

## 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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A synthesis of the novel tyrosine analogue (2*S*)-2-methyl-3-(2,6-dimethyl-4-carbamoylphenyl)propanoic acid [(2*S*)-Mdcp] (**15**) was developed. In (2*S*)-Mdcp, the amino and hydroxyl groups of 2',6'-dimethyltyrosine are replaced by a methyl and a carbamoyl group, respectively, and its substitution for Tyr<sup>1</sup> in opioid agonist peptides resulted in compounds showing antagonism at all three opioid receptors. The cyclic peptide (2*S*)-Mdcp-c[D-Cys-Gly-Phe(*p*NO<sub>2</sub>)-D-Cys]NH<sub>2</sub> (**1**) was a potent and selective  $\mu$  antagonist, whereas (2*S*)-Mdcp-c[D-Pen-Gly-Phe(*p*F)-Pen]-Phe-OH (**3**) showed subnanomolar  $\delta$  antagonist activity and extraordinary  $\delta$  selectivity.

### Introduction

Substitution of 2',6'-dimethyltyrosine (Dmt<sup>a</sup>) for the Tyr<sup>1</sup> residue in opioid peptides generally increases agonist potency by 1–2 orders of magnitude,<sup>1</sup> presumably due to additional hydrophobic binding interactions of the two methyl groups with the receptor. Deletion of the N-terminal amino group in Dmt<sup>1</sup>-opioid peptide analogues or its replacement with a methyl group produced potent opioid antagonists.<sup>2–4</sup> This was achieved through substitution of 3-(2,6-dimethyl-4-hydroxyphenyl)propanoic acid (Dhp) or (2*S*)-2-methyl-3-(2,6-dimethyl-4-hydroxyphenyl)propanoic acid ((2*S*)-Mdp, Figure 1) for Dmt<sup>1</sup>. Replacement of the Tyr<sup>1</sup> hydroxyl group in opioid peptides with a carbamoyl (–CONH<sub>2</sub>) group resulted in compounds that retained high opioid agonist potency.<sup>5,6</sup> This interesting observation prompted the replacement of the hydroxyl group of Dhp<sup>1</sup> in opioid peptide antagonists with a carbamoyl group, as achieved by substitution of 3-(2,6-dimethyl-4-carbamoylphenyl)propanoic acid (Dcp) for Dhp.<sup>7</sup> The Dcp<sup>1</sup>-analogue of the cyclic enkephalin agonist peptide H-Tyr-c[D-Cys-Gly-Phe(*p*NO<sub>2</sub>)-D-Cys]NH<sub>2</sub><sup>8</sup> showed high  $\mu$  opioid antagonist activity, whereas

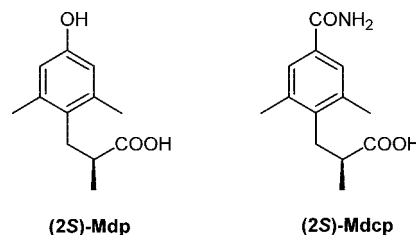


Figure 1. Chemical structures of (2*S*)-Mdp and (2*S*)-Mdcp.

[Dcp<sup>1</sup>]dynorphin A(1–11)-NH<sub>2</sub> was a moderately potent  $\kappa$  opioid antagonist.<sup>7</sup>

Because (2*S*)-Mdp<sup>1</sup>-analogues of opioid peptides are more potent antagonists than their corresponding Dhp<sup>1</sup>-analogues,<sup>3</sup> it is of interest to determine how the replacement of the (2*S*)-Mdp hydroxyl group in (2*S*)-Mdp<sup>1</sup>-containing opioid peptide antagonists with a –CONH<sub>2</sub> group would affect the in vitro opioid activity profile. This requires the substitution of (2*S*)-2-methyl-3-(2,6-dimethyl-4-carbamoylphenyl)propanoic acid [(2*S*)-Mdcp] for (2*S*)-Mdp (Figure 1). Here, we describe the stereoselective synthesis of (2*S*)-Mdcp. The (2*S*)-Mdcp<sup>1</sup>-analogues of the nonselective cyclic opioid peptide H-Tyr-c[D-Cys-Gly-Phe(*p*NO<sub>2</sub>)-D-Cys]NH<sub>2</sub>, the  $\delta$ -selective enkephalin analogue H-Tyr-c[D-Pen-Gly-Phe(*p*F)-Pen]-Phe-OH<sup>9</sup> and dynorphin A(1–11)-NH<sub>2</sub> ( $\kappa$ -selective) were prepared and pharmacologically characterized in vitro.

The preparation of Mdcp (**15**) is illustrated in Scheme 1. 3,5-Dimethyl-4-nitrobenzoic acid **7** was synthesized from mesitylene in two steps following literature procedures.<sup>10,11</sup> Subsequent esterification and reduction afforded ethyl 4-amino-3,5-dimethylbenzoate (**8**),<sup>12</sup> which was next converted to ethyl 4-iodo-3,5-dimethylbenzoate (**9**) via a Sandmeyer reaction.<sup>13</sup> Basic hydrolysis and re-esterification provided the advanced intermediate benzyl 4-iodo-3,5-dimethyl benzoate (**11**). The organozinc reagent, prepared by sonicating a mixture of (*R*)-methyl 3-iodo-2-methylpropanoate and Zn–Cu couple, underwent palladium-mediated coupling reaction with **11** to afford diester (**12**).<sup>14</sup> Catalytic hydrogenolysis of **12** yielded free benzoic acid **13**, which was converted to amide **14**. Final basic hydrolysis gave

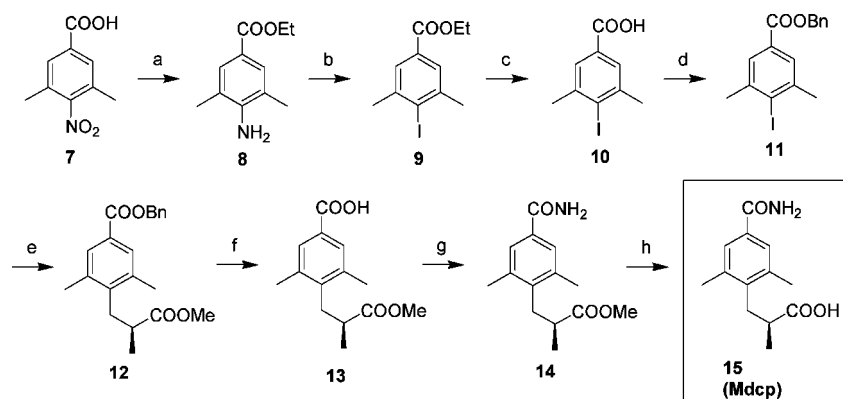
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<sup>a</sup> Abbreviations: Acn, acetamidomethyl; CTOP, H-D-Phe-c[Cys-Tyr-D-Trp-Orn-Thr-Pen]-Thr-NH<sub>2</sub>; DAMGO, H-Tyr-D-Ala-Gly-NaMePhe-Gly-OH; Dcp, 3-(2,6-dimethyl-4-carbamoylphenyl)propanoic acid; Dhp, 3-(2,6-dimethyl-4-hydroxyphenyl)propanoic acid; DIC, diisopropylcarbodiimide; DIEA, *N,N*-diisopropylethylamine; Dmt, 2',6'-dimethyltyrosine; DPDPE, H-Tyr-c[D-Pen-Gly-Phe-D-Pen]OH; DSLET, H-Tyr-D-Ser-Gly-Phe-Leu-Thr-OH; Dyn A, dynorphin A; GPI, guinea pig ileum; HBTU, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; (2*S*)-Mdcp, (2*S*)-2-methyl-3-(2,6-dimethyl-4-carbamoylphenyl)propanoic acid; (2*S*)-Mdp, (2*S*)-2-methyl-3-(2,6-dimethyl-4-hydroxyphenyl)propanoic acid; Mob, methoxybenzyl; MVD, mouse vas deferens; NMM, *N*-methylmorpholine; Pen, penicillamine; TAPP, H-Tyr-D-Ala-Phe-Phe-NH<sub>2</sub>; TFA, trifluoroacetic acid; Tic, tetrahydroisoquinoline-3-carboxylic acid; U50,488, *trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]-benzeneacetamide; U69,593, (5 $\alpha$ ,7 $\alpha$ ,8 $\beta$ -(–)-*N*-methyl-*N*-[7-pyrrolidinyl]-1-oxaspiro[4.5]dec-8-yl]benzeneacetamide.

Scheme 1<sup>a</sup>

<sup>a</sup> Reagents and conditions: (a) EtOH, dry HCl(g), reflux 6 h, followed by Sn powder, 40 °C, 4 h, 82%; (b) NaNO<sub>2</sub>, conc HCl, KI, acetone, 83%; (c) LiOH, THF-MeOH-H<sub>2</sub>O, 0 °C to rt, 95%; (d) K<sub>2</sub>CO<sub>3</sub>, BnBr, DMF, rt, 6 h, 92%; (e) Zn-Cu couple, (*R*)-methyl 3-iodo-2-methylpropanoate, PdCl<sub>2</sub>[P(*o*-Tol)<sub>3</sub>]<sub>2</sub>, benzene, 52%; (f) Pd/C, H<sub>2</sub>(g), MeOH, rt, overnight, 96%; (g) (COCl)<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>, rt, 2 h then NH<sub>4</sub>OH (25%), 0 °C to rt, 2 h, 87%; (h) LiOH (1N)-THF (1:1), 0 °C, 2.5 h, 78%.

**Table 1.** Antagonist Potencies ( $K_e$  Values) of (2*S*)-MdcP<sup>1</sup>- and (2*S*)-Mdp<sup>1</sup>-Analogues of Opioid Peptides<sup>a</sup>

| no. | antagonist   | GPI                            |                                   | MVD                               | $K_e$ ratio         |                     |
|-----|--|--------------------------------|-----------------------------------|-----------------------------------|---------------------|---------------------|
|     |  | $K_e^\mu$<br>(nM) <sup>b</sup> | $K_e^\kappa$<br>(nM) <sup>c</sup> | $K_e^\delta$<br>(nM) <sup>d</sup> | $\mu/\delta/\kappa$ | $\delta/\mu/\kappa$ |
| 1   | (2 <i>S</i> )-MdcP-c[D-Cys-Gly-Phe( <i>p</i> NO <sub>2</sub> )-D-Cys]NH <sub>2</sub>             | 1.24 ± 0.23                    | 18.9 ± 0.3                        | 9.78 ± 1.16                       | 1/8/15              |                     |
| 2   | (2 <i>S</i> )-Mdp-c[D-Cys-Gly-Phe( <i>p</i> NO <sub>2</sub> )-D-Cys]NH <sub>2</sub> <sup>e</sup> | 1.48 ± 0.18                    | 6.41 ± 0.18                       | 3.48 ± 0.28                       | 1/2/4               |                     |
| 3   | (2 <i>S</i> )-MdcP-c[D-Pen-Gly-Phe( <i>p</i> F)-Pen]-Phe-OH                                      | 1170 ± 100                     | 9980 ± 1650                       | 0.326 ± 0.030                     |                     | 1/3590/30600        |
| 4   | (2 <i>S</i> )-Mdp-c[D-Pen-Gly-Phe( <i>p</i> F)-Pen]-Phe-OH <sup>f</sup>                          | 668 ± 40                       | 4370 ± 860                        | 0.785 ± 0.135                     |                     | 1/851/5570          |
| 5   | [(2 <i>S</i> )-MdcP <sup>1</sup> ]Dyn A(1-11)-NH <sub>2</sub>                                    | 401 ± 62                       | 5.96 ± 0.80                       | 843 ± 88                          | 1/2/0.015           |                     |
| 6   | [(2 <i>S</i> )-Mdp <sup>1</sup> ]Dyn A(1-11)-NH <sub>2</sub> <sup>g</sup>                        | 925 ± 94                       | 3.92 ± 0.65                       | 3320 ± 520                        | 1/4/0.004           |                     |

<sup>a</sup> Values represent means of 3–6 determinations. <sup>b</sup> Determined with TAPP as  $\mu$  agonist. <sup>c</sup> Determined with U50488 as  $\kappa$  agonist. <sup>d</sup> Determined with DPDPE as  $\delta$  agonist. <sup>e</sup> Data taken from Schiller et al.<sup>2</sup> <sup>f</sup> Data taken from Schiller et al.<sup>4</sup> <sup>g</sup> Data taken from Lu et al.<sup>3</sup>

**Table 2.** Opioid Receptor Binding Affinities of (2*S*)-MdcP<sup>1</sup>- and (2*S*)-Mdp<sup>1</sup>-Analogues of Opioid Peptides<sup>a</sup>

| no. | antagonist   | $K_i^\mu$<br>(nM) | $K_i^\kappa$<br>(nM) | $K_i^\delta$<br>(nM) | $K_i$ ratio         |                     |
|-----|--|-------------------|----------------------|----------------------|---------------------|---------------------|
|     |  |                   |                      |                      | $\mu/\delta/\kappa$ | $\delta/\mu/\kappa$ |
| 1   | (2 <i>S</i> )-MdcP-c[D-Cys-Gly-Phe( <i>p</i> NO <sub>2</sub> )-D-Cys]NH <sub>2</sub>             | 1.02 ± 0.15       | 185 ± 32             | 19.0 ± 1.5           | 1/19/181            |                     |
| 2   | (2 <i>S</i> )-Mdp-c[D-Cys-Gly-Phe( <i>p</i> NO <sub>2</sub> )-D-Cys]NH <sub>2</sub> <sup>b</sup> | 2.23 ± 0.08       | 92.8 ± 5.3           | 3.61 ± 0.41          | 1/2/42              |                     |
| 3   | (2 <i>S</i> )-MdcP-c[D-Pen-Gly-Phe( <i>p</i> F)-Pen]-Phe-OH                                      | 5850 ± 300        | 3650 ± 650           | 2.92 ± 0.23          |                     | 1/2000/1250         |
| 4   | (2 <i>S</i> )-Mdp-c[D-Pen-Gly-Phe( <i>p</i> F)-Pen]-Phe-OH <sup>c</sup>                          | 406 ± 21          | 3710 ± 400           | 2.32 ± 0.40          |                     | 1/175/1600          |
| 5   | [(2 <i>S</i> )-MdcP <sup>1</sup> ]Dyn A(1-11)-NH <sub>2</sub>                                    | 29.6 ± 6.8        | 26.0 ± 4.1           | 27.2 ± 1.0           | 1/1/1               |                     |
| 6   | [(2 <i>S</i> )-Mdp <sup>1</sup> ]Dyn A(1-11)-NH <sub>2</sub> <sup>d</sup>                        | 213 ± 50          | 0.823 ± 0.162        | 163 ± 15             | 1/1/0.004           |                     |

<sup>a</sup> Values represent means of 3–6 determinations. <sup>b</sup> Data taken from Schiller et al.<sup>2</sup> <sup>c</sup> Data taken from Schiller et al.<sup>4</sup> <sup>d</sup> Data taken from Lu et al.<sup>3</sup>

the desired (2*S*)-MdcP (**15**). Peptides were prepared by standard solid-phase and solution synthesis techniques.

## Results and Discussion

The cyclic pentapeptide analogue (2*S*)-MdcP-c[D-Cys-Gly-Phe(*p*NO<sub>2</sub>)-D-Cys]NH<sub>2</sub> (**1**) displayed antagonist activity at all three opioid receptors ( $\mu$ ,  $\delta$ ,  $\kappa$ ), as determined in the functional in vitro assays (Table 1). In the GPI assay, it showed very high  $\mu$  opioid antagonist activity ( $K_e^\mu = 1.24 \pm 0.23$  nM) and considerably lower antagonist potency at the  $\kappa$  opioid receptor ( $K_e^\kappa = 18.9 \pm 0.3$  nM). Its  $\delta$  antagonist activity determined in the MVD assay ( $K_e^\delta = 9.78 \pm 1.16$  nM) was also weaker. As indicated by the calculated  $K_e$  ratios (Table 1), compound **1** turned out to be a quite selective  $\mu$  opioid antagonist. In agreement with its high  $\mu$  opioid antagonist activity determined in the GPI assay, cyclic peptide **1** showed high  $\mu$  opioid receptor binding affinity in the rat brain membrane binding assay ( $K_i^\mu = 1.02 \pm 0.15$  nM) (Table 2). The  $\mu$  receptor selectivity of this compound was confirmed by its relatively weak  $\delta$  and  $\kappa$  receptor affinities determined in the binding assays. These results indicate that (2*S*)-MdcP-c[D-Cys-Gly-Phe(*p*NO<sub>2</sub>)-D-Cys]NH<sub>2</sub> (**1**) is a

slightly more potent  $\mu$  antagonist than (2*S*)-Mdp-c[D-Cys-Gly-Phe(*p*NO<sub>2</sub>)-D-Cys]NH<sub>2</sub> (**2**) (Tables 1 and 2). Furthermore, the (2*S*)-MdcP<sup>1</sup>-analogue (**1**) is  $\mu$ -selective, whereas the (2*S*)-Mdp<sup>1</sup>-analogue has almost no  $\mu$  vs  $\delta$  selectivity.

The cyclic hexapeptide analogue (2*S*)-MdcP-c[D-Pen-Gly-Phe(*p*F)-Pen]-Phe-OH (**3**) showed subnanomolar  $\delta$  opioid antagonist activity ( $K_e^\delta = 0.326 \pm 0.30$  nM) in the MVD assay (Table 1). It displayed very weak  $\mu$  and  $\kappa$  antagonist activity in the GPI assay. As indicated by the selectivity ratios based on the  $K_e$  values, it is an extraordinarily selective  $\delta$  opioid antagonist with higher  $\delta$  selectivity than the corresponding (2*S*)-Mdp<sup>1</sup> analogue (**4**).<sup>4</sup> In agreement with these results, peptide **3** showed very high  $\delta$  receptor binding affinity and extraordinary  $\delta$  receptor selectivity in the opioid receptor binding assays (Table 2). The selectivity ratios based on the receptor binding constants ( $K_i$  values) indicate that the (2*S*)-MdcP<sup>1</sup>-analogue (**3**) has higher  $\delta$  selectivity than the (2*S*)-Mdp<sup>1</sup>-analogue (**4**), in confirmation of its higher  $\delta$  selectivity established in the functional assays ( $K_e$  value ratios).

In the functional assays, [(2*S*)-MdcP<sup>1</sup>]Dyn A(1-11)-NH<sub>2</sub> (**5**) showed slightly lower  $\kappa$  antagonist potency than [(2*S*)-

Mdp<sup>1</sup>]Dyn A(1–11)-NH<sub>2</sub> (**6**) and somewhat lower  $\kappa$  receptor selectivity, as determined from the  $K_e$  selectivity ratios (Table 1). In the opioid receptor binding assays, compound **5** displayed about 30-fold lower  $\kappa$  receptor binding affinity than **6** and, unlike **6**, was nonselective. The opioid receptor binding affinities of [(2*S*)-Mdcpl]Dyn A(1–11)-NH<sub>2</sub> (**5**) are similar to those of [Dcp<sup>1</sup>]Dyn A(1–11)-NH<sub>2</sub>, which also lacks opioid receptor binding selectivity.<sup>7</sup> The discrepancies between the antagonist activities ( $K_e$  values) of [(2*S*)-Mdcpl]Dyn A(1–11)-NH<sub>2</sub> determined in the functional assays and its receptor affinities measured in the binding assays may be due to differences in structural requirements for ligand binding between opioid receptors in the peripheral tissue preparations and brain opioid receptors. Such discrepancies have previously been observed on several occasions.

In conclusion, replacement of Tyr<sup>1</sup> with (2*S*)-Mdcpl in three opioid agonist peptides with different opioid receptor selectivity resulted in compounds with antagonist activity at all three opioid receptors ( $\mu$ ,  $\delta$ ,  $\kappa$ ). It is noteworthy that (2*S*)-Mdcpl substitution produced opioid receptor selectivity profiles that were distinct among the three peptides investigated and, in some cases, were also distinct from the profiles shown by the corresponding (2*S*)-Mdp<sup>1</sup>-analogues. This may be due to differences between the –OH and –CONH<sub>2</sub> substituent of the 1-position residue with regard to steric bulk or the H-bonding pattern with receptor moieties. The cyclic peptide (2*S*)-Mdcpl-c[D-Cys-Gly-Phe(*p*NO<sub>2</sub>)-D-Cys]NH<sub>2</sub> (**1**) is another example of an opioid peptide-derived  $\mu$  antagonist. It shows high  $\mu$  antagonist potency and marked  $\mu$  receptor binding selectivity (Table 2) but is less  $\mu$ -selective than the somatostatin-derived  $\mu$  opioid antagonists (e.g., CTOP; IC<sub>50</sub> $\mu$  = 2.8 nM, IC<sub>50</sub> $\delta$  = 13500 nM<sup>15</sup>). It has 2–3-fold higher  $\mu$  antagonist activity than Dcp-c[D-Cys-Gly-Phe(*p*NO<sub>2</sub>)-D-Cys]NH<sub>2</sub><sup>7</sup> and similar  $\mu$  receptor binding selectivity, and is a more potent and more selective  $\mu$  antagonist than Dhp-c[N <sup>$\epsilon$</sup> ,N <sup>$\beta$</sup> -carbonyl-D-Lys<sup>2</sup>,Dap<sup>5</sup>]enkephalinamide ( $K_i^\mu$  = 15.5 nM,  $K_i^\delta$  = 273 nM).<sup>4</sup> Furthermore, compound **1** has higher  $\mu$  antagonist potency and higher  $\mu$  receptor binding selectivity than the endomorphin-derived  $\mu$  antagonists antanal-1 ([Dmt<sup>1</sup>,D-2-Nal<sup>4</sup>]endomorphin-1;  $K_i^\mu$  = 2.38 nM,  $K_i^\delta$  = 17.4 nM) and antanal-2 ([Dmt<sup>1</sup>,D-2-Nal<sup>4</sup>]endomorphin-2;  $K_i^\mu$  = 1.52 nM,  $K_i^\delta$  = 7.74 nM),<sup>16</sup> and higher  $\mu$  antagonist potency but lower  $\mu$ -selectivity than the tetrapeptide H-Dmt-Sar-Phe-D-Nal-NH<sub>2</sub> ( $K_e^\mu$  = 2.34 nM,  $K_e^\delta$  = 305 nM).<sup>17</sup> These various opioid peptide-derived  $\mu$  antagonists are of interest because their mode of binding to the  $\mu$  opioid receptor is likely to be different from that of the somatostatin-derived  $\mu$  antagonists. The cyclic hexapeptide (2*S*)-Mdcpl-c[D-Pen-Gly-Phe(*p*F)-Pen]-Phe-OH (**3**) is a  $\delta$  opioid antagonist with very high  $\delta$  antagonist potency and extraordinary  $\delta$  opioid receptor selectivity. Together with some of the Tic<sup>2</sup>-containing opioid antagonists (for a review, see ref 18) and naltrindole,<sup>19</sup> it ranks among the most potent and most selective  $\delta$  opioid antagonists reported to date. Finally, it should be pointed out that the novel opioid peptide antagonists described here are more lipophilic than their corresponding parent peptides because they contain a methyl group in place of the positively charged N-terminal amino group. For this reason, they can be expected to have an improved ability to cross the blood-brain barrier.

## Experimental Section

**Chemistry. Synthesis of Mdcpl (15).** Ethyl 4-Amino-3,5-dimethylbenzoate (**8**). 3,5-Dimethyl-4-nitrobenzoic acid (**7**) (12.3 g, 63.1 mmol) was dissolved in absolute ethanol (75 mL), and

dry HCl gas was passed into the solution for 20 min. The resulting mixture was refluxed for 5 h, then cooled and passed with HCl gas for another 20 min and refluxed for 5 h. After the reaction mixture was cooled down to room temperature, tin (22.5 g, 189.4 mmol) was added slowly, maintaining the temperature of the mixture between 35 and 40 °C. The mixture was then allowed to stand for 4 h and basified with aqueous NaOH (30%) and extracted with Et<sub>2</sub>O (3 × 300 mL). The combined ether layers were washed with brine, dried, and concentrated. The residue was purified by flash column chromatography to give **8** as a light-yellow solid (10 g, 82%); mp 67.3–68.4 °C. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  7.65 (s, 2H), 4.27–4.34 (q, 2H,  $J$  = 7.2 Hz), 2.18 (s, 6H), 1.33–1.38 (t, 3H,  $J$  = 7.08 Hz). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  167.1, 147.2, 129.9, 120.3, 119.1, 60.1, 17.3, 14.3. HRMS (ESI) *m/e* calcd for C<sub>11</sub>H<sub>14</sub>O<sub>2</sub>N [M – H]<sup>–</sup> 192.1024; obsd, 192.1026.

**Ethyl 4-Iodo-3,5-dimethylbenzoate (9).** To a solution of **8** (7.65 g, 39.6 mmol) in acetone (50 mL) was added concentrated HCl (250 mL) at room temperature. The resulting solution was cooled to 0 °C, and a solution of NaNO<sub>2</sub> (3.58 g, 51.9 mmol) in H<sub>2</sub>O (25 mL) was added dropwise. The mixture was stirred at 0 °C for 2 h, then a solution of KI (19.72 g, 118.8 mmol) in H<sub>2</sub>O (50 mL) was added. After 6 h, the solution was extracted with Et<sub>2</sub>O (3 × 300 mL). The combined ether layers were washed with brine, dried, and concentrated. The residue was purified by flash column chromatography to afford **9** as a yellowish solid (10 g, 83%); mp 41.2–43.2 °C. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  7.68 (s, 2H), 4.32–4.39 (q, 2H,  $J$  = 7.05 Hz), 2.51 (s, 6H), 1.36–1.41 (t, 3H,  $J$  = 7.05 Hz). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  166.3, 142.4, 129.6, 127.3, 114.3, 61.0, 29.6, 14.2. HRMS (ESI) *m/e* calcd for C<sub>11</sub>H<sub>14</sub>O<sub>2</sub>I [M + H]<sup>+</sup> 305.0039; obsd, 305.0033.

**4-Iodo-3,5-dimethylbenzoic acid (10).** To a solution of **9** (9.39 g, 30.9 mmol) in a mixture of THF (45 mL) and MeOH (30 mL) at 0 °C was added LiOH (2.22 g, 92.6 mmol) dissolved in H<sub>2</sub>O (30 mL). The resultant solution was allowed to warm up to room temperature. After stirring for 4 h, the organic solvents were removed and the aqueous phase was neutralized with precooled aqueous HCl (1 N) at 0 °C, and extracted with EtOAc (2 × 100 mL). The combined EtOAc extracts were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated to yield **10** as a white solid (8.1 g), which was directly used for the next step without further purification.

**Benzyl 4-Iodo-3,5-dimethylbenzoate (11).** To a solution of crude **10** obtained from the previous step in dry DMF (40 mL) was added K<sub>2</sub>CO<sub>3</sub> (6.0 g, 44.0 mmol), followed by benzyl bromide (3.6 mL, 29.9 mmol) at room temperature under nitrogen atmosphere. The resulting mixture was stirred for 6 h. Water (350 mL) was then added, and the mixture was extracted with EtOAc (2 × 200 mL). The combined EtOAc extracts were washed with brine, dried, and concentrated. The crude product was purified by flash column chromatography to afford **11** as a light-yellow solid (9.8 g, 87% for two steps); mp 56.8–57.9 °C. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  7.76 (s, 2H), 7.30–7.48 (m, 5H), 5.39 (s, 2H), 2.55 (s, 6H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  166.2, 142.5, 135.9, 129.3, 128.5, 128.3, 128.2, 127.4, 114.7, 66.7, 29.6. HRMS (ESI) *m/e* calcd for C<sub>16</sub>H<sub>15</sub>O<sub>2</sub> [M<sup>+</sup>] 366.0117; obsd, 366.0127.

**(*S*)-Benzyl 4-(3-Methoxy-2-methyl-3-oxopropyl)-3,5-dimethylbenzoate (12).** Preparation of (*R*)-methyl 3-iodo-2-methylpropanoate: To a solution of (*S*)-methyl 2-(hydroxymethyl)propanoate (1.18 g, 10 mmol) in dichloromethane (30 mL) at 0 °C were added triphenylphosphine (10.49 g, 40 mmol), imidazole (2.72 g, 40 mmol), and iodine (7.62 g, 30 mmol). The mixture was warmed up to room temperature and stirred for 4 h. The mixture was then washed with aqueous sodium thiosulfate solution (5%) and extracted with ethyl acetate (2 × 40 mL). The combined organic extracts were concentrated, and the residue was purified by silica gel flash chromatography (5% hexane in ethyl acetate) to afford the desired iodide as a colorless oil (1.80 g, 79%). [ $\alpha$ ]<sub>D</sub> = +24.0° ( $c$  = 2.1, CHCl<sub>3</sub>). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  3.72 (s, 3H), 3.23–3.40 (m, 2H),



2.76–2.83 (m, 1H), 1.27 (d, 3H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  173.7, 52.0, 42.1, 18.1, 6.8.

A solution of (*R*)-methyl 3-iodo-2-methylpropanoate (6.60 g, 28.9 mmol) in anhydrous benzene (96 mL) and DMA (6.4 mL) was added to a dry nitrogen-purged flask charged with zinc–copper couple (3.47 g). The resulting mixture was sonicated under nitrogen for 40 min. Bis(*tri-*o**-tolylphosphine) palladium dichloride (1.12 g, 1.28 mmol) was added followed by **11** (8.82 g, 24.1 mmol). The resulting mixture was stirred under nitrogen at 55 °C for 2 h and then allowed to cool down to room temperature. Ethyl acetate (400 mL) was added and the mixture filtered into a separating funnel. The mixture was washed with aqueous hydrochloric acid (0.1 N) (400 mL) and distilled water (3 × 100 mL), and dried, filtered, and concentrated. The crude product was purified by flash chromatography to afford the desired product **12** as a light-yellow oil (4.27 g, 52%).  $[\alpha]_{\text{D}}^{20} +39.5^\circ$  (*c* 0.22,  $\text{CHCl}_3$ ).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.71 (s, 2H), 7.36–7.43 (m, 7H), 5.34 (s, 2H), 3.63 (s, 3H), 3.05–3.12 (a, 1H), 2.71–2.86 (m, 2H), 2.36 (s, 6H), 1.14 (d, 3H, *J* = 6.9 Hz).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ) 176.5, 166.6, 141.9, 137.1, 136.2, 129.4, 128.5, 128.19, 129.17, 127.7, 66.5, 51.7, 39.1, 33.2, 20.2, 16.5. HRMS (ESI) *m/e* calcd for  $\text{C}_{21}\text{H}_{24}\text{O}_4\text{Na}$  [*M* + *Na*] $^+$  363.1573; obsd, 363.1573.

**(S)-4-(3-Methoxy-2-methyl-3-oxopropyl)-3,5-dimethylbenzoic acid (13)**. To a solution of **12** (4.1 g, 12.1 mmol) in MeOH (20 mL), 10% Pd/C (240 mg) was added, and the mixture was stirred overnight under atmospheric pressure of  $\text{H}_2$ . The reaction mixture was then filtered through a short pad of celite, and the filter cake was washed with EtOAc (2 × 20 mL). The filtrate and washings were combined and concentrated in vacuo to give **13** as a white solid (2.9 g, 96%); mp 128.4–129.9 °C.  $[\alpha]_{\text{D}}^{20} +57.3^\circ$  (*c* 0.20,  $\text{CHCl}_3$ ).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.78 (s, 2H), 3.68 (s, 3H), 3.11–3.18 (q, 1H), 2.77–2.92 (m, 2H), 2.42 (s, 6H), 1.20 (d, 3H, *J* = 6.72 Hz).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  176.5, 172.1, 142.8, 137.2, 129.9, 126.9, 51.7, 39.0, 33.2, 20.2, 16.5. HRMS (ESI) *m/e* calcd for  $\text{C}_{14}\text{H}_{17}\text{O}_4$  [*M* – *H*] $^-$  249.1205; obsd, 249.1126.

**(S)-Methyl 3-(4-Carbamoyl-2,6-dimethylphenyl)-2-methylpropanoate (14)**. Oxalyl chloride (2.76 mL, 31.7 mmol) was added slowly to a solution of **13** (2.64 g, 10.6 mmol) in  $\text{CH}_2\text{Cl}_2$  (20 mL) at room temperature under nitrogen atmosphere, and slow gas formation was observed. DMF (20  $\mu\text{L}$ ) was then added, accelerating gas evolution considerably. After stirring at room temperature for 2 h, organic solvent was removed, and the brown oily residue was dissolved in THF (20 mL). Aqueous  $\text{NH}_4\text{OH}$  (25%) (23 mL) was then added to the reaction mixture at 0 °C and stirred at the same temperature for 30 min. The reaction mixture was then acidified with HCl (1 N) at 0 °C, and extracted with EtOAc (2 × 50 mL). After concentration, the residue was purified by flash chromatography to give **14** as a white solid (2.3 g, 87%); mp 91.8–93.2 °C;  $[\alpha]_{\text{D}}^{20} +58.5^\circ$  (*c* 0.22,  $\text{CHCl}_3$ ).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.44 (s, 2H), 6.0 (br s, 1H), 5.76 (br s, 1H), 3.63 (s, 3H), 3.05–3.10 (q, 1H), 2.74–2.85 (m, 2H), 2.36 (s, 6H), 1.15 (d, 3H, *J* = 6.75 Hz).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  176.5, 169.5, 140.8, 137.3, 130.8, 127.1, 51.7, 39.1, 33.1, 20.3, 16.5. HRMS (ESI) *m/e* calcd for  $\text{C}_{14}\text{H}_{19}\text{O}_3\text{NNa}$  [*M* + *Na*] $^+$  272.1263; obsd, 272.1256.

**(2S)-2-Methyl-3-(2,6-dimethyl-4-carbamoylphenyl)propanoic Acid [(2S)-MdcP] (15)**. To a solution of **14** (2.03 g, 8.16 mmol) in THF (50 mL) was added an aqueous solution of LiOH (1N, 50 mL) at 0 °C. After stirring at this temperature for 2.5 h, the organic solvent was removed and the aqueous phase was neutralized with precooled HCl (1 N) at 0 °C, and extracted with EtOAc (2 × 75 mL). The combined EtOAc extracts were washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , and concentrated to yield **15** as a white solid (1.5 g, 78%); mp 224.1–225.9 °C.  $[\alpha]_{\text{D}}^{20} +64.2^\circ$  (*c* 0.25, MeOH);  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  12.17 (br s, 1H), 7.80 (s, 1H), 7.51 (s, 2H), 7.18 (s, 1H), 2.96–3.03 (q, 1H), 2.50–2.74 (m, 2H), 2.32 (s, 6H), 1.04 (d, 3H, *J* = 6.72 Hz).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  177.0, 167.9, 139.8, 136.3,

131.6, 127.1, 79.1, 32.6, 19.9, 16.6. HRMS (ESI) *m/e* calcd for  $\text{C}_{13}\text{H}_{17}\text{O}_3\text{NNa}$  [*M* + *Na*] $^+$  258.1106; obsd, 258.1088.

**Peptide Synthesis. (2S)-MdcP-c[D-Cys-Gly-Phe(pNO<sub>2</sub>)-D-Cys]NH<sub>2</sub> (1)**. The cyclic tetrapeptide H-c[D-Cys-Gly-Phe(pNO<sub>2</sub>)-D-Cys]NH<sub>2</sub> was prepared by the manual solid-phase technique as described elsewhere.<sup>7</sup> To a solution of (2S)-MdcP (**15**) (37.7 mg, 0.16 mmol), HBTU (62.7 mg, 0.16 mmol), and *N,N*-diisopropylethylamine (DIEA) (91.0  $\mu\text{L}$ , 0.82 mmol) in 5 mL DMF were added H-c[D-Cys-Gly-Phe(pNO<sub>2</sub>)-D-Cys]NH<sub>2</sub> × TFA (90.0 mg, 0.15 mmol) and NMM (16.5  $\mu\text{L}$ , 0.15 mmol). After stirring for 30 min, the solvent was evaporated to dryness in vacuo, and the residue was extracted with 20 mL of AcOEt. After washing with 5%  $\text{KHSO}_4$ , saturated  $\text{NaHCO}_3$ , and brine, the organic phase was dried ( $\text{MgSO}_4$ ), filtered, and evaporated to dryness in vacuo. The peptide was purified by reversed-phase HPLC. HPLC *K'* 3.75; TLC *R<sub>f</sub>* 0.65 (I), *R<sub>f</sub>* 0.86 (II), *R<sub>f</sub>* 0.16 (III); MS [*M* + *H*] $^+$  688.

**(2S)-MdcP-c[D-Pen-Gly-Phe(pF)-Pen]-Phe-OH (3)**. The linear precursor peptide was prepared by the manual solid-phase technique using Boc-protection of the  $\alpha$ -amino group and Mob protection of the Pen residues and DIC/HOBt as coupling agents. The peptide was assembled on a polystyrene-divinylbenzene (1%) resin (200–400 mesh) (Boc-Phe-resin, 0.65 equiv/g, Bachem Bioscience, King of Prussia, PA) according to a published protocol.<sup>3</sup> The peptide was cleaved from the resin and deprotected by HF/anisole treatment in the usual manner. After evaporation of the HF, the resin was extracted three times with  $\text{Et}_2\text{O}$  and subsequently three times with glacial AcOH. The peptide was obtained in solid form through lyophilization of the acetic acid extract. For disulfide bond formation, the peptide (150 mg) dissolved in 20 mL MeOH was slowly added to a solution of  $\text{K}_3\text{Fe}(\text{CN})_6$  (293 mg) in 750 mL ammonium acetate buffer (0.05N, pH 8.5) over a period of 20 h. After lowering the pH to 4.5 by addition of AcOH, the solution was treated with Amberlite IRA-400 (Cl). After subsequent filtration, solvent evaporation, and dissolution of the residue in AcOH, the product was obtained in solid form through lyophilization and was purified by preparative reversed-phase HPLC. HPLC *K'* 5.83; TLC *R<sub>f</sub>* 0.88 (I), *R<sub>f</sub>* 0.81 (II), *R<sub>f</sub>* 0.30 (III), MS [*M* + *H*] $^+$  865.

**[(2S)-MdcP]<sup>1</sup>Dyn A(1–11)-NH<sub>2</sub> (5)**. The peptide was prepared by the manual solid-phase technique by using the protocol described above for the synthesis of the linear precursor peptide of **3**. Side chain protection was as follows: tosyl (Arg) and 2-chlorobenzoyloxycarbonyl (Lys). HPLC *K'* 3.78; TLC *R<sub>f</sub>* 0.38 (II), *R<sub>f</sub>* 0.32 (IV); MS [*M* + *H*] $^+$  1414.

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**Supporting Information Available:** Experimental details and refs 20–26. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## References

- (1) Hansen, D. W., Jr.; Stapelfeld, A.; Savage, M. A.; Reichman, M.; Hammond, D. L.; Haaseth, R. C.; Mosberg, H. I. Systemic Analgesic Activity and  $\delta$ -Opioid Selectivity in [2,6-Dimethyl-Tyr<sup>1</sup>,D-Pen<sup>2</sup>,D-Pen<sup>5</sup>]Enkephalin. *J. Med. Chem.* **1992**, *35*, 684–687.
- (2) Schiller, P. W.; Lu, Y.; Weltrowska, G.; Berezowska, I.; Wilkes, B. C.; Nguyen, T. M.-D.; Chung, N. N.; Lemieux, C. A General New Concept for the Development of Opioid Peptide Derived  $\mu$ -,  $\delta$ - and  $\kappa$  Antagonists. In *Peptides: The Wave of the Future, Proceedings of the 2nd International Peptide Symposium/17th American Peptide Symposium*; Lebl, M., Houghten, R. A., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2001; pp 676–678.
- (3) Lu, Y.; Nguyen, T. M.-D.; Weltrowska, G.; Berezowska, I.; Lemieux, C.; Chung, N. N.; Schiller, P. W. [2',6'-Dimethyltyrosine]dynorphin A(1–11)-NH<sub>2</sub> Analogues Lacking an N-Terminal Amino Group: Potent and Selective  $\kappa$  Opioid Antagonists. *J. Med. Chem.* **2001**, *44*, 3048–3053.
- (4) Schiller, P. W.; Weltrowska, G.; Nguyen, T.M.-D.; Lemieux, C.; Chung, N. N.; Lu, Y. Conversion of  $\delta$ -,  $\kappa$ - and  $\mu$ -Receptor Selective Opioid Peptide Agonists into  $\delta$ -,  $\kappa$ - and  $\mu$ -Selective Antagonists. *Life Sci.* **2003**, *73*, 691–698.

- (5) Dolle, R. E.; Machaut, M.; Martinez-Teipel, B.; Belanger, S.; Cassel, J. A.; Stabley, G. J.; Graczyk, T. M.; DeHaven, R. N. (4-Carboxamido)phenylalanine is a Surrogate for Tyrosine in Opioid Receptor Peptide Ligands. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3545–3548.
- (6) Weltrowska, G.; Lemieux, C.; Chung, N. N.; Schiller, P. W. Cyclic Enkephalin Analogues Containing Various *para*-Substituted Phenylalanine Derivatives in Place of Tyr<sup>1</sup> are Potent Opioid Agonists. *J. Pept. Res.* **2005**, *65*, 36–41.
- (7) Lu, Y.; Lum, T. K.; Augustine, Y. W. L.; Weltrowska, G.; Nguyen, T. M.-D.; Lemieux, C.; Chung, N. N.; Schiller, P. W. Replacement of the *N*-terminal Tyrosine Residue in Opioid Peptides with 3-(2,6-Dimethyl-4-carbamoylphenyl)propanoic Acid (Dcp) Results in Novel Opioid Antagonists. *J. Med. Chem.* **2006**, *49*, 5382–5385.
- (8) Schiller, P. W.; Nguyen, T. M.-D.; DiMaio, J.; Lemieux, C. Comparison of  $\mu$ -,  $\delta$ - and  $\kappa$ -Receptor Binding Sites Through Pharmacologic Evaluation of *p*-Nitrophenylalanine Analogs of Opioid Peptides. *Life Sci.* **1983**, *33* (1), 319–322.
- (9) Hruby, V. J.; Bartosz-Bechowski, H.; Davis, P.; Slaninova, J.; Zalewska, T.; Stropova, D.; Porreca, F.; Yamamura, H. I. Cyclic Enkephalin Analogues with Exceptional Potency and Selectivity for Delta-Opioid Receptors. *J. Med. Chem.* **1997**, *40*, 3957–3962.
- (10) Garfield, P.; Johnson, F. R. *Org. Synth. Coll.* **1943**, *2*, 449.
- (11) Goldstein, S. L.; McNelis, E. Migrations in Oxidations of Mesidine. *J. Org. Chem.* **1984**, *49*, 1613–1620.
- (12) Arthur, J. H.; Mildred, V. C. Some Amidines of the Holocaine Type II. Ester Substituted Amidines. *J. Am. Chem. Soc.* **1926**, *48*, 3214–3219.
- (13) Wang, L.; Wang, G. T.; Wang, X.; Tong, Y.; Sullivan, G.; Park, D.; Leonard, N. M.; Li, Q.; Cohen, J.; Gu, W.-Z.; Zhang, H.; Bauch, J. L.; Jakob, C. G.; Hutchins, C. W.; Stoll, V. S.; Marsh, K.; Rosenberg, S. H.; Sham, H. L.; Lin, N. H. Design, Synthesis, and Biological Activity of 4-[(4-Cyano-2-arylbenzyloxy)-(3-methyl-3*H*-imidazol-4-yl)methyl]benzotrioles as Potent and Selective Farnesyltransferase Inhibitors. *J. Med. Chem.* **2004**, *47*, 612–626.
- (14) Jackson, R. F. W.; Wishart, N.; Wood, A.; James, K.; Wythes, M. J. Preparation of Enantiomerically Pure Protected 4-Oxo- $\alpha$ -Amino Acids and 3-Aryl- $\alpha$ -Amino Acids from Serine. *J. Org. Chem.* **1992**, *57*, 3397–3404.
- (15) Pelton, J. T.; Kazmierski, W.; Gulya, K.; Yamamura, H. I.; Hruby, V. J. Design and Synthesis of Conformationally Constrained Somatostatin Analogues with High Potency and Specificity for Mu Opioid Receptors. *J. Med. Chem.* **1986**, *29*, 2370–2375.
- (16) Fichna, J.; do-Rego, J.-C.; Chung, N. N.; Lemieux, C.; Schiller, P. W.; Vanden Broeck, J.; Costentin, J.; Janecka, A. Synthesis and Characterization of Potent and Selective  $\mu$ -Opioid Receptor Antagonists, [Dmt<sup>1</sup>,D-2-Nal<sup>4</sup>]endomorphin-1 (Antanal-1) and [Dmt<sup>1</sup>,D-2-Nal<sup>4</sup>]endomorphin-2 (Antanal-2). *J. Med. Chem.* **2007**, *50*, 512–520.
- (17) Fichna, J.; do-Rego, J.-C.; Janecki, T.; Staniszewska, R.; Poels, J.; Vanden Broeck, J.; Costentin, J.; Schiller, P. W.; Janecka, A. Novel Highly Potent  $\mu$ -Opioid Receptor Antagonist Based on Endomorphin-2 Structure. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1350–1353.
- (18) Schiller, P. W.; Weltrowska, G.; Berezowska, I.; Nguyen, T. M.-D.; Wilkes, B. C.; Lemieux, C.; Chung, N. N. The TIPP Opioid Peptide Family: Development of  $\delta$  Antagonists,  $\delta$  Agonists, and Mixed  $\mu$  Agonist/ $\delta$  Antagonists. *Biopolymers (Peptide Sci.)* **1999**, *51*, 411–425.
- (19) Portoghese, P. S.; Sultana, M.; Takemori, A. E. Design of Peptidomimetic  $\delta$  Opioid Receptor Antagonists Using the Message-Address Concept. *J. Med. Chem.* **1990**, *33*, 1714–1720.

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