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Syntheses of Optically Active Amino Acids by Reduction of Schiff Bases with Sodium Borohydride[1)]

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Several nonenzymatic asymmetric syntheses of α-amino acids from their corresponding α-keto acids have been reported.[2-12)] An asymmetric synthesis of α-amino acids by catalytic hydrogenation and subsequent hydrogenolysis of the Schiff bases of

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8) R. G. Hiskey and R. C. Northrop, *J. Amer. Chem. Soc.*, **89**, 1753 (1965).

9) K. Harada and K. Matsumoto, *J. Org. Chem.*, **32**, 1794 (1967).

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Table 1. Amino acids prepared from α-keto acid esters

	Keto acid[a]	Amine[b]	Solvent	$NaBH_4$ (mole)	Amino acid	Yield[d] (%)	Confign.	DNP-Amino acid $[\alpha]_D^{25}$ 1N NaOH[f]	DNP-Amino acid Optical purity[g]
1	Et-Py	(−)-Me	Diglyme	0.01	ala	13	S	+36.2 (0.51)	25
2	Et-Py	(+)-Me	Diglyme	0.01	ala	15	R	−34.9 (0.49)	24
3	Et-Py	(−)-Me	THF	0.01	ala	18	S	+41.5 (0.46)	29
4	Et-Py	(+)-Me	THF	0.01	ala	18	R	−40.8 (0.56)	28
5	Bz-Py	(−)-Me	Diglyme	0.01	ala	16	S	+42.1 (0.49)	29
6	Bz-Py	(+)-Me	Diglyme	0.01	ala	17	R	−40.3 (0.45)	28
7	Bz-Py	(−)-Et	Diglyme	0.01	ala	10	S	+23.6 (0.51)	16
8	Bz-Py	(+)-Et	Diglyme	0.01	ala	11	R	−24.5 (0.46)	17
9	Bz-Py	(−)-Me	THF[c]	0.01	ala	12	S	+52.7 (0.49)	37
10	Bz-Py	(+)-Me	THF[c]	0.01	ala	11	R	−49.5 (0.43)	34
11	Bz-Py	(−)-Et	THF[c]	0.01	ala	9	S	+32.3 (0.52)	22
12	Bz-Py	(+)-Et	THF[c]	0.01	ala	8	R	−30.9 (0.54)	22
13	Bz-Py	(−)-Me	EtOH	0.01	ala	5	S	+34.6 (0.44)	24
14	Bz-Py	(+)-Me	EtOH	0.01	ala	5	R	−32.3 (0.45)	23
15	Bz-Py	(−)-Me	THF[c]	0.01	ala	12(8)[e]	S	+50.1 (0.49)	35(33)[h]
16	Bz-Py	(+)-Me	THF[c]	0.01	ala	12(9)[e]	R	−51.2 (0.50)	36(37)[h]
17	Bz-Py	(−)-Me	THF[c]	0.005	ala	12(9)[e]	S	+48.2 (0.51)	34(34)[h]
18	Bz-Py	(+)-Me	THF[c]	0.005	ala	12(9)[e]	R	−51.8 (0.61)	36(35)[h]
19	Bz-Py	(−)-Me	THF[c]	0.0025	ala	11(8)[e]	S	+48.4 (0.51)	34(35)[h]
20	Bz-Py	(+)-Me	THF[c]	0.0025	ala	12(7)[e]	R	−49.6 (0.49)	34(36)[h]
21	Et-Bu	(−)-Me	THF[c]	0.01	NH_2-but	23	S	+15.3 (0.42)	16
22	Et-Bu	(+)-Me	THF[c]	0.01	NH_2-but	27	R	−15.2 (0.52)	15
23	Et-Bu	(−)-Et	THF[c]	0.01	NH_2-but	22	S	+ 9.1 (0.39)	9.2
24	Et-Bu	(+)-Et	THF[c]	0.01	NH_2-but	26	R	− 8.4 (0.58)	8.5
25	Bz-Bu	(−)-Me	THF[c]	0.01	NH_2-but	23	S	+19.3 (0.56)	20
26	Bz-Bu	(+)-Me	THF[c]	0.01	NH_2-but	20	R	−19.4 (0.55)	21
27	Bz-Bu	(−)-Et	THF[c]	0.01	NH_2-but	23	S	+15.9 (0.49)	16.1
28	Bz-Bu	(+)-Et	THF[c]	0.01	NH_2-but	21	R	−16.0 (0.40)	16.2

a) Et-Py, ethyl pyruvate; Bz-Py, benzyl pyruvate; Et-Bu, ethyl α-ketobutyrate; Bz-Bu, benzyl α-ketobutyrate.
b) (−)-Me, (S)-(−)-α-methylbenzylamine ($[\alpha]_D^{25}$ −42.3°, benzene); (+)-Me, (R)-(+)-α-methylbenzylamine ($[\alpha]_D^{25}$ +41.5°, benzene); (−)-Et, (S)-(−)-α-ethylbenzylamine ($[\alpha]_D^{25}$ 21.0°, benzene); (+)-Et, (R)-(+)-α-ethylbenzylamine ($[\alpha]_D^{25}$ +21.7°, benzene).
c) THF, tetrahydrofuran.
d) Yields are calculated from α-keto acids.
e) Yields represent alanine (reactions 15—20) prepared at room temperature and at 0°C (with parentheses).
f) Optical rotations were measured in 1N sodium hydroxide.
g) Defined as ($[\alpha]_D$ observed/$[\alpha]_D$ lit) × 100. DNP-(S)-ala, $[\alpha]_D$ +143.9° (1N NaOH); DNP-(S)-α-NH_2-but, $[\alpha]_D$ +98.8° (1N NaOH): K. R. Rao and H. A. Sober, *J. Amer. Chem. Soc.*, **76**, 1228 (1954).
h) Optical purities represent alanine (reactions 15—20) prepared at room temperature and at 0°C (with parentheses).

α-keto acids with optically active amines were studied[4] and the steric course of the asymmetric synthesis was studied and a chelated intermediate structure of the substrate with the catalyst was suggested.[9,10]

Several methods of hydrogenation of the azomethine group have been reported. These are: catalytic hydrogenation,[13] reduction using metals,[14] lithium aluminum hydride,[15] sodium borohydride,[16] dimethylamine-borane,[17] formic acid,[18]

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16) G. N. Walker, M. A. Moor and B. N. Weaver, *ibid.*, **26**, 2740 (1961).
17) J. H. Billman and J. W. McDowell, *ibid.*, **26**, 1437 (1961).
18) R. Baltzly and O. Kauder, *ibid.*, **16**, 173 (1951).

Table 2. Amino acids prepared from sodium salts of α-keto acids

Keto acid[a]	Amine[b]	Solvent	$NaBH_4$ (mole)	Amino acid	Yield[c] (%)	Confign.	DNP-Amino acid $[\alpha]_D$, 1N NaOH[d]	DNP-Amino acid Optical purity[e] (%)
Na-Py	(−)-Me	MeOH–H_2O (1:3)	0.01	ala	6	S	+39.2 (0.59)	27
Na-Py	(+)-Me	MeOH–H_2O (1:3)	0.01	ala	7	R	−37.0 (0.44)	26
Na-Py	(−)-Et	MeOH–H_2O (1:3)	0.01	ala	6	S	+36.2 (0.52)	25
Na-Py	(+)-Et	MeOH–H_2O (1:3)	0.01	ala	8	R	−40.0 (0.59)	25
Na-Bu	(−)-Me	MeOH–H_2O (1:3)	0.01	NH_2-but	19	S	+ 9.0 (0.48)	9
Na-Bu	(+)-Me	MeOH–H_2O (1:3)	0.01	NH_2-but	18	R	−10.8 (0.51)	11
Na-Bu	(−)-Et	MeOH–H_2O (1:3)	0.01	NH_2-but	17	S	+ 8.2 (0.52)	8
Na-Bu	(+)-Et	MeOH–H_2O (1:3)	0.01	NH_2-but	18	R	− 9.1 (0.50)	9

a) Na-Py, sodium pyruvate; Na-Bu, sodium α-ketobutyrate.
b) (−)-Me, (S)-(−)-α-methylbenzylamine ($[\alpha]_D^{25}$ −42.3°, benzene); (+)-Me, (R)-(+)-methylbenzylamine ($[\alpha]_D^{25}$ +41.5°, benzene); (−)-Et, (S)-(−)-α-ethylbenzylamine ($[\alpha]_D^{25}$ −21.0°, benzene); (+)-Et, (R)-(+)-α-ethylbenzylamine ($[\alpha]_D^{25}$ +21.7°, benzene).
c) Yields are calculated from α-keto acids.
d) Optical rotations were measured in 1N sodium hydroxide.
e) Defined as ($[\alpha]_D$ observed/$[\alpha]_D$ lit)×100. DNP-(S)-ala, $[\alpha]_D$ +143.9° (1N NaOH); DNP-(S)-α-NH_2-but, $[\alpha]_D$ +98.8° (1N NaOH): K. R. Rao and H. A. Sober, *J. Amer. Chem. Soc.*, **76**, 1328 (1954).

and electrolytic reduction.[19] In the present study, the Schiff bases of α-keto acids with optically active α-alkylbenzylamines were reduced with sodium borohydride. The reduced compounds were further hydrogenolyzed and hydrolyzed to afford α-amino acids. The α-keto acid esters used were: ethyl pyruvate, ethyl α-ketobutyrate, and benzyl pyruvate. The optically active α-alkylbenzylamines used were: S(−)-α- and R(+)-α-methylbenzylamine, S(−)-α- and R(+)-α-ethylbenzylamine. Solvents used were: diglyme, tetrahydrofuran, and ethanol. The Schiff base was dissolved in a solvent and was reduced with sodium borohydride at room temperature (or at 0°C). After the reaction was over, the boron complex was decomposed with hydrochloric acid. The resulting *N*-alkyl amino acid ester was hydrolyzed and then hydrogenolyzed by the use of palladium hydroxide on charcoal. A part of the reduction product was applied to an amino acid analyzer to estimate the yield of amino acid. The other part was desalted and then applied to a Dowex 50×2 column (H form). Free amino acids were obtained by elution with aqueous ammonia. The resulting amino acids still contained some impurities, so that the measurements of optical rotation at this stage were meaningless. The crude amino acids were converted to their corresponding DNP-amino acids by the use of 2,4-dinitrofluorobenzene in a conventional manner.[20] The resulting DNP-amino acids were purified without fractionation of optical isomers by the use of Celite column chromatography,[21] as reported in the previous papers.[5,6] Results are summarized in Tables 1 and 2.

The yields of the asymmetric synthesis were rather low (5—27%). The optical purity of synthesized amino acids was in a range of 9—36%. When (S)-α-alkylbenzylamines were used, (S)-amino acids were synthesized. Yields of amino acids prepared at lower temperature (0°C) were smaller than that prepared at room temperature. However, optical purities of amino acids prepared at 0°C and at room temperature (25°C) were found to be the same (Table 1, 15—20). Yields of amino acids were almost constant dependent on the amount of sodium borohydride used (Table 1, 15—20). When α-methylbenzylamine was used, higher optical activity of amino acid was obtained than by the use of α-ethylbenzylamine. The optical purities of alanine were generally higher than that of α-amino butyric acid. These results are similar to those obtained by the hydrogenolytic asymmetric transamination of α-keto acids,[9,10] or α-keto acid esters,[12] with optically active α-alkylbenzylamine.

Experimental

Amino Acids from Benzyl Esters of α-Keto Acids. Benzyl pyruvate (1.78 g, 0.01 mol) and S(−)-α-methylbenzylamine (1.21 g, 0.01 mol) were dissolved in 50 ml of benzene and the solution was gently refluxed for 30 min using a Dean and Stark distillation tube. After dehydration was over, benzene was evaporated under reduced pressure. The residual syrup was dissolved in 30 ml of tetrahydrofuran. To this, sodium

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20) F. Sanger, *Biochem. J.*, **39**, 507 (1945).
21) J. C. Perrone, *Nature*, **167**, 513 (1951).

borohydride (0.380 g, 0.01 mol) was added under stirring at room temperature. No heat generation was observed. After 2 hr of stirring, 10 m*l* of 2N hydrochloric acid was added to decompose unreacted sodium borohydride and boron complex. The solvent was removed under reduced pressure. The residual material was dissolved in a mixture of 10 m*l* of water and 5 m*l* of methanol. Sodium hydrogencarbonate was added to the solution to adjust the pH to about 8.0. Palladium hydroxide on charcoal (0.50 g) was added to the solution and hydrogenolysis was carried out for 30 hr. After hydrogenolysis was over, the catalyst was filtered and washed with water. A part of the combined solution was used for amino acid analysis to determine the yield of amino acid.

To the main reaction mixture, 10 m*l* of 2N hydrochloric acid was added and the solution was evaporated to dryness under reduced pressure. To the residual material, absolute ethanol (20 m*l*) was added to extract amino acid hydrochloride. Undissolved sodium chloride was removed by filtration. The alcoholic solution was evaporated to dryness *in vacuo* and the residue was dissolved in 10 m*l* of water. The amino acid solution was applied to a Dowex 50×2 column (H form, 2× 15 cm). The column was washed with water until the effluent became neutral. Then amino acid was eluted with 5% pyridine (*v*/*v*). Fractions containing alanine were combined and evaporated, 0.16 g (18%).

The crude alanine was treated with 2,4-dinitrofluorobenzene in a conventional manner.[20] The resulting DNP-alanine was purified by the use of a Celite column treated with pH 7.0 buffer as described in the earlier reports.[5,6] Results are listed in Table 1.

DNP-(S)-Alanine (reaction 3): $[\alpha]_D^{25}$ +41.5° (*c* 0.46, 1N NaOH).

Found: C, 42.48; H, 3.63; N, 16.38%. Calcd for $C_9H_9N_3O_6$: C, 42.36; H, 3.55; N, 16.47%.

DNP-(R)-Alanine (reaction 4): $[\alpha]_D^{25}$ −40.8° (*c* 0.56, 1N NaOH).

Found: C, 42.53; H, 3.67; N, 16.30%. Calcd for $C_9H_9N_3O_6$: C, 42.36; H, 3.55; N, 16.47%.

Amino Acids from Ethyl Esters of α-Keto Acids. When ethyl esters of α-keto acids were used, the reaction mixture was hydrolyzed with 50 m*l* of 2N hydrochloric acid for 3 hr after reduction and evaporation of the solvent.

DNP-(S)-α-Aminobutyric acid (reaction 21): $[\alpha]_D^{25}$ +15.3° (*c* 0.42, 1N NaOH).

Found: C, 44.68; H, 4.18; N, 15.58%. Calcd for $C_{10}H_{11}N_3O_6$: C, 44.62; H, 4.12; N, 15.61%.

DNP-(R)-α-NH_2-Butyric acid (reaction 22): $[\alpha]_D^{25}$ −15.2° (*c* 0.52, 1N NaOH).

Found: C, 44.73; H, 4.13; N, 15.57%. Calcd for $C_{10}H_{11}N_3O_6$: C, 44.62; H, 4.12; N, 15.61%.

Amino Acids from Sodium Salts of α-Keto Acids. The experimental procedures using α-keto acid sodium salt are similar to those described above. The results are summarized in Table 2.

Amino Acid Analysis of the Products. Synthesized amino acids were analyzed by the use of a Phoenix 5000 K automatic amino acid analyzer. Alanine and α-aminobutyric acid appeared in the ranges of elution volume 148—159 m*l* and 157—159 m*l*. In each of the reactions using benzyl and ethyl esters of α-keto acid and also the α-keto acid sodium salt, few unknown peaks signifying trace amounts were observed in both long (for acidic and neutral amino acid) and short column (for basic amino acid) analyses. The elution volume of alaninol is in a range of 88—90 m*l* in the short column. Because trace amounts of a peak which might correspond to alaninol were found in the reduction products, the low yield of amino acid in this reduction could not be due to the formation of alaninol.

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