Synthesis and structure of *cis*-peptidyl prolinamide mimetics based upon 1,2,5-triazepine-3,6-diones

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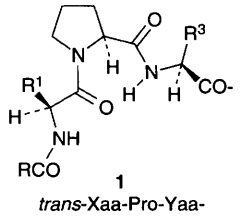
The synthesis of novel constrained *cis*-peptidyl prolinamide mimetics for the dipeptide amides glycyl (2*S*)-prolinamide, (2*S*)-alanyl (2*S*)-prolinamide and (2*R*)-alanyl (2*S*)-prolinamide and some analogues, based upon fused 1,2,5-triazepine-3,6-diones, is described; these are suitable for elaboration into larger peptides at both the carboxy and amino termini, X-ray crystal structures for some of the compounds and intermediates are also presented

Proline is unique amongst proteogenic amino acids in possessing a secondary amino group. Acyl prolines possess no amide hydrogen atoms and, therefore, the energies of the *cis*- and *trans*-isomeric forms are similar.[1] In nature, the Xaa-Pro peptide bond can exist in both stable conformations, **1** and **2**, and both forms occur in proteins, as in ribonuclease,[2] and in bioactive peptides, such as bradykinin.[3] The interconversions of the *cis*- and *trans*-forms of small peptides are quite slow[4] in water (k = 10^{-1}–10^{-3} s^{-1} at 30 °C) and peptidylprolyl *cis*–*trans*-isomerases (rotamases), *e.g.* cyclophilin[5] and FK506 binding protein,[6] exist to speed-up the isomerisation rates.[7] In recent years there has been immense interest in these enzyme activities and it is now believed that the slow uncatalysed isomerisation rate is important and may play a role in controlled proteolysis.

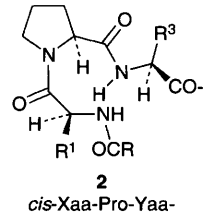
Given that some proline-containing bioactive peptides including angiotensin and thyroliberin are believed to bind to their receptors with the Pro residue fixed in the *cis*-form[8] it has been of interest to synthesise constrained *cis*-peptidyl prolinamides which might serve as high affinity ligands. In all examples of previously reported constrained *cis*-peptidyl proline analogues, the constraining modification had caused a significant increase in the steric size of the system, for example, the C^{α}-C^{α} bridged Gly-Pro mimetics recently described by Robinson[9] and Curran.[10] We wished to introduce the smallest modification possible in order to retain the maximum potential for biological activity in the finished peptides and/or structural motifs of proteins and, therefore, opted to link the N^{α}-atom of the residue preceding Pro to the N^{α}-atom of the residue following Pro. Such a modification differs from the parent system **2** by just two H-atoms, but fixes the stereochemistry of the acyl proline amide bond in its *cis*- or (*E*)-configuration through the formation of a novel 4,5-fused 1,2,5-triazepine-3,6-dione system **3**. It was envisaged that the other stereocentres could be controlled during synthesis.

To synthesise the simplest 4,5-fused 1,2,5-triazepine-3,6-dione **4**, (2*S*)-proline methyl ester was treated with bromoacetic acid activated as its mixed anhydride to give the required bromoacetamide **5**, Scheme 1. Treatment with hydrazine hydrate in ethanol was expected to give the α-bromoacetyl proline hydrazide **6** which would cyclise through the nucleophilic displacement of bromide ion by the non-acylated hydrazine N-atom to give the required *cis*-glycyl (2*S*)-prolinamide analogue **4**. In the event, after refluxing for 1.5 h, a compound was isolated which gave the expected mass and showed the existence of two major conformational isomers in its ^1H and ^{13}C NMR spectra in [$^2H_6$]-Me_2SO.† The compound failed to form a hydrazone adduct upon treatment with benzaldehyde and together these results indicate that a 7-membered triazepine rather than a 6-membered piperazine ring had been formed.

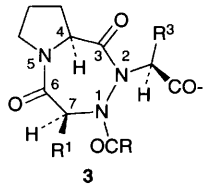
Each of the diastereoisomeric *cis*-(2*R*)-and *cis*-(2*S*)-alanyl (2*S*)-proline ester homologues, **5a** and **5b**, were prepared in a similar manner, starting from the appropriate chiral 2-bromopropanoic acids, which were themselves prepared *via* the diazotisation-bromination of (2*R*)- and (2*S*)-alanine. Each diastereoisomeric ester **5a** and **5b** was obtained as a crystalline



1
trans-Xaa-Pro-Yaa-



2
cis-Xaa-Pro-Yaa-



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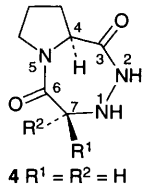
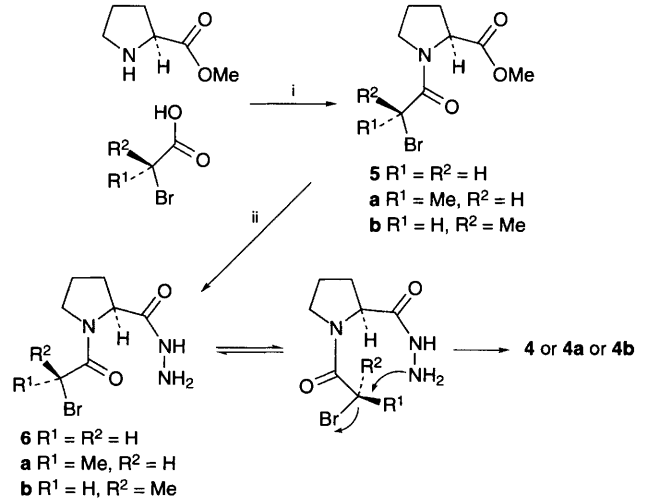


4 R^1 = R^2 = H
a R^1 = Me, R^2 = H
b R^1 = H, R^2 = Me



5 R^1 = R^2 = H
a R^1 = Me, R^2 = H
b R^1 = H, R^2 = Me

6 R^1 = R^2 = H
a R^1 = Me, R^2 = H
b R^1 = H, R^2 = Me

Scheme 1 *Reagents and conditions*: i, NMM, *iso*-butyl chloroformate, THF/DMF, −40 °C; ii, $H_2NNH_2.H_2O$, EtOH, reflux

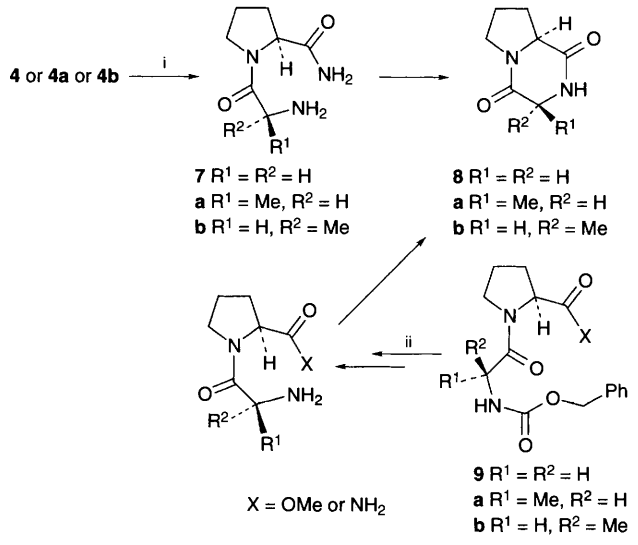
solid† in moderate yield but examination of the NMR spectra of the crude reaction mixtures for each of the coupling reactions indicated that some epimerisation had occurred. This was largely prevented by lowering the temperature of the reactions from −10 to −40 °C. For the compound **5a**, that derived from (2*R*)-2-bromopropanoic acid, the stereochemistry at C-2 of the 2-bromopropanoyl moiety was verified by X-ray crystallography, Fig. 1.‡

Treatment of each of the individual esters **5a** and **5b** with hydrazine hydrate in refluxing ethanol gave a new product almost immediately, as judged by TLC and by NMR spectroscopy, but in each case, reaction to give a second new product was not complete until 16 h had elapsed. Thus, it appeared that for each of the homologues **6a** and **6b** the cyclisation step was significantly slower than for the non-methylated derivative **6**. These observations are almost certainly explained by; (i) the low equilibrium concentration of the *cis*-isomers of **6a** and **6b** which are required for the cyclisation, due to the steric effects of the extra methyl groups, (compared to hydrazide **6**), and; (ii) the fact that the cyclisation itself occurs *via* S_N2 attack on a secondary α-bromoacylamide instead of a primary α-bromoacylamide, as in the case for hydrazide **6**. Nevertheless, after prolonged reaction, each of the triazepines **4a** and **4b** were obtained in excellent yield (>90%).†

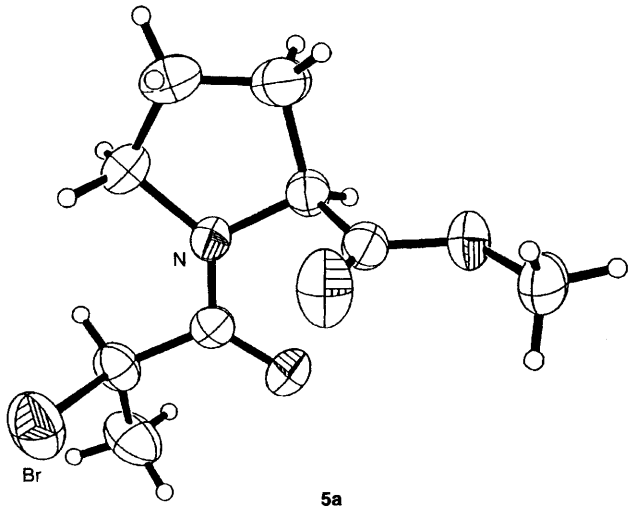
To confirm that the cyclisations had proceeded with inversion of configuration, the fused triazepines **4a** and **4b** were each subjected to dissolving metal reduction (Scheme 2) to give the alanyl prolinamides **7a** and **7b**. These underwent spontaneous cyclisation to give the diketopiperazines **8a** and **8b** through the displacement of ammonia from the Pro carboxamide moiety. The same diketopiperazines **8a** and **8b** were also prepared by deprotecting the terminal N-atom of N–Z proline esters[11] (**9a** and **9b**, X = OMe) or N–Z prolinamides (**9a** and **9b**, X = NH_2) (Z = benzyloxycarbonyl). Comparison of the ^1H and ^{13}C NMR spectra of the diketopiperazines indicated that the cyclisation of **6a** and **6b** to form the triazepines **4a** and **4b** occurred with inversion at the secondary carbon centre. Thus, it was possible to synthesise *cis*-aminoacyl prolinamide mimetics of defined stereochemistry.

To assess the possibility that such triazepines might be extended to provide longer and more elaborate peptide mimetics, each of the α-bromoacyl (2*S*)-proline methyl esters **5**, **5a** and **5b** were treated with methylhydrazine. As expected, the ester methoxy group was displaced by the less hindered primary amino group and subsequent cyclisation occurred with inversion to give the N^1-methyl-1,2,5-triazepine diones **10**, **10a** and **10b**.† Since these compounds were resistant to reduction, the structures of compounds **10** and **10b** were verified by X-ray crystallography using the known stereochemistry at C-2 of proline for reference, Figs. 2 and 3.‡ Thus the use of monoalkylated hydrazines should provide access to N^1- alkyl-1,2,5-triazepine diones. Treatment of **4**, **4a** and **4b** with acetic anhydride gave the N^1-acetyl-1,2,5-triazepine-3,6-diones **11**, **11a** and **11b**.† Similarly treatment of **12** with activated N–Z-(2*S*)-phenylalanine gave the corresponding N^1-phenylalanyl derivative,† indicating that extension at the *N*-terminal of the peptide mimetic was possible.

Extension at the Pro carboxamide terminal proved to be more difficult because substitution at the N^2-position of the 1,2,5-triazepine-3,6-dione system required the synthesis of unsymmetrical alkylhydrazines protected on the primary amino group. Nevertheless, N^2-*tert*-butoxycarbonyl-N^1-methylhydrazine was prepared from methylhydrazine in three steps and the reaction of this with the mixed anhydride of chloroacetyl (2*S*)-proline, Scheme 3, gave the required proline hydrazide **13** in 73% yield.† Removal of the *tert*-butoxycarbonyl protecting group with hydrogen chloride gave the salt which upon treatment with *N*-methylmorpholine (NMM) cyclised to give the desired N^2-methyl-1,2,5-triazepine-3,6-dione **14**.† Using similar protocols we have now also prepared the *cis*-glycyl prolyl phenylalanine methyl ester mimetic **15**† which is part of the sequence of the self-cleaving polypeptide from the foot and mouth disease virus 2A region.[12] Full details will be reported in due course.[13] Thus,

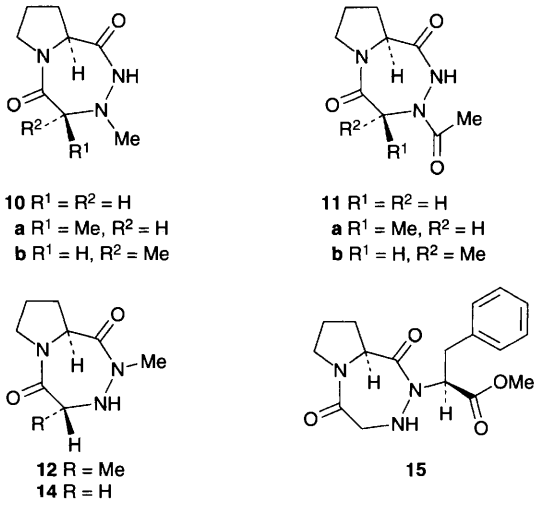


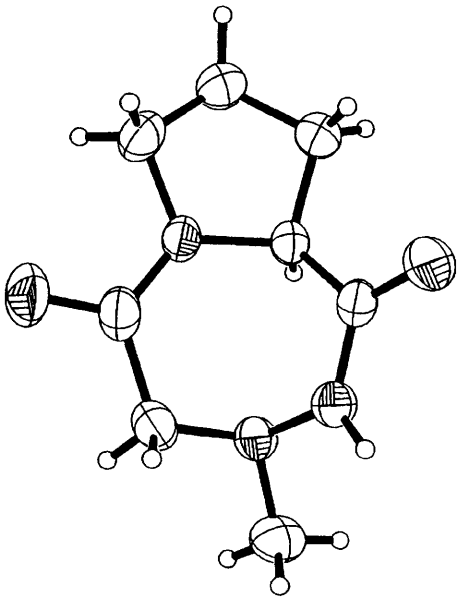
Scheme 2 *Reagents and conditions*: i, $Na/NH_{3(liq)}$, −68 °C; ii, $H_{2(g)}$/Pd/C, MeOH, 22 °C



5a

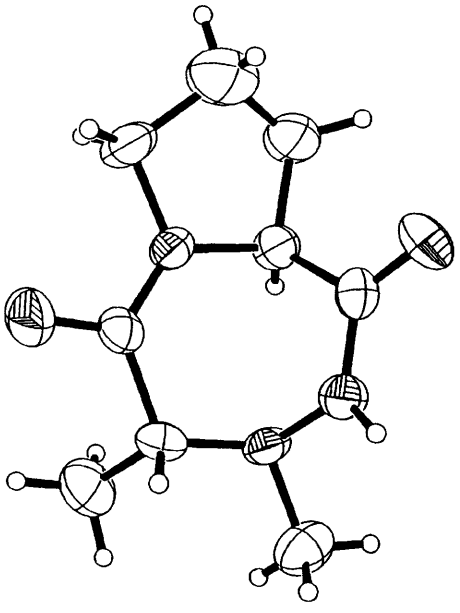
Fig. 1





10

Fig. 2



10b

Fig. 3

we have demonstrated that this new *cis*-aminoacyl prolinamide mimetic, the fused 1,2,5-triazepine-3,6-dione system can be extended at the amino terminal (N^1- of the triazepine) or the carboxy terminal (N^2- of the triazepine) to provide more elaborate mimetics.

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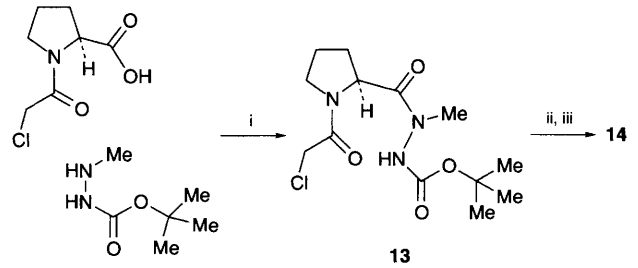

13

Scheme 3 *Reagents and conditions*: i, NMM, *iso*-butyl chloroformate, THF, −10 °C, 12 h, 73%; ii, HCl, EtOAc, 22 °C; 15 min., 100%; iii, NMM, MeOH, 22 °C, 5 min., 79%

Footnotes

† All compounds were fully characterised and gave the expected spectral and analytic data.

‡ *Crystal data* for **5a**; $C_9H_{14}NO_3Br$, M = 264.12, trigonal, space group $P3_2$, a = 9.7930(10), c = 9.978(2) Å, V = 828.7(2) Å^3, Z = 3, D_c = 1.588 g cm^{-3}, T = 293 K, $F(000)$ = 402, R_1 = 0.0343 [940 reflections with $F_o > 4\sigma(F_o)$], wR_2 = 0.0944 for 1029 independent reflections corrected for absorption [μ(Mo-Kα) = 3.703 mm^{-1}] and 129 parameters. For **10**; $C_8H_{13}N_3O_2$, M = 183.21, monoclinic, space group $P2_1$, a = 9.039(3), b = 10.664(3), c = 9.496(3) Å, Å, β = 101.85(2)°, V = 895.8(4) Å^3, Z = 4, D_c = 1.358 g cm^{-3}, T = 293 K, $F(000)$ = 392, R_1 = 0.0299 [2466 reflections with $F_o > 4\sigma(F_o)$], wR_2 = 0.0800 for 2641 independent reflections [μ(Mo-Kα) = 0.100 mm^{-1}] and 253 parameters. For **10b**; $C_9H_{15}N_3O_2$, M = 197.24, monoclinic, space group $P2_1$, a = 6.5861(5), b = 10.5475(10), c = 7.3384(7) Å, β = 97.934(7)°, V = 504.90(8) Å^3, Z = 2, D_c = 1.297 g cm^{-3}, T = 293 K, $F(000)$ = 212, R_1 = 0.0347 [1691 reflections with $F_o > 4\sigma(F_o)$], wR_2 = 0.0998 for 1767 independent reflections [μ(Mo-Kα) = 0.094 mm^{-1}] and 134 parameters.

Atomic coordinates, bond lengths and angles and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Information for Authors, Issue No. 1.

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