[Chem. Pharm. Bull.
30(2) 581—585 (1982)]

Amino Acids and Peptides. VII.[1,2] Synthesis of Methionine-Enkephalin using Transfer Hydrogenation

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(Received July 24, 1981)

Catalytic transfer hydrogenation was examined with several proton donors for removal of the Z group from Z–Met–OBu^t. *cis*-Decalin as well as cyclohexene was a good proton donor for transfer hydrogenation. Met-enkephalin was synthesized by the same route as Leu-enkephalin in combination with Z group and *tert*-butyl ester group for protection, with removal of the Z group by transfer hydrogenation. The biological activity of the synthetic peptides as determined by measuring the inhibition of electrically evoked contraction of the guinea pig ileum was in good agreement with the results reported with the natural materials, indicating that this method may be useful for the synthesis of Met-containing biologically active peptides.

Keywords——transfer hydrogenation; proton donor; sulfur-containing amino acid; peptide; chemical synthesis; enkephalin; biological activity

Previously, it was reported that catalytic transfer hydrogenation was useful for the removal of protecting groups commonly used in peptide synthesis, for instance, *N*-benzyloxycarbonyl, benzyl ester, nitro of nitroarginine, benzyl of N$^\tau$-benzylhistidine, and benzyl ether of *O*-benzyltyrosine or serine, and that this method was useful for the removal of the *N*-benzyloxycarbonyl group from sulfur-containing peptide.[3,4] Sivanandiah and Grusiddappa reported that formic acid was suitable as a hydrogen donor to remove the Z group from sulfur-containing peptides.[5] However, *tert*-butyloxycarbonyl (*tert*-Boc) and *tert*-butyl ester are susceptible to formic acid treatment and it is sometimes convenient to synthesize a peptide using the Z group as an α-amino protecting group in combination with acid-labile protecting groups. In view of these considerations, Anwer and Spatola synthesized Leu-enkephalin using ammonium formate as the hydrogen donor for transfer hydrogenation; under the conditions used, acid-labile protecting groups were not affected.[6] On the contrary, Wünsch *et al.*,[7] stated that the Z group on the α-amino function of Met-containing peptide was not completely removed by transfer hydrogenation using 1,4-cyclohexadiene as the proton donor in the synthesis of cholecystokinin-pancreozymin.

This report deals with the rate of removal of the Z group from Z–Met–OBu^t by transfer hydrogenation using several proton donors and with the synthesis of Met-enkephalin using transfer hydrogenation as well as the synthesis of Leu-enkephalin using conventional catalytic hydrogenation.

The rate studies on the removal of Z group from the sulfur-containing amino acid were carried out by measuring the absorbance at 570 nm with a chromatoscanner after thin-layer chromatography on silica gel and by following the reaction with ninhydrin as a function of time. As shown in Fig. 1, *cis*-decalin as well as cyclohexene[3] gave effective removal of the Z group from Z–Met–OBu^t, but tetralin and *trans*-decalin were not suitable as proton donors. It was also found that *cis*-decalin and cyclohexene could not remove the Z group of Z–Cys(Bzl)–OH or the Bzl group of Boc–Glu(OBzl)–Cys(MBzl)–OMe (Y. Okada and N. Ohta, in preparation).

Next, Met-enkephalin and Leu-enkephalin were synthesized by the same route as shown in Fig. 2, using transfer hydrogenation and conventional catalytic hydrogenation, respectively. As the hydrogen donor, cyclohexene was used instead of *cis*-decalin, because it was easily

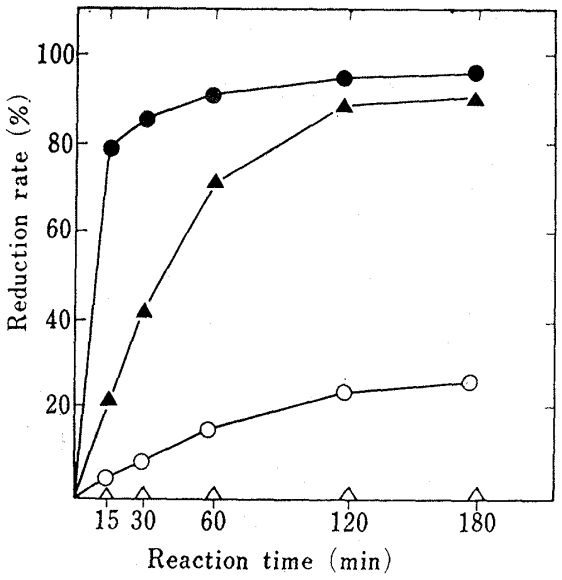
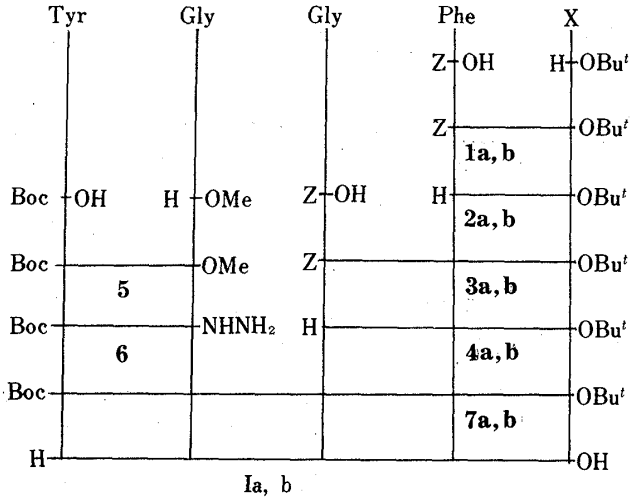


Fig. 1. Catalytic Transfer Hydrogenation of Z–Met–OBu^t

Proton donor ; 50 mmol,
Protected amino acid ; Z–Met–OBu^t 1 mmol,
Solvent ; dry EtOH 10 ml,
Catalyst ; palladium-black 250 mg,
●—●; *cis*-decalin, ▲—▲; cyclohexene,
○—○; tetralin, △—△; *trans*-decalin.



Ia, b

a; X=Met, b; X=Leu

Fig. 2. Synthetic Route for Enkephalins

removed by evaporation. The α-amino function of amino acids except for the N-terminal Tyr residue was protected with the Z group. The C-terminal carboxyl group was protected as its *tert*-butyl ester. In the case of Met-enkephalin, Z–Met–OH was *tert*-butyl-esterified by the isobutylene method.[8] The Z group was removed by transfer hydrogenation in EtOH over a palladium catalyst using cyclohexene as a proton donor to afford H–Met–OBu^t. Z–Phe–OH and H–Met–OBu^t were coupled by the DCC method to give Z–Phe–Met–OBu^t (**1a**). **1a** was dissolved in dry EtOH containing cyclohexene. The solution was refluxed in the presence of palladium black to give the dipeptide amine. This amine was coupled with Z–Gly–OH by the DCC–HOBt method[9] to give Z–Gly–Phe–Met–OBu^t (**3a**), which was purified by silica gel column chromatography using 1% MeOH in $CHCl_3$ as the eluant. Boc–Tyr–Gly–OMe (**5**) was prepared from Boc–Tyr–OH[10] and H–Gly–OMe by the DCC method and converted to the corresponding hydrazide (**6**) by treatment with hydrazine hydrate. After removal of the Z group of **3a** by transfer hydrogenation, the resulting amine was coupled with Boc–Tyr–Gly–N_3, prepared from **6** by treatment with isopentyl nitrite to give Boc–Tyr–Gly–Gly–Phe–Met–OBu^t (**7a**), which was purified by column chromatography on silica gel. The protecting groups were removed by treatment with trifluoroacetic acid (TFA) containing thioanisole to give crude pentapeptide trifluoroacetate. This salt was converted to the acetate form by treatment with Amberlite CG 400 (acetate form) and purified by gel-filtration on Sephadex G-25, equilibrated and eluted with 5% AcOH.

Leu–Enkephalin was synthesized by the same route as described above (Fig. 2) using catalytic hydrogenation to remove the Z group. Starting with H–Phe–Leu–OBu^t,[11] Boc–Tyr–Gly–Gly–Phe–Leu–OBu^t (**7b**) was prepared and the desired pentapeptide acetate (Ib) was obtained by the same method as described above. In the column chromatography, the eluted material was monitored by following the absorbance at 275 nm. Both purified peptides, (Ia) and (Ib) were homogeneous upon thin-layer chromatography on silica gel. Amino acid ratios in acid and enzymatic (AP–M)[12] hydrolysates were in good agreement with the theoretically expected values. The biological activity of Met-enkephalin (Ia) and Leu-enkephalin

(Ib) obtained above was determined by measurement of the inhibition of electrically evoked contraction of guinea pig ileum.[13] Values were expressed as negative logarithms of ED50 values and were as follows: morphine (6.96±0.07), Met-enkephalin (7.67±0.17) and Leu-enkephalin (7.04±0.15). These values are in good agreement with those reported for the natural materials,[14] indicating that transfer hydrogenation may be used for the synthesis of Met-containing biologically active peptides.

Experimental

The melting points are uncorrected. Optical rotations were measured with an automatic polarimeter, model DIP-180 (Japan Spectroscopic Co., Ltd.). Amino acid compositions of acid and enzymatic hydrolysates were determined with a JEOL JLC-6AH amino acid analyzer. The absorbance at 570 nm was measured with a Shimazu CS-910 chromatoscanner. On thin-layer chromatography (Kieselgel G, Merck), Rf^1, Rf^2 and Rf^3 values refer to the systems of $CHCl_3$, MeOH and AcOH (90: 8: 2), *n*-butanol, AcOH, pyridine and H_2O (4: 1: 1: 2) and $CHCl_3$, MeOH and H_2O (8: 3: 1, lower phase), respectively.

General Transfer Hydrogenation Procedure for Examination of Proton Donors——Z–Met–OBu^t (340 mg, 1 mmol) was dissolved in dry EtOH (10 ml). Palladium black (250 mg), which has been washed with dry EtOH, and proton donor (50 mmol) were added. This reaction mixture was refluxed. Ten μl of the mixture was spotted on a silica gel plate. The plate was developed using a solvent of $CHCl_3$: MeOH: H_2O (8: 3: 1, lower phase). After removal of the Z group of unreacted Z–Met–OBu^t by HBr/AcOH treatment on the thin-layer, ninhydrin solution was sprayed. The absorbance at 570 nm was measured with a chromatoscanner as a function of time.

Z-Phe-Met-OBu^t (1a)——A solution of Z–Met–OBu^t (1.1 g) in dry EtOH (20 ml) and cyclohexene (10 ml) was refluxed for 3 h in the presence of palladium black (200 mg) which had been washed thoroughly with dry EtOH. After removal of the palladium and the solvent, H–Met–OBu^t was obtained as an oily material. The material and Z–Phe–OH (0.97 g) were dissolved in CH_3CN (15 ml) and the solution was cooled to −10°C. DCC (0.7 g) was added and the resulting mixture was stirred overnight. The urea derivative and the solvent were removed and the residue was extracted with AcOEt. The extract was washed with 5% Na_2CO_3 and water, dried over Na_2SO_4 and concentrated. The oily residue in $CHCl_3$ (3 ml) was applied to a silica gel column (2×12 cm) equilibrated with $CHCl_3$ and the column was eluted with the same solvent. Individual fractions (50 ml each) were collected. The eluate (Nos. 2—4) was concentrated and petroleum ether was added to the residue to afford a crystalline material; yield 700 mg (45.0%); mp 85—87°C; $[\alpha]_D^{27}$ −17.2° (c=1.0, MeOH), Rf^1 0.80. *Anal.* Calcd for $C_{26}H_{34}N_2O_5S$: C, 64.2; H, 7.04; N, 5.8. Found: C, 64.4; H, 7.10; N, 5.7.

Z-Gly-Phe-Met-OBu^t (3a)——A solution of **1a** (243 mg) in dry EtOH (8 ml) and cyclohexene (4 ml) was refluxed for 30 min over a palladium catalyst (50 mg). After removal of the palladium and the solvent, the resulting amine, H–Phe–Met–OBu^t (Rf^1 0.27) and Z–Gly–OH (100 mg) were dissolved in CH_3CN (10 ml) and DMF (10 ml) and the solution was cooled to −10°C. DCC (120 mg) and 1-hydroxybenzotriazole (HOBt, 81 mg) were added to the solution and the reaction mixture was stirred overnight. After removal of the urea derivative and the solvent, the residue was dissolved in AcOEt. The extract was washed with 5% Na_2CO_3 and water, dried over Na_2SO_4 and concentrated. The oily residue in $CHCl_3$ (3 ml) was applied to a silica gel column (1.3×15 cm). The column was eluted with $CHCl_3$ (200 ml) and then 1% MeOH in $CHCl_3$ (200 ml). The desired peptide was obtained from the latter eluate and recrystallized from ether and petroleum ether; yield 160 mg (59%), mp 78—81°C, $[\alpha]_D^{27}$ −25.0° (c=0.5, MeOH), Rf^1 0.55. *Anal.* Calcd for $C_{28}H_{37}N_3O_6S$: C, 61.9; H, 6.86; N, 7.7. Found: C, 62.0; H, 6.68; N, 7.7.

Boc-Tyr-Gly-OMe (5)——Boc–Tyr–OH (14 g) and H–Gly–OMe (prepared from 6.3 g of H–Gly–OMe·HCl and 7 ml of triethylamine) were dissolved in CH_3CN (100 ml). DCC (12.1 g) was added to the above solution at −10°C. The reaction mixture was stirred overnight. After removal of urea derivative and the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and concentrated. Petroleum ether was added to the residue to afford a solid material, which was collected by filtration and recrystallized from AcOEt and ether; yield 10.5 g (60%), mp 105—110°C, $[\alpha]_D^{27}$ +2° (c=1.0, MeOH), Rf^1 0.58. *Anal.* Calcd for $C_{17}H_{24}N_2O_6$: C, 57.9; H, 6.87; N, 8.0. Found: C, 57.7; H, 6.80; N, 8.1.

Boc-Tyr-Gly-NHNH$_2$ (6)——Hydrazine hydrate (80%, 5 ml) was added to a solution of **5** (7 g) in MeOH (40 ml). The solution was kept at room temperature overnight. After removal of the solvent, ether was added to the residue to give a crystalline material, which was collected by filtration and recrystallized from MeOH and ether; yield 2.5 g (36%), mp 117—119°C, $[\alpha]_D^{27}$ +21.8° (c=1.0, MeOH). *Anal.* Calcd for $C_{16}H_{24}N_4O_5 \cdot 1/2H_2O$: C, 53.2; H, 6.97; N, 15.5. Found: C, 53.2; H, 6.97; N, 15.7.

Boc-Tyr-Gly-Gly-Phe-Met-OBu^t (7a)——A solution of **3a** (130 mg) in dry EtOH (8 ml) and cyclohexene (4 ml) was refluxed over a palladium catalyst for 30 min. After removal of the palladium, the solvent was evaporated off to give the corresponding amine. Boc–Tyr–Gly–N_3 (prepared from 88 mg of **6** with isopentyl nitrite in the usual manner) in DMF (5 ml) was added to a cold solution of the tripeptide amine

prepared above in DMF (5 ml). The reaction mixture was stirred overnight at 4°C and concentrated. The residue was extracted with AcOEt. The extract was washed with water, dried over Na_2SO_4 and concentrated. The residue in $CHCl_3$ (5 ml) was applied to a silica gel column (1.2×18 cm). The column was eluted with $CHCl_3$ (200 ml) followed by 1% MeOH in $CHCl_3$ (500 ml) and 3% MeOH in $CHCl_3$ (200 ml). The eluate with 3% MeOH in $CHCl_3$ provided the desired pentapeptide (**7a**); yield 80 mg (46%), mp 125—130°C, $[\alpha]_D^{27}$ −11.0° (c=1.0, DMF), Rf^1 0.38. *Anal.* Calcd for $C_{36}H_{51}N_5O_9S$: C, 59.2; H, 7.04; N, 9.6. Found: C, 59.5; H, 7.06; N, 9.5. Amino acid ratios in an acid hydrolysate: Gly 2.00; Met 0.72; Tyr 0.91; Phe 1.15 (average recovery 88%).

H-Tyr-Gly-Gly-Phe-Met-OH (Ia)——A solution of Boc–Tyr–Gly–Gly–Phe–Met–OBu^t (**7a**, 50 mg) in TFA (0.5 ml) containing thioanisole (0.1 ml) was kept at room temperature for 1 h. Ether was added to the solution to form a precipitate, which was collected by centrifugation, washed with ether and dried over KOH pellets *in vacuo*. The precipitate was dissolved in water (12 ml) and treated with Amberlite CG 400 (acetate form) to convert the trifluoroacetate to the corresponding acetate. This acetate was purified by gel-filtration on Sephadex G-25 (2.5×135 cm) equilibrated with 5% AcOH, and the column was eluted with the same solvent. The eluted material was monitored by measuring the absorbance at 275 nm. Fractions (6 g each) were collected and the solvent of the desired effluent fractions (tube Nos. 57—70) was evaporated off. The residue was lyophilized to afford a white fluffy powder; yield 33 mg (74%), $[\alpha]_D^{25}$ −24.0° (c=0.2, DMF) (lit.[15] $[\alpha]_D^{22}$ −21.9° (c=1.0, DMF)), Rf^2 0.70. *Anal.* Calcd for $C_{27}H_{35}N_5O_7S\cdot CH_3COOH\cdot 1/2H_2O$: C, 52.7; H, 6.40; N, 10.6. Found: C, 52.4; H, 6.18; N, 10.9. Amino acid ratios in an acid hydrolysate: Gly 2.10; Met 0.94; Tyr 0.93; Phe 1.00 (average recovery 85%), amino acid ratios in an AP-M digest: Gly 2.15; Met 0.95; Tyr 1.00; Phe 1.00 (average recovery 88%).

Z-Gly-Phe-Leu-OBu^t (3b)——Z–Gly–OH (2.1 g) and H–Phe–Leu–OBu^t[6,11] (3.3 g) were dissolved in DMF (50 ml). DCC (2.5 g) was added to the solution at −10°C. This reaction mixture was stirred at room temperature overnight. After removal of the urea derivative and the solvent, the residue was dissolved in AcOEt. The AcOEt layer was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. The residue was crystallized from ether and petroleum ether; yield 4.3 g (82%), mp 115—116°C, $[\alpha]_D^{25}$ −25.0° (c=1.0, MeOH), Rf^1 0.67. *Anal.* Calcd for $C_{29}H_{39}N_3O_6$: C, 66.3; H, 7.48; N, 8.0. Found: C, 66.4; H, 7.54; N, 8.2.

Boc-Tyr-Gly-Gly-Phe-Leu-OBu^t (7b)——H–Gly–Phe–Leu–OBu^t (prepared from 1.0 g of **3b** by catalytic hydrogenation over palladium) was dissolved in DMF (5 ml). Boc–Tyr–Gly–N_3 (prepared from 0.7 g of **6** with isopentyl nitrite) in DMF (5 ml) was added to the cold DMF solution of the tripeptide amine prepared above. The reaction mixture was stirred at 4°C overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with water, dried over Na_2SO_4 and concentrated. The residue in $CHCl_3$ (5 ml) was applied to a silica gel column (2×50 cm). The column was eluted with $CHCl_3$ (1000 ml) followed by 3% MeOH in $CHCl_3$ (300 ml). The desired peptide was obtained from the latter eluate, yield 0.71 g (53%), mp 119—123°C, $[\alpha]_D^{25}$ −9.4° (c=1.0, MeOH), Rf^3 0.75. *Anal.* Calcd for $C_{37}H_{53}N_5O_9$: C, 62.4; H, 7.51; N, 9.8. Found: C, 62.7; H, 7.64; N, 9.8. Amino acid ratios in an acid hydrolysate: Gly 2.11; Leu 1.00; Tyr 1.04; Phe 1.08 (average recovery 84%).

H-Tyr-Gly-Gly-Phe-Leu-OH (Ib)——A solution of **7b** (100 mg) in TFA (1 ml) containing anisole (0.1 ml) was kept at room temperature for 1 h. After removal of TFA by evaporation, ether was added to the residue to afford a white precipitate, which was collected by centrifugation, washed with ether and dried over KOH pellets *in vacuo*. The trifluoroacetate was dissolved in water (15 ml) and treated with Amberlite CG 400 (acetate form). After removal of the resin, the solution was concentrated to 3 ml and applied to a Sephadex G-25 column (3×140 cm) equilibrated and eluted with 5% AcOH. The eluted material was detected by measuring the absorbance at 275 nm. Fractions (6 g each) were collected and the solvent of the desired effluent fractions (tube Nos. 59—71) was removed. The residue was lyophilized to give a purified fluffy powder, 70 mg (79%), $[\alpha]_D^{25}$ −14.0° (c=0.2, DMF) (lit.[16] $[\alpha]_D^{20}$ −15.3° (c=0.3, DMF)), Rf^2 0.70. *Anal.* Calcd for $C_{28}H_{37}N_5O_7\cdot CH_3COOH\cdot H_2O$: C, 56.9; H, 6.84; N, 11.1. Found: C, 56.9; H, 6.61; N, 11.5. Amino acid ratios in an acid hydrolysate: Gly 2.00; Leu 1.10; Tyr 1.02; Phe 0.90 (average recovery 84%), amino acid ratios in an AP-M digest: Gly 2.00; Leu 1.10; Tyr 1.02; Phe 0.85 (average recovery 90%).

Acknowledgement The authors are greatly indebted to Dr. Y. Kiso, Faculty of Pharmaceutical Sciences, University of Tokushima, for carrying out tests of biological activity and for valuable discussions throughout this investigation. They also thank Mr. T. Hirai for technical assistance.

References and Notes

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