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Research Article

Asymmetric synthesis of L-[4-^{13}C]glutamic acid and glutamine

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Summary

L-[4-^{13}C]Glutamic acid (**1**) and L-[4-^{13}C]glutamine (**2**) were synthesized from sodium [2-^{13}C]acetate (**5**) and Dellaria's oxazinone **3** as a chiral glycine equivalent. Sodium [2-^{13}C]acetate (**5**) was converted to [2-^{13}C]acrylate **4**. Diastereoselective Michael addition of the enolate of **3** to the acrylate **4** proceeded with high diastereoselectivity to give the adduct **12**. Reductive cleavage of the C–S bond, ethanolysis, hydrogenolysis and hydrolysis gave L-[4-^{13}C]glutamic acid (**1**). L-[4-^{13}C]Glutamine (**2**) was synthesized from **1** in 4 steps. Copyright © 2006 John Wiley & Sons, Ltd.

Key Words: L-[4-^{13}C]glutamic acid; L-[4-^{13}C]glutamine; labeled amino acid; chiral glycine equivalent; oxazinone

Introduction

Glutamic acid is an important chemical mediator in the brain, and metabotropic glutamate receptors modulate neuronal function. Glutamic acid discharged from the neurons is collected by astrocytes, and metabolized to glutamine. Glutamine is then returned to the neurons, where it is reconverted into glutamic acid, and used for neurotransmission.[1] L-[4-^{13}C]Glutamic acid (**1**) and L-[4-^{13}C]glutamine (**2**) are required for detailed NMR studies of this glutamate–glutamine cycle. We have already reported asymmetric syntheses of various specifically ^{13}C-labeled amino acids from Dellaria's oxazinone **3**[2] or its ^{13}C-labeled form as a chiral optically active glycine equivalent.[3–6] In this paper, we describe an asymmetric chemical synthesis of L-[4-^{13}C]glutamic acid (**1**) and L-[4-^{13}C]glutamine (**2**) by diastereoselective Michael addition of the oxazinone **3** to ^{13}C-labeled acrylate **4** bearing a phenylsulfonyl group as an

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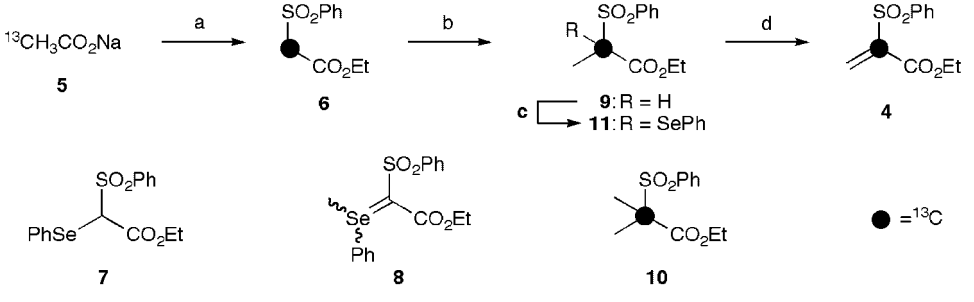
electron-withdrawing group to inhibit polymerization in the Michael reaction. Enzymatic or chemo-enzymatic syntheses of **1** have already been reported by other groups.[7,8]

Results and discussion

The ^{13}C-labeled acrylate **4** corresponding to C3–C5 of glutamic acid was prepared from sodium [2-^{13}C]acetate (**5**) (Scheme 1). Sodium [2-^{13}C]acetate (**5**, 99 at.% ^{13}C) was converted to ethyl bromo[2-^{13}C]acetate by using the method previously reported,[4,9] and then treatment with sodium benzenesulfinate gave ethyl 2-(phenylsulfonyl)[2-^{13}C]acetate (**6**) in 78% yield from **5**. Conversion from **6** to **4** was performed in three steps: methylation, selenylation and oxidative elimination of the selenyl group. The order of methylation and selenylation was investigated using non-labeled **6**. Initially, selenylation of the non-labeled form of **6** was carried out by treatment with phenylselenyl bromide and NaH in THF-HMPA, and gave **7** in quantitative yield. Subsequent methylation with NaH and methyl iodide in THF unfortunately afforded the ylide **8**, methylated at the selenyl group, in quantitative yield. Therefore, the conversion from **6** to **4** was conducted by applying methylation first, then selenylation. Methylation of **6** was performed by enolization with NaH in THF followed by addition of the resulting enolate to excess methyl iodide in THF at 0°C, to give the desired ethyl 2-(phenylsulfonyl)[2-^{13}C]propionate (**9**) in 69% yield together with the over-methylated product **10** in 12% yield and the recovered starting material **6** in 14% yield. Subsequent selenylation of **9** with NaH and phenylselenyl bromide in HMPA-THF gave **11** in 88% yield.[10] Oxidative elimination of the selenyl group with H_2O_2 gave the ^{13}C-labeled acrylate **4** in quantitative yield.

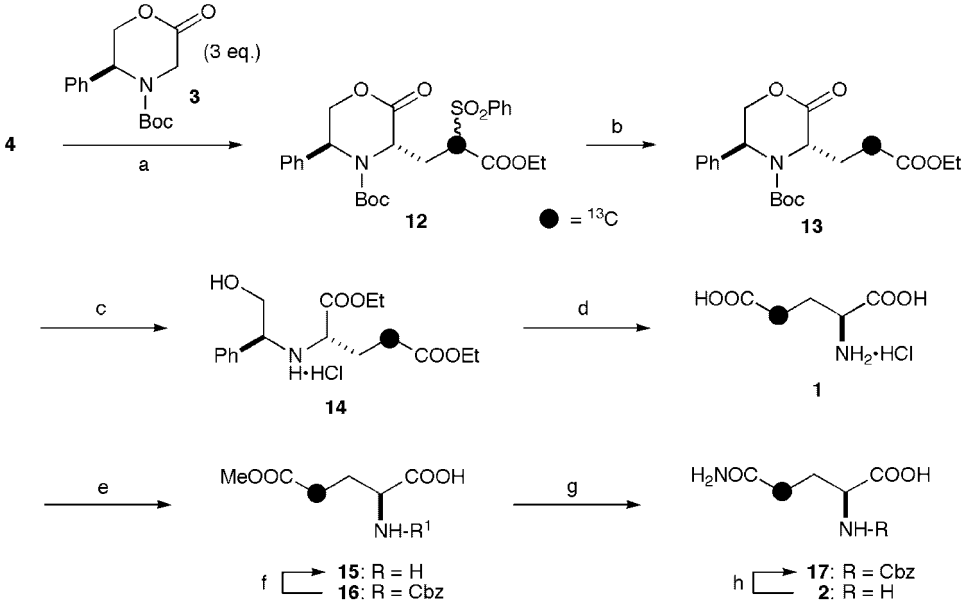
Michael addition of Dellaria's oxazinone **3** to the ^{13}C-labeled acrylate **4** was performed by enolization of excess **3** with sodium bis(trimethylsilyl)amide (NaHMDS) in THF, followed by addition of **4** at −78°C to give the



Scheme 1. Reagents and conditions: (a) (i) PhCOBr, $PhCO_2H$, (ii) Br_2, (iii) EtOH, (iv) $PhSO_2Na$, 78% from 5; (b) NaH, MeI, 69%; (c) NaH, PhSeBr, HMPA, 88%; (d) H_2O_2, 0°C quant

corresponding Michael adduct **12** as a 1:1 diastereomeric mixture relative to the phenylsulfonyl group in 49% yield from **11** (Scheme 2). Reductive cleavage of the C–S bond of **12** with SmI_2 gave **13** as a single isomer in 65% yield. Ethanolysis of the oxazinone ring and removal of the Boc group of **13** were simultaneously carried out by heating at 85°C in ethanol-saturated HCl gas to give **14**. Hydrogenolysis for removal of the chiral auxiary of **14** with catalytic $Pd(HO)_2$ in ethanol gave the diethyl glutamate. Hydrolysis with HCl gave L-[4-^{13}C]glutamic acid hydrochloride (**1**) in 49% yield from **13**. The enrichment ratio was 98 at.% ^{13}C, determined from the integration ratio of the signals at 2.49 ppm, assigned to the 4-position of **1** and the residual non-labeled form, in the ^{1}H-NMR spectrum.

The resulting L-[4-^{13}C]glutamic acid hydrochloride (**1**) was converted to ^{13}C-glutamine **2** using the following sequence. Selective esterification of **1** in dry methanol and protection of the amino group of **15** with Cbz-Cl gave **16** in 65% yield. Transamidation of **16** with ammonium hydroxide and removal of the Cbz group of **17** by hydrogenolysis gave L-[4-^{13}C]glutamine (**2**) in 48% yield. The ^{13}C-NMR spectrum of **2** showed the enriched signal at 33.6 ppm, assigned to the 4-position of glutamine. The optical purity was >96% ee and the absolute configuration was L, as determined by HPLC-CD analysis using a chiral column.[11]



Scheme 2. Reagents and conditions: (a) NaHMDS then 4, THF, −78°C, 49% from 11; (b) SmI_2, 65%; (c) saturated HCl-EtOH; (d) (i) H_2, $Pd(OH)_2$, (ii) 6M HCl, 49% from 13; (e) MeOH, 97%; (f) Cbz-Cl, Na_2CO_3, 67%; (g) NH_4OH, 62%; (h) H_2, Pd-C, 78%

In summary, we have synthesized L-glutamic acid (**1**) and L-glutamine (**2**) ^{13}C-labeled at the 4-position, from sodium [2-^{13}C]acetate (**5**) by using diastereoselective Michael addition of Dellaria's oxazinone **3** to the acrylate **4**. Compounds **1** and **2** will be useful for biochemical experiments.

Experimental

Sodium [2-^{13}C]acetate (**5**, 99 at.% ^{13}C) was supplied by Cambridge Isotope Laboratories. Melting points were measured on a Yanaco micro melting point apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Gemini-2000 (^{1}H, 300 MHz: ^{13}C, 75.4 MHz) Fourier-transform spectrometer. The chemical shifts are reported in δ values relative to tetramethylsilane (TMS) at 0 ppm in $CDCl_3$ and [1,l,l,3,3,3-D_6]acetone, or sodium 3-(trimethylsilyl)[2,2,3,3-D_4]propionate (TSP) at 0 ppm in D_2O for ^{1}H-NMR, and relative to $CDCl_3$, at 77.0 ppm, [1,1,1,3,3,3-D_6]acetone at 29.8 ppm or TSP at 0 ppm in D_2O for ^{13}C-NMR. IR spectra were recorded on a JASCO VALOR-III Fourier-transform spectrometer. EI- and HR-FAB-MS were obtained with a JEOL JMS-700 double-focusing spectrometer. CD spectra were recorded on a JASCO J-720 CD spectrometer. HPLC-CD analysis was carried out on a JASCO 800 Series HPLC system with a JASCO 875-UV detector and a JASCO J-720 CD spectrometer as detectors. The column was a Crown Pak CR(-) column (150 mm × 4 mm i.d.), purchased from Daicel.

Ethyl 2-(phenylsulfonyl)[2-^{13}C]acetate (**6**)

Ethyl bromo[2-^{13}C]acetate was prepared from sodium [2-^{13}C]acetate (**5**, 5.01 g, 60.3 mmol) by using the method previously reported.[4,9] To a solution of benzenesulfinate dihydrate (13.29 g, 66.4 mmol) in DMF (50 ml) was added dropwise a solution of the crude ethyl bromo[2-^{13}C]acetate in DMF (18 ml) at room temperature, and the mixture was stirred for 3 d. The mixture was diluted with ether, and the solution was washed with water 5 times. The combined aqueous layers were extracted with ether 5 times. The combined organic layers were washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (hexane : ethyl acetate = 1:1) to give the ethyl 2-(phenylsulfonyl)[2-^{13}C]acetate **6** (11.80 g) in 78% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.20 (t, J = 7.1 Hz, 3H), 4.11 (d, J_{C-H} = 140.1 Hz, 2H), 4.15 (q, J = 7.1 Hz, 2H), 7.59 (m, 2H), 7.70 (m, 1H), 7.96 (m, 2H). ^{13}C-NMR ($CDCl_3$) δ: 61.0. IR (neat) cm^{-1}: 3067, 2988, 2940, 1741, 1449, 1327, 1312, 1276, 1155, 1085, 1025. HR-MS calculated for $C_9^{13}CH_{12}O_4S$: m/z = 229.0490. Found: m/z = 229.0488 (M^+). EI-MS m/z (%): 229 (M^+, 0.4), 184 (21), 165 (100), 141 (97), 92 (88), 77 (76).

Ethyl 2-(phenylsulfonyl)[2-^{13}C]propionate (**9**)

To a stirred suspension of NaH (60% dispersion in mineral oil, 1.16 g, 29.00 mmol) in THF (70 ml) was added dropwise at 0°C over 30 min a solution of **6** (7.00 g, 30.55 mmol) in THF (15 ml). The suspension was stirred for 10 min, and the precipitate was dissolved by addition of further THF (42 ml). The solution was added dropwise to a stirred solution of methyl iodide (48 ml, 0.77 mol) in THF (53 ml) over 1.5 h at 0°C through a cannula. The suspension was stirred at 4°C overnight. The reaction was quenched with saturated NH_4Cl. The aqueous layer was extracted with ether. The combined organic layers were washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (hexane : ethyl acetate = 5:1) to give **9** (5.13 g) in 69% yield. ^{1}H-NMR ($CDCl_3$,) δ: 1.20 (t, $J = 7.1$ Hz, 3H), 1.58 (dd, $^2J_{C-H} = 4.7$ Hz, $J = 7.1$ Hz, 3H), 4.05 (dq, $J_{C-H} = 140.9$ Hz, $J = 7.1$ Hz, 1H), 4.12 (q, $J = 7.1$ Hz, 2H), 7.58 (m, 2H), 7.69 (m, 1H), 7.91 (m, 2H). ^{13}C-NMR ($CDCl_3$) δ: 65.4. IR (neat) cm^{-1}: 2986, 2943, 1737, 1449, 1323, 1311, 1150. HR-MS Calculated for $C_{10}\,^{13}CH_{14}O_4S$: $m/z = 243.0647$. Found: $m/z = 243.0653$ (M^+). EI-MS m/z (%): 243 (M^+, 0.3), 198 (20), 187 (20), 179 (81), 159 (22), 141 (72), 125 (22), 106 (34), 77 (100).

Ethyl 2-(phenylselenyl)-2-(phenylsulfonyl)[2-^{13}C]propionate (**11**)

To a stirred suspension of NaH (60% dispersion in mineral oil, 1.27 g, 31.64 mmol) in THF (64 ml) was added dropwise at 0°C over 30 min a solution of **9** (5.13 g, 21.09 mmol) in THF (29 ml). The mixture was stirred for 10 min, HMPA (4.4 ml, 25.3 mmol) was added dropwise over 10 min, and the mixture was stirred for 30 min. A solution of phenylselenyl bromide (5.47 g, 23.2 mmol) in THF (29 ml) was added dropwise at 0°C over 30 min, and the mixture was stirred for 30 min. The reaction was quenched with saturated NH_4Cl, and the aqueous layer was extracted with ether. The combined organic layers were washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (hexane : ethyl acetate = 1:1) to give **11** (7.36 g) in 88% yield, mp. 96.2–97.3°C. ^{1}H-NMR ($CDCl_3$) δ: 1.19 (t, $J = 7.1$ Hz, 3H), 1.65 (d, $^2J_{C-H} = 5.0$ Hz, 3H), 4.08 (q, $J = 7.1$ Hz, 2H), 7.33 (m, 2H), 7.44 (m, 1H), 7.55 (m, 2H), 7.65–7.73 (m, 3H), 7.98 (m, 2H). ^{13}C-NMR ($CDCl_3$) δ: 70.7. IR (KBr) cm^{-1}: 3010, 2976, 2933, 1723, 1447, 1325, 1307, 1233, 1153, 1083, 752, 696. HR-MS Calculated for $C_{16}\,^{13}CH_{18}O_4SSe$: $m/z = 399.0125$. Found: $m/z = 399.0131$ (M^+). EI-MS m/z (%): 399 (M^+, 0.01), 258 (100), 212 (32), 184 (51), 125 (13), 104 (10), 77 (30).

Ethyl 2-(phenylsulfonyl)[2-^{13}C]acrylate (**4**)

To a stirred solution of selenylpropionate **11** (4.26 g, 10.69 mmol) in CH_2Cl_2 (107 ml) was added H_2O_2 (30%, 3.05 ml, 26.85 mmol) at 0°C.

The heterogeneous mixture was stirred vigorously 15 min and cold saturated $NaHCO_3$ was added at 0°C. The aqueous layer was extracted with cold CH_2Cl_2. The combined organic layers were dried over Na_2SO_4, and the solvent was removed at 0°C under reduced pressure to give the acrylate **4** (2.75 g) in quantitative yield. ^{1}H-NMR ($CDCl_3$) δ: 1.12 (t, $J = 7.1$ Hz, 3H), 4.18 (q, $J = 7.1$ Hz, 2H), 7.03 (d, $^2J_{C-H} = 0.8$ Hz, 1H), 7.15 (d, $^2J_{C-H} = 3.8$ Hz, 1H), 7.55 (m, 2H), 7.64 (m, 1H), 7.98 (m, 2H). ^{13}C-NMR ($CDCI_3$) δ: 143.4. IR (neat) cm^{-1}: 1336, 3068, 2986, 2940, 1728, 1586, 1449, 1322, 1268, 1160, 1079, 748, 688.

(3S,5S)-tert-Butyl 3-(3-ethoxy-3-oxo-2-(phenylsulfonyl)[2-^{13}C]propyl)-2-oxo-5-phenylmorpholine-4-carboxylate (**12**)

To a solution of oxazinone **3** (8.94 g, 32.22 mmol) in THF (110 ml) was added dropwise NaHMDS (1 M solution in hexane, 31.1 ml, 31.1 mmol) at −78°C under nitrogen. The mixture was stirred for 1 h, then a solution of the acrylate **4** (2.75 g, 11.40 mmol) in THF (34 ml) was added dropwise over 30 min through a cannula. The reaction mixture was stirred at −78°C for 1 h. The reaction was quenched with saturated NH_4Cl, and the mixture was extracted five times with ether. The combined organic layers were washed with saturated NaCl, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (hexane : ethyl acetate = 5:1) to give **12** (2.75 g) as a 1:1 diastereomeric mixture in 49% yield from **11**. ^{1}H-NMR ($CDCl_3$, 55°C) δ: 1.07–1.34 (m, 12H), 2.50–3.03 (m, 2H), 3.95–4.60 (m, 4H), 4.80–5.09 (m, 3H), 7.04 (m, 2H), 7.25–7.39 (m, 3H), 7.57 (m, 2H), 7.69 (m, 1H), 7.89 (m, 2H). mp. 119.2–120.4°C. ^{13}C-NMR ($CDCI_3$, 55°C) δ: 66.8, 67.61. IR (KBr) cm^{-1}: 2980, 2932, 1753, 1701, 1450, 1371, 1326, 1261, 1149, 1125, 1084, 1047, 1028. HR-MS Calculated for $C_{25}\,^{13}CH_{31}NO_8S$: $m/z = 518.1804$. Found: $m/z = 518.1799$ (M^+). EI-MS m/z (%): 518 (M^+, 1), 418 (36), 231 (43), 104 (100), 77 (13).

3-(3-ethoxy-3-oxo[2-^{13}C]propyl)-2-oxo-5-phenylmorpholine-4-carboxylate (**13**). To a 0.1 M solution of SmI_2 in THF (700 ml) was added dropwise over 1 h through a cannula a solution of **12** (2.99 g, 5.77 mmol) in a mixture of THF (42 ml) and MeOH (4.8 ml) at 0°C. The mixture was stirred for 30 min, and 20% K_2CO_3, (70 ml) was added. The mixture was filtered through Celite. The aqueous layer of the filtrate was separated and extracted with ether. The combined organic layers were washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel (hexane : ethyl acetate = 2:1) to give **13** (1.43 g) in 65% yield, mp. 135.2–136.0°C. ^{1}H-NMR ($CDCl_3$, 55°C) δ: 1.26 (t, $J = 7.1$ Hz, 3H, and s, 9H), 2.17 (m, 1H), 2.32–2.57 (m, 1H), 2.56 (dt, $J_{C-H} = 129.6$ Hz, $J = 7.1$ Hz, 2H), 4.07–4.23 (m, 2H), 4.40 (dd, $J = 1.1, 11.8$ Hz), 4.80–4.88 (m, 1H), 4.86 (dd, $J = 3.0$, 11.8 Hz, 1H),

5.02 (m, 1H), 7.07 (m, 2H), 7.23–7.36 (m, 3H). ^{13}C-NMR ($CDCl_3$, 55°C) δ: 30.6. IR (KBr) cm^{-1}: 2978, 2934, 1741, 1684, 1486, 1471, 1457, 1450, 1375, 1336, 1301, 1250, 1218, 1194, 1122, 1098, 1080, 888. HR-MS Calculated for C_{19} $^{13}CH_{27}NO_6$: $m/z = 378.1872$. Found: $m/z = 378.1866$ (M^+). EI-MS m/z (%): 378 (M^+, 5), 278 (51), 233 (22), 104 (100), 57 (39).

L-*[4-^{13}C]Glutamic acid hydrochloride* (**1**)

A solution of **13** (1.43 g, 3.77 mmol) in saturated HCl in ethanol (18 ml) was stirred at 85°C overnight, and the mixture was evaporated. The residual diethyl ester **14** was dissolved in EtOH (18 ml), and 20% $Pd(OH)_2$ (2.65 g, 3.77 mmol) was added. The mixture was stirred at room temperature overnight under a hydrogen atmosphere. The catalyst was removed by filtration through Celite, and the filtrate was evaporated to give diethyl glutamate. The glutamate was dissolved in 6 M HC1 (60 ml). The mixture was heated at 80°C for 12 h, then washed with chloroform and ether. The aqueous layer was evaporated, and the residue was chromatographed on Amberlite IRA904 resin (OH form) with **1** M HCl to give 1 (343 mg) in 49% yield from **13**. ^{1}H-NMR (D_2O) δ: 1.99–2.16 (m, 2H), 2.49 (dt, $J_{C-H} = 128.6$ Hz, $J = 7.1$ Hz, 2H), 3.89 (dt, $^3J_{C-H} = 4.4$ Hz, $J = 6.6$ Hz, 1H). ^{13}C-NMR (D_2O) δ: 32.4. IR (KBr) cm^{-1}: 3379, 3200–2200 (br), 1725, 1671, 1612, 1509, 1421, 1275, 1254, 1213, 1078, 996, 824. HR-FAB-MS (glycerol) Calculated for C_4 $^{13}CH_{10}NO_4$: $m/z = 149.0643$. Found: $m/z = 149.0637$ (MH^+).

L-*[4-^{13}C]Glutamic acid 5-methyl ester hydrochloride* (**15**)

A solution of L-[4-^{13}C]glutamic acid hydrochloride (**1**, 220 mg, 1.19 mmol) in dry MeOH (5 ml) was stirred at room temperature for 42 h. The mixture was evaporated to give the 5-methyl ester **15** (230 mg) in 97% yield. ^{1}H-NMR (D_2O) δ: 2.02–2.15 (m, 2H), 2.49 (dt, $J_{C-H} = 129.4$ Hz, $J = 7.4$ Hz, 2H), 3.62 (s, 3H), 3.73 (m, 1H). ^{13}C-NMR (D_2O) δ: 32.5. IR (KBr) cm^{-1}: 3700–2300 (br), 1725, 1637, 1509, 1441, 1221. HR-FAB-MS (glycerol) Calculated for C_5 $^{13}CH_{12}NO_4$,: $m/z = 162.0800$. Found: $m/z = 162.0799$ (MH^+).

L-*N-Benzyloxycarbonyl[4-^{13}C]glutamic acid 5-methyl ester* (**16**)

Cbz-Cl (0.19 ml. 1.32 mmol) was added to a solution of **15** (218 mg, 1.10 mmol) in water (1 ml) and acetonitrile (1.5 ml). To the mixture was added dropwise a solution of Na_2CO_3 (181 mg, 1.70 mmol) in water (1.5 ml) over 45 min at room temperature. The mixture was vigorously stirred for 1 h, then further Cbz-Cl (0.15 ml, 1.05 mmol) and a solution of Na_2CO_3 (135 mg, 1.27 mmol) in water (2.0 ml) was added. The mixture was stirred for 1 h, and evaporated to remove acetonitrile. The mixture was washed with ether. The organic layer was extracted with water 3 times. The combined

aqueous layers were acidified with 3 M HC1, and extracted with ethyl acetate 5 times. The combined organic layers were washed with brine, dried over Na_2SO_4, and evaporated to give **16** (216 mg) in 67% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.90–2.70 (m, 4H), 3.67 (s, 3H), 4.43 (m, 1H), 5.12 (s, 2H), 5.54 (d, $J = 7.1$ Hz, 1H), 7.25–7.37 (m, 5H). ^{13}C-NMR ($CDCl_3$) δ: 30.1. IR (neat) cm^{-1}: 3333, 3034, 2955, 1736, 1531, 1440, 1256, 1251, 1177, 1050. HR-MS Calculated for $C_{13}\,{}^{13}CH_{17}NO_6$: $m/z = 296.1089$. Found: $m/z = 296.1095$ (M^+). EI-MS m/z (%): 296 (M^+, 5), 251 (6), 207 (12), 108 (48), 91 (100), 85 (19), 79 (17).

L-*N*-*Benzyloxycarbonyl[4-^{13}C]glutamine* (**17**)

The 5-methyl ester **16** (214 mg, 0.712 mmol) was dissolved in concentrated ammonium hydroxide (5 ml), and the solution was stirred for 4 h at room temperature. The solution was evaporated, the residue was dissolved in water, and the solution was acidified with 3 M HCl. The mixture was extracted with ethyl acetate 5 times. The combined organic layers were washed with brine, dried over Na_2SO_4, and evaporated to give the amide **17** (125 mg) in 62% yield. ^{1}H-NMR ([l,l,l,3,3,3-D_6]acetone) δ: 1.90–2.70 (m, 4H), 4.26 (m, 1H), 5.08 (s, 2H), 6.29 (brs, 1H), 6.77 (d, $J = 7.4$ Hz, 1H), 6.91 (brs, 1H), 7.25–7.40 (m, 5H). ^{13}C-NMR ([l,l,l,3,3,3-D_6]acetone) δ: 32.1. IR (KBr) cm^{-1}: 3331, 3067, 3034, 2959, 1711, 1655, 1541, 1455, 1418, 1348, 1248, 1055. HR-MS Calculated for $C_{12}\,{}^{13}CH_{16}N_2O_5$: $m/z = 281.1093$. Found: $m/z = 281.1105$ (M^+). EI-MS m/z (%): 281 (M^+, 4), 263 (7), 236 (6), 174 (18), 129 (13), 108 (55), 91 (100), 85 (25), 79 (21).

L-*[4-^{13}C]Glutamine* (**2**)

A mixture of **17** (118 mg, 0.418 mmol) and 10% Pd-C (44 mg, 0.042 mmol) in dry MeOH (5 ml) was stirred for 4 h at room temperature under a hydrogen atmosphere. The catalyst was removed by filtration through Celite, and the Celite pad was washed with water. The combined filtrates were evaporated. The residue was treated with activated carbon in water, then evaporated to give crude L-[4-^{13}C]glutamine (**2**). Crystallization from water : EtOH = 1:5 gave **2** (48 mg) in 78% yield, mp. 172.4–173.1°C. ^{1}H-NMR (D_2O) δ: 1.99–2.19 and 2.54–2.62 (m, 4H), 3.67 (dt, $^3J_{C-H} = 4.9$ Hz, $J = 6.0$ Hz, 1H). ^{13}C-NMR (D_2O) δ: 33.6. IR (KBr) cm^{-1}: 3411, 3300–2200 (br), 1686, 1638, 1630, 1483, 1412, 1333, 1315, 1160. HR-FAB-MS (glycerol) Calculated for $C_4\,{}^{13}CH_{11}N_2O_3$: $m/z = 148.0803$. Found: $m/z = 148.0800$ (MH^+). CD (pH 1.5 $HClO_4$) λ nm (Δε): 208 (+1.61). HPLC-CD analysis: the eluent was pH 2.0 $HClO_4$, the flow rate was 0.8 ml/min, the detection wavelength was 208 nm (UV and CD), the temperature was 0°C, and other conditions were as

described in a previous report,[11] Rt = 2.0 min (authentic D-form gave Rt = 4.3 min).

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