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Synthesis of Enkephalin Analog with Leucinthiol at C-Terminus as Probe for Thiol Group in Opiate Receptors

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Enkephalin analog with thiol group at C-terminus, [D-Ala^2, Leu(CH_2SH)^5]enkephalin, was synthesized as possible probe for the essential thiol group in opiate receptors. In order to protect the free SH group of leucinthiol, oxidized Boc-L-leucinthiol dimer was prepared from L-leucinol (2-amino-4-methyl-1-pentanol) to elongate the enkephalin sequence. SH-Containing monomeric enkephalin analog was obtained from dimer by treatment with zinc in 50% AcOH, and it exhibited a high affinity for mu opiate receptors.

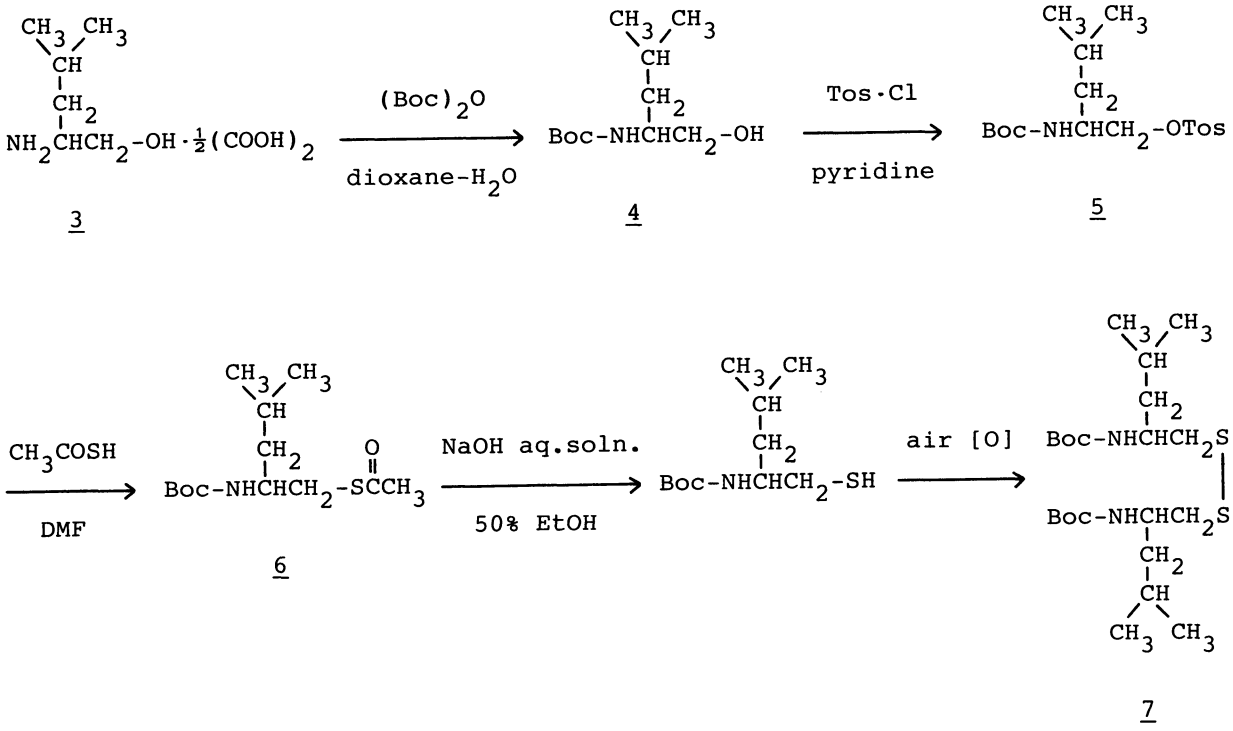
It has recently been suggested that opiate receptors contain the essential thiol (SH) group which may participate in the structural stabilization of receptors and/or their environment required for showing biological activity.[1-4] In order to elucidate a role of such SH group in the molecular mechanism of interaction between opioid ligand and receptor, we synthesized here an enkephalin analog containing leucinthiol[5] at C-terminus, [D-Ala^2, Leu(CH_2SH)^5]-enkephalin (1) which can be expected to interact with opiate receptor through disulfide bond formation or disulfide exchange. As an intermediate of this

H-Tyr-D-Ala-Gly-Phe-Leu(CH_2SH) 1

H-Tyr-D-Ala-Gly-Phe-Leu(CH_2S)
|
H-Tyr-D-Ala-Gly-Phe-Leu(CH_2S) 2

synthesis, we also prepared [D-Ala^2, Leu(CH_2S-)^5]enkephalin dimer (2).[6]

Although Chan[7] reported the synthesis of leucinthiol from N-trifluoroacetyl-L-leucinol, for elongation of peptide sequence toward N-terminal side, we have found the difficulty of deprotection of trifluoroacetyl group because of unstability of thiol group under alkaline conditions. In addition, Chan described no detail experimental data. Thus, as a synthetic strategy of enkephalin analog, we chose t-butoxycarbonyl (Boc) group for N-protection and dimerized the protected leucinthiol by air-oxidation to convert unstable SH group completely into the stable disulfide bond for protection during peptide synthesis. L-Leucinthiol was purified as the oxalate[8] 3 (76%; mp 172-173 °C; $[\alpha]_{589}^{20}$ +9.7° (c 2.0, H_2O)). Boc-leucinol (4) was obtained as an oily material (97%; $[\alpha]_{589}^{20}$ -39.3° (c 2.0, $CHCl_3$)), and 4 was converted to the tosyl derivative (5) in an 80% yield. The compound 5 was treated with thioacetic acid to afford thioester derivative (6) (91%; mp 50-53 °C; $[\alpha]_{589}^{20}$ -44.3°(c 2.0, $CHCl_3$); MS m/e 275 (M^+)). Alkaline hydrolysis of this thioester and the following air-oxidation monitored by 5,5'-dithio-bis(2-nitrobenzoic acid)[9] gave the protected leucinthiol dimer (7) (83%; mp 134-135 °C; $[\alpha]_{589}^{20}$ -68.7° (c 2.0, $CHCl_3$); MS m/e 465 (M^+)).



3 4 5 6 7

Fig. 1. Synthesis of Boc-leucinthiol dimer (7).

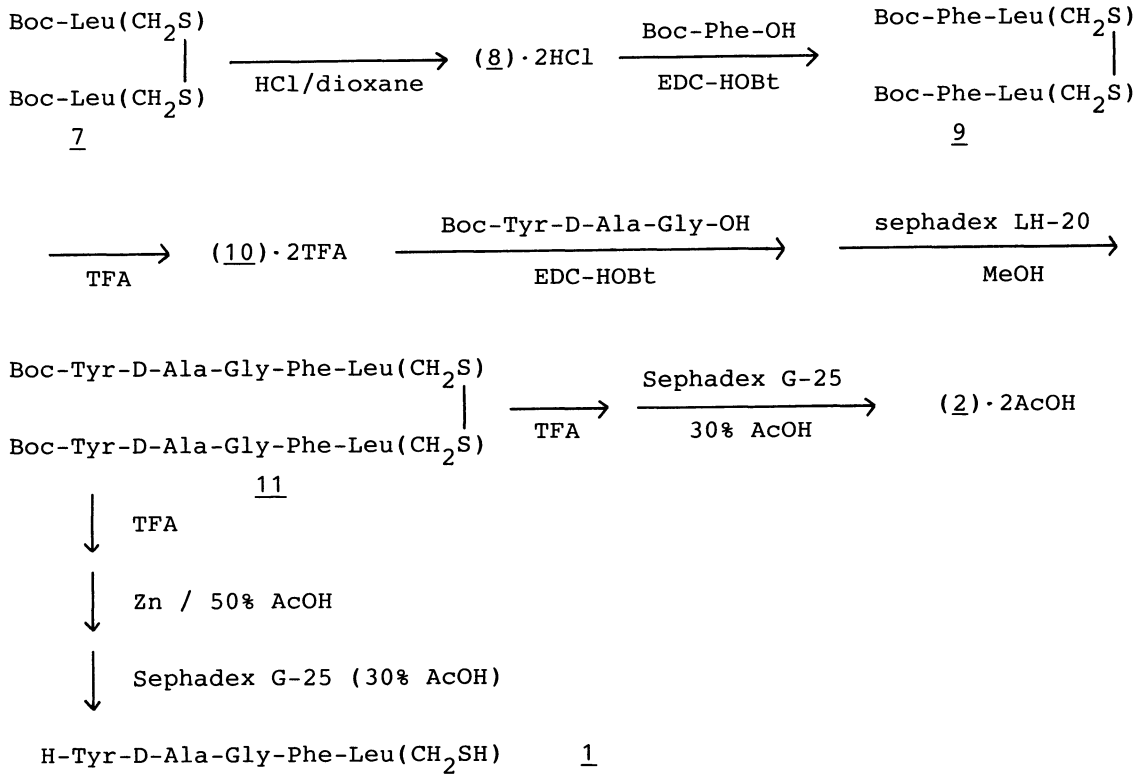


Fig. 2. Synthesis of [D-Ala^2, Leu(CH$_2$SH)^5]enkephalin (1) and [D-Ala^2, Leu(CH$_2$S-)^5]enkephalin dimer (2).

The Boc group of 7 was removed by HCl/dioxane, and the resulting salt (8) was coupled with Boc-Phe-OH by utilizing 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide and 1-hydroxybenzotriazole (EDC-HOBt) method to give protected dipeptide dimer (9) (88%; mp 197-198 °C; $[\alpha]_{589}^{20}$ -43.2° (c 2.0, $CHCl_3$)). After deprotection of 9 with trifluoroacetic acid, compound 10 was elongated with Boc-Tyr-D-Ala-Gly-OH using EDC-HOBt method to afford Boc-pentapeptide dimer (11) (80%; mp 137-139 °C; $[\alpha]_{589}^{20}$ +31.5° (c 1.0, MeOH); MW 1340 by Hitachi Osmometer Type 117 with MeOH, calcd 1342). The dimeric enkephalin analog (2) was obtained by deprotection of 11 and then purified by gel filtration (Sephadex G-25) to lyophilize (85%; mp 146-150 °C; $[\alpha]_{589}^{20}$ +81.2° (c 2.0, 50% AcOH)). In order to obtain the desired monomeric analog, reduction of dimer (2) was performed by Zn-dust in 50% AcOH. Purification was carried out twice by gel filtration and lyophilization of eluate containing 1 afforded pure material of leucinthiol^5-enkephalin (1) (76%; mp 215-220 °C (decomp.); $[\alpha]_{589}^{20}$ +16.2° (c 1.0, MeOH)). The homogeneity of each intermediate was confirmed by thin-layer chromatography,

elemental analysis, and NMR. In addition, the final products 1 and 2 were examined for their purity by using paper electrophoresis and reverse-phased high performance liquid chromatography. Amino acid analyses of their acid hydrolyzates gave the satisfactory results.

In the radio-ligand binding assay using rat brain membrane preparations, the monomer (1) and its dimeric analog (2) showed a considerably high receptor binding affinity for both delta and mu receptors. Especially for mu receptors, the monomer exhibited almost the same affinity level as usual enkephalins. Thus, in order to examine a possibility of leucinthiol[5]-enkephalin to interact with an essential SH group in the opiate receptors, detailed biological assays using the isolated smooth muscles are in progress.

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10) Abbreviations according to biochemical nomenclature by IUPAC-IUB Joint Commission, Eur. J. Biochem., 138, 9 (1984), are used throughout.

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