

THE SYNTHESIS OF [ $^{14}\text{C}$ ]BRL 26830A,  
A NOVEL  $\beta$ -ADRENOCEPTOR AGONIST.

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SUMMARY

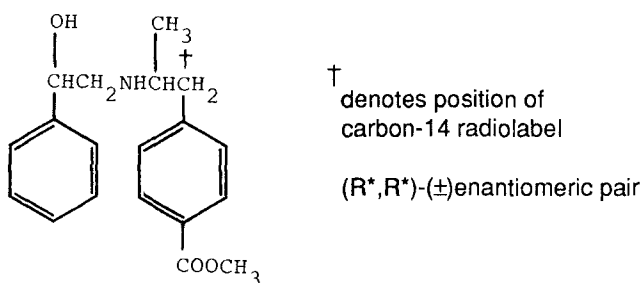
BRL 26850A has been extensively reported <sup>1</sup> as a novel  $\beta$ -adrenoceptor agonist. In order to perform metabolism studies, carbon-14 radiolabelled BRL 26830A was synthesised from potassium [ $^{14}\text{C}$ ]cyanide. As BRL 26830A contains two asymmetric centres, the initial product required resolution of the two enantiomeric pairs. This was achieved by sequential fractional crystallisation of the free base and then the hydrobromide salt to obtain the required ( $R^*$ ,  $R^*$ )-( $\pm$ ) enantiomeric pair before final conversion to the hemifumarate salt ([ $^{14}\text{C}$ ]BRL 26830A).

Key words: [ $^{14}\text{C}$ ]BRL 26830A, potassium [ $^{14}\text{C}$ ]cyanide, resolution, fractional crystallisation, chemical synthesis.

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## INTRODUCTION

BRL 26830A ((R\*,R\*)-(±)-methyl 4-[(2-[(2-hydroxy-2-phenylethyl)amino][1-<sup>14</sup>C]-propyl]benzoate,(E)-2-butenedioate(2:1)salt) is a β-adrenoceptor agonist with a novel type of β-receptor selectivity that may find clinical utility in the treatment of both obesity and type II diabetes<sup>1</sup>.



[<sup>14</sup>C]BRL 26830A (hemifumarate salt) (1)

[<sup>14</sup>C]BRL 26830 (free base) (3)

[<sup>14</sup>C]BRL 26830B (hydrobromide salt) (4)

An efficient multistage synthesis of [<sup>14</sup>C]BRL 26830A (1) was developed in order to provide material for metabolic studies. We required a facile introduction of the radiolabel, into a metabolically stable position in the molecule. A carbon atom adjacent to an aromatic ring was considered to fulfil this requirement. In addition, a radiolabel at this position could be readily introduced using a simple radiolabelled precursor.

We therefore decided to synthesise [<sup>14</sup>C]BRL 26830A (1) by introducing the carbon-14 label into the activated position para to the carboxymethyl by displacement of the corresponding bromide with copper(I) [<sup>14</sup>C]cyanide(2).

As BRL 26830 contains two asymmetric centres, the initial product required separation of the two enantiomeric pairs. This was achieved by sequential fractional

crystallisation of the free base and then the hydrobromide salt to obtain the required (R\*, R\*)-(±) enantiomeric pair ([<sup>14</sup>C]BRL 26830B) (4).

## DISCUSSION

A strategy was adopted, which facilitated a high specific activity carbon-14 radiolabelled synthesis of [<sup>14</sup>C]BRL 26830A (1) using potassium [<sup>14</sup>C]cyanide (5) as an inexpensive carbon-14 precursor. The radiolabel was incorporated as detailed in the scheme.

Copper(II), as the sulphate, was reduced to copper(I) by the addition of sodium metabisulphite. Alkaline potassium [<sup>14</sup>C]cyanide (5) was added to produce copper(I) [<sup>14</sup>C]cyanide (2). Reaction of copper (I) [<sup>14</sup>C]cyanide (2) with methyl 4-bromobenzoate (6) in 1-methyl-2-pyrrolidinone afforded methyl 4-[<sup>14</sup>C]cyanobenzoate (7), using the method of Newman and Boden<sup>2</sup>, in combination with an improved work up procedure developed by Friedman and Shechter<sup>3</sup>.

Methyl 4-[<sup>14</sup>C]formylbenzoate (8) was prepared by reduction of methyl 4-[<sup>14</sup>C]cyanobenzoate (7), with nickel/aluminium alloy in aqueous formic acid utilising the conditions reported by vanEs and Staskun<sup>4</sup>. Preparation of a Schiff's base intermediate was achieved with *n*-butylamine in refluxing benzene and this intermediate was then reacted, without isolation, with nitroethane and acetic acid to produce 1-(4-carbomethoxyphenyl)-2-nitro [1-<sup>14</sup>C]prop-1-ene (9).

To this stage, the overall yield from potassium [<sup>14</sup>C]cyanide (5) was 56%.

Reduction of 1-(4-carbomethoxyphenyl)-2-nitro [1-<sup>14</sup>C]prop-1-ene (9) using iron powder/concentrated hydrochloric acid in methanol yielded 1-(4-carbomethoxyphenyl)[1-<sup>14</sup>C]propan-2-one (10) in 95% yield.

The ketone (10) was coupled with 1-phenyl-2-aminoethanol (11) in refluxing benzene to remove the condensate. The resulting imine (12) was reduced to the (R\*,R\*)-(±), (R\*,S\*)-(±) diastereoisomeric mixture (13) with sodium borohydride in methanol in 75% yield from (10), after purification by column chromatography.

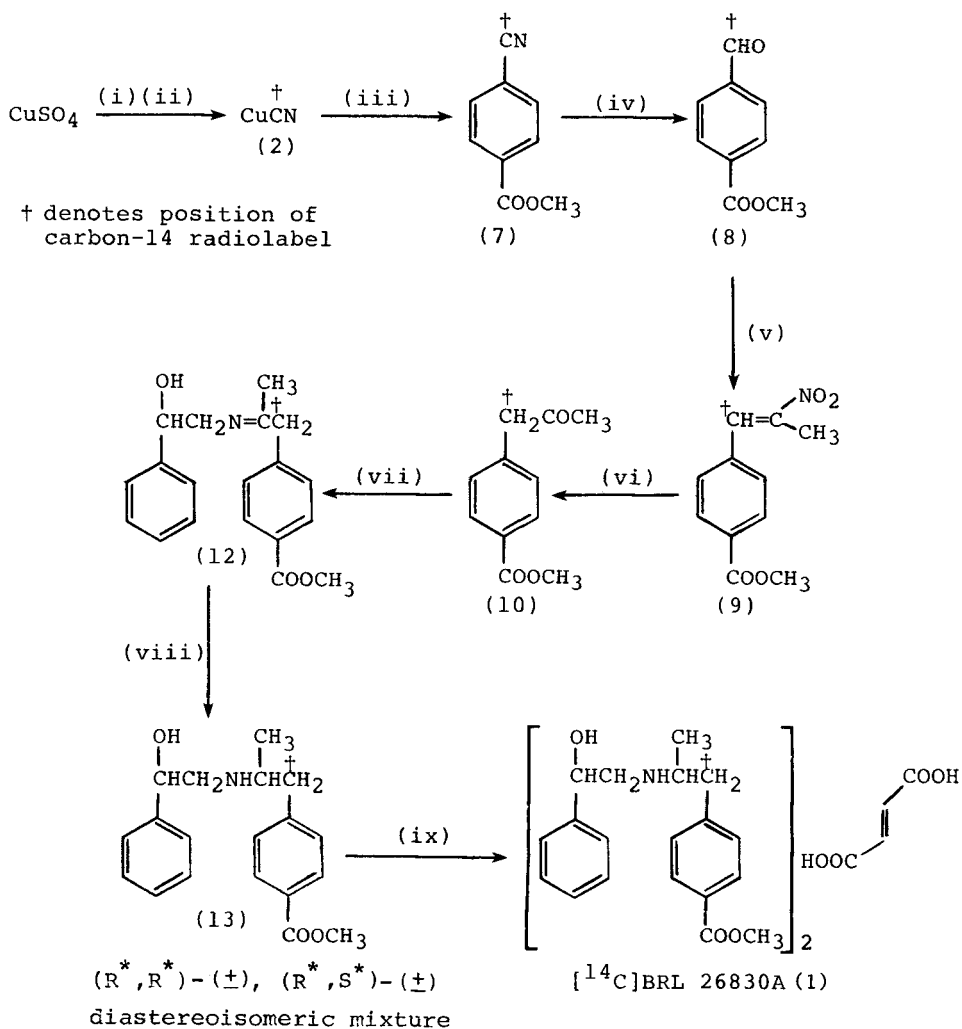
The major remaining problem was to achieve an efficient high yielding separation of the required (R\*, R\*)-(±) enantiomeric pair ([<sup>14</sup>C]BRL 26830 (3)). Initially, partial resolution was achieved by removing most (ca 75%) of the unwanted (R\*, S\*)-(±) enantiomeric pair, in one fractional crystallisation of the free base from methanol.

The required (R\*, R\*)-(±) enantiomeric pair ([<sup>14</sup>C]BRL 26830) (3) which remained in the methanolic filtrate was further purified by fractional crystallisation of the hydrobromide salt ([<sup>14</sup>C]BRL 26830B) (4), following the addition of ethereal hydrogen bromide to the filtrate. Overall, the (R\*, R\*)-(±) enantiomeric pair ([<sup>14</sup>C]BRL 26830B) was isolated in 64% yield from the diastereoisomeric mixture.

The hydrobromide salt ([<sup>14</sup>C]BRL 26830B) (4) was converted to the free base ([<sup>14</sup>C]BRL 26830) (3) and then to the hemifumarate salt ([<sup>14</sup>C]BRL 26830A) (1), after the addition of a stoichiometric amount of fumaric acid. [<sup>14</sup>C]BRL 26830A (1) was obtained from potassium [<sup>14</sup>C]cyanide (5) in a highly satisfactory 12% overall radiochemical yield and 11% chemical yield at a radiochemical purity of 97.4% (hplc), a chemical purity of 99.3% (hplc) and a stereochemical purity of 99.0% ((R\*, R\*)-(±) enantiomeric pair).

A preparative scale hplc purification method was also developed which was suitable for the further purification of [<sup>14</sup>C]BRL 26830A (1), or the purification of stocks that had undergone radiolytic decomposition on storage.

## SCHEME



Reagents: (i)  $\text{Na}_2\text{S}_2\text{O}_5$ ; (ii)  $\overset{+}{\text{KCN}}$  (5); (iii)  $p\text{-Br-C}_6\text{H}_4\text{COOCH}_3$  (6)

(iv)  $\text{Ni/Al, HCOOH}$ ; (v) a)  $\text{CH}_3(\text{CH}_2)_3\text{NH}_2$ ;

b)  $\text{CH}_3\text{CH}_2\text{NO}_2, \text{CH}_3\text{COOH}$ ; (vi)  $\text{Fe, HCl, CH}_3\text{OH}$ ;

vii)  $\text{PhCH(OH)CH}_2\text{NH}_2$  (11); (viii) a)  $\text{NaBH}_4$ ;

b) Fractional crystallisation (removal of  $(R^*, S^*)-(\pm)$  enantiomeric pair); b)  $\text{HBr}$ /fractional

crystallisation; (ix) a) Aqueous  $\text{Na}_2\text{CO}_3$ ; b) Fumaric acid.

## EXPERIMENTAL

General

Potassium [ $^{14}\text{C}$ ]cyanide (5) was supplied by Amersham International plc., methyl 4-bromobenzoate was supplied by Cambrian Chemicals Ltd. and 1-phenyl-2-aminoethanol (11) was obtained from the Aldrich Chemical Company Limited. 1-Methyl-2-pyrrolidinone was distilled immediately prior to use and benzene and methanol were dried with 3A molecular sieve.

Thin layer chromatography (tlc) was performed on Merck silica gel 60 F254 plates (part no. 5735). Column chromatography was performed on Machery and Nagle silica (60 $\mu\text{m}$ ). The radiochemical purity was determined by scanning the developed chromatogram (Berthold Scanner type LB 2722-2) and comparing the radioactivity associated with the zone containing [ $^{14}\text{C}$ ]BRL 26830A (1) to that of the complete chromatogram. The stereochemical purity of the pure ( $R^*$ ,  $R^*$ )-( $\pm$ ) enantiomeric pair ([ $^{14}\text{C}$ ]BRL 26830B) (4) was determined using capillary gas chromatography on a Carlo Erba Fractovap Series 4160 gas chromatograph, using a WCOT glass capillary column, (0.3 mm internal diameter with a 0.1  $\mu\text{m}$  film) supplied by "GC<sup>2</sup> (Chromatography) Ltd.", Northwich, England. The sample was prederivatised with tert-butyldimethylchlorosilane/imidazole in pyridine, followed by addition of trifluoroacetyl/imidazole reagent at 135°C.

Analytical scale high performance liquid chromatography (hplc) was performed on Waters  $\mu\text{BONDAPAK C}_{18}$  (10 $\mu\text{m}$ ) stationary phase (stainless steel column: 4.6 mm id x 250 mm) using a Perkin Elmer Series 3B pump (flow rate: 1.5  $\text{ml}\cdot\text{min}^{-1}$ ), a Rheodyne 7125 injector, a Cecil Instruments Variable Wavelength UV Monitor Model 2102 at 238 nm and eluant containing 0.1M sodium acetate buffer, pH 4.5:methanol (1:1 v/v). The radiochemical purities were determined by

fractionation of the eluate from analytical scale hplc, the radioactivity in each fraction of eluate being determined by liquid scintillation counting. The radiochemical purity was calculated by comparing the radioactivity collected in the eluant fraction corresponding to BRL 26830 to that of the complete chromatographic run. The chemical purity was determined by comparison of the UV peak height of the product with respect to reference material using a suitable internal standard.

The specific activity of [<sup>14</sup>C]BRL 26830A (1) was determined by measurement of the radioactivity by liquid scintillation counting of a known mass of [<sup>14</sup>C]BRL 26830A (1).

Liquid scintillation counting was performed using a Packard Tri-Carb 2660 liquid scintillation counter, and Packard ES 299 scintillant. Autoradiography was performed with Kodak Blue Brand X ray film using Pentalex developer and Perfix fixative.

All intermediates and products were characterised during preliminary non-isotopically labelled syntheses using standard spectroscopic and chromatographic methods.

#### Copper(I) [<sup>14</sup>C]cyanide (2)

Copper(II) sulphate pentahydrate (1.785 g, 7.15 mmol) was dissolved in water (8 ml) at 40°C. This solution was vigorously stirred and a solution of sodium metabisulphite (665 mg, 3.50 mmol) in water (2 ml) was added dropwise. Potassium [<sup>14</sup>C]cyanide (5) (200 mCi at 55.5 mCi.mmol<sup>-1</sup>, 241 mg, 3.60 mmol) was mixed with non-radioactive potassium cyanide (105 mg, 1.61 mmol) and potassium

hydroxide (248 mg, 4.42 mmol). This mixture was dissolved in water (3 ml) and added dropwise to the copper(I) solution, immediately forming a white precipitate which was vigorously stirred at 40°C for 1 h. The solid was separated by filtration, washed with water, and transferred whilst still moist. The copper(I) [ $^{14}\text{C}$ ]cyanide (2) was dried overnight under high vacuum over phosphorus pentoxide. Yield 448 mg.

Methyl 4- $^{14}\text{C}$ cyanobenzoate (7)

Methyl 4-bromobenzoate (6) (1.078 g, 5.02 mmol) in 1-methyl-2-pyrrolidinone (8.5 ml) was added to copper(I) [ $^{14}\text{C}$ ]cyanide (2) (448 mg, 5.00 mmol). The solution was heated under reflux for 5 h, allowed to cool, and a solution of iron(III) chloride hexahydrate (4.0 g, 14.8 mmol) in concentrated hydrochloric acid (1 ml) and water (5.5 ml) was added. The solution was stirred for 45 min at 65°C and extracted with diethyl ether (40 ml and 2 x 20 ml). The organic extracts were combined, washed with water (2 x 25 ml) and dried over anhydrous magnesium sulphate. Filtration and evaporation *in vacuo* yielded methyl 4- $^{14}\text{C}$ cyanobenzoate (7) as a yellow solid (1.057 g) which was used without further purification.

Methyl 4- $^{14}\text{C}$ formylbenzoate (8)

Methyl 4- $^{14}\text{C}$ cyanobenzoate (7) (1.057 g) was dissolved in aqueous formic acid (11 ml, formic acid : water 75:25 v/v) to which was added nickel/aluminium alloy (1/1 composition, 825 mg). The suspension was heated under reflux for 1 h, cooled, filtered and the residual alloy rinsed with ethanol (2 ml), chloroform (40 ml) and diethyl ether (5 ml). The combined organic extract was separated from the aqueous, which was further extracted with chloroform (20 ml and 10 ml). The organic extracts were combined and washed with water (15 ml) and saturated



sodium hydrogen carbonate solution (15 ml). The water and sodium hydrogen carbonate washes, were individually back-extracted with chloroform (10 ml) followed by diethyl ether (10 ml). The organic extracts were combined, dried over anhydrous magnesium sulphate, filtered and evaporated in vacuo to yield a semi-solid (1.3 g), which was used immediately without purification to prepare 1-(4-carbomethoxyphenyl)-2-nitro[1-<sup>14</sup>C]prop-1-ene (9).

1-(4-Carbomethoxyphenyl)-2-nitro[1-<sup>14</sup>C]prop-1-ene (9)

Methyl 4-[<sup>14</sup>C]formylbenzoate (8) (1.3 g) was dissolved in dry benzene (12 ml) to which was added n-butylamine (1.1 ml, 11 mmol). The solution was heated under reflux for 3 h under a small soxhlet extractor containing 3A molecular sieve in order to absorb the condensate. The cooled solution was evaporated in vacuo and glacial acetic acid (2.5 ml) followed by nitroethane (0.75 ml, 9.9 mmol) were added. This solution was stirred at 115°C for 45 min and cooled to ambient temperature overnight. The resultant crystalline material was separated by filtration. Further crops were obtained by addition of methanol to the filtrate and concentration by evaporation in vacuo. The total yield (three crops) of 1-(4-carbomethoxyphenyl)-2-nitro[1-<sup>14</sup>C]prop-1-ene (9) was 645 mg (2.92 mmol, 56% from potassium [<sup>14</sup>C]cyanide (5)).

1-(4-Carbomethoxyphenyl)[1-<sup>14</sup>C]propan-2-one (10)

1-(4-Carbomethoxyphenyl)-2-nitro[1-<sup>14</sup>C]prop-1-ene (9) (645 mg, 2.92 mmol) and iron powder (1 g, 17.9 mmol) were suspended in methanol (11 ml) and heated to reflux. Concentrated hydrochloric acid (5 ml) was added dropwise over 5 min and the suspension heated under reflux for a further 3 h. The cooled solution was

evaporated *in vacuo* and extracted into diethyl ether (60 ml and 2 x 20 ml). The diethyl ether extracts were combined, washed with water (2 x 15 ml) and saturated sodium hydrogen carbonate solution (10 ml) and dried over anhydrous magnesium sulphate. Filtration and evaporation *in vacuo* afforded

1-(4-carbomethoxyphenyl)[1-<sup>14</sup>C]propan-2-one (10) (532 mg, 2.77 mmol, 95% from 1-(4-carbomethoxyphenyl)-2-nitro-[1-<sup>14</sup>C]prop-1-ene).

Methyl 4-[2-[(2-hydroxy-2-phenylethyl)amino][1-<sup>14</sup>C]propyl]benzoate (13)

1-(4-Carbomethoxyphenyl)-[1-<sup>14</sup>C]propan-2-one (10) (532 mg, 2.77 mmol) was dissolved in dry benzene (15 ml) containing 1-phenyl-2-aminoethanol (11) (410 mg, 3.01 mmol). This solution was heated to reflux for 4 h under a soxhlet extractor containing 3A molecular sieve to remove the condensate. The benzene was removed by evaporation *in vacuo* and the resultant oil redissolved in dry methanol (15 ml) and cooled to 0°C. Sodium borohydride (1 g, 26.5 mmol) was added in portions to the stirred solution and the resulting suspension was stirred for 1 h at 0°C. The methanol was partially removed by evaporation *in vacuo* and the remaining mixture was dissolved in dichloromethane (50 ml) and water (20 ml). The dichloromethane was separated and the aqueous further extracted with dichloromethane (2 x 20 ml). The organic solutions were combined and washed with saturated brine (20 ml) and dried over anhydrous magnesium sulphate. Filtration and evaporation *in vacuo* yielded crude methyl 4-[2-[(2-hydroxy-2-phenylethyl) amino][1-<sup>14</sup>C]propyl]benzoate (13) as an oil (990 mg). The product (13) was purified by column chromatography (eluant; 3% grading to 5% methanol : dichloromethane) to yield methyl 4-[2-[(2-hydroxy-2-phenylethyl)amino][1-<sup>14</sup>C]propyl]benzoate (13) (651 mg, 2.08 mmol, 75% as a diastereoisomeric mixture from the ketone (10)).

[<sup>14</sup>C]BRL 26830B (4) ((R\*,R\*)-(±) enantiomeric pair)

Methyl 4-[2-[(2-hydroxy-2-phenylethyl)amino]][1-<sup>14</sup>C]propyl]benzoate (13) (651 mg, 2.08 mmol) was dissolved in methanol (2 ml), a seed crystal of the (R\*,S\*)-(±) enantiomeric pair was added and the solution stored at 4°C for 48 h. The resultant (R\*,S\*)-(±)-methyl 4-[2-[(2-hydroxy-2-phenylethyl)-amino]][1-<sup>14</sup>C]propyl]benzoate, (247 mg, 0.788 mmol) was separated by filtration. The filtrate was evaporated in vacuo, redissolved in diethyl ether (ca 2 ml) and stirred at 0°C. Ethereal hydrogen bromide (ca 35 ml) was added and the resulting suspension stirred at 0°C for 1 h. The supernatant was removed and the remaining solid suspended in ethyl acetate (4.5 ml) and stirred at 0°C for 1 h. The resultant solid was separated by filtration and dried under high vacuum to yield [<sup>14</sup>C]BRL 26830B (4) (362 mg, 0.918 mmol) at a stereochemical purity of 84% (R\*,R\*)-(±) enantiomeric pair. The solid was redissolved in ethyl acetate (7 ml), heated to reflux for 15 min, allowed to cool to ambient temperature and stored at 4°C overnight. The resultant crystalline solid was separated by filtration, rinsed with ethyl acetate and dried under high vacuum to yield [<sup>14</sup>C]BRL 26830B (4) (325 mg, 0.824 mmol). Analysis of this material showed little improvement of the stereochemical purity. The solid was therefore resuspended in ethyl acetate (10 ml) and methanol (0.5 ml) and heated to reflux for 15 min. The suspension was allowed to cool to ambient temperature over 2 h and the resultant solid was filtered, rinsed with ethyl acetate and dried under high vacuum to yield [<sup>14</sup>C]BRL 26830B (4) (262 mg, 0.664 mmol, 64% from (R\*, R\*)-(±) enantiomeric pair contained in methyl 4-[2-[(2-hydroxy-2-phenylethyl)amino]][1-<sup>14</sup>C]propyl]benzoate (13) diastereoisomeric mixture), at a stereochemical purity of 99% (R\*,R\*)-(±) enantiomeric pair.

### [<sup>14</sup>C]BRL 26830A (1)

[<sup>14</sup>C]BRL 26830B (4) (262 mg, 0.664 mmol) was converted to the free base ([<sup>14</sup>C]BRL 26830) (3), by addition of sodium carbonate (146 mg, 1.38 mmol) in water (5 ml) and partitioning into dichloromethane (20 ml). The aqueous layer was further extracted with dichloromethane (2 x 10 ml). The dichloromethane extracts were combined, washed with water, and dried over anhydrous magnesium sulphate. Filtration and evaporation *in vacuo* yielded an oil (203 mg, 0.648 mmol), which was dissolved in propan-2-ol (1.5 ml) and heated under reflux. Fumaric acid (36 mg, 0.31 mmol) in methanol (0.5 ml) was added, followed by rinsings of propan-2-ol (2 x 1 ml) and heating at reflux was maintained for a further 10 min. A seed crystal of BRL 26830A (1) was then added and crystallisation immediately commenced. The suspension was stored at 4°C overnight. The crystalline solid was filtered, washed with diethyl ether and dried under high vacuum over phosphorus pentoxide to yield [<sup>14</sup>C]BRL 26830A (1) (221 mg, 0.297 mmol, 89%, 23.7 mCi, 107.2 µCi.mg<sup>-1</sup>). The overall radiochemical yield was 12% and the overall chemical yield was 11%.

The radiochemical purity of the product was 97.4% as determined by hplc and 98.0% by tlc (eluants:- chloroform: propan-2-ol:0.88 ammonia solution (90:10:1 by volume) and chloroform:methanol:acetic acid (85:15:1 by volume)).

The stereochemical purity was assayed as 99% (R<sup>\*</sup>, R<sup>\*</sup>)-(±) enantiomeric pair by capillary gc. The chemical purity was 99.3% hemifumarate salt, as determined by hplc.

### Purification of [<sup>14</sup>C]BRL 26830A (1) by preparative scale high performance liquid chromatography (hplc)

Preparative scale high performance liquid chromatography was performed using a

Waters  $\mu$ BONDAPAK C<sub>18</sub> (10  $\mu$ m) reverse phase silica column (stainless steel column: 8 mm id x 300 mm) with same equipment as used for the analytical scale hplc. The eluant used was 0.1M sodium phosphate buffer, pH 7.0:methanol (45:55 v/v) at a flow rate of 7.5 ml.min<sup>-1</sup> with UV detection at 260 nm.

A typical purification by preparative scale hplc was as follows:-

[<sup>14</sup>C]BRL 26830A (1) (138 mg, 0.186 mmol) was dissolved in eluant (9 ml). The solution was divided into two aliquots (4.5 ml) and chromatographed in two separate runs. The eluate containing [<sup>14</sup>C]BRL 26830 (3) was collected and the methanol removed by evaporation *in vacuo*. Potassium carbonate (78 mg, 0.56 mmol) was added and the aqueous extracted with chloroform (30 ml). The organic phase was separated and the aqueous further extracted with chloroform (30 ml). The chloroform extracts were combined and dried over anhydrous magnesium sulphate. Filtration and removal of solvent by evaporation *in vacuo* yielded [<sup>14</sup>C]BRL 26830 (3), (75 mg, 0.24 mmol) as an oil. The oil was dissolved in propan-2-ol (1 ml), fumaric acid (14 mg, 0.12 mmol) was added and the mixture was heated at 90°C for 5 min. The crystalline product which formed was allowed to cool to ambient temperature and was stored at 4°C for 12 h. The solid was separated by filtration, washed with ethanol (1 ml) and diethyl ether (2 x 1 ml) and dried under high vacuum, to yield [<sup>14</sup>C]BRL 26830A (1) (63 mg, 0.085 mmol, 46%). By using the above procedure batches with radiochemical purities of up to 99% were obtained.

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