N-Hydroxy Amides. III.[1] Active Esters of Polystyrene-bound 1-Hydroxy-2-pyrrolidinone and Their Use in Peptide Synthesis

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A polymer-bond cyclic *N*-hydroxy amide has been prepared by reaction of aminomethylated copoly(styrene-2% divinylbenzene) with 1-hydroxy-5-oxo-3-pyrrolidinecarboxylic acid using dicyclohexylcarbodiimide (DCC) as a condensing agent. Several *N*-blocked α-amino acids have been loaded on this resin by use of DCC. The amino acid resin can be utilized for peptide synthesis. Repetitive usage of the resin in the form of esters and facile synthesis of a biologically active pentapeptide, Leu[5]-enkephalin, show the usefulness of the polymer-bound 1-hydroxy-2-pyrrolidinone.

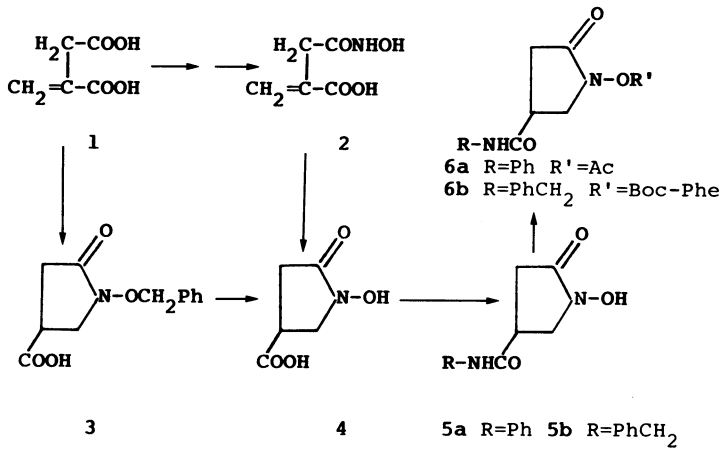
It is well documented that polymer-bound reagents are useful and economical in syntheses where repetitive procedures are required.[2-5] Thus a number of polymeric active esters have been prepared, including recent examples, and used in peptide synthesis.[6-11] *N*-Hydroxy ester-type polymers are prefered to a phenolic ester type to suppress racemization.[12] For recycling, however, *N*-hydroxysuccinimide polymers[7] are of limited utility because of a side reaction between *N*-hydroxysuccinimide (HOSu) and dicyclohexylcarbodiimide (DCC).[13] Polymers of highly active 1-hydroxybenzotriazole esters[8] are recognized to be sensitive to moisture and alcohols.[10]

In the course of our study,[14] it was found that 1-hydroxy-5-oxo-3-pyrrolidinecarboxylic acid (**4**) is readily obtainable from 2-methylenesuccinic acid. A pyrrolidinone ring is more stable than a HOSu ring due to the lack of one of the two carbonyl groups; yet esters of the *N*-hydroxypyrrolidinone are expected to retain a high reactivity, as many hydroxamate esters have been used as an acylating agent.[15,16] It is necessary to explore various functional groups in order to provide practical polymers. No polymer having a cyclic hydroxamic moiety has been tested so far. This paper describes the preparation and use of polymer-bound 1-hydroxy-2-pyrrolidinone as a recyclable polymeric hydroxy component together with synthesis of a biologically active peptide, Leu[5]-enkephalin[17].

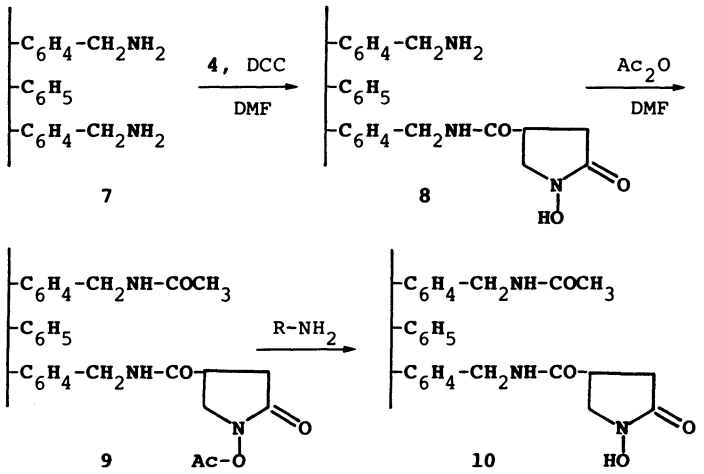
Results and Discussion

1-Hydroxy-2-pyrrolidinone Derivatives. Mild heating of *N*-hydroxy-3-carboxy-3-butenamide (**2**) gave 1-hydrpxy-5-oxo-3-pyrrolidinecarboxylic acid (**4**). The compound was also obtained *via* a reaction of 2-methylenesuccinic acid with *O*-benzylhydroxylamine. Scheme 1 shows these transformations. Reaction of **4** with aniline or benzylamine in the presence of DCC yielded the corresponding amides **5a** and **5b**. Acetic anhydride converted the amide **5a** to the acetoxy pyrrolidinone **6a** whose characteristic ester absorption is observed at 1800 cm^{-1}. The acetoxy amide **6a** reacted with cyclohexylamine to give *N*-cyclohexylacetamide. An amino acid ester **6b** can be obtained and used as an active ester. However, compound **4** is more suitable as a polymeric ligand, since problems such as solubility and crystallinity encountered with monomeric derivatives can be avoided through attachment to a polymer.

Polymer-bound 1-Hydroxy-2-pyrrolidinone and Its



Scheme 1.



Scheme 2.

Amino Acid Esters. Commercial beads of styrene–2% divinylbenzene (DVB) copolymer were used as the starting material. A 2% content of DVB is adequate for a base polymer in several respects.[5] Aminomethyl resin **7** (1.41—3.73 mmol/g) was prepared by phthalimidomethylation of the starting polymer beads.[18] The key step in the resin preparation was introduction of the hydroxy pyrrolidinone unit to the polymer **7** (Scheme 2). The aminomethyl resin **7** reacted with **4** in *N*,*N*-dimethylformamide (DMF) in the presence of DCC, without any protection of the hydroxamic acid moiety. The extent of the reaction was in the range of 60—80%, and complete removal of unchanged **4** was difficult at this stage. Exhaustive acetylation of the resin blocked the *N*-hydroxy group and any remaining free amino groups of polymer **8**. The *O*-acetyl group of polymer **9** is readily removed by aminolysis, affording the desired *N*-hydroxypyrrolidinone resin **10** (0.85—3.17 mmol/g). The resin **7** similarly reacted with 1-benzyloxy-5-oxo-3-pyrrolidinecarboxylic acid (**3**), but subsequent debenzylation was difficult to perform. The content of the *N*-hydroxypyrrolidinone unit was estimated by aminolysis of the *O*-acetyl group.[8,19]

The pyrrolidinone resin has the good mechanical property of the starting beads and swells well in DMF, THF, $CHCl_3$, CH_2Cl_2, and benzene. The bead-type resin can be easily filtered and washed with these and alcoholic solvents.

Molecular models suggest that the *N*-hydroxy amide moiety of the pyrrolidinone ring extends from the polymer backbone due to the trans configuration of the linking amide linkage.

Preparation of polymer-bound active esters was performed by reaction of appropriate *N*-protected amino acids with resin **10** in DMF in the presence of DCC. The esterified resin showed a characteristic peak at 1800 cm^{-1}. The amino acid content was determined by titration.[8,19] Figure 1 shows the progress of a loading reaction of Boc-Phe-OH, chosen as an example. It takes 40 h with DCC alone, but addition of 1-hydroxybenzotriazole accelerates the reaction. The symmetric anhydride technique is effective in shortening the reaction time. Table 1 shows typical results obtained with DCC alone. The polymers are stable for months when stored in a desiccator.

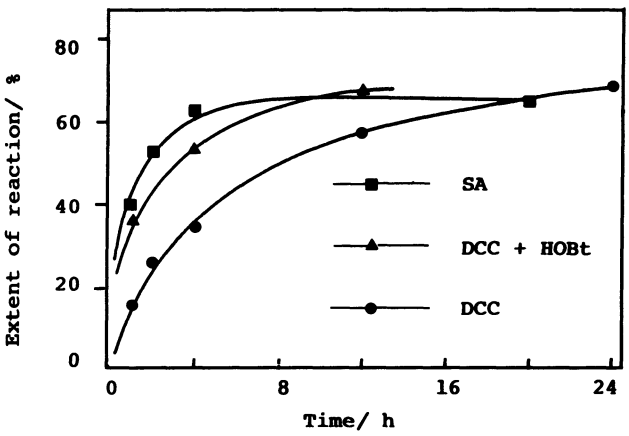
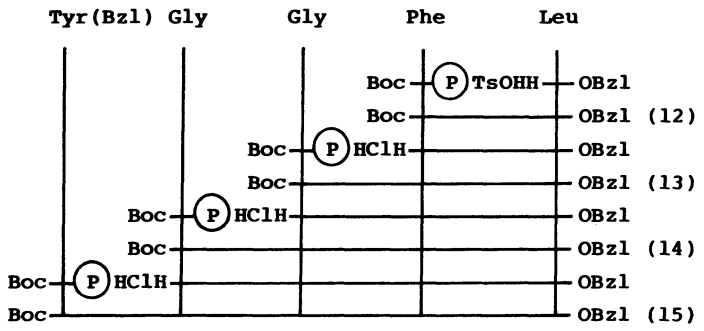


Fig. 1. Progress of the loading reaction of Boc-Phe-OH on the *N*-OH resin (**10**); 2 mol reagents were used for 1 mol *N*-OH unit, in DMF at room temperature; SA, symmetric anhydride method; HOBt, 1-hydroxybenzotriazole. The extent of the reaction was followed by aminolysis.

Peptide Synthesis by Use of Polymer Reagents. The coupling procedure to form a peptide bond is simple; addition of an amino component to an appropriate resin suspension and removal of a wasted polymer after a suitable reaction time. The usual work-up gives a peptide. In a coupling between Boc-Phe resin and H-Leu-OBzl, a rather prolonged time (48 h) for completion was observed in contrast to



Scheme 3.

the case of HOSu esters, where the coupling usually completes within 20 h at room temperature.[7] Nevertheless, a routine coupling was carried out in DMF at 35 °C for 24 h to give a practical yield.

Repetitive usage is an important factor for polymer reagents. This was examined using the same resin repeatedly. Good reproducible results in the peptide formation are notable (Table 2). Further use was not attempted since some of the polymer beads became powdered.

Table 1. Polymer-bound active esters of *N*-blocked amino acids[a]

N-Blocked amino acid bound to polymer	Content of ester mmol/g
Boc-Ala	0.93
Boc-Gly	1.05
Boc-Phe	0.88
Boc-Pro	0.56
Boc-Val	0.64
Cbz-Ala[b]	0.70
Cbz-Gly[b]	0.55
Cbz-Leu[b]	0.65
Cbz-Tyr(Bzl)	0.97

a) Polymer-bound 1-hydroxy-2-pyrrolidinone having an *N*-OH content = 1.27 mmol/g was used; the content of ester was assayed by the titration method. b) Polymer of an *N*-OH content = 1.04 mmol/g was used; the ester content was estimated from weight gain.

Table 2. Results of repetitive use of a single polymer

Peptide formed	Recycle run[a]	Yield/%
Boc-Phe-Leu-OBzl[b]	1	94
	2	87
	3	94
Boc-Gly-Phe-Leu-OBzl[c]	4	69
	5	71
	6	68

a) Boc-Phe resin (*ca.* 4 g) was used as a starting polymer. b) Reaction of the Boc-Phe resin with H-Leu-OBzl gave *ca.* 1.5 g of the peptide. c) After complete detachment of Boc-Phe residue with cyclohexylamine, the resin was loaded with Boc-Gly and subjected to aminolysis with H-Phe-Leu-OBzl to give *ca.* 1.0 g of the peptide.

Leu^5*-Enkephalin.* In an extension of these couplings, synthesis of the protected peptide of Leu^5-enkephalin was carried out as depicted in Scheme 3. This coupling order of amino acids is rather few, since the Gly^2-Gly^3 sequence is usually prepared separately.[17] Removal of the protective groups, followed by column chromatography, gave the free petide (**16**), Leu^5-enkephalin. The peptide was digested almost completely by an aminopeptidase and was physiologically effective.

The present results show that the 1-hydroxy-2-pyrrolidinone unit is a useful functional moiety when utilized as a polymeric ligand, although aminolysis of its esters is rather slow.

Experimental

All the melting points are uncorrected. IR spectra were obtained with a JASCO 403G spectrophotometer and ^{1}H NMR spectra were recorded on a JEOL C-60HL spectrometer with TMS as internal standard. Optical rotations were measured with a JASCO ORD/UV-5 spectrometer. HPLC was carried out with a JASCO model twincle apparatus using a column packed with Finepak SIL C_{18}. Microanalysis was performed by the Analytical Section, Institute of Physical and Chemical Research, Saitama. TLC was carried out using Merck precoated silica gel $60F_{254}$ plates: R_f^1, EtOAc; R_f^2, $CHCl_3$-MeOH-C_6H_6 (6:1:3); R_f^3, THF-hexane (1:1); R_f^4, $CHCl_3$-MeOH-AcOH-H_2O (30:20:4:6). Aminopeptidase M (Sigma) was used as received. 1 M = 1 mol dm^{-3}.

1-Benzyloxy-5-oxo-3-pyrrolidinecarboxylic Acid (3). A mixture of 2-methylenesuccinic acid (30 g, 0.24 mol), *O*-benzylhydroxylamine (25.6 g, 0.24 mol) and water (240 mL) was refluxed for 1 h, and the hot, aqueous layer removed by decantation. The residue was recrystallized from water to give 43 g (78%) of **3**, 151—152 °C; IR, 1725 (-CO_2H) and 1635 (-CO-N-) cm^{-1}. Found: C, 61.19; H, 5.55; N, 5.94%. Calcd for $C_{12}H_{13}NO_4$: C, 61.27; H, 5.57; N, 5.96%.

1-Hydroxy-5-oxo-3-pyrrolidinecarboxylic Acid (4). (A): *N*-Hydroxy-3-carboxy-3-butenamide (**2**)[14] (0.50 g, 3.4 mmol) in 1-butanol (30 mL) was refluxed for 0.5 h and evaporated

to give an oil which crystallized on cooling (0.30 g, 60%). Recrystallization from acetone–hexane gave a sample, mp 145—146 °C; IR, 1700 (-CO_2H) and 1630 (-CO-N) cm^{-1}; ^1H NMR (DMSO-d_6) δ=3.62 (2H, m, -CH_2-N), 3.20 (1H, m, -CH-COO) and 2.44 (2H, d, -CH_2-CO-N, J=7.2 Hz). Found: C, 40.99; H, 4.84; N, 9.60%. Calcd for $C_5H_7NO_4$: C, 41.38; H, 4.84; N, 9.60%.

(*B*): A solution of **3** (6.0 g, 25 mmol) in MeOH (160 mL) was hydrogenated with H_2 in the presence of 10% Pd/C (0.20 g) under the atmospheric pressure for 2 d to give, after recrystallization, 2.8 g (88%) of **4**, mp 150—151 °C.

*1-Hydroxy-4-phenylcarbamoyl-2-pyrrolidinone (**5a**).* To a chilled solution of **4** (1.00 g, 6.9 mmol) and aniline (0.96 g, 10 mmol) in DMF (20 mL) was added a solution of dicyclohexylcarbodiimide (DCC) (1.42 g, 7 mmol) in DMF (3 mL); the mixture was stirred overnight at room temperature. *N,N'*-Dicyclohexylurea (DCU) was filtered off and the solvent evaporated. The residue was recrystallized from acetone–hexane to yield 1.06 g (70%) of **5a**, mp 183—184 °C (decomp); IR, 1685 (-CO-N(OH)-) and 1665 (-CONH-) cm^{-1}. Found: C, 59.93; H, 5.69; N, 12.92%. Calcd for $C_{11}H_{12}N_2O_3$: C, 59.99; H, 5.49; N, 12.72%.

*1-Hydroxy-4-benzylcarbamoyl-2-pyrrolidinone (**5b**).* *N*-Ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide (WSC) (1.60 g, 8.5 mmol) was added to a solution containing **4** (1.0 g, 6.9 mmol) and benzylamine (0.84 g, 7.8 mmol) in DMF (15 mL), and the mixture stirred for 24 h at room temperature. The usual work-up gave 0.25 g (15%) of **5b**, mp 185—186 °C. Found: C, 61.78; H, 6.10; N, 11.75%. Calcd for $C_{12}H_{14}N_2O_3$: C,61.52; H, 6.02; N, 11.96%.

*1-Acetoxy-4-phenylcarbamoyl-2-pyrrolidinone (**6a**).* A suspension mixture of **4** (0.50 g, 2.3 mmol) and acetic anhydride (0.35 g, 3.4 mmol) in THF (7 mL) was stirred for 5 h at room temperature, the mixture evaporated. The residue was recrystallized from THF–hexane to give 0.38 g (68%) of **6a**, mp 111—112 °C; IR, 1795 (CH_3CO), 1715 (-CO-N) and 1665 (-CONH-) cm^{-1}. Found: C, 59.59; H, 5.41; N, 10.69%. Calcd for $C_{13}H_{14}N_2O_4$: C, 59.53; H, 5.38; N, 10.68%.

*Acetylation with **6a**:* A mixture of **6a** (0.26 g, 1 mmol) and cyclohexylamine (0.10 g, 1 mmol) in THF (3 mL) was stirred for 2 h at room temperature to give *N*-cyclohexylacetamide (0.70 g, 50%), mp 99—100 °C (104 °C for a pure sample).

*1-[N-(t-Butoxycarbonyl)phenylalanyloxy]-4-benzylcarbamoyl-2-pyrrolidinone (**6b**).* To a chilled solution containing **5b** (0.18 g, 0.75 mmol) and Boc-Phe-OH (0.20 g, 0.76 mmol) in DMF (9 mL) was added WSC (0.20 g, 1.0 mmol). The mixture was stirred for 18 h at room temperature and evaporated. Work-up and recrystallization from THF–hexane gave 0.18 g (45%) of **6b**, mp 103—106 °C. Found: C, 64.69; H, 6.40; N, 8.65%. Calcd for $C_{26}H_{31}N_3O_6$: C, 64.85; H, 6.49; N. 8.73%.

*Aminomethylated Polystyrene (**7**).* Commercial copoly(styrene-2% divinylbenzene) beads (100—200 mesh) were washed and subjected to phthalimidomethylation.[18] The following is as example: Resin beads (15.6 g) and *N*-(chloromethyl)phthalimide (15.3 g, 78 mmol) in CH_2Cl_2 (140 mL) containing $SnCl_4$ (9.45 g, 36 mmol) were stirred for 15 h at room temperature. The resin was washed with CH_2Cl_2, EtOH, MeOH, and Et_2O, and dried, yielding 27.2 g. The content of phthalimido group (IR 1775 and 1715 cm^{-1}) was 2.51 mmol/g from microanalysis; 2.66 mmol/g from weight gain.

The resulting phthalimidomethyl resin (26.9 g) was hydrolyzed in aqueous methylamine solution (60 mL; 40%) at first for 100 h and additionally for 20 h at room temperature, to give 19.6 g of **7** (aminomethyl content, 3.73 mmol/g; no peak at 1775 and 1715 cm^{-1}). By a similar procedure polymers (**7**) having different aminomethyl contents (1.41—3.73 mmol/g) were obtained.

*Preparation of Polymer-bound 1-Hydroxy-2-pyrrolidinone (**10**).* A mixture of aminomethyl polystyrene (**7**) (5.00 g; 3.73 mmol/g) and the pyrrolidinone **4** (3.00 g, 21 mmol) in DMF (50 mL) was kept for 2 h at room temperature with stirring and chilled. To this was added DCC (4.25 g, 21 mmol) in DMF (20 mL), and the suspension kept at 0 °C for 2 h and at room temperature for 18 h. The resin was filtered off, washed with DMF and hot MeOH alternatively 3 times, then with ether, and dried under vacuum.

The dried resin was allowed to react with acetic anhydride (16.3 g, 160 mmol) in DMF (50 mL) for 40 h at room temperature. The resin was filtered, washed with CH_2Cl_2 and MeOH alternatively 4 times, then with ether, and dried under vacuum.

The dried acetylated resin (**9**) was swollen in AcOEt–CH_2Cl_2 solution (60 mL; 5:7 v/v). To this was added cyclohexylamine (2.60, 26 mmol) and stirred for 45 h at room temperature. The resin was filtered off, washed with DMF, MeOH, CH_2Cl_2, and ether, and dried under vacuum, affording the *N*-hydroxypyrrolidinone resin (**10**) (6.67 g; 3.17 mmol/g).

The Acyl Group Content of the Polymers. Titration: *O*-Acyl resin (*ca.* 35 mg) was heated with benzylamine in toluene (1 mL; 0.30 M) at 80 °C for 1 h. After cooling the mixture was titrated with $HClO_4$ in AcOH (0.01 M) using Methylene Blue as an indicator.

HPLC Procedure: Excess benzylamine unconsumed in the aminolysis was analyzed by HPLC. The column was eluted with MeOH–0.1% phosphate buffer (65:35 v/v).

*Preparation of Polymer-bound Amino Acid Esters (**11**).* A mixture of polymer-bound pyrrolidinone (**10**) (3.47 g; 1.27 mmol/g) and an *N*-protected amino acid (8.81 mmol) in DMF (30 mL) was stirred for 2 h at room temperature and chilled. To this was added DCC (8.8 mmol) in DMF (10 mL) at 0 °C; the suspension was stirred for 2 h at 0 °C and for 22 h at room temperature. The resin was filtered off and washed with DMF (2×20 mL), MeOH–$CHCl_3$ (2×20 mL, 1:1 v/v), and Et_2O, and then dried under vacuum. The content of the resin was determined by titration using a part of the resin. Typical data were summarized in Table 1. 1-Hydroxybenzotriazole-DCC procedure was similarly carried out as above. In a symmetric anhydride procedure, an anhydride derived from Boc-Phe-OH (2 equiv) and DCC (1 equiv) in DMF was added *in situ* to a resin suspension at room temperature.

Synthesis of Protected Peptides with Polymer-bound Reagents. General Procedure: Boc-amino acid resin (3.6 mmol) was swollen in DMF (30 mL) for 1 h. A mixture of a hydrochloride or tosylate of an amino component (3.0 mmol) and Et_3N (0.303 g, 3.0 mmol) in DMF (10 mL) was added to the swollen suspension and stirred for 30 h at 35 °C. The resin was filtered off and washed with DMF (3×20 mL). The combined filtrate and washings were evaporated to give a residue which was diluted with EtOAc (60 mL). The EtOAc layer was washed with 5% $NaHCO_3$

(3×20 mL), water, 5% citric acid (3×20 mL) and water, and dried (Na_2SO_4). Evaporation of the solvent gave a product.

Boc-Phe resin and TsOH·H-Leu-OBzl gave *Boc-Phe-Leu-OBzl* (**12**), 1.32 g (94%); mp 84—85 °C. Recrystallization from Et_2O-petroleum ether, mp 85—86 °C (lit, [17e] 85.5—86.5 °C); $[\alpha]_D$ −16.3° (THF); R_f^1=0.86, R_f^2=0.91, R_f^3=0.90.

Boc-Gly resin (3.15 g; 1.17 mmol/g) and HCl·H-Phe-Leu-OBzl (1.34 g, 3.31 mmol) produced *Boc-Gly-Phe-Leu-OBzl* (**13**) as an oil; 1.16 g (68%); R_f^1=0.80, R_f^2=0.67, R_f^3=0.75. The peptide **13** gave HCl·H-Gly-Phe-Leu-OBzl by treatment with HCl-dioxane. Neutralization of the hydrochloride and recrystallization from Et_2O gave the free peptide, mp 103—104 °C; $[\alpha]_D$ −16° (THF). Found: C, 67.68; H, 7.38; N, 9.85%. Calcd for $C_{24}H_{31}N_3O_4$: C, 67.74; H, 7.34; N, 9.88%.

Boc-Gly resin (4.82 g; 1.10 mmol/g) and HCl·H-Gly-Phe-Leu-OBzl (2.20 g, 4.76 mmol) gave *Boc-Gly-Gly-Phe-Leu-OBzl* (**14**), 2.20 g (79%). Recrystallization from THF-petroleum ether gave mp 160—161.5 °C (lit,[17d] mp 162.5—163.5 °C); $[\alpha]_D$ −16.7° (THF); R_f^1=0.27, R_f^2=0.56, R_f^3=0.41.

Boc-Try(Bzl)-Gly-Gly-Phe-Leu-OBzl (**15**) was produced from Boc-Tyr(Bzl) resin (2.26 g; 0.62 mmol/g) and HCl·H-Gly-Gly-Phe-Leu-OBzl (0.648 g, 1.25 mmol); 0.853 g (81%), mp 181—182 °C. Recrystallization from MeOH-petroleum ether gave a sample of mp 187—188 °C; $[\alpha]_D$ −17.4° (DMF); R_f^2=0.52, R_f^3=0.17. Found: C, 66.87; H, 6.83; N, 8.26%. Calcd for $C_{47}H_{57}N_5O_9 \cdot 1/2H_2O$: C, 66.80; H, 6.92; N, 8.29%.

Leu⁵-Enkephalin. The protective groups of **15** were removed by hydrogenation and then by treatment with HCl-dioxane, followed by column chromatography,[17b] to give H-Tyr-Gly-Gly-Phe-Leu-OH (**16**); 0.29 g (42%) from 0.76 g of **15**, mp 200—201 °C (lit,[17b-d] mp 206—208 °C); $[\alpha]_D$ −22.5° (DMF) (lit, [17b,d] $[\alpha]_D$ −23.4°); R_f^4=0.76.

Biological Assay: The peptide (**16**) (3 mg) was incubated with aminopeptidase M (0.016 mg) in phospate buffer (1 mL; pH=7.2) at 36 °C; 8 μL aliquots of the mixture were analyzed for the peptide using HPLC at appropriate time intervals. The peptide was virtually digested with the pseudo-first-oder rate within 1.5 h. *In vivo* analgesic activity was observed for 5-week-old male mice with the peptide in dimethyl sulfoxide solution (16.7 mg/mL). 10 μL (300 nmol) of the solution was injected intracerebroventricularly and, in a few minutes, the analgesic state was carefully watched for the mouse. The state lasted for *ca.* 15 min.

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