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An Efficient Synthesis of 4(5)-Benzyl-L-Histidines Employing Catalytic Transfer Hydrogenolysis at Elevated Temperatures

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Abstract

An efficient two-step synthesis of 4(5)-benzyl-L-histidine from L-histidine was developed. A Pictet-Spengler reaction between L-histidine and benzaldehyde in the presence of excess strong base yielded 4-phenylspinacine within one hour. Catalytic transfer hydrogenolysis in methanol at reflux using ammonium formate rapidly converted 4-L-phenylspinacine to 4(5)-benzyl-L-histidine within five minutes. No racemization of the final product 4(5)-benzyl-L-histidine was observed using the Marfey reagent. To show the utility of this methodology, a series of fluorinated benzylhistidines is presented.

Graphical abstract

Keywords

amino acids; benzylation; Pictet-Spengler reaction; catalytic transfer hydrogenolysis; electrophilic aromatic substitution

Calcitonin gene-related peptide (CGRP) is a 37 amino acid residue neuropeptide of the central and peripheral nervous systems that causes potent vasodilation of blood vessels¹. The N-truncated fragment, CGRP(8–37), is the prototypical competitive antagonist of CGRP receptors^{2–4}. Benzylation of the His¹⁰ residue of N-terminally derivatized analogues of CGRP(8-37) substantially increases antagonist receptor affinity⁵ and spawned the

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development of a new class of potent, competitive and irreversible antagonists of CGRP receptors⁵⁻⁷.

The discovery that a benzylated histidyl residue in position 10 of CGRP(8-37) increases antagonist receptor affinity arose serendipitously from the preparation of N- α -benzyl-CGRP(8-37)⁵. Benzylation of the resin-bound, side chain protected peptide with benzyl bromide under basic conditions yielded two products, the desired mono-benzylated peptide in a 55% yield and a di-benzylated peptide side product in a 45% yield. Amino acid analysis indicated the second benzylation occurring on the histidyl residue in position 10 and long range coupling constants from ¹H-NMR experiments were consistent with benzylation of the C4 carbon. This was particularly surprising because the histidyl side chain had been protected with the benzyloxymethyl group⁸ on the π -nitrogen during the benzylation reaction. Subsequent pharmacological studies revealed the potent antagonist effects of N- α -benzyl-[benzylHis¹⁰]CGRP(8-37)⁵.

As part of ongoing studies to confirm the spectroscopically derived structure of the benzylated histidyl residue and to improve the isolated yields of CGRP antagonists containing benzylated histidyl residues, 4(5)-benzyl-L-histidine was prepared in two steps using previously reported methods^{9, 10}. In the first step, L-histidine underwent a Pictet-Spengler reaction with benzaldehyde to yield 4-phenylspinacine. Then, hydrogenolysis of 4-phenylspinacine over a palladium catalyst cleaved a doubly benzylic C-N bond in the second step to yield 4(5)-benzyl-L-histidine. Unfortunately, both steps proved challenging to reproduce in our hands. We now report optimized reaction conditions for the Pictet-Spengler reaction and the use of catalytic transfer hydrogenolysis at elevated temperatures to quantitatively cleave the doubly benzylic C-N bond, for the preparation of 4(5)-benzyl-L-histidine and a series of fluorinated benzylhistidines. In addition, we show for the first time that the stereochemical integrity of the starting material L-histidine is preserved in the final product, 4(5)-benzyl-L-histidine. The modified two-step synthesis of 4(5)-benzyl-L-histidine is shown in Scheme 1. Initially, the monohydrochloride salt of L-histidine underwent a Pictet-Spengler reaction with benzaldehyde to yield 4-L-phenylspinacine. Using two equivalents of KOH and a reaction time of one hour, 4-L-phenylspinacine was repeatedly obtained in a 60% yield, much lower than the 81% previously reported⁹. The reaction mixture distinctly smelled of almonds after one-hour indicating that benzaldehyde remained and the reaction had not gone to completion. This was confirmed by analytical RP-HPLC, which revealed histidine was present in the isolated crude product (Figure 1). Time course experiments, using RP-HPLC to monitor for the presence of L-histidine in the reaction mixture, showed the reaction required 24 hours to reach completion.

Recently, substituted phenylspinaceamines and phenylspinacines were prepared in good yields using the Pictet-Spengler reaction in the presence of excess base^{10, 11}. For histidine to undergo a Pictet-Spengler reaction it is essential that the side chain remain deprotonated for addition of the intermediate imine (Scheme 1) because protonated imidazoles are deactivated towards electrophilic addition. Since histidine is used as its mono-hydrochloride salt, only a stoichiometric amount of KOH (2 eq.) was previously used. When the Pictet-Spengler reaction was repeated using excess KOH (3 eq.), RP-HPLC showed that the L-histidine was consumed within one hour and the yield of 4-phenylspinacine improved to 91%.

4-Phenylspinacine is produced as a mixture of the *S,R* (*trans*) and *S,S* (*cis*) diastereoisomers, which are readily separated by RP-HPLC (Figure 1). Even though both diastereoisomers are substrates for the subsequent hydrogenolysis reaction to produce 4(5)-benzyl-L-histidine, the specific identity of each diastereoisomer eluting from the RP-HPLC column was determined. A sample of the *S,R* diastereoisomer was obtained by fractional crystallization of the crude product from water and identified by its melting point⁹. The structure of each diastereoisomer was confirmed by ¹H-NMR where NOE effects were observed between the doubly benzylic β-hydrogen and the α-hydrogen in the *cis* diastereoisomer but not observed in the *trans* diastereoisomer (Figure 2). Predictably the less soluble *trans* diastereoisomer was last to elute from the RP-HPLC column whereas the more soluble *cis* diastereoisomer eluted first from the column (Figure 1).

To further establish the utility of this reaction for the preparation of phenylspinacines, six fluorophenylspinacines were prepared using two and three equivalents of KOH with a reaction time of one hour. The isolated yields for each preparation are listed in Table 1. Use of a stoichiometric amount of KOH resulted in generally poor yields of the fluorinated phenylspinacines with one exception, D,L-4-*o*-fluorophenylspinacine that was obtained in an 80% yield. When the reaction was repeated in the presence of excess KOH, all fluorinated phenylspinacines were obtained in good to excellent yields. Interestingly, Pictet-Spengler cyclization using fluorobenzaldehydes proceeded diastereoselectively, with predominant formation of the *cis*-spinacine products. To examine if the observed diastereoselectivity was a consequence of inductively enhanced base-catalyzed epimerization of the *trans*-4-fluorophenylspinacines to their more thermodynamically stable *cis* isomers during Pictet-Spengler cyclization, a sample of 4-L-*p*-fluorophenylspinacine was dissolved in hot deuterium oxide, exchanging all NH and OH hydrogens for deuterium. This exchanged material was dissolved in 1 M 98%-*d*NaOD. After 8 h at room temperature, ¹H NMR spectroscopy revealed no detectable exchange of deuterium for hydrogen at position 4 of the spinacine. Diastereoselection, then, must occur during the electrophilic cyclization step of the Pictet-Spengler reaction.

In the second step, hydrogenolysis of the doubly benzylic C-N bond of phenylspinacine was surprisingly slow with the reaction being incomplete after 24 hours when using hydrogen gas at 40 psi over a 10% palladium on carbon (Pd/C) catalyst (Scheme 1). Interestingly, more forcing conditions have been reported for the hydrogenolysis of the structurally similar compound phenylspinaceamine where either the hydrogen gas needs to be at 100 atm pressure when using 10% Pd/C¹² or 25% Pd/C is required when using hydrogen gas at atmospheric pressure^{10,13}. These latter conditions were also effective for the reduction of a variety of substituted phenylspinacines¹⁰.

25% Pd/C is not readily available and so modifying reaction conditions to use the commercially available catalyst, 10% Pd/C, were explored further. Performing the hydrogenolysis under catalytic transfer conditions¹⁴ where ammonium formate is substituted for hydrogen gas also resulted in an incomplete reaction after 24 hours with 20% of unreacted 4-phenylspinacine still present. Repeating the catalytic transfer hydrogenolysis in refluxing solvents¹⁵ dramatically increased the rate of the reduction; phenylspinacine was consumed within five minutes yielding 4(5)-benzyl-L-histidine as the only product when

methanol, trifluoroethanol and water were used as solvents. Identical catalytic transfer hydrogenolysis of the fluorophenylspinacines in refluxing methanol resulted in a mixture of 4(5)-(fluorophenylmethyl)histidine and 4(5)-benzylhistidine, the latter forming from competitive hydrodefluorination of the desired (fluorophenylmethyl)histidine, the starting fluorophenylspinacine, or both. Efforts to mitigate this unexpected^{16–18} defluorination by use of stoichiometric amounts of ammonium formate, triethylamine and formic acid in methanol^{14, 19–21}, and hydrogen gas at one atmosphere or 40 psi with 10% Pd/C in methanol at room temperature were unsuccessful. On the other hand, use of a weight of 5% platinum on carbon equal to that of the fluorophenylspinacine with ammonium formate in water at reflux for 2h^{21, 22} afforded the corresponding fluorinated benzylhistidines in good yields.

In order to establish if the chiral integrity of the starting material L-histidine is preserved during the modified reaction conditions employing excess KOH in the Pictet-Spengler reaction, the enantiomeric excess of 4(5)-benzyl-L-histidine was determined using the Marfey reagent²³. Consistent with previous reports using histidine^{24–26}, reaction of 4(5)-benzyl-L-histidine with (1-fluoro-2,4-dinitrophenyl)-5-L-alaninamide (FDAA), otherwise known as the Marfey reagent, yielded two derivatives, a major one resulting from the addition of the dinitrophenyl-L-alaninamide group onto the α -amino group (mono- α -derivative) and a minor product resulting from addition of a second dinitrophenyl-L-alaninamide group onto the side chain benzylated-imidazole group (bis-derivative). Both FDAA-derivatives were readily separated by RP-HPLC with the mono- α -derivative eluting first. The retention times of the corresponding FDAA-derivatives of the D-isomer were identified using a sample of 4(5)-benzyl-D-histidine that in turn was prepared from D-histidine.

The mono- α -derivative of 4(5)-benzyl-L-histidine eluted from the RP-HPLC column before the corresponding D-derivative (L $\rightarrow$ D), which is in common with the elution order of most FDAA derivatives of amino acids but in contrast to the elution order of the mono- α -derivatives of histidine (D $\rightarrow$ L)²⁵. The separation mechanism of FDAA amino acid derivatives in RP-HPLC is based on differences in hydrophobicity²⁵ and so this result suggests that the added hydrophobic bulk of the benzyl group in 4(5)-benzyl-histidine overcomes the polar properties of the positively charged, protonated imidazole group that is present in the acidic (pH 3.0) chromatography conditions that caused the elution order of mono- α -derivatives of histidine to be reversed. The elution order of the bis-derivatives of 4(5)-benzyl-histidine was the same as that of the mono- α -derivatives of 4(5)-benzyl-histidine and the bis-derivatives of histidine (L $\rightarrow$ D)^{25, 26}.

Enantiomeric excess (ee) of two samples of 4(5)-benzyl-L-histidine, one prepared using two equivalents of KOH and the other prepared using three equivalents of KOH, and a sample of the batch of L-histidine that was used for the preparation of 4(5)-benzyl-L-histidine are listed in Table 2. The identical ee values of both samples of 4(5)-benzyl-L-histidine show that the modified reaction conditions for this preparation do not cause racemization of the final product. Furthermore, the ee value of both 4(5)-benzyl-L-histidine samples was also identical to the ee value of the starting material L-histidine, which shows that no racemization occurs during the two-step preparation of 4(5)-benzyl-L-histidine employing a

Pictet-Spengler reaction and catalytic transfer hydrogenolysis. Enantiomeric excesses of the 4(5)-(fluorophenylmethyl)-L-histidines fully corroborated these observations (Table 2).

Summary

In summary, 4(5)-benzyl-L-histidine was readily prepared from L-histidine in two steps employing a Pictet-Spengler reaction and catalytic transfer hydrogenolysis using ammonium formate. Key to the success of this synthesis was the use of analytical RP-HPLC to monitor the progress of each step. In the first step, excess strong base was required for the Pictet-Spengler reaction to be complete within one hour. In the second step, the hydrogenolysis was complete within five minutes only when the reaction was performed under reflux. Using these reaction conditions, 4(5)-benzyl-L-histidine was obtained in an excellent two-step isolated yield of 81% (91% for the Pictet-Spengler reaction and 89% for the catalytic transfer hydrogenolysis), a significant improvement over the 62% two-step isolated yield that would be obtained using previously published methods (i.e. an otherwise unattainable 81% for the Pictet-Spengler reaction⁹ and 77% for the hydrogenolysis¹⁰). Finally, use of excess strong base in the Pictet-Spengler reaction did not cause racemization of the final product 4(5)-benzyl-L-histidine.

All reactions were performed in dried glassware under atmospheric conditions (unless otherwise noted). L-Histidine hydrochloride monohydrate was from Acros Organics (Geel, Belgium). Ethanol (95%) was from Aaper Alcohol (Shelbyville, KY) and all fluorobenzaldehydes from Oakwood Products (West Columbia, SC). The *meta*-fluorobenzaldehyde was received as a 4:1 mixture of *meta*- and *ortho*-isomers, respectively and was used as such. Benzaldehyde and all other solvents were from Fisher Scientific (Pittsburg, PA). The Marfey reagent was from Thermo Scientific (Rockford, IL). All benzaldehydes were redistilled, and all other solvents and reagents were used as supplied. Thin layer chromatography was performed on precoated silica gel F₂₅₄ glass plates from Whatman (Clifton, NJ) or Analtech (Newark, DE) with visualization by staining with ninhydrin in ethanol or UV light. Melting and decomposition points were collected using a digital melting point apparatus from Electrothermal (Southend, England) or a Mel-temp apparatus from Laboratory Devices (Cambridge, MA) and are uncorrected. Infrared data was collected on Nicolet Avatar 370 and Thermo/Nicolet iS5 FTIR spectrometers and absorptions are reported in wavenumbers (cm⁻¹). HPLC analyses were performed on a Waters 600E system comprised of a 600E system controller, 600 pump, 484 UV detector, and HP 3395 Integrator. Analytes were eluted from a Spherisorb 5 ODS(1) column (250 × 4.6 mm, 5µm silica, C₁₈) from Phenomenex (Torrance, CA) using mixtures of 100 mM potassium phosphate buffer, pH 3.0 (A), water (B) and acetonitrile (C) as noted. The eluent was monitored at 214 or 254 nm. ¹H NMR spectra were recorded at 300 MHz on an INOVA Unity 300-NB instrument from Varian (Palo Alto, CA) with chemical shifts (δ) quoted in parts per million (ppm); ¹³C NMR spectra were recorded at 75 MHz with chemical shifts (δ) quoted in ppm, and ¹⁹F NMR spectra were recorded at 282 MHz with chemical shifts (δ) quoted in ppm using potassium fluoride (δ -125.3³¹) as an internal reference. Mass spectra were obtained using a Voyager-DE Pro MALDI-TOF instrument from ABI (Framingham, MA) in linear or reflector positive modes with 2,5-dihydroxybenzoic acid as the matrix.

4-L-Phenylspinacine (1a)⁹

Solutions of KOH (10.1 g, 180 mmol) in water (30 mL) and benzaldehyde (6.4 g, 60 mmol) in ethanol (30 mL) were sequentially added dropwise to a stirred suspension of L-histidine monohydrochloride (12.6 g, 60 mmol) in water (30 mL) and the resulting mixture was heated to reflux. After 1 hour, ethanol was removed by rotary evaporation under reduced pressure and the pH of the aqueous solution was adjusted to 6.8 by dropwise addition of HCl (6M). The resulting white precipitate was stored at 0 °C overnight, filtered, and dried. The filtrate was concentrated and stored at 0 °C overnight to yield a second crop. This procedure was repeated twice more and the crops were combined to afford a mixture of *cis* and *trans* diastereoisomers of **1** as a white powder.

Yield: 13.4 g (91%); $R_f = 0.27$ (n-butanol:acetic acid:water, 4:1:1).

RP-HPLC (20% A, 80% B isocratic) R_t , 5.3 min (*cis* isomer), 7.5 min (*trans* isomer).

IR (ATR): 3543, 3044, 1625, 1604, 1500, 1246, 1093 cm^{-1} .

^1H NMR (300 MHz, D_2O): $\delta = 7.47$ (s, 0.6 H, *trans* isomer), 7.39 (s, 0.4 H, *cis* isomer), 7.31–7.12 (m, 5 H), 5.07 (s, 0.7 H, *trans* isomer), 4.87 (s, 0.3 H, *cis* isomer), 3.50 (m, 1H), 2.95–2.21 (m, 2 H).

^{13}C NMR (75 MHz, $\text{D}_2\text{O}/\text{NaOD}$): $\delta = 180.3, 180.1, 142.3, 141.5, 137.3, 132.9, 131.1, 129.1, 128.9, 128.8, 128.7, 128.6, 128.5, 128.3, 127.9, 58.9, 58.2, 55.0, 53.1, 27.2, 26.2$.

MS (MALDI): m/z $[\text{M} + \text{H}]^+$ calcd for $[\text{C}_{13}\text{H}_{14}\text{N}_3\text{O}_2]^+$: 244.11; found: 244.08.

Recrystallization of a sample from hot water gave pure white crystals of *trans*-4-L-phenylspinacine, 99.5% diastereomerically pure by HPLC.

Mp 262–264 °C, (lit.⁹ 255–257 °C, lit.²⁷ 267–268 °C)

^1H NMR (300 MHz, D_2O): $\delta = 7.71$ (s, 1 H), 7.41–7.36 (m, 3 H), 7.26–7.23 (m, 2 H), 5.71 (s, 1 H), 4.00 (dd, $J=8.9, 5.7$ Hz, 1 H), 3.30 (ddd, $J=16.7, 5.7, 0.7$ Hz, 1 H), 3.04 (ddd, $J=16.7, 8.9, 1.3$ Hz, 1 H).

^{13}C NMR (75 MHz, $\text{D}_2\text{O}/\text{NaOD}$): $\delta = 180.2, 142.1, 136.5, 130.9, 128.8, 128.5, 128.2, 128.1, 54.9, 53.1, 25.9$.

4-L-*o*-Fluorophenylspinacine (1b)

Solutions of KOH (5.02 g, 89.4 mmol) in water (15 mL) and freshly distilled *o*-fluorobenzaldehyde (3.1 mL, 29.8 mmol) in absolute ethanol (15 mL) were sequentially added dropwise to a stirred suspension of L-histidine monohydrochloride (6.24 g, 29.8 mmol) in water (15 mL) and the resulting mixture was heated to reflux. After 1 hour, ethanol was removed by rotary evaporation under reduced pressure and the pH was adjusted to 6.8 by dropwise addition of H_2SO_4 (0.5 M). The resulting white precipitate was stored at 0 °C overnight, filtered, and dried. The filtrate was concentrated and stored at 0 °C overnight to

yield a second crop. This procedure was repeated once more and the crops were combined to afford the *cis* isomer of 4-L-*o*-fluorophenylspinacine as a white powder.

Yield: 6.93 g (89%); mp 201–203 °C (dec). R_f = 0.31 (n-butanol:acetic acid:water, 4:1:1).

IR (ATR): 3500–2400, 2822, 1651, 1632, 780 cm^{-1} .

^1H NMR (300 MHz, D_2O): δ = 7.70 (s, 1 H), 7.49–7.41 (m, 1 H), 7.25–7.16 (m, 3 H), 5.84 (s, 1 H), 4.17 (dd, J =9.3, 5.4 Hz, 1 H), 3.30 (ddd, J =16.6, 1.6, 1.5 Hz, 1 H), 3.08–2.98 (m, 1 H).

^{13}C NMR (75 MHz, $\text{NaOD}/\text{D}_2\text{O}$): δ = 177.6, 159.0 (d, J =245 Hz), 134.0, 130.1, 128.1, (d, J =13 Hz), 128.0, 125.6, 128, 125.4 (d, J =13 Hz), 122.6 (d, J =4 Hz), 113.7 (d, J =22 Hz), 56.6, 49.4 (d, J =4 Hz), 56.8, 55.6, 25.2.

^{19}F NMR (282 MHz, $\text{D}_2\text{O}/\text{NaOD}$): δ = –123.0 (m).

MS (MALDI): m/z $[\text{M} + \text{H}]^+$ calcd for $[\text{C}_{13}\text{H}_{14}\text{FN}_3\text{O}_2]^+$: 262.10; found: 262.08.

In a similar manner, D,L-histidine monohydrochloride (6.25 g, 29.8 mmol) afforded 4-D,L-*o*-fluorophenylspinacine (**1c**) as a white powder.

Yield: 7.12 g (91%).

4-L-*m*-Fluorophenylspinacine (**1d**)

Solutions of KOH (5.02 g, 89.4 mmol) in water (15 mL) and freshly distilled 80% *m*-fluorobenzaldehyde (3.2 mL, 29.8 mmol) in absolute ethanol (15 mL) were sequentially added dropwise to a stirred suspension of L-histidine monohydrochloride (6.25 g, 29.8 mmol) in water (15 mL) and the resulting mixture was heated to reflux. After 1 hour, ethanol was removed by rotary evaporation under reduced pressure and the pH was adjusted to 6.8 by dropwise addition of H_2SO_4 (0.5 M). The resulting white precipitate was stored at 0 °C overnight, filtered, and dried. The filtrate was concentrated and stored at 0 °C overnight to yield a second crop. This procedure was repeated once more and the crops were combined to afford a 78:21 mixture of *cis* isomers of 4-L-*m*-fluorophenylspinacine (**1d**) and 4-L-*o*-fluorophenylspinacine (**1b**) as a white powder.

Yield: 6.54 g (84%); R_f = 0.41 (*meta* isomer), 0.33 (*ortho* isomer) (n-butanol:acetic acid:water, 4:1:1).

IR (ATR): 3400–2300, 3024, 2979, 2819, 1650, 1592, 1379, 1356, 780 cm^{-1} .

4-L-*m*-Fluorophenylspinacine (**1d**)

^1H NMR (300 MHz, D_2O): δ = 7.70 (s, 1 H), 7.47–7.39 (m, 1 H), 7.23–7.06 (m, 3 H), 5.55 (s, 1 H), 4.18–4.12 (m, 1 H), 3.33–3.26 (m, 1 H), 3.08–2.99 (m, 1 H).

^{13}C NMR (75 MHz, $\text{NaOD}/\text{D}_2\text{O}$): δ = 178.0, 158.7 (d, J =245 Hz), 136.1, 130.6, 128.0, 127.9, 127.8 (d, J =11 Hz), 126.8, 125.9 (d, J =13 Hz), 122.6, (d, J =3 Hz), 56.8, 49.6, 25.1.

^{19}F NMR (282 MHz, NaOD/D₂O): $\delta = -116.8$ (m).

MS (MALDI): m/z [M + H]⁺ calcd for [C₁₃H₁₄FN₃O₂]⁺: 262.10; found: 262.06.

In a similar manner, D,L-histidine monohydrochloride (6.22 g, 29.7 mmol) afforded a mixture of 4-D,L-*m*-fluorophenylspinacine (**1e**) and 4-D,L-*o*-fluorophenylspinacine (**1c**) as a white powder.

Yield: 6.23 g (80%).

4-L-*p*-Fluorophenylspinacine (**1f**)¹⁰

Solutions of KOH (5.02 g, 89.4 mmol) in water (15 mL) and freshly distilled *p*-fluorobenzaldehyde (3.2 mL, 29.8 mmol) in absolute ethanol (15 mL) were sequentially added dropwise to a stirred suspension of L-histidine monohydrochloride (6.24 g, 29.8 mmol) in water (15 mL) and the resulting mixture was heated to reflux. After 1 hour, ethanol was removed by rotary evaporation under reduced pressure and the pH was adjusted to 6.8 by dropwise addition of H₂SO₄ (0.5 M). The resulting white precipitate was stored at 0 °C overnight, filtered, and dried. The filtrate was concentrated and stored at 0 °C overnight to yield a second crop. This procedure was repeated once more and the crops were combined to afford predominantly the *cis* isomer of 4-L-*p*-fluorophenylspinacine¹⁰ as a white powder.

Yield: 7.63 g (98%); mp 199–201 °C, (lit.¹⁰ 219–221 °C for the dihydrochloride salt); $R_f = 0.42$ (n-butanol:acetic acid:water, 4:1:1).

IR (ATR): 3580–2100, 3534, 3240, 1629, 1605, 1511, 1389, 1370, 842 cm⁻¹.

^1H NMR (300 MHz, D₂O): $\delta = 7.67$ (s, 1 H), 7.37–7.32 (m, 2 H), 7.17–7.11 (m, 2 H), 5.55 (s, 1 H), 4.15 (dd, $J=11.9$, 5.2 Hz, 1 H), 3.30 (ddd, $J=11.2$, 5.3, 1.5 Hz, 1 H), 3.08–3.00 (m, 1 H).

^{13}C NMR (75 MHz, NaOD/D₂O): $\delta = 178.0$, 160.2 (d, $J=243$ Hz), 136.6, 135.5 (d, $J=4$ Hz), 131.2, 128.2 (d, $J=8$ Hz), 127.4, 113.2 (d, $J=22$ Hz), 25.2.

^{19}F NMR (282 MHz, D₂O/NaOD): $\delta = -118.3$ (m).

MS (MALDI): m/z [M + H]⁺ calcd for [C₁₃H₁₄FN₃O₂]⁺: 262.10; found: 262.10⁺.

In a similar manner, D,L-histidine monohydrochloride (6.21 g, 29.7 mmol) afforded 4-D,L-*p*-fluorophenylspinacine (**1g**) as a white powder.

Yield: 7.77 g (100%).

4(5)-Benzyl-L-histidine (**2a**)¹⁰

10% Pd/C (0.65 g) and ammonium formate (0.86 g, 13.6 mmol) were added with stirring to a suspension of **1a** (0.64 g, 2.67 mmol) in methanol (20 mL) and the mixture was heated to reflux. After five minutes the reaction mixture was cooled to room temperature and filtered

through celite before methanol was removed by rotary evaporation under reduced pressure to afford **2a** as tan coloured, crystals.

Yield: 0.58g (89%); R_f (n-butanol:acetic acid:water, 4:1:1, v:v:v) 0.37.

RP-HPLC, R_t , (20% A, 80% B, 0% C to 20% A, 20% B, 60% C over 30 min) 9.22 min.

IR (ATR): 3031, 2896, 1667, 1496, 1192, 1134 cm^{-1} .

^1H NMR (300 MHz, D_2O): δ = 7.99 (s, 1 H), 6.83–6.60 (m, 5 H), 3.51 (dd, J =17.7, 17.1 Hz, 2 H), 3.31 (t, J =7.2 Hz, 1 H), 2.70 (ddd, J =7.4, 7.2, 3.3 Hz, 2 H).

^{13}C NMR (75 MHz, $\text{D}_2\text{O}/\text{CD}_3\text{COOD}$): δ = 172.6, 136.8, 133.2, 130.4, 129.2, 128.6, 127.5, 123.8, 53.7, 29.1, 25.2.

MS (MALDI): m/z $[\text{M} + \text{H}]^+$ calcd for $[\text{C}_{13}\text{H}_{16}\text{N}_3\text{O}_2]^+$: 246.13; found: 246.12.

An analytical sample was recrystallized from a 7/1 (v/v) ethanol water mixture.

Mp 223–225 $^\circ\text{C}$ (dec), (lit.¹⁰ 222–224 $^\circ\text{C}$, dec).

4(5)-(o-Fluorophenylmethyl)-L-histidine (2b)

5% Pt/C (0.513 g) and ammonium formate (0.636 g, 10.1 mmol) were added with stirring to a suspension of 4-L-*m*-fluorophenylspinacine (0.509 g, 1.9 mmol) in water (13 mL) and the mixture was heated to reflux. After 2 hours the reaction mixture was cooled to room temperature and filtered through celite before water was removed by rotary evaporation under reduced pressure. Drying in an evacuated desiccator (0.75 mm of Hg) overnight afforded 4(5)-(o-fluorophenylmethyl)-L-histidine as a buff-colored solid, 0.784 g, R_f (n-butanol:acetic acid:water, 4:1:1, v:v:v) 0.59, typically suitable for use without further purification. Recrystallization from 60% aqueous isopropyl alcohol returned buff-colored crystals.

Yield: 0.177 g (35%); mp 125 $^\circ\text{C}$ (dec with prior softening and shrinking).

IR (ATR): 3350–2200, 3033, 1585, 1490, 1341, 1230, 758 cm^{-1} .

^1H NMR (300 MHz, D_2O): δ = 8.30 and 8.16 (two s, 1 H), 7.26–6.97 (m, 4 H), 3.94 (s, 2 H), 3.78 (t, J =7.0 Hz, 1 H), 3.11 (ddd, J =7.2, 7.0, 5.6 Hz, 2 H).

^{13}C NMR (75 MHz, D_2O): δ = 170.8 and 169.0, 158.7 (d, J =244 Hz), 131.8, 128.6 (d, J =4 Hz), 127.4 (d, J =8 Hz), 127.0, 122.8, 122.7 (d, J =4 Hz), 122.1 (d, J =16 Hz), 113.5 (d, J =22 Hz), 51.9, 23.5, 21.0 (d, J =4 Hz).

^{19}F NMR (282 MHz, D_2O): δ = –122.1(m).

MS (MALDI): m/z $[\text{M} + \text{H}]^+$ calcd for $[\text{C}_{13}\text{H}_{15}\text{FN}_3\text{O}_2]^+$: 264.12; found: 264.15, $[\text{M} + \text{Na}]^+$ calcd for $[\text{C}_{13}\text{H}_{14}\text{FNaN}_3\text{O}_2]^+$: 286.10; found 286.12.

In a similar manner, 4-D,L-*o*-fluorophenylspinacine (0.261 g, 1.00 mmol) afforded 4(5)-(*o*-fluorophenyl)methyl-D,L-histidine (**2c**) as a buff-colored solid.

Yield: 0.356 g (135%).

4(5)-(*m*-Fluorophenylmethyl)-L-histidine (**2d**)

5% Pt/C (0.538 g) and ammonium formate (0.663 g, 10.5 mmol) were added with stirring to a suspension of a 78:21 mixture of 4-L-*m*-fluorophenylspinacine and 4-L-*o*-fluorophenylspinacine (0.536 g, 2.0 mmol) in water (14 mL) and the mixture was heated to reflux. After 2 hours the reaction mixture was cooled to room temperature and filtered through celite before water was removed by rotary evaporation under reduced pressure. Drying in an evacuated desiccator (0.75 mm of Hg) overnight afforded 0.679 g of a white, crystalline mixture of 4(5)-(*m*-fluorophenylmethyl)-L-histidine (**2d**) and 4(5)-(*o*-fluorophenylmethyl)-L-histidine (**2b**), R_f (n-butanol:acetic acid:water, 4:1:1, v:v:v) 0.59, typically suitable for use without further purification. Recrystallization from 60% aqueous isopropyl alcohol returned buff-colored crystals that were a 70:30 mixture of the *meta* and *ortho* isomers as determined by ^{19}F NMR.

Yield: 0.145 g (27%).

IR (ATR): 3700–2200, 3037, 1588, 1489, 1340, 760 cm^{-1} .

MS (MALDI): m/z $[\text{M} + \text{H}]^+$ calcd for $[\text{C}_{13}\text{H}_{15}\text{FN}_3\text{O}_2]^+$: 264.12; found: 264.16, $[\text{M} + \text{Na}]^+$ calcd for $[\text{C}_{13}\text{H}_{14}\text{FNaN}_3\text{O}_2]^+$: 286.10; found 286.12.

4(5)-(*m*-Fluorophenylmethyl)-L-histidine (**2d**)

^1H NMR (300 MHz, D_2O): δ = 8.26 and 8.24 (2 s, 1 H), 7.29–6.82 (m, 4 H), 3.95 (s, 2 H), 3.78 (t, J =7.0 Hz, 1 H), 3.21–3.05 (m, 2 H).

^{13}C NMR (75 MHz, D_2O): δ = 170.7 and 169.0, 160.8 (d, J =244 Hz), 137.6 (d, J =8 Hz), 131.6, 128.6 (d, J =9 Hz), 127.5, 122.8, 122.3 (d, J =3 Hz), 113.6, 111.9 (d, J =21 Hz), 51.8, 26.7 (d, J =2 Hz), 23.4.

^{19}F NMR (282 MHz, D_2O): δ = –117.8 (m).

In a similar manner, 4-D,L-*m*-fluorophenylspinacine (0.790 g, 3.03 mmol) afforded a mixture of 4(5)-(*m*-fluorophenyl)methyl-D,L-histidine (**2e**) and of 4(5)-(*o*-fluorophenyl)methyl-D,L-histidine (**2c**) as a white powder.

Yield: 0.885 g (111 %).

4(5)-(*p*-Fluorophenylmethyl)-L-histidine (**2f**)¹⁰

5% Pt/C (0.789 g) and ammonium formate (0.961 g, 15.2 mmol) were added with stirring to a suspension of 4-L-*p*-fluorophenylspinacine (0.789 g, 3.0 mmol) in water (20 mL) and the mixture was heated to reflux. After 2 hours the reaction mixture was cooled to room temperature and filtered through celite before water was removed by rotary evaporation

under reduced pressure. Drying in an evacuated desiccator (0.75 mm of Hg) overnight afforded white, crystalline 4(5)-(p-fluorophenylmethyl)-L-histidine, typically suitable for use without further purification.

Yield: 0.874 g (111%); R_f (n-butanol:acetic acid:water, 4:1:1) 0.62; mp 202–205 °C (dec with prior softening and shrinking) (lit.¹⁰ mp 206–208 °C, (dec) for the dihydrochloride salt).

IR (ATR): 3550–2350, 3013, 2888, 1633, 1600, 1509, 1223 cm^{-1} .

^1H NMR (300 MHz, D_2O): δ = 8.51 (s, 1 H), 7.20–7.14 (m, 2 H), 7.06–7.00 (m, 2 H), 4.00 (s, 2 H), 3.85 (t, $J=7.3$ Hz, 1 H), 3.21 (d, $J=7.5$ Hz, 2 H).

^{13}C NMR (75 MHz, D_2O): δ = 172.7, 162.0 (d, $J=243$ Hz), 133.5, 132.7, 130.4 (d, $J=8$ Hz), 130.3, 124.7, 115.8 (d, $J=22$ Hz), 53.8, 28.3, 25.3.

^{19}F NMR (282 MHz, D_2O): δ = –115.5 (m).

MS (MALDI): m/z $[\text{M} + \text{H}]^+$ calcd for $[\text{C}_{13}\text{H}_{15}\text{FN}_3\text{O}_2]^+$: 264.12; found: 264.17.

In a similar manner, 4-D,L-p-fluorophenylspinacine (0.791 g, 3.0 mmol) afforded 4(5)-(p-fluorophenyl)methyl-D,L-histidine (**2g**) as a white powder.

Yield: 0.950 g (119%).

Determination of enantiomeric excess

A solution of L-histidine monohydrochloride or **2a** in water (100 μL , 10 mg/mL) and NaHCO_3 in water (40 μL , 1M) were added to a solution of 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide in acetone (200 μL , 1% w/v) and the mixture was heated to 40 °C on a water bath. After 1 hour, hydrochloric acid (20 μL , 2 M) was added and the mixture was reduced to dryness in a vacuum. The resulting residue was dissolved in DMSO (1mL)²⁸ and an aliquot (5 μL) was loaded onto a YMC-Pack ODS AM column (50 $\times$ 4.6 mm, 3 μm silica, C_{18}) from YMC America Inc. (Allentown, PA) previously equilibrated at a flow rate of 0.67 mL/min with a mixture of 50 mM TEAP buffer pH 3.0 and acetonitrile (90/10, v/v)^{23, 29}. 4(5)-benzyl-L-histidine derivatives were separated and eluted from the column by increasing the percentage of acetonitrile in the eluent from 10% to 40% over 60 minutes. The effluent was monitored at 340 nm. The mono- α -derivative of 4(5)-benzyl-L-histidine eluted in 17.6 minutes and the *bis*-derivative of 4(5)-benzyl-L-histidine eluted in 25.9 minutes, the mono- α -derivative of 4(5)-benzyl-D-histidine eluted in 18.7 minutes and the *bis*-derivative of 4(5)-benzyl-D-histidine eluted in 27.7 minutes. Histidine derivatives were loaded onto the same YMC-Pack ODS AM column that was previously equilibrated with a mixture of 50 mM TEAP buffer pH 3.0 and acetonitrile (88/12, v/v) and eluted from the column by holding the percentage of acetonitrile in the eluent at 12% for 10 minutes before increasing it to 50% over 30 minutes. The mono- α -derivative of L-histidine eluted in 10.1 minutes and the *bis*-derivative of L-histidine eluted in 32.1 minutes, the mono- α -derivative of D-histidine eluted in 9.4 minutes and the *bis*-derivative of D-histidine eluted in 33.9 minutes. Percent enantiomeric excess was calculated from the area under the peaks of each derivative using

the formula [(area of mono- α -L-derivative + area of *bis*-L-derivative) - (area of mono- α -D-derivative + area of *bis*-D-derivative)] / [(area of mono- α -L-derivative + area of *bis*-L-derivative) + (area of mono- α -D-derivative + area of *bis*-D-derivative)] $\times$ 100%.

Similarly, 4(5)-(fluorophenylmethyl)-L-histidine and 4(5)-(fluorophenylmethyl)-D,L-histidine derivatives were prepared as above. Aliquots (10 μ L) of each were loaded onto an Agilent Zorbax SB-C18 column (150 $\times$ 4.6 mm, 5 μ m silica, C18) from Agilent Technologies, Inc. (Santa Clara, CA) previously equilibrated at a flow rate of 0.50 mL/min with a mixture of 0.1% trifluoroacetic acid in water and 0.1% trifluoroacetic acid in acetonitrile (90/10, v/v). The derivatives were eluted from the column by increasing the percentage of acetonitrile in the eluent from 10% to 60% over 90 minutes. The effluent was monitored at 340 nm. Retention times of the derivatives were found to be 28.7 minutes for 4(5)-(*o*-fluorophenylmethyl)-L-histidine, 33.4 minutes for 4(5)-(*o*-fluorophenylmethyl)-D-histidine, 30.0 minutes for 4(5)-(*m*-fluorophenylmethyl)-L-histidine, 33.0 minutes for 4(5)-(*m*-fluorophenylmethyl)-D-histidine, 31.0 minutes for 4(5)-(*p*-fluorophenylmethyl)-L-histidine and 34.4 minutes for 4(5)-(*p*-fluorophenylmethyl)-D-histidine. Percent enantiomeric excess was calculated as above.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Analytical RP-HPLC of crude reaction mixture from Pictet-Spengler reaction after one hour. HPLC Conditions: Eluent: 20mM potassium phosphate buffer, pH 3.0, 1 mL/min, Spherisorb 5 ODS(1) (250 × 4.6 mm), $\lambda = 214$ nm.

Figure 2.
NOE coupling in *cis* and *trans* isomers of 4-L-phenyl-spinacine.

Scheme 1.

Preparation of 4(5)-benzyl-L-histidine. Reagents and conditions: a) benzaldehyde, KOH (3 eq.), refluxing ethanol/water (2/1, v/v). b) ammonium formate, 10% Pd/C, refluxing methanol.

Table 1

Isolated yields of 4-phenylspinacines.

Entry	4-Phenylspinacine	Yield (%) 2 eq. KOH	Yield (%) 3 eq. KOH
1		81 ^a	91
	1a		
2		26	89
	1b		
3		80	91
	1c		
4		12	84
	1d		
5		14	80
	1e		
6		26	98
	1f		

Entry	4-Phenylspinacine	Yield (%) 2 eq. KOH	Yield (%) 3 eq. KOH
7	1g	66	100

^aReference ⁹

Table 2

Enantiomeric excess (EE) values of histidine and 4(5)-benzyl-L-histidines.

Entry	Amino acid	Eq. of KOH	ee (%)
1		—	99.1
2	2a	2	99.1
3	2a	3	99.1
4	2b	3	99.0
5	2d	3	99.0
6	2f	3	99.0