JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2002; **45**: 569–576.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.583

Research Article

Synthesis of 5-[4,5-$^{13}C_2$]- and 5-[1,5-$^{13}C_2$]aminolevulinic acid

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Summary

5-[4,5-$^{13}C_2$]- and 5-[1,5-$^{13}C_2$]Aminolevulinic acid (ALA) have been synthesized by the Gabriel condensation of potassium phthalimide with ethyl bromo [1,2-$^{13}C_2$]acetate (derived from [1,2-$^{13}C_2$]acetic acid) or ethyl bromo[2-^{13}C]-acetate (derived from sodium [2-^{13}C]acetate), followed by conversion to the chloride, coupling reaction with 2-ethoxycarbonylethylzinc iodide derived from ethyl 3-iodopropionate or 2-methoxy[^{13}C]carbonylethylzinc iodide derived from methyl 3-iodo[1-^{13}C]propionate (generated from potassium [^{13}C]cyanide), and hydrolysis.

Key Words: 5-[4,5-$^{13}C_2$]aminolevulinic acid; 5-[1,5-$^{13}C_2$]aminolevulinic acid; [1,2-$^{13}C_2$]acetic acid; sodium [2-^{13}C]acetate; potassium [^{13}C]cyanide

Introduction

We have developed various methods for the regioselective synthesis of singly ^{13}C-labeled 5-aminolevulinic acid (ALA),[1,2] and have used ^{13}C-ALA thus synthesized for studies on the biosynthesis of porphyrins.[3–5] We present here a synthesis of ^{13}C-double-labeled ALA, [4,5-$^{13}C_2$]- and [1,5-$^{13}C_2$]ALA, using [1,2-$^{13}C_2$]acetic acid, sodium [2-^{13}C]acetate and potassium [^{13}C]cyanide as isotope sources by modification of the methods described in our previous paper[2] reported by Campbell *et al.*[6]

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Received 11 December 2001
Revised 12 February 2002
Accepted 25 February 2002

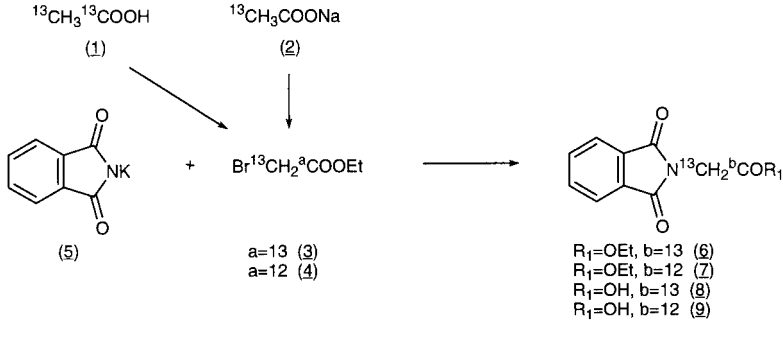
Results and discussion

Synthesis of 2-phthalimido[1,2-$^{13}C_2$]acetic acid (8) and 2-phthalimido[2-^{13}C]acetic acid (9)

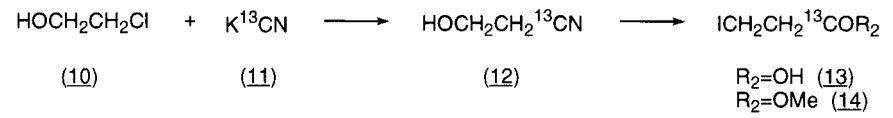
As shown in Scheme 1, bromination of [1,2-$^{13}C_2$]acetic acid (1) with bromine catalyzed by red phosphorus, and esterification with ethanol gave ethyl bromo[1,2-$^{13}C_2$]acetate (3) in 44% yield. Bromination of sodium [2-^{13}C]acetate (2) with benzoyl bromide, benzoic acid and acetyl bromide, followed by bromination of the resulting [2-^{13}C]acetyl bromide with bromine and esterification with ethanol gave ethyl bromo[2-^{13}C]acetate (4) in 69% yield. The Gabriel condensation of potassium phthalimide (5) and ethyl bromo[1,2-$^{13}C_2$]acetate (3) or ethyl bromo[2-^{13}C]acetate (4) in dry dimethylformamide gave ethyl 2-phthalimido[1,2-$^{13}C_2$]acetate (6) or ethyl 2-phthalimido[2-^{13}C]acetate (7), respectively, in 77% yield. The hydrolysis of ethyl 2-phthalimido[1,2-$^{13}C_2$]acetate (6) or ethyl 2-phthalimido[2-^{13}C]acetate (7) in acetic acid and 6 M hydrochloric acid gave 2-phthalimido[1,2-$^{13}C_2$]acetic acid (8) or 2-phthalimido[2-^{13}C]acetic acid (9), respectively, in 81% yield.

Synthesis of methyl 3-iodo[1-^{13}C]propionate (14)

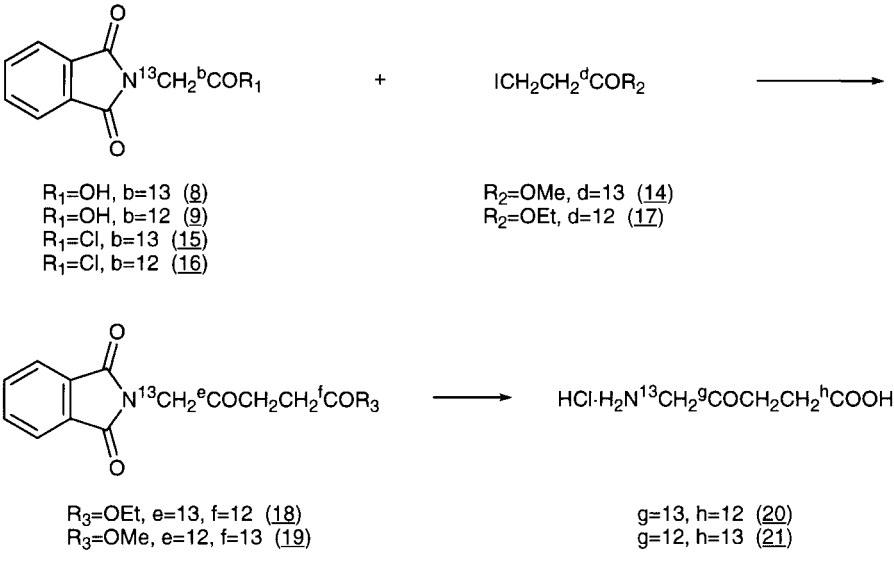
As shown in Scheme 2, cyanation of ethylene chlorohydrin (10) by potassium [^{13}C]cyanide (11) gave ethylene [^{13}C]cyanohydrin (12) in 87% yield. The hydrolysis and iodination of ethylene [^{13}C]cyanohydrin (12) with 55% hydroiodic acid gave 3-iodo[1-^{13}C]propionic acid (13) in 85% yield. The methyl esterification of 3-iodo[1-^{13}C]propionic acid (13) with diazomethane gave methyl 3-iodo[1-^{13}C]propionate (14) in 96% yield.



Scheme 1.



Scheme 2.



Scheme 3.

Synthesis of [4,5-$^{13}C_2$]ALA hydrochloride (20) and [1,5-$^{13}C_2$]ALA hydrochloride (21)

As shown in Scheme 3, the coupling reaction[2,6–8] of 2-phthalimido[1,2-$^{13}C_2$]acetyl chloride (15) (derived from 2-phthalimido[1,2-$^{13}C_2$]-acetic acid (8) and thionyl chloride) and 2-ethoxycarbonylethylzinc iodide (derived from ethyl 3-iodopropionate (17)[2,6] treated with zinc–copper couple) catalyzed by tetrakis(triphenylphosphine) palladium (0) in dry toluene and dry *N*,*N*-dimethylacetamide gave ethyl 5-phthalimido[4,5-$^{13}C_2$]levulinate (18) in 92% yield. The same coupling reaction of compounds derived from 2-phthalimido[2-^{13}C]-acetic acid (9) and methyl 3-iodo[1-^{13}C]propionate (14) was carried out to give methyl 5-phthalimido[1,5-$^{13}C_2$]levulinate (19). Finally, hydrolysis of ethyl 5-phthalimido[4,5-$^{13}C_2$]levulinate (18) or methyl 5-phthalimido[1,5-$^{13}C_2$]levulinate (19) in 6 M hydrochloric acid gave

[4,5-$^{13}C_2$]ALA hydrochloride (20) or [1,5-$^{13}C_2$]ALA hydrochloride (21), respectively, in 93% yield.

Experimental

Materials

[1,2-$^{13}C_2$]Acetic acid (90 at% ^{13}C) and sodium [2-^{13}C]acetate (98 at% ^{13}C) were purchased from CIL, Inc., Potassium [^{13}C]cyanide (90 at% ^{13}C) was purchased from Isotec, Inc., All other chemicals were of analytical grade.

Instruments

Melting point determinations were carried on a Yanaco micro-melting point apparatus, Model MP; values are uncorrected. IR spectra were recorded on a Jasco VALOR-III FT-IR spectrometer. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded on a Jeol GSX-400 spectrometer. EI- and FAB-MS spectra were obtained on a Jeol DX302 spectrometer.

Ethyl bromo[1,2-$^{13}C_2$]acetate (3)

Bromine (7.2 ml, 75.4 mmol) was added dropwise to [1,2-$^{13}C_2$]acetic acid (1) (1.0 g, 16.1 mmol) and dry red phosphorus (0.20 g, 64.6 mmol), and the mixture was heated at 120°C for 5 h. After cooling, excess bromine was blown off with argon gas. To the residue, dry EtOH (5.0 ml, 85.2 mmol) was added at −78°C, and the whole was stirred at room temperature for 12 h. The reaction was neutralized with saturated $NaHCO_3$ and the mixture was extracted with ether. The combined extracts were washed with water, dried over anhydrous $MgSO_4$, and evaporated. Distillation of the crude product gave ethyl bromo[1,2-$^{13}C_2$]acetate (3) (1.21 g, 44%), b.p. 85°C (53 mmHg); ^{1}H-NMR ($CDCl_3$) δ: 1.31 (t, 3 H, $J = 7.2$ Hz), 3.83 (dd, 2H, $J = 153.2$ Hz, $^2J = 4.6$ Hz), 4.24 (dq, 2H, $J = 7.2$ Hz, $^3J = 3.1$ Hz); ^{13}C-NMR ($CDCl_3$) δ: 25.9 (d, $J = 64.5$ Hz), 167.2 (d, $J = 64.5$ Hz); FT-IR (neat) cm^{-1}: 1690; EI-MS m/z (rel. int. %): 169 (M^+, 10).

Ethyl bromo[2-^{13}C]acetate (4)

Benzoyl bromide (7.2 ml, 61.1 mmol) was added dropwise to sodium [2-^{13}C]acetate (2) (1.0 g, 12.2 mmol) and benzoic acid (1.32 g, 10.8 mmol), and the mixture was heated at 120°C for 5 h. The resulting [2-^{13}C]acetyl bromide was distilled at 80°C under 28 mmHg. Bromine (3.0 ml, 31.4 mmol) was added dropwise to [2-^{13}C] acetyl bromide at −78°C, and the mixture was stirred at room temperature for 5 min, and then refluxed at 60°C for 5 h. It was allowed to cool to room temperature, then excess bromine was blown off with argon gas. To the residue, dry EtOH (3.0 ml, 51.1 mmol) was added at −78°C, and the whole stirred at room temperature for 12 h. The reaction mixture was neutralized with saturated $NaHCO_3$ and extracted with ether. The combined extracts were washed with water, dried over anhydrous $MgSO_4$ and evaporated. Distillation of the crude product gave ethyl bromo[2-^{13}C]acetate (4) (1.41 g, 69%), b.p. 85°C (56 mmHg); ^{1}H-NMR ($CDCl_3$) δ: 1.31 (t, 3H, J = 7.2 Hz), 3.83 (d, 2H, J = 142.2 Hz), 4.24 (q, 2 H, J = 7.2 Hz); ^{13}C-NMR ($CDCl_3$) δ: 25.9; FT-IR (neat) cm^{-1}: 1735; EI-MS m/z (rel. int. %): 168 (M^+, 5).

Ethyl 2-phthalimido[1,2-$^{13}C_2$]acetate (6) and ethyl 2-phthalimido[2- ^{13}C]acetate (7)

A solution of ethyl bromo[1,2-$^{13}C_2$]acetate (3) (497.0 mg, 3.0 mmol) and potassium phthalimide (5) (500.0 mg, 2.7 mmol) in dry dimethylformamide (7 ml) was heated at 90°C for 3 h. The reaction was neutralized with 0.1 M NaOH and the mixture was extracted with $CHCl_3$. The combined extracts were washed with water, dried over anhydrous $MgSO_4$, and evaporated. The residue was recrystallized from EtOH to give ethyl 2-phthalimido[1,2-$^{13}C_2$]acetate (6) (537.2 mg, 77%), m.p. 109–110°C; ^{1}H-NMR ($CDCl_3$) δ: 1.29 (t, 3 H, J = 7.2 Hz), 4.23 (dq, 2H, J = 7.2 Hz, ^3J = 3.1 Hz), 4.44 (dd, 2 H, J = 142.2 Hz, ^2J = 6.2 Hz), 7.74–7.90 (m, 4 H); ^{13}C-NMR ($CDCl_3$) δ: 38.9 (d, J = 63.1 Hz), 167.2 (d, J = 63.1 Hz); FT-IR (KBr) cm^{-1}: 1470, 1740; EI-MS m/z (rel. int. %): 276 (M^+, 17).

Similar procedures using ethyl bromo[2-^{13}C]acetate (4) in place of ethyl bromo[1,2-$^{13}C_2$]acetate (3) afforded ethyl 2-phthalimido[2-^{13}C] acetate (7), m.p. 109–111°C; ^{1}H-NMR ($CDCl_3$) δ: 1.29 (t, 3H, J = 7.2 Hz), 4.23 (q, 2H, J = 7.2 Hz), 4.44 (d, 2H, J = 142.2 Hz),

7.74–7.90 (m, 4H); ^{13}C-NMR ($CDCl_3$) δ: 39.0; FT-IR (KBr) cm^{-1}: 1470, 1720; EI-MS *m/z* (rel. int. %): 275 (M^+, 15).

2-Phthalimido[1,2-$^{13}C_2$]acetic acid (8) and 2-phthalimido[2-^{13}C] acetic acid (9)

A solution of ethyl 2-phthalimido[1,2-$^{13}C_2$]acetate (6) (537.2 mg, 2.3 mmol) in acetic acid (6 ml) and 6 M HCl (2 ml) was heated at 90°C for 1.5 h. The reaction mixture was diluted with water, and then lyophilized. The residue was recrystallized from water to give 2-phthalimido[1,2-$^{13}C_2$]acetic acid (8) (382.6 mg, 81%), m.p. 189–195°C; ^{1}H-NMR ($CDCl_3$) δ: 4.49 (dd, 2H, $J = 142.2$ Hz, $^2J = 5.9$ Hz), 7.74–7.90 (m, 4H); ^{13}C-NMR ($CDCl_3$) δ: 38.5 (d, $J = 1.6$ Hz), 171.1 (d, $J = 61.6$ Hz); FT-IR (KBr) cm^{-1}: 1470, 1770; EI-MS *m/z* (rel. int. %): 207 (M^+, 4).

Similar treatment of ethyl 2-phthalimido[2-^{13}C]acetate (7) afforded 2-phthalimido[2-^{13}C]acetic acid (9), m.p. 193–195°C; ^{1}H-NMR ($CDCl_3$) δ: 4.50 (d, 2 H, $J = 142.2$ Hz), 7.74–7.90 (m, 4 H); ^{13}C-NMR ($CDCl_3$) δ: 38.5; FT-IR (KBr) cm^{-1}: 1465, 1760; EI-MS *m/z* (rel. int. %): 206 (M^+, 15).

Ethylene [^{13}C]cyanohydrin (12)

Ethylene chlorohydrin (10) (6.6 ml, 98.4 mmol) was added to a suspension of potassium [^{13}C]cyanide (11) (3.05 g, 46.1 mmol) in dry MeOH (5 ml), and the mixture was heated at 80°C for 4 h. It was allowed to cool to room temperature, and the crystals were removed by filtration. The filtrate was evaporated. Distillation of the crude product gave ethylene [^{13}C]cyanohydrin (12) (2.88 g, 87%), b.p. 115°C (18 mm Hg); ^{1}H-NMR ($CDCl_3$) δ: 2.61 (dt, 2H, $J = 6.2$ Hz, $^2J = 9.5$ Hz), 3.87 (dt, 2 H, $J = 6.2$ Hz, $^3J = 6.2$ Hz); ^{13}C-NMR ($CDCl_3$) δ: 118.2.

3-Iodo[1-^{13}C]propionic acid (13)

A solution of ethylene [^{13}C]cyanohydrin (12) (751.1 mg, 10.6 mmol) in 55% HI (10.0 ml, 43.0 mmol) was refluxed at 130°C for 2 h. The reaction mixture was diluted with water and extracted with ether. The combined extracts were washed with brine, dried over anhydrous $MgSO_4$, and evaporated. Chromatography of the crude product on silica gel with ethyl acetate:hexane (1:1) gave 3-iodo[1-^{13}C]propionic acid (13) (1.80 g,

85%), ^{1}H-NMR ($CDCl_3$) δ: 3.06 (dt, 2H, $J = 7.2$ Hz, $^{2}J = 7.2$ Hz), 3.32 (dt, 2 H, $J = 7.2$ Hz, $^{3}J = 4.9$ Hz); ^{13}C-NMR ($CDCl_3$) δ: 117.5.

Methyl 3-iodo[1-^{13}C]propionate (14)

A solution of diazomethane in ether was added dropwise to a solution of 3-iodo[1-^{13}C]propionic acid (13) (1.80 g, 9.0 mmol) in ether (10 ml) at 0°C, until the color of the reaction solution become yellow. Evaporation afforded a crude product, which was distilled to give methyl 3-iodo[1-^{13}C]propionate (14) (1.85 g, 96%), b.p. 70°C (7 mmHg); ^{1}H-NMR ($CDCl_3$) δ: 2.99 (dt, 2H, $J = 7.2$ Hz, $^{2}J = 7.2$ Hz), 3.33 (dt, 2H, $J = 7.2$ Hz, $^{3}J = 4.9$ Hz), 3.73 (d, 3 H, $^{3}J = 3.9$ Hz); ^{13}C-NMR ($CDCl_3$) δ: 171.5.

Ethyl 5-phthalimido[4,5-$^{13}C_2$]levulinate (18) and methyl 5-phthalimido[1,5-$^{13}C_2$]levulinate (19)

A mixture of 2-phthalimido[1,2-$^{13}C_2$]acetic acid (8) (1.01 g, 4.9 mmol) and thionyl chloride (6.0 ml, 82.3 mmol) was refluxed at 80°C for 4 h. The excess thionyl chloride was removed in *vacuo*. The residue was dissolved in dry toluene (3 ml) and evaporated. This process was repeated twice, and the resulting 2-phthalimido[1,2-$^{13}C_2$]acetyl chloride (15) was dissolved in dry toluene (4 ml) under argon. Ethyl 3-iodopropionate (17) (1.66 g, 7.8 mmol) was added to a suspension of Zn–Cu couple (634.5 mg) in dry toluene (0.8 ml) and dry *N*, *N*-dimethylacetamide (9 ml) under argon, and the mixture was heated at 60°C for 4.5 h. To this suspension, a suspension of $Pd(PPh_3)_4$ (153.4 mg, 132.7 μmol) in dry toluene (4 ml) under argon was added, and the whole was stirred at 60°C for 5 min. Then, a solution of 2-phthalimido[1,2-$^{13}C_2$]acetyl chloride (15) was added, and the whole was stirred at 60°C for 25 min.The reaction was diluted with ethyl acetate, and the whole was washed with 1 M HCl, saturated $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, and evaporated. Chromatography of the crude product on silica gel with ethyl acetate:hexane (1:1) gave ethyl 5-phthalimido[4,5-$^{13}C_2$]levulinate (18) (1.31 g, 92%), m.p. 69–71°C; ^{1}H-NMR ($CDCl_3$) δ: 1.26 (t, 2H, $J = 7.2$ Hz), 2.66 (m, 2 H), 2.85 (m, 2H), 4.14 (q, 2H, $J = 7.2$ Hz), 4.56 (dd, 2H, $J = 139.4$ Hz, $^{2}J = 4.1$ Hz), 7.74–7.90 (m, 4H); ^{13}C-NMR ($CDCl_3$) δ: 46.5 (d, $J = 41.1$ Hz), 200.7 (d, $J = 41.1$ Hz); FT-IR (KBr) cm^{-1}: 1410, 1770; EI-MS m/z (rel. int. %): 291 (M^+, 2).

A similar procedure starting from 2-phthalimido[2-^{13}C]acetic acid (9) via methyl 3-iodo[1-^{13}C]propionate (14) afforded methyl 5-phthalimido[1,5-$^{13}C_2$]levulinate (19), ^{1}H-NMR ($CDCl_3$) δ: 2.66 (m, 2H), 2.85 (m, 2H), 3.70 (d, 2H, $^2J = 3.9$ Hz), 4.56 (d, 2H, $J = 139.4$ Hz), 7.74–7.90 (m, 4H); ^{13}C-NMR ($CDCl_3$) δ: 46.5, 172.5.

[4,5-$^{13}C_2$]ALA hydrochloride (20) and [1,5-$^{13}C_2$]ALA hydrochloride (21)

A solution of ethyl 5-phthalimido[4,5-$^{13}C_2$]levulinate (18) (1.02 g, 3.5 mmol) in 6 M HCl (5 ml) was heated under reflux for 14 h. The reaction mixture was cooled to room temperature and filtered. The filtrate was washed with ether and evaporated. The residue was recrystallized from EtOH–ether to give [4,5-$^{13}C_2$]ALA hydrochloride (20) (1.86 g, 93%), m.p. 146–149°C; ^{1}H-NMR (D_2O) δ: 2.74 (m, 2H), 2.91 (m, 2H), 4.15 (dd, 2H, $J = 143.5$ Hz, $^2J = 4.1$ Hz); ^{13}C-NMR (D_2O) δ: 49.9 (d, $J = 39.6$ Hz), 207.0 (d, $J = 41.1$ Hz); FT-IR (KBr) cm^{-1}: 1727; FAB-MS (glycerol) m/z: 134 (MH^+-HCl).

Similar treatment of methyl 5-phthalimido[1,5-$^{13}C_2$]levulinate (19) afforded [1,5-$^{13}C_2$]ALA hydrochloride (21), m.p. 155–157°C; ^{1}H-NMR (D_2O) δ: 2.74 (m, 2H), 2.91 (m, 2H), 4.15 (d, 2 H, $J = 143.5$ Hz); ^{13}C-NMR (D_2O) δ: 49.9, 179.7; FT-IR (KBr) cm^{-1}: 1725; FAB-MS (glycerol) m/z: 134 (MH^+-HCl).

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