

Solid Phase Synthesis of Cyclic Peptides with a Guanidine Bridge

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Introduction

Cyclisation through covalent linkage between two side-chains has often proved to be efficient in producing peptide analogues with improved biological properties, i.e. metabolic stability, potency, and target selectivity. Cyclisation can be achieved by the formation of a disulfide bond, an amide bond, ring closing metathesis,...

As part of our efforts in developing solid phase strategies for the synthesis of arginine derivatives [1,2], we are interested in developing peptides bridged through a guanidine function involving an arginine side-chain [3] (Fig. 1).

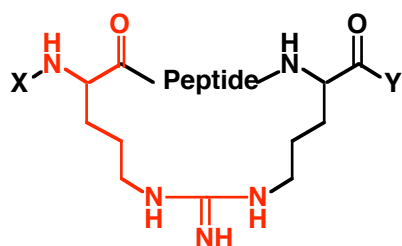


Fig. 1. Schematic structure of a cyclic peptide with a guanidine bridge. The arginine residue is shown in red.

In addition to the constraints due to cyclisation, it would also limit the mobility of the arginine side-chain. To our knowledge, only a similar approach (cyanoguanidine bridge for a GnRH analogue) has yet been reported in a patent by Jean Rivier's team [4].

Results and Discussion

The principle of guanidine formation is shown in figure 2.

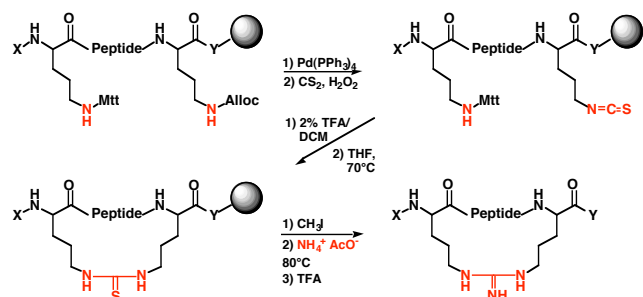


Fig. 2. Synthesis of cyclic peptides with a guanidine bridge.

First, the peptide is assembled on a solid support following classical Fmoc chemistry but using a low loaded resin (0.2 mmol/g) in order to favour intramolecular cyclisation. It contains two orthogonally protected

diaminoacyl residues, at least one of them being an ornithine. After assembly, the alloc protecting group is removed and the free amine is converted into isothiocyanate. This step was primarily done with success by reaction with CS₂ followed by desulfurization with H₂O₂. However, the latter reagent might be harsh conditions for the polymeric support, leading to some degradation and resin loss. We now use di(2-pyridyl) thionocarbonate as a very convenient alternative. The Mtt protecting group of the second diaminoacyl residue is then removed. We found that the classical procedure (i.e. repeated treatments with a dilute TFA solution) resulted in significant loss of peptide. We replaced it by repeated treatments with DCM/TFE/AcOH (7/2/1), a mixture usually employed for the cleavage of trityl resins. Intramolecular cyclisation is performed in THF, leading to the cyclic peptide bridged through a thiourea group. Finally, guanidinylation is performed in two steps: i) S-methylation of the thiourea with MeI, followed by ii) treatment with a 2M solution of ammonium acetate in DMSO at 80°C. Cleavage with TFA affords the cyclic peptide.

We also showed that it was possible to obtain cyclic peptides with a substituted guanidine bridge by reacting the S-methyl-isothiurea intermediate with alkyl-amines (Fig. 3).

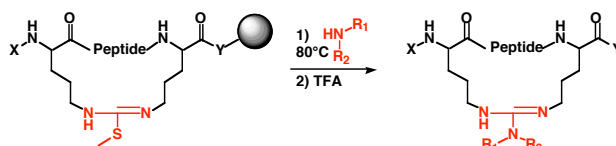


Fig. 3. Synthesis of cyclic peptides with a substituted guanidine bridge.

The best results were obtained with small primary amines and secondary cyclic amines such as pyrrolidine or N-methyl-piperazine. Thus, starting from the S-methyl-isothiurea precursor, it is possible to obtain a series of variously substituted cyclic peptides.

We are working on two model peptides: i) a fragment of the hypothalamic hormone, GnRH (Ac-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂), and ii) a fragment of the HIV Tat protein containing the RGD motif involved in recognition by integrins (Ac-Arg-Gly-Asp-Pro-Thr-NH₂). The residues in bold and blue have been replaced by a diaminoacyl residue (Ornithine or Diaminopropyl), which is involved in the cyclisation process with an ornithine precursor of the bridged arginine (in red). Figure 4 shows the HPLC

profiles of two crude cyclic peptides analogues of GnRH, the first one with a regular guanidine bridge and the second with a substituted bridge.

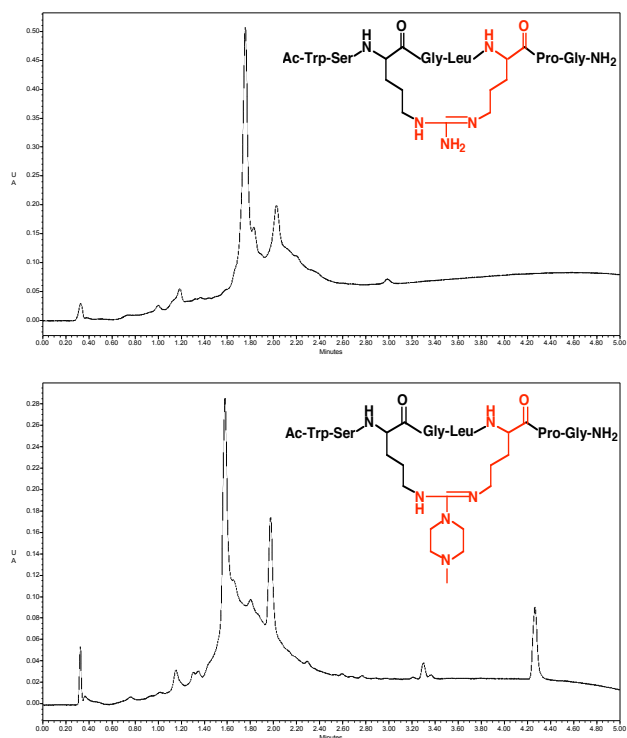


Fig. 4. HPLC analyses of crude cyclic GnRH analogues

LC-MS analyses showed that the major peak contains the expected mass while the minor peak appears to be due to dimerisation through the side chains of two peptide molecules, indicating that the 0.2 mmol/g loading of the resin is not sufficiently low to avoid intermolecular reactions. These analyses also showed the absence of thiourea and *S*-methyl-isothiurea intermediates, and a very low content of non cyclised form, indicating that the steps of isothiocyanate formation, cyclisation and guanidinylation are quite quantitative. Figure 5 shows a similar HPLC profile for a crude cyclic Tat analogue with a substituted bridge.

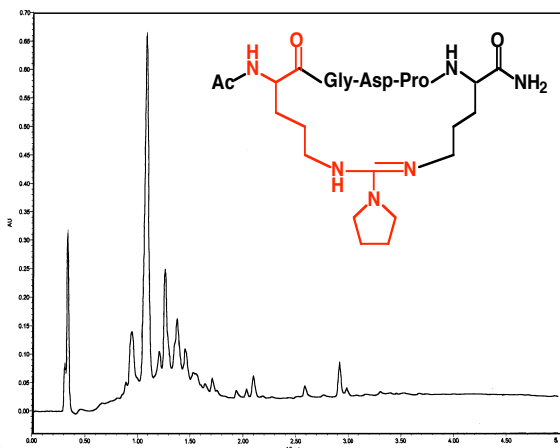


Fig. 5. HPLC analysis of a cyclic RGD analogue.

2D ^1H NMR experiments (500 MHz, DMSO- d_6 , 300 K) were performed on a cyclic RGD-containing analogue. They confirmed the expected structure, but also showed the presence of two conformers in rapid equilibrium. These originate from the isomerization of the Aspartyl-Proline peptide bond. Figure 6 presents the $\alpha\text{H}/\alpha\text{NH}$ region of a COSY spectrum, showing two series of signals, one corresponding to the trans Asp-Pro conformer (blue) and the other to the cis form (red). Quantitation of the relative abundance of trans and cis forms gave a ratio of 40/60.

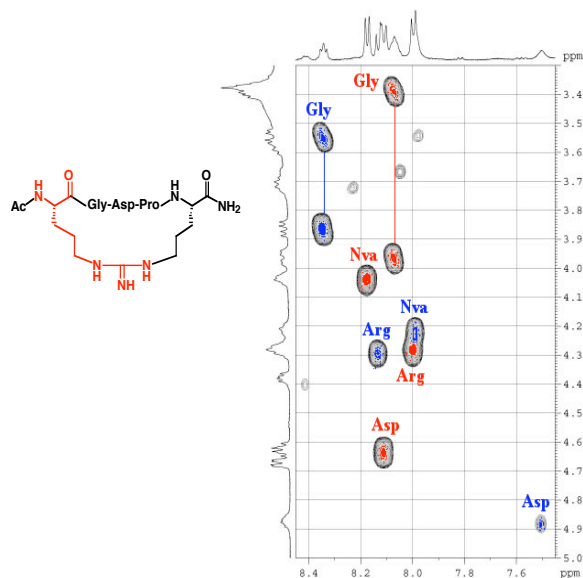


Fig. 6. Extended region of a 2D COSY spectrum of a cyclic RGD-containing analogue.

In conclusion, we were able to obtain cyclic peptides containing a guanidine bridge substituted or not using a solid-phase strategy. In order to diminish the amount of dimeric species, we consider now to use resins with a lower loading. Then, we found that the basic and high temperature conditions of the guanidinylation step induced aspartimide side-reaction in the case of peptides containing an aspartyl followed by a residue other than proline. We are currently exploring a similar strategy, which uses less rigorous conditions for guanidine formation.

Acknowledgments

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References

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