Synthesis of ^{14}C-Labelled Cefluprenam (E1077), a Novel Parenteral Cephalosporin Antibiotic.

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SUMMARY

Cefluprenam (E1077) is a new parenteral cephalosporin with a well-balanced antibacterial spectrum and potent antibacterial activity. It was synthesized labelled in the side chain at the 3-position of the cephem with carbon-14, starting from potassium [^{14}C]cyanide, according to the method illustrated in Schemes **1**,**2**. [^{14}C]Cefluprenam having a specific activity of 3.9 MBq/mg, was obtained in 28.6 % overall radiochemical yield, with a radiochemical purity of more than 97.3 %.

Key Words ; [^{14}C]cefluprenam, E1077, a fourth generation cephalosporin, potassium [^{14}C]cyanide, [[2-^{14}C]carbamoylmethyl]ethylmethylamine

INTRODUCTION

Cefluprenam is a novel parenteral cephalosporin with a wide spectrum and potent antibacterial activity against aerobic and

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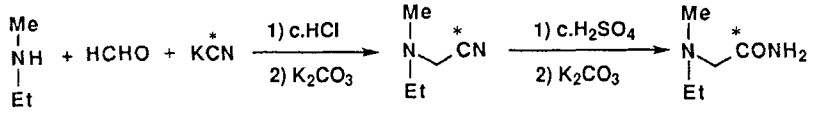
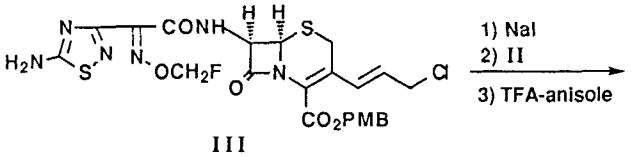
anaerobic gram-positive bacteria, including staphylococci and enterococci, and gram-negative bacteria, including *Pseudomonas aeruginosa* and *Bacteroides fragilis* (1,2,3). Its activity is more than twice as high as that of cefpirome , a so-called fourth generation cephalosporin. We have already reported the synthesis of [^{14}C]E1077, prepared labelled in the side chain at the 7-position of the cephem (4). In this paper we report the synthesis of ^{14}C-labelled ceftuprenam, labelled in the side chain at the 3-position of the cephem. The compound was required for pharmacokinetic studies.

Scheme 1

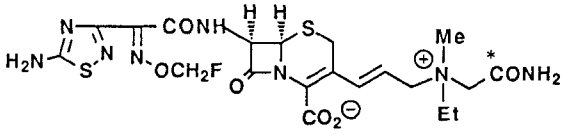


I II

Scheme 2



III



IV

* = position of label
TFA = Trifluoroacetic acid
PMB = p-Methoxybenzyl

RESULTS AND DISCUSSION

The reaction of ethylmethylamine, formaldehyde and potassium [^{14}C]cyanide gave tertiary amine(**I**). Hydrolysis of (**I**) afforded [[2-^{14}C]carbamoylmethyl]ethylmethylamine(**II**), 6.07 GBq in 65.6% radiochemical yield based on potassium [^{14}C]cyanide.

p-Methoxybenzyl *(6R,7R)* -7-[*(Z)* -2-(5-amino-1,2,4-thiadiazol -3- yl) -2- fluoromethoxyiminoacetamide] -3- [*(E)*-3-chloro-1-propenyl] -8-oxo -5- thia -1- azabicyclo[4.2.0]oct -2- ene -2- carboxylate(**III**) was condensed with amine(**II**) in the presence of sodium iodide, giving the quaternary salt. Removal of the protecting group was carried out using trifluoroacetic acid. The product of this reaction was purified by passage through an ODS column, to produce [^{14}C]cefluprenam (**IV**) labelled in the side chain at the 3-position of the cephem, as an aqueous solution. The overall radiochemical yield from potassium [^{14}C]cyanide was 28.6%. All experimental conditions were optimized using non-radioactive materials.

EXPERIMENTAL

All chemicals used in the synthesis were purchased, and were used without purification. All solvents were either distilled or were of analytical reagent quality.

Analysis of purity and determination of yield were performed on a reverse-phase column (YMC A-301-3 S3 120A ODS, 4.6mm (I.D.) x 100mm). The mobile phase was 10mM sodium dodecyl sulfate solution containing acetonitrile, water and phosphoric acid (300 : 700 : 5 v/v/v). The retention time was 13.9 min at 4°C at a flow rate of 1 ml/min. (The retention time was dependent upon the column temperature. It was 9.3 min at 20°C.)

Thin layer chromatography (TLC) was carried out on silica gel plates (Kieselgel 60F_{254}, No.5715, Merck) using either of two developing systems: A. acetone : water : acetic acid : 28% aqueous ammonia (30 : 20 : 20 : 2.5 v/v/v/v) ; B. acetonitrile : ethyl acetate : water : acetic acid (3 : 2 : 2 : 1 v/v/v/v).

[[2-^{14}C]cyanomethyl]ethylmethylamine(**I**)

To a stirred solution of N-ethylmethylamine (0.39ml, 4.54mmol) in water (1.1ml) at 0°C was added 37% aqueous formaldehyde

solution (0.385ml, 5.14mmol) and potassium [^{14}C]cyanide (305mg, 4.56mmol, 9.25GBq). After the reaction mixture had been stirred for 1 hour at 0°C, conc. hydrochloric acid (0.44ml) was added. The reaction mixture was allowed to warm to room temperature and was stirred for a further 48 hours. Potassium carbonate (312mg, 2.26mmol) was added to the reaction mixture, which was then extracted with dichloromethane (3 x 20ml). The combined organic layers were dried over potassium carbonate, then filtered and the solvent was removed by distillation at atmospheric pressure. This crude product (**I**) was used without further purification

[[2-^{14}C]carbamoylmethyl]ethylmethylamine(**II**)

Conc. sulfuric acid (1.25ml) was added slowly to the amine (**I**) at -30°C and the mixture was stirred at room temperature for 48 hours. The solution was cooled to 0°C and water (7ml), ethyl acetate (5.7ml) and potassium carbonate (3.7g) were added. The reaction mixture was filtered, and the solid residue was washed with ethyl acetate (200ml). It was then dissolved in water (15ml) and extracted with chloroform (4 x 50ml). All organic layers were combined and dried over potassium carbonate, filtered, and then the solvent was removed *in vacuo*. The residue was purified by distillation under vacuum to afford (**II**) (6.07GBq, 53mCi/mmol, 65.6% radiochemical yield over two steps) as a white crystalline solid.

(-)-*(RS)*-[*(E)*-3-[*(6R,7R)*-7-[*(Z)*-2-(5-amino-1,2,4-thiadiazol-3-yl) - 2-fluoromethoxyiminoacetamide] - 2 - carboxy - 8 - oxo - 5-thia-1 - aza - bicyclo [4.2.0] oct - 2 - ene - 3 - yl] -2-propenyl] [[2-^{14}C] (carbamoylmethyl)]ethylmethylammonium hydroxide inner salt (**IV**, [^{14}C]cefluprenam)

p-Methoxybenzyl *(6R,7R)*-7-[*(Z)*-2-(5-amino-1,2,4-thiadiazol-3-yl) -2-fluoromethoxyiminoacetamide]-3-[*(E)*-3-chloro-1-propenyl]-8-oxo -5- thia -1- azabicyclo[4.2.0]oct -2- ene -2- carboxylate (**III**)

(829mg, 1.39mmol) was added to a dimethylformamide (8.5ml) solution of amine(**II**) (156mg, 1.32mmol, 2.59GBq) and sodium iodide (218mg, 1.45mmol). After the reaction mixture had been stirred at room temperature for 2.5 hours, the solvent was removed *in vacuo*. The residue was dissolved with an ice-cooled mixture of trifluoroacetic acid(4.25ml) - anisole(4.25ml), and this solution was sonicated for 20 min. The reaction mixture was added dropwise to a diisopropyl ether (56ml) and ethyl acetate (56ml) mixed solvent. After cooling the mixture with ice, the precipitate was collected by filtration, washed with diisopropyl ether, and dried under vacuum. Sodium acetate (700 mg) and water (25ml) was added to the solid residue, and the mixture was sonicated for 20 min. The precipitate was then filtered off. Methanol (3ml) was added to the filtrate and the solution was concentrated to a volume of 5ml *in vacuo*. After filtering the solution, the filtrate was placed on an ODS column (YMC SH-343-7 AM S7 120A, 20mm(I.D.) x 250mm). The column was eluted first with water (200 ml), then with 0.8% tetrahydrofuran-water. The fraction containing the desired compound was concentrated at < 40°C under reduced pressure to a volume of 5ml, and purified again using the ODS column under the same conditions to afford (**IV**), (291 mg based on non labelled ceftuprenam), as a 10 ml solution, 1.13 GBq, 28.6% radiochemical yield based on potassium [^{14}C]cyanide. The specific activity was 3.9 MBq/mg (58.7 mCi/mmol). The radiochemical purity was 99.0% by HPLC analysis and 97.3 %(A), 97.9 %(B) respectively by TLC analysis (A: Rf=0.75, B: Rf=0.45). The [^{14}C]ceftuprenam solution was frozen and kept at -80°C prior to being used for pharmacokinetic studies.

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