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REVIEW

Incorporation of Conformationally Constrained β-Amino Acids into Peptides

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Abstract: The use of norbornene units to induce the formation of β-sheet and β-turn type structures in peptides is discussed. The norbornene unit is readily prepared by a desymmetrization reaction and is easily incorporated into a peptide chain. Depending upon the exact nature of the norbornene unit, it is possible to form structures which resemble parallel β-sheets, antiparallel β-sheets or β-turns. Similar peptide analogues incorporating a *cis*-2-amino-cyclopropane carboxylic acid unit can also be prepared. As an illustration of the application of this chemistry, a short, asymmetric synthesis of conformationally constrained metalloprotease inhibitors is presented. Copyright © 2000 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: conformationally constrained; β-amino acids; norbornene units

INTRODUCTION

It is more than a century ago that Fischer [1] first appreciated that the conformation of a peptide or protein is important in determining the biological activity of the protein. This is expressed in the so called 'lock and key' description of the interaction between an enzyme and its substrate [2]. Although the 'lock and key' description is now appreciated to be an oversimplification with an 'induced fit' between the substrate and receptor being more realistic [3,4], there is a considerable demand for peptides and peptide analogues which are constrained to adopt specific conformations [5–8]. Such compounds have medical applications (inhibiting enzymes or activating receptors) and are of use in bio-organic chemistry to determine the biologically active conformation of a native peptide.

A number of approaches have been developed for the preparation of conformationally constrained peptide analogues. These include:

- The use of a conformation inducing template: for example the α-helix inducer (Figure 1) developed by Kemp *et al.* [9,10]. Such templates tend to influence the global conformation adopted by the peptide.
- The use of peptidomimetics which restrict the local conformation near to individual amide bonds [11]. The work of Norwick *et al.* [12] on the use of urea based peptidomimetics to induce β-sheet conformations is a good illustration of this technique.
- The use of conformationally constrained amino acids. This is exemplified by the work of Toniolo *et al.* on α,α-disubstituted amino acids where the requirement for individual amino acids to adopt specific conformations results in the peptide as a whole being forced to adopt a defined conformation [13].
- The use of side chain linking groups such as disulphides [14] or amide bonds [15] to join together the side chains of amino acids within a peptide, thus restricting the conformation. A related area is the use of salt bridges (usually

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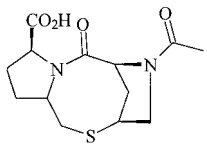


Figure 1 Kemp's α-helix inducer.

involving the side chains of aspartic or glutamic acid and lysine) to encourage the amino acids to adopt a conformation in which they are close to one another. This has been used with most success in the synthesis of helical peptides [16].

Proteins are constructed exclusively from α-amino acids, though natural peptides may contain β-amino, γ-amino, or other unusual amino acids. Recently there has been considerable interest in the synthesis and characterization of peptide analogues derived from β- or γ-amino acids (Figure 2). These amino acids contain two or three carbon atoms between the carbonyl and amino groups of the backbone, each of which can be a stereocentre, thus giving more regio- and stereo-isomeric structures compared to the corresponding α-amino acids. A number of methodologies have been developed for the asymmetric synthesis of β- and γ-amino acids [17], with the Arndt-Eistert homologation of α-amino acids being the most common [18]. It has been found that peptides derived exclusively from β-amino acids adopt helical or sheet conformations reminiscent of the corresponding conformations adopted by α-amino acid derived peptides. The structure adopted by peptides derived from β-amino acids depends upon which of the two backbone carbon atoms are substituted, and the relative stereochemistry of the substituents if both backbone carbon atoms are substituted [19,20].

In addition to preparing peptide derivatives containing just α-, β-, or γ-amino acids, it is feasible to prepare peptide analogues derived from a mixture of these amino acids. It seemed attractive to us, to incorporate a single conformationally constrained β-amino acid into a peptide comprised exclusively of α-amino acids. In this way, we hoped to exploit the fixed conformation of the β-amino acid to induce a preferred conformation on the whole structure, whilst keeping the side chains of most of the

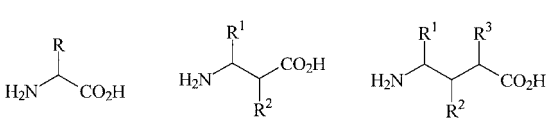


Figure 2 General structures of α-, β- and γ-amino acids.

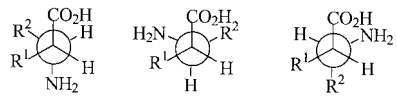


Figure 3 The three staggered conformations of a β-amino acid.

amino acids in the positions that they would adopt in a peptide derived only from α-amino acids.

It is known [21] that most β-amino acids adopt a staggered conformation around the two sp^3 hybridized backbone carbon atoms (Figure 3). However, we chose to investigate structures in which the β-amino acid was constrained to an eclipsed conformation. This structure seemed to be ideal for inducing chain reversal in a peptide containing a single such conformationally constrained β-amino acid. By varying the nature of the attachment between the β-amino acid and α-amino acids, it should be possible to prepare structures which resemble the parallel or anti-parallel β-sheet conformations found in proteins composed of only α-amino acids (Figure 4). To constrain the β-amino acid to the desired conformation, we chose to investigate two structures (Figure 5), a bicyclic β-amino acid incorporating a norbornene ring, and *cis*-2-amino cyclopropane carboxylic acid derivatives.

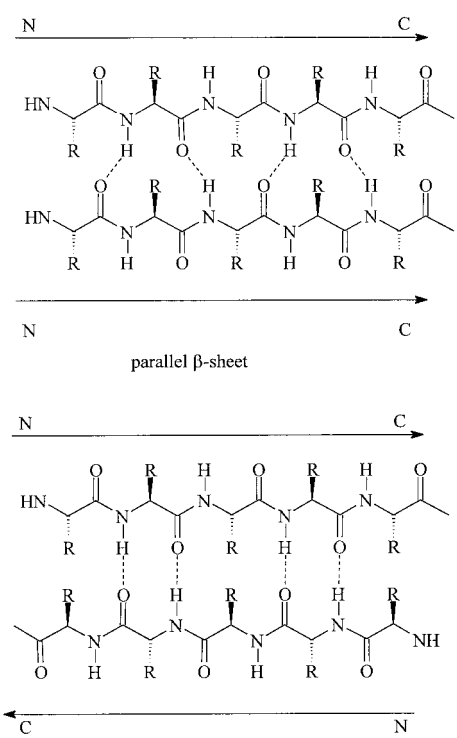


Figure 4 Parallel and antiparallel β-sheets.

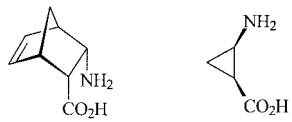


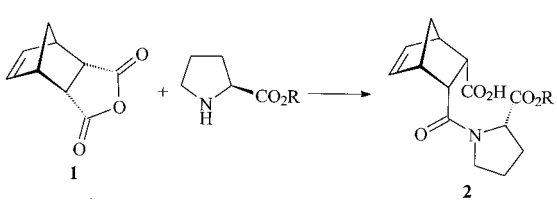
Figure 5 Two β-amino acids constrained to eclipsed conformations.

SYNTHESIS OF PEPTIDES INCORPORATING A NORBORNENE-DERIVED β-AMINO ACID

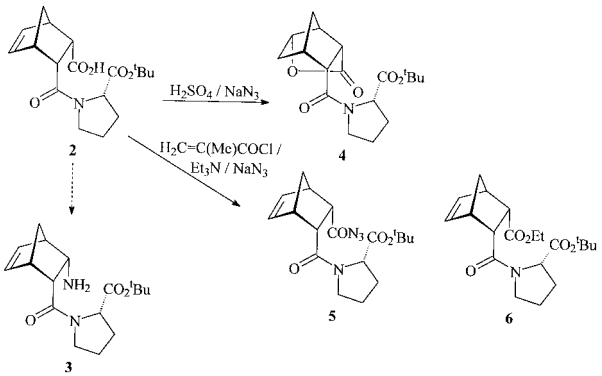
As a first goal, we aimed to prepare a heptapeptide in which the central amino acid was the β-amino acid *endo*-2-amino-3-carboxy-norborn-5-ene. If the conformationally constrained β-amino acid did induce the formation of a β-sheet in the three α-amino acids on each side of it, then this would give the most basic antiparallel β-sheet structure, a β-hairpin [22].

The desymmetrization of meso-anhydrides by a chiral alcohol or amine, leading to chiral, non-racemic esters or amides is a well established process [23]. At the start of this project, we found that esters of (S)-proline could be used to achieve this transformation [24–26], and that *endo*-norborn-5-ene-2,3-dicarboxylic anhydride **1** was one of the best substrates, leading to stereoisomerically pure amido-acids **2** (Scheme 1). This desymmetrization procedure is limited to the use of derivatives of the secondary amino acid proline, since esters of other amino acids lead to achiral cyclic imides [27]. Amido acids **2** have been found to be versatile starting materials for the synthesis of highly substituted, stereochemically defined cyclopentane derivatives [28,29], and we were also attracted to the potential of using these compounds as precursors of conformationally constrained β-amino acids.

Amido acid **2** (R = ^{t}Bu) was envisaged to be a precursor of dipeptide **3** via a Curtius rearrangement. In the event, considerable experimentation was required before the Curtius rearrangement could be effected [30]. Attempts to form the acyl azide under acidic conditions [31] led to lactone **4**, whilst activation of the carboxylic acid as an acid



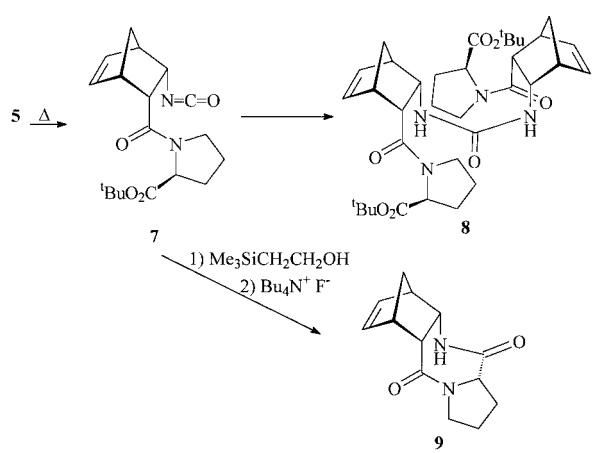
Scheme 1



Scheme 2

chloride resulted in recyclization leading back to anhydride **1**. Eventually, treatment of amido acid **2** with isopropenyl chloroformate [32] followed by aqueous sodium azide was found to produce the desired acyl azide **5** as shown in Scheme 2. The same transformation could be accomplished using ethyl chloroformate, though in this case, the acyl azide **5** was always contaminated with ethyl ester **6**, presumably formed by reaction between acyl azide **5** and the ethanol by-product from the chloroformate.

The conversion of acyl azide **5** into the corresponding isocyanate **7** was accomplished without difficulty, however all attempts to hydrolyse isocyanate **7** to dipeptide derivative **3** were unsuccessful, leading only to urea **8** (Scheme 3). Similarly, indirect conversion of the isocyanate to an amine via formation of the 2-trimethylsilylethyl urethane derivative was unsuccessful, as subsequent removal of the 2-trimethylsilylethyloxycarbonyl protecting group resulted in cyclization to give cyclic



Scheme 3

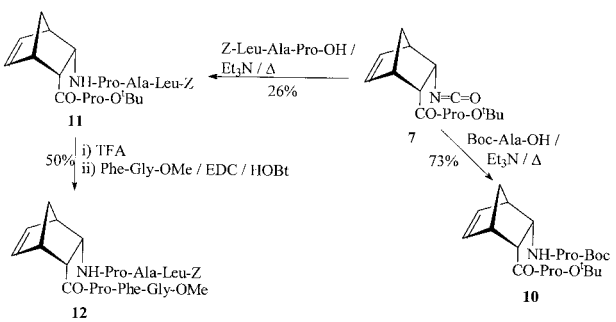
dipeptide **9**. Thus, all attempts to achieve the transformation of acid **2** into amine **3** were unsuccessful.

Fortunately, amine **3** was found not to be essential for the completion of the project; the only reason for preparing the amine was so that it could subsequently be reacted with an *N*-protected amino acid to form an amide bond. It had previously been reported [33] that isocyanates react with carboxylic acids to give amides directly without the need to first convert the isocyanate into the corresponding amine. This reaction had only previously been applied to simple achiral isocyanates and acids, but we decided to investigate whether it could be applied to our more complex systems without loss of stereochemical integrity. In the event, reaction of isocyanate **7** with Boc-proline in the presence of triethylamine gave the desired tripeptide derivative **10** in a respectable 73% yield and with no evidence of epimerization at any of the stereocentres by ^{1}H- or ^{13}C-NMR spectroscopy (Scheme 4). Similarly, reaction of the isocyanate with the tripeptide Z-Leu-Ala-Pro gave pentapeptide **11** in an unoptimized 26% yield. Compound **11** was further elongated to the target heptapeptide **12** by cleavage of the *tert*-butyl ester and coupling to Phe-Gly-OMe.

The conformation of compound **12** was studied in chloroform solution by a combination of NMR, infrared and CD techniques [34,35]. The CD spectrum showed a maximum at 190 nm and a minimum at 225 nm, typical of a β-sheet [36]. The infrared spectrum of compound **12** showed both hydrogen bonded (3326 cm^{-1}) and non-hydrogen bonded (3411 cm^{-1}) NH stretches of equal intensity [37,38], and was concentration independent between 7 and 30 mM. A more detailed picture of the conformation was obtained from the ^{1}H-NMR spectrum of compound **12**. Each of the signals in the spectrum could be assigned by a combination of COSY and TOCSY spectra, then the conformation was determined from the NOESY, ROESY, and variable temperature spectra [39–41]. The latter indicated that the norbornene, Phe and Gly NHs were involved in intramolecular hydrogen bonds [37,38,42,43]. A number of nOe/rOes were detected in the NOESY/ROESY spectra, as illustrated in Figure 6. In particular, one of the Pro δ-Hs of the Pro-Ala-Leu chain showed nOes to both the α- and β-hydrogens of the Phe-residue in the other chain, and one of the Leu δ-CH_3 groups showed an nOe to one of the Gly α-CH_2 hydrogens. Finally, both norbornene alkene hydrogens showed nOes to the Ala-β-CH_3 group, indicating that the peptide chains were folded back underneath the norbornene ring.

The conformational analysis of peptide **12** thus showed that the two tripeptide chains were approximately parallel to one another, held together by two intramolecular hydrogen bonds. This conformation approximates a simple antiparallel β-sheet (a β-hairpin [22]), though the intramolecular hydrogen-bonding pattern is slightly different to that usually found in β-sheets. The unusual hydrogen bonding may be due to distortions caused by the presence of the norbornene ring and/or the two proline residues which are unable to act as hydrogen bond donors. Work is currently in progress to investigate how far along a peptide chain a single norbornene residue can induce the formation this conformation, and to see whether the hydrogen bonding adopts a more 'normal' pattern further from the norbornene ring. The construction of more complex β-sheet structures is also under investigation.

In addition to reacting with the carboxylic acid group of a peptide, it was anticipated that



Scheme 4

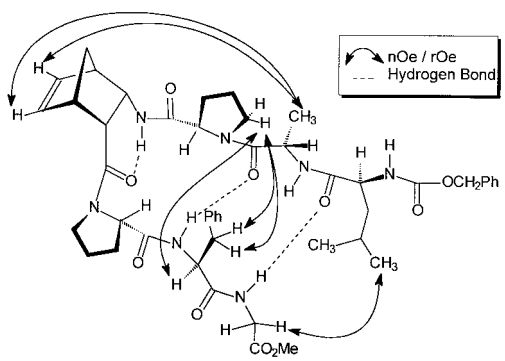


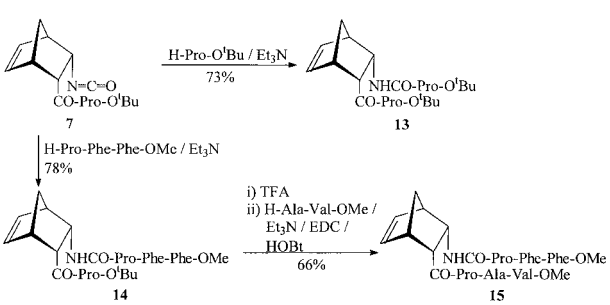
Figure 6 Hydrogen bonds and nOes observed for compound **12**.

isocyanate **7** would react with the amino group of a suitably protected peptide, leading to *pseudo*-peptides incorporating a urea unit [44]. In these *pseudo*-peptides, the two peptide chains attached to the norbornene ring would both be attached at their *N*-terminus, and the urea would have the effect of offsetting the two chains so that the carbonyl groups of one chain were opposite the NHs of the other chain. Thus, it appeared that this motif may be able to induce the formation of a parallel β-sheet (Figure 4).

In the event, treatment of isocyanate **7** with proline-*tert*-butyl ester led to *pseudo*-tripeptide **13** (Scheme 5) and reaction of the isocyanate with the tripeptide H-Pro-Phe-Phe-OMe gave *pseudo*-pentapeptide **14** which could be deprotected and coupled to H-Ala-Val-OMe to give *pseudo*-heptapeptide **15** [30].

The conformational analysis of *pseudo*-peptide **15** was not as straight-forward as that of peptide **12** [34,35]. The NH-stretching region of the infrared spectrum was concentration dependent, indicating that at high concentrations (ca. 30 mM) aggregation of the *pseudo*-peptide occurs in chloroform solution [37,38]. The presence of a urea NH in compound **15** also complicates the interpretation of the spectrum. However, at concentrations below 15 mM, the infrared spectrum was concentration independent and a hydrogen bonded NH stretch (at 3300 cm^{-1}) was still present in these spectra. The CD spectrum of *pseudo*-peptide **15** showed that the compound adopted a preferred conformation, but could not be further interpreted [36].

The ^{1}H-NMR spectrum of compound **15** (in $CDCl_3$) was complicated by the presence of two conformers (10:1 relative populations), however it was possible to determine the conformation of the major conformer using the same methods described above for peptide **12**. Variable temperature spectra suggested that the Ala and Val NHs were involved in



Scheme 5

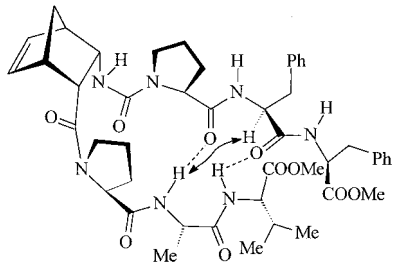
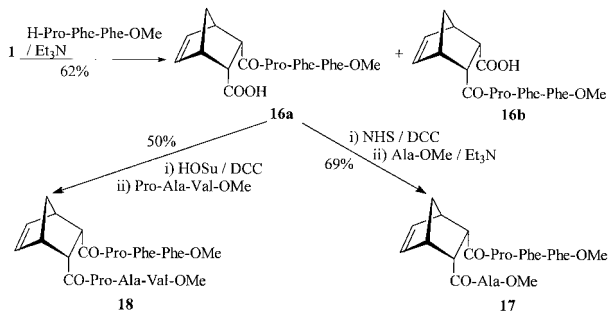


Figure 7 Hydrogen bonds and the interchain nOes observed for compound **15**.

intramolecular hydrogen bonds whilst the other NH groups were not hydrogen bonded. Only one interchain ROESY crosspeak was observed for compound **15**, between the NH of the alanine residue and the α-CH of the central phenylalanine residue. However, a large number of ROESY and NOESY cross peaks were observed between the NH, α-CH and β-CHs of adjacent residues along each chain which is consistent with the formation of a β-sheet type structure. In addition, all of the ^3J (NH-α-CH) coupling constants had magnitudes typical of those found in β-sheets [39–41,45–48]. Based on this evidence, the conformation of compound **15** was deduced to be as shown in Figure 7, with the two peptide chains running approximately parallel to one another and connected by two hydrogen bonds. As in the case of peptide **12** however, the hydrogen bonds are not those which would be formed in a traditional parallel β-sheet, an effect which may again be due to distortions caused by the norbornene ring, proline residues and urea units. Ongoing work aimed at the synthesis of longer *pseudo*-peptides analogous to compound **15** should clarify this issue.

SYNTHESIS OF PEPTIDES INCORPORATING A NORBORNENE DERIVED DICARBONYL UNIT

Another way in which it was anticipated that a norbornene unit could be used to link two peptide chains running in parallel directions was by building the peptides directly onto the acid and ester groups of compound **2**. This would give *pseudo*-peptides analogous to compound **15** but without the urea group. One effect of this would be to alter the juxtaposition of the two peptide chains so that the carbonyl groups of one chain were no longer located opposite the NHs of the other chain. This was anticipated to have an effect on the inter-chain



Scheme 6

hydrogen bonding and hence on the conformation adopted by the resulting *pseudo*-peptide.

Preliminary studies showed that is would be convenient to develop a convergent synthesis of this type of *pseudo*-peptide as shown in Scheme 6. Thus, reaction of anhydride **1** with H-Pro-Phe-Phe-OMe gave a 1:1 mixture of diastereomeric amido-acids **16a**,**b**. The reason for the lack of stereoselectivity in this ring-opening reaction is not clear, but acid **16a** could be separated from its diastereomer by flash chromatography. At this stage, the absolute configurations of the two acids was unknown, but reaction of acid **16a** with alanine methyl ester gave *pseudo*-pentapeptide **17** which was found to be crystalline and suitable for X-ray analysis. The resulting X-ray structure (Figure 8) allowed the absolute configuration of acid **16a** to be determined to be as shown in Scheme 6, and also gave useful conformational information (*vide infra*). Acid **16a** was subsequently coupled to H-Pro-Ala-Val-OMe, giving *pseudo*-heptapeptide **18**.

The X-ray structure of compound **17** provided valuable information on the solid state conformation of the compound. The Pro-Phe-Phe chain was found to form two consecutive β-turns (a nascent 3_{10} helix [49]) stabilized by two intramolecular hydrogen bonds between the two Phe NHs and the two carbonyls of the norbornene unit (Figure 9). β-Turns are classified according to the dihedral angles of the backbone atoms of the residues involved in the β-turn [50,51]. In the case of compound **17** however, the observed dihedral angles do not fit those of any of the standard subclasses of β-turns, probably due to the distortion imposed by the norbornene dicarbonyl unit. Although *pseudo*-peptide **17** adopts a well defined conformation in the solid state, NMR, infrared and CD spectroscopy all showed no evidence for the presence of β-turns or any other preferred conformation in chloroform solution.

Unlike *pseudo*-pentapeptide **17**, the longer peptide derivative **18** was not crystalline and so its conformation was studied exclusively in chloroform solution. The CD spectrum of compound **18** was suggestive of an ordered conformation [36], but could not be further interpreted due to the presence of the norbornene unit. The NH-stretching region of the infrared spectrum of the *pseudo*-peptide was concentration independent (30–4 mM) and showed a strong hydrogen bonded NH-stretch (3293 cm^{-1}) and only a weak non-hydrogen bonded NH-stretch (3420 cm^{-1}). The ^{1}H-NMR spectrum of of compound **18** showed the presence of two conformations in a 6:1 ratio. It was possible to assign all of the peaks in both conformations (using a combination of COSY and TOCSY experiments) and

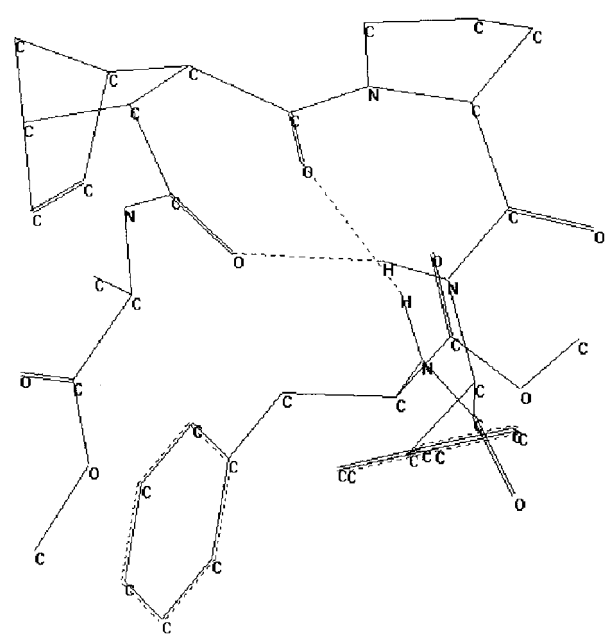


Figure 8 Representation of the X-ray structure of *pseudo*-peptide **17**.

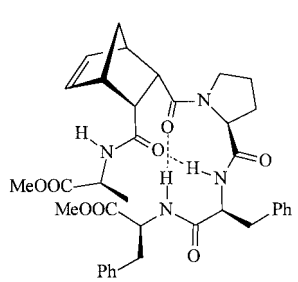


Figure 9 Representation of the solid state structure of *pseudo*-peptide **17**.

subsequently to determine the structure of both conformations from variable temperature and NOESY/ROESY experiments.

For the major conformation, the variable temperature spectra indicated that the NHs of the valine and *C*-terminal phenylalanine residues are involved in intramolecular hydrogen bonds. A number of informative nOes were observed in the ROESY spectrum of *pseudo*-peptide **18**, in particular between one of the norbornene alkene hydrogens and one of the β-hydrogens of the *C*-terminal phenylalanine residue which indicates that the Pro-Phe-Phe chain is folded under the norbornene ring. A similar close contact between these two protons is present in the X-ray structure of compound **17**. The aromatic protons of the *C*-terminal phenylalanine residue also show nOes to the α-hydrogen of the proline residue in the Pro-Ala-Val chain. All of the ^3J NH-αCH coupling constants have values between 6.9 and 8.8 Hz, which is consistent with β-turns involving all of the amino acid residues. Based on this evidence, the structure of the major conformation was determined to be as shown in Figure 10 in which both peptide chains adopt a β-turn conformation.

The minor conformation of *pseudo*-peptide **18** exhibited a different hydrogen bonding pattern, variable temperature NMR experiments indicated that both phenylalanine NHs were involved in intramolecular hydrogen bonds. This is the hydrogen bonding observed in the X-ray structure of compound **17** and immediately suggested that the two *pseudo*-peptides may have similar structures. Further evidence for the formation of a β-turn involving the Pro-Phe-Phe chain came from the ROESY spectrum which showed nOes between the β-hydrogens of one phenylalanine residue and the NH of the other phenylalanine, consistent with a turn structure. The ^3J NH-αCH coupling constants for the phenylalanine residues were >7.5 Hz, consistent

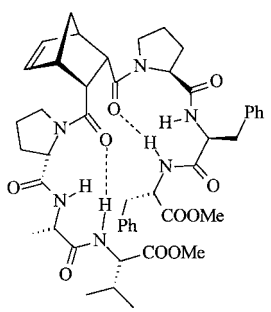


Figure 10 The conformation of the major conformer of *pseudo*-peptide **18**.

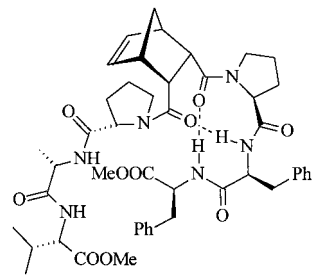


Figure 11 The conformation of the minor conformer of *pseudo*-peptide **18**.

with a β-turn conformation for this part of the molecule, whilst the corresponding coupling constants for the alanine and valine residues were <7.0 Hz, consistent with a random structure for that part of the molecule. Thus, the minor conformation of compound **18** was found to have a structure resembling the solid state structure of *pseudo*-peptide **17** as shown in Figure 11.

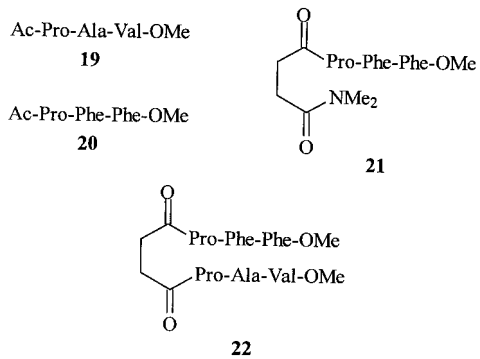
Comparison of the conformations adopted by *pseudo*-peptide **18** with that adopted by compound **15** clearly illustrates the importance of having the correct location of carbonyl and NH-groups to enable β-sheet formation. The two analogues are derived from the same amino acids and differ only by a single NH group, yet adopt completely different conformations.

The major conformation of *pseudo*-peptide **18** is not available to compound **17** which lacks one of the peptide chains. This may explain why compound **18** adopts well defined conformations in solution, but compound **17** does so only in the solid state. Subsequent to our work, the synthesis and conformational analysis of a variety of norbornene containing peptide derivatives analogous to compounds **17** and **18** has been reported, and again the formation of β-turn conformations was observed [52].

SYNTHESIS OF ANALOGUES OF THE PSEUDO-PEPTIDES

Pseudo-peptides **15**, **17**, and **18** all contain two phenylalanine residues and aromatic amino acids are often found within natural β-sheets [53,54]. It was thus possible that the formation of β-sheet and β-turn type structures in compounds **15**, **17**, and **18** was due to the nature of the amino acids rather than to the presence of the norbornene unit. To investigate this possibility, model compounds

19–22 were prepared. Compounds **19** and **20** were readily available by the acetylation of the corresponding tripeptide methyl esters, and compound **21** was prepared by reaction between H-Pro-Phe-Phe-OMe and *N*,*N*-dimethylsuccinamide. Compound **22** was prepared by the reaction between H-Pro-Phe-Phe-OMe and succinic anhydride, followed by coupling of the resulting acid to H-Pro-Ala-Val-OMe.

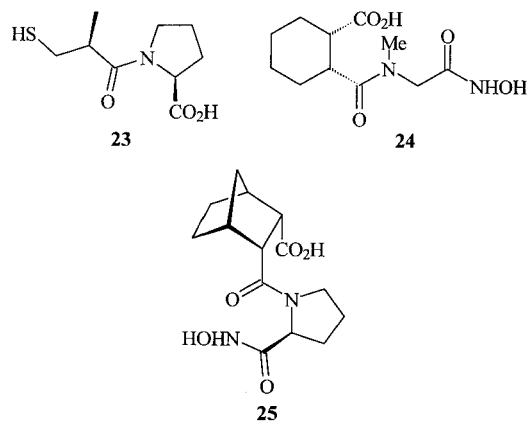


Conformational analysis of compounds **19–21** showed that they all adopted a disordered structure in chloroform solution. Thus, no evidence for intramolecular hydrogen bonding was observed in the ^{1}H-NMR spectra or in the infrared spectra of these compounds. Compound **22** was more complex to analyse, the infrared spectrum showed only non-hydrogen bonded NH-stretches at low concentration (2 mM), but hydrogen bonded NH-stretches were observed at higher concentrations and became dominant at 30 mM. Thus, compound **22** appears to be susceptible to aggregation in solution. The ^{1}H-NMR spectrum of compound **22** was also complicated, showing the presence of at least four conformations, and variable temperature spectra did not give a linear variation of the NH signals, indicative of conformational changes occurring as the temperature changes. It appears likely, that the conformations observed for this compound are due to *cis*/*trans* rotomers around the proline tertiary amide bonds [55], and not to the formation of any preferred secondary structure. It should be noted however, that *cis*/*trans* isomerism was not observed for the norbornene derivatives, for which the ROESY spectra clearly showed that only trans amide bonds were present.

The analysis of model compounds **19–22** showed that the conformations adopted by the norbornene containing peptides were not due to the nature of the amino acids from which they were constructed. In addition, compounds **21** and **22** showed that an unconstrained succinimide unit was not sufficient to induce secondary structure, rather a conformationally constrained norbornene unit in which the two peptide chains are forced to eclipse one another is required.

SYNTHESIS OF A CONFORMATIONALLY CONSTRAINED ANALOGUE OF AN ACE INHIBITOR

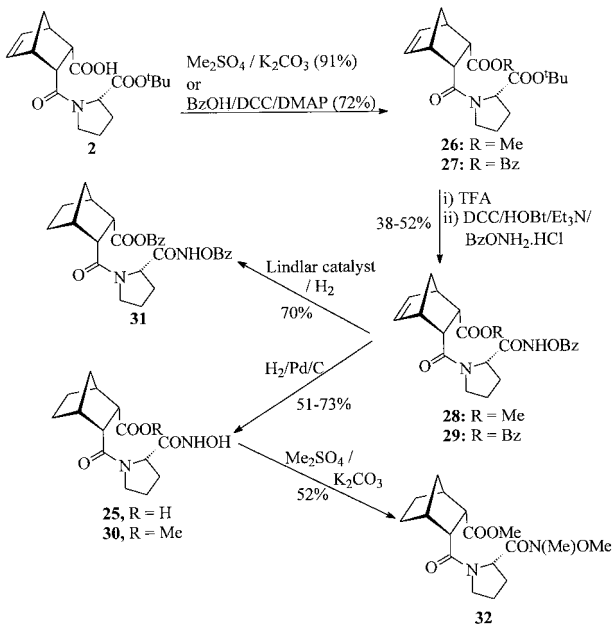
Angiotensin converting enzyme (ACE) is a zinc-metalloprotease which is involved in the final stage of the biosynthesis of angiotensin(II), a human vasoconstrictor [56]. Inhibition of the biosynthesis of angiotensin(II) has been shown to provide an effective method for the control of high blood pressure, a medical condition which is a causative agent of diseases such as heart disease, hypertension, renal failure and haemorrhage. Considerable pharmaceutical effort has thus been directed at the development of ACE inhibitors to reduce the concentration of angiotensin(II) *in vivo* and thus lower human blood pressure. The biological funtion of ACE is to cleave the two *C*-terminal amino acids (histidine and leucine) from a biologically inactive decapeptide (angiotensin(I)) which is the immediate precursor of angiotensin(II). Thus, ACE inhibitors tend to consist of a dipeptide (or peptidomimetic of a dipeptide unit) attached to a good zinc binding ligand. The dipeptide unit provides the specificity for ACE in the presence the multitude of other protease enzymes present *in vivo*, and the zinc binding ligand provides reversible inhibition of the enzyme.



Probably the best known commercial ACE inhibitor is captopril **23** [57,58], which consists of an Ala-Pro dipeptide analogue and uses a thiol as the zinc binding unit. Idrapril **24** is another ACE inhibitor and has been shown to exhibit a similar

potency to captopril both *in vitro* and *in vivo* [59]. Within idrapril, the dipeptide is mimicked by the Gly-cyclohexane unit, and a hydroxamic acid group is used to coordinate to the zinc atom of ACE [60]. Both captopril and idrapril are conformationally flexible molecules since there are a number of bonds about which rotation can occur and in idrapril there are a number of possible conformations for the cyclohexane ring. Idrapril has also been shown to exist in solution as an equilibrium mixture of *cis* and *trans* isomers about the tertiary amide bond [61]. Conformationally constrained analogues of these ACE inhibitors would be useful in mapping the biologically active conformations of the compounds and hence allowing more potent ACE inhibitors to be developed. Our desymmetrization methodology seemed to be appropriate for the synthesis of some conformationally constrained analogues of idrapril. In particular, we decided to undertake the synthesis of compound **25** in which two conformational constraints have been introduced: the sarcosine residue has been constrained by conversion to a proline residue, and the cyclohexane ring has been constrained to a boat conformation by conversion to a norbornane unit [62]. The latter constraint forces the two carbonyl groups attached to the cyclohexane ring to adopt conformations in which they are eclipsed to one another, rather than the staggered conformations which will dominate in the low energy conformations of idrapril [62]. Thus, the activity or lack of activity present in compound **25** may provide an indication of the importance of the location of these two carbonyl groups.

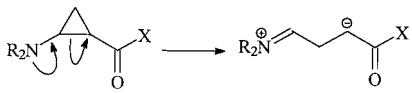
The synthesis of compound **25** and related protected compounds was achieved as shown in Scheme 7. Thus, amido acid **2** was esterified to give methyl ester **26** or benzyl ester **27**. Acidolysis of compounds **26** and **27** removed the *tert*-butyl esters, and coupling of the acids to *O*-benzyl hydroxylamine gave *O*-benzyl hydroxamates **28** and **29**. Attempts to optimize the chemical yield of this coupling reaction by formation of the *para*-nitrophenyl active ester derived from compound **26** and reaction of this with *O*-benzyl hydroxylamine gave the desired hydroxamate **28** in higher chemical yield, but as a 1:1 mixture of epimers at the proline stereocentre. Hydrogenation of compounds **28** and **29** led to the desired idrapril analogue **25** and its methyl ester **30** respectively. Hydrogenation of compound **29** using Lindlar catalyst [63] resulted



Scheme 7

in hydrogenation of the alkene whilst leaving the benzyl protecting groups untouched giving diprotected analogue **31**. Finally, methylation of compound **25** with dimethyl sulphate gave the fully methylated analogue **32**. NMR spectroscopy showed that compound **3** existed in chloroform as a slowly interconverting mixture of *cis*- and *trans*-isomers about the proline amide bond. This seems to be a common feature of all compounds which contain an *endo*-acid along with the proline residue on the norbornane skeleton. Esterification of the acid (as in compounds **28**-**32**) however, resulted in a single set of peaks being observed in the NMR spectra, indicating that rotation around the tertiary amide bond was now more rapid.

Compounds **25**, **30**, and **32** were tested (by British Biotech. Ltd.) for activity against a range of metalloproteinases. None of the compounds showed any activity against ACE suggesting that the two carbonyl groups were being forced into a conformation which is incompatible with the active site of the enzyme. The compounds did however, show low levels of inhibition (10–20% at 100 μmol concentration) against MMP-1, MMP-2 and MMP-3 which suggests that there is scope for the use of norbornane derivatives such as these in constructing other enzyme inhibitors.

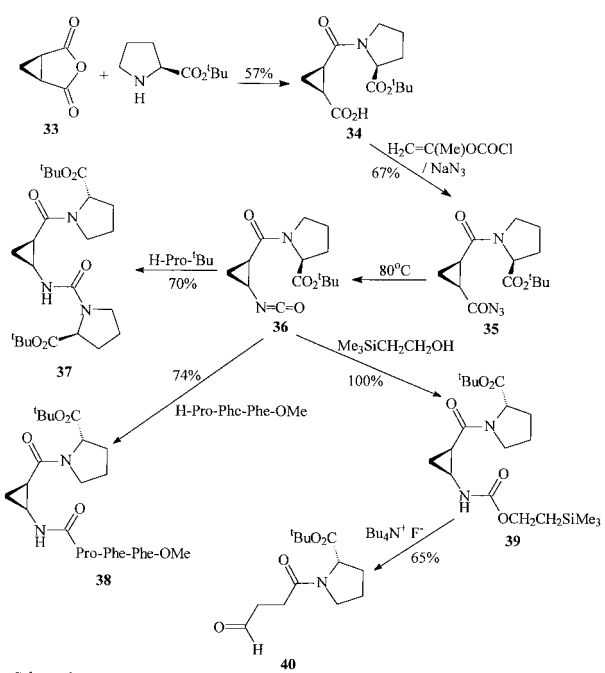


Scheme 8

SYNTHESIS OF CYCLOPROPANE-CONTAINING *PSEUDO*-PEPTIDES

The desymmetrization methodology is not limited to norbornene derived anhydride **1**, a wide range of cyclic anhydrides can be employed [24–26]. It appeared attractive to investigate the synthesis of cyclopropane containing peptide derivatives using this methodology since there is significant interest in the synthesis of cyclopropane derivatives of amino acids and peptides [8,64,65]. Most work to date has concentrated on α-aminocyclopropane carboxylic acid and its derivatives [66], rather than β-aminocyclopropane carboxylic acid which, like all cyclopropanes bearing electron withdrawing and electron donating substituents on adjacent ring positions, is prone to ring-opening [67–70] as shown in Scheme 8. Peptides derived from β-aminocyclopropane carboxylic acid should be stable however, due to delocalization of the nitrogen lone pair of electrons [71,72].

As it turned out, ring-opening of cyclopropane-1,2-dicarboxylic anhydride **33** with (S)-proline-*tert*-butyl ester gave a 3:1 ratio of diastereomeric amido acids from which the major isomer **34** could easily be obtained by trituration (Scheme 9). The stereochemistry of the major product was established to be as shown in Scheme 8 by X-ray crystallography (Figure 12). Conversion of acid **34** to acyl azide **35** and subsequently to isocyanate **36** proceeded by the same route as that developed for the norbornene derivatives. Isocyanate **36** reacted with the amino termini of amino acids and peptides to give *pseudo*-peptides **37** and **38**, respectively, thus demonstrating that *pseudo*-peptides containing both a *cis*-2-amino-cyclopropane-carboxylic acid and a urea group could be prepared by this methodology. As expected, it did not prove possible to hydrolyse isocyanate **36** to the corresponding amine, either directly or via urethane **39**. As soon as the free amino group was generated, it ring opened and underwent hydrolysis, giving aldehyde **40** as the isolated product.



Scheme 9

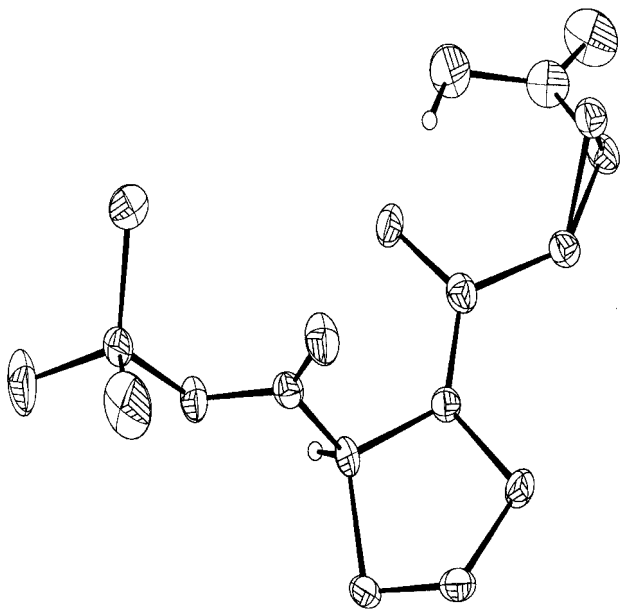


Figure 12 ORTEP diagram of the X-ray structure of compound **34**.

CONCLUSIONS

The work summarized in this review started with a project aimed at developing new methodology for the asymmetric synthesis of natural and unnatural products containing a cyclopentane ring. From this

purely synthetic start, the project developed and has allowed the synthesis of peptides and *pseudo*-peptides containing conformationally constrained β-amino acids which adopt conformations which mimic the β-sheet and β-turn conformations which are common in proteins. To date, we have only started to explore the possibilities in peptide design opened up by the ability to rapidly prepare conformationally constrained β-amino acids in a highly stereocontrolled manner and incorporate them into peptides composed of α-amino acids. Based on the results of the last 3 years, this is an area which has much to offer peptide chemistry in the 21st century.

Acknowledgements

The work described in this review was carried out by a talented group of students and postdoctoral fellows who are cited in the references. The author is grateful for funding from EPSRC and Peboc-Eastman UK Ltd., and to British Biotech. for carrying out the biological assays.

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