Articles

Synthesis of Foroxymithine, a Microbial Fermentation Product and Angiotensin 1 Converting Enzyme Inhibitor

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The first total synthesis of the microbial fermentation product, ferric ion chelator and angiotensin converting enzyme inhibitor foroxymithine 1 is described. In addition to firmly establishing the reported structure of 1, a flexible synthetic strategy was employed which will allow the future syntheses of various derivatives and conjugates. Use of the chiral building block L-glutamic acid and conversion to the protected δ-hydroxynorvaline 3, followed by synthesis of the protected hydroxamic acids 4 and 5, afforded the precursor fragments needed. A series of peptide coupling reactions, including the problems encountered and conditions devised for the formation of the diketopiperazine 13 in good yield, are discussed. Finally, coupling of the left- and right-hand fragments, acetylation, deprotection, and hydrogenolysis gave foroxymithine 1.

The formohydroxamic acid containing microbial fermentation product foroxymithine 1 was first isolated[1] from *Streptomyces nitrosporeus*. Its pharmacological activity as a ferric ion chelator[2] potentiating the antineoplastic agent erbstatin and its ability to inhibit angiotensin converting enzyme and other proteases[3] have been reported. Although no specific studies have been published to date to establish whether foroxymithine is indeed a microbial siderophore necessary for ferric ion transport, a large body of evidence[4] has established that many low molecular weight hydroxamic acids excreted by microorganisms serve this function. We have previously synthesized and studied a number of other siderophores and analogues with the long-term goals of gaining a better understanding of the essential aspects of microbial iron metabolism and demonstrating the therapeutic potential of microbial iron chelator (siderophore) analogues and their conjugates with antimicrobial agents.[5] Recently, we completed the synthesis[5a] of several albomycin-like siderophore-carbacephalosporin antibiotic conjugates that appear to be transported by the ferrichrome iron transport system.[6] This work also established that considerable structural diversity of the conjugates can be tolerated by the ferrichrome iron transport system. Foroxymithine and various conjugates would be especially interesting since foroxymithine has demonstrated broad activity and, most notably, is orally effective. Due to the success in transporting β-lactams via iron transport, we felt that investigation of the possibility of expanding this mode of drug delivery to other microbes was warranted. The utilization of siderophores for iron assimilation has been clearly demonstrated for some microbes (*Pseudomonas*,[7] *Aspergillus*,[8] *Cryptococcus*,[9] *Histoplasma*[10]) yet only implicated in others (e.g., *Candida albicans*, *Toxoplasma*, *Pneumocystis*, and others). For these reasons, we set out to devise a flexible synthesis of foroxymithine 1 which would allow one to synthesize various derivatives, including antimicrobial conjugates, for screening against the various microorganisms cited above. In this paper we report the total synthesis of foroxymithine 1.

In devising an approach to the synthesis of 1, we hoped to begin with the protected version of N^5-hydroxy-L-ornithine 5 utilized previously to synthesize several siderophores in our laboratory,[11] since it could be produced readily from L-glutamic acid in large quantities in optically pure form. As in all total syntheses, selective manipulation of protecting groups throughout was important. In addition, it was necessary to devise a means to attach other molecules (an acetyl group in the case of 1; antimicrobial

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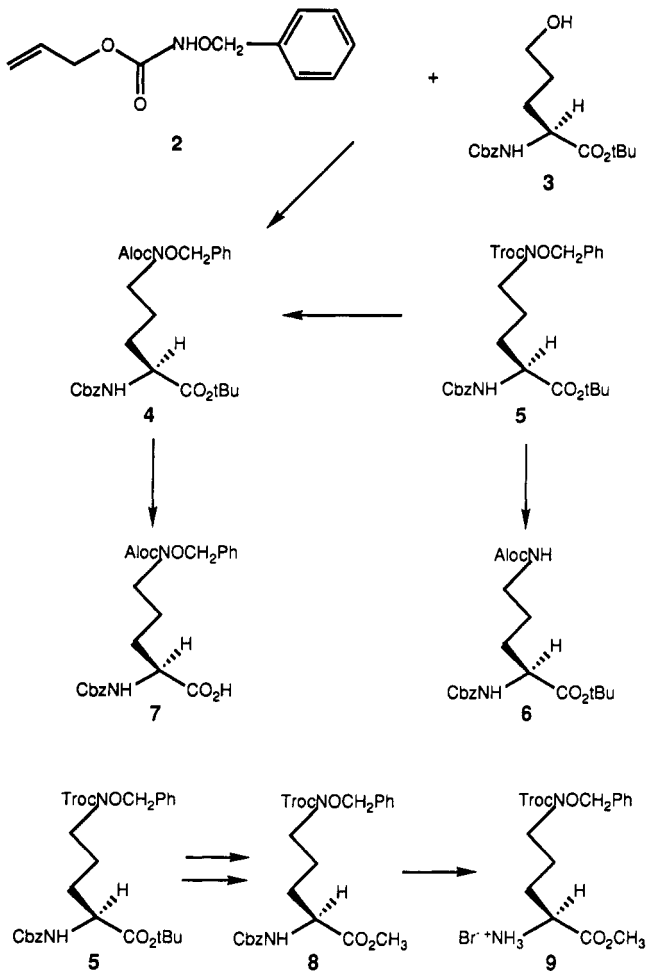
0022-3263/91/1956-0492$02.50/0

Scheme I



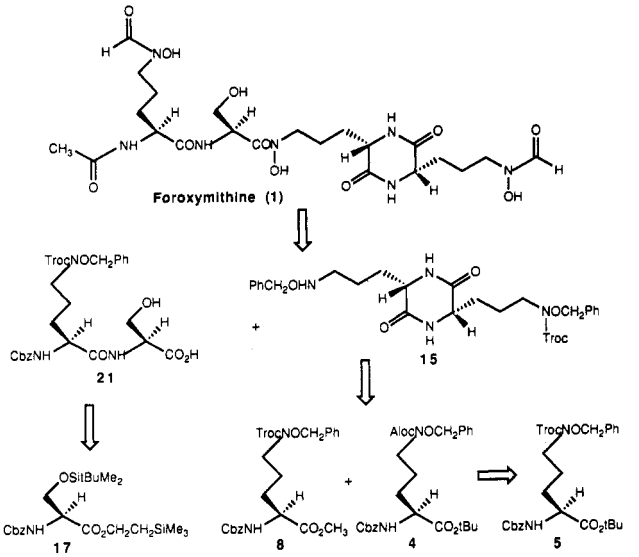
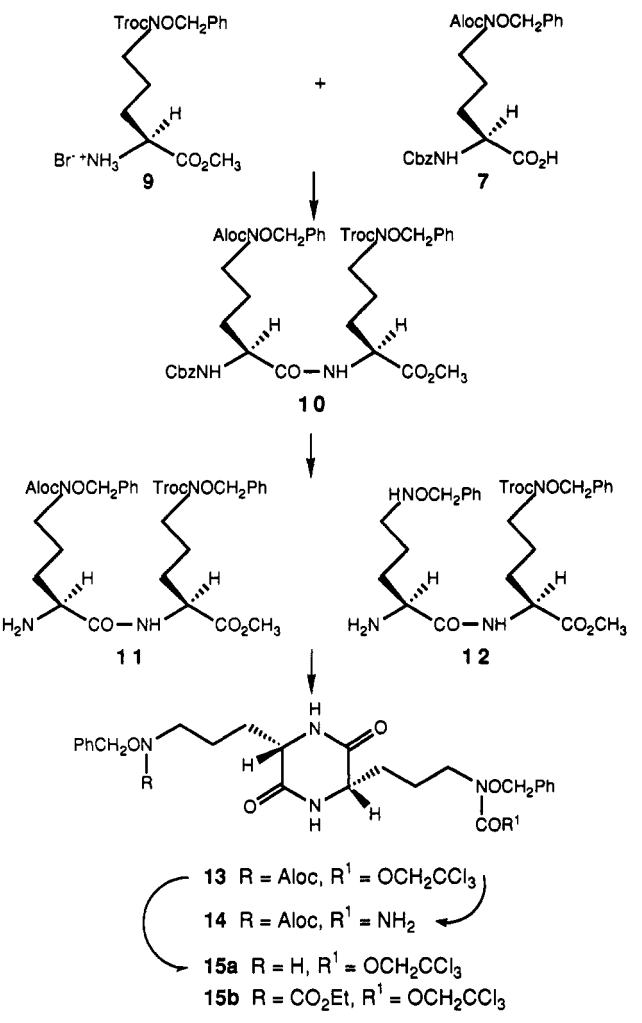


Figure 1.

Scheme II



agents in future planned syntheses of conjugates) to the foroxymithine skeleton as late as possible in the synthetic sequence. Purification of hydroxamate type siderophores is often difficult due to their high affinity for metal ions; therefore, hydrogenolysis was chosen to liberate **1** in the final deprotection so that all byproducts arising would be volatile.

A retrosynthetic analysis for the planned synthesis is shown in Figure 1. Disconnection of **1** at the amide bond between the serine and ornithine residue provides the seryl-ornithine dipeptide **21** and the diketopiperazine **15**. Disconnection of the left-hand dipeptide **21** amide provides the two protected amino acids **17** and **5** derived from L-serine and N^5-hydroxy-L-ornithine, respectively. Disconnection of the right-hand diketopiperazine fragment affords the two protected hydroxamates **4** and **8**, each of which can be produced from **5** or independently synthesized from the norvaline derivative **3** (Scheme I).

Synthesis of the Right-Hand Diketopiperazine Fragment (15). As shown in Scheme I the allyloxycarbonyl-protected hydroxamate **4** can be synthesized by two possible routes. Initially, Mitsunobu[12] reaction of *N*-(allyloxycarbonyl)-*O*-benzylhydroxylamine (**2**) and the norvaline derivative **3**[5] produced the desired hydroxamate **4**; however, purification of this material was impossible on a large scale since reduced azodicarboxylate cochromatographed with **4**. In an attempt to obtain pure **4**, conversion of the trichlorethoxycarbonyl (Troc) protected hydroxamate **5**[5] to **4** was attempted. Treatment of **5** with activated zinc in glacial acetic acid followed by treatment with (allyloxy)chloroformate produced pure **4** in 39% yield, plus the N–O bond reduction product, **6**, in 33% yield. It was later determined that although compound **4** as derived

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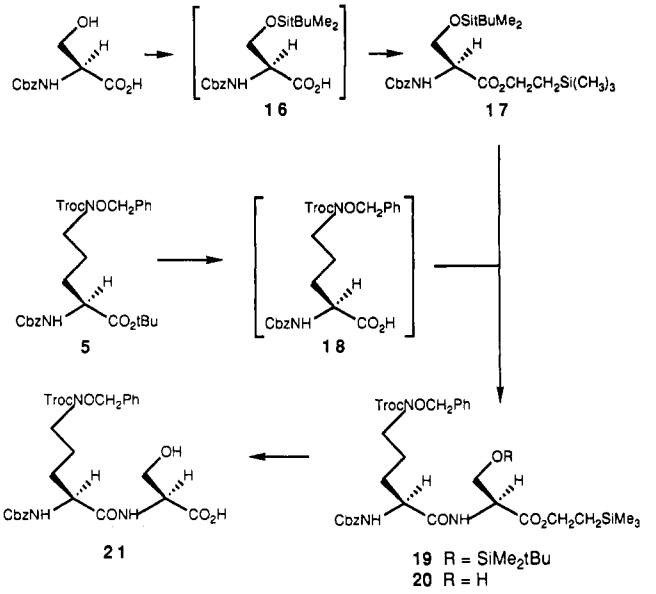
from the Mitsunobu reaction was impure, the subsequent coupling of the corresponding acid **7** with amine **9** produced protected dipeptide **10** (Scheme II), which could be easily purified. Thus, deprotection of the *tert*-butyl group from **4** was accomplished using trifluoroacetic acid in dichloromethane affording the acid **7**, which was used immediately without purification.

Manipulation of the Troc-protected hydroxamate **5** to produce the methyl ester **8** was a straightforward process. Treatment of **5** with trifluoroacetic acid in dichloromethane to remove the *tert*-butyl ester, followed by evaporation and treatment with diazomethane, afforded the methyl ester **8** in 82% yield. The carbobenzyloxy (Cbz) group was removed by brief treatment with 30% hydrobromic acid in acetic acid and dichloromethane to produce the crude bromide salt **9**. Coupling of fragments **7** and **9** (Scheme II) with EEDQ[13] in dichloromethane afforded the dipeptide **10** in 61% yield. Analysis of the ^{13}C NMR of **10** revealed the presence of only one diasteromer since no signal doubling was evident. Conversion of dipeptide **10** to the diketopiperazine **13**, however, was not straightforward. Following our precedented procedure,[12a] treatment of this peptide **10** briefly with 30% hydrobromic acid in acetic acid with the cosolvent dichloromethane followed by evaporation and addition of methanol saturated with ammonia failed to give the desired diketopiperazine **13**. Instead of producing product with an intact Troc carbamate group, substituted urea **14** was obtained as a white amorphous solid in addition to several other products, as analyzed by TLC (thin-layer chromatography). Apparently, **14** arises by displacement of trichloroethanol from **13** by ammonia. This was subsequently verified when the desired product **13** was treated with methanol and ammonia and monitored by TLC. In fact, **13** was found to be converted to the unusual product **14** slowly over a 3-h period.

Since several other products appeared to form from this reaction in addition to **14**, the reaction sequence was investigated in detail. Peptide **10** was treated with hydrobromic acid as described above followed by evaporation, neutralization, extraction, and chromatography to afford two primary products, monoamine **11** (44%) and diamine **12** (28%), resulting from hydrolysis of the Aloc protecting group. Several other products arising from *acetylation* of **11** and **12** were detected apparently arising from either acetyl bromide or acetic anhydride present in the hydrobromic acid in acetic acid. However, these accounted for a loss of only 15% of the starting peptide **10**.

Despite considerable literature precedent[14] for the spontaneous formation of diketopiperazines from dipeptide amine esters, this was not the case for either of the two peptides **11** or **12**. Use of other bases (potassium carbonate, triethylamine, (dimethylamino)pyridine (DMAP), DMAP plus DMAP–HCl[15] or pyridine) in methanol or other solvents either produced difficult to separate mixtures of the monoamine **11** and desired diketopiperazine **13** or resulted in decomposition. Weinreb's method[16] utilizing trimethylaluminum resulted only in decomposition. Eventually, treatment of **11** and **12** with 10 equiv of imidazole in anhydrous methanol for approximately 100 h was found to produce both the *N*-Aloc-*N*-Troc-diketopiperazine **13** and the Troc-diketopiperazine **15a** in 77% and 70% yields, respectively, as precipitated solids which required no further purification. Deprotection of the allyloxycarbonyl group from **13** was accomplished using tetrakis(triphenylphosphine)palladium(0)[17] in anhydrous chloroform using 10 equiv of *O*-benzylhydroxylamine as an allyl cation scavenger affording **15a** in 74% yield.

Scheme III



Synthesis of the Left-Hand Segment (21). Synthesis of dipeptide **21** is shown in Scheme III. Treatment of *N*-(carbobenzyloxy)-L-serine with an excess of *tert*-butyldimethylsilyl chloride and imidazole in dimethylformamide[18] resulted in bis-silylation. Following aqueous workup, the residue was hydrolyzed with potassium carbonate in aqueous tetrahydrofuran. After a mildly acidic aqueous workup, the resulting crude free acid **16** was treated with *O*-[(trimethylsilyl)ethyl]diisopropylisourea[19] in refluxing tetrahydrofuran. Following filtration and chromatography, the appropriately protected serine **17** was isolated in 42% yield overall from Cbz-L-serine. Treatment of **17** with acetic acid, methanol, 10% palladium on carbon, and hydrogen removed the Cbz group producing the amine salt.

Deprotection of **5** as described previously using trifluoroacetic acid provided the crude acid **18**, which immediately was coupled to the amine salt of **17** using the EEDQ approach previously described. This produced the dipeptide **20** in 35% yield with loss of the *tert*-butyldimethylsilyl group from the serine hydroxyl. Not surprisingly, none of the peptide **19** possessing the *tert*-butyldimethylsilyl group was isolated since the TBDMS group is susceptible to removal in the acidic solvent used during the hydrogenation of **17**. Removal of the (trimethylsilyl)ethyl ester was accomplished using excess tetrabutylammonium fluoride in tetrahydrofuran to afford the free acid **21** in 83% yield.

Coupling of the Left- and Right-Hand Fragments 15a and 21. Assembly of fragments **15a** and **21** is shown in Scheme IV. Initial use of EEDQ as a coupling agent produced the desired tetrapeptide **22** in only 28% yield. In addition to **22**, apparent attack by the amino group of

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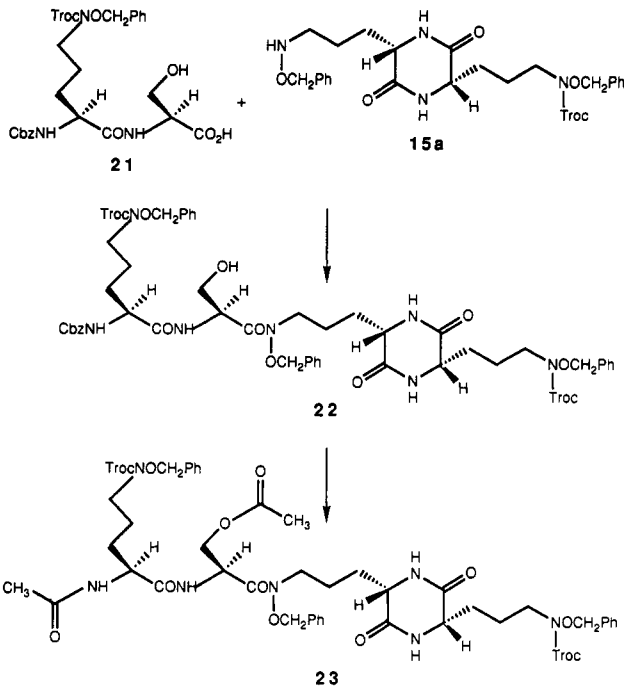
(15) Boden, E. P.; Keck, G. E. *J. Org. Chem.* **1985**, *50*, 2394.
(16) Basha, A.; Lipton, M.; Weinreb, S. M. *Tetrahedron Lett.* **1977**, 4171.
(17) Jeffrey, P. D.; McCombie, S. W. *J. Org. Chem.* **1982**, *47*, 587.
(18) Corey, E. J.; Venkateswarlu, A. *J. Am. Chem. Soc.* **1972**, *94*, 6190.
(19) Mathias, L. J. *Synthesis* **1979**, 561.

Scheme IV

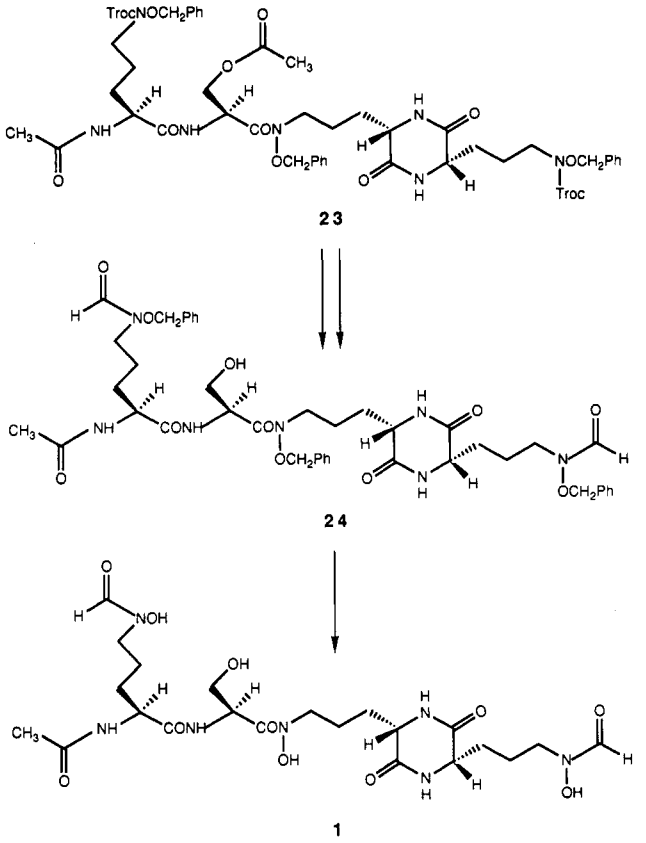


15a on the undesired ethyl carbonate portion of the EEDQ mixed anhydride produced the corresponding ethyl carbamate **15b**. As an alternative, acid **21** was treated with *N*-hydroxysuccinimide and dicyclohexylcarbodiimide in tetrahydrofuran. The *N*-hydroxysuccinimide active ester was not isolated, but filtered directly into a solution of the amine diketopiperazine **15a** in anhydrous chloroform. After 4 h at room temperature, an aqueous workup and chromatography produced the tetrapeptide **22** as an oil in 56% yield.

At this point, we envisioned that selective removal of the Cbz group followed by coupling with an activated carbonyl compound would allow us to proceed with a total syntheses of foroxymithine by acetylation or to eventually attach antimicrobial agents for our planned syntheses of conjugates. To test this approach, tetrapeptide **22** was treated with 30% hydrobromic acid in acetic acid followed by evaporation. To this crude bromide salt in anhydrous dichloromethane was added acetic anhydride followed by 1 equiv of triethylamine to produce the diacetate **23** in 76% yield.

Treatment of **23** with activated zinc dust in the presence of formyl acetic anhydride in tetrahydrofuran resulted in removal of the Troc protecting groups and formylation of the amino groups as expected (Scheme V). Removal of the acetate ester with catalytic potassium bicarbonate in anhydrous methanol at room temperature for 48 h, followed by chromatography, afforded tribenzyl-protected foroxymithine **24** in 49% yield overall from **23** as an oil. Inspection of the proton and carbon spectra of this protected version (**24**) of foroxymithine revealed evidence of the restricted rotation of the formyl groups as observed by broadening of the signals in the proton NMR and signal multiplicity in the carbon spectrum. Similar spectral behavior is observed in a protected version of the amino acid N^5-formyl-N^5-hydroxy-L-ornithine.[20] Hydrogenolysis of **24** followed by filtration and lyophilization afforded a 98% yield of foroxymithine **1**. The synthetic material had IR and proton and carbon NMR data in excellent agreement with those reported.[1,21]

Scheme V



In conclusion, we have devised a flexible synthetic route to the angiotensin 1 converting enzyme inhibitor foroxymithine **1** which will allow the syntheses of various analogues. In addition, this synthesis has firmly established the structure of **1** as that reported. Further work involving the synthesis of antimicrobial agent–foroxymithine conjugates and their biological activity will be reported in the future.

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 1420 spectrophotometer. TF refers to thin film and KBr refers to potassium bromide for infrared spectra. Proton and carbon-13 spectra were obtained on a General Electric GN-300 spectrometer. ^{1}H NMR chemical shifts are reported in ppm relative to tetramethylsilane for deuteriochloroform and for deuterium oxide the signal for 1,4-dioxane (3.55 ppm). For ^{13}C NMR, references were the center peak of deuteriochloroform (77.0 ppm) or for deuterium oxide the signal for 1,4-dioxane (66.5 ppm). Peak assignments for ^{13}C NMR were made with the help of the distortionless enhanced polarization transfer (DEPT) pulse program.[22] Electron-impact mass spectra, chemical-ionization mass spectra, and fast atom bombardment were recorded on an AEI Scientific Apparatus MS 902, Du Pont DP 102, and Finnigan MAT Model 8430 spectrometers. Optical rotations were obtained using a Rudolf Research Autopol III polarimeter with spectral grade solvents. Analytical TLC was carried out using 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 silica gel chromatography was

(20) Detailed NMR studies of these hydroxamate-containing amino acids, with and without added metals, will be reported separately.

(21) We sincerely thank Professor Takaaki Aoyagi of the Institute of Microbial Chemistry, Tokyo, Japan, for copies of the proton and carbon nuclear magnetic resonance spectra of foroxymithine.

(22) Doddrell, D. M.; Pegg, D. T.; Bendall, M. R. *J. Magn. Reson.* 1981, *45*, 8.

performed using a Harrison Research Chromatotron Model 7924, and flash silica gel column chromatography was conducted using Merck silica gel 60. Solvents used were dried and purified by standard methods.[23] The term "dried" refers to the drying of an organic solution over anhydrous magnesium sulfate.

N^2-(Benzyloxycarbonyl)-5-hydroxy-L-norvaline 1-*tert*-butyl ester (3) was synthesized by the method of Dolence, Lin, and Miller.[11]

N^2-(Benzyloxycarbonyl)-N^5-(benzyloxy)-N^5-(2,2,2-trichloroethoxycarbonyl)-L-ornithine 1-*tert*-butyl ester (5) was prepared by the method of Dolence, Lin, and Miller.[11]

N-(Allyloxycarbonyl)-O-benzylhydroxylamine (2). To a solution of O-benzylhydroxylamine (9.234 g, 75.0 mmol) in 75 mL of anhydrous dichloromethane at 0 °C under nitrogen was added pyridine (6.1 mL, 75.0 mmol) followed by dropwise addition of allyl chloroformate (8.2 mL, 77.32 mmol). The ice bath was removed, and the solution was stirred for 1 h. This solution was transfered to a separatory funnel, washed with water, 0.2 M acetic acid, and brine, dried, and filtered. Evaporation afforded 14.998 g (96%) of 2 as a clear oil which was nearly homogeneous by TLC (25% ethyl acetate–75% hexanes). This oil was used as is for further reactions without purification: IR (TF) 3290, 2950, 1720 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 4.50–4.60 (m, 2 H, allylic H), 4.82 (s, 2 H, benzylic H), 5.15–5.35 (m, 2 H, olefinic H), 5.80–5.95 (m, 1 H, olefinic H), 7.28–7.40 (m, 5 H, aromatic H), 7.86 (br s, 1 H, NH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 66.00, 78.33, 118.14, 128.22, 128.28, 131.80, 135.27, 157.06; MS (CI, NH_3) m/z 208 (M + H).

N^2-(Benzyloxycarbonyl)-N^5-(benzyloxy)-N^5-(allyloxycarbonyl)-L-ornithine 1-*tert*-Butyl Ester (4). To a solution of alcohol 3 (6.00 g, 18.67 mmol) in 62 mL of THF was added N-(allyloxycarbonyl)-O-benzylhydroxylamine (2) (3.23 g, 15.56 mmol) and solid triphenylphosphine (12.25 g, 46.69 mmol). Diethyl azodicarboxylate (7.4 mL, 46.69 mmol) was added dropwise to the reaction mixture under nitrogen and stirred at room temperature for 13 h. After evaporation, the crude oil was purified by flash chromatography, eluting with 25% ethyl acetate–75% hexanes to provide 6.57 of crude 4 as an oil. This material was rechromatographed, eluting with 20% ethyl acetate–80% hexanes but failed to yield clean homogeneous product. A pure sample could be obtained by careful application as a narrow band of 0.24 g of the above material to four analytical (20 cm × 20 cm) aluminum-backed silica gel TLC plates and eluted twice with 25% ethyl acetate–75% hexanes to afford 0.15 g of pure 4 as a clear oil: $[\alpha]^{23}_D$ = +7.1° (c = 1.5, $CHCl_3$); IR (TF) 3450, 2980, 1720 (br) cm^{-1}; ^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), 3.46 (t, 2 H, J = 6 Hz, CH_2N), 4.20–4.32 (m, 1 H, NCHCO), 4.60–4.75 (m, 2 H, allylic H), 4.84 (s, 2 H, benzylic H), 5.08 (s, 2 H, benzylic H), 5.20–5.45 (m, 3 H, olefinic H and NH), 5.86–6.10 (m, 1 H, olefinic H), 7.25–7.45 (m, 10 H, aromatic H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 22.69, 27.80, 29.86, 49.13, 53.93, 66.43, 66.70, 79.95, 81.96, 117.97, 127.89, 127.93, 128.30, 128.33, 128.44, 129.18, 132.23, 135.24, 136.25, 155.69, 156.84, 174.10; MS (CI, isobutane) m/z 513 (M + H), 457 (M – $C(CH_3)_3$ +H).

Method B. To a solution of the Troc-protected hydroxamate 5 (0.50 g,0.83 mmol) in 4.0 mL of glacial acetic acid was added freshly activated zinc dust. The mixture was sonicated for 2 h and filtered, and the acetic acid was evaporated. To the residue was added 1.0 mL of THF, 1.0 mL of water, and potassium bicarbonate (0.143 g, 1.03 mmol) followed quickly by (allyloxy)chloroformate (90 μL, 0.85 mmol). The mixture was stirred overnight, diluted with ethyl acetate, and washed with 1.0 M HCl and brine, followed by drying, filtration, and evaporation. The residue was purified by radial silica gel chromatography to afford two fractions of R_f 0.47 and 0.28 (25% ethyl acetate–75% hexanes). The R_f 0.47 material was desired product 4 weighing 0.165 g (39%) having the same spectral data described above. The R_f 0.28 material weighed 0.111 g (33%) and was shown to be the product 6 resulting from N–O bond reduction: IR (TF) 3340, 2980, 1710 (br) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.42 (s, 9 H, $C(CH_3)_3$), 1.50–1.90 (m, 4 H, CH_2), 3.00–3.25 (m, 2 H, CH_2N), 4.20–4.30 (m, 1 H, NCHCO), 4.50–4.60 (m, 2 H, allylic H), 5.02 (br t, 1 H, NH), 5.10 (s, 2 H, benzylic H), 5.18–5.35 (m, 2 H, olefinic H), 5.50 (d, 1 H, J = 8 Hz, NH), 5.80–6.00 (m, 1 H, olefinic H), 7.25–7.40 (m, 5 H, aromatic H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 25.55, 27.83, 29.92, 40.36, 53.89, 65.29, 66.74, 82.08, 117.40, 127.95, 127.98, 128.35, 132.85, 136.18, 155.80, 156.15, 171.23; MS (CI, ammonia) m/z 424 (M + NH_4), 407 (M + H).

N^2-(Benzyloxycarbonyl)-N^5-(benzyloxy)-N^5-(2,2,2-trichloroethoxycarbonyl)-L-ornithine 1-Methyl Ester (8). To a solution of the *tert*-butyl ester 5 (2.50 g, 4.140 mmol) in 5.0 mL of anhydrous dichloromethane under nitrogen was added 5 mL of trifluoroacetic acid. The mixture was stirred for 6 h, evaporated, diluted with anhydrous toluene, and evaporated. This residue was treated with an excess of diazomethane in diethyl ether until the yellow color of the diazomethane persisted. Several drops of glacial acetic acid were added to decompose the excess diazomethane, and the ether solution was washed with water, saturated sodium bicarbonate, and brine, dried, filtered, and evaporated. The residue was purified by flash silica gel chromatography, eluting with 25% ethyl acetate–75% hexanes to produce 1.91 g (82%) of 8 as a clear oil: $[\alpha]^{22}_D$ = +6.8° (c = 1.09, $CHCl_3$); IR (TF) 3350, 2950, 1725 (br) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.50–1.80 (m, 4 H, CH_2), 3.52 (t, 2 H, J = 6 Hz, CH_2N), 3.69 (s, 3 H, CH_3), 4.25–4.41 (m, 1 H, NCHCO), 4.82 (s, 2 H, benzylic H), 4.90 (s, 2 H, CH_2CCl_3), 5.09 (s, 2 H, benzylic H), 5.40 (d, 1 H, J = 8 Hz, NH), 7.25–7.45 (m, 10 H, aromatic H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 22.88, 2967, 49.07, 52.27, 53.41, 66.90, 75.05, 77.14, 95.19, 127.96, 128.06, 128.41, 128.43, 128.74, 129.40, 134.73, 136.12, 154.96, 155.76, 172.43; MS (CI, isobutane) m/z 563 (M + H), 565 (M + H + 2).

N^2-[N^2-(Benzyloxycarbonyl)-N^5-(benzyloxy)-N^5-(allyloxycarbonyl)-L-ornithinyl]-N^5-(benzyloxy)-N^5-(2,2,2-trichloroethoxycarbonyl)-L-ornithine 1-Methyl Ester (10). To a solution of the Aloc-protected hydroxamate 4 (0.125 g, 0.245 mmol) in 1.0 mL of anhydrous dichloromethane under nitrogen was added 1.0 mL of trifluoroacetic acid. After 1 h, TLC revealed an absence of starting 4, and the solution was evaporated. The residue was diluted with anhydrous benzene and evaporated for a total of three times to produce the crude acid 7. In the mean time, a solution of Troc-protected hydroxamate 8 (0.136 g, 0.245 mmol) in 0.5 mL of anhydrous dichloromethane under a drying tube was treated with 0.5 mL of 30% hydrobromic acid in acetic acid. This mixture was stirred for 10 min, evaporated, diluted with anhydrous benzene, and evaporated to give the crude salt 9. To this residue was added 0.5 mL of anhydrous dichloromethane, Et_3N (34 μL, 0.245 mmol) followed by immediate addition of the Aloc-carboxylic acid 7 in a total of 1.0 mL of anhydrous dichloromethane, EEDQ (0.079 g, 0.318 mmol), and then another 34 μL of Et_3N. The mixture was stirred overnight, diluted with ethyl acetate, washed with two portions of 0.5 M HCl and brine, dried, filtered, and evaporated to give a yellow oil. This oil was purified by radial silica gel chromatography eluting with 50% ethyl acetate–50% hexanes to afford 0.130 g (61%) of 10 as a clear oil: $[\alpha]^{27}_D$ = +4.4 (c = 1.3, $CHCl_3$); IR (TF) 3340, 2960, 1720 (br), 1650 (br) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.40–1.90 (m, 8 H, CH_2), 3.35–3.45 (m, 1 H, CH_2N), 3.51 (t, 2 H, J = 6 Hz, CH_2N), 3.64 (s, 3 H, CH_3), 3.67–3.80 (m, 1 H, CH_2N), 4.20–4.40 (m, 1 H, NCHCO), 4.50–4.60 (m, 1 H, NCHCO), 4.64 (br d, 2 H, J = 6 Hz, allylic H), 4.81 (s, 2 H, benzylic H), 4.85 (s, 2 H, benzylic H), 4.91 (s, 2 H, CH_2CCl_3), 5.06 (s, 2 H, benzylic H), 5.20–5.40 (m, 2 H, olefinic H), 5.53 (d, 1 H, J = 8 Hz, NH), 5.85–6.05 (m, 1 H, olefinic H), 6.83 (d, 1 H, J = 8 Hz, NH), 7.25–7.45 (m, 15 H, aromatic H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 22.89, 23.04, 29.06, 30.11, 48.12, 48.87, 51.66, 52.15, 53.37, 66.58, 66.81, 75.05, 77.09, 95.18, 118.01, 127.85, 127.96, 128.36, 128.40, 128.53, 128.69, 129.25, 129.38, 132.22, 134.69, 135.14, 136.19, 154.94, 156.08, 157.17, 171.59, 172.01; MS (positive ion FAB, glycerol–m-nitrobenzyl alcohol matrix) m/z 867 (M + H); exact mass (EI) calcd for $C_{40}H_{47}N_4O_{11}Cl_3$ 866.2288, found 866.2275.

N^2-[N^5-(Benzyloxy)-N^5-(allyloxycarbonyl)-L-ornithinyl]-N^5-(benzyloxy)-N^5-(2,2,2-trichloroethoxycarbonyl)-L-ornithine 1-Methyl Ester (11) and N^2-[N^5-(Benzyloxy)-L-ornithinyl]-N^5-(benzyloxy)-N^5-(2,2,2-trichloroethoxycarbonyl)-L-ornithine 1-Methyl Ester (12). To a solution of the dipeptide 10 (1.00 g, 1.15 mmol) in 10 mL of dichloromethane under nitrogen was added 10 mL of 30% hydrobromic acid in acetic acid. This mixture was stirred for 10

(23) Gordon, A. J.; Ford, R. A. *The Chemist's Companion—a Handbook of Practical Data, Techniques, and References*; John Wiley & Sons: New York, 1972; pp 408–455.

min, evaporated, and diluted with chloroform and saturated sodium carbonate solution. The aqueous layer was repeatedly extracted with chloroform, and the pooled organic layers were washed with brine, dried, filtered, and evaporated to give an oil. This oil was immediately purified by radial silica gel chromatography, eluting successively with chloroform, 2% methanol–98% chloroform, and finally 10% methanol–90% chloroform to afford 0.371 g (44%) of the monoamine **11** (R_f 0.60 in 10% methanol–90% chloroform) and 0.208 g (28%) of the diamine **12** (R_f 0.36 in 10% methanol–90% chloroform) as viscous oils. Spectral data for the monoamine **11**: IR (TF) 3320, 3040, 2960, 1740, 1720, 1675 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.35–1.95 (m, 8 H, CH_2), 3.33 (dd, 2 H, J = 4 and 8 Hz, NH_2), 3.42–3.49 (m, 2 H, CH_2N), 3.50–3.56 (m, 2 H, CH_2N), 3.69 (s, 3 H, CH_3), 4.50–4.60 (m, 2 H, NCHCO), 4.66 (dt, 2 H, J = 1.4 and 5.6 Hz, allylic H), 4.83 (s, 2 H, benzylic H), 4.86 (s, 2 H, benzylic H), 4.91 (s, 2 H, CH_2CCl_3), 5.20–5.40 (m, 2 H, olefinic H), 5.84–6.05 (m, 1 H, olefinic H), 7.25–7.46 (m, 15 H, aromatic H), 7.65 (d, 1 H, J = 8 Hz, NH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 23.05, 23.39, 29.53, 32.03, 48.96, 49.11, 51.35, 52.30, 54.50, 66.63, 75.11, 77.10, 77.17, 95.25, 118.18, 128.46, 128.52, 128.63, 128.83, 129.36, 129.48, 132.30, 134.72, 135.31, 154.99, 157.06, 172.52, 174.78; MS (Cl, isobutane) m/z 731 (M + H). Spectral data for the diamine **12**: IR (TF) 3340, 3040, 2960, 1740, 1720 1675 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.40–2.00 (m, 8 H, CH_2), 2.80–3.00 (m, 2 H, CH_2N), 3.33–3.43 (m, 1 H, NH_2), 3.48–3.58 (m, 2 H, CH_2N), 3.70 (s, 3 H, CH_3), 4.50–4.63 (m, 2 H, NCHCO), 4.69 (s, 2 H, benzylic H), 4.82 (s, 2 H, benzylic H), 4.91 (s, 2 H, CH_2CCl_3), 7.25–7.50 (m, 10, H, aromatic H), 7.73 (d, 1 H, J = 8 Hz, NH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 23.04, 23.65, 29.56, 32.60, 49.00, 51.36, 51.58, 52.32, 54.83, 75.12, 76.12, 77.18, 95.25, 118.18, 127.79, 128.36, 128.51, 128.57, 128.83, 129.48, 129.54, 134.74, 137.93, 155.01, 172.55, 174.58; MS (Cl, NH_3) m/z 647 (M + H).

(3*S*,6*S*)-3-[3-[*N*-(Allyloxycarbonyl)-*N*-(benzyloxy)amino]propyl]-6-[3-[*N*-(2,2,2-trichloroethoxycarbonyl)-*N*-(benzyloxy)amino]propyl]-2,5-piperazinedione (13). To a solution of the monoamine **11** (0.371 g, 0.508 mmol) in 2 mL of anhydrous methanol under nitrogen was added imidazole (0.346 g, 5.082 mmol). This mixture was stirred for 96 h at room temperature at which time a fine white solid had percipitated from solution. This solid was removed by filtration using a HPLC organic solvent filter pad (Millipore, Type FH, 0.5 μm) and washed with ether to afford 0.209 g of **13**. The mother liquor was evaporated, another 1 mL of anhydrous methanol was added, and the mixture was stirred overnight, again producing a white precipitate (0.044 g) which was collected as described above. The mother liquor was evaporated, diluted with chloroform, washed with water and brine, dried, filtered, and evaporated. The residue was purified by preparative silica gel chromatography, eluting twice with 5% methanol–95% chloroform to afford another 0.021 g of **13**. The total yield of combined **13** was 0.274 g (77%) as a white amorphous solid: mp 107–109 °C (acetone–hexanes); $[\alpha]^{27}_D$ = (c = 0.2, $CHCl_3$); IR (TF) 3200, 3065, 2960, 1715, 1680 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.40–2.00 (m, 8 H, CH_2), 3.40–3.58 (m, 4 H, CH_2N), 3.84–3.98 (m, 2 H, NCHCO), 4.45 (dt, 2 H, J = 1.2 and 5.7 Hz, allylic H), 4.82 (s, 2 H, benzylic H), 4.85 (s 2 H, benzylic H), 4.92 (s, 2 H, CH_2CCl_3), 5.22–5.40 (m, 2 H, olefinic H), 5.87–6.01 (m, 1 H, olefinic H), 6.70–6.90 (br m, 2 H, NH), 7.32–7.47 (m, 10 H, aromatic H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 22.61, 22.66, 30.99, 48.88, 48.98, 54.37, 54.39, 66.69, 75.11, 77.12, 77.25, 95.19, 118.28, 128.32, 128.47, 128.53, 128.67, 128.86, 129.37, 129.52, 132.19, 134.71, 135.22, 155.09, 157.05, 167.81, 168.02; MS (positive ion FAB, chloroform–m-nitrobenzyl alcohol matrix) m/z cluster at 701 (M + H). Anal. Calcd for $C_{31}H_{37}N_4O_8Cl_3$: C, 53.19; H, 5.33; N, 8.00. Found: C, 53.39; H, 5.28; N, 8.01.

(3*S*,6*S*)-3-[3-[*N*-(Allyloxycarbonyl)-*N*-(benzyloxy)amino]propyl]-6-[3-[*N*-(aminocarbonyl)-*N*-(benzyloxy)amino]propyl]-2,5-piperazinedione (14). To a solution of the dipeptide **10** (0.117 g, 0.1351 mmol) in 0.5 mL of dry dichloromethane was added 0.5 mL of 30% hydrobromic acid in acetic acid. This was stirred under a drying tube for 10 min, evaporated, diluted with anhydrous benzene, and evaporated to afford a light amber oil. To this oil was added 2.0 mL of ammonia saturated anhydrous methanol, and the mixture was stoppered and stirred for 22 h. The solution was evaporated and the residue was dissolved in chloroform and chromatographed on a preparative silica gel TLC plate, eluting with 20% methanol–80% chloroform to afford 0.057 g (74%) of **14** as a white amorphous solid: IR (KBr) 3220 (br), 2940, 2880, 1675 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.50–2.00 (m, 8 H, CH_2), 3.30–3.62 (m, 4 H, CH_2N), 3.84–4.00 (m, 2 H, NCHCO), 4.45 (dt, 2 H, J = 1.2 and 5.4 Hz, allylic H), 4.76 (t, 2 H, J = 11.4 and 11.7 Hz, benzylic H), 4.84 (s, 2 H, benzylic H), 5.22–5.40 (m, 2 H, olefinic H), 5.80 (br s, 2 H, NH_2), 5.86–6.00 (m, 1 H, olefinic H), 7.02 (br s, 1 H, NH), 7.20–7.45 (m, 10 H, aromatic H), 7.80 (br s, 1 H, NH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 22.34, 22.71, 30.86, 30.96, 47.55, 49.07, 54.42, 54.45, 66.65, 76.83, 77.11, 118.20, 128.44, 128.62, 128.68, 128.84, 129.18, 129.35, 132.25, 135.01, 135.29, 157.02, 161.23, 168.29, 168.44; MS (positive ion FAB, m-nitrobenzyl alcohol matrix) m/z 590 (M + Na), 568 (M + H).

(3*S*,6*S*)-3-[3-[*N*-(Benzyloxy)amino]propyl]-6-[3-[*N*-(2,2,2-trichloroethoxycarbonyl)-*N*-(benzyloxy)amino]propyl]-2,5-piperazinedione (15a). To a solution of the diamine **12** (0.208 g, 0.322 mmol) in 2 mL of anhydrous methanol under nitrogen was added imidazole (0.220 g, 3.22 mmol). This mixutre was stirred 96 h and, following filtration, workup, and chromatography as described above for **13**, afforded a total of 0.138 g (70%) of **15a** as a white amorphous solid: mp 143–145 °C; $[\alpha]^{25}_D$ = –41.5° (c = 0.2, $CHCl_3$); IR (TF) 3280, 3200, 3060, 2980, 1730, 1675 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.55–2.06 (m, 8 H, CH_2), 2.91 (t, 2 H, J = 5.4 and 6.6 Hz, CH_2N), 3.53 (t, 2 H, J = 5.7 and 6.0 Hz, CH_2N), 3.78–4.00 (2 br m, 2 H, NCHCO), 4.70 (s, 2 H, benzylic H), 4.82 (s, 2 H, benzylic H), 4.92 (s, 2 H, CH_2CCl_3), 5.90 (br s, 1 H, ONH), 6.60 (br d, 1 H, NH), 7.00 (br d, 1 H, NH), 7.20–7.50 (m, 10 H, aromatic H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 22.62, 22.98, 31.09, 32.06, 49.04, 51.17, 54.41, 54.72, 75.10, 76.08, 77.25, 95.19, 127.85, 128.35, 128.44, 128.52, 128.85, 129.50, 134.71, 137.71, 155.08, 167.99, 168.53; MS (positive ion FAB, chloroform–m-nitrobenzyl alcohol matrix) m/z cluster at 617 (M + H), 629 (M + Na). Anal. Calcd for $C_{27}H_{33}N_4O_6Cl_3$: C, 52.65; H, 5.40; N, 9.10. Found: C, 52.57; H, 5.49; N, 9.20.

Palladium(0)-Mediated Deprotection of the Allyloxycarbonyl Group from 13 To Afford 15a. To a solution of the Aloc diketopiperazine **13** (0.240 g, 0.343 mmol) and *O*-benzylhydroxylamine (0.422 g, 3.43 mmol) in 10 mL of anhydrous chloroform under nitrogen in a foil-covered flask was added, as a solid, tetrakis(triphenylphosphine)palladium (0.040 g, 0.0343 mmol). This solution was stirred for 18 h and then evaporated to approximately 2 mL. This residue was chromatographed by radial silica gel chromatography, eluting successively with chloroform, 2% methanol–98% chloroform, 5% methanol–95% chloroform, and finally 10% methanol–90% chloroform. This afforded 0.149 g (71%) of the aminodiketopiperazine **15a**, which had spectral data identical with that which is described above.

N^2-(Benzyloxycarbonyl)-*O*-(*tert*-butyldimethylsilyl)-L-serine 1-(Trimethylsilyl)ethyl Ester (17). To a solution of Cbz-L-serine (5.00 g, 20.90 mmol) in 40 mL of dimethylformamide was added imidazole (7.12 g, 104.50 mmol) and *tert*-butyldimethylsilyl chloride (12.60 g, 83.60 mmol). The solution was stirred under nitrogen overnight. The mixture was dissolved in ethyl acetate, washed repeatedly with water and brine, dried, filtered, and evaporated to give an oil. In order to hydrolyze the silyl ester, this oil was dissolved in a mixture of 40 mL of water and 40 mL of THF. Solid potassium carbonate (0.29 g, 2.09 mmol) was added, and the hydrolysis was followed by TLC (20% ethyl acetate–80% hexanes) until only base-line UV-active material was observed. The THF was evaporated, and the mixture was diluted with ethyl acetate, acidified to a pH of 2–3, and repeatedly extracted with ethyl acetate. The organic layer was washed with brine, dried, and filtered followed by evaporation to provide an oil. To this crude silyl acid **16** in 20 mL of anhydrous THF was added *O*-[(trimethylsilyl)ethyl]diisopropylisourea (5.11 g, 20.9 mmol) under nitrogen, and the mixture was refluxed for a total of 4 h. The THF solution was cooled to 0 °C with an ice bath, and the percipitated diisopropylurea was removed by filtration through Celite. The filtrate was evaporated, and the residual oil was purified by flash silica gel chromatography eluting with 10% ethyl acetate–90% hexanes to provide 3.99 g (42% from Cbz-L-serine) of **17** as a clear oil: $[\alpha]^{25}_D$ = +8.6° (c = 1.0, $CHCl_3$); IR (TF) 3360, 3040, 2960, 1725 (br), 1500 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.01 (s, 3 H, $SiCH_3$), 0.02 (s, 3 H, $SiCH_3$), 0.04 (s, 9 H, $Si(CH_3)_3$), 0.85 (s, 9 H, $SiC(CH_3)_3$), 1.00 (t, 2 H, J = 8 Hz, CH_2Si), 3.84 (dd, 1 H, J = 10 Hz, CH_2OSi), 4.07 (dd, 1 H, J = 10 Hz,

CH_2OSi), 4.15–4.30 (m, 2 H, OCH_2), 4.34–4.44 (m, 1 H, NCHCO), 5.13 (s, 2 H, benzylic H), 5.60 (d, 1 H, J = 8 Hz, NH), 7.27–7.45 (m, 5 H, aromatic H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ −5.68, −5.56, −1.58, 17.39, 18.16, 25.69, 56.08, 63.65, 63.85, 66.92, 128.09, 128.11, 128.48, 136.35, 155.94, 170.48; MS (CI, isobutane) m/z 454 (M + H), 397 (M − $C(CH_3)_3$).

[N^2-(Benzyloxycarbonyl)-N^5-(benzyloxy)-N^5-(2,2,2-trichloroethoxycarbonyl)-L-ornithinyl]-L-serine 1-(Trimethylsilyl)ethyl Ester (20). To a solution of the Cbz bis(silyl)serine **17** (3.00 g, 6.61 mmol) in 30 mL of methanol was added acetic acid (0.38 mL, 6.61 mmol) and 10% Pd/C (0.25 g). Hydrogen was then bubbled through the solution with TLC monitoring until all starting **17** had been consumed. The catalyst was removed by filtration through a Celite pad and washed with methanol. The solvent was removed by evaporation, and the residue was placed under high vacuum.

To a solution of the Cbz Troc *tert*-butyl ester **5** (3.99 g, 6.61 mmol) in 8 mL of anhydrous dichloromethane under nitrogen was added 8 mL of trifluoroacetic acid. The solution was stirred with TLC monitoring until all of starting **5** had been consumed. The solvents were removed by evaporation, and the residue was repeatedly evaporated from benzene to removed all traces of trifluoroacetic acid to afford the crude carboxylic acid **18** as an oil. To this oil under nitrogen was added 5 mL of anhydrous dichloromethane followed by triethylamine (0.92 mL, 6.61 mmol) and quickly the deprotected serine derivative in a total of 7 mL of anhydrous dichloromethane. Immediately, EEDQ (2.13 g, 8.60 mmol) was added as a solid, and the mixture was stirred overnight under nitrogen. The reaction mixture was diluted with ethyl acetate and washed with 1 M HCl and brine, then dried, filtered, and evaporated to afford an oil. This oil was purified by flash silica gel chromatography, eluting with 50% ethyl acetate–50% hexanes to afford 1.71 g (35%) of the dipeptide **20** as a thick viscous oil: $[\alpha]^{25}_D$ = +6.8° (c = 5.37, $CHCl_3$); IR (TF) 3325, 3040, 2960, 1720 (br), 1660 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.03 (s, 9 H, $Si(CH_3)_3$), 0.90–1.10 (m, 2 H, CH_2Si), 1.50–1.90 (m, 4 H, CH_2), 3.30–3.55 (m, 2 H, diastereotopic CH_2O and OH), 3.62–3.78 (m, 1 H, diastereotopic CH_2O), 3.80–4.00 (m, 2 H, CH_2N), 4.15–4.30 (m, 2 H, OCH_2), 4.35–4.45 (m, 1 H, NCHCO), 4.50–4.62 (m, 1 H, NCHCO), 4.84 (AB q, 2 H, J = 16 Hz, benzylic H), 4.92 (s, 2 H, CH_2CCl_3), 5.04 (AB q, 2 H, J = 12 Hz, benzylic H), 5.70 (d, 1 H, J = 9 Hz, NH), 7.28–7.45 (m, 10 H, aromatic H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ −1.82, 17.10, 22.70, 29.73, 48.19, 53.61, 54.57, 62.52, 63.95, 66.83, 75.06, 76.37, 95.02, 127.76, 127.87, 128.23, 128.26, 128.58, 129.29, 134.47, 135.85, 155.18, 156.27, 169.95, 171.84; MS (positive ion FAB, glycerol–*m*-nitrobenzyl alcohol) m/z 736 (M + H), 738 (M (^{37}Cl) + H).

[N^2-(Benzyloxycarbonyl)-N^5-(benzyloxy)-N^5-(2,2,2-trichloroethoxycarbonyl)-L-ornithinyl]-L-serine (21). To a solution of the ester **20** (0.40 g, 0.551 mmol) in 1.3 mL of anhydrous THF under nitrogen was added a solution of 1 M tetrabutylammonium fluoride in THF (0.69 mL, 0.689 mmol). After stirring overnight, starting ester **20** remained as monitored by TLC; therefore, another 0.69 mL of TBAF was added. After stirring for 2 h, all starting ester **20** had been consumed. The THF was evaporated, and the mixture was diluted with ethyl acetate, washed with two portions of 1 M HCl and brine, dried, filtered, and evaporated to provide 0.29 g (83%) of **21** as a light tan foam. TLC analysis revealed that the material was homogeneous (5% acetic acid–95% ethyl acetate). Attempts to crystallize the dichlorohexylamine salt of **21** failed: $[\alpha]^{24}_D$ = +19.6° (c = 2.80, $CHCl_3$); IR (KBr) 3700–2400 (br), 3040, 2950, 1720 (br), 1650 (br) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.50–1.90 (m, 4 H, CH_2), 3.30–3.60 (m, 2 H, CH_2N), 3.80 (br d, 1 H, J = 10 Hz, diastereotopic CH_2O), 4.00 (br d, 1 H, J = 10 Hz, diastereotopic CH_2O), 4.30–4.45 (m, 1 H, NCHCO), 4.50–4.62 (m, 1 H, NCHCO), 4.70–4.80 (m, 2 H, benzylic H), 4.87 (s 2 H, CH_2CCl_3), 5.04 (AB q, 2 H, J = 12 Hz, benzylic H), 6.04 (d, 1 H, J = 8 Hz, NH), 7.20–7.42 (m, 10 H, aromatic H), 7.54–7.46 (br d, 1 H, NH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 22.94, 29.79, 48.67, 54.08, 54.47, 62.38, 67.17, 75.19, 77.17, 77.19, 95.11, 127.92, 128.12, 128.44, 128.47, 128.81, 129.47, 134.54, 135.88, 155.30, 156.66, 172.37, 172.43; MS (positive ion FAB, *m*-nitrobenzyl alcohol) m/z 638 (M (^{37}Cl) + H), 636 (M + H), 591 (M − CO_2H + H).

(3*S*,6*S*)-3-[3-[*N*-[*N*-[N^2-(Benzyloxycarbonyl)-N^5-(benzyloxy)-N^5-(2,2,2-trichloroethoxycarbonyl)-L-ornithinyl]-L-seryl]-*N*-(benzyloxy)amino]propyl]-6-[3-[*N*-(2,2,2-trichloroethoxycarbonyl)-*N*-(benzyloxy)amino]propyl]-2,5-piperazinedione (22). To a solution of the protected ornithine–serine acid **21** (0.154 g, 0.2419 mmol) and *N*-hydroxysuccinimide (0.028 g, 0.2419 mmol) in 1.0 mL of anhydrous tetrahydrofuran at 0 °C under nitrogen was added dicyclohexylcarbodiimide (0.049 g, 0.2419 mmol). This mixture was stirred for 30 min, and then filtered directly into a solution of the diketopiperazine **15a** (0.149 g, 0.2419 mmol) in anhydrous chloroform. The solvents were removed under vacuum, and 2.0 mL of anhydrous chloroform was added. The mixture was stirred for 4 h under nitrogen, diluted with chloroform, and washed with water. The aqueous layer was repeatedly extracted with chloroform. The pooled organic layers were washed with brine, dried, filtered, and evaporated to give an oil. This oil was purified by preparative silica gel chromatography, eluting twice with 5% methanol–95% chloroform to afford 0.168 g (56%) of **22** as an oil: $[\alpha]^{27}_D$ = −8.6° (c = 0.57, $CHCl_3$); IR (TF) 3260, 3040, 2960, 1715, 1675 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.50–1.95 (m, 12 H, CH_2), 3.20–4.05 (m, 10 H, CH_2N, CH_2O and NCHCO), 4.35–4.47 (m, 1 H, NCHCO), 4.79 (s, 2 H, benzylic H), 4.82 (s, 2 H, benzylic H), 4.84 (s 2 H, benzylic H), 4.89 (s 4 H, CH_2CCl_3), 4.94–5.02 (m, 1 H, NCHCO), 5.05 (br q, 2 H, benzylic H), 6.07 (d, 1 H, J = 8 Hz, NH), 6.65 (br s, 1 H, NH), 7.20–7.45 (m, 20 H, aromatic H), 7.60 (br s, 1 H, NH), 7.69 (d, 1 H, J = 7 Hz, NH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 21.86, 22.48, 23.03, 30.07, 30.18, 30.54, 44.12, 48.57, 48.95, 51.90, 53.52, 53.80, 54.28, 61.88, 66.81, 75.10, 75.15, 76.75, 77.19, 95.19, 95.26, 127.88, 127.98, 128.40, 128.48, 128.51, 128.79, 128.85, 129.15, 129.38, 129.52, 133.70, 134.67, 136.32, 155.04, 155.20, 156.35, 168.20, 168.38, 171.02, 172.31; MS (positive ion FAB, *m*-nitrobenzyl alcohol–glycerol) m/z cluster at 1233 (M + H), 615, 618.

(3*S*,6*S*)-3-[3-[*N*-[*N*-[N^2-Acetyl-N^5-(benzyloxy)-N^5-(2,2,2-trichloroethoxycarbonyl)-L-ornithinyl]-3-acetoxy-L-seryl]-*N*-(benzyloxy)amino]propyl]-6-[3-[*N*-(2,2,2-trichloroethoxycarbonyl)-*N*-(benzyloxy)amino]propyl]-2,5-piperazinedione (23). To a solution of **22** (0.200 g, 0.1623 mmol) in 2 mL of anhydrous dichloromethane under a drying tube was added 2 mL of 30% hydrobromic acid in acetic acid, and the mixture was stirred for 13 min. The solvent was evaporated. The residue was diluted with anhydrous benzene and evaporated to remove traces of acetic acid. This process was repeated three times, producing an amber oil which was placed under high vacuum. To this residue under nitrogen was added 2 mL of anhydrous dichloromethane followed by acetic anhydride (17 μL, 0.1804 mmol). This was stirred for 5 min, and TLC monitoring revealed the presence of ninhydrin positive material remaining as a minor product. Therefore, 6 μL of acetic anhydride (0.0600 mmol) was added (total acetic anhydride is 23 μL (0.2403 mmol)). The reaction solution was stirred for 18 h, evaporated to approximately 1 mL, and applied to six analytical (20 cm × 20 cm) silica gel plates as a narrow band. These plates were eluted with 5% methanol–95% chloroform, affording 0.146 g (76%) of the bis-acetate **23** as an amorphous foam that resisted crystallization: $[\alpha]^{24}_D$ = −23.2° (c = 0.34, $CHCl_3$); IR (TF) 3280 (br), 3080, 2960, 1740, 1730, 1720, 1715, 1675, 1655 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.45–1.90 (m, 12 H, CH_2), 1.97 (s, 3 H, CH_3CO), 2.07 (s, 3 H, CH_3CO), 3.35–3.57 (m, 4 H, CH_2N), 3.73–3.90 (m, 2 H, CH_2N), 3.92–3.98 (m, 1 H, NCHCO), 4.00–4.10 (m, 2 H, NCHCO and diastereotopic CH_2O), 4.23 (dd, 1 H, J = 7.5 and 17.7 Hz, diastereotopic CH_2O), 4.71 (t, 1 H, J = 9.3 and 9.6 Hz, NCHCO), 4.82 (s, 2 H, benzylic H), 4.86 (s, 2 H, benzylic H), 4.88 (s, 2 H, benzylic H), 4.92 (s, 2 H, CH_2CCl_3), 4.93 (s, 2 H, CH_2CCl_3), 5.09 (AB q, 1 H, J = 10.8 and 11.1 Hz, NCHCO), 6.39 (br s, 1 H, NH), 7.21 (d, 1 H, J = 5.1 Hz, NH), 7.32–7.46 (m, 15 H, aromatic H), 7.51 (d, 1 H, J = 9.0 Hz, NH), 7.95 (br s, 1 H, NH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.761, 21.10, 22.34, 22.79, 22.90, 28.92, 29.69, 29.75, 45.72, 47.91, 48.95, 49.42, 50.83, 53.67, 54.04, 62.33, 74.99 (br), 76.82, 77.10, 95.12, 95.14, 128.43, 128.70, 128.76, 129.08, 129.35, 129.42, 133.65, 134.46, 134.60, 154.95, 155.29, 168.54, 168.73, 170.31, 170.52, 170.67, 173.20; MS (positive ion FAB, chlorform–glycerol matrix) m/z cluster at 1182 (M + H), 615, 617.

(3*S*,6*S*)-3-[3-[*N*-[*N*-[N^2-Acetyl-N^5-(benzyloxy)-N^5-formyl-L-ornithinyl]-L-seryl]-*N*-(benzyloxy)amino]-propyl]-6-[3-[*N*-formyl-*N*-(benzyloxy)amino]propyl]-2,5-piperazinedione (24). To a solution of **23** (0.140 g, 0.118 mmol)

in 1.0 mL of anhydrous tetrahydrofuran was added a solution of 0.41 M formic–acetic anhydride (2.42 mL, 0.993 mmol) and activated zinc dust (0.233 g, 3.556 mmol). The reaction was monitered by TLC over a period of 3 h. The zinc was removed by filtration and washed with tetrahydrofuran, and the filtrate was evaporated. This was dissolved in chloroform, applied to a preparative TLC plate, and chromatographed, eluting with 17% methanol–83% chloroform to afford 0.085 g of the acetate ester as an oil as observed by the infrared absorption at 1740 cm^{-1} and by proton NMR. This ester was dissolved in anhydrous methanol and stirred with a catalytic amount of potassium bicarbonate under nitrogen with TLC monitoring for a period of 48 h. The methanol was evaporated, the residue was dissolved in chloroform, washed with water and brine, and then dried, and the solvents were removed by evaporation. The remaining residue was purified on two analytical (20 cm × 20 cm) silica gel plates, eluting with 17% methanol–83% chloroform to give 0.049 g (49%) of **24** as an oil: $[\alpha]^{25}_D = -25°$ (c = 0.020, CH_3OH); IR (TF) 3260 (br), 3070, 2940, 2880, 1665 (br), 1545, 1455, 910, 735, 695 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.45–1.85 (m, 12 H, CH_2), 1.97 (s, 3 H, CH_3CO), 3.20–3.90 (m, 8 H, CH_2N and NCHCO), 3.92–4.10 (2 br s, 2 H, CH_2O), 4.60–4.74 (m, 1 H, NCHCO), 4.80 (br s, 2 H, benzylic H), 4.82 (br s, 2 H, benzylic H), 4.86 and 4.87 (2 br s, 2 H, benzylic H), 4.89–5.00 (m, 1 H, NCHCO), 6.67 (br s, 1 H, NH), 7.20–7.50 (m, 15 H, aromatic H), 7.67 (br s, 1 H, NH), 7.95 (br s, 2 H, NH), 8.12 (br s, 1 H, CHO), 8.18 (br s, 1 H, CHO); ^{13}C NMR ($CDCl_3$, 75 MHz, all signals reported as observed at 20 °C) δ 21.40, 21.43, 22.28, 22.8–22.94 (m), 23.00, 23.55, 23.59, 22.39, 29.42, 29.48, 29.67, 29.94, 30.01, 43.02 (m), 43.58 (m), 45.04 (m), 51.07, 51.21, 51.26, 52.28, 53.57, 54.20, 61.62, 61.64, 61.67, 76.86, 77.20, 77.67, 128.40, 128.46, 128.81, 129.14, 129.41, 129.50, 133.91, 134.0–134.2 (m), 163.26, 163.28, 163.32, 163.68, 163.70, 168.40, 168.60, 170.79, 170.83, 171.55, 172.98; MS (positive ion FAB, chloroform–glycerol matrix) m/z 846 (M + H).

Foroxymithine: (3*S*,6*S*)-3-[3-[*N*-[*N*-(*N*²-Acetyl-*N*⁵-formyl-*N*⁵-hydroxy-L-ornithinyl)-L-seryl]-*N*-hydroxyamino]propyl]-6-[3-(*N*-formyl-*N*-hydroxyamino)propyl]-2,5-piperazinedione (1). To a solution of **24** (0.0175 g, 0.0207 mmol) in 0.5 mL of methanol and 0.5 mL of deionized distilled water was added 10% palladiumon carbon (0.005 g). This mixture was exposed to hydrogen for 2.5 h, filtered, and lyophilized to afford 0.0117 g (98%) of foroxymithine (**1**) as a glass. This synthetic material proved to be one spot (1% ferric chloride spray indication) by silica gel TLC (R_f 0.32) using a 2:1 mixture of ethanol–28% ammonium hydroxide as a solvent system in excellent agreement with the literature[1] R_f value of 0.33: $[\alpha]^{25}_D = -38°$ (c = 0.034, deionized distilled water) [lit.[1] $[\alpha]^{25}_D = -44°$ (c = 1.00, water)]; IR (KBr) 3420 (br), 3260 (br), 2940, 2880, 1660 (br), 1540, 1460, 875 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 1.35–1.80 (m, 12 H, CH_2), 1.83 (s, 3 H, CH_3CON), 3.00–3.12 (m, 1 H, diastereotopic CH_2N), 3.30–3.45 (m, 3 H, CH_2N), 3.46–3.55 (m, 2 H, NCHCO), 3.60–3.75 (m, 2 H, CH_2N), 3.95–4.05 (m, 2 H, CH_2O, 4.20–4.30 (m, 1 H, NCHCO), 4.88 (t, 1 H, J = 5.1 and 5.4 Hz, NCHCO), 7.76, 7.83, 8.11 (3 s, 2 H, CHO): ^{13}C NMR ($CDCl_3$, 75 MHz) δ 21.28, 21.57, 22.54, 27.94, 28.14, 30.02, 30.39, 37.27, 45.94, 46.09, 48.00, 49.90, 52.31, 53.24, 53.98, 54.04, 60.42, 159.54, 163.76, 164.21, 164.23, 169.82, 169.87, 169.92, 170.06, 173.65, 174.19; MS (positive ion FAB, glycerol matrix) m/z 598 (M + Na), 576 (M + H), 561 (M − CH_3CO + CHO), 517 (M − 2CHO).

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Supplementary Material Available: ^1H NMR, ^{13}C NMR, and DEPT spectra of new compounds (55 pages). Ordering information is given on any current masthead page.

Syntheses and Rearrangements of Spirocyclic Oxaziridines Derived from Unsymmetrical Ketones

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Oxaziridines provide useful alternatives to the Beckmann rearrangement and Schmidt reaction for ring enlargement of cyclic ketones. The procedure involves the condensation of the ketone in question with optically active α-methylbenzylamine, oxidation of the resultant imine, and photolysis to afford ring-expanded lactams. The α-phenylethyl substituent can be removed after photolysis to yield the N-unsubstituted lactam. When a distal ketone substituent is present, the oxaziridines can be synthesized stereoselectively. Thus, optically active ketones can be converted to either ring-expanded lactam by choice of either enantiomer of optically active α-methylbenzylamine. Ketones bearing adjacent substitution are generally not amenable to such regiocontrol because the resident substituent is the key stereocontrol element for the oxaziridine synthesis, although a notable exception is 2-methoxycyclohexanone. Stereogenic centers present in such compounds undergo epimerization during the couse of the reaction sequence; in addition, substrates containing substantial amounts of enamine give rise to novel doubly oxygenated products upon oxidation. Finally, the conformational behavior of the side chains in both oxaziridines and their product lactams permits some key stereochemical assignments to be made, on the basis of chemical shift trends in the NMR spectra of these materials.

The conversions of cyclic ketones to heterocycles using ring-expansion reactions have been featured in the preparations of such disparate materials as nylon (cyclohexanone → caprolactam[1]) and the plant growth promoter brassenolide (Baeyer–Villiger reaction on a 6-keto steroid[2]). The more sophisticated applications of such reactions require careful planning with respect to a number of chemical issues, chief among these being regioselectivity. In the formal insertion of a nitrogen substituent into a carbocyclic ring, the regiochemistry is controllable only in cases in which the ketone undergoing reaction is locally dissymmetric. For example, the standard Beckmann and Schmidt sequences afford primarily products which result from the migration of the most highly substituted group.[3]

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