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A CONVENIENT SYNTHESIS OF THE CONFORMATIONALLY CONSTRAINED AMINO ACID 5,5-DIMETHYLPROLINE

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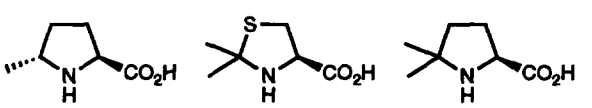
Abstract: A convenient synthesis of Boc-DL-5,5-dimethylproline methyl ester is described. This conformationally-constrained amino acid may be expected to exist predominantly in the *cis* peptide bond isomer when incorporated into peptides. The peptide bond in the model peptide Boc-Phe-Me_2Pro-OMe is shown to be 90% in the *cis* isomer.

While most peptide amide bonds occur predominantly as the *trans* rotamer, there is the possibility of *cis-trans* isomerism for X–Pro or X–N-methylamino acid peptide bonds because the energies of the *cis* and *trans* isomers differ by only about 0.5 kcal/mol.[1] The occurrence of *cis* peptide bonds is becoming recognized as an increasingly important structural feature in both peptides and proteins. X-ray crystal structures of several proteins have revealed specific *cis* X-Pro peptide bonds[2] and proline isomerization has been implicated as a slow step in protein folding.[3] Cyclophilin, the binding protein for the immunosuppressive peptide cyclosporin A, has been shown to possess peptidyl-prolyl *cis-trans* isomerase (PPIase) activity[4,5] and cyclosporin A itself has been shown to contain a *cis* peptide bond both in solution and X-ray crystal structures.[6]

In linear peptides, *cis-trans* isomerism about X–Pro bonds is an equilibrium process and the amount of *cis* isomer present varies both with the peptide sequence[7,8] and solvent conditions.[9] Investigators have employed variously substituted proline analogs[10] or modifications to the peptide sequence to alter the *cis/trans* ratio in peptides. For example, the *anti*-5-methylproline **1** has been shown to exhibit a relatively high percentage (30-50%) of *cis* isomer in model compounds,[11] and the *cis* content of PPIase substrates of the sequence Suc-Ala-X-Pro-Phe-pNA has been altered by varying X.[12] In both cases, the peptides still exist as an equilibrium mixture of *cis* and *trans* isomers and the fraction of *cis* isomer is still fairly modest. In contrast, 2,2-dimethylthiazolidine carboxylic acid **2** has been shown to give rise exclusively to the *cis* peptide bond isomer in a model peptide.[13] The dimethylthiazolidine nucleus is very acid-labile, however, and is not well-suited to incorporation into peptide analogs.

We were interested in a proline analog which would be stable to the conditions of peptide synthesis, could readily be incorporated into different peptides and which would exist in peptides substantially in the *cis* peptide bond isomer. We selected 5,5-dimethylproline (**3**) which should be chemically stable and, based on molecular modelling considerations, should be very similar to **2** in its conformational preferences.[14]

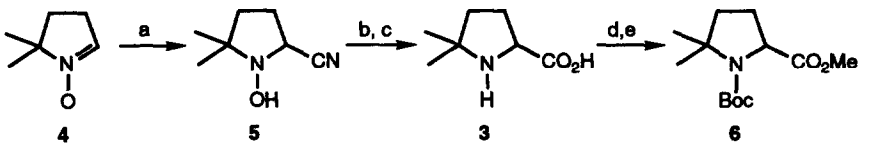


1 **2** **3**

5,5-Dimethylproline (**3**) has been previously prepared by acid-catalyzed addition of cyanide to 5,5-dimethyl-1-pyrroline, which was itself prepared in four steps from 4-methyl-4-nitropentan-1-al.[15] Acid hydrolysis of the resulting nitrile gave the free acid **3** as a D,L mixture. We wish to report a more convenient synthesis of 5,5-dimethylproline derivatives suitable for peptide synthesis, as shown in Scheme 1, and incorporation into a model dipeptide.

Nitrones readily undergo acid-catalyzed addition of cyanide to yield N-hydroxynitriles. Thus the commerically available 5,5-dimethylpyrroline-1-oxide **4** was treated with KCN in 2N HCl to give N-hydroxy-2(*RS*)-cyano-5,5-dimethylpyrrolidine **5**.[16, 21] This material was then hydrolyzed to the somewhat labile N-hydroxyamino acid which was immediately reduced to racemic **3** by catalytic hydrogenation[17] without isolation. While the amino acid **3** could be isolated at this point, it proved more convenient to convert it to a diprotected derivative which could be isolated and purified more readily. Therefore **3** was first converted to the methyl ester hydrochloride by treatment with HCl/methanol. Then the Boc group was introduced by refluxing the methyl ester hydrochloride with di-*t*-butyldicarbonate and N-methylmorpholine in methylene chloride. We have found this to be an efficient method for preparing Boc derivatives of sterically hindered amino acids which react poorly under Schotten-Baumann conditions. The hydrolysis, reduction and protection can all be carried out without isolation of the intermediates, yielding the diprotected product **6** in 38% overall yield after purification by flash silica gel chromatography.[22] Conversion of the amino acid to the diprotected derivative is a convenient, one-pot procedure which allows us to minimize handling of the poorly soluble amino acid and amino ester intermediates.

Scheme I



a. 2N HCl, KCN; b. conc. HCl; c. H_2/Pd, MeOH; d. HCl, MeOH; e. Boc_2O, N-methylmorpholine, CH_2Cl_2, reflux.

In order to determine the effect of 5,5-dimethylproline on peptide bond *cis-trans* isomerism, the Boc methyl ester **6** was deblocked with HCl/dioxane and coupled to Boc-L-Phe using DCC/HOBt in DMF to give the dipeptide Boc-L-Phe-DL-5,5-Me_2Pro-OMe as a mixture of diastereomers in 78% yield after extractive work-up and initial purification by silica gel flash chromatography. It was not possible to separate the two diastereomers either by normal phase or reverse phase hplc. An analytical sample of the peptide was purified by reverse phase hplc, giving a 4:1 mixture of diastereomers[18] as determined by chiral analysis of the acid hydrolysate using tetraacetyl glucose isothiocyanate.[19, 20] The ROESY spectrum taken at 20° C in MeOH showed two pairs of signals arising from the Phe NH's. The two signals in each pair were connected by an off-diagonal cross-peak with positive intensity, indicating that each pair represented the *cis* and *trans* isomers of one of the diastereomeric dipeptides. The ratio of intensities in each pair of signals was 9:1. In the case of the more abundant diastereomer, the major rotamer was determined to be *cis* due to the presence of an NOE between the α-protons of the Phe and Me_2Pro residues associated with the major isomer NH signal, which is only consistent with a *cis*-peptide bond conformation. It was not possible to unambiguously detect the same

NOE in the less abundant diastereomer because the signal intensities were too low, but *cis-trans* isomerism in such a small, unconstrained peptide is unlikely to be affected by the optical configuration of the dimethylproline residue. In addition, both the ratio of intensity and the relative chemical shifts of the Phe NH signals were similar for both diastereomers, suggesting that the *cis-trans* isomer ratio is likewise similar in each diastereomer.

In conclusion, we have developed a simple and convenient method for the preparation of protected 5,5-dimethylproline analogs suitable for peptide synthesis. In a model dipeptide, this conformationally-constrained amino acid exists 90% as the *cis* peptide bond isomer. We expect that this amino acid will prove useful in assessing the role of *cis* X-Pro peptide bonds in biologically active peptides.

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14. Theoretical energy calculations on both the *cis* and *trans* rotamers of the N-acetyl-N'-methylamide derivatives of **2** and **3** were carried out using the MM2 forcefield implementation in Macromodel v3.3.1 (Mohamadi, F.; Richards, N. G. T.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comp. Chem.*, **1990**, *11*, 440-467). For acetyl-**2**-N-methylamide, the *cis* isomer is 3.85 kcal/mol lower in energy than the *trans* isomer. For acetyl-**3**-N-methylamide, the *cis* isomer is 1.92 kcal/mol lower in energy than the *trans* isomer.
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18. We have previously observed unexpectedly strong diastereoselectivity in the coupling of the monosubstituted 5-methylprolines as well.

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20. Reaction of DL-5,5-dimethylproline with GITC gave two peaks of equal intensity upon hplc analysis, indicating that there is no diastereoselectivity in the derivatization reaction.

21. **1-Hydroxy-2-(*RS*)-cyano-5,5-dimethylpyrrolidine (5).** Potassium cyanide (5.74 g, 88.4 mmol) was dissolved in 10 mL water and cooled to -10° C. To this was added 5,5-dimethyl-1-pyrroline N-oxide (5.0 g, 44.2 mmol) in 10 mL of water. 2 N HCl (125 mL) was added dropwise over one hour, then stirred at 0° C for 3 hours and at room temperature overnight. The pH was adjusted to 11 with 6 N KOH and the solution was extracted with ethyl ether. The ether extracts were dried over $MgSO_4$ and evaporated to yield 3.65 g of a white powder (59%), m.p. 86-87° (lit. 92°, recrystallized[16]); ^1H NMR ($CDCl_3$): δ 1.0 (6H, s); 1.6–2.2 (4H, m); 3.9 (1H, t); 6.1 (1H, s); DCI mass spectrum (CH_4): m/z 141.1 (MH^+); 114.1 ($M\text{-}HCN^+$).

22. **Boc-DL-5,5-dimethylproline methyl ester (6).** The nitrile **5** (3.5 g, 25 mmol) was hydrolyzed in conc. HCl (10 mL) at 50° C for 5 hours and evaporated to a white solid. After evaporating from water two times to remove traces of HCl, the residue was dissolved in methanol/water 1:1 and hydrogenated over 10% Pd/C in a Parr shaker for 4 hours. The catalyst was removed by filtration and the solvent evaporated. The resulting dimethylproline was dissolved in methanol and dry HCl gas was passed through the solution for 1 hour, then sealed and allowed to stand at room temperature overnight. After removal of solvent and evaporation from methanol two times to remove residual HCl, the resultant methyl ester hydrochloride was dissolved in methylene chloride. Di-*t*-butyldicarbonate (6.0 g, 27.5 mmol) and N-methylmorpholine (3 mL, 25 mmol) were added and the mixture was heated at reflux for 48 hours. The solution was cooled, washed with water, dried over $MgSO_4$ and evaporated to a colorless oil which was purified by flash silica gel chromatography ($CHCl_3$) to yield 2.45 g of a colorless oil (38% based on starting nitrile); ^1H NMR ($CDCl_3$): δ 1.6–1.7 (15H, overlapping s); 1.4–1.8 (4H, m); 3.8 (3H, s); 4.2–4.3 (1H, m); DCI mass spectrum (NH_3): m/z 258.1 (MH^+); 158.1 ($M\text{+}H\text{-}Boc^+$).

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