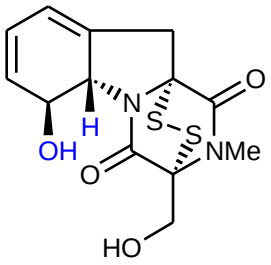
Case Study 4

Question CS4.1
Gliotoxin contains a hexadiene ring with an alcohol group present. Dehydration should result in a stable aromatic ring, yet this does not happen. Explain why this is the case.

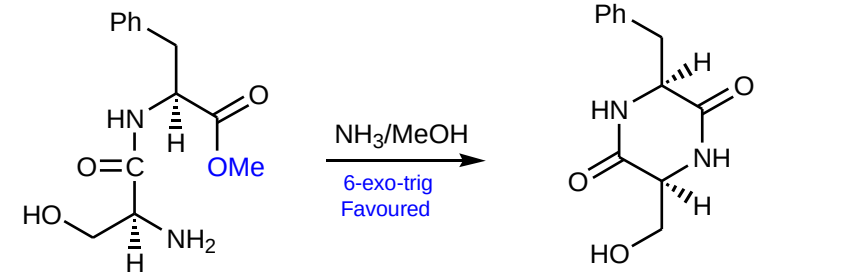
Answer
For a dehyration to occur, the OH and H coloured blue would have to be lost. However, both groups are pointing in the same direction and so this would rule out any possibility of a concerted E2 elimination mechanism.



Gliotoxin

Question CS4.2
Consider the intramolecular cyclisation reaction shown in figure CS4.3. Analyse the cyclisation using Baldwin's rules (section 4.11) and state whether it is favoured or not favoured.

Answer
The reaction is defined as 6-exo-trig, which is a favoured reaction.



L-Seryl-L-phenylalanine methyl ester

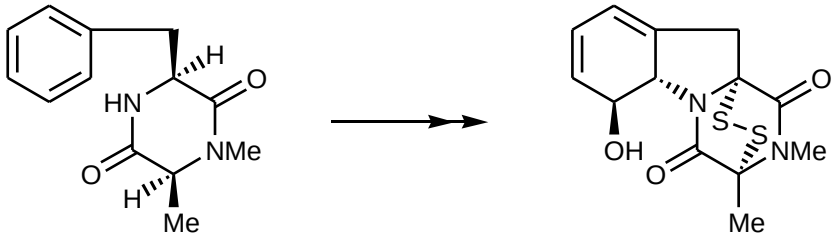
cyclo-(L-PhenylalanylL-seryl)

Question CS4.3
In section CS4.8, it was stated that the radiolabelled *N*-methylated cyclic dipeptide was not converted to gliotoxin. However, it was also stated that it was an 'unlikely' intermediate. Can you suggest why these results do not conclusively rule out this structure as a biosynthetic intermediate.

Answer
A couple of possible reasons could be the following;
*The radiolabelled structure failed to enter fungal cells.
*A multienzyme complex might be involved in the final stages of the biosynthesis. *N*-Methylation of *cyclo*-L-(phenylalanyl-L-seryl) might occur but the structure is immediately transferred to enzymes catalysing cyclisation and incorporation of the disulphide bridge. Such a multienzye complex might not recognise or accept externally administered *N*-methylated cyclic dipeptide.

Question CS4.4
Considering the successful biosynthesis of a gliotoxin analogue described in section CS4.12, can you identify any other analogues that might be successfully generated in this way? Which analogues would have the best chance of success?

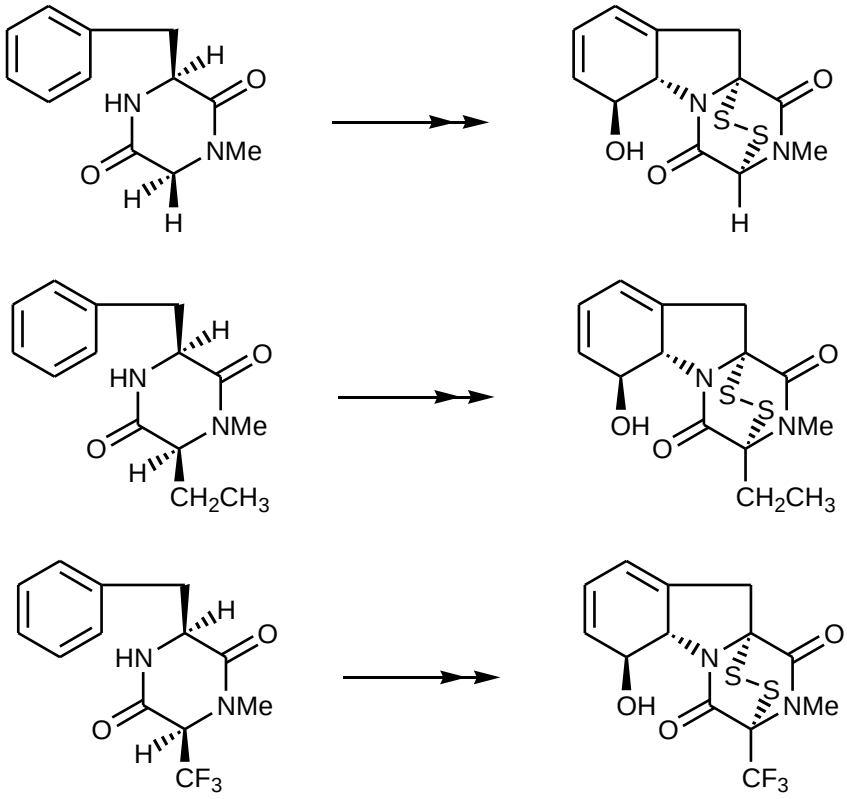
Answer
The successful analogue involved the use of alanine in the cyclic dipeptide, rather than serine in the 'southern' part of the molecule.



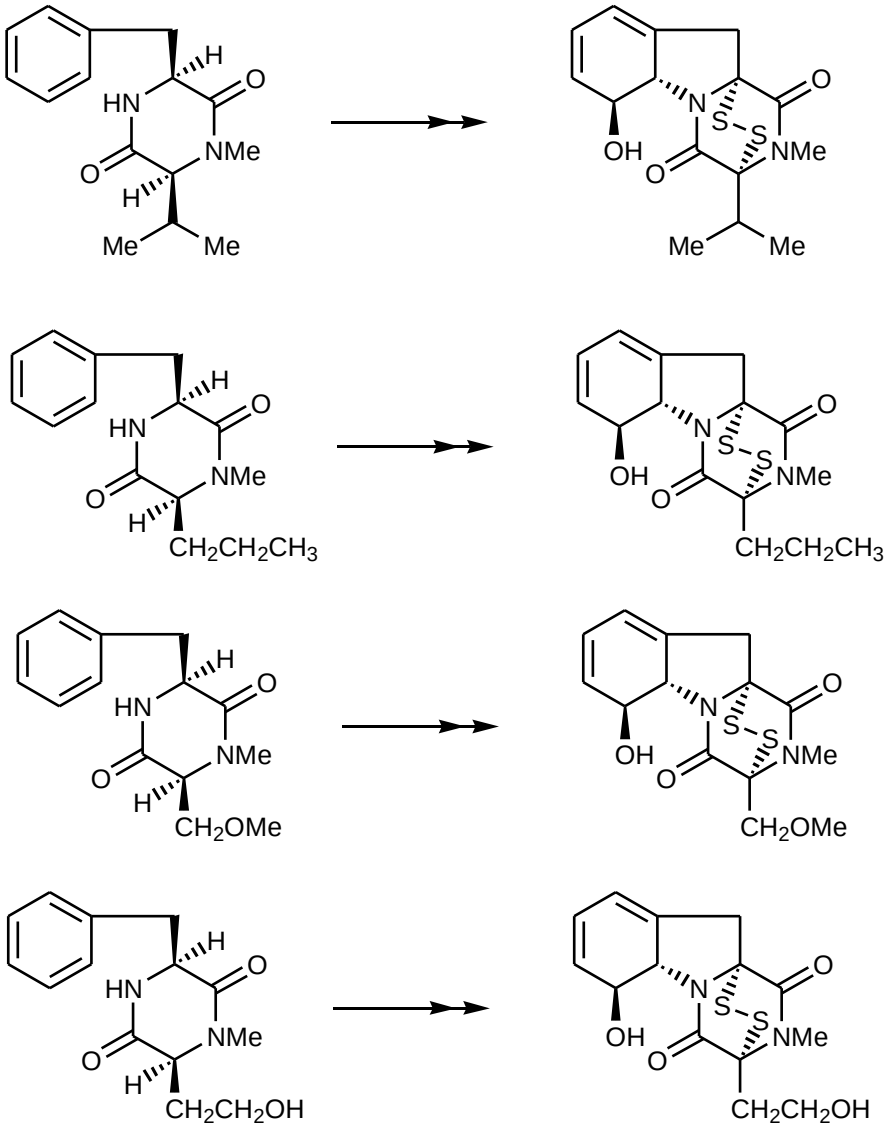
cyclo-(L-Alanyl-L-seryl)

Gliotoxin analogu

There are a huge number of natural and unnatural amino acids which could be tried instead of serine or alanine. The ones most likely to succeed are those that have side chains that are similar in size to the side chains of serine or alanine. Therefore, the following might be worth trying. The first of these would involve the natural amino acid glycine.



If these proved successful, one could try slightly longer or bulkier chains such as the following;



The first example of these would involve the amino acid valine. The last example, is where the hydroxyl group derived from serine has been 'extended' by one carbon unit. The success or otherwise of synthesising such analogues would provide indirect evidence of the steric restrictions imposed by the enzymes involved in the biosynthesis of gliotoxin in that region of the molecule.
It would also be of interest to see whether phenylalanine could be replaced with amino acids containing a substituted aromatic ring.