Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXVII, No. 5

Radiosynthesis of [^{11}C]Nifedipine and [^{11}C]Nicardipine

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Summary

The radiosyntheses of the dihydropyridine (DHP) calcium channel blockers [^{11}C]nifedipine and [^{11}C]nicardipine by alkylation of the appropriate DHP 3-monocarboxylic acid anion with [^{11}C]iodomethane are described. Isolated radiochemical yields of 30-50% were obtained 25 minutes after the end-of-bombardment, with specific activities of 400-1400 mCi/μmol at the end-of-synthesis. A variety of positron emitting DHPs with different ester side chains in the 3 and 5 positions on the DHP ring may be prepared by this approach using currently available labelled alkylating agents.

Key Words: carbon-11, dihydropyridine derivatives, calcium channel blocker, radiotracer, nicardipine, nifedipine.

Introduction

1,4-Dihydropyridine derivatives (DHP's) have received much attention recently as potent calcium channel blockers with clinical efficacy in the treatment of oxygen deficiency diseases of the heart (1). Investigations using tritium labelled DHP's such as nifedipine **1** and closely related analogues have resulted in the identification of high affinity, saturable, and stereoselective binding sites in various tissues *in vitro* and *in vivo* (2-5). ^{14}C labelled DHP's, designed for metabolic studies (6,7), and an ^{125}I labelled analogue (8) have also been reported. The non-invasive imaging and

0362–4803/89/050589–10$05.00

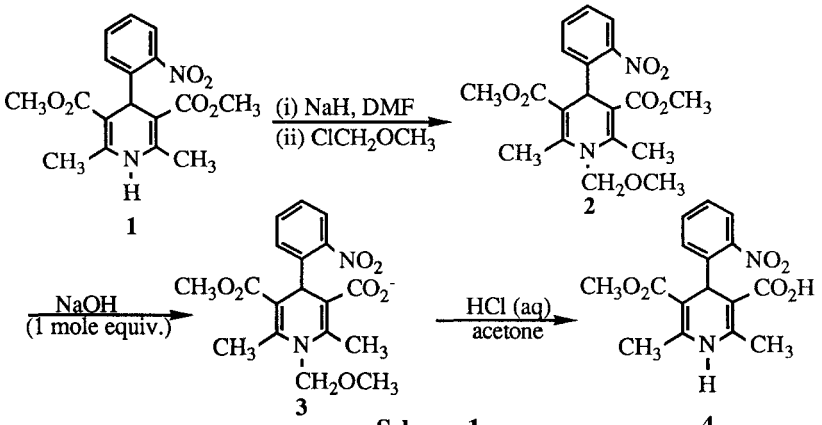
Received November 8, 1988

quantification of these putative calcium channels in living human tissues by positron emission tomography (PET) requires the development of positron-emitting DHP radiotracers.

Structure-activity relationships for DHP's suggest that the most lucrative site for introducing a positron emitting radionuclide is within the ester side-chains (9). Much variation is possible in this region without adverse loss of potency and the side chains are synthetically more accessible for labelling with short-lived radionuclides than other portions of the DHP structure. We (10) and others (11) have shown that the alkylation of carboxylic acid anions by [^{11}C]iodomethane is a facile reaction for the preparation of ^{11}C methyl esters. Recently, a variety of ^{11}C labelled alkylating agents (iodomethane, propyl iodide, benzyl iodide, etc.) (12) and of ^{18}F labelled agents (fluoroethyl tosylate, fluoropropyl bromide, etc.) (13) have been developed. A synthetic strategy which could take advantage of this wealth of electrophilic reagents was devised. We report here the synthesis of two DHP's, nifedipine **1** and nicardipine **10** (8), labelled with carbon-11 using methods which may be applied to the preparation of other DHP's labelled with fluorine-18 or carbon-11.

Results

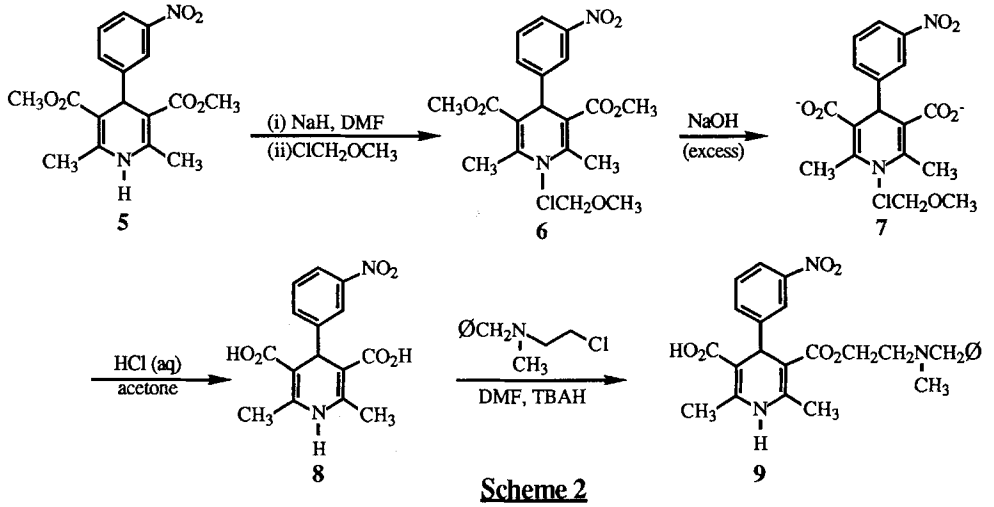
The precursor for radiolabelling nifedipine, the monocarboxylic acid **4**, was prepared as outlined in Scheme 1. Dihydropyridines of the Hantszch type (14) are reported to be peculiarly resistant to acid, base, or nucleophilic catalysed hydrolysis of the ester groups (15). However Iwanami *et al.* (7) have reported that the N-methoxymethyl derivatives of DHP's may be hydrolysed by base catalysis.



Scheme 1

Thus, treatment of the N-methoxymethyl derivative of nifedipine **2** with one equivalent of sodium hydroxide in methanol resulted in the carboxylic acid **3** which generated the desired precursor **4** upon removal of the methoxymethyl group by mild acid hydrolysis.

Unlike nifedipine **1**, nicardipine **10** has unsymmetrical ester side-chains and the preparation of the precursor **9** in this case required the synthesis of the key intermediate dicarboxylic acid **8**. Treatment of **6** with excess sodium hydroxide in

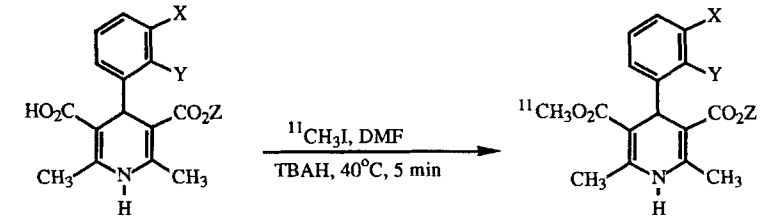


Scheme 2

methanol produced **7** which gave the dicarboxylic acid **8** upon removal of the N-methoxymethyl moiety with aqueous acid (Scheme 2). The desired precursor **9** to radiolabelled nicardipine was obtained by reaction of this dicarboxylic acid with one equivalent of N-(2-chloroethyl)-N-methyl-benzylamine and tetrabutylammonium hydroxide in dimethylformamide (DMF).

Modelling reactions were carried out to determine appropriate conditions for the alkylation of carboxylic acid **4** with [^{11}C]iodomethane. In DMF, at 25 °C, the reaction proved to be essentially quantitative with a 2nd order rate constant of 6 x 10^3 $M^{-1}s^{-1}$.

Scheme 3 depicts the synthesis of both [^{11}C]nicardipine and [^{11}C]nifedipine. [^{11}C]Iodomethane was produced as described previously (10, 15) and trapped at -78 °C in 200 μL of DMF containing 1-2 mg of the appropriate precursor. Upon addition of one equivalent of tetrabutylammonium hydroxide, the solution was warmed to 40 °C in a water bath for 5 min and the labelled DHP isolated by preparative HPLC. Yields were 40-50% for [^{11}C]nifedipine, and 30-40 % for [^{11}C]nicardipine (uncorrected for



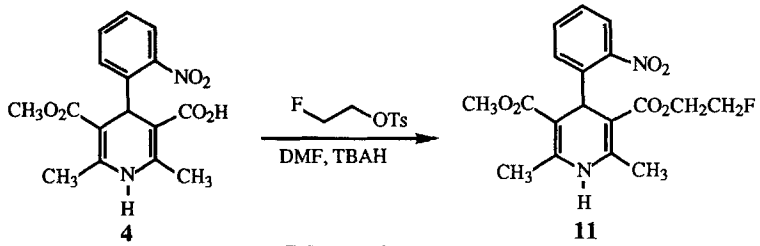
4 X=H, Y=NO_2, Z=CH_3
9 X=NO_2, Y=H, Z=$CH_2CH_2N(CH_3)CH_2Ø$

1 X=H, Y=NO_2, Z=CH_3
10 X=NO_2, Y=H, Z=$CH_2CH_2N(CH_3)CH_2Ø$

Scheme 3

decay, based on [^{11}C]iodomethane) with specific activities of 400-1400 mCi/μmol at the end-of-synthesis.

Finally, using fluoroethyl tosylate as the alkylating agent, the monocarboxylic acid **4** was esterified, yielding the DHP derivative **11** (Scheme 4). This process was carried out in good yield under conditions compatible with the requirements of ^{18}F labelling.



4 **11**

Scheme 4

Discussion

The model reactions of **4** with iodomethane demonstrated that alkylation of the carboxylate anion was fast even at 25 °C, being essentially complete after 7 min, compatible with the short half-life (20.4 min) of ^{11}C. Nifedipine was the only product observed by HPLC from these reactions unless excess base was added whereupon small amounts of two other products were detected, possibly from N-methylation of nifedipine and **4**.

The facility of the alkylation of the carboxylic acid anions of **4** and **9** with [^{11}C]iodomethane was borne out by the successful radiosyntheses of [^{11}C]nifedipine and [^{11}C]nicardipine. The reactions were clean and rapid with minimal amounts of radioactive side-products and gave good yields of radiochemically pure (>99.5%), high specific activity material. The precursors **4** and **9** are much more hydrophilic than

nifedipine and nicardipine respectively which allows for a complete and rapid separation of product from excess starting material by semi-preparative HPLC.

The approach used for the radiosynthesis of [^{11}C]nifedipine and [^{11}C]nicardipine should be applicable to the preparation of a host of other DHP's labelled with ^{11}C or ^{18}F. The monocarboxylic acid **4** may be used to prepare labelled analogues which contain a methyl ester side chain with the other ester side-chain containing the radionuclide. Carboxylic acid anions are potent nucleophiles, especially in dipolar aprotic solvents (17), and should combine quickly with positron emitting alkylating agents which are less reactive than iodomethane. The preparation of the fluoroethyl compound **11** (equipotent with nifedipine) depicted in Scheme 4 illustrates the feasibility of this approach as [^{18}F]fluoroethyl tosylate has been reported and used as an alkylating agent (13,18).

A more general approach would make use of the dicarboxylic acid **8**. Different ester side-chains may be attached by using the appropriate electrophile to monoalkylate **8** as shown by the preparation of the nicardipine precursor **9** in Scheme 3. Reaction with the appropriate radiolabelled alkylating agent may then be carried out to generate the desired positron emitting dihydropyridine derivative.

Experimental

NMR spectra were obtained on an IBM NR/80 using $(CH_3)_4$ Si as an internal standard, and IR spectra were recorded on a Perkin-Elmer 399B. DMF was stirred overnight with BaO then distilled under reduced pressure from BaO. Nifedipine and nicardipine were purchased from Sigma. Wherever possible, reactions were carried out in the dark or in dim red light as some DHP's are known to be light sensitive. Elemental analyses were performed by Atlantic Microlab, Atlanta, Georgia. All new compounds gave satisfactory analyses (C,H,N $\pm$ 0.4%). Purification and analyses of radioactive mixtures were performed with a previously described system (16). The HPLC columns used were either A - Alltech Econosil C_{18} (250mm x 10mm), or B - Alltech Econosil C_{18} (250mm x 4.4mm). Peak areas were measured using Hewlett-Packard 3390A recording integrators. Isolated radiochemical yields were determined with a dose-calibrator (Capintec CRC-5R).

Dimethyl 2,6-dimethyl-1-methoxymethyl-4-(2-nitrophenyl)-1,4-dihydro-pyridine-3,5-dicarboxylate 2. A variation of the method of Iwanami (7) was followed. A slurry of nifedipine (7.0 g, 20.3 mmol) in DMF (20 mL) was vigorously stirred at ambient temperature under nitrogen whilst NaH (60% in oil, 1.2 g, 30 mmol) was added over 5 min. The resultant solution was stirred for 90 min , cooled to -5 °C , and a solution of chloromethylmethyl ether (3.5 mL, 46.1 mmol) in DMF (10 mL) was added dropwise. The mixture was stirred for 30 min at ambient temperature then quenched with aqueous $NaHCO_3$ (5%, 200 mL) and extracted with CH_2Cl_2 (2 x 100 mL). The combined organic extracts were washed with water (2 x 150 mL), dried (Na_2SO_4), and filtered. The solvent removed to leave an oily red liquid which was taken up in CH_3CN (100 mL) and filtered using paper which had been saturated with CH_3CN to remove the hydrocarbon oil. The product was isolated by column chromatography (silica gel, toluene/ethyl acetate 4:1) as a yellow solid (4.6 g, 59%). A sample was recrystallised from hexane/THF : mp 113-117 °C; IR (KBr) 1705, 1530, 1195, and 1165 cm^{-1}; ^{1}H-NMR ($CDCl_3$) ∂ 7.2-7.6 (m, 4H), 5.62 (s, 1H), 4.83 (s, 2H), 3.64 (s, 6H), 3.38 (s, 3H), and 2.46 (s, 6H). Anal. (C, H, N).

1-Methoxymethyl-5-methoxycarbonyl-2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3-carboxylic acid 3. A solution of **2** (7.37 g, 18.9 mmol) and KOH (87.1 %, 1.40 g, 19 mmol) in methanol (39 mL) was heated to reflux for 48 hr then cooled. Water (80 mL), and ethyl acetate (80 mL) was added, followed by aqueous HCl (1N, 13.6 mL) with good stirring. The aqueous layer was extracted with ethyl acetate (100 mL) and the combined organic extracts washed with water (100 mL), dried (Na_2SO_4), and filtered. The solvent was removed to leave a sticky red solid (6.96 g, 98%). A small portion was recrystallised from hexane/THF to give a buff amorphous solid: mp 164-165 °C; IR (KBr) 1705, 1655, 1530, and 1210 cm^{-1}; ^{1}H-NMR ($CDCl_3$) ∂ 7.2-7.5 (m, 4H), 5.65 (s, 1H), 4.81 (s, 2H), 3.65 (s, 3H), 3.37 (s, 3H), 2.51 (s, 3H), and 2.43 (s, 3H). Anal. (C, H, N).

5-Methoxycarbonyl-2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydro- pyridine-3-carboxylic acid 4. Crude **3** (6.76 g, 18 mmoL) was stirred in acetone (205 mL) at 0 °C and aqueous HCl (1N, 40.8 mL) added dropwise. The solution was then stirred for 30 min at ambient temperature and water (500 mL) added. The mixture was extracted with ethyl acetate (2 x 300 mL) and the combined organic extracts were washed with

water (200 mL), dried (Na_2SO_4), and filtered. Evaporation of the solvent gave a brown oil which solidified upon standing Trituration with hot chloroform/ethyl acetate (9:1) gave a yellow powder (2.13 g, 31%): mp 185-186.5 °C. Recrystallisation from methanol gave dark orange crystals but did not raise the melting point. IR (KBr) 3290, 1700, 1690, 1650, and 1215 cm^{-1}; ^{1}H-NMR (DMSO-d_6) ∂ 11.65 (br s, 1H), 8.79 (br s, 1H), 7.3-7.8 (m, 4H), 5.52 (s, 1H), 3.45 (s, 3H), 2.24 (s, 6H). Anal. (C, H, N).

Dimethyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-di-carboxylate 5. Yellow crystals were obtained by the method of Hantzsch (19): mp 208-210 °C lit. (20) 209-210 °C.

N-(2-Chloroethyl)-N-methylbenzylamine hydrochloride 12. The method of Ehrenpries (21) gave a white solid after two recrystallisations from dioxane : mp 137-138 °C lit. (21) 136-138 °C.

2,6-Dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid 8. Starting from **5** the synthesis of **8** was the same as that of **4** from **1** except that excess (3 molar equivalents) KOH in methanol was used in the saponification of **6** to **7**. Upon removal of the methoxymethyl group with aqueous hydrochloric acid in acetone, the product was isolated by vacuum filtration and washed with copious amounts of water followed by ethyl acetate to give **8** as a yellow solid (34 % from **5**): mp 177 °C; IR (KBr) 3360, 2950, 1675, 1520, 1345, 1215 cm^{-1}; ^{1}H-NMR (DMSO-d_6) ∂ 11.70 (br s, 2H), 8.81 (br s, 1H), 7.5-8.1 (m, 4H), 5.04 (s, 1H), 2.32 (s, 6H); ^{13}C-NMR (DMSO-d_6) ∂ 168.4, 150.3, 147.6, 145.8, 133.9, 129.3, 121.6, 120.7, 101.3, 39.3, 18.1; Anal. (C, H, N).

5-[2-(N-benzyl-N-methylamino)]ethoxycarbonyl-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3-carboxylic acid 9. A stirred solution of **8** (4.0 g, 11.5 mmol) in DMF (80 mL) was treated with **12** (2.0 g, 9.1 mmol) followed by aqueous tetrabutylammonium hydroxide (40%, 14 mL, 21.6 mmol). The resultant mixture was stirred at 60 °C for 2 hr, cooled, and water (600 mL) added. The mixture was extracted with CH_2Cl_2 (2 x 200 mL), dried (Na_2SO_4), filtered, and the solvent removed to leave a red oil. Column chromatography (silica gel, ethyl acetate/toluene 1:1) gave a partially purified product which was not successfully recrystallised. Final purification was achieved using reverse-phase flash chromatography (Waters prep

Bondapak (55-105 μm), methanol/H_2O + 0.1 N NH_4HCO_2 , 50:50) which gave pure **9** as a yellow powder (0.80 g, 20 %): mp 85-89 °C: IR (KBr) 3310, 1675, 1530, and 1210 cm^{-1}; ^{1}H-NMR ($CDCl_3$) ∂ 7.2-8.1 (m, 9H), 6.45 (br s, 1H), 5.14 (s, 1H), 4.18 (t, 2H), 3.54 (s, 2H), 2.67 (t, 2H), 2.33 (s, 6H), 2.20 (s, 3H); ^{13}CNMR ($CDCl_3$) ∂ 176.2, 166.8, 149.5, 148.2, 146.3, 146.1, 145.0, 137.5, 134.3, 129.1, 128.5, 128.1, 127.1, 122.7, 121.1, 103.1, 61.9, 61.1, 55.1, 41.7, 39.5, 19.5, 19.3; Anal. (C, H, N).

Modelling Reactions 1. A solution of **4** (25.0 mg, 75.2 μmol) in DMF (4.0 mL) was treated with aqueous tetrabutylammonium hydroxide (190 μL, 0.4 M, 76 μmol) at 25 °C. The solution was stirred and the required amount of iodomethane added by syringe, either neat or as a 10% (w/v) solution in DMF. Aliquots (100 μL) were removed periodically and injected into an ethanolic solution of acetic acid (1 mL, 10%) to quench the reaction. These solutions were then analysed by HPLC (column B) and the yields of nifedipine calculated by comparing peak areas to a standard curve.

Modelling Reactions 2. Methyl 2-fluoroethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydro-pyridine -3,5-dicarboxylate 11. A stirred solution of **4** (1.0 g, 3.01 mmol) and fluoroethyl tosylate (0.657 g, 3.3 mmol) in DMF (20 mL) was treated with a methanolic solution of tetrabutylammonium hydroxide (1 M, 3.1 mL) dropwise. The solution was heated to 60 °C for 45 min, cooled and H_2O (200 mL) added. Extraction with ethyl acetate (3 x 50 mL) followed by drying (Na_2SO_4), filtration, and evaporation left a dark yellow oil. This was chromatographed on silica gel (toluene/ethyl acetate 4:1) to give a yellow oil (0.53 g, 46.5%). A portion was recrystallised from ethyl acetate/hexane to give a dark yellow solid: mp 120-122 °C; IR (KBr) 3320, 1680, 1530, and 1215 cm^{-1}; ^{1}H-NMR ∂ 7.2-7.8 (m, 4H), 5.80 (s, 1H), 5.6 (br s, 1H), 3.8-4.85 (m, 4H), 3.57 (s, 3H), 2.36 (s, 3H), 2.31 (s, 3H); ^{13}C-NMR ∂ 167.2, 166.7, 145.2, 144.3, 141.9, 132.5, 131.0, 124.9, 123.9, 103.9, 103.3, 85.3, 63.1, 62.1, 50.8, 34.8, 19.5, 19.4, 14.0.

Radiosyntheses [^{11}C]CH_3I, produced as previously described (10, 15), was swept by a stream of nitrogen gas into a solution of 12 mg of **4** or **9** in DMF (200 μL) cooled to -78 °C. A solution of tetrabutylammonium hydroxide (10 μL, 0.4 M) was then added and the solution heated to 40 °C for 5 min. HPLC buffer (250 μL) was then added, and the mixture was injected onto HPLC semi-preparative column A, and eluted with 78/22 MeOH/H_2O + 0.1N NH_4HCO_2 at 5 mL/min ($k'_{nifedipine}$ = 4.1, $k'_{nicardipine}$ = 4.7). The

desired fraction was collected, evaporated to dryness, and the residue taken up in 6 mL of sterile saline and 1 mL of ethanol (7 mL of saline only for [^{11}C]nicardipine). This was passed through a sterile 0.22 μm filter into a sterile, pyrogen free bottle and aqueous sodium bicarbonate (3 mL, 8.4%) was added. The radiochemical purity and specific activity of the final solutions were determined by analytical HPLC using column B; 78/22 MeOH/H_2O + 0.1N NH_4HCO_2, 3 mL/min ($k'_{nifedipine}$ = 4.3, $k'_{nicardipine}$ = 5.0). The total synthesis time for both labelled DHPs was approximately 25 mins from the end-of-bombardment.

Acknowledgements

This work was supported in part by USPHS No. CA 32845 and NS 15080.

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