

## Supporting Information

### **6-*N,N*-Dimethylamino-2,3-Naphthalimide a New Environment-Sensitive Fluorescent**

### **Probe in $\delta$ -Selective and $\mu$ -Selective Opioid Peptides**

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### In Solution Peptide Synthesis of Compound 3

**Boc-Tyr-Pro-Phe-Phe-OBzl.** To a solution of Boc-Tyr-Pro-OH<sup>27</sup> (0.37 g, 0.97 mmol) and TFA·H-Phe-Phe-OBzl<sup>28</sup> (0.5 g, 0.97 mmol) in DMF (10 mL) at 0 °C, NMM (0.11 mL, 0.97 mmol), HOBt (0.16 g, 1.07 mmol), and WSC (0.2 g, 1.07 mmol) were added. The reaction mixture was stirred for 3 h at 0 °C and 24 h at room temperature. After DMF was evaporated, the residue was dissolved in EtOAc and washed with citric acid (10% in H<sub>2</sub>O), NaHCO<sub>3</sub> (5% in H<sub>2</sub>O), and brine. The organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated to dryness. The residue was precipitated from Et<sub>2</sub>O/Pe (1:9, v/v): yield 0.66 g (89%); *R<sub>f</sub>*(B) 0.91; HPLC *K'* 9.14; mp 133-135 °C;  $[\alpha]_D^{20}$  -22.6; MH<sup>+</sup> 764; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ 1.40 (s, 9H), 1.92-2.34 (m, 4H), 2.92-3.29 (m, 6H), 3.41-3.51 (m, 2H), 4.40-4.92 (m, 4H), 5.34 (s, 2H), 6.68-7.21 (m, 19H).

**Boc-Tyr-Pro-Phe-Phe-OH.** To a solution of Boc-Tyr-Pro-Phe-Phe-OBzl (0.66 g, 0.86 mmol) in methanol (30 mL) was added Pd/C (10%, 0.1 g), and H<sub>2</sub> was bubbled for 1 h at room temperature. After filtration, the solution was evaporated to dryness. The residue was crystallized from Et<sub>2</sub>O/Pe (1:9, v/v): yield 0.56 g (96%); *R<sub>f</sub>*(B) 0.82; HPLC *K'* 7.39; mp 142-144 °C;  $[\alpha]_D^{20}$  -25.1; MH<sup>+</sup> 674.

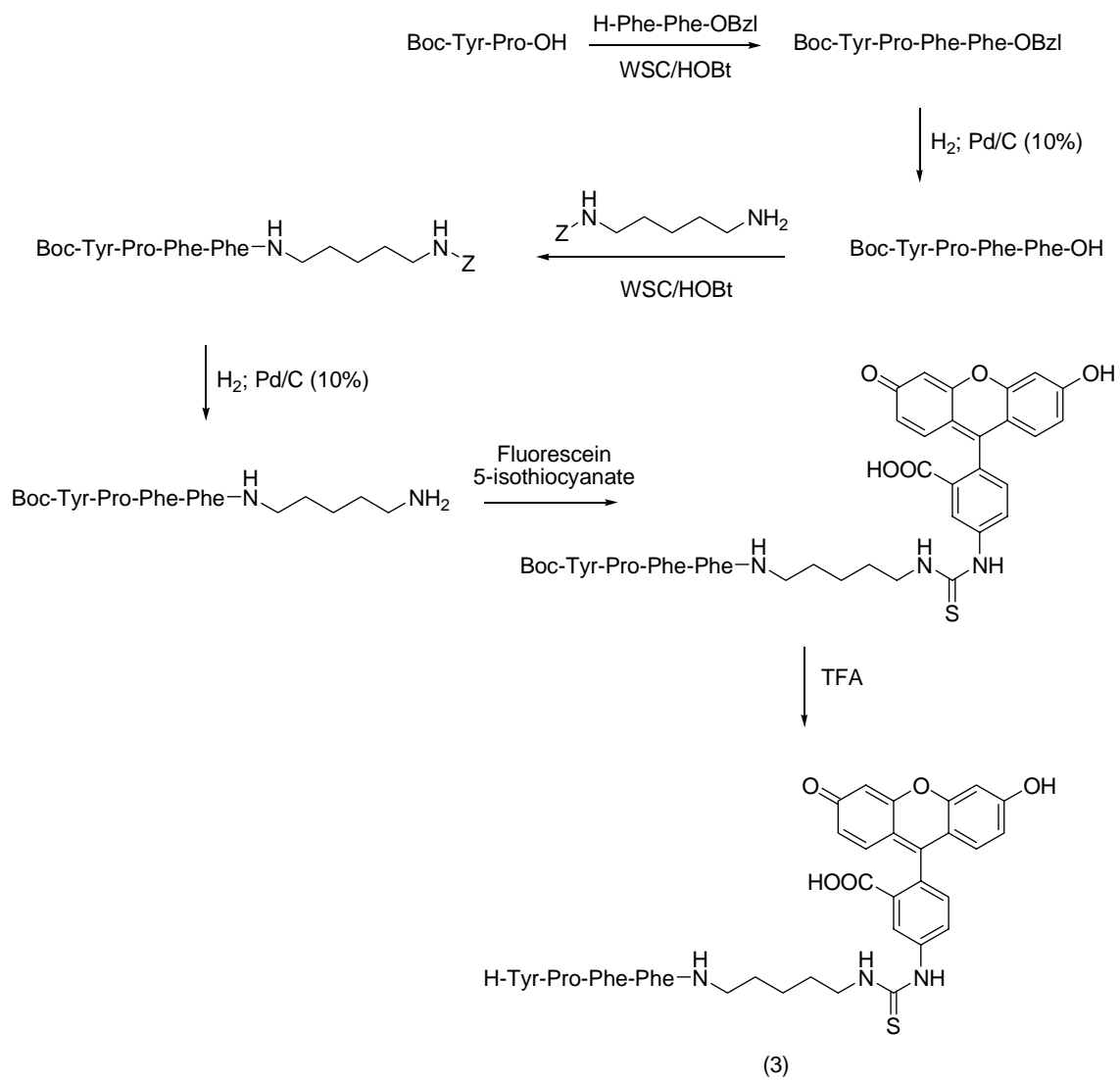
**Boc-Tyr-Pro-Phe-Phe-NH-(CH<sub>2</sub>)<sub>5</sub>-NH-Z.** To a solution of Boc-Tyr-Pro-Phe-Phe-OH (0.64 g, 0.95 mmol) and TFA·H<sub>2</sub>N-(CH<sub>2</sub>)<sub>5</sub>-NH-Z (0.26 g, 0.95 mmol) in DMF (10 mL) at 0 °C, NMM (0.10 mL, 0.95 mmol), HOBt (0.16 g, 1.05 mmol), and WSC (0.2 g, 1.05 mmol) were added. The reaction mixture was stirred for 3 h at 0 °C and 24 h at room temperature. After DMF was evaporated, the residue was dissolved in EtOAc and washed with citric acid (10% in H<sub>2</sub>O), NaHCO<sub>3</sub> (5% in H<sub>2</sub>O), and brine. The organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated to dryness. The residue was precipitated from Et<sub>2</sub>O/Pe (1:9, v/v): yield 0.70 g (83%); *R<sub>f</sub>*(B) 0.95; HPLC *K'* 9.81; mp 125-127 °C;  $[\alpha]_D^{20}$  -18.4; MH<sup>+</sup> 892; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ 1.29-1.55 (m, 15H), 1.92-2.34 (m, 4H), 2.92-3.29 (m, 10H), 3.41-3.51 (m, 2H),

4.40-4.92 (m, 4H), 5.34 (s, 2H), 6.68-7.21 (m, 19H).

**Boc-Tyr-Pro-Phe-Phe-NH-(CH<sub>2</sub>)<sub>5</sub>-NH<sub>2</sub>.** To a solution of Boc-Tyr-Pro-Phe-Phe-NH-(CH<sub>2</sub>)<sub>5</sub>-NH-Z (0.70 g, 0.79 mmol) in methanol (30 mL) was added Pd/C (10%, 0.1 g), and H<sub>2</sub> was bubbled for 1 h at room temperature. After filtration, the solution was evaporated to dryness. The residue was crystallized from Et<sub>2</sub>O/Pe (1:9, v/v): yield 0.50 g (83%); *R<sub>f</sub>*(B) 0.76; HPLC *K'* 7.30; mp 138-140 °C;  $[\alpha]_D^{20}$  -20.1; MH<sup>+</sup> 758.

**Boc-Tyr-Pro-Phe-Phe-NH-(CH<sub>2</sub>)<sub>5</sub>-NH-(C=S)-NH-fluorescein.** With stirring at 25 °C under argon, fluorescein isothiocyanate isomer I (0.06 g, 0.15 mmol) was added to a mixture of Boc-Tyr-Pro-Phe-Phe-NH-(CH<sub>2</sub>)<sub>5</sub>-NH<sub>2</sub> (0.11 g, 0.15 mmol) and triethylamine (2.5 mL) in freshly distilled THF (10 mL) and absolute ethanol (15 mL). The mixture was stirred in the dark, at room temperature for 24 h. After solvent evaporation, the residue was purified by preparative HPLC: yield 0.09 g (50%); *R<sub>f</sub>*(B) 0.92; HPLC *K'* 9.12; mp 149-151 °C;  $[\alpha]_D^{20}$  -13.4; MH<sup>+</sup> 1147; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ 1.29-1.55 (m, 15H), 1.92-2.34 (m, 4H), 2.92-3.29 (m, 8H), 3.41-3.51 (m, 4H), 4.40-4.92 (m, 4H), 6.11-7.28 (m, 23H).

**TFA·H-Tyr-Pro-Phe-Phe-NH-(CH<sub>2</sub>)<sub>5</sub>-NH-(C=S)-NH-fluorescein (3).** Boc-Tyr-Pro-Phe-Phe-NH-(CH<sub>2</sub>)<sub>5</sub>-NH-(C=S)-NH-fluorescein (0.09 g, 0.08 mmol) was treated with 66% TFA in H<sub>2</sub>O (1 mL) for 30 min at room temperature. Et<sub>2</sub>O/Pe (1:5, v/v) was added to the solution until the product precipitated: yield 0.07 g (90%); *R<sub>f</sub>*(A) 0.66; HPLC *K'* 7.26; mp 153-155 °C;  $[\alpha]_D^{20}$  -15.1; MH<sup>+</sup> 1047; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ 1.29-1.55 (m, 6H), 1.92-2.34 (m, 4H), 2.92-3.29 (m, 8H), 3.41-3.51 (m, 4H), 3.95-4.92 (m, 4H), 6.11-7.28 (m, 23H).



**Scheme 1. Synthesis of compound (3).**

**Table 3.** Elemental analysis of compounds **1-5**.<sup>a</sup>

Comp.	Formula	MH <sup>+</sup> , <i>m/z</i>		C	H	N
1	C <sub>53</sub> H <sub>60</sub> F <sub>6</sub> N <sub>8</sub> O <sub>14</sub>	920	Calc	55.49	5.27	9.77
			Found	55.33	5.20	9.60
2	C <sub>47</sub> H <sub>49</sub> F <sub>6</sub> N <sub>7</sub> O <sub>13</sub>	807	Calc	54.60	4.78	9.48
			Found	54.89	4.94	9.27
3	C <sub>60</sub> H <sub>60</sub> F <sub>3</sub> N <sub>7</sub> O <sub>12</sub> S	1047	Calc	62.11	5.21	8.45
			Found	61.98	5.06	8.31
4	C <sub>59</sub> H <sub>65</sub> F <sub>6</sub> N <sub>9</sub> O <sub>13</sub>	995	Calc	57.98	5.36	10.31
			Found	58.24	5.50	10.43
5	C <sub>44</sub> H <sub>45</sub> F <sub>6</sub> N <sub>7</sub> O <sub>11</sub>	735	Calc	54.94	4.72	10.19
			Found	54.78	4.65	10.03

<sup>a</sup> Only the analysis of the new compounds, detailed in the **Experimental Section**, are included.

### References

- (27) Salvadori, S.; Marastoni, M.; Balboni, G.; Borea, P.; Tomatis, R. Opioid peptides: synthesis and biological properties of dermorphin related hexapeptides. *Eur. J. Med. Chem.* **1990**, *25*, 171-177.
- (28) Oya, M.; Takahashi, T. The steric hindrance of the stepwise reaction of N-carboxy alpha amino acid anhydride with the alpha amino acid ester *Bull. Chem. Soc. Japan* **1981**, *54*, 2705-2707.