JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
*J Label Compd Radiopharm* 2006; **49**: 229–235.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.1038

**Research Article**

# Convenient synthesis of deuterated glutamic acid, proline and leucine via catalytic deuteration of unsaturated pyroglutamate derivatives

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## Summary

Novel 3,4-didehydropyroglutamate derivatives, the key intermediates in this synthesis, were obtained from a protected pyroglutamate by the well-known selenenylation-oxidative deselenenylation method and were catalytically deuterated using a slow-addition technique. The obtained deuterated pyroglutamates were converted to [3,4-$^2H_2$]glutamic acid, [3,4,5-$^2H_3$]proline, and [3,4,5,5,5-$^2H_5$]leucine via an appropriate functional group interconversions followed by the standard deprotection procedure. Copyright © 2006 John Wiley & Sons, Ltd.

**Key Words:** deuterated amino acids; catalytic deuteration; unsaturated pyroglutamate

## Introduction

Recently, we have been engaged in the asymmetric synthesis of regio- and stereoselectively deuterium-labeled amino acids for structure analysis of proteins by means of NMR spectroscopy,[1–6] in which L-pyroglutamic acid (**1**) has been often used as a chiral starting material. We previously reported the synthesis of deuterated proline and leucine using deuterated L-pyroglutamate derivative **3** as a common intermediate, where a catalytic deuteration of unsaturated L-pyroglutaminol **2** derived from L-pyroglutamic acid (**1**) was used as a key reaction to introduce deuterium atoms into the $\beta$- and $\gamma$-positions of the amino acid side chains (Scheme 1).[3,5] However, the procedure necessitates reduction of the $\alpha$-carboxyl group of the L-pyroglutamic acid and

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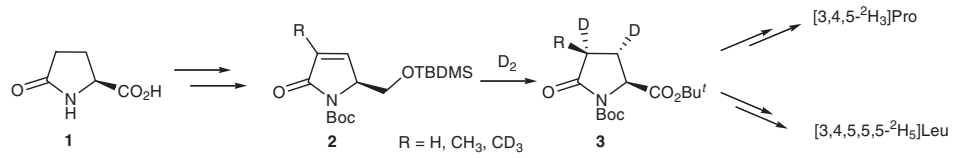
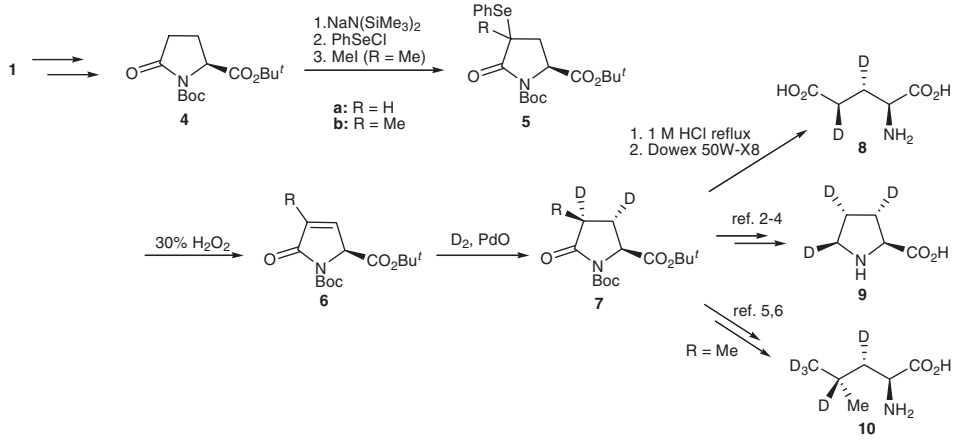
Contract/grant sponsor: Japan Science and Technology Corporation



*Received 25 October 2005*
*Revised 22 November 2005*
*Accepted 22 November 2005*




**Scheme 1. Synthesis of deuterated proline and leucine via deuteration of unsaturated pyroglutaminol derivatives 2**




**Scheme 2. Synthesis of deuterated glutamic acid, proline, and leucine via deuteration of unsaturated pyroglutamate derivatives 6**

its regeneration after the incorporation of deuterium atoms has been achieved. In fact, the overall yield of compound **3** (R = H) from **1** was 23% for 10 steps.

If the catalytic deuteration of an unsaturated L-pyroglutamate derivative such as compound **6** in Scheme 2 can be realized, the protocol would become more straightforward. However, only a few reports regarding the reactivity of the 3,4-didehydropyroglutamates can be seen in the literature.[7–9] Furthermore, it is well known that 3,4-didehydropyroglutamates are prone to racemize and isomerize via a double-bond shift. In 1995, Ezquerra and co-workers reported the first trapping reaction of the olefin with cyclopentadiene where the Diels-Alder adduct was obtained in 50% ee.[8] To our knowledge, there are no examples where a catalytic reduction is performed with the olefin. In this paper, we describe a convenient synthesis of [3,4-$^2H_2$]glutamic acid (**8**), [3,4,5-$^2H_3$]proline (**9**), and [3,4,5,5,5-$^2H_5$]leucine (**10**) via catalytic deuteration of novel unsaturated pyroglutamate derivatives **6** (Scheme 2).

## Results and discussion

3,4-Didehydropyroglutamate **6a** (R = H) is a new compound and can be obtained from *tert*-butyl *N*-(*tert*-butoxycarbonyl)pyroglutamate (**4**) by the well-established procedure involving phenylselenenylation and oxidative deselenenylation by hydrogen peroxide. In our case, olefin **6a** was obtained as the sole product in almost pure form; and the predicted side reactions associated with the use of organoselenium compounds as reported by Ezquerra *et al.*[8] could not be observed. Since the unsaturated pyroglutamate **6a** was chromatographically unstable, olefin **6a** was prepared from the purified phenyl selenide **5a** and used in the next step without further purification.

First of all, olefin **6a** was subjected to the conventional hydrogenation conditions. When a mixture of **6a** and 10% palladium on carbon in MeOD was stirred at room temperature under an atmosphere of deuterium gas, the deuterated pyroglutamate **7a** was obtained with considerable hydrogen–deuterium scrambling along with partial methanolysis to give an open-chain by-product. It was found that a slow addition of a dilute solution of olefin **6a** to the suspension where the deuterium gas was pre-adsorbed on the catalyst improved the extent of the hydrogen–deuterium scrambling. To explore the optimization of this deuteration reaction, we surveyed other transition metal catalysts. Finally, the use of palladium(II) oxide as a catalyst proved advantageous, resulting in quantitative formation of [3,4-$^2H_2$]pyroglutamate **7a**. Consequently, it has become feasible to obtain the deuterated pyroglutamate **7a** from **1** in 61% yield for **7** steps.

The pyroglutamate **7a** was then deprotected in refluxing 1 M HC1 and subsequent ion exchange purification afforded (2*S*,3*S*,4*R*)-[3,4-$^2H_2$]glutamic acid (**8**) in 84% yield. Although the relative stereochemistry of the three contiguous stereocenters within the deuterated glutamic acid **8** was established, the HPLC analysis using a chiral column showed that partial racemization occurred at the α-position (63% ee), probably due to the high acidity of the α-proton in compound **6a**.

According to the procedure described in our previous papers,[2–4] the deuterated pyroglutamate **7a** was also converted to [3,4,5-$^2H_3$]proline (**9**) in 46% yield for 3 steps. The cis selective deuteration of the δ-position was achieved by a stepwise reduction of the lactam carbonyl group with $LiEt_3BH$ followed by $Bu_3SnD$ in the presence of $BF_3.OEt_2$.[10]

In order to obtain [3,4,5,5,5-$^2H_5$]leucine (**10**), unsaturated 4-methylpyroglutamate **6b** is necessary. Introduction of a 4-methyl group into the 3,4-didehydropyroglutamate framework could be achieved simply by quenching the α-phenylseleno enolate, the reaction intermediate in the phenylselenenylation of the pyroglutamate derivative, with iodomethane followed by the usual oxidative deselenenylation reaction using hydrogen peroxide. Olefin **6b** was obtained as the sole product and the isomeric exomethylene compound could

not be detected in the reaction mixture. As compared with **6a**, the methylated olefin **6b** was less stable and should be immediately used in the next step without further purification. Thus, a catalytic deuteration of olefin **6b** in the presence of palladium(II) oxide in MeOD was carried out using the slow-addition technique to give 4-methyl[3,4-$^2H_2$]pyroglutamate **7b** in quantitative yield. According to the procedure described in our previous paper,[5,6] chemoselective ring-opening of pyroglutamate **7b**, reductive deuteration of the terminal carboxyl group, and standard deprotection procedure afforded [3,4,5,5,5-$^2H_5$]leucine (**10**) in 27% yield for 5 steps.

As with [3,4-$^2H_2$]glutamic acid (**8**), the relative stereochemistry of deuterium substitution in [3,4,5-$^2H_3$]proline (**9**) and [3,4,5,5,5-$^2H_5$]leucine (**10**) was established; however, their optical purities determined by HPLC were 14 and 18%ee, respectively, and the values were not reproducible on repeated runs. It seems that the extent of the racemization was varied according to the reaction conditions employed in the olefin synthesis.

## Conclusion

We have demonstrated a simple route to [3,4-$^2H_2$]glutamic acid, [3,4,5-$^2H_3$]proline, and [3,4,5,5,5-$^2H_5$]leucine via a catalytic deuteration of novel 3,4-didehydropyroglutamate derivatives. Although the obtained deuterated amino acids are partially racemized, the protocol described here still has some advantages. We are now seeking alternative protective groups for the carboxyl functionality which improve the stability of the unsaturated pyroglutamate derivatives.

## Experimental

$^1H$ 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 ($\delta_H$ 7.26), sodium 3-(trimethylsilyl)[2,2,3,3-$^2H_4$]propionate ($\delta_H$ 0.00), or the central peak of $CDCl_3$ ($\delta_c$ 77.0). High-resolution mass spectra (HRMS) were determined using perfluorokerosene as an internal standard. Optical purity was determined on an HPLC system equipped with a chiral MCIGEL CRS10W column using 2 mM $CuSO_4$ solution as an eluent.

*tert-Butyl (2S)-N-tert-Butoxycarbonyl-3,4-didehydropyroglutamate* **(6a)**

A solution of 1 M $NaN(SiMe_3)_2$ in THF (39.0 ml, 39.0 mmol) was treated with *N*,*N*′-dimethylpropyleneurea (5 ml) at 0°C under an argon atmosphere for 10 min, cooled to −78°C, and treated with a solution of *tert*-butyl (2*S*)-(*N*-*tert*-butoxycarbonyl)pyroglutamate (**4**, 5.06 g, 17.7 mmol) in THF (40 ml). After 0.5 h, a solution of PhSeCl (3.64 g, 19.0 mmol) in THF (30 ml) was added and the mixture was stirred for 2 h. The reaction was quenched with saturated aqueous $NH_4Cl$ and the mixture was extracted with ethyl acetate. The organic

layer was washed with water and brine, dried over $MgSO_4$, and concentrated. The residue was chromatographed on silica gel (hexane/ethyl acetate = 88/12) to give the corresponding 4-phenylselenopyroglutamate **5a** (7.81 g) as a mixture of diastereomers in quantitative yield.

To a solution of the obtained phenylselenide **5a** (4.39 g, 9.97 mmol) in THF (30 ml) was added dropwise 30% $H_2O_2$ (20 ml) at 0°C and the reaction mixture was stirred at room temperature for 2 h. The mixture was extracted with ethyl acetate and the organic layer was washed with saturated aqueous $NaHCO_3$ and brine and dried over $MgSO_4$. Concentration of the solution gave quantitative yield of the title compound **6a** (2.82 g) as an oil, which is used in the next step without further purification. $^1$H NMR ($CDCl_3$) δ 1.47 (s, 9 H), 1.53 (s, 9 H), 5.04 (dd, $J$ = 2.4 and 2.2 Hz, 1 H), 6.21 (dd, $J$ = 3.7 and 2.2 Hz, 1 H), 7.05 (dd, $J$ = 3.7 and 2.4 Hz, 1 H). $^{13}$C NMR ($CDCl_3$) δ 27.75, 27.82, 65.31, 83.23, 83.34, 128.46, 143.47, 148.40, 165.09, 168.43. HRMS (EI, 30 eV) $m/z$ 284.1519 [$(M + H)^+$, calculated for $C_{14}H_{22}NO_5$ 284.1498].

*tert-Butyl (2S)-N-tert-Butoxycarbonyl-3,4-didehydro-4-methylpyroglutamate* **(6b)**

A solution of 1 M $NaN(SiMe_3)_2$ in THF (21.0 ml, 21.0 mmol) was treated with *N*,*N*′-dimethylpropyleneurea (3 ml) at 0°C under an argon atmosphere for 10 min, cooled to −78°C, and treated with a solution of *tert*-butyl (2*S*)-(*N*-*tert*-butoxycarbonyl)pyroglutamate (**4**, 2.87 g, 10.1 mmol) in THF (20 ml). After 0.5 h, a solution of PhSeCl (2.11 g, 11.0 mmol) in THF (15 ml) was added and the mixture was stirred for 2 h. Then, $CH_3I$ (2.01 g, 14.2 mmol) was added. The reaction was quenched with saturated aqueous $NH_4Cl$ and the mixture was extracted with ethyl acetate. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated. The residue was chromatographed on silica gel (hexane/ethyl acetate = 50/50) to give the corresponding 4-methyl-4-phenylselenopyroglutamate **5b** (3.54 g) as a mixture of diastereomers in 77% yield.

To a solution of the obtained phenylselenide **5b** (1.00 g, 2.20 mmol) in THF (10 ml) was added dropwise 30% $H_2O_2$ (2.5 ml) at 0°C and the reaction mixture was stirred at room temperature for 1 h. The mixture was extracted with ethyl acetate and the organic layer was washed with saturated aqueous $NaHCO_3$ and brine and dried over $MgSO_4$. Concentration of the solution gave quantitative yield of the title compound **6b** (778 mg) as an oil, which is used in the next step without further purification. $^1$H NMR ($CDCl_3$) δ 1.47 (s, 9 H), 1.53 (s, 9 H), 1.90 (dd, $J$ = 2.1 and 1.7 Hz, 3 H), 4.89 (dq, $J$ = 2.5 and 2.1 Hz, 1 H), 6.68 (dq, $J$ = 2.5 and 1.7 Hz, 1 H). $^{13}$C NMR ($CDCl_3$) δ 11.12, 28.00, 28.07, 63.30, 83.14, 83.20, 136.20, 136.84, 148.86, 166.07, 169.30.

*tert-Butyl (2S,3S,4R)-N-tert-Butoxycarbonyl-[3,4-$^2H_2$]pyroglutamate* **(7a)**

A suspension of PdO (120 mg) in MeOD (10 ml) was stirred at room temperature for 30 min under an atmosphere of deuterium gas. To the mixture was added dropwise a solution of olefin **6a** (510 mg, 1.80 mmol) in MeOD (20 ml) and the mixture was stirred for 30 min. After removal of the catalyst using a Celite pad, evaporation of the solvent afforded quantitative yield of the title compound **7a** (513 mg) as an oil. $^1$H NMR ($CDCl_3$) δ 1.47 (s, 9 H), 1.49 (s, 9 H), 1.96 (dd, $J$ = 10 and 2 Hz, 1 H), 2.57 (d, $J$ = 10 Hz, 1H), 4.46 (d, $J$ = 2 Hz, 1 H). $^{13}$C NMR ($CDCl_3$) δ 21.19 (t, $J$ = 21 Hz), 27.85 (6 C), 30.64 (t, $J$ = 21 Hz), 59.47, 82.10, 83.09, 149.37, 170.32, 173.11. HRMS (EI, 70 eV) $m/z$ 288.1778 [$(M+H)^+$, calculated for $C_{14}H_{22}D_2NO_5$ 288.1780].

*tert-Butyl(2S,3S,4S)-N-tert-Butoxycarbonyl-4-methyl[3,4-$^2H_2$]pyroglutamate* **(7b)**

According to the procedure for the preparation of compound **7a**, deuteration of olefin **6b** (1.09 g, 3.67 mmol) in MeOD (100 ml) in the presence of PdO (193 mg) gave quantitative yield of the title compound **7b** (1.14 g) as an oil. $^1$H NMR ($CDCl_3$) δ 1.25 (s, 3 H), 1.48 (s, 9 H), 1.51 (s, 9 H), 1.57 (d, $J$ = 6 Hz, 1 H), 4.38 (d, $J$ = 6 Hz, 1 H). $^{13}$C NMR ($CDCl_3$) δ 16.66, 28.11 (6 C), 29.42 (t, $J$ = 21 Hz), 37.56 (t, $J$ = 18 Hz), 58.24, 82.32, 83.52, 149.81, 170.86, 176.29. HRMS (EI, 70 eV) $m/z$ 301.1876 ($M^+$, calculated for $C_{15}H_{23}D_2NO_5$ 301.1858).

*(2S,3S,4R)-[3,4-$^2H_2$]Glutamic Acid* **(8)**

A mixture of compound **7a** (48.0 mg, 1.67 mmol) in 1 M HC1 (30 ml) was heated to reflux overnight. The cooled aqueous solution was washed with chloroform and concentrated to dryness. The residue was submitted to ion-exchange column chromatography on Dowex 50W-X8 and elution with 1 M $NH_4OH$ gave the ammonium salt of compound **8** (232 mg, 84%) as a colorless solid, m.p. 183–185°C (lit,[11] 205°C dec). The optical purity (63%ee) was determined by HPLC. $^1$H NMR ($D_2O$) δ 1.89 (dd, $J$ = 8 and 8 Hz, 1 H), 2.25 (d, $J$ = 8 Hz, 1 H), 3.59 (d, $J$ = 8 Hz, 1 H). $^{13}$C NMR (5% NaOD in $D_2O$) δ 34.06 (t, $J$ = 19 Hz), 36.42 (t, $J$ = 19 Hz), 58.50, 185.64, 185.79. MS (FAB, glycerol) $m/z$ 150 [$(M+H)^+$].

*(2S,3S,4R,5S)-[3,4,5-$^2H_3$]Proline* **(9)**

According to the procedure described in our previous paper,[3,4] reduction and deprotection of compound **7a** (2.99 g, 10.4 mmol) gave 46% (3 steps) yield of the title compound **9** (565 mg) as colorless solids, m.p. 215–220°C dec (lit,[12] 228°C dec). The optical purity (14%ee) was determined by HPLC. The spectral data were identical with those reported by us.[4]

$^1$H NMR ($D_2O$) δ 1.97 (dd, $J = 7$ and 7 Hz, 1 H), 2.05 (dd, $J = 7$ and 7 Hz, 1 H), 3.32 (d, $J = 7$ Hz, 1 H), 4.13 (d, $J = 7$ Hz, 1 H).

*(2S,3S,4R)-[3,4,5,5,5-$^2H_5$]Leucine* **(10)**

According to the procedure described in our previous paper,[5,6] ring-opening, reduction of the terminal carboxyl group, and deprotection of compound **7b** (1.14 g, 3.78 mmol) gave 27% (5 steps) yield of the title compound **10** (140 mg) as colorless solids, m.p. $> 250^\circ$C (lit,[13] $> 300^\circ$C). The optical purity (18%ee) was determined by HPLC. $^1$H NMR (5% NaOD in $D_2O$) δ 0.89 (s, 3 H), 1.36 (d, $J = 8$ Hz, 1 H), 3.26 (d, $J = 8$ Hz, 1 H). $^{13}$C NMR (5% NaOD in $D_2O$) δ 24.80, 24.92 (m), 26.16 (t, $J = 19$ Hz), 46.22 (t, $J = 19$ Hz), 57.07, 187.12. HRMS (EI, 70 eV) $m/z$ 137.1317 [$(M + H)^+$, calculated for $C_6H_9D_5NO_2$ 137.1338].

**Acknowledgements**

This work has been supported by CREST (Core Research for Evolutional Science and Technology) of Japan Science and Technology Corporation (JST). We also thank Professor Masatsune Kainosho of Tokyo Metropolitan University for helpful discussions.

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