Novel Deltorphin Heptapeptide Analogs with Potent δ Agonist, δ Antagonist, or Mixed μ Antagonist/δ Agonist Properties

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A series of deltorphin (DLT: Tyr-D-Met-Phe-His-Leu-Met-Asp-NH_2) analogs in which Leu^5 and/or Met^6 were mainly replaced by *t*-Leu(Tle) and/or N$^\alpha$-alkylated glycine were synthesized and examined for their receptor binding properties and *in vitro* bioactivities by guinea pig ileum (GPI) and mouse vas deferens (MVD) assays. [Tle^5]DLT(**2**) showed a dramatic decrease in the MVD potency when compared to the parent peptide and was found to have a potent δ receptor antagonist activity against various δ agonists with K_e values of 16–311 nM. Interestingly, the antagonist potency of **2** against DPDPE as agonist was 20-fold weaker than that against deltorphins or Leu-enkephalin. Among the analogs in which Met^6 was replaced by an N$^\alpha$-alkylated Gly residue, [N^α-isobutyl-Gly^6]DLT(**5**) behaved as a mixed μ antagonist/δ agonist while its isomeric analogs in which the N^α-alkyl is *n*-butyl (**4**) or (*R* or *S*) *sec*-butyl (**6a**,**b**) were very potent δ receptor agonists. Analogs **2**, **4**, **6a**, and **6b** were highly stable against rat brain and rat plasma enzymes and thus may represent a starting point for the development of novel receptor-specific compounds useful as ligands for studies of opioid receptors.

Introduction[1]

The recent discovery of a new family of opioid peptides from the skin of South American frogs of the genus *Phyllomedusa* caused much excitement in the structure–opioid receptor selectivity correlation studies. Although these naturally occurring heptapeptides have high homology in their N-terminal tripeptide portions, they exhibit enormous differences in receptor selectivity. That is, dermorphin[2] (Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2) behaves as the most potent and selective natural ligand for the μ opioid receptor while deltorphin[3] (or dermenkephalin,[4] DLT, Tyr-D-Met-Phe-His-Leu-Met-Asp-NH_2) and other deltorphins,[5] Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH_2 (DL-I) and Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH_2 (DL-II), behave as potent and selective δ opioid receptor agonists. Structure–activity studies have revealed that the existence of two lipophilic residues in deltorphins (Leu^5-Met^6 in DLT and Val^5-Val^6 in DL-I and DL-II) is an important factor for δ receptor affinity and selectivity.[6–12]

Several studies that have focused on these lipophilic residues have indicated that the lipophilicity and properly hindered or oriented side chains are needed in these residues for the high δ affinity and selectivity.[13–18] Previously, we reported that substitution of Leu^5 in DLT with a sterically hindered residue, *t*-Leu(Tle), led to an analog with high stability against degradation enzymes.[19] However, this analog showed a reduced δ receptor affinity and a dramatically low potency in the bioassay based on electrically induced smooth muscle contraction of mouse vas deferens when compared with the parent peptide.[19] Such extraordinarily low potency profiles of [Tle^5]DLT led us to examine further the structure–activity correlations focused on residues 5 and 6. We report here the δ antagonist properties of [Tle^5]DLT (**2**) and some novel analogs containing a N$^\alpha$-alkylated glycine residue at position 6 with a high δ agonist potency or a mixed μ antagonist/δ agonist activity.

Results and Discussion

The new analogs were designed to retain hydrophobicity and enzymatic stability on the basis of the recent evidence that the Leu^5-Met^6 bond in DLT is the most labile to degradation enzymes.[19] Consequently, two analogs containing a highly hindered Tle residue at position 5 or 6 (**2** and **3**) and seven analogs containing an N$^\alpha$-alkylated Gly residue at position 6 (**4**–**9**) were expected to improve enzymatic stability.

The synthetic DLT analogs were examined for their binding properties to rat brain μ and δ opioid receptors and *in vitro* bioactivities by their abilities to inhibit electrically induced contraction of mouse vas deferens (MVD assay) and guinea pig ileum (GPI assay). Both assays are usually used to test for bioactivities at δ and μ receptors, respectively. In the δ receptor binding assay, a potent μ ligand, [*N*-MePhe^3,D-Pro^4]morphiceptin, was used to suppress peptide binding to the μ receptor[6,20] because the [^{3}H]DADLE radioligand used has relatively low δ (*vs* μ) selectivity.[21] Table 1 shows the results of the binding assays. The replacement of Leu^5 by Ala (**1**) caused a 10-fold reduction in δ receptor affinity and a slight increase in μ affinity resulting in a considerably low δ selectivity. These findings are compatible with the data reported by Charpentier *et al.*[17] and also support the view that the lipophilic residue at position 5 in deltorphins is of critical importance for δ affinity and selectivity.[6–18] Interestingly, the introduction of the highly hindered residue, Tle, in place of Leu^5 (**2**) resulted in a 50-fold reduction in δ affinity while the same replacement in residue 6 (**3**) caused only a slight reduction in δ affinity and selectivity. To examine further the combinatorial effects of residues 5 and 6, seven analogs with an N$^\alpha$-alkylated Gly residue introduced in place of Met^6 (**4**–**9**) were prepared. Analog **4** which contains N^α-*n*-butyl-Gly (nBuG) in place of Met^6 showed a very high δ affinity over that of DLT,

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Table 1. Opioid Receptor Binding Affinities of Synthetic Deltorphin Analogs

compound	K_i (nM) [^{3}H]DAGO (μ)	[^{3}H]DADLE (δ)	K_i (μ)/K_i (δ)
[Ala^5]DLT (**1**)	133 ± 31.0	1.71 ± 0.33	78
[Tle^5]DLT (**2**)	382 ± 21.4	9.45 ± 0.98	40
[Tle^6]DLT (**3**)	271 ± 19.7	0.46 ± 0.02	589
[nBuG^6]DLT (**4**)	820 ± 6.4	0.04 ± 0.01	18231
[isoBuG^6]DLT (**5**)	58 ± 6.7	0.85 ± 0.01	68
[(*R* or *S*)-secBuG^6]DLT (**6a**)	856 ± 36.4	0.10 ± 0.07	8734
[(*R* or *S*)-secBuG^6]DLT (**6b**)	798 ± 31.4	0.09 ± 0.09	9689
[Tle^5,nBuG^6]DLT (**7**)	152 ± 41.0	12.4 ± 0.12	12
[ψ(2−3)^a,nBuG^6]DLT (**8**)	143 ± 18.7	1.89 ± 0.41	76
[ψ(2−3)^a,Tle^5,nBuG^6]DLT (**9**)	157 ± 3.6	2.56 ± 0.12	61
DLT	219 ± 29.5	0.19 ± 0.06	1152
TIPPψ	9921 ± 951	2.05 ± 0.12	4839

[a] Peptide bond between D-Met^2 and Phe^3 was replaced by CH_2NH bond.

Table 2. *In Vitro* Bioactivities of Synthetic Deltorphin Analogs

compound	IC_{50}, nM GPI	MVD	IC_{50} ratio (GPI/MVD)
[Ala^5]DLT (**1**)	216 ± 23	406 ± 21	0.5
[Tle^5]DLT (**2**)	1198 ± 132	2432 ± 451	0.5
[Tle^6]DLT (**3**)	325 ± 21	1.28 ± 0.21	254
[nBuG^6]DLT (**4**)	578 ± 46	0.56 ± 0.04	1032
[isoBuG^6]DLT (**5**)	3870 ± 245	2.36 ± 0.44	1639
[(*R* or *S*)-secBuG^6]DLT (**6a**)	684 ± 36	0.88 ± 0.06	777
[(*R* or *S*)-secBuG^6]DLT (**6b**)	642 ± 34	0.76 ± 0.09	844
[Tle^5,nBuG^6]DLT (**7**)	1678 ± 211	1911 ± 124	0.9
[ψ(2−3)^a,nBuG^6]DLT (**8**)	422 ± 31	26 ± 5.3	16
[ψ(2−3)^a,Tle^5,nBuG^6]DLT (**9**)	995 ± 20	42 ± 2.4	23
DLT	632 ± 38	0.93 ± 0.08	679

[a] Peptide bond between D-Met^2 and Phe^3 was replaced by CH_2NH bond.

and its μ affinity was 4-fold lower than that of DLT, resulting in an extremely high δ selectivity. Analog **5** with the introduced N^{α}-*iso*-butyl-Gly (isoBuG) showed a moderate δ affinity and a 4-fold higher μ affinity resulting in a very low δ selectivity. Similar replacement of residue 6 with N^{α}-(*R* or *S*)-*sec*-butyl-Gly (secBuG) also gave analogs (**6a** or **6b**) with a 4-fold lower μ affinity than DLT, resulting in a very high δ selectivity comparable to that of DLT. These findings indicate that subtle structural changes based on the *N*-alkyls in residue 6 are responsible for the varying μ affinity without a major change in δ affinity. Because combination of the highly hindered Tle residue at position 5 with nBuG^6 (**7**) resulted in a dramatic reduction in δ affinity and consequently a very low δ selectivity, a limited degree of conformational constraint between residues 5 and 6 may be an important factor in the interaction with the receptors. The low δ binding affinity of **2** and **7** may be caused by hampering of proper topographical requirements of the whole molecule at the δ receptor by the highly hindered Tle^5 residue as suggested by the assumption that the C-terminal tripeptide portion of DLT may serve to stabilize bioactive conformations at the receptor site.[13] Two analogs (**8** and **9**) in which the peptide bond of D-Met^2-Phe^3 in **4** and **7**, respectively, was replaced by an isosteric CH_2NH bond that has been proved to be an effective modification for the design of antagonists[22-29] showed a moderate δ affinity and selectivity.

In the *in vitro* bioassay (Table 2), analogs **1**, **2**, and **7** unexpectedly showed a dramatic reduction in MVD potencies in view of their δ receptor binding affinities. Because these analogs were assumed to have significant δ affinity but impaired signal transduction, their antagonist activities were examined in the MVD assay.[30] As Table 3 shows, **2** possessed potent antagonist properties against various δ agonists with K_e values of 16−310 nM. Analog **1** also showed very weak antagonist properties, but **7** did not behave as an antagonist. Thus, **2** is a potent and selective δ antagonist with very low δ agonist activity. Although the δ antagonist activity of **2** is weaker than that of the nonpeptide antagonist naltrindole[31] or the peptidic antagonist TIPPψ, which was recently found to be a potent δ antagonist,[32] this analog had unique features of the antagonism; it showed an exceptionally low antagonist potency (K_e = 311 nM) against DPDPE while against other δ agonists it showed a high potency(K_e = 16−18 nM). Recently, two δ receptor subtypes, δ1 and δ2, have been identified in the brain;[33] DPDPE is a selective agonist for δ1 while DL-II is for δ2.[34] However, in the MVD tissue, there is no clear evidence of δ receptor subtypes.[35,36] The divergent action of **2** may be an indication that δ receptor subtypes in MVD tissue differ from those in brain tissue.[37-40] Furthermore, it should also be noted that the potency profiles of the isoBuG analog (**5**) and the nBuG analog (**4**) in μ receptor affinity were reversed in the GPI assay: **5** possessed an extraordinarily low potency in the GPI assay. Surprisingly, as Table 4 shows, **5** behaved as a moderately potent μ antagonist against the μ agonists dermorphin and DAGO with K_e values of 138−165 nM. This compound is the first reported deltorphin-related peptide with a mixed μ antagonist/δ agonist property. In this context, Schiller *et al.* have recently reported some cyclic casomorphin analogs[41] and Tic-containing peptides[43] with mixed μ agonist/δ antagonist properties. Analog **7**, which possessed very low GPI and MVD potencies, also showed a weak μ antagonism against both agonists. No significant μ antagonist activity was observed with **2** in the GPI assay. Analog **4** showed a very high MVD potency over that of DLT and a low GPI potency as expected from the receptor binding properties. Both diastereomeric peptides, **6a** and **6b**, also showed high MVD potencies comparable to that of DLT. The two ψCH_2-NH analogs, **8** and **9**, showed potency profiles similar to their binding profiles and behaved as moderately potent δ agonists.

Table 3. K_e Values Determined for δ Antagonists against Various δ Agonists in the MVD Assays

antagonist	K_e, nM[a] DLT	Leu-enkephalin	DL-II	DADLE	DPDPE
[Ala^5]DLT (**1**)	632 ± 21	721 ± 56	–[b]	598 ± 84	–[b]
[Tle^5]DLT (**2**)	16.1 ± 0.86	18.4 ± 0.43	17.2 ± 0.45	15.8 ± 2.43	311 ± 45
TIPPψ	3.28 ± 0.56	4.51 ± 0.65	4.08 ± 0.22	3.98 ± 0.81	4.12 ± 0.13
naltrindole	0.65 ± 0.21	0.94 ± 0.12	0.67 ± 0.10	0.67 ± 0.05	0.65 ± 0.08

[a] Mean of three determinations ± SE. [b] Not determined.

Table 4. K_e Values Determined for μ Antagonists against Dermorphin and DAGO in the GPI Assay

antagonist	$K_{e,nM}$[a] dermorphin	DAGO
[isoBuG^6]DLT (**5**)	165 ± 42	138 ± 34
[Tle^5,nBuG^6]DLT (**7**)	554 ± 11	520 ± 135
naloxone	1.38 ± 0.23	1.45 ± 0.31
naltrindole	151 ± 31	52.2 ± 11

[a] Mean of three determinations ± SE.

Several δ antagonists having a Tic residue have recently been reported.[32,42-46] Their antagonist actions appear to be conferred by the conformational constraints and chirality of Tic in residue 2. On the other hand, the antagonist action of **2** can be assumed to be related to a reduced flexibility around the Tle^5 residue, which may impair required conformations for the signal transduction. Moreover, the introduction of an N-alkylated Gly residue (or "peptoid"[47,48] monomer) at position 6 of DLT, which may confer a local conformational restriction by virtue of effects on the peptide backbone as well as metabolic stability to the peptide, afforded analogs with significantly different receptor recognition or activation properties. Analog **2** and those analogs with an N$^\alpha$-alkylated Gly replacement in residue 6 (**4**–**9**) were highly stable to degradation enzymes in the rat brain and plasma as mentioned in the Experimental Section. This is due to the fact that the peptide bond between residues 5 and 6 in DLT is the primary cleavage site by degradation enzymes.[19]

In conclusion, the modification of residue 5 and/or 6 of DLT with Tle or N$^\alpha$-alkylated Gly afforded potent δ agonists (**4**, **6a**, and **6b**), δ antagonists with very low agonist potency (**1** and **2**), and analogs with a mixed μ antagonist/δ agonist property (**5** and **7**). The present study also indicated that the modifications mentioned above are advantageous for improving the enzymatic stability of the peptide. These analogs (**2**, **4**, **6a**, and **6b**) may represent a starting point for the development of novel receptor-specific compounds useful as ligands for the studies of opioid receptors.

Experimental Section

Peptide Synthesis. All peptides except for the ψ(CH$_2$NH) analogs (**8** and **9**) were synthesized by the solid phase technique with DIPCI/HOBt-mediated Fmoc strategy, starting with a Fmoc-NH-SAL-MBHA resin[49] (0.39 mequiv/g, 100–200 mesh, Watanabe Chem. Ind. Ltd., Hiroshima). The following schedule was employed: (1) DMF wash (×3), (2) 30% piperidine/DMF (×2, 5 and 20 min), (3) DMF wash (×6), (4) Fmoc-amino acid (4 equiv)/HOBt (4 equiv)/DIPCI (4 equiv) in DMF–CH$_2$Cl$_2$ (1:1) (120 min), (5) DMF wash (×3), and (6) 2-propanol wash (×2). The side chain protecting groups used were trityl for His and *tert*-butyl for Asp and Tyr. For the preparation of analogs **4**–**9**, N$^\alpha$-alkylated amino acids were prepared by a reductive alkylation of corresponding alkylamines with glyoxylic acid monohydrate in water using catalytic (10% Pd/C) hydrogenolysis according to the method of Simon *et al.*[47] and converted to the Fmoc derivatives: Fmoc-nBuG, mp 102–104 °C; FAB-MS 354 (M + H)$^+$; Fmoc-isoBuG, oily residue; FAB-MS 354 (M + H)$^+$; Fmoc-(*R* and *S*)-secBuG, oily residue; FAB-MS 354 (M + H)$^+$. After incorporation of the *N*-alkyl glycine residue, a subsequent coupling reaction (Tle or Leu) on the solid support was carried out by the PyBOP/HOBt method.[50] The protected peptide SAL-resin was treated with a mixture of TFA/phenol/ethanedithiol (10:0.5:0.25) and then purified using a medium-pressured HPLC as reported previously.[16] The diastereomeric peptides (**6a** and **6b**) were isolated separately by preparative HPLC. **6a** denotes a faster eluted product and **6b** is the later one on HPLC.

In the case of the synthesis of **8** and **9**, the DIPCI/HOBt-mediated BOC strategy[16] was employed except that the incorporation of N$^\alpha$-alkylated residues and subsequent coupling reactions were carried out as described above, starting with a MBHA resin (0.48 meq/g, 100–200 mesh, Watanabe Chem. Ind. Ltd., Hiroshima). The side chain protecting groups used were tosyl for His and benzyl for Tyr and Asp. The ψ(CH$_2$-NH) peptide bond isostere was introduced using Boc-D-methioninal and NaCNBH$_3$ by the method of Sasaki and Coy.[23] Peptides were cleaved from the resin by treatment with a mixture of HF–anisole (9:1) and purified as reported previously.[16] The purity of all final compounds was checked by HPLC (Waters LC Module I) on a YMC ODS (AM-303–10) column (4.6 × 250 mm) with a mixture of 80% acetonitrile containing 0.06% TFA (solvent A) and 0.06% TFA (soluvent B) as eluent (flow rate, 1 mL/min) with UV detection at 220 nm. All peptides synthesized had a purity of more than 95% on the HPLC and gave satisfactory FAB MS and amino acid analytical data as shown in Table 5.

Receptor Binding Assay. Crude synaptosomal fractions (P2) were prepared from decerebrated rat whole brain as reported previously.[9,51] [^{3}H]DAGO (Amersham Corp.) and [^{3}H]-DADLE (Amersham Corp.) were used as μ and δ radioligands, respectively. The binding experiments were carried out by incubating an aliquot of the crude rat brain synaptosomal fraction (600 μg of protein/mL) in an assay mixture containing 500 μg of bovine serum albumin, 50 μg of bacitracin, 10 μg of bestatin, 20 μg of soybean trypsin inhibitor, and 2 nM radioligand in a final volume of 500 μL(50 mM Tris-HCl buffer

Table 5. Physicochemical Properties of Synthetic Peptides

analog[a]	$[\alpha]^{22}_D$[b] (deg)	TLC[c] I	TLC[c] II	HPLC (K'[d])	FAB MS (MH$^+$)	amino acid analysis[e] Tyr	Met	Phe	His	Leu	Asp	other amino acid
1	−16.8	0.36	0.79	6.22	912	0.83	1.80	1.00	1.10		1.02	1.00 (Ala)
2	−8.0	0.41	0.76	7.23	955	0.84	1.67	1.00	0.89		0.90	1.05 (Tle[f])
3	−7.3	0.41	0.76	7.65	937	0.75	0.86	1.00	1.01	1.02	1.15	0.90 (Tle[f])
4	+3.6	0.50	0.80	8.87	937	0.86	0.85	1.00	0.99		1.02	+ (nBuG[g])
5	−0.3	0.49	0.79	8.56	937	0.89	0.78	1.00	1.03		1.05	+ (isoBuG[h])
6a	−3.4	0.50	0.76	8.48	937	0.90	0.88	1.00	1.04	0.95	0.99	+ (secBuG[i])
6b	−2.8	0.45	0.77	8.69	937	0.96	0.95	1.00	1.09	0.97	1.04	+ (secBuG[i])
7	+3.2	0.52	0.82	8.45	937	0.84	0.76	1.00	1.01		1.10	1.10 (Tle[f]), + (nBuG[g])
8	+2.7	0.47	0.78	6.61	923	0.75			1.00	1.07	1.16	+ (nBuG[g])
9	+1.5	0.41	0.80	7.00	923	0.83			1.00		1.10	1.05 (Tle[f]), + (nBuG[g])

[a] All analogs were at least 95% pure as assessed by analytical HPLC peak integrations. [b] Optical rotations were measured in 50% ethanol (c = 0.5) using a 10-cm path length cell in a JASCO DIP-40 polarimeter. [c] Silica gel plates (Merck, Kiesel gel 60F$_{254}$, 5 × 10 cm were used with the following solvent systems: I, 1-BuOH–AcOH–water (4:1:5, upper phase); II, 1-BuOH–pyridine–AcOH–water (15:10:3:12). [d] Capacity factor (K') was determined using YMC ODS (AM-303-10) column (4.6 × 250 mm) with following solvent systems: A, 0.06% TFA; B, 80% acetonitrile containing 0.06% TFA. A linear gradient from 20% B to 60% B over 40 min at a flow rate of 1.0 mL/min was used and the eluate was monitored at 220 nm. [e] Amino acid analysis was performed using a HITACHI 9500 amino acid analyzer after 6 N HCl hydrolysis at 110 °C for 22 h. [f] Eluted just before Met. [g] Eluted at the same position as Val. [h] Eluted just before Gly. [i] Eluted at the same position as Gly.

at pH 7.4). A specific μligand, [N^{α}-MePhe^3,D-Pro^4]morphiceptin (2.6 μM), was included in the δ binding assay. Nonspecific binding was determined in the presence of excess (1 μM) unlabeled ligand. After 60 min at 25 °C, the reaction mixture in each tube was filtered through GF/B filters (presoaked in 0.1% polyethylenimine) with 2 × 4 mL of ice-cold Tris-HCl buffer. The radioactivity on the filters was counted in a Beckman liquid scintilation counter after overnight extraction with liquid scintilation fluid (3 mL). The IC_{50} values were obtained from a log dose−displacement curve, and inhibition constants (K_i) were calculated from the IC_{50} value by the equation of Cheng and Prusoff.[52] The K_d values of [^{3}H]DAGO and [^{3}H]DADLE used were 1.509 and 1.500, respectively.

***In Vitro* Bioactivity Assay.** The GPI and MVD assays were performed as reported previously[16] in detail using isolated longitudinal muscle strips of Hartley strain guinea pig (250−300 g) ileum and vas deferens of male ddY strain mouse (25−35 g), respectively. In both assays, log dose−response curves were constructed and IC_{50} values were determined. K_e values for the antagonists were determined from the ratio of IC_{50} values obtained in the presence and absence of a fixed antagonist concentration.[30] Antagonist concentrations used were as follows: naloxone, 5 nM; naltrindole, 5 nM and 50 nM for δ and μ antagonist assays, respectively; TIPPψ, 50 nM; analogs 1, **2**, **5**, and **7**, 50 nM.

Enzymatic Stability of Peptides. Peptide (50 nmol) was incubated with a crude preparation of rat brain membranes[51] (1.9 mg of protein/mL, 150 μL) or rat plasma (150 μL) in a total volume of 250 μL containing 10 mM Tris-HCl buffer (pH 7.6) at 37 °C for 2 h. The mixture was heated to 98 °C for 5 min, and then 0.2 N HCl (10 μL of I, in the case of brain homogenate) or 10% trichloroacetic acid (40 μL, in the case of plasma) was added. After centrifugation at 3 °C for 10 min (10 000 rpm), the supernatant was analyzed by HPLC. The degradation rate was estimated from the relative peak area of residual intact peptide to that of the peptide at zero time on HPLC using a Chromatocorder 12 integrator (System Instruments). Consequently, the degradation rate of analogs tested (**2**, **4**−**9**) was less than 5% in both brain membrane and plasma hydrolysates. By contrast, 35−37% of hydrolysis occurred with DLT in both hydrolysates.

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(1) The following abbreviations have been used: DICI = *N,N'*-diisopropylcarbodiimide, HOBt = 1-hydroxybenztriazole, Boc = *tert*-butyloxycarbonyl, PyBOP = (benztriazol-1-yloxy)tris(pyrrolidino)phosphonium hexafluorophosphate, Fmoc = fluorenylmethoxycarbonyl, Fmoc-SAL-MBHA resin = [[4-[(2',4'-dimethoxyphenyl)-Fmoc-aminomethyl]phenoxy]acetoamido]-*p*-methylbenzhydrylamine resin (super acid labile MBHA resin), DLT = Tyr-D-Met-Phe-His-Leu-Met-Asp-NH_2, DL-I = Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH_2, DL-II = Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH_2, DAGO = [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin, DADLE = [D-Ala^2,D-Leu^5]enkephalin, DPDPE = [D-Pen^2,D-Pen^5]enkephalin (Pen: penicillamine), Tle = *tert*-leucine, nBuG = N^{α}-*n*-butylglycine, isoBuG = N^{α}-isobutylglycine, secBuG = N^{α}-*sec*-butylglycine, Tic = 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, TFA = trifluoroacetic acid, DMF = dimethylformamide. Other abbreviations used are those recommended by the IUPAC−IUB Commission (*Eur. J. Biochem.* **1984**, *138*, 9−37).

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