Formation of the 7-Oxa-1,4,10-triazatricyclo[8.2.2^{5,12}]tetradecane-2,14-dione Ring System: Misrouted Synthesis of a Peptidomimetic

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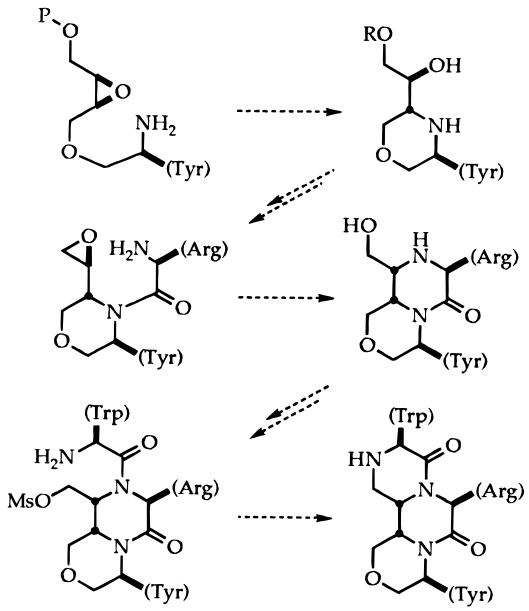
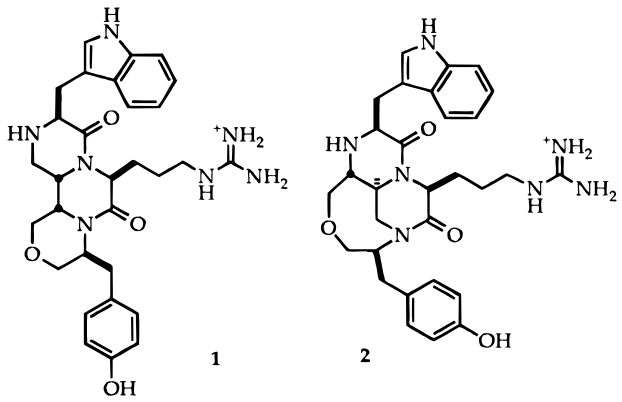
An attempted synthesis of the tricyclic peptidomimetic **1**, designed to imitate a β-turn tripeptide in tendamistat, afforded instead the 6,6,8-ring system of **2**. The key step in the synthesis entailed acylation of the hindered α,α'-disubstituted morpholine **4.2**, which was approached by acylative ring opening of the 3,6-oxazabicyclo[4.2.0]octane **4.3**. However, transannular rather than exocyclic cleavage occurred, giving the 1,6-oxazacyclooctane isomer **4.5**. Subsequent ring closures to form the bi- and tricyclic intermediates **7.3** and **8.5** were difficult because of the strain being built into the ring systems. After completion of the synthesis, the structures of the intermediates and final product were elucidated by NMR, with three-bond, heteronuclear multiple-bond correlation experiments providing unambiguous evidence for the ring connectivity, and by molecular modeling, which allowed assignment of the stereochemistry. Compound **2** is a modest inhibitor of the target enzyme α-amylase ($K_i = 170\ \mu$M in 5% DMSO/water), binding with similar affinity to the tripeptide Ac-Trp-Arg-Tyr-OMe. Although the side-chain attachment points in the ring system of **2** correspond closely to the relative Cα-positions in tendamistat (rmsd = 0.24 Å), the alignment of the Cα−Cβ bonds is poor, illustrating the importance of side-chain *orientation* in a peptidomimetic.

The tricyclic peptidomimetic **1** was designed as a mimic of the α-amylase inhibitor tendamistat.[1] The 6,6,6-ring system can be readily identified in CAVEAT[2] searches as a rigid template able to hold the side chains in the same orientation observed around the β-turn at positions 18−20 in tendamistat.[3,4] This Trp^{18}Arg^{19}Tyr^{20} loop is central to the interaction between the two proteins,[5] and mimics incorporating this sequence are effective inhibitors of α-amylase.[6,7] A synthesis of this compound was planned with the key ring closures as shown in Scheme 1. Execution of this route proved to be a significant challenge and, indeed, went awry at a key step, leading to a final product with the isomeric 6,6,8-ring system of **2**. In this report, we detail the chemistry involved and proof of structure of the compound produced.

Tricycle **1** incorporates the Trp-Arg-Tyr tripeptide unit as well as a four-carbon backbone. To our knowledge, no compound containing the tricyclic ring system represented by **1** has been reported, although several strategies can be envisaged for its assembly. We considered two primary routes: the "bottom-up" sequence depicted in Scheme 1 and a "top-down" approach for stitching the four-carbon backbone directly to a tripeptidyl unit. We regarded the tryptophan indole as the most sensitive functionality in the molecule and therefore wanted to introduce it toward the end of the synthesis. This consideration, as well as concern that the "top-down" sequence could result in O- as opposed to N-alkylation in the amide cyclization steps, led us to pursue the "bottom-up" route. The side-chain configurations on the

Scheme 1





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(1) Vertesy, L.; Oeding, V.; Bender, R.; Zepf, R. H.; Nesemann, G. *Eur. J. Biochem.* **1984**, *141*, 505.
(2) Lauri, G.; Bartlett, P. A. *J. Comput.-Aided Mol. Des.* **1994**, *8*, 51.
(3) Pflugrath, J. W.; Wiegand, G.; Huber, R. *J. Mol. Biol.* **1986**, *189*, 383.
(4) Kline, A. D.; Braun, W.; Wüthrich, K. J. *J. Mol. Biol.* **1988**, *204*, 675.
(5) Wiegand, G.; Epp, O.; Huber, R. *J. Mol. Biol.* **1995**, *247*, 99.
(6) Etzkorn, F. A.; Guo, T.; Lipton, M. A.; Goldberg, S. D.; Bartlett, P. A. *J. Am. Chem. Soc.* **1994**, *116*, 10412.
(7) Matter, H.; Kessler, H. *J. Am. Chem. Soc.* **1995**, *117*, 3347.

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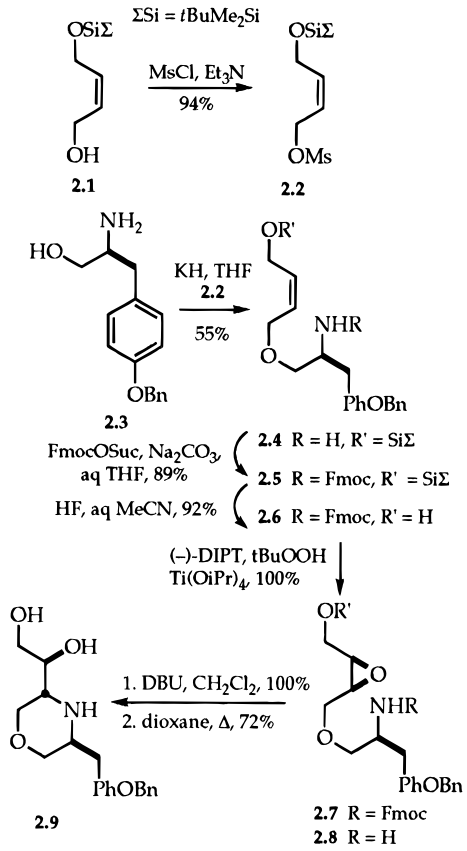
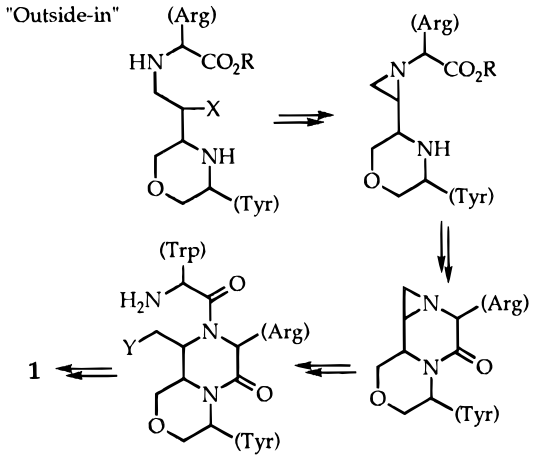
Scheme 2



tricyclic system would originate with the L-amino acid starting materials, and asymmetric epoxidation of an allylic alcohol intermediate[8] was to be used to control the stereochemistry of the backbone itself. Accordingly, our first goal was to assemble the allylic ether from *cis*-2-butene-1,4-diol and tyrosinol.

Synthesis and Attempted Acylation of Morpholine 2.9 (Scheme 2). The mono-*tert*-butyldimethylsilyl ether of *cis*-2-butene-1,4-diol, **2.1**, prepared according to McDougal's procedure,[9] was converted to the mesylate **2.2** and coupled with O-benzyl-L-tyrosinol, **2.3**,[10,11] generated by reduction of the corresponding methyl ester.[12] The use of sodium hydride or potassium *tert*- butoxide as base led to mixtures of O- and N-alkylated material; however, potassium hydride afforded the desired ether **2.4** as the exclusive isomer, albeit in 55% isolated yield on a preparative scale. We undertook a number of experiments designed to effect cyclization of the morpholine ring from *N*-acylamino epoxides. The substrates were prepared from **2.4** by N-acylation, O-deprotection, Sharpless epoxidation[8,13] of the allylic alcohol, and, finally, silylation or mesylation of the hydroxyl group. Unfortunately, attempts to form the morpholine ring from such substrates under a variety of basic conditions failed. We presume that steric hindrance prevents cyclization to the desired product, in which severe $A^{1,3}$-strain cannot be avoided. However, cyclization of the

Scheme 3



amino epoxide **2.8** was successful; this material was prepared by the sequence depicted in Scheme 2. The amino epoxide **2.8** could be isolated and purified; it cyclized slowly at room temperature but was converted cleanly to the morpholine **2.9** in refluxing dioxane.

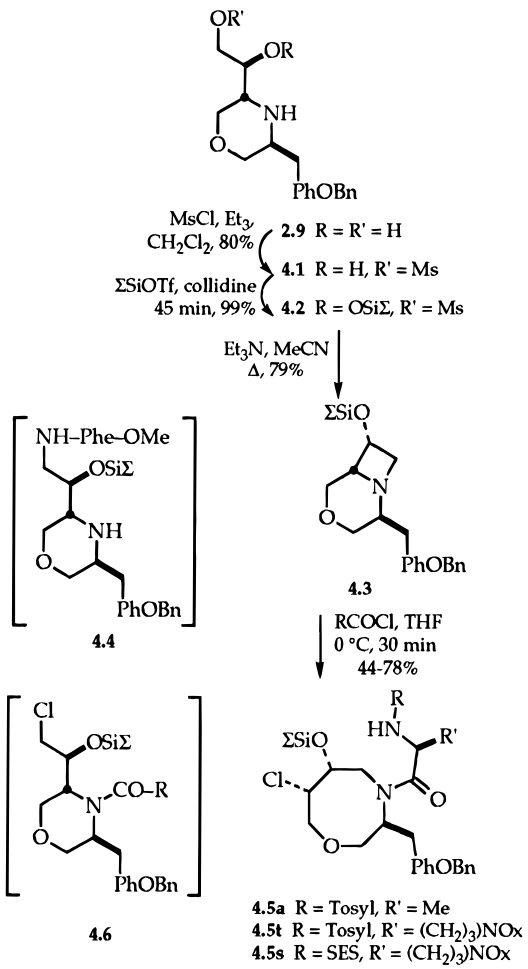
Further evidence of steric encumbrance around the nitrogen of the *trans*-substituted morpholine was its resistance to acylation even under forcing conditions. For example, conventional peptide coupling reagents had no effect on di-O-protected derivatives of **2.9**, and trifluoroacetic anhydride was required even for formation of the trifluoroacetamide. Despairing of carrying the morpholine on, we explored alternative strategies for assembly of the tricyclic system that could be characterized as "outside-in" sequences (Scheme 3). Ultimately, the acyl bond to the morpholine was to be formed by lactamization, after establishment of the backbone−nitrogen bond; that linkage would also be formed intramolecularly by walking it in from the 1° carbon via the aziridine.

Formation and Ring Opening of Azetidine 4.3 (Scheme 4). In one model study, attempted alkylation of phenylalanine methyl ester with the 1° mesylate **4.2** failed to give the desired 2° amine **4.4** but led instead to the 3-oxa-6-azabicyclo[4.2.0]octane **4.3**. When prepared intentionally, this material could be formed in 79% yield with triethylamine in refluxing acetonitrile. Although formation of azetidines by ring closure of linear precursors is usually difficult,[14] with alkyl substitution and especially with a monocyclic amine, high yields can be obtained, as exemplified by the synthesis of conidine from α-(2-chloroethyl)piperidine.[15] Although the bicyclic azetidine **4.3** did not lie on a planned route to the desired tricycle, its formation represented one of the few transformations in which a new bond could be formed to the morpholine nitrogen and it was a structure in which the reactivity of the nitrogen itself was significantly altered.

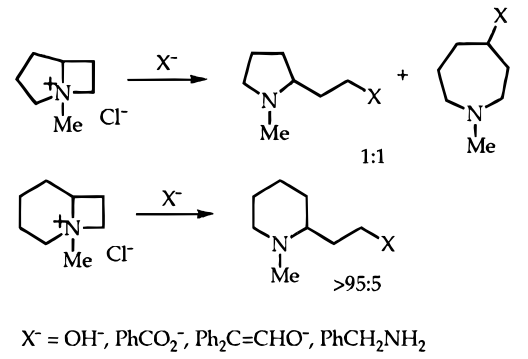
There is considerable precedent for the ring opening of strained, quaternary ammonium ions by nucleophilic attack, and significant regioselectivity is observed for unsymmetrical aziridinium and azetidinium ions.[14−20] Most relevant is a study of the cleavage pattern of the quaternized 1-azabicyclo[4.2.0]heptane and 1-azabicyclo-

(8) Hanson, R. M.; Sharpless, K. B. *J. Org. Chem.* **1986**, *51*, 1922.
(9) McDougal, P. G.; Rico, J. G.; Oh, Y.-I.; Condon, B. D. *J. Org. Chem.* **1986**, *51*, 3388.
(10) Itsuno, S.; Hirao, A.; Nakahama, N. *J. Chem. Soc., Perkin Trans. 1* **1983**, 1673.
(11) Rinaldi, P. L.; Wilk, M. *J. Org. Chem.* **1983**, *48*, 2141.
(12) Ramachadran, J.; Berger, A.; Katchalski, E. *Biopolymers* **1971**, *10*, 1829.
(13) Gao, Y.; Hanson, R. M.; Klunder, J. M.; Ko, S. Y.; Masamune, H.; Sharpless, K. B. *J. Am. Chem. Soc.* **1987**, *109*, 5765.
(14) Ballard, S. A.; Melstrom, D. S. In *Heterocyclic Compounds*; R. C. Elderfield, R. C.; Ed.; Wiley: New York, 1950; Vol. 1; p 78.
(15) Moore, J. A. In *The Chemistry of Heterocyclic Compounds*; Weissberger, A., Ed.; Wiley: New York, 1964; Vol. 19, Part 2, p 885.
(16) Tshiamala, K.; Vebrel, J.; Laude, B.; Mercier, R. *Bull. Soc. Chim. Fr.* **1990**, *127*, 584.
(17) DiVona, M. L.; Illuminati, G.; Lilloci, C. *J. Chem. Soc., Perkin Trans. 2* **1985**, 1943.
(18) Deyrup, J. A. In *The Chemistry of Heterocyclic Compounds*; Hassner, A., Ed.; Wiley: New York, 1983; Vol. 42, Part 1, p 1.

Scheme 4



Scheme 5



[3.2.0]octane systems in the presence of various nucleophiles: Ebnöther reported that the former affords comparable amounts of the isomeric azepine and pyrrolidine products, while in reaction of the latter the azetidine ring is cleaved exclusively at the primary carbon to give the piperidine (Scheme 5).[21] Less precedent exists for the reactivity of azetidines toward acylating agents; the one example we found was for an intramolecular system with no regiochemical issue.[22]

The premise of ring opening the strained azetidine **4.3** with concomitant acylation was tested with several reagents. Although acetic anhydride gave a complex mixture after formation of a highly polar intermediate, treatment of the bicyclic azetidine with acetyl chloride in methylene chloride at 0 °C led rapidly to a ring-cleaved and chlorine-substituted acetamide in 79% yield. Fmoc-alanine acid chloride[23] was not sufficiently stable under the reaction conditions, forming the Leuch's anhydride,[24] but the *N*-tosyl analog reacted with **4.3** to generate an acylated compound in 44% yield. This product was initially assigned the structure **4.6**.

Hindsight. The spectral differences to be expected for the isomeric cleavage products from azetidine **4.3** are subtle, since they have identical masses and identical ^{1}H NMR spin systems. Moreover, the presence of amide conformers further complicated an already congested NMR spectrum. From the precedent cited above, reinforced by our expectation that the mild cleavage conditions would favor S_N2-like attack at the primary carbon of the azetidine ring, we assigned the structure of the cleavage products as **4.6**, in preference to the eight-membered rings **4.5**. As detailed below, this assignment was incorrect.

We completed the synthesis of a tricyclic product, incorporating the tyrosine, arginine, and tryptophan side chains, with the belief that we were following the route depicted in Scheme 1. With hindsight, many of the difficulties we encountered in a number of the steps, in particular the ring-closure reactions, are understandable, but it was not until we had a fully elaborated tricyclic ring system, with no conformational ambiguities, that NOESY spectra suggested, and heteronuclear multiple-bond correlation (HMBC) experiments confirmed, that we had made the wrong assignment.

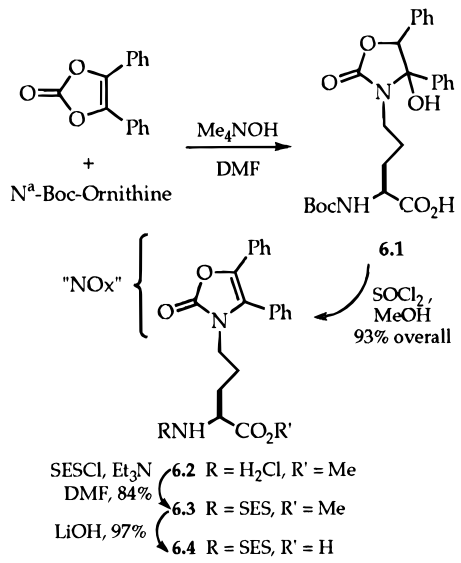
A Robust Protection Scheme for Ornithine. The requirement of an acid chloride for the acylative ring-opening step placed severe limitations on the manner in which the arginine residue could be introduced and on the choice of protecting groups. Rather than carry the guanidino function through the synthesis, we elected to incorporate a protected ornithine at this stage and introduce the guanidine at the end. The protecting group on the α-nitrogen had to be stable to acid and strong acylating conditions, tolerate the adjacent acid chloride without formation of Leuch's anhydride or the azlactone, and yet be readily removed. These requirements were satisfied by the recently described [2-(trimethylsilyl)-ethyl]sulfonyl (SES) group,[25] which can be fragmented with fluoride ion. The protection on the δ-nitrogen additionally had to survive strong base (for subsequent ring closures) and fluoride ion (for cleavage of the α-protecting group); moreover, it could not retain any NH moiety or cyclization on the acid chloride would occur. We chose the seldom used diphenyloxazolone (Ox) moiety, which can be removed under oxidative or reductive conditions.[26]

The protected ornithine **6.4** was prepared in 48% yield for four steps from the commercially available N^{α}-Boc derivative, as shown in Scheme 6.

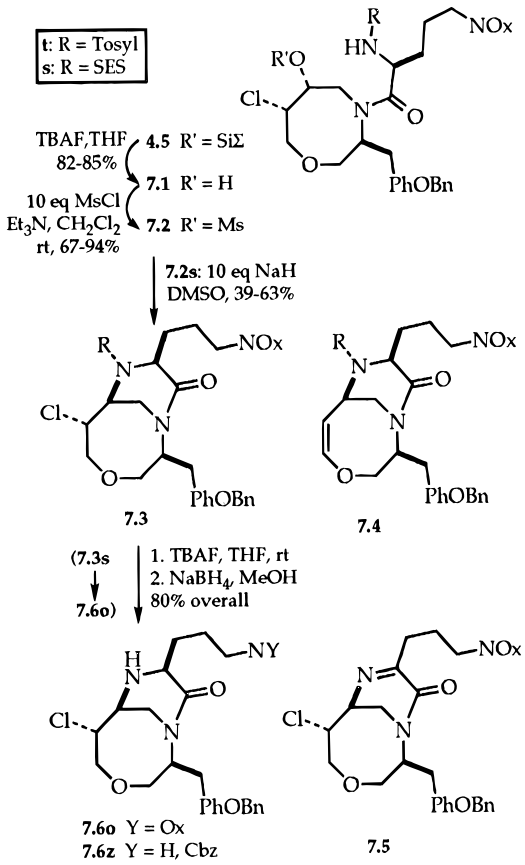
Azetidine Opening with the Protected Ornithine Acid Chloride. Initial experiments involving azetidine acylation and cyclization of the second ring were carried

(19) Crist, D. R.; Leonard, N. J. *Angew. Chem., Int. Ed. Engl.* **1969**, *81*, 962.
(20) Fruton, J. S. In *Heterocyclic Compounds*; Elderfield, R. C., Ed.; Wiley: New York, 1950; Vol. 1, p 61.
(21) Ebnöther, A.; Jucker, E. *Helv. Chim. Acta* **1964**, *47*, 745.
(22) Bartholomew, D.; Stocks, M. J. *Tetrahedron Lett.* **1991**, *32*, 4799.
(23) Bodanzsky, M.; Bodanzsky, A. *The Practice of Peptide Synthesis*, Vol. 21; Springer Verlag: Berlin, 1984.
(24) Bodanszky, M. *Principles of Peptide Synthesis*; Springer-Verlag: New York, 1984.
(25) Weinreb, S. M.; Demko, D. M.; Lessen, T. A. *Tetrahedron Lett.* **1986**, *27*, 2099.
(26) Sheehan, J. C.; Guzier, F. J., Jr. *J. Org. Chem.* **1973**, *38*, 3034.

Scheme 6



Scheme 7



out with the N^{α}-tosyl analog of **6.4**. When the acid chloride of this derivative was isolated and added to the azetidine **4.3**, low yields (ca. 10%) of acylated material were invariably obtained. However, if the acid chloride (3 equiv) was generated *in situ* with excess PCl_5 (8 equiv), and then combined with diisopropylethyl amine (DIEA) and the azetidine, ring-opened material **4.5t** could be isolated in 49% yield, with 46% recovery of starting azetidine.

Removal of the silyl ether with tetrabutylammonium fluoride (TBAF) afforded the chloro alcohol **7.1t** as a mixture of amide rotamers (Scheme 7). This material was not cyclized under Mitsunobu conditions, nor was the mesylate **7.2t** cyclized in the presence of DIEA in refluxing acetonitrile or dioxane. Ring closure of **7.2t** to the bicyclic lactam **7.3t** was effected in the more polar solvent DMSO at 110 °C with DIEA. Interestingly, sodium hydride in DMSO was ineffective in inducing the desired cyclization. In contrast to the two monocyclic precursors **7.1t** and **7.2t**, the product did not display a mixture of amide rotamers in the NMR spectrum, indicating that a rigid, bicyclic structure had formed.

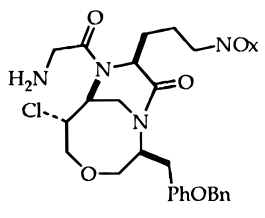
When the SES-protected analog **6.4** was employed in this sequence, 55−78% yields of the acylated product **4.5s** were obtained, and formation and cyclization of the corresponding mesylate **7.2s** proceeded in an overall yield of 40−60%. However, in contrast to the tosylamide, the SES derivative required more forcing conditions for cyclization: with DIEA as base, an 8-h period at 130 °C in DMSO was necessary, giving cyclic material **7.3s** in 38% yield. The best conditions we found for cyclization of **7.2s** employed sodium hydride in DMSO, although these strongly basic conditions also produced the eliminated products **7.4s** and **7.5**.

Completion of the Synthesis. Removal of the SES group from the bicyclic intermediate **7.3s** proceeded with 1 M TBAF in THF at room temperature, surprisingly mild conditions in comparison to those typically reported for this transformation.[25] We attribute this behavior, along with the difficulty encountered in the preceding cyclization step, to the steric crowding around the B-ring, which is alleviated as the N−S bond is broken.

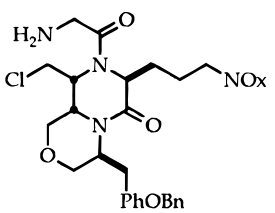
A significant amount of the eliminated byproduct **7.5** was also generated during removal of the SES group with TBAF. This material could be recycled to the desired product with sodium borohydride ($NaBH_4$), reduction occurring from the *exo* direction to restore the lost stereocenter. Thus, treatment of sulfonamide **7.3s** consecutively with TBAF and $NaBH_4$ afforded the amine **7.6o** in 80% yield. The imine **7.5** presumably results from β-elimination of 2-(trimethylsilyl)ethyl sulfinate as another consequence of the relatively weak N−S bond.

In spite of the apparent steric crowding of the B-ring nitrogen, the amine **7.6o** reacted readily with unhindered acylating agents. The glycyl derivatives were formed either from the acid chloride of *N*-tosylglycine or from bromoacetyl bromide followed by ammonia. However, neither of these derivatives could be induced to cyclize under a variety of basic conditions.[27] Consequently, our approach to introduction of the C-ring involved displacement of the halide first and ring closure to the lactam last. The steric congestion of the SES amide prevented substitution of the chloride, even under Finkelstein conditions, but displacement with iodide proceeded in quantitative yield with amine **7.6o**. However, attempted replacement of the iodide in turn with tryptophan methyl ester (Trp-OMe) in refluxing acetonitrile only gave aziridine **8.2**; iodide-induced epimerization is presumably

(27) While the resistance of **i** to cyclization can be understood in the context of the strained bicyclic skeleton, it was puzzling when we believed we were working with the fused ring system of **ii**. Moreover, in this system there would not have been any stereochemical consequences of the double displacement process for introduction of the tryptophan moiety (R-Cl → R-I → R-Trp-OMe).

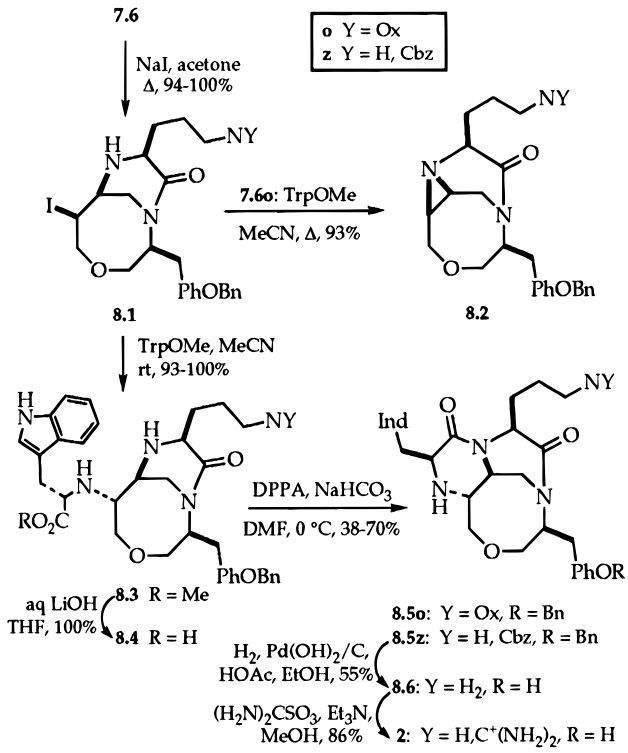


i



ii

Scheme 8

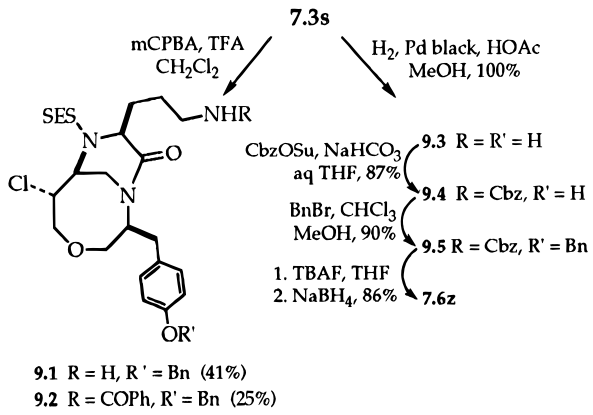


responsible for the double inversion required for this displacement.

The challenge at this point was to favor an intermolecular reaction (**8.1** → **8.3**) over an intramolecular reaction (**8.1** → **8.2**) (Scheme 8). Increased nucleophile concentration was one factor that we addressed, of course, but the most important proved to be temperature. We reasoned that the entropically favored *intra*molecular process would be *less* favored at lower temperature. Indeed, when iodide **8.1o** was treated with an excess of Trp-OMe in acetonitrile at room temperature, the alkylated product **8.3o** was produced *exclusively*! Because the tricyclic bislactam **8.5o** is quite strained, it is not surprising that cyclization of the amino ester **8.3o** does not occur simply on heating. However, the amino acid **8.4o** cyclizes in 70% yield with bromotris(pyrrolidino)phosphonium hexafluorophosphate (PyBrOP)[28,29] or diphenylphosphoryl azide (DPPA)[30,31] in DMF.

From tricycle **8.5o**, only deprotection steps remained, but they were not to be accomplished directly. In spite of a number of precedents for removal of the diphenyloxazolinone group (Ox) under mild conditions,[26,32−35] we were unable to accomplish this step in the presence of the indole moiety. Under hydrogenolytic conditions, the phenolic benzyl ether was cleaved first, and under the more forcing conditions required for removal of the Ox group, the indole was also attacked. Dissolving metal reduction of **8.5o** yielded material in which a lactam bond had been cleaved. Although the Ox group is susceptible to oxidative cleavage as well (e.g., MCPBA in TFA),[26] this approach was also precluded by the oxidative sensitivity of the indole moiety.

Scheme 9



The Ox group apparently had to be replaced prior to introduction of the tryptophan unit. From examination of the synthetic route, it was clear that exchange after installation of the B-ring was the most advantageous prospect: by this point, the most demanding reactions had been executed and subsequent transformations would tolerate a Boc or Cbz group. From intermediate **7.3s**, we initially examined oxidative processes in order to preserve the phenolic benzyl ether (Scheme 9). However, a variety of oxidative conditions provided the desired product **9.1** in less than 50% yield; the remainder of the material was recovered starting material and benzamide **9.2** from Baeyer−Villiger oxidation of one of the intermediates during breakdown of the Ox group.

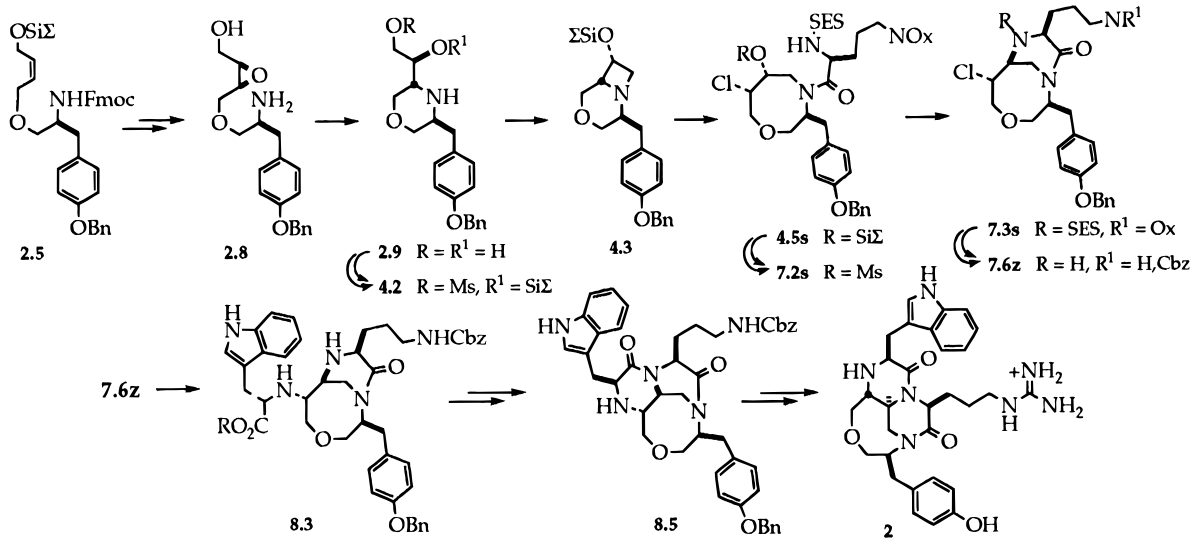
The reductive approach proved to be more successful. Hydrogenolysis of **7.3s** in acidic methanol over palladium black afforded phenolic amine **9.3** in quantitative yield. Protection of the amine with *N*-((benzyloxycarbonyl)oxy)-succinimide (CbzOSu) afforded the *N*-Cbz derivative in 87% yield after hydrolysis of the small amount of *O*-Cbz byproduct, and re-benzylation proceeded smoothly with benzyl bromide.[36] What this roundabout approach lacked in elegance it more than made up in efficiency, providing the Cbz derivative **9.5** in 78% overall yield from the Ox analog **7.3s**.

With the more felicitous Cbz group in place, the same steps were executed to introduce the C-ring as described for the Ox-protected series. The Trp-OMe adduct **8.3z** and subsequent intermediates proved to be fairly sensitive to acidic conditions, including halogenated solvents and silica chromatography, as indicated by the development of a red-orange color; this may be due to the propensity of the indole nucleus to dimerize in acid.[37] After conversion to the tricyclic product **8.5z**, removal of the protecting groups was accomplished by hydrogenolysis, and the guanidino moiety of the arginine side chain was introduced with aminoiminomethanesulfonic acid in the presence of triethylamine (Scheme 8).[38,39] Reverse phase HPLC then afforded the final compound **2**. The overall sequence is summarized in Scheme 10.

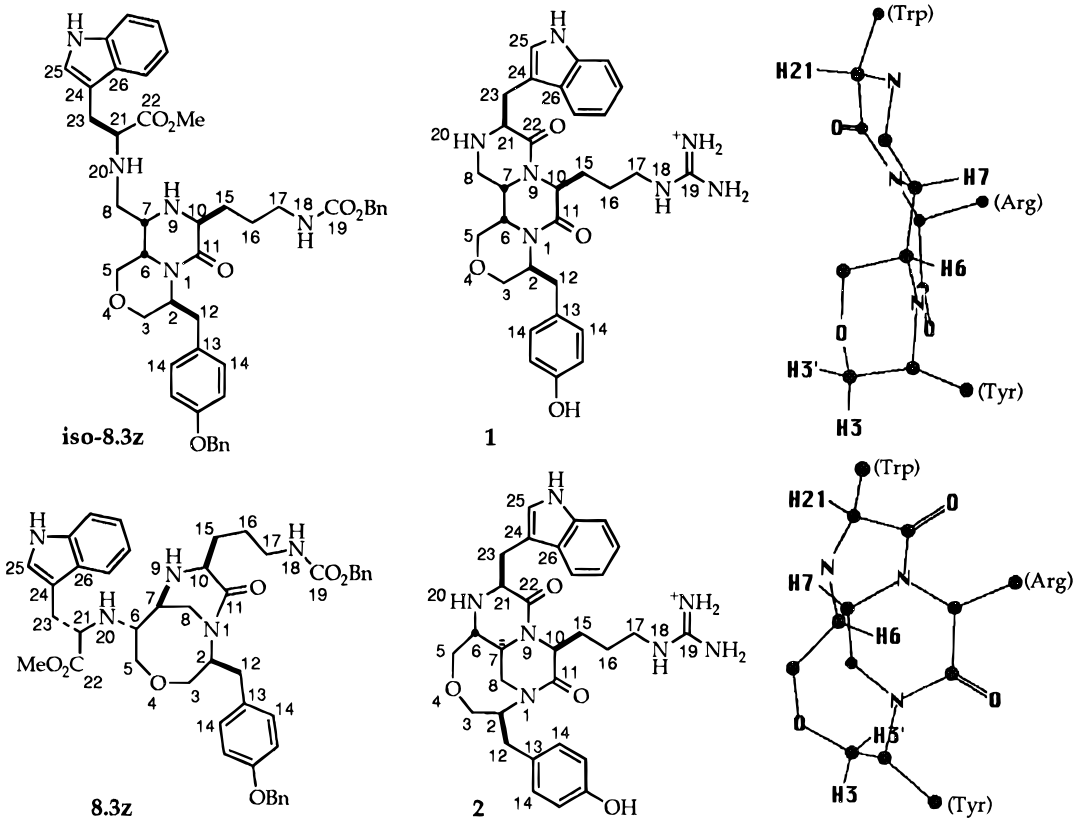
Assignment of Intermediate and Final Structures. Several observations led us to suspect that the

(28) Castro, B.; Dormoy, J. R. *Tetrahedron Lett.* **1973**, *35*, 3243.
(29) Coste, J.; Frerot, E.; Jouin, P. *Tetrahedron Lett.* **1991**, *32*, 1967.
(30) Brady, S. F.; Paleveda, W. J.; Arison, B. H.; Saperstein, R.; Brady, E. J.; Raynor, K.; Reisine, T.; Veber, D. F.; Freidinger, R. M. *Tetrahedron* **1993**, *49*, 3449.
(31) Laszlo, S. E.; Ley, S. V.; Porter, R. A. *J. Chem. Soc., Chem. Commun.* **1986**, 344.
(32) Pansare, S. V.; Vederas, J. C. *J. Org. Chem.* **1987**, *52*, 4804.
(33) Salituro, G. M.; Townsend, C. A. *J. Am. Chem. Soc.* **1990**, *112*, 760.
(34) Basak, A.; Salowe, S. P.; Townsend, C. A. *J. Am. Chem. Soc.* **1990**, *112*, 1654.
(35) Sreenivasan, U.; Mishra, R. K.; Johnson, R. L. *J. Med. Chem.* **1993**, *36*, 256.
(36) Schmidhammer, H.; Brossi, A. *J. Org. Chem.* **1983**, *48*, 1469.
(37) Houlihan, W. J., Ed. *Indoles Part I*, Vol. 25; Wiley: New York, 1972.
(38) Miller, A. E.; Bischoff, J. J. *Synthesis* **1986**, 777.
(39) Kim, K.; Lin, Y. T.; Mosher, H. S. *Tetrahedron Lett.* **1988**, *29*, 3183.

Scheme 10



Scheme 11



final product might not have the desired structure **1**. First, the difficulties we encountered in forming the B- and C-rings were not in accord with our expectations for the fused-ring structures. Second, the final material we produced proved to be only a modest inhibitor of α-amylase, comparable to the Trp-Arg-Tyr tripeptide. Most significantly, cross peaks expected in the ROESY spectrum of **1**, such as between H6 and H7, were missing, while unanticipated and strong cross peaks were observed between H3′ and H6 and between H7 and H21 (see Scheme 11 for numbering scheme). We therefore reexamined our structural assignments for key intermediates in the sequence. The immediate products from opening of the azetidine ring, amides **4.5**, gave broad peaks in the ^{1}H NMR spectrum that did not coalesce satisfactorily at higher temperature nor resolve at lower temperature. These observations are consistent with conformational interconversions that are slow on the NMR time scale, of which both **4.5** and **4.6** are capable. We therefore used the bicyclic intermediate **8.3z** and the final tricyclic product **2** for NMR studies.

The aromatic region of the spectra is not intrinsic to the ring systems and hence will not be discussed in relation to the structural elucidation. However, the data for the aromatic region are consistent with the assignments, confirming the presence and connectivity of one indole and three phenyl groups for **8.3z** and one indole and one phenyl group for **2**.

COSY and TOCSY spectra of the bicyclic molecule identified four spin systems consistent with either structure **iso-8.3** or **8.3** (Scheme 11): CH−CH_2−CH_2−CH_2−NH (10−15−16−17−18), CH−CH_2 (21−23), CH_2−CH−

Table 1. Proton−Carbon Long-Range Correlations for 8.3z

H	two bonds to carbon (H−X−*C*)	three bonds to carbon[a] (H−X−Y−*C*)
2	3, 12	**8**, **11**
3	2	**5**
5	6	**3**, 7
6	7	8, **21**
7		5
8	7	**2**, 6, **11**
10	11, 15	**7**, 16
12	2, 13	3, 14
15	10, 16	11, 17
16	15, 17	10
17	16	15, 19
21	22, 23	**6**, 24
23	21, 24	22, 25, 26

[a] Correlations which establish the ring system are in boldface type.

CH_2 (3−2−12), and CH_2−CH−CH−CH_2 (5−6−7−8 or 8−7−6−5). All protons were assigned, although the inherent ambiguity for the CH_2-CH-CH-CH_2 spin system could not be resolved until a two- and three-bond proton−carbon correlation experiment was carried out, as described below. The carbons were assigned from the multiplicity observed in DEPT experiments and from one-bond correlations with their respective hydrogens identified by an HSQC (heteronuclear single quantum coherence) experiment. A two- and three-bond proton−carbon correlation experiment was carried out to establish the connectivity of the spin systems rigorously. The HMBC (heteronuclear multiple-bond correlation) experiment was performed in such a way as to filter out one-bond proton−carbon correlations, which would have complicated data interpretation. The three-bond correlation data enabled the two possible assignments for the CH_2−CH−CH−CH_2 spin system to be distinguished, with a number of key correlations across the heteroatoms (Table 1) consistent only with structure **8.3z**.

In addition to the three-bond correlation data, the coupling constants of the ring protons and the NOEs observed are more in accord with structure **8.3z** than **iso-8.3z**. These data in turn allowed us to determine the stereochemistry of the bicyclic product and the course of the cyclization reaction (**7.6** → **8.1** → **8.3**), which involved iodide catalysis in displacement of the chloride. Two inversions from **7.6** would provide the *trans* isomer **8.3**, while multiple displacements with iodide in formation of **8.1** or in the next step, as postulated to explain formation of the aziridine **8.2**, could have resulted in the *cis* isomer **epi-8.3** (Scheme 12).

The two ring systems were modeled with methyl groups substituted for the side chains. The *cis* isomer **epi-8.3m** is lower in energy than the *trans* conformers, **8.3m**, and it is less flexible conformationally. Low-energy structures of the former are very similar, with the ether oxygen of the eight-membered ring exhibiting the greatest mobility. The *trans* isomer has two major low-energy conformational sets which differ in the dihedral angle between H6 and H7 and are designated as *trans-gauche* and *trans-anti* (Figure 1). The *trans-anti* conformations of **8.3m** are generally higher in energy than the *trans-gauche*, and both sets contain members with slightly different conformations of the eight-membered ring near the ether oxygen. The coupling constants and NOEs observed for **8.3** are most consistent with the geometry and interatomic distances predicted for the *trans-gauche* model of **8.3m** (Tables 2 and 3). The discrepancies in the data arise from the flexibility of the eight-membered ring about the ether oxygen and a minor population of the *trans-anti* conformer.

Scheme 12

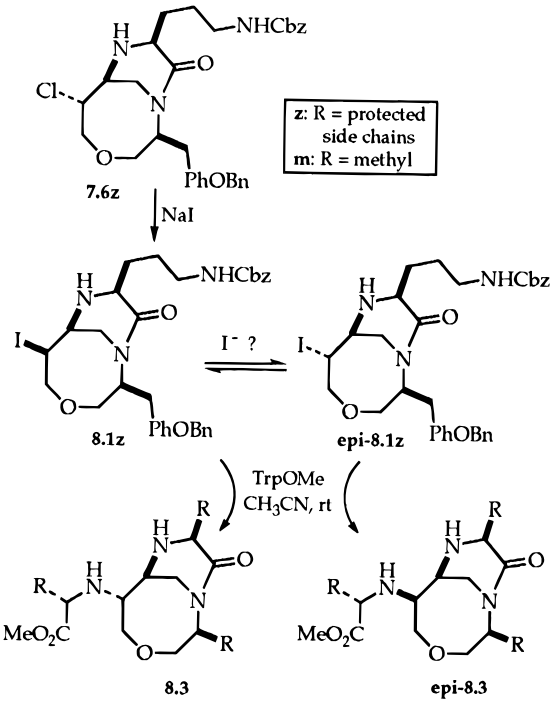


Table 2. Calculated and Observed Vicinal Coupling Constants for Bicycle 8.3z

spin system	obsd	calcd coupling constants (Hz)[a] *trans-gauche*	*trans-anti*	cis
2−3	3.5	**4.6**	3.5	4.7
2−3′	10.6	**11.2**	10.9	11.3
5′−6	<1.0	**0.9**	5.1	1.9
5−6	4.5	**4.4**	11.0	9.4
6−7	3.9	**2.6**	9.9	3.2
7−8′	5.0	**5.7**	3.8	5.3
7−8	1.4	**1.3**	2.1	1.3

[a] Calculated in Macromodel according to ref 55.

Table 3. Calculated Distances and NOEs Observed for Bicycle 8.3z

spin system	NOE obsd	calcd distance (Å) *trans-gauche*	*trans-anti*	*cis*
3−12	weak	**2.6**	2.6	2.6
3′−5′	obscured[a]	**2.2**	2.4	2.2
3−8	very weak	**4.0**[b]	3.9	4.0
2−8	obscured[a]	**2.3**	2.4	2.3
5−8	very weak	**2.3**	3.1	4.2
6−7	medium	**2.5**	3.1	2.4
6−21	medium	<**3.0**	<3.0	<3.0
7−21	medium	**2.2**	<4.0	<4.0
8′−10	medium	**2.3**	2.4	2.3

[a] The expected cross peaks are obscured by overlapping resonances. [b] The eight-membered ring containing H1 is conformationally mobile, so this NOE could be due to another conformation.

Determination of the stereochemistry and conformation of the tricyclic product **2** confirmed the above assignments in the bicyclic series. COSY and TOCSY spectra identified the four aliphatic spin systems in the tricyclic molecule and enabled assignment of all the protons. The two possible arrangements of the CH_2−CH−CH−CH_2 spin system were distinguished in this case by the distinctive coupling patterns of H5 and H8, as described below. 1D-decoupling experiments revealed an H6−H7 coupling of 9.5 Hz, reflecting either an *anti* relationship or an almost totally eclipsing *syn* arrange-

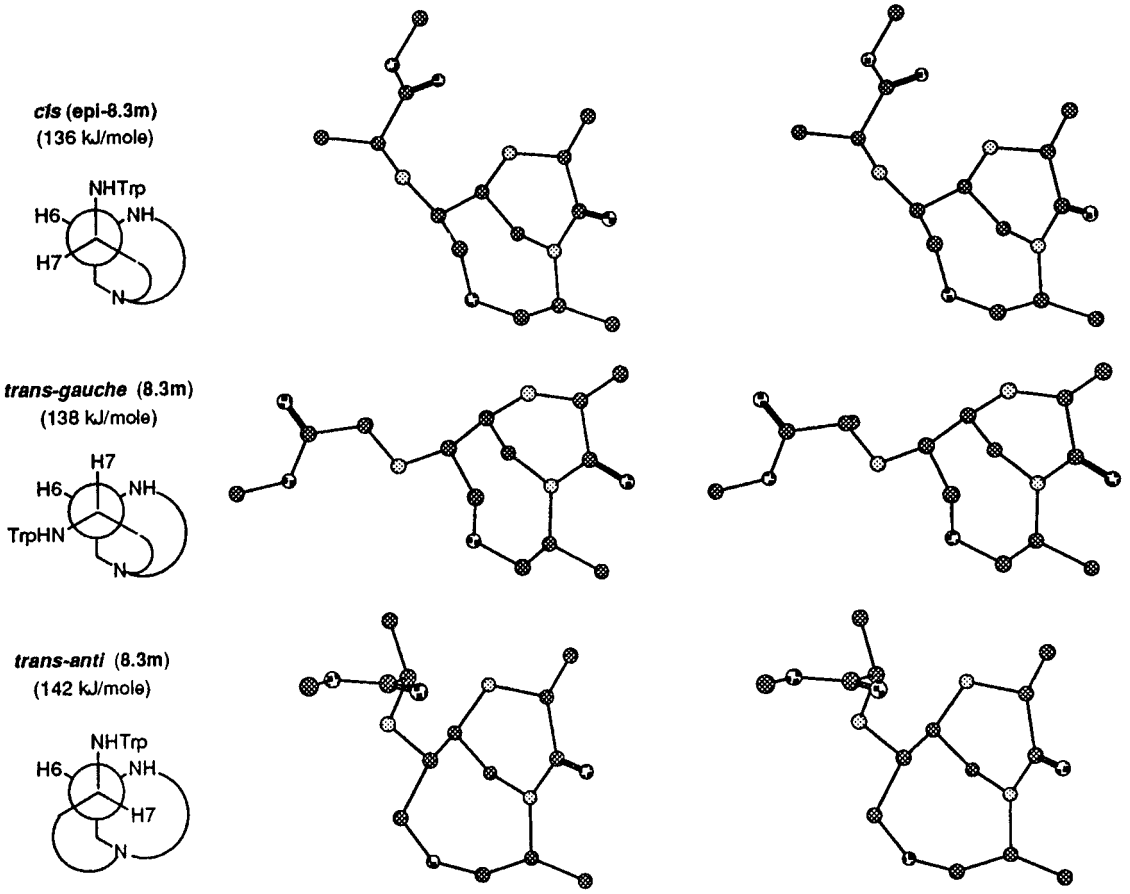


Figure 1. Minimum-energy conformations for the bicyclic models **epi-8.3m** and **8.3m** (energy values calculated in MacroModel using MM2* force field).

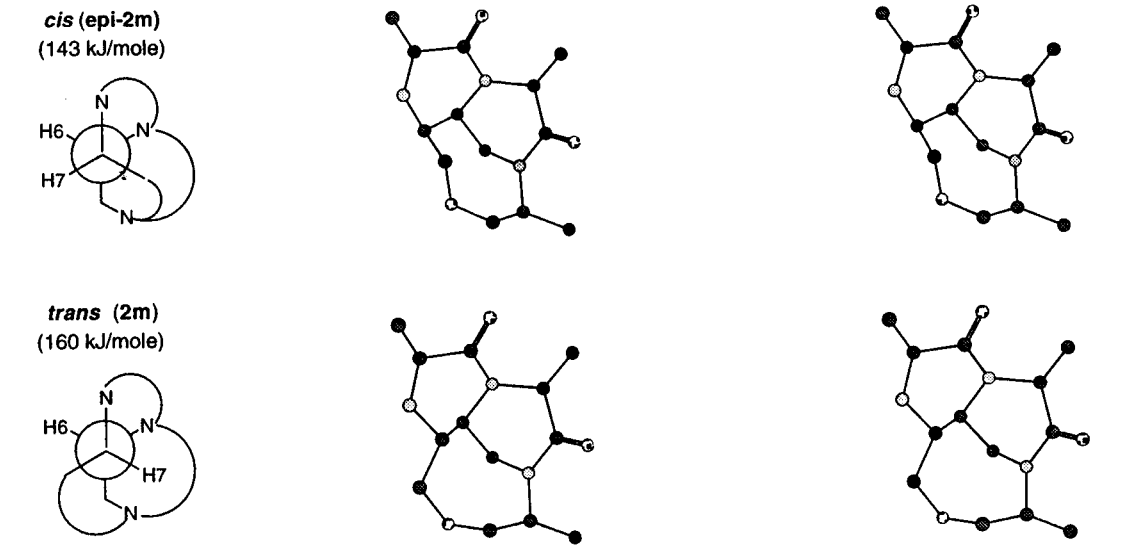


Figure 2. Minimum-energy conformations for the tricyclic models **epi-2m** and **2m** (energy values calculated in MacroModel using MM2* force field).

ment between these hydrogens. Because the size of **2** (MW = 530) prevented observation of NOE cross peaks, a ROESY spectrum was recorded to identify through-space relationships. The absence from this spectrum of a cross peak between H6-H7 argued for an *anti* arrangement, but the peaks that actually were observed provided more definitive evidence. The strongest ROESY cross peaks (aside from geminal relationships) were between H3′ and H6, between H7 and H21, and between H8′ and H10, which could not be reconciled with any conformation or stereoisomer of **1**, but are completely consistent with the structure of **2**.

Both the *cis* and *trans* stereoisomers of **2** were modeled with methyl groups substituted for the side chains (Figure 2). In this series as well, the *cis* isomers were found to be much lower in energy than the *trans* compounds; both tricycles have very little flexibility, with the only apparent mobility confined to the ether moiety. As above, calculations of the coupling constants and interatomic distances for the methyl side-chain analogs

Table 4. Calculated and Observed Vicinal Coupling Constants for Tricycle 2

		calcd coupling constants (Hz)[a]	
spin system	obsd	*trans*	*cis*
2−3′	10.1	**10.8**	11.3
2−3	3.0	**3.3**	5.1
5′−6	5.0	**5.0**	2.6
5−6	11.0	**11.0**	10.2
6−7	9.5	**9.6**	4.4
7−8′	2.2	**3.1**	4.9
7−8	2.2	**2.8**	1.5

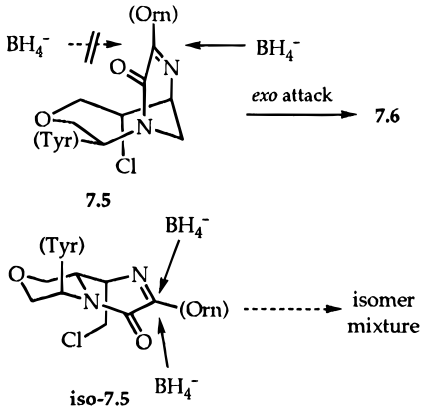
[a] Calculated in Macromodel according to ref 55.

Table 5. Calculated Distances and ROEs Observed for Tricycle 2

		calcd distances (Å)	
spin system	ROE	trans	cis
3−12	medium	**2.5**	2.7
3′−5′	obscured[a]	**2.5**	2.2
3′−6	medium	**2.2**	4.4
3−8	weak	**3.9**[b]	3.9
2−8	medium	**2.4**	2.3
6−7	absent	**3.1**	2.4
6−21	absent	**3.6**	3.1
7−21	strong	**2.3**	2.3
8′−10	strong	**2.3**	2.4

[a] This portion of the spectrum was obscured by contaminating TOCSY cross peaks which have the opposite sign of ROESY cross peaks and effectively cancel. [b] The eight-membered ring containing H3 is mobile so this distance could be less in some conformations.

Scheme 13



of **2m** and **epi-2m** indicate that the *trans* structure is the most consistent with the experimental coupling constants and observed ROEs (Tables 4 and 5).

Assignment of the tricyclic structure of the final product as **2** explains some of the side reactions and synthetic difficulties observed along the route. For example, the difficulty of the ring closure reactions is now understandable, given the strain that is being built into the tricylic system. We had also found it disconcerting that reduction of the imine **7.5** is so stereoselective; this byproduct, formed by elimination during the deprotection of **7.3**, is reduced to a material identical to the normal deprotection product **7.6**. While such stereoselectivity in reduction of the bicyclo[4.4.0] ring of **iso-7.6** would be unexpected, it is quite consistent with *exo* approach to the bicyclo[5.3.1] structure (Scheme 13).

Inhibition of α-Amylase. The ability of the tricyclic compound **2** to inhibit α-amylase was determined in 5% DMSO/water using a modified assay with *p*-nitrophenyl maltotrioside as substrate.[6] The inhibition constants for previously reported peptide analogs were redetermined in this solvent for comparison (Table 6). These values were found to be twice as large as in water, reflecting the importance that desolvation of the hydrophobic regions of these analogs plays in their affinity. Compound **2** binds to α-amylase with the same affinity as the capped tripeptide, Ac-Trp-Arg-Tyr-OMe, in spite of the fact that its skeleton is quite different from that in the original design **1**.

Table 6. Inhibitors of α-Amylase[a]

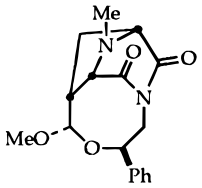
inhibitor	K_i (μM)
Trp-Arg-Tyr	870 ± 30
Ac-Trp-Arg-Tyr-OMe	190 ± 10
tricycle **2**	170 ± 20
cyclo[*D*PFAWRY]	30[b]

[a] Determined in 5% DMSO/water at 25 °C, pH 7.6 [b] Estimated from two-point determination.

Fortuitously, the ring systems of both **1** and **2** locate the side-chain attachment points (corresponding to the Cα carbons) in a similar fashion to the *β*-turn region of tendamistat itself (Figure 3). The rms deviations for these three carbons in the modeled structures of **1** and **2** from the corresponding Cα carbons in tendamistat−α-amylase complex[5] are 0.05 and 0.24 Å, respectively (Figure 3). However, the simple distance relationships between these points is less important than the relative orientations of the side chains, which are aligned quite differently in **2** than in **1**. Moreover, the skeleton of **2** is a poor match to the intervening atoms of the peptide backbone, in contrast to **1**. Whereas the lactams in **1** follow the zig-zag of the amides in the tendamistat *β*-turn, those of **2** are perpendicular; as a result, side-chain conformations that are staggered in tendamistat would be eclipsed in **2**.

Conclusion

The tricyclic structure of **2** embodies a novel complex ring system. Little or no precedent for compounds of this type exist according to structural searches of the Chemical Abstracts Service database. Although many examples of the all-carbon skeleton exist, the 4-oxa-1,7,10-triazatricyclo[8.2.2.$0^{6,11}$]tetradecane ring system itself appears to be unknown. The closest heterocyclic structure we found is **3**, derived from an intramolecular azomethine ylide cycloaddition.[40]



3

The route to the tricyclic compound **2** entails 31 steps from the component pieces *O*-Bn-L-Tyr, but-2-ene-1,4-diol, α-*N*-Boc-L-ornithine, and L-Trp-OMe with an overall yield from *O*-Bn-L-Tyr of 0.54%. The synthesis is semi-convergent with the longest linear sequence consisting of 25 steps. The challenge of this molecule lies in the large amount of functionality that is exposed during the synthesis, including seven nitrogens and four oxygens, most of which require protection given the current state of synthetic technology. In particular, the troublesome indole and guanidine functionalities were predictably

(40) Garner, P.; Sunitha, K.; Ho, W.-B.; Youngs, W. J.; Kennedy, V. O.; Djebli, A. *J. Org. Chem.* **1989**, *54*, 2041.

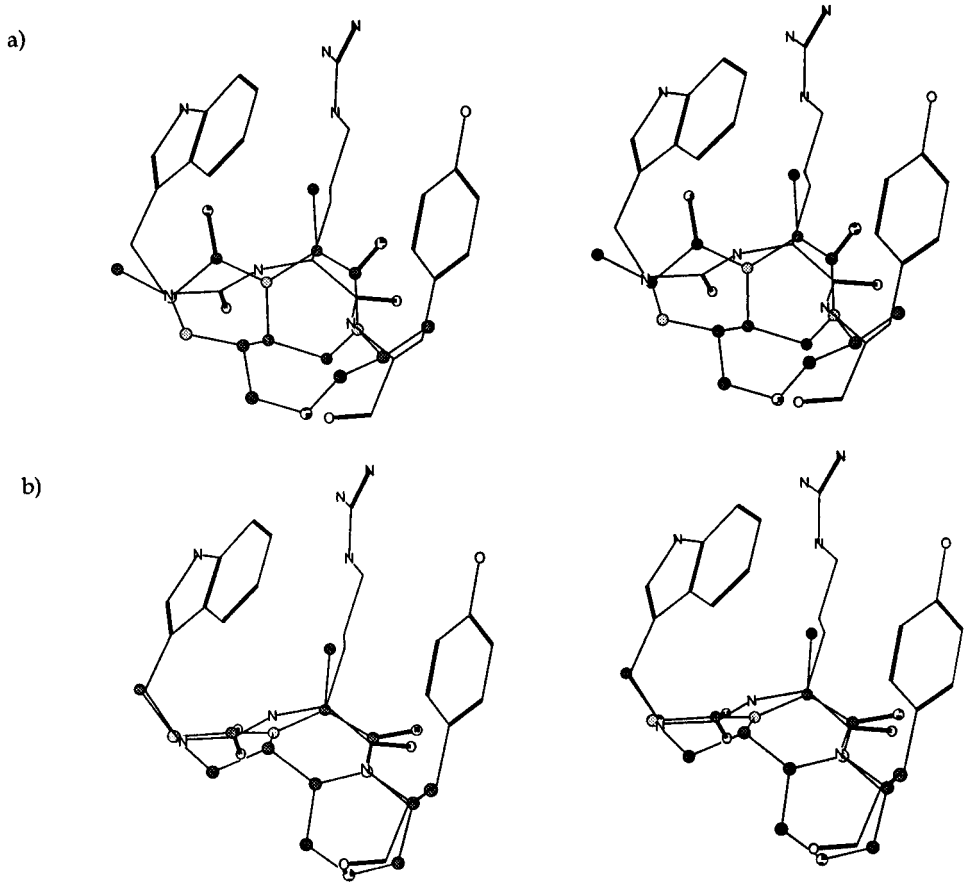


Figure 3. Superposition of the tricyclic ring systems of **2** (a) and **1** (b) on the $Trp^{18}Arg^{19}Tyr^{20}$ loop of tendamistat.

difficult to reconcile with the required reaction conditions. The synthesis of **2** provides solutions to many of these challenges, while highlighting the structural issue that must still be surmounted in assembling its elusive isomer **1**.

Experimental Section[41]

Synthesis. (*Z*)-4-(*tert*-Butyldimethylsiloxy)but-2-en-1-ol (2.1). NaH (60% oil dispersion, 9.60 g, 0.24 mol) was washed with hexanes (3 × 50 mL) and suspended in ether (400 mL). (*Z*)-2-Butene-1,4-diol (16.5 mL, 0.20 mol) was added dropwise to this suspension and the mixture was stirred for 1 h at rt. The mixture was then cooled to 0 °C and *tert*-butyldimethylsilyl chloride (30.1 g, 0.2 mol) was added in portions. After 3 h, the reaction mixture was diluted with ether (1.5 L), washed with saturated $NaHCO_3$ (3 × 600 mL), and dried over $MgSO_4$. Removal of the solvent gave the desired compound (40.7 g, 100%) as a beige oil. The crude product was usually carried on directly, but analytical samples could be obtained by chromatography with 30% EtOAc/hexanes which gave the silyl ether **2.1** as a clear oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.69 (m, 2), 4.25 (d, 2, J = 5.3), 4.20 (d, 2, J = 5.3), 1.68 (s, 1), 0.91 (s, 9), 0.09 (s, 6); ^{13}C NMR (100 MHz, $CDCl_3$) δ 131.2, 130.0, 59.5, 58.7, 25.8, 18.3, −5.3; IR (film) 3375 cm^{-1}. Anal. Calcd for $C_{10}H_{22}O_2Si$: C, 59.35; H, 10.96. Found: C, 59.54; H, 10.92.

(*Z*)-4-(*tert*-Butyldimethylsiloxy)but-2-enyl Mesylate (2.2). Mesyl chloride (4.20 mL, 54.4 mmol) was added dropwise to a 0 °C solution of alcohol **2.1** (10.0 g, 49.4 mmol) and triethylamine (7.60 mL, 54.4 mmol) in CH_2Cl_2 (250 mL). The ice bath was allowed to warm to rt, and after 3.5 h, the mixture was diluted with ether (500 mL), washed with saturated NaCl (3 × 250 mL), dried over $MgSO_4$, and concentrated. The brown oil was purified by chromatography with 30% ether/hexanes to afford the mesylate **2.2** (13.0 g, 94%) as a clear oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.82 (m, 1), 5.61 (m, 1), 4.85 (d, 2, J = 6.9), 4.27 (d, 2, J = 5.2), 2.99 (s, 3), 0.88 (s, 9), 0.06 (s, 6); IR (film) 1370, 1185 cm^{-1}. Anal. Calcd for $C_{11}H_{24}O_4SSi$: C, 47.11; H, 8.63; S, 11.43. Found: C, 47.50; H, 8.75; S, 11.05.

4-[(2*S*)-2-Amino-3-hydroxypropyl]-1-(phenylmethoxy)-benzene (2.3).[10,11] *O*-Bn-L-Tyr (49.6 g, 0.18 mol) was dissolved in methanol (1.2 L) and cooled to −10 °C with an ice/salt/water bath. Thionyl chloride (32 mL, 0.44 mol) was added dropwise, causing a highly exothermic reaction. After the addition, the ice bath was removed and the mixture warmed to rt. After 3 d, the mixture was concentrated to a yellow solid, which was precipitated from hot methanol with two volumes of ether to afford *O*-Bn-L-TyrOMe•HCl[12] (57.8 g, 98%) as a white solid which was used without further purification.

A suspension of *O*-Bn-L-TyrOMe•HCl (28.3 g, 88 mmol) in EtOAc (290 mL) was cooled to 0 °C, and triethylamine (13.5 mL, 97 mmol) was added. After 1 h, the precipitated salt was removed by filtration and the filtrate was concentrated. The remaining oil was dissolved in THF (500 mL), and the solution was added dropwise over 1 h to a suspension of $LiAlH_4$ (10.0 g, 260 mmol) in THF (90 mL). After stirring for an additional 1 h, the mixture was quenched cautiously at 0 °C by successive addition of water (10 mL), 20% aqueous NaOH (10 mL), and water (30 mL). After the solution was stirred vigorously for 1 h at rt, the slurry was concentrated, filtered through Celite, and rinsed with CH_2Cl_2 (3 × 350 mL). The filtrate was washed with saturated NaCl (600 mL) and dried over Na_2SO_4. Removal of the solvent afforded the amino alcohol **2.3** (22.2 g, 98%) as fine white crystals: mp 97−99 °C (lit.[10] mp 95−97 °C); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43 (d, 2, J = 6.9), 7.39 (t, 2, J = 7.0), 7.32 (t, 1, J = 7.1), 7.10 (d, 2, J = 8.6), 6.93 (d, 2, J = 8.6), 5.05 (s, 2), 3.63 (dd, 1, J = 3.9, 10.6), 3.36 (dd, 1,

(41) **General**. Aminoiminomethanesulfonic acid[38,39] and (trimethylsilyl)ethanesulfonyl chloride (SESCl)[25] were prepared by following literature procedures. Column chromatography was performed by the method of Still, Kahn, and Mitra using 60-mesh silica gel.[42] Mass spectra and combustion analyses were obtained from the Mass Spectrometry Laboratory and the Microanalytical Laboratory, Department of Chemistry, University of California, Berkeley.

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

J = 7.2, 10.6), 3.08 (m, 1), 2.73 (dd, 1, J = 5.3, 13.6), 2.47 (dd, 1, J = 8.5, 13.6), 1.65 (br s, 3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.4, 137.0, 130.9, 130.1, 128.6, 127.9, 127.4, 114.9, 70.0, 66.2, 54.2, 39.9; IR (CH_2Cl_2) 3620, 3400 cm^{-1}. Anal. Calcd for $C_{16}H_{19}NO_2$: C, 74.68; H, 7.44; N, 5.44. Found: C, 74.39; H, 7.32; N, 5.45.

(*Z*)-4-[(2*S*)-2-Amino-3-(4-(phenylmethoxy)phenyl)propoxy]-1-(*tert*-butyldimethylsiloxy)but-2-ene (2.4). Alcohol **2.3** (6.00 g, 23.3 mmol) was added to a suspension of KH (35% oil dispersion, 5.87 g, 51.3 mmol), washed with hexanes (3 × 60 mL), in ether (180 mL). After the generation of H_2 ceased, the mixture was cooled to 0 °C and a solution of mesylate **3** (7.84 g, 28.0 mmol) in ether (50 mL) was added dropwise. The ice bath was allowed to warm to rt and after 3 h, the reaction mixture was diluted with saturated NH_4Cl (300 mL) and extracted with CH_2Cl_2 (3 × 500 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated, and the residue was purified by chromatography with 5% methanol/CH_2Cl_2 to afford the amino ether **2.4** (6.65 g, 55%) as a clear oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (m, 5), 7.11 (d, 2, J = 8.6), 6.91 (d, 2, J = 8.6), 5.69 (ddddd, 1, J = 1.3, 1.3, 5.8, 5.8, 11.4), 5.59 (ddddd, 1, J = 1.5, 1.5, 6.2, 6.2, 12.5), 5.04 (s, 2), 4.24 (dd, 2, J = 1.0, 5.8), 4.05 (d, 2, J = 6.0), 3.41 (dd, 1, J = 3.8, 8.8), 3.25 (dd, 1, J = 7.2, 8.7), 3.18 (m, 1), 2.49 (dd, 1, J = 5.4, 13.6), 1.74 (s, 2), 0.90 (s, 9), 0.07 (s, 6); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.3, 137.0, 132.5, 131.0, 130.1, 128.5, 127.9, 127.4, 126.8, 114.8, 74.9, 70.0, 66.9, 59.5, 52.4, 39.7, 25.9, 18.3, −5.2; IR (film) 3300, 1615 cm^{-1}. Anal. Calcd for $C_{26}H_{39}NO_3Si$: C, 70.70; H, 8.90; N, 3.17. Found: C, 70.74; H, 8.81; N, 3.23.

(*Z*)-1-(*tert*-Butyldimethylsiloxy)4-[(2*S*)-2-[(fluorenylmethoxycarbonyl)amino]-3-(4-(phenylmethoxy)phenyl)-propoxy]but-2-ene (2.5). Amino ether **2.4** (6.26 g, 14.2 mmol) and FmocOSu (7.17 g, 21.2 mmol) were dissolved in THF (120 mL) and aqueous 10% Na_2CO_3 (22 mL) was added. After 23 h, the THF was removed. The resultant slurry was diluted with water (250 mL) and extracted with CH_2Cl_2 (5 × 500 mL). The combined organic extracts were dried with $MgSO_4$ and concentrated, and the residue was purified by chromatography with 15% EtOAc/hexanes to afford the protected product **2.5** (8.41 g, 89%) as a white powder: mp 68.5−69.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (rotamers) 7.79 (d, 2, J = 7.0), 7.59 (d, 2, J = 6.5), 7.36 (m, 9), 7.15 (m, 2), 6.93 (d, 2, J = 6.7), 5.74 (m, 1), 5.65 (m, 1), 5.11 (m, 1), 5.04 (s, 2), 4.46 (m, 1), 4.36 (m, 1), 4.25 (m, 3), 4.08 (m, 2), 4.03 (m, 1), 3.40 (m, 2), 2.88 (m, 2), 0.95 (s, 9), 0.12 (s, 6); ^{13}C NMR (100 MHz, $CDCl_3$) δ (rotamers) 157.4, 155.8, 143.9, 141.2, 137.0, 132.7, 130.3, 130.2, 128.5, 127.9, 127.6, 127.4, 127.0, 126.6, 125.0, 119.9, 114.8, 70.0, 69.9, 66.9, 66.5, 59.5, 52.1, 47.2, 36.8, 25.9, 18.3, −5.2; IR (film) 3410, 3330, 2950, 2925, 2855, 1720, 1610, 1510, 1450, 1240, 1075, 840, 775, 740 cm^{-1}. Anal. Calcd for $C_{41}H_{49}NO_5Si$: C, 74.17; H, 7.44; N, 2.11. Found: C, 73.82; H, 7.45; N, 2.04.

(*Z*)-4-[(2*S*)-2-[(Fluorenylmethoxycarbonyl)amino]-3-(4-(phenylmethoxy)phenyl)propoxy]-2-buten-1-ol (2.6). HF (40% in water, 11 mL) was added to a solution of silyl ether **2.5** (14.64 g, 22.0 mmol) in acetonitrile (110 mL) contained in a polypropylene bottle. After 20 min, the reaction mixture was poured into saturated $NaHCO_3$ (400 mL) and extracted with $CHCl_3$ (5 × 500 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated, and the residue was purified by chromatography with a step gradient of 20−50% EtOAc/hexanes to afford the alcohol **2.6** (11.06 g, 92%) as a white solid: mp 97−98 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (d, 2, J = 7.4), 7.56 (t, 2, J = 7.6), 7.38 (m, 9), 7.11 (d, 2, J = 7.8), 6.90 (br d, 3, J = 7.8), 5.79 (m, 1), 5.40 (m, 1), 5.15 (d, 1, J = 8.0), 5.00 (s, 2), 4.42 (t, 1, J = 10.1), 4.33 (t, 1, J = 10.1), 4.25−3.90 (m, 6), 3.38 (br s, 2), 2.81 (d, 2, J = 6.7), 2.26 (s, 1); ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.5, 156.0, 144.0, 141.4, 137.1, 132.4, 130.4, 130.1, 128.6, 128.0, 127.7, 127.6, 127.1, 125.4, 125.1, 120.0, 114.9, 70.4, 70.0, 66.6, 58.6, 52.5, 47.3, 43.5, 36.5; IR (CH_2Cl_2) 3600, 3440, 1725, 1515 cm^{-1}. Anal. Calcd for $C_{35}H_{35}NO_5$: C, 73.36; H, 7.70; N, 4.28. Found: C, 73.18; H, 7.65; N, 4.22.

(2*R*,3*S*)-3-[[(2*S*)-2-((Fluorenylmethoxycarbonyl)amino)-3-(4-(phenylmethoxy)phenyl)propoxy]methyl]oxiranemethanol (2.7). A suspension of powdered 4Å sieves (0.510 g, dried in vacuo at 150 °C) and D-(−)-diisopropyl tartrate (1.546 g, 6.60 mmol) in CH_2Cl_2 (40 mL) was cooled to −30 °C. Titanium(IV) isopropoxide (1.475 g, 5.19 mmol) was added, and the mixture was stirred at −30 °C for 45 min. *t*-BuOOH (4.71 mL of 3.0 M solution in isooctane, 14.14 mmol) was added, and the mixture was stirred for an additional 15 min at −30 °C. A solution of allylic alcohol **2.6** (2.592 g, 4.71 mmol) in CH_2Cl_2 (10 mL) was added, and the mixture was stirred at −15 °C. After 37 h, water (20 mL) was added and the resultant emulsion was stirred at rt for 1 h. The mixture was then filtered through Celite, and the solids were thoroughly rinsed with CH_2Cl_2 (500 mL). The combined filtrates were washed with water (250 mL), and the aqueous layer was extracted with CH_2Cl_2 (3 × 250 mL). The combined organic layers were dried with $MgSO_4$ and concentrated, and the residue was purified by chromatography with a gradient of 50−80% EtOAc/hexanes to afford the epoxy alcohol **2.7** (2.667 g, 100%) as a white solid: mp 95−97 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (d, 2, J = 7.5), 7.58 (t, 2, J = 7.6), 7.37 (m, 9), 7.13 (d, 2, J = 8.0), 6.91 (d, 2, J = 8.0), 5.40 (d, 1, J = 8.6), 4.99 (s, 2), 4.45 (t, 1, J = 6.9), 4.36 (t, 1, J = 6.9), 4.20 (t, 1, J = 6.5), 4.03 (s, 1), 3.73 (d, 2, J = 5.0), 3.67 (dd, 1, J = 3.4, 11.3), 3.54 (dd, 1, J = 5.7, 10.8), 3.44 (m, 2), 3.20 (m, 3), 2.82 (d, 1, J = 6.9); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.5, 156.0, 143.8, 141.2, 136.9, 130.2, 129.8, 128.5, 127.9, 127.6, 127.4, 127.0, 125.0, 119.9, 114.8, 71.5, 69.9, 69.0, 66.4, 60.4, 55.6, 54.8, 52.0, 47.2, 36.7; IR (CH_2Cl_2) 3600, 3440, 1727, 1517, 920 cm^{-1}. Anal. Calcd for $C_{35}H_{35}NO_6$: C, 74.31; H, 6.24; N, 2.48. Found: C, 74.26; H, 6.29; N, 2.48.

(2*R*,3*S*)-3-[[(2*S*)-2-Amino-3-(4-(phenylmethoxy)phenyl)propoxy]methyl]oxiranemethanol (2.8). DBU (0.62 mL, 4.12 mmol) was added to a solution of epoxide **2.7** (2.12 g, 3.74 mmol) in CH_2Cl_2 (19 mL). After 1.5 h, the solvent was removed, and the resultant oil was purified by chromatography with a gradient of 10−20% methanol/CH_2Cl_2 to afford the amino epoxide **2.8** (1.29 g, 100%) as a clear oil, which slowly cyclized to the morpholine **2.9** at rt: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, 2, J = 7.5), 7.38 (t, 2, J = 7.6), 7.32 (t, 1, J = 7.6), 7.09 (d, 2, J = 8.6), 6.91 (d, 2, J = 8.6), 5.03 (s, 2), 3.78 (dd, 1, J = 5.7, 12.3), 3.71 (dd, 1, J = 5.3, 12.3), 3.67 (dd, 2, J = 3.6, 5.2), 3.48 (dd, 1, J = 3.7, 9.4), 3.39 (dd, 1, J = 7.1, 9.3), 3.22 (m, 2), 2.77 (br s, 4), 2.72 (dd, 1, J = 5.6, 13.7), 2.53 (dd, 1, J = 8.2, 13.6); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.5, 137.0, 130.1, 130.0, 128.5, 127.9, 127.4, 114.9, 75.0, 70.0, 68.9, 60.0, 55.9, 54.7, 52.4, 39.3; IR (film) 3340, 3280, 3150, 2900, 2850, 1795, 1605, 1565, 1505, 1230, 1100 cm^{-1}.

(2*S*,6*R*)-6-[(1*S*)-1,2-Dihydroxyethyl]-2-[[4-(phenylmethoxy)phenyl]methyl]morpholine (2.9). A solution of amino epoxide **2.8** (1.28 g, 3.73 mmol) in dry dioxane (20 mL) was heated to reflux. After 9 h, the solvent was removed and the resultant oil was purified by chromatography with 15% methanol/ CH_2Cl_2 to afford the morpholine **2.9** (0.917 g, 72%) as a white solid: mp 85−86 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (m, 5), 7.09 (d, 2, J = 8.6), 6.91 (d, 2, J = 8.6), 5.02 (s, 2), 3.70 (m, 4), 3.59 (dd, 1, J = 4.4, 11.0), 3.50 (dd, 1, J = 4.4, 11.4), 3.37 (dd, 1, J = 6.4, 11.3), 3.10 (ddd, 1, J = 3.0, 6.9, 13.5), 3.00 (m, 1), 2.71 (m, 2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.3, 136.8, 130.1, 129.9, 128.4, 127.8, 127.3, 114.8, 70.4, 69.8, 68.9, 67.8, 63.9, 51.4, 51.4, 36.7; IR (CH_2Cl_2) 3680, 3600, 3340 cm^{-1}; MS (EI) *m*/*z* 344 (MH^+), 282, 146. Anal. Calcd for $C_{20}H_{25}NO_4$: C, 69.95; H, 7.34; N, 4.08. Found: C, 69.72; H, 7.36; N, 3.98.

(2*S*,6*R*)-6-[(1*S*)-1-Hydroxy-2-(methanesulfonyloxy)ethyl]-2-[[4-(phenylmethoxy)phenyl]methyl]morpholine (4.1). Diol **2.9** (0.659 g, 1.92 mmol) was dissolved in CH_2Cl_2 (100 mL), and triethylamine (0.294 mL, 2.11 mmol) was added. The mixture was cooled to −5 °C, and MsCl was added (0.156 mL, 2.01 mmol) over 1 h. After an additional 10 min, the mixture was diluted with CH_2Cl_2 (200 mL), washed with saturated NaCl (2 × 100 mL), dried with $MgSO_4$, and concentrated, and the residue was purified by chromatography with 5% methanol/CH_2Cl_2 to afford the hydroxy mesylate **4.1** (0.649 g, 80%) as a white powder: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 (d, 2, J = 7.1), 7.36 (t, 2, J = 7.2), 7.31 (t, 1, J = 7.2), 7.08 (d, 2, J = 8.5), 6.90 (d, 2, J = 8.5), 5.01 (s, 2), 4.29 (dd, 1, J = 2.9, 11.0), 4.14 (dd, 1, J = 5.2, 11.0), 4.00 (ddd, 1, J = 3.0, 5.0, 7.9), 3.71 (dd, 1, J = 3.2, 11.4), 3.68 (dd, 1, J = 3.3, 11.9), 3.60 (dd, 1, J = 4.3, 11.9), 3.31 (dd, 1, J = 7.3, 11.2), 3.08 (ddd, 1, J = 3.0, 7.1,

10.1), 2.98 (s, 3), 2.92 (dd, 1, J = 3.9, 7.9), 2.64 (d, 2, J = 7.1); ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.4, 136.9, 130.0, 129.9, 128.4, 127.8, 127.4, 114.9, 71.2, 71.1, 69.9, 67.3, 66.3, 51.1, 50.8, 37.3, 37.1; IR (film) 3500, 3380, 3300, 3020, 2900, 2850, 1605, 1505, 1450, 1345, 1235, 1170, 1100, 955 cm^{-1}; HRMS (FAB) m/z calcd for $C_{21}H_{28}NO_6S$ 422.1637, found 422.1649, 422 (MH^+), 326, 307, 282, 224, 154, 136.

(2*S*,6*R*)-6-[(1*S*)-1-(*tert*-Butyldimethylsiloxy)-2-((methanesulfonyloxy)ethyl]-2-[[4-(phenylmethoxy)phenyl]-methyl]morpholine (4.2). Note: use of 1 equiv of TBDMSOTf results in formation of the azetidine via intramolecular reaction of the desired product. Compound **4.1** (0.645 g, 1.53 mmol) was dissolved in CH_2Cl_2 (15 mL), and collidine (0.81 mL, 6.12 mmol) and TBDMSOTf (1.05 mL, 4.59 mmol) were added. After 75 min, the mixture was diluted with CH_2Cl_2 (200 mL), washed with saturated NaCl (2 × 80 mL), dried with $MgSO_4$, and concentrated, and the residue was purified by chromatography with 2.5% methanol/CH_2Cl_2 to afford the silyl ether **4.2** (0.811 g, 99%) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (m, 5), 7.07 (d, 2, J = 8.6), 6.89 (d, 2, J = 8.6), 5.02 (s, 2), 4.29 (dd, 1, J = 3.8, 10.5), 4.09 (dd, 1, J = 5.1, 10.5), 3.90 (m, 1), 3.79 (dd, 1, J = 3.2, 11.3), 3.62 (dd, 1, J = 3.2, 11.2), 3.49 (d, 1, J = 11.2), 3.48 (dd, 1, J = 2.5, 11.1), 3.08 (ddd, 1, J = 3.2, 6.8, 6.8), 3.02 (m, 1), 2.96 (s, 3), 2.79 (dd, 1, J = 8.3, 13.6), 2.67 (dd, 1, J = 6.5, 13.6), 1.95 (s, 1), 0.83 (s, 9), 0.09 (s, 3), −0.02 (s, 3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.4, 137.1, 130.9, 130.1, 128.5, 127.8, 127.3, 115.0, 70.8, 70.4, 70.0, 70.0, 68.4, 52.1, 52.0, 37.3, 36.8, 25.6, 17.9, −4.4, −5.0; IR (film) 3420, 2930, 2855, 1610, 1515, 1190, 1175, 840 cm^{-1}; HRMS (FAB) m/z calcd for MH^+ $C_{27}H_{42}NO_6SiS$ 536.2502, found 536.2502, 536 (56), 478 (10), 440 (100), 281 (6), 242 (56), 197 (8), 115 (9).

(2*S*,6*R*,7*R*)-7-(*tert*-Butyldimethylsiloxy)-2-[[4-(phenylmethoxy)phenyl]methyl]-4,1-oxazabicyclo[4.2.0]octane (4.3). Mesylate **4.2** (0.870 g, 1.62 mmol) and triethylamine (1.13 mL, 8.12 mmol) were dissolved in acetonitrile (33 mL), and the solution was heated to reflux. After 10 h, the reaction mixture was concentrated and the oil was purified by chromatography with 40% EtOAc/hexanes to afford the bicyclic azetidine **4.3** (0.568 g, 79%) as a white crystalline solid: mp 49−51 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (m, 5), 7.11 (d, 2, J = 8.5), 6.90 (d, 2, J = 8.5), 5.04 (s, 2), 4.56 (dd, 1, J = 6.4, 12.8), 4.22 (dd, 1, J = 11.5, 11.5), 3.96 (dddd, 1, J = 1.2, 6.0, 6.0, 11.4), 3.68 (dd, 1, J = 6.0, 11.4), 3.66 (dd, 1, J = 3.4, 8.2), 3.53 (m, 2), 3.47 (dd, 1, J = 2.9, 11.6), 2.75 (dd, 1, J = 8.9, 13.5), 2.70 (dd, 1, J = 6.3, 13.6), 2.55 (m, 1), 0.88 (s, 9), 0.03 (s, 6); ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.2, 137.2, 131.8, 130.3, 128.5, 127.8, 127.4, 114.7, 70.0, 64.0, 63.3, 61.8, 60.4, 59.2, 55.8, 37.7, 25.6, 17.9, −5.2, −5.3; IR (film) 2920, 2850, 1610, 1510, 1335, 840 cm^{-1}; MS (FAB) m/z 441 (MH_2^+), 440 (MH^+), 439, 438, 281, 242, 197, 115. Anal. Calcd for $C_{26}H_{37}NO_3Si$: C, 71.03; H, 8.48; N, 3.19. Found: C, 69.91; H, 8.30; N, 3.37.

Methyl (2*S*)-5-(2,3-Dihydro-2-oxo-4,5-diphenyl-1,3-oxazol-3-yl)-2-aminopentanoate (6.2). A mixture of Me_4NOH (20% in methanol w/v, 7.0 mL, 15.3 mmol) and N^{α}-Boc-L-ornithine (3.56 g, 15.3 mmol) was triturated with absolute EtOH (3 × 30 mL). The residual solvent was removed to yield the tetramethylammonium salt as a white powder. The salt was dissolved in dry DMF (15 mL), and 4,5-diphenyl-1,3-dioxolone (4.02 g, 16.8 mmol) was added. After being stirred for 3 h, the reaction mixture was acidified to pH 1 with 2 N HCl, diluted with EtOAc (200 mL), washed with 0.5 N HCl (70 mL) and saturated NaCl (2 × 70 mL), and dried over $MgSO_4$. Removal of the solvent yielded the hydroxyoxazolidinone **6.1** as a white foam.

The crude hydroxyoxazolidinone was dissolved in methanol (340 mL) and cooled to −5 °C. Thionyl chloride (7.5 mL, 102.5 mmol) was added dropwise. The cooling bath was then removed, and the reaction mixture was allowed to warm to rt. After 23 h, the solvent was removed exhaustively at 50 °C with a rotary evaporator. The resultant oil was dissolved in saturated $NaHCO_3$ (100 mL), and the mixture was extracted with EtOAc (3 × 200 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated, and the residue was purified by chromatography with a gradient of 2.5−10% methanol/CH_2Cl_2 to afford the N^{δ}-protected ornithine analog **6.2** (5.12 g, 93%) as a clear colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (m, 3), 7.43 (m, 2), 7.16−7.26 (m, 5), 3.68 (s, 3), 3.52 (dd, 2, J = 7.0, 7.0), 3.34 (dd, 1, J = 4.8, 7.7), 1.60 (m, 3), 1.43 (m, 3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.9, 154.5, 134.4, 130.4, 130.1, 129.5, 128.4, 127.7, 127.6, 127.2, 124.2, 123.1, 53.8, 51.9, 41.6, 31.4, 25.0; IR (film) 3370, 3300, 3050, 2940, 1750, 1600, 1435, 1355, 1050 cm^{-1}. Anal. Calcd for $C_{21}H_{22}N_2O_4$: C, 68.84; H, 6.05; N, 7.65. Found: C, 68.59; H, 6.05; N, 7.40.

Methyl (2*S*)-5-(2,3-Dihydro-2-oxo-4,5-diphenyl-1,3-oxazol-3-yl)-2-[[2-(trimethylsilyl)ethyl]sulfonamido]pentanoate (6.3). Compound **6.2** (1.06 g, 2.9 mmol) and triethylamine (4.0 mL, 29.0 mmol) were dissolved in DMF (7 mL), and the mixture was cooled to 0 °C. 2-(Trimethylsilyl)-ethanesulfonyl chloride (0.87 g, 4.35 mmol) dissolved in DMF (8 mL) was added dropwise to the above mixture over 30 min. After the reaction mixture was stirred an additional 2.5 h at 0 °C, the mixture was diluted with water (100 mL) and extracted with ether (4 × 200 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated, and the residue was purified by chromatography with 40% EtOAc/hexanes to afford compound **6.3** (1.30 g, 84%) as a white solid: mp 49−50 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (m, 3), 7.38−7.41 (m, 2), 7.12−7.22 (m, 5), 5.28 (d, 1, J = 9.2), 3.98 (m, 1), 3.70 (s, 3), 3.53 (dd, 2, J = 6.3, 6.3), 2.87 (m, 2), 1.76 (m, 1), 1.58 (m, 3), 1.02 (m, 2), 0.01 (s, 9); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3, 154.5, 134.4, 130.3, 130.1, 129.5, 128.3, 127.5, 127.5, 126.9, 124.1, 123.0, 55.3, 52.5, 49.5, 41.1, 29.8, 24.7, 10.2, −2.2; IR (film) 3250, 3050, 2940, 2245, 1740, 1600 cm^{-1}. Anal. Calcd for $C_{26}H_{34}N_2O_6SiS$: C, 58.84; H, 6.46; N, 5.28. Found: C, 58.81; H, 6.38; N, 5.03.

(2*S*)-5-(2,3-Dihydro-2-oxo-4,5-diphenyl-1,3-oxazol-3-yl)-2-[[2-(trimethylsilyl)ethyl]sulfonamido]pentanoic Acid (6.4). Compound **6.3** (5.60 g, 10.6 mmol) was dissolved in THF (100 mL) and water (50 mL), 2 N LiOH (53 mL, 106 mmol) was added, and the mixture was stirred at rt. After 2.5 h, the mixture was diluted with water (300 mL), acidified to pH 1 with concentrated HCl, and extracted with CH_2Cl_2 (4 × 400 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated, and the residue was purified by chromatography with a gradient of 5−10% methanol/CH_2Cl_2 to afford acid **6.4** (5.27 g, 97%) as a white powder: mp 140−42 °C; ^{1}H NMR (400 MHz, 1:1 $CDCl_3$/CD_3OD) δ 7.46 (m, 3), 7.28 (m, 2), 7.13 (m, 5), 3.82 (s, 1), 3.51 (m, 1), 3.31 (m, 1), 2.87 (dd, 2, J = 8.8, 8.8), 1.77 (s, 1), 1.59 (s, 3), 0.94 (m, 2), −0.04 (s, 9); ^{13}C NMR (100 MHz, 1:1 $CDCl_3$/ CD_3OD) δ 177.9, 154.7, 134.5, 130.0, 129.9, 129.2, 128.0, 127.4, 127.0, 126.1, 123.8, 123.2, 56.2, 53.0, 41.0, 29.7, 24.5, 9.6, −2.8; IR (KBr) 3460, 3250, 3030, 2945, 1745, 1590, 1580, 1440, 1385, 1315, 1255, 1130, 855, 840, 750, 690 cm^{-1}; HRMS (FAB) m/z calcd for MH^+ $C_{25}H_{32}N_2O_6SiS$ 516.1750, found 516.1746, 555 (MK^+), 539 (MNa^+), 516 (M^+), 493, 425, 366, 237, 172; UV−Vis (CH_2Cl_2) λ_{max} 220, 284 nm.

(3*S*,4*R*,7*S*)-4-(*tert*-Butyldimethylsiloxy)-3-chloro-6-[(2*S*)-5-(2,3-dihydro-2-oxo-4,5-diphenyl-1,3-oxazol-3-yl)-2-[[2-(trimethylsilyl)ethyl]sulfonamido]pentanoyl]-7-[[4-(phenylmethoxy)phenyl]methyl]-1,6-oxazacyclooctane (4.5s). Azetidine **4.3** (0.292 g, 0.66 mmol) and acid **6.4** (1.028 g, 1.99 mmol) were suspended in THF (6.6 mL), and the solution was cooled to 0 °C. PCl_5 (1.105 g, 5.31 mmol) was added, and the solution was stirred at 0 °C. After 1 h 45 min, DIEA (0.11 mL, 0.64 mmol) was added and stirring was continued at 0 °C. After an additional 15 min, the solution was made basic with DIEA, diluted with EtOAc (200 mL), and washed with 0.5 N HCl (2 × 70 mL) and saturated $NaHCO_3$ (2 × 70 mL). The organic layer was dried with $MgSO_4$ and concentrated, and the residue was purified by chromatography with a gradient of 20−30% EtOAc/hexanes to afford the ring-opened compound **4.5s** (0.429 g, 66%) as a white foam: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (m, 3), 7.29−7.43 (m, 7), 7.17−7.25 (m, 5), 7.00 (d, 2, J = 8.6), 6.90 (d, 2, J = 8.6), 5.11 (d, 1, J = 9.6), 5.05 (d, 1, J = 11.7), 5.00 (d, 1, J = 11.7), 4.29 (t, 1, J = 3.9), 4.20 (t, 1, J = 10.9), 4.09 (m, 2), 4.01 (m, 2), 3.83 (m, 1), 3.48 (t, 3, J = 6.8), 3.38 (d, 1, J = 14.0), 3.26 (dd, 1, J = 10.2, 13.6), 2.91 (m, 3), 2.60 (dd, 1, J = 4.7, 13.6), 1.69 (m, 2), 1.56 (m, 1), 1.26 (m, 1), 1.05 (m, 2), 0.92 (s, 9), 0.12 (s, 3), 0.04 (s, 3), 0.03 (s, 9); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 157.4, 154.6, 136.9, 134.5, 130.3, 130.3, 130.2, 129.9, 129.7, 128.4,

128.4, 127.8, 127.6, 127.4, 127.3, 126.9, 124.2, 123.0, 114.8, 74.0, 70.5, 70.2, 69.9, 62.2, 54.1, 53.8, 49.6, 41.2, 33.1, 29.9, 26.4, 25.7, 25.2, 17.9, 10.1, −2.1, -4.4, −5.2; IR (film) 3240, 2951, 2858, 1754, 1648, 1510, 1250 cm^{-1}; HRMS (FAB) *m/z* calcd for MH^+ $C_{51}H_{69}ClN_3O_8Si_2S$ 974.4032, found 974.4053, 996 (MNa^+), 974 (MH^+), 956, 946, 916, 882, 808, 737, 684, 643, 573, 502, 476.

(3*S*,4*R*,7*S*)-3-Chloro-6-[(2*S*)-5-(2,3-dihydro-2-oxo-4,5-diphenyl-1,3-oxazol-3-yl)-2-[[2-(trimethylsilyl)ethyl]sulfonamido]pentanoyl]-4-hydroxy-7-[[4-(phenylmethoxy)-phenyl]methyl]-1,6-oxazacyclooctane (7.1s). Compound **4.5s** (0.220 g, 0.23 mmol) was dissolved in THF (5 mL), and TBAF (1.0 M in THF, 0.23 mL, 0.23 mmol) was added. After 30 min, the solution was diluted with CH_2Cl_2 (100 mL). The organic layer was washed with 0.5 N HCl (2 × 50 mL) and saturated $NaHCO_3$ (2 × 50 mL), dried with $MgSO_4$, and concentrated, and the residue was purified by chromatography with 40% EtOAc/hexanes to afford alcohol **7.1s** (0.159 g, 82%) as a white foam: ^1H NMR (400 MHz, $CDCl_3$, rotamers 1:1) δ 7.52 (m, 3), 7.37 (m, 7), 7.21 (m, 5), 7.07 (d, 1, J = 8.5), 6.99 (d, 1, J = 8.6), 6.88 (d, 1, J = 8.6), 6.82 (d, 1, J = 8.6), 5.18 (d, 1, J = 9.2), 4.96 (m, 2), 4.38 (m, 2), 4.28 (m, 1), 4.17 (m, 1), 4.07 (m, 2), 3.88 (m, 1), 3.73 (m, 2), 3.56 (m, 1), 3.40 (m, 2), 2.86 (m, 4), 1.71 (m, 2), 1.42 (m, 2), 1.00 (m, 2), 0.04 (s, 5), 0.01 (s, 4); ^{13}C NMR (100 MHz, $CDCl_3$, amide rotamers 1:1, the rotameric carbons are listed parenthetically) δ 174.7 (173.0), 157.5 (157.2), 154.7 (154.3), 136.8 (136.6), 134.6 (134.1), 130.2, 130.2, 130.0, 129.9, 129.4 (129.4), 128.2, 128.2, 127.6 (127.6), 127.5 (127.5), 127.4 (127.3), 127.1 (127.1), 126.7 (126.6), 124.1 (123.9), 123.0 (122.9), 114.9 (114.5), 72.2 (72.0), 70.3 (69.9), 69.6 (69.5), 64.5 (62.0), 58.9, 53.3 (52.4), 49.8, 44.7 (44.2), 40.6, 34.1 (33.4), 29.3 (29.0), 26.8 (26.2), 24.9 (24.5), 10.1 (9.9), −2.2 (-2.2); IR (CH_2Cl_2) 3450, 3350, 3061, 2954, 1750, 1612, 1511, 1140 cm^{-1}; HRMS (FAB) *m/z* calcd for MH^+ $C_{45}H_{55}ClN_3O_8SiS$ 860.3167, found 860.3159, 860 (MH^+), 832, 796, 768, 620, 570, 459.

(3*S*,4*R*,7*S*)-3-Chloro-6-[(2*S*)-5-(2,3-dihydro-2-oxo-4,5-diphenyl-1,3-oxazol-3-yl)-2-[[2-(trimethylsilyl)ethyl]sulfonamido]pentanoyl]-4-(methanesulfonyloxy)-7-[[4-(phenylmethoxy)phenyl]methyl]-1,6-oxazacyclooctane (7.2s). A solution of alcohol **7.1s** (0.169 g, 0.20 mmol) in CH_2Cl_2 (5 mL) was cooled to 0 °C; triethylamine (0.30 mL, 2.16 mmol) and mesyl chloride (0.15 mL, 1.96 mmol) were added and the cooling bath was removed. After 1.5 h, the solution was diluted with CH_2Cl_2 (100 mL), the organic layer was washed with saturated NaCl (2 × 50 mL), dried, and concentrated, and the oily product was chromatographed (40% EtOAc/hexanes) to afford 0.173 g (94%) of mesylate **7.2s** as a white foam: ^1H NMR (400 MHz, $CDCl_3$, 6:1 ratio of rotamers; data reported only for major rotamer) δ 7.51 (m, 3), 7.35 (m, 7), 7.19 (m, 5), 6.99 (d, 2, J = 8.5), 6.88 (d, 2, J = 8.6), 5.39 (m, 1), 5.02 (d, 1, J = 11.7), 4.98 (d, 1, J = 11.8), 4.96 (m, 1), 4.37 (m, 1), 4.15 (m, 2), 4.08 (m, 2), 3.65 (m, 2), 3.38 (m, 3), 3.26 (dd, 1, J = 11.0, 13.8), 3.14 (s, 3), 2.94 (t, 2, J = 9.0), 2.86 (m, 1), 2.75 (dd, 1, J = 5.7, 13.9), 1.68 (m, 2), 1.57 (m, 1), 1.47 (m, 1), 1.01 (m, 2), 0.01 (s, 9); ^{13}C NMR (100 MHz, $CDCl_3$, rotamers 1:1, the rotameric carbons are listed paranthetically) δ 172.7 (171.8), 157.5 (157.2), 154.3, 136.8 (136.6), 134.2 (134.1), 130.1, 130.0, 129.9 (129.8), 129.7, 129.4 (129.2), 128.2 (128.0), 127.6, 127.4, 127.2, 127.1, 126.7, 124.0 (123.9), 123.0 (122.9), 114.9 (114.6), 78.0, 69.6, 69.5, 62.1, 53.6 (53.3), 50.6 (50.2), 49.0, 41.0 (40.9), 39.2, 37.7, 34.0, 33.6, 31.3, 29.1, 25.2 (24.5), 9.9 (9.7), −2.2 (−2.3); IR (film) 3257, 2948, 2350, 1754, 1657, 1511, 1361, 1317, 1171 cm^{-1}; HRMS (FAB) *m/z* calcd for MH^+ $C_{46}H_{57}ClN_3O_{10}SiS_2$ 938.2943, found 938.2940, 960 (MNa^+), 938 (MH^+), 910, 874, 860, 846, 772, 537, 471, 440.

(2*S*,6*S*,7*S*,9*S*)-6-Chloro-9-[3-(2,3-dihydro-2-oxo-4,5-diphenyl-1,3-oxazol-3-yl)propyl]-10-oxo-2-[[4-(phenylmethoxy)phenyl]methyl]-8-[2-(trimethylsilyl)ethanesulfonyl]-4,1,8-oxadiazabicyclo[5.3.1]undecane (7.3s). Mesylate **7.2s** (0.061 g, 0.065 mmol) was dissolved in DMSO (3.3 mL), and NaH (60% dispersion, 0.026 g, 0.65 mmol) was added. Two such reactions were performed simultaneously and were combined for the workup and purification. After 2 h, the transparent light-orange solutions were diluted with EtOAc (200 mL). The organic layer was washed with water (40 mL) and saturated NaCl (2 × 80 mL), dried with $MgSO_4$, and concentrated, and the residue was purified by chromatography with 30% EtOAc/hexanes to afford the bicyclic product **7.3s** (0.062 g, 57%) as a white foam: ^1H NMR (400 MHz, $CDCl_3$) δ 7.51 (m, 5), 7.29−7.43 (m, 5), 7.24 (m, 2), 7.18 (m, 3), 7.02 (d, 2, J = 8.6), 6.86 (d, 2, J = 8.7), 5.02 (s, 2), 4.17 (s, 1), 4.08 (dd, 1, J = 5.2, 13.4), 4.02 (m, 1), 3.89 (m, 2), 3.81 (m, 2), 3.70 (d, 1, J = 13.4), 3.63 (dd, 1, J = 3.6, 14.5), 3.59 (dd, 1, J = 6.8, 14.2), 3.55 (dd, 1, J = 6.7, 14.2), 3.38 (m, 2), 2.96 (ddd, 1, J = 1.4, 9.7, 9.7, 2), 2.81 (m, 1), 2.02 (m, 2), 1.78 (m, 1), 1.52 (m, 1), 0.97 (m, 2), 0.08 (s, 9); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.7, 157.4, 154.7, 136.9, 134.3, 130.6, 130.6, 130.0, 129.5, 129.4, 128.5, 128.4, 127.9, 127.9, 127.4, 127.4, 127.3, 124.2, 123.4, 114.9, 70.6, 70.1, 70.0, 70.0, 61.3, 59.5, 57.7, 49.8, 49.6, 41.8, 33.7, 27.4, 25.3, 9.9, −2.0; IR (CH_2Cl_2) 3061, 2954, 1750, 1677, 1510, 1343, 1255 cm^{-1}; HRMS (FAB) *m/z* calcd for M^+ $C_{45}H_{52}ClN_3O_7SiS$ 841.2983, found 841.2977, 864 (MNa^+), 842 (MH^+), 814, 767, 750, 676, 605, 586, 515.

(2*S*,6*S*,7*S*,9*S*)-9-(3-Aminopropyl)-6-chloro-2-(4-(hydroxyphenyl)methyl)-10-oxo-8-[2-(trimethylsilyl)ethanesulfonyl]-4,1,8-oxadiazabicyclo[5.3.1]undecane (9.3). A solution of compound **7.3s** (0.153 g, 0.181 mmol), Pd black (0.096 g, 0.906 mmol), and HOAc (0.52 mL, 9.06 mmol) in absolute EtOH (5 mL) and 5% water/methanol (52 mL) was purged with H_2 three times and stirred under an H_2 atmosphere. After 2 d, the reaction mixture was filtered through Celite and concentrated. The resultant yellow oil was suspended in water and lyophilized to remove the toluene and benzil formed during the reaction. This procedure afforded the amine phenol **9.3** (0.097 g, 100%) as a pale yellow oil which was used without further purification: ^1H NMR (400 MHz, CD_3OD) δ 7.01 (d, 2, J = 8.4), 6.69 (d, 2, J = 8.4), 4.16 (m, 1), 4.08 (m, 3), 4.01 (d, 1, J = 10.9), 3.84 (dd, 1, J = 3.7, 12.2), 3.78 (m, 3), 3.53 (m, 1), 3.30 (d, 1, J = 10.0), 3.12 (m, 2), 2.91 (t, 2, J = 7.3), 2.78 (dd, 1, J = 6.0, 13.8), 2.13 (m, 2), 1.92 (m, 1), 1.78 (m, 1), 0.99 (m, 2), 0.06 (s, 9); ^{13}C NMR (100 MHz, CD_3OD) δ 169.4, 157.1, 130.8, 130.6, 116.3, 71.9, 71.8, 71.1, 63.9, 61.2, 58.9, 49.8, 46.5, 40.9, 34.6, 28.3, 24.8, 10.3, −2.0; IR (film) 3410, 3182, 2924, 2851, 1668 cm^{-1}; HRMS (FAB) *m/z* calcd for MH^+ $C_{23}H_{39}ClN_3O_5SiS$ 532.2068, found 532.2057, 544 (MNa^+), 532 (MH^+), 498, 366, 349, 307.

(2*S*,6*S*,7*S*,9*S*)-6-Chloro-2-((4-hydroxyphenyl)methyl)-10-oxo-9-[3-[[(phenylmethoxy)carbonyl]amino]propyl]-8-[2-(trimethylsilyl)ethanesulfonyl]-4,1,8-oxadiazabicyclo-[5.3.1]undecane (9.4). $NaHCO_3$ (1 M, 8 mL) was added to a solution of compound **9.3** (0.096 g, 0.181 mmol) and benzylsuccinimidyl dicarbonate (0.452 g, 1.81 mmol) in THF (30 mL) and water (8 mL). After 18 h, the THF was removed by rotary evaporation and the reaction mixture was diluted with saturated $NaHCO_3$ (10 mL). The mixture was extracted with CH_2Cl_2 (3 × 40 mL), the combined organic layers were dried over $MgSO_4$ and concentrated, and the residue was purified by chromatography with 40% EtOAc/hexanes to afford the Cbz derivative **9.4** (0.105 g, 87%) as a white foam, as well as a small amount of the di-Cbz compound which could be hydrolyzed with LiOH in aqueous THF to give more of the desired product: ^1H NMR (400 MHz, $CDCl_3$) δ 7.35 (m, 5), 6.97 (d, 2, J = 8.5), 6.75 (d, 2, J = 8.5), 6.23 (s, 1), 5.24 (t, 1, J = 4.6), 5.11 (s, 2), 4.20 (s, 1), 4.09 (dd, 1, J = 5.4, 13.3), 4.01 (d, 1, J = 10.5), 3.94 (t, 1, J = 4.3), 3.89 (m, 2), 3.82 (dd, 1, J = 1.5, 14.7), 3.76 (d, 1, J = 13.4), 3.65 (dd, 1, J = 2.7, 14.6), 3.38 (m, 2), 3.24 (dd, 2, J = 6.1, 12.0), 2.96 (t, 2, J = 7.7), 2.81 (dd, 1, J = 5.6, 13.1), 2.05 (m, 2), 1.76 (m, 1), 1.63 (m, 1), 1.01 (m, 2), 0.05 (s, 9); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.1, 156.7, 154.7, 136.6, 130.0, 129.7, 128.5, 128.0, 128.0, 115.5, 70.7, 70.2, 70.0, 66.6, 61.2, 59.8, 57.8, 50.1, 49.7, 40.7, 33.7, 27.6, 25.7, 9.9, −2.0; IR (film) 3347, 2950, 1703, 1673, 1515, 1250 cm^{-1}; HRMS (FAB) *m/z* calcd for MH^+ $C_{31}H_{45}ClN_3O_7SiS$ 666.2436, found 666.2448, 688 (MNa^+), 666 (MH^+), 622, 574, 532, 500, 466, 351.

(2*S*,6*S*,7*S*,9*S*)-6-Chloro-10-oxo-9-[3-[[(phenylmethoxy)-carbonyl]amino]propyl]-2-[[4-(phenylmethoxy)phenyl]-methyl]-8-[2-(trimethylsilyl)ethanesulfonyl]-4,1,8-oxadiazabicyclo[5.3.1]undecane (9.5). Benzyl bromide (72 μL, 0.603 mmol) was added to a suspension of compound **9.4** (0.040 g, 0.060 mmol) and K_2CO_3 (0.100 g, 0.724 mmol) in $CHCl_3$ (3 mL) and methanol (3 mL), and the mixture was heated to reflux. After 37 h, the reaction mixture was diluted

with EtOAc (40 mL), washed with 1 N HCl (15 mL) and saturated $NaHCO_3$ (15 mL), dried over $MgSO_4$, and concentrated, and the residue was purified by chromatography with a gradient of 30−40% EtOAc/hexanes to afford the benzyl ether **9.5** (0.041 g, 90%) as a clear oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (m, 10), 7.05 (d, 2, J = 8.6), 6.88 (d, 2, J = 8.6), 5.12 (s, 1), 5.10 (s, 2), 5.03 (s, 2), 4.21 (s, 1), 4.10 (dd, 1, J = 5.6, 13.4), 4.03 (d, 1, J = 10.4), 3.91 (m, 3), 3.83 (dd, 1, J = 1.4, 14.7), 3.78 (d, 1, J = 13.5), 3.66 (dd, 1, J = 2.6, 14.8), 3.40 (m, 2), 3.26 (dd, 2, J = 5.7, 11.6), 2.97 (t, 2, J = 7.8), 2.84 (m, 1), 2.08 (m, 2), 1.71 (m, 2), 1.02 (m, 2), 0.06 (s, 9); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.1, 157.5, 156.5, 137.0, 136.8, 130.7, 129.6, 128.5, 128.4, 128.0, 128.0, 127.9, 127.4, 115.0, 70.7, 70.2, 70.1, 70.1, 66.5, 61.2, 59.8, 57.8, 50.2, 49.8, 40.7, 33.8, 27.7, 25.9, 10.0, −2.0; IR (film) 3364, 3032, 2950, 1716, 1667, 1510, 1248 cm^{-1}; HRMS (FAB) m/z calcd for MH^+ $C_{38}H_{51}ClN_3O_7SiS$ 756.2906, found 756.2890, 778 (MNa^+), 756 (MH^+), 712, 664, 622, 590.

(2*S*,6*S*,7*S*,9*S*)-6-Chloro-10-oxo-9-[3-[[(phenylmethoxy)carbonyl]amino]propyl]-2-[[4-(phenylmethoxy)phenyl]methyl]-4,1,8-oxadiazabicyclo[5.3.1]undecane (7.6z). TBAF (82 μL of a 1.0 M solution in THF, 0.082 mmol) was added to a solution of compound **9.5** (0.031 g, 0.041 mmol) in THF (4 mL). After 2 h, methanol (4 mL) and $NaBH_4$ (0.016 g, 0.408 mmol) were added. After an additional 1 h, the reaction mixture was cooled to 0 °C and concentrated HCl was added to give a pH 1 solution, which was poured slowly into saturated $NaHCO_3$ (20 mL). This mixture was extracted with CH_2Cl_2 (3 × 40 mL), the combined organic layers were dried over $MgSO_4$ and concentrated, and the residue was purified by chromatography with 60% EtOAc/hexanes to afford the amine **7.6z** (0.021 g, 86%) as a white foam: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42 (d, 2, J = 7.1), 7.36 (m, 8), 7.06 (d, 2, J = 8.6), 6.87 (d, 2, J = 8.6), 5.11 (s, 1), 5.10 (s, 2), 5.03 (s, 2), 4.09 (dd, 1, J = 3.8, 13.1), 3.94 (m, 2), 3.88 (dd, 1, J = 3.0, 12.2), 3.68 (s, 2), 3.56 (s, 1), 3.47 (s, 1), 3.41 (m, 2), 3.34 (t, 1, J = 5.4), 3.26 (m, 1), 3.18 (m, 1), 2.85 (m, 1), 1.89 (m, 1), 1.61 (m, 2), 1.41 (m, 1); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 157.2, 156.4, 137.0, 136.6, 131.2, 129.6, 128.5, 128.4, 128.0, 127.9, 127.8, 127.4, 114.7, 72.5, 70.0, 69.9, 69.8, 66.5, 63.0, 55.5, 55.1, 49.0, 40.7, 34.1, 27.3, 26.3; IR (film) 3337, 3031, 2929, 1716, 1661, 1511, 1241 cm^{-1}; HRMS (FAB) m/z calcd for MH^+ $C_{33}H_{39}ClN_3O_5$ 592.2578, found 592.2575, 592 (MH^+), 556, 487, 456, 307.

(2*S*,6*R*,7*S*,9*S*)-6-Iodo-10-oxo-9-[3-[[(phenylmethoxy)carbonyl]amino]propyl]-2-[[4-(phenylmethoxy)phenyl]methyl]-4,1,8-oxadiazabicyclo[5.3.1]undecane (8.1z). A solution of compound **7.6z** (0.055 g, 0.093 mmol) and NaI (1.39 g, 9.29 mmol) in acetone (20 mL) was heated to reflux. After 21 h, the reaction mixture was cooled, diluted with CH_2Cl_2 (80 mL), washed with 50% saturated NaCl (20 mL) and saturated NaCl (20 mL), dried over $MgSO_4$, and concentrated, and the residue was purified by chromatography with a gradient of 50−60% EtOAc/hexanes to afford the iodide **8.1z** (0.059 g, 94%) as a white foam: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42 (d, 2, J = 7.1), 7.36 (m, 8), 7.06 (d, 2, J = 8.6), 6.87 (d, 2, J = 8.6), 5.14 (t, 1, J = 5.8), 5.09 (s, 2), 5.02 (s, 2), 4.04 (d, 1, J = 9.6), 4.01 (d, 1, J = 7.9), 3.85 (dd, 2, J = 2.7, 12.0), 3.75 (t, 1, J = 13.6), 3.69 (s, 1), 3.68 (s, 1), 3.40 (m, 2), 3.25 (t, 2, J = 6.2), 3.17 (m, 2), 2.84 (dd, 1, J = 5.1, 12.4), 1.88 (m, 1), 1.61 (quin, 2, J = 7.0), 1.40 (m, 1); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.0, 157.3, 156.5, 137.1, 136.7, 131.4, 129.7, 128.5, 128.4, 128.0, 128.0, 127.9, 127.4, 114.8, 71.6, 70.6, 70.3, 70.0, 66.5, 57.0, 56.0, 50.3, 40.8, 39.9, 34.2, 27.4, 26.4; IR (film) 3337, 3031, 2932, 1718, 1653, 1509, 1240 cm^{-1}; HRMS (FAB) m/z calcd for MH^+ $C_{33}H_{39}IN_3O_5$ 684.1934, found 684.1923, 684 (MH^+), 664, 556, 307.

(2*S*,6*S*,7*S*,9*S*)-6-[(1*S*)-2-Indolyl-1-[(methoxycarbonyl)ethyl]amino]-10-oxo-9-[3-[[(phenylmethoxy)carbonyl]amino]propyl]-2-[[4-(phenylmethoxy)phenyl]methyl]-4,1,8-oxadiazabicyclo[5.3.1]undecane (8.3z). L-TrpOMe·HCl (0.864 g, 3.39 mmol) was added to saturated $NaHCO_3$ (4 mL) and extracted with ether (3 × 6 mL). The organic extracts were dried over $MgSO_4$, and the solvent was removed to yield L-TrpOMe as a clear oil which was used immediately without further purification.

A solution of the L-TrpOMe in acetonitrile (8 mL) was added to iodide **8.1z** (0.058 g, 0.085 mmol) with a cannula. After being stirred for 41 h at rt, the solution was evaporated and the resultant oil was purified by chromatography with EtOAc to afford the adduct **8.3z** (0.061 g, 93%) as a white foam: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1), 7.59 (d, 1, J = 7.8), 7.44 (d, 2, J = 7.2), 7.39 (t, 2, J = 7.3), 7.34 (m, 7), 7.17 (dt, 1, J = 1.0, 7.6), 7.09 (dt, 1, J = 1.0, 7.5), 7.03 (s, 1), 7.02 (d, 2, J = 8.5), 6.88 (d, 2, J = 8.6), 5.15 (s, 1), 5.09 (s, 2), 5.04 (s, 2), 3.94 (dd, 1, J = 4.0, 12.3), 3.84 (dd, 1, J = 10.7, 12.2), 3.72 (dd, 1, J = 3.3, 12.3), 3.63 (s, 3), 3.59 (m, 2), 3.34 (dd, 1, J = 9.0, 13.8), 3.20 (m, 3), 3.10 (m, 4), 3.00 (dd, 1, J = 7.8, 14.4), 2.94 (t, 1, J = 3.7), 2.79 (dd, 1, J = 6.6, 13.9), 2.09 (t, 1, J = 3.4), 1.82 (m, 1), 1.57 (quin, 2, J = 7.2), 1.34 (m, 1); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.5, 172.1, 157.2, 156.5, 137.1, 136.7, 136.1, 131.6, 129.7, 128.6, 128.5, 128.0, 128.0, 127.9, 127.5, 127.4, 122.7, 122.1, 119.4, 118.8, 114.7, 111.9, 111.2, 72.0, 70.0, 70.0, 69.7, 66.5, 60.9, 60.2, 55.5, 52.7, 51.8, 49.2, 40.8, 34.2, 29.6, 27.3, 26.5; IR (film) 3415, 3333, 3033, 2928, 1716, 1651, 1511, 1239 cm^{-1}; HRMS (FAB) m/z calcd for MH^+ $C_{45}H_{52}N_5O_7$ 774.3867, found 774.3856, 774 (MH^+), 556, 512.

(2*S*,6*S*,7*S*,9*S*)-6-[[(1*S*)-1-Carboxy-2-indolylethyl]amino]-10-oxo-9-[3-[[(phenylmethoxy)carbonyl]amino]propyl]-2-[[4-(phenylmethoxy)phenyl]methyl]-4,1,8-oxadiazabicyclo[5.3.1]undecane (8.4z). Two identical reactions were performed simultaneously and were combined after the cyclization. Aqueous LiOH (2 N, 100 μL) and water (1 mL) were added to a solution of ester **8.3z** (16.0 mg, 20.7 μmol) in THF (2 mL). After 3 h, 2 N HCl (120 μL) and water (10 mL) were added. The resulting solution was lyophilized to afford the free acid **8.4z** as a white powder which was used without further purification: ^{1}H NMR (500 MHz, CD_3OD) δ 7.62 (d, 1, J = 7.7), 7.42 (d, 2, J = 7.2), 7.36 (t, 2, J = 7.6), 7.31 (m, 7), 7.25 (s, 1), 7.11 (t, 1, J = 7.1), 7.04 (t, 1, J = 7.3), 7.02 (d, 2, J = 8.5), 6.90 (d, 2, J = 8.5), 5.05 (s, 2), 4.92 (s, 2), 4.10 (m, 1), 3.95 (m, 1), 3.76 (m, 2), 3.52 (m, 2), 3.43 (m, 2), 3.21 (m, 2), 3.12 (t, 2, J = 6.5), 2.92 (s, 1), 2.85 (d, 1, J = 13.8), 2.69 (dd, 1, J = 5.8, 13.6), 2.04 (m, 1), 1.88 (m, 1), 1.58 (m, 2), 1.51 (m, 1).

(3*S*,6*S*,8*S*,12*S*,14*S*)-2,5-Dioxo-6-(indolylmethyl)-3-[3-[[(phenylmethoxy)carbonyl]amino]propyl]-12-[[4-(phenylmethoxy)phenyl]methyl]-10,1,4,7-oxatriazatricyclo[6.4.2.0^{4,14}]tetradecane (8.5z). DPPA (45 μL, 413 μmol) was added to a 0 °C solution of the free acid **8.4z** (crude, ∼20.7 μmol) and $NaHCO_3$ (0.070 g, 827 μmol) in DMF (6 mL, 3 μM), and the solution was stirred vigorously at 0 °C. After 4 h, more DPPA (45 μL, 413 μmol) was added. After an additional 11 h, the two reaction mixtures were combined, diluted with EtOAc (160 mL), washed with saturated $NaHCO_3$ (2 × 60 mL), dried over $MgSO_4$, and concentrated. The crude mixture was partially purified by chromatography with EtOAc to afford the tricyclic product **8.5z** (11.8 mg, 38%) approximately 50−80% pure.

This material was usually used directly in the next reaction, since this compound is particularly sensitive to acid and degrades on silica or in the presence of CH_2Cl_2 or $CHCl_3$. This decomposition is especially noticeable since an orange color develops due to dimerization of the indole groups. For purposes of characterization, the material was subjected to several purifications by preparative silica TLC, with EtOAc as the eluent, to afford the **8.5z** (5.9 mg, 19%) in pure form as a clear oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (s, 1), 7.59 (d, 1, J = 7.7), 7.38 (m, 2), 7.15 (m, 2), 7.08 (d, 2, J = 8.5), 6.87 (d, 2, J = 8.5), 5.62 (t, 1, J = 5.1), 5.19 (d, 1, J = 12.2), 5.11 (d, 1, J = 12.2), 5.04 (s, 2), 4.11 (d, 1, J = 15.5), 3.91 (m, 2), 3.77 (m, 3), 3.55 (m, 3), 3.44 (quin, 2, J = 7.2), 3.32 (m, 2), 3.25 (dd, 1, J = 5.6, 14.7), 3.14 (dd, 1, J = 4.6, 14.9), 3.08 (dd, 1, J = 4.6, 10.3), 2.93 (dd, 1, J = 8.1, 14.7), 2.75 (m, 1), 2.20 (m, 1), 1.76 (m, 1), 1.62 (m, 1), 1.31 (m, 1); HRMS (FAB) m/z calcd for MH^+ $C_{44}H_{48}N_5O_6$ 742.3605, found 742.3601, 742 (MH^+), 712, 698, 643, 612, 487, 429.

(3*S*,6*S*,8*S*,12*S*,14*S*)-3-(3-Aminopropyl)-2,5-dioxo-6-(indolylmethyl)-12-[4-(phenylmethoxy)-10,1,4,7-oxatriazatricyclo[6.4.2.0^{4,14}]tetradecane (8.6). $Pd(OH)_2$/C (Degussa wet, 20% Pd, 7.0 mg, 27.0 μmol) was added to a solution of crude compound **8.5z** (50−80% pure, 10.0 mg, 13.5 μmol) and HOAc (20 μL, 674 μmol) in absolute EtOH (1 mL) and 5% water/methanol (5 mL). The mixture was purged three times with H_2 and stirred under an H_2 atmosphere. After 1 h 40 min, the reaction mixture was filtered through Celite with

methanol and water. The methanol was removed by rotary evaporation at rt, and the water was lyophilized. The resultant yellow powder was purified by HPLC using a 10 μm C_{18} reverse phase preparative column (25 cm × 25 mm). A gradient of 0−50% acetonitrile/water (0.1% TFA) over 30 min was used with a flow rate of 8 mL/min. Detection at 280 nm revealed that the product eluted at 22.4 min. Lyophilization afforded the amine **8.6** (3.8 mg, 55%) as a white flocculent powder, which was only partially soluble in water, methanol, or acetonitrile but was completely soluble in 50% methanol/water or 50% acetonitrile/water: ^{1}H NMR (400 MHz, 50% CD_3CN/D_2O) δ 7.53 (d, 1, J = 7.9), 7.35 (d, 1, J = 8.1), 7.10 (s, 1), 7.09 (t, 1, J = 7.3), 7.03 (d, 2, J = 8.4), 7.00 (t, 1, J = 7.2), 6.68 (d, 2, J = 8.5), 3.98 (m, 2), 3.83 (m, 2), 3.71 (m, 2), 3.52 (m, 4), 3.36 (dd, 1, J = 8.7, 14.0), 3.21 (dd, 1, J = 5.5, 14.8), 3.03 (m, 1), 2.92 (m, 2), 2.84 (dd, 1, J = 1.8, 7.0), 2.80 (d, 1, J = 6.9), 2.53 (m, 1), 2.00 (m, 1), 1.58 (m, 2), 1.34 (m, 1); HRMS (FAB) m/z calcd for MH^+ $C_{29}H_{36}N_5O_4$ 518.2767, found 518.2775, 518 (MH^+), 451, 429, 388, 307.

(3*S*,6*S*,8*S*,12*S*,14*S*)-2,5-Dioxo-3-(3-guanidinopropyl)-6-(indolylmethyl)-12-[[4-(phenylmethoxy)phenyl]methyl]-10,1,4,7-oxatriazatricyclo[6.4.2.0^{4,14}]tetradecane (2). Compound **8.6** (2.6 mg, 5.0 μmol) and aminoiminomethanesulfonic acid[38,39] (1.2 mg, 10.0 μmol) were dissolved in methanol (3 mL) and triethylamine (3.5 μL, 25 μmol) was added. After being stirred for 2.5 h, the solution was concentrated and the residue was dissolved in methanol. The crude mixture was purified by HPLC using a 10 μm C_{18} reverse phase preparative column (25 cm × 25 mm) eluted with a gradient of 0−50% acetonitrile/water (0.1% TFA) over 30 min at a flow rate of 8 mL/min. Detection at 280 nm revealed that the product eluted at 23.8 min. Lyophilization afforded the final product **2** (2.4 mg, 86%) as a white powder, which was insoluble in water or acetonitrile but soluble in 20% DMSO/water, 50% methanol/ water, and 50% acetonitrile/water: ^{1}H NMR (400 MHz, 50% CD_3CN/D_2O) δ 7.53 (d, 1, J = 8.0), 7.38 (d, 1, J = 8.1), 7.16 (s, 1), 7.12 (t, 1, J = 7.5), 7.04 (t, 1, J = 7.4), 7.03 (d, 2, J = 8.4), 6.68 (d, 2, J = 8.4), 4.00 (m, 4), 3.79 (m, 3), 3.67 (dd, 1, J = 4.8, 12.6), 3.55 (m, 1), 3.39 (m, 2), 3.22 (m, 1), 3.11 (t, 2, J = 6.9), 3.05 (dd, 1, J = 7.1, 14.3), 2.96 (dd, 1, J = 8.4, 14.7), 2.81 (dd, 1, J = 6.8, 14.2), 2.50 (m, 1), 1.99 (m, 1), 1.44 (m, 1), 1.36 (m, 1); HRMS (FAB) m/z calcd for MH^+ $C_{30}H_{38}N_7O_4$ 560.2985, found 560.3001, 560 (MH^+), 465, 451, 441, 429, 373, 325.

Stucture Determination. General. Modeling was performed using Macromodel with the MM2* forcefield and no solvent.[43] Conformations of various compounds were found using a Monte Carlo search procedure, and conformational space was considered well-searched if each conformation was found five or more times.

NMR spectra were recorded with a Bruker AM 500 spectrometer and were processed on a Bruker X-32 data station running UXNMR. Time proportional phase incrementation (TPPI)[44] was used to obtain phase-sensitive spectra. T1 relaxation times were measured using an inversion−recovery experiment.

Bicyclic Compound 8.3z. Spectra of compound **8.3z** were recorded at 27 °C in d_6-acetone. Chemical shifts were referenced to acetone at δ 2.04 ppm for protons and δ 29.8 ppm for carbons. A 1D ^{1}H NMR spectrum was recorded and resolution enhanced to allow for accurate measurement of individual coupling constants (Table 7). A 1D ^{13}C spectrum was recorded as well as DEPT 90 and DEPT 135 spectra (Table 8).

A TOCSY spectrum was recorded using the MLEV-17 mixing sequence[45] of 100 ms duration. All $\pi/2$ pulses were 39.2 μs. An acquisition time per scan of 524 ms, a size of 4 K, and a spectral width of 3906 Hz in F2 and F1 were used; 320 experiments of four scans were performed with a relaxation delay of 1.4 s. Zero filling was performed in F2 and F1 to 4K complex × 1K real. Apodization in both dimensions used a squared sinebell shifted by $\pi/2.5$ in F2 and $\pi/2$ in F1.

Table 7. Aliphatic Proton Assignments for 8.3z

H	chem shift (δ)	mult	coupling constants (Hz)
2	3.15	m	
3	3.66	dd	3.5, 12.1
3′	3.81	dd	10.6, 12.1
5	3.88	dd	4.5, 12.2
5′	3.65	dd	<1, 12.4
6	2.13	br t	(<1, 4, 4.5)
7	3.02	br t	(1.4, 4, 5.0)
8	3.22	dd	1.4, 14.2
8′	3.27	dd	5.0, 14.5
10	3.17	dd	6, 7
12	2.74	dd	6.2, 13.7
12′	3.38	dd	9.6, 13.6
15	1.34	ddt	7, 7, 14
15′	1.80	ddt	7, 7, 14
16	1.58	quintet	7.2
17	3.12	m	
21	3.61	dd	6.1, 7.1
23	3.10	ddd	0.7, 6.0, 14.3
23′	3.00	ddd	0.7, 7.1, 14.3

[a] Spectrum recorded in d_6-acetone at 27 °C.

Table 8. Aliphatic Carbon Assignments for 8.3z

H	H chem shift (δ)	correlated carbon chem shift (δ)
2	3.15	70.6
3	3.66, 3.81	72.5
5	3.65, 3.88	70.6
6	2.13	61.5
7	3.02	53.7
8	3.22, 3.27	50.1
10	3.17	56.4
12	2.74, 3.38	34.6
15	1.34, 1.80	28.4
16	1.58	27.4
17	3.12	41.6
21	3.61	62.0
22	3.00, 3.10	29.3

[a] Spectrum recorded in d_6-acetone at 27 °C.

A DQF-COSY spectrum was obtained following the method of Derome.[46] All $\pi/2$ pulses were 13.3 μs. An acquisition time per scan of 740 ms, a size of 8 K, and a spectral width of 5556 Hz in F2 and F1 were used; 512 experiments of eight scans were performed with a relaxation delay of 1.6 s. Zero filling was performed in F2 and F1 to 4K complex × 1K real. Apodization consisted of exponential multiplication with 1.2 Hz of line broadening in F2 and a squared sinebell shifted by $\pi/2$ in F1.

A NOESY spectrum was obtained using the pulse sequence described by Bodenhausen[47] with a mixing time of 600 ms. All $\pi/2$ pulses were 13.3 μs. An acquisition time per scan of 524 ms, a size of 4 K, and a spectral width of 3906 Hz in F2 and F1 were used; 492 experiments of eight scans were performed with a relaxation delay of 3 s. Zero filling was performed in F2 and F1 to 4K complex × 1K real. Apodization in both dimensions used a squared sinebell shifted by $\pi/2$.

An HSQC (heteronuclear single quantum coherence) spectrum was obtained with refocusing and ^{13}C decoupling[48] using a GARP sequence.[49] Protons bound to ^{12}C were suppressed using a BIRD sequence followed by an inversion−recovery delay of 190 ms using the method of Bax et al.[50] Proton $\pi/2$ pulses were 22.5 μs, ^{13}C $\pi/2$ pulses were 19 μs, and a $\pi/2$ pulse of 59 μs was used for ^{13}C during the GARP sequence. An acquisition time per scan of 260 ms, a size of 2K, and a spectral width of 3937 Hz in F2 and 6410, centered on the aliphatic region, in F1 were used; 512 experiments of eight scans were performed with a relaxation delay of 100 ms. Delays were

(43) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.
(44) Marion, D.; Wüthrich, K. *Biochem. Biophys. Res. Commun.* **1983**, *113*, 967.
(45) Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *65*, 355.
(46) Derome, A. E.; Williamson, M. P. *J. Magn. Reson.* **1990**, *88*, 177.
(47) Bodenhausen, G.; Kogler, H.; Ernst, R. R. *J. Magn. Reson.* **1984**, *58*, 370.
(48) Norwood, T. J.; Boyd, J.; Heritage, J. E.; Soffe, N.; Campbell, I. D. *J. Magn. Reson.* **1990**, *87*, 488.
(49) McCoy, M. A.; Mueller, L. *J. Magn. Reson.* **1993**, *101*, 122.
(50) Bax, A.; Subramanian, S. *J. Magn. Reson.* **1986**, *67*, 565.

Table 9. Aliphatic Proton Assignments for 2

H	chem shift (δ)	multiplicity	coupling constants (Hz)
2	3.72	m	
3	3.87	dd	3.0, 13.0
3′	3.93	dd	10.1, 13.1
5	3.70	m	
5′	3.66	m	
6	3.04	ddd	5.0, 9.5, 11.0
7	3.99	ddd	2.2, 2.2, 9.4
8	4.08	m	
10	4.11	m	
12	2.89	dd	6.3, 14.7
12′	3.45	dd	9.8, 14.6
15	2.58	m	
15′	2.12	m	
16	1.48	m	
17	3.21	dt	2.2, 7.1
21	3.88	dd	5.6, 6.9
23	2.90	dd	5.8, 15.0
23′	3.27	dd	6.6, 15.0

[a] Spectrum recorded in 20% d_6-DMSO/D_2O at 28 °C.

optimized for a coupling constant of 131 Hz. Zero filling was performed in F2 and F1 to 2K complex × 1K real. Apodization consisted of exponential multiplication with 4 Hz of line broadening in F2 and a squared sinebell shifted by $\pi/2$ in F1.

An HMBC (heteronuclear multiple-bond correlation) spectrum was obtained using the method of Bax.[51] The experiment was recorded in mixed mode using TPPI[44] in F1. Proton $\pi/2$ pulses were 17.1 μs, and ^{13}C $\pi/2$ pulses were 7.2 μs. Delays were optimized for a long-range coupling constant of 8.3, allowing one-bond C−H couplings to be filtered out. An acquisition time per scan of 520 ms, a size of 4 K, and a spectral width of 3937 Hz in F2 and 20,833 Hz in F1 were used; 800 experiments of 16 scans were performed with a relaxation delay of 1.4 s. Data were processed using a magnitude calculation in the F2 dimension only. Zero filling was performed in F2 and F1 to 2K complex × 1K real. Apodization consisted of exponential multiplication with 2 Hz of line broadening in F2 and a squared sinebell shifted by $\pi/2$ in F1.

Tricyclic Compound 2. Spectra of compound **2** were recorded at 28 °C in 20% d_6-DMSO/D_2O. Chemical shifts were referenced to HDO at δ 4.74 ppm. All 2D spectra were obtained with an acquisition time per scan of 490 ms, a size of 4K, and a spectral width of 4167 Hz in F2 and F1. Zero filling was performed in F2 and F1 to 4K complex × 1K real. Apodization in both dimensions used a square sinebell shifted by $\pi/3$ in F2 and $\pi/2$ in F1.

A 1D ^{1}H NMR of 1K scans was recorded and resolution enhanced to allow for accurate measurement of individual coupling constants (Table 9). A 1D decoupling experiment was also performed to elucidate the magnitude of a critical coupling constant. Irradiation at 3.06 ppm collapsed the doublet of triplets at 4.00 ppm to a triplet, positively indicating that the coupling constant between these two protons was 9.2 Hz.

A TOCSY spectrum was recorded using the MLEV-17 mixing sequence[45] of 100 ms duration. All $\pi/2$ pulses were 34.8 μs; 512 experiments of 24 scans were performed with a relaxation delay of 450 ms.

A DQF-COSY spectrum was obtained following the method of Derome.[46] All $\pi/2$ pulses were 13.3 μs; 512 experiments of 48 scans were performed with a relaxation delay of 1.0 s.

A ROESY spectrum was obtained using the pulse sequence described by Rance with a *Z*-filtered pulsed spinlock of 300 ms duration and an effective field strength 2.2 kHz.[52] All $\pi/2$ pulses were 22.5 μs; 588 experiments of 32 scans were performed with a relaxation delay of 1.3 s.

Enzymology. Porcine pancreatic α-amylase (type 1-A) was obtained from Sigma; *p*-nitrophenyl maltotrioside (*p*-NPG_3) was obtained as a generous gift from Boehringer-Mannheim. All solutions were prepared using doubly distilled deionized water and were passed through a 0.45-μm filter. All assays were performed at 30 °C. The forward reaction rate was measured by monitoring the liberation of *p*-nitrophenol from the *p*-NPG_3 substrate at 405 nm ($\Delta\epsilon$ = 9500 M^{-1} cm^{-1}).[6] Enzyme dilutions were made with buffer containing 50 mM HEPES (pH 7.0), 60 mM NaCl, 2 mM $CaCl_2$, and 0.1 mg/mL BSA. The assay mixture contained 40 nM enzyme in 25 mM HEPES (pH 7.0), 30 mM NaCl, and 1 mM $CaCl_2$ in 5% DMSO/H_2O in a total volume of 1 mL; the solution was equilibrated for 3 min at 30 °C prior to addition of substrate.

Five substrate concentrations were used (0.40, 0.50, 0.70, 1.0, 3.0 mM) with two independent determinations done at each concentration to determine K_m. K_i measurements were performed with substrate concentration equal to K_m. Inhibitor concentrations from DMSO stock solutions were measured spectrophotometrically using the absorbance values for one tyrosine and one tryptophan (λ = 280 nm, ϵ = 6000 M^{-1} cm^{-1}).[53] After substrate addition, 20 absorption points were recorded in a 10-min period; the reaction was less than 2% complete at this point and good first-order kinetics were observed. Initial rates were calculated using the kinetics module of the Uvikon 860. No background correction was necessary, since a background rate was not observed when the assay was run either without substrate or enzyme. K_m was computed using the Enzfitter program.[54] K_i values were calculated by a Dixon analysis, assuming competitive inhibition, performed in Enzfitter using the equation $V_o/V_i = [I]/K_i(1 + [S]/K_m) + 1$.

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Supporting Information Available: ^{1}H NMR spectra for compounds not submitted to elemental analysis (17 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(51) Bax, A.; Marion, D. *J. Magn. Reson.* **1988**, *78*, 186.
(52) Rance, M. *J. Magn. Reson.* **1987**, *74*, 557.
(53) Wetlaufer, D. B. In *Advances in Protein Chemistry*, Vol. 17; Anfinsen, C. B., Jr., Anson, M. L., Bailey, K., Edsall, J. T., Eds.; Academic Press: New York, 1962; p 303.
(54) Leatherbarrow, R. J. *ENZFITTER*; Elsevier Science: 1987.
(55) Haasnoot, C. A. G.; De Leeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783.