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CYCLIZATION UNDER MILD CONDITIONS OF CYSTEINE CONTAINING PEPTIDES

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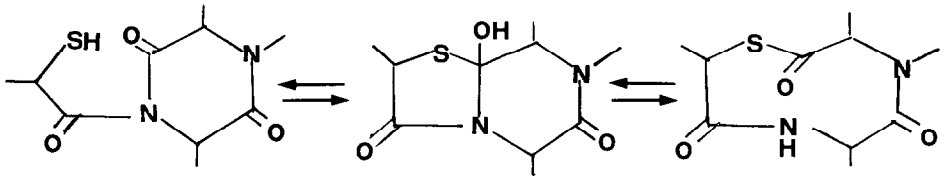
Summary: Cyclization of di- and tripeptides containing cysteine as N-terminal residue is reported. The preferred cyclization patterns and the nature of the products (diketopiperazine, aza-cyclol, peptide thiolactone) are discussed.

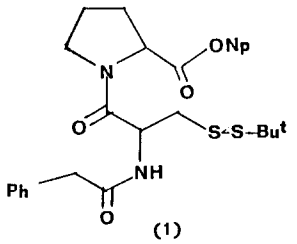
In order to investigate the endoannular interactions in medium sized cyclopeptides, we reported previously on the cyclization of N-(α-mercaptoacyl)-dipeptides containing C-terminal proline[1]. These experiments led to the isolation of the tetrahedral intermediates which connect, through an acyl transfer reaction, 9-membered thiolactones to N-(α-mercaptoacyl)-diketopiperazines (Scheme 1).



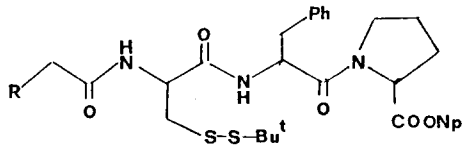
Scheme 1

In view of our continuing interest in this field, we started to investigate the cyclization in mild aqueous conditions of N-(β-mercaptoacyl)-peptides. Carboxy activated N-acyl-Cys-$(X)_n$-Pro-OH (X = α-aminoacid residue) were selected as suitable models for preliminary experiments. The cyclization tendency induced by the C-terminal proline together with the presence of polyfunctional N-terminal residue, should combine favourably to provide information on the preferred cyclization patterns and on the tendency of the CO-S group to participate to intramolecular reactions.

Here is a preliminary account on the cyclization experiments performed on



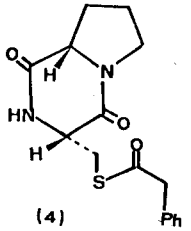
(1)



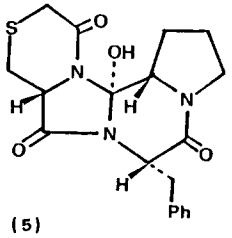
(2) R=Cl
(3) R=Ph

active esters (1) - (3). No urethane type groups were used to protect the N-terminal residues, in order to restrict to the amido NH-groups the interactions involved in the cyclization step[2]. Starting linear peptides were prepared by acylating Pro-O^{t}Bu or Phe-Pro-O^{t}Bu with the appropriate R-CH_2-CO-Cys(S-Bu^{t})-OH. Selective deprotection at the carboxylic end (CF_3COOH) followed by activation (DCCI/pNO_2phenol) gave the desired active esters (1) - (3)[3]. S-t-Butylmercapto group[4] was used throughout the work for the protection of cysteine thiol function since it is selectively removable in aqueous medium by using tri-n-butyl-phosphine[5]. Cyclization and unmasking of the SH was performed at the same time and under very mild conditions by treating (8-10 h, room temperature) a $2.5 \cdot 10^{-3}$ M solution of the active esters in n-PrOH - H_2O (2:1) (Method A) or in n-PrOH - H_2O-0.1M $NaHCO_3$ (2:1:0.2) (Method B) with 1.0 - 1.5 equivalents of Bu_3P.

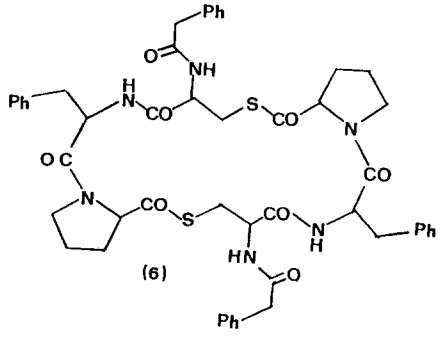
Deprotection of the thiol group of (1) (Method A) led to the isolation of a cyclic compound $C_{16}H_{18}N_2O_3S$ [60% yield, m.p. 110-11° (AcOEt-Ether), $[\alpha]_D^{20}$ - 200° (c, 1.0 $CHCl_3$)] whose chemical and spectroscopic properties did not correspond to the N-phenylacetyl 7-membered thiolactone originated from the attack of the SH on the activated proline carboxyl. Treatment of the product with methanolic NH_2-NH_2.H_2O gave in fact $PhCH_2CONHNH_2$ and cyclo(-L-Cys-L-Pro-): in ^{1}H and ^{13}C n.m.r. spectra, the methylene signals of the benzylic chain appear downfield shifted (ca. 0.35 and 8.0 ppm respectively) in accordance with the presence of directly bonded CO-S group. Structure (4) was then assigned and the assignment was confirmed by X-ray crystallographic analysis.



(4)



(5)



(6)

By unmasking the thiol function of (2) (Method B), a non halogenated compound $C_{19}H_{21}N_3O_4S$ [50% yield, m.p. 211-14° (AcOEt), $[\alpha]_D^{20}$ - 118° (c, 0.7 $CHCl_3$)] was obtained. Structure (5) was assigned to this product on the basis of the following data: ^{13}C n.m.r. spectrum ($CDCl_3$, 50.3 MHz) showed only three amide carbonyl signals (163.80, 164.42, 167.03 δ) and a singlet at 96.11 δ, characteristic of a non protonated carbon bonded to three heteroatoms. In the ^1H n.m.r. spectrum (DMSO-d_6, 90 MHz) the exchangeable proton appeared as sharp singlet at 8.0 δ, the i.r. spectrum ($CHCl_3$) exhibited absorptions at 3550-3200, 1730, 1650 cm^{-1} and no amide II bands. The mass spectrum showed the M^+ at 387 m/z and abundant fragments at 369 ($M^+ - H_2O$), 250, 244 and 91 (100%). Treatment of (5) with methanolic NH_2-NH_2.H_2O gave cyclo(-L-Phe-L-Pro-).

Treatment of (3) with Bu_3P (Method B) afforded a neutral compound $(C_{25}H_{27}N_3O_4)_n$ [35% yield, m.p. 210-11° (MeOH-H_2O), $[\alpha]_D^{20}$ - 53° (c, 1.0 $CHCl_3$)]. In the ^{13}C n.m.r. spectrum ($CDCl_3$, 50.3 MHz) three amide carbonyls (171.05, 170.9, 168.3 δ) and a singlet attributable to a CO-S (199.3δ) were observed; the Pro-Cα resonance appeared sensibly shifted at low field (65.6δ) in accordance with the presence of a directly bonded CO-S group. The ^1H n.m.r. spectrum ($CDCl_3$, 200 MHz) showed three $C_\alpha H$ multiplets centered at 3.60, 4.67 and 5.00δ;each of the two last signals being coupled to an NH doublet located at 6.85δ (J = 8.90 Hz) and 7.85δ (J = 8.20 Hz) respectively. Cleavage with methanolic NH_2-NH_2.H_2O gave a single product which, after treatment with Me_3C-SH was identified as Ph-CH_2CO-Cys(S-Bu^t)-Phe-Pro-$NHNH_2$. On the basis of these data and of the observation of the molecular ion using fast atom bombardment mass spectrometry (MH^+ = 931, MNa^+ = 953 m/z), the dimeric thiolactone structure (6) has been assigned to this compound.The appearance in the n.m.r. spectrum of only one set of resonances for each pair of aminoacids, indicates that (6) adopts in solution a C_2-symmetric conformation.

Although more data are necessary to define the pathways followed by the above reported cyclization reactions, the common tendency toward the formation of intermediate acyl-diketopiperazines should be underlined.Thus cyclization of (2) can be interpreted through the known pattern of equilibria (Scheme 1) associated with α-amidoacyl-incorporation of the N-acyl-cysteinyl residue into the cyclo (-Phe-Pro-) ring; in this case the intramolecular alkylation of the SH by the chloroacetyl group plays an important role giving rise to the formation of a stable form, i.e. the rigid polycyclic skeleton of the aza-cyclol (5)[6]. Cyclization of (3), on the other hand, shows that all the prototropic tautomers involved in α-amidoacyl- and in β-mercaptoacyl-incorporation equilibria, are not favoured and that cyclodimerization prevails. The unusual N to S acyl

migration observed during the cyclization of (1) can also be the result of the intermediacy of N-phenyacetyl-cyclo(-Cys-Pro-); the tendency of this class of compounds to undergo acyl tranfer reactions is in fact well known[7]. In the present case, however, initial formation of the 7-membered thiolactone (N-phenyl-acetyl-Cys-Pro) may play a role.

References and Notes

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