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# Synthesis and binding properties of deltorphin I analogues containing (*R*) and (*S*)-$\alpha$-hydroxymethylnaphtylalanine★✪

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**New analogues of deltorphin I (DT I), in which the phenylalanine residue in position 3 is substituted with amphiphilic $\alpha$,$\alpha$-disubstituted amino acid enantiomers, (*R*) and (*S*)-$\alpha$-hydroxymethylnaphtylalanine, were synthesized and tested for $\mu$ and $\delta$ opioid receptor affinity and selectivity. Although both analogues have lower affinity to $\delta$ receptors than DT I, they both expressed specificity to $\delta$ receptors.**

Deltorphins are constructed from two major parts. First, the N-terminal tripeptide is responsible for high opioid agonist properties of deltorphins. This part itself possesses a strong selectivity for $\mu$ opioid receptors. The C-terminal fragments play a dual function, increasing the affinity to $\delta$ receptors, and decreasing affinity to $\mu$ receptors. As a result, very selective $\delta$ opioid ligands are formed. It has been proposed that the selectivity of deltorphins is a result of a special amphiphilic topography ("hot-dog" shape) [1]. In such a conformation, the hydrophilic strain formed by $NH_2$(Tyr$^1$)...COOH(Asp$^4$)...$CONH_2$(Gly$^7$) is on the top of dominating liphophilic shells. Changing the chirality of Phe$^3$ resulted in

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**Abbreviations:** Boc, *tert*-butyloxycarbonyl; DIEA, diisopropylethylamine; DT I, Tyr-D-Ala-Phe-Asp-Val-Val-Gly-$NH_2$; HOBt, 1-hydroxybenzotriazole; HmNal, $\alpha$-hydroxymethylnaphtylalanine; TBTU, *O*-(benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate; TFA, trifluoroacetic acid.

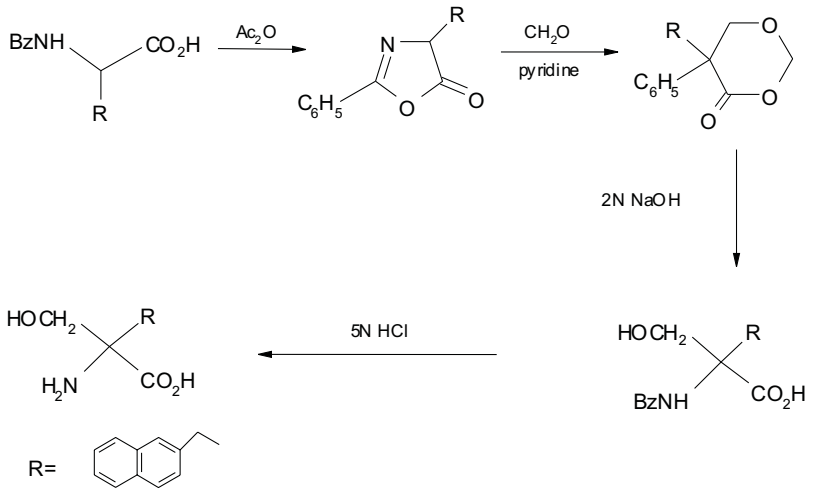
some reduction of receptor affinity but with retained receptor selectivity for $\delta$ receptors. This may suggests that the analogue may modify conformation(s) to adopt changes of chirality in position 3 without significant changes in the overall topography of the molecule. Introducing in this position an amphiphilic amino acid related to D-Phe resulted in the analogue [(*S*)HmPhe$^3$]DT I, with high receptor affinity and exceptional selectivity for $\delta$ type receptor [2]. In the proposed model of "hot-dog" conformation of deltorphin the additional hydrophilic hydroxymethyl group may interact with other elements of the hydrophilic strain and thus stabilise the active conformation.

We now report the synthesis and binding properties of two analogues of deltorphin I, in which phenylalanine residue in position 3 was replaced with (*S*) or (*R*)-$\alpha$-hydroxymethylnaphtylalanine.

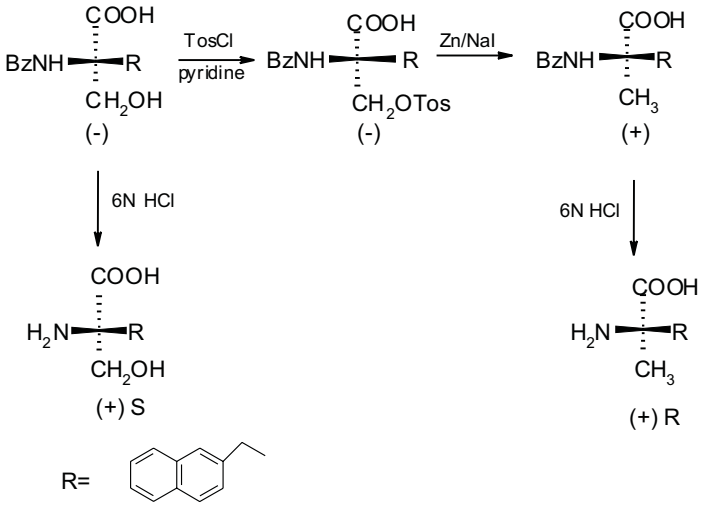
## MATERIALS AND METHODS

***Amino acids synthesis: (R)- or (S)-N$^\alpha$(tert-butyloxycarbonyl)-$\alpha$-hydroxy methylnaphtylalanine.*** (*R*)- and (*S*)-$\alpha$-hydroxymethylnaphtylalanine were obtained from *N*-benzoylnaphtylalanine by selective hydroxymethylation (Scheme 1) [3, 4] and resolved by fractional crystallization of their diastereomeric salts with (–)-quinine. The absolute configuration of laevorotatory $\alpha$-hydroxymethylnaphtylalanine was elucidated, using method previously described [5] (Scheme 2), to be (*S*) by the chemical correlation with (+)-$\alpha$-methylnaphtylalanine, for which the (*R*)-configuration was established by Schöllkopf *et al* [6]. The Boc protecting group was introduced using *tert*-butyl pyrocarbonate (Fluka) as described elsewhere [6].

***Peptide synthesis: [(R)HmNal$^3$]DT I and [(S)HmNal$^3$] DT I.*** These compounds were synthesized by the manual solid-phase method using a 4-methylbenzhydrylamine resin × HCl (100–200 mesh, 0.8 mmole/g, Novabiochem). Standard N$^\alpha$-Boc-protected amino acids were obtained from commercial sources. Starting with 0.25 g (0.2 mmole) of resin the protected amino acids were added in a stepwise fashion to the growing peptide chain. Amino acids were coupled in a 3-fold excess using TBTU (3 equiv.) and HOBt (3 equiv.) in the presence of DIEA (6 equiv.). For 'difficult' coupling, when HmNal was acylated or used as an acylating component, prolonged reaction times (8 and 16 h for repeated coupling) were necessary. Removal of the Boc protecting group was performed with 50% (v/v) TFA in $CH_2Cl_2$ for 5 and 20 min, followed by $CH_2Cl_2$ washes (3 × 1 min), neutralization with 10% DIEA in $CH_2Cl_2$ for 5 and 10 min and $CH_2Cl_2$ washes (3 × 1 min). Completion of coupling reactions was monitored by the ninhydrin test [7]. The peptides were cleaved from the resin with anhydrous HF (5 ml/g resin) with anisole added as scavenger (1




**Scheme 1.** **$\alpha$-Hydroxymetylation of naphtylalanine.**




**Scheme 2. Chemical correlation of absolute configuration.**

ml/g resin) for 1 h at 0°C. After evaporation of the HF, the resin was extracted three times with anhydrous ethyl ether and subsequently with a 50% aqueous solution of acetic acid. The crude peptides were obtained in solid form by lyophilization of the acetic acid extracts and were purified by preparative reversed-phase HPLC on a Vydac $C_{18}$ column (25 × 2.2 cm) with a linear 20–50% gradient of B at a flow rate of 12 ml/min. Each peptide was > 98% pure as determined by analytical reversed-phase HPLC on a Vydac $C_{18}$ column (25 × 0.46 cm) using a linear gradient of 20–60% B in 25 min at a flow rate of 1 ml/min, with UV detection at 220 nm. Solvents: (A) 0.05% trifluoroacetic acid in water and (B) 0.038% trifluoroacetic acid in acetonitrile/$H_2O$ (90 : 10, v/v). Relative molecular masses were confirmed by FAB-MS (Table 1).

bed [1], but with using [$^3$H]naltrexon or [$^3$H]deltorphin I as radioligands.

## RESULTS AND DISCUSSION

Topographical relationships of lipophilic, aromatic and alkyl residues and hydrophilic groups (amines and hydroxyls) are the most important features of the structure of bioactive peptides, including opioids. The analogue of the opioid peptide deltorphin I with Phe$^3$ replaced with the amphiphilic HmPhe showed both an increased receptor affinity and selectivity to $\delta$ receptors. The analogue in which the phenylalanine residue in position 3 was replaced with a bicyclo aromatic ring (naphtylalanine) retained the affinity to $\mu$ opioid receptors but lost by almost hun-

**Table 1. Analytical parameters of DT I analogues**

| Compound | HPLC[a] | | FAB-MS[b] | |
|---|---|---|---|---|
| | $t_R$ min | purity % | $M_r$ | $(M+H)^+$ |
| Tyr-D-Ala-(*R*)HmNal-Asp-Val-Val-Gly-$NH_2$ | 13.4 | 99.90 | 848.7 | 849.6 |
| Tyr-D-Ala-(*S*)HmNal-Asp-Val-Val-Gly-$NH_2$ | 15.6 | 98.50 | 848.7 | 849.7 |

[a]Linear gradient 20–60% B over 25 min at flow rate of 1 ml/min; [b] calc. for $C_{42}H_{56}O_{11}N_8$.

The new analogues were tested for receptor affinity and selectivity for $\mu$ and $\delta$ opioid receptors (Table 2). The receptor binding was performed by the method previously descri-

dred-fold the affinity to $\delta$ receptors when compared to parent compound [8]. The presented results show that incorporation of an $\alpha$-hydroxymethyl group into Nal$^3$ with HmNal slightly

**Table 2. Receptor binding properties of deltorphin I analogues**

| Compound | $IC_{50}$ (nM) | | Ratio |
|---|---|---|---|
| | $\delta$ | $\mu$ | $\mu/\delta$ |
| Tyr-D-Ala-(*R*)HmNal-Asp-Val-Val-Gly-$NH_2$ | 770 | >10000 | – |
| Tyr-D-Ala-(*S*)HmNal-Asp-Val-Val-Gly-$NH_2$ | 776 | >10000 | – |
| Tyr-D-Ala-Nal-Asp-Val-Val-Gly-$NH_2$ [a] | 110 | 3724 | 33.8 |
| Tyr-D-Ala-(*S*)HmPhe-Asp-Val-Val-Gly-$NH_2$ [b] | 1.8 | >10000 | – |
| Tyr-D-Ala-Phe-Asp-Val-Val-Gly-$NH_2$ (DT I) | 0.6 | 2140 | 3566 |

[a] [8]; [b] [2]

(sixfold) decreases the affinity to $\delta$ receptors and significantly reduces the affinity to $\mu$ receptors. Unexpectedly, the absolute configuration of the chiral center of the $\alpha$-hydroxymethylphenylalanine in position 3 has no impact on the affinity and selectivity. Low affinities of peptide analogues containing bicyclo aromatic amino acids in position 3 may suggests that naphtyl ring is too large for optimal fitting with respective receptor pocket of $\delta$ opioid receptors. The hydroxymethyl group, which was shown to stabilize "$\delta$ type" conformation, is not able to improve the affinity to $\delta$ receptors but effectively decreases the affinity to $\mu$ ones.

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