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Design, synthesis, and conformational analysis of eight-membered cyclic peptidomimetics prepared using ring closing metathesis

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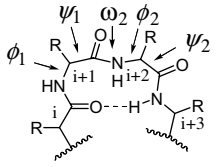
The authors wish to dedicate this manuscript to the memory of Professor Murray Goodman with respect and gratitude

Abstract—As part of a program to identify novel scaffolds that adopt defined secondary structure when incorporated into peptides, we have designed and prepared a library of constrained eight-membered ring lactams based upon 7-amino-8-oxo-1,2,3,6,7-pentahydroazocine-2-carboxylic acid. Ring closing metathesis (RCM) was employed as the key step, proceeding in high yields to afford the *Z* olefin. In this reaction sequence, the first generation benzylidene ruthenium RCM catalyst was superior to the second-generation imidazoline catalyst, which gave extensive oligomerization at higher concentrations. Conformational analysis of the 2*S*,7*S* and 2*R*,7*S* stereoisomers revealed that the 2*R*,7*S* isomer is a Type VIa β-turn in the solid state (X-ray crystal structure) and in water (NMR analysis). The Type VIa β-turn is relatively rare, typically bearing the *cis* amide bond found in proline-containing sequences. The 2*S*,7*S* diastereomer has an extended geometry of the pendent amide chains. The corresponding saturated derivatives (7-amino-8-oxoazocane-2-carboxylic acid) were also synthesized and investigated. The 2*S*,7*S* azocane bears an extended geometry and mimics the C^+ conformer of *ox*-[Cys-Cys], found in a variety of naturally occurring peptides. The scaffolds described here are useful for the design of constrained peptidomimics with defined secondary structure.

1. Introduction

Understanding and controlling the secondary structure of natural and synthetic peptides and proteins is essential for mediating the overall topology required to establish proper biological function.[1] Among the common structural elements found in nature, the β-turn is the primary reverse turn and has been extensively studied. The β-turn consists of four amino acids oriented in such a way that the peptide backbone changes direction by ca. 180° (Table 1).[2] A 10-membered ring hydrogen bond is usually formed between the carbonyl oxygen of the first or *i* residue, and the hydrogen on the amide of the fourth or $i+3$ residue. Also, the distance between the α-carbons of the *i* and $i+3$ residues should vary between 4 and 7 Å. The characterization and classification of the dihedral angles of the backbone atoms are used to categorize different types of β-turns with at most a 30° difference from standard angles, except that one angle can deviate by as much as 45°. A recent study looking at structures deposited in the Protein Data Bank

Table 1. β-Turns and their prevalence.[3]



β-Turn type	Position $i+1$: ϕ_1	Position $i+1$: ψ_1	Position $i+2$: ϕ_2	Position $i+2$: ψ_2	Abundance (%)
I	−60	−30	−90	0	34
I′	60	30	90	0	4
II	−60	120	80	0	13
II′	60	−120	−80	0	4
VIa	−60	120	−90	0	<1
VIb	−120	120	−60	0	1
IV	−60	10	−50	20	35
VIII	−60	−30	−120	120	9

Keywords: Type VI β-turns; Ring closing metathesis; Conformational analysis; Peptidomimetics.
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(http://www.rcsb.org/pdb/) has provided insight as to which β-turns are found in nature in the solid state.[3] The Type I, II, and IV β-turns are the most prevalent, with the I′ and II′ designations referring to the exact opposite value for each of the dihedral angles. Type VI β-turns are unique in that they bear a *cis*-amide bond between the $i+1$ and $i+2$ residues, and thus typically have a proline at the $i+3$ residue. Peptidomimetics are often designed and prepared in order to restrict the backbone dihedral angles to the values for specific β-turns, with the best mimics being those in which all of the dihedral angles fall within the appropriate ranges. The Type VIa mimics that have been described fall into two broad structural classes: modified proline derivatives[4] and rigid cyclic structures.[5] The Type VI β-turn has been implicated in several important biological functions such as the thrombin-catalyzed cleavage of the V_3 loop of HIV gp120[6] and substrate recognition of peptidyl prolyl isomerases.[7] We here describe the use of the ring closing metathesis reaction to prepare a series of eight-membered ring, constrained dipeptide derivatives, one of which is a Type VIa β-turn both in aqueous solution and the solid state.

We have been interested in the conformational preferences of *ox*-[Cys-Cys] **1**,[8] an unusual dipeptide found in the catalytic domain of mercuric ion reductase,[9] malformin[10] and in the N-terminal extracellular domain of most nicotinic acetylcholine receptor (nAChR) protein subunits (Fig. 1).[11] We have designed mimetics of **1** based upon the 7-amino-8-oxoazocane-2-carboxylic acid scaffold **2**, such as **3**–**6**.[12]

In addition to being closely related to *ox*-[Cys-Cys], compounds derived from scaffold **2** may be useful for studying the structure activity relationships of a variety of biologically relevant peptides including somatostatin,[13] malformin, Arg-Gly-Asp (RGD) peptides,[14] and enkephalins.[15] Also, **2** is an attractive building block for the design and synthesis of polymeric, supermolecular structures that may have interesting physical properties.[16] We here provide a full report on the design and synthesis of **3**–**6**, as well as a thorough conformational analysis using both X-ray crystallography and NMR spectroscopy in water. During the course of this work, we have fully characterized **5** as a Type VIa β-turn mimic in both water and the solid state.

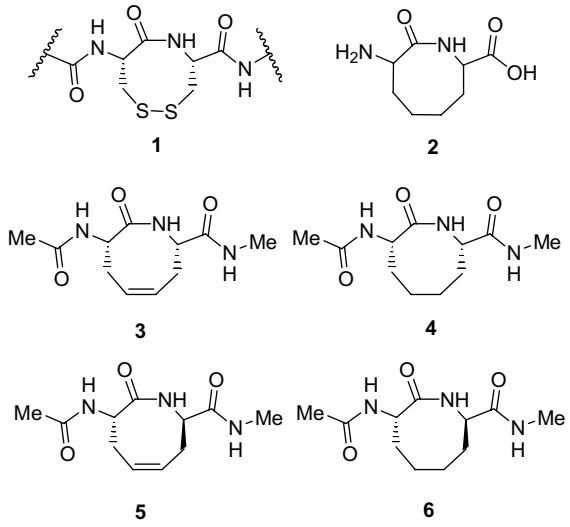
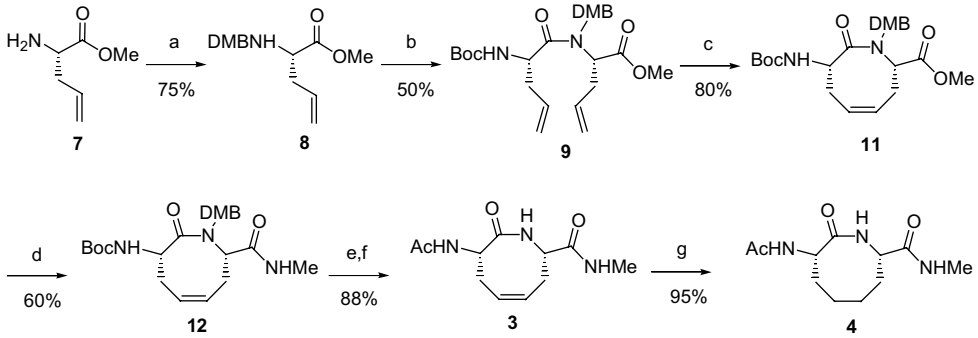


Figure 1. Constrained eight-membered ring dipeptides.

2. Results and discussion

We employed ring closing metathesis (RCM)[17] as the key step for the preparation of the required eight-membered ring starting from two adjacent allylglycine (Agy) residues. It had been reported that Agy-Agy did not undergo intramolecular RCM presumably because the thermodynamically stable *s-trans* conformation of the amide bond did not allow for the necessary orientation of the pendant olefins.[18] Therefore, we added the acid labile 2,4-dimethoxybenzyl group (DMB) onto the amide nitrogen to facilitate the formation of the required *s-cis* form (Scheme 1). The DMB group was chosen because it can be readily removed under acidic conditions, and the chemistry could be applied to a dialkoxybenzaldehyde resin for immobilization onto a solid support. L-Allylglycine methyl ester **7** was reductively alkylated with 2,4-dimethoxybenzaldehyde to give **8**, which was coupled with *N*-(Boc)-L-allylglycine using HATU and HOAt to afford dipeptide **9**.



Scheme 1. DMB = 2,4-$(MeO)_2PhCH_2$. Reagents and conditions: (a) 2,4-dimethoxybenzaldehyde, $Na(AcO)_3BH$; (b) Boc-L-allylglycine, HOAt/HATU, *N*-ethyl morpholine; (c) $Cl_2Ru(PCy_3)_2=CHPh$ (**10**), DCM, reflux (0.003 M substrate), 1 day; (d) 2 M $MeNH_2$/MeOH; (e) TFA, Et_3SiH; (f) acetic anhydride; (g) H_2, Pd/C.

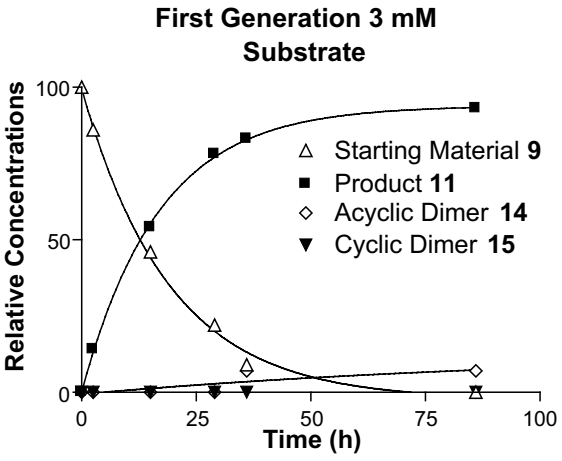
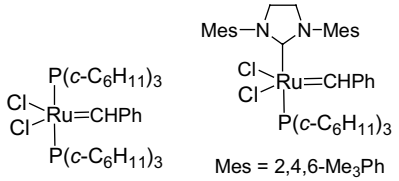


Figure 2. Ring closing metathesis of **9** to give primarily **11** using the first generation Grubbs catalyst **10**.

Ring closing metathesis of **9** (3 mM) using first-generation Grubbs catalyst **10** reproducibly provided protected olefin **11** in high yield (80%). This reaction was monitored with time at ambient temperature using a 3 mM concentration of **9**, which revealed that a high conversion to **11** was achieved over the course of 2–3 days and that very few other products were observed (Fig. 2).



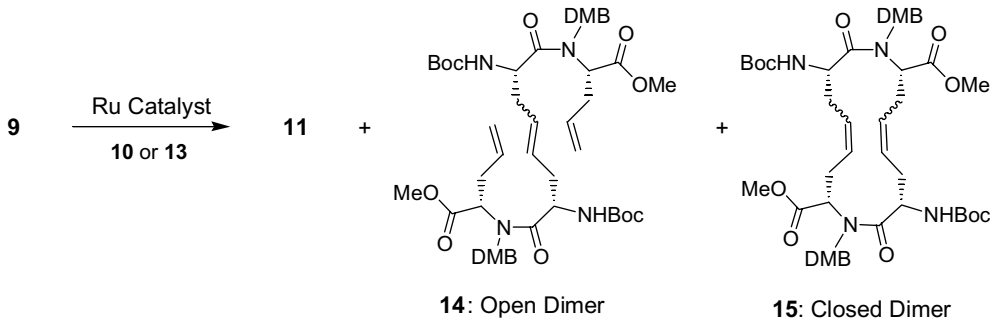
10 **13**

To complete the synthesis, methyl ester **11** was treated with methylamine to form amide **12**. Acidolysis of the Boc and 2,4-dimethoxybenzyl groups followed by treatment of the resultant TFA salt with acetic anhydride and base provided target **3**, and hydrogenation of **3** (Pd/C) afforded the corresponding saturated derivative **4**. A similar reaction sequence starting with D-allylglycine methyl ester instead of **7** gave **5** and **6**. Also, we have prepared the D,D- and D,L-variants of **3** for incorporation into peptides for biological evaluation, which will be described in due course.

Use of the second-generation imidazoline ruthenium benzylidene complex **13**[19] for the conversion of **9** to **11** proved to be much less satisfactory than what we had seen for **10**, as a time-dependent oligomerization was observed. We investigated the RCM reaction of **9** with catalysts **10** and **13** in more detail because we required >10 g quantities of **3** and **4** and wanted to increase the concentration. Under certain conditions, we observed the formation of acyclic and cyclic dimers **14** and **15**, respectively, as mixtures of six or eight possible geometric and regioisomers (Scheme 2).

The RCM reaction of **9** was conducted at concentrations of 12, 50, and 100 mM with 10 mol% of either **10** or **13**, and monitored over the period of ca. 1 day in methylene chloride at ambient temperature (Fig. 3). The reaction of **9** at 12 mM with first-generation catalyst **10** was similar to that observed at 3 mM except that open dimer **14** was formed to a substantial extent (ca. 10%) after 23 h. Furthermore, more **14** was produced at concentrations of 50 and 100 mM, being found at the same level as product **11** at 100 mM after 23 h. The use of second-generation catalyst **13** also afforded a substantial amount of **14**, but dimer **15** was the predominant product after 22 h at all concentrations. In these experiments, using either catalyst, the expected monocyclic product **11** appeared rapidly during the first five hours, and then the levels did not increase further. We propose that the reaction manifold may initially start with formation of **11**, which then undergoes an additional metathesis with another molecule of **11** to afford **14**. For the more powerful catalyst **13**, the reaction proceeds further to give closed dimer **15** as the terminal and prevalent product.

An X-ray crystal structure for **3** was obtained revealing *cis* olefin and amide geometry, and the eight-membered ring is in a folded twist-boat–boat conformation similar to a low energy form of cyclooctadiene (Fig. 4).[20] The peptide backbone is in an extended conformation, with the dihedral angles of the backbone residues listed in Table 2.



Scheme 2.

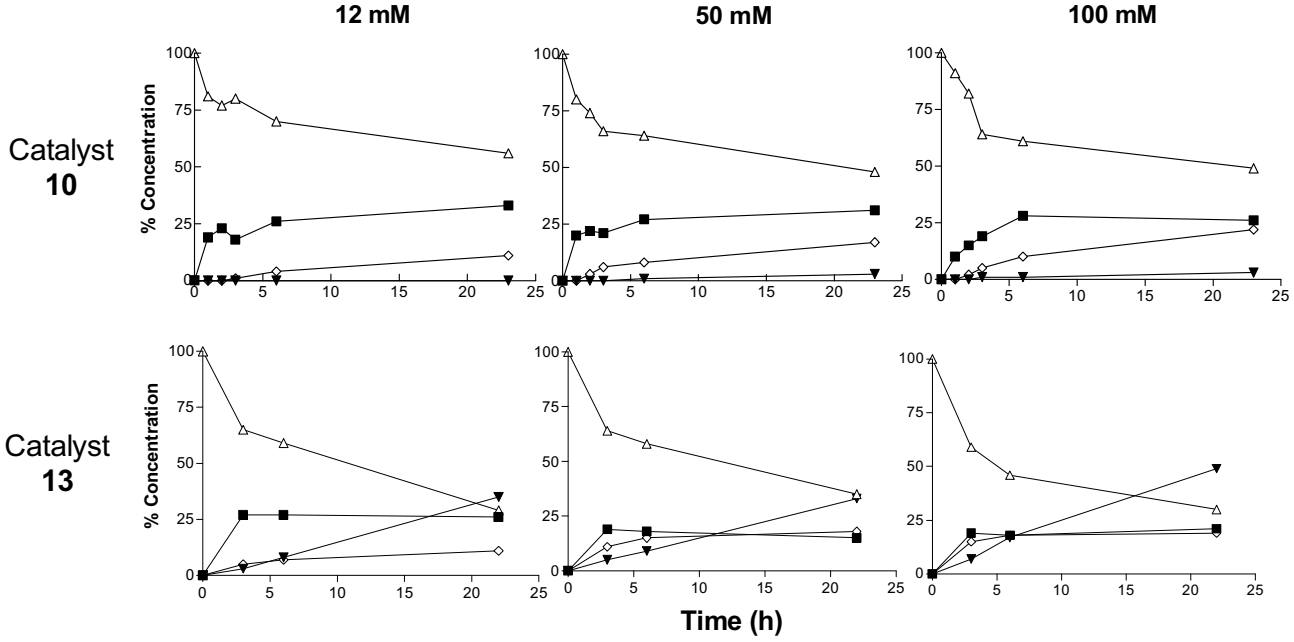


Figure 3. Conversion of **9** (△) to **11** (■), **14** (◇), and **15** (▼) using either the first generation Grubbs catalyst **10** (top) or the second-generation catalyst **12** (bottom) at concentrations of 12, 50, or 100 mM in methylene chloride at ambient temperature varying over time.

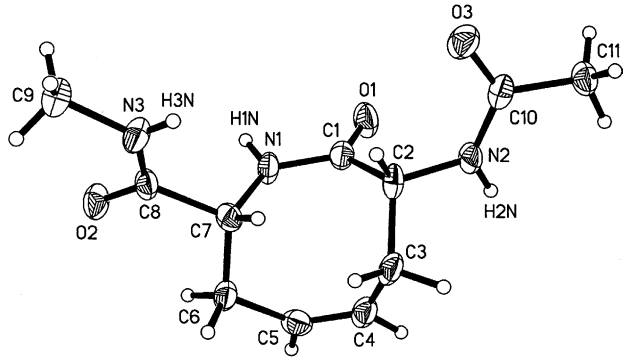
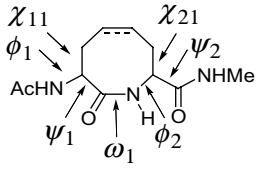


Figure 4. Crystal structure of **3** showing the *cis* olefin, *s-cis* amide bond, and extended orientation of the peptide backbone.

The X-ray crystal structure of saturated-derivative **4** (crystals prepared in ethyl ether) revealed an *s-cis* amide bond, but the eight-membered ring was no longer folded upon itself and the orientation of the two pendant groups of the peptide backbone was relatively more compact than for compound **3** (Fig. 5). The dihedral angles for **4** mimic very closely those found for the C^+ conformer of *ox*-[Cys-Cys] **1**.[8] Therefore, compounds in which **4** is incorporated would be expected to serve as close surrogates for **1**, which suffers the limitations imparted by the disulfide bond such as the possibility of reduction to the corresponding ring-opened Cys-Cys dipeptide unit.

The X-ray crystal structure for diastereomer **5** was also obtained (Fig. 6). This compound contains an *s-cis*

Table 2. Dihedral angles for **3**–**5** and those expected for a Type VIa β-turn



Dihedral	**3** (X-ray)	**4** (X-ray)	**5** (X-ray)	**5** (^{1}H NMR)	Type V1a β-turn[3]
ϕ_1	−93	−70	−64	−63	−60
ϕ_2	−148	−148	−94	−85	−90
ψ_1	177	152	146	[a]	120
ψ_2	90	166	19	[a]	0
ω_1	−12	−7	4	0	0
χ_{11}	−101.5	175.3	169	162	—
χ_{21}	45.3	−66.9	42	43	—

[a] These dihedrals cannot be determined by NMR without ^{15}N enrichment.

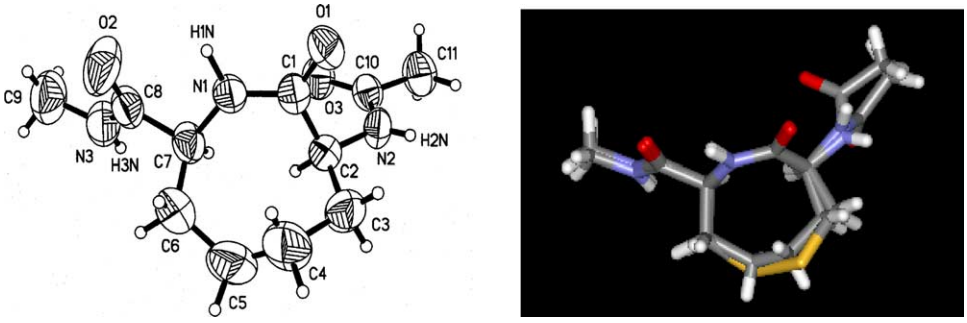


Figure 5. Left: ORTEP representation of the X-ray crystal structure of **4**. Right: superimposition of the C^+ conformer of *ox*-[Cys-Cys][8] and **4** (yellow atoms are the sulfurs in *ox*-[Cys-Cys] **1**).

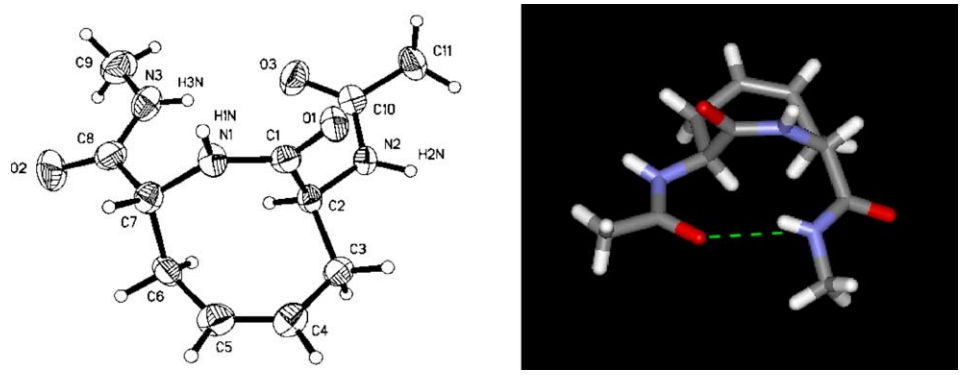


Figure 6. Left: ORTEP representation of the X-ray crystal structure of **5**. Right: compound **5** has been rotated to highlight the 10-membered β-turn hydrogen bond.

amide and *cis* olefin, and the eight-membered ring is in a folded boat–boat conformation. Unlike **3** and **4**, the pendant substituents on **5** are in a compact, turn orientation, and the distance between C9 and C11 is 5.9 Å, consistent with the β-turn designation.[21] The carbonyl oxygen O3 of the acetyl moiety is involved in a hydrogen bond to the NH of the terminal *N*-methyl amide as part of a 10-membered ring.

To complement the X-ray analysis, ^1H–^1H and ^1H–^{13}C coupling constants were determined for **5** in water, and the dihedral angles ϕ_1, ϕ_2, ω_2, χ_{11}, and χ_{21} were calculated based upon the Karplus equation (Table 2).[22] Extensive 2-D NMR analysis was performed using HMQC and ROESY techniques, to assign atom connectivity and coupling constants as appropriate. The ϕ, ψ, and ω dihedral angles for **5** from both the solid state and in water indicate that it is a Type VIa β-turn. The ψ_1 and ψ_2 angles for **5** could not be derived using NMR techniques, so it is important to note that the χ_{11} and χ_{21} values from both methods are also very similar. The dihedral angles obtained from X-ray and NMR are all within 10° of each other, showing high concordance between the solid state and solution conformations.

Nuclear Overhauser enhancements (NOE) were also determined in aqueous solution, and were consistent with the interatomic H–H distances observed in the crystal structure for **5**. In all cases in which the interatomic H–H distances obtained from the crystal structure were below 3.0 Å an NOE was also observed in water, with the exception of the 1.6 Å value for the $H6^R$ and $H6^S$ protons, which cannot be pulsed in the NOE experiment due to the proximity of their chemical shift (Table 3). In addition, the proton on C5 exhibited a relatively weak NOE to the H1N and HC7 protons, indicating that the folded boat–boat conformation of the eight-membered ring is also present in solution. A strong NOE was observed between the H2N and pro R HC3 protons, a weak NOE between the H2N and HC2 protons, and a weak, ambiguous NOE between the H2N and pro S HC3 protons. These data indicate that atoms N2, C10, O3, and C11 are in a similar orientation as that shown by the ORTEP diagram in Figure 6. Finally a relatively strong NOE between the H3N and HC2 protons was detected whereas H3N exhibited weak NOE's to the H1N and HC7 protons. These observations support the same alignment of the C-terminal portion of **5** as seen in the crystal structure.

We reduced **5** to **6** using standard hydrogenation conditions. During the course of our work, **6** was independently described by Holmes and co-workers, prepared via a ca. 10-step procedure.[5b] Because of the route employed, these authors did not have access to olefin **5**, but characterized **6** as a Type VIb β-turn by 2-D NMR analysis in solution, and were able to obtain an X-ray crystal structure of a synthetic precursor earlier in the pathway, which supported the Type VI β-turn conformation at that stage. Consistent with our observations, they were not able to prepare crystals of **6** suitable for X-ray diffraction. In an accompanying

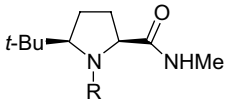
Table 3. Interatomic H–H distances from the crystal structure of **5**, and NOE effects observed (shaded boxes)

	HC11	H2N	HC2	HC3^{R}	HC3^{S}	HC4	H1N	HC7	HC6^{R}	HC6^{S}	HC5	H3N	HC9
HC11		2.5	4.1	4.7	5.0	6.8	5.7	7.1	6.0	7.5	7.8	4.6	5.9
H2N	2.5		2.8	2.3	2.7	4.4	4.7	5.9	4.6	6.0	5.7	4.5	6.2
HC2	4.1	2.8		2.9	2.3	3.8	3.5	3.7	1.9	3.4	4.0	2.6	4.2
HC3^{R}	4.7	2.3	2.9		1.6	2.3	4.4	5.1	3.9	4.9	5.0	5.1	6.9
HC3^{S}	5.0	2.7	2.3	1.6		2.6	4.9	5.1	3.0	4.3	3.8	4.9	6.5
HC4	6.8	4.4	3.8	2.3	2.6		4.5	4.5	3.6	4.0	2.2	5.8	7.5
H1N	5.7	4.7	3.5	4.4	4.9	4.5		2.2	3.8	4.0	4.0	2.9	4.6
HC7	7.1	5.9	3.7	5.1	5.1	4.5	2.2		2.9	2.3	3.0	3.3	4.4
HC6^{R}	6.0	4.6	1.9	3.9	3.0	3.6	3.8	2.9		1.6	2.8	3.2	4.4
HC6^{S}	7.5	6.0	3.4	4.9	4.3	4.0	4.0	2.3	1.6		2.3	4.1	4.9
HC5	7.8	5.7	4.0	5.0	3.8	2.2	4.0	3.0	2.8	2.3		5.3	6.7
H3N	4.6	4.5	2.6	5.1	4.9	5.8	2.9	3.3	3.2	4.1	5.3		2.0
HC9	5.9	6.2	4.2	6.9	6.5	7.5	4.6	4.4	4.4	4.9	6.7	2.0	

Red color indicates protons that overlap with each other.
Of the 23 observed NOE's, 8 are ambiguous.
Distances to methyl protons are measured from the carbon atom.

paper, Holmes and co-workers described the synthesis of the enantiomer of **4** using a 12-step procedure with an overall yield of 22–27% from a serine aldehyde derivative.[5a] They obtained crystals of *ent*-**4** from chloroform–hexane and found the pendant groups to be in an extended orientation consistent with our findings, but their unit cell included two slightly different conformers. We observed a single conformer in the structure of **4**, which could be due to polymorphic forms of **4** and *ent*-**4** caused by different solvents and conditions in the crystallization.

Halab and Lubell have described a series of 5-*t*-BuPro derivatives that are Type VIa β-turns.[4a–c] High (>80%) proportions of the *cis*-Pro rotamer with this conformation were seen in solution by NMR and CD techniques, if the Pro was appropriately N- and C-substituted.[4a] X-ray structures were determined for dipeptides **18** and **19**, and both fit the criteria for Type VIa β-turn form.



18 R = Ac-L-Tyr
19 R = Ac-L-Leu

3. Conclusion

We prepared a series of compounds based upon core scaffold 7-amino-8-oxo-1,2,3,6,7-pentahydroazocine-2-carboxylic acid **2**. The key step in the synthesis was the ring closing metathesis of two sequential allylglycine residues bearing *N*-(2,4-dimethoxy)benzyl amide substitution, which allowed for the pendent olefins to be in close proximity. After removing the protecting groups, an acetyl and a methyl amide at the *N*- and *C*-termini of each scaffold were incorporated, respectively. The synthetic approach we have described is compact and versatile, providing **4** in ca. 17% overall yield after only six synthetic steps starting from L-allylglycine methyl ester. The olefin found in **3** and **5** also offers an attractive synthetic handle for further elaboration. Intermediate **11** bearing latent amino and carboxylic acid functionality has been converted to diketopiperazines, which have interesting conformational properties and a tape crystal packing orientation in the solid state.[23]

Conformational analysis of the target structures by X-ray crystallography and 2-D NMR techniques has proved to be particularly informative. Compounds **3** and **4** adopt an extended orientation of the pendant substitution. For example, **4** mimics the C^{+} conformer of *ox*-[Cys-Cys][8] and may be useful in the design of small molecules that interfere with the binding of ligands such as bungarotoxin and acetylcholine to nicotinic acetylcholine receptors. Our serendipitous discovery of 2*S*,7*R* isomer **5** as a Type VIa β-turn based on both X-ray in the solid state and 2-D NMR in aqueous solution complements the structure determined for **6** as a Type VIb β-turn based upon NMR analysis.[5b] Given the importance of β-turn secondary structures in bioorganic chemistry, the identification of this unique Type VIa β-turn provides another useful tool for the design of novel, constrained peptidomimetics.

4. Experimental section

4.1. General methods

^{1}H NMR spectra were collected on Bruker 300-MHz NMR spectrometer unless otherwise specified. Chemical shifts are reported relative to the resonance of tetramethylsilane (TMS) or the corresponding solvent peak. During the conformational studies, NMR experiments were carried out at 14.1 T using approximately 10 mg of test compound dissolved in 0.5 mL of water with 0.05 mL D_2O for lock stabilization. The pH was adjusted to 6.5, and the data was acquired at 1 °C. Low temperature was used to shift the water resonance to lower field to avoid overlap with one of the alpha protons and also to sharpen the amide signals. Proton and carbon spectra were referenced to TSP. The 600-MHz

^{1}H NMR spectrometer that was employed as indicated was used with a triple resonance (H, C, N) and triple axis gradient probe. The gradient amplifier delivered 10 amps per channel. Standard experiments were used to assign the proton and carbon resonances, and measurement of the heteronuclear couplings was done as previously described.[6] Mass spectra were obtained with a single quadrapole and fitted with an electrospray source. Purifications were conducted by preparative thin layer chromatography using 20×20 cm tapered silica gel plates or by flash column chromatography on silica gel (230–400 mesh). Reagents and solvents were purchased and used without further purification. Crystallographic data (excluding structure factors) for the compounds reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publications CCDC-176870, CCDC-187971, and CCDC 189564. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: +44-1223-336-033; deposite@ccdc.cam.ac.uk).

4.1.1. *N*-2,4-Dimethoxybenzyl L-allylglycine methyl ester (8). A suspension of L-allylglycine (15 gm, 130 mmol) in methanol (260 mL) was cooled with an ice bath to 0 °C and thionyl chloride (14.26 mL, 195 mmol) was added dropwise via an addition funnel. Once the thionyl chloride addition was completed, the ice bath was removed and the reaction allowed to proceed overnight. The next morning the solvent was removed under reduced pressure. The corresponding oil was triturated for 4 h in an 95:5 ethyl ether/ethyl acetate mixture. The resulting solid (**7**) was collected on a glass-fritted funnel (9.5 g, 57.54 mmol), and placed into a separatory funnel and treated with excess 10% Na_2CO_3 in water. The free base was extracted from the aqueous layer with ethyl ether. The organics were dried with anhydrous $MgSO_4$, and the ethyl ether was removed under reduced pressure. The residue (7.27 g, 56.32 mmol) was dissolved in 1,2-dichloroethane (200 mL), and an equimolar amount of 2,4-dimethoxybenzaldehyde was added (9.36 g, 56.32 mmol) followed by sodium triacetoxyborohydride (16.71 g, 78.85 mmol). The reaction was allowed to proceed for 12 h at ambient temperature, and the excess borohydride was quenched with 10% Na_2CO_3 in water followed by removal of DCE under reduced pressure. Ethyl ether was added to the reaction and the organic and aqueous layers were poured into a separatory funnel. The ether layer was washed 3X with 10% aqueous Na_2CO_3 and dried with anhydrous $MgSO_4$, and ethyl ether was removed under reduced pressure to provide a clear yellow oil. The desired compound was purified using flash chromatography (65:35 hexanes/ethyl acetate) yielding 11.8 g of a clear, colorless, oil of **8** (75%), which is best kept for long-term storage as the HCl salt. ^{1}H NMR, free base: ($CDCl_3$) δ 7.1 (d, 1H, $J = 8.6$ Hz), 6.4 (m, 2H), 5.7 (m, 1H), 5.1 (m, 2H), 3.5–3.9 (m, 10H), 2.4 (m, 2H), 2.0 (1H, broad).

4.1.2. Boc-L-allylglycine-*N*-(2,4-dimethoxybenzyl)-L-allylglycine methylester (9). Compound **8** (7.79 g, 27.9 mmol) was dissolved in methylene chloride (90 mL), and Boc-L-allylglycine (6.0 g, 27.9 mmol), HOAt (3.8 g, 27.9 mmol), HATU (10.6 g, 27.9 mmol), and *N*-ethylmorpholine (3.6 mL, 27.9 mmol) were added to the solution. After 12 h at ambient temperature, the solvent was removed, and the crude mixture was suspended in diethyl ether and then filtered through a pad of Celite. The filtrate was poured into a separatory funnel and washed 3X with 10% Na_2CO_3 in water, 1X with saturated brine solution, 3X with 1 N HCl and 1X with saturated brine. The organic layer was dried with anhydrous $MgSO_4$ and the solvent was removed under reduced pressure, and purification was accomplished by flash chromatography (3:1 hexanes/ethyl acetate) to provide **9** as a clear, colorless oil (6.5 g, 50%). ^{1}H NMR ($CDCl_3$) δ 7.12 (d, 1H, 9 Hz), 6.45, (m, 2H), 5.7 (m, 1H), 5.35 (d, 1H, 9 Hz), 5.0 (m, 5H); 4.65 (d, 1H, 15 Hz), 4.35 (d, 1H, 15 Hz), 4.05 (m, 1H), 3.75 (m, 6H), 3.55 (s, 3H), 2.7 (m, 1H); 2.55 (m, 2H), 2.35 (m, 1H), 1.5 (s, 9H). MS m/z (% relative intensity, ion): 477 (30, M+H$^+$), 499 (30, M+Na$^+$) 151 (100, M+ of DMB).

4.1.3. (2*S*,7*S*)-7-*N*-(Boc)amino-1-(2,4-dimethoxybenzyl)-8-oxo-1,2,3,6,7-pentahydroazocine-2-carboxylic acid methyl ester (11). Intermediate **9** (3.0 g, 6.25 mmol) was dissolved in methylene chloride (200 mL) and added to a 5 L flask equipped with a condenser. An additional 1.8 L of methylene chloride was added to the reaction followed by the addition of ruthenium catalyst **10** (0.514 g, 0.625 mmol). The reaction was heated to reflux for 60 h under a stream of dry nitrogen. After the formation of compound **11** was complete as determined by LC/MS, the solvent was removed and the crude product purified by flash chromatography (3:1 hexanes/ethyl acetate) to provide 2.25 g of a clear, light brown oil of **11** (80%): ^{1}H NMR ($CDCl_3$) δ 7.09 (d, 1H, $J = 8$ Hz), 6.40 (m, 2H), 6.20 (d, 1H, $J = 5$ Hz), 5.72 (m, 1H), 5.45 (m, 1H), 4.95 (m, 2H), 4.60 (d, 1H, $J = 15$ Hz), 4.28 (d, 1H, $J = 15$ Hz), 3.80 (s, 3H), 3.80 (s, 3H), 3.44 (s, 3H), 3.00 (m, 1H), 2.70 (m, 2H), 2.35 (ddd, 1H, $J = 16$, 8, 3 Hz), 1.45 (s, 9H). HPLC Rt = 4.06 min. MS m/z (% relative intensity, ion): 449 (20, M+H$^+$), 471 (20, M+Na$^+$) 151 (100, M+ of DMB). Anal. Calcd for $C_{23}H_{32}N_2O_7$: C, 61.59; H, 7.19; N, 6.25. Found: C, 61.82; H, 7.20; N, 5.96.

4.1.4. (2*S*,7*S*)-7-*N*-(Boc)amino-1-(2,4-dimethoxybenzyl)-8-oxo-1,2,3,6,7-pentahydroazocine-2-carboxylic acid methyl amide (12). Lactam **11** (0.5 g 1.11 mmol) was dissolved in a 2 M solution of methylamine in MeOH (20 mL) and the reaction vessel was sealed with a rubber septum. After 48 h, the solvent was then removed under reduced pressure and the resulting oil purified by flash chromatography (3:1 ethyl acetate/hexanes) to yield 0.3 g of **12** a white solid (60%). ^{1}H NMR (DMSO-d_6) δ 8.35 (m, 1H), 6.85 (d, 1H, $J = 8$), 6.58 (d, 1H $J = 6$), 6.45 (d, 1H, $J = 2$), 6.35 (dd, 1H, $J_a = 6$, $J_b = 2$), 5.7 (m, 1H), 5.55 (m, 1H), 5.15 (m, 1H), 4.85 (m, 1H), 4.31 (d, 1H, $J = 15$), 4.03 (d, 1H, $J = 15$), 3.75 (s, 3H), 3.70 (s, 3H), 3.32 (s, 3H), 3.10 (m, 1H), 2.68 (m, 1H), 2.35 (m, 1H), 2.30 (d, 3H, $J = 4$), 2.05 (m, 1H), 1.40 (s, 9H). MS

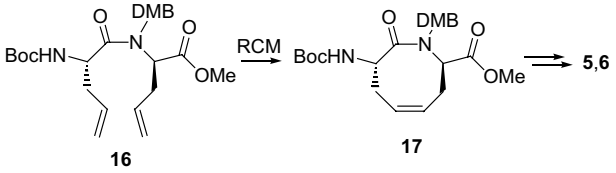
(m/z, % relative intensity, ion) 470 (10%, M+Na$^+$) 448 (10, M+H$^+$), 151 (100, M+ of DMB).

4.1.5. (2*S*,7*S*)-7-Acetylamino-8-oxo-1,2,3,6,7-pentahydroazocine-2-carboxylic acid methyl amide (3). Compound **12** (0.04 g, 0.08 mmol) was treated with a solution of triethylsilane (0.020 mL) and water (0.010 mL) in TFA (0.200 mL). The reaction was allowed to proceed for 16 h after which the solvent was removed and the crude oil was treated with excess ethyl ether. The product was triturated in ether for 4 h and then collected on a glass-fritted funnel (0.025 g, >95%). This solid (0.025 g, 0.080 mmol) was then suspended in methylene chloride (0.240 mL) and treated with triethylamine (0.022 mL 0.160 mmol) and acetic anhydride (0.008 mL 0.080 mmol). After 3 h, the solvent was removed by reduced pressure and the compound purified by flash chromatography (95:5 chloroform/methanol) yielding 0.017 g of **3** a white solid (88%). ^{1}H NMR (600 MHz, H_2O) δ 8.47 (d, 1H, $J = 6.6$), 8.18 (q, 1H, 4.2), 7.50 (d, 1H, $J = 7.8$), 5.7 (m, 1H), 5.5 (1H, m), 4.9 (m, 1H), 4.70 (m, 1H), 2.90 (m, 2H), 2.68 (d, 3H, $J = 4.2$), 2.30 (m, 1H), 2.13 (m, 1H), 1.98 (s, 3H). ^{13}C NMR (151 MHz, H_2O) δ 175.6, 173.6, 173.0, 127.2, 127.1, 53.5, 51.2, 32.1, 30.8, 26.1 (2C).

4.1.6. X-ray diffraction of lactam 3. Single crystals of $C_{11}H_{17}N_3O_3$ are, at -80 ± 2 °C, orthorhombic, space group $P2_12_12_1$-D (No. 19) with $a = 7.538(1)$ Å, $b = 11.511(2)$ Å, $c = 13.981(2)$ Å, $V = 1213.2(3)$ Å^3 and $Z = 4$ molecules $d_{calcd} = 1.310$ g cm^{-3}; μ_a (MoK) = 0.097 mm^{-1}. A full hemisphere of diffracted intensities (omega scan width of 0.30°) was measured using graphite-monochromated MoKα radiation on a Siemens/Bruker SMART CCD Single Crystal Diffraction System. X-rays were provided by a normal-focus sealed X-ray tube operated at 50 kV and 40 mA. Lattice constants were determined with the Siemens/Bruker SAINT software package using peak centers for 855 reflections. A total of 5535 integrated reflection intensities having $2\Theta(\text{MoK}) < 46.50°$ were produced using the Siemens/Bruker program SAINT. A total of 1737 of these were independent and gave $R_{int} = 0.110$. The Siemens/Bruker SHELXTL-PC software package was used to solve the structure using 'direct methods' techniques. All stages of weighted full-matrix least-squares refinement were conducted using F_o^2 data with the SHELXTL-PC Version 5 software package. Final agreement factors at convergence are: R_1 (unweighted, based on F) = 0.049 for 1013 independent reflections having $2\Theta(\text{MoK}) < 46.50°$ and $I > 2\sigma(I)$; R_1 (unweighted, based on F) = 0.120 and wR_2 (weighted, based on F^2) = 0.069 for all 1737 independent reflections having $2\Theta(\text{MoK}) < 46.50°$. The structural model incorporated anisotropic thermal parameters for all nonhydrogen atoms and isotropic thermal parameters for all hydrogen atoms. Amine hydrogen atoms H_{1N}, H_{2N} and H_{3N} were located from a difference Fourier map and refined as independent isotropic atoms. The remaining hydrogen atoms were included in the structure factor calculations as idealized atoms (assuming sp^2- or sp^3-hybridization of the carbon atoms and C–H bond lengths of 0.95–1.00 Å) 'riding' on their respective carbon atoms. The two methyl groups (C_9, C_{11} and their hydrogens) were refined as rigid rotors (using idealized sp^3-hybridized geometry and a C–H bond length of 0.96 Å) with three rotational parameters for each in least-squares cycles. The refined values of these rotational parameters gave N–C–H and C–C–H angles, which ranged from 108° to 112°. The isotropic thermal parameters for H_{1N}, H_{2N}, and H_{3N} refined to final U_{iso} values of 0.02(1), 0.03(1), and 0.05(2) Å^2, respectively. The isotropic thermal parameters of the remaining hydrogen atoms were fixed at values 1.2 (nonmethyl) or 1.5 (methyl) times the equivalent isotropic thermal parameters of the carbon atoms to which they are covalently bonded.

4.1.7. (2*S*,7*S*)-7-Acetylamino-8-oxoheptahydroazocine-2-carboxylic acid methyl amide (4). Compound **3** (0.040 g, 0.1 mmol) was dissolved in a mixture of methanol (3.0 mL) and water (1.0 mL). To this solution was added approximately 10 mg of 10% Pd/C and the reaction mixture was treated with 1 atm of hydrogen and allowed to react for 4 h at room temperature. After 4 h, the reaction mixture was filtered over a pad of Celite and washed 3X with methanol. The organics were combined and the solvent removed under reduced pressure to yield **4** as a white solid (0.040 g, >95%). ^{1}H NMR (600 MHz, H_2O) δ 8.1 (d, 1H, $J = 5.4$), 7.95 (m, 1H), 7.43 (d, 1H, $J = 6.6$), 4.42 (m, 1H), 4.36 (m, 1H), 2.58 (d, 3H, $J = 4.8$), 1.85 (s, 3H), 1.85 (m, 3H), 1.52 (m, 6H), 1.30 (m, 1H). ^{13}C NMR (151 MHz, H_2O) δ 179.1, 176.4, 176.1, 57.4, 53.3, 37.8, 36.4, 28.9, 26.4, 25.5, 24.3.

In a similar manner as for **11**, **12**, **3**, and **4**, compounds **16**, **17**, **5**, and **6** were also prepared.



4.1.8. Boc-L-allylglycine-*N*-2,4-dimethoxybenzyl-D-allylglycine methylester (16). 50–80% yield. ^{1}H NMR ($CDCl_3$) δ 7.15 (d, 9.3), 6.42 (m, 2H), 5.75 (m, 1 H), 5.5 (m, 1H), 5.40 (d, 8.7), 5.12 (m, 2 H), 5.04 (m, 1H), 4.90 (m, 2H), 4.68 (d, 1H, $J = 1.6$), 4.30 (d, 1H, $J = 15.4$), 4.11 (m, 2H), 3.80 (s, 3H), 3.79 (s, 3H), 3.55 (s, 3H), 2.78 (m, 1H), 2.42 (m, 3H), 1.44 (s, 9H). Crystals were obtained from diethyl ether for X-ray diffraction studies.

4.1.9. (2*R*,7*S*)-7-*N*-(Boc)amino-1-(2,4-dimethoxybenzyl)-8-oxo-1,2,3,6,7-pentahydroazocine-2-carboxylic acid methyl ester (17). 70–80% yield. ^{1}H NMR ($CDCl_3$) δ 7.22 (d, 1H, $J = 8.1$), 6.40 (m, 2H), 5.64 (m, 2H), 5.30 (m. 1H), 4.90 (m, 1H), 4.78 (d, 1H, $J = 14.1$), 4.36 (d, 1H, $J = 14$), 3.79 (s, 3H), 3.77 (s, 3H), 3.69 (s, 3H), 2.85 (m, 2H), 2.65 (m, 1H), 2.10 (m, 1H), 1.43 (s, 9H).

Crystals were obtained from diethyl ether for X-ray diffraction studies.

4.1.10. (2*R*,7*S*)-7-Acetylamino-8-oxo-1,2,3,6,7-pentahydroazocine-2-carboxylic acid methyl amide (5). 50–70% overall yield. ^{1}H NMR (600 MHz, H_2O) δ 8.47 (d, 1H $J = 3.6$), 8.40 (m, 1H), 8.02 (d, 1H, $J = 7.8$), 5.77 (m, 1H), 5.70 (m, 1H), 4.86 (m, 1H), 4.02 (m, 1H), 2.78 (m, 3H), 2.76 (d, 3H, $J = 4.8$), 2.2 (m, 1H), 2.0 (s, 3H). ^{13}C NMR (151 MHz, H_2O) δ 180.5, 176.9, 176.6, 132.0, 129.6, 57.5, 54.4, 33.0, 32.7, 29.1, 23.9. Crystals were obtained from diethyl ether for X-ray diffraction studies.

4.1.11. X-ray diffraction of lactam 5. Single crystals of $C_{11}H_{17}N_3O_3$ are, at -80 ± 2 °C, orthorhombic, space group $P2_12_12_1$-D (No. 19) with $a = 6.992(2)$ Å, $b = 9.099(3)$ Å, $c = 17.814(6)$ Å, $V = 1133.3(7)$ Å^3 and $Z = 4$ molecules $\{d_{calcd} = 1.402\,g\,cm^{-3}$; $\mu_a(MoK) = 0.104\,mm^{-1}\}$. A full hemisphere of diffracted intensities (omega scan width of 0.30°) was measured using graphite-monochromated MoKα radiation on a Bruker SMART CCD Single Crystal Diffraction System. X-rays were provided by a normal-focus sealed X-ray tube operated at 50 kV and 40 mA. Lattice constants were determined with the Bruker SAINT software package using peak centers for 550 reflections. A total of 4959 integrated reflection intensities having $2\Theta(MoK) < 46.52°$ were produced using the Bruker program SAINT. A total of 1628 of these were independent and gave $R_{int} = 0.123$. The Bruker SHELXTL-PC software package was used to solve the structure using 'direct methods' techniques. All stages of weighted full-matrix least-squares refinement were conducted using F_o^2 data with the SHELXTL-PC Version 5 software package. Final agreement factors at convergence are: R_1 (unweighted, based on F) = 0.051 for 1055 independent reflections having $2\Theta(MoK) < 46.52°$ and $I > 2\sigma(I)$; R_1 (unweighted, based on F) = 0.099 and wR_2 (weighted, based on F^2) = 0.112 for all 1628 independent reflections having $2\Theta(MoK) < 46.52°$. The structural model incorporated anisotropic thermal parameters for all nonhydrogen atoms and isotropic thermal parameters for all hydrogen atoms. Amide hydrogen atoms H_{1N}, H_{2N} and H_{3N} were located from a difference Fourier map and refined as independent isotropic atoms. The remaining hydrogen atoms were included in the structure factor calculations as idealized atoms (assuming sp^2- or sp^3-hybridization of the carbon atoms and C–H bond lengths of 0.95–1.00 Å) 'riding' on their respective carbon atoms. The two methyl groups (C_9, C_{11} and their hydrogens) were refined as rigid rotors (using idealized sp^3-hybridized geometry and a C–H bond length of 0.96 Å) with three rotational parameters for each in least-squares cycles. The refined values of these rotational parameters gave N–C–H and C–C–H angles, which ranged from 104° to 115°. The isotropic thermal parameters for H_{1N}, H_{2N}, and H_{3N} refined to final U_{iso} values of 0.04(2), 0.05(1), and 0.06(2) Å^2, respectively. The isotropic thermal parameters of the remaining hydrogen atoms were fixed at values 1.2 (nonmethyl) or 1.5 (methyl) times the equivalent isotropic thermal parameters of the carbon atoms to which they are covalently bonded.

4.1.12. (2*R*,7*S*)-7-Acetylamino-8-oxoheptahydroazocine-2-carboxylic acid methyl amide (6). >95% yield. ^{1}H NMR (600 MHz, H_2O 500 μL, D_2O 50 *rmu*L, 7 μL 1 N HCl, pH 6.2, $T = 285$ K) δ 7.89 (d, 1H, 5.41 Hz), 7.75 (m, 1H), 7.5 (m, 1H), 4.22 (d, 1H, 9.75 Hz), 4.21, (m, 1H), 2.37 (d, 3H, 4.76 Hz), 1.61 (s, 3H), 1.60 (m, 2H), 1.30 (m, 4H), 1.10 (m, 2H). This spectrum was similar to that previously reported.[5b]

4.1.13. X-ray diffraction of lactam 6. Single crystals of $C_{11}H_{19}N_3O_3$ are, at 20 ± 2 °C, orthorhombic, space group $P2_12_12_1$-D (No. 19) with $a = 9.661(1)$ Å, $b = 14.836(1)$ Å, $c = 18.431(3)$ Å, $V = 2641.7(5)$ Å^3 and $Z = 8$ formula units $\{d_{calcd} = 1.213\,g\,cm^{-3}$; $\mu_a(MoK) = 0.089\,mm^{-1}\}$. A total of 2735 reflections having $2\Theta(MoK) < 45.8°$ (the equivalent of 0.6 limiting CuK sphere) were collected on a computer-controlled Bruker P4 Single Crystal Diffractometer using 0.90°-wide ω scans and graphite-monochromated MoK radiation; 2551 of these reflections were independent. X-rays were provided by a normal-focus sealed X-ray tube operated at 50 kV and 40 mA. Lattice constants were determined with the Bruker XSCANS software package using 54 centered reflections. The Bruker SHELXTL-PC (version 5) software package was used to solve and refine the structure. 'Direct methods' techniques were used to solve the structure and the resulting structural parameters have been refined with F^2 data to convergence using counter-weighted full-matrix least-squares techniques and a structural model, which incorporated anisotropic thermal parameters for all nonhydrogen atoms and isotropic thermal parameters for all hydrogen atoms. Final agreement factors are: R_1 (unweighted, based on F) = 0.057 for 1729 independent reflections having $2\Theta(MoK) < 45.8°$ and $I > 2\sigma(I)$; R_1 (unweighted, based on F) = 0.096 and wR_2 (weighted, based on F^2) = 0.148 for all 2551 independent reflections having $2\Theta(MoK) < 45.8°$. Amide hydrogen atoms H_{1N}, H_{2N}, H_{3N}, H_{21N}, H_{22N}, and H_{23N} were located from a difference Fourier map and refined as independent isotropic atoms. The four methyl groups (C_9, C_{11}, C_{29}, C_{31} and their hydrogens) were refined as rigid rotors (using idealized sp^3-hybridized geometry and a C–H bond length of 0.96 Å), which were allowed to rotate about their N–C or C–C bonds in least-squares cycles. The remaining hydrogen atoms were included in the structure factor calculations as idealized atoms (assuming sp^3-hybridization of the carbon atoms and C–H bond lengths of 0.97 or 0.98 Å) 'riding' on their respective carbon atoms. The isotropic thermal parameters for H_{1N}, H_{2N}, H_{3N}, H_{21N}, H_{22N}, and H_{23N} refined to final U_{iso} values of 0.07(2), 0.05(2), 0.08(3), 0.10(2), 0.04(2), and 0.03(2) Å^2, respectively. The isotropic thermal parameters of the remaining hydrogen atoms were fixed at values 1.2 (nonmethyl) or 1.5 (methyl) times the equivalent isotropic thermal parameters of the carbon atoms to which they are covalently bonded. The second crystallographically independent molecule in the asymmetric unit

appears to be slightly disordered in the lattice with carbon atoms C_{24} and C_{25} having substantially larger equivalent isotropic thermal parameters than the remaining nonhydrogen atoms in the asymmetric unit. This disorder is presumably responsible for the short C_{24}–C_{25} bond length of 1.288(11) Å. Analytical and spectroscopic data for the compound indicate the presence of a single species with no C=C double bonds.

4.2. Concentration studies of the ring closing metathesis reaction

Compound **9** was added to four 10 mL round bottom flasks (0.025 g, 0.052 mmol), and treated with an increasing amount (0.55, 1.1, 2.2, and 4.3 mL) of dichloromethane corresponding to 100, 50, 24, and 12 mM solutions. To each reaction vessel first-generation Grubbs catalyst **10** was added (0.004 g, 0.0052 mmol), and this time point was taken to be t_0. Each vessel was equipped with a rubber septum and purged with N_2. Periodically, a 20 μL aliquot of each reaction was removed via syringe and diluted with 20 μL of methanol. From this mixture, 2 μL was injected on an LC-MS instrument (MS for **14**: $C_{48}H_{68}N_4O_{14}$, *m/e* 925.1, M+1; for **15**: $C_{46}H_{64}N_4O_{14}$, *m/e* 897.0, M+1). The same procedure was used for studies involving second-generation catalyst **13** (0.004 g, 0.0052 mmol).

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