

Enantioselective Synthesis of a Phenylalanine Library Containing Alkyl Groups on the Aromatic Moiety: Confirmation of Stereostructure by X-Ray Analysis

Tingyou LI,^a Yuko TSUDA,^a Katsuhiko MINOURA,^b Yasuko IN,^b Toshimasa ISHIDA,^b Lawrence H. LAZARUS,^c and Yoshio OKADA^{*a}

^a The Graduate School of Food and Medicinal Sciences and Faculty of Pharmaceutical Sciences, Kobe Gakuin University; Nishi-ku, Kobe 651–2180, Japan; ^b Osaka University of Pharmaceutical Sciences; Takatsuki, Osaka 569–1094, Japan; and ^c Medicinal Chemistry Group, Laboratory of Pharmacology and Chemistry, National Institute of Environmental Health Sciences; Research Triangle Park, NC 27709, U.S.A.

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Six phenylalanine analogues containing 2'-methyl-, 2',6'-dimethyl-, 2'-ethyl-6'-methyl-, 2'-isopropyl-6'-methyl-, 2',4',6'-trimethyl-, and 3',5'-dimethyl-L-phenylalanine were synthesized enantioselectively through asymmetric hydrogenation of acetamidoacrylate derivatives. Enzymatic digestion and X-ray analysis supported the L-configuration of the phenylalanine derivatives obtained.

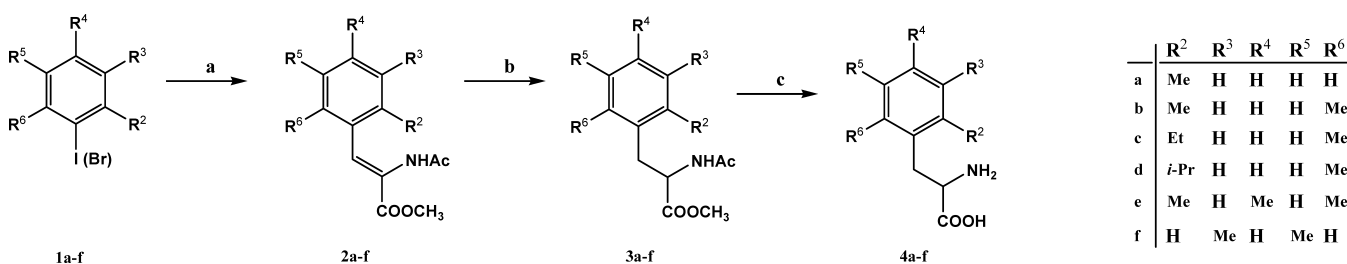
Key words phenylalanine analogue; asymmetrical hydrogenation; L-configuration; enzymatic digestion; X-ray analysis

Introduction of unnatural amino acid into biologically active peptides is one of the most powerful approaches to development of peptides and peptidomimetics with unique properties. It permits an examination of the topographical requirements for peptide bioactivities,^{1–3} improved enzymatic stability,⁴ constrained spatial conformation,⁵ and the elicitation of novel biological properties.^{6,7}

In opioid peptides for example, the introduction of unnatural amino acids, such as 2',6'-dimethyl-L-tyrosine (Dmt) as an analogue of tyrosine and 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic) as a conformationally constrained phenylalanine analogue, play critical roles in the development of unique ligands for the investigation of structure–activity relationships of δ - and μ -opioid ligands.^{1–3} Similarly, opioid studies indicated that 2',6'-dimethyl-L-phenylalanine (Dmp) is an effective surrogate of phenylalanine in enkephalin (H-Tyr-Gly-Gly-Phe-Leu-OH),⁸ dermorphin (H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH₂), deltorphin II (H-Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH₂),⁹ YrFB (H-Tyr-D-Arg-Phe- β -Ala-NH₂),¹⁰ and endomorphin-2 (H-Tyr-Pro-Phe-Phe-NH₂).¹¹ More interestingly, substitution of Tyr¹ by Dmp in deltorphin II and enkephalin, yielded analogues which were nearly as effective as the parent peptides.¹² In order to study the structure–activity relationships of this unnatural amino acid, phenylalanine analogues bearing different substituent(s) on the aromatic ring were required. These analogues consist of 2'-methyl- (Mmp), 2',6'-dimethyl- (Dmp), 2'-ethyl-6'-

methyl- (Emp), 2'-isopropyl-6'-methyl- (Imp), 2',4',6'-trimethyl- (Tmp), and 3',5'-dimethyl-L-phenylalanine (^{3,5}Dmp). Until now, 2'-methyl-L-phenylalanine was synthesized through optical resolution with enzymes¹³; 2',6'-dimethyl-L-phenylalanine was prepared by separation of Ac-D,L-Dmp-Arg-OMe in preparative HPLC followed by acid hydrolysis⁸ or asymmetric alkylation of glycine equivalent^{14–17}; 3',5'-dimethyl-L-phenylalanine was synthesized through asymmetric alkylation of glycine equivalent without physicochemical data¹⁶; and 2',4',6'-trimethyl-D,L-phenylalanine was obtained by alkylation of diethyl acetamidomalonate with the corresponding alkyl halide, however the racemic mix failed to be optically resolved by enzymatic treatment.^{13,18} Although several methods have been developed for preparing optically pure amino acids, the asymmetric catalytic hydrogenation is still a very convenient method due to the commercially availability of chiral catalysts and simple starting materials. In our report, the phenylalanine analogues were synthesized by the method described by Dygos *et al.* for synthesis of Dmt through [Rh(1,5-COD)(*R,R*-DIPAMP)]BF₄ mediated asymmetric catalytic hydrogenation of acetamidoacrylate (Chart 1).¹⁹ During the synthesis, Z-configuration of acetamidoacrylate after Heck reaction and L-configuration of the final phenylalanine analogues were confirmed by X-ray analysis.

According to the Chart 1, L-phenylalanine analogues were prepared. The starting materials, 2-iodotoluene (**1a**; Sigma-



Reagents and conditions: a) methyl acetamidoacrylate, Pd(OAc)₂, tri-*o*-tolylphosphine, Et₃N, CH₃CN, reflux 10 h; b) H₂ (60 psig), [Rh(1,5-COD)(*R,R*-DIPAMP)]BF₄, 60 °C, 12 h; c) 12 mol/l HCl conducted under reflux conditions for 6 h.

Chart 1. Synthetic Procedure for Phenylalanine Analogues

* To whom correspondence should be addressed. e-mail: okada@pharm.kobegakuin.ac.jp

Aldrich Co., St. Louis, U.S.A.), 2,6-dimethylbromobenzene (**1b**; Wako Pure Chemical Industries, Ltd., Osaka, Japan), and 3,5-dimethyliodobenzene (**1f**; Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan) are commercially available. 2-Ethyl-6-methyliodobenzene (**1c**), 2-isopropyl-6-methyliodobenzene (**1d**), and 2,4,6-trimethyliodobenzene (**1e**) were prepared from 2-ethyl-6-methylaniline, 2-isopropyl-6-methylaniline, and 2,4,6-trimethylaniline, respectively, by diazotization of the aniline followed by replacement with iodide. [Rh(1,5-COD)(*R,R*-DIPAMP)]BF₄ was purchased from Strem Chemicals (U.S.A.).

The Heck reaction was employed to produce (*Z*)-dehydroamino acid derivatives (**2a–f**).¹⁹ In this reaction, more than 95% *Z*-isomer over the corresponding *E*-isomer was constructed through a complex between substrate and catalyst. Addition of hexane to the condensed reaction mixture precipitated the desired pure *Z*-isomer. In order to establish the stereochemistry about the olefinic bond of **2a–f**, the proton coupled ¹³C-NMR spectra were examined. The magnitude of the long range coupling constants (*J*_{CH}) between the vinyl proton and the carbonyl carbon on the acrylic ester moiety of **2a–f** ranging from 3.8–4.3 Hz²⁰ are consistent with the desired *Z*-configuration.¹⁹ The *Z*-configuration of **2c** was also confirmed by X-ray analysis (Fig. 1). The Heck reaction of **1a** and **1c–f** gave dehydroamino acids **2a** and **2c–f**, respectively, with over 60% yields. It was reported that aryl bromide is a good substrate in the Heck reaction,²¹ but in this experimental protocol, the Heck reaction with 2,6-dimethylbromobenzene (**1b**) only gave **2b** in low yield (38%), probably due to the rapid deactivation of the catalyst which

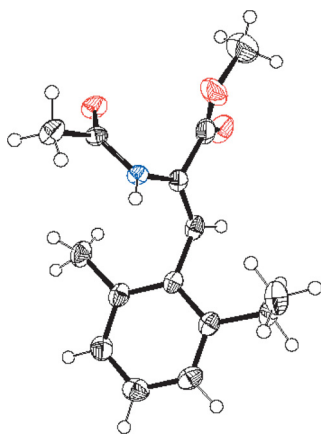


Fig. 1. X-Ray Structure of Molecule **2c**

The nitrogen and oxygen atoms are shown with blue and red colors, respectively.

precipitated out from the homogeneous solution under reflux conditions (60 min). The dehydroamino acids were then reduced with [Rh(1,5-COD)(*R,R*-DIPAMP)]BF₄ mediated asymmetric catalytic hydrogenation to give the protected amino acids **3a–f**. The protecting groups of **3a–f** were removed by concentrated HCl to yield the final amino acid hydrochloride salts **4a–f**. Racemic phenylalanine analogues were also prepared through the reduction of dehydroamino acids **2a–f** with Pd/C catalyzed hydrogenation followed by deprotection with concentrated HCl. The *D* and *L* configurations of the phenylalanine analogues were determined by digestion of the racemic phenylalanine analogues using *L*-amino acid oxidase according to a method described by Toth *et al.*²² After digestion of each phenylalanine analogue, the isomer which exhibited longer retention time in a Chiralpak WH column disappeared, and the one with shorter retention time remained intact. This result demonstrated that the isomer with the longer retention time was the *L*-configuration, and the other was the *D*-antipode. The retention times and the enantiomeric excesses of **3a–f** determined by Chiralcel OD-H column, as well as the retention times and the enantiomeric excesses of **4a–f** determined by Chiralpak WH column are summarized in Tables 1 and 2, respectively; the *L*-form of **4c** was also confirmed by X-ray analysis. As shown in Tables 1 and 2, [Rh(1,5-COD)(*R,R*-DIPAMP)] is an excellent catalyst for preparing **4b** (Dmp), **4c** (Emp), **4d** (Imp), and **4e** (Tmp), which bear alkyl groups at the 2' and 6' positions, while it is moderately effective for **4a** (Mmp), with a single alkyl group at the 2' or 6' position, and poor for the synthesis of **4f** (^{3,5}Dmp), with its absence of alkyl groups at the 2' and 6' positions. Studies indicated that the *re* face of (*Z*)- α -acylaminocinnamate interacts with [Rh(1,5-COD)(*R,R*-DIPAMP)]BF₄ producing a *L*-configuration prod-

Table 1. Retention Times and the Enantiomeric Excesses of Compounds **3a–f**^{a,b}

Compd.	Yield (%)	<i>t</i> _R (<i>D</i> -isomer, min)	<i>t</i> _R (<i>L</i> -isomer, min)	ee (%)
3a Mmp	97.5	10.96	12.86	89.1
3b Dmp	94.0	9.97	12.19	100
3c Emp	94.6	10.49	11.27	100
3d Imp	93.7	8.74	9.49	98.3
3e Tmp	95.7	9.95	11.39	100
3f ^{3,5} Dmp	87.8	ND ^c	ND	ND

^a) Enantiomeric excesses were determined with Chiralcel OD-H column (4.6 mm×250 mm), and the products were eluted with hexane: *i*-PrOH=4:1 containing 0.1% TFA at a flow rate of 0.5 ml/min. ^b) The main peaks which have longer retention time are tentatively assigned as *L*-isomers. ^c) *DL*-**3f** cannot be separated by this condition.

Table 2. Retention Times and Enantiomeric Excesses of the Analogues **4a–f**^a

Compd.	<i>t</i> _R (<i>D</i> -isomer, min)	<i>t</i> _R (<i>L</i> -isomer, min)	Before recrystallization		After recrystallization	
			Yield (%)	ee (%)	Yield (%) ^b	ee (%)
4a Mmp	16.73	25.03	100	87.3	79.0	96.6
4b Dmp	21.37	25.48	95.2	97.0		
4c Emp	23.30	28.21	87.6	95.0	86.2	>99
4d Imp	29.03	32.10	88.5	>99		
4e Tmp	23.95	29.84	94.6	>99		
4f ^{3,5} Dmp	18.51	57.40	90.7	76.4	35.7 ^c	90.0

^a) Enantiomeric excesses were determined with Chiralpak WH column (4.6 mm×250 mm), and the products were eluted with H₂O: MeOH=4:1 containing 1 mmol/l CuSO₄ at 50 °C at a flow rate of 1.5 ml/min. ^b) Refers to the recovery of recrystallized product. ^c) Refers to the sum of two batches of crystals that have ee over 90.0%.

uct, in contrast, when its *si* face interacts with the catalyst a D-configurational product results.²³ A molecular model of the catalyst²³ shows that the edge-exposed phenyl ring of the catalyst prevents the substrate from closely approaching the metal with its *si* face, such that any group which increases the steric hindrance in the phenyl ring of the catalyst or in the phenyl ring in the substrate will increase enantiomeric selectivity. From these results, we can deduce that the alkyl groups at the 2' and 6' positions (**4b–e**) are more effective in improving enantioselectivity than only one group at the 2' or 6' position (**4a**), or two groups at the 3' and 5' positions (**4f**). Comparing with the ee data in the Tables 1 and 2, we find that partial racemization occurred in **4a–c**, and with high probability in **4f**, during the deprotection process by using concentrated HCl, which was also observed in the deacylation for other amino acids.²⁴

In summary, we synthesized a phenylalanine library with various alkyl groups on the aromatic ring through enantioselective catalytic hydrogenation reaction to yield high enantiomeric purity. Following the Heck reaction, (*Z*)-dehydroamino acids were obtained and their stereostructures supported by NMR studies and X-ray analysis. L-Configuration of the final phenylalanine derivatives was confirmed by enzymatic digestion and X-ray analysis. The study on the structure–activity relationship of these phenylalanine analogues by incorporation of these unnatural amino acids to the third position of endomorphin-2 (H-Tyr-Pro-Phe-Phe-NH₂) is under progress.

Experimental

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_f*, *R_f*₂, *R_f*₃, and *R_f*₄ values refer to hexane, AcOEt:hexane (2:1), *n*-BuOH:AcOH:Py:H₂O (4:1:1:2), and *n*-BuOH:AcOH:H₂O (4:1:5, upper layer), respectively. $[\alpha]_D$ values were measured with a DIP-1000 automatic polarimeter (Japan Spectroscopic Co.) and are given in 10⁻¹ deg cm² g⁻¹. Analytical RP-HPLC was performed with a Waters Delta 600 with a COSMOSIL C18 column (4.6 mm×250 mm). The solvents for analytical HPLC are: A, 0.05% TFA 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; the retention time was reported as *t_R* (min). The enantiomeric ratio was determined by using a Chiralcel OD-H column (Daisel Chemical Industries, Ltd., 4.6 mm×250 mm) or a Chiralpak WH column (Daisel Chemical Industries, Ltd., 4.6 mm×250 mm). ¹H- (400 MHz) and ¹³C- (100 MHz) NMR spectra except **2a–f** were recorded on a Bruker DPX-400 spectrometer at 25 °C. For **2a–f**, ¹H- (500 MHz) and ¹³C- (125 MHz) NMR spectra were recorded on a Varian unity INOVA500 spectrometer at 25 °C. Chemical shift values are expressed as ppm downfield from tetramethylsilane. Elemental analyses were performed at the Laboratory for Organic Elemental Microanalysis, Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan.

2-Ethyl-6-methyliodobenzene (1c) To a suspension of 2-ethyl-6-methylaniline (14.9 g, 110 mmol) in conc. HCl (50 ml) and 30 g of ice, a solution of NaNO₂ (7.95 g, 115 mmol) in H₂O (35 ml) was added dropwise over 30 min at 0 to 5 °C. The solution was stirred for another 30 min at the same temperature. Then, a solution of KI (24.9 g, 150 mmol) in H₂O (35 ml) was added dropwise over 20 min at 0 to 5 °C. The reaction mixture was allowed to warm to room temperature during which time an exothermic reaction occurred with gas evolution. The resulting red colored solution was stirred for 18 h. The mixture was extracted with ethyl acetate (AcOEt, 100 ml×3). The combined extracts were washed with 15% sodium thiosulfate solution (80 ml×2), brine solution and dried over MgSO₄. After removal of MgSO₄, the solvent was removed under vacuum. The colored residue was purified by silica gel flash chromatography to give pure 2-ethyl-6-methyliodobenzene (**1c**). Yield 14.0 g (51.8%), colorless oil, *R_f*₁=0.77. *Anal.* Calcd for C₉H₁₁I: C, 43.9; H, 4.51; I, 51.6. Found: C, 44.1; H, 4.43; I, 51.5. ¹H-NMR (CDCl₃, 400 MHz) δ: 1.23 (3H, t, *J*=7.5 Hz), 2.49 (3H, s), 2.81 (2H, q, *J*=7.5 Hz), 7.05–7.10 (2H, m), 7.15 (1H, t, *J*=7.4 Hz).

2-Isopropyl-6-methyliodobenzene (1d) Title compound was prepared from 2-isopropyl-6-methylaniline (14.9 g, 110 mmol) using the same method described for the synthesis of 2-ethyl-6-methyliodobenzene (**1c**). Yield 7.90 g (27.6%), yellowish oil, *R_f*₁=0.77. *Anal.* Calcd for C₁₀H₁₃I: C, 46.2; H, 5.04; I, 48.8. Found: C, 46.1; H, 4.80; I, 49.0. ¹H-NMR (CDCl₃, 400 MHz) δ: 1.23 (6H, d, *J*=6.8 Hz), 2.49 (3H, s), 3.37 (1H, hept, *J*=6.8 Hz), 7.00–7.20 (3H, m).

2,4,6-Trimethyliodobenzene (1e) Title compound was prepared from 2,4,6-trimethylaniline (14.9 g, 110 mmol) using the same method described for the synthesis of 2-ethyl-6-methyliodobenzene (**1c**). Yield 15.2 g (56.3%), colorless oil, *R_f*₁=0.77. *Anal.* Calcd for C₉H₁₁I: C, 43.9; H, 4.51; I, 51.6. Found: C, 44.0; H, 4.40; I, 51.8. ¹H-NMR (CDCl₃, 400 MHz) δ: 2.23 (3H, s), 2.42 (6H, s), 6.87 (2H, s).

General Procedure for Synthesis of Methyl (Z)-2-Acetamido-3-(alkylphenyl)-2-propenoate (2a–f) A mixture of aryl halide (34.9 mmol), methyl-2-acetamidoacrylate (34.9 mmol), tri-*o*-tolylphosphine (1.85 mmol), triethylamine (69.9 mmol) and Pd(OAc)₂ (0.65 mmol) in CH₃CN (50 ml) was subjected to reflux conditions for 10 h. The mixture was cooled to room temperature and filtered. The solvent was removed *in vacuo* and the residue was diluted with water (50 ml). The aqueous phase was extracted with AcOEt (100 ml×3). The combined organic phase was washed with saturated NaCl solution (50 ml×3), dried (Na₂SO₄) and treated with Florisil® (10 g) overnight.

Methyl (Z)-2-Acetamido-3-(2'-methylphenyl)-2-propenoate (2a) Starting from 2-methyliodobenzene (7.62 g, 34.9 mmol), after the reaction, workup, and removal of Florisil® by filtration, the filtrate was concentrated to about 60 ml. To this solution, hexane (80 ml) was added. The crystals appeared were collected by filtration and dried *in vacuo*, yield 5.35 g. Another 770 mg of the product was obtained from the filtrate by flash chromatography (SiO₂, AcOEt). Total yield 6.12 g (75.0%), mp 129–130 °C, *R_f*₂=0.44. *Anal.* Calcd for C₁₃H₁₅NO₃: C, 66.9; H, 6.48; N, 6.00. Found: C, 67.0; H, 6.51; N, 5.96. ¹H-NMR (500 MHz, CDCl₃) δ: 2.03 (3H, s), 2.33 (3H, s), 3.86 (3H, s), 6.85 (1H, s), 7.13–7.40 (5H, m). ¹³C-NMR (125 MHz, CDCl₃) δ: 19.9 (2'-CH₃), 23.0 (2-NHCOCH₃), 52.6 (1-OCH₃), 125.7 (4'-C), 125.9 (2-C), 127.8 (6'-C), 128.9 (5'-C), 129.9 (3-C), 130.3 (3'-C), 132.9 (1'-C), 137.2 (2'-C), 165.5 (2-NHCO), 168.8 (1-CO).

Methyl (Z)-2-Acetamido-3-(2',6'-dimethylphenyl)-2-propenoate (2b) Starting from 2,6-dimethylbromobenzene (15.5 g, 84.0 mmol), after the reaction, workup, and removal of Florisil® by filtration, the filtrate was concentrated to about 60 ml. To this solution, hexane (80 ml) was added. The crystals appeared were collected by filtration and dried *in vacuo*, yield 5.08 g. Another 1.50 g of the product was obtained from the filtrate by flash chromatography (SiO₂, AcOEt:hexane=1:1). Total yield 6.58 g (38.0%), mp 131–132 °C, *R_f*₂=0.52. *Anal.* Calcd for C₁₄H₁₇NO₃: C, 68.0; H, 6.93; N, 5.66. Found: C, 68.3; H, 6.94; N, 5.61. ¹H-NMR (500 MHz, CDCl₃) δ: 1.92 (3H, s), 2.21 (6H, s), 3.88 (3H, s), 6.62 (1H, br), 7.05 (1H, s), 7.07 (1H, s), 7.12–7.18 (2H, m). ¹³C-NMR (125 MHz, CDCl₃) δ: 20.1 (2',6'-CH₃), 22.8 (2-NHCOCH₃), 52.5 (1-OCH₃), 127.5 (3',5'-C), 128.0 (4'-C), 128.3 (2-C), 128.5 (3-C), 132.2 (1'-C), 136.0 (2',6'-C), 164.8 (1-CO), 168.3 (2-NHCO).

Methyl (Z)-2-Acetamido-3-(2'-ethyl-6'-methylphenyl)-2-propenoate (2c) Starting from 2-ethyl-6-methyliodobenzene (8.60 g, 34.9 mmol), after the reaction, workup, and removal of Florisil® by filtration, the filtrate was concentrated to about 50 ml. To this solution, hexane (50 ml) was added. The crystals were collected by filtration and dried *in vacuo*, yield 6.27 g. Filtrate was concentrated to about 20 ml, and hexane (20 ml) was added, and crystals (500 mg) were collected by filtration. Total yield 6.77 g (74.1%), mp 147–148 °C, *R_f*₂=0.56. *Anal.* Calcd for C₁₅H₁₉NO₃: C, 68.9; H, 7.33; N, 5.36. Found: C, 68.8; H, 7.34; N, 5.41. ¹H-NMR (500 MHz, CDCl₃) δ: 1.15 (3H, t, *J*=7.6 Hz), 1.95 (3H, s), 2.20 (3H, s), 2.53 (2H, q, *J*=7.6 Hz), 3.88 (3H, s), 6.49 (1H, s), 7.00–7.20 (4H, m). ¹³C-NMR (125 MHz, CDCl₃) δ: 14.9 (2'-CH₂CH₃), 20.1 (6'-CH₃), 22.8 (2-NHCOCH₃), 26.5 (2'-CH₂CH₃), 52.6 (1-OCH₃), 125.7 (3'-C), 127.7 (5'-C), 127.8 (3-C), 128.3 (4'-C), 128.8 (2-C), 131.4 (1'-C), 136.0 (6'-C), 142.2 (2'-C), 164.8 (1-CO), 168.5 (2-NHCO).

Methyl (Z)-2-Acetamido-3-(2'-isopropyl-6'-methylphenyl)-2-propenoate (2d) Starting from 2-isopropyl-6-methyliodobenzene (7.80 g, 30.0 mmol), after the reaction, workup, and removal of Florisil® by filtration, the filtrate was concentrated to about 30 ml. To this solution, hexane (70 ml) was added. The crystals appeared were collected by filtration and dried *in vacuo*. Yield 5.60 g (67.9%), mp 149–150 °C, *R_f*₂=0.62. *Anal.* Calcd for C₁₆H₂₁NO₃: C, 69.8; H, 7.69; N, 5.09. Found: C, 69.8; H, 7.64; N, 5.19. ¹H-NMR (500 MHz, CDCl₃) δ: 1.16 (6H, d, *J*=6.8 Hz), 1.95 (3H, s), 2.20 (3H, s), 2.95 (1H, hept, *J*=6.8 Hz), 3.88 (3H, s), 6.44 (1H, br), 7.05–7.26 (4H,

m). ^{13}C -NMR (125 MHz, CDCl_3) δ : 20.2 (6'- CH_3), 22.8 (2- NHCOCH_3), 23.7 (2'- $\text{CH}(\text{CH}_3)_2$), 30.3 (2'- $\text{CH}(\text{CH}_3)_2$), 52.6 (1- OCH_3), 122.8 (3'-C), 127.6 (5'-C), 127.8 (3-C), 128.5 (4'-C), 129.0 (2-C), 130.6 (1'-C), 135.8 (6'-C), 146.9 (2'-C), 164.7 (1-C), 168.3 (2- NHCO).

Methyl (Z)-2-Acetamido-3-(2',4',6'-trimethylphenyl)-2-propenoate (2e) Starting from 2,4,6-trimethyliodobenzene (8.60 g, 34.9 mmol), after the reaction, workup, and removal of Florisil[®] by filtration, the filtrate was concentrated to about 60 ml. To this solution, hexane (50 ml) was added. The crystals were collected by filtration and dried *in vacuo*, yield 4.59 g. Another 1.90 g of the product was obtained from the filtrate by flash chromatography (SiO_2 , AcOEt:hexane=2:1). Total yield 6.45 g (70.6%), mp 165–166 °C, R_f =0.57. Anal. Calcd for $\text{C}_{15}\text{H}_{19}\text{NO}_3$: C, 68.9; H, 7.33; N, 5.36. Found: C, 69.0; H, 7.26; N, 5.35. ^1H -NMR (500 MHz, CDCl_3) δ : 1.95 (3H, s), 2.17 (6H, s), 2.28 (3H, s), 3.87 (3H, s), 6.57 (1H, s), 6.89 (2H, s), 7.13 (1H, br). ^{13}C -NMR (125 MHz, CDCl_3) δ : 20.0 (2',6'- CH_3), 20.9 (4'- CH_3), 22.8 (2- NHCOCH_3), 52.5 (1- OCH_3), 128.2 (2-C), 128.4 (3',5'-C), 128.6 (3-C), 129.2 (1'-C), 135.9 (2',6'-C), 137.7 (4'-C), 164.9 (1-C), 168.2 (2- NHCO).

Methyl (Z)-2-Acetamido-3-(3',5'-dimethylphenyl)-2-propenoate (2f) Starting from 3,5-dimethyliodobenzene (5.00 g, 21.6 mmol), after the reaction, workup, and removal of Florisil[®] by filtration, the filtrate was concentrated to about 50 ml. To this solution, hexane (50 ml) was added. The crystals appeared were collected by filtration and dried *in vacuo*, yield 2.96 g. Another 370 mg of the product was obtained from the filtrate by flash chromatography (SiO_2 , EtOAc:hexane=2:1). Total yield 3.30 g (62.1%), mp 139–139 °C, R_f =0.45. Anal. Calcd for $\text{C}_{14}\text{H}_{17}\text{NO}_3$: C, 68.0; H, 6.93; N, 5.66. Found: C, 67.9; H, 6.94; N, 5.68. ^1H -NMR (500 MHz, CDCl_3) δ : 2.13 (3H, s), 2.31 (6H, s), 3.84 (3H, s), 6.88 (1H, s), 6.96 (1H, s), 7.08 (2H, s), 7.31 (1H, s). ^{13}C -NMR (125 MHz, CDCl_3) δ : 21.2 (2- NHCOCH_3), 23.2 (3',5'- CH_3), 52.5 (1- OCH_3), 124.13, 133.44 (2-C, 1'-C), 127.4 (2',6'-C), 131.2 (4'-C), 132.8 (3-C), 138.0 (3',5'-C), 165.8 (1-CO), 168.9 (2- NHCO).

General Procedure for Synthesis of N-Acetylalkylphenylalanine Methyl Ester (3a–f) An N_2 -purged reaction vessel was charged with a slurry of $[\text{Rh}(1,5\text{-COD})(R,R\text{-DIPAMP})]\text{BF}_4$ (0.46 mmol), methyl (Z)-2-acetamido-3-(alkylphenyl)-2-propenoate (25.7 mmol) and AcOEt (60 ml). The vessel was repeatedly aspirated to boiling of the AcOEt and pressurized to 60 psig with H_2 (five times). The last reaction mixture was pressurized to 60 psig with H_2 and stirred vigorously at 60 °C for 12 h. The mixture was cooled to room temperature, vented with N_2 , filtered, and the filtrate was treated with Florisil[®] (12 g) overnight. The mixture was filtered, and the filtrate was concentrated, and the product was precipitated with hexane.

N-Acetyl-2'-methyl-L-phenylalanine Methyl Ester (3a) Yield 5.85 g (97.5%), oil, $[\alpha]_D^{25}$ -3.06° ($c=0.53$, MeOH), R_f =0.32. Anal. Calcd for $\text{C}_{13}\text{H}_{17}\text{NO}_3$: C, 66.4; H, 7.28; N, 5.95. Found: C, 66.5; H, 7.31; N, 6.24. ^1H -NMR (400 MHz, CDCl_3) δ : 1.96 (3H, s), 2.24 (3H, s), 3.04 (1H, dd, $J=14.1$, 6.9 Hz), 3.15 (1H, dd, $J=14.1$, 6.8 Hz), 3.68 (3H, s), 4.86 (1H, q, $J=8.0$ Hz), 5.91 (1H, d, $J=7.3$ Hz), 6.97–7.04 (1H, m), 7.10–7.20 (3H, m).

N-Acetyl-2',6'-dimethyl-L-phenylalanine Methyl Ester (3b) Yield 5.62 g (94.0%), mp 127–128 °C, $[\alpha]_D^{25}$ $+4.09^\circ$ ($c=0.55$, MeOH), R_f =0.43. Anal. Calcd for $\text{C}_{14}\text{H}_{19}\text{NO}_3$: C, 67.5; H, 7.68; N, 5.62. Found: C, 67.2; H, 7.54; N, 5.63. ^1H -NMR (400 MHz, CDCl_3) δ : 1.96 (3H, s), 2.34 (6H, s), 3.08 (1H, dd, $J=14.0$, 8.2 Hz), 3.13 (1H, dd, $J=14.0$, 7.7 Hz), 3.61 (3H, s), 4.82 (1H, q, $J=8.0$ Hz), 6.05 (1H, d, $J=7.4$ Hz), 6.90–7.10 (3H, m).

N-Acetyl-2'-ethyl-6'-methyl-L-phenylalanine Methyl Ester (3c) Yield 5.68 g (94.6%), mp 94–95 °C, $[\alpha]_D^{25}$ $+8.37^\circ$ ($c=0.57$, MeOH), R_f =0.44. Anal. Calcd for $\text{C}_{15}\text{H}_{21}\text{NO}_3$: C, 68.4; H, 8.04; N, 5.32. Found: C, 68.2; H, 8.07; N, 5.17. ^1H -NMR (400 MHz, CDCl_3) δ : 1.20 (3H, t, $J=7.6$ Hz), 1.96 (3H, s), 2.35 (3H, s), 2.67 (2H, q, $J=7.5$ Hz), 3.08 (1H, dd, $J=14.0$, 7.6 Hz), 3.15 (1H, dd, $J=14.0$, 7.6 Hz), 3.60 (3H, s), 4.79 (1H, q, $J=7.9$ Hz), 6.02 (1H, d, $J=7.2$ Hz), 6.98–7.12 (3H, m).

N-Acetyl-2'-isopropyl-6'-methyl-L-phenylalanine Methyl Ester (3d) Yield 5.00 g (93.7%), mp 97–98 °C, $[\alpha]_D^{25}$ $+3.96^\circ$ ($c=0.55$, MeOH), R_f =0.46. Anal. Calcd for $\text{C}_{16}\text{H}_{23}\text{NO}_3$: C, 69.3; H, 8.36; N, 5.05. Found: C, 68.9; H, 8.32; N, 4.97. ^1H -NMR (400 MHz, CDCl_3) δ : 1.22 (3H, d, $J=7.0$ Hz), 1.24 (3H, d, $J=6.8$ Hz), 1.96 (3H, s), 2.34 (3H, s), 3.07–3.28 (3H, m), 3.61 (3H, s), 4.77 (1H, q, $J=8.0$ Hz), 5.98 (1H, d, $J=7.4$ Hz), 6.93–7.00 (1H, m), 7.12–7.14 (2H, m).

N-Acetyl-2',4',6'-trimethyl-L-phenylalanine Methyl Ester (3e) Yield 5.78 g (95.7%), mp 141–142 °C, $[\alpha]_D^{25}$ $+12.2^\circ$ ($c=0.55$, MeOH), R_f =0.37. Anal. Calcd for $\text{C}_{15}\text{H}_{19}\text{NO}_3$: C, 68.4; H, 8.04; N, 5.32. Found: C, 68.0; H, 7.98; N, 5.20. ^1H -NMR (400 MHz, CDCl_3) δ : 1.95 (3H, s), 2.24 (3H, s), 2.30 (6H, s), 3.00–3.12 (2H, m), 3.63 (3H, s), 4.78 (1H, q, $J=7.9$ Hz), 5.98 (1H, d, $J=7.5$ Hz), 6.83 (2H, s).

N-Acetyl-3',5'-dimethyl-L-phenylalanine Methyl Ester (3f) Yield

2.81 g (87.8%), mp 72–74 °C, $[\alpha]_D^{25}$ $+15.7^\circ$ ($c=0.54$, MeOH), R_f =0.36. Anal. Calcd for $\text{C}_{14}\text{H}_{19}\text{NO}_3$: C, 67.5; H, 7.68; N, 5.62. Found: C, 67.3; H, 7.69; N, 5.54. ^1H -NMR (400 MHz, CDCl_3) δ : 1.99 (3H, s), 2.27 (6H, s), 3.00 (1H, dd, $J=13.8$, 5.9 Hz), 3.06 (1H, dd, $J=13.8$, 5.9 Hz), 3.73 (s, 3H), 4.84 (1H, q, $J=7.8$ Hz), 5.87 (1H, d, $J=7.2$ Hz), 6.70 (2H, s), 6.88 (1H, s).

2'-Methyl-L-phenylalanine Hydrochloride (4a) N-Acetyl-2-methyl-L-phenylalanine methyl ester (3a, 4.87 g, 20.7 mmol) in concentrated HCl (27.6 ml, 331 mmol) was subjected to reflux conditions for 6 h. After the temperature of the solution was automatically decreased to room temperature, the solution became a solid mass. The solid was dried directly *in vacuo*, yield 4.84 g (100%); ee 87.3%. The optically crude sample (4.00 g) was dissolved in MeOH (10 ml), Et₂O (35 ml) was added to precipitate the product. After 1 h, the crystals were collected by filtration and dried *in vacuo*. Yield 3.16 g (79.0%), mp 226–227 °C, $[\alpha]_D^{25}$ -3.63° ($c=1.0$, H₂O), ee 96.6%, R_f =0.54, R_f =0.29, t_R =12.45 min. Anal. Calcd for $\text{C}_{10}\text{H}_{13}\text{NO}_2\cdot\text{HCl}$: C, 55.7; H, 6.54; N, 6.49. Found: C, 55.5; H, 6.40; N, 6.45. ^1H -NMR (400 MHz, DMSO-*d*₆) δ : 2.30 (3H, s), 3.03–3.20 (2H, m), 3.99 (1H, br), 7.10–7.23 (4H, m), 8.57 (3H, br), 13.73 (1H, br).

2',6'-Dimethyl-L-phenylalanine Hydrochloride (4b) N-Acetyl-2,6-dimethyl-L-phenylalanine methyl ester (3b, 5.50 g, 22.0 mmol) in concentrated HCl (30 ml, 360 mmol) was subjected to reflux conditions for 6 h. After cooling to room temperature, the solvent was removed *in vacuo* to afford 4b as a white solid. Yield 5.19 g (95.2%), mp 251–253 °C, $[\alpha]_D^{25}$ $+59.7^\circ$ ($c=0.5$, MeOH), ee 97.0%, R_f =0.45, R_f =0.29, t_R =12.78 min. Anal. Calcd for $\text{C}_{11}\text{H}_{15}\text{NO}_2\cdot\text{HCl}\cdot0.5\text{H}_2\text{O}$: C, 55.4; H, 7.18; N, 5.87. Found: C, 55.2; H, 7.14; N, 5.84. ^1H -NMR (400 MHz, CD_3OD) δ : 2.36 (6H, s), 3.17 (1H, dd, $J=14.4$, 7.8 Hz), 3.39 (1H, dd, $J=14.4$, 8.2 Hz), 4.07 (1H, t, $J=8.0$ Hz), 7.00–7.10 (3H, m).

2'-Ethyl-6'-methyl-L-phenylalanine Hydrochloride (4c) N-Acetyl-2-methyl-6-ethyl-L-phenylalanine methyl ester (3c, 5.60 g, 21.3 mmol) in concentrated HCl (28 ml, 340 mmol) was subjected to reflux conditions for 6 h. The temperature of the solution was automatically decreased to room temperature, and the crystals were collected by filtration and dried *in vacuo*, yield 4.88 g (87.6%), ee 95.0%. The optically crude product (4.70 g) was recrystallized from 95 ml of hot hydrochloric acid solution (5 mol/l) to give optically pure 4c 4.05 g (86.2%), mp 233–235 °C, $[\alpha]_D^{25}$ $+62.5^\circ$ ($c=1.0$, H₂O), ee >99%, R_f =0.57, R_f =0.35, t_R =12.89 min. Anal. Calcd for $\text{C}_{12}\text{H}_{17}\text{NO}_2\cdot\text{HCl}\cdot0.1\text{H}_2\text{O}$: C, 58.7; H, 7.47; N, 5.70. Found: C, 58.6; H, 7.40; N, 5.70. ^1H -NMR (400 MHz, DMSO-*d*₆) δ : 1.12 (3H, t, $J=7.5$ Hz), 2.29 (3H, s), 2.55–2.68 (2H, m), 3.12–3.25 (2H, m), 3.79 (1H, dd, $J=9.9$, 6.2 Hz), 6.95–7.03 (2H, m), 7.06–7.11 (1H, m), 8.74 (3H, br), 13.49 (1H, br).

2-Isopropyl-6-methyl-L-phenylalanine Hydrochloride (4d) N-Acetyl-2-isopropyl-6-methyl-L-phenylalanine methyl ester (3d, 4.41 g, 15.9 mmol) in concentrated HCl (21 ml, 252 mmol) was subjected to reflux conditions for 6 h. The temperature of the solution was automatically decreased to room temperature, and the crystals were collected by filtration and dried *in vacuo*. Yield 3.88 g (88.5%), mp 192–194 °C, $[\alpha]_D^{25}$ $+56.7^\circ$ ($c=0.94$, H₂O), ee >99%, R_f =0.58, R_f =0.39, t_R =14.00 min. Anal. Calcd for $\text{C}_{13}\text{H}_{19}\text{NO}_2\cdot\text{HCl}\cdot\text{H}_2\text{O}$: C, 56.6; H, 8.04; N, 5.08. Found: C, 56.4; H, 7.89; N, 5.08. ^1H -NMR (400 MHz, DMSO-*d*₆) δ : 1.15 (6H, t, $J=6.9$ Hz), 2.29 (3H, s), 3.10–3.27 (3H, m), 3.70–3.80 (1H, m), 6.94–7.00 (1H, m), 7.09–7.15 (2H, m), 8.76 (3H, br), 13.42 (1H, br).

2',4',6'-Trimethyl-L-phenylalanine Hydrochloride (4e) N-Acetyl-2,4,6-trimethyl-L-phenylalanine methyl ester (3e, 4.66 g, 17.7 mmol) in concentrated HCl (23.6 ml, 283 mmol) was heated and subjected to reflux conditions. After 30 min, the solution turned into a gel, at which time HCl (6 mol/l, 50 ml) was added, and reflux conditions were continued for total 6 h. The temperature of the solution was automatically decreased to room temperature, and the resulting crystals were collected by filtration and dried *in vacuo*. Yield 4.38 g (94.6%), mp 270–275 °C (dec.), $[\alpha]_D^{25}$ $+64.6^\circ$ ($c=0.50$, MeOH), ee >99%, R_f =0.56, R_f =0.35, t_R =13.24 min. Anal. Calcd for $\text{C}_{12}\text{H}_{17}\text{NO}_2\cdot\text{HCl}\cdot0.5\text{H}_2\text{O}$: C, 57.0; H, 7.58; N, 5.54. Found: C, 57.0; H, 7.52; N, 5.57. ^1H -NMR (400 MHz, DMSO-*d*₆) δ : 2.19 (3H, s), 2.24 (6H, s), 3.06–3.17 (2H, m), 3.79 (1H, t, $J=8.0$ Hz), 6.81 (2H, s), 8.68 (3H, br), 13.45 (1H, br).

3',5'-Dimethyl-L-phenylalanine Hydrochloride (4f) N-Acetyl-3,5-dimethyl-L-phenylalanine methyl ester (3f, 2.71 g, 10.9 mmol) in concentrated HCl (14.5 ml, 174 mmol) was heated under reflux conditions. After 1 h, crystals precipitated, HCl (6 mol/l, 25 ml) was added, and the reflux conditions were continued for total 6 h. The temperature of the solution was automatically decreased to room temperature, and the resulting crystals were collected by filtration and dried *in vacuo*, yield 2.44 g (90.7%), ee 76.4%. The optically crude compound (2.38 g) was dissolved in MeOH (10 ml), the product was precipitated with Et₂O (10 ml), and the crystals formed were

collected by filtration and dried *in vacuo*, yield 740 mg, ee 90.0%. The filtrate was evaporated to dryness, and the residue was dissolved again in MeOH (10 ml), and acetone (22 ml) was added. The solution was kept at 7 °C overnight. The crystals that appeared were collected by filtration and dried *in vacuo*. Yield 110 mg, mp 212–214 °C, $[\alpha]_D^{25} -13.5^\circ$ ($c=0.48$, H₂O), ee >99%, $R_f=0.57$, $R_f=0.35$, $t_R=12.45$ min. *Anal.* Calcd for C₁₁H₁₅NO₂·HCl·0.33H₂O: C, 56.1; H, 7.13; N, 5.94. Found: C, 56.0; H, 6.97; N, 5.93. ¹H-NMR (400 MHz, DMSO-*d*₆) δ : 2.24 (6H, s), 3.01–3.12 (2H, m), 4.08 (1H, t, $J=6.2$ Hz), 6.88 (2H, s), 6.90 (1H, s), 8.49 (3H, br), 13.73 (1H, br).

General Procedure for Enzymatic Digestion of Phenylalanine Analogues Racemic phenylalanine analogues (0.3 mg) were dissolved in 1 ml of Tris–HCl buffer (0.1 mol/l, pH 7.5), and 0.3 mg of L-amino acid oxidase (*Bothrops atrox* crude dried venom, Sigma) was added. The solutions were incubated for 24 h at 37 °C. After 24 h fresh enzyme was added and the incubation continued for another 24 h. The digestion was stopped by addition of HCl (1 ml, 0.1 mol/l) and further denaturation of the enzyme by heating the solution in boiling water for 3 min. The solutions were filtered, lyophilized, and the residue dissolved in water (300 μ l) for chiral HPLC analysis.

X-Ray Structure Determination The X-ray diffraction data were collected with a Bruker AXS SMART APEX CCD camera using graphite-monochromated MoK α radiation ($\lambda=0.71073$ Å) at 120 K for **2c** and 278 K for **4c**. The crystal structures were solved by a direct method using the SHELXS97 program.²⁵ Atomic scattering factors were taken from International Tables for X-Ray Crystallography.²⁶ Positional parameters of non-H atoms were refined by a full matrix least squares method with anisotropic thermal parameters using the SHELXL97 program.²⁷ The structural data were deposited with the following designations: **2c**: CCDC 288681, **4c**: CCDC 288682. These can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U.K.; fax: (+44)1223-336-033; or deposit@ccdc.cam.ac.uk).

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