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Systematic Study of the Synthesis of Macrocyclic Dipeptide β-Turn Mimics Possessing 8-, 9-, and 10- Membered Rings by Ring-Closing Metathesis

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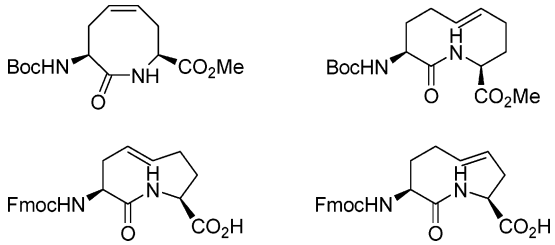
Received December 21, 2004



A systematic study was performed to establish general synthesis protocols for forming enantiomerically pure macrocyclic dipeptide lactams. Focusing on macrocycles of 8-, 9-, and 10-membered rings, effective syntheses were achieved by a sequence featuring peptide coupling of allyl- and homoallyl-glycine building blocks followed by ring-closing metathesis. The 8-membered lactam possessing *cis*-amide and *cis*-olefin geometry as well as 9- and 10-membered lactams having *trans*-amide and *trans*-olefin configurations were effectively prepared by a general strategy employing the respective protected dipeptide, the first generation Grubbs' catalyst, and temporary protection of the central amide as a benzyl derivative.

Introduction

β-turns in peptides and proteins are of interest because of their structure and biological activity.[1] Polar in nature, these secondary structures generally occupy the surfaces of protein molecules, where they are involved in recognition and binding.[2] β-turns have also been shown to be important for receptor affinity in biologically active peptides, such as somatostatin,[3] MSH,[4] bradykinin,[5] and LHRH.[6] Mimics of β-turns are thus desirable tools for studying the structure−activity relationships responsible for protein and peptide biology.[7]

Strategies to design β-turn mimics have often constrained the peptide backbone dihedral angles. For example, fused bicyclic systems, such as azabicyclo[X,Y,0]-alkanone amino acids **1**−**4**, of varying ring sizes have been used as rigid dipeptide surrogates[7] that structurally constrain three contiguous dihedral angles, ϕ, ψ, and ω, of a β-turn segment within the body of the heterocycle (Figure 1). Conformational analysis of bicyclic lactams **1**−**4** has demonstrated their propensity to favor type II and II′ β-turns[8,9] contingent on their stereochemistry, as evident from X-ray diffractometry, IR, and NMR spectroscopy as well as theoretical calculations.[10] Because differences in the type II turn dihedral angle have been

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10.1021/jo0477648 CCC: $30.25
Published on Web 03/22/2005

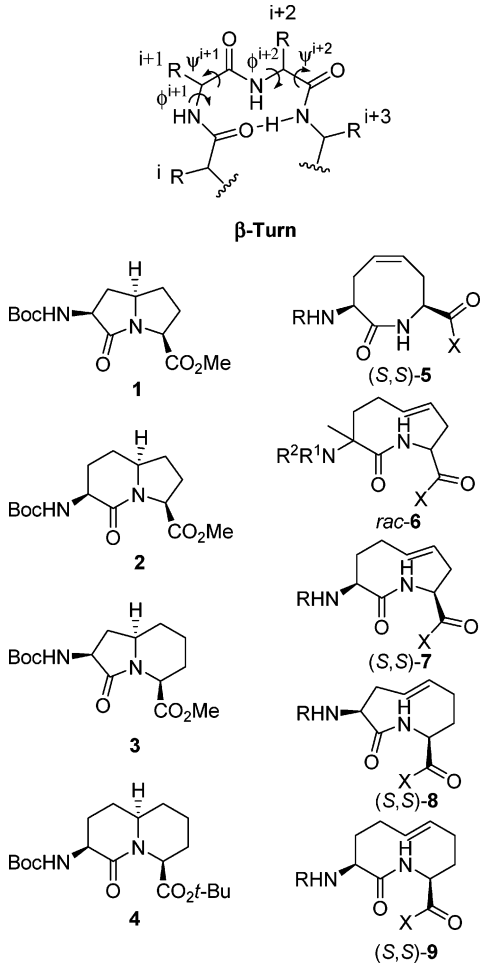

FIGURE 1. Representative β-turn structure as well as bicyclic and macrocyclic constrained dipeptide β-turn mimics.

observed after variation of the ring sizes in these bicyclic mimics, application of sets of related azabicycloalkane amino acids has thus proven effective for studying the effect of turn geometry on the biological activity of native peptides.[11,12]

Alternative scaffolds are needed to cover a wider range of turn geometries in order to better mimic natural diversity and to enhance success in peptide mimicry. Macrocyclic dipeptide lactams of 8-, 9-, and 10-members have been less well investigated relative to their bicyclic cousins, in part due to the difficulties inherent in synthesizing such medium-sized ring systems.[13]

The syntheses of eight-membered macrocycles, such as **5**, have been accomplished by ring-closing metathesis. Constrained dipeptides (*S*,*S*)-**5** and (*R*,*S*)-**5**, (R = Ac, X = NHMe) were shown to adopt conformations similar to an ideal type VIa β-turn, as demonstrated by NMR spectroscopy and the X-ray crystal structure of (*S*,*S*)-**5**.[14] Although the type VI β-turn did not possess proline, the eight-membered macrocycle forced the amide between the $i + 1$ and $i + 2$ residues to adopt the cis-isomer geometry, as illustrated by X-ray structural analysis, which also revealed a *cis*-olefin in a folded twist-boat-boat conformation.[14] The saturated analogue of eight-membered lactam (*R*,*R*)-**5** possessed a *cis*-amide geometry and type VIb β-turn conformation; however, dimerization took place over time in solution and in the solid state, as revealed by NMR dilution experiments and X-ray crystallographic studies.[15,16] Their *cis*-amide geometry and their propensity to favor type VI β-turns makes eight-membered lactams (**5**) structurally comparable to other type VI β-turn mimics, such as 5-*tert*-butylproline-containing peptides.[17]

Among the few syntheses of nine-membered macrocyle dipeptide lactams, an Ugi-multicomponent reaction followed by ring-closing metathesis has been used to prepare unsaturated nine-membered lactam **6** ($R^1 = CH_3$-CO, $R^2 = PhCH_2$, X = OEt), albeit as a racemic isomeric mixture.[18] The ring geometry and conformational preferences of nine-membered macrocyclic lactams such as **6**−**8** have, however, yet to be characterized.

The 10-membered macrocycle dipeptide lactam (**9**, R = Boc, X = OMe) has also been synthesized by ring-closing metathesis and shown to possess a *trans*-olefin geometry and φ, ψ, and ω torsional angles similar to that of an ideal type I β-turn, as demonstrated by X-ray and NMR analyses.[19]

With the precedent that 8- and 10-membered macrocyclic peptide lactams adopted type VI and type I β-turns, respectively, and with potential for their nine-membered counterparts to serve similarly as turn mimics, we sought to develop a general means for constructing the set of heterocyclic dipeptides (**5** and **7**−**9**, R = Boc or Fmoc, X = OH). We envisioned that this set will serve in studies of biologically active peptides as well as intermediates for trans-annular cyclizations to prepare azabicyclo-[X,Y,0]alkanone amino acids related to **1**−**4**.

Medium-sized macrocyclic lactams of 8-, 9-, and 10-members have been traditionally harder to form than their smaller and larger-sized counterparts;[13] however, ring-closing metathesis has significantly advanced their synthesis. For example, *N*-alkyl lactams of 6−10 members have been prepared by RCM.[20] Transient *N*-alkylation of the central amide with a 2,4-dimethoxy benzyl group (Dmb) was also shown to be essential to favor a *cis*-amide geometry and facilitate metathesis in the synthesis of eight-membered lactams (**5**).[14,15] Moreover,

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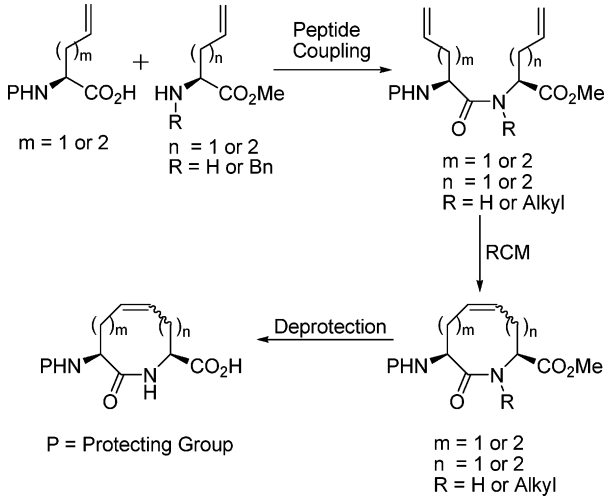
SCHEME 1. General Scheme for the Synthesis of Macrocyclic Dipeptides



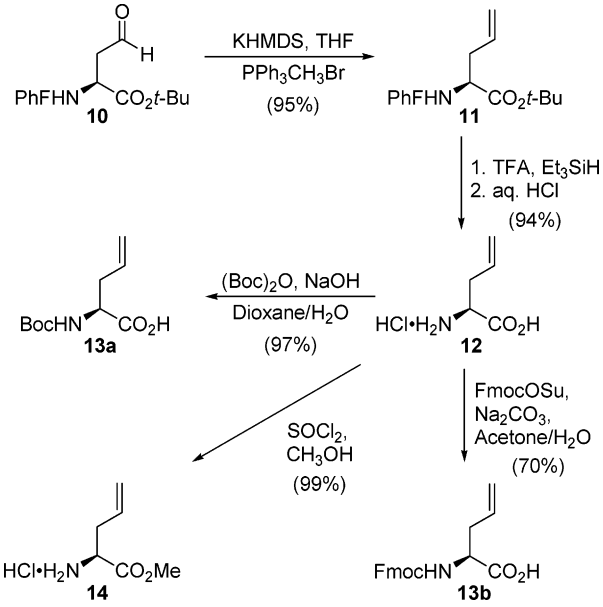
RCM has been used effectively to make larger ring systems that have constrained peptide conformations.[21]

Considering these results and the need for material on a suitable scale and in a protected form for peptide synthesis, we undertook a detailed study to prepare macrocyclic dipeptide lactams of 8−10 members by a general strategy featuring RCM (Scheme 1).

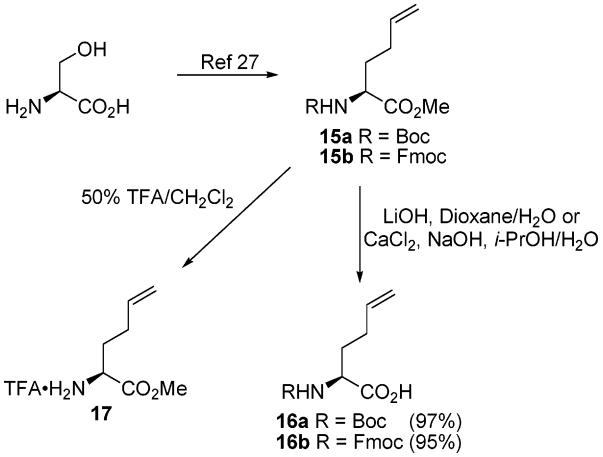
Results and Discussion

Dipeptide Precursor Synthesis. The construction of 8-, 9-, and 10-membered ring sizes necessitated the synthesis and coupling of allylglycine and homoallylglycine units prior to RCM. Allylglycine had been synthesized by a variety of methods;[22] however, our expertise with α-*tert*-butyl *N*-(PhF)aspartate β-aldehyde (**10**, PhF = 9-phenylfluren-9-yl)[23] served as motivation to employ this chiral educt in the synthesis of allylglycine (Scheme 2). Aldehyde **10** was synthesized on a 7-g scale from aspartic acid [23b,24] in five steps and 47% overall yield. *N*-(PhF)Allylglycine *tert*-butyl ester **11** was obtained in 95% yield from the Wittig reaction of aldehyde **10** and the ylide generated from treatment of methyltriphenylphosphonium bromide with KHMDS in THF.[25] Allylglycine hydrochloride **12** was quantitatively prepared from the treatment of **11** with TFA, which cleaved both the *tert*-butyl ester and PhF groups, followed by conversion of the trifluoroacetate salt to the hydrochloride using aq HCl and lyophilization. *N*-(Boc)- and *N*-(Fmoc)allylglycines **13a** and **13b** were then prepared by the protection of **12** with $(Boc)_2O$[26a] and FmocOSu,[26b] respectively, under basic conditions. Allylglycine methyl ester hydrochloride **14** was quantitatively obtained by the treatment of **12** with methanol and thionyl chloride. The higher *N*-(Boc)- and *N*-(Fmoc)-protected homologues (homoallylglycines **16a** and **16b**) were synthesized respectively in five steps from serine using reported procedures (Scheme 3).[27] Briefly, serine was converted to its methyl ester hydrochloride, which was protected using Boc_2O or FmocOSu and transformed to *N*-(Boc)- or *N*-(Fmoc)iodoalanine methyl ester using iodine, PPh_3, and imidazole.[28] The iodide was then converted to a zincate and coupled to allyl chloride

SCHEME 2. Synthesis of Allylglycine Components 13 and 14



SCHEME 3. Synthesis of Homoallylglycine Components 16 and 17



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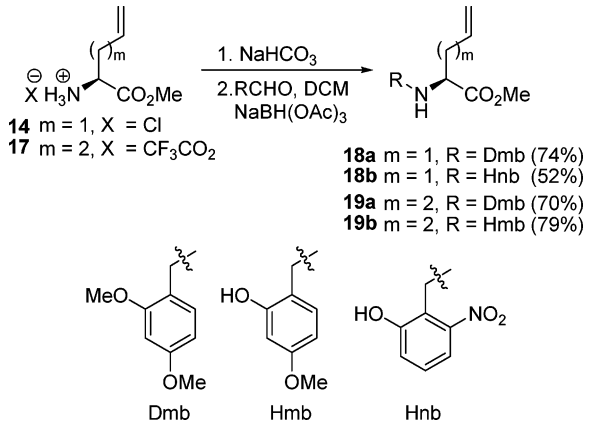
SCHEME 4. Synthesis of *N*-Benzyl Components 18 and 19



using CuBr·DMS. *N*-(Boc)amino ester **15a** was hydrolyzed with LiOH and deprotected with 50% TFA in CH_2Cl_2 to provide *N*-(Boc)homoallylglycine **16a** and homoallylglycine methyl ester trifluoroacetate **17**, respectively, as components for peptide coupling. *N*-(Fmoc)Homoallylglycine **16b** was obtained by hydrolysis of *N*-(Fmoc)-amino ester **15b** using 0.8 M calcium chloride and 0.5 M sodium hydroxide in *i*PrOH/H_2O (7:3).[29]

Considering that rotation around the amide bound would influence the rate of RCM of the dipeptides, we synthesized an array of secondary benzyl amino esters to prepare tertiary amides having a lower barrier for isomerization about the amide bond (Scheme 4). This hypothesis was supported by earlier studies of the synthesis of eight-membered lactams that demonstrated the utility of the acid labile 2,4-dimethoxybenzyl (Dmb) group in the RCM step.[15b] The related 2-hydroxy-4-methoxybenzyl (Hmb) group has been used to surmount difficult couplings in solid-phase peptide synthesis,[30] and we envisioned that secondary amino esters bearing this group could have better reactivity than their Dmb cousins. Similary, the 2-hydroxy-6-nitrobenzyl (Hnb) group was considered as a photocleavable protection reported to favor coupling by O to N acyl transfer in a similar way as the Hmb auxiliary.[31] Reductive aminations of imine prepared with the respective benzaldehyde and allyl and homoallylglycine methyl esters (**14** and **17**) were performed using $NaBH(OAc)_3$ to furnish Dmb-, Hmb-, and Hnb-protected amino esters **18** and **19** in yields varying between 52% and 79%.

Coupling of the Fragments. The set of dipeptides **21a**−**23a** was assembled first by combining allylglycine and homoallylglycine methyl ester salts **14** and **17** as amine components and *N*-(Boc)allyl and *N*-(Boc)homoallylglycines **13a** and **16a** as acid components using TBTU as the coupling agent in 85−87% yields (Table 1). Coupling of the Dmb secondary amino esters proved more difficult, and many coupling conditions were tried;[32] however, only HATU as the coupling agent with *N*-ethylmorpholine as the base was found to be effective for making *N*-Boc-protected dipeptides **20b**−**23b** bearing a 2,4-dimethoxybenzyl tertiary amide bond in yields varying between 71% and 86%. Changing from Boc protection to Fmoc did not affect coupling, and dipeptides **21c** and **22c** were obtained in 85% and 82% yields, respectively. The use of HATU could be avoided by employing *N*-(Hmb)homoallylglycine methyl ester **19b** and the symmetrical anhydride generated from *N*-(Boc)homoallylglycine **16a**, respectively, in situ to furnish **23c** in 86% yields. Attempts to apply the symmetrical anhydride conditions using *N*-(Hnb)allylglycine **18b** gave **22d** in 87% yield; however, this route was not pursued further because of the difficulties in making the requisite 2-hydroxy-6-nitrobenzaldhyde.[33]

Ring-Closing Metathesis. The importance of the rate of amide bond isomerization on the ring-closing metathesis was realized in the synthesis of nine-membered macrocyles, which could not be formed without a tertiary amide. When cyclization by olefin metathesis was examined on dipeptides bearing no benzyl group at the amide nitrogen, secondary amides **21a** and **22a** failed to cyclize using both the first and second generation Grubbs' catalyst in a variety of solvents at reflux and room temperature. Dipeptide **21a** also did not cyclize to a nine-membered ring in the presence of Ti(O-*i*Pr)$_4$, which has been used previously to prevent the formation of a cyclic chelate between the catalyst and an amide.[34] As reported,[19] 10-membered macrocyclic dipeptide **27a** was formed on cyclization of dipeptide **23a** using the first generation of Grubbs' catalyst at high dilution (0.7 mM).

Macrocycles of eight and nine members were synthesized from dipeptides bearing only a benzyl group on the nitrogen of the amide. Dmb-analogues **20b**, **21b** and **c**, **22b** and **c**, and **23b** reacted with the first generation Grubbs' catalyst to provide 8−10 membered macrocycles **24a**, **25b** and **c**, **26b** and **c,** and **27b**, respectively, in 71−81% yields (Table 2). The 10-membered macrocyle (**27b**) was formed in 87% yield from tertiary amide **23b** employing the same conditions used to cyclize secondary amide **23a** at a concentration four and a half times higher. The phenol of the Hmb group was also tolerated by the catalyst, and 10-membered macrocyle **27c** was prepared in 86% yield. The tertiary amide facilitated amide bond rotation and favored conformers in which the ring-closing metathesis was possible. In the smaller-ring cases, without the benzyl group, the *cis*-amide isomer was disfavored energetically, and the barrier for isomerization was higher such that macrocylization was inhibited.

The Dmb and Hmb groups were reported to be easily cleaved under acidic conditions.[30,35] However, *N*-Boc-protected-9- and 10-membered lactams **25b**, **26b**, and

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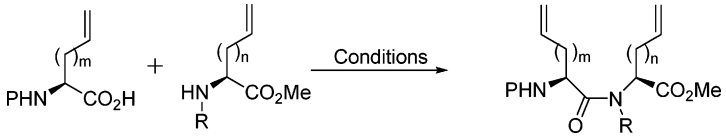
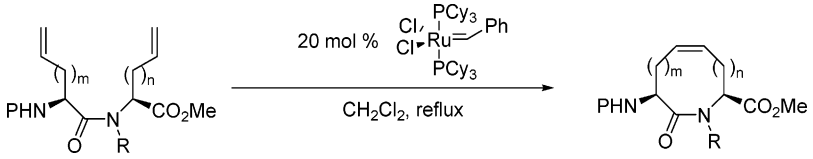
TABLE 1. Dipeptide Assembly



acid	*m*	P	amine	*n*	R	conditions[a]	amide	yield (%)
13a	1	Boc	**18a**	1	Dmb	A	**20b**	71
13a	1	Boc	**17**	2	H	B	**21a**	86
13a	1	Boc	**19a**	2	Dmb	A	**21b**	86
13b	1	Fmoc	**19a**	2	Dmb	A	**21c**	85
16a	2	Boc	**14**	1	H	B	**22a**	85
16a	2	Boc	**18a**	1	Dmb	A	**22b**	81
16b	2	Fmoc	**18a**	1	Dmb	A	**22c**	82
16a	2	Boc	**18b**	1	Hnb	C	**22d**	87
16a	2	Boc	**17**	2	H	B	**23a**	87
16a	2	Boc	**19a**	2	Dmb	A	**23b**	74
16a	2	Boc	**19b**	2	Hmb	C	**23c**	86

[a] Condition A: HATU, *N*-ethylmorpholine, CH_2Cl_2, rt; condition B: TBTU, DIEA, CH_2Cl_2, rt; condition C: symmetric anhydride, benzylic amino ester, CH_2Cl_2, rt.

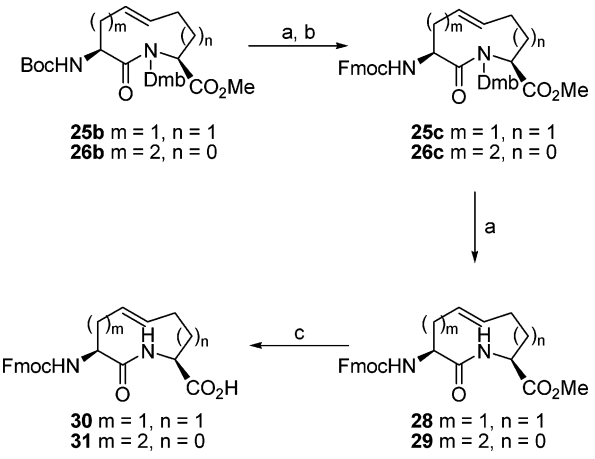
TABLE 2. Macrocycle Formation



amide	P	*m*	*n*	R	macrocycle	yield (%)
20b	Boc	1	1	Dmb	**24a**	78
21a	Boc	1	2	H	**25a**	0
21b	Boc	1	2	Dmb	**25b**	80
21c	Fmoc	1	2	Dmb	**25c**	75
22a	Boc	2	1	H	**26a**	0
22b	Boc	2	1	Dmb	**26b**	81
22c	Fmoc	2	1	Dmb	**26c**	71
23a	Boc	2	2	H	**27a**	77
23b	Boc	2	2	Dmb	**27b**	87
23c	Boc	2	2	Hmb	**27c**	86

27b and **c** bearing Dmb and Hmb groups were recovered as deprotected amines without the loss of the benzylic tertiary amide after prolonged treatment with 50% TFA in CH_2Cl_2. Alternatively, both the Boc and Dmb groups were removed upon exposure of eight-membered lactam **24a** to the same TFA in CH_2Cl_2 conditions as reported.[15b] In contrast to the *N*-Boc-protected lactams, *N*-Fmoc-protected lactams **25c** and **26c** were quantitatively converted in 30 min to their secondary amide derivatives **28** and **29** by treatment with 50% TFA in CH_2Cl_2 (Scheme 5). This behavior confirms the hypothesis[36] that difficulties in cleaving Dmb groups are due to the formation of a neighboring positively charged ammonium ion upon deprotection of the Boc moiety, which inhibits protonation of the amide and shuts down the reaction. Conversion of *N*-Boc-protected macrocycles **25b** and **26b** to *N*-Fmoc-derivatives **25c** and **26c** gave average yields of 35−45%, illustrating an advantage in starting with *N*-Fmoc-protected amino acids. Finally, esters **28** and **29** were hydrolyzed using 0.5 M NaOH and 0.8 M $CaCl_2$ in *i*PrOH/water (7:3) to obtain the protected amino acids (**30** and **31**) suitable for incorporation into peptides by solid-phase synthesis.[29]

SCHEME 5. Synthesis of Macrocycle *N*-(Fmoc)-dipeptides 30 and 31



[a] Reagents and conditions: (a) 50% TFA/CH_2Cl_2; (b) FmocOSu, Na_2CO_3, acetone/H_2O; (c) 0.8 M $CaCl_2$/0.5 M NaOH, *i*-PrOH/H_2O.

The olefin geometry was determined based on the vicinal coupling constant of the vinyl protons. In the case of eight-membered lactam (*S*, *S*)-**5**, a coupling-constant value of 10.4 Hz was observed and assigned to *cis*-olefin

(36) Dinsmore, C. J.; Bergman, J. M.; Bogusky, M. J.; Culberson, J. C.; Hamilton, K. A.; Graham, S. L. *Org. Lett.* **2001**, *3*, 865.

geometry. In the case of 9- and 10-membered lactams **7**−**9**, coupling-constant values ranged from 18.5 to 19.1 Hz and were assigned to *trans*-olefins.

The amide isomer geometry of 8- and 10-membered lactams **5** and **9** were assigned cis and trans, respectively, based on analogy to literature compounds **5**[14] and **9**,[19] for which X-ray structures were obtained. The assignment of the amide isomers of nine-membered lactams **30** and **31** was made based on 2D NMR experiments. Initially, COSY spectra of **30** and **31** were performed in pyridine-d_5 and $CDCl_3$, respectively, to assign the protons for each compound. Subsequently, NOESY spectra were performed to measure long-distance transfers of magnetization. In the NOESY spectrum of both **30** and **31**, no transfer of magnetization between the $C^{\alpha}H$ protons was observed using a variety of mixing times. Because an amide cis isomer would be expected to exhibit strong NOE between the neighboring $C^{\alpha}H$ protons, the lack of such a transfer of magnetization leads us to assign the amide trans isomer geometry for **30** and **31**.

Conclusions

A general methodology has been developed for synthesizing enantiomerically pure 8-, 9-, and 10-membered macrocyclic dipeptides. This methodology has provided the first syntheses of nine-membered lactams **7** and **8**, as well as a more practical route for making 10-membered lactam **9**. The importance of transient *N*-alkylation of the central amide was demonstrated to facilitate ring-closing metathesis, such that higher concentrations (3.15 mM) could be used without evoking dimer or polymer formation. As reported, the *cis*-olefin geometry was found in eight-membered lactam **5**. The 9- and 10-membered lactams (**7**−**9**) possessed *trans*-olefin geometry. The amide bond geometry was shown to be cis in the case of **5** and trans for macrocycles **7**−**9**. A more detailed investigation of the conformation of **7** and **8** in peptide analogues is presently under study. In light of the ability of these constrained dipeptide surrogates to adopt conformations similar to natural β-turns, this practical methodology should be of general utility for research in peptide science and medicinal chemistry.

Experimental Section

***tert*-Butyl (2*S*)-2-[(*N*-(PhF)Amino]pent-4-enoate (11).** A rt solution of methyltriphenylphosphonium bromide (13.2 g, 37 mmol) in THF (103 mL) was treated with a 0.5 M solution of KHMDS in toluene (67 mL, 33 mmol), stirred for 30 min, and treated dropwise with aldehyde **10** (7.64 g, 18.5 mmol, prepared according to ref 23) in THF (103 mL) over 10 min. The reaction mixture was stirred for 1 h, quenched with 200 mL of a saturated solution of NH_4Cl, and the phases were separated. The aqueous layer was extracted twice with 150 mL of Et_2O. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated. Chromatography (7% EtOAc/hexanes) afforded allylglycine **11** (7.22 g, 95% yield) as a clear oil: $[\alpha]^{20}_D$ +163.7° (*c* 1.0, $CHCl_3$), lit.[37] $[\alpha]^{20}_D$ 168.7° (*c* 1.0, $CHCl_3$). The ^1H and ^{13}C NMR spectral data were consistent with the literature.[37]

(2*S*)-2-Aminopent-4-enoic Acid Hydrochloride (12). TFA (60 mL) was added dropwise to a solution of allylglycine **11** (7.22 g, 17.5 mmol) in CH_2Cl_2 (60 mL). Once the addition was completed, Et_3SiH (7 mL, 43.8 mmol) was added to the solution, which was stirred for 15 h. The volatiles were evaporated, and the residue was dissolved in 3:1 hexanes/Et_2O (50 mL) and treated with 0.5 N HCl (25 mL). The phases were separated, and the organic layer was extracted twice with 0.5 N HCl. The combined aqueous layers were lyophilized to give amino acid hydrochloride **12** (2.50 g, 94% yield) as a white solid: mp 205−207 °C; ^1H NMR (CD_3OD): δ 5.79 (m, 2H), 5.29 (m, 2H), 4.06 (dd, 1H, *J* = 7.1, 5.1 Hz), 2.68 (m, 2H); ^{13}C NMR (CD_3OD): δ 169.4, 130.1, 119.7, 51.6, 34.0. MS (ESI, *m/z*): 116.0 $(MH)^+$.

(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]pent-4-enoic Acid (13a). A 1:1 dioxane/H_2O solution (33 mL) containing hydrochloride **12** (750 mg, 4.95 mmol) was treated with NaOH (436 mg, 9.90 mmol). After the NaOH dissolved, Boc_2O (1.30 g, 5.94 mmol) was added to the mixture in three portions over 10 min. The mixture was stirred for 18 h. The dioxane was removed by evaporation. The crude mixture was diluted with H_2O and acidified to pH 3−4 with a 10% $KHSO_4$ solution. This aqueous solution was extracted with Et_2O (three times). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated to give *N*-(Boc)allylglycine **13a** (1.03 g, 97% yield) as a thick clear oil: $[\alpha]^{20}_D$ +14.5° (*c* 1.28, $CHCl_3$); The ^1H and ^{13}C NMR were consistent with the literature.[22c] HRMS calcd for $C_{10}H_{17}NO_4Na$, 238.10498; found, 238.10538.

(2*S*)-2-[(9*H*-Fluoren-9-ylmethoxycarbonyl)amino]pent-4-enoic Acid (13b). A 1:1 acetone/H_2O solution (67 mL) containing hydrochloride **12** (1.5 g, 10 mmol) was treated with Na_2CO_3 (2.1 g, 20 mmol) and FmocOSu (3.3 g, 10 mmol). The mixture was stirred for 18 h at room temperature and concentrated. The crude mixture was diluted with H_2O and acidified to pH 3−4 with a 10% $KHSO_4$ solution. This aqueous solution was extracted with EtOAc (three times). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated to give *N*-(Fmoc)allylglycine **13b** (2.36 g, 70% yield): mp 134−135 °C; $[\alpha]^{20}_D$ +10.6° (*c* 0.93, $CHCl_3$); ^1H NMR (CD_3OD): δ 7.68 (d, 2H, *J* = 9.9), 7.59 (m, 2H), 7.32 (t, 2H, *J* = 9.7), 7.23 (t, 2H, *J* = 9.9), 5.64 (m, 1H), 5.09 (m, 3H), 4.25 (m, 2H), 4.12 (m, 2H), 2.57 (m, 1H), 2.44 (m, 1H); ^{13}C NMR (CD_3OD): δ 175.2, 158.5, 145.3, 145.1, 142.5, 134.7, 128.8, 128.1, 126.3, 120.8, 118.7, 68.0, 55.1, 37.1; HRMS calcd for $C_{20}H_{19}NO_4Na$, 360.12063; found, 360.12080.

Methyl (2*S*)-2-Aminopent-4-enoate Hydrochloride (14). $SOCl_2$ (0.55 mL, 19.8 mmol) was added dropwise to a solution of allylglycine **12** (2.0 g, 13.2 mmol) in MeOH (22 mL) at 0 °C. The mixture was allowed to warm to room temperature, stirred overnight, and evaporated to give methyl ester **14** (2.16 g, 99% yield) as a white solid: mp 91−92 °C; $[\alpha]^{20}_D$ +8.3° (*c* 1.07, CH_3OH); ^1H NMR (CD_3OD): δ 5.76 (m, 1H), 5.28 (m, 2H), 4.15 (dd, 1H, *J* = 6.8, 5.5), 3.83 (s, 3H), 2.69 (m, 2H); ^{13}C NMR (CD_3OD): δ 168.8, 130.1, 120.1, 52.1, 52.0, 34.1; HRMS $(MH)^+$ calcd for $C_6H_{11}NO_2$, 130.08626; found, 130.08636.

(2S)-2-[(*tert*-Butoxycarbonyl)amino]hex-5-enoic Acid (16a). A solution of *N*-(Boc)homoallylglycine methyl ester (**15a**, 400 mg, 1.64 mmol prepared according to ref 27) in 1:1 H_2O/dioxane (16 mL) was treated with $LiOH{\cdot}H_2O$ (103 mg, 2.47 mmol), stirred for 3 h, and evaporated to a residue that was partitioned between H_2O (20 mL) and EtOAc (20 mL). The aqueous phase was acidified with 0.1 M HCl to pH 4 and extracted twice with EtOAc (20 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered, and concentrated to afford acid **16a** (0.364 g, 97% yield) as a colorless oil: $[\alpha]^{20}_D$ −1.1° (*c* 1.31, CH_3OH); ^1H NMR (400 MHz, CD_3OD): δ 5.82 (m, 1H), 5.03 (m, 3H), 4.08 (m, 1H), 2.15 (m, 2H), 1.88 (m, 1H), 1.73 (m, 1H), 1.44 (s, 9H); ^{13}C NMR (75 MHz, CD_3OD): δ 175.2, 157.1, 137.4, 115.0, 79.4, 53.2, 31.1, 30.0, 27.7; HRMS calcd for $C_{11}H_{19}NO_4Na$, 252.12063; found, 252.12089.

General Procedure A: Reductive Aminations. Allylglycine methyl ester hydrochloride **14** or homoallylglycine methyl ester trifluoroacetate **17** (3 mmol) was treated with 20 mL of a saturated $NaHCO_3$ solution and extracted with 3

(37) Kaul, R.; Broullette, Y.; Sajjadi, Z.; Hansford, K. A.; Lubell, W. D. *J. Org. Chem.* **2004**, *69*, 6131.

× 25 mL of CH_2Cl_2. The combined organic layers were washed with 20 mL of brine and dried over $MgSO_4$, filtered, and concentrated to a volume of 30 mL. The selected benzaldehyde derivative (3.3 mmol) and $NaBH(OAc)_3$ (4.5 mmol) were added to the mixture, which was stirred for 18 h at room temperature, treated with 20 mL of saturated $NaHCO_3$, and stirred for 30 min. The aqueous layer was separated and washed with 3 × 20 mL of CH_2Cl_2. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated to a residue that was purified by chromatography.

Methyl (2*S*)-2-[(2,4-Dimethoxybenzyl)amino]pent-4-enoate (18a). Chromatography of the product from **14** (20 mmol) using 30:70 EtOAc/hexanes as the eluant gave **18a** as a yellow oil (74% yield): ^{1}H NMR: δ 7.10 (d, 1H, J = 7.8), 6.39 (m, 2H), 5.69 (m, 1H), 5.08 (m, 2H), 3.81−3.64 (m, 11H), 3.11 (t, 1H, J = 6.6), 2.39 (t, 2H, J = 6.5), 2.22 (bs, 1H); ^{13}C NMR: δ 174.8, 160.0, 158.4, 133.6, 130.2, 120.0, 117.6, 103.5, 98.2, 60.0, 55.1, 55.0, 51.4, 46.8, 37.5. MS (ESI, *m/z*): 280.1 $(MH)^+$.

General Procedure B: Peptide Coupling using HATU. The selected *N*-protected amino acid (1.5 equiv) and *N*-benzyl amino ester (1.0 equiv) were dissolved in CH_2Cl_2 (0.07 M), treated with *N*-ethylmorpholine (1.5 equiv) and HATU (1.5 equiv), stirred for 24h, and diluted with water. The aqueous layer was extracted with CH_2Cl_2 (3 times). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated to a residue that was purified by chromatography.

***N*-(Fmoc)-L-Allylglycinyl-*N*-(2,4-dimethoxybenzyl)-L-homoallylglycine Methyl Ester (21c).** Chromatography of the product from **13b** (1.5 mmol) and **19a** (1.0 mmol) using 30:70 EtOAc/hexanes as the eluant gave **21c** (85% yield):^{1}H NMR: δ 7.76 (d, 2H, J = 7.5), 7.62 (dd, 2H, J = 3.3, 3.4), 7.39 (t, 2H, J = 7.5), 7.30 (t, 2H, J = 7.4), 7.07 (d, 1H, J = 8.0), 6.40 (dd, 2H, J = 2.1, 2.3), 5.81−5.65 (m, 3H), 5.14 (m, 2H), 5.05 (m, 1H), 4.98 (m, 2H), 4.67 (d, 1H, J = 15.8), 4.46−4.29 (m, 3H), 4.24−4.22 (t, 1H, J = 7.1), 4.13 (m, 1H), 3.76 (s, 6H), 3.57 (s, 3H), 2.59 (m, 1H), 2.45 (m, 1H), 2.11 (m, 1H), 2.03 (m, 2H), 1.80 (m, 1H); ^{13}C NMR: δ 171.6, 171.0, 160.9, 158.6, 155.3, 143.7, 141.1, 137.3, 132.3, 130.2, 127.5, 126.8, 125.0, 124.9, 119.8, 118.7, 115.8, 115.4, 103.5, 98.3, 66.7, 57.7, 55.2, 55.0, 51.8, 50.7, 47.0, 37.8, 30.4, 28.1. MS (ESI, *m/z*): 635.2 $(MNa)^+$.

General Procedure C: Ring-Closing Metathesis. In a flame dried flask, dipeptide (1.0 equiv) was dissolved in dry CH_2Cl_2 (3 mM). The mixture was heated for 10 min at 35 °C, treated with bis(tricyclohexylphosphonium)benzylidine ruthenium (IV) dichloride ($RuCl_2$(=CHPh)$(PCy_3)_2$, 20 mol %), heated at reflux for 72h, and concentrated. The crude residue was purified by chromatography to afford the unsaturated lactam.

Methyl (*E*, 3*S*, 9*S*)-3-*N*-(Fmoc)Amino-1-(2,4-dimethoxybenzyl)-2-oxo-2,3,4,5,8,9-hexahydro-1*H*-Azonine-9-carboxylate (25c). Chromatography of the product from **21c** (0.4 mmol) using 20:80 EtOAc/hexanes as the eluant gave **25c** (75% yield) as a brown solid: mp 96−101 °C; $[\alpha]^{20}_D$ −37.8° (*c* 0.93, $CHCl_3$); ^{1}H NMR: δ 7.76 (d, 2H, J = 7.5), 7.62 (d, 2H, J = 7.4), 7.40 (t, 2H, J = 7.4), 7.30 (t, 2H, J = 7.3), 7.23(d, 1H, J = 8.3), 6.49 (d, 1H, J = 6.6), 6.45−6.36 (m, 2H), 6.09 (dd, 1H, J = 9.1, 18.0), 5.61 (dd, 1H, J = 9.1, 18.0), 4.66−4.35 (m, 5H), 4.23 (t, 1H, J = 7.1), 3.79 (s, 3H), 3.77 (s, 3H), 3.46 (s, 3H), 2.67 (m, 1H), 2.30−2.15 (m, 2H), 1.90 (m, 2H), 1.75−1.65 (m, 2H); ^{13}C NMR: δ 173.3, 170.4, 159.8, 157.4, 155.2, 143.8, 141.1, 130.9, 129.9, 129.0, 127.5, 126.9, 125.0, 125.0 119.8, 117.5, 104.0, 97.9, 66.8, 56.9, 55.1, 52.0, 51.7, 47.0, 39.9, 35.0, 27.8, 22.0; HRMS $(MH)^+$ calcd for $C_{34}H_{37}N_2O_7$, 585.25953; found, 585.25918.

General Procedure D: Removal of Dmb. A stirred solution of Fmoc-protected dipeptide lactam (0.2 mmol) in CH_2Cl_2 (8 mL) was treated dropwise with TFA (2 mL), stirred for 18 h, and evaporated to a residue that was purified by chromatography.

Methyl (3*S*,9*S*)-3-*N*-(Fmoc)Amino-2-oxo-2,3,4,7,8,9-hexahydro-1*H*-azonine-9-carboxylate (28). Chromatography of the product from **25c** (0.5 mmol) using EtOAc as the eluant gave **28** (95% yield) as a brown gum: ^{1}H NMR: δ 7.76 (d, 2H, J = 7.4), 7.60 (d, 2H, J = 7.3), 7.39 (t, 2H, J = 7.4), 7.30 (t, 2H, J = 7.4), 6.35 (d, 1H, J = 7.0), 6.25 (d, 1H, J = 11.6), 6.08 (dd, 1H, J = 8.8, 18.9), 5.65 (ddd, 1H, J = 6.04, 10.95, 10.84), 4.39−4.38 (m, 2H), 4.25 (m, 3H), 3.73 (s, 3H), 2.70 (m, 1H), 2.30 (dd, 2H, J = 8.5, 8.7), 2.12 (m, 1H), 1.87 (m, 1H), 1.75 (m, 1H); ^{13}C NMR: δ 172.7, 172.0, 155.2, 143.7, 143.6, 141.1, 130.1, 128.7, 127.5, 126.9, 124.9, 119.8, 66.8, 52.5, 52.1, 47.0, 34.1, 33.8, 22.5; HRMS $(MH)^+$ calcd for $C_{25}H_{27}N_2O_5$, 435.19145; found, 435.19184.

General Procedure E: Methyl Ester Hydrolysis. A stirred solution of methyl ester (0.5 mmol) in 0.8 M $CaCl_2$ in a 7:3 *i*-PrOH/H_2O solution (10 mL) was treated with 0.5 M NaOH solution (2 mL). After 2 h, ether was added, and the phases were separated. The aqueous layer was acidified with 1.0 N HCl and extracted with EtOAc (three times). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated to give the acid.

(*E*,3*S*,9*S*)-3-*N*-(Fmoc)Amino-2-oxo-2,3,4,7,8,9-hexahydro-1*H*-azonine-9-carboxylic Acid (30). Hydrolysis of **28** (0.4 mmol) gave **30** (99% yield) as a white solid: mp 190−193 °C; ^{1}H NMR (400 MHz, pyridine-d_5): δ 8.90 (d, 1H, J = 11.0), 8.38 (d, 1H, J = 7.1), 7.8 (m, 3H), 7.7 (t, 1H, J = 7.5), 7.35 (m, 3H), 7.25 (t, 1H, J = 9.0), 6.04 (dd, 1H, J = 8.6, 18.5), 5.75 (bs, 1H), 5.56 (dd, 1H, J = 8.7, 18.5), 4.84 (t, 1H, J = 7.8), 4.69 (m, 1H), 4.55 (d, 2H, J = 7.16), 4.33 (t, 1H, J = 6.8), 2.95−2.88 (m, 1H), 2.5 (m, 1H), 2.35 (m, 1H), 2.20 (m, 1H), 1.9 (m, 2H); ^{13}C NMR: δ 175.3, 174.1, 156.7, 145.2, 144.9, 142.1, 131.5, 129.3, 128.5, 127.9, 126.1, 120.8, 67.3, 52.9, 48.2, 41.4, 34.8, 31.0; HRMS $(MH)^+$ calcd for $C_{24}H_{25}N_2O_5$, 421.17580; found, 421.17599.

Acknowledgment. This work was supported by grants from Fonds Québécois de la Recherche sur la Nature et les Technologies (FQRNT), Valorisation-Recherche Québec (VRQ), the Natural Sciences and Engineering Research Council of Canada (NSERC), the Canadian Society for Chemistry (CSC), Merck Frosst Canada, Boehringer Ingelheim Recherche Inc., Shire Biochem., and AstraZeneca Canada for financial support. We thank Hassan Iden for assistance in making starting materials, Dr. Alexandra Frutos and Mr. Dalbir Sekhon for mass spectral analysis, and Sylvie Bilodeau and Dr. M. T. Pham Viet of the Regional High-field NMR Laboratory for their assistance in running 2D NOESY/COSY experiments.

Supporting Information Available: General experimental section, general procedures for TBTU and symmetric anyhydride couplings, ^{1}H and ^{13}C NMR data for **18b**, **19a** and **b**, **21a** and **b**, **22a**−**d**, **23b** and **c**, **25b**, **26b** and **c**, **27b** and **c**, **29**, and **31**, ^{1}H and ^{13}C NMR spectra of **12**, **13b**, **14**, **16a**, **18a** and **b**, **19a** and **b**, **21a**−**c**, **22a**−**d**, **23b** and **c**, **25b** and **c 26b** and **c**, **27b** and **c**, and **28**−**31**, 2D COSY and NOESY spectra of **30** and **31**, and HPLC profiles of **30** and **31**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO0477648