JOC Article

Synthesis and Conformational Studies of Dipeptides Constrained by Disubstituted 3-(Aminoethoxy)propionic Acid Linkers

D. Srinivasa Reddy, David Vander Velde, and Jeffrey Aubé*

Department of Medicinal Chemistry, 1251 Wescoe Hall Drive, Malott Hall, Room 4070, University of Kansas, Lawrence, Kansas 66045-7582

jaube@ku.edu

Received November 15, 2003

A series of cyclic compounds with dimethyl-substituted 3-(aminoethoxy)propionic acid linkers have been prepared as potential β-turn mimics. The desired linkers were prepared from disubstituted pyrones, which were coupled with dipeptides and then subjected to macrocyclization using diethylcyanophosphonate to furnish cyclic compounds **1**−**5**. Conformational analysis was carried out using NMR and X-ray crystallography. All of the five cyclic compounds were found to exist in type I or type II β-turn conformations.

Peptidomimetics based on β-turns are important, as many peptides are required to adopt such a conformation while effecting a biological response. A variety of structural motifs have been utilized as turn mimics, ranging from substituted heterocycles to scaffolds derived from steroids or carbohydrates.[1,2] More recent work has emphasized the generation of libraries for β-turn models using solid-phase synthesis, in which a linker is attached to a solid support.[3] The use of 6-aminocaproic acid (Aca) as a dipeptide linker was introduced by Woody and Scheraga, who showed that such macrocycles adopt a β-turn around the dipeptide unit.[4] They also established that the type of β-turn formed (type I or type II) depended on the stereochemistry of the dipeptide used. Our laboratory has studied the design and synthesis of dipeptides constrained by substituted Aca linkers.[5] These studies showed that the position and stereochemistry of Aca linker substitution had an effect on the conformation of the macrocycles.

As a part of ongoing research in this area, we were interested in designing more effective linkers able to constrain simple dipeptides into particular subtypes of β-turn conformations. X-ray analysis of cyclic compounds prepared using a variety of all-carbon Aca linkers showed that one of the C−H bonds of the C4-methylene group occupies an inside position in the macrocycle (Figure 1c), disrupting a potential hydrogen bond between the Aca carbonyl and its amide nitrogen. We hypothesized that

(1) Selected reviews on β-turn mimics: (a) Rose, G. D.; Gierasch, L. M.; Smith, J. A. In *Advances in Protein Chemistry*; Anfinsen, C. B., Edsall, J. T., Richards, F. M., Eds.; Academic: Orlando, FL, 1985; Vol. 37, pp 1−109. (b) Giannis, A.; Kolter, T. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1244−1267. (c) Nagai, U.; Sato, K.; Nakamura, R.; Kato, R. *Tetrahedron* **1993**, *49*, 3577−3592. (d) Olson, G. L.; Bolin, D. R.; Bonner, M. P.; Bös, M.; Cook, C. M.; Fry, D. C.; Graves, B. J.; Hatada, M.; Hill, D. E.; Kahn, M.; Madison, V. S.; Rusiecki, V. K.; Sarabu, R.; Sepinwall, J.; Vincent, G. P.; Voss, M. E. *J. Med. Chem.* **1993**, *36*, 3039−3049. (e) Mueller, G. *Angew. Chem., Int. Ed. Engl.* **1997**, *35*, 2767−2769. (f) MacDonald, M.; Aubé, J. *Curr. Org. Chem.* **2001**, *5*, 417−438. (g) Hanessian, S.; McNaughton-Smith, G.; Lombart, H.-G.; Lubell, W. D. *Tetrahedron* **1997**, *53*, 12789−12854. (h) Burgess, K. *Acc. Chem. Res.* **2001**, *34*, 826−835. (i) Nubbemeyer, U. *Top. Curr. Chem.* **2001**, *216*, 125−196. (j) Souers, A. J.; Ellman, J. A. *Tetrahedron* **2001**, *57*, 7431−7448. (k) Eguchi, M.; Kahn, M. *Mini-Rev. Med. Chem.* **2002**, *2*, 447−462. (l) Hruby, V. J. *Nat. Rev. Drug Discovery* **2002**, *1*, 847−858. (m) Suat Kee, K.; Jois, S. D. S. *Curr. Pharm. Des.* **2003**, *9*, 1209−1224. (n) Ahn, J.-M.; Boyle, N. A.; MacDonald, M. T.; Janda, K. D. *Mini-Rev. Med. Chem.* **2002**, *2*, 463−473.

(2) Selected recent papers on β-turn mimics: (a) Zhang, J.; Xiong, C.; Ying, J.; Wang, W.; Hruby, V. J. *Org. Lett.* **2003**, *5*, 3115−3118. (b) Lee, H. B.; Pattarawarapan, M.; Roy, S.; Burgess, K. *Chem. Commun.* **2003**, 1674−1675. (c) Xiong, C.; Zhang, J.; Davis, P.; Wang, W.; Ying, J.; Porreca, F.; Hruby, V. J. *Chem. Commun.* **2003**, 1598−1599. (d) Baek, B.; Lee, M.; Kim, K.; Cho, U.; Boo, D. W.; Shin, I. *Org. Lett.* **2003**, *5*, 971−974. (e) Boruah, A.; Rao, I. N.; Nandy, J. P.; Kumar, S. K.; Kunwar, A. C.; Iqbal, J. *J. Org. Chem.* **2003**, *68*, 5006−5008. (f) Luppi, G.; Lanci, D.; Trigari, V.; Garavelli, M.; Garelli, A.; Tomasini, C. *J. Org. Chem.* **2003**, *68*, 1982−1993. (g) Kaul, R.; Deechongkit, S.; Kelly, J. W. *J. Am. Chem. Soc.* **2002**, *124*, 11900−11907. (h) Han, Y.; Giragossian, C.; Mierke, D. F.; Chorev, M. *J. Org. Chem.* **2002**, *67*, 5085−5097. (i) Wels, B.; Kruijtzer, J. A. W.; Liskamp, R. M. J. *Org. Lett.* **2002**, *4*, 2173-2176. (j) Grieco, P.; Campiglia, P.; Gomez-Monterrey, I.; Novellino, E. *Tetrahedron Lett.* **2002**, *43*, 1197−1199. (k) Gibbs, A. C.; Bjorndahl, T. C.; Hodges, R. S.; Wishart, D. S. *J. Am. Chem. Soc.* **2002**, *124*, 1203−1213. (l) Hoffmann, T.; Lanig, H.; Waibel, R.; Gmeiner, P. *Angew. Chem., Int. Ed.* **2001**, *40*, 3361−3364. (m) Sukopp, M.; Marinelli, L.; Heller, M.; Brandl, T.; Goodman, S. L.; Hoffmann, R. W.; Kessler, H. *Helv. Chim. Acta* **2002**, *85*, 4442−4452. (n) Halab, L.; Lubell, W. D. *J. Am. Chem. Soc.* **2002**, *124*, 2474−2484.

(3) (a) Wallace, O. B.; Whitehouse, D. L.; Dodd, D. S. *Adv. Amino Acid Mimetics Peptidomimetics* **1999**, *2*, 1−51. (b) Feng, Y.; Pattarawarapan, M.; Wang, Z.; Burgess, K. *Org. Lett.* **1999**, *1*, 121−124. (c) Feng, Y.; Burgess, K. *Chem.—Eur. J.* **1999**, *5*, 3261−3272. (d) Eguchi, M.; Lee, M. S.; Stasiak, M.; Kahn, M. *Tetrahedron Lett.* **2001**, *42*, 1237−1239. (e) Virgilio, A. A.; Schurer, S. C.; Ellman, J. A. *Tetrahedron Lett.* **1996**, *37*, 6961−6964. (f) Virgilio, A. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1994**, *116*, 11580−11581. (g) Virgilio, A. A.; Bray, A. A.; Zhang, W.; Trinh, L.; Snyder, M.; Morrissey, M. M.; Ellman, J. A. *Tetrahedron* **1997**, *53*, 6635−6644.

(4) (a) Bandekar, J.; Evans, D. J.; Krimm, S.; Leach, S. J.; Lee, S.; McQuie, J. R.; Minasian, E.; Némethy, G.; Pottle, M. S.; Scheraga, H. A.; Stimson, E. R.; Woody, R. W. *Int. J. Pept. Protein Res.* **1982**, *19*, 187−205. (b) Deslauriers, R.; Evans, D. J.; Leach, S. J.; Meinwald, Y. C.; Minasian, E.; Némethy, G.; Rae, I. D.; Scheraga, H. A.; Somorjai, R. L.; Stimson, E. R.; Van Nispen, J. W.; Woody, R. W. *Macromolecules* **1981**, *14*, 985−996. (c) Némethy, G.; McQuie, J. R.; Pottle, M. S.; Scheraga, H. A. *Macromolecules* **1981**, *14*, 975−985. (d) Maxfield, F. R.; Bandekar, J.; Krimm, S.; Evans, D. J.; Leach, S. J.; Némethy, G.; Scheraga, H. A. *Macromolecules* **1981**, *14*, 997−1003.

(5) (a) Kitagawa, O.; Vander Velde, D.; Dutta, D.; Morton, M.; Takusagawa, F.; Aubé, J. *J. Am. Chem. Soc.* **1995**, *117*, 5169−5178. (b) MacDonald, M.; Vander Velde, D.; Aubé, J. *Org. Lett.* **2000**, *2*, 1653−1655. (c) MacDonald, M.; Vander Velde, D.; Aubé, J. *J. Org. Chem.* **2001**, *66*, 2636−2642.

10.1021/jo035683q CCC: $27.50
Published on Web 02/10/2004

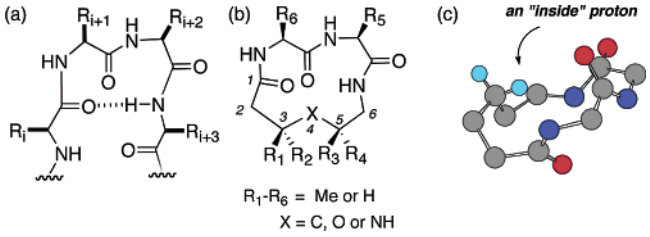
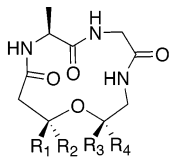


FIGURE 1. (a) A generalized β-turn. (b) Dipeptides constrained with Aca linkers. (c) Ball-and-stick model showing the location of the "inside" proton.



	R_1	R_2	R_3	R_4
1	Me	H	Me	H
2	H	Me	H	Me
3	Me	H	H	Me
4	H	Me	Me	H
5	H	H	H	H

FIGURE 2. Cyclic compounds with oxa-Aca linkers.

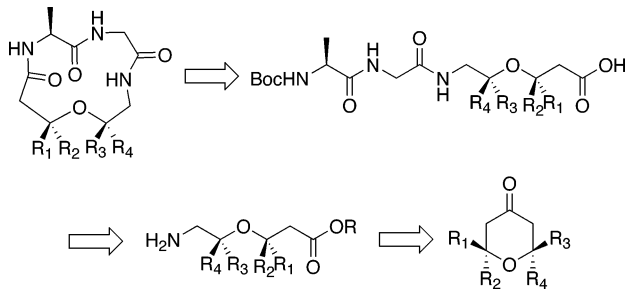
this space might be freed up by replacement of the C4-methylene group with an oxygen atom or, alternatively, with an NH group containing an additional hydrogen-bonding partner. In so doing, it seemed possible that tighter macrocycles might be obtained, leading to better conformational control by the hetero-Aca linkers. In this paper, we describe the synthesis and conformational analysis of a set of ether-containing β-turn mimics (Figure 2). In this study, we concentrated on linkers bearing 1,3-dimethyl substitution as a conformational controlling feature.[6]

Synthesis

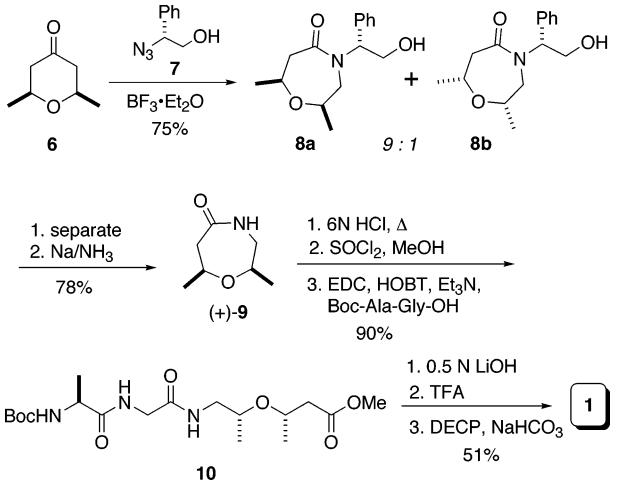
Retrosynthetically, our target macrocycles **1**−**5** were envisioned to arise from the corresponding acyclic precursors, obtained from coupling the linkers with commercially available dipeptides (Scheme 1).

A synthetic route for the enantiopure *syn*-dimethyl linkers was devised on the basis of a ring-expansion method recently developed in our laboratory. Thus, the Lewis acid-mediated azido-Schmidt reaction[7] of chiral azido alcohol **7** with ketone **6** furnished a 9:1 diastereomeric mixture of ring-expanded lactams **8a** and **8b** in 75% yield (Scheme 2). The lactams were separated cleanly using chromatography, and the major product was converted to lactam (+)-**9** by reductive removal of the phenethyl group using metal ammonia. The absolute

SCHEME 1



SCHEME 2



configuration of (+)-**9** was confirmed by comparing its rotation with the literature value.[8] Hydrolysis of the amide bond of (+)-**9** using 6 N HCl followed by esterification with $SOCl_2$−MeOH resulted in the desired *syn*-dimethyl linker in 95% yield. The crude *syn*-dimethyl linker was condensed with commercially available Boc-Ala-Gly-OH under standard peptide coupling conditions[9] to give **10**. Ester hydrolysis and deprotection of the Boc group was followed by macrocyclization under dilute conditions with diethylcyanophosphonate (DECP)[10] to furnish the target macrocycle **1** in 51% yield. The spectral data of **1** were consistent with its assigned structure.

For the synthesis of an *anti*-dimethyl oxa linker, we prepared *trans*-2,6-dimethyl tetrahydropyrone (**12**) from the known dihydropyrone **11**[11] via methylcuprate addition. However, when we subjected **12** to asymmetric azido-Schmidt conditions, the same mixture of lactams **8a** and **8b** as obtained above was exclusively isolated. Control experiments showed that BF_3 promoted the conversion of *trans*-dimethyl pyrone **12** into the thermodynamically more stable *cis*-isomer **6** prior to ring expansion (Scheme 3). The use of the more reactive three-carbon tethered chiral azido alcohol was also unsuccessful and resulted in several diastereomers resulting from both *cis*- and *trans*-dimethyl groups.

Our inability to secure the *trans* lactams corresponding to **8** forced us to pursue an alternative approach. Thus,

(6) (a) Hoffmann, R. W.; Schopfer, U.; Muller, G.; Brandl, T. *Helv. Chim. Acta* **2002**, *85*, 4424−4441. (b) Hoffmann, R. W.; Gottlich, R.; Schopfer, U. *Eur. J. Org. Chem.* **2001**, 1865−1871. (c) Hoffmann, R. W. *Angew. Chem., Int. Ed. Engl.* **2000**, *39*, 2055−2070. (d) Stenkamp, D.; Hoffmann, R. W.; Gottlich, R. *Eur. J. Org. Chem.* **1999**, 2929−2936. (e) Hoffmann, R. W.; Stenkamp, D.; Trieselmann, T.; Gottlich, R. *Eur. J. Org. Chem.* **1999**, 2915−2927.

(7) (a) Furness, K.; Aubé, J. *Org. Lett.* **1999**, *1*, 495−497. (b) Sahasrabudhe, K.; Gracias, V.; Furness, K.; Smith, B. T.; Katz, C. E.; Reddy, D. S.; Aubé, J. *J. Am. Chem. Soc.* **2003**, *125*, 7914−7922.

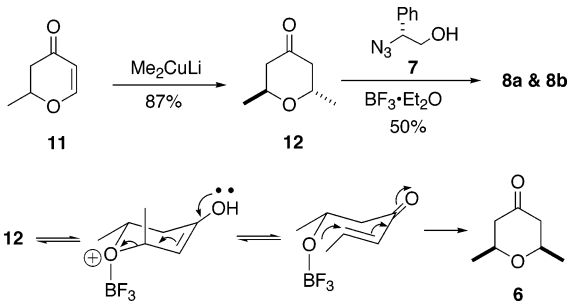
(8) Sato, M.; Kuroda, H.; Kaneko, C.; Furuya, T. *J. Chem. Soc., Chem. Commun.* **1994**, 687−688.

(9) Sheehan, J. C.; Preston, J.; Cruickshank, P. A. *J. Am. Chem. Soc.* **1965**, *87*, 5468−5469.

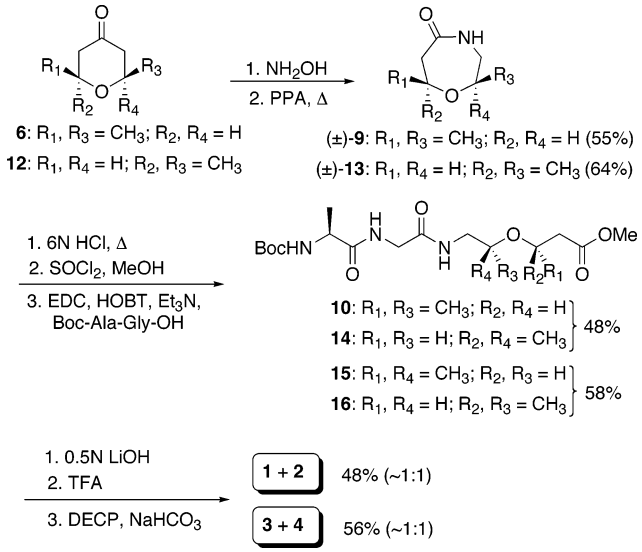
(10) Hayashi, K.; Hamada, Y.; Shioiri, T. *Tetrahedron Lett.* **1992**, *33*, 5075−5076.

(11) Danishefsky, S.; Kerwin, J. F. J. *J. Org. Chem.* **1982**, *47*, 1597−1598.

SCHEME 3



SCHEME 4



crystalline lactams (±)-**9** and (±)-**13** were prepared from **6** and **12**, respectively, using the standard Beckmann rearrangement of the corresponding oximes. With lactams in hand, hydrolysis of the amide bond in (±)-**9** followed by esterification resulted in the desired linker. The crude linker was reacted with Boc-Ala-Gly-OH under standard peptide coupling conditions to give the corresponding 1:1 diastereomeric mixture of **14** and **10**; no attempt was made to separate the diastereomers at this stage (Scheme 4). Removal of the methyl ester and Boc groups followed by macrocyclization produced the desired target macrocycles **1** and **2** in an ~1:1 ratio. Compounds **1** and **2** were separated chromatographically. The spectral data of **1** showed this compound to be identical with that prepared in Scheme 2 using the asymmetric azido-Schmidt reaction. Similarly, we prepared the *anti* series comprised of **3** and **4** from (±)-**13** (Scheme 4). Tentatively, we have assigned the stereochemistry of dimethyl groups for compounds **3** and **4**, as drawn on the basis of high-field 2D NMR and molecular modeling.

For comparison purposes, we also synthesized the parent macrocycle **5** (Scheme 5). In this case, linker **18** was obtained by the treatment of Boc-protected ethanolamine **17** with ethyl acrylate in the presence of a catalytic amount of Na in THF.[12] After the successful synthesis of five new cyclic compounds containing an oxygen heteroatom, we explored several ways of introducing an NH unit in place of oxygen, but none succeeded. Some of the unsuccessful routes explored for the synthesis of aza-

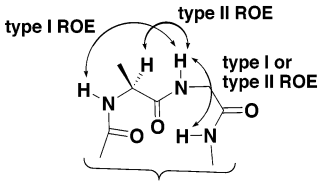
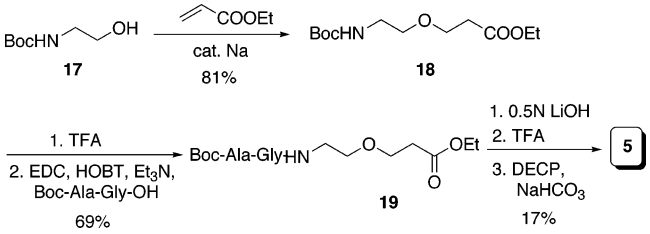


FIGURE 3. Characteristic ROEs in predicting type I vs type II β-turns.

SCHEME 5



Aca cyclic compounds are disclosed in the Supporting Information.

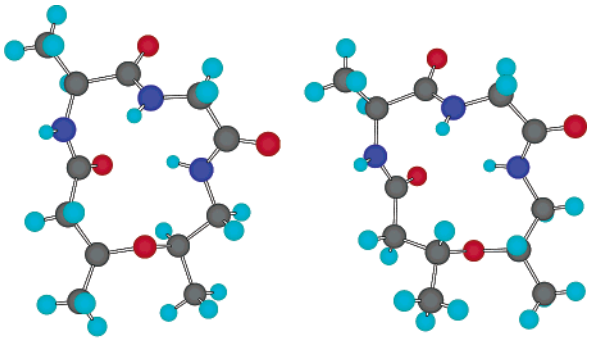
Conformational Analysis

The systematic conformational analysis of the cyclic peptidomimetics was carried out in solution phase using nuclear magnetic resonance (NMR) and in the solid state using X-ray crystallography. All of the protons in the NMR spectra of each cyclic compound were assigned using ^{1}H−^{1}H COSY and ROESY experiments. It is well-established in these systems that a strong rotating-frame (nuclear) Overhauser enhancement (ROE) between the glycine NH and the linker NH indicates that a compound can exist in either a type I or a type II β-turn, and ROE between the alanine NH and the glycine NH is characteristic for type I turns.[5] Similarly, a cross-peak between the alanine α-hydrogen and the glycine NH indicates a type II β-turn (Figure 3). On the basis of these characteristics, it appears that compounds **1**, **4**, and **5** take up substantial type II conformations. On the other hand, compound **2** showed significant type I character in the NMR. We were unable to further analyze compound **3** using NMR because of overlapping proton signals.

In addition, we obtained X-ray crystal structures for compounds **1** and **2**, both of which indicate type I β-turn conformers (Figure 4). We also note the crystallographic observation of hydrogen bonding between the linker carbonyl and Gly-NH units in these structures. We had anticipated that the oxygen atom present in the linker would occupy an inside position, and perhaps engage in hydrogen bonding, but an examination of the two crystal structures showed that this simplistic situation did not apply. In fact, neither compound **1** nor **2** took up this expected conformation; rather, the plane containing the C−O−C atoms of the ethereal linkage is essentially perpendicular to the plane approximately defined by the macrocycle in both cases. Overall, no drastic structural changes were observed in this series of structures in

(12) Hashimoto, M.; Yang, J.; Holman, G. D. *ChemBioChem* **2001**, *2*, 52−59.

(13) X-ray analysis of compound **2** showed the presence of two closely related conformers in a single unit cell. For clarity, only one of the conformers is shown in Figure 4; see the Supporting Information for full details.



Ideal Type I β-turn	Compound **1**	Compound **2**
ϕ_{i+1} = - 60°	ϕ_{i+1} = - 67.4°	ϕ_{i+1} = - 64.0°
Ψ_{i+1} = - 30°	Ψ_{i+1} = - 24.3°	Ψ_{i+1} = - 30.9°
ϕ_{i+2} = - 90°	ϕ_{i+2} = - 104.1°	ϕ_{i+2} = - 113.7°
Ψ_{i+2} = 0°	Ψ_{i+2} = + 8.4°	Ψ_{i+2} = + 13.7°

FIGURE 4. Ball-and-stick representations of the X-ray structures and their dihedral angles of **1** and **2**.[13]

comparison to the all-carbon series previously reported. In part, we attribute the different conformations observed in solution versus solid state to crystal packing forces. However, it is also clear that these compounds have a fair degree of conformational mobility. The bottom line is that the tether substituents in this series are unlikely to control subtype populations. The upside of this is that these positions can be freed up to accommodate additional side chain proxies for potential binding interactions.

Summary

We have accomplished the synthesis of macrocycles **1**−**5** constrained with dimethyl-substituted 3-(aminoethoxy)propionic acid linkers which were, in turn, generated from the readily available dimethyl tetrahydropyrones. Conformational analysis showed that compounds **1**−**5** all adopt β-turn conformations but with complex results with respect to subtypes. Despite our original design hypothesis, there appears to be no conformational benefit to including the oxygen in the linker relative to the all-carbon case.

Acknowledgment. We thank the National Science Foundation and the National Institutes of Health for the support of this work and Doug Powell for X-ray crystallography.

Supporting Information Available: Experimental procedures, characterization of compounds, a summary of unsuccessful routes for the synthesis of the aza cyclic analogues, and CIF data. This material is available free of charge via the Internet at http://pubs.acs.org.

JO035683Q