

Supporting Information

Bifunctional [2',6'-Dimethyl-L-tyrosine¹]Endomorphin-2 Analogues Substituted at Position 3 with Alkylated Phenylalanine Derivatives Yield Potent Mixed μ -Agonist/ δ -Antagonist and Dual μ -/ δ -Agonist Opioid Ligands

Tingyou Li,¹ Kimitaka Shiotani,¹ Anna Miyazaki,[†] Yuko Tsuda,^{1,†}, Akihiro Ambo,[#]
Yusuke Sasaki,[#] Yunden Jinsmaa,[‡] Ewa Marczak,[‡] Sharon D. Bryant,[‡]
Lawrence H. Lazarus,^{‡,*} and Yoshio Okada^{1,†,*}

¹*The Graduate School of Food and Medicinal Sciences and [†]Faculty of Pharmaceutical Sciences, Kobe Gakuin University, Nishi-ku, Kobe 651-2180, Japan, [#]Department of Biochemistry, Tohoku Pharmaceutical University, Aoba-ku, Sendai 981-8558, Japan, and [‡]Medicinal Chemistry Group, Laboratory of Pharmacology and Chemistry, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709, U.S.A.*

*Corresponding authors: Y. Okada: Tel: +81-78-974-1551, fax: +81-78-974-5689. E-mail: okada@pharm.kobegakuin.ac.jp. L. H. Lazarus: Tel: +1-919-541-3238, fax: +□-919-541- 5737. E-mail: lazarus@niehs.nih.gov.

^aAbbreviations: Dmp: 2',6'-dimethyl-L-phenylalanine; ^{3,5}Dmp: 3',5'-dimethyl-L-phenylalanine; Dmt: 2',6'-dimethyl-L-tyrosine; Emp: 2'-ethyl-6'-methyl-L-phenylalanine; Imp: 2'-isopropyl-6'-methyl-L-phenylalanine; Mmp: 2'-methyl-L-phenylalanine; Tmp: 2',4',6'-trimethyl-L-phenylalanine Xaa: phenylalanine analogue;

Contents of Supporting Information: detailed elemental analysis data summarized in Table 1-4.

Table 1. Elemental analysis data of the Boc-Xaa-OH compounds.

	Calcd.	Found	Formula
Boc-Mmp-OH	C, 64.50; H, 7.58; N, 5.01	C, 64.22; H, 7.63; N, 4.94	C ₁₅ H ₂₁ NO ₄
Boc- ^{3,5} Dmp-OH	C, 65.51; H, 7.90; N, 4.77	C, 65.29; H, 7.89; N, 4.75	C ₁₆ H ₂₃ NO ₄
Boc-Dmp-OH	C, 65.51; H, 7.90; N, 4.77	C, 65.24; H, 8.09; N, 4.80	C ₁₆ H ₂₃ NO ₄
Boc-Tmp-OH	C, 66.43; H, 8.20; N, 4.56	C, 66.14; H, 8.26; N, 4.41	C ₁₇ H ₂₅ NO ₄
Boc-Emp-OH	C, 66.43; H, 8.20; N, 4.56	C, 66.26; H, 8.33; N, 4.52	C ₁₇ H ₂₅ NO ₄
Boc-Imp-OH	C, 67.26; H, 8.47; N, 4.36	C, 67.18; H, 8.52; N, 4.35	C ₁₈ H ₂₇ NO ₄

Table 2. Elemental analysis data of the Boc-Xaa-Phe-NH₂ compounds.

Compound	Calcd.	Found	Formula
Boc-Mmp-Phe-NH ₂	C, 67.74; H, 7.34; N, 9.87	C, 67.45; H, 7.51; N, 9.64	C ₂₄ H ₃₁ N ₃ O ₄
Boc- ^{3,5} Dmp-Phe-NH ₂	C, 67.62; H, 7.60; N, 9.46	C, 67.51; H, 7.51; N, 9.40	C ₂₅ H ₃₃ N ₃ O ₄ ·0.25H ₂ O
Boc-Dmp-Phe-NH ₂	C, 68.31; H, 7.57; N, 9.56	C, 68.08; H, 7.61; N, 9.45	C ₂₅ H ₃₃ N ₃ O ₄
Boc-Dmt-Phe-NH ₂	C, 65.27; H, 7.34; N, 9.13	C, 65.19; H, 7.33; N, 9.03	C ₂₅ H ₃₃ N ₃ O ₅ ·0.25H ₂ O
Boc-Tmp-Phe-NH ₂	C, 68.17; H, 7.81; N, 9.17	C, 68.03; H, 7.71; N, 9.14	C ₂₆ H ₃₅ N ₃ O ₄ ·0.25H ₂ O
Boc-Emp-Phe-NH ₂	C, 68.85; H, 7.78; N, 9.26	C, 68.92; H, 7.59; N, 9.25	C ₂₆ H ₃₅ N ₃ O ₄
Boc-Imp-Phe-NH ₂	C, 68.69; H, 8.01; N, 8.90	C, 68.59; H, 8.00; N, 8.83	C ₂₇ H ₃₇ N ₃ O ₄ ·0.25H ₂ O

Table 3. Elemental analysis data of the Boc-Tyr/Dmt-Xaa-Phe-NH₂ compounds.

Peptide	Calcd.	Found	Formula
Boc-Tyr-Pro-Mmp-Phe-NH ₂	C, 65.97; H, 6.94; N, 10.12	C, 65.92; H, 6.83; N, 10.12	C ₃₈ H ₄₇ N ₅ O ₇ ·0.33H ₂ O
Boc-Dmt-Pro-Mmp-Phe-NH ₂	C, 66.46; H, 7.25; N, 9.69	C, 66.74; H, 7.24; N, 9.73	C ₄₀ H ₅₁ N ₅ O ₇ ·0.5H ₂ O
Boc-Tyr-Pro- ^{3,5} Dmp-Phe-NH ₂	C, 65.25; H, 7.16; N, 9.76	C, 65.25; H, 7.28; N, 9.58	C ₃₉ H ₄₉ N ₅ O ₇ ·1.0H ₂ O
Boc-Dmt-Pro- ^{3,5} Dmp-Phe-NH ₂	C, 65.39; H, 7.47; N, 9.30	C, 665.38; H, 7.21; N, 9.12	C ₄₁ H ₅₃ N ₅ O ₇ ·1.2H ₂ O
Boc-Dmt-Pro-Dmp-Phe-NH ₂	C, 65.23; H, 7.48; N, 9.28	C, 65.00; H, 7.07; N, 9.20	C ₄₁ H ₅₃ N ₅ O ₇ ·1.5H ₂ O
Boc-Tyr-Pro-Dmt-Phe-NH ₂	C, 62.68; H, 7.08; N, 9.37	C, 62.70; H, 6.92; N, 9.07	C ₃₉ H ₄₉ N ₅ O ₈ ·1.75H ₂ O
Boc-Dmt-Pro-Dmt-Phe-NH ₂	C, 64.63; H, 7.28; N, 9.19	C, 64.71; H, 7.28; N, 8.98	C ₄₁ H ₅₃ N ₅ O ₈ ·1.0H ₂ O
Boc-Tyr-Pro-Tmp-Phe-NH ₂	C, 66.46; H, 7.25; N, 9.69	C, 66.68; H, 7.13; N, 9.74	C ₄₀ H ₅₁ N ₅ O ₇ ·0.5H ₂ O
Boc-Dmt-Pro-Tmp-Phe-NH ₂	C, 66.78; H, 7.54; N, 9.27	C, 66.82; H, 7.50; N, 9.18	C ₄₂ H ₅₅ N ₅ O ₇ ·0.75H ₂ O
Boc-Tyr-Pro-Emp-Phe-NH ₂	C, 67.30; H, 7.20; N, 9.81	C, 67.06; H, 7.05; N, 9.84	C ₄₀ H ₅₁ N ₅ O ₇
Boc-Dmt-Pro-Emp-Phe-NH ₂	C, 64.84; H, 7.64; N, 9.00	C, 64.49; H, 7.11; N, 9.07	C ₄₂ H ₅₅ N ₅ O ₇ ·2.0H ₂ O
Boc-Tyr-Pro-Imp-Phe-NH ₂	C, 67.65; H, 7.34; N, 9.62	C, 67.45; H, 7.22; N, 9.55	C ₄₁ H ₅₃ N ₅ O ₇
Boc-Dmt-Pro-Imp-Phe-NH ₂	C, 66.98; H, 7.61; N, 9.09	C, 66.75; H, 7.52; N, 9.13	C ₄₃ H ₅₇ N ₅ O ₇ ·0.8H ₂ O

Table 4. Elemental analysis data of the H-Tyr/Dmt-Xaa-Phe-NH₂ compounds.

No.	Peptide	Calcd.	Found	Formula
2	H-Tyr-Pro-Mmp-Phe-NH ₂	C, 59.41; H, 6.80; N, 10.50	C, 59.04; H, 6.38; N, 10.50	C ₃₃ H ₄₀ N ₅ O ₅ ·HCl·2.5H ₂ O
2'	H-Dmt-Pro-Mmp-Phe-NH ₂	C, 59.69; H, 7.16; N, 9.94	C, 59.68; H, 6.83; N, 9.81	C ₃₅ H ₄₄ N ₅ O ₅ ·HCl·3.0H ₂ O
3	H-Dmt-Pro- ^{3,5} Dmp-Phe-NH ₂	C, 60.20; H, 7.30; N, 9.75	C, 60.15; H, 6.82; N, 9.68	C ₃₆ H ₄₆ N ₅ O ₅ ·HCl·3.0H ₂ O
3'	H-Tyr-Pro- ^{3,5} Dmp-Phe-NH ₂	C, 59.95; H, 6.95; N, 10.28	C, 59.67; H, 6.48; N, 10.31	C ₃₄ H ₄₂ N ₅ O ₅ ·HCl·2.5H ₂ O
4'	H-Dmt-Pro-Dmp-Phe-NH ₂	C, 60.20; H, 7.30; N, 9.75	C, 60.49; H, 6.83; N, 9.77	C ₃₆ H ₄₆ N ₅ O ₅ ·HCl·3.0H ₂ O
5	H-Tyr-Pro-Dmt-Phe-NH ₂	C, 57.82; H, 6.85; N, 9.92	C, 57.59; H, 6.39; N, 9.90	C ₃₄ H ₄₂ N ₅ O ₆ ·HCl·3.0H ₂ O
5'	H-Dmt-Pro-Dmt-Phe-NH ₂	C, 59.62; H, 7.09; N, 9.66	C, 59.29; H, 6.60; N, 9.65	C ₃₆ H ₄₆ N ₅ O ₆ ·HCl·2.5H ₂ O
6	H-Tyr-Pro-Tmp-Phe-NH ₂	C, 59.69; H, 7.16; N, 9.94	C, 59.53; H, 6.74; N, 9.99	C ₃₅ H ₄₄ N ₅ O ₅ ·HCl·3.0H ₂ O
6'	H-Dmt-Pro-Tmp-Phe-NH ₂	C, 61.06; H, 7.41; N, 9.62	C, 60.97; H, 6.95; N, 9.58	C ₃₇ H ₄₈ N ₅ O ₅ ·HCl·2.75H ₂ O
7	H-Tyr-Pro-Emp-Phe-NH ₂	C, 60.46; H, 7.10; N, 10.07	C, 60.25; H, 6.61; N, 10.08	C ₃₅ H ₄₄ N ₅ O ₅ ·HCl·2.5H ₂ O
7'	H-Dmt-Pro-Emp-Phe-NH ₂	C, 60.68; H, 7.43; N, 9.56	C, 60.41; H, 6.96; N, 9.49	C ₃₇ H ₄₈ N ₅ O ₅ ·HCl·3.0H ₂ O
8	H-Tyr-Pro-Imp-Phe-NH ₂	C, 60.96; H, 7.25; N, 9.87	C, 60.68; H, 6.85; N, 9.84	C ₃₆ H ₄₆ N ₅ O ₅ ·HCl·2.5H ₂ O
8'	H-Dmt-Pro-Imp-Phe-NH ₂	C, 61.15; H, 7.56; N, 9.38	C, 61.23; H, 7.28; N, 9.47	C ₃₈ H ₅₀ N ₅ O ₅ ·HCl·3.0H ₂ O