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Research Article

Synthesis of ^{3}H, ^{14}C and $^{13}C_6$ labelled Sch 58235

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Summary

^{3}H-Sch 58235 was prepared at a specific activity of 29.1 Ci/mmol by $Ir(COD)(Cy_3P)PyPF_6$ catalysed exchange with tritium gas. ^{14}C-Sch 58235 was prepared in three steps from *p*-hydroxy[ring-U-^{14}C]benzaldehyde with an overall radiochemical yield of 21%. $^{13}C_6$-Sch 58235 was similarly prepared in three steps from *p*-hydroxy[ring-U-$^{13}C_6$]benzaldehyde in an overall yield of 41%.

Key Words: Sch 58235; tritium; carbon-13; carbon-14; synthesis

Introduction

Sch 58235[1,2] inhibits the absorption of dietary cholesterol and is currently in Phase II clinical trials as a successor to the earlier candidate Sch 48461[3]. The preparation of labelled Sch 48461 compound has already been reported.[4]

As is typical during the progression of a compound from the discovery to development phase, ^{3}H-Sch 58235 was initially requested to conduct preliminary AME studies. Once the compound had progressed to the development phase, ^{14}C-Sch 58235 and SIL-Sch 58235 were prepared. This paper thus describes the synthesis of each labelled form.

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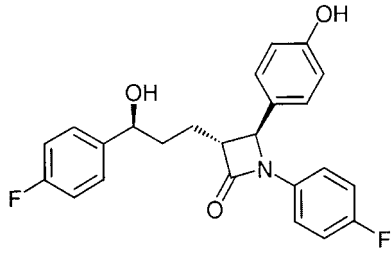
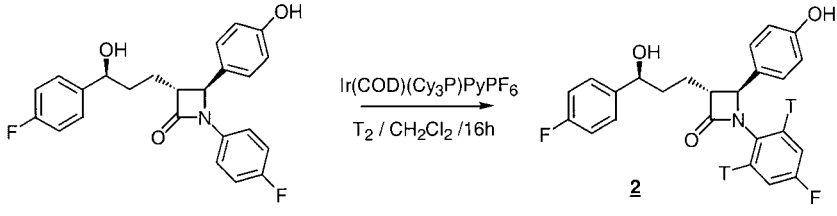


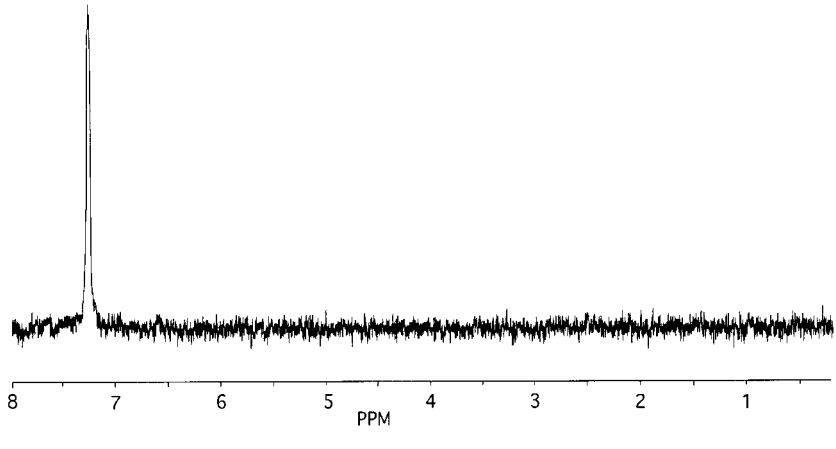
Sch 58235 **1**

Results and discussion

^{3}H-Sch 58235 was synthesized by Ir(COD)(Cy_3P)$PyPF_6$ (Crabtree's Catalyst)[5] catalysed exchange with tritium gas.



^{3}H NMR analysis showed tritium was exclusively located in the fluoroaniline ring and ortho to the β-lactam.

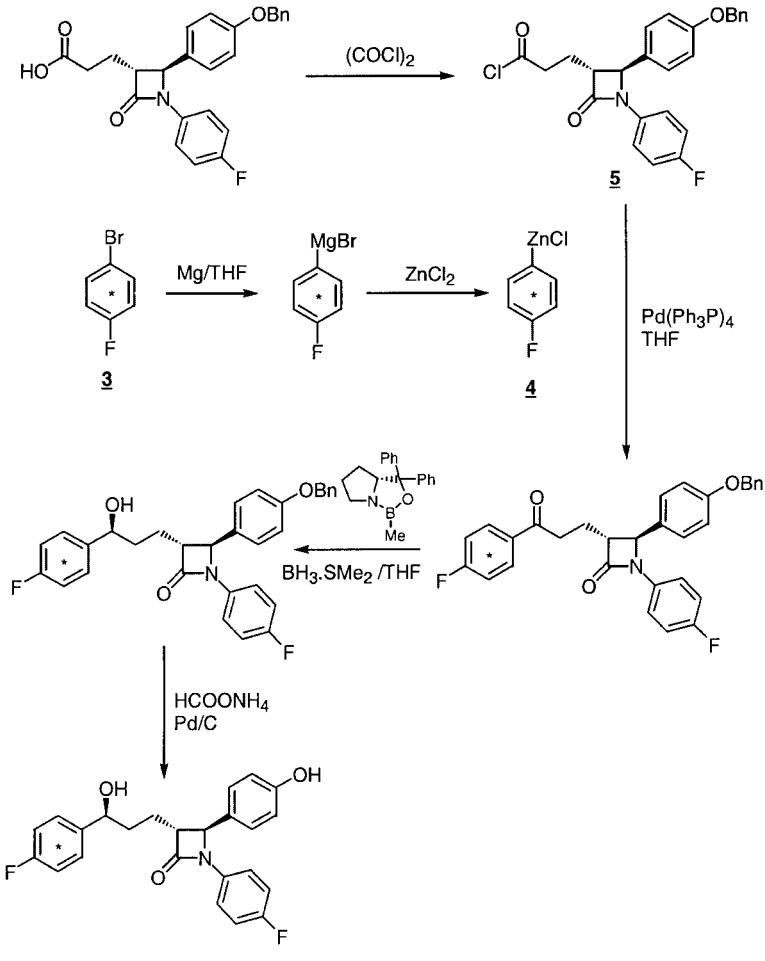


320 MHz proton decoupled ^{3}H NMR of ^{3}H-Sch 58235 in d6-DMSO.

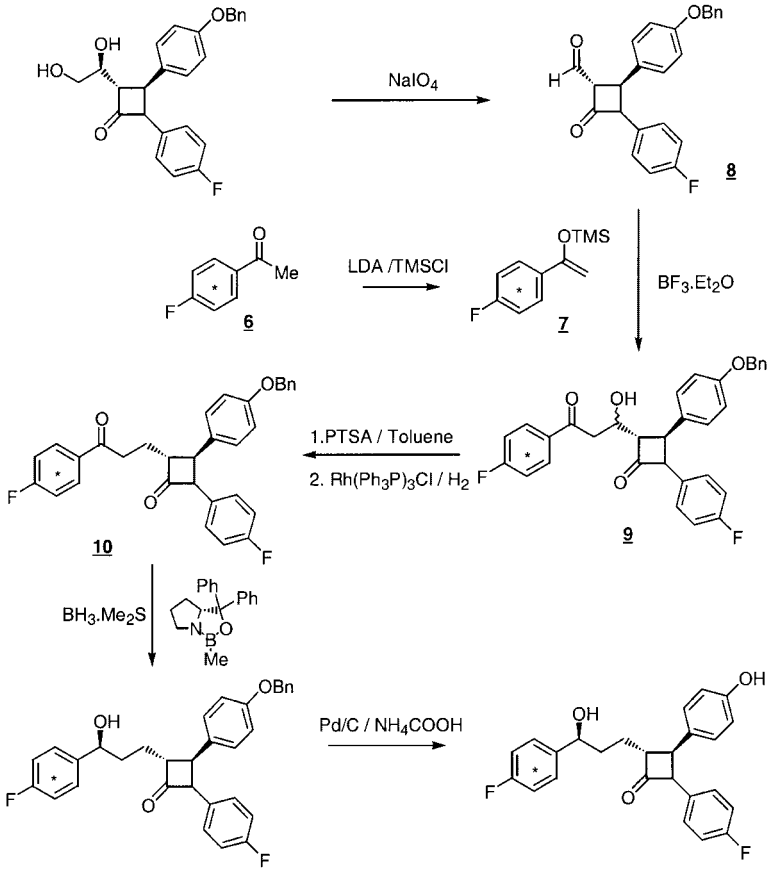
Such a finding is consistent with earlier deuterium labelling results in model acetanilides and ^{2}H-Sch 48461, suggesting the formation of a

6-membered cyclometallated species with the ortho position and the carbonyl in the β-lactam[6,7].

The first proposed route to ^{14}C-Sch 58235 is shown below:



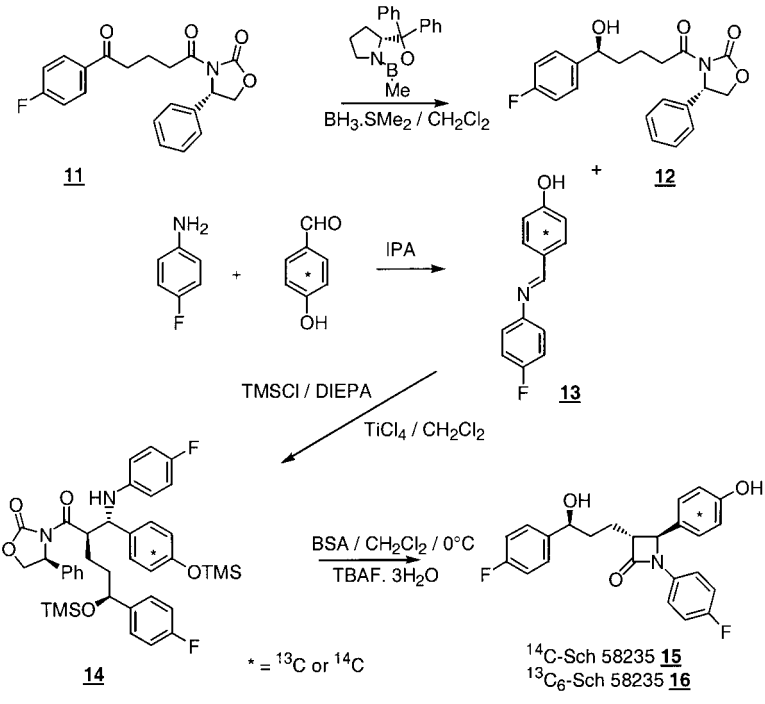
The label was to be introduced from *p*-bromo-[ring-U-^{14}C]-fluorobenzene **3** by firstly making the Grignard reagent and converting this to the zinc complex **4**. The freshly prepared ^{14}C-zincate would be then reacted with the acid chloride **5** in the presence of Pd(0). Although this method was viable using unlabelled commercially available Grignard reagent, the reaction failed when ^{14}C-*p*-bromofluorobenzene was the starting material. Lack of formation of the desired ^{14}C Grignard and hence the ^{14}C-zincate was the most likely cause. This route was abandoned in favour of an alternative route shown below.



In this route, *p*-fluoro-[ring-U-^{14}C]-acetophenone **6** was converted to the TMS enol ether **7** and reacted with the freshly prepared chiral aldehyde **8**. The alcohol **9** was obtained in 27% yield, with the low yield likely due to competing enolisation of the aldehyde. Dehydration with PTSA proceeded in low yield and attempts to reduce the small amount of product with Wilkinson's catalyst gave none of the desired ketone **10**. This route was abandoned in favour of the route shown below.

This route, which has become the working Chemical Development synthesis, used *p*-hydroxy-[ring-U-^{14}C]-benzaldehyde as the starting material. This route was also used for the preparation of $^{13}C_6$-Sch 58235:

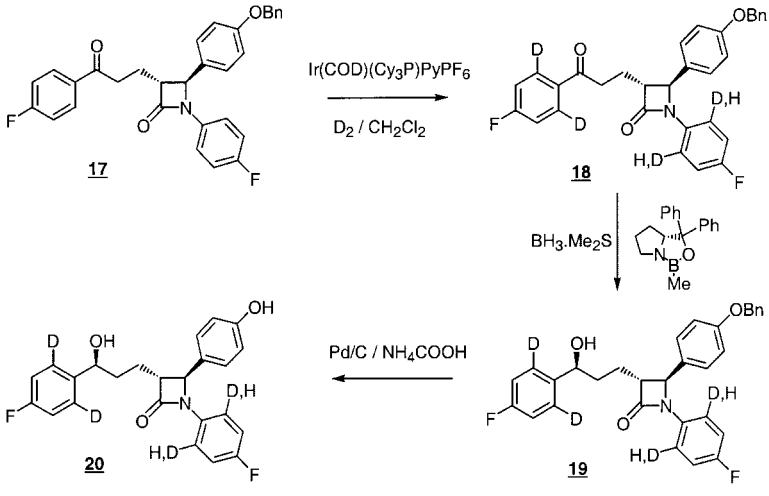
The initial steps involved preparation of S alcohol **12** via CBS reduction of the ketone **11** supplied by Chemical Development and preparation of the ^{14}C-imine **13** in 91% yield by heating *p*-fluoroaniline



with *p*-hydroxy-[ring-U-^{14}C]-benzaldehyde in isopropanol. The S alcohol **12** and 1.3 equivalents of the ^{14}C-Schiff base **13** were silylated with TMS chloride and then treated with titanium tetrachloride to yield intermediate **14** in 39% radiochemical yield with the desired R, S configuration. Attempts to use ^{14}C-Schiff base **13** stoichiometrically led to incomplete reaction and still lower yields. Intermediate **14** was then re-silylated with N,O-bis-(trimethylsilyl) acetamide (BSA) and then cyclized with catalytic tetrabutyl ammonium fluoride to yield crude ^{14}C-Sch 58235. Recrystallisation from isopropanol and water yielded 19 mCi of ^{14}C-Sch 58235 in 21% overall radiochemical yield.

For the ^{13}C-synthesis, the imine was prepared in 97% yield from $^{13}C_6$-*p*-hydroxybenzaldehyde and *p*-fluoroaniline. The chiral aldol proceeded in 40% yield after recrystallization to generate **14**. Finally, cyclization and removal of the chiral auxillary resulted in $^{13}C_6$-Sch 58235 **16** in 90% yield after recrystallization.

Initially it was attempted to prepare SIL-Sch 58235 with the use of deuterium and Crabtree's catalyst, as shown in the following scheme:



Starting from intermediate **17**, exchange of the protons ortho to the ketone and ortho in the aromatic attached to the lactam nitrogen, was attempted with Crabtree's catalyst. Although the protons ortho to the ketone were totally exchanged, three successive exchange reactions afforded a M + 3 and M + 4 cluster **18**. After chiral reduction to alcohol **19**, attempts at further exchange were also unsuccessful. Finally after de-benzylation to form ^{2}H-Sch 58235 **20** a final exchange was attempted, with no further deuterium enrichment seen. Hence as a SIL standard with clean enrichment was desired, this approach was abandoned in favour of $^{13}C_6$-Sch 58325.

Experimental

Materials

p-Hydroxy[ring-U-^{14}C]benzaldehyde was purchased from Amersham plc. Compound **11** and unlabelled Sch 58235 **1** were obtained from Schering Plough Research Institute, Chemical Development. $^{13}C_6$-*p*-hydroxybenzaldehyde was obtained from Cambridge Isotope Laboratories. (Tricyclohexylphosphine) (1-5-cyclooctadiene) (pyridine) iridium (I) hexafluorophosphate was purchased from the Aldrich Chemical Company. All remaining reagents and solvents were purchased from Aldrich or Acros Organics and were used as received. All ^{14}C and ^{13}C-synthetic steps were carried out under an atmosphere of 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) 5×20 cm, 0.25 mm plates. The plates were scanned on a Bioscan 1000 linear analyser. The following systems were used:

1. Ethyl acetate:hexane (1:1)
2. Ethyl acetate:hexane (6:4)

High performance liquid chromatography

^{3}H and ^{14}C-Sch 58235 were analysed by hplc for radiochemical, chemical and chiral purity. $^{13}C_6$-Sch 58235 was assayed for chemical purity alone. A Waters 600E system controller was used with a Waters 712 WISP auto injector. Chemical purity was determined using a Waters 486 single channel UV 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. Phase-Separations Spherisorb S5 ODS2, 10 cm $\times$ 4.6 mm ID, 240 nm, methanol:water (1:1) at 1.5 ml/min for about 15 min followed by a gradient to acetonitrile.

2. Keystone scientific PVA-silica, 15 cm $\times$ 4.6 mm ID, 240 nm, hexane: ethanol (90:10) at 1.5 ml/min followed by a gradient to 100% ethanol.

3. Supelco ABZ, 15 cm $\times$ 4.6 mm ID, 248 nm, acetonitrile:water (35:65) to (58:42) over 20 min to (95:5) over 10 min at 1.5 ml/min.

4. Daicel Chiralcel OD-R, 25 cm $\times$ 4.6 mm ID, 248 nm, acetonitrile:-water (45:55) at 0.4 ml/min.

5. Daicel Chiralcel OD-H, 25 cm $\times$ 4.6 mm ID, 254 nm, hexane:ethanol (70:30) at 1 ml/min.

Synthesis of ^{3}H-Sch 58235 **(2)**

Sch 58235 (12 mg) and $Ir(COD)(Cy_3P)(Py)PF_6$ (1.2 mg) were dissolved in dry methylene chloride (2 ml) and stirred with tritium gas (20 Ci) at

room temperature for 16 h. At the completion of the reaction, the contents were diluted in ethanol and evaporated to dryness. The product was redissolved and the evaporation repeated twice more. A total of 1.3 Ci was isolated with a radiochemical purity as determined in tlc system 1 of 80%.

40 mCi of the crude product was purified by hplc system 2 to yield 10.4 mCi of ^{3}H-Sch 58235 at a specific activity of 29.1 Ci/mmol. The radiochemical purity as determined by hplc systems 1 and 2 was $>99\%$. ^{3}H NMR of ^{3}H-Sch 58235, δ7.25 ppm.

Synthesis of ^{14}C-Sch 58235 **(15)**

3−[5−(p-fluorophenyl)-5-hydroxy-1-oxopentyl]-4-phenyl-2-oxazolidinone **12**. R−(+)-α,α-Diphenyl-2-prolinol (759 mg, 3 mmol) and trimethylboroxine (510 µl, 3.66 mmol) were dissolved in toluene (100 ml) and distilled. Upon completion of the distillation, a further 100 ml of toluene was added and the distillation repeated. The formed catalyst was dissolved in anhydrous methylene chloride (10 ml), borane dimethyl sulphide (1 ml, 10.54 mmol) was added and the solution was cooled to 0°C. A solution of 3-[5-(1, 5-Dioxo-5-(*p*-fluorophenyl) pentyl]-4-phenyl-2-oxazolidinone (3.56 g, 10 mmol) in methylene chloride (10 ml) was then added dropwise over 40 min and the reaction continued for 2 h at 0°C.

After checking for complete reaction by tlc system 1, methanol (3 ml) was added and the reaction stirred at ice temperature for 10 min before 1.5 ml of 30% hydrogen peroxide was added followed by 15 ml of water after a further 10 min. The organic layer was separated and washed with 5% sodium sulphite solution, 1 M sulphuric acid and brine. After drying over anhydrous sodium sulphate, filtration and drying, a quantitative yield of 12 was obtained. Analysis in hplc system 5 showed $>99\%$ ee of the desired SS product.

4−[[(p-fluorophenol) imino] methyl] [ring-U-^{14}C] phenol **13**. *p*-Hydroxy[ring-U-^{14}C]benzaldehyde (90 mCi, 93 mg, 0.73 mmol) and *p*-hydroxybenzaldehyde (185 mg, 1.51 mmol) were mixed and suspended in isopropanol (1 ml). The suspension was heated until complete solution was obtained and *p*-fluoroaniline (220 µl, 2.36 mmol) was added dropwise. The reaction was heated at 50–60°C for 1 h during which a yellow precipitate formed. The reaction was diluted with isopropanol (300 µl) and stirred at room temperature for a further 30 min before the product was

collected by filtration and dried under vacuum. A yield of 82 mCi, 441 mg (91%) of **13** was obtained which was used directly in the next step.

3−[5−(4−fluorophenyl)-2-[[4-fluorophenyl) amino] [4-[(trimethylsilyl) - oxy] [ring-U-^{14}C] phenyl] methyl- 1- oxo- 5- [trimethylsilyl) oxy] pentyl-2-oxzaolidinone **14**. A solution of 12 (566 mg, 1.58 mmol) in methylene chloride (11 ml) was transferred via cannula into a flame dried flask containing **13** (82 mCi, 441 mg, 2.05 mmol). Diisopropylethylamine (1.65 ml, 9.46 mmol) was added and the solution cooled to 0–5°C. Trimethylsilyl chloride (660 µl, 5.23 mmol) was added and the reaction stirred for 75 min at this temperature before it was cooled to between −35 and −30°C.

Titanium tetrachloride (180 µl, 1.66 mmol) was added dropwise and the reaction was stirred for 4 h at this temperature before it was quenched by the addition of acetic acid (450 µl) while maintaining the temperature below −25°C. The reaction was stirred for 15 min and poured into 5% aqueous tartaric solution (11.6 ml) at 0°C. Stirring was continued at 0°C for 15 min and at room temperature for a further hour. Sodium bisulphite (566 mg) was then added. The organic layer was split off and the aqueous layer extracted with methylene chloride (5 ml). The combined organic layers were washed with water (12 ml) and evaporated to dryness.

The solid was dissolved in methylene chloride (3.5 ml), N,O-Bis-(trimethylsilyl)acetamide (BSA) (460 µl) was added and the solution refluxed for 30 min. The reaction was evaporated to dryness and the resulting solid residue recrystallized from ethyl acetate and hexane to yield 32 mCi, 576 mg, of **14** (39%). The radiochemical purity as determined by tlc system 2 was 89%.

1−(4−fluorophenyl-3-[3-(4-fluorophenyl-3-hydroxypropyl-4-([ring-U-^{14}C])-4−hydroxy phenyl-2-azetidinone, ^{14}C-Sch 58235 **15**. BSA (440 µl, 1.75 mmol) was added to a solution of 14 (32 mCi, 576 mg, 0.8 mmol) in anhydrous methylene chloride (3.8 ml) under argon. After stirring for 15 min at room temperature, the reaction was cooled to 0°C and tetrabutyl ammonium fluoride trihydrate (31.4 mg, 0.10 mmol) added. The reaction was stirred at 0°C for 2 h and then evaporated to an oil.

The oil was dissolved in isopropanol (4.2 ml) and sulphuric acid (1 M, 1.2 ml) was added. After stirring for 1 h, water was added dropwise until a precipitate was observed. The mixture was cooled to 0°C and the precipitate collected by filtration. The crude product was recrystallized

twice from isopropanol:water to yield 19.1 mCi, 196 mg (60%) of ^{14}C-Sch 58235 at a specific activity of 40.5 mCi/mmol. The radiochemical purity determined by reverse phase hplc (hplc system 3) and chiral hplc (hplc system 4) was in excess of 99%.

Synthesis of $^{13}C_6$-Sch 58235 **(16)**

4−[[(p-fluorophenol) imino] methyl] [$^{13}C_6$] phenol **13**. *p*-Hydroxy[$^{13}C_6$]benzaldehyde (989 mg, 7.71 mmol)) was suspended in isopropanol (4 ml) and heated until complete solution was obtained. *p*-Fluoroaniline (760 µl, 8.1 mmol) was then added dropwise and reaction was heated at 50–60°C for 1 h during which a yellow precipitate formed. The product was collected by filtration and dried under vacuum. A yield of 1650 mg (97%) of **13** was obtained.

3−[5−(4−fluorophenyl)-2-[[4-fluorophenyl) amino] [4-[(trimethylsilyl)-oxy] [$^{13}C_6$] phenyl] methyl- 1- oxo- 5- [trimethylsilyl) oxy] pentyl-2-oxzaolidinone **14**. A solution of **12** (1.03 g, 2.89 mmol) in methylene chloride (5 ml) was transferred via cannula into a flame dried flask containing **13** (795 mg, 3.6 mmol) in methylene chloride (18 ml). Diisopropylethylamine (3.2 ml, 15.2 mmol) was added and the solution cooled to 0–5°C. Trimethylsilyl chloride (1.2 ml, 9.6 mmol) was added and the reaction stirred for 60 min at this temperature before it was cooled to between −35 and −30°C.

Titanium tetrachloride (332 µl, 3.03 mmol) was added dropwise and the reaction was stirred for 3 h at this temperature before it was quenched by the addition of acetic acid (800 µl) while maintaining the temperature below −25°C. The reaction was stirred for 15 min and poured into 5% aqueous tartaric solution (25 ml) at 0°C. Stirring was continued at 0°C for 15 min and at room temperature for a further hour. Sodium bisulphite (1 g) was then added. The organic layer was split off and the aqueous layer extracted with methylene chloride (3 × 20 ml). The combined organic layers were washed with water (12 ml) and evaporated to dryness.

The solid was dissolved in methylene chloride (4.5 ml), N,O-Bis-(trimethylsilyl)acetamide (BSA) (800 µl) was added and the solution refluxed for 30 min. The reaction was evaporated to dryness and the resulting solid residue recrystallized from ethyl acetate and hexane to yield 995 mg, of **14** (47%).

1−(4−fluorophenyl)-3-[3-(4-fluorophenyl)-3-hydroxypropyl]-4-([$^{13}C_6$]-4-hydroxyphenyl) -2-azetidinone, $^{13}C_6$-Sch 58235 **16**. BSA (480 µl, 1.75 mmol) was added to a solution of **14** (490 mg, 0.77 mmol) plus (an additional 148 mg , 0.2 mmol of **14** from an earlier synthesis) in anhydrous methylene chloride (3.5 ml) under argon. After stirring for 15 min at room temperature, the reaction was cooled to 0°C and tetrabutyl ammonium fluoride trihydrate (35 mg, 0.11 mmol) added. The reaction was stirred at 0°C for 90 min and then evaporated to an oil.

The oil was dissolved in isopropanol (2.2 ml) and sulphuric acid (1 M, 0.32 ml) was added. After stirring for 1 h, water was added dropwise until a precipitate was observed. The mixture was cooled to 0°C and the precipitate collected by filtration. The product was vacuum dried to yield 330 mg (90%) of $^{13}C_6$-Sch 58235 The chemical purity as determined by reverse phase hplc (hplc system 3) and chiral hplc (hplc system 4) was in excess of 98%.

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