Sterically Controlled Syntheses of Optically Active Organic Compounds. XIV. Syntheses of Dipeptides from *N*-(α-Ketoacyl)-α-amino Acid Esters[1]

Kaoru HARADA and Kazuo MATSUMOTO

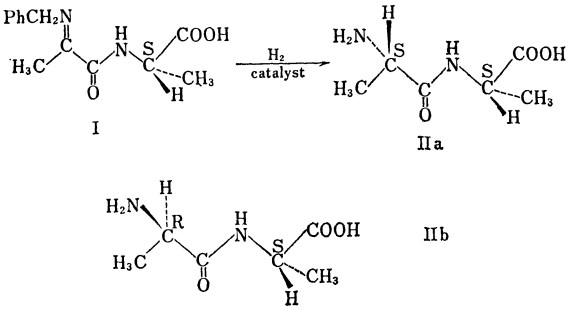
Institute of Molecular Evolution and Department of Chemistry, University of Miami, Coral Gables, Florida 33134, U.S.A.

(Received August 10, 1970)

Optically active α-amino acids were synthesized from azomethines of *N*-α-ketoacyl-amino acid esters by catalytic hydrogenation. When optically active (S)-alanine was used, (R)-amino acyl-(S)-alanine ester was obtained. When alkyl groups of optically active (S)-amino acids in the *N*-α-ketoacyl-amino acid esters were larger than ethyl group, the configurations of newly formed amino acids were found to be (S). From these results possible steric courses of the asymmetric syntheses are discussed.

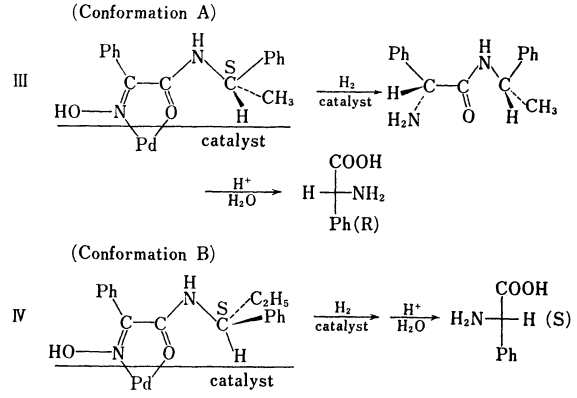
Several nonenzymatic asymmetric syntheses of α-amino acids from α-keto acids have been reported.[2-14] Hiskey and Northrop[4] published a description of a stereospecific synthesis of dipeptide from benzylamine Schiff bases of *N*-pyruvoyl-(S)-alanine. They discussed the question of whether (S)-alanyl-(S)-analine would result (Scheme I), if the catalytic hydrogenation of the Schiff base were to follow the "Prelog Rule".[15] However, the ratio of resulting dipeptide, (R)-ala-(S)-ala: (S)-ala-(S)-ala, was found to be 2 : 1. The results indicate that the catalytic hydrogenation does not follow the Prelog rule.

In order to clarify the stereochemical course of the sterically controlled synthesis, several reactions were carried out.[8] Oximes of (S)- and (R)-*N*-phenylglyoxyl-oyl-α-methylbenzylamine and *N*-(S)- and (R)-ethyl-



Scheme 1

benzoylformamide were hydrogenated and the hydrogenated products were hydrolyzed. When optically active (S)- or (R)-α-methylbenzylamine was used, the configuration of the resulting phenylglycine was (R) and (S), respectively,[8] which agreed with the results obtained by Hiskey and Northrop.[4] However, when (S)- or (R)-α-ethylbenzylamine was used, the resulting phenylglycine was (S) or (R) respectively, which agreed with the configurations expected by the formal application of the Prelog rule. The clear inversion of the configuration of the products depending on the α-methyl- and α-ethylbenzylamines could not be explained by an electronic effect. These results suggest that the reaction would be controlled by steric factors. In order to solve the problem, it has been proposed that the substrate might form a five-membered chelate ring structure with the catalyst (Scheme 2).[8] When (S)-α-methylbenzylamine was used as an optically active moiety of benzoylformamide, the conformation was proposed as structure III (conformation A). However, when (S)-α-ethylbenzylamine was used, the conformation of the substrate could be structure IV (conformation B). Since the ethyl group is bulkier than the methyl group, the ethyl group could reach the catalyst surface if the substrate were to take on con-



Scheme 2

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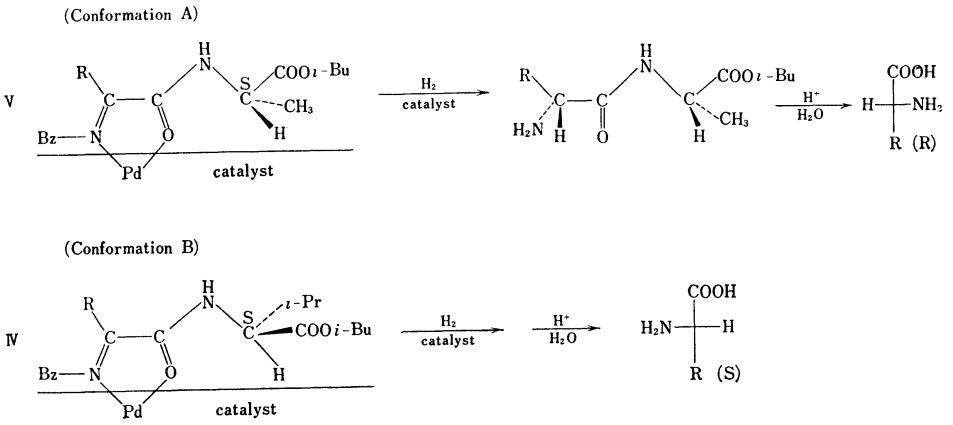
15) V. Prelog, *Helv. Chim. Acta*, **36**, 308 (1953). The Prelog rule was originally proposed for homogeneous reactions. Therefore, the Prelog rule might not be applicable for catalytic hydrogenation of the Schiff base of α-keto acid amides. It is important to consider that the configurational agreement of the final product does not always mean that the conformation of the substrate follows the Prelog rule, because several possible conformations of the substrate would result in the specific configuration predicted by the Prelog rule.

Table 1. Sterically controlled syntheses of alanyl dipeptides

$$CH_3\text{-}\underset{\underset{\underset{CH_2Ph}{|}}{N}}{\overset{\overset{}{||}}{C}}\text{-}CONH\text{-}\overset{*}{\underset{\underset{R}{|}}{CH}}\text{-}COOiBu \xrightarrow[\text{catalyst}]{H_2} \text{Alanyl dipeptides}$$

R	Asymmetric moiety	Yield of alanine % a)	Ratio of diastereomeric dipeptides	Newly formed alanine: Config'n	Newly formed alanine: $[\alpha]_D$ of DNP-Alanine (1 N NaOH, c=0.3—0.6)	Newly formed alanine: Optical purity % d)	Conform'n of substrate
CH_3	(S)-ala-*i*-Bu	15	R-S:S-S=82:18	R	b)	64	A
	(S)-ala-Me	25	76:24	R	b)	52	A
Et	(S)-α-NH_2-*n*-Bu-*i*-Bu	16	29:71	S	c)	41	B
i-Pr	(S)-val-*i*-Bu	11	34:66	S	+45.5	32	B
	(R)-val-*i*-Bu	12	34:66	R	−45.3	32	B
	(S)-val-Me	12	42:58	S	+24.6	17	B
i-Bu	(S)-leu-*i*-Bu	19	32:68	S	+56.7	39	B
	(S)-leu-Me	18	41:59	S	+25.2	18	B
Benzyl	(S)-ph-ala-*i*-Bu	26	37:63	S	+36.4	25	B
$-CH_2COOiBu$	(S)-asp-di-*i*-Bu	11	37:63	S	+36.0	25	B
ϕ	(R)-ph-gly-*i*-Bu	27	50:50	±	0	0	B

a) Yields are calculated from pyruvic acid.
b) The ratio of diastereomeric dipeptides was measured by amino acid analyzer after saponification. From these results optical purities were calculated.
c) The optical activity of alanine was calculated from the results of $[\alpha]_D$ of free amino acid mixture and the composition of amino acids.
d) Optical purity defined as ($[\alpha]_D$ observed/$[\alpha]_D$ literature)×100, DNP-(S)-alanine, $[\alpha]_D$=+143.9° (1 N NaOH).



Scheme 3

formation A. Therefore, the most probable conformation is structure IV (conformation B) when the R-group of the amine is larger than the ethyl group. Then the cyclic intermediate complex could be adsorbed on the less bulky side of the molecule and the hydrogenation reaction would take place (Scheme II)[8] (two step mechanism).

In order to confirm further the proposed steric course of the asymmetric synthesis, a series of dipeptide syntheses were carried out in the study reported here. When benzylamine Schiff base of *N*-pyruvoyl-(S)-alanine isobutyl ester was used as a starting material, the configuration of the newly formed alanine was (R), (R)-ala-(S)-ala : (S)-ala-(S)-ala=82 : 18. When isobutyl (S)-α-aminobutyrate was used as an asymmetric center, (S)-alanine was obtained, (R)-ala-(S)-NH_2-Bu : (S)-ala-(S)-NH_2-Bu=29 : 71. In this series of reactions, also the inversion of configuration of the resulting amino acid was observed when asymmetric moieties containing methyl or ethyl residues were used. Results are summarized in Table 1. These results agreed with those obtained in the phenylcine synthesis.[8] The possible steric course of the reactions is shown in Scheme 3.

When the alkyl group of the asymmetric moiety is methyl (alanine isobutyl ester), structure A could be the preferred conformation. When the alkyl group is larger than the ethyl group (isobutyl α-aminobutyrate), structure B could be the major conformation in the sterically controlled syntheses of dipeptides. Intermediates A and B were then absorbed on the catalyst at the less bulky side and the hydrogenation reaction could take place. Optical purities of the newly formed alanine using valine and leucine isobutyl ester

TABLE 2. STERICALLY CONTROLLED SYNTHESES OF α-AMINOBUTYRYL PEPTIDES

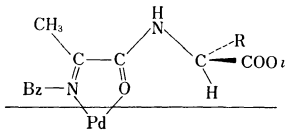
$$C_2H_5\text{-}C(=N\text{-}CH_2Ph)\text{-}CONH\text{-}CH(R)\text{-}COO\text{-}i\text{-}Bu \xrightarrow[\text{catalyst}]{H_2} \xrightarrow[H_2O]{H^+} \alpha\text{-Aminobutyric Acid}$$

Asymmetric moiety	Yield a) %	Newly formed α-amino-*n*-butyric acid: Config'n	Newly formed α-amino-*n*-butyric acid: [α] of DNP-Derivative (1N NaOH, $c=0.3$—0.6)	Newly formed α-amino-*n*-butyric acid: Optical purity b)	Conform'n of substrate
(R)-φ-gly-*i*-Bu (oxime)	67	—	0	0	B
(S)-φ-ala-*i*-Bu (oxime)	56	S	+12.1	12	B
(S)-φ-ala-*i*-Bu	65	S	+8.8	9	B
(S)-asp-di-*i*-Bu	53	S	+25.7	26	B

a) Yields are calculated from α-ketobutyric acid.
b) Optical purity defined as ($[\alpha]_D$ observed/$[\alpha]_D$ literature)×100. DNP(S)-α-amino-*n*-butyric acid, $[\alpha]=+98.8°$ (1 N NaOH)

are larger than those of alanine obtained by the use of valine or leucine methyl ester. This finding may also support the fact that structure B could be the major conformation in these reactions.

If structure B is the preferred conformation when the R group is larger than the ethyl group, one could state an order of effective bulkiness of the R group in this reaction by the use of the optical purity of newly formed alanine. When R is phenyl, the optical purity of newly formed alanine is zero, so that the effective bulkiness of the phenyl group and of the –COO–*i*-Bu group are the same. In the same way, the bulkiness of the benzyl group and of the $-CH_2COO$–*i*-Bu group are almost the same. However, these are smaller than those of the phenyl or –COO–*i*-Bu groups. The order of effective bulkiness of the R group could be arranged as shown below.



(Conformation B)

$$-COO\text{-}i\text{-}Bu \sim Ph > -CH_2Ph \sim -CH_2COO\text{-}i\text{-}Bu > i\text{-}Pr > i\text{-}Bu > Et$$

The order of bulkiness does not agree with the order of residue weight. In the order of effective bulkiness, the phenyl group is larger than the benzyl group and also the isopropyl group is larger than the isobutyl group. This relationship can be explained on the basis that the phenyl group and isopropyl group are rigid and branched and that these groups also cannot be bent. On the other hand, benzyl and isobutyl groups are flexible; these are not branched at the α-carbon to which these groups are attached. Therefore, the effective bulkiness of rigid and branched phenyl and isopropyl groups does not follow the order of residual weight.

Table II describes α-aminobutyryl peptides. The reaction products have all S–S and R–R structures. The R groups used were phenyl, benzyl, and $-CH_2$–COO–*i*-Bu. Therefore, structure B could be the major conformation in these reactions.

In summary, (a) a chelation hypothesis in the sterically controlled synthesis of dipeptide is proposed; (b) an order of effective bulkiness of side chains is assigned by the use of optical purity of the newly formed amino acids; (c) the dimension of the space between substrate and catalyst surface is inferred by the use of chemical data; (d) the possibility is advanced that one can determine the configurations of structurally unknown primary amines by the use of these results, when the proposed stereochemical course is further established.

Experimental

Amino Acid Esters. Amino acid methyl ester hydrochlorides were prepared by the thionyl chloride method.[16] Amino acid isobutyl ester *p*-toluenesulfonates (amino acid ester PTS) were prepared by the use of the azeotropic method in benzene with a Dean and Stark separation tube.[17] Yields were between 70 to 80%, (Table 3).

N-α-Ketoacyl-α-amino Acid Esters. Experimental procedures in the syntheses of dipeptide esters are similar to those described earlier.[8] α-Keto acid (0.03 mol) and α-amino acid ester (hydrochloride or *p*-toluenesulfonate), (0.03 mol) were dissolved in anhydrous tetrahydrofurane (150 m*l*) or freshly purified chloroform (100 m*l*). The solution was cooled to about −15°C. To this, 2.75 m*l* (0.03 mol) of phosphorus oxychloride was added dropwise in a period of 10 min. The mixture was kept at −10°C for 1 hr and at 0°C for 30 min. Then the mixture was again cooled to −15°C. To this solution 7.5 m*l* (0.09 mol) of pyridine was added dropwise in a period of 30 min at −15——10°C. After the addition, the solution was kept at −10——5°C for 1 hr, then the solution was brought to room temperature. Solvent was removed under reduced pressure. The residual syrup was dissolved in ethyl acetate and the solution was washed with 1 N hydrochloric acid, 5% sodium hydrogen carbonate, and then with water. The solution was dried with anhydrous sodium sulfate. Ethyl acetate was evaporated under reduced pressure. The attempt to crystallyze the residual yellow syrup was unsuccessful. The syrup was

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Table 3. Elemental analyses of amino acid esters

	Mp °C	$[\alpha]_D^{25}$ (EtOH)		C%	H%	N%
(S)-Ala *i*-Bu PTS	120—121	−1.1	Found	53.04	7.36	4.42
			Calcd	52.98	7.30	4.41
(S)-NH_2-Bu *i*-Bu HCl	124—125	+6.5	Found	49.29	9.36	7.11
			Calcd	49.05	9.27	7.15
(R)-Val *i*-Bu PTS	146—147	−9.1	Found	55.82	8.00	4.08
			Calcd	55.62	7.87	4.05
(S)-Leu *i*-Bu PTS	137—138	+10.3	Found	57.07	8.33	4.03
			Calcd	56.79	8.13	3.89
(S)-Phe *i*-Bu PTS	165—166	+24.9	Found	60.89	6.99	3.56
			Calcd	61.04	6.91	3.55
(R)-Phgly *i*-Bu PTS	185—186	−61.8	Found	60.16	6.74	3.74
			Calcd	60.13	6.64	3.69
(S)-Asp di-*i*-Bu PTS	128—129	+11.5	Found	54.64	7.61	3.40
			Calcd	54.65	7.48	3.35

used for further experiment.

Alanyl and α-Aminobutyryldipeptide Isobutyl Esters. *N*-α-Ketoacyl-α-amino acid ester and an equimolar amount of benzylamine were dissolved in benzene. The solution was gently refluxed for 20 minutes with a Dean-Stark separator. The benzene was removed under reduced pressure. The residual syrup was dissolved in absolute methanol. Hydrogenation and hydrogenolysis were carried out with 10% palladium on charcoal at room temperature, by the use of a Parr 3910 shaker type hydrogenation apparatus. After the reaction was over, the catalyst was removed and the methanol was evaporated under reduced pressure. The residue was dissolved in ethyl acetate and the solution was extracted with 1 N hydrochloric acid. To the aqueous solution, sodium hydrogencarbonate was added to bring the pH to 8. The solution was extracted with ethyl acetate. The solution was dried with anhydrous sodium sulfate and the solvent was evaporated under reduced pressure. The residual product (dipeptide ester) was used for further experiments (for saponification or for hydrolysis).

Optical Purity of Newly Formed Amino Acid Residues. Dipeptide esters were refluxed with 6 N hydrochloric acid for 8 hr. The hydrolyzate was evaported to dryness *in vacuo*. The residue was dissolved in a small amount of water and the solution was applied to a Dowex 50×2 column (hydrogen form, 100—200 mesh, 20×1.8 cm). After washing with water, amino acid mixture was eluted with 1 N aqueous ammonia. After evaporation of water and ammonia under reduced pressure, a mixture of free amino acids was obtained. The amino acid mixture was treated with 2,4-dinitrofluorobenzene in a conventional manner.[18] The resulting DNP-amino acids were separated by the use of a Celite column treated with pH 7.0 citrate buffer[19] without fractionation of optical isomer.[8,12]

In the synthesis of alanylphenylalanine in Table 1, DNP-(S)-alanine was isolated and analyzed.

Found: C, 42.42; H, 3.66; N, 16.64%. Calcd for $C_9H_9O_6N_3$; C, 42.36; H, 3.55: N, 16.47%.

In the synthesis of alanylaspartic acid in Table 1, free (S)-alanine was isolated by the use of Dowex 50×2 column.

Found: C, 40.68; H, 8.01; N, 15.58%. Calcd for $C_3H_7O_2N$: C, 40.44; H, 7.92; N, 15.72%.

Optical activities of separated DNP-amino acids were measured in 1 N sodium hydroxide by the used of a Rudolph model 80 polarimeter with PEC-101 photometer. In the case of alanyl-α-aminobutyric acid synthesis, the optical purity of alanine was determined by the measurement of specific rotation and composition of free amino acid mixture which was obtained by acid hydrolysis. The amino acid compositions were determined by the used of a Phoenix model K-5000 automatic amino acid analyzer.

This work was supported by Grant NGR 10-007-052 from the U.S. National Aeronautics and Space Administration.

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