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Research Article

Synthesis of ^{13}C and ^{15}N multilabeled 5-aminolevulinic acid

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Summary

5-[4-^{13}C,^{15}N]- and 5-[5-^{13}C,^{15}N]Aminolevulinic acid (ALA) were simply synthesized in four steps by the condensation of [1-^{13}C,^{15}N]- or [2-^{13}C,^{15}N]glycine, respectively, with phthalic anhydride, followed by conversion to the chloride, coupling reaction with a three-carbon unit and hydrolysis. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: 5-[4-^{13}C,^{15}N]aminolevulinic acid; 5-[5-^{13}C,^{15}N]aminolevulinic acid; [1-^{13}C,^{15}N]glycine; [2-^{13}C,^{15}N]glycine

Introduction

We have developed various methods for the synthesis of regioselectively stable-isotope-labeled 5-aminolevulinic acid (ALA),[1,2] which is a precursor of porphyrins, for use in studies on the biosynthesis of porphyrins.[3–5] Synthetic methods of ALA isotope-labeled on C-5 have been reported by Shemin *et al.*,[6] Battersby *et al.*[7] and from our laboratory,[1] but involve many steps between introduction of the labeled carbon and formation of the final product. We present here a simple synthesis of [5-^{13}C,^{15}N]ALA from [2-^{13}C,^{15}N]glycine, and of [4-^{13}C,^{15}N]ALA from [1-^{13}C,^{15}N]glycine.

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Results and discussion

As shown in Scheme 1, the condensation of [2-^{13}C,^{15}N]glycine (**1**) and phthalic anhydride (**2**) gave 2-[^{15}N]phthalimido[2-^{13}C]acetic acid (**3**) in 86% yield. The transhalogenation of ethyl 3-bromopropionate (**5**) with sodium iodide in 2-butanone gave ethyl 3-iodopropionate (**6**) in 95% yield. The coupling reaction[8,9] of 2-[^{15}N]phthalimido[2-^{13}C]acetyl chloride (**4**), which was derived from 2-[^{15}N]phthalimido[2-^{13}C]acetic acid (**3**) and thionyl chloride, with 2-ethoxycarbonylethylzinc iodide, which was derived from ethyl 3-iodopropionate (**6**) treated with zinc–copper couple, catalyzed by tetrakis(triphenylphosphine)palladium (0) in dry toluene and dry *N,N*-dimethylacetamide gave ethyl 5-[^{15}N]phthalimido[5-^{13}C]levulinate (**7**) in 92% yield. Finally, hydrolysis of ethyl 5-[^{15}N]phthalimido[5-^{13}C]levulinate (**7**) in 6 N hydrochloric acid gave [5-^{13}C,^{15}N]ALA (**8**) in 89% yield. [4-^{13}C,^{15}N]ALA was similarly synthesized from [1-^{13}C,^{15}N]glycine in four steps.

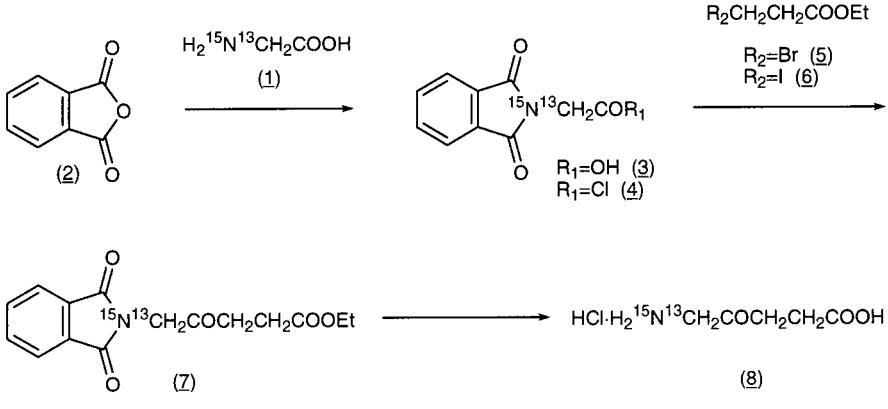
Experimental

Materials

[1-^{13}C,^{15}N]- and [2-^{13}C,^{15}N]Glycine (99 at% ^{13}C, 99 at % ^{15}N) were purchased from Masstrace, 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



Scheme 1. Synthesis of [5-^{13}C,^{15}N]ALA (8)

recorded on a Jasco VALOR-III FT-IR spectrometer. ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) spectra were recorded on a Varian GEMINI-300 spectrometer. EI- and FAB-MS spectra were obtained on a Jeol DX302 spectrometer.

2-[^{15}N]Phthalimido[2-^{13}C]acetic acid (3)

[2-^{13}C,^{15}N]Glycine (**1**) (1.0 g, 13.0 mmol) and phthalic anhydride (**2**) (2.0 g, 13.5 mmol) were heated at 160°C, until the whole was melted. After cooling, the residue was recrystallized from water to give 2-[^{15}N]phthalimido[2-^{13}C]acetic acid (**3**) (2.3 g, 86%), m.p. 193–195°C; ^{1}H-NMR ($CDCl_3$) δ : 4.49 (dd, 2H, J = 142.3 Hz, ^{2}J = 1.1 Hz), 7.75–7.92 (m, 4H); ^{13}C-NMR ($CDCl_3$) δ :38.4 (d, J = 13.3 Hz); FT-IR (KBr) cm^{-1}: 1745, 2928; EI-MS m/z (rel. int. %): 207 (M^+, 4), 162 (100).

Analytical data of 2-[^{15}N]phthalimido[1-^{13}C]acetic acid, m.p. 191–194°C; ^{1}H-NMR ($CDCl_3$) δ : 4.50 (dd, 2H, ^{2}J = 5.8 Hz, ^{2}J = 1.1 Hz), 7.75–7.92 (m, 4H); ^{13}C-NMR ($CDCl_3$) δ : 171.6; FT-IR (KBr) cm^{-1}; 1726, 2926; EI-MS m/z (rel. int. %): 207 (M^+, 4) 161 (100).

Ethyl 3-iodopropionate (6)

A suspension of ethyl 3-bromopropionate (**5**) (10 ml, 78.0 mmol) and NaI (17 g, 113.4 mmol) in 2-butanone (100 ml) was refluxed at 90°C for 16 h under argon. After cooling, the crystals were removed by filtration. The filtrate was evaporated, diluted with ether, and washed with brine. Distillation of the crude product gave ethyl 3-iodopropionate (**6**) (33.7 g, 95%), b.p. 105°C (20 mm Hg); ^{1}H-NMR ($CDCl_3$) δ :1.29 (t, 3H, J = 7.1 Hz), 2.97 (t, 2H, J = 7.1 Hz), 3.34 (t, 2H, J = 7.1 Hz), 4.19 (q, 2H, J = 7.1 Hz); FT-IR (neat) cm^{-1}: 1730; EI-MS m/z (rel. int. %): 228 (M^+, 100), 155 (61), 101 (92), 73 (47).

Ethyl 5-[^{15}N]phthalimido[5-^{13}C]levulinate (7)

A mixture of 2-[^{15}N]phthalimido[2-^{13}C]acetic acid (**3**) (2.3 g, 11.1 mmol) and thionyl chloride (30 ml) was refluxed at 80°C for 18 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-[^{15}N]phthalimido[2-^{13}C]acetyl chloride (**4**) was dissolved in dry toluene (5 ml) under argon. Ethyl 3-iodopropionate (**6**) (2.82 g, 12.4 mmol) was added to a suspension of Zn–Cu couple (1.0 g) in dry toluene (1.2 ml) and dry *N*, *N*-dimethylacetamide (14 ml) under argon, and the mixture was heated at 60°C for 15 h. To this suspension, a suspension of $Pd(PPh_3)_4$ (530 mg, 459 μmol) in dry toluene (5 ml) under argon was added, and the whole was stirred at 60°C for 5 min. To this suspension, a solution of 2-[^{15}N]phthalimido[2-^{13}C]acetyl chloride (**4**) was added, and the whole was stirred at 60°C for 25 min. The reaction was diluted with ethyl acetate (300 ml), and the whole was washed with 1 N HCl, sat. $NaHCO_3$ and brine, dried over dry $MgSO_4$, and evaporated. Chromatography of the crude product on silica gel with ethyl acetate:hexane (1 : 1) gave ethyl 5-[^{15}N]phthalimido[5-^{13}C]levulinate (**7**) (2.98 g, 92%), m.p. 75–77°C; ^{1}H-NMR ($CDCl_3$) δ : 1.26 (t, 3H, $J = 7.1$ Hz), 2.66 (t, 2H, J = 6.6 Hz), 2.85 (t, 2H, $J = 6.6$ Hz), 4.15 (q, 2H, $J = 7.1$ Hz), 4.56 (d, 2H, $J = 139.8$ Hz), 7.73–7.89 (m, 4H); ^{13}C-NMR ($CDCl_3$) δ: 46.5 (d, $J = 12.2$ Hz); FT-IR (KBr) cm^{-1}: 1725, 1771, 2920; EI-MS m/z (rel. int. %): 291 (M^+, 2), 162 (44), 129 (100), 101 (75).

Analytical data of ethyl 5-[^{15}N]phthalimido[4-^{13}C]levulinate, m.p. 73–76°C; ^{1}H-NMR ($CDCl_3$) δ: 1.26 (t, 3H, $J = 7.1$ Hz) 2.67 (q, 2H, $J = 6.6$ Hz), 2.86 (q, 2H, $J = 6.6$ Hz), 4.15 (q, 2H, $J = 7.1$ Hz), 4.57 (dd, 2H, $^2J = 3.8$ Hz, $^2J = 1.1$ Hz), 7.73–7.89 (m, 4H); ^{13}C-NMR ($CDCl_3$) δ: 200.8; FT-IR (KBr) cm^{-1}: 1726, 1770, 2916; EI-MS m/z (rel. int. %): 291 (M^+, 1), 161 (100), 130 (50), 102 (37).

[5-^{13}C,^{15}N]ALA hydrochloride (8)

A solution of ethyl 5-[^{15}N]phthalimido[5-^{13}C]levulinate (**7**) (1.38 g, 4.7 mmol) in 6 N HCl (6 ml) was refluxed at 110°C for 14 h. The reaction mixture was cooled to room temperature and filtrated. The filtrate was washed with ether and evaporated. The residue was recrystallized from EtOH–ether to give [5-^{13}C,^{15}N]ALA hydrochloride (**8**) (717 mg, 89%), m.p. 144–147°C; ^{1}H-NMR (D_2O) δ: 2.72 (t, 2H, $J = 6.2$ Hz), 2.90 (t, 2H, $J = 6.2$ Hz), 4.13 (d, 2H, $J = 143.9$ Hz); ^{13}C-NMR (D_2O) δ: 49.8 (d, $J = 7.7$ Hz); FT-IR (KBr) cm^{-1}: 1695, 1725, 2927; FAB-MS (glycerol) m/z: 134 (MH^+-HCl).

Analytical data of [4-^{13}C,^{15}N]ALA hydrochloride, m.p. 146–149°C; ^{1}H-NMR (D_2O) δ: 2.72 (q, 2H, $J = 6.0$ Hz), 2.90 (q, 2H, $J = 6.0$ Hz),

4.13 (d, 2H, $J = 4.2$ Hz); ^{13}C-NMR (D_2O) δ: 206.8; FT-IR (KBr) cm^{-1}; 1685, 1725, 3005; FAB-MS (glycerol) m/z: 134 (MH^+-HCl).

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