# SYNTHESIS OF N-[2-(2-METHYLPROPOXY)ETHYL]-N-{2-[(4-METHOXY) PHENYLMETHYLTHIO]PHENYL}-1-PYRROLIDINEETHANAMINE (E)-2-BUTENEDIOATE (1:1) SALT (CERM 12816): A POTENTIAL ANTI-ANGINAL DRUG

Emmanuelle Mounetou[1], Claude Poisson[2], André Monteil[2] and Jean-Claude Madelmont[1]*

[1]*Institut National de la Recherche Médicale Unité 71, BP 184, 63005 Clermont-Ferrand Cedex, France and* [2]*Riom Laboratoires-CERM, 10 rue des Boules, BP 140, 63203 Riom Cedex, France.*

## SUMMARY

CERM 12816, which was required for metabolic studies, was labelled with [$^{14}$C] in the methylene carbon of the benzyl group. This involved eight steps starting from barium [$^{14}$C] carbonate and 4-methoxyphenylmagnesium bromide, with a 20% overall yield. The specific activity was 208 MBq mmol$^{-1}$ (5.6 mCi mmol$^{-1}$).

**Key words :** Carbon-14, barium [$^{14}$C] carbonate, anti-anginal drug.

## INTRODUCTION

CERM 12816 (N-[2-(2-methylpropoxy)ethyl]-N-{2-[(4-methoxy)phenylmethylthio]phenyl}-1-pyrrolidineethanamine (E)-2-butenedioate (1:1) salt) is a potential anti-anginal drug, combining potent intracellular calcium antagonism with pronounced effect upon platelet function. It possesses additional attractive features as a free radical scavenger activity and anti-ischaemic properties. As development of this drug candidate has progressed, a radiolabelled analog was required for *in vivo*

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metabolic examination. The prefered isotope for such experiments is [$^{14}$C] because of its long half life and suitable decay energy. Based on metabolic and synthetic considerations, the methylene carbon atom of the benzyl group was identified as the optimal position for the radiolabel.

This paper reports the synthesis of the [$^{14}$C] labelled version of CERM 12816.

## RESULTS AND DISCUSSION

The synthetic pathway for the preparation of [$^{14}$C] CERM 12816 is outlined in Scheme1. The initial step was the carbonation of 4-methoxyphenylmagnesium bromide with [$^{14}$C] carbon dioxide, generated from barium [$^{14}$C] carbonate and sulfuric acid, leading to 4-methoxy[$^{14}$C-carboxy]benzoïc acid (**2**)[1]. On reduction with lithium aluminium hydride in tetrahydrofuran (THF) **2** gave the corresponding alcohol **3**, further converted into the unstable chloride **4** by hydrochloric acid treatment[2]. Both reactions were quantitative. 4-Methoxy[$^{14}$C]benzylchloride (**4**) was rapidly used and underwent nucleophilic displacement with 2-aminothiophenol according to a phase transfer catalyst procedure affording **5** in 80% yield.

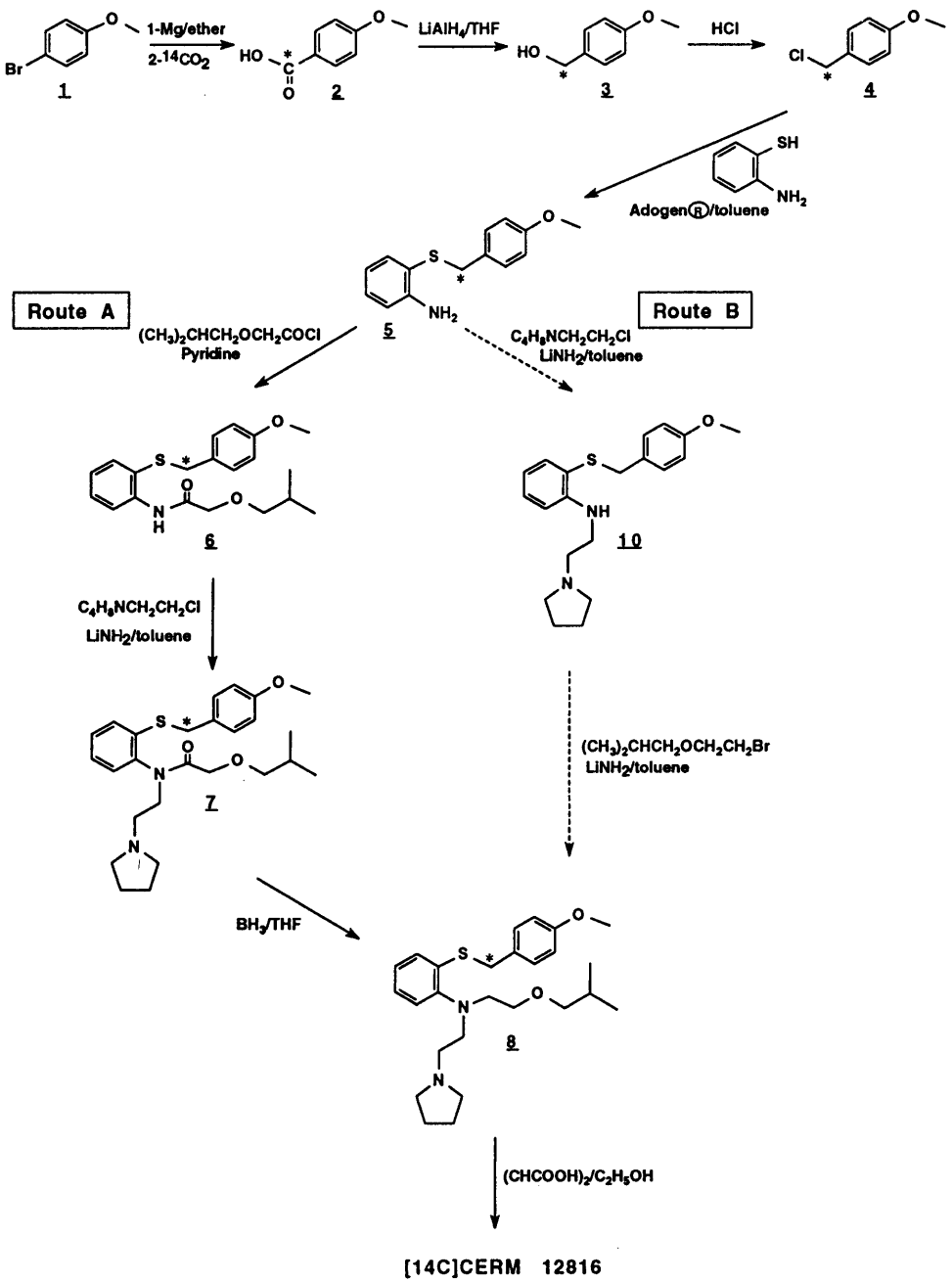
Two synthetic routes could then be considered (Scheme 1). Method B, based on the preliminary introduction of the ethylpyrrolidine moiety followed by an N-alkylation with (2-methylpropoxy)ethylbromide, has been studied with cold materials. Although this path saved one step, method A, leading to [$^{14}$C] CERM 12816 with a higher purity as amine **8** was more readily separated from amide **7** than from amine **10**, was prefered. Subsequent acylation of the aniline **5** with 2-methylpropoxyacetyl chloride, in pyridine, followed by N-alkylation with 2-chloroethylpyrrolidine, in the presence of lithium amide, in refluxing toluene, provided [$^{14}$C] acetanilide **7** in good yields.

Reduction of **7** with borane/THF complex afforded the amine **8** with a moderate yield. Attempts to use lithium aluminium hydride as reducing agent were not successful, leading to the deacylated compound even with extended reaction times (8 to 10 h). Nevertheless, an alternative route shown in scheme 2 was explored. The acetanilide **6** was reduced to the aniline **9**, in 96% yield, with borane/THF complex according to the procedure previously described. Unfortunately, alkylation of **9** with 2-chloroethylpyrrolidine failed.

After chromatographic purification, conversion of **8** into its fumarate salt formed the final [$^{14}$C] CERM 12816. The overall yield of the labelling sequence was 20% based on barium [$^{14}$C] carbonate. The radiochemical and chemical purities of the sample were determined to be better than 99% by thin layer chromatography analysis. The specific activity was 208 MBq mmol$^{-1}$ (5.6 mCi mmol$^{-1}$).

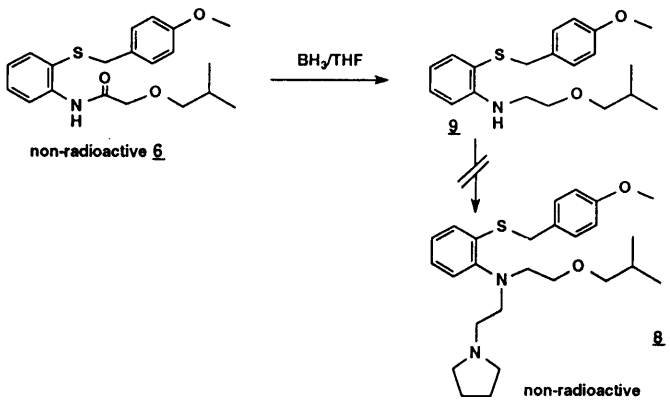
 

**Scheme 1**




*: [$^{14}$C] radiolabelled atom

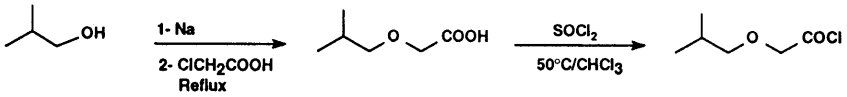
**Scheme 2**




## EXPERIMENTAL

**General comments.** Barium [$^{14}$C] carbonate was purchased from Amersham International (France). All chemicals were from commercial suppliers and used as received. Proton nuclear magnetic resonance ($^{1}$H-NMR) spectra were performed on a Brücker AM 200 (4.5T) spectrometer. Chemical shifts (δ) are reported in parts per million relative to the internal tetramethylsilane standard. Infrared (IR) spectra were recorded on a Perkin Elmer 398 spectrometer. Melting points (mp) were determined on an Electrothermal digital apparatus. Analytical thin layer chromatography (TLC) was conducted on precoated silca gel plates (Merck $60F_{254}$, 0.2 mm thick and Merck RP 18 $F_{254}S$, 0.25 mm thick) with detection by ultra violet light at 254 nm, visualization by iodine and radioactivity evaluation by a Berthold LB 2832 analyser. Silica gel 60 (Chromagel, 230-400 mesh, SDS) was used for medium pressure chromatography using the indicated solvent mixture expressed as volume/volume ratios. Radioactive samples were measured using a Packard 4530 liquid scintillation counter.

2-Methylpropoxyacetyl chloride was freshly prepared using the route outlined :




**4-Methoxy[$^{14}$C-carboxy]benzoïc acid (2).**

Magnesium turnings (1.20 g, 50 mmol) in anhydrous ether (10 mL) were treated with a solution of 4-methoxyphenyl bromide (1) (9.34 g, 50 mmol) in ether (70 mL) and stirred for 1 h at

room temperature. After titration[1], 4-methoxyphenylmagnesium bromide (14 mmol) was carbonated with [$^{14}$C] carbon dioxide, generated from barium [$^{14}$C] carbonate (2.37g, 12 mmol, 2035 MBq, 55 mCi) and sulfuric acid (1N) (20mL). The mixture was stirred for 1 h at -40°C and for another 2 h at room temperature.

After hydrolysis with hydrochloric acid (1N) (20 mL), the organic phase was extracted with sodium hydroxide (2N) (25 mL). The aqueous fraction was then made acidic and re-extracted with ether. The etheral phase was dried over magnesium sulfate, filtered and evaporated under reduced pressure to give **2** as a white solid (1.55 g, 56.0 mCi, 2072 MBq, 85%).

mp 182-185°C ;

TLC *Rf* 0.26 (ethyl acetate/hexane, 50/50) ;

Specific activity : 202 MBq mmol$^{-1}$ (5.5 mCi mmol$^{-1}$) ;

IR (KBr) ν (cm$^{-1}$) : 3600-2500 COOH ; 1680 C=O ; 1600-1450 ArH ; 1255 =C-O- ;

RMN $^1$H ($CDCl_3$) δ : 3.86 (s, 3H, $OCH_3$); 6.90-8.07 (m, 4H, ArH).

**4-Methoxyphenyl [$^{14}$C] methanol (3).**

To a stirred solution of acid **2** (1.30 g, 8.50 mmol) in THF (10 mL) was added dropwise, under nitrogen atmosphere, at 0°C, lithium aluminium hydride (1M solution in THF, 11 mL, 11 mmol). The mixture was stirred overnight at room temperature. After hydrolysis, the precipitate was filtered and washed with ethyl acetate. The filtrate when evaporated to dryness afforded **3** as a colorless oil (1.2 g, 56.0 mCi, 2072 MBq, quantitative).

TLC *Rf* 0.60 (ethyl acetate/hexane, 50/50) ;

Specific activity : 202 MBq mmol$^{-1}$ (5.5 mCi mmol$^{-1}$) ;

IR (KBr) ν (cm$^{-1}$) : 3500-3100 OH ; 3000-2800 CH alkyl ; 1600-1450 ArH ; 1255 =C-O- ;

RMN $^1$H ($CDCl_3$) δ : 1.90 (bs, 1H, OH) ; 3.78 (s, 3H, $OCH_3$); 4.57 (s, 2H, $CH_2$) ; 6.84-7.28 (m, 4H, ArH).

**4-Methoxyphenyl [$^{14}$C] methyl chloride (4).**

Alcohol **3** (1.2 g, 8.50 mmol) was stirred with concentrated hydrochloric acid (2.1 mL) for 20 min. The mixture was extracted with dichloromethane and the organic layer dried over magnesium

sulfate, filtered and evaporated under reduced pressure to give **4** as an oil (1.34 g, 56.0 mCi, 2072 MBq, quantitative).

TLC *Rf* 0.80 (ethyl acetate/hexane, 50/50) ;

Specific activity : 202 MBq $mmol^{-1}$ (5.5 mCi $mmol^{-1}$) ;

IR (KBr) ν ($cm^{-1}$) : 3000-2800 CH alkyl ; 1600-1450 ArH ; 1255 =C-O- ;

RMN $^1H$ ($CDCl_3$) δ : 3.73 (s, 3H, $OCH_3$); 4.51 (s, 2H, $CH_2$) ; 6.80-7.28 (m, 4H, ArH).

**2-[(4-Methoxy)phenyl [$^{14}C$] methylthio]aniline (5).**

A mixture of 2-aminothiophenol (1.28 g, 8.50 mmol), sodium hydroxide (0.57 g, 14.2 mmol), water (6 mL), Adogen 464 ® (1.28 g, 10.2 mmol), toluene (5 mL) and 4-methoxyphenyl [$^{14}C$] methyl chloride (**4**) (1.34 g, 8.50 mmol) was vigorously stirred at 80°C for 6 h. The reaction mixture was extracted with ether, the organic layer dried over magnesium sulfate, filtered and evaporated under reduced pressure. The solid residue was chromatographed on silica gel with a gradient of ethyl acetate/hexane (5/95 to 10/90) as eluent to afford **5** as a white solid (1.72 g, 37.8 mCi, 1399 MBq, 73%).

mp 67-69°C ;

TLC *Rf* 0.20 (ethyl acetate/hexane, 10/90) ;

TLC *Rf* 0.95 (n-butanol/water/acetic acid (upper layer), 40/50/10) ;

Specific activity : 200 MBq $mmol^{-1}$ (5.4 mCi $mmol^{-1}$) ;

IR (KBr) ν ($cm^{-1}$) : 3500, 3300 NH ; 3030, 1600-1450 ArH ; 3000-2800 CH alkyl ; 1240 =C-O- ;

RMN $^1H$ ($CDCl_3$) δ : 3.77 (s, 3H, $OCH_3$) ; 3.85 (s, 2H, $CH_2$) ; 6.74-7.08 (m, 4H, ArH).

**2'-[(4-Methoxy)phenyl [$^{14}C$] methylthio]-2-(2-methylpropoxy)acetanilide (6).**

To a solution of aniline **5** (1.72 g, 7.0 mmol) in pyridine (3 mL) was added dropwise, at 0°C, 2-methylpropoxyacetyl chloride (1.21g, 8.2 mmol). The mixture was stirred at room temperature for 3 h. Pyridine was evaporated under reduced pressure and the residue extracted with ether. The organic layer was washed with water, dried over magnesium sulfate, filtered and evaporated under reduced pressure. The crude product was chromatographed on silica gel with a gradient of ethyl acetate/hexane (30/70 to 40/60) as eluent to yield **6** as an oil (2.50 g, 37.0 mCi, 1369 MBq, 99%).

TLC *Rf* 0.45 (ethyl acetate/hexane, 30/70) ;

TLC *Rf* 0.95 (n-butanol/water/acetic acid (upper layer), 40/50/10) ;

Specific activity : 197 MBq $mmol^{-1}$ (5.3 mCi $mmol^{-1}$) ;

IR (KBr) ν ($cm^{-1}$) : 3300 NH ; 3030, 1600-1450 ArH ; 3000-2800 CH alkyl ; 1670 CONH ; 1240 =C-O- ;

RMN $^1H$ ($CDCl_3$) δ : 1.00 (d, 6H, *J* = 6.7 Hz, $(CH_3)_2$) ; 2.00 (m, 1H, CH) ; 3.35 (d, 2H, *J* = 6.5 Hz, $CHCH_2O$); 3.76 (s, 3H, $OCH_3$); 3.84 (s, 2H, $SCH_2$) ; 4.00 (s, 2H, $OCH_2C0$) ; 6.71-8.50 (m, 8H, ArH) ; 9.60 (bs, 1H, NH).

**2'-[(4-Methoxy)phenyl [$^{14}C$] methylthio]-2-(2-methylpropoxy)-N-ethylpyrrolidine-acetanilide (7).**

To a stirred solution of the amide 6 (2.50 g, 6.9 mmol) in toluene (5mL) was added 2-chloroethylpyrrolidine (1.20 g, 9.0 mmol), freshly prepared from 1-(2-chloroethyl)pyrrolidine hydrochloride, and lithium amide (0.24 g, 10 mmol). The mixture was refluxed for 4 h, filtered and evaporated to dryness under reduced pressure. The residue was diluted in water and extracted with ether. The organic layer was washed with water, dried over magnesium sulfate, filtered and evaporated under reduced pressure. The residue was chromatographed on silica gel with dichloromethane/ethanol (90/10) as eluent to afford 7 as an oil (2.50 g, 29.0 mCi, 1072 MBq, 80%).

TLC *Rf* 0.34 (dichloromethane/ethanol, 90/10) ;

TLC *Rf* 0.20 (n-butanol/water/acetic acid (upper layer), 40/50/10) ;

Specific activity : 196 MBq $mmol^{-1}$ (5.3 mCi $mmol^{-1}$) ;

IR (KBr) ν ($cm^{-1}$) : 3030, 1600-1450 ArH ; 3000-2800 CH alkyl ; 1670 CONH ; 1240 =C-O- ;

RMN $^1H$ ($CDCl_3$) δ : 0.88 (d, 6H, *J* = 6.6 Hz, $(CH_3)_2$) ; 1.71 (m, 4H, H-3, H-4 pyrrolidine ) ; 1.84 (m, 1H, CH) ; 2.47 (m, 4H, H-2, H-5 pyrrolidine ) ; 2.62 (t, 2H, *J* = 5.7, 5.9 Hz, $CH_2N$ pyrrolidine) ; 3.05-3.29 (m, 3H, $CHCH_2O$, $CONCHCH_2$pyrrolidine) ; 3.30-3.50 (2s, 2H; $OCH_2CO$) ; 3.75 (s, 3H, $OCH_3$) ; 4.10 (s, 2H, $SCH_2$) ; 4.30 (m, 1H, $CONCHCH_2$pyrrolidine) ; 6.71-8.50 (m, 8H, ArH).

**N-[2-(2-methylpropoxy)ethyl]-N-{2-[(4-methoxy)phenyl[$^{14}$C]methylthio]phenyl}-1-pyrrolidineethanamine (8).**

To a stirred solution of the amide 7 (2.50 g, 5.4 mmol) in THF (5 mL) was added dropwise borane/THF complex (1M) (33 mL, 33 mmol), at 0°C, under argon. The reaction mixture was refluxed 24 h at room temperature, treated with hydrochloric acid (10%) (20 mL) and evaporated at atmospheric pressure. The aqueous phase was basified at 0°C and extracted with dichloromethane. The organic solution was dried over magnesium sulfate, filtered and evaporated under reduced pressure. The crude residue was chromatographed on silica gel with dichloromethane/ethanol (50/50) as eluent to afford 8 as a yellowish oil (0.92 g, 11.2 mCi, 414 MBq, 40%).

TLC *Rf* 0.20 (dichloromethane/ethanol, 50/50) ;

TLC *Rf* 0.35 (n-butanol/water/acetic acid (upper layer), 40/50/10) ;

Specific activity : 208 MBq mmol$^{-1}$ (5.6 mCi mmol$^{-1}$) ;

IR (KBr) ν (cm$^{-1}$) : 3030, 1600-1450 ArH ; 3000-2800 CH alkyl ; 1240 =C-O- ;

RMN $^{1}$H ($CDCl_3$) δ : 0.84 (d, 6H, *J* = 6.6 Hz, $(CH_3)_2$) ; 1.72 (m, 5H, H-3, H-4 pyrrolidine, CH) ; 2.51 (m, 6H, H-2, H-5, $CH_2N$ pyrrolidine) ; 3.10 (d, 2H, *J* = 6.7 Hz, $CHCH_2O$) ; 3.27 (m, 4H, 2 $NCH_2$) ; 3.44 (m, 2H, $OCH_2CO$) ; 3.78 (s, 3H, $OCH_3$) ; 4.03 (s, 2H, $SCH_2$) ; 4.30 (m, 1H, $CONCHCH_2$pyrrolidine) ; 6.80-7.30 (m, 8H, ArH).

RMN $^{13}$C ($CDCl_3$) δ : 19.34 ($(CH_3)_2$) ; 23.42 (C-3, C-4 pyrrolidine) ; 28.45 (CH) ; 35.74 ($SCH_2$) ; 53.30, 53.68 (2 $CH_2N$) ; 54.10 ($CH_2N$ pyrrolidine) ; 54.34 (C-2, C-5 pyrrolidine) ; 55.25 ($OCH_3$) ; 69.22 ($OCH_2CH_2$) ; 78.09 ($OCH_2CH$) ; 113.90, 123.20, 124.47, 125.29, 126.88, 129.18, 130.02, 135.82, 148.40, 158.72 (Ar).

**N-[2-(2-methylpropoxy)ethyl]-N-{2-[(4-methoxy)phenyl[$^{14}$C]methylthio]phenyl}-1-pyrrolidineethanamine (E)-2-butenedioate (1:1) salt ([$^{14}$C] CERM 12816).**

To a stirred solution of amine 8 (0.92 g, 2.1 mmol) in refluxed ethanol (3 mL) was added fumaric acid (0.24 g, 2.1 mmol). The solvent was evaporated and the fumarate salt crystallised from isopropyl ether as a white chromatographically pure solid (1.06 g, 10.6 mCi, 393 MBq, 92%).

mp 108-110°C ;

TLC *Rf* 0.25 (dichloromethane/ethanol, 50/50) ;

TLC *Rf* 0.48 (n-butanol/water/acetic acid (upper layer), 40/50/10) ;

Specific activity : 208 MBq mmol$^{-1}$ (5.6 mCi mmol$^{-1}$).

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