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Syntheses of a radiolabelled CXCR2 antagonist AZD5069 and its major human metabolite

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The CXCR2 antagonist AZD5069 has been synthesized in tritium and carbon-14-labelled forms. [^{3}H]AZD5069 was prepared via reductive dehalogenation of an iodinated precursor with tritium gas to provide material with a specific activity of 25.1 Ci/mmol. [^{14}C]AZD5069 was labelled in the pyrimidine ring from [^{14}C]thiourea in an overall radiochemical yield of 18%. In addition, a synthetic route to the major metabolite of AZD5069 was developed. The synthesis of this metabolite was achieved from AZD5069 using a chemoselective Lindgren–Pinnick reaction in order to minimize oxidation of the sulphide group.

Keywords: carbon-14; tritium; AZD5069; Lindgren–Pinnick

Introduction

The CXC chemokine receptor-2 (CXCR2) is a Gprotein-coupled receptor expressed on a variety of inflammatory cells (monocytes, macrophages, neutrophils) and is known to be elevated in several inflammatory diseases, including chronic obstructive pulmonary disease,[1] rheumatoid arthritis[2] and psoriasis.[3] Inhibition of the CXCR2 chemokine receptor, therefore, represents an attractive strategy for the treatment of inflammatory disorders. To support a programme aimed at developing CXCR2 antagonists,[4] AZD5069 (**1**) (Figure 1) was identified as a potent, orally active small molecule that demonstrated selective reversible antagonism of human CXCR2. To investigate the pharmacokinetic and metabolism profile of the compound, the preparations of tritium and carbon-14-labelled AZD5069 were conducted. Metabolism studies of AZD5069 in human identified the carboxylic acid (**14**) as a major circulating metabolite. In order to develop a liquid chromatography mass spectrometry (LCMS) method for quantitative bioanalysis of the metabolite, the synthesis of an authentic reference standard and a stable isotope-labelled internal standard were also prepared.

Results and discussion

Tritium labelling

Our initial approach to the preparation of [^{3}H]AZD5069, [^{3}**H**]-**1** was to introduce the tritium label into the pyrimidine ring. However, deuterium labelling experiments found this position to be labile rendering it unsuitable for tritium labelling. Stirring a solution of AZD5069 (0.5 mg) in deuterated ethanol (0.5 mL) for 24 h revealed 16% isotope incorporation into the pyrimidine ring by ^{1}H NMR. Addition of triethylamine (5 μL) increased the deuterium incorporation to 80%. Therefore, a more traditional synthetic approach was adopted using 2,3-difluoro-6-iodobenzyl bromide (**3**) to prepare a suitable iodinated precursor (**6**) that could then be reductively dehalogenated in the presence of tritium gas. The synthesis is outlined in Scheme 1. The route also provided a versatile method for preparing tritium-labelled compounds where variations to the substituents on the S-benzyl group were introduced. The use of an iodine as the halogen precursor was preferred over bromine for reasons of selectivity, higher conversion and higher isotopic incorporation.[5,6] Protection of the acetal synthetic intermediate (**4**) with *p*-methoxybenzyl bromide followed by oxidation of the sulphide with 3-chloroperbenzoic acid (mCPBA) furnished the sulphone (**5**) in 69% yield. Addition of sodium hydrosulphide (NaSH) from a new, previously unopened bottle, to the sulphone in dimethyl sulfoxide generated the thiol *in situ*, which was smoothly alkylated with 2,3-difluoro-6-iodobenzyl bromide (**3**) to give the desired iodinated precursor (**6**) in good yield after trifluoroacetic acid deprotection. 2,3-Difluoro-6-iodobenzyl bromide (**3**) was conveniently prepared using a directed lithiation approach. Deprotonation of 1,2-difluoro-4-iodobenzene with freshly prepared lithium diisopropylamide at −78°C followed by quenching with solid carbon dioxide and acidic workup afforded 2,3-difluoro-6-iodobenzoic acid (**2**) in 67% yield as a single product. The hindered acid was reduced to the corresponding

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The preparation of the CXCR2 antagonist AZD5069 is described.

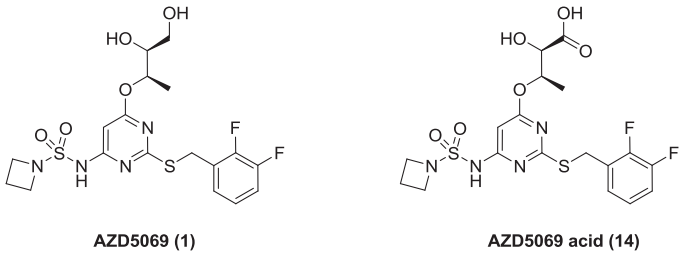
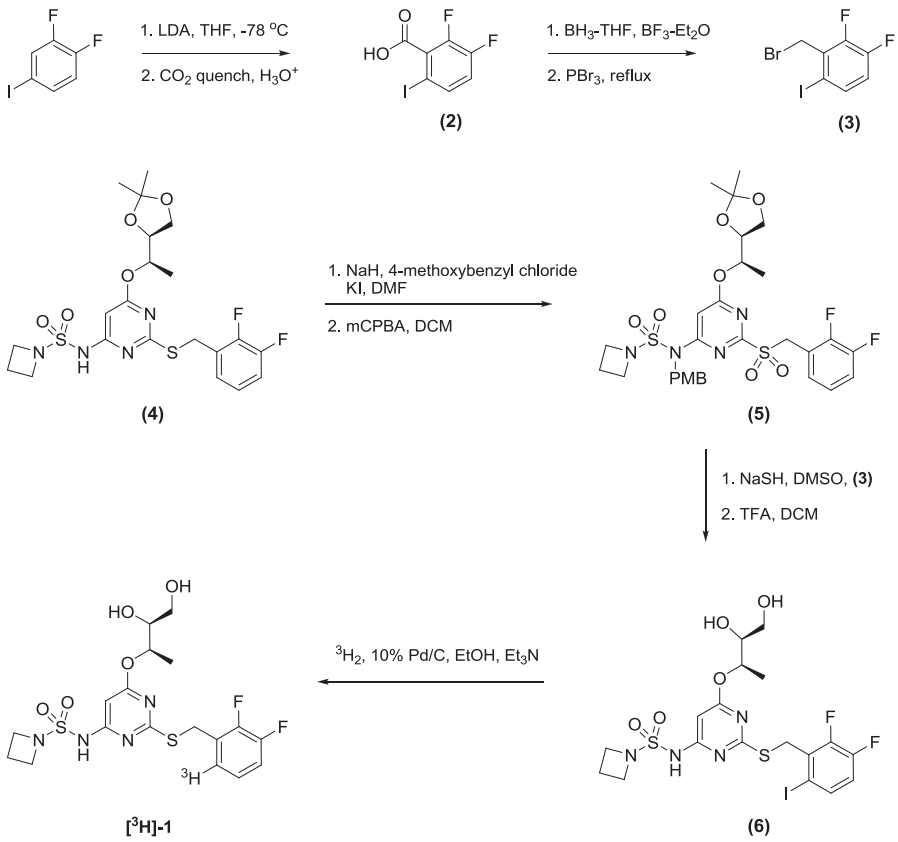


Figure 1. Structural formula of AZD5069 and the major human metabolite.



Scheme 1. Synthesis of [^{3}H]AZD5069, [^{3}**H**]-**1**.

benzyl alcohol in the presence of borane and boron trifluoride etherate,[7] and conversion to the benzyl bromide (**3**) was achieved by refluxing with phosphorous tribromide. With the iodinated precursor (**6**) in hand, reductive dehalogenation in the presence of tritium gas and 10% Pd/C showed complete conversion after 2 h to furnish the crude product with a radiochemical purity of 96%. Initial efforts to purify the material by reverse phase HPLC using a 10 mM ammonia/acetonitrile gradient were hampered due to the slow formation of two closely related impurities in the HPLC eluant. The two impurities were identified as isomeric products resulting from a Smiles rearrangement[8] of [^{3}H] AZD5069 under basic conditions (Figure 2). The rate of formation of the two impurities was accelerated as the pH increased. To avoid the formation of these

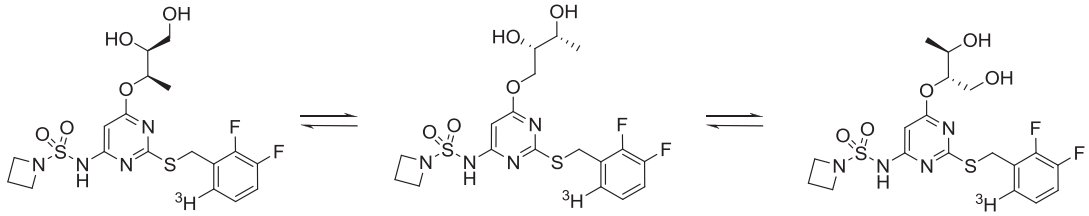


Figure 2. Smiles rearrangement of [^{3}H]AZD5069, [^{3}**H**]-**1** in basic HPLC eluant.

impurities, the crude material was subsequently purified by reverse phase HPLC under acidic conditions using an aqueous trifluoroacetic acid/acetonitrile gradient. Crude [^{3}H]AZD5069 [**^{3}H**]-**1** was successfully purified to give the final material with a radiochemical purity of >99% and a specific activity of 25.1 Ci/mmol. The material was stored as an ethanol solution at −20°C to minimize radiochemical decomposition.

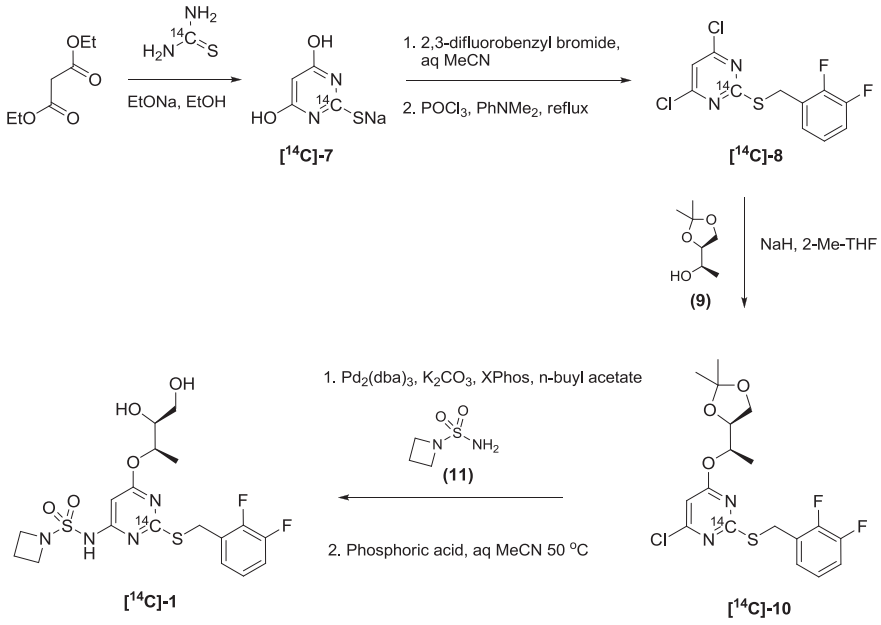
Carbon-14 labelling

The synthesis of [^{14}C]AZD5069 [**^{14}C**]-**1** is outlined in Scheme 2. Introduction of the carbon-14 isotope into the metabolically stable pyrimidine ring could be achieved through condensation of either [^{14}C]thiourea or [^{14}C]diethylmalonate, both of which were commercially available. Using a slight modification to the published procedure by Mathew *et al.*,[9] we were able to maximize the radiochemical yield of this condensation by using[^{14}C]thiourea as the labelled starting material. The reaction of [^{14}C]thiourea with diethylmalonate in the presence of freshly prepared sodium ethoxide and ethanol at 80°C for 4 h produced [2-^{14}C]thiobarbituric acid [**^{14}C**]-**7** as the sodium salt. The salt was isolated from the reaction mixture by filtration and the filtrate, which contained predominately unreacted [^{14}C]thiourea, was evaporated and recycled by reacting with fresh diethyl malonate and sodium ethoxide in ethanol. After heating at 80°C for 3 h, a further batch of [2-^{14}C]thiobarbituric acid sodium salt [**^{14}C**]-**7** was isolated to give a total radiochemical yield of 83.4%. The combined solids [**^{14}C**]-**7** were alkylated with one equivalent of 2,3-difluoro benzyl bromide in aqueous acetonitrile. After 24 h, the reaction mixture was filtered to remove mono and bis O-alkylated impurities, and the filtrate was evaporated to furnish the pyrimidine diol as a white solid with a radiochemical purity of 97%. Without purification, the diol was heated at 105°C in the presence of $POCl_3$ and *N*,*N*-dimethylaniline for 10 h to give the pyrimidine dichloride [**^{14}C**]-**8** (52.3 mCi, 53% radiochemical yield from [^{14}C]thiourea) after silica gel flash chromatography. The material had a radiochemical purity of 97.4%. (*R*)-1-((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl)ethanol (**9**) was treated with sodium hydride in 2-methyl tetrahydrofuran, and the resulting anion was reacted with [**^{14}C**]-**8** affording [**^{14}C**]-**10** in 56% radiochemical yield after workup and purification by flash chromatography. Palladium-catalysed cross-coupling[10] of [**^{14}C**]-**10** with azetidine-1-sulfonamide (**11**) in the presence of potassium carbonate and Xphos, followed by purification by flash chromatography, provided the penultimate compound in 84% radiochemical yield. It was important to carry out this cross-coupling reaction in degassed solvent under a nitrogen atmosphere accompanied with rapid stirring to ensure that the conversion proceeded in high yield. Finally, deprotection with phosphoric acid in aqueous acetonitrile provided crude [^{14}C]AZD5069 [**^{14}C**]-**1** with a radiochemical purity of >98%. A portion of the crude material was further purified by reverse phase HPLC in aqueous trifluoroacetic acid/acetonitrile to give [^{14}C]AZD5069 [**^{14}C**]-**1** as a white solid. The radiochemical purity was measured at 99.8% with a specific activity determined by gravimetric analysis of 122.1 μCi/mg.

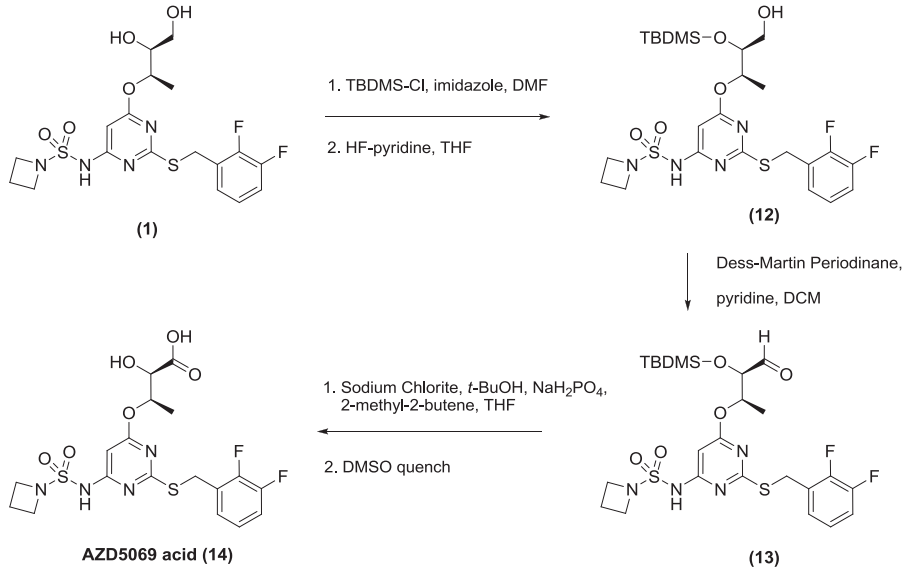
Following the success in preparing carbon-14-labelled AZD5069, an identical synthetic route was used to synthesize the stable labelled internal standard of AZD5069. Condensation of commercially available [$^{13}C_3$]diethylmalonate with [^{13}C,$^{15}N_2$] thiourea and subsequent alkylation with 2,3-difluorobenzyl bromide provided a bulk stock of the key [M+6]dihydroxy-pyrimidine intermediate. Chlorination, followed by insertion of the alcohol and azetidine sulphonamide moieties, gave [**$^{13}C_4$,$^{15}N_2$**]-**1** with the required six mass units higher than the parent and was successfully used to support MS method development.

Metabolite synthesis

The synthetic route used to prepare the AZD5069 acid metabolite (**14**) is outlined in Scheme 3. Initially, we investigated the synthesis through direct oxidation of the parent compound (**1**). Not surprisingly, attempts to prepare the metabolite by oxidation of the primary alcohol to the carboxylic acid proved challenging due to concomitant oxidation of the sulphide or



Scheme 2. Synthesis of [^{14}C]AZD5069, [**^{14}C**]-**1**.



Scheme 3. Synthesis of AZD5069 acid metabolite, (**14**).

cleavage of the diol. The problem was neatly solved through the synthesis of the aldehyde precursor that was then selectively oxidized using Lindgren–Pinnick oxidation conditions.[11] Protection of the AZD5069 (**1**) diol as silyl ethers followed by removal of the primary silyl protecting group[12] produced (**12**) in a modest 68% yield. Subsequent Dess–Martin periodinane oxidation cleanly furnished the aldehyde (**13**) in 95% yield after purification with no oxidation of the sulphide being observed. Finally, chemoselective oxidation of the aldehyde with sodium chlorite followed by *in situ* deprotection and dimethyl sulfoxide (DMSO) quench gave the crude acid metabolite (**14**) with $<$10% of sulphoxide and sulphone being observed. Purification of the crude product by reverse phase preparative chromatography afforded pure AZD5069 acid metabolite (**14**).

Using the conditions developed in the preceding texts, the stable labelled internal standard [$^{13}C_4$,$^{15}N_2$] AZD5069 acid metabolite was successfully prepared from [$^{13}C_4$,$^{15}N_2$]AZD5069 for use in quantitative bioanalysis studies.

Experimental

Materials and methods

[^{14}C]Thiourea (specific activity 0.75 mCi/mg) was purchased from Quotient Bioresearch (Radiochemicals), Cardiff, UK. All other reagents and anhydrous solvents were obtained from Sigma-Aldrich and were used without further purification. Azetidine-1-sulphonamide, (*R*)-1-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)ethanol and authentic reference standards were prepared by AstraZeneca Medicinal Chemistry. ^{1}H NMR spectra were recorded on a Varian Unity Inova 400 MHz NMR spectrometer unless otherwise stated. Chemical shifts (δ) in ppm are quoted relative to $(CD_3)_2S{=}O$ (δ 2.50) or $CDCl_3$ (δ 7.26). Flash column chromatography was performed using prepacked SiliSep^{TM} silica gel cartridges (SiliCycle, Quebec, Canada). Analytical thin-layer chromatography (TLC) was carried out on Merck 5785 Kieselgel 60F_{254} fluorescent plates. Liquid chromatography mass spectrometry (LCMS) and specific activity measurements were obtained by electrospray ionization using a Waters Acquity UPLC with a Waters Micromass ZQ ESCi probe mass detector. Specific activity was calculated by comparison of the ratio of carbon-14/carbon-12 for the tracer against the unlabelled reference. Radiochemical reaction monitoring and purity checks were determined on a Waters 2695 alliance analytical HPLC fitted with a 996 Diode Array Detector and a Lab Logic Radioflow Detector Beta-Ram Model 3. The following conditions were used: Waters Xbridge C_{18}, 5.0 µm, 4.6 × 150 mm, column temperature 40°C, 5% acetonitrile : water (0.1% trifluoroacetic acid) to 95% acetonitrile : water (0.1% trifluoroacetic acid) 10 min linear gradient, 1 mL/min, UV 254 nm. Quantification of radioactivity was performed using a Perkin-Elmer TRI-CARB 2500 liquid scintillation analyser, with Ultima Gold^{TM} cocktail.

*2,3-Difluoro-6-iodobenzoic acid (**2**)*

A solution of diisopropylamine (5.46 mL, 4.21 g, 41.6 mmol) in dry tetrahydrofuran (200 mL) was stirred at −78°C under nitrogen. *n*-Butyllithium 2.5 M in tetrahydrofuran (16.6 mL, 41.6 mmol) was added dropwise, and the mixture was stirred for 10 min. 1,2-Difluoro-4-iodobenzene (10 g, 41.6 mmol) was added over 15 min, and the yellow solution was stirred at −78°C for 1.5 h. The mixture was poured on to solid carbon dioxide and left to attain room temperature. The mixture was partitioned between 1 N aqueous sodium hydroxide (60 mL) and diethyl ether (60 mL). The basic aqueous extract was acidified to pH 2 with concentrated hydrochloric acid and extracted with diethyl ether (3 × 60 mL). The combined organic phases were washed with brine (50 mL), dried over magnesium sulphate, filtered and evaporated *in vacuo* to yield the acid (**2**) as a white solid (7.9 g, 67%).

^{1}H NMR ($CDCl_3$) δ 7.05 (td, J = 8.6, 8.7 Hz, 1H), 7.66 (ddd, J = 8.8, 4.4, 1.9 Hz, 1H), 10.87 (s, 1H)

*2,3-Difluoro-6-iodobenzyl bromide (**3**)*

To a solution of 2,3-difluoro-6-iodobenzoic acid (**2**) (3 g, 10.56 mmol) in dry tetrahydrofuran (50 mL) at 0°C under nitrogen was added dropwise borane-tetrahydrofuran complex 1.0 M (38 mL, 38 mmol) followed by boron trifluoride etherate (1.33 mL, 1.49 g, 10.56 mmol). The mixture was heated at 60°C for 2 h and left to stand at room temperature for 48 h. The mixture was quenched by dropwise addition of methanol (30 mL). The solution was evaporated to an oil and partitioned between 1 N aqueous sodium hydroxide (30 mL) and diethyl ether (2 × 50 mL). The combined organic extracts were washed with brine (30 mL), dried over magnesium sulphate, filtered and evaporated *in vacuo* before pumping under high vacuum to constant weight. The alcohol was obtained as a white solid (2.45 g, 86%).

^{1}H NMR ($CDCl_3$) δ 4.83 (d, J = 2.3 Hz, 2H), 7.59 (ddd, J = 8.6, 4.5, 1.9 Hz, 1H), 7.66 (dd, J = 8.8 Hz, 1H)

A mixture of 2,3-difluoro-6-iodobenzyl alcohol (1.5 g, 5.56 mmol) and phosphorous tribromide (2 mL, 5.7 g, 21.1 mmol) was heated at 80°C for 1 h. The dark mixture was cooled to room temperature, and saturated

sodium bicarbonate (40 mL) was carefully added dropwise. The mixture was extracted with diethyl ether (2 × 50 mL), and the combined organic extracts were washed with water (50 mL) and brine (30 mL), dried over magnesium sulphate, filtered and evaporated *in vacuo* to yield (**3**) as a white solid (1.61 g, 87%).

^{1}H NMR ($CDCl_3$) δ 4.62 (d, J = 2.5 Hz, 2H), 6.92 (m, 1H), 7.60 (ddd, J = 8.8, 4.7, 2.0 Hz, 1H); ^{13}C NMR (76 MHz, $CDCl_3$) δ 29.9, 93.1, 119.0, 130.61, 134.9, 148.8, 150.4.

N-(2-(2,3-Difluorobenzylsulfonyl)-6-((*R*)-1-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)ethoxy)pyrimidin-4-yl)-*N*-(4-methoxybenzyl) azetidine-1-sulfonamide (**5**)

Sodium hydride (60% in mineral oil) (0.511 g, 23.23 mmol) was added to a solution of *N*-(2-(2,3-difluorobenzylthio)-6-((*R*)-1-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)ethoxy)pyrimidin-4-yl)azetidine-1-sulfonamide (**4**) (6.0 g, 11.61 mmol) in *N*,*N*-dimethylformamide (60 mL) at 0°C under an atmosphere of nitrogen. The reaction mixture was stirred for 15 min; then, 4-methoxybenzyl chloride (3.16 mL, 23.23 mmol) was added followed by potassium iodide (2.03 g, 12.2 mmol). After stirring at room temperature for 18 h, the reaction mixture was diluted with ethyl acetate (50 mL) and washed with water (50 mL). The aqueous was further extracted with ethyl acetate (2 × 50 mL), and the combined organic phases were washed with brine (50 mL) and dried over magnesium sulphate, filtered and evaporated to give a yellow oil. The crude product was purified by flash silica chromatography, eluting with a 5 to 20% ethyl acetate/isohexane gradient. Pure fractions were evaporated to dryness to afford *N*-(2-(2,3-difluorobenzylthio)-6-((*R*)-1-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)ethoxy)pyrimidin-4-yl)-*N*-(4-methoxybenzyl)azetidine-1-sulfonamide (5.61 g, 76%) as a yellow gum that was used directly in the next step.

3-Chloroperbenzoic acid (mCPBA) (70%) (0.774 g, 3.14 mmol) was added to *N*-(2-(2,3-difluorobenzylthio)-6-((*R*)-1-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)ethoxy)pyrimidin-4-yl)-*N*-(4-methoxybenzyl)azetidine-1-sulfonamide (1 g, 1.57 mmol) in dichloromethane (40 mL). The resulting mixture was stirred at room temperature for 24 h. The reaction mixture was diluted with dichloromethane (50 mL) and washed successively with aqueous sodium thiosulphate solution (3 × 50 mL, 10 g/100 mL), saturated aqueous sodium bicarbonate (2 × 50 mL) and brine (50 mL). The organic phase was dried over magnesium sulphate, filtered and evaporated, and the crude residue was triturated with diethyl ether and filtered to give *N*-(2-(2,3-difluorobenzylsulfonyl)-6-((*R*)-1-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)ethoxy)pyrimidin-4-yl)-*N*-(4-methoxybenzyl) azetidine-1-sulfonamide (**5**) (0.96 g, 91%) as a white solid. The material was 90% pure by HPLC and used directly in the next step. LCMS (ES^+) *m/z*: 669 $[M+H]^+$.

N-(2-(2,3-Difluoro-6-iodobenzylthio)-6-((*R*)-1-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)ethoxy)pyrimidin-4-yl)-*N*-(4-methoxybenzyl) azetidine-1-sulfonamide (**6**)

Sodium hydrosulphide hydrate (0.025 g, 0.45 mmol) was added to a solution of *N*-(2-(2,3-difluorobenzylsulfonyl)-6-((*R*)-1-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)ethoxy)pyrimidin-4-yl)-*N*-(4-methoxybenzyl)azetidine-1-sulfonamide (**5**) (0.15 g, 0.22 mmol) and stirred in anhydrous DMSO (5 mL) under nitrogen. After 30 min, 2,3-difluoro-6-iodobenzyl bromide (**3**) (0.223 g, 0.67 mmol) was added, and the reaction mixture was stirred for 1 h, before it was diluted with diethyl ether (50 mL) and washed with saturated aqueous ammonium chloride (3 × 50 mL) and brine (50 mL). The organic phase was dried over magnesium sulphate, filtered and evaporated to give a colourless oil. The crude product was purified by flash silica chromatography, eluting with isohexane 67%/ethyl acetate 33%. The pure fractions were combined and evaporated to dryness to afford *N*-(2-(2,3-difluoro-6-iodobenzylthio)-6-((*R*)-1-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)ethoxy)pyrimidin-4-yl)-*N*-(4-methoxybenzyl)azetidine-1-sulfonamide (0.107 g, 63%) as a white solid. LCMS (ES^+) *m/z*: 763 $[M+H]^+$. The solid was dissolved in trifluoroacetic acid (2.0 mL) and dichloromethane (2.0 mL) and stirred at room temperature for 2 h before evaporating to dryness. The crude product was purified by preparative HPLC (Xterra C8 RP column, 150 × 19 mm, 5 to 95% acetonitrile/water (0.1% trifluoroacetic acid) gradient over 15 min, 20 mL/min, 254 nm). Pure factions were combined and freeze dried to afford *N*-(2-(2,3-difluoro-6-iodobenzylthio)-6-((2*R*,3*S*)-3,4-dihydroxybutan-2-yloxy)pyrimidin-4-yl)azetidine-1-sulfonamide (**6**) (0.030 g, 35.5%) as a white solid.

^{1}H NMR ($(CD_3)_2SO$) δ 1.27 (d, 3H) 2.14 (quin, 2H) 3.39 (t, 2H) 3.70 (m, 1H) 3.93 (t, 4H) 4.59 (m, 3H) 4.92 (d, 1H) 5.29 (m, 1H) 6.13 (s, 1H) 7.27 (m, 1H) 7.77 (m, 1H) 11.11 (br. s, 1H). LCMS (ES^+) *m/z*: 603 $[M+H]^+$.

N-(2-(2,3-Difluoro-[6-^{3}H]benzylthio)-6-((2*R*,3*S*)-3,4-dihydroxybutan-2-yloxy)pyrimidin-4-yl)azetidine-1-sulfonamide, [^{3}H]AZD5069, [^{3}H]-1

N-(2-(2,3-Difluoro-6-iodobenzylthio)-6-((2*R*,3*S*)-3,4-dihydroxybutan-2-yloxy)pyrimidin-4-yl)azetidine-1-sulfonamide (**6**) (5.3 mg, 8.30 μmol) was dissolved in ethanol (1.0 mL), and triethylamine (20 μL, 133 μmol) was added followed by 10% palladium on carbon (2.5 mg, 2.35 μmol). The reaction mixture was cooled to liquid nitrogen temperature, degassed twice by a freeze-thaw cycle and placed under a partial atmosphere of tritium gas (73 mbar, 2.5 Ci) for 2 h. The reaction mixture was filtered, and the labile tritium was removed through lyophilization with ethanol (2 × 5 mL) to leave crude [^{3}H]AZD5069, [^{3}**H**]-**1**. Ethanol (10 mL) was added, and the solution (240 mCi, 24.0 mCi/mL) was stored at −20°C. The radiochemical purity was measured at 96%.

An ethanol solution of *N*-(2-(2,3-difluoro-[6-^{3}H]benzylthio)-6-((2*R*,3*S*)-3,4-dihydroxybutan-2-yloxy)pyrimidin-4-yl)azetidine-1-sulfonamide [^{3}**H**]-**1** (24 mCi) was evaporated and dissolved in 0.1% aq trifluoroacetic acid/acetonitrile 50/50% (0.2 mL) and purified by reverse phase preparative chromatography (Xterra C8 RP column, 150 × 7.8 mm, 40% acetonitrile/water (0.1% trifluoroacetic acid) isocratic, then acetonitrile gradient flush, 3.0 mL/min, 254 nm). The pure fractions were combined and freeze dried to give [^{3}H]AZD5069 [^{3}**H**]-**1** that was dissolved in ethanol (10 mL). The activity was measured at 18.4 mCi, and the radiochemical purity was 99% by HPLC with a specific activity of 25.1 Ci/mmol. LCMS (ES^+) *m/z*: 477, 479 $[M+H]^+$

[2-^{14}C]Thiobarbituric acid sodium salt, [^{14}C]-7

Into a dried 5 mL reactivial fitted with a sure seal septum and stirrer bar was placed diethyl malonate (282 mg, 1.76 mmol), ethanol (2 mL) and [^{14}C]thiourea (98.8 mCi, 131 mg, 1.68 mmol). The mixture was stirred at 80°C in a heat block, and after 10 min, a solution was observed. Freshly prepared sodium ethoxide (0.8 mL, 1.74 mmol; made from 0.19 g sodium in 3.8 mL ethanol) was injected in one portion, and the yellow mixture was heated at 80°C for 4 h. A cream-white solid precipitated. The mixture was cooled and left at room temperature overnight for 16 h. The solid was filtered and washed with ice-cold ethanol (0.5 mL) and ether (0.5 mL), air-dried and collected to give [2-^{14}C]thiobarbituric acid sodium salt [^{14}**C**]-**7** as a cream-white solid (157 mg). The mother liquor was evaporated to dryness and heated at 80°C in ethanol (1 mL) with diethyl malonate (141 mg, 0.88 mmol) and sodium ethoxide (0.4 mL, 0.87 mmol; made from 0.19 g sodium in 3.8 mL ethanol) for 3 h, cooled and left at room temperature overnight. The solid was filtered and washed with ice-cold ethanol (0.5 mL) and ether (0.5 mL), air-dried and collected to give [2-^{14}C]thiobarbituric acid sodium salt [^{14}**C**]-**7** as a cream solid (78 mg). The two solids were combined (235 mg, 83.4%) and used directly in the next reaction.

4,6-Dichloro-2-(2,3-difluorobenzylthio)[2-^{14}C]pyrimidine, [^{14}C]-8

[2-^{14}C]Thiobarbituric acid sodium salt [^{14}**C**]-**7** (235 mg, 1.40 mmol) was dissolved in acetonitrile (3.0 mL) and water (3.0 mL) and stirred under nitrogen at room temperature. 2,3-Difluorobenzyl bromide (289 mg, 1.40 mmol) in acetonitrile (1.5 mL) was added dropwise, and the mixture was stirred at room temperature for a total of 24 h. A white solid precipitated. The mixture was cooled in an ice bath, then filtered. The solid contained mostly peralkylated material. The filtrate was evaporated *in vacuo* and repeated with 3 × 4 mL of acetonitrile. The sample was dried at 40°C under high vacuum to a white solid 395 mg that was taken on directly to the next reaction. The material co-chromatographed with an

authentic sample by HPLC and had a radiochemical purity of 93%. A mixture of (2,3-difluorobenzylthio)[2-^{14}C]pyrimidine-4,6-diol (395 mg, 1.05 mmol), phosphorous oxychloride (1.7 mL, 18.2 mmol) and *N*,*N*-dimethylaniline (0.31 mL, 2.46 mmol) were heated at 105°C for 10 h, and the dark mixture was left to cool overnight. A small aliquot was quenched in water/acetonitrile (1:1). HPLC analysis showed no starting material remaining. The reaction mixture was carefully quenched by adding to warm water (15 mL) and extracted with ethyl acetate (3 × 15 mL). The combined extracts were washed with brine, dried (magnesium sulphate) and concentrated to a brown oil, dissolved in dichloromethane (3 mL) and purified by flash chromatography on silica eluting with 1% ethyl acetate in isohexane. Fractions containing product were combined and evaporated yielding a pale yellow oil [**^{14}C**]-**8** (276 mg, 52.3 mCi, 53% radiochemical yield from [^{14}C]thiourea). The material co-chromatographed with an authentic sample by HPLC and had a radiochemical purity of 97.4%.

4-Chloro-2-(2,3-difluorobenzylthio)-6-((R)-1-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)ethoxy) [2-^{14}C]pyrimidine, [^{14}C]-10

A mixture of (*R*)-1-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)ethanol (**9**) (98 mg, 0.67 mmol) and 4,6-dichloro-2-(2,3-difluorobenzylthio)[2-^{14}C]pyrimidine [**^{14}C**]-**8** (~39.4 mCi, 206 mg, 0.67 mmol) were stirred at 0°C in 2-methyl tetrahydrofuran (6 mL) under nitrogen. Sodium hydride in oil (34.6 mg, 0.87 mmol) was added, and the mixture was stirred for 18 h overnight at room temperature. TLC (isohexane 96%/ethyl acetate 4%) showed no starting material and major product as monochloride. The mixture was treated with brine (30 mL) and extracted with ethyl acetate (2 × 30 mL), evaporated to an oil and purified by flash chromatography on silica eluting with isohexane 96%/ethyl acetate 4%. Pure fractions were combined and evaporated *in vacuo* to [**^{14}C**]-**10** as a clear oil (156 mg, 21.9 mCi, 56% radiochemical yield). The material co-chromatographed with an authentic sample by HPLC and had a radiochemical purity of 98.2%. LCMS (ES^+) *m/z*: 419 $[M+H]^+$.

N-(2-(2,3-Difluorobenzylthio)-6-((2*R*,3*S*)-3,4-dihydroxybutan-2-yloxy)[^{14}C]pyrimidin-4-yl)azetidine-1-sulfonamide, [^{14}C]AZD5069, [^{14}C]-1

Into a dry flask under nitrogen was added 4-chloro-2-(2,3-difluorobenzylthio)-6-((*R*)-1-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)ethoxy)[2-^{14}C] pyrimidine [**^{14}C**]-**10** (21.9 mCi, 156 mg, 0.37 mmol), azetidine-1-sulfonamide (**11**) (102 mg, 0.75 mmol), potassium carbonate (77 mg, 0.56 mmol), tris(dibenzylideneacetone)dipalladium(0) (34 mg, 0.04 mmol), 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (35 mg, 0.07 mmol) and butyl acetate (5 mL). The red mixture was degassed twice and stirred vigorously under nitrogen for 10 min at room temperature, then for 1 h at 100°C during which time the reaction mixture became a pale brown colour. The reaction mixture was cooled, butyl acetate (10 mL) added followed by brine. The mixture was filtered through 0.5 μ filter, and the layers were separated. The organic layer was washed with 1 M aqueous hydrochloric acid, saturated sodium bicarbonate solution, brine and dried over magnesium sulphate, concentrated under vacuum to a dark red oil and purified by flash chromatography on silica eluting with isohexane 90%/ethyl acetate 10%. Pure fractions were collected, combined and concentrated to furnish a pale yellow oil (162 mg, 18.4 mCi, 84% radiochemical yield). The material had a radiochemical purity of 98.6%. LCMS (ES^+) *m/z*: 519 $[M+H]^+$.

The oil (18.4 mCi, 162 mg, 0.31 mmol) was dissolved in acetonitrile (2 mL), water (0.4 mL) and phosphoric acid (36.0 mg, 0.31 mmol) and was heated under nitrogen at 50°C for 24 h. The pale yellow solution was evaporated under reduced pressure, and the residue was dissolved in ethanol (10 mL). The crude solution of [^{14}C]AZD5069 [**^{14}C**]-**1**, which had a total radioactivity of 18.4 mCi by liquid scintillation counting and a radiochemical purity of 98%, was stored in the fridge. LCMS (ES^+) *m/z*: 479 $[M+H]^+$.

A portion of the ethanol solution (6.60 mL, 12.1 mCi) was concentrated to dryness, dissolved in 0.1% aq trifluoroacetic acid 50%/acetonitrile 50% (8 mL) and subjected to preparative chromatography (Xterra C8 RP column, 150 × 19 mm, 40% acetonitrile in water (0.1% trifluoroacetic acid) isocratic, then acetonitrile gradient flush, 20 mL/min, 10 × 0.66 mL injections). Pure factions were combined and freeze dried to give [^{14}C] AZD5069 [**^{14}C**]-**1** as a white solid (89.2 mg, 122.1 μCi/mg, 10.9 mCi) with a radiochemical purity of 99.8% by HPLC and a specific activity of 59.3 mCi/mmol by mass spectrometry.

^{1}H NMR ($(CD_3)_2SO$) δ 1.21 (d, *J* = 6.36 Hz, 3H), 2.14 (quin, *J* = 7.68 Hz, 2H), 3.39 (br. s, 2H), 3.63–3.70 (m, 1H), 3.92 (t, *J* = 7.70 Hz, 4H), 4.42–4.56 (m, 2H), 4.62 (t, 1H), 4.92 (d, *J* = 5.17 Hz, 1H), 5.18–5.25 (m, 1H), 6.12 (s, 1H), 7.13–7.19 (m, 1H), 7.29–7.44 (m, 2H), 11.11 (br. s, 1H). LCMS (ES^+) *m/z*: 479 $[M+H]^+$.

N-(6-((2*R*,3*S*)-3-(*tert*-Butyldimethylsilyloxy)-4-hydroxybutan-2-yloxy)-2-(2,3-difluorobenzylthio)pyrimidin-4-yl)azetidine-1-sulfonamide (**12**)

AZD5069 (**1**) (4.23 g, 8.88 mmol), *tert*-butylchlorodimethylsilane (5.35 g, 35.51 mmol), and imidazole (3.02 g, 44.38 mmol) were dissolved in *N*,*N*-dimethylformamide (30 mL) to give a colourless solution. The mixture was stirred overnight. TLC (isohexane 75%/ethyl acetate 25%) showed complete disappearance of the starting material and formation of a single product. The reaction mixture was partitioned between ethyl acetate (200 mL) and 0.5 M aqueous hydrochloric acid (200 mL). The organic phase was collected, and the aqueous phase was extracted with another portion of ethyl acetate (150 mL). The combined organic phases were washed with water (2 × 100 mL), brine (50 mL) and dried over sodium sulphate. Filtration and evaporation of the solvent *in vacuo* gave 8 g of crude product as a yellow syrup. The crude product was taken up in dichloromethane (20 mL), applied onto a silica column and eluted with isohexane 88%/ethyl acetate 12% to isohexane 84%/ethyl acetate 16%. Fractions containing product were combined and concentrated under reduced pressure to yield the bis-silyl ether, 5.70 g, 91% as a clear oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 0.03 (2s, 6H), 0.07 (2s, 6H), 0.89 (2s, 18H), 1.25 (d, 3H), 2.25 (p, 2H), 3.55 (ddd, 2H), 3.94 (td, 1H), 4.01 (t, 4H), 4.39 (dd, 2H), 5.42 (qd, 1H), 6.26 (s, 1H), 6.95 (bs, 1H), 6.99–7.1 (m, 2H), 7.21 (t, 1H).

The oil (5.70 g, 8.08 mmol) was dissolved with pyridine (4.6 mL, 56.87 mmol) and tetrahydrofuran (55 mL) in a 50-mL plastic bottle to give a colourless solution. To this solution was added HF-pyridine (4.6 mL, 30.63 mmol). The bottle was closed, and the contents were stirred for 2.5 h, monitoring the reaction by TLC (isohexane 67%/ethyl acetate 33%). The reaction was stopped at 95% conversion by partitioning the reaction mixture between ethyl acetate and 1 M aqueous hydrochloric acid (150 mL/150 mL). The organic phase was collected, and the aqueous phase was extracted with another portion of ethyl acetate (150 mL). The combined organic extracts were washed with 0.5 M aqueous hydrochloric acid (100 mL) and a solution of saturated sodium carbonate (50 mL) and brine before drying over sodium sulphate. Filtration and evaporation *in vacuo* yielded a pale yellow oil. The oil was dissolved in 25 mL of dichloromethane, applied onto a silica column and eluted with isohexane 84%/ethyl acetate 16% to isohexane 75%/ethyl acetate 25%. Pure fractions were combined and evaporated to give (**12**) as a white solid foam (3.60 g, 75%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 0.11 (s, 3H), 0.12 (s, 3H), 0.93 (s, 9H), 1.29 (d, 3H), 1.89 (t, 1H), 2.26 (p, 2H), 3.61 (t, 2H), 3.86 (q, 1H), 3.98–4.05 (m, 4H), 4.33–4.43 (m, 2H), 5.25–5.33 (m, 1H), 6.27 (s, 1H), 6.96 (bs, 1H), 7–7.1 (m, 2H), 7.21–7.26 (m, 1H).

N-(6-((2*R*,3*R*)-3-(*tert*-Butyldimethylsilyloxy)-4-oxobutan-2-yloxy)-2-(2,3-difluorobenzylthio)pyrimidin-4-yl)azetidine-1-sulfonamide (**13**)

A solution of *N*-(6-((2*R*,3*S*)-3-(*tert*-butyldimethylsilyloxy)-4-hydroxybutan-2-yloxy)-2-(2,3-difluorobenzylthio)pyrimidin-4-yl)azetidine-1-sulfonamide (**12**) (3.6 g, 6.09 mmol) and pyridine (0.49 mL, 6.09 mmol) in dichloromethane (60 mL) was stirred to give a colourless solution. To this solution was added solid Dess–Martin periodinane (3.10 g, 7.31 mmol). The yellow solution was stirred at room temperature for 1 h to give a milky solution. Diethyl ether (200 mL) was added followed

by saturated sodium thiosulphate solution (100 mL) and saturated sodium hydrogen carbonate solution (100 mL). The mixture was stirred vigorously for 30 min, and the phases were allowed to separate. The organic phase was collected, and the aqueous phase was further extracted with ether (100 mL). The combined organic phases were washed with 0.5 M aqueous hydrochloric acid (2 × 100 mL), saturated sodium carbonate solution (50 mL) and brine (50 mL). The organic solution was dried over sodium sulphate and filtered. Removal of the solvent under reduced pressure gave a red oil 3.70 g that became more and more coloured over time. The oil was dissolved in dichloromethane (20 mL) and applied onto a silica column. The column was eluted with isohexane 85%/ethyl acetate 15% to isohexane 80%/ethyl acetate 20%. Pure fractions were combined and evaporated to give (**13**) as a clear gum (2.90 g, 95%) which was used directly in the next reaction.

^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 0.01 (s, 3H), 0.03 (s, 3H), 0.87 (s, 9H), 1.20 (d, 3H), 2.07–2.17 (m, 2H), 3.90 (t, 4H), 4.44 (d, 1H), 4.48 (d, 2H), 5.54 (qd, 1H), 6.11 (s, 1H), 7.13–7.19 (m, 1H), 7.3–7.37 (m, 1H), 7.40 (t, 1H), 9.57 (s, 1H), 11.16 (s, 1H).

(2R,3R)-3-(6-(Azetidine-1-sulfonamido)-2-(2,3-difluorobenzylthio) pyrimidin-4-yloxy)-2-hydroxybutanoic acid, AZD5069 acid (**14**)

A solution of *N*-(6-((*2R,3R*)-3-(*tert*-butyldimethylsilyloxy)-4-oxobutan-2-yloxy)-2-(2,3-difluorobenzylthio)pyrimidin-4-yl)azetidine-1-sulfonamide (**13**) (1.60 g, 2.72 mmol) in *tert*-butanol (9 mL) was stirred to give a colourless solution. The reaction mixture was diluted with water (3 mL), and sodium dihydrogen phosphate monohydrate (750 mg, 5.44 mmol) and 2-methyl-2-butene (2 M in tetrahydrofuran) (2.72 mL, 5.44 mmol) were added. The solution was cooled in an ice bath and stirred for 5 min before sodium chlorite (614 mg, 5.44 mmol) was added. An immediate colour change from colourless to yellow/green was observed. After 1 h, DMSO (4 mL) was added, and the mixture was filtered. The crude product was purified by reverse phase preparative chromatography using the following conditions: Kromasil C8 RP column, 10 μm, 5 to 50% acetonitrile in water (0.2% acetic acid) over 20 min, 254 nm. The pure fractions containing product were combined and freeze-dried to yield AZD5069 acid (**14**) as off-white solid (0.9 g, 67%) with a chemical purity of 99.5%.

^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 1.21 (d, 3H), 2.13 (p, 2H), 3.91 (t, 4H), 4.25 (d, 1H), 4.48 (q, 2H), 5.38–5.45 (m, 1H), 5.60 (bs, 1H), 6.10 (s, 1H), 7.16 (dd, 1H), 7.34 (dd, 1H), 7.41 (t, 1H), 11.14 (bs, 1H), 12.74 (bs, 1H). LCMS (ES^+) *m/z*: 491 $[M+H]^+$.

Conclusion

The synthesis of radiolabelled AZD5069 has been described. [^{3}H]AZD5069 was prepared through reductive dehalogenation of an iodinated precursor. The reduction with tritium gas was rapid, clean and high yielding with ~87% incorporation of the tritium isotope. The synthetic route was also applicable for preparing high specific activity tritium labels where changes to the S-benzyl group substituents were made. The synthesis of [^{14}C]AZD5069 was performed in six steps from [^{14}C]thiourea with an overall radiochemical yield of 18%. The synthetic route used was also applied to the synthesis of an M+6 stable labelled isotopomer using [$^{13}C_3$]diethylmalonate with [^{13}C,$^{15}N_2$]thiourea to achieve the desired mass increase. Finally, chemoselective oxidation of an aldehyde precursor under Lindgren–Pinnick oxidizing conditions furnished the AZD5069 acid metabolite.

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