Efficient Synthesis of a New Amino Acid, (2*S*,3*R*,4*R*)-4-Chloro-3-hydroxyproline

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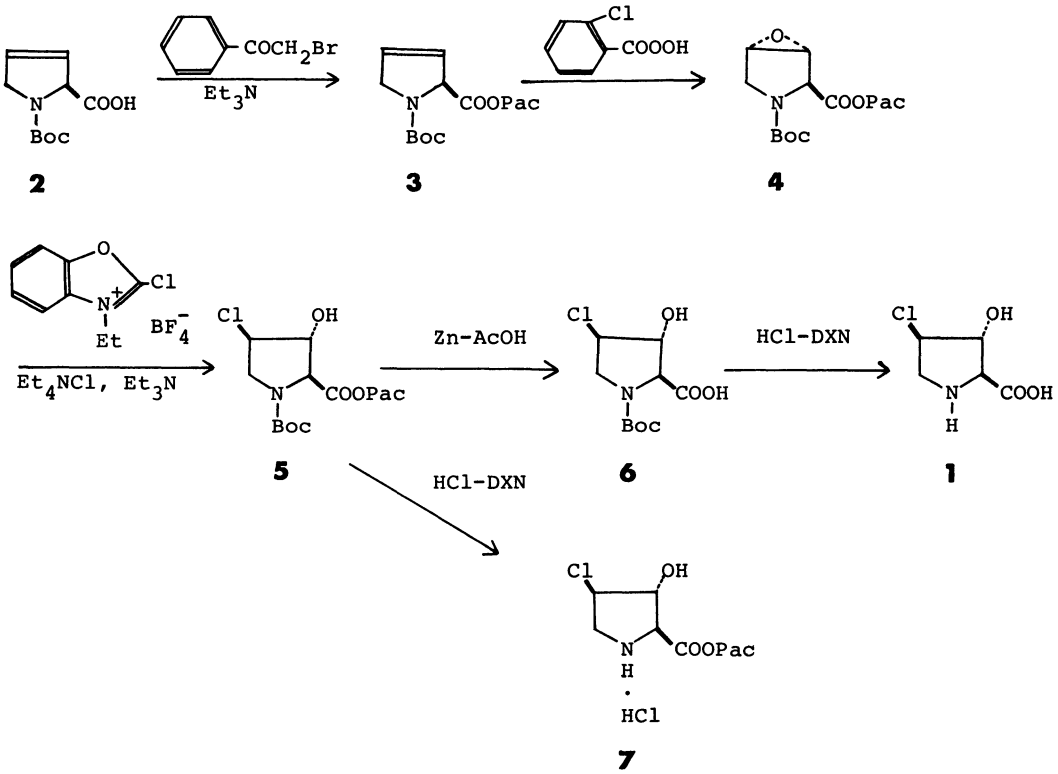
An unusual new amino acid, (2*S*,3*R*,4*R*)-4-chloro-3-hydroxyproline, was stereospecifically synthesized through a four-intermediate from Boc-3,4-dehydro-L-proline. The obtained product was stable in a mild alkaline solution.

The 3,4-disubstituted prolines occur in natural cyclic peptide toxins, e.g., virotoxin[1] and cyclochlorotine.[2] The two diastereomeric 3,4-dihydroxy-L-prolines in virotoxin were synthesized from *N*-tosyl-3,4-dehydro-L-proline methyl ester by Kahl and Wieland.[3] (2*S*,3*S*,4*R*)-Dichloroproline in cyclochlorotine has neither been isolated in a free state nor synthesized because of its instability under acid hydrolytic and mild basic conditions. In the course of studying the structure-activity relationship of cyclochlorotine, we found that both chlorine moieties in 3,4-dichloroproline are essential regarding toxic activity,[4] although attempts to synthesize 3,4-dichloroproline were unsuccesful.

Here, we report the synthesis of an interesting proline derivative, (2*S*,3*R*,4*R*)-4-chloro-3-hydroxyproline (**1**) through a stereoselective route (Scheme 1). The phenacyl group, which is deprotected by zinc powder in an aqueous acetic acid, was selected for the protection of the carboxyl group in order to avoid alkaline conditions.[5] Boc-3,4-dehydro-L-proline (**2**) was converted to its phenacyl ester (**3**) in high yield. Compound **3** was treated with *m*-chloroperbenzoic acid and an obtained crude product was purified by silica gel chromatography to give the corresponding epoxide (**4**). The stereochemistry in this epoxide was not directly clarified but a TLC of the reaction mixture showed only two main spots of the epoxide and the starting material, except for a few trace spots. This result suggests that the configuration of the obtained epoxide is a 2,3-*trans* form in consideration of the stereoselective epoxidation of *m*-chloroperbenzoic acid.[6] A treatment of **4** with 2-chloro-3-ethylbenzoxazolium tetrafluoroborate was expected to give Boc-3,4-dichloro-L-proline phenacyl ester,[7] but yielded Boc-3-hydroxy-4-chloro-L-proline phenacyl ester (**5**) instead. A deprotection of the phenacyl group using zinc powder in acetic acid gave Boc-3-hydroxy-4-chloro-L-proline (**6**), which was converted to 3-hydroxy-4-chloro-L-proline (**1**) with HCl in dioxane. Compound **5** was treated with HCl in dioxane to

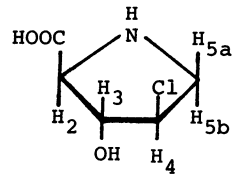


Scheme 1.

Table 1. Chemical Shifts (δ) and Coupling Constants of **1** and Comparison with 3,4-Dihydroxy-L-proline (**8**)

H[a]	δ of **1** (in DMSO-d_6)	Coupling constant (Hz)			
			1 (in DMSO-d_6)	(2*S*,3*R*,4*R*)-**8**[3] (in D_2O)	(2*S*,3*S*,4*S*)-**8**[3] (in D_2O)
2	4.13	J(2, 3)	1.5	1.5	4.0
3	4.58	J(3, 4)	1.1	1.3	1.3
4	4.50	J(4, 5a)	1.5	1.5	
5a	3.55	J(4, 5b)	4.2	3.5	4.0
5b	3.78	J(5a, 5b)	13.4	12.4	12.9

a) Protones of **1** were defined as described below.



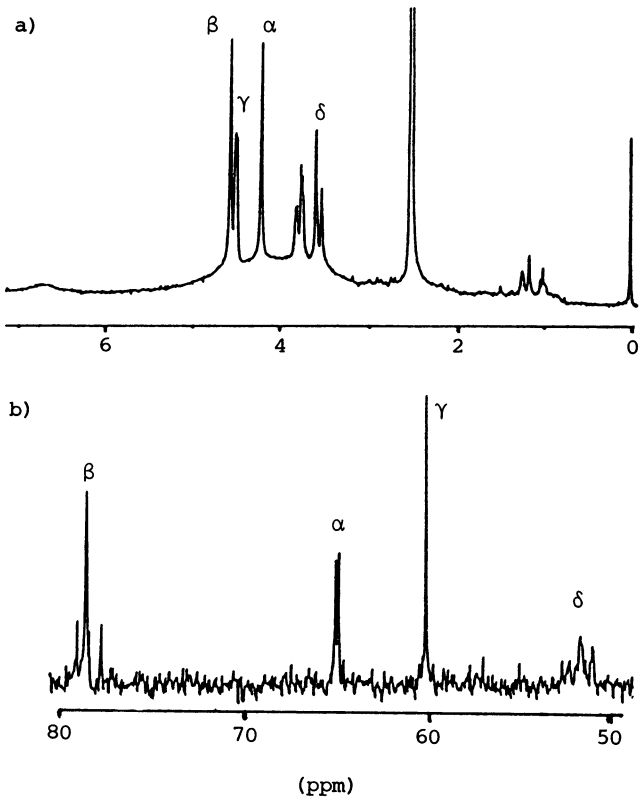


Fig. 1. ^{1}H-NMR spectrum of **1** (a) and ^{13}C-NMR spectrum of **1** resulting from selective irradiation of γH (b). The peak of δC showed a coupling with γH.

give 3-hydroxy-4-chloro-L-proline phenacyl ester (**7**). Compounds **6** and **7** can be used as acid and amine components, respectively, in a peptide synthesis.

The substituted positions of 3-hydroxy and 4-chloro groups in the proline was determined by a ^{13}C{^{1}H} selective decoupling technique of NMR (Fig. 1). The stereochemistry of 3-hydroxy and 4-chloro groups was determined to be 2,3-*trans* and 3,4-*trans*, respectively, compared to the coupling constants of 3,4-dihydroxy-L-proline diastereoisomer (Table 1). These results suggest that the ring opening of epoxide **3** arose from its trans side, attacking (exclusively) position 4.

This 3-hydroxy-4-chloro-L-proline was stable under ammoniacal alkaline conditions the same conditions under which dichloroproline in cyclochlorotine decomposes to a pyrrole derivative with the release of hydrogen chloride.[2] This result means that 3-hydroxy-4-chloro-L-proline derivatives can be used for a conventional peptide synthesis. Proline analogs with a variety of functional groups have been developed for use in peptide and protein chemistry since proline often plays an important role for maintaining conformation and biological activities. This new amino acid may be useful for structure-activity studies as a proline analog having both hydrophobic and hydrophilic functional groups.

Experimental

All the melting points are uncorrected. TLC was carried out on silica gel G (Merck). Optical rotations were measured on a Union high-sensitivity polarimeter PM-71. ^{1}H and ^{13}C NMR spectra were recorded in a DMSO-d_6 solution on a JEOL FX-200 spectrometer at 29°C. Tetramethylsilane was used as an internal reference; sample concentration, 10—30 mg ml^{-1}. Amino acid analyses were performed using a Yanagimoto automatic analyzer LCA-8.

Boc-3,4-dehydro-L-proline Phenacyl Ester (3): To a solution of Boc-3,4-dehydro-L-proline (**2**)[8] (6.39 g, 30 mmol) and Et_3N (4.06 ml) in EtOAc was added phenacyl bromide (5.77 g, 29 mmol) at room temperature. Stirring was continued for 6 h and the precipitate was filtered off. The filtrate was diluted with EtOAc (100 ml). The solution was washed with water, 4% $NaHCO_3$ and water, and dried over Na_2SO_4. The filtrate was evaporated in vacuo to give an yellow oil; yield, 9.47 g (98%); R_f=0.66 ($CHCl_3$–EtOH, 9:1).

***Trans*-Boc-3,4-epoxy-L-proline Phenacyl Ester (4):** To a solution of **3** (9.47 g, 28 mmol) and 2,6-di-*t*-butyl-*p*-cresol in $CHCl_3$ (100 ml), *m*-chloroperbenzoic acid (6.79 g, 28 mmol of 80% assay) dissolved in $CHCl_3$ (50 ml) was added dropwise over a period of 2 h with vigorous stirring. The mixture

was heated to reflux for 3 h. The content of the flask was cooled in an ice bath and the precipitate was removed by filtration. The filtrate was washed with 10% $NaHSO_3$, 4% $NaHCO_3$, and water, and dried over Na_2SO_4. The filtrate was then evaporated to give an oil (9.80 g). A solution of the oil in $CHCl_3$ was applied to a silica gel column (4×62 cm); cyclohexane–EtOAc (4:1) was used as an eluant and a 7.5 ml fraction was collected. Fractions 358—490 were collected and evaporated to produce an oil; yield, 2.12 g (22%); R_f=0.35 (cyclohexane–EtOAc, 4:1). A portion of starting material **3** (4.01 g) was recovered from fractions 235—335.

Boc-(2*S*,3*R*,4*R*)-4-chloro-3-hydroxyproline Phenacyl Ester (5): To a stirred suspension of **4** (335 mg, 1 mmol), tetraethylammonium chloride (165 mg, 1 mmol) and 2-chloro-3-ethylbenzoxazolium tetrafluoroborate (269 mg, 1 mmol) in CH_2Cl_2 (4 ml) was added (dropwise) a solution of Et_3N (0.17 ml) in CH_2Cl_2 (2 ml) at 0 °C under a nitrogen atmosphere.[7] The resulting mixture was stirred at room temperature for 48 h. After evaporation of the solvent, the residue was applied to a LiChroprep Si 60 (40—63 μm) column (1×20 cm); $CHCl_3$–EtOAc (9:1) was used as an eluant, 2 ml fraction. Fractions 10—13 were collected and evaporated to give an oil; yield 120 mg (32%); R_f=0.58 ($CHCl_3$–EtOAc, 9:1).

Boc-(2*S*,3*R*,4*R*)-4-chloro-3-hydroxyproline (6): To a stirred solution of **5** (250 mg, 0.67 mmol) in 90% acetic acid was added zinc powder (30 mg) at room temperature. After 2 h, the precipitate was filtered off, and the filtrate was evaporated in vacuo. The residue was acidified with 10% citric acid and extracted with EtOAc. The extract was dried over Na_2SO_4 and evaporated to give an oil; yield 150 mg (84%). R_f=0.17 ($CHCl_3$–MeOH, 5:1).

(2*S*,3*R*,4*R*)-4-Chloro-3-hydroxyproline Phenacyl Ester Hydrochloride (7): Compound **5** (690 mg, 1.8 mmol) was treated with 3.5 M HCl (1 M=1 mol dm^{-3}) in dioxane (5.1 ml) for 2 h and the solution was evaporated. The obtained product was collected with the aid of ether. Recrystallization from MeOH–ether gave 463 mg (80%); mp 168—169 °C; R_f=0.38 ($CHCl_3$–MeOH, 9:1).

Found: C, 48.73; H, 4.83; N, 4.29%. Calcd for $C_{13}H_{15}O_4NCl_2$: C, 48.76; H, 4.72; N, 4.37%.

(2*S*,3*R*,4*R*)-4-Chloro-3-hydroxyproline (1): Compound **6** (30 mg, 0.1 mmol) was treated with 4.7 M HCl in dioxane (2 ml) for 2 h at room temperature. The solution was evaporated. The residual oil was applied to a Dowex 50 (H^+ form); 10% pyridine was used as an eluant. The fraction containing the product was evaporated to give a solid; yield, 12 mg (68%); $[\alpha]_D^{20}$ −18.1° (*c* 0.25, MeOH); R_f=0.34 (1-butanol–AcOH–pyridine–H_2O, 4:1:1:2).

Found: C, 34.39; H, 5.15; N, 7.75%. Calcd for $C_5H_8O_3NCl \cdot 1/2H_2O$: C, 34.39; H, 5.19; N, 8.02%.

Stability of 1: To a solution of **1** (5.0 mg) in water (2 ml), Et_3N was added to adjust at pH 10.5. The solution was left to stand for 2 d at room temperature. An aliquot of the solution was applied on the amino acid analyzer (column size; 0.4×12.5 cm, pH of buffer solution; 3.25). Only a single peak of **1**, eluted for 6.0 min (cf Pro, at 12 min), was detected.

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