THE SYNTHESIS OF (-), (+) AND (±)-[^{14}C] SK&F 94836 A SELECTIVE PDE III INHIBITOR

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SUMMARY

SK&F 94836, a selective PDE III inhibitor is a positive inotropic agent with vasodilator activity. The syntheses of (-)-[N-methyl-^{14}C]SK&F 94836, (+)-[N-methyl-^{14}C]SK&F 94836 and [carbonyl-^{14}C] SK&F 94836 are described . The racemate, labelled in the dihydropyridazinone heterocycle was obtained in 24.6% overall yield from K[^{14}C]CN. The chiral compounds were radiolabelled by reacting a resolved precursor with [^{14}C]methylamine.

Keywords: PDE III Inhibitor, Inotrope, Vasodilator, [^{14}C]dihydropyridazinone, [^{14}C]cyanoguanidine, h.p.l.c.

INTRODUCTION

2-Cyano-1-methyl-3-[4-(4-methyl-6-oxo-1,4,5,6-tetrahydropyridazin-3-yl) phenyl]guanidine, SK&F 94836 (6), a selective PDE III Inhibitor, is a positive inotropic agent with vasodilator activity[2], under investigation in our laboratories for the treatment of congestive heart failure.

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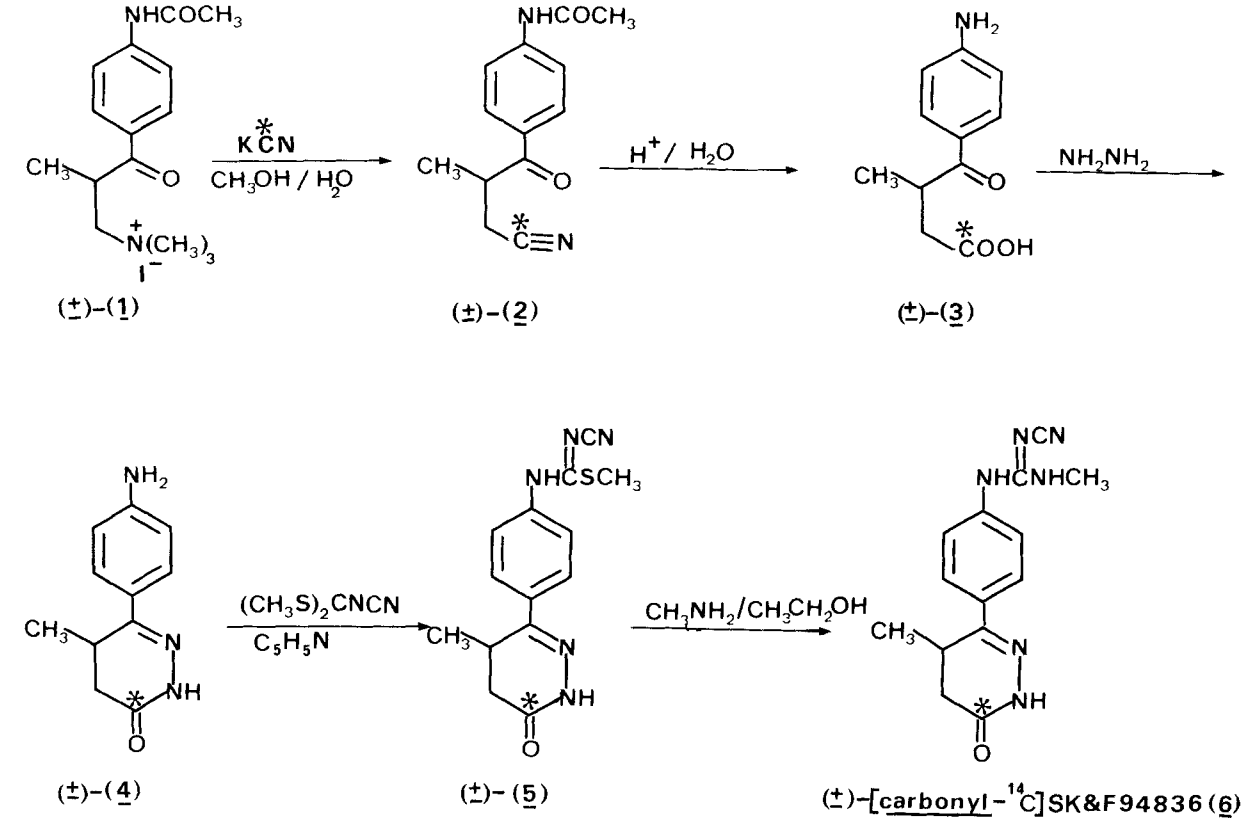
Scheme I

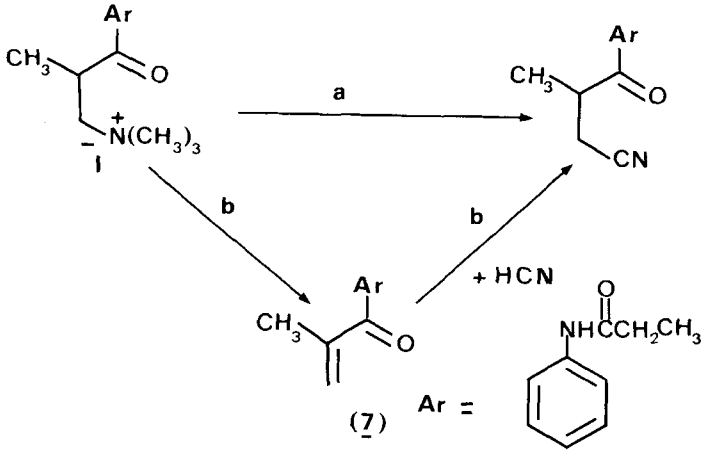

NHCOCH3
CH3
O
N(CH3)3
I−
(±)-(1)
K*CN
CH3OH / H2O
C*N
(±)-(2)
H+ / H2O
NH2
C*OOH
(±)-(3)
NH2NH2
NH2
N
NH
(±)-(4)
(CH3S)2CNCN
C5H5N
NCN
NHCSCH3
(±)-(5)
CH3NH2/CH3CH2OH
NHCNHCH3
(±)-[carbonyl-14C]SK&F94836 (6)

We describe here the syntheses of (±)-[carbonyl-^{14}C]SK&F 94836, (+)-[N-methyl-^{14}C]SK&F 94836 and (-)-[N-methyl-^{14}C]SK&F 94836 which were required for drug metabolism studies and for radioimmunoassay validation.

DISCUSSION

We required, initially for drug metabolism studies, the racemic modification of SK&F 94836 radiolabelled with carbon-14. We envisaged that incorporation of a radiolabel into the carbonyl group of the dihydropyridazinone heterocycle could be readily achieved, and importantly, that this position would be metabolically stable. The synthetic sequence we utilised is illustrated in Scheme 1. Racemic 3-(4-acetanilido)-2-methyl-3-oxo-1-trimethylammoniopropane iodide (1), an intermediate in the large scale synthesis of SK&F 94836, was readily available, which on treatment with K[^{14}C]CN (310 mCi 55.8 mCi mmol), in aqueous methanol, at room temperature for 18h, gave the required 4-(4-acetanilido)-3-methyl-4-oxo-[cyano-^{14}C]butyronitrile (2) in 80.9% radiochemical yield. This reaction which overall results in the substitution of a trimethylammonio group by cyanide, can proceed by two distinct mechanisms[3] (Scheme II), namely

Scheme II



direct substitution (S_N2) (path a) or by an elimination/addition process (E_2), (Path b). The corresponding S_N1/E_1 reaction modes, which involve the intermediacy of a primary carbonium ion, would appear unlikely. We were able to isolate, by treatment of the corresponding quaternary ammonium salt with 10% aqueous sodium carbonate 3-(4-propionanilido)-3-oxo-2-methylprop-1-ene (7). The isolated 3-(4-propionanilido)-3-oxo-2- methylprop-1-ene (7) was then, in a separate experiment, reacted with K[^{14}C]CN cleanly affording 4-(4-propionanilido)-3-methyl-4-oxo[1-^{14}C]-butyronitrile in high yield (77%), within 4h. We were also able to obtain t.l.c. evidence for the intermediacy of this enone (7) in the reaction between the corresponding quaternary ammonium salt and KCN, and we believe therefore, that the reaction procedes to a large part via an elimination/addition mechanism, in which trimethylamine is eliminated (cyanide may act as a base here) followed by conjugate addition of hydrogen cyanide to the resultant enone (7).

Treatment of the [^{14}C]nitrile (2) with 5M hydrochloric acid under reflux gave 4-(4-anilino)-4-oxo-3-methyl[1-^{14}C]butyric acid (3), the product of both nitrile and amide hydrolysis. The dihydropyridazinone heterocycle was readily formed, under standard conditions,[4] without isolation of the intermediate acid, by reaction with a large excess of hydrazine, in 75.8% radiochemical yield from (2).

The final two steps in the synthesis, which put in place the N-methyl-N^1-cyanoguanidine functionality were readily achieved in good yield. Treatment of the [^{14}C]amine (4) with two molar equivalents of dimethyl-N-cyanodithioiminocarbonate[5] in pyridine, under reflux, gave the intermediate thiomethyl derivative (5).

This was further reacted, without purification, with a large excess of methylamine in ethanol furnishing the required 2-cyano-1-methyl-3-[4-

(4-methyl-[6-^{14}C]-6-oxo-1,4,5,6-tetrahydro- pyridazin-3-yl)phenyl]-guanidine, (±)-[carbonyl-^{14}C]SK&F 94836 (6). This two stage protocol is a widely applicable and efficient method for the preparation of substituted N-cyanoguanidines.[6]

Purification of the crude (±)-[carbonyl-^{14}C]SK&F 94836 (6) was readily achieved by semi-preparative h.p.l.c., followed by recrystallisation from methanol/water, yielding 52.9 mg (195.4 μCi.mg^{-1}, 11.355 mCi) and of 97.2% radiochemical purity as assessed by t.l.c. A second batch of (±)-[carbonyl-^{14}C]SK&F 94836 was obtained by dilution of the mother liquors of recrystallisation and h.p.l.c. residues, with unlabelled SK&F 94836, 92.3 mg (53.0 μCi.mg^{-1}, 4.892mCi). The overall radiochemical yield of this synthesis was 24.6% from K[^{14}C]CN.

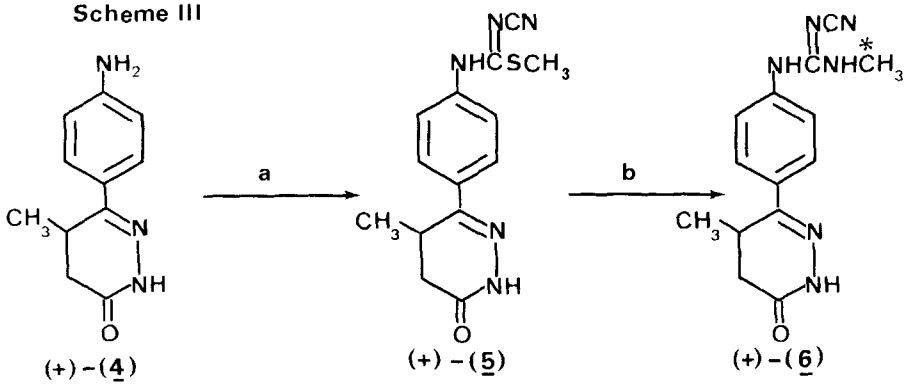
We now turned our attention to radiolabelled syntheses of each enantiomer of SK&F 94836. As these compounds were required for the validation of enantiospecific radioimmunoassays this removed the necessity of labelling in the core of the molecule, i.e. potential metabolic loss of the label was not an issue. We chose, therefore, to introduce the radiolabel at the last stage in the syntheses, i.e. via [^{14}C]methylamine.

Racemic 6-(4-anilino)-4,5-dihydro-5-methyl-pyridazine-3-(2H)-one (4) can be readily resolved[7] by a chiral h.p.l.c. method. (+)-[N-Methyl-^{14}C] SK&F 94836 was prepared from the corresponding (+)-(4) (>98.0% ee[8]) by a directly analogous method to the racemic modification. The thiomethyl derivative (+)-(5) (53.9 mg, 0.179 mmol) was reacted with [^{14}C]methylamine hydrochloride (5 mCi, 12.1 mg, 0.176 mmol) in the presence of triethylamine as before (Scheme III), giving, following h.p.l.c. purification (+)-[N-methyl-^{14}C]SK&F 94836 (13.9mg, 85.7 μCi.mg^{-1}, 1.32 mCi) (6) in 26.4% radiochemical yield. The enantiomeric excess[8] of this material was 90%. The radiochemical yield of this transformation was rather low, thus it would appear that a large excess

of methylamine is required for efficient preparation of SK&F 94836 from the thiomethyl precursor.

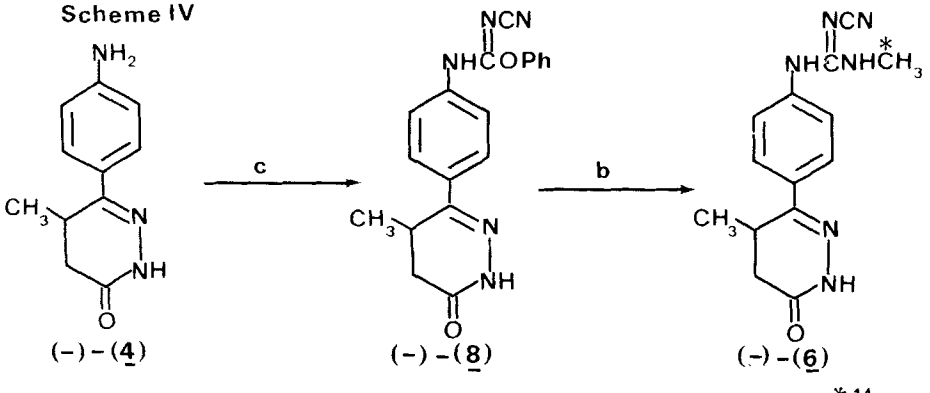
In the light of the above discussion we prepared the (-)-[N-methyl-^{14}C] SK&F 94836 (6) by an alternative, but closely related method. It has been reported [9] that displacement of a phenoxy group occurs under much milder conditions than the thiomethyl functionality. Thus treatment of (-)-(4) (>98.0% ee[8]) with diphenyl-N-cyanoiminocarbonate[10] (Scheme IV) in dimethylformamide under reflux, gave, following recrystallisation from acetonitrile, the required phenoxy analogue of (5), (-)-6-[4-N^2-cyano-O-phenyl-N^1-isouriedo)phenyl]-5-methyl-4,5-dihydropyridazin-3(2H)-one (8). This, on reaction with [^{14}C]methylamine

Scheme III



(+)-(4) (+)-(5) (+)-(6)

Scheme IV



(-)-(4) (-)-(8) (-)-(6)

*^{14}C

a) $(CH_3S)_2CNCN$; b) $^{*}CH_3NH_2 \cdot HCl$, C_5H_5N, $(C_2H_5)_3N$;

c) $(PhO)_2CNCN$

hydrochloride (10 mCi) as before, gave the required (-)-[N-methyl-^{14}C] SK&F 94836 (6.62 mCi, 66.2% radiochemical yield) of >99.0% radiochemical purity, and 97.8% ee[8].

EXPERIMENTAL

Potassium [^{14}C]cyanide was obtained from Amersham International plc and [^{14}C]methylamine hydrochloride from ICI, Physics and Radiosotopes Services. Semi-preparative h.p.l.c. was carried out with a Gilson preparative system and analytical h.p.l.c. on a Beckman 344 liquid chromatograph system equipped with Reeve radio h.p.l.c. detector. Radiochemical purities were determined by t.l.c. on Analtech 02511 silica gel utilising a Berthold LB2832 Automatic Linear Analyser in the following t.l.c. systems i) ethyl acetate/methanol/conc. ammonium hydroxide (5:1:1 by volume) ii) dichloromethane/methanol/conc. ammonium hydroxide (10:1:1 by volume) and chloroform/methanol/glacial acetic acid (10:1:1 by volume). Liquid scintillation counting was performed on a Beckman LS6800 counter. The identity of all radiolabelled compounds was confirmed by t.l.c. comparison to authentic samples.

(±)-4-(4-Acetanilido)-3-methyl-4-oxo-[1-^{14}C]butyronitrile (2)

To a stirred suspension of 3-(4-acetanilido)-2-methyl-3-oxo-1-trimethylammoniopropane iodide (1, 2.06g, 5.278 mmol) in methanol (15 ml) was added potassium [^{14}C]cyanide (310.0 mCi 354.3 mg, 58.6 mCi.mmol^{-1}) in water (7.5 ml) and the mixture stirred at room temperature for 18h. The methanol was distilled off under reduced pressure, water (5 ml) was added and the mixture extracted (x4) with ethyl acetate. The combined organics were dried over $MgSO_4$, filtered and evaporated to dryness to yield (2, 991.2 mg, 80.9%, 250.79 mCi).

Isolation of 3-(4-propionanilido)-2-methyl-3-oxo-prop-1-ene (7) and reaction with potassium [^{14}C]cyanide

To a solution of 3-(4-propionanilido)-2-methyl-3-oxo-1-trimethyl-

ammoniopropane iodide (10 g) in chloroform (100 ml) was added 10% aqueous sodium carbonate (100 ml) and the two phase system vigourously stirred for 3h. The layers were separated, the organic layer dried over $MgSO_4$, filtered and evaporated to dryness. The residue was purified by column chromatography (silica, chloroform) to give 3-(4-propionanilido)-2-methyl-3-oxo-prop-1-ene (7, 4.4 g; 81.9%; m.pt. 64-65°C; Anal. calculated for $C_{13}H_{15}NO_2$ C 71.87%, H 6.96%, N 6.45% found C 71.67%, H 6.85% N 6.33%, nmr ($CDCl_3$) signals at δ1.25 (3H, t., J=8 Hz), δ2.06 (3H, s), δ2.41 (2H, q, J=8 Hz), δ5.58 (2H, bs), δ5.86 (2H, bs), δ7.61 (2H, d, J=8 Hz), δ7.68 (1H, bs), δ7.75 (2H, d, J=8 Hz); ir νmax ($CHCl_3$) 1700 cm^{-1}, 1660 cm^{-1}, 1620 cm^{-1}). To a solution of (7) (1.375 g in methanol (5 ml) was added potassium[^{14}C]cyanide (259 mg, 187.13 mCi) and carrier potassium cyanide (201 mg) and the mixture stirred at room temperature for 4h, when water (50 ml) was added and the precipitated 4-(4-propionanildo)-3-methyl- 4-oxo[1-^{14}C]-butyronitrile (1.190 g, 143.90 mCi, 76.9%) isolated by filtration.

(±)-6-(4-Anilino)-4,5-dihydro-5-methyl-[carbonyl-^{14}C]pyridazin-3(2H)-one (4).

4-(4-Acetanilido)-4-oxo-3-methyl-[1-^{14}C]butyronitrile (2, 991.2 mg, 4.268 mmol, 250.79 mCi) was dissolved in concentrated hydrochloric acid (5 ml) and water (5 ml) and stirred under reflux for 2.5h. On cooling the pH of the solution was adjusted to 8 with 50% aqueous sodium hydroxide. Hydrazine hydrate (402.5 mg, 390 µl, 8.039 mmol) was added and the mixture stirred under reflux for 2h. The reaction mixture was kept at 4°C for 18h, the resultant precipitate filtered off, washed with water and thoroughly dried in vacuo to yield (4), (568.8 mg, 281.8 µCi.mg^{-1}, 160.29 mCi, 64.0% radiochemical yield).

A second, lower specific activity, crop of (4) was obtained from the mother liquors of filtration. The volume of the mother liquors was reduced to ~5 ml, acidified to pH = 1 with concentrated hydrochloric acid

and carrier (4), (214 mg) added. The pH was adjusted to 8 with 50% aqueous sodium hydroxide, the solution kept at 4°C for 72h and the resultant solid filtered off and dried in vacuo (272.6 mg, 102.0 $\mu Ci.mg^{-1}$, 27.81 mCi). The overall radiochemical yield from (2) was 75.0%.

(±)-[Carbonyl-^{14}C]SK&F 94836 (6)

A solution of (±)-6-(4-anilino)-4,5-dihydro-5-methyl-[carbonyl-^{14}C] pyridazin-3-(2H)-one (4, 142.6 mg 40.185 mCi, 0.695 mmol) and dimethyl N-cyanodithioiminocarbonate (203.2 mg, 1.389 mmol) in pyridine (1.2 ml) was stirred, under reflux for 4h, when t.l.c. (silica, dichloromethane/methanol/ammonia 90:10:1 by vol) showed no remaining (4). The solvent was removed under reduced pressure, and the residue,(5), dissolved in a 33% solution of methylamine in ethanol (6.5 ml) and heated at reflux for 2.5h. On evaporation of the solvent, the residue was purified by semi-preparative h.p.l.c. (Spherisorb 10μ silica 22.4 mm i.d. x 20 cm column, eluted with dichloromethane/methanol/conc. ammonium hydroxide (94:6:0.6 by volume) at 10 $ml.min^{-1}$, UV detection at 330nm), followed by recrystallisation from methanol/water giving (±)-[carbonyl-^{14}C]SK&F 94836 (6) as a light-buff solid (59.2 mg, 195.4 $\mu Ci.mg^{-1}$, 11.355mCi). The radiochemical purity of this material was determined by t.l.c., systems i) and ii) to be 97.2%. The residue from h.p.l.c. purification and the mother liquors of crystallisation were combined and carrier SK&F 94836 (~ 100 mg) added. This was purified by h.p.l.c. (as above) to furnish a second batch of (±)-[carbonyl-^{14}C]SK&F 94836 (6) (92.3 mg, 52.99 $\mu Ci.mg^{-1}$, 4.939mCi), of radiochemical purity >97.0% by t.l.c., systems i and ii. The radiochemical yield for the conversion of (4) to (±)-(6), (±)-[Carbonyl-^{14}C] SK&F 94836 was 40.5% and the overall radiochemical yield for this synthesis was 24.6%.

(+)-[N-Methyl-^{14}C]SK&F 94836 (6)

A stirred solution of dimethyl N-cyanodithioiminocarbonate (362 mg 2.48

mmol), (+)-6-(4-anilino)4,5-dihydro-5-methyl-pyridazine-3(2H)-one (248 mg 1.22 mmol >98.0% ee[8]) in pyridine 1.5 ml was heated, under reflux for 4h. On cooling the precipitated (+)-(5) was filtered off and dried (203.8 mg, 55.5% yield).

A stirred solution of [^{14}C]methylamine hydrochloride (12.1 mg, 0.176 mmol, 413 μCi.mg^{-1}, 5 mCi), (+)-6-[4-(N^2-cyano-S-methyl-N^1-isothioiuriedo)phenyl]-5-methyl-4,5-dihydro-pyridazin-3(2H)-one (5) (53.9 mg, 0.179 mmol) and triethylamine (25 μl, 0.176 mmol) in pyridine (1 ml) was heated at 80°C in a sealed vial for 3h. On cooling the solvent was removed under vacuum and the residue purified by semi-preparative h.p.l.c., (system as above) furnishing (+)-[N-methyl-^{14}C]SK&F 94836 (13.9 mg, 85.7 μCi.mg^{-1}, 1.32 mCi, 26.4% yield) of 90% ee as determined by h.p.l.c.[8] The radiochemical purity was determined by t.l.c. to be system i) 98.9%, system ii) 98.7%, system iii) 98.9% and by h.p.l.c. (μ-Bondapak C18 eluted at 1 ml.min^{-1} with 12 -> 40% acetonitrile in 0.1M ammonium acetate (pH=6.0) over 20 minutes, u.v. detection at 300 nm) to be >99.0%.

(-)-[N-methyl-^{14}C]SK&F 94836 (6)

A stirred solution of diphenyl N-cyanoiminocarbonate (293 mg, 1.23 mmol) and (-)-6-(4-anilino)-4,5-dihydro-5-methyl-pyridazin-3(2H)-one (4) (250 mg, 1.23 mmol, >98.0% ee[8]) in DMF (3 ml) was heated, under reflux for 5h. On cooling the solvent was removed under vacuum and the residue recrystallised twice from acetonitrile furnishing (-)-6-[4-N^2-cyano-O-phenyl-N^1-isouriedo)phenyl]-5-methyl-4,5-dihydro-pyridazin-3(2H)-one (8). (180 mg, 42.2%). A stirred solution of (-)-(8) (59.0 mgs, 0.170 mmol) and [^{14}C]methylamine hydrochloride (11.77 mg, 0.170 mmol, 10 mCi) in pyridine (1 ml) containing triethylamine (100 μl) as heated at 80°C for 2h. On cooling the solvent was removed under vacuum and the residue purified by semi-preparative h.p.l.c. (see above) furnishing

(-)-[N-methyl-^{14}C]SK&F 94836 (40.7 mg). To this was added non-radioactively labelled (-) SK&F 94836 (35.0 mg) and the mixture recrystallised from propan-1-ol/water to give (-)-(6), 62.5 mg of specific activity 108.0 μCi.mg^{-1} (6.75 mCi, 67.5% yield) and radiochemical purity by t.l.c. system i) >99.0% and system ii) >99.0%. The enantiomeric excess[8] was 97.8%.

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