, filtered, and evaporated at ambient temperature to provide an oil. This oil was immediately dissolved in 27 mL of anhydrous dichloromethane and transferred to a flask containing acid **11** (5.51 g, 13.37 mmol) and EEDQ (4.30 g, 17.37 mmol). The mixture was stirred under nitrogen overnight. The reaction mixture was dissolved in ethyl acetate, washed with 1.0 M HCl, brine, dried, filtered, and evaporated to give 10.06 g of a yellow oil. The oil was purified by column chromatography eluting first with ethyl acetate–hexanes (3:2 then 96:4) to give 5.25 g (55%) of **18** as a clear oil: TLC (ethyl acetate–hexanes, 25:1) R_f = 0.33; IR (TF) 3300, 1740, 1670 cm^{-1}; $[\alpha]^{27}_D$ = +4.2° (c = 2.50, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.40–1.95 (m, 8 H, CH_2), 2.00–2.15 (m, 6 H, CH_3CON), 3.40–3.80 (m, 4 H, CH_2N), 4.10–4.30 (m, 1 H, NCHCO), 4.40–4.60 (m, 3 H, allylic H and NCHCO), 4.70–4.85 (m, 4 H, benzylic H), 5.05 (s, 2 H, benzylic H), 5.27 (dd, 2 H, J = 11 and 17 Hz, olefinic H), 5.55 (d, 1 H, J = 9 Hz, NH), 5.75–5.90 (m, 1 H, olefinic H), 7.20–7.40 (m, 15 H, aromatic H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.13, 22.63, 22.94, 28.60, 30.27, 43.0–44.5 (2 br m), 51.68, 52.64, 65.39, 66.40, 75.97, 118.29, 127.59, 127.67, 128.13, 128.38, 128.64, 128.91, 131.36, 134.05, 134.13, 136.18, 156.02, 171.19, 171.87, 172.69 (br); MS (CI, isobutane) m/z 716 (M^+). Anal. ($C_{39}H_{48}N_4O_9$) C, H, N.

N^5-Acetyl-N^5-(benzyloxy)-N^2-(benzyloxycarbonyl)-L-ornithyl-N^5-acetyl-N^5-(benzyloxy)-L-ornithine (19). To dipeptide **14** (5.00 g, 7.02 mmol) was added *O*-benzylhydroxylamine (0.95 g, 7.72 mmol) and $Pd(PPh_3)_4$ (0.81 g, 0.70 mmol) in

(37) Bryan, D. B.; Hall, R. F.; Holden, K. G.; Huffman, W. F.; Gleason, J. G. *J. Am. Chem. Soc.* **1977**, *99*, 2353.

40 mL of dichloromethane under nitrogen in a foil-covered flask. The reaction was stirred with TLC monitoring for 5 h followed by evaporation of the solvent. The residue was dissolved in ethyl acetate and washed with 1 M HCl and brine, then dried, filtered, and evaporated to give a yellow oil. The oil was purified by chromatography eluting first with ethyl acetate then 1:10:2 acetic acid–ethyl acetate–methanol. After evaporation of the solvents, the residue, containing some acetic acid, was dissolved in ethyl acetate and washed with 1 M HCl, water, and brine, then dried, filtered, and concentrated to give a pale yellow solid. Recrystallization from hot ethyl acetate gave 3.13 g (66%) of **19** as a white solid: mp 129–130 °C; TLC (acetic acid–ethyl acetate–methanol, 1:10:2) R_f = 0.54; $[\alpha]^{27}_D$ = +11.4° (c = 1.00, $CHCl_3$); IR (KBr) 3320, 1720, 1695, 1665, 1645 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 1.50–1.95 (m, 8 H, CH_2), 2.01 (s, 3 H, CH_3CON), 2.02 (s, 3 H, CH_3CON), 3.50–3.80 (m, 4 H, CH_2N), 4.10–4.27 (m, 1 H, NC*H*CO), 4.33–4.50 (m, 1 H, NC*H*CO), 4.75–4.94 (m, 4 H, benzylic H), 4.98–5.10 (m, 2 H, benzylic H), 7.20–7.45 (m, 15 H, aromatic H); ^{13}C NMR (CD_3OD, 75 MHz) δ 20.48, 24.29, 24.41, 29.86, 30.54, 45.0–46.0 (br m), 53.10, 55.66, 67.60, 77.16, 128.72, 128.93, 129.43, 129.66, 129.94, 130.65, 135.99, 138.12, 158.27, 174.0–174.4 (br m), 174.54, 174.69; MS (CI, isobutane) m/z 677 (M^+). Anal. ($C_{36}H_{44}N_4O_9$) C, H, N.

N^5-Acetyl-N^5-hydroxy-L-ornithyl-N^5-acetyl-N^5-hydroxy-L-ornithine (20). To a solution of **19** (0.250 g, 0.372 mmol) in 10 mL of methanol and 5 mL of deionized distilled water was added 0.050 g of 10% Pd/C. Hydrogen was bubbled through the solution for 4 h with stirring. The catalyst was removed by filtration and the methanol was removed by evaporation at ambient temperature. The aqueous residue was extracted with ethyl acetate and the aqueous phase was lyophilized to give 0.132 g (98%) of **20** as a off-white solid: $FeCl_3$ positive (red-purple); mp 120–121 °C; paper chromatography (Whatman No. 1, water–2-propanol 1:2) R_f = 0.55; $[\alpha]^{27}_D$ = +18.2° (c = 1.14, deionized distilled water), IR (KBr) 3700–2700 (br), 1620 (br) cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 1.30–1.80 (m, 8 H, CH_2), 1.85–2.00 (m, 6 H, CH_3CON), 3.40–3.55 (m, 4 H, CH_2N), 3.80–3.90 (m, 1 H, NC*H*CO), 3.93–4.03 (m, 1 H, NC*H*CO); ^{13}C NMR (D_2O, 75 MHz, all signals observed at 20 °C reported) δ 19.29, 19.57 sh (methyl signals), 21.36, 21.85, 21.99, 22.57, 23.11, 23.73, 24.92, 27.98, 28.21, 28.45, 28.71, 38.45, 38.92, 46.97, 47.46, 50.69, 51.05 (methylene signals), 52.73, 55.28 (methine signals), 168.89, 169.15 sh (br m), 173.61, 173.80, 173.88, 173.94, 177.84 (carbonyl signals); MS (positive ion FAB, glycerol) m/z 363 (M + 1), 385 (M + Na). Anal. ($C_{14}H_{26}N_4O_7$) C, H, N.

Allyl N^5-Acetyl-N^5-(benzyloxy)-N^2-(benzyloxycarbonyl)-L-ornithyl-N^5-acetyl-N^5-(benzyloxy)-L-ornithyl-N^5-acetyl-N^5-(benzyloxy)-L-ornithinate (21). To a solution of **12** (1.86 g, 4.11 mmol) in 10 mL of dichloromethane under nitrogen was added 10 mL of 30% HBr in acetic acid. After stirring for 10 min, the mixture was evaporated and diluted with ethyl acetate and water. The organic phase was discarded and the aqueous phase was adjusted to pH 8–9 with saturated sodium carbonate in the presence of ethyl acetate. The aqueous phase was reextracted twice with ethyl acetate; the organic layers were pooled and washed with brine, dried, filtered, and evaporated. Immediately 20 mL of anhydrous dichloromethane was added and the solution transferred to a mixture of the acid **19** (2.78 g, 4.11 mmol) and EEDQ (1.32 g, 5.34 mmol) under nitrogen. The mixture was stirred overnight, evaporated, diluted with ethyl acetate, and washed with 1 M HCl and brine, dried, filtered, and evaporated to give 4.47 g of an oil. The oil was purified by repeated flash silica gel column chromatography eluting with 1:30 methanol–ethyl acetate to provide 3.13 g (78%) of **21** as a thick viscous oil: TLC (methanol–ethyl acetate, 1:30) R_f = 0.41; IR (TF) 3300, 1735, 1720, 1655 cm^{-1}; $[\alpha]^{28}_D$ = −3.1° (c = 1.00, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) δ 1.40–1.95 (m, 12 H, CH_2), 2.05 (s, 3 H, CH_3CON), 2.08 (s, 3 H, CH_3CON), 2.10 (s, 3 H, CH_3CON), 3.40–3.80 (m, 4 H, CH_2N), 3.95–4.20 (m, 2 H, CH_2N), 4.35–4.70 (m, 5 H, allylic H and NC*H*CO), 4.75–4.90 (m, 6 H, benzylic H), 5.10 (s, 2 H, benzylic H), 5.15–5.30 (m, 2 H, olefinic H), 5.70–5.90 (m, 1 H, olefinic H), 7.15 (d, 1 H, J = 9 Hz, N*H*), 7.23–7.40 (m, 20 H, aromatic H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.28, 22.93, 23.11, 28.71, 29.49, 30.32, 43.0–45.0 (3 br m), 51.24, 51.87, 53.25, 65.53, 66.61, 76.14, 118.42, 127.79, 127.84, 128.28, 128.53, 128.78, 129.02, 131.50, 134.21, 136.27, 156.19, 171.34, 171.59, 172.00, 172.89 (br m); MS (CI, isobutane) m/z 938 (M − C_3H_5). Anal. ($C_{53}H_{66}O_{12}N_6$) C, H, N.

N^5-Acetyl-N^5-(benzyloxy)-N^2-(benzyloxycarbonyl)-L-ornithyl-N^5-acetyl-N^5-(benzyloxy)-L-ornithyl-N^5-acetyl-N^5-(benzyloxy)-L-ornithine (22). To tripeptide **21** (3.03 g, 3.09 mmol) and O-benzylhydroxylamine (0.42 g, 3.40 mmol) under nitrogen in a foil-covered flask was added $(Ph_3P)_4Pd$ (0.36 g, 0.31 mmol) and 30 mL of anhydrous dichloromethane. The reaction was monitored by TLC and after 5 h evaporated, diluted with ethyl acetate, and washed with 1 M HCl and brine, then dried, filtered, and evaporated to give a yellow oil. The oil was purified by flash silica gel column chromatography eluting successively with ethyl acetate, 1:30 methanol–ethyl acetate, and then 1:10:2 acetic acid–ethyl acetate–methanol. Following evaporation of the appropriate fractions, the solid was dissolved in ethyl acetate, washed with 1 M HCl, water, and brine, then dried, filtered, and evaporated. The off-white solid was recrystallized from hot ethyl acetate–hexanes to provide 2.10 g (74%) of **22** as a white amorphous solid: mp 118–119 °C; TLC (acetic acid–ethyl acetate–methanol, 1:10:2) R_f = 0.50; IR (TF) 3300, 3420–2700 (br), 1720, 1655 (br) cm^{-1}; $[\alpha]^{28}_D$ = +3.6° (c = 1.00, $CHCl_3$); ^1H NMR (CD_3OD, 300 MHz) δ 1.50–1.90 (m, 12 H, CH_2), 2.00 (s, 6 H, CH_3CON), 2.10 (s, 3 H, CH_3CON), 3.50–3.80 (m, 6 H, CH_2N), 4.10–4.25 (m, 1 H, NC*H*CO), 4.30–4.50 (m, 2 H, NC*H*CO), 4.75–4.96 (m, 6 H, benzylic H), 5.05 (AB q, 2 H, J = 16 Hz, benzylic H), 7.20–7.46 (m, 20 H, aromatic H), 7.88 (s, 1, N*H*), 8.00–8.20 (m, 2 H, N*H*); ^{13}C NMR (CD_3OD, 75 MHz) δ 20.50, 24.35, 29.77, 30.39, 44.5–45.0 (br m), 53.10, 53.76, 55.71, 67.57, 77.09, 128.68, 128.88, 129.39, 129.61, 129.88, 130.59, 135.92, 138.02, 158.23, 173.63, 174.19 (m), 174.33, 174.61; MS (positive ion FAB, matrix: glycerol–methanol) m/z 940 (M + 1). Anal. ($C_{50}H_{62}O_{12}N_6$) C, H, N.

N^5-Acetyl-N^5-hydroxy-L-ornithyl-N^5-acetyl-N^5-hydroxy-L-ornithyl-N^5-acetyl-N^5-hydroxy-L-ornithine (23).[4d] To protected tripeptide **22** (0.250 g, 0.267 mmol) in 10 mL of methanol and 5 mL of deionized distilled water was added 0.050 g of 10% Pd/C. After hydrogen was bubbled through the solution for 3 h, the catalyst was removed by filtration and the methanol was removed by evaporation at ambient temperature. The aqueous residue was extracted with ethyl acetate and the aqueous layer was lyophilized to provide 0.144 g (100%) of **23** as a off-white solid: $FeCl_3$ positive (red-purple); mp 73–75 °C; $[\alpha]^{24}_D$ = +4.0° (c = 0.100, deionized distilled water); IR (KBr) 3700–2300 (br), 1620 (br) cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 1.30–1.80 (m, 12 H, CH_2), 1.90–2.00 (m, 9 H, CH_3CON), 3.38–3.53 (m, 6 H, CH_2N), 3.84 (t, 1 H, J = 6 Hz, NC*H*CO), 3.90–4.05 (m, 1 H, NC*H*CO), 4.15–4.28 (m, 1 H, NC*H*CO); ^{13}C NMR (D_2O, 75 MHz, all signals observed at 20 °C reported) δ 19.29 (methyl signal), 21.67, 21.88, 22.47, 22.64, 23.89, 24.70, 24.76, 28.13, 28.22, 28.35, 29.10, 29.32, 38.40, 46.5–48.5 (br m) (methylene signals), 52.91, 53.83, 54.82 (methine signals), 169.69, 171.75, 173.0–174.0 (br m), 177.46 (carbonyl signals); MS (positive ion FAB, glycerol) m/z 535 (M + 1), 557 (M + Na). Anal. ($C_{21}H_{38}N_6O_{10}$) C, H, N.

Benzyl N^5-Acetyl-N^5-(benzyloxy)-N^2-(benzyloxycarbonyl)-L-ornithyl-N^5-acetyl-N^5-(benzyloxy)-L-ornithyl-N^5-acetyl-N^5-(benzyloxy)-L-ornithyl-L-serinate (24). To a solution of tripeptide **22** (0.250 g, 0.267 mmol) and the free amine of L-serine benzyl ester [freshly prepared from its hydrochloride salt (0.062 g, 0.267 mmol)] in 2 mL of anhydrous dichloromethane was added EEDQ (0.660 g, 0.267 mmol) and the mixture stirred overnight under nitrogen. The mixture was evaporated, diluted with ethyl acetate, and washed with 1 M HCl and brine, then dried, filtered, and evaporated to give 0.263 g of a white solid. Radial chromatography, eluting with 7.5% methanol–92.5% ethyl acetate, followed by recrystallization from ethyl acetate–ether gave 0.200 g (67%) of **24** as a white, amorphous solid: mp 108–110 °C; TLC (methanol–ethyl acetate, 2:25) R_f = 0.30; $[\alpha]^{26}_D$ = −10.0° (c = 1.86, $CHCl_3$); IR (KBr) 3300, 1740, 1720, 1690, 1660, 1650, 1630 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.50–1.90 (m, 12 H, CH_2), 2.00 (s, 3 H, CH_3CON), 2.04 (s, 3 H, CH_3CON), 2.05 (s, 3 H, CH_3CON), 3.02 (br s, 1 H, CH_2OH), 3.45–3.55 (m, 4 H, CH_2N), 3.60–4.00 (m, 4 H, CH_2N and CH_2OH), 4.20–4.40 (m, 2 H, NC*H*CO), 4.45–4.65 (m, 2 H, NC*H*CO), 4.68–4.82 (m, 6 H, benzylic H), 4.95–5.20 (m, 4 H, benzylic H), 6.05 (d, 1 H, J = 8 Hz, N*H*), 7.20–7.40 (m, 25 H, aromatic H), 7.50–7.70 (br m, 3 H, N*H*); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 19.97, 20.03, 22.82, 22.95, 28.53, 29.57, 43.5–44.5 (br

m), 51.78, 51.89, 52.25, 52.37, 53.70, 53.80, 54.53, 54.61, 62.06, 62.18, 66.44, 66.55, 75.87, 76.37, 127.50, 127.56, 127.74, 128.05, 128.29, 128.54, 128.80, 133.92, 135.09, 135.93, 156.18, 169.69, 171.09, 171.17, 171.43, 171.50, 172.0–173.0 (br m); MS (positive ion FAB, glycerol) m/z 1117 (M + 1). Anal. ($C_{60}H_{73}N_7O_{14}$) C, H, N.

N^5-Acetyl-N^5-hydroxy-L-ornithyl-N^5-acetyl-N^5-hydroxy-L-ornithyl-N^5-acetyl-N^5-hydroxy-L-ornithyl-L-serine (25). To protected tetrapeptide **24** (0.184 g, 0.165 mmol) was added 20 mL of methanol, 5 mL of deionized distilled water, and 0.037 g of 10% Pd/C. Hydrogen was bubbled through the solution for 3 h. The catalyst was removed by filtration and the methanol was removed by evaporation at ambient temperature. The aqueous residue was extracted with ethyl acetate, and the aqueous phase was lyophilized to give 0.103 g (100%) of **25** as a light tan solid: $FeCl_3$ positive (red-purple); mp 163–165 °C dec; $[a]^{26}_D = -8.2°$ (c = 0.086, deionized distilled water); IR (KBr) 3700–2300 (br), 1625 (br) cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 1.30–1.80 (m, 12 H, CH_2), 1.85–2.00 (m, 9 H, CH_3CON), 3.35–3.50 (m, 6 H, CH_2N), 3.64 (d, 2 H, J = 4 Hz, CH_2OH), 3.82–3.92 (m, 1 H, NCHCO), 4.02–4.10 (m, 1 H, NCHCO), 4.15–4.30 (m, 2 H, NCHCO); ^{13}C NMR (D_2O, 75 MHz, all signals observed at 20 °C reported) δ 19.32, 19.56 sh (methyl signals), 21.37, 21.89, 22.30, 22.41, 22.95, 22.98, 23.01, 28.01, 28.16, 38.43, 38.64, 38.69, 46.94, 47.19, 50.5–51.0 (m) (methylene signals), 52.51, 53.42, 56.98 (methine signals), 62.05 (methylene signal), 169.19, 169.43, 172.42, 172.45, 172.54, 172.80, 172.92, 173.75, 173.88, 175.61 (carbonyl signals); MS (positive ion FAB, glycerol) m/z 622 (M + 1). Anal. ($C_{24}H_{43}N_7O_{12}$) C, H, N.

N^2-(*tert*-Butyloxycarbonyl)-L-seryl-1,4-dibenzyl-D-aspartate (26). To a solution of N^2-(*tert*-butyloxycarbonyl)-L-serine (0.513 g, 2.50 mmol) and 1,3-dibenzyl-D-aspartate (0.875 g, 2.50 mmol, freshly prepared from the HCl salt by neutralization and extraction) in 10 mL of anhydrous dichloromethane was added EEDQ (0.619 g, 2.50 mmol). The mixture was stirred under nitrogen overnight. The solution was evaporated, diluted with ethyl acetate, washed with 1 M HCl and brine, then dried, filtered, and evaporated to provide an oil. Crystallization from ether–hexanes gave 0.701 g (56%) of **26** as a white solid: mp 72–74 °C; TLC (ethyl acetate–hexanes 2:1) R_f = 0.50; $[\alpha]^{27}_D = -33.0°$ (c = 1.00, $CHCl_3$); IR (KBr) 3350, 1750, 1740, 1690, 1660 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.43 (s, 9 H, $C(CH_3)_3$), 2.89 (dd, 1 H, J = 5 and 17 Hz, CH_2), 3.04 (dd, 1 H, J = 5 and 17 Hz, CH_2), 3.20–3.40 (br s, 1 H, CH_2OH), 3.55–3.70 (m, 1 H, CH_2), 3.90–4.05 (m, 1 H, CH_2), 4.10–4.30 (m, 1 H, NCHCO), 4.90–4.98 (m, 1 H, NCHCO), 4.99–5.16 (m, 4 H, benzylic H), 5.68 (d, 1 H, J = 7 Hz, NH), 7.20–7.38 (m, 10 H, aromatic H), 7.55–7.65 (br m, 1 H, NH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 28.16, 36.00, 48.75, 55.52, 62.78, 66.80, 67.55, 80.29, 128.11, 128.28, 128.36, 128.47, 134.93, 135.18, 155.80, 170.23, 170.39, 171.16; MS (CI, ammonia) m/z 501 (M + 1), 518 (M + NH_4^+). Anal. ($C_{26}H_{32}N_2O_8$) C, H, N.

N^5-Acetyl-N^5-(benzyloxy)-N^2-(benzyloxycarbonyl)-L-ornithyl-N^5-acetyl-N^5-(benzyloxy)-L-ornithyl-N^5-acetyl-N^5-(benzyloxy)-L-ornithyl-L-seryl-1,4-dibenzyl-D-aspartate (27). To a solution of **26** (0.134 g, 0.267 mmol) in 1.0 mL of anhydrous dichloromethane was added 1.0 mL of trifluoroacetic acid under nitrogen. The mixture was stirred for 1 h, evaporated, neutralized with saturated sodium carbonate, and extracted with ethyl acetate. The organic layer was washed with brine, then dried, filtered, and evaporated. To the free amine residue was added tripeptide acid **22** (0.250 g, 0.267 mmol), 2.0 mL of anhydrous dichloromethane, and EEDQ (0.066 g, 0.267 mmol). The mixture was stirred under nitrogen overnight, evaporated, diluted with ethyl acetate, washed with 1 M HCl and brine, then dried, filtered, and evaporated to give 0.314 g of a white solid. Radial chromatography, eluting with 7.5% methanol–92.5% ethyl acetate, gave 0.191 g (54%) of **27** as a white solid: mp 110–112 °C; TLC (methanol–ethyl acetate, 2:25) R_f = 0.44; IR (KBr) 3700–3200 (br), 3290, 1745, 1710, 1655, 1630 cm^{-1}; $[\alpha]^{26}_D = -15.0$ (c = 1.50, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) δ 1.40–1.85 (m, 12 H, CH_2), 2.02 (s, 3 H, CH_3CON), 2.03 (s, 3 H, CH_3CON), 2.05 (s, 3 H, CH_3CON), 2.66–2.77 (br s, 1 H, CH_2OH), 2.84–3.00 (m, 2 H, CH_2), 3.40–4.00 (m, 8 H, CH_2N and CH_2OH), 4.30–4.65 (m, 4 H, NCHCO), 4.75 (s, 6 H, benzylic H), 4.85–5.12 (m, 7 H, NCHCO and benzylic H), 6.05–6.15 (br s, 1 H, NH), 7.00–7.40 (m, 30 H, aromatic H), 7.45–7.80 (m, 4 H, NH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.30, 23.18, 23.39, 23.47, 23.64, 28.84, 29.56, 29.75, 36.15, 44.0–44.5 (br m), 48.82, 52.93, 53.20, 54.36, 55.03, 62.57, 66.62, 66.81, 67.20, 76.19, 127.78, 127.96, 128.14, 128.19, 128.36, 128.60, 128.87, 129.11, 134.20, 135.15, 135.39, 136.21, 156.61, 170.18, 170.25, 171.82, 172.08, 172.5–173.0 (m); MS (positive ion FAB, matrix: glycerol, m-nitrobenzyl alcohol, and methanol) m/z 1322 (M + 1). Anal. ($C_{71}H_{84}N_8O_{17}$) C, H, N.

N^5-Acetyl-N^5-hydroxy-L-ornithyl-N^5-acetyl-N^5-hydroxy-L-ornithyl-N^5-acetyl-N^5-hydroxy-L-ornithyl-L-seryl-D-aspartic Acid (28). To 157 mg of fully protected pentapeptide **27** was added 20 mL of methanol, 5 mL of deionized distilled water, and 0.032 g of 10% Pd/C. Hydrogen was bubbled through the solution for 3 h. The catalyst was removed by filtration and the methanol was removed by evaporation at ambient temperature. The aqueous residue was extracted with ethyl acetate and the aqueous phase was lyophilized to provide 0.084 g (97%) of **28** as a light tan solid: $FeCl_3$ positive (red-purple); mp 162–164 °C dec; $[\alpha]^{26}_D = -31.9°$ (c = 0.342, deionized distilled water); IR (KBr) 3700–2300 (br), 1630 (br) cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 1.30–1.80 (m, 12 H, CH_2), 1.94 (s, 9 H, CH_3CON), 2.48–2.68 (m, 2 H, CH_2), 3.40–3.50 (m, 6 H, CH_2N), 3.62–3.68 (m, 2 H, CH_2OH), 3.82–3.92 (m, 1 H, NCHCO), 4.14–4.40 (m, 4 H, NCHCO); ^{13}C NMR (D_2O, 75 MHz, all signals observed at 20 °C reported) δ 19.30, 19.57 sh (methyl signals), 21.36, 21.88, 22.20, 22.41, 22.8–23.0 (m), 27.96, 28.09, 37.68, 46.94, 47.13, 47.23, 50.5–51.0 (m) (methylene signals), 51.48, 52.50, 53.34, 55.46 (methine signals), 61.29 (methylene signal), 169.35, 170.52, 172.97, 173.29, 173.79, 173.91, 176.16, 176.50 (carbonyl signals); MS (positive ion FAB, glycerol) m/z 737 (M + 1). Anal. ($C_{28}H_{50}N_9O_{15}\cdot H_2O$) C, H, N.

N-(Triphenylmethyl)-D-phenylglycine (29). To a suspension of D-(−)-phenylglycine (0.302 g, 2.0 mmol) in 2.0 mL of DMF was added trimethylsilyl chloride (290 μL, 2.1 mmol) under nitrogen. After stirring for 10 min a thick white paste had formed to which was added triphenylmethyl chloride (0.586 g, 2.1 mmol) in 1.0 mL of DMF. Distilled Et_3N (0.59 mL, 4.2 mmol) was added dropwise, resulting in formation of a tan solution with a fine, white solid in suspension. The mixture was stirred for 2 h, diluted with water and ether, and acidified to pH 3–4 and the aqueous phase was extracted with ether. The organic phases were washed with brine, then dried, filtered, and evaporated to give 0.794 g of a crude white foam. Flash chromatography, eluting first with ethyl acetate–hexanes (1:2) then with methanol–ethyl acetate (1:4), followed by evaporation, gave an oil which was redissolved in ether and repeatedly evaporated to remove any residual methanol. The result was 0.485 g (62%) of **29** as a white foam: TLC (ethyl acetate–hexanes 1:2) R_f = 0.50; IR (KBr) 3700–2300 (br), 3320, 1720 cm^{-1}; $[\alpha]^{21}_D = -134°$ (c = 2.70, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) δ 4.29 (s, 1 H, NCHCO), 6.95–7.50 (m, 20 H, aromatic H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 60.40, 71.95, 126.58, 127.09, 127.81, 128.55, 128.82, 139.40, 145.54, 178.59; MS (CI, ammonia) 394 (M + 1).

Benzyl N-(Triphenylmethyl)-D-phenylglycinate (31). To a solution of **29** (2.00 g, 5.08 mmol) in 10.0 mL of DMF was added potassium carbonate (0.773 g, 5.59 mmol) and benzyl bromide (0.67 mL, 5.59 mmol). The mixture was stirred overnight under nitrogen. The reaction was diluted with ether and washed with water and brine, then dried, filtered, and evaporated to provide 2.51 g of a cream solid. Recrystallization from ethyl acetate–methanol gave 1.47 g (60%) of **31** as a white, amorphous solid: mp 136–138 °C; TLC (ethyl acetate–hexanes 1:16) R_f = 0.29; $[\alpha]^{27}_D = -100°$ (c = 1.00, $CHCl_3$); IR (KBr) 3350, 3040, 2880, 1740 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.40 (d, 1 H, J = 10 Hz, NH), 4.48 (AB q, 2 H, J = 12 and 13 Hz, benzylic H), 4.47 (d, 1 H, J = 9 Hz, benzylic H), 6.96–7.54 (m, 25 H, aromatic H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 60.10, 66.69, 72.03, 126.44, 127.06, 127.50, 127.73, 127.80, 127.94, 128.26, 128.47, 128.89, 135.25, 139.92, 145.82, 173.17; MS (CI, ammonia) m/z 484 (M + 1). Anal. ($C_{34}H_{29}NO_2$) C, H, N.

Benzyl N^5-Acetyl-N^5-(benzyloxy)-N^2-(benzyloxycarbonyl)-L-ornithyl-N^5-acetyl-N^5-(benzyloxy)-L-ornithyl-N^5-acetyl-N^5-(benzyloxy)-L-ornithyl-D-phenylglycinate (33). To a solution of **31** (0.129 g, 0.267 mmol) in 1.0 mL of anhydrous dichloromethane was added 0.5 mL of trifluoroacetic acid to give a bright yellow solution. TLC analysis showed no starting **31** remaining and the solution was evaporated. THF was added followed by water and the yellow dissipated. This solution was stirred for 10 min and evaporated and the residue was dissolved in ethyl acetate and saturated sodium carbonate. The organic

phase was washed with brine, then dried, filtered, and evaporated to provide an oil. This oil was immediately dissolved in 2.0 mL of anhydrous dichloromethane and transferred to a flask containing tripeptide acid **22** (0.250 g, 0.267 mmol) and EEDQ (0.066 g, 0.267 mmol). The mixture was stirred overnight under nitrogen and then evaporated. The residue was dissolved in ethyl acetate, washed with 1 M HCl and brine, then dried, filtered, and evaporated to give 0.342 g of an oil. Radial chromatography, eluting with 3% methanol–97% ethyl acetate, gave 0.265 g (85%) of **33** as a foam. This foam resisted recrystallization from several solvent systems: mp 42–44 °C; TLC (methanol–ethyl acetate, 1:30) R_f = 0.34; $[\alpha]^{23}_D$ = −32.0° (*c* = 2.38, $CHCl_3$); IR (KBr) 3300, 3040, 2940, 1745, 1720, 1655 and 1635 (br); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.50–1.85 (m, 12 H, CH_2), 1.95 (s, 3 H, CH_3CON), 2.05 (s, 3 H, CH_3CON), 2.06 (s, 3 H, CH_3CON), 3.20–3.60 (m, 2 H, CH_2N), 3.70–4.05 (m, 4 H, CH_2N), 4.35–4.50 (m, 1 H, NC*H*CO), 4.55–4.85 (m, 8 H, benzylic H and NC*H*CO), 4.95–5.20 (m, 5 H, benzylic H), 5.55 (d, 1 H, *J* = 7 Hz, N*H*), 5.97 (d, 1 H, *J* = 8 Hz, N*H*), 7.05–7.40 (m, 30 H, aromatic H), 7.47 (d, 1 H, *J* = 7 Hz, N*H*), 7.77 (d, 1 H, *J* = 7 Hz, N*H*); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.10, 20.12, 20.18, 22.94, 23.08, 29.05, 29.25, 30.08, 43.5–44.5 (br m), 51.72, 52.01, 53.50, 56.33, 66.49, 66.71, 75.91, 76.01, 77.20, 127.11, 127.42, 127.62, 127.70, 127.81, 128.05, 128.10, 128.16, 128.41, 128.47, 128.64, 128.93, 128.97, 134.14, 135.05, 136.01, 136.18, 156.20, 169.92, 170.81, 171.74, 172.12, 172.4–172.8 (br m); MS (positive ion FAB, matrix: glycerol, *m*-nitrobenzyl alcohol, and acetone) *m*/*z* 1163 (M + 1). Anal. ($C_{65}H_{75}N_7O_{13}$) C, H, N.

N^5-Acetyl-N^5-hydroxy-L-ornithyl-N^5-acetyl-N^5-hydroxy-L-ornithyl-N^5-acetyl-N^5-hydroxy-L-ornithyl-D-phenylglycine (35). To a solution of **33** (0.226 g, 0.194 mmol) in 10 mL of methanol and 5 mL of deionized distilled water was added 0.046 g of 10% Pd/C. Hydrogen was bubbled through this solution for 3 h and the catalyst was removed by filtration. The methanol was evaporated at ambient temperature and the aqueous residue was extracted with ethyl acetate. The aqueous phase was lyophilized to provide 0.108 g (83%) of **35** as a light tan solid: $FeCl_3$ positive (red-purple); mp 172–174 °C dec; $[\alpha]^{23}_D$ = −84.0° (*c* = 0.100, deionized distilled water); ^{1}H NMR (D_2O, 300 MHz) δ 1.30–1.85 (m, 12 H, CH_2), 1.91 (s, 6 H, CH_3CON), 1.94 (s, 3 H, CH_3CON), 3.30–3.50 (m, 6 H, CH_2N), 3.80–3.95 (m, 1 H, NC*H*CO), 4.15–4.30 (m, 2 H, NC*H*CO), 5.00 (s, 1 H, benzylic H), 7.19 (s, 5 H, aromatic H); ^{13}C NMR (D_2O, 75 MHz, all signals observed at 20 °C reported) δ 19.31, 19.50 sh (methyl signals), 21.35, 22.33, 27.94, 28.06, 28.29, 46.92, 47.12 (methylene signals), 52.50, 53.31, 53.47, 59.11 (methine signals except aromatics), 127.02, 127.97, 128.80, 138.47 (aromatic signals), 169.31, 171.88, 172.88, 173.59, 173.75, 173.90, 175.50 (carbonyl signals); MS (positive ion FAB, glycerol and water) *m*/*z* 668 (M + 1), 690 (M + Na). Anal. ($C_{29}H_{45}N_7O_{11}$) C, H, N.

***N*-(*tert*-Butyoxycarbonyl)-D-4-hydroxyphenylglycine Benzyl Ester (32).** To a solution of *N*-Boc-4-hydroxyphenylglycine (**30**; 6.75 g, 25.2 mmol) in 10 mL of DMF was added potassium bicarbonate (2.53 g, 25.3 mmol) and benzyl bromide (4.32 g, 25.2 mmol). The mixture was stirred overnight under nitrogen and the DMF was removed under high vacuum. The residue was diluted with ethyl acetate and washed with water and brine, then dried, filtered, and evaporated to provide an oil. Crystallization from ethyl acetate–hexanes gave 5.27 g (58%) of **32** as a white solid: mp 103–105 °C; TLC (ethyl acetate–hexanes 1:2) R_f = 0.42; IR (KBr) 3440, 3360, 1740, 1675 cm^{-1}; $[\alpha]^{25}_D$ = −77.3° (*c* = 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.43 (s, 9 H, $C(CH_3)_3$), 5.00–5.20 (m, 2 H, benzylic H), 5.26 (d, 1 H, *J* = 7 Hz, benzylic NC*H*CO), 5.35–5.45 (br s, 1 H, phenol O*H*), 5.65 (d, 1 H, *J* = 7 Hz, N*H*), 6.68 (d, 2 H, *J* = 8 Hz, aromatic H), 7.11 (d, 2 H, *J* = 8 Hz, aromatic H), 7.15–7.40 (m, 5 H, aromatic H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 28.25, 57.23, 67.28, 80.53, 115.74, 127.83, 128.22, 128.38, 135.07, 155.10, 156.41, 171.32; exact MS (EI) calcd for $C_{20}H_{23}NO_5$ (M^+) 357.1576, found 357.1571. Anal. ($C_{20}H_{23}NO_5$) C, H, N.

Benzyl N^5-Acetyl-N^5-(benzyloxy)-N^2-(benzyloxycarbonyl)-L-ornithyl-N^5-acetyl-N^5-(benzyloxy)-L-ornithyl-N^5-acetyl-N^5-(benzyloxy)-L-ornithyl-D-4-hydroxyphenylglycinate (34). To a solution of **32** (0.096 g, 0.267 mmol) in 1.0 mL of anhydrous dichloromethane was added 1.0 mL of trifluoroacetic acid under nitrogen. The mixture was stirred for 0.25 h at which time TLC analysis showed an absence of starting **32**. The mixture was evaporated and 2.0 mL of anhydrous dichloromethane was added followed by triethylamine (37 μL, 0.267 mmol). Immediately, tripeptide acid **22** (0.250 g, 0.267 mmol) and EEDQ (0.066 g, 0.267 mmol) were added. The mixture was stirred overnight under nitrogen. The solvent was evaporated and the residue was diluted with ethyl acetate, washed with 1 M HCl and brine, then dried, filtered, and evaporated to afford 0.321 g of a white foam. Radial chromatography, eluting with methanol–ethyl acetate (3:97), gave 0.202 g (64%) of **34** as a white foam which resisted all recrystallization attempts: mp 50–52 °C; TLC (methanol–ethyl acetate 1:30) R_f = 0.24; $[\alpha]^{23}_D$ = −30.3° (*c* = 2.03, $CHCl_3$); IR (KBr) 3700–3100 (br), 3300, 1740, 1720, 1655 and 1640 (br) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.40–1.80 (m, 12 H, CH_2), 1.95 (s, 3 H, CH_3CON), 2.03 (s, 3 H, CH_3CON), 2.05 (s, 3 H, CH_3CON), 2.95–3.10 (br s, 1 H, phenol O*H*), 3.40–3.60 (m, 2 H, CH_2N), 3.65–4.00 (m, 4 H, CH_2N), 4.30–4.41 (m, 1 H, NC*H*CO), 4.47–4.63 (m, 2 H, NC*H*CO), 4.65–4.80 (m, 6 H, benzylic H), 4.90–5.10 (m, 5 H, benzylic H), 5.39 (d, 1 H, *J* = 7 Hz, N*H*), 5.88 (d, 1 H, *J* = 8 Hz, N*H*), 6.72 (d, 2 H, *J* = 8 Hz, aromatic H), 7.06–7.50 (m, 27 H, aromatic H), 7.68 (d, 1 H, *J* = 6 Hz, N*H*), 8.50 (s, 1 H, N*H*); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.17, 20.24, 23.02, 23.06, 23.17, 28.93, 29.11, 29.14, 29.40, 29.97, 30.04, 43.0–45.0 (br m), 52.13, 52.30, 53.69, 56.14, 66.71, 76.04, 76.13, 77.20, 115.71, 126.64, 127.49, 127.75, 127.85, 128.20, 128.27, 128.53, 128.78, 129.04, 129.08, 134.12, 135.23, 136.17, 156.33, 157.05, 170.41, 170.93, 171.93, 172.29, 172.38–173.00 (br m); MS (positive ion FAB, matrix: glycerol, *m*-nitrobenzyl alcohol, and acetone) *m*/*z* 1179 (M + 1). Anal. ($C_{65}H_{75}N_7O_{14}$) C, H, N.

N^5-Acetyl-N^5-hydroxy-L-ornithyl-N^5-acetyl-N^5-hydroxy-L-ornithyl-N^5-acetyl-N^5-hydroxy-L-ornithyl-D-4-hydroxyphenylglycine (36). To a solution of protected peptide **34** (0.170 g, 0.144 mmol) in 10 mL of methanol and 5 mL of deionized distilled water was added 0.034 g of 10% Pd/C. Hydrogen was bubbled through the solution for 3 h and the catalyst was removed by filtration. The methanol was evaporated at ambient temperature and the aqueous residue was extracted with ethyl acetate. The aqueous phase was lyophilized to provide 0.092 g (94%) of **36** as a light tan solid: $FeCl_3$ positive (red-purple); mp 158–160 °C dec; IR (KBr) 3700–2300 (br), 1620 (br) cm^{-1}; $[\alpha]^{23}_D$ = −91.0° (*c* = 0.100, distilled deionized water); ^{1}H NMR (D_2O, 300 MHz) δ 1.20–1.80 (m, 12 H, CH_2), 1.91 (s, 6 H, CH_3CON), 1.94 (s, 3 H, CH_3CON), 3.20–3.50 (m, 6 H, CH_2N), 3.86 (t, 1 H, *J* = 6 Hz, NC*H*CO), 4.10–4.30 (m, 2 H, NC*H*CO), 4.90 (s, 1 H, benzylic NC*H*CO), 6.66 (d, 2 H, *J* = 8 Hz, aromatic H), 7.04 (d, 2 H, *J* = 8 Hz, aromatic H); ^{13}C NMR (D_2O, 75 MHz, all signals observed at 20 °C reported) δ 19.28, 19.40, 19.47, 19.53 (methyl signals), 21.35, 22.33, 22.93, 27.86, 27.98, 28.02, 28.29, 46.91, 47.12 (methylene signals), 52.49, 53.36, 53.46, 58.66 (methine signals except aromatics), 115.49, 128.54, 130.67, 155.09 (aromatic signals), 169.39, 171.74, 172.89, 173.61, 173.77, 173.90, 176.15 (carbonyl signals); MS (positive ion FAB, glycerol and water) *m*/*z* 684 (M + 1). Anal. ($C_{29}H_{45}N_7O_{12}$) C, H, N.

Bioassay Test Procedure. Strain SA240 (SA 100 *iuc*D:Tn5), a siderophore biosynthesis mutant of *S. flexneri* 2a strain SA 100, was grown overnight in Luria broth. Luria agar containing the iron chelator ethylenediamine bis(*o*-hydroxyphenylacetic acid) (EDDA; 250 μg/mL) was seeded with the bacterial strain at a concentration of 10^3/mL, poured into plates, and allowed to solidify.

Compounds were prepared as 10 mM stock solutions in water and were diluted in water to give appropriate test concentrations. Sterile Sensi-disks (BBL) containing 10 μL of the compound test solutions were placed on the surface of the seeded agar and the plates were incubated at 37 °C for 18 h. Diameters of zones of stimulation were measured.

This assay was also completed with two *E. coli* strains, RW193, an *E. coli* K12 *ent*A, *fhu*A positive organism, and the AN193 strain, a *ent*A, *fhu*A negative mutant (deficient in the ferrichrome receptor system).

The growth curve procedure using *E. coli* X580 was completed as follows: The preformed iron complex of each respective siderophore peptide was added by filtration through an Acro-Disc 0.2-μ filter assembly to sterile Luria broth containing EDDA (either 0.1 mg/mL or 1.0 mg/mL) to give solutions of 10 and 50 μM final concentration in each case. Immediately, 20 μL of a 26-hour-old Luria broth culture of *E. coli* X580 was added. The

culture flasks were then shaken at 37 °C at 300 rpm. Aliquots were removed every 2 h for culture turbidity measurements at 600 nm.

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Synthesis and Siderophore and Antibacterial Activity of N^5-Acetyl-N^5-hydroxy-L-ornithine-Derived Siderophore–β-Lactam Conjugates: Iron-Transport-Mediated Drug Delivery

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N^5-Acetyl-N^5-hydroxy-L-ornithyl-N^5-acetyl-N^5-hydroxy-L-ornithyl-N^5-acetyl-N^5-hydroxy-L-ornithine, the functionally instrumental component of the albomycins and ferrichromes, has been incorporated as a "carrier" substructure into both carbacephalosporin and oxamazin type β-lactam antibiotics. The previously synthesized protected version of this tripeptide (**14**) was coupled with various β-lactam analogues **17**, **19**, **24**, and **25** to give protected conjugates **21**, **22**, **26**, and **27**. Final deprotection by hydrogenolysis provided the deprotected siderophore–β-lactam antibiotic conjugates **1**–**4**. The growth-promoting ability of each has been evaluated using either the siderophore-deficient mutant *Shigella flexneri* SA 100 or *S. flexneri* SA240 (SA 100 *iuc*D:Tn5). Measurement of the growth-promoting activity using two isogenic *Escherichia coli* strains differing only in the presence or absence of *fhu*A (hydroxamate ferrichrome receptor) suggests uptake by the hydroxamate iron-transport system. The antibacterial activity of these conjugates has been investigated, and the potential for use of the ferrichrome iron-transport system as a means of drug delivery is discussed.

The albomycins (Figure 1) are natural siderophores and antibiotics first isolated in 1947 from *Streptomyces griseus* and given the name grisein.[1] Several years later, another microbial iron-transport agent, named albomycin,[2] was isolated from *Streptomyces subtropicus* and subsequently determined to have the same structure as grisein.[3] Although early extensive structural studies seemed to indicate that albomycin was an iron-chelating siderophore based on the tripeptide N^5-acetyl-N^5-hydroxy-L-ornithyl-N^5-acetyl-N^5-hydroxy-L-ornithyl-N^5-acetyl-N^5-hydroxy-L-ornithine, similar to the ferrichromes[4] (Figure 1), it was not until 1982 that Benz and co-workers[5] firmly established the structure of the albomycins. It has been demonstrated[6] that the linear tripeptide of N^5-acetyl-N^5-hydroxy-L-ornithine is the hexadenate, octahedral ligand for ferric ion responsible for intracellular transport of iron as determined by its growth-promoting ability in *S. flexneri* SA240 (SA 100 *iuc*D:Tn5). This tripeptide apparently utilizes the same ferrichrome iron-transport system[7] as implicated in the case of the albomycins.

The albomycins are not the only family of antibiotics and drugs based on iron-containing natural siderophores.[8] Siderophores and analogues have other potential uses, such as the treatment of iron metabolism disorders. The drug of choice[9] for treatment of the iron overload resulting from

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