

Application of tandem Ugi multi-component reaction/ring closing metathesis to the synthesis of a conformationally restricted cyclic pentapeptide

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A conformationally restricted cyclic pentapeptide, containing an unsaturated 9-membered lactam as a semi-rigid scaffold, was prepared in a very convergent manner, through tandem Ugi reaction/ring closing metathesis.

Introduction

The interaction of peptides with receptors¹ or with other proteins² has a strong influence on several physiological processes. Therefore, small molecules have been used as antagonists of protein function in the pharmaceutical industry since its very beginning. The effectiveness of protein–receptor or protein–protein interaction is strongly dependent on the secondary and tertiary structure of the peptides. Thus, small oligopeptides characterized by the same recognition sequence, but lacking the conformational restrictions typical of the natural macromolecules, may be completely inactive. Among the most important secondary motifs, the reverse-turns (*e.g.* the β -turns, the hairpins and so on) play a particularly important role. For this reason various research groups have tried to design and synthesize reverse-turn mimetics.

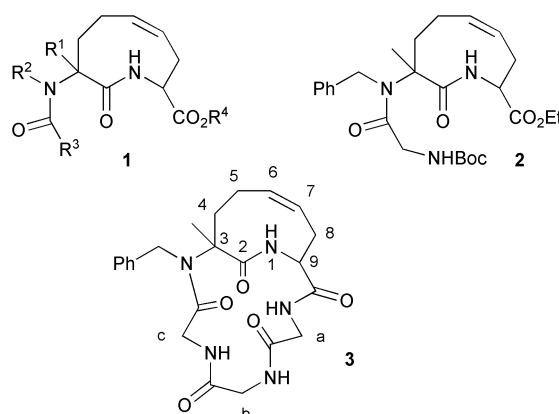
This has been successfully accomplished by using cyclic penta- or hexapeptides,^{3,4} obtained by assembling proteinogenic amino acids. However, turn mimics composed of only amino acids are not ideal, because compounds of this kind may have unfavourable bioavailability properties.²

Another strategy involves the attachment of small peptide chains to an unnatural rigid or semi-rigid template (scaffold). In some cases this template represents the reverse-turn itself (and has been defined as “internal reverse-turn mimetic”).⁵ In other cases, the rigid scaffold serves to force an attached cyclic peptide chain to adopt the desired reverse-turn conformation (and has been defined as an “external reverse-turn mimetic”).⁵ This latter strategy seems particularly promising. The fact that the scaffold is “unnatural” should grant it a higher metabolic stability, compared to the cyclic penta- or hexapeptides. On the other hand, the fact that the recognition sequence is not placed on the template itself (as for “internal reverse-turn scaffolds”)^{2,6,7} allows for a higher degree of freedom in testing several different oligopeptides or peptidomimetics as recognition motifs.

In the past, various conformationally restricted reverse-turn scaffolds have been designed and synthesized. Most of them were fused bicyclic^{8–10} lactams, but also bridged bicyclic,^{11,12} spirocyclic,¹³ or monocyclic (with ring sizes from 3 to 7)^{14–18} lactams have been explored. However, only a few reports dealt with mesocyclic compounds.^{19–21}

A drawback connected to most of the reverse turn scaffolds reported to date is that the synthetic approaches to them are poorly flexible. Even small variations of the substituents decorating the basic scaffold, which could be useful in order to finely tune the biological activity, require *de novo* syntheses. In the course of a project on the exploitation of isocyanide-based multi-

component reactions followed by secondary transformations^{22–26} in diversity-oriented synthesis,²⁷ we decided to design and synthesize a new family of “external” reverse-turn scaffolds characterized by an unsaturated 9-membered lactam (general formula **1**, Scheme 1). According to our plan, various diverse members of this family could be prepared convergently in two steps by coupling a multi component reaction (the Ugi 4CR) with a subsequent ring closing metathesis (RCM). In this way, not only could the scaffolds be synthesized in just 2 steps, but several different substituents could be placed at will, as R¹, R², and R³. Although coupling of Ugi MCR with RCM has also been recently described by other groups,^{17,28–31} the synthesis of 9-membered rings by this strategy was unprecedented. Also a related tandem Passerini/RCM was reported.³⁰ In a preliminary communication³² we have described the successful synthesis of a series of compounds of general formula **1** through this approach. Now we report full details on the synthesis of a particular member of this family of scaffolds (namely **2**) by two alternative synthetic routes, and its transformation into the first cyclic pentapeptides **3**. The conformations of both **2** and **3** will be also thoroughly discussed, on the basis of NMR data.



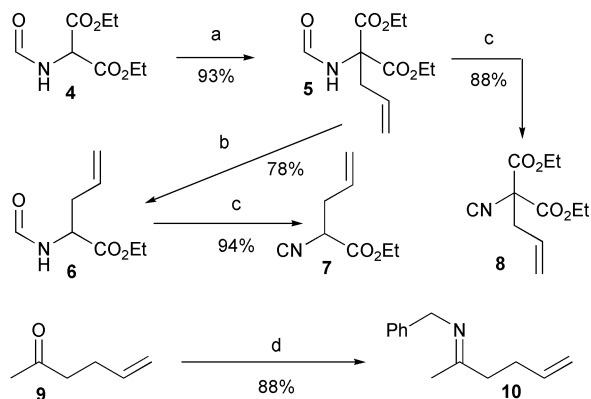
Scheme 1

Results and discussion

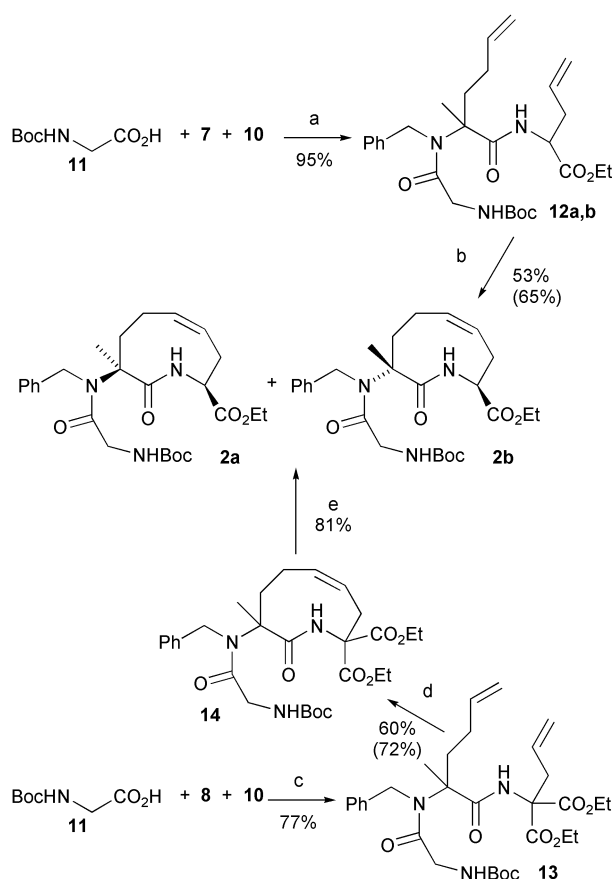
Synthesis

For the preparation of the required scaffold **2**, we first utilized the 2-step strategy already reported in our preliminary communication. It involved Ugi condensation of Boc-glycine **11**

with isocyanoacetate **7** and the preformed imine derived from 5-hexen-2-one and benzylamine, followed by RCM of the resulting diene **12**. The required isocyanide **7** was prepared, as a racemic mixture, in 4 high-yielding steps, starting from commercially available diethyl formamidomalonate, as described in Scheme 2. The Ugi condensation (Scheme 3) proceeded in high yield, but with no stereoselectivity at all, giving an inseparable mixture of the two diastereoisomers in a 1 : 1 ratio.



Scheme 2 Reagents and conditions: a) NaH, allyl-Br, r.t. b) 1. NaOH, EtOH; 2. Dioxane, reflux. c) POCl₃, Et₃N, -30 °C. d) BnNH₂, benzene, Dean-Stark.



Scheme 3 Reagents and conditions: a) EtOH, r.t. b) 4 mM in CH₂Cl₂, PhCH=Ru(PCy₃)₂Cl₂, reflux. c) EtOH, 65 °C. d) 6.5 mM in CH₂Cl₂, PhCH=Ru(PCy₃)₂Cl₂, reflux. e) 1. NaOH, EtOH; 2. Dioxane, reflux. Yields in parentheses are based upon recovered starting material.

More critical was the ensuing ring closing metathesis. There were no examples in the literature of RCM that gives 9-membered secondary lactams. It is well known that the analogous cyclization of secondary amides to afford 8-membered lactams by RCM is unfeasible.^{28,33} This behaviour has been

explained by the insufficient equilibrium concentration of the *syn* rotamer at the amide bond in the acyclic diene (only this rotamer is able to cyclize). It was suggested that, for the same reason, also secondary 9-membered lactams could not be obtained by RCM.²⁸ On the other hand, both 8-membered^{28,33,34} and 9-membered lactams²⁸ can be obtained starting from tertiary amides; in this case the relative abundance of the acyclic rotamers is expected to be closer to 1 : 1. However, while both secondary and tertiary unsaturated 8-membered lactams exist as the *syn* conformers, the previous report on the synthesis of tertiary unsaturated 9-membered lactams²⁸ does not give information on the actual conformational equilibrium between *syn* and *anti* rotamers in the cyclic compounds.

On the basis of preliminary computer aided analysis,³² we expected the *anti* rotamer at the amide to be more stable than the *syn* one in secondary 9-membered unsaturated lactams **1**.³⁵ Therefore, the *anti* rotamer of the acyclic diene should, in this case, undergo cyclization even faster than the *syn* one. This prediction was later confirmed by NMR conformational analysis on the metathesis products (*vide infra*).

The yield of RCM was relatively good when carried out with Grubbs' 1st generation catalyst in refluxing CH₂Cl₂, even when at a concentration not dramatically low (4 mM). It is worth noting that formation of a 10-membered lactam by RCM was reported to afford satisfactory yields only at much lower concentrations (0.8 mM).¹⁹ It was difficult to drive the reaction to completion, mainly because the catalyst tended to become deactivated after 24–48 hours (the colour of the solution turned from pink-orange to black). This may be caused, as previously suggested,^{29,36} by interaction of the catalyst with one of the polar amidic groups. The overall 53% yield was raised to 65% if based upon recovered starting material. We tried to optimize the reaction by changing the solvent in order to increase the reaction temperature. However, in refluxing benzene, dichloroethane, or THF, the catalyst was rapidly deactivated. At lower temperatures these solvents were less satisfactory than CH₂Cl₂ anyway. We also tested Grubbs' 2nd generation catalyst. The reaction was indeed faster, and it was possible now to drive it to near-completion. However, the relative percentage of intermolecular products was increased. An analogous outcome was recently reported by Creighton and Reitz.³⁴

The reaction was completely stereoselective with regard to the double bond, giving only *Z* compounds. The two diastereoisomers **2a,b** were easily separated from each other, but were contaminated by several acyclic dimeric by-products derived from intermolecular processes, easily identified as such in the ¹³C NMR-DEPT spectra by the presence of terminal double bonds. It is worth noting that, even if only the less encumbered double bond (the one derived from the ketone), is involved in dimerization, and only *E* double bonds are formed, 6 different diastereoisomeric acyclic dimers may be obtained! Thus, in order to obtain **2a** and **2b** in pure form, 2–3 chromatographies with different eluents were necessary.

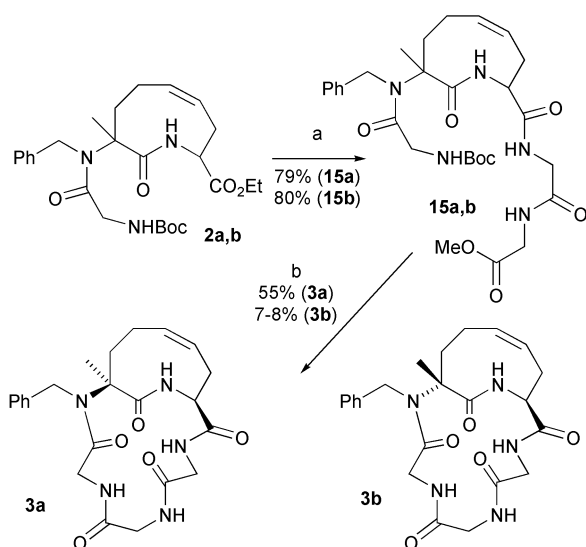
For this reason we decided to explore an alternative strategy, using this time, as the isocyanide component, the achiral malonate **8**. In this approach the second stereogenic centre was planned to be generated only after the RCM reaction, thus reducing the number of diastereoisomeric products and by-products, and therefore simplifying the purification process. The Ugi condensation with **8** was slower, as expected, and required a higher temperature. However, the yield was still high enough. On the other hand, the yield and conversion of RCM were remarkably better in this case. Interestingly, the catalyst seemed to be much more stable and the solution did not change colour even after 3–4 days. The percentage of intermolecular products was also lower, and this allowed us, for preparative purposes, to increase the concentration to 6.5 mM. Purification of the product was much easier as well, since we had now only one (racemic) product, and only 2 by-products (reasonably well separated from **14**) were detected by TLC. In this case, if only the

less encumbered double bond is involved in dimerization, and *E* double bonds are formed, only 2 diastereoisomeric dimers are expected. Compound **14** was converted into a 6 : 4 mixture of **2a,b** by saponification followed by decarboxylation.

The advantages of this alternative route are: a) The purification of RCM product **14** is much easier than that of RCM products **2a,b**. b) A higher concentration may be used in the RCM step. c) The decarboxylation is moderately stereoselective favouring the *cis* isomer **2a**, while from RCM of **12** a slightly higher percentage of the less wanted *trans* isomer **2b** is obtained. e) the overall yield of the pure *cis* compound **2a** from **5** is 24% instead of 20%; as discussed below, the *cis* scaffold is the most useful one.

With a good methodology for the rapid obtention of both **2a** and **2b** in hand, we went on to transform these scaffolds into cyclic peptides. Since **2a** and **2b** were obtained in racemic forms, we chose, as the first example, to attach an achiral dipetide formed by two additional glycine units. Although the cyclic peptide **3** is not very attractive from the point of view of its biological properties, it has allowed us to check the ease of macrocycle formation for the two different diastereoisomers and to do a first preliminary conformational analysis in the absence of additional biases derived from side-chains of more complex amino acids.

Both **2a** and **2b** were elaborated to the acyclic triglycine derivatives **15a,b**. Removal of the terminal protecting groups followed by cyclization in DMF-CH₂Cl₂ at 6 mM with HATU/collidine afforded the two diastereoisomeric cyclopeptides **3a** and **3b** (Scheme 4). In the case of the *cis* compound, the yield of **3a** was quite good (55%) and the compound could be obtained in high purity by silica gel chromatography, as evidenced by NMR and HPLC/MS analysis. On the other hand, formation of the *trans* adduct **3b** was much more troublesome. A crude product giving nearly a single spot in tlc was isolated in only 13% yield. Moreover, HPLC-MS analysis showed that, although the expected monomer **3b** was the main component, the 2 diastereoisomers of the cyclodimer were also present (38%), as well as a small amount of **3a** (8%). The actual yield of **3b** was therefore only 7–8%. This result is not unexpected, since conformational analysis of **2b** (*vide infra*) has indicated that the virtual dihedral angle between the nitrogen attached at C-3 and the carboxyl attached at C-9 as well as the distance between these two atoms is not favourable for cyclopeptide cyclization. Therefore, formation of **3b** is probably disfavoured both enthalpically and entropically. The presence of **3a** is probably due to traces of *cis* isomer not completely separated



Scheme 4 Reagents and conditions: a) 1. NaOH, EtOH, r.t.; 2. H-GlyGlyOMe, BOP, Et₃N, CH₂Cl₂. b) 1. LiOH, THF-H₂O; 2. TFA; 3. HATU, *sym*-collidine, DMF-CH₂Cl₂ (6 mM).

at the level of **2b**. Because of the difference in cyclization yield, about 1% of **2a** contaminating **2b** may justify the presence of 8% **3a** in crude **3b**.

Conformational analysis

The double bond configuration in metathesis adducts **2** and **14** as well as in their derivatives was demonstrated to be *Z* by the CH=CH vicinal coupling constants (which were around 9–10 Hz). In compounds **2a** and **2b** only one set of signals was detected by NMR at r.t. This means that only one of the two possible rotamers around the ring amide bond is present. This rotamer is in both cases the *anti* one, as demonstrated by the presence of a strong NOE between the NH and the *ortho* hydrogens of the benzyl group in **2a** and of a very strong NOE between the NH and the CH₃ bonded at C-3 in **2b**. These NOEs are obviously possible only for the *anti* rotamer and also allow us to determine without doubt the relative configuration of the two diastereoisomers.

From the coupling constants and NOEDIFF experiments it is also possible to get a fairly accurate idea of the preferred conformation of the 9-membered ring. In order to get some hints on the possible conformations of these tetrahydroazoninones we carried out an MM2 minimization on the parent compound with the software Chem3D (v. 4.5) from CSC, Cambridge (USA), looking for all local minima. These compounds were found to be not completely rigid. Four conformations with a comparable energy were found and three of them are depicted in Fig. 1. In the absence of substituents on the ring, conformations **16a** and **16b** are enantiomeric and therefore isoenergetic. The same applies for **17a,b** (only one of the two conformations is shown). The difference between **16** and **17** is the position of the double bond. In **17** it is on the same side of NH; in **16** it is on the opposite side.

In the following discussion, for the sake of clarity, we will consider the (9*S*) enantiomers of **2a** and **2b**, as depicted in Scheme 3. An examination of the NMR data indicates that, in both cases, a conformation analogous to **16a**, placing the CO₂Et in *pseudo*-equatorial (β) position, is actually preferred (see also Scheme 5). For example, in **2a**, there is a high $J_{1,9}$ of 9.3 Hz, which excludes conformation **16b**, with the CO₂Et in *pseudo*-axial position. The $J_{8,9}$ (3.9 and 7.2 Hz) and $J_{7,8}$ (6.7 and 9.0 Hz) are perfectly compatible with conformations **16**, but not with **17**. In **17** the dihedral angles related to $J_{8,9}$ should be 41° and 76°, whereas those related to $J_{7,8}$ should be 64° and 31° and so they are clearly not in agreement with the detected values. Moreover, NOEs between the ring NH and one of the H-8 and one of the H-5, and an NOE between H-9 and H-7 were detected. They are possible in **16**, but not in **17**. Also, other J values and NOEs are in agreement with the proposed conformation (see the Experimental section).

For the *trans* isomer **2b**, similar arguments demonstrate that also in this case the preferred conformation is **16a**, with the CO₂Et group in the *pseudo*-equatorial position.

The equilibrium between **16a** and **16b** is expected to be slow enough to allow detection of both conformations at r.t., as demonstrated in the case of malonate **14** (*vide infra*). The fact that only one set of signals is evident in the ¹H and ¹³C NMR means that the preference for the conformations having the CO₂Et in the *pseudo*-equatorial position should be quite high in both diastereoisomers.

It would be interesting to know which of the two diastereoisomers **2a** and **2b** is more stable. At first sight, the *trans* compound **2b** is expected to be more stable, since the bulkier nitrogen substituent is in the *pseudo*-equatorial position. On the other hand, the fact that the nitrogen is planar (while CH₃ is tetrahedral) and that a hydrogen bond may be formed between the carbonyl bonded to it and the ring NH could favour a *pseudo*-axial position of the nitrogen. The 1 : 1 ratio of the two conformers in malonate **14** (*vide infra*) and the only moderate

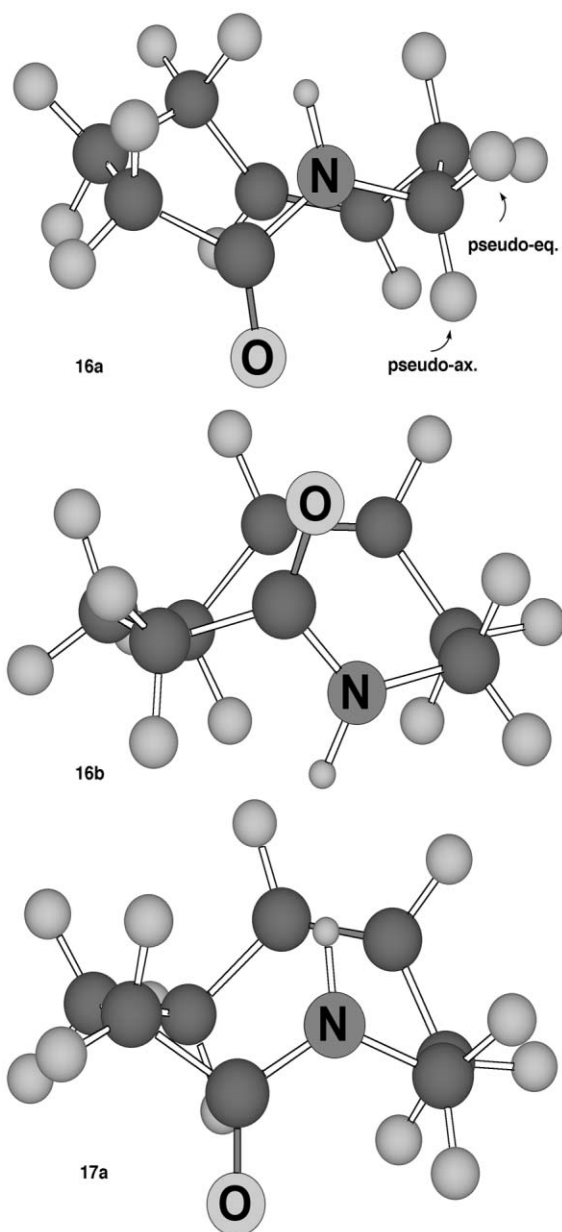
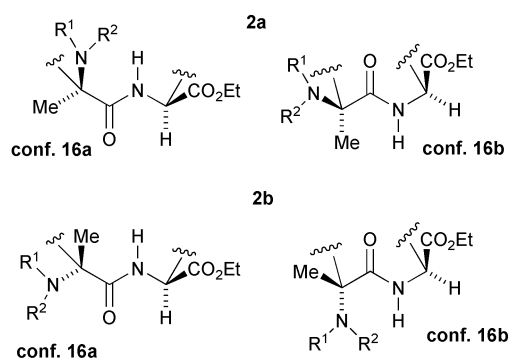


Fig. 1 Conformations of the parent tetrahydroazoniinones as minimized with Chem3D (MM2).

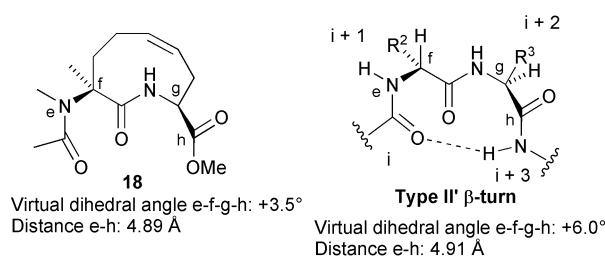


Scheme 5 Simplified alternative conformations of **2a** and **2b**.

stereoselectivity in the decarboxylation reaction suggest that the two stereoisomers should be quite close in energy.

Having established by NMR spectroscopy the approximate preferred conformation of the ring in **2a** and **2b** it was possible to check if they are well suited or not to act as external reverse-turn mimetics. Using Chem3D (MM2), we applied to **18** (a simplified analogue of **2a**) the ring conformation **16a** and carried

out minimization, noticing that addition of the substituents does not change considerably the various bond angles and bond lengths of the ring (Scheme 6). We then compared the minimized structure with various types of β -turns. β -Turns are usually defined by two φ and two ψ angles. However, our templates have been designed in order to function as “external” reverse-turn scaffolds. So the coincidence of all of these 4 angles is not mandatory. On the contrary, it is more important to compare the virtual dihedral angles between *NHAc*, *C-3*, *C-9* and *CO₂Me*, as well as the distances between *NHAc* and *CO₂Me* (Scheme 6). These values turned out to be quite similar to those determined for a type II' β -turn (obviously if we consider the enantiomer of **18** they will be similar to a type II β -turn). Kessler has shown that a II' β -turn may be ideal as an “external” template in preparing conformationally restricted RGD peptides to be employed as integrin ligands. In one of the most effective cyclic pentapeptides, this II' β -turn was assured by a D-Phe-L-Val unit bonded to the RGD recognition sequence.³⁷



Scheme 6 Conformational comparison of a simplified analogue of scaffold **2a** and type II' β -turn. The conformation of **18** was minimized with Chem3D and corresponds to conformation **16a** (Fig. 1). Virtual dihedral angles and distances a–d for the type II' β -turn were determined by applying standard bond length and angles and the dihedral angles typical of this β -turn.

Using the same approach we calculated, for the *trans* (3*R*,9*S*) isomer of **18**, a virtual dihedral angle and a distance of about $+115^\circ$ and 5.98 Å. While the angle is not far from the one typical of a type III' β -turn, the distance is 1.4–1.5 Å higher. From these data it is clear that *cis* scaffold **2a** is the most promising among the two isomers as an external reverse-turn mimetic. On the other hand, the low yield in the cyclization of *trans* **15b** may be explained by the excessive distance between the nitrogen at *C-3* and the carbonyl at *C-9* (which are the two attachment points for the tripeptide chain).

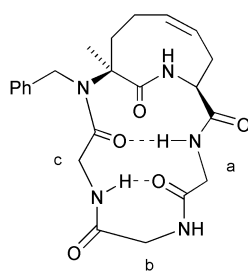
While **2a** and **2b** showed just one set of signals in the ¹H and ¹³C NMR spectra, the malonate **14** gave two sets of signals, in a 1 : 1 ratio. These are most probably due to a slow conformational equilibrium between **16a** and **16b**. An alternative hypothesis would be a conformational equilibrium between the two rotamers at the tertiary amide, but in our opinion this is unlikely because: a) we do not see the reason why this equilibrium should be present in **14** and not in **2a,b**; b) the equilibrium at the amide bond is not expected to influence in such a strong way the δ values of the ring protons and carbons; and finally, c) the NOEDIFF experiments showed, for example, that in one conformer the ring *NH* is close to the *CH₃* at *C-3*, while in the other one it is close to the aromatic protons. These NOEs are quite similar to those detected for **2a** and **2b**. All other NMR data (NOEs and *J* values) are in agreement with an equilibrium between the two conformations **16a** and **16b**. The fact that the ratio is 1 : 1 shows that the two groups at *C-3* (*CH₃* and the nitrogen substituent) occupy the *pseudo*-equatorial and the *pseudo*-axial positions equally, suggesting also a similar energy for **2a** and **2b**.

Finally, the *cis* cyclic peptide **3a** was carefully studied by NMR spectroscopy with the aid of NOEDIFF, COSY, and double resonance experiments, as well as measuring the difference in δ between *d*₆-DMSO and CDCl₃. A first important fact is that only one set of signals is present at r.t., either in *d*₆-DMSO or

in CDCl₃. As already stated above, it is very unlikely that the two ring conformations related to **16a** and **16b** may interconvert fast enough at r.t. to give single signals. Also the two *syn* and *anti* rotamers of the tertiary amide would surely give different signals. The presence of a single set of signals means therefore that the equilibrium strongly favours a single conformer at the ring and at the tertiary amide bond.

Once again the *J* values and the NOEs related to the ring hydrogens gave results quite similar to those collected for the acyclic scaffolds **2a** and **14**. It seems therefore that the conformation of the 9-membered lactam is not strongly modified by the attachment to the tripeptide.

The signals of the NH groups of the three glycines were assigned thanks to NOEDIFF experiments: there is a NOE between the NH of glycine b and the CH₂N of glycine a, and a (small) NOE between the NH of glycine c and the CH₂N of glycine b (Scheme 7). The presence of a NOE between one of the CH₂N groups of glycine c and the aromatic protons is consistent with an *anti* conformation around the tertiary amide bond.



3a

Scheme 7

On passing from d₆-DMSO to CDCl₃ (0.01 M) the behaviour of the four NH protons is strikingly different: while the ring NH and the NH of glycine b displayed a lower δ in CDCl₃ (−1.15 and −0.23 ppm respectively), for the NH of glycines c and a we observed a surprisingly higher δ in CDCl₃ than that in DMSO (+0.91 compared to +1.00 ppm).

In order to gain further insight, we also measured the temperature dependence of these NH chemical shifts (in DMSO).³⁸ The thermal coefficients (negative parts per billion per K) turned out to be 3.9 for ring NH, 4.9 for glycine b, 1.7 for glycine c, and −1.0 for glycine a. Moreover, the ring NH undergoes a very slow exchange with D₂O (in DMSO). It takes more than 1 hour for the signal to disappear completely at r.t., while the signals of the other three NH disappear in less than 10 minutes.

The negative thermal coefficient for glycine a, and the higher δ in CDCl₃ than in DMSO suggest its participation in a strong intramolecular hydrogen bond. Also for the NH of glycine c, the particularly high δ in CDCl₃ and the low thermal coefficient may indicate the involvement in an intramolecular hydrogen bond. Finally, all data (including NOEs) suggest an insignificant participation of NH of glycine b in intramolecular hydrogen bonds.

As for the ring NH, both the temperature coefficient, as well as the CDCl₃ – DMSO δ difference, point towards its non-involvement in hydrogen bonds. The very slow D₂O exchange is somehow in contradiction with this assumption. However, this may be simply due to the fact that this NH is rather buried inside the molecule. Actually, a hydrogen bond between this NH and the carbonyl of glycine c or b would indeed prevent the observed NOEs with H-5 and H-8.

A tentative favoured conformation that seems in agreement with the collected NMR data is shown in Scheme 7 and involves a strong β -turn between glycines c (acting as amino acid *i*) and a (acting as amino acid *i* + 3) (in this β -turn the amino acids *i* + 1 and *i* + 2 are those included in the scaffold) and a γ -turn between glycines c (*i* + 2) and a (*i*).

Conclusions

The cyclic peptidomimetic **3a**, including a conformationally biased scaffold, has been straightforwardly prepared in only 5 steps thanks to a tandem Ugi reaction–ring closing metathesis. NMR studies have allowed us to suggest a preferred conformation for the scaffolds **2a,b** and for the cyclic compound **3a**. The formation of the *trans* cyclic peptide **3b** in only low yield confirms that the corresponding scaffold is not a good external reverse-turn mimetic. On the other hand, the *cis* scaffold **2a** mimics well a type II' β -turn and appears to be well suited for the synthesis of conformationally biased cyclopeptides or cyclopeptidomimetics. The promising properties of **2a** have been demonstrated by the good yield achieved in macrocyclization, by the fact that the macrocyclic peptide **3a** seems to be conformationally defined, and by the probable presence of strong intramolecular hydrogen bonds corresponding to β - and γ -turns.

Although all the compounds described in this work are racemic, and GGG is not a biologically relevant recognition sequence, these results open the way to the preparation of other cyclic peptides including enantiomerically pure scaffolds **1** and more useful tripeptide sequences (such as the RGD one) attached to it. Studies directed toward this goal are in progress in our laboratory.

Experimental

NMR spectra were taken at 200 or 300 MHz (¹H), and 50 or 75 MHz (¹³C), using as internal standard: TMS for ¹H NMR in CDCl₃, the central peak of DMSO (at 2.506 ppm) for ¹H NMR in d₆-DMSO, the central peak of CDCl₃ (at 77.02 ppm) for ¹³C NMR in CDCl₃, the central peak of DMSO (at 39.429 ppm) for ¹³C NMR in d₆-DMSO. Chemical shifts are reported in ppm (δ scale), coupling constants are reported in hertz. Peak assignment in ¹H NMR spectra was also made with the aid of double resonance and COSY experiments. In some cases coupling constants were not directly determined from normal spectra, but with the aid of double resonance or D₂O exchange experiments. In these cases they are indicated with a * symbol. In AB systems, the proton A is considered downfield and B upfield. Peak assignment in ¹³C spectra was made with the aid of DEPT experiments.

GC-MS were carried out on an HP-5971A instrument, using an HP-1 column (12 m long, 0.2 mm wide), electron impact at 70 eV, and a mass temperature of about 170 °C. Only *m/z* > 33 were detected. All analyses were performed with a constant He flow of 0.9 mL min^{−1}, and (unless otherwise stated) with initial temperature of 100 °C, initial time 2 min, rate 20 °C min^{−1}, final temperature 260 °C, final time 4 min, injection temperature 250 °C, detector temperature 280 °C. *R_f* are in min.

HPLC-MS were carried out with an Agilent 1100 LC/MSD Trap SL instrument (electrospray ion trap analysis) with a C18 reverse phase Polarity column (Waters Corporation, MA, USA). In all cases, before introducing the eluent in the MS, a detection at 220 nm was performed using a diode array detector integrated in the system. The MS electrospray ion source parameters were set to maximize, from time to time, the interesting *m/z* ratios.

IR spectra were measured with a Perkin–Elmer 881 instrument as CHCl₃ solutions. Melting points were measured on a Büchi 535 apparatus and are uncorrected. TLC analyses were carried out on silica gel plates and developed with I₂ vapour. *R_f* values were measured after an elution of 7–9 cm. Chromatographies were carried out on 220–400 mesh silica gel using the “flash” methodology. Petroleum ether (40–60 °C) is abbreviated as PE. In extractive work-up, aqueous solutions were always reextracted thrice with the appropriate organic solvent. Organic extracts were washed with brine, dried over Na₂SO₄ and filtered before evaporation of the solvent under reduced pressure. Dry solvents were purchased from Fluka. All reactions

employing dry solvents were carried out under a nitrogen (or argon when specified) atmosphere. The purity of all compounds was established by TLC, ^1H and ^{13}C NMR, and by GC-MS or HPLC or HPLC-MS. Diethyl formamidomalonate, 5-hexen-2-one, Boc-glycine, sodium hydride and Grubbs' catalyst were all purchased from Fluka or Aldrich and used as such.

Diethyl (allyl)(formamido)malonate **5**

Sodium hydride (60% in mineral oil) (1.652 g, 41.31 mmol) was suspended in dry dimethylformamide (DMF) (40 mL) and cooled to 0 °C. A solution of diethyl formamidomalonate (7.994 g, 39.34) in dry DMF (20 mL) was slowly added over 5 min. After 10 min, the cooling bath was removed and the mixture stirred for 30 min at r.t. Allyl bromide (3.57 mL, 4.997 g, 41.31 mmol) was then added *via* a syringe. After stirring for 3 h, and cooling in an ice bath, the reaction was quenched with saturated aqueous NH_4Cl (100 mL). The mixture was extracted with Et_2O (70 mL + 50 mL) and with AcOEt (2 × 50 mL). The united organic extracts were washed with H_2O and evaporated to give an oil. It was taken up with CH_2Cl_2 and *n*-octane and evaporated again. After removing the solvent overnight at 8×10^{-2} mbar, a solid (9.92 g) was obtained. Trituration with *n*-pentane gave pure **5** as a white solid (8.904 g, 93%). R_f 0.57 (PE–AcOEt 1 : 1); mp 64.5–65.8 °C. Found: C, 54.45; H, 7.0; N, 5.7%. $\text{C}_{11}\text{H}_{17}\text{NO}_5$ requires: C, 54.31; H, 7.04; N, 5.76%; GC-MS R_t 5.48; m/z 243 (M^+ , 1.8), 198 (28.2), 174 (25.7), 170 (47.1), 151 (3.4), 146 (5.9), (44.1), 124 (80.1), 123 (7.1), 118 (6.6), 114 (7.3), 112 (6.2), 97 (8.2), 96 (43.4), 74 (5.6), 69 (13.8), 68 (100.0), 67 (11.0), 56 (8.2), 47 (16.2), 43 (9.4), 42 (14.9), 41 (65.7), 39 (19.0). ^1H NMR (CDCl_3 , 200 MHz): δ 8.17 [1 H, s, CHO]; 6.93 [1 H, s, NH]; 5.58 [1 H, ddt, $\text{CH}=\text{CH}_2$, J_d 9.4, 17.2, J_t 7.4]; 5.27–5.08 [2 H, m, $\text{CH}_2=\text{CH}$]; 4.27 [4 H, q, CH_2CH_3 , J 7.1]; 3.10 [2 H, d, $\text{CH}_2\text{CH}=\text{CH}_2$, J 7.4]; 1.27 [6 H, t, CH_3 , J 7.1].

Ethyl (allyl)(formamido)acetate **6**

A solution of diethyl (allyl)(formamido)malonate **5** (8.33 g, 34.24 mmol) in 96% EtOH (300 mL), was treated, at r.t., with 6 M aqueous NaOH (6.85 mL, 41.09 mmol) and stirred for 140 min. Then a mixture of 32% HCl (2.69 mL, 27.39 mmol) and 96% EtOH (10 mL) was added. After evaporation of nearly all the EtOH, the residue was taken up with 0.5 M citric acid (20 mL), saturated NaCl (50 mL) and Et_2O (100 mL). The phases were separated and the aqueous one re-extracted twice with Et_2O and twice with AcOEt. The organic phases gave, on evaporation, a white solid (6.876 g). It was taken up in 1,4-dioxane and refluxed for 6 h. Evaporation gave an oil that was chromatographed on 90 g of silica gel (PE–AcOEt 1 : 1 to 4 : 6) to give pure **6** as a slightly yellow oil (4.629 g, 79%). R_f 0.62 (PE–AcOEt 1 : 1 + 5% AcOH). Found: C, 56.1; H, 7.75; N, 7.9%. $\text{C}_8\text{H}_{13}\text{NO}_3$ requires: C, 56.13; H, 7.65; N, 8.18%; GC-MS R_t 3.54; m/z : 171 (M^+ , 1.5), 130 (59.1), 126 (45.2), 125 (16.3), 102 (16.4), 98 (100.0), 97 (12.9), 81 (9.6), 74 (86.4), 71 (6.4), 70 (44.4), 68 (8.7), 54 (6.0), 53 (6.7), 47 (5.6), 46 (21.4), 43 (23.3), 42 (12.0), 41 (19.5), 39 (15.7). ^1H NMR (CDCl_3 , 200 MHz): δ 8.21 [1 H, s, CHO]; 6.20 [1 H, broad s, NH]; 5.69 [1 H, ddt, $\text{CH}=\text{CH}_2$, J_d 9.0, 17.6, J_t 7.1]; 5.25–5.08 [2 H, m, $\text{CH}_2=\text{CH}$]; 4.76 [1 H, dt, CHNH, J_d 8.0, J_t 6.0]; 4.32–4.12 [2 H, m, CH_2CH_3]; 2.73–2.46 [2 H, m, $\text{CH}_2\text{CH}=\text{CH}_2$]; 1.30 [3 H, t, CH_3 , J 7.2].

Ethyl (allyl)(isocyano)acetate **7**

A solution of **6** (2.983 g, 17.4 mmol) in dry CH_2Cl_2 (50 mL) was cooled to –30 °C and treated with Et_3N (8.2 mL, 59.4 mmol) and POCl_3 (1.75 mL, 19.2 mmol). After 25 min the reaction was quenched with saturated aqueous NaHCO_3 (80 mL). The mixture was allowed to warm to r.t., extracted with Et_2O , and evaporated to dryness. Chromatography (PE– Et_2O 8 : 2) gave pure **7** as a colourless oil (2.510 g, 94%). R_f 0.54 (PE– Et_2O 6 : 4). Found: C, 62.45; H, 7.3; N, 9.0%. $\text{C}_8\text{H}_{11}\text{NO}_2$ requires: C,

62.73; H, 7.24; N, 9.14%; GC-MS R_t 1.55; m/z : 125 (M^+ – 28, 16.8), 124 (3.9), 108 (28.4), 107 (20.2), 98 (28.4), 97 (28.0), 85 (8.3), 84 (20.0), 81 (27.2), 80 (74.8), 79 (27.7), 70 (22.6), 69 (19.0), 68 (10.1), 67 (8.5), 57 (7.0), 56 (12.9), 55 (21.1), 54 (88.5), 53 (100.0), 52 (25.2), 51 (17.1), 50 (7.3), 45 (6.3), 43 (13.6), 42 (30.5), 41 (38.7), 39 (71.5). ^1H NMR (CDCl_3 , 200 MHz): δ 5.81 [1 H, ddt, $\text{CH}=\text{CH}_2$, J_d 9.8, 17.2, J_t 7.2]; 5.32–5.19 [2 H, m, $\text{CH}_2=\text{CH}$]; 4.28 [2 H, q, CH_2CH_3 , J 7.2]; 2.76–2.50 [2 H, m, $\text{CH}_2\text{CH}=\text{CH}_2$]; 1.32 [3 H, t, CH_3CH_2 , J 7.2]. IR: ν_{max} 2151, 1754, 1433, 1371, 1331, 1270, 1242, 1190, 1020, 925 cm^{-1} .

Diethyl (allyl)(isocyano)malonate **8**

Prepared from **5** (6.386 g, 26.25 mmol) following the same procedure employed for **7**. Yield after chromatography: 5.192 g (88%) of a colourless oil that had some tendency to darken on standing, R_f 0.56 (PE–AcOEt 8 : 2). Found: C, 58.8; H, 6.9; N, 6.0%. $\text{C}_{11}\text{H}_{15}\text{NO}_4$ requires: C, 58.66; H, 6.71; N, 6.22%; GC-MS R_t 4.04; m/z 226 (M^+ + 1, 0.1), 199 (M^+ – 26, 1.1), 198 (1.0), 197 (2.7), 169 (64.5), 153 (15.8), 152 (21.9), 151 (100.0), 134 (9.1), 133 (73.9), 129 (8.1), 125 (27.5), 124 (61.6), 123 (10.7), 111 (35.9), 110 (5.0), 108 (16.4), 107 (17.1), 106 (28.9), 105 (16.7), 101 (8.0), 99 (5.7), 98 (9.8), 97 (19.2), 96 (32.2), 81 (24.3), 89 (68.5), 79 (45.5), 78 (48.7), 69 (45.0), 68 (75.7), 67 (22.7), 57 (67.0), 54 (43.6), 53 (71.5), 52 (60.3), 41 (60.4), 39 (44.3). ^1H NMR (CDCl_3 , 200 MHz): δ 5.79 [1 H, ddt, $\text{CH}=\text{CH}_2$, J_d 9.8, 17.2, J_t 7.2]; 5.35–5.20 [2 H, m, $\text{CH}_2=\text{CH}$]; 4.32 [4 H, q, CH_2CH_3 , J 7.1]; 2.90 [2 H, d, $\text{CH}_2\text{CH}=\text{CH}_2$, J 7.0]; 1.32 [3 H, t, CH_3CH_2 , J 7.1]. IR: ν_{max} 2142, 1752, 1368, 1298, 1192, 1147, 1095, 1036, 924 cm^{-1} .

N-(Hex-5-en-2-ylidene)(phenyl)methanamine **10**

A solution of 5-hexenone (10.16 mL, 87.73 mmol) and benzylamine (8.547 g, 79.76 mmol) in benzene was refluxed in a Dean–Stark apparatus for 4 h. Evaporation of benzene at 16 mbar, followed by distillation at 0.55 mbar, afforded **10** (pure by GC-MS) as a colourless liquid (bp 85–90 °C), which tended to darken if left in the light (13.20 g, 88%). NMR spectroscopy indicated a *trans* : *cis* ratio of 3.9 : 1. GC-MS (injection temperature: 200 °C, initial temperature: 45 °C, initial time: 2 min, rate: 20 °C min^{-1}); R_t 7.97; m/z 187 (M^+ , 5.1), 186 (15.0), 172 (4.5), 104 (19.6), 91 (100.0), 65 (13.6), 42 (5.9), 41 (4.9), 39 (5.5). ^1H NMR (200 MHz): δ 7.33 (*cis*), 7.31 (*trans*) [5 H, s, arom.]; 5.98–5.70 [1 H, m, $\text{CH}=\text{CH}_2$]; 5.14–4.93 [2 H, m, $\text{CH}=\text{CH}_2$]; 4.52 (*cis*), 4.49 (*trans*) [2 H, s, CH_2Ph]; 2.50–2.27 [4 H, m, $\text{CH}_2\text{CH}_2\text{CH}=\text{CH}_2$]; 2.09 (*cis*), 1.91 (*trans*) [3 H, s, CH_3].

Ugi adducts **12a,b**

A solution of isocyanide **7** (1.784 g, 11.66 mmol) in absolute EtOH (12 mL), was treated at room temperature with imine **10** (2.609 g, 13.93 mmol) and with Boc-glycine **11** (2.442 g, 13.94 mmol). After stirring under N_2 for 48 h, the solvent was evaporated, and the crude product chromatographed (PE–AcOEt 1 : 1 to 4 : 6) to give a pure 1 : 1 mixture of **12a,b** as a foam (5.714 g, 95%). Found: C, 64.9; H, 8.1; N, 7.95%. $\text{C}_{28}\text{H}_{41}\text{N}_3\text{O}_6$ requires: C, 65.22; H, 8.01; N, 8.15%. ^1H NMR (CDCl_3 , 200 MHz): δ 7.53–7.24 [5 H, m, arom.]; 6.35 [0.5 H, d, NH, J 7.3]; 6.32 [0.5 H, d, NH, J 7.3]; 5.84–5.68 [2 H, m, $\text{CH}=\text{CH}_2$]; 5.43 [1 H, broad s, NHBoc]; 5.20–4.90 [4 H, m, $\text{CH}=\text{CH}_2$]; 4.73–4.54 [3 H, m, CHN and CH_2Ph]; 4.30–4.12 [2 H, m, CH_2CH_3]; 4.03–3.91 [1.5 H, CH_2NHBoc]; 3.84 [0.5 H, dd, CHNHBoc, J 4.0, 17.2]; 2.70–2.48 [2 H, m, allylic CH_2]; 2.30–1.70 [4 H, m, allylic CH_2 and $\text{CH}_2\text{CH}_2\text{CH}=\text{CH}_2$]; 1.45 and 1.44 [3 H, 2 s, CH_3]; 1.40 [9 H, s, (CH_3) $_3\text{C}$]; 1.284 [1.5 H, t, CH_3CH_2 , J 7.2]; 1.280 [1.5 H, t, CH_3CH_2 , J 7.2]. ^{13}C NMR (50 MHz, CDCl_3) (the number of diastereoisomers is given in brackets): δ 173.23 (1d), 173.01 (1d), 171.84 (2d), 169.94 (2d) [C=O]; 155.65 (2d) [urethane C=O]; 137.51 (2d) [quat. arom.]; 137.38 (2d), 132.51 (2d) [CH=CH $_2$]; 129.19 (2d), 127.61 (2d), 125.96 (2d) [arom. CH]; 119.04 (2d), 115.21 (2d) [CH=CH $_2$]; 79.54

(2d) [C(CH₃)₃]; 65.66 (1d), 65.59 (1d) [quat. C–N]; 61.48 (2d) [CH₂CH₃]; 51.74 (1d), 51.70 (1d) [CHN]; 47.58 (1d), 47.35 (1d) [CH₂Ph]; 43.36 (2d) [CH₂N]; 36.50 (1d), 36.43 (1d), 35.32 (1d), 34.93 (1d) [allylic CH₂]; 28.32 (2d) [C(CH₃)₃ + CH₂CH₂CH=]; 21.45 (1d), 20.99 (1d) [CH₃]; 14.20 (2d) [CH₃CH₂].

Ugi adduct 13

A solution of isocyanide **8** (3.869 g, 17.18 mmol) in absolute EtOH (17 mL), was treated at room temperature with imine **10** (2.0875 g, 11.15 mmol) and with Boc-glycine **11** (2.000 g, 11.41 mmol). After stirring under N₂ at 65 °C for 11 h, and at r.t. for 48 h, the solvent was evaporated, and the crude product chromatographed (PE–AcOEt 6 : 4 to 1 : 1) to give pure **13** as a foam (5.130 g, 77%). Found: C, 63.05; H, 7.8; N, 7.05%. C₃₁H₄₅N₃O₈ requires: C, 63.35; H, 7.72; N, 7.15%. ¹H NMR (CDCl₃, 200 MHz): 7.52–7.22 [5 H, m, arom.]; 7.05 [1 H, s, NH]; 5.92–5.62 [2 H, m, CH=CH₂]; 5.41 [1 H, broad s, NHBoc]; 5.24–4.91 [4 H, m, CH=CH₂]; 4.60 and 4.56 [2 H, AB system, CH₂Ph, *J* 19.4]; 4.30–4.15 [4 H, m, CH₂CH₃]; 4.02 and 3.80 [2 H, AB part of ABX system, CH₂NHBoc, *J*_{AB} 17.2, *J*_{AX} 4.4, *J*_{BX} 4.0]; 3.10 [2 H, d, C–CH₂CH=, *J* 7.4]; 2.37–1.70 [4 H, m, CH₂CH₂CH=]; 1.45 [3 H, s, CH₃]; 1.40 [9 H, s, C(CH₃)₃]; 1.25 and 1.23 [6 H, 2 t, CH₃CH₂, *J* 7.2]. ¹³C NMR (CDCl₃, 50 MHz): δ 172.11, 169.78, 167.82, 167.71 [C=O]; 155.61 [urethane C=O]; 137.53 [quat. arom.]; 137.40, 132.05 [CH=CH₂]; 129.19, 127.50, 125.92 [arom. CH]; 119.53, 115.28 [CH=CH₂]; 79.56 [C(CH₃)₃]; 66.30, 65.39 [quat. CN]; 62.48, 62.38 [CH₂CH₃]; 47.64 [CH₂Ph]; 43.31 [CH₂NHBoc]; 36.97, 34.99 [allylic CH₂]; 28.34 [C(CH₃)₃]; 28.20 [CH₂CH₂CH=]; 21.36, 14.02 [CH₃].

(*Z*)-*rac*-3-(((*N*-Benzyl-*t*-butoxycarbonyl)amino)acetamido)-9,9-bis(ethoxycarbonyl)-3-methyl-4,5,8,9-tetrahydro-1*H*-azonin-2(3*H*)-one 14

A solution of diene **13** (2.250 g, 3.83 mmol) in dry CH₂Cl₂ (600 mL) was placed under argon, and treated with benzyldiene bis(tricyclohexylphosphine)ruthenium dichloride (393 mg, 0.478 mmol). In order to ensure removal of all oxygen present, a series of vacuum/argon cycles was carried out. The orange solution was refluxed for 48 h. Et₃N (1 mL) was added, and most of the solvent was evaporated, until about 100 mL were left. This solution was introduced in a column of silica gel packed with CH₂Cl₂. Elution with CH₂Cl₂–AcOEt–EtOH–Et₃N 74 : 20 : 5 : 1 to 69 : 25 : 5 : 1 gave a black crude product containing both substrate and products (2.362 g). It was chromatographed with AcOEt–PE–EtOH 34.5 : 64.5 : 0.5 to 64.5 : 34.5 : 0.5 to give pure **14** as a grey foam (1.278 g, 60%). Also 382 mg of starting material (17%) were recovered. Yield based on recovered starting material was 72%. Apart from **14** and the substrate **13**, the crude product showed the presence of 2 impurities (faster eluting than **14** in PE–AcOEt). They were both intermolecular dimers, characterized by the presence of terminal double bonds (clearly detected with ¹³C/DEPT NMR spectroscopy). *R*_f 0.18 (AcOEt–PE 6 : 4), 0.74 (AcOEt–PE 9 : 1). Found: C, 62.2; H, 7.4; N, 7.45%. C₂₉H₄₁N₃O₈ requires: C, 62.24; H, 7.38; N, 7.51%. ¹H NMR (CDCl₃, 300 MHz) (the spectrum shows 2 distinct conformers in a 1 : 1 ratio): δ 7.52–7.22 [5 H, m, arom.]; 6.89, 6.74 [1 H, 2 s, NH of 2 conf.]; 5.74–5.55 [1 H, m, *H*-6 of 2 conf.]; 5.52 [1 H, broad s, NHBoc]; 5.55–5.42 [0.5 H, m, *H*-7 of 1 conf.]; 5.14 [0.5 H, dt, *H*-7 of 1 conf., *J*_d 7.2, *J*_t 10.3]; 4.62 and 4.55 [1 H, slightly broad AB system, CH₂Ph of 1 conf., *J*_{AB} 17.1]; 4.50 [1 H, s, CH₂Ph of 1 conf.]; 4.46–4.22 [4 H, m, CH₂CH₃]; 4.15–4.01 [1 H, m, CHHNHBoc of 2 conf.]; 3.85 [0.5 H, broad d, CHHNHBoc of 1 conf., *J* 15.9]; 3.50 [0.5 H, slightly broad dd, CHHNHBoc of 1 conf., *J* 1.9, 17.0]; 3.39 [0.5 H, t, *pseudo*-eq. *H*-8 of 1 conf., *J* 13.8]; 3.35 [0.5 H, t, *pseudo*-eq. *H*-8 of 1 conf., *J* 13.8]; 2.59 [0.5 H, dd, *pseudo*-ax. *H*-8 of 1 conf., *J* 6.9, 13.8]; 2.50 [0.5 H, dd, *pseudo*-ax. *H*-8 of 1 conf., *J* 6.5, 13.8]; 2.45–2.34 [0.5 H, m, *H*-5 of 1 conf.]; 2.10–1.80 [3.5 H, m, *H*-5, *H*-4]; 1.67 [1.5 H, s, CH₃ of 1 conf.]; 1.41 [6 H, s, CH₃ of 1

conf. + C(CH₃)₃ of 1 conf.]; 1.37 [4.5 H, s, C(CH₃)₃ of 1 conf.]; 1.36–1.23 [6 H, m, CH₃CH₂]. From COSY we could establish that the signal at δ 5.14 is coupled with those at δ 3.39 and 2.59. Irradiating the NH at δ 6.74 there was an NOE of 3.2% on the methyl at δ 1.67, and an NOE of 0.5% on *H*-8 at δ 2.50. On irradiating the NH at δ 6.89 there was a NOE of 7% on the *ortho* aromatic *H* (doublet at δ 7.28 with *J* 7.0) and a NOE of 1% on *H*-7 at δ 5.14 ppm. Therefore the signals at δ 6.89, 5.14, 3.39, 2.59, 1.41 are all due to the conformer with the CH₃ on the opposite side of the ring NH; the signals at δ 6.74, 5.55–5.42, 3.35, 2.50, 1.67 are due to the conformer with the CH₃ group on the same side as the ring NH. ¹³C NMR (CDCl₃, 50 MHz): 171.68, 171.25, 170.26, 169.51, 168.60, 168.00, 167.25, 166.96 [C=O]; 155.64 [urethane C=O, 2 conf.]; 137.53 [quat. arom., 2 conf.]; 137.31, 135.71 [CH=CH]; 129.48, 129.10, 127.74, 127.37, 125.71, 125.39 [arom. CH]; 124.04, 121.75 [CH=CH]; 79.50, 79.27 [C(CH₃)₃]; 65.48, 64.80, 64.19, 64.08 [quat. CN]; 63.00, 62.76, 62.17 (2 conf.) [CH₂CH₃]; 46.99, 46.07 [CH₂Ph]; 43.51, 42.93 [CH₂NHBoc]; 38.62, 37.78 [CH₂CH=]; 31.92, 29.19 [CH₂CH=]; 28.30 [C(CH₃)₃]; 23.84, 19.88 [CH₂CH₂CH=]; 22.40, 20.50 [CH₃]; 14.03, 13.98 [CH₃CH₂].

(*Z*,3*R**,9*R**) and (*Z*,3*R**,9*S**)-3-(((*N*-Benzyl-*t*-butoxycarbonyl)amino)acetamido)-9-(ethoxycarbonyl)-3-methyl-4,5,8,9-tetrahydro-1*H*-azonin-2(3*H*)-ones 2a and 2b

Method A: RCM of 12. A solution of dienes **12a,b** (1.549 g, 3.00 mmol) in dry CH₂Cl₂ (750 mL) was placed under argon, and treated with benzyldiene bis(tricyclohexylphosphine)ruthenium dichloride (489 mg, 0.591 mmol). In order to ensure removal of all oxygen present, a series of vacuum/argon cycles was carried out. The orange solution was refluxed for 62 h, by which time it had become black. Et₃N (1 mL) was added, and most of the solvent was evaporated, until about 100 mL was left. This solution was introduced in a column of silica gel packed with CH₂Cl₂. Elution with CH₂Cl₂–AcOEt–EtOH–Et₃N 74 : 20 : 5 : 1 to 69 : 25 : 5 : 1 gave a crude product containing both substrate and products. Two other chromatographies (PE–AcOEt 3 : 7 + 2% 96% EtOH to PE–AcOEt 15 : 85 + 2% 96% EtOH) separated the substrate, the *cis* product, and the *trans* product. The *cis* compound was still contaminated with an intermolecular by-product and was further purified by chromatography using CH₂Cl₂–AcOEt 6 : 4 + 2% 95% EtOH. Overall, substrate **12a,b** (270 mg, 17.4%, grey foam), *cis* adduct **2a** (351 mg, 24.0%, slightly grey foam), and *trans* adduct **2b** (432 mg, 29.5%, grey foam) were obtained. The overall yield based upon recovered starting material was 65%.

Method B: decarboxylation of 14. A solution of malonate **14** (505 mg, 0.902 mmol) in abs. EtOH (11.0 mL), was cooled to 0 °C, and treated with 2.96 N aq. NaOH (385 μL, 1.14 mmol). The solution was stirred for 2 h. Solid (NH₄)H₂PO₄ (100 mg) was added. The mixture was allowed to warm to r.t., diluted with 5% aq. (NH₄)H₂PO₄ (20 mL), with 1 N HCl (1 mL), and with AcOEt. The aqueous phase was saturated with NaCl. After separation of the phases, the aqueous phase was extracted 3 more times with AcOEt. The solution was evaporated, taken up in 1,4-dioxane (5 mL) and refluxed for 3 h. After evaporation, the crude product was chromatographed with PE–AcOEt–EtOH 34.5 : 64.5 : 0.5 to give pure *cis* diastereoisomer **2a** (202.4 mg, 46.0%), and pure *trans* diastereoisomer **2b** (152.9 mg, 34.8%). Overall yield: 81%. The diastereoisomeric ratio was determined by ¹H NMR spectroscopy on the crude product and turned out to be 60 : 40 (*cis* : *trans*).

2a (*cis*): *R*_f 0.26 (PE–AcOEt 3 : 7). Found: C, 63.9; H, 7.7; N, 8.4%. C₂₆H₃₇N₃O₆ requires: C, 64.05; H, 7.65; N, 8.62%. ¹H NMR (300 MHz, CDCl₃): δ 7.42 [2 H, t, *meta*-*H*, *J* 7.5]; 7.32 [1 H, t, *para*-*H*, *J* 7.5]; 7.25 [2 H, d, *ortho*-*H*, *J* 7.7]; 6.30 [1 H, d, *NH*, *J* 9.3]; 5.54 [1 H, broad s, NHBoc]; 5.54–5.40 [1 H, m, *H*-6]; 5.33 [1 H, slightly broad q, *H*-7, *J* ≈ 9.0]; 4.74–4.52 [2 H, m, CH₂Ph]; 4.58 [1 H, ddd, CHN, *J* ≈ 3.9, 7.2, 9.3]; 4.22

[2 H, q, CH_2CH_3 , J 7.2]; 4.10 and 4.02 [2 H, AB part of ABX system, CH_2NHBOc , J_{AB} 16.9, J_{AX} 4.7, J_{BX} 3.9]; 2.79 [1 H, broad ddd, $H-8 \alpha$, $J_{7-8} \approx 9.0^*$]; 2.17 [1 H, dt, $H-8 \beta$, J_d 13.5, J_t 6.7]; 2.10–1.88 [3 H, m, $H-5$ (2) and $H-4$ (1)]; 1.88–1.74 [1 H, m, $H-4$ (1)]; 1.44 [3 H, s, CH_3C]; 1.41 [9 H, s, $(\text{CH}_3)_3\text{C}$]; 1.30 [3 H, t, CH_3CH_2 , J 7.1]. NOEDIFF: on irradiating the NH at δ 6.30: 5.0% overall NOE on benzyl *ortho-H*; 2.4% NOE on $H-8 \beta$; 4.6% NOE on one of the $H-5$; no NOE on $H-8 \beta$ or on CH_3C . On irradiating the CH_3 at δ 1.44: 1.2% NOE on benzyl *ortho-H*; 0.9% NOE on $H-6$; 1.2% on $H-4$ downfield; no NOE on NH . On irradiating $H-7$: 1.2% NOE on $H-9$. ^{13}C NMR (75 MHz, CDCl_3): δ 171.68, 171.56, 170.39 [C=O]; 155.65 [urethane C=O]; 136.92 [quat. arom.]; 135.28 [CH=CH]; 129.36, 127.92, 125.71 [arom. CH]; 122.51 [CH=CH]; 79.57 [C(CH₃)₃]; 65.68 [CNBn]; 61.47 [CH₂CH₃]; 51.12 [CHN]; 47.45, 43.65 [CH₂N]; 38.83 [CH₂CH=]; 29.86 [CH₂CH=]; 28.26 [C(CH₃)₃]; 21.91 [CH₂CH₂CH=]; 21.56 [CH₃]; 14.11 [CH₃CH₂]. IR: ν_{max} 3427, 2971, 1728, 1680, 1489, 1448, 1402, 1369, 1163, 1054 cm^{-1} .

2b (*trans*): R_f 0.18 (PE–AcOEt 3 : 7). Found: C, 63.85; H, 7.6; N, 8.35%. $\text{C}_{26}\text{H}_{37}\text{N}_3\text{O}_6$ requires: C, 64.05; H, 7.65; N, 8.62%. ^1H NMR (300 MHz, CDCl_3): δ 7.54 [2 H, d, *ortho-H*, J 7.6]; 7.38 [2 H, t, *meta-H*, J 7.3]; 7.26 [1 H, t, *para-H*, J 7.4]; 6.18 [1 H, d, NH , J 9.6]; 5.65–5.30 [3 H, m, $\text{CH}_2\text{CH}_2\text{CH}=\text{CH}$ and $NHBoc$]; 4.71 [1 H, dt, CHN , J_d 3.0, J_t 9.8]; 4.56 [2 H, s, CH_2Ph]; 4.32–4.06 [3 H, m, CH_2CH_3 and $CHHNHBOc$]; 3.59 [1 H, dd, $CHHNHBOc$, J 3.0, 17.2] 2.62–2.42 [1 H, m, $H-8 \alpha$, small J with $H-9$ (from COSY)]; 2.40–2.10 [2 H, m, $H-8 \beta$ + $H-5$]; 2.10–1.80 [3 H, m, $H-5$ (1) and $H-4$ (2)]; 1.67 [3 H, s, CH_3C]; 1.37 [9 H, s, $(\text{CH}_3)_3\text{C}$]; 1.31 [3 H, t, CH_3CH_2 , J 7.1]. NOEDIFF: on irradiating the NH at δ 6.18: 3.4% NOE on C– CH_3 ; 5.0% NOE on $H-8 \beta$. On irradiating the CH_3 at δ 1.65: 1.1% NOE on benzyl *ortho-H*; 17.5% NOE on NH ; 6.0% NOE on one of the $H-5$. ^{13}C NMR (50 MHz, CDCl_3): δ 172.26, 171.62, 169.73 [C=O]; 155.65 [urethane C=O]; 137.57 [quat. arom.]; 134.40 [CH=CH]; 129.05, 127.36, 125.79 [arom. CH]; 124.06 [CH=CH]; 79.33 [C(CH₃)₃]; 64.22 [CNBn]; 61.65 [CH₂CH₃]; 51.66 [CHN]; 45.87, 42.99 [CH₂N]; 38.14 [CH₂CH=]; 33.00 [CH₂CH=]; 28.26 [C(CH₃)₃]; 23.02 [CH₂CH₂CH=]; 20.90 [CH₃]; 14.13 [CH₃CH₂].

(Z,3*R,9*R**)-3-(((N-Benzyl-*t*-butoxycarbonyl)amino)acetamido)-9-(((methoxycarbonylmethyl)amino)carbonylmethyl)amino)carbonyl)-3-methyl-4,5,8,9-tetrahydro-1*H*-azonin-2(3*H*)-one 15a**

N-Boc-glycylglycine methyl ester (179.4 mg, 0.728 mmol) was dissolved in CH_2Cl_2 (1.2 mL), and treated, at r.t., with $\text{CF}_3\text{CO}_2\text{H}$ (400 μL). After 1 h 15 min, the solvent was evaporated to dryness. The residue was taken up in CH_2Cl_2 and evaporated again three times to give crude glycylglycine methyl ester TFA salt. Meanwhile, a solution of ester **2a** (213.2 mg, 0.437 mmol) in EtOH (5.5 mL), was treated, at r.t., with 3 N aq. NaOH (190 μL , 0.57 mmol). After stirring for 30 min, the solution was poured into 5% aq. $(\text{NH}_4)_2\text{PO}_4$ (30 mL) + 1 N HCl (1.3 mL). After saturation with NaCl, the mixture was extracted 4 times with AcOEt. Evaporation furnished the crude acid. It was taken up in CH_2Cl_2 (3 mL) and added to the flask containing the crude glycylglycine methyl ester. Et_3N (273 μL , 1.96 mmol) and BOP (benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate) (298.2 mg, 0.674 mmol) were added in this order. The solution was stirred at r.t. overnight and then poured into 5% aq. $(\text{NH}_4)_2\text{PO}_4$ saturated with NaCl (30 mL), and extracted with AcOEt (3 times). The organic layer was washed with saturated aq. NaHCO_3 and evaporated to dryness. Chromatography with CH_2Cl_2 –AcOEt–EtOH 46.5 : 46.5 : 7 to 45 : 45 : 10 followed with another chromatography with AcOEt–MeOH 98 : 2 gave pure **15a** as a white solid (203 mg, 79%). R_f 0.52 (AcOEt– CH_2Cl_2 –MeOH 69 : 30 : 1). Found: C, 59.1; H, 7.1; N, 11.8%. $\text{C}_{29}\text{H}_{41}\text{N}_5\text{O}_8$ requires: C, 59.27; H, 7.03; N, 11.92%. ^1H NMR (DMSO, 300 MHz): δ 8.58 [1 H, t, NH , J 5.7]; 7.75 [1 H, d, NH , J 10.2]; 7.50–7.25 [5 H, m, arom.]; 7.09 [1 H, broad t, NH];

6.87 [1 H, t, NH , J 5.7]; 5.43 [1 H, slightly broad q, $CH=\text{CH}$, J 9.0]; 5.25 [1 H, dt, $CH=\text{CH}$, J_d 3.5, J_t 11.1]; 4.98 [1 H, d, $CHHPh$, J 18.3]; 4.26 [1 H, d, $CHHPh$, J 18.3]; 4.16–3.78 [7 H, m, CHN and CH_2N]; 3.64 [3 H, s, OCH_3]; 2.60–2.40 [2 H, m, CH_2]; 2.35–1.95 [4 H, m, CH_2]; 1.74–1.57 [1 H, m, CH_2]; 1.38 [9 H, s, C(CH₃)₃]; 1.15 [3 H, s, CH_3]. ^{13}C NMR (DMSO, 50 MHz): δ 172.86, 170.88, 169.93, 169.06, 168.80 [C=O]; 156.17 [urethane C=O]; 137.94 [quat. arom.]; 133.39 [CH=CH]; 128.56, 127.29, 126.60 [arom. CH]; 124.56 [CH=CH]; 77.74 [C(CH₃)₃]; 64.40 [quat. CN]; 52.37 [CHN]; 51.68 [CH₃O]; 47.82 [CH₂Ph]; 43.25, 41.98, 40.37 [CH₂N]; 38.28, 29.67 [CH₂CH=]; 28.09 [(CH₃)₃C]; 23.01 [CH₂CH₂CH=]; 22.80 [CH₃]. IR: ν_{max} 3360 (broad), 2953, 2864, 2816, 1740, 1650, 1543, 1487, 1448, 1368, 1326, 1279, 1145, 1079, 1037, 983, 949 cm^{-1} .

(Z,3*R,9*R**)-3-(((N-Benzyl-*t*-butoxycarbonyl)amino)acetamido)-9-(((methoxycarbonylmethyl)amino)carbonylmethyl)amino)carbonyl)-3-methyl-4,5,8,9-tetrahydro-1*H*-azonin-2(3*H*)-one 15b**

Prepared in 80% yield by the same procedure described above for **15a**. R_f 0.45 (AcOEt– CH_2Cl_2 –MeOH 69 : 30 : 1). Found: C, 58.9; H, 7.2; N, 11.6%. $\text{C}_{29}\text{H}_{41}\text{N}_5\text{O}_8$ requires: C, 59.27; H, 7.03; N, 11.92%. ^1H NMR (CDCl_3 , 200 MHz): δ 8.41 [1 H, t, NH , J 5.9]; 8.27 [1 H, broad s, NH]; 7.62 [2 H, d, *ortho-H*, J 7.4]; 7.44–7.19 [3 H, m, *meta*- and *para-H*]; 6.75 [1 H, d, ring NH , J 10.0]; 6.57 [1 H, broad t, $NHBoc$]; 5.43 [1 H, slightly broad q, $CH=\text{CH}$, J 9.1]; 5.21–5.02 [1 H, broad m, centre 5.11, $CH=\text{CH}$]; 4.62 [2 H, s, CH_2Ph]; 4.41 [1 H, t, CHN , J 9.9]; 3.95–3.77 [4 H, m, CH_2N]; 3.64 [3 H, s, OCH_3]; 3.70–3.60 [1 H, m, $CHHNHBOc$]; 3.29 [1 H, dd, $CHHNHBOc$, J 5.4, 16.7]; 2.50–2.16 [3 H, m, CH_2]; 2.00–1.85 [2 H, m, CH_2]; 1.74–1.57 [1 H, m, CH_2]; 1.59 [3 H, s, CH_3]; 1.33 [9 H, s, C(CH₃)₃]. ^{13}C NMR (DMSO, 50 MHz): δ 172.31, 170.89, 170.15, 169.06, 168.80 [C=O]; 155.35 [urethane C=O]; 139.50 [quat. arom.]; 133.53 [CH=CH]; 128.35, 126.55, 125.87 [arom. CH]; 124.83 [CH=CH]; 77.72 [C(CH₃)₃]; 63.74 [quat. CN]; 52.20 [CHN]; 51.62 [CH₃O]; 44.80 [CH₂Ph]; 42.25, 41.63, 40.45 [CH₂N]; 37.77, 32.24 [CH₂CH=]; 28.06 [(CH₃)₃C]; 21.94 [CH₂CH₂CH=]; 19.97 [CH₃].

Cyclic peptide 3a

A solution of compound **15a** (110.1 mg, 0.187 mmol) in tetrahydrofuran (5 mL), was treated at r.t. with 0.5 M aq. LiOH (1.13 mL, 0.565 mmol) and stirred for 1 h. The solution was poured into 5% aq. $(\text{NH}_4)_2\text{PO}_4$ (15 mL) + 1 N HCl (0.5 mL). After saturation with NaCl, the mixture was extracted 3 times with AcOEt and evaporated to dryness. It was taken up in CH_2Cl_2 (1 mL) and treated with $\text{CF}_3\text{CO}_2\text{H}$ (250 μL). After stirring for 90 min at r.t., the solvents were evaporated. In order to remove completely the $\text{CF}_3\text{CO}_2\text{H}$, the residue was taken up twice with *n*-heptane and evaporated again. Then it was finally taken up in dry DMF (13 mL) and dry CH_2Cl_2 (17 mL). *Sym*-collidine (104 μL , 0.786 mmol) and HATU [(*O*-7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate] (150.6 mg, 0.396 mmol) were added in sequence and the mixture stirred for 2 days at r.t. The solvents were evaporated. Then the residue was taken up in CHCl_3 , and washed with 5% aq. $(\text{NH}_4)_2\text{PO}_4$ saturated with NaCl, and with 10% K_2CO_3 (15 mL) + 0.1 M NaOH (5 mL). Evaporation and chromatography (CH_2Cl_2 –EtOH 9 : 1) gave pure **3a** as a white solid (47.2 mg, 55%). R_f 0.42 (CH_2Cl_2 –MeOH 9 : 1). Found: C, 60.4; H, 6.4; N, 15.25%. $\text{C}_{23}\text{H}_{29}\text{N}_5\text{O}_8$ requires: C, 60.65; H, 6.42; N, 15.37%.

^1H NMR (DMSO, 300 MHz): δ 8.70 [1 H, t, NH -b, J 5.7]; 7.64 [1 H, d, ring NH , J 13.2]; 7.46–7.35 [3 H, m, *meta-H* and NH -c]; 7.34–7.26 [3 H, m, *ortho*- and *para-H*]; 6.92 [1 H, d, NH -a, J 7.3]; 5.43 [1 H, slightly broad q, $H-7$, J 9.0]; 5.27 [1 H, dt, $H-6$, J_d 5.2, J_t 10.8]; 5.00 [1 H, d, $CHHPh$, J 18.3]; 4.38 [1 H, dd $CHHN$ of glycine c, J 5.1, 15.3*] 4.35 [1 H, d, $CHHPh$, J 18.3]; 4.15–4.03 [2 H, m, $H-9$ and $CHHN$ of glycine a]; 3.81 [1 H, dd, $CHHN$ of glycine b, J 6.0, 15.6]; 3.60* [1 H, dd, $CHHN$

of glycine c, J_{AB} 15.6*]; 3.57* [1 H, dd, CHHN of glycine b, J_{AB} 15.6*]; 3.35* [1 H, dd, CHHN of glycine a, J_{AB} 14.1*]; 2.47 [1 H, ddd, $H-8\ \alpha$, $J \approx 2.0, 7.8, 12.6$]; 2.55–2.10 [3 H, m, $H-5\ \beta$, $H-8\ \beta$, $H-4$ (1)]; 2.10–1.95 [1 H, m, $H-5\ \alpha$]; 1.33 [1 H, dd, $H-4$ (1), J 9.9, 15.6]; 1.18 [3 H, s, CH_3]. NOEDIFF: on irradiating the signal at δ 3.65 (H of glycines c and b): 3.3% NOE on *ortho-H* (doublet at δ 7.27); on irradiating the signal at δ 3.81 (H of glycine b): 1% NOE of NH of glycine c; on irradiating the ring NH at δ 7.64: 4.44% NOE on $H-5\ \beta$ (at δ 2.35) and 3.33% NOE on $H-8\ \beta$ (at δ 2.16); on irradiating the signal at δ 4.10 (CHN and CH of glycine a): 3% NOE on $H-7$, 1.4% NOE on NH of glycine b; on irradiating NH of glycine b (at δ 8.70): 1.9% NOE on CHHN of glycine a at $\delta \approx 4.06$ ppm and 1.5% NOE on CHHN of glycine a at δ 3.35 ppm. On irradiating the signal of the methyl at δ 1.18 ppm: 3.7% NOE on *ortho-H* at δ 7.27 and 1% on *meta-H* at δ 7.36. 1H NMR (anhydrous $CDCl_3$, 300 MHz, 0.01 M) (attribution of ring NH is certain (COSY). Attribution of glycine a-c NH is only guessed): δ 8.47 [1 H, broad t, NH b, $J \approx 5.0$]; 8.31 [1 H, broad s, NH c]; 7.92 [1 H, d, NH a, J 8.1]; 7.42 [2 H, t, *meta-H*, J 7.2]; 7.36–7.24 [3 H, m, *ortho-* and *para-H*]; 6.49 [1 H, d, ring NH , J 9.9]; 5.55 [1 H, slightly broad q, $H-7$, J 9.1]; 5.34 [1 H, dt, $H-6$, J_a 4.5, J_b 10.8]; 5.29 [1 H, d, CHHPh, J 18.0]; 4.61 [1 H, d, CHHPh, J 18.0]; 4.66–4.36 [4 H, m, $H-9$ + 3 CHH glycines]; 3.52 [1 H, dd, CHH glycine b, J 4.5, 15.3]; 3.42 [1 H, slightly broad d, CHH glycine a or c, J 14.0]; 3.41 [1 H, slightly broad d, CHH glycine a or c, J 14.1]; 2.59 [1 H, slightly broad q, $H-8\ \beta$, J 11.4]; 2.42–2.22 [3 H, m, (from left to right): $H-5$, $H-4\ \beta$, $H-8\ \alpha$]; 2.06–1.90 [1 H, m, $H-5$]; 1.58 [1 H, dd, $H-4\ \alpha$, J 10.5, 15.9]; 1.47 [3 H, s, CH_3]. $\Delta\delta$ ($CDCl_3$ – DMSO): ring NH : –1.15; NH -a: +0.91; NH -b: –0.23; NH -c: +1.00.

^{13}C NMR (DMSO, 75 MHz): δ 172.01, 170.84, 170.21, 169.57, 168.96 [amidic C=O]; 137.91 [quat. arom.]; 135.51 [CH=CH]; 128.60, 127.31, 126.76 [arom. CH]; 124.35 [CH=CH]; 64.95 [quat. CN]; 51.80 [CHN]; 48.37 [CH_2 Ph]; 43.44, 43.32, 41.77 [CH_2 N]; 38.15, 28.89 [allylic CH_2]; 22.92 [CH_2]; 22.42 [CH_3]. ^{13}C NMR ($CDCl_3$, 75 MHz): δ 172.53, 172.44, 172.28, 171.90, 171.23 [amidic C=O]; 137.27 [quat. arom.]; 133.26 [CH=CH]; 129.25, 127.96, 126.07 [arom. CH]; 124.47 [CH=CH]; 65.74 [quat. CN]; 51.91 [CHN]; 49.28 [CH_2 Ph]; 44.50, 42.73, 42.48 [CH_2 N]; 39.02, 30.03 [allylic CH_2]; 24.08 [CH_2]; 23.04 [CH_3].

HPLC-MS was performed with the following eluent: 5 min isocratic 100% A (0.1% TFA in H_2O); then linear gradient from 0% to 70% B (0.1% TFA in CH_3CN) over 20 min. Temperature: 15 °C for 15 min; then gradient to 5 °C in 1 min; then 5 °C for the rest of analysis; flow: 0.35 mL min^{-1} for 16 min; then 0.1 mL min^{-1} . Detection by TIC and DAD (220, 254 nm) showed only 1 peak, with $R_t = 31.4$ min; m/z (positive mode): 456.24 ($M + H^+$) (base peak) (calculated mass: 456.22). MS/MS of this peak: 428.0 (62%), 411.0 (44), 399.9 (100), 382.8 (9), 370.9 (8), 353.7 (13), 320.8 (54), 296.7 (5), 292.8 (12), 284.8 (12), 263.7 (14), 228.8 (13), 211.8 (16), 206.9 (11), 178.8 (5), etc. In addition, other smaller peaks (<10%) were detected: $m/z = 478$ ($M + Na^+$), 494 ($M + K^+$), 911 (dimeric artifact), 933 and 949 (Na^+ and K^+ of dimeric artifact). The fact that the 911 peak was an instrument artifact is demonstrated by the following evidence: a) This dimeric artifact was present also in the *trans* cyclic peptide **3b**, where the real dimer was detected at different R_t (see below). b) The fragmentation energy used to perform MS/MS analysis on the 911 ion co-eluting with the monomers was lower (0.6 V) and therefore consistent with a non-covalent interaction, than that needed to fragment the 911 ion of the real *trans* dimer (9 V). c) The obtained MS/MS mass spectrum of the dimeric artifact revealed a poor fragmentation: 456.0 (100%), 428.0 (31), 411.0 (4), 399.9 (8); on the contrary, the MS/MS spectrum of the real *trans* dimers gave a rich fragmentation (see below). d) The dimeric artifact gave a poorly resolved isotopic cluster, probably due to the presence of multicharged (e.g. tetramers) instrumental overlapping signals.

HRMS was carried out on an ibrid q-TOF geometry tandem mass spectrometer (Q-STAR XL MS/MS system – Applied

Biosystems MSD Sciex, Toronto, Canada) equipped with a MALDI ion source. 2,5-Dihydroxybenzoic acid at a final concentration of 5 mg mL^{-1} in 70 : 30 0.1% TFA in H_2O –0.05% TFA in CH_3CN was used as matrix. All the measures were carried out mixing 500 fmol of an in-house obtained hexapeptide (ALELFR, MW = 747.4272) with 500 fmol of sample. Internal instrument calibration was performed using the main mono-charged matrix fragment at $m/z = 137.0239$ (from DHB) and mono-charged ion at $m/z = 748.4352$ of the above mentioned hexapeptide. The main peak obtained was that of the monosodium adduct of the monomer at $m/z = 478.2034$. Calculated for $C_{23}H_{29}N_5O_5Na$: 478.2066 (accuracy: 6 ppm)

Cyclic peptide **3b**

Prepared from **15b** following the same procedure above described for **3a**. After careful chromatographic purification, a product apparently giving a single spot in tlc (R_f 0.38 (CH_2Cl_2 –MeOH 9 : 1)) was obtained in 13% yield. Both 1H and ^{13}C NMR showed the presence of **3b** as the major product, but it was contaminated by other three similar substances. HPLC-MS (see **3a** above for the conditions) showed a main sharp peak corresponding to the expected monomer **3b** (R_t 32.2 min), a peak corresponding to the *cis* isomer **3a** (R_t 31.4 min), and two broader peaks (R_t 37.5 and 39.5 min), corresponding to the 2 diastereoisomeric cyclodimers. Attempts to isolate pure **3b** by silica gel chromatography with various eluents failed. The relative ratio of **3b**, **3a** and cyclodimers in this mixture, determined by HPLC/UV, is 55 : 8 : 37. Therefore the yield of **3b** is 7%.

HPLC-MS [for the conditions see **3a**]: the peak at R_t 32.2 gave m/z (positive mode): 456.22 ($M + H^+$) (base peak). Also other smaller peaks (<10%) were detected: 478 ($M + Na^+$), 494 ($M + K^+$), 911 (dimeric artifact), 933 and 949 (Na^+ and K^+ of dimeric artifact). MS/MS of the peak at 456: 428.0 (42%), 411.0 (100), 399.9 (69), 382.8 (18), 370.9 (17), 353.7 (15), 320.8 (26), 296.7 (10), 292.8 (11), 284.8 (26), 263.7 (26), 251.7 (10), 246.6 (10), 228.8 (12), 211.8 (10), 206.9 (19), 178.8 (18), etc. MS/MS of the peak at 911 of the cyclodimer with R_t 37.5: 740.6 (100), 712.6 (10), 696.6 (40), 563.3 (20), 456.0 (38), 428.0 (11), 411.0 (5), 399.9 (13), 284.8 (14). MS/MS of the peak at 911 of the cyclodimer with R_t 39.5: 740.6 (100), 712.6 (10), 696.6 (44), 563.3 (24), 456.0 (52), 428.0 (13), 411.0 (4), 399.9 (13), 284.8 (12). HRMS: (for conditions see **3a**) the main peak obtained was that of the monosodium adduct of the monomer at $m/z = 478.2118$. Calculated for $C_{23}H_{29}N_5O_5Na$: 478.2066 (accuracy: 10 ppm).

We tried to separate a mixture of **3b** and **3a** (8 : 2) from the cyclodimers by reverse-phase preparative HPLC (conditions were similar to those employed for analytical HPLC). The separation was successful but, surprisingly, on evaporation to dryness (at 30–40 °C), extensive decomposition of **3b** was observed giving hydrolysed adducts (as demonstrated by the presence in the MS of peaks with $m/z = 474$). After a further silica gel chromatography, we isolated a small amount of an 83 : 17 mixture of **3a** and **3b**. Thus it seems that **3b** is much more sensitive to hydrolysis than **3a**.

From careful analysis of the NMR spectra of the above mentioned mixtures we could extract the signals of **3b**: 1H NMR (DMSO, 300 MHz): δ 8.44 [1 H, t, NH , J 6.1]; 7.74 [1 H, d, NH , J 6.3]; 7.48–7.22 [6 H, m, arom. and NH]; 7.11 [1 H, d, NH , J 6.9]; 5.66–5.36 [2 H, m, $CH=CH$]; 4.71 and 4.59 [2 H, AB system, CH_2Ph , J 17.7]; 4.45–3.60 [6 H, m, CHN and CH_2N]; 3.43–3.30 [1 H, m, $CHHN$]; 2.50–1.60 [5 H, m, allylic CH_2 and other CHH]; 1.40–1.20 [1 H, m, CHH]; 1.01 [3 H, s, CH_3]. ^{13}C NMR (DMSO, 75 MHz): δ 171.68, 171.35, 170.94, 170.17, 169.21 [amidic C=O]; 138.11 [quat. arom.]; 134.57 [CH=CH]; 128.68, 127.28, 126.81 [arom. CH]; 125.87 [CH=CH]; 64.39 [quat. CN]; 53.02 [CHN]; 46.04 [CH_2 Ph]; 44.72, 43.90, 42.15 [CH_2 N]; 36.62, 23.11 [allylic CH_2]; 19.63 [CH_3]; 19.23 [CH_3].

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