SYNTHESES OF RADIOLABELLED FORMS OF A NOVEL HISTAMINE H_1 ANTAGONIST (SK&F 93944)

M M Cashyap, M B Mitchell, D C Osborne and D Saunders
Smith Kline and French Research Limited
The Frythe, Welwyn, Hertfordshire, England, AL6 9AR

SUMMARY

A novel histamine H_1 antagonist SK&F 93944[1], 2-[4-(5-bromo-3-methylpyrid-2-yl)butylamino]-5-(6-methylpyrid-3-yl-methyl)-4(3H)-pyrimidone, has been labelled with carbon-14 in two different positions for use in metabolism and pharmacokinetic studies. The four stage synthesis of [pyrimidone-2-^{14}C] SK&F 93944 (7) from barium [^{14}C]cyanamide is described. The overall radiochemical yield was 38%. [butylamino-4-^{14}C] SK&F 93944 (14) was synthesised in four steps from [^{14}C]methyl iodide. The key steps in this synthesis were alkylation of 2-lithio-3-methyl pyridine with [^{14}C]methyl iodide and the bromination of 4-(3-methyl-2-pyridyl)[4-^{14}C]butylamine in a liquid sulphur trioxide/freon mixture. The overall radiochemical yield was 1.6% from [^{14}C]methyl iodide.

Keywords: preparative hplc, halogen-metal exchange, bromination, [2-^{14}C]-2-methylthio-4(3H)-pyrimidone, 2-[methyl-^{14}C]methyl pyridine.

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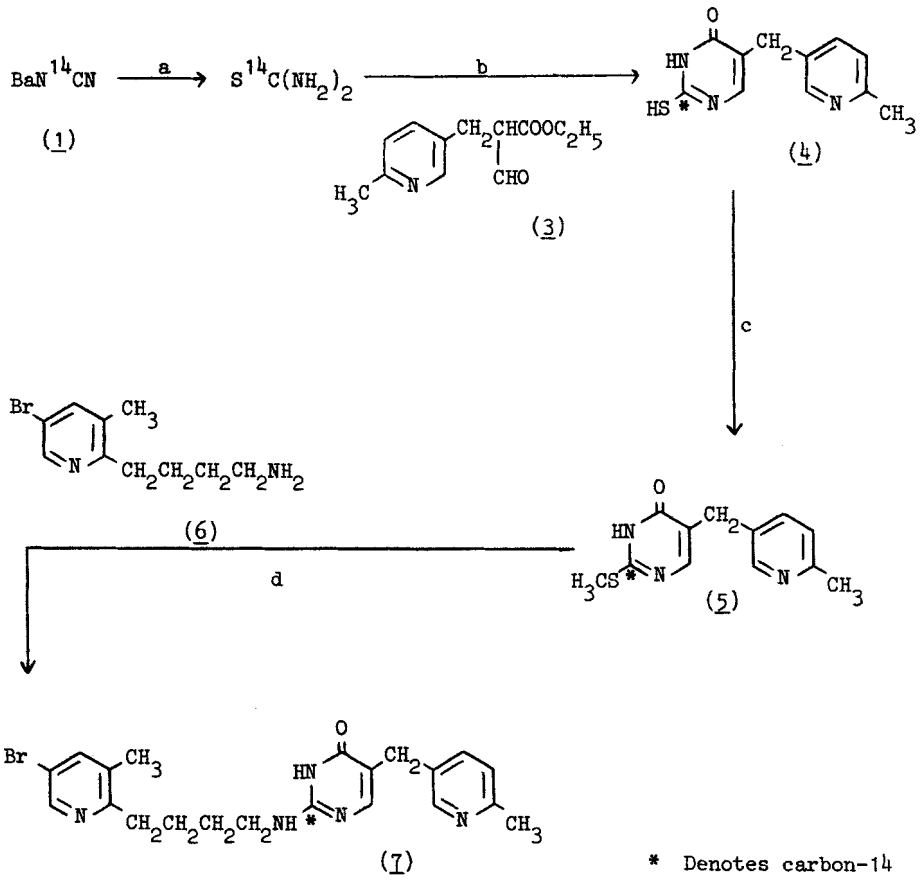
INTRODUCTION

SK&F 93944 is a novel histamine H_1-antagonist currently under investigation within Smith Kline & French. Drugs which block H_1-receptors are useful in the symptomatic treatment of various allergic diseases in which histamine is an important mediator, most notably allergic rhinitis or hay fever. SK&F 93944 shows similar or increased efficiency with respect to mepyramine in a number of assays. Furthermore it does not enter the central nervous system, thus avoiding the side effects of drowsiness observed with many H_1-antagonist drugs presently on the market. We were required to prepare carbon-14 labelled SK&F 93944 for metabolic and other studies. We initially labelled C-2 of the pyrimidone ring, and subsequently labelled α to the pyridine ring (containing the bromine group). The synthesis of these two radiolabelled forms of SK&F 93944 are described in this paper.

DISCUSSION

The synthesis of [pyrimidone-2-^{14}C] SK&F 93944 (7) is outlined in Scheme 1. Barium [^{14}C]cyanamide (1) was converted into [^{14}C]thiourea (2) by the method of Bills and Ronzio[2]. Base catalysed cyclisation with the α formyl ester (3) gave the thiouracil (4)[3, 4], which was methylated to provide the 2-methyl -thio-[2-^{14}C]pyrimid-4(3H)-one (5). Displacement of the methylthio group by the butylamine (6) gave the crude product (7). Purification by preparative layer chromatography and recovery of the compound by hydrofluoric acid digestion[5] of the silica, basification and extraction provided [pyrimidone-2-^{14}C]-SK&F 93944 (radiochemical purity 98.9%, overall 38% radiochemical yield from barium [^{14}C]cyanamide).

Scheme 1



* Denotes carbon-14

[pyrimidone-2-^{14}C]SK&F 93944

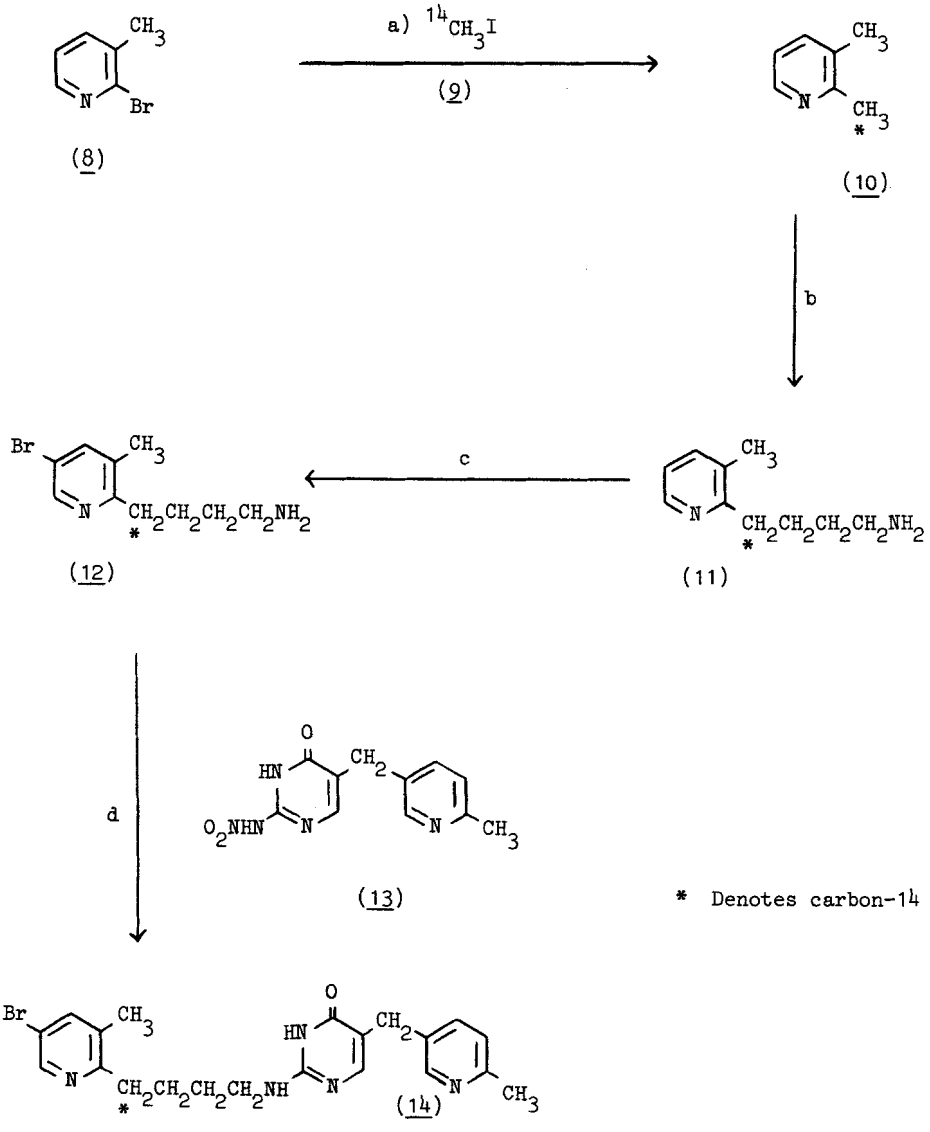
a) i) $Ba(OH)_2$, H_2O, H_2S, reflux ii) CO_2

b) $NaOC_2H_5$, ethanol

c) CH_3I, NaOH

d) Pyridine, reflux.

Scheme 2



a) n-Butyl lithium, THF, -78°C, $^{14}CH_3I$

b) $NaNH_2$/Liquid NH_3, $Cl(CH_2)_3NH_3Cl$

c) Br_2/SO_3/Freon, 0.5eq.H_2O

d) Pyridine, reflux.

The preparation of the [butylamino-4-^{14}C] SK&F 93944 (14) posed a more difficult problem. The synthesis used is shown in Scheme 2. It was known[1] that SK&F 93944 could be prepared from 2,3-dimethylpyridine (10) by alkylation with chloropropylamine hydrochloride to give the butylamine (11), bromination with elemental bromine in a mixture of oleums to give (12) followed by reaction with a pyrimid-4(3H)-one (13) bearing a suitable leaving group at C-2. The first problem was to prepare 2,3-[2-methyl-^{14}C]dimethylpyridine (10). We chose to synthesise the required dimethylpyridine (10) by alkylation of 2-lithio-3-methylpyridine with [^{14}C]methyl iodide. The preparation of aryl lithiums from the corresponding aryl bromides and their subsequent alkylation is well documented[6]. 2-[Carboxyl-^{14}C]picolinic acid has been prepared by quenching 2-lithiopyridine, prepared from 2-bromo pyridine, with [^{14}C]carbon dioxide[7]. Similarly 5-[methyl-^{14}C]methyluracil was prepared from a protected 5-bromouracil derivative *via* metal-halogen exchange with butyllithium followed by alkylation with [^{14}C]methyl iodide[8]. Thus treatment of 2-bromo-3-methylpyridine (8)[9] with n-butyl lithium followed by alkylation with [^{14}C]methyl iodide provided [2-methyl-^{14}C]-2,3-dimethylpyridine (10) (crude radiochemical yield 53%). The unexpectedly low recovery of activity in this step[10] meant that the product was contaminated with unlabelled 3-methylpyridine. This was confirmed by gas-liquid chromatography. Treatment of the mixture with sodamide in liquid ammonia generated the 2-sodiomethyl anion of (10) which was alkylated with chloropropylamine hydrochloride. Preparative layer chromatography purification of the crude product which contained starting material (10) and a dialkylated compound gave the 4-(3-methyl-2-pyridyl)[4-^{14}C]butylamine (11) in 13% radiochemical yield from [^{14}C]methyl iodide.

On a large scale, bromination of 4-(3-methyl-2-pyridyl)butylamine (11) may be performed using bromine in a mixture of oleums[1, 11], but the concentration of sulphur trioxide is critical in order to prevent the formation of large amounts of dibrominated material. On a small scale this method was unworkable. The bromination was eventually carried out using bromine in a sulphur trioxide/freon[12] mixture containing 0.5eq of water. The water serves to protonate the primary amine function and protect it from further reaction. The 4-(5-bromo-3-methyl-2-pyridyl)[4-^{14}C]butylamine (12) was obtained in a 33% radiochemical yield after purification by preparative layer chromatography. Reaction with the 2-nitroaminopyrimidone (13)[1] and purification by normal phase high performance liquid chromatography gave the required [butylamino-4-^{14}C] SK&F 93944 (14) (radiochemical purity >99.5%, overall 1.6% radiochemical yield from [^{14}C]methyl iodide). This is the first reported synthesis of 2,3-[2-methyl-^{14}C]-dimethylpyridine (10). Furthermore the meta bromination of substituted pyridines in a sulphur trioxide/freon mixture as exemplified here is an ideal alternative to the use of oleum[11] for small scale synthesis. This is also the first reported synthesis of 2-^{14}C-labelled isocytosines from readily available [^{14}C]thiourea via displacement of the 2-methylthio group of 4-pyrimidones with a suitable amine.

EXPERIMENTAL

Barium [^{14}C]cyanamide was obtained from ICI Physics and Radioisotope Services, Billingham, and [^{14}C]methyl iodide from Amersham International plc. Hplc purifications were carried out using Arcksil 10μm silica columns (250mmx id 22mm) with a Perkin Elmer Series 2 pump and Perkin Elmer LC55 UV/VIS

Spectrophotometer. Radiochemical purities were determined on Analtech 02511 silica gel plates followed by radiochromatogram scanning on a Berthold Linear Analyser.

[^{14}C]Thiourea (2)

Barium [^{14}C]cyanamide (1) (310mg, 1.75 mmol, 57.1mCi.mmol^{-1}, 100 mCi) was combined with barium hydroxide (1.02g, 5.94 mmol) and dissolved in water (40ml). Hydrogen sulphide gas was passed through the solution for 5h, and the resultant solution refluxed for 16h. Carbon dioxide was passed through the cooled solution. The precipitated barium carbonate was separated by filtration using "hi-flo" filter aid, and the residue washed with water (10ml) followed by ethanol (10ml). The filtrate and washings were combined and the solvent removed by rotary evaporation under reduced pressure to give [^{14}C]thiourea (2) (276mg, >100%, assumed quantitative).

2-Mercapto-5-(6-methyl-3-pyridylmethyl)-4-[2-^{14}C]pyrimidone (4)

Sodium metal (40mg, 1.74 mmol) was dissolved in ethanol (3ml) and ethyl 2-formyl-3-(6-methyl-3-pyridyl)propionate (3) (386mg, 1.75 mmol) was added. The mixture was stirred at room temperature for 1h, and the crude [^{14}C]thiourea (1.75 mmol) prepared above, was added. The resulting mixture was stirred and refluxed for 20h. The solvent was removed by rotary evaporation under reduced pressure, the residue was dissolved in water (4ml) and the solution was acidified to pH6 using glacial acetic acid. After standing overnight the crystalline solid was separated by filtration to give 2-mercapto-5-(6-methyl-3-pyridylmethyl)-4-[2-^{14}C]pyrimidone (4) (417mg, 1.75 mmol).

5-(6-Methyl-3-pyridylmethyl)-2-methylthio-4-[2-^{14}C]pyrimidone (5)

2-Mercapto-5-(6-methyl-3-pyridylmethyl)-4-[2-^{14}C]pyrimidone (4) (417mg, 1.79mmol) was stirred in a solution of sodium hydroxide (143mg, 3.58mmol) in water (4ml). Methyl iodide (254mg, 1.79mmol) in ethanol (4ml) was added and the resulting solution was heated at 50°C for 3h. The reaction mixture was cooled and the solvent was removed by rotary evaporation under reduced pressure. Water (3ml) was added and the pH was adjusted to 5 with glacial acetic acid. The precipitate was separated and dried to give 5-(6-methyl-3-pyridylmethyl)-2-methylthio-4-[2-^{14}C]pyrimidone (5) (307mg, 1.24mmol, 57.0mCi.mmol^{-1}, 70.7mCi, overall radiochemical yield 71% from barium [^{14}C]cyanamide).

[pyrimidone-2-^{14}C] SK&F 93944 (7)

5-(6-Methyl-3-pyridylmethyl)-2-methylthio-4-[2-^{14}C]pyrimidone (5) (95mg, 0.385mmol 21.9mCi) and 4-(5-bromo-3-methyl-2-pyridyl) butylamine (6) (113mg, 0.465mmol) were refluxed in pyridine (2ml) for 16h. Pyridine was removed by rotary evaporation under reduced pressure and unlabelled SK&F 93944 (110mg, 0.276mmol) was added. The crude reaction mixture was purified by preparative layer chromatography (4 silica plates, 20cm x 20cm x 1mm; ethyl acetate: methanol:conc. ammonium hydroxide, (5:1:1, by volume) as the developing solution). The bands corresponding to SK&F 93944 were scraped off and the silica digested using aqueous hydrofluoric acid solution (10ml). The resulting solution was basified with solid sodium bicarbonate and extracted with ethyl acetate (3 x 10ml) to give [pyrimidone-2-^{14}C] SK&F 93944 (7) (171.1mg, 0.387mmol, 11.67mCi, 53% radiochemical yield, specific activity 68.2μCi.mg^{-1}, 30.1mCi.mmol^{-1}, overall 38% radiochemical yield

from barium [^{14}C]cyanamide). The purity of the final material was 98.9% in two different tlc systems, ((i) ethyl acetate: methanol:ammonia (SG 0.88), (5:1:1, by vol), (ii) n-propanol: ammonia (SG 0.88), (7:3, v/v), both systems on Analtech 02511 254GF, 250μm silica plates).

2,3-[2-methyl-^{14}C]Dimethylpyridine (10)

2-Bromo-3-picoline (8) (1.433g, 8.33mmol) was dissolved in dry tetrahydrofuran (25ml) under nitrogen and cooled to -70°C.n-Butyl lithium in hexane (5.21ml, 1.6M, 8.33mmol) was added dropwise to the stirred solution. The resulting mixture was stirred at -70°C for 1.5h, frozen in liquid nitrogen and [^{14}C]methyl iodide (9) (8.33mmol, 500mCi, 60mCi.mmol^{-1}) was added by vacuum line transfer. The reaction mixture was stirred at -70°C for 3h, aqueous ammonium chloride (20ml, 1M) was added, the mixture was allowed to warm to 0°C, concentrated hydrochloric acid (15ml) was added and the aqueous solution extracted with hexane (1 x 30ml, 2 x 15ml). The aqueous layer was basified with concentrated ammonium hydroxide (15ml) and extracted with ether (1 x 30ml, 2 x 15ml). The combined ether extracts were dried ($MgSO_4$). Gas chromatographic analysis of the ether extract showed there to be 52% 2,3-dimethylpyridine, the principal contaminant being 3-picoline.

4-(3-Methyl-2-pyridyl)[4-^{14}C]butylamine (11)

The above ether extract containing 2,3-[2-methyl-^{14}C]-dimethylpyridine (10) was filtered and the solvent removed by distillation under atmospheric pressure. The residue was dissolved in liquid ammonia (14ml) and sodamide (464mg, 11.9mmol) was added. After stirring under reflux under an inert atmosphere of nitrogen for 0.5h, 3-chloropropylamine hydrochloride (676mg,

5.20mmol) was added in portions. The reaction mixture was stirred for 2h under reflux and then quenched by the cautious addition of water (10ml). The resulting aqueous solution was extracted with ethyl acetate (6 x 10ml).

The combined extracts were dried ($MgSO_4$), filtered and the solvent removed by rotary evaporation under reduced pressure. The crude product was purified by preparative layer chromatography on silica plates using ethyl acetate:methanol:conc. ammonium hydroxide (5:1:1, by volume) as the developing solution. The band corresponding to the required product was removed, and the product repeatedly extracted using methanol:conc. ammonium hydroxide (50:1, v/v, 5 x 20ml). The combined extracts were evaporated to dryness, dissolved in ethyl acetate (60ml) and dried ($MgSO_4$). The solution was filtered and the solvent removed by rotary evaporation under reduced pressure to give the purified 4-(3-methyl-2-pyridyl)[4-^{14}C]butylamine (11) (194mg, 1.18mmol, 64mCi, radiochemical purity 92%).

4-(5-Bromo-3-methyl-2-pyridyl)[4-^{14}C]butylamine (12)

Water (10µl, 0.55mmol) was added to the 4-(3-methyl-2-pyridyl)-[4-^{14}C]butylamine (11) (194mg, 1.18mmol), followed by freon (3.5ml). The mixture was cooled to -20°C and sulphur trioxide (1.9ml) was added to the vigorously stirred mixture. The two-phase solution was allowed to warm to room temperature and bromine (73µl, 226mg, 1.42mmol) was added. The reaction mixture was stirred at room temperature in a sealed container for 21h, added to ice (10ml), conc. ammonium hydroxide (15ml) was added and the aqueous layer extracted with ethyl acetate (5 x 10ml). The combined extracts were dried ($MgSO_4$), filtered, and the solvent removed by rotary evaporation under reduced pressure to give the crude product (203mg).

This was purified by preparative layer chromatography on a silica plate using ethyl acetate:methanol:conc. ammonium hydroxide, (5:1:1, v/v, 5 x 20ml). The combined extracts were evaporated to dryness, dissolved in ethyl acetate and dried ($MgSO_4$). The solution was filtered and evaporated to give 4-(5-bromo-3-methyl-2-pyridyl)[4-^{14}C]butylamine (12) (78mg, 0.324mmol, 20.2mCi, 4.0% overall radiochemical yield from [^{14}C]methyl iodide).

[butylamino-4-^{14}C] SK&F 93944 (14)

4-(Bromo-3-methyl-2-pyridyl)[4-^{14}C]butylamine (12) (78mg, 0.324mmol) and 5-(6-methyl-3-pyridylmethyl)-2-nitroamino-4-pyrimidone (13) (88mg, 0.340mmol) were refluxed together in pyridine (1ml) for 16h. The reaction mixture was evaporated to dryness and purified by preparative scale normal phase hplc (10µm silica, 22.5mm id x 250mm, acetonitrile:methanol:conc. ammonium hydroxide, (90:10:1, by volume)). This gave [butylamino-4-^{14}C]-SK&F 93944 (14) (63mg, 0.143mmol, 8.03mCi, 127.6µCi.mg^{-1}, 56.4mCi.mmol^{-1}) in 1.6% overall radiochemical yield from [^{14}C]methyl iodide. The purity of the final material was >99% in two different tlc systems, ((i) ethyl acetate:methanol:ammonia (SG 0.88), (5:1:1, by vol), (ii) n-propanol:ammonia (SG 0.88), (7:3, v/v), both systems on Analtech 02511 254GF, 250µm silica plates).

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REFERENCES

1. European Patent Application EP0068833, 25 June (1982).
2. Bills C.W. and Ronzio A.R. - J. Amer. Chem. Soc. 72:5510, (1950).

3. Review - Brown D.J. - Chem. Heterocycl. Compd. 20: 16-51, (1970).

4. For the use of radiolabelled thiourea in similar cyclisations, see Jezdic V., Razumenic N., Skakun M., Albahari S. and Odavic-Josic J. - J. Label. Compds. 6:88, (1970); Szabales A., Kruppa G. and Sagi J. - J. Label. Compds. Radiopharm. 14:713, (1978).

5. Solvent extraction of the compound from the silica gave a poor recovery. This hydrofluoric acid digestion method has proved useful for a number of compounds prepared in this laboratory which have proved difficult to extract from silica.

6. Negishi E.I. - Organometallics in Organic Synthesis, vol 1, pp36, Wiley - Interscience (1980).

7. Merril E.J. and Vernice G.G. - J. Label. Compds. 8:589, (1972).

8. Pichat L., Deschamps J., Masse B. and Dufay P. - Bull. Soc. Chim. Fr. 2110, (1971).

9. Fieser L.F. and Pechet M.M. - J Amer. Chem. Soc. 68:2577, (1946).

10. Due to faulty manifold.

11. Misetich R.G. in Heterocyclic Compounds, Ed Abramovitch R.A. vol 14, Pyridine and its derivatives, Supplement Part 2, pg 312, Wiley-Interscience (1974).

12. The "Freon" (DuPont) used was 1,1,2-trichloro-1,2,2-trifluoroethane.