Synthesis of Carbon-14 and Carbon-13 labelled (R)-(-)-2[[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]methyl]-3,4-dihydro-2,5,7,8-tetramethyl-2H-1-benzopyran-6-ol

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SUMMARY

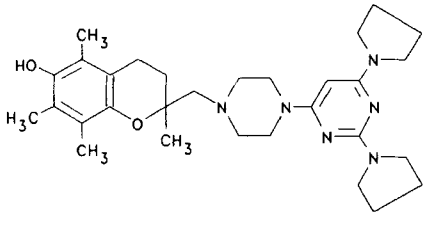
This paper describes the synthesis and characterisation of 2[[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-[^{14}C]-methyl]-3,4-dihydro-2,5,7,8-tetramethyl-2H-1-benzopyran-6-ol and 2[[4-(2,6-di-1-pyrrolidinyl-4-[$^{13}C_2$]-pyrimidinyl)-1-piperazinyl]-[^{13}C]-methyl]-3,4-dihydro-2,5,7,8-tetramethyl-2H-1-benzopyran-6-ol. Intermediates in the synthesis included R-(+)-[^{13}C]-Trolox® (R-(+)-6-hydroxy-2,5,7,8-tetramethylchroman-[2-^{13}C]-carboxylic acid), [2,5-$^{13}C_2$]-barbituric acid, 2,4,6-trichloro-[2,5-$^{13}C_2$]-pyrimidine, 2,6-di-pyrrolidinyl-4-chloro-[2,5-$^{13}C_2$]-pyrimidine and 4-(1-piperazinyl)-2,6-di-1-pyrrolidinyl-[2,5-$^{13}C_2$]-pyrimidine.

Keywords: lazaroid, R-(+)-[^{13}C]-Trolox®, [2,5-$^{13}C_2$]-barbituric acid, 2,4,6-trichloro-[2,5-$^{13}C_2$]-pyrimidine, 2,6-di-pyrrolidinyl-4-chloro-[2,5-$^{13}C_2$]-pyrimidine, 4-(1-piperazinyl)-2,6-di-1-pyrrolidinyl-[2,5-$^{13}C_2$]-pyrimidine, potassium-[^{14}C]-cyanide.

INTRODUCTION

Oxygen radical generation and lipid peroxidation are thought to play an important part in the pathophysiology of many illnesses and traumatic or ischemic injuries. For instance in brain and spinal chord injury, lipid peroxidation has been recognised as an important degradative process in the irreversible loss of neuronal tissue[1]. These processes are also thought to be important in the pathophysiology of asthma, and to this end the title compound (1, figure 1) has been under development within Upjohn Laboratories as an anti-asthmatic for clinical use.

Figure 1.



(-) enantiomer

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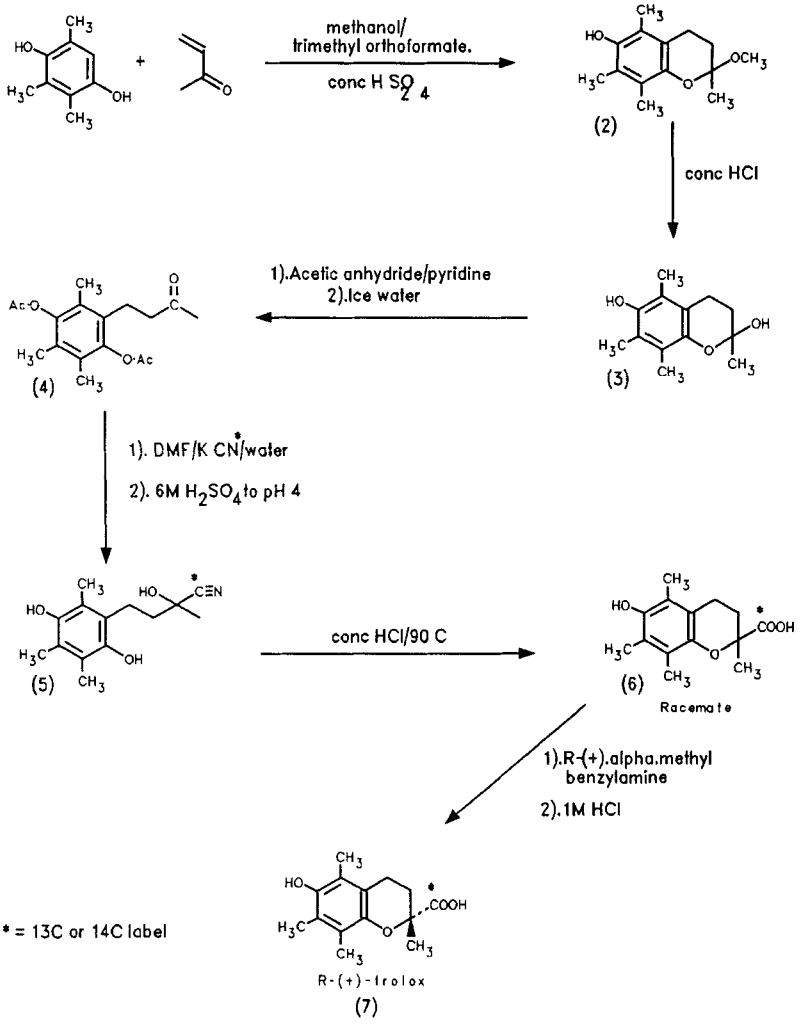
The benzopyran-6-ol fragment of the compound is an analogue of vitamine-E, a natural free radical scavenger. The di-pyrrolidinyl-pyrimidine portion has also been noted for its free radical scavenging abilities in connection with an Upjohn aminosteroid analogue 21-[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-16α-methylpregna-1,4,9(11)-triene-3,20-dione[1] also under development within Upjohn Laboratories.

The title compound has recently been tritiated in the pyrrolidine ring[2] for preliminary drug metabolism studies. However there were concerns that the compound may cleave metabolically in the central portion during ADME studies, resulting in an unlabelled benzopyran fragment that could not be traced. It seemed pertinent to locate a ^{14}C label centrally in the compound to accommodate this possibility and complement the tritium labelled compound. Also, in order to assist the identification of metabolites by mass spectral analysis, an isotopomer of the compound containing three ^{13}C-labels was also required, preferably distributed throughout the molecule.

DISCUSSION

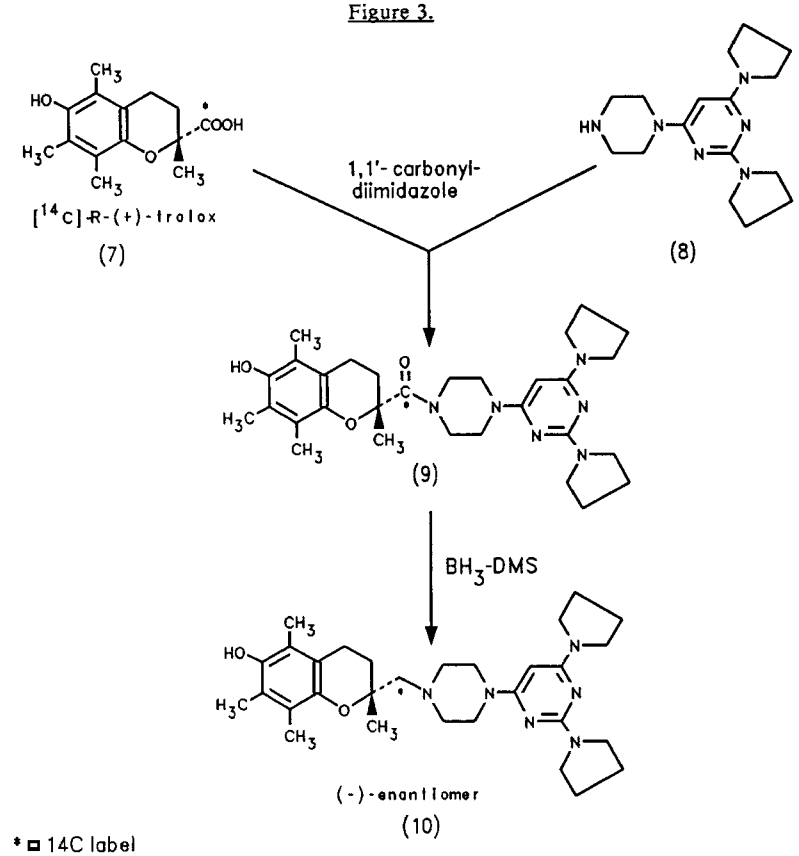
A consideration of the possible synthetic approaches and expected metabolic stability of the candidate suggested that label could be incorporated into the methylene bridge at a central position within the molecule (10; figure 3). In the original synthesis, the benzopyran portion of the molecule was derived from commercially available (±)-Trolox®[3], after resolution into the (R) form as the (R)-(+)-α-methylbenzylamine salt[4]. However for our purposes it was necessary to synthesise the labelled Trolox® precursor. There are elegant approaches to the chiral synthesis of Trolox® and vitamine-E in the literature[5]. However for our purposes the method of J.W.Scott *et al*[4] was particularly useful, as it utilised a cyanohydrin intermediate (5; figure 2) thus providing a suitable method for incorporation of ^{13}C or ^{14}C cyanide. Hence 4-(2,5-diacetoxy-3,4,6-trimethylphenyl)- butan-2-one (4) was prepared as per the literature[4] and treated with ^{14}C-potassium cyanide (10 mCi; 44 mCi mmole^{-1}) in DMF/water, followed by acidification to yield 2-[^{14}C]cyano-4-(2,5-diacetoxy-3,4,6-trimethylphenyl)butan-2-ol (5; 8.68 mCi; 86.8% radiochemical yield). Compound (5) was then treated with concentrated hydrochloric acid at 90°C to give (±)-6-hydroxy-2,5,7,8-tetramethylchroman-[2-^{14}C]-2-carboxylic acid (6) which was resolved into the (R) form with (R)-(+)-α-methyl-benzylamine[4]. The (R)-(+)-[2-^{14}C]-Trolox® (7; 1.89 mCi; 38% radiochemical yield)

Figure 2.



assayed as 99.7% enantiomerically pure by HPLC using a chiral assay developed within Upjohn Control Division (see experimental). (R)-(+)-[2-^{14}C]-Trolox• (7; 1.89 mCi) was coupled to unlabelled 4-(1-piperazinyl)-2,6-di-1-pyrrolidinyl-[2,5-$^{13}C_2$]-pyrimidine(8; figure 3) to give (R)-(-)-2-[[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-[^{14}C]-carbonyl]-3,4-dihydro-2,5,7,8-tetramethyl-2H-1-benzopyran-6-ol (9; 1.41 mCi; 75% radiochemical yield) by treatment with 1,1'-carbonyldiimidazole. Treatment of '9' with borane-dimethyl sulfide complex followed by purification gave the title compound

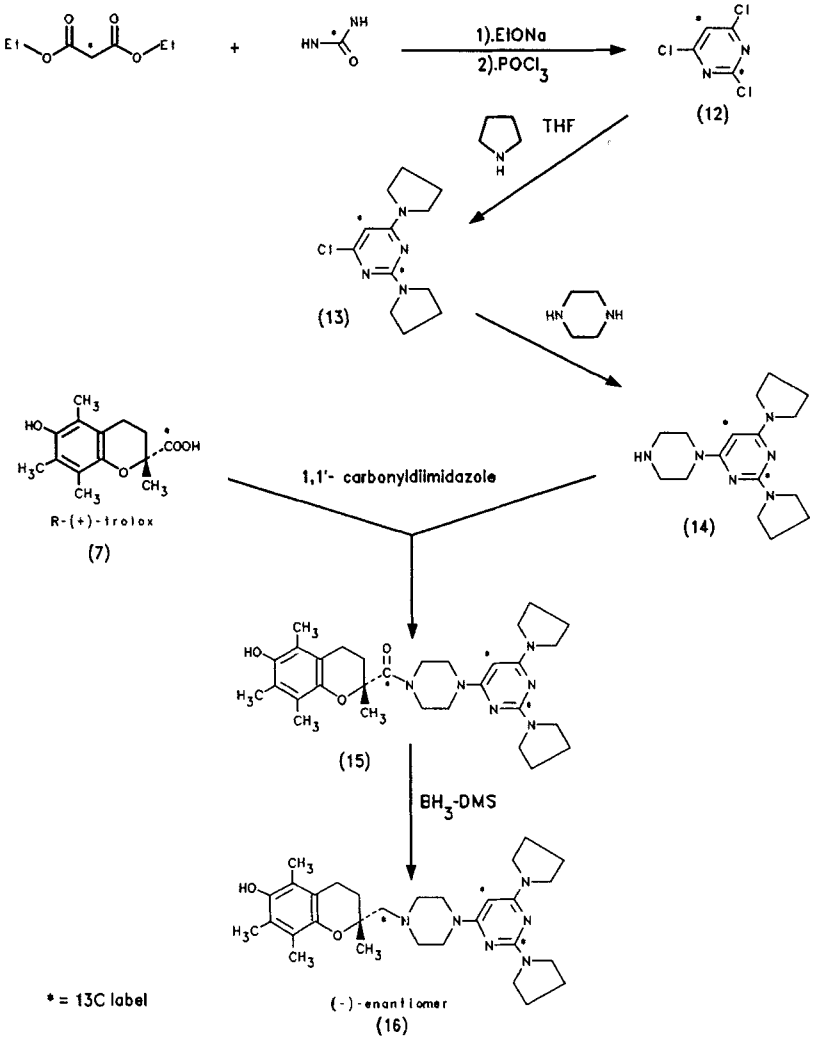
Figure 3.



(R)-(-)-2-[[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-[^{14}C]-methyl]-3,4-dihydro-2,5,7,8-tetramethyl-2H-1-benzopyran-6-ol (10; 0.753 mCi; specific activity 9.78 Ci mole^{-1}) with radiochemical purity > 98% and enantiomeric purity > 99% by HPLC.

4-(1-Piperazinyl)-2,6-di-1-pyrrolidinyl-[2,5-$^{13}C_2$]-pyrimidine was prepared as in figure 4. Diethyl-[2-^{13}C]-malonate was treated with sodium ethoxide and [2-^{13}C]-urea to give [2,5-$^{13}C_2$]-barbituric acid. Treatment of this with phosphorus oxychloride gave 2,4,6-trichloro-[2,5-$^{13}C_2$]-pyrimidine (12) which reacted efficiently with two moles of pyrrolidine to give 2,6-di-1-pyrrolidinyl-4-chloro-[2,5-$^{13}C_2$]-pyrimidine (13). Under more rigorous conditions '13' reacted with piperazine to give 4-(1-piperazinyl)-2,6-di-1-pyrrolidinyl-[2,5-$^{13}C_2$]-pyrimidine (14). Compound '14' was then coupled with R-(+)-[2-^{13}C]-Trolox (7; prepared in a similar manner to the ^{14}C material) to give '15'. Treatment of

Figure 4



'15' with borane-methyl sulfide complex gave (R)-(-)-2-[[4-(2,6-di-1-pyrrolidinyl-4-[2,5-$^{13}C_2$]-pyrimidinyl)-1-piperazinyl]-[^{13}C]-methyl)-3,4-dihydro-2,5,7,8-tetramethyl-2H-1-benzopyran-6-ol (16).

The ^{13}C-labelled compounds were characterised by ^{1}H-NMR, ^{13}C-NMR, mass spectrometry, infrared, optical rotation (where appropriate) and microanalysis. ^{13}C-NMR spectra of the pyrimidine ring in compounds (13), (14), (15), and (16) gave doublets corresponding to the coupled C-2 and C-5 aromatic carbons (J_{C-C} = 8.05, 8.25, 7.95 and

8.85 Hz respectively). The proton coupled ^{13}C-NMR of (15) showed a proton-carbon coupling at the C-5 carbon (J_{C-H} = 161.9 Hz). The ^{13}C-NMR Spectrum of R-(+)-[2-^{13}C]-Trolox® gave doublets corresponding to the C-2 and carboxylic acid coupled carbons (J_{C-C} = 59.8 Hz).

METHODS & MATERIALS

^{14}C-Potassium cyanide was obtained from Cambridge Research Biochemicals (Billingham, Cleveland, UK). Unlabelled di-pyrrolidinyl-pyrimidine was obtained from Chemical Research Preparations (Upjohn, Kalamazoo, Michigan, USA). Solvents were obtained from F.S.A Laboratory Supplies (Loughborough, Leics, UK). Diethyl-[2-^{13}C]-malonate, [^{13}C]-urea and all other reagents were obtained from the Aldrich Chemical Co (Gillingham, Dorset, UK).

Melting points were obtained on a Koeffler hot stage microscope (Reichert, Austria).

Normal phase chromatography was performed according to the method of W.C. Still *et al*,[6] commonly known as 'flash chromatography'. Silica gel (230-400 mesh ASTM, E.Merck, Darmstadt, Germany obtained through Anderman & Co) was employed as the stationary phase.

Thin layer chromatography was performed on silica gel (Kieselgel 60F 254; 20 x 20cm plates; 0.25mm coating; glass mounted; E.Merk) and visualised by u.v lamp (UV GL 58; 254nm, UVG Inc, San Gabriel, California, USA) and the radioactivity detected by a Ray Test RITA linear analyzer (Raytest, Sheffield, UK).

Chiral analysis of labelled Trolox® was performed with a Waters Associates chromatography pump (Millipore (UK) Ltd, Waters Chromatography Division, Harrow, Middlesex, UK), Chiral AGP column (100 x 4 mm; Chrom Tech AB, Norsberg, Sweden; distributed by J.T.Baker Inc, New Jersey, USA), LDC Milton Roy Spectromonitor-D variable wavelength detector, Spectraphysics Integrator (Spectraphysics, St Albans, Herts) and a Berthold LB503 Radioactivity Monitor (Berthold Labs, D-7547 Wildbad 1,

Germany). Solvent system: 2.5% acetonitrile in aqueous 15 mM piperazine acetate, pH 6.0. Flow rate 0.8 ml min^{-1}, detection 290 nm; 20μl injections, 0.8μg on column. Typical retention times were 3.4 mins ((R)-(+)-isomer) and 4.6 mins ((S)-(-)-isomer).

Chiral analysis of labelled products (10 and 16) were performed using the aforementioned equipment. Solvent system: 20.5% acetonitrile in aqueous 15 mM piperazine acetate, pH 6.0. Flow rate 0.8 ml min^{-1}, detection 290 nm; 20μl injections, 0.8μg on column. Typical retention times were 8.1 mins (S-(+)-isomer) and 9.6 mins (R-(-)-isomer).

Scintillation counting was performed using Optiphase scintillant (3ml) and an LKB 1218 Rackbeta scintillation counter (Raytek Scientific Ltd, Sheffield, UK). The external standard ratio method was used to correct for quenching.

Proton and carbon spectra were obtained on a Bruker AC 400 NMR spectrometer operating at 400 MHz (^{1}H) and 100 MHz (^{13}C).

Mass spectra were obtained on a Finnigan Mat 4610B quadrupole mass spectrometer (Finnigan MAT, Hemel Hempstead, Hertfordshire, UK).

Infrared spectra were run on a 157G grating infra red spectrometer (Perkin Elmer, Beaconsfield, Bucks, UK). The samples were prepared in chloroform and air dried.

Microanalyses were performed by Butterworth Laboratories Ltd (Teddington, Middlesex, UK). The water contents were determined on a Mitsubishi Moisturemeter CA-05 (Anachem, Luton, Bedfordshire).

Resolution of (±)-Trolox• to R-(+)-Trolox• for dilution of radiolabel.

Commercial racemic Trolox• (10 g; 0.04 moles) in ethanol (28 ml) was treated with (R)-(+)-α-methylbenzylamine (5.2 ml; 0.04 moles) and ether (284 ml) added. After cooling (-20 °C) overnight this gave white amorphous crystals which were collected, washed with ether and dried to give the (R)-(+)-Trolox• (R)-(+)-α-methylbenzylamine salt in 91% enantiomeric excess according to the above HPLC procedure. The material was redissolved

in ethanol (12 ml) and ether (117 ml) added. After cooling overnight (-20 °C) the white crystals were again collected, washed with ether and dried to give (R)-(+)-Trolox● (R)-(+)-α-methylbenzylamine salt (7.34 g) in greater than 99% enantiomeric excess. The solids were slurried into aqueous hydrochloric acid (1 M; 75 ml) and extracted with ethyl acetate (3 x 75 ml). The combined organic layers were washed with aqueous hydrochloric acid (2 x 10 ml), water (10 ml) and aqueous saturated brine (10 ml), dried (sodium sulphate), filtered and the solvent removed *in vacuo* to give (R)-(+)-Trolox● (4.5 g). MP 159-162°C (lit., MP 159-160°C). $[\alpha]_D^{25}$ = (+) 63.04 (c=0.1; ethanol).

Preparation of [2-^{14}C]-2-cyano-4-(2,5-diacetoxy-3,4,6-trimethylphenyl)butan-2-ol (5).

4-(2,5-Diacetoxy-3,4,6-trimethylphenyl)butan-2-one (0.2 g; 6.53 x 10^{-4} moles) was dissolved in dimethylformamide (0.850 ml) and cooled to 0°C in an ice/water bath. A solution of ^{14}C-potassium cyanide (14.8 mg; 2.27 x 10^{-4} moles; 10 mCi; 44 mCi mmole^{-1}) in distilled water (0.065 ml) was added dropwise over 5 minutes to the above mixture and stirred for 10 minutes. Unlabelled potassium cyanide (30 mg; 4.61 x 10^{-4} moles) in distilled water (0.065 ml) was also added dropwise to the mixture over 5 minutes and the whole stirred for a further 10 minutes at 0°C. Aqueous sulphuric acid (6 M; 0.140 ml) was added dropwise over 15 minutes and the reaction stirred for a further 30 minutes at 0°C. The reaction was poured into ice/water (10 ml) which was extracted with diethyl ether (3 x 7 ml). The combined ether layers were washed with saturated aqueous sodium chloride solution (10 ml), dried over magnesium sulphate and filtered. The product had an identical retention time to that of a ^{13}C reference sample (solvent system: chloroform/methanol 19:1; Rf 0.6) and appeared as a single radioactive peak (8.68 mCi; 86.8% radiochemical yield).

Preparation of (R)-(+)-6-hydroxy-2,5,7,8-tetramethylchroman-[2-^{14}C]-2-carboxylic acid ((R)-(+)-[2-^{14}C]-Trolox●; 7).

A solution of [2-^{14}C]-2-cyano-4-(2,5-diacetoxy-3,4,6-trimethylphenyl)butan-2-ol (8.68 mCi) in ether was blown to dryness (N_2) and dissolved in concentrated hydrochloric acid (2.25 ml). The solution was heated at 90°C under a nitrogen atmosphere for 50 hours and then cooled. The mixture was extracted with ether (3 x 4 ml) and the pooled layers washed with saturated brine (2 x 4 ml). The product was extracted from the ether layer into saturated sodium bicarbonate solution (15 ml) over 1 hour and the aqueous phase washed

with ether (8 ml). The aqueous phase was acidified to pH 2.0 (3 M hydrochloric acid) and extracted with ether (3 x 7 ml), the combined ether layers were washed with saturated aqueous brine solution (2 x 5 ml), dried over magnesium sulphate and filtered. The solvent was removed *in vacuo* to yield a white solid (0.114 g; 4.56 x 10^{-4} mole). To this was added the previously prepared unlabelled R-(+)-Trolox• (0.0625 g; 2.5 x 10^{-4} moles) and the mixture was dissolved in ethanol (0.280 ml) and ether (2.8 ml). (R)-(+)-α-Methylbenzylamine (0.106 ml; 0.0996 g; 8.22 x 10^{-4} moles) was added and the mixture stirred at room temperature for 2 hours. The mixture was left overnight at -20°C to give a white crystalline solid (4.43 mCi; 93% enantiomeric excess), which was collected and washed with ether (3 x 1.0 ml), dried and redissolved in ethanol (10 ml). The material was recrystallised again (x2) as above to give material of 99.7% enantiomeric excess. The solvent was removed *in vacuo* and the residue dissolved in hydrochloric acid (1 M; 2 ml) and stirred for 10 minutes. The aqueous layer was extracted with ethyl acetate (3 x 2 ml), washed with hydrochloric acid (1 M, 2 ml), water (2 ml) and saturated brine (2 ml), dried (sodium sulphate), filtered and the solvent removed *in vacuo*. The residue was redissolved in Helium de-gassed ethyl acetate (10 ml) to give (R)-(+)-[2-^{14}C]-Trolox• (7; 1.89 mCi; 99.7% enantiomeric excess by HPLC) in 44% radiochemical yield. The product had an identical HPLC retention time to that of authentic Trolox•.

Preparation of (R)-(-)-2-[[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-[^{14}C]-carbonyl]-3,4-dihydro-2,5,7,8-tetramethyl-2H-1-benzopyran-6-ol (9).

(R)-(+)-[2-^{14}C]-Trolox• (1.89 mCi) and 1,1'-carbonyldiimidazole (0.033 g; 2.04 x 10^{-4} moles) were dissolved in anhydrous tetrahydrofuran (0.9 ml) and stirred for 1 hour at room temperature under a nitrogen atmosphere. A solution of 4-(1-piperazinyl)-2,6-di-1-pyrrolidinylpyrimidine (0.055 g; 1.99 x 10^{-4} moles) in anhydrous chloroform (0.46 ml) was added to the reaction mixture over 5 minutes and stirred at room temperature for 16 hours. A further addition of 1,1'-carbonyldiimidazole (0.006 g; 3.7 x 10^{-5} moles) and 4-(1-piperazinyl)-2,6-di-1-pyrrolidinylpyrimidine (0.011 g; 4 x 10^{-5} moles) was made and the reaction stirred for a further 24 hours. When the reaction was complete the solvent was removed *in vacuo* and the residue partitioned between dichloromethane and saturated sodium bicarbonate solution (1:1; 8 ml). The aqueous phase was extracted further with dichloromethane (2 x 4 ml) and the combined organic phases washed with a solution of

saturated sodium chloride (10 ml), dried (magnesium sulphate) and then filtered. The solvent was removed *in vacuo* and subjected to flash chromatography (10 x 150 mm column; solvent hexane/ethyl acetate; 1:1; 1 ml fraction size) to yield (R)-(-)-2-[[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-[^{14}C]-carbonyl]-3,4-dihydro-2,5,7,8-tetramethyl-2H-1-benzopyran-6-ol (1.41 mCi) in fractions 4-7 (75% yield). The fractions were combined and the solvent removed *in vacuo* and the residue re-dissolved in dichloromethane (10 ml). The product had identical TLC characteristics (solvent hexane/ethyl acetate; 1:1) to that of an authentic sample and was 98% pure by HPLC analysis.

Preparation of (R)-(-)-2[[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-[^{14}C]-methyl]-3,4-dihydro-2,5,7,8-tetramethyl-2H-1-benzopyran-6-ol (10).

(R)-(-)-2-[[4-(2,6-Di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-[^{14}C]-carbonyl]-3,4-dihydro-2,5,7,8-tetramethyl-2H-1-benzopyran-6-ol (1.41 mCi) was dissolved in a 2.0 M borane-methyl sulphide THF complex (1.6 ml; 3.23 x 10^{-3} moles) and stirred at room temperature for 72 hours. A solution of aqueous hydrochloric acid (1 M) was added to the reaction until effervescence ceased and the mixture then adjusted to pH 7.0 by the addition of sodium hydroxide (1.0 M). The mixture was extracted with chloroform (4 x 3 ml) and the combined organic phases were washed with saturated sodium bicarbonate (8 ml) and then dried over magnesium sulphate and filtered. The solvent was removed *in vacuo* and the residue submitted to flash chromatography (10 x 150 mm column; solvent hexane/ethyl acetate; 2:1; 5 ml fractions) to give (R)-(-)-2[[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-[^{14}C]-methyl]-3,4-dihydro-2,5,7,8-tetramethyl-2H-1-benzopyran-6-ol in fractions 4-7 (0.753 mCi; specific activity 9.78 Ci mole^{-1}; purity 98.86% by TLC in solvent hexane/ethyl acetate/triethylamine, 12:8:2; 99.73% by tlc in solvent butan-1-ol/water/acetic acid, 12:6:3; enantiomeric excess > 99% by HPLC). The compound was stored in methanol (5 ml; 53% yield).

Preparation of [2,5-$^{13}C_2$]-barbituric acid.

Sodium (0.292 g; 0.0127 moles) was dissolved in ethanol (6.3 ml). To this was added [2-^{13}C]-diethylmalonate (2.0 g; 0.0124 moles) and [1-^{13}C]-urea (0.76 g; 0.0124 moles). The mixture was well mixed and then refluxed for 16 hours. Water (13 ml) was added and the

mixture acidified (conc.hydrochloric acid). The white solid was collected and washed with a further portion of water (10 ml). The precipitate was dried in a vacuum oven (50°C; 1 hour) to give [2,5-$^{13}C_2$]-barbituric acid (1.01 g). Mp 249-253°C (lit[5]., 248-252 °C for barbituric acid). Found: C, 36.81; H, 3.07; N, 21.07. $^{13}C_2$ C_2 H_4 O_3 N_2. 0.25 H_2O requires: C, 37.18; H, 3.37; N, 20.81. ^{13}C-NMR (d_6-dimethyl sulphoxide) δ 151.44 (-$^{13}CH_2$-), 167.55 (-^{13}C(O)-NH-). MS m/z 130 (M^+). IR (nujol) 1170 (w), 1245 (m), 1540 (w), 1690 (s), 1750 (s) 3070 (m), 3200 (m) cm^{-1}.

Preparation of 2,4,6-trichloro-[2,5-$^{13}C_2$]-pyrimidine (12).

Dried [2,5-$^{13}C_2$]-barbituric acid (1.01 g; 7.77 x 10^{-3} moles) was treated dropwise with a mixture of phosphorus oxychloride (3.12 ml) and N,N-dimethylaniline (0.77 ml) and the reaction refluxed for 50 mins. The mixture was cooled to -30°C and excess water slowly added. The aqueous mix was left at room temperature for 1 hour and then extracted with ether (4 x 30 ml). The combined organics were washed with water (2 x 5 ml), saturated sodium chloride (5 ml), dried (sodium sulphate) and the solvent removed *in vacuo* to give 2,4,6-trichloro-[2,5-$^{13}C_2$]-pyrimidine as an oil (1.34 g). The compound was stored in tetrahydrofuran (6.6 ml) over molecular sieves prior to use. Bp 209-214°C (lit[3]., 210-215°C for trichloropyrimidine). MS m/z 87 (80%) 121 (20%), 149 (60%), 184 (100%), 186 (90%), 188 (40%) and 190 (10%). IR (thin film) 810 (m), 830 (s), 850 (m) 1525 (s) cm^{-1}.

Preparation of 2,6-di-pyrrolidinyl-4-chloro-[2,5-$^{13}C_2$]-pyrimidine (13).

A solution of pyrrolidine (2.15 ml; 0.026 moles) in tetrahydrofuran (2 ml) was cooled to 0°C and stirred under a nitrogen atmosphere. A solution of 2,4,6-trichloro-[2,5-$^{13}C_2$]-pyrimidine (1.34 g) in tetrahydrofuran (6.6 ml) was added to the mixture over a period of 30 minutes. After a further 1 hour the reaction was allowed to warm slowly to room temperature. After 4 hours, pyridine (2 ml) was added and the reaction left for a further 16 hours. The solvent was reduced *in vacuo* and the residue partitioned between dichloromethane (40 ml) and saturated sodium bicarbonate solution (40 ml). The layers were separated and the aqueous phase extracted with dichloromethane (3 x 40 ml). The organic phases were pooled, dried (sodium sulphate) and the sample reduced to an oil *in vacuo*. Flash chromatography (20 x 150 mm column; solvent: 10% ethyl acetate/ hexane; 10

ml fractions) gave product in fractions 4-14. The product was recrystallised from hexane (x 3) to give 2,6-di-pyrrolidinyl-4-chloro-[2,5-$^{13}C_2$]-pyrimidine (1.27 g) as a white solid. Mp 77-78°C (lit[1]., 77-79°C). Found: C, 57.46; H, 7.08; N, 21.71. $^{13}C_2$ C_{10} H_{17} N_4 Cl requires: C, 57.36; H, 6.83; N, 21.99. ^{1}H-NMR ($CDCl_3$) δ 1.91 (8H, m, $-CH_2-$), 3.5 (8H, m, $-CH_2-N$), 5.61 (1H, s, Ar-H). ^{13}C-NMR ($CDCl_3$) δ 25.22, 25.47 (ss, $-CH_2-$), 46.18, 46.57 (ss, $-CH_2N-$), 90.62 (d, J_{c-c}= 8.05 Hz; 5-^{13}C-), 158.98 (s, 6-C), 159.88 (d, J_{c-c}= 8.05 Hz; 2-^{13}C), 161.44 (s, 4-C) ppm. MS m/z 70 (50%), 122 (30%), 226 (80%), 254 (M+; 100%). IR (thin film) 760 (m), 860 (w), 1340 (m), 1440 (s), 1470 (m), 1555 (s) cm^{-1}.

Preparation of 4-(1-piperazinyl)-2,6-di-1-pyrrolidinyl-[2,5-$^{13}C_2$]-pyrimidine (14).

A solution of 2,6-di-pyrrolidinyl-4-chloro-[2,5-$^{13}C_2$]-pyrimidine (1.27 g; 4.99 x 10^{-3} moles) and piperazine (2.3 g; 0.027 moles) in pyridine (13 ml) was gently refluxed under a nitrogen atmosphere for 48 hours. The reaction was concentrated *in vacuo* and partitioned between dichloromethane (50 ml) and saturated sodium bicarbonate solution (20 ml). The layers were separated and the aqueous phase extracted with dichloromethane (3 x 50 ml). The organic phases were pooled, dried (sodium sulphate) and the solvents removed *in vacuo* to yield a yellow solid. The material was flash chromatographed (40 x 150 mm column; solvent: dichloromethane/methanol/triethylamine, 19.8:0.2:1; 30 ml fractions) to give 4-(1-piperazinyl)-2,6-di-1-pyrrolidinyl-[2,5-$^{13}C_2$]-pyrimidine (1.245 g) as a white solid. Mp 175-180°C (lit[1]., 177-178°C). Found: C, 62.06; H, 8.69; N, 26.93. $^{13}C_2$ C_{14} H_{26} N_6· 0.5 H_2O requires: C, 61.95; H, 8.68; N, 26.81. ^{1}H-NMR ($CDCl_3$) δ 1.90 (8H; m; $-CH_2-$), 2.93 (4H; dd; piperazinyl-$(CH_2)_2$-NH), 3.5 (12H; m; $-CH_2-N-$), 4.84 (1H; d; J_{C-H}=162 Hz; Ar-H). ^{13}CNMR ($CDCl_3$) δ 20.6, 20.61 (ss; $-CH_2-$), 40.6, 40.61, 40.62 (sss; $-CH_2-N$), 70.22 (d; J_{C-C}= 8.25 Hz; 2-^{13}C), 160.1 (d; J_{C-C}= 8.25 Hz; 5-^{13}C). MS m/z 122 (18%), 235 (40%), 248 (100%), 262 (8%), 276 (4%), 304 (M^+; 35%). IR (nujol) 775 (m), 960 (w), 1015 (w), 1240 (m), 1540 (s), cm^{-1}.

Preparation of R-(+)-6-hydroxy-2,5,7,8-tetramethylchroman-[2-^{13}C]-carboxylic acid ([2-^{13}C]-Trolox®; 7).

A solution of ^{13}C-potassium cyanide (0.68 g; 0.01 moles) in water (1.5 ml) was added dropwise over 10 minutes to a solution of 4-(2,5-diacetoxy-3,4,6-trimethylphenyl)butan-2-one (2.24 g; 7.31 x 10^{-3} moles) in dimethylformamide (1 ml) at 0°C. After stirring for

10 minutes, a solution of 6 M aqueous sulphuric acid (1.2 ml) was added dropwise over 15 minutes. The mixture was left for 30 minutes and further acidified with 6 M aqueous sulphuric acid (0.36 ml). The reaction was poured into ice water (200 ml), stirred and extracted with 20% ethyl acetate/ether (4 x 100 ml), washed with saturated aqueous sodium chloride solution (3 x 20 ml), dried (sodium sulphate) and the solvent removed *in vacuo* to give a yellow oil (2.7 g). This was immediately treated with concentrated hydrochloric acid (30 ml) at 90°C for 4 hours, during which a heavy precipitate formed. The mixture was cooled, extracted with ether (4 x 30 ml) and the pooled organics washed with saturated sodium chloride solution (10 ml). The ether layer was extracted with aqueous saturated sodium bicarbonate solution (3 x 30 ml) and the aqueous layer washed with ether (2 x 20 ml). The aqueous layer was acidified with concentrated hydrochloric acid and re-extracted with ether (4 x 30 ml). The pooled organics were then washed with saturated sodium chloride (2 x 20 ml), dried (sodium sulphate) and the solvents removed *in vacuo* to give a white solid (1.47 g). The racemic Trolox• (1.47 g; 5.86 x 10^{-3} moles) was dissolved in ethanol (2.4 ml) and R-(+)-α-methylbenzylamine (0.9 ml) added. Ether (23.5 ml) was slowly added with rapid stirring to give white crystals. The material was collected and washed with ether (3 x 6 ml) to give product (1.19 g). The product was recrystallised twice more by dissolving in ethanol (2.4 ml) and adding ether (23.5 ml), collecting the precipitate and washing with ether (3 x 6 ml). This procedure gave R-(+)-Trolox• as the R-(+)-α-methylbenzylamine salt (0.65 g). The solid was partitioned between aqueous hydrochloric acid (1 M; 20 ml) and ethyl acetate (20 ml) and the aqueous layer further extracted with ethyl acetate (2 x 20 ml). The combined organics were washed with 1 M aqueous hydrochloric acid (3 ml), water (3 ml) and saturated aqueous sodium chloride (3 ml), dried (sodium sulphate) and the solvents removed to give a white solid. This was recrystallised by dissolving in ether (9 ml) and adding hexane (90 ml) with rapid stirring. The mixture was then cooled to -20°C and the crystals collected. This procedure was repeated twice more to give R-(+)-6-hydroxy-2,5,7,8-tetramethylchroman-[2-^{13}C]-carboxylic acid (0.3 g; > 99% enantiomeric excess). Mp 159-160°C. α_D = +64.5° (c = 0.13, ethanol). Found: C, 66.79; H, 7.49. $^{13}C\ C_{13}\ H_{18}\ O_4$. 0.12 H_2O requires: C, 66.74; H, 7.25. ^{1}H-NMR ($CDCl_3/d_6$-DMSO, 4:1) δ 1.57 (3H, d, $J_{^{13}C-H}$ = 3.73 Hz, 2-CH_3), 1.81 (1H, m, 3-CH-), 2.05 (3H, s, -CH_3), 2.12, 2.13 (6H, ss, -CH_3), 2.40 (1H, m, 3-CH-), 2.58 (2H, m, 4-CH_2). ^{13}C-NMR

($CDCl_3/d_6$-DMSO, 4:1) δ 16.0 (s,-CH_3), 16.1 (s, -CH_2-), 17.0 (s, -CH_2-C-O-), 20.0 (s, Ar-CH_3), 24.5 (s, Ar-CH_3), 30.0 (s, Ar-CH_3), 75.8 (d, J_{C-C} = 59.8 Hz, 2-C), 116.0 (s, Ar-C), 119.0 (s, Ar-C), 121.0 (s, Ar-C), 122.0 (s, Ar-C), 144.5 (s, Ar-C-O), 145.0 (s, Ar-C-O), 175.0 (d, J_{C-C} = 59.8 Hz; -^{13}COOH). MS m/z 121 (25%), 164 (100%), 175 (15%), 189 (20%), 205 (65%), 218 (8%), 233 (5%), 251 (M^+; 100%). IR (nujol) 1085 (w), 1120 (w), 1140 (w), 1250 (m), 1665 (s), 2800 (m) cm^{-1}.

Preparation of (R)-(-)-2[[4-(2,6-di-1-pyrrolidinyl-4-[2,5-$^{13}C_2$]-pyrimidinyl)-1-piperazinyl]-[^{13}C]-carbonyl)-3,4-dihydro-2,5,7,8-tetramethyl-2H-1-benzopyran-6-ol (15).

To a stirred solution of R-(+)-[^{13}C]-Trolox® (0.2 g; 7.96 x 10^{-4} moles) in tetrahydrofuran (3 ml) under a nitrogen atmosphere was added 1,1'-carbonyldiimidazole (0.132 g; 8.12 x 10^{-4} moles). The mixture was stirred at room temperature for 1 hour and then a solution of 4-(1-piperazinyl)-2,6-di-1-pyrrolidinyl-[2,5-$^{13}C_2$]-pyrimidine (0.22 g; 7.95 x 10^{-4} moles) in dry chloroform (3 ml) was slowly added. After 16 hours, the solvents were removed *in vacuo*, the gum dissolved in dichloromethane (50 ml), washed with a solution of saturated sodium bicarbonate (3 x 10 ml), dried over sodium sulphate, filtered, and the solvent removed *in vacuo* to give an oil (0.65 g). Flash chromatography (20 x 150 mm column; solvent system: ethyl acetate/hexane, 7:3; 10 ml fractions) gave (R)-(-)-2-[[4-(2,6-di-1-pyrrolidinyl-4-[2,5-$^{13}C_2$]-pyrimidinyl)-1-piperazinyl]-[^{13}C]-carbonyl)-3,4-dihydro-2,5,7,8-tetramethyl-2H-1-benzopyran-6-ol (0.16 g) as a colourless oil in fractions 6-9. ^{1}H-NMR ($CDCl_3$) δ 1.5 (3H, s, CH_3-C-), 1.7 (1H, m, 3-CH_2-), 1.9 (8H, m, pyrrolidine-CH_2), 2.1, 2.15, 2.2 (9H, s, Ar-CH_3), 2.6 (2H, m, 4-CH_2), 2.8 (1H, m, 3-CH_2), 3.5 (14H, m, piperidine and pyrrolidine-CH_2-N), 4.1 (2H, br s, -CH_2-N), 4.8 (1H, d, J_{C-H}=161.0 Hz, Ar-5-CH-). ^{13}C-NMR ($CDCl_3$) δ 11.0 (s, -CH_3), 12.0 (s, -CH_2-), 12.2 (s, -CH_2-C-O), 21.0 (s, Ar-CH_3), 25.5 (s, Ar-CH_3), 26.0, 26.1 (s,s, pyrrolidine -CH_2-), 31.5 (s, Ar-CH_3), 45.5, 45.6 (s,s, pyrrolidine and piperidine -CH_2-N-), 72.5 (dd, J_{C-H} = 161.9 Hz; J_{C-C} = 7.95 Hz; Ar-5-^{13}CH), 78.5 (s, quaternary-C-), 118.0, 119.0, 121.5, 122.0 (s, Trolox Ar-C), 144.5, 145.5 (s, Ar-C-O), 160.0 (d, J_{C-C} = 7.95 Hz, pyrimidine 2-^{13}C), 162.0, 163.5 (s, pyrimidine Ar-C), 172.0 (s, -^{13}C(O)-N). MS m/z 122 (10%, 248 (25%), 374 (100%), 537 (M+; 10%). IR (nujol) 1680 (s), 1585 (m), 3400 (m) cm^{-1}.

Preparation of (R)-(-)-2-[[4-(2,6-di-1-pyrrolidinyl-4-[2,5-$^{13}C_2$]-pyrimidinyl)-1-piperazinyl]-[^{13}C]-methyl)-3,4-dihydro-2,5,7,8-tetramethyl-2H-1-benzopyran-6-ol hydrochloride (16).

(R)-(-)-2-[[4-(2,6-di-1-pyrrolidinyl-4-[2,5-$^{13}C_2$]-pyrimidinyl)-1-piperazinyl]-[^{13}C]-carbonyl)-3,4-dihydro-2,5,7,8-tetramethyl-2H-1-benzopyran-6-ol (160 mg; 1.13 x 10^{-4} moles) was treated with a solution of borane-methyl sulphide complex (2.0 M, 4.0 ml) in tetrahydrofuran for 16 hours. Aqueous hydrochloric acid (2.0 M) was added dropwise until effervescence ceased and then the solution was neutralised (pH 7) to litmus paper by dropwise addition of a saturated solution of sodium bicarbonate. The reaction mixture was reduced in volume to remove tetrahydrofuran and the aqueous layer extracted with ethyl acetate (4 x 20 ml). The pooled organics were dried over sodium sulphate, filtered and the solvent removed *in vacuo* to give a colourless gum (341 mg). Flash chromatography (30 x 150 mm column; solvent system: 35% ethyl acetate/hexane; 20 ml fractions) gave pure product in fractions 8-16 as a white solid. The product was dissolved in methanol (5 ml), filtered, and acidified to pH <4.0 with aqueous hydrochloric acid (2.0 M). The solution was concentrated to an oil and then azeotroped with acetone (2 x 3 ml) until a solid was obtained. The compound was precipitated by redissolving in a small amount of methanol and reducing to a gum; acetone (5 ml) was added with rapid stirring and the white flocculent precipitate stirred overnight. The above precipitation was repeated twice more to yield (R)-(-)-2-[[4-(2,6-di-1-pyrrolidinyl-4-[2,5-$^{13}C_2$]-pyrimidinyl)-1-piperazinyl]-[^{13}C]-methyl)-3,4-dihydro-2,5,7,8-tetramethyl-2H-1-benzopyran-6-ol hydrochloride (110 mg; > 99% enantiomeric excess). Mp 251°C. Found: C, 59.25; H, 7.40; N, 13.76. $^{13}C_3$ C_{27} H_{44} N_6 O_2. 2 HCl. 0.92 H_2O requires: C, 59.24; H, 7.80; N, 13.71. ^{1}H-NMR (d_6-DMSO) δ 1.2 (3H, s, CH_3-C-), 1.7 (1H, m, 3-CH_2-), 1.85 (8H, m, pyrrolidine-CH_2), 1.95, 2.01, 2.03 (9H, s, Ar-CH_3), 2.5 (2H, m, 4-CH_2), 2.6 (1H, m, 3-CH_2), 3.4 (16H, m, piperidine and pyrrolidine-CH_2-N), 4.8 (1H, d, J_{C-H}= 161.0 Hz, Ar-5-CH-). ^{13}C-NMR (D_2O) δ 10.0 (s, -CH_3), 11.0 (s, -CH_3), 12.2 (s, -CH_3), 18.0 (s, -CH_2-), 21.0 (s, 2-CH_3), 24.0 (s, pyrrolidine -CH_2-), 28.5 (s, 3-CH_2), 40.0, (s, piperidine -CH_2-N-), 46.7 (s, pyrrolidine -CH_2-), 52.3 (s, piperidine -CH_2-N), 62.3 (s, J_{C-H} = 132 Hz, trolox$^{\bullet}$-$^{13}CH_2$-N), 71.5 (s, J_{C-C} = 8.85 Hz, J_{C-H} = 162 Hz, Ar-5-^{13}CH-), 73.5 (s, quat-C-), 118.0, 121.5, 122.0, 123.5 (s, Trolox Ar-C), 142.5, 144.5 (s, Ar-C), 149.2, 152.0, (s, pyrimidine Ar-C), 160 (d, J_{C-C} = 8.85 Hz, Ar-2-^{13}CH). MS m/z 248 (100%), 318 (93%), 360 (12%), 523 (M^+; 20%). IR (nujol) 725(s), 1250 (m), 1310 (m), 1545 (s), 1600 (s), 1615 (s), 3200 (m) cm^{-1}.

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