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SYNTHESIS OF CARBON-14 LABELLED NK-1 RECEPTOR ANTAGONISTS GR203040 AND GR205171

Karl M Cable,* Guy N Wells and Derek R Sutherland
Chemical Development Division, Glaxo Wellcome Medicines Research Centre, Stevenage, Hertfordshire SG1 2NY. UK.

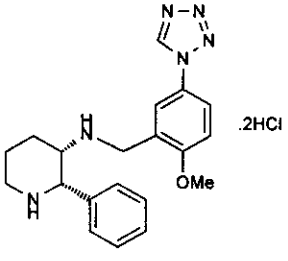
SUMMARY

Syntheses of carbon-14 labelled versions of NK-1 receptor antagonists GR203040 and GR205171 are described. The carbon-14 atoms were introduced by palladium (0) catalysed cyanation of iodoaromatic substrates.

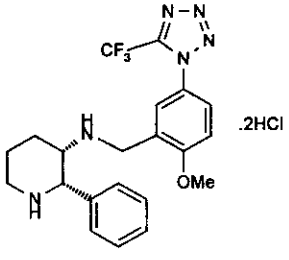
Keywords: carbon-14, cyanation, NK-1 receptor antagonist, GR203040, GR205171

INTRODUCTION

GR203040 (1a) and GR205171 (2a) are high affinity, selective NK-1 receptor antagonists with potent anti-emetic activity (1-6). Carbon-14 labelled versions of



(1a) GR203040



(2a) GR205171

* Author for correspondence

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these compounds were required for pharmacokinetic and metabolism studies as part of a development programme in this therapeutic area.

GR205171 (2a) contains a 1,5-disubstituted tetrazole bearing a trifluoromethyl group whereas GR203040 (1a) has a hydrogen at the tetrazole 5-position. This small difference in structure has a marked effect on the relative reactivity of the two compounds. It is known that *5H*-tetrazoles can be deprotonated using strong base and then rapidly decompose by loss of nitrogen to give cyanamides. The acidity of the 5-hydrogen in (1a) has been exploited in the synthesis of a series of 5-aminotetrazole derivatives of GR203040 using this methodology (7). Thermal decomposition of tetrazoles by loss of nitrogen is also precedented (8). The difference in reactivity of the tetrazole groups in (1a) and (2a) had a major influence on the efficacy of the labelling process.

It was known that both (1a) and (2a) could be synthesised by reductive amination of aromatic aldehydes with a chiral diamine (8) as shown in Schemes 1 and 2 (1, 5). Since labelling of the chiral diamine would involve a wasteful resolution step, methods for incorporating carbon-14 atoms into metabolically stable positions on the aldehyde fragments were sought. Initial metabolism work with unlabelled (1a) showed evidence for aromatic hydroxylation and O-demethylation. Based on this evidence and predicted routes of metabolism, the central benzylic methylene carbons in (1a) and (2a) were chosen as acceptable sites for isotopic labelling. This approach required the synthesis of two aromatic aldehydes labelled at their carbonyl carbons. An attractive strategy was to insert the carbon-14 atoms by cyanation of iodoaromatic substrates with labelled cyanide followed by reduction to the aldehydes (9). Aromatic nitriles are versatile synthetic intermediates which can be conveniently prepared from the corresponding iodides (or bromides) by a variety of cyanation methods (10). The cyanide source is normally a salt such as potassium cyanide. The advantages of using labelled inorganic cyanides are that they are readily available, relatively inexpensive radiochemicals and are non-volatile solids which can be handled easily.

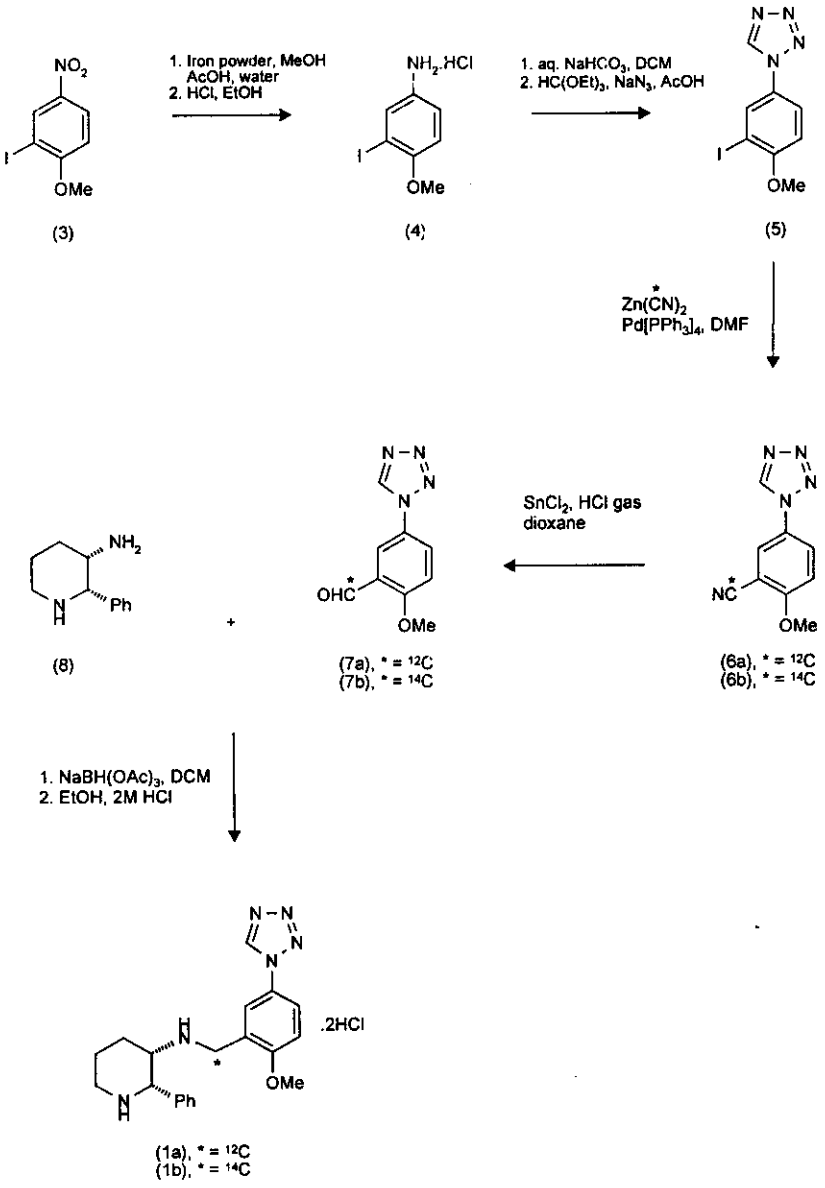
RESULTS AND DISCUSSION

[^{14}C]GR203040

Key iodo intermediate (5) was prepared in two steps from 2-iodo-1-methoxy-4-nitrobenzene (3) (Scheme 1). Reduction with iron powder in aqueous acetic acid gave the corresponding aniline which was isolated as its hydrochloride salt (4). Treatment of the free base with triethyl orthoformate and sodium azide in glacial acetic acid gave (5) in 38% overall yield from (3) (5). It was anticipated that cyanation of iodide (3) would be successful under conditions previously developed in our laboratory (11). However, reaction of (3) with potassium cyanide and copper (I) iodide in refluxing dimethylformamide resulted in decomposition. Other cyanation methods, such as copper (I) cyanide in dimethylformamide at 120° (12) and potassium cyanide with tetrakistriphenylphosphine palladium (0) in tetrahydrofuran at reflux (13), gave poor yields (<20%) of nitrile (6a). The tetrazole moiety in (1a) was subsequently found to be unstable at elevated temperatures (8). It is also known that demethylation of anisoles occurs on heating with sodium cyanide in dimethylsulphoxide (14). A procedure involving the use of zinc cyanide with palladium (0) catalysis at 80° was finally successful (15). Nitrile (6a) was obtained in 68-73% yield using this relatively mild cyanation method. Attempted reduction of (6a) using diisobutylaluminium hydride in dichloromethane or Raney nickel in formic acid afforded poor yields of aldehyde (7a) (16). Stephen reduction with tin (II) chloride and anhydrous hydrogen chloride was more successful but yields were variable (30-70%) (17). It was not possible to force the reduction to completion by addition of excess tin (II) chloride. The reason for the variability in yield was unclear, but it is known that traces of atmospheric moisture can have a significant effect on the progress of the Stephen reduction (18). Since unreacted nitrile (6a) could not be readily separated from (7a) at this stage, the reductive amination was carried out using this mixture. The chiral diamine (8) was liberated from its di-p-toluoyl-L-tartrate salt immediately prior to the reductive amination step (5). Reductive amination of (7a) with (8) followed by salt formation typically gave GR203040 dihydrochloride

(1a) in 70-75% yield (over two steps). Unreacted nitrile (6a) was recovered efficiently by column chromatography of GR203040 free base. Using the same methodology the corresponding radiosynthesis afforded [^{14}C]GR203040 dihydrochloride (1b) in 26% overall yield from zinc [^{14}C]cyanide.

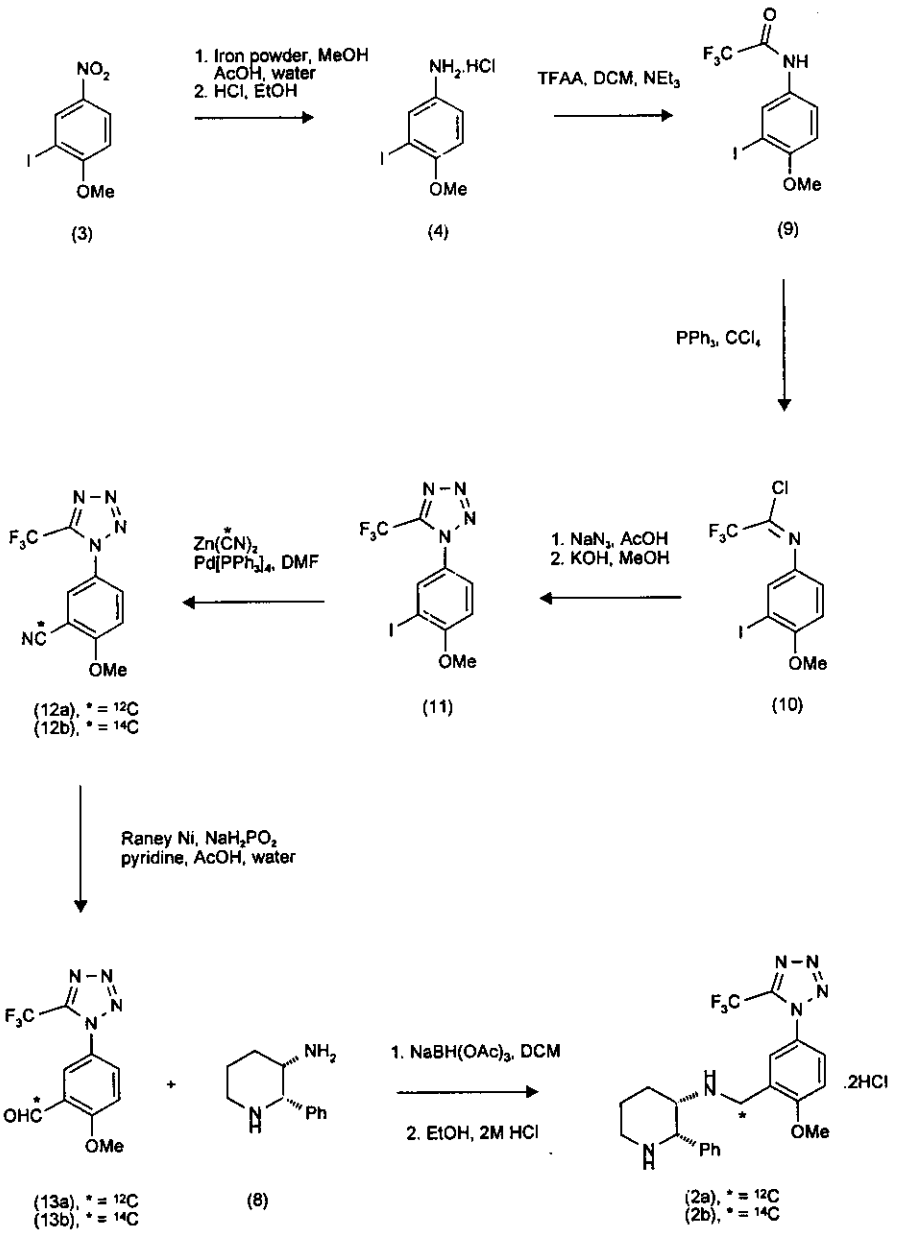
Scheme 1



[^{14}C]GR205171

Key iodo intermediate (11) was prepared in 35% overall yield from 2-iodo-1-methoxy-4-nitrobenzene (3) (Scheme 2). Aniline hydrochloride (4) was obtained by reduction of (3) as in the preparation of (1b). The free base was liberated *in situ* and treated with trifluoroacetic anhydride to give trifluoroacetamide (9) in high yield. The tetrazole was assembled *via* the imidoyl chloride (10) which was isolated in crude form and treated immediately with sodium azide to give (11) in 59% yield (over two steps) (5). However some hydrolysis of imidoyl chloride (10) back to trifluoroacetamide (9) was observed by TLC during this procedure. The reaction mixture was treated with potassium hydroxide in aqueous methanol to hydrolyse any trifluoroacetamide (9) to the corresponding aniline. The unwanted aniline was then removed efficiently by column chromatography. Palladium (0) catalysed cyanation of (11) with zinc cyanide typically gave nitrile (12a) in >90% yield (15). Cyanation under alternative conditions (potassium cyanide with copper (I) iodide in N-methylpyrrolidinone at 150° for 8h) afforded a 51% yield of (12a) (11). These experimental results demonstrated the relative stability of the 1,5-disubstituted tetrazole (11), compared to the mono-substituted tetrazole (5), under the reaction conditions. Reduction of (12a) with Raney nickel and sodium hypophosphite in aqueous buffer afforded the aldehyde (13a) in 80% yield (19). In contrast to the Stephen reduction of (6a) during the preparation of GR203040, the reduction of (12a) proceeded smoothly to completion (<3% nitrile remaining by HPLC). It was subsequently found that nitrile (6a) could also be reduced with buffered Raney nickel in 50% yield. Reductive amination of (13a) with (8) followed by salt formation typically gave GR205171 dihydrochloride (2a) in 75-80% yield (over two steps). Using the same methodology the corresponding radiosynthesis afforded [^{14}C]GR205171 dihydrochloride (2b) in 65% overall yield from zinc [^{14}C]cyanide.

Scheme 2



CONCLUSION

NK-1 receptor antagonists GR203040 (1b) and GR205171 (2b) labelled in the metabolically stable central benzylic methylene position were prepared from zinc [^{14}C]cyanide in 26% and 65% overall yields respectively. Superior yields were obtained in the cyanation and reduction steps during the synthesis of GR205171. This was attributed to the difference in reactivity between the 1,5-disubstituted tetrazole in GR205171 and the mono-substitutedtetrazole in GR203040, under the reaction conditions.

EXPERIMENTAL

General Methods: ^{1}H nuclear magnetic resonance (NMR) spectra were recorded on Bruker AC250 and Varian Unity 400 spectrometers. Low and high resolution mass spectrometry was performed using Hewlett Packard HP5989B Engine and VG Autospec Q spectrometers respectively. All column chromatography was carried out over Merck Kieselgel 60 (9385) silica gel. Melting points are uncorrected. Thin layer chromatography (TLC) was carried out using Merck Kieselgel 60 F_{254} (5714 and 5715) plates. Radiochemicalpurities were determined either by TLC using a Berthold linear analyser, or by HPLC using a Canberra Packard Radiomatic Flo-One βeta detector with liquid scintillation counting. IUPAC names were generated using the ACD/Name Pro 3.6 program.

3-Iodo-4-methoxyaniline hydrochloride (4)

Iron powder (10g, 179mmol) was added to a solution of 2-iodo-1-methoxy-4-nitrobenzene (3) (5g, 17.92mmol) in methanol (250ml) containing water (8ml) and glacial acetic acid (8ml). The reaction was stirred under nitrogen and heated at 40° for 1h. The mixture was filtered through a celite pad and concentrated under reduced pressure to a solid which was re-suspended in dichloromethane (400ml). The suspension was filtered through silica gel (70g) and the filtrate was washed with 8% sodium bicarbonate solution (2x100ml). The aqueous layer was re-extracted with dichloromethane (100ml) and the organic extracts were combined,

dried over magnesium sulphate and concentrated under reduced pressure to give a brown oil (3.2g). The oil was dissolved in diethyl ether (60ml) and to this solution was added anhydrous 2M hydrogen chloride in methanol (10ml) giving a cream suspension. The solid was collected by filtration, washed with diethyl ether (25ml) and dried under vacuum at 20° for 17h to give the title compound (3.23g, 63%) as a cream solid with melting point 263-265°; δ_H (250MHz, DMSO-d_6) 7.80 (1H, d, 2-H), 7.38 (1H, m, 6-H), 7.09 (1H, m, 5-H), 3.87 (3H, s, OCH_3); m/z (Thermospray +ve) 250 (MH^+, 100%) (Found: [Electrospray +ve] 249.973570 [MH+]. C_7H_9INO requires 249.972891).

1-(3-Iodo-4-methoxyphenyl)-1H-tetrazole (5)

3-Iodo-4-methoxyaniline hydrochloride (4) (8.7g, 30.47mmol) was suspended in dichloromethane (1000ml) and 8% sodium bicarbonate solution (300ml) and the mixture was stirred at 20° for 90min. The aqueous layer was separated and extracted with more dichloromethane (400ml). The combined organic extracts were washed with saturated sodium chloride solution (300ml), dried over magnesium sulphate and concentrated under reduced pressure to a solid which was dried under vacuum for 1h (7.53g). The solid was dissolved in glacial acetic acid (42ml) and N,N-dimethylformamide (8.5ml). Sodium azide (2.97g, 45.69mmol) and triethyl orthoformate (7.6ml, 6.77g, 45.7mmol) were added and the stirred suspension was heated at 80° under nitrogen for 2h. The reaction was cooled to 20° , treated with a solution of sodium nitrite (4g, 57.97mmol) in water (32ml) and stirred for 20min. The mixture was partitioned between water (300ml) and ethyl acetate (400ml). The aqueous layer was re-extracted with ethyl acetate (400ml) and the combined organic extracts were washed with 1M hydrochloric acid (400ml) and saturated sodium chloride solution (300ml). The organic extracts were dried over magnesium sulphate and concentrated under reduced pressure to give a solid. The solid was dissolved in ethyl acetate (250ml), adsorbed onto silica gel (20g) and purified by chromatography over silica gel (270g) eluting with dichloromethane-ethyl acetate (98:2) to give the title compound (5.5g, 60%) as a

buff solid with melting point 156-158°; δ_H (250MHz, DMSO-d_6) 10.00 (1H, s, tetrazole H), 8.32 (1H, d, aromatic 3-H), 7.92 (1H, m, aromatic 5-H), 7.25 (1H, m, aromatic 6-H), 3.93 (3H, s, OCH_3); m/z (Thermospray +ve) 303 (MH^+, 100%) (Found: [Electrospray +ve] 302.975666 [MH+]. $C_8H_8IN_4O$ requires 302.974288).

2-Methoxy-5-(1H-tetrazol-1-yl)benzo[^{14}C]nitrile (6b)

To a suspension of zinc [^{14}C]cyanide (115mg, 0.95mmol; 104mCi at 109mCi/mmol) in anhydrous N,N-dimethylformamide (3ml) was added tetrakis(triphenylphosphine)palladium(0) (123mg, 107μmol, 5.6mol%) and 1-(3-iodo-4-methoxyphenyl)-1H-tetrazole (5) (574mg, 1.90mmol). The reaction was stirred under nitrogen and heated at 80° for 5.25h. The reaction was cooled to 20°, diluted with ethyl acetate (220ml) and washed with water (3x80ml). The aqueous washes were extracted with ethyl acetate (95ml). The combined organic extracts were dried with magnesium sulphate and concentrated under reduced pressure to give a solid. The solid was dissolved in dichloromethane (30ml), adsorbed onto silica gel (2g) and purified by chromatography over silica gel (60g) eluting with dichloromethane-ethyl acetate (95:5) to give the title compound (293mg, 76%, 79mCi) as a cream solid. A sample of unlabelled title compound (6a) had been prepared similarly as a cream solid with melting point 189-191°; δ_H (250MHz, $CDCl_3$) 8.99 (1H, s, tetrazole-H), 7.93 (2H, m, aromatic 4-H and 6-H), 7.22 (1H, m, aromatic 3-H), 4.07 (3H, s, OCH_3); m/z (Thermospray +ve) 219 (MNH_4^+, 100%).

2-Methoxy-5-(1H-tetrazol-1-yl)benz[^{14}C]aldehyde (7b)

2-Methoxy-5-(1H-tetrazol-1-yl)benzo[^{14}C]nitrile (6b) (307mg, 1.51mmol, 82mCi) was dissolved in 1,4-dioxane (7ml) and added to a suspension of tin(II)chloride (1.7g, 8.97mmol) in 1,4-dioxane (30ml) which had been previously saturated with hydrogen chloride gas. The reaction was stirred under nitrogen and heated at 80° for 20h. Analysis of the reaction mixture by HPLC showed ca.30% nitrile (6b) remaining (on-line radiochemical detection). The reaction was cooled to 20° and concentrated under reduced pressure using a rotary evaporator and a high vacuum

pump. The resulting brown oil was treated with water (45ml) and heated at 90° for 5min when a suspension formed. After cooling to 20° over 40 min, water (15ml) and ethyl acetate (150ml) was added, the mixture was saturated with solid sodium chloride and stirred vigorously for 20min. The organic layer was washed with water (100ml) and saturated brine (100ml). The aqueous washes were extracted with ethyl acetate (100ml) and the combined organic extracts were dried over magnesium sulphate and concentrated under reduced pressure to a brown solid. The solid was dissolved in ethyl acetate (20ml) and adsorbed onto silica gel (1g). Chromatography over silica gel (30g) eluting with dichloromethane-ethyl acetate (95:5) gave a 7:3 mixture of the title compound (142mg, 0.7mmol, 37.9mCi) and nitrile (6b) (57mg, 0.28mmol, 15.1mCi) respectively, as a white solid. HPLC: Dynamax C18 column (25 x 0.46cm) eluted isocratically with 20% solvent B for 40min. Solvent A = water-TFA (100: 0.1). Solvent B = acetonitrile-water-TFA (95: 5: 0.1). Flow rate 1ml/min, UV detection at 254nm. Retention times: aldehyde (7b) = 20.2min, nitrile (6b) = 28.7min. Ratio aldehyde:nitrile = 7:3 (on-line radiochemical detection). A sample of unlabelled title compound (7a) was prepared by an alternative procedure (20) as a cream solid with melting point 177-179°; δ_H (250MHz, $CDCl_3$) 10.55 (1H, s, C<u>H</u>O), 9.03 (1H, s, tetrazole-<u>H</u>), 8.08 (1H, d, aromatic 6-<u>H</u>), 8.00 (1H, m, aromatic 4-<u>H</u>), 7.27 (1H, d, aromatic 3-<u>H</u>), 4.06 (1H, s, OC<u>H</u>$_3$); m/z (Thermospray +ve) 205 (MH^+, 100%).

(2S,3S)-N-[2-Methoxy-5-(1H-tetrazol-1-yl)[^{14}C]benzyl]-2-phenyl-3-piperidinamine dihydrochloride (1b)

(2S,3S)-2-Phenyl-3-piperidinamine (-)-di-p-toluoyl-L-tartrate (520mg, 0.92mmol) was added to a mixture of concentrated ammonia solution (30ml) and water (30ml) and the resulting solution was extracted with dichloromethane (3 x 30ml). The combined organic layers were dried over magnesium sulphate and concentrated to dryness to give (2S,3S)-2-phenyl-3-piperidinamine (8) (150mg, 93%). A portion of this material (129mg, 0.74mmol) was dissolved in anhydrous dichloromethane (10ml) and the solution was added to crude 2-methoxy-5-(1H-tetrazol-1-yl)benz[^{14}C]aldehyde (7b) (a mixture of aldehyde (7b), 142mg, 0.70mmol,

37.9mCi, and nitrile (6b), 57mg, 0.28mmol, 15.1mCi). To this mixture was added glacial acetic acid (3 drops) and the mixture was stirred at 20° under nitrogen for 5min. To the resulting solution was added sodiumtriacetoxyborohydride (234mg, 1.1mmol) and the mixture was stirred at 20° under nitrogen for 2.3h. More sodium triacetoxyborohydride (234mg, 1.1mmol) was added and stirring was continued for a further 18h. HPLC analysis indicated 66% conversion to [^{14}C]GR203040 with 2% [^{14}C]aldehyde (7b) and 28% [^{14}C]nitrile (6b) remaining (on-line radiochemical detection). The mixture was concentrated under reduced pressure and the residue was diluted with 2M sodium carbonate solution (25ml) and extracted with ethyl acetate (3 x 25ml). The combined organic layers were dried over magnesium sulphate and concentrated under reduced pressure to a solid. Column chromatography over silica gel (30g) eluting with dichloromethane-ethanol-concentrated ammonia solution (95:5:1) gave [^{14}C]GR203040 free base (237mg, 0.65mmol, 35.2mCi) and recovered [^{14}C]nitrile (6b) (52mg, 0.26mmol, 13.9mCi). To a solution of the [^{14}C]GR203040 free base in refluxing ethanol (4.6ml) was added 2M hydrochloric acid (0.72ml, 1.44mmol). The mixture was allowed to cool to 20° and crystallisation occurred. After standing at 20° for 15h the crystalline solid was collected by filtration and washed with ethanol (10ml) before drying under vacuum at 20° to give the title compound (231mg, 0.53mmol, 28.5mCi). HPLC: Dynamax C18 column (25 x 0.46 cm) eluted isocratically with 20% solvent B for 40 min. Solvent A = water-TFA (100: 0.1). Solvent B = acetonitrile-water-TFA (95: 5: 0.1), flow rate 1ml/min, retention time = 6.5min. Chemical purity >98% (UV detection at 215nm and 255nm). Radiochemical purity 99.3% (on-line radiochemical detection). Specific activity (gravimetric) 123μCi/mg, 54.1mCi/mmol. δ_H (400MHz, D_2O) 9.56 (1H, s, tetrazole-H), 7.82 (1H, m, Ar-H), 7.60 (1H, m, Ar-H), 7.50 (3H, m, phenyl-H), 7.35 (2H, m, phenyl-H), 7.15 (1H, dd, Ar-H), 4.96 (1H, d, piperidine 2-H), 4.35 (1H, d, benzyl-H), 4.12 (1H, d, benzyl-H), 4.00 (1H, m, piperidine 3-H), 3.74 (1H, m, NCH_2), 3.70 (3H, s, OCH_3), 3.35 (1H, m, NCH_2), 2.53 (1H, m, piperidine CH_2), 2.26 (1H, m, piperidine

$C\underline{H}_2$), 2.13 (2H, m, piperidine $C\underline{H}_2$); m/z (Electrospray +ve) 365 (21.9%), 366 (6.2%), 367 (MH^+, 100%), 368 (18.9%), 369 (2.5%).

2,2,2-Trifluoro-N-(3-iodo-4-methoxyphenyl)acetamide (9)

To a stirred suspension of 3-iodo-4-methoxyaniline hydrochloride (4) (6.30g, 22.1mmol) in anhydrous dichloromethane (105ml) was added triethylamine (7.08ml, 5.14g, 50.8mmol). To the resulting solution at 0-5° under nitrogen was added trifluoroacetic anhydride (3.42ml, 5.08g, 24.2mmol) over ten minutes. The mixture was allowed to warm to 20° and stirring was continued for 19h. The mixture was poured into diethyl ether (210ml) and washed with 0.5M hydrochloric acid (140ml), water (100ml) and brine (100ml). The organic layer was dried over magnesium sulphate and concentrated under reduced pressure to give the title compound as a white solid (7.04g, 93%) with melting point 163-165°; δ_H (250MHz, $CDCl_3$) 7.95 (1H, d, 2-H), 7.75 (1H, br, NH), 7.60 (1H, dd, 5-H), 6.85 (1H, d, 6-H), 3.90 (3H, s, $OC\underline{H}_3$); m/z (Thermospray +ve) 363 (MNH_4^+, 100%) (Found: [Electrospray +ve] 362.982537 [MNH_4^+]. $C_9H_{11}IF_3N_2O_2$ requires 362.981739).

1-(3-Iodo-4-methoxyphenyl)-5-(trifluoromethyl)-1H-tetrazole (11)

A mixture of 2,2,2-trifluoro-N-(3-iodo-4-methoxyphenyl)acetamide (9) (6.98g, 20.2mmol) and triphenylphosphine (polymer-bound, containing 3mmol of triphenylphosphine per gram of resin, 16.9g, 50.6mmol) in carbon tetrachloride (270ml) was heated at reflux under nitrogen for 24h. The mixture was allowed to cool to 20° and then filtered. The resin was washed withdichloromethane (100ml) followed by diethyl ether (100ml). The filtrate was concentrated under reduced pressure to give 2,2,2-trifluoro-N-(3-iodo-4-methoxyphenyl)ethanimidoyl chloride (10) (5.85g, 80%) as a brown oil. This imidoyl chloride was used immediately in the next step. To a stirred solution of (10) (5.85g, 16.1mmol) in glacial acetic acid (78ml) was added sodium azide (6.57g, 101mmol). The mixture was heated at 70° under nitrogen for 4h. TLC analysis indicated >80% conversion totetrazole (11) with ca. 10% trifluoroacetamide (9) as the major by-product (TLC mobile phase: dichloromethane). The mixture was poured into water (230ml) and extracted with

dichloromethane (3 x 90ml). The combined organic layers were washed with 8% sodium bicarbonate solution (150ml) and brine (100ml). After drying over magnesium sulphate the organic layer was concentrated under reduced pressure to a brown residue. The residue was dissolved in methanol (200ml) and to the solution was added 1M potassium hydroxide solution (20.2ml, 20.2mmol). The mixture was stirred at 20° for 20h, when TLC analysis indicated complete hydrolysis of residual trifluoroacetamide (9) to the corresponding aniline. After concentration under reduced pressure to remove methanol, the residue was dissolved in dichloromethane (200ml) and washed with 1M hydrochloric acid (2 x 100ml), water (100ml), 8% sodium bicarbonate solution (100ml) and brine (100ml). The organic layer was dried over magnesium sulphate and concentrated under reduced pressure. The residue was chromatographed over silica (Merck 9385, 300g) eluting with dichloromethane-cyclohexane (1:1) to give a solid which was triturated with cyclohexane (50ml) at 20° for 5min. The resulting yellow solid was collected by filtration to give the title compound (4.42g, 59% overall yield from trifluoroacetamide (9)) with melting point 73-75°; δ_H (250MHz, $CDCl_3$) 7.93 (1H, d, 6-H), 7.48 (1H, dd, 4-H), 6.98 (1H, d, 3-H), 4.00 (3H, s, OCH_3); m/z (Electrospray +ve) 371 (MH^+, 100%) (Found: [Electrospray +ve] 370.961133 [MH^+]. $C_9H_7IF_3N_4O$ requires 370.961673).

2-Methoxy-5-[5-(trifluoromethyl)-1H-tetrazol-1-yl]benzo[^{14}C]nitrile (12b)

To a suspension of zinc [^{14}C]cyanide (119mg, 0.98mmol; 107mCi at 109mCi/mmol) in anhydrous N,N-dimethylformamide (3ml) was added tetrakis(triphenylphosphine)palladium(0) (108mg, 93μmol) and 1-(3-iodo-4-methoxyphenyl)-5-(trifluoromethyl)-1H-tetrazole (11) (696mg, 1.88mmol). The reaction was stirred under nitrogen and heated at 80° for 20h. The reaction was cooled to 20°, diluted with ethyl acetate (220ml) and washed with water (3x80ml). The aqueous washes were extracted with ethyl acetate (110ml). The combined organic extracts were dried with magnesium sulphate and concentrated under reduced pressure to give a solid. The solid was chromatographed over silica gel (50g) eluting with dichloromethane to give the title compound (507mg, 99%,

106mCi) as a white solid. A sample of unlabelled title compound (12a) had been prepared similarly as a white solid with melting point 124-126°; δ_H (250MHz, $CDCl_3$) 7.70 (2H, m, aromatic 4-H and 6-H), 7.27 (1H, d, aromatic 3-H), 4.07 (3H, s, OCH_3)

2-Methoxy-5-[5-(trifluoromethyl)-1H-tetrazol-1-yl]benz[^{14}C]aldehyde (13b)

To a solution of 2-methoxy-5-[5-(trifluoromethyl)-1H-tetrazol-1-yl] benzo[^{14}C]nitrile (12b) 505mg, 1.86mmol, 105.5mCi) in pyridine-acetic acid-water (2:1:1, 38ml) was added sodium hypophosphite (3.27g, 37.2mmol) followed by Raney nickel (642mg). The mixture was stirred at 80° under nitrogen for 19h. More sodium hypophosphite (3.27g, 37.2mmol) and Raney nickel (642mg) were added and stirring at 80° was continued for a further 44h. Analysis of the reaction mixture by HPLC showed <3% nitrile (12b) remaining (on-line radiochemical detection). After cooling to 20° the mixture was poured into 2M hydrochloric acid (114ml) and extracted with ethyl acetate (3 x 95ml). The combined organic layers were washed with water (95ml), 8% sodium bicarbonate solution (95ml) and brine (95ml). The organic layer was dried with magnesium sulphate and concentrated under reduced pressure to give a solid. Chromatography over silica gel (50g) eluting with dichloromethane gave the title compound (403mg, 79%,83.4mCi) as a white solid. HPLC: Spherisorb S5-ODS2 column (25 x 0.46cm) eluted isocratically with 40% solvent B for 30min. Solvent A = water-TFA (100: 0.1). Solvent B = acetonitrile-water-TFA (95: 5: 0.1). Flow rate 1ml/min. Retention times: aldehyde (13b) = 16.5min, nitrile (12b) = 17.8min. Chemical purity 99.4% (UV detection at 255nm). Radiochemical purity 97.6% (on-line radiochemical detection). A sample of unlabelled title compound (13a) had been prepared similarly as a white solid with melting point 115-117°; δ_H (400MHz, $CDCl_3$) 10.50 (1H, s, CHO), 7.98 (1H, d, aromatic 6-H), 7.67 (1H, dd, aromatic 4-H), 7.25 (1H, d, aromatic 3-H), 4.08 (1H, s, OCH_3); m/z (Electrospray +ve) 273 (MH^+, 10%), 562 ($[2M+NH_4]^+$, 100%).

(2S,3S)-N-[2-Methoxy-5-[5-(trifluoromethyl)-1H-tetrazol-1-yl][^{14}C]benzyl]-2-phenyl-3-piperidinamine dihydrochloride (2b)

(2S,3S)-2-Phenyl-3-piperidinamine (-)-di-p-toluoyl-L-tartrate (1.10g, 1.96mmol) was added to a mixture of concentrated ammonia solution (76ml) and water (76ml) and the resulting solution was extracted with dichloromethane (3 x 76ml). The combined organic layers were dried over magnesium sulphate and concentrated to dryness to give (2S,3S)-2-phenyl-3-piperidinamine (8) (311mg, 90%). A portion of this material (286mg, 1.63mmol) was dissolved in anhydrous dichloromethane (24ml) and the solution was added to 2-methoxy-5-[5-(trifluoromethyl)-1H-tetrazol-1-yl]benz[^{14}C]aldehyde (13b) (402mg, 1.47mmol, 83.3mCi). To this mixture was added glacial acetic acid (8 drops) and the mixture was stirred at 20° under nitrogen for 5min. To the resulting solution was added sodium triacetoxyborohydride (534mg, 2.52mmol) and the mixture was stirred at 20° under nitrogen for 0.5h. More sodium triacetoxyborohydride (534mg, 2.52mmol) was added and stirring was continued for a further 16.5h. The mixture was concentrated under reduced pressure and the residue was diluted with 2M sodium carbonate solution (50ml) and extracted with ethyl acetate (3 x 50ml). The combined organic layers were dried over magnesium sulphate and concentrated under reduced pressure to a solid. Column chromatography over silica gel (50g) eluting with dichloromethane-ethanol-concentrated ammonia solution (95:5:1) gave [^{14}C]GR205171 free base (560mg, 1.29mmol, 73.2mCi. To a solution of the [^{14}C]GR205171 free base in refluxing ethanol (10.3ml) was added 2M hydrochloric acid (1.62ml, 3.24mmol). The mixture was allowed to cool to 20° and crystallisation occurred. After standing at 20° for 18h the crystalline solid was collected by filtration and washed with ethanol (10ml) before drying under vacuum at 20° to give the title compound (613mg, 1.21mmol, 69.3mCi). HPLC:Dynamax C18 column (25 x 0.46 cm) eluted isocratically with 40% solvent B for 30 min. Solvent A = water-TFA (100: 0.1). Solvent B = acetonitrile-water-TFA (95: 5: 0.1), flow rate 1ml/min, retention time = 4.6min. Chemical purity >98.5% (UV detection at 215nm and 255nm). Radiochemical purity 99.5% (on-line

radiochemical detection). Specific activity (gravimetric) 113μCi/mg, 57.3mCi/mmol. δ_H (400MHz, D_2O) 7.70 (1H, dd, Ar-H), 7.55 (1H, m, Ar-H), 7.50 (3H, m, phenyl-H), 7.30 (2H, m, phenyl-H), 7.12 (1H, d, Ar-H), 4.96 (1H, d, piperidine 2-H), 4.40 (1H, d, benzyl-H), 4.10 (1H, d, benzyl-H), 3.97 (1H, m, piperidine 3-H), 3.70 (1H, m, NCH_2), 3.67 (3H, s, OCH_3), 3.32 (1H, m, NCH_2), 2.50 (1H, m, piperidine CH_2), 2.28 (1H, m, piperidine CH_2), 2.08 (2H, m, piperidine CH_2); m/z (FAB +ve) 433 (19.7%), 434 (6.8%), 435 (100%, MH^+), 436 (26.8%), 437 (4.3%).

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