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Conformational Study and Enantioselective, Regiospecific Syntheses of Novel Aminoxy *trans*-Proline Analogues Derived from an Acylnitroso Diels−Alder Cycloaddition

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The cis/trans isomerization of the proline amide bond has many implications in biological processes. The conformations of representative acylnitroso-derived proline analogues derived from cyclopentadiene were shown to exist exclusively as the *E* or trans conformation in CD_2Cl_2. The energetically favored conformations were determined using COSMO self-consistent reaction field calculations at the B3LYP/6-31G* level of theory in addition to low temperature ^{1}H NMR studies. The syntheses of the acylnitroso-derived peptides utilized two methods to selectively functionalize either of two chemically similar esters in the acylnitroso-derived amino acids. A novel transpeptidation of the amino acid that controlled the absolute stereochemistry in the acylnitroso Diels−Alder cycloaddition took advantage of an activated aminoxy amide linkage to control regiochemistry. Alternatively, an enantioselective and regiospecific enzymatic resolution of a racemic dimethyl ester provided a novel aminoxy acid.

Introduction

Conformationally restricted molecules are used to represent bioactive conformations of substrates for a particular biological targets. The design and syntheses of peptide mimetics that are conformationally restricted through covalent bonds[1] is a rapidly expanding area. Likewise, geometrically favored intramolecular hydrogen bonds[2−5] can bias a substrate into a given conformation. Medicinally, this approach has great potential for the design of new therapeutic agents.[1]

The cis/trans isomerization of the Xaa-proline amide bond has many implications in biological systems.[6−10] Prolines and 4-hydroxy prolines are important constitutents of collagen.[6] In addition, proline is integral to protein folding,[7] regulatory switches,[8] peptide antigens,[9] and peptide hormones.[10] Since *N*-acylated prolines are tertiary amides, they are unique among naturally occurring amino acids in that they exist as mixtures of cis and trans isomers about the N−CO bond.[11] In large proteins, approximately 10% of the Xaa-pro bonds are in the *cis* conformation.[11a] Nature has chosen peptidyl-prolyl isomerases[12] such as the FK506 binding protein and the cyclophilins to catalyze cis/trans isomerization processes intracellularly. The design and syntheses of small conformationally restricted mimetics that exist preferentially as *cis*- or *trans*-proline analogues is, therefore, of interest.[13]

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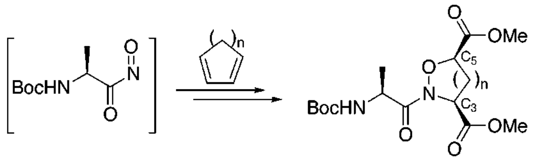
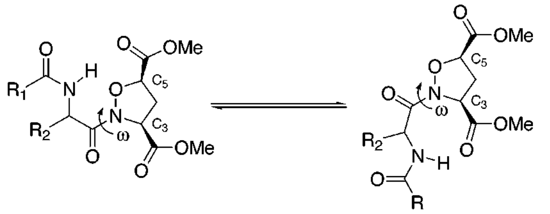
Previously, we have reported the synthesis of a novel cyclic disubstituted aminoxy dipeptide **2**[14] (eq 1, $n = 1$) utilizing the cycloadduct obtained from the Diels–Alder reaction[15] between cyclopentadiene and acylnitroso species **1**, generated transiently from the corresponding L-alanine-derived hydroxamic acid.[14a,16] The cyclic aminoxy acid[17] obtained from the acylnitroso Diels–Alder cycloaddition possesses three sites for functionalization, which include (1) the disubstituted hydroxylamine nitrogen, which is already functionalized in **2** with Boc-L-ala, (2) the C-3 ester, and (3) the C-5 ester. The relative stereochemistry of newly generated *syn*-stereocenters of **2** are controlled in the acylnitroso cycloaddition. Moreover, the use of larger dienes[18] allows control of the ring size ($n = 1-3$) that makes up the disubstituted acylated hydroxylamine.



(1)

1 **2**

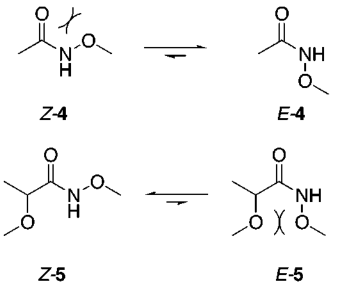
Acylnitroso-derived peptides, represented in a general sense as **3**, may exist as *E*-**3** ($w \approx 180°$) or *Z*-**3** ($w \approx 0°$) with respect to the aminoxy amide (eq 2).[19] The conformation of the aminoxy amide would presumably determine whether noncovalent interactions, such as hydrogen bonding, could occur with functionalities attached at C-3 or C-5. Intuition would favor predominance of *E*-**3** ($w \approx 180°$) due to the presence of the repelling dipoles of the aminoxy amide, therefore allowing it to represent a *trans*-proline analogue.[19b] In fact, Ac-5-oxa-Pro has been shown to exist exclusively in the trans conformation.[20]



(2)

E-**3** *Z*-**3**

However, when heteroatom containing substitutents, such as OMe, are in the α-position of aminoxy amides, the *Z*-configurations are more stable (eq 3).[21] For example, the relative energies of the *Z* and *E* conformations of aminoxy amides **4** and **5** have been determined using density functional calculations.[21c] The calculations indicated that *E*-**4** was more stable than *Z*-**4** by 0.8 kcal/mol in $CHCl_3$ due to electron/electron repulsions between the oxygen atoms of the aminoxy amide in *Z*-**4**, as would be expected. However, in **5** the α-methoxy group renders *Z*-**5** more stable than *E*-**5** by 2.8 kcal/mol in $CHCl_3$. The electrostatic repulsion between the methoxy groups in *E*-**5** is apparently more destabilizing than the repulsion between the oxygen atoms of the aminoxy amide in *Z*-**5**.



(3)

Herein, we report reproducible, regiospecific, and enantioselective methodology to synthesize peptides related to **2** or **3**. Regioselective functionalization of either of the methyl esters in an acylnitroso peptide such as **3** would be necessary before rationally designed *trans*- or *cis*-proline-like systems could be obtained. In addition, *E*,*Z*-conformational analyses of representative *N*-acylated acylnitroso-derived cyclic aminoxy amides utilizing density functional calculations and variable temperature ^{1}H NMR are described.[19]

Results and Discussion

Synthesis. We envisioned two methods to synthesize the desired peptides in a regio- and stereocontrolled fashion (Scheme 1). Both of these methods rely on an acylnitroso-derived diester represented as **6**.[14a] If diester **6** was formed utilizing an L-Ala-derived acylnitroso Diels–Alder cycloaddition, deprotection, and cyclization should lead regiospecifically to diketopiperazine **7**. Then, upon methanolysis of the activated aminoxy amide, the intermediate, substituted hydroxylamine **8**, should be

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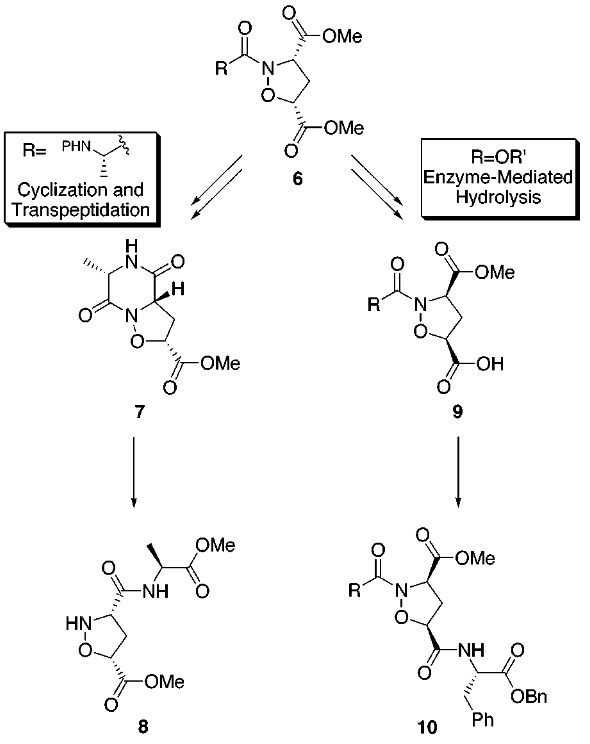
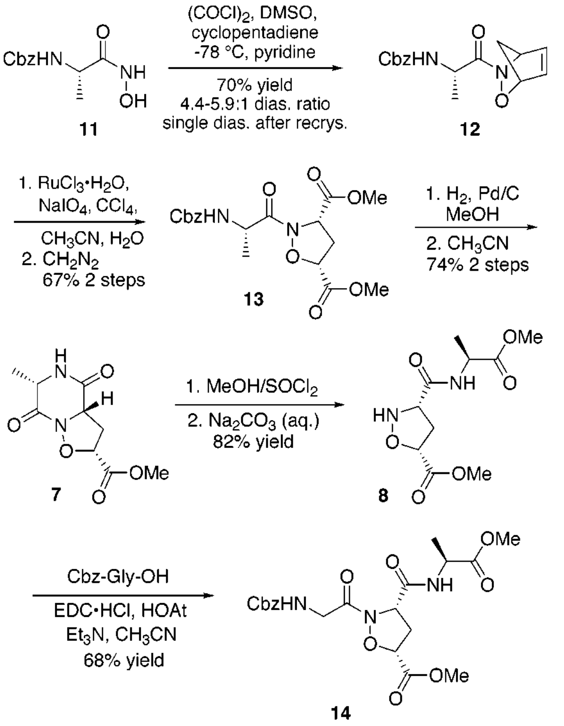
Scheme 1



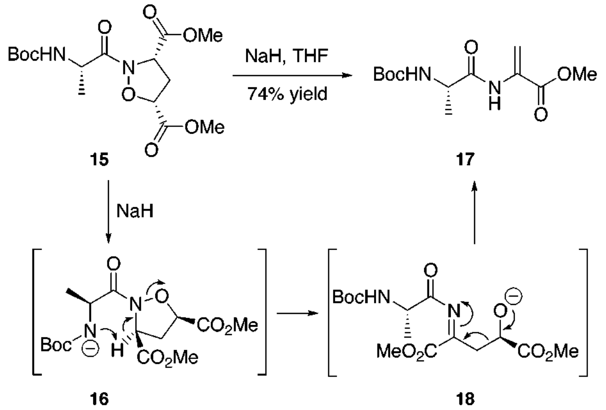
obtained. The net reaction would constitute a novel transpeptidation of the amino acid initially utilized to control both newly generated stereogenic centers.[18] Another route would be to employ an enzyme-mediated simultaneous enantio- and regioselective hydrolysis of (±)-**6** (R = OR′) to provide an acid/ester such as **9**. The carboxylic acid in **9** would be available for coupling reactions to provide an ester/amide such as **10**.

Implementation of the nonenzymatic, transpeptidation route is shown in Scheme 2. Oxidation of hydroxamic acid **11** under Swern conditions[22] in the presence of cyclopentadiene provided the previously reported cycloadduct[16b] **12** in 70% combined yield as a 4.1:1 to 5.9:1 mixture of diastereomers, as determined by HPLC analyses.[16c] Recrystallization provided **12** as a single diastereomer. Oxidation of **12** with RuO_4, generated in situ from $NaIO_4$/$RuCl_3{\cdot}H_2O$,[14a,23] provided dimethyl ester **13** in 67% yield after treatment with ethereal CH_2N_2. Removal of the Cbz protecting group with Pd/C under a H_2 atmosphere provided the corresponding free amine that was dissolved in CH_3CN to provide diketopiperazine **7** in 74% yield for the deprotection–cyclization sequence. It should be pointed out that diketopiperazine **7** represents a conformationally restricted *cis*-γ-substituted proline mimetic. Upon treating **7** with premixed $SOCl_2$/MeOH, opening of the diketopiperazine occurred to provide **8** in 82% yield after neutralization with aqueous Na_2CO_3. The transpeptidation of the amino acid to the C-3 ester proceeded in 61% combined yield for four steps beginning with dimethyl ester **13**. As a representative example, disubstituted hydroxylamine **8** was coupled with Cbz-Gly, utilizing 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) in CH_3CN in the presence of Et_3N and 1-hydroxy-7-azabenzotriazole (HOAt),[24] to give **14** in 68% yield.

Scheme 2



Scheme 3



During the course of these studies, an interesting N–O bond cleavage was discovered (Scheme 3). In an attempt to cyclize Boc amide **15** directly to the corresponding diketopiperazine by deprotonation with NaH in THF, dehydroalanine-containing dipeptide **17**[25] was isolated in 74% yield. NMR and mass spectral data were consistent with the structure of **17**. The NMR also matched literature data[25b] and was similar to spectra of related dehydroalanine derivatives previously prepared in our laboratory.[25d] Although this pathway is speculative, this unexpected product might arise by initial deprotonation of the Boc-carbamate to give anion **16**. Proton transfer with simultaneous N–O bond cleavage would then give imine

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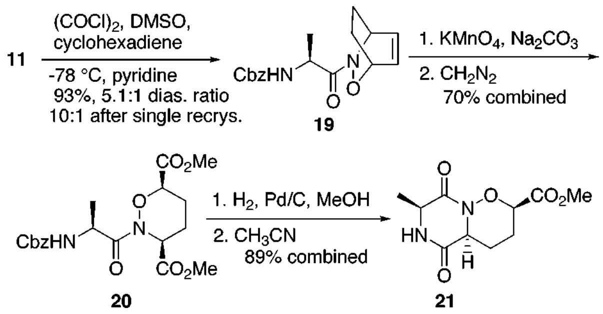
Scheme 4



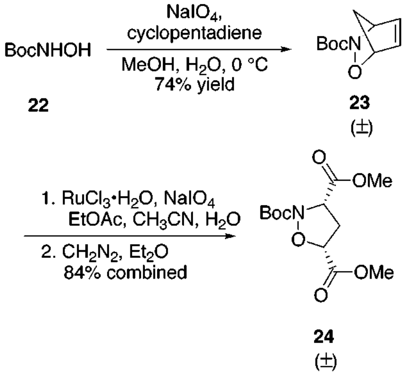
18,[26] and subsequent loss of methyl glyoxylate would give **17** after workup. We have observed similar anion-induced N−O bond cleavage reactions in our earlier work on substituted *N*-hyroxy *β*-lactams.[26a]

To increase the size of the ring containing the disubstituted hydroxylamine, a larger diene such as cyclohexadiene was used. As shown in Scheme 4, oxidation of hydroxamic acid **11** under Swern conditions in the presence of 1,3-cyclohexadiene provided cycloadduct **19** in 93% combined yield as a 5.1:1 diastereomeric mixture, as determined by ^{1}H NMR analysis of the crude reaction mixture. The diastereomeric ratio was enhanced to 10:1 by a single recrystallization. While further manipulations were performed on the 10:1 diastereomeric mixture, subsequent recrystallizations were required to provide cycloadduct **19** as a single diastereomer, at which point an X-ray crystal structure determination confirmed the expected stereochemistry. The RuO_4 protocol utilized previously afforded low yields of **20**. However, oxidation of the 10:1 diastereomeric mixture with $KMnO_4$ in the presence of Na_2CO_3, followed by treatment with CH_2N_2, provided **20** in a 70% combined yield as a mixture of diastereomers. Without separation, the diastereomeric mixture was subjected to Pd/C under a H_2 atmosphere, yielding the diastereomeric free amines. After dissolving the diastereomeric amines in CH_3CN, diketopiperazine **21** was isolated as a single diastereomer in 89% combined yield for both steps. The deprotected amine derived from the minor diastereomer did not cyclize under the reaction conditions. Thus, intramolecular diketopiperazine formation readily differentiates the two methyl esters of the elaborated acylnitroso Diels−Alder cycloadducts **6** and homologues.

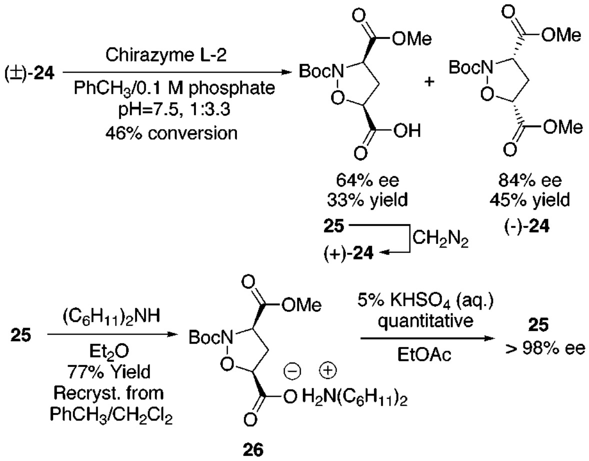
To pursue the proposed enzymatic methyl ester differentiation, racemic dimethyl ester **24** was utilized (Scheme 5).[14b] Oxidation of BocNHOH (**22**) with $NaIO_4$ in $MeOH/H_2O$ at 0 °C in the presence of cyclopentadiene provided (±)-**23** in 74% yield.[27] Oxidative cleavage of alkene (±)-**23** with RuO_4 in $EtOAc/CH_3CN/H_2O$, generated in situ from $NaIO_4/RuCl_3{\cdot}H_2O$, provided (±)-**24** in 84% combined yield after treatment with ethereal CH_2N_2.

Although dimethyl ester **24** was subjected to reactions with a variety of enzymes, the best results were obtained with Chirazyme L-2[28] in toluene and 0.1 M phosphate buffer at pH = 7.5 (Scheme 6). Under these conditions acid **25** was obtained in 33% yield (64% ee) along with diester (−)-**24** in 45% yield (84% ee) at 46% conversion. If the reaction was quenched at 25% conversion, an 18% yield of acid **25** was obtained in 68% ee. To purify and increase the enantiomeric purity of **25**, dicyclohexylamine salt **26** was generated in Et_2O and precipitated in 77% yield. After a single recrystallization from $PhCH_3/CH_2Cl_2$ (64% recovery), followed by regeneration of the corresponding free acid utilizing 5% aqueous $KHSO_4$, **25** was obtained in >98% ee. From (±)-**24**, **25** was obtained in an overall 16% yield (32% based on only (+)-**24** in (±)-**24**) in >98% ee as a single regioisomer. Analysis of the X-ray crystal structure ($CH_2Cl_2/PhCH_3$) of **26** confirmed the regiochemistry as shown. To determine enantiomeric excesses, (+)-**24** was first obtained by treatment of **25** with CH_2N_2. Then, the Boc protecting groups of dimethyl esters (−)-**24** and (+)-**24** were individually removed with HCl(g)/EtOAc. Formation of the corresponding Mosher's amides then allowed % ee determinations by ^{19}F NMR.

Scheme 5



Scheme 6



The absolute configuration of acid **25** was determined indirectly by determination of the absolute configuration of (−)-**24** as shown in Scheme 7. Conversion of optically enriched (−)-**24** to the corresponding free amine occurred in quantitative yield by removal of the Boc protecting group with HCl(g) in EtOAc, followed by partitioning of the resulting HCl salt between 5% Na_2CO_3 (aq) and EtOAc. Coupling of the free amine, derived from the recovered starting diester, with Cbz-L-Ala, mediated by *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU)[24] in CH_3CN in the presence of Et_3N, provided diester **13** and the minor diastereomer

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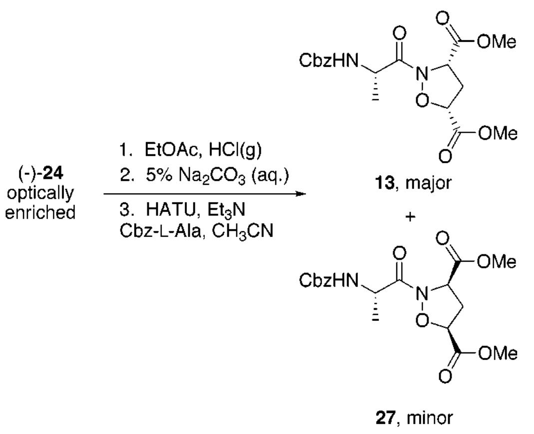
[26] (a) Lee, B. H.; Biswas, A.; Miller, M. J. *J. Org. Chem.* **1986**, *51*, 106. (b) Endo, Y.; Uchida, T.; Hizatate, S.; Shudo, K. *Synthesis* **1994**, 1096. (c) Wu, M.; Begley, T. P. *Org. Lett.* **2000**, *2*, 1345. (d) Desai, M. C.; Doty, J. L.; Stephens, L. M.; Brighty, K. E. *Tetrahedron Lett.* **1993**, *34*, 961.

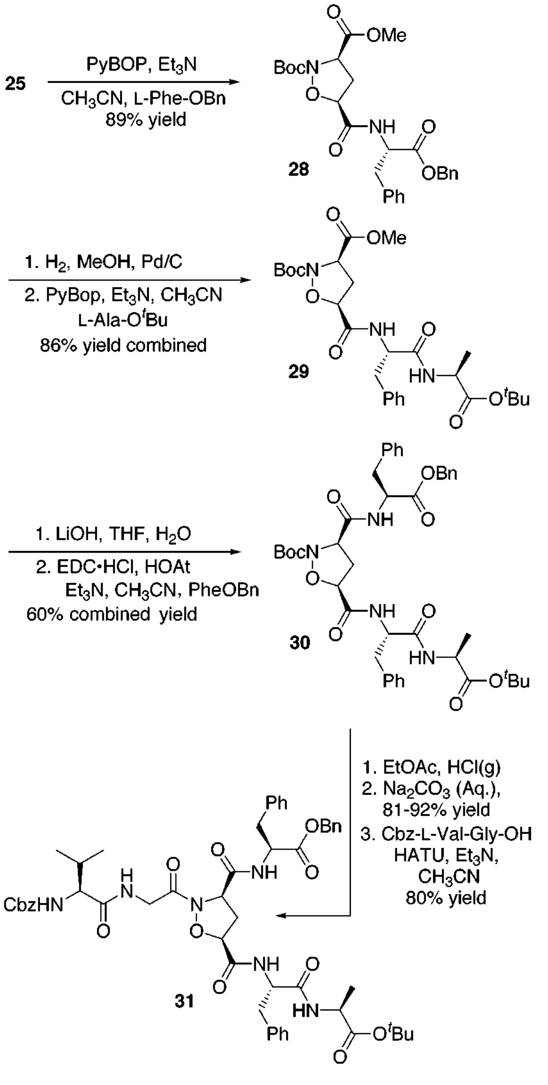
[27] Zhang, D.; Miller, M. J. *J. Org. Chem.* **1998**, *63*, 755.

[28] Purchased from Boehringer Mannheim which is now Roche Molecular Biochemicals. The active enzyme in Chirazyme L-2, lyo. is *Candida antarctica* F-B.

Scheme 7



Scheme 8



27. The major diastereomer **13** was identical to the diester obtained from the amino acid-derived acylnitroso Diels–Alder cycloaddition in Scheme 2, by TLC and ^{1}H NMR analyses both of which readily distinguished diastereomers **13** and **27**.

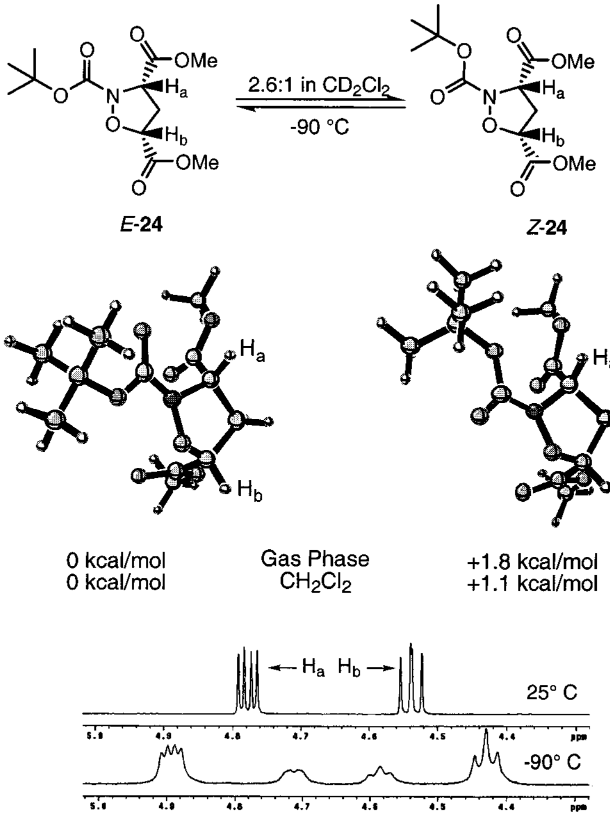


Figure 1. Computational and NMR Studies of **24**.

With enantiomerically pure **25** in hand, an arbitrary target peptide **31** was chosen to further demonstrate the methodology (Scheme 8). Activation of acid **25** with benzotriazol-1-yl-oxytripyrrolidine phosphonium hexafluorophosphate (PyBOP)[29] in the presence of the free base of L-Phe-OBn and Et_3N in CH_3CN provided differentially protected diester **28** in 89% yield. Hydrogenation, utilizing Pd/C in MeOH under a H_2 atmosphere provided, in quantitative yield, the corresponding acid that was used without further purification. Treatment of the acid with PyBOP in CH_3CN in the presence of HCl•L-Ala-OC$(CH_3)_3$ and Et_3N provided peptide **29** in 86% combined yield for the two steps. Saponification of **29** using 4.0 equiv[30] of 0.1 N LiOH (aq) in THF at −10 °C provided the corresponding acid in 99% crude mass recovery. Coupling of the acid derived from **29** with L-Phe-OBn using EDC•HCl in CH_3CN in the presence of HOAt and Et_3N provided peptide **30** in 60% overall yield. Selective deprotection of the Boc aminoxy carbamate in preference to the *tert*-butyl ester of **30**, with HCl(g) in EtOAc, gave the corresponding free amine in 81–92% yield. The free amine was then coupled with Cbz-L-Val-Gly-OH mediated by HATU in the presence of Et_3N in CH_3CN, to give the target peptide **31** in 80% yield.

Conformational Studies. Determination of the *E* to *Z* conformation of representative acylated acylnitroso-derived amino acid derivatives in solution was determined using variable temperature ^{1}H NMR[19] and quantum mechanical calculations. For example, at −90 °C in CD_2Cl_2, the ratio of conformers of diester **24** was demonstrated to be 2.6:1 by ^{1}H NMR (Figure 1). A ratio of 2.6:1 would correspond to an energy difference of 0.35 kcal/mol at −90 °C. One might expect only a small difference in stability between the two conformers since

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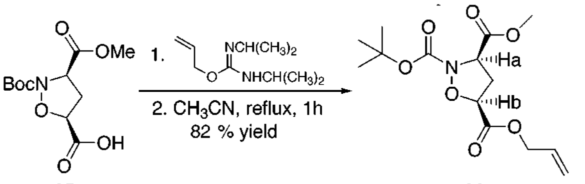
[29] Coste, J.; Le-Nguyen, D.; Castro, D. *Tetrahedron Lett.* **1990**, *31*, 205.

[30] When 1.0 equiv of LiOH was utilized a 3:1 mixture of product to an unidentified unseparable impurity was obtained. We found that the equivalency of LiOH had an effect. Therefore, to avoid this impurity, 4.0 equiv of LiOH must be utilized.

both *E*-**24** and *Z*-**24** have an oxygen of the carbamate (either the carbonyl O or the O−C(CH$_3$)$_3$) that can interact with the oxygen lone pairs of electrons of the N−O bond. In accordance with the literature,[19b] H$_a$ was shifted downfield in the *E* conformation.

The assignment of the proton labeled as H$_a$ as the downfield absorption was made by analogy to methyl/allyl ester **32** (eq 4). Treatment of acid **25** with *O*-allyl-*N*,*N'*-diisopropyl isourea[31] followed by refluxing in CH$_3$CN provided allyl/methyl ester **32** in 81% yield. Long-range HMBC NMR experiments using **32** in CD$_2$Cl$_2$ confirmed H$_a$ as the downfield absorption by correlation of H$_a$ with the carbonyl carbon of the methyl ester. Moreover, proton H$_a$ showed a cross-peak with the carbamate carbonyl carbon of the Boc protecting group. Additionally, a cross-peak was present between H$_b$ and the carbonyl carbon of the allyl ester functionality.



25 **32** (4)

To gain additional insights into the conformational assignment, density functional calculations were performed using the G98 series of programs.[32] The geometries of the *E* and *Z* conformers of **24**, **33**, and **34** were fully optimized at the B3LYP/6-31G* level of theory. Energies were corrected for zero-point energy, obtained from normal-mode analysis at the same level of theory. Relative energies in solution were evaluated by self-consistent reaction field single-point calculations using the gas-phase geometries. The B3LYP/6-31+G** level of theory and the conductor-like screening solvation model (COSMO)[33] were used to account for the electrostatic effects of solvation. The dipole moment of the solvent continuum was chosen to reproduce the dielectric constant of CH$_2$Cl$_2$. The calculations confirmed that *E*-**24** was more stable. The energy differences were 1.8 kcal/mol in gas phase and 1.1 kcal/mol in CH$_2$Cl$_2$. This would correspond to a 6.4:1 ratio of conformers at room temperature, thus slightly overestimating the experimentally observed ratio.

Since carbamates are known to exist as mixtures of rotamers,[34] the carbamate was replaced with an acetamide (Figure 2). Treatment of **24**, with HCl(g) in EtOAc provided the corresponding HCl salt. Acetylation with AcCl in CH$_2$Cl$_2$ in the presence of Et$_3$N and (dimethylamino)pyridine (DMAP) provided aminoxy acetamide **33** in 61% combined yield. Only a single conformer was observed at −85 °C in CD$_2$Cl$_2$. Density functional calculations were in agreement with these results. The calculations indicated that *E*-**33** was 6.0 kcal/mol more stable than the Z-**33** in the gas phase. When the dielectric constant of the continuum was chosen to represent CH$_2$Cl$_2$ as a solvent, the electrostatic repulsion between the carbonyl and the ring oxygens gets screened by the dielectricum. *E*-**33** was therefore calculated to be only 3.7 kcal/mol more stable than the Z-**33**.

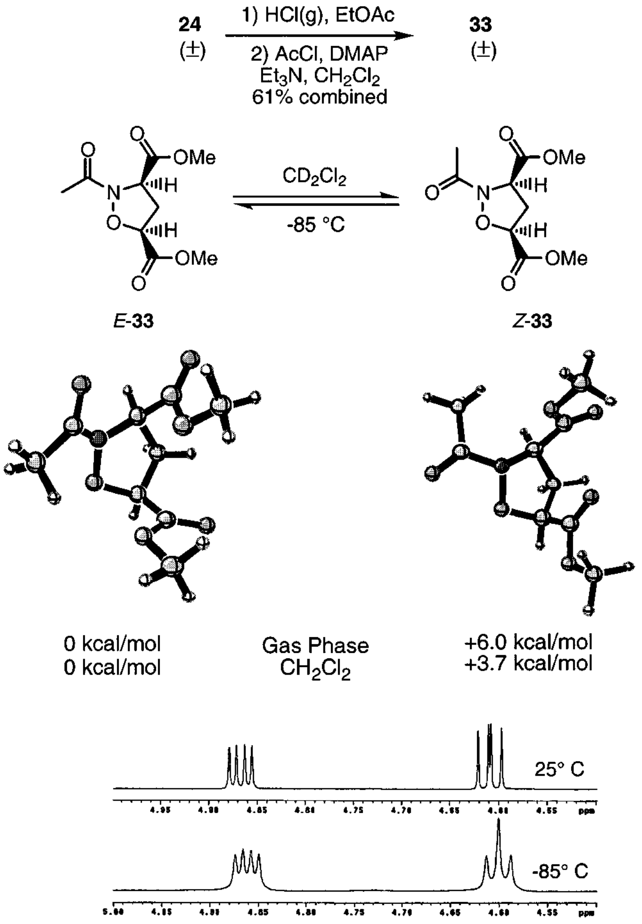


Figure 2. Computational and NMR Studies of **33**.

A question that still remained was whether a NH at the α-position of acetamide **34** would stabilize either of the conformers. As previously discussed, literature precedent indicates that the *Z*-conformer is more stable when an α-OMe is incorporated into aminoxy amides.[21a−c] Deprotection of **24** with HCl(g) in EtOAc followed by neutralization provided the corresponding free disubstituted hydroxylamine (Figure 3). The Gly derived peptide **34** was obtained in 51% yield after the HOSu active ester of Ac-Gly was treated with the free amine obtained from **24** in the presence of HOAt. Density functional calculations indicated that *E*-**34** was 9.1 kcal/mol more stable than the Z-**34** in the gas phase. When the dielectric was chosen to represent CH$_2$Cl$_2$ as solvent, *E*-**34** was 7.0 kcal/mol more stable than the Z-**34**. At least a portion of the

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(31) Mathias, L. J. *Synthesis* **1979**, 561.

(32) Gaussian98, Revision A9: Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, Jr., J. A.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. Gaussian, Inc., Pittsburgh, PA, 1998.

(33) a) Barone, V.; Cossi, M. *J. Phys. Chem. A* **1998**, *102*, 1995. For recent studies of the performance of the model, compare: (b) Rablen, P. R. *J. Org. Chem.* **2000**, *65*, 7930. (c) Nielsen, P. A.; Norrby, P. O.; Liljefors, T.; Rega, N.; Barone, V. *J. Am. Chem. Soc.* **2000**, *122*, 3151.

(34) (a) Kost, D.; Kornberg, N. *Tetrahedron Lett.* **1978**, *35*, 3275. (b) Marcovici-Mizrahi, D.; Gottlieb, H. E.; Marks, V.; Nudelman, A. *J. Org. Chem.* **1996**, *61*, 8402. (c) Moraczewski, A. L.; Banaszynski, L. A.; From, A. M.; White, C. E.; Smith, B. D. *J. Org. Chem.* **1998**, *63*, 7258.

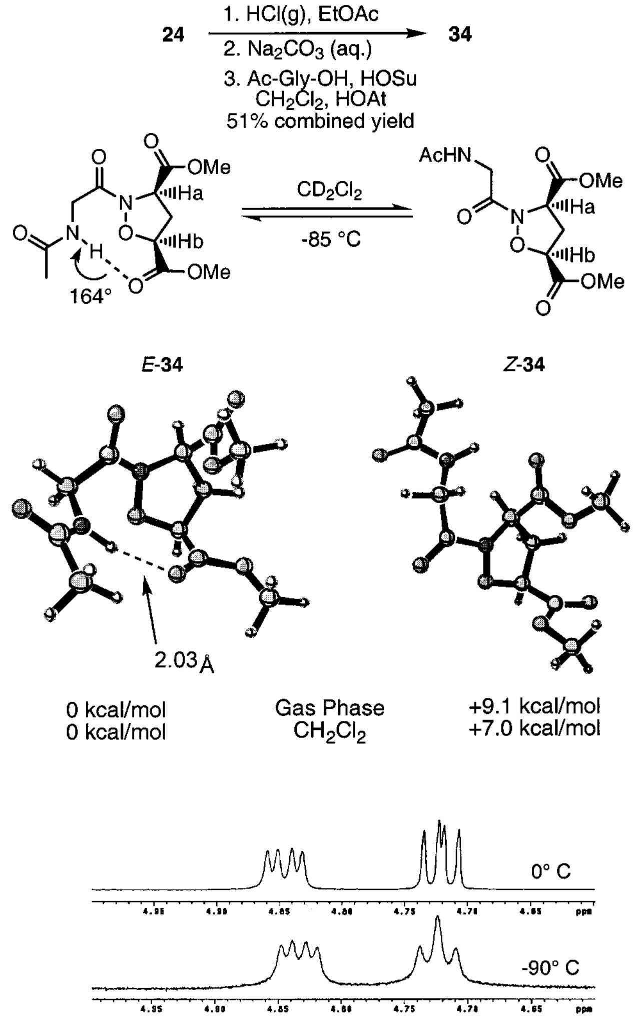

Figure 3. Computational and NMR Studies of **34**.

added stabilization compared to *E*-**33** was attributed to a hydrogen bond between the AcNH and the C-5 methyl ester as indicated in Figure 3. The hydrogen bond distance according to the calculations was 2.03 Å with an NHO angle of 164°. However, experimentally this hydrogen bond was not observed by ^{1}H NMR titration studies and FT-IR analysis[35] in CH_2Cl_2. It is possible that this hydrogen bond is an artifact of the geometry optimization in the gas phase, where no alternative hydrogen bond donors or acceptors are available. This leads in many cases to the well-known globulization of polar compounds in gas-phase calculations. Nevertheless, the repulsion between the carbonyl and ring oxygens still favors *E*-**34** by several kcal/mol, as is evident from the ^{1}H NMR spectrum shown in Figure 3.

When the ^{1}H NMR of **34** was measured in CD_2Cl_2 at 0 °C the spectrum shown in Figure 3 was obtained. Upon cooling of the sample to −90 °C, the chemical shifts of the protons labeled H_a and H_b were essentially the same. This indicates the presence of a single conformer.

Conclusions

In conclusion, we have demonstrated two methods for selective functionalization of chemically similar methyl esters of an acylnitroso-derived amino acid. A novel transpeptidation of the amino acid that controlled the absolute stereochemistry in the subsequently prepared and described peptides was utilized taking advantage of the activated aminoxy amide linkage. Alternatively, an enantioselective regiospecific, enzymatic resolution of a racemic dimethyl ester provided a novel amino acid. The conformations of representative acylnitroso-derived amino acid derivatives were shown to exist exclusively as the *E* or trans conformers. These types of derivatives may then be considered *trans*-proline mimetics. With the conformation of the aminoxy linkage determined in CD_2Cl_2 and the regioselective functionalization of the acylnitroso-derived peptide demonstrated, access to rationally designed conformationally restricted molecules is now possible. Further conformational studies in more physiologically relevant aqueous solvents will provide more insight on the potential of these new proline analogues for the design of conformationally controlled peptides. The utility of these analogues for use as scaffolds in combinatorial chemistry for development of biologically active compounds also is under active consideration.

Experimental Section

General Methods. DMF and DMSO were distilled from $MgSO_4$ or 3 Å molecular sieves onto 3 Å molecular sieves and stored under Ar. Et_3N, CH_3CN, and CH_2Cl_2 were distilled directly before use from CaH_2 under Ar. THF was distilled from Na/benzophenone ketyl under Ar. Melting points were performed on a Thomas-Hoover Capillary Melting Point Apparatus and are uncorrected. ^{1}H NMR were taken at 300, 500, or 600 MHz as indicated on Varian Unity spectrometers. ^{13}C NMR were taken at 75, 125, or 150 MHz on Varian Unity spectrometers. ^{1}H NMR are reported in parts per million (ppm) relative to tetramethylsilane (0.00 ppm), residual $CHCl_3$ (7.26 ppm), or residual CD_3SOCD_2H (2.49 ppm). ^{13}C NMR are reported in ppm relative to $CDCl_3$ (77.0 ppm), or C_2D_6SO (39.5 ppm). Coupling constants are reported in Hz. Optical rotations were obtained utilizing a Rudolph Research Autopol III polarimeter with a 1.0 dm cell length at ambient temperature. TLC was performed utilizing silica gel 60 F_{254}. Visualization was typically performed by UV light, $KMnO_4$, ninhydrin, or PMA stain. Flash chromatography was performed utilizing silica gel 60 (30−70 mm irregular particles). HPLC was performed on a Alltech Econosil column (5μ, 4.6 × 250 mm) with monitoring at 254 nm.

General Procedure for the Amino Acid-Derived Acylnitroso Diels−Alder Reactions.[16b−c,18] A solution of *N*-protected hydroxamic acid (12.6 mmol) in CH_2Cl_2/DMSO (5.1:1, 0.25 M) was cooled to −78 °C, and 300 mol % cyclopentadiene or 100 mol % cyclohexadiene was added. In a separate flask, $(COCl)_2$ (6.40 g, 4.40 mL, 50.4 mmol) in CH_2Cl_2 (57.0 mL) was cooled to −78 °C and DMSO (5.91 g, 5.37 mL, 75.6 mmol) was added. After 3 min, the $(COCl)_2$/DMSO solution was transferred by cannula into the hydroxamic acid solution. After the transfer was complete, the solution was allowed to stir at −78 °C for 15 min. Pyridine (9.98 g, 10.2 mL, 126.0 mmol) was added dropwise over 10 min. The reaction was allowed to warm slowly to room temperature, and Et_2O (150 mL) was added followed by 1 N HCl (150 mL). The layers were separated, and the aqueous layer was extracted with Et_2O (2 × 100 mL). The combined organic layers were washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give a residue that was purified on silica gel followed by recrystallization from EtOAc/hexanes.

1(*R*),4(*S*)-*N*-[*N*-(Carbobenzyloxy)-L-alanyl]-2,3-oxazabicyclo[2.2.1]hept-5-ene (12).[16b] Following the general procedure, **11** (5.94 g, 25.0 mmol) was oxidized in the presence of freshly generated cyclopentadiene (300 mol %) to give 5.25 g (70%) of a 4.1:1−5.9:1 diastereomeric mixture of **12** as a white solid after chromatography on silica utilizing 65% EtOAc/hexanes. The major diastereomer was further purified by

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[35] Blank, J. T.; Guerin, D. J.; Miller, S. J. *Org. Lett.* **2000**, *2*, 1247.

recrystallization from EtOAc/hexanes to give a single diastereomer whose spectral characteristics matched those previously reported.[16b] HPLC (10% *i*PrOH/hexanes, 1.5 mL/min) t_R 12.3 (major) and 9.7 (minor) min; mp 119−120 °C; R_f 0.44 (70% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.12 (m, 3H), 1.87 (d, J = 8.7 Hz, 1H), 2.02 (d, J = 8.4 Hz, 1H), 4.57 (dq, J = 6.9, 7.5 Hz, 1H), 5.09 (s, 2H), 5.36 (m, 2H), 5.52 (d, J = 7.8 Hz, 1H), 6.40 (m, 1H), 6.55 (m, 1H), 7.34 (s, 5H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 16.0, 48.3, 48.6, 61.8, 66.6, 84.6, 128.0, 128.4, 133.0, 1361, 136.4, 155.5, 173.3.

1(*R*),4(*S*)-*N*-[*N*-(Carbobenzyloxy)-L-alanyl]-2,3-oxazabicyclo[2.2.2]oct-5-ene (19). Following the general procedure, **11** (3.00 g, 12.6 mmol) was oxidized in the presence of cyclohexadiene (100 mol %) to give 3.69 g (93%) of a 5:1 diastereomeric mixture of **19** as a white solid after chromatography on silica gel utilizing 65% EtOAc/hexanes. The major diastereomer was further purified by recrystallization from EtOAc/hexanes to give a 10:1 diastereomeric ratio. An analytical sample was obtained by an additional recrystallization to provide a single diastereomer for characterization. HPLC (10% *i*PrOH/hexanes, 1.0 mL/min) t_R 12.3 (major) and 13.1 (minor) min; major diasteromer: mp 103−104 °C; R_f 0.29 (60% EtOAc in hexanes); IR (KBr) 3278, 3046, 1720, 1643, 1539, 1458, 1252 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.17 (d, J = 6.9 Hz, 3H), 1.50 (m, 1H) 1.65 (m, 1H), 2.19 (m, 2H), 4.64 (m, 1H), 4.80 (m, 1H), 5.09 (s, 2H), 5.23 (m, 1H), 5.62 (d, J = 7.8 Hz), 7.35 (m, 5H); ^{13}C NMR (DMSO-d_6, 300 MHz) δ 14.7, 20.5, 22.9, 46.8, 47.1, 65.2, 71.3, 127.7, 127.7, 128.3, 131.7, 132.6, 137.0, 155.6, 172.4; HRMS−FAB (*m*/*z*) [M + H]$^+$ calcd for $C_{17}H_{21}N_2O_4$, 317.1501; found, 317.1514.

2-[*N*-(Carbobenzyloxy)-L-alanyl]-3(*S*),5(*R*)-[dicarbomethoxy]oxazole (13). To cycloadduct **12** (0.056 g, 0.187 mmol) in EtOAc/CH_3CN (1:1, 3.0 mL) was added $NaIO_4$ (0.179 g, 0.840 mmol) in H_2O (1.0 mL), followed by $RuCl_3{\cdot}H_2O$ (0.090 g, 0.434 mmol). The reaction was allowed to stir at room-temperature open to the air for 2 h. The reaction was partitioned between EtOAc and brine. The layers were separated and the aqueous layer was extracted with EtOAc (4×). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give the crude diacid as a brown oil.

The brown oil was dissolved in THF (5.0 mL) and treated with excess CH_2N_2 in Et_2O at 0 °C. After 1 h, excess CH_2N_2 was quenched with 5% AcOH in Et_2O. The reaction mixture was washed with saturated $NaHCO_3$. The aqueous layer was extracted with Et_2O (5.0 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give a clear oil. The residue was chromatographed on silica gel utilizing 60% EtOAc/hexanes to give 0.080 g (66%) of **13** as a clear oil whose spectral characteristics matched those previously reported.[14a] R_f 0.52 (70% EtOAc/hexanes); IR (thin film) 3334, 2956, 1748, 1714 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.48 (d, J = 6.9 Hz, 3H), 2.79 (m,1H), 2.92 (m, 1H), 3.76 (s, 3H), 3.81 (s, 3H), 4.80 (m, 2H), 4.99 (dd, J = 4.5, 9.9 Hz, 1H), 5.05 (d, J = 12.0 Hz, 1H), 5.11 (d, J = 12.3 Hz, 1H), 5.38 (d, J = 7.5 Hz, 1H), 7.34 (s, 5H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 18.4, 34.9, 47.6, 52.7, 52.9, 56.6, 66.9, 77.8, 128.0, 128.1, 128.5, 136.3, 155.8, 168.2, 169.2, 173.6; HRMS−FAB (*m*/*z*) [M + H]$^+$ calcd for $C_{18}H_{23}N_2O_8$, 395.1454; found, 395.1453.

General Procedure for Hydrogenation of Cbz Protecting Groups and Cyclization to Diketopiperazines. To *N*-Cbz-protected dimethyl ester in MeOH (0.1 M), purged with Ar, was added 10 wt % Pd/C. The flask was purged with Ar followed by the addition of H_2 by a balloon. The reaction was monitored closely (TLC, 100% EtOAc) until all starting material was consumed and an intense ninhydrin positive baseline spot was present. The H_2 balloon was then removed and the reaction mixture was again purged with Ar. The reaction mixture was filtered through a short pad of Celite and concentrated under reduced pressure. The crude product was dissolved in CH_3CN (0.1 M) and allowed to stir at room temperature for 1 h. The volatiles were removed under reduced pressure to give a yellow solid that was purified by silica gel flash chromatography.

Diketopiperazine 7. Following the general procedure, **13** (0.047 g, 0.119 mmol) was deprotected and cyclized to give a residue which was purified on silica gel utilizing 5% MeOH/CH_2Cl_2 to give 0.020 g (74%) of **7** as a white solid. mp 239.5−241 °C, R_f 0.22 (5% MeOH in EtOAc); IR (KBr) 3269, 3001, 1743, 1682, 1660, 1405 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.22 (d, J = 6.9 Hz, 3H), 2.66 (ddd, J = 3.8, 5.8, 9.4 Hz, 1H), 2.88 (m, 1H), 3.60 (s, 3H), 4.18 (q, J = 6.3 Hz, 1H), 4.63 (dd, J = 5.7, 9.9 Hz, 1H), 4.90 (dd, J = 3.6, 8.4 Hz, 1H), 8.38 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 15.4, 34.1, 48.9, 52.2, 58.0, 75.4, 161.8, 166.6, 169.7; HRMS−FAB (*m*/*z*) [M + H]$^+$ calcd for $C_9H_{13}N_2O_5$, 229.0824; found, 229.0820.

Diketopiperazine 21. Following the general procedure, **20** (0.117 g, 0.285 mmol) was hydrogenated and cyclized to give a yellow solid that was chromatographed on silica gel utilizing 10% *i*-PrOH/CH_2Cl_2 to give 0.062 g (89%) of **21** as a white solid. mp 148−150 °C; R_f 0.36 (10% *i*-PrOH/CH_2Cl_2); IR (KBr) 3292, 2939, 1752, 1687, 1439, 993; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.54 (d, J = 6.5 Hz, 3H), 1.91 (m, 1H), 2.16 (m, 1H), 2.35 (m, 2H), 3.77 (s, 3H), 4.13 (m, 1H), 4.27 (m, 1H), 4.76 (bd, J = 5.1 Hz, 1H), 6.67 (bs, 1H);^{13}C NMR ($CDCl_3$, 75 MHz) δ 21.7, 24.0, 24.8, 50.4, 52.4, 59.1, 76.3, 162.8, 165.8, 169.2; HRMS−FAB (*m*/*z*) [M + H]$^+$ calcd for $C_{10}H_{15}N_2O_5$, 243.0981; found, 243.0995.

2-[*N*-(Carbobenzyloxy)-glycyl]-3(*S*)-[carboxy-*N*-L-alanyl-*O*-methyl]-5(*R*)-carbomethoxy-2-oxazole (14). To MeOH (1.0 mL) at 0 °C was added $SOCl_2$ (0.082 g, 0.050 mL, 0.689 mmol) dropwise. Diketopiperazine **7** (0.030 g, 0.132 mmol) was added, and the heterogeneous mixture was allowed to stir at 0 °C for 15 min and then allowed to warm to room temperature. The reaction was refluxed for 30 min and cooled to room temperature. The reaction was partitioned between EtOAc (5.0 mL) and 5% Na_2CO_3 (aq) until the aqueous layer remained basic. The layers were separated, and the aqueous layer was saturated with NaCl and extracted with EtOAc (4×). The combined organics were washed with brine, dried ($MgSO_4$), filtered, and concentrated to give an oil. Chromatography on silica gel utilizing 100% EtOAc provided 0.028 g (82%) of **8** as a clear residue that was utilized immediately in the next step. R_f 0.18 (100% EtOAc); HRMS−FAB (*m*/*z*) [M + H]$^+$ calcd for $C_{10}H_{17}N_2O_6$, 261.1087; found, 261.1066.

To disubstituted hydroxyamine **8** (0.028, 0.108 mmol) in CH_3CN (1.0 mL) was added Cbz-Gly-OH (0.025 g, 0.118 mmol) followed by Et_3N (0.012 g, 0.016 mL, 0.118 mmol), HOAt (0.016 g, 0.118 mmol), and EDC·HCl (0.024 g, 0.118 mmol). After 6 h, the reaction was partitioned between Et_2O and 1 N HCl. The layers were separated and the aqueous layer was extracted with Et_2O (1×). The combined organic layers were washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give a yellow solid. Chromatography on silica gel utilizing 100% EtOAc afforded 0.033 g (68%) of **14** as a clear glass. R_f 0.36 (100% EtOAc); IR (thin film) 3337, 2955, 1744, 1717, 1684, 1532, 1456 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.40 (d, J = 7.5 Hz, 3H), 2.81 (ddd, J = 4.0, 13.0, 18.0 Hz, 1H), 3.05 (ddd, J = 4.2, 10.2, 13.0 Hz, 1H), 3.74 (s, 3H), 3.79 (s, 3H), 4.19 (dd, J = 4.5, 18.5 Hz, 1H), 4.35 (dd, J = 6.0, 18.5, 1H), 4.51 (dq, J = 8.4, 8.4 Hz, 1H), 4.72 (dd, J = 6.5, 8.0 Hz, 1H), 4.91 (dd, J = 4.0, 9.5 Hz, 1H), 5.13 (AB$_q$, J = 14.7 Hz, 2H), 5.42 (m, 1H), 7.20 (d, J = 7.0 Hz, 1H), 7.30−7.37 (m, 5H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 18.0, 34.2, 42.4, 48.5, 52.5, 52.8, 58.8, 67.1, 77.7, 128.1, 128.2, 128.5, 136.2, 156.4, 167.0, 168.3, 171.3, 172.7; HRMS−FAB (*m*/*z*) [M + H]$^+$ calcd for $C_{20}H_{26}N_3O_9$, 452.1669; found, 452.1687.

2-(2-*tert*-Butoxycarbonylamino-propionylamino)acrylic Acid Methyl Ester (17).[25] To dimethyl ester **15**[14a] (0.122 g, 0.34 mmol) in THF (3.4 mL) at 0 °C was added NaH (60% in mineral oil, 0.014 g, 0.34 mmol). After 2 h at 0 °C followed by 14 h at room temperature, the reaction was partitioned between Et_2O (10.0 mL) and 1/2 saturated NH_4Cl (10.0 mL). The layers were separated, and the aqueous layer was extracted with Et_2O (2 × 10.0 mL). The combined organics were washed with brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give a clear residue that was chromatographed on silica gel utilizing 40% EtOAc/hexanes to give 0.070 g (75%) of **17** as a clear oil whose spectral characteristics were consistent with the known compound.[25b] R_f 0.42 (40% EtOAc/hexanes); ^{1}H NMR (CDCl$_3$, 300 MHz) δ 1.40 (d, *J* = 7.20 Hz, 3H), 1.45 (s, 9H), 3.84 (s, 3H), 4.25 (m, 1H), 4.97 (m, 1H), 5.90 (d, *J* = 1.8 Hz, 1H), 6.59 (s, 1H), 8.44 (s, 1H); ^{13}C NMR (CDCl$_3$, 75 MHz) δ 18.0, 28.3, 51.0, 53.0, 80.6, 109.2, 130.9, 155.1, 164.3, 171.5; HRMS−FAB (*m/z*) $[M + H]^+$ calcd for $C_{12}H_{20}N_2O_5$, 273.1450; found, 273.1453.

2-[*N*-(Carbobenzyloxy)-L-alanyl]-3(*S*),6(*R*)-[dicarbomethoxy]oxazine (20). To cycloadduct **19** (0.100 g, 0.316 mmol) in acetone/*t*-BuOH (44:1, 4.5 mL) was added Na_2CO_3 (0.030 g, 0.284 mmol). The flask was cooled to −10 °C and $KMnO_4$ (0.140 g, 0.885 mmol) was added portionwise such that the internal temperature of the reaction did not exceed 0 °C. The reaction was kept at −10 °C for 1 h and then allowed to warm to room temperature and stir an additional 18 h. EtOAc (5.0 mL) was added followed by 10% aqueous $Na_2S_2O_5$ (5.0 mL). The aqueous layer was acidified to pH = 3 (pH paper) with 1 N HCl. The layers were separated, and the aqueous layer was saturated with NaCl and extracted with EtOAc (3 × 5 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated to give a yellow residue that was dissolved in THF (5.0 mL) and treated with excess CH_2N_2 in Et_2O at 0 °C. After 1 h, excess CH_2N_2 was removed with a stream of Ar, that was passed through an AcOH trap. The reaction mixture was concentrated under reduced pressure and chromatographed on silica gel utilizing 55% EtOAc/hexanes to give 0.088 g (68%) of **20** as a clear oil. R_f 0.28 (50% EtOAc/hexanes); IR (thin film) 3331, 2956, 1732, 1682, 1520, 1455, 1210, 1026 cm^{-1}; ^{1}H NMR (CDCl$_3$, 300 MHz) δ 1.42 (d, *J* = 6.9 Hz, 3H), 1.98 (m, 3H), 2.49 (bd, *J* = 11.40 Hz, 1H), 3.76 (s, 3H), 3.79 (s, 3H), 4.52 (m, 1H), 4.81 (m, 1H), 5.06 (d, *J* = 12.3 Hz, 1H), 5.13 (d, *J* = 12.6 Hz, 1H), 5.21 (m, 1H), 5.45 (d, *J* = 7.8 Hz, 1H), 7.35 (s, 5H); ^{13}C NMR (CDCl$_3$, 75 MHz) δ 17.4, 23.0, 24.3, 47.5, 52.5, 52.6, 52.8, 66.8, 79.8, 128.0, 128.1, 128.5, 136.3, 155.8, 168.1, 168.9, 172.4; HRMS−FAB (*m/z*) $[M + H]^+$ calcd for $C_{19}H_{25}N_2O_8$, 409.1611; found, 409.1637.

(±)-*N*-(Carbo-*tert*-butoxy)-2,3-oxazabicyclo[2.2.1]hept-5-ene (23). To BocNHOH (15.0 g, 113.0 mmol) in MeOH (1100 mL) cooled to 0 °C equipped with an overhead stirrer was added freshly generated cyclopentadiene (33.0 g, 500.0 mmol, 40.0 mL). Then, $NaIO_4$ (26.6 g, 124.3 mmol) in H_2O (300 mL) was added dropwise over 20 min. After stirring at 0 °C for 30 min, the reaction mixture was partitioned between brine (1500 mL) and EtOAc (1500 mL). The layers were separated, and the aqueous layer was saturated with NaCl and extracted with EtOAc (3 × 300 mL). The combined organic layers were washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to a brown oil that was chromatographed on silica gel utilizing 30% EtOAc/hexanes to give 16.4 g (74% yield) of (±)-**23**[27] as a light brown solid that could be further purified by recrystallization from hexanes. mp 43−45 °C; R_f 0.5 (50% EtOAc/hexanes); ^{1}H NMR (CDCl$_3$, 300 MHz) δ 1.47 (s, 9H), 1.73 (d, *J* = 8.6 Hz, 1 H), 1.99 (ddd, *J* = 1.8, 1.8, 8.6 Hz, 1H), 4.98 (m, 1H), 5.21 (m, 1H), 6.41 (m, 2H); ^{13}C NMR (CDCl$_3$, 75 MHz) δ 28.1, 48.1, 65.0, 81.9, 83.5, 132.9, 134.1, 158.5; HRMS−FAB (*m/z*) $[M + H]^+$ calcd for $C_{10}H_{16}NO_3$, 198.1130; found, 198.1137.

(±)-2-Carbo-*tert*-butoxy-3,5-*syn*-dicarbomethoxy-2-oxazole (24). To (±)-**23** (8.0 g, 40.6 mmol) in EtOAc/CH_3CN (1:1, 220.0 mL) at 0 °C was added $NaIO_4$ (39.0 g, 182.0 mmol) in H_2O (440.0 mL) followed by $RuCl_3 \cdot H_2O$ (1.5 g, 7.2 mmol). The mixture was allowed to stir at 0 °C open to the air for 15 min and then room temperature for 2 h. The reaction mixture was suction filtered through a short pad of Celite, and the aqueous layer was saturated with NaCl. The layers were separated, and the aqueous layer was extracted with EtOAc (3 × 100 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated to give the crude diacid as a brown oil.

The brown oil was dissolved in Et_2O (80.0 mL) and treated with excess CH_2N_2 in Et_2O at 0 °C. After 1 h, the excess CH_2N_2 was quenched with 5% AcOH in Et_2O. The reaction mixture was washed with saturated $NaHCO_3$. The aqueous layer was extracted with Et_2O (1×). The combined organic layers were then dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give a white solid that was chromatographed on silica gel utilizing 50% EtOAc/hexanes to give 9.87 g (84%) of (±)-**24** as a white solid. R_f 0.35 (50% EtOAc/hexanes); mp 77.5−79 °C; IR (KBr) 3040, 3006, 2978, 2952, 1752, 1730, 1716 cm^{-1}; ^{1}H NMR (CDCl$_3$, 300 MHz) δ 1.49 (s, 9H), 2.81 (m, 2H), 3.78 (s, 3H), 3.79 (s, 3H), 4.60 (dd, *J* = 6.9, 8.1 Hz, 1H), 4.81 (dd, *J* = 8.1, 5.1 Hz, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 27.7, 35.2, 52.3, 52.4, 59.7, 76.6, 82.2, 155.2, 168.5, 170.1; HRMS−FAB (*m/z*) $[M + H]^+$ calcd for $C_{12}H_{20}NO_7$, 290.1240; found, 290.1265.

Representative Procedure for the Resolution of 2-Carbo-*tert*-butoxy-3(*R*)-carbomethoxy-5(*S*)-carboxy-2-oxazole (25) Using Chirazyme L-2.[28] To **24** (2.11 g, 7.3 mmol) in $PhCH_3$/0.1 N phosphate pH = 7.8 (1:3.3, 100 mL) equipped with a pH probe was added Chirazyme L-2 (0.026 g). The reaction was stirred rapidly, and the pH was maintained between 7.6 and 7.8 with the slow addition of 0.2 N NaOH (ca. 8 h). After 14.3 mL of the NaOH solution had been added, the reaction was extracted with Et_2O (3×).

The combined Et_2O layers were washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give a yellow solid that was chromatographed on silica gel utilizing 50% EtOAc/hexanes to give (−)-**24** as a white solid whose spectral characteristics matched that of (±)-**24** except for optical rotation, $[\alpha]^{20}_D$ −44.4° (*c* = 1.05 in CHCl$_3$) after recrystallization from EtOAc/hexanes. An optically enriched (ca. 80% ee) sample of **25** was treated with CH_2N_2 to give the corresponding diester (+)-**24**. $[\alpha]^{20}_D$ +34.8 ° (*c* = 0.52 in CHCl$_3$).

The aqueous layer from above was acidified to pH = 1.5 with 6 N HCl under a blanket of EtOAc, saturated with NaCl, and extracted with EtOAc (2×). The combined EtOAc layers were washed with brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give **25** as a light yellow oil that was further purified and characterized as the corresponding dicyclohexyamine salt **26**.

2-Carbo-*tert*-butoxy-3(*R*)-carbomethoxy-5(*S*)-carboxy-2-oxazole Dicyclohexylammonium Salt (26). To acid **25** (0.985 g, 3.59 mmol) in Et_2O (10.0 mL) at 0 °C was added dicyclohexylamine (0.65 g, 0.71 mL, 3.59 mmol) in Et_2O (3.0 mL) over 20 min. Then, without stirring, the solution was allowed to stand at 0 °C for 1 h. The resulting solid was filtered and washed with Et_2O to give 1.26 g (77%) of dicyclohexylamine salt **26**. The solid was then recrystallized from $PhCH_3$/CH_2Cl_2 (5:1, 30.0 mL) to give 0.790 g (63% recovery) of a white solid; mp 174−177 °C (dec); IR (KBr) 3432, 2931, 2860, 1761, 1713, 1613, 1451, 1333 cm^{-1}; ^{1}H NMR (CDCl$_3$, 300 MHz) δ 1.20−1.32 (m, 6H), 1.47 (s, 9H), 1.47−1.65 (m, 8H), 1.78−1.82 (m, 4H), 2.03−2.07 (m, 4H), 2.54 (ddd, *J* = 6.5, 9.3, 12.6 Hz, 1H), 2.96 (ddd, *J* = 7.2, 9.3, 12.6 Hz), 3.05−3.12 (m, 2H), 3.74 (s, 3H), 4.39 (dd, *J* = 7.2, 9.6 Hz, 1H), 4.67 (dd, *J* = 6.6, 9.3 Hz, 1H); ^{13}C NMR (CDCl$_3$, 75 MHz) δ 24.8, 25.1, 28.1, 29.2, 37.2, 52.4, 53.0, 60.3, 79.7, 82.5, 156.0, 170.9, 171.9.

A heterogeneous mixture of EtOAc and dicyclohexylamine salt **26** was washed with 5% $KHSO_4$ (2×). The combined aqueous layers were then extracted with EtOAc and the combined organic layers were washed with brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give **25** as a clear residue in quantitative recovery. R_f 0.31 (77% CHCl$_3$, 20% MeOH, 3% AcOH); IR (thin film) 3411-(broad), 2982, 1746, 1716, 1370, 1160 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.42 (s, 9H), 2.45 (ddd, *J* = 5.1, 7.5, 12.6 Hz, 1H), 2.88 (ddd, *J* = 8.1, 9.0, 12.3 Hz, 1H), 3.67 (s, 3H), 4.51 (dd, *J* = 7.8, 8.1 Hz, 1H), 4.78 (dd, *J* = 5.0, 9.3 Hz, 1H), 13.3 (bs, 1H); HRMS−FAB (*m/z*) $[M + H]^+$ calcd for $C_{11}H_{18}NO_7$, 276.1083; found, 276.1080.

Representative Mosher's Amide Analysis of (±)-24. To (±)-**24** (0.010 g, 0.035 mmol) in EtOAc (1.0 mL) at 0 °C was bubbled HCl (g) until TLC analysis indicated starting material was consumed. The reaction was concentrated under reduced pressure, redissolved in CH_2Cl_2 (0.1 M), and cooled to 0 °C. Then, Et_3N (200 mol %) and DMAP (150 mol %) were added followed by Mosher's-Cl (150 mol %) in CH_2Cl_2 (0.100 g in 2.0 mL of CH_2Cl_2). After 1 h, the reaction was partitioned between Et_2O and 1 N HCl. The layers were separated, and the aqueous layer was extracted with Et_2O. The combined organic layers were washed with saturated $NaHCO_3$, brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give a clear to slightly yellow oil that was analyzed by ^{19}F NMR without further purification.

Determination of Absolute Configuration (−)-24. To optically enriched diester (−)-**24** (0.200 g, 0.692 mmol) in EtOAc (2.0 mL) cooled to 0 °C was bubbled HCl(g) until starting material was consumed. The reaction was concentrated under reduced pressure and partitioned between EtOAc and 5% Na_2CO_3 (aq). The aqueous layer was saturated with NaCl, then extracted with EtOAc (3×). The combined organic layers were washed with brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give a clear oil that was dissolved in CH_3CN (6.0 mL). Cbz-L-Ala (0.139 g, 0.624 mmol) was added followed by Et_3N (0.063 g, 0.087 mL, 0.624 mmol) and HATU (0.237 g, 0.624 mmol). After 18 h, the reaction was partitioned between EtOAc and 1 N HCl. The layers were separated and the aqueous layer was extracted with EtOAc (1×). The combined organic layers were washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), filtered, and concentrated to a light yellow oil. Separation of the diastereomers by chromatography on silica gel utilizing 70% EtOAc/hexanes provided **13** as the major diastereomer whose spectral characteristics matched **13** obtained in Scheme 2.

2-*tert*-Butoxycarbonyl-3(*R*)-carbomethoxy-5(*S*)-[carboxy-*N*-L-phenylalanyl-*O*-benzyl]-2-oxazole (28). *p*-TsOH-L-Phe-OBn (0.855 g, 2.0 mmol) was partitioned between CH_2Cl_2 and 5% Na_2CO_3 (aq). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (1×). The combined organic layers were dried ($MgSO_4$), filtered and concentrated to give L-Phe-OBn as a clear oil that was dissolved in CH_3CN (15.0 mL) and added to **25** (0.428 g, 1.56 mmol). Then Et_3N (0.174 g, 1.72 mol, 0.240 mL) was added followed by PyBOP reagent (0.895 g, 1.72 mmol). After 10 h, the reaction was partitioned between Et_2O (20.0 mL) and 1 N HCl (10.0 mL). The layers were separated, and the aqueous layer was extracted with Et_2O (20.0 mL). The combined organic layers were washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure affording a faint yellow residue. Chromatography on silica gel utilizing 60% EtOAc/hexanes provided 0.714 g (89%) of **28** as a clear oil. $[\alpha]^{20}_D$ +18.4° (c = 0.97 in $CHCl_3$); R_f 0.40 (60% EtOAc/hexanes); IR (thin film) 3407, 3355, 1746, 1686, 1523 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 1.41 (s, 9H), 2.15 (ddd, J = 5.8, 8.4, 13.2 Hz, 1H), 2.78 (m, 1H), 2.99−3.10 (m,2H), 3.66 (s, 3H), 4.37 (dd, J = 7.8, 7.8 Hz, 1H), 4.59 (dd, J = 8.1, 14.2 Hz, 1H), 4.69 (dd, J = 5.5, 9.1 Hz, 1H), 5.10 (s, 2H), 7.17−7.36 (m, 10H), 8.44 (d, J = 7.8 Hz, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 27.7, 36.1, 52.4, 53.2, 59.9, 66.1, 78.0, 82.2, 126.6, 127.9, 128.0, 128.2, 128.4, 129.1, 135.7, 137.0, 155.4, 167.1, 170.2, 170.7; HRMS−FAB (m/z) $[M + H]^+$ calcd for $C_{27}H_{33}N_2O_8$, 513.2237; found, 513.2249.

2-*tert*-Butoxycarbonyl-3(*R*)-carbomethoxy-5(*S*)-[carboxy-*N*-L-phenylalanyl-L-alanyl-*O*-*tert*-butyl]-2-oxazole (29). To benzyl ester **28** (0.110 g, 0.214 mmol) in MeOH (2.0 mL) purged with Ar was added 10 mol % Pd/C. The flask was purged with Ar followed by addition of a H_2 balloon. After all starting material was consumed, the H_2 balloon was then removed and the reaction mixture was purged with Ar. The reaction mixture was filtered through a short pad of Celite and concentrated under reduced pressure to give 0.088 g of a clear oil (98%) that was utilized without further purification.

To the acid (0.079 g, 0.187 mmol) from above in CH_3CN (2.0 mL) was added HCl·L-Ala-OC$(CH_3)_3$ (0.037 g, 0.206 mmol), followed by Et_3N (0.040 g, 0.054 mL 0.393 mmol) and PyBOP reagent (0.107 g, 0.206 mmol). After 7 h, the reaction mixture was partitioned between EtOAc and 1 N HCl. The layers were separated and the aqueous layer was extracted with EtOAc (1×). The combined organic layers were washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to afford a clear residue. Chromatography on silica gel using 70% EtOAc/hexanes provided 0.088 g (86%) of **29** as a clear residue. R_f 0.32 (70% EtOAc/hexanes); $[\alpha]^{20}_D$ = +5.8° (c = 1.12 in $CHCl_3$); IR (thin film) 3380, 3317, 1737, 1664, 1154 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) d 1.26 (d, J = 7.2 Hz, 1H), 1.39 (s, 9H), 1.41 (s, 9H), 2.13 (ddd, J = 5.4, 8.4, 12.4 Hz, 1H), 2.74−2.80 (m, 1H, overlapping with 2.84 ppm), 2.84 (dd, J = 9.4, 13.6 Hz, 1H), 3.02 (dd, J = 4.0, 13.6 Hz, 1H), 3.67 (s, 3H), 4.12 (dq, J = 7.2, 7.2 Hz, 1H), 4.31 (dd, J = 8.1, 8.1 Hz, 1H), 4.58 (ddd, J = 4.1, 9.0, 9.0 Hz, 1H), 4.75 (dd, J = 5.4, 9.6 Hz, 1H), 7.16−7.24 (m, 5H), 7.98 (d, J = 8.4 Hz, 1H), 8.50 (d, J = 6.9 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 18.5, 27.9, 28.1, 36.1, 37.8, 48.7, 52.8, 54.3, 59.5, 77.8, 81.7, 82.0, 83.7, 127.0, 128.6, 129.4, 136.3, 155.4, 168.9, 169.2, 170.2, 171.4; MS m/z (MH^+) calcd for $C_{27}H_{40}N_3O_9$ 550.2765, found 550.2762.

2-*tert*-Butoxycarbonyl-3(*R*)-[carboxy-*N*-L-phenylalanyl-*O*-benzyl]-5(*S*)-[carboxy-*N*-L-phenylalanyl-L-alanyl-*O*-*tert*-butyl]-2-oxazole (30). To ester **29** (0.088 g, 0.160 mmol) in THF (1.6 mL) at −10 °C was added 0.1 N LiOH (3.0 mL). After 10 min, the solution had solidified and was allowed to warm to room temperature. The reaction was partitioned between EtOAc and 1 N HCl. The layers were separated, and the aqueous layer was saturated with NaCl and extracted with EtOAc. The combined organic layers were washed with brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to afford 0.085 g (99% mass recovery) of a clear residue.

To the residue from above was added L-Phe-OBn (0.045 g, 0.176 mmol) in CH_3CN (2.0 mL) followed by Et_3N (0.016 g, 0.022 mL, 0.155 mmol) and PyBOP (0.081 g, 0.156 mmol). After 3 h, the reaction was partitioned between EtOAc and 1 N HCl. The layers were separated and the aqueous layer was extracted with EtOAc (1×). The combined organic layers were washed with brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to afford a clear residue. Chromatography on silica gel utilizing 70% EtOAc/hexanes provided 0.066 g (60%) of **30** as a clear residue. R_f 0.30 (70% EtOAc/hexanes); IR (thin film) 3325, 2980, 1738, 1668, 1524 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.32 (d, J = 7.2 Hz, 3H), 1.42 (s, 9H), 1.50 (s, 9H), 2.59 (ddd, J = 3.9, 7.3, 13.2 Hz, 1H), 2.78 (ddd, J = 9.3, 9.3, 13.2 Hz, 1H), 2.97 (dd, J = 7.5, 13.5 Hz, 1H), 3.08 (dd, J = 6.6, 13.2 Hz, 1H), 3.13 (dd, J = 3.4, 5.8 Hz, 2H), 4.33 (dq, J = 7.3, 7.3 Hz, 1H), 4.46 (dd, J = 7.0, 9.1 Hz, 1H), 4.52 (dd, J = 7.5, 15.3 Hz, 1H), 4.69 (dd, J = 3.7, 9.4, 1H), 4.92 (ddd, J = 5.7, 5.7, 8.7 Hz, 1H), 5.10 (d, J = 12.1 Hz, 1H), 5.22 (d, J = 12.1 Hz, 1H), 6.26 (d, J = 6.9 Hz, 1H), 6.81 (d, J = 8.4 Hz, 1H), 7.0−7.35 (overlapping NH and aromatic, m, 16 H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 16.9, 27.5, 27.6, 27.6, 36.4, 36.9, 37.8, 48.3, 53.0, 53.6, 61.0, 66.1, 78.2, 80.4, 82.0, 126.3, 126.5, 127.9, 128.2, 128.4, 129.1, 129.3, 135.6, 137.0, 137.3, 156.0, 166.6, 169.5, 170.3, 170.9, 171.5; HRMS−FAB (m/z) $[M + H]^+$ calcd for $C_{42}H_{53}N_4O_{10}$, 773.3762; found, 773.3734.

2-[*N*-(Carbobenzyloxy)-L-valyl-glycyl]-3(*R*)-[carboxy-*N*-L-phenylalanyl-*O*-benzyl]-5(*S*)-[carboxy-*N*-L-phenylalanyl-L-alanyl-*O*-*tert*-butyl]-2-oxazole (31). To **30** (0.066 g, 0.085 mmol) at 0 °C was added EtOAc saturated with HCl(g) until TLC analysis indicated no starting material was present. The reaction mixture was concentrated under reduced pressure, and the resulting residue was partitioned between EtOAc and 5% Na_2CO_3 (aq). The aqueous layer was saturated with NaCl and extracted with EtOAc (2×). The combined organic layers were washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to afford 0.052 g (91%) of a clear residue that was chromatographed on silica utilizing 100% EtOAc (R_f 0.19) and utilized in the next reaction.

To the disubstituted hydroxylamine (0.009 g, 0.013 mmol) in CH_3CN (0.2 mL) was added Cbz-L-Val-Gly-OH (0.006 g, 0.019 mmol) followed by Et_3N (0.002 mL, 0.015 mmol) and HATU (0.007 g, 0.019 mmol). After 24 h, the reaction was partitioned between EtOAc and 1 N HCl. The layers were separated, and the aqueous layer was extracted with EtOAc (1×). The combined organic layers were washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give a clear residue that was chromatographed on silica utilizing 10% ^{i}PrOH/CH_2Cl_2 to give 0.010 g of **31** (80% yield) as an amorphous white solid. R_f 0.45 (10% ^{i}PrOH/CH_2Cl_2); ^1H NMR (DMSO-d_6, 600 MHz) d 0.86 (d, J = 6.6 Hz, 3H), 0.90 (d, J = 7.2 Hz, 3H), 1.27 (d, J = 7.8

Hz, 3H), 1.39 (s, 9H), 1.95−2.00 (m, 1H), 2.67−2.71 (m, 1H), 2.80 (dd, J = 9.9, 13.5 Hz, 1H), 2.95 (dd, J = 9.0, 13.8 Hz, 1H), 2.93−3.07 (m, 4H), 3.97 (dd, J = 7.2, 8.4 Hz, 1H), 4.02−4.09 (m, 2H), 4.13 (dq, J = 7.2, 7.2 Hz, 1H), 4.43 (dd, J = 6.6, 9.6 Hz, 1H), 4.52 (ddd, J = 6.2, 8.4, 8.4 Hz, 1H), 4.58 (ddd, J = 4.0, 9.3, 9.3 Hz, 1H), 4.68 (dd, J = 8.0, 8.0 Hz), 5.03 (dd, J = 12.3, 17.7 Hz, 2H), 5.08 (dd, J = 12.6, 14.4 Hz, 2H), 7.12−7.36 (m, 21H, overlapping aromatic and NH), 8.08 (t, J = 5.1 Hz, 1H), 8.25 (d, J = 8.4 Hz, 1H), 8.54 (m, 2H). ^{13}C NMR (DMSO-d_6, 150 MHz) δ 18.8, 18.1, 19.2, 27.6, 30.4, 36.1, 36.6, 37.7, 48.4, 53.3, 53.7, 58.5, 60.1, 65.4, 66.1, 79.2, 80.4, 126.4, 126.6, 127.7, 127.8, 127.9, 128.0, 128.3, 128.4, 128.4, 129.2, 129.4, 135.7, 136.9, 137.1, 137.4, 165.7, 168.9, 170.5, 170.9, 171.6, 171.7; HRMS−FAB (*m*/*z*) [M + H]$^+$ calcd for $C_{52}H_{62}N_6O_{12}$, 963.4504; found, 963.4498.

Variable Temperature NMR Experiments. Variable temperature NMRs were measured using 0.005−0.010 g of sample in CD_2Cl_2 (0.75 mL) at intervals of 15 °C beginning at 25 °C and cooling stepwise to −90 °C.

2-Carbo-*tert*-butoxy-3(*R*)-carbomethoxy-5(*S*)-carboallyloxy-2-oxazole (32). To acid **25** (0.54 g, 1.96 mmol) was added *O*-allyl-*N*,*N*′-diisopropylisourea (0.379 g, 0.437 mL, 2.06 mmol).[31] The mixture was allowed to stir at room temp for 6 h. Then CH_3CN (1.0 mL) was added, and the reaction was brought to reflux for 1h. The reaction was cooled to room temperature, Et_2O was added, and the reaction was filtered through a short pad of silica gel. The solution was concentrated under reduced pressure to give a red oil that was chromatographed utilizing 40% EtOAc/hexanes to give 0.509 g (82%) of **32** as a clear oil. R_f 0.38 (40% EtOAc/hexanes); IR (thin film) 2981, 1744, 1370, 1207, 1160 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.49 (s, 9H), 2.81 (m, 2H), 3.77 (s, 3H), 4.60 (dd, J = 7.5, 7.5 Hz, 1H), 4.67 (ddd, J = 1.3, 1.3, 5.8 Hz, 2H), 4.74 (dd, J = 5.8, 7.0, 1H), 5.27 (m, 1H), 5.34 (ddd, J = 1.26, 3.0, 17.4 Hz, 1H), 5.92 (ddt, J = 5.8, 10.5, 17.1 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 28.1, 35.7, 52.9, 59.9, 66.3, 83.4, 119.2, 131.2, 156.0, 168.1, 170.1; HRMS−FAB (*m*/*z*) [M + H]$^+$ calcd for $C_{14}H_{22}NO_7$, 316.1396; found, 316.1416.

(±)-2-Acetyl-3,5-dicarbomethoxy-2-oxazole (33). To (±)-**24** (0.250 g, 0.865 mmol) in EtOAc (4.0 mL) at 0 °C was bubbled HCl (g) until TLC indicated starting material was consumed. The reaction was concentrated under reduced pressure and redissolved in CH_2Cl_2 (4.0 mL) and cooled to 0 °C. Then Et_3N (0.087 g, 0.726 mmol) was added dropwise over 5 min followed by DMAP (0.127 g, 1.04 mmol) and acetyl chloride (0.075 g, 0.072 mL, 0.952 mmol) dropwise over 5 min. After 30 min, the reaction was partitioned between EtOAc and 1 N HCl. The layers were separated, and the aqueous layer was extracted with EtOAc (1×). The combined organic layers were washed with brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give a residue that was purified, utilizing 70% EtOAc/hexanes to give 0.127 (64%) of **33** as a clear oil. R_f 0.47 (100% EtOAc); IR (thin film) 2958, 1749, 1670, 1438, 1219 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.23 (s, 3H), 2.78−2.96 (m, overlapping methylenes, 2H), 3.77 (s, 3H), 3.82 (s, 3H), 4.64 (dd, J = 6.1, 7.9 Hz, 1H), 4.93 (dd, J = 4.5, 9.3 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.4, 35.6, 52.8, 52.9, 56.2, 77.3, 168.5, 169.6, 171.0; HRMS−FAB (*m*/*z*) [M + H]$^+$ calcd for $C_9H_{14}NO_6$, 232.0821; found, 232.0840.

(±)-2-[*N*-(Acetyl)-glycyl]-3,5-*syn*-[dicarbomethoxy]oxazole (34). The Boc group of (±)-**24** (0.150 g, 0.520 mmol) in EtOAc (2.5 mL) at 0 °C was removed as in the case of **33**.

To Ac-Gly (0.073 g, 0.620 mmol) in CH_2Cl_2 (3.0 mL) was added HOSu (0.071 g, 0.620 mmol) followed by EDC•HCl (0.119 g, 0.620 mmol). After 3.5 h, Boc-deprotected **34** in CH_2Cl_2 (2.0 mL) was added followed by HOAt (0.036 g, 0.265 mmol). After 15 h, the reaction was partitioned between EtOAc and 1 N HCl. The layers were separated, and the aqueous layer was saturated with NaCl and extracted with CH_2Cl_2 (4×). The combined organics were washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), filtered, and concentrated to give a residue that was chromatographed on silica, utilizing 7% MeOH in CH_2Cl_2 to give 0.076 mg (51%) of **34** as a white solid that could be further purified by recrystallization from EtOAc. R_f 0.24 (7% MeOH/CH_2Cl_2); mp 121−123 °C; IR (thin film) 3364 (br), 2958, 1744, 1659, 1435, 1283 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.04 (s, 3H), 2.84 (ddd, J = 4.0, 5.5, 13.0 Hz, 1H), 2.93 (ddd, J = 8.2, 9.7, 13.0 Hz, 1H), 3.77 (s, 3H), 3.81 (s, 3H), 4.19 (dd, J = 4.2, 18.7 Hz, 1H), 4.41 (dd, J = 5.5, 18.5 Hz, 1H), 4.87 (dd, J = 5.5, 8.0 Hz, 1H), 4.87 (dd, J = 3.5, 9.5 Hz, 1H), 6.21 (bs, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 23.0, 35.4, 41.0, 53.0, 53.1, 56.6, 77.4, 168.3, 168.6, 169.0, 170.3; HRMS−FAB (*m*/*z*) [M + H]$^+$ calcd for $C_{11}H_{17}N_2O_7$, 289.1036; found, 289.1029.

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Supporting Information Available: ^{1}H NMR and ^{13}C NMR for compounds **7**, **12**−**14**, **17**, **19**−**21**, **23**, **24**, **26**, and **28**−**34** as well as coordinates and energies of the calculated structures of *E*- and *Z*-**24**, **33**, and **34** are included. This material is available free of charge via the Internet at http://pubs.acs.org.

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