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# Synthesis of δ-[$^{15}$N]Aminolevulinic Acid Hydrochloride

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**Summary**

δ-[$^{15}$N]Aminolevulinic acid (ALA) hydrochloride was synthesized by the condensation of potassium [$^{15}$N]phthalimide and tetrahydrofurfuryl bromide, followed by ruthenium oxidation and hydrolysis, in high yield. Relevant $^{15}$N-NMR spectral data are presented.

**Key words:** δ-[$^{15}$N]aminolevulinic acid, potassium [$^{15}$N]phthalimide, tetrahydrofurfuryl bromide, ruthenium oxidation, $^{15}$N-NMR.

## Introduction

δ-Aminolevulinic acid (ALA) is a precursor of porphyrins and corrins. We have already developed methods for the synthesis of regioselectively $^{13}$C-labelled δ-ALAs[1], which were used for studies on the biosynthesis of porphyrins and vitamin $B_{12}$.[2] Further, a simple synthetic route to δ-[$^{15}$N]ALA is available,[3] via Gabriel condensation of potassium [$^{15}$N]phthalimide and methyl δ-chlorolevulinate to afford methyl δ-[$^{15}$N]phthalimidyl levulinate in 71 % yield (80 % for the unlabelled compound). Hydrolysis (yield not reported) then gave δ-[$^{15}$N]ALA. We

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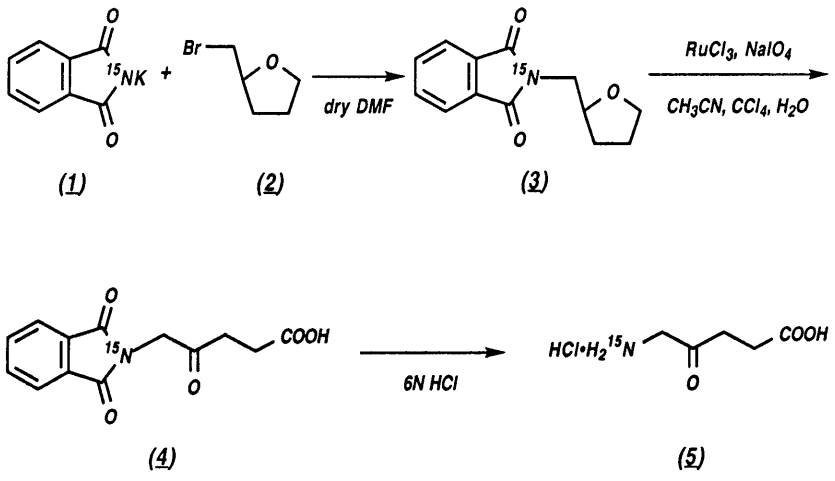
present here a higher-yield, simple synthesis of δ-[$^{15}$N]ALA based on a combination of this method with the method developed for unlabelled δ-ALA synthesis by Kawakami *et al.*.[4] Relevant $^{15}$N-NMR spectral data are presented.

## Results and Discussion

Kawakami *et al.* employed the condensation of phthalic anhydride and tetrahydrofurfurylamine to give *N*-tetrahydrofurfuryl phthalimide in 95 % yield, followed by ruthenium oxidation to give δ-phthalimidyl levulinic acid in 59 % yield, and hydrolysis to afford unlabelled δ-ALA hydrochloride in 64 % yield. We modified this method to synthesize $^{15}$N-labelled δ-ALA. As shown in scheme 1, the first step was changed to Gabriel condensation of potassium [$^{15}$N]phthalimide (1) and tetrahydrofurfuryl bromide (2) in dimethylformamide to give *N*-tetrahydrofurfuryl [$^{15}$N]phthalimide (3) in 89 % yield. Ruthenium oxidation was optimized to give δ-[$^{15}$N]phthalimidyl levulinic acid (4) in 95 % yield. Our strategy to this point, despite having one additional step, gives a higher yield than that of Neuberger and Scott.[3]




(1) (2) (3) (4) (5)

**Scheme 1; Synthesis of δ-[$^{15}$N]Aminolevulinic Acid Hydrochloride (5).**

Finally, hydrolysis of δ-[15N]phthalimidyl levulinic acid (4) gave δ-[15N]ALA hydrochloride (5) in 93 % yield. Its 15N-NMR spectrum is shown in figure 1 together with that of ammonium [15N]nitrate as an external standard.

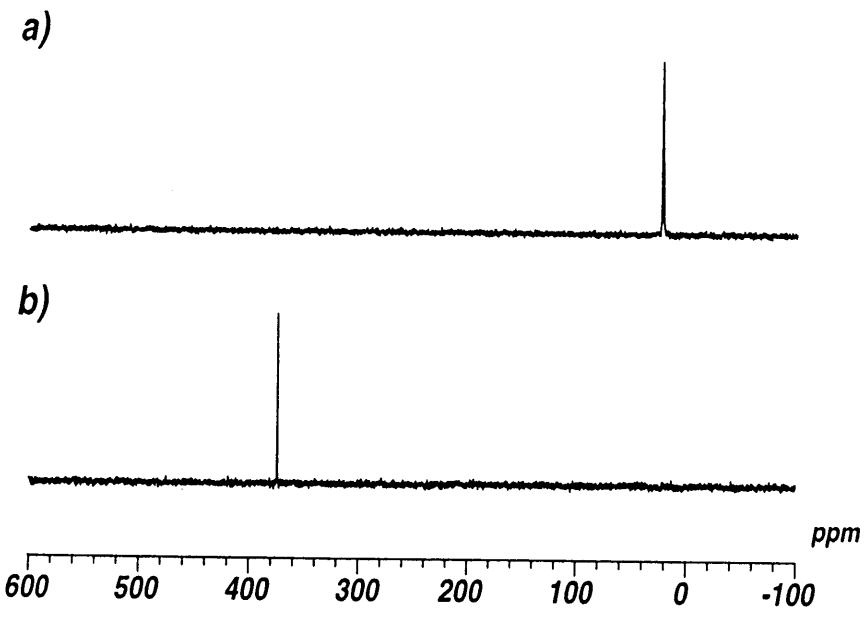



*Figure 1; 15N-NMR Spectra of a); δ-[15N]Aminolevulinic Acid Hydrochloride (5) (22.2 ppm) and b); Ammonium [15N]Nitrate (347 ppm) as an External Standard.*

## Experimental

### Materials

Potassium [15N]phthalimide (99.6 atom % 15N) was purchased from Shoko Co., Ltd.. Tetrahydrofurfuryl bromide and ruthenium (III) chloride *n*-hydrate were obtained from Wako Ltd..

### 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. 1H, 13C and 15N-NMR

spectra were recorded on a JEOL GSX-400 ($^{1}H$: 400 MHz, $^{13}C$: 100 MHz and $^{15}N$: 40 MHz) spectrometer. The signal (374.0 ppm) of nitrate nitrogen of ammonium [$^{15}N$]nitrate (figure 1) was used as an external standard for measurement of $^{15}N$-NMR spectra. EI and FAB-MS were obtained on a Fisons Instruments VG Analytical AutoSpec spectrometer with a DEC VAX-4000 Model 60 data system.

### *N-Tetrahydrofurfuryl [$^{15}N$]Phthalimide (3)*

Tetrahydrofurfuryl bromide (2) (6.96 g, 42.2 mmol) was added dropwise to a suspension of potassium [$^{15}N$]phthalimide (1) (6.01 g, 32.3 mmol) in dry dimethylformamide (15 ml) over 12 min at 0 °C under argon, and the whole was heated under reflux for 1 hr. The reaction was quenched with water (10 ml), and the mixture was extracted with chloroform (60 ml x 3). The combined extracts were washed with water (50 ml x 2) and brine (50 ml), dried over magnesium sulfate and evaporated. Chromatography of the residue on silica gel and elution with ethyl acetate:hexane (1:5-1:3) gave *N*-tetrahydrofurfuryl [$^{15}N$]phthalimide (3) (6.64 g, 89 %), m.p. 82.6~86.3 °C; $^{1}H$-NMR ($CDCl_3$) δ: 1.68 (m, 1H, $C\underline{H}_2CH_2O$), 1.84~2.08 (m, 3H, $C\underline{H}_2C\underline{H}_2CH_2O$), 3.66 (ddd, 1H, $J_{1H15N}$=1.2 Hz, $J_{1H1H}$=4.7, 13.9 Hz, $^{15}NC\underline{H}_2$), 3.75 (dt, 1H, $J_{1H1H}$=6.2, 7.8 Hz, $C\underline{H}_2O$), 3.81 (ddd, 1H, $J_{1H15N}$=1.0 Hz, $J_{1H1H}$=8.2, 13.9 Hz, $^{15}NC\underline{H}_2$), 3.92 (dt, 1H, $J_{1H1H}$=6.2, 7.2 Hz, $C\underline{H}_2O$), 4.27 (m, 1H, $^{15}NCH_2C\underline{H}$), 7.70~7.88 (m, 4H, phenyl proton); $^{13}C$-NMR ($CDCl_3$) δ: 25.3 (s, $\underline{C}H_2CH_2O$), 29.1 (s, $\underline{C}H_2CH_2CH_2O$), 41.7 (d, $J_{13C15N}$=8.8 Hz, $^{15}N\underline{C}H_2$), 67.8 (s, $\underline{C}H_2O$), 76.1 (s, $^{15}NCH_2\underline{C}H$), 123.2 (s, phenyl carbon), 132.0 (s, phenyl carbon), 133.8 (s, phenyl carbon), 168.3 (d, $J_{13C15N}$=13.2 Hz, $^{15}N\underline{C}O$); $^{15}N$-NMR ($CDCl_3$) δ: 157.2 ($^{15}\underline{N}CH_2$); FT-IR (KBr) $cm^{-1}$: 1719 (C=O), 1401 ($^{15}NCH_2$), 1100 (C-O-C); EI-MS *m/z* (rel. int. %): 232 ($M^+$, 9.24), 71 ($M^+$-[$^{15}N$]phthalimidyl-$CH_2$, 100).

### *δ-[$^{15}N$]Phthalimidyl Levulinic Acid (4)*

Sodium metaperiodate (24.10 g, 112.7 mmol) and ruthenium (III) chloride *n*-hydrate (136.0 mg) were added to a solution of *N*-tetrahydrofurfuryl [$^{15}N$]phthalimide (3) (6.50 g, 28.0 mmol) in

tetrachloromethane (20 ml), acetonitrile (100 ml) and water (30 ml), and the whole was heated at 80 °C for 2 hr, then evaporated. The residue was taken up in 3 N hydrochloric acid (300 ml) and extracted with chloroform (200 ml x 6). The combined extracts were dried over magnesium sulfate and evaporated. Chromatography of the residue on silica gel and elution with ethyl acetate:hexane (1:2-1:0) gave δ-[$^{15}$N]phthalimidyl levulinic acid (4) (6.98 g, 95 %), m.p. 158.9~161.7 °C; $^{1}$H-NMR ($CDCl_3$:DMSO-$d_6$=9:1) δ: 2.60 (t, 2H, $J_{1H1H}$=6.5 Hz, C$\underline{H}_2$COOH), 2.84 (t, 2H, $J_{1H1H}$=6.5 Hz, C$\underline{H}_2$CH$_2$COOH), 4.58 (s, 2H, $^{15}$NC$\underline{H}_2$), 7.78~7.88 (m, 4H, phenyl proton); $^{13}$C-NMR ($CDCl_3$:DMSO-$d_6$=9:1) δ: 27.1 (s, $\underline{C}$H$_2$COOH), 34.0 (s, $\underline{C}$H$_2$CH$_2$COOH), 45.9 (d, $J_{13C15N}$=13.2 Hz, $^{15}$N$\underline{C}$H$_2$), 122.8 (s, phenyl carbon), 131.3 (s, phenyl carbon), 133.7 (s, phenyl carbon), 166.9 (d, $J_{13C15N}$=13.2 Hz, $^{15}$N$\underline{C}$O), 173.4 (s, $\underline{C}$OOH), 200.7 (s, CH$_2\underline{C}$OC$_2$H$_4$); $^{15}$N-NMR ($CDCl_3$:DMSO-$d_6$=9:1) δ: 151.4 ($^{15}\underline{N}$CH$_2$); FT-IR (KBr) cm$^{-1}$: 2929 (COOH), 1719 (C=O), 1402 ($^{15}$NCH$_2$); EI-MS *m/z* (rel. int. %): 262 ($M^+$, 0.52), 162 ($MH^+$-COC$_2$H$_4$COOH, 100).

### *δ-[$^{15}$N]ALA Hydrochloride (5)*

A solution of δ-[$^{15}$N]phthalimidyl levulinic acid (4) (3.10 g, 11.8 mmol) in 6 N hydrochloric acid (40 ml) was heated under reflux for 14 hr. The reaction mixture was cooled to room temperature and filtered. The filtrate was washed with ether (80 ml x 4) and evaporated. The residue was recrystallized from ethyl alcohol-ether to give δ-[$^{15}$N]ALA hydrochloride (5) (1.86 g, 93 %), m.p. 144.3~147.6 °C; $^{1}$H-NMR ($D_2O$) δ: 2.60 (t, 2H, $J_{1H1H}$=6.3 Hz, C$\underline{H}_2$COOH), 2.78 (t, 2H, $J_{1H1H}$=6.3 Hz, C$\underline{H}_2$CH$_2$COOH), 4.01 (s, 2H, $^{15}$NH$_2$C$\underline{H}_2$); $^{13}$C-NMR ($D_2O$) δ: 29.7 (s, $\underline{C}$H$_2$COOH), 36.7 (s, $\underline{C}$H$_2$CH$_2$COOH), 49.4 (d, $J_{13C15N}$=7.3 Hz, $^{15}$NH$_2\underline{C}$H$_2$), 179.2 (s, $\underline{C}$OOH), 206.7 (s, CH$_2\underline{C}$OC$_2$H$_4$); $^{15}$N-NMR ($D_2O$) δ: 22.2 ($^{15}\underline{N}$H$_2$CH$_2$); FT-IR (KBr) cm$^{-1}$: 3007 ($^{15}$NH), 1727 (C=O); FAB-MS (glycerol) *m/z*: 133 ($MH^+$-HCl).

## References

1) Kurumaya K., Okazaki T., Seido N., Akasaka Y., Kawajiri Y., Kajiwara M. and Kondo M. -J. Label. Compds. Radiopharm. 27: 217 (1989)

2) a) Okazaki T., Kurumaya K. and Kajiwara M. -Chem. Pharm. Bull. 38: 1727 (1990), b) Kajiwara M., Hara K., Mizutani M. and Kondo M. -Chem. Pharm. Bull. 40: 3321 (1992), c) Spencer J. B., Stolowich N. J., Santander P. J., Pichon C., Kajiwara M., Tokiwa S., Takatori K. and Scott A. I. -J. Am. Chem. Soc. 116; 4991 (1994)

3) Neuberger A. and Scott J. J. -J. Chem. Soc. 1820 (1954)

4) Kawakami H., Ebata T. and Matsushita H. -Agric. Biol. Chem. 55: 1687 (1991)