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SYNTHESIS OF ^{3}H AND ^{14}C LABELLED SCH 48461

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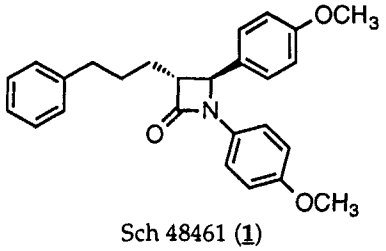
SUMMARY

^{3}H-Sch 47949, racemic ^{3}H-Sch 48461, was prepared at a specific activity of 40 mCi/mmole by Pt catalysed exchange with tritiated water. ^{3}H-Sch 48461 was prepared at a specific activity of 64.6 Ci/mmole by a Pd/C catalysed reduction of an olefinic intermediate. ^{14}C-Sch 48461 was prepared in 8 steps from ^{14}C-potassium cyanide with an overall radiochemical yield of 18.5%.

INTRODUCTION

Sch 48461 (**1**), is a drug candidate currently under development as an inhibitor of dietary cholesterol absorption.[1,2]



Sch 48461 (**1**)

During the early discovery phase, the tritiated racemate, Sch 47949 was prepared to conduct preliminary AME studies in rodents. After the compound had progressed to the development phase, all work was carried out on the single enantiomer, Sch 48461. Hence ^{14}C-Sch 48461 was prepared for metabolism studies, and high specific activity ^{3}H-Sch 48461 for plasma protein binding and mechanism of action studies. The paper describes the synthesis of each labelled form.

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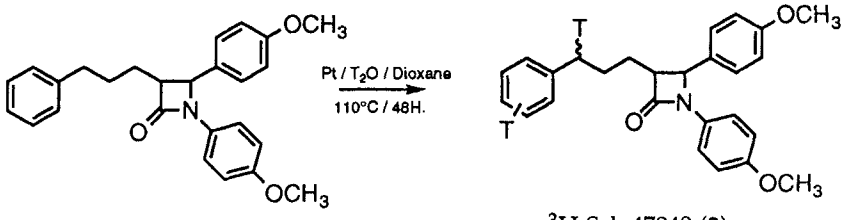
RESULTS AND DISCUSSION



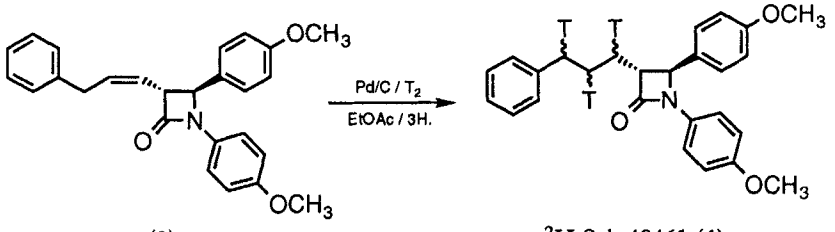
^{3}H-Sch 47949 (2)

Scheme 1

^{3}H-Sch 47949 (2) was synthesized by Pt catalysed exchange with high specific activity water (Scheme 1), with the conditions being worked out with D_2O. A portion of the compound was analysed by ^{3}H-NMR which showed 85% of the label in the *m* and *p*-positions in the free phenyl ring with the remaining 15% in the benzylic sites. This is in agreement with previous platinum catalysed exchange work in substituted benzenes.[3]

In order to supply samples of both enantiomers for preliminary metabolism studies, a portion of the racemate was resolved into its enantiomers by preparative chromatography on cellulose triacetate.

High specific activity ^{3}H-Sch 48461 (4), was prepared by reduction of the olefinic intermediate (3) as shown in Scheme 2.

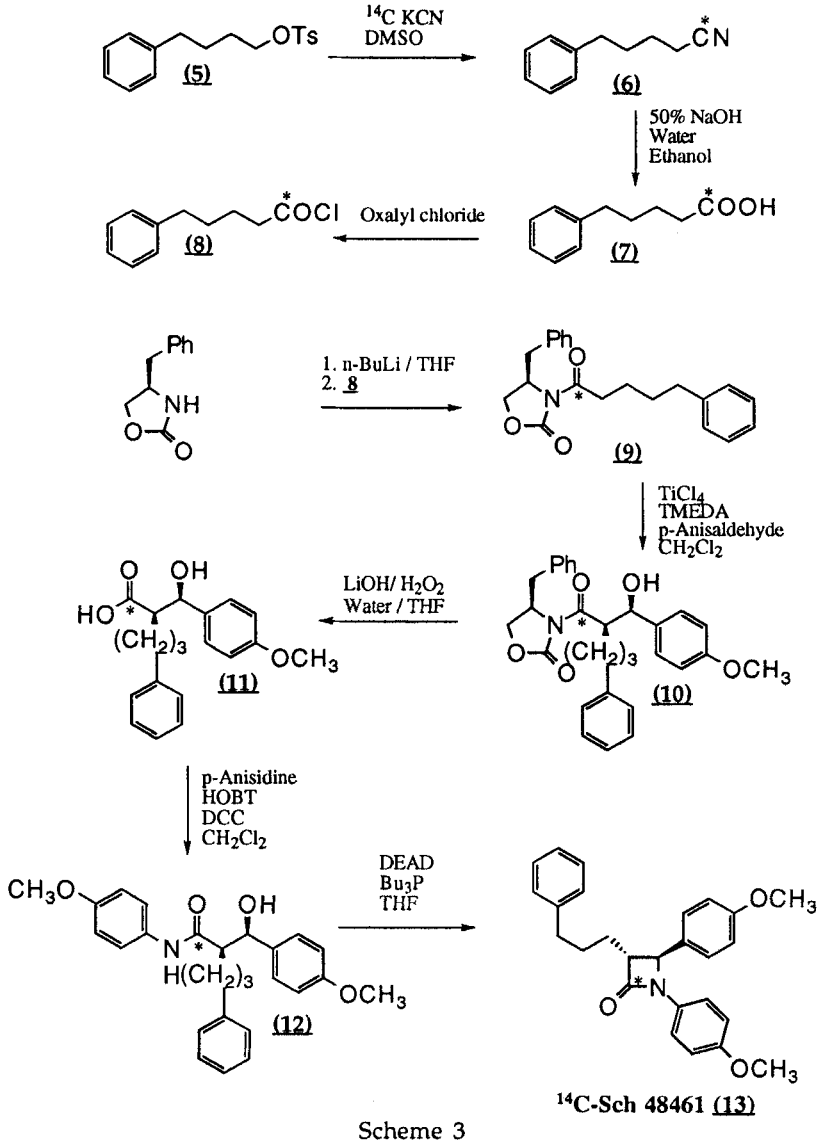


(3) ^{3}H-Sch 48461 (4)

Scheme 2

Analysis by ^{3}H-NMR showed approximately 67% of the tritium was present in the expected sites β and γ to the phenyl ring, with the remaining 33% in the benzylic sites. Such a result is not unexpected as scrambling of the double bond with platinum is well known, and in addition the benzylic sites are susceptible to exchange under the conditions used for the reduction.[4]

The synthesis of ^{14}C-Sch 48461 is shown in Scheme 3.



Scheme 3

The label was introduced by simple nucleophilic substitution of ^{14}C-cyanide on the tosylate (5), which proceeded in quantitative yield. Cyanide (6) was hydrolyzed to phenyl valeric acid (7). The acid was converted to the acid chloride (8) with oxalyl chloride and this was reacted with the Evans chiral auxiliary[5] to form (9), which was purified by column chromatography. The titanium enolate[6] of (9) was then formed and reacted with *p*-anisaldehyde, giving the aldol addition product, (10), with the desired R,R configuration at the newly formed chiral centres. The chiral auxiliary was removed by hydrolysis with lithium hydroxide and hydrogen peroxide to form the free acid (11). This was coupled with *p*-anisidine via HOBT and DCC to give (12),

which was purified by column chromatography. The β-lactam ring was formed, with clean inversion of stereochemistry, using Mitsunobu[7] conditions to give ^{14}C-Sch 48461 (13). The overall yield from K^{14}CN was 18.5%.

EXPERIMENTAL

Materials

^{14}C-Potassium Cyanide was purchased from Amersham plc and was used without further purification. [(4-methylphenyl)-sulphonyl]-butylbenzene, unlabelled Sch 47949 and (3R,4S)-2-Azetidinone 1,4-Bis(*p*-methoxyphenyl)-3-(3-phenyl-1-propylene) were obtained from Schering-Plough Research Institute, Chemical Research. All reagents and solvents were reagent grade and were also used without further purification. All ^{14}C-synthetic steps were carried out under argon.

Liquid Scintillation Counting

Quantitation of radioactivity was performed using a Packard 2200CA liquid scintillation analyser, with Scintiverse BD cocktail used throughout.

Thin layer chromatography

Thin layer chromatography was performed using Whatman LK6DF (silica gel 60) 5 x 20 cm, 0.25 mm plates. The plates were scanned on a Bioscan 1000 linear analyser. The following systems were used:

1. Ethyl acetate: hexane (3: 7)

2. Methylene chloride: hexane (3: 2).

3. Methylene chloride: methanol: acetic acid (98: 1.5: 0.5).

4. Ethyl acetate: hexane (3: 17).

5. Ethyl acetate: hexane (1: 3).

6. Methylene chloride: methanol: acetic acid (96: 3.5: 0.5).

7. Ethyl acetate: hexane (2: 3).

High Performance Liquid Chromatography

^{3}H and ^{14}C-Sch 48461 were analysed by hplc for radiochemical, chemical and chiral purity. A Waters 600E system controller was used with a Waters 712 WISP auto-injector. Chemical purity was determined using a Waters 490 programmable multiwavelength detector and radiochemical purity using a Radiomatic Flow 1 radioflow detector with Radiomatic Flo-Scint III liquid scintillation cocktail. The following systems were used:-

1. Whatman Partisil 5 ODS-3 column, 10 cm x 4.6 mm I.D., 254 nm, methanol: water (3:1), at 0.6 mL/min. for about 15 minutes followed by a gradient to acetonitrile.

2. Daicel Chiralcel OD column, 25 cm x 4.6 mm I.D., 254 nm, hexane: isopropanol (96:4), at 2 mL/min.

3. YMC PVA silica column, 15 cm x 4.6 mm I.D., 254 nm, hexane: ethyl acetate (9: 1) at 1 mL/min.

Synthesis of ^{3}H-Sch 47949

^{3}H-Sch 47949 (2)

To a stirred suspension of platinum dioxide (50 mg) in water (2 mL) was added sodium borohydride (200 mg) over 10 minutes. After the effervescence had ceased, the flask was heated to 80°C to ensure complete hydrolysis of the borohydride. The Pt catalyst was collected on Millipore 5μm LS and washed with water. Sch 47979 (20 mg) was then added to the tube in dioxane (200 μL), and tritiated water (20 Ci) was distilled in. The tube was sealed and heated to 110°C for 24 hours. At the completion of the reaction, the tube was opened and evaporated to dryness. The solid was taken up in ethanol, filtered to remove the catalyst and evaporated to remove labile tritium. A yield of 160 mCi was obtained. The Radiochemical purity by radio-tlc (system 1) was 36%. Crude ^{3}H-Sch 47949 (2) was cleaned up by two silica gel chromatography columns using ethyl acetate: hexane (3: 7) as eluent on the first, and toluene: MTBE (17: 3) on the second. Further purification was achieved by lowering the specific activity by the addition of unlabelled Sch 47949 and recrystallizing from ethyl acetate: hexane. A total of 27 mCi of ^{3}H-Sch 47949 (2) was obtained with a specific activity of 40 mCi/mmole. The radiochemical purity as determined by hplc systems 1 and 3 was >96%. ^{3}H NMR of ^{3}H-Sch 47949, δ7.29 ppm (55% intensity), δ7.18 ppm (30% intensity) and δ2.58 ppm (15% intensity). A portion of ^{3}H-Sch 47949 was resolved into its enantiomeric pair, ^{3}H-Sch 48461 and ^{3}H-Sch 48462 by chromatography on cellulose triacetate. About 100 g of micro crystalline bulk cellulose triacetate powder was boiled in ethanol and packed into a 1" x 2' glass column. ^{3}H-Sch 47949 (55.5 mg, 5.5 mCi) was applied in ethanol and eluted by both gravity and nitrogen pressure with methanol: water (95:5) at 0.5 mL/min. ^{3}H-Sch 48462 eluted first followed by ^{3}H-Sch 48461 two hours later. A batch of 2.3 mCi of ^{3}H-Sch 48462 at a radiochemical purity of 98.2% on hplc system 2 was obtained.

Synthesis of ^{3}H-Sch 48461

^{3}H-Sch 48461 (4)

(3R,4S)-2-Azetidinone 1,4-Bis(*p*-methoxyphenyl)-3-(3-phenyl-1-propylene) (3) (25 mg), was dissolved in ethyl acetate (2 mL) and added to 10% Pd/C (50 mg). To this was added 80 Ci of carrier free tritium gas and the reaction was allowed to stir for 3 hours at room temperature. An uptake of 1.5 mL of tritium was observed. At the completion of the reaction, the labile tritium was removed with ethanol, and the catalyst was filtered off. A yield of 2.998 Ci of crude (4) was obtained. 150 mCi of crude product was purified by hplc using a Whatman Partisil 5 silica column, 10 cm x 9.4 mm, using hexane: ethyl acetate (9: 1) at 3 mL/minute, to yield 124 mCi of (4) at a specific activity of 64.6 Ci/mmole. The radiochemical purity determined in hplc systems 1 and 2 was greater than 99%. ^{3}H NMR of ^{3}H-Sch 48461, δ1.70 ppm (67% intensity) and δ2.60 ppm (33% intensity).

Synthesis of [^{14}C]-Sch 48461 (13)

[^{14}C]-5-Phenylvalerylnitrile, (6)

^{14}C Potassium cyanide (0.303 g, 4.51 mmoles, 248 mCi) was added to a solution of 4-[(4-methylphenyl)-sulphonyl]-butylbenzene (5) (1.41 g, 4.62 mmoles) in DMSO (10 mL). The solution was stirred at 60°C for 8 hours and at room temperature for 12 hours. The reaction solution was then partitioned between water (10 mL) and ethyl acetate (20 mL). The layers were separated and the organic phase washed with water (10 mL). The combined aqueous layers were then extracted with ethyl acetate (10 mL). The combined organic phases were washed with brine (5 mL), dried with anhydrous sodium sulphate, filtered and concentrated to dryness. A quantitative yield (248 mCi) of (6) was obtained. The radiochemical purity as determined by tlc system 2 was 97%.

[^{14}C]-5-Phenylvaleric acid, (7)

To a solution of (6) (2.45 mmoles, 135 mCi) in ethanol (5 mL) was added 50% sodium hydroxide (1 mL). The reaction was then heated at 65°C for approximately 16 hours. After cooling, the reaction was acidified with 4M HCl and extracted with ethyl acetate (2 x 5 mL). The organic phase was washed with brine (10 mL), dried over sodium sulphate, filtered and concentrated to dryness. The yield of (7) was 128 mCi and the radiochemical purity as determined by tlc system 3 was 93%.

[^{14}C]-5-Phenylvaleryl chloride, (8)

Oxalyl chloride (6.9 mmoles, 0.6 mL) was added to a solution of (7) (128 mCi, 2.33 mmoles) in benzene (2 mL), containing 1 drop of dimethylformamide. The reaction was heated at 55°C for two hours. After cooling, the reaction mixture was concentrated to a liquid and diluted with a further 2 mL of benzene. The reaction mixture was re-concentrated and used directly in the next step.

(R)-2-Oxazolidinone, 3-([^{14}C]-1-oxo-5-phenyl-pentyl)-4-(phenylmethyl) (9)

(R)-4-Benzyl-2-oxazolidinone (3.95 mmoles, 0.699 g) was dissolved in 10 mL of anhydrous tetrahydrofuran, cooled to -78°C and 1.6M n-butyl-lithium in hexane (3.5 mmoles, 2.18 mL) was added dropwise over a period of approximately 15 minutes. The reaction was stirred at -78°C for one hour after which (8) (128 mCi, 2.33 mmoles), dissolved in 3 mL of anhydrous tetrahydrofuran, was added. The reaction was stirred at -78°C for one hour before being allowed to warm to 0°C over an additional hour. The reaction was quenched with sodium carbonate (1M, 7 mL), concentrated to one-third its volume and extracted with ethyl acetate (3 x 10 mL). The combined organic extracts were washed with brine (10 mL), dried over anhydrous sodium sulphate, filtered and evaporated to dryness. Purification of (9) was carried out by silica gel chromatography using ethyl acetate: hexane (3: 17) to yield 90 mCi at a radiochemical purity of 98% (tlc system 4).

(R,R,R)-2-Oxazolidinone, 3-[2-[hydroxy (4-methoxyphenyl)methyl]-[^{14}C]-1-oxo-5-phenylpentyl-4-(phenylmethyl) (10)

To a solution of (9) (0.553 g, 1.64 mmoles, 90 mCi) in anhydrous methylene chloride (3 mL) at -20°C, titanium tetrachloride in methylene chloride (1M, 1.63 mL, 1.63 mmoles) was added dropwise over approximately a five minute period. N,N,N'N'-Tetramethylethylenediamine (0.492 mL, 3.26 mmoles) was added and the resulting mixture was stirred at -20°C for one hour. *p*-Anisaldehyde (0.397 mL, 3.26 mmoles) was then added and the reaction

continued for a further hour at -20°C, before it was allowed to warm to 15°C and subsequently quenched with 10 mL of 10% tartaric acid. Crude (10) was extracted with ethyl acetate (2 x10 mL) and the combined organic extracts washed with 5 mL of water, saturated sodium bicarbonate and brine. The organic phase was dried over anhydrous sodium sulphate, filtered and evaporated to dryness. The crude compound (10) was re crystallized from ethyl acetate: hexane (1:2) to yield 81 mCi of product, at a radiochemical purity determined by tlc system 5 of 99%.

(R,R)-Benzene-1-[^{14}C]-pentanoic acid α-[hydroxy (4-methoxyphenyl)methyl] (11)

30% Hydrogen peroxide (8.94 mmoles, 1 mL) was slowly added to a cooled (about 0°C) solution of (10) (1.47 mmoles, 81 mCi) in a mixture of tetrahydrofuran (7.5 mL) and water (1 mL). 2M Lithium hydroxide solution (1.75 mL) was slowly added, and the reaction was stirred at 0°C for 3 hours. The reaction was then quenched by the careful addition of sodium sulphite (8.94 mmoles, 1.13 g) in water (3.5 mL), and then concentrated to about one third its original volume. The reaction mixture was partitioned between toluene (15 mL) and 1M sodium carbonate (5 mL). The organic layer was washed with a further 5 mL 1M sodium carbonate and the combined aqueous layers were acidified with 4M hydrochloric acid. The product was extracted into 20 mL ethyl acetate, dried over anhydrous sodium sulphate, filtered and concentrated. Yield of (11) was 75 mCi, with a radiochemical purity 95% (tlc system 6).

(R,R)-Benzene-1-[^{14}C]-pentanamide α-[hydroxy(4-methoxyphenyl)methyl]-N-(4-methoxyphenyl) (12)

To a solution of (11) (75 mCi, 1.36 mmoles) in methylene chloride (2.5 mL), *p*-anisidine (0.502 g, 4.08 mmoles), 1-hydroxybenzotriazole hydrate (0.203 g, 1.5 mmoles) and N,N-dicyclohexylcarbodiimide (0.310 g, 1.5 mmoles) were added. The reaction mixture was stirred for 3 hours at room temperature, before it was concentrated and suspended in ethyl acetate (20 mL). The suspension was filtered and washed with 5 mL each of 1M hydrochloric acid, saturated sodium bicarbonate and brine. The organic phase was dried over anhydrous sodium sulphate, filtered and evaporated to dryness to give the product, which was purified by silica gel chromatography using ethyl acetate: hexane (3:7) to give 37 mCi of (12). The radiochemical purity as determined by tlc system 7 was ≈90%.

^{14}C Sch 48461; (3R,4S)-2-[^{14}C]-Azetidinone 1,4-Bis(4-methoxyphenyl)-3-(3-phenylpropyl), (13)

A solution of (12) (37 mCi, 0.67 mmoles) in anhydrous tetrahydrofuran (8 mL) was cooled to -78°C. Diethyl azodicarboxylate (1.35 mmoles, 0.213 mL) in tetrahydrofuran (1.78 mL) and tributylphosphine (1.35 mmoles, 0.336 mL) in tetrahydrofuran (1.66 mL) were added simultaneously using syringe pumps at about 1.5 mL/hour. At the completion of the addition, the reaction mixture was stirred for 4 hours at -78°C and was subsequently warmed to room temperature and concentrated. The crude ^{14}C Sch 48461 (13) was purified by silica gel chromatography using ethyl acetate: hexane (1:9) to yield 25 mCi of product at a specific activity of 55 mCi/mmole. The radiochemical purity determined by reverse phase (hplc system 1) and chiral hplc (hplc system 2) was in excess of 99%.

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