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Articles

Bifunctional [2′,6′-Dimethyl-L-tyrosine[1]]endomorphin-2 Analogues Substituted at Position 3 with Alkylated Phenylalanine Derivatives Yield Potent Mixed μ-Agonist/δ-Antagonist and Dual μ-Agonist/δ-Agonist Opioid Ligands

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Endomorphin-2 (H-Tyr-Pro-Phe-Phe-NH_2) and [Dmt[1]]EM-2 (Dmt = 2′,6′-dimethyl-L-tyrosine) analogues, containing alkylated Phe[3] derivatives, 2′-monomethyl (**2**, **2′**), 3′,5′- and 2′,6′-dimethyl (**3**, **3′**, and **4′**, respectively), 2′,4′,6′-trimethyl (**6**, **6′**), 2′-ethyl-6′-methyl (**7**, **7′**), and 2′-isopropyl-6′-methyl (**8**, **8′**) groups or Dmt (**5**, **5′**), had the following characteristics: (i) [Xaa[3]]EM-2 analogues exhibited improved μ- and δ-opioid receptor affinities. The latter, however, were inconsequential (K_i^{δ} = 491−3451 nM). (ii) [Dmt[1],-Xaa[3]]EM-2 analogues enhanced μ- and δ-opioid receptor affinities (K_i^{μ} = 0.069−0.32 nM; K_i^{δ} = 1.83−99.8 nM) without κ-opioid receptor interaction. (iii) There were elevated μ-bioactivity (IC_{50} = 0.12−14.4 nM) and abolished δ-agonism (IC_{50} > 10 μM in **2′**, **3′**, **4′**, **5′**, **6′**), although **4′** and **6′** demonstrated a potent mixed μ-agonism/δ-antagonism (for **4′**, IC_{50}^{μ} = 0.12 and pA_2 = 8.15; for **6′**, IC_{50}^{μ} = 0.21 nM and pA_2 = 9.05) and **7′** was a dual μ-agonist/δ-agonist (IC_{50}^{μ} = 0.17 nM; IC_{50}^{δ} = 0.51 nM).

Introduction

Endomorphin-1 (EM-1 = H-Tyr-Pro-Trp-Phe-NH_2) and endomorphin-2 (EM-2 = H-Tyr-Pro-Phe-Phe-NH_2) are endogenous opioid peptides with remarkably high selectivity for μ-opioid receptors.[1] Since the endomorphins express pharmacological properties similar to those of morphine and are thought to inhibit pain without its undesirable side effects,[2,3] extensive studies have been performed in order to clarify their pharmacological characteristics[4−9] and bioactive conformation[10−14] and to improve biological activity in vitro and in vivo by chemical modification.[15−25]

The aromatic amino acid residue in position 3 is the defining structural determinant between EM-1 (Trp[3]) and EM-2 (Phe[3]).[1,26] Moreover, extensive studies previously indicated that Dmt[a] in lieu of the N-terminal Tyr dramatically enhanced receptor affinity and bioactivity of numerous opioid agonists and antagonists,[27,28] and data on endomorphin-2 analogues modified at position 1 with tyrosine analogues alkylated on the tyramine ring improved in vitro biological parameters.[29] Similarly, an alkylated Phe analogue, 2′,6′-dimethylphenylalanine (Dmp), was an effective surrogate for phenylalanine in several opioid

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[a] Abbreviations: 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.

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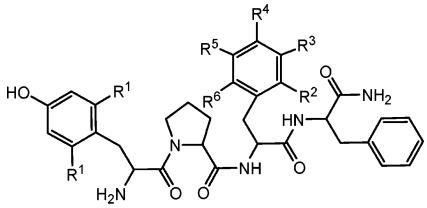

no.	R^1	R^2	$R^3 = R^5$	R^4	R^6
1	H	H	H	H	H
1'	Me	H	H	H	H
2	H	Me	H	H	H
2'	Me	Me	H	H	H
3	H	H	Me	H	H
3'	Me	H	Me	H	H
4	H	Me	H	H	Me
4'	Me	Me	H	H	Me
5	H	Me	H	OH	Me
5'	Me	Me	H	OH	Me
6	H	Me	H	Me	Me
6'	Me	Me	H	Me	Me
7	H	Et	H	H	Me
7'	Me	Et	H	H	Me
8	H	*i*-Pr	H	H	Me
8'	Me	*i*-Pr	H	H	Me

Figure 1. Schematic structure of endomorphin-2 with alkylation sites (R^1-R^6) on Tyr^1 and Phe^3.

peptides such as dynorphin A,[30] [Leu^5]enkephalin,[31] dermorphin, deltorphin II,[32] YrFB (H-Tyr-D-Arg-Phe-βAla-NH_2),[33] and endomorphin-2.[34] Furthermore, the replacement of Tyr^1 by Dmp in deltorphin II and enkephalin also yielded analogues that were surprisingly nearly as effective as the parental peptides,[35] suggesting that alkylation of the aromatic ring enhances hydrophobicity and stability and/or limits rotational freedom in its solution conformation.

Rationale

It is well-known that a subtle change in both the hydrophobicity and spatial conformation of an opioid ligand contributes not only to a modification of its overall activity profile (receptor affinity, bioactivity)[30,32,34,35] and physicochemical properties (stability, conformation)[14,18,21,31] but also to its biological efficacy (in vitro, in vivo) as an antinociceptive agent[27,28,36] with an enhanced ability to transit through the blood−brain barrier.[36,37] In this regard, N,N-alkylation converted a potent δ-opioid antagonist into an inverse agonist[38] and led to the appearance of elevated μ-opioid receptor affinity and increased δ-antagonism among numerous analogues of the Dmt-Tic (1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid) pharmacophoric compounds.[39] On the other hand, the acquisition of a greater degree of hydrophobicity through alkylation of the tyramine ring of Tyr^1 permitted further differentiation of the biological properties between the structurally related endomorphin-1 and -2 molecules.[29,36] In light of the ability of the dialkylated derivative of phenylalanine (Dmp) to substitute for Tyr^1 in deltorphin II[35] and for Phe in other opioids,[30−35] the importance of the residue in the third position of the endomorphins, which is known to affect their receptor selectivity and bioactivity,[1−4,6,7] should be amenable to alterations in the hydrophobic milieu of the aromatic moiety of Phe. An overall change in conformation or reduced freedom of rotation imparted by the alkyl substituents should provide a gauge of the potential effect of the substitution on receptor interaction and functional bioactivity parameters (Figure 1). Furthermore, in light of the importance of μ-opioid receptors in modulating pain, appetite, and alcohol-induced elevation of spontaneous inhibition of postsynaptic currents (IPSC)[40] and the involvement of δ-opioid receptors in ameliorating the morphine tolerance and dependence due to μ-opioid receptors,[41] this study focused exclusively on these two important and interrelated opioid systems. Furthermore, as our data reveal (infra vide), these EM-2 analogues are essentially devoid of activity toward κ-opioid receptors. In fact, the combination of Dmt^1 and an alkylated Phe^3 substitution provides unique evidence for the change in the bioactivities of several analogues, including identification of potent bifunctional μ-opioid receptor agonists containing δ-antagonist or δ-agonist properties.

Chemistry

Dmt was synthesized by following the method described by Dygos et al.[42] The Phe analogues (Figure 1) containing 2′-methyl (Mmp) (R^2), 3′,5′-dimethyl (^{3,5}Dmp) (R^3,R^5), 2′,6′-dimethyl (Dmp) (R^2,R^6), 2′,4′,6′-trimethyl (Tmp) (R^2,R^4,R^6), 2′-ethyl-6′-methyl (Emp) (R^2,R^6), and 2′-isopropyl-6′-methyl (Imp) (R^2,R^6) were prepared as reported, through [Rh(1,5-COD)(*R*,*R*-DIPAMP)]BF_4, [(*R*,*R*)-(−)-1,2-bis[(*o*-methoxyphenyl)(phenyl)phosphino]ethane(1,5-cyclooctadiene)rhodium(I) tetrafluoroborate] mediated asymmetric catalytic hydrogenation of the corresponding acetamidoacrylate.[43] Boc(*tert*-butyloxycarbonyl)-Tyr-Pro-OH[44] and Boc-Dmt-Pro-OH[40] were prepared as reported. Tyr/Dmt-Pro-Xaa-Phe-NH_2 (Xaa = Mmp, ^{3,5}Dmp, Dmp, Dmt, Tmp, Emp, Imp) was formed by segment condensation method in solution. Briefly, after deprotection of Boc-Xaa-Phe-NH_2 with HCl/dioxane, the resulting H-Xaa-Phe-NH_2 was condensed with Boc-Tyr/Dmt-Pro-OH using PyBop (benzotriazol-1-yloxytrispyrrolidinophosphonium hexafluorophosphate) as the coupling reagent. The final Boc protecting group was removed with HCl/dioxane in the presence of anisole, and the resulting peptide was purified by semipreparative RP (reversed phase)-HPLC.

The identification and purity of the final compounds and their intermediates were verified using MS, NMR, analytical HPLC, and elemental analysis. Some analytical data of the final compounds are summarized in Table 1. The final compounds exhibiting greater than 98% purity were used for all biological assays. Supporting Information provides detailed elemental analysis results of all the compounds.

Results and Discussion

Opioid Receptor Affinity. Improved affinities for μ-opioid receptors were observed in the [Xaa^3]EM-2 compounds (**2**, **4**, **6**−**8**) compared to the parent peptide (**1**), except **3** and **5** which exhibited weaker affinities (Table 2). Introduction of a single methyl group at the 2′-position of Phe (**2**) enhanced μ-opioid receptor affinity 6-fold; a second methyl group at the 6′-position (**4**) induced a further 5-fold increase. Sterically bulky groups, such as a third methyl group at the 4′-position (**6**) or an ethyl (**7**) or isopropyl (**8**) at the 6′-position (Figure 1), reduced receptor affinity by a factor of 10 relative to [Dmp^3]EM-2 (**4**), which exhibits the highest μ-selectivity of any known μ-opioid agonist;[1,34] nonetheless, the μ-affinities of **6**−**8** were still 3-fold greater than that of the naturally occurring EM-2 (**1**) (Table 2). Another dimethylated Phe derivative, [^{3,5}Dmp^3]EM-2 (**3**), displayed the lowest μ-opioid receptor affinity of all the analogues (Table 2), being 141-fold lower than [Dmp^3]EM-2 (**4**) and about 5-fold less than **1**, suggesting that the positions of the alkyl groups on the Phe^3 aromatic ring were a critical factor for receptor affinity.

Among the [Dmt^1,Xaa^3]EM-2 ligands (**1′**−**8′**) (Table 2), alkylated Phe^3 analogues essentially enhanced the affinities for both μ- and δ-opioid receptors, although minor discrepancies in μ-opioid receptor affinity were noted for **6′**, **7′**, and **8′** in comparison to the [Xaa^3]EM-2 substances. Although they

Table 1. Analytical Data of H-Tyr/Dmt-Pro-Xaa-Phe-NH_2 Endomorphin-2 Analogues

					TOF mass [M + 1]		HPLC (min)	
compd	peptide	$[\alpha]_D$ (deg)	*c* in H_2O	TLC[a] R_f	calcd	found	t_R[b]	t_R[c]
2	H-Tyr-Pro-Mmp-Phe-NH_2	−38.63	0.26	0.76	586.7	586.8	15.27	27.53
2′	H-Dmt-Pro-Mmp-Phe-NH_2	−4.18	0.23	0.76	614.8	615.0	16.08	29.50
3	H-Tyr-Pro-^{3,5}Dmp-Phe-NH_2	−36.0	0.24	0.76	600.7	600.7	16.44	30.57
3′	H-Dmt-Pro-^{3,5}Dmp-Phe-NH_2	−1.51	0.22	0.78	628.8	628.9	17.33	32.59
4′	H-Dmt-Pro-Dmp-Phe-NH_2	−2.71	0.42	0.72	628.8	628.8	16.69	30.25
5	H-Tyr-Pro-Dmt-Phe-NH_2	−34.22	0.34	0.75	616.7	616.8	13.67	23.26
5′	H-Dmt-Pro-Dmt-Phe-NH_2	0.88	0.45	0.71	644.8	644.8	14.47	25.29
6	H-Tyr-Pro-Tmp-Phe-NH_2	−42.67	0.23	0.77	614.8	615.0	16.63	31.10
6′	H-Dmt-Pro-Tmp-Phe-NH_2	0.98	0.21	0.80	642.8	642.7	17.35	32.59
7	H-Tyr-Pro-Emp-Phe-NH_2	−36.53	0.24	0.79	614.8	614.8	16.39	30.25
7′	H-Dmt-Pro-Emp-Phe-NH_2	−1.97	0.24	0.80	642.8	642.9	17.19	32.70
8	H-Tyr-Pro-Imp-Phe-NH_2	−33.25	0.21	0.76	628.8	628.9	17.26	32.81
8′	H-Dmt-Pro-Imp-Phe-NH_2	1.75	0.25	0.77	656.8	656.7	18.09	34.51

[a] Solvent: *n*-BuOH/H_2O/AcOH/pyridine = 4:1:1:2. [b] The column was eluted at a flow rate of 1 mL/min with a linear gradient of 90% solvent A to 10% solvent A in 30 min. [c] The column was eluted at a flow rate of 1 mL/min with a linear gradient of 90% solvent A to 50% solvent A in 40 min.

Table 2. Receptor Affinities of H-Tyr/Dmt-Pro-Xaa-Phe-NH_2 Endomorphin-2 Analogues[a]

compd	peptide sequence	K_i^μ (nM)[b]	*n*	K_i^δ (nM)[c]	*n*	K_i^δ/K_i^μ	K_i^κ (nM)[d]	n	K_i^κ/K_i^μ
1	*Tyr-Pro-Phe-Phe-NH_2*	1.33 ± 0.15	3	6085 ± 1215	3	4575	>4000		>3000
1′	*Dmt-Pro-Phe-Phe-NH_2*[e]	0.26 ± 0.01	4	99.2 ± 7.9	3	382	489 ± 135	3	1880
2	Tyr-Pro-Mmp-Phe-NH_2	0.22 ± 0.05	3	1741 ± 177	5	7878	ND		
2′	Dmt-Pro-Mmp-Phe-NH_2	0.18 ± 0.015	3	4.61 ± 0.3	3	26	176 ± 64	3	994
3	Tyr-Pro-^{3,5}Dmp-Phe-NH_2	6.19 ± 0.24	5	3451 ± 624	5	558	ND		
3′	Dmt-Pro-^{3,5}Dmp-Phe-NH_2	0.11 ± 0.005	3	11.6 ± 1.6	5	105	830 ± 148	3	7480
4	*Tyr-Pro-Dmp-Phe-NH_2*[e]	0.044 ± 0.003	3	1440 ± 94	3	32730	>4000		>91000
4′	Dmt-Pro-Dmp-Phe-NH_2	0.069 ± 0.008	3	2.27 ± 0.44	4	33	94.2 ± 10.5	3	1365
5	Tyr-Pro-Dmt-Phe-NH_2	2.63 ± 0.25	3	3020 ± 161	5	1148	ND		
5′	Dmt-Pro-Dmt-Phe-NH_2	0.092 ± 0.015	3	80.8 ± 6.2	4	878	998 ± 363	3	10850
6	Tyr-Pro-Tmp-Phe-NH_2	0.44 ± 0.04	4	1358 ± 128	4	3093	ND		
6′	Dmt-Pro-Tmp-Phe-NH_2	0.18 ± 0.004	3	1.83 ± 0.22	3	10	172 ± 35	3	945
7	Tyr-Pro-Emp-Phe-NH_2	0.46 ± 0.071	3	491 ± 14	4	1074	ND		
7′	Dmt-Pro-Emp-Phe-NH_2	0.21 ± 0.025	3	3.03 ± 0.09	3	14	278 ± 66	3	1320
8	Tyr-Pro-Imp-Phe-NH_2	0.45 ± 0.02	4	543 ± 42	4	1221	ND		
8′	Dmt-Pro-Imp-Phe-NH_2	0.32 ± 0.027	3	4.61 ± 0.3	3	14	205 ± 73	3	641

[a] The italicized compounds represent parental opioid peptides. [b] Versus [^{3}H]DAMGO. [c] Versus [^{3}H]deltorphin-II. [d] Versus [^{3}H]U-69,593. *n* is the number of independent repetitions conducted for each analogue using five to eight doses of peptide. ND: not determined. [e] These data are nearly identical to the μ- and δ-opioid affinites and selectivities previously published for **1′** and **4** (refs 29 and 34, respectively).

displayed improved δ-opioid receptor affinity, μ-opioid selectivity remained. As published previously, the presence of Dmt[1] in opioids enhanced δ-opioid receptor affinity by orders of magnitude;[27−29,45] however, with μ-opioid selective agonists, an N-terminal Dmt residue enhanced affinity to δ-sites to a greater extent than found with the prototype δ-opioid antagonists containing the Dmt-Tic pharmacophore,[45] as seen by the 634-fold increase in δ-opioid affinity in **4′** relative to **4** with the concomitant loss of μ-selectivity by 3 orders of magnitude (Table 2). In these Dmt derivatives, the highest μ-opioid selectivity occurred with [Dmt^{1,3}]EM-2 (**5′**) (K_i^δ/K_i^μ = 878), which was less (37-fold) than the most selective [Xaa^3]EM-2 compound (**4**) but nearly 3-fold greater than **1′** (Table 2). One analogue of considerable interest is Tmp^3 (**6′**); with a 44-fold greater interaction than Dmt^3 (**5′**) toward δ-opioid receptors, the data suggest that the hydrogen donor capability of the hydroxyl group of Dmt is apparently less effective in affecting receptor interaction when situated within the peptide than the hydrophobicity of the 4′ methyl group; i.e., the hydroxyl group may contribute a negative influence as an internal residue supporting the original study on the endomorphins.[1]

As seen in Table 2, κ-opioid receptor affinities for the Dmt derivatives (**1′**−**8′**) and parental compounds (**1**, **4**) were quite weak relative to the interaction of these peptides to both μ- and δ-opioid receptors, although the control U-69593 demonstrated good affinity (1.04 ± 0.45 nM). Owing to the lack of high κ-opioid affinity, none of the analogues were investigated for their κ-functional bioactivity.

Functional Bioactivity. The functional bioactivities (Table 3) of the [Xaa^3]EM-2 analogues exhibited considerable variation in μ-agonism relative to EM-2 (**1**); analogues **2** and **6**−**8** were 2- to 5-fold more active, while **3** and **5** were inactive. The increase in hydrophobicity and positioning of the methyl groups on Phe^3 changed μ-agonism relative to EM-2 (**1**); i.e., while alkylation at the 3′ and 5′ positions of Phe^3 inactivated the molecule (**3**), substitutions at positions 2′ and 6′ were very well tolerated (**4**) producing dual μ-agonism/δ-agonism.[34] Moreover, trimethylation (**6**) yielded a highly selective μ-agonist.

The [Dmt^1,Xaa^3]EM-2 analogues (Table 3) generally doubled μ-opioid bioactivities (**2′**, **4′**), remained essentially unchanged (**5′**, **6′**, **7′**, **8′**), or lost bioactivity (**3′**) relative to **1′**.[29] Compound **3′** was an anomaly; the biopotency of this analogue correlated with μ-opioid receptor affinity, suggesting that the methyl groups at positions 3′ and 5′ (**3**, **3′**) presented an unfavorable conformation for activation of the μ-opioid receptor, whereas the dimethyl alkylation at positions 2′ and 6′ in Phe^3 (**4′** and **4**[34]) was permitted. This was equally true for the trimethyl- (**6′**), ethylmethyl- (**7′**), and isopropylmethyl-Phe^3 (**8′**) derivatives. This observation was repeated in the [Xaa^3]EM-2 series (**6**−**8**), reflecting a change that presumably enabled the ligands to adopt a more compatible ligand−receptor conformation to trigger a biological response. Substitution of Phe^3 by Dmt^3 only yielded a bioactive compound in the presence of Dmt^1 (compare **5** and **5′**). Moreover, replacement of the 4′ OH of Dmt^3 (**5′**) by a methyl group to yield Tmp^3 (**6′**) displayed slightly enhanced μ-agonism and significantly improved δ-antagonism. Tmp^3 (**6′**)

Table 3. Functional Bioactivities of H-Tyr/Dmt-Pro-Xaa-Phe-NH_2 Endomorphin-2 Analogues[a]

			MVD assay[b]	
compd	peptide	GPI assay[b] IC_{50} (nM)	IC_{50} (nM)[c]	pA_2[d]
1	*Tyr-Pro-Phe-Phe-NH_2*	6.9 ± 0.9	344 ± 93	
1′	*Dmt-Pro-Phe-Phe-NH_2*[e]	0.26 ± 0.038	0.59 ± 0.18	
2	Tyr-Pro-Mmp-Phe-NH_2	1.33 ± 0.27	15.7 ± 5.1	
2′	Dmt-Pro-Mmp-Phe-NH_2	0.16 ± 0.041	>10000 (0.00%)	6.59
3	Tyr-Pro-^{3,5}Dmp-Phe-NH_2	389 ± 166	>10000 (8.0%)	
3′	Dmt-Pro-^{3,5}Dmp-Phe-NH_2	14.4 ± 5.4	>10000 (7.1%)	6.77
4	*Tyr-Pro-Dmp-Phe-NH_2*[f]	0.38 ± 0.104	1.39 ± 0.17	
4′	Dmt-Pro-Dmp-Phe-NH_2	0.12 ± 0.020	>10000 (15.6%)	8.15
5	Tyr-Pro-Dmt-Phe-NH_2	541 ± 178	978 ± 113	
5′	Dmt-Pro-Dmt-Phe-NH_2	1.94 ± 0.21	>10000 (<1%)	7.06
6	Tyr-Pro-Tmp-Phe-NH_2	1.90 ± 0.49	>10000 (36.2%)	
6′	Dmt-Pro-Tmp-Phe-NH_2	0.21 ± 0.077	>10000 (22.2%)	9.05
7	Tyr-Pro-Emp-Phe-NH_2	2.35 ± 0.53	277 ± 29	
7′	Dmt-Pro-Emp-Phe-NH_2	0.17 ± 0.072	0.51 ± 0.24	
8	Tyr-Pro-Imp-Phe-NH_2	2.74 ± 1.20	>10000 (17.7%)	
8′	Dmt-Pro-Imp-Phe-NH_2	0.20 ± 0.050	5.56 ± 2.47	

[a] The italicized compounds represent parental opioid peptides. [b] The data are the mean of over five independent repetitions used in different isolated tissue preparations. IC_{50} is the concentration required for 50% inhibition of the electrically induced contraction in muscle derived from a dose-response curve. [c] Values in parentheses indicate maximal inhibition of the tissue contraction at a concentration of 10 000 nM. [d] Negative log of the molar concentration required to double the deltorphin II concentration to achieve the original response. [e] Data cited from ref 29. [f] Data cited from ref 34.

became a mixed μ-agonist/δ-antagonist with δ-antagonism being 2 orders of magnitude greater than that for Dmt[3] (**5′**).

Recently, Dmt-Pro-Trp-Phe-NH_2 ([Dmt[1]]EM-1) was reported to be a mixed μ-opioid agonist/δ-antagonist (GPI IC_{50} = 0.272 nM; MVD pA_2 = 8.60).[36b] On the other hand, [Dmt[1]]EM-2 (**1′**) is a dual μ-opioid agonist/δ-opioid agonist.[29] The only difference between [Dmt[1]]EM-1 and **1′** is Trp[3] or Phe[3]. This indicates that the bulky side chain of Trp in combination with Dmt[1] causes either a steric hindrance in the conformation of the peptide or a shift in hydrophobicity to potentiate the induction of δ-opioid receptor antagonism.

In terms of the δ-functional bioactivities, the majority of the ligands failed to elicit substantial δ-agonism except **7′** and to a lesser extent both **8′** (10-fold loss) and **2** (30-fold decrease), in which the bioactivity profile of **7′** resembled the bioactivity profiles of the reference compounds (**1′** (cf. ref 24) and **4**[34]). Nonetheless, the [Dmt[1],Xaa[3]]EM-2 compounds established bioactive profiles distinctly different from the profiles of the [Xaa[3]]EM-2 analogues: (i) the appearance of δ-antagonism (**2′**, **3′**, **4′**, **5′**, **6′**) ranging from pA_2 = 6.59 (**2′**) to a potent pA_2 = 9.05 (**6′**) and (ii) the appearance of δ-agonism with **7′** and **8′**. The δ-opioid antagonism of [Dmt[1],Tmp[3]]EM-2 (**6′**) is about 8-fold more potent than the prototype δ-antagonist H-Dmt-Tic-OH (pA_2 = 8.48).[45] Furthermore, [Xaa[3]]EM-2 analogues remained μ-agonists except for the inactive ligands **3** and **5** and the dual μ-agonism/δ-agonism of **2**. An N-terminus Dmt in **4′** eliminated δ-agonism of **4** with the appearance of δ-antagonism (**4′**). In fact, the loss of δ-agonism in the [Dmt[1],Xaa[3]]EM-2 analogues was associated with the δ-antagonism (**6′** > **4′** > **5′**, **3′**, **2′**).

Substitution of Phe[3] in EM-2 by other phenylalanine analogues, such as Hfe (homophenylalanine), Phg (phenylglycine), D-Phg,[46] *N*-methyl-Phe,[22] D-2-Nal [D-3-(2-naphthyl)-alanine],[23] L- and D-α-aminoxy-Phe,[21] β-methyl-Phe,[18] 4′-NH_2-Phe, and 4′-NCS-Phe,[47] failed to improve the affinity toward the μ-opioid receptor or provide unique functional bioactivities in the resultant ligands. Our results clearly indicated that modification of Phe[3] with alkyl groups not only enhnaced receptor affinity but also transformed the functional bioactivity to elicit pharmacological properties as bifunctional opioid ligands (dual μ-agonists/δ-agonists and mixed μ-agonists/δ-antagonists).

Conclusion

This study demonstrated that alkylated Phe[3] residues incorporated into the third position of EM-2 act as effective amino acid surrogates, in particular the [Dmt[1],Xaa[3]]EM-2 analogues, which exhibited significantly higher affinity for both μ- and δ-opioid receptors and yet retained μ-opioid bioactivity. The lack of interaction of the analogues with κ-opioid receptors revealed that these compounds maintained the selectivity of endomorphin for μ-opioid receptors. The most intriguing observation was the unique appearance of δ-antagonism in the endomorphins, which normally lack interaction with δ-opioid receptors and have high μ-opioid receptor selectivity and prominent μ-opioid agonism.[1] These bifunctional molecules are targets in the design of new antinociceptive opioids that could potentially alleviate acute or chronic pain with low physical dependence and tolerance,[41] especially due to the acquisition of δ-opioid antagonism. Pharmacological and biochemical evidence further reveal that μ- and δ-opioid receptors readily form heterodimers[48] and that the δ-opioid receptors modulate the function of μ-opioid receptors in this complex.[49] Thus, our opioid derivatives displaying dual μ-agonism/δ-agonism (**7′**) and mixed μ-agonism/δ-antagonism (**6′**, **4′**) characteristics that are shown to exhibit a low tendency to develop dependence and tolerance[50] and verified using δ-receptor knockout mice[51] and a δ-receptor antagonist[52] might be applicable for antinociception with a low degree of dependence and tolerance. Furthermore, structural clarification of the subtle differences among these interrelated opioid ligands[14,23] should provide insight on the molecular mechanism of action between agonists and antagonists.[53]

Experimental Section

Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. TLC was performed on precoated plates of silica gel F254 (Merk, Darmstadt, Germany). R_{f1}, R_{f2}, R_{f3}, and R_{f4} values refer to $CHCl_3$/H_2O/AcOH (acetic acid) (90:8:2), AcOEt (ethyl acetate), AcOEt/MeOH (methanol) (20:1), and *n*-BuOH (butanol)/H_2O/AcOH/pyridine (4:1:1:2), respectively. Optical rotations were measured with a DIP-1000 automatic polarimeter (Japan Spectroscopic Co.). A Waters Delta 600 with COSMOSIL C18 column (4.6 mm × 250 mm) was used for analytic RP-HPLC. The solvents for analytical HPLC were the

following: solvent A, 0.05% TFA (trifluoroacetic acid) in water; solvent B, 0.05% TFA in CH_3CN. Elution was at a flow rate of 1 mL/min with a linear gradient of 90% A to 10% A in 30 min and with a linear gradient of 90% A to 50% A in 40 min. The retention time was reported as t_R (min). ^1H and ^{13}C NMR spectra were measured on a Bruker DPX-400 spectrometer at 25 °C. Chemical shift values are expressed as ppm downfield from tetramethylsilane.

General Procedure for Boc Protected Phe Analogues Boc-Xaa-OH (Xaa = Mmp, ^{3,5}Dmp, Dmp, Dmt, Tmp, Emp, Imp). $(Boc)_2O$ (di-*tert*-butyl dicarbonate, l5.5 mmol) in dioxane (15 mL) was added to a solution of amino acid (5.0 mmol) in H_2O (15 mL) containing TEA (triethylamine, 15.0 mmol). The reaction mixture was stirred at 0 °C for 15 min and then at room temperature for 2 h, and the solvent was removed under vacuum. The residue was adjusted to pH 2−3 by using cooled 10% citric acid; the precipitated product was extracted with AcOEt. The combined extract was washed with saturated NaCl solution, dried over Na_2SO_4, filtered, and concentrated. The residue was diluted with hexane, and the solid was collected by filtration and dried under vacuum. Elemental analysis results of Boc-Xaa-OH are summarized in Supporting Information (Table 1).

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-2′-methyl-L-phenylalanine.** Yield 1.25 g (89.2%); mp 118−119 °C; R_{f1} = 0.56; $[\alpha]_D^{25}$ −15.54° (*c* 1.03, MeOH). ^1H NMR (400.1 MHz, $CDCl_3$) δ: 7.20−7.05 (m, 4H), 6.63 (br, 0.38H), 4.93 (br, 0.62H), 4.60−4.37 (m, 1H), 3.33−3.20 (m, 1H), 3.07−2.80 (m, 1H), 2.36 (s, 3H), 1.40, 1.17 (2s, 9H).

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-3′,5′-dimethyl-L-phenylalanine.** Yield 0.71 g (96.5%); mp 115−116 °C; R_{f1} = 0.56; $[\alpha]_D^{25}$ +16.09° (*c* 0.67, MeOH). ^1H NMR (400.1 MHz, $CDCl_3$) δ: 6.89 (s, 1H), 6.79 (s, 2H), 4.89 (br, 1H), 4.52 (br, 1H), 3.12 (dd, 1H, *J* = 5.34, 13.84 Hz), 3.05−2.91 (m, 1H9, 2.28 (s, 3H), 1.42 (s, 9H).

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-2′,6′-dimethyl-L-phenylalanine.** Yield 1.3 g (93.0%); mp 135−136 °C; R_{f1} = 0.40; $[\alpha]_D^{25}$ −17.85° (*c* 0.41, MeOH). ^1H NMR (400.1 MHz, $CDCl_3$) δ: 7.13−6.95 (m, 3.50H), 5.03−4.92 (br, 0.4H), 4.58−4.46 (m, 1H), 3.25−3.02 (m, 2H), 2.40, 2.37 (2s, 6H), 1.36, 1.06 (2s, 9H).

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-2′,4′,6′-trimethyl-L-phenylalanine.** Yield 1.37 g (88.2%); mp 153−154 °C; R_{f1} = 0.56; $[\alpha]_D^{25}$ −13.26° (*c* 0.94, MeOH). ^1H NMR (400.1 MHz, $CDCl_3$) δ: 7.03 (br, 0.60H), 6.84 (s, 2H), 4.97 (br, 0.40H), 4.53−4.40 (m, 1H), 3.23−3.13 (m, 1H), 3.05 (dd, 1H, *J* = 9.95, 14.10 Hz), 2.35 (s, 6H), 2.24 (s, 3H), 1.37, 1.08 (2s, 9H).

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-2′-ethyl-6′methyl-L-phenylalanine.** Yield 1.32 g (85.7%); mp 117−118 °C; R_{f1} = 0.56; $[\alpha]_D^{25}$ −14.33° (*c* 1.0, MeOH). ^1H NMR (400.1 MHz, $CDCl_3$) δ: 7.26 (br, 0.77H), 7.14−7.00 (m, 3H), 4.97 (br, 0.23H), 4.60−4.50 (m, 1H), 3.28−3.17 (m, 1H), 3.17−3.06 (m, 1H), 2.86−2.65 (m, 2H), 2.42, 2.39 (2s, 3H), 1.36 (s, 2.2H), 1.22 (t, 3H, *J* = 7.51 Hz), 1.05 (s, 6.8H).

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-2′-isopropyl-6′-methyl-L-phenylalanine.** Yield 1.45 g (90.3%); mp 121−122 °C; R_{f1} = 0.56; $[\alpha]_D^{25}$ −12.78° (*c* 0.93, MeOH). ^1H NMR (400.1 MHz, $CDCl_3$) δ: 7.26 (br, 0.76H), 7.17−7.07 (m, 2H), 7.05−6.95 (m, 1H), 4.95 (br, 0.24H), 4.56−4.45 (m, 1H), 3.47−3.30 (m, 0.76H), 3.30−3.05 (m, 2.24H), 2.43, 2.40 (2s, 3H), 1.35 (s, 2.3H), 1.25 (d, 3H, *J* = 6.73 Hz), 1.18 (d, 3H, *J* = 6.42 Hz), 1.05 (s, 6H).

General Procedure for Synthesis of Boc-Xaa-Phe-NH_2 (Xaa = Mmp, ^{3,5}Dmp, Dmp, Dmt, Tmp, Emp, Imp). The H-Phe-NH_2 hydrochloride salt (0.25 g, 1.25 mmol) was dissolved in DMF (*N*,*N*-dimethylformamide, 10 mL) containing DIPEA (diisopropylethylamine, 0.50 mL, 2.87 mmol), and to this solution Boc-Xaa-OH (1.14 mmol) and PyBop (0.65 g, 1.25 mmol) were added. The reaction mixture was stirred at 0 °C for 10 min, then at room temperature for 4 h. After removal of solvent, the residue was diluted with AcOEt (80 mL). The diluted mixture was washed with ice-cooled 10% citric acid (3 × 15 mL), 5% Na_2CO_3 (3 × 15 mL), and saturated NaCl (3 × 20 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, AcOEt). The compound was precipitated with hexane, collected by filtration, and dried under vacuum. Elemental analysis results of Boc-Xaa-Phe-NH_2 are summarized in Supporting Information (Table 2).

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-2′-methyl-L-phenylalanylphenylalanylamide.** Yield 470 mg (97.0%); mp 164−166 °C; R_{f2} = 0.73; $[\alpha]_D^{25}$ −21.04° (*c* 0.34, MeOH). ^1H NMR (400.1 MHz, DMSO-d_6) δ: 7.89 (d, 0.15H, *J* = 6.80 Hz, Phe NH), 7.79 (d, 0.84H, *J* = 8.25 Hz, Phe NH), 7.45, 7.36 (2s, 1.0H, $CONH_2$), 7.30−6.96 (m, 10.9H, $CONH_2$, Mmp NH, Phe Ar−H, Mmp Ar−H), 6.52 (d, 0.1H, *J* = 6.8 Hz, Mmp NH), 4.60−4.43 (m, 1.0H, Phe αH), 4.11 (dt, 1.0H, *J* = 4.45, 9.80 Hz, Mmp αH), 3.03 (dd, 1.0H, *J* = 4.87, 13.72 Hz, Phe βH), 2.90−2.70 (m, 2H, Phe βH, Mmp βH), 2.65 (dd, 1.0H, *J* = 10.15, 14.12 Hz, Mmp βH), 2.24 (s, 3H, Mmp CH_3), 1.29, 1.14 (2s, 9.0H, Boc). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 172.45, 171.11, 155.01, 137.58, 136.01 (5q), 129.73, 129.25, 127.89, 126.13, 125.39 (5t, Mmp and Phe Ar−C), 78.16 (q, Boc), 4.71 (t, Mmp αC), 53.29 (t, Phe αC), 37.67 (s, Phe βC), 34.78 (s, Mmp βC), 28.00, 27.57 (2p, Boc), 18.90 (p, Mmp *C*H_3).

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-3′,5′-dimethyl-L-phenylalanylphenylalanylamide.** Yield 493 mg (98.6%); mp 193−195 °C; R_{f2} = 0.74; $[\alpha]_D^{25}$ −16.02° (*c* 0.39, MeOH). ^1H NMR (400.1 MHz, DMSO-d_6) δ: 8.0−7.92 (br, 0.14H, Phe NH), 7.86 (d, 0.80H, *J* = 8.23 Hz, Phe NH), 7.45, 7.35 (2s, 1H, $CONH_2$), 7.30−7.14 (m, 5H, Phe Ar−H), 7.14−6.96 (m, 1H, $CONH_2$), 6.91−6.71 (m, 3.75H, ^{3,5}Dmp Ar−H, ^{3,5}Dmp NH), 6.40−6.32 (br, 0.12H, ^{3,5}Dmp NH), 4.36−4.39 (m, 1H, Phe αH), 4.12−3.98 (m, 1H, ^{3,5}Dmp αH), 3.01 (dd, 1.0H, *J* = 5.04, 13.75 Hz, Phe βH), 2.84 (dd, 1.0H, *J* = 8.65, 13.75 Hz, Phe βH), 2.77 (dd, 1.0H, *J* = 4.26, 13.68 Hz, ^{3,5}-Dmp βH), 2.59 (dd, 1.0H, *J* = 10.09, 13.68 Hz, ^{3,5}Dmp βH), 2.21 (s, 6H, ^{3,5}Dmp CH_3), 1.31, 1.16 (2s, 9H, Boc). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 172.54, 171.14, 155.02, 137.77, 137.62, 136.65 (6q), 129.20, 127.91 (2t, Phe Ar−C), 127.49, 126.77 (2t, ^{3,5}Dmp Ar−C), 126.13 (t, Phe Ar−C), 78.04 (q, Boc), 55.88 (t, ^{3,5}Dmp αC), 53.36 (t, Phe αC), 37.79 (s, Phe βC), 37.16 (s, ^{3,5}Dmp βC), 28.01 (p, Boc), 20.81 (p, ^{3,5}Dmp *C*H_3).

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-2′,6′-dimethyl-L-phenylalanylphenylalanylamide.** Yield 491 mg (98.0%); mp 177−179 °C; R_{f2} = 0.73; $[\alpha]_D^{25}$ −15.28° (*c* 0.58, MeOH). ^1H NMR (400.1 MHz, DMSO-d_6) δ: 7.95 (d, 0.2H, *J* = 7.51 Hz, Phe NH), 7.83 (d, 0.75H, *J* = 8.48 Hz, Phe NH), 7.45 (br, 0.23H, $CONH_2$), 7.33−7.21 (m, 4.68H, $CONH_2$ and Ar−H), 7.21−7.13 (m, 1H, Ar−H), 7.08 (s, 1.0H, $CONH_2$), 7.0−6.86 (m, 3.73H, Ar−H and Dmp NH), 6.54 (d, 0.14H, *J* = 9.54 Hz, Dmp NH), 4.58−4.45 (m, 1.0H, Phe αH), 4.19−4.03 (m, 1.0H, Dmp αH), 3.02 (dd, 1.0H, *J* = 5.05, 13.62 Hz, Phe βH), 2.88−2.55 (m, 3.0H, Phe βH and Dmp βH), 2.07 (s, 6.0H, Dmp CH_3), 1.26, 1.04 (2s, 9.0H, Boc). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 172.48, 170.91, 154.64, 137.66, 136.61, 134.68 (6q), 129.28, 127.86, 127.78, 126.12, 125.85 (5t, Ar−C), 78.13 (q, Boc), 54.91 (t, Dmp αC), 53.35 (t, Phe αC), 37.84 (s, Phe βC), 32.30 (s, Dmp βC), 27.97, 27.34 (2p, Boc), 19.80 (p, Dmp *C*H_3).

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-2′,6′-dimethyl-L-tyrosylphenylalanylamide.** Yield 483 mg (93.2%); mp 184−186 °C; R_{f2} = 0.93; $[\alpha]_D^{25}$ −19.08° (*c* 0.33, MeOH). ^1H NMR (400.1 MHz, DMSO-d_6) δ: 8.66 (s, 1H, Dmt OH), 7.91 (d, 0.20H, *J* = 8.18 Hz, Phe NH), 7.79 (d, 0.78H, *J* = 8.44 Hz, Phe NH), 7.47, 7.32 (2s, 1.0H, $CONH_2$), 7.29−7.02 (m, 6H, Phe Ar−H, $CONH_2$), 6.86 (d, 0.74H, *J* = 9.30 Hz, Dmt NH), 6.43 (d, 0.17H, *J* = 9.69 Hz, Dmt NH), 6.34 (s, 2H, Dmt Ar−H), 4.58−4.43 (m, 1.0H, Phe αH), 4.10−3.93 (m, 1.0H, Dmt αH), 3.02 (dd, 1.0H, *J* = 5.09, 13.60 Hz, Phe βH), 2.81 (dd, 1.0H, *J* = 9.01, 13.60 Hz, Phe βH), 2.65 (dd, 1.0H, *J* = 5.14, 14.40 Hz, Dmt βH), 2.55 (dd, 1.0H, *J* = 9.16, 14.40 Hz, Dmt βH), 2.13 (s, 6.0H, Dmt CH_3), 1.28, 1.09 (2s, 9.0H, Boc). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 172.49, 171.13, 154.90, 154.69, 137.65 (5q), 129.26, 127.83, 126.09 (3t, Phe Ar−C), 124.96 (q), 114.61 (t, Dmt Ar−C), 78.05 (q, Boc), 55.38 (t, Dmt αC), 53.30 (t, Dmt αC), 37.82 (s, Phe βC), 31.58 (s, Dmt βC), 27.99, 27.39 (2p, Boc), 19.95 (p, Dmt *C*H_3).

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-2′,4′,6′-trimethyl-L-phenylalanylphenylalanylamide.** Yield 483 mg (93.5%); mp 224−225 °C; R_{f2} = 0.76; $[\alpha]_D^{25}$ −16.38° (*c* 0.41, MeOH). ^1H NMR (400.1 MHz,

DMSO-d_6) δ: 7.94 (d, 0.18H, J = 7.62 Hz, Phe NH), 7.83 (d, 0.79H, J = 8.46 Hz, Phe NH), 7.43 (br, 0.20H, $CONH_2$), 7.30−7.13 (m, 5.80H, $CONH_2$ and Tyr Ar−H), 6.92 (d, 0.77H, J = 9.29 Hz, Tmp NH), 6.73 (s, 2H, Tmp Ar−H), 6.48 (d, 0.15H, J = 9.46 Hz, Tmp NH), 4.60−4.40 (m, 1H, Phe αH), 4.15−3.96 (m, 1H, Tmp αH), 3.02 (dd, 1H, J = 5.03, 13.54 Hz, Phe βH), 2.81 (dd, 1H, J = 9.05, 13.54 Hz, Phe βH), 2.72 (dd, 1H, J = 5.31, 14.15 Hz, Tmp βH), 2.63 (dd, 1H, J = 9.22, 14.10 Hz, Tmp βH), 2.18, 2.15 (2s, 9H, Tmp CH_3), 1.27, 1.06 (2s, 9H, Boc). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 172.49, 171.00, 154.67, 153.62, 137.68, 136.40, 134.50, 131.56 (8q), 129.26 (t, Phe Ar−C), 128.48 (t, Tmp Ar−C), 127.84, 126.09 (2t, Phe Ar−C), 78.11 (q, Boc), 55.04 (t, Tmp αC), 53.34 (t, Phe αC), 37.79 (s, Phe βC), 31.91 (s, Tmp βC), 27.96, 27.31 (2p, Boc), 20.33, 19.71 (2p, Tmp CH_3).

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-2′-ethyl-6′-methyl-L-phenylalanylphenylalanylamide.** Yield 481 mg (93.2%); mp 208−210 °C; R_{f2} = 0.78; $[\alpha]_D^{25}$ −15.16° (c 0.35, MeOH). ^{1}H NMR (400.1 MHz, DMSO-d_6) δ: 7.93 (d, 0.16H, J = 7.63 Hz, Phe NH), 7.81 (d, 0.77H, J = 8.46 Hz, Phe NH), 7.50−6.88 (m, 10.87H, $CONH_2$, Emp NH, Phe Ar−H, Emp Ar−H), 6.55 (d, 0.16H, J = 9.29 Hz, Emp NH), 4.57−4.40 (m, 1H, Phe αH), 4.16−3.97 (m, 1H, Emp αH), 3.03 (dd, 1H, J = 5.02, 13.62 Hz, Phe βH), 2.85−2.53 (m, 5H, Phe βH, Emp βH, Emp CH_2CH_3), 2.24 (s, 3H, Emp Ar−CH_3), 1.26 (s, 7.38H, Boc), 1.10 (t, 3H, J = 7.42 Hz, Emp CH_2CH_3), 1.04 (s, 1.62H, Boc). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 172.46, 170.89, 154.65, 142.64, 137.62, 136.65, 133.90 (7q), 129.29, 127.83 (2t, Phe Ar−C), 127.67 (t, Emp Ar−C), 126.10 (t, Phe Ar−C and Emp Ar−C), 125.94 (t, Emp Ar−C), 78.12 (q, Boc), 55.56 (t, Emp αC), 53.28 (t, Phe αC), 37.88 (s, Phe βC), 31.44 (s, Emp βC), 27.95, 27.35 (2p, Boc), 25.03 (s, Emp, CH_2CH_3), 19.90 (p, Emp Ar-CH_3) 15.03 (p, Emp CH_2CH_3).

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-2′-isopropyl-6′-methyl-L-phenylalanylphenylalanylamide.** Yield 495 mg (93.0%); mp 109−110 °C; R_{f2} = 0.72; $[\alpha]_D^{25}$ −22.11° (c 0.33, MeOH). ^{1}H NMR (400.1 MHz, DMSO-d_6) δ: 7.93 (d, 0.17H, J = 7.29 Hz, Phe NH), 7.83 (d, 0.76H, J = 8.56 Hz, Phe NH), 7.51, 7.43 (2s, 0.87H, $CONH_2$), 7.30−6.86 (m, 10H, $CONH_2$, Ar−H, Imp NH), 6.60 (d, 0.17H, J = 9.47 Hz, Imp NH), 4.60−4.48 (m, 1H, Phe αH), 4.10−3.97 (m, 1H, Imp αH), 3.22 (hept, 1H, J = 6.68 Hz, $CH(CH_3)_2$), 3.04 (dd, 1H, J = 4.98, 13.52 Hz, Phe βH), 2.83 (dd, 1H, J = 9.14, 13.52 Hz, Phe βH), 2.78−2.58 (m, 2H, Imp βH), 2.25 (s, 3H, Imp Ar−CH_3), 1.23 (s, 7H, Boc), 1.17 (d, 3H, J = 6.68 Hz, $CH(CH_3)_2$), 1.10 (d, 3H, J = 6.68 Hz, $CH(CH_3)_2$), 1.02 (s, 2H, Boc). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 172.53, 170.85, 154.60, 147.38, 137.59, 136.48, 133.01 (7q), 129.34, 127.81, 127.39, 126.22, 126.11, 122.74 (6t, Ar−C), 78.10 (q, Boc), 55.74 (t, Imp αC), 53.19 (t, Phe αC), 38.03 (s, Phe βC), 30.97 (s, Imp βC), 27.92 (t, $CH(CH_3)_2$; p, Boc), 27.36 (p, Boc), 24.37, 23.73 (2p, $CH(CH_3)_2$), 20.13 (p, Imp Ar-CH_3).

General Procedure for Synthesis of Boc-Tyr/Dmt-Pro-Xaa-Phe-NH_2 (Xaa = Mmp, ^{3,5}Dmp, Dmp, Dmt, Tmp, Emp, Imp). Boc-Xaa-Phe-NH_2 (0.44 mmol) was treated with 7.7 M HCl/dioxane (1.15 mL, 8.8 mmol) to remove the Boc group at room temperature for 30 min. The product was precipitated with ether, filtered, and dried under vacuum. The resulting hydrochloride salt was dissolved in DMF (10 mL) containing DIPEA (184 μL, 1.06 mmol), and to this solution Boc-Xaa-Pro-OH (0.44 mmol) and PyBop (252 mg, 0.49 mmol) were added. The reaction mixture was stirred at 0 °C for 10 min, then at room temperature for 4 h. After removal of solvent, the residue was diluted with AcOEt. The dilution was washed with ice-cooled 10% citric acid (3 × 15 mL), 5% Na_2CO_3 (3 × 15 mL), and saturated NaCl (3 × 20 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, AcOEt/MeOH = 10:1). The compound was precipitated with hexane, filtered, and dried under vacuum. Elemental analysis results of Boc-Tyr/Dmt-Pro-Xaa-Phe-NH_2 are summarized in Supporting Information (Table 3).

***N*$^{\alpha}$-*tert*-Butyloxycarbonyltyrosylprolyl-2′-methyl-L-phenylalanylphenylalanylamide.** Yield 289 mg (95.6%); mp 138−140 °C; R_{f3} = 0.64; $[\alpha]_D^{25}$ −50.30° (c 0.64, MeOH). ^{1}H NMR (400.1 MHz, DMSO-d_6) δ: 9.21 (s, 0.19H, Tyr cis-OH), 9,15 (s, 0.76H, Tyr trans-OH), 8.46 (d, 0.16H, J = 8.39 Hz, Phe cis-NH), 8.01 (d, 0.76H, J = 7.57 Hz, Phe trans-NH), 7.86 (d, 0.78H, J = 8.20 Hz, Mmp trans-NH), 7.76 (d, 0.18H, J = 8.12 Hz, Mmp cis-NH), 7.28−7.14 (m, 5.0H, Phe Ar−H), 7.14−6.93 (m, 8.9H, Tyr Ar−H, Mmp Ar−H, $CONH_2$, Tyr NH), 6.68−6.57 (m, 2.1H, Tyr Ar−H, Tyr NH), 4.48−4.34 (m, 2.0H, Phe αH, Tyr αH), 4.34−4.10 (m, 1.77H, Pro trans-αH, Mmp αH), 3.82 (d, 0.17H, J = 7.67 Hz, Pro cis-αH), 3.64−3.53 (m, 0.65H, Pro trans-δH), 3.53−3.37 (m, 0.96H, Pro trans-δH), 3.24−2.54 (m, 6.39H, Pro cis-δH, Phe βH, Tyr βH, Mmp βH), 2.25 (s, 3.0H, Mmp CH_3), 1.98−1.87 (m, 0.70H, Pro trans-βH), 1.87−1.66 (m, 2.66H, Pro cis-βH, Pro trans-βH, Pro trans-γH), 1.48−1.38 (m, 0.27H, Pro cis-γH), 1.38−1.14 (m, 9.2H, Boc, Pro cis-βH), 1.0−0.86 (m, 0.15H, Pro cis-γH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 172.39, 171.56, 170.86, 170.65, 170.35, 170.25, 155.96, 155.67, 155.36, 155.24, 137.84, 137.65, 136.04, 135.79 (14q), 130.30, 130.09, 129.77, 129.05, 128.94, 127.94, 126.44, 126.20, 126.12, 125.53, 114.89, 114.77 (12t, Ar−C), 78.32, 77.87 (2q, Boc), 59.52 (t, Pro trans-αC), 59.39 (t, Pro cis-αC), 54.10 (t, Tyr trans-αC), 53.88 (t, Tyr cis-αC), 53.71 (t, Phe αC), 53.49 (t, Mmp cis-αC), 53.16 (t, Mmp trans-αC), 46.62 (s, Pro trans-δC), 46.04 (s, Pro cis-δC), 37.60 (s, Phe cis-βC), 37.17 (s, Phe trans-βC), 36.85 (s, Tyr cis-βC), 35.45 (s, Tyr trans-βC), 34.23 (s, Mmp trans-βC), 33.77 (s, Mmp cis-βC), 29.99 (s, Pro cis-βC), 28.54 (s, Pro trans-βC), 28.06 (p, trans-Boc), 27.75 (p, cis-Boc), 24.39 (s, Pro trans-γC), 21.01 (s, Pro cis-γC), 19.01 (p, Mmp trans-CH_3), 18.91 (p, Mmp cis-CH_3).

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-2′,6′-dimethyl-L-tyrosylprolyl-2′-methyl-L-phenylalanylphenylalanylamide.** Yield 266 mg (84.5%); mp 222−228 °C; R_{f3} = 0.71; $[\alpha]_D^{25}$ −45.81° (c 0.36, MeOH). ^{1}H NMR (400.1 MHz, DMSO-d_6) δ: 9.05 (s, 0.45H, Dmt cis-OH), 8.86 (s, 0.43H, Dmt trans-OH), 8.33 (d, 0.44H, J = 8.22 Hz, Phe NH), 8.10 (d, 0.15H, J = 7.22 Hz, Phe NH), 8.03 (d, 0.33H, J = 7.52 Hz, Phe NH), 7.83 (d, 0.48H, J = 8.14 Hz, Mmp NH), 7.65 (d, 0.43H, J = 8.06 Hz, Mmp NH), 7.29−7.14 (m, 5.5H, Phe Ar−H, $CONH_2$), 7.14−6.95 (m, 6.0H, Mmp Ar−H, $CONH_2$, Dmt NH), 6.75 (d, 0.33H, J = 8.54 Hz, Dmt NH), 6.43−6.28 (m, 2.1H, Dmt Ar−H, Dmt NH), 4.48−4.23 (m, 3.0H, Pro trans-αH, Mmp αH, Phe αH, Dmt trans-αH), 4.17−4.08 (m, 0.46H, Dmt cis-αH), 3.52−3.40 (m, 0.49H, Pro trans-δH), 3.20−2.65 (m, 8.0H, Pro cis-αH, Pro cis-δH, Pro trans-δH, Phe βH, Mmp βH, Dmt βH), 2.25 (s, 3.0H, Mmp CH_3), 2.16, 2.09 (2s, 6.0H, Dmt CH_3), 1.96−1.62 (m, 2.53H, Pro cis-βH, Pro trans βH, Pro trans-γH), 1.45−1.07 (m, 9.50H, Boc, Pro cis-γH), 1.05−0.91 (m, 0.51H, Pro cis-βH), 0.86−0.70 (m, 0.47H, Pro cis-γH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 172.36, 172.19, 171.42, 171.06, 170.63, 170.26, 170.14, 155.51, 155.41, 154.92, 154.65, 138.08, 137.85, 137.81, 137.53, 136.05, 136.01, 135.85 (18q), 129.79, 129.75, 129.07, 129.01, 128.89, 127.94, 126.13, 125.53, 125.50 (9t, Ar−C), 124.24, 122.95 (2q), 114.83, 114.62 (3t, Ar−C), 78.54, 77.88 (2q, Boc), 59.78 (t, Pro trans-αC), 59.14 (t, Pro cis-αC), 53.74 (t, Mmp cis-αC), 53.45 (t, Phe αC), 53.18 (t, Mmp trans-αC), 51.47 (t, Dmt αC), 46.55 (s, Pro trans-δC), 46.18 (s, Pro cis-δC), 37.89 (s, Phe cis-βC), 37.20 (s, Phe trans-βC), 34.12 (s, Mmp cis-βC), 33.48 (s, Mmp trans-βC), 29.93 (s, Pro cis-βC), 28.61 (s, Pro trans-βC), 28.18, 27.98, 27.45 (3p, Boc), 24.34 (s, Pro trans-γC), 20.99 (s, Pro cis-γC), 20.11 (p, Dmt trans-CH_3), 19.43 (p, Dmt cis-CH_3), 18.99, 18.94 (2p, Mmp CH_3).

***N*$^{\alpha}$-*tert*-Butyloxycarbonyltyrosylprolyl-3′,5′-dimethyl-L-phenylalanylphenylalanylamide.** Yield 282 mg (91.4%); mp 126−128 °C; R_{f3} = 0.67; $[\alpha]_D^{25}$ −44.94° (c 0.48, MeOH). ^{1}H NMR (400.1 MHz, DMSO-d_6) δ: 9.21 (s, 0.19H, Tyr cis-OH), 9.13 (s, 0.80H, Tyr trans-OH), 8.28 (d, 0.17H, J = 7.94 Hz, Phe cis-NH), 7.94 (d, 0.81H, J = 8.23 Hz, Phe trans-NH), 7.82−7.72 (m, 0.96H, ^{3,5}Dmp NH), 7.30−6.88 (m, 9.90H, Phe Ar−H, Tyr Ar−H, Tyr NH, $CONH_2$), 6.77 (s, 3.0H, ^{3,5}Dmp Ar−H), 6.71−6.54 (m, 2.1H, Tyr Ar−H, Dmt NH), 4.47−4.07 (m, 3.83H, Pro trans-αH, ^{3,5}Dmp αH, Phe αH, Tyr αH), 3.76 (d, 0.17H, J = 7.72 Hz, Pro cis-αH), 3.64−3.53 (m, 0.78H, Pro trans-δH), 3.53−3.47 (m, 1.0H, Pro trans-δH), 3.27−3.16 (m, 0.22H, Pro cis-δH), 3.10−2.52 (m, 6.0H,

Phe βH, ^{3,5}Dmp βH, Tyr βH), 2.20, 2.18 (2s, 6.0H, ^{3,5}Dmp CH_3), 2.0−1.65 (m, 3.47H, Pro cis-βH, Pro trans-βH, Pro trans-γH), 1.53−1.42 (m, 0.33H, Pro cis-γH), 1.35, 1.29, 1.23 (3s, 9.0H, Boc), 1.11−0.94 (m, 0.1H, Pro cis-γH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 172.51, 171.31, 170.89, 170.56, 170.39, 170.27, 170.19, 155.97, 155.68, 155.34, 155.25, 137.81, 137.61, 137.25, 136.76 (15q), 130.25, 130.08, 129.03, 127.95, 127.91, 127.60, 126.80, 126.64, 126.13, 114.92, 114.78 (11t, Ar−C), 78.28, 77.85 (2q, Boc), 59.56 (t, Pro αC), 54.79, 54.10, 54.06, 53.68, 53.42 (5t, ^{3,5}Dmp αC, Phe αC, Tyr αC), 46.60 (s, Pro δC), 37.33 (s, Phe βC), 36.81 (s, ^{3,5}Dmp βC), 35.47 (s, Tyr βC), 28.56 (s, Pro βC), 28.07 (p, trans-Boc), 27.75 (p, cis-Boc), 24.28 (s, Pro γC), 20.79 (p, ^{3,5}Dmp trans-CH_3), 20.75 (p, ^{3,5}Dmp cis-CH_3).

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-2′,6′-dimethyl-L-tyrosylprolyl-3′,5′-dimethyl-L-phenylalanylphenylalanylamide.** Yield 281 mg (87.6%); mp 208−210 °C; R_{f3} = 0.73; $[\alpha]_D^{25}$ −35.02° (*c* 0.31, MeOH). ^{1}H NMR (400.1 MHz, DMSO-d_6) δ: 9.06 (br, 0.47H, Dmt cis-OH), 8.89 (br, 0.39H, Dmt trans-OH), 8.17 (d, 0.47H, J = 7.83 Hz, ^{3,5}-Dmp cis-NH), 7.93−7.83 (m, 0.67H, ^{3,5}Dmp trans-NH, Phe trans-NH), 7.74 (d, 0.81H, J = 7.98 Hz, Phe cis-NH), 7.30−6.95 (m, 7.68 H, Phe Ar−H, $CONH_2$, Dmt NH), 6.85−6.72 (m, 3.0H, ^{3,5}-Dmp Ar−H), 6.69 (d, 0.34H, J = 8.49 Hz, Dmt NH), 6.39, 6.40 (2s, 2.0H, Dmt Ar−H), 6.24 (d, 0.10H, J = 8.52 Hz, Dmt NH), 4.48−4.19 (m, 3.0H, Pro trans-αH, ^{3,5}Dmp αH, Phe αH, Dmt trans-αH), 4.19−4.10 (m, 0.50H, Dmt cis-αH), 3.55−3.44 (m, 0.50H, Pro trans-δH), 3.20−2.64 (m, 8.0H, Pro cis-αH, Pro trans-δH, Pro cis-δH, Phe βH, ^{3,5}Dmp βH, Dmt βH), 2.21, 2.19 (2s, ^{3,5}Dmp CH_3), 2.17, 2.09 (2s, Dmt CH_3), 1.96−1.83 (m, 0.50H, Pro trans-βH), 1.83−1.60 (m, 2.0H, Pro cis-βH, Pro trans-βH, Pro trans-γH), 1.46−1.06 (m, 9.54H, Boc, Pro cis-γH), 1.06−0.92 (m, 0.47H, Pro cis-βH), 0.92−0.77 (m, 0.49H, Pro cis-γH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 172.46, 172.25, 171.22, 171.08, 170.71, 170.40, 170.22, 170.08, 155.51, 155.43, 154.96, 154.62, 138.06, 137.85, 137.76, 137.51, 137.39, 136.79, 136.78 (19q), 129.04, 127.95, 127.92, 127.57, 127.48, 126.70, 126.61, 126.13 (8t, Ar−C), 124.25, 122.99 (2q), 114.84, 114.66 (2t, Ar−C), 78.46, 77.89 (2q, Boc), 59.78 (t, Pro trans-αC), 59.11 (t, Pro cis-αC), 55.02 (t, ^{3,5}Dmp trans-αC), 54.22 (t, ^{3,5}Dmp cis-αC), 53.72 (t, Phe cis-αC), 53.43 (t, Phe trans-αC), 51.44 (t, Dmt cis-αC), 51.35 (t, Dmt trans-αC), 46.51 (s, Pro trans-δC), 45.94 (s, Pro cis-δC), 37.94 (s, Phe cis-βC), 37.56 (s, Phe trans-βC), 36.61 (s, ^{3,5}Dmp cis-βC), 36.00 (s, ^{3,5}Dmp trans-βC), 31.26 (s, Dmt cis-βC), 30.82 (s, Dmt trans-βC), 30.03 (s, Pro cis-βC), 28.63 (s, Pro trans-βC), 28.19, 27.97, 27.42 (3p, Boc), 24.18 (s, Pro trans-γC), 20.94 (s, Pro cis-γC), 20.78 (p, ^{3,5}Dmp, cis-CH_3), 20.76 (p, ^{3,5}Dmp trans-CH_3), 20.13 (p, Dmt trans-CH_3), 19.39 (p, Dmt cis-CH_3).

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-2′,6′-dimethyl-L-tyrosylprolyl-2′,6′-dimethyl-L-phenylalanylphenylalanylamide.** Yield 284 mg (84.9%); mp 145−146 °C; R_{f3} = 0.88; $[\alpha]_D^{25}$ −39.32° (*c* 0.52, MeOH). ^{1}H NMR (400.1 MHz, DMSO-d_6) δ: 9.05, 8.86 (2s, 1H, Dmt OH), 8.35 (d, 0.45H, Dmp NH), 8.10−7.94 (m, 0.55H, Dmp NH), 7.88−7.55 (m, 0.40H, Phe NH), 7.70−7.54 (m, 0.60H, Phe NH), 7.40−6.61 (m, 10.9H, Phe Ar−H, Dmp Ar−H, $CONH_2$, Dmt NH), 6.50−6.22 (m, 2.1H, Dmt Ar−H, Dmt NH), 4.65−4.28 (m, 2.80H, Pro trans-αH, Phe αH, Dmp αH, Dmt trans-αH), 4.28−3.98 (m, 0.53H, Dmt cis-αH), 3.57−3.44 (m, 0.47H, Pro trans-δH), 3.28−2.63 (m, 8.20H, Pro cis-αH, Pro trans-δH, Pro cis-δH, Phe βH, Dmp βH, Dmt βH), 2.42−1.98 (m, 12.0H, Dmt CH_3, Dmp CH_3), 1.98−1.54 (m, 2.54H, Pro cis-βH, Pro trans-βH, Pro trans-γH), 1.54−1.00 (m, 9.75H, Boc, Pro cis-γH), 1.00−0.82 (m, 0.41H, Pro cis-βH), 0.73−0.53 (m, 0.32H, Pro cis-γH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 172.21, 172.09, 171.16, 170.97, 170.70, 170.12, 169.98, 155.60, 155.42, 154.92, 138.11, 137.88, 137.82, 137.56, 136.78, 136.54, 134.57, 134.11 (18q), 129.12, 129.00, 128.95, 127.96, 127.91, 127.82, 126.13, 126.07, 125.98, 125.90, 114.83, 114.63 (12t, Ar−C), 78.64, 77.90 (2q, Boc), 59.65 (t, Pro trans-αC), 59.30 (t, Pro cis-αC), 53.82 (t, Phe trans-αC), 53.53 (t, Phe cis-αC), 53.05 (t, Dmp trans-αC), 52.43 (t, Dmp cis-αC), 51.47 (t, Dmt αC), 46.53 (s, Pro trans-δC), 46.34 (s, Pro cis-δC), 37.93 (s, Phe trans-βC), 37.17 (s, Phe cis-βC), 31.89 (s, Dmp cis-βC), 31.40 (s, Dmp trans-βC), 30.93 (s, Dmt trans-βC), 30.65 (s, Dmt cis-βC), 29.66 (s, Pro cis-βC), 28.66 (s, Pro trans-βC), 28.17, 28.00, 27.44 (3p, Boc), 24.35 (s, Pro trans-γC), 20.90 (s, Pro cis-γC), 20.11, 19.84, 19.53 (3p, Dmt CH_3, Dmp CH_3).

***N*$^{\alpha}$-*tert*-Butyloxycarbonyltyrosylprolyl-2′,6′-dimethyl-L-tyrosylphenylalanylamide.** Yield 307 mg (97.2%); mp 154−156 °C; R_{f3} = 0.60; $[\alpha]_D^{25}$ −43.85° (*c* 0.33, MeOH). ^{1}H NMR (400.1 MHz, DMSO-d_6) δ: 9.21, 9.15 (2s, 0.96H, Tyr OH), 8.89, 8.84 (2s, 0.95H, Dmt OH), 8.44 (d, 0.21H, J = 8.88 Hz, Dmt cis-NH), 7.91 (d, 0.76H, J = 7.83 Hz, Dmt trans-NH), 7.79 (d, 0.77H, J = 8.10 Hz, Phe trans-NH), 7.69 (d, 0.22H, J = 8.07 Hz, Phe cis-NH), 7.57−7.12 (m, 2.15H, Tyr Ar−H, Tyr NH), 7.10−6.83 (m, 4.84H, Tyr Ar−H, Tyr NH, $CONH_2$), 6.79−6.53 (m, 2.15H, Tyr Ar−H, Tyr NH), 6.35−6.33 (m, 2.0H, Dmt Ar−H), 4.52−4.12 (m, 3.79H, Pro trans-αH, Tyr αH, Phe αH, Dmt αH), 3.88 (d, 0.21H, J = 7.66 Hz, Pro cis-αH), 3.66−3.55 (m, 0.65H, Pro trans-δ), 3.55−3.38 (m, 0.85H, Pro trans-δH), 3.24−3.10 (m, 0.50H, Pro cis-δH), 3.10−2.94 (m, 1.24H, Phe trans-βH, Dmt trans-βH), 2.94−2.54 (m, 4.73H, Phe trans-βH, Phe cis-βH, Tyr βH, Dmt trans-βH), 2.14 (s, 6.0H, Dmt CH_3), 2.01−1.88 (m, 0.76H, Pro trans-βH), 1.88−1.77 (m, 1.55H, Pro trans-βH), 1.77−1.64 (m, 0.97H, Pro trans-βH, Pro cis-βH), 1.50−1.40 (m, 0.29H, Pro cis-γH), 1.31, 1.24 (2s, 9.0H, Boc), 1.20−1.06 (m, 0.37H, Pro cis-βH), 0.98−1.83 (m, 0.16H, Pro cis-γH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 172.34, 172.26, 171.13, 170.93, 170.79, 170.42, 170.18, 155.97, 155.68, 155.46, 155.24, 154.99, 154.90, 137.85, 137.66 (15q), 130.39, 130.11, 129.02, 128.97, 127.91, 126.36, 126.07 (7t, Ar−C), 124.82, 124.72 (2q), 114.86, 114.77, 114.69 (3t, Ar−C), 78.41, 77.87 (2q, Boc), 59.45 (t, Pro αC), 54.12 (t, Tyr αC), 53.81 (t, Phe αC), 53.54 (t, Dmt trans-αC), 52.62 (t, Dmt cis-αC), 46.62 (s, Pro trans-δC), 46.20 (s, Pro cis-δC), 37.15 (s, Phe trans-βC), 36.82 (s, Phe cis-βC), 35.56 (s, Tyr βC), 31.24 (s, Dmt trans-βC), 30.24 (s, Dmt cis-βC), 29.79 (s, Pro cis-βC), 28.58 (s, Pro trans-βC), 28.08 (p, trans-Boc), 27.77 (p, cis-Boc), 24.42 (s, Pro trans-γC), 21.05 (s, Pro cis-γC), 20.00 (p, Dmt, trans-CH_3), 19.96 (p, Dmt cis-CH_3).

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-2′,6′-dimethyl-L-tyrosylprolyl-2′,6′-dimethyl-L-tyrosylphenylalanylamide.** Yield 257 mg (80.3%); mp 160−162 °C; R_{f3} = 0.63; $[\alpha]_D^{25}$ −34.23° (*c* 0.53, MeOH). ^{1}H NMR (400.1 MHz, DMSO-d_6) δ: 9.06, 8.87 (2br, 2H, Dmt OH), 8.24 (d, 0.48H, J = 8.25 Hz, Dmt^3 NH), 8.03−7.87 (m, 0.52H, Dmt^3 NH), 7.82−7.70 (m, 0.49H, Phe NH), 7.53 (d, 0.51H, J = 7.66 Hz, Phe NH), 7.34−6.81 (m, 7.7H, Phe Ar−H, $CONH_2$, Dmt^1 NH), 6.73 (d, 0.30H, J = 8.13 Hz, Dmt^1 NH), 6.48−6.30 (m, 4.0H, Dmt Ar−H), 4.53−4.03 (m, 3.40H, Pro trans-αH, Dmt^3 αH, Phe αH, Dmt^1 αH), 3.56−3.43 (m, 0.40H, Pro trans-δH), 3.28−3.17 (m, 0.60H, Pro cis-αH), 3.17−2.62 (m, 7.60H, Pro trans-δH, Pro cis-δH, Phe βH, Dmt^3 βH, Dmt^1 βH), 2.30−1.98 (m, 12.0H, Dmt CH_3), 1.95−1.83 (m, 0.40H, Pro trans-βH), 1.83−1.57 (m, 2.0H, Pro cis-βH, Pro trans-γH), 1.47−1.04 (m, 9.65H, Boc, Pro cis-γH), 1.04−0.88 (m, 0.51H, Pro cis-βH), 0.84−0.65 (m, 0.45H, Pro cis-γH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 172.26, 172.07, 171.25, 170.96, 170.91, 170.58, 170.29, 170.00, 155.64, 155.44, 154.98, 154.95, 154.65, 138.12, 137.87, 137.81, 137.53 (19q), 129.01, 128.96, 127.95, 127.90, 126.12, 126.05 (6t, Phe Ar−C), 124.80, 122.93 (2q), 114.84, 114.66 (2t, Dmt Ar−C), 78.63, 77.89 (2q, Boc), 59.70 (t, Pro trans-αC), 59.26 (t, Pro cis-αC), 53.84 (t, Dmt^3 cis-αC), 53.56 (t, Dmt^3 trans-αC), 53.48 (t, Phe cis-αC), 53.14 (t, Phe trans-αC), 51.50 (t, Dmt^1 cis-αC), 51.41 (t, Dmt^1 trans-αC), 46.48 (s, Pro trans-δC), 46.39 (t, Pro cis-δC), 38.01 (s, Phe cis-βC), 37.17 (s, Phe trans-βC), 31.31 (s, Dmt^3 trans-βC), 31.19 (s, Dmt^3 cis-βC), 30.95 (s, Dmt^1 trans-βC), 29.98 (s, Dmt^1 cis-βC), 29.80 (s, Pro cis-βC), 28.65 (s, Pro trans-βC), 28.19, 27.99, 27.47 (3p, Boc), 24.35 (s, Pro trans-γC), 20.95 (s, Pro cis-γC), 20.11, 19.99, 19.51 (3p, Dmt CH_3).

***N*$^{\alpha}$-*tert*-Butyloxycarbonyltyrosylprolyl-2′,4′,6′-trimethyl-L-phenylalanylphenylalanylamide.** Yield 237 mg (75.3%); mp 215−217 °C; R_{f3} = 0.76; $[\alpha]_D^{25}$ −46.69° (*c* 0.35, MeOH). ^{1}H NMR (400.1 MHz, DMSO-d_6) δ: 9.20 (s, 0.23H, Dmt cis-OH), 9.15 (s, 0.72H, Dmt trans-OH), 8.50 (s, 0.20H, J = 8.93 Hz, Phe cis-NH),

7.96 (d, 0.75H, J = 7.87 Hz, Phe trans-NH), 8.22 (d, 0.74H, J = 8.22 Hz, Tmp trans-NH), 7.75 (d, 0.22H, J = 8.16 Hz, Tmp cis-NH), 7.27−7.12 (m, 5.0H, Phe Ar−H), 7.10−6.97 (m, 3.2H, Tyr Ar−H, Tyr NH), 6.94 (d, 0.70H, J = 8.17 Hz, Tyr NH), 6.80 (br, 0.24H, cis-$CONH_2$), 6.76−6.55 (m, 4.8H, Tmp Ar−H, Tyr Ar−H, $CONH_2$, Tyr NH), 4.58−4.49 (m, 0.23H, Phe cis-αH), 4.43−4.20 (m, 3.38H, Pro trans-αH, Tyr trans-αH, Tmp αH, Phe trans-αH), 4.38−4.10 (m, 0.13H, Tyr cis-αH), 3.92 (d, 0.21H, J = 7.67 Hz, Pro cis-αH), 3.65−3.54 (m, 0.70H, Pro trans-δH), 3.54−3.37 (m, 0.80H, Pro trans-δH), 3.20−3.10 (m, 0.50H, Pro cis-δH), 3.10−2.85 (m, 2.3H, Phe βH, Tmp βH), 2.85−2.53 (m, 3.7H, Phe βH, Tyr βH, Tmp βH), 2.28−2.08 (m, 9.0H, Tmp CH_3), 2.0−1.87 (m, 0.71H, Pro trans-βH), 1.87−1.76 (m, 1.56H, Pro trans-γH), 1.76−1.63 (m, 1.0H, Pro cis-βH, Pro trans-βH), 1.48−1.37 (m, 0.23H, Pro cis-γH), 1.30, 1.24 (2s, 9.0H, Boc), 1.20−1.10 (m, 0.29H, Pro cis-βH), 0.97−0.82 (m, 0.17H, Pro cis-γH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 172.33, 172.24, 171.12, 170.77, 170.23, 170.32, 170.16, 155.95, 155.68, 155.36, 155.23, 137.88, 137.73, 136.59, 136.44, 134.69, 134.54, 131.21 (18q), 130.39, 130.11, 128.99, 128.94, 128.57, 128.48, 127.90, 126.37, 126.05, 114.77 (10t, Ar−C), 78.34, 77.86 (2q, Boc), 59.37 (t, Pro αC), 54.10 (t, Tyr αC), 53.74 (t, Tmp trans-αC), 53.53 (t, Tmp cis-αC), 43.14 (t, Phe trans-αC), 52.16 (t, Phe cis-αC), 46.61 (s, Pro trans-δC), 46.21 (s, Pro cis-δC), 37.42 (s, Phe cis-βC), 37.10 (s, Phe trans-βC), 36.88 (s, Tyr cis-βC), 35.55 (s, Tyr trans-βC), 31.61 (s, Tmp trans-βC), 30.72 (s, Tmp cis-βC), 29.87 (s, Pro cis-βC), 28.58 (s, Pro trans-βC), 28.08 (p, trans-Boc), 27.78 (p, cis-Boc), 24.42 (s, Pro trans-γC), 20.95 (s, Pro cis-γC), 20.36, 20.32, 19.75, 19.71 (4p, Tmp CH_3).

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-2′,6′-dimethyl-L-tyrosylprolyl-2′,4′,6′-trimethyl-L-phenylalanylphenylalanylamide.** Yield 250 mg (76.4%); mp 148−150 °C; R_{f3} = 0.80; $[\alpha]_D^{25}$ −35.74° (c 0.35, MeOH). ^{1}H NMR (400.1 MHz, DMSO-d_6) δ: 9.05 (br, 0.51H, Dmt cis-OH), 8.86 (br, 0.43H, Dmt trans-OH), 8.31 (d, 0.48H, J = 8.76 Hz, Phe NH), 8.06−7.93 (m, 0.51H, Phe NH), 7.86−7.76 (m, 0.49H, Tmp NH), 7.60 (d, 0.49H, J = 8.14 Hz, Tmp NH), 7.28−7.10 (m, 5.59H, Phe Ar−H, Dmt NH, $CONH_2$), 7.06, 6.98 (2s, 1.41H, $CONH_2$), 6.80−6.65 (m, 2.84H, Tmp Ar−H, Dmt NH, $CONH_2$), 6.45−6.28 (m, 2.15H, Dmt Ar−H, Dmt NH), 4.50−4.22 (m, 3.0H, Pro trans-αH, Tmp αH, Phe αH, Dmt trans-αH), 4.16−4.06 (m, 0.49H, Phe cis-αH), 3.55−3.43 (m, 0.56H, Pro trans-δH), 3.27−2.66 (m, 8.55H, Pro cis-αH, Pro cis-δH, Phe βH, Tmp βH, Dmt βH), 2.28−2.05 (m, 15.0H, Dmt CH_3, Tmp CH_3), 1.95−1.58 (m, 2.74H, Pro cis-βH, Pro trans-βH, Pro trans-γH), 1.14−1.08 (m, 9.47H, Boc, Pro cis-γH), 1.05−0.9 (m, 0.42H, Pro cis-βH), 0.78−0.63 (m, 0.42H, Pro cis-γH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 172.25, 172.10, 171.16, 170.95, 170.76, 170.54, 170.20, 169.98, 155.53, 155.41, 154.92, 154.65, 138.11, 137.86, 137.57, 136.57, 136.32, 134.67, 134.60, 131.42, 131.27 (21q), 128.99, 128.95, 128.55, 128.50, 127.95, 127.90, 126.11, 126.06 (8t, Ar−C), 124.22, 122.95 (2q), 114.83, 114.63 (2t, Ar−C), 78.60, 77.90 (2q, Boc), 59.64 (t, Pro trans-αC), 59.23 (t, Pro cis-αC), 53.78, 53.49 (2t, Tmp αC), 53.20, 52.68 (2t, Phe αC), 51.45 (t, Dmt αC), 46.53 (t, Pro trans-δC), 46.40 (t, Pro cis-δC), 37.90 (s, Phe cis-βC), 37.12 (s, Phe trans-βC), 31.59 (s, Tmp cis-βC), 31.46 (s, Tmp trans-βC), 30.97 (s, Dmt trans-βC), 30.41 (s, Dmt cis-βC), 29.81 (s, Pro cis-βC), 28.66 (s, Pro trans-βC), 28.17, 28.00, 27.47 (3p, Boc), 24.36 (s, Pro trans-γC), 20.87 (s, Pro cis-γC), 20.36, 20.31 (2p, Tmp CH_3), 20.11 (p, Dmt trans-CH_3), 19.75 (p, Tmp CH_3), 19.52 (p, Dmt cis-CH_3).

***N*$^{\alpha}$-*tert*-Butyloxycarbonyltyrosylprolyl-2′-ethyl-6′-methyl-L-phenylalanylphenylalanylamide.** Yield 242 mg (76.7%); mp 222−224 °C; R_{f3} = 0.66; $[\alpha]_D^{25}$ −48.52° (c 0.34, MeOH). ^{1}H NMR (400.1 MHz, DMSO-d_6) δ: 9.20 (s, 0.26H, Tyr cis-OH), 9.15 (s, 0.73H, Tyr trans-OH), 8.50 (d, 0.23H, J = 8.97 Hz, Phe cis-NH), 7.99 (d, 0.75H, J = 7.87 Hz, Phe trans-NH), 7.81 (d, 0.75H, J = 8.13 Hz, Emp trans-NH), 7.73 (d, 0.24H, J = 8.10 Hz, Emp cis-NH), 7.27−7.13 (m, 5.0H, Phe Ar−H), 7.10−6.88 (m, 7.12H, Emp Ar−H, Tyr Ar−H, $CONH_2$), 6.82−6.72 (m, 0.7H, $CONH_2$), 6.70−6.57 (m, 2.10H, Tyr Ar−H, Tyr NH), 4.60−4.52 (m, 0.26H, Phe cis-αH), 4.46−4.10 (m, 3.5H, Pro trans-αH, Phe trans-αH, Tyr αH, Emp αH), 3.90 (d, 0.24H, J = 7.62 Hz, Pro cis-αH), 3.66−3.60 (m, 0.62H, Pro trans-δH), 3.40−3.38 (m, 0.77H, Pro trans-δH), 3.23−3.08 (m, 0.62H, Pro cis-δH), 3.08−2.53 (m, 8.0H, Phe βH, Tyr βH, Emp βH, Emp CH_2CH$_3$), 2.26 (s, 3.0H, Emp Ar−CH_3), 2.02−1.88 (m, 0.75H, Pro trans-βH), 1.88−1.77 (m, 1.5H, Pro trans-γH), 1.77−1.63 (m, 1.0H, Pro trans-βH, Pro cis-βH), 1.47−1.20 (m, 9.35H, Pro cis-γH, Boc), 1.20−1.02 (m, 3.25H, Pro cis-βH, Emp CH_2CH_3), 0.9−0.75 (m, 0.15H, Pro cis-γH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 172.33, 172.18, 171.21, 170.79, 170.61, 170.21, 170.14, 155.96, 155.69, 155.46, 155.24, 142.80, 142.69, 137.84, 137.67, 136.89, 136.75, 133.76, 133.59 (19q), 130.40, 130.10, 129.01, 127.91, 127.79, 126.37, 126.27, 126.07, 126.00, 114.83, 114.78 (11t, Ar−C), 78.39, 77.86 (2q, Boc), 59.43 (t, Pro αC), 54.13 (t, Tyr αC), 53.77 (t, Emp αC), 53.62 (t, Phe trans-αC), 52.67 (t, Phe cis-αC), 46.62 (s, Pro trans-δC), 46.14 (s, Pro cis-δC), 37.54 (s, Phe cis-βC), 37.14 (s, Phe trans-βC), 36.80 (s, Tyr cis-βC), 35.56 (s, Tyr trans-βC), 31.06 (s, Emp trans-βC), 30.13 (s, Emp cis-βC), 29.76 (s, Pro cis-βC), 28.58 (s, Pro trans-βC), 28.06, 27.76 (2p, Boc), 25.18 (s, Emp CH_2CH$_3$), 24.43 (s, Pro trans-γC), 21.00 (s, Pro cis-γC), 19.95 (p, Emp trans-Ar-CH_3), 19.91 (p, Emp cis-Ar-CH_3), 15.56 (p, Emp CH_2CH_3).

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-2′,6′-dimethyl-L-tyrosylprolyl-2′-ethyl-6′-methyl-L-phenylalanylphenylalanylamide.** Yield 280 mg (85.6%); mp 135−137 °C; R_{f3} = 0.75; $[\alpha]_D^{25}$ −38.20° (c 0.39, MeOH). ^{1}H NMR (400.1 MHz, DMSO-d_6) δ: 9.06 (br, 0.44H, Dmt cis-OH), 8.87 (br, 0.36H, Dmt trans-OH), 8.30 (d, 0.50H, J = 8.77 Hz, Phe NH), 8.09−7.97 (m, 0.50H, Phe NH), 7.86−7.74 (m, 0.50H, Emp NH), 7.56 (d, 0.50H, J = 8.10 Hz, Emp NH), 7.30−7.10 (m, 5.70H, Phe Ar−H, $CONH_2$, Dmt NH), 7.10−6.87 (m, 4.41H, Emp Ar−H, $CONH_2$), 6.84−6.68 (m, 0.73H, $CONH_2$, Dmt NH), 6.47−6.27 (m, 2.1H, Dmt Ar−H, Dmt NH), 4.50−4.23 (m, 3.0H, Pro trans-αH, Tmp αH, Phe αH, Dmt cis-αH), 4.16−4.04 (m, 0.50H, Dmt cis-αH), 3.23 (d, 0.50H, J = 7.37 Hz, Pro cis-αH), 3.17−2.52 (m, 10.0H, Pro δH, Phe βH, Emp βH, Dmt βH, Emp CH_2CH$_3$), 2.25 (s, 3.0H, Emp Ar−CH_3), 2.15, 2.11 (2s, 6.0H, Dmt CH_3), 1.97−1.84 (m, 0.49H, Pro trans-βH), 1.84−1.58 (m, 2.1H, Pro cis-βH, Pro trans-βH, Pro trans-γH), 1.47−1.10 (m, 9.67H, Dmt CH_3, Emp Ar−CH_3, Pro cis-γH), 1.10−1.00 (m, 3.0H, Emp CH_2CH_3), 1.10−0.84 (m, 0.42H, Pro cis-βH), 0.70−0.53 (m, 0.38H, Pro cis-γH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 172.17, 172.05, 171.13, 171.02, 170.59, 170.02, 155.63, 155.43, 154.90, 154.65, 142.77, 142.58, 138.11, 137.86, 137.81, 137.51, 136.86, 136.59, 133.74, 133.62 (20q), 128.90, 128.96, 127.96, 127.89, 127.76, 126.24, 126.14, 126.07, 125.96 (9t, Ar−C), 124.21, 122.93 (2q), 114.83, 114.61 (2t, Ar−C), 78.65, 77.89 (2q, Boc), 59.68 (t, Pro trans-αC), 59.26 (t, Pro cis-αC), 53.80 (t, Tmp cis-αC), 53.66 (t, Tmp trans-αC), 53.50 (t, Phe cis-αC), 53.09 (s, Phe trans-αC), 51.48 (t, Dmt αC), 46.54 (s, Pro trans-δC), 46.31 (s, Pro cis-δC), 38.00 (s, Phe cis-βC), 37.16 (s, Phe trans-βC), 31.32 (s, Emp cis-βC), 31.11 (s, Emp trans-βC), 30.09 (s, Dmt trans-βC), 29.77 (s, Dmt cis-βC), 29.71 (s, Pro cis-βC), 28.68 (s, Pro trans-βC), 28.16, 27.99, 27.46 (3p, Boc), 25.20 (s, Emp cis-CH_2CH$_3$), 25.14 (s, Emp trans-CH_2CH$_3$), 24.37 (s, Pro trans-γC), 20.88 (s, Pro cis-γC), 20.10 (p, Emp trans-Ar-CH_3), 19.96 (p, Emp cis-Ar-CH_3), 19.95 (p, Dmt trans-CH_3), 19.50 (p, Dmt cis-CH_3), 15.58 (p, Emp CH_2CH_3).

***N*$^{\alpha}$-*tert*-Butyloxycarbonyltyrosylprolyl-2′-isopropyl-6′-methyl-L-phenylalanylphenylalanylamide.** Yield 269 mg (83.8%); mp 222−224 °C; R_{f3} = 0.62; $[\alpha]_D^{25}$ −45.51° (c 0.31, MeOH). ^{1}H NMR (400.1 MHz, DMSO-d_6) δ: 9.21 (s, 0.21H, Tyr cis-OH), 9.15 (s, 0.76H, Tyr trans-OH), 8.46 (d, 0.21H, J = 8.89 Hz, Phe cis-NH), 7.84 (d, 0.76H, J = 7.84 Hz, Phe trans-NH), 7.73 (d, 0.77H, J = 8.12 Hz, Imp trans-NH), 7.66 (d, 0.21H, J = 8.07 Hz, Imp cis-NH), 7.30−7.10 (m, 5.0H, Phe Ar−H), 7.10−6.83 (m, 7.9H, $CONH_2$, Imp Ar−H, Tyr Ar−H, Dmt NH), 6.68−6.56 (m, 2.1H, Tyr Ar−H, Dmt NH), 4.58−4.47 (m, 0.21H, Phe cis-αH), 4.45−4.10 (m, 3.60H, Pro trans-αH, Imp αH, Tyr αH, Phe trans-αH), 3.90 (d, 0.19H, J = 7.67 Hz, Pro cis-αH), 3.67−3.50 (m, 0.65H, Pro trans-δH), 3.55−3.40 (m, 0.73H, Pro trans-δH), 3.28−3.11 (m, 1.62H, Pro cis-δH, CH(CH_3)$_2$), 3.11−2.92 (m, 2.0H, Phe βH, Imp βH), 2.92−2.67 (m, 3.0H, Phe βH, Tyr βH, Imp βH),

2.67−2.55 (m, 0.82H, Tyr trans-αH), 2.25 (s, 3.0H, Imp Ar−CH_3), 2.05−1.90 (m, 0.81H, Pro trans-βH), 1.90−1.77 (m, 1.63H, Pro trans-γH), 1.77−1.63 (m, 0.96H, Pro trans-βH), 1.47−1.36 (m, 0.18H, Pro cis-γH), 1.36−1.02 (m, 15.23H, Pro cis-βH, Boc, Imp CH(CH_3)$_2$), 0.90−0.88 (m, 0.18H, Pro cis-γH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 172.27, 172.20, 171.39, 170.75, 170.51, 170.27, 170.02, 155.96, 155.67, 155.47, 155.25, 147.38, 137.76, 137.57, 136.68, 132.99 (16q), 130.38, 130.06, 129.07, 129.00, 127.90, 127.51, 126.39, 126.09, 122.79, 114.77 (10t, Ar−C), 78.38, 77.86 (2q, Boc), 59.50 (t, Pro αC), 54.32 (t, Imp αC), 54.17 (t, Tyr αC), 53.71 (t, Phe trans-αC), 53.46 (t, Phe cis-αC), 46.62 (s, Pro trans-δC), 46.14 (s, Pro cis-δC), 37.61 (s, Pro cis-βC), 37.24 (s, Pro trans-βC), 36.74 (s, Tyr cis-βC), 35.53 (s, Tyr trans-βC), 30.48 (s, Imp trans-βC), 29.88 (s, Imp cis-βC), 29.74 (s, Pro cis-βC), 28.66 (s, Pro trans-βC), 28.07 (p, trans-Boc), 27.95 (t, *C*H-(CH$_3$)$_2$), 27.73 (p, cis-Boc), 24.43 (s, Pro trans-γC), 24.31, 23.76, 23.65 (3p, CH(*C*H$_3$)$_2$), 21.00 (s, Pro cis-γC), 20.46 (p, Imp cis-Ar-*C*H$_3$), 20.23 (p, Imp trans-Ar-*C*H$_3$).

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-2′,6′-dimethyl-L-tyrosylprolyl-2′-isopropyl-6′-methyl-L-phenylalanylphenylalanylamide.** Yield 252 mg (75.6%); mp 143−145 °C; $R_{f3} = 0.67$; $[\alpha]_D^{25}$ −35.88° (*c* 0.44, MeOH). ^{1}H NMR (400.1 MHz, DMSO-d_6) δ: 9.06 (br, 0.35H, Dmt cis-OH), 8.86 (br, 0.37H, Dmt trans-OH), 8.25 (d, 0.37H, J = 8.71 Hz, Phe cis-NH), 8.17−7.98 (m, 0.47H, Phe trans-NH), 7.77−7.53 (m, 0.47H, Imp trans-NH), 7.50 (d, 0.38H, J = 8.09 Hz, Imp cis-NH), 7.30−7.12 (m, 5.5H, Phe Ar−H, Dmt NH), 7.12−6.86 (m, 5.0H, Imp Ar−H, CONH$_2$), 6.75 (d, 0.40H, J = 8.46 Hz, Dmt cis-NH), 6.44−6.30 (m, 2.1H, Dmt Ar−H, Dmt NH), 4.50−4.25 (m, 2.46H, Pro trans-αH, Phe trans-αH, Imp αH, Dmt trans-αH), 4.25−4.17 (m, 0.51H, Phe cis-αH), 4.15−4.07 (m, 0.45H, Dmt cis-αH), 3.56−3.42 (m, 0.56H, Pro cis-δH), 3.26−2.62 (m, 9.0H, Pro cis-αH, Pro trans-δH, Pro cis-δH, Phe βH, Dmt βH, Imp βH, Imp C*H*(CH$_3$)$_2$), 2.36−2.00 (m, 9.0H, Imp Ar−C*H*$_3$, Dmt Ar−C*H*$_3$), 2.00−1.85 (m, 0.59H, Pro trans-βH), 1.85−1.57 (m, 2.0H, Pro cis-βH, Pro trans-βH, Pro trans-γH), 1.45−1.00 (m, 15.52H, Boc, Imp CH(C*H*$_3$)$_2$, Pro cis-γH), 1.00−0.85 (m, 0.51H, Pro cis-βH), 0.70−0.55 (m, 0.38H, Pro cis-γH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 172.32, 172.01, 171.22, 170.50, 170.10, 169.94, 155.63, 155.45, 154.90, 154.68, 147.37, 147.12, 138.11, 137.85, 137.75, 137.44, 136.64, 136.53, 132.95, 132.85 (20q), 129.08, 128.99, 127.96, 127.89, 127.50, 127.40, 126.37, 126.30, 126.15, 126.11 (10t, Ar−C), 124.20 (q), 122.92, 122.79, 114.83, 114.61 (4t, Ar−C), 78.63, 77.91 (2q, Boc), 59.77 (t, Pro trans-αC), 59.24 (t, Pro cis-αC), 54.36 (t, Phe cis-αC), 53.84 (t, Phe trans-αC), 53.76 (t, Imp trans-αC), 53.45 (t, Imp cis-αC), 51.53 (t, Dmt cis-αC), 51.49 (t, Dmt, trans-αC), 46.55 (s, Pro cis-δC), 46.35 (s, Pro trans-δC), 38.04 (s, Phe cis-βC), 37.24 (s, Phe trans-βC), 31.30 (s, Dmt cis-βC), 30.85 (s, Dmt trans-βC), 30.49 (s, Imp trans-βC), 29.82 (s, Pro cis-βC), 29.40 (s, Imp cis-βC), 28.73 (s, Pro trans-βC), 28.17 (p, Boc), 27.98 (p, Boc; t, *C*H(CH$_3$)$_2$), 27.45 (p, Boc), 24.37 (s, Pro trans-γC), 24.33, 23.78, 23.70 (3p, CH-(*C*H$_3$)$_2$), 20.89 (s, Pro cis-γC), 20.58 (p, Imp cis-Ar-*C*H$_3$), 20.21 (p, Imp trans-Ar-*C*H$_3$), 20.07 (p, Dmt cis-Ar-*C*H$_3$), 19.49 (p, Dmt trans-Ar-*C*H$_3$).

General Procedure for Synthesis of H-Tyr/Dmt-Pro-Xaa-Phe-NH$_2$·HCl (Xaa = Mmp, ^{3,5}Dmp, Dmp, Dmt, Tmp, Emp, Imp). Boc-Tyr/Dmt-Pro-Xaa-Phe-NH$_2$ (0.25 mmol) was treated with TFA (0.6 mL, 7.79 mmol) and anisole (40 μL) for 1 h at room temperature. The reaction solution was diluted with hexane, and the solid was collected by filtration, dried over KOH pellets, and purified by semipreparative RP-HPLC. The purified peptide was lyophilized from water (3 × 10 mL) containing 1 M HCl (0.25 mL, 0.25 mmol) to give amorphous powder. Elemental analysis results of H-Tyr/Dmt-Pro-Xaa-Phe-NH$_2$ are summarized in Supporting Information (Table 4).

Tyrosylprolyl-2′-methyl-L-phenylalanylphenylalanylamide Hydrochloride (2). Yield 136.5 mg (83.7%); mp 170−172 °C. ^{1}H NMR (400.1 MHz, DMSO-d_6) δ: 9.51 (s, 0.36H, Tyr cis-OH), 9.41 (s, 0.64H, Tyr trans-OH), 8.49−8.43 (m, 0.36H, Phe cis-NH), 8.43−8.07 (m, 4.0H, NH_3^+, Phe trans-NH, Mmp cis-NH), 8.07−7.98 (m, 0.64H, Mmp trans-NH), 7.47−7.40 (m, 0.36H, cis-CONH$_2$), 7.34−7.02 (m, 11.90H, Ar−H, CONH$_2$), 6.95, 6.93 (2s, 0.75H, Ar−H), 6.74−6.67 (m, 2.0H, Ar−H), 4.50−4.33 (m, 2.66H, Pro trans-αH, Mmp αH, Phe αH), 4.15 (t, 0.64H, J = 6.40 Hz, Tyr trans-αH), 3.60−3.48 (m, 1.36H, Pro cis-αH, Tyr cis-αH, Pro trans-δH), 3.35−3.20 (m, 0.72H, Pro cis-δH), 3.10−2.77 (m, 6.64H, Pro trans-δH, Phe βH, Tyr βH, Mmp βH), 2.29, 2.26 (2s, 3.0H, Mmp CH$_3$), 2.0−1.88 (m, 0.64H, Pro trans-βH), 1.82−1.56 (m, 2.28H, Pro cis-βH, Pro trans-βH, Pro γH), 1.56−1.38 (m, 0.72H, Pro cis-βH, Pro cis-γH), 1.34−1.18 (m, 0.36H, Pro cis-γH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 172.95, 172.38, 170.66, 170.60, 169.99, 167.00, 166.85, 156.67, 156.45, 137.74, 136.06, 135.99, 135.68, 135.60 (14q), 130.75, 130.26, 129.86, 129.75, 129.25, 129.16, 129.12, 127.91, 127.83, 126.37, 126.20, 126.10, 125.58, 125.45 (14t), 124.51, 123.94 (2q), 115.34, 115.18 (2t), 59.44 (t, Pro trans-αC), 59.12 (t, Pro cis-αC), 53.67 (t, Mmp αC), 53.20 (t, Phe αC), 52.36 (t, Tyr trans-αC), 52.31 (t, Tyr cis-αC), 46.70 (s, Pro trans-δC), 46.44 (s, Pro cis-δC), 37.45 (s, Phe cis-βC), 37.24 (s, Phe trans-βC), 36.21 (s, Tyr cis-βC), 35.21 (s, Tyr trans-βC), 34.68 (s, Mmp trans-βC), 34.25 (s, Mmp cis-βC), 31.04 (s, Pro cis-βC), 28.25 (s, Pro trans-βC), 24.32 (s, Pro trans-γC), 21.23 (s, Pro cis-γC), 19.03 (p, Mmp trans-*C*H$_3$), 18.95 (p, Mmp cis-*C*H$_3$).

2′,6′-Dimethyl-L-tyrosylprolyl-2′-methyl-L-phenylalanylphenylalanylamide Hodrochloride (2′). Yield 153.8 mg (93.9%); mp 180−182 °C. ^{1}H NMR (400.1 MHz, DMSO-d_6) δ: 9.30 (s, 0.7H, Dmt cis-OH), 9.15 (s, 0.3H, Dmt trans-OH), 8.53 (br, 3.0H, NH_3^+), 8.30−8.20 (m, Mmp NH, Phe cis-NH), 7.94 (d, 0.3H, J = 8.22 Hz, Phe trans-NH), 7.62 (s, 0.7H, cis-CONH$_2$), 7.34−7.00 (m, 10.3H, CONH$_2$, Ar−H), 6.43 (s, 2.0H, Dmt Ar−H), 4.51 (dt, 0.7H, J = 4.77, 8.98 Hz, Phe cis-αH), 4.47−4.32 (m, 1.6H, Pro trans-αH, Mmp αH, Phe trans-αH), 4.10 (dd, 0.3H, J = 5.23, 10.08 Hz, Dmt trans-αH), 3.61 (dd, 0.7H, J = 4.49, 10.82 Hz, Dmt cis-αH), 3.38−3.24 (m, 1.0H, Pro cis-δH, Pro trans-δH), 3.24−3.13 (m, 0.7H, Pro cis-δH), 3.08−2.75 (m, 6.7H, Pro cis-αH, Phe βH, Mmp βH, Dmt βH), 2.35−2.20 (m, 3.3H, Pro trans-δH, Mmp Ar−CH$_3$), 2.17, 2.05 (2s, 6.0H, Dmt Ar−CH$_3$), 1.87−1.74 (m, 0.3H, Pro trans-βH), 1.67−1.50 (m, 1.7H, Pro cis-βH, Pro trans-βH, Pro trans-γH), 1.50−1.37 (m, 0.7H, Pro cis-γH), 1.25−1.0 (m, 1.3H, Pro cis-βH, Pro cis-γH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 173.13, 172.36, 170.76, 170.55, 170.33, 169.91, 167.83, 167.28, 155.93, 155.62, 138.43, 138.15, 137.68, 137.64, 135.97, 135.84, 135.77, 135.70 (18q), 129.87, 129.71, 129.32, 129.11, 129.01, 128.72, 127.89, 127.81, 126.29, 126.12, 125.59, 125.48 (12t, Ar−C), 121.38, 120.98 (2q), 115.05, 114.94 (2t, Ar−C), 59.63 (t, Pro trans-αC), 59.04 (t, Pro, cis-αC), 53.71 (t, Mmp cis-αC), 53.54 (t, Phe αC), 53.06 (t, Mmp trans-αC), 49.74 (t, Dmt αC), 46.61 (s, Pro cis-δC), 46.12 (s, Pro trans-δC), 37.59 (s, Phe cis-βC), 37.29 (s, Phe trans-βC), 34.78 (s, Mmp trans-βC), 33.55 (s, Mmp cis-βC), 31.04 (s, Pro cis-βC), 30.70 (s, Dmt cis-βC), 30.01 (s, Dmt trans-βC), 28.85 (s, Pro trans-βC), 24.14 (s, Pro trans-γC), 21.18 (s, Pro cis-γC), 20.02 (p, Dmt trans-*C*H$_3$), 19.29 (p, Dmt cis-*C*H$_3$), 19.00 (p, Mmp trans-*C*H$_3$), 18.91 (p, Dmt cis-*C*H$_3$).

Tyrosylprolyl-3′,5′-dimethyl-L-phenylalanylphenylalanylamide Hydrochoride (3). Yield 142.5 mg (87.2%); mp 170−172 °C. ^{1}H NMR (400.1 MHz, DMSO-d_6) δ: 9.53 (s, 0.34H, Tyr cis-OH), 9.43 (s, 0.66H, Tyr trans-OH), 8.40−8.16 (m, 3.68H, NH_3^+, ^{3,5}Dmp cis-NH, Phe cis-NH), 8.12 (d, 0.66H, J = 8.22 Hz, ^{3,5}Dmp trans-NH), 7.88 (d, 0.66H, J = 7.91 Hz, Phe trans-NH), 7.47 (s, 0.34H, cis-CONH$_2$), 7.33−7.05 (m, 8.0H, Ar−H, CONH$_2$), 6.94 (d, 0.66H, J = 8.31 Hz, Ar−H), 6.83−6.67 (m, 5.0H, Ar−H), 4.50−4.30 (m, 2.66H, Pro trans-αH, ^{3,5}Dmp αH, Phe αH), 4.15 (t, 0.66H, J = 6.53 Hz, Tyr trans-αH), 3.70 (dd, 0.36H, J = 5.68, 8.92 Hz, Tyr cis-αH), 3.60−3.48 (m, 0.66H, Pro trans-δH), 3.48−3.43 (m, 0.34H, Pro cis-αH), 3.35−3.20 (m, 0.68H, Pro cis-δH), 3.07−2.70 (m, 6.66H, Pro trans-βH, Phe βH, ^{3,5}Dmp βH, Tyr βH), 2.20 (s, 6.0H, ^{3,5}Dmp CH$_3$), 2.0−1.87 (m, 0.66H, Pro trans-βH), 1.78−1.59 (m, 1.98H, Pro trans-βH, Pro trans-γH), 1.59−1.44 (m, 0.68H, Pro cis-βH, Pro cis-γH), 1.44−1.32 (m, 0.34H, Pro cis-βH), 1.32−1.16 (m, 0.34H, Pro cis-γH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 173.01, 172.55, 170.67, 170.44, 170.36, 169.97, 167.12, 167.08, 156.70, 156.49, 137.76, 137.50, 137.15, 136.80, 136.76 (15q), 130.69, 130.20, 129.25, 129.12, 127.93, 127.86,

127.61, 126.89, 126.66, 126.12 (10t, Ar−C), 124.63, 123.98 (2q), 115.38, 115.24 (2t, Ar−C), 59.60 (s, Pro trans-αC), 59.35 (t, Pro cis-αC), 55.12 (t, ^{3,5}Dmp cis-αC), 54.05 (t, ^{3,5}Dmp trans-αC), 53.76 (t, Phe αC), 52.43 (t, Tyr trans-αC), 52.36 (t, Tyr cis-αC), 46.74 (s, Pro trans-δC), 46.40 (s, Pro cis-δC), 37.50 (s, Phe cis-βC), 37.38 (s, Phe trans-βC), 37.29 (s, ^{3,5}Dmp trans-βC), 36.63 (s, ^{3,5}Dmp cis-βC), 36.16 (s, Tyr cis-βC), 35.30 (s, Tyr trans-βC), 30.97 (s, Pro cis-βC), 28.85 (s, Pro trans-βC), 24.21 (s, Pro trans-γC), 21.13 (s, Pro cis-γC), 20.81 (p, ^{3,5}Dmp *C*H$_3$).

2′,6′-Dimethyl-L-tyrosylprolyl-3′,5′-dimethyl-L-phenylalanylphenylalanylamide Hydrochloride (3′). Yield 141.6 mg (86.3%); mp 180−182 °C. ^{1}H NMR (400.1 MHz, DMSO-d_6) δ: 9.30 (s, 0.71H, Dmt cis-OH), 9.18 (s, 0.29H, Dmt trans-OH), 8.50 (br, 3.0H, NH_3^+), 8.36 (d, 0.71H, J = 8.47 Hz, Phe cis-NH), 8.12 (d, 0.71H, J = 8.35 Hz, ^{3,5}Dmp cis-NH), 8.01 (d, 0.29H, J = 8.22 Hz, Phe trans-NH), 7.84 (d, 0.29H, J = 7.99 Hz, ^{3,5}Dmp trans-NH), 7.63 (s, 0.71H, cis-$CONH_2$), 7.37−7.05 (m, 6.29H, $CONH_2$, Ar−H), 6.84−6.75 (m, 3.0H, Ar−H), 6.45, 6.43 (2s, 2.0H, Dmt Ar−H), 4.51 (dt, 0.71H, J = 4.90, 8.75 Hz, Phe cis-αH), 4.46−4.33 (m, 0.87H, Pro trans-αH, ^{3,5}Dmp trans-αH, Phe trans-αH), 4.33−4.22 (m, 0.71H, ^{3,5}Dmp cis-αH), 4.11 (dd, 0.29H, J = 5.61, 9.50 Hz, Dmt trans-αH), 3.66 (dd, 0.71H, J = 4.01, 11.08 Hz, Dmt cis-αH), 3.44−3.28 (m, 0.8H, Pro cis-δH), 3.25−3.14 (m, 0.8H, Pro cis-δH), 3.10−2.68 (m, 6.7H, Pro cis-αH, Dmt βH, ^{3,5}Dmp βH, Phe βH), 2.88−2.80 (m, 0.4H, Pro trans-δH), 2.20, 2.06 (2s, 12.0H, Dmt and ^{3,5}Dmp CH_3), 1.87−1.75 (m, 0.29H, Pro trans-βH), 1.67−1.40 (m, 2.29H, Pro cis-βH, Pro trans-βH, Pro trans-γH, Pro cis-γH), 1.28−1.14 (m, 0.71H, Pro cis-βH), 1.14−1.0 (m, 0.71H, Pro cis-γH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 173.17, 172.48, 170.83, 170.31, 170.25, 169.98, 167.94, 167.60, 155.95, 138.43, 138.14, 137.74, 137.65, 137.25, 136.78 (15q), 129.28, 129.11, 127.92, 127.82, 127.59, 126.73, 126.61 (8t, Ar−C), 121.43, 121.02 (2q), 115.05 (t, Ar−C), 59.81 (t, Pro trans-αC), 59.08 (t, Pro cis-αC), 55.67 (t, ^{3,5}Dmp cis-αC), 54.02 (t, ^{3,5}Dmp trans-αC), 53.61 (t, Phe αC), 49.81 (t, Dmt αC), 46.65 (s, Pro cis-δC), 46.21 (s, Pro trans-δC), 37.60 (s, Phe cis-βC), 37.41 (s, Phe trans-βC), 37.24 (s, ^{3,5}-Dmp trans-βC), 36.01 (s, ^{3,5}Dmp cis-βC), 31.11 (s, Pro cis-βC), 30.58 (s, Dmt cis-βC), 30.03 (s, Dmt trans-βC), 28.83 (s, Pro trans-βC), 23.98 (s, Pro trans-γC), 21.09 (s, Pro cis-γC), 20.81 (p, ^{3,5}-Dmp *C*H$_3$), 20.07 (p, Dmt trans-*C*H$_3$), 19.23 (p, Dmt cis-*C*H$_3$).

2′,6′-Dimethyl-L-tyrosylprolyl-2′,6′-dimethyl-L-phenylalanylphenylalanylamide Hydrochloride (4′). Yield 168 mg (69.5%); mp 194−196 °C. ^{1}H NMR (400.1 MHz, DMSO-d_6) δ: 9.33 (s, 0.6H, Dmt cis-OH), 9.15 (s, 0.4H, Dmt trans-OH), 8.58, 8.40 (2br, 3.0H, $-NH_3^+$), 8.25 (d, 1.6H, J = 8.62 Hz, Dmp NH, Phe cis-NH), 7.95 (d, 0.4H, J = 8.38 Hz, Phe trans-NH), 7.45 (s, 0.6H, cis-$CONH_2$), 7.37−7.09 (m, 5.6H, Ar−H, $CONH_2$), 7.03 (s, 0.4H, trans-$CONH_2$), 6.98−6.84 (m, 3.0H, Ar−H), 6.68 (s, 0.4H, trans-$CONH_2$), 6.46 (s, 1.2H, Dmt cis-CH_3), 6.40 (s, 0.8H, Dmt trans-CH_3), 4.50 (dt, 0.6H, J = 4.71, 8.95 Hz, Phe cis-βH), 4.44−4.28 (m, 1.8H, Pro trans-αH, Dmt αH, Phe trans-αH), 4.10 (dd, 0.4H, J = 5.02, 9.59 Hz, Dmt trans-αH), 3.63 (dd, 0.6H, J = 4.30, 10.43 Hz, Dmt cis-αH), 3.38−3.28 (m, 0.6H, Pro cis-δH), 3.28−3.17 (m, 0.6H, Pro cis-δH), 3.08−2.70 (m, 6.6H, Pro cis-αH, Phe βH, Dmt βH, Dmp βH), 2.37−2.0 (m, 12.6H, Pro cis-αH, Dmt and Dmp CH_3), 1.87−1.74 (m, 0.4H, Pro trans-βH), 1.67−1.43 (m, 2.4H, Pro cis-βH, Pro trans-βH, Pro trans-γH, Pro cis-γH), 1.30−1.10 (m, 1.2H, Pro cis-βH, Pro cis-γH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 172.97, 172.20, 170.69, 170.61, 169.93, 169.81, 167.92, 167.20, 155.99, 155.58, 138.44, 138.15, 137.77, 137.70, 136.76, 136.52, 134.66, 134.30 (18q), 129.30, 129.02, 127.84, 127.79, 126.07, 126.04, 126.02, 125.98 (8t, Ar−C), 121.34, 121.03 (2q), 115.08, 114.90 (2t, Ar−C), 59.62 (t, Pro trans-αC), 59.09 (t, Pro cis-αC), 54.26 (t, Dmp cis-αC), 53.63 (t, Phe αC), 53.01 (t, Dmp trans-αC), 49.72 (t, Dmt αC), 37.47 (s, Pro cis-βC), 37.24 (s, Pro trans-βC), 32.48 (s, Dmp trans-βC), 31.09 (s, Dmp cis-βC), 31.03 (s, Pro cis-βC), 30.69 (s, Dmt cis-βC), 30.10 (s, Dmt trans-βC), 28.90 (s, Pro trans-βC), 24.17 (s, Pro trans-βC), 21.30 (s, Pro cis-βC), 20.04 (p, Dmp *C*H$_3$), 19.81 (p, Dmt trans-*C*H$_3$), 19.40 (p, Dmt cis-*C*H$_3$).

Tyrosylprolyl-2′,6′-dimethyl-L-tyrosylphenylalanylamide Hydrochloride (5). Yield 143.0 mg (87.3%); mp 188−190 °C. ^{1}H NMR (400.1 MHz, DMSO-d_6) δ: 9.52 (s, 0.28H, Tyr cis-OH), 9.41 (s, 0.69H, Tyr trans-OH), 9.00 (s, 0.90H, Dmt OH), 8.40−8.10 (m, 4.0H, $-NH_3^+$, Dmt NH, Phe cis-NH), 7.95 (d, 0.73H, J = 8.18 Hz, Phe trans-NH), 7.30−7.04 (m, 8.0H, $CONH_2$, Tyr and Phe Ar−H), 6.99 (d, 0.64H, J = 8.36 Hz, Tyr Ar−H), 6.93 (s, 0.68H, trans-$CONH_2$), 6.73 (d, 0.62H, J = 8.22 Hz, Tyr cis-Ar−H), 6.69 (d, 1.40H, J = 8.31 Hz, Tyr trans-Ar−H), 6.38 (s, 2.0H, Dmt Ar−H), 4.50−4.33 (m, 2.0H, Pro trans-αH, Phe αH, Dmt cis-αH), 4.30 (q, 0.73H, J = 7.30, 14.84 Hz, Dmt trans-αH), 4.15 (t, 0.70H, J = 6.52 Hz, Tyr trans-αH), 3.72−3.65 (m, 0.35H, Pro cis-αH), 3.65−3.50 (m, 1.0H, Tyr cis-αH, Pro trans-βH), 3.10−2.55 (m, 6.7H, Pro trans-δH, Phe βH, Tyr βH, Dmt βH), 2.19, 2.16 (2s, 6.0H, Dmt C*H*$_3$), 2.04−1.90 (m, 0.70H, Pro trans-βH), 1.83−1.69 (m, 1.40H, Pro trans-γH), 1.69−1.36 (m, 1.9H, Pro cis-βH, Pro trans-βH, Pro cis-γH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 172.70, 172.78, 170.96, 170.69, 170.32, 169.90, 167.04, 166.84, 156.67, 156.43, 155.02, 154.98, 137.87, 137.82, 137.77, 137.75 (16q), 130.78, 130.36, 129.18, 129.09, 127.87, 127.80, 126.04 (9t, Ar−C), 124.74, 124.49, 124.04 (3q), 115.32, 115.17, 114.76 (3t, Ar−C), 59.48 (t, Pro trans-αC), 59.07 (t, Pro cis-αC), 53.80, 53.70 (2s, Phe αC and Dmt αC), 52.40 (s, Tyr αC), 46.73 (s, trans Pro δC), 46.63 (s, Pro cis-δC), 37.31 (s, Phe cis-βC), 37.25 (s, Phe trans-βC), 36.18 (s, Tyr cis-βC), 35.25 (s, Tyr trans-βC), 31.60 (s, Dmt trans-βC), 31.14 (s, Dmt cis-βC, Pro cis-βC), 28.83 (s, Pro trans-βC), 24.41 (s, Pro trans-γC), 21.41 (s, Pro cis-γC), 20.13 (p, Dmt cis-*C*H$_3$), 20.04 (p, Dmt trans-*C*H$_3$).

2′,6′-Dimethyl-L-tyrosylprolyl-2′,6′-dimethyl-L-tyrosylphenylalanylamide Hydrochloride (5′). Yield 130 mg (67.8%); mp 209−211 °C. ^{1}H NMR (400.1 MHz, DMSO-d_6) δ: 9.31 (s, 0.57H, Dmt^1 cis-OH), 9.12 (s, 0.39H, Dmt^1 trans-OH), 8.99, 8.96 (2s, 0.97H, Dmt^3 OH), 8.47 (br, 2.8H, NH_3^+), 8.20−8.07 (m, 1.58H, Dmt^3 NH, Phe cis-NH), 7.86 (d, 0.38H, J = 8.13 Hz, Phe trans-NH), 7.48 (s, 0.54H, cis-$CONH_2$), 6.50−6.30 (m, 4.0H, Dmt^1 and Dmt^3 Ar−H), 4.49 (td, 0.59H, J = 4.89, 8.81 Hz, Phe cis-αH), 4.43−4.32 (m, 0.82H, Pro trans-αH, Phe trans-αH), 4.32−4.20 (m, 1.0H, Dmt^3 βH), 4.10 (dd, 0.39H, J = 5.09, 10.0 Hz, Dmt^1 trans-αH), 3.68−3.57 (m, 0.54H, Dmt^1 cis-αH), 3.40−3.16 (m, 1.18H, Pro cis-δH), 3.10−2.60 (m, 6.6H, Pro cis-αH, Phe βH, Dmt^1 βH, Dmt^3 βH), 2.30−2.20 (m, 12.82H, Pro trans-δH, Dmt^1 and Dmt^3 C*H*$_3$), 1.87−1.73 (m, Pro trans-βH), 1.67−1.38 (m, 2.64H, Pro cis-βH, Pro trans-βH, Pro trans-γH, Pro cis-γH), 1.32−1.07 (m, 1.24H, Pro cis-βH, Pro cis-γH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 172.94, 172.22, 170.92, 170.80, 169.91, 169.82, 167.94, 167.21, 155.99, 155.59, 155.01, 154.98, 138.47, 138.16, 137.80, 137.67, 137.55 (18q), 129.29, 129.04, 127.84, 127.80, 126.08, 126.02 (6t, Ar−C), 124.94, 124.69, 121.37, 121.07 (4q), 115.08, 114.90, 114.75 (3t, Ar−C), 59.67 (t, Pro trans-αC), 59.08 (t, Pro cis-αC), 54.83 (t, Dmt^1 αC), 53.56 (t, Phe αC), 49.77 (t, Dmt^3 αC), 46.81 (s, Pro cis-δC), 46.08 (s, Pro trans-δC), 37.51 (s, Phe cis-βC), 37.28 (s, Phe trans-βC), 31.08 (s, Dmt^3 trans-βC), 31.04 (s, Pro cis-βC), 30.73 (s, Dmt^1 cis-βC), 30.48 (s, Dmt^3 cis-βC), 30.09 (s, Dmt^1 trans-βC), 28.89 (s, Pro trans-βC), 24.20 (s, Pro trans-γC), 21.37 (s, Pro cis-γC), 20.09, 20.02, 19.99, 19.36 (4p, Dmt *C*H$_3$).

Tyrosylprolyl-2′,4′,6′-trimethyl-L-phenylalanylphenylalanylamide Hydrochloride (6). Yield 156.1 mg (95.3%); mp 179−181 °C. ^{1}H NMR (400.1 MHz, DMSO-d_6) δ: 9.53 (s, 0.3H, Tyr cis-OH), 9.41 (s, 0.7H, Tyr trans-OH), 8.49 (d, 0.3H, J = 8.75 Hz, Tmp cis-NH), 8.40−8.10 (br, 4.0H, NH_3^+, Tmp trans-NH, Phe cis-NH), 8.00 (d, 0.7H, J = 8.30 Hz, Phe trans-NH), 7.30−6.91 (m, 8.3H, Ar−H, $CONH_2$), 6.80−6.60 (m, 4.7H, Ar−H, $CONH_2$), 4.50−4.32 (m, 2.7H, Pro trans-αH, Tmp αH, Phe αH), 4.15 (t, 0.7H, J = 6.36 Hz, Tyr trans-αH), 3.82−3.75 (m, 0.3H, Pro cis-αH), 3.69 (t, 0.3H, J = 7.08 Hz, Tyr cis-αH), 3.60−3.50 (m, 0.7H, Pro trans-δH), 3.40−3.25 (m, 0.6H, Pro cis-δH), 3.10−2.72 (m, 6.7H, Pro trans-δH, Phe βH, Tyr βH, Tmp βH), 2.30−2.10 (m, 9.0H, Tmp Ar−CH_3), 2.02−1.90 (m, 0.7H, Pro trans-βH), 1.85−1.69 (m, 1.4H, Pro trans-γH), 1.69−1.38 (m, 1.9H, Pro cis-βH, Pro trans-βH, Pro cis-γH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 172.66, 172.25, 170.76, 170.54, 170.32, 169.97, 166.94, 166.78, 156.69, 156.44, 137.87, 137.81, 136.64, 136.49, 134.71, 131.16, 131.06 (18q), 129.15, 129.04, 128.62, 127.86, 127.79, 126.02 (6t,

Ar−C), 124.40, 124.01 (2q), 115.31, 115.15 (2t, Ar−C), 59.41 (t, Pro trans-αC), 59.04 (t, Pro cis-αC), 53.67 (t, Phe αC), 53.43 (t, Pro cis-αC), 53.29 (t, Tmp trans-αC), 52.32 (t, Tyr αC), 46.72 (s, Pro trans-δC), 46.64 (s, Pro cis-δC), 37.15 (s, Phe βC), 36.05 (s, Tyr cis-βC), 35.21 (s, Tyr, trans-βC), 32.01 (s, Tmp trans-βC), 31.62 (s, Tmp cis-βC), 31.13 (s, Pro cis-βC), 28.85 (s, Pro trans-βC), 24.39 (s, Pro trans-γC), 21.39 (s, Pro cis-γC), 20.36, 19.86, 19.78 (3p, Tmp Ar-*C*H$_3$).

2′,6′-Dimethyl-L-tyrosylprolyl-2′,4′,6′-trimethyl-L-phenylalanylphenylalanylamide Hydrochloride (6′). Yield 137.6 mg (80.6%); mp 184−186 °C. ^{1}H NMR (400.1 MHz, DMSO-d_6) δ: 9.32 (s, 0.6H, Dmt cis-OH), 9.14 (s, 0.4H, Dmt trans-OH), 8.48 (br, 3.0H NH_3^+), 8.24−8.14 (m, 1.6H, Tmp NH, Phe cis-NH), 7.93 (d, 0.4H, J = 8.38 Hz, Phe trans-NH), 7.40 (s, 0.60H cis-$CONH_2$), 7.33−7.10 (m, 5.6H, Phe Ar−H, cis-$CONH_2$), 7.05 (s, 0.4H, trans-$CONH_2$), 6.75, 6.74 (2s, Tmp Ar−H), 6.60 (s, 0.4H, trans-$CONH_2$), 6.46 (s, 1.2H, Dmt cis-Ar−CH_3), 6.40 (s, 0.8H, Dmt trans-Ar−CH_3), 4.49 (td, 0.6H, J = 4.76, 8.9 Hz, Phe cis-αH), 4.42−4.24 (m, 1.8H, Pro trans-αH, Tmp αH, Phe trans-αH), 4.00 (dd, 0.4H, J = 5.08, 10.06 Hz, Dmt trans-αH), 3.64 (dd, 0.6H, J = 4.36, 10.88 Hz, Dmt cis-αH), 3.46−3.27 (m, 1.4H, Pro cis-δH, Pro trans-δH), 3.27−3.18 (m, 0.6H, Pro cis-δH), 3.08−2.68 (m, 6.6H, Pro cis-βH, Phe βH, Tmp βH, Dmt βH), 2.30−2.00 (m, 15.4H, Pro trans-δH, Dmt and Tmp Ar−C*H*$_3$), 1.87−1.75 (m, 0.4H, Pro trans-βH), 1.70−1.43 (m, 2.4H, Pro cis-βH, Pro trans-βH, Pro trans-γH, Pro cis-γH), 1.30−1.15 (m, 1.2H, Pro cis-βH, Pro cis-γH). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 172.96, 172.23, 170.74, 170.68, 169.91, 169.83, 167.98, 167.22, 156.00, 155.59, 138.45, 138.16, 137.75, 136.57, 136.33, 134.73, 134.66, 131.55, 131.16 (19q), 129.29, 129.02, 128.59, 127.84, 127.79, 126.07, 126.01 (7t, Ar−C), 121.37, 121.07 (2q), 115.09, 114.90 (2t, Ar−C), 59.62 (t, Pro trans-αC), 59.09 (t, Pro cis-αC), 54.50 (t, Tmp cis-αC), 53.59 (t, Phe αC), 53.10 (t, Tmp trans-αC), 49.78 (t, Dmt cis-αC), 49.73 (t, Dmt trans-αC), 46.80 (s, Pro cis-δC), 46.10 (s, Pro trans-δC), 37.47 (s, Phe cis-βC), 37.18 (s, Phe trans-βC), 32.21 (s, Tmp trans-βC), 31.03 (s, Pro cis-βC), 30.86 (s, Tmp cis-βC), 30.74 (s, Dmt trans-βC), 30.12 (s, Dmt cis-βC), 28.92 (s, Pro trans-βC), 24.18 (s, Pro trans-γC), 21.36 (s, Pro cis-γC), 20.38 (p, Tmp trans-Ar-*C*H$_3$), 20.35 (p, Tmp, cis-Ar-*C*H$_3$), 20.01 (p, Tmp trans-Ar-*C*H$_3$), 19.94 (p, Dmt trans-Ar-*C*H$_3$), 19.73 (p, Tmp cis-Ar-*C*H$_3$), 19.36 (p, Dmt cis-Ar-*C*H$_3$).

Tyrosylprolyl-2′-ethyl-6′-methyl-L-phenylalanylphenylalanylamide Hydrochloride (7). Yield 158.3 mg (96.6%); mp 167−169 °C. ^{1}H NMR (400.1 MHz, DMSO-d_6) δ: 9.53 (s, 0.33H, Tyr cis-OH), 9.41 (s, 0.67H, Tyr trans-OH), 8.43 (d, 0.33H, J = 8.96 Hz, Emp cis-NH), 8.37−8.10 (m, 4.0H, NH_3^+, Emp trans-NH, Phe cis-NH), 7.97 (d, 0.67H, J = 8.30 Hz, Phe trans-NH), 7.30−6.90 (m, 11.33H, Ar−H, $CONH_2$), 6.80−6.64 (m, 2.67H, Ar−H, trans-$CONH_2$), 4.52−4.33 (m, 2.67H, Pro trans-αH, Emp αH, Phe αH), 4.14 (t, 0.67H, J = 6.26 Hz, Tyr trans-αH), 3.68−3.63 (m, 0.33H, Pro cis-αH), 3.60−3.50 (m, 1.0H, Tyr cis-αH, Pro trans-δH), 3.36−3.24 (m, 0.66H, Pro cis-δH), 3.08−2.76 (m, 6.67H, Pro trans-δH, Phe βH, Tyr βH, Emp βH), 2.67−2.55 (m, 2.0H, Emp C*H*$_2$CH$_3$), 2.31, 2.28 (2s, 3.0H, Emp Ar−CH_3), 2.03−1.90 (m, 0.67H, Pro trans-γH), 1.83−1.70 (m, 1.34H, Pro trans-γH), 1.70−1.30 (m, 2.0H, Pro cis-βH, Pro trans-βH, Pro cis-γH), 1.14−1.03 (m, 3.0H, Emp CH$_2$C*H*$_3$). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 172.71, 172.18, 170.62, 170.42, 170.38, 169.86, 166.99, 166.85, 156.70, 156.44, 142.84, 142.71, 137.83, 137.75, 136.95, 136.80, 133.53, 133.44 (18q), 130.76, 130.34, 129.21, 130.34, 127.85, 127.78, 126.38, 126.30, 126.04 (9t, Ar−C), 124.47, 123.96 (2q), 115.33, 115.16 (2t, Ar−C), 59.45 (t, Pro trans-αC), 59.08 (t, Pro cis-αC), 53.79 (t, Emp αC), 53.67 (t, Phe αC), 52.38 (t, Tyr, trans-αC), 52.29 (t, Tyr cis-αC), 46.71 (s, Pro trans-δC), 46.58 (s, Pro cis-δC), 37.34 (s, Phe cis-βC), 37.22 (s, Phe, trans-βC), 36.15 (s, Tyr cis-βC), 35.32 (s, Tyr trans-βC), 31.47 (s, Emp trans-βC), 31.13 (s, Emp cis-βC), 30.95 (s, Pro cis-βC), 28.84 (s, Pro trans-βC), 25.30 (s, Emp cis-*C*H$_2$CH$_3$), 25.12 (s, Emp trans-*C*H$_2$CH$_3$), 24.38 (s, Pro trans-γC), 21.32 (s, Pro, cis-γC), 20.09 (p, Emp cis-Ar-*C*H$_3$), 19.99 (p, Emp, trans-Ar-*C*H$_3$), 15.06 (p, Emp CH$_2$*C*H$_3$).

2′,6′-Dimethyl-L-tyrosylprolyl-2′-ethyl-6′-methyl-L-phenylalanylphenylalanylamide Hydrochloride (7′). Yield 143.4 mg (87.0%); mp 176−178 °C. ^{1}H NMR (400.1 MHz, DMSO-d_6) δ: 9.32 (s, 0.65H, Dmt cis-OH), 9.14 (s, 0.35H, Dmt trans-OH), 8.52 (br, 3.0H, NH_3^+), 8.27−8.17 (m, 1.65H, Emp NH, Phe cis-NH), 7.89 (d, 0.35H, Phe trans-NH), 7.51 (s, 0.65H, cis-$CONH_2$), 7.36−7.28 (m, 1.30H, Ar−H), 7.25−7.12 (m, 4.6H, Ar−H), 7.06−6.89 (m, 3.1H, $CONH_2$, Ar−H), 6.65 (s, 0.35H, trans-$CONH_2$), 6.45, 6.40 (2s, 2.0H, Dmt Ar−H), 4.51 (dt, 0.65H, J = 4.75, 9.02 Hz, Phe cis-αH), 4.43−4.26 (m, 1.70H, Pro trans-αH, Emp αH, Phe trans-αH), 4.10 (dd, 0.35H, J = 5.15, 10.02 Hz, Dmt trans-αH), 3.64 (dd, 0.65H, J = 4.01, 10.94 Hz, Dmt cis-αH), 3.36−3.18 (m, 1.65H, Pro cis-δH, Pro trans-δH), 3.10−2.50 (m, 8.65H, Pro cis-αH, Phe βH, Emp βH, Dmt βH, Emp C*H*$_2$CH$_3$), 2.34−2.18 (m, 3.35H, Emp Ar−CH_3, Pro trans-δH), 2.14, 2.09 (2s, 6.0H, Dmt Ar−CH_3), 1.87−1.76 (m, 0.35H, Pro trans-βH), 1.68−1.43 (m, 2.35H, Pro cis-βH, Pro trans-βH, Pro trans-γH, Pro cis-γH), 1.27−1.02 (m, 4.30H, Pro cis-βH, Pro cis-γH, Emp CH$_2$C*H*$_3$). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 173.03, 172.15, 170.58, 169.95, 169.83, 167.96, 167.20, 156.01, 142.77, 142.64, 138.46, 138.15, 137.74, 137.68, 136.87, 136.61, 133.90, 133.44 (14q), 129.34, 129.02, 127.84, 127.77, 126.31, 126.26, 126.11, 126.02 (8t, Ar−C), 121.36, 121.04 (2q), 115.08, 114.90 (2t, Ar−C), 59.63 (t, Pro trans-αC), 59.09 (t, Pro cis-αC), 54.93 (t, Emp αC), 53.57 (t, Phe αC), 49.76 (t, Dmt αC), 46.76 (s, Pro cis-δC), 46.10 (s, Pro trans-δC), 37.52 (s, Phe cis-βC), 37.24 (s, Phe trans-βC), 31.70 (s, Emp trans-βC), 31.02 (s, Pro cis-βC), 30.68 (s, Dmt cis-βC), 30.27 (s, Emp cis-βC), 30.10 (s, Dmt trans-βC), 28.95 (s, Pro trans-βC), 25.41 (s, Emp cis-*C*H$_2$CH$_3$), 25.04 (s, Emp trans-*C*H$_2$CH$_3$), 24.18 (s, Pro trans-γC), 21.30 (s, Pro cis-γC), 20.13 (p, Emp trans-CH$_2$*C*H$_3$), 20.01 (p, Emp cis-CH$_2$*C*H$_3$), 19.97 (p, Dmt cis-Ar-*C*H$_3$), 19.36 (p, Dmt trans-Ar-*C*H$_3$).

Tyrosylprolyl-2′-isopropyl-6′-methyl-L-phenylalanylphenylalanylamide Hydrochloride (8). Yield 155.7 mg (94.9%); mp 173−175 °C. ^{1}H NMR (400.1 MHz, DMSO-d_6) δ: 9.53 (s, 0.3H, Dmt cis-OH), 9.40 (s, 0.7H, Dmt trans-OH), 8.44 (d, 0.3H, J = 8.81 Hz, Imp cis-NH), 8.37−8.05 (m, 4.0H, NH_3^+, Imp trans-NH, Phe cis-NH), 7.91 (d, 0.7H, J = 8.05 Hz, Phe trans-NH), 7.32−6.87 (m, 12.0H, Ar−H, $CONH_2$), 6.64−6.76 (m, 2.0H, Ar−H), 4.51−4.36 (m, 2.0H, Pro trans-αH, Imp cis-αH, Phe αH), 4.32 (dd, 0.7H, J = 7.23, 15.13 Hz, Imp trans-αH), 4.13 (t, 0.7H, J = 6.33 Hz, Tyr trans-αH), 3.67−3.50 (m, 1.3H, Pro cis-αH, Tyr cis-αH, Pro trans-δH), 3.37−3.16 (m, 1.6H, Pro cis-δH, Imp C*H*(CH$_3$)$_2$), 3.10−2.78 (m, 6.7H, Pro trans-δH, Phe ββH, Tyr βH, Imp βH), 2.31, 2.29 (2s, 3.0H, Imp Ar−CH_3), 2.07−1.96 (m, 0.7H, Pro trans-βH), 1.82−1.64 (m, 2.1H, Pro trans-βH, Pro trans-γH), 1.64−1.51 (m, 0.6H, Pro cis-βH, Pro cis-γH), 1.51−1.41 (m, 0.3H, Pro cis-βH), 1.41−1.27 (m, 0.3H, Pro cis-γH), 1.20−1.04 (m, 6.0H, CH(C*H*$_3$)$_2$). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 172.75, 172.23, 170.57, 17052, 170.37, 169.94, 167.11, 166.82, 156.70, 156.44, 147.45, 147.24, 137.81, 137.65, 136.76, 136.68, 132.97, 132.71 (18q), 130.73, 130.33, 129.25, 129.17, 127.84, 127.79, 127.56, 127.47, 126.48, 126.41, 126.06 (11t, Ar−C), 124.55, 124.00 (2q), 122.83, 115.33, 115.18 (3t, Ar−C), 59.49 (t, Pro trans-αC), 59.17 (t, Pro cis-αC), 54.46 (t, Imp αC), 53.66 (t, Phe cis-αC), 53.56 (t, Phe trans-βC), 52.44 (t, Tyr trans-βC), 52.32 (t, Tyr cis-βC), 46.72 (s, Pro trans-δC), 46.53 (s, Pro cis-δC), 37.39 (s, Phe βC), 36.20 (s, Tyr cis-βC), 35.34 (s, Tyr trans-βC), 31.05 (s, Pro cis-βC), 30.88 (s, Imp trans-βC), 30.45 (s, Imp cis-βC), 28.93 (s, Pro trans-βC), 27.87 (t, Imp *C*H(CH$_3$)$_2$), 24.42 (s, Pro trans-γC), 24.36 (p, Imp trans-Ar−*C*H$_3$), 23.86 (p, Imp cis-Ar−*C*H$_3$), 21.33 (s, Pro cis-γC), 20.61 (p, Imp cis-CH(*C*H$_3$)$_2$), 20.27 (p, Imp trans-CH(*C*H$_3$)$_2$).

2′,6′-Dimethyl-L-tyrosylprolyl-2′-isopropyl-6′-methyl-L-phenylalanylphenylalanylamide Hydrochloride (8′). Yield 131.3 mg (79.6%); mp 185−187 °C. ^{1}H NMR (400.1 MHz, DMSO-d_6) δ: 9.31 (s, 0.54H, Dmt cis-OH), 9.12 (s, 0.46H, Dmt trans-OH), 8.47 (br, 3.0H, NH_3^+), 8.30 (0.46H, d, J = 8.57 Hz, Imp trans-NH), 8.21 (d, 1.08H, J = 8.72 Hz Imp cis-NH, Phe cis-NH), 7.82 (d, 0.46H, J = 8.31 Hz, Phe trans-NH), 7.55 (s, 0.46H, trans-$CONH_2$), 7.34, 7.32 (2s, 1.08H, Ar−H), 7.26−6.85 (m, 8.46H, Ar−H, $CONH_2$), 6.45, 6.39 (2s, Dmt Ar−H), 4.51 (dt, 0.54H, J = 4.71,

8.98 Hz, Phe cis-αH), 4.45−4.24 (m, 1.92H, Pro trans-αH, Imp αH, Phe trans-αH), 4.09 (dd, 0.46H, J = 5.30, 9.98 Hz, Dmt trans-αH), 3.69 (dd, 0.54H, J = 4.08, 11.20 Hz, Dmt cis-αH), 3.37−3.10 (m, 2.54H, Pro cis-δH, Pro trans-δH, Imp C*H*$(CH_3)_2$), 3.10−2.75 (m, 6.54H, Pro cis-αH, Phe βH, Imp βH, Dmt βH), 2.34−2.17 (m, 3.46H, Pro trans-δH, Imp Ar−CH_3), 2.11, 2.09 (2s, 6.0H, Dmt Ar−CH_3), 1.90−1.75 (m, 0,46H, Pro trans-βH), 1.68−1.40 (m, 2.46H, Pro cis-βH, Pro trans-βH, Pro trans-γH, Pro cis-γH), 1.27−1.00 (m, 7.08H, Pro cis-βH, Pro cis-γH, Imp CH(C*H*$_3)_2$). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ: 173.06, 172.18, 170.54, 170.50, 170.03, 169.85, 168.05, 167.17, 156.03, 155.55, 147.35, 147.14, 138.44, 138.13, 137.74, 137.57, 136.69, 136.50, 133.12, 132.72 (20q), 129.35, 129.11, 127.83, 127.78, 127.53, 127.45, 126.42, 126.36, 126.09, 126.05, 122.87, 122.82 (12t, Ar−C), 121.34, 121.05 (2q), 115.08, 114.90 (2t, Ar−C), 59.59 (t, Pro trans-αC), 59.12 (t, Pro cis-αC), 55.36 (t, Imp cis-αC), 54.12 (t, Imp trans-αC), 53.62 (t, Phe cis-αC), 53.42 (t, Phe trans-αC), 49.80 (t, Dmt, cis-αC), 49.72 (t, Dmt, trans-αC), 46.72 (s, Pro cis-δC), 46.06 (s, Pro, cis-δC), 37.49 (s, Phe, cis-βC), 37.40 (s, Phe trans-βC), 31.11 (s, Imp, trans-βC), 30.95 (s, Pro trans-βC), 30.67 (s, Imp, cis-βC), 30.09 (s, Dmt, trans-βC), 29.87 (s, Dmt cis-βC), 28.94 (s, Pro, cis-βC), 27.93 (t, Imp cis-*C*H$(CH_3)_2$), 27.83 (t, Imp trans-*C*H$(CH_3)_2$), 24.41 (p, Imp trans-CH$(CH_3)_2$), 24.17 (p, Imp cis-CH$(CH_3)_2$), 24.12 (s, Pro trans-γC), 23.89 (p, Imp trans-CH$(CH_3)_2$), 23.82 (p, Imp cis-CH$(CH_3)_2$), 21.34 (s, Pro cis-γC), 20.73 (p, Imp cis-Ar-CH_3), 20.24 (p, Imp trans-Ar-CH_3), 19.96 (p, Dmt trans-Ar-CH_3), 19.34 (p, Dmt cis-Ar-CH_3).

Opioid Receptor Binding Assays. Opioid receptor affinities were determined under equilibrium conditions (2.5 h at room temperature (22 °C)) in a competition assay using brain P_2 synaptosomal membranes prepared from 150−160 g Sprague-Dawley rats.[29,40] Synaptosomes were preincubated to remove endogenous opioids for 60 min at 22 °C, washed in excess ice-cold buffer (50 mM Tris-HCl, pH 7.5) containing protease inhibitor (soybean trypsin inhibitor), and stored in a 20% glycerol-containing buffer (50 mM HEPES, pH 7.5) with protease inhibitor at −80 °C as described previously.[29,40] The δ- and μ-opioid receptors were radiolabeled with 1.9 nM [^{3}H]deltorphin-II (Tyr-D-Ala-Phe-Glu-Val−Val-Gly-NH_2) and 3.5 nM [^{3}H]DAMGO ([D-Ala^2,*N*-Me-Phe^4,-Gly-ol^5]enkephalin), respectively,[29,36,40] and excess unlabeled peptide (2 μM) established the level of nonspecific binding. After incubation, the radiolabeled membranes containing both radioligands were rapidly filtered on Whatman GF/C glass fiber filters presoaked in 0.1% polyetheneimine in order to optimize the signal-to-noise ratio, washed with ice-cold Tris-HCl−BSA (bovine serum albumin) buffer, and dried at 75−80 °C for 1 h. Radioactivity was determined using EcoLume (ICN, Costa Mesa, CA). All compounds were analyzed in duplicate using five to eight peptide dosages and several synaptosomal preparations in independent repetitions (n values noted in Table 2) to ensure statistical significance. The affinity constants (K_i) were calculated according to Cheng and Prusoff[54] using published K_d values for [^{3}H]deltorphin-II (1.4 nM) and [^{3}H]DAMGO (3.5 nM).

κ-Receptor binding assays were carried out using P_2 synaptosomal membranes prepared from guinea pig cerebellum (Hartley, male, 260−310 g).[30,55] They were labeled with 2.0 nM [^{3}H]U-69,-593[56] [(5a,7a,8b)-(+)-*N*-methyl-*N*-[7-(1-pyrrolidiniyl)-1-oxaspiro-[4.5]dec-8yl]-benzeneacetamide, 1.75 TBq/mmol, NEN], and nonspecific binding was estimated in the presence of unlabeled compound (1 μM). Filtration used Whatman GF/B glass fiber filters presoaked in 0.1% polyetheneimine, and free radiolabeled compound was washed with ice-cold Tris-HCl buffer. The K_d value for [^{3}H]U-69,593 was 1.3 nM.

Biological Activity in Isolated Tissue Preparation. The myenteric plexus longitudinal muscle preparations (2−3 cm segments) from the small intestine of male Hartley strain guinea pigs (GPI) measured μ-opioid receptor agonism, and a single mouse vas deferens (MVD) was used to determine δ-opioid receptor agonism as described previously.[33−35] The isolated tissues were suspended in organ baths containing balanced salt solutions in a physiological buffer, pH 7.5. Agonists were tested for the inhibition of electrically evoked contraction and expressed as IC_{50} (nM) obtained from the dose-response curves. The IC_{50} values represent the mean ± SE of five to six separate assays. δ−Opioid antagonist potencies in the MVD assay were determined against the δ agonist deltorphin-II and are expressed as pA_2 determined using the Schild plot.[57] The Schild slopes of all analogues were 0.88−1.07.

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Supporting Information Available: Tables 1−4 listing elemental analysis results of all the new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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