SYNTHESIS OF HIGH SPECIFIC ACTIVITY TRITIATED DIHYDROPYRIDINES: NICARDIPINE-^{3}H*

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SUMMARY

The synthesis of high specific activity 3-nitrobenzaldehyde-[4,6-^{3}H], a key intermediate in the general synthesis of tritiated 4-aryldihydropyridines, is described. This substance was then used to prepare the calcium channel entry blocker, nicardipine-[4',6'-^{3}H] at a specific activity of 51 Ci/mmole via the Hantzsch process.

Key Words: Dihydropyridines-^{3}H, nicardipine-^{3}H, m-nitrobenzaldehyde-^{3}H, calcium channel entry blockers.

INTRODUCTION

Certain dihydropyridine (DHP) derivatives act as powerful antihypertensive agents by attenuating smooth muscle and cardiac muscle contractions (1,2,3). These properties derive from the fact that DHPs block the influx of calcium ions into vascular smooth muscle cells (2). Nicardipine (8) (2,3,4), a compound currently marketed by Syntex Corp. in the United States for the treatment of hypertension and angina, is particularly effective in this regard because of its selectivity. Its effect on smooth muscle is approximately ten times greater than on cardiac muscle (5). It is, therefore, an effective antihypertensive agent which exhibits minimal inotropic properties.

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Although we had recently reported the synthesis of nicardipine-^{14}C (6) for use in metabolism studies, a high specific activity tritiated analog was needed for radioimmunoassay development, for receptor binding and autoradiography studies, as well as for additional metabolism work. We report herein a synthesis of nicardipine-^{3}H (at 51 Ci/mmol) which can be applied to the production of a variety of other high specific activity tritiated 4-aryldihydropyridine analogs.

DISCUSSION

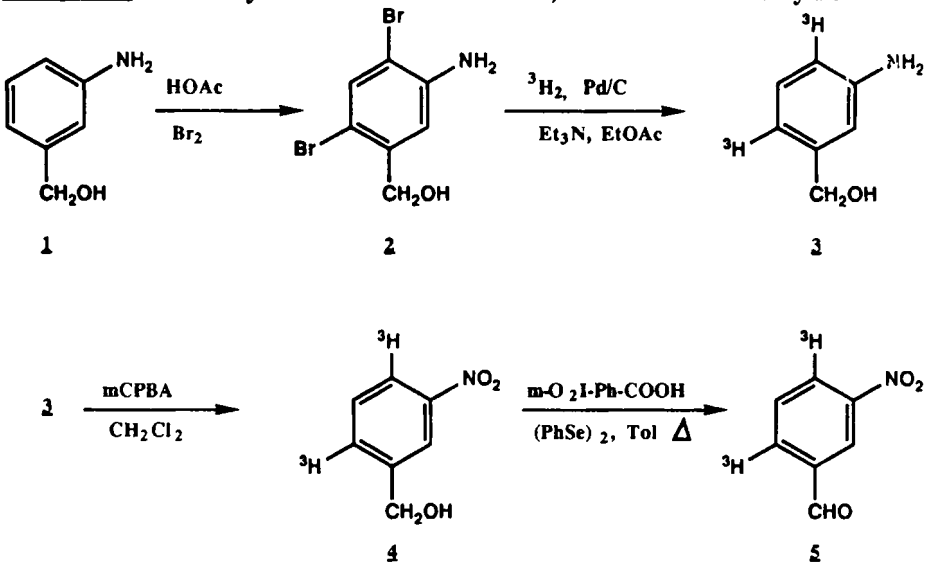
We recently described (6) the synthesis of C-14 labelled nicardipine. That work described the synthesis of the key labelled intermediate m-nitrobenzaldehyde-^{14}C and its use in the general synthesis of C-14 labelled 4'-nitrophenyl-DHPs by means of the Hantzsch process (7,8). Our need for high specific activity tritiated nicardipine served as an impetus to develop a synthesis of tritiated m-nitrobenzaldehyde (5) and thereby extend the generality of this intermediate for the preparation of labelled DHPs.

Two additional considerations were important in selecting (5) as our tritiated target. The physical form and volatility of carrier free tritiated intermediates is an important factor in the design of any tritium labelled synthesis. This safety consideration made (5) particularly suitable as our labelled reagent since this material is a nonvolatile crystalline solid. Other possible labelled intermediates in this synthesis are volatile and would present potential health hazards during isolation and purification procedures.

The second point concerns the metabolic stability of the label. The metabolism of 4-aryl-DHPs, and Nicardipine in particular, has been extensively studied (4,9,10). All known metabolites are primarily the result of side chain degradation and/or oxidation of the DHP to the pyridine form. More to the point, there are no known metabolites resulting from hydroxylation of the aromatic ring. These results suggest that the tritium in aryl labelled DHPs would be metabolically stable.

Introduction of tritium by reductive dehalogenation was an obvious approach to the synthesis of labelled (5). This presented a problem, however, since reductive dehalogenation conditions would also reduce the nitro group (11). This problem was overcome by the strategy shown in *scheme 1*.

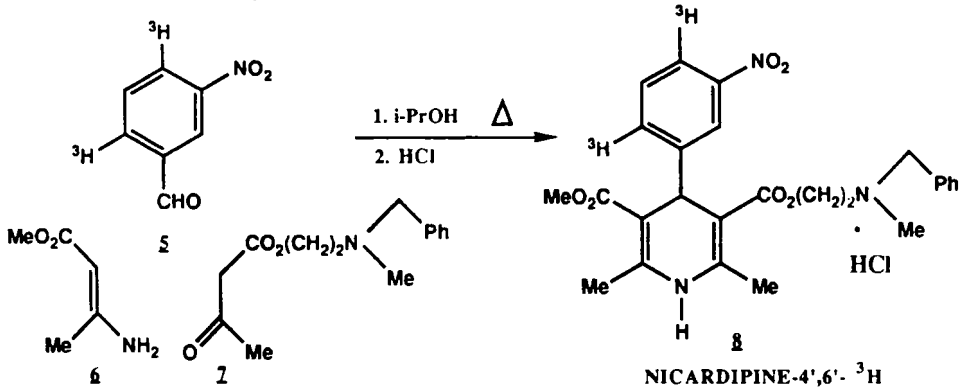
scheme 1: **Synthesis of 3-nitro-4,6-^{3}H-benzaldehyde**



The precursor for tritiation, 2,4-dibromo-5-hydroxymethylaniline (2) was prepared by bromination of 3-hydroxymethylaniline (1) in acetic acid. Reduction of (2) with 10 Ci of carrier free tritium gas in the presence of 10% Pd/C in ethyl acetate containing triethylamine afforded 1100 mCi (37%) of tritiated hydroxymethylaniline (3) after chromatographic purification. The amino group was selectively oxidized with m-chloroperbenzoic acid (12) to the corresponding nitro compound (4) in 57% yield. Further oxidation using m-iodoxybenzoic acid/diphenyldiselenide (13) afforded the key labelled intermediate 3-nitro-4,6-^{3}H-benzaldehyde (5) in 74% yield. This oxidizing system is particularly effective and practical. Alcohols are oxidized to aldehydes without a trace of over-oxidation. Furthermore, the reactions are clean and product isolation is very simple (14). The m-iodoxybenzoic acid is removed by a basic extraction and the diphenyldiselenide (present in catalytic amount) is extremely non-polar and is removed at the solvent front during chromatographic purification of the product. Hantzsch condensation of (5) with methylaminocrotonate (6) and the acetoacetate (7) (6) in i-propanol at 80° followed by aqueous acidic workup gave nicardipine-[4'6'-^{3}H] hydrochloride (8) as depicted in ***scheme 2***. Final purification on a Chromatotron afforded pure (8) in 26% yield. The specific activity was found to be 51 Ci/mmole by uv analysis and radioassay. Although this specific activity is somewhat lower than theoretical, it is nevertheless quite high for a reductive debromination process based on our experience. There is no satisfactory explanation, to our knowledge, for the source of tritium dilution in such reductions.

The methodology developed herein was focussed on the synthesis of 3-nitro-4,6-^{3}H-benzaldehyde as the key labelled intermediate since the 4-[3'-nitrophenyl] group was a constant in various dihydropyridine derivatives of interest in our laboratory. However, because of the selectivity of the steps in this sequence, our approach can be applied to the synthesis of a wide variety of highly labelled aromatic aldehydes. Furthermore, the dihydropyridine side chain can be easily varied by the synthesis of different of acetoacetates. This can be accomplished by condensation of a desired alcohol with diketene (6).

scheme 2 : **Syntesis of Nicardipine-[4'6'-^{3}H]**



The general potential of our strategy, combined with its use of nonvolatile labelled intermediates and its operational simplicity, should make it useful for the preparation of a wide variety of high specific activity tritiated dihydropyridines.

EXPERIMENTAL

Carrier free tritium gas was purchased from DuPont NEN Research Products. Cold reagents were purchased from Aldrich Chemical Co. and used without purification. Solvents were HPLC grade. "Chromatotron" is a radial chromatography apparatus manufactured by Harrison Research, Inc., Palo Alto, CA. Radiochromatography was performed on a Bioscan 200 scanner. Radioassays were obtained using a Packard 4000 liquid scintillation counter. UV spectra were obtained using a Hitachi UV-265 spectrophotometer. NMR spectra were recorded using a Varian EM 390 spectrometer.

2,4-Dibromo-5-hydroxymethylaniline (2)

A solution of bromine (509 mg, 0.16 mL, 3.18 mmole) in 2 mL of acetic acid was added to a solution of 3-hydroxymethylaniline (370 mg, 3.0 mmole) in 10 mL of acetic acid at such a rate that the

bromine color in the reaction dissipated before the next drop was added. When the bromine color persisted, further addition was stopped and the reaction stirred for 15 min. The reaction was poured onto ice chips and extracted with ethyl acetate. The organic phase was washed with sodium bisulfite, sodium bicarbonate, water, brine and then dried over sodium sulfate. Purification by column chromatography (silica gel, hexane-ethyl acetate 2:1) afforded the desired product (2) (193 mg, 0.69 mmole) in 23% yield along with a mixture of monobromo compounds (216 mg, 1.07 mmole). The structure of (2) was consistent with its nmr (DMSO, δ7.0, s, 1H; δ7.4, s, 1H; δ4.3, m, 2H, δ4.3-4.5, m, 3H) and mass spectra (M^+ 279, 281, 283).

3-Hydroxymethyl-4,6-^{3}H-aniline (3)

A 10 cc side arm septum flask was charged with 50 mg of 5% Pd-C, connected to a vacuum line and evacuated. A solution of (2) 14 mg, 0.05 mmole) in 1 mL of ethyl acetate containing 0.2 mL of triethylamine was injected and the system was de-gassed. The reaction flask was immersed in liquid nitrogen and 10 Ci (0.167 mmole) carrier free tritium gas was transferred into it by means of a Toeppler pump. The system was brought to ambient temperature and stirred overnight. Labile radioactivity was removed by vacuum transfer into a waste bulb connected to the vacuum line. The residue was dissolved in methanol, filtered trough a teflon filter connected to a disposable syringe and taken to dryness. Three additional evaporations from methanol ensured complete removal of exchangeable tritium. Purification on the Chromatotron (silica gel, 30-50% gradient of ethyl acetate) afforded 1100 mCi of pure (3).

3-Hydroxymethyl-4,6-^{3}H-nitrobenzene (4)

A 90 mCi portion of (3) was evaporated to dryness. A solution of m-chloperbenzoic acid (5 mg, 0.03 mmole) in 2 mL of chloroform was added all at once. The reaction was heated at reflux for 30 min., diluted with 50 mL of chloroform, washed successively with sodium bicarbonate, water, brine, and dried over sodium sulfate. Purification on the Chromatotron (silica gel, 30-50% gradient of ethyl acetate-hexane) afforded 51 mCi (56%) of (4). The purity as determined by TLC (ethyl acetate-hexane 1:1) and radioscanning was >98%. Analysis by uv (ethanol, λ_{max} 264nm, ε_{max} 7100) and radioassay showed the specific activity to be 51 Ci/mmole.

3-Nitro-4,6-^{3}H-benzaldehyde (5)

A suspension of m-iodoxybenzoic acid (167 mg, 0.93 mmole) and

diphenyldiselenide (11.2 mg, 0.036 mmole) in 3 mL of toluene was heated at reflux until the yellow color dissipated. (about 30 min.). A solution containing 170 mCi of labelled alcohol (4) in 5 mL toluene was treated with one mL of the oxidation suspension and the reaction was stirred at reflux fro 1 hr. After cooling to ambient temperature, the reaction was diluted with ethyl acetate and washed sequentially with, sodium bicarbonate, water, brine, and dried over sodium sulfate. Purification on the Chromatotron (silica gel, hexane-ethyl acetate 5:1) gave 126 mCi (74%) of pure aldehyde(5).

Nicardipine-[4'6'-^{3}H] hydrochloride (8)

Solutions of m-nitrobenzaldehyde-4,6-^{3}H] (90 mCi, 0.0018 mmole), methyl 3-aminocrotonate (0.58 mg, 0.0051 mmole), and 2-(N-methyl-N-benzylamino)ethyl acetoacetate (6), each in 0.1 mL of i-propanol, were combined and heated at 80°. Additional portions of crotonate and acetoacetate were added after 4 hrs. and 20 hrs. After 28 hrs. the reaction was evaporated to dryness. The crude residue was dissolved in dichloromethane and applied directly to the Chromatotron. Elution with a 1-2% gradient of methanol in dichloromethane gave 25 mCi of partially purified product which contained the pyridine analog as a close running impurity. Two additional purifications on the Chromatotron (silica gel, 5-10% gradient of i-propanol in hexane) furnished 23 mCi of pure nicardipine-4'6'-^{3}H free base. The chromatography solvent was replaced with ethanol and 1 equivalent of conc HCl was added to give the title compound. The specific activity was determined by uv analysis (ethanol, λ_{max} 353 nm, ε 4600) and radioassay to be 51 Ci/mmole. The purity was confirmed to be >99% by radiochroamtography under the following conditions:

1. silica gel: a) dichloromethane-methanol (95:5)
   b) toluene-acetone-acetonitrile (8:2:1)
   c) hexane-i-propanol (9:1)
2. RPC-18 (Whatman KC-18F) acetone-water (4:1)

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