

## SYNTHESIS OF PENTAMIDINE LABELLED WITH TRITIUM AND CARBON-14

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### SUMMARY

Tritium labelled pentamidine has been prepared with a specific activity of 90 mCi mmol<sup>-1</sup> using a one-step exchange reaction between the unlabelled drug and tritiated water. The labelling utilised a homogeneous rhodium trichloride catalyst and yielded pentamidine regiospecifically labelled in the positions *ortho* to the amidine groups. Carbon-14 labelled pentamidine was prepared via a seven-step procedure in which the isotope was introduced via a nucleophilic substitution of 4-bromophenol with copper(I) [<sup>14</sup>C]cyanide.

**Keywords:** Pentamidine, synthesis, carbon-14, tritium.

### INTRODUCTION

Pentamidine (I) has been widely used as a drug to treat protozoal diseases, such as malaria, amoebic dysentery and trypanosomiasis<sup>1,2</sup>. More recently, it has also been shown to be effective, when administered in a nebulised form, for both the treatment and the prophylaxis of pneumocystic carinii pneumonia (PCP), a potentially fatal, secondary infection often contracted by AIDS sufferers<sup>3,4</sup>.

In the course of developing pentamidine for the latter application, a radiolabelled form of the drug was required for metabolic, pharmacokinetic and analytical purposes. Two approaches, rational synthesis and exchange labelling, were investigated for the synthesis of the labelled drug. As

a result of these investigations, pentamidine labelled with  $^{14}\text{C}$  in the amidine groups was prepared by a rational synthetic route starting from labelled cyanide. In addition, the drug was conveniently labelled with tritium via a simple one-step metal-catalysed exchange procedure.

#### RESULTS AND DISCUSSION

Previous studies<sup>5,6</sup> have shown that aromatic compounds containing functional groups such as carboxyl, amide, aralkylamine and anilide can be regiospecifically deuteriated ortho to the functional group, by employing a rhodium trichloride catalyst in a single-step exchange reaction with deuterium oxide. Subsequent work has shown that the same high regiospecificity for ortho exchange is maintained when the tritium isotope is used<sup>7,8</sup>. Moreover, a series of pharmacologically-active compounds, containing at least one of these functional groups, have subsequently been labelled in a similar fashion with both deuterium and tritium<sup>7,9</sup>. Since the amidine functional group possesses the required stereoelectronic features necessary to function as a directing group, it was considered likely that pentamidine would prove a suitable candidate for this type of exchange labelling procedure.

Indeed, [ $^3\text{H}$ ]pentamidine was obtained at a specific activity of  $90 \text{ mCi mmol}^{-1}$ , using tritiated water at  $900 \text{ mCi mmol}^{-1}$ . This specific activity is in the same range as that found for other drugs labelled by this method eg nedocromil sodium and sodium cromoglycate, using tritiated water at the same specific activity<sup>10,11</sup>. The proton-decoupled  $^3\text{H}$ -nmr spectrum (Figure 1) contains only one signal corresponding to the positions ortho to the amidine groups, confirming that labelling has occurred regiospecifically as predicted.

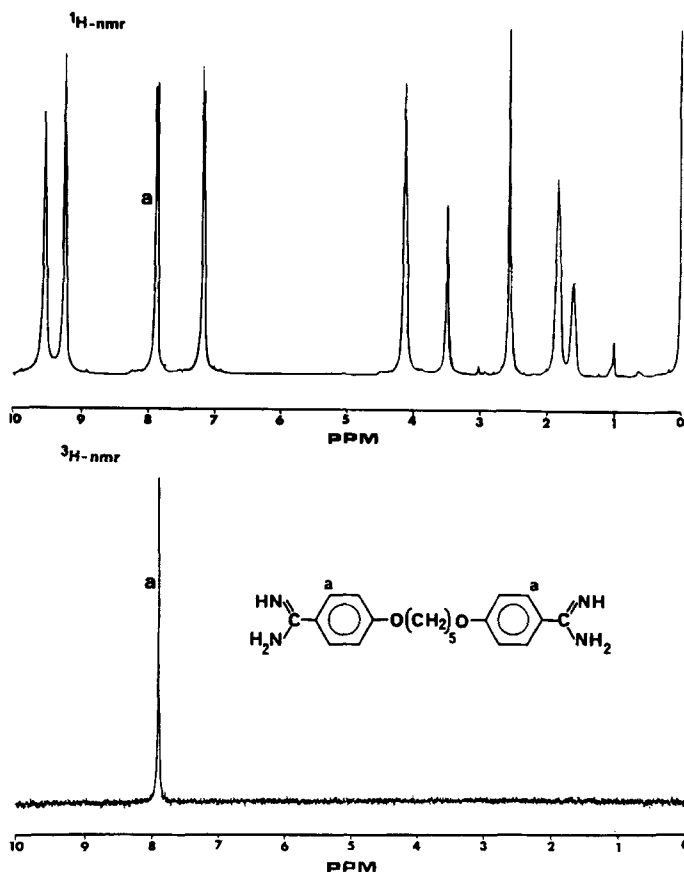


Figure 1

The synthesis of pentamidine labelled with  $^{14}\text{C}$  in both the amidine and the central pentamethylene chain has been reported, in part, previously<sup>12</sup>. In the former synthesis the [amidine- $^{14}\text{C}$ ] material was prepared, in 12% overall yield, via the key 4-hydroxybenzo[ $^{14}\text{C}$ ]nitrile intermediate. This intermediate was obtained by the reaction of a neutral solution of the diazonium salt of 4-aminophenol with nickel(II) sulphate/ potassium [ $^{14}\text{C}$ ]cyanide solution. This particular approach was felt to be unsuitable due to the need to carry out the reaction at neutral pH to avoid loss of cyanide as  $\text{H}^{14}\text{CN}$ . This, together with the resulting reduction in the reactivity of the diazonium salt that is

brought about by the high pH, was felt to be responsible for the low yield. Therefore, an alternative route (Figure 2) was employed<sup>13</sup>, via the nucleophilic exchange of

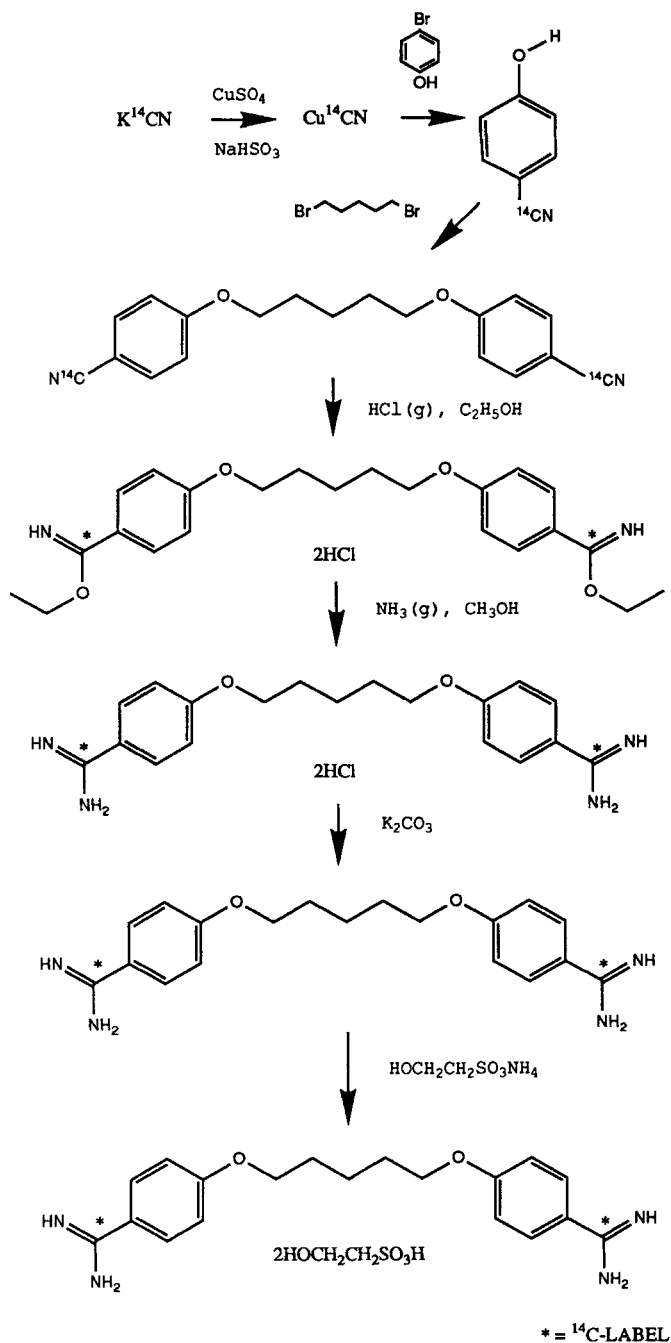


Figure 2

4-bromophenol and copper(I) [<sup>14</sup>C]cyanide. [<sup>14</sup>C]Pentamidine isethionate was obtained from the resulting synthesis in 19% overall yield from potassium [<sup>14</sup>C]cyanide and with a radiochemical purity of 98.9%.

## EXPERIMENTAL

### Materials

Authentic pentamidine isethionate was obtained from the Department of Analytical Chemistry, Fisons plc, Pharmaceutical Division, R&D Laboratories, Loughborough, Leicestershire, UK. Rhodium trichloride was obtained from the Aldrich Chemical Company Ltd., Gillingham, Dorset, UK. Tritiated water was obtained from Amersham International plc, Amersham, Bucks., UK. Potassium [<sup>14</sup>C]cyanide was purchased from ICI Tracerco, ICI Chemicals and Polymers Group, Billingham, Cleveland, UK. All other reagents were of reagent quality.

### Methods

#### <sup>3</sup>H-Nmr Analysis.

<sup>1</sup>H- and <sup>3</sup>H-nmr spectra were recorded at 300.13 MHz and 320.13 MHz respectively, on a Bruker AC-300 nmr spectrometer fitted with a 5 mm dual <sup>1</sup>H/<sup>3</sup>H probe. A solution of [<sup>3</sup>H]pentamidine in [<sup>2</sup>H]dimethyl sulphoxide (100 mm<sup>3</sup>), together with an internal reference (tetramethylsilane, TMS) were injected into a 3 mm microsample tube (Wilmad SK-1374B). The sample was frozen in liquid nitrogen, the tube evacuated, sealed, fitted with PTFE spacer-rings and inserted into a standard 5 mm nmr tube. The annular space around the inner tube, was filled with CCl<sub>4</sub>.

<sup>1</sup>H- and <sup>3</sup>H-nmr free induction decay (FID) data were collected into 32K of 24 bit words (including zero filling) using 30° flip-angle pulses and acquisition times of 3.4 s and 1.6 s for <sup>1</sup>H and <sup>3</sup>H respectively, with spectral widths of 15 ppm

and 12 ppm. FID data were processed according to the standard Fourier transformation methods. The resulting  $^3\text{H}$ -nmr spectrum was 'ghost-referenced' to the frequency of notional, monotrinitated TMS.

Under these conditions an activity of 40  $\mu\text{Ci}$ , present in one site and giving one sharp nmr line (as in the case of pentamidine) could be easily detected in a 16 hour overnight accumulation whilst at the mCi level, satisfactory results were obtained in minutes.

#### Chromatography.

Column chromatography was performed using Merck silica gel 60 (230-400 mesh ASTM) using an appropriate combination of solvents as the eluting mobile phase.

Preparative and analytical hplc were performed using the following chromatographic conditions:-

Column:	Preparative - Dynamax C-18 250 x 21.4 mm Analytical - Dynamax C-18 250 x 5.0 mm	
Solvents:	Pump A - methanol containing trifluoroethanoic acid (65 mmol dm <sup>-3</sup> ) Pump B - water containing trifluoroethanoic acid (65 mmol dm <sup>-3</sup> )	
Flowrate:	Preparative - 9 cm <sup>3</sup> min <sup>-1</sup> Analytical - 1 cm <sup>3</sup> min <sup>-1</sup>	
Gradient:	<u>Time</u>	<u>%A concentration</u>
	Initial	30
	10 min	50
	25 min	50
	30 min	100
	40 min	100
Detection:	UV wavelength - 280 nm	

Thin-layer chromatography was performed using Merck precoated silica gel 60 F<sub>254</sub> 100 x 50 x 0.25 mm tlc plates obtained from BDH Ltd., Poole, Dorset, UK. Tlc plates were quantitated using a Berthold LB284/500HR linear analyser (Berthold Instruments (UK) Ltd., St. Albans, Herts., UK).

The tlc systems used are listed below:-

<u>Solvent system</u>	<u>Parts by Volume</u>
1. hexane/ethyl ethanoate	1:1
2. dichloromethane/ethyl ethanoate	19:1
3. trichloromethane/ethanol	40:1
4. hexane/ethyl ethanoate	3:2
5. hexane/ethyl ethanoate	4:1
6. trichloromethane	1
7. ethyl ethanoate/propan-2-ol/water	10:7:6
8. ethyl ethanoate/butanone/methanoic acid/ water	5:3:1:1
9. methylbenzene/ethanoic acid	3:1
10. trichloromethane/diethyl ether/ methanoic acid	7:2:1

#### Synthesis of [<sup>3</sup>H]pentamidine

Pentamidine hydrochloride (8 mg) and rhodium trichloride (5 mg) were weighed into a 0.5 cm<sup>3</sup> thick-walled reaction vessel and dissolved in *N,N*-dimethylformamide (30 mm<sup>3</sup>). Tritiated water (3 mm<sup>3</sup>, 50 Ci cm<sup>-3</sup>, 150 mCi) was added and the tube cooled in liquid nitrogen, evacuated and sealed; the reaction was heated at 110° for 18 hrs. After cooling, the vessel was opened and the contents removed and diluted with methanol (1 cm<sup>3</sup>). The solvent was evaporated under reduced pressure to ensure removal of labile tritium; this procedure was repeated on a further two occasions. The labelled pentamidine was purified by reversed-phase hplc using the preparative conditions described previously, to afford a white solid. The specific activity was determined gravimetrically and was found to be 90 mCi mmol<sup>-1</sup>.

#### Synthesis of copper(I) [<sup>14</sup>C]cyanide

Copper(II) sulphate pentahydrate (1248 mg, 5 mmol) was dissolved in water (2.6 cm<sup>3</sup>) and heated at 60° whilst being stirred. A solution of sodium bisulphite (434.8 mg, 4.17 mmol) in water (1.3 cm<sup>3</sup>) was added dropwise over a one minute period followed immediately by a solution of potassium [<sup>14</sup>C]cyanide (217.6 mg, 15 mCi mmol<sup>-1</sup>, 50 mCi) in water (1 cm<sup>3</sup>) to give a white precipitate. The reaction was

stirred at 60° for a further 10 minutes after which the solid was filtered, washed with boiling water, followed by ethanol to yield copper(I) [<sup>14</sup>C]cyanide (270.1 mg; chemical yield = 90.5 %).

#### Synthesis of 4-[<sup>14</sup>C]cyanophenol

4-Bromophenol (438.6 mg, 2.535 mmol) was dissolved in *N,N*-dimethylformamide (1.5 cm<sup>3</sup>) and added to the copper(I) [<sup>14</sup>C]cyanide (270.1 mg, 3.016 mmol) and the suspension left to stir at 160° for 4 hours. The reaction was allowed to cool and the *N,N*-dimethylformamide was removed under reduced pressure to leave an oil, which was redissolved in hydrochloric acid (6 mol dm<sup>-3</sup>, 6 cm<sup>3</sup>) and stirred at room temperature for 30 minutes. Iron(III) chloride hydrate (2 mg) in water (1 cm<sup>3</sup>) was then added and stirring continued for a further 30 minutes at 85°. The dark brown solution was diluted with water (40 cm<sup>3</sup>), extracted with diethyl ether (4 x 50 cm<sup>3</sup>) and the organic solvent removed under reduced pressure to afford an oily solid. Following purification using column chromatography and dichloromethane/ethyl ethanoate, 50:1 by volume as the eluting solvent, 4-[<sup>14</sup>C]-cyanophenol (213.8 mg, 138.8 μCi mg<sup>-1</sup>, 29.7 mCi) was obtained in 54% overall chemical yield from potassium [<sup>14</sup>C]cyanide. The radiochemical purity was determined by tlc in systems 1 and 2 and was found to be >98%.

#### Synthesis of 1,5-di(4-[<sup>14</sup>C]cyanophenoxy)pentane

4-[<sup>14</sup>C]Cyanophenol (214 mg, 1.798 mmol), 1,5-dibromopentane (206.6 mg, 0.898 mmol) and anhydrous potassium carbonate (273 mg, 1.978 mmol) were suspended in *N*-methyl-2-pyrrolidinone (6 cm<sup>3</sup>) with the aid of stirring, and heated at 130° for 4 hours. The reaction was allowed to cool and poured into water (60 cm<sup>3</sup>) to give a beige-coloured precipitate. The aqueous suspension was extracted with ethyl ethanoate (4 x 50

cm<sup>3</sup>) and the organic extracts combined and washed with water (100 cm<sup>3</sup>). The organic solvent was removed under reduced pressure to leave the crude product (517.9 mg, 57.8 μCi mg<sup>-1</sup>, 29.9 mCi) as a fawn-coloured solid. Tlc in system 1 gave an estimate of the radiochemical purity of >94%. Following purification by column chromatography using hexane/ethyl ethanoate as the eluting solvent 1,5-di(4-[<sup>14</sup>C]cyano-phenoxy)pentane (242.1 mg, 115.7 μCi mg<sup>-1</sup>, 28.0 mCi; chemical yield = 88.1%) was obtained as a white solid. The radiochemical purity was determined by tlc in systems 4, 5 and 6 and was found to be >97%.

Synthesis of 1,5-di(4-(ethyl[<sup>14</sup>C]carboximido)phenoxy)pentane dihydrochloride

1,5-Di(4-[<sup>14</sup>C]cyanophenoxy)pentane (242 mg, 0.791 mmol) was dissolved in 1,2-dichlorobenzene (8 cm<sup>3</sup>) containing dry ethanol (1.6 cm<sup>3</sup>, 27.3 mmol) and cooled to 5° with stirring. Dry hydrogen chloride gas was passed through for 4 hours and the reaction allowed to warm to room temperature and stand for 72 hours. The solution was warmed slightly to redissolve the solid that had crystallised. Addition of ethyl ethanoate (30 cm<sup>3</sup>) resulted in crystallisation of the dihydrochloride salt. After standing at 4° for 6 hours, the crystals were filtered to afford 1,5-di(4-(ethyl[<sup>14</sup>C]carboximido)phenoxy)-pentane dihydrochloride (395.5 mg, 58.3 μCi mg<sup>-1</sup>, 23.1 mCi; chemical yield = 106%). The radiochemical purity was determined by tlc in systems 7, 8, 9 and 10 and was found to be >97%.

Synthesis of [<sup>14</sup>C]pentamidine dihydrochloride

1,5-Di(4-(ethyl[<sup>14</sup>C]carboximido)phenoxy)pentane dihydrochloride (395 mg, 0.839 mmol) was dissolved in methanolic ammonia (20 cm<sup>3</sup>; containing 13.6% w/v ammonia) and heated at 55° for 24 hours, with stirring. The solvent was removed

under reduced pressure to leave a white solid which was suspended in hydrochloric acid ( $2 \text{ mol dm}^{-3}$ ,  $35 \text{ cm}^3$ ) and refluxed for one hour, after which time a clear solution was obtained. The solution was cooled to  $4^\circ$  and the resulting crystalline material was filtered and dried to afford the crude [ $^{14}\text{C}$ ]pentamidine hydrochloride ( $309.6 \text{ mg}$ ,  $75.7 \text{ } \mu\text{Ci mg}^{-1}$ ,  $23.5 \text{ mCi}$ ). The radiochemical purity was determined by analytical hplc and found to be 83.6%; the remaining radioactivity was accounted for by less polar impurities.

#### Purification of [ $^{14}\text{C}$ ]pentamidine dihydrochloride

The crude [ $^{14}\text{C}$ ]pentamidine dihydrochloride ( $309 \text{ mg}$ ) was dissolved in methanol/water/trifluoroethanoic acid, 100:100:1 by volume ( $16 \text{ cm}^3$ ) and 7 injections of equal volume were made using the preparative hplc conditions described previously. The fractions containing the required material were collected and pooled and the solvent removed under reduced pressure to afford [ $^{14}\text{C}$ ]pentamidine ( $354 \text{ mg}$ ,  $54.8 \text{ } \mu\text{Ci mg}^{-1}$ ,  $19.4 \text{ mCi}$ ; chemical yield = 74%) which was isolated as the di(trifluoroethanoate) salt. The radiochemical purity was determined using analytical hplc and was found to be 99.5%.

#### Synthesis of [ $^{14}\text{C}$ ]pentamidine

[ $^{14}\text{C}$ ]Pentamidine di(trifluoroethanoate) ( $354 \text{ mg}$ ,  $0.623 \text{ mmol}$ ) was dissolved in *N,N*-dimethylformamide ( $6 \text{ cm}^3$ ), with the aid of warming, to give a clear solution. Aqueous potassium carbonate ( $554.2 \text{ mg}$ ,  $4.02 \text{ mmol}$ ; in  $12 \text{ cm}^3$  of water) was added followed by a further portion of water ( $12 \text{ cm}^3$ ). The solution was stirred and allowed to stand at  $4^\circ$  for 6 hours; the resulting white crystalline solid was filtered, washed with water and dried to afford [ $^{14}\text{C}$ ]pentamidine ( $143.4 \text{ mg}$ ,  $70.6 \text{ } \mu\text{Ci mg}^{-1}$ ,  $10.1 \text{ mCi}$ ; chemical yield = 67.7%). The radiochemical purity was determined using analytical hplc and found to be 97.8%.

Synthesis of [<sup>14</sup>C]pentamidine isethionate

[<sup>14</sup>C]Pentamidine (143 mg, 0.421 mmol) was suspended in hot *N,N*-dimethylformamide (2.5 cm<sup>3</sup>) and ammonium isethionate (168.6 mg, 1.179 mmol) dissolved in water (2.5 cm<sup>3</sup>) was added to give an immediate clear solution. The solution was stirred for one hour at 60° after which, propanone (50 cm<sup>3</sup>) was added, and the isethionate salt allowed to crystallise at 4° overnight. The white crystalline solid was filtered, washed with propanone and dried at 65°, under vacuum, to afford [<sup>14</sup>C]pentamidine isethionate (189.1 mg, 51.8 μCi mg<sup>-1</sup>; overall chemical yield from potassium [<sup>14</sup>C]cyanide = 19%). The radiochemical purity was examined by analytical hplc and was found to be 98.9%.

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