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THE SYNTHESIS OF ISOTOPOLOGUES OF AZD7307. A SELECTIVE β_2-ADRENORECEPTOR AGONIST.

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The preparation of the β_2-adrenoreceptor agonist AZD7307 is described.
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Introduction

Belonging to the G-protein-coupled receptor family (GPCR), adrenoreceptors can be divided into two major sub families, α and β[1]. Further subtype division of the β group gives three members: β_1, β_2 and β_3 adrenoreceptors of which the β_2 adrenoreceptor is expressed mainly on smooth muscle cells, particularly in the respiratory tract[2]. Agonism of the β_2 receptor on airway smooth muscle moderates bronchodilation and is effective against asthma and chronic obstructive pulmonary disease (COPD)[3]. This action has generated considerable interest in the development of β_2 adrenoreceptor agonists which can be historically divided into three generations. First generation agonists were short acting (SABAs) and they required multiple daily dosing (e.g. salbutamol). Improvements in the class were made to form a second generation of compounds (e.g. salmeterol) which are longer acting and require only twice daily dosing (LABAs). Third generation LABA compounds have an increased duration of action and can be dosed once daily.

AstraZeneca has had a long-standing interest in the investigation of compounds which target asthma and COPD in a single daily dosing regimen[4]. A research programme discovered several candidates and the compound AZD7307 was identified as a development candidate. To progress its development, several labelled compounds were required – namely a stable isotopically labelled (SIL) isotopologue to support bioanalytical studies and a radiolabelled species to investigate the fate of the compound in the body (ADME).

As the project evolved, a number of similar compounds were under investigation which shared a common structural motif. Recognising this, a strategy was devised utilising simple precursors which enabled the synthesis of common structural fragments; these could be elaborated further to afford the target structure.

Results and Discussion

The structure and retrosynthetic analysis of AZD7307 **(1)** is shown in **Scheme 1**. A standard disconnection approach identified several fragments which gave reasonable starting points for radiosynthesis. The benzoxazinone and cyclohexylamine fragments were common to several other compounds of interest and it was these moieties that were concentrated upon.
There were several factors that dictated the final labelling strategy. There was a desire within the project to track the fate of the benzoxazinone fragment, so it was pertinent to incorporate the radiolabel within this portion of the molecule. Time constraints within the project meant that there was insufficient time to develop an aromatic ring labelled intermediate. Moreover, radiolabelled chloroacetyl chloride was readily available and several key intermediates were readily accessible in amounts amenable for route development.

Synthesis [^{14}C]AZD7307.

Dropwise addition of [^{14}C]chloroacetyl chloride to a methyl isobutyl ketone suspension of (**2**) containing two equivalents of potassium hydrogen carbonate formed the intermediate amide. Addition of a further 2.5 equivalents of base with heating at 89 °C for 90 minutes gave excellent conversion to the oxazinone which precipitated on cooling. Further washing and extraction of the aqueous filtrate afforded **(3)** with a total recovery of 87% with respect to total starting radioactivity. Oxidative chlorination with benzyltrimethylammonium dichloroiodate afforded the α-chloroketone **(4)** in 87% yield with a radiochemical purity of 96%. The product was filtered directly from the reaction and was used directly in the next step. Conversion to the

azide **(5)** proceeded smoothly at room temperature in 95% yield giving a product with a radiochemical purity of greater than 95%.

Reduction of the keto azide and subsequent removal of the benzyl protecting group was very sluggish and incomplete after four hours. To drive the reaction to completion, several catalyst recharges were required in conjunction with running the reaction at elevated temperatures. Filtration of the catalyst and concentration of the resultant filtrate gave the product as the hydrochloride salt in 97% yield and 99% radiochemical purity.
Reductive amination of **(6)** with the aldehyde **(7)** gave the crude product which was purified initially on silica to afford the product as the free base. Recrystallisation with fumaric acid in methanol further enhanced the purity giving [^{14}C]AZD7307 **(8)** in 15% yield with a radiochemical purity of 99%. The recovery of the hemi fumarate product was low, however further batches of material were produced by HPLC purification of the mother liquors and subsequent reformation of the hemi fumarate salt, this work is not described in this report.

[^{13}C]AZD7307 Isotopologue

Many more options existed for the stable isotope labelling of AZD7307 as there was no restriction due to metabolism and a larger pool of labelled precursors available. Thus, label incorporation in either of the aromatic rings, the ethyl propionic acid side chain or the cyclohexylamine ring were considered. Incorporation of the label into the benzoxazinone ring was less desirable due to the relative cost of labelled trisubstituted aromatics and, to introduce the labelled moiety at as late stage as possible, the cyclohexylamine ring portion was targeted as the labelled precursor.
Acetal **(10)** was prepared from the amine (**9)** in 60% yield and coupled with the acid chloride derived from carboxylic acid **(11)** to afford **(12)**. As previously described, *in-situ* deprotection of the acetal **(12)** to give the aldehyde and reductive amination with **(13)** afforded **(14)** in 19% yield.

Conclusion

The synthesis of [^{14}C]AZD7307 was completed in six linear steps with an overall yield of 10%. [^{13}C]AZD7307 was completed in four linear steps with a yield of 12%. In addition, by careful selection of common intermediate fragments, the development of both labelled intermediates were used to produce several related analogues which reduced both the synthesis and project lead times.

Experimental

Materials and methods

[^{14}C]Chloroacetyl chloride (2.6 GBq mmol^{-1}) was purchased from Pharmaron UK Ltd, Cardiff, UK. All other reagents and anhydrous solvents were obtained from Sigma Aldrich and were used without further purification.
Intermediates **(2)**, **(7)**, **(11)** and **(13)** were prepared by AstraZeneca Medicinal Chemistry unless otherwise stated.

^{1}H-NMR spectra were recorded on a Varian Unity Inova 400MHz 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 pre-packed SiliSep^{TM}

silica gel cartridges (SiliCycle, Quebec, Canada). LCMS 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 to assess radiochemical purity: Waters Xbridge C_{18}, 5.0 µm, 4.6 x 150 mm, column temperature 40 ºC, 5% acetonitrile: water (0.1% TFA) to 95% acetonitrile: water (0.1% TFA) 20 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™ cocktail.

8-acetyl-5-(benzyloxy)-2H-benzo[e][1,4]oxazin-3-[3-^{14}C]-(4H)-one (3)

A suspension of 1-(3-amino-4-(benzyloxy)-2-hydroxyphenyl)ethanone **(2),** (400 mg, 1.55 mmol) and potassium hydrogen carbonate (311 mg, 3.11 mmol) in methyl isobutyl ketone (2.5 mL) was heated at 50 °C under a nitrogen atmosphere as a methyl isobutyl ketone solution (2 mL) of chloro[1-^{14}C]acetyl chloride (197 mg, 1.71 mmol) added dropwise by syringe. After addition, the reaction was heated at 50 °C for 45 minutes. A second portion of potassium hydrogen carbonate (389 mg, 3.89 mmol) dissolved in water (2.2 mL) was added dropwise to the reaction and the mixture heated at 89 °C for 90 minutes. The reaction was cooled, and the resulting slurry stirred overnight. The slurry was filtered and was partitioned between ethyl acetate (40 mL) and brine (20 mL). The organic phase was removed and dried over magnesium sulphate; the solid filtered and the organics removed *in vacuo* to afford **(3)** as a white solid (447 mg, 3212 MBq, 87% from total radioactivity). The radiochemical purity was assessed and found to be 99.9%. LCMS (ES^+) *m/z*: 300 $[M+H]^+$.

5-(benzyloxy)-8-(2-chloroacetyl)-2H-benzo[e][1,4]oxazin-3-[3-^{14}C]-(4H)-one (4)

Compound **(3)**, (337.4 mg, 1.13 mmol) was suspended in ethanol (3 mL) and benzyltrimethylammonium dichloroiodate (785 mg, 2.25 mmol) added. The reaction was heated to 78 °C under nitrogen for 60 minutes. The reaction was allowed to cool to 50 °C and water (3 mL) added. The reaction temperature was lowered to 20 °C and allowed to stand for 120 minutes. The resultant precipitate was filtered and washed with water (1 mL) and ethanol (1 mL). The solid was returned to a round bottomed flask, dissolved in ethyl acetate (6.5 mL) and heated to 77 °C for 60 minutes. The reaction was cooled to room temperature and left to stand under nitrogen for 72 hours.

The precipitate was filtered, washed with ethyl acetate (1.5 mL) and dried under vacuum at 50 °C to afford **(4)** (329 mg, 87%), The product co-eluted with an unlabelled standard of the product: radiochemical purity 96%.

8-(2-azidoacetyl)-5-(benzyloxy)-2H-benzo[e][1,4]oxazin-3-[3-^{14}C]-(4H)-one (5)

An N-methyl-2-pyrorolidine (1 mL) suspension of compound **(4)**, (328.9 mg, 0.99 mmol) was stirred under a nitrogen atmosphere at room temperature. Sodium azide (96 mg, 1.48 mmol) was added in one portion and the yellow suspension allowed to stir for 4 hours, after this time

HPLC showed full conversion. Water (6 mL) was added and the reaction left to stir for 30 minutes, the resultant yellow solid was filtered, washed with water (600 µL), isopropyl alcohol (3x 600 µL) and dried under vacuum overnight to afford **(5)** as a yellow solid (320 mg, 95%), radiochemical purity 97%.

8-(2-aminoethyl)-5-hydroxy-2H-benzo[e][1,4]oxazin-3-[3-^{14}C]-(4H)-one hydrochloride (6)

A mixture of **(5)** (344 mg, 1.01 mmol) and 10% Pd/C (159 mg) was added to a solution of glacial acetic acid (5.75 mL)/ concentrated hydrochloric acid (2.4 mL) and stirred under a hydrogen atmosphere (3 bar) for 24 hours. Radio HPLC indicated a conversion of 50%. Two further catalyst recharges were required (each under a hydrogen atmosphere of 3 bar with additional heating at 45 °C) to force the reaction to completion. The reaction was cooled, the catalyst removed by filtration and the filter cake washed with methanol (20 mL) and methanol/water 1:1 (20 mL) solutions. The filtrate was concentrated *in vacuo* to afford the product **(6)** as a cream solid, (240 mg, 97%). Radiochemical purity 99%, LCMS (ES^+) *m/z*: 211 $[M+H]^+$.

N-cyclohexyl-N-(2-((2-(5-hydroxy-3-oxo-3,4-dihydro-2H-benzo[e][1,4]oxazin-8-yl-[3-^{14}C])ethyl)amino)ethyl)-3-(3-(1-methyl-1H-pyrazol-4-yl)phenethoxy)propanamide hemi fumarate (8)

A solution of *p*-toluenesulphonic acid (124 mg, 0.65 mmol) and **(7)** (182 mg, 0.41 mmol) in tetrahydrofuran (1.6 mL) was stirred for 70 minutes at room temperature. LC/MS showed one peak which corresponded to the aldehyde intermediate. This was used in the reductive amination without further purification.

The aldehyde was added dropwise over 10 minutes to a pre-formed suspension of **(6)**, (100 mg, 0.41 mmol) in N-methyl-2-pyrrolidone (1.0 mL)/water (0.11 mL) and sodium hydrogen carbonate (111 mg, 1.32 mmol). Once addition was complete, the reaction was stirred for 15 minutes before sodium triacetoxyborohydride (234 mg, 1.10 mmol) was added in three portions over a 5-minute period. Acetic acid (26 µl, 0.45 mmol) added and the reaction stirred for 120 minutes at room temperature.

The reaction mixture was added to a stirred aqueous sodium bicarbonate solution (0.7 M, 10 mL) and the mixture extracted with dichloromethane (2x 20 mL). The combined organic extracts were washed with water (20 mL), 20% w/w brine (20 mL), dried over magnesium sulphate and the filtrate evaporated. The residue was purified by flash silica chromatography, eluting with 0 to 10% methanol in dichloromethane, to afford the product as the free base (107 mg).

The crude product was dissolved in methanol (2 ml) and heated. Fumaric acid (9 mg, 0.08 mmol) added and the solution allowed to cool overnight. The precipitate that formed was filtered, the solid washed with methanol (2x 200 µl), ether (2x 200 µl) and dried under vacuum at 45 °C overnight to give **(8)** as the hemi fumarate salt, (40 mg, 15%).
LCMS (ES^+) *m/z*: 592 $[M+H]^+$. ^{1}H NMR (500 MHz, DMSO) δ 0.99 – 1.76 (m, 10H), 2.52 – 2.82 (m, 10H),[3.25, t, *J* = 7.48 Hz, 2H] 3.29 (t, *J* = 7.48, 2H), [4.06, tt, *J* = 3.85, 11.88 Hz, 1H] 3.56 – 3.62 (m, 1H), 3.62 – 3.67 (m, 4H), 3.85 (s, 3H), [4.48, s, 2H], 4.50 (s, 2H), [6.44, d, *J* = 8.26 Hz, 1H] 6.45 (d, *J* = 8.27 Hz, 1H), 6.48 (s, 2H), [6.62, d, *J* = 8.31 Hz, 1H] 6.63 (d, *J* = 8.31 Hz, 1H), [7.02 d, *J* = 7.64 Hz, 1H] 7.04 (d, *J* = 7.64 Hz, 1H), [7.22, t, J=7.61,7.61 Hz,

1H] 7.24 (t, *J* = 7.61, 7.61 Hz, 1H), [7.35 ,d, *J* = 6.6 Hz, 1H] 7.36 (d, *J* = 6.6 Hz, 1H), [7.40 s, 1H] 7.42 (s, 1H), 7.81 (s, 1H) [7.82 s, 1H], 8.08 (s, 1H) [8.09, s, 1H], 9.84 (s, 1H). Figures in square brackets refer to the rotameric form in an approximate ratio of 4:3 if resolved. ^{13}C NMR (126 MHz, DMSO, 30°C) δ 24.75 (25.02), 25.20 (25.54) , 27.72 (28.75), (30.11) 30.91, 33.15 (33.34), 35.48 (35.53), 38.58, 42.28, 47.27 (48.50), (48.98) 49.17, (52.97) 55.97, 66.60, 66.92, 71.01 (71.05), (108.99) 109.04 , (115.54) 115.56, 116.81 (117.61), 121.91, (122.61) 122.65, 123.29, 125.36, 126.36 (126.39) , 127.66, (128.55) 128.60, 132.45 (132.46), 134.99 (broad),135.92, 139.46 (139.5), 142.54, (143.71) 143.91, 167.62, (170.08) 170.33 (Figures in brackets refer to rotameric form if resolved.)

[$^{13}C_6$]-N-(2,2-dimethoxyethyl)cyclohexanamine (10)

A suspension of [$^{13}C_6$]cyclohexylamine hydrochloride **(9)** (7.67 g, 54.19 mmol) and potassium carbonate (18.22 g, 131.86 mmol) in dimethylformamide (20 mL) was stirred for 5 minutes at room temperature under a nitrogen atmosphere. 2-Chloro-1,1-dimethoxyethane (4.13 mL, 36.13 mmol) was added and the reaction heated at 120 °C for 20 hours. The reaction was cooled to room temperature and an aqueous solution of sodium hydroxide (1.25 M, 40 mL) added. The mixture was stirred at room temperature for 10 minutes and the layers separated. The product was purified by distillation, boiling at 86-88 °C under a vacuum of 5.5 mm mercury to afford **(10)** in 60% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82-2.62 (m, 12H), 2.75 (dd, J = 5.6, 2.6 Hz, 2H), 3.38 (s, 6H), 4.46 (t, J = 5.6 Hz, 1H).

[cylcohexyl-$^{13}C_6$]-N-cyclohexyl-N-(2.2-dimethylethyl)-3-(3-(1-methyl-1H-pyrazol-4-yl)phenethoxy)propanamide (12)

A suspension of 3-(3-(1-methyl-1H-pyrazol-4-yl)phenethoxy)propanoic acid **(11)** (2 g, 7.29 mmol) in dichloromethane (10 mL) was stirred under nitrogen. Oxalyl chloride (0.96 mL, 10.94 mmol) was added dropwise, followed by dimethylformamide (2 drops) and the reaction stirred at room temperature for 1.5 hours. The solvent was evaporated, azeotroped with toluene (2 x 10 mL) and the residue dissolved in dichloromethane (10 mL). The dichloromethane solution was added dropwise to a preformed solution of [$^{13}C_6$]-N-(2,2-dimethoxyethyl)cyclohexanamine (1.34 g, 6.93 mmol) and triethylamine (2.24 mL, 16.04 mmol) in dichloromethane (10 mL) cooled to 5 °C. Once addition was complete, the reaction was warmed to room temperature and stirred for a further 90 minutes.

Water (40 mL) was added and the organic portion separated, washed with aqueous saturated sodium hydrogen carbonate solution (40 mL), water (40 mL) and dried over magnesium sulphate. After filtration, the organics were concentrated *in vacuo* to afford **(12)** as an orange oil, (3.51 g, 107%).

[$^{13}C_6$]-N-cyclohexyl-N-(2-(2-(5-hydroxy-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-8-yl)ethylamino)ethyl)-3-(3-(1-methyl-1H-pyrazol-4-yl)phenethoxy)propanamide hemi fumarate (14)

The acetal **(12)** (3.40 g, 7.56 mmol) in tetrahydrofuran (30 mL) and p-toluenesulphonic acid (2.42 g, 12.74 mmol) were stirred for 70 minutes at room temperature. LC/MS showed a single peak which corresponded to the aldehyde. The resultant solution was added dropwise over 10 minutes to a pre-formed mixture of 8-(2-aminoethyl)-5-hydroxy-2H-benzo[b][1,4]oxazin-3(4H)-one hydrochloride **(13)**, (1.95 g, 7.96 mmol) in N-methyl-2-pyrrolidone(18 mL)/ water (1.8 mL) and sodium hydrogen carbonate (2.00 g, 23.88 mmol). Once addition was complete,

the reaction was stirred for 15 minutes before sodium triacetoxyborohydride (4.22 g, 19.90 mmol) was added in three portions over 5 minutes. Acetic acid (0.51 mL, 8.84 mmol) was added and the reaction stirred for 120 minutes at room temperature. The reaction mixture was added to a stirred aqueous sodium hydrogen carbonate solution (0.7 M, 200 mL) and the mixture extracted with dichloromethane (3x 30 mL). The combined organic extracts were washed with water, 20% brine and dried over magnesium sulphate. The filtrate was evaporated under reduced pressure to give a light tan oil.

The residue was purified by silica flash chromatography, eluting with 0-10% methanol in dichloromethane to give an off-white solid (1.80 g).

The solid was dissolved in hot methanol (10 mL) and fumaric acid added (147 mg, 1.26 mmol). The precipitate from the cooled reaction was filtered, washed with methanol (0.5 mL), ether (0.5 mL) and dried under vacuum to afford compound **(14)**, (946.8 mg, 19%). ^{1}H NMR (400 MHz, DMSO) δ 1.04 – 1.88 (m, 10H), 2.52 – 2.86 (m, 10H), 3.52 – 3.67 (m, 7H), 3.83 (s, 3H), 4.50 (s, 2H), 6.42 (s, 1H), 6.46 (d, *J* = 8.28 Hz, 1H), 6.64 (d, *J* = 8.33 Hz, 1H), 7.03 (d, *J* = 7.46 Hz, 1H), 7.23 (t, *J* = 7.62, 7.62 Hz, 1H), 7.35 (d, *J* = 7.76 Hz, 1H), 7.40 (s, 1H), 7.81 (s, 1H), 8.07 (s, 1H), the minor rotameric form is not reported. LCMS (ES^+) *m/z*: 596 $[M+H]^+$.

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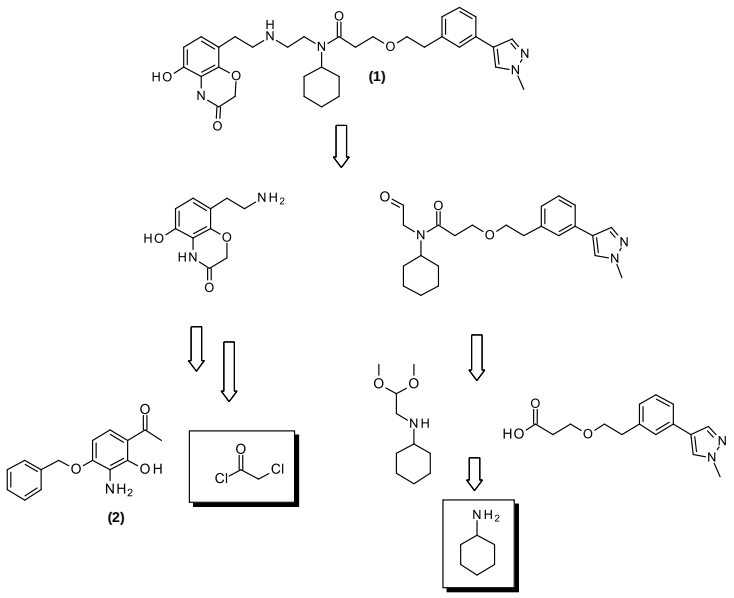
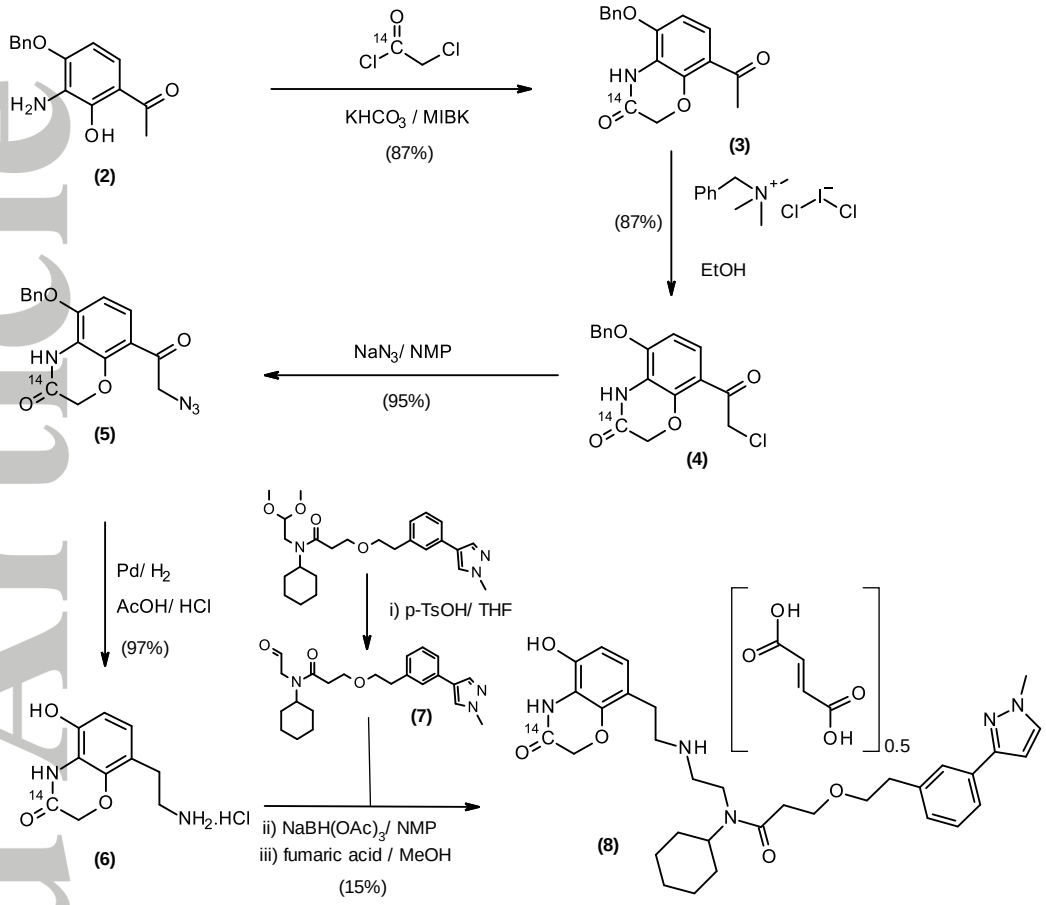
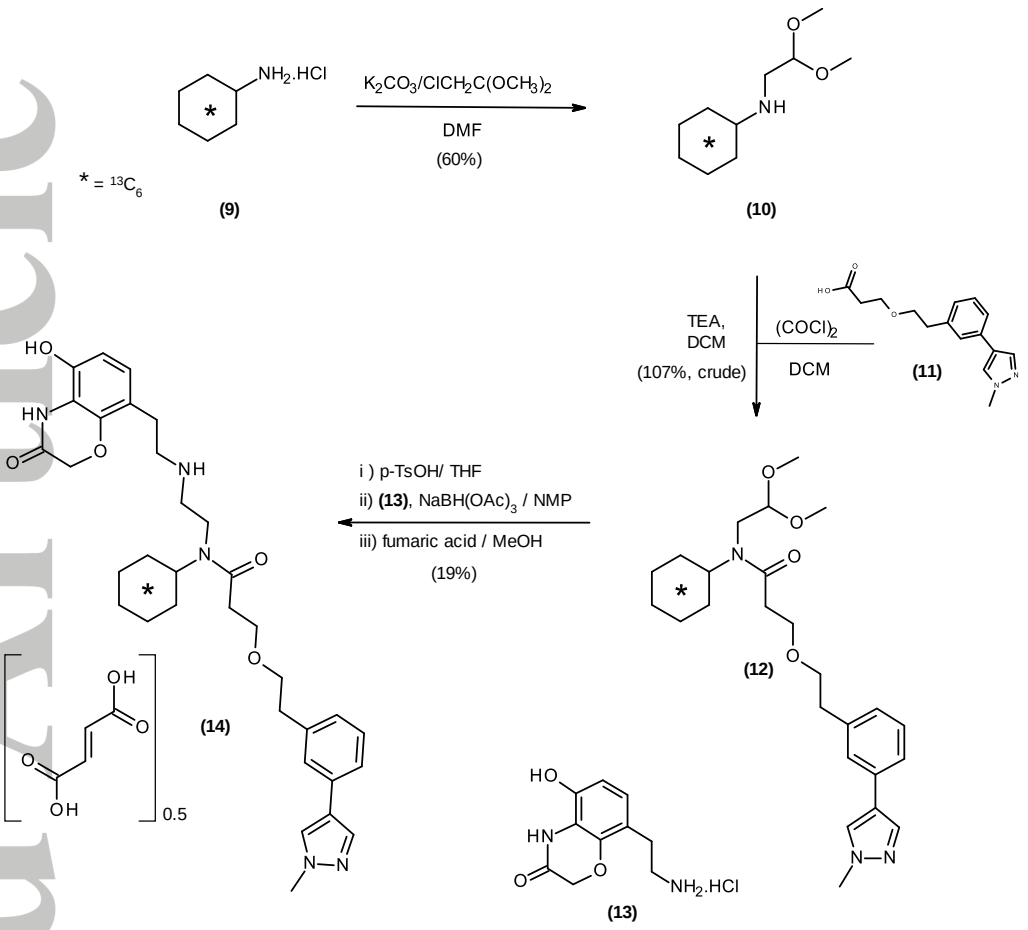


Scheme 1. Retrosynthetic analysis of AZD7307.



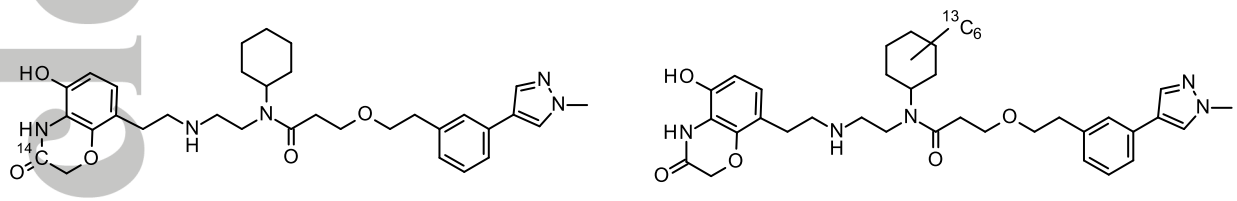
Scheme 2. Synthesis of [^{14}C]AZD7307



Scheme 3. Synthesis of [^{13}C]AZD7307

Graphical Abstract

To support the development of a β_2-adrenoreceptor agonist, the syntheses of carbon-13 and carbon-14 labelled AZD7307 are described.



AZD7307

The β_2-adrenoreceptor agonist AZD7307 has been synthesised radiolabelled with carbon-14 in six linear steps from [^{14}C]chloroacetyl chloride in an overall radiochemical yield of 10%. In addition, the synthetic route of a stable labelled isotopomer of AZD7307 is also described and synthesised in four linear steps from [$^{13}C_6$]cyclohexylamine hydrochloride in an overall yield of 12%.