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PREPARATION OF REMACEMIDE HYDROCHLORIDE LABELLED WITH CARBON-14, CARBON-13, DEUTERIUM AND TRITIUM

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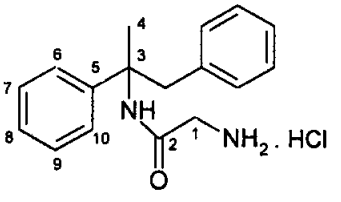
SUMMARY

The anti-epileptic agent remacemide hydrochloride has been prepared labelled with ^{14}C, from [carbonyl-^{14}C]acetophenone, and with ^{13}C from [$^{13}C_6$]benzene, [1,2-^{13}C]acetyl chloride and [1-^{13}C]glycine. [2-^{3}H]Glycine was utilised to prepare remacemide labelled with tritium at low specific activity. In addition other ^{2}H- and high specific activity ^{3}H-isotopomers of the drug, and of an active metabolite of the drug, were prepared by hydrogen isotope exchange methodology. The R-^{12}C/S-^{14}C and S-^{12}C/R-^{14}C pseudoracemic drugs were also prepared by a synthesis involving resolution of a ^{14}C-labelled amine intermediate via fractional crystallisation of the dibenzoyltartrate salts.

Key words: Remacemide, NMDA receptor, Ritter reaction, Friedel-Crafts acylation, [$^{13}C_6$]acetophenone, $RhCl_3$, Crabtree's catalyst, ^{3}H-exchange.

INTRODUCTION

Remacemide hydrochloride (1) is a novel drug with anti-convulsant properties(1). The drug has demonstrated broad spectrum anticonvulsant activity in animal models, whilst studies in patients with treatment-refractory epilepsy have shown it to be effective and well tolerated. Remacemide possesses an asymmetric carbon atom and exists as a racemate, both enantiomers of which are active. Currently, the compound is in advanced clinical development for the treatment of epilepsy. This report describes the synthesis of the various isotopomers of the drug which were required during the development programme.



Remacemide hydrochloride, (1)

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The ^{14}C-labelled remacemide utilised for all the basic drug metabolism and pharmacokinetic studies in animals and in man was prepared in five steps from [carbonyl-^{14}C]acetophenone (Reaction Scheme, reactions 2-6). In addition, the separate ^{14}C-labelled enantiomers of the drug were also prepared via a synthesis involving the resolution of intermediate (6). The preparation of both enantiomers allowed the preparation of pseudoracemates in which one of the enantiomers was ^{14}C-labelled whilst the other enantiomer was unlabelled. This enabled studies of the biological fate of each enantiomer in the presence of the other.

Also described are the syntheses of remacemide hydrochloride variously labelled with ^{13}C. These materials were required for studies of the bioavailability of the drug in man ($^{13}C_6/^{14}C$ dual label study via the oral and intravenous routes) and metabolism in rodents (^{13}C-labelled compound from [1-^{13}C]glycine). Remacemide hydrochloride, $^{13}C_2$- labelled from [1,2-^{13}C]acetophenone was also prepared for use as an internal standard in mass spectrometric assays.

[1-^{3}H]Remacemide was prepared for use in brain uptake and biotransformation studies (2), whilst higher specific activity [6,10-^{3}H]remacemide, and the analogous tritiated active metabolite (8), were prepared for *in vitro* metabolism and receptor binding/typing studies (3) respectively.

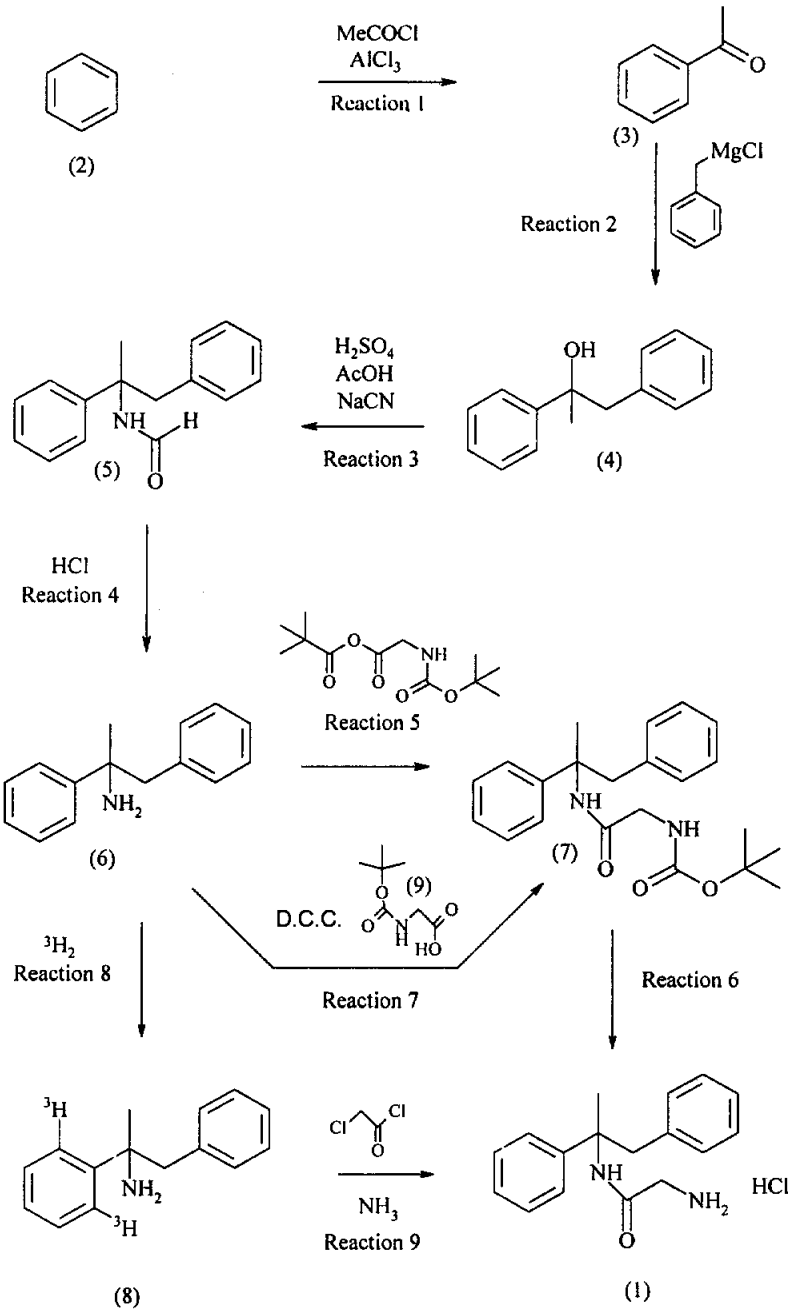
RESULTS AND DISCUSSION

The reactions carried out in the various syntheses are summarised in the Reaction Scheme:-

Carbon-14 syntheses.

The carbon-14 labelled drug was prepared from commercial [carbonyl-^{14}C]acetophenone (3) in five steps (reactions 2-6 above) on several occasions in overall radiochemical yields of around 30-34 %. Yields from the Grignard reaction ranged from 93-100 % whilst the Ritter reaction was variable and lower yielding, in the range 29-58 %. The lowest yield in this reaction was partly a result of formation of a substantial by-product, (1,2-diphenyl[2-^{14}C]propene). Fortunately this compound could itself be recovered and re-utilised as a substrate in the Ritter reaction. Hydrolysis of the formamide (5) to give ^{14}C-labelled 6 proceeded in a straightforward fashion in yields of 75-78 %. The mixed-anhydride acylation of 6 was very effective, proceeding in ca. 95 % yield. Removal of the *t*-BOC protecting group and purification gave ^{14}C-labelled remacemide hydrochloride again in high yield (83-100 %) and with good radiochemical and chemical purity.

Reaction Scheme



The synthesis of 1,2-diphenyl[2-^{14}C]prop-2-ylamine (6) as described above, afforded material of an adequate radiochemical purity (97.3 %) for the separation of the individual enantiomers. This was achieved via fractional recrystallisation of the salts of 6 with dibenzoyltartaric acid. The R-enantiomer was isolated as the dibenzoyl-*l*-tartrate salt and the S-enantiomer as the dibenzoyl-*d*-tartrate salt. The resolved materials were characterised as their respective free base forms, via chiral HPLC, the R-form yielding 99.3 % enantiomeric purity whilst the S-form gave a value of >96 %. The derived maleate salts had radiochemical and chemical purities of >99 %. Following conversion, via reactions 5 and 6, the individual remacemide enantiomers were obtained with chemical and radiochemical purities of >98 %. Samples of each of the two labelled enantiomers were subsequently mixed with an equivalent amount of the unlabelled enantiomer of opposite configuration to afford two ^{14}C/^{12}C-labelled pseudoracemate analogues of remacemide hydrochloride. These materials allowed tracer investigations of the disposition of each enantiomer of remacemide in the presence of the other enantiomer, a situation which accurately mimics dosing with remacemide itself.

Carbon-13 syntheses

To support a human pharmacokinetic study involving the administration of both ^{14}C- and ^{13}C-labelled drug, a batch of [$^{13}C_6$]remacemide hydrochloride was prepared from [$^{13}C_6$]benzene via reactions 1-6. The identity of the product was determined by mass spectrometry and NMR spectroscopy and by comparison with authentic unlabelled remacemide hydrochloride. From the mass spectrum, the atom % abundance of ^{13}C in the phenyl ring (97.3 %.) was consistent with the atom % abundance of the starting benzene, ca. 99.5 %/position. The chemical purity of the compound was also ascertained by HPLC analysis which showed the purity of the batch to be 99.6 % with no single impurity present at levels >0.3 %.

In addition to the above [3,4-$^{13}C_2$]remacemide hydrochloride was synthesised via reactions 1-6, this time introducing the label from [$^{13}C_2$]acetyl chloride. The material was utilised as a mass spectrometric internal standard for the above study and for many other studies with unlabelled, [^{14}C] and [$^{13}C_6$]remacemide.

Lastly, [2-^{13}C]remacemide was prepared from 6 and protected [1-^{13}C]glycine to facilitate studies relating to metabolic transformations of the glycine residue.

Tritium and deuterium syntheses

Remacemide was also prepared at low specific activity labelled with tritium in the glycine moiety. Thus, carbodiimide coupling of commercial *t*-BOC-protected ^{3}H-glycine with 6 and subsequent deprotection gave ^{3}H-labelled remacemide hydrochloride in 27 % overall radiochemical yield.

Since the (6) possesses a benzylamine sub-structure, the compound presents an opportunity for labelling via *ortho*-exchange processes (4) . Indeed ^{2}H-studies showed that the compound could be labelled most effectively via $RhCl_3$-catalysed exchange with isotopic water (5,6) and, somewhat less efficiently, by iridium complex catalysed exchange with deuterium gas (7,8). Since it was possible to handle tritium gas at high atom % abundance 'in-house', the tritiation was carried out using an iridium-based catalyst. ^{3}H-Nmr of the product showed a clean tritiation (98 %) of the *ortho*-positions of the benzylamine sub-structure whilst labelling of the *ortho*-positions of the alternative phenethylamine sub-structure constituted less than 2 % of the labelling. The result is as expected for exchange-labelling via the favoured five-membered or the much less favoured six-membered metallocycles respectively.

Subsequently the labelled 6 required conversion to remacemide hydrochloride. An approach involving condensation with phthalimide protected glycine, followed by deprotection was initially considered. However it proved feasible to use a simpler one-pot procedure in which reaction with chloroacetyl chloride followed by *in situ* quenching of the chloroamide product with ammonia gave labelled remacemide in 47 % radiochemical yield. In this case the small scale of the ^{3}H reaction proved fortuitous: the potential complication of secondary amine formation was obviated by the opportunity to employ a high dilution and a high mole ratio of ammonia.

Attempts to prepare a trideuteriomethyl analogue of remacemide, [4,4,4-$^{2}H_3$]remacemide, from [2,2,2,-$^{2}H_3$]acetyl chloride via the reactions in the Reaction Scheme failed. Despite the expected stability of the intermediary tertiary benzylic carbocation in the Ritter reaction, sufficient isotopic scrambling took place to render the deuteriated product unsuitable for its intended use as a mass spectrometric internal standard.

EXPERIMENTAL

Reagents

Labelled precursors [^{14}C]Acetophenone and *t*-BOC-protected [2-^{3}H]glycine were obtained from NEN Research Products. [U-^{13}C]Benzene was obtained from MSD via Cambrian Gases at K & K Greeff Ltd. [$^{13}C_2$]Acetyl chloride was obtained from the Sigma Chemical Company. Tritium gas was obtained from RC Tritec Ltd, Switzerland, whilst deuterium gas was obtained from the Aldrich Chemical Company. *t*-Boc[1-^{13}C]glycine was obtained from Cambridge Isotope Laboratories Inc.

Other reagents Unlabelled (6) and the enantiomers thereof were obtained from AstraZeneca PR&D Avlon/Charnwood, whilst authentic remacemide hydrochloride was obtained from Pharmaceutical and Analytical R&D, AstraZeneca R&D Charnwood, Crabtree's catalyst, (tricyclohexylphosphine)(1,5-cyclooctadiene)(pyridine)iridium(I) hexafluorophosphate was obtained from the Aldrich Chemical Company. All other reagents were obtained from recognised chemical suppliers and were used as received.

Chromatography

The HPLC purity of remacemide samples was determined using Waters Novapak Phenyl (150 x 3.9 mm) columns with a mobile phase of acetonitrile in potassium phosphate buffer (pH 4.5, 0.05M). The flowrate was 1.5 ml/min and the following gradient was employed (% acetonitrile / minutes) 0/20, 5/20, 20/70, 25/90, 30/90. Detection was by ultraviolet absorbance at 210nm.

REACTION STEPS

Reaction 1

[$^{13}C_6$]Acetophenone. A solution of [U-^{13}C]benzene (2.50 g, 29.9 mmol) and aluminium chloride (4.77 g, 35.8 mmol) in carbon disulphide (12.0 ml) was prepared. Acetyl chloride (2.55 ml, 35.8 mmol) was added in 100 µl portions over 80 minutes. The mixture was refluxed for 5 hours, left at room temperature overnight and quenched with water (15.0 ml). The product was extracted into ether, washed with dilute HCl, water, sodium bicarbonate solution and again with water. After drying ($MgSO_4$) the solvent was removed to leave 3.48 g (93 %) of an oil. The mass spectrum and NMR (^{1}H and ^{13}C) were consistent with the proposed structure,

[$^{13}C_2$]Acetophenone. This preparation was performed as above from [$^{13}C_2$]acetyl chloride (1.00g, 12.4 mmol) and benzene (1.32 ml, 14.8 mmol) to yield the title product (904 mg, 61%). The mass spectrum and NMR (^{1}H and ^{13}C) were consistent with the proposed structure.

Reaction 2

1,2-Diphenyl[2-^{14}C]propan-2-ol. [1-^{14}C]Acetophenone (nominal 1 mmol, 1.97 GBq) was diluted with unlabelled acetophenone (1.12 ml, 9.58 mmol) in anhydrous THF (9.30 ml) and added dropwise over 15 minutes to benzyl magnesium chloride (2M in THF, 5.40 ml) in a further portion of anhydrous THF (18.6 ml), at <0°C . The reaction was left to stir overnight, quenched by pouring onto a mixture of concentrated hydrochloric acid (5.00 ml) and ice (50.0 g), and stirred for one hour. The resulting solution was extracted with dichloromethane (3 x 50.0 ml) and the solvent removed to yield 2.25 g of crude 1,2-diphenyl[2-^{14}C]propan-2-ol (0.863 MBq mg^{-1}, 1.94 GBq, 98 % radiochemical yield) . TLC (silicagel F_{254}, hexane/diethyl ether 3:2 v/v) revealed a radiochemical purity of ca. 90%.

2-[U-^{13}C]Phenyl-1-phenylpropan-2-ol. The reaction was carried out as for the ^{14}C-compound from benzyl magnesium chloride (33.1 mmol) and [$^{13}C_6$]acetophenone (27.6 mmol) to yield 2-[U-^{13}C]phenyl-1-phenylpropan-2-ol (6.14 g, 28.1 mmol).

2,3-[$^{13}C_2$]-1,2-Diphenylpropan-2-ol. A similar reaction in which the Grignard reagent was prepared *in situ* from benzyl chloride (10 mmol) and reacted with [$^{13}C_2$] acetophenone (7.4 mmol) gave 2,3-[$^{13}C_2$]-1,2-diphenylpropan-2-ol (1.59 g, 7.4 mmol). The mass spectrum and NMR (^{1}H and ^{13}C) were consistent with the proposed structure.

Reaction 3

N-{1,2-Diphenyl[2-^{14}C]prop-2-yl}-formamide. Acetic acid (10.0 ml) was stirred under dry nitrogen whilst sodium cyanide (2.67g, freshly ground) was added keeping the temperature below 16 ° C . Concentrated sulphuric acid (10.0 ml) was then added dropwise over 15 minutes, followed by 1,2-diphenyl[2-^{14}C]propan-2-ol (3.9 g, 3.98 GBq.) in di-*n*-butyl ether (12.6 ml), maintaining the temperature below 22 °C. After 3.5 hours the reaction was poured onto ice (78.0

g), extracted with dichloromethane (3 x 50 ml), washed with sodium hydroxide solution (2M, 50 ml), brine (30.0 ml) and dried (Na_2SO_4). Removal of the solvent and trituration with hexane (16 ml) yielded N-{1,2-diphenyl[2-^{14}C]prop-2-yl}formamide (2.58 g, 2.33 GBq, 58% radiochemical yield) with a purity of ca. 90%.

N-{1,2-Diphenyl[2-^{14}C]prop-2-yl}formamide from 1,2-diphenyl[2-^{14}C]propene. 1,2-Diphenyl[2-^{14}C]propene (814 mg, 4.19 mmol, 680 kBq mg^{-1}, 554 MBq) was converted into N-{1,2-diphenyl[2-^{14}C]prop-2-yl}formamide (178 mg, 737 kBq mg^{-1}, 131 MBq) in 24% radiochemical yield by a procedure identical to the above reaction. The radiochemical purity of the product was 97.2%

N-{2-[U-^{13}C]Phenyl-1-phenylprop-2-yl}-formamide. A similar reaction was carried out with 2-[U-^{13}C]phenyl-1-phenylpropan-2-ol (6.14 g, 28.2 mmol) to yield N-{2-[U-^{13}C]phenyl-1-phenylprop-2-yl}formamide (2.9 g, 11.8 mmol, 42%, after flash chromatography of the product on silicagel eluted with chloroform).

N-{[2,3-$^{13}C_2$]1,2-Diphenylprop-2-yl}formamide. A similar reaction carried out with [2,3-$^{13}C_2$]-1,2-diphenylpropan-2-ol (1.61 g, 7.5 mmol) gave crystalline N-{[2,3-$^{13}C_2$]-1,2-diphenylprop-2-yl}formamide (949 mg, 3.97mmol, 53%).

Reaction 4

1,2-Diphenyl-[2-^{14}C]prop-2-ylamine. N-{[2-^{14}C]-1,2-Diphenylprop-2-yl}formamide (3.74 g, 15.7 mmol, 3.38 GBq) was refluxed with a solution of conc. hydrochloric acid (10.8 ml) in water (97.2 ml) for 1.75 hours. The reaction was cooled and washed with toluene. The aqueous layer was basified to pH 11 with sodium hydroxide solution and then the layer was extracted with dichloromethane (3 x 110 ml). The organic extracts were washed with brine (45 ml), dried ($MgSO_4$), filtered, and the solvent removed to yield 1,2-diphenyl[2-^{14}C]prop-2-ylamine as a yellow oil (2.56 g, 12.1 mmol, 2.540 GBq, 75.1% radiochemical yield). The radiochemical purity by radio TLC (silicagel F_{254}, methanol/dichloromethane, 5:95 v/v) was 93%.

2-[U-^{13}C]Phenyl-1-phenylprop-2-ylamine. N-{2-[U-^{13}C]Phenyl-1-phenylprop-2-yl}formamide (2.9 g, 11.8 mmol) was similarly hydrolysed to yield 2-[U-^{13}C]-phenyl-1-phenylprop-2-ylamine (2.5 g, 11.7 mmol, 99 %).

[2,3-$^{13}C_2$]-1,2-Diphenylprop-2-ylamine. N-{[2,3-$^{13}C_2$]-1,2-Diphenylprop-2-yl}formamide (0.949 g, 3.9 mmol) was hydrolysed in a similar fashion to give [2,3-$^{13}C_2$]-1,2-diphenylprop-2-ylamine (0.78 g, 3.66 mmol, 94 %).

Reactions 5

2-{N-*t*-Butyloxycarbonylamino}-N-{1,2-diphenyl-[2-^{14}C]prop-2-yl}acetamide. N-(*t*-Butoxycarbonyl)glycine (2.78 g, 15.9 mmol) was dissolved in dichloromethane (67.0 ml), triethylamine (2.21 ml) added and the solution cooled to -4°C. A solution of pivaloyl chloride (1.95 ml) in dichloromethane (14 ml) was added dropwise over 15 minutes and the mixture stirred for 2.5 hours at 0°C. To the resulting mixed anhydride solution was added a solution of 1,2-diphenyl[2-^{14}C]prop-2-ylamine (2.56 g ,12.1 mmol, 2.56 GBq) in dichoromethane (36 ml) over a period of 20 minutes. The mixture was left at 0°C for 3.5 hours and then allowed to warm to room temperature overnight. The resulting reaction mixture was washed with aqueous sodium hydrogen carbonate (5% w/v, 120 ml), dilute hydrochloric acid (ca. 4M, 120 ml), brine (120 ml), dried ($MgSO_4$)and evaporated to yield 2-{N-t-butyloxycarbonylamino}-N-{1,2 diphenyl[2-^{14}C]prop-2-yl}acetamide (5.21 g, 2.42 GBq , 94.5% radiochemical yield) as a yellow gum with a purity by radio-TLC (silicagel F_{254}, methanol/dichloromethane, 5:95 v/v) of 95%.

2-{N-*t*-Butyloxycarbonylamino}-N-{2-[U-^{13}C]phenyl-1-phenylprop-2-yl}acetamide. In a similar fashion 2-[U-^{13}C]phenyl-1-phenylprop-2-ylamine hydrochloride (2.35 g, 9.27 mmol) yielded 2-{N-t-butyloxycarbonylamino}-N-{2-[U-^{13}C]phenyl-1-phenylprop-2-yl}acetamide (2.99 g, 7.99 mmol, 86 %).

2-{N-*t*-Butyloxycarbonylamino}-N-{[2,3-$^{13}C_2$]-1,2-diphenylprop-2-yl}-acetamide. Likewise [2,3-$^{13}C_2$]-1,2-diphenylprop-2-ylamine (0.693 g, 3.2 mmol) yielded an unpurified batch of 2-{N-t-butyloxycarbonylamino}-N-{[2,3-$^{13}C_2$]-1,2-diphenylprop-2-yl}acetamide (1.53 g, 127 %).

2-{N-*t*-Butyloxycarbonylamino}-N-{1,2-diphenylprop-2-yl}-[1-^{13}C]acetamide. As above *t*-Boc[1-^{13}C]glycine (500 mg, 2.8 mmol) and 1,2-diphenylprop-2-ylamine (6, 599mg, 2.84 mmol) yielded 2-{N-*t*-butyloxycarbonylamino}-N-{1,2-diphenylprop-2-yl}-[1-^{13}C]acetamide (995 mg, 2.69 mmol, 96%) which was deprotected without purification.

Reaction 6

2-Amino-N-(1,2-diphenyl-[2-^{14}C]prop-2-yl)acetamide hydrochloride, [3-^{14}C]remacemide hydrochloride. 2-{N-*t*-Butyloxycarbonylamino}-N-{1,2 diphenyl[2-^{14}C]prop-2-yl}acetamide (5.21 g, 14.1 mmol, 2.41 GBq) was dissolved in methanol (161 ml) and concentrated hydrochloric acid (8.20 ml) added. The resulting solution was refluxed for 1 hour, filtered and the filtrate concentrated under reduced pressure and repeatedly azeotroped with ethanol (3 x 120 ml) to yield a white solid. The solid was washed with diethyl ether (2 x 25ml), dried at 30°C under vacuum and then crystallised from a mixture of ethanol and diethyl ether to yield 2-amino-N-(1,2-diphenyl-[2-^{14}C]prop-2-yl)-acetamide hydrochloride, (2.83 g, 9.29 mmol, 2.00 GBq, 83.3% radiochemical yield) with a chemical purity of 99.6% and a radiochemical purity of 98.8%.

2-Amino-N-(2-[U-^{13}C]phenyl-1-phenylprop-2-yl)acetamide hydrochloride, [5,6,7,8,9,10-$^{13}C_6$]remacemide hydrochloride. In a similar fashion, 2-{N-*t*-butyloxycarbonylamino}-N-{2-[U-^{13}C]phenyl-1-phenylprop-2-yl}-acetamide (2.99 g, 7.99 mmol) was hydrolysed by refluxing in methanol (60.0 ml) and concentrated hydrochloric acid (95.0 ml). After removal of the solvent and azeotroping with ethanol the resulting solid (2.45 g) was crystallised from ethanol/ether and then from methanol/isopropanol to yield 1.95 g of crystalline product. This material was further purified by preparative HPLC (Column: Dynamax C-18 300 x 21.4 mm, mobile phase: acetonitrile:water, 35:65 v/v containing 0.5% trifluoroacetic acid; flowrate: 20.25 ml min^{-1}; detection: 235 nm) and converted to the hydrochloride salt to yield pure 2-amino-N-(2-[U-^{13}C]phenyl-1-phenylprop-2-yl)-acetamide hydrochloride (1.36 g, 4.38 mmol, 55 %).

2-Amino-N-(2,3-[$^{13}C_2$]-1,2-diphenylprop-2-yl)acetamide hydrochloride, [3,4-$^{13}C_2$]remacemide hydrochloride. As above 2-{N-*t*-butyloxycarbonylamino}-N-{[2,3-$^{13}C_2$]-1,2-diphenylprop-2-yl}-acetamide (1.53 g, 4.14 mmol) was deprotected and crystallised from ethanol/ether to yield 2-amino-N-(2,3-[$^{13}C_2$]-1,2-diphenylprop-2-yl)-acetamide hydrochloride, as a white solid (216 mg, 0.704 mmol, 17%). The mother liquors from the recrystallisation yielded a further 290 mg, 0.945 mmol, 23%) of less pure material after removal of the solvent under reduced pressure and washing the residue with diethyl ether. The mass spectrum and NMR (^{1}H and ^{13}C) spectra of both batches were consistent with the proposed structure.

2-Amino-N-(1,2-diphenylprop-2-yl)-[1-^{13}C]acetamide hydrochloride, [2-^{13}C]remacemide hydrochloride. 2-{N-*t*-Butoxycarbonylamino}-N-{1,2-diphenylprop-2-yl}-[1-^{13}C]acetamide (995 mg, 2.69 mmol) similarly gave 2-amino-N-(1,2-diphenylprop-2-yl)-[1-^{13}C]acetamide hydrochloride (654 mg, 2.14 mmol, 79%).

Reaction 7

2-Amino-N-(1,2-diphenylprop-2-yl)-[2-^{3}H]acetamide hydrochloride, [1-^{3}H]remacemide hydrochloride. To a mixture of unlabelled *t*-BOC-glycine (87.5 mg, 0.548 mmol) and 1,2-diphenylprop-2-ylamine (106 mg, 0.501 mmol) in dichloromethane (4.0 ml) was added BOC-[2-^{3}H]glycine (1.67 TBq mmol^{-1}, 0.097 mg, 0.541 μmol, 914 MBq). To the resulting solution was added dicyclohexylcarbodiimide (103 mg, 0.500 mmol) and the reaction allowed to stir at room temperature overnight. The dicyclohexylurea was filtered and the solvent removed under reduced pressure to yield crude 2-{N-*t*-butoxycarbonylamino}-N-{1,2-diphenylprop-2-yl}-[2-^{3}H]acetamide (222 mg, 0.603 mmol). This was dissolved in methanol (1.90 ml) and conc. hydrochloric acid (110 μl) and the solution refluxed for 15 minutes. Removal of the solvents under reduced pressure yielded the crude deprotected material which was dried over phosphorus pentoxide for 18 hours and recrystallised from methanol (860 μl) and isopropanol (2.15 ml) to give 2-amino-N-(1,2-diphenylprop-2-yl)-[2-^{3}H]acetamide hydrochloride (98.5 mg, 0.323 mmol, 849 MBq mmol^{-1}, 274 MBq, radiochemical purity 99.6%)

Reaction 8

2-[2,6-$^2H_2$]Phenyl-1-phenylprop-2-ylamine via $RhCl_3$ catalysis. 1,2-Diphenylprop-2-ylamine hydrochloride (8.25 mg, 33.3 μmol) and rhodium chloride trihydrate (2.80 mg, 10.6 μmol) were dissolved in a mixture of deuterium oxide (9 μl) and dimethylformamide (9 μl) and heated at 107°C for 18 hours. After this time the mixture was acidified with dilute hydrochloric acid solution (3.5 M, 9 μl) and washed with diethyl ether. The remaining solution was basified with sodium hydroxide solution (2 M, 50 μl) and extracted with diethyl ether (1.0 ml), the ether was dried over magnesium sulphate, filtered and the solvent removed under reduced pressure to yield 2-[2,6-^{2}H]phenyl-1-phenylethylamine as an oil. Mass spectroscopic analysis showed that the material had incorporated 1.2 atoms of deuterium/molecule.

2-[2,6-^{2}H]Phenyl-1-phenylprop-2-ylamine via iridium complex catalysis. (Tricyclohexylphosphine)(1,5-cyclooctadiene)(pyridine)iridium(I) hexafluorophosphate, Crabtree's catalyst, (1.00 mg, 1.24 μmol) and 1,2-diphenylprop-2-ylamine (2.00 mg, 9.48 μmol) were dissolved in dichloromethane (0.50 ml) and stirred under an atmosphere of deuterium gas (ca. 0.40 ml) for 24 hours. The solvent was evaporated and the residue dissolved in ethanol (10.0 ml) and filtered through a 1.0 cm bed of silica gel, contained in a Pasteur pipette, to yield crude 2-[2,6-$^2H_2$]phenyl-1-phenylprop-2-ylamine with 0.82 atoms of deuterium/molecule.

2-[2,6-$^3H_2$]Phenyl-1-phenylprop-2-ylamine. Crabtree's catalyst (1.00 mg, 1.24 μmol) and 1,2-diphenylprop-2-ylamine (2.00 mg, 9.48 μmol) were dissolved in dichloromethane (0.50 ml) and stirred under an atmosphere of tritium gas (37 GBq) for 24 hours. The labile tritium was removed by evaporation of the solvent and by subsequently twice dissolving the residue in ethanol (5.0 ml) and removing the ethanol under vacuum. The residue was dissolved in ethanol (10.0 ml) and filtered through a 1.0 cm bed of silica gel contained in a Pasteur pipette to yield crude 2-[2,6-$^3H_2$]phenyl-1-phenylprop-2-ylamine (4.92 GBq) with a radiochemical purity of ca. 90%. A portion of the above material was further purified using HPLC (Column: Novapak C-18, 150 x 3.9 mm, mobile phase: acetonitrile:water, 22:78 containing 0.35% trifluoroacetic acid, flowrate: 1.0 ml min^{-1}, detection: 235 nm) in three portions (each prepared by evaporating 1.0 ml aliquots of the ethanol solution and reconstituting in HPLC mobile phase, for injection). This procedure yielded pure 2-[2,6-$^3H_2$]phenyl-1-phenylprop-2-ylamine, 648 GBq/mmol.

The ^{3}H-NMR spectrum (533 MHz, $^2H_6$-DMSO) of the product showed two resonances at δ 6.83 and 7.42 in the ratio 2:98 corresponding to the *ortho* positions of the benzyl and phenyl groups respectively.

The purified material was stored at 4° C in HPLC mobile phase diluted with ethanol to a radioactive concentration of 61.8 MBq ml^{-1}.

Reaction 9

2-Amino-N-{2-[2,6-$^3H_2$]phenyl-1-phenylprop-2-yl}acetamide, [6,10-^{3}H]remacemide. The stock solution of 2-[2,6-$^3H_2$]phenyl-1-phenylprop-2-ylamine (2.00 ml, 984 MBq) was reduced to dryness under a stream of nitrogen and then further dried under high vacuum for 30 minutes at room temperature. To the residue was added a solution of pyridine (2% v/v in dichloromethane, 200 µl) and chloroacetyl chloride (1% v/v solution in dichloromethane, 200 µl) and the reaction allowed to stand for 10 minutes. To the resulting solution was added concentrated ammonia solution (0.880 density NH_3 diluted 1:1 with ethanol, 1.40 ml) and the reaction heated at 60° for 3.0 hours and allowed to stand at room temperature for three days. The solvent was then removed under a stream of nitrogen and the compound purified using preparative HPLC (as above but with an isocratic mobile phase of acetonitrile / 0.5% w/v ammonium acetate solution 20:80 v/v). The product (463 MBq) had a specific radioactivity (HPLC-MS) of 710 GBq $mmol^{-1}$. ^{3}H-NMR showed a single resonance at δ 7.34.

The purified material was stored at 4° C in HPLC mobile phase diluted with ethanol to a radioactive concentration of 29 MBq ml^{-1}.

Resolution of enantiomers of 1,2-diphenyl[2-^{14}C]prop-2-ylamine as the dibenzoyl-l-tartrate salts. Racemic 1,2-diphenyl[2-^{14}C]prop-2-ylamine (1.523 g, 7.22 mmol) was dissolved in 95% ethanol (15.0 ml) and heated at reflux. Dibenzoyl-*l*-tartaric acid (2.58 g, 7.22 mmol) dissolved in hot 95% ethanol (15.0 ml) was then added and reflux continued for a further fifteen minutes. The solution was cooled to 4° C overnight and the white crystals (1.54 g) of the R-enriched tartrate salt were collected and dried. The mother liquors (2.18 g) were predominantly the S-enriched enantiomer. The enantiomeric purity of the R-enriched salt was determined by chiral HPLC (Column: Astec Cyclobond I, 250 x 2.5 mm, mobile phase: 8% v/v acetonitrile / aqueous phosphate buffer (0.1 M), 0.5 ml min^{-1}, 4°C, detection by u.v. at 210 nm and by radioactivity and was found to be 95.5%. A second recrystallisation from 95% ethanol (20 ml) yielded the pure enantiomer (1.288 g, enantiomeric purity 99 %).

Preparation of (R)- and (S)-1,2-diphenyl[2-^{14}C]prop-2-ylamine. (R)-1,2-Diphenyl[2-^{14}C]prop-2-ylamine dibenzoyl-*l*-tartrate salt (1.288 g, 2.26 mmol) was slurried with water (50.0 ml) and dichloromethane (50.0 ml) and the pH was adjusted to 11 with concentrated ammonia. The aqueous layer was extracted with a further portion of dichloromethane (50.0 ml), the organic extracts combined and the solvent removed under reduced pressure. The resultant oil was azeotroped with ethanol and dried under vacuum to yield (R)-1,2-diphenyl[2-^{14}C]prop-2-ylamine (459 mg, 96% recovery; 877 kBq mg^{-1}, 402 MBq, enantiomeric purity 99.3%, radiochemical purity 99.5%).

In a similar fashion, the S-enriched dibenzoyl-*l*-tartrate salt (2.18 g, 3.83 mmol) was converted to the free base by treatment with aqueous ammonia and solvent extraction to yield 795.5 mg of predominantly (S)-1,2-diphenyl[2-^{14}C]prop-2-ylamine. This was resolved with dibenzoyl-*d*-tartaric acid monohydrate (1.56 g, 4.15 mmol) to yield 1.50 g of the S-enriched tartrate salt. Analysis of the product by chiral HPLC revealed an enantiomeric purity of greater than 98%. The material was converted to the free base form as for the R-enantiomer to yield (S)-1,2-diphenyl[2-^{14}C]prop-2-ylamine (561 mg, 0.86 MBq mg^{-1}, 483 MBq, enantiomeric purity 96%, radiochemical purity 98.9%).

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