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Synthesis of Cyclic Proline-Containing Peptides via Ring-Closing Metathesis

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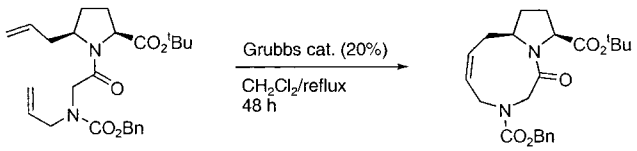
ABSTRACT



19 1:1 *cis*:*trans* (Pro) **21** 46% [100% *trans* (Pro)]

Several dienes embedded in di- and tripeptides which incorporate proline have been prepared and subjected to ring-closing metathesis. Bicyclic peptides of well-defined amide geometry and of varying ring sizes were prepared. Several limitations of the cyclization step were revealed.

The use of cyclic peptides provides an elegant approach for the synthesis of peptides of rigid geometry that can be used to probe the bioactive conformation of a given peptide. This strategy can be used to establish the structure of a more potent analogue.[1]

A common method for the synthesis of cyclic peptides[2] relies on construction of an acyclic precursor, typically a diene, that then undergoes cyclization with use of Grubbs ring-closing metathesis (RCM).[3] The work reported herein involves the synthesis of dienes from suitable amino acid precursors bearing an allyl group at C(2) or C(5) of proline. RCM of these dienes affords cyclic proline-containing peptides of defined rotamer geometry about the proline amide bond. Several cyclic peptides of varying ring sizes have been prepared and the effect of both stereochemistry and heteroatom location on the metathesis reaction has been investigated.

Synthetic Strategy. We were interested in synthesizing a range of conformationally restricted macrocyclic di- and tripeptides containing proline, which adopt either cis or trans stereochemistry about the peptide bond, depending on the substitution on the proline ring.[4] Given that *cis*/*trans* isomerase enzymes often deliver the bioactive conformation of a peptide,[5] peptidomimetics of defined conformation are useful for incorporation into a peptide scaffold. Etzkorn et al.[6] have recently synthesized amide bond isosteres of *cis*- and *trans*-prolines for incorporation into peptide synthesis.

To prepare a series of *cis* and *trans* bicyclic dipeptides incorporating proline, it was decided to introduce an allyl group at either the C(2) or C(5) position. We chose a glycylprolylglutamic acid scaffold onto which were also

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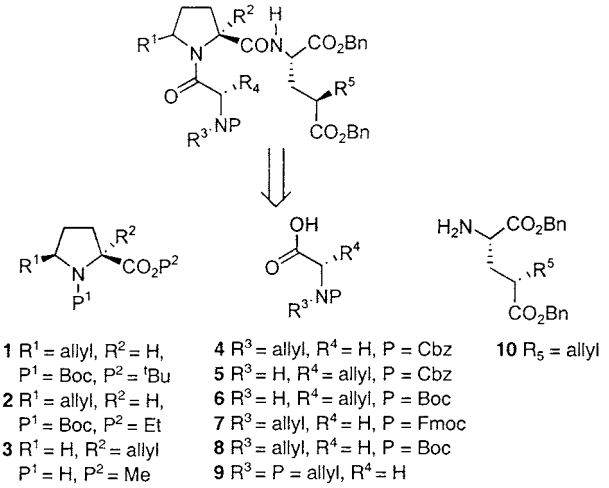
placed an allyl group on either the glycine or glutamate residue (Scheme 1). Introduction of an allyl group onto the

Scheme 1. General Synthesis of Diene Precursors for RCM Based on a Glyclyprolylglutamate Scaffold

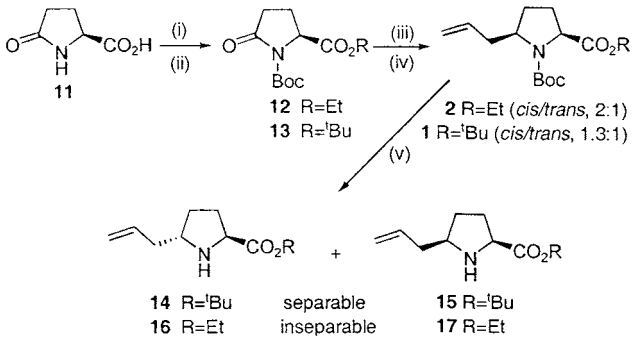


1 R^1 = allyl, R^2 = H, P^1 = Boc, P^2 = ^{t}Bu
2 R^1 = allyl, R^2 = H, P^1 = Boc, P^2 = Et
3 R^1 = H, R^2 = allyl P^1 = H, P^2 = Me
4 R^3 = allyl, R^4 = H, P = Cbz
5 R^3 = H, R^4 = allyl, P = Cbz
6 R^3 = H, R^4 = allyl, P = Boc
7 R^3 = allyl, R^4 = H, P = Fmoc
8 R^3 = allyl, R^4 = H, P = Boc
9 R^3 = P = allyl, R^4 = H
10 R_5 = allyl

α-carbon and the nitrogen atom of glycine was deemed straightforward. Glutamic acid was chosen in view of the well-established methodology for the stereoselective introduction of an allyl group at C(4).[7]

The synthesis of proline derivatives bearing an allyl group at C(5) has been reported[8] (Scheme 2). Thus, the ethyl ester[9]

Scheme 2. Synthesis of 5-Allylprolines[a]



11
12 R=Et
13 R=^{t}Bu
2 R=Et (*cis/trans*, 2:1)
1 R=^{t}Bu (*cis/trans*, 1.3:1)
14 R=^{t}Bu
16 R=Et
separable
inseparable
15 R=^{t}Bu
17 R=Et

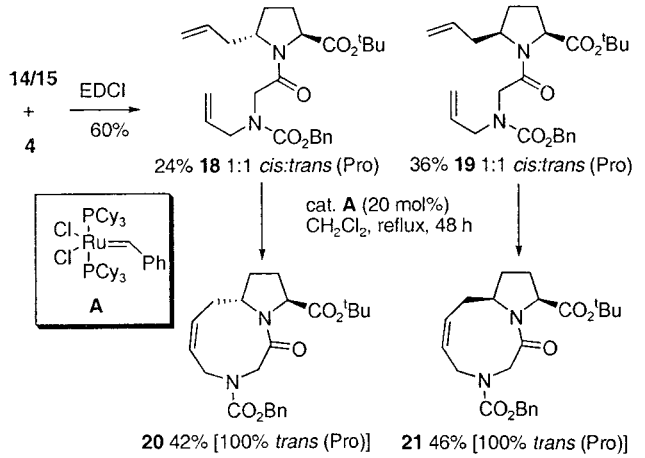
[a] Reagents and conditions: (i) for **12**, EtOH/$SOCl_2$, for **13**, 70% $HClO_4$, *t*-BuOAc (56%); (ii) Boc_2O, DMAP (85% for **2**, 95% for **1**); (iii) $LiEt_3BH$; (iv) for **1**, allyltributylstannane, Me_3SiOTf (70%), for **2**, allyltributylstannane, $BF_3{\cdot}Et_2O$ (54%); (v) for **1**, 4 M HCl, dioxane 72% (**14** 31%, **15** 41%), for **2**, CF_3CO_2H, ca. 80%.

and *tert*-butyl ester[10] of readily available (*S*)-pyroglutamic acid **11** were protected as their respective Boc carbamates **12**[11] and **13**.[12] Selective reduction of the lactam carbonyl with $LiEt_3BH$ yielded aminols, which underwent *C*-allylation with allyltributylstannane to afford 5-allylprolines **2**[13] (*cis/trans*, 66:33) and **1**[8] (*cis/trans*, 57:43) in good yield. Neither set of diastereomers could be separated; however, removal of the Boc group with HCl in dioxane allowed small-scale separation of the *trans* and *cis tert*-butyl esters **14** and **15**. The stereochemistry of **14** and **15** was determined with use of nOe experiments and was supported by literature analogues[8,10] which suggested that the *cis* isomer **15** would be the major product.

The preparation of (*S*)-2-allylproline methyl ester **3**,[14] *N*-allylglycine carbamates **4**,[15] **7**,[17b] and **8**,[16] of *C*-allylglycines[17] **5**[18] and **6**,[19] and of glutamate **10**[7] followed literature procedures or variations thereof.

Coupling of *N*-allylglycine derivative **4** with the mixture of diastereoisomeric 5-allylprolines **14** and **15** was effected with EDCI to yield the separable dienes **18** and **19** in moderate yield (60%). The *cis/trans* ratio of the newly created amide (Pro) bond in both these flexible acyclic dipeptides was 1:1. Ring-closing metathesis of the individual 1,10-dienes **18** and **19** was accomplished with use of Grubbs catalyst **A** to give the expected cyclononenes **20** and **21** in moderate yield (Scheme 3). The ring closure was not affected

Scheme 3



24% **18** 1:1 *cis:trans* (Pro)
36% **19** 1:1 *cis:trans* (Pro)
A
20 42% [100% *trans* (Pro)]
21 46% [100% *trans* (Pro)]

by the stereochemistry of the allyl group at C(5) on the proline moiety, both reactions giving similar yields with

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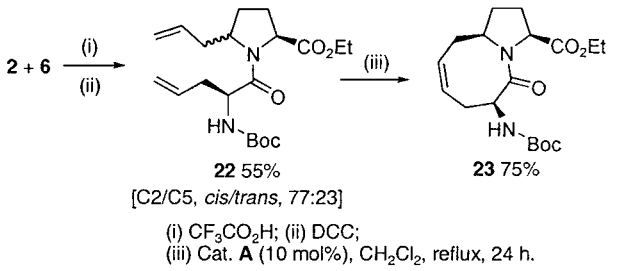
comparable reaction times and catalyst loadings. Very little deallylation/isomerization occurred (cf. *N*- or *O*-allyl systems).[20] In contrast to their respective precursors, both macrocycles **20** and **21** adopted only the *trans* conformation about the peptide bond.

Subsequent transformations of **20** and **21** proved to be problematic due to the presence of the *tert*-butyl ester and a benzylcarbamate, hence our attention was turned to the use of an ethyl ester with a Boc as a protecting group. Due to difficulties in separating the ethyl esters **16** and **17**, the mixture of diastereomers **2** was coupled with the C(2)-allylglycine **6** with use of DCC to afford an inseparable mixture of dipeptides **22** [C(2)/C(5), *cis/trans*, 77:23, *cis/trans* (Pro) ratio undetermined due to complex NMR spectra] in 55% yield (Scheme 4). Subjecting the diene mixture to

Scheme 4[a]



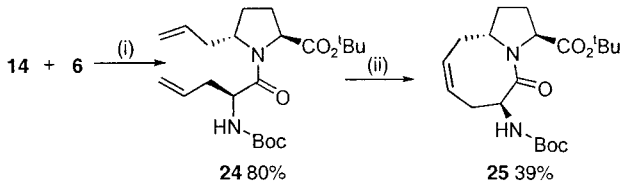
[a] Reagents and conditions: (i) CF_3CO_2H; (ii) DCC; (iii) cat. **A** (10 mol %), CH_2Cl_2, reflux, 24 h.

the standard RCM conditions yielded only one macrocycle **23** in excellent yield (75%). On the basis of the fact that the *cis* diastereomer was the major component in the precursor, the cyclic metathesis product was assigned as *cis* C(2)/C(5) stereochemistry. Clearly the minor *trans* isomer cyclizes extremely slowly relative to the *cis* isomer, a trend that was not observed earlier in the metathesis of the *N*-allyl dienes **18** and **19** (Scheme 3). Gratifyingly cyclooctene **23** adopted only *trans* stereochemistry about the peptide bond.

To access the C2/C5 *trans* diastereomer **25** of **23** it was necessary to use amine **14**; coupling with C(2)-allylglycine **6** with use of DCC yielded the diene **24** in 80% yield [Scheme 5, *cis:trans* (Pro) ratio undetermined due to complex NMR spectra]. As anticipated, ring closure of **24** was extremely slow, required high catalyst loading, and afforded a lower yield of the cyclooctene **25**. However, the peptide bond within macrocycle **25** adopted only a *trans* conformation.

Grubbs et al.[21] have reported that certain *cis*-substituted cyclohexane derivatives undergo metathesis under more

Scheme 5[a]

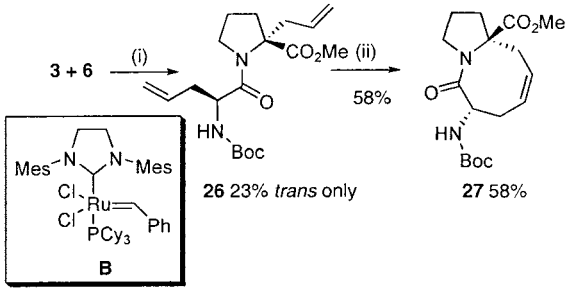


[a] Reagents and conditions: (i) DCC; (ii) cat. **A** (30 mol %), CH_2Cl_2, reflux, 116 h.

forcing conditions and in lower yield than the corresponding *trans* isomers. In the present work it was observed that placing the allyl moieties closer together (*C*-allyl vs *N*-allyl) and in certain orientations (*cis* vs *trans*) resulted in significant differences in reactivity.

The above metathesis reactions had involved an allyl group at C(5) on the proline and an allyl unit on the glycine, forming macrocycles containing a *trans* (Pro) amide bond. To obtain macrocycles with a *cis* (Pro) amide bond, metathesis between an allyl group at C(2) on proline and an allyl group on the glycine was required. Thus metathesis of **26** with the second generation Grubbs's catalyst (**B**)[22] yielded bicyclic cyclooctene **27**[2h] as a single (*cis*) rotamer in good yield (Scheme 6).

Scheme 6[a]



[a] Reagents and conditions: (i) DCC, HOBt; (ii) cat. **B** (10 mol %), C_6H_6, 45 °C, 48 h.

All attempts to effect metathesis of the corresponding C(2) allyl/*N*-allyl derivatives **28**, **29**, or **30** (Scheme 7) gave complex mixtures probably due to competing deallylation, which is accelerated by the electron-withdrawing Boc, CO_2-Bn, or Fmoc carbamates. *N*,*N*′-Diallylglycine[23] derivative **31** was prepared in an attempt to avoid this effect; however, only starting material was recovered after attempted RCM, possibly due to quenching of the catalyst by the reaction

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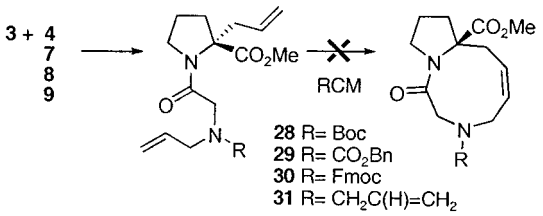
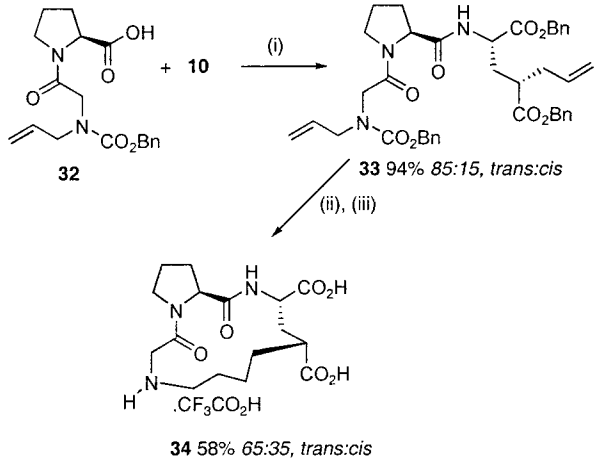
Scheme 7



with the more basic nitrogen.[24] Conversion of diallylamine **31** into its hydrochloride salt[25] followed by attempted RCM was also unsuccessful.

The ring-closing metathesis of dienes contained within a Gly.Pro.Glu scaffold was also examined. The substrate was synthesized (Scheme 8) from coupling **32** with **10** to yield

Scheme 8[a]

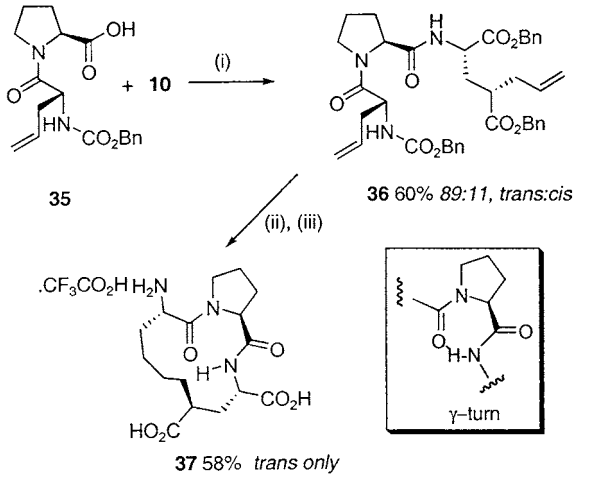


[a] Reagents and conditions: (i) EtOCOCl; (ii) cat. **B** (30 mol %), C_6H_6, 45 °C, 65 h; (iii) H_2, 10% Pd/C.

N-allylglycyl diene **33**, predominantly as the *trans* (Pro) rotamer. Exposure of **33** to Grubbs catalyst **B** followed by hydrogenation gave macrocycle **34** in 58% yield after purification by HPLC. Cyclotetradecene **34** existed as a 65:35 mixture of *trans*:*cis* (Pro) rotamers. The increased proportion of the *cis* rotamer may reflect increased flexibility of the Pro amide bond when it is embedded in a larger 14-membered ring.

Metathesis of the corresponding *C*-allylglycyl dipeptide **36** and subsequent global deprotection yielded the 13-membered macrocycle **37** (58%) as a single *trans* rotamer (Scheme 9). The observation of only the *trans* amide rotamer may be a consequence of the smaller ring size and/or

Scheme 9[a]



[a] Reagents and conditions: (i) EtOCOCl; (ii) cat. **A** (20 mol %), CH_2Cl_2, reflux, 48 h; (iii) H_2, 10% Pd/C.

stabilization of the structure by the formation of a γ-turn. A peptide γ-turn is a structural motif present in many biologically active cyclic peptides that occurs to reverse the orientation of the peptide chain. γ-Turns contain three residues held together in a seven-membered cyclic conformation by an intramolecular hydrogen bond.[26]

The synthesis and ring closure of a number of di- and tripeptides incorporating proline has been accomplished via construction of appropriate diallylated peptide precursors and subsequent olefin metathesis. The proline-containing peptides cyclized to afford cyclic products that adopted a single conformation about the Gly-Pro amide bond (with the exception of **34**). *N*-Allyl dienes **18** and **19** afforded cyclononenes **20** and **21** with similar yields under identical reaction conditions. The *cis* isomer [C(2)/C(5)] of *C*-allyl diene **22** underwent smooth cyclization to cyclooctene **23** in good yield in contrast to the *trans* isomer **24** that required high catalyst loading to achieve a moderate yield of cyclooctene **25**. In the two cases involving cyclization of dienes in which one allyl group was located at the more hindered C(2) position on proline, C-allyl diene **26** readily formed the RCM product **27** whereas *N*-allyl diene **28** failed to undergo cyclization to the analogous diazabicyclic product (Scheme 7).

Acknowledgment. The authors thank NeuronZ Ltd for financial support.

Supporting Information Available: Experimental procedures and spectral data for compounds **20**, **23**, and **37**. This material is available free of charge via the Internet at http://pubs.acs.org.

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