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Synthesis of Phenylalanines Regiospecifically Labelled with Deuterium in the Aromatic Ring

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Summary: Phenylalanines regiospecifically labelled with deuterium in the aromatic ring can be prepared through the hydrogenolysis of tyrosine tetrazolyl ethers using D_2 gas and a medium pressure catalytic hydrogenator.

Key words: ^{2}H-labelled *L*-phenylalanines, ^{2}H-labelled *L*-tyrosines, tyrosine tetrazolyl ether, hydrogenolysis, catalytic deuteration

Introduction

Recently, structural analytical techniques for amino acids, peptides, and proteins using nuclear magnetic resonance spectrometry have been developed by using positive (^{13}C, ^{15}N) or negative (^{2}H) stable isotope labelling methodologies. For elucidation of their functions, it is very important that the nmr signals of both protons and carbons are properly assigned. Though these signals are considered to be simplified by introducing D-atom(s) into appropriate positions in the compounds, it is not easy to incorporate these labels into such compounds directly. As one of our recent interests in synthesis of labelled compounds, we attempted to prepare a set of phenylalanines which are regioselectively labelled in the aromatic ring with D-atom(s), and incorporate biochemically these phenylalanines into proteins. For a synthetic approach to these compounds, we intended to realize a deoxygenation and a simultaneous deuteration of aromatic hydroxy compounds. Phenol tetrazolyl ethers have been reported to be hydrogenolyzed to the corresponding

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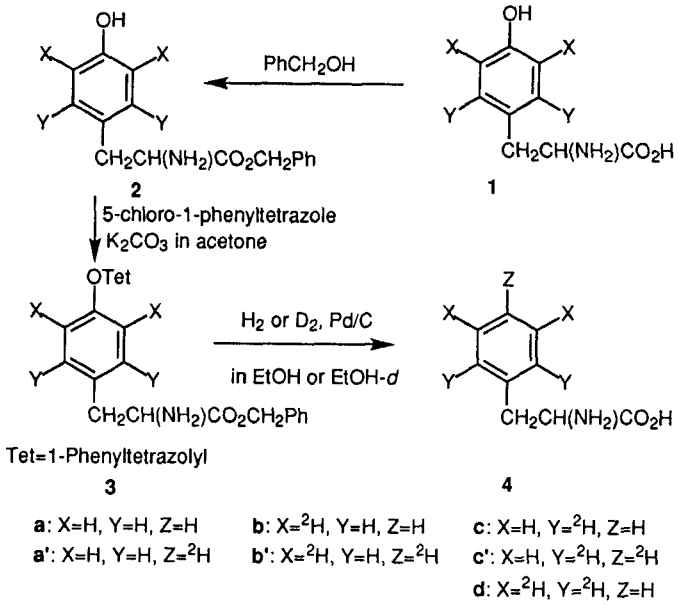
deoxygenated aromatic compounds and tetrazolone derivative in good yields,[1] but there have been no detailed reports on the hydrogen pressure and the deoxygenation from the aromatic hydroxyl group of amino acids such as tyrosine **1**. We have reported a convenient synthesis of 1-alkyltetrazoles from alkyl aldehydes and silyl azide,[2] and an application of these tetrazole derivatives to deoxygenation of phenolic hydroxy groups but they are not applicable to tyrosine.[3] On the other hand, Hruby *et al.* have reported a conversion of *L*-tyrosine (**1a**) into *L*-phenylalanine (**4a**) via 1-phenytetrazolyl ether of *L*-tyrosine benzyl ester (**3a**) in refluxing ethanol using a large excess of Pd/C.[4] We tried to apply the procedure to the regioselective deuteration of the aromatic ring of phenylalanine, but no effective deuteration was achieved. Therefore, we would like to report a new regioselective deuteration of phenylalanine in this paper.

Results and Discussion

According to Hruby's method,[4] we examined the reaction of **3a** in ethanol-*d* to obtain [4'-^{2}H]phenylalanine (**4a'**) because ethanol was considered to be at least one of the hydrogen donors in the reaction. However, the nmr spectrum of the products was complicated and the deuterium content could not be estimated with confidence. By refluxing in 6*N*-DCl/D_2O, *L*-tyrosine (**1a**) was easily converted into [3',5'-$^2H_2$]-*L*-tyrosine (**1b**), which was carefully esterified to give **2b** (without any H-D exchange) using benzyl alcohol and *p*-TsOH as described in the experimental section. Then, we carried out a similar reaction of [3',5'-$^2H_2$]-*L*-tyrosine benzyl ester (**3b**), which was obtained from **2b** and 5-chloro-1-phenyltetrazole in a very good yield, in order to simplify the nmr spectrum of phenylalanine(s). A treatment of 1-phenyltetrazolyl ether of **3b** with cyclohexene and Pd/C in ethanol-*d* was found to give a mixture of [3',5'-$^2H_2$]-*L*-phenylalanine (**4b**) and [3',4',5'-$^2H_3$]-*L*-phenylalanine (**4b'**) in 70% yield (**4b** : **4b'**=7 : 3). This suggests that the reaction is unstuitable for the regioselective deuteration of the aromatic ring of phenylalanine. Then, we tried to investigate another selective and effective conversion method of tyrosine tetrazolyl ether **3** into ^{2}H-labelled phenylalanine **4**.

When **3a** was subjected to hydrogenolysis in the presence of a catalytic amount (2-5%) of 10%-Pd/C at a pressure of less than 2-3 kgf/cm^2 of hydrogen, only debenzylation of **3a** occurred to give 1-phenyltetrazolyl ether of tyrosine and no phenylalanine was formed. However under a pressure of 5kgf/cm^2 of hydrogen, **3a** was converted into *L*-phenylalanine

(**4a**), which was extracted with *1N*-HCl from the reaction mixture and purified through ion-exchange chromatography on DOWEX 50W-X8, in 85% yield.



a: X=H, Y=H, Z=H **b**: X=^{2}H, Y=H, Z=H **c**: X=H, Y=^{2}H, Z=H
a': X=H, Y=H, Z=^{2}H **b'**: X=^{2}H, Y=H, Z=^{2}H **c'**: X=H, Y=^{2}H, Z=^{2}H
d: X=^{2}H, Y=^{2}H, Z=H

The product was spectroscopically identified by comparing with an authentic sample. The optical purity of the product was also checked by a chiral column chromatography. The reaction was found to proceed with predominant retention (>99%) of configuration and no racemization occurred under the reaction conditions. With D_2 gas, [4'-^{2}H]-*L*-phenylalanine (**4a'**) was obtained from **3a** in a similar yield. The deuterium content of the compound was more than 97% by nmr spectroscopic analysis. For compound **4a'**, the aromatic proton signals were shown at δ 7.30 and 7.40 as a set of AA'BB' quartet, and the quartet was different from H_{ar} of the starting material or tyrosine itself. Similarly, regioselectively ^{2}H-labelled phenylalanines **4b** and **4b'** were synthesized from **3b** using H_2 gas or D_2 gas, respectively. Two singlets were recorded at 7.32 ($H_{2'}$ and $H_{6'}$) and 7.37 ($H_{4'}$) ppm for **4b**, and only a singlet was shown at 7.32 ppm for the protons at 2'- and 6'-positions of **4b'**. These findings show that the regioselectively deuterated tyrosines can be easily converted into the regioselectively ^{2}H-labelled phenylalanines via a catalytic hydrogenolysis (H_2 or D_2) of the corresponding tyrosine tetrazolyl ether. Any D-atom scrambling, which often occur under acid- or base-catalyzed reaction conditions, did not occur under the reaction conditions.

For the synthesis of other ^{2}H-labelled phenylalanines, [2',3',5',6'-$^2H_4$]-*L*-tyrosine (**1d**) was prepared from phenol-d_6, sodium pyruvate, and ammonium acetate in H_2O in the presence of *E. herbicola* by a modification of the reported procedure.[5] [2',3',5',6'-$^2H_4$]-*L*-Tyrosine (**1d**) was refluxed to be quantitatively converted into [2',6'-$^2H_2$]-*L*-tyrosine (**1c**) in *6N*-HCl. Respective synthesis of **3c** and **3d** from tyrosine **1c** and **1d** was achieved by careful esterification with benzyl alcohol followed by etherification with 1-phenyltetrazole as described above. By catalytic hydrogenations, **3c** and **3d** were transformed into **4c** and **4d** in 85-95% yields, similar to the yield of **4a**. With D_2 gas, **4c'** was obtained from **3c**. As expected, the aromatic proton nmr spectrum for **4c** showed an AB_2 spin-spin coupling pattern, and the aromatic proton signals for **4c'** and **4d** were singlets at δ 7.42 and 7.37, respectively. It became clear that the regioselectivity of the reaction was complete and the stereochemistry was retained under the reaction conditions used.

Syntheses of some proteins using these labelled phenylalanines are now in progress in our laboratory.

Experimental

Melting points were determined on a Yamato MP-21 melting point apparatus and are uncorrected. ^{1}H NMR spectra were measured in $CDCl_3$ or D_2O on a Varian UNITY-400 spectrometer. All chemical shifts are reported as δ values (ppm) relative to tetramethylsilane (0 ppm) or sodium 3-(trimethylsilyl)propanesulfonate (0 ppm). High resolution mass spectra were obtained on a JEOL JMS-AX-500 spectrometer with DA7000 data system using perfluorokerosene as an internal standard. For ion-exchange chromatography, DOWEX 50W-X8 activated with *1N*-HCl was used. Optical purities were determined on a Senshu SSC-3100 high-pressure liquid chromatography system equipped with chiral MCIGEL CRS10W column from Mitsubishi Kasei Co. and 2mM-$CuSO_4$ solution as an eluent. Catalytic hydrogenation was performed in an Ishii CHA-S medium-pressure catalytic hydrogenator. All reagents were commercial products and were used without further purification.

Synthesis of [2',3',5',6'-$^2H_4$]-*L*-Tyrosine (**1d**).

As a modification to the reported method,[5] a mixture of sodium pyruvate (4.5 g, 40 mmol), CH_3CONH_4 (5.0 g, 65 mmol), sodium sulfite (0.2 g, 1.5 mmol), EDTA (0.3 g, 0.8 mmol), and pyridoxal phosphate (20 mg, 0.1 mmol) in H_2O (100 mL) was suspended with dry cells of *Erwinia herbicola* (2.5 g). Then the pH was adjusted to 8.0 by adding 10%-NaOH solution. The reaction mixture was incubated at 37 °C for 10 h. At hourly intervals during the incubation, phenol-d_6 (2.64 g, 26.4 mmol) in H_2O (17 mL) was added to maintain the initial concentration. After an additional incubation of 10 h, the pH of the reaction

mixture was adjusted to ~1 by adding concentrated HCl solution and the insoluble materials were filtered off. Precipitation of [2',3',5',6'-$^2H_4$]-*L*-tyrosine (**1d**) was achieved by adjusting the pH to its isoelectric point (5.7), and the resulting precipitates were collected by suction; yield 4.2 g (86%).

^1H NMR (D_2O + NaOD): δ=2.66 (dd, 1H, *J*=13.7, 7.3), 2.84 (dd, 1H, *J*=13.7, 5.2 Hz), and 3.39 (dd, 1H, *J*=7.3, 5.2 Hz).

Preparation of [3',5'-$^2H_2$]-*L*-Tyrosine (**1b**) and [2',6'-$^2H_2$]- (**1c**).

L-Tyrosine (5.4 g, 30 mmol) was heated to reflux in *6N*-DCl (100 mL) for 6 h. After cooling to room temperature, the mixture was evaporated to dryness and D_2O added repeatedly until the distillate became neutral. The yield was almost quantative (5.4 g).

^1H NMR (D_2O + NaOD): δ=2.66 (dd, 1H, *J*=13.7, 7.3 Hz), 2.84 (dd, 1H, *J*=13.7, 5.2 Hz), 3.39 (dd, 1H, *J*=7.3, 5.2 Hz), and 6.98 (s, 2H).

A similar treatment of [2',3',5',6'-$^2H_4$]-*L*-tyrosine (5.4 g, 30 mmol) in *6N*-HCl (100 mL) gave [2',6'-$^2H_2$]-*L*-tyrosine (**1c**) in a quantitative yield.

^1H NMR (D_2O + NaOD): δ=2.66 (dd, 1H, *J*=13.7, 7.3 Hz), 2.84 (dd, 1H, *J*=13.7, 5.2 Hz), 3.39 (dd, 1H, *J*=7.3, 5.2 Hz), and 6.57 (s, 2 H).

Preparation of Benzyl Ester of Labelled *L*-Tyrosine (**2**).

A representative procedure of the preparations for [3',5'-$^2H_2$]-*L*-tyrosine benzyl ester (**2b**) is as follows: In order to prevent H-D exchange at the 3',5'-positions of the aromatic ring, [3',5'-$^2H_2$]-*L*-tyrosine (**1b**, 4.0 g, 20 mmol) and *p*-toluenesulfonic acid (6.0 g, 30 mmol) were lyophilized three times from D_2O (35 g) and benzyl alcohol (3.2 g, 30 mmol) was extracted from D_2O (100 mL) suspension before use. A mixture of these three compounds was heated at 100 °C to give a clear solution and then benzene (150 mL) was added. After azeotropical removal of water using a Dean-Stark trap, the mixture was evaporated and triturated with ether (400 mL). The precipitated solid was treated with an excess of triethylamine, filtered, washed with water, and dried to afford 3.5 g (60%) of **2b**.

2b: ^1H NMR ($CDCl_3$): δ=2.84 (dd, 1H, *J*=13.7, 7.4 Hz), 3.03 (dd, 1H, *J*=13.7, 5.3 Hz), 3.76 (dd, 1H, *J*=7.4, 5.3 Hz), 5.14, 5.18 (ABq, 2H, *J*=12.1 Hz), 6.94 (s, 2H), and 7.31-7.40 (m, 5H).

HRMS: *m/z*, calcd for $C_{16}H_{15}O_3ND_2$, 273.1334. Found: 273.1344.

2c: ^1H NMR ($CDCl_3$): δ=2.84 (dd, 1H, *J*=13.7, 7.4 Hz), 3.03 (dd, 1H, *J*=13.7, 5.3 Hz), 3.76 (dd, 1H, *J*=7.4, 5.3 Hz), 5.14, 5.18 (ABq, 2H, *J*=12.1 Hz), 6.67 (s, 2H), and 7.31-7.40 (m, 5 H).

HRMS: *m/z*, calcd for $C_{16}H_{15}O_3ND_2$, 273.1334. Found: 273.1384.

2d: ^1H NMR ($CDCl_3$): δ=2.84 (dd, 1H, *J*=13.7, 7.4 Hz), 3.03 (dd, 1H, *J*=13.7, 5.3 Hz), 3.76 (dd, 1H, *J*=7.4, 5.3 Hz), 5.14, 5.18 (ABq, 2H, *J*=12.1 Hz), and 7.31-7.40 (m, 5H).

HRMS: *m/z*, calcd for $C_{16}H_{13}O_3ND_4$, 275.1460. Found: 275.1453.

1-Phenyltetrazolyl Ether of Labelled *L*-Tyrosine Benzyl Ester (**3**).

General Procedure: According to the method reported by Viswanatha and Hruby,[4] a mixture of labelled *L*-tyrosine benzyl ester (**2**, 3 mmol), 5-chloro-1-phenyltetrazole (0.5 g, 3

mmol), and anhydrous potassium carbonate (2.5 g, 18 mmol) in acetone (80 mL) was heated to reflux overnight. After removal of the solvent, water (100 mL) was added and stirred for 3 h. The precipitated solid was collected by filtration and dried to give **3** in 83-87% yield. An analytical sample was obtained by recrystallization from ethyl acetate.

3b; mp 88-90 °C. ^{1}H NMR ($CDCl_3$): δ=2.93 (dd, 1H, *J*=13.6, 7.4 Hz), 3.10 (dd, 1H, *J*=13.6, 5.6 Hz), 3.77 (dd, 1H, *J*=7.4, 5.6 Hz), 5.13, 5.17 (ABq, 2H, *J*=12.0 Hz), 7.22 (s, 2H), and 7.30-7.82 (m, 10H).

HRMS: *m/z*, calcd for $C_{23}H_{19}O_3N_5D_2$, 417.1769. Found: 417.1754.

3c; mp 88-90 °C. ^{1}H NMR ($CDCl_3$): δ=2.93 (dd, 1H, *J*=13.6, 7.4 Hz), 3.10 (dd, 1H, *J*=13.6, 5.6 Hz), 3.77 (dd, 1H, *J*=7.4, 5.6 Hz), 5.13, 5.17 (ABq, 2H, *J*=12.0 Hz), 7.31 (s, 2H), and 7.30-7.82 (m, 10H).

HRMS: *m/z*, calcd for $C_{23}H_{19}O_3N_5D_2$, 417.1769. Found: 417.1763.

3d; mp 89-91°C. ^{1}H NMR ($CDCl_3$): δ=2.93 (dd, 1H, *J*=13.6, 7.4 Hz), 3.10 (dd, 1H, *J*=13.6, 5.6 Hz), 3.77 (dd, 1H, *J*=7.4, 5.6 Hz), 5.13, 5.17 (ABq, 2H, *J*=12.0 Hz), and 7.30-7.82 (m, 10H).

HRMS: *m/z*, calcd for $C_{23}H_{17}O_3N_5D_4$, 419.1895. Found: 419.1865.

Catalytic Hydrogenation of 1-Phenyltetrazolyl Ether of Labelled *L*-Tyrosine Benzyl Ester (**3**).

A typical procedure of these reactions for [4'-^{2}H]phenylalanine (**4a'**) is as follows. A mixture of **3a** (0.2 g, 0.48 mmol) and 10%-Pd/C (0.06 g) in EtOD (50 mL) was subjected to hydrogenolysis at a pressure of 5 kgf/cm^2 of deuterium gas for 24 h. It was then evaporated to dryness, and the residue was extracted with *1N*-HCl and insoluble substances were filtered off. The clear filtrate was submitted to DOWEX 50W-X8 column and washed with water. Elution with *1N*-ammonia solution afforded 69.2 mg (87%) of pure **4a'**. The optical purities of the obtained phenylalanines were checked by HPLC analysis using commercially available chiral column and found to be more than 99% enantiomeric excess.

4a': ^{1}H NMR ($CDCl_3$): δ = 3.12 (dd, 1H, *J*=14.5, 8.0), 3.28 (dd, 1H, *J*=14.5, 5.2), 3.98 (dd, 1H, *J*=8.0, 5.2), and 7.30, 7.40 (AA'BB'q, 4H,*J*=8.0).

HRMS: *m/z*, calcd for $C_9H_{10}O_2ND$, 166.0852. Found: 166.0793.

4b: ^{1}H NMR ($CDCl_3$): δ = 3.12 (dd, 1H, *J*=14.5, 8.0), 3.28 (dd, 1H, *J*=14.5, 5.2), 3.98 (dd, 1H, *J*=8.0, 5.2), 7.32 (s, 2 H), and 7.37 (s, 2H).

HRMS: *m/z*, calcd for $C_9H_9O_2ND_2$, 167.0913. Found: 167.0916.

4b': ^{1}H NMR ($CDCl_3$): δ = 3.12 (dd, 1H, *J*=14.5, 8.0), 3.28 (dd, 1H, *J*=14.5, 5.2), 3.98 (dd, 1H, *J*=8.0, 5.2), and 7.32 (s, 2H).

HRMS: *m/z*, calcd for $C_9H_8O_2ND_3$, 168.0978. Found: 168.0955.

4c: ^{1}H NMR ($CDCl_3$): δ = 3.12 (dd, 1H, *J*=14.5, 8.0), 3.28 (dd, 1H, *J*=14.5, 5.2), 3.98 (dd, 1H, *J*=8.0, 5.2), and 7.35-7.43 (AB_2, 3H).

HRMS: *m/z*, calcd for $C_9H_9O_2ND_2$, 167.0913. Found: 167.0915.

4c': ^{1}H NMR ($CDCl_3$): δ = 3.12 (dd, 1H, *J*=14.5, 8.0), 3.28 (dd, 1H, *J*=14.5, 5.2), 3.98 (dd, 1H, *J*=8.0, 5.2), and 7.42 (s, 2H).

HRMS: *m/z*, calcd for $C_9H_8O_2ND_3$, 168.0978. Found: 168.0900.

4d: ^{1}H NMR ($CDCl_3$): δ = 3.12 (dd, 1H, *J*=14.5, 8.0), 3.28 (dd, 1H, *J*=14.5, 5.2), 3.98 (dd, 1H, *J*=8.0, 5.2), and 7.37 (s, 1H).
HRMS: *m/z*, calcd for $C_9H_7O_2ND_4$, 169.1045. Found: 169.1041.

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