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Dimeric Dermorphin Analogues Containing β³-*homo*-Amino Acids: Synthesis, Binding Affinities and Metabolic Stability

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Introduction

Opioids are widely used for the treatment of moderate to severe chronic pain, although they exhibit a large number of side effects. There are three major types of opioid receptors: μ (MOR), δ (DOR), and κ (KOR). The main target for analgesia is the MOR. Opioid receptors can form homo- and heterodimers. Specially designed ligands capable of penetrating the BBB are used to study the physiological consequences of opioid receptor homo- and heterodimerization, and are also applied as new analgesics.

Dermorphin (Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2) is a heptapeptide first isolated from the skin of South American frogs of the genus *Phyllomedusa* [1]. The peptide is a natural opioid that binds as an agonist to the μ opioid receptor with high potency and selectivity. Dermorphin is about 30-40 times more potent than morphine but is theoretically less likely to produce drug tolerance and addiction, due to its high potency [2].

Dimeric dermorphin analogues linked by a bridge of varying length were described by Lazarus, et al. [3]. Several dimeric dermorphin pentapeptides connected "tail-to-tail" by a hydrazine bridge exhibit higher μ-affinities than either dermorphin or DAGO. The most active in this series is (Tyr-D-Ala-Phe-Gly-Tyr-$NH)_2$.

In the present study, we designed and synthesized a new dimeric dermorphin pentapeptides containing β³-*homo*-amino acids. The synthesis of α/β hybrid peptides, containing homologated proteinogenic amino acids is a useful tool in drug design. Various β³-*homo*-amino acids have been used in the design of several classes of ligands, including opioid peptides [4]. The incorporation of β³-*homo*-phenylalanine or its analogues at the 4,4' positions of biphalin resulted in good opioid receptor affinities and antinociceptive activity [5].

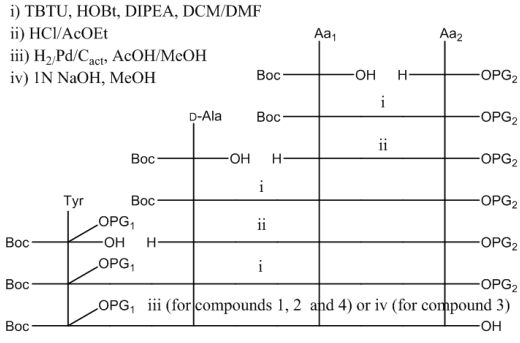


Fig. 1. Synthesis of N,O-protected tetrapeptides.

Results and Discussion

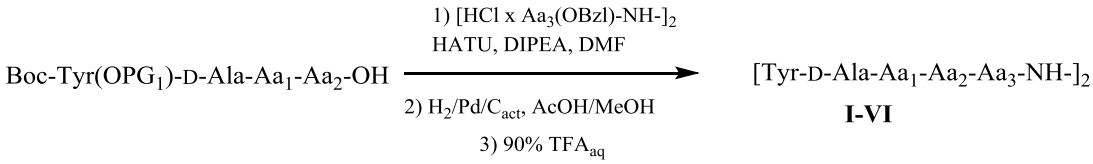
Enantiomerically pure, protected β³-*homo*-amino acids were synthesized from protected proteinogenic α-amino acids in two-step Arndt–Eistert homologation. Dimeric dermorphin analogs **I-V** were synthesized using a convergent synthetic strategy (Figure 2). The tetrapeptide precursors were obtained by stepwise elongation starting from glycine methyl or benzyl ester (Figure 1). *N*-Protected bridged dimers were synthesized by one-pot cross-coupling reaction of hydrazine, Boc-α- or β³-*homo*-Tyr(Bzl) and TBTU/HOBt/DIPEA in DMF. Cross-coupling reactions were conducted between *N*-unprotected bridged dimers and appropriate tetrapeptides to yield the corresponding *N,O*-protected dimeric dermorphin analogs. After deprotection, the crude peptides were purified on a preparative RP-HPLC column. The purity of the final TFA salts of analogs **I-V** was assessed by analytical RP-HPLC and FAB-MS.

Table 1. Biological activity of dimeric dermorphin analogues.

	Peptide	IC50 (nM)		δ/μ
		μ vs DAMGO	δ vs DELT	
	Dermorphin	1.22±0.13 [3]	178.6±18 [3]	146.4
I	(Tyr-D-Ala-Phe-Gly-Tyr-NH)$_2$	0.243±0.005 0.27±0.04 [3]	8.754±0.26 7.88±0.07 [3]	36.0 29.2
II	(Tyr-D-Ala-β^3-*h*-Phe-Gly-Tyr-NH)$_2$	18.20 ± 0.43	203.7 ± 9.4	11.2
III	(Tyr-D-Ala-Phe-β^3-*h*-Gly-Tyr-NH)$_2$	3.543 ± 0.169	85.34 ± 1.83	24.1
IV	(Tyr-D-Ala-Phe-Gly-β^3-*h*-Tyr-NH)$_2$	0.302 ± 0.006	20.55 ± 1.22	68.0
V	(Tyr-D-Ala-β^3-*h*-Phe-Gly-β^3-*h*-Tyr-NH)$_2$	11.2 ± 0.68	>10000	-
VI	(Tyr-D-Ala-Phe-β^3-*h*-Gly-β^3-*h*-Tyr-NH)$_2$	40.7 ± 2.9	645 ± 45.8	15.8

The new dermorphin analogues were tested for selectivity and affinity to μ- and δ-opioid receptors. Binding affinities to μ- and δ-opioid receptors were determined by displacing [^{3}H]-DAMGO and [^{3}H]-DELT in standard displacement tests on receptors in rat brain homogenates. The results are shown in Table 1. Peptides **I** and **IV** are the most active and show higher affinity for MOR and DOR than dermorphin. The introduction of two additional methylene groups at positions 5,5' is well tolerated. On the other hand, the presence of four additional methylene groups (**V**, **VI**) leads to a mild reduction of affinity to MOR and a significant reduction of affinity for DOR.

The stability of peptides is important if a drug candidate is to be considered for therapeutic use. Compounds **I** and **IV**, the two most active peptides in the series, were selected for a study of their stability in human serum (Figure 3). The activity of the serum was tested on endomorphin-2. Z-Val was used as an internal standard. EM-2 underwent degradation in just 20 minutes. Stability studies of peptides **I** and **IV** were done in triplicate to ascertain accuracy. Both analogues are highly stable in human serum, but the peptide containing β^3-*h*-Tyr at positions 5,5`(**IV**) is extremely stable over 96 h.



I Aa_1=Phe, Aa_2=Gly, Aa_3=Tyr
II Aa_1=β^3-*homo*-Phe, Aa_2=Gly, Aa_3=Tyr
III Aa_1=Phe, Aa_2=β^3-*homo*-Gly, Aa_3=Tyr
IV Aa_1=Phe, Aa_2=Gly, Aa_3=β^3-*homo*-Tyr
V Aa_1=β^3-*homo*-Phe, Aa_2=Gly, Aa_3=β^3-*homo*-Tyr
VI Aa_1=Phe, Aa_2=β^3-*homo*-Gly, Aa_3=β^3-*homo*-Tyr

Fig. 2. Synthesis of dimeric dermorphin analogs.

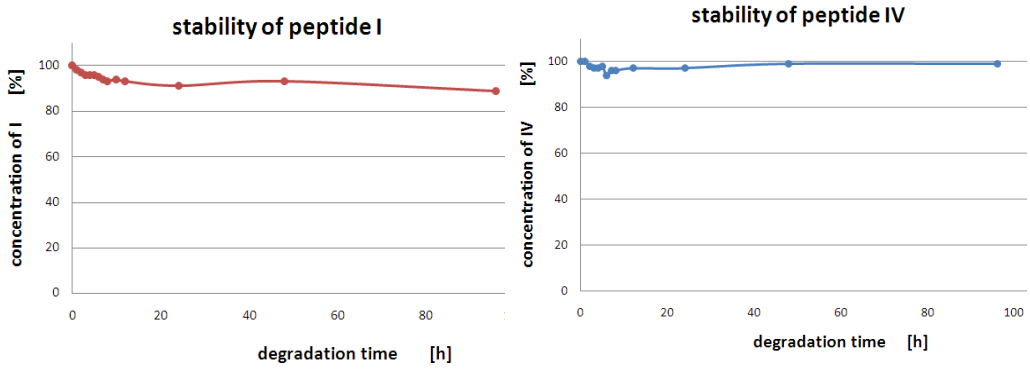


Fig. 3. The stability of peptide I and IV in human serum.

Acknowledgments

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