SYNTHESIS OF A NOVEL ANTIINFLAMMATORY AGENT, BTS 71321, SINGLY LABELLED WITH CARBON-14 IN TWO POSITIONS

Maurice W Baker, Michael V Mewse, Stephen W Shutler, Lucyna M Taylor and Roger B Titman*

Knoll Pharmaceuticals, Research & Development Department,

Pennyfoot Street, Nottingham NG1 1GF, UK

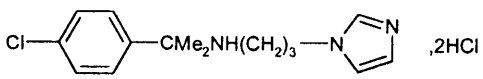
SUMMARY

The synthesis of the antiinflammatory compound, BTS 71321, singly labelled with carbon-14 in two positions, is described. Labelling of the imidazole ring of the compound was achieved using [2-^{14}C]imidazole, prepared from potassium [^{14}C]cyanide *via* potassium [^{14}C]thiocyanate, whereas labelling of the tertiary carbon atom in the chain was effected by means of a procedure starting from barium [^{14}C]carbonate and involving an organocerium reagent.

***Key Words**: carbon-14, BTS 71321, antiinflammatory, antiasthmatic, [2-^{14}C]imidazole, organocerium reagent*

INTRODUCTION

The new antiinflammatory agent, BTS 71321, which acts by a novel mechanism involving arachidonic acid release, may have utility in the treatment of asthma. As part of a development programme, the compound was required singly labelled with carbon-14 in different regions of the molecule for the purpose of metabolism and pharmacokinetic studies. Suitable positions for labelling were identified as the 2-position in the imidazole ring and the tertiary carbon atom in the chain linking the imidazole and benzene rings.



BTS 71321

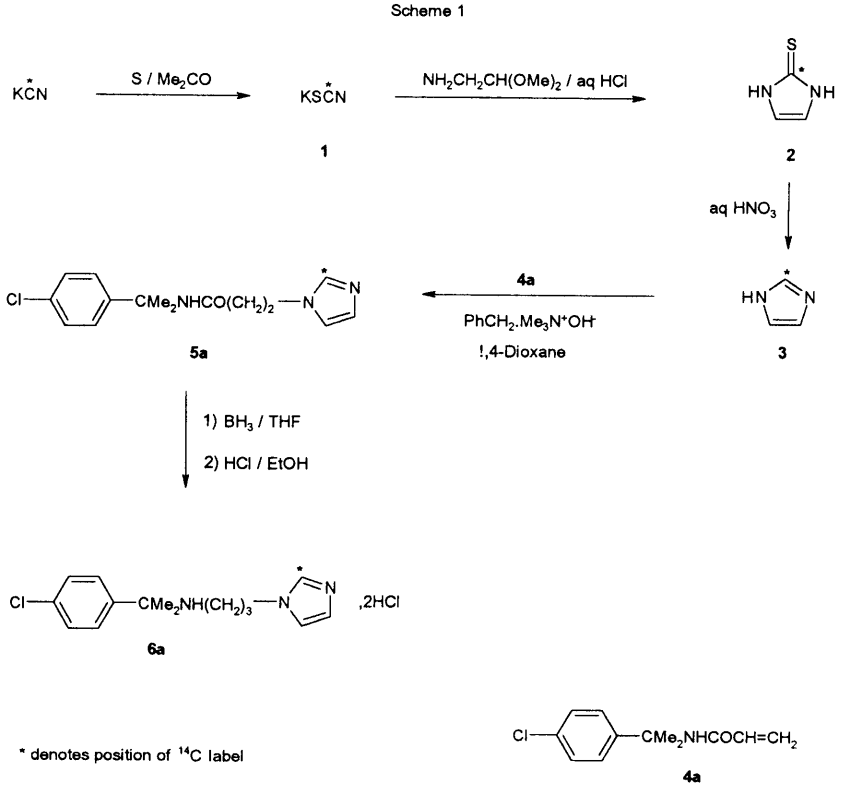
CCC 0362–4803/97/080685–09$17.50

Received 22 March 1997
Revised 8 April 1997

RESULTS AND DISCUSSION

The route used for labelling the imidazole ring in BTS 71321 is given in Scheme 1. Labelling this ring was an attractive option as in-house methodology existed for preparing BTS 71321 from imidazole and the amide **4a** (1), so the remaining requirement was to prepare labelled imidazole. This was readily obtained, labelled in the 2-position, in approximately 60% radiochemical yield by an adaptation of Marckwald's method (2). In this, potassium [^{14}C]thiocyanate, prepared from potassium [^{14}C]cyanide, was reacted with aminoacetaldehyde dimethyl acetal in a single step, by heating with dilute hydrochloric acid, to give [2-^{14}C]imidazole-2-thione (**2**), which was then dethiolated. Michael addition of the resulting [2-^{14}C]imidazole (**3**) to the amide **4a** followed by reduction gave imidazole ring labelled BTS 71321 (**6a**) in an overall radiochemical yield of 20% from potassium [^{14}C]cyanide.

Scheme 1

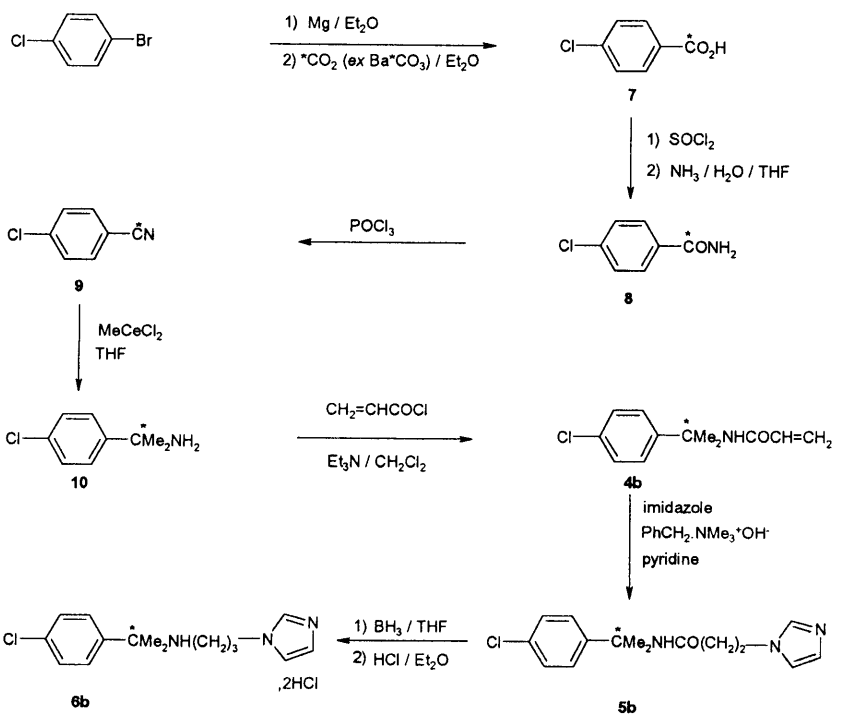


The next requirement was to label a carbon atom at the other end of the molecule from the imidazole ring. The most easily labelled carbon atoms were either the tertiary carbon atom or those in the methyl groups, and from these possibilities the tertiary carbon atom was selected in view of its more likely metabolic stability.

The route used to label the tertiary carbon atom is shown in Scheme 2. A key stage was the reaction of the benzonitrile **9** with an organocerium reagent derived from methyllithium and anhydrous cerium (III) chloride to give the amine **10**, using the methodology reported by Ciganek (3). This procedure had been used in-house to prepare the corresponding unlabelled amine (4). The amine **10** could then be treated with acryloyl chloride to give the amide **4b** and the synthesis then followed the same path as used in preparing imidazole ring labelled BTS 71321. The labelled benzonitrile **9** was prepared by routine procedures from 1-bromo-4-chlorobenzene as shown in Scheme 2, using barium [^{14}C]carbonate as the source of the label.

Although preliminary work with unlabelled materials gave BTS 71321 in an overall chemical yield of approximately 10%, when the preparation was carried out with labelled materials a problem, attributed to the quality of the borane reagent used on this occasion, resulted in a very low yield from

Scheme 2



* denotes position of ^{14}C label

the reduction of the amide **5b** to the final product. Difficulty was experienced in driving the reaction to completion and the isolated mixture, containing starting material and product, was recycled and subjected to treatment with a large excess of the reducing agent in order to achieve complete consumption of starting material. This led to a significant loss of material with the result that labelled BTS 71321 was obtained in an overall radiochemical yield of only 2.4% from barium [^{14}C]carbonate.

CONCLUSION

The reported work has provided routes to carbon-14 labelled BTS 71321, singly labelled in two regions of the molecule, starting from potassium [^{14}C]cyanide or barium [^{14}C]carbonate as appropriate. [2-^{14}C]Imidazole, which was prepared from potassium [^{14}C]cyanide *via* potassium [^{14}C]thiocyanate, was used in one of these routes.

EXPERIMENTAL

Radiochemical purities were determined by HPLC using a Canberra Packard Radiomatic Flo-one\Beta detector with liquid scintillation detection or by TLC using a Lablogic RITA linear analyser. Chemical purities were determined by HPLC using a Severn Analytical uv/vis SA 6500 detector, set at 225 nm unless otherwise stated.

Potassium [^{14}C]thiocyanate (1)

A mixture of potassium [^{14}C]cyanide (280 mg, 230 mCi; 55 mCi/mmol), unlabelled potassium cyanide (95 mg), sulphur (190 mg) and acetone (15 ml) was heated under reflux for 1.5 h, more sulphur (95 mg) being added after the first hour. On evaporation of the mixture to dryness under reduced pressure, the product was obtained as a pale brown solid.

[2-^{14}C]Imidazole-2-thione (2)

The product from the previous stage was dissolved in water (10 ml) and residual sulphur was removed by filtration. To the filtrate was added aminoacetaldehyde dimethyl acetal (0.5 ml) and 2.5M hydrochloric acid (10 ml), and the resulting solution was heated on a steam bath for 7 h. Evaporation of the solution at 45 °C under reduced pressure gave a solid residue which was dried at ambient temperature under reduced pressure to give the product as a solid (890 mg).

[2-^{14}C]Imidazole (3)

The product from the previous stage (890 mg) was dissolved in water (8 ml) in a 500 ml flask and heated on a steam bath. To the hot solution was added during 1 min concentrated nitric acid (2.5 ml), giving a vigorous reaction. The resulting solution was heated on the steam bath for 2 h, after which it

was cooled and basified to pH 12 with saturated aqueous sodium carbonate solution and subjected to continuous extraction with diethyl ether (350 ml) for about 16 h. The ether extract was dried over sodium sulphate and evaporated at 40 °C under slightly reduced pressure to give a pale yellow oil which was purified by sublimation at 70 °C/1 mmHg to give the product as an off-white solid (236 mg).

_N_-[1-(4-Chlorophenyl)-1-methylethyl]-3-([2-^{14}C]imidazol-1-yl)propionamide (5a)

The product from the previous stage (236 mg) in 1,4-dioxane (8 ml) was treated with _N_-[1-(4-chlorophenyl)-1-methylethyl]acrylamide (4a; 771 mg; for preparation see reference 1 or the procedure used for the preparation of the corresponding labelled compound 4b below) and a solution of benzyltrimethylammonium hydroxide in methanol (0.1 ml; 40 w.% solution). The resulting solution was heated on a steam bath for about 16 h. After evaporation to dryness, the residue was dissolved in dichloromethane (40 ml) and the solution was extracted with 5M hydrochloric acid (3 x 10 ml). The combined aqueous extracts were basified to pH 14 with 10M aqueous sodium hydroxide solution to deposit an oil which was extracted into dichloromethane (3 x 20 ml). The combined extracts were washed with water (10 ml), dried over sodium sulphate and evaporated to give an oil which solidified. Drying this solid at ambient temperature under reduced pressure gave the product as a colourless solid (737 mg). HPLC: S5-C8 eluted with acetonitrile-water-triethylammonium formate (40+60+0.2); radiochemical purity 99%, chemical purity (220 nm) 98%.

_N_-[1-(4-Chlorophenyl)-1-methylethyl]-3-([2-^{14}C]imidazol-1-yl)propylamine dihydrochloride (6a)

To a solution of the product from the previous stage (737 mg) in dry THF (30 ml) under nitrogen was added borane-tetrahydrofuran complex in THF (22 ml; 1.0M solution) during 5 min. The resulting solution was heated under reflux for 16 h and then evaporated to dryness under reduced pressure. The residue was treated with M hydrochloric acid (12 ml) and the solution obtained was heated on a steam bath for 1.5 h after which it was cooled, washed with dichloromethane (5 ml) and basified to pH 14 with 5M aqueous sodium hydroxide solution. The deposited oil was extracted into dichloromethane (3 x 10 ml) and the combined extracts were washed with water (5 ml), dried over magnesium sulphate and evaporated to give the free base of the product as a semi-solid (714 mg). This contained an impurity (9%) that appeared from hplc analysis to be starting material, but retreatment with borane-tetrahydrofuran complex as above only reduced its level to 7%. The recovered material was dissolved in a saturated solution of hydrogen chloride in ethanol (50 ml). Evaporation of the solution under reduced pressure followed by trituration of the residual gum with acetonitrile gave a colourless solid which was recrystallised (hot filtration) from a mixture of ethanol (3 ml) and ethyl acetate (3ml) to

give the product as colourless needles (437 mg; specific activity 106 mCi/g). The overall radiochemical yield for the sequence from potassium [^{14}C]cyanide was 20%. HPLC: S5-CN eluted with acetonitrile-water-triethylammonium formate (10+90+0.2) and S5-ODS2 eluted with methanol-acetonitrile-water-triethylammonium formate (20+20+60+0.2); radiochemical purity >99%, chemical purity (220 nm) >99%. TLC: kieselgel developed with ethanol-aqueous ammonia (d=0.88) (99.5+0.5) and with isopropanol-triethylamine-water (8+1+1); radiochemical purity >99%.

4-Chloro[*carboxy*-^{14}C]benzoic acid (7)

1-Bromo-4-chlorobenzene (1.03 g) in dry diethyl ether (17 ml) was heated under reflux with magnesium turnings (130 mg) until the magnesium had dissolved (1.5 h). The solution obtained was cooled in liquid nitrogen and treated in a vacuum manifold with [^{14}C]carbon dioxide generated by the action of concentrated sulphuric acid (6 ml) on barium [^{14}C]carbonate (817 mg, 230 mCi; 56 mCi/mmol). The temperature was raised and the reaction mixture was stirred at -35 °C for 15 min and -25 °C for 45 min. After releasing the vacuum, 2M hydrochloric acid (10 ml) was added to the reaction mixture. The separated ether layer, and ether extracts (3 x 10 ml) of the aqueous layer, were combined and extracted with M aqueous sodium hydroxide solution (3 x 15 ml). The combined extracts were acidified wth concentrated hydrochloric acid to precipitate a solid which was collected and dried under reduced pressure to give the product as a pale pink solid (331 mg). HPLC: S5-C8 eluted with acetonitrile-water-triethylammonium formate (50+50+0.1); radiochemical purity >99%, chemical purity 96%.

4-Chloro[*carbamoyl*-^{14}C]benzamide (8)

A mixture of the product from the previous stage (328 mg) and thionyl chloride (5 ml) was heated under reflux for 16 h after which evaporation gave an oil which was dried at 50 °C/150 mmHg for 30 min. The oil, dissolved in dry THF (2 ml), was added during 5 min with cooling to a mixture of aqueous ammonia (6 ml; d = 0.88) and water (6 ml). After stirring at ambient temperature for 16 h the reaction mixture was extracted with ether (3 x 20 ml followed by 4 x 15 ml). Evaporation of the extracts under reduced pressure gave a light brown solid (188 mg) which was diluted with unlabelled 4-chlorobenzamide to give the product (750 mg). HPLC: S5-C8 eluted with acetonitrile-water-triethylammonium formate (50+50+0.1); radiochemical purity >99%, chemical purity 96%.

4-Chloro[*cyano*-^{14}C]benzonitrile (9)

The product from the previous stage (736 mg) was stirred with phosphoryl chloride (6 ml) at 100 °C for 2 h, a solution gradually being obtained. It was then poured on to iced water and the mixture was extracted with ether (3 x 20 ml). The combined ether extracts were washed with water (20 ml), dried

over magnesium sulphate and evaporated under reduced pressure to give the product as a beige solid (577 mg). HPLC: S5-C8 eluted with acetonitrile-water-triethylammonium formate (50+50+0.1); radiochemical purity 99%, chemical purity 99%.

1-(4-Chlorophenyl)-1-methyl[1-^{14}C]ethylamine (10)

Anhydrous cerium (III) chloride was obtained from its heptahydrate (4.77 g) by drying at 150 °C/0.9-2 mmHg. After cooling it was treated, in an iced water bath under nitrogen, with dry precooled THF (26 ml) by syringe through a septum. The mixture was then stirred at ambient temperature for 45 min, cooled in a bath at -78 °C and treated dropwise with methyllithium (8.4 ml; 1:1 complex with lithium bromide; 1.5M solution in diethyl ether), keeping the temperature below -65 °C. After the addition was complete the mixture was stirred at -70 °C for 30 min. A solution of the product from the previous stage (577 mg) in dry THF (8 ml) was added dropwise to it at the same temperature, maintaining this temperature for a further 30 min after the addition was complete prior to allowing it to warm up to ambient temperature. Aqueous ammonia (8 ml; d=0.88) was added dropwise and the mixture was stirred at ambient temperature for 16 h. After filtration the residue was washed with ether (10 ml) and the combined filtrate and washings were evaporated under reduced pressure. The residue obtained was dissolved in ether and extracted with dilute hydrochloric acid (3 x 20 ml). The combined acid extracts were washed with ether (3 x 20 ml), basified to pH 11 with aqueous sodium hydroxide and extracted with ether (3 x 20 ml). The combined ether extracts were washed with water (20 ml), dried with magnesium sulphate and evaporated under reduced pressure to give the product as a green oil (456 mg). HPLC: S5-C8 eluted with acetonitrile-water-triethylammonium formate (50+50+0.1); radiochemical purity 83%, chemical purity 74%.

***N*-{1-(4-Chlorophenyl)-1-methyl[1-^{14}C]ethyl}acrylamide (4b)**

To a stirred suspension of the product from the previous stage (456 mg) in triethylamine (1.14 ml) and dichloromethane (3.8 ml) under nitrogen was added dropwise a solution of acryloyl chloride (0.17 ml) in dichloromethane (2 ml), keeping the temperature at -15 to -20 °C. The mixture was then stirred at ambient temperature for 16 h. Following this it was treated with 2M aqueous sodium hydroxide (8 ml) and extracted with dichloromethane (3 x 10 ml). The combined extracts were washed with water (10 ml), dried over magnesium sulphate and evaporated under reduced pressure to give a brown oil which crystallized on standing. The solid obtained was placed on a short Florisil ® column (100mm long, 25mm diameter) and eluted with dichloromethane (approx. 1500 ml). Evaporation of this under reduced pressure gave the product as a dark yellow solid (404 mg). HPLC: S5-C8 eluted with acetonitrile-water-triethylammonium formate (50+50+0.1); radiochemical purity 97%, chemical purity 95%.

N-{1-(4-Chlorophenyl)-1-methyl[1-^{14}C]ethyl}-3-(imidazol-1-yl)propionamide (5b)

A mixture of the product from the previous stage (404 mg), imidazole (123 mg), benzyltrimethylammonium hydroxide (0.016 ml of a 40% solution in methanol) and pyridine (1.5 ml) was heated under reflux for 6 h and then kept at ambient temperature for 16 h. The solid obtained by evaporation of the solvent was dissolved in dichloromethane (15 ml) and extracted with 2M hydrochloric acid (3 x 15 ml). The combined extracts were washed with dichloromethane (15 ml) and, following basification with 5M aqueous sodium hydroxide to pH 14, were extracted with dichloromethane (3 x 20 ml). These combined extracts were washed with water (20 ml), dried over magnesium sulphate and evaporated under reduced pressure to give the product as an off-white solid (420 mg). HPLC: S5-C8 eluted with acetonitrile-water-triethylammonium formate (50+50+0.1); radiochemical purity 96%, chemical purity 96%.

N-{1-(4-Chlorophenyl)-1-methyl[1-^{14}C]ethyl}-3-(imidazol-1-yl)propylamine dihydrochloride (6b)

To a solution of the product from the previous stage (366 mg) in dry THF (10 ml) was added borane-tetrahydrofuran complex (5.0 ml; 1M solution in THF) dropwise during 5 min under nitrogen. After the addition, the reaction mixture was heated under reflux for 3 h and then kept at ambient temperature for 16 h. The solvent was evaporated under reduced pressure and the residue kept at 65 °C/10 mmHg for 0.75 h. Following dropwise treatment under ice-bath cooling with 1M hydrochloric acid (12 ml), the resulting mixture was heated at 100 °C for 2 h and then kept at ambient temperature for 16 h. It was then washed with dichloromethane (3 x 15 ml), basified to pH 14 with 5M aqueous sodium hydroxide and extracted with dichloromethane (3 x 15 ml). The combined extracts were washed with brine, dried over magnesium sulphate and evaporated under reduced pressure to give an oil (313 mg). Radiochemical analysis showed this to be a mixture mainly of the required product (74%) and starting material (22%). This oil (311 mg) in dry THF (10ml) was heated again with borane-tetrahydrofuran complex (5 ml) as above and two further aliquots of the reagent (5 ml) were added to the reaction mixture at approximately 5 h intervals; after heating for a further 6 h no starting material remained. Work-up in a similar manner to the above gave the free base of the product as an oil (157 mg; radiochemical purity 97%, chemical purity 96%). To a solution of this oil in ether (5 ml) was added dropwise ethereal hydrogen chloride to precipitate a solid which turned oily. Decantation of the solvent and repeated trituration of this oil with dry ether (10 ml) gave a solid which was dried over phosphorus pentoxide *in vacuo* to give the product as an off-white solid (156 mg; specific activity 30 mCi/g). The overall radiochemical yield for the sequence from barium [^{14}C]carbonate was 2.4%. HPLC: S5-C8 eluted with methanol-water-triethylammonium formate (40+60+0.2); radiochemical purity 95%, chemical purity 97%.

REFERENCES

1. Clegg L. S., Hunneyball I. M., Jones C. G. P., Rafferty P. and Steele L. - WO 93/13075 (1993)

2. Marckwald W. - Ber. 25: 2354 (1892)

3. Ciganek E. - J. Org. Chem. 57: 4521 (1992)

4. Calderwood D. J., Fisher A. J., Jeffery J. E., Jones C. G. P. and Rafferty P. - WO 95/00493 (1995)