TRITIUM LABELLING OF ENKEPHALIN ANALOGS : TYR-D-ALA-GLY-NH-CH(CH_3)-CH_2-CH(CH_3)$_2$ (TRIMU 4), TYR-D-SER-GLY-PHE-LEU-THR (DSLET), TYR-D-THR-GLY-PHE-LEU-THR (DTLET), μ AND δ OPIATE-RECEPTORS SPECIFIC LIGANDS.

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SUMMARY

Unambiguous biochemical characterization of the different opiate receptors (i.e., μ and δ) imperatively requires fully specific and radiolabelled ligands. Therefore, in this paper, we report the synthesis of the 3,5-dibromotyrosyl precursors of three highly selective peptides : Tyr-D-Ala-Gly-NH-CH(CH_3)-CH_2-CH(CH_3)$_2$, TRIMU 4 (μ) ; Tyr-D-Ser-Gly-Phe-Leu-Thr, DSLET (δ) and Tyr-D-Thr-Gly-Phe-Leu-Thr, DTLET (δ). Reductive tritiation by exchange with $^3H_2$ leads to the labelled enkephalins analogs in high yields and with specific activities between 45 to 60 Ci/mmole.

Key words : peptides synthesis, tritiated enkephalins, specific μ and δ agonists, opiate-receptors.

INTRODUCTION

The multiple pharmacological responses elicited upon central administration of morphine would be related to the interaction of this compound with several receptor subtypes exhibiting binding preferences for classical opiates (μ-receptor) or for peptide structures (enkephalin or δ-receptor) (1,2). μ-receptors seems to be preferentially involved in analgesia while δ-receptors stimulation would produce various behavioural responses (3,4). However these assumptions were

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put forward from pharmacological assays performed with non-selective agonists. Therefore, unambiguous characterization of physiological responses associated with specific μ or δ receptors stimulation as well as localisation in brain tissue and binding properties of both receptor subtypes require fully selective ligands. Using a rational approach (5) we have recently prepared a μ specific agonist : Tyr-D-Ala-Gly-NH-CH(CH_3)-CH_2-CH(CH_3)$_2$ (TRIMU 4) and two δ selective hexapeptides : Tyr-D-Ser-Gly-Phe-Leu-Thr (DSLET) and Tyr-D-Thr-Gly-Phe-Leu-Thr (DTLET) (6-8). For biochemical investigations it was necessary to use labelled derivatives of these compounds obtained from their 3,5-dibromotyrosyl precursors. Synthesis of these latter and tritium exchange of their bromine atoms are reported in this paper. DSLET is considered at this time as the most specific δ-agonist for binding and pharmacological assays (9).

EXPERIMENTAL PART

Materials

Protected aminoacids are from Bachem (Switzerland). The peptides were prepared by the liquid-phase method using tert-butyloxycarbonyl (Boc), benzyloxycarbonyl (Z), methyl and benzyl esters as protecting groups and dicyclohexylcarbodiimide (DCC) with hydroxybenzotriazole (HOBt) as coupling reagents. The structure of the compounds and of all the intermediates were confirmed by ^{1}H NMR spectroscopy (Bruker WH 270 MHz). The purity was checked by thin layer chromatography (Merck) on silicagel plates in the following solvent systems (V/V) : A, chloroform-methanol (9:1) ; B, BuOH-AcOH-H_2O (4:1:1) ; C, chloroform-methanol (7:3) and by HPLC at 220 nm on a Waters Apparatus (μ-Bondapak C_{18} column) with CH_3CN/NH_4 AcO buffer (pH 4.2) as solvent. Melting points of crystallized products are reported uncorrected.

The reduction catalyst was palladium oxyde prepared by Engelhard (France). Tritium gas was made by the Commissariat à l'Energie Atomique (France). The automatic gas transfer unit for catalytic tritiation was previously described (10). The catalyst was separated from the reacting solution by filtration over Millipore (Millex F.G., 0.2 μ, USA). The final ^{3}H-peptides were purified by thin-layer chromatography on silicagel (Merck 11845, W-Germany) and by HPLC. ^{3}H-Scannings

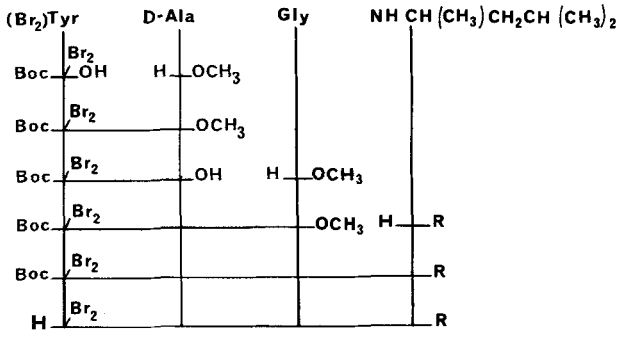
of TLC plates were performed with a Berthols Scanner II (W-Germany). Peptide weight determinations were carried out after acidic hydrolysis with an aminoacids autoanalyser LKB 4400 (U.K.). Radioactive countings(^3H) were determined in a liquid scintillation counter SL 3000 Intertechnique (France). All other chemical and solvents were of analytical grade from Prolabo (France), Merck (W-Germany) or Fluka (Switzerland).

The following abbreviations are used : THF, tetrahydrofuran ; MeOH, methanol ; $CHCl_3$, chloroform ; EtOAc, ethylacetate ; DCC, dicyclohexylcarbodiimide ; HOBt, 1-hydroxybenzotriazole ; TFA, trifluoroacetic acid ; Boc, tert-butyloxycarbonyl and Z, benzyloxycarbonyl.

METHODS

I - Peptide synthesis.

1. Synthesis of (R,S)-3,5-dibromo-L-tyrosyl-D-alanyl-glycine-1,3-dimethyl butyl amide.



N-(tert-butyloxycarbonyl)-3,5-dibromo-L-tyrosyl-D-alanine methyl ester.

To a solution of Boc-3,5-dibromo-L-tyrosine (1.63 g, 3.5 mmol) in anhydrous THF (30 ml), cooled at 0°C, were added successively a solution of D-alanine methyl ester hydrochloride (0.49 g, 3.5 mmol) and triethylamine (0.49 ml) in $CHCl_3$ (30 ml), a solution of HOBt (0.540 g, 3.5 mmol) in THF (15 ml), a solution of DCC (0.72 g, 3.5 mmol) in $CHCl_3$ (15 ml). After 1 h, the mixture was allowed to come to room temperature and stirred overnight. After removal of dicyclohexylurea

and evaporation of solvents in vacuo, the residue was dissolved in EtOAc (40 ml) ans washed successively with a satured solution of NaCl (20 ml), a 10% solution of citric acid (3 x 20 ml), water (20 ml), a 10% solution of $NaHCO_3$ (3 x 20 ml) and finally, with a satured solution of NaCl (20 ml). The solvent was dried on Na_2SO_4 and evaporated in vacuo. This procedure is designated as standard treatment. The protected dipeptide was obtained as a white solide : yield 1.80 g (97%) ; TLC Rf(A) = 0.57.

N-(tert-butyloxycarbonyl)-3,5-dibromo-L-tyrosyl-D-alanine.

To a solution of the preceding compound (1.64 g, 3.1 mmol) in MeOH (10 ml) cooled at 0°C, 7 ml of 1 N NaOH was added. The mixture was stirred at 0°C for 1 h and at room temperature for 2 h. The solution was concentrated in vacuo, diluted with 10 ml of water, filtered and acidified to pH 2 with 1 N HCl. After extraction of the aqueous solution by EtOAc, the organic layer was dried and evapored in vacuo. This treatment is designated as "standard procedure for alkaline hydrolysis". The white solid was recrystallized from EtOAc, yielding 1.45 g (90%) of the pure protected dipeptide, m.p. 248-250°C ; TLC Rf(B) = 0.87.

N-(tert-butyloxycarbonyl)-3,5-dibromo-L-tyrosyl-D-alanylglycyl methyl ester.

To a solution of Boc-3,5-dibromo-L-Tyr-D-Ala (1.02 g, 2 mmol) in anhydrous THF (10 ml), cooled in an ice-water bath, were added successively a mixture of glycine methyl ester hydrochloride (0.25 g, 2 mmol) and triethylamine (0.28 ml) in $CHCl_3$ (10 ml) a solution of HOBt (0.31 g, 2 mmol) in THF (5 ml) and a solution of DCC (0.41 g, 2 mmol) in $CHCl_3$ (5 ml). After 1 h at 0°C, the mixture was allowed to come to room temperature and stirred for 18 h. The reaction was worked up follwing the standard treatment and yielded a white solide ; 0.98 g (84%) ; m.p. 160-161°C ; TLC Rf(A) = 0.38.

(R,S)-N-(tert-butyloxycarbonyl)-3,5-dibromo-L-tyrosyl-D-alanyl-glycine-1,3-dimethylbutyl amide.

To a solution of the preceding compound (0.58 g, 1 mmol) in MeOH (5 ml) was

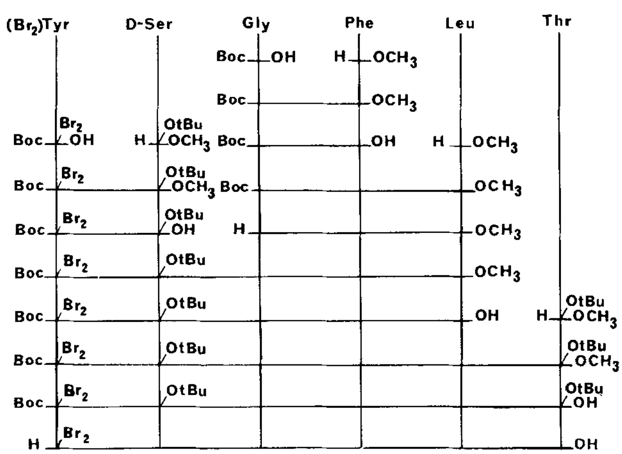
added 7 ml of (R,S)-1,3-dimethylbutyl amine . The mixture was stirred at room temperature for 10 days. After removal in vacuo of both the solvent and the excess of amine, the residue was triturated with ether until a white solid was obtained. This was collected, washed with ether, and dried. The product weighed 0.65 g (100%) ; TLC Rf(A) = 0.21.

(R,S)-3,5-dibromo-L-tyrosyl-D-alanylglycine, 1-3-dimethylbutyl amide trifluoroacetate.

The preceding compound (0.19 g, 0.3 mmol) was dissolved in TFA (0.5 ml) at 0°C. After 30 min, the mixture was allowed to come to room temperature and stirred for 30 min. The addition of ether (60 ml) led to the precipitation of the crude compound. The white solid was washed with ether (5 x 80 ml) and dried in vacuo ; yield 0.195 g (98%). The purity of the compound was checked both by TL. (single spot ; Rf(B) = 0.57) and by HPLC on a Waters apparatus (reversed phase μ-Bondapak C_{18}) ; solvent CH_3CN/NH_4AcO buffer pH 4.2, 45/55, flow rate 1.2 ml/min, retention time of the single peak, 1090 s).

NMR (Me_2SO-d_6) , δ : 3.95 ($TyrH_\alpha$), 2.83 ($CH_2\beta$), 7.40 (Ar), 4.37 ($AlaH_\alpha$), 1.08 (CH_3), 3.62 ($GlyCH_2$) ; chain protons, δ: 3.75 (CH-NH), 1.22 (CH_2), 1.53 (CH-isopropyl), 0.95 (CH_3-CH), 0.78 (CH_3-isopropyl).

2. <u>Synthesis of 3,5-dibromo-L-tyrosyl-D-serylglycyl-L-phenylalanyl-L-leucyl-L-threonine.</u>



N-(tert-butyloxycarbonyl)-3,5-dibromo-L-tyrosyl-O-tert-butyl-D-serine methyl ester.

To a solution of N-(tert-butyloxycarbonyl)-3,5 dibromotyrosine (1.10 g, 2.5 mmol) in THF (15 ml), cooled at 0°C, were added successively a solution of O-tert-butyl-D-serine methyl ester hydrochloride (0.53 g, 2.5 mmol) and triethylamine (0.35 ml) in $CHCl_3$ (15 ml), a solution of HOBt (0.38 g, 2.5 mmol) in THF (10 ml) and a solution of DCC (0.52 g, 2.5 mmol) in $CHCl_3$ (10 ml). After 1 h at 0°C, the mixture was allowed to come to room temperature and then stirred overnight. The reaction was treated as usual, and a white solid was obtained : yield 1.41 g (95%), TLC Rf(A) = 0.82.

N-(tert-butyloxycarbonyl)-3,5 - dibromo-L-tyrosyl-O-tert-butyl-D-serine.

To a solution of the preceding compound (1.28 g, 2.2 mmol) in MeOH (5 ml) was added 4.4 ml of 1 N NaOH at 0°C. The reaction mixture was stirred at 0°C for 1 h and then at room temperature for 6 h. The reaction was treated following the standard procedure for alkaline hydrolysis and produced a white solid : yield 1.10 g (85 %), TLC Rf(C) = 0.53.

N-(tert-butyloxycarbonyl)-3,5-dibromo-L-tyrosyl-O-tert-butyl-D-serylglycyl-L-phenylalanyl-L-leucine methyl ester.

To a solution of the preceding compound (0.93 g, 1.6 mmol) in anhydrous THF (10 ml), cooled in an ice water bath, were added successively a solution of a precedently described compound (8) : glycyl-L-phenylalanyl-L-leucine methyl ester trifluoroacetate (0.74 g, 1.6 mmol) and triethylamine (0.25 ml) in $CHCl_3$ (10 ml) a solution of HOBt (0.25 g, 1.16 mmol) in THF (5 ml) and a solution of DCC (0.33 g, 1.6 mmol) in $CHCl_3$ (5 ml). After 1 h at 0°C, the mixture was allowed to come to room temperature and then stirred overnight. The reaction was treated following the standard procedure and a white solid was obtained ; yield 1.23 g (85%) TLC Rf(A) = 0.65.

N-(tert-butyloxycarbonyl)-3,5-dibromo-L-tyrosyl-O-tert-butyl-D-serylglycyl-L-phenylalanyl-L-leucine.

To a solution of the preceding compound (1.20 g, 1.30 mmol) in MeOH (10 ml) cooled in an ice-water bath was added 2.6 ml of 1 N NaOH. The mixture was stirred at 0°C for 1 h and then at room temperature for 20 h. Then the reaction was treated following the standard procedure for alkaline hydrolysis and the product was obtained as a white solid ; yield 0.95 g (80%) ; TLC Rf(C) = 0.62.

N-(tert-butyloxycarbonyl)-3,5-dibromo-L-tyrosyl-O-tert-butyl-D-serylglycyl-L-phenylalnyl-L-leucyl-O-tert-butyl-L-threonine methyl ester.

To a solution of N-(tert-butyloxycarbonyl)-3,5-dibromo-L-tyrosyl-O-tert-butyl-D-serylglycyl-L-phenylalanyl-L-leucine (0.90 g, 1 mmol) in THF (5 ml) cooled in a ice-water bath, were added successively a solution of O-tert-butyl-L-threonine methyl ester hydrochloride (0.23 g, 1 mmol) and triethylamine (0.14 ml) in $CHCl_3$ (5 ml), a solution of HOBt (0.15 g, 1 mmol) in THF (5 ml) and a solution of DCC (0.21 g, 1 mmol) in $CHCl_3$ (5 ml). After 1 h at 0°C, the mixture was allowed to come to room temperature and then stirred overnight. The reaction was treated following the standard procedure and a white solid (0.93 g, 87 %) was obtained, which showed a single spot on TLC Rf(A) = 0.65.

N-(tert-butyloxycarbonyl)-3,5-dibromo-L-tyrosyl-O-tert-butyl-D-serylglycyl-L-phenylalanyl-L-leucyl-O-tert-butyl-L-threonine.

To a solution of the preceding compound (0.80 g, 0.75 mmol) in MeOH cooled at 0°C was added 1.6 ml of 1 N NaOH. The mixture was stirred at 0°C for 30 min and then at room temperature for 24 h. Then an excess of 1 N NaOH (1 ml) was added and stirring continued for 24 h. The reaction mixture was treated following the standard procedure for alkaline hydrolysis and yielded the N,O-protected hexapeptide ; 0.5 g (63%) ; TLC Rf(C) = 0.66.

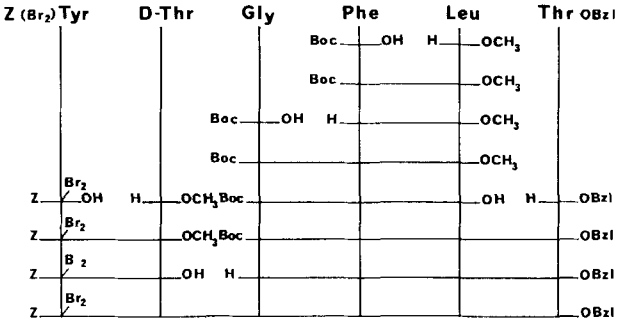
3,5-dibromo-L-tyrosyl-D-serylglycyl-L-phenylalanyl-L-leucyl-L-threonine.

The preceding compound (0.5 g, 0.47 mmol) was dissolved at 0°C in TFA (1.5

(1.5 ml). The reaction mixture was stirred at 0°C during 30 min and then at room temperature overnight. Ether (50 ml) was added to the well-stirred mixture. The precipitate was collected and washed with ether (5 x 50 ml). The crude product was purified by gel filtration on LH 20 (Pharmacia) with MeOH as eluent. Fractions containing the pure hexapeptide were evaporated in vacuo and lyophilized to yield 0.25 g (50%). The purity of the dibromo hexapeptide was checked both by TLC (single spot, Rf(B) = 0.55) and by HPLC on a Waters apparatus (reversed phase μ-Bondapak C_{18} ; solvent CH_3CN/NH_4AcO, buffer pH 4.2, 25/75, flow rate 1.2 ml/min, retention time of the sigle peak 720 s).

NMR (Me_2SO-d_6), δ : 3.90 ($TyrH_\alpha$), 2.65 and 2.94 (H_β), 7.41 (ArH), 4.25 ($SerH_\alpha$), 3.45 and 3.50 (H_β), 3.65 ($GlyH_\alpha$), 4.45 ($PheH_\alpha$), 2.95 and 2.70 (H_β), 7.20 (ArH), 4.25 ($LeuH_\alpha$), 1.45 (H_β and H_γ), 0.80 (CH_3), 4.04 ($ThrH_\alpha$), 4.04 (H_β), 0.98 (CH_3).

3. Synthesis of N-(benzyloxycarbonyl)-3,5-dibromo-L-tyrosyl-D-threonylglycyl-L-phenylalanyl-L-leucyl-L-threonine benzyl ester.



N-(benzyloxycarbonyl)-3,5-dibromo-L-tyrosine.

To a solution of 3,5 dibromo tyrosine (2 g, 6 mmol) in 2 N NaOH (6 ml), cooled at 0°C, was added slowly and simultaneously 1.5 ml of 4 N NaOH and benzylchloroformiate (1.02 g, 6 mmol). The reaction mixture is stirred at 0°C for 15 min, washed with ether and acidified by 12 N HCl to pH 1. The white precipitate is collected, washed with cold-water and dried ; yield 2.78 g (98%) ; TLC Rf(B) = 0.93.

N-(benzyloxycarbonyl)-3,5-dibromo-L-tyrosyl-D-threonine benzyl ester.

To a solution of Z-dibromo-L-tyrosine (0.47 g, 1 mmol) in THF (10 ml), cooled at 0°C, were added successively a solution of D-threonine benzyl ester hemioxalate (0.30 g, 1 mmol) and triethylamine (0.14 ml) in $CHCl_3$ (10 ml), a solution of HOBt (0.15 g, 1 mmol) in THF (5 ml) and a solution of DCC (0.20 g, 1 mmol) in $CHCl_3$ (5 ml). After 1 h at 0°C, the mixture was allowed to come to room temperature and then stirred overnight. The reaction was treated as usual, and a white solid was obtained ; yield 0.53 g (80%) ; TLC Rf(A) = 0.56.

N-(benzyloxycarbonyl)-3,5-dibromo-L-tyrosyl-D-threonine.

To a solution of the preceding compound (0.53 g, 0.8 mmol) in MeOH (5 ml) was added 2 ml of 1 N NaOH at 0°C and the reaction mixture was stirred at 0°C for 30 min and then at room temperature for 2 h. The reaction was treated following the standard procedure for alkaline hydrolysis and produced a white solid ; yield 0.37 g (80%) ; TLC Rf(A) = 0.38.

N-(tert-butyloxycarbonyl)-glycyl-L-phenylalanyl-L-leucine.

To a solution of N-(tert-butyloxycarbonyl)-glycyl-L-phenylalanyl-L-leucine methyl ester (8) (2.1 g, 4.6 mmol) in MeOH (15 ml) at 0°C was added 4.6 ml of 1 N NaOH. The mixture was stirred at 0°C for 1 h and at room temperature for 6 h. Then the reaction was treated following the standard procedure for alkaline hydrolysis. The product was recrystallized from EtOAc ; yield 1.9 g (95%) ; TLC Rf (B) = 0.83. m.p. 158-160°C.

N-(tert-butyloxycarbonyl)-glycyl-L-phenylalanyl-L-leucyl-L-threonine benzyl ester.

To a solution of the preceding compound (1.9 g, 4.4 mmol) in THF (20 ml), cooled at 0°C, were added successively a solution of L-threonine benzyl ester hemioxalate (1.3 g, 4.4 mmol) and triethylamine (0.6 ml) in $CHCl_3$ (20 ml), a solution of HOBT (0.76 g, 4.4 mmol) in THF (10 ml) and a solution of DCC (0.99 g, 4.4 mmol) in $CHCl_3$ (10 ml). After 1 h at 0°C, the mixture was allowed to come to

room temperature and then stirred overnight. The reaction was treated as usual and the pure protected tetrapeptide was obtained ; yield 2.2 g (80%) ; TLC Rf(A) = 0.65 ; m.p. 170-172°C.

Glycyl-L-phenylalanyl-L-leucyl-L-threonine benzyl ester trifluoroacetate.

The preceding compound (2.2 g, 3.5 mmol) was dissolved in TFA (5.3 ml) at 0°C. After 30 min, the mixture was allowed to come to room temperature and stirred for 30 min. The addition of ether (100 ml) led to the precipitation of the crude product which is washed with ether (5 x 100 ml) and dried in vacuo ; yield 1.7 g (75%) ; TLC Rf(B) = 0.41.

N-(benzyloxycarbonyl)-3,5-dibromo-L-tyrosyl-D-threonylglycyl-L-phenylalanyl-L-leucyl-L-threonine benzyl ester.

To a solution of N-(benzyloxycarbonyl)3,5-dibromo-L-tyrosyl-D-threonine (0.29 g, 0.5 mmol) in THF (5 ml), cooled at 0°C, were added successively a solution of glycyl-L-phenylalanyl-L-leucyl-L-threonine benzyl ester trifluoroacetated (0.32 g, 0.5 mmol) and triethylamine (0.07 ml) in $CHCl_3$ (5 ml), a solution of HOBT (0.76 g, 0.5 mmol) in THF (2.5 ml) and a solution of DCC (0.11 g, 0.5 mmol) in $CHCl_3$ (2.5 ml). After 1 h at 0°C, the mixture was allowed to come to room temperature and then stirred overnight. The reaction was treated as usual and the crude product was obtained and purified by "flash" chromatography on Kieselgel 60 with $CHCl_3$/MeOH, 20/1, as eluent. Fractions containing pure diprotected hexapeptide ; yielded 0.30 g (55%). The purity of the compound was checked both by TLC (single spot, Rf(A) = 0.40) and by HPLC (reversed phase μ Bondapak C_{18} ; solvent CH_3CN/NH_4AcO, buffer pH 4.2, 55/45, flow rate 1.2 ml/min, retention time of the single peak : 730 s).

NMR (Me_2SO-d_6), : 4.23 ($TyrH_\alpha$), 7.41 (ArH), 2.50 and 2.79 (H_β), 4.10 (D-$ThrH_\alpha$), 3.83 (H_β), 0.87 (CH_3), 3.75 ($GlyH_\alpha$), 4.44 ($PheH_\alpha$), 7.20 (ArH), 2.63 and 2.90 (H_β), 4.30 ($LeuH_\alpha$), 1.37 (H_β), 1.50 (H_γ), 0.75 (CH_3), 4.32 (L-$ThrH_\alpha$), 4.10 (H_β), 1.00 (CH_3)

II - Tritiation.

According a method described elsewhere (10), Tyr-D-Ala-Gly-1,3-dimethylbutyl-amide (TRIMU 4), Tyr-D-Ser-Gly-Phe-Leu-Thr (DSLET) and Z-Tyr-D-Thr-Gly-Phe-Leu-Thr-OBzl (DTLET) dibromo-derivatives (2 to 3 µmoles) were dissolved in 1 ml of pure MeOH with 1 µl of triethyl amine and solutions were then frozen. The catalyst (20-25 mg of palladium oxyde : 10 times) was then dispersed on the surface and the reacting vial was connected to the automatic tritium gas transfer unit (11). After a vacuum of 10^{-4} Torr was reached, pure tritium gas (80 Curies) was introduced and compressed until 1-1.1 bar and the catalyst was flushed 15 min into the still frozen solution. After thawing, the reaction mixture was kept at ambient temperature under constant magnetic stirring for 3 h. The absorption of tritium gas produced a pressure reduction of about 0.45, 0.8 and 0.8 bar for TRIMU 4, DSLET and DTLET respectively. PdO was easily separated from the reacting mixture by filtration over Millipore and labile tritium atoms eliminated by successive flash rotative evaporations with a large volume of dilute methanol ($MeOH/H_2O$, 50/50, v/v). Total radioactivities recovered were : 93.2 mCi (TRIMU 4), 81 mCi (DSLET) and 29.7 mCi (DTLET). The ^3H enkephalin analogs reaction mixture were analysed using thin layer chromatography (TLC) on silicagel with B.A.W. solvent system : 4/1/1 (v/v/v). The autoradiochromatogram and ^3H-scanning performed on each analog revealed a major peak. Rf = 0.80 (TRIMU 4) , Rf = 0.58 (DSLET) , Rf = 0.33 (DTLET) commigrating with the deshalogenated analog and corresponding to the spot detectable with 4,4'-tetramethyldimino-diphenyl methane (TDM). Then the chromatograms (preparative TLC) were separated in successive strip and the peptide extracted from silicagel powder with 6 x 10 ml of pure methanol. After centrifugation and concentration, the U.V. spectrum of tritiated analogs were found to be exactly the same as that of the original meterial (λ_{max} : 275 nm). The purity and homogenity was also checked by high performance liquid chromatography (HPLC) (µ-Bondapak C_{18} column, 1 ml/min, solvent system A : ammonium acetate (10^{-2}M, pH 4.2) / acetonitrile, 55/45 (v/v) flow rate : 1.2 ml/min ; TRIMU 4 retention time : 7.6 min. Solvent system B : ammonium acetate (10^{-2}M, pH 4.2) / acetonitrile, 75/25 (v/v), flow rate : 1.2 ml/min. DSLET retention

time : 5.2 min ; DTLET retention time : 7.8 min.

After acid hydrolysis of an aliquot (5 mmoles) of the labelled compounds (6 N HCl, 110°, 16-17 h) quantitative and comparative estimations indicated that the specific radioactivities were found to be : 55-60 Ci/mmole (TRIMU 4), 55-60 Ci/mmole (DSLET) and 45-50 Ci/mmol (DTLET).

After sith months of storage in liquid nitrogen, ^{3}H-TRIMU 4, ^{3}H-DSLET and ^{3}H-DTLET retained both their chemical and biological properties.

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