chromatography (1:1, ethyl acetate/hexanes), giving **10** as a white solid (0.118 g, 80% from 9), which was crystallized from acetone, affording colorless needles, mp 221-3 °C. The isomers ((2*S*)-**10** and (2*R*)-**10**) were separated by HPLC, as described above.

(2*S*)-**10**: ^{1}H NMR (CD_3CN; see Figure 2) δ 2.43 (dd, J = 10.9, 9 Hz, 1 H, axial C-3 H, overlapped with dd, J = 10.9, 10.6 Hz, 0.38 Hz, axial C-3 H of minor C-4 epimer), 4.28 (d, J = 10.6 Hz, 0.38 H, axial C-4 H of minor epimer), 4.45 (d, J = 9 Hz, 0.63 H, equatorial C-4 H of major epimer), 4.78 (dd, J = 10.9, 8 Hz, 1 H, C-2 H), 7.94 (br d, 1 H, NH), 8.96 (d, J = 1.9 Hz, 2 H, C-2′,6′ H), 9.04 (t, J = 1.9 Hz, 1 H, C-4′ H).

(2*R*)-**10**: ^{1}H NMR (CD_3CN; see Figure 2) δ 2.58 (m, 1 H, overlapping equatorial C-3 H of both epimers), 4.28 (d, J = 6.5 Hz, 0.63 H, axial C-4 H), 4.44 (s, 0.38 H, equatorial C-4 H of the minor epimer), 4.78 (dd, J = 9.1, 8 Hz, 1 H, C-2 H), 7.94 (br d, 1 H, NH), 8.95 (d, J = 2 Hz, 2 H, C-2′,6′ H), 9.04 (t, J = 2 Hz, 1 H, C-4′ H).

***N*-[*benzoyl*-^{18}O]Benzoylhomoserine Lactone (9).** A solution of homoserine (0.13 g, 1.1 mmol) in 3 N hydrochloric acid (4 mL) was heated at reflux for 30 min and then was cooled. The resulting lactone solution was neutralized with aqueous sodium bicarbonate and was then added to [^{18}O]benzoyl chloride (0.16 g, 1.1 mmol; made from [$^{18}O_2$]benzoic acid[53] by reaction with thionyl chloride). Sodium bicarbonate (0.42 g, 5.0 mmol) was added, and the mixture was stirred at room temperature for 1 h. The white solid that separated during the reaction was removed by filtration and dissolved in ethyl acetate. The organics were washed with water, dried, and concentrated, providing [*benzoyl*-^{18}O]**9**: ^{1}H NMR δ 2.27 (dddd, J = 12.6, 11.2, 9, 11.5 Hz, 1 H, C-3 H, cis to NH), 2.86 (dddd, J = 12.6, 5.9, 8.7, 1.3 Hz, 1 H, C-3 H, trans to NH), 4.30 (ddd, J = 11.2, 5.9, 9 Hz, 1 H, C-4 H, trans to NH), 4.47 (ddd, J = 9, 9, 1.3 Hz, 1 H, C-4 H, cis to NH), 4.75 (ddd, J = 11.5, 8.7, 6.2 Hz, 1 H, C-2 H), 7.08 (d, J = 6.2 Hz, 1 H, NH), 7.4-7.9 (m, 5 H, ArH); MS (70 eV, EI), m/z (% of base peak) 208 (10), 207 (10), 198 (1), 188 (5), 179 (1), 163 (3), 149 (2), 123 (4), 107 (100). Authentic, unlabeled **9**: MS (70 eV, EI), m/z (% of base peak) 206 (8), 205 (10), 196 (1), 188 (4), 176 (1), 161 (3), 147 (2), 121 (4), 105 (100).

Acid Hydrolysis of [*benzoyl*-^{18}O]-9. Derivatization and Analysis of the Resulting Homoserine Lactone. [*benzoyl*-^{18}O]-**9** was subjected to acid hydrolysis, as described above. The resulting homoserine lactone was converted to the 3,5-dinitrobenzamide **10**, which was crystallized from acetone (mp 222-3 °C). The ^{1}H NMR spectrum (CD_3CN) was identical with that of unlabeled **10**, described above. MS (CI, isobutane), m/z 298 (4), 296 (100), 282 (2), 266 (4). Authentic unlabeled **10**: MS (CI, isobutane), m/z 298 (2), 296 (100), 282 (3), 266 (5).

Acknowledgment. We gratefully acknowledge the NIH for generous financial support, via Grant GM36285. The 470-MHz ^{1}H NMR spectra were obtained at Purdue at the PUBMRL/NSF Center for Biomolecular NMR, Structure and Design, supported by Grants RR01077 (NIH) and 8714258 (NSF). We also thank Profs. Jack Baldwin and Ronald Woodard for preliminary communication of their results, Prof. Marc Loudon for helpful discussions, and Jeffrey A. Moore for obtaining some of the analytical data.

Supplementary Material Available: Deuterium NMR spectrum of the chirally labeled 2-phenylethyl (1*S*)-camphanate ester derivatives of the chirally labeled oxirane enantiomers; a summary of general experimental procedures; a modification of the published procedure for the preparation of [^{18}O]benzoyl chloride (5 pages). Ordering information is given on any current masthead page.

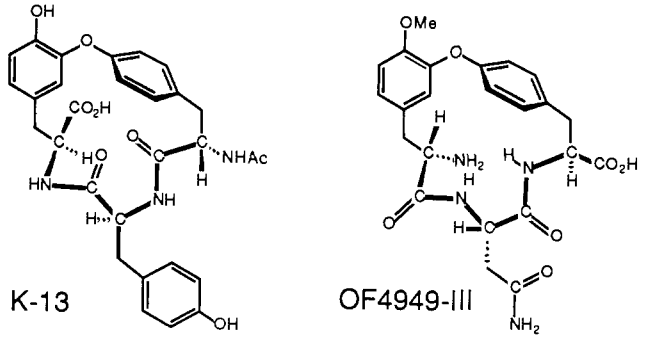
(53) Kobayashi, M.; Kiritani, R. *Bull. Chem. Soc. Jpn.* **1966**, *39*, 1782-1784.
(54) Ramalingam, K.; Woodard, R. W. *J. Labelled Compd. Radiopharm.* **1987**, *24*, 369-376.
(55) Ramalingam, K.; Woodard, R. W. *J. Org. Chem.* **1988**, *53*, 1900-1903.

The Total Syntheses of the Isodityrosine-Derived Cyclic Tripeptides OF4949-III and K-13. Determination of the Absolute Configuration of K-13

David A. Evans* and Jonathan A. Ellman[1]

Contribution from the Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138. *Received July 18, 1988*

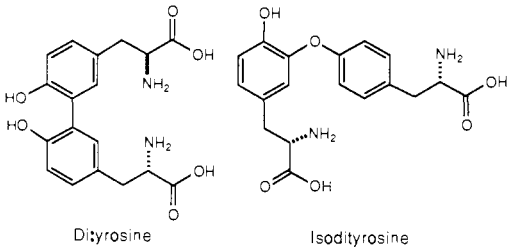
Abstract: The asymmetric syntheses of the two cyclic tripeptides OF4949-III and K-13 have been completed. The absolute stereochemical assignment of the former compound has been confirmed, while the absolute configuration of the latter has been established for the first time. The expedient bidirectional synthesis of a fully differentiated isodityrosine, a common



intermediate to both molecules, was achieved by employing the recently developed direct electrophilic azidation of chiral imide enolates. In completing these syntheses, the utility of the azide as an amine-protecting group in peptide-coupling reactions and in peptide cyclizations was also evaluated. These studies have established that α-azido carboxylic acids are practical N-protected α-amino acid synthons and may be used as such in "racemization-free" peptide synthesis.

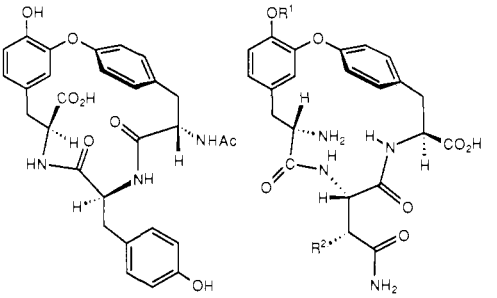
A diverse array of amino acids and peptides containing oxidatively coupled aromatic nuclei exist in nature. These compounds range from the tyrosine-derived peptides (thyroxine,[2] dityrosine,[3] isodityrosine,[4] trityrosine,[5] isotrityrosine,[6] piperazinomycin,[7]

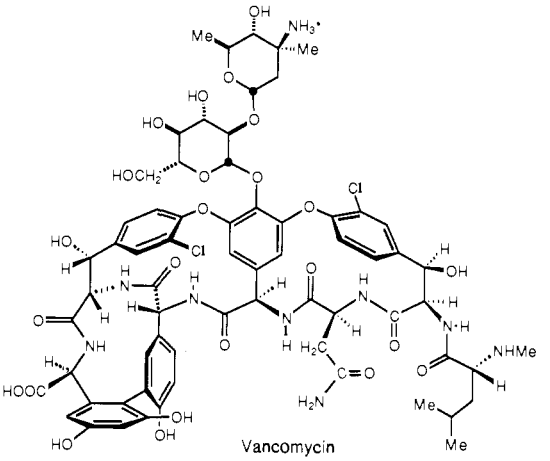
0002-7863/89/1511-1063$01.50/0



OF4949 I-IV,[8] K-13,[9] bouvardin,[10] deoxybouvardin,[11] and RA I-VII[12]) to the exceedingly structurally complex glycopeptide antibiotics exemplified by vancomycin, teicoplanin, and ristocetin.[13] Most of these molecules elicit significant effects upon biological systems. For example, vancomycin is an important antibiotic which is widely used in the treatment of staphylococcal infections, while K-13 has recently been reported to be an inhibitor of angiotensin converting enzyme,[9a] and OF4949 I-IV are inhibitors of aminopeptidase B from Erlich ascites carcinoma cells and stimulate the cell-mediated immune response.[8e-g]

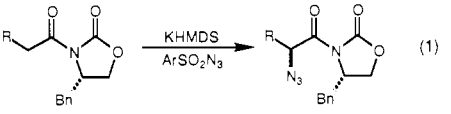
Collectively, these oxidatively cross-linked cyclic peptides are challenging synthesis targets which have provided us with the impetus for the development of new methodology for the construction of complex amino acids. The issue of architectural complexity becomes quite evident when contemplating an approach to the synthesis of members of the vancomycin class of antibiotics.





In conjunction with long-range objectives which are being directed toward the eventual achievement of a vancomycin synthesis, we have become attracted to the recently isolated cyclic tripeptides OF4949 I-IV and K-13. By inspection it is clear that these structures contain several obvious structural elements common to the more complex glycopeptides such as vancomycin which might be addressed in conjunction with the syntheses of these target structures. In particular, we were interested in determining whether the amino acid synthesis methodology under development in this laboratory might be applicable to the synthesis of highly functionalized amino acids. In addition, these cyclic tripeptides offer the opportunity to carefully examine those macrocyclization methods which might be applicable to the synthesis of not only OF4949 I-IV and K-13 but also the more complex glycopeptides as well.

With the above goals in mind, we elected to develop asymmetric syntheses of both OF4949-III and K-13. In our approach to the syntheses of these structures, the common synthon, isodityrosine, in fully differentiated form, became the initial objective. From this intermediate it was hoped that the appropriate tripeptides might be cyclized to their 17-membered congeners and elaborated to the desired target structures. In dealing with the stereochemical issues in this exercise we elected to rely upon the diastereoselective electrophilic azidation of the illustrated chiral imide enolates to prepare all the requisite α-amino acids (eq 1).[14] After this study had been completed, two independent and quite complementary approaches to the synthesis of OF4949-III have appeared.[8c,d] In the following discussion, successful approaches to both of the target tripeptides will be presented.



(1)

(1) National Science Foundation Fellow, 1984–1987.

(2) Isolation: (a) Kendall, *J. Am. Med. Assoc.* **1915**, *64*, 2042. (b) Kendall, *J. Am. Med. Assoc.* **1919**, *39*, 125. Structure: (c) Harrington, C. R. *Biochem. J.* **1926**, *20*, 283–300. Synthesis: (d) Harrington, C. R.; Borger, G. *Ibid.* **1927**, *21*, 169–181. (e) Canzanelli, A.; Guild, R.; Harrington, C. R. *Ibid.* **1935**, *29*, 1617–1619. (f) Ginger U.S. patent 289,364 (1959 to Baxter Labs). (g) Nahm, H.; Siedel, W. *Chem. Ber.* **1963**, *96*, 1–9.

(3) Isolation: (a) Anderson, S. O. *Biochim. Biophys. Acta* **1964**, *93*, 213–215. Structure: (b) Gross, A. J.; Sizer, I. W. *J. Biol. Chem.* **1959**, *234*, 1611–1614. Synthesis: (c) Amado, R.; Aeschbach, R.; Neukom, H. *Methods Enzymol.* **1984**, *107*, 377–388.

(4) Isolation and Structure: (a) Fry, S. C. *Biochem. J.* **1982**, *204*, 449–455. Synthesis: (b) Fry, S. C. *Methods Enzymol.* **1984**, *107*, 388–397.

(5) Isolation: (a) Fujimoto, D. *Comp. Biochem. Physiol.* **1975**, *51B*, 205–207. Structure: Gross, A. J.; Sizer, I. W. *J. Biol. Chem.* **1959**, *234*, 1611–1614.

(6) Isolation and Structure: Fujimoto, D.; Horiuchi, K.; Hirama, M. *Biochem. Biophys. Res. Commun.* **1981**, *99*, 637–643.

(7) Isolation: (a) Tamai, S.; Kaneda, M.; Nakamura, S. *J. Antibiot.* **1982**, *35*, 1130–1137. Structure: (b) Kaneda, M.; Tamai, S.; Nakamura, S.; Hirata, T.; Kushi, Y.; Suga, T. *J. Antibiot.* **1982**, *35*, 1137–1140. Synthesis: (c) Nishiyama, S.; Nakamura, K.; Suzuki, S.; Yamamura, S. *Tetrahedron Lett.* **1986**, *27*, 4481–4484.

(8) Isolation: (a) Sano, S.; Ikai, K.; Kuroda, H.; Nakamura, T.; Obayashi, A.; Ezure, Y.; Enomoto, H. *J. Antibiot.* **1986**, *39*, 1674–1684. Structure: (b) Sano, S.; Ikai, K.; Katayama, K.; Takesako, K.; Nakamura, T.; Obayashi, A.; Ezure, Y.; Enomoto, H. *J. Antibiot.* **1986**, *39*, 1685–1696. Synthesis: (c) Nishiyama, S.; Suzuki, Y.; Yamamura, S. *Tetrahedron Lett.* **1988**, *29*, 559–562. (d) Schmidt, U.; Weller, D.; Holder, A.; Lieberknecht, A. *Ibid.* **1988**, *29*, 3227–3230. Biological Studies: (e) Sano, S.; Ueno, M.; Katayama, K.; Nakamura, T.; Obayashi, A. *J. Antibiot.* **1986**, *39*, 1697–1703. (f) Sano, S.; Ikai, K.; Yoshikawa, Y.; Nakamura, T.; Obayashi, A. *J. Antibiot.* **1987**, *40*, 512–518. (g) Sano, S.; Kuroda, H.; Ueno, M.; Yoshikawa, Y.; Nakamura, T.; Obayashi, A. *J. Antibiot.* **1987**, *40*, 519–525.

(9) Isolation: (a) Kase, H.; Kaneko, M.; Yamada, K. *J. Antibiot.* **1987**, *40*, 450–454. Structure: (b) Yasuzawa, T.; Shirahata, K.; Sano, H. *J. Antibiot.* **1987**, *40*, 455–458.

(10) Isolation and Structure: Jolad, S. D.; Hoffman, J. J.; Torrance, S. J.; Weidhopf, R. M.; Cole, J. R.; Arora, S. K.; Bates, R. B.; Gargiulo, R. L.; Kriek, G. R. *J. Am. Chem. Soc.* **1977**, *99*, 8040–8044.

(11) Isolation and Structure: (a) As in footnote 10. Synthesis: (b) Inaba, T.; Umezawa, I.; Yuasa, M.; Inoue, T.; Mihashi, S.; Itokawa, H.; Ogura, K. *J. Org. Chem.* **1987**, *52*, 2958–2960.

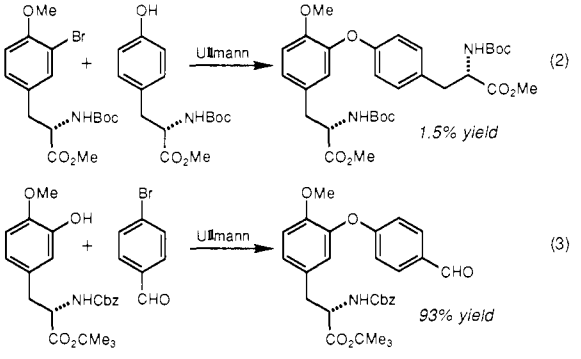
(12) Isolation: (a) Itokawa, H.; Takeya, K.; Mori, N.; Himanaka, T.; Sonobe, T.; Mihara, K. *Chem. Pharm. Bull.* **1984**, *32*, 284–290. Structure: (b) Itokawa, H.; Takeya, K.; Mihara, K.; Mori, N.; Hamanaka, T.; Sonobe, T.; Iitaka, Y. *Chem. Pharm. Bull.* **1983**, *31*, 1424. Synthesis: as in 11b.

(13) For a recent review on the glycopeptide antibiotics, see: Williams, D. H. *Acc. Chem. Res.* **1984**, *17*, 364–369.

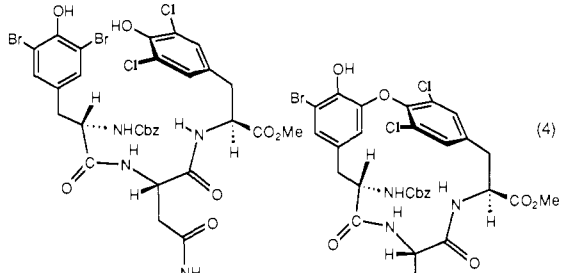
(14) Evans, D. A.; Britton, T. C. *J. Am. Chem. Soc.* **1987**, *109*, 6881–6883. For the preparation of 2,4,6-triisopropylbenzenesulfonyl azide (trisyl azide), see: Harmon, R. E.; Wellman, G.; Gupta, S. K. *J. Org. Chem.* **1973**, *38*, 11–16.

Results and Discussion

Asymmetric Synthesis of a Fully Differentiated Isodityrosine. The synthesis of isodityrosine has been examined by a number of research groups not only because isodityrosine is an essential constituent of many important structural proteins such as the plant cell wall protein elastin[4a] but also because it is incorporated into a number of peptide natural products such as OF4949 I-IV,[8] K-13,[9] piperazinomycin[7] as well as deoxybouvardin[11] and several of the structually related RA peptides.[12] Isodityrosine poses a challenge in synthesis because the amino acid functionality is not compatible with the harsh reaction conditions required in the standard methods for construction of diphenyl ether bonds. For example, the classical Ullmann reaction[15] has been employed in the synthesis of an isodityrosine derivative (eq 2);[9b] however, the harsh conditions of this process resulted in a disappointing 1.5% yield of product in undifferentiated form. More recently, Schmidt and co-workers have employed more optimal Ullmann coupling partners (eq 3), in conjunction with asymmetric dehydroamino acid hydrogenation methodology, to produce an isodityrosine derivative which is differentially protected at both carboxyl and amino functionalities in an overall yield of ca. 34%.[8d]



One promising method for the synthesis of isodityrosine-derived peptides is the oxidative phenolic coupling methodology of Yamamura as manifested in the recent syntheses of piperazinomycin[7c] and of OF4949-III.[8c] In the latter system, oxidation with thallium trinitrate afforded modest yields (25%) of cyclic products which were transformed to the target in an 18% yield for the two-step procedure (eq 4). Similar yields were reported for the analogous



steps of the piperazinomycin synthesis. Although this methodology is appealing in that it probably mimics a related step in the biosynthesis of these structures, the low yields for macrocyclization detract from the practical utility of these reactions.

We elected to follow a different approach to these isodityrosine derivatives by employing an Ullmann reaction to form the diphenyl ether subunit prior to the construction of *either* of the amino acid side chains. We feel that this methodology will also be amenable to the synthesis of the more complex diphenyl ether crosslinked amino acids in vancomycin. The carbon framework of the fully differentiated isodityrosine was synthesized in 90–91% yield through Ullmann coupling of 1.0 equiv of an *E*/*Z* mixture of **1** with 1.2 equiv of **2** according to the procedure of Tomita[15] (Scheme I). This reaction is notable in that, at the high temperature of the reaction (145 °C, 24 h), the benzyl ester remains intact in the presence of the phenoxide and pyridine nucleophiles, the *tert*-butyl ester is not lost by thermolysis, and the esters do not undergo transesterification.

With the carbon framework in place, attention was directed to the introduction of the first amine-bearing stereogenic center. Catalytic hydrogenation of **3** (10% Pd/C) reduced both double bonds with concomitant hydrogenolysis of the benzyl ester. The unpurified mono acid was then treated with pivaloyl chloride and triethylamine to obtain the mixed anhydride, which upon reaction with 1.8 equiv of lithiated 4-(4*S*)-(phenylmethyl)-2-oxazolidinone[16] at −78 °C, afforded the carboximide **4** as a white crystalline solid, mp 88–89 °C, in 85–90% overall yield from **3**. The subsequent selective direct azidation[14] of **4** was critical to the success of this bidirectional synthesis. In previous studies, we have relied upon the activated nature of the carboximide relative to a methyl ester to selectively enolize the carboximide moiety with dibutylboron triflate and triethylamine[17] as well as with sodium bis(trimethylsilyl)amide.[18] In the present instance, selective enolization of the carboximide in the presence of the *tert*-butyl ester was achieved by the rapid addition of 1.02 equiv of potassium bis(trimethylsilyl)amide to **4** at −78 °C. The rapid addition of the base was necessary in order to prevent Claisen condensation. After reaction of the enolate with 2,4,6-triisopropylbenzenesulfonyl azide (trisyl azide)[14] followed by the usual acetic acid quench according to established procedure, the unpurified α-azidocarboximide **5** was isolated as a 97.5/2.5 ratio of diastereomers as determined by HPLC. Purification by flash chromatography afforded 83–86% yields of diastereomerically pure material (>99%).

α-Azidocarboximide **5** was then readily converted into a suitably protected amino acid derivative. We again relied upon the activated nature of the carboximide to selectively transesterify **5** with titanium tetrabenzyloxide in benzyl alcohol according to the procedure of Seebach.[19] The product α-azido benzyl ester **6**, mp 70–71 °C, was isolated in 90–93% yield without any transesterification of the *tert*-butyl ester. This transformation nicely illustrates the "active ester" properties of these *N*-acyl oxazolidinones which may be exploited in such selective acyl transfer processes. Previous work has shown that less than 0.5% epimerization occurs during the titanium tetrabenzyloxide transesterifications of an analogous α-azidocarboximide.[20] The selective reduction of the azide moiety in **6** in the presence of the benzyl ester was then accomplished by hydrogenation at atmospheric pressure with W-2 Raney nickel catalyst and 1:1 acetic acid/methylene chloride as solvent. Stannous chloride in alcoholic solvents is also an effective azide reducing agent which has enjoyed considerable popularity in this laboratory;[21] however in the present instance, the conditions of this reaction were shown to promote a small amount (ca. 5%) of benzyl ester transesterification. After flash chromatography of the amine derived from **6**, the *tert*-butyl ester moiety was converted into the derived acid by reaction with trifluoroacetic acid in the presence of thioanisole as cation scavenger.[22] Protection of the resulting amino acid with *tert*-butyl pyrocarbonate[23] followed by chromatography provided the mono acid **7** in 82–87% overall yield from **6**.

(15) Tomita, M.; Fujitani, K.; Aoyagi, Y. *Chem. Pharm. Bull.* **1965**, *13*, 1341–1345.

(16) For full experimental details for the synthesis of this compound, see: Evans, D. A.; Weber, A. E. *J. Am. Chem. Soc.* **1986**, *108*, 6757–6761.

(17) Evans, D. A.; Bartroli, J.; Shih, T. L. *J. Am. Chem. Soc.* **1981**, *103*, 2127–2129.

(18) Evans, D. A.; Morrissey, M. M.; Dorow, R. L. *J. Am. Chem. Soc.* **1985**, *107*, 4346–4348.

(19) Seebach, D.; Hungerbuhler, E.; Naef, R.; Schurrenberger, P.; Weidmann, B.; Zuger, M. *Synthesis* **1982**, 138–141.

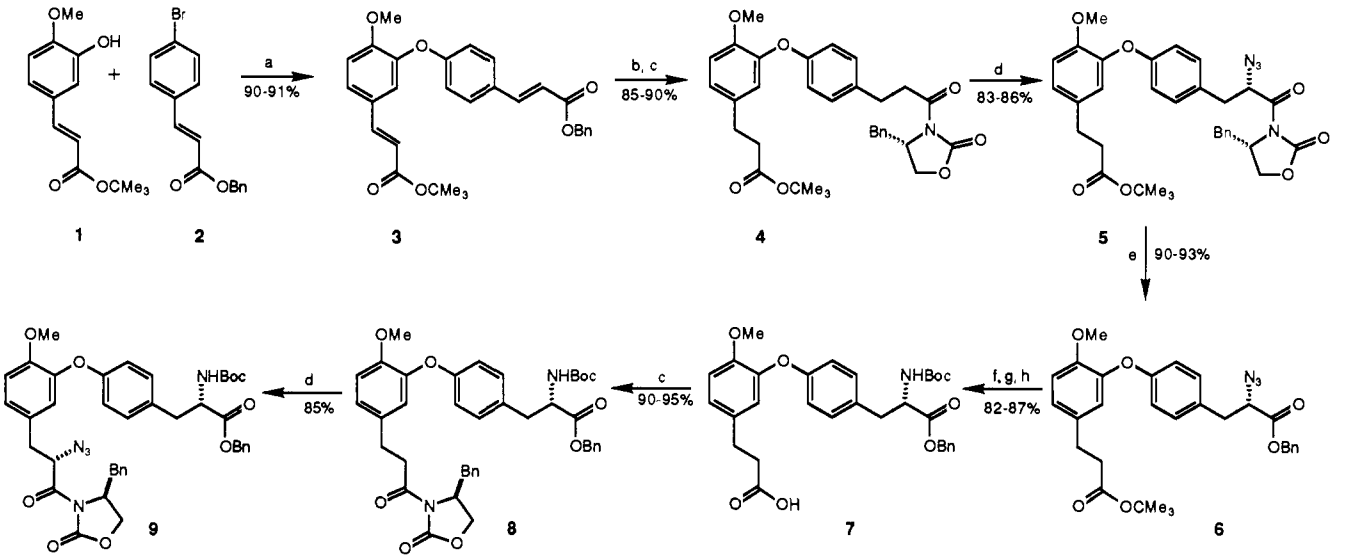
(20) Evans, D. A.; Ellman, J. A.; Dorow, R. L. *Tetrahedron Lett.* **1987**, *28*, 1123–1126.

(21) (a) Maiti, S. N.; Singh, M. P.; Micetich, R. G. *Tetrahedron Lett.* **1986**, *27*, 1423–1424. (b) Morrissey, M. M. Ph.D. Dissertation, Harvard University, 1986.

(22) Fujii, N.; Otaka, A.; Ikemura, O.; Konichi, A.; Funakoshi, S.; Hayashi, Y.; Kuroda, Y.; Yajima, H. *J. Chem. Soc., Chem. Commun.* **1987**, 274–275.

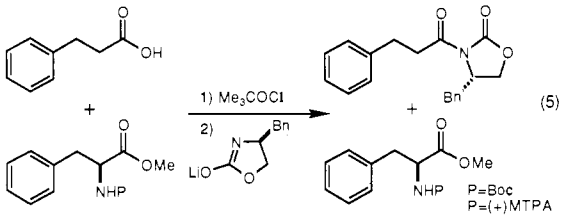
(23) Tarbell, D. F.; Yamamoto, Y.; Pope, B. M. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 730–732.

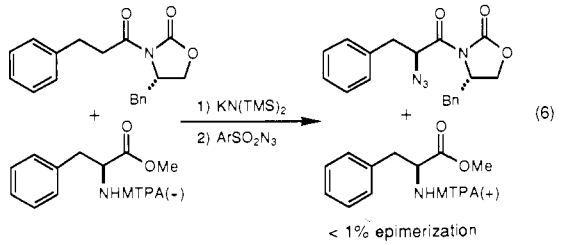
Scheme I[a]



[a](a) 2 equiv of CuO, K_2CO_3, pyr, 145 °C; (b) H_2, 10% Pd/C; (c) pivaloyl chloride, Et_3N, 0 °C, lithiated oxazolidinone; (d) KHMDS, −78 °C, trisyl azide; (e) $Ti(OBn)_4$, benzyl alcohol; (f) Raney Ni, H_2; (g) TFA, thioanisole; (h) Boc_2O, $NaHCO_3$.

While the incorporation of the first amine required that the introduction of the oxazolidinone and the direct azidation of the resulting carboximide be executed in the presence of an enolizable ester, the incorporation of the second amine-bearing stereogenic center requires that these transformations be executed in the presence of a racemization prone N-protected α-amino acid ester. Two control experiments were therefore conducted in order to assay the extent of racemization of a protected amino ester during both the introduction of the chiral auxiliary (eq 5) and during the direct azidation of a carboximide enolate (eq 6).

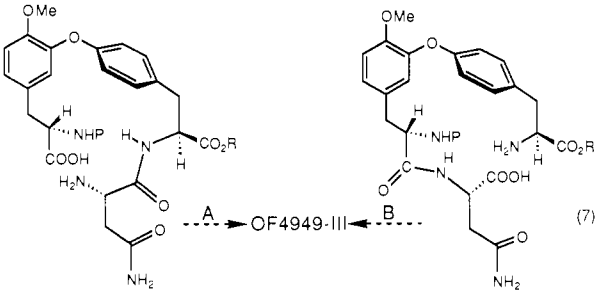




In the first control experiment, hydrocinnamic acid was converted to hydrocinnamoylcarboximide in the presence of 1 equiv of *N*-Boc-phenylalanine methyl ester (eq 5). Straightforward conversion of the recovered *N*-Boc amino ester into the (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetamide [(+)-MTPA amide][24] methyl ester followed by gas chromatographic analysis revealed that <1% racemization had occurred. In the second control reaction the direct azidation of hydrocinnamoylcarboximide was carried out in the presence of 1 equiv of the (+)-MTPA amide methyl ester of phenylalanine (eq 6). Again, gas chromatographic analysis of the recovered (+)-MTPA amide methyl ester revealed that <1% epimerization had occurred to the added methyl ester during the electrophilic azidation process.

Upon successful completion of the above experiments, **7** was converted into a fully differentiated isodityrosine derivative. The mono acid **7** was treated with pivaloyl chloride and triethylamine to yield the mixed anhydride, which after reaction with 2 equiv of lithiated oxazolidinone at −78 °C afforded the carboximide **8** in 90–95% yield. The dianion of **8**, formed by addition of a THF solution of **8** to 2.2 equiv of potassium bis(trimethylsilyl)amide at −78 °C, was then treated with 2.4 equiv of trisyl azide followed by a quench with 2.8 equiv of acetic acid. After chromatographic purification, the fully differentiated isodityrosine derivative was isolated in 85% yield as a 99:1 ratio of azide diastereomers as determined by analytical HPLC. Thus, the fully differentiated isodityrosine derivative **9** was constructed in 38–42% overall yield in ten steps from achiral starting materials **1** and **2**. This key intermediate is ideally protected for expedient conversion to both OF4949-III and K-13.

The Synthesis of OF4949-III. In designing a route to OF4949-III from the fully differentiated isodityrosine derivative **9**, we chose to cyclize the peptide through carboxyl activation of the isodityrosine (eq 7, option A) rather than through carboxyl

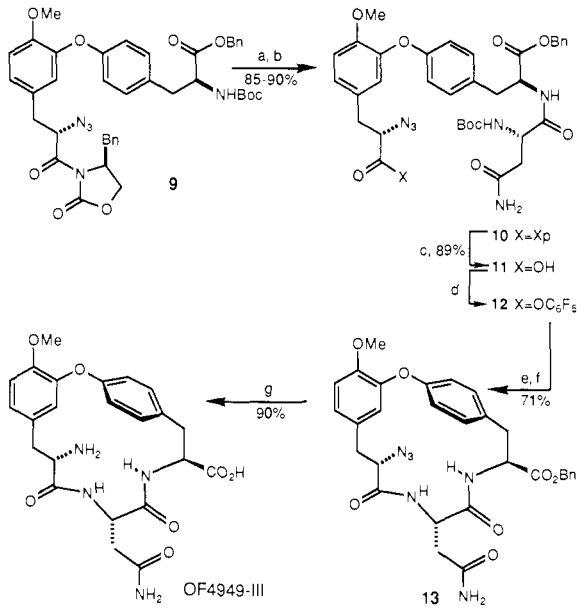


activation of the *N*-acyl asparagine moiety (eq 7, option B). The former course of action was pursued in order to avoid subjecting the carboxyl-activated asparagine dipeptide to the conditions that are required in a peptide macrocyclization. It is well known that carboxyl activated asparagine derivatives readily cyclize to form both succinimide and β-cyanoalanine derivatives.[25] In the successful complementary study recently reported by Schmidt and co-workers,[8d] option B was selected on a related *aspartic acid congener* to avoid the reactivity problems posed by the pendant carboxamide functionality. The yield of the macrocyclization step reported in this study was 40%.

(24) Dale, J. A.; Dull, D. J.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543–2549.

(25) Bodansky, M.; Martinez, J. In *The Peptides*; Gross, E., Meienhofer, J., Eds.; 1983; Vol. 5, pp 152–156.

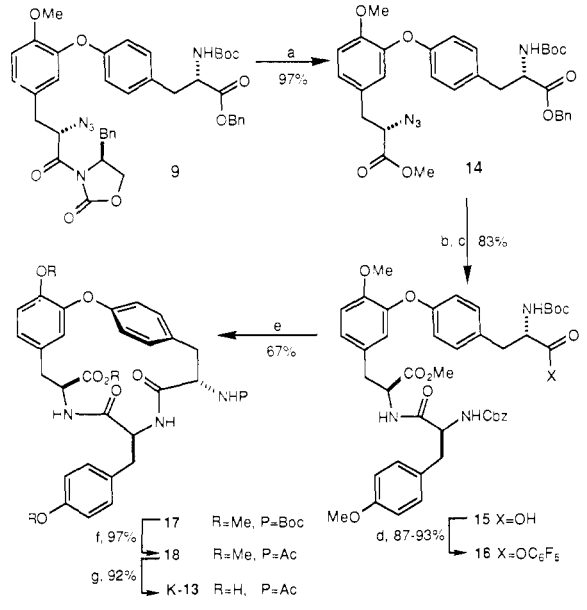
Scheme II[a]



[a](a) TFA, thioanisole; (b) *N*-Boc-Asn-OH, EDC, HOBt; (c) LiOOH; (d) pentafluorophenol, DCC; (e) TFA, thioanisole; (f) 20% pyridine/dioxane, 90 °C; (g) H_2, Pd(0).

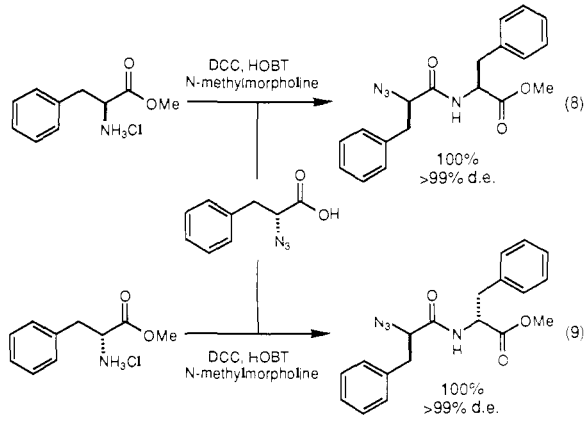
In the reduction of the macrocyclization via option A to practice, **9** was treated with trifluoroacetic acid in the presence of thioanisole to remove the Boc protecting group (Scheme II). After base extraction and coupling of the resulting free amine with *N*-Boc-asparagine using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC)[26] and hydroxybenzotriazole, **10**, was isolated in 85–90% yield. Selective removal of the chiral auxiliary (χ_P) in the presence of the benzyl ester was then accomplished by reaction with lithium hydrogen peroxide according to the procedure established in these laboratories[27] to provide in 89% yield the α-azido acid **11**, mp 156.5–157.5 °C. Among the various activation methods available for macrocyclic amide constructions, we elected to employ the *p*-nitrophenyl/pentafluorophenyl active ester macrocyclization procedure utilized by Rapoport[28] and also by Schmidt in the syntheses of several of the peptide alkaloids.[29] Of the two options, we chose to employ the latter method due to the inherently higher reactivity of the pentafluorophenyl active ester.[30] At this point, the most expedient approach to the completion of the synthesis was to attempt to carry the azide moiety, unmodified, through the remaining steps in the synthesis. Control experiments to be described shortly suggested that this was indeed a viable option. Accordingly, reaction of **11** with dicyclohexylcarbodiimide and pentafluorophenol afforded the pentafluorophenyl ester **12** as illustrated below (Scheme II). Due to the inherent lability of **12**, it was immediately treated with trifluoroacetic acid in the presence of thioanisole to furnish the amine salt which was dissolved in dixoane and added via syringe pump over 5 h to a solution of 20% pyridine/dioxane maintained at 90 °C. Purification by chromatography followed by recrystallization provided **13** in 71% overall yield from **11** as a white granular solid, mp 246–247 °C. Hydrogenation of **13** with palladium black reduced the azide and concomitantly hydrogenolyzed the benzyl ester to afford synthetic OF4949-III in 95–97% yield identical with natural material by spectroscopic (NMR and IR) and chromatographic criteria and corresponding quite closely in optical rotation ($[\alpha]^{30}_D$ −35° (*c* 1.14, 1 N HCl); literature $[\alpha]^{27}_D$ −38.2 °C (*c* 1.06, 1 N HCl)).[8c]

Scheme III[a]



[a](a) LiOOH, CH_2N_2; (b) H_2, 10% Pd/C; (c) *N*-Cbz-4-*O*-methyl-Tyr-OC_6F_5, $NaHCO_3$; (d) pentafluorophenol, DCC; (e) H_2, Pd(0), *N*-methylmorpholine, 2% EtOH/dioxane, 90 °C; (f) 1. TFA, thioanisole; 2. Ac_2O, pyridine; (g) $AlBr_3$, EtSH.

In the preceding synthesis, the pivotal amide bond forming macrocyclization involved the use of an α-azido acid as an N-protected amino acid synthon. Since enantiomerically pure α-azido acids have not been utilized previously in peptide synthesis, the issue of racemization during amide formation had not yet been evaluated in the literature. In order to address this issue, we independently assessed the viability of the azide moiety as an amine-protecting group during conventional peptide coupling reactions. Accordingly, α-azidohydrocinnamic acid was coupled with L-phenylalanine methyl ester by using EDC, hydroxybenzotriazole, and *N*-methylmorpholine to afford the dipeptide product, mp 66–67 °C, in quantitative yield (eq 8). α-Azidohydrocinnamic acid was also coupled with D-phenylalanine methyl ester to obtain the corresponding diastereomeric dipeptide, mp 87.5–88.5 °C, in comparable yield (eq 9). HPLC analysis of the dipeptide products from each experiment did not reveal any of the diastereomeric cross-contaminant (>99% diastereomeric purity).



The Synthesis of K-13. The fully differentiated isodityrosine derivative **9** also served as an ideal intermediate in the synthesis of K-13 (Scheme III). Reaction of **9** with 1.05 equiv of lithium hydrogen peroxide according to established procedure selectively hydrolyzed the carboximide to the desired mono acid which was

(26) Sheehan, J. C.; Cruickshank, P. A.; Boshart, G. L. *J. Org. Chem.* **1961**, *26*, 2525–2528.
(27) Evans, D. A.; Britton, T. C.; Ellman, J. A. *Tetrahedron Lett.* **1987**, *29*, 6141–6144.
(28) Lagarias, J. C.; Houghten, R. A.; Rapoport, H. *J. Am. Chem. Soc.* **1978**, *100*, 8202–8209.
(29) Schmidt, U.; Griesser, H.; Lieberknecht, A.; Talbiersky, J. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 280–281.
(30) Kovacs, J.; Cover, R.; Jham, G.; Hsieh, Y.; Kalas, T. In *Peptides: Chemistry, Structure and Biology*; Walter, R., Meienhofer, J., Eds.; Ann Arbor Science: Ann Arbor, MI, 1975; pp 317–324.

treated with diazomethane to give the methyl ester **14** in 97% yield for the two steps. Concomitant reduction of the azide and hydrogenolysis of the benzyl ester were next accomplished by catalytic hydrogenation at atmospheric pressure in 5% acetic acid/ethanol with 10% Pd/C as catalyst. The resulting amino acid was then coupled with the pentafluorophenyl ester of *N*-Cbz-*O*-methyltyrosine in a dilute aqueous sodium bicarbonate–dioxane solution to afford **15** in 81–85% overall yield. The subsequent cyclization of **16** was initially carried out according to the procedure developed by Schmidt.[29] Thus, reaction of **15** with pentafluorophenol and dicyclohexylcarbodiimide under standard conditions afforded the active ester **16**, mp 79–80 °C, in 87–93% yield. A solution of active ester **16** in dioxane was then added via syringe pump over 5 h to a vigorously stirred suspension of 5% Pd/C and 1 equiv of pyrrolidinopyridine in a solution of 2% ethanol in dioxane. The suspension was maintained at 90 °C, while hydrogen was bubbled continuously through the slurry via a gas dispersion tube. This procedure furnished the cyclic tripeptide **17**, albeit in only 29% yield. Optimization of the reaction conditions by replacement of the pyrrolidinopyridine with the milder base *N*-methylmorpholine increased the yield to 44%, and substitution of the 5% Pd/C with palladium black further increased the yield to 67–70% (presumably the carbon support in 5% Pd/C absorbs either the polyaromatic starting material or cyclic product). The removal of the Boc protecting group from the cyclic product **17** was accomplished by treatment with trifluoroacetic acid. After acetylation, **18** was isolated in 97% yield, mp > 300 °C.

The final step in the synthesis of K-13 involved the tris-demethylation of **18**. In this reaction we hoped to find conditions under which both aryl methyl ethers as well as the methyl ester moieties might be cleaved with a common reagent. Initial attempts at the execution of this reaction using the boron tribromide procedure utilized in the deprotection of amino acids and peptides as developed by Felix[31] proved unsuccessful due to the slow reaction rates. Presumably, the DMF addend which is necessary to solubilize **18** deactivated the boron tribromide through complexation. On the other hand, demethylation of **18** with excess aluminum tribromide and ethanethiol according to the procedure of Fujita[32] cleanly provided K-13 in 92% yield as a white powdery solid which was identical with natural K-13 using spectroscopic (^{13}C NMR, 500 MHz ^{1}H NMR, and IR) and analytical chromatographic (reverse phase HPLC and TLC) methods. The optical rotation of synthetic K-13 at $[\alpha]_D$ −6.5° (*c* 0.46, MeOH) was somewhat higher than the reported optical rotation of natural K-13 at $[\alpha]_D$ −3.4° (*c* 0.60, MeOH).[9b] Unfortunately, only 1 mg of impure natural K-13 was provided to us, thus precluding a direct comparison of the optical rotation of natural and synthetic material under *identical* conditions. In the initial structural elucidation studies the absolute configurations of the isodityrosine chiral centers were not determined; hence, this first total synthesis of K-13 establishes that the configuration at all stereogenic centers is (S).

Conclusions

The total syntheses of OF4949-III and K-13 from completely achiral starting materials clearly demonstrates the utility of the direct azidation of imide enolates in complex polyfunctional molecules. The introduction of the oxazolidinone chiral auxiliary and the direct azidation of the imide enolate are mild and high yielding processes. The α-azidocarboximide not only serves as a useful protected amino acid derivative but can also readily be converted to a variety of other amino acid derivatives in the presence of labile functionality.

Experimental Section

General Methods. Melting points are uncorrected. Combustion analyses were performed by Spang Microanalytical Laboratory (Eagle Harbor, MI). Fast atom bombardment (FAB) mass spectra were determined on a Kratos MS-50 spectrometer with solutions in the indicated solvent and with xenon as the ionization gas. Liquid chromatography was performed by using forced flow (flash chromatography) of the indicated solvent system on EM Reagents silica gel 60 (230–400) mesh. Tetrahydrofuran (THF), diethyl ether, and dioxane were distilled from sodium metal–benzophenone ketyl. Dichloromethane (CH_2Cl_2), pyridine, diisopropylamine, triethylamine, and acetonitrile were distilled from calcium hydride. Dimethylformamide (DMF) was distilled under reduced pressure from calcium hydride and stored over activated 5Å molecular sieves under a nitrogen atmosphere. Ethanethiol was fractionally distilled from calcium chloride and stored under nitrogen atmosphere. Benzyl alcohol and pivaloyl chloride were fractionally distilled under reduced pressure and stored under nitrogen atmosphere. All other reagents were used as received. Air and/or moisture sensitive reactions were carried out under an atmosphere of nitrogen with flame-dried glassware. In the ^{1}H NMR spectral assignments of isodityrosine, the carbons of the less substituted and of the more substituted phenylalanine moieties are labeled with the superscripts 1 and 2, respectively.

(*E*,*E*)-1,1-Dimethylethyl 3-[4-[3-Oxo-3-(phenylmethoxy)propenyl]-phenoxy]-4-methoxybenzenepropenoate (3). To a flame-dried Schlenk flask fitted with a stirring bar was added 8.39 g (32.5 mmol) of (*E*/*Z*)-*tert*-butyl 3-hydroxy-4-methoxycinnamate (**1**), 13.0 g (40.2 mmol, 1.2 equiv) of (*E*)-benzyl 4-bromocinnamate (**2**), and 9.26 g (67.0 mmol, 2.0 equiv) of anhydrous potassium carbonate. After evacuating the reaction flask and flushing it with nitrogen successively three times, 40 mL of freshly distilled pyridine was added by syringe. The yellow reaction slurry was warmed to 90 °C, and 6.66 g (83.8 mmol, 2.5 equiv) of cupric oxide was added under positive nitrogen flush. A reflux condenser was affixed to the flask under positive nitrogen flush, and the black slurry was heated at reflux (oil bath, 145 °C) under nitrogen with vigorous stirring for 24 h. After cooling to ambient temperature, the reaction slurry was diluted with 300 mL of CH_2Cl_2, and the solid material was removed by filtration. The filtrate was concentrated in vacuo to give a red oil. The oil was diluted with 300 mL of CH_2Cl_2, washed twice with 1 N aqueous sodium hydrogen sulfate and then once with brine/saturated aqueous sodium bicarbonate, dried over sodium sulfate, and concentrated in vacuo to give a red, viscous residue. Purification by flash chromatography (8 cm × 60 cm silica gel, eluted with 25:75 ethyl acetate/hexane) followed by reflashing mixed fractions (same column) with 20:80 ethyl acetate/hexane yielded 14.55 g (91%) of the title compound as a clear viscous oil [*E*/*Z* ratio of olefin isomers in product corresponds to *E*/*Z* ratio for **1**]. R_f 0.34 (*E*), 0.3 (*Z*) (ethyl acetate/hexane); IR (CH_2Cl_2) 3120–2820, 1705, 1678, 1611, 1603, 1590, 1508 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 (d, *J* = 16.0 Hz, 0.8 H, C_2^1-*H* (*E*)), 7.68 (d, *J* = 16 Hz, 0.2 H, C_3^1-*H* (*Z*)), 7.22–7.51 (m, 9 H, aromatic Hs), 6.89–7.01 (m, 3 H, aromatic Hs), 6.70 (d, *J* = 12.6 Hz, 0.2 H, C_3^2-*H* (*Z*)), 6.38 (d, *J* = 16.0 Hz, 0.8 H, C_2^1-*H* (*E*)), 6.37 (d, *J* = 16.0 Hz, 0.2 H, C_2^1-*H* (*Z*)), 6.19 (d, *J* = 15.9 Hz, 0.8 H, C_2^2-*H* (*E*)), 5.78 (d, *J* = 12.6 Hz, 0.2 H, C_2^2-*H* (*Z*)), 5.25 (s, 2 H, OCH_2Ph), 3.84 (s, 2.4 H, OCH_3 (*E*)), 3.83 (s, 0.6 H, OCH_3 (*Z*)), 1.51 (s, 7.2 H, $OC(CH_3)_3$ (*E*)), 1.43 (s, 1.8 H, $OC(CH_3)_3$ (*Z*)). Anal. Calcd for $C_{30}H_{30}O_6$: C, 74.06; H, 6.21. Found: C, 73.97; H, 6.23.

(*S*)-1,1-Dimethylethyl 3-[4-[3-Oxo-3-[2-oxo-4-(phenylmethyl)-3-oxazolidinyl]propyl]phenoxy]-4-methoxybenzenepropanoate (4). To a solution of 12.84 g (25.2 mmol) of **3** dissolved in 300 mL of 5:1 ethanol/acetic acid was added 1.5 g of 10% Pd/C. The resulting black slurry was stirred under hydrogen at atmospheric pressure for 10 h. The slurry was then filtered through Celite, and the Celite filter cake was washed with several portions of ethanol. The combined filtrate was concentrated in vacuo to give a clear oil. The clear oil was diluted with toluene and concentrated in vacuo two times to remove any remaining acetic acid. A solution of the clear oil in 200 mL of freshly distilled THF was cooled to −78 °C, and 4.52 mL (3.28 g, 32.4 mmol, 1.3 equiv) of freshly distilled triethylamine followed by 3.37 mL (3.30 g, 27.4 mmol, 1.1 equiv) of distilled pivaloyl chloride were added by syringe with stirring. The resulting slurry was stirred at −78 °C for 15 min and 0 °C for 45 min and then recooled to −78 °C. In a separate flask, 7.94 g (44.8 mmol, 1.8 equiv) of (4*S*)-4-(phenylmethyl)-2-oxazolidinone was dissolved in 100 mL of freshly distilled THF and cooled to −78 °C. To this solution was added via cannula 18.39 mL (44.8 mmol, 1.8 equiv) of 1.6 M *n*-butyllithium in hexanes. The metalated oxazolidinone was added via cannula to the white slurry prepared as described above. The resulting slurry was stirred for 15 min at −78 °C and then warmed to room temperature over 1.5 h. The reaction was quenched by addition of 200 mL of 1 N aqueous sodium bisulfate. The THF was removed in vacuo, and the remaining aqueous mixture was extracted with three 100-mL portions of CH_2Cl_2. The combined organic extracts were washed successively with dilute aqueous sodium bicarbonate and with dilute brine, dried over sodium sulfate, and concentrated in vacuo to give a viscous oil. Purification by flash chromatography (8 cm × 25 cm silica gel, eluted with 28:72 ethyl

(31) Felix, A. M. *J. Org. Chem.* **1974**, *39*, 1427–1429.
(32) Node, M.; Nishide, K.; Fuji, K.; Fujita, E. *J. Org. Chem.* **1980**, *45*, 4275–4277.

acetate/hexane) gave 12.8 g (90%) of the title compound as a white, crystalline solid: mp 88–89 °C; R_f 0.26 (30:70 ethyl acetate/hexane); IR (CH_2Cl_2) 3680, 3100–2820, 1785, 1720, 1705, 1607, 1585, 1507 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.14–7.32 (m, 7 H, aromatic Hs), 6.78–6.94 (m, 5 H, aromatic Hs), 4.61–4.75 (m, 1 *H*, C_2^{xp}-*H*), 4.14–4.22 (m, 2 H, C_1^{xp}-H_2), 3.77 (s, 3 CH_3), 3.07–3.35 (m, 3 H, C_3^{xp}-*H*H overlapping C_2^1-H_2), 2.93–2.98 (m, 2 H, C_3^1-H_2), 2.70–2.80 (m, 3 H, C_3^{xp}-*H*H overlapping C_2^2-H_2), 2.44 (t, J = 7.84 Hz, 2 H, C_3^2-H_2), 1.37 (s, 9 H, $OC(CH_3)_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 172.4, 172.0, 156.4, 153.3, 149.8, 145.0, 135.2, 134.3, 133.9, 129.5, 129.3, 128.9, 127.3, 124.3, 121.0, 117.1, 113.0, 80.2, 66.1, 56.1, 55.0, 37.8, 37.2, 37.1, 30.2, 29.5, 28.0; $[\alpha]_{Hg\ 365}$ + 157.5° (*c* 0.900, CH_2Cl_2). Anal. Calcd for $C_{33}H_{37}O_7N$: C, 70.82; H, 6.66. Found: C, 70.89; H, 6.77.

[*S*-(*R,*R**)]-1,1-Dimethylethyl 3-[4-[2-Azido-3-oxo-3-[2-oxo-4-(phenylmethyl)-3-oxazolidinyl]propyl]phenoxy]-4-methoxybenzenepropanoate (5).** To a stirred solution of 940 mg (1.68 mmol) of **4** in 40 mL of freshly distilled THF at −78 °C was added via syringe over 15 s 3.07 mL (1.69 mmol, 1.00 equiv) of 0.55 M potassium bis(trimethylsilyl)amide in toluene. The resulting solution was stirred for 15 min at −78 °C followed by treatment, via Teflon cannula, with a precooled (−78 °C) solution of 649 mg (2.10 mmol, 1.25 equiv) of trisyl azide in 8 mL of freshly distilled THF. The solution was stirred for 2 min at −78 °C and then quenched by rapid addition of 0.289 mL (303 mg, 5.04 mmol, 3 equiv) of glacial acetic acid with immediate warming to 30 °C with a water bath. After stirring the white slurry at ambient temperature for 1.5 h, it was diluted with 250 mL of CH_2Cl_2, washed successively with dilute brine and with dilute aqueous sodium bicarbonate, dried over sodium sulfate, and concentrated in vacuo to give a clear and colorless oil (HPLC diastereomer analysis revealed a 97.5:2.5 ratio of diastereomers). Purification by flash chromatography (300 g silica gel, gradient eluent 30:70:4 ethyl acetate/hexane/CH_2Cl_2) gave 0.867 g (86%) of product: HPLC diastereomer analysis (4.5 mm × 25.0 cm Zorbax column, 25:75 ethyl acetate/isooctane, 2 mL/min, 270 nm, t_r minor = 5.05 min, t_r major = 5.58) revealed a greater than 99:1 ratio of diastereomers; R_f 0.26 (4:30:70 ethyl acetate/hexane/CH_2Cl_2 to 6:10:90 ethyl acetate/hexane/CH_2Cl_2); IR (CH_2Cl_2) 3110–2820, 2102, 1784, 1715, 1608, 1508 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.20–7.38 (m, 7 H, aromatic Hs), 6.82–6.98 (m, 2 H, aromatic Hs), 5.25 (dd, J = 5.7, 8.9 Hz, 1 H, C_2^1-*H*), 4.56–4.63 (m, 1 H, C_2^{xp}-*H*), 4.09–4.22 (m, 2 H, C_1^{xp}-H_2), 3.78 (s, 3 H, OCH_3), 3.33 (dd, J = 3.1, 13.4 Hz, 1 H, C_3^{xp}-*H*H), 3.16 (dd, J = 5.7, 13.6 Hz, 1 H, C_3^1-*H*H), 3.03 (dd, J = 8.9, 13.6 Hz, 1 H, C_3^1-*H*H), 2.78–2.86 (m, 3 H, C_3^{xp}-H_2 overlapping with C_3^2-*H*H), 2.47 (t, J = 7.7 Hz, 2 H, C_3^2-H_2), 1.38 (s, 9 H, $OC(CH_3)_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 172.0, 170.5, 157.4, 152.4, 149.9, 144.5, 134.7, 134.0, 130.4, 129.4, 129.3, 129.0, 127.5, 124.6, 121.3, 117.1, 113.1, 80.3, 66.5, 61.3, 56.1, 55.4, 37.6, 37.1, 37.0, 30.2, 28.0; $[\alpha]_{Hg\ 365}$ + 232° (*c* 1.01, CH_2Cl_2). Anal. Calcd for $C_{33}H_{36}O_7N_4$: C, 65.99; H, 6.04. Found: C, 65.82; H, 6.11.

(*S*)-1,1-Dimethylethyl 3-[4-[2-Azido-3-oxo-3-(phenylmethoxy)-propyl]phenoxy]-4-methoxybenzenepropanoate (6). To a flame-dried, 100-mL Schlenk flask fitted with a stirring bar was added 20 mL of distilled benzyl alcohol. Titanium tetraisopropoxide (0.728 mL, 0.694 g, 2.39 mmol, 0.5 equiv) was added by syringe. The isopropyl alcohol was then distilled off by stirring the pale yellow solution under reduced pressure (1 mm) for 1 h. The resulting titanium tetrabenzyloxide solution was added via cannula to 2.87 g (4.78 mmol) of **5**. The yellow solution was stirred under nitrogen atmosphere at 70 °C for 2 h. After cooling the reaction solution to ambient temperature, the solution was diluted with 250 mL of ethyl ether, washed with 1 N aqueous hydrochloric acid and then brine, dried over sodium sulfate, and concentrated in vacuo to give a yellow oil. The remaining benzyl alcohol was removed by Kugelrohr distillation (0.1 mm) at 70 °C. Purification by flash chromatography (4 cm × 30 cm silica gel, 18:82 ethyl acetate/hexane) gave 2.32 g (91%) of the title compound as a white crystalline solid: mp 70–71 °C; R_f 0.69 (40:60 ethyl acetate/hexane); IR (CH_2Cl_2) 3140–2820, 2107, 1730, 1610, 1585, 1510 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.82–7.38 (m, 12 H, aromatic Hs), 5.18 (s, 2 H, OCH_2Ph), 4.06 (dd, J = 5.6, 8.4 Hz, 1 H, C_2^1-*H*), 3.78 (s, 3 H, OCH_3), 3.11 (dd, J = 5.6, 14.1 Hz, 1 H, C_3^1-*H*H), 2.98 (dd, J = 8.4, 14.1 Hz, 1 H, C_3^1-*H*H), 2.81 (t, J = 7.7 Hz, 2 H, C_2^2-H_2), 2.47 (t, J = 7.7 Hz, 2 H, C_3^2-H_2), 1.38 (s, 9 H, $OC(CH_3)_3$; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 172.0, 169.7, 157.4, 149.9, 144.7, 135.0, 134.0, 130.3, 129.5, 128.6, 128.4, 124.6, 121.4, 117.1, 113.1, 67.5, 63.4, 56.1, 37.1, 36.9, 30.2, 28.1; $[\alpha]_{Hg\ 365}$ −90.2° (*c* 1.06, CH_2Cl_2). Anal. Calcd for $C_{30}H_{33}O_6N_3$: C, 67.78; H, 6.26. Found: C, 67.77; H, 6.29.

(*S*)-3-[4-[2-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-oxo-3-(phenylmethoxy)propyl]phenoxy]-4-methoxybenzenepropionic Acid (7). To a solution of 549 mg (1.03 mmol) of **6** in 10 mL of 1:1 acetic acid/CH_2Cl_2 was added 30 mg of Aldrich activity-1 Raney nickel (washed successively with water (2×), methanol (2×), and CH_2Cl_2 (2×)). After stirring the reaction slurry under a hydrogen atmosphere for 6.5 h, the slurry was filtered through Celite and concentrated in vacuo to yield a viscous green oil. Purification by flash chromatography (3 cm × 20 cm silica gel, eluted with 50:50 ethyl acetate/hexane followed by 90:10 ethyl acetate/triethylamine) gave 486 mg of a clear oil which was used directly in the subsequent reaction. The colorless oil was diluted with 5 mL of 5:1 CH_2Cl_2/thioanisole, and after cooling the resulting solution to 0 °C, 5 mL of trifluoroacetic acid was added slowly, dropwise with stirring. After stirring for 1 h with warming to ambient temperature, the solution was concentrated in vacuo to give the amino acid. Any residual trifluoroacetic acid was removed by diluting with heptane and concentrating in vacuo. The unpurified amino acid was diluted with 40 mL of dioxane, and a solution of 323 mg (3.85 mmol, 4.0 equiv) of sodium bicarbonate in 1 mL of distilled water was added followed by addition of 631 μL (600 mg, 2.89 mmol, 3.0 equiv) of *tert*-butylpyrocarbonate. The clear, colorless solution was stirred 2 h at ambient temperature. After adding an additional equiv of *tert*-butylpyrocarbonate, the solution was stirred an additional hour. The solvent was removed in vacuo, and the residue was diluted with CH_2Cl_2, washed with 1 N aqueous sodium hydrogen sulfate, dried over sodium sulfate, and concentrated in vacuo to give a pale orange oil. Purification by flash chromatography (3 cm × 25 cm silica gel, eluted with 40:60:1 ethyl acetate/hexane/acetic acid) gave 497 mg (88%) of the title compound as a clear and colorless oil: R_f 0.23 (40:60:1 ethyl acetate/hexane/acetic acid); IR (CH_2Cl_2) 3680, 3440, 2820–3110, 1740, 1718, 1665, 1610, 1590, 1510 cm^{-1}; ^1H NMR (300 MHz, methanol-d_4) δ 6.68–7.33 (m, 11 H, aromatic Hs), 5.06–5.11 (m, 2 H, OCH_2Ph), 4.20–4.36 (m, 1 H, C_2^1-*H* (both Boc rotamers)), 3.69 (s, 3 H, OCH_3), 2.99 (dd, J = 6.2, 13.8 Hz, 1 H, C_3^1-*H*H), 2.86 (dd, J = 8.4, 13.8 Hz, 1 H, C_3^1-*H*H), 2.79 (t, J = 7.5 Hz, 2 H, C_2^2-H_2), 2.50 (t, J = 7.5 Hz, C_3^2-H_2), 1.26–1.35 (m, 9 H, $OC(CH_3)_3$ (both Boc rotamers); $[\alpha]_{Hg\ 365}$ −19.1° (*c* 0.770, MeOH-d_4). Anal. Calcd for $C_{31}H_{35}NO_8$: C, 67.74; H, 6.42. Found: C, 67.51; H, 6.51.

(*S*)-3-(1′-Oxo-3-phenylpropyl)-4-(phenylmethyl)-2-oxazolidinone (Control Experiment; Introduction of Oxazolidinone). A solution of 80 mg (0.533 mmol) of hydrocinnamic acid and 149 mg (0.533 mmol, 1.0 equiv) of *N*-Boc-L-phenylalanine methyl ester in 10 mL of freshly distilled THF was cooled to −78 °C, and 111 μL (81 mg, 0.799 mmol, 1.5 equiv) of freshly distilled triethylamine followed by 79 μL (77 mg, 0.640 mmol, 1.2 equiv) of distilled pivaloyl chloride were added by syringe with stirring. The resulting slurry was stirred at −78 °C for 15 min, 0 °C for 45 min, and then recooled to −78 °C. In a separate flask, 189 mg (1.07 mmol, 2.0 equiv) of (4*S*)-4-(phenylmethyl)-2-oxazolidinone was dissolved in 5 mL of freshly distilled THF and cooled to −78 °C. To this solution was added via cannula 413 μL (1.00 mmol, 1.9 equiv) of 1.6 M *n*-butyllithium in hexanes. The resulting metalated oxazolidinone was added via cannula to the white slurry prepared as described above. The resulting slurry was stirred for 30 min at −78 °C and then quenched by addition of 50 mL of 1 N aqueous sodium bisulfate. The THF was removed by concentrating in vacuo, and the resulting aqueous mixture was extracted with three 25-mL portions of CH_2Cl_2. The combined organic extracts were washed with dilute aqueous sodium bicarbonate and then with dilute brine, dried over sodium sulfate, and concentrated in vacuo to give a viscous oil.

The unpurified product mixture containing hydrocinnamoylcarboximide and recovered *N*-Boc-L-phenylalanine methyl ester was dissolved in 1 mL of CH_2Cl_2. Thioanisole (20 μL) was added followed by 1 mL of trifluoroacetic acid. The clear solution was stirred at ambient temperature for 1 h and then concentrated to give a viscous oil. The oil was diluted with 25 mL of 1 N aqueous hydrochloride followed by extraction with three 25-mL portions of ethyl ether. The aqueous phase was then adjusted to pH 10 with sodium carbonate and extracted with two 25-mL portions of CH_2Cl_2. The combined organic phase was dried over sodium sulfate and concentrated in vacuo to give phenylalanine methyl ester. To 17 mg (0.09 mmol) of the above unpurified phenylalanine methyl ester dissolved in 2 mL of CH_2Cl_2 was added 50 μL (0.37 mg, 0.36 mmol, 3.6 equiv) of triethylamine and 37 μL (48 mg, 1.9 mmol, 2.0 equiv) of (+)-MTPA chloride. After stirring at ambient temperature for 1 h the solution was diluted with 50 mL of CH_2Cl_2, washed with 1 N aqueous sodium bisulfate and then with saturated aqueous bicarbonate, dried over sodium sulfate, and concentrated in vacuo to give the unpurified (+)-MTPA amide methyl ester of phenylalanine. Capillary GC analysis (DB-1, 200 °C, 10 PSI) of the unpurified product revealed the ratio of (+)-MTPA amide methyl ester of D-phenylalanine (t_r = 6.83) to the (+)-MTPA amide methyl ester of L-phenylalanine (t_r = 7.05) to be less than 0.5:99.5.

[*S*-(*R,*R**)]-3-(2′-Azido-1′-oxo-3-phenylpropyl)-4-(phenylmethyl)-2-oxazolidinone (Control Experiment; Direct Azidation).** A precooled (−78 °C) solution of 60 mg (0.194 mmol) of hydrocinnamoylcarboximide and 77 mg (0.194 mmol) of (+)-MTPA amide methyl ester of L-phenylalanine in 4 mL of freshly distilled THF was added via cannula to a solution of 749 μL (0.427 mmol, 2.2 equiv) of potassium bis(trimethylsilyl)amide in 3 mL of THF. The resulting solution was stirred for 15

min at −78 °C, followed by treatment, via Teflon cannula, with a precooled (−78 °C) solution of 150 mg (0.485 mmol, 2.5 equiv) trisyl azide in 2 mL of freshly distilled THF. The solution was stirred for 2 min at −78 °C and then quenched by rapid addition of 49 μL (51 mg, 0.854 mmol, 4.4 equiv) of glacial acetic acid with immediate warming to 30 °C with a 30 °C water bath. After stirring the white slurry at ambient temperature for 1.5 h, it was diluted with 75 mL of CH_2Cl_2, washed successively with dilute brine and then dilute aqueous sodium bicarbonate, dried over sodium sulfate, and concentrated in vacuo to give a clear and colorless oil. Filtration through silica (2 cm × 20 cm silica gel, eluted with 20:80 ethyl acetate/hexane) gave a clear oil containing the recovered (+)-MTPA amide methyl ester and the product α-azidocarboximide. Capillary GC analysis (DB-1, 200 °C, 10 PSI) of the product mixture revealed the ratio of (+)-MTPA amide methyl ester of D-phenylalanine (t_r = 6.74) to the (+)-MTPA amide methyl ester of L-phenylalanine (t_r = 7.18) to be 0.8:99.2.

(*S*)-*N*-[(1,1-Dimethylethoxy)carbonyl]-*O*-[2-methoxy-5-[3-oxo-3-[2-oxo-4-(phenylmethyl)-3-oxazolidinyl]propyl]phenyl]-L-tyrosine Phenylmethyl Ester (8). To a stirred solution of 2.55 g (4.64 mmol) of **7** in 100 mL of freshly distilled THF cooled to −78 °C was added 970 μL (704 mg, 6.90 mmol, 1.5 equiv) of freshly distilled triethylamine followed by 629 μL (616 mg, 5.10 mmol, 1.1 equiv) of distilled pivaloyl chloride. The reaction slurry was warmed to 0 °C over 20 min and then recooled to −78 °C. In a separate flask, 1.73 g (9.74 mmol, 2.1 equiv) of (4*S*)-4-(phenylmethyl)-2-oxazolidinone was dissolved in 50 mL of freshly distilled THF and cooled to −78 °C. To this solution was added via syringe 3.79 mL (9.28 mmol, 2.0 equiv) of 2.45 M *n*-butyllithium in hexanes. The metalated oxazolidinone was then added via cannula to the white slurry prepared as described above. The resulting slurry was stirred for 20 min at −78 °C and then was quenched by addition of 1 N aqueous sodium bisulfate. The THF was removed in vacuo, and the remaining aqueous mixture was extracted three times with CH_2Cl_2 (600 mL total). The combined organic extracts were washed with saturated aqueous sodium bicarbonate, dried over sodium sulfate, and concentrated in vacuo to give a viscous oil. Purification by flash chromatography (4 × 30 cm silica gel, eluted with 35:65 ethyl acetate/hexane) gave 3.12 g (95%) of pure product as a white solid: R_f 0.26 (40:60 ethyl acetate/hexane) IR (CH_2Cl_2) 3440, 2820–3110, 1785, 1742, 1610, 1585, 1510 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.77–7.37 (m, 17 H, aromatic Hs), 5.15 (d, J = 12.3 Hz, 1 H, OC*H*HPh), 5.12 (d, J = 12.3 Hz, 1 H, OC*H*HPh), 4.98 (d, J = 8.3 Hz, 1 H, N-*H*), 4.54–4.70 (m, 2 H, C_2^1-*H* overlapping with C_2^{Xp}-*H*), 4.11–4.18 (m, 2 H, C_1^{Xp}-H_2), 3.80 (s, 3 H, OCH_3), 3.14–3.29 (m, 2 H, C_3^2-H_2), 3.14–3.19 (m, 3 H, C_3^{Xp}-*H*H overlapping with C_2^2-H_2), 3.01–3.03 (m, 2 H, C_3^1-H_2), 2.90–2.96 (m, 2 H, C_3^2-H_2), 2.73 (dd, J = 9.5, 13.3 Hz, 1 H, C_3^{Xp}-*H*H), 1.41 (br s, 3 H, $OC(CH_3)_3$); $[\alpha]_{Hg\ 365}$ + 124.5° (*c* 0.715, CH_2Cl_2). Anal. Calcd for $C_{41}H_{44}N_2O_9$: C, 69.48; H, 6.26. Found: C, 69.47; H, 6.22.

[*S*-(*R,*R**)]-*N*-[(1,1-Dimethylethoxy)carbonyl]-*O*-[2-methoxy-5-[2-azido-3-oxo-3-[2-oxo-4-(phenylmethyl)-3-oxazolidinyl]propyl]phenyl]-L-tyrosine Phenylmethyl Ester (9).** A precooled (−78 °C) solution of 1.464 g (2.06 mmol) of **8** in 25 mL of freshly distilled THF was added via Teflon cannula to a precooled (−78 °C) solution of 7.89 mL (4.34 mmol, 2.1 equiv) of 0.55 M potassium bis(trimethylsilyl)amide in toluene diluted with 30 mL of freshly distilled THF. After 20 min, the resulting solution of the potassium enolate was treated, via Teflon cannula, with a −78 °C solution of 1.59 g (5.15 mmol, 2.5 equiv) of trisyl azide dissolved in 15 mL of freshly distilled THF. After stirring the clear solution for 1.5 min, the reaction was quenched by addition of 519 μL (544 mg, 9.06 mmol, 4.4 equiv) of glacial acetic acid. The white slurry was immediately warmed to 30 °C with a water bath and was stirred at this temperature for 2 h. The reaction slurry was then partitioned between 200 mL of dilute brine and 200 mL of CH_2Cl_2, and the aqueous phase was washed twice with 100 mL of CH_2Cl_2. The combined organic extracts were washed with dilute aqueous sodium bicarbonate, dried over sodium sulfate, and concentrated in vacuo to give a yellow oil. Purification by flash chromatography (300 g silica gel, eluent gradient 20:80 to 50:50 ethyl acetate/hexane) gave 1.34 g (87%) of the title compound isolated as a white foam. HPLC diastereomer analysis (4.5 mm × 25.0 cm Zorbax column, 30:70 ethyl acetate/hexane, 2 mL/min, 270 nm, t_r minor 5.16 min, t_r major 6.16 min) revealed a 99/1 mixture of the (*S*) and (*R*) isomers: R_f 0.28 (40:60 ethyl/hexane); IR (CH_2Cl_2) 3440, 3120–2820, 1785, 1742, 1712, 1609, 1587 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.67–7.38 (m, 17 H, aromatic Hs), 5.20 (dd, J = 5.1, 9.1 Hz, 1 H, C_2^2-*H*), 5.15 (d, J = 12.1 Hz, OC*H*HPh), 5.12 (d, J = 12.1 Hz, OC*H*HPh), 4.99 (d, J = 8.2 Hz, 1 H, N-*H*), 4.55–4.65 (m, 2 H, C_2^1-*H* overlapping with C_2^{Xp}-*H*), 4.19 (dd, J = 2.8, 9.1 Hz, 1 H, C_1^{Xp}-*H*H), 4.14 (dd, J = 16.8, 9.1 Hz, 1 H, C_1^{Xp}-*H*H), 3.81 (s, 3 H, OCH_3), 3.31 (dd, J = 3.2, 13.4 Hz, 1 H, C_3^{Xp}-*H*H), 3.12 (dd, J = 5.1, 13.8 Hz, 1 H, C_3^2-*H*H), 3.02–3.10 (m, 2 H, C_3^1-H_2), 2.94 (dd, J = 9.2, 13.8 Hz, 1 H, C_3^2-*H*H), 2.82 (dd, J = 9.5, 13.4 Hz, 1 H, C_3^{Xp}-*H*H), 1.42 (s, 9 H, $OC(CH_3)_3$); $[\alpha]_{Hg\ 365}$ +157.3° (*c* 0.935, CH_2Cl_2). Anal. Calcd for $C_{41}H_{43}N_5O_9$: C, 65.68; H, 5.78. Found: C, 65.73; H, 5.84.

[*S*-(*R,*R**)]-*O*-[5-[2-Azido-3-oxo-3-[2-oxo-4-(phenylmethyl)-3-oxazolidinyl]propyl]-2-methoxyphenyl]-*N*-[*N*-[(1,1-dimethylethoxy)carbonyl]-L-asparaginyl]-L-tyrosine Phenylmethyl Ester (10).** To 297 mg (0.396 mmol) of **9** dissolved in 11 mL of CH_2Cl_2 was added 697 μL (738 mg, 5.94 mmol, 15 equiv) of thioanisole. The clear and colorless solution was cooled to 0 °C, and 6 mL of trifluoroacetic acid was added slowly, dropwise with stirring. The solution was warmed to ambient temperature with stirring over 2 h and then was concentrated in vacuo to give a pale yellow oil. Any remaining trifluoroacetic acid was removed by diluting with toluene and concentrating in vacuo. The resulting oil was diluted with CH_2Cl_2 and washed twice with aqueous sodium bicarbonate solution, dried over sodium sulfate, and concentrated in vacuo to give a clear oil. The unpurified free amine was dissolved in 4 mL of distilled *N*,*N*-dimethylformamide. Hydroxybenzotriazole monohydrate (118 mg, 0.871 mmol, 2.2 equiv) and 184 mg (0.792 mmol, 2.0 equiv) of Boc-asparagine were added with stirring. The solution was cooled to 0 °C and 152 mg (0.792 mmol, 2.0 equiv) of 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride was added in one portion. The resulting solution was stirred at 0 °C for 2 h and at room temperature for 10 h, and then concentrated in vacuo (1 mm) to remove the *N*,*N*-dimethylformamide. The residue was diluted with 50 mL of CH_2Cl_2, washed with 1 N aqueous sodium bisulfate and then saturated aqueous sodium bicarbonate followed by dilute brine, dried over sodium sulfate, and concentrated in vacuo to give a clear oil. Purification by flash chromatography (3 cm × 30 cm silica gel, 75:25:2 ethyl acetate/hexane/acetic acid) gave 308 mg (90%) of the title compound as a white powdery solid: mp 87.5–88.5 °C (phase change to amorphous solid); R_f 0.18 (75:25:2 ethyl acetate/hexane/acetic acid); IR (CH_2Cl_2) 3540, 3410, 2840–3110, 2115, 1785, 1747, 1710, 1690, 1610, 1593 cm^{-1}; ^1H NMR δ (300 MHz, methanol-d_4) 7.—7.36 (m, 14 H, aromatic Hs), 6.92 (d, J = 1.7 Hz, 1 H, C_{5a}^2-*H*), 6.71 (d, J = 8.5 Hz, 2 H, C_{6a}^1-H_2), 5.14 (dd, J = 4.6, 9.5, Hz, 1 H, C_2^2-*H*), 5.09 (s, 2 H, OCH_2Ph), 4.61–4.70 (m, 2 H, C_2^1-*H* overlapping with C_2^{Xp}-*H*), 4.43 (t, J = 7.5 Hz, 1 H, C_2^{Asn}-*H*), 4.20–4.26 (m, 2 H, C_1^{Xp}-H_2), 3.74 (s, 3 H, OCH_3), 3.15 (dd, J = 4.6, 14.0 Hz, 1 H, C_3^2-*H*H), 3.13 (dd, 3.3, 13.7 Hz, 1 H, C_3^{Xp}-*H*H), 2.94–3.04 (m, 3 H, C_3^{Xp}-*H*H overlapping with C_3^1-H_2), 2.87 (dd, J = 9.5, 14.0 Hz, 1 H, C_3^2-*H*H), 2.43–2.63 (m, 2 H, C_3^{Asn}-H_2), 1.39 (s, 9 H, $OC(CH_3)_3$); $[\alpha]_{Hg\ 363}$ +141.5° (*c* 0.685, ethanol). Anal. Calcd for $C_{45}H_{49}O_{11}N_7$: C, 62.56; H, 5.72. Found: C, 62.78; H, 5.90.

(*S*)-*O*-[5-(2-Azido-2-carboxyethyl)-2-methoxyphenyl]-*N*-[*N*-[(1,1-dimethylethoxy)carbonyl]-L-asparaginyl]-L-tyrosine α-(Phenylmethyl) Ester (11). To a precooled (0 °C) solution of 328 mg (0.380 mmol) of **10** dissolved in 12 mL of THF was added slowly dropwise with stirring an aqueous lithium hydrogen peroxide solution [prepared from 2.00 mL (0.399 mmol, 1.05 equiv) of 0.20 M aqueous lithium hydroxide, 193 μL (214 mg, 1.90 mmol, 5 equiv) of 30% aqueous hydrogen peroxide, and 2 mL of distilled water]. After stirring the clear and colorless solution for 1 h at 0 °C, the reaction was quenched by dropwise addition of a solution of 395 mg (3.80 mmol, 10 equiv) of sodium hydrogen sulfite in 2 mL of water. After stirring the resulting slurry for 15 min at 0 °C, the organic solvent was removed in vacuo, and the remaining aqueous mixture was extracted three times with 2% THF in ethyl acetate (200 mL total). The combined organic phase was dried over sodium sulfate and concentrated in vacuo to give a white solid. Purification by flash chromatography (3 cm × 25 cm silica gel, eluted with 2:98 acetic acid/ethyl acetate followed by 5:95 acetic acid/ethyl acetate; unpurified product applied to column preadsorbed on silica gel) gave 240 mg (89%) of the title compound as a white solid [to prevent the loss of the Boc protecting group during concentration, the fractions containing product were concentrated in vacuo (1 mm) at <10 °C after addition of toluene to azeotrope off the acetic acid]: mp 156.5–157.5 °C; R_f 0.19 (100:2 ethyl acetate/acetic acid); IR (KBr) 3700–2400 (b), 2115 (s), 1760–1650 (b), 1610, 1590 cm^{-1}; ^1H NMR (300 MHz, 2:1 methanol-d_4/$CDCl_3$) δ 6.97–7.35 (m, 9 H, aromatic Hs), 6.86 (d, J = 1.7 Hz, 1 H, C_{5a}^2-*H*), 6.73 (d, J = 8.6 Hz, 2 H, C_6^2-H_2), 5.10 (d, J = 12.2 Hz, 1 H, OC*H*HPh), 5.08 (d, J = 12.2 Hz, 1 H, OC*H*HPh), 4.68 (t, J = 6.7 Hz, 1 H, C_2^{Asn}-*H*), 4.42 (t, J = 6.3 Hz, 1 H, C_2^1-*H*), 3.99 (dd, J = 4.7, 8.7 Hz, 1 H, C_2^2-*H*), 3.75 (s, 3 H, OCH_3), 3.09 (dd, J = 4.9, 14.1 Hz, 1 H, C_3^2-*H*H), 2.95–3.10 (m, 2 H, C_3^1-H_2), 2.85 (dd, J = 8.7, 14.1 Hz, 1 H, C_3^2-*H*H), 2.53–2.57 (m, 2 H, C_3^{Asn}-H_2), 1.39 (s, 9 H, $OC(CH_3)_3$); $[\alpha]_{Hg\ 365}$ −91° (*c* 0.275, acetonitrile). Anal. Calcd for $C_{35}H_{40}O_{10}N_6$: C, 59.65; H, 5.72. Found: C, 59.60; H, 5.64.

Methyl [2*R*,2(2*R*)]-2-[2-Azido-1-oxo-3-phenylamino]-3-phenylpropionate. To a precooled (0 °C) solution of 55 mg (0.286 mmol) of (2*R*)-2-azidohydrocinnamic acid, 92 mg (0.429 mmol, 1.5 equiv) of D-phenylalanine hydrochloride methyl ester, 46 mg (0.343 mmol, 1.2 equiv) of hydroxybenzotriazole monohydrate, and 47 μL (0.429 mmol, 1.5 equiv) of *N*-methylmorpholine in 2 mL of dry *N*,*N*-dimethylform-

amide was added 66 mg (0.343 mmol, 1.2 equiv) of 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride. After the resulting slurry was stirred for 2 h at 0 °C and for 24 h at ambient temperature, the solvent was removed in vacuo. The resulting solid was diluted with 50 mL of CH_2Cl_2, washed with 1.0 N aqueous sodium bisulfate and with saturated aqueous sodium bicarbonate/brine, dried over sodium sulfate, and concentrated in vacuo. Filtration through silica gel (2 cm × 20 cm) with 30:70 ethyl acetate/hexane as eluent afforded 101 mg (quantitative yield) of the titled compound as a white crystalline solid. HPLC diastereomer analysis (4.5 mm × 25.0 cm Zorbax column, 40:60 *tert*-butyl methyl ether/isooctane, 2.0 mL/min, 254 nm) showed >99:1 diastereomeric purity (t_r 3.99 min): mp 87.6–88.6 °C; R_f (40:60 ethyl acetate/hexane); IR (CH_2Cl_2) 3405, 3110–2840, 2110, 1746, 1680, 1520 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.21–7.36 (m, 8 H, aromatic Hs), 7.86–6.90 (m, 2 H, aromatic Hs), 6.69 (d, J = 8.1 Hz, 1 H, N-*H*), 4.84–4.90 (m, 1 H, C_2-*H*), 4.19 (dd, J = 8.0, 4.1 Hz, 1 H, C_2'-*H*), 3.71 (s, 3 H, CH_3), 3.27 (dd, J = 14.1, 4.1 Hz, 1 H, C_3'-*H*H), 2.92–3.08 (m, 3 H, C_3-H_2 overlapping with C_3-*H*H); $[\alpha]_{Hg\ 365}$ = −346 (c 0.785, CH_2Cl_2). Anal. Calcd for $C_{19}H_{20}O_3N_4$: C, 64.75; H, 5.72. Found: C, 64.61; H, 5.66.

Methyl [2*R*,2(2*S*)]-2-[2-Azido-1-oxo-3-phenylamino]-3-phenylpropionate. To a precooled (0 °C) solution of 45 mg (0.234 mmol) of (2*R*)-2-azidohydrocinnamic acid, 76 mg (0.351 mmol, 1.5 equiv) of L-phenylalanine hydrochloride methyl ester, 38 mg (0.281 mmol, 1.2 equiv) of hydroxybenzotriazole monohydrate, and 36 μL (0.351 mmol, 1.5 equiv) of *N*-methylmorpholine in 2 mL of dry *N*,*N*-dimethylformamide was added 54 mg (0.281 mmol, 1.2 equiv) of 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride. After stirring the reaction slurry for 2 h at 0 °C and for 24 h at ambient temperature, the solvent was removed in vacuo. The resulting solid was diluted with 50 mL of CH_2Cl_2, washed with 1.0 N aqueous sodium bisulfate and with saturated aqueous sodium bicarbonate/brine, dried over sodium sulfate, and concentrated in vacuo. Filtration through silica gel (2 cm × 25 cm) with 30:70 ethyl acetate/hexane as eluent afforded 82 mg (quantitative yield) of the titled compound as a white crystalline solid. HPLC diastereomer analysis (4.5 mm × 25.0 cm Zorbax column, 40:60 *tert*-butyl methyl ehter/isooctane, 2.0 mL/min, 254 nm) showed >99:1 diastereomeric purity (t_r 3.23 min): mp 66.0–67.0 °C; R_f 0.42 (40:60 ethyl acetate/hexane); IR (CH_2Cl_2) 3405, 3110–2840, 2110, 1747, 1684, 1518 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.24–7.35 (m, 8 H, aromatic Hs), 7.01–7.05 (m, 2 H, aromatic Hs), 6.63 (d, J = 7.7 Hz, 1 H, N-*H*), 4.81–4.88 (m, 1 H, C_2-*H*), 4.10 (dd, J = 8.8, 4.5 Hz, 1 H, C_2'-*H*), 3.71 (s, 3 H, CH_3), 3.31 (dd, J = 14.0, 4.5 Hz, 1 H, C_3'-*H*H), 3.10 (dd, J = 5.9, 2.3 Hz, 2 H, C_3-CH_2), 2.93 (dd, J = 14.0, 8.8 Hz, 1 H, C_3-*H*H); $[\alpha]_{Hg\ 365}$ = +127.0° (c 0.805, CH_2Cl_2). Anal. Calcd for $C_{19}H_{20}O_3N_4$: C, 64.75; H, 5.72. Found: C, 64.90; H, 5.72.

[9*S*-[9*R,12*R**(*S**),15*R**]]-Phenylmethyl 12-(2-Amino-2-oxoethyl)-9-azido-4-methoxy-10,13-dioxo-2-oxa-11,14-diazatricyclo-[15.2.2.1]docosa-3,5,7(22),17,19,20-hexaene-15-carboxylate (13).** To a precooled (0 °C) solution of 82 mg (0.117 mmol) of **11** in 5 mL of freshly distilled THF was added 39 μL (64.6 mg, 0.351 mmol, 3.0 equiv) of pentafluorophenol followed by 38 mg (0.176 mmol, 1.5 equiv) of dicyclohexylcarbodiimide. The colorless solution was stirred at 0 °C for 2 h and then at ambient temperature for 12 h. The resulting slurry was filtered through glass wool and concentrated in vacuo to give a pale yellow oil containing the active ester **12**. The oil was diluted with 8 mL of CH_2Cl_2, and after cooling the solution to 0 °C, thioanisole (274 μL, 291 mg, 2.34 mmol, 2.0 equiv) and then 6 mL of trifluoroacetic acid were added. After stirring 1 h at 0 °C the solution was concentrated in vacuo to give the unpurified amine salt as a pale yellow oil. The amine salt was immediately diluted with 10 mL of dioxane and added by syringe pump over 3.5 h to a 5:1 dioxane/pyridine solution maintained at 90 °C and under nitrogen atmosphere. After the addition was complete, the reaction solution was stirred at 90 °C for 1 h. The solution was then cooled to ambient temperature and concentrated in vacuo to give a pale yellow solid. Purification by flash chromatography (3 cm × 25 cm silica gel, 4:96 methanol/CH_2Cl_2; compound loaded onto column preadsorbed on silica gel) gave greater than 95% pure material which after recrystallization with 4:1 acetonitrile/H_2O gave 38 mg of pure title compound as fine white needles. The mother liquors were concentrated in vacuo, and the residue was purified by chromatography (2 cm × 10 cm silica gel, 4:96 methanol/CH_2Cl_2) and recrystallization to give 12 mg of pure title compound. The combined yield was 71%: mp 246–247 °C; R_f 0.19 (5:95 methanol/CH_2Cl_2); IR (Nujol) 3435, 3392 (w), 3305, 2580 (w), 2480 (w), 2417 (w), 2100, 1750, 1645, 1617, 1513, 1462, 1380 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 8.44 (d, J = 9.9 Hz, 1 H, N-*H*), 8.07 (d, J = 8.7 Hz, 1 H, N-*H*), 7.41–7.32 (m, 7 H, aromatic Hs overlapping with N-*H*), 7.22 (dd, J = 1.9, 8.2 Hz, 1 H, aromatic H), 7.16 (s, 1 H, N-*H*), 6.95–6.90 (m, 2 H, aromatic Hs), 6.79 (dd, J = 1.9, 8.3 Hz, 1 H, aromatic H), 6.74 (dd, J = 2.5, 8.3 Hz, 1 H, aromatic H), 5.90 (d, J = 1.8 Hz, 1 H, C_{5a}^2-*H*), 5.21 (s, 2 H, CH_2Ph), 4.67–4.60 (m, 2 H, C_2^1-*H* overlapping with C_2^{Asn}-*H*), 3.91 (dd, J = 2.1, 7.0 Hz, 1 H, C_2^2-*H*), 3.79 (s, 3 H, OCH_3), 3.25 (dd, J = 3.4, 12.8 Hz, 1 H, C_3^1-*H*H), 2.91 (dd, m, J = 7.0, 14.2 Hz, 1 H, C_3^2-*H*H), 2.78 (unresolved dd, J at 12.6 Hz, 1 H, C_3^2-*H*H), 2.65 (t, J = 12.6 Hz, 1 H, C_3^1-*H*H), 2.35–2.30 (m, 2 H, C_3^{Asn}-H_2); $[\alpha]_{Hg\ 365}$ −171° (c 0.29, DMF); MS (FAB, *m*-nitrobenzyl alcohol) m/z 587 (M + H). Anal. Calcd for $C_{30}H_{30}O_7N_6$: C, 61.43; H, 5.16. Found: C, 61.15; H, 5.24.

OF4949-III. To a colorless solution of 26.9 mg (0.046 mmol) of **13** in 15 mL of 2:1 acetic acid/distilled water was added 60 mg of palladium black. The reaction flask was flushed with nitrogen and then hydrogen and stirred under a hydrogen atmosphere overnight. The reaction mixture was filtered through a plug of glass wool and concentrated in vacuo. The resulting white solid was lyophilized with 4 × 10 mL of distilled water in order to remove residual acetic acid thus yielding 21 mg (97%) of OF4949-III as a white powdery solid. By HPLC analysis synthetic OF4949-III coeluted with natural OF4949-III and was greater than 98% diastereomerically pure [2.5 mm × 12.5 cm Whatman 5μ partisil ODS-3 column, 20:80 methanol/3% aqueous acetic acid, 1.5 mL/min, 275 nm, t_r 4.88 min]: mp 219–225 °C dec (natural OF4949-III 217–225 °C dec); R_f 0.42 (2:1 *n*-propanol/ammonium hydroxide); IR (KBr) 3600–2500 (br), 3400, 1670, 1590 (br), 1510, 1400, 1265, 1235, 1131 cm^{-1}; ^1H NMR (500 MHz, D_2O, chemical shifts sensitive to concentration/trace amounts of acetic acid) δ 7.34 (dd, 1 H, J = 1.7, 8.3 Hz, aromatic *H*), 7.11 (dd, 1 H, J = 1.7, 8.3 Hz, aromatic *H*), 6.94 (d, 2 H, J = 8.2 Hz, aromatic *Hs*), 6.79 (dd, 1 H, J = 2.4, 8.3 Hz, aromatic *H*), 6.72 (d, 1 H, J = 8.1 Hz, aromatic *H*), 5.74 (s, 1 H, C_{5a}^5-*H*), 4.69 (dd, 1 H, J = 3.9, 10.0 Hz, C_2^{Asn}-*H*), 4.31 (dd, 1 H, 3.4, 12.4 Hz, C_2^1-*H*), 4.03 (d, 1 H, J = 3.9 Hz, C_2^2-*H*), 3.79 (s, 3 H, OCH_3), 3.26 (dd, 1 H, J = 3.3, 13.2 Hz, C_3^1-*H*H), 2.99 (d, 1 H, J = 14.9 Hz, C_3^2-*H*H), 2.91 (dd, 1 H, J = 5.8, 15.0 Hz, C_3^2-*H*H), 2.72 (dd, 1 H, J = 3.9, 15.4 Hz, C_3^{Asn}-*H*H), 2.51 (t, 1 H, J = 12.8 Hz, C_3^1-*H*H), 2.46 (dd, 1 H, J = 10.1, 15.3 Hz, C_3^{Asn}-*H*H); $[\alpha]^{30}_D$ −35° (c 1.14, 1 N aqueous HCl), [literature $[\alpha]^{27}_D$ −38.2° (c 1.06, 1 N aqueous HCl)]; MS (FAB, *m*-nitrobenzyl alcohol) m/z 493 (M + Na), 471 (M + H).

(*S*)-*O*-[5-(2-Azido-3-methoxy-3-oxopropyl)-2-methoxyphenyl]-*N*-[(1,1-dimethylethoxy)carbonyl]-L-tyrosine Phenylmethyl Ester (14). To a precooled (0 °C) solution of 528 mg (0.704 mmol) of **9** dissolved in 20 mL of THF was added slowly dropwise with stirring an aqueous lithium hydrogen peroxide solution [prepared from 3.70 mL (0.739 mmol, 1.05 equiv) of 0.20 M lithium hydroxide, 358 μL (398 mg, 3.52 mmol, 5.0 equiv) of 30% aqueous hydrogen peroxide, and 5 mL of distilled water]. After stirring the clear and colorless solution for 1 h at 0 °C, the reaction was quenched by dropwise addition of a solution of 732 mg (7.04 mmol, 10 equiv) of sodium hydrogen sulfite in 8 mL of distilled water. After stirring the resulting slurry for 15 min at 0 °C, the organic solvent was removed in vacuo. The remaining aqueous mixture was diluted with 1 N aqueous sodium bisulfate and extracted three times with CH_2Cl_2. The combined organic phase was dried over sodium sulfate and concentrated in vacuo to give the unpurified acid as a clear oil. The unpurified acid was diluted with 15 mL of ethyl ether and treated with excess diazomethane in ether. After 15 min, the excess diazomethane was removed by bubbling nitrogen through the solution (in the fume hood). The resulting colorless solution was concentrated in vacuo to give a clear oil. Purification by flash chromatography (3 cm × 25 cm silica gel, eluted with 30:70 ethyl acetate/hexane) gave 413 mg (97%) of the title compound as a clear viscous oil: R_f 0.24 (30:70 ethyl acetate/hexane); IR (CH_2Cl_2) 3420, 3110–3820, 2106, 1750 (b), 1717, 1610, 1508 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.28–7.39 (m, 5 H, aromatic Hs), 6.92–7.01 (m, 4 H, aromatic Hs), 6.77–6.82 (m, 3 H, aromatic Hs), 5.15 (d, J = 12.3 Hz, 1 H, OC*H*HPh), 5.13 (d, J = 12.3 Hz, 1 H, OC*H*HPh), 4.97 (d, J = 8.1 Hz, 1 H, N-*H*), 4.53–4.61 (m, 1 H, C_2^1-*H*), 4.01 (dd, J = 5.4, 8.4 Hz, 1 H, C_2^2-*H*), 3.80 (s, 3 H, CH_3), 3.72 (s, 3 H, CH_3), 2.97–3.09 (m, 3 H, C_3^1-H_2 overlapping C_3^2-*H*H), 2.91 (dd, J = 8.4, 14.1 Hz, 1 H, C_3^2-*H*H), 1.40 (s, 9 H, $OC(CH_3)_3$); $[\alpha]_{Hg\ 365}$ −58.5° (c 0.800, CH_2Cl_2). Anal. Calcd for $C_{32}H_{36}N_4O_8$: C, 63.56; H, 6.00. Found: C, 63.41; H, 6.03.

(*S*)-3-[4-[2-Carboxy-2-[[(1,1-dimethylethoxy)carbonyl]amino]ethyl]-phenoxy]-*O*-methyl-*N*-[*O*-methyl-*N*-[(phenylmethoxy)carbonyl]-L-tyrosyl]-L-tyrosine α-Methyl Ester (15). A solution of 246 mg (4.08 mmol) of **14** dissolved in 3 mL of ethyl ether was diluted with 12 mL of 8:1 methanol/acetic acid. Ten percent Pd/C (180 mg) was added to the solution, and the resulting reaction slurry was stirred under a hydrogen atmosphere for 0.5 h. The reaction slurry was then filtered through Celite, and the Celite filter cake was washed with several portions of methanol. In order to remove the acetic acid without resulting in the loss of the Boc protecting group, the combined filtrate was concentrated in vacuo to 30 mL followed by azeotropic removal of the acetic acid in vacuo (1 mm) and at <10 °C with 20 mL of heptane. Any residual acetic acid was removed by diluting with heptane and concentrating in

vacuo (1 mm) to give the amino acid as a white solid. The amino acid was dissolved in 8 mL of dioxane, and 86 mg (1.02 mmol, 2.5 equiv) of sodium bicarbonate dissolved in 1.5 mL of water followed by 410 mg (0.816 mmol, 2.0 equiv) of the pentafluorophenyl ester of 4-*O*-methyltyrosine dissolved in 2 mL of dioxane were added slowly dropwise with stirring. After the resulting clear and colorless solution was stirred 3 h at ambient temperature, the solution was partitioned between 75 mL of ethyl acetate and 50 mL of aqueous 1 N sodium bisulfate. The organic phase was collected, and the aqueous phase was extracted twice with 75 mL of ethyl acetate. The combined organic phase was dried over sodium sulfate and concentrated in vacuo to give a clear oil. Purification by flash chromatography (3 cm × 25 cm silica gel, eluted with 50:50:1 ethyl acetate/hexane/acetic acid followed by 100:2 ethyl acetate/acetic acid) gave 270 mg (83%) of the title compound as a white foam (The fractions containing product were diluted with heptane and concentrated in vacuo (1 mm) while maintaining the solution at less than 10 °C. Any residual acetic acid was removed by diluting with heptane and concentrating in vacuo.): R_f 0.28 (10:90 methanol/CH_2Cl_2); IR (CH_2Cl_2) 3690, 3410, 2820–3110, 1740 (s), 1715, 1680, 1610, 1510 cm^{-1}; ^1H NMR (300 MHz, methanol-d_4) δ 7.21–7.33 (m, 4 H, aromatic Hs), 7.06–7.15 (m, 5 H, aromatic Hs), 6.70–6.98 (m, 5 H, aromatic Hs), 5.02 (d, J = 12.5 Hz, 1 H, C*H*HPh), 4.97 (d, J = 12.5 Hz, C*H*HPh), 4.57–4.63 (m, 1 H, C_2-*H*), 4.20–4.41 (m, 2 H, C_2-*H* overlapping with C_2-*H*), 3.73 (s, 3 H, OCH_3), 3.69 (s, 3 H, OCH_3), 3.59 (s, 3 H, OCH_3), 2.70–3.15 (m, 6 H, C_3^2-H_2 overlapping with C_3^1-H_2 and C_3^3-H_2), 1.37 (s, 9 H, $OC(CH_3)_3$); $[\alpha]_{Hg\ 365}$ +27.6° (c 0.540, methanol). Anal. Calcd for $C_{43}H_{49}N_3O_{12}$: C, 64.57; H, 6.17. Found: C, 64.54; H, 6.15.

(*S*)-3-[4-[2-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-oxo-3-(pentafluorophenoxy)propyl]phenoxy]-*O*-methyl-*N*-[*O*-methyl-*N*-[(phenylmethoxy)carbonyl]-L-tyrosyl]-L-tyrosine α-Methyl Ester (16). To 201 mg (0.251 mmol) of **15** dissolved in 2 mL of freshly distilled THF was added 82 μL (139 mg, 0.754 mmol, 3.0 equiv) of pentafluorophenol. After cooling the solution to 0 °C, 58 μL (48 mg, 0.377 mmol, 1.5 equiv) of diisopropylcarbodiimide was added by syringe, and the reaction solution was stirred for 2 h at 0 °C and then for 15 h at ambient temperature. The resulting slurry was concentrated in vacuo, diluted with 50 mL of CH_2Cl_2, washed twice with 1 N aqueous potassium carbonate and once with brine, dried over sodium sulfate, and concentrated in vacuo to give an oily solid. The oily solid was filtered through silica gel (2 cm × 12 cm) with 48:52 ethyl acetate/hexane as eluent to give 226 mg (93%) of pure active ester **16**. The active ester **16** was used immediately in the subsequent reaction: mp 79–80 °C; R_f 0.40 (50:50 ethyl acetate/hexane); IR (CH_2Cl_2) 3420, 3110–2840, 1790, 1740, 1720, 1685, 1611 cm^{-1}; ^1H NMR (300 MHz, 2:1 methanol-d_4/$CDCl_3$) δ 6.77–7.71 (m, 16 H, aromatic Hs), 5.05 (d, J = 12.6 Hz, 1 H, C*H*HPh), 5.00 (d, J = 12.6 Hz, 1 H, C*H*HPh), 4.61–4.82 (m, 2 H, C_2^1-*H* overlapping C_2-*H*), 4.32 (dd, J = 6.1, 8.2 Hz, 1 H, C_2-*H*), 3.76 (s, 3 H, OCH_3), 3.75 (s, 3 H, OCH_3), 3.62 (s, 3 H, OCH_3), 3.22 (dd, J = 5.8, 14.0 Hz, 1 H, C_3^1-*H*H), 2.96–3.10 (m, 3 H, C_3^1-*H*H overlapping with C_3^2-*H*H and C_3^{Tyr}-*H*H), 2.90 (dd, J = 7.8, 14.0 Hz, 1 H, C_3-*H*H), 2.78 (dd, J = 8.6, 13.9 Hz, 1 H, C_3-*H*H), 1.42 (s, 9 H, $C(CH_3)_3$).

[9*S*-(9*R,12*R**,15*R**)]-Methyl 15-[[(1,1-Dimethylethoxy)carbonyl]amino]-4-methoxy-12-[(4-methoxyphenyl)methyl]-11,14-dioxo-2-oxa-10,13-diazatricyclo[15.2.2.1^{3,7}]docosa-3,5,7(22),17,19,20-hexaene-9-carboxylate (17).** Active ester **16** (226 mg, 0.234 mmol) dissolved in 10 mL of dioxane was added by syringe pump over 6 h to a vigorously stirred suspension (maintained at 90 °C) containing 250 mg of Pd(0), 26 μL (24 mg, 0.234 mmol, 1.0 equiv) of *N*-methylmorpholine, 3.6 mL of absolute ethanol, and 200 mL of freshly distilled dioxane. Hydrogen was bubbled continuously through the slurry via a gas dispersion tube, and the reaction apparatus was fitted with a reflux condenser to prevent loss of solvent. After the addition of the active ester was complete, hydrogen was bubbled through the slurry with vigorous stirring for an additional 1 h. The slurry was cooled under a nitrogen flush and then was filtered through Celite. The Celite was washed with three 50-mL portions of 1:1 CH_2Cl_2/methanol, and the combined filtrate was concentrated in vacuo to give a solid residue. Purification by flash chromatography (2 cm × 25 cm silica gel, eluted with 40:60:2 ethyl acetate/hexane/CH_2Cl_2 followed by 60:40:2 ethyl acetate/hexane/CH_2Cl_2) gave 101 mg (67%) of the title compound as a white crystalline solid: mp 245–246 °C; R_f 0.21 (50:50 ethyl acetate/hexane); IR (CH_2Cl_2) 3400, 3120–2840, 1745, 1712, 1670, 1611, 1585, 1515 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.25–7.30 (m, 1 H, aromatic Hs), 7.00–7.13 (m, 4 H, aromatic Hs), 6.72–6.81 (m, 4 H, aromatic Hs), 6.50 (dd, J = 2.0, 8.3 Hz, 1 H, C_{6a}^1-*H*), 6.19 (s (b), 1 H, N-*H*), 6.06 (d, J = 1.7 Hz, 1 H, C_{5a}^2-*H*), 5.47 (d, J = 5.0 Hz, 1 H, N-*H*), 5.28 (d, J = 8.1 Hz, 1 H, N-*H*), 4.31–4.36 (m, 1 H, C_2-*H*), 4.10–4.20 (m, 1 H, C_2-*H*), 3.85–3.95 (m, 1 H, C_2-*H*), 3.88 (s, 3 H, OCH_3), 3.74 (s, 3 H, OCH_3), 3.68 (s, 3 H, OCH_3), 3.10–3.31 (m, 3 H, C_3^1-*H*H overlapping with C_3^2-*H*H and C_3^{Tyr}-*H*H), 2.65–2.90 (m, 3 H, C_3^1-*H*H overlapping with C_3^2-*H*H and C_3^{Tyr}-*H*H), 1.49 (s (b), 9 H, $OC(CH_3)_3$); $[\alpha]_{Hg\ 365}$ +213.0° (c 0.355, CH_2Cl_2). Anal. Calcd for $C_{32}H_{35}N_3O_8$: C, 65.18; H, 5.98. Found: C, 64.90; H, 6.26.

[9*S*-(9*R,12*R**,15*R**)]-Methyl 15-[Acetylamino]-4-methoxy-12-[(4-methoxyphenyl)methyl]-11,14-dioxo-2-oxa-10,13-diazatricyclo-[15.2.2.1^{3,7}]docosa-3,5,7(22),17,19,20-hexaene-9-carboxylate (18).** To 149 mg (0.230 mmol) of **17** dissolved in 12 mL of CH_2Cl_2 was added 810 μL (857 mg, 6.90 mmol, 30 equiv) of thioanisole. The solution was cooled to 0 °C, and 6 mL of trifluoroacetic acid was added slowly dropwise with stirring. The resulting solution was stirred 15 min at 0 °C and warmed to ambient temperature over 1.5 h. The solution was concentrated in vacuo, and the residual trifluoroacetic acid was removed by diluting with 20 mL of toluene and concentrating in vacuo. The resulting amine salt was dissolved in 20 mL of 5:1 CH_2Cl_2/pyridine, and 2.0 mL of acetic anhydride was added slowly dropwise with stirring. After stirring at ambient temperature for 1 h, the solution was concentrated in vacuo to give an oily, white solid. Purification by flash chromatography (2 cm × 25 cm silica gel, 5:95 methanol/CH_2Cl_2) followed by rechromatography of the fractions which were contaminated with thioanisole (2 cm × 25 cm silica gel, eluted with 2:98 methanol/CH_2Cl_2 followed by 95:5 methanol/CH_2Cl_2) gave 131 mg (97%) of pure title compound as a white solid: mp >300 °C; R_f 0.21 (5:95 methanol/CH_2Cl_2); IR (CH_2Cl_2) 3350, 3295, 3110–2880, 2838, 1740, 1665, 1637, 1586, 1515 cm^{-1}; ^1H NMR ($CDCl_3$/methanol-d_4, 300 MHz) δ 7.30 (dd, J = 2.1, 8.3 Hz, 1 H, C_{5b}^1-*H*), 7.09 (dd, J = 2.1, 8.3 Hz, 1-H, C_{6b}^1-*H*), 7.03 (d, J = 8.6 Hz, 2 H, C_5^3-H_2), 7.02 (dd, J = 2.1, 8.3 Hz, 1 H, C_{5a}^1-*H*), 6.89 (d, J = 8.4 Hz, 1 H, C_{6a}^2-*H*), 6.75 (d, J = 8.6 Hz, 2 H, C_6^3-H_2), 6.74 (dd, J = 2.1, 8.4 Hz, 1 H, C_{5a}^2-H), 6.70 (dd, J = 2.1, 8.3 Hz, 1 H, C_{6a}^1-H), 6.28 (dd, J = 2.0 Hz, 1 H, C_{5a}^2-*H*), 4.51 (dd, J = 3.3, 7.5 Hz, 1 H, C_2^2-*H*), 4.44 (dd, J = 5.4, 11.9 Hz, 1 H, C_2^1-*H*), 4.08 (dd, J = 5.1, 7.5 Hz, 1 H, C_2^{Tyr}-*H*), 3.90 (s, 3 H, OCH_3), 3.75 (s, 3 H, OCH_3), 3.72 (s, 3 H, OCH_3), 3.19 (dd, J = 3.3, 15.4 Hz, 1 H, C_3^2-*H*H), 3.05 (dd, J = 5.3, 12.3 Hz, 1 H, C_3^1-*H*H), 2.97 (dd, J = 8.3, 13.9 Hz, C_3^{Tyr}-*H*H), 2.94–2.77 (m, 3 H, C_3^1-*H*H overlapping with C_3^2-*H*H and C_3^{Tyr}-*H*H), 2.06 (s, 3 H, $COCH_3$); $[\alpha]_D$ −11° (c 0.09, DMF): lit. $[\alpha]_D$ −20° (c 0.10, DMF). $[\alpha]_D$ for $C_{32}H_{35}N_3O_8$: C, 65.18; H, 5.98. Found: C, 64.98; H, 5.85.

K-13. A slurry of 58.4 mg (0.099 mmol) of **18** dissolved in 11 mL of distilled CH_2Cl_2 was added via cannula to a solution of 2.97 mL (2.97 mmol, 30 equiv) of 1.0 M aluminum tribromide in dibromoethane in 4 mL of distilled ethanethiol. The resulting clear orange solution was stirred at ambient temperature for 10 h (a white solid began to precipitate out of solution after several minutes). The reaction was quenched by pouring the orange slurry into H_2O precooled to 0 °C. After 10 min methanol was added to the mixture until it became homogeneous. The solution was concentrated in vacuo (in a well ventilated fume hood) to give an orange residue. Purification by chromatography (3 cm × 25 cm silica gel, eluted with 85:7:5 CH_2Cl_2/methanol/acetic acid, loaded on column preadsorbed to silica) followed by rechromatography (3 cm × 25 cm silica gel, eluted with 93:7 ethyl acetate/acetic acid) gave 51 mg (92%) of the title compound as a white solid. By HPLC analysis synthetic K-13 was greater than 98% diastereomerically pure and coeluted with natural K-13 (2.5 mm × 12.5 cm Whatman 5 μ Partisil C-8 column, 30:70 methanol/0.2% aqueous trifluoroacetic acid, 270 nm, 2 mL/min, t_r = 4.88 min); mp 260–270 °C dec (lit. 265–270 °C dec);[9b] R_f 0.33 (80:15:5 CH_2Cl_2/methanol/acetic acid); IR (KBr) 3650–2350 (br), 33408 1730, 1670–1590 (br), 1520, 1445, 1220, 1140 cm^{-1}; ^1H NMR (300 MHz, methanol-d_4) δ 7.29 (dd, 1 H, J = 2.1, 8.3 Hz, C_{5b}^1-*H*), 7.05 (dd, 1 H, J = 2.5, 8.2 Hz, C_{6b}^1-*H*), 6.98 (dd, 1 H, J = 2.0, 8.4 Hz, C_{5a}^1-*H*), 6.95 (d, 2 H, J = 8.4 Hz, C_5^{Tyr}-H_2), 6.75 (d, 1 H, J = 8.2 Hz, C_{6b}^2-*H*), 6.71 (dd, 1 H, J = 1.5, 8.3 Hz, C_{5b}^2-*H*), 6.66 (dd, 1 H, J = 2.5, 8.4 Hz, C_{6a}^1-*H*), 6.58 (d, 2 H, J = 8.4 Hz, C_6^{Tyr}-H_2), 6.34 (d, 1 H, J = 1.5, C_{5a}^2-*H*), 4.42 (dd, 1 H, J = 5.3, 11.8 Hz, C_2^1-*H*), 4.23 (dd, 1 H, J = 3.0–3.3, 7.8 Hz, C_2^2-*H*), 4.12 (dd, 1 H, J = 5.3, 6.0 Hz, C_2^{Tyr}-*H*), 3.15 (dd, 1 H, J = 3.1, 15.3 Hz, C_{3a}^2-*H*H), 3.01 (dd, 1 H, J = 5.3, 12.3 Hz, C_3^1-*H*H), 2.84–2.96 (m, 2 H, C_3^1-*H*H overlapping with C_3^2-*H*H), 2.79 (t, 2 H, J = 12.2 Hz, C_3^{Tyr}-H_2); ^{13}C NMR (75.5 MHz, methanol-d_4) δ 177.8, 172.8, 172.2, 171.0, 158.1, 157.0, 147.1, 133.0, 132.1, 132.0, 131.5, 131.1, 128.4, 125.6, 122.4, 121.2, 119.2, 117.3, 116.0, 57.5, 56.5, 56.3, 39.1, 38.8, 37.2, 22.4; $[\alpha]_D$ −6.5° (c 0.46, methanol) (natural $[\alpha]_D$ −3.4° (c 0.6, methanol)); MS (FAB, *m*-nitrobenzyl alcohol) m/z 570 (M + Na), 548 (M + H).

Acknowledgment. Support has been provided by the National Institutes of Health, Eli Lilly, and Merck. The NIH BRS Shared Instrumentation Grant Program 1S10 RR01748-01A1 is also acknowledged for providing NMR facilities. We thank Dr. Susumo Sano (Takara Shuzo Co.) for a sample of OF4949-III and Dr. Hiroshi Kase (Tokyo Research Laboratories) for a sample of K-13.