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A simple convenient synthesis of L-[4-^{13}C] glutamine

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L-[4-^{13}C]Glutamine was synthesized from sodium [2-^{13}C]acetate in 12 steps and 18% overall yield. A Wittig reaction of (R)-benzyl 4-formyl-2,2-dimethyloxazolidine-3-carboxylate and ethyl 2-(triphenylphosphoranylidene)[2-^{13}C]acetate prepared from D-serine and sodium [2-^{13}C]acetate, respectively, gave (4S)-4-(2-ethoxycarbonyl[2-^{13}C]vinyl)-2,2-dimethyloxazolidine-3-carboxylic acid α,β-isopropylidene group, oxidation of the resulting hydroxyl group to a carboxyl group and transamidation of the ester moiety gave L-*N*-Cbz-[4-^{13}C]glutamine (Cbz = benzyloxycarbonyl). Finally, removal of the Cbz group gave L-[4-^{13}C]glutamine. L-[4-^{13}C]Glutamine can be prepared in fewer steps and higher yield by this method compared with previously reported methods.

Keywords: stable labeled synthesis; C-13; glutamine; serine; amino acid; Wittig reaction

Introduction

Glutamic acid (glutamate) is a major excitatory neurotransmitter that regulates high-level cognitive functions such as memory and learning in the mammalian central nervous system.[1] An excessive extracellular concentration of glutamate causes neuronal cytotoxicity called glutamate excitotoxicity through excessive activation of glutamate receptors and has been implicated in many psychiatric disorders.[2] Glutamate released from neurons is taken up by astrocytes around the synapse and is metabolized to glutamine. Glutamine is then sent from astrocytes to neurons, converted into glutamate again, and used for neurotransmission.[3] This neuron–astrocyte/glutamate–glutamine cycle is usually studied by using ^{13}C-labeled glucose[4-8] or uniform-^{13}C-labeled glutamic acid and glutamine.[9-11] For detailed in vivo nuclear magnetic resonance (NMR) study of this cycle, for example, to confirm that glutamine is used as an energy source in neurons, single-^{13}C-labeled L-glutamine (**1**) is required. To monitor the metabolic turnover of the labeled carbon during the tricarboxylic acid cycle, labeling at the 4-position of glutamine is preferable because this position is converted to a methylene carbon that produces a strong ^{13}C-NMR signal without ^{13}C–^{13}C coupling in the uniform-^{13}C-labeled form.

We have already reported asymmetric syntheses of various specific ^{13}C-labeled amino acids from Dellaria's oxazinone[12] or its ^{13}C-labeled form as the chiral optically active glycine equivalent.[13-16] For example, asymmetric syntheses of L-[4-^{13}C] glutamic acid and L-[4-^{13}C]glutamine (**1**) were achieved by using diastereoselective Michael addition of the oxazinone to a ^{13}C-labeled acrylate, which was prepared from sodium [2-^{13}C]acetate in a 16 total steps and 2% overall yield.[17] In this method, it is possible to label L-glutamine with ^{13}C at specific positions—C1, C2, C4, and/or C5—but many steps are required, including the laborious preparation of labeled acrylate for ^{13}C labeling at just the 4-position. Here, we describe the efficient and convenient synthesis of L-[4-^{13}C]glutamine (**1**) for specific labeling at the 4-position. In our synthetic plan, the glutamine carbons originate from unlabeled D-serine (**2**) and sodium acetate, which is a commercially available ^{13}C-labeled compound. In the proposed method, the reactions are simple, and glutamine can be efficiently prepared in higher yield and fewer steps than in the previous method.

Results and discussion

Oxazolidine **5** was synthesized using a known method[18] starting from D-serine (**2**) (Scheme 1). The ester moiety in oxazolidine **5** was reduced to an aldehyde with diisobutylaluminium hydride, giving **6**. Phosphonium ylide **3** was derived from sodium [2-^{13}C]acetate (**4**, 99 atom % ^{13}C) via ethyl bromo[2-^{13}C]acetate in 68% yield from **4** by using a previously reported method.[16] The Wittig reaction of ^{13}C-labeled phosphonium ylide **3** and aldehyde **6** gave α,β-unsaturated ester **7** in 57% yield based on **3**. 1,4-Reduction of α,β-unsaturated ester **7** with $NaBH_4$ and $CoCl_2$, removal of the isopropylidene group in **8**, followed by oxidation of the resulting hydroxy group in **9,** gave L-*N*-Cbz-[4-^{13}C]glutamic acid 5-ethyl ester (**10**; Cbz = benzyloxycarbonyl) in 80% yield over three steps. The ester moiety in **10** was

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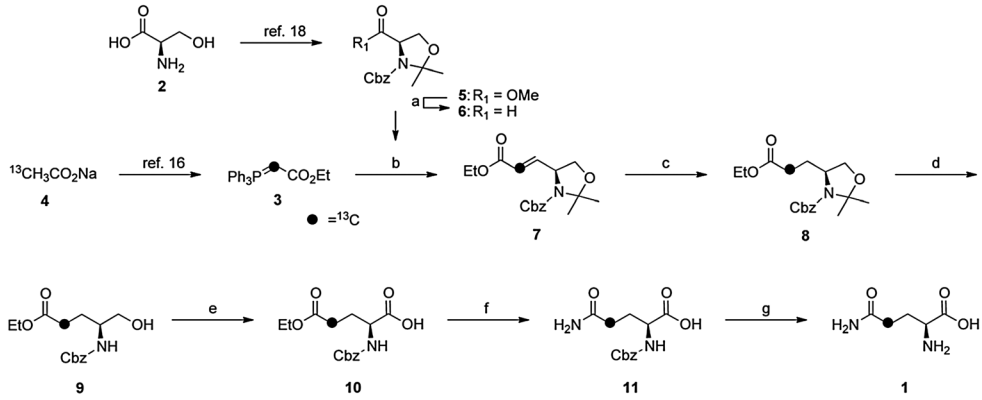

Scheme 1. Reagents and conditions. (a) diisobutylaluminium hydride, 91%; (b) benzene, 57% from **3**; (c) $NaBH_4$, $CoCl_2$·$6H_2O$, 94%; (d) TsOH, H_2O, 90%; (e) Jones reagent, 94%; (f) NH_4OH, 83%; (g) H_2, Pd-C 70%.

transamidated with ammonium hydroxide, and removal of the Cbz group of the resulting L-*N*-Cbz-[4-^{13}C]glutamime (**11**) by hydrogenolysis gave L-[4-^{13}C]glutamine (**1**) in 58% yield over two steps. The ^{13}C-NMR spectrum of **1** showed an enriched signal at 33.7 ppm, which was assigned to the 4-position of glutamine (Figure 1). The optical purity was 99% ee, and the absolute configuration was L, as determined by HPLC-circular dichroism (CD) analysis using a chiral column.[19]

Conclusion

In summary, we have synthesized L-[4-^{13}C]glutamine (**1**) from sodium [2-^{13}C]acetate (**4**) and D-serine (**2**) by using the Wittig reaction in 18% overall yield over 12 steps from **4**. This method has better efficiency than the previous methods in terms of the yield and number of steps for specific labeling at the 4-position of L-glutamine. The product can be used for NMR studies of the glutamine metabolism in the brain.

Experimental

Sodium [2-^{13}C]acetate (99 atom % ^{13}C) was supplied by Cambridge Isotope Laboratories. Melting points were measured on a Yanaco micro melting point apparatus (Yanaco New Science Inc., Nishiokenoi-cho, Takeda, Fushimi-ku, Kyoto, Japan) and were uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a JEOL JMN-Lambda-500 (^{1}H: 500 MHz; ^{13}C: 125 MHz) (JEOL Ltd., Akishima, Tokyo, Japan). The chemical shifts are reported as δ values relative to tetramethylsilane at 0 ppm in $CDCl_3$, to acetone at 2.04 ppm in CD_3COCD_3, or to sodium 3-trimethylsilylpropionate-d_4 (TSP) at 0 ppm in D_2O for ^{1}H-NMR and relative to $CDCl_3$ at 77.0 ppm, to CD_3COCD_3 at 29.8 ppm, or to TSP at 0 ppm in D_2O for ^{13}C-NMR. Infrared spectra (IR) were recorded on a JASCO FT/IR 4100 Series spectrometer (JASCO Corporation, Ishikawamachi, Hachioji-shi, Tokyo, Japan). High resolution fast atom bombardment mass spectra (HR-FAB-MS) were performed with a JEOL JMS-700 double-focusing spectrometer. Purity was determined by HPLC-UV analysis (at 254 nm), shown by the percent region of interest for the UV area under the curve. HPLC-UV analysis was carried out on a JASCO LC-2000 Plus Series HPLC system with a JASCO PU-2089 Plus pump and a JASCO UV-2075 Plus UV spectrophotometer as a detector. The column was a COSMOSIL 5SL-II (250 × 4.6 mm i.d.) purchased from Nacalai Tesque (Nijo Karasuma, Nakagyo-ku, Kyoto, Japan) or a CAPCELL PAK C_{18} (150 × 4.6 mm i.d.) purchased from Frontier Science Business Division, Shiseido Co. Ltd. (Higashi-shimbashi, Minato-ku, Tokyo, Japan). HPLC-CD analysis was carried out on a JASCO 800 Series HPLC system with a JASCO J-720 CD spectrophotometer as a detector. The column was a Crown Pak CR(-) column (150 × 4 mm i.d.) purchased from Daicel (Umeda, Kita-ku, Osaka, Japan), and pH 2.0 $HClO_4$ was used as the eluent. The other conditions have been described previously.[19]

(4S)-4-(2-Ethoxycarbonyl[2-^{13}C]vinyl)-2,2-dimethyloxazolidine-3-carboxylic acid benzyl ester (7)

A 1.02 M solution of diisobutylaluminium hydride in toluene (9.80 mL, 10.0 mmol) was added dropwise to a stirred solution of ester **5**[18]

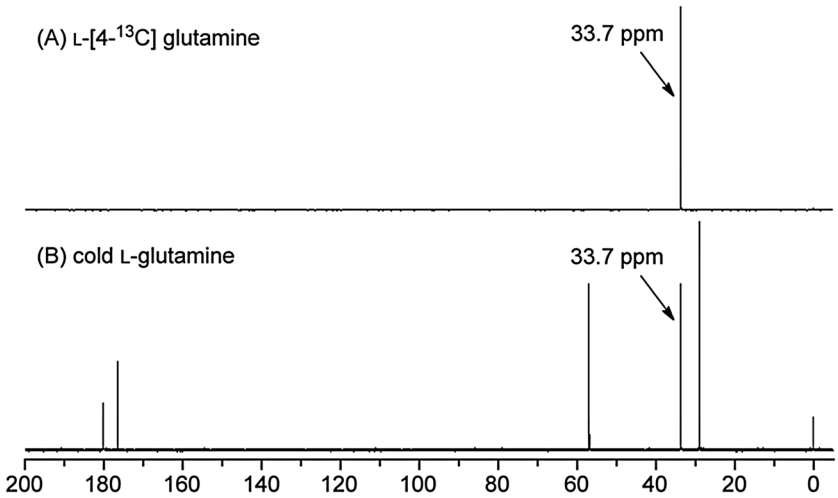

Figure 1. ^{13}C-nuclear magnetic resonance spectra of l-[4-^{13}C]glutamine (A) and cold l-glutamine (B).

(1.89 g, 6.44 mmol) in dry toluene (43 mL) over 10 min at −78 °C, and the mixture was then stirred for 1 h. The reaction was quenched with methanol (4.0 mL), and then diethyl ether (500 mL) and brine (10 mL) were added to precipitate the aluminous salt. The precipitate was filtered off, and the filtrate was evaporated. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate = 3:1 to 1:1) to give aldehyde **6** (1.54 g) in 91% yield.

^{13}C-Labeled phosphonium ylide **3** (1.82 g, 5.20 mmol) was added to a solution of **6** (1.43 g, 5.43 mmol) in benzene (16 mL). The mixture was stirred for 20 h at room temperature, and then a mixture of hexane and diethyl ether (1:1, 400 mL) was added. The precipitated phosphine oxide was filtered off, and the filtrate was evaporated. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate = 4:1 to 3:1) to give **7** (995 mg) in 57% yield from **3**. The purity was 98.7% (column: COSMOSIL 5SL-II; eluent: hexane/ethyl acetate = 4:1; flow rate: 0.7 mL/min; temperature: 25 °C; R_t = 13.7 min). ^{1}H-NMR ($CDCl_3$) δ: 1.30 (t, J = 7.1 Hz, 3H), 1.46–1.71 (m, 6H), 3.84 (dd, J = 2.1, 9.2 Hz, 1H), 4.11 (dd, J = 6.4, 9.2 Hz, 1H), 4.20 (q, J = 7.1 Hz, 2H), 4.47–4.72 (m, 1H), 5.02–5.22 (m, 2H), 5.84 and 5.99 (each dd, J_{C-H} = 163.3 Hz, J = 15.6 Hz and J_{C-H} = 162.7 Hz, J = 15.3 Hz, 1H, these signals originated from Cbz rotomers), 6.78–6.90 (m, 1H), 7.27–7.39 (m, 5H). ^{13}C-NMR ($CDCl_3$) δ: 122.8. IR (neat): 2985, 2941, 2883, 1757, 1709, 1634, 1407, 1350, 1264, 1179, 1094. HR-FAB-MS (glycerol) calcd for $C_{17}{}^{13}CH_{24}NO_5$: *m/z* 335.1688. Found: *m/z* 335.1689 (MH^+).

(4S)-4-(2-Ethoxycarbonyl[2-^{13}C]ethyl)-2,2-dimethyloxazolidine-3-carboxylic acid benzyl ester (8)

$CoCl_2{\cdot}6H_2O$ (172 mg, 0.722 mmol) was added to α,β-unsaturated ester **7** (965 mg, 2.89 mmol) dissolved in ethanol (20 mL), and the mixture was cooled to 0 °C. $NaBH_4$ (546 mg, 14.4 mmol) was added to the mixture, and the mixture was stirred at 0 °C for 1 h. The reaction was quenched with saturated NH_4Cl (2 mL). The catalyst was removed by filtration through Celite, and the Celite pad was washed with ethyl acetate (50 mL). The combined filtrates were evaporated to a volume of about 5 mL. The resulting mixture was diluted with ethyl acetate (200 mL) and washed with H_2O (20 mL) three times. The combined aqueous layers were extracted with ethyl acetate (150 mL) three times. The combined organic layers were washed with brine (50 mL) and dried over Na_2SO_4. After evaporation, the residue was chromatographed on silica gel (hexane/ethyl acetate = 4:1) to give **8** (908 mg) in 94% yield. The purity was 98.7% (column: COSMOSIL 5SL-II; eluent: hexane/ethyl acetate = 4:1; flow rate: 0.7 mL/min; temperature: 25 °C; R_t = 13.2 min). ^{1}H-NMR ($CDCl_3$) δ: 1.18–1.27 (m, 3H), 1.44, 1.51, 1.56, 1.64, (each s, total 6H, these signals originated from Cbz rotomers), 1.87–2.05 (m, 2H), 2.18–2.52 (m, 2H), 3.77 (d, J = 8.2 Hz, 1H), 3.93–4.17 (m, 4H), 5.06–5.21 (m, 2H), 7.28–7.39 (m, 5H). ^{13}C-NMR ($CDCl_3$) δ: 30.9. IR (neat): 3066, 3034, 2984, 2941, 2889, 1733, 1705, 1407, 1352, 1097, 1074 cm^{-1}. HR-FAB-MS (glycerol) calcd for $C_{17}{}^{13}CH_{26}NO_5$: *m/z* 337.1845. Found: *m/z* 337.1853 (MH^+).

(4S)-4-Benzyloxycarbonylamino-5-hydroxy[2-^{13}C]pentanoic acid ethyl ester (9)

Acetonide **8** (870 mg, 2.59 mmol) was dissolved in ethanol (90 mL), and *p*-toluenesulfonic acid (TsOH; 148 mg, 0.777 mmol) and purified water (0.47 mL) were added. The mixture was stirred at 60 °C for 22 h. The resulting mixture was cooled to room temperature, basified with saturated $NaHCO_3$ (10.0 mL), and evaporated to remove the ethanol. The residue was extracted with ethyl acetate (200 mL) three times. The combined organic layers were washed with brine (50 mL) and dried over Na_2SO_4. After evaporation, the residue was chromatographed on silica gel (hexane/ethyl acetate = 1:1) to give **9** (689 mg) in 90% yield. The purity was 99.8% (column: COSMOSIL 5SL-II; eluent: hexane/2-propanol = 4:1; flow rate: 0.7 mL/min; temperature: 25 °C; R_t = 9.0 min). ^{1}H-NMR ($CDCl_3$) δ: 1.23 (t, J = 7.1 Hz, 3H), 1.72–1.96 (m, 2H), 2.39 (dt, J_{C-H} = 128.2 Hz, J = 6.7 Hz, 2H), 2.66 (br, 1H), 3.46–3.74 (m, 3H), 4.12 (q, J = 7.0 Hz, 2H), 5.09 (s, 2H), 5.20 (each br, 3H), 7.27–7.37 (m, 5H). ^{13}C-NMR ($CDCl_3$) δ: 30.8. IR (KBr): 3313, 3068, 3034, 2973, 1728, 1686, 1546, 1259, 1188, 1060 cm^{-1}. HR-FAB-MS (glycerol) calcd for $C_{14}{}^{13}CH_{22}NO_5$: *m/z* 297.1532. Found: *m/z* 297.1532 (MH^+).

(2S)-2-Benzyloxycarbonylamino[4-^{13}C]pentanedioic acid 5-ethyl ester (10)

Alcohol **9** (644 mg, 2.18 mmol) was dissolved in acetone (93 mL), and the mixture was cooled to 0 °C. Jones reagent (2.67 M, 2.01 mL) was added, and the mixture was stirred at 0 °C for 1.5 h. The reaction was quenched with 2-propanol (0.6 mL) and neutralized with saturated $NaHCO_3$ (11.0 mL). The precipitate was removed by filtration through Celite, and the Celite pad was washed with ethyl acetate (50 mL). The combined filtrates were evaporated to about one-fifth of their original volume. The residue was diluted with ethyl acetate (25 mL) and extracted with $NaHCO_3$ aqueous solution (15 mL) prepared by threefold dilution of saturated $NaHCO_3$ aqueous solution. The combined aqueous layers were acidified with 3 M HCl (6.0 mL) and extracted with ethyl acetate (100 mL) five times, and the combined organic layers were washed with brine (30 mL), dried over Na_2SO_4, and evaporated. The residue was chromatographed on silica gel (chloroform/methanol = 10:1) to give **10** (634 mg) in 94% yield. The purity was 98.8% (column: CAPCELL PAK C_{18}; eluent: 20 mM sodium phosphate buffer (pH 2.5)/acetonitrile = 13:7; flow rate: 1.0 mL/min; temperature: 25 °C; R_t = 7.4 min). ^{1}H-NMR ($CDCl_3$) δ: 1.24 (m, 3H), 1.98–2.09 (m, 1H), 2.18–2.40 (m, 2H), 2.48–2.68 (m, 1H), 4.13 (q, J = 7.1 Hz, 2H), 4.42 (m, 1H), 5.11 (s, 2H), 5.53 (d, J = 7.3, 1H), 7.28–7.37 (m, 5H). ^{13}C-NMR ($CDCl_3$) δ: 30.3. IR (KBr): 3323, 3032, 2990, 1729, 1691, 1541, 1450, 1326, 1292, 1072 cm^{-1}. HR-FAB-MS (glycerol) calcd for $C_{14}{}^{13}CH_{20}NO_6$: *m/z* 311.1324. Found: *m/z* 311.1321 (MH^+).

Cbz-L-[4-^{13}C]Glutamine (11)

Carboxylic acid **10** (597 mg, 1.93 mmol) was dissolved in concentrated ammonium hydroxide (15.0 mL), and the solution was stirred for 5 h at room temperature. The solution was evaporated to a volume of about 5 mL and then acidified with 3 M HCl (3.0 mL). The mixture was extracted with ethyl acetate (20 mL) five times. The combined organic layers were washed with brine (5 mL), dried over Na_2SO_4, and evaporated. The residue was chromatographed on silica gel (hexane/ethyl acetate/acetic acid = 2:10:1) to give **11** (449 mg) in 83% yield. The purity was 98.0% (column: CAPCELL PAK C_{18}; eluent: 20 mM sodium phosphate buffer (pH 2.5)/acetonitrile = 13:7; flow rate: 1.0 mL/min; temperature: 25 °C; R_t = 8.2 min). ^{1}H-NMR (CD_3COCD_3, δ): 1.90–2.05 (m, 1H), 2.13–2.24 (m, 1H), 2.24–2.64 (m, J_{C-H} = 126.6 Hz, 2H), 4.18–4.33 (m, 1H), 5.07 (s, 2H), 6.52 (brs, 1H), 6.74 (d, J = 7.3 Hz, 1H), 7.02 (brs, 1H), 7.24–7.38 (m, 5H). ^{13}C-NMR (CD_3COCD_3) δ: 32.1. IR (KBr): 3465, 3336, 1752, 1691, 1529, 1454, 1412, 1339, 1305, 1276, 1244, 1215 cm^{-1}. HR-FAB-MS (glycerol) calcd for $C_{12}{}^{13}CH_{17}N_2O_5$: *m/z* 282.1171. Found: *m/z* 282.1172 (MH^+).

L-[4-^{13}C]Glutamine (1)

Cbz-L-[4-^{13}C]Glutamine (**11**, 411 mg, 1.46 mmol) was dissolved in dry methanol (15.0 mL), and 10% Pd-C (78 mg, 0.073 mmol) was added. The mixture was stirred for 1.5 h under 1 atm of hydrogen at room temperature. The catalyst was removed by filtration through Celite, and the Celite pad was washed with water (50 mL). The combined filtrates were evaporated to give crude L-[4-^{13}C]glutamine (**1**). Crystallization from water/EtOH (3.2 mL/7.6 mL) gave **1** (150 mg) in 70% yield. The purity was 99.2% as determined by an amino acid analyzer (Hitachi L-8800A Amino Acid Analyzer [Hitachi High-Tech Science Co., Nishishimbashi, Minato-ku, Tokyo, Japan]). mp 174.6–185.7 °C. ^{1}H-NMR (D_2O) δ: 2.08–2.23 (m, 2H), 2.27–2.65 (m, J_{C-H} = 128.5 Hz, 2H), 3.79 (dt, $^3J_{CH}$ = 4.9 Hz, J = 6.0 Hz, 1H). ^{13}C-NMR (D_2O) δ: 33.7. IR (KBr): 3410, 3215, 2956, 2043, 1686, 1638, 1586, 1483, 1412, 1333, 1315, 1160 cm^{-1}. HR-FAB-MS (glycerol) calcd for $C_4{}^{13}CH_{11}N_2O_3$: *m/z* 148.0803. Found: *m/z* 148.0808 (MH^+). HPLC-CD analysis was performed as described previously[19] but with the following modifications: eluent, pH 2.0 $HClO_4$; flow rate, 0.4 mL/min; detection wavelength, 208 nm (UV and CD); temperature, 4 °C; R_t = 2.9 min (authentic D-glutamine gave R_t = 4.8 min).

Conflict of Interest

The authors did not report any conflict of interest.

References

[1] S. Nakanishi, Y. Nakajima, M. Masu, Y. Ueda, K. Nakahara, D. Watanabe, S. Yamaguchi, S. Kawabata, M. Okada, *Brain Res. Rev.* **1998**, *26*, 230.
[2] D. W. Choi, *Neuron* **1988**, *1*, 623.
[3] A. K. Bouzier-Sore, S. Serres, P. Canioni, M. Merle, *Biochimie* **2003**, *85*, 841.
[4] N. Becmann, I. Turkalj, J. Seeling, U. Keller, *Biochimistry* **1991**, *30*, 6362.
[5] T. Kanamatsu, Y. Tsukada, *Brain Res.* **1999**, *841*, 11.
[6] J. Shen, K. F. Petersen, K. L. Behar, P. Brown, T. W. Nixon, G. F. Mason, O. A. C. Petroff, G. I. Shulman, R. G. Shulman, D. L. Rothman, *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 8235.
[7] A. B. Patel, R. A. de Graaf, G. F. Mason, T. Kanamatsu, D. L. Rothman, R. G. Shulman, K. L. Behar, *J. Cereb. Blood Flow Metab.* **2004**, *24*, 972.
[8] T. Kanamatsu, R. Watanabe, S. Takase-Yoden, *J. Vet. Med. Sci.* **2006**, *68*, 259.
[9] E. Olstad, H. Qu, U. Sonnewald, *J. Cereb. Blood Flow Metab.* **2007**, *27*, 811.
[10] L. Peng, L. Gu, H. Zhang, X. Huang, E. Hertz, L. Hertz, *J. Neurosci. Res.* **2007**, *85*, 3480.
[11] L. K. Bak, E. Ziemińska, H. S. Waagepetersen, A. Schousboe, J. Albrecht, *Neurochem. Res.* **2008**, *33*, 273.
[12] J. F. Dellaria Jr., B. D. Santarsiero, *J. Org. Chem.* **1989**, *54*, 3916.
[13] K. Takatori, M. Nishihara, Y. Nishiyama, M. Kajiwara, *Tetrahedron* **1998**, *54*, 15861.
[14] K. Takatori, M. Nishihara, M. Kajiwara, J. Labelled *Compd. Radiopharm.* **1999**, *42*, 701.
[15] K. Takatori, S. Toyama, S. Narumi, S. Fujii, M. Kajiwara, J. Labelled *Compd. Radiopharm.* **2004**, *47*, 91.
[16] K. Takatori, A. Hayashi, M. Kajiwara, J. Labelled *Compd. Radiopharm.* **2004**, *47*, 787.
[17] K. Takatori, T. Sakamoto, M. Kajiwara, J. Labelled *Compd. Radiopharm.* **2006**, *49*, 445.
[18] J. M. Delacotte, H. Galons, D. Schott, J. L. Morgat, J. Labelled *Compd. Radiopharm.* **1991**, *29*, 1141.
[19] K. Takatori, S. Toyama, S. Fujii, M. Kajiwara, *Chem. Pharm. Bull.* **1995**, *43*, 1797.

