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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

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Abstract

Endomorphin-2 (H-Tyr-Pro-Phe-Phe-NH₂) and [Dmt¹]EM-2 (Dmt = 2',6'-dimethyl-L-tyrosine) analogues were synthesized containing alkylated Phe³ 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'**). They had the following characteristics: (i) [Xaa³]EM-2 analogues improved μ - and δ -opioid receptor affinities, the latter were inconsequential (K_i^δ = 491–3,451 nM); (ii) [Dmt¹,Xaa³]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) and lacked interaction with κ -opioid receptors, and (iii) elevated μ -bioactivity (IC₅₀ = 0.12–14.4 nM) and abolished δ -agonism (IC₅₀ > 10 μ M; **2'**, **3'**, **4'**, **5'**, **6'**); however, **4'** and **6'** exhibited mixed μ -agonism/ δ -antagonism (**4'**: IC₅₀ ^{μ} = 0.12, pA₂ = 8.15; **6'**: IC₅₀ ^{μ} = 0.21 nM, pA₂ = 9.05), and **7'** was a dual μ -/ δ -agonist (IC₅₀ ^{μ} = 0.17 nM; IC₅₀ ^{δ} = 0.51 nM). Alteration of EM-2 activity by Dmt¹ and alkylated Phe³ residues retained μ -receptor bioactivity and formed dual μ -/ δ -agonists and mixed μ -agonists/ δ -antagonists.

Introduction

Endomorphin-1 (EM-1: H-Tyr-Pro-Trp-Phe-NH₂) and endomorphin-2 (EM-2: H-Tyr-Pro-Phe-Phe-NH₂) are endogenous opioid peptides with remarkably high selectivity for μ -opioid receptors.¹ Since the endomorphins express pharmacological properties similar to 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} bioactive conformation,^{10–14} and to improve biological activity in vitro and in vivo by chemical modification.^{15–25}

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The aromatic amino acid residue in position 3 is the defining structural determinant between EM-1 (Trp³) and EM-2 (Phe³).^{1,26} Moreover, extensive studies previously indicated that Dmt (2',6'-dimethyl-L-tyrosine) 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 peptides, such as dynorphin A,³⁰ [Leu⁵]enkephalin,³¹ dermorphin, deltorphin II,³² YrFB (H-Tyr-D-Arg-Phe-βAla-NH₂),³³ and endomorphin-2.³⁴ Furthermore, the replacement of Tyr¹ by Dmp in deltorphin II and enkephalin also yielded analogues which were surprisingly nearly as effective as the parental peptides,³⁵ suggesting that alkylation of the aromatic ring enhances hydrophobicity, 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 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 Tyr¹ permitted further differentiation of the biological properties between the structurally related endomorphin-1 and -2 molecules.^{29,36} In light of ability of the dialkylated derivative of phenylalanine (Dmp) to substitute for Tyr¹ in deltorphin II³⁵ 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. A change in overall change in conformation or reduced freedom of rotation imparted by the alkyl substituents should provide a gauge on 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 the μ-opioid system,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, our data reveal that the combination of Dmt¹ and an alkylated Phe³ 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) (R²), 3',5'-dimethyl (^{3,5}Dmp) (R³,R⁵), 2',6'-dimethyl (Dmp) (R²,R⁶), 2',4',6'-trimethyl (Tmp) (R²,R⁴,R⁶), 2'-ethyl-6'-methyl (Emp) (R²,R⁶) and 2'-isopropyl-6'-methyl (Imp) (R²,R⁶) were prepared as reported through [Rh(1,5-COD)](*R,R*-DIPAMP)]BF₄, [(*R,R*)-(-)-1,2-Bis-[(*o*-methoxyphenyl)(phenyl)phosphino]ethane(1,5-cyclooctadiene)rhodium(I) tetrafluoroborate] mediated asymmetric catalytic hydrogenation of corresponding acetamidoacrylate.⁴³ Boc(*tert*-butoxycarbonyl)-Tyr-Pro-OH⁴⁴ and Boc-

Dmt-Pro-OH⁴⁰ were prepared as reported. Tyr/Dmt-Pro-Xaa-Phe-NH₂ (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-NH₂ with HCl/dioxane, the resulting H-Xaa-Phe-NH₂ 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 semi-preparative 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 analyses of all the compounds.

Results and Discussion

Opioid Receptor Affinity

Improved affinities for μ -opioid receptors were observed in the [Xaa³]EM-2 compounds (**2**, **4**, **6–8**) compared to the parent peptide (**1**), except **3** and **5** that 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 third methyl group at the 4'-position (**6**), an ethyl (**7**) or isopropyl (**8**) at the 6'-position (Figure 1) reduced receptor affinity by a factor of 10 relative to [Dmp³]EM-2 (**4**), which exhibits the highest μ -selectivity of any known μ -opioid agonist;^{1,34} nonetheless, the μ -affinities of **6**, **7** and **8** were still 3-fold greater than the naturally occurring EM-2 (**1**) (Table 2). Another dimethylated Phe derivative, [^{3,5}Dmp³]EM-2 (**3**), displayed the lowest μ -opioid receptor affinity of all the analogues (Table 2), being 141-fold lower than [Dmp³]EM-2 (**4**) and *ca.* 5-fold less than **1**, suggesting that the position of the alkyl groups on the Phe³ aromatic ring was a critical factor for receptor affinity.

Among the [Dmt¹,Xaa³]EM-2 ligands (**1'–8'**) (Table 2), alkylated Phe³ 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 [Xaa³]EM-2 substances. Although they displayed improved δ -opioid receptor affinity, μ -opioid selectivity remained. As published previously, the presence of Dmt¹ in opioids enhanced δ -opioid receptor affinity by orders of magnitude;^{27–29,45} however, with μ -opioid selective agonists, a 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 three orders of magnitude (Table 2). In these Dmt-derivatives, the highest μ -opioid selectivity occurred with [Dmt^{1,3}]EM-2 (**5'**) ($K_i^{\delta}/K_i^{\mu} = 878$), which was less (37-fold) than the most selective [Xaa³]EM-2 compound (**4**), but nearly 3-fold greater than **1'** (Table 2). One analogue of considerable interest is Tmp³ (**6'**): with a 44-fold greater interaction than Dmt³ (**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

Supporting Information Available: The elemental analyses of all the new compounds are summarized in Table 1–4. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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 functional bioactivity.

Functional Bioactivity

The functional bioactivities (Table 3) of the [Xaa³]EM-2 analogues exhibited considerable variation in μ -agonism relative to EM-2 (**1**): analogues **2**, **6**, **7** and **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 Phe³ changed μ -agonism relative to EM-2 (**1**); i.e., while alkylation at the 3' and 5' positions of Phe³ inactivated the molecule (**3**), substitutions at positions 2' and 6' were very well tolerated (**4**) producing dual μ -/ δ -agonism.³⁴ Moreover, trimethylation (**6**) yielded a highly selective μ -agonist.

The [Dmt¹,Xaa³]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 Phe³ (**4'**) (and **4**)³⁴ was permitted. This was equally true for the trimethyl- (**6'**), ethylmethyl- (**7'**) and isopropylmethyl-Phe³ (**8'**) derivatives; this observation was repeated in the [Xaa³]EM-2 series (**6**, **7**, **8**) reflecting a change that presumably enabled the ligands to adopt a more compatible ligand-receptor conformation to trigger a biological response. Substitution of Phe³ by Dmt³ only yielded a bioactive compound in the presence of Dmt¹ (compare **5** and **5'**). Moreover, replacement of the 4' OH of Dmt³ (**5'**) by a methyl group to yield Tmp³ (**6'**) displayed slightly enhanced μ -agonism and significantly improved δ -antagonism. Tmp³ (**6'**) became a mixed μ -agonist/ δ -antagonist with δ -antagonism being two orders of magnitude greater than Dmt³ (**5'**).

Recently, Dmt-Pro-Trp-Phe-NH₂ ([Dmt¹]EM-1) was reported to be a mixed μ -opioid agonist/ δ -antagonist (GPI IC₅₀ = 0.272 nM; MVD pA₂ = 8.60);^{36b} on the other hand, [Dmt¹]EM-2 (**1'**) is a dual μ -/ δ -opioid agonist.²⁹ The only difference between [Dmt¹]EM-1 and **1'** is Trp³ or Phe³, respectively. This indicates that the bulky side chain of Trp in combination with Dmt¹ 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 **7'** resembled the bioactivity profiles of the reference compounds (**1'** *cf.* ref. ²⁴, and **4**)³⁴). Nonetheless, the [Dmt¹,Xaa³]EM-2 compounds established a distinctly different bioactive profile than that of the [Xaa³]EM-2 analogues: (i) the appearance of δ -antagonism (**2'**, **3'**, **4'**, **5'**, **6'**) ranging from pA₂ = 6.59 (**2'**) to a potent pA₂ = 9.05 (**6'**), and (ii) exhibited δ -agonism with **7'** and **8'**. The δ -opioid antagonism of [Dmt¹,Tmp³]EM-2 (**6'**) is ca. 8-fold more potent than the prototype δ -antagonist H-Dmt-Tic-OH (pA₂ = 8.48).⁴⁵ Furthermore, [Xaa³]EM-2 analogues remained μ -agonists except for the inactive ligands **3** and **5**, and the dual μ -/ 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 [Dmt¹,Xaa³]EM-2 analogues was associated with the δ -antagonism (**6'** > **4'** > **5'**, **3'**, **2'**).

Substitution of Phe³ 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'-NH₂-Phe and 4'-NCS-

Phe,⁴⁷ failed to improve either the affinity toward the μ -opioid receptor or provide unique functional bioactivities in the resultant ligands. Our results clearly indicated that modification of Phe³ with alkyl groups not only enhanced receptor affinity, but also transformed the functional bioactivity to elicit pharmacological properties as bifunctional opioid ligands (dual μ -/ δ -agonists and mixed μ -agonists/ δ -antagonists).

Conclusion

This study demonstrated that alkylated Phe³ residues incorporated into the third position of EM-2 act as effective amino acid surrogates, in particular the [Dmt¹,Xaa³]EM-2 analogues, which exhibited significantly higher affinity for both μ - and δ -opioid receptors, 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 a 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 heterodimers⁴⁸ and that the δ -opioid receptors modulate the function of μ -opioid receptors in this complex.⁴⁹ Thus, our opioid derivatives displaying dual μ -/ δ -agonism (**7'**) and mixed μ -agonism/ δ -antagonism (**6'**, **4'**) characteristics which are shown to exhibit a low tendency to develop dependence and tolerance,⁵⁰ and verified using δ -receptor knockout mice⁵¹ and a δ -receptor antagonist⁵² 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 CHCl₃:H₂O:AcOH (acetic acid) (90:8:2), AcOEt (ethyl acetate), AcOEt:MeOH (methanol) (20:1), and *n*-BuOH (butanol):H₂O:AcOH:Pyridine (4:1:1:2), respectively. Optical rotations were measured with a DIP-1000 automatic polarimeter (Japan Spectroscopic Co.). Analytic RP-HPLC used a Waters Delta 600 with COSMOSIL C18 column (4.6 mm $\times$ 250 mm). The solvents for analytical HPLC were: A, 0.05% TFA (trifluoroacetic acid) in water; B, 0.05% TFA in CH₃CN. The column was eluted at a flow rate of 1 mL/min with a linear gradient: 90% A to 10% A in 30 min and with a linear gradient: 90% A to 50% A in 40 min; the retention time was reported as t_R (min). ¹H and ¹³C 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: 15.5 mmol) in dioxane (15 mL) was added to the solution of amino acid (5.0 mmol) in H₂O (15 mL) containing TEA (triethylamine: 15.0 mmol). The reaction mixture was stirred at 0 °C for 15 min, then 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 Na₂SO₄, filtered and concentrated. The residue was

diluted with hexane, the solid was collected by filtration and dried under vacuum. Elemental analyses of Boc-Xaa-OH are summarized in Supporting Information (Table 1).

***N*^α-*tert*-Butyloxycarbonyl-2'-methyl-L-phenylalanine**—Yield 1.25 g (89.2%); mp

118–119 °C; $R_{f1} = 0.56$; $[\alpha]_D^{25} -15.54^\circ$ ($c = 1.03$, MeOH). $^1\text{H-NMR}$ (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*^α-*tert*-Butyloxycarbonyl-3',5'-dimethyl-L-phenylalanine**—Yield 0.71 g (96.5%); mp

115–116 °C; $R_f = 0.56$; $[\alpha]_D^{25} + 16.09^\circ$ ($c = 0.67$, MeOH). $^1\text{H-NMR}$ (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, 1H), 2.28 (s, 3H), 1.42 (s, 9H).

***N*^α-*tert*-Butyloxycarbonyl-2',6'-dimethyl-L-phenylalanine**—Yield 1.3 g (93.0%); mp

135–136 °C; $R_f = 0.40$; $[\alpha]_D^{25} -17.85^\circ$ ($c = 0.41$, MeOH). $^1\text{H-NMR}$ (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*^α-*tert*-Butyloxycarbonyl-2',4',6'-trimethyl-L-phenylalanine**—Yield 1.37 g (88.2%);

mp 153–154 °C; $R_{f1} = 0.56$; $[\alpha]_D^{25} -13.26^\circ$ ($c = 0.94$, MeOH). $^1\text{H-NMR}$ (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*^α-*tert*-Butyloxycarbonyl-2'-ethyl-6'methyl-L-phenylalanine**—Yield 1.32 g (85.7%);

mp 117–118 °C; $R_{fl} = 0.56$; $[\alpha]_D^{25} -14.33^\circ$ ($c = 1.0$, MeOH). $^1\text{H-NMR}$ (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*^α-*tert*-Butyloxycarbonyl-2'-isopropyl-6'-methyl-L-phenylalanine**—Yield 1.45 g

(90.3%); mp 121–122 °C; $R_{f1} = 0.56$; $[\alpha]_D^{25} - 12.78^\circ$ ($c = 0.93$, MeOH). $^1\text{H-NMR}$ (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-NH₂ (Xaa = Mmp, ^{3,5}Dmp, Dmp, Dmt, Tmp, Emp, Imp)

The H-Phe-NH₂ 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 room temperature for 4 h. After remove of solvent, the residue was diluted with AcOEt (80 mL). The dilution was washed with ice cooled 10% citric acid (3 × 15 mL), 5% Na₂CO₃ (3 × 15 mL) and saturated NaCl (3 × 20 mL), dried over Na₂SO₄ and evaporated to dryness. The residue was purified by flash chromatography (SiO₂, AcOEt). The compound was precipitated with hexane, collected by filtration and dried under vacuum. Elemental analyses of Boc-Xaa-Phe-NH₂ are summarized in Supporting Information (Table 2).

***N*^α-*tert*-Butyloxycarbonyl-2'-methyl-L-phenylalanylphenylalanylamide—Yield**

470 mg (97.0%); mp 164–166 °C; $R_D = 0.73$; $[\alpha]_D^{25} -21.04^\circ$ ($c = 0.34$, 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, CONH₂), 7.30–6.96 (m, 10.9H, CONH₂, 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 α H), 4.11 (dt, 1.0H, J = 4.45, 9.80 Hz, Mmp α H), 3.03 (dd, 1.0H, J = 4.87, 13.72 Hz, Phe β H), 2.90–2.70 (m, 2H, Phe β H, Mmp β H), 2.65 (dd, 1.0H, J = 10.15, 14.12 Hz, Mmp β H), 2.24 (s, 3H, Mmp CH₃), 1.29, 1.14 (2s, 9.0H, Boc). ¹³C 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 α C), 53.29 (t, Phe α C), 37.67 (s, Phe β C), 34.78 (s, Mmp β C), 28.00, 27.57 (2p, Boc), 18.90 (p, Mmp CH₃).

N^α-tert-Butyloxycarbonyl-3',5'-dimethyl-L-phenylalanylphenylalanylamide—

Yield 493 mg (98.6%); mp 193–195 °C; R_f = 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, CONH₂), 7.30–7.14 (m, 5H, Phe Ar-H), 7.14–6.96 (m, 1H, CONH₂), 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 α H), 4.12–3.98 (m, 1H, ^{3,5}Dmp α H), 3.01 (dd, 1.0H, J = 5.04, 13.75 Hz, Phe β H), 2.84 (dd, 1.0H, J = 8.65, 13.75 Hz, Phe β H), 2.77 (dd, 1.0H, J = 4.26, 13.68 Hz, ^{3,5}Dmp β H), 2.59 (dd, 1.0H, J = 10.09, 13.68 Hz, ^{3,5}Dmp β H), 2.21 (s, 6H, ^{3,5}Dmp CH₃), 1.31, 1.16 (2s, 9H, Boc). ¹³C 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 α C), 53.36 (t, Phe α C), 37.79 (s, Phe β C), 37.16 (s, ^{3,5}Dmp β C), 28.01 (p, Boc), 20.81 (p, ^{3,5}Dmp CH₃).

N^α-tert-Butyloxycarbonyl-2',6'-dimethyl-L-phenylalanylphenylalanylamide—

Yield 491 mg (98.0%); mp 177–179 °C; R_f = 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, CONH₂), 7.33–7.21 (m, 4.68H, CONH₂ and Ar-H), 7.21–7.13 (m, 1H, Ar-H), 7.08 (s, 1.0H, CONH₂), 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 α H), 4.19–4.03 (m, 1.0H, Dmp α H), 3.02 (dd, 1.0H, J = 5.05, 13.62 Hz, Phe β H), 2.88–2.55 (m, 3.0H, Phe β H and Dmp β H), 2.07 (s, 6.0H, Dmp CH₃), 1.26, 1.04 (2s, 9.0H, Boc). ¹³C 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 α C), 53.35 (t, Phe α C), 37.84 (s, Phe β C), 32.30 (s, Dmp β C), 27.97, 27.34 (2p, Boc), 19.80 (p, Dmp CH₃).

N^α-tert-Butyloxycarbonyl-2',6'-dimethyl-L-tyrosylphenylalanylamide—

Yield 483 mg (93.2%); mp 184–186 °C; R_f = 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, CONH₂), 7.29–7.02 (m, 6H, Phe Ar-H, CONH₂), 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 α H), 4.10–3.93 (m, 1.0H, Dmt α H), 3.02 (dd, 1.0H, J = 5.09, 13.60 Hz, Phe β H), 2.81 (dd, 1.0H, J = 9.01, 13.60 Hz, Phe β H), 2.65 (dd, 1.0H, J = 5.14, 14.40 Hz, Dmt β H), 2.55 (dd, 1.0H, J = 9.16, 14.40 Hz, Dmt β H), 2.13 (s, 6.0H, Dmt CH₃), 1.28, 1.09 (2s, 9.0H, Boc). ¹³C 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 α C), 53.30 (t, Dmt α C), 37.82 (s, Phe β C), 31.58 (s, Dmt β C), 27.99, 27.39 (2p, Boc), 19.95 (p, Dmt CH₃).

N^α-tert-Butyloxycarbonyl-2',4',6'-trimethyl-L-phenylalanylphenylalanylamide—

Yield 483 mg (93.5%); mp 224–225 °C; R_f = 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, CONH₂), 7.30-7.13 (m, 5.80H, CONH₂ 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 CH₃), 1.27, 1.06 (2s, 9H, Boc). ¹³C 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 CH₃).

N^α-tert-Butyloxycarbonyl-2'-ethyl-6'-methyl-L-phenylalanylphenylalanylamide

—Yield 481 mg (93.2%); mp 208–210 °C; *R*_f = 0.78; [α]_D²⁵ –15.16° (*c* = 0.35, MeOH). ¹H 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, CONH₂, 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 CH₂CH₃), 2.24 (s, 3H, Emp Ar-CH₃), 1.26 (s, 7.38H, Boc), 1.10 (t, 3H, *J* = 7.42 Hz, Emp CH₂CH₃), 1.04 (s, 1.62H, Boc). ¹³C 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, CH₂CH₃), 19.90 (p, Emp Ar-CH₃) 15.03 (p, Emp CH₂CH₃).

N^α-tert-Butyloxycarbonyl-2'-isopropyl-6'-methyl-L-phenylalanylphenylalanylamide

—Yield 495 mg (93.0%); mp 109–110 °C; *R*_f = 0.72; [α]_D²⁵ –22.11° (*c* = 0.33, MeOH). ¹H 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, CONH₂), 7.30-6.86 (m, 10H, CONH₂, 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(CH₃)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-CH₃), 1.23 (s, 7H, Boc), 1.17 (d, 3H, *J* = 6.68 Hz, CH(CH₃)₂), 1.10 (d, 3H, *J* = 6.68 Hz, CH(CH₃)₂), 1.02 (s, 2H, Boc). ¹³C 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(CH₃)₂; p, Boc), 27.36 (p, Boc), 24.37, 23.73 (2p, CH(CH₃)₂), 20.13 (p, Imp Ar-CH₃).

General Procedure for Synthesis of Boc-Tyr/Dmt-Pro-Xaa-Phe-NH₂ (Xaa = Mmp, ^{3,5}Dmp, Dmp, Dmt, Tmp, Emp, Imp)

Boc-Xaa-Phe-NH₂ (0.44 mmol) was treated with 7.7 mol/L HCl/dioxane (1.15 mL, 8.8 mmol) to remove the Boc group at room temperature for 30 minutes. The product was precipitated with ether, filtered and dried under vacuum. The resulted 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 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% Na₂CO₃ (3 × 15 mL) and saturated NaCl (3 × 20 mL), dried over Na₂SO₄ and evaporated to dryness. The residue was purified by flash chromatography (SiO₂, AcOEt:MeOH = 10:1). The compound was precipitated with hexane, filtered and dried under vacuum. Elemental analyses of Boc-Tyr/Dmt-Pro-Xaa-Phe-NH₂ are summarized in Supporting Information (Table 3).

***N*^α-*tert*-Butyloxycarbonyltyrosylprolyl-2'-methyl-L-phenylalanylphenylalanylamide**—Yield 289 mg (95.6%); mp 138–140 °C; *R*_f = 0.64;

[α]_D²⁵ –50.30° (*c* = 0.64, MeOH). ¹H 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, CONH₂, Tyr NH), 6.68–6.57 (m, 2.1H, Tyr Ar-H, Tyr NH), 4.48–4.34 (m, 2.0H, Phe α H, Tyr α H), 4.34–4.10 (m, 1.77H, Pro trans- α H, Mmp α H), 3.82 (d, 0.17H, *J* = 7.67 Hz, Pro cis- α H), 3.64–3.53 (m, 0.65H, Pro trans- δ H), 3.53–3.37 (m, 0.96H, Pro trans- δ H), 3.24–2.54 (m, 6.39H, Pro cis- δ H, Phe β H, Tyr β H, Mmp β H), 2.25 (s, 3.0H, Mmp CH₃), 1.98–1.87 (m, 0.70H, Pro trans- β H), 1.87–1.66 (m, 2.66H, Pro cis- β H, Pro trans- β H, Pro trans- γ H), 1.48–1.38 (m, 0.27H, Pro cis- γ H), 1.38–1.14 (m, 9.2H, Boc, Pro cis- β H), 1.0–0.86 (m, 0.15H, Pro cis- γ H). ¹³C 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- α C), 59.39 (t, Pro cis- α C), 54.10 (t, Tyr trans- α C), 53.88 (t, Tyr cis- α C), 53.71 (t, Phe α C), 53.49 (t, Mmp cis- α C), 53.16 (t, Mmp trans- α C), 46.62 (s, Pro trans- δ C), 46.04 (s, Pro cis- δ C), 37.60 (s, Phe cis- β C), 37.17 (s, Phe trans- β C), 36.85 (s, Tyr cis- β C), 35.45 (s, Tyr trans- β C), 34.23 (s, Mmp trans- β C), 33.77 (s, Mmp cis- β C), 29.99 (s, Pro cis- β C), 28.54 (s, Pro trans- β C), 28.06 (p, trans-Boc), 27.75 (p, cis-Boc), 24.39 (s, Pro trans- γ C), 21.01 (s, Pro cis- γ C), 19.01 (p, Mmp trans-CH₃), 18.91 (p, Mmp cis-CH₃).

***N*^α-*tert*-Butyloxycarbonyl-2',6'-dimethyl-L-tyrosylprolyl-2'-methyl-L-phenylalanylphenylalanylamide**—Yield 266 mg (84.5%); mp 222–228 °C; *R*_f = 0.71;

[α]_D²⁵ –45.81° (*c* = 0.36, MeOH). ¹H 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, CONH₂), 7.14–6.95 (m, 6.0H, Mmp Ar-H, CONH₂, 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- α H, Mmp α H, Phe α H, Dmt trans- α H), 4.17–4.08 (m, 0.46H, Dmt cis- α H), 3.52–3.40 (m, 0.49H, Pro trans- δ H), 3.20–2.65 (m, 8.0H, Pro cis- α H, Pro cis- δ H, Pro trans- δ H, Phe β H, Mmp β H, Dmt β H), 2.25 (s, 3.0H, Mmp CH₃), 2.16, 2.09 (2s, 6.0H, Dmt CH₃), 1.96–1.62 (m, 2.53H, Pro cis- β H, Pro trans β H, Pro trans- γ H), 1.45–1.07 (m, 9.50H, Boc, Pro cis- γ H), 1.05–0.91 (m, 0.51H, Pro cis- β H), 0.86–0.70 (m, 0.47H, Pro cis- γ H). ¹³C 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- α C), 59.14 (t, Pro cis- α C), 53.74 (t, Mmp cis- α C), 53.45 (t, Phe α C), 53.18 (t, Mmp trans- α C), 51.47 (t, Dmt α C), 46.55 (s, Pro trans- δ C), 46.18 (s, Pro cis- δ C), 37.89 (s, Phe cis- β C), 37.20 (s, Phe trans- β C), 34.12 (s, Mmp cis- β C), 33.48 (s, Mmp trans- β C), 29.93 (s, Pro cis- β C), 28.61 (s, Pro trans- β C), 28.18, 27.98, 27.45 (3p, Boc), 24.34 (s, Pro trans- γ C), 20.99 (s, Pro cis- γ C), 20.11 (p, Dmt trans-CH₃), 19.43 (p, Dmt cis-CH₃), 18.99, 18.94 (2p, Mmp CH₃).

***N*^α-*tert*-Butyloxycarbonyltyrosylprolyl-3',5'-dimethyl-L-phenylalanylphenylalanylamide**—Yield 282 mg (91.4%); mp 126–128 °C; *R*_f = 0.67;

[α]_D²⁵ –44.94° (*c* = 0.48, MeOH). ¹H 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, CONH₂), 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- α H, 3,5 Dmp α H, Phe α H, Tyr α H), 3.76 (d, 0.17H, J = 7.72 Hz, Pro cis- α H), 3.64–3.53 (m, 0.78H, Pro trans- δ H), 3.53–3.47 (m, 1.0H, Pro trans- δ H), 3.27–3.16 (m, 0.22H, Pro cis- δ H), 3.10–2.52 (m, 6.0H, Phe β H, 3,5 Dmp β H, Tyr β H), 2.20, 2.18 (2s, 6.0H, 3,5 Dmp CH₃), 2.0–1.65 (m, 3.47H, Pro cis- β H, Pro trans- β H, Pro trans- γ H), 1.53–1.42 (m, 0.33H, Pro cis- γ H), 1.35, 1.29, 1.23 (3s, 9.0H, Boc), 1.11–0.94 (m, 0.1H, Pro cis- γ H). ^{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 α C), 54.79, 54.10, 54.06, 53.68, 53.42 (5t, 3,5 Dmp α C, Phe α C, Tyr α C), 46.60 (s, Pro δ C), 37.33 (s, Phe β C), 36.81 (s, 3,5 Dmp β C), 35.47 (s, Tyr β C), 28.56 (s, Pro β C), 28.07 (p, trans-Boc), 27.75 (p, cis-Boc), 24.28 (s, Pro γ C), 20.79 (p, 3,5 Dmp trans-CH₃), 20.75 (p, 3,5 Dmp cis-CH₃).

***N*^α-*tert*-Butyloxycarbonyl-2',6'-dimethyl-L-tyrosylprolyl-3',5'-dimethyl-L-phenylalanylphenylalanylamide**—Yield 281 mg (87.6%); mp 208–210 °C; R_f = 0.73;

$[\alpha]_D^{25}$ –35.02° (c = 0.31, MeOH). ^1H 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, CONH₂, 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- α H, 3,5 Dmp α H, Phe α H, Dmt trans- α H), 4.19–4.10 (m, 0.50H, Dmt cis- α H), 3.55–3.44 (m, 0.50H, Pro trans- δ H), 3.20–2.64 (m, 8.0H, Pro cis- α H, Pro trans- δ H, Pro cis- δ H, Phe β H, 3,5 Dmp β H, Dmt β H), 2.21, 2.19 (2s, 3,5 Dmp CH₃), 2.17, 2.09 (2s, Dmt CH₃), 1.96–1.83 (m, 0.50H, Pro trans- β H), 1.83–1.60 (m, 2.0H, Pro cis- β H, Pro trans- β H, Pro trans- γ H), 1.46–1.06 (m, 9.54H, Boc, Pro cis- γ H), 1.06–0.92 (m, 0.47H, Pro cis- β H), 0.92–0.77 (m, 0.49H, Pro cis- γ H). ^{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- α C), 59.11 (t, Pro cis- α C), 55.02 (t, 3,5 Dmp trans- α C), 54.22 (t, 3,5 Dmp cis- α C), 53.72 (t, Phe cis- α C), 53.43 (t, Phe trans- α C), 51.44 (t, Dmt cis- α C), 51.35 (t, Dmt trans- α C), 46.51 (s, Pro trans- δ C), 45.94 (s, Pro cis- δ C), 37.94 (s, Phe cis- β C), 37.56 (s, Phe trans- β C), 36.61 (s, 3,5 Dmp cis- β C), 36.00 (s, 3,5 Dmp trans- β C), 31.26 (s, Dmt cis- β C), 30.82 (s, Dmt trans- β C), 30.03 (s, Pro cis- β C), 28.63 (s, Pro trans- β C), 28.19, 27.97, 27.42 (3p, Boc), 24.18 (s, Pro trans- γ C), 20.94 (s, Pro cis- γ C), 20.78 (p, 3,5 Dmp cis-CH₃), 20.76 (p, 3,5 Dmp trans-CH₃), 20.13 (p, Dmt trans-CH₃), 19.39 (p, Dmt cis-CH₃).

***N*^α-*tert*-Butyloxycarbonyl-2',6'-dimethyl-L-tyrosylprolyl-2',6'-dimethyl-L-phenylalanylphenylalanylamide**—Yield 284 mg (84.9%); mp 145–146 °C; R_f = 0.88;

$[\alpha]_D^{25}$ –39.32° (c = 0.52, MeOH). ^1H 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, CONH₂, Dmt NH), 6.50–6.22 (m, 2.1H, Dmt Ar-H, Dmt NH), 4.65–4.28 (m, 2.80H, Pro trans- α H, Phe α H, Dmp α H, Dmt trans- α H), 4.28–3.98 (m, 0.53H, Dmt cis- α H), 3.57–3.44 (m, 0.47H, Pro trans- δ H), 3.28–2.63 (m, 8.20H, Pro cis- α H, Pro trans- δ H, Pro cis- δ H, Phe β H, Dmp β H, Dmt β H), 2.42–1.98 (m, 12.0H, Dmt CH₃, Dmp CH₃), 1.98–1.54 (m, 2.54H, Pro cis- β H, Pro trans- β H, Pro trans- γ H), 1.54–1.00 (m, 9.75H, Boc, Pro cis- γ H), 1.00–0.82 (m, 0.41H, Pro cis- β H), 0.73–0.53 (m, 0.32H, Pro cis- γ H). ^{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- α C), 59.30 (t, Pro cis- α C), 53.82 (t, Phe trans- α C), 53.53 (t, Phe cis- α C), 53.05 (t,

Dmp trans- α C), 52.43 (t, Dmp cis- α C), 51.47 (t, Dmt α C), 46.53 (s, Pro trans- δ C), 46.34 (s, Pro cis- δ C), 37.93 (s, Phe trans- β C), 37.17 (s, Phe cis- β C), 31.89 (s, Dmp cis- β C), 31.40 (s, Dmp trans- β C), 30.93 (s, Dmt trans- β C), 30.65 (s, Dmt cis- β C), 29.66 (s, Pro cis- β C), 28.66 (s, Pro trans- β C), 28.17, 28.00, 27.44 (3p, Boc), 24.35 (s, Pro trans- γ C), 20.90 (s, Pro cis- γ C), 20.11, 19.84, 19.53 (3p, Dmt CH₃, Dmp CH₃).

***N*^α-*tert*-Butyloxycarbonyltyrosylprolyl-2',6'-dimethyl-L-**

tyrosylphenylalanylamine—Yield 307 mg (97.2%); mp 154–156 °C; $R_f = 0.60$; $[\alpha]_D^{25} -43.85^\circ$ ($c = 0.33$, MeOH). ¹H 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, CONH₂), 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- α H, Tyr α H, Phe α H, Dmt α H), 3.88 (d, 0.21H, $J = 7.66$ Hz, Pro cis- α H), 3.66–3.55 (m, 0.65H, Pro trans- δ), 3.55–3.38 (m, 0.85H, Pro trans- δ H), 3.24–3.10 (m, 0.50H, Pro cis- δ H), 3.10–2.94 (m, 1.24H, Phe trans- β H, Dmt trans- β H), 2.94–2.54 (m, 4.73H, Phe trans- β H, Phe cis- β H, Tyr β H, Dmt trans- β H), 2.14 (s, 6.0H, Dmt CH₃), 2.01–1.88 (m, 0.76H, Pro trans- β H), 1.88–1.77 (m, 1.55H, Pro trans- β H), 1.77–1.64 (m, 0.97H, Pro trans- β H, Pro cis- β H), 1.50–1.40 (m, 0.29H, Pro cis- γ H), 1.31, 1.24 (2s, 9.0H, Boc), 1.20–1.06 (m, 0.37H, Pro cis- β H), 0.98–1.83 (m, 0.16H, Pro cis- γ H). ¹³C 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 α C), 54.12 (t, Tyr α C), 53.81 (t, Phe α C), 53.54 (t, Dmt trans- α C), 52.62 (t, Dmt cis- α C), 46.62 (s, Pro trans- δ C), 46.20 (s, Pro cis- δ C), 37.15 (s, Phe trans- β C), 36.82 (s, Phe cis- β C), 35.56 (s, Tyr β C), 31.24 (s, Dmt trans- β C), 30.24 (s, Dmt cis- β C), 29.79 (s, Pro cis- β C), 28.58 (s, Pro trans- β C), 28.08 (p, trans-Boc), 27.77 (p, cis-Boc), 24.42 (s, Pro trans- γ C), 21.05 (s, Pro cis- γ C), 20.00 (p, Dmt, trans-CH₃), 19.96 (p, Dmt cis-CH₃).

***N*^α-*tert*-Butyloxycarbonyl-2',6'-dimethyl-L-tyrosylprolyl-2',6'-dimethyl-L-**

tyrosylphenylalanylamine—Yield 257 mg (80.3%); mp 160–162 °C; $R_f = 0.63$; $[\alpha]_D^{25} -34.23^\circ$ ($c = 0.53$, MeOH). ¹H 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, Dmt³ NH), 8.03–7.87 (m, 0.52H, Dmt³ 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, CONH₂, Dmt¹ NH), 6.73 (d, 0.30H, $J = 8.13$ Hz, Dmt¹ NH), 6.48–6.30 (m, 4.0H, Dmt Ar-H), 4.53–4.03 (m, 3.40H, Pro trans- α H, Dmt³ α H, Phe α H, Dmt¹ α H), 3.56–3.43 (m, 0.40H, Pro trans- δ H), 3.28–3.17 (m, 0.60H, Pro cis- α H), 3.17–2.62 (m, 7.60H, Pro trans- δ H, Pro cis- δ H, Phe β H, Dmt³ β H, Dmt¹ β H), 2.30–1.98 (m, 12.0H, Dmt CH₃), 1.95–1.83 (m, 0.40H, Pro trans- β H), 1.83–1.57 (m, 2.0H, Pro cis- β H, Pro trans- γ H), 1.47–1.04 (m, 9.65H, Boc, Pro cis- γ H), 1.04–0.88 (m, 0.51H, Pro cis- β H), 0.84–0.65 (m, 0.45H, Pro cis- γ H). ¹³C 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- α C), 59.26 (t, Pro cis- α C), 53.84 (t, Dmt³ cis- α C), 53.56 (t, Dmt³ trans- α C), 53.48 (t, Phe cis- α C), 53.14 (t, Phe trans- α C), 51.50 (t, Dmt¹ cis- α C), 51.41 (t, Dmt¹ trans- α C), 46.48 (s, Pro trans- δ C), 46.39 (t, Pro cis- δ C), 38.01 (s, Phe cis- β C), 37.17 (s, Phe trans- β C), 31.31 (s, Dmt³ trans- β C), 31.19 (s, Dmt³ cis- β C), 30.95 (s, Dmt¹ trans- β C), 29.98 (s, Dmt¹ cis- β C), 29.80 (s, Pro cis- β C), 28.65 (s, Pro trans- β C), 28.19, 27.99, 27.47 (3p, Boc), 24.35 (s, Pro trans- γ C), 20.95 (s, Pro cis- γ C), 20.11, 19.99, 19.51 (3p, Dmt CH₃).

***N*^α-*tert*-Butyloxycarbonyltyrosylprolyl-2',4',6'-trimethyl-L-**

phenylalanylphenylalanylamine—Yield 237 mg (75.3%); mp 215–217 °C; $R_f = 0.76$;

$[\alpha]_D^{25} -46.69^\circ$ ($c = 0.35$, MeOH). ^1H 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-CONH₂), 6.76-6.55 (m, 4.8H, Tmp Ar-H, Tyr Ar-H, CONH₂, Tyr NH), 4.58-4.49 (m, 0.23H, Phe cis- α H), 4.43-4.20 (m, 3.38H, Pro trans- α H, Tyr trans- α H, Tmp α H, Phe trans- α H), 4.38-4.10 (m, 0.13H, Tyr cis- α H), 3.92 (d, 0.21H, $J = 7.67$ Hz, Pro cis- α H), 3.65-3.54 (m, 0.70H, Pro trans- δ H), 3.54-3.37 (m, 0.80H, Pro trans- δ H), 3.20-3.10 (m, 0.50H, Pro cis- δ H), 3.10-2.85 (m, 2.3H, Phe β H, Tmp β H), 2.85-2.53 (m, 3.7H, Phe β H, Tyr β H, Tmp β H), 2.28-2.08 (m, 9.0H, Tmp CH₃), 2.0-1.87 (m, 0.71H, Pro trans- β H), 1.87-1.76 (m, 1.56H, Pro trans- γ H), 1.76-1.63 (m, 1.0H, Pro cis- β H, Pro trans- β H), 1.48-1.37 (m, 0.23H, Pro cis- γ H), 1.30, 1.24 (2s, 9.0H, Boc), 1.20-1.10 (m, 0.29H, Pro cis- β H), 0.97-0.82 (m, 0.17H, Pro cis- γ H). ^{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 α C), 54.10 (t, Tyr α C), 53.74 (t, Tmp trans- α C), 53.53 (t, Tmp cis- α C), 43.14 (t, Phe trans- α C), 52.16 (t, Phe cis- α C), 46.61 (s, Pro trans- δ C), 46.21 (s, Pro cis- δ C), 37.42 (s, Phe cis- β C), 37.10 (s, Phe trans- β C), 36.88 (s, Tyr cis- β C), 35.55 (s, Tyr trans- β C), 31.61 (s, Tmp trans- β C), 30.72 (s, Tmp cis- β C), 29.87 (s, Pro cis- β C), 28.58 (s, Pro trans- β C), 28.08 (p, trans-Boc), 27.78 (p, cis-Boc), 24.42 (s, Pro trans- γ C), 20.95 (s, Pro cis- γ C), 20.36, 20.32, 19.75, 19.71 (4p, Tmp CH₃).

***N*^α-tert-Butyloxycarbonyl-2',6'-dimethyl-L-tyrosylprolyl-2',4',6'-trimethyl-L-phenylalanylphenylalanylamide**—Yield 250 mg (76.4%); mp 148–150 °C; $R_f = 0.80$;

$[\alpha]_D^{25} -35.74^\circ$ ($c = 0.35$, MeOH). ^1H 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, CONH₂), 7.06, 6.98 (2s, 1.41H, CONH₂), 6.80-6.65 (m, 2.84H, Tmp Ar-H, Dmt NH, CONH₂), 6.45-6.28 (m, 2.15H, Dmt Ar-H, Dmt NH), 4.50-4.22 (m, 3.0H, Pro trans- α H, Tmp α H, Phe α H, Dmt trans- α H), 4.16-4.06 (m, 0.49H, Phe cis- α H), 3.55-3.43 (m, 0.56H, Pro trans- δ H), 3.27-2.66 (m, 8.55H, Pro cis- α H, Pro cis- δ H, Phe β H, Tmp β H, Dmt β H), 2.28-2.05 (m, 15.0H, Dmt CH₃, Tmp CH₃), 1.95-1.58 (m, 2.74H, Pro cis- β H, Pro trans- β H, Pro trans- γ H), 1.14-1.08 (m, 9.47H, Boc, Pro cis- γ H), 1.05-0.9 (m, 0.42H, Pro cis- β H), 0.78-0.63 (m, 0.42H, Pro cis- γ H). ^{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- α C), 59.23 (t, Pro cis- α C), 53.78, 53.49 (2t, Tmp α C), 53.20, 52.68 (2t, Phe α C), 51.45 (t, Dmt α C), 46.53 (t, Pro trans- δ C), 46.40 (t, Pro cis- δ C), 37.90 (s, Phe cis- β C), 37.12 (s, Phe trans- β C), 31.59 (s, Tmp cis- β C), 31.46 (s, Tmp trans- β C), 30.97 (s, Dmt trans- β C), 30.41 (s, Dmt cis- β C), 29.81 (s, Pro cis- β C), 28.66 (s, Pro trans- β C), 28.17, 28.00, 27.47 (3p, Boc), 24.36 (s, Pro trans- γ C), 20.87 (s, Pro cis- γ C), 20.36, 20.31 (2p, Tmp CH₃), 20.11 (p, Dmt trans-CH₃), 19.75 (p, Tmp CH₃), 19.52 (p, Dmt cis-CH₃).

***N*^α-tert-Butyloxycarbonyltyrosylprolyl-2'-ethyl-6'-methyl-L-phenylalanylphenylalanylamide**—Yield 242 mg (76.7%); mp 222–224 °C; $R_f = 0.66$;

$[\alpha]_D^{25} -48.52^\circ$ ($c = 0.34$, MeOH). ^1H 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, CONH₂), 6.82-6.72 (m, 0.7H, CONH₂), 6.70-6.57 (m, 2.10H, Tyr Ar-H, Tyr NH), 4.60-4.52 (m, 0.26H, Phe cis- α H), 4.46-4.10 (m, 3.5H, Pro trans- α H, Phe trans- α H, Tyr α H, Emp α H), 3.90 (d, 0.24H, J = 7.62 Hz, Pro cis- α H), 3.66-3.60 (m, 0.62H, Pro trans- δ H), 3.40-3.38 (m, 0.77H, Pro trans- δ H), 3.23-3.08 (m, 0.62H, Pro cis- δ H), 3.08-2.53 (m, 8.0H, Phe β H, Tyr β H, Emp β H, Emp CH₂CH₃), 2.26 (s, 3.0H, Emp Ar-CH₃), 2.02-1.88 (m, 0.75H, Pro trans- β H), 1.88-1.77 (m, 1.5H, Pro trans- γ H), 1.77-1.63 (m, 1.0H, Pro trans- β H, Pro cis- β H), 1.47-1.20 (m, 9.35H, Pro cis- γ H, Boc), 1.20-1.02 (m, 3.25H, Pro cis- β H, Emp CH₂CH₃), 0.9-0.75 (m, 0.15H, Pro cis- γ H). ¹³C 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 α C), 54.13 (t, Tyr α C), 53.77 (t, Emp α C), 53.62 (t, Phe trans- α C), 52.67 (t, Phe cis- α C), 46.62 (s, Pro trans- δ C), 46.14 (s, Pro cis- δ C), 37.54 (s, Phe cis- β C), 37.14 (s, Phe trans- β C), 36.80 (s, Tyr cis- β C), 35.56 (s, Tyr trans- β C), 31.06 (s, Emp trans- β C), 30.13 (s, Emp cis- β C), 29.76 (s, Pro cis- β C), 28.58 (s, Pro trans- β C), 28.06, 27.76 (2p, Boc), 25.18 (s, Emp CH₂CH₃), 24.43 (s, Pro trans- γ C), 21.00 (s, Pro cis- γ C), 19.95 (p, Emp trans-Ar-CH₃), 19.91 (p, Emp cis-Ar-CH₃), 15.56 (p, Emp CH₂CH₃).

N^α-tert-Butyloxycarbonyl-2',6'-dimethyl-L-tyrosylprolyl-2'-ethyl-6'-methyl-L-phenylalanylphenylalanylamide—Yield 280 mg (85.6%); mp 135–137 °C; R_f = 0.75;

$[\alpha]_D^{25}$ –38.20° (c = 0.39, MeOH). ¹H 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, CONH₂, Dmt NH), 7.10-6.87 (m, 4.41H, Emp Ar-H, CONH₂), 6.84-6.68 (m, 0.73H, CONH₂, Dmt NH), 6.47-6.27 (m, 2.1H, Dmt Ar-H, Dmt NH), 4.50-4.23 (m, 3.0H, Pro trans- α H, Tmp α H, Phe α H, Dmt cis- α H), 4.16-4.04 (m, 0.50H, Dmt cis- α H), 3.23 (d, 0.50H, J = 7.37 Hz, Pro cis- α H), 3.17-2.52 (m, 10.0H, Pro δ H, Phe β H, Emp β H, Dmt β H, Emp CH₂CH₃), 2.25 (s, 3.0H, Emp Ar-CH₃), 2.15, 2.11 (2s, 6.0H, Dmt CH₃), 1.97-1.84 (m, 0.49H, Pro trans- β H), 1.84-1.58 (m, 2.1H, Pro cis- β H, Pro trans- β H, Pro trans- γ H), 1.47-1.10 (m, 9.67H, Dmt CH₃, Emp Ar-CH₃, Pro cis- γ H), 1.10-1.00 (m, 3.0H, Emp CH₂CH₃), 1.10-0.84 (m, 0.42H, Pro cis- β H), 0.70-0.53 (m, 0.38H, Pro cis- γ H). ¹³C 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- α C), 59.26 (t, Pro cis- α C), 53.80 (t, Tmp cis- α C), 53.66 (t, Tmp trans- α C), 53.50 (t, Phe cis- α C), 53.09 (s, Phe trans- α C), 51.48 (t, Dmt α C), 46.54 (s, Pro trans- δ C), 46.31 (s, Pro cis- δ C), 38.00 (s, Phe cis- β C), 37.16 (s, Phe trans- β C), 31.32 (s, Emp cis- β C), 31.11 (s, Emp trans- β C), 30.09 (s, Dmt trans- β C), 29.77 (s, Dmt cis- β C), 29.71 (s, Pro cis- β C), 28.68 (s, Pro trans- β C), 28.16, 27.99, 27.46 (3p, Boc), 25.20 (s, Emp cis-CH₂CH₃), 25.14 (s, Emp trans-CH₂CH₃), 24.37 (s, Pro trans- γ C), 20.88 (s, Pro cis- γ C), 20.10 (p, Emp trans-Ar-CH₃), 19.96 (p, Emp cis-Ar-CH₃), 19.95 (p, Dmt trans-CH₃), 19.50 (p, Dmt cis-CH₃), 15.58 (p, Emp CH₂CH₃).

N^α-tert-Butyloxycarbonyltyrosylprolyl-2'-isopropyl-6'-methyl-L-phenylalanylphenylalanylamide—Yield 269 mg (83.8%); mp 222–224 °C; R_f = 0.62;

$[\alpha]_D^{25}$ –45.51° (c = 0.31, MeOH). ¹H 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, CONH₂, 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- α H), 4.45-4.10 (m, 3.60H, Pro trans- α H, Imp α H, Tyr α H, Phe trans- α H), 3.90 (d, 0.19H, J = 7.67 Hz, Pro cis- α H), 3.67-3.50 (m, 0.65H, Pro trans- δ H), 3.55-3.40 (m, 0.73H, Pro trans- δ H), 3.28-3.11 (m, 1.62H, Pro cis- δ H, CH(CH₃)₂), 3.11-2.92 (m, 2.0H, Phe β H, Imp β H), 2.92-2.67 (m, 3.0H, Phe β H, Tyr β H, Imp β H), 2.67-2.55 (m, 0.82H, Tyr trans- α H), 2.25 (s, 3.0H, Imp Ar-CH₃), 2.05-1.90 (m, 0.81H, Pro trans- β H), 1.90-1.77 (m, 1.63H, Pro trans- γ H), 1.77-1.63 (m, 0.96H, Pro trans- β H), 1.47-1.36 (m, 0.18H, Pro cis- γ H), 1.36-1.02 (m, 15.23H, Pro cis- β H, Boc, Imp CH(CH₃)₂), 0.90-0.88 (m, 0.18H, Pro cis- γ H). ¹³C 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 α C), 54.32 (t, Imp α C), 54.17 (t, Tyr α C), 53.71 (t, Phe trans- α C), 53.46 (t, Phe cis- α C), 46.62 (s, Pro trans- δ C), 46.14 (s, Pro cis- δ C), 37.61 (s, Pro cis- β C), 37.24 (s, Pro trans- β C), 36.74 (s, Tyr cis- β C), 35.53 (s, Tyr trans- β C), 30.48 (s, Imp trans- β C), 29.88 (s, Imp cis- β C), 29.74 (s, Pro cis- β C), 28.66 (s, Pro trans- β C), 28.07 (p, trans-Boc), 27.95 (t, CH(CH₃)₂), 27.73 (p, cis-Boc), 24.43 (s, Pro trans- γ C), 24.31, 23.76, 23.65 (3p, CH(CH₃)₂), 21.00 (s, Pro cis- γ C), 20.46 (p, Imp cis-Ar-CH₃), 20.23 (p, Imp trans-Ar-CH₃).

N^α-tert-Butyloxycarbonyl-2',6'-dimethyl-L-tyrosylprolyl-2'-isopropyl-6'-methyl-L-phenylalanylphenylalanylamide—Yield 252 mg (75.6%); mp 143–145 °C; R_f = 0.67; $[\alpha]_D^{25}$ –35.88° (c = 0.44, MeOH). ¹H 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, CONH₂), 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- α H, Phe trans- α H, Imp α H, Dmt trans- α H), 4.25-4.17 (m, 0.51H, Phe cis- α H), 4.15-4.07 (m, 0.45H, Dmt cis- α H), 3.56-3.42 (m, 0.56H, Pro cis- δ H), 3.26-2.62 (m, 9.0H, Pro cis- α H, Pro trans- δ H, Pro cis- δ H, Phe β H, Dmt β H, Imp β H, Imp CH(CH₃)₂), 2.36-2.00 (m, 9.0H, Imp Ar-CH₃, Dmt Ar-CH₃), 2.00-1.85 (m, 0.59H, Pro trans- β H), 1.85-1.57 (m, 2.0H, Pro cis- β H, Pro trans- β H, Pro trans- γ H), 1.45-1.00 (m, 15.52H, Boc, Imp CH(CH₃)₂, Pro cis- γ H), 1.00-0.85 (m, 0.51H, Pro cis- β H), 0.70-0.55 (m, 0.38H, Pro cis- γ H). ¹³C 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- α C), 59.24 (t, Pro cis- α C), 54.36 (t, Phe cis- α C), 53.84 (t, Phe trans- α C), 53.76 (t, Imp trans- α C), 53.45 (t, Imp cis- α C), 51.53 (t, Dmt cis- α C), 51.49 (t, Dmt, trans- α C), 46.55 (s, Pro cis- δ C), 46.35 (s, Pro trans- δ C), 38.04 (s, Phe cis- β C), 37.24 (s, Phe trans- β C), 31.30 (s, Dmt cis- β C), 30.85 (s, Dmt trans- β C), 30.49 (s, Imp trans- β C), 29.82 (s, Pro cis- β C), 29.40 (s, Imp cis- β C), 28.73 (s, Pro trans- β C), 28.17 (p, Boc), 27.98 (p, Boc; t, CH(CH₃)₂), 27.45 (p, Boc), 24.37 (s, Pro trans- γ C), 24.33, 23.78, 23.70 (3p, CH(CH₃)₂), 20.89 (s, Pro cis- γ C), 20.58 (p, Imp cis-Ar-CH₃), 20.21 (p, Imp trans-Ar-CH₃), 20.07 (p, Dmt cis-Ar-CH₃), 19.49 (p, Dmt trans-Ar-CH₃).

General Procedure for Synthesis of H-Tyr/Dmt-Pro-Xaa-Phe-NH₂-HCl (Xaa = Mmp, ^{3,5}Dmp, Dmp, Dmt, Tmp, Emp, Imp)

Boc-Tyr/Dmt-Pro-Xaa-Phe-NH₂ (0.25 mmol) was treated with TFA (0.6 mL, 7.79 mmol) and anisole (40 μ L) for 1 h at room temperature. The reaction solution was diluted with hexane, the solid was collected by filtration, dried over KOH pellets and purified by semi-preparative RP-HPLC. The purified peptide was lyophilized from water (3 $\times$ 10 mL) containing 1 mol/L HCl (0.25 mL, 0.25 mmol) to give amorphous powder. Elemental analyses of H-Tyr/Dmt-Pro-Xaa-Phe-NH₂ are summarized in Supporting Information (Table 4).

Tyrosylprolyl-2'-methyl-L-phenylalanylphenylalanylamine Hydrochloride (2)—

Yield 136.5 mg (83.7%); mp 170–172 °C. ¹H 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, NH₃⁺, Phe trans-NH, Mmp cis-NH), 8.07–7.98 (m, 0.64H, Mmp trans-NH), 7.47–7.40 (m, 0.36H, cis-CONH₂), 7.34–7.02 (m, 11.90H, Ar-H, CONH₂), 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 CH₃), 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). ¹³C 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-CH₃), 18.95 (p, Mmp cis-CH₃).

2',6'-Dimethyl-L-tyrosylprolyl-2'-methyl-L-phenylalanylphenylalanylamine Hydrochloride (2')—

Yield 153.8 mg (93.9%); mp 180–182 °C. ¹H 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, NH₃⁺), 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-CONH₂), 7.34–7.00 (m, 10.3H, CONH₂, 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-CH₃), 2.17, 2.05 (2s, 6.0H, Dmt Ar-CH₃), 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). ¹³C 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-CH₃), 19.29 (p, Dmt cis-CH₃), 19.00 (p, Mmp trans-CH₃), 18.91 (p, Dmt cis-CH₃).

Tyrosylprolyl-3',5'-dimethyl-L-phenylalanylphenylalanylamine Hydrochloride (3)

—Yield 142.5 mg (87.2%); mp 170–172 °C. ¹H 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, NH₃⁺, ^{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-CONH₂), 7.33–7.05 (m, 8.0H, Ar-H, CONH₂), 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 CH₃), 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). ¹³C 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 CH₃).

2',6'-Dimethyl-L-tyrosylprolyl-3',5'-dimethyl-L-phenylalanylphenylalanyl amide Hydrochloride (3')—Yield 141.6 mg (86.3%); mp 180–182 °C. ¹H 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, NH₃⁺), 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-CONH₂), 7.37-7.05 (m, 6.29H, CONH₂, 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 CH₃), 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). ¹³C 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 CH₃), 20.07 (p, Dmt trans-CH₃), 19.23 (p, Dmt cis-CH₃).

2',6'-Dimethyl-L-tyrosylprolyl-2',6'-dimethyl-L-phenylalanylphenylalanyl amide Hydrochloride (4')—Yield 168 mg (69.5%); mp 194–196 °C. ¹H 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, -NH₃⁺), 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-CONH₂), 7.37-7.09 (m, 5.6H, Ar-H, CONH₂), 7.03 (s, 0.4H, trans-CONH₂), 6.98-6.84 (m, 3.0H, Ar-H), 6.68 (s, 0.4H, trans-CONH₂), 6.46 (s, 1.2H, Dmt cis-CH₃), 6.40 (s, 0.8H, Dmt trans-CH₃), 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 CH₃), 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). ¹³C 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- β C), 31.03 (s, Pro cis- β C), 30.69 (s, Dmt cis- β C), 30.10 (s, Dmt trans- β C), 28.90 (s, Pro trans- β C), 24.17 (s, Pro trans- β C), 21.30 (s, Pro cis- β C), 20.04 (p, Dmp CH₃), 19.81 (p, Dmt trans-CH₃), 19.40 (p, Dmt cis-CH₃).

Tyrosylprolyl-2',6'-dimethyl-L-tyrosylphenylalanylamine Hydrochloride (5)—

Yield 143.0 mg (87.3%); mp 188–190 °C. ¹H 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, -NH₃⁺, Dmt NH, Phe cis-NH), 7.95 (d, 0.73H, *J* = 8.18 Hz, Phe trans-NH), 7.30–7.04 (m, 8.0H, CONH₂, Tyr and Phe Ar-H), 6.99 (d, 0.64H, *J* = 8.36 Hz, Tyr Ar-H), 6.93 (s, 0.68H, trans-CONH₂), 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- α H, Phe α H, Dmt cis- α H), 4.30 (q, 0.73H, *J* = 7.30, 14.84 Hz, Dmt trans- α H), 4.15 (t, 0.70H, *J* = 6.52 Hz, Tyr trans- α H), 3.72–3.65 (m, 0.35H, Pro cis- α H), 3.65–3.50 (m, 1.0H, Tyr cis- α H, Pro trans- β H), 3.10–2.55 (m, 6.7H, Pro trans- δ H, Phe β H, Tyr β H, Dmt β H), 2.19, 2.16 (2s, 6.0H, Dmt CH₃), 2.04–1.90 (m, 0.70H, Pro trans- β H), 1.83–1.69 (m, 1.40H, Pro trans- γ H), 1.69–1.36 (m, 1.9H, Pro cis- β H, Pro trans- β H, Pro cis- γ H). ¹³C 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- α C), 59.07 (t, Pro cis- α C), 53.80, 53.70 (2s, Phe α C and Dmt α C), 52.40 (s, Tyr α C), 46.73 (s, trans Pro δ C), 46.63 (s, Pro cis- δ C), 37.31 (s, Phe cis- β C), 37.25 (s, Phe trans- β C), 36.18 (s, Tyr cis- β C), 35.25 (s, Tyr trans- β C), 31.60 (s, Dmt trans- β C), 31.14 (s, Dmt cis- β C, Pro cis- β C), 28.83 (s, Pro trans- β C), 24.41 (s, Pro trans- γ C), 21.41 (s, Pro cis- γ C), 20.13 (p, Dmt cis-CH₃), 20.04 (p, Dmt trans-CH₃).

2',6'-Dimethyl-L-tyrosylprolyl-2',6'-dimethyl-L-tyrosylphenylalanylamine Hydrochloride (5')—

Yield 130 mg (67.8%); mp 209–211 °C. ¹H NMR (400.1 MHz, DMSO-*d*₆) δ : 9.31 (s, 0.57H, Dmt¹ cis-OH), 9.12 (s, 0.39H, Dmt¹ trans-OH), 8.99, 8.96 (2s, 0.97H, Dmt³ OH), 8.47 (br, 2.8H, NH₃⁺), 8.20–8.07 (m, 1.58H, Dmt³ NH, Phe cis-NH), 7.86 (d, 0.38H, *J* = 8.13 Hz, Phe trans-NH), 7.48 (s, 0.54H, cis-CONH₂), 6.50–6.30 (m, 4.0H, Dmt¹ and Dmt³ Ar-H), 4.49 (td, 0.59H, *J* = 4.89, 8.81 Hz, Phe cis- α H), 4.43–4.32 (m, 0.82H, Pro trans- α H, Phe trans- α H), 4.32–4.20 (m, 1.0H, Dmt³ β H), 4.10 (dd, 0.39H, *J* = 5.09, 10.0 Hz, Dmt¹ trans- α H), 3.68–3.57 (m, 0.54H, Dmt¹ cis- α H), 3.40–3.16 (m, 1.18H, Pro cis- δ H), 3.10–2.60 (m, 6.6H, Pro cis- α H, Phe β H, Dmt¹ β H, Dmt³ β H), 2.30–2.20 (m, 12.82H, Pro trans- δ H, Dmt¹ and Dmt³ CH₃), 1.87–1.73 (m, Pro trans- β H), 1.67–1.38 (m, 2.64H, Pro cis- β H, Pro trans- β H, Pro trans- γ H, Pro cis- γ H), 1.32–1.07 (m, 1.24H, Pro cis- β H, Pro cis- γ H). ¹³C 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- α C), 59.08 (t, Pro cis- α C), 54.83 (t, Dmt¹ α C), 53.56 (t, Phe α C), 49.77 (t, Dmt³ α C), 46.81 (s, Pro cis- δ C), 46.08 (s, Pro trans- δ C), 37.51 (s, Phe cis- β C), 37.28 (s, Phe trans- β C), 31.08 (s, Dmt³ trans- β C), 31.04 (s, Pro cis- β C), 30.73 (s, Dmt¹ cis- β C), 30.48 (s, Dmt³ cis- β C), 30.09 (s, Dmt¹ trans- β C), 28.89 (s, Pro trans- β C), 24.20 (s, Pro trans- γ C), 21.37 (s, Pro cis- γ C), 20.09, 20.02, 19.99, 19.36 (4p, Dmt CH₃).

Tyrosylprolyl-2',4',6'-trimethyl-L-phenylalanylphenylalanylamine Hydrochloride (6)—

Yield 156.1 mg (95.3%); mp 179–181 °C. ¹H 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, NH₃⁺, 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, CONH₂), 6.80–6.60 (m, 4.7H, Ar-H, CONH₂), 4.50–4.32 (m, 2.7H, Pro trans- α H, Tmp α H, Phe α H), 4.15 (t, 0.7H, *J* = 6.36 Hz, Tyr trans- α H), 3.82–3.75 (m, 0.3H, Pro cis- α H), 3.69 (t, 0.3H, *J* = 7.08 Hz, Tyr cis- α H), 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-CH₃), 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). ¹³C 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-CH₃).

2',6'-Dimethyl-L-tyrosylprolyl-2',4',6'-trimethyl-L-phenylalanylphenylalanylamide Hydrochloride (6')—Yield 137.6 mg (80.6%); mp 184–186 °C. ¹H 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 NH₃⁺), 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-CONH₂), 7.33-7.10 (m, 5.6H, Phe Ar-H, cis-CONH₂), 7.05 (s, 0.4H, trans-CONH₂), 6.75, 6.74 (2s, Tmp Ar-H), 6.60 (s, 0.4H, trans-CONH₂), 6.46 (s, 1.2H, Dmt cis-Ar-CH₃), 6.40 (s, 0.8H, Dmt trans-Ar-CH₃), 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-CH₃), 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). ¹³C 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-CH₃), 20.35 (p, Tmp cis-Ar-CH₃), 20.01 (p, Tmp trans-Ar-CH₃), 19.94 (p, Dmt trans-Ar-CH₃), 19.73 (p, Tmp cis-Ar-CH₃), 19.36 (p, Dmt cis-Ar-CH₃).

Tyrosylprolyl-2'-ethyl-6'-methyl-L-phenylalanylphenylalanylamide Hydrochloride (7)—Yield 158.3 mg (96.6%); mp 167–169 °C. ¹H 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, NH₃⁺, 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, CONH₂), 6.80-6.64 (m, 2.67H, Ar-H, trans-CONH₂), 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 CH₂CH₃), 2.31, 2.28 (2s, 3.0H, Emp Ar-CH₃), 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 CH₂CH₃). ¹³C 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- δ C), 37.34 (s, Phe cis- β C), 37.22 (s, Phe, trans- β C), 36.15 (s, Tyr cis- β C), 35.32 (s, Tyr trans- β C), 31.47 (s, Emp trans- β C), 31.13 (s, Emp cis- β C), 30.95 (s, Pro cis- β C), 28.84 (s, Pro trans- β C), 25.30 (s, Emp cis-CH₂CH₃), 25.12 (s, Emp trans-CH₂CH₃), 24.38 (s, Pro trans- γ C), 21.32 (s, Pro, cis- γ C), 20.09 (p, Emp cis-Ar-CH₃), 19.99 (p, Emp, trans-Ar-CH₃), 15.06 (p, Emp CH₂CH₃).

2',6'-Dimethyl-L-tyrosylprolyl-2'-ethyl-6'-methyl-L-phenylalanylphenylalanylamide Hydrochloride (7')—Yield 143.4 mg (87.0%); mp 176–178 °C. ¹H 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, NH₃⁺), 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-CONH₂), 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, CONH₂, Ar-H), 6.65 (s, 0.35H, trans-CONH₂), 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 CH₂CH₃), 2.34-2.18 (m, 3.35H, Emp Ar-CH₃, Pro trans-δH), 2.14, 2.09 (2s, 6.0H, Dmt Ar-CH₃), 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 CH₂CH₃). ¹³C 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-CH₂CH₃), 25.04 (s, Emp trans-CH₂CH₃), 24.18 (s, Pro trans-γC), 21.30 (s, Pro cis-γC), 20.13 (p, Emp trans-CH₂CH₃), 20.01 (p, Emp cis-CH₂CH₃), 19.97 (p, Dmt cis-Ar-CH₃), 19.36 (p, Dmt trans-Ar-CH₃).

Tyrosylprolyl-2'-isopropyl-6'-methyl-L-phenylalanylphenylalanylanilamide Hydrochloride (8)—Yield 155.7 mg (94.9%); mp 173–175 °C. ¹H 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, NH₃⁺, 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, CONH₂), 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 CH(CH₃)₂), 3.10–2.78 (m, 6.7H, Pro trans-δH, Phe βH, Tyr βH, Imp βH), 2.31, 2.29 (2s, 3.0H, Imp Ar-CH₃), 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(CH₃)₂). ¹³C NMR (100.6 MHz, DMSO-*d*₆) δ: 172.75, 172.23, 170.57, 170.52, 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 CH(CH₃)₂), 24.42 (s, Pro trans-γC), 24.36 (p, Imp trans-Ar-CH₃), 23.86 (p, Imp cis-Ar-CH₃), 21.33 (s, Pro cis-γC), 20.61 (p, Imp cis-CH(CH₃)₂), 20.27 (p, Imp trans-CH(CH₃)₂).

2',6'-Dimethyl-L-tyrosylprolyl-2'-isopropyl-6'-methyl-L-phenylalanylphenylalanylamide Hydrochloride (8')—Yield 131.3 mg (79.6%); mp 185–187 °C. ¹H 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, NH₃⁺), 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-CONH₂), 7.34, 7.32 (2s, 1.08H, Ar-H), 7.26–6.85 (m, 8.46H, Ar-H, CONH₂), 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 CH(CH₃)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-CH₃), 2.11, 2.09 (2s, 6.0H, Dmt Ar-CH₃), 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(CH₃)₂). ¹³C 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-CH(CH₃)₂), 27.83 (t, Imp trans-CH(CH₃)₂), 24.41 (p, Imp trans-CH(CH₃)₂), 24.17 (p, Imp cis-CH(CH₃)₂), 24.12 (s, Pro trans-γC), 23.89 (p, Imp trans-CH(CH₃)₂), 23.82 (p, Imp cis-CH(CH₃)₂), 21.34 (s, Pro cis-γC), 20.73 (p, Imp cis-Ar-CH₃), 20.24 (p, Imp trans-Ar-CH₃), 19.96 (p, Dmt trans-Ar-CH₃), 19.34 (p, Dmt cis-Ar-CH₃).

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 P₂ 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 [³H]deltorphin-II (Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH₂) and 3.5 nM [³H]DAMGO ([D-Ala², N-Me-Phe⁴, Gly-ol⁵]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 hr. Radioactivity was determined using EcoLume (ICN, Costa Mesa, CA). All compounds are analyzed in duplicate using 5–8 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 Prusoff⁵⁴ using published *K*_d values for [³H]deltorphin-II (1.4 nM) and [³H]DAMGO (3.5 nM).

κ-Receptor binding assays were carried out using P₂ synaptosomal membranes prepared from guinea pig cerebellum (Hartley, male, 260–310 g).^{30,55} They were labeled with 2.0 nM [³H] U-69,593⁵⁶ [(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 [^3H]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 $\pm$ 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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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>i</i> -Pr	H	H	Me
8'	Me	<i>i</i> -Pr	H	H	Me

Figure 1.
Schematic Structure of Endomorphin-2 with Alkylation Sites (R¹–R⁶) on Tyr¹ and Phe³.

Table 1.
Analytical Data of H-Tyr/Dmt-Pro-Xaa-Phe-NH₂ Endomorphin-2 Analogues

No.	peptide	[α] _D	<i>c</i> in H ₂ O	TLC ^a <i>R_f</i>	tof-mass[M+1]		HPLC (min)	
		(deg)			Calcd	Found	<i>t_R^b</i>	<i>t_R^c</i>
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:H₂O: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% A to 10% A in 30 min.

^c The column was eluted at a flow rate of 1 mL/min with a linear gradient of 90% A to 50% A in 40 min.

Table 2
Receptor Affinities of H-Tyr/Dmt-Pro-Xaa-Phe-NH₂ Endomorphin-2 Analogues

no.	Peptide sequence	K_1^{μ} (nM) ^a	(n)	K_1^{δ} (nM) ^b	(n)	K_1^{δ}/K_1^{μ}	K_1^{κ} (nM) ^c	(n)	K_1^{κ}/K_1^{μ}
<i>1</i>	<i>Tyr-Pro-Phe-Phe-NH₂</i>	1.33	(3)	6085	(3)	4575	>4000	(3)	>3000
<i>1'</i>	<i>Dmt-Pro-Phe-Phe-NH₂</i> ^d	0.26	(4)	99.2	(4)	382	489	(3)	1880
2	Tyr-Pro-Mmp-Phe-NH ₂	0.221	(3)	1741	(5)	7878	ND	(3)	994
2'	Dmt-Pro-Mmp-Phe-NH ₂	0.177	(3)	4.61	(3)	26	176	(3)	994
3	Tyr-Pro- ^{3,5} Dmp-Phe-NH ₂	6.19	(5)	3451	(5)	558	ND	(3)	994
3'	Dmt-Pro- ^{3,5} Dmp-Phe-NH ₂	0.111	(3)	11.6	(5)	105	830	(3)	7480
<i>4</i>	<i>Tyr-Pro-Dmp-Phe-NH₂</i> ^d	0.044	(3)	1440	(3)	32730	>4000	(3)	>91000
<i>4'</i>	<i>Dmt-Pro-Dmp-Phe-NH₂</i>	0.069	(3)	2.27	(4)	33	94.2	(3)	1365
5	Tyr-Pro-Dmt-Phe-NH ₂	2.63	(3)	3020	(5)	1148	ND	(3)	1365
5'	Dmt-Pro-Dmt-Phe-NH ₂	0.092	(3)	80.8	(4)	878	998	(3)	10850
6	Tyr-Pro-Tmp-Phe-NH ₂	0.439	(4)	1358	(4)	3093	ND	(3)	945
6'	Dmt-Pro-Tmp-Phe-NH ₂	0.182	(3)	1.83	(3)	10	172	(3)	945
7	Tyr-Pro-Emp-Phe-NH ₂	0.457	(3)	491	(4)	1074	ND	(3)	1320
7'	Dmt-Pro-Emp-Phe-NH ₂	0.211	(3)	3.03	(3)	14	278	(3)	1320
8	Tyr-Pro-Imp-Phe-NH ₂	0.445	(4)	543	(4)	1221	ND	(3)	641
8'	Dmt-Pro-Imp-Phe-NH ₂	0.32	(3)	4.61	(3)	14	205	(3)	641

The italicized compounds represent parental opioid peptides.

^aVersus [³H]DAMGO.

^bVersus [³H]Deltorphin-II.

^cVersus [³H]U-69,593. (n) is the number of independent repetitions conducted for each analogue using 5–8 doses of peptide. ND: not determined.

^dThese data are nearly identical to the μ - and δ -opioid affinities and selectivities previously published for **1'** and **4** (ref. 29 and 34, respectively).

Table 3
Functional Bioactivities of H-Tyr/Dmt-Pro-Xaa-Phe-NH₂ Endomorphin-2 Analogues

no.	peptide	GPI assay ^a		MVD assay ^a		pA ₂ ^c
		IC ₅₀ (nM)		IC ₅₀ (nM) ^b		
<i>1</i>	<i>Tyr-Pro-Phe-Phe-NH₂</i>	6.9	±	0.9	344	± 93
<i>1'</i>	<i>Dmt-Pro-Phe-Phe-NH₂</i> ^d	0.261	±	0.038	0.59	± 0.18
<i>2</i>	<i>Tyr-Pro-Mmp-Phe-NH₂</i>	1.33	±	0.27	15.7	± 5.1
<i>2'</i>	<i>Dmt-Pro-Mmp-Phe-NH₂</i>	0.162	±	0.041	>10000 (0.00%)	6.59
<i>3</i>	<i>Tyr-Pro-^{3,5}Dmp-Phe-NH₂</i>	389	±	166	>10000 (8.0%)	6.77
<i>3'</i>	<i>Dmt-Pro-^{3,5}Dmp-Phe-NH₂</i>	14.4	±	5.4	>10000 (7.1%)	0.17
<i>4</i>	<i>Tyr-Pro-Dmp-Phe-NH₂</i> ^e	0.378	±	0.104	1.39	± 8.15
<i>4'</i>	<i>Dmt-Pro-Dmp-Phe-NH₂</i>	0.123	±	0.020	>10000 (15.6%)	7.06
<i>5</i>	<i>Tyr-Pro-Dmt-Phe-NH₂</i>	541	±	178	978	± 113
<i>5'</i>	<i>Dmt-Pro-Dmt-Phe-NH₂</i>	1.94	±	0.21	>10000 (<1%)	9.05
<i>6</i>	<i>Tyr-Pro-Tmp-Phe-NH₂</i>	1.90	±	0.49	>10000 (36.2%)	
<i>6'</i>	<i>Dmt-Pro-Tmp-Phe-NH₂</i>	0.212	±	0.077	>10000 (22.2%)	
<i>7</i>	<i>Tyr-Pro-Emp-Phe-NH₂</i>	2.35	±	0.53	277	± 29
<i>7'</i>	<i>Dmt-Pro-Emp-Phe-NH₂</i>	0.170	±	0.072	0.51	± 0.24
<i>8</i>	<i>Tyr-Pro-Imp-Phe-NH₂</i>	2.74	±	1.20	>10000 (17.7%)	
<i>8'</i>	<i>Dmt-Pro-Imp-Phe-NH₂</i>	0.204	±	0.050	5.56	± 2.47

The italicized compounds represent parental opioid peptides

^aThe data are the means of over five independent repetitions used different isolated tissue preparations. IC₅₀: concentration required for 50% inhibition of the electrically induced contraction in muscle derived from a dose-response curve.

^bValues in parentheses indicate maximal inhibition of the tissue contraction at the concentration of 10,000 nM.

^cNegative log of the molar concentration required to double the deltorphin II concentration to achieve the original response.

^dData cited from ref. 29.

^eData cited from ref. 34.