Application of Ring-Closing Metathesis to the Synthesis of Rigidified Amino Acids and Peptides

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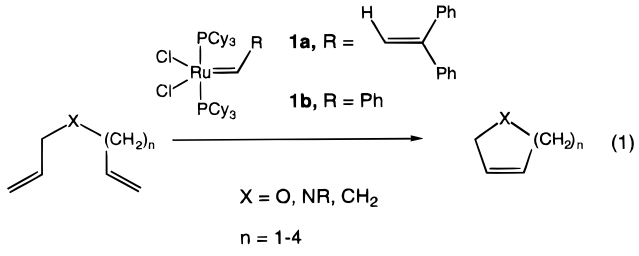
Abstract: Ruthenium complexes **1a** and **1b** have been applied to the ring-closing metathesis (RCM) reactions of a number of dienic substrates. The substrate scope includes rings of 6 to 20 members. In addressing macrocyclic peptides, a class of tetrapeptide disulfides inspired the synthesis of the carbon−carbon bond analogs. Replacement of cysteine residues with allylglycines resulted in the acyclic precursors which were subjected to RCM to afford the corresponding macrocycles. In addition, several macrocycles were prepared which were not based upon disulfide-bridge-containing species found in nature. The method was found to function on dienic peptides which were either dissolved in organic solvents or bound to solid supports.

Introduction

Previous reports from these laboratories have demonstrated that ruthenium complexes **1a** and **1b** efficiently catalyze ring-closing metathesis (RCM) reactions to form five-, six-, seven-, and eight-membered carbocycles and heterocycles (eq 1).[1−3] Metathesis-based strategies for carbocycle and heterocycle synthesis, based on alkylidenes of ruthenium and molybdenum, are now being applied with some regularity in natural products synthesis.[4] The extraordinary functional group tolerance of the ruthenium-based catalysts **1** has also enabled the synthesis of cyclic amino acids and peptides, containing multiple heteroatoms, acidic protons, and hydrogen bonds.[5] In addition, Ghadiri and co-workers have recently demonstrated that these structural features can be engineered into systems to allow for efficient cross-metathesis processes, enabling efficient dimerization and template driven inter- and intramolecular olefin metathesis.[6] In this paper, we describe our findings concerning the scope of RCM in the synthesis of cyclic amino acids and peptides.



(1)

Given the prominent role of conformationally restricted amino acids and peptides in the design of peptidomimetics, we sought to apply the functional group tolerant catalysts **1** to the synthesis of this class of molecules.[7] Many approaches to the synthesis of a vast array of these compounds have been described. Amide linkage modification, side chain modification, and *de novo* synthesis of particular structural motifs are among the strategies employed to date. Cyclic substructures are likewise frequently incorporated since these motifs serve to restrict the conformational space of the molecules. Such modifications often result in increased affinity for a given biological receptor, with simultaneously diminished sensitivity to cellular peptidases. Because RCM based on catalysts **1a** and **1b** was emerging as a powerful method for carbocycle and heterocycle synthesis in

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(1) For a recent reviews on applications of olefin metathesis and related processes in organic synthesis, see: (a) Grubbs, R. H.; Pine, S. H. In *Comprehensive Organic Synthesis*; Trost, B. M., Ed.; Pergamon: New York, 1991; Vol. 5, Chapter 9.3. (b) Grubbs, R. H.; Miller, S. J.; Fu, G. C. *Acc. Chem. Res.* **1995**, *28*, 446−552. (c) Schmalz, H.-G. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1833−1836.

(2) For previous reports on RCM from this laboratory, see: (a) Fu, G. C.; Grubbs, R. H. *J. Am. Chem. Soc.* **1992**, *114*, 5426−5427. (b) Fu, G. C.; Grubbs, R. H. *J. Am. Chem. Soc.* **1992**, *114*, 7324−7325. (c) Fu, G. C.; Grubbs, R. H. *J. Am. Chem. Soc.* **1993**, *115*, 3800−3801. (d) Fu, G. C.; Nguyen, S. T.; Grubbs, R. H. *J. Am. Chem. Soc.* **1993**, *115*, 9856−9857. (e) Fujimura, O.; Fu, G. C.; Grubbs, R. H. *J. Org. Chem.* **1994**, *59*, 4029−4031. (f) Kim, S. H.; Bowden, N.; Grubbs, R. H. *J. Am. Chem. Soc.* **1994**, *115*, 10680−10681. (g) Miller, S. J.; Kim, S. H.; Chen, Z.-R.; Grubbs, R. H. *J. Am. Chem. Soc.* **1995**, *117*, 2108−2109. (h) Coates, G. W.; Grubbs, R. H. *J. Am. Chem. Soc.* **1996**, *118*, 229−230. (i) Fujimura, O.; Grubbs, R. G. *J. Am. Chem. Soc.* **1996**, *118*, 2499−2500. (j) Kim, S. H.; Zuercher, W. J.; Bowden, N. B.; Grubbs, R. H. *J. Org. Chem.* **1996**, *61*, 1073−1081.

(3) For the preparation and characterization of catalyst **1a**, see: (a) Nguyen, S. T.; Johnson, L. K.; Grubbs, R. H.; Ziller, J. W. *J. Am. Chem. Soc.* **1992**, *114*, 3974−3975. (b) Nguyen, S. T.; Grubbs, R. H.; Ziller, J. W. *J. Am. Chem. Soc.* **1993**, *115*, 9858−9859. For the preparation and characterization of catalyst **1b**, see: (c) Schwab, P.; Grubbs, R. H.; Ziller, J. W. *J. Am. Chem. Soc.* **1996**, *118*, 100−110. (d) Schwab, P.; France, M. B.; Ziller, J. W.; Grubbs, R. H. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2039−2041.

(4) For recent applications of metathesis-based methods to natural product synthesis, see: (a) Martin, S. F.; Liao, Y.; Rein, T. *Tetrahedron Lett*. **1994**, *35*, 691−694. (b) Borer, B. C.; Deerenberg, S.; Bieraugel, H.; Pandit, U. K. *Tetrahedron Lett*. **1994**, *35*, 3191−3194. (c) Morken, J. P.; Didiuk, M. T.; Visser, M. S.; Hoveyda, A. H. *J. Am. Chem. Soc.* **1994**, *116*, 3123−3124. (d) Martin, S. F.; Wagman, A. S. *Tetrahedron Lett.* **1995**, *36*, 1169−1170. (e) Houri, A. F.; Xu, Z.; Cogan, D.; Hoveyda, A. H. *J. Am. Chem. Soc.* **1995**, *117*, 2943−2944. (f) Huwe, C. M.; Blechert, S. *Tetrahedron Lett.* **1995**, *36*, 1621−1624. (g) Overkleeft, H. S.; Pandit, U. K. *Tetrahedron Lett.* **1996**, *37*, 547−550. (h) Randall, M. L.; Tallarico, J. A.; Snapper, M. L. *J. Am. Chem. Soc.* **1995**, *117*, 9610−9611. (i) Huwe, C. M.; Kiehl, O. C.; Blechert, S. *Synlett* **1996**, 65−66. (j) Fürstner, A.; Langemann, K. *J. Org. Chem.* **1996**, *61*, 3942−3943.

(5) (a) Miller, S. J.; Grubbs, R. H. *J. Am. Chem. Soc.* **1995**, *117*, 5855−5856. (b) Garro-Héllion, F.; Guibé, F. *J. Chem. Soc., Chem. Commun*. **1996**, 641−642.

(6) Clark, T. D.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1995**, *117*, 12364−12365.

(7) For an overview of the field, see: (a) Gante, J. *Angew. Chem., Int. Ed. Engl.* **1994,** *33*, 1699−1720. (b) *Tetrahedron Symposia-in-Print Number 50*, M. Kahn, Ed.; 1993; Vol. 49, number 17. (c) Giannis, A.; Kolter, T. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1244−1267. (d) Hruby, V. J.; Al-Obeidi, F.; Kazmierski, W. *Biochem. J.* **1990**, *268*, 249−262. (e) Toniolo, C. *Int. J. Pept. Protein Res.* **1990**, *35*, 287−300.

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the presence of a wide range of functional groups, we endeavored to apply this strategy to the synthesis of peptide-based structures.

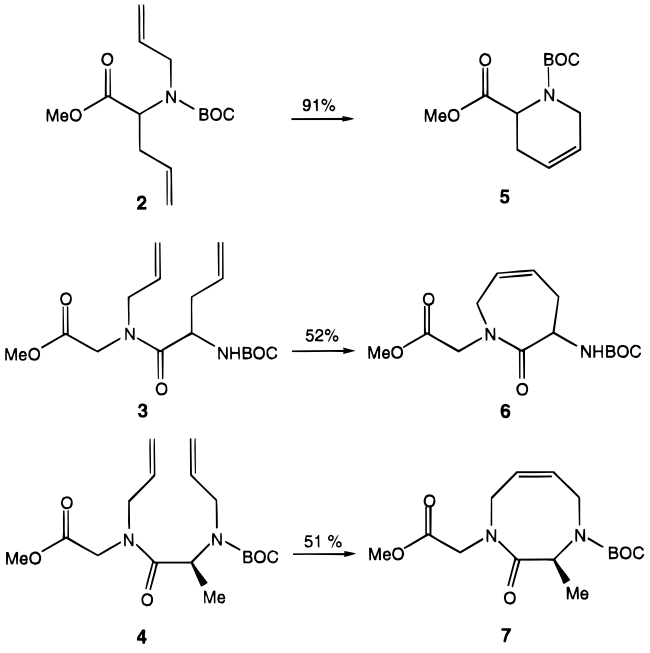
Results and Discussion

Cyclic Amino Acids. Our initial objective was the synthesis of simple amino acid derivatives containing various ring sizes which might subsequently be introduced into peptides. Depending on the desired ring size, our approach required the introduction of alkene functionality at either C_α or at the amide nitrogen of the amino acid. Alkene incorporation at C_α is facilitated by the commercial availability of (+/−)-, (+)-, and (−)-allylglycine. In addition, highly efficient methodology for the asymmetric synthesis of this unnatural amino acid is also available.[8] Introduction of alkene functionality at the amide nitrogens has also been addressed. In particular, Seebach has shown that peptides can be converted to the poly(*N*-allylamides) upon exposure to allyl bromide in the presence of the P4-phosphazene base.[9] Synthesis of the RCM precursors **2**−**4** then proceeded in a straightforward fashion employing conventional peptide-coupling and derivatization techniques (see Experimental Section).

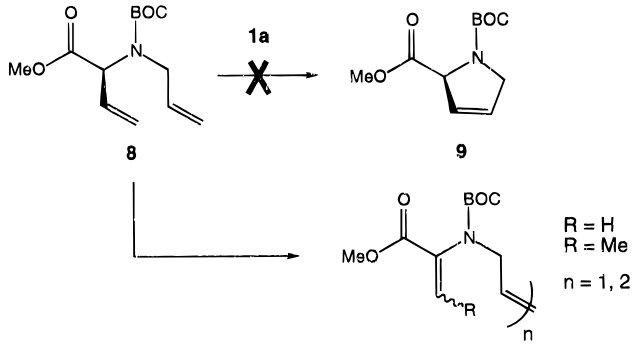
The results of the RCM experiments are shown in Scheme 1.[10] Treatment of modified amino acid **2** with catalyst **1a** under conditions analogous to those described in our earlier reports (5 mol % **1a**, 0.20 M, $CHCl_3$, 25 °C) furnished the dehydropipicolinate **5** in good yield (91%) within 1 h. In contrast, substrates **3** and **4** required more vigorous conditions for ring closure, and the isolated yields for the corresponding cyclizations were reduced. Nevertheless, subjection of acyclic dienes **3** and **4** to the above RCM conditions afforded the seven-membered ring **6** in 52% yield and the eight-membered ring **7** in 51% yield, respectively. The latter two transformations appear to be limited by the ring strain inherent to the cyclic product, which requires the reactions be run at high dilution to minimize competing intermolecular oligomerization processes.[11]

A notable limitation of the RCM strategy employing **1** for the synthesis of cyclic amino acids is illustrated by our unsuccessful attempt to prepare the dehydroproline **9** by this method (Scheme 2). Initially, the vinyl glycine derivative **8** was projected to undergo facile, rapid RCM with **1a** to afford the 5-membered ring in analogy to the other 5-membered rings we had studied.[12] However, upon exposure to the ruthenium-based catalyst **1a**, only acyclic, α,β-unsaturated esters were recovered. Although no rigorous mechanistic studies were carried out, we attribute this undesired reactivity to the labile acidic C_α proton associated with the vinylglycine structure. The synthesis of the dehydroproline derivative was not pursued further.

Scheme 1

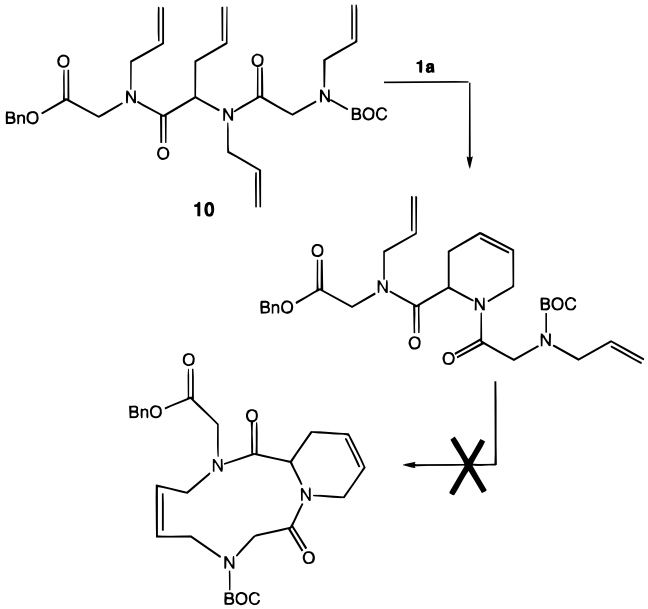


Scheme 2



Macrocyclic Peptides. At this stage, we turned our attention to the synthesis of macrocyclic peptide structures. Our initial approach to the problem was based on the incorporation of alkene functionality at the amide nitrogens (Scheme 3). Synthesis of *N*,*N*,*N*-(triallyl)peptide **10** was accomplished by conventional peptide-coupling chemistry, followed by allylation

Scheme 3



(8) Myers, A. G.; Gleason, J. L.; Yoon, T. Y. *J. Am. Chem. Soc.* **1995**, *117*, 8488−8489 and references therein.

(9) Pietzonka, T.; Seebach, D. *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 1481−1482.

(10) Compounds were identified on the basis of their ^{1}H NMR, ^{13}C NMR, IR, and mass spectral characteristics (see Experimetal Section). The cases involving *C*-allylglycine in Scheme 1 were performed on the racemic mixtures.

(11) We have discussed this issue in more detail in ref 2g.

(12) Vinyl glycine derivative **8** was prepared by derivatization of *N*-allyl-*N*-BOC-methionine methyl ester in analogy to the procedure of Rapoport, see: (a) Afzali-Ardakani, A.; Rapoport, H. *J. Org. Chem.* **1980**, *45*, 4817−4820. (b) Meffre, P.; Voquang, L.; Voquang, Y.; Le Goffic, F. *Synth. Commun.* **1989**, *19*, 3457−3468.

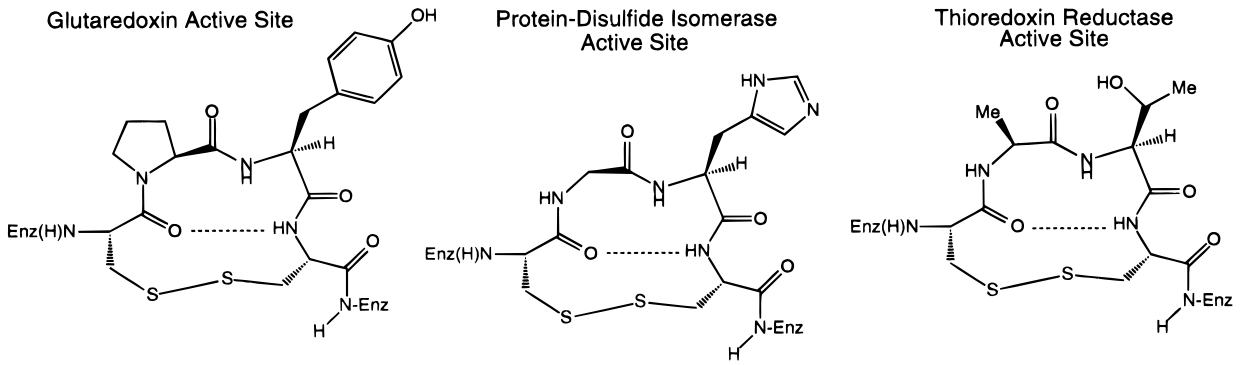


Figure 1. Naturally occurring disulfide-stabilized β-turns.

with the P4-phosphazene base. Our initial projection was that the substrate, upon treatment with the metathesis catalyst, would undergo a bicyclization reaction wherein a rapid six-membered ring closure would be followed by a macrocyclization. However, treatment of **10** with **1a** resulted in only monocyclization and no macrocyclic compounds were obtained.[13] While the reasons for this lack of macrocyclization were not definitively established, we attributed the low reactivity of the structure to either the ring strain inherent to the bicyclic product or kinetic problems associated with its formation. As a result, we sought an alternative approach to macrocyclic peptide formation.

Dicarba Analogs of Naturally Occurring Macrocyclic Disulfides. In order to contend with the potential thermodynamic and kinetic problems associated with peptide macrocyclizations, we turned our attention to examples of macrocyclic peptides which were found in nature. While examples of such peptides are numerous, we were attracted to a class of disulfide-stabilized tetrapeptides found in a number of redox active proteins (Figure 1).[14] Importantly, each member of this class contains a disulfide bridge which locks the tetrapeptide into a β-turn type structure.[15]

Our objective was to replace the disulfide bridges in these known systems with carbon−carbon bonds. So-called "dicarba" peptides of this nature have themselves been prepared previously in peptidomimetic research.[16] Replacement of the disulfide bridge in biologically active compounds can provide drugs which have increased metabolic stability, as the covalent cross-link is inert to conditions which can reduce disulfide-based cross-links.[17] Our approach was to design a laboratory model for the oxidation of cysteines to disulfide bridges. Replacement of cysteines with allylglycines in acyclic peptides sets up RCM instead of thiol oxidation. In the presence of a metathesis catalyst, a carbon−carbon bond is then formed in place of the disulfide bridge.

Balaram and co-workers have reported the disulfide-stabilized β-turn **11** as an analog of the redox active β-turns above, and this substrate stimulated us to attempt to prepare the analogous tetrapeptide olefin (Figure 2).[18] Significantly, Balaram has shown that both **11** and the corresponding acyclic bis(benzyl) thioether **12** possess the illustrated hydrogen bond in $CHCl_3$ solvent, and we felt that this feature might help dispose the acyclic diene analog toward a conformation suitable for ring closure.

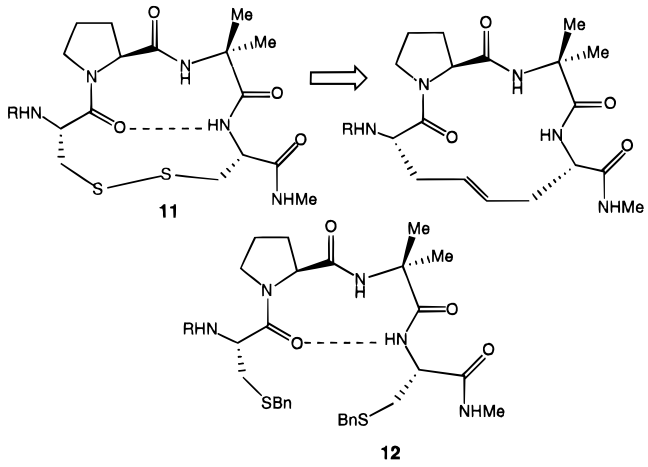


Figure 2. Balaram's disulfide-stabilized β-turn abd tge C=C bond analog.

Since disulfide bridges possess different dihedral angle requirements relative to olefins,[19] it was not obvious at the outset of our study whether (*S*)-allylglycine would be the optimal stereoisomer for replacement of the L-cysteine. Therefore, in our initial study of this system, we prepared a statistical mixture of the four stereoisomers of the acyclic dienes **13**. As reported in our earlier communication, when this mixture of the four diastereomers was treated with catalyst **1a** (20 mol %, 0.002 M, CH_2Cl_2, 40 °C), a single macrocycle (**14** diastereomer was obtained. The majority of the reaction mixture was composed of unreacted dienes. When diastereomerically pure (*S*,*S*,*S*)-**13** was subjected to the reaction conditions, (*S*,*S*,*S*)-**14** was obtained in 60% yield and the product was identical to that obtained from the analogous experiment on the mixture (eq 2).

In order to explore the scope of the process, and in order to assay the role of preorganization in facilitating ring closure, we prepared additional tetrapeptides where we systematically removed the conformationally constrained amino acids proline and aminoisobutyric acid (Aib). The Pro-Aib sequence is

(13) A monocyclized product was identified by FABMS. No ions corresponding to the bicyclization product could be detected. The monocyclized product was formed at approximately the same rate as the six-membered cyclic product **5**, and no other product was formed after elongated reaction time. The formation of cyclic product **5** was previously found to be fast and effectively irreversible. Thus, we believe that treatment of substrate **10** under the analogous reaction conditions leads to the six-membered monocyclization product as opposed to the 11-membered monocyclization product.

(14) (a) Musiol, H.-J.; Siedler, F.; Quarzago, D.; Moroder, L. *Biopolymers* **1994**, *34*, 1553−1562. (b) Siedler, F.; Quarzago, D.; Rudolph-Bohner, S.; Moroder, L. *Biopolymers* **1994**, *34*, 1563−1572.

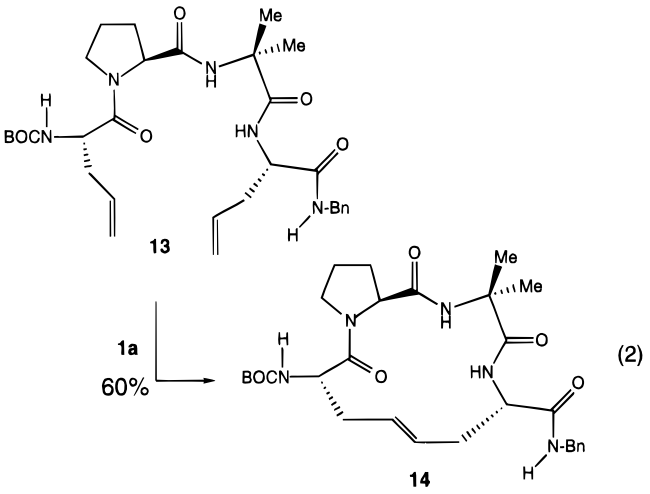
(15) For discussions of β-turns in proteins, see: (a) Rizo, J.; Gierasch, L. M. *Ann. Rev. Biochem.* **1992**, *61*, 387−418. (b) Rose, G. D.; Gierasch, L. M.; Smith, J. A. *Adv. Protein Chem.* **1985**, *37*, 1−109. For a review of β-turn mimetics, see: ref 7b.

(16) Nutt, R. F.; Strachan, R. G.; Veber, D. F.; Holly, F. W. *J. Org. Chem. Am. Chem. Soc.* **1980**, *45*, 3078−3080.

(17) Nutt, R. F.; Veber, D. F.; Saperstein, R. *J. Am. Chem. Soc.* **1980**, *102*, 6539−6545.

(18) Ravi, A.; Balaram, P. *Tetrahedron* **1984**, *40*, 2577−2583.

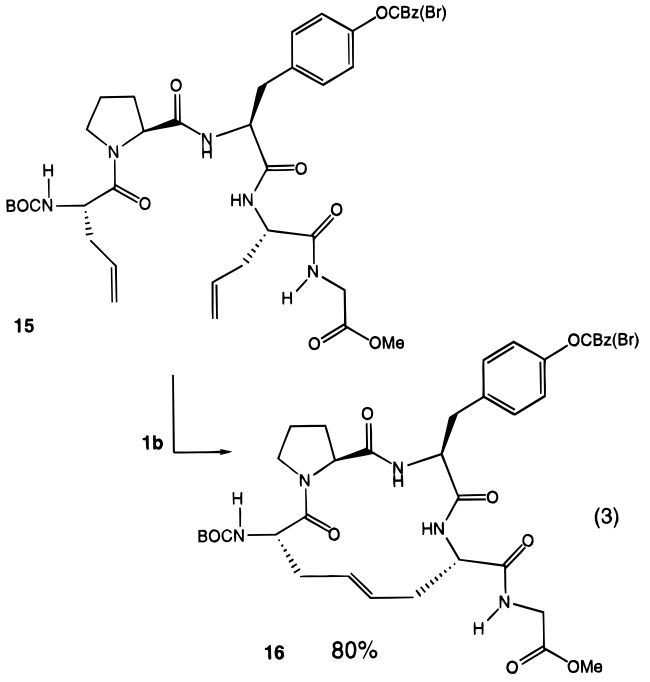
(19) The disulfide dihedral angle in **10** has been shown to be 82° with right-handed chirality in the solid state. Ravi. A.; Prasad, B. V.; Balaram, P. *J. Am. Chem. Soc.* **1983**, *105*, 105−109.



(2)

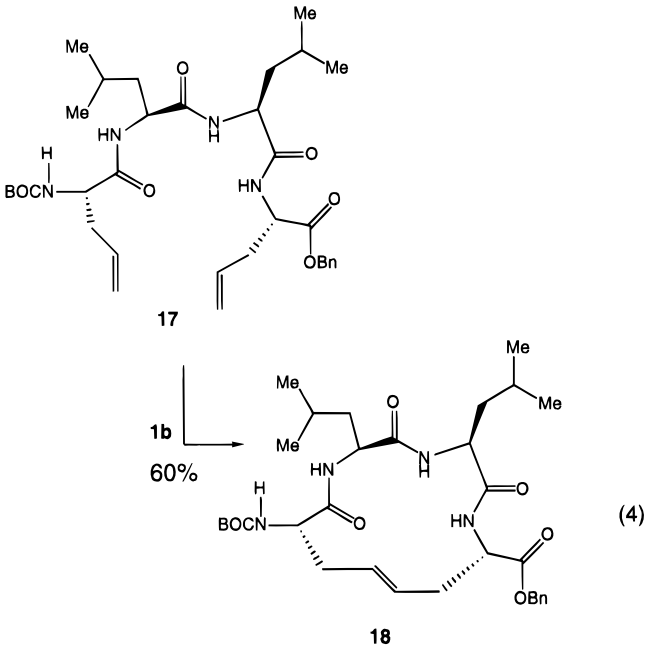
known to restrict the conformational space of peptides.[20] Therefore, we examined substrates where these amino acids were replaced with less rigidifying residues.

Replacement of the Aib residue with protected tyrosine resulted in the formation of peptide **15**. The Pro-Tyr dipeptide sequence spans the cysteine residues in the glutaredoxin active site. Compound **15** therefore represented an attempt to synthesize a carbon−carbon bond mimic of the active site of this protein. Exposure of **15** to the ruthenium catalyst **1b** under our standard macrocyclization conditions (0.004 M, CH_2Cl_2, 40 °C) resulted in clean formation of the macrocycle **16** in 80% yield (eq 3).[21] This experiment demonstrates that two consecutive conformationally constrained amino acids are not required for the synthesis of tetrapeptide macrocycles by RCM.



(3)

Replacement of both the proline and the Aib residues with leucines resulted in peptide **17**, which is devoid of conformationally restricted amino acids in the bridging positions. Once again, exposure to the catalyst **1b** resulted in very efficient macrocyclization to afford cyclic tetrapeptide **18** (eq 4). These



(4)

results establish that there is no rigorous requirement for the Pro-Aib sequence as the bridging residues for the synthesis of tetrapeptide macrocycles by RCM.

Conformational Analysis of Cyclic Dicarbatetrapeptides. In order to ascertain the extent to which the macrocyclic products containing carbon−carbon double bonds resemble their disulfide bridge analogs, an investigation of the solution conformations of the cyclic tetrapeptides was undertaken. As indicated above, Balaram's Cys-Pro-Aib-Cys system **11** was found by a series of IR and NMR experiments to possess an intramolecular, transannular H-bond. The corresponding data on the carbon−carbon bond analogs of these systems is discussed below.

Table 1 lists the NH region of the IR spectra of the cyclic peptides **14**, **16**, and **18**. In each case, the characteristic bands for both non-hydrogen-bonded NH groups (>3400 cm^{-1}) and intramolecular C═O···H−N hydrogen bonds appear (<3400 cm^{-1}).[22] Although it is difficult to definitively establish from the IR data alone which of the amide N−H groups is involved in intramolecular hydrogen bonding, these data are consistent with the possibility of transannular intramolecular H-bonding in the cyclic structures.

Table 1. IR Data (cm^{-1}) for Cyclic Tetrapeptides (Concentration ~0.002 M, CH_2Cl_2)

14	**16**	**18**
3424	3426	3419
3324	3354	3339

To further understand the H-bonding network in the cyclic tetrapeptides, we turned to ^1H NMR techniques (Figure 3). Compound **14** was studied to identify the presence of any transannular hydrogen bonding. The participation of specific NH groups in hydrogen bonds was established by examining the temperature dependence of the NH chemical shifts ($-\Delta\delta/$

(20) (a) Venkatachalapathi, Y. V.; Balaram, P. *Biopolymers* **1981**, *20*, 1137−1145. (b) Prasad, B. V. V.; Balaram, H.; Balaram, P. *Biopolymers* **1982**, *21*, 1261−1273.

(21) A related cyclization was performed with unprotected tyrosine which afforded the analogous macrocycle in 70% yield. The free phenol therefore does not interfere with catalyst activity.

(22) For interpretation of N−H stretches in CH_2Cl_2, see: (a) Gellman, S. H.; Dado, G. P.; Liang, G.-B.; Adams, B. R. *J. Am. Chem. Soc.* **1991**, *113*, 1164−1173. (b) Liang, G.-B.; Desper, J. M.; Gellman, S. H. *J. Am. Chem. Soc.* **1993**, *115*, 925−938. (c) Gardner, R. R.; Liang, G.-B.; Gellman, S. H. *J. Am. Chem. Soc.* **1995**, *117*, 3280−3281. (d) Rao, C. P.; Nagaraj, R.; Rao, C. N. R.; Balaram, P. *Biochemistry* **1980**, *19*, 425−431.

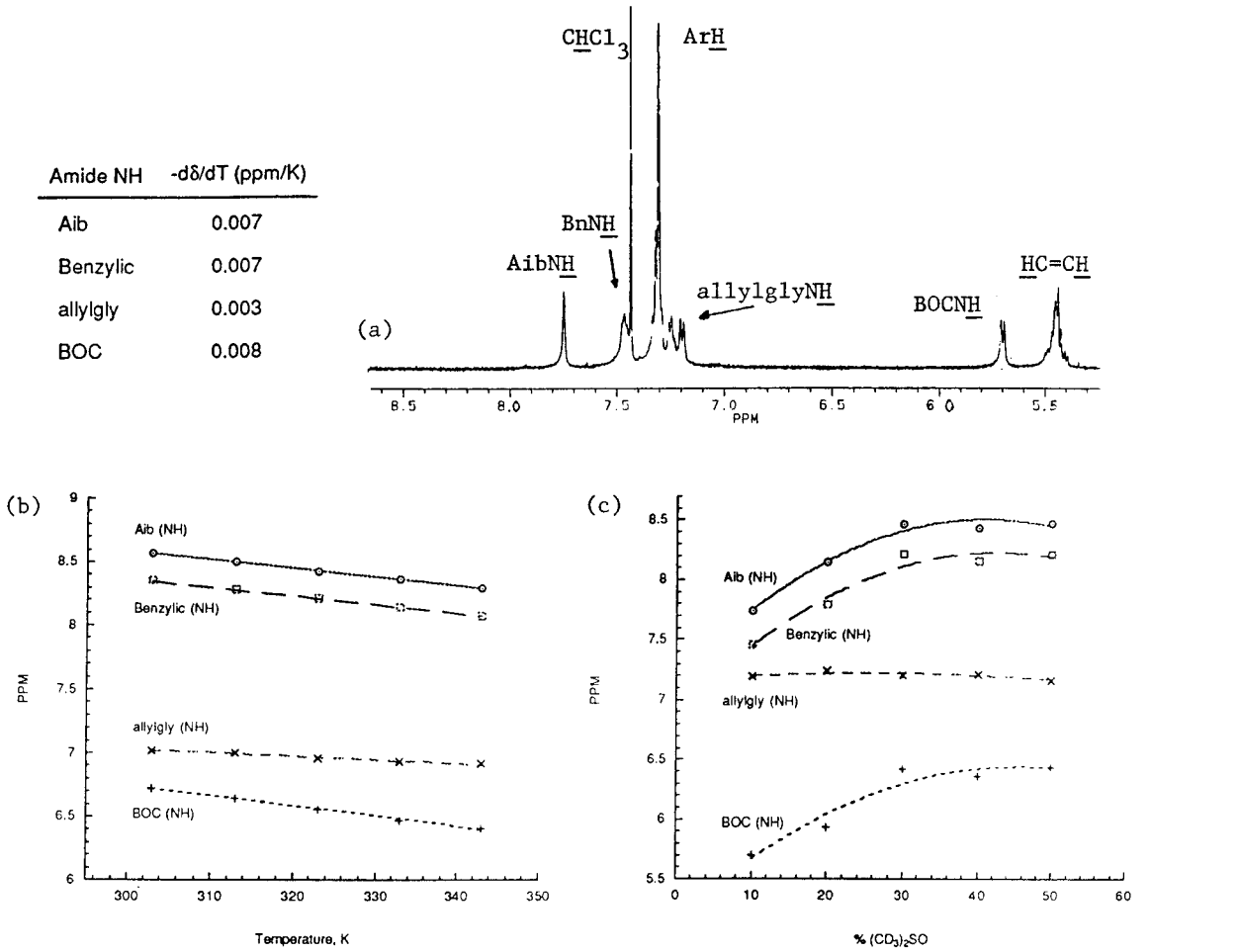
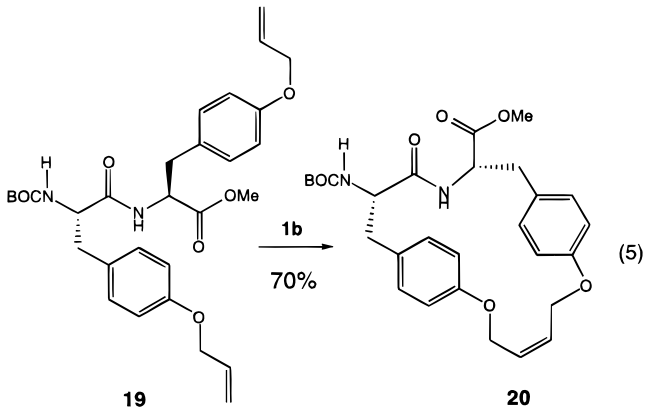


Figure 3. (a) Amide NH and olefin region of the 500 MHz ^{1}H NMR spectrum of **14** in 10% $(CD_3)_2SO/CDCl_3$. (b) Temperature dependence of chemical shift for amide protons in $(CD_3)_2SO$ solution $(-\Delta\delta/\Delta T)$. (c) Dependence of chemical shift for amide protons in $CDCl_3$ solution with increasing $(CD_3)_2SO$ concentration.

ΔT) in $(CD_3)_2SO$ and the solvent dependence of the chemical shifts in $CDCl_3/(CD_3)_2SO$ mixtures (Figure 3).[23] Proton assignments are shown on the illustrated ^{1}H NMR plot showing the olefinic and amide regions of the spectrum and were established using conventional decoupling techniques. For cyclic peptide **14**, an intramolecular transannular H-bond was identified. The temperature dependence $(-\Delta\delta/\Delta T)$ of the key allylglycine NH was found to be 0.003 ppm/K. In contrast, the $(-\Delta\delta/\Delta T)$ for all the other amide resonances are >0.007 ppm/K, reflecting full solvent exposure. Likewise, in the solvent titration measurements, the allylglycine NH shows very minor changes in chemical shift with increasing $(CD_3)_2SO$ concentration, while the other amide resonances shift steadily downfield. These results are very similar to the observations of Balaram for the disulfide **11**, where the illustrated Cys(C═O···H−N)-Cys was established by these methods.[24]

Additional Examples. In an effort to understand the generality of the method for introducing carbon−carbon bond cross-links into peptide systems, we have explored several examples which are not based on the tetrapeptide framework. In these cases, we have also studied examples wherein alkene functionality is not solely based on allylglycine. For example, the bis(*O*-allyl)tyrosine dimer **19** undergoes efficient RCM to produce the 20-membered ring macrocycle **20** in 70% yield.[25] It is interesting to note that acyclic peptide **19** has no apparent structural preorganization by intramolecular hydrogen bonding. Nevertheless, at high dilution (0.002 M) it undergoes successful RCM to yield macrocyclic peptide **20**.

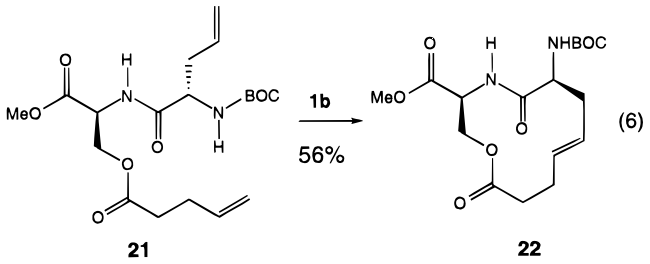


(5)

Likewise the serine-allylglycine dipeptide derivative **21** can be converted to macrocycle **22** by RCM in 56% yield (eq 6). Interestingly, this system required extremely high-dilution conditions to minimize dimer formation. When this cyclization was performed at conventional cyclization concentration (0.002 M), dimeric products were observed. However, further dilution to the somewhat extreme level of 0.0005 M resulted in the exclusion of dimer.

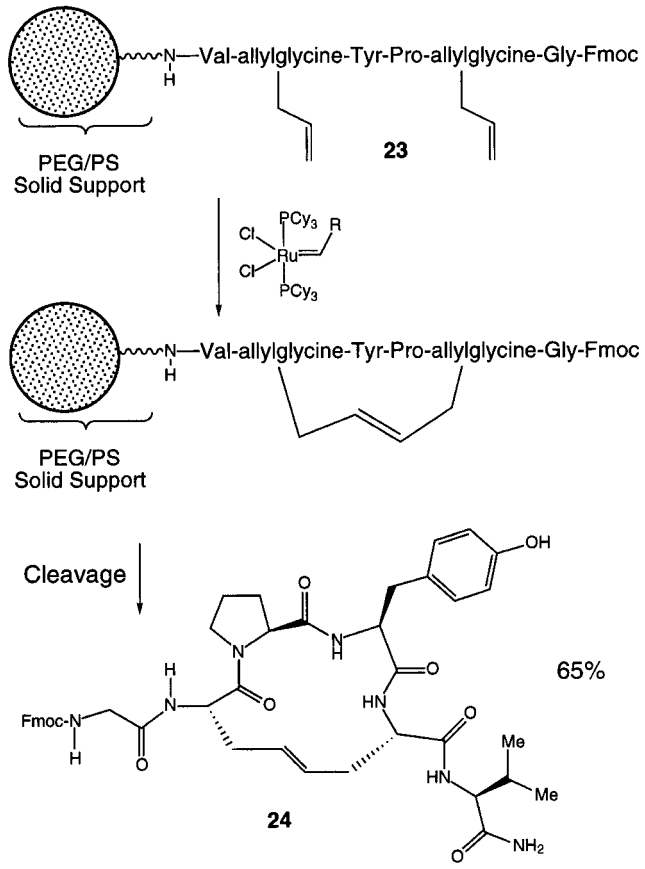
Solid Support. Our initial efforts to study the RCM of alkene-containing peptides wherein we incorporate >5 amino acid residues have been thwarted by the low solubility of the

(23) See ref 19.
(24) See ref 18.
(25) The olefin geometry of **22** was not assigned.



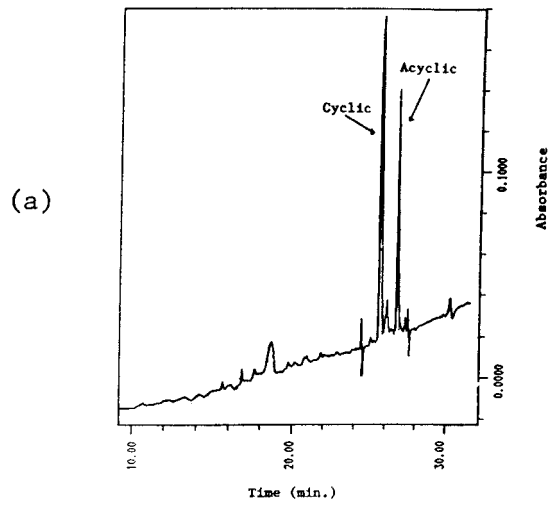
substrates in the organic solvents where catalysts **1** display their highest activity. As a result, we have begun to examine the feasibility of performing the ring-closing reactions on solid-support-bound peptides.[26] To demonstrate the compatibility of the catalyst with solid phase techniques, we revisited one of the tetrapeptide macrocycles that was found to undergo efficient RCM in solution (Scheme 4). We resynthesized the solid-

Scheme 4



support-bound analog **23** of tetrapeptide **15** using conventional SPPS (solid phase peptide synthesis) techniques. To effect RCM, the beads were swelled in CH_2Cl_2, the solvent of choice for maximum activity of **1**. Catalyst **1b** was then introduced, and the mixture was heated to 40 °C for 22 h, in analogy to the conditions for the solution phase RCM reaction. When the

(26) For literature on cyclization reactions performed on solid-support-bound substrates, see: (a) Hiroshige, M.; Hauske, J. R.; Zhou, P. *J. Am. Chem. Soc.* **1995**, *117*, 11590−11591. (b) Andreu, D.; Albericio, F.; Sole, N. A.; Munson, M. C.; Ferrer, M.; Barany, G. *Methods Mol. Biol. (Totowa, NJ)* **1994**, *35* (Peptide Synthesis Protocols), 91. (c) Bunin, B. A.; Plunkett, M. J.; Ellman, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4708−4712. (d) Virgilio, A. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1994**, *116*, 11580−11581. (e) Zhao, Z.; Felix, A. M. *Pept. Res.* **1994**, *7*, 218−223. (f) Albericia, F.; Hammer R. P.; Garciaechevaerria, C.; Mollins, M. A.; Chang, J. L.; Munson, M. C.; Pons, M.; Giralt, E.; Barany, G. *Int. J. Pept. Protein Res.* **1991**, *37*, 402−413. (g) Apsimon, J. W.; Dixit, D. M. *Can. J. Chem.* **1982**, *60*, 368−370.



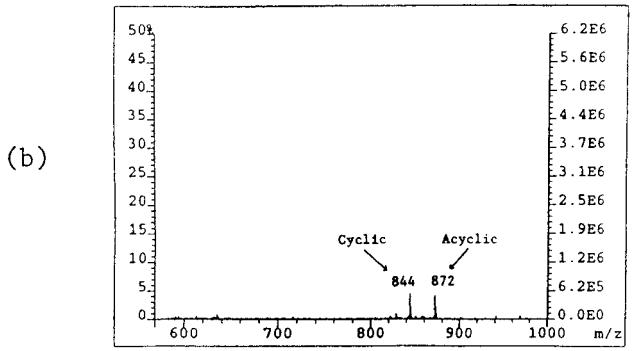


Figure 4. (a) HPLC trace of mixture of acyclic and cyclic glutaredoxin analogs synthesized on solid support. (b) Low-resolution FABMS of mixture of acyclic and cyclic glutaredoxin analogs.

substrate was cleaved from the resin, HPLC analysis revealed the presence of two peaks in a 3:2 ratio, the minor peak corresponding to the acyclic peptide (Figure 4a). Analysis by FABMS confirmed the identity of the two peaks as the starting material and the ring-closed product **24** (Figure 4b). This experiment demonstrates that catalysts **1** react efficiently with solid-support-bound substrates. We now intend to investigate the scope of this method for the synthesis of larger conformationally rigidified systems.

Conclusion

Ruthenium alkylidenes have been shown to demonstrate high metathesis activity for the RCM of amino acids and peptides containing olefins. A range of substrates was examined and ring sizes ranging from 6 to 20 were prepared. The method was found to be particularly well-suited for the synthesis of tetrapeptide, carbon−carbon double bond mimics of naturally occurring tetrapeptide disulfides. Solution conformational analysis of one of the cyclic tetrapeptides revealed the presence of intramolecular H-bonding which was analogous to that reported for the corresponding disulfide-bridged system. In addition, RCM using ruthenium-based alkylidenes was demonstrated to be compatible with substrates bound to solid supports. These experiments illustrate a very straightforward synthetic strategy for the synthesis of conformationally rigidified cyclic peptides which contain carbon−carbon bond cross-links.

Experimental Section

General. NMR spectra were recorded on a General Electric QE-300 or Bruker AM-500 spectrometer. Chemical shifts are reported in parts per million (ppm) downfield form tetramethylsilane (TMS) with

reference to internal solvent. Multiplicities are abbreviated as follows: singlet (s), doublet (d), triplet (t), quartet (q), and multiplet (m). Major rotamer peaks are reported in spectra that are not fully coalesced. The olefin configurations for **14**, **16**, **18**, and **22** were assigned by sequential irradiation of the C_β protons and analysis of the resulting patterns. The notation d(m) refers to the value of the primary *J* value (coupling constant) extracted from this analysis. Infrared spectra were obtained on a Perkin-Elmer 1600 Series FT-IR. Optical rotations were recorded on a Jasco DIP-181 digital polarimeter at 589 nm and are reported as $[\alpha]_\lambda$ (concentration in grams/100 mL of solvent). Low- and high-resolution mass spectra were provided by either the Chemistry and Biology Mass Spectrometry Facility (Caltech) or the Southern California Mass Spectrometry Facility (University of California, Riverside).

Analytical thin-layer chromatography (TLC) was performed using silica gel 60 F254 precoated plates (0.25 mm thickness) with a fluorescent indicator. Flash column chromatography was performed using silica gel 60 (230−400 mesh) from EM Science.[27] Catalysts **1a**,**b** were prepared according to the published procedures.[28]

Peptide Synthesis. Peptides **13**, **15**, and **17** were synthesized by standard solution phase peptide-coupling protocols, using *N*,*N*-dicyclohexylcarbodiimide (DCC)/1-hydroxybenzotriazole (HOBT) as peptide-coupling agent.[29]

All ring-closing metathesis (RCM) reactions were carried out under an argon atmosphere with dry, degassed solvents under anhydrous conditions.

***N*-Allyl-*N*-(*tert*-butoxycarbonyl)-4,5-didehydro-DL-norvaline, Methyl Ester (2).** To a 0 °C solution of (+/−)-*N*-BOC-allylglycine methyl ester (1.4 g, 6.1 mmol) in 30 mL of DMF was added allyl bromide (581 μL, 6.7 mmol) follwed by sodium hydride (160 mg, 6.7 mmol). Gas evolution was observed, and the reaction mixture assumed a pale yellow color. The solution was stirred for 1.5 h at 0 °C and 30 min at 25 °C before the reaction was quenched by addition of 20 mL of dilute aqueous NH_4Cl. The product was extracted with three 20 mL portions of Et_2O, dried over $MgSO_4$, and purified by flash chromatography (1.5 cm × 12 cm of silica gel, 20% EtOAc/hexanes) to afford 900 mg (55%) of **2** as a colorless oil: TLC R_f 0.50 (30% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 500 MHz, not coalesced) δ 5.77−5.60 (br m, 2H), 5.10−4.95 (br m, 4H), 4.5−3.6 (br m, 3H), 3.65 (s, 3H), 2.80−2.65 (br m, 1H), 2.60−2.45 (br m, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz, not coalesced) δ 171.7, 155.4, 154.6, 135.0, 134.5, 134.4, 134.2, 120.4, 117.6, 117.3, 116.1, 80.5, 80.4, 59.0, 58.2, 51.9, 50.5, 49.0, 34.7, 33,8, 28.2; IR (neat, cm^{-1}) 3079, 2978, 1745, 1697, 1643, 1453; HRMS calcd for $C_{14}H_{24}N_1O_4$ (MH^+) 270.1705, found 270.1698.

1-*tert*-Butyl 2-Methyl (±)-3,6-Dihydro-1,2(2*H*)-pyridinedicarboxylate (5). To a 25 °C solution of acyclic diene **2** (170 mg, 0.636 mmol) in 7 mL of C_6H_6 was added ruthenium catalyst **1a** (29 mg, 0.032 mmol, 5 mol %). The orange-brown solution was stirred at this temperature for 2 h before it was concentrated and applied directly to a silica gel column. Chromatography (1.5 cm × 12 cm silica gel, solvent gradient from 5% EtOAc/hexane to 20% EtOAc/hexane) afforded 139 mg (91%) of **5** as a clear oil: TLC R_f 0.40 (30% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz, not coalesced) δ 5.77−5.65 (br m, 1H), 5.01−4.80 (br m, 1H), 4.20−3.70 (br m, 2H), 3.71, 3.70 (2 × s, 3H), 2.65−2.50 (br m, 2H), 1.49, 1.46 (2 × s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz, not coalesced) δ 172.3, 155.9, 124.5, 124.2, 122.4, 122.0, 80.4, 52.4, 52.3, 51.0, 42.3, 41.6, 28.4, 26.7, 26.6; IR (neat, cm^{-1}) 2976, 1746, 1694, 1454, 1403; HRMS calcd for $C_{12}H_{18}N_1O_4$ (M − H) 240.1236, found 240.1236.

***N*-Allyl-*N*-[*N*-(*tert*-butoxycarbonyl)-4,5-didehydro-DL-norvalyl]glycine, Methyl Ester (3).** To a solution of *N*-allyl-*N*-BOC-Gly methyl ester (1.17 g, 5.10 mmol) in 20 mL of CH_2Cl_2 was added 10 mL of trifluoroacetic acid (TFA). The mixture was stirred for 1 h before it was concentrated and redissolved in 25 mL of CH_2Cl_2. The solution was washed with 100 mL of saturated $NaHCO_3$ solution, dried over Na_2SO_4, filtered, and concentrated. The unpurified amine was then dissolved in 15 mL of CH_2Cl_2 and treated with (+/−)-*N*-BOC-allylglycine (400 mg, 1.86 mmol), DCC (383 mg, 1.86 mmol), and 4-(dimethylamino)pyridine (DMAP) (25 mg, 0.121 mmol). The mixture was stirred for 1 h as a white precipitate formed. The mixture was filtered, concentrated, and chromatographed (1.5 cm × 10 cm silica gel, 20% EtOAc/hexanes to 50% EtOAc/hexane) to afford 566 mg (93%) of **3** as a clear oil: TLC R_f 0.40 (30% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 500 MHz, not coalesced) δ 5.81−5.70 (m, 2H), 5.29−5.04 (m, 5H), 4.65 and 4.40 (q, J = 8.2 Hz, 1H), 4.21 (d, J = 7.2 Hz, 1H), 4.05−3.80 (m, 3H), 3.82 (d, J = 7.2 Hz, 1H), 3.71 and 3.68 (2 × s, 3H), 2.50−2.40 (m, 1H), 2.34−2.30 (m, 1H), 1.38 and 1.37 (2 × s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz, not coalesced) δ 172.3, 172.1, 169.5, 169.3, 155.2, 155.0, 132.9, 132.6, 132.2, 118.5, 118.4, 118.1, 79.6, 79.5, 52.3, 52.0, 51.2, 49.9, 49.7, 49.4, 48.2, 46.9, 37.6, 37.3, 28.2, 28.1; IR (neat, cm^{-1}) 3320, 2979, 1747, 1713, 1650, 1514, 1454; HRMS calcd for $C_{16}H_{27}N_2O_5$ (MH^+) 327.1920, found 327.1914.

Methyl (±)-3-[(*tert*-Butoxycarbonyl)amino]-2,3,4,7-tetrahydro-2-oxo-1*H*-azepine-1-acetate (6). To a 50 °C solution of acyclic diene **3** (160 mg, 0.491 mmol) in 60 mL of $CHCl_3$ was added ruthenium catalyst **1a** (29 mg, 0.032 mmol, 5 mol %). The orange-brown solution was stirred at this temperature for 4 h before it was concentrated and applied directly to a silica gel column. Chromatography (1.5 cm × 12 cm silica gel, 25% EtOAc/hexane) afforded 76 mg (52%) of **6** as a clear oil: TLC R_f 0.15 (30% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.76−5.68 (m, 2H), 4.91 (m, 1H), 4.52 (br d, J = 17 Hz, 1H), 4.35 (d, J = 17.4 Hz, 1H), 4.03 (d, J = 17.4 Hz, 1H), 3.70 (s, 3H), 3.70 (m, 1H), 3.32 (dd, J = 17.6, 7.2 Hz, 1H), 2.63 (dd, J = 18.1, 4.1 Hz, 1H), 2.23 (m, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.9, 169.4, 154.9, 129.9, 123.6, 79.5, 52.2, 50.1, 50.0, 47.3, 33.2, 28.3; IR (neat, cm^{-1}) 3272, 2973, 1759, 1715, 1650, 1538, 1487, 1454; HRMS calcd for $C_{14}H_{23}N_2O_5$ (MH^+) 299.1607, found 299.1603.

***N*-Allyl-*N*-[*N*-allyl-*N*-(*tert*-butoxycarbonyl)-L-alanyl]glycine, Methyl Ester (4).** To a −78 °C solution of *N*-BOC-Ala-Gly methyl ester (180 mg, 0.66 mmol) in 5 mL of THF was added allyl bromide (125 mL, 1.45 mmol) followed by P4-phosphazene base (968 μL, 1.38 mmol). The mixture was stirred at −78 °C for 1 h and then warmed to 25 °C for 30 min. The reaction mixture was concentrated and purified by chromatography (1.5 cm × 12 cm silica gel, 25% EtOAc/Hexane) to afford 44 mg (20%) of **4** as a colorless oil: TLC R_f 0.55 (50% EtOAc/hexane); ^{1}H NMR (toluene-d_8, 300 MHz, 80 °C, not fully coalesced) δ 5.8 (br m, 1H), 5.65 (br m, 1H), 5.30−4.80 (m, 5H), 4.20−3.70 (m, 6H), 3.40 (s, 3H), 1.40 (s, 9H), 1.28 (d, J = 6.8 Hz, 3H); ^{13}C NMR (toluene-d_8, 125 MHz, 80 °C, not fully coalesced) δ 172.0, 169.7, 155.8, 136.9, 134.1, 117.3, 115.7, 80.1, 51.4, 47.6, 46.5, 28.6, 16.4; IR (neat, cm^{-1}) 2980, 1754, 1660, 1450; HRMS calcd for $C_{17}H_{29}N_2O_5$ (MH^+) 341.2076, found 341.2064.

Methyl (3*S*,6*Z*)-4-(*tert*-Butoxycarbonyl)-3,4,5,8-tetrahydro-3-methyl-2-oxo-1,4-diazocine-1(2*H*)-acetate (7). C_6H_6 was added ruthenium catalyst **1a** (7 mg, 0.008 mmol, 16 mol %) as a solution in 5 mL of C_6H_6. The orange-brown solution was stirred at this temperature for 24 h before it was concentrated and applied directly to a silica gel column. Chromatography (1.5 cm × 12 cm silica gel, 25% EtOAc/Hexane) afforded 8 mg (51%) of **7** as a clear oil: TLC R_f 0.30 (50% EtOAc/hexane); ^{1}H NMR (toluene-d_8, 300 MHz, 80 °C, not fully coalesced) δ 5.60 (m, 1H), 5.37 (m, 1H), 5.00 (br m, 1H), 4.40−3.00 (br m, 6H), 3.35 (s, 3H), 1.40 (br d, J = 7 Hz), 1.38 (s, 9H); ^{13}C NMR (C_6D_6, 125 MHz, 70 °C, not fully coalesced) δ 171.2, 169.3, 154.3, 134.4 (br), 125.2 (br), 79.9, 51.2, 49.9, 45.4, 43.2 (br), 30.0, 28.4, 17.1; IR (neat, cm^{-1}) 2926, 1755, 1693, 1659, 1436, 1393; HRMS calcd for $C_{15}H_{24}O_5N_2$ (M^+) 312.1685, found 312.1678.

***N*-Benzyl-*N*^{2}-[*N*-[1-[*N*-(*tert*-butoxycarbonyl)-4,5-didehydro-L-norvalyl]-L-prolyl]-2-methylalanyl]-4,5-didehydro-L-norvalinamide (13).** Tetrapeptide **13** was prepared according to the standard solution protocol described in the general experimental above. For **13**: TLC R_f 0.45 (100% EtOAc); $[\alpha]_D$ −53.0 (*c* 0.7, CH_2Cl_2); ^{1}H NMR (CD_3CN, 500 MHz) δ 7.74 (br t, 1H), 7.29 (m, 5H), 7.22 (m, 1H), 7.0 (br s, 1H), 5.83−5.78 (m, 2H), 5.55 (br d, 1H), 5.18−5.05 (m, 4H), 4.42−4.21 (m, 5H), 3.99 (br t, 1H), 3.76 (m, 1H), 3.60 (m, 1H), 2.75 (m, 1H), 2.5−1.1.7 (m, 8H), 1.40 (s, 12H), 1.35 (s, 3H); ^{13}C NMR (CD_3CN, 125 MHz) δ 175.5, 174.0, 173.5, 172.6, 157.0, 140.8, 136.6, 135.0, 129.7, 128.7, 128.1, 119.3, 118.1, 80.5, 63.6, 58.3, 54.8, 53.8, 49.0, 43.9, 36.9, 36.6, 30.0, 29.0, 27.6, 26.3, 24.8; IR (CH_2Cl_2, cm^{-1}) 3425,

(27) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923−2925.

(28) See ref 3.

(29) Bodansky, M. *Peptide Chemistry*; Springer-Verlag: New York, 1988, pp 55−146 and references therein.

3339, 2982, 2934, 1668, 1498, 1439; HRMS calcd for $C_{31}H_{46}N_5O_6$ (MH^+) 584.3448, found 584.3471.

***tert*-Butyl (6*S*,8*E*,11*S*,16a*S*)-6-(Benzylcarbamoyl)-1,2,3,4,5,6,7,10,-11,12,14,15,16,16a-tetradecahydro-3,3-dimethyl-1,4,12-trioxopyrrolo[1,2-*a*][1,4,7]-triazacyclotetradecine-11-carbamate (14).** To a solution of acyclic diene **13** (210 mg, 0.360 mmol) in 80 mL of CH_2Cl_2 was added via cannula a solution of ruthenium catalyst **1a** (67 mg, 0.072 mmol) predissolved in 20 mL of CH_2Cl_2. The orange-brown solution was heated to 40 °C and stirred at this temperature for 20 h. The solution was then concentrated under reduced pressure to afford an oily brown mixture. Purification by chromatography (1.5 cm × 15 cm silica gel, solvent gradient from 50% EtOAc/hexane to 100% EtOAc) afforded 120 mg (60%) of **14** as an off-white powder. Macrocycle **14** can be recrystallized by dissolving the powder in CH_2Cl_2 and layering the solution with hexanes. White needles and prisms result. However, upon removal of solvent the amorphous white powder is reobtained. For **14**: TLC R_f 0.20 (100% EtOAc); $[\alpha]_D$ +63.8 (*c* 0.73, CH_2Cl_2); ^{1}H NMR (CD_2Cl_2, 500 MHz) δ 7.33−7.21 (m, 5H), 7.01 (br d, J = 7.2 Hz, 1H), 6.91 (br t, 1H), 6.53 (br s, 1H), 5.58 (d, J = 7.8Hz, 1H), 5.51−5.42 (d(m), J = 15 Hz, 1H), 5.39−5.30 (d(m), J = 15 Hz, 1H), 4.65−4.59 (m, 2H), 4.50 (q, J = 6 Hz, 1H), 4.29 (dd, J = 15, 5.0 Hz, 1H), 4.20 (t, J = 7.2 Hz, 1H), 3.69 (m, 1H), 3.58 (m, 1H), 2.6−1.88 (m, 8H), 1.50 (s, 3H), 1.41 (s, 9H), 1.35 (s, 3H); ^{13}C NMR (CD_2Cl_2, 125 MHz) δ 175.4, 172.5, 172.1, 172.0, 155.0, 139.9, 131.0, 129.6, 128.9, 128.5, 128.3, 80.5, 62.2, 61.9, 58.4, 53.4, 48.7, 44.2, 34.9, 34.4, 29.3, 29.2, 26.8, 23.9; IR (CH_2Cl_2, cm^{-1}) 3424, 3324, 3054, 2985, 1691, 1632, 1498, 1444; HRMS calcd for $C_{29}H_{42}N_5O_6$ (MH^+) 556.3135, found 556.3145.

***N*-[*N*-[[*O*-[(*o*-Bromobenzyl)oxy]carbonyl]-*N*-[1-[*N*-(*tert*-butoxycarbonyl)-4,5-didehydro-L-norvalyl]-L-prolyl]-L-tyrosyl]-4,5-didehydro-L-norvalyl]glycine, Methyl Ester (15).** Pentapeptide **15** was prepared according to the standard solution protocol described in the general experimental above.[30] For **15**: TLC R_f 0.27 (83% EtOAc/hexane); $[\alpha]_D$ −38.0 (*c* 1.1, CH_2Cl_2); ^{1}H NMR ($(CD_3)_2SO$, 500 MHz) δ 8.34 (br t, J = 5 Hz, 1H), 7.94 (d, J = 8 Hz, 1H), 7.89 (d, J = 8 Hz, 1H), 7.71 (d, J = 8 Hz, 1H), 7.57 (d, J = 8 Hz, 1H), 7.46 (t, J = 8 Hz, 1H), 7.36 (t, J = 8 Hz, 1H), 7.29 (amide NH obscured, apparent d, J = 8 Hz, 3H), 7.13 (d, J = 7 Hz, 2H), 6.91 (d, J = 8 Hz, 1H), 5.83−5.70 (m, 2H), 5.32 (s, 2H), 5.12- 5.01 (m, 4H), 4.48 (br q, J = 9 Hz, 1H), 4.40−4.30 (br m, 2H), 4.21 (br q, J = 7 Hz, 1H), 3.91−3.80 (m, 2H), 3.63 (s, 3H), 3.60−3.51 (m, 2H), 3.06 (dd, J = 14, 4 Hz, 1H), 2.87 (dd, J = 14, 9 Hz, 1H), 2.40 (m, 1H), 2.33 (m, 2H), 2.22 (m, 1H), 1.95 (m, 1H), 1.82−1.71 (br m, 3H), 1.36 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz, 25 °C, not fully coalesced) δ 172.1, 172.0, 171.4, 170.9, 170.3, 155.6, 153.3, 150.2, 134.8, 134.4, 133.3, 127.7, 123.5, 120.0, 118.8, 118.5, 79.8, 69.6, 63.0, 60.7, 55.9, 52.9, 52.2, 47.6, 41.3, 36.9, 36.5, 28.7, 28.4, 25.2; IR (CH_2Cl_2, cm^{-1}) 3415, 3333, 2923, 2851, 1759, 1672, 1605, 1503, 1441, 1364; HRMS calcd for $C_{40}H_{51}N_5O_{11}Br$ (MH^+) 856.2768, found 856.2794.

***tert*-Butyl (3*S*,6*S*,8*E*,11*S*,16a*S*)-3-[*p*-[[[(*o*-Bromobenzyl)oxy]carbonyl]oxy]benzyl]-1,2,3,4,5,6,7,10,11,12,14,15,16,16a-tetradecahydro-6-[[(methoxycarbonyl)methyl]carbamoyl]-1,4,12-trioxypyrrolo[1,2-*a*][1,4,7]-triazacyclotetradecine-11-carbamate (16).** To a solution of acyclic diene **15** (200 mg, 0.234 mmol) in 53 mL of CH_2Cl_2 was added via syringe a solution of ruthenium catalyst **1b** (58 mg, 0.072 mmol) predissolved in 10 mL of CH_2Cl_2. The purple solution was heated to 45 °C and turned orange-brown in color over a 30 min period. The solution was stirred at 45 °C for 23 h. The solution was then concentrated under reduced pressure to afford an oily brown mixture. Purification by chromatography (3 cm × 15 cm silica gel, solvent gradient from 80% EtOAc/hexane to 100% EtOAc) afforded 155 mg (80%) of **16** as an off-white powder: TLC R_f 0.21 (83% EtOac/hexane); $[\alpha]_D$ −29.0 (*c* 1.1, CH_2Cl_2); ^{1}H NMR ($(CD_3)_2SO$, 500 MHz) δ 8.22 (br t, J = 6 Hz, 1H), 7.88 (br d, J = 9 Hz, 1H), 7.71 (d, J = 8 Hz, 1H), 7.57 (d, J = 7 Hz, 1H), 7.46 (t, J = 7 Hz, 1H), 7.36 (t, J = 7 Hz, 2H), 7.22 (d, J = 8 Hz, 2H), 7.13 (amide NH obscured, apparent d, J = 8 Hz, 3H), 6.69 (d, J = 9 Hz, 1H), 5.46 (d(m), J = 15 Hz, 1H), 5.36 (d(m), J = 15 Hz, 1H), 5.31 (s, 2H), 4.58 (m, 2H), 4.47 (m, 1H), 3.97 (m, 1H), 3.85 (m, 2H), 3.68 (m, 1H), 3.64 (s, 3H), 3.36 (m, 2 H), 2.80 (m, 1H), 2.46 (m, 2 H), 2.11 (m, 2H), 1.84 (m, 1H), 1.71 (m, 2H), 1.37 (s, 9H), 1.23 (m, 1H); ^{13}C NMR ($(CD_3)_2SO$, 75 MHz) δ 171.8, 170.8, 170.2, 170.0, 168.9, 155.0, 152.7, 149.1, 136.5, 134.0, 132.7, 130.7, 130.6, 130.3, 130.1, 128.0, 127.4, 122.9, 120.5, 78.2, 69.2, 60.8, 53.1, 51.7, 51.2, 50.7, 46.5, 35.3, 34.8, 28.5, 28.0, 24.4; IR (CH_2Cl_2, cm^{-1}) 3426, 3354, 2954, 2923, 2851, 1759, 1687, 1621, 1508, 1446, 1369, 1164; HRMS calcd for $C_{38}H_{47}N_5O_{11}Br$ (MH^+) 828.2443, found 828.2455.

***N*-[*N*-[*N*-[*N*-(*tert*-Butoxycarbonyl)-4,5-didehydro-L-norvalyl]-L-leucyl]-L-leucyl]-4,5-didehydro-L-norvaline, Benzyl Ester (17).** Tetrapeptide **17** was prepared according to the standard solution protocol described in the general experimental above. For **17**: TLC R_f 0.41 (80% CH_2Cl_2/EtOAc); $[\alpha]_D$ −29.5 (*c* 1.2, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz, 25 °C, not fully coalesced) δ 7.33 (m, 5H), 6.91 (apparent s, 1H), 6.73 (apparent s, 1H), 6.64 (d, J = 7 Hz, 1H), 5.74−5.63 (m, 2H), 5.19−5.02 (m, 6H), 4.66 (q, J = 7 Hz, 1H), 4.49 (q, J = 9 Hz, 1H), 4.39 (q, J = 8 Hz, 1H), 4.11 (m, 1H), 2.61−2.38 (m, 4H), 1.73−1.46 (m, 6H), 1.43 (s, 9H), 0.96−0.86 (m, 12H); ^{13}C NMR ($CDCl_3$, 125 MHz, 25 °C, not fully coalesced) δ 171.9, 171.8, 171.4, 135.7, 133.1, 132.6, 128.8, 128.6, 128.5, 80.7, 67.2, 52.3, 51.9, 41.3, 41.1, 36.8, 36.5, 34.2, 29.9, 28.5, 25.9, 25.1, 25.0, 24.9, 23.2, 23.0, 22.2, 22.1; IR (CH_2Cl_2, cm^{-1}) 3414, 3339, 2961, 2929, 1740, 1694, 1505, 1456, 1365, 1169; HRMS calcd for $C_{34}H_{53}N_4O_7$ (MH^+) 629.3914, found 629.3914.

***tert*-Butyl (3*S*,6*S*,9*S*,11*E*,14*S*)-14-[(Benzyloxy)carbonyl]-3,6-diisobutyl-2,5,8-trioxo-1,4,7-triazacyclotetradec-11-ene-9-carbamate (18).** To a solution of acyclic diene **17** (285 mg, 0.453 mmol) in 100 mL of CH_2Cl_2 was added via syringe a solution of ruthenium catalyst **1b** (112 mg, 0.136 mmol) predissolved in 22 mL of CH_2Cl_2. The purple solution was heated to 45 °C and turned orange-brown in color over 20 min. The solution was stirred at 45 °C for 21 h. The solution was then concentrated under reduced pressure to afford an oily brown mixture. Purification by chromatography (3 cm × 15 cm silica gel, 80% CH_2Cl_2/EtOAc) afforded 163 mg (60%) of **18** as an off-white powder: TLC R_f 0.24 (80% CH_2Cl_2/EtOAc); $[\alpha]_D$ −114.0 (*c* 1.0, CH_2Cl_2); ^{1}H NMR ($(CD_3)_2SO$, 500 MHz) δ 7.90 (d, J = 9, 1H), 7.80 (d, J = 8, 1H), 7.37 (m, 5H), 7.24 (d, 8 Hz, 1H), 5.46 (d(m), J = 15 Hz, 1H), 5.16 (d(m), J = 15 Hz, 1H), 5.13 (s, 2H), 4.48 (m, 1H), 4.21 (m, 2H), 4.03 (m, 1H), 2.45−2.12 (br m, 4H), 1.66−1.45 (m, 6H), 1.38 (s, 9H), 0.87 (apparent d, J = 6 Hz, 6H), 0.81 (apparent t, J = 6 Hz, 6H); ^{13}C NMR ($(CD_3)_2CO$, 75 MHz) δ 171.9, 170.8, 170.6, 130.3, 128.1, 127.7, 127.6, 127.5, 78.7, 66.0, 53.4, 52.8, 50.6, 50.0, 40.2, 39.7, 34.6, 33.7, 24.4, 24.2, 24.1, 22.5, 22.2, 20.4, 20.3; IR (CH_2Cl_2, cm^{-1}) 3419, 3339, 2958, 2929, 1740, 1671, 1602, 1515, 1365, 1158; HRMS calcd for $C_{32}H_{49}N_4O_7$ (MH^+) 601.3601, found 601.3610.

***O*-Allyl-*N*-[*O*-allyl-*N*-(*tert*-butoxycarbonyl)-L-tyrosyl]-L-tyrosine, Methyl Ester (19).** To a solution of *N*-BOC-dityrosine methyl ester (3.26 g, 7.10 mmol) in 30 mL of acetone were added allyl bromide (1.71 mL, 19.8 mmol) and finely powdered K_2CO_3 (2.94 g, 21.3 mmol). The reaction mixture was stirred for 48 h at 25 °C before being filtered through a Celite pad. Purification of the residue by chromatography (3 cm × 12 cm silica gel, solvent gradient from 20% EtOAc/hexane to 50% EtOAc/hexane) afforded **19** as a white solid: TLC R_f 0.50 (50% EtOAc/hexane); $[\alpha]_D$ +20.7 (*c* 2.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.06 (d, J = 8.4 Hz, 2H), 6.87 (d, J = 8.4 Hz, 2H), 6.79 (d, J = 8.4 Hz, 2H), 6.75 (d, J = 8.4 Hz, 2H), 6.38 (br d, J = 7.5 Hz, 1H), 6.00 (m, 2H), 5.35 (dd, J = 17.1, 0.6 Hz, 2H), 5.23 (br d, J = 9.3 Hz, 2H), 4.92 (br s, 1H), 4.71 (br d, J = 6.2 Hz, 1H), 4.46 (br t, J = 4.1 Hz, 4H), 4.29 (br s, 1H), 3.63 (s, 3H), 2.95 (m, 4H), 1.38 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.3, 170.7, 157.6 (br d), 155.1, 133.2, 130.2, 130.1, 128.6, 127.8, 117.3, 117.2, 114.8, 114.7, 79.9, 68.7, 68.6, 55.8, 53.3, 51.9, 37.3, 37.0, 28.1; IR (CH_2Cl_2, cm^{-1}) 3420, 2981, 2932, 1742, 1713, 1681, 1610, 1510, 1361; HRMS calcd for $C_{30}H_{39}N_2O_7$ (MH^+) 539.2757, found 539.2766.

***tert*-Butyl (13*S*,16*S*)-13-(Methoxycarbonyl)-15-oxo-2,7-dioxa-14-azatricyclo[16.2.2.2^{8,11}]tetracosa-4,8,10,18,20,21,23-heptaene-16-carbamate (20).** To a 50 °C solution of acyclic diene **19** (130 mg, 0.241 mmol) in 100 mL of CH_2Cl_2 was added ruthenium catalyst **1b** (29 mg, 0.072 mmol). Within 5 min, the purple solution became orange-brown, and the solution was stirred for an additional 2.5 h, when TLC analysis showed full disappearance of starting material. Triethylamine (1 mL)

(30) A minor impurity remains in compound **15** even after repeated column chromatography and recrystallization. Peptide **15** is greater than 95% pure by ^{1}H NMR. This impurity is not present in the cyclized product **16**.

was added to the solution to deactivate any remaining active catalyst. The solution was then concentrated to afford an oily brown mixture. Purification by chromatography (3 cm × 12 cm silica gel, 50% EtOAc/hexane) afforded 83 mg (68%) of **21** as a white powder: olefin configuration not assigned; TLC R_f 0.45 (50% EtOAc/hexane); $[\alpha]_D$ +46.4 (*c* 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.04 (br s, 2H), 6.77 (br s, 2H), 6.68 (t, J = 8.5 Hz, 4H), 5.90 (br m, 1H), 5.83 (s, 2H), 5.25 (br s, 1H), 4.85 (br s, 1H), 4.66 (m, 4H), 4.39 (br t, 1H), 3.70 (s, 3H, minor rotomer at 3.77), 3.34 (br d, J = 12.6 H, 1H), 2.98 (br s, 2H), 2.63 (dd, J = 14.0, 8.9 Hz, 1H), 1.49 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz, 25 °C, not fully coalesced) δ 171.2, 171.1, 156.8, 156.6, 155.5, 130.5, 130.2, 130.0, 129.7, 129.5, 129.2, 127.4, 115.6, 115.4, 115.3, 80.9, 67.0, 66.7, 64.7, 64.1, 55.6, 52.7, 52.1, 37.4, 36.2, 28.3; IR (CH_2Cl_2, cm^{-1}) 3683, 3411, 2933, 1744, 1713, 1676, 1611, 1511, 1484; HRMS calcd for $C_{28}H_{35}N_2O_7$ (MH^+) 511.2444, found 511.2437.

***N*-[*N*-(*tert*-Butoxycarbonyl)-4,5-didehydro-L-norvalyl]-*O*-4-pentenoyl-L-serine, Methyl Ester (21).** *N*-BOC-serine methyl ester (1.20 g, 5.47 mmol) was dissolved in 75 mL of CH_2Cl_2 and treated with 4-pentenoic acid (559 μL, 5.47 mmol), DCC (1.13 g, 5.47 mmol), and DMAP (100 mg, 0.82 mmol). A white precipitate formed immediatley, and the solution was stirred for 12 h. The mixture was filtered and washed with 50 mL of a 10% citric acid solution, followed by 50 mL of saturated $NaHCO_3$ solution. The solution was dried over $MgSO_4$ and concentrated to afford 1.65 g of the crude esterified product as a pale yellow oil with some crystalline domains. To the crude product (1.65 g, theoretical 5.47 mmol) dissolved in 15 mL of CH_2Cl_2 was added an excess of TFA (7.67 mL, 99.6 mmol). The solution was allowed to stir at room temperature for 2.5 h after which the solution was concentrated to an orange oil and dried under high vacuum. The oil was then taken up in 100 mL of CH_2Cl_2 and treated with triethylamine (915 μL, 6.56 mmol) at room temperature. After 15 min of stirring, *N*-BOC-allylglycine (1.18 g, 5.47 mmol), HOBT (1.11 g, 8.21 mmol), and DCC (1.13 g, 5.47 mmol) were added to the solution. A white precipitate formed immediately, and the solution was allowed to stir at room temperature for 9 h. The mixture was then filtered, washed with 75 mL of a 10% citric acid solution, and subsequently washed with 75 mL of a saturated $NaHCO_3$ solution. The product was dried over $MgSO_4$ and concentrated to yield a yellow oil. Purification by column chromatography (4 cm × 15 cm silica gel, solvent gradient from 25% EtOAc/hexane to 50% EtOAc/hexane) afforded 1.19 g (55%) of **21** as a clear oil: TLC R_f 0.21 (75% hexane/EtOAc); $[\alpha]_D$ +13.1 (*c* 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.92 (br d, J = 7 Hz, 1H), 5.81−5.68 (m, 2H), 5.15−4.97 (m, 5H), 4.80−4.78 (m, 1H), 4.42 (dd, J = 11, 4 Hz, 1H), 4.36 (dd, J = 11, 3 Hz, 1H), 4.17 (m, 1H), 3.73 (s, 3H), 2.54−2.43 (m, 2H), 2.38 (m, 2H), 2.32 (m, 2H), 1.41 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.6, 171.6, 169.7, 155.6, 136.6, 133.1, 119.2, 115.8, 80.4, 63.8, 52.9, 52.0, 36.8, 34.1, 33.7, 28.8, 28.4; IR (CH_2Cl_2, cm^{-1}) 3680, 3427, 2981, 1746, 1716, 1685, 1494, 1438; HRMS calcd for $C_{19}H_{31}N_2O_7$ (MH^+) 399.2131, found 399.2140.

***tert*-Butyl (3*S*,6*S*,8*E*)-3-(Methoxycarbonyl)-5,12-dioxo-1-oxa-4-azacyclododec-8-ene-6-carbamate (22).** To a solution of acyclic diene **21** (50 mg, 0.125 mmol) in 230 mL of CH_2Cl_2 was added via syringe a solution of ruthenium catalyst **1b** (30 mg, 0.036 mmol) predissolved in 20 mL of CH_2Cl_2. The purple solution was heated to 45 °C and turned orange-brown in color over 20 min. The solution was stirred at 45 °C for 20 h. The solution was then concentrated under reduced pressure to afford an oily brown mixture. Purification by chromatography (3 cm × 10 cm silica gel, (50% EtOAc/hexane) afforded 28 mg (56%) of **22** as an off-white powder: TLC R_f 0.30 (50% EtOAc/hexane); $[\alpha]_D$ +41.1 (*c* 1.0, CH_2Cl_2); ^{1}H NMR ($(CD_3)_2SO$, 500 MHz, 60 °C, not fully coalesced) δ 7.47 (br d, J = 8 Hz, 1H), 6.03 (apparent s, 1H), 5.48 (d(m), J = 15 Hz, 1H), 5.32 (d(m), J = 15 Hz, 1H), 4.72 (m, 1H), 4.52 (apparent t, J = 11 Hz, 1H), 4.16 (dd, J = 11, 4 Hz, 1H), 4.07 (m, 1H), 3.64 (s, 3H), 2.44−2.18 (m, 6H), 1.41, 1.40 (2 × s, 9H); ^{13}C NMR ($(CD_3)_2CO$, 75 MHz, 25 °C, not fully coalesced) δ 172.2, 170.5, 169.5, 155.0, 131.7, 126.0, 78.5, 61.0, 55.0, 52.3, 51.9, 50.1, 35.0, 34.0, 28.1; IR (CH_2Cl_2, cm^{-1}) 3691, 3422, 2929, 1736, 1720, 1683, 1517, 1485; HRMS calcd for $C_{17}H_{27}N_4O_7$ (MH^+) 371.1818, found 371.1812.

Solid Phase Synthesis of 23. Peptide **23** was prepared by manual solid phase peptide synthesis.[31] Fmoc-Pal-PEG-PS resin (substitution 0.20 mmol/g) was used to afford C-terminal primary amides. Commerically available reagents and starting materials were purchased from Sigma Chemical Co., Applied Biosystems, Peptides International, and PerSeptive Biosystems. N_α-(Fluorenylmethoxy)carbonyl (Fmoc) protection was employed for all amino acids in the solid phase synthesis, with the tyrosine phenol protected as a *tert*-butyl ester. Each amino acid was coupled sequentially to the peptide chain grown from the C-terminal amino acid using *N*,*N*-diisopropylcarbodiimide/1-hydroxybenzotriazole. A complete coupling in each step was monitored by a quantitative ninhydrin test.[32] Unreacted N-termini were acetylated using an acetic anyhdride/HOBT/diisoprolpylethylamine capping protocol. Fmoc groups were cleaved with 20% piperidine in dimethylformamide (DMF). The peptide was deprotected and cleaved form the resin by treatment with with a solution of TFA/anisole/thioanisole (90:5:5) for 2 h.

Heterogeneous RCM Protocol. To a suspension of solid-support-bound peptide **23** (300 mg of resin, 0.06 mmol (theoretical) of bound peptide) in 22 mL of CH_2Cl_2 was added via syringe a solution of ruthenium catalyst **1b** (25 mg, 0.030 mmol) predissolved in 5 mL of CH_2Cl_2. The solution turned from pink to orange-brown over 3 h. The suspension was heated to 40 °C and gently stirred for 22 h. The beads were then filtered, rinsed with CH_2Cl_2, DMF, and MeOH, respectively, and dried under high vacuum. To 270 mg of the dried resin was added 3 mL of a solution of TFA/anisole/thioanisole (90:5:5). The suspension was shaken gently at room temperature for 2 h, after which the beads were filtered and rinsed with a minimal amount of TFA. The filtrate was reduced in volume to ~0.5 mL to yield a brown oil. Trituration with 2:1 ether/hexane afforded the crude peptide mixture as an off-white solid. The solid was dissolved in deionized H_2O and freeze dried to afford a cream powder which was a mixture of 60% **24** and 40% **23**.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of **2** and **5**, ^{1}H NMR and GC-MS spectra of **4** and **7**, and ^{1}H NMR spectra of **3**, **4**, **6**, and **13**−**22** (20 pages). See any current masthead for ordering and Internet access information.

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(31) Merrified, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 1476−1482.
(32) Sarin, V. K.; Kent, S. B. H.; Tam, J. P.; Merrifield, R. B. *Anal. Biochem.* **1981**, *117*, 147−157.