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Research Article

Synthesis of [^{14}C]-labelled repinotan hydrochloride and its major metabolite M-6

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Summary

For studies of pharmacokinetics and drug metabolism of the new 5-HT$_{1A}$ agonist repinotan, the ^{14}C-labelled version was synthesized. Starting from [U-^{14}C]phenol, a 10-step synthesis led to 457 mg (1.58 GBq) of [U-^{14}C]repinotan hydrochloride, labelled uniformly in the aromatic ring of the chromane moiety. For a study in man, a mono-carbon-14 labelled substance was required. Therefore a 7-step synthesis was performed starting from [carbonyl-^{14}C]2-hydroxy-acetophenone. The yield was 106 mg (0.396 GBq) of [4-chromane-^{14}C]repinotan hydrochloride. The carbon-14 labelled major metabolite, hydroxylated in the 6-position of the chromane moiety, was synthesised as reference compound. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: chromane; uniform labelling; mono ^{14}carbon-labelling; optical resolution; semi-preparative chromatography

Introduction

Repinotan is a new, enantiomerically pure aminomethyl chromane derivative with a saccharinylbutyl substituent. It demonstates neuroprotective properties, especially for the treatment and prevention of ischemic diseases, such as stroke.[1]

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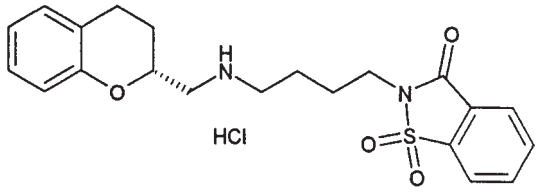


(A)

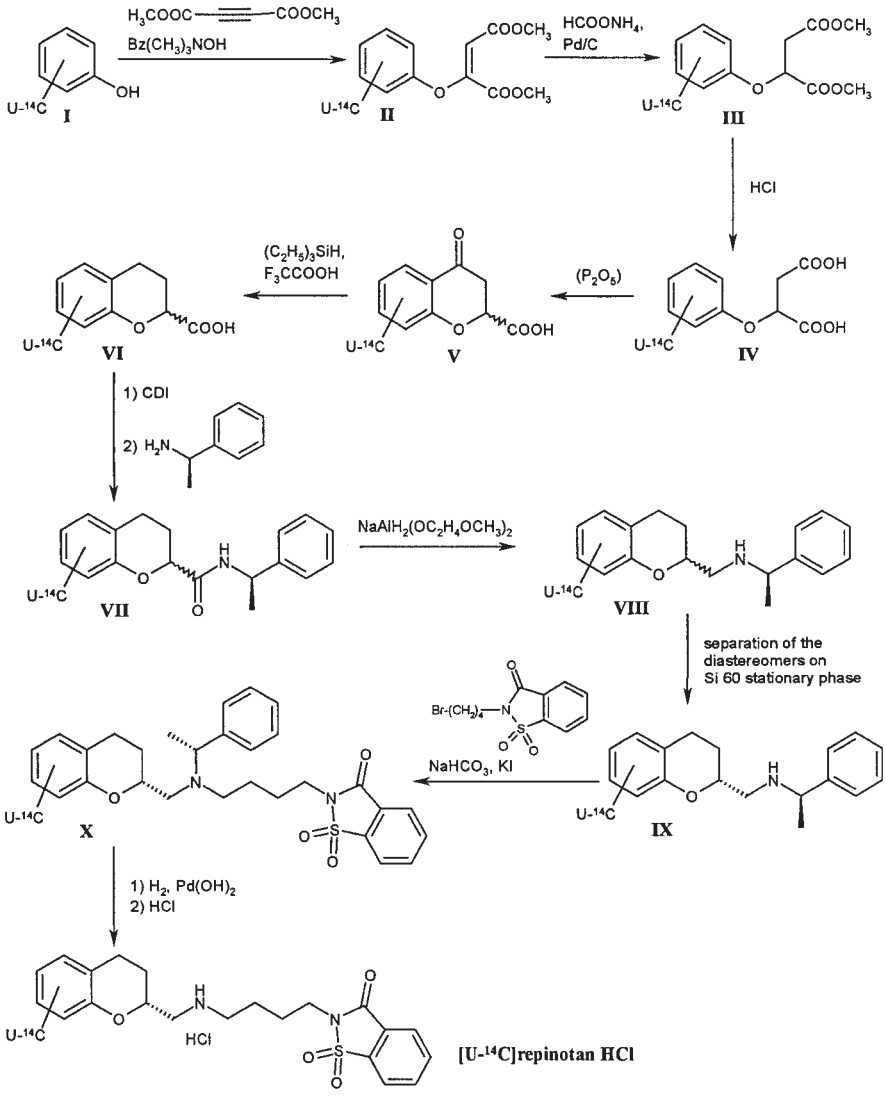
For studies of pharmacokinetics and drug metabolism of repinotan, the version with a metabolically stable ^{14}carbon-label in the chromane moiety was required. A first radiosynthesis was planned giving uniform labelling in the aromatic ring of the chromanyl part of the molecule.

Repinotan is administered in a very low dose. In order to facilitate mass spectral analysis of metabolites in the course of an ADME study in healthy volunteers, mono-labelling with ^{14}carbon was needed.[2] So a second radiosynthesis of repinotan hydrochloride (A) was performed, locating the label in the 4-position of the chromane moiety.

For evaluation and interpretation of the study the ^{14}carbon labelled metabolite M-6 of repinotan, hydroxylated in the 6-position of the chromane moiety, was synthesised as the hydrochloride. The synthesis was started with an appropriate intermediate of the second radiosynthesis.

Results and discussion

Uniformly labelled [^{14}C]-phenol is a readily available starting compound. In the first labelling synthesis it was used for the known preparation of chromane rings via Michael adduct formation with dimethyl acetylene-dicarboxylate, hydrogenation of the dimethyl phenoxyfumarate/maleate, hydrolysis of the ester and cyclization to the chromanone system. The Michael addition is described in the literature using non-labelled compounds under different conditions.[3] In some cases the resulting 1-phenoxy-ethylene-1,2-dicarboxylates were saponified and cyclized under dehydrating conditions.[4] Cox and coworkers[4] pointed out that only the fumaric acid derivative underwent cyclization. To avoid this selectivity problem, the double bond was hydrogenated to obtain the corresponding dimethyl phenoxy-succinate (III) (Reaction scheme 1) which was hydrolysed and subsequently cyclized. The ^{14}carbon-labelled 4-oxo-4*H*-chromane-2-carboxylic acid (V)

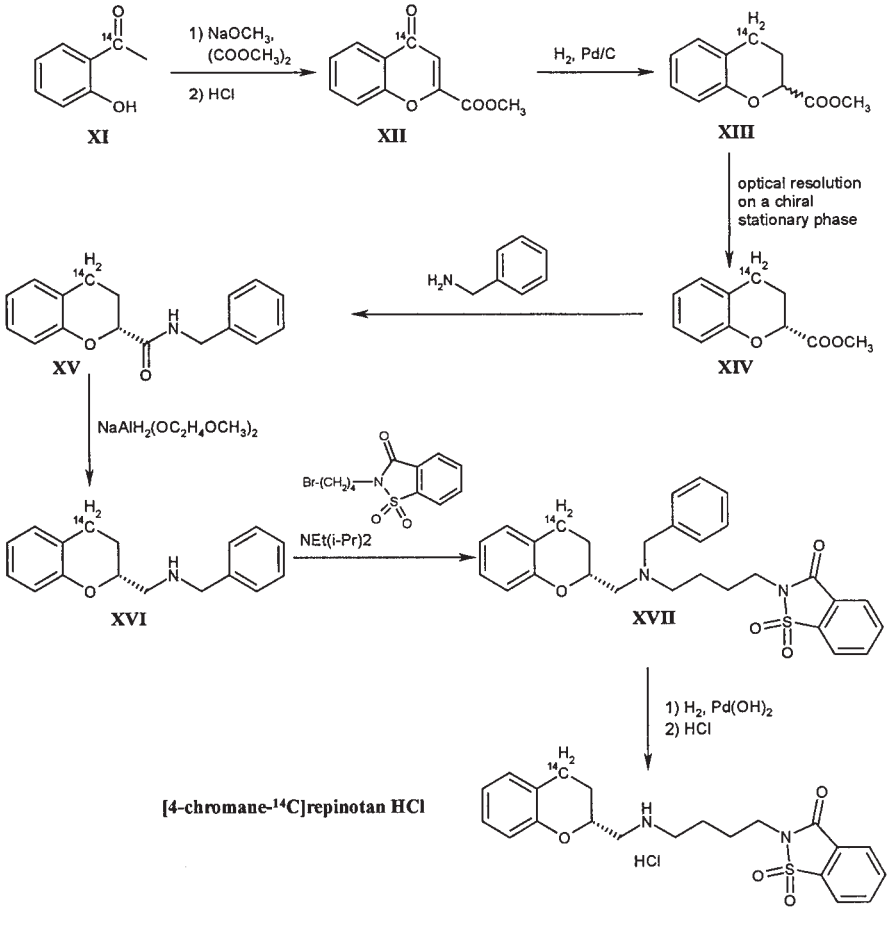


Reaction scheme 1.

was reduced with triethylsilane/trifluoroacetic acid as described for other systems by Swenton and Raynolds.[5] For optical resolution the amide VII was formed with the optically active (1R)-1-phenylethylamine, the amide was reduced to the corresponding amine (VIII) and the diastereomers were separated by chromatography on silica gel. In the penultimate step alkylation with 4-bromobutylsaccharine led to the tertiary amine X. The reaction was performed solvent free, using

sodium bicarbonate as HBr trapping agent. Critical in this step was the liability of the saccharine moiety towards hydrolysis. The crude substance was purified chromatographically on silica gel. The final product was obtained by cleavage of the phenethyl–N bond and acidic work up of the labelled secondary amine; purification was achieved via recrystallization from ethanol.

The second labelling route was started from commercially available [carbonyl-^{14}C]-2-hydroxy-acetophenone (XI) (Reaction scheme 2). Condensation with dimethyl oxalate and subsequent ring closure gave compound XII. The synthesis of the non-labelled compounds have been reported.[6] So also has the hydrogenation to the chromane-2-carboxylate.[7] In this second synthesis optical resolution was achieved at the

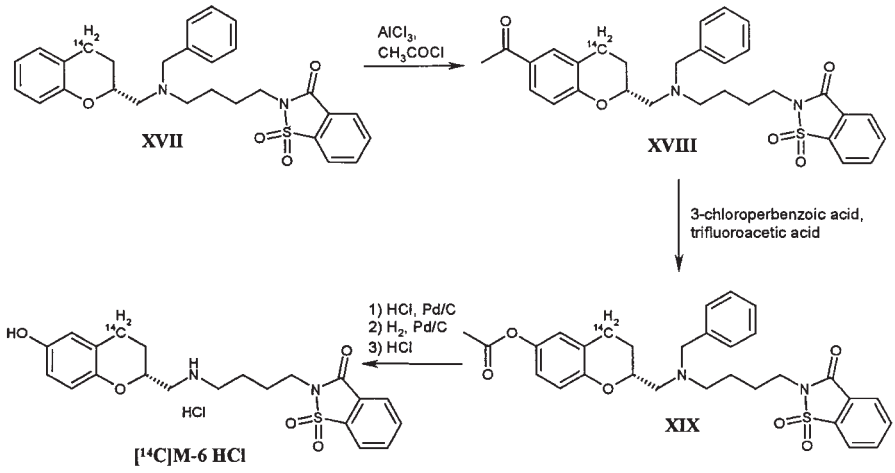


Reaction scheme 2.

stage of the chromane-2-carboxylate. This procedure had the advantage that the undesired enantiomer can be racemized and again used in the enantiomeric separation. The separation of the enantiomers by semi-preparative HPLC on a chiral stationary phase was changed from reversed phase to normal phase conditions during the synthesis, because of steam-volatility of the methyl ester. The amine XV was obtained in excellent yield by aminolysis of the methyl ester without any loss of enantiomeric excess. Lithium iodide was used as catalyst. The corresponding amine was obtained by reduction with a complex aluminium hydride as in the first synthesis. The same amine in racemic and non-labelled form was prepared by Meshaw and co-workers by alkylation of chroman-2-ylmethylamine with benzylchloride,[8] but the yield was poor. Van Lommen and co-workers used similar route[9] alkylating benzylamine with 3,4-dihydro-2*H*-chromen-2-ylmethyl tosylate. The optically active, but non-labelled *N*-benzyl-*N*-(3,4-dihydro-2*H*-chromen-2-ylmethyl)amine was prepared by the same group by reductive amination of the (2R)-3,4-dihydro-2*H*-chromene-2-carbaldehyde.[9] Both synthetic routes were not attractive because of poor yields. Some experiments were performed for optimisation of the alkylation of chroman-2-ylmethylamine with bromobutylsaccharin to obtain the final product more easily. However the preparation of chroman-2-ylmethylamine gave a lower yield than the synthesis of the benzyl protected amine, and furthermore double alkylation and side reactions of the saccharin moiety with the primary amine were observed.

In the present synthesis the alkylation of XVI with bromobutylsaccharin proceeded at a lower temperature and more rapidly than in the case of the phenethyl substitution, probably due to steric reasons. The final product was formed by hydrogenolytical debenzylation and subsequent addition of hydrochloric acid. The presence of several by-products required a two-fold chromatographic purification, indicating that purification of the precursor XVII is advantageous prior to the hydrogenolysis.

To aid in the interpretation of the results of the study with [4-chromane-^{14}C]repinotan hydrochloride in humans the ^{14}carbon-labelled 6-hydroxy metabolite [^{14}C]M-6 was required as reference compound. The synthesis started from the labelled intermediate XVII (Reaction scheme 3). After chromatographic purification XVII was subjected to a Friedel–Crafts acylation with acetyl chloride. The acetylation took place very selectively in position 6 of the chromane ring. Subsequent



Reaction scheme 3.

Baeyer–Villiger oxidation with 3-chloroperbenzoic acid/trifluoroacetic gave intermediate XIX, the *O*-acetyl and *N*-benzyl protected metabolite. The deacetylation and the debenzylation steps were carried out using HCl, Pd/C in the first and H_2, Pd/C in the second step. The desired product was obtained after purification by semi-preparative HPLC in good quality.

Experimental

General methods

The ^{1}H NMR spectra were recorded on a Bruker AM 300 nuclear magnetic resonance spectrometer. Radiochemical GC purities were determined with the GC system HP 5890 equipped with radioactivity detector FHT 7000. HPLC analyses of the final products were performed on a HP 1050 Series II instrument. For non-chiral analysis Nucleosil® 100 C-18 was used as stationary phase and acetonitrile/phosphate buffer (pH 2.5) as mobile phase. For chiral analysis Chiralpak AD® was used as stationary phase and *n*-heptane/ethanol (0.2% NEt_3) as mobile phase. Radioactivity and UV signals (radioactivity detector Ramona® 92) were recorded by the work station of the chromatograph. The radioactivity signal was digitised by the A/D-converter HP 35900 and evaluated by the work station. Radiochemical counting was performed on a Liquid Scintillation Analyzer TRI-CARB® 2500 TR using Ultima Gold™ cocktail. Radio thin layer

chromatography was performed using silica gel plates Si 60 F_{254} and a Raytest Rita®-3200 analyser. Mass spectrometric analysis was performed on a PE/Sciex API III mass spectrometer with MacIntosh Quadra® 900. Semipreparative chromatography was performed with Latek-P 402 in the first synthesis and WellChrom Maxi-Star® K 1000 in the second synthesis and in the labelling synthesis of the metabolite, Knauer Variable Wavelength monitor and Merck/Hitachi D-2500A Chromato-Integrator.

First labelling procedure

Dimethyl (2E,Z)-2-[U-^{14}C]phenoxy-2-butenedioate, (II). [U-^{14}C]-Phenol (I) was obtained from Forschungszentrum Rossendorf e.V. with a specific radioactivity of 2.49 GBq/mmol and a total quantity of 6.07 mmol. Both the chemical and radiochemical purities were 98%, as established by GC and radio-GC, respectively.

Non-labelled phenol (3.92 mmol) was added to the labelled compound. The resulting specific activity was calculated to be 1.51 GBq/mmol.

A solution of the labelled phenol, 2.29 g (16 mmol) of dimethyl acetylenedicarboxylate and 6 drops of Triton B® (40% solution in methanol) in 8 ml of dioxane was refluxed for 40 minutes. The brown solution was cooled in an ice bath, diluted with 35 ml of water, acidified with 25% hydrochloric acid and further diluted with 20 ml of water. The product was extracted with diethyl ether (4×25 ml). The combined organic layers were washed with water (5×10 ml), dried over sodium sulphate and evaporated to dryness to yield 3.5 g of an oily residue with a chemical purity (GC) of $\geq 91\%$ and a radiochemical purity (radio-GC) of $\geq 99\%$.

For purification the crude II was dissolved in 23 ml of acetonitrile and purified in 7 runs by low pressure chromatography on Lobar® column, LiChroprep® RP-18, size B with the eluent acetonitrile/water 70:30 and a flow of 5 ml/min. The product containing fractions were evaporated under reduced pressure. The residue was extracted with diethyl ether. The organic layer was dried over sodium sulphate and evaporated to dryness.

Yield: 2.71 g of II, chemical purity (GC): $\geq 99\%$.

Dimethyl 2-[U-^{14}C]phenoxysuccinate, (III). To 400 mg of 10% palladium on charcoal and 5 g of ammonium formate in 8 ml of methanol,

a solution of II (2.71 g) in 4 ml of methanol was added dropwise and the reaction mixture was stirred for 1 h at room temperature. For work up the mixture was filtered over kieselguhr and the filter cake was washed several times with methanol. The filtrate was evaporated under reduced pressure. To the concentrated solution 40 ml of water was added and the mixture was extracted several times with 15 ml portions of diethyl ether. The ether layers were washed with water, dried over sodium sulphate and evaporated to dryness under reduced pressure.

Yield: 2.57 g (8.85 mmol with respect to the purity) of compound III, chemical purity (GC): $\geq 82\%$, radiochemical purity (radio-GC): $\geq 83\%$. This amount corresponds to 88% of the theoretical related to the starting phenol.

The crude product was used without further purification in the next step.

2-*[U-^{14}C]Phenoxysuccinic acid, (IV)*. The ester III (2.57 g) was dissolved in 30 ml of 25% hydrochloric acid and stirred for 3 h at 130°C bath temperature. After cooling to room temperature 50 ml of water was added and the product was extracted with 20 ml of diethyl ether. The aqueous layer was saturated with 1 g of sodium chloride. The organic layer was removed and the extraction was repeated twice, each with 10 ml of diethyl ether. For purification 5 ml of water was added to the combined organic layers and basified with sodium carbonate. The organic layer was discarded and the aqueous layer was washed with diethyl ether. For liberation of the desired acid IV the aqueous solution was acidified with concentrated hydrochloric acid and the product was extracted with diethyl ether (4×15 ml). The organic layers were dried over sodium sulphate and evaporated to dryness under reduced pressure.

Yield: 2.84 g of compound IV, chemical purity (GC): 96%, radiochemical purity (radio-GC): 98%.

4-*Oxo-[U-^{14}C]chromane-2-carboxylic acid, (V)*. Polyphosphoric acid (30 g) was mixed with 2 ml of xylene (isomeric mixture) and heated to 70°C. Compound IV (2.84 g) was dissolved in 4 ml of hot xylene and added to the mixture. The reaction mixture was stirred intensively for 5 h at 70°C. Then water was added until two layers were obtained. After cooling to room temperature the product was extracted with diethyl ether (4×20 ml). Purification and isolation were performed by acidic–alkaline treatment as described above.

Yield: 1.49 g (6.8 mmol with respect to the purity) of compound V, chemical purity (GC): $\geq$ 88%, radiochemical purity (radio-GC): $\geq$ 87%. This corresponds to 77% of the theory over 2 steps.

The crude product was used without further purification in the next step.

(2R,S)-[U-^{14}C]Chromane-2-carboxylic acid, (VI). Compound V (1.49 g) was dissolved in 6 ml of trifluoroacetic acid. Triethylsilane (2 ml) was added and the solution was stirred overnight at room temperature. Addition of 2 ml of triethylsilane was repeated twice every 25 h. For work up trifluoroacetic acid and excessive triethylsilane were removed by evaporation at aspirator pressure. To the residue 10 ml of water and 20 ml of diethyl ether were added. The aqueous layers were extracted with diethyl ether and the desired compound VI was purified and isolated by acidic-alkaline treatment as described above.

Yield: 1.61 g of compound VI, chemical purity (GC): 92%, radiochemical purity (radio-GC): 86%.

The crude product was used without further purification in the next step.

N-[(1R)-1-Phenylethyl]-(2R,S)-[U-^{14}C]chromane-2-carboxamide, (VII). Compound VI (1.61 g) was dissolved in 6 ml of THF and *N*,*N*′-carbonyldiimidazole (2.27 g) was added in portions. After stirring for one hour at reflux the mixture was cooled in an ice bath and 2 ml of (1R)-1-phenylethylamine was added. After stirring overnight at room temperature 20 ml of water and 10 ml of diethyl ether were added. The aqueous layer was extracted with diethyl ether and the organic layer was dried over sodium sulphate and evaporated to dryness under reduced pressure.

Yield: 2.8 g of compound VII, chemical purity (GC): 77%, radiochemical purity (radio-GC): 98%.

The crude product was used without further purification in the next step.

(2R,S)-2-{N-[(1R)-1-Phenylethyl]}aminomethyl-[U-^{14}C]chromane, (VIII). The labelled VII (2.8 g) was dissolved in 6 ml of toluene and 10 ml of sodium bis(methoxyethoxy)aluminumhydride (70%in toluene, Red-Al®) were added under ice bath cooling. The reaction mixture was stirred for one hour at reflux. After cooling to room temperature the reaction mixture was added carefully to 20 ml of a 16% sodium hydroxide solution. The mixture was

diluted with water and the desired amine was extracted with diethyl ether. The organic layer was washed with water, dried over sodium sulphate and evaporated to dryness under reduced pressure.

Yield: 2.88 g of compound VIII, chemical purity (GC): 51% radiochemical purity (radio-GC): 97%.

The crude product was used without further purification in the optical resolution.

(2R)-2-{N-[(1R)-1-Phenylethyl]}aminomethyl-[U-^{14}C]chromane, (IX). The mixture of the diastereomers VIII (2.88 g) was dissolved in 18 ml of cyclohexane/isopropanol 14:1. The chromatographic separation of the diastereomers was performed in 11 runs on a column Lobar® Si 60 size B using *n*-heptane/isopropanol 40:1 as eluent, a flow of 6 ml/min and UV detection at 230 nm. The desired R,R-diastereomer was eluted first. The product containing fractions were combined and evaporated to dryness under reduced pressure.

Yield: 0.745 g (2.79 mmol) of compound IX. The diastereomeric excess was determined by GC (Chiraldex®) as 98.4%.

2-(4-{[(2R)-3,4-dihydro-2H-[U-^{14}C]chromen-2-ylmethyl][(1R)-1-phenylethyl]amino}butyl)-1,1-dioxo-1,2-benzisothiazol-3(2H)-one, (X). Compound IX (0.745 g, 2.79 mmol) was mixed with 1.77 g (5.59 mmol) of 4-bromobutylsaccharin, 0.704 g (8.39 mmol) of sodium bicarbonate and 0.24 g (1.42 mmol) of powdered potassium iodide. The mixture was stirred for 6.5 h at a bath temperature of 145°C. Then the mixture was cooled to 100°C and 10 ml of pinacolone were added. After cooling to room temperature the salts were removed by filtration over quartz wool. The filter was washed colourless with pinacolone. The filtrate was evaporated under reduced pressure and 3 ml of ethyl acetate and 15 ml of 25% hydrochloric acid were added to the brownish residue. After extraction with diethyl ether (4×15 ml) the organic layers were discarded and the acidic layer was neutralised with 45% sodium hydroxide solution to pH 6.5. The product was extracted with ethyl acetate (4×15 ml). The organic layer was dried over sodium sulphate and evaporated to dryness under reduced pressure. Crude X was obtained as a residue of 1.43 g.

For purification the crude product was dissolved in 6 ml of THF/isopopanol 1:1. Chromatographic purification was performed in 4 runs on a column Lobar® Si 60 size B using *n*-heptane/isopropanol 9:1 as eluent, a flow of 6 ml/min. and UV detection at 230 mm. The

product-containing fractions were combined and evaporated to dryness under reduced pressure.

Yield: 1.09 g (2.16 mmol) of compound X. This amount corresponds to a yield of 77%. The radiochemical purity was determined by radio TLC as 97%.

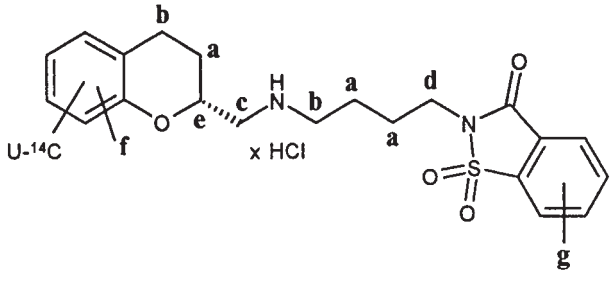
(-)-R-2-{4-[([U-^{14}C]Chroman-2-ylmethyl)amino]butyl}-1,1-doxo-1,2-benzisothiazole-3(2H)-one monohydrochloride, [U-^{14}C]repinotan hydrochloride. Compound X (1.09 g) was dissolved in 16 ml of methanol and 8 ml of acetic acid and 1.1 g of Pearlman's catalyst ($Pd(OH)_2$) was added. Hydrogen was bubbled through the solution for 4 h under intensive stirring. Then the mixture was filtered over kieselguhr and the filtrate was evaporated under reduced pressure. The residue was dissolved in 15 ml of concentrated hydrochloric acid and the product was extracted with dichloromethane. The organic layer was evaporated to dryness leading to 0.843 g of crude product with a purity of 90%, it was recrystallised from 3 ml of ethanol. The product was filtered off, washed with ethanol and dried at 70°C under vacuum.

Yield: 0.457 g of [U-^{14}C]repinotan hydrochloride (1.05 mmol), radiochemical purity: $\geq 99\%$ by radio-HPLC and $> 98\%$ by radio-TLC (ROI), chemical purity (HPLC): $\geq 98\%$. The optical purity was determined as 99% e.e.

Determination of the specific radioactivity:

For determining the specific radioactivity a weighed amount of the labelled compound was dissolved in acetonitrile/water and the total radioactivity was determined by LS counting. By this method the specific activity was determined as 1.51 GBq/mmol (3.46 MBq/mg).

The total radioactivity of 1.581 GBq corresponds to a yield of 10% of the theory, based on the starting [U-^{14}C]phenol.



NMR (methanol-d_4) δ (ppm); 1.93 (m, 6H, **a**), 2.86 (m, 4H, **b**), 3.15 (m, 2H, **c**), 3.89 (t, 2H, **d**), 4.30 (m, 1H, **e**), 6.87 + 7.08 (multiplets, 4H, **f**), 8.01 (m, 4H, **g**).

Second labelling procedure

Methyl 4-oxo-4H-[4-^{14}C]chromene-2-carboxylate, (XII). The starting [carbonyl-^{14}C]2-hydroxyacetophenone (XI) was obtained from Isotopchim, France, (batch no. 97113) with a total radioactivity of 11.1 GBq and a specific radioactivity of 1.903 GBq/mmol, corresponding to 5.8 mmol (0.789 g). The chemical and radiochemical purities were 97%, as established by HPLC and radio-HPLC, respectively.

The labelled 2-hydroxyacetophenone (5.8 mmol) was added to a solution of 0.85 g (7.2 mmol) dimethyl oxalate in 2.2 ml of methanol. This solution was added to solution of 1.177 g (21.8 mmol) sodium methoxide in 4 ml of methanol within 1.5 h at a temperature of 60°C. The thick yellow suspension was stirred for 3 h at reflux. After cooling to room temperature 4.8 ml of concentrated hydrochloric acid was added without external cooling. A new slightly coloured solid was obtained. After stirring for 30 min at room temperature 16 ml of water was added and stirring was continued for 45 min in an ice bath. The product was filtered off, washed intensively with water and dried in an evacuated desiccator over blue gel. A first product batch of 1.03 g was obtained.

A second precipitate was obtained from the mother liquor overnight which was filtered over the frit containing the first product batch. The combined solids were washed with water and dried at reduced pressure over blue gel, leading to 1.2 g of product. By GC analysis 83% of the desired methyl ester and 16.2% of the corresponding carboxylic acid was detected. Complete conversion to the desired ester was performed with diazomethane.

Yield: 1.0 g (4.9 mmol) of XII. The chemical purity was determined as 99% by GC. The amount corresponds to 84% of the theory related to the starting labelled 2-hydroxyacetophenone.

Methyl [4-^{14}C]chroman-2-carboxylate, (XIII). Compound XII (1.0 g) was dissolved in 75 ml of acetic acid. Palladium catalyst (2 g of 10% palladium on charcoal) and hydrogen were added. The mixture was filtered over kieselguhr and the filter was washed with methanol (4 × 15 ml). The filtrate was evaporated to dryness.

Yield: 0.73 g (3.8 mmol) of compound XIII. The chemical purity was determined as 97.4% by GC. The amount corresponds to 77.6% of the theory related to XII.

Methyl (-)-(2R)-[4-^{14}C]chroman-2-carboxylate, (XIV). The first optical resolution by semi-preparative HPLC on Chiralcel® OD (250×20 mm) with the eluent acetonitrile/water 50:50 (v/v) failed due to isolation problems. Unexpectedly the chromane ester was found to be very steam-volatile.

Interestingly the chiral stationary phase could also be used under normal phase conditions. The racemic ester was dissolved in *n*-heptane/ethanol 1:1 (v/v) and chromatographed in 10 runs on Chiralcel® OD, 250×20 mm with the eluent *n*-heptane/ethanol 35:65 (v/v), a flow of 8 ml/min. and UV detection at 230 nm. The desired R-enantiomer was eluted at approximately 21 min and the S-enantiomer at approximately 10 min. The fractions of both enantiomers were combined separately and evaporated under reduced pressure.

Yield: 0.403 g of the R-enantiomer XIV (2.1 mmol). In addition, 0.288 g of the S-enantiomer was isolated.

The S-enantiomer (0.288 g) was dissolved in methanol and racemized with 30% sodium methoxide solution at 50°C. The obtained racemate was resolved into the enantiomers (2 runs) as described above. This recycling was repeated once with the new S-enantiomer.

Total yield: 0.538 g (2.8 mmol) of compound XIV. The chemical purity was determined as 99% by HPLC. The optical purity was determined as 99% e.e. by HPLC. The amount corresponds to 73% of the amount of the racemate XIII.

(-)-(2R)-[4-^{14}C]Chromane-2-carboxylic acid benzylamide,(XV). Compound XIV (0.538 g), benzylamine (1.53 ml, 14 mmol) and lithium iodide (54 mg) were stirred for 2 h at 100°C. For work up the mixture was cooled to room temperature and 6 ml of 0.1 M hydrochloric acid added. After dilution of the suspension with 3 ml of 0.1 M hydrochloric acid the desired amide was filtered off, washed with 0.1 M hydrochloric acid (2×2 ml) and dried in an evacuated desiccator over blue gel.

Yield: 0.702 g (2.63 mmol) of XV, chemical purity (GC): 93%, radiochemical purity (radio-GC): 99%. The optical purity was determined as $>99\%$ e.e. by HPLC. The structure was confirmed by GC/MS with $m/z = 269$ [^{14}C-M]$^+$ and 267 [^{12}C-M]$^+$. The amount corresponds to 94% of theory.

(-)-(2R)-2-(Benzylaminomethyl)-[4-^{14}C]chromane, (XVI). Compound XV (0.702 g) was suspended in 6 ml of toluene. Red-Al® (1.44 ml, 4.8 mmol) was added and the reaction mixture was stirred for 30 min at room temperature, 30 min at 50°C and subsequently 1 h at 60°C. Then an additional 0.7 ml and subsequently 0.4 ml of Red-Al® were added and stirring was continued at 60°C. The conversion was still incomplete, but due to the formation of by-products the reaction was stopped by cooling to room temperature and careful addition of 7 ml of 2 M sodium hydroxide solution. The organic layer was washed with water (2 × 4 ml) and evaporated to dryness under reduced pressure.

Yield: 0.52 g of compound XVI (1.64 mmol), chemical purity (GC): 79%, radiochemical purity (radio-GC): 72%. This corresponds to 62% of the theory.

The crude product was used without further purification in the next step.

2-(4-{Benzyl[(-)-(2R)-3,4-dihydro-2H-[4-^{14}C]chromen-2-ylmethyl]amino} butyl)-1,1-dioxo-1,2-benzisothiazole-3(2H)-one, (XVII). A solution of compound XVI (0.52 g, 1.64 mmol), 4-bromobutylsaccharin (0.79 g, 2.47 mmol) and *N*-ethyl-diisopropylamine (0.538 ml, 3.09 mmol) in 2.5 ml of *N*-methylpyrrolidinone was stirred for 3 h at 120°C. After cooling to room temperature 3 ml of water was added. The clear supernatant was removed and the remaining oily residue was dissolved in 3 ml of dichloromethane. The solution was washed with water (2 × 3 ml) and evaporated to dryness under reduced pressure. For aceotropic drying 10 ml of ethanol was added and also removed by evaporation.

Yield: 1.5 of compound XVII. The chemical purity was determined by HPLC as 67%.

An aliquot of the crude substance was used without further purification in the next chemical step.

(-)-R-2-{4-[([4-^{14}C]Chroman-2-ylmethyl)amino]butyl}-1,1-doxo-1,2-benzisothiazole-3(2H)-one monohydrochloride, [4-chromane-^{14}C]repinotan hydrochloride. The major part of XVII (1 g) was dissolved in 14 ml of methanol and 7 ml of acetic acid. Pearlman's catalyst ($Pd(OH)_2$; 1 g) was added. Hydrogen was bubbled through the solution under intensive stirring. After 4 hours an additional 0.2 g of Pearlman's catalyst was added and hydrogenolysis was continued for 3 h. Then the mixture was filtered over kieselguhr and the filter was washed with methanol

(3×6 ml). The filtrate was evaporated under reduced pressure. The oily residue was dissolved in 20 ml of concentrated hydrochloric acid and extracted with dichloromethane (3×10 ml). The HCl layer was discarded. The organic layers were combined and evaporated to dryness giving 0.39 g of crude product.

A first purification was performed by low pressure liquid chromatography on Lobar® RP-18 size B with the eluent acetonitrile/0.1 M hydrochloric acid 40:60 (v/v).

Final purification

Final purification was performed by low pressure liquid chromatography on Lobar® Si 60 size B with the eluent dichloromethane/methanol 96:4 (v/v), a flow of 10 ml/min. and UV detection at 230 nm. The substance (0.222) g was dissolved in 10 ml of dichloromethane and purified in 24 runs. To each 0.7 ml of the product solution was added 1 drop of 2 M sodium hydroxide solution to liberate the free base. The organic layer was injected. The basic compound was eluted at approximately 21 min. To the combined product an excess of hydrochloric acid (4 M dioxane) was added and the solution evaporated to dryness.

Yield: 0.106 g of [4-chromane-^{14}C]repinotan hydrochloride with a radiochemical purity of 99.4% and a chemical purity of 99.4%. The optical purity was determined by HPLC on Chiralpak AD® as $>99\%$ e.e.

Determination of the specific radioactivity:

The specific radioactivity was determined by two different methods. A weighed amount of the labelled compound was dissolved in acetonitrile/water and the total radioactivity was determined by LS counting. On the other hand the concentration of [4-chromane-^{14}C]repinotan hydrochloride in a solution was determined by HPLC/UV quantification using non-labelled reference compound and the total radioactivity was also measured by LSC. The specific radioactivity was determined as 1.63 GBq/mmol (3.74 MBq/mg).

With respect to the use of only 2/3 of the yield of the precursor XVII and disregarding the resolution of the ester XIII the obtained total radioactivity of 396 MBq corresponds to a yield of 6.3% of theory, related to carbon-14 labelled 2-hydroxyacetophenone.

Synthesis of the labelled metabolite M-6

2-(4-{Benzyl[(-)-(2R)-6-acetyl-3,4-dihydro-2H-[4-^{14}C]chromen-2-ylmethyl]amino}butyl)-1,1-dioxo-1,2-benzisothiazole-3(2H)-one, (XVIII). The crude XVII (450 mg) was dissolved in 60 ml of acetonitrile/water 75:25 and purified in 16 runs on Nucleosil® 100 C-18 (120 × 16 mm, 7 µm) with the eluent acetonitrile/water 75:25 (v/v), a flow of 6 ml/min. and UV detection at 254 nm. The desired product was eluted at approximately 15 minutes. The product containing fractions were evaporated to dryness giving 138 mg of VXII.

A solution of XVII (138 mg) in 1.1 ml of dichloromethane was cooled in an ice bath and 171 mg (1.28 mmol) of anhydrous aluminium chloride was added. The mixture was stirred for 20 minutes at room temperature, then it was cooled again and 55 µl (0.77 mmol) of acetyl chloride added. The mixture was refluxed for 2 h and worked up with careful addition of 1.4 g of ice and 1.4 ml of water under ice bath cooling. The organic layer was washed twice with water (2 × 1.6 ml), subsequently with 1.1 ml of 2.2% sodium bicarbonate solution and finally with water (2 × 1.1 ml). Then the organic layer was evaporated to dryness yielding 164 mg of XVIII. The purity was not determined.

(2R)-2-({Benzyl[4-(1,1-dioxido-3-oxo-1,2-benzisothiazole-2(3H)-yl)-butyl]amino}methyl)-3,4-dihydro-2H-[4-^{14}C]chromen-6-yl acetate, (XIX). Compound XVIII (164 mg) was dissolved in 1.7 ml dichloromethane and 170 mg of 3-chloroperbenzoic acid (57–86%) was added. The mixture was cooled in an ice bath and 22.3 µl of trifluoroacetic acid added. The reaction mixture was stirred for 18 h at room temperature in the dark. For work up 1 ml of dichloromethane was added and the mixture washed with 1.7 ml of saturated sodium bicarbonate solution. The dichloromethane layer was washed with of 0.1 M sodium hydroxide solution (2 × 6.1 ml) and subsequently with water (2 × 6.1 ml). The organic layer was evaporated to dryness giving 124 mg of XIX. The purity was not determined.

(-)-R-2-{4-[(6-Hydroxy-[4-^{14}C]chroman-2-ylmethyl)amino]butyl}-1,1-dioxo-1,2-benzisothiazole-3(2H)-one monohydrochloride, [^{14}C]M-6 HCl. Compound XIX (124 mg) was dissolved in 3 ml THF. Concentrated hydrochloric acid (1 ml) and 10% palladium (30 mg) on charcoal were added. The mixture was stirred for 2.5 h at room temperature and filtered over kieselguhr. The filter was washed with THF and the filtrate was evaporated to dryness. The oily residue was dissolved in 2 ml of methanol

and 1 ml acetic acid, and 124 mg of Pearlman's catalyst (20% $Pd(OH)_2$ on charcoal) were added. Hydrogen was bubbled through the solution for 4 hours at room temperature. The mixture was filtered over Kieselguhr, the filter washed with methanol and the filtrate evaporated to dryness. The oily residue was dissolved in 5 ml of concentrated hydrochloric acid and extracted with dichloromethane (2×4 ml and 2×2.5 ml). The aqueous layer was neutralised with saturated sodium bicarbonate solution and extracted with dichloromethane (3×8 ml). The dichloromethane layer was washed with water (2×5 ml) and evaporated to dryness. To remove water the evaporation was repeated twice with each 5 ml of ethanol giving 86 mg of crude [^{14}C]M-6 HCl with a radiochemical purity (radio-HPLC) of 87.6%.

Purification

The product was dissolved in acetonitrile/water and chromatographed in 6 runs on Nucleosil® 7 C-18 (250×10 mm, 5 µm) with the eluent acetonitrile/0.05 M hydrochloric acid 30:70 (v/v), a flow of 5 ml/min and UV detection at 230 nm. The desired product eluted at approximately 9 min. The product containing fractions were evaporated to dryness.

Yield: 28 mg of [^{14}C]M-6 HCl, radiochemical purity (radio-HPLC): 97.8%, chemical purity (HPLC): 98.5%.

Determination of the specific radioactivity

The specific radioactivity was determined by two different methods. First the content of [^{14}C]M-6 HCl in a solution was determined by HPLC/UV quantification using non-labelled reference compound and the total radioactivity was also measured by LSC. The specific radioactivity was found to be 1.614 GBq/mmol (3.568 MBq/mg). This value was confirmed by electrospray mass spectrometry. The specific radioactivity calculated from the ratio of the ^{14}C-molecular ion $m/z = 419$ $[M+H]^+$ and the ^{12}C-molecular ion $m/z = 417$ $[M+H]^+$ was found to be 1.665 GBq/mmol (3.680 MBq/mg).

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