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# A short expedient synthesis of [$^{14}$C]Ticlopidine

Michael J. Hickey,* Lee P. Kingston, Paul H. Allen, Tim Johnson, and David J. Wilkinson

**To support the development of a reactive metabolite strategy, the preparation of several radiolabelled compounds such as [$^{14}$C] Ticlopidine was required. In this report, we describe a facile and rapid synthesis of [$^{14}$C] Ticlopidine starting from [$^{14}$C] carbon dioxide. The compound was radiolabelled in the 2-chloromethyl portion of the molecule with a specific activity of 53.4 mCi/mmol and with a radiochemical purity of 98.5%. Storage stability was best as the hydrochloride salt in an ethanol solution.**

**Keywords:** carbon-14; ticlopidine; metal-halogen exchange

## Introduction

Ticlopidine [5-(2-chlorophenyl)methyl-4,5,6,7-tetrahydrothieno[3,2-*c*] pyridine Figure 1] is a well-known, potent and long-acting inhibitor of platelet aggregation.[1] The mechanism of action of ticlopidine is by inhibition of the $P2Y_{12}$ purinergic receptor and prevention of adenosine diphosphate binding to the $P2Y_{12}$ receptor. Ticlopidine has proved effective in the treatment of atherosclerosis and for the prevention of atherothrombosis.[2] Nevertheless, occasional life threatening adverse haematological and hepatotoxic events induced by ticlopidine have been reported.[3] The bioactivation of ticlopidine to reactive metabolites and their subsequent covalent binding to cellular macromolecules has been implicated in the rare idiosyncratic hepatotoxicity in patients.[4]

In order to develop an *in vitro* approach to investigate the potential for risk of idiosyncratic adverse reactions from candidate drugs, several radiolabelled compounds including [$^{14}$C]ticlopidine were required to quantitatively assess the irreversible (i.e. covalent) binding of the molecules to human hepatocytes.[5]

Metabolic studies on ticlopidine appear to suggest that *N*-dealkylation, hydroxylation, *N*-oxidation and oxidation of the thiophene ring, followed by ring opening, are the main routes of metabolism.[6] However, more recently, *in vitro* metabolism studies and the use of high-resolution mass spectrometry coupled with glutathione trapping experiments identified the formation of several additional metabolites which could be the reactive intermediates implicated in the idiosyncratic reactions. It is suspected that the formation of reactive metabolites **1a** and **1b** (Figure 2**)** and their subsequent irreversible covalent binding might be responsible for ticlopidine induced idiosyncratic adverse reactions.[7,8]

The synthesis of [$^{14}$C]ticlopidine has been accomplished in nine steps from [$^{14}$C]carbon dioxide in an overall radiochemical yield of 24%.[9] The synthesis demonstrated the use of the Wolff rearrangement to prepare the intermediate thiophen-2-yl[1-$^{14}$C] acetic acid that was further elaborated to provide [$^{14}$C] ticlopidine. In addition, a one-pot synthesis of ticlopidine from *para*formaldehyde has been reported in 52% yield.[10] The method uses 1.8 equivalents of *para*formaldehyde and requires depolymerisation before use. In both syntheses, the position of the carbon-14 radiolabel would be placed in the tetrahydrothieno[3,2-*c*]pyridine ring.

Our approach to label ticlopidine is outlined in Scheme 1. Here, the carbon-14 radiolabel is positioned in the 2-chlorophenylmethyl portion of the molecule *via* 2-chloro-[*carboxyl*-$^{14}$C] benzoic acid **2** that is readily accessible from [$^{14}$C] carbon dioxide. This method provides a convenient route to [*methylene*-$^{14}$C] ticlopidine HCl **1**. Given that the reactive metabolites **1a** and **1b** are suspected in the covalent binding, we believed this alternative radiolabel would be suitable for use in an *in vitro* covalent binding assay.

## Results and discussion

The synthesis of [*methylene*-$^{14}$C]ticlopidine.HCl **1** was achieved following the route depicted in Scheme 1. Lithium chloride mediated metallation of 1-bromo-2-chlorobenzene with *iso*propyl magnesium chloride at 0°C followed by reaction with 1 equiv of [$^{14}$C]carbon dioxide and acidic workup afforded 2-chloro-[*carboxyl*-$^{14}$C]benzoic acid **2**. The use of *iso*propyl magnesium chloride lithium chloride under conditions developed by Knochel[11] avoided the use of low temperatures and selectivity issues observed with organolithium reagents in the previous syntheses of 2-chloro-[*carboxyl*-$^{14}$C]benzoic acid.[12,13] Subsequent conversion of carboxylic acid **2** to [*methylene*-$^{14}$C]ticlopidine was accomplished in a one-pot procedure *via* the amide **3**. Reaction of *N*,*N*-carbonyldiimidazole with 2-chloro-[*carboxyl*-$^{14}$C]benzoic acid **2** followed by reaction with commercially available 4,5,6,7-tetrahydro thieno[3,2-*c*]pyridine hydrochloride in the presence of triethylamine afforded the amide **3**. Treatment of **3** with borane–tetrahydrofuran at 55°C provided crude [*methylene*-$^{14}$C]ticlopidine with a radiochemical purity of 96% after workup. Purification by flash chromatography on silica gel followed by concentration under reduced pressure provided pure [*methylene*-$^{14}$C]ticlopidine as a clear oil. However,

*Isotope Chemistry, AstraZeneca R&D, Alderley Park, Alderley Edge, SK10 4TG, UK*

**Correspondence to: Michael J. Hickey, AstraZeneca R&D, Mereside, Alderley Park, Alderley Edge, Cheshire SK10 4TG UK.*
*E-mail: michael.j.hickey@astrazeneca.com*

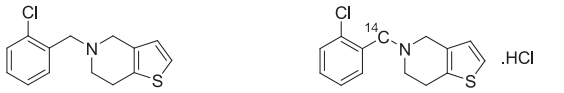



**Figure 1.** Ticlopidine structure.

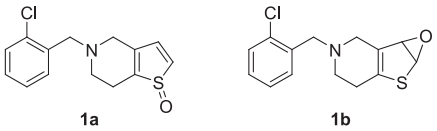



**Figure 2.** Ticlopidine metabolites identified from glutathione trapping experiments.

the material was found to be unstable as the free base with the formation of a 3% radiochemical impurity after storing for 1 h at room temperature; the MS of the impurity showed a mass decrease of two when compared with the parent compound, but was not further characterised. Consequently, [*methylene*-$^{14}$C] ticlopidine was converted to the hydrochloride salt directly after silica gel purification to provide 55 mCi of [*methylene*-$^{14}$C]ticlopidine HCl **1** (47% overall radiochemical yield) with a specific activity of 53.4 mCi/mmol and a radiochemical purity of 98.5%. The hydrochloride salt was stored as an ethanol solution (1 mCi/ml) at −20°C. Under these conditions, the rate of radiochemical degradation was measured to be 1.4% over 6 months.

## Experimental

### Materials and methods

[$^{14}$C]Carbon dioxide (specific activity 57 mCi/mmol) was generated from a [$^{14}$C]carbon dioxide manifold system purchased from RC TRITEC AG, Teufen, Switzerland. All other reagents and anhydrous solvents were obtained from Sigma Aldrich and were used without further purification. $^{1}$H and $^{13}$C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker (500 MHz). Chemical shifts (δ) in parts per million are quoted relative to $CDCl_3$ (δ = 7.26). Flash column chromatography was performed using pre-packed SiliSep™ silica gel cartridges (SiliCycle, Quebec, Canada). Analytical thin layer chromatography was carried out on Merck 5785 Kieselgel 60$F_{254}$ fluorescent plates. LCMS data were obtained on a Waters Acquity ultra performance liquid chromatography with a Waters Micromass ZQ ESCi probe mass detector. Radiochemical purity checks were determined on an Agilent series 1100 HPLC system coupled to a β-Ram Flow Scintillation Analyser (Lab Logic, UK) using the following system: Waters Sunfire $C_{18}$, 3.5 μm, 4.6 × 100 mm, column temperature: 40°C, flow rate: 1.2 mL/min, eluent; A: 0.1% aq TFA, B: 0.1% TFA in acetonitrile, gradient: 0 min 5% B, 2 min 5% B, 18 min 95% B, 20 min 95% B, UV 254 nm. Quantification of radioactivity was performed using a Perkin–Elmer TRI-CARB 2500 liquid scintillation analyzer, with Ultima Gold™ cocktail.

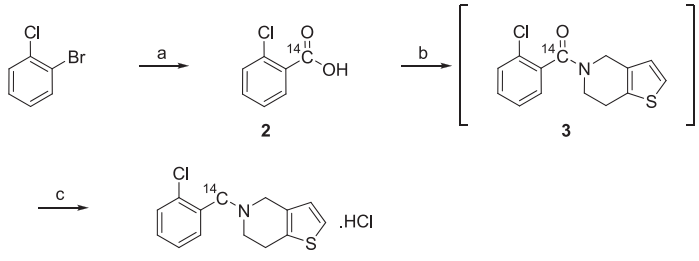
### 2-Chloro-[*carboxyl*-$^{14}$C] benzoic acid (2)

To a solution of 1-bromo-2-chlorobenzene (396 mg, 2.07 mmol) in dry tetrahydrofuran (4 mL) at 0°C, was added dropwise, *i*PrMgCl·LiCl complex (1.3 M in tetrahydrofuran) (1.59 mL, 2.07 mmol). The solution was stirred at 0°C for 1 h, submerged in liquid nitrogen and degassed twice by a freeze-thaw cycle. The reaction flask was evacuated and [$^{14}$C]carbon dioxide (118 mCi, 2.07 mmol) gas was transferred *in vacuo* to the reaction mixture. The mixture was warmed to 0°C and after stirring for 1 h, a mixture of aq 2M HCl (30 mL) and methyl *t*-butyl ether (30 mL) was added. The aqueous was extracted with methyl *t*-butyl ether (30 mL) and combined organic layers were washed with water (30 mL), sat. NaCl (30 mL) and dried over $MgSO_4$. After filtering, the solvent was removed under reduced pressure to give the product as a white solid (0.24 g, 81 mCi, 69% radiochemical yield) that was used directly in the next reaction. LCMS *m/z*: 159 ($[M + H]^+$); the material cochromatographed with an authentic sample by HPLC (Rt = 9.0 min).

### [*methylene*-$^{14}$C]Ticlopidine hydrochloride (1)

2-Chloro-[*carboxyl*-$^{14}$C]benzoic acid **2** (0.24 g, 1.51 mmol) in tetrahydrofuran (4 mL) was treated with *N,N*-carbonyldiimidazole (0.26 g, 1.59 mmol) and stirred at room temperature for 2 h. 4,5,6,7-Tetrahydro thieno[3,2-*c*]pyridine hydrochloride (0.29 g, 1.67 mmol) was added followed by triethylamine (0.63 mL, 4.53 mmol). The mixture was heated at 55°C for 16 h, cooled to 0°C and borane–tetrahydrofuran complex (1M) (12 mL, 12.0 mmol) was added dropwise. The mixture was heated at 55°C under nitrogen for 6 h and cooled to room temperature. 2M aq HCl (30 mL) was added and the solution stirred at 55°C for 18 h. The volatiles were evaporated *in vacuo*, and the residue diluted with water (30 mL) and washed with methyl *t*-butyl ether (30 mL). The aqueous layer was basified to pH 12 with aq 2M NaOH, extracted twice with methyl *t*-butyl ether (30 mL each), and the combined organic layers were washed with water (30 mL), sat. NaCl (30 mL) and dried over $MgSO_4$. The slurry was then filtered and the filtrate concentrated under reduced pressure. The crude product (292 mg) was dissolved in dichloromethane (2 mL) and purified by flash chromatography on silica (70 g) eluting with 5% ethyl acetate in *iso*hexane to give a clear oil that was immediately dissolved in ethanol (20 mL). 4M HCl in dioxane (3 mL) was added, and the solution was evaporated *in vacuo* to afford a white solid (312 mg, 55 mCi, 68% radiochemical yield, 98.5% RCP by HPLC). The solid was dissolved in ethanol (55 mL) and stored under nitrogen at −20°C.

1H NMR ($CDCl_3$) δ 13.25 (br s, 1H) 8.40 (dd, *J* = 7.57, 1.63 Hz, 1H), 7.47 (m, 2H), 7.43 (dd, *J* = 7.64, 1.74 Hz, 1H), 7.25 (d, *J* = 5.22 Hz, 1H), 6.75 (d, *J* = 5.22 Hz, 1H), 4.58 (m, 2H), 4.41 (d, *J* = 15.25 Hz, 1H), 4.04 (dd, *J* = 15.14, 6.31 Hz, 1H), 3.78 (m, 1H), 3.62 (m, 1H), 3.35 (m, 1H), 3.13 (m, 1H)




**Scheme 1.** a) *i*PrMgCl·LiCl, THF, 0°C then [$^{14}$C]carbon dioxide; b) *N,N*-carbonyldiimidazole (CDI), THF, then 4,5,6,7-tetrahydro thieno[3,2-*c*]pyridine hydrochloride, $Et_3N$, 55 °C; c) $BH_3$-THF, 55°C then 2M aq HCl, purification, 4M HCl in dioxane.

$^{13}$C NMR ($CDCl_3$) 135.2, 134.4, 131.7, 131.1, 130.1, 128.5, 126.6, 126.3, 125.7, 124.7, 49.8, 49.5, 21.5.

LCMS *m/z*: ($[M+H]^+$) 264 (15.7%), 266 (100%), 268 (40.1%)

The material cochromatographed with an authentic sample of the parent by HPLC (Rt = 8.0 min).

The specific activity of this material was measured at 53.4 mCi/mmol by mass spectrometry.

## Conclusion

[*methylene*-$^{14}$C]Ticlopidine. HCl **1** was synthesised in two steps from [$^{14}$C]carbon dioxide in 47% overall radiochemical yield with a specific activity of 53.4 mCi/mmol. The radiolabelled product was observed to be unstable as the free base and was stored as the hydrochloride salt as an ethanol solution. *In vitro* covalent binding of [*methylene*-$^{14}$C]ticlopidine **1** to human hepatocytes highlighted significant levels of covalent binding demonstrating its suitability for use in the covalent binding assay.

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