

Synthesis of Deramciclane* labelled with radiocarbon in various positions

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Summary

[U-¹⁴C]Bromobenzene, prepared from [¹⁴C]barium carbonate according to literature procedures, was transformed (Li / diethylether) into [¹⁴C]phenyllithium which was condensed with camphor giving rise to [U-¹⁴C-phenyl]borneol: 2. The latter, after conversion into its sodium salt, was reacted with dimethylaminoethyl chloride to give [¹⁴C-Ar]Deramciclane, which was isolated as the fumarate salt. This 8 step synthesis from [¹⁴C]BaCO₃ gave an overall yield of 12 %.

[1-¹⁴C]Sodium acetate, through a standard 5 step literature procedure, was converted to N,N-dimethylaminoethyl-1-¹⁴C chloride 5 which was condensed with the sodium derivative of 2-phenylborneol giving rise to [1-¹⁴C-side chain]Deramciclane, again isolated as the fumarate. This 6 step synthesis from [¹⁴C]BaCO₃ gave an overall yield of 8 %.

The specific radioactivities from the two syntheses were respectively 40 mCi/mmol and 21 mCi/mmol; chemical and radiochemical purities were better than 98 %.

Key words: carbon-14, anxiolytic agents, * 1R,2S,4R-(-)-2-(dimethylaminoethoxy)-2-phenyl-1,7,7-trimethylbicyclo[2.2.1]heptane, [U-¹⁴C-phenyl]Deramciclane, [1-¹⁴C-side chain] Deramciclane

Introduction

Deramciclane (EGIS-3886)⁽¹⁾ (4,7) developed by EGIS Pharmaceuticals Ltd. is a new nonbenzodiazepine type potential anxiolytic agent free of sedative and muscle relaxant side effects. The pharmacokinetic and metabolism studies of this compound require the synthesis of [¹⁴C-Ar]Deramciclane and [1-¹⁴C-side chain]Deramciclane. Radioactive labelling of the various positions (aromatic ring and side chain) were needed for the in vitro and in vivo studies to investigate various aspects of Deramciclane metabolism.

Discussion

Labelling of the desired molecule in the benzene ring may lead to a product of high specific activity. The other possibility is labelling position 1 of the side chain of the molecule. These syntheses resulted in the formation of [¹⁴C-Ar]Deramciclane and [1-¹⁴C-side chain]Deramciclane compounds, respectively. The syntheses of these molecules were accomplished using the synthetic results⁽²⁾ supplied by EGIS Pharmaceuticals Ltd..

In our procedure, the synthesis of [¹⁴C-Ar]Deramciclane from [¹⁴C]barium carbonate was realized as shown in Fig. 1. The preparation of [¹⁴C]benzene^{(3a)(3b)} and [¹⁴C]bromobenzene⁽⁴⁾

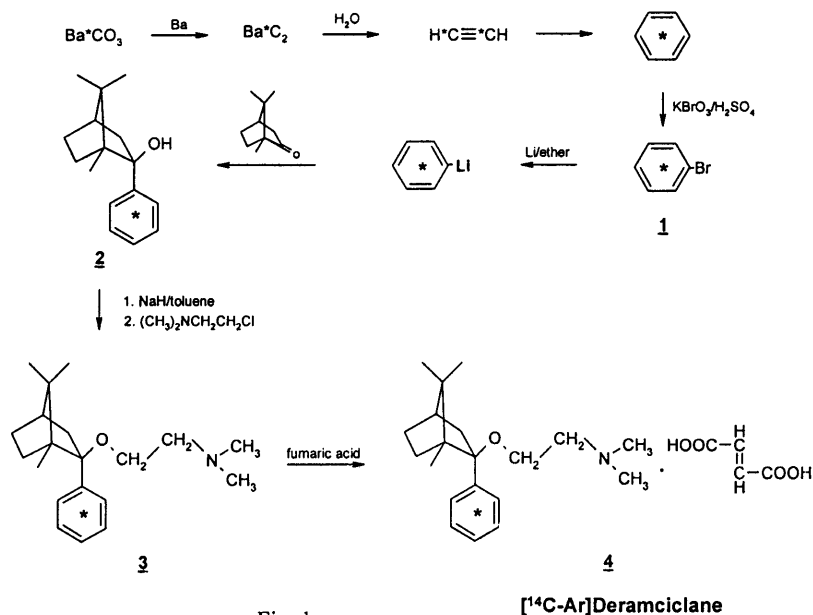


Fig. 1.

have been reported. The additional steps in the synthesis were carried out using the procedure⁽²⁾ described earlier for unlabelled Deramciclane.

The synthesis of [1-¹⁴C-side chain]Deramciclane was performed according to the reaction steps shown in Fig. 2. The preparation of N,N-dimethylaminoethyl-1-¹⁴C chloride was achieved in a similar manner to N,N-diethylaminoethyl-1-¹⁴C chloride⁽⁵⁾. The Williamson method was employed for the synthesis of the specifically labelled product.

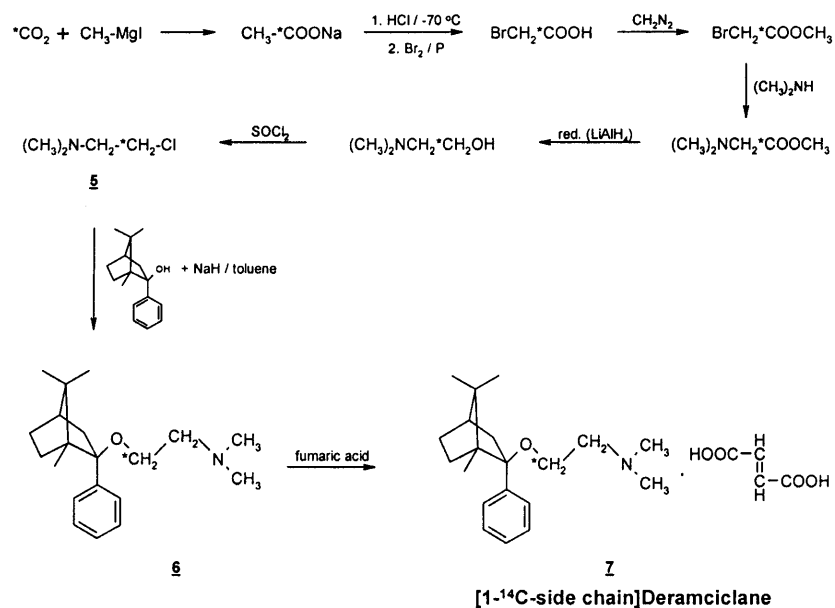


Fig. 2.

Experimental

Several reaction steps in the synthesis of [¹⁴C-Ar]Deramciclane involve work with volatile radioactive intermediates and byproducts. Therefore to comply with safety problems, these reactions were carried out in a glove-box under a slight stream of N₂.

[U-¹⁴C]Bromobenzene (1)

Cyclotrimerisation of [U-¹⁴C]acetylene prepared from Ba¹⁴CO₃ (2 g, 7.4 GBq) to [U-¹⁴C]benzene (0.120 g, 6.66 GBq) with the use of 20 g of activated "KC - Perlkatalysator Neu "

was realized as reported ^{(3a)(3b)}. A cooled solution of 0.34 ml of concentrated sulfuric acid, 1.1 ml of acetic acid and 1.2 ml water was added to [U-¹⁴C]benzene (0.120g). Then 0.320 g of potassium bromate was added in a single portion. The vessel was sealed with a glass stopper and the reaction mixture was stirred overnight at room temperature. The reaction mixture was cooled to 0 °C, and diluted successively with 5 ml of light petroleum ether, 5 ml of water, and then made alkaline with a sodium hydroxide solution. The organic phase was separated and the water phase was further extracted with 4x2 ml light petroleum ether. The organic phases were pooled and the solvent was removed by distillation with a Vigreux column (bath temperature: 45-50 °C). The residue was diluted twice with "cold" bromobenzene (2 x 0.157 g) and lyophilized under vacuum. The resulting [U-¹⁴C]bromobenzene (4.1 GBq) contained a few per cent solvent.

[U-¹⁴C-Phenyl]borneol (2)

Under a slight stream of He gas 0.128g of 30% oil suspension of Li (5.5 mmol) was placed in a round-bottomed flask fitted with a magnetic stirrer, dropping funnel and reflux condenser connected to a trap cooled in liquid N₂. To this suspension 4.1 GBq of [U-¹⁴C]bromobenzene (1) in 1.4 ml of ether was added and refluxed for an hour, then the reaction mixture was cooled to room temperature and 0.334 g of (+)-camphor was added and refluxed for a further 3.5 hours. After being cooled, the reaction mixture was decomposed with 2 ml of water and 3 ml of 2N sulfuric acid. The organic layer was separated, the inorganic phase was re-extracted with 1 ml of ether containing 0.1 ml of benzene. The organic layers were collected and lyophilized under vacuum. The volatile fraction of this solution contained 2.1 GBq [U-¹⁴C]benzene as a byproduct of the reaction. The residue containing [U-¹⁴C-phenyl]borneol (2) was purified by column chromatography (20 g Kieselgel 60, eluent : chloroform). The pure fractions were combined and evaporated. Yield: 0.240 g, 1.43 GBq (35% based on [U-¹⁴C]bromobenzene).

[¹⁴C-Ar]Deramciclane (4)

To the solution of [U-¹⁴C-phenyl]borneol (2) (0.240 g) in 3.3 ml of dry toluene, 0.110 g of sodium hydride in mineral oil (50%) was added and refluxed for 10 minutes. Then

dimethylaminoethyl chloride (3.3 mmol) was added in 1.6 ml of dry toluene under reflux and the mixture was refluxed for a further 3 hours. The reaction mixture was cooled in an ice water bath and decomposed with 0.2 ml of methanol and 0.2 ml of methanol-water 1: 1, then evaporated on a rotary evaporator. The crude product (**3**) was purified by column chromatography, using 20 g of silica gel and chloroform:methanol:ammonia = 97:3:0.3 as eluent. The pure fractions were combined and evaporated. The residue was dissolved in 2 ml of ethanol and 0.086 g of fumaric acid in 3.5 ml of ethanol was added. The solution was concentrated to about 1 ml and the precipitated mixture was stirred at 110°C for one hour, and after cooling, stored for some hours in a refrigerator. The product (**4**) was filtered and recrystallised from 1.3 ml of dimethylformamide. Yield : 0.250 g, (0.89 GBq) [¹⁴C-Ar]Deramciclane. Chemical and radiochemical purities were >98 %.

N,N-Dimethylaminoethyl-1-¹⁴C chloride hydrochloride (5**)**

Sodium acetate-1-¹⁴C was prepared by carbonation of methylmagnesium iodide (2.5 mmol) in 15 ml of ether at -30 °C with ¹⁴CO₂ liberated from [¹⁴C]-barium carbonate (0.40 g, 4.40 GBq) with concentrated H₂SO₄ in a vacuum manifold system. To sodium acetate-1-¹⁴C, unlabelled acetic acid (0.1 ml) and about 5 mmol of dry HCl gas were added and the reaction mixture was stirred at -70 °C in a sealed flask for one hour. To the acetic-1-¹⁴C acid lyophilized from the salt mixture, acetic anhydride (0.05 ml), red phosphorus (10 mg) and bromine (0.25 ml) were added and warmed at 100 °C for two hours. After 3 ml of dry benzene was distilled off from the reaction mixture, the residue was dissolved in 5 ml of ether and esterified with diazomethane at 0 °C. Most of the solvent was distilled under atmospheric pressure and dimethylamine (0.5 g) in 10 ml of ether was added to the residue and stirred for two hours at room temperature. The precipitate was filtered and the mother liquor was dried over Na₂SO₄, filtered and evaporated. The methyl dimethylaminoacetate-1-¹⁴C (1.95 GBq) obtained was used in the next reaction step without further purification.

To a solution of lithium aluminium hydride (0.38 g) in 10 ml of ether, methyl dimethylaminoacetate-1-¹⁴C in 5 ml of ether was added at room temperature, then the reaction mixture was stirred and refluxed for one hour. The reaction mixture was decomposed with ethyl acetate (1 ml), ice cold water (5 ml) and 2 N sulfuric acid (15 ml) successively. The organic solvent was distilled, the residue was made alkaline with 40 % sodium hydroxide and steam-distilled. About 100 ml of distillate were collected, which were acidified and evaporated on a rotary evaporator. The residue was refluxed with thionyl chloride (1 ml) for 30 minutes, then evaporated to dryness with a rotary evaporator. Yield: 0.160 g (0.83 GBq) dimethylaminoethyl-1-¹⁴C chloride hydrochloride (5).

[1-¹⁴C-side chain]Deramciclane (7)

To the solution of phenylborneol (0.280 g) in 3 ml of dry toluene in a glass-tube, 0.120 g of sodium hydride in mineral oil (50 %) was added and refluxed for 10 minutes. The base of (5) was liberated in water solution with sodium hydroxide, extracted with toluene (3x1 ml) and dried on K₂CO₃. The toluene solution was added to the above phenylborneol reaction mixture, the glass tube was sealed and warmed for 3 hours at 110 °C. After cooling the glass tube was opened, the reaction mixture processed and purified as described for [¹⁴C-Ar]Deramciclane. Yield: 0.192 g (0.36 GBq). Chemical and radiochemical purities were >98 %.

Analysis of Deramciclane labelled with ¹⁴C

Radioactivity measurements and determination of chemical and radiochemical purity of [¹⁴C-Ar]Deramciclane and [1-¹⁴C-side chain]Deramciclane were performed by the following methods.

Radioactivities of samples were measured by liquid scintillation counting with RackBeta Liquid Scintillation Counter Model 1217-001 (LKB-Wallac) in a cocktail of benzyl alcohol (1000 ml) - ethanol (200 ml) and PPO (6 g).

Chemical and radiochemical purities of the products were checked by thin layer (TLC) and high-performance liquid (HPLC) chromatographies, on the basis of comparison with Deramciclane standard.

Thin layer chromatography:

TLC-sheet: DC Alufolien Kieselgel 60 F₂₅₄ (Merck)

Eluent: chloroform : methanol : ammonia = 95 : 5 : 0,5

Detection: UV light and DigitalAutoradiograph [(EG&G Berthold LB 287, WinDAR Software),

Run time: 30 min., Gas flow: 5 ml/min, Counter voltage: 1100 V].

R_f-value for Deramciclane: 0.4.

High-performance liquid chromatography:

ISCO HPLC-system was used under the following conditions:

Column: Hypersil ODS (5µm , 250x4mm ID)

Mobile phase: acetonitrile : water = 6 : 4 + 0.1 % 70 % perchloric acid + 0.11 % triethylamine

Detection: at 220 nm by UV detector

Radioactivity was measured by a HPLC-Radioactivity Monitor (Hewlett-Packard

1090M-EG&G Berthold LB506-C-1), Parameters: Scintillator cocktail Quickscint

Flow 301 (Zinsser Analytic), Detector cell type: Z-1000 4 (1 ml)

Acknowledgement

This work was partially supported by grants N^o T 022623 provided by the Hungarian Research Fund (OTKA) and EGIS Pharmaceuticals Ltd.

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