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An improved synthesis of radiolabeled 4-(acetoxymethylnitrosamino)-1-(3-pyridyl)-1-butanone

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Summary

The synthesis of high specific activity tritium-labeled 4-(acetoxymethylnitrosamino)-1-(3-pyridyl)-1-butanone is reported. This compound was prepared by tritiation of 4-(acetoxymethylnitrosamino)-1-[3-(5-bromo)-pyridyl]-1-butanone (5-BrNNKOAc) in the presence of 10% Pd/C catalyst, yielding a product with a specific activity of 11.9 Ci/mmol. This pathway represents a major improvement over previously published methods. Copyright © 2001 John Wiley & Sons, Ltd.

Key Words: Tobacco-specific nitrosamines; NNK; NNN and NNKOAc

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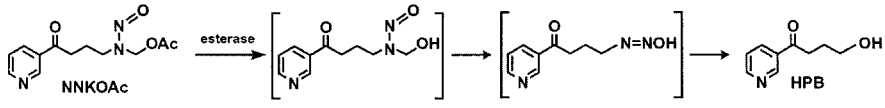
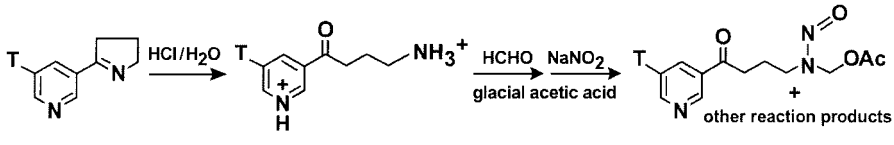
Introduction

The tobacco-specific nitrosamines, N′-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) are among the most potent carcinogens present in tobacco smoke and tobacco products.[1] DNA alkylation is thought to initiate the carcinogenic process for both these nitrosamines. NNN pyridyloxobutylates DNA[2]



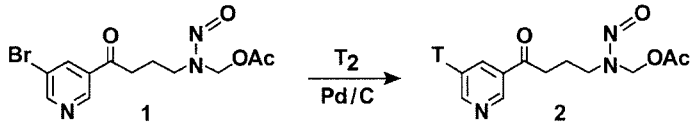
whereas NNK methylates and pyridyloxobutylates DNA.[3] The available data suggest that DNA pyridyloxobutylation by NNN or NNK contributes to the carcinogenic and mutagenic properties of these nitrosamines.[1] An invaluable tool in these studies has been 4-(acetoxymethylnitrosamino)-1-(3-pyridyl)-1-butanone (NNKOAc). This compound, in the presence of esterase, generates *in situ* the same pyridyloxobutylating species formed upon 2′-hydroxylation of NNN or methyl hydroxylation of NNK. NNKOAc has been used in a variety of *in vivo* and *in vitro* assays as a model compound for the pyridyloxobutylation pathway.[3–9] Tritiated NNKOAc has been invaluable to studies investigating the carcinogenic properties of pyridyloxobutylating nitrosamines.[3,5,6]



Scheme 1. Previous synthetic pathway for [5-^{3}H]NNKOAc

The previously reported synthesis started with [5-^{3}H]myosmine and required several synthetic steps with an overall yield of less than 4% (Scheme 1).[6] The early introduction of tritium and the poor yield required working with large amounts of radioactivity (500–1000 mCi). Another limitation was the radiochemical instability of [5-^{3}H]myosmine, which restricted the specific activity of [5-^{3}H]NNKOAc to less than 1 Ci/mmol. In addition, the purification of the radiolabeled compound was cumbersome, necessitating HPLC purification of

the final product. In this paper, we report the synthesis of 4-(acetoxymethylnitrosamino)-1-[3-(5-bromo)-pyridyl]-1-butanone (5-BrNNKOAc, 1) which is then subjected to reductive tritiation to yield [5-^{3}H]NNKOAc (2) in one step. Purification was achieved by flash chromatography with silica gel. This method allows for the rapid preparation of [5-^{3}H]NNKOAc of high specific activity (Scheme 2).



Scheme 2. New synthetic pathway for [5-^{3}H]NNKOAc

Results and discussion

Hydrogen substitution of the bromine in 5-BrNNKOAc proceeded rapidly in ethyl acetate in the presence of Pd/C catalyst, with greater than 90% conversion to product within 30 min. The reaction did not proceed in the presence of triethylamine, a surprising observation given published studies with similar compounds.[10] TLC purification of the reaction product followed by NMR analysis confirmed the production of the desired product, NNKOAc.

The tritiolysis reaction proceeded more slowly; starting material remained after 3.5 h. The identity of the purified reaction product was confirmed by HPLC analysis with radioflow detection; 93% of the radioactivity co-migrated with NNKOAc standard. [5-^{3}H]NNKOAc (specific activity 11.9 Ci/mmol) was stable for more than 6 months when stored in 5% toluene in methylene chloride at –80°C as judged by periodic HPLC analysis with radiochemical detection.

Experimental

General

^{1}H-NMR spectra were recorded on a Bruker AM360WB NMR spectrometer. UV spectra were recorded on a Hewlett-Packard Model 8452A diode array spectrometer. HPLC analyses were performed with a

Waters 510 system with a Shimadzu SPD-UV-visible detector and a β-Ram radioflow detector.

4-(acetoxymethylnitrosamino)-1-[3-(5-bromo)-pyridyl]-1-butanone

5-BrNNKOAc was prepared from 5-bromomyosmine[11] by a modification of a previously published method for NNKOAc.[4] Upon conversion of 5-bromomyosmine (1.0 g, 4.4 mmol) to 4-amino-1-[3-(5-bromo)-pyridyl]-1-butanone dihydrochloride, it was combined with paraformaldehyde (135 mg, 4.4 mmol) in 40 ml glacial acetic acid and stirred at 65°C for 3 h. Sodium nitrite (460 mg, 6.7 mmol) in water was added to the ice-cooled mixture over 30 min. After 30 min at room temperature, the acetic acid was removed under reduced pressure. The residue was dissolved in saturated sodium bicarbonate solution and extracted four times with chloroform. The combined organic layers were washed with saturated NaCl, dried (sodium carbonate), filtered and concentrated. The product was purified using silica gel column chromatography. The column was eluted successively with methylene chloride (500 ml), 10% ethyl acetate in methylene chloride (500 ml) and 20% ethyl acetate in methylene chloride (500 ml). 5-BrNNKOAc eluted in the last fraction. It was further purified by preparative TLC on silica gel developing with 50% ethyl acetate in hexane. Product ($R_f = 0.2$) was eluted from the silica gel with acetone. It was approximately 95% pure (12.9 mg, 37.5 µmol, 1% yield). NMR ($CDCl_3$) δ: 9.0 (s. 1 H, 2-pyrH), 8.9 (1 H, m, 6-pyrH), 8.3 (1 H, m, 4-pyr), 6.2 and 5.4 (2 H, s, CH_2OAc), 4.4 and 3.7 (2 H, t, C4), 3.1 and 2.9 (2 H, t, C2), 2.3 and 1.9 (2 H, m, C3), 2.1 and 2.0 (3 H, s, $COCH_3$).

Reductive tritiation

5-BrNNKOAc (4.3 mg, 12.5 µmol) and 10% Pd/C (9.7 mg) in ethyl acetate (1.0 ml) in a 10 ml reaction vessel were degassed via three freeze/thaw evacuation cycles prior to the introduction of 10.0 Ci of carrier-free tritium gas (0.172 mmol, 58 Ci/mmol). Reaction progress was monitored by TLC analysis (ethyl acetate). After 3.5 h, the catalyst was removed by filtration through a short column of Celite (0.5×3 cm) which was washed with 2 ml ethyl acetate and 3 ml ethanol. The solvents were removed under reduced pressure and labile tritium was removed by co-distillation with methanol (3×1 ml). The residue was dissolved in

20 ml ethyl acetate to give 65 mCi of crude product. The solvent was evaporated and the residue dissolved in 10 ml toluene.

[5-^{3}H]NNKOAc (24 mCi) was purified with a 5 ml disposable pipette packed with Florisil in toluene. The column was eluted sequentially with 10 ml each of methylene chloride, 10% ethyl acetate in methylene chloride, 25% ethyl acetate in methylene chloride, 50% ethyl acetate in methylene chloride and 100% ethyl acetate. TLC analysis (ethyl acetate) indicated that 5-BrNNKOAc eluted in the 25% ethyl acetate fraction and [5-^{3}H]NNKOAc eluted in the last two fractions. Solvent was evaporated and the residue was dissolved in 5% toluene in methylene chloride for storage at -80°C (total activity: 15.7 mCi). Radiochemical purity (93%) was established by HPLC analysis with radiochemical detection (see below). Specific activity (11.9 Ci/mmol) was determined by measuring UV absorption (NNKOAc: $\varepsilon_{232\,nm} = 14\,750$ in 15 mM sodium citrate, pH 7) and liquid scintillation counting.

Structural confirmation was achieved by HPLC with radiochemical detection. A C18 column (Phenomenex) was eluted with a linear gradient from 100% sodium phosphate buffer, pH 7.0, to 50% sodium phosphate buffer, 50% methanol over 50 min. The radioactive peak co-eluted with unlabeled NNKOAc standard (47 min). In the presence of esterase, the radioactive peak for NNKOAc disappeared. A new radioactive peak (32 min) co-eluted with 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB), the major hydrolysis product of NNKOAc.

Acknowledgements

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