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DERMORPHIN SEQUENCE WITH HIGH δ-AFFINITY BY FIXING THE PHE SIDECHAIN TO TRANS AT χ_1

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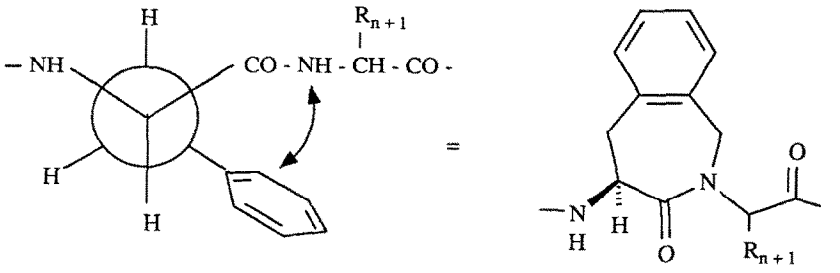
Abstract : The Phe sidechain in dermorphin was fixed into the trans conformation by linking the aromatic ring to the Gly nitrogen through a methylene bridge. The compound has high μ- and δ-opioid activities.

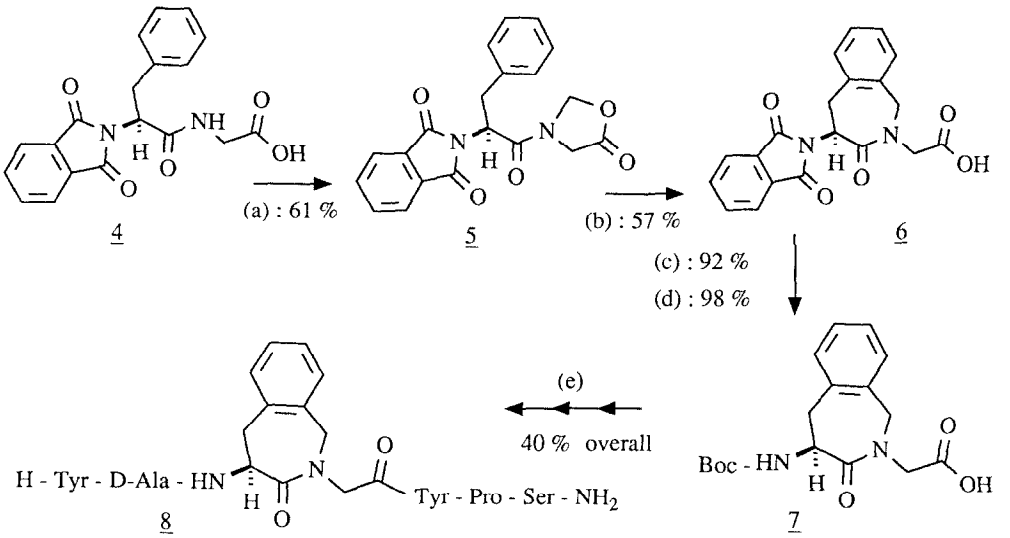
The naturally occuring peptides dermorphin H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2 1 and deltorphin (e.g. deltorphin II : H-Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH_2 2) display different selectivities towards the opioid receptor subtypes. Whereas 1 is μ-selective, 2 is δ-selective, which is in agreement with the hypothesis of a common N-terminal "message" part, and a different C-terminal "address" part (1), the latter being responsible for the receptor selection. On the other hand, the importance of the relative orientation of the Tyr and Phe sidechains for determining receptor selectivity and affinity has been emphasized in several studies of opioid peptides containing Phe at positions 3 or 4 (2-6).

For linear peptides such as 1 or 2, the flexibility of the backbone and especially of the sidechain moieties makes determination of the important conformational features responsible for biological activity very difficult. Therefore conformational flexibility has been reduced by cyclization of the peptide backbone (7). Alternatively, the sidechain orientation can be fixed by cyclization as has been done for Phe or Tyr by using a tetrahydroisoquinoline-3-carboxylic acid (Tic) structure (4,8,9). The use of Tic constrains the sidechain to the gauche(-) (χ_1 = -60°) or gauche(+) (χ_1 = +60°) staggered conformations (8). We now report fixing the Phe sidechain in 1 into the trans (χ_1 = 180°) conformation. This can be achieved by bridging the phenyl ring of Phe and the nitrogen atom of the succeeding amino acid by a methylene group (Fig. 1).

Figure 1



SCHEME 1



4 5 6 7 8

(a) : CH_2=O, p-TosOH, toluene, reflux 1.5 h ; (b) : CF_3SO_3H, room temp., overnight ;
(c) : NH_2NH_2, EtOH, reflux 1.5 h ; (d) Boc_2O, NEt_3, CH_2Cl_2, room temp., overnight ;
(d) : solid phase peptide synthesis

The required 4-amino-tetrahydro-2-benzazepin-3-one 3 (R_{n+1} = H) for substitution of Phe-Gly in dermorphin has been prepared by adaptation of the method described by Flynn (10, 11) and de* Laszlo (12) (Scheme 1) (13). Incorporation of the Boc-protected dipeptide mimetic 7 into the dermorphin sequence was performed by solid phase synthesis (14) using a p-methyl-benzhydrylamine resin, DCC/HOBt couplings and HF cleavage. The overall yield of the purified peptide was 40%.

The affinity of peptide 8 for μ- and δ-opioid receptors in rat forebrain using [^{3}H]sufentanil as the μ-selective ligand and [^{3}H]DPDPE as the δ-selective ligand and its ability to inhibit electrically induced contractions in the guinea pig ileum (GPI) and mouse vas deferens (MVD) (15) are collected in Table 1.

Table 1 Biological activities (IC_{50} values, nM)

	[^{3}H]sufentanil	[^{3}H]DPDPE	GPI	MVD	MVD/GPI
1	2.4 ± 1.1	295 ± 31	1.06 ± 0.06	17.80 ± 2.10	16.7(1)
2	-	-	≥ 3000	0.32 ± 0.05	≤ 1 10^{-4}(1)
8	11.0 ± 0.8	17.4 ± 1.0	10.8 ± 1.6	0.66 ± 0.13	0.06

Clearly, compared to 1, μ-affinity and activity of 8 are decreased by a factor 4 and 10, respectively, while δ-affinity and activity are increased by a factor 17 and 27 respectively.
As a result of the fixing of the Phe sidechain in the trans conformation at χ_1 the strongly μ-selective dermorphin loses its specificity, mainly by an increase in δ-affinity. The high potency of 8 contrasts to the low potency observed for [Tic^3]dermorphin (9). In the Tic structure, the Phe sidechain is fixed by bridging the phenyl ring and its own α-nitrogen atom by a methylene group. This results in a gauche(+) orientation of the sidechain when Tic is in a non N-terminal position (8). This result further illustrates the importance of the Phe sidechain orientation, and hence the Tyr/Phe relative orientation, for determining receptor selectivity. It corroborates the theoretical model for the δ-receptor bound conformation of H-Tyr-D-Met-Phe-His-Leu-Met-Asp-NH_2 (16) and DPDPE (6) which places the Phe aromatic ring in the trans rotameric position. Thus, the N-terminal tripeptide is not just a "message", but through its conformation it also contributes to receptor selection (17). The potential of the sidechain constrained pseudodipeptide 7 for inducing δ-selectivity wil be investigated by incorporation into other opioid peptides.

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13. All new compounds gave analytical and spectroscopic data in full accord with their structures :

5 : mp. : 174-175°; FAB-MS : 365(M+1); NMR (270 MHz, DMSO) : 7.81(4H, H^{arom}Pht), 7.11(5H, H^{arom}Phe), 5.55(1H, N-CH-O), 5.27(1H, N-CH'-O), 5.19(1H, CHαPhe), 4.07(2H, CHαα'Gly), 3.32(2H, CHββ'Phe);

6 : mp. : 193.5-196.5°; FAB-MS : 365(M+1); NMR(270 MHz, DMSO) : 11.43(1H, COOH), 7.90(4H, H^{arom}Pht), 7.26(5H, H^{arom}Phe), 5.36(1H, CHαPhe), 4.95(1H, CH^{α}Gly), 4.54(1H, CHα'Gly), 4.12(1H, CH^{ε}), 4.02(1H, $CH^{\varepsilon'}$), 3.32(2H, CHββ'Phe);

7 : FAB-MS : 335(M+1); NMR(270 MHz, DMSO) : 7.15(4H, H^{arom}Phe), 6.73(1H, NH), 5.19(1H, CHε), 4.98(1H, CH^{α}Phe), 4.21(1H, CH^{α}Gly), 4.12(1H, $CH^{\varepsilon'}$) 3.91(1H, $CH^{\alpha'}$Gly), 3.14(1H CHβPhe), 2.94(1H, $CH^{\beta'}$Phe), 1.40(9H, tBu);

8 : FAB-MS : 814(M+1); NMR(500 MHz, DMSO) : a complete analysis has been performed and will be reported separately.

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