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SERINE DERIVED OXAZOLIDINES AS SECONDARY STRUCTURE DISRUPTING, SOLUBILIZING BUILDING BLOCKS IN PEPTIDE SYNTHESIS

Thomas Haack and Manfred Mutter*
Séction de Chimie, Université de Lausanne, Rue de la Barre 2, CH - 1005 Lausanne (Switzerland)

Abstract: *The incorporation of serine as oxazolidine protected " pseudo proline "in a host peptide results in the disruption of secondary structure formation thus increasing the solvation of the peptide chain. The use of this protection technique for serine, threonine or cysteine represents a powerful tool to modulate the conformational and physico- chemical properties of peptides.*

The elucidation of the relationship between preferred conformation of a growing peptide chain and its physico-chemical properties is of considerable practical interest in todays peptide synthesis[1-3]. Most notably, the formation of secondary structures, e.g. β-sheeted conformations, is often paralleled by a significant decrease in the solvation of the peptide chain, resulting in a low solubility of hydrophobic peptides, a problem of long standing in peptide chemistry. We have shown previously, that the incorporation of a proline residue in a potentially secondary structure forming peptide disrupts the formation of helical as well as of β-sheet conformations and increases the solubility and coupling kinetics of peptides in a number of solvents[4,5]. As shown in Figure 1, the simultaneous protection of the α- amino and the side chain groups of Ser, Thr (or Cys) via cyclization with an aldehyde or ketone results in oxazolidines (thiazolidines) exhibiting analogous structural features as Pro itself. Consequently, this temporary protection technique should give rise to conformationally induced effects similar to Pro. In the present communication, we explore the use these "pseudo- prolines" as a new tool to overcome the notorious solubility problem of fully protected peptides as e.g. needed in convergent peptide synthesis[6].

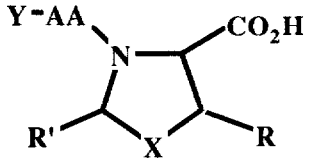
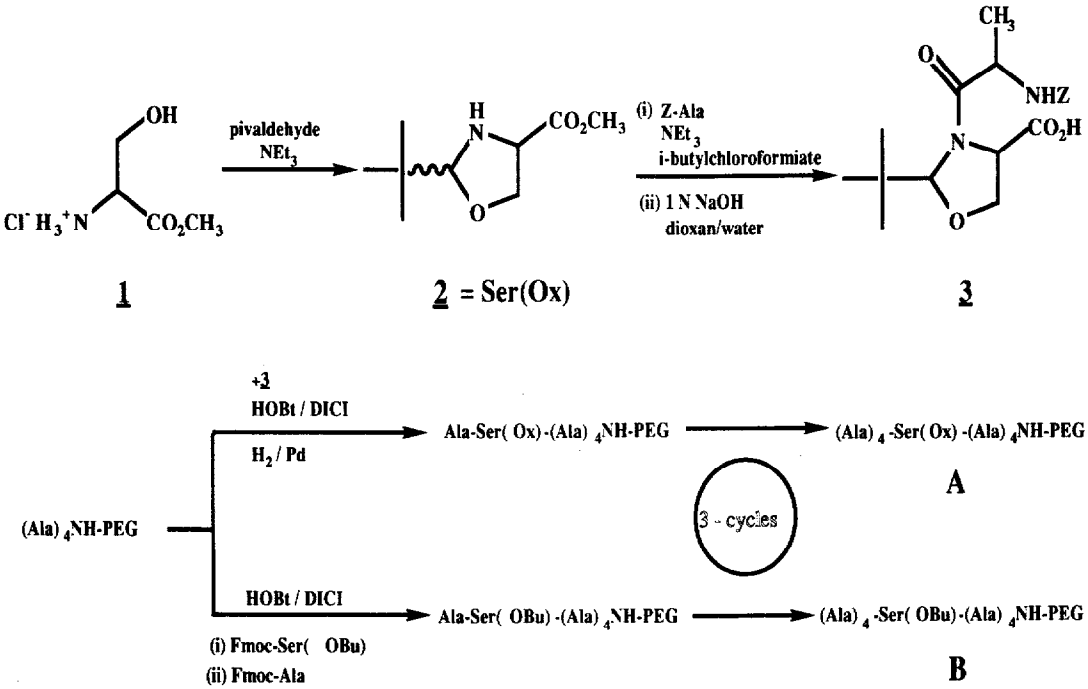


Figure 1 Serine (X = O; R = H) and threonine (X = O; R = CH_3) derived oxazolidines (cysteine- thiazolidines; X = S; R = H) as building blocks for peptide synthesis. (Y: amino acid (AA) N^{α} protecting groups; R' e.g. alkyl, phenyl)

To this end, host - guest peptide **A** and **B** (Scheme 1) were synthezised according to the liquid - phase method[7]; the efficient incorporation of the serine derived oxazolidine[8] (designated as Ser(Ox)) was achieved by prior synthesis of the corresponding dipeptide derivative (Figure 1) CbO-Ala-Ser(Ox)-OH (3) from 2 (35% yield) as outlined in Scheme 1.



Scheme 1 Synthesis of the dipeptide derivative CbO-Ala-Ser(Ox)-OH 3 and of the peptides **A** and **B**

The differential effect of a Ser guest residue protected as Ser(Ox) in peptide **A** and Ser(OBu) in peptide **B** upon the preferred conformation of the host - Ala - peptide was evaluated by CD- and IR- studies.
As depicted in Figure 2, peptides **A** and **B** show pronounced differences in their conformational behavior. Peptide **B** (containing the regularly protected Ser(OBu)) adopts a β-sheet (characterised by a negative cotton effect at 218 nm, crossover at 208 nm and a positive cotton effect at 198 nm) in methanol and a partial α-helical conformation (negative Cotton effects at 222 nm and 204 nm,) in trifluoroethanol. In contrast, peptide **A** (containing the conformationally constrained ring system) can no longer adopt an ordered conformation under identical experimental conditions. The CD spectra in methanol (Figure 2a) and in trifluoroethanol (not shown) is indicative for an unordered conformation. The disruption of a regular secondary structure by the guest residue in peptide **A** is also observed in the solid state: Whereas peptide **B** shows an amide I band at 1624 cm^{-1} in the IR- spectra typical for a β-sheet structure[9], this absorption band is shifted to 1665 cm^{-1} in peptide **A** (Figure 2b), in harmony with the adoption of a random coil conformation.

This drastic difference in the preferred conformation of peptide **A** and **B** is reflected in the physico- chemical properties of the model peptides. In chain elongation (Scheme 1) peptide **A** exhibited much higher solubility in

organic solvents such as DCM, DMF or MeOH compared to peptide **B**; also, the coupling reactions proceeded significantly faster in the stepwise synthesis of peptide **A** compared to the conventionally protected peptide **B**.

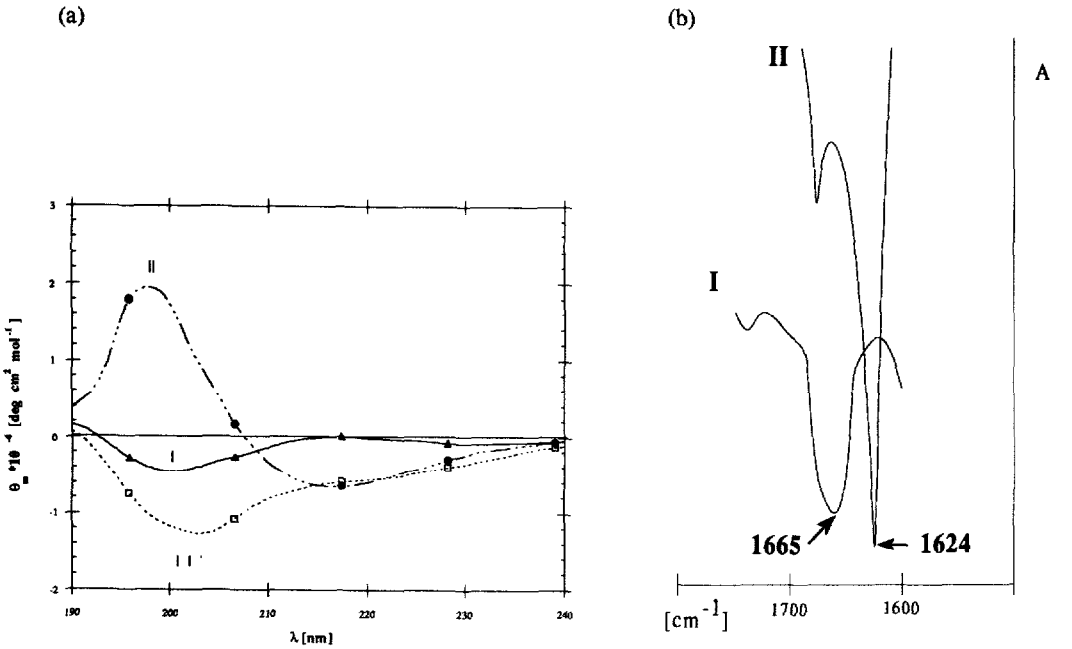


Figure 2 (a) CD - spectra of peptide **A** in MeOH (I), peptide **B** in MeOH (II) and in TFE (II'), c = 10^{-4} M
(b) IR- spectra (amide I region) of peptide **A** (I) and peptide **B** (II) in the solid state (KBr)

The present findings can be rationalized by considering the steric constraints exerted by the oxazolidine ring system (Figure 3). In analogy to the situation in a proline containing sequence, the conformational space of the residue preceeding the Ser(Ox) is dramatically reduced[10]. Due to steric overlaps, the φ/ψ– values necessary for the formation of a helical conformation are no longer accessible in residue i - 1. Moreover, restriction of φ_i by ring formation in Ser(Ox) renders the adoption of a regular β-sheet structure energetically unfavorable. Conformational energy calculations suggest[10], that a cis configuration is enforced in the Ala_{i-1} - $Ser(Ox)_i$ peptide bond, resulting in a kink within the polypeptide backbone. Preliminary studies indicate, that the oxazolidine ring can be cleaved under acidic conditions, restoring the regular peptide sequence as outlined schematically in Figure 3.

In conclusion, the incorporation of serine- derived oxazolidines into a peptide chain results in a disruption of secondary structure formation, simultaneously improving the solvation and couplings kinetics of the growing peptide. Similar effects can be predicted for the pseudo- proline systems derived from Thr or Cys (Figure 1).

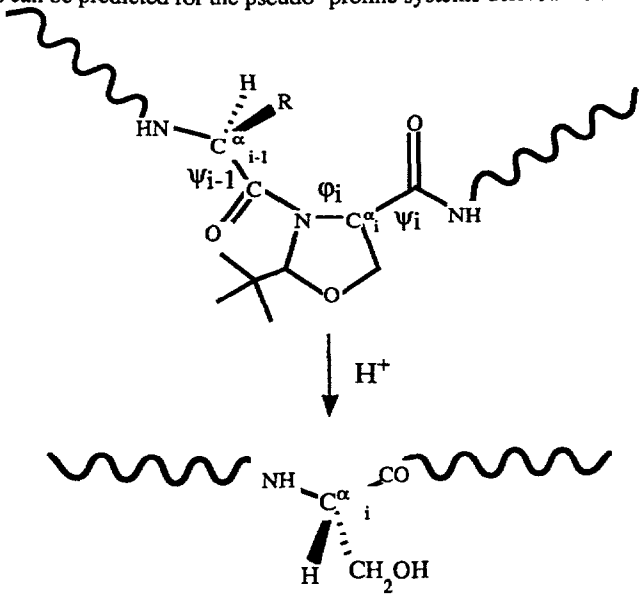


Figure 3 Schematic representation of the effect induced by a Ser(Ox) residue on the conformational properties of a polypeptide chain and its transformation to the deprotected Ser - residue by acid hydrolysis

The application of these protection techniques as a new tool in peptide synthesis to modulate the conformational, physico- chemical and biological properties of peptides is presently under investigation in our laboratory.

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References and notes:

Dedicated to Professor Ernst Bayer on the occasion of his 65 th birthday.

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