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Amino Acids and Peptides. VII.[1] Preparation and Application of a Water-Soluble Active Ester, *p*-Trimethylammoniophenyl Ester

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A water-soluble active ester, *p*-trimethylammoniophenyl ester iodide, and its nitro derivative were prepared from *N*-protected amino acid and the corresponding phenol derivative by the dicyclohexylcarbodiimide method. The synthetic esters were applied to the synthesis of Leu-enkephalin.

Keywords——water-soluble active ester; *p*-trimethylammoniophenyl ester iodide; Leu-enkephalin; peptide synthesis

The active ester method is a useful tool in peptide synthesis, but the removal of unreacted active ester and of the hydroxyl compound resulted from the reaction is difficult in some instances. A water-soluble active ester, like water-soluble carbodiimide,[2] might be advantageous for easy purification of the product by an extraction procedure. Mitin[3] reported that the treatment of benzyloxycarbonylamino acid *p*-dimethylaminophenyl ester (Z–X–ODAP) with methyl iodide gave an active ester, Z-amino acid *p*-trimethylammoniophenyl ester iodide (Z–X–OTAP). We examined its suitability as a water-soluble active ester and studied its preparation and application to peptide synthesis in some detail.

p-Methoxybenzyloxycarbonylglycine *p*-trimethylammoniophenyl ester iodide [Z-(OMe)–Gly–OTAP] was prepared by Mitin's procedure and the dicyclohexylcarbodiimide (DCC) procedure from Z(OMe)–glycine and *p*-trimethylammoniophenol iodide as shown in Fig. 1. The total yield based on Z(OMe)–Gly–OH was 11% by Mitin's procedure and 48% by the DCC procedure. *p*-Dimethylaminophenol[4] used in Mitin's procedure was not commercially available, and it was prepared from *p*-trimethylammoniophenol iodide[4] used in the DCC procedure. The above results show that the DCC procedure is superior to Mitin's procedure. A disadvantage of the DCC procedure is the need to remove the unreacted phenol from the product. The yield of ester by the DCC procedure was almost quantitative judging from thin-layer chromatography (TLC) of the reaction mixture, but the actual yield of analytically pure product after recrystallization was decreased to 48%. Contamination by unreacted phenol did not disturb the subsequent coupling reaction of the active ester with an amine component when the crude active ester was tested in a coupling reaction.

Next, application of the *p*-trimethylammoniophenyl ester iodide to peptide synthesis was examined. Leu-enkephalin was synthesized by this method and the result was compared with that by the mixed anhydride method,[5] as shown in Fig. 2. The coupling reactions of the *p*-trimethylammoniophenyl ester iodide with amine components were done in dimethylformamide at room temperature, and the protected synthetic intermediates were purified easily by extraction. No side reaction was observed and the yields were comparable with those of the mixed anhydride method. Z–Tyr(Bzl)–OTAP was not readily soluble in water but it could be removed by washing with water and recrystallization from methanol.

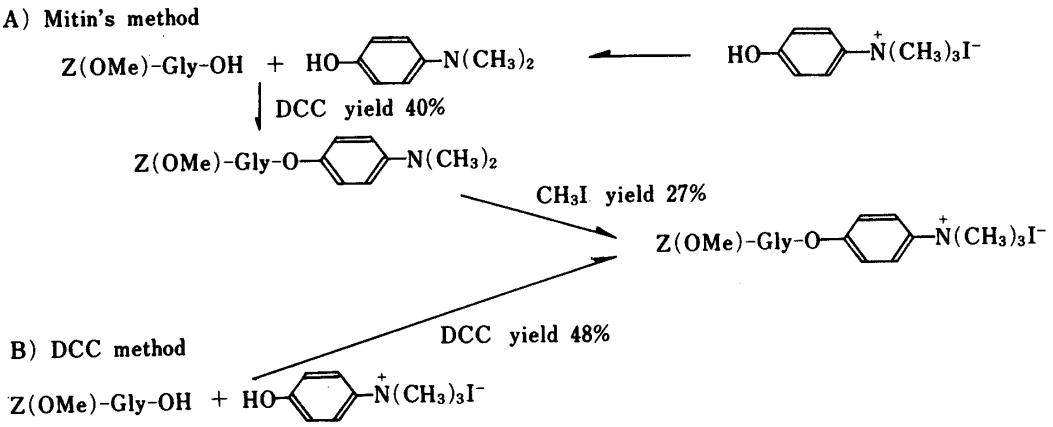

Fig. 1. Preparation of Z(OMe)–Gly–OTAP

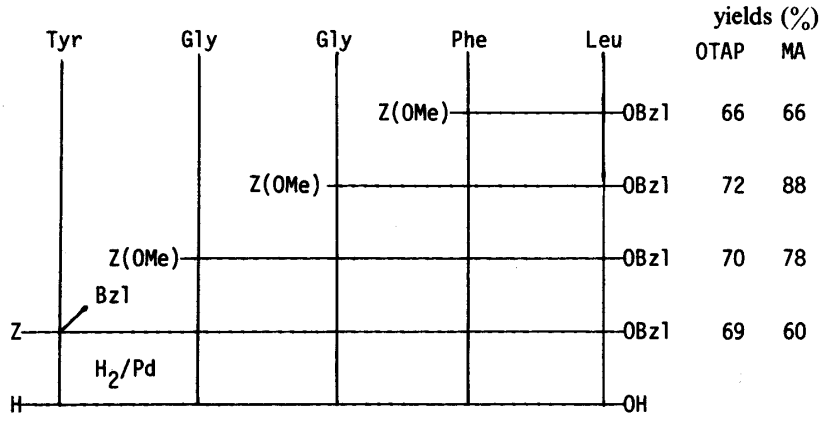

Fig. 2. Synthetic Scheme for Leu-enkephalin

MA: mixed anhydride method. OTAP: *p*-trimethylammoniophenyl ester method. Z(OMe) groups were removed by TFA treatment prior to the coupling reaction.

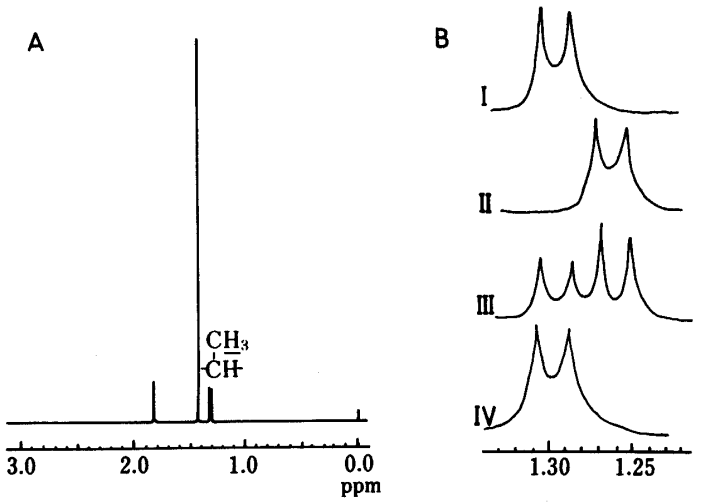

Fig. 3. A. NMR Spectrum Showing the Methyl Signals of Ala in Boc–Ala–Phe–OMe (L-L) Solvent: $CDCl_3$

B. Enlarged Methyl Signals of Ala in Boc–Ala–Phe–OMe [I, L-L; II, D-L; III, L-L + D-L (1 : 1); IV, Prepared by the OTAP Method]

To test the occurrence of racemization in the *p*-trimethylammoniophenyl ester procedure by nuclear magnetic resonance (NMR) spectroscopy according to the method reported by Halpern *et al.*,[6] *tert*-butoxycarbonylalanylphenylalanine methyl ester (Boc–Ala–Phe–OMe)[7] was prepared. The coupling reaction of Boc–Ala–OTAP and H–Phe–OMe was slow at 20°C,

so it was done at 40°C; the yield was 62%.

o-Nitro-*p*-trimethylammoniophenyl ester iodide [OTAP(NO_2)] was also employed for the preparation of Boc–Ala–Phe–OMe. The synthetic Boc–Ala–OTAP(NO_2) was an unstable compound and decomposed gradually at room temperature. Analytically pure Boc–Ala–OTAP(NO_2) could not be obtained but crude Boc–Ala–OTAP(NO_2) reacted much faster than Boc–Ala–OTAP. The reaction was done at 20°C and the yield was 88%. Racemization was not detectable by examining the NMR spectrum of the product in comparison with that of Boc–D-Ala–Phe–OMe prepared by the mixed anhydride method.

The *p*-trimethylammoniophenyl ester is a water-soluble active ester that should be useful for peptide synthesis. The disadvantage of this method lies in the difficulty of purification of the ester. Removal of *p*-trimethylammoniophenol iodide from the ester prepared by the DCC method was difficult in some instances, but the crude ester may be used for coupling reaction without difficulty.

Experimental

Melting points are uncorrected. Solvent systems for ascending TLC on Silica gel G (type 60, E. Merck) are indicated as follows: Rf^1 = BuOH–AcOH–H_2O(4 : 1 : 5, upper phase), Rf^2 = BuOH–pyridine–AcOH–H_2O(4 : 1 : 2), Rf^3 = $CHCl_3$–MeOH–H_2O (8 : 3 : 1, lower phase), Rf^4 = AcOEt–benzene (1 : 1). NMR spectra were taken in $CDCl_3$ on a Brucker AM-400 (400 MHz).

***o*-Nitro-*p*-trimethylammoniophenol Iodide**——CH_3I (6.6 ml) was added to a mixture of *o*-nitro-*p*-aminophenol[8] (5 g), Na_2CO_3 (3.5 g) and H_2O (55 ml) and the whole was refluxed for 8 h. The reaction mixture was washed with AcOEt and concentrated. The separated crystalline product was collected by filtration and recrystallized twice from H_2O and MeOH. Yield 5.74 g (55%), mp 246°C (dec.), Rf^1 0.11. *Anal.* Calcd for $C_9H_{13}IN_2O_3$: C, 33.4; H, 4.1; N, 8.6. Found: C, 33.3; H, 4.1; N, 8.7.

Z(OMe)–Gly–ODAP——Prepared from Z(OMe)–Gly–OH(4.65 g) and *p*-dimethylaminophenol (2.66 g) by DCC (4 g) in DMF (35 ml) in the usual manner. The product was purified by silica gel column chromatography. The material was obtained from the 1% MeOH/$CHCl_3$ eluate. Yield 2.8 g (40%), mp 74—77 °C, Rf^4 0.82. *Anal.* Calcd for $C_{19}H_{22}N_2O_5$: C, 63.7; H, 6.2; N, 7.8. Found: C, 63.9; H, 6.1; N,7.9.

General Procedure for Preparation of *p*-Trimethylammoniophenyl Ester Iodide by the DCC Method——DCC was added to a DMF solution of *N*-protected amino acid *p*-trimethylammoniophenol iodide at −10 °C and the mixture was stirred for 15 h in a cold room. The solvent was evaporated off and AcOEt and H_2O were added to the residue. The insoluble material was filtered off and the aqueous layer was lyophilized. The material was recrystallized from MeOH.

Z(OMe)–Gly–OTAP——1) The DCC Procedure: Prepared according to the general procedure. Yield 2.3 g (46%), mp 167—168 °C, Rf^3 0.21. *Anal.* Calcd for $C_{20}H_{25}IN_2O_5$: C, 48.0; H, 5.0; N, 5.6. Found: C, 48.0; H, 5.1; N, 5.4.

2) From Z(OMe)–Gly–ODAP: CH_3I (0.87 ml) was added to a MeOH solution of Z(OMe)–Gly–ODAP (1 g) and the mixture was refluxed for 12 h. The solvent was evaporated off and the residue was dissolved in H_2O. The H_2O layer was washed with AcOEt and lyophilized. The residue was recrystallized from MeOH. Yield 382 mg (27%), mp 166—168 °C, Rf^3 0.21. The material was identical with Z(OMe)–Gly–OTAP prepared by the DCC procedure described above as judged by mixed melting point determination, and TLC, liquid chromatographic and infrared absorption spectral comparisons.

Z(OMe)–Phe–OTAP——Prepared by the DCC method according to the general procedure. Yield 41%, mp 110—115 °C, Rf^3 0.31, $[\alpha]_D^{27}$ −50.5 °(c = 1.0, DMF). *Anal.* Calcd for $C_{27}H_{31}IN_2O_5I$: C, 54.9; H, 5.3; N, 4.8. Found: C, 55.2; H, 5.2; N, 4.9.

Z–Tyr(Bzl)–OTAP——Prepared by the DCC method according to the general procedure. The material that separated out from the aqueous extract during the extraction procedure was collected by filtration and recrystallized from MeOH. Yield 71%, mp 187—190 °C, Rf^1 0.26, Rf^2 0.38, $[\alpha]_D^{33}$ −7.3 ° (c = 1.0, DMF). *Anal.* Calcd for $C_{33}H_{35}IN_2O_5$: C, 59.5; H, 5.3; N, 4.2. Found: C, 59.8; H 5.3; N, 4.4.

Boc–Ala–OTAP——Prepared by the DCC method according to the general procedure. Yield 36%, mp 159—161 °C, Rf^3 0.41, $[\alpha]_D^{33}$ −40.0°(c = 1.0, H_2O). *Anal.* Calcd for $C_{17}H_{27}IN_2O_4$: C, 45.3; H, 6.1; N, 6.2. Found: C, 45.1; H, 6.0; N, 6.1.

Boc–Ala–OTAP(NO_2)——Prepared by the DCC method according to the general procedure.The material was unstable and could not be obtained in analytically pure form. After lyophilization, the crude material was used for the next coupling reaction without purification.

General Procedure for Peptide Synthesis by the *p*-Trimethylammoniophenyl Ester Method——*N*-Protected amino acid *p*-trimethylammoniophenyl ester iodide (20% excess) and an amino component were reacted in DMF at room

temperature for 16 h. The solvent was evaporated off and the residue was extracted with AcOEt. The AcOEt layer was washed with 5% Na_2CO_3, 5% citric acid and H_2O, and dried over Na_2SO_4. After evaporation of the solvent, the residue was recrystallized from a suitable solvent. Z(OMe) groups on the amino components were removed by TFA-treatment prior to the coupling reactions.

General Procedure for Peptide Synthesis by the Mixed Anhydride Method—— A mixed anhydride [prepared from an *N*-protected amino acid (20% excess over an amino component), Et_3N and iso-butylchloroformate in DMF] was reacted with an amino component in DMF for 6 h and the solvent was evaporated off. The residue was extracted with AcOEt and the extract was washed successively with 5% Na_2CO_3, 5% citric acid and H_2O. The AcOEt was evaporated off and the residue was recrystallized from the same solvent as used for recrystallization of the same peptide synthesized by the active ester (OTAP) method.

Z(OMe)–Phe–Leu–OBzl——1) OTAP Method: Recrystallized from AcOEt/petroleum ether, yield 66%, mp 78—80 °C, Rf^4 0.88, $[\alpha]_D^{31}$ −25.1 °(c=1.0, MeOH). *Anal.* Calcd for $C_{31}H_{36}N_2O_6$: C, 69.9; H, 6.8; N, 5.3. Found: C, 69.6; H, 6.8; N, 5.1. Amino acid ratios in an acid hydrolysate: Leu 1.04, Phe 1.00 (recovery of Phe 91%).

2) The Mixed Anhydride Method: Yield 66%, mp 78—80 °C, Rf^4 0.88, $[\alpha]_D^{31}$ −24.8 ° (c=1.0, MeOH). Amino acid ratio in an acid hydrolysate: Leu 0.99, Phe 1.00 (recovery of Phe 89%).

Z(OMe)–Gly–Phe–Leu–OBzl——1) OTAP Method: Recrystallized from AcOEt/petroleum ether. Yield 72%, mp 93—96 °C, Rf^4 0.62, $[\alpha]_D^{28}$ −18.6 ° (c=1.0, MeOH). *Anal.* Calcd for $C_{33}H_{39}N_3O_7$: C, 67.2; H, 6.7; N, 7.1. Found: C, 67.1; H, 6.6; N, 6.9. Amino acid ratios in an acid hydrolysate: Gly 1.00, Phe 0.96, Leu 1.03(recovery of Gly 89%).

2) The Mixed Anhydride Method: Yield 88%, mp 91—94 °C, Rf^4 0.62, $[\alpha]_D^{28}$ −20.8 ° (c=1.0, MeOH). Amino acid ratios in an acid hydrolysate: Gly 1.00, Phe 1.04, Leu 1.11 (recovery of Gly 84%).

Z(OMe)–Gly–Gly–Phe–Leu–OBzl——1) OTAP Method: Recrystallized from AcOEt/petroleum ether. Yield 70%, mp 132—136 °C, Rf^3 0.69, $[\alpha]_D^{32}$ −16.0 °(c=1.0, MeOH). *Anal.* Calcd for $C_{35}H_{42}N_4O_8$: C, 65.0; H, 6.6; N, 8.7. Found: C, 64.8; H, 6.5; N, 8.6. Amino acid ratios in an acid hydrolysate: Gly 2.00, Phe 0.98, Leu 0.99 (recovery of Gly 91%).

2) The Mixed Anhydride Method: Yield 78%, mp 133—138 °C, Rf^3 0.69, $[\alpha]_D^{32}$ −17.0 ° (c=1.0, MeOH). Amino acid ratios in an acid hydrolysate: Gly 2.00, Phe 0.94, Leu 0.96 (recovery of Gly 94%).

Z–Tyr(Bzl)–Gly–Gly–Phe–Leu–OBzl——1) OTAP Method: After the coupling reaction, the solvent was evaporated off and the residue was washed with 5% Na_2CO_3, 5% citric acid and H_2O in a mortar. The material was recrystallized from MeOH. Yield 69%, mp 146—151 °C, Rf^3 0.90, $[\alpha]_D^{26}$ −23.6 ° (c=1.0, DMF). *Anal.* Calcd for $C_{50}H_{55}N_5O_9$: C, 69.0; H, 6.4; N, 8.1. Found: C, 69.1; H, 6.3; N, 8.0. Amino acid ratios in an acid hydrolysate: Tyr 0.83, Gly 2.00, Phe 0.97, Leu 1.07 (recovery of Gly 83%).

2) The Mixed Anhydride Method: After the coupling reaction, the material was purified in the same manner as described in the OTAP method. Yield 60%, mp 144—150 °C, Rf^3 0.88, $[\alpha]_D^{30}$ −22.4 °(c=1.0, DMF). Amino acid ratios in an acid hydrolysate: Tyr 0.80, Gly 2.00, Phe 1.03, Leu 1.06 (recovery of Gly 87%).

H–Tyr–Gly–Gly–Phe–Leu–OH——Z–Tyr(Bzl)–Gly–Gly–Phe–Leu–OBzl (500 mg) was hydrogenated in a mixture of AcOH (5 mg) and MeOH (20 ml) over Pd catalyst for 20 h. The solvent was evaporated off and the residue was purified by Sephadex LH-20 column (2.5 × 75 cm) chromatography using MeOH as the eluent. Yield 220 mg (79%), mp 202—206 °C [lit.: mp 206—208 °C,[9, 10] mp 142—143 °C[11]], Rf^1 0.76, Rf^3 0.28, $[\alpha]_D^{26}$ −25.2 ° (c=1.0, DMF) [lit.: $[\alpha]_D^{22}$ −23.4 ° (c=1.0, DMF)[9]]. *Anal.* Calcd for $C_{28}H_{37}N_5O_7$: C, 60.5; H, 6.7; N, 12.6. Found: C, 60.1; H, 6.8; N, 12.3. Amino acid ratios in an acid hydrolysate: Tyr 0.79, Gly 2.00, Phe 0.95, Leu 1.01 (recovery of Gly 81%).

Boc–D-Ala–Phe–OMe——Prepared from Boc–D-Ala–OH[12] (1.9 g) and H–Phe–OMe·HCl (1.8 g) by the mixed anhydride method in the usual manner. Recrystallized from ether/petroleum ether. Yield 2.1 g (60%), mp 88 °C, Rf^4 0.65, $[\alpha]_D^{33}$ +24.4 ° (c=1.0, MeOH). *Anal.* Calcd for $C_{18}H_{26}N_2O_5$: C, 61.7; H, 7.5; N, 8.0. Found: C, 61.6; H, 7.6; N, 7.8.

References and Notes

1) Standard abbreviations are used for amino acids, protecting groups, and peptides [*Eur. J. Biochem.*, **138**, 9 (1984)]. Other abbreviations include: OTAP=*p*-trimethylammoniophenyl ester iodide; ODAP=*p*-dimethylaminophenyl ester; TFA=trifluoroacetic acid, DMF=dimethylformamide.
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