The Synthesis of [^{14}C]SK&F 97426A

A novel bile acid sequestrant

Kenneth W M Lawrie* , Pravina Aggarawal and David Saunders

SmithKline Beecham Pharmaceuticals Research and Development
Coldharbour Road, The Pinnacles, Harlow, Essex CM19 5AD, U.K.

Summary

SK&F 97426A (10), a 1% crosslinked 11-trimethylammonioundecylmethacrylate chloride ethylene glycol dimethacrylate co-polymer bile acid sequestrant, has been prepared in two carbon-14 labelled forms, namely in the 1-position of the undecyl side chain and the pendant N-methyl group. 11-Bromo[1-^{14}C]undecyl methacrylate has been prepared and conditions developed for its small scale suspension polymerisation.

Keywords: [^{14}C]bile acid sequestrant, [^{14}C]polymer, [1-^{14}C]undecyl methacrylate, suspension polymerisation, hypercholesterolaemia

Introduction

The use of bile acid sequestrants (BAS) for the treatment of hypercholesterolaemia is well established as they have been used in the clinic for around twenty years (ref.1). Sequestrants are typically anion exchange resins, which reduce plasma cholesterol levels by binding bile acids in the gut. This in turn increases fecal bile acid secretion and so reduces their recirculation to the liver. This has the effect of increasing bile acid synthesis, from cholesterol, and a consequent reduction of plasma cholesterol levels (ref. 2). SK&F 97426A (10) is a 1% cross linked 11-trimethylammonioundecylmethacrylate chloride ethylene glycol dimethacrylate co-polymer BAS under development by SmithKline Beecham for the treatment of hypercholesterolaemia (ref. 3). In order to investigate its metabolic fate we required SK&F 97426A (10) radiolabelled with carbon-14 in the N-methyl position and in the C-1 position of the undecyl side chain. The former compound can be prepared by functionalisation of a polymeric precursor with [^{14}C]trimethylamine, but the latter requires the synthesis of a carbon-14 labelled monomer and subsequent, small scale, polymerisation.

0362–4803/94/020165–08$09.00

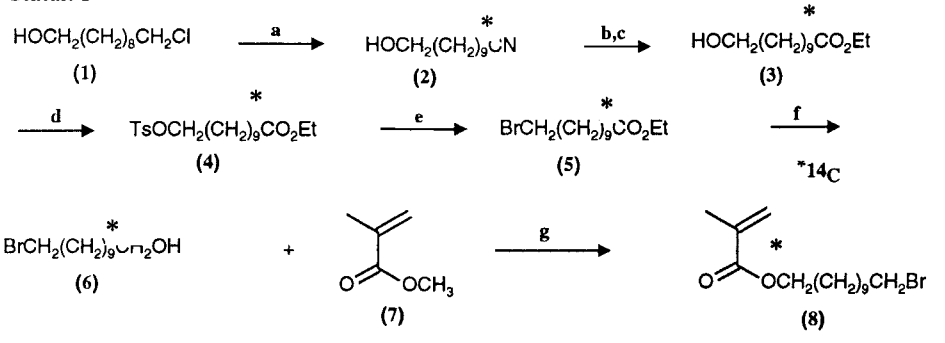
Received 27 August, 1993
Revised 4 October, 1993

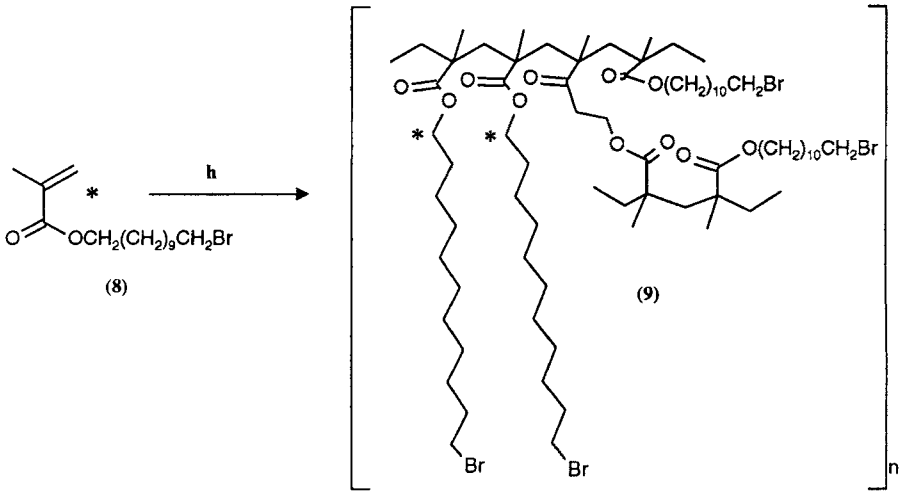
Discussion

The syntheses of [*undecyl*-1-^{14}C]SK&F 97426A and [N-*methyl*-^{14}C]SK&F 97426A are illustrated in Schemes 1 and 2. The strategy adopted for the preparation of the required [^{14}C]monomer, 11-bromo[1-^{14}C]undecylmethacrylate (8), was that of transesterification of 11-bromo[1-^{14}C]undecanol (6) and methyl methacrylate (7).

The radiolabel was introduced into the 1-position of 11-bromoundecanol (6) by the following sequence. Treatment of 10-chlorodecanol (1)with potassium [^{14}C]cyanide in DMSO at 60°C gave the corresponding 10-cyano[*cyano*-^{14}C]decanol (2) in virtually quantitative yield, which on alkaline hydrolysis and esterification (ethanol/HCl) smoothly furnished ethyl 11-hydroxy[*carboxyl*-^{14}C]undecanoate (3) in 64.5% overall yield from potassium cyanide.

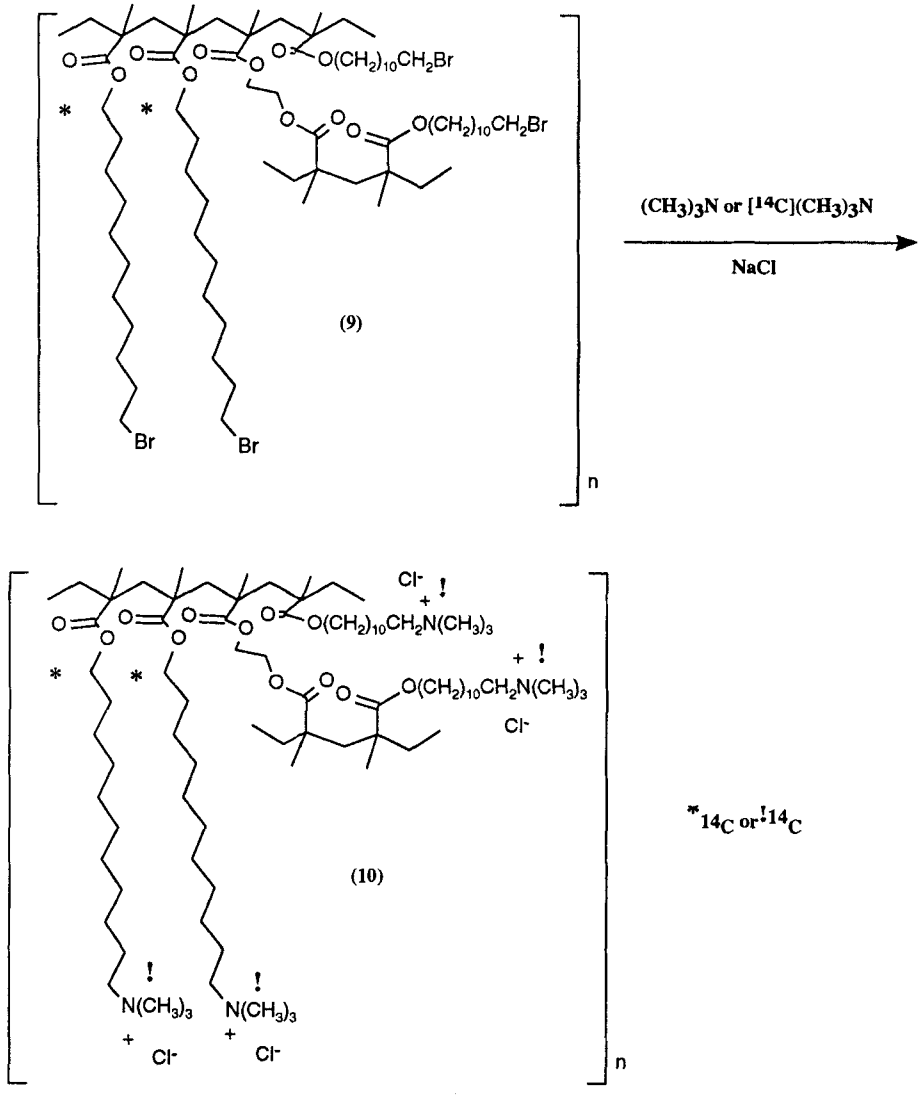
Scheme 1





a : K[^{14}C]CN , DMSO , 60 ° C b : NaOH c : EtOH/HCl d : TsCl/DMAP/Et_3N e : LiBr f : $LiAlH_4$ g : $Ti(^iPrO)_4$. h : see text for polymerisation conditions .

Scheme 2



*[*undecyl*-^{14}C]SK&F 97426A !**[N-*methyl*-^{14}C]SK&F 97426 A (10)**

Introduction of the 11-bromo substituent could be achieved either directly, by reaction of ethyl 11-hydroxyundecanoate (3)with CBr4/Ph3P, or in a two step sequence [i) TsCl, DMAP, Et3N ii) LiBr, CH3CN 79.2%]. In practice, the latter approach proved experimentally more convenient, although the product generally contained a few percent of ethyl 11-chloroundecanoate (identified by GCMS), presumably formed during the tosylation reaction by substitution of the tosyl group with chloride ion generated *in situ*. Reduction of the ester group (LiAlH4/Et2O, 87.4%) completed the synthesis of 11-bromo[1-^{14}C]undecanol (6)in 34.7% overall yield from potassium [^{14}C]cyanide.

Reaction 11-bromo[1-^{14}C]decanol (6) with a large excess of methyl methacrylate (7), and a catalytic amount of titanium tetraisopropoxide, at reflux, furnished the transesterification product, 11-bromo[1-^{14}C]undecyl methacrylate (8), in 80.3% yield following purification by column chromatography. Gratifyingly, this radiolabelled monomer proved quite stable and could be stored at room temperature for several weeks without any significant decomposition. It had been a concern that this [^{14}C] material would spontaneously polymerise, due to radiolytic activation, however this was not the case. The specific activity of this batch of 11-bromo[1-^{14}C]undecyl methacrylate (8) was low (~900μCi/mmol) however, and the stability of high specific activity [^{14}C]monomer remains in doubt.

The ability to polymerise efficiently, and reproducibly, 11-bromoundecyl methacrylate (8), on a small scale (<5g), was crucial for the success of this synthesis. Consequently, a great deal of effort was directed to this end. An apparatus for small scale suspension polymerisation has been described by Arshady and Ledwith (ref. 4), and for our experiments we used a minor modification of their design (see Fig. 1), introducing a flanged joint two thirds of the way up the body of the reaction flask (to facilitate filtration of the product). Glass stirring rods proved too fragile and so a metal stirrer was used. Following extensive experimentation, conditions were developed which allowed reproducible, high yielding (>80%) polymerisations giving beads of consistent particle size, to be achieved on a 3-4g scale : i) stirring rate 550rpm, ii) reaction temperature 80±10°C, iii) volume of solvent (PVA/water) 60-80ml, iv) the monomers were added dropwise (over ~30s) directly into the vortex formed by the stirrer, v) reaction time 6-8h and vi) initiator; dimethyl 2,2'-azobis-2-methylpropionate. Polymerisation of 11-bromo[1-^{14}C]undecyl methacrylate (8) proceeded uneventfully, under these conditions, giving the required [^{14}C]bromo polymer in 88.7% yield. Quaternisation ($(CH_3)_3N/CH_3OH$) and exchange of bromide for chloride (NaCl) completed the synthesis of [*undecyl*-1-^{14}C]SK&F 97426A (10). SK&F 97426A is in the form of free flowing off white beads which are insoluble in aqueous or organic solvents, and so is not amenable to conventional radiochemical analysis. Consequently [*undecyl*-1-^{14}C]SK&F 97426A was characterised by CH&N analysis, and the specific activity (79.2kBq/mg, 2.14μCi/mg) determined by combustion in a sample oxidiser. Combustion, and counting of the resultant $[^{14}C]CO_2$, proved to be the best method for quantifying radioactivity in samples of SK&F 97426A. The beads could not be conveniently solubilised, and if counted by liquid scintillation as a suspension in cocktail considerable inaccuracies resulted, presumably due to absorption of the β particles within the body of the bead.

[N-*Methyl*-^{14}C]SK&F 97426A was readily prepared (see Scheme 2) by quaternisation of the bromo polymer (9) with a three fold excess of [^{14}C]trimethylamine, in methanol at reflux, followed by exchange of bromide for chloride, and was characterised as above.

Experimental

Potassium [^{14}C]cyanide and [^{14}C]trimethylamine hydrochloride were supplied by Amersham International plc and ICI-Cambridge Research Biochemicals, Billingham

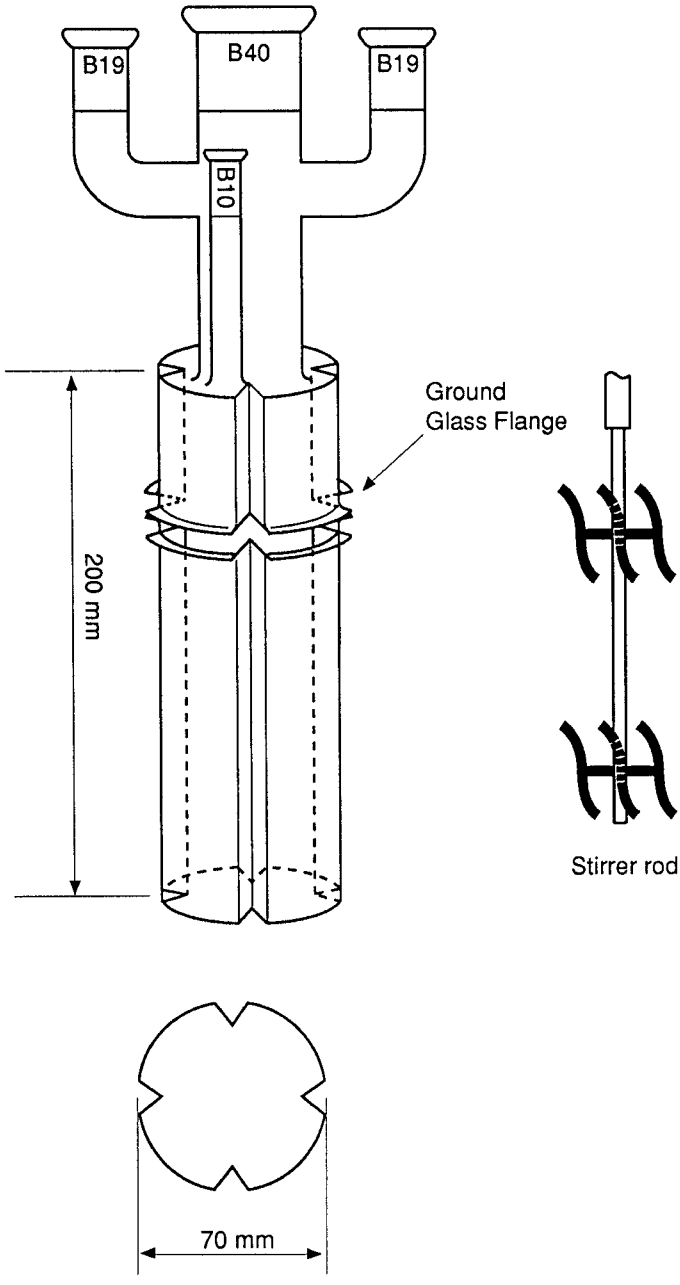


Fig. 1. Small scale suspension polymerisation apparatus after Arshady and Ledwith (ref. 4)

respectively. Radiochemical purities were determined by TLC using a Berthold 2832 Linear Analyser, integration by Berthold Chroma software. Quantification of radioactivity was by use of a Beckman LS6800 liquid scintillation counter. A Packard Tricarb sample oxidiser

was used for combustion analysis. Radioactive intermediates were characterised by chromatographic comparison to authentic samples.

10-Cyano[*cyano*-^{14}C]decanol (2)

To a stirred solution of 10-chlorodecanol (1) (5.003g, 26.1mmol) in DMSO (10ml) was added K[^{14}C]CN (90mg, 1.38mmol, 833μCi/mg, 74970μCi) and carrier KCN (1.603g, 24.7mmol). The mixture was heated at 60°C for 36h. On cooling, ethyl acetate (30ml) was added and the solution thoroughly extracted with water. the organic layer was dried over $MgSO_4$, filtered and evaporated to dryness furnishing the title compound (2) (4.713g, 25.7mmol, 98.5% yield).

Ethyl 11-hydroxy[*carboxyl*-^{14}C]undecanoate (3)

10-Cyano[*cyano*-^{14}C]decanol (2) (4.713g, 25.7mmol) was dissolved in ethanol (80ml) containing 10M sodium hydroxide (14ml) and the mixture heated at reflux for 2h. The volume was reduced to ~15ml, water (30ml) added and the solution extracted with ethyl acetate (x1). The aqueous layer was acidified (pH=1) and extracted thoroughly with ethyl acetate. These combined extracts were dried over $MgSO_4$, filtered and evaporated. The residue was dissolved in ethanol (110ml), containing conc. HCl (0.5ml) and heated at reflux with azeotropic removal of water for 2h. On cooling the volume of the solution was reduced to~10ml, diluted with ether (50ml), washed sequentially with 5M NaOH (10ml) and saturated brine (10ml), dried over $MgSO_4$, filtered and evaporated to dryness. The crude product was purified by column chromatography (silica ether/60:80 pet. ether 1:1 v/v) furnishing the title compound 3.864g (64.5% from KCN).

Ethyl 11-bromo[*carbonyl*-^{14}C]undecanoate (5)

Ethyl 11-hydroxy[*carbonyl*-^{14}C]undecanoate (3) (2.975g, 10.9mmol), p-toluenesulfonyl chloride (2.55g, 13.4mmol), triethylamine (1.86ml, 13.4mmol) and 4-N,N-dimethylaminopyridine (DMAP, 204mg, 1.67mmol) were stirred at ambient temperature in CH_2Cl_2 (35ml) for 6h., when a further 250mg (1.31mmol) of p-toluenesulfonyl chloride, 180μl (1.34mmol) of triethylamine and 20mg (0.16mmol) of DMAP were added and reaction continued for 18h. The solution was washed with water, dried over $MgSO_4$, filtered and evaporated to dryness furnishing ethyl 11-tosyloxy[*carbonyl*-^{14}C]undecanoate (4) (6.69g, >100%). This was dissolved in acetonitrile (50ml) containing anhydrous LiBr (11.31g, 130mmol) and stirred at ambient temperature for 18h. The solids were filtered off and the filtrate reduced to ~10ml, diluted with ether (60ml), washed with water, dried over $MgSO_4$, filtered and evaporated to dryness. The crude product was purified by column chromatography (silica ether/n-pentane 1:4) furnishing the title compound 3.043g (80.3%) as a colourless oil.

11-Bromo[1-^{14}C]undecanol (6)

Lithium aluminium hydride (403mg, 10.9mmol) was suspended in anhydrous ether (10ml, distilled from sodium) and cooled in an ice bath. To this was added, dropwise, a solution of ethyl-11-bromo[*carbonyl*-^{14}C]undecanoate (5) (1.065g, 3.63mmol) in anhydrous ether

(2ml). The ice bath was removed after 0.5h, and reaction continued at ambient temperature for 1.75h, when an additional portion of $LiAlH_4$ (150mg, 4.09mmol) was added. Following a further 1.25h, the mixture was cooled in an ice bath and saturated aqueous NH_4Cl added. The layers were separated and the aqueous layer extracted with ether (x2). The combined organics were dried over $MgSO_4$, filtered and evaporated to dryness furnishing the title compound 0.859g (94.2%). In a separate experiment a further 2.111g of the title compound were prepared. The two batches were combined and recrystallised from 60:80 pet. ether furnishing 2.472g (9.84mmol) (83.2%) at 8.80μCi/mg, which was diluted with carrier 11-bromoundecanol to a specific activity of 3.50μCi/mg.

11-Bromo[1-^{14}C]undecyl methacrylate (8)

11-Bromo[1-^{14}C]undecanol (6) (1.05g, 4.18mmol, 3675mCi) and methyl methacrylate (2.46g, 23mmol) were degassed with nitrogen. Titanium isopropoxide (63μl, 0.21mmol) was added and the mixture heated, at reflux, for 1.0h. Approximately 1ml of the methyl methacrylate was distilled off and saturated brine (5ml) and ether (10ml) added to the residue. A gummy precipitate formed and the water decanted off. The precipitate was dissolved in CH_2Cl_2 (30ml), the solution filtered, dried over $MgSO_4$, filtered and evaporated to dryness. The residue was purified by column chromatography (silica, ether/n-pentane 1:4v/v) furnishing the title compound (1.04g, 78.2%). This was repeated using 2.82g of 11-bromo[1-^{14}C]undecanol giving a further 2.98g (83.2%) of 11-bromo[1-^{14}C]undecyl methacrylate (8).

[*Undecyl*-1-^{14}C]SK&F 97426A (10)

Polyvinyl alcohol (82mg, 125,000MWt) was dissolved in hot water (75ml), placed in the suspension polymerisation apparatus (Fig. 1) and degassed with nitrogen. The solution was stirred at 559RPM and heated in an oil bath at 80-85°C. To this was added, dropwise over ~30s directly into the vortex formed by the stirrer, an intimate mixture of 11-bromo[1-^{14}C]undecyl methacrylate (8) (4.05g, 12.7mmol), dimethyl 2,2'-azobis-2-methylpropionate (56.1mg, 0.24mmol) and ethylene glycol dimethacrylate (40mg, 0,202mmol) and the reaction mixture heated for 6h. The solvent was decanted and the [^{14}C]bromo polymer (9) washed onto a sintered funnel with cold water. The product was washed successively with cold water (50ml), hot water (~60°C, 300ml), acetone (1,000ml) and ether (500ml). The resultant off white sticky polymer was dried at 40°C, 0.1mm Hg, for 18h furnishing (9) 3.592g, (88.7%). The [^{14}C]bromo polymer (9) was suspended in methanol (100ml) and trimethylamine (26.4ml of a 4.2M solution in ethanol) added. The reaction mixture was heated, at reflux, for 18h, when a further 13ml of the trimethylamine solution added and reaction continued for 6h. The product was filtered off, washed thoroughly successively with methanol, acetone and 10% aqueous sodium chloride (to exchange bromide for chloride) and dried under vacuum, giving [*undecyl*-^{14}C]SK&F 97426 (10) (3.343g, %) as off white beads of specific activity 2.14μCi/mg (79.2kBq/mg). (Found %C 58.98, 58.67 %H 10.66, 10.43, %N 3.43,3.53 %Cl 9.60 %Br - none detected)

[N-*Methyl*-^{14}C]SK&F 97426A (10)

To a stirred suspension of the non-radioactively labelled bromo polymer (9) (10g) in methanol (100ml) was added a solution of [^{14}C]trimethylamine hydrochloride (9.78mCi, 361/.9MBq, 33.4mg, 0,35mmol) and carrier trimethylamine (22.0ml of a 4.2M solution in ethanol, 9.24mmol) in methanol (50ml). The volume of solvent was made up to 200ml with methanol, and the stirred suspension heated at reflux for 20h. On cooling, the suspension was filtered and the filter cake washed thoroughly successively with methanol, 20% aqueous sodium chloride , water and acetone. The product was dried under vacuum, furnishing the title compound as off white beads (10.07g) of specific activity 297μC/mg (10.98MBq/mg). (Found %C 59.02, 58.75 %H 10.23, 10.09 %N 3.78, 3.92 %Cl 10.14 %Br - none detected).

Acknowledgement

The authors would like to thank Drs Deirdre Hickey (SB Welwyn) and Norman Lewis (SB Tonbridge) for helpful suggestions.

References

1. D B Hunninghake - J. Drug Dev. **3 (supp. 1)** 205 (1990)

2. C C Schwartz, M Bergman Z R Vlahcevic, L G Halloran, D H Gregory and L Swell - J. Clin. Invest. - **61** 408 (1978)

3. G M Benson, D R Alston, B C Bond, AN Gee, A Glen, C Haynes, D M B Hickey, S Iqual, B Jackson, A A Jaxa-Chamiec, M R Johnson, M G Roberts, B P Slingsby, C M Whittaker and K E Suckling - Atherosclerosis **101** 51 (1993)

4. R Arshady and A Ledwith - Reactive Polymers **1** 159 (1983)