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

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Six phenylalanine analogues containing 2'-methyl-, 2',6'-dimethyl-, 2'-ethyl-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 1-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-O-Ala-Phe-Gly-Tyr-Pro-Ser-NH₂), 9) deltorphin II (H-Tyr-O-Ala-Phe-Glu-Val-Val-Gly-NH₂), 9) YrFB (H-Tyr-O-Arg-Phe-BAla-NH₂), 10) and endomorphin-2 (H-Tyr-Pro-Phe-Phe-NH₂). 11) More interestingly, substitution of Tyr 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 (6-Dmp). Until now, 2'-methyl-l-phenylalanine was synthesized through optical resolution with enzymes 13); 2',6'-dimethyl-l-phenylalanine was prepared by separation of Acc-D-Am-Dmp-Arg-OMe in preparative HPLC followed by acid hydrolysis 14) or asymmetric alkylation of glycine equivalent without 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). 15) 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-

![Chart 1. Synthetic Procedure for Phenylalanine Analogues](image-url)

Reagents and conditions: a) methyl acetamidoacrylate, Pd(OAc)₂, tri-o-tolyolphosphine, Et₃N, CH₂CN, 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.

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Aldrich Co., St. Louis, U.S.A.), 2,6-dimethylbromobenzene (1b; Wako Pure Chemical Industries, Ltd., Osaka, Japan), and 3,5-dimethylidendobenzene (1f; Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan) are commercially available. 2-Ethyl-6-methylidendobenzene (1c), 2-isopropyl-6-methylidendobenzene (1d), and 2,4,6-trimethylidendobenzene (1e) were prepared from 2-ethyl-6-methylnitoline, 2-isopropyl-6-methylnitoline, and 2,4,6-trimethylnitoline, 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)-dehydiamino acid derivatives (2a—f). 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 (JZ) between the vinyl proton and the carbonyl carbon on the acrylic ester ranging from 3.8—4.3 Hz are consistent with the desired Z-configuration. The Z-configuration of 2c was also confirmed by X-ray analysis (Fig. 1). The Heck reaction of 1a and 1c—f gave dehydiamino acids 2a and 2c—f, respectively, with over 60% yields. It was reported that allyl bromide is a good substrate in the Heck reaction, 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 precipitated out from the homogeneous solution under reflux conditions (60 min). The dehydiamino 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 dehydiamino 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. 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 ODH 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-configuration of 4a—f 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-acetylaminoinnamate interacts with [Rh(1,5-COD)(R,R-DIPAMP)]BF₄ producing a l-configuration product.

**Table 1. Retention Times and the Enantiomeric Excesses of Compounds 3a—f**

<table>
<thead>
<tr>
<th>Compd.</th>
<th>Yield (%)</th>
<th>tₖ (Z-isomer, min)</th>
<th>tₖ (L-isomer, min)</th>
<th>ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>97.5</td>
<td>10.96</td>
<td>12.86</td>
<td>89.1</td>
</tr>
<tr>
<td>3b</td>
<td>94.0</td>
<td>9.97</td>
<td>12.19</td>
<td>100</td>
</tr>
<tr>
<td>3c</td>
<td>94.6</td>
<td>10.49</td>
<td>11.27</td>
<td>100</td>
</tr>
<tr>
<td>3d</td>
<td>93.7</td>
<td>8.74</td>
<td>9.49</td>
<td>98.3</td>
</tr>
<tr>
<td>3e</td>
<td>95.7</td>
<td>9.95</td>
<td>11.39</td>
<td>100</td>
</tr>
<tr>
<td>3f</td>
<td>87.8</td>
<td>ND⁹</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

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) n-3f cannot be separated by this condition.

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

<table>
<thead>
<tr>
<th>Compd.</th>
<th>Yield (%)</th>
<th>tₖ (Z-isomer, min)</th>
<th>tₖ (L-isomer, min)</th>
<th>ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>Mmp</td>
<td>16.73</td>
<td>25.03</td>
<td>100</td>
</tr>
<tr>
<td>4b</td>
<td>Dmp</td>
<td>21.37</td>
<td>25.48</td>
<td>95.2</td>
</tr>
<tr>
<td>4c</td>
<td>Emp</td>
<td>23.30</td>
<td>28.21</td>
<td>87.6</td>
</tr>
<tr>
<td>4d</td>
<td>Imp</td>
<td>29.03</td>
<td>32.10</td>
<td>88.5</td>
</tr>
<tr>
<td>4e</td>
<td>Tmp</td>
<td>23.95</td>
<td>29.84</td>
<td>94.6</td>
</tr>
<tr>
<td>4f</td>
<td>3,5-Dmp</td>
<td>18.51</td>
<td>57.40</td>
<td>90.7</td>
</tr>
</tbody>
</table>

Before recrystallization | After recrystallization

<table>
<thead>
<tr>
<th>Yield (%)</th>
<th>ee (%)</th>
<th>Yield (%)</th>
<th>ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>79.0</td>
<td>96.6</td>
<td>96.6</td>
</tr>
<tr>
<td>4b</td>
<td>86.2</td>
<td>&gt;99</td>
<td>&gt;99</td>
</tr>
<tr>
<td>4c</td>
<td>35.7</td>
<td>90.0</td>
<td>90.0</td>
</tr>
</tbody>
</table>

a) Enantiomeric excesses were determined with Chiralcel OD-H 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%.

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

The nitrogen and oxygen atoms are shown with blue and red colors, respectively.
uct, in contrast, when its $s$ face interacts with the catalyst a $d$-configurational product results.23) A molecular model of the catalyst21 shows that the edge-exposed phenyl ring of the catalyst prevents the substrate from closely approaching the metal with its $s$ 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 enantioselectivity. 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 dea-
cyclation for other unnatural 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 enantio-
metric purity. Following the Heck reaction, (Z)-dehydroamino acids were obtained and their stereocenters supported by NMR studies and X-ray analysis. $l$-Configuration of the final phenylalanine derivatives was confirmed by enzy-
matic digestion and X-ray analysis. The study on the struc-
ture–activity relationship of these phenylalanine analogues by incorporation of these unnatural amino acids to the third position of endomorphin-2 (H-Tyr-Pro-Phe-NH$_2$) is under progress.

Experimental

Methyl (2c)-2-Acetamido-3-(2′-isopropyl-6-methylanilino)propenoate (2c)

Starting from methyl-2-acetamidoacrylate (34.9 mmol), tri-o-tolyolphosphate (1.85 mmol), triethylamine (69.9 mmol) and Pd(OAc)$_2$ (0.65 mmol) in CH$_3$CN (10 g) overnight.

$\text{C}_2\text{H}_7\text{I}$, using concentrated HCl, which was also observed in the dea-
chyromatography to give pure 2-ethyl-6-
methyliodobenzene (1e).

1.95 (3H, s), 2.18 (3H, s), 2.55 (2H, t, $J_\text{H2CH}_3$ = 7.5 Hz), 1.95 (3H, s), 2.55 (2H, t, $J_\text{H2CH}_3$ = 7.5 Hz). 13C-NMR (125 MHz, CDCl$_3$) $\delta$: 20.3 (CH), 129.7 (5-C), 129.3 (3-C), 129.2 (1′-C), 157.2 (2-C), 165.2 (NHCO), 168.8 (CO).

Methyl (Z)-2-Acetamido-3-(2′-6′-dimethylphenyl)propenoate (2b)

Starting from 2,6-dimethylbromobenzene (15.5 g, 84.0 mmol), after the re-
action, 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 (SiO$_2$, AcOEt). Total yield 6.12 g (75.0%), mp 129—130°C, $R_f$=0.44.

Anal. Found: C, 68.8; H, 7.34; N, 5.61. $\text{C}_2\text{H}_7\text{I}$-NMR (500 MHz, CDCl$_3$) $\delta$: 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, CDCl$_3$) $\delta$: 19.9 (2′-CH$_2$), 23.0 (2-NHCO$_2$), 52.6 (1-OCH$_3$), 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), 157.2 (2-C), 165.5 (2-NHCO), 168.8 (CO).

Methyl (Z)-(2-Acetamido-3′-(2′-ethyl-6′-methylphenyl))propenoate (2e)

Starting from 2-ethyl-6-methylbromobenzene (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 5.08 g. Another 1.50 g of the product was obtained from the filtrate by flash chromatography (SiO$_2$, AcOEt): hexane=1:1. Total yield 6.58 g (38.0%), mp 131—132°C, $R_f$=0.52. Anal. Calcld for $\text{C}_7\text{H}_8\text{NO}_2$: C, 68.6; H, 6.93; N, 5.66. Found: C, 68.3; H, 6.94; N, 5.61. $\text{C}_2\text{H}_7\text{I}$-NMR (500 MHz, CDCl$_3$) $\delta$: 1.92 (3H, s), 2.21 (6H, s), 3.83 (3H, s), 6.62 (1H, br), 7.05 (1H), 7.07 (1H), 7.12—7.15 (12H, m). 13C-NMR (125 MHz, CDCl$_3$) $\delta$: 20.3 (CH), 129.5 (2-C), 127.8 (6′-C), 128.9 (5′-C), 129.9 (3′-C), 130.3 (3′-C), 132.9 (1′-C), 157.3 (2-C), 165.3 (2-NHCO), 168.8 (CO).

Methyl (Z)-(2-Acetamido-3′-(2′-ethyl-6′-methylphenyl))propenoate (2d)

Starting from 2-ethyl-6-methylbromobenzene (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 6.27 g. Filterate was concentrated to about 20 ml, and hexane (20 ml) was added. The crystals appeared were collected by filtration and dried in vacuo, yield 2.03 g. Another 0.77 g of the product was obtained from the filtrate by flash chromatography (SiO$_2$, AcOEt): hexane=1:1. Total yield 2.80 g (34.0%), mp 147—148°C, $R_f$=0.56. Anal. Calcld for $\text{C}_8\text{H}_{10}\text{NO}_2$: C, 68.9; H, 7.73; N, 5.36. Found: C, 68.8; H, 7.34; N, 5.41. $\text{C}_2\text{H}_7\text{I}$-NMR (500 MHz, CDCl$_3$) $\delta$: 1.15 (3H, t, $J_{\text{HCH}_3}$ = 7.6 Hz), 1.95 (3H, s), 2.20 (3H, s), 2.53 (2H, q, $J_{\text{H2CH}_3}$ = 7.6 Hz), 3.88 (3H, s), 6.49 (1H, s), 7.00—7.20 (4H, m). 13C-NMR (125 MHz, CDCl$_3$) $\delta$: 24.9 (2′-CH$_2$), 22.8 (2-NHCO$_2$), 52.6 (2′-CH$_2$), 22.8 (2-NHCO$_2$). 165.5 (2-NHCO), 168.8 (CO), 168.5 (CO).
**Methyl (2)-2-Acetamido-3-(2',4',6'-trimethylphenyl)-2-propenoate (2e)**

Starting from 2,4,6-trimethylidobenzone (8.0 g, 34.9 mmol), after the reaction, workup, and removal of Florisil® by filtration, the filtrate was concentrated about to 50 ml. To this solution (ca. 50 ml) was added 15 ml of concentrated HCl (30% w/v) and H2O: 1.5 ml. The mixture was automatically decreased to room temperature, the solution became a solid mass. The solid was dried directly in vacuo, yield 4.84 g (70%, ca. 87.3%). The optically pure sample (4.0 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 3.67 g (79%), mp 116—117 °C, 7.98; N, 5.20. 1H-NMR (400 MHz, CDCl3):

\[ \text{Rf} = 0.54, \text{Rf} = 0.29, \text{t} = 12.85 \text{min.} \]

Anal. C15H19NO3: C, 68.9; H, 7.33; N, 5.36. Found: C, 68.4; H, 7.1; N, 5.85. 1H-NMR (400 MHz, CDCl3): δ 1.93 (3H, s), 2.21 (3H, s), 3.42 (3H, s), 3.44 (3H, s), 4.84 (1H, q, J = 7.3 Hz), 6.97—7.04 (1H, m), 7.10—7.20 (3H, m).

2',6'-Dimethyl-1-phenylalanine Hydrochloride (4b)

N-Acetyl-2,6-di-methyl-1-phenylalanine methyl ester (3b, 5.0 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 solution was removed in vacuo to afford 4b (27.6 mm, 33%). Yield 25.7 mmol). The filtrate was flash chromatographed (SiO2, EtOAc:hexane = 1:1). Total yield 3.0 g (62.1%), mp 139—149 °C, Rf = 0.45. Anal. Calcd for C15H19NO3: C, 68.9; H, 7.33; N, 5.36. Found: C, 67.9; H, 6.94; N, 5.64. 1H-NMR (500 MHz, CDCl3): δ 1.23 (3H, s), 2.31 (6H, s), 3.64 (2H, s), 3.96 (2H, s), 7.31 (1H, s). 1C-NMR (125 MHz, CDCl3): δ 21.2 (2-CH2-CH2-), 23.2 (3',5'-CH2-), 52.5 (1-OCH3), 128.2 (2'-C), 128.4 (3'-C'), 129.6 (3C), 135.9 (2'-C'), 137.7 (4'-C), 164.9 (1'-C), 168.2 (2-NCHO). Anal.

2',6'-Dimethyl-1-phenylalanine Hydrochloride (4c)

N-Acetyl-2-ethyl-6-methyl-1-phenylalanine methyl ester (3c, 5.6 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%), ca. 95.0%. The optically pure 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, [α]D25 +62.0° (+1.0, H2O). ee >99%. Rf = 0.57, Rf = 0.35, t = 12.89 min. Anal. Calcd for C15H19NO3·HCl: C, 55.4; H, 7.18; N, 5.70. Found: C, 55.4; H, 7.14; N, 5.84. 1H-NMR (400 MHz, CD2OD): δ 2.36 (6H, s), 3.17 (1H, d, J = 14.4, 7.8 Hz), 3.91 (3H, s), 14.4, 8.2 Hz, 4.07 (1H, t, J = 8.0 Hz), 7.00—7.10 (3H, m).

2',4'-6'Trimethyl-1-phenylalanine Hydrochloride (4d)

N-Acetyl-2,4,6-trimethyl-1-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, the crystals were collected by filtration and dried in vacuo, yield 4.88 g (87.6%), ca. 95.0%. The optically pure product (4.70 g) was recrystallized from 95 ml of hot hydrochloric acid solution (5 mol/l) to give optically pure 4d 4.05 g (86.2%), mp 233—235 °C, [α]D25 +62.0° (+1.0, H2O). ee >99%. Rf = 0.57, Rf = 0.35, t = 12.89 min. Anal. Calcd for C15H19NO3·HCl: C, 56.6; H, 8.04; N, 5.08. Found: C, 56.4; H, 7.89; N, 5.08. 1H-NMR (400 MHz, DMSO-d6): δ 1.15 (6H, d, J = 7.6 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 (3H, m), 8.74 (3H, br), 13.49 (1H, br).

2',6'-Dimethyl-1-phenylalanine Hydrochloride (4e)

N-Acetyl-2-ethyl-6-methyl-1-phenylalanine methyl ester (3e, 4.46 g, 17.7 mmol) in concentrated HCl (23 ml, 283 mmol) was heated under reflux conditions for 1 h. 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.), [α]D25 +66.4° (+0.50, MeOH). ee >99%. Rf = 0.56, Rf = 0.35, t = 13.24 min. Anal. Calcd for C15H19NO3·HCl: C, 57.0; H, 7.50; N, 5.54. Found: C, 57.0; H, 7.52; N, 5.57. 1H-NMR (400 MHz, DMSO-d6): 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).

2',3',5'-Trimethyl-1-phenylalanine Hydrochloride (4f)

N-Acetyl-3,5-dimethyl-1-phenylalanine methyl ester (3f, 2.71 g, 10.0 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 pure 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, [α]D 25 13.5° (c=0.48, H2O), ee >99%. Rf 0.57, Rf 0.35. λ 12.45 min. Anal. Calcd for C11H15NO2 · HCl · 0.33H2O: C, 56.1; H, 7.13; N, 5.93. Found: C, 56.0; H, 7.0; N, 6.97; N, 5.93. 1H-NMR (400 MHz, DMSO-d6) 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-α-amino acid oxidase (Bothrops atrox crude dried venom, Sigma) was added. The solutions were incubated for 24 h at 37°C. After 24 h 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 µl) 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 Mo Kα radiation (λ=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 Positional parameters of non-H atoms were refined by a full matrix least squares method with anisotropic thermal parameters using the SHELXL97 program.26 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)1223-336-043; or deposit@ccdc.cam.ac.uk).

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References and Notes