

A SIMPLE METHOD FOR SYNTHESIS OF AMIDES
AND PEPTIDES THROUGH ACYL CHLORIDES.
A RAPID SYNTHESIS OF THYROTROPIN RELEASING HORMONE

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Abstract - By improvement of the classical SOCl_2 -pyridine method for the preparation of acid chlorides, amides and optically pure peptides were synthesized rapidly in a simple manner from DCHA (dicyclohexylammonium) salts of carboxylic acids. This modified SOCl_2 -pyridine method was applied to a rapid synthesis of TRH.

For the preparation of acid chlorides by the classical SOCl_2 -pyridine method, a long reaction period was usually used.² However, it turned out that carboxylic acids were converted into the corresponding acid chlorides rapidly (usually within 6 min) and almost quantitatively with a nearly stoichiometric amount of SOCl_2 in the presence of pyridine in CH_2Cl_2 at room temp as previously described.³ Further investigations on this reaction have shown that DCHA (dicyclohexylammonium) salts of carboxylic acids are activated more rapidly (within 1 min) under similar conditions. On the basis of this observation, such improvement of the classical SOCl_2 -pyridine method was applied to rapid one-pot synthesis of amides and optically pure peptides. We report here this modified method and its application to a rapid synthesis of TRH.

First, synthesis of simple amides by this method was studied. DCHA salts of carboxylic acids were activated sufficiently within 1 min employing 1.2 equiv of SOCl_2 and 1.3 equiv of pyridine in CH_2Cl_2 at room temp (Step 1). After 1 min, the reaction mixture was treated *in situ* with 0.77 equiv of amines in the presence of 1.5 equiv of DMAP or DBU as a base at room temp for 20 min to give amides in excellent yields (Step 2), as shown in Table 1. In the absence of pyridine activation of DCHA salts under the same conditions was not satisfactory and amides were obtained in only low yields.

Application of this method to peptide synthesis was next examined. Peptide bond was produced under the conditions similar to those of simple amides in good yields, employing DCHA salts of benzyloxycarbonylamino acids.⁴ Several dipeptides were prepared rapidly employing DMAP as a base for Step 2 (Table 2). In 400 MHz ¹H-NMR spectra of crude Z-L-Phe-L-Ala-OMe and Z-L-Ala-L-Phe-OMe, peaks due to DL-isomers were not observed.⁵ In the Young test,⁶ although complete racemization was anticipated, the present method gave at -14° Bz-Leu-Gly-OEt having $[\alpha]_D^{20} -3.90^\circ$ (2.00, EtOH, L-isomer 11%) in 26% yield from Gly-OEt·HCl. Coupling of free Bz-L-Leu instead of Bz-L-Leu·DCHA with Gly-OEt·HCl under the same conditions resulted in almost complete racemization. Therefore, this modified SOCl_2 -pyridine method seems to be superior to the classical one in regard to racemization.

Table 1 Synthesis of Amides

Amide	Base for Step 2	Yield ^a (%)	mp (°C)
PhCH ₂ CH ₂ CONHCH ₂ Ph	DMAP	93	83 - 84
PhCH ₂ CH ₂ CONHt-Bu	DBU	86	oil
PhCH ₂ CH ₂ CON(n-Bu) ₂	DBU	85	oil
c-C ₆ H ₁₁ CONHPh	DMAP	90	145 - 146
c-C ₆ H ₁₁ CONHCH ₂ Ph	DMAP	92	103 - 104
c-C ₆ H ₁₁ CON(CH ₂) ₄	DMAP	95	66 - 67
PhCONHs-Bu	DBU	86	74 - 75
PhCON(CH ₂) ₄	DBU	91	oil

a) From amine.

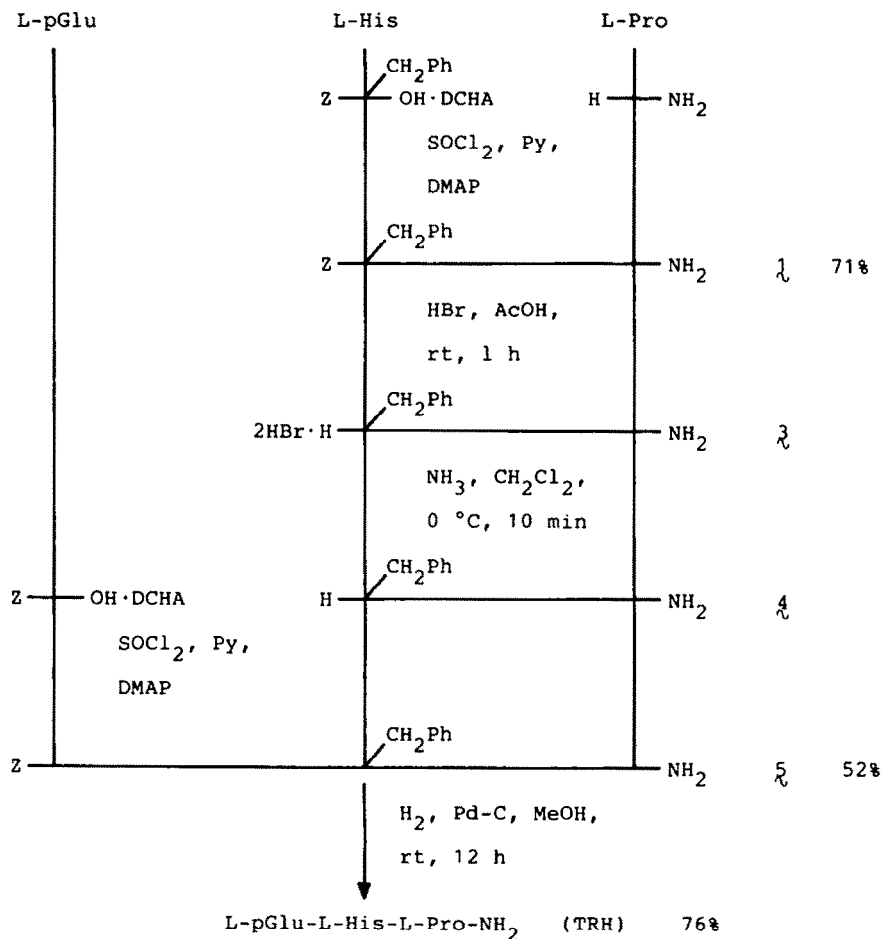
Table 2 Synthesis of Dipeptides

Dipeptide	Yield ^a (%)	mp (°C)	[α] _D (°C, Conc, Solv)
Z-L-Ala-Gly-OEt	85	97 - 98	-20.9° (24, 1.00, EtOH)
Z-L-Phe-Gly-OEt	83	105 - 106	-15.7° (24, 1.00, EtOH)
Z-L-Val-Gly-OEt	73	164 - 165	-17.5° (24, 1.00, EtOH)
Z-L-Leu-Gly-OEt	82	98 - 99	-26.5° (20, 1.55, EtOH)
Z-L-Ala-L-Phe-OMe	80	100 - 101	-9.24° (22, 1.00, EtOH)
Z-L-Phe-L-Ala-OMe	94	126 - 127	-22.9° (25, 1.25, EtOH)
Z-L-Val-L-Val-OMe	85	102 - 103	-21.2° (23, 1.00, EtOH)
Z-L-Leu-L-Phe-OMe	76	87 - 88	-22.4° (22, 2.00, MeOH)
Z-L-pGlu-Gly-OEt	84	130 - 131	-45.1° (25, 1.15, AcOH)
Z-L-Glu(OEt)-Gly-OEt	85	97 - 98	-15.6° (25, 2.00, EtOH)

a) From L-amino acid ester hydrochloride.

Based on these results, this method was applied to a rapid synthesis of TRH. The synthetic route is outlined in Fig 1. Z-L-His(CH₂Ph)⁷ was employed and the aptitude of racemization was examined. Rapid coupling of Z-L-His(CH₂Ph)·DCHA and L-Pro-NH₂⁸ by the modified SOCl₂-pyridine method afforded after separation by silica gel chromatography Z-L-His(CH₂Ph)-L-Pro-NH₂ **1** in 85% yield from L-Pro-NH₂, along

Fig 1 Synthesis of TRH



with a small amount of racemized Z-D-His(CH₂Ph)-L-Pro-NH₂ 2 in 6% yield. The extent of racemization in the coupling of Z-L-His(CH₂Ph)·DCHA with L-Ala-OMe·HCl by 400 MHz ¹H-NMR.⁹ The ratio of LL-isomer to DL-isomer was 93 to 7. Dipeptides, 1 and 2 were identified with authentic samples, prepared by the coupling of Z-L-His(CH₂Ph) or Z-D-His(CH₂Ph) with L-Pro-NH₂ by the DCC-HOSu method (DMF, rt, 20 h). After recrystallization from AcOEt-MeOH (83%), the dipeptide 1 was deprotected selectively with saturated soln of HBr in glacial AcOH to give L-His(CH₂Ph)-L-Pro-NH₂·2HBr 2. Treatment of 2 with gaseous NH₃ in CH₂Cl₂ gave L-His(CH₂Ph)-L-Pro-NH₂ 4, which without isolation was coupled directly with Z-L-pGlu·DCHA¹² to give practically pure Z-L-pGlu-L-His(CH₂Ph)-L-Pro-NH₂ 5¹⁰ in 61% overall yield from 1. Homogeneity of 5 was shown by TLC and 400MHz ¹H-NMR spectrum. After recrystallization from ether-CHCl₃-AcOEt (85%), the tripeptide 5 was subjected to catalytic hydrogenolysis to remove the benzyl and benzyloxycarbonyl group¹⁰ to yield TRH in 76% yield. The synthetic TRH was identical with an authentic sample in all respects (400 MHz ¹H-NMR, TLC mobilities with several different solv systems, and EP mobilities with several different buffer systems).

As illustrated in this paper, this modification of the classical SOCl₂-pyridine method for the preparation of acid chlorides should be useful for synthesis of peptides.

EXPERIMENTAL

Mps were determined in open capillaries and were uncorrected. Optical rotations were determined on a JASCO DIP-SL instrument. IR spectra were recorded on a JASCO IR-S instrument and were calibrated with 1603 cm^{-1} absorption of polystyrene. $^1\text{H-NMR}$ spectra were measured at 60 MHz on a Hitachi R20B instrument and at 400 MHz on a JEOL JNM-FX 400 instrument. Chemical shifts are reported in δ units relative to TMS as internal standard. Low and high resolution mass spectra were run on a JEOL JMS-D300 instrument (EI-MS) and JEOL JMS-OISG-2 instrument (FD-MS). Elemental analyses were performed at Laboratory for Instrumental Analysis of Hokkaido University.

Preparation of DCHA Salts of Carboxylic Acids. According to the procedure for the preparation of DCHA salts of N-acylamino acids,⁴ DCHA salts of simple carboxylic acids were prepared. A typical experiment is illustrated for the preparation of dicyclohexylammonium 3-phenylpropionate.

Dicyclohexylammonium 3-Phenylpropionate. To a soln of 3-phenylpropionic acid (1.0 g, 6.7 mmol) in AcOEt (5.0 ml) was added dicyclohexylamine (1.3 g, 7.2 mmol) at room temp. After stirring at room temp for 5 min, the precipitate was collected, washed with ether, and recrystallized from ether- CHCl_3 to give 1.9 g (86%) of dicyclohexylammonium 3-phenylpropionate: mp $111\text{--}112^\circ$; IR (Nujol) $1625, 1400\text{ cm}^{-1}$; $^1\text{H-NMR}$ (CDCl_3) δ 0.70-2.25 (20H, m), 2.25-3.20 (6H, m), 7.14 (5H, s), 7.58 (2H, bs). (Found: C, 75.95; H, 10.11; N, 4.43%. Calc for $\text{C}_{21}\text{H}_{33}\text{NO}_2$: C, 76.09; H, 10.03; N, 4.23%.)

Dicyclohexylammonium Cyclohexanecarboxylate: mp $153\text{--}154^\circ$ (recrystallized from ether- CHCl_3); IR (Nujol) $1625, 1405\text{ cm}^{-1}$; $^1\text{H-NMR}$ (CDCl_3) δ 0.80-2.40 (31H, m), 2.55-3.20 (2H, m), 9.41 (2H, bs). (Found: C, 73.85; H, 11.32; N, 4.38%. Calc for $\text{C}_{19}\text{H}_{33}\text{NO}_2$: C, 73.73; H, 11.40; N, 4.53%.)

Dicyclohexylammonium Benzoate: mp $210\text{--}202^\circ$ (recrystallized from ether- CHCl_3); IR (Nujol) $1630, 1550, 1415\text{ cm}^{-1}$; $^1\text{H-NMR}$ (CD_3OD) δ 0.80-2.30 (20H, m), 2.80-3.50 (2H, m), 7.15-7.50 (3H, m), 7.75-8.05 (2H, m). (Found: C, 75.11; H, 9.67; N, 4.70%. Calc for $\text{C}_{19}\text{H}_{29}\text{NO}_2$: C, 75.20; H, 9.63; N, 4.62%.)

Synthesis of Amides.¹¹ A typical procedure is described for the reaction of dicyclohexylammonium 3-phenylpropionate and dibutylamine.

N,N-Dibutyl-3-phenylpropionamide. To a stirred soln of dicyclohexylammonium 3-phenylpropionate (245 mg, 0.74 mmol) and pyridine (76 mg, 0.96 mmol) in CH_2Cl_2 (1.0 ml) was added SOCl_2 (106 mg, 0.89 mmol) at room temp under Ar atmosphere. The resulting mixture was stirred at room temp for 1 min. To the mixture was added a soln of dibutylamine (74 mg, 0.57 mmol) and DBU (172 mg, 1.1 mmol) in CH_2Cl_2 (1.0 ml) at room temp. The reaction mixture was stirred at the same temp for 20 min. After brine (5.0 ml) was poured into the mixture, the product was extracted with AcOEt. The organic layers were washed successively with 3N-HCl, 1N-NaOH, and brine and dried over Na_2SO_4 . Evaporation of the solv *in vacuo* gave a crude product, which was purified by chromatography on silica gel (hexane-AcOEt, 90:10) to give 127 mg (85%, from dibutylamine) of N,N-dibutyl-3-phenylpropionamide: IR (neat) 1645 cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3) δ 0.70-1.80 (14H, m), 2.40-2.70 (2H, m), 2.80-3.50 (6H, m), 7.19 (5H, s); EI-MS m/z 261 (M^+). (Exact Mass Found: 261.2089. Calc for $\text{C}_{17}\text{H}_{27}\text{NO}$: 261.2093.)

N-Benzyl-3-phenylpropionamide: mp $83\text{--}94^\circ$; (Nujol) $3320, 1640, 1545\text{ cm}^{-1}$; $^1\text{H-NMR}$ (CDCl_3) δ 2.49, 2.90 (each 2H, bt, 7 Hz), 4.30 (2H, d, 6 Hz), 6.00 (1H, bs), 7.14 (5H, s); EI-MS m/z 239 (M^+). (Exact Mass Found: 239.1300. Calc for $\text{C}_{16}\text{H}_{17}\text{NO}$: 239.1310.)

N-tert-Butyl-3-phenylpropionamide: IR (neat) $3300, 1645, 1555\text{ cm}^{-1}$; $^1\text{H-NMR}$ (CDCl_3) δ 1.26 (9H, s), 2.35, 2.93 (each 2H, bt, 5 Hz), 5.15 (1H, bs), 7.17 (5H, s); EI-MS m/z 205 (M^+). (Exact Mass Found: 205.1455. Calc for $\text{C}_{13}\text{H}_{19}\text{NO}$: 205.1467.)

Cyclohexanecarboxanilide: mp $145\text{--}146^\circ$; IR (Nujol) $3340, 1665, 1605, 1535\text{ cm}^{-1}$; $^1\text{H-NMR}$ (CDCl_3) δ 0.90-2.50 (11H, m), 6.95-7.75 (10H, m); EI-MS m/z 203 (M^+). Lit: mp 145° .

N-Benzylcyclohexanecarboxamide: mp $103\text{--}104^\circ$; IR (Nujol) $3300, 1645, 1555\text{ cm}^{-1}$; $^1\text{H-NMR}$ (CDCl_3) δ 0.90-2.40 (11H, m), 4.36 (2H, d, 6 Hz), 5.92 (1H, bs), 7.20 (5H, s); EI-MS m/z 217 (M^+). Lit: $105\text{--}106^\circ$.

N-(Cyclohexylcarbonyl)pyrrolidine: mp $66\text{--}67^\circ$; IR (Nujol) 1630 cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3) δ 0.90-2.70 (15H, m), 3.15-3.80 (4H, m); EI-MS m/z 181 (M^+). (Exact Mass Found: 181.1477. Calc for $\text{C}_{11}\text{H}_{19}\text{NO}$: 181.1467.)

N-sec-Butylbenzamide: mp $74\text{--}75^\circ$; IR (Nujol) $3340, 1635, 1605, 1580, 1540\text{ cm}^{-1}$; $^1\text{H-NMR}$ (CDCl_3) δ 0.98 (3H, t, 7 Hz), 1.24 (3H, d, 7 Hz), 1.52 (2H, sextet, 7 Hz), 4.11 (1H, heptet, 7 Hz), 7.20-7.55 (3H, m), 7.55-7.90 (2H, m); EI-MS m/z 177 (M^+). Lit: mp $72\text{--}73^\circ$.

N-Benzoylpyrrolidine: IR (neat) $3500, 1630, 1605, 1575\text{ cm}^{-1}$; $^1\text{H-NMR}$ (CDCl_3) δ 1.50-2.40 (4H, m), 3.20-3.95 (4H, m), 7.20-7.70 (5H, m); EI-MS m/z 175 (M^+).

Synthesis of Dipeptides.¹² A typical procedure is described for the synthesis of Z-L-Phe-L-Ala-OMe.

Z-L-Phe-L-Ala-OMe. To a stirred soln of Z-L-Phe-DCHA⁴ (356 mg, 0.74 mmol) and pyridine (76 mg, 0.96 mmol) in CH_2Cl_2 (1.0 ml) was added SOCl_2 (106 mg, 0.89 mmol) at room temp under Ar atmosphere. The resulting mixture was stirred at room temp for 1 min. To the reaction mixture was added a soln of L-Ala-OMe-HCl (80 mg,

0.57 mmol) and DMAP (141 mg, 1.2 mmol) in CH_2Cl_2 (1.0 ml) at room temp. The mixture was stirred at the same temp for 20 min. After brine (5.0 ml) was poured into the reaction mixture, the product was extracted with AcOEt. The organic layers were washed successively with 10% citric acid aq, saturated NaHCO_3 aq, and brine and dried over Na_2SO_4 . Evaporation of the solv *in vacuo* gave 220 mg (99%, from L-Ala-OMe·HCl) of Z-L-Phe-L-Ala-OMe, which was employed for detection of racemization as such. On the other hand the crude product was purified by recrystallization from petroleum ether-AcOEt to give 208 mg (94%, from L-Ala-OMe·HCl) of Z-L-Phe-L-Ala-OMe: mp 126-127°; $[\alpha]_D^{25}$ -22.9° (1.25, EtOH); $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 1.33 (3H, d, 7.1 Hz), 3.05, 3.19 (each 1H, dd, 7.1 and 13.9 Hz), 3.71 (3H, s), 4.41 (1H, q, 7.1 Hz), 4.50 (1H, quintet, 7.1 Hz), 5.09 (2H, s), 5.30, 6.29 (each 1H, bd, 7.1 Hz). Lit: mp 130-131° (recrystallized from petroleum ether-AcOEt).

Z-L-Ala-Gly-OEt: mp 97-98° (recrystallized from petroleum ether-AcOEt); $[\alpha]_D^{24}$ -20.9° (1.00, EtOH); $^1\text{H-NMR}$ (CDCl_3) δ 1.24 (3H, t, 7 Hz), 1.38 (3H, d, 7 Hz), 3.97 (2H, d, 5 Hz), 4.15 (2H, q, 7 Hz), 4.32 (1H, quintet, 7 Hz), 5.07 (2H, s), 5.38 (1H, bd, 7 Hz), 7.02 (1H, bt, 5 Hz), 7.27 (5H, s). Lit: mp 97-99° (recrystallized from petroleum ether-AcOEt); $[\alpha]_D^{25}$ -19.8° (1, EtOH).

Z-L-Phe-Gly-OEt: mp 105-106° (recrystallized from petroleum ether-AcOEt); $[\alpha]_D^{24}$ -15.7° (1.00, EtOH); $^1\text{H-NMR}$ (CDCl_3) δ 1.23 (3H, t, 7 Hz), 3.07 (2H, d, 7 Hz), 3.92 (2H, d, 5 Hz), 4.13 (2H, q, 7 Hz), 4.52 (1H, q, 7 Hz), 5.00 (2H, s), 5.26 (1H, bd, 7 Hz), 6.83 (1H, bt, 5 Hz), 7.16, 7.23 (each 5H, s). Lit: mp 108-110° (recrystallized from petroleum ether-AcOEt); $[\alpha]_D^{25}$ -14.8° (1, EtOH).

Z-L-Val-Gly-OEt: mp 164-165° (recrystallized from ether); $[\alpha]_D^{24}$ -17.5° (1.00, EtOH); $^1\text{H-NMR}$ (CDCl_3) δ 0.93, 0.98 (each 3H, d, 7 Hz), 1.26 (3H, t, 7 Hz), 2.15 (1H, octet, 7 Hz), 3.95 (2H, d, 5 Hz), 4.15 (2H, q, 7 Hz), 5.05 (2H, s), 5.63 (1H, bd, 9 Hz), 6.83 (1H, bt, 5 Hz), 7.26 (5H, s). Lit: mp 163-165° (recrystallized from ether); $[\alpha]_D^{25}$ -16.7° (1, EtOH).

Z-L-Leu-Gly-OEt: mp 98-99° (recrystallized from EtOH); $[\alpha]_D^{20}$ -26.5° (1.55, EtOH); $^1\text{H-NMR}$ (CDCl_3) δ 0.93 (6H, d, 6 Hz), 1.25 (3H, t, 7 Hz), 3.93 (2H, d, 5 Hz), 4.13 (2H, t, 7 Hz), 5.05 (2H, s), 5.56 (1H, bd, 9 Hz), 6.89 (1H, bt, 5 Hz), 7.26 (5H, s). Lit: mp 103° (recrystallized from EtOH); $[\alpha]_D^{25}$ -25.5±1.5° (1.568, EtOH).

Z-L-Ala-L-Phe-OMe: mp 100-101° (recrystallized from petroleum ether-AcOEt); $[\alpha]_D^{22}$ -9.24° (1.00, EtOH); $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 1.34 (3H, d, 6.8 Hz), 3.08, 3.15 (each 1H, dd, 6.1 and 13.8 Hz), 3.73 (3H, s), 4.21 (1H, quintet, 6.8 Hz), 4.85 (1H, dt, 6.8 and 6.1 Hz). Lit: 99-100° (recrystallized from petroleum ether-AcOEt); $[\alpha]_D^{22}$ -9.3° (1, EtOH).

Z-L-Val-L-Val-OMe: mp 102-103° (recrystallized from petroleum ether-AcOEt); $[\alpha]_D^{23}$ -21.2° (1.00, EtOH); $^1\text{H-NMR}$ (CDCl_3) δ 1.39, 1.41, 1.42, 1.45 (each 3H, d, 7 Hz), 1.80-2.40 (2H, m), 3.68 (3H, s), 4.16 (1H, t, 7 Hz), 4.52 (1H, dd, 5 and 9 Hz), 5.09 (2H, s), 5.76 (1H, bd, 9 Hz), 6.90 (1H, bd, 7 Hz), 7.26 (5H, s). Lit: mp 100-103° (recrystallized from petroleum ether-AcOEt); $[\alpha]_D^{22}$ -21.0° (1, EtOH).

Z-L-Leu-L-Phe-OMe: mp 87-88° (recrystallized from hexane-AcOEt); $[\alpha]_D^{22}$ -22.4° (2.00, MeOH); $^1\text{H-NMR}$ (CDCl_3) δ 0.90 (6H, d, 5 Hz), 3.07 (2H, d, 7 Hz), 3.65 (3H, s), 4.00-4.50 (1H, m), 4.85 (1H, q, 7 Hz), 5.05 (2H, s), 5.51, 6.79 (each 1H, bd, 7 Hz), 7.14 (5H, bs), 7.27 (5H, s). Lit: mp 92.0° (recrystallized from hexane-AcOEt); $[\alpha]_D^{25}$ -20±1° (2.00, MeOH).

Z-L-pGlu-Gly-OEt: mp 130-131° (recrystallized from EtOH); $[\alpha]_D^{25}$ -45.1° (1.15, AcOH); $^1\text{H-NMR}$ (CDCl_3) δ 1.24 (3H, t, 7 Hz), 1.90-3.00 (4H, m), 3.95 (2H, d, 5 Hz), 4.15 (2H, q, 7 Hz), 4.68 (1H, dd, 2 and 4 Hz), 5.17 (2H, s), 7.28 (5H, s), 7.36 (1H, bt, 5 Hz). Lit: mp 131-132° (recrystallized from EtOH); $[\alpha]_D^{25}$ -44.1° (1.15, AcOH).

Z-L-Glu(OEt)-Gly-OEt: mp 97-98° (recrystallized from hexane-AcOEt); $[\alpha]_D^{25}$ -15.6° (2.00, EtOH); $^1\text{H-NMR}$ (CDCl_3) δ 1.22, 1.25 (each 3H, t, 7 Hz), 1.80-2.65 (4H, m), 3.96 (2H, d, 5 Hz), 4.07, 4.13 (each 2H, q, 7 Hz), 5.07 (2H, s), 5.96 (1H, bd, 9 Hz), 7.10 (1H, bt, 5 Hz), 7.26 (5H, s). Lit: mp 97-99° (recrystallized from hexane-AcOEt); $[\alpha]_D^{25}$ -15.8° (2.0, EtOH).

Bz-L-Leu·DCHA. On the same treatment as dicyclohexylammonium 3-phenylpropionate, Bz-L-Leu⁶ (1.0 g, 4.0 mmol) gave, after recrystallization from ether-EtOH, 1.4 g (83%) of Bz-L-Leu·DCHA: mp 156-157°; $[\alpha]_D^{25}$ +24.0° (1.00, CHCl_3); $^1\text{H-NMR}$ (CDCl_3) δ 0.98, 1.01 (each 3H, d, 6 Hz), 4.25-4.70 (1H, m), 7.16 (1H, bd, 8 Hz), 7.20-7.55 (3H, m), 7.60-7.95 (2H, m), 8.44 (2H, bs). (Found: C, 72.15; H, 9.62; N, 6.68%. Calc for $\text{C}_{25}\text{H}_{40}\text{N}_2\text{O}_3$: C, 72.07; H, 9.68; N, 6.73%.)

Bz-Leu-Gly-OEt (L-isomer 11%⁶). To a stirred soln of Bz-L-Lue·DCHA (307 mg, 0.74 mmol) and pyridine (76 mg, 0.96 mmol) in CH_2Cl_2 (1.0 ml) cooled to -14° was added SOCl_2 (106 mg, 0.87 mmol) under Ar atmosphere. The resulting mixture was stirred at -14° for 1 min. To the mixture was added a soln of Gly-OEt·HCl (80 mg, 0.57 mmol) and DMAP (141 mg, 1.2 mmol) in CH_2Cl_2 (1.0 ml) at -14°. The temp was allowed to raise to room temp and the resulting mixture was stirred at room temp for additional 2 h. Similar treatment as above gave a crude product, which was purified by chromatography on silica gel (PhH-AcOEt, 70:30) to yield 50 mg (26%, from Gly-OEt·HCl) of Bz-Leu-Gly-OEt: $[\alpha]_D^{20}$ -3.90° (2.00, EtOH); $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 0.94, 0.96 (each 3H, d, 6.8 Hz), 1.25 (2H, t, 7.3 Hz), 1.67-1.80 (5H, m), 3.96, 4.06 (each 1H, dd, 5.4 and 18.1 Hz), 4.18 (2H, q, 7.3 Hz), 4.79 (1H, dt, 5.9 and 8.3 Hz), 6.95 (1H, d, 8.3 Hz), 7.09 (1H, t, 5.4 Hz).

Z-L-His(CH_2Ph)·DCHA. To a stirred suspension of Z-L-His(CH_2Ph)⁷ (2.5 g, 6.6 mmol)

in CH_2Cl_2 (150 ml) was added dicyclohexylamine (1.3 g, 7.2 mmol) at room temp. After all solid materials dissolved, the solv was removed *in vacuo*. The residue was recrystallized from ether-EtOH to give 3.1 g (84%) of Z-L-His(CH_2Ph) \cdot DCHA: mp 100-102° dec; $[\alpha]_{\text{D}}^{20}$ +7.40° (1.00, EtOH); $^1\text{H-NMR}$ (CDCl_3) δ 4.10-4.50 (1H, m), 4.93, 4.99 (each 2H, s), 5.90 (1H, bd, 8 Hz), 6.70 (1H, bs), 7.24 (10H, s). (Found: C, 70.47; H, 8.02; N, 10.05%. Calc for $\text{C}_{33}\text{H}_{44}\text{N}_4\text{O}_4$: C, 70.68; H, 7.91; N, 9.99%.)

Z-L-His(CH_2Ph)-L-Pro-NH₂ 1. To a stirred soln of Z-L-His(CH_2Ph) \cdot DCHA (414 mg, 0.74 mmol) and pyridine (76 mg, 0.96 mmol) in CH_2Cl_2 (1.0 ml) was added SOCl_2 (106 mg, 0.89 mmol) at room temp under Ar atmosphere. The resulting mixture was stirred at room temp for 1 min. To the mixture was added a soln of L-Pro-NH₂ (65 mg, 0.57 mmol) and DMAP (70 mg, 0.57 mmol) in DME (1.0 ml) at room temp and the reaction mixture was stirred at the same temp for 20 min. After brine (5.0 ml) was poured into the reaction mixture, the product was extracted with CHCl_3 . The organic layers were washed successively with saturated NaHCO_3 aq and brine, dried over Na_2SO_4 , and evaporated *in vacuo*. Chromatography of the crude product on silica gel (CHCl_3 -MeOH, 98:2) gave 230 mg (85%, from L-Pro-NH₂) of 1 (less polar epimer) and 16 mg (6%, from L-Pro-NH₂) of Z-D-His(CH_2Ph)-L-Pro-NH₂ 2 (more polar epimer). The dipeptide 1 thus obtained was further purified by recrystallization from AcOEt-MeOH to yield 191 mg (83%) of 1.

Z-L-His(CH_2Ph)-L-Pro-NH₂ 1: mp 133-134°; $[\alpha]_{\text{D}}^{25}$ -21.4° (2.00, CHCl_3); $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 3.01 (1H, dd, 3.9 and 14.2 Hz), 3.07 (1H, dd, 8.1 and 14.2 Hz), 4.60 (1H, dd, 3.2 and 8.5 Hz), 4.63 (1H, dt, 3.9 and 8.1 Hz), 5.03, 5.04 (each 1H, d, 15.6 Hz), 5.08, 5.09 (each 1H, d, 13.4 Hz), 5.40 (1H, s), 5.38 (1H, d, 8.1 Hz), 6.73, 8.95 (each 1H, s); FD-MS m/z 475 (M^+). (Exact Mass Found: 475.2223. Calc for $\text{C}_{26}\text{H}_{29}\text{N}_5\text{O}_4$: 475.2219.)

Z-D-His(CH_2Ph)-L-Pro-NH₂ 2: mp 200-202° dec; $[\alpha]_{\text{D}}^{25}$ -55.1° (2.00, CHCl_3); $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 2.93, 2.94 (each 1H, dd, 7.1 and 14.4 Hz), 4.45 (1H, d, 7.9 Hz), 4.62 (1H, q, 7.1 Hz), 5.03 (4H, s), 5.22 (1H, s), 5.86 (1H, d, 7.1 Hz), 6.72, 6.93, 7.51 (each 1H, s); FD-MS m/z 475 (M^+). (Exact Mass Found: 475.2227. Calc for $\text{C}_{26}\text{H}_{29}\text{N}_5\text{O}_4$: 475.2219.)

Z-L-pGlu-L-His(CH_2Ph)-L-Pro-NH₂ 5. To 1 (191 mg, 0.40 mmol) was added a saturated soln of HBr in glacial AcOH (7.5 ml) at room temp. After stirring at room temp for 1 h, the reaction mixture was treated with ether (20 ml). The solv was decanted off and the crystals of L-His(CH_2Ph)-L-Pro-NH₂ \cdot 2HBr 3 was washed with ether.

The HBr salt 3 was suspended in CHCl_3 (7.5 ml) and cooled to 0°. Gaseous NH_3 was bubbled through the suspension at 0° for 15 min. The NH_4Br was filtered off and the filtrates were concentrated *in vacuo* to give 97 mg (71% from 1) of L-His(CH_2Ph)-L-Pro-NH₂ 4.

To a stirred soln of Z-L-pGlu \cdot DCHA¹² (164 mg, 0.37 mmol) and pyridine (38 mg, 0.48 mmol) in CH_2Cl_2 (0.5 ml) was added SOCl_2 (53 mg, 0.45 mmol) at room temp under Ar atmosphere. The resulting mixture was stirred at room temp for 1 min. To the mixture was added a soln of 4 (97 mg, 0.28 mmol) and DMAP (35 mg, 0.29 mmol) in CH_2Cl_2 (0.5 ml) at room temp. After stirring at the same temp for 20 min, the reaction mixture was processed as above to give a crude product. Chromatography of the crude product on silica gel (CHCl_3 -MeOH, 98:2) gave 143 mg (86%, from 4) of 5, which was further purified by recrystallization from ether- CHCl_3 -AcOEt to yield 122 mg (85%) of 5: mp 149-150°; $[\alpha]_{\text{D}}^{20}$ -24.6° (0.90, CHCl_3); $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 2.48 (1H, ddd, 3.4, 9.8, and 17.6 Hz), 2.72 (1H, dt, 17.6 and 10.1 Hz), 2.87 (1H, dd, 3.6 and 14.4 Hz), 2.97 (1H, dd, 8.2 and 14.4 Hz), 4.55 (1H, dd, 3.4 and 8.8 Hz), 4.59 (1H, dd, 2.7 and 9.0 Hz), 4.78 (1H, dt, 3.6 and 8.2 Hz), 5.03 (2H, s), 5.17, 5.28 (each 1H, d, 12.5 Hz), 5.66, 6.74 (each 1H, s), 7.40 (1H, d, 8.2 Hz), 9.17 (1H, s); FD-MS m/z 587 (M^+H), 586 (M^+). Lit¹⁰: mp 149-152° (recrystallized from ether- CHCl_3 -AcOEt); $[\alpha]_{\text{D}}^{25}$ -25.3° (0.87, CHCl_3).

TRH. The tripeptide 5 (122 mg, 0.21 mmol) in MeOH (20 ml) was hydrogenated at room temp and 4 atm pressure in the presence of 5% Pd-C (270 mg). After 12 h, the reaction mixture was filtered and filtrates were evaporated *in vacuo*. Chromatography of the residue on silica gel (CHCl_3 -MeOH, 70:30) afforded 57 mg (76%) of TRH. The synthetic TRH was identical with an authentic sample in all respects (400 MHz $^1\text{H-NMR}$, TLC mobilities with several different solvent systems, and EP mobilities with several different buffer systems).

TRH: $^1\text{H-NMR}$ (D_2O , DSS as internal standard, 400 MHz) δ 3.17 (1H, dd, 8.1 and 15.3 Hz), 3.27 (1H, dd, 6.1 and 15.3 Hz), 3.59, 3.76 (each 1H, dt, 10.1 and 6.5 Hz), 4.32 (1H, dd, 6.1 and 8.1 Hz), 7.35 (1H, s), 8.64 (1H, d, 1.5 Hz).

REFERENCES AND NOTES

1. Present address: Sagami Chemical Research Center, 4-4-1, Nishi-Ohnuma, Sagamihara, Kanagawa 229, Japan.
2. S. S. Pizey, "Synthetic Reagents," John Wiley & Sons, New York, Vol. I, Ch. 4, p 335 (1974). For example, in the case of the one-pot method for the preparation of amides of half esters of phthalic acid, 1 h was required to activate the carboxylic acids in ether at room temp [J. P. E. Human and J. A. Mills, *Nature*, **158**, 877 (1946)].
3. F. Matsuda, M. Yanagiya, and T. Matsumoto, *Tetrahedron Letters*, **23**, 4043

- (1982).
4. DCHA salts of acylamino acids: E. Klieger, E. Schröder, and H. Gibian, *Liebigs Ann. Chem.*, 640, 157 (1961).
 5. B. Halpern, L. Chew, and B. Weinstein, *J. Am. Chem. Soc.*, 89, 5051 (1967).
 6. M. W. Williams and G. T. Young, *J. Chem. Soc.*, 881 (1963).
 7. Z-L-His(CH₂Ph) is very susceptible to racemization: S. Terada, A. Kawabata, N. Mitsuyasu, H. Aoyagi, and N. Izumiya, *Bull. Chem. Soc. Jpn.*, 51, 3409 (1978). Z-L-His(CH₂Ph): V. Du Vigneaud and O. K. Begrens, *J. Bio. Chem.*, 117, 27 (1937); D. Theodoropoulos and G. Flösch, *Acta Chem. Scand.*, 12, 1955 (1958).
 8. L-Pro-NH₂: R. W. Chambers and F. H. Carpenter, *J. Am. Chem. Soc.*, 77, 1522 (1955).
 9. B. Weinstein and A. E. Prichard, *J. Chem. Soc., Perkin Trans. I*, 1015 (1955).
 10. Z-L-pGlu-L-His(CH₂Ph)-L-Pro-NH₂ 5: J.-K. Chang, H. Sievertsson, C. Bogentoft, B. Currie, K. Folkers, and G. D. Daves, Jr., *J. Med. Chem.*, 14, 481 (1971).
 11. Cyclohexanecarboxanilide: M. Regitz and J. Rüter, *Chem. Ber.*, 103, 1263 (1971). N-Benzylcyclohexanecarboxamide: P. Lorenz, C. Rüdhardt, and E. Schacht, *Chem. Ber.*, 104, 3429 (1971). N-sec-Butylbenzamide: C. C. Price and C. A. Sears, *J. Am. Chem. Soc.*, 75, 3275 (1953). N-Benzoylpyrrolidine: M. Mitzlaff, K. Warning, and H. Jensen, *Liebigs Ann. Chem.*, 1713 (1978).
 12. Z-L-Phe-L-Ala-OMe: W. Grassmann, E. Wunsch, and A. Riedel, *Chem. Ber.*, 91, 455 (1958). Z-L-Ala-Gly-OEt, Z-L-Phe-Gly-OEt, and Z-L-Val-Gly-OEt: E. Schnabel, *Liebigs Ann. Chem.*, 688, 238 (1965). Z-L-Leu-Gly-OEt: R. Glatthard and M. Matter, *Helv. Chim. Acta*, 87, 795 (1963). Z-L-Ala-L-Phe-OMe: E. Schröder, *Liebigs Ann. Chem.*, 674, 207 (1964). Z-L-Val-L-Val-OMe: J. Hinman, E. L. Caron, and H. N. Christensen, *J. Am. Chem. Soc.*, 72, 1620 (1950). Z-L-Leu-L-Phe-OMe: I. Tomita, J. Ohashi, T. Tokuda, and M. Nakajima, *Nippon Nogeikagaku Kaishi*, 39, 378 (1965). Z-L-pGlu•DCHA and Z-L-pGlu-Gly-OEt: H. Gibian and E. Klieger, *Liebigs Ann. Chem.*, 640, 145 (1961). Z-L-Glu(OEt)-Gly-OEt: Y. Takeuchi and S. Yamada, *Chem. Pharm. Bull.*, 22, 841 (1974).