SYNTHESES OF ISOTOPICALLY LABELLED ANGIOTENSIN II RECEPTOR ANTAGONIST GR138950X

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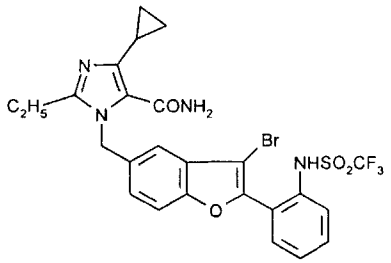
Summary

Syntheses of [^{13}C] and [^{14}C]-labelled versions of angiotensin II receptor antagonist GR138950X, labelled in the imidazole carboxamide residue, are described. These involved preparation of an iodoimidazole substrate by a novel iododecarboxylation procedure, followed by cyanation with a mixture of carbon-labelled potassium cyanide and copper (I) iodide in DMF at high temperature. The preparation of a mass-labelled (M+5) version of GR138950X is also described. This involved the synthesis of an [$^{13}C_3$,$^{15}N_2$]-labelled imidazole from a 1,2,3-tricarbonyl compound, [$^{13}C_3$]propionaldehyde and [^{15}N]ammonia. The labelled imidazole was further elaborated into multiply-labelled GR138950X.

Key Words Angiotensin, carbon-labelled, cyanation, imidazole, iododecarboxylation, triflamide.

Introduction

GR138950X (1) is a non-peptidic angiotensin II (ATI subtype) receptor antagonist, formerly under investigation as a therapeutic agent for the treatment of hypertension and congestive heart failure (1, 2).

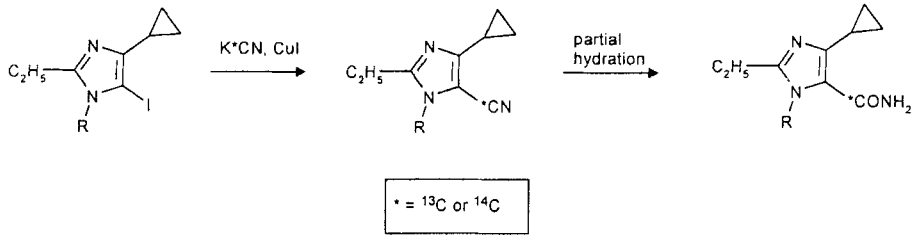


(1) GR138950X

CCC 0362–4803/96/050453–18

Received 24 November 1995
Revised 11 December 1995

A [^{14}C]-labelled version of GR138950X was required for ADME studies. Based on metabolic profiling of a similar compound (3), the carboxamide moiety of (1) was considered to be a suitable site for a carbon label. Recently, we have reported (4) the efficient and selective cyanation of a variety of iodoarenes, utilising mixtures of potassium [^{13}C]cyanide and copper (I) iodide, thereby obviating synthesis of the classical cyanation reagent, copper (I) cyanide. Consequently, a synthetic strategy for the preparation of carbon-labelled GR138950X (1) was envisaged, where cyanation of a suitable iodoimidazole substrate would give a labelled cyanoimidazole. Partial hydration of the cyano group would then afford a labelled carboxamide (Scheme 1). The labelling route was developed using [^{13}C]-labelled derivatives.



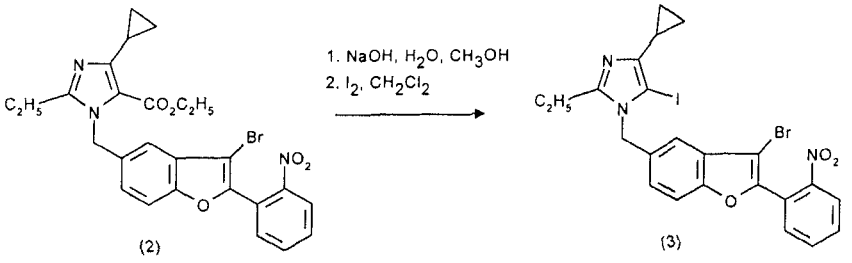
Scheme 1

In addition to the [^{14}C]-labelled version of GR138950X (1), a mass-labelled version of (1) was required as an internal standard for mass spectrometric assay of the drug. Since GR138950X (1) contains a bromine atom, a standard with a molecular weight 5amu higher than the unlabelled version was required to ensure complete separation of labelled and unlabelled molecular ion clusters. The strategy adopted utilised the synthesis of an imidazole from a 1,2,3-tricarbonyl compound by reaction with a mixture of an aldehyde and ammonia (5). By using [$^{13}C_3$]propionaldehyde and [^{15}N]ammonia (from [^{15}N]ammonium acetate and triethylamine), it was anticipated that a mass-labelled (M+5)-imidazole could be synthesised (Scheme 4) and subsequently converted into (M+5)-GR138950X (1c) (Scheme 5).

Results and Discussion

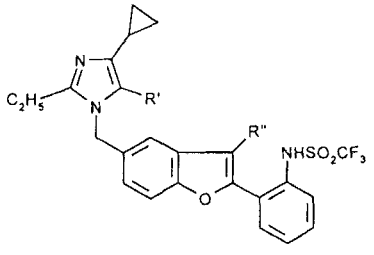
Syntheses of Carbon-Labelled GR138950X (1a) and (1b)

Hydrolysis of imidazolecarboxylic ester (2) (6) with sodium hydroxide in aqueous methanol at reflux gave the carboxylate salt. The resulting solution (at *ca.* pH12) was treated *in situ* with a solution of iodine in dichloromethane, whereupon a rapid iododecarboxylation reaction occurred. The resulting iodoimidazole (3) passed into the organic phase to be isolated in 88% yield from (2) (Scheme 2).



Scheme 2

Reaction of (3) with potassium [^{13}C]cyanide (0.8 equivalents) and copper (I) cyanide (0.2 equivalents) in DMF at 150°C for 15h gave cyanoimidazole (8a) in 81% yield (Scheme 3). There was no evidence for cyanation of the bromobenzofuran. An alternative, potentially shorter labelling route *via* nitrile (5a) was unsuccessful, as attempts to convert iodotriflamide (4) into (5a), using similar cyanation conditions, afforded intractable mixtures.



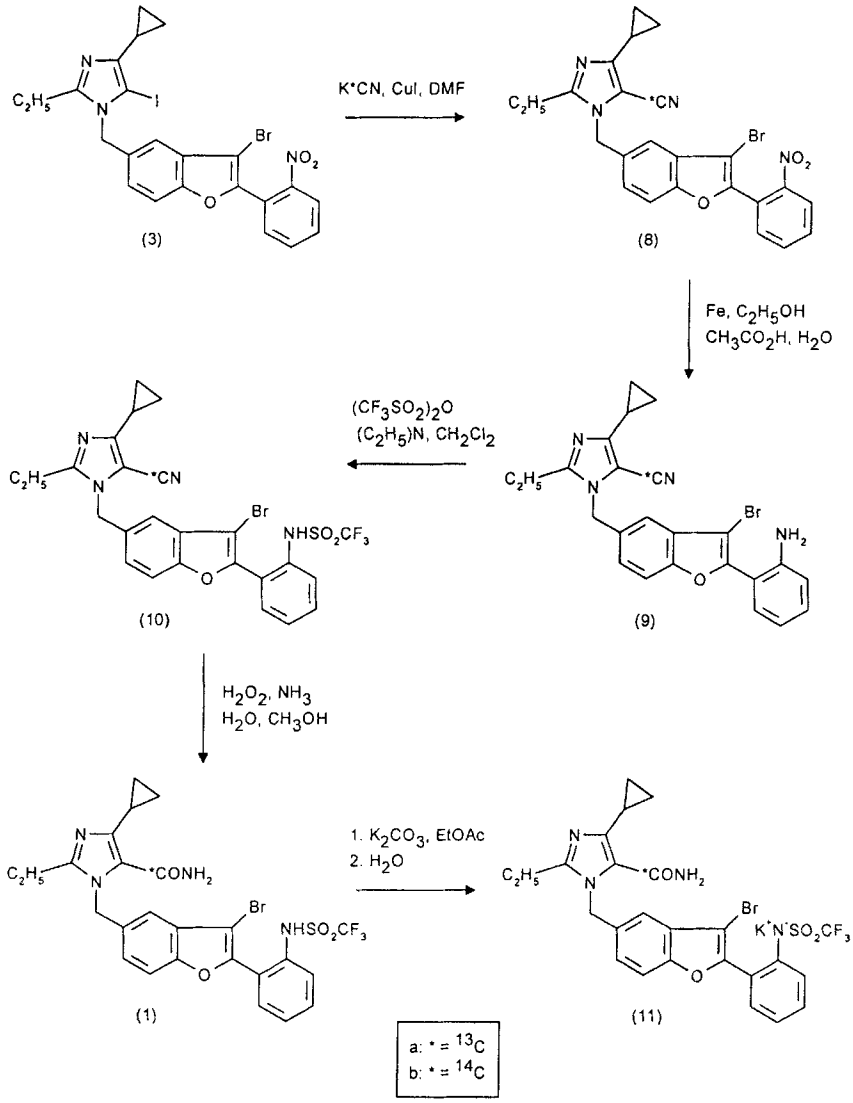
(4) R' = I, R" = Br
(5) R' = *CN, R" = Br
(6) R' = *$CONH_2$, R" = I
(7) R' = *$CONH_2$, R" = H

a: * = ^{13}C
b: * = ^{14}C

Nitro compound (8a) was reduced to aniline (9a) in quantitative yield with iron filings in ethanol-acetic acid-water at reflux. Under these conditions significant hydrodebromination was not detected. Triflamide (10a) was prepared in quantitative yield from (9a) by

treatment with triflic anhydride and triethylamine in dichloromethane at -78°C. Finally, partial hydration of (10a) was achieved using hydrogen peroxide and aqueous ammonia-methanol at 22°C to give (1a) in 64% yield after recrystallisation (Scheme 3). Some batches of (1a) prepared by the route described were shown to contain up to 10% (HPLC, by area) of an impurity that was not resolved by TLC. LCMS and combustion analysis indicated that this was iodobenzofuran (6a). Presumably, iodide anion liberated during the formation of (8a) from (3), displaced bromine from (8a) in a copper-catalysed nucleophilic substitution. The resulting impurity must then have undergone reduction, triflamidation and hydration to give (6a). Chromatographic methods employed had not revealed the presence of these putative impurities. Recrystallisation of contaminated (1a) failed to remove (6a). However, exhaustive treatment of impure (1a) with iron filings in ethanol-acetic acid-water at reflux resulted in selective hydrodeiodination of (6a) to give the more polar deshalobenzofuran (7a), which was readily removed chromatographically to give pure (1a). It was also shown that by increasing the amount of iron filings used in the reduction of (8a) to (9a) and by increasing the reaction time, the amount of (6a) in the derived (1a) could be reduced. Here, hydrodeiodination of the iodo analogue of (9a) presumably occurred to give the deshalo compound which was further elaborated to (7a). Using the latter procedure, pure [^{13}C]GR138950X (1a) was obtained in overall yield of 43% from potassium [^{13}C]cyanide.

The radiosynthesis and purification of [^{14}C]GR138950X (1b) from potassium [^{14}C]cyanide was performed using the methodology developed for the preparation of (1a). In this case, HPLC analysis revealed the presence of 5.8% iodo impurity (6b). This was removed by treatment with iron in ethanol as described above, to give (1b) in overall radiochemical yield of 30% from potassium [^{14}C]cyanide. A portion of (1b) was combined with unlabelled (1) before recrystallisation. Treatment of the resulting diluted (1b) with aqueous potassium carbonate and ethyl acetate, gave the ethyl acetate solvate of the triflamide potassium salt. Addition of a small volume of water then gave hydrated crystalline potassium salt (11b) in 40% radiochemical yield from undiluted (1b).

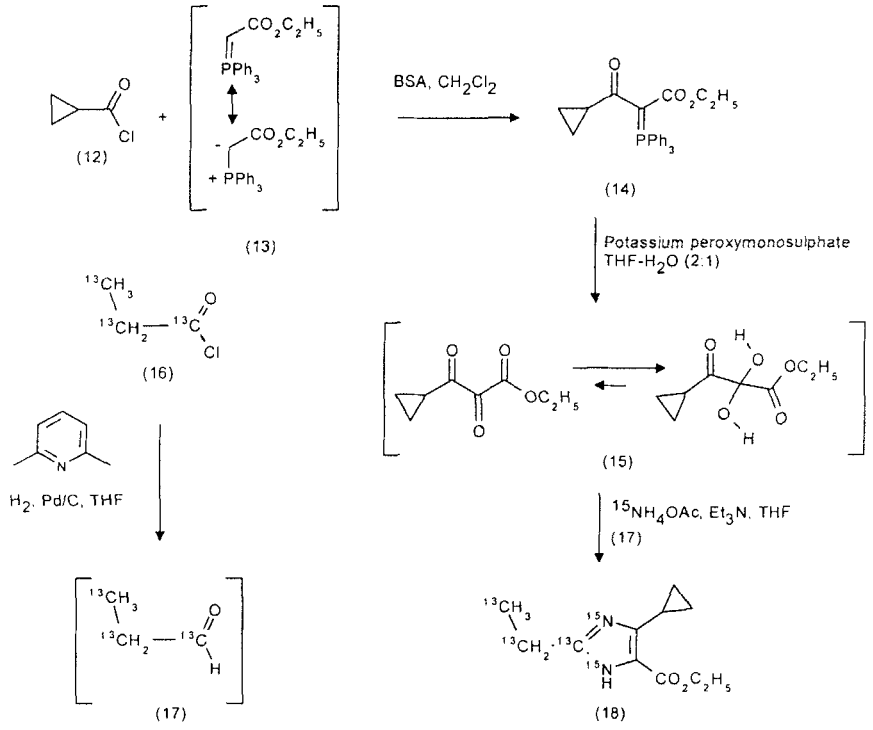


Scheme 3

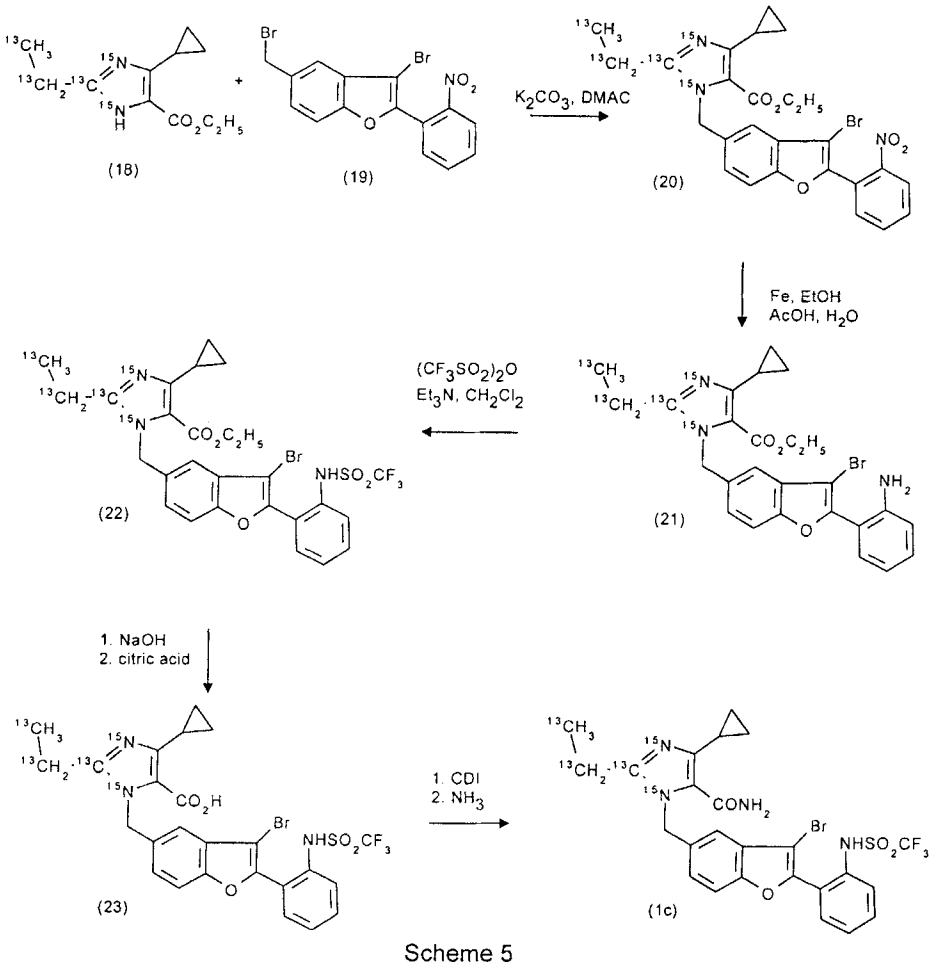
Synthesis of (M+5) Mass-Labelled Version of GR138950X

The required 1,2,3-tricarbonyl compound (15) was prepared as shown (Scheme 4). Reaction of cyclopropylcarbonyl chloride (12) with phosphorane (13) and *N,O*-bis(trimethylsilyl)acetamide (BSA) as acid scavenger, gave (14) in 78% yield. Oxidative cleavage of (14) with potassium peroxymonosulphate (7) in tetrahydrofuran-water (2:1) at 20°C afforded (15) (as its stable monohydrate) in 71% yield. [$^{13}C_3$]Propionaldehyde (17) was prepared by the catalytic hydrogenation of [$^{13}C_3$]propionyl chloride (16) in

tetrahydrofuran, using 10% palladium-on-charcoal as catalyst and 2,6-lutidine as base (8). After removal of catalyst, the solution of (17) was immediately treated with [^{15}N]ammonium acetate, triethylamine and (15) to give imidazole (18) in 60% overall yield from [$^{13}C_3$]propionyl chloride (16). Imidazole (18) was converted into [$^{13}C_3$,$^{15}N_2$]GR138950X (1c) using the methodology developed for the synthesis of unlabelled (1) (Scheme 5) (6). Coupling of bromomethylbenzofuran (19) with imidazole (18) afforded (20) in 94% yield. This was reduced with iron in ethanol-acetic acid-water to give aniline (21) in 90% yield. Treatment of (21) with triflic anhydride in ethyl acetate gave triflamide ester (22) in 91% yield. Alkaline hydrolysis of (22) gave a quantitative yield of carboxylic acid (23), which was activated with 1,1'-carbonyldiimidazole. The resulting imidazolide was treated with ammonia to give (1c) in 75% yield.



Scheme 4



Scheme 5

Conclusion

A novel iododecarboxylation reaction afforded a suitable precursor for the syntheses of [^{13}C] and [^{14}C]-labelled versions of GR138950X in high yield from labelled potassium cyanide in four stages. A mass labelled imidazole (M+5) was prepared from a 1,2,3-tricarbonyl compound, [$^{13}C_3$]propionaldehyde and [^{15}N]ammonia and converted to [$^{13}C_3$,$^{15}N_2$]GR138950X in good yield.

Experimental

General Methods: ^{1}H nuclear magnetic resonance (NMR) spectra were recorded on Bruker AC250, Varian XL200 and Varian Unity 400MHz spectrometers. ^{13}C NMR spectra were recorded on Bruker AC250 and Varian Unity 400 spectrometers. Infra Red (IR) spectra were recorded on a Nicolet 20SXB FT-IR spectrometer. Low and high resolution mass spectrometry (MS) were performed using Finnigan 4600 quadrupole and VG Autospec spectrometers respectively. Elemental analyses were carried out using Carlo Erba 1106 and Perkin-Elmer 240C microanalysers. Merck silica gel Kieselgel 60 (9385) was used throughout for flash column chromatography. Melting points are uncorrected.

1-[3-Bromo-2-(2-nitrophenyl)benzofuran-5-ylmethyl]-4-cyclopropyl-2-ethyl-5-iodo-1H-imidazole (3): A stirred mixture of imidazolecarboxylic ester (2) (6.90g, 12.82mmol), 1M aqueous sodium hydroxide (128ml) and methanol (350ml) was heated at reflux for 135min. The solution was allowed to cool and methanol removed by evaporation. Saturated brine (260ml) and dichloromethane (350ml) were added and the aqueous phase adjusted from pH13.2 to pH12.0 by addition of 5M hydrochloric acid. A solution of iodine (3.25g, 12.80mmol) in dichloromethane (100ml) was added dropwise to the stirred mixture whilst keeping the aqueous phase between pH11.5 and pH12.5 by simultaneous dropwise addition of 1M aqueous sodium hydroxide. The mixture was then stirred at 20°C for 10min and the aqueous phase adjusted to pH 7.0 by addition of 5M hydrochloric acid. Saturated aqueous sodium metabisulphite was added dropwise to destroy excess iodine, and the dichloromethane phase isolated. The aqueous phase was re-extracted with dichloromethane (150ml) and the combined extracts clarified with saturated brine (400ml) then dried over exsiccated magnesium sulphate. Solvent was removed by evaporation and the residue purified chromatographically on silica (300g) eluted with dichloromethane-methanol (40:1) then dichloromethane-methanol (20:1). The product was re-dissolved in dichloromethane (150ml) and diisopropyl ether (250ml) added. Dichloromethane was removed by evaporation and further diisopropyl ether (50ml) added. The resulting precipitate was isolated by filtration, and dried *in vacuo* to give the *title compound* as a crystalline solid (6.67g, 87.9%), m.p. 177-178°C (dec.). ν_{max} (Nujol)/cm^{-1} 1530 (NO_2, asy), 1462 (C=N), 1353 (NO_2, sym); δ_H (250MHz, DMSO-d_6) 8.23 (1H, d, nitrophenyl 6H), 8.02-7.90 (2H, m, nitrophenyl 3H and 4H), 7.86 (1H, m, nitrophenyl 5H), 7.72 (1H, d, benzofuran 7H), 7.28 (1H, s, benzofuran 4H), 7.10 (1H, d, benzofuran 6H), 5.34 (2H, s, NCH_2), 2.65 (2H, q, CH_2CH_3), 1.77 (1H, m, cyclopropyl CH), 1.08 (3H, t, CH_2CH_3), 0.90-0.70 (4H, m, cyclopropyl CH_2CH_2); m/z (Thermospray) 592/594 (MH^+,

100%), 466/468 ($MH\text{-}I^+$, 21%); (Found: C, 46.7; H, 3.2; N, 7.1, Br 13.7; I, 21.6%. $C_{23}H_{19}BrIN_3O_3$ requires: C, 46.6; H, 3.2; N, 7.1; Br, 13.5; I, 21.4%).

3-[3-Bromo-2-(2-nitrophenyl)-benzofuran-5-ylmethyl]-5-cyclopropyl-2-ethyl-3H-imidazole-[4-^{13}C]-carbonitrile (8a):

A stirred mixture of (3) (573mg, 0.968mmol), potassium [^{13}C]cyanide (51mg, 0.775mmol) and finely-ground copper (I) iodide (29mg, 0.150mmol) in DMF (4ml) was heated at 150°C for 21h. After cooling, the reaction mixture was diluted with ethyl acetate (80ml). The resulting solution was washed with 1% w/v aqueous iron (III) chloride (160ml) and the aqueous phase re-extracted with ethyl acetate (40ml). The combined organic extracts were then sequentially washed with water (160ml), sodium metabisulphite (1g) in water (160ml), water (160ml) and saturated brine (160ml) before being dried over exsiccated magnesium sulphate. Solvent was removed by evaporation and the residue purified chromatographically on silica (80g) eluted with ethyl acetate-cyclohexane (1:2). The resulting oil was triturated with diethyl ether to give the *title compound* as a crystalline solid (306mg, 80.5% from potassium [^{13}C]cyanide) m.p. 116-118°C; ν_{max} (Nujol)/cm^{-1} 2160 ($^{13}C{\equiv}N$), 1531 (NO_2, asy), 1355 (NO_2, sym); δ_H (250MHz, DMSO-d_6) 8.22 (1H, d, nitrophenyl 6H), 8.05-7.95 (2H, m, nitrophenyl 3H and 4H), 7.84 (1H, m, nitrophenyl 5H), 7.74 (1H, d, benzofuran 7H), 7.44 (1H, s, benzofuran 4H), 7.24 (1H, dd, benzofuran 6H), 5.43 (2H, s, NCH_2), 2.74 (2H, q, CH_2CH_3), 1.98 (1H, m, cyclopropyl CH), 1.16 (3H, t, CH_2CH_3), 1.05-0.80 (4H, m, cyclopropyl CH_2CH_2); δ_C (63MHz, DMSO-d_6) 113.3 (^{13}CN enhanced); (Found: m/z [LSIMS +ve] 494.072800 [MH^+]. $C_{23}{}^{13}C_1H_{20}{}^{81}Br_1N_4O_3$ requires 494.073186).

3-[3-Bromo-2-(2-nitrophenyl)-benzofuran-5-ylmethyl]-5-cyclopropyl-2-ethyl-3H-imidazole-[4-^{14}C]-carbonitrile (8b)

Iodoimidazole (3) underwent cyanation with potassium [^{14}C]cyanide (125mCi at 56.3mCi/mmol) and copper (I) iodide using the methodology described for the preparation of (8a), to give the *title compound* (82.9mCi, radiochemical yield 66.3%).

3-[2-(2-Aminophenyl)-3-bromo-benzofuran-5-ylmethyl]-5-cyclopropyl-2-ethyl-3H-imidazole-4-[^{13}C]carbonitrile (9a).

A mixture of (8a) (289mg, 0.586mmol), iron filings (10.19g, 180.3mmol), acetic acid (2.2ml), water (2.2ml) and ethanol (54ml) was stirred rapidly at reflux for 6.0h. The mixture was allowed to cool

then filtered through a bed of Celite J2 which was in turn washed with dichloromethane (90ml). Solvents were removed by evaporation and the residue re-dissolved in dichloromethane (110ml). Iron salts were removed by filtration and the filtrates washed with saturated aqueous sodium hydrogen carbonate (100ml) which was in turn extracted with dichloromethane (45ml). The combined organic extracts were dried over exsiccated magnesium sulphate and solvent removed by evaporation. The residue was purified chromatographically on silica (80g), eluted with dichloromethane-ethyl acetate (20:1). The product was recrystallised from boiling acetonitrile (1ml) and the product dried *in vacuo* giving the *title compound* as a crystalline solid (219.9mg, 81.1%). m.p. 164-166°C.; ν_{max} (Nujol)/cm^{-1} 3440, 3393 (NH_2), 2162 (C≡N); δ_H (200MHz, DMSO-d_6) 7.68 (1H, d, benzofuran 7H), 7.40-7.10 (4H, m, benzofuran 4H and 6H, aniline 3H and 5H), 6.81 (1H, d, aniline 6H), 6.64 (1H, t, aniline 4H), 5.48 (2H, br s, NH_2), 5.41 (2H, s, NCH_2), 2.72 (2H, q, CH_2CH_3), 1.95 (1H, m, cyclopropyl CH), 1.15 (3H, t, CH_2CH_3), 1.05-0.80 (4H, m, cyclopropyl CH_2CH_2); δ_C (63MHz, DMSO-d_6) 111.9 (^{13}CN enhanced); m/z (Thermospray) 462/464 (MH^+, 100%); (Found: m/z [Electrospray +ve] 462.101175 [MH^+]. $C_{23}{}^{13}C_1H_{22}{}^{79}Br_1N_4O$ requires 462.101052).

3-[2-(2-Aminophenyl)-3-bromo-benzofuran-5-ylmethyl]-5-cyclopropyl-2-ethyl-3H-imidazole-4-[^{14}C]carbonitrile (9b).

Nitroarene (8b) (82.7mCi) was reduced using the methodology described for the preparation of (8a) to give the *title compound* (70.1mCi, radiochemical yield 84.8%).

N-{2-[3-Bromo-5-(5-[^{13}C]cyano-4-cyclopropyl-2-ethyl-imidazol-1-ylmethyl)-benzofuran-2-yl]-phenyl}-C,C,C-trifluoromethanesulphonamide (10a)

A solution of (9a) (202mg, 0.436mmol) and triethylamine (44mg, 0.436mmol) in dichloromethane (33ml) was cooled to -78°C under nitrogen. Trifluoromethanesulphonic anhydride (123mg, 0.436mmol) in dichloromethane (2ml) was added. After 10min TLC (silica, dichloromethane-methanol 20:1) indicated *ca.* 50% reaction. Further trifluoromethanesulphonic anhydride (92mg, 0.33mmol) in dichloromethane (1.3ml) was added followed by triethylamine (17mg, 0.17mmol) in dichloromethane (1.1ml). After a further 10min, TLC indicated that reaction was complete. Water (4ml) was added and the mixture allowed to warm to 20°C. After addition of water (50ml) the organic phase was isolated. The aqueous phase was re-extracted with dichloromethane (25ml) and the combined extracts dried over exsiccated magnesium sulphate. Solvent was removed by

evaporation giving the *title compound* as an amorphous solid (266mg, 102.5% uncorrected); ν_{max} (Nujol)/cm^{-1} 2162 (C≡N), 1560 (C=N), 1377 and 1144 ($NH\underline{S}O_2CF_3$); δ_H (400MHz, $CDCl_3$) 7.85 (1H, dd, phenyltriflamide 3$\underline{H}$), 7.69 (1H, dd benzofuran 6$\underline{H}$), 7.54 (1H, d, benzofuran 7$\underline{H}$), 7.60-7.45 (2H, m, phenyltriflamide 4$\underline{H}$ and 5$\underline{H}$), 7.37 (1H, d, benzofuran 4$\underline{H}$), 7.18 (1H, dd, phenyltriflamide 6$\underline{H}$), 5.27 (2H, s, $NC\underline{H}_2$), 2.67 (2H, q, $C\underline{H}_2CH_3$), 2.02 (1H, m, cyclopropyl C$\underline{H}$), 1.24 (3H, t, $CH_2C\underline{H}_3$), 1.05-0.95 (4H, m, cyclopropyl $C\underline{H}_2C\underline{H}_2$); δ_C (63MHz, DMSO-d_6) 112.4 ($^{13}\underline{C}N$ enhanced); m/z (Thermospray +ve) 594/596 (MH^+, 100%), 632/634 (53%); (Found: m/z [Electrospray +ve] 594.049784 [MH^+]. $C_{24}{}^{13}C_1H_{21}{}^{79}Br_1F_3N_4O_3S$ requires 594.050338).

N-{2-[3-Bromo-5-(5-[^{14}C]cyano-4-cyclopropyl-2-ethyl-imidazol-1-ylmethyl)-benzofuran-2-yl]-phenyl}-C,C,C-trifluoromethanesulphonamide (10b)

Aniline (9b) (70.1mCi) was converted to the triflamide using the methodology described for the preparation of (10a) to give the *title compound* (65.8mCi, 93.9% radiochemical yield).

3-{3-Bromo-2-[2-(Trifluoromethanesulphonylamino)-phenyl]-benzofuran-5-ylmethyl}-5-cyclopropyl-2-ethyl-3H-imidazole-4-[^{13}C]-carboxylic amide (1a)

To (10a) (253mg, 0.425mmol) were added water (13ml), aqueous ammonia (S.G. 0.880, 13ml), methanol (5ml) and hydrogen peroxide (27.5% w/v, 11ml). The mixture was stirred at 20°C for 80min. Ethyl acetate (100ml) and water (100ml) were added and the aqueous phase adjusted from pH10.8 to pH2.0 by addition of 11M hydrochloric acid (*ca.* 20ml). The organic phase was isolated and the aqueous phase re-extracted with ethyl acetate (50ml). The combined extracts were washed sequentially with a solution of sodium metabisulphite (9g) in water (100ml), water (100ml), and saturated brine (100ml) then dried over exsiccated magnesium sulphate. Solvent was removed by evaporation and the residue purified chromatographically on silica (33g) eluted with dichloromethane-methanol (10:1). The product was recrystallised from boiling ethanol-water (1:1, 14ml) giving the *title compound* (167mg, 64.2%). m.p. 208-210°C. ν_{max} (Nujol)/cm^{-1} 1648 (carboamide C=O), 1377, 1154 ($NH\underline{S}O_2CF_3$); δ_H (250MHz, DMSO-d_6) 7.96 (2H, s, $CONH_2$), 7.57 (1H, d, benzofuran 7$\underline{H}$), 7.55-7.30 (4H, m, benzofuran 4$\underline{H}$, phenyltriflamide 3$\underline{H}$, 4$\underline{H}$ and 5$\underline{H}$), 7.19 (1H, dd, benzofuran 6$\underline{H}$), 7.09 (1H, br s, phenyltriflamide 6$\underline{H}$), 5.68 (2H, s, $NC\underline{H}_2$), 2.93 (2H, q, $C\underline{H}_2CH_3$), 2.17 (1H, m, cyclopropyl C$\underline{H}$), 1.15 (3H, t, $CH_2C\underline{H}_3$), 1.10-0.80 (4H, m, cyclopropyl $C\underline{H}_2$); δ_C (63MHz, DMSO-d_6) 160.1 ($^{13}\underline{C}ONH_2$ enhanced) m/z (Thermospray) 612/614 (MH^+,

100%); (Found: C, 48.7; H, 3.6; N, 9.0, Br 12.8; F, 9.2%. $C_{24}{}^{13}C_1H_{22}BrF_3N_4O_4S$ requires: C, 49.1; H, 3.6; N, 9.2; Br, 12.8; F, 9.3%.

3-{3-Bromo-2-[2-(Trifluoromethanesulphonylamino)-phenyl]-benzofuran-5-ylmethyl}-5-cyclopropyl-2-ethyl-3H-imidazole-4-[^{14}C]-carboxylic amide (1b)

Nitrile (10b) (65.8mCi) was converted to carboxamide (1b), using the methodology described for the preparation of (1a), giving, after chromatographic purification, impure *title compound* (58.2mCi) shown by HPLC to contain 5.8% a/a iodobenzofuran (6b). Absolute ethanol (70ml), acetic acid (2ml), water (2ml) and iron powder (10g) were added and the mixture stirred rapidly at reflux for 6h. Solids were removed by filtration through Celite J2. Solvents were removed from the filtrate by evaporation and the residue re-dissolved in ethyl acetate (100ml). The solution was stirred with saturated aqueous sodium hydrogen carbonate (100ml) and the mixture again filtered through Celite J2. The organic phase was isolated and the aqueous phase re-extracted with ethyl acetate (100ml and 30ml). The combined extracts were dried over exsiccated magnesium sulphate then purified chromatographically on silica (400g) eluted with dichloromethane-methanol (10:1). The product was recrystallised from boiling ethanol-water (1:1) (20ml) to give pure *title compound* (438mg, 37.7mCi at 52.8mCi/mmol, 57.3% radiochemical yield).

3-{3-Bromo-2-[2-(Trifluoromethanesulphonylamino)-phenyl]-benzofuran-5-ylmethyl}-5-cyclopropyl-2-ethyl-3H-imidazole-4-[^{14}C]-carboxylic amide potassium salt (11b)

A mixture of (1b) (152mg, 13.1mCi) and (1) (100mg) was recrystallised twice from boiling ethanol-water (1:1) (19.2ml and 16.0ml) to give (1b) (207mg, 82.1%, 10.7mCi at 31.7mCi/mmol,). To a solution of anhydrous potassium carbonate (620mg, 4.50mmol) in water (0.7ml) at 20°C were added diluted (1b) (205mg, 0.330mmol, 10.6mCi) and ethyl acetate (1.7ml) with stirring. The organic phase was isolated and the aqueous phase re-extracted with ethyl acetate (2 x 0.9ml). The combined extracts were dried over anhydrous potassium carbonate (500mg) and concentrated to 0.6ml by evaporation. After 10min tert-butyl methyl ether (1.36ml) was added and the mixture stirred at 20°C for 18h. The crystalline precipitate was isolated by filtration, washed with tert-butyl methyl ether (2ml), and dried *in vacuo* to give the potassium salt ethyl acetate solvate (198mg, 80%). This was dissolved in water (0.3ml) at 35°C and the solution allowed to cool to 20°C then seeded with (11a). The mixture was stirred at 20°C for 2h then kept for 18h at 5°C. The resulting precipitate was isolated by filtration, washed with water at 5°C (5 x 0.35ml),

and dried for 4h at 20° *in vacuo* giving the *title compound* (108mg, 5.2mCi, 40% radiochemical yield from (1b).

3-Cyclopropyl-3-oxo-2-(triphenylphosphanylidene)-propionic acid ethyl ester (14)

To a stirred mixture of carboethoxymethylene triphenylphosphorane (11.28g, 32.38mmol) and bis(trimethylsilyl)- acetamide (7.90g, 38.85mmol) in dichloromethane (150ml) under nitrogen at 10°C, was added a solution of cyclopropanecarbonyl chloride (3.38g, 32.37mmol) in dichloromethane (10ml). The mixture was allowed to warm to 20°C then stirred for 120min and washed with water (300ml). The aqueous phase was re-extracted with dichloromethane (150ml, 2 x 100ml) and solvent removed from the combined organic phases by evaporation. The residue was redissolved in methanol (50ml) which was in turn removed by evaporation. Methanol (60ml) was added and the solution warmed to 50°C. Water (30ml) was added with rapid stirring whereupon crystallisation proceeded spontaneously. The mixture was kept undisturbed for 5min at 40°C and more water (60ml) added. After cooling to 5°C, the solid product was isolated by filtration, washed with methanol-water (3:2, 30ml), then water (30ml), and finally dried for 17h at 40°C *in vacuo* to give the *title compound* as a white, crystalline solid, (10.55g, 78.2%) m.p. 151-153°C. ν_{max} (Nujol)/cm^{-1} 1664 (conj. C=O); δ_H (400MHz, DMSO-d_6) 7.75-7.40 (15H, m, $C_6\underline{H}_5$), 3.73 (2H, q, $C\underline{H}_2CH_3$), 3.37 (1H, m, cyclopropyl C<u>H</u>), 0.85-0.70 (4H, m, cyclopropyl $C\underline{H}_2$), 0.65 (3H, t, $CH_2C\underline{H}_3$); m/z (Thermospray) 417 (MH^+, 100%); (Found: C, 74.9; H, 5.9%. $C_{26}H_{25}O_3P$ requires: C, 75.0; H, 6.1%).

3-Cyclopropyl-2,2-dihydroxy-3-oxo-propionic acid ethyl ester (15)

To a stirred solution of (14) (6.03g, 14.49mmol) in tetrahydrofuran-water (2:1, 90ml) at 20°C was added potassium peroxymonosulphate (Oxone®, 13.35g, 21.72mmol). After 4.75h water (450ml) and dichloromethane (200ml) were added and the organic phase isolated. The aqueous phase was re-extracted with dichloromethane (2 x 150ml) and the combined extracts clarified with saturated brine (400ml) then dried over exsiccated magnesium sulphate. Solvent was removed almost completely by evaporation and the resulting concentrate purified chromatographically on silica (300g) eluted with ethyl acetate giving the *title compound* as an oil (1.94g, 71.2%). ν_{max} (Nujol)/cm 1737 (ester C=O), 1702 (ketonic C=O); δ_H (250MHz, $CDCl_3$) 5.08 (2H, s, O<u>H</u>), 4.35 (2H, q, $C\underline{H}_2CH_3$), 2.16 (1H, m, cyclopropyl C<u>H</u>), 1.32 (3H, t, $CH_2C\underline{H}_3$), 1.30-1.10 (4H, m,

cyclopropyl C$\underline{H}_2$); δ_C (100MHz, $CDCl_3$) 203.4 ($C_3H_7\underline{C}O$), 169.4 ($\underline{C}O_2C_2H_5$), 92.6 ($\underline{C}(OH)_2$), 63.3 ($\underline{C}H_2CH_3$), 15.9 ($CH_2\underline{C}H_3$), 13.9 (cyclopropyl $\underline{C}H_2$), 13.2 (cyclopropyl $\underline{C}H$).

5-Cyclopropyl-2-[1,2-^{13}C]-ethyl-3H-[1,3-^{15}N, 2-^{13}C]-imidazole-4-carboxylic acid ethyl ester (18)

To 10% palladium on charcoal catalyst (75mg) under nitrogen were added [$^{13}C_3$]propionyl chloride (500mg, 5.24mmol) in THF (20ml) and 2,6-lutidine (561mg, 5.24mmol) in THF (30ml). The mixture was stirred under hydrogen at 20°C and atmospheric pressure for 2h then filtered through a bed of Hyflo Supercel. The bed was washed with THF (10ml) and the filtrates combined. To this solution of [$^{13}C_3$]propionaldehyde (17) were added (15) (1145mg, 6.09mmol), [^{15}N]ammonium acetate (1.947g, 24.94mmol) and triethylamine (2.523g, 24.93mmol). The mixture was stirred at 65°C for 60min. Solvent was removed by evaporation. Saturated brine (250ml) and dichloromethane (250ml) were added and the organic phase isolated. The aqueous phase was re-extracted with dichloromethane (2 x 125ml) and the combined extracts dried over anhydrous sodium sulphate. Solvent was removed by evaporation and the residue re-dissolved in dichloromethane then purified chromatographically on silica (300g), eluted with dichloromethane-ethyl acetate (1:1), to give the *title compound* as a white, crystalline solid (664mg, 60% from [$^{13}C_3$]propionyl chloride) m.p. 198-200°C. ν_{max} (Nujol)/cm^{-1} 1698 (conj. ester C=O); δ_H (250MHz, $CDCl_3$) 9.46 (1H, d, N$\underline{H}$, J^1H-^{15}N 98Hz), 4.36 (2H, q, $CO_2C\underline{H}_2CH_3$), 2.76 (2H, m, $^{13}CH_3{}^{13}C\underline{H}_2$, J^1H-^{13}C 112Hz), 2.45 (1H, m, cyclopropyl C$\underline{H}$), 1.37 (3H, t, $CO_2CH_2C\underline{H}_3$), 1.28 (3H, m, $^{13}C\underline{H}_3{}^{13}CH_2$, J^1H-^{13}C 133Hz), 1.10-0.90 (4H, m, cyclopropyl $\underline{C}H_2$); δ_C (100MHz, $CDCl_3$) 152.3 (imidazole $^{13}\underline{C}2$ enhanced, $J^{13}C$-^{13}C 50Hz), 22.1 ($^{13}\underline{C}H_3$ enhanced, $J^{13}C$-^{13}C 50Hz, 34Hz), 12.8 ($^{13}\underline{C}H_3$ enhanced, $J^{13}C$-^{13}C 34Hz); m/z (Chemical Ionisation) 214 (M^+, 100%); (Found: m/z [Electrospray] 214.133159 [MH^+]. $C_8{}^{13}C_3H_{17}{}^{15}N_2O_2$ requires 214.133137).

3-[3-Bromo-2-(2-nitrophenyl)--5-benzofuran-5-ylmethyl]-5-cyclopropyl-2-[1,2-^{13}C]-ethyl-3H-[1,3-^{15}N, 2-^{13}C]-imidazole-4-carboxylic acid ethyl ester (20)

To a suspension of (18) (622mg, 2.91mmol) in N,N-dimethylacetamide (4ml) were added (19) (1.438g, 3.50mmol) and finely-ground potassium carbonate (967mg, 7.00mmol). The mixture was stirred for 20h at 20°C then diluted with ethyl acetate (200ml). The solution was extracted sequentially with water (200ml), a mixture of water (200ml) and saturated brine (50ml), and finally, saturated brine (200ml) then dried over anhydrous sodium sulphate. Solvent was removed by

evaporation under reduced pressure and the residue purified chromatographically on silica (300g) eluted with dichloromethane-ethyl acetate (8:1) giving the *title compound*, as a yellow foam (1.492g, 94.2% from 18). ν_{max} (Nujol)/cm^{-1} 1689 (conj. ester C=O), 1530 (asy. NO_2), 1352 (sy NO_2). δ_H (400MHz, $CDCl_3$) 8.08, (1H, d, nitrophenyl 6H), 7.85 (1H, d, nitrophenyl 3H), 7.74 (1H, t, nitrophenyl 5H), 7.63 (1H, t, nitrophenyl 4H), 7.42 (1H, d, benzofuran 7H), 7.22 (1H, s, benzofuran 4H), 7.01 (1H, d, benzofuran 6H), 5.64 (2H, d, J^1H-^{15}N 2Hz, NCH_2), 4.28 (2H, q, $CO_2CH_2CH_3$), 2.68 (2H, m, J^1H-^{13}C 115Hz, $^{13}CH_2$), 2.63 (1H, m, cyclopropyl CH), 1.34 (3H, t, $CO_2CH_2CH_3$), 1.22 (3H, m, J^1H-^{13}C 130Hz, $^{13}CH_3$), 1.15-0.90 (4H, m, cyclopropyl CH_2); δ_C (100MHz, $CDCl_3$) 152.9 (imidazole $^{13}C_2$ enhanced, $J^{13}C$-^{13}C 50Hz), 20.0 ($^{13}CH_2$ enhanced, $J^{13}C$-^{13}C 50Hz, 34Hz), 11.8 ($^{13}CH_3$ enhanced, $J^{13}C$-^{13}C 34Hz). (Found: m/z [Electrospray +ve] 543.102146 [MH^+]. $C_{23}{}^{13}C_3H_{25}{}^{79}Br_1N^{15}N_2O_5$ requires 543.101892).

3-[2-(2-Aminophenyl)-3-bromo-benzofuran-5-ylmethyl]-5-cyclopropyl-2-[1,2-^{13}C]-ethyl-3H-[1,3-^{15}N, 2-^{13}C]-imidazole-4-carboxylic acid ethyl ester (21)

To a solution of (20) (1.448g, 2.66mmol) in absolute ethanol (30ml) were added glacial acetic acid (800mg, 0.76ml, 13.3mmol), water (2.9ml) and iron filings (325 mesh, 744mg, 13.3mmol). The mixture was stirred rapidly at reflux for 1h then allowed to cool to 20°C and filtered through a bed of Hyflo Supercel which was in turn washed with dichloromethane (60ml). Solvent was removed from the combined filtrates by evaporation. Dichloromethane (100ml) and saturated aqueous sodium hydrogen carbonate (200ml) were added to the residue and the mixture stirred vigorously. The organic phase was isolated and the aqueous phase re-extracted with dichloromethane (2 x 50ml). The combined extracts were dried over anhydrous sodium sulphate and solvent removed by evaporation. The residue was purified chromatographically on silica (220g) eluted with dichloromethane-ethyl acetate (8:1). The resulting oil was re-dissolved in diethyl ether (20ml) and the solution ultrasonicated to induce crystallisation. Solvent was removed by evaporation giving the *title compound* as a colourless, crystalline solid (1.223g, 89.5%) m.p. 129-131°C. ν_{max} (Nujol)/cm^{-1} 3309, 3187 (NH_2), 1692 (conj. ester C=O); δ_H (400MHz, $CDCl_3$) 7.57 (1H, d, anilino 3H), 7.41 (1H, d, benzofuran 7H), 7.30-7.20 (2H, m, anilino 5H and benzofuran 4H), 6.95 (1H, d, benzofuran 6H), 6.83 (1H, m, anilino 4H), 6.79 (1H, d, anilino 6H), 5.62 (2H, d, J^1H-^{15}N 2Hz, $^{15}NCH_2$), 4.33 (2H, s, NH_2), 4.27 (2H, q, CO_2CH_2), 2.64 (2H, m, J^1H-^{13}C 115Hz, $^{13}CH_2$), 2.62 (1H, m, cyclopropyl CH), 1.34 (3H, t, $CO_2CH_2CH_3$), 1.21 (3H, m, J ^1H-^{13}C, 130Hz, $^{13}CH_3$), 1.10-0.90 (4H, m, cyclopropyl CH_2); δ_C (100MHz, $CDCl_3$) 155.5 (imidazole $^{13}C2$ enhanced, J ^{13}C-^{13}C

50Hz), 22.7 ($^{13}\underline{C}H_2$ enhanced, J ^{13}C-^{13}C 50Hz, 34Hz), 14.2 ($^{13}\underline{C}H_3$ enhanced, J ^{13}C-^{13}C 34Hz); (Found: m/z [Electrospray +ve] 513.127723 [MH^+]. $C_{23}{}^{13}C_3H_{27}{}^{79}Br_1N{}^{15}N_2O_3$ requires 513.127713).

3-{3-Bromo-2-[2-(trifluoromethanesulphonylamino)-phenyl]-5-benzofuran-5-ylmethyl}-5-cyclopropyl-2-[1,2-^{13}C]-ethyl-3H-[1,3-^{15}N, 2-^{13}C]-imidazole-4-carboxylic acid ethyl ester (22).

To a solution of (21) (1.173g, 2.29mmol) in ethyl acetate (30ml) was added triethylamine (11.8mg, 0.12mmol) in ethyl acetate (1.0ml). The stirred solution was cooled to 5°C under nitrogen. Trifluoromethanesulphonic anhydride (725mg, 2.57mmol) in iso-octane (7.4ml) was added over 15min.. After 10min TLC indicated almost complete reaction. A solution of citric acid (2.0g) in water (20ml) was added, and the aqueous phase re-adjusted from pH 3.1 to pH 4.25 by dropwise addition of 2M aqueous sodium hydroxide. The organic phase was isolated and the aqueous phase re-extracted with ethyl acetate (25ml). The combined extracts were clarified with saturated brine (25ml), and dried over anhydrous sodium sulphate. Solvent was removed by evaporation and the residue purified chromatographically on silica (220g), eluted with dichloromethane-methanol (10:1). The product was triturated with diethyl ether (25ml) giving the *title compound* as a colourless foam (1.346g, 91.2%). ν_{max} (Nujol)/cm^{-1} 1692 (conj. ester C=O), 1377, 1135 ($NH\underline{S}O_2CF_3$); δ_H (250MHz, DMSO-d_6) 7.75-7.45 (5H, m, phenyltriflamide 3H, 5H, 6H, and benzofuran 6H), 7.38 (1H, t, phenyltriflamide 4H), 7.22 (1H, s, benzofuran 4H), 7.08 (1H, d, benzofuran 7H), 5.70 (2H, d, J^1H-^{15}N 2Hz, $^{15}NCH_2$), 4.23 (2H, q, CO_2CH_2), 2.63 (2H, m, J^1H-^{13}C 115Hz, $^{13}CH_2$), 2.60 (1H, m, cyclopropyl CH), 1.20 (3H, t, $CO_2CH_2CH_3$), 1.09 (3H, m, J^1H-^{13}C 130Hz, $^{13}CH_3$), 1.00-0.80 (4H, m, cyclopropyl CH_2); δ_C (100MHz, $CDCl_3$) 153.9 (imidazole $^{13}\underline{C}2$ enhanced, J ^{13}C-^{13}C 50Hz), 20.4 ($^{13}\underline{C}H_2$ enhanced, J ^{13}C-^{13}C 50Hz, 34Hz), 12.7 ($^{13}\underline{C}H_3$ enhanced, J ^{13}C-^{13}C 34Hz): (Found: m/z [Electrospray +ve] 654.075300 [MH^+]. $C_{24}{}^{13}C_3H_{26}{}^{79}Br_1FN{}^{15}N_2O_5S$ requires 645.076998).

3-{3-Bromo-2-[2-(trifluoromethanesulphonylamino)-phenyl]-5-benzofuran-5-ylmethyl}-5-cyclopropyl-2-[1,2-^{13}C]-ethyl-3H-[1,3-^{15}N, 2-^{13}C]-imidazole-4-carboxylic acid (23)

To a solution of (22) (1.306g, 2.02mmol) in methanol (7.5ml) was added 2M aqueous sodium hydroxide (8ml, 16mmol). After stirring at reflux for 105min, the mixture was allowed to cool to 20°C and methanol removed by evaporation. Water (25ml) and t-butyl methyl ether (25ml) were added and the aqueous phase adjusted from pH13.0 to pH4.0 by addition of saturated aqueous

citric acid (10ml). The mixture was stirred for 15min and the organic phase isolated. The aqueous phase was re-extracted with t-butyl methyl ether (2 x 25ml) and the combined extracts dried over anhydrous sodium sulphate before removal of solvent by evaporation. The residue was triturated with diethyl ether (2 x 20ml) giving the *title compound* as a colourless, amorphous solid (1.346g, 107.8% uncorr.). ν_{max} (Nujol)/cm^{-1} 1709 (CO_2H), 1377, 1143 ($NHSO_2CF_3$); δ_H (400MHz, DMSO-d_6) 7.65-7.20 (5H, m, phenyltriflamide 3H, 4H, 5H , 6H and benzofuran 4H, 6H), 7.28 (1H, d, benzofuran 7H), 5.75 (2H, d, J^1H-^{15}N 2Hz, $^{15}NCH_2$), 2.68 (2H, m, J^1H-^{13}C 115Hz, $^{13}CH_2$), 2.62 (1H, m, cyclopropyl CH), 1.06 (3H, m, J^1H-^{13}C 130Hz, $^{13}CH_3$), 1.00-0.80 (4H, m, cyclopropyl CH_2); δ_C (100MHz, $CDCl_3$) 154.8 (imidazole $^{13}C2$ enhanced, J ^{13}C-^{13}C 50Hz), 21.5 ($^{13}CH_2$ enhanced, J ^{13}C-^{13}C 50Hz, 34Hz), 14.0 ($^{13}CH_3$ enhanced, J ^{13}C-^{13}C 34Hz) (Found: m/z [Electrospray +ve] 617.044502 [MH^+]. $C_{22}{}^{13}C_3H_{22}{}^{79}Br_1F_3N^{15}N_2O_5S$ requires 617.045698).

3-{3-Bromo-2-[2-(trifluoromethanesulphonylamino)-phenyl]-benzofuran-5-ylmethyl}-5-cyclopropyl-2-[1,2-^{13}C]-ethyl-3-H-[1,3-^{15}N, 2-^{13}C]-imidazole-4-carboxylic acid amide (1c)

A stirred solution of (23) (1.310g, 2.12mmol) in THF (25ml) at 20°C, under nitrogen, was treated with 1,1'-carbonyl- diimidazole (1.032g, 6.36mmol). After 2.5h the mixture was cooled to 5°C and ammonia bubbled through in a steady stream for 0.5h. The mixture was then allowed to warm to 20°C, and periodically re-saturated with ammonia. After a total of 86h, TLC indicated complete reaction. Solvent was removed by evaporation and ethyl acetate (100ml) and water (80ml) added. The aqueous phase was adjusted to pH4.6 by addition of saturated aqueous citric acid (13ml) to the stirred mixture. The organic phase was isolated and the aqueous phase re-extracted with ethyl acetate (2 x 50ml). The combined extracts were clarified with saturated brine (100ml) and dried over anhydrous sodium sulphate. Solvent was removed by evaporation and the residue purified chromatographically on silica (220g) eluted with dichloromethane-methanol (10:1). The product was crystallised from boiling ethanol (30ml) and water (40ml) giving *title compound* (1.017g). This was recrystallised from boiling ethanol (40ml) and water (40ml) to give the *title compound* as a colourless, crystalline solid (972mg, 74.3% overall) m.p. 207-210°C. ν_{max} (Nujol)/cm^{-1} 3456 (NH), 1679 (carboxamide C=O); δ_H (400MHz, DMSO-d_6) 7.84 (2H, s, $CONH_2$), 7.55 (1H, d, benzofuran 6H), 7.43 (1H, d, phenyltriflamide 3H), 7.40-7.25 (3H, m, phenyltriflamide 4H and 5H and benzofuran 4H), 7.15 (1H, d, benzofuran 7-H), 7.03 (1H, br s, phenyltriflamide 6H), 5.65 (2H, d, J^1H-^{15}N 2Hz, $^{15}NCH_2$), 2.91 (2H, m, J^1H-^{13}C 115Hz, $^{13}CH_2$),

2.13 (1H, m, cyclopropyl C<u>H</u>), 1.09 (3H, m, $J^{1}H$-^{13}C 130Hz, $^{13}C\underline{H}_3$), 1.05-0.80 (4H, m, cyclopropyl $C\underline{H}_2$); δ_C (100MHz, $CDCl_3$) 151.7 (imidazole $^{13}\underline{C}2$ enhanced, J ^{13}C-^{13}C 50Hz), 20.3 ($^{13}\underline{C}H_2$ enhanced, J ^{13}C-^{13}C 50Hz, 34Hz), 13.8 ($^{13}\underline{C}H_3$ enhanced, J ^{13}C-^{13}C 34Hz); m/z (Electrospray) 617/619 (MH^+, 100) (Found: m/z [LSIMS +ve] 616.062729 [MH^+]. $C_{22}{}^{13}C_1H_{23}{}^{79}Br_1F_3N_2{}^{15}N_2O_4S$ requires 616.061683).

Acknowledgements

The authors thank Dr Bob Lancaster for NMR and IR data, Keith Brinded for MS data and Miss Pat McDonough for microanalyses.

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