# SYNTHESIS OF α-ACETOXY-N-NITROSO-4[$^3$H]-PYRROLIDINE

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## SUMMARY

Tritium labeled α-acetoxy-N-nitroso-4[$^3$H]-pyrrolidine 3, an important model compound used in the study of the metabolism of nitrosopyrrolidine, has been prepared from commercially available 4[$^3$H]-ℓ-proline. The synthesis involves two steps; first the nitrosation of the aminoacid to N-nitroso-4[$^3$H]-ℓ-proline 2, followed by the oxidative decarboxylation with lead tetraacetate in dichloromethane with 1.2 equivalents of pyridine to α-acetoxy-N-nitroso-4[$^3$H]-pyrrolidine in 23% overall yield. The compound was further purified by HPLC.

Key Words: α-Hydroxy nitrosamines, α-Acetoxy-N-nitroso-4[$^3$H]-pyrrolidine, Nitrosation, Oxidative decarboxylation, Lead tetraacetate

## INTRODUCTION

The synthesis of α-hydroxy nitrosamines or their esters has recently been the subject of considerable attention (1-4). Evidence has been presented that α-hydroxylation is an important step in the metabolism of cyclic nitrosamines by rat liver microsomes (5,6). Since α-hydroxy nitrosamines have short half-lives, α-acetoxy nitrosamines have been used as model compounds for the study of nitrosamine metabolism (5) and mutagenesis (7,8). In the studies of Hecht *et al.* (5) unlabeled α-acetoxy nitrosopyrrolidine was used to obtain the metabolite 2-hydroxy tetrahydrofuran 4.

During the course of our studies on the chemistry and mechanism of carcinogenesis of nitrosopyrrolidine, it became necessary to prepare tritium-labeled α-acetoxy-N-nitroso-4[$^3$H]-pyrrolidine as a precursor to 2-hydroxy-4[$^3$H]-tetrahydrofuran. Preliminary investigations into the synthesis of α-oxygenated nitrosamines were reported in a previous communication (9). The method involved

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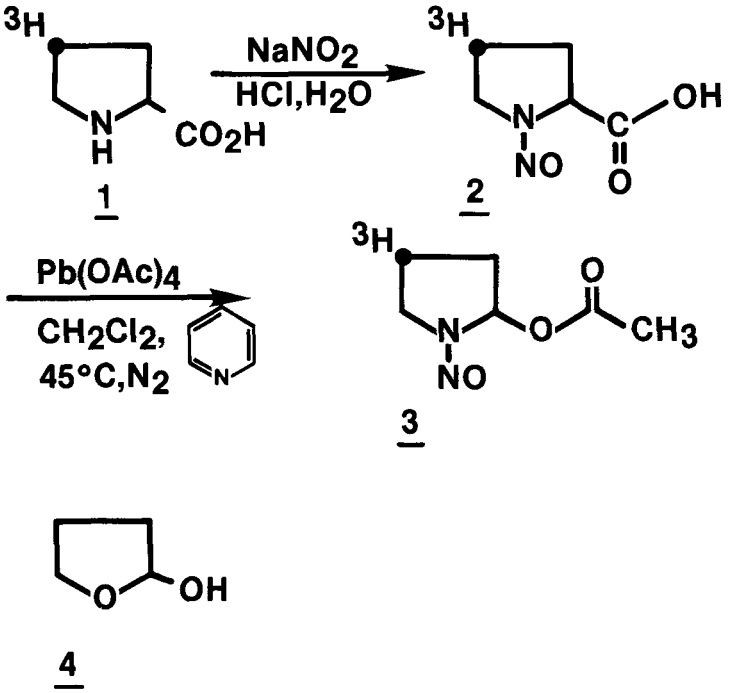
the oxidative decarboxylation of a nitrosoamino acid with lead tetraacetate then substitution by an acetate ion. Application of this method to a radiolabeled system has been successful. We report here the two-step synthesis of 3 from ℓ-proline-4[$^3$H].

## RESULTS AND DISCUSSION

ℓ-Proline-4[$^3$H] 1 was nitrosated in aqueous hydrochloric acid by the method of Lijinsky et al., (10) to N-nitroso-4[$^3$H]-ℓ-proline 2. Decarboxylation of 2 with lead tetraacetate in methylene chloride at 45°C for 10 hours in 1.2 equivalents of pyridine gave 3. The product was purified by Silica Gel column chromatography and was further purified before use for metabolic studies by high pressure liquid chromatography.



The progress of the reaction of 2 with oxidizing agents could be followed by measuring the evolution of carbon dioxide. The addition of pyridine enhanced the rate of reaction almost three fold. This increase in the reaction rate may be due to the coordination of pyridine to the lead (IV) carboxylate intermediate (11), thus facilitating the decarboxylation step. Detailed mechanistic studies on the oxidative decarboxylation of nitrosoamino acids will be discussed elsewhere.

## EXPERIMENTAL

Radioactive disintegrations were measured on a Nuclear-Chicago Isocap/300 or a Packard 3350 liquid scintillation counter. Mass spectra were taken on a Finnegan 3300 mass spectrometer equipped with a Finnegan 6000 MS data system. Proton magnetic resonance spectra were taken on a Varian XL-100 spectrometer using $CDCl_3$ as the solvent, with 0.5% tetramethyl silane as the internal standard. The IR spectra were obtained on a Perkin-Elmer 467 spectrometer. Ultraviolet spectra were taken in aqueous solutions using a Beckman Acta MVI spectrophotometer. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter. Melting points were determined on an Electrothermal capillary melting point apparatus. Purifications by HPLC were carried out on a Waters Associates apparatus equipped with a 440 absorption detector ($\lambda$=254). A 4.6 mm x 25 cm Dupont Zorbas ODS column was used, the product was eluted with 28% methanol in water. ℓ-Proline was obtained from Aldrich Chemical Co., Milwaukee, WI. 4[$^3$H]-ℓ-Proline was purchased from New England Nuclear, Boston, MA.

### N-Nitroso-4[$^3$H]-ℓ-Proline 2

A solution of 1.763 g (15.333 mmol) of inactive ℓ-proline in 10 ml of 10% aqueous hydrochloric acid was mixed with 1 ml of a solution which contained 0.0042 mg of ℓ-proline-4[$^3$H] (0.98 mCi) in 0.01N hydrochloric acid. The final solution has a specific activity of 0.0639 mCi/mmol. The solution was cooled

to 5°C, then 2.07 g (30 mmol) of sodium nitrite was added in small lots over a period of 30 mins. The cooling bath was removed and stirring continued at room temperature for 2 hours. The solvent was evaporated and the residue extracted with acetone. The acetone was filtered, the solvent removed on a rotary evaporator, and the residue recrystallized from chloroform to give 1.467 g (66.5%), 0.637 mCi of 2 (Sp. Act. 0.0624 mCi/mmol): M.P. 98-100° (lit (10) 99-100°C); $[\alpha]_D^{25}$ = -188° (C-0.231, EtOH).

α-Acetoxy-N-Nitroso-4[$^3$H]-Proline 3

To a 0.5M solution of 1.467 g (10.18 mmol) N-nitroso-4[$^3$H]-ℓ-proline 2 in degassed dichloromethane was added 1.2 equivalents of anhydrous pyridine, and 5.4 g (12.2 mmol) of lead tetraacetate. The solution was stirred under nitrogen at 45°C for 10 hours. The mixture was allowed to cool to room temperature, and the insoluble lead salts removed by filtration. The solvent was evaporated under a stream of dry nitrogen, followed by extraction of the residue with ether. The ether extract was washed with ice-cold 2% aqueous hydrochloric acid, then with ice-cold 2% sodium bicarbonate solution, dried over sodium sulfate, filtered through a pad of magnesium sulfate and the solvent removed on a rotary evaporator. The product was purified by column chromatography on Silica Gel 60 (E.M. Reagents, E. Merck, Darmstadt, F.R.G.) in a methylene chloride gradient giving 560 mg (35%), 0.243 mCi of 3 (Sp. Act 0.068 mCi/mmol). Further purification was carried out by high pressure liquid chromatography: nmr ($CDCL_3$) δ 2.0-2.3 (m, 4H), δ 2.12 (S, 3H), δ 3,3-3.8 (m, 2H), 7.4 (m, 1H); IR (film) 1745 $cm^{-1}$, 1450 $cm^{-1}$, 1370 $cm^{-1}$, 1225 $cm^{-1}$; uv: $\lambda_{max}^{H_2O}$ (ε) 350 (75), 231 (4,834); Mass Spectrum: m/e 158 (5.36%), m/e 99 (28.41%), m/e 69 (18.93%); $[\alpha]_D^{25}$ = 0 (C=0.3, EtOH).

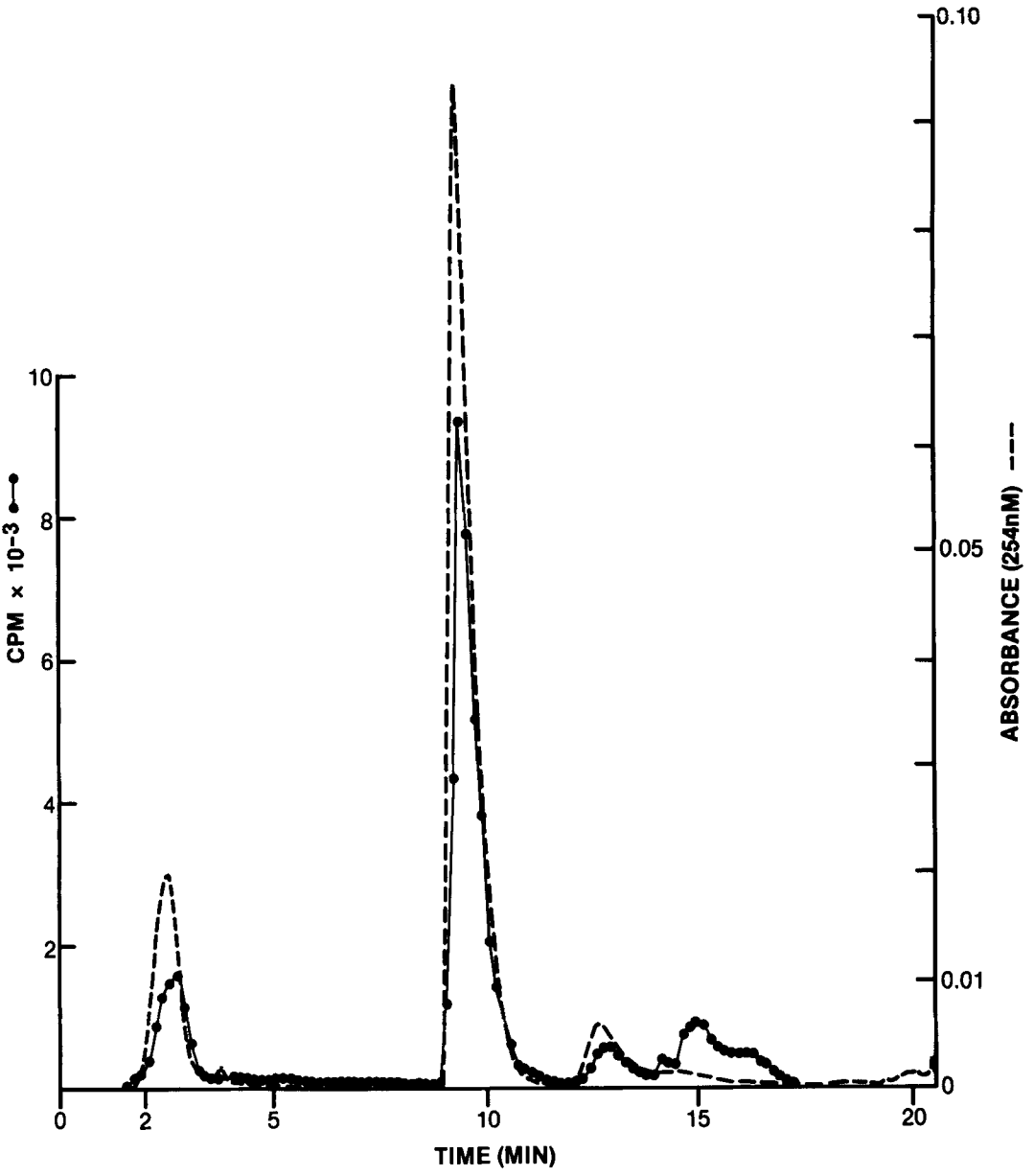



**Figure 1. HPLC Chromatogram of α-acetoxy-N-nitroso-4 [$^3$H]-pyrrolidine 3. 0.2μl of 3 dissolved in 20μl of 28% methanol-water was loaded on a Dupont Zorbax-ODS column and eluted with 28% methanol-water at 1.5ml/min.**

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