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Research Article

Stereoselective synthesis of L-[2,3,4,5-D_4] ornithine

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Summary

Synthesis of L-[2,3,4,5-D_4]ornithine in which all of the diastereotopic hydrogens were stereoselectively labeled with deuterium was investigated. The chirally deuterated 3-aminopropanal derivative, a key intermediate in this synthesis, was prepared by a catalytic deuteration of an unsaturated γ-lactone derived for L-glutamic acid followed by several functional group interconversions. Condensation of the obtained deuterium-labeled 3-aminopropanal derivative with a chiral glycine template afforded unsaturated ornithine. The dehydroornithine was then subjected to a catalytic deuteration followed by deprotection to give the L-[2,3,4,5-D_4]ornithine. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: deuterium labeling; ornithine; catalytic deuteration

Introduction

Stable isotope-assisted NMR techniques have become more promising methods of predicting the overall and local structures of proteins.[1,2] In particular, the regio-and stereoselectively isotope-labeled amino acids are excellent probes for collecting precise structural information such as

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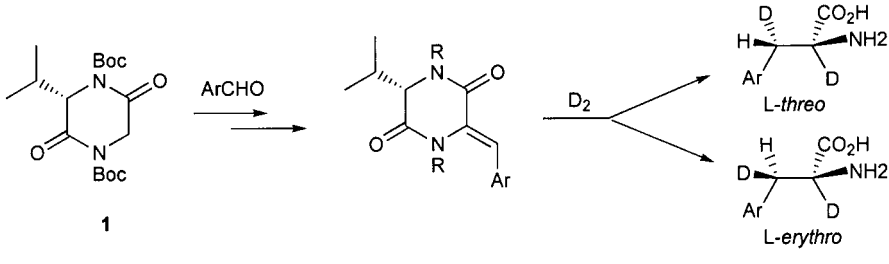
the side-chain conformations about specific residues. We have recently explored the methods for stereoselective isotopic substitution of only one of the diastereotopic methyl groups and methylene protons of amino acid side-chains.[3–11] However, investigation of long-chain amino acids, such as lysine or arginine, remains untouched despite their importance in protein function. In this paper we disclose the first example of [2,3,4,5-D_4]ornithine, in which all the diastereotopic methylene protons are stereoselectively labeled with deuterium.

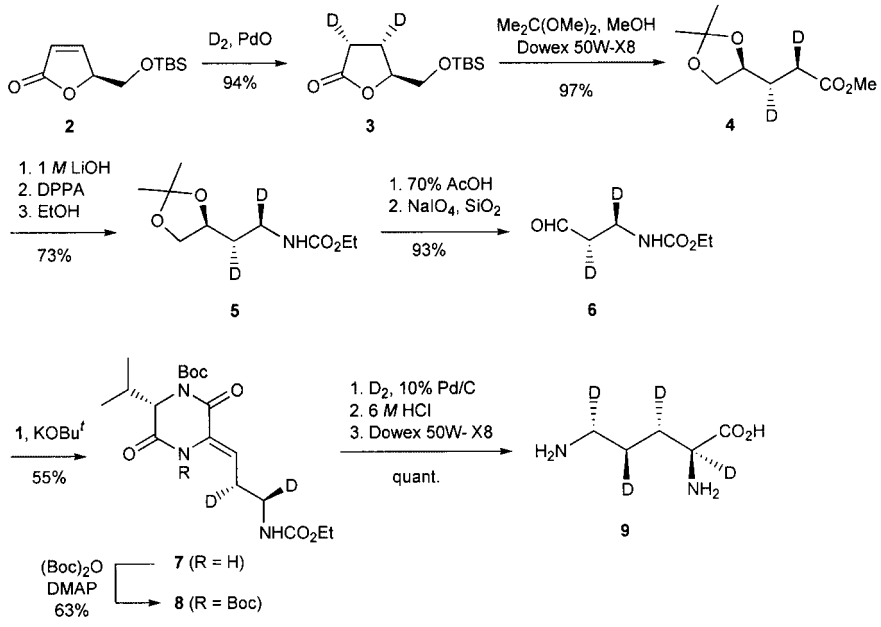
The non-proteinogenic amino acid, ornithine, is important not only as a precursor of arginine but also as a constituent of certain antibiotics and alkaloids. In connection with the stereochemical studies on the biosynthesis of antibiotic clavulanic acid, sinefungin, blasticidine, and streptothricin F, some stereoselectively deuterium- or tritium-labeled ornithine was prepared.[12–16] The syntheses involved asymmetric deuteration of dehydroornithine catalyzed by a chiral rhodium complex or the reduction of [D]- or [T]aldehyde with *S*- or *R*-Alpine borane.

Results and discussion

The asymmetric hydrogenation of dehydroamino acids has provided a useful approach to chiral amino acids. We previously reported the stereoselective synthesis of L-*threo*- and L-*erythro*-[2,3-D_2]amino acids by an asymmetric deuteration of dehydroamino acids prepared by condensation of aldehydes with a novel chiral glycine template **1** (Scheme 1).[3,4] Production of the desired [2,3,4,5-D_4]ornithine may be feasible if an appropriately deuterium-labeled 3-aminopropanal is available. As shown in Scheme 2, we adopted an optically active butenolide **2**, easily prepared from L-glutamic acid, as the starting material.



Scheme 1.



Scheme 2.

When a catalytic deuteration of the butenolide **2** in the presence of palladium oxide was performed in MeOD using deuterium gas at medium pressure (5 kgf/cm^2), deuterated γ-lactone **3** was obtained in 94% yield as an almost single diastereomer. Removal of the silyl group and ring-opening of the lactone **3** and subsequent protection of the liberated carboxyl and 1,2-diol functions were simultaneously conducted in methanol and acetone dimethyl acetal in the presence of Dowex 50W-X8 to give the protected ester **4** in 97% yield. After hydrolysis of the ester **4** with 1 M LiOH, the resultant carboxylic acid was subjected to the modified Curtius rearrangement.[17] Treatment of the free acid and diphenylphosphoryl azide (DPPA) and subsequent ethanolysis of the intermediate isocyanate afforded carbamate **5** in 73% yield. It is well-known that migration proceeds with retention of configuration when a chiral group rearranges. It was not until the isolation of dehydroornithine **7** that the ratio of the (*R*) and (*S*) configuration at the carbon atom α to the carbamate group turned out to be 86:16, because the ^{1}H NMR signals of those protons can be discriminated only in the compound **7**.

The acetonide group of compound **5** was removed by 70% aqueous acetic acid and the 1,2-diol so formed was oxidatively cleaved by sodium

periodide to give aldehyde **6** in 93% yield. Aldolization of the aldehyde **6** with the chiral dioxopiperazine **1** afforded dehydroornithine **7** in 55% with the loss of one Boc group by acyl transfer to the proximal incipient alkoxide followed by elimination. Since the olefin **7** was obtained as a 96:4 mixture of (*Z*)- and (*E*)-isomers, chromatographic separation was carried out and the (*Z*)-isomer was used in the next step. For the sake of higher degree of asymmetric induction in the hydrogenation step, reprotection of the dehydroornithine **7** to the compound **8** was carried out. Finally, a catalytic deuteration of the dehydroornithine **8** followed by acidic hydrolysis furnished the aimed L-[2,3,4,5-D_4]ornithine (**9**) in quantitative yield with 92% ee determined by a chiral HPLC.

The 400 MHz ^{1}H NMR spectrum of the deuterated ornithine **9** is shown in Figure 1. Compared to that of the unlabeled ornithine, the signals assigned to 2-, *3S*-, and *4R*-protons are diminished and the coupling patterns of the remaining protons, i.e. doublets for 5- and *3R*-protons and doublet of doublets for *4S*-protron, indicate the stereoselective incorporation of the deuterium atoms.

In conclusion, we have achieved the synthesis of (*2S,3S,4R,5R*)-[2,3,4,5-D_4] ornithine (**9**) based on the face-selective deuteration of the olefins **2** and **8** although there remains room for improving the stereoselectivity of the deuterium incorporation. The starting amino acids, L-glutamic acid and glycine uniformly labeled with ^{13}C and ^{15}N are now commercially available. The present protocol has opened vast possibilities for the use of long-chain amino acids labeled with stable isotopes as probes for protein structural analysis.

Experimental

^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively. All chemical shifts are reported as δ values (ppm) relative to residual chloroform (δ_H 7.26), sodium 3-(trimethylsilyl)[2,2,3,3-D_4] propionate (δ_H 0.00), or the central peak of $CDCl_3$ (δ_c 77.0). High resolution mass spectra (HRMS) were determined using perfluorokerosene as an internal standard. Optical purity was determined on a HPLC system equipped with a chiral SUMICHIRAL OA-6100 column using 2 mM $CuSO_4$ solution as an eluent.

2,5-Dioxopiperazine **1** was prepared as described before.[3,4] All other reagents were of commercial grade and used as supplied.

*(3R,4S,5S)-5-tert-Butyldimethylsiloxymethyl[3,4-D_2]tetrahydrofuran-2-one (**3**)*

A mixture of the olefin **2** (4.83 g, 21.3 mmol) and PdO (483 mg) in EtOD (150 ml) was stirred at room temperature for 1 h under medium pressure (5 kgf/cm^2) of deuterium gas. After removal of the catalyst using a Celite pad, the concentrated reaction mixture was chromatographed on silica gel. Elution with a mixture of hexane and ethyl acetate (95:5) gave the title compound **3** (4.63 g, 94%) as an oil. ^{1}H NMR ($CDCl_3$) δ 0.065 (s, 3 H), 0.071 (s, 3 H), 0.89 (s, 9 H), 2.16 (m, 1 H), 2.58 (br d, $J=10$ Hz, 1 H), 3.70 (dd, $J=11$ and 3 Hz, 1 H), 3.86 (dd, $J=11$ and 3 Hz, 1 H), 4.58 (m, 1 H). ^{13}C NMR ($CDCl_3$) $\delta -5.6$, -5.5, 18.2, 23.1(t, $J=20$ Hz), 25.8, 28.1 (t, $J=20$ Hz), 64.9, 80.0, 177.6. HRMS (EI, 30 eV) m/z 233.1568 (M^+, calcd for $C_{11}H_{21}D_2O_3Si$ 233.1542).

*Methyl (2R,3S,4S)-4,5-O-Isopropylidene[2,3-D_2]pentanoate (**4**)*

A mixture of the lactone **3** (4.63 g, 20.0 mmol), methanol (20 ml), and Dowex 50W-X8 (800 mg) in 2,2-dimethoxypropane (60 ml) was stirred at room temperature overnight. After removal of the resin using a Celite pad, the concentrated reaction mixture was chromatographed on silica gel. Elution with a mixture of hexane and ethyl acetate (85:15) gave the title compound **4** (3.66 g, 97%) as an oil. ^{1}H NMR ($CDCl_3$) δ 1.29 (s, 3 H), 1.35 (s, 3 H), 1.78 (br dd, $J=8$ and 8 Hz, 1 H), 2.34 (br d, $J=8$ Hz, 1 H), 3.50 (dd, $J=8$ and 7 Hz 1 H), 3.63 (s, 3 H), 4.00 (dd, $J=8$ and 6 Hz, 1 H), 4.07 (m, 1 H). ^{13}C NMR ($CDCl_3$) δ 25.5, 26.8 28.3 (t, $J=20$ Hz), 29.7 (t, $J=20$ Hz), 51.5, 68.9, 74.7, 108.9, 173.6. MS (CI) m/z 191 [$(M+H)^+$]. HRMS (EI, 30 eV) m/z 175.0978 [$(M-Me)^+$, calcd for $C_8H_{11}D_2O_4$ 175.0939].

*(2R,3S,4S)-N-Ethoxycarbonyl-3,4-O-isopropylidene[1,2-D_2]-butylamine (**5**)*

To a solution of the ester **4** (3.66 g, 19.3 mmol) in THF (20 ml) was added dropwise 1 M LiOH (20 ml) at 0°C over a period of 15 min. After being stirred for an additional 30 min, the mixture was acidified to pH 4 with 10% aqueous citric acid and extracted with ethyl acetate. The organic layer was dried over $MgSO_4$ and the solvent was evaporated to afford the corresponding acid (3.39 g, quant.) as an oil. ^{1}H NMR ($CDCl_3$) δ 1.34 (s, 3 H), 1.41 (s, 3 H), 1.84 (br dd, $J=8$ and 8 Hz, 1 H), 2.45 (br d, $J=8$ Hz, 1 H), 3.56 (dd, $J=8$ and 7 Hz, 1 H), 4.06 (dd, $J=8$ and 6 Hz, 1 H), 4.15 (m, 1 H). ^{13}C NMR ($CDCl_3$) δ 25.3, 26.6, 27.8

(t, $J = 20$ Hz), 29.5 (t, $J = 20$ Hz), 68.7, 74.5, 109.0, 178.5. MS (CI) m/z 177 $[(M+H)^+]$. HRMS (EI, 30 eV) m/z 161.0786 $[(M-Me)^+$, calcd for $C_7H_9D_2O_4$ 161.0783].

A solution of the obtained acid (4.77 g, 27.1 mmol), triethylamine (3.01 g, 29.8 mmol), and diphenylphosphoryl azide (8.20 g, 29.8 mmol) in benzene (80 ml) was refluxed under an argon atmosphere for 1 h. To the refluxed reaction mixture was added ethanol (12.5 g, 271 mmol) and the solution was refluxed for an additional 3.5 h. After removal of the solvent, the residue was extracted with ethyl acetate and the organic layer was washed with 5% HCl, water, saturated $NaHCO_3$, and brine. The dried organic layer was evaporated and residue was chromatographed on silica gel. Elution with a mixture of hexane and ethyl acetate (65:34) afforded the title compound **5** (4.35 g, 73%) as an oil. ^{1}H NMR ($CDCl_3$) δ 1.23 (t, $J = 7$ Hz, 3 H) 1.35 (s 3 H), 1.41 (s, 3 H), 1.68 (br dd, $J = 7$ and 7 Hz, 1 H), 3.31 (br m, 1 H), 3.55 (dd, $J = 8$ and 8 Hz, 1 H), 4.06 (dd, $J = 8$ and 8 Hz, 1 H), 4.09–4.17 (m, 3 H), 5.00 (br s, 1 H). ^{13}C NMR ($CDCl_3$) δ 14.4, 25.3, 26.6, 32.8 (t, $J = 20$ Hz), 37.6 (t, $J = 20$ Hz), 60.3, 68.9, 74.1, 108.7, 156.5. MS (CI) m/z 220 $[(M+H)^+]$. HRMS (EI, 30 eV) m/z 204.1178 $[(M-Me)^+$, calcd for $C_9H_{14}D_2NO_4$ 204.1205].

*(2S,3R)-3-Ethoxycarbonylamino[2,3-D$_2$]propanal (**6**)*

A solution of the carbamate **5** (1.73 g, 7.90 mmol) in 70% acetic acid (9.3 ml) was stirred at 50°C overnight. After removal of the solvent, the obtained 1,2-diol was dissolved in CH_2Cl_2 (22.6 ml). To the solution was added 0.65 M $NaIO_4$ (22.6 ml) and silica gel (22.6 g), and the mixture was stirred at room temperature for 30 min. After removal of silica gel using a Celite pad, the filtrate was diluted with chloroform and the organic layer was washed with water. The dried organic layer was evaporated to give the title compound **6** (1.08 g, 93%) as an oil. The crude aldehyde was used for the next step without further purification. ^{1}H NMR ($CDCl_3$) δ 1.22 (t, $J = 7$ Hz, 3 H), 2.70 (br m, 1 H), 3.45 (br m, 1 H), 4.09 (q, $J = 7$ Hz, 2 H), 5.02 (br s, 1 H), 9.81 (s, 1 H).

(Z,S)-3-[(2R,3R)-3-Ethoxycarbonylamino[2,3-D$_2$]propylidene]-1-(tert-butoxycarbonyl)-5-(1-methylethyl)-2,5-piperazinedione (7)

To a chilled solution of dioxopiperazine **1** (2.62 g, 7.35 mmol) and the aldehyde **6** (1.08 g, 7.35 mmol) in THF (30 ml) was added *t*-BuOK

(825 mg, 7.35 mmol), and the reaction mixture was stirred at room temperature overnight. Then, the reaction mixture was partitioned between ethyl acetate and aqueous NH_4Cl, and the organic layer was dried over $MgSO_4$. After evaporation of the solvent, the residue was chromatographed on silica gel. Elution with a mixture of hexane and ethyl acetate (40:60) afforded the title compound **7** (1.55 g, 55%) as an oil. ^{1}H NMR ($CDCl_3$) δ 1.00 (d, J = 7 Hz, 3 H), 1.05 (d, J = 7 Hz, 3 H), 1.23 (t, J = 7 Hz, 3 H), 1.54 (s, 9 H), 2.10 (m, 1 H), 2.40 (br m, 1 H), 3.29 (br m, 1 H), 4.13 (q, J = 7 Hz, 2 H), 4.59 (d, J = 7 Hz, 1 H), 5.01 (br s, 1 H), 6.24 (d, J = 9 Hz, 1 H), 8.73 (br s, 1 H). ^{13}C NMR ($CDCl_3$) δ 14.6, 18.4, 19.1, 26.3 (t, J = 18 Hz), 27.9, 34.0, 39.4 (t, J = 18 Hz), 61.1, 63.7, 84.3, 119.2, 129.4, 151.1, 157.0, 159.2, 166.6.

*(Z,S)-3-[(2R,3R)-3-Ethoxycarbonylamino[2,3-D_2]propylidene]-1,4-(di-tert-butoxycarbonyl)-5-(1-methylethyl)-2,5-piperazinedione (**8**)*

To a mixture of **7** (1.55 g, 4.02 mmol) and $(Boc)_2O$ (1.05 g, 4.82 mmol) in DMF (8 ml) was added DMAP (589 mg, 4.82 mmol), and the reaction mixture was stirred at room temperature for 1 h. Then, the reaction mixture was diluted by ethyl acetate, washed with aqueous $KHSO_4$ and dried over $MgSO_4$. After removal of the solvent, the crude product was purified by flash chromatography on silica gel, eluting with a mixture of hexane and ethyl acetate (75:25), to give 1.23 g (63%) of **8** as an oil. ^{1}H NMR ($CDCl_3$) δ 0.95 (d, J = 7 Hz, 3 H), 1.02 (d, J = 7 Hz, 3 H), 1.20 (t, J = 7 Hz, 3 H), 1.52 (s, 18 H), 1.90 (m, 1 H), 2.25 (br m, 1 H), 3.29 (br m, 1 H), 4.07 (q, J = 7 Hz, 2 H), 4.50 (d, J = 10 Hz, 1 H), 4.83 (br s, 1 H), 6.56 (d, J = 5 Hz, 1 H). ^{13}C NMR ($CDCl_3$) δ 14.6, 19.3, 19.5, 27.8, 27.9, 28.8 (t, J = 18 Hz), 3.17, 38.8 (t, J = 21 Hz), 60.9, 65.5, 84.5, 85.5, 129.8, 132.9, 148.8, 150.6, 156.5, 161.2,165.1.

*(2S,3S,4R,5R)-[2,3,4,5-D_4]Ornithine (**9**)*

To a suspension of 10% Pd/C (240 mg) in MeOD (20 ml) was added a solution of olefin **8** (240 mg, 0.495 mmol) in MeOD (20 ml) over a period of 2 h under an atmosphere of deuterium gas. After removal of the catalyst using a Celite pad, the solution was evaporated to give deuterated dioxopiperazine derivative and the product was directly subjected to acidic hydrolysis with refluxing 6 M HCl (20 ml) at 120°C for 18 h to afford a mixture of valine and deuterated ornithine. Treatment of the mixture with Dowex 50W-X8 furnished

(*2S,3S,4R,5R*)-[2,3,4,5-D_4]ornithine (**9**, 70.0 mg, quant.). ^{1}H NMR (10% DCl in D_2O) δ 1.97 (br dd, $J = 10$ and 10 Hz, 1 H), 2.08 (br d, $J = 10$ Hz, 1 H), 3.15 (br d, $J = 10$ Hz, 1 H).

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