Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXV, No. 6

SYNTHESIS OF [^{14}C]-LABELLED DIHYDROPYRIDINE CALCIUM CHANNEL ENTRY BLOCKERS: NICARDIPINE-[4-^{14}C] AND RS-93522-[4-^{14}C]*

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SUMMARY

The Hantzsch synthesis has been applied to the general preparation of 4-aryl-dihydropyridines labelled in the metabolically stable 4-position of the dihydropyridine ring. The synthesis is based on the preparation of a key common intermediate, m-nitrobenzaldehyde-[formyl-^{14}C], in high yield from $Ba^{14}CO_3$.

Key Words: Dihydropyridines-[^{14}C], Nicardipine-^{14}C, RS-93522-^{14}C, m-nitrobenzaldehyde-^{14}C, calcium channel entry blockers.

INTRODUCTION

Various dihydropyridines (DHPs) have been shown to be calcium channel entry blocker (CEBs)[1,2] and, as such, they are antihypertensive agents. Two such compounds currently under development at Syntex Research are Nicardipine[2] (11) and RS-93522[16] (12). These compounds are known to be extensively metabolized via degradation of the side chain at the 5-position of the dihydropyridine ring[3,4,5]. In order to study the metabolism, bioavailability, and absorption of these compounds, labelled analogs were prepared in which the label was in a metabolically stable position, namely the dihydropyridine ring.

DISCUSSION

A variety of synthetic methods are available for the construction of substituted DHPs[6]. The most efficient and versatile approach, however, remains the Hantzsch synthesis which was described over 100 years ago[7]. Earlier use of this method for the synthesis of labelled Nicardipine[2,8] relied on the preparation of labelled methyl acetoacetate followed by reaction with ammonia to produce the required 3-methylamino crotonate-[^{14}C]. Use of this intermediate in the Hantzsch synthesis gave 6-[^{14}C]-Nicardipine in 46% yield from commercially purchased ethyl acetoacetate-[^{14}C][3]. Since large quantities of labelled DHPs were required, purchase of acetoacetate-[^{14}C] was not a viable option. Alternatively, literature procedures for the synthesis of ethyl acetoacetate-[^{14}C] from $^{14}CO_2$indicate that the product is available at low specific activity in yields of about 50%[9]. Such a process would afford Nicardipine-[^{14}C] in 23% from $^{14}CO_2$.

Furthermore, isolation of labelled acetoacetate requires aqueous workup. We felt this would be a hazardous operation because of the volatility of the product.

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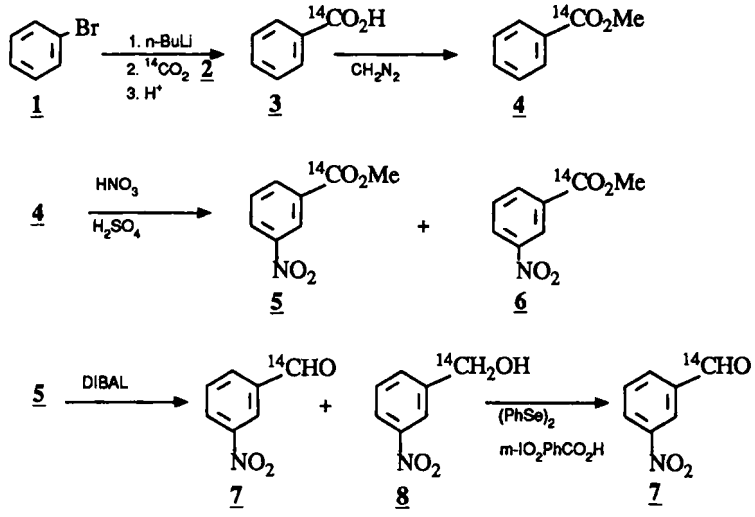
0362–4803/88/060621–06$05.00

Received July 22, 1987
Revised October 5, 1987

In order to make the synthesis of DHP analogs operationally safer and more economical, we have developed a synthesis for, and used m-nitrobenzaldehyde-[formyl-^{14}C] (7) as the key labelled intermediate in the Hantzsch process. This non-volatile compound can be easily isolated and purified in large scale and at high specific activity. An additional benefit is that this aryl substituent is a constant in our various DHP derivatives so that a single synthesis of (7) may be used to prepare a variety of DHP CEBs having different side chains at C-3 by using appropriate acetoacetate derivatives.

The use of a labelled arylaldehyde in the Hantzsch process has been reported for the synthesis of Nifedipine-[^{14}C][10]. In that case o-trifluoromethylbenzaldehyde-[formyl-^{14}C] was prepared by displacement of the corresponding iodide with Cu^{14}CN followed by diisobutylaluminum hydride (DIBAL) reduction. However, neither experimental details nor yields were given.

We generally prefer a sequence in which the label is incorporated in a carbonation step. This tends to be a very economical and high yielding approach which has the additional advantage that the carboxyl group can be easily transformed into a variety of other functionalities[11] (the formyl group, in this case). The most direct sequence to the desired (7) using this strategy would involve carbonation of m-nitrobromobenzene. Although o-nitrobromobenzene affords o-nitrobenzoic acid in high yield using a lithiation/carbonation sequence, the corresponding meta and para substituted compounds cannot be lithiated[12]. The less highly efficient route to (7) which was, therefore, developed is shown in **Scheme 1**.

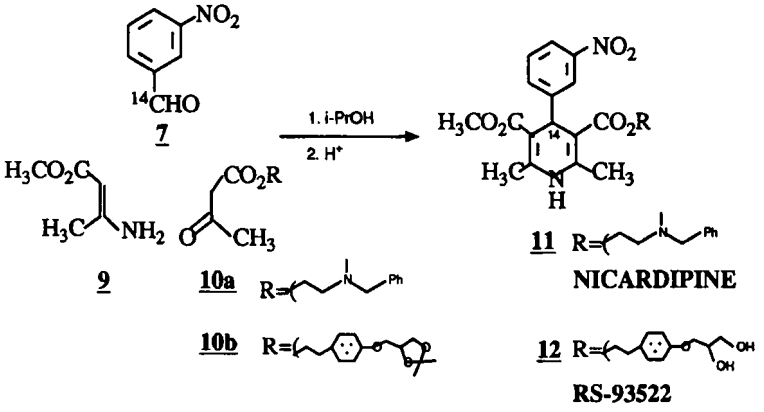


Scheme 1

Carbonation of bromobenzene (1) with high specific activity $^{14}CO_2$ (2) afforded benzoic [^{14}C] acid (3) in quantitative yield. It has been reported that nitration of methyl benzoate gives higher yields and a more favorable meta/ortho product ratio than benzoic acid itself[13]. Therefore, (3) was isolated by ether extraction, dried, and treated directly with diazomethane in ether to give methyl [^{14}C]-benzoate (4), again, in quantitative yield. The ether was evaporated at water aspirator vacuum using an ice bath to prevent possible evaporation of (4). The neat methyl [^{14}C]-benzoate thus obtained was cooled in ice, dissolved in ice cold conc. H_2SO_4, and treated with a 1:1 mixture of ice cold conc. HNO_3/H_2SO_4. Aqueous workup afforded mixture of m-nitromethyl [^{14}C]-benzoate (5) and o-nitromethyl [^{14}C]-benzoate (6) in quantitative yield. This result verified the fact that no (4) was lost upon evaporation during the previous step. These two isomers were effectively separated using radial silica gel chromatography on a Chromatotron (1-5% EtOAc-hexane) and the desired meta isomer was isolated in 77% yield. Reduction of (5) without affecting the nitro group was accomplished using DIBAL in toluene. The reduction could not be

controlled, however, to give the desired aldehyde (7) without overreduction to the benzyl alcohol (8) even when less than one equivalent of DIBAL was used at -100°. The reduction to the aldehyde/alcohol mixture was quantitative, however. This mixture was, therefore, oxidized directly without purification using catalytic diphenyldiselenide and m-iodoxybenzoic acid in refluxing toluene[14]. This oxidation was found to be far superior to the Collins oxidation[15] since it avoids the severe problems associated with the recovery of the product from gummy chromium salts. Furthermore, the reaction is exceptionally easy to work up. A basic extraction serves to remove m-iodoxybenzoic acid and the catalytic amount of diphenyldiselenide, which is extremely non-polar, is removed at the solvent front during chromatographic purification. The key labelled intermediate, m-nitrobenzaldehyde-[formyl-^{14}C] was thus isolated in 88% yield. The overall yield for the five step sequence from $Ba^{14}CO_3$ was 68%.

Scheme 2 depicts the use of aldehyde (7) in the synthesis of the two DHPs (11) and (12). Hantzsch condensation of (7) (diluted with carrier to 18 mCi/mmol) with methyl 3-aminocrotonate (9) and the acetoacetate (10a) in i-propanol gave 21 mCi of Nicardipine-[4-^{14}C] (11) in 71 % yield (48% from $Ba^{14}CO_3$) after purification on the Chromatotron. This represents a significant improvement in the previously reported yield of 46% from methyl acetoacetate-[^{14}C] (probably no more than 25% from $Ba^{14}CO_3$). In a similar manner, using the acetoacetate derivative (10b) and the undiluted aldehyde (7), the DHP RS-93522-[4-^{14}C] (12) was prepared in 52 % yield at a specific activity of 54 mCi/mmol.



Scheme 2

The synthesis and use of the labelled arylaldehyde (7) in the Hantzsch process affords a safe, versatile, and high yielding method for the synthesis of high specific activity [4-^{14}C]-dihydropyridine calcium channel entry blockers.

EXPERIMENTAL

$Ba^{14}CO_3$ was purchased from Atomic Energy of Canada. Nitrosomethylurea was purchased from Columbia Organic Chemicals. Other cold reagents were purchased from Aldrich Chemical Co. Solvents were reagent grade or better and were used without purification. "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. Products were identified by radiochromatographic mobility compared to authentic standards on Analtech silica gel plates.

BENZOIC-[^{14}C] ACID

A side-arm septum flask charged with bromobenzene (1090 mg; 7 mmol) was connected to a high vacuum line and evacuated. The flask was cooled to -78° and about 25 ml of THF was distilled in from $LiAlH_4$. The solution was stirred and n-BuLi equiv. of n-BuLi (5.76 mmol; 3.6 ml of a 1.6M soln.) was injected through the septum. After 10 min. $Ba^{14}CO_3$ (279 mCi; 54 mCi/mmol; 1030 mg; 5.17 mmol), contained in a 50 ml side-arm septum flask was acidified with H_2SO_4 and the liberated $^{14}CO_2$ was vacuum transferred into the reaction vessel. Stirring was continued for an additional 20 min. at which time 1 ml of a satd. Na_2CO_3 soln. was injected to quench the reaction. Following removal of labile radioactivity, the reaction mixture was partitioned between aqueous $NaHCO_3$ and ether to remove neutrals. The aqueous phase was acidified with conc. HCl, extracted with ether, and dried over Na_2SO_4 to give radiochemically pure benzoic acid (280 mCi) in 100% yield.

METHYL BENZOATE-[^{14}C]

A portion of the ether soln. of benzoic-[^{14}C] acid (138 mCi) obtained above, was cooled in ice and treated with a slight excess of freshly prepared diazomethane (nitrosomethylurea/aqu. KOH/ether).[13a] The ether soln. of methyl benzoate-[^{14}C] was taken to dryness using a water aspirator and a cold water bath. The product was isolated in quantitative yield and used directly in the next step.

METHYL m-NITROBENZOATE-^{14}C]

The neat methyl benzoate-[^{14}C] (138 mCi) obtained above was flushed with N_2, cooled in ice and treated with 3 ml of conc. H_2SO_4 which had been previously cooled to O°. After 5 min., 1.5 ml of a 1:1 solution of HNO_3/H_2SO_4 (previously cooled to O°) was added dropwise with rapid stirring Radiochromatography (silica gel; EtOAc-hexane, 1:9) showed complete reation to a 4:1 mixture of methyl m-nitrobenzoate [^{14}C] and methyl o-nitrobenzoate-[^{14}C]. The reaction was quenched with ice water followed by $NaHCO_3$, then extracted with EtOAc. The organic phase was washed with water, brine, and dried over Na_2SO_4. Separation of the meta and ortho isomers was effected by radial chromatography on a Chromatotron using a 2 mm silica gel rotor which was eluted with a 1-5% gradient of EtOAc in hexane. The desired methyl m-nitrobenzoate-[^{14}C] (106 mCi) was obtained in 77% radiochemical yield.

m-NITROBENZALDEHYDE-[FORMYL-^{14}C]

A solution of methyl m-nitrobenzoate-[^{14}C] (106mCi; 54 mCi/mmol; 1.96 mmol)in 20 ml of toluene was cooled to -78° and treated with 3.9 ml of a 1.5M solution of DIBAL (5.85 mmol). After 1 hr. all the starting material was consumed and the reaction consisted of a 7:3 mixture of m-nitrobenzyl-[^{14}C] alcohol and m-nitrobenzaldehyde-[formyl-^{14}C]. The reaction was quenched with 10% HCl and the products were extracted with toluene and dried over sodium sulfate.

A heterogenous mixture of diphenyldiselenide (112 mg; 0.36 mmol) and m-iodoxybenzoic acid (1.4g, 5 mmol) was heated at reflux in toluene until the yellow color of the diphenyldiselenide disappeared (about 30 min). This mixture was then added to a solution of the above aldehyde/alcohol mixture also in toluene. The reaction was stirred at reflux for about 2 hrs. at which time radio-tlc analysis showed complete conversion to m-nitrobenzaldehyde-[formyl-^{14}C]. Standard aqueous $NaHCO_3$ workup followed by purification on the Chromatotron (2 mm silica gel rotor eluted with a 5-30% gradient of ethyl acetate-hexane) afforded 93 mCi of the pure title compound. The radiochemical yield was 67% from $Ba^{14}CO_3$.

2-(N-METHYL-N-BENZYLAMINO)ETHYL ACETOACETATE

Neat 2-(N-methyl-N-benzyl)aminoethanol (2.96 g; 17.9 mmol) was cooled in an ice bath and diketene (1.66 g; 19.6 mmol) was added dropwise with stirring. The ice bath was removed and the reaction was stirred at 80°for 1 hr, then at room temperature overnight. The reaction was quenched with water and extracted with ethyl acetate. The organic phase was washed with brine and dried over Na_2SO_4. Column chromatography (silica gel eluted with 10% EtOAc-hexane followed by 2% MeOH-CH_2Cl_2) afforded the pure title compound (4.23 g; 17 mmol) in 95% yield.

NICARDIPINE-[4-^{14}C]

An ethyl acetate solution of m-nitrobenzaldehyde-[formyl-^{14}C] (29.5 mCi) prepared above, was diluted with carrier to a specific activity of 18.1 mCi/mmol (1.63 mmol) and the solution was evaporated to dryness. Methyl-3-aminocrotonate (226 mg; 1.96 mmol) and 2-(N-methyl-1-N-benzylamino)ethyl acetoacetate were added sequentially in 2ml i-propanol each. The reaction was heated at reflux for 6 hrs. then stirred at ambient temperature overnight. The reaction was evaporated to dryness, reconstituted in methylene chloride and purified by Chromatotron chromatography (silica gel, 4mm rotor, eluted with 2% MeOH-CH_2Cl_2. A total of 21 mCi (71%) of pure title compound was isolated. The specific activity was determined by uv analysis and radioassay to be 17.13 mCi/mmol.

uv(EtOH): λ_{max} 253 nm, ε 6582.
Radiochromatography: silica gel, CH_2Cl_2-dioxane (1:4); CH_2Cl_2-MeOH (98:2). RPC-18, acetone-water (4:1).

2,6-DIMETHYL-4-(3-NITROPHENYL)-1,4-DIHYDROPYRIDINE-[4-^{14}C]-3,5-DICARBOXYLIC ACID 2[4-(2,3-DIHYDROXYPROPOXY)PHENYL]-ETHYL METHYL ESTER (RS-93522)

An ethyl acetate solution of m-nitrobenzaldehyde-[formyl-^{14}C] (20 mCi; 54 mCi/mmol; 0.37 mmol) was evaporated to dryness. The solid residue was treated with methyl-3-aminocrotonate (60 mg; 0.52mmol) and 2[4-(2,3-dihydroxypropoxy-2,3-acetonide) phenylethyl acetoacetate, each in 1.5 ml of i-propanol. The reaction was heated at reflux for 6 hrs. At this point radio-tlc (EtOAc-hexane 1:1) showed about 30% unreacted ^{14}C-aldehyde. An additional 30 mg of crotonate and 90 mg of acetoacetate were added and the reaction was heated for an additional 2.5 hrs then allowed to stir at ambient temperature overnight. The i-propanol was evaporated and the residue was treated with 4 ml of THF and 1.0 ml of 10% HCl. Heating at reflux for 1 hr. resulted in complete hydrolysis of the acetonide (silica gel, CH_2Cl_2-MeOH 9:1). The THF was evaporated and the reaction was partitioned between CH_2Cl_2 and $NaHCO_3$. The organic phase was washed with water, brine, and dried over Na_2SO_4. Three purifications on the Chromatotron (silica gel, 4 mm rotor, 5% MeOH-CH_2Cl_2) afforded 10.3 mCi (52% yield) of the pure title compound. The specific activity was determined by uv analysis and radioassay to be 54.4 mCi/mmol.

uv(MeOH) λ_{max} 228 nm, ε 29,876.
Radio-tlc: silica gel, CH_2Cl_2-MeOH (95:5); CH_2Cl_2-EtOH-EtOAc (18:1:1); RPC-18, MeOH-water (3:1).

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