# SYNTHESIS AND STUDY OF OPIOID ACTIVITY OF MODIFIED ANALOGUES OF DAla$^2$, DLeu$^2$-ENKEPHALIN

M. M. Sobirov, Sh. Kh. Khalikov, S. V. Zaitsev, S. S. Saidov, and S. D. Varfolomeev

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DAla$^2$, DLeu$^5$-enkephalin (DADLE), is a widely used highly specific δ-ligand of opioid receptors that is resistant to the action of proteases [1].

In order to study the laws of structural-functional relationships, we have synthesized the four new analogues of DAla$^2$, DLeu$^5$-enkephalin (II-V) the formulas of which were given in Table 1.

The new analogues differ from the initial DADLE by the fact that chains have been added to the C-terminal amino ester of this peptide consisting of glycine and ethylene diamine residues of the type of —Gly → Gly → NH—$CH_2$—$CH_2$—NH ← Gly—. The addition of such a fragment to the enkephalin molecule leads to an enhancement of the basic properties of the desired peptide (II). The further modification of the peptide was carried out in the direction of decreased basicity ((III) and (IV)).

Peptides (II-V) were synthesized by the stepwise growth of the peptide chain in solution using activated esters. The use of a combination of selectivity eliminable benzyloxycarbonyl (Z—) and t-butoxycarbonyl (Boc-) protective groups permitted the growth of the peptide chain from either end. The final products were purified with the aid of HPLC on an Ultrasphere-ODS semipreparative column (10 mm × 25 cm) with a gradient of methanol in 1% aqueous $CF_3COOH$ solution.

The new analogues of DAla$^2$, DLeu$^5$-enkephalin that had been synthesized were investigated for opioid activity by the radioreceptor method of analysis on lyophilized rat brain membrane [2]. [$^3$H]Tyr-DAla-Gly-MePhe-Gly-ol and [$^3$H]-DADLE were used as the radioactive μ and δ-ligands, respectively. Opioid activity was determined from the inhibition of the binding of radioactive ligands by 50% ($IC_{50}$).

With the exception of compound (IV), all the peptides exhibited opioid activity. As can be seen from the figures given in Table 1, modification of the molecule of initial peptide (DADLE) led to some lowering of the affinity for the binding centers of the opioid receptors in all cases (II-V). It must be mentioned that in all the cases given there was a reversal of the selectivity that is characteristic of DADLE. The new active analogues ((II), (III), and (V)) exhibited μ-selectivity. The Results obtained create the prerequisites for the further study of structure-activity relationships among the enkephalins.

TABLE 1. Opioid Activities of Modified DAla$^2$, DLeu$^5$-Enkephalin

| Peptide | Formula | $IC_{50}$, nM | |
|---|---|---|---|
| | | μ | δ |
| I | Tyr-DAla-Gly-Phe-DLeu (DADLE) | 18 | 3,5 |
| II | Tyr→DAla→Gly→Phe→DLeu→Gly→Gly→NH-$(CH_2)_2$-NH←Gly | 34 | 100 |
| III | Tyr→DAla→Gly→Phe→DLeu→Gly→Gly→NH-$(CH_2)_2$-NH←Gly-NH-$COCH_3$ | 150 | 450 |
| IV | Tyr→DAla→Gly→Phe→DLeu→Gly→Gly→NH-$(CH_2)_2$-NH←Gly-NH-CO-$(CH_2)_2$-COOH | 10000 | 10000 |
| V | Tyr→DAla→Gly→Phe→DLeu→Gly→Gly→NH-$(CH_2)_2$-NH←Gly←DLeu←Phe←Gly←DAla←Tyr | 47 | 84 |

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# USE OF VARIOUS MODIFYING AGENTS FOR INTRODUCTION OF AN ISOTOPIC LABEL INTO CELLULOSE

N. I. Parshina, Sh. K. Kasymov, O. N. Veshkurova, A. A. Takanaev, and Sh. I. Salikhov

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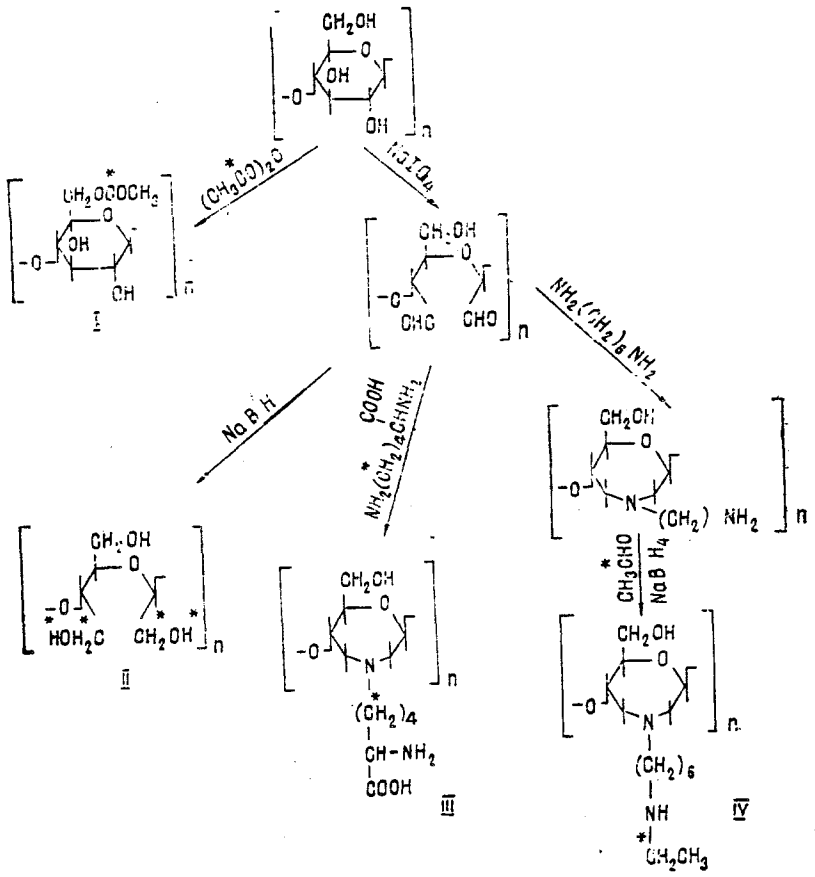
The introduction into the cellulose molecule of a number of radioactive modifying agents makes it possible to investigate various aspects of its interaction with biospecific systems of the organism. [1-$^{14}$C]Acetic anhydride, [1-$^{14}$C]acetaldehyde, [2-$^{14}$C]lysine, and sodium [$^{3}$H]tetrahydroborate have been used as labeled modifying agents. A 1% cellulose [sic] from cotton lint was used in the synthesis. The reaction was performed by the following scheme:



Cellulose [$^{14}$C]acetate was obtained by the acetylation with [1-$^{14}$C]acetic anhydride in the presence of catalytic amounts of zinc chloride of 20 ml of a suspension of cellulose from cotton lint with a molecular mass of 50,000-70,000 Da. The specific radioactivity of the product obtained was 600-800 MBq/g, which corresponds to 14% of modified groups in the cellulose suspension.

To obtain compounds (II), (III), and (IV), 15, 25, and 20 ml, respectively, of a 1% suspension of cellulose previously activated with a 0.2 M solution of sodium periodate for 1 h were taken.

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