Supporting Information

Novel Opioid Peptide Derived Antagonists Containing (2*S*)-2-Methyl-3-(2,6-dimethyl-4-carbamoylphenyl)propanoic Acid [(2*S*)-Mdcp]

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Experimental Section

General Methods. Molecular masses of the compounds were determined by electrospray mass spectrometry on a Hybrid Q-Tof mass spectrometer interfaced to a Mass Lynx 4.0 data system or on a Finnigan/MAT 95XL-T spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity 400 spectrometer or a Bruker Model *Advance* 300 MHz or DPX-300 NMR spectrometer, and referenced with respect to the residual signals of the solvent. The following abbreviations were used in reporting spectra: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Peptides were purified on a Vydac 218-TP1022 column (22 x 250 mm) with a linear gradient of 20-80% MeOH in 0.1% TFA/H_2O over 30 min at a flow rate of 12 mL/min (peptides **1** and **3**) or with a linear gradient of 20-65% MeOH in 0.1% TFA/H_2O over 50 min at a flow rate of 12 mL/min (peptide **5**). Analytical reversed-phase HPLC was performed on a Vydac 218-TP54 column (5 x 250 mm) with a linear gradient of 20-80% acetonitrile in 0.1% TFA/H_2O at a flow rate of 1 mL/min. The same column was also used for the determination of the capacity factors K' under the same conditions. Precoated plates (silica gel 60 F_{254}, 250 μm, Merck Darmstadt, Germany) were used for ascending TLC in the following solvent systems (all v/v): (I) *n*-BuOH/AcOH/H_2O (4:1:1), (II) n-BuOH/pyridine/AcOH/H_2O (15:10:3:12), (III) CH_3Cl/MeOH/CH_3COOH (85:10:5), (IV) CH_3 $COOH/H_2O$ (7:13).

In Vitro Bioassays and Receptor Binding Assays. The GPI[20] and MVD[21] bioassays were carried out as reported in detail elsewhere.[22,23] K_e values for antagonists were determined from the ratio of IC_{50} values obtained with an agonist in the presence and absence of a fixed antagonist concentration.[24] μ antagonist K_e values of compounds were determined in the GPI assay against the μ agonist TAPP[25] using antagonist concentrations ranging from 10 to 1000 nM. κ antagonist K_e values of compounds were also measured in the GPI assay against the κ agonist U50,488, using antagonist concentrations ranging from 10 to 2000 nM. δ antagonist K_e values of compounds were determined in the MVD assay against the δ agonist DPDPE using antagonist concentrations ranging from 2 to 4000 nM.

Opioid receptor binding studies were performed as described in detail elsewhere.[22] Binding affinities for μ and δ receptors were determined by displacing, respectively, [^{3}H]DAMGO (Multiple Peptide Systems, San Diego, CA) and [^{3}H]DSLET (Multiple Peptide Systems) from rat brain membrane binding sites, and κ opioid receptor affinities were measured by displacement of [^{3}H]U69,593 (Amersham) from guinea pig brain membrane binding sites. Incubations were performed for 2h at 0°C with [^{3}H]DAMGO, [^{3}H]DSLET and [^{3}H]U69,593 at respective concentrations of 0.72, 0.78 and 0.80 nM. IC_{50} values were determined from log-dose displacement curves, and K_i values were calculated from the obtained IC_{50} values by means of the equation of Cheng and Prusoff,[26] using values of 1.3, 2.6 and 2.9 nM for the dissociation constants of [^{3}H]DAMGO, [^{3}H]DSLET, and [^{3}H]U69,593, respectively.

References

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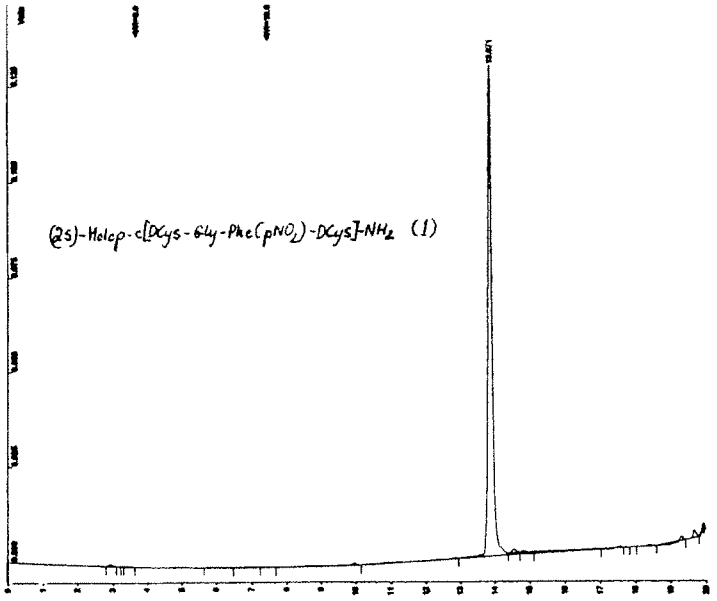
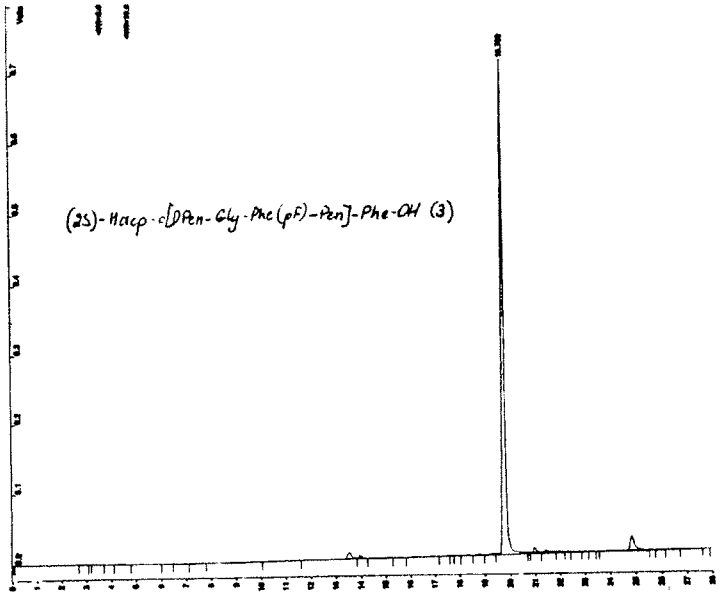
Purity of target compounds 1, 3 and 5

Analytical reversed-phase HPLC was performed on a Varian 9010/9050 system using a Vydac 218-TP54 column (5 x 250 mm) with a linear gradient of 20-80% acetonitrile in 0.1% TFA/H_2O at a flow rate of 1 mL/min (λ = 254 nm).

Compound		K'	Purity
1	(2*S*)-Mdcp-c[D-Cys-Gly-Phe(*p*NO_2)-D-Cys]NH_2	3.75	> 98%
3	(2*S*)-Mdcp-c[D-Pen-Gly-Phe(*p*F)-Pen]-Phe-OH	5.83	> 98%
5	[(2*S*)-Mdcp[1]]Dyn A(1-11)-NH_2	3.78	> 95%



Compound 1



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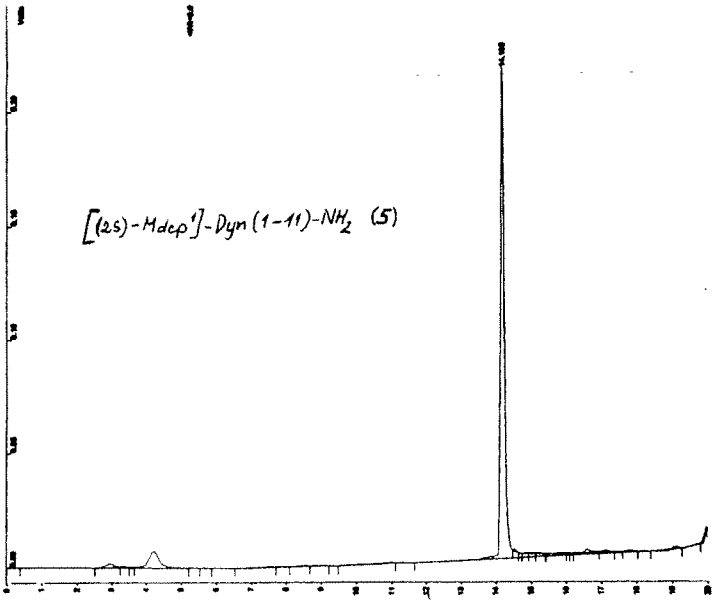

Compound 5