

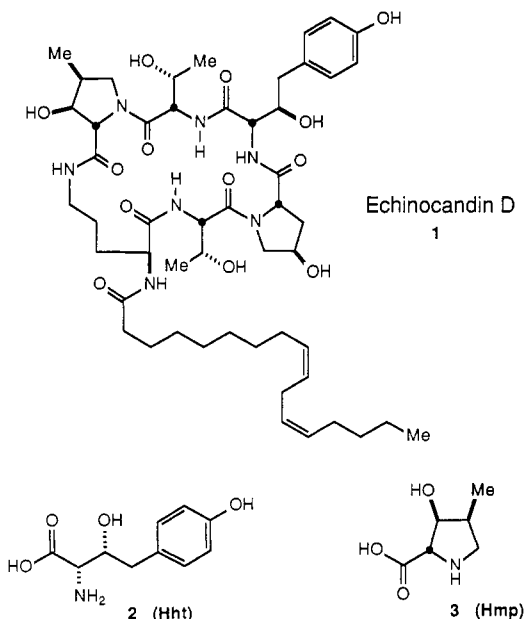
Synthesis of the Cyclic Hexapeptide Echinocandin D. New Approaches to the Asymmetric Synthesis of β -Hydroxy α -Amino Acids

David A. Evans* and Ann E. Weber

Contribution from the Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138. Received June 1, 1987

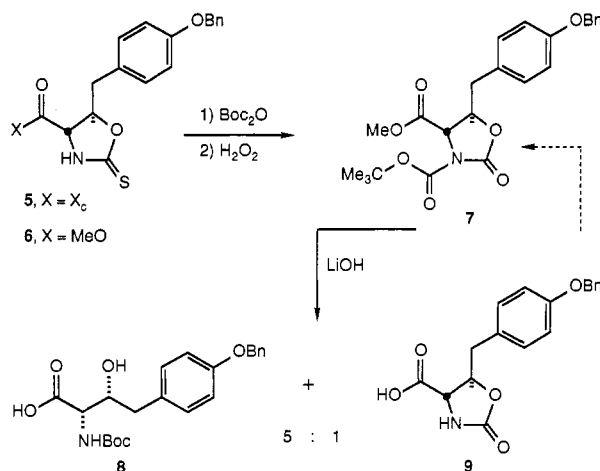
Abstract: Derivatives of the two unusual β -hydroxy amino acids in echinocandin D (**1**) have been synthesized by employing asymmetric glycine enolate aldol methodology. The *N*-Boc, *O*-benzyl derivative of (2*S*,3*R*)-3-hydroxyhomotyrosine (Hht) (**2**) and the methyl ester of (2*S*,3*S*,4*S*)-3-hydroxy-4-methylproline (Hmp) (**3**) have been synthesized in four steps each from (isothiocyanatoacetyl)oxazolidinone **4** and (bromoacetyl)oxazolidinone **10**, respectively. In both syntheses, asymmetric aldol addition reactions have been employed to establish the absolute stereochemical relationships at both hydroxyl and nitrogen-bearing asymmetric centers. In conjunction with the synthesis of Hmp (**3**) a new approach to the construction of nitrogen heterocycles via the intramolecular cycloalkylation of olefinic azides has been presented. Each of these amino acids has been integrated into a synthesis of echinocandin D.

Echinocandin D (**1**), isolated from *Aspergillus rugosus*, is a member of a family of lipopeptides possessing high antifungal activity.¹ This structure is unusual in that five of the six amino acids in this cyclic hexapeptide are hydroxylated. In addition to two threonine moieties and a 4-hydroxyproline, echinocandin D contains two rare β -hydroxy amino acids, (2*S*,3*R*)-3-hydroxyhomotyrosine (Hht) (**2**) and (2*S*,3*S*,4*S*)-3-hydroxy-4-methylproline (Hmp) (**3**). Both of these entities have recently been



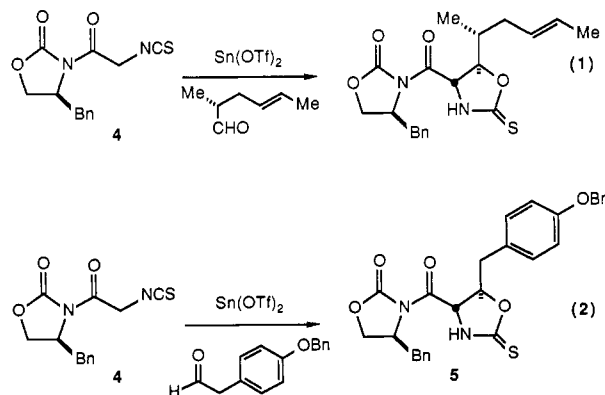
synthesized by Kurokawa and Ohfuné in conjunction with their total synthesis of echinocandin D.² Our own interest in the development of efficient methods for the asymmetric synthesis of both syn and anti β -hydroxy α -amino acids has attracted us to echinocandin D as a target for synthesis. Recent publications from this laboratory have described approaches to the asymmetric synthesis of both syn and anti β -hydroxy α -amino acids.^{3,4} Herein

Scheme I



we report the application of this asymmetric glycine enolate aldol methodology to the stereoselective synthesis of both Hht (**2**) and Hmp (**3**), as suitably protected derivatives, and the incorporation of these two amino acids into an efficient synthesis of echinocandin D (**1**).

The most direct approach to the construction of Hht (**2**) would be via the appropriate stereoregulated aldol addition reaction, and with the recent development of such bond constructions, such an approach to the synthesis of this family of α -amino acids may now be entertained. Based upon the precedent established in our recent synthesis of the cyclosporine amino acid MeBmt (eq 1),³ the



desired stereochemical relationship in the syn β -hydroxy amino acid Hht (**2**) was readily generated by the stannous triflate-me-

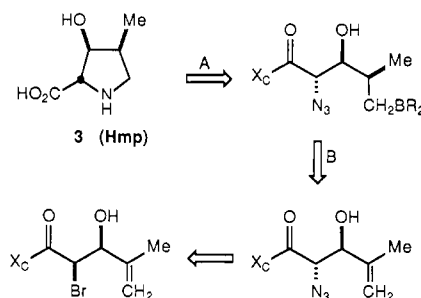
(1) (a) Traber, v. R.; Keller-Juslen, C.; Loosli, H.-R.; Kuhn, M.; v. Wartburg, A. *Helv. Chim. Acta* **1979**, *62*, 1252. (b) Keller-Juslen, C.; Kuhn, M.; Loosli, H.-R.; Petcher, T. J.; Weber, H. P.; v. Wartburg, A. *Tetrahedron Lett.* **1976**, 4147. (c) Benz, v. F.; Knusel, F.; Nuesch, J.; Treichler, H.; Voser, W.; Nyfeler, R.; Keller-Schierlein, W. *Helv. Chim. Acta* **1974**, *57*, 2459.
(2) (a) Kurokawa, N.; Ohfuné, Y. *J. Am. Chem. Soc.* **1986**, *108*, 6041.
(b) Kurokawa, N.; Ohfuné, Y. *Ibid.* **1986**, *108*, 6043.
(3) Evans, D. A.; Weber, A. E. *J. Am. Chem. Soc.* **1986**, *108*, 6757.
(4) Evans, D. A.; Sjogren, E. B.; Weber, A. E.; Conn, R. E. *Tetrahedron Lett.* **1987**, 28, 39.

diated⁵ aldol reaction of (isothiocyanatoacetyl)oxazolidinone **4** and *p*-(benzyloxy)phenylacetaldehyde. This stereoselective aldol process (eq 2) afforded adduct **5** in 72% yield as a colorless crystalline solid, mp 167–168 °C, after chromatographic purification and established both the carbon framework and the requisite stereochemical relationships found in Hht (**2**). The fact that this particular aldol reaction proceeds in good yield is noteworthy. It has been our experience that phenylacetaldehyde derivatives are not “conventional” aldehyde constituents in the aldol process, presumably due to their enhanced acidity. This point has surfaced in a synthesis of the β -lactam PS-5 recently reported from this laboratory.⁶

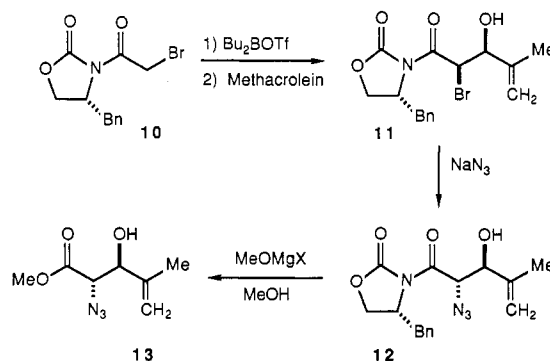
Conversion of the aldol adduct **5** to the N-protected Hht derivative **8** was carried out according to the plan outlined in Scheme I. Removal of the chiral auxiliary proceeded smoothly with magnesium methoxide in 1:1 methanol/methylene chloride at 0 °C to give the corresponding ester **6** in 95% yield as a nicely crystalline solid, mp 120–121 °C. All that remained for the completion of the synthesis was the hydrolytic removal of the oxazolidinone ring masking both oxygen and nitrogen functional groups. While these heterocycles may be hydrolyzed under vigorous conditions with refluxing concentrated hydrochloric acid, a milder protocol for the execution of the transformation is certainly desirable. The procedure devised to achieve this objective is illustrated below. Methyl ester **6** was first acylated with di-*tert*-butyl pyrocarbonate in the presence of (dimethylamino)pyridine. After the reaction was judged complete by TLC, a solution of 30% aqueous hydrogen peroxide and formic acid was added to effect the exchange of sulfur for oxygen,⁷ affording a 95% yield of *N*-Boc oxazolidinone **7**. Regioselective hydrolysis of carboximide **7** was then achieved with excess 2 N aqueous lithium hydroxide in dioxane at room temperature overnight to give a 5:1 mixture of **8** and **9**, respectively, the products of endocyclic and exocyclic carbonyl attack by hydroxide ion. Under these conditions the methyl ester was also hydrolyzed and the desired Boc-Hht(OBn)-OH **8** was isolated in 83% yield, mp 143–144 °C. This represents a 75% overall yield from aldol adduct **5**. In addition, the deacylated product **9** (17% yield) may be recycled by esterification with diazomethane or acidic methanol, followed by acylation as described above. The success of this hydrolysis procedure depends upon the selection of a sterically demanding N-protecting group such as Boc. The importance of this decision surfaces in the basic hydrolysis of the *N*-acyl oxazolidinone intermediate **7**, which can hydrolyze via exocyclic carbonyl attack to give the desired N-protected amino acid or, alternatively, by endocyclic attack to nonproductively deacylate the oxazolidinone ring. The above experiments demonstrate that the Boc moiety largely, but not exclusively, controls the regioselectivity of this process.⁸

The synthesis plan for the hydroxyproline derivative Hmp (**3**) is shown in Scheme II. The most speculative aspect of this undertaking was the projected intramolecular cycloalkylation of the illustrated azido borane (transform A). Although Brown and co-workers have demonstrated that alkyl azides react with trialkylboranes to give secondary amines,⁹ intramolecular variants of this reaction have not been investigated. The successful execution of this notion demands that olefin hydroboration to form the desired azido borane precedes the intermolecular reaction of the dialkylborane with the azide moiety. With regard to the stereoselective construction of the methyl-bearing stereocenter (transform B), we anticipated that the analogies provided by Still and Barrish¹⁰ for the related hydroborations of allylic alcohol

Scheme II



Scheme III



derivatives provided good precedent for the stereochemical course of this reaction. The successful execution of this series of reactions is described below.

The required 2(*S*) and 3(*S*) stereochemical relationships for Hmp (**3**) were established via the stereoselective bromoacetate aldol reaction illustrated in Scheme III.⁴ The (bromoacetyl)-oxazolidinone **10**, mp 41–42 °C, was prepared from the lithiated (4*R*)-4-(phenylmethyl)-2-oxazolidinone³ and bromoacetyl bromide in 87% yield. Enolization of **10** with dibutylboryl triflate¹¹ and triethylamine and its subsequent reaction with methacrolein afforded the crystalline aldol adduct **11**, mp 94–95 °C, as the predominant diastereomer, which was obtained in 50% yield after purification by flash chromatography.¹² Careful analysis of the unpurified reaction mixture by ¹H NMR spectroscopy revealed that the reaction diastereoselectivity was 97%. As has been noted in our recent publication on this reaction,⁴ the mass balance in this process was largely accounted for by recovered bromoacetate **10**. The requisite α -amino substituent was then introduced by nucleophilic azide displacement. Treatment of **11** with sodium azide at room temperature in dimethyl sulfoxide afforded a 9:1 mixture of azide **12** and, surprisingly, the dehydrohalogenated β -keto imide formed by loss of HBr. The desired crystalline azide, mp 94–95 °C, was isolated by direct crystallization of the reaction mixture in 82% yield. The oxazolidinone chiral auxiliary was then removed by treatment of **12** with 1.1 equiv of magnesium methoxide in 1:1 methanol/methylene chloride at 0 °C for 2 min to afford an 87% yield of methyl ester **13**.

Initial experiments directed toward an evaluation of the proposed hydroboration–cycloalkylation sequence outlined in Scheme II (transforms A and B) were carried out with 9-borabicyclononane (9-BBN)¹³ and the silylated methyl ester **14**.¹⁴ This experiment was carried out in benzene-*d*₆ and was directly monitored by ¹H NMR spectroscopy. When treated with 9-BBN, **14** was cleanly transformed into a single product whose spectrum was fully

(5) See ref 3 for the synthesis of stannous triflate. Reaction diastereoselection is dependent on the quality of reagent used. It must be washed thoroughly with anhydrous diethyl ether to remove all traces of triflic acid following its synthesis and has a shelf life of approximately 6 months when stored at room temperature under nitrogen.

(6) Evans, D. A.; Sjogren, E. *Tetrahedron Lett.* **1986**, 27, 3119.

(7) Hoppe, D.; Follmann, R. *Chem. Ber.* **1976**, 109, 3047.

(8) For the use of a similar strategy in the hydrolysis of cyclic amides, see: Flynn, D. L.; Zelle, R. E.; Greico, P. A. *J. Org. Chem.* **1983**, 48, 2424.

(9) Suzuki, A.; Sono, S.; Itoh, M.; Brown, H. C.; Midland, M. M. *J. Am. Chem. Soc.* **1971**, 93, 4329.

(10) Still, W. C.; Barrish, J. C. *J. Am. Chem. Soc.* **1983**, 105, 2487.

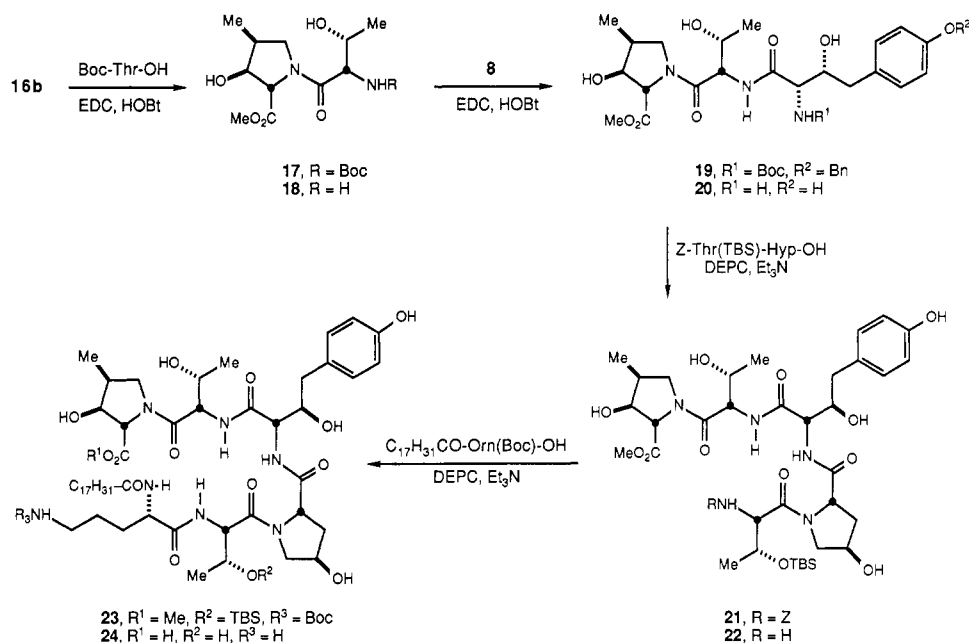
(11) For the synthesis of this reagent, see: Evans, D. A.; Nelson, J. V.; Vogel, E.; Taber, T. R. *J. Am. Chem. Soc.* **1981**, 103, 3099. Note: The quality of commercially available reagent is often insufficient to ensure high diastereoselectivity in the aldol reaction.

(12) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, 43, 2923.

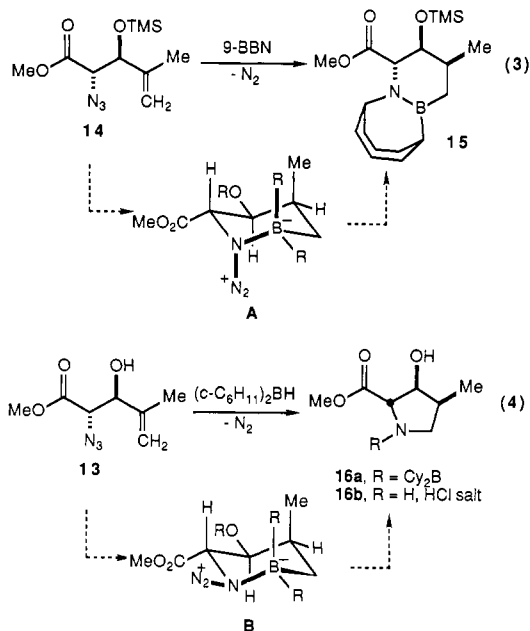
(13) Brown, H. C.; Chen, J. C. *J. Org. Chem.* **1981**, 46, 3978.

(14) The silyl group has no effect on the outcome of the reaction but was used to simplify the NMR spectra taken to follow the course of the reaction.

Scheme IV



consistent with the endocyclic aminoborane **15**, the product of cyclooctyl migration (eq 3). During the course of this reaction



there was no spectroscopic evidence for the buildup of any intermediates. Although the intervention of the undesired migration of the cyclooctyl ligand was unexpected, it was subsequently discovered that this side reaction has been preceded by Brown and coworkers in the analogous bimolecular process.¹⁵ In contrast, when azido olefin **13** (or its silylated counterpart **14**) was treated with dicyclohexylborane¹⁶ at room temperature for 5 h, the desired migratory insertion was observed (eq 4). The exocyclic aminoborane **16a** was hydrolyzed with dilute hydrochloric acid in the extractive workup to give the desired proline derivative **16b**, as its hydrochloride salt, mp 186–187 °C, in 72% yield as a single diastereomer by 500-MHz ¹H NMR.¹⁷ Thus, in one step the

C-4 stereochemistry is established, the azide is reduced, and the ring is closed, giving Hmp-OMe hydrochloride directly from the azido olefin. The proposed sense of asymmetric induction in the hydroboration, as predicted from the studies of Still and Barrish,¹⁰ was confirmed by comparison of the ¹H NMR spectrum of the diacetate derivative of **4** with that previously reported.^{1c}

It is difficult at best to rationalize the course of each of the reactions illustrated above. Given the reasonable assumption that there is a stereoelectronic requirement for the migration process, the two intermediates A and B possess the required anti disposition of migrating alkyl and departing diazonium moieties to give the insertion products **15** and **16**, respectively. Unfortunately, a full analysis of the impact of ring strain and steric effects on ligand migratory aptitudes in organoborane insertion reactions is beyond the scope of this study. Nonetheless, the precedent established for the illustrated cycloalkylation process should prove to be relevant to the development of related cyclic α -amino acids. The generalization of this concept will be reported in due course.

Further confirmation of the identities of both amino acid derivatives Hht (**2**) and Hmp (**3**) was gained by their incorporation into echinocandin D (Scheme IV). Thus, Hmp-OMe (**16b**) was coupled to *N*-Boc-threonine using ethyl((dimethylamino)propyl)carbodiimide (EDC) and hydroxybenzotriazole (HOBt)¹⁸ to give dipeptide **17** in 80% yield as a crystalline solid, mp 153–156 °C. After deprotection with trifluoroacetic acid (TFA), the resultant dipeptide methyl ester **18** was coupled with Boc-Hht-(Bn)-OH (**8**) using EDC/HOBt to provide a 94% yield of tripeptide **19**. This compound was deprotected in two steps (H₂, 10% Pd-C; TFA; quantitative yield), affording peptide **20**, whose spectral properties were identical with those reported.^{2b} The synthesis was completed following the procedure of Kurokawa and Ohfuné.^{2b} Thus, peptide **20** was coupled to *Z*-Thr(TBS)-Hyp-OH by using diethylphosphoryl cyanide (DEPC)¹⁹ to give pentapeptide **21**²⁰ in 86% yield. Following deprotection (H₂, 10% Pd-C), **22** and *N*^ω-linoleyl-*N*^ω-Boc-ornithine were coupled by using DEPC, and hexapeptide **23**²⁰ was isolated in 81% yield. After two-step deprotection (1 N NaOH, methanol; TFA-water), cyclization of **24** was achieved with diphenylphosphoryl azide²¹ to afford a 50% yield of echinocandin D (**1**), [α]_D -43° (c 0.82,

(15) Brown, H. C.; Midland, M. M.; Levy, A. B. *J. Am. Chem. Soc.* **1972**, *94*, 2114.

(16) Brown, H. C. *Organic Synthesis via Boranes*; Wiley: New York, 1975; pp 28–29.

(17) Any product formed by transfer of the cyclohexyl group to nitrogen remains in the organic phase during extractive workup and is thus separated from the desired product.

(18) Sheehan, J. C.; Cruickshank, P. A.; Boshart, G. L. *J. Org. Chem.* **1961**, *26*, 2525.

(19) Yamada, S.-I.; Kasai, Y.; Shioiri, T. *Tetrahedron Lett.* **1973**, 1595.

(20) The spectral properties of this compound were identical with those recorded by Kurokawa and Ohfuné.^{2b}

(21) Shioiri, T.; Yamada, S.-I. *Chem. Pharm. Bull. Jpn.* **1974**, *22*, 859.

MeOH). This compound was identical (500-MHz ^1H NMR, IR, MS, TLC) with a sample independently synthesized by Kurokawa and Ohfuné.^{2b} In addition, **1** was converted to its tetrahydro derivative^{1a} and found to be identical (500-MHz ^1H NMR, IR, UV, HPLC, MS, $[\alpha]_D -42^\circ$ (c 0.59, MeOH)) with a sample obtained from the natural product.

In conclusion, as part of a total synthesis of echinocandin D, Boc-Hht(Bn)-OH (**8**) has been synthesized in four steps from (isothiocyanocetyl)oxazolidinone **4**, and Hmp-OMe (**16a**) has been synthesized in four steps from (bromoacetyl)oxazolidinone **10**, further demonstrating the versatility of these glycine enolate aldol synthons.

Experimental Section

Tetrahydrofuran, diethyl ether, and *N*-ethylpiperidine were distilled from sodium metal/benzophenone ketyl. Methylene chloride, triethylamine, and diisopropylamine were distilled from calcium hydride. Methanol was distilled from magnesium methoxide. Dimethyl sulfoxide was distilled from calcium hydride and stored over 4-Å sieves. Dimethylformamide was dried over 4-Å sieves, distilled, and stored over 4-Å sieves. Methacrolein was distilled and used immediately. All other reagents were used as received. Unless otherwise noted, all nonaqueous reactions were carried out under a dry nitrogen atmosphere using flame-dried glassware. Melting points are uncorrected.

(4S)-3-(((4'S,5'R)-5'-((4''-(Phenylmethoxy)phenyl)methyl)-2'-thiooxo-4'-oxazolidinyl)carbonyl)-4-(phenylmethyl)-2-oxazolidinone (5). To a -78°C suspension of 3.66 g (8.77 mmol, 1.1 equiv) of stannous triflate^{3,5} and 1.18 g (1.43 mL, 10.4 mmol, 1.3 equiv) of *N*-ethylpiperidine in 30 mL of tetrahydrofuran (THF) was added via canula a solution of 2.21 g (7.98 mmol, 1.0 equiv) of 3-(isothiocyanocetyl)-2-oxazolidinone³ **4** in 10 mL of THF. The pale yellow solution was stirred at -78°C for 1.5 h, and then a solution of 2.16 g (9.55 mmol, 1.2 equiv) of 4-(phenylmethoxy)phenylacetaldehyde²² in 5 mL of methylene chloride was added via canula. After the reaction mixture was stirred at -78°C for 2.5 h, it was quenched by the addition of 20 mL of aqueous pH 7 buffer. The resultant slurry was filtered through Celite. The filtrate was diluted with 100 mL of 1 N aqueous sodium bisulfate and extracted with three 125-mL portions of methylene chloride. The combined organic phases were dried over anhydrous sodium sulfate and concentrated to give a white foam. Purification by MPLC (Chromoflex 2 in. \times 30 cm column, 35% ethyl acetate/hexane) yielded 2.91 g (72%) of the title compound as a white crystalline solid. An analytical sample was prepared by recrystallization from methylene chloride/carbon tetrachloride: R_f 0.37 (40% ethyl acetate/hexane); mp $167-168^\circ\text{C}$; IR (CH_2Cl_2) 3430, 3150-2810, 1782, 1712, 1514, 1471, 1300 cm^{-1} ; ^1H NMR (250 MHz, CDCl_3) δ 7.50-6.90 (m, 10 H, aromatic H's, NH), 5.58 (dt, 1 H, $J = 4.0, 6.2$ Hz, C(S)OCH), 5.04 (s, 2 H, OCH_2Ph), 4.82 (dd, 1 H, $J = 1.5, 4.0$ Hz, C(S)NHCH), 4.70-4.60 (m, 1 H, $\text{C}_4\text{-H}$), 4.36-4.28 (m, 2 H, $\text{C}_5\text{-H}_2$), 3.23-3.15 (m, 2 H, CHHAr, CHHPh), 3.05 (dd, 1 H, $J = 6.5, 14.4$ Hz, CHHAr), 2.88 (dd, 1 H, $J = 8.6, 13.6$ Hz, CHHPh); ^{13}C NMR (62.9 MHz, CDCl_3) δ 188.7, 166.6, 158.1, 153.6, 136.8, 134.1, 130.6, 129.3, 129.1, 128.5, 127.9, 127.7, 127.4, 126.4, 115.1, 84.4, 69.9, 67.5, 61.6, 55.2, 38.8, 37.4; $[\alpha]_D +141^\circ$ (c 1.01, CH_2Cl_2).

Anal. Calcd for $\text{C}_{28}\text{H}_{26}\text{N}_2\text{O}_5\text{S}$: C, 66.91; H, 5.21. Found: C, 66.35; H, 4.97.

Methyl (4S,5R)-5-((4'-(Phenylmethoxy)phenyl)methyl)-2-thiooxo-oxazolidine-4-carboxylate (6). To a 0°C solution of 1.07 g (2.13 mmol) of aldol adduct **5** in 10 mL of anhydrous methanol and 10 mL of methylene chloride was added via canula a suspension formed by the addition of 0.73 mL (2.34 mmol, 1.1 equiv, 3.2 M in diethyl ether) of methylmagnesium bromide to 5 mL of anhydrous methanol. After the reaction mixture was stirred for 3 min, it was quenched by the addition of 10 mL of 1 N aqueous sodium bisulfate. Volatiles were removed in vacuo. The residue was dissolved in 100 mL of 1 N aqueous sodium bisulfate and extracted with three 125-mL portions of methylene chloride. The combined organic phases were dried over anhydrous sodium sulfate and concentrated to give 1.18 g (104% mass balance) of a pale yellow oil. Purification by flash chromatography (35 \times 150 mm silica gel, 40% ethyl acetate/hexane) afforded 723 mg (95%) of the title compound as a white crystalline solid. An analytical sample was prepared by recrystallization from ethyl acetate/hexane: R_f 0.33 (40% ethyl acetate/hexane); mp $120-121^\circ\text{C}$; IR (CH_2Cl_2) 3440, 3040-2840, 1756, 1514, 1487, 1240, 1175 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ 7.72 (br s, 1 H, NH), 7.44-7.32 (m, 5 H, aromatic H's), 7.17 (d, 2 H, $J = 8.6$ Hz, aromatic H's), 6.94 (d, 2 H, $J = 8.6$ Hz, aromatic H's), 5.19 (q, 1 H, $J = 5.8$ Hz, $\text{C}_5\text{-H}$), 5.04 (s, 2 H, OCH_2Ph), 4.25 (d, 1 H, $J = 6.1$ Hz, $\text{C}_4\text{-H}$), 3.76

(s, 3 H, OCH_3), 3.18-3.05 (m, 2 H, $\text{C}_5\text{-CH}_2$); ^{13}C NMR (75.5 MHz, CDCl_3) δ 188.8, 168.6, 158.1, 136.8, 130.7, 128.4, 127.8, 127.3, 126.0, 115.2, 85.3, 69.9, 60.6, 53.1, 38.9; $[\alpha]_D +32.6^\circ$ (c 1.15, CH_2Cl_2).

Anal. Calcd for $\text{C}_{19}\text{H}_{19}\text{NO}_4\text{S}$: C, 63.85; H, 5.36. Found: C, 63.69; H, 5.27.

Methyl (4S,5R)-3-((tert-Butyloxy)carbonyl)-5-((4'-(phenylmethoxy)phenyl)methyl)-2-oxazolidine-4-carboxylate (7). To a room-temperature solution of 723 mg (2.02 mmol) of methyl ester **6** in 10 mL of methylene chloride was added 486 mg (0.51 mL, 2.23 mmol, 1.1 equiv) of di-*tert*-butyl pyrocarbonate and 12 mg (0.10 mmol, 0.05 equiv) of (dimethylamino)pyridine. After the reaction mixture was stirred for 30 min, it was cooled to 0°C and 5 mL of 30% aqueous hydrogen peroxide and 5 mL of 95% formic acid were added. The resultant two-phase mixture was stirred vigorously for 30 min and then poured into 150 mL of 1 M aqueous potassium carbonate. The aqueous solution was extracted with three 75-mL portions of methylene chloride. The combined organic phases were washed with 100 mL of 1 M aqueous potassium carbonate, dried over anhydrous sodium sulfate, and concentrated to give 1.01 g (114% mass balance) of a yellow oil. Purification by flash chromatography (35 \times 150 mm silica gel, 30% ethyl acetate/hexane) gave 849 mg (95%) of the title compound as a viscous oil: R_f 0.51 (40% ethyl acetate/hexane); IR (CH_2Cl_2) 3090-2820, 1828, 1805, 1758, 1730, 1515, 1372, 1332, 1245, 1221, 1178, 1153, 1070 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ 7.44-6.93 (m, 9 H, aromatic H's), 5.04 (s, 2 H, OCH_2Ph), 4.59 (dt, 1 H, $J = 4.3, 5.8$ Hz, $\text{C}_5\text{-H}$), 4.44 (d, 1 H, $J = 4.3$ Hz, $\text{C}_4\text{-H}$), 3.74 (s, 3 H, OCH_3), 3.02 (d, 2 H, $J = 5.8$ Hz, $\text{C}_5\text{-CH}_2$), 1.46 (s, 9 H, $\text{C}(\text{CH}_3)_3$); ^{13}C NMR (75.5 MHz, CDCl_3) δ 169.0, 158.2, 150.5, 148.4, 136.8, 130.6, 128.5, 127.9, 127.4, 125.6, 115.2, 84.5, 75.8, 69.9, 60.0, 52.9, 39.7, 27.7; $[\alpha]_D +29.2^\circ$ (c 2.60, CH_2Cl_2).

Anal. Calcd for $\text{C}_{24}\text{H}_{27}\text{NO}_7$: C, 65.29; H, 6.16. Found: C, 65.36; H, 6.22.

(2S,3R)-3-Hydroxy-2-(((tert-butyloxy)carbonyl)amino)-4-((phenylmethoxy)phenyl)butanoic Acid (8). To a room-temperature solution of 710 mg (1.61 mmol) of methyl ester **7** in 40 mL of dioxane was added 4 mL (8.00 mmol, 5 equiv) of freshly prepared 2 N aqueous lithium hydroxide solution. The resultant suspension was stirred at room temperature overnight. Volatiles were removed in vacuo. The residue was dissolved in 100 mL of 1 N aqueous sodium bisulfate and extracted with three 75-mL portions of methylene chloride. The combined organic phases were dried over sodium sulfate and concentrated to give 747 mg (116% mass balance) of a pale yellow foam. Purification by flash chromatography (35 \times 150 mm silica gel slurry-packed with 5% methanol/methylene chloride, eluted with 500 mL of 0.5:10:90 and 800 mL of 2:10:90 acetic acid/methanol/methylene chloride) gave 96 mg (17%) of the de-Boc acid **9** as a foam (R_f 0.22 (88:8:4 methylene chloride/methanol/acetic acid)) and 557 mg (83%) of the title compound as a white foam, which crystallized. An analytical sample was prepared by recrystallization from ethyl acetate/toluene: R_f 0.49 (88:8:4 methylene chloride/methanol/acetic acid); mp $143-144^\circ\text{C}$; IR (Nujol) 3600-2300 (br), 3430, 1769, 1675, 1514, 1241, 1159, 1067 cm^{-1} ; ^1H NMR (250 MHz, CD_3OD) δ 7.42-6.89 (m, 9 H, aromatic H's), 5.04 (s, 2 H, OCH_2Ph), 4.28 (br t, 1 H, $J = 7.0$ Hz, $\text{C}_3\text{-H}$), 4.09 (br s, 1 H, $\text{C}_2\text{-H}$), 2.74 (d, 2 H, $J = 7.0$ Hz, $\text{C}_4\text{-H}_2$), 1.47 (s, 9 H, $\text{C}(\text{CH}_3)_3$); ^{13}C NMR (62.9 MHz, CD_3OD) δ 174.8, 159.0, 138.9, 131.7, 131.4, 129.5, 128.8, 128.5, 116.1, 80.9, 74.2, 71.2, 58.2, 40.8, 28.8; $[\alpha]_D +3.74^\circ$ (c 1.07, MeOH). Anal. Calcd for $\text{C}_{22}\text{H}_{27}\text{NO}_6$: C, 65.82; H, 6.78. Found: C, 65.86; H, 6.85.

(4R)-3-(Bromoacetyl)-4-(phenylmethyl)-2-oxazolidinone (10). To a -78°C solution of 10.9 g (61.5 mmol) of (2R)-4-(phenylmethyl)-2-oxazolidinone³ in 200 mL of tetrahydrofuran was added 38 mL (61.5 mmol, 1 equiv, 1.63 M in hexane) of *n*-butyllithium, followed by 13.7 g (5.9 mL, 67.7 mmol, 1.1 equiv) of bromoacetyl bromide. The resultant bright yellow solution was stirred at -78°C for 10 min, and then the cooling bath was removed. After 20 min, the reaction was quenched by the addition of 100 mL of saturated aqueous ammonium chloride solution and volatiles were removed by rotary evaporation. The residue was extracted with three 200-mL portions of methylene chloride. The combined organic phases were dried over sodium sulfate and concentrated. The resultant dark yellow oil was filtered through silica gel (50 \times 150 mm, CH_2Cl_2) to give 17.4 g (95%) of a yellow oil, which crystallized. Further purification by recrystallization from diethyl ether at low temperature (two crops) and flash chromatography of the mother liquors gave 15.9 g (87%) of the title compound as a white crystalline solid: R_f 0.33 (25% ethyl acetate/hexane); mp $41-42^\circ\text{C}$; IR (CH_2Cl_2) 3110-2860, 1783, 1715, 1389, 1327, 1201, 702 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ 7.37-7.19 (m, 5 H, aromatic H's), 4.75-4.67 (m, 1 H, $\text{C}_4\text{-H}$), 4.57 (d, 1 H, $J = 12.8$ Hz, CHHBr), 4.52 (d, 1 H, $J = 12.8$ Hz, CHHBr), 4.28-4.21 (m, 2 H, $\text{C}_5\text{-H}_2$), 3.33 (dd, 1 H, $J = 3.3, 13.4$ Hz, CHHPh), 2.81 (dd, 1 H, $J = 9.6, 13.4$ Hz, CHHPh); ^{13}C NMR (75.5 MHz, CDCl_3) δ 166.0, 152.9, 134.8, 129.3, 129.0, 127.5, 66.7, 55.4, 37.5,

27.9; $[\alpha]_D +75.4^\circ$ (*c* 2.30, CH₂Cl₂).

Anal. Calcd for C₁₂H₁₂BrNO₃: C, 48.34; H, 4.06. Found: C, 48.20; H, 4.01.

(4R)-3-((2'R,3'S)-2'-Bromo-3'-hydroxy-4'-methyl-4'-pentenoyl)-4-(phenylmethyl)-2-oxazolidinone (11). To a -78 °C suspension of 3.77 g (12.7 mmol) of 3-(bromoacetyl)-2-oxazolidinone **10** in 30 mL of diethyl ether were added 1.79 g (2.50 mL, 17.75 mmol, 1.4 equiv) of triethylamine and 3.81 g (3.50 mL, 13.9 mmol, 1.10 equiv) of di-*n*-butylboryl triflate. The cooling bath was removed and the solution was stirred at room temperature for 1.5 h. The resultant two-phase brown mixture was gradually cooled to -78 °C with vigorous stirring, and 1.33 g (1.57 mL, 19.0 mmol, 1.5 equiv) of methacrolein was added neat. After the reaction mixture was stirred at -78 °C for 30 min and 0 °C for 2 h, it was diluted with 150 mL of ether, washed with two 100-mL portions of 1 N aqueous sodium bisulfate and one 100-mL portion of water, and concentrated. The residue was dissolved in 30 mL of ether and cooled to 0 °C. To this solution was added dropwise 30 mL of 1:1 methanol/30% aqueous hydrogen peroxide. The mixture was stirred at 0 °C for 1 h, then poured into 200 mL of saturated aqueous sodium bicarbonate, and extracted with two 250-mL portions of ether. The combined organic phases were washed with two 150-mL portions of saturated aqueous sodium bicarbonate, dried over magnesium sulfate, and concentrated to give 5.00 g (107% mass balance, 97:3 mixture of isomers by 250-MHz ¹H NMR) of a yellow oil. Purification by flash chromatography (50 × 150 mm silica gel, 3% ethyl acetate/methylene chloride) gave 2.32 g (50%) of the title compound as a white crystalline solid. An analytical sample was prepared by recrystallization from ethyl acetate/hexane: *R*_f 0.20 (2% ethyl acetate/methylene chloride); mp 94–95 °C; IR (CH₂Cl₂) 3550 (br), 1787, 1708, 1400, 1201 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 7.38–7.23 (m, 5 H, aromatic H's), 5.93 (d, 1 H, *J* = 5.1 Hz, *CHBr*), 5.93 (br s, 1 H, *C=CHH*), 5.06 (t, 1 H, *J* = 0.5 Hz, *C=CHH*), 4.76–4.68 (m, 1 H, *C₄-H*), 4.51 (br d, 1 H, *J* = 4.9 Hz, *CHOH*), 4.29–4.22 (m, 2 H, *C₅-H₂*), 3.31 (dd, 1 H, *J* = 3.3, 13.5 Hz, *CHPh*), 3.10 (br s, 1 H, *OH*), 2.82 (dd, 1 H, *J* = 9.5, 13.5 Hz, *CHPh*), 1.80 (d, 3 H, *J* = 0.5 Hz, *CH₃*); ¹³C NMR (75.5 MHz, CDCl₃) δ 168.5, 152.4, 142.2, 134.6, 129.4, 129.0, 127.5, 114.7, 73.7, 66.3, 55.2, 48.6, 37.0, 18.5; $[\alpha]_D -60.4^\circ$ (*c* 1.01, CH₂Cl₂).

Anal. Calcd for C₁₆H₁₈BrNO₄: C, 52.19; H, 4.93. Found: C, 52.24; H, 4.82.

(4R)-3-((2'S,3'S)-2'-Azido-3'-hydroxy-4'-methyl-4'-pentenoyl)-4-(phenylmethyl)-2-oxazolidinone (12). A solution of 2.62 g (7.11 mmol) of aldol adduct **11** and 925 mg (14.2 mmol, 2 equiv) of sodium azide in 25 mL of dimethyl sulfoxide was stirred at room temperature for 5 h. The resultant yellowish orange solution was diluted with 1750 mL of 2:1 hexane/methylene chloride, washed with four 250-mL portions of water, dried over sodium sulfate, and concentrated to give a pale yellow oil, which crystallized. Purification by recrystallization from ethyl acetate/hexane gave 1.92 g (82%) of the title compound as white crystals: *R*_f 0.30 (2% ethyl acetate/methylene chloride); mp 94–95 °C; IR (CH₂Cl₂) 3600, 3100–2900, 2115, 1787, 1708, 1390, 1288, 1213 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 7.38–7.24 (m, 5 H, aromatic H's), 5.21 (d, 1 H, *J* = 0.6 Hz, *C=CHH*), 5.17 (d, 1 H, *J* = 8.7 Hz, *CHN₃*), 5.13 (t, 1 H, *J* = 1.4 Hz, *C=CHH*), 4.79–4.71 (m, 1 H, *C₄-H*), 4.52 (br d, 1 H, *J* = 7.5 Hz, *CHOH*), 4.31–4.21 (m, 2 H, *C₅-H₂*), 3.34 (dd, 1 H, *J* = 3.4, 13.5 Hz, *CHPh*), 2.79 (dd, 1 H, *J* = 9.6, 13.5 Hz, *CHPh*), 2.71 (br s, 1 H, *OH*), 1.89 (d, 3 H, *J* = 1.4 Hz, *CH₃*); ¹³C NMR (75.5 MHz, CDCl₃) δ 170.1, 153.6, 143.1, 134.8, 129.4, 129.0, 127.4, 115.6, 76.3, 66.6, 59.5, 55.6, 37.5, 17.0; $[\alpha]_D -11.2^\circ$ (*c* 1.12, CH₂Cl₂).

Anal. Calcd for C₁₆H₁₈N₄O₄: C, 58.17; H, 5.49. Found: C, 58.35; H, 5.41.

Methyl (2S,3S)-2-Azido-3-hydroxy-4-methyl-4-pentenoate (13). To a 0 °C solution of 1.15 g (3.49 mmol) of azide **12** in 8 mL of anhydrous methanol and 8 mL of methylene chloride was added via canula a suspension formed by the addition of 1.20 mL (3.84 mmol, 1.1 equiv, 3.2 M in diethyl ether) of methylmagnesium bromide to 5 mL of anhydrous methanol. After the reaction mixture was stirred for 2 min, it was quenched by the addition of 20 mL of 1 N aqueous sodium bisulfate. Volatiles were removed in vacuo. The residue was dissolved in 100 mL of 1 N aqueous sodium bisulfate and extracted with three 100-mL portions of methylene chloride. The combined organic phases were dried over anhydrous sodium sulfate and concentrated to give 1.39 g (110% mass balance) of a pale yellow oil. Purification by flash chromatography (35 × 150 mm silica gel, 3% ethyl acetate/methylene chloride) gave 559 mg (87%) of the title compound as a clear oil: *R*_f 0.20 (2% ethyl acetate/methylene chloride); IR (neat) 3500 (br), 3090, 3010–2850, 2105, 1756, 1653, 1439, 1353, 1260, 1228, 1203, 1178, 1025, 913 cm⁻¹; ¹H NMR (250 MHz, CDCl₃) δ 5.12 (s, 1 H, *C₅-HH*), 5.05 (t, 1 H, *J* = 1.3 Hz, *C₅-HH*), 4.44 (br t, 1 H, *J* = 5.8 Hz, *C₃-H*), 3.93 (d, 1 H, *J* = 7.0 Hz, *C₂-H*), 3.82 (s, 3 H, *OCH₃*), 2.62 (br d, 1 H, *J* = 5.1 Hz, *OH*), 1.80 (s, 3 H, *C₄-CH₃*); ¹³C NMR (62.9 MHz, CDCl₃) δ 169.4, 142.5, 114.8,

75.5, 63.7, 52.7, 17.8; $[\alpha]_D -0.34^\circ$ (*c* 1.16, CH₂Cl₂).

Anal. Calcd for C₇H₁₁N₃O₃: C, 45.50; H, 5.99. Found: C, 45.91; H, 6.19.

(2S,3S,4S)-3-Hydroxy-4-methylproline Methyl Ester Hydrochloride (16b). To a 0 °C suspension of 1.55 g (8.68 mmol, 3 equiv) of dicyclohexylborane in 10 mL of methylene chloride was added via canula a 0 °C solution of 534 mg (2.89 mmol) of azido olefin **13** in 5 mL of methylene chloride. The cooling bath was removed and the reaction mixture was stirred for 5 h at room temperature. The resultant bright yellow solution was partitioned between 150 mL of methylene chloride and 150 mL of 1 N aqueous hydrochloric acid, freshly prepared from doubly distilled water. The aqueous phase was concentrated to give 408 mg (72%, >97% pure by 500-MHz ¹H NMR) of a white crystalline solid. An analytical sample was prepared by recrystallization from ethanol/ethyl acetate: mp 186–187 °C; IR (KBr pellet) 3600–2400 (br), 3300, 2900, 1736, 1589, 1462, 1432, 1371, 1348, 1337, 1305, 1266, 1238, 1148, 1045, 1010, 975, 909 cm⁻¹; ¹H NMR (250 MHz, CD₃OD) δ 4.41 (d, 1 H, *J* = 3.8 Hz, *C₃-H*), 4.32 (s, 1 H, *C₂-H*), 3.86 (s, 3 H, *OCH₃*), 3.55 (dd, 1 H, *J* = 8.1, 11.0 Hz, *C₅-HH*), 3.06 (t, 1 H, *J* = 11.3 Hz, *C₅-HH*), 2.33–2.17 (m, 1 H, *C₄-H*), 1.10 (d, 3 H, *J* = 6.7 Hz, *C₄-CH₃*); ¹³C NMR (62.9 MHz, CD₃OD) δ 169.2, 76.1, 69.3, 54.2, 50.8, 38.2, 10.5; $[\alpha]_D +6.7^\circ$ (*c* 0.70; MeOH).

Anal. Calcd for C₇H₁₄ClNO₃: C, 42.97; H, 7.21. Found: C, 42.91; H, 7.24.

Boc-Thr-Hmp-OMe (17). To a 0 °C solution of 199 mg (1.02 mmol) of hydroxymethylproline methyl ester hydrochloride **16b** and 245 mg (1.12 mmol, 1.1 equiv) of *N*-Boc-threonine in 4 mL of dimethylformamide (DMF) were added 103 mg (0.14 mL, 1.02 mmol, 1.0 equiv) of triethylamine, 158 mg (1.17 mmol, 1.15 equiv) of hydroxybenzotriazole monohydrate, and 224 mg (1.17 mmol, 1.15 equiv) of 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride. After the reaction mixture was stirred for 2 h at 0 °C and 20 h at room temperature, the DMF was removed under reduced pressure. The residue was partitioned between 100 mL of ethyl acetate and 20 mL of water. The organic phase was washed with 20-mL portions each of 1 N aqueous sodium bisulfate, water, saturated aqueous sodium bicarbonate, and saturated aqueous sodium chloride, dried over magnesium sulfate, and concentrated to give 332 mg (91% mass balance) of a crystalline solid. Purification by flash chromatography (20 × 150 mm silica gel, ethyl acetate) gave 292 mg (80%) of the title compound as a white crystalline solid. An analytical sample was prepared by recrystallization from methylene chloride/carbon tetrachloride: *R*_f 0.38 (ethyl acetate); mp 153–156 °C; IR (Nujol) 3430, 3350 (br), 3265, 1748, 1690, 1636 cm⁻¹; ¹H NMR (500 MHz, CD₃OD, major conformer) δ 4.40 (s, 1 H, *Hmp C₂-H*), 4.30 (d, 1 H, *J* = 5.8 Hz, *Thr C₂-H*), 4.15 (dd, 1 H, *J* = 1.1, 4.3 Hz, *Hmp C₃-H*), 3.95–3.90 (m, 2 H, *Thr C₃-H*, *Hmp C₅-H*), 3.76 (s, 3 H, *OCH₃*), 3.51 (t, 1 H, *J* = 9.8 Hz, *Hmp C₅-H*), 2.40–2.34 (m, 1 H, *Hmp C₄-H*), 1.43 (s, 9 H, *C(CH₃)₃*), 1.23 (d, 3 H, *J* = 6.3 Hz, *Thr C₄-H₃*), 1.07 (d, 3 H, *J* = 6.8 Hz, *Hmp C₄-CH₃*); $[\alpha]_D -63.2^\circ$ (*c* 1.07, MeOH); MS (FAB, *m*-nitrobenzyl alcohol) *m/z* 361 (*M* + 1), 305, 261.

Anal. Calcd for C₁₆H₂₈N₂O₇: C, 53.32; H, 7.83. Found: C, 53.45; H, 7.96.

Boc-Hht(Bn)-Thr-Hmp-OMe (19). A solution of 183 mg (0.508 mmol) of dipeptide **17** in 2 mL of trifluoroacetic acid was stirred at 0 °C for 20 min and concentrated. The amorphous solid was dissolved in methanol and concentrated several times, suspended in toluene and concentrated, and dried over P₂O₅ under vacuum for 2 h. The resultant deprotected dipeptide was dissolved in 2.5 mL of dimethylformamide (DMF) and cooled to 0 °C. To this solution were added 204 mg (0.508 mmol, 1.0 equiv) of Boc-Hht(Bn)-OH (**8**), 51.4 mg (56 μL, 0.508 mmol, 1.0 equiv) of *N*-methylmorpholine, 75.5 mg (0.559 mmol, 1.1 equiv) of hydroxybenzotriazole monohydrate, and 107 mg (0.559 mmol, 1.1 equiv) of 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride. After the reaction mixture was stirred for 2 h at 0 °C and 20 h at room temperature, the DMF was removed under reduced pressure. The residue was partitioned between 100 mL of ethyl acetate and 30 mL of water. The organic phase was washed with 30-mL portions each of 1 N aqueous sodium bisulfate, water, saturated aqueous sodium bicarbonate, and saturated aqueous sodium chloride, dried over magnesium sulfate, and concentrated to give 380 mg (116% mass balance) of a white foam. Purification by flash chromatography (20 × 150 mm silica gel, 150 mL of ethyl acetate and 250 mL of 2% methanol/ethyl acetate) gave 308 mg (94%) of the title compound as a white foam: *R*_f 0.23 (2% methanol/ethyl acetate); IR (CH₂Cl₂) 3600, 3420, 1745, 1716 (br), 1645, 1514 cm⁻¹; ¹H NMR (300 MHz, CD₃OD, major conformer) δ 7.42–7.25 (m, 5 H, aromatic H's), 7.12 (d, 2 H, *J* = 8.5 Hz, aromatic H's), 6.90 (d, 2 H, *J* = 8.5 Hz, aromatic H's), 5.04 (s, 2 H, *CH₂Ph*), 4.64 (d, 1 H, *J* = 6.2 Hz, *Thr C₂-H*), 4.37 (s, 1 H, *Hmp C₂-H*), 4.18–4.13 (m, 2 H, *Hht C₃-H*, *Hmp C₅-H*), 4.08 (d, 1 H, *J* = 2.5 Hz, *Hht C₂-H*), 4.01 (qn, 1 H, *J* = 6.3 Hz, *Thr C₃-H*), 3.93 (dd, 1 H, *J* = 8.1, 9.3 Hz, *Hmp C₅-H*), 3.71

(s, 3 H, OCH₃), 3.49 (t, 1 H, *J* = 9.9 Hz, Hmp C₅-H), 2.69 (d, 2 H, *J* = 6.8 Hz, Hht C₄-H₂), 2.40–2.30 (m, 1 H, Hmp C₄-H), 1.47 (s, 9 H, C(CH₃)₃), 1.24 (d, 3 H, *J* = 6.4 Hz, Thr C₄-H₃), 1.04 (d, 3 H, *J* = 6.8 Hz, Hmp C₄-CH₃); [α]_D²⁵ -42.7° (c 1.25, MeOH); MS (FAB, *m*-nitrobenzyl alcohol) *m/z* 644 (M + 1), 417, 307, 289.

Anal. Calcd for C₃₃H₄₅N₃O₁₀: C, 61.57; H, 7.05. Found: C, 61.59; H, 7.08.

Hht-Thr-Hmp-OMe (20). A solution of 180 mg (0.279 mmol) of tripeptide **19** in 4 mL of 1:1 ethanol/acetic acid was stirred over 10% palladium on carbon under an atmosphere of hydrogen for 5 h. The suspension was filtered through Celite and concentrated. The resultant amorphous solid (*R_f* 0.41 (10% methanol/methylene chloride)) was dissolved in 2 mL of trifluoroacetic acid, stirred at 0 °C for 30 min, and concentrated to give a quantitative yield of the deprotected tripeptide, as its trifluoroacetic acid salt. For comparison purposes this was converted to the hydrochloric salt as follows. The tripeptide was dissolved in 5 mL of 3% methanolic hydrogen chloride (formed by the addition of 1 mL of acetyl chloride to 19 mL of methanol) and concentrated. This process was repeated several times to give the tripeptide hydrochloride salt as a white amorphous solid: IR (Nujol) 3400 (br), 1741, 1680, 1640 (br) cm⁻¹; ¹H NMR (250 MHz, CD₃OD, major conformer) δ 7.07 (d, 2 H, *J* = 8.5 Hz, aromatic H's), 6.71 (d, 2 H, *J* = 8.5 Hz, aromatic H's), 4.62 (d, 1 H, *J* = 6.2 Hz, Thr C₂-H), 4.39 (d, 1 H, *J* = 1.1 Hz, Hmp C₂-H), 4.17 (dd, 1 H, *J* = 1.1, 4.2 Hz, Hmp C₃-H), 4.04–3.93 (m, 3 H, Hht C₃-H, Thr C₃-H, Hmp C₅-H), 3.85 (d, 1 H, *J* = 5.6 Hz, Hht C₂-H), 3.71 (s, 3 H, OCH₃), 3.56 (t, 1 H, *J* = 9.0 Hz, Hmp C₅-H), 2.76 (dd, 1 H, *J* = 3.1, 14.0 Hz, Hht C₄-H), 2.60 (dd, 1 H, *J* = 9.8, 14.0 Hz, Hht C₄-H), 2.42–2.30 (m, 1 H, Hmp C₄-H), 1.30 (d, 3 H, *J* = 6.4 Hz, Thr C₄-H₃), 1.07 (d, 3 H, *J* = 6.8 Hz, Hmp C₄-CH₃); [α]_D²⁵ -16.8° (c 2.67, MeOH) (lit.^{2b} [α]_D²⁵ -11.8° (c 1.7, MeOH)); MS (FAB, *m*-nitrobenzyl alcohol) *m/z* 454 (M + 1), 307.

Z-Thr(TBS)-Hyp-Hht-Thr-Hmp-OMe (21). This reaction was carried out according to the procedure of Kurokawa and Ohfuné.^{2b} To a 0 °C solution of 159 mg (0.279 mmol) of tripeptide **20** and 141 mg (0.293 mmol, 1.05 mmol) of Z-Thr(TBS)-Hyp-OH^{2b} in 2.5 mL of dimethylformamide (DMF) was added via canula a 0 °C solution of 54.7 mg (50.9 μL, 0.335 mmol, 1.2 equiv) of diethyl cyanophosphonate in 0.5 mL of DMF, followed by 57.9 mg (79.8 μL, 0.573 mmol, 2.05 equiv) of triethylamine. The resultant solution was stirred for 16 h, during which time the ice/water cooling bath was allowed to warm to room temperature. The reaction mixture was then diluted with 200 mL of 1:1 ethyl acetate/toluene, washed with 50-mL portions each of water, 1 N aqueous sodium bisulfate, water, saturated aqueous sodium bicarbonate, and saturated aqueous sodium chloride, dried over magnesium sulfate, and concentrated to give 249 mg (97% mass balance) of a white foam. Purification by flash chromatography (20 × 150 mm silica gel, 5% methanol/methylene chloride) gave 220 mg (86%) of the title compound as an amorphous solid: *R_f* 0.25 (10% methanol/methylene chloride); IR (CHCl₃) 3600 (br), 3020–2860, 1750–1700, 1640 (br) cm⁻¹; ¹H NMR (500 MHz, DMSO-*d*₆, major conformer) δ 9.07 (s, 1 H, PhOH), 8.17 (d, 1 H, *J* = 8.8 Hz, NH), 7.47 (d, 1 H, *J* = 9.4 Hz, NH), 7.36–7.28 (m, 6 H, aromatic H's, NH), 6.96 (d, 2 H, *J* = 8.4 Hz, aromatic H's), 6.57 (d, 2 H, *J* = 8.4 Hz, aromatic H's), 5.47 (d, 1 H, *J* = 4.5 Hz, OH), 5.16 (d, 1 H, *J* = 3.6 Hz, OH), 5.01 (d, 1 H, *J* = 5.1 Hz, OH), 4.99 (s, 2 H, OCH₂Ph), 4.72 (d, 1 H, *J* = 5.0 Hz, OH), 4.56 (t, 1 H, *J* = 7.9 Hz, Hyp C₂-H), 4.41 (dd, 1 H, *J* = 6.0, 7.7 Hz, Thr C₂-H), 4.35 (br s, 1 H, Hyp C₄-H), 4.24 (t, 1 H, *J* = 8.8 Hz, Thr C₂-H), 4.18 (s, 1 H, Hyp C₂-H), 4.12 (dd, 1 H, *J* = 2.2, 8.8 Hz, Hht C₃-H), 4.00–3.99 (m, 2 H, Hht C₃-H, Hmp C₃-H), 3.92–3.86 (m, 2 H, Thr C₃-H, Hmp C₅-H), 3.81–3.73 (m, 2 H, Thr C₃-H, Hyp C₅-H), 3.65 (br d, 1 H, *J* = 10.6 Hz, Hyp C₅-H), 3.61 (s, 3 H, OCH₃), 3.28 (t, 1 H, *J* = 11.4 Hz, Hmp C₅-H), 2.68 (dd, 1 H, *J* = 7.9, 13.3 Hz, Hht C₄-H), 2.52 (dd, 1 H, *J* = 5.9, 13.3 Hz, Hht C₄-H), 2.23–2.17 (m, 1 H, Hmp C₄-H), 2.11 (br t, 1 H, *J* = 10.4 Hz, Hyp C₃-H), 1.88 (ddd, 1 H, *J* = 4.7, 8.4, 13.1 Hz, Hyp C₃-H), 1.17 (d, 3 H, *J* = 6.2 Hz, Thr C₄-H₃), 1.08 (d, 3 H, *J* = 6.3 Hz, Thr C₄-H₃), 0.92 (d, 3 H, *J* = 6.8 Hz, Hmp C₃-CH₃), 0.81 (s, 9 H, C(CH₃)₃), 0.04 (s, 3 H, Si(CH₃)₃), 0.01 (s, 3 H, Si(CH₃)₃); [α]_D²⁵ -82.8° (c 1.37, MeOH) (lit.^{2b} [α]_D²⁵ -83.9° (c 1.27, MeOH)); MS (FAB, *m*-nitrobenzyl alcohol) *m/z* 916 (M + 1), 350, 261.

Linoleyl-Orn(Boc)-Thr(TBS)-Hyp-Hht-Thr-Hmp-OMe (23). This reaction was carried out according to the procedure of Kurokawa and Ohfuné.^{2b} A solution of 132 mg (0.144 mmol) of pentapeptide **21** in methanol was stirred over 10% palladium on carbon under an atmosphere of hydrogen for 4 h. The suspension was filtered through Celite, concentrated, dissolved in 1.0 mL of dimethylformamide (DMF) along with 78.3 mg (0.158 mmol, 1.1 equiv) of *N*^α-linoleyl *N*^ω-Boc-ornithine,^{2b} and cooled to 0 °C. To the resultant solution was added via canula a 0 °C solution of 28.2 mg (26.2 μL, 0.173 mmol, 1.2 equiv) of diethyl cyanophosphonate in 0.5 mL of DMF, followed by 16.0 mg (22.0 μL, 0.158 mmol, 1.1 equiv) of triethylamine. The resultant solution was stirred for

16.5 h, during which time the ice/water cooling bath was allowed to warm to room temperature. The reaction mixture was then diluted with 100 mL of 1:1 ethyl acetate/toluene, washed with 25-mL portions each of water, 1 N aqueous sodium bisulfate, water, saturated aqueous sodium bicarbonate, and saturated aqueous sodium chloride, dried over magnesium sulfate, and concentrated to give 168 mg (93% mass balance) of a glass. Purification by flash chromatography (20 × 100 mm silica gel, 500 mL of 5% and 350 mL of 10% methanol/methylene chloride) gave 146 mg (81%) of the title compound as an amorphous solid: *R_f* 0.29 (10% methanol/methylene chloride); IR (CHCl₃) 3460 (br), 3310 (br), 3090, 2940, 2865, 1745, 1655 (br), 1632, 1520 cm⁻¹; ¹H NMR (500 MHz, CD₃OD, major conformer) δ 7.07 (d, 2 H, *J* = 8.5 Hz, aromatic H's), 6.66 (d, 2 H, *J* = 8.5 Hz, aromatic H's), 5.38–5.28 (m, 4 H, olefinic H's), 4.67 (d, 1 H, *J* = 6.7 Hz, Thr C₂-H), 4.65–4.62 (overlapping d and t, 2 H, Hyp C₂-H, Thr C₂-H), 4.49 (br d, 1 H, *J* = 4.7 Hz, Hyp C₄-H), 4.43 (dd, 1 H, *J* = 5.2, 8.8 Hz, Orn C₂-H), 4.38 (s, 1 H, Hmp C₂-H), 4.34 (d, 1 H, *J* = 2.3 Hz, Hht C₂-H), 4.20 (dt, 1 H, *J* = 2.3, 7.0 Hz, Hht C₃-H), 4.13 (d, 1 H, *J* = 4.2 Hz, Hmp C₃-H), 4.09 (qn, 1 H, *J* = 6.4 Hz, Thr C₃-H), 4.01 (qn, 1 H, *J* = 6.1 Hz, Thr C₃-H), 3.94–3.87 (m, 2 H, Hyp C₅-H, Hmp C₅-H), 3.83 (br d, 1 H, *J* = 10.9 Hz, Hyp C₅-H), 3.71 (s, 3 H, OCH₃), 3.45 (t, 1 H, *J* = 10.0 Hz, Hmp C₅-H), 3.01 (br m, 2 H, Orn C₅-H₂), 2.81 (dd, 1 H, *J* = 7.7, 13.6 Hz, Hht C₄-H), 2.76 (t, 2 H, *J* = 6.6 Hz, CH=CHCH₂CH=CH), 2.74 (dd, 1 H, *J* = 6.5, 13.6 Hz, Hht C₄-H), 2.38–2.28 (m, 2 H, Hyp C₃-H, Hmp C₄-H), 2.22 (t, 2 H, *J* = 7.5 Hz, COCH₂), 2.09 (ddd, 1 H, *J* = 4.5, 8.5, 13.4 Hz, Hyp C₃-H), 2.05 (q, 4 H, *J* = 6.9 Hz, allylic H's), 1.79–1.74 (m, 1 H, Orn C₃-H), 1.62–1.54 (br m, 3 H, Orn C₃-H, Orn C₄-H₂), 1.51–1.46 (m, 2 H, COCH₂CH₂), 1.41 (s, 9 H, OC(CH₃)₃), 1.31 (br s, 14 H, linoleyl aliphatic H's), 1.26 (d, 3 H, *J* = 6.1 Hz, Thr C₄-H₃), 1.24 (d, 3 H, *J* = 6.4 Hz, Thr C₄-H₃), 1.04 (d, 3 H, *J* = 6.8 Hz, Hmp C₃-CH₃), 0.90 (t, 3 H, *J* = 6.9 Hz, linoleyl CH₃), 0.88 (s, 9 H, Si(CH₃)₃), 0.11 (s, 6 H, Si(CH₃)₂); [α]_D²⁵ -63.5° (c 1.07, MeOH) (lit.^{2b} [α]_D²⁵ -25.0° (c 1.07, MeOH)); MS (FAB, *m*-nitrobenzyl alcohol) *m/z* 1259 (M + 1), 1159.

Echinocandin D (1). This reaction was carried out according to the procedure of Kurokawa and Ohfuné.^{2b} To a solution of 50.1 mg (0.0398 mmol) of hexapeptide **23** in 0.2 mL of methanol under an atmosphere of nitrogen was added 87.6 μL of 1 N aqueous sodium hydroxide solution. After the resultant solution was stirred for 5 h at room temperature, it was partitioned between 10 mL of 1 N aqueous sodium bisulfate and 30 mL of ethyl acetate. The aqueous phase was extracted with two 30-mL portions of ethyl acetate and one 30-mL portion of methylene chloride. The combined organic phases were dried over magnesium sulfate and concentrated. The resultant glass was stirred at 0 °C with 1 mL of trifluoroacetic acid for 30 min, and then 0.2 mL of water was added. After 30 min, the pale yellow solution was concentrated. Trace amounts of acid and water were removed azeotropically with methanol and toluene. The resultant white solid was dried over P₂O₅ under vacuum for 2 h prior to use.

To a 0 °C solution of the deprotected peptide in 16 mL of anhydrous dimethylformamide (DMF) were added dropwise via a canula a 0 °C solution of 21.9 mg (17.2 μL, 0.0796 mmol, 2.0 equiv) of diphenylphosphoryl azide in 2 mL of DMF and a 0 °C solution of 8.9 mg (12.2 μL, 0.0876 mmol, 2.2 equiv) of triethylamine in 2 mL of DMF. The resultant clear solution was stirred at 0 °C for 3 h, 6–7 °C for 66 h, and room temperature for 20 h. It was then concentrated. The residue was diluted with 100 mL of 3:1 ethyl acetate/toluene, washed with 25-mL portions each of water, 1 N aqueous sodium bisulfate, water, saturated aqueous sodium bicarbonate, and saturated aqueous sodium chloride, dried over magnesium sulfate, and concentrated to give 37.9 mg (94% mass balance) of a white foam. Purification by flash chromatography (15 × 100 mm silica gel, 10% methanol/chloroform) gave 19.5 mg (50%) of the title compound as an amorphous solid: *R_f* 0.34 (20% methanol/methylene chloride); IR (Nujol) 3680–2500 (br), 1690–1620 (br), 1555–1510, 1350 (br), 1240 (br), 1085, 720 cm⁻¹; ¹H NMR (500 MHz, CD₃OD) δ 7.00 (d, 2 H, *J* = 8.4 Hz, aromatic H's), 6.69 (d, 2 H, *J* = 8.4 Hz, aromatic H's), 5.38–5.28 (m, 4 H, olefinic H's), 4.89 (d, 1 H, *J* = 3.9 Hz, Thr C₂-H), 4.88 (d, 1 H, Thr C₂-H), 4.64 (dd, 1 H, *J* = 7.0, 11.4 Hz, Hyp C₂-H), 4.56 (br s, 1 H, Hyp C₄-H), 4.48–4.44 (m, 1 H, Thr C₃-H), 4.41 (br s, 1 H, Hht C₂-H), 4.40–4.36 (m, 2 H, Orn C₂-H, Hht C₃-H), 4.30 (d, *J* = 2.7 Hz, 1 H, Hmp C₂-H), 4.26–4.22 (m, 1 H, Thr C₂-H), 4.14 (dd, 1 H, *J* = 2.7, 4.4 Hz, Hmp C₃-H), 4.00 (dd, 1 H, *J* = 3.3, 11.0 Hz, Hyp C₅-H), 3.83 (dd, 1 H, *J* = 7.5, 9.2 Hz, Hmp C₅-H), 3.80 (d, 1 H, *J* = 11.0 Hz, Hyp C₅-H), 3.49–3.43 (m, 1 H, Orn C₅-H), 3.38 (t, 1 H, *J* = 9.2 Hz, Hmp C₅-H), 2.99–2.94 (m, 1 H, Orn C₅-H), 2.77 (t, 2 H, *J* = 6.6 Hz, CH=CHCH₂CH=CH), 2.64 (dd, 1 H, *J* = 6.4, 13.7 Hz, Hht C₄-H), 2.55 (dd, 1 H, *J* = 7.8, 13.7 Hz, Hht C₄-H), 2.49–2.43 (m, 2 H, Hyp C₃-H, Hmp C₄-H), 2.23 (two overlapping dt, 2 H, *J* = 7.4, 15.0 Hz, COCH₂), 2.15–2.04 (m, 2 H, Orn C₃-H, Hyp C₃-H), 2.05 (q, 4 H, *J* = 6.5 Hz, allylic H's), 1.72–1.66 (m, 2 H, Orn C₄-H₂), 1.62–1.51 (m, 3 H, Orn C₃-H, COCH₂CH₂), 1.45–1.28 (m,

14 H, linoleyl aliphatic H's), 1.21 (d, 3 H, $J = 6.3$ Hz, Thr C₄-H₃), 1.18 (d, 3 H, $J = 6.4$ Hz, Thr C₄-H₃), 1.04 (d, 3 H, $J = 6.9$ Hz, Hmp C₃-CH₃), 0.90 (t, 3 H, $J = 6.9$ Hz, linoleyl CH₃); $[\alpha]_D -43^\circ$ (c 0.82, MeOH) (lit.^{1a} $[\alpha]_D -45.5^\circ$ (c 0.81, MeOH)); MS (FAB, *m*-nitrobenzyl alcohol) m/z 1012 (M + 1).

Tetrahydroechinocandin D. This reaction was carried out according to the procedure of v. Wartburg and co-workers.^{1a} A solution of 25.4 mg of echinocandin D in 3 mL of ethanol was stirred over 10% Pd-C under an atmosphere of hydrogen for 3 h. It was then filtered through Celite and concentrated to give a glass: R_f 0.34 (20% methanol/methylene chloride); HPLC (Vydak, 10% water/methanol, 1.5 mL/min, 280-nm detection) t_r 5.20 min; IR (Nujol) 3700-2500 (br), 1690-1620 (br), 1537, 1518, 1350 (br), 1240 (br), 1077, 720 cm⁻¹; ¹H NMR (500 MHz, CD₃OD) δ 6.99 (d, 2 H, $J = 8.4$ Hz, aromatic H's), 6.68 (d, 2 H, $J = 8.4$ Hz, aromatic H's), 4.89 (overlapping d, 2 H, $J = 3.9$ Hz, Thr C₂-H, Thr C₂-H), 4.64 (dd, 1 H, $J = 7.0$, 11.4 Hz, Hyp C₂-H), 4.57 (br s, 1 H, Hyp C₄-H), 4.48-4.44 (m, 1 H, Thr C₃-H), 4.41 (br s, 1 H, Hht C₂-H), 4.40-4.36 (m, 2 H, Orn C₂-H, Hht C₃-H), 4.31 (d, $J = 2.5$ Hz, 1 H, Hmp C₂-H), 4.26-4.22 (m, 1 H, Thr C₂-H), 4.14 (dd, 1 H, $J = 2.5$, 4.4 Hz, Hmp C₃-H), 4.00 (dd, 1 H, $J = 3.3$, 11.0 Hz, Hyp C₅-H), 3.85 (dd, 1 H, $J = 7.5$, 9.2 Hz, Hmp C₅-H), 3.80 (d, 1 H, $J = 11.0$ Hz, Hyp C₅-H), 3.49-3.43 (m, 1 H, Orn C₅-H), 3.38 (t, 1 H, $J = 9.2$ Hz, Hmp

C₅-H), 2.99-2.95 (m, 1 H, Orn C₅-H), 2.64 (dd, 1 H, $J = 6.4$, 13.7 Hz, Hht C₄-H), 2.55 (dd, 1 H, $J = 7.8$, 13.7 Hz, Hht C₄-H), 2.49-2.43 (m, 2 H, Hyp C₃-H, Hmp C₄-H), 2.23 (two overlapping dt, 2 H, $J = 7.4$, 15.0 Hz, COCH₂), 2.15-2.04 (m, 2 H, Orn C₃-H, Hyp C₃-H), 1.72-1.66 (m, 2 H, Orn C₄-H₂), 1.62-1.51 (m, 3 H, Orn C₃-H, COCH₂CH₂), 1.41-1.28 (m, 2 H, COCH₂CH₂CH₂), 1.28 (s, 26 H, stearyl H's), 1.22 (d, 3 H, $J = 6.3$ Hz, Thr C₄-H₃), 1.18 (d, 3 H, $J = 6.4$ Hz, Thr C₄-H₃), 1.05 (d, 3 H, $J = 6.8$ Hz, Hmp C₃-CH₃), 0.89 (t, 3 H, $J = 6.9$ Hz, stearyl CH₃); $[\alpha]_D -42^\circ$ (c 0.59, MeOH) (natural: $[\alpha]_D -42^\circ$ (c 0.59, MeOH)); MS (FAB, *m*-nitrobenzyl alcohol) m/z 1016 (M + 1).

Acknowledgment. We thank Dr. Y. Ohfune, Suntory Institute, for kindly providing a sample of echinocandin D, as well as spectroscopic data for **20**, **21**, **23**, echinocandin D, and tetrahydroechinocandin D. We also thank Dr. A. v. Wartburg, Sandoz AG, for providing a generous sample of tetrahydroechinocandin D. Support from the National Science Foundation (Predoctoral Fellowship (1982-1985) for A.E.W.) and the National Institutes of Health is gratefully acknowledged. The NIH BRS Shared Instrumentation Grant Program 1 S10 RR01748-01A1 is acknowledged for providing NMR facilities.

X-ray Absorption Spectroscopy of Metal-Histidine Coordination in Metalloproteins. Exact Simulation of the EXAFS of Tetrakis(imidazole)copper(II) Nitrate and Other Copper-Imidazole Complexes by the Use of a Multiple-Scattering Treatment

Richard W. Strange,^{1a} Ninian J. Blackburn,^{1a} P. F. Knowles,^{1b} and S. Samar Hasnain*^{1c}

Contribution from the Department of Chemistry, University of Manchester Institute of Science and Technology, Manchester, M60 1QD, United Kingdom, and S.E.R.C. Daresbury Laboratory, Warrington, Cheshire, WA4 4AD, United Kingdom. Received January 26, 1987

Abstract: Histidine coordination occurs in many metalloproteins, but analysis of the contributions to the EXAFS of the outer shells of atoms of the imidazole rings has, in the past, proved difficult. An exact method for simulating the raw experimental EXAFS over the complete energy range ($k = 2-16 \text{ \AA}^{-1}$) is reported and applied to the simulation of tetrakis(imidazole)copper(II) nitrate, tetrakis(imidazole)copper(II) perchlorate, and aquatris(imidazole)copper(II) sulfate. It is shown that strong multiple-scattering contributions are present in the EXAFS over an extended range above the absorption edge and these contributions are necessary to fix the third-shell atoms of the imidazole groups at their correct positions. Furthermore, by including multiple scattering in the EXAFS analysis, it is possible to extend the low-energy fitting range to include the XANES region of the spectrum below $k = 3$, the general shape of this part of the spectrum being well reproduced. In favorable circumstances, the multiple-scattering approach can provide the basis for determining the number of histidine ligands in a mixed-ligand complex and can clearly distinguish between two and four coordinated imidazole groups, although distinction between three and four histidines is probably unrealistic for a metalloprotein site of unknown structure.

In recent years, X-ray absorption spectroscopy has been used to probe the environment of transition metals at the active sites of metalloproteins and other biologically important molecules. By analyzing the high-energy (EXAFS) region of the X-ray spectrum, useful information concerning the distance, type, and number of atoms coordinated at the metal site has been obtained.²⁻⁴ For

metalloenzymes whose X-ray absorption spectrum is dominated by the presence of histidine ligands, this information has often been restricted to the first- and sometimes second-shell coordination spheres, extending ca. 3.2 Å from the absorbing atom. Atoms belonging to imidazole rings lying beyond this distance have proved more difficult to simulate. Methods employing parameterization of the amplitudes and phase-shift functions,³⁻⁶

(1) (a) Department of Chemistry, University of Manchester Institute of Science and Technology, UK. (b) Department of Biophysics, University of Leeds, UK. (c) S.E.R.C. Daresbury Laboratory, Warrington, Cheshire, UK.

(2) (a) *EXAFS and Near Edge Structure III, Proceedings of the International Conference, Stanford*; Hodgson, K. O., Hedman, B. O., Penner-Hahn, J. E., Eds.; Springer: Berlin, 1983; *Springer Proc. Phys.* **1983**, *1*. (b) Teo, B. K. In *EXAFS Spectroscopy: Techniques and Applications*; Teo, B. K., Joy, D. C., Eds.; Plenum: New York, 1981; pp 13-58. (c) Cramer, S. P.; Hodgson, K. O. *Prog. Inorg. Chem.* **1979**, *25*, 1-39. (d) Garner, C. D.; Helliwell, J. R. *Chem. Br.* **1986**, *22*, 835-840.

(3) Cramer, S. P.; Hodgson, K. O.; Stiefel, E. I.; Newton, W. E. *J. Am. Chem. Soc.* **1978**, *100*, 2748-2761.

(4) (a) Co, M. S.; Scott, R. A.; Hodgson, K. O. *J. Am. Chem. Soc.* **1981**, *103*, 986-988. (b) Co, M. S.; Hodgson, K. O. *J. Am. Chem. Soc.* **1981**, *103*, 3200-3201. (c) Co, M. S.; Hodgson, K. O.; Eccles, T. K.; Lontie, R. *J. Am. Chem. Soc.* **1981**, *103*, 984-986.

(5) (a) Brown, J. M.; Powers, L.; Kincaid, B.; Larrabee, J. A.; Spiro, T. G. *J. Am. Chem. Soc.* **1980**, *102*, 4210-4216. (b) Woolery, G. L.; Powers, L.; Winkler, M.; Solomon, E. I.; Spiro, T. G. *J. Am. Chem. Soc.* **1984**, *106*, 86-92. (c) Woolery, G. L.; Powers, L.; Winkler, M.; Solomon, E. I.; Lerch, K.; Spiro, T. G. *Biochim. Biophys. Acta* **1984**, *788*, 155-161. (d) Woolery, G. L.; Powers, L.; Peisach, J.; Spiro, T. G. *Biochemistry* **1984**, *23*, 3428-3434.