SYNTHESIS OF ISOTOPICALLY LABELLED PYRIDOINDOLONE 5-HT_3 RECEPTOR ANTAGONISTS (1)

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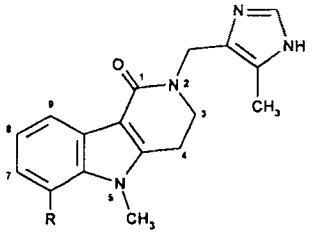
Summary

Syntheses of labelled versions of 5-HT_3 receptor antagonists, Alosetron and Lurosetron, are described. [^{14}C]Alosetron was prepared by routes utilizing either Fischer indolisation of an amidohydrazine or palladium-mediated cyclisation of an aryl enaminone as key steps. ^{2}H and ^{13}C versions of Alosetron were prepared from suitably labelled imidazoles. Lurosetron was labelled in either the methylene bridge carbon or carbonyl carbon, using ^{14}C-labelled paraformaldehyde or phosgene, respectively.

Key Words: 5-HT_3 receptor antagonists, tetrahydropyrido[4,3-b]indolones, Fischer indolisation, palladium mediated cyclisation, Friedel-Crafts cyclisation, ^{13}C and ^{2}H imidazoles.

Introduction

5-HT_3 receptors existing in the peripheral and central nervous system are known (2) to regulate the release of neurotransmitters such as acetylcholine, noradrenaline, dopamine and 5-hydroxytryptamine. Therefore, selective antagonists of 5-HT_3 receptors have clinical utility in the treatment of, for example, psychiatric disorders and chemotherapy induced emesis. Alosetron (1a) and its fluoro analogue, Lurosetron (2a), both (imidazolylmethyl)tetrahydropyrido[4,3b]indolones, are potent 5-HT_3 receptor antagonists (3,4). Isotopically labelled versions were required for metabolite profiling and excretion balance studies, and for *in vitro* biotransformation studies.



(1a) R = H, Alosetron
(2a) R = F, Lurosetron

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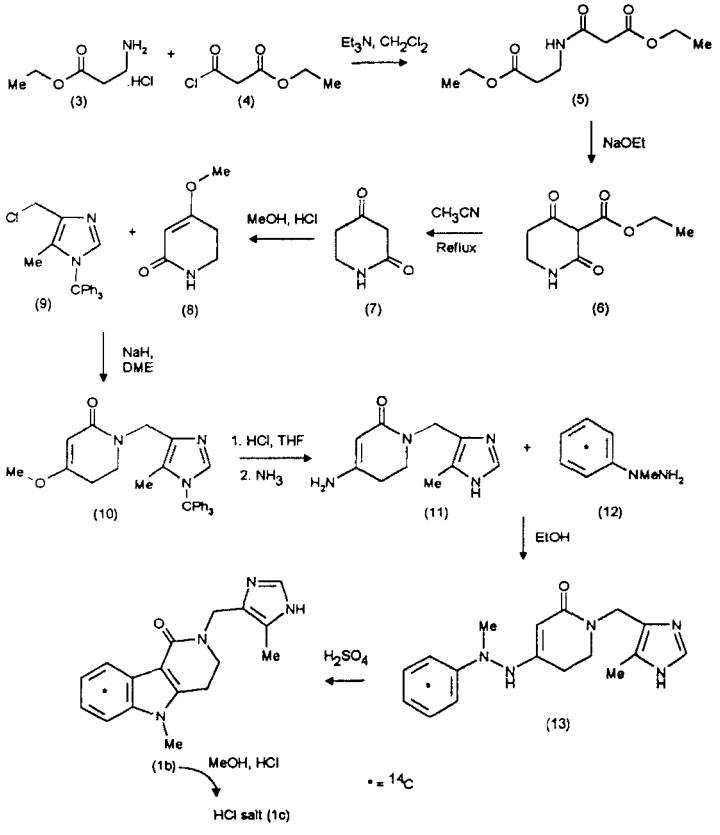
Results and Discussion

[^{14}C] Labelled Alosetron

Key steps employed during the preparations of Alosetron have been (a) Fischer indolization reaction, (b) Friedel-Crafts cyclization of a substituted carbamoyl chloride, and (c) palladium-mediated cyclization of an aryl enaminone (3,4). All these routes were utilised for incorporation of carbon-14 into Alosetron.

Scheme 1

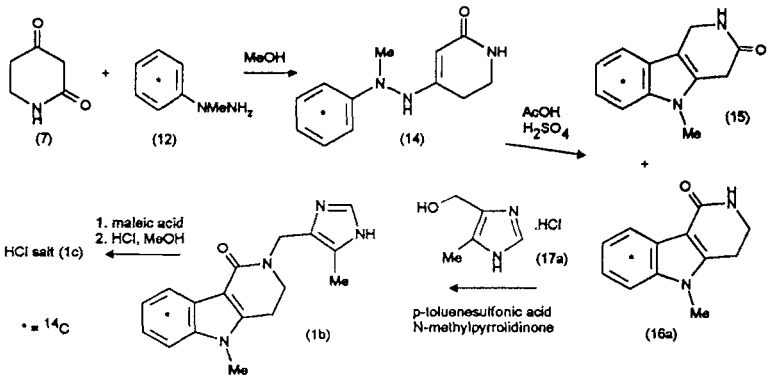


The first synthesis (Scheme 1) employed Fischer cyclisation at a late stage of the synthesis, utilising N-methyl-N-[U-^{14}C]phenylhydrazine (12) (5) as the radiolabelled precursor. The required unlabelled lactam (11) was prepared as shown in Scheme 1 (3). Reaction of (11) with (12) in refluxing ethanol afforded hydrazine (13), which was treated briefly with concentrated sulphuric acid to give Alosetron (1b). Conversion to hydrochloride salt gave (1c) in 28% overall radiochemical yield. N-Methyl-N-[U-^{14}C]phenylhydrazine (12) was relatively unstable, the radiochemical purity falling by *ca* 15% over 4 weeks.

A second synthesis of [^{14}C]Alosetron (Scheme 2) utilised Fischer cylisation at an earlier stage. Lactam (7) was allowed to react with N-methyl-N-[U-^{14}C]phenylhydrazine (12) in

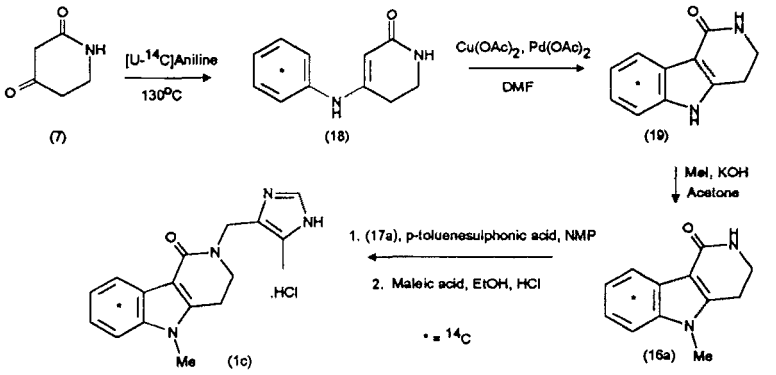
methanol, and the resulting hydrazine (14) treated with glacial acetic acid and concentrated sulphuric acid. Isomeric impurity (15) was readily removed by crystallisation to give indole (16a) in 42% overall radiochemical yield. Condensation of indole (16a) with hydroxymethylimidazole (17a) in N-methylpyrrolidinone (NMP), in the presence of p-toluenesulfonic acid at 125-130°C gave [^{14}C]Alosetron (1b). Hydrochloride salt (1c) was prepared by initial conversion of the free base to maleate salt, followed by treatment of the latter with concentrated hydrochloric acid. In this case the overall radiochemical yield of (1c) from (12) was 12%.

Scheme 2



As a result of the relatively poor yields for the routes decribed above, an alternative strategy was utilised during the third synthesis. Intramolecular cyclization of enaminones involving catalytic arylpalladium complexes has been reported to provide carbazoles in good yields (6). Thus, treatment of the lactam (7) with the readily available [U-^{14}C]aniline afforded enaminone (18), which underwent the cyclisation reaction using palladium acetate and cupric acetate in DMF to provide indole (19), in 52% radiochemical yield (Scheme 3). Alkylation of (19) with methyl iodide gave (16a), which was elaborated to [^{14}C]Alosetron (1c), as described above. The overall radiochemical yield from [^{14}C]aniline was 29%.

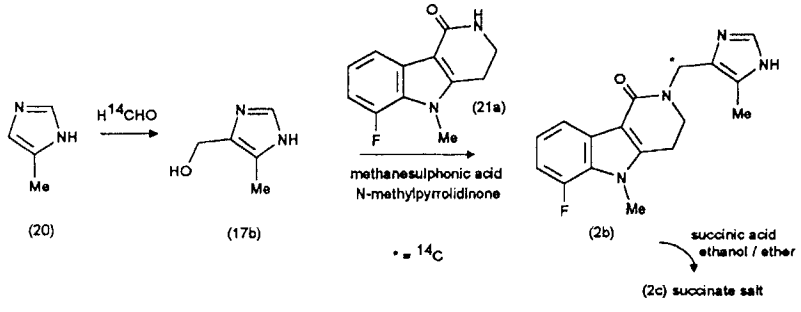
Scheme 3



[Methylene-^{14}C]Lurosetron

The radiosynthetic methodology developed during preparation of labelled Alosteron was not easily extendable to its fluoro analogue, Lurosetron (2a). Although 2-fluoroaniline and 2,4-dioxopiperidine are precursors of choice for preparation of Lurosetron (4), multi-step syntheses would be required to prepare their ^{14}C labelled versions. Therefore, as a quick method of providing radiolabelled Lurosetron for preliminary metabolism studies, the methylene carbon between pyridoindole and imidazole rings was chosen as the labelling site. Base catalyzed hydroxymethylation of 4-methylimidazole (20) (Scheme 4) with 10% aqueous [^{14}C]formaldehyde solution gave (17b), which was coupled to lactam (21a), utilising methanesulphonic acid as catalyst. The resulting (2b) was converted to its succinate salt (2c). The overall radiochemical yield was only 9%. The conversion of (20) into (17b) was only *ca* 30%, compared with the 65% yield observed during optimisation experiments with unlabelled formalin solution.

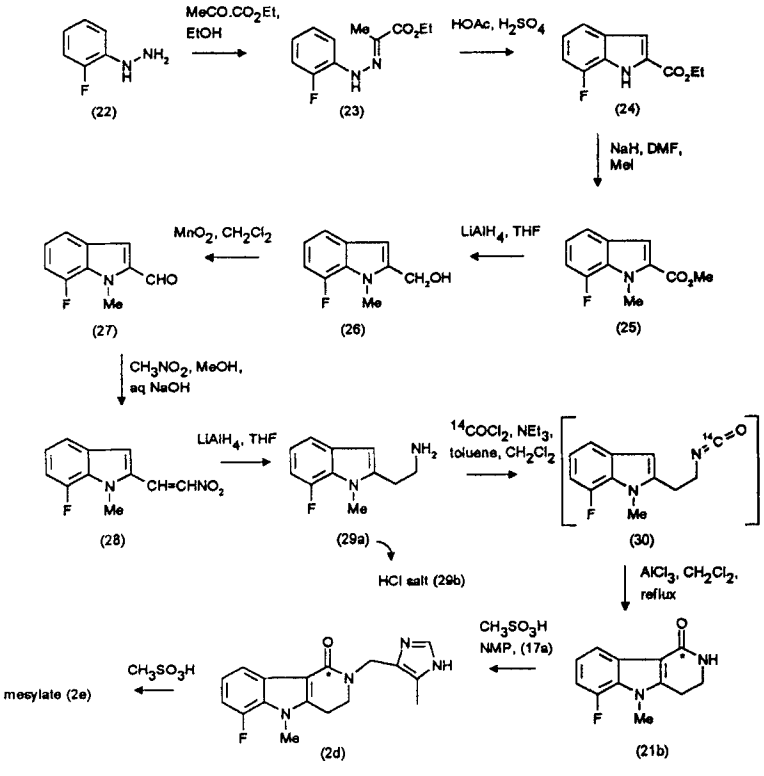
Scheme 4



[Carbonyl-^{14}C]Lurosetron

There was a concern that the ^{14}C label in (2b) might be vulnerable to loss by metabolic processes, and therefore an alternative site of labelling was investigated. 2-Aminoethylindole (29a) was prepared from 2-fluorophenylhydrazine by the route shown in Scheme 5 (4). Amine (29a) was either purified immediately before use, or isolated as its stable hydrochloride salt (29b). Addition of [^{14}C]phosgene (1.2 equivalents) to (29a) in dichloromethane, in the presence of triethylamine, followed by treatment of resulting isocyanate (30) with aluminum chloride in dichloromethane at reflux temperature, afforded (21b) in 39% radiochemical yield, after preparative HPLC purification. Nearly 20% of the radioactivity in the crude product was associated with an unidentified non-polar product. Investigation of the same sequence of reactions using [^{13}C]phosgene afforded by-products, identified as ureas containing two and three units of amine (29). Lactam (21b) was converted to [carbonyl-^{14}C]Lurosetron (2d), and isolated as its mesylate salt (2e). The overall radiochemical yield of (2e) from [^{14}C]phosgene was 22%.

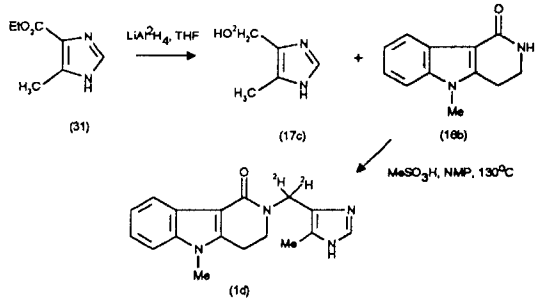
Scheme 5



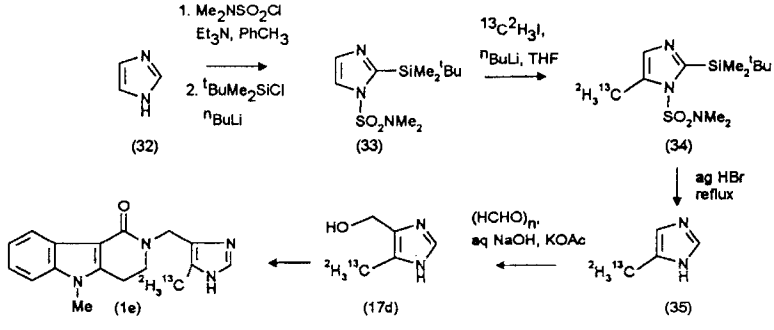
^{13}C and ^{2}H Labelled Alosetron

Formation of novel oxidative metabolites, involving migration of imidazole methyl group of Alosetron and Lurosetron, has been observed during *in vitro* and *in vivo* metabolism studies. In order to elucidate the mechanism of formation of these metabolites using NMR and MS (7), several specifically labelled versions of Alosetron were prepared. The required hydroxymethylimidazoles (17c), (17d) and (17e) were prepared by the methods shown in Schemes 6, 7 and 8. Subsequent coupling reactions with lactam (16b) afforded required Alosetron derivatives (1d), (1e) and (1f). Imidazole (17e) was prepared by two routes utilising either chloro compound (37) (8) or oxime (39) (9), the latter affording superior yields of (1f).

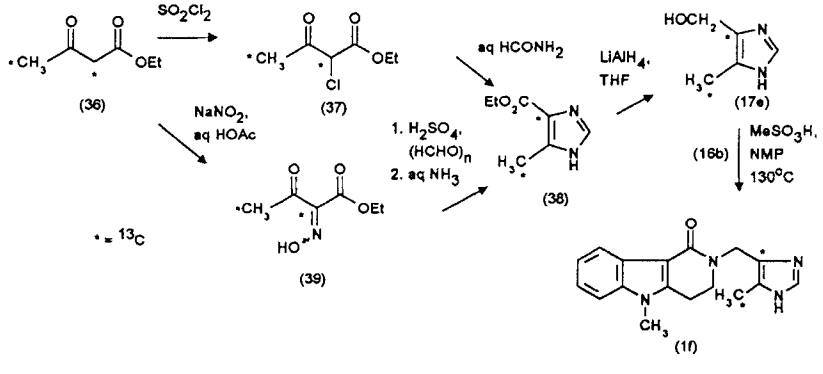
Scheme 6



Scheme 7



Scheme 8



Experimental

NMR spectra were recorded on Varian 200, 300 or 400MHz, and Brucker 250MHz spectrometers. Mass spectra were recorded on Finnigan TSQ-70, 4600, or VG Autospec instruments. Radiochemical purities (RCPs) were determined using Ramona 90 or Berthold LB 507A or Radiomatic on-line radioactivity flow detectors, Berthold linear analyzer and autoradiography. Unless otherwise stated, HPLC analyses were performed using 25 x 0.46 cm columns and details of the analyses are provided in the following format, column, mobile phase,

and flow rate. TLC analyses were carried out using Whatman silica gel 60 A K6F or Merck silica plates. Column chromatography was performed using Merck silica gel Kieselgel 60 (9385).

Preparation of [^{14}C]Alosetron: Method 1.

1-[(5-Methyl-1H-imidazol-4-yl)methyl]-4-(N'-methyl-N'-[U-^{14}C]phenylhydrazino-5,6-dihydro-1H-pyridin-2-one (13). A solution of N-methyl-N-[U-^{14}C] phenylhydrazine (12) (125mCi, 70mCi/mmol, radiochemical purity 90%) and lactam (11) (419mg, 2.03mmol) in ethanol (45ml) was stirred at reflux for 5h. More lactam (11) (21mg) was added, and the mixture stirred at reflux for a further 2h. The mixture was cooled and evaporated to dryness. The residue was purified by column chromatography, eluting with dichloromethane-ethanol-ammonia (200:8:1 to 50:8:1) to afford the *title compound* (13), as pale yellow foam (425mg, 88% radiochemical yield based on 90% pure phenylhydrazine). TLC: Dichloromethane-ethanol-ammonia (50:8:1), Rf 0.4, RCP 96.5%.

2,3,4,5-Tetrahydro-5-Methyl-2-[(5-methyl-1H-imidazol-4-yl)methyl]-5a,6,7,8,9,9a[^{14}C]-1H-pyrido[4,3-b]indol-1-one hydrochloride (1c). Hydrazine (13) (424.7 mg, 1.353mmol, *ca* 99 mCi) was dissolved in concentrated sulphuric acid (2ml) and allowed to stand at 20°C for 5min. The mixture was then cautiously added to saturated sodium bicarbonate solution (1200ml), and thoroughly extracted with methanol-dichloromethane (1:9) (3x1200ml). The extracts were combined, dried over magnesium sulphate and evaporated to dryness. The resulting oil was purified by column chromatography eluting with dichloromethane-ethanol-ammonia (200:8:1 to 100:8:1). The fractions containing (1b) were combined and evaporated to dryness. The residue (203mg) was diluted with unlabelled Alosetron (1a) (30mg), dissolved in hot methanol (10ml), cooled to 20°C and treated with saturated hydrogen chloride in diethyl ether (20ml). The mixture was evaporated to dryness, the residue re-dissolved in warm methanol and treated with diethyl ether, benzene and cyclohexane. The resulting oil was stirred at 20°C and treated with a dropwise addition of methanol until a solid formed. The suspension was cooled to 0°C and the solid was collected by filtration, washed with diethyl ether, and dried under vacuum at 20°C to provide the *title compound* (1c) as a colourless solid (169mg, 27.7mCi, 28% radiochemical yield). TLC: dichloromethane-ethanol-ammonia (100:8:1), Rf 0.5, RCP 98%. HPLC: Spherisorb cyano (200 x 4.6mm), hexane-ethanol-dichloromethane-ammonia (800:120:80:1), 1.5ml/min, UV detection at 235 nm, Rt 10min, RCP >97%. Mass Spectrum: CI (+); 0.9 x ^{14}C per molecule.

Preparation of [^{14}C]Alosetron: Method 2.

4-(N'-Methyl-N'-[U-^{14}C]phenylhydrazino)-5,6-dihydro-1H-pyridin-2-one (14). N-Methyl-N-[U-^{14}C]phenylhydrazine (12) (150mCi at 74.3mCi/mmol) was dissolved in deoxygenated methanol (0.5ml). This solution was added to a suspension of lactam (7) (0.25g, 2.2mmol) in deoxygenated methanol (0.5ml) at 0°C under nitrogen over 10min. The mixture was stirred under nitrogen at 0°C for 0.8h, then at 20°C for 2.2h Water (1.25ml) was added dropwise over 10min with stirring. More water (1.25ml) was added dropwise until the solution became cloudy. Crystallisation was initiated by adding seed crystals. After 10min, more water (2.5ml) was added

dropwise over 5min with stirring. The suspension was stirred at 0°C for 45min and the solid was removed by filtration. The cream coloured solid was washed with water (3x3ml) and then dried under vacuum at 20°C for 18h to give the *title compound* (14) (0.38g, 80%, 109mCi).

2,3,4,5-Tetrahydro-5-methyl-5a,6,7,8,9,9a-[^{14}C]-1H-pyrido[4,3-b]indol-1-one (16a). To (14) (0.59g, 2.55mmol, 159mCi) was added glacial acetic acid (1.0ml), and the mixture stirred under nitrogen until the solid dissolved. The resulting brown solution was added dropwise to concentrated sulphuric acid (1.1ml) with stirring at 0°C under nitrogen over 10min. The colour of the mixture changed from purple to orange. The mixture was stirred at 20°C under nitrogen for 3.75h. The reaction mixture was cautiously added to ice-cold 6N ammonia solution (10ml). The brown solid was dissolved by acidifying to pH1 with concentrated sulphuric acid and the pH was finally adjusted to 7.0 with 6N and 3N ammonia solutions. The grey precipitate was collected by filtration, and dried under vacuum for 18h to give crude product mixture (0.38g, 74%). To this was added ethanol (2.5ml), and the mixture heated to reflux for 5min with stirring. The mixture was cooled to 0°C over 1.5h. The solid was collected by filtration and washed with ethanol (3x1ml), before drying under vacuum for 18h to give the *title compound* (16a) (0.25g, 48%, 77 mCi).

2,3,4,5-Tetrahydro-5-Methyl-2-[(5-methyl-1H-imidazol-4-yl)methyl]-5a,6,7,8,9,9a[^{14}C]-1H-pyrido[4,3-b]indol-1-one (1b). A mixture of (16a) (240mg, 1.19mol, 74.6mCi), p-toluenesulphonic acid monohydrate (57mg, 0.30mmol), hydroxymethylimidazole (17a) (306mg, 2.06mmol) in deoxygenated N-methyl-2-pyrrolidinone (NMP) (1.2ml) was heated to 140°C for 1.25h with stirring under nitrogen. The mixture was stirred and gradually cooled to 110°C over 2.5h, and then heated to 140°C again for 1.1h. The reaction mixture was allowed to cool to 20°C for 1.5h with stirring. The mixture was left to stand at 20°C for 16h. Water (0.9ml) and then sodium bicarbonate solution (8% w/w, 1.1ml) were added over 30min. A white solid formed and the suspension was stirred for 30min. More 8% sodium bicarbonate solution (1.1ml) was added over 20 min. The suspension was cooled to 0°C, and stirred for 1h. The solid was collected by filtration and washed with water (10ml), before drying for 18h under vacuum to give the *title compound* (1b) (207mg, 59%, 43.7mCi).

2,3,4,5-Tetrahydro-5-Methyl-2-[(5-methyl-1H-imidazol-4-yl)methyl]-5a,6,7,8,9,9a[^{14}C]-1H-pyrido[4,3-b]indol-1-one hydrochloride (1c). A mixture of (1b) (168mg, 0.57mmol, 35.7mCi) and maleic acid (66mg, 0.57mmol) in methanol (1.7ml) was heated to 65°C for 1.15h with stirring under nitrogen. The solution was allowed to cool to 20°C over 1h. The solution was stirred and reheated to 60°C over 10 min and concentrated hydrochloric acid (0.06ml) was added dropwise over 5min. The mixture was stirred at 60°C for 30min and then cooled to 20°C over 2h. A few drops of ethanol were added, and crystallisation was initiated by scratching with a spatula. After standing at 20°C for 30min the white solid was collected by filtration and washed with methanol (2x2ml). The solid was dried under vacuum for 18 h to give the *title compound* (1c) (105mg, 55%, 19.7mCi); δ_H (200MHz, DMSO-d6) 9.00 (1H, s, imidazole H), 8.01 (1H, d, aromatic H), 7.57 (1H, d, aromatic H), 7.23 (2H, m, aromatic H), 4.71 (2H, s, NCH_2), 3.79 (3H, s, NCH_3), 3.78 (2H, m, NCH_2CH_2), 3.19 (2H, m, NCH_2CH_2), 2.42 (3H, s, imidazole CH_3); m/z (thermospray +ve) 295

(MH^+, 100%), 296 (20.5%), 297 (2.1%), 298 (0.1%), 299 (0.1%), 301 (1.1%); specific activity (gravimetric) 188mCi/mg (62.6mCi/mmol). A portion of (1b) (31 mg, 0.11 mmol, 6.6 mCi) was diluted with (1a) (234mg, 0.79mmol) and was converted to hydrochloride salt (12b) as described above to afford a product of low specific activity (187mg, 62%, 4.20mCi). (Found: C, 61.57; H, 5.72; N, 16.80. $C_{17}H_{19}N_4OCl$ requires C, 61.72; H, 5.79; N, 16.94); specific activity (gravimetric) 22.5μCi/mg (7.43mCi/mmol).

Preparation of [^{14}C]Alosetron: Method 3.

2,3,4,5-Tetrahydro-5a,6,7,8,9,9a-[^{14}C]-1H-pyrido-[4,3-b]-indol-1-one (18). The contents of a vial of [U-^{14}C]aniline hydrochloride (150mCi, 131mCi/mmol) were transferred to a separating funnel using 2N sodium carbonate (9ml) and dichloromethane (4ml). The lower layer was separated and unlabelled aniline hydrochloride (170mg, 1.312mmol) added to the aqueous phase, which was then extracted with more dichloromethane (4x2.5ml). The combined organic layers were treated with the lactam (7) (325mg, 2.87mmol), concentrated by distillation at atmospheric pressure and the residual oil heated at 125°-130°C for 15min. The mixture was allowed to cool, treated with dimethylformamide (12ml), cupric acetate monohydrate (1g, 5mmol) and palladium acetate (105mg, 0.47mmol), and the reaction flask reheated at 125-130°C for 1.5h. On cooling, the solvent was removed under reduced pressure, and the residue purified by chromatography on silica gel (100g), eluted with dichloromethane-ethanol-ammonia (100:8:1 to 100:10:1). Fractions containing pure (18) were combined, evaporated under reduced pressure, and redissolved in methanol (200ml). Fractions containing a close running more polar component were chromatographed on a column of alumina (Merck 1077, 30g), eluted with dichloromethane-ethanol-triethylamine (100:7:1). Fractions containing pure (18) were combined, evaporated, and redissolved in methanol (100ml). The two batches were combined to give *title compound* (18) (77.3mCi, 51.5%).

2,3,4,5-Tetrahydro-5-methyl-5a,6,7,8,9,9a-[^{14}C]-1H-pyrido-[4,3-b]-indol-1-one (19). The solution of (18) (77.3mCi, 1.28mmol) in methanol (300ml) was evaporated to dryness. The residual gum was stirred with iodomethane (300μl, 4.82mmol) and powdered potassium hydroxide (200mg, 3.5 mmol) in acetone (15ml) at 20°C for 1.5h. The reaction mixture was then added to aqueous ammonium chloride (75ml), and extracted with ethyl acetate (3x30ml). The combined extracts were dried over anhydrous sodium sulphate, filtered, and evaporated under reduced pressure to give *title compound* (19) (77.3mCi, 100%) as a fawn solid, which was redissolved in methanol (200ml).

2,3,4,5-Tetrahydro-5-methyl-2-[(5-methyl-1H-imidazol-4-yl)methyl]-5a,6,7,8,9,9a[^{14}C]-1H-pyrido[4,3-b]indol-1-one hydrochloride (16a). The methanol solution of the lactam (19) (75.9mCi, 1.259mmol) was concentrated under reduced pressure. The residue was heated at 130-135°C with hydroxymethylimidazole (17a) (439mg, 2.954mmol), and p-toluenesulphonic acid monohydrate (83mg, 0.436mmol), in NMP (1.6ml), for 2h under a nitrogen atmosphere. On cooling to 35°C, water (1.5ml) was added followed by dropwise addition of 1N sodium hydroxide solution (3.5ml). After a few minutes crystallisation commenced, and the mixture was allowed to

age at 20°C for 2h, and then at 4°C for 15h. The solid was collected by filtration, washed with water (4x1ml), and dried under vacuum to give the free base (1b) (243mg), as a cream solid. This material was suspended in absolute ethanol (3ml) and maleic acid (101mg, 0.867mmol) added. The mixture was heated to 70°C giving a clear solution. Ethanolic hydrogen chloride (0.8ml, 2.2mmol; prepared by the addition of acetyl chloride (1ml) to ethanol (5ml) and ageing the solution for 1h) was added resulting in rapid crystallisation of the hydrochloride salt. The mixture was allowed to cool, and aged at 4°C for 3h. The solid was collected by filtration and dried at 20°C under vacuum for 8h to give *title compound* (1c) (38.5mg, 43.2mCi, 57%): Specific activity 181μCi/mg. TLC: dichloromethane-ethanol-ammonia (100:10:1), RCP 98.1%; HPLC: Spherisorb ODS2, 25% 0.05M ammonium dihydrogen phosphate (pH 2.3) in acetonitrile, RCP 98.9%; δ_H (250 MHz, DMSO-d_6) 8.91 (1H, s, imidazole-2-H), 7.96 (1H, d, 9-H), 7.52 (1H, d, 6-H), 7.19 (2H, m,7-H, 8-H), 4.66 (2H, s, 2-CH_2), 3.73 (3H, s, 5-CH_3), 3.68 (2H, t, 3-H_2), 3.12 (2H, s, 4-H_2), 2.36 (3H, s, imidazole-5-CH_3).

Preparation of [Methylene-^{14}C]Lurosetron.

4-Methyl-5-imidazole[^{14}C]methanol (17b). [^{14}C]Formaldehyde solution (10% aqueous w/w, 0.32ml, 1mmol, 55mCi) was treated with 4-methylimidazole (0.36g, 4.3mmol) and sodium hydroxide (5.8mg) in methanol (0.6ml). The reaction vial was sealed, and the mixture allowed to stand at room temperature for 4 days. Isopropanol (3ml) was added, and the mixture diluted with a solution of potassium carbonate (4g) in water (5ml). The organic layer was separated, and the aqueous further extracted with isopropanol (2x3ml). The organic extracts were combined, evaporated under reduced pressure and the residue purified by chromatography on silica gel (20g), eluting with hexane-methanol (7:3). The appropriate fractions were combined, and evaporated to give *the title compound* (17b) as a yellow solid (37.7mg, 0.33mmol).

6-Fluoro-2,3,4,5-tetrahydro-5-methyl-2-[(5-methyl-1H-imidazol-4-yl)[^{14}C]methyl]-1H-pyrido-[4,3-b]indol-1-one succinate (2c). NMP (2ml) was degassed by bubbling nitrogen gas through the liquid, whilst being heated at 125°C for 1h. The degassed NMP (0.5ml) was then added to the solid imidazole (17b) (37.7mg, 0.33mmol), followed by methanesulphonic acid (0.03ml, 0.46mmol), and lactam (21a) (83mg, 0.38mmol). The mixture was heated at 125°C for 5h under nitrogen, and left to cool overnight. Water (0.33ml) was added, and the mixture stirred for 20min. 1N sodium hydroxide (0.253ml) was added, and the mixture seeded with unlabelled Lurosetron free base (2a; 1mg). After 3min a further portion of 1N sodium hydroxide (0.253ml) was added. The solid produced after ageing for 1h was collected by filtration, and washed with water (5x3ml). The filtrate was saturated with solid potassium carbonate, and extracted with ethanol (3x10ml). The ethanol solution was combined with the collected solid, and the mixture evaporated under reduced pressure to give crude Lurosetron base (2b). The crude base was purified by chromatography over silica gel (20g), eluted with dichloromethane-ethanol-ammonia (100:8:1). The appropriate fractions were combined and evaporated under reduced pressure. To the residue was added succinic acid (23mg, 0.195mmol) and ethanol (1.2ml). The mixture was heated to boiling to obtain a clear solution, which was allowed to cool. The suspension was aged at 4°C overnight and the solid collected by filtration to give a white solid (46.3mg). The filtrate

from the crystallisation was evaporated, and the residue crystallised from isopropanol (0.5ml). The two crops of solid were combined, and recrystallised from ethanol (1ml) to give a white powder (56.2mg). A mixture of this succinate (56.2mg) and unlabelled Lurosetron succinate (56mg) was recrystallised from ethanol (2ml) and ether (0.5ml). The product was dried under vacuum for 4h to give (2c) as an off-white powder (99.3mg, 5.0mCi): mp 182-84°C; specific activity 50.7μCi/mg; δ_H (250MHz, DMSO-d6) 7.80 (1H, d, 9-H), 7.51 (1H, s, imidazole-2-H), 7.1 (1H, m, 8-H), 7.0 (1H, m, 7-H), 4.52 (2H, s, 2-CH2), 3.87 (3H, s, 5-CH3), 3.63 (2H, t, 4-H2), 3.04 (2H, t, 3-H2), 2.44 (4H, s, succinate-CH2), 2.21 (3H, s, imidazole-5-CH3). TLC: dichloromethane: ethanol: ammonium hydroxide (100:8:1), RCP >97%. HPLC: Capital Cartridge 5μ nitrile column (15 x 0.46cm), methanol: 0.02 M sodium dihydrogen orthophosphate (pH 6) (6:4), 0.5ml/min, UV detection at 220 nm, Rt 7.0 min, RCP >99%.

Preparation of [Carbonyl-^{14}C]Lurosetron.

2-(7-Fluoro-1-methyl-1H-indol-2-yl)-ethylamine hydrochloride (29b). To a stirred suspension of lithium aluminium hydride (1.75g, 46mmol) in dry tetrahydrofuran (100ml) at 5°C under nitrogen was added a solution of 7-fluoro-1-methyl-2-(2-nitroethenyl)-1H-indole (4) (1.0g, 4.54mmol) in dry tetrahydrofuran (40ml) over 10min. The stirred mixture was refluxed under nitrogen for 1.6h. To the stirred mixture at 5°C was added tetrahydrofuran-water (9:1; 10ml), followed by water (1ml), and stirring was continued for 30min. Magnesium sulphate (20g) was added, the solids were removed by filtration through a sinter, and washed with tetrahydrofuran (40ml). The filtrate was concentrated under reduced pressure to leave a brown residue. The residue was chromatographed on silica gel (150g), eluting with dichloromethane-methanol-ammonia (190:10:1) to give (29a) as an oil (355mg, 41%). To this oil was added a 1.34M soluton of hydrogen chloride in ethanol (5ml, 6.7mmol). Diethyl ether (10ml) was added, and the mixture was stored at 5°C for 4h. The solid was collected by filtration, and washed with diethyl ether-ethanol (9:1; 40ml), before being dried under vacuum for 18h to give the *title compound* (29b) as a white solid (352mg, 34%): δ_H (250 MHz, D_2O) 7.39 (1H, d, indole 6H), 7.1-6.9 (2H, m, indole 4H and 5H), 6.46 (1H, s, indole 3H), 3.87 (3H, s, NCH_3), 3.39 (2H, t, NCH_2CH_2), 3.16 (2H, t, NCH_2CH_2); m/z (thermospray +ve) 193 (MH^+, base peak); (Found: C, 56.02; H, 6.26; N, 11.82. $C_{11}H_{14}N_2FCl$ requires C, 57.77; H, 6.17; N, 12.25%).

6-Fluoro-2,3,4,5-tetrahydro-5-methyl-1H-pyrido[4,3-b]indol-1-[^{14}C]-one (21b).
[^{14}C]Phosgene (0.90 mmol, 55 mCi/mmol, 50 mCi, 10% in toluene) was transferred *via* a high vacuum manifold (0.01mm of Hg) into a cooled (liquid nitrogen) flask (100ml) containing indole (29a) (143mg, 0.744mmol), triethylamine (260μl, 1.86mmol) and anhydrous dichloromethane (6.6ml). The reaction mixture was slowly warmed to 0°C, and stirred in an ice-water bath for 1h. The solvent and unreacted phosgene were vacuum transferred into a cooled (liquid nitrogen) trap containing toluene (4ml) and 20% sodium hydroxide (1.5ml), attached to the manifold. The resulting solid (30) was dissolved in anhydrous dichloromethane (12ml) and treated with aluminum chloride (236mg, 1.76mmol). The dark coloured solution was refluxed for 4h under nitrogen. The mixture was cooled, quenched with 2M NaOH, and extracted with dichloromethane (3x15ml). The combined extracts were dried over sodium sulphate, and

concentrated under vacuum to yield the crude product (34.4mCi). TLC analysis (ethyl acetate-methanol 9:1) showed a radioactivity profile with 64% of the radioactivity associated with the desired product, and 20% with an unidentified non polar product. Preparative HPLC (Zorbax Sil, 21.2 x 250 mm, dichloromethane-ethanol-ammonia 100:7:1, 10ml/min, UV detection at 280nm) purification of the mixture gave two fractions, fraction 1, (21b) (77 mg, 19.8 mCi, RCP 97%, 39%) and fraction 2, (21b) (1.9 mCi, RCP 88%). Fraction 1: ν_{max} CH_2Cl_2/KBr 1648 (amide $^{12}C{=}O$), 1598cm^{-1} (amide $^{14}C{=}O$); δ_H (300 MHz, $CDCl_3$) 7. 91 (1H, d, 9-H), 7.12 (1H, m, 8-H), 6.92 (1H, dd, 7-H), 3.93 (1H, d, 5-CH_3), 3.7 (2H, t, 3-CH_2), 3.01 (2H, t, 4-CH_2) ; MS (EI) m/z, 220 (218) , 191 (189) (220-$NHCH_2$), 161 (220-$CH_2NH^{14}CO$), ^{14}C abundance, 80-82%.

6-Fluoro-2,3,4,5-tetrahydro-5-methyl-2-[5-Methyl--1H-Imidazol-4-yl)methyl]-1H-pyrido [4,3-b]-indol-1-[^{14}C]-one (2d). To a solution of lactam (21b) (77mg, 0.353mmol, 19.8mCi) in NMP (6ml, previously degassed by bubbling nitrogen through the liquid while being heated at 125°C for 1 hour) were added methanesulphonic acid (28ml, 0.423mmol) and hydroxymethylimidazole hydrochloride (17a) (49.7mg, 0.335mmol). The mixture was heated under nitrogen at 125°C for 11h, during which time two further portions of (17a) (14mg, 0.094mmol and 6mg, 0.040mmol) were added after 3 and 6h respectively. The progress of the reaction was monitored by HPLC [Hypersil BDS C18, CH_3CN-0.05M NH_4OAC (pH4) 35:65, UV detection at 280nm]. The reaction mixture was cooled, treated with water (2.0ml) and 1N NaOH (4.0ml), and extracted with ethyl acetate (5x15ml). The combined organic extracts were dried over sodium sulphate, and concentrated to yield *title compound* (2d) (17mCi; RCP 88% by HPLC. Preparative HPLC (Zorbax Sil, 21.2 x 250 mm, dichloromethane-ethanol-ammonia 200:8:1, 10ml/min, UV detection at 280nm) purification of this product provided pure free base (2d) (13mCi, 73.2mg, 66%): ν_{max} dichloromethane/KBr 1632 ($^{12}C{=}O$), 1580cm^{-1} ($^{14}C{=}O$); MS(EI) m/z 314, MS/MS of m/z 314 ion, 233, 220, 191, 176 , 96, 95; ^{14}C abundance 78%. TLC system 1, ethyl acetate: 2-propanol-water-ammonia (25:7:1:1); system 2, dichloromethane-ethanol-ammonia (100:8:1), RCP 98%. HPLC: Hypersil BDS C8, CH_3CN-0.05M NH_4OAC (pH4) 25:75, UV detection at 280nm, RCP >98%.

6-Fluoro-2,3,4,5-tetrahydro-5-methyl-2-[5-Methyl-1H-Imidazol-4-yl)methyl]-1H-pyrido[4,3-b] indol-1-[^{14}C]-one mesylate dihydrate (2e). 1-Propanol containing 4% water (1.6ml) was added to the free base (2d) (72mg, 0.23mmol, 11.5mCi) and the mixture was heated to 70°C to give a clear solution. The solution was cooled to room temperature and isopropyl ether (1.6ml) was added dropwise to initiate crystallisation. The mixture was cooled, stirred at 3-5°C for 45min and filtered. The solid was washed with isopropyl ether-1-propanol (3:1, 0.29ml) and isopropyl ether (0.65ml), and dried under vacuum for 4h to yield an off-white coloured solid (100mg, 11.2mCi, 22% radiochemical yield from phosgene). Specific activity (gravimetric) 112μCi/mg, 49.9mCi/mmol. δ_H (300MHz, CD_3OD) 8.72 (1H, s, imidazole 2-H), 7.81 (1H, d, 9-H), 7.09 (1H, m, 8-H), 6.91 (1H, dd, 7-H), 4.74 (2H, s, bridge CH_2), 3.91 (3H, s, CH_3), 3.73 (2H, t, 3-CH_2), 3.13 (2H, t, 4-CH_2), 2.68 (3H, s, CH_3 SO_3H), 2.42 (3H, s, imidazole CH_3). RCP by TLC and HPLC >97 %. A portion of the mesylate salt (81.8mg, 9.2mCi) was diluted with unlabelled mesylate salt (46.7mg) and crystallised from 1-propanol-isopropyl ether as described above to afford crystalline (2e) (125.6mg, 8.9mCi): λ_{max} 218, 286, nm; Specific activity (gravimetric)

71.5μCi/mg, 31.9mCi/mmol; Specific activity (UV) 72.8-73.2μCi/mg. TLC: ethyl acetate-2-propanol-water-ammonia (25:7:1:1), RCP > 98%. HPLC: Hypersil BDS C8, CH_3CN-0.05M NH_4OAc (pH4) (25:75), UV detection at 280nm, RCP >98%.

Carbon-13 and Deuterium Labelled Alosetron.

5-Methyl-3H-imidazol-4-yl)-[$^2H_2$]methanol (17c). Imidazole ester (31) (1.84g, 11.9mmol) was reduced using the method of Villani *et al* (10) with lithium aluminium deuteride (1.0g, 23.8mmol) to give crude (17c), which was converted to the hydrochloride by dissolution in hot isopropanol (12ml) and treatment with concentrated hydrochloric acid (1.5ml, 16.8mmol). The solution was concentrated under reduced pressure to a volume of 5ml and acetone (12ml) was added. This solution was allowed to cool to 20°C and the resulting suspension was stirred for 30 min, collected by filtration, washed with acetone (2x2ml), and dried under vacuum at 35°C for 17h to give the hydrochloride of the *title compound* (17c) (1.05g, 50%); (Found: m/z (EI +ve) 114.076646 $[M]^+$ $C_5H_6{}^2H_2N_2O$ requires 114.076216); δ_H (400MHz, D_2O) 11.8 (1H, br, OH), 8.5 (1H, s, 2-H), 2.3 (3H, s, CH_3). Triethylamine (0.68g, 0.94ml, 6.7mmol) was added to a suspension of (17c) hydrochloride (0.5g, 3.36mmol) in isopropanol (5ml). The mixture was heated to 70°C and the resulting solution allowed to cool slowly to 20°C. The precipitated triethylamine hydrochloride was removed by filtration and washed with isopropanol (3x5ml). The filtrate and washes were concentrated under reduced pressure to a small volume (1ml). Acetone (5ml) and isopropanol (2ml) were added dropwise to give a solution which crystallised on sonication. The product was collected by filtration, washed with isopropanol (2x2ml), and dried under vacuum at 30°C for 17h to give the *title compound* (17c) as a white solid (0.33g, 86%); Found: m/z (FAB +ve) 115 (MH^+100%); δ_H (400MHz, DMSO-d_6) 7.6 (1H, s, 2-H), 2.1 (3H, s, CH_3).

5-Methyl-2-(5-methyl-1H-imidazol-4-yl-[$^2H_2$]methyl)-2,3,4,5-tetrahydro-pyrido[4,3-b]indol-1-one (1d). Imidazole (17c) (280mg, 2.46mmol) was coupled to (16b) (591mg, 2.95mmol), using the method described below for the preparation of (1e), to give the *title compound* (1d) as a cream solid (449mg, 62%); (Found: m/z (LSIMS +ve) 297.168852 $[M]^+$ $C_{17}H_{16}{}^2H_2N_4O$ requires 297.168440); δ_H (400MHz, DMSO-d_6) 11.7 (1H, br, NH), 7.9 (1H, m, indole aromatic), 7.4 (1H, m, indole aromatic), 7.38 (1H, s, imidazole 2-H), 7.11 (2H, m, indole aromatic), 4.5 (2H, s, N-CH_2), 3.7 (3H, s, NCH_3), 3.6 (2H, br, N-CH_2CH_2), 3.0 (2H, br , N-CH_2CH_2), 2.2 (3H, s, imidazole CH_3); δ_C (100MHz, DMSOd_6) 41.1(m, $^{13}C^2H_2$).

[4-$^{13}C^2H_3$]Methyl-1H-imidazole (35). Sulphonamide (33) (5.0g, 17.3mmol), prepared by the method of Chadwick (11), was converted to methylimidazole (34) using the method of Ngochindo (12) with iodo[$^{13}C^2H_3$]methane (3.07ml, 48mmol) to give a colourless oil (5.25g, 99%), which was used without further purification. Imidazole (34) (3.3g, 11.1mmol) was suspended in 48% aqueous hydrobromic acid (25ml, 150mmol) and heated at reflux for 22h. The reaction mixture was cooled to 20°C, adjusted to pH11 with 40% aqueous potassium hydroxide (30ml), and concentrated under reduced pressure to a solid, which was extracted with methanol-ethyl acetate (1:9; 2x250ml). The organic extracts were dried over magnesium sulphate, and concentrated under reduced pressure. The residue was purified by chromatography over silica gel (75g),

eluted with methanol-dichloromethane-ammonia (10:90:0.5) to give the *title compound* (35) as a colourless oil (0.8g, 83%); Found: m/z (Thermospray +ve) 87 (MH^+,100%); δ_H (400MHz, $CDCl_3$) 6.7 (1H, s, 4-H), 7.5 (1H, s, 2-H), 10.1 (1H, s, 1-H). δ_C (100MHz, $CDCl_3$) 10.4 (m, $^{13}C^2H_3$).

([5-$^{13}C^2H_3$]Methyl-3H-imidazol-4-yl)-methanol (17d). Imidazole (35) (0.9g, 10.45mmol,) was dissolved in water (1.2ml) by warming to 40°C. Potassium acetate (1.39g, 14.1mmol) and sodium hydroxide (0.11g, 2.7mmol) were added, followed by paraformaldehyde (0.31g, 10.45mmol) and water (0.2ml). The mixture was warmed to 30°C, then allowed to cool to 20°C, and stirred for 68h. The solution was chilled at 5°C for 23h, and the resulting suspension stirred at 5°C for 4h. The product was collected by filtration, washed with chilled saturated sodium chloride solution (4ml), and dried under vacuum at 45°C for 17h to give the *title compound* (17d) as a cream solid (0.56g, 46%); (Found: m/z (CI +ve) 117.093491 $[MH]^+$. $C_4{}^{13}CH_6{}^2H_3N_2O$ requires 117.093673); δ_H(400MHz, DMSO-d_6)11.8 (1H,br,OH), 7.4 (1H, s, 2-H), 4.8 (1H, br, NH), 4.3 (2H, s, CH_2OH). δ_C (100MHz, DMSO-d_6) 9.55 (m, $^{13}C^2H_3$).

5-Methyl-2-([5-$^{13}C^2H_3$]methyl-1H-imidazol-4-ylmethyl)-2,3,4,5-tetrahydropyrido[4,3-b]indol-1-one (1e). A solution of (17d) (230mg, 1.98mmol) and methanesulphonic acid (129µl, 1.98mmol) in de-oxygenated NMP (1.1ml), was added dropwise to a solution of lactam (16b) (476mg, 2.38mmol) and methanesulphonic acid (46µl, 0.9mmol) in de-oxygenated NMP (1.5ml) at 135°C under nitrogen. The reaction mixture was stirred at 135°C for 4h, before it was allowed to cool to 20°C for 17h. Water (1.6ml) was added and the solution was then cooled to 5°C. Sodium hydroxide (1M, 2.7ml) was added and the resulting suspension was stirred at 5°C for 1.25h. The solid was collected by filtration washed with water (4x1.5ml) and dried under vacuum at 45°C for 1.5h to give crude (1e) (365mg, 62%), which was purified by chromatography over silica (31g) eluted with dichloromethane-ethanol-ammonia (200:16:1). The solid obtained was triturated with diethyl ether (5ml), collected by filtration, washed with diethyl ether (3ml), and dried under vacuum at 40°C for 17h to give the *title compound* (1e) as a cream solid (308mg, 52%); (Found: m/z (FAB +ve) 299.177999. $[M]^+$ $C_{16}{}^{13}CH_{16}{}^2H_3N_4O$ requires 299.178072); δ_H (400MHz, DMSO-d_6) 11.7(1H, br, NH), 7.9 (1H, m, indole aromatic), 7.4 (1H, m, indole aromatic), 7.38 (1H, s, 2-H imidazole), 7.11 (2H, m, indole aromatic), 4.5 (2H, s, N-CH_2), 3.7 (3H, s, NCH_3), 3.6 (2H, br, CH_2CH_2-N), 3.0 (2H, t, CH_2CH_2-N); δ_C (100MHz, DMSOd$_6$) 8.4 (m, $^{13}C^2H_3$).

[5-^{13}C]Methyl-[4-^{13}C]-1H-imidazole-4-carboxylic acid ethyl ester (38). A mixture of ethyl acetoacetate (4g, 30.74mmol) and ethyl [2,4-$^{13}C_2$]acetoacetate (1g, 7.57mmol) was converted into the *title compound* (38) using the method of Graboyes (9). (38) was isolated as colourless oil (5.26g, 85%): m/z (Thermospray +ve) 155 (MH^+ unlabelled, 100%), 157 (MH^+ labelled, 25%); δ_H (400MHz, DMSOd$_6$) 7.6 (1H, s, 2-H), 4.2 (2H, q, CH_3CH_2), 2.4 (3H, s, CH_3), 1.3 (3H, t, CH_3CH_2); δ_C (100 MHz, DMSO-d$_6$) 127.4 (br, ^{13}C-4), 10.2 (br, $^{13}CH_3$).

[5-^{13}C]Methyl-3H-imidazol-[4-^{13}C]yl)-methanol (17e). Ester (38) (1.5 g, 9.62 mmol) was reduced with lithium aluminium hydride (1.13 g, 28.9 mmol), using the method of Villani (10) to give the *title compound* (17e) as a white solid (785mg, 72%): m/z (FAB +ve) 113 (MH^+, 100%),

115(24.3%); 2x^{13}C incorp 16.6%; δ_H (400MHz, DMSO-d6) 7.4 (1H, s, 2-H), 4.3 (2H, s, CH$_2$OH), 2.1 (3H, s, CH$_3$); δ_C (100MHz, DMSO-d$_6$) 130.9 (^{13}C-4), 10.4(^{13}CH$_3$).

2,3,4,5-Tetrahydro-5-methyl-2-[([5-^{13}C]methyl-1H-imidazol-[4-^{13}C]yl)methyl]-1H-pyrido[4,3-b]indol-1-one (1f). Imidazole (17e) (280 mg, 2.46 mmol) was coupled to lactam (16b) (591 mg, 2.95 mmol), using the method described above for preparation of (1e) to give the *title compound* (1f) as a cream solid (535 mg, 74%): m/z (LSIMS +ve) 295 (MH$^+$, 100%), 297 (23.3%), ^{13}C incorporation 15%; δ_H (400 MHz, DMSO-d$_6$) 11.7(1H, br, NH), 7.9 (1H, m, indole aromatic), 7.4 (1H, m, indole aromatic), 7.38 (1H, s, imidazole 2-H), 7.11 (2H, m, indole aromatic), 4.5 (2H, s, N-CH$_2$), 3.7 (3H, s, NCH$_3$), 3.6 (2H, br, N-CH$_2$CH$_2$), 3.0 (2H, t, N-CH$_2$CH$_2$), 2.2 (3H, s, ^{13}CH$_3$, J C-H = 129Hz); δ_C (100 MHz, DMSO-d6) 10.0 (^{13}CH$_3$, imidazole).

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