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Syntheses and Separations of Diastereomers of Protected-Δ^1-, Δ^2-, and Δ^3-Dehydrotripeptides

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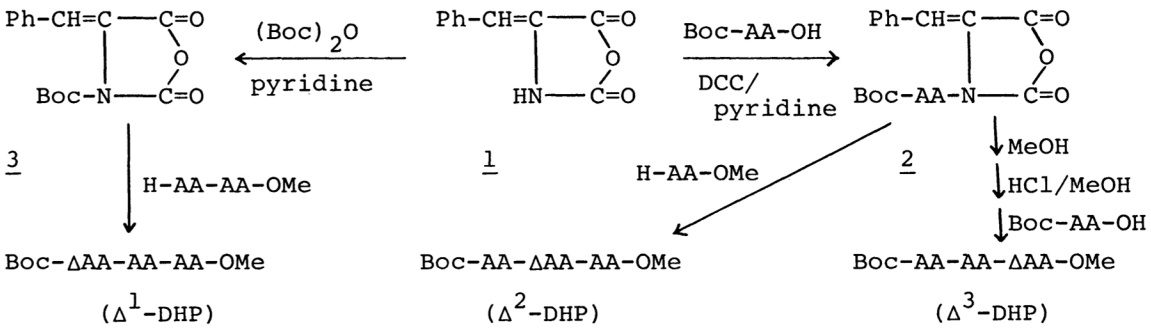
Total eighteen kinds of Δ^1-, Δ^2-, and Δ^3-dehydrotripeptides (DHP) containing of a ΔPhe and two chiral α-amino acid residues were synthesized and the diastereomeric mixture of DHP were subjected to the HPLC separation. From the results and the ^{1}H NMR spectral data, it was found to be significant relationship between the separation and the conformational structure of DHP.

Recently, the study on the separation of the diastereomers of tripeptides containing an uncommon amino acid and N-terminal racemic α-amino acid (AA) residues has been reported.[1] Up to now, however, the similar investigation of the diastereomeric mixtures of dehydrotripeptides (DHP) has never been reported. More recently, it was found that the close relationship between the separation and the conformation of diastereomeric DHPs was also very rigorously related to the asymmetric hydrogenation ability.[2] Therefore, here, first we would like to report on the high yield syntheses of eighteen kinds of Δ^1-, Δ^2-, and Δ^3-dehydrotripeptides (DHP)[3] by various combinations of (L)- and (D)-alanine (Ala), leucine (Leu), and (Z)-dehydrophenylalanine (ΔPhe) residues and the correlation between the conformational structures and the separation factor of the diastereomeric mixtures of the obtained DHP.

All the substrates DHP could be newly synthesized by the N-carboxy α-dehydroamino acid anhydride (ΔNCA) method,[4-6] according to the procedures reported previously,[7] as illustrated in Scheme 1. Thus, Δ^3-DHP was derived successively by the coupling of an N-carboxy dehydrophenylalanine anhydride (ΔPhe·NCA; 1)[4] with t-butoxycarbonyl (Boc)-AA-OH and by the ring cleavage of the resulting



Scheme 1.

Table 1. Boc-Δ^1-, Δ^2-, and Δ^3-Dehydrotripeptide-OMe Containing a Dehydrophenylalanine Residue

Compound Δ^3-DHP	Yield %	$[\alpha]_D^{25}$ a) /°	Compound Δ^2-DHP	Yield %	$[\alpha]_D^{25}$ a) /°	Compound Δ^1-DHP	Yield %	$[\alpha]_D^{25}$ a) /°
-A-L-ΔF-	65	-52.0	-A-ΔF-L-	80	-44.4	-ΔF-A-L-	80	-50.0
-(D)-A-L-ΔF-	60	55.5	-(D)-A-ΔF-L-	83	-17.6	-ΔF-(D)-A-L-	81	-125.7
-A-(D)-L-ΔF-	68	-55.0	-A-ΔF-(D)-L-	72	18.2	-ΔF-A-(D)-L-	82	124.8
-L-A-ΔF-	70	-50.7	-L-ΔF-A-	85	-16.2	-ΔF-L-A-	84	-49.7
-(D)-L-A-ΔF-	73	63.0	-(D)-L-ΔF-A-	78	-4.7	-ΔF-(D)-L-A-	85	-15.6
-L-(D)-A-ΔF-	70	-65.0	-L-ΔF-(D)-A-	83	4.5	-ΔF-L-(D)-A-	78	14.9

A=Alanine. L=Leucine. D=Dextrorotatory. ΔF=Dehydrophenylalanine.
a) Recorded in methanol (c 1.00).

Boc-AA-ΔPhe·NCA (2) with MeOH and finally, after deprotecting Boc-Δ^2-dehydrodipeptide methyl ester formed as an intermediate, by the elongation of the obtained H-AA-ΔPhe-OMe with Boc-AA-OH. On the other hand, treatment of 2 with H-AA-OMe as an amine component gave Δ^2-DHP. In addition, Δ^1-DHP was also obtained by the direct coupling of dipeptide methyl ester with Boc-ΔPhe·NCA (3), derived from 1 and di-t-butyl dicarbonate [$(Boc)_2O$]. As was summarized in Table 1, it can be seen that the high yield syntheses of Δ^1-, Δ^2-, and Δ^3-DHP are successful.

Subsequently, in order to acquire the separation factor (α value) of Δ^1-, Δ^2-, and Δ^3-DHP containing a (DL)-Ala residue, total twelve variations of exactly equimolar mixture of (L)-(L) and (D)-(L) or (L)-(D) sequential structures listed

Table 2. Separation of DHP Containing a Racemic Alanine Residue by HPLC

MeOH : H_2O (v/v, %)	A-L Sequence, α value a)			L-A Sequence, α value a)		
	-A-L-ΔF-	-A-ΔF-L-	-ΔF-A-L-	-L-A-ΔF-	-L-ΔF-A-	-ΔF-L-A-
75 : 25	1.00	1.00	1.08	1.00	1.00	1.00
70 : 30	1.00	1.00	1.12	1.00	1.00	1.05
65 : 35	1.00	1.00	1.19	1.00	1.00	1.07
60 : 40	1.00	1.05	1.28	1.00	1.05	1.13
	(1.00	1.05	1.28) b)	(1.00	1.05	1.13) b)
55 : 45	1.00	1.05	1.25	1.00	1.04	1.20
50 : 50	1.00	1.08	1.20	1.00	1.04	1.12

A=Racemic alanine residue. a) Calculated by $^tR_{(L-D)}-{}^tR_0/{}^tR_{(L-L)}-{}^tR_0$ equation.
b) In case using racemic leucine residue.

in Table 1 were prepared and then subjected to the high performance liquid chromatography (HPLC). The separation of the diastereomers of DHP was carried out on an HPLC column [250 x 4.5 (i.d.)mm] packed Lichrosorb RP-18 (Merck) using a mixture of MeOH and H_2O as the eluent. The volume ratio of the eluent was altered from 75 : 25 to 50 : 50 (v/v, %) as shown in Table 2. As a result, in the case of Boc-ΔPhe-(DL)-Ala-Leu-OMe [-ΔF-(DL)-A-L-], the conditions determined as optimum for obtaining the maximum of α value (1.28) were found that a mixture of MeOH and H_2O (60 : 40) was made to flow the column at the rate of 1.0 ml/min at the column temperature of 25 °C. As illustrated in Fig. 1, the separation profiles of Δ^1-, Δ^2-, and Δ^3-DHP respectively are appreciably different from each other.

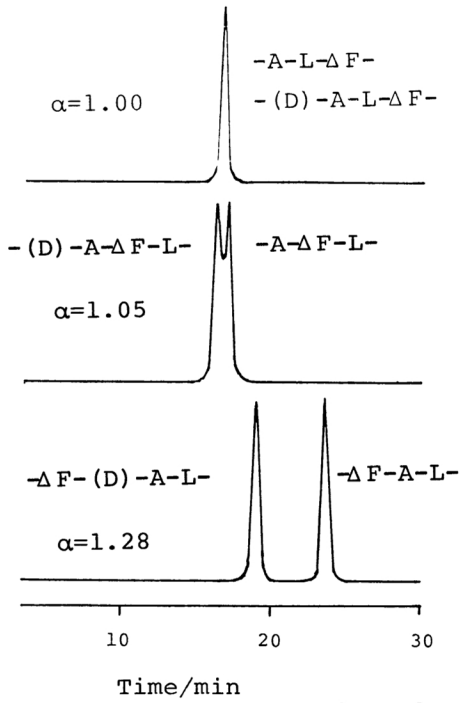


Fig. 1. Separation of Δ^3-, Δ^2-, and Δ^1-DHP containing racemic alanine residue. Column: 250 mm x 4.5 mm, RP-18.

In addition, even when the racemic AA residue was present at C-terminus [Boc-ΔPhe-Leu-(DL)-Ala-OMe], the similar tendency for the separation [α=1.20 by MeOH and H_2O (55 : 45 (v/v)] was observed. Interestingly, it was found that the DHP with (D)-AA residue was eluted faster than that with (L)-AA.

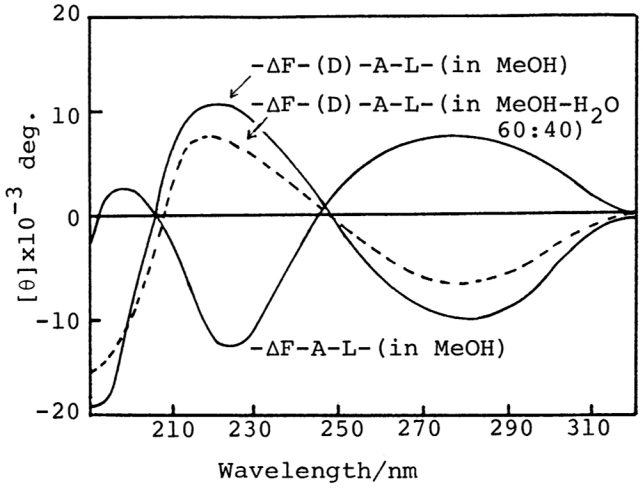


Fig. 2. CD spectra of the diastereomers (Δ^1-DHP) in MeOH and in a mixture of MeOH-H_2O.

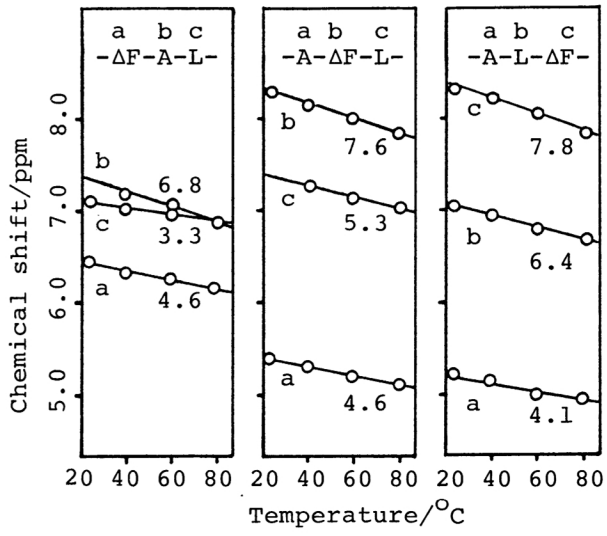


Fig. 3. Temperature dependence of amide protons of the major conformer in DHP (in $CDCl_3$). Numbers on the lines refer to 10^3 x temperature efficient in ppm $^oC^{-1}$.

Furthermore, circular dichroism (CD) spectra of Δ^1-DHP containing (L)- and (D)-Ala residues respectively are shown in Fig. 2. As the figure shows, in the cases of Boc-ΔPhe-(D)-Ala-Leu-OMe and Boc-ΔPhe-Ala-Leu-OMe, it can be seen that the CD spectrum bands at about 220 nm and 280 nm give rise to two strong Cotton effects. On the other hand, Boc-(D)- and (L)-Ala-ΔPhe-Leu-OMe and -Ala-Leu-ΔPhe-OMe showed weak Cotton effect or not showed any dichroic band. Consequently, the CD spectra of Δ^1-DHP are opposite to each other in the wavelength region shorter than 310 nm, whereas those of each diastereomer of Δ^2-DHP and Δ^3-DHP are faintly opposite and random, respectively.

From the above results and the fact that $\Delta^{1,2}$-dehydrotripeptide containing C-terminal (L)-AA residue assumed β-turn structure,[8,9] particularly, Δ^1-DHP derivatives, thus obtained, also seem to have a rigidly fixed conformation with the similar β-turn in solution. In addition, based on the ^{1}H NMR spectral data shown in Fig. 3, the above assumption was further supported from the temperature dependence of the amide proton of C-terminal amino acid residue in Δ^1-DHP. That is to say, the slope of the c line by the chemical shift was found to be very small (3.3) due to the effect of the slightest temperature dependence of Δ^1-DHP, comparing with those of Δ^2- and Δ^3-DHPs (5.3 and 7.8).

Accordingly, the highest CD band seems to indicate the strong β-turn preference of the sequence, probably being stabilized by the intramolecular hydrogen bond between N-protecting acyl carbonyl group and the hydrogen atom of C-terminal peptide bond.

In conclusion, it is worth noting that the diastereomers of Δ^1-DHP can be readily separated on a usual silica-gel column. Moreover, it was found that the separation procedure was widely applicable to various kinds of Δ^1-DHP containing (DL)-uncommon amino acid, such as α,β-diamino acid and pipecolic acid. These results will be reported in detail elsewhere.

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3) In this paper, the symbols Δ^1, Δ^2, Δ^3, and $\Delta^{1,2}$ indicate the position numbers of double bonds of DHA residues from the N-terminus in sequence.
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