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EFFICIENT ASYMMETRIC SYNTHESIS OF α-AMINO ACIDS THROUGH HYDROGENATION OF α,β-DEHYDROAMINO ACID RESIDUE IN CYCLIC DIPEPTIDES

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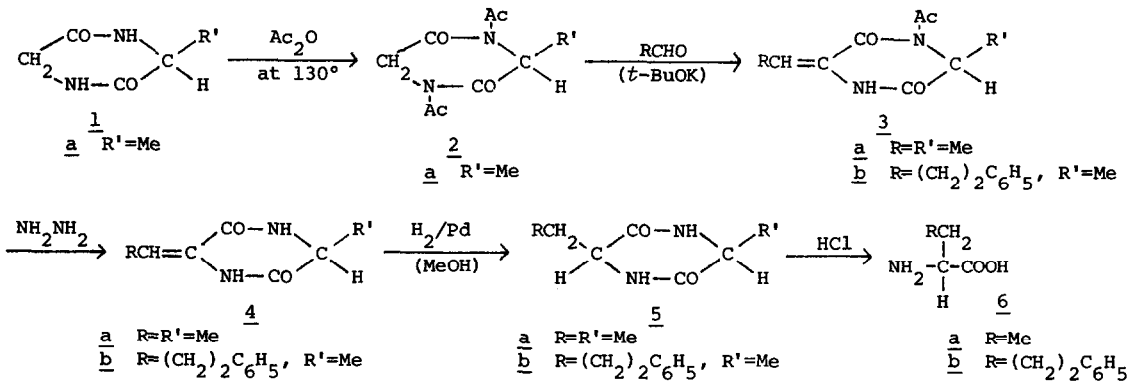
Summary: A series of *cyclo*(Δaminoacyl-L-Ala) (4) (Δ=α,β-dehydro) were prepared from *cyclo*(Gly-L-Ala) and corresponding aldehyde, and hydrogenated with Pd black in MeOH. Chiral inductions producing *cyclo*(L-aminoacyl-L-Ala) (5) from 4 were 96-99% in the case of L-Aba (2-aminobutanoic acid), L-Val, L-Leu, and L-App (2-amino-5-phenylpentanoic acid) as an L-aminoacyl moiety in 5. Pure L-Leu, L-Aba, and L-App were synthesized in preparative scale from corresponding 4 through asymmetric hydrogenation and acid-hydrolysis.

We reported high asymmetric hydrogenation of ΔAla (Δ=α,β-dehydro) residue in *cyclo*(ΔAla-L-Leu) and preparation of pure L-alanine through acid-hydrolysis of *cyclo*(L-Ala-L-Leu) obtained.[1,2] The key intermediate, *cyclo*(ΔAla-L-Leu), was synthesized from *cyclo*(L-Ser-L-Leu) by Photaki's method,[3] namely tosylation of Ser residue and successive detosylation by the action of Et_2NH. In respect to practical preparation of L-alanine, however, this route suffers from disadvantage to consume precious serine. Nevertheless, we attempted to synthesize *cyclo*(ΔPhe-L-Leu) by the action of Et_2NH to *cyclo*(DL-Hyphe(*O*-Tos)-L-Leu) (Hyphe=phenylserine), however, we could not convert the Hyphe(*O*-Tos) residue to ΔPhe presumably due to the presence of bulky side chain in Hyphe residue. Recently, we developed a new route to synthesize pure *cyclo*(Δaminoacyl-L-aminoacyl) (4) in moderate yield as shown in Scheme 1. This paper reports synthesis of 4, asymmetric hydrogenation of Δamino acid residue in 4, and efficient synthesis of pure L-amino acid (6) in good yield from hydrogenated cyclic dipeptide (5).

We describe the synthesis of *cyclo*(ΔAba-L-Ala) (4a) (Aba=2-aminobutanoic acid) as an example. We observed appreciable racemization for synthesis of *cyclo*(Gly-L-Ala) by Fischer's method using methanolic NH_3 on H-Gly-L-Ala-OMe,[4] then we developed a method to synthesize a cyclic dipeptide without racemization as follows. Reflux of H-Gly-L-Ala-OMe in MeOH for 24 h afforded pure *cyclo*(Gly-L-Ala) (1a), 86%, mp 228-230° dec, $[\alpha]_D^{20}$ -21.3° (DMF). Treatment of 1a (10 mmol) with Ac_2O (30 ml) at 130° for 8 h yielded 2a, 90%, mp 69-71°; unexpectedly almost no racemization of L-Ala residue in 2a was detected in spite of that the conditions to produce 2a were quite drastic. Since Gallina and Liberatori reported that *cyclo*(*N*-Ac-Gly-*N*-Ac-Gly) reacts easily with any aldehyde in the presence of *t*-BuOK, we followed their procedure.[5] A mixture of

Scheme 1



2a (1 equiv) and CH_3CHO (4 equiv) in DMF was treated *t*-BuOK (1 equiv) in *t*-BuOH at 25° for 5 h to yield oily *cyclo*(ΔAba-*N*-Ac-L-Ala) (3a) quantitatively. Treatment of 3a (1 equiv) with $NH_2NH_2 \cdot H_2O$ (4 equiv) in DMF at 25° for 3 h afforded *cyclo*(ΔAba-L-Ala) (4a), 48% from 1a, mp 285-287° dec, $[\alpha]_D^{20}$ -10.8° (AcOH), R_f 0.64 (TLC with $CHCl_3$:MeOH=5:1). NMR of 4a revealed that ΔAba residue was *Z*, and presence of *E* isomer could not be detected. To confirm further the structure of 4a and configuration of *Z* in 4a, we prepared *Z* and *E* isomers as follows. H-L-Thr-L-Ala-OMe was refluxed in MeOH affording *cyclo*(L-Thr-L-Ala), 70%, mp 243-244° dec, R_f 0.30, which was converted to *cyclo*(*Z*-ΔAba-L-Ala) (7), 41%, mp 287-289° dec, $[\alpha]_D^{21}$ -11.3° (AcOH), R_f 0.64, by Photaki's method. *cyclo*(*E*-ΔAba-L-Ala) (8), 16%, mp 262-264° dec, $[\alpha]_D^{21}$ +8.1° (AcOH), R_f 0.64, was prepared from *cyclo*(L-aThr-L-Ala), mp 201-203° dec, R_f 0.30. Thus, properties of 4a was identical with *cyclo*(*Z*-ΔAba-L-Ala) (7).

A series of *cyclo*(Gly-L-aminoacyl) (1), in which aminoacyls are Ala, Val, Leu, and Lys(ε-Ac) residues, were synthesized from corresponding dipeptide ester. Then, 1 was converted to cyclic dehydrodipeptide (4) by condensing with corresponding aldehyde or acetone and by successive treatment with NH_2NH_2. Yields of 4 are listed in Table 1. Extent of racemization of L-aminoacyl residue in 4 was determined as follows. A portion of 4 was hydrolyzed with 6 M HCl at 110° for 24 h, and racemization was determined by modified Manning's method;[6] presence of very small amount (0-2%) of D-amino acid in 4 was observed. NMR measurements revealed that Δamino acid residues except ΔLeu in 4 were practically *Z* form, while the presence of *Z*-ΔLeu (70%) and *E*-ΔLeu (30%) residues was recognized in *cyclo*(ΔLeu-L-aminoacyl).

We carried out hydrogenation of 4a (0.1 mmol) at 1-atm pressure of H_2 with Pd black (2-4 mg) in MeOH (4 ml) at 25° for 1-2 h; yield of *cyclo*(L-Aba-L-Ala) (5a) was quantitative. Modified Manning's method revealed that presence of D-Aba residue in 5a was about 0.5% after correction as literature,[2] and chiral induction was claculated as 99%.[7] The chiral induction in hydrogenation of *cyclo*(*E*-ΔAba-L-Ala) (8) was also 99%, indicating that geometric difference between *Z*-ΔAba (in 7 and 4a) and *E*-ΔAba (in 8) residues gave no effect on asymmetric induction. The results suggest that pure L-amino acid originated from Δaminoacyl residue will be prepared from *cyclo*(Δaminoacyl-L-aminoacyl) which is a mixture of *Z* and *E* isomer such as *cyclo*(ΔLeu-L-Ala). Similarly hydrogenations using MeOH were carried out for a series of *cyclo*(Δaminoacyl-L-aminoacyl) (4), and their chiral inductions are shown in Table 1. Excellent chiral inductions

Table 1. Hydrogenation of Cyclic Dehydrodipeptides in Several Solvents Using Pd Black

Entry	Cyclic dehydrodipeptide (4)	Yield of 4 (%)[a]	Solvent	Chiral induction (%)[b]
1	*cyclo*(ΔAba-L-Ala)	48	MeOH	99
2	*cyclo*(Z-ΔAba-L-Ala)[c]	41[c]	MeOH	99
3	*cyclo*(E-ΔAba-L-Ala)[d]	16[d]	MeOH	99
4	*cyclo*(ΔAba-L-Lys(ε-Ac))	45	MeOH	97
5	"		AcOH	72
6	*cyclo*(ΔVal-L-Ala)	8	MeOH	96
7	*cyclo*(ΔLeu-L-Ala)	47	MeOH	98
8	"		DMF	98
9	"		AcOH	97
10	*cyclo*(ΔLeu-L-Val)	61	MeOH	>99
11	"		DMF	99
12	"		AcOH	99
13	*cyclo*(ΔLeu-L-Leu)	69	MeOH	98
14	"		DMF	96
15	"		AcOH	95
16	*cyclo*(ΔLeu-L-Lys(ε-Ac))	22	MeOH	95
17	"		DMF	96
18	"		AcOH	87
19	*cyclo*(ΔPhe-L-Ala)	56	MeOH	88
20	"		DMF	93
21	"		AcOH	63
22	*cyclo*(ΔPhe-L-Val)	63	MeOH	94
23	*cyclo*(ΔPhe-L-Leu)	52	MeOH	90
24	*cyclo*(ΔPhe-L-Lys(ε-Ac))	26	MeOH	77
25	"		DMF	69
26	*cyclo*(ΔApp-L-Ala)	55	MeOH	98
27	"		AcOH	84
28	*cyclo*(ΔApp-L-Leu)	52	MeOH	97
29	*cyclo*(ΔTrp-L-Ala)	49	MeOH	71
30	*cyclo*(ΔTrp-L-Leu)	18	MeOH	66

[a] Calculated from *cyclo*(Gly-L-aminoacyl) except *c* and *d*. [b] Defined as % new L-amino acid minus % new D-amino acid in *cyclo*(new aminoacyl-L-aminoacyl) derived from *cyclo*(ΔaminoacyL-L-aminoacyl). [c] From *cyclo*(L-Thr-L-Ala). [d] From *cyclo*(L-aThr-L-Ala).

(entries 1, 6, 7, and 26) are observed in 4 containing ΔAba, ΔVal, ΔLeu and ΔApp (App=2-amino-5-phenylpentanoic acid) residues. On the contrary, hydrogenation of ΔPhe and ΔTrp residues caused slightly low (entries 19 and 23) and appreciably low (entries 29 and 30) inductions. For example, *cyclo*(ΔTrp-L-Leu) (9)[8] yielded a mixture of 83% L-L isomer and 17% D-L isomer of *cyclo*(Trp-L-Leu).[9] Variations of L-aminoacyl residues in *cyclo*(ΔaminoacyL-L-aminoacyl) (4) gave no significant influence for chiral inductions (entries 7, 10, and 16) though L-Val residue in extremely effective.

In respect to mechanism of chiral induction, we proposed previously the planar structure of cyclic dipeptide containing ΔAla residue as an important factor inducing high asymmetric hydrogenation.[1,2] We propose here the similar conformation for *cyclo*(ΔLeu-L-Ala) because ΔLeu residue is hydrogenated asymmetrically as ΔAla is. In cyclic dipeptide with ΔTrp or ΔPhe, however, diketopiperazine-ring and aromatic side chain are not coplanar by slightly twisted form; we assume, therefore, that poor stereoselectivity in adsorption of diketopiperazine-ring on catalyst lowered the degree of asymmetric hydrogenation.

We examined influence of variations of solvents, catalysts, and temperatures. MeOH, DMF, and AcOH were examined (Table 1); MeOH and DMF were similarly effective for asymmetric hydrogenation (e.g., entries 7 and 8), however, AcOH lowered the induction in some cases

(e.g., entry 27). Several catalysts were tested with *cyclo*(ΔLeu-L-Ala) (10) in MeOH. Chiral inductions were 98%, 94%, and 91% with Pd black, 5% Pd charcoal, and PtO, respectively; Pd black was most effective. At different temperatures, 10 was hydrogenated with Pd black in MeOH. Chiral inductions were 99%, 98% and 97% at 0°, 25°, and 50°, respectively; change of temperature gave almost no influence. The results show that a combination of MeOH or DMF and Pd black at room temperature is best for our hydrogenation system.

We synthesized common and unusual L-amino acids in preparative scale. We could isolate pure L-leucine in good yield from 10 through hydrogenation. Even pure L-phenylalanine was obtained in moderate yield as follows. *cyclo*(ΔPhe-L-Ala) in DMF afforded *cyclo*(Phe-L-Ala) quantitatively by hydrogenation which was hydrolyzed with 6 M HCl at 110° for 12 h. After evaporation and neutralization, the residue was recrystallized several times from hot water; 61% of L-phenylalanine, $[\alpha]_D^{20}$ -6.9° (5 M HCl). Preparation of unusual amino acids attracted especially our interest because these amino acids are often present in nature. For instance, L-2-aminobutanoic acid (6a) and D-2-aminobutanoic acid (11) are present in ophthalmic acid[10] and an antibiotic,[11] respectively. L-2-Amino-5-phenylpentanoic acid (6b) is a constituent of AM-toxin II.[12] Then, 6a was synthesized as follows. *cyclo*(ΔAba-L-Lys(ε-Ac)) (12) was hydrogenated in MeOH and hydrolyzed with 6 M HCl. The residue was treated with Dowex 50 and eluted with 1 M pyridine. Pure 6a was obtained by recrystallization from H_2O-EtOH, 72% from 12, $[\alpha]_D^{20}$ +21.2° (5 M HCl). Pure 11 will be synthesized from *cyclo*(Gly-D-Lys(ε-Ac)). Similarly *cyclo*(ΔApp-L-Ala) (4b) was prepared through 2 and 3b, and afforded a mixture of 6b and L-alanine *via* 5b. The mixture was dissolved in 1 M HCl and neutralized with Et_3N to precipitate pure 6b, 78% from 4b, $[\alpha]_D^{20}$ +18.5° (1 M HCl).

References and Notes

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7. Ratio of diastereomeric isomers obtained by hydrogenation of *cyclo*(ΔTrp-L-Ala) or *cyclo*(ΔTrp-L-Leu) was directly determined by Hitachi model 635A high performance liquid chromatograph.
8. We assume that 9 will be obtained from *cyclo*(L-Trp-L-Leu) by the action of an enzyme isolated from *Pseudomonus*. Y. Noda, K. Takai, T. Tokuyama, H. Narumiya, H. Ushiro and O. Hayaishi (*J. Biol. Chem.*, 252, 4413 (1977)) isolated the enzyme, and observed that the enzyme catalyses the formation of ΔTrp residue from L-Trp residue in many peptides.
9. We observed that the mixture of *cyclo*(Trp-L-Leu) possesses a bitter taste in threshold value of 0.015 mg/ml; *cyclo*(ΔTrp-L-Leu) and an authentic *cyclo*(L-Trp-L-Leu) showed exactly the same bitterness. A. Yasutake, K. Miyazaki, H. Aoyagi, T. Kato and N. Izumiya (*FEBS Lett.* 100, 241 (1979)) reported that both diastereomeric *cyclo*(L-Trp-L-Hmp) (Hmp=2-hydroxy-4-methylpentanoic acid) and *cyclo*(L-Trp-D-Hmp) possess the same bitterness. T. Shiba and K. Nunami (*Tetrahedron Lett.*, 509 (1974)) elucidated the structure of a bitter principle in nature as *cyclo*(L-Trp-L-Leu).
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