

**Supporting Information for:**

**Structure-Activity Study on the Phe Side-Chain Arrangement of Endomorphins Using Conformationally Constrained Analogs**

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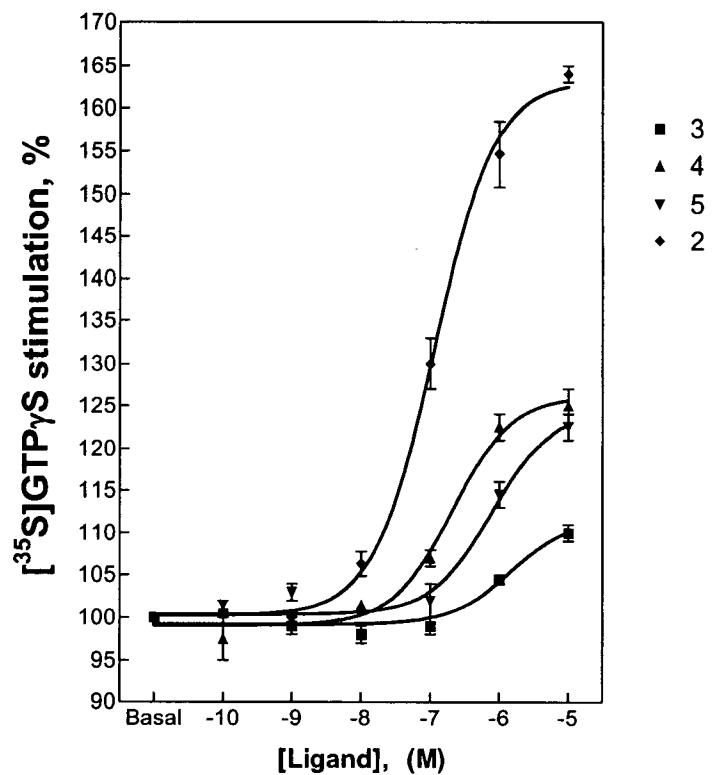
**Figure 1.** Concentration – Effect Curve of 2-5 Stimulated [<sup>35</sup>S]GTPγS Binding in Rat Brain Membrane Fraction

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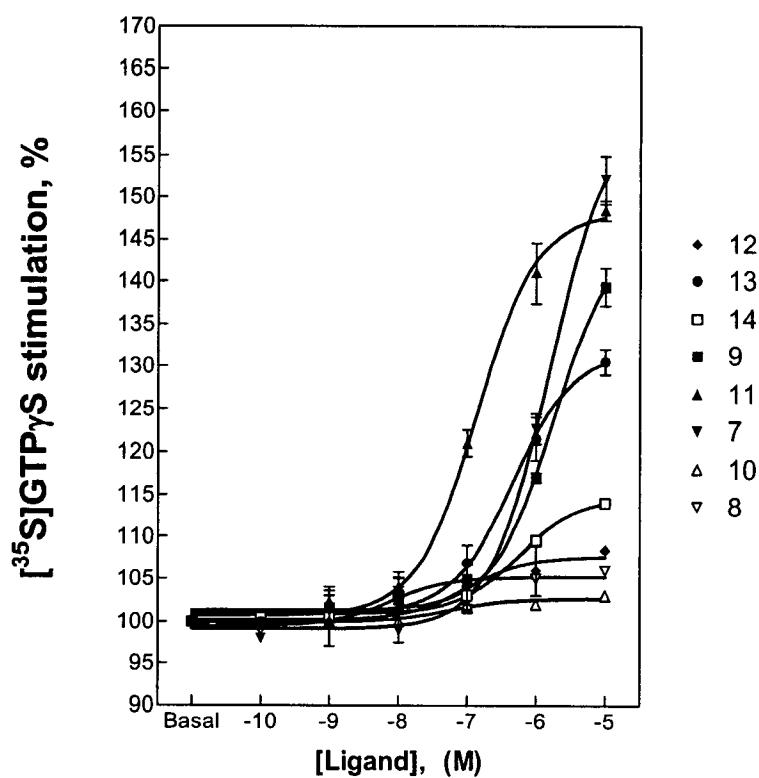
**Receptor-mediated  
G-protein activation in rat  
brain membranes**



**Figure 1.** Stimulation of  $[^{35}\text{S}]GTP\gamma\text{S}$  binding by  $\beta\text{-MePhe}^4$ -endomorphin-1 isomers **2-5**.

Membranes were incubated with 0.05 nM  $[^{35}\text{S}]GTP\gamma\text{S}$ , 100  $\mu\text{M}$  GDP and various concentration of **2-5**. Incubations were carried out at 30°C for 60 min. Stimulation is given as percent of the specific binding, data are mean values  $\pm$  S.E.

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**Figure 2.** Stimulation of  $[^{35}\text{S}]$ GTP $\gamma$ S binding by  $\beta$ -MePhe<sup>3</sup>- and  $\beta$ -MePhe<sup>4</sup>-endomorphin-2 isomers 7-14. Membranes were incubated with 0.05 nM  $[^{35}\text{S}]$ GTP $\gamma$ S, 100  $\mu\text{M}$  GDP and various concentration of 7-14. Incubations were carried out at 30°C for 60 min. Stimulation is given as percent of the specific binding, data are mean values  $\pm$  S.E.

**Table 8.**  $^1\text{H}$  NMR Chemical Shifts<sup>a</sup> (ppm) and Coupling Constants ( $J$  in Hz) and Temperature Coefficients (ppb/K) in Brackets for **12**

Residue	Tyr-Pro <sup>b</sup>	NH	$\text{H}^\alpha$	$\text{H}^\beta$	$\text{H}^\gamma,\gamma'$	$\text{H}^\delta,\delta'$
Tyr <sup>1</sup>	<i>trans</i>	8.00	4.17, $J_{\alpha\beta}=5.9$ , $J_{\alpha\beta}=7.2$	2.94 ( $\beta$ ), 2.76 ( $\beta$ ), $J_{\beta\beta}=14.3$		
	<i>cis</i>	8.00	3.27, $J_{\alpha\beta}=7.5$ , $J_{\alpha\beta}=8.6$	2.85 ( $\beta$ ), 2.74 ( $\beta$ ), $J_{\beta\beta}=14.0$		
Pro <sup>2</sup>	<i>trans</i>	NA	4.33	1.94, 1.63	1.72	3.56, 3.10
	<i>cis</i>		3.44	1.57, 1.54	1.47, 1.41	3.39, 3.17
Phe <sup>3</sup>	<i>trans</i>	7.54, $J_{\text{NH}\alpha}=8.0$ , (-2.6)	4.48, $J_{\alpha\beta}=4.9$ , $J_{\alpha\beta}=8.6$	2.43 ( $\beta$ ), 2.30 ( $\beta$ ), $J_{\beta\beta}=13.8$		
	<i>cis</i>	8.06, $J_{\text{NH}\alpha}=9.2$ , (-4.5)	4.47, $J_{\alpha\beta}=4.3$ , $J_{\alpha\beta}=11.2$	2.40 ( $\beta$ ), 2.18 ( $\beta$ ), $J_{\beta\beta}=13.8$		
$\beta$ -MePhe <sup>4</sup>	<i>trans</i>	8.16, $J_{\text{NH}\alpha}=8.9$ , (-10.0)	4.46, $J_{\alpha\beta}=10.3$	3.03, $J_{\beta\gamma}=7.1$	1.18	
	<i>cis</i>	8.35, $J_{\text{NH}\alpha}=9.2$ , (-11.0)	4.53, $J_{\alpha\beta}=10.3$	3.04, $J_{\beta\gamma}=7.1$	1.19	

<sup>a</sup> Proton chemical shifts are referenced to the residual solvent signal of DMSO-d<sub>6</sub> at 2.49 ppm.  $^1\text{H}$  NMR signals of aromatic protons for Tyr<sup>1</sup> (6.68/7.11) in *trans* **12** and Tyr<sup>1</sup> (6.68/6.88) in *cis* **12**; for Phe<sup>3</sup> and (2*R*,3*R*)- $\beta$ -MePhe<sup>4</sup> (7.0-7.3). <sup>b</sup> Tyr-Pro conformer.

**Table 9.**  $^1\text{H}$  NMR Chemical Shifts<sup>a</sup> (ppm) and Coupling Constants ( $J$  in Hz) and Temperature Coefficients (ppb/K) in Brackets for **13**

Residue	Tyr-Pro <sup>b</sup>	NH	$\text{H}^\alpha$	$\text{H}^\beta$	$\text{H}^\gamma$	$\text{H}^\delta,\text{s}$
Tyr <sup>1</sup>	<i>trans</i>	8.03	4.18, $J_{\alpha\beta}=6.3$ , $J_{\alpha\beta}=7.5$	2.95 (β), 2.80 (β), $J_{\beta\beta}=14.5$		
	<i>cis</i>	8.17	3.33, $J_{\alpha\beta}=6.6$ , $J_{\alpha\beta}=8.1$	2.85 (β), 2.79 (β), $J_{\beta\beta}=13.6$		
Pro <sup>2</sup>	<i>trans</i>	NA	4.41	1.98, 1.74	1.76	3.57, 3.09
	<i>cis</i>		3.62	1.68	1.58, 1.49	3.40, 3.25
Phe <sup>3</sup>	<i>trans</i>	7.97, $J_{\text{NH}\alpha}=7.8$ , (-2.7)	4.60, $J_{\alpha\beta}=5.1$ , $J_{\alpha\beta}=8.5$	2.99 (β), 2.86 (β), $J_{\beta\beta}=14.0$		
	<i>cis</i>	8.26, $J_{\text{NH}\alpha}=8.6$ , (-3.8)	4.62, $J_{\alpha\beta}=4.9$ , $J_{\alpha\beta}=10.0$	3.03 (β), 2.83 (β), $J_{\beta\beta}=14.1$		
$\beta$ -MePhe <sup>4</sup>	<i>trans</i>	7.99, $J_{\text{NH}\alpha}=9.1$ , (-7.1)	4.51, $J_{\alpha\beta}=8.2$	3.13, $J_{\beta\gamma}=7.3$	1.19	
	<i>cis</i>	8.10, $J_{\text{NH}\alpha}=9.2$ , (-7.6)	4.52, $J_{\alpha\beta}=8.1$	3.13, $J_{\beta\gamma}=7.2$	1.17	

<sup>a</sup> Proton chemical shifts are referenced to the residual solvent signal of DMSO-d<sub>6</sub> at 2.49 ppm.  $^1\text{H}$  NMR signals of aromatic protons for Tyr<sup>1</sup> (6.70/7.14) in *trans* **13** and Tyr<sup>1</sup> (6.70/6.90) in *cis* **13**; for Phe<sup>3</sup> and (2S,3R)- $\beta$ -MePhe<sup>4</sup> (7.1-7.3). <sup>b</sup> Tyr-Pro conformer.

**Table 10.**  $^1\text{H}$  NMR Chemical Shifts<sup>a</sup> (ppm) and Coupling Constants ( $J$  in Hz) and Temperature Coefficients (ppb/K) in Brackets for **14**

Residue	Tyr-Pro <sup>b</sup>	NH	$\text{H}^\alpha$	$\text{H}^\beta$	$\text{H}^{\gamma,\gamma'}$	$\text{H}^{\delta,\delta'}$
Tyr <sup>1</sup>	<i>trans</i>	8.02	4.20, $J_{\alpha\beta}=6.0$ , $J_{\alpha\beta}=7.5$	2.96 ( $\beta$ ), 2.78 ( $\beta$ ), $J_{\beta\beta}=14.3$		
	<i>cis</i>	8.13	3.34, $J_{\alpha\beta}=6.0$ , $J_{\alpha\beta}=8.0$	2.86 ( $\beta$ ), 2.77 ( $\beta$ ), $J_{\beta\beta}=14.0$		
Pro <sup>2</sup>	<i>trans</i>		4.38	1.98, 1.68	1.76	3.59, 3.13
	<i>cis</i>	NA	3.51	1.59	1.51, 1.44	3.40, 3.20
Phe <sup>3</sup>	<i>trans</i>	7.76, $J_{\text{NH}\alpha}=8.1$ , (-2.6)	4.71, $J_{\alpha\beta}=5.2$ , $J_{\alpha\beta}=8.6$	2.87 ( $\beta$ ), 2.65 ( $\beta$ ), $J_{\beta\beta}=14.0$		
	<i>cis</i>	8.18, $J_{\text{NH}\alpha}=9.2$ , (-3.9)	4.71, $J_{\alpha\beta}=4.9$ , $J_{\alpha\beta}=10.6$	2.96 ( $\beta$ ), 2.58 ( $\beta$ ), $J_{\beta\beta}=13.8$		
$\beta$ -MePhe <sup>4</sup>	<i>trans</i>	8.20, $J_{\text{NH}\alpha}=9.2$ , (-6.6)	4.49, $J_{\alpha\beta}=6.4$	3.25, $J_{\beta\gamma}=7.2$	1.16	
	<i>cis</i>	8.39, $J_{\text{NH}\alpha}=9.2$ , (-8.6)	4.57, $J_{\alpha\beta}=6.2$	3.24, $J_{\beta\gamma}=7.6$	1.17	

<sup>a</sup> Proton chemical shifts are referenced to the residual solvent signal of DMSO-d<sub>6</sub> at 2.49 ppm.  $^1\text{H}$  NMR signals of aromatic protons for Tyr<sup>1</sup> (6.71/7.13) in *trans* **14** and Tyr<sup>1</sup> (6.70/6.91) in *cis* **14**; for Phe<sup>3</sup> and (2R,3S)- $\beta$ -MePhe<sup>4</sup> (7.0-7.4). <sup>b</sup> Tyr-Pro conformer.

**Table 11.**  $^{13}\text{C}$  NMR Chemical Shifts (ppm) for 11-14

Residue	Tyr-Pro <sup>a</sup>	$\text{C}_\alpha$	$\text{C}_\beta$	$\text{C}_\gamma$	$\text{C}_\delta$
Tyr <sup>1</sup>	<i>trans</i>	53.4	53.5	53.3	36.1
	<i>cis</i>	53.3	53.3	53.4	36.2
Pro <sup>2</sup>	<i>trans</i>	60.2	60.5	60.6	29.7
	<i>cis</i>	59.6	59.9	59.8	60.1
Phe <sup>3</sup>	<i>trans</i>	54.8	54.0	54.7	54.3
	<i>cis</i>	54.8	54.0	54.7	54.3
$\beta$ -MePhe <sup>4</sup>	<i>trans</i>	57.9	58.4	58.0	58.3
	<i>cis</i>	58.0	58.2	58.0	58.1

<sup>a</sup> Tyr-Pro conformer.

$^{13}\text{C}$  Chemical shifts for aromatic ring of Tyr<sup>1</sup> (131.6/115.9) in *trans* 11 and Tyr<sup>1</sup> (131.2/115.9) in *cis* 11, for Phe<sup>3</sup> and (2S,3S)- $\beta$ -MePhe<sup>4</sup> (127-130);  $^3J_{\text{CH}\alpha} = 2.0 \pm 0.3$  Hz in (2S,3S)- $\beta$ -MePhe<sup>4</sup> of *trans* 11 and  $^3J_{\text{CH}\alpha} = 2.0 \pm 0.3$  Hz in (2S,3S)- $\beta$ -MePhe<sup>4</sup> of *cis* 11.

<sup>13</sup>C chemical shifts for aromatic ring of Tyr<sup>1</sup> (130.0/116.0) in *trans* **12** and Tyr<sup>1</sup> (131.0/116.0) in *cis* **12**, for Phe<sup>3</sup> and (2*R*,3*R*)-β-MePhe<sup>4</sup> (126-131); <sup>3</sup>J<sub>CγHα</sub>= 1.0 ± 0.3 Hz in (2*R*, 3*R*)-β-MePhe<sup>4</sup> of *trans* **12** and <sup>3</sup>J<sub>CγHα</sub>= 2.2 ± 0.3 Hz in (2*R*, 3*R*)-β-MePhe<sup>4</sup> of *cis* **12**.

<sup>13</sup>C chemical shifts for aromatic ring of Tyr<sup>1</sup> (131.5/116.1) in *trans* **13** and Tyr<sup>1</sup> (131.1/116.1) in *cis* **13**, for Phe<sup>3</sup> and (2*S*,3*R*)-β-MePhe<sup>4</sup> (127-130); <sup>3</sup>J<sub>CγHα</sub>= 3.5 ± 0.3 Hz in (2*S*,3*R*)-β-MePhe<sup>4</sup> of *trans* **13** and <sup>3</sup>J<sub>CγHα</sub>= 4.2 ± 0.3 Hz in (2*S*,3*R*)-β-MePhe<sup>4</sup> of *cis* **13**.

<sup>13</sup>C chemical shifts for aromatic ring of Tyr<sup>1</sup> (131.5/116.1) in *trans* **14** and Tyr<sup>1</sup> (131.1/116.1) in *cis* **14**, for Phe<sup>3</sup> and (2*R*,3*S*)-β-MePhe<sup>4</sup> (127-130); <sup>3</sup>J<sub>CγHα</sub>= 3.5 ± 0.3 Hz in (2*R*,3*S*)-β-MePhe<sup>4</sup> of *trans* **14** and <sup>3</sup>J<sub>CγHα</sub>= 4.0 ± 0.3 Hz in (2*R*,3*S*)-β-MePhe<sup>4</sup> of *cis* **14**.