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Synthesis of the NK3 receptor antagonist AZD2624 in C-14-, H-3- and C-13-labeled forms

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In support of a program to develop an antipsychotic treatment for schizophrenia, three labeled forms of the NK3 receptor antagonist AZD2624 have been prepared. [$^{3}H_2$]AZD2624 was synthesized by tritiodehalogenation for use in receptor occupancy and autoradiographic studies. [$^{13}C_6$]AZD2624 was prepared for use as an internal standard through the intermediacy of [$^{13}C_6$]isatin, and two C-14 isotopomers of AZD2624 were prepared from [^{14}C]benzoic acid and [^{14}C]isatin for a variety of DMPK studies.

Keywords: NK3 receptor antagonist; isatin; AZD2624

Introduction

Schizophrenia is a serious psychiatric illness characterized by positive symptoms such as distorted perception of reality, hallucinations, delusions, disordered thinking, and paranoia; negative symptoms such as anergia (lack of energy), anhedonia (lack of joy in life), and avolition (lack of desire); and cognitive symptoms such as attention and memory deficits.[1,2] Schizophrenia often leads to social isolation or withdrawal. Approximately 1% of the population develops schizophrenia during their lifetime and while it affects both genders in equal proportions, it has an earlier onset age in males. Many treatments are currently marketed that provide some efficacy versus the positive symptoms of schizophrenia. Unfortunately, current therapies, including second generation antipsychotics, do not have much efficacy in treating the negative or cognitive symptoms and are sometimes associated with side effects including: weight gain, extrapyamidal effects, CV effects, and liability for type II diabetes.[2] These treatments are generally thought to function by reducing dopamine neurotransmission through modulation of D2 receptors and tend to possess activity at multiple other targets including adrenergic, muscarinergic, histaminergic, and serotonergic (in particular 5-HT_{2A}) receptors.[3,4] Because of this lack of efficacy especially in treating the negative and cognitive aspects of the disease and because of the side effect liabilities of these substances, considerable research effort has been focused on the discovery of alternative therapeutic targets beyond D2 receptor modulators.[5] One such mechanistic alternative is the NK3 receptor. Extensive preclinical evidence exists to show that NK3 activity modulates monoaminergic and amino acid neurotransmission and furthermore, that NK3 receptor antagonists are active in a range of animal models.[6] Two chemically distinct, high-affinity, selective NK3 receptor antagonists have been evaluated clinically and have been claimed to show efficacy in Phase II trials. However, both compounds have been dropped from development and there is considerable debate regarding the therapeutic value of NK3 receptor antagonists in treating schizophrenia.

To address these questions, we developed AZD2624 as a selective, high-affinity NK3 receptor antagonist. In support of the pre-clinical and clinical studies, we isotopically labeled this compound with tritium, C-14, and C-13, and these syntheses will be detailed herein.

Experimental

General

(1*R*,2*S*)1-phenyl-2-hydroxy-propylamine was prepared in two steps according to the method of Russell.[7] [Carbonyl-^{14}C]benzoic acid and methyl [carbonyl-^{14}C]benzoate were prepared from $Ba^{14}CO_3$ using standard methodology.[8,9] Handling of $^{3}H_2$ was done on a RC Tritec tritium gas manifold.[10] AZD2624 was provided by AstraZeneca CNS-Chemistry. [$^{13}C_6$]aniline was obtained from Cambridge Isotopes Laboratories, and $K^{14}CN$ (54 mCi/mmol) and $Ba^{14}CO_3$ (54 mCi/mmol) were obtained from American Radiolabeled Chemicals, Inc. All other reagents were obtained from Acros and Sigma-Aldrich and were used without purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance 500 spectrometer and/or an Avance 600 with a ^{1}H, ^{13}C Dual Cryoprobe spectrometer in DMSO, $CDCl_3$, or CD_3OD and were referenced to the residual solvent peak. Assignment of the protons of AZD2624 and **1** was done using a combination of COSY, NOESY, and HMBC. LC/MS analyses were performed on an HP MSD-1100 using a 4.6 × 100 mm Luna-C18(2) column, with a 10–100% gradient of MeCN-0.1% aq formic acid over 10 min and electrospray ionization. HPLC analyses were performed using a

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Agilent 1100 series HPLC system using either: method A (4.6 × 100 mm Phenomenex Luna C-18(2), 10–100% MeCN-0.1% TFA over 20 min), method B (4.6 × 100 mm Phenomenex Luna C-18(2), 30–50% MeCN-0.1% TFA over 20 min), method C (4.6 × 100 mm Phenomenex Luna C-18(2), 10–100% MeCN-0.1% TFA over 10 min), or method D (2.1 × 50 mm Zorbax C-8, 5–85% MeCN-0.1% formic acid over 12 min). All HPLC analyses were conducted using a flow rate of 1 mL/min on columns heated to 30°C and concluded with a 5 min wash of 100% MeCN. Preparative HPLC was performed using the following methodology: method E (21.2 × 250 mm Phenomenex Luna C-18(2), 50–100% MeOH-0.1% TFA over 40 min, 15 mL/min), method F (10 × 250 mm Phenomenex Luna C-18(2), 30–90% MeCN-0.1% TFA over 40 min, 3 mL/min), method G (30 × 100 mm Phenomenex Gemini C18, 15–42% over 7 min then 42–90% over 5 min MeCN-5 mM NH_4HCO_3, 51 mL/min,), method H (21.2 × 250 mm Phenomenex Luna C-18(2), 20–30% MeCN-0.1% TFA over 40 min, 15 mL/min), or method I (21.2 × 250 mm Phenomenex Luna C-18(2), 25–70% MeCN-0.1% TFA over 40 min, 15 mL/min). GC/MS analyses were conducted with a Hewlett Packard 6890 GC system and 5973 Mass Selective Detector using a temperature gradient of 70–260°C over 20 min.

(S)-N-(1-(3,4-diiodophenyl)propyl)-3-(methylsulfonamido)-2-phenylquinoline-4-carboxamide (**1**): A solution of 5 mg (0.011 mmol) of AZD2624 in 0.5 mL of TFA was degassed by bubbling Ar through the solution and then 10 mg (0.044 mmol) of *N*-iodosuccinimide was added and the solution stirred under Ar overnight. The solution was then concentrated to dryness and the residue purified by preparative HPLC (method E) to give 4 mg of a white solid (52%). LC/MS: 712 (100%), 713 (29.8%), 714 (9.0%). ^{1}H NMR (600 MHz, $CDCl_3$)δppm 0.96 (t, J = 7.2 Hz, 3 H), 1.89 (m, 1 H), 2.07 (d, J = 5.7 Hz, 1 H), 2.18 (s, 3 H), 5.01 (q, J = 6.4 Hz, 1 H), 7.17 (br. s., 1 H), 7.34 (m, 3 H), 7.67 (br. s., 3 H), 7.79 (t, J = 7.4 Hz, 3 H), 7.88 (d, J = 7.9 Hz, 1 H), 8.00 (br. s., 1 H), 8.18 (d, J = 6.0 Hz, 1 H).

(S)-N-(1-([3,4-$^3H_2$]phenyl)propyl)-3-(methylsulfonamido)-2-phenylquinoline-4-carboxamide ([$^3H_2$]AZD2624): A slurry of 0.35 mg (0.60 μmol) of iodide **1**, 10 μL (0.072 mmol) of NEt_3 and 0.45 mg of 5% Pd/C in 0.5 mL of DMF was degassed using freeze–thaw methodology and then 38 mBar (0.011 mmol, 624 mCi) of $^3H_2$ was added to the $N_{2(l)}$-cooled solution. The solution was warmed to room temperature and was stirred for 2 h. The remaining $^3H_2$ was recovered and the volatiles were transferred into a waste container. The residue was taken up in 1 mL of EtOH and this slurry was filtered through a syringe filter to afford 10 mCi of [$^3H_2$]AZD2624 with a radiochemical purity of 94.5% (method A). Purification of 2.5 mCi of the crude material by semi-preparative HPLC (method F) and isolation by Oasis HLB SepPak gave 1.67 mCi at a radiochemical purity of > 99.5% (method B) and a specific activity of 37 Ci/mmol. LC/MS: 462 (100%), 464 (99%), 460 (26%). ^{1}H NMR (500 MHz, CD_3OD)δppm 7.31 (m, 0.21 H), 7.40 (dt, J = 7.8, 3.8 Hz, 1.5 H), 7.47 (d, J = 7.7 Hz, 2 H), 7.55 (m, 4 H), 7.68 (d, J = 8.2 Hz, 1 H), 7.74 (dd, J = 7.9, 1.4 Hz, 2 H), 7.81 (m, 1 H), 8.09 (d, J = 8.4 Hz, 1 H). ^{3}H NMR (534 MHz, CD_3OD)δppm 7.32 (m, 1 H) 7.40 (m, 1 H) 7.46 (s, 0.03 H). ^{3}H {proton decoupled} NMR (534 MHz, CD_3OD)δppm 7.31 (s, 1 H), 7.31 (d, J = 8.0 Hz, 1 H), 7.40 (d, J = 8.0 Hz, 1 H), 7.40 (s, 1 H), 7.47 (s, 0.13 H).

Dimethylsulfoxonium [2-^{14}C]2-phenyl-2-oxoethylide (**[^{14}C]-4**): A solution of 1.54 g (12.0 mmol) of trimethylsulfoxonium chloride in 12 mL of THF was stirred as 12.2 mL of 1 M (12.2 mmol) KOtBu in THF was added. The solution was then warmed to 70°C for 2 h under N_2. After cooling to rt, 130 mCi (2.4 mmol, 54 mCi/mmol) of methyl [^{14}C]benzoate was added in 5 mL of THF and the solution was stirred overnight. The reaction mixture was diluted with 50 mL of EtOAc and 50 mL of water and the layers separated. The aqueous layer was then extracted twice with 25 mL of EtOAc and the organic extracts were combined to give 94 mCi. HPLC assay (method C) showed a 50% radiochemical purity with 37% of residual methyl [^{14}C]benzoate. The volatiles were removed *via* vacuum distillation and the residue was taken up in 10 mL of THF to give 32 mCi (23% radiochemical yield) of **[^{14}C]-4** (purity of 95%, method C). The volatiles contained 25 mCi (19%) of methyl [carbonyl-^{14}C]benzoate which was of sufficient quality to be recycled in this procedure without further purification.

[1-^{14}C]2-chloroacetophenone (**[^{14}C]-5**): A solution of 5.7 mCi (0.11 mmol, 54 mCi/mmol) of **[^{14}C]-4** in 0.5 mL of THF was stirred at room temperature as 30 μL (0.12 mmol) of 4 M HCl in dioxane was added and then the solution was heated at 90°C for 2 h. The solvent was removed by distillation at reduced pressure and the residue was partitioned between 5 mL of water and 5 mL of MTBE. The layers were separated and the aqueous layer was extracted two additional times with 5 mL of MTBE. The organic layers were combined to give 5.2 mCi and the solvent was removed by distillation. HPLC analysis showed a radiochemical purity of 35% (method C).

(1H-imidazol-1-yl) [carbonyl-^{14}C]benzoate (**[^{14}C]-7**): A solution of 12.5 mCi (0.23 mmol) of [carbonyl-^{14}C]benzoic acid in 2 mL of CH_2Cl_2 was stirred as 0.10 mL (1.2 mmol) of oxalyl chloride and 0.01 mL of DMF were added sequentially and the resulting solution was stirred for 3 h. The solution was concentrated to dryness under a stream of N_2, and the residue was dissolved in 2 mL of CH_2Cl_2 and was then added to a solution of 76 mg (1.1 mmol) of imidazole in 1 mL of THF. The reaction mixture was stirred for 16 h at room temperature and was then washed four times with 2 mL of water. The organic layer was then dried ($MgSO_4$) and filtered to give 12.1 mCi of **[^{14}C]-7** (97%, radiochemical purity 95.5%, method C).

[1-^{14}C]2-nitro-acetophenone (**[^{14}C]-8**): To a stirred solution of 12.1 mCi (0.22 mmol) of **[^{14}C]-7** in 2 mL of THF was added $NaCH_2NO_2$ (formed from 86 mg (1.4 mmol) of $MeNO_2$ and 96 mg (1.4 mmol) of NaOEt in 0.5 mL of EtOH). The resulting slurry was warmed to 75°C and stirred overnight. The reaction mixture was diluted with 15 mL of water and was acidified to pH 1 with conc HCl. The resulting solution was extracted three times with 20 mL of EtOAc. The combined organic layers were dried ($MgSO_4$), filtered, and concentrated to give 10.8 mCi (56% radiochemical yield) of **[^{14}C]-8** with a radiochemical purity of 63% (method C, assay showed 35% of residual starting material).

[1-^{14}C]2-amino-acetophenone (**[^{14}C]-6**): A solution of 155 mg (0.69 mmol) of stannous chloride dihydrate in 0.23 mL of conc HCl and 0.18 mL of EtOH was added to 10.8 mCi (0.13 mmol, 63% radiochemical purity) of **[^{14}C]-8** under N_2 and the resulting solution was stirred at 70°C for 2 h. The solution was diluted with 5 mL of water and was then extracted five times with 1 mL of CH_2Cl_2. The organic extracts were discarded. The aqueous solution was then basified with NaOH and was extracted five times with 2 mL of CH_2Cl_2. The combined organic layers were dried (Na_2SO_4), filtered, and concentrated to approximately 2 mL to give 5.3 mCi (78% radiochemcial yield) of **[^{14}C]-6** (99.5% radiochemical purity, method C). LC/MS: 138 (100%), 136 (15.5%), 137 (7.7%).

[2-^{14}C]N-(2-oxo-2-phenylethyl)methanesulfonamide (**[^{14}C]-2**): A stirred solution of 5.3 mCi (0.10 mmol) of **[^{14}C]-6** in 2 mL of

CH_2Cl_2 was cooled to 0°C and 0.07 mL (0.5 mmol) of NEt_3 was added followed by dropwise addition of 0.2 mL (2.5 mmol) of methanesulfonyl chloride over 5 min. The solution was stirred at 0°C for 1 h and then at room temperature for 1 h. After cooling to 0°C, the solution was diluted with 3 mL of water and the layers were separated. The aqueous layer was extracted five times with 10 mL of CH_2Cl_2. The combined organic layers were washed with 5 mL of saturated aq NaCl and were then dried (Na_2SO_4). The drying agent was removed by filtration to give a solution containing 3.8 mCi (64% radiochemical yield) of (**[^{14}C]-3** (90% radiochemical purity, method C).

3-(Methylsulfonamido)-2-phenyl[2-^{14}C]quinoline-4-carboxylic (**[2-^{14}C]-9**): A solution of 22 mg (0.15 mmol) of isatin and 60 mg (1.5 mmol) of NaOH in 0.50 mL of water was stirred at room temperature as 1.65 mCi (0.031 mmol) of **[^{14}C]-2** in 1.25 mL of EtOH, 0.25 mL of THF and 1.25 mL of water was added dropwise over 30 min. The resulting solution was heated at 85°C for 16 h. The organics were removed under a stream of N_2 and the solution was applied to an Oasis HLB SepPak. The compound was eluted from the seppak with MeCN to give 1.5 mCi (42% radiochemical purity) which was purified by Silica Gel chromatography (1–10% MeOH-CH_2Cl_2) to give 0.65 mCi (39% radiochemical yield) of **[2-^{14}C]-9** (97% radiochemical purity, method C).

(S)-3-(Methylsulfonamido)-2-phenyl-N-(1-phenylpropyl)[2-^{14}C]-quinoline-4-carboxamide ([2-^{14}C]AZD2624): A solution of 0.7 mCi (0.013 mmol) **[2-^{14}C]-9**,15 mg (0.11 mmol) of HOBT and 0.040 mL (0.36 mmol) of *N*-methylmorpholine in 1 mL of CH_2Cl_2 was stirred as 21 mg (0.11 mmol) of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and 15 mg (0.11 mmol) of (*S*)-1-phenyl propylamine were added sequentially. After 22 h, the material was diluted with 0.1% aqueous TFA and purified by preparative HPLC (method I) to give 0.12 mCi (17% radiochemical yield) of [^{14}C]AZD2624 (97.3% radiochemical purity, method C) with a specific activity of 54 mCi/mmol. LC/MS (M+1): 462 (100%), 463 (29.8%), 460 (13.9%).

t-Butyl 2-bromophenylcarbamate: A slurry of 512 mg (12.8 mmol) of 60% NaH in oil and 70 mL of THF was stirred as 2.0 g (11.6 mmol) of 2-bromoaniline was added. The solution was heated at reflux for 1 h and after cooling to room temperature, 3.04 g (14 mmol) di-*t*-butyl carbonate was added. The resulting slurry was stirred for 30 min and another 512 mg (12.8 mmol) of 60% NaH in oil was added. The slurry was heated at reflux for 2 h and at 50°C for 72 h. Then the reaction mixture was partitioned between 50 mL of water and 75 mL of Et_2O. The layers were separated and the aqueous layer was extracted twice with 75 mL of Et_2O. The combined organic layers were washed with 50 mL of saturated aqueous NH_4Cl and 50 mL of saturated aq. $NaHCO_3$ and were then dried (Na_2SO_4). The drying agent was removed by filtration and the solvent removed at reduced pressure. The residue was purified by silica gel chromatography (5% Et_2O in hexane) and the product containing fractions were combined to give 1.1 g (36%) of *t*-butyl 2-bromophenylcarbamate (>98% UV area % purity, method C). LC/MS(M+1-56): 215.9(100%), 217.9 (92%). ^{1}H NMR (500 MHz, $CDCl_3$)δppm 1.54 (m, 9 H), 6.89 (td, *J* = 7.6, 1.5 Hz, 1 H), 6.98 (br. s., 1 H), 7.28 (ddd, *J* = 8.5, 7.1, 1.5 Hz, 1 H), 7.49 (dd, *J* = 7.9, 1.5 Hz, 1 H), 8.14 (d, *J* = 7.3 Hz, 1 H). ^{13}C NMR (126 MHz, $CDCl_3$)δppm 28.3, 81.1, 112.4, 120.1, 123.8, 128.3, 132.2, 136.3, 152.4.

Ethyl [^{14}C]cyanoformate: A solution of 119 mg (99 mCi, 1.77 mmol) of K^{14}CN in 0.8 mL of water was cooled to 0°C as 0.22 mL (0.22 mmol) of 1 M NH_4OH in MeOH and 288 mg (2.65 mmol) of ethyl chloroformate in 0.8 mL of CH_2Cl_2 were added. The bi-phasic mixture was stirred vigorously for 45 min at 0°C and the layers were then separated. The aqueous layer was extracted twice with 0.5 mL of CH_2Cl_2 and the combined organic layers were dried ($MgSO_4$). The drying agent was removed by filtration to give 60 mCi (61%) of ethyl [^{14}C]cyanoformate in CH_2Cl_2 which was used directly in the next reaction without further characterization.

Diethyl [^{14}C]oxalate: A solution of 60 mCi (1.1 mmol) of ethyl [^{14}C]cyanoformate in 1.5 mL of CH_2Cl_2 was added to 0.6 mL (10 mmol) of EtOH and $HCl_{(g)}$ was bubbled through the solution for 30 min. The solvent was then carefully removed under vacuum. The residue was taken up in 1 mL of water and the resulting solution was extracted five times with 1 mL of CH_2Cl_2. The combined organic layers were dried (Polycarbonate-$MgSO_4$) and the solution was then purified by silica gel (100% CH_2Cl_2) to give 45 mCi (75% radiochemical yield) of diethyl [^{14}C]oxalate (91% radiochemical purity, method C).

[Carbonyl-^{14}C]ethyl 2-(2-(tert-butoxycarbonylamino)phenyl)-2-oxoacetate (**[^{14}C]-10**): A solution of 280 mg (1.03 mmol) of *t*-butyl 2-bromophenylcarbamate in 2 mL of Et_2O and 2 mL of THF under Ar was stirred as 0.06 mL (0.09 mmol) of 1.6 M MeLi in Et_2O was added dropwise. The solution was stirred for 30 min at room temperature and was then cooled to −78°C as 1.24 mL (2.1 mmol) of 1.7 M *t*-butyllithium in pentane was added dropwise and the resulting solution was stirred for 90 min. To this solution was added 13 mg (0.09 mmol, 5 mCi) of diethyl [^{14}C]oxalate in 2 mL of Et_2O and the solution was stirred for 0.5 h. The reaction was then diluted with 10 mL of Et_2O and 5 mL of water and the layers were separated. The organic layer was extracted four times with 1 M NaOH and the combined aqueous layers were acidified with 5 M HCl. The resulting solution was extracted five times with 10 mL of CH_2Cl_2. The combined organic layers were dried (Na_2SO_4) and the drying reagent removed by filtration to give 3.0 Ci (87% radiochemical purity, method C) of **[^{14}C]-10**.

[Carbonyl-^{14}C]Isatin (**[^{14}C]-3**): A solution of 21 mg (3.6 mCi, 0.071 mmol) of **[^{14}C]-10** in 1 mL of dimethoxyethane was stirred as 0.35 mL of conc. HCl was added and the resulting solutiuon was stirred at 80°C for 2 h. The solvent was removed and the residue taken up in 5 mL of water. The aqueous solution was applied to a 1 g Oasis HLB SepPak column and was washed with 10 mL of 5% $NaHCO_3$ and 20 mL of water prior to eluting the compound with 20 mL of MeCN to give 2.9 mCi (74%) of **[^{14}C]-3** (90% radiochemical purity, method C).

3-(Methylsulfonamido)-2-phenyl[4-^{14}C]quinoline-4-[^{14}C]carboxylic acid, (**[carbonyl, 4-^{14}C]-9**): A solution of 2.9 mCi (0.071 mmol) of isatin and 15 mg (0.070 mmol) of *N*-(2-oxo-2-phenylethyl)methanesulfonamide in 0.1 mL of EtOH was heated for 3 min with a heat gun. The red gum was diluted with 0.8 mL of water and 0.2 mL of MeCN and the resulting solution purified by preparative HPLC (method H). Product containing fractions were concentrated to remove the MeCN and then applied to Oasis SepPak to afford 2.3 mCi (79% radiochemical yield) of **[carbonyl, 4-^{14}C]-9** (95% radiochemical purity, method C) in EtOH. LC/MS (M+1): 345 (100%), 343 (15.6%), 346 (18.0%).

(S)-3-(Methylsulfonamido)-2-phenyl-N-(1-phenylpropyl) [4-^{14}C]quinoline-4-^{14}C]carboxamide ([carbonyl, 4-^{14}C]AZD2624): A solution of 2.3 mCi (0.043 mmol) of **[carbonyl, 4-^{14}C]-9,** 12 mg (0.082 mmol) of HOBT, and 0.022 mL (0.20 mmol) of *N*-methylmorpholine in 1.5 mL of CH_2Cl_2 was stirred for 5 min and then 15 mg (0.081 mmol) of (*S*)-1-phenylpropylamine was added. After stirring 10 min, 11 mg (0.081 mmol) of (*S*)-1-phenylpropan-1-amine was added and the solution was stirred overnight at room temperature. The solution was concentrated to near dryness and was then purified by

preparative HPLC (method I). The product containing fractions were pooled, concentrated to remove the MeCN, and applied to an Oasis HLB SepPak. Elution with 10 mL of EtOH afforded 1.8 mCi (78% radiochemical yield) of [carbonyl, 4-^{14}C]AZD2426 with a specific acidity of 53 mCi/mmol and radiochemical purity of 99.5% (method C). LC/MS (M+1): 462 (100%), 463 (28%), 460 (15%), 463 (8.7%). ^{1}H NMR (500 MHz, $CDCl_3$)δppm 0.94 (t, J = 7.4 Hz, 3 H), 1.95 (d of quintet, J = 13.7, 7.8, 7.8, 7.8, 7.8 Hz, 1 H), 2.14 (s, 1 H), 2.17 (m, 3 H), 5.18 (m, 1 H), 7.03 (d, J = 7.6 Hz, 1 H), 7.19 (s, 1 H), 7.31 (m, 1 H), 7.39 (m, 4 H), 7.47 (m, 3 H), 7.53 (t, J = 7.6 Hz, 1 H), 7.68 (dd, J = 7.8, 1.4 Hz, 2 H), 7.74 (m, 2 H), 8.13 (d, J = 8.4 Hz, 1 H). ^{13}C NMR (126 MHz, $CDCl_3$)δppm 10.6, 28.8, 41.8, 56.7, 123.4, 124.4, 125.1, 127.1, 127.7, 128.1, 128.7, 128.8, 129.2, 129.8, 129.9, 130.7, 138.8, 141.1, 143.2, 147.2, 158.1, 165.0.

2-(Hydroxyimino)-N-[$^{13}C_6$]phenylacetamide (**[$^{13}C_6$]-11**): A solution of 1.00 g (10.1 mmol) of [$^{13}C_6$]aniline in 10 mL of water and 1 mL of concentrated HCl was stirred as 2.01 g (13.7 mmol) of chloral in 30 mL of water and 30 g (211 mmol) of Na_2SO_4 in 80 mL of water were added sequentially. After stirring 20 min, 2.40 g (72.7 mmol) of hydroxylamine in 10 mL of water was added and the solution warmed to 105°C. After 2 h, the solution was cooled to 0°C and the resulting precipitate was removed by filtration and was allowed to air dry for 12 h to give 2 g (133%) of **[$^{13}C_6$]-11** as white solid.

[$^{13}C_6$]Isatin (**[$^{13}C_6$]-3**): A solution of 1.0 g (5.1 mmol, containing 33% water) of **[$^{13}C_6$]-11** in 2 mL of concentrated H_2SO_4 was heated at 65°C for 1 h. The solution was poured slowly into 100 mL of ice and the orange solid removed by filtration. The solid was dissolved in EtOAc and the resulting solution was dried ($MgSO_4$). The dying agent was removed by filtration and the solvent was removed at reduced pressure to give 0.42 g (56% yield from [$^{13}C_6$]aniline) of **[$^{13}C_6$]-3** as an orange solid. LC/MS (M+1): 154 (100%), 153 (5.6%), 155 (5.5%). ^{1}H NMR (500 MHz, $CDCl_3$)δppm 6.91 (dq, J = 164, 6.7 Hz, 1 H), 7.13 (dq, J = 165, 7.6 Hz, 1 H), 7.56 (dquin, J = 162, 9.2 Hz, 1 H), 7.62 (dq, J = 166, 8.2 Hz, 1 H), 8.01 (br. s., 4 H). ^{13}C NMR (126 MHz, $CDCl_3$)δppm 112.2 (dddd, J = 67, 57, 6.4, 2.7 Hz), 118.2 (ddd, J = 62, 58, 8.5 Hz), 124.0 (tdd, J = 56, 9.1, 1.8 Hz), 125.8 (ddd, J = 64, 56, 5.5 Hz), 138.6 (td, J = 57, 8.2 Hz), 149.0 (ddd, J = 67, 58, 10 Hz).

3-(Methylsulfonamido)-2-phenyl[4a, 5, 6, 7, 8, 8a-$^{13}C_6$]quinoline-4-carboxylic acid (**[$^{13}C_6$]-9**): A solution of 0.57 g (3.35 mmol) of [$^{13}C_6$]isatin, 1.54 g (37.5 mmol) of NaOH and 3 mL of water was stirred at 85°C for 20 min. A solution of 0.90 g (4.1 mmol) of *N*-(2-oxo-2-phenylethyl)methanesulfonamide in 20 mL of EtOH, 20 mL of water, and 10 mL of THF was added dropwise and was stirred overnight. The organic solvents were removed under reduced pressure and the aqueous solution was washed twice with 20 mL of ether. The aqueous layer was acidified to pH 1 with concentrated HCl and was then extracted twice with 100 mL of EtOAc. The organic layer was dried ($MgSO_4$) and then filtered. The organic solution was concentrated to dryness and was then recrystallized from a minimum of MeCN to give 0.67 g (52%) of **[$^{13}C_6$]-9** as a yellow solid. LC/MS (M+1): 349 (100%), 350 (14.2%), 348 (5.7%). ^{1}H NMR (500 MHz, CD_3OD)δppm 2.51 (s, 3 H), 7.53 (m, 4 H), 7.71 (dm, J = 165 Hz, 1 H), 7.76 (m, 1 H), 7.86 (dm, J = 165 Hz, 1 H), 8.07 (d, J = 163.0 Hz, 1 H), 8.12 (d, J = 165 Hz, 1 H). ^{1}H {C-13 decoupled} NMR (500 MHz, CD_3OD)δppm 2.51 (s, 3 H), 7.54 (m, 3 H), 7.71 (t, J = 7.8 Hz, 1 H), 7.76 (d, J = 6.7 Hz, 1 H), 7.86 (t, J = 7.0 Hz, 1 H), 8.07 (d, J = 8.5 Hz, 1 H), 8.12 (d, J = 8.5 Hz, 1 H). ^{13}C NMR (126 MHz, CD_3OD)δppm 123.8 (m), 125.5 (m), 127.8 (m), 128.0 (m), 130.7 (m), 145.9 (m).

(S)-3-(Methylsulfonamido)-2-phenyl-N-(1-phenylpropyl)[4a, 5, 6, 7, 8, 8a-$^{13}C_6$]quinoline-4-carboxamide ([$^{13}C_6$]AZD2624): A solution of 100 mg (0.29 mmol) of **[$^{13}C_6$]-9** and 70 mg (0.52 mmol) of HOBT in 0.2 mL (1.8 mmol) of *N*-methylmorpholine and 5 mL of CH_2Cl_2 was stirred under N_2 as 84 mg (0.54 mmol) of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide was added. After 5 min, 55 mg (0.41 mmol) of (*S*)-1-phenylpropylamine was added and the solution was stirred overnight. The solution was diluted with 10 mL of CH_2Cl_2 and was washed with 10 mL of water, 5 mL of 0.5 M HCl, and 10 mL of sat. aq. $NaHCO_3$. The organic layer was dried ($MgSO_4$) and the drying agent removed by filtration. The organic solution was then concentrated to dryness and purified by preparative HPLC (method G) to give 51 mg (38 %) of [$^{13}C_6$]AZD2624 (99.5% UV area%, method D) as a white solid. LC/MS (M+1): 465 (5.6%), 466 (100%), 467 (24.7%), 468 (7.9%). ^{1}H NMR (500 MHz, DMSO-d_6)δppm 0.92 (t, J = 7.3 Hz, 3 H), 1.84 (d of septet, J = 65, 6.8 Hz, 2 H), 2.37 (s, 3 H), 5.01 (q, J = 7.5 Hz, 1 H), 7.29 (m, 1 H), 7.38 (t, J = 7.6 Hz, 2 H), 7.48 (m, 5 H), 7.78 (d, J = 6.7 Hz, 3 H), 7.81 (d, J = 155 Hz, 1 H), 8.06 (d, J = 161 Hz, 1 H), 9.16 (d, J = 7.3 Hz, 1 H), 9.31 (s, 1 H). ^{13}C NMR (126 MHz, DMSO-d_6)δppm 124.2 (t, J = 56 Hz), 125.2 (t, J = 55 Hz), 127.2 (t, J = 55 Hz), 128.8 (t, J = 59 Hz), 130.3 (t, J = 54 Hz), 146 (t, J = 58 Hz).

N-((1R,2S)-2-hydroxy-1-phenylpropyl)-3-(methylsulfonamido)-2-phenyl[4a, 5, 6, 7, 8, 8a-$^{13}C_6$]quinoline-4-carboxamide (**[$^{13}C_6$]-12**): A solution of 100 mg (0.29 mmol) of **[$^{13}C_6$]-9** and 70 mg (0.52 mmol) of HOBT in 0.2 mL (1.8 mmol) of *N*-methylmorpholine and 5 mL of CH_2Cl_2 was stirred under N_2 as 84 mg (0.54 mmol) of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide was added. After 5 min, 54 mg (0.36 mmol) of (*R*)-1-amino-1-phenylpropan-2-ol was added and the solution was stirred overnight. LC/MS assay indicated substantial starting material remaining; therefore, 70 mg (0.52 mmol) of HOBT, 0.2 mL (1.8 mmol) of *N*-methylmorpholine, 84 mg (0.54 mmol) of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and 54 mg (0.36 mmol) of (*R*)-1-amino-1-phenylpropan-2-ol were added and the solution was stirred 3 h longer. The solution was diluted with 10 mL of CH_2Cl_2 and was washed with 10 mL of water, 5 mL of 0.5 M HCl, and 10 mL of sat. aq. $NaHCO_3$. The organic layer was dried ($MgSO_4$) and the drying agent removed by filtration. The solution was concentrated to dryness to give 130 mg (94%) of **[$^{13}C_6$]-12** which was carried directly into the next reaction. LC/MS (M+1): 482 (100%), 483 (24.6%), 481 (7.8%), 480 (0.1%).

(R)-3-(methylsulfonamido)-N-(2-oxo-1-phenylpropyl)-2-phenyl [4a, 5, 6, 7, 8, 8a-$^{13}C_6$]quinoline-4-carboxamide (**[$^{13}C_6$]-13**): A solution of ca 130 mg (0.29 mmol) of **[$^{13}C_6$]-12** in 5 mL of CH_2Cl_2 was stirred as 220 mg (1.0 mmol) of pyridium chlorochromate was added. After 1 h, the reaction was concentrated to dryness and then taken up in 5 mL of CH_2Cl_2. The resulting solution was filtered. Purification by preparative HPLC (method G) afforded 21 mg (15% yield from **[$^{13}C_6$]-9**) of **[$^{13}C_6$]-13** (96.5% UV area% purity, method D) as a white solid. LC./MS: 480 (100%), 481 (24%), 479 (8.4%), 478 (0.3%). ^{1}H NMR (500 MHz, DMSO-d_6)δppm 2.20 (s, 3 H), 2.42 (s, 3 H), 5.86 (d, J = 6.1 Hz, 1 H), 7.45 (m, 8 H), 7.82 (d, J = 155.0 Hz, 1 H), 7.80 (d, J = 6.7 Hz, 3 H), 8.06 (d, J = 155.0 Hz, 1 H), 7.96 (d, J = 145.0 Hz, 1 H), 9.43 (d, J = 6.1 Hz, 1 H). ^{13}C NMR (126 MHz, DMSO-d_6)δppm 124.2 (t, J = 52 Hz), 125.5 (td, J = 56, 3.6 Hz), 127.3 (ddd, J = 56, 52, 8.0 Hz), 128.7 (t, J = 60 Hz), 130.3 (t, J = 53 Hz), 145.9 (t, J = 59 Hz).

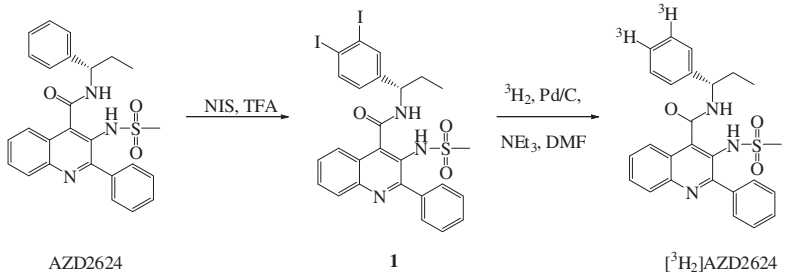
Results

[$^3H_2$]AZD2624 was required for use in receptor occupancy and autoradiographic studies. The site of labeling was not critical so long as the tritium label was in a non-exchangable location with

a specific activity of > 30 Ci/mmol. Unlabeled AZD2624 was iodinated with *N*-iodosuccinimide in TFA and the iodide (**1**) subjected to tritiodehalogenation (Scheme 1). Purification by semi-preparative HPLC gave [$^3H_2$]AZD2624 with a specific activity of 37 Ci/mmol. The sites of tritiation were confirmed by ^{3}H NMR. As can be seen from the ^{3}H NMR (Figure 1), the ratio of tritium is an approximately 1:1:1:1 split between the para and meta positions of the phenylpropyl ring. The proton decoupled ^{3}H NMR shows a mixture of two singlets and two doublet in approximately 1:1:1:1 ratio which correspond to meta and para monotritiated AZD2624 and meta and para ditritiated AZD2624.

We next turned our efforts to the synthesis of C-14-labeled AZD2624. The medicinal chemistry approach offered two fragments that were considered for C-14 labeling: methanesulfonamide **2** and isatin, **3** (Scheme 2). We investigated two routes leading to [^{14}C]sulfonamide **2** both of which originated from [^{14}C]benzoic acid. In route A, benzoic acid would be converted to an α-chloroketone *via* the sulfur ylide and subsequently to **[^{14}C]-2** *via* nucleophilic displacement. In route B, benzoic acid would be coupled with nitromethane (offering an alternative site of label incorporation) to give the α-nitroketone which could be reduced and then converted to **[^{14}C]-2**. Route C which targeted label incorporation in isatin was envisioned as consisting of the coupling of aniline and [^{14}C]diethyloxalate.

We chose to first investigate Route A. Methyl [carbonyl-^{14}C]-benzoate was reacted with trimethylsulfoxonium chloride to



Scheme 1. Preparation of [$^3H_2$]AZD2624.

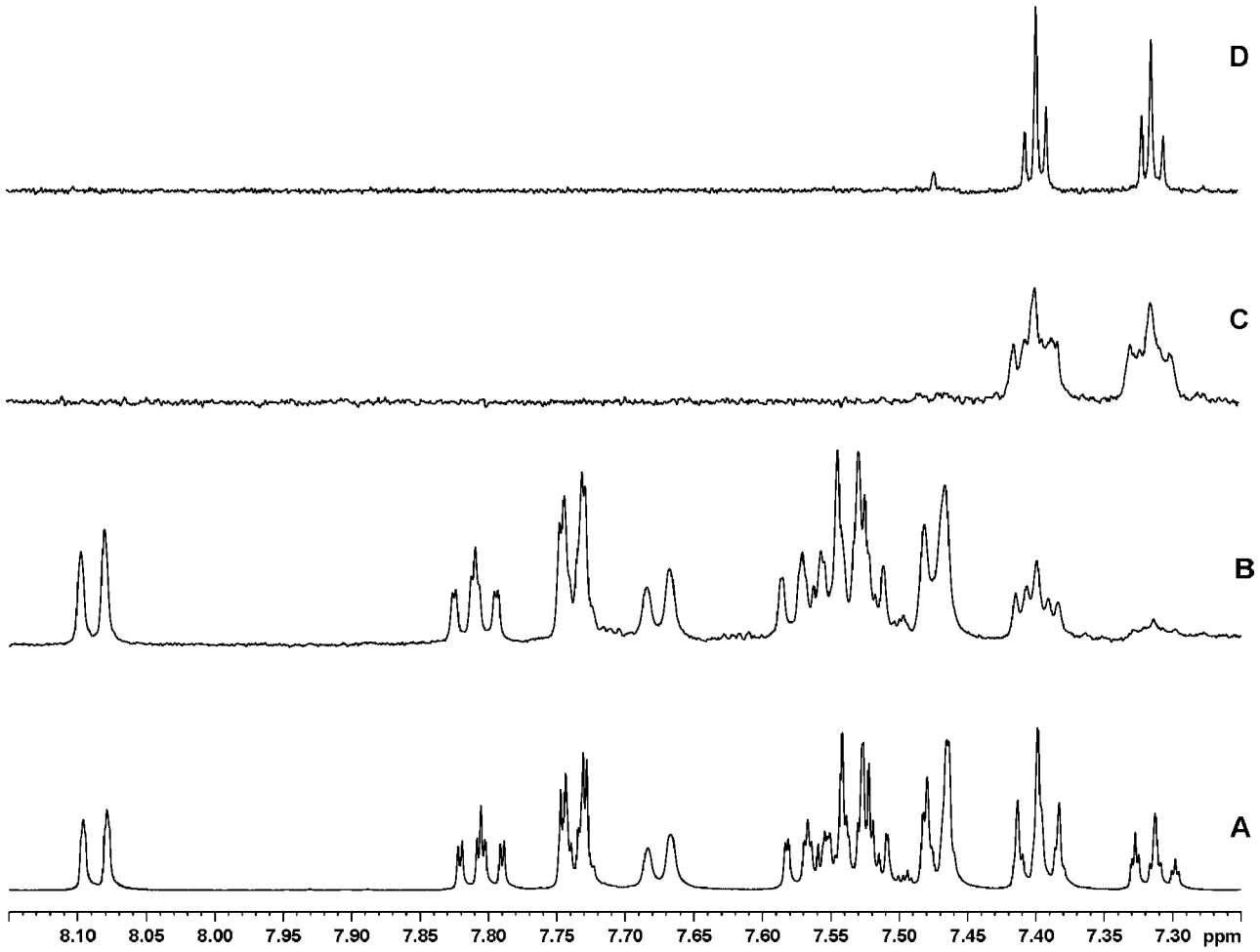
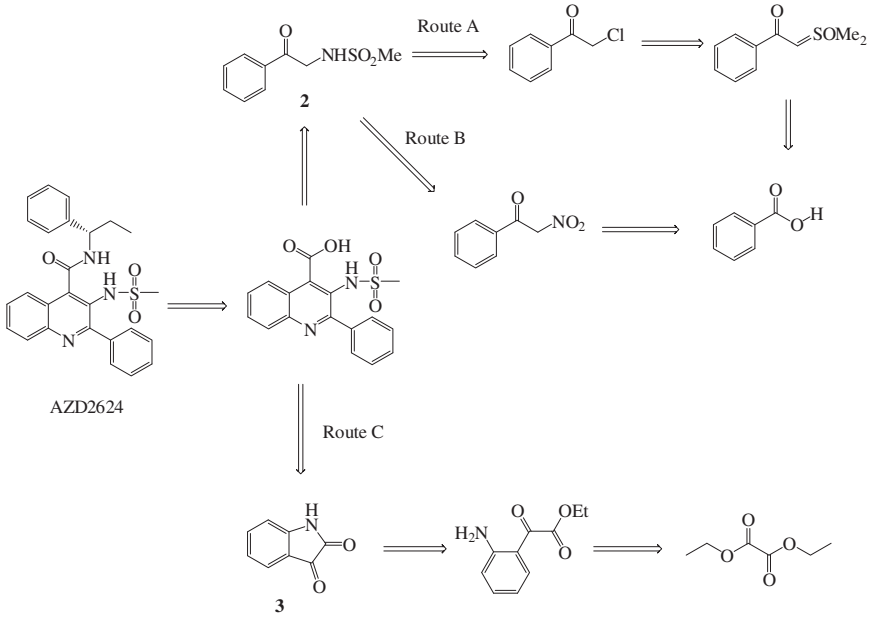
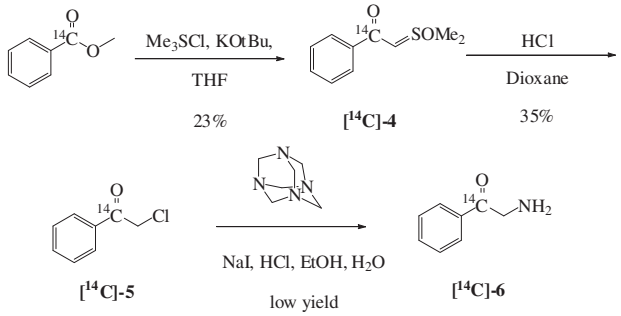


Figure 1. A stacked plot of (A) ^{1}H NMR of unlabeled AZD2624 (B) ^{1}H NMR of [$^3H_2$]AZD2624 (C) ^{3}H NMR of [$^3H_2$]AZD2624 (D) proton decoupled ^{3}H NMR of [$^3H_2$]AZD2624.



Scheme 2. Retrosynthetic analysis of C-14-labeled AZD2624.



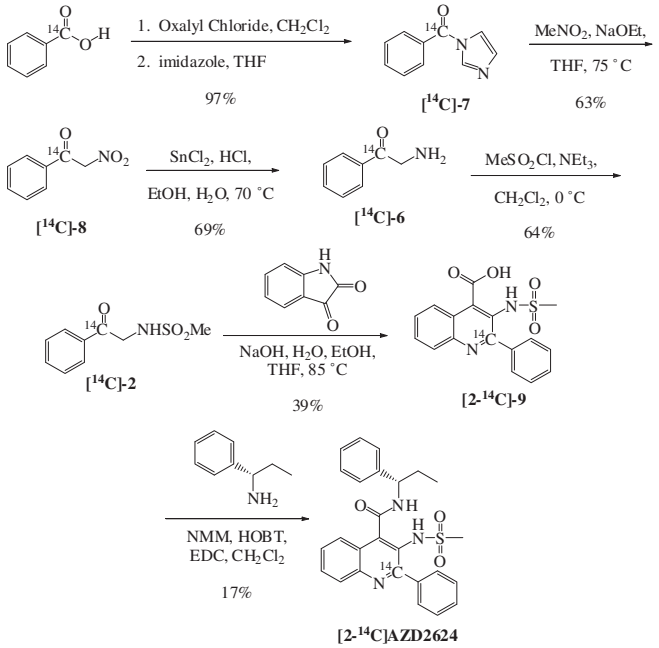
Scheme 3. Investigation of Route A towards C-14-labeled AZD2624.

give β-keto sulfur ylide **[^{14}C]-4**, albeit in poor yield (Scheme 3).[11] A considerable amount of methyl [carbonyl-^{14}C]benzoate was recovered during the reaction, accounting for the poor yield. Ylide **[^{14}C]-4** was then reacted with anhydrous HCl in dioxane to give the corresponding chloride **[^{14}C]-5** in 35% yield.[11] The reaction produced many radiochemical impurities and purification was difficult due to the impurity profile and the reactivity of **[^{14}C]-5**. Conversion of **[^{14}C]-5** to amine **[^{14}C]-6** was attempted on the crude reaction mixture using hexamine in EtOH,[12] but a low yield was observed likely due to the low purity of the starting material. As the radiochemical yields of this route were low and the purifications difficult, we sought an alternate route.

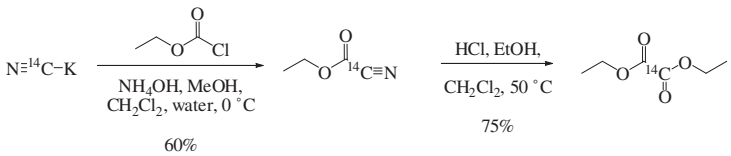
Next we investigated Route B. [Carbonyl-^{14}C]benzoic acid was activated as the imidazole amide (**[^{14}C]-7**)[13] and subsequently reacted with nitromethane to give nitro adduct **[^{14}C]-8** (Scheme 4).[14] Reduction of the nitro to give amine **[^{14}C]-6** with $SnCl_2$ in concentrated HCl proceeded in modest yield,[15] but isolation of the amine proved to be problematic. Multiple extractions were required to remove the majority of the compound from the aqueous layer, and if the compound was warmed above room temperature or concentrated to near dryness it decomposed, presumably *via* self condensation. To minimize the decomposition of amine **[^{14}C]-6**, the isolation and subsequent conversion of **[^{14}C]-6** to the sulfonamide **[^{14}C]-2** were performed on the same day. Careful temperature control was required during the sulfonylation to minimize decomposition and provided the target compound in 64% radiochemical yield. Sulfonamide **[^{14}C]-2** was then reacted with isatin to give acid **[^{14}C]-9** in 39% radiochemical yield. Peptide coupling of **[^{14}C]-9** to give [2-^{14}C]AZD2624 occurred in low yield but provided an initial batch of [2-^{14}C]AZD2624 for DMPK studies. The difficulties encountered in the handling of **[^{14}C]-6** made Route B unattractive. Therefore, we turned our attention to Route C.

Lastly we investigated Route C which goes *via* [^{14}C]isatin. There are multiple reports of labeled isatins in the literature[16–18]; three of these procedures couple an aniline or Boc-protected aniline with chloral or ethyl oxalate. Diethyl [^{14}C]oxylate was prepared in two steps using a modification of the procedure reported by Wilmes (Scheme 5).[19] K^{14}CN was coupled with ethyl chloroformate in a biphasic mixture of dichloromethane and water to afford the ethyl [^{14}C]cyanoformate in 60% yield. Ethanolysis of the cyanoformate to diethyl [^{14}C]oxylate proceeded in 75% radiochemical yield after purification by silica gel chromatography. Ortho lithiation of Boc-protected aniline followed by reaction with diethyl oxylate gave poor results in our hands,[17, 20–22] but halogen-metal exchange of *t*-butyl carbamate protected 2-bromoaniline with *t*-butyllithium to generate the anion followed by coupling with diethyl [^{14}C]oxylate afford diketone **[^{14}C]-10** in 60% yield on a small scale (Scheme 6). The yield of the reaction dropped to 20% when the reaction scale was increased from 5 mCi to 50 mCi perhaps due to loss of temperature control. The diketone was then cyclized to [^{14}C]isatin **[^{14}C]-3** in 74% radiochemical yield. Coupling with N-(2-oxo-2-phenylethyl)methanesulfonamide afforded acid **[carbonyl, 4-^{14}C]-9** in 79% radiochemical yield. This time the final peptide coupling was smoothly accomplished to give a 78% radiochemical yield of the target compound after HPLC purification.

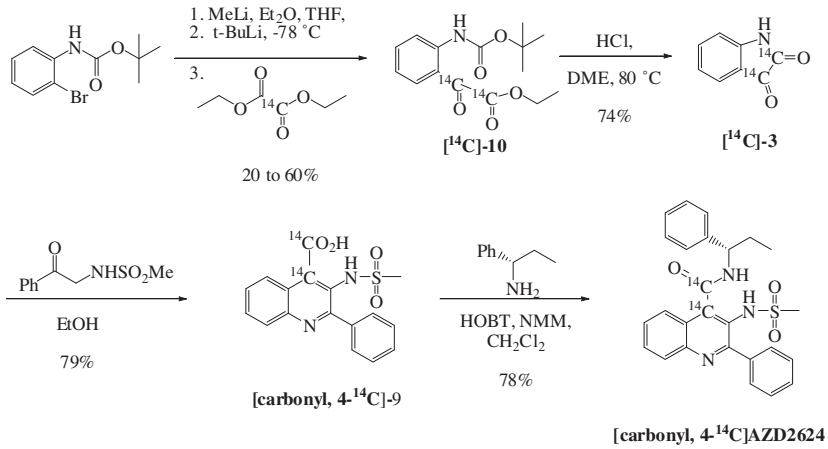
Finally we turned our attention to the preparation of stable isotope-labeled AZD2624 which was required for use as an internal standard for mass spectrometry to allow quanititation of AZD2624 in biological samples. A minimum mass increase of



Scheme 4. Synthesis of [2-^{14}C]AZD2624 *via* Route B.

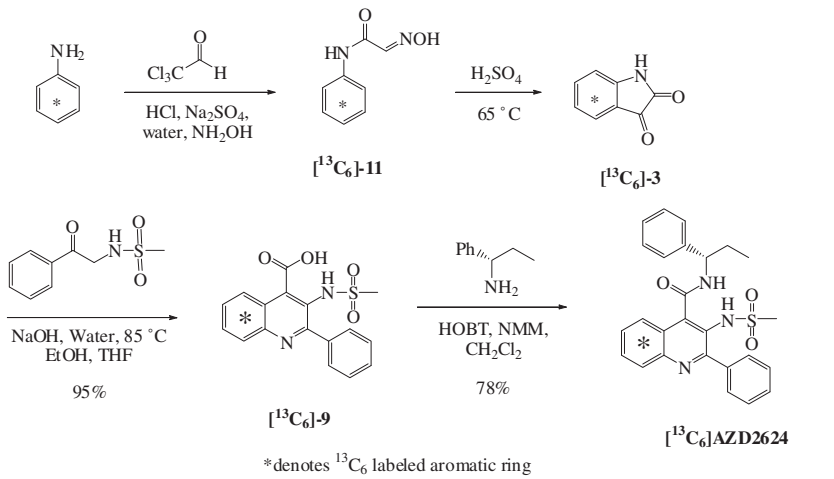


Scheme 5. Preparation of diethyl [^{14}C]oxylate using a modification of the procedure reported by Wilmes.[15]

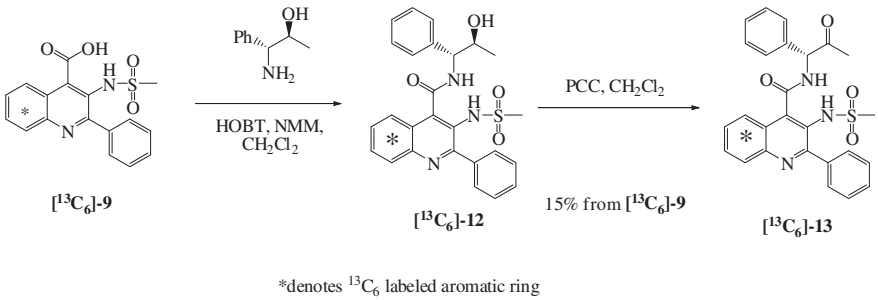


Scheme 6. Preparation of [carbonyl, 4-^{14}C]AZD2624 *via* [^{14}C]isatin (Route C).

four daltons and no detectable amount of the unlabeled material was needed. Based on our previous experience, we elected to target the isatin portion for label incorporation. Isatin has been labeled with N-15,[23] and deuterium[18] previously and our selected route was very similar to those reports. The reaction of [$^{13}C_6$]aniline with chloral gave acetanilide **[$^{13}C_6$]-11** which was converted to [$^{13}C_6$]isatin by heating in sulfuric acid (Scheme 7). Isatin ([**$^{13}C_6$]-3** was then converted to acid **[$^{13}C_6$]-9** as previously described. At this point the material was split: a portion being converted to [$^{13}C_6$]AZD2624 as previously described for the C-14-labeled material and a portion being converted to the ketone metabolite of AZD2624 (Scheme 8). The metabolite was prepared by peptide coupling of acid **[$^{13}C_6$]-9** to give alcohol **[$^{13}C_6$]-12** which was reacted with PCC to give ketone **[$^{13}C_6$]-13** in 15% isolated yield after preparative HPLC.



Scheme 7. Preparation of [$^{13}C_6$]AZD2624.



Scheme 8. Preparartion of [$^{13}C_6$]-13.

Conclusion

In support of the AZD2624 program, we synthesized three different labeled forms of AZD2624: $^{3}H_2$, $^{13}C_6$ and two ^{14}C isotopomers. The tritiation was accomplished in a straighforward manner as was the C-13 labeling. The C-14 labeling, however, required considerable development and ultimately three routes to arrive at a sufficient amount of material and to progress the project were developed.

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