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Stereospecific Synthesis of (2*S*)-2-Methyl-3-(2′,6′-dimethyl-4′-hydroxyphenyl)-propionic Acid (Mdp) and its Incorporation Into an Opioid Peptide

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Abstract—To examine the effect of replacing the N-terminal amino group in opioid peptides with a methyl group on biological activity, a stereospecific synthesis of the tyrosine analogue (2*S*)-2-methyl-3-(2′,6′-dimethyl-4′-hydroxyphenyl)-propionic acid (Mdp) was performed. The enkephalin analogue (2*S*)-Mdp-D-Ala-Gly-Phe-Leu-NH_2 turned out to be a quite potent δ opioid antagonist and a somewhat less potent μ antagonist, indicating that a positively charged N-terminal amino group is not a *conditio sine qua non* for the binding of opioid peptides to δ and μ receptors but may be required for signal transduction.

Until recently it has been assumed that the presence of a positively charged nitrogen atom in opioid compounds represents an absolute requirement for their interaction with opioid receptors.[1] The general assumption was that the positive charge on the ligand would engage in an electrostatic interaction with the negatively charged side chain of an Asp residue located in the *trans*-membrane domain III of opioid receptors. Alternatively, it was suggested that a non-ionic interaction, such as chelation of the quaternary ammonium group by multiple aromatic residues, may be important for the binding of opioid compounds to the δ opioid receptor.[2] Recently, somatostatin-derived cyclic hexapeptide analogues lacking a positive charge were shown to be δ opioid antagonists with significant δ receptor binding affinity ($K_i^{\delta} = 150$–1070 nM).[3] A neutral des-amino analogue of a cyclic β-casomorphin analogue also turned out to be a δ antagonist with relatively modest δ receptor affinity ($K_i^{\delta} = 109$ nM).[3]

To further investigate the role of the positive charge on the N-terminal amino group of opioid peptides in the interaction with their receptors, we decided to prepare and pharmacologically characterize an enkephalin analogue in which the amino group is replaced with the neutral and almost isosteric methyl group. As parent peptide we chose the potent enkephalin analogue H-Dmt-D-Ala-Gly-Phe-Leu-NH_2 because substitution of Dmt (2′,6′-dimethyltyrosine) for Tyr[1] in opioid peptides is known to generally increase δ and μ receptor binding affinity by at least one order of magnitude.[4] The replacement of the amino group in this analogue with a methyl group required the development of a stereospecific synthesis of (2*S*)-2-methyl-3-(2′,6′-dimethyl-4′-hydroxyphenyl)-propionic acid (Mdp).

The stereospecific synthesis of (2*S*)-Mdp is outlined in Scheme 1. Iodination of 3,5-dimethylphenol **1** using a literature procedure[5] afforded 3,5-dimethyl-4-iodophenol **2** (79% yield), which was then protected as the Boc derivative (98% yield). Heck coupling of **3** with methyl acrylate[6] gave **4** in 61% yield. The *trans* configuration of **4** was established by measurement of the coupling constant (16.4 Hz) between the two alkene protons. Subsequent catalytic hydrogenation, followed by basic hydrolysis afforded acid **6** in 88% yield. Incorporation of Evans' chiral auxiliary (*S*)-(−)-4-benzyl-2-oxazolidinone was performed in the standard manner[7] to yield **7** (81% yield). Asymmetric methylation[8] then furnished **8** as a single diastereomer[9] in 71% yield after chromatographic purification. The chiral oxazolidinone auxiliary and Boc group were then removed to give Mdp (**10**)[10] in 96% yield.

Peptides were prepared by manual solid-phase synthesis using a *p*-methylbenzhydrylamine resin and Boc

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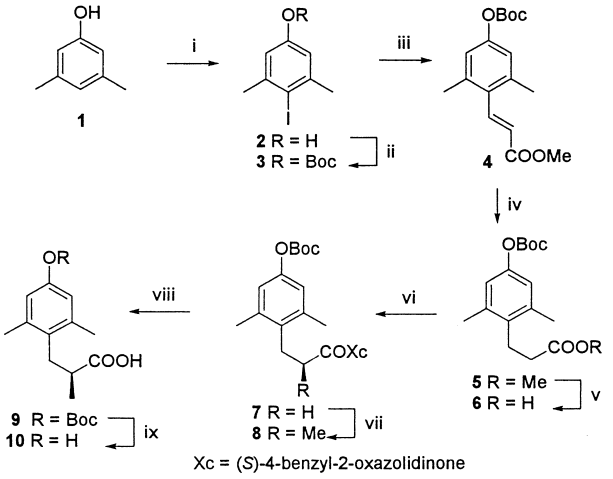
protection according to a protocol published elswhere.[11] After cleavage from the resin by HF/anisole treatment, the crude peptides were purified by preparative reversed-phase HPLC. Their purity and structural identity were established by analytical HPLC and FAB-MS.



Scheme 1. (i) KI, KIO_3, MeOH, HCl; (ii) $(Boc)_2O$, Et_3N, DMAP, H_2O/THF; (iii) CH_2=CHCOOMe, $Pd(OAc)_2$, Et_3N, $(p\text{-}MePh)_3P$, CH_3CN, reflux; (iv) H_2, Pd/C, 70 psi, 50 °C, EtOAc; (v) 1 N aq NaOH/THF; (vi) Et_3N, PvCl, ether, −78 to 0 °C, then treated with *n*-BuLi, Xc, THF, −78 to 0 °C; (vii) NaHMDS, THF, MeI, −78 to −25 °C; (viii) LiOH, H_2O_2, THF/H_2O; (ix) TFA, CH_2Cl_2.

Pharmacological Results and Discussion

Mu, δ and κ opioid receptor binding affinities of peptides **11** and **12** were determined in rat and guinea pig brain membrane binding assays as described elsewhere.[11] For the determination of their in vitro opioid activities, compounds were tested in bioassays based on inhibition of electrically evoked contractions of the guinea pig ileum (GPI) and the mouse vas deferens (MVD) according to published protocols.[3] The GPI and MVD assays are representative for μ and δ opioid receptor interactions, respectively. In the rat brain membrane binding assay, the enkephalin analogue (2*S*)-Mdp-D-Ala-Gly-Phe-Leu-NH_2 (**11**) showed quite high δ receptor binding affinity ($K_i^\delta = 11.7$ nM) (Table 1). Its affinity for δ receptors was only about 5 times lower than that of [Leu^5]enkephalin and 36 times lower than that of the extremely potent parent peptide H-Dmt-D-Ala-Gly-Phe-Leu-NH_2 (**12**). On the other hand, **11** displayed only modest μ receptor binding affinity ($K_i^\mu = 192$ nM). Thus, replacement of the N-terminal amino group in **12** with a methyl group lowered the binding affinity to a much larger extent at μ receptors than at δ receptors and, consequently, peptide **11** turned out to be about 10 times more δ-selective ($K_i^\mu/K_i^\delta = 16.4$) than **12** ($K_i^\mu/K_i^\delta = 1.53$). In fact, peptide **11** is 4 times more δ-selective than [Leu^5]enkephalin (Table 1). Both **11** and **12** showed no significant binding affinity for κ receptors ($K_i^\kappa > 10$ μM). Both in the GPI assay and in the MVD assay, peptide **12** displayed subnanomolar agonist potency with IC_{50}s of 0.586 nM and 0.212 nM, respectively (Table 2). Interestingly, the (2*S*)-Mdp[1]-analogue (**11**) was found to be a moderately potent μ opioid antagonist in the GPI assay ($K_e = 154$ nM) and an even more potent δ antagonist in the MVD assay ($K_e = 28.1$ nM). Whereas a neutral des-amino analogue of a μ agonist peptide had previously been found to be a μ opioid antagonist,[3] the data obtained with compound **11** demonstrated for the first time that elimination of the positive charge converted a δ agonist peptide into a δ opioid antagonist as well.

These results indicate that elimination of the positive charge through substitution of the N-terminal amino group with a methyl group resulted in a neutral ligand which was no longer capable of binding to and stabilizing an active conformation of the μ or δ receptor. Obviously, the presence on the ligand of a positively charged amino group capable of engaging in an electrostatic or amino–aromatic interaction with an appropriate receptor moiety or domain is required for the induction or stabilization of an active receptor conformation. There is good agreement between the receptor binding affinities of compounds **11** and **12** and their K_e and IC_{50} values determined in the functional assays. Peptide **11** is the most potent neutral δ opioid antagonist reported to date. Its δ receptor binding affinity is about 15 times higher than that of the well known δ opioid antagonist ICI 174,864 (*N,N*-diallyl-Tyr-Aib-Aib-Phe-Leu-OH).[12]

Table 1. Binding affinities of opioid peptide analogues at μ and δ receptors

Compound	K_i^μ [nM][a]	K_i^δ [nM][a]	K_i^μ/K_i^δ
11 (2*S*)-Mdp-D-Ala-Gly-Phe-Leu-NH_2	192±2	11.7±2.7	16.4
12 H-Dmt-D-Ala-Gly-Phe-Leu-NH_2	0.492±0.075	0.322±0.059	1.53
[Leu^5]enkephalin	9.43±2.07	2.53±0.35	3.73

[a]Mean of 3 determinations±SEM.

Table 2. GPI and MVD assays of opioid peptide analogues

Compound	GPI		MVD	
	IC_{50} [nM][a]	K_e [nM][a,b]	IC_{50} [nM][a]	K_e [nM][a,c]
11 (2*S*)-Mdp-D-Ala-Gly-Phe-Leu-NH_2		154±25		28.1±2.5
12 H-Dmt-D-Ala-Gly-Phe-Leu-NH_2	0.586±0.085		0.212±0.51	
[Leu^5]enkephalin	246±39		11.4±1.1	

[a]Mean of 3–6 determinations±SEM.
[b]Determined against the μ agonist TAPP (H-Tyr-D-Ala-Phe-Phe-NH_2).
[c]Determined against the δ agonist DPDPE (H-Tyr-c[D-Pen-Gly-Phe-D-Pen]OH).

A non-stereospecific synthesis of racemic (*R*,*S*)-Mdp and its substitution for Dmt in the dipeptide δ opioid antagonist H-Dmt-Tic-OH has recently been reported.[13] H-(*R*,*S*)-Mdp-Tic-OH showed very low δ and μ receptor binding affinities and it was not characterized in the functional GPI and MVD assays. The very low receptor binding affinities of this compound suggest that replacement of the N-terminal amino group with a methyl group has a more detrimental effect in the case of opioid peptides containing the N-terminal H-Dmt-Tic-pharmacophore than in the case of opioid peptides with a non-cyclic D-amino acid in the 2-position of the peptide sequence.

Acknowledgements

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References and Notes

1. Rees, D. C.; Hunter, J. C. In *Comprehensive Medicinal Chemistry*; Emmet, J. C., Ed.; Pergamon: New York, 1990; pp 805–846.
2. Befort, K.; Tabbara, L.; Bausch, S.; Chavkin, C.; Evans, C.; Kieffer, B. *Mol. Pharmacol.* **1996**, *49*, 216.
3. Schiller, P. W.; Berezowska, I.; Nguyen, T. M.-D.; Schmidt, R.; Lemieux, C.; Chung, N. N.; Falcone-Hindley, M. L.; Yao, W.; Liu, J.; Iwama, S.; Smith, A. B., III.; Hirschmann, R. *J. Med. Chem.* **2000**, *43*, 551.
4. Hansen, D. W.; Stapelfeld, A.; Savage, M. A.; Reichmann, M.; Hammond, D. L.; Haaseth, R. C.; Mosberg, H. I. *J. Med. Chem.* **1992**, *35*, 684.
5. Hünig, S.; Schwarz, H. *Liebigs Ann. Chem.* **1956**, *599*, 131.
6. Dygos, J. H.; Yonan, E.; Scaros, M. G.; Goodmonson, O. J.; Getman, D. P.; Periana, R. A.; Beck, G. R. *Synthesis* **1992**, 741.
7. Evans, D. A.; Gage, J. R.; Leighton, J. L. *J. Am. Chem. Soc.* **1992**, *114*, 9434.
8. Evans, D. A.; Ennis, M. D.; Mathre, D. J. *J. Am. Chem. Soc.* **1982**, *104*, 1737.
9. A single diastereomer of **8** was confirmed by HPLC analysis. Analytical reversed-phase HPLC chromatography was carried out on a Vydac 218-TP column (22×250 mm) with a linear gradient of 50–95% MeOH in aq 0.1% TFA at a flow rate of 1.0 mL/min (retention time = 24.46 min).
10. Physical data for **10**: $[\alpha]_D^{20}$ +45.9° (c 0.93, CH_2Cl_2); ^1H NMR (400 MHz, CD_3COCD_3) δ 6.5 (s, 2H), 2.94–3.00 (m, 1H), 2.61–2.68 (m, 2H), 2.24 (δ, 6H), 1.08 (d, 3H, J = 6.8 Hz); ^{13}C NMR (100.6 MHz, CD_3COCD_3) δ 177.7, 155.9, 138.6, 128.1, 115.8, 40.2, 33.0, 20.5, 16.8; HRMS (FAB) *m*/*e* calcd for $C_{12}H_{16}O_3$ $[M]^+$ 208.1099, found: 208.1095.
11. Schiller, P. W.; Fundytus, M. E.; Merovitz, L.; Weltrowska, G.; Nguyen, T. M.-D.; Lemieux, C.; Chung, N. N.; Coderre, T. J. *J. Med. Chem.* **1999**, *42*, 3520.
12. Corbett, A. D.; Gillan, M. G. C.; Kosterlitz, H. W.; McKnight, A. T.; Paterson, S. J.; Robson, L. E. *Br. J. Pharmacol.* **1984**, *83*, 271.
13. Salvadori, S.; Balboni, G.; Guerrini, R.; Tomatis, R.; Bianchi, C.; Bryant, S. D.; Cooper, P. S.; Lazarus, L. H. *J. Med. Chem.* **1997**, *40*, 3100.