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SYNTHESIS OF A NEW PROTECTED LACTAM-BRIDGED DIPEPTIDE

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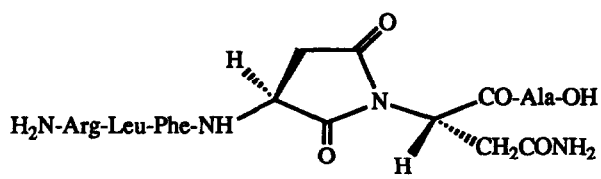
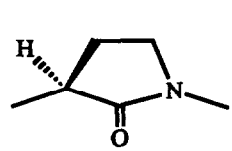
Abstract. A method has been developed for the synthesis of a new protected γ-lactam-bridged dipeptide. This R,S lactam has been prepared in good yield and incorporated into a peptide which is analogous to hGH (7-13) using standard solid phase peptide synthesis methodolgy. The peptide showed insulin-potentiating activity of the same order as those containing an imide in place of the lactam.

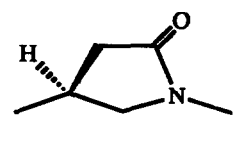
Our laboratories have recently been interested in structure-function studies on hypoglycaemic peptide analogues modelled on the N-terminus of human growth hormone (hGH). Studies by Robson *et al.*[1] showed that the hexapeptide Arg-Leu-Phe-Asp-Asn-Ala [hGH (8-13)] incorporating an aspartimide ring (1) at position 11 was responsible for the previously reported biological activity of this peptide[2, 3]. The activity of the aspartimide analogue of hGH (8-13) suggests that synthetic peptides might have clinical potential in the treatment of type 2 diabetes (aspartimide analogues of hGH (6-13) and (7-13) were also active). The facile hydrolytic opening of the imide ring prompted us to test peptides containing more stable ring structures. The γ-lactam ring (2a) pioneered by Freidinger *et al.*[4] was one logical replacement for the imide. It is more stable to chemical attack and, like the imide[5], the γ-lactam[6, 7] rings have been shown to induce type II' ß-turns. Synthesis and biological testing of hGH (6-13) peptide analogues incorporating the Freidinger lactam in place of the imide formed part of our earlier studies[8]. We were also interested in the isomeric γ-lactam (2b) but found that it was unreported in the literature. We have investigated several routes to this lactam using aspartic acid as the chiron so as to retain the L configuration throughout the peptide



(1)

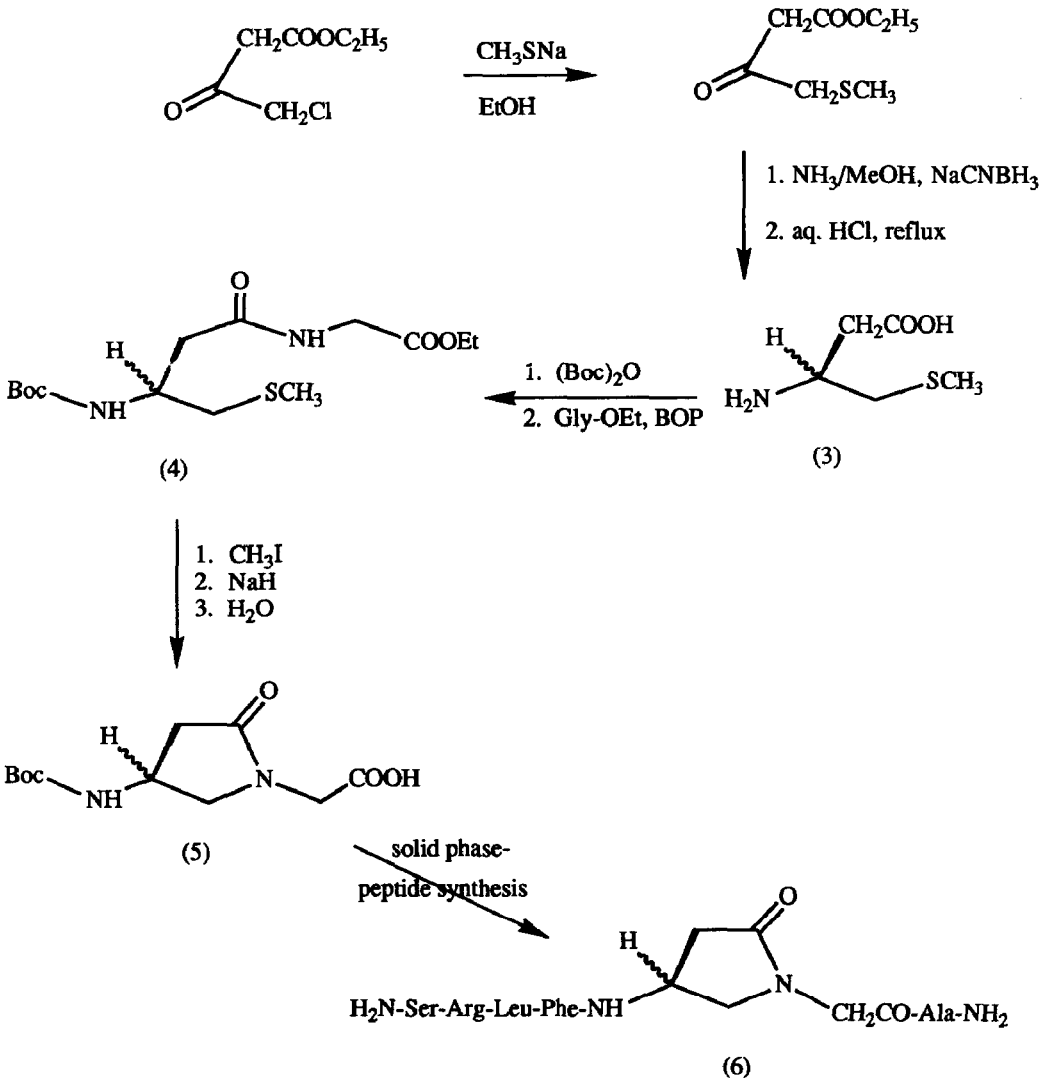


(2a)



(2b)

As yet we have not been able conveniently to synthesize the L-γ-lactam from L-aspartic acid in a form amenable to solid phase peptide synthesis, but we communicate here a simple synthetic route to the racemic γ-lactam incorporating glycine as the carboxy terminal amino acid. Like Freidinger's γ-lactam (2a), the ring closure in the synthesis of our γ-lactam (2b) involves displacement of Me_2S from a sulfonium salt and is compatible with the incorporation of amino acids other than glycine at the carboxy terminal. This new lactam provides another type of conformational restraint which can be used for general application in structure-function studies on peptides. We have incorporated this new lactam into a peptide (6) which is analogous to hGH (7-13) and report here its synthesis and some initial biological data.



The starting material was the isomer of methionine, 3-amino-4-methylthio-butanoic acid (3) (ß-methionine), previously prepared by Griffith *et al.*[9] for conversion to a methionine sulfoximine analogue. Protection of the amine with di-tert-butyl dicarbonate yielded (R,S)-Boc-ß-methionine in 88% yield as a hygroscopic white solid which was converted to its dicyclohexylamine salt for analysis[10]. Condensation of the protected amino acid with BOP reagent[11] and glycine ethyl ester gave the protected ß-methionine-glycine dipeptide (4) in 90% yield. Reaction of the dipeptide (4) with methyl iodide gave the dimethylsulfonium salt, which following treatment with sodium hydride yielded the γ-lactam (5) in 82% yield[12]. The dipeptide lactam was obtained in a form amenable to solid phase peptide synthesis due to the concurrent saponification of the ethyl ester to the corresponding carboxylic acid during aqueous workup. The peptide hGH (7-13) (lactam type 2b) was assembled on benzhydrylamine resin employing a standard solid phase protocol for Boc amino acids[13] (Ala,(5) and Ser(OBzl)) and Fmoc amino acids[14] (Phe, Leu and Arg(Mts)). The peptide was cleaved from the resin with 1M CF_3SO_3H/thioanisole-ethanedithiol in CF_3COOH. The crude peptide was purified by preparative h.p.l.c. Purity was confirmed by analytical h.p.l.c., amino acid analysis and ^1H n.m.r.[15]. Both the R and S diastereomers were evident in the h.p.l.c. but separation was not possible. In addition to this, proton resonances of those residues past phenylalanine were duplicated, especially those of the lactam. This can be seen clearly in the COSY n.m.r. spectrum.

The solution conformation of peptide (5) was investigated using NOESY n.m.r. spectroscopy. Strong n.O.e.'s between the Phe ring protons and the pseudo-Gly methylene, the Ala methine and Ala methyl protons indicate bending of the C-terminus. NOESY spectra of hGH (6-13) (imide) and hGH (6-13) (lactam type 2a) show identical n.O.e.'s between the Phe ring protons and those past the ring analogues. We attribute this to the presence of a type II'ß-turn in each of these peptides. Intravenous insulin tolerance testing[2] was carried out on overnight fasted male albino Wistar rats of 130-150g. The peptide (6), at a dose of 3mg/Kg body weight, significantly potentiated exogenously administered insulin for more than 90 minutes. For example, at 90 minutes, blood glucose levels of test rats had decreased by 40-50% while those of control rats had returned to basal levels. We have found both lactam analogues to be longer lasting in their activity than the imide analogue and we attribute this to the greater stability of the lactam rings to physiological degradation.

In conclusion we believe this γ-lactam provides an interesting variation in conformational restraint for use in synthetic peptides. The results bear out our prediction that a lactam of type (2b) would be geometrically a suitable substitute for the imide and that its effects would be more persistant because of its hydrolytic stability. It should also find application in other studies of conformational restraint.

References and Notes

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10. All new compounds gave satisfactory spectral data and elemental analyses.

11. Benzotriazoyloxytris[dimethylamino]phosphonium hexafluorophosphate.

12. *(R,S)-3-[[(1,1-dimethylethoxy)carbonyl]amino]-5-oxo-1-pyrrolidine acetic acid* (5) as a white solid, (1.38g, 82%). m.p 186°-186.5°. (Found: C, 51.5; H, 6.7; N, 10.7. $C_{11}H_{18}N_2O_5$ requires C, 51.2; H, 7.0; N, 10.8%). ν max (Nujol) 3320s, 1751s, 1681s, 1636s cm^{-1}. ^1H n.m.r. (300MHz, DMSO) 1.38, s, $C(CH_3)_3$; 2.21, dd, J = 16.9, 6.1, C4H; 2.50, concealed dd, C4H; 3.19, dd, J = 9.6, 4.9, C2H; 3.62, dd, J = 9.8, 7.1, C2H; 3.84, d, and 3.97, d, J = 17.6, CH_2; 4.08, m, C3H; 7.71, d, J = 5.6, NH; 11.34, b, COOH. ^{13}C n.m.r. (75MHz, DMSO) 28.18, $C(CH_3)_3$; 36.38, C4; 43.33, C2; 43.86, C3; 53.67, Gly CH_2; 78.08, $C(CH_3)_3$; 155.20, urethane CO; 170.16, C1; 172.23, COOH.

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