A New Peptide Synthesis Using 2-Fluoro-1,3,5-trinitrobenzene. Syntheses of Thyrotropin Releasing Hormone and Leucine-Enkephalin

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2-Fluoro-1,3,5-trinitrobenzene has been found to be a useful new condensing reagent for peptide synthesis. A variety of dipeptides have been prepared from the corresponding amino acids with the reagent. The Young test under certain conditions did not cause racemization during the procedures. Subsequent application of this reagent enabled the syntheses of thyrotropin releasing hormone and leucine-enkephalin in good yields.

It has been reported[1,2] that the readily available 2-fluoro-1,3,5-trinitrobenzene(FTNB) is a useful new condensing reagent for the preparation of amides, esters, and thiocarboxylic *S*-esters. In this paper, the syntheses of various dipeptides and biologically active peptides, thyrotropin releasing hormone(TRH) and leucine-enkephalin are described.

As a preliminary, several *N*-benzyloxycarbonyl(Z) dipeptide esters were prepared by the reaction of *N*-benzyloxycarbonyl amino acids with amino acid esters using FTNB as a condensing reagent, as shown in Table 1. To an equimolar solution of *N*-benzyloxycarbonyl amino acid and FTNB in acetonitrile was added an acetonitrile solution of pyridine(one equivalent) at 0 °C under a nitrogen atmosphere. After stirring for 3 h at 0 °C, the amino acid ester(one equivalent) was added to the reaction mixture, followed by the addition of a solution of pyridine(one equivalent) in acetonitrile. The solution was stirred for several hours and then worked up to give the desired dipeptide **1a**—**g** in good yield (Table 1).

In a similar manner, the *N*-*t*-butoxycarbonyl(Boc) dipeptide esters(**2a**—**e**) were obtained in excellent yields, the results of which are given in Table 2.

Compound **1g** was obtained in good yield with no side reactions such as *O*-acyl derivatives[3] without protection of the hydroxyl group of *N*-(benzyloxycarbonyl)serine. The condensation reaction of amino acids containing bulky substituents (**2b**,**c**) also proceeded smoothly giving the desired products in good yields. FTNB is then an excellent condensing reagent for peptide synthesis.

The reaction appears to proceed *via* a mechanism similar to the formation of amides[1] and esters[2] as illustrated in the following scheme: First, the anionic sigma complex **3** is formed by the reaction of the amino acid with FTNB in the presence of pyridine followed by the elimination of the picrate to produce an intermediate acyl fluoride **4**, which then reacts with amino acid esters to give the corresponding dipeptide **5**.

Fragment condensation is frequently used to synthesize the more complex peptides, however, the problem of racemization of the acyl peptide exists. Consequently the Young test[4] was applied to determine the applicability of the FTNB method to fragment condensation. The results of which are given in Table 3. The

TABLE 1. PREPARATION OF BENZYLOXYCARBONYL DIPEPTIDE ESTERS

FTNB + Z-A.A.-OH + Py $\xrightarrow[\text{in } CH_3CN]{0\,^\circ C,\ 3\,h}$ $\xrightarrow[0\,^\circ C,\ r.t.,\ 3\,h]{H\text{-}A.A.\text{-}OR,\ Py}$ Z-dipeptide-OR

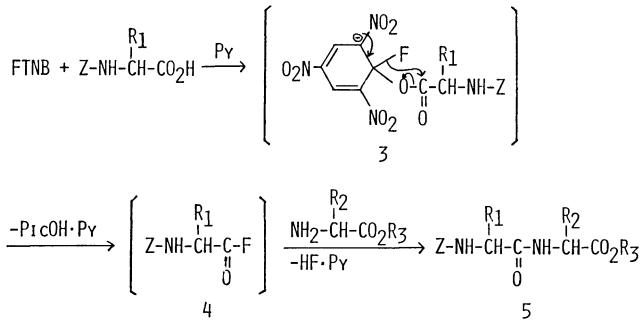
	Product	Yield/%	Mp/°C (lit)	$[\alpha]_D$/° (lit)	Ref.
1a	Z-Ile-Gly-OEt	85	155—156 (155—156)	−25.9 (0.65, EtOH) (−25.6)	13
1b	Z-Gly-Gly-OEt	85	80—81 (80)		14
1c	Z-Ala-Gly-OEt	85	99—100 (99—100)	−22.3 (3.65, EtOH) (−22.2)	13
1d	Z-Val-Gly-OEt	85	163—164 (162—164)	−27.2 (0.77, EtOH) (−27.0)	13
1e	Z-Met-Gly-OEt	82	96—97 (94—96)	−19.7 (3.50, EtOH) (−19.8)	15
1f	Z-Cys(Bzl)-Gly-OEt	80	97—98 (97—99)	−28.9 (5.86, AcOEt) (−27.0)	15
1g	Z-Ser-Thr-OMe	76	128—130 (126—127)	+7.1 (1.98, DMF) (+6.9)	16

TABLE 2. PREPARATION OF *t*-BUTOXYCARBONYL DIPEPTIDE ESTERS

FTNB $\xrightarrow[0\,^\circ C,\ 1.5\,h,\ \text{in } CH_3CN]{\text{Boc-A.A.-OH, Py}}$ $\xrightarrow[0\,^\circ C,\ r.t.,\ 3\,h]{H\text{-}A.A.\text{-}OMe,\ Py}$ Boc-dipeptide-OMe

	Product	Yield/%	Mp/°C (lit)	$[\alpha]_D$/° (lit)	Ref.
2a	Boc-Phe-Val-OMe	85	117—118 (118—119)	−11.0 (1.89, DMF) (−11.6)	17
2b	Boc-Leu-Leu-OMe	81	141—142 (141—142)	−50.0 (0.39, MeOH) (−50.4)	18
2c	Boc-Ile-Ile-OMe	81	157—158 (158—159)	−36.5 (0.51, MeOH) (−33.3)	19
2d	Boc-Ala-Ala-OMe	76	105—106 (105—108)	−63.6 (0.66, MeOH) (−63.8)	17
2e	Boc-Ala-Val-OMe[a]	83	63—64	−49.5 (0.31, MeOH)	

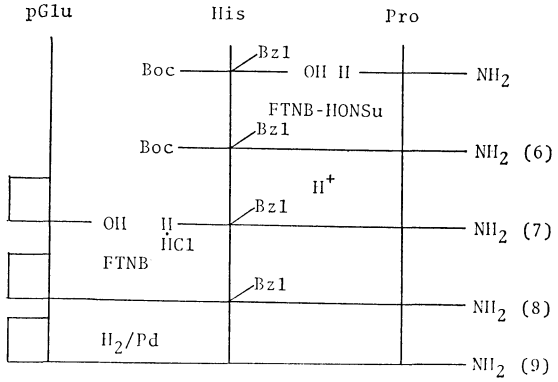
a) **2e** exhibited NMR spectral data and elemental analysis in accordance with assigned structure.



product obtained from the same procedures as described in the preparation of the dipeptides using only FTNB was perfectly optically inactive, regardless of solvent(Entry 1, 2). Accordingly, the reaction conditions which did not induce racemization were investigated; this difficulty was satisfactorily overcome by employing the Eintopf method reported by Wünsch *et al.*[5] As may be seen from the Table, the coexistence of additives such as *N*-hydroxysuccinimide(HONSu) and *N*-hydroxy-5-norbornene-2,3-dicarboximide (HONB) effectively suppressed racemization, twomolar amounts of additive being required. Furthermore, dichloromethane proved to be the best solvent among the solvents investigated. With respect to base, collidine was especially effective. The reaction conditions making use of collidine as a base in the presence of two molar amounts of HONSu in dichloromethane gave optimum results with 98% optical purity(Entry 10).

The results may be explained as follows. In the absence of additive, the intermediary amino acid fluoride is rapidly converted into the oxazolone regarded as an intermediate for racemization.[6,7] In the presence of an additive, the amino acid fluoride reacts more rapidly with HONSu to give the so-called "active ester" of HONSu,[8] which then reacts with the amine component to give the optically pure product.

The optimum conditions for suppression of racemization were utilized to synthesize the biologically active peptides, TRH and leucine-enkephalin uisng the FTNB method.



Scheme 1. Synthesis of thyrotropin releasing hormone.

The synthesis of TRH was conducted as illustrated in Scheme 1. α-*t*-Butoxycarbonyl(Boc)-N^{im}-benzyl (Bzl)histidine was condensed with prolinamide in the presence of two molar amounts of HONSu using the FTNB method to give the dipeptide **6** (81% yield after purification by column chromatography). Cleavage of the *t*-butoxycarbonyl group of the protected dipeptide **6** with 5 M-hydrogen chloride in ethyl acetate gave the deprotected product **7** (nearly quantitative yield), which was condensed with pyroglutamic acid using the FTNB method to give the desired protected tripeptide **8** (56% yield after purification by ion-exchange resin column chromatography followed by preparative TLC). Finally, debenzylation of the protected tripeptide **8** by catalytic hydrogenation over palladium black followed by purification of the reaction mixture by preparative TLC gave the desired product, pyroglutamyl-histidyl-prolinamide (**9**) in 75% yield. The specific rotation was in accordance with the literature.[9]

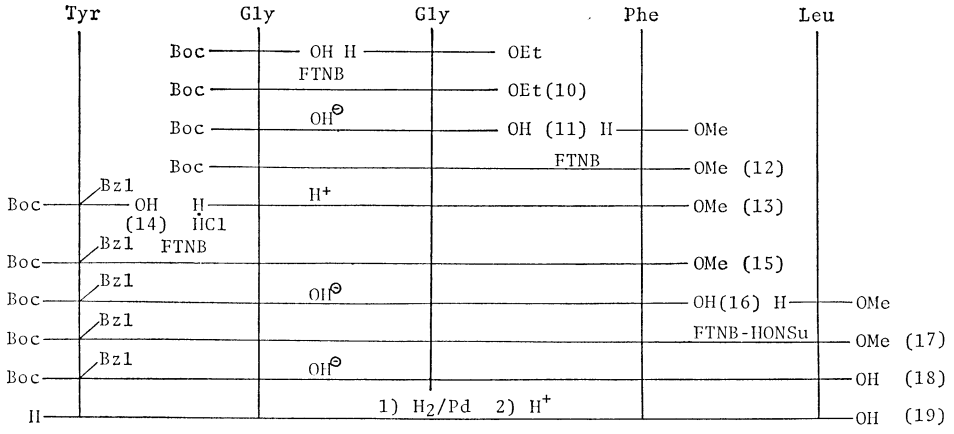
The synthesis of leucine-enkephalin with opiate-

Table 3. The results of the Young Test

FTNB + Bz-Leu-OH (I) + Additive (II) —Base, Solvent; Time/h→ —H-Gly-OEt→ Bz-Leu-Gly-OEt

Entry	Additive	Molar ratio II/I	Reaction conditions: Base	Reaction conditions: Time/h	Solvent[a]	Yield %	Optical purity/%[b]
1	none		Py	2	CH_3CN	60.0	0
2	none		Py	2	CH_3Cl_2	68.8	0
3	HONSu	1	Py	3	CH_3CN	45.6	39.5
4	HONSu	1	Py	3	THF	61.2	34.1
5	HONSu	1	Py	3	$CHCl_3$	65.0	59.6
6	HONSu	1	Py	2	CH_2Cl_2[c]	60.0	76.5
7	HONSu	2	Py	2	CH_2Cl_2[c]	60.0	95.0
8	HONSu	2	2,4-Lutidine	3	CH_2Cl_2[c]	58.0	72.6
9	HONSu	2	2,6-Lutidine	3	CH_2Cl_2[c]	59.3	89.4
10	HONSu	2	Collidine	3	CH_2Cl_2[c]	63.7	98.3
11	HONB	1	Py	2	CH_2Cl_2[c]	50.0	89.0
12	HONB	2	Py	2	CH_2Cl_2[c]	45.0	94.5

a) 5—6 ml of solvent was used. b) Based on $[\alpha]_D^{20}$ −34.0° (3.1, EtOH) reported by M. W. Williams and G. T. Young.[4] c) 1 ml of CH_3CN was added in addition to the solvent to dissolve the additive.



Scheme 2. Synthesis of leucine-enkephalin.

agonist activity was conducted according to Scheme 2. Saponification of the dipeptide **10**, derived from the coupling of *N*-(*t*-butoxycarbonyl)glycine with ethyl glycinate using the FTNB method, gave the dipeptide **11** which was condensed with methyl phenylalaninate using the FTNB method to give the protected ester **12** in 76% yield. Treatment of **12** with 5 M-hydrogen chloride in ethyl acetate gave the desired deprotected tripeptide ester hydrochloride **13** in 81% yield. The melting point and specific rotation were however in disagreement with the values reported by Voelter *et al.*[10] In order to clarify the discrepancy, **12** was prepared by two other methods, the azide and DCC-HONSu methods, and subsequent deprotection of the *t*-butoxycarbonyl group of compound **12** was conducted under the same conditions as described for the FTNB method. It was found that the physical constants of the products obtained from the two methods were consistent with the values obtained from the FTNB method. α-*t*-Butoxycarbonyl-*O*-benzyltyrosine (**14**) was condensed with the tripeptide **13** to give the tetrapeptide ester **15** in 81% yield, which was saponified to give the deprotected tetrapeptide **16** in 86% yield. The desired pentapeptide ester **17** was obtained from the coupling of **16** with methyl leucinate in the presence of two molar amounts of HONSu thereby avoiding racemization (71% yield). Saponification of **17** gave the pentapeptide **18** in 81% yield, which was hydrogenated over 10%-palladium on charcoal followed by cleavage of the *t*-butoxycarbonyl group to give the desired product, leucine-enkephalin (**19**) in 44% yield after treatment with ion-exchange resin column chromatography. The melting point and specific rotation of the product were in accordance with the reported values.[11]

Experimental

All melting points are uncorrected. The NMR spectra were recorded on a JEOL/MH-60. The chemical shifts are reported on the δ scale relative to TMS as an internal standard. The IR spectra were measured with a JASCO IRA-1 diffraction grating infrared spectrometer. The optical rotation values were measured with a JASCO DIP-SL polarimeter.

Materials. All solvents were distilled according to the usual methods and stored over a drying agent. Thin-layer chromatography (TLC) and column chromatography were performed on Merck's Silica gel 60 PF_{254} (Type 7749), on Alumina Woelm (Act. 1), and on Merck's Aluminium oxide 90 (Type 1077).

*Z–Ile–Gly–OEt (**1a**).* To a solution of FTNB (116 mg, 0.5 mmol) and Z–Ile–OH (113 mg, 0.5 mmol) in CH_3CN (5 ml) was added a solution of pyridine(Py) (40 mg, 0.5 mmol) in CH_3CN (1 ml) dropwise at 0 °C under a nitrogen atmosphere. After stirring for 3 h at 0 °C, H–Gly–OEt·HCl (70 mg, 0.5 mmol) was added to the reaction mixture, followed by the addition of a solution of Py (79 mg, 1 mmol) in CH_3CN (1 ml). The solution was stirred at 0 °C for 1.5 h and for additional hours at room temperature. The reaction mixture was evaporated to dryness under reduced pressure and the residue partitioned between AcOEt and water. The AcOEt layer was washed successively with 1M-HCl, 10%-$NaHCO_3$, and a saturated solution of NaCl, dried over Na_2SO_4 and evaporated to dryness *in vacuo*. The residue was subjected to neutral alumina column chromatography using benzene–AcOEt (1:1 v/v) as solvent to give a crystalline product. Recrystallization from benzene–hexane gave the desired product **1a** in 85% yield (149 mg).

In a similar manner, the dipeptides **1b—g** were obtained as shown in Table 1.

*Boc–Phe–Val–OMe (**2a**).* To a solution of FTNB (116 mg, 0.5 mmol) in CH_3CN (5 ml) was added slowly a mixed solution of Boc–Phe–OH (113 mg, 0.5 mmol) and Py (40 mg, 0.5 mmol) in CH_3CN (5 ml) at 0 °C. After stirring for 1.5 h at 0 °C, H–Val–OMe·HCl (84 mg, 0.5 mmol) was added to the solution followed by the addition of a solution of Py (79 mg, 1 mmol) in CH_3CN (0.5 ml). The reaction mixture was stirred for 3 h at room temperature and worked up as described for **1a**. The residue was subjected to neutral alumina column chromatography using benzene–AcOEt (1:1 v/v) as solvent, followed by preparative TLC using benzene–AcOEt (3:2 v/v) as solvent to afford the desired product. Recrystallization from benzene–hexane gave pure **2a** in 85% yield.

By a similar procedure, the dipeptides **2b—e** were obtained as shown in Table 2. The physical constants of **2e** were in disagreement with the reported values,[17] but the structure was confirmed by elemental analysis and NMR data as shown below: NMR ($CDCl_3$) δ 0.93 (d, 6H, J=7 Hz), 1.35 (d, 3H, J=6 Hz), 1.44 (s, 9H), 1.80—2.40 (m, 1H), 3.70 (s, 3H), 4.00—4.67 (m, 2H), 4.95—5.30 (bd, 1H, J=8 Hz), 6.45—6.90 (bd, 1H, J=8 Hz). Found: C, 55.59; H, 8.70; N, 9.27%. Calcd for $C_{14}H_{26}N_2O_5$: C, 55.61; H, 8.67; N, 9.27%.

Young Test (Bz–Leu–Gly–OEt). *Entry 1*: To a solu-

tion of FTNB (116 mg, 0.5 mmol) and Bz–Leu–OH (118 mg, 0.5 mmol) in CH_3CN (5 ml) was added dropwise a solution of Py (40 mg, 0.5 mmol) in CH_3CN (1 ml) at 0 °C under a nitrogen atmosphere. After stirring for 3 h at 0 °C, a solution of H–Gly–OEt (52 mg, 0.5 mmol) in CH_3CN (2 ml) was added to the solution at 0 °C, followed by the addition of a solution of Py (40 mg, 0.5 mmol) in CH_3CN (1 ml). The reaction mixture was allowed to stand overnight at room temperature and evaporated to dryness. The residue was dissolved in AcOEt and the solution washed successively with 10%-$NaHCO_3$, 1M-HCl, and a saturated solution of NaCl and dried over Na_2SO_4. After evaporation of the solvent, the residue was subjected to neutral alumina column chromatography using benzene–AcOEt (1:1 v/v) as solvent followed by preparative TLC using benzene–AcOEt (3:2 v/v) as solvent to afford the product in 60% yield (96 mg): mp 146—147 °C; $[\alpha]_D^{20}$ 0° (2.98, EtOH). *Entry 10*: To a solution of Bz–Leu–OH (118 mg, 0.5 mmol), FTNB (116 mg, 0.5 mmol), and HONSu (115 mg, 1 mmol) in CH_3CN (1 ml) was added CH_2Cl_2 (5 ml) at 0 °C, followed by the addition of a solution of collidine (112 mg, 1 mmol) in CH_2Cl_2 (1 ml). After stirring for 3 h at 0 °C, a solution of H–Gly–OEt (52 mg, 0.5 mmol) in CH_2Cl_2 (2 ml) was added at 0 °C to the solution. The reaction mixture was allowed to stand overnight and worked up as described for Entry 1. Yield, 108 mg (64%): mp 153—154 °C; $[\alpha]_D^{29}$ −33.4° (2.15, EtOH).

*Boc–His(Bzl)–Pro–NH_2 (**6**).* To a solution of FTNB (116 mg, 0.5 mmol) and HONSu (115 mg, 1 mmol) in CH_3CN (1 ml) was added CH_2Cl_2 (5 ml). To the resulting clear solution was added dropwise a solution of Boc–His(Bzl)–OH (173 mg, 0.5 mmol) and triethylamine(TEA) (101 mg, 1 mmol) in CH_2Cl_2 (2 ml) at 0 °C. After stirring for 3 h at 0 °C, H–Pro–NH_2·HCl (76 mg, 0.5 mmol) was added to the solution followed by the addition of a solution of TEA (51 mg, 0.5 mmol) in CH_2Cl_2 (1 ml). The reaction mixture was allowed to stand overnight at room temperature. After evaporation of the solvent, the residue was subjected to ion-exchange resin chromatography (Amberlite IRA-410) using MeOH as solvent and basic alumina column chromatography using benzene–EtOH (8:1 v/v) as solvent to give a solid material. This was separated by preparative TLC using chloroform–MeOH (10:1 v/v) as solvent to give the desired product in 81% yield (179 mg); $[\alpha]_D^{28.5}$ −23.4° (0.64, MeOH); NMR (CD_3OD) δ 1.37 (s, 9H), 1.70—2.20 (m, 4H), 2.70—3.00 (m, 2H), 3.10—3.50 (m, 2H), 4.20—4.60 (m, 1H), 5.06 (s, 2H), 6.90 (s, 1H), 7.20 (s, 5H), 7.53 (s, 1H).

*H–His(Bzl)–Pro–NH_2·2HCl (**7**).* A solution of **6** in AcOEt (2 ml) was treated with 6M-HCl (6 ml) in AcOEt, and the solution allowed to stand at room temperature for 30 min. To the reaction mixture was added dry ether (30 ml). The supernatant liquid was decanted, and the residue washed with dry ether (4×30 ml) to give the salt **7** which was dried *in vacuo* in the presence of solid potassium hydroxide and phosphorous pentoxide, yield, 164 mg. The product was used without further purification in the subsequent reaction.

*pGlu–His(Bzl)–Pro–NH_2 (**8**).* A solution of FTNB (70 mg, 0.3 mmol) in CH_2Cl_2 (1 ml) was cooled at 0 °C and to the solution was added dropwise a mixed solution of pGlu–OH (39 mg, 0.3 mmol) and TEA (31 mg, 0.3 mmol) in CH_2Cl_2 (2 ml). After stirring for 2 h at 0 °C, **7** (120 mg, 0.3 mmol) was added to the reaction mixture followed by the addition of a solution of TEA (91 mg, 0.9 mmol) in CH_2Cl_2 (1 ml). The reaction mixture was allowed to stand overnight at room temperature. The solvent was evaporated *in vacuo*, and the residual oil purified by ion-exchange resin chromatography using MeOH as solvent followed by preparative TLC using chloroform–MeOH (3:1 v/v) as solvent to afford the product in 56% yield (59 mg).

*pGlu–His–Pro–NH_2 (**9**).* The protected tripeptide **8** (57 mg, 0.13 mmol) was dissolved in EtOH (25 ml) and hydrogenated for 12 h over palladium black (20 mg) at room temperature under atmospheric pressure. The catalyst was filtered off followed by evaporation of the filtrate. The crude product was subjected to preparative TLC using chloroform–MeOH (3:1 v/v) to afford the desired TRH (**9**) in 75% yield (35 mg): $[\alpha]_D^{28.5}$ −40.7° (1.00, MeOH) [lit,[8] −42.4° (1.00, MeOH)]. The product gave a positive Pauli test and showed a single spot on TLC in several solvent systems.

*Boc–Gly–Gly–OEt (**10**).* To an CH_3CN solution (6 ml) of FTNB (232 mg, 1 mmol) cooled at −30 °C, was added a solution of Boc–Gly–OH (175 mg, 1 mmol) and TEA (101 mg, 1 mmol) in CH_3CN (5 ml). After stirring for 2 h at 0 °C, H–Gly–OEt·HCl (140 mg, 1 mmol) and then a solution of TEA (202 mg, 2 mmol) in CH_3CN (1 ml) were added to the reaction mixture. The solution was allowed to stand overnight at room temperature and worked up as described for **1a**. After evaporation of the solvent, the residue was subjected to basic alumina column chromatography using benzene–EtOH (1:2 v/v) as solvent and to preparative TLC using benzene–EtOH (8:1 v/v) as solvent to give the desired product as an oil in 82% yield (212 mg).

*Boc–Gly–Gly–OH (**11**).* To a solution of **10** (1.30 g, 5 mmol) in MeOH–H_2O (5 ml) (4:1 v/v), was added 1M-NaOH (5 ml) at 0 °C and the reaction mixture maintained at 0 °C for 2 h and then allowed to stand overnight. The MeOH was removed *in vacuo* and water (5 ml) added to the solution. The resulting aqueous solution was washed with AcOEt and neutralized with 1M-HCl (5 ml). The separated oil was extracted with AcOEt after salting-out, and the organic layer dried over Na_2SO_4 and evaporated to dryness *in vacuo* to give a crystalline product. Recrystallization from AcOEt gave an 87% yield (1.01 g): mp 133—134 °C; Found: C, 46.70; H, 7.02; N, 12.18%. Calcd for $C_9H_{16}N_2O_5$: C, 46.54; H, 6.94; N, 12.06%.

*Boc–Gly–Gly–Phe–OMe (**12**).* To a solution of FTNB (232 mg, 1 mmol) in CH_3CN (2 ml) was added slowly a solution of **11** (232 mg, 1 mmol) and TEA (101 mg, 1 mmol) in CH_3CN (3 ml) at 0 °C. After stirring for 2 h at 0 °C, H–Phe–OMe·HCl (215 mg, 1 mmol) was added to the solution followed by the addition of a solution of TEA (202 mg, 2 mmol) in CH_3CN (1 ml). The reaction mixture was allowed to stand overnight and worked up as described in the preparation of **1a**. The desired product was separated from the residue by basic alumina column chromatography using benzene–EtOH (1:1 v/v) as solvent followed by preparative TLC using benzene–EtOH (8:1 v/v) as solvent; 76% yield as an oil (297 mg): NMR ($CDCl_3$) δ 1.43 (s, 9H), 3.05 (d, 2H, J=6.0 Hz), 3.65 (s, 3H), 3.75 (d, 2H, J=6.3 Hz), 3.87 (d, 2H, J=6.3 Hz), 4.84 (t, 1H, J=6.0 Hz), 5.38—5.65 (m, 1H), 6.85—7.16 (bs, 2H), 7.00—7.43 (m, 5H).

*H–Gly–Gly–Phe–OMe·HCl (**13**).* *Method A*: The product **12** obtained from the FTNB method was dissolved in AcOEt, and 5M-HCl (5 ml) in AcOEt added to the solution at room temperature. After 3 h, the solvent was evaporated to dryness *in vacuo* to give a crude product which was recrystallized from 1-propanol in 81% yield (202 mg): mp 179—180 °C; $[\alpha]_D^{21.5}$ +31.4° (1.05, AcOH). Found: C, 50.81; H, 6.15; N, 12.96%. Calcd for $C_{14}H_{20}N_3O_4Cl$: C, 50.98; H, 6.06; N, 12.74%.

Method B: Boc–Gly–Gly–NH–NH_2 derived from the reac-

tion of **10** with hydrazine was condensed with H–Phe–OMe using the azide method to give the desired tripeptide **12** in 66% yield. **12** was deprotected with hydrogen chloride according to method A. Recrystallization from 1-propanol gave the product **13** in 81% yield: mp 178—179 °C; $[\alpha]_D^{21.5}$ +31.4° (1.05, AcOH).

Method C: **12** was obtained from the reaction of **11** with H–Phe–OMe using the DCC–HONSu method in 89% yield, which was treated according to Method A. Recrystallization from 1-propanol gave the product **13** in 76% yield; mp 178—179 °C; $[\alpha]_D^{23}$ +30.4° (0.99, AcOH).

*Boc–Tyr(Bzl)–Gly–Gly–Phe–OMe (**15**).* To a solution of FTNB (232 mg, 1 mmol) in CH_3CN (4 ml) was added slowly a mixed solution of Boc–Tyr(Bzl)–OH (**14**) (371 mg, 1 mmol) and TEA (101 mg, 1 mmol) in CH_3CN (2 ml) at 0 °C. After stirring at 0 °C for 2 h, **13** (330 mg, 1 mmol) was added to the solution followed by the addition of a solution of TEA (202 mg, 2 mmol) in CH_3CN (2 ml). The reaction mixture was allowed to stand at room temperature, and worked up as described for **1a**. After evaporation of the solvent, the crude product was purified by gradient elution on basic alumina using benzene–EtOH (8:1 v/v to 1:1 v/v). Recrystallization from AcOEt–hexane gave the pure product in 81% yield: mp 112—113 °C; $[\alpha]_D^{32.5}$ +13.8° (1.23, MeOH). Found: C, 64.69; H, 6.64; N, 8.70%. Calcd for $C_{19}H_{27}N_3O_6$: C, 65.00; H, 6.55; N, 8.70%.

*Boc–Tyr(Bzl)–Gly–Gly–Phe–OH (**16**).* To a solution of **15** (406 mg, 0.63 mmol) in MeOH–H_2O (5 ml) (4:1 v/v) was added with stirring 1M-NaOH (0.8 ml) at 0 °C and worked up as described in the preparation of **11**. Recrystallization from chloroform–hexane gave the desired product in 84% yield (341 mg): mp 83—84 °C; $[\alpha]_D^{31}$ +17.3° (1.38, MeOH). Found: C, 63.56; H, 6.59; N, 8.89%. Calcd for $C_{34}H_{40}N_4O_8 \cdot 1/2H_2O$: C, 63.65; H, 6.39; N, 8.74%.

*Boc–Tyr(Bzl)–Gly–Gly–Phe–Leu–OMe (**17**).* To a mixed solution of **16** (327 mg, 0.51 mmol), FTNB (120 mg, 0.52 mmol), and HONSu (120 mg, 1.04 mmol) in CH_3CN (1 ml) was added CH_2Cl_2 (5 ml) at 0 °C followed by the addition of a solution of collidine (126 mg, 1.04 mmol) in CH_2Cl_2 (2 ml). After stirring at 0 °C for 3 h, H–Leu–OMe·HCl (95 mg, 0.52 mmol) was added followed by the addition of a solution of TEA (53 mg, 0.52 mmol) in CH_2Cl_2 (1 ml). The reaction mixture was allowed to stand overnight at room temperature, and then worked up as described for **12**. Recrystallization from chloroform–hexane gave the pure product in 72% yield (280 mg): mp 153—154 °C; $[\alpha]_D^{22}$ −11.4° (1.74, MeOH). Found: C, 64.55; H, 7.08; N, 9.26%. Calcd for $C_{41}H_{53}N_5O_9$: C, 64.80; H, 7.03; N, 9.22%.

*Boc–Tyr(Bzl)–Gly–Gly–Phe–Leu–OH (**18**).* To a solution of **17** (400 mg, 0.53 mmol) in MeOH–H_2O (5 ml) (4:1 v/v) was added with stirring 1M-NaOH (0.6 ml) at 0 °C and worked up as described in the preparation of **11**. Recrystallization from AcOEt gave the desired product in 81% yield (321 mg): mp 149—150 °C; $[\alpha]_D^{28}$ +1.6° (1.26, MeOH). Found: C, 64.53; H, 6.95; N, 9.43%. Calcd for $C_{40}H_{51}N_5O_9$: C, 64.51; H, 6.89; N, 9.39%.

*H–Tyr–Gly–Gly–Phe–Leu–OH (**19**).* The protected pentapeptide **18** (90 mg, 0.12 mmol) was dissolved in MeOH (15 ml), and hydrogenated over 10% palladium on charcoal (250 mg) for 2 h at room temperature under atmospheric pressure. The catalyst was filtered off and the filtrate evaporated to dryness *in vacuo* to give the debenzylated product which was dissolved in 5M-HCl (1 ml) in dioxane. The reaction mixture was allowed to stand for 6 h at room temperature. Evaporation of the solvent gave a residue which was washed with dry ether. The residual product was dissolved in a small amount of water and the aqueous solution passed through an ion-exchange resin column (Amberlite IR-120B) using 5%-NH_4OH as solvent. The eluate was evaporated to dryness *in vacuo* to give the desired leucine-enkephalin (**19**) in 44% yield (30 mg): mp 208—210 °C (dec); $[\alpha]_D^{25}$ −24.0° (1.0, DMF) [lit,[11] mp 206 (dec), $[\alpha]_D^{23}$ −23.4° (1.0, DMF)]. Amino acid analysis (after acidic hydrolysis) showed the presence of tyrosine, glycine, phenylalanine, and leucine in the ratio 1.00:2.15:1.26:0.98.

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