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Design and Synthesis of a *cis*-Gly-Pro, Type-VI Turn, Dipeptide Mimetic and its Use in Fmoc-Solid Phase Peptide Synthesis

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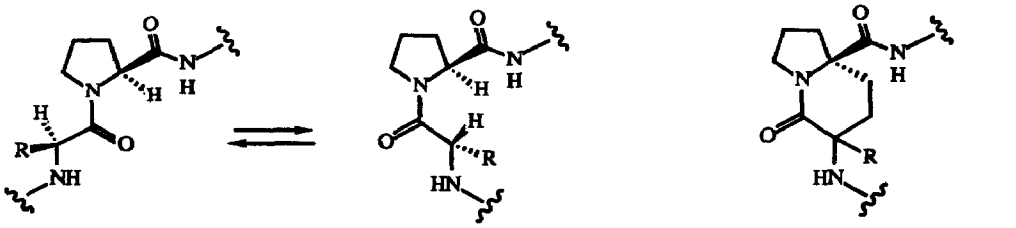
Abstract : The Fmoc-protected bicyclic molecules **7** and **8** have been produced as *cis*-Gly-Pro peptide mimetics in nine synthetic steps starting from optically pure (R)-2-allylproline. Their use in solid-phase peptide synthesis has been demonstrated by their incorporation into analogues of *cis*-Gly^6-Pro^7-bradykinin.

Peptide secondary structure mimetics are proving to be important tools in the exploration of structure-activity relationships in biologically active peptides and proteins[1]. One important feature in many peptide hormones is the occurrence of *cis*-peptide bonds, which although rarely observed in short linear[2] and cyclic peptides[3] without proline, can become significantly populated in aqueous solution in proline-containing peptides. The *cis-trans* Xaa-Pro (Xaa = any proteinogenic amino acid) conformer distribution is determined on the one hand by steric interactions between the C_α centres of the two amino acid residues in the *cis* isomer, and between the C_α centre of Xaa and the δ-position of proline in the *trans* rotomer (see Figure); differences in *cis* and *trans* peptide bond solvation energies appear not to have an overriding influence[4]. On the other hand, the subtle long range effects which may also influence the relative population of *cis*- and *trans*-Xaa-Pro conformers are often less well characterized. The importance of this relatively slow[5] ($k \approx 10^{-3}$ - 10^{-1} s^{-1}) isomerization on protein folding pathways[6], receptor-mediated transmembrane signalling[7], and the mode of action of immunosuppressive drugs[8], has attracted great interest recently.

We are interested in structural mimetics of dipeptide *cis*-Xaa-Pro units, which might, for example, be valuable to assess whether such conformations are important for biological activity. Interest in *cis*-peptide bond surrogates has grown recently, and substituted 1,5-tetrazoles[9], 1,2-disubstituted pyrroles[10], simple *o*-aminomethylphenylacetic acid derivatives[11], ß-lactam derivatives[12], and cyclic peptides[13] have been developed for this purpose. We describe here a new *cis*-Xaa-Pro mimetic **1** (see Figure), which according to molecular modelling (data not shown) allows retention of the peptide backbone and amino acid side chains in positions closely similar to those likely to occur in native *cis*-Xaa-Pro type-VI turn conformations[14]. We focussed initially on a synthesis of the *cis*-Gly-Pro mimetic **1** where R = H. The incorporation of this unit into the peptide hormone bradykinin using Fmoc-solid phase methods is also described below.

(*R*)-Allylproline, prepared by known methods[15], was transformed in six steps on a multi-gram scale into the optically pure bicyclic molecule **4**, as shown in Scheme-1[16]. Upon treatment of the lithium enolate derived from **4** with di-t-butylazodicarboxylate the two diastereomers **5** and **6** were formed in 1:9 ratio, respectively. These were treated directly with excess lithium diisopropylamide (LDA), and the enolate was quenched with pivalic acid to afford a 2:3 ratio of **5** and **6**, which were readily separated by silica chromatography (eluant, n-hexane : EtOAc, 7 : 3). It was anticipated that upon quenching the enolate of **4**, the *trans*-isomer **6** should on steric

FIGURE

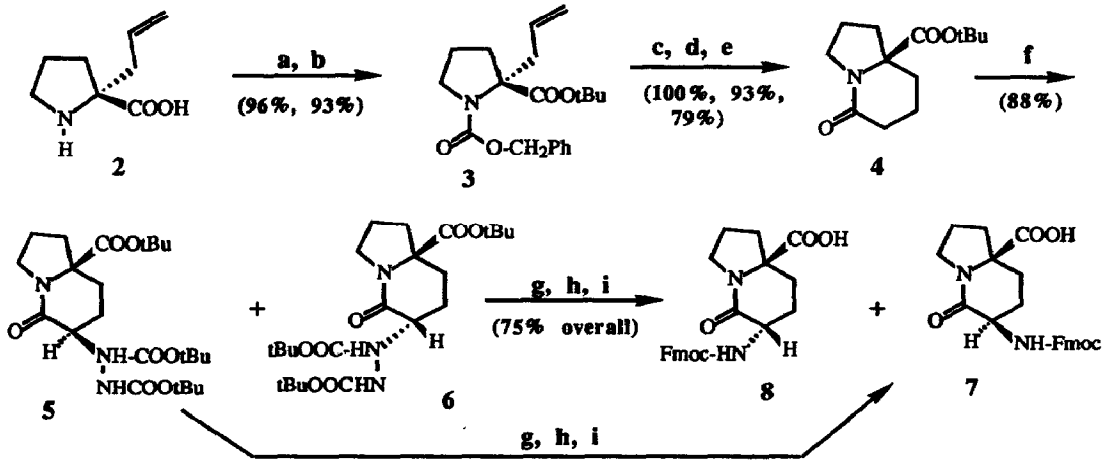


trans Xaa-Pro *cis* Xaa-Pro *cis* Xaa-Pro mimetic (1)

grounds be formed as the major product. Indeed, the *cis* configuration of the minor product was confirmed by converting **5** in three steps (i, TFA, CH_2Cl_2; ii, PtO_2, H_2 ; iii, $SOCl_2$) into the tricyclic molecule **9**. Evidence for the *trans* configuration of **6** was obtained from ^1H 2D-ROESY[17] spectra after its incorporation into a peptide (vide infra).

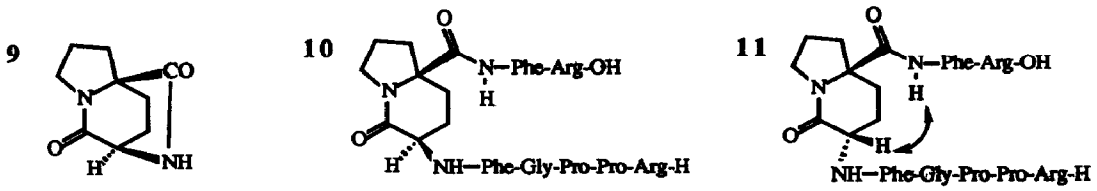
Thereafter, **5** and **6** were converted into **7** and **8**, respectively, by treatment with TFA, reductive cleavage of the hydrazine moiety, and protection of the free amine with Fmoc-chloride. In this form, the two mimetics **7** and **8** can be incorporated into peptides using the standard Fmoc solid-phase method of peptide synthesis[18].

To illustrate this we chose to incorporate the mimetics **7** and **8** into analogues of the peptide hormone bradykinin (Arg^1-Pro^2-Pro^3-Gly^4-Phe^5-Ser^6-Pro^7-Phe^8-Arg^9), a potent mediator of blood vessel dilation, smooth muscle contraction, pain, inflammation and vascular permeability[19]. NMR studies of bradykinin in aqueous solution[20] indicate that no stable, folded secondary structure is significantly populated on the NMR time-scale, i.e. the peptide exists primarily in a disordered state. However, a significant population (10%) of the *cis*-Ser-Pro conformer has been detected by ^1H NMR, and replacement of Ser^6 by glycine leads to a significant



SCHEME-1

Reagents : a), $PhCH_2OCOCl$, aq. NaOH, EtOH ; b), Isobutene, c.H_2SO_4, CH_2Cl_2 ; c), O_3, CH_2Cl_2, -78°, then Ph_3P=CH-COOEt ; d), H_2, Pd/C ; e), DMAP cat., toluene, reflux ; f), LDA, THF-hexane, then (tBuOOC-N)$_2$= ; g), TFA, CH_2Cl_2 ; h), H_2, PtO_2, H_2O ; i), Fmoc-Cl, Na_2CO_3, aq. dioxan.



increase (to 35%) in the population of the *cis*-Gly^6-Pro^7 rotomer[21]. The aim, therefore, was to use **7** and **8** in syntheses of bradykinin analogues **10** and **11**, which may be viewed as mimics of *cis*-Gly^6-Pro^7-bradykinin.

The synthesis of **10** was initiated with p-alkoxybenzylalcohol resin (Wang resin[22]) preloaded with Fmoc-Arg(Pmc)[23] on a 0.25 mmol scale. After removal of the Fmoc group (20% piperidine in N-methylpyrrolidone), subsequent amino acids (4 x excess) were activated with *o*-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate in N-methylpyrrolidone and condensed onto the free amino group of the peptide chain. Each coupling proceeded >99% to completion based on Kaiser or Isatin tests as appropriate. The bicycle **7** was used in 2-fold excess and coupled to ≈ 90% completion. After capping with acetic anhydride, the assembly of the peptide was completed, the resin was treated with TFA/H_2O/thioanisole/ethanedithiol/phenol (82;5;5;3;5), and the product was purified by reverse-phase h.p.l.c., to afford product **10** (>99% by reverse phase-h.p.l.c.) in 53% overall yield. The analogue **11** was prepared in a similar manner, and again no problems were encountered in using the bicycle **8** in the usual automated cycles of peptide synthesis.

In contrast to Gly^6-bradykinin, the 600MHz ^{1}H 1D-NMR spectra of **10** and **11** showed that only a single major rotomeric form (≈ 95%) of each is present in aqueous solution at pH 4. These correspond to a conformer with *trans* peptide bonds at Arg^1-Pro^2 and Pro^2-Pro^3, since strong NOEs were evident in phase sensitive 2D-ROESY spectra[17] between the δ-protons of each proline ring and the α-protons of the preceding residue (see Figure). The low-field peptide-NH-region of each spectrum, however, contained weak doublets typical of a small proportion (≈ 5%) of at least one *cis*-Xaa-Pro conformer, most likely arising from conformational heterogeneity at the Arg^1-Pro^2 and/or Pro^2-Pro^3 peptide bonds. Resonances from the major conformers in the ^{1}H NMR spectra of **10** and **11** were assigned using standard methods[24]. All significant off-diagonal cross-peaks in 2D-ROESY spectra could be assigned to rotating frame NOE's arising between protons located either within one residue, or in adjacent residues in the sequence. No longer range NOE's were apparent in these spectra, indicating that **10** and **11** do not adopt stable secondary structure in water on the NMR timescale. The 2D-ROESY spectrum of **11**, however, showed a cross peak between the protons indicated by the arrow on structure **11**, which confirmed the *trans*-relationship of the carboxyl- and amido-substituents on the bicyclic system.

One motivation to prepare **10** and **11** was to test their affinity for the bradykinin B_1 and B_2 receptors. When assayed for their ability to displace ^{3}H-bradykinin from the guinea pig ileum receptors, both analogues were 10^2 - 10^3 fold less active than the known[19b] bradykinin antagonist D-Arg-[Hyp^3,Thi^5, D-Tic^7, Oic^8] bradykinin (Hoe 140). Recently, another bradykinin antagonist, containing a 1,5-disubstituted tetrazole dipeptide as a *cis*-Ser^6-Pro^7 mimetic, was shown[9c] to be inactive at the bradykinin B_2 receptor. While it cannot be concluded from these data that the *cis*-Ser^6-Pro^7 conformation is not recognized by these receptors, recent results from Kyle and coworkers[19a], who have developed a new series of potent bradykinin antagonists, suggest that the active conformation of bradykinin comprises a C-terminal ß-turn with the *trans*-Ser^6-Pro^7 geometry. However, the dipeptide mimetics of type **1** (R = H) reported here can now be produced by an

efficient synthetic route, and may be incorporated into a wide variety of peptide sequences. Hence, they may find general use in the development of new receptor antagonists and enzyme inhibitors. The elaboration of this system into other Xaa-Pro mimetics (1, R = alkyl) and peptide-turn templates is currently under investigation.

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16. Selected analytical data for compounds **4**, **7**, and **8** are as follows : **4**, colourless solid m.p. 65-66°; [α] -44.0° (c 1.0, CH_2Cl_2) ; υ_{max} 2950, 1725, 1630 cm^{-1}; δ (^1H, $CDCl_3$) 3.67(1H, m), 3.52(1H, m), 2.49(1H, m), 2.45(1H, m), 2.41(1H, m), 2.29(1H, m), 1.90(1H, m), 1.84(1H, m), 1.70(2H, m), 1.60(1H, m), 1.47(1H, m), 1.45(9H, s); CI-MS: (butane) 240 (100, M+H); **7**, colourless glass; [α] -23.1° (c 0.7, MeOH); υ_{max} 3350, 3050, 2950, 1705, 1650 cm^{-1} ; δ (^1H, DMSO) 7.84(2H, d), 7.67(2H, d), 7.40(2H, t), 7.31(2H, t), 6.92(1H, br d), 4.33(2H, m), 4.23(1H, m), 3.94(1H, q), 3.60(1H, m), 3.30(1H, m), 2.40(2H, m), 2.00(1H, m), 1.6-1.9(5H, m). **8**, colourless solid, m.p. >220° dec.; [α] -10.1° (c 0.85, DMSO) ; υ_{max} 3340, 3050, 2950, 1715, 1600, 1580 cm^{-1} ; δ (^1H, DMSO) 7.84(2H, d), 7.70(2H, d), 7.40(2H, t), 7.31(2H, t), 7.02(1H, br d), 4.34-4.20(3H, m), 4.00(1H, m), 3.52(1H, m), 3.35(1H, m), 2.45(1H, m), 2.30(1H, m),1.62-2.02(6H, m).
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