SYNTHESIS OF ^{15}N-1 NICOTINAMIDE. A GENERAL, ONE STEP SYNTHESIS OF ^{15}N LABELED PYRIDINE HETEROCYCLES.

Norman J. Oppenheimer*, Terry O. Matsunaga and Bernard L. Kam

Department of Pharmaceutical Chemistry, School of Pharmacy
University of California, San Francisco
San Francisco, California, 94143.

Received November 3, 1977

Summary

A one step, high yield synthesis is reported of ^{15}N-1 nicotinamide from the reaction of ^{15}N ammonia with 1-N-(2,4-dinitrobenzene)-3-carbamoyl-pyridinium chloride. This reaction represents a specific example of a general and facile method for the synthesis of a wide variety of ^{15}N labeled pyridine heterocycles.

Key words: ^{15}N-1 Nicotinamide

Introduction

Biologically active derivatives of pyridine are common, for example, the coenzymes nicotinamide adenine dinucleotide and the pyridoxals. There are also many pharmacologically active alkaloids such as nicotine and quinoline derivatives. The incorporation of ^{15}N into such molecules is important both as a nonradioactive tracer for metabolic studies and increasingly for ^{15}N nuclear magnetic resonance studies.

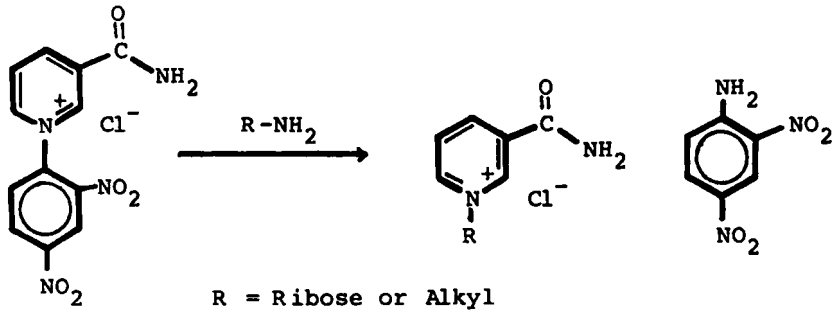
In the coenzymes sited above, the heteroatom has an intimate role in their chemical mechanism by acting as an electron source or sink. Hence, an ^{15}N label should prove a highly sensitive probe to their interaction with, and activation by enzymes as studied by ^{15}N NMR. Because of the interest in this laboratory in studies of the mechanism of pyridine nucleotide dependent dehydrogenases we have investigated the synthesis of ^{15}N-1 nicotinamide.

Previous syntheses of ^{15}N-1 pyridines have involved *de novo* formation of the ring by a Hantzsch type reaction of ^{15}N ammonia with a specially

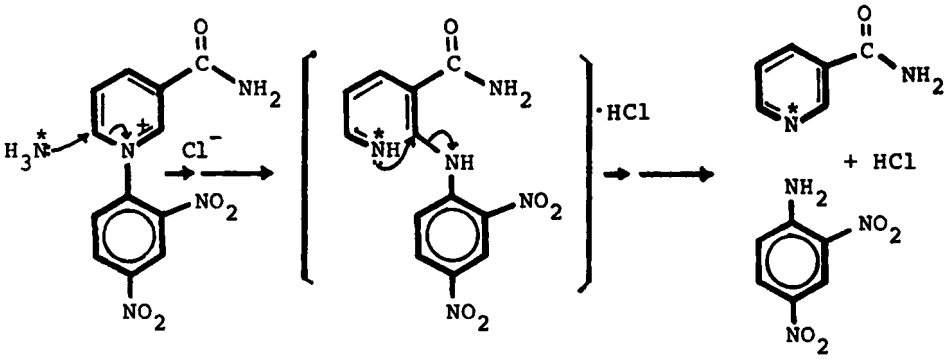
*To whom reprint request should be addressed.

0362-4803/78/0015-0191$01.00

synthesized dicarbonyl precursor. The labeled dihydropyridine intermediate must then be oxidized, with subsequent chemical modifications yielding the desired product. The synthesis of ^{15}N-2-bromopyridine *via* reaction of ammonia and 2-hydroxy-4-pyrone-5-carboxylate is such a series of reactions (1). With the exception of the synthesis of ^{15}N pyridine itself, which is a one step reaction with a 50-55% yield (2), these reactions are difficult, time consuming and have a poor overall efficiency of ^{15}N utilization.



Scheme 1



Scheme 2

Recently, we have been employing a method developed by Atkinson et al. (3) for the synthesis of nicotinamide nucleosides. This reaction shown in scheme 1 is an example of the Zincke reaction in which a primary amine attacks a Zincke salt, an 1-N-(2,4-dinitrobenzene)pyridinium halide, to give an N-substituted pyridinium and dinitroaniline (4). The mechanism proposed for this reaction (scheme 2) involves a nucleophilic addition of a primary amine or ammonia followed by ring opening, ring closing and finally elimination of dinitroaniline (4) with the resulting incorporation of the exogenous nitrogen as the heteroatom. Therefore, this reaction has potential as an alternative to the Hantzsch type method for incorporation of ^{15}N into a variety of pyridine heterocycles. The reaction sequence would involve synthesis of the Zincke salt from the desired unlabeled pyridine derivative and then in a one step reaction with ^{15}N ammonia the free heterocycle would be regenerated but with an ^{15}N label at the heteroatom. In order to test this method and to generate a precursor for ^{15}N NMR studies of pyridine coenzymes we report the synthesis of ^{15}N-1 nicotinamide from the reaction of ^{15}N ammonia with 1-N-(2,4-dinitrobenzene)-3-carbamoylpyridinium chloride.

Experimental

^{15}N-1 Nicotinamide: Into 600 mL of anhydrous methanol at 0°C in a one liter Erlenmeyer flask were added ^{15}N ammonium chloride (0.93 g, 17 mmol, 99% Korr Isotopes Cambridge, MA) and freshly distilled triethylamine (3.35 g, 33.2 mmol). After stirring for twenty minutes a solution of 1-N-(2,4-dinitrobenzene)-3-carbamoylpyridinium chloride (5.28 g, 16.3 mmol prepared according to ref. 5) dissolved in 100 mL of anhydrous methanol was added dropwise over 1 hr. Addition of the Zincke salt produced an immediate deep red color, characteristic of the ring-opened form. After addition, the solution was allowed to come to room temperature and was

stirred for three days, until the color of the solution had become yellow. The methanol was removed by rotoevaporation and the yellow precipitate was suspended in 100 mL of water and filtered. The yellow solid of dinitroaniline was further washed with cold water, 40 mL total, and the combined filtrates were concentrated in vacuo. The dried residue was dissolved in 80 mL of acetonitrile, which upon standing gave long needles of triethylamine-HCl. After filtering off the crystals the filtrate was again concentrated to dryness, dissolved in minimal water and applied to a 2.5 x 40 cm C-2 silica gel reverse phase column. The column was initially eluted with 100 mL of water followed by a 1.5 liter water:absolute ethanol linear gradient. The collected crude ^{15}N-nicotinamide fractions were rotoevaporated to a pale yellow powder (melting range of 118°-123°) and the nicotinamide was separated from residual dinitroaniline on a 2 x 30 cm silica gel column eluted with acetonitrile:ethanol:dichloromethane (5:1:1; v:v:v). The pooled ^{15}N-1 nicotinamide fractions were concentrated to dryness and crystallized from acetonitrile; total yield 1.65 g, 13.4 mmol (82.5%) M.P. 128°-129° uncorrected. ^{1}H-NMR (D_2O pD = 7.1 δ from tetramethylammonium) 5.74 (H-2), 5.51 (H-6), 5.05 (H-4) and 4.40 (H-5) with the following nitrogen-proton coupling constants in Hz: $^{2}J_{1-2}$ = 9.6, $^{2}J_{1-6}$ = 10.1, $^{3}J_{1-5}$ = 1.8, $^{4}J_{1-4}$ < 0.3. Isotopic purity was 99.0 $\pm$ 0.3% as determined by chemical ionization mass spectrometry.

^{1}H-NMR spectra were obtained on a Varian XL-100 nuclear magnetic resonance spectrometer equipped with a Nicolet technologies Fourier transform system. Mass spectra were obtained on a Associated Electrical Industries MS-902 mass spectrometer with a direct inlet system and modified for chemical ionization.

Results and Discussion

The specific reaction of ammonia with 1-N-(2,4-dinitrobenzene)-3-carbamoyl-pyridinium chloride is shown in Scheme 2. Note that this reaction liberates

a mole of HCl for each mole of ammonia consumed. In contrast the reaction of a primary amine (Scheme 1) yields a pyridinium ion and no acid. Thus, if only ammonia gas is used directly for the reaction, a two fold excess is required, one mole to react, a second to neutralize the HCl. This is an inefficient use of label although the ^{15}N ammonium chloride generated would be easily recovered as we have shown for triethylamine-HCl. We have found that using buffers is not without problems. Such buffers as phosphate or carbonate cause an irreversible formation of a bright red, insoluble polymer at the high concentrations of the Zincke salt that have been used by previous investigators for syntheses of pyridinium compounds (3,4). This latter problem can be circumvented by using a triethylamine buffer and keeping the concentration of the reactants low. In this manner, recovered yields in excess of 80%, based on ^{15}N ammonium chloride, were routinely achieved.

The Zincke reaction should prove to be of general use in the synthesis of most ^{15}N labeled pyridines since there is ample precedence for the formation of alkyl and glycosyl pyridinium compounds using Zincke salts of pyridiniums substituted with various electron withdrawing groups (6). Less reactive Zincke salts like 1-N-(2,4-dinitrobenzene) pyridinium chloride have been found to be unable to ring close and eliminate the dinitroaniline (3,4). Nonetheless, through modification of the reaction conditions we expect that even the less reactive pyridines should be easily labeled by this method.

Acknowledgements

This work was in part supported by NIH grant GM-22982 and utilized the UCSF NMR Research Resource Center supported by the NIH Grant RR-00892.

References

1. Hertog, H. J. den, Boer, H., Streef, J. W., Vekemans, F. C. A. and Zoest, W. J. van, Recueil J. Royal Netherlands Chem. Soc. 93, 195 (1974).

2. Whaley, T. W. and Ott, D. G., J. Labelled Compounds 10, 283 (1974).

3. Atkinson, M. R., Morton, R. K. and Naylor, R., J. Chem. Soc. (London), 610 (1965).

4. Zincke, T., Annalen, 341, 371 (1905).

5. Lettre, V. H., Haede, W. and Ruhbaum, E., Annalen 579, 123 (1953).

6. Freyne, E. J., Lepoivre, J. A. and Alderweireldt, F. C., J. Carbohydrates Nucleosides Nucleotides 3, 113 (1976).