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Amino Acids and Peptides. VIII.¹⁾ A Water-Soluble Active Ester, Phenolsulfonic Acid Derivative

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N-Protected amino acid 4-phenolsulfonic acid derivative esters, such as 2,6-dibromo-4-sulfophenyl ester sodium salt, 2,6-dichloro-4-sulfophenyl ester potassium salt and 2-nitro-4-sulfophenyl ester sodium salt, were prepared. These esters are water-soluble active esters, and were applied for the synthesis of Leu-enkephalin.

Keywords—water-soluble active ester; 2,6-dichloro-4-sulfophenyl ester; 2,6-dibromo-4-sulfophenyl ester; 2-nitro-4-sulfophenyl ester; peptide synthesis; Leu-enkephalin

Water-soluble active esters, such as water-soluble carbodiimide,²⁾ may be advantageous in peptide synthesis for easy purification of the product by an extraction procedure. In the preceding paper,³⁾ *p*-trimethylammonio-phenyl ester was examined as a water-soluble active ester. The ester had good reactivity, but its purification was rather difficult and it was somewhat unstable. Here we report water-soluble active esters, substituted 4-sulfophenyl esters, which are easier to purify and more stable than *p*-trimethylammonio-phenyl ester. During our study, Aldwin and Nitecki⁴⁾ reported the use of 2-nitro-4-sulfophenyl ester for a protein-protein crosslinking reaction in the pH range of 6–8 in aqueous media. They prepared *N*-maleimido-6-aminocaproic acid 2-nitro-4-sulfophenyl ester and used it as a crosslinking reagent.

Since 4-phenolsulfonic acid is a strong acid and may cleave acid-labile protecting groups, its salt was examined as an ester component. *tert*-Butoxycarbonylalanine (Boc-Ala-OH) was easily deblocked by 4-phenolsulfonic acid but was stable to sodium 4-phenolsulfonate. Boc-Ala 4-sulfophenyl ester sodium salt [Boc-Ala-OPS (Na)] was first prepared and was reacted with phenylalanine methyl ester. No reaction was observed, which suggested that an electron-attracting group should be introduced onto the phenyl ring to increase the reactivity of the 4-sulfophenyl ester. 2,6-Dichloro-4-sulfophenyl ester potassium salt [OPS (Cl,K)], 2,6-dibromo-4-sulfophenyl ester sodium salt [OPS (Br, Na)] and 2-nitro-4-sulfophenyl ester sodium salt [OPS (NO₂,Na)], each of which has an electron-attracting group on the phenyl ring, were examined as water-soluble active esters.

Potassium 3,5-dichloro-4-phenolsulfonate,⁵⁾ sodium 3,5-dibromo-4-phenolsulfonate⁶⁾ and sodium 3-nitro-4-phenolsulfonate⁷⁾ were prepared from potassium (or sodium) 4-phenolsulfonate according to the known methods. Esterification of benzyloxycarbonylalanine (Z-Ala-OH) and the 4-phenolsulfonic acid derivative was done with dicyclohexylcarbodiimide (DCC)⁸⁾ in dimethylformamide (DMF) to afford Z-Ala-OPS (Cl, K), Z-Ala-OPS (Br, Na) and Z-Ala-OPS (NO₂, Na). All these esters were water-soluble and had good reactivity. Each ester was reacted with phenylalanine methyl ester to form Z-Ala-Phe-OMe in reasonable yield as shown in Table I. No side reaction was observed and the product was easily purified by extraction with ethyl acetate followed by washing out of the unnecessary

TABLE I. Yields of Z-Ala-Phe-OMe Prepared from Z-Ala Ester and H-Phe-OMe

Esters	Yields (%)
Z-Ala-O- SO ₃ Na	0
Z-Ala-O- SO ₃ K	72
Z-Ala-O- SO ₃ Na	66
Z-Ala-O- SO ₃ Na	74

Fig. 1. NMR Spectra Showing the Methyl Signals of Ala in Z-Ala-Phe-OMe

Solvent: CDCl₃. A: standard L-L. B: standard D-L. C: standard L-L + standard D-L. D: prepared by the OPS (Cl, K) method. E: prepared by the OPS (Br, Na) method. F: prepared by the OPS (NO₂, Na) method.

material with 5% sodium carbonate, 5% citric acid and water.

Nuclear magnetic resonance (NMR) spectra of the synthetic Z-Ala-Phe-OMe were measured to examine the occurrence of racemization during the reactions according to the method reported by Weinstein and Pritchard.⁹⁾ The methyl signal of Ala in Z-Ala-Phe-OMe was compared with those of standard Z-L-Ala-L-Phe-OMe^{9,10)} and Z-D-Ala-L-Phe-OMe⁹⁾ as shown in Fig. 1. The methyl signal of Ala in Z-L-Ala-L-Phe-OMe appeared at 1.34 ppm, while the methyl signal of Ala in Z-D-Ala-L-Phe-OMe appeared at 1.29 ppm. A mixture of Z-L-Ala-L-Phe-OMe and Z-D-Ala-L-Phe-OMe showed signals at 1.32 and 1.29 ppm. All methyl signals of Ala in Z-Ala-Phe-OMe prepared by three different phenolsulfonic acid derivative ester procedures appeared at 1.32 ppm and no signal was observed at 1.29 ppm. The results suggested that these phenolsulfonic acid derivative esters are applicable to peptide synthesis.

Next, application of the phenolsulfonic acid derivative ester was examined. Leucine-

Fig. 2. Synthetic Scheme for Leu-enkephalin

MA: mixed anhydride method. Boc and Z(OMe) groups were removed by TFA treatment prior to the coupling reaction.

enkephalin was synthesized by these active ester methods and the coupling yields were compared with those of the mixed anhydride method¹¹⁾ reported in the preceding paper,³⁾ as shown in Fig. 2. The coupling reactions of the esters with amine components were done in DMF 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. *N*-Benzyloxycarbonyl-*O*-benzyltyrosine 2,6-dichloro-4-sulfophenyl ester potassium salt [Z-Tyr(Bzl)-OPS(Cl, K)] was not readily soluble in water but it could be removed from the reaction mixture by washing with water and recrystallization from methanol.

The reactivity of the phenolsulfonic acid derivative esters in aqueous media was also examined. Z-Ala-OPS(Cl, K), Z-Ala-OPS(Br, Na) and Z-Ala-OPS(NO₂, Na) were each reacted with glycine methyl ester in water to give Z-Ala-Gly-OMe. The precipitation of the product occurred immediately after the addition of the active ester to the aqueous solution of glycine methyl ester. The coupling yields were 73% by the OPS(Cl, K) method, 65% by the OPS(Br, Na) method and 79% by the OPS(NO₂, Na) method, and the products were identical with an authentic sample prepared by the known method.¹²⁾

The phenolsulfonic acid derivative esters may be useful not only for peptide synthesis but also for the acylation of proteins in aqueous media.

Experimental

Melting points are uncorrected. Solvent systems for ascending thin-layer chromatography (TLC) on Silica gel G (type 60, E. Merck) are indicated as follows: R_f^1 = BuOH-AcOH-H₂O (4:1:5, upper phase), R_f^2 = BuOH-pyridine-AcOH-H₂O (4:1:1:2), R_f^3 = CHCl₃-MeOH-H₂O (8:3:1, lower phase), R_f^4 = AcOEt-benzene (1:1). NMR spectra were taken in CDCl₃ on a Bruker AM-400 (400 MHz).

Treatment of Boc-Ala-OH with 4-Phenolsulfonic Acid—Boc-Ala-OH (100 mg, 0.53 mm) dissolved in MeOH (0.5 ml) and 4-phenolsulfonic acid (92 mg, 0.53 mm) dissolved in MeOH (5 ml) were combined and the mixture was stirred at room temperature. To examine the deblocking of Boc-Ala-OH, the mixture was checked by TLC (R_f^3 system). The spot of Boc-Ala-OH disappeared within 2 h and the spot of H-Ala-OH appeared.

Boc-Ala-OH was also treated with sodium 4-phenolsulfonate under the same conditions and the deblocking of Boc-Ala-OH was not observed.

General Procedure for Preparation of *N*-Protected Amino Acid 4-Phenolsulfonic Acid Derivative Ester—DCC (4.94 g, 24 mm) was added to a DMF solution (30 ml) of *N*-protected amino acid (20 mm) at -10 °C and the mixture was stirred for 20 min. 4-Phenolsulfonic acid derivative (20 mm) dissolved in DMF (30 ml) was then added to the mixture and the whole was stirred overnight in a cold room. The resulting precipitate was filtered off and the filtrate was evaporated down. The residue was extracted with H₂O, and the aqueous layer was washed with ether and lyophilized. The residue was recrystallized from a suitable solvent.

Z-Ala-OPS (Na)—Prepared according to the general procedure. Recrystallized from ethanol. Yield 74%, mp > 300 °C, R_f^3 0.45, $[\alpha]_D^{25}$ -32.0° (c = 1.0, DMF). Anal. Calcd for C₁₇H₁₆NNaO₅S: C, 50.9; H, 4.0; N, 3.5. Found:

C, 50.9; H, 4.0; N, 3.4.

Z-Ala-OPS (Br, Na)—Prepared according to the general procedure. Recrystallized from propanol. Yield 78%, mp 243 °C (dec), $R_f^{3.0}$ 0.38, $[\alpha]_D^{25} - 12.2^\circ$ ($c = 1.0$, DMF). *Anal.* Calcd for $C_{17}H_{14}Br_2NNaO_7S$: C, 36.5; H, 2.5; N, 2.5. Found: C, 36.7; H, 2.7; N, 2.7.

Z-Ala-OPS (Cl, K)—Prepared according to the general procedure. Recrystallized from a mixture of MeOH and EtOH. Yield 69%, mp > 300 °C, $R_f^{3.0}$ 0.43, $[\alpha]_D^{25} - 18.1^\circ$ ($c = 1.0$, DMF). *Anal.* Calcd for $C_{17}H_{14}Cl_2KNO_7S$: C, 42.0; H, 2.9; N, 2.9. Found: C, 41.7; H, 3.1; N, 2.7.

Z-Ala-OPS (NO₂, Na)—Prepared according to the general procedure. Recrystallized from MeOH. Yield 65%, mp > 300 °C, $R_f^{3.0}$ 0.35, $[\alpha]_D^{25} - 31.2^\circ$ ($c = 1.0$, DMF). *Anal.* Calcd for $C_{17}H_{15}N_2NaO_9S$: C, 45.7; H, 3.4; N, 6.3. Found: C, 45.6; H, 3.5; N, 6.0.

Boc-Phe-OPS (Br, Na)—Prepared according to the general procedure. Recrystallized from a mixture of MeOH and EtOH. Yield 66%, mp > 300 °C, $R_f^{3.0}$ 0.57, $[\alpha]_D^{25} - 44.2^\circ$ ($c = 1.0$, DMF). *Anal.* Calcd for $C_{20}H_{20}Br_2NNaO_7S$: C, 36.5; H, 2.5; N, 2.0. Found: C, 36.6; H, 2.7; N, 1.8.

Z(OMe)-Gly-OPS(Cl, K)—Prepared according to the general procedure. Recrystallized from EtOH. Yield 81%, mp > 300 °C, $R_f^{3.0}$ 0.45. *Anal.* Calcd for $C_{17}H_{14}Cl_2KNO_8S$: C, 40.6; H, 2.8; N, 2.8. Found: C, 40.4; H, 3.0; N, 2.5.

Z(OMe)-Gly-OPS(Br, Na)—Prepared according to the general procedure. Recrystallized from EtOH. Yield 68%, mp > 300 °C, $R_f^{3.0}$ 0.45. *Anal.* Calcd for $C_{17}H_{14}BrNNaO_8S \cdot H_2O$: C, 34.4; H, 2.7; N, 2.4. Found: C, 34.3; H, 2.7; N, 2.5.

Z(OMe)-Gly-OPS(NO₂, Na)—Prepared according to the general procedure. Recrystallized from a mixture of MeOH and EtOH. Yield 77%, mp > 300 °C, $R_f^{3.0}$ 0.43. *Anal.* Calcd for $C_{17}H_{15}N_2NaO_{10}S \cdot 1/2H_2O$: C, 43.3; H, 3.4; N, 5.9. Found: C, 43.4; H, 3.4; N, 6.1.

Z-Tyr(Bzl)-OPS (Cl, K)—Z-Tyr (Bzl)-OH and HOPS (Cl, K) were reacted with DCC according to the general procedure. After removal of the precipitated urea, the solvent was evaporated off and the residue was extracted with H₂O. Precipitation occurred when the aqueous layer was washed with ether and the precipitate was collected by filtration and recrystallized from MeOH. Yield 66%, mp > 300 °C, $R_f^{3.0}$ 0.71, $[\alpha]_D^{25} - 41.0^\circ$ ($c = 1.0$, DMF). *Anal.* Calcd for $C_{30}H_{24}Cl_2KNO_8S$: C, 53.9; H, 3.6; N, 2.1. Found: C, 53.7; H, 3.9; N, 1.9.

General Procedure for Peptide Synthesis with 4-Sulfophenyl Ester Derivative—An *N*-protected amino acid 4-sulfophenyl ester derivative and an amino component (1 : 1 in molar ratio) were dissolved in DMF and the solution was stirred at room temperature for 15 h. The solvent was evaporated off and the residue was extracted with AcOEt. The AcOEt layer was washed successively with 5% citric acid, 5% Na₂CO₃ and H₂O, and dried over Na₂SO₄. The AcOEt was evaporated off and the residue was recrystallized from a suitable solvent.

Z-Ala-Phe-OMe—prepared according to the general procedure. Recrystallized from AcOEt–petroleum ether. a) OPS (Cl, K) method: Yield 72%, mp 101–103 °C, $R_f^{4.0}$ 0.87, $[\alpha]_D^{32} - 8.1^\circ$ ($c = 1.0$, EtOH). *Anal.* Calcd for $C_{21}H_{24}N_2O_5$: C, 65.6; H, 6.3; N, 7.3. Found: C, 65.8; H, 6.3; N, 7.1. Amino acid ratios in an acid hydrolysate: Ala 1.00, Phe 0.96 (recovery of Ala, 84%). b) OPS (Br, Na) method: Yield 66%, mp 103–104 °C, $R_f^{4.0}$ 0.87, $[\alpha]_D^{28} - 8.4^\circ$ ($c = 1.0$, EtOH). Amino acid ratio in an acid hydrolysate, Ala 1.00, Phe 0.97 (recovery of Ala, 89%). c) OPS(NO₂, Na) method: Yield 74%, mp 102–103 °C, $R_f^{4.0}$ 0.87, $[\alpha]_D^{29} - 8.9^\circ$ ($c = 1.0$, EtOH). Amino acid ratio in an acid hydrolysate, Ala 1.00, Phe 1.05 (recovery of Ala, 89%). [lit.¹²⁾ mp 99–100 °C, $[\alpha]_D^{22} - 9.3^\circ$ ($c = 1.0$, EtOH)].

Z-Ala-OPS (Na) was also reacted with H-Phe-OMe but no reaction was observed.

Boc-Phe-Leu-OBzl—Boc-Phe-OPS (Br, Na) (1.67 g, 2.2 mm) and H-Leu-OBzl [prepared from its tosylate (787 mg, 2 mm) by treatment with Et₃N] were reacted according to the general procedure. Recrystallized from a mixture of ether and petroleum ether. Yield 688 mg (73%). mp 86–87 °C, $R_f^{3.0}$ 0.95, $R_f^{4.0}$ 0.86, $[\alpha]_D^{24} - 23.0^\circ$ ($c = 1.0$, MeOH). *Anal.* Calcd for $C_{27}H_{36}N_2O_5$: C, 69.2; H, 7.7; N, 6.0. Found: C, 69.0; H, 7.7; N, 5.9. Amino acid ratio in an acid hydrolysate: Phe 1.00, Leu 1.06 (recovery of Phe, 86%).

Boc-Phe-Leu-OBzl was also prepared from Boc-Phe-OH (2 g 7.5 mm) and H-Leu-OBzl tosylate (2.68 g, 6.8 mm) in DMF by the mixed anhydride method in the usual manner.¹¹⁾ Yield 2.36 g (74%), mp 87–88 °C, $R_f^{3.0}$ 0.86, $[\alpha]_D^{27} - 25.7^\circ$ ($c = 1.0$, MeOH). Amino acid ratios in an acid hydrolysate: Ala 1.00, Leu 0.99 (recovery of Ala, 89%).

Z(OMe)-Gly-Phe-Leu-OBzl—Z(OMe)-Gly-OPS (Cl, K) (1 g, 2 mm) was reacted with H-Phe-Leu-OBzl [prepared from 852 mg of Boc-Phe-Leu-OBzl (1.8 mm) by trifluoroacetic acid (TFA) treatment followed by Et₃N treatment] according to the general procedure. Recrystallized from AcOEt–petroleum ether. Yield 787 mg (71%), mp 136–137 °C, $R_f^{3.0}$ 0.93, $R_f^{4.0}$ 0.76, $[\alpha]_D^{28} - 10.3^\circ$ ($c = 1.0$, MeOH). *Anal.* Calcd for $C_{33}H_{36}N_3O_7$: C, 67.2; H, 6.7; N, 7.1. Found: C, 67.1; H, 6.7; N, 6.8. Amino acid ratios in an acid hydrolysate: Gly 1.00, Phe 1.02 (recovery of Gly, 88%).

Z(OMe)-Gly-Phe-Leu-OBzl was also prepared by the OPS (Br, Na) method and the OPS (NO₂, Na) method. The yields were 69% by the OPS (Br, Na) method and 79% by the OPS (NO₂, Na) method. The products were identical with those prepared by the OPS (Cl, K) method and the mixed anhydride method.³⁾

Z(OMe)-Gly-Gly-Phe-Leu-OBzl—Z(OMe)-Gly-OPS (Cl, K) (603 mg, 1.2 mm) was reacted with H-Gly-Phe-Leu-OBzl [prepared from 590 mg of Z(OMe)-Gly-Phe-Leu-OBzl (1 mm) by TFA treatment followed by Et₃N treatment] according to the general procedure. Recrystallized from a mixture of AcOEt and petroleum ether. Yield 445 mg (69%), mp 147–150 °C, $R_f^{3.0}$ 0.69, $[\alpha]_D^{28} - 15.8^\circ$ ($c = 1.0$, MeOH). *Anal.* Calcd for $C_{35}H_{42}N_4O_8$: C, 65.0; H, 6.6;

N, 8.7. Found: C, 64.9; H, 6.5; N, 8.5. Amino acid ratios in an acid hydrolysate: Gly 2.00, Phe 0.99, Leu 1.03 (recovery of Gly, 84%).

Z-Tyr(Bzl)-Gly-Gly-Phe-Leu-OBzl—Z-Tyr (Bzl)-OPS (Cl, K) (620 mg, 0.93 mm) and H-Gly-Gly-Phe-Leu-OBzl [prepared from 500 mg of Z(OMe)-Gly-Phe-Leu-OBzl (0.77 mm) by TFA treatment followed by Et₃N treatment] were reacted in DMF (8 ml) at 30 °C for 48 h. The solvent was evaporated off and the residue was washed successively with 5% Na₂CO₃, 5% citric acid and H₂O. The material was purified by Sephadex LH 20 column (2.5 × 80 cm) chromatography using a mixture of MeOH and dioxane (1:1) as the eluent. Yield 424 mg (63%), mp 146–150 °C, *R*_f³ 0.91, $[\alpha]_D^{25} -24.2^\circ$ (*c* = 1.0, DMF) *Anal.* Calcd for C₅₀H₅₅N₅O₉: C, 69.0; H, 6.4; N, 7.9. Found: C, 69.0; H, 6.3; N, 7.9. Amino acid ratios in an acid hydrolysate: Tyr 0.86, Gly 2.00, Phe 0.96, Leu 1.02 (recovery of Gly, 88%).

H-Tyr-Gly-Gly-Phe-Leu-OH—Prepared from its protected derivative (435 mg) by catalytic hydrogenation as described in the preceding paper.³⁾ Yield 220 mg (79%), mp 201–205 °C, *R*_f¹ 0.76, $[\alpha]_D^{26} -24.9^\circ$ (*c* = 1.0, DMF). *Anal.* Calcd for C₂₈H₃₇N₅O₇: C, 60.5; H, 6.7; N, 12.6. Found: C, 60.2; H, 6.8; N, 12.2. Amino acid ratios in an acid hydrolysate: Tyr 0.81, Gly 2.00, Phe 1.04, Leu 1.06 (recovery of Gly, 88%). [lit.³⁾: mp 202–206 °C, $[\alpha]_D^{22} -23.4^\circ$ (*c* = 1.0, DMF)].

Preparation of Z-Ala-Gly-OMe by the Phenolsulfonic Acid Derivative Ester Methods in H₂O—Z-Ala phenolsulfonic acid derivative ester (2.1 mm) was added to a solution of H-Gly-OMe (2.1 mm, prepared from its HCl salt by addition of Et₃N) in H₂O (5 ml). Precipitation of the product occurred immediately and the mixture was stirred over night. The product was extracted from the mixture with AcOEt and the AcOEt layer was washed successively with 5% citric acid, 5% Na₂CO₃ and H₂O, and dried over Na₂SO₄. The solvent was evaporated off and the residue was recrystallized from a mixture of AcOEt and petroleum ether.

a) OPS (Cl, K) Method: Yield 73%, mp 98–100 °C, $[\alpha]_D^{29} -23.5^\circ$ (*c* = 1.0, MeOH), *R*_f⁴ 0.49. Amino acid ratio in an acid hydrolysate, Ala 1.03, Gly 1.00 (recovery of Gly, 86%). *Anal.* Calcd for C₁₄H₁₈N₂O₅: C, 57.1; H, 6.2; N, 9.5. Found: C, 57.0; H, 6.2; N, 9.6.

b) OPS (Br, Na) Method: Yield 65%, mp 98–100 °C, $[\alpha]_D^{27} -27.0^\circ$ (*c* = 1.0, MeOH), *R*_f⁴ 0.49. Amino acid ratios in an acid hydrolysate, Ala 0.98, Gly 1.00 (recovery of Gly, 91%).

c) OPS (NO₂, Na) Method: Yield 79%, mp 98–101 °C, $[\alpha]_D^{28} -27.8^\circ$ (*c* = 1.0, MeOH), *R*_f⁴ 0.49. Amino acid ratio in an acid hydrolysate, Ala 1.04, Gly 1.00 (recovery of Gly, 88%). (lit.¹²⁾ Z-Ala-Gly-OMe: mp 98–99 °C, $[\alpha]_D^{15} -25.1^\circ$ (*c* = 5, MeOH)].

References and Notes

- 1) Standard abbreviations are used for amino acids, protecting groups, and peptides [*Eur. J. Biochem.*, **138**, 9 (1984)].
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