Synthesis of [^{14}C]E1077, a Novel Parenteral Cephalosporin Antibiotic.

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SUMMARY

(-)-*(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-azabicyclo[4.2.0]oct-2-ene-3-yl]-2-propenyl](carbamoylmethyl) ethylmethylammonium hydroxide inner salt (E1077), a new parenteral cephalosporin with well-balanced antibacterial spectrum and potent antibacterial activity, was labelled with carbon-14, starting from potassium [^{14}C]thiocyanate which on reaction with *(Z)* - 2 - (*N* - chloroamidino) - 2 - fluoromethoxyiminoacetic acid (I) gave *(Z)*-2-(5-amino-1,2,4-[5-^{14}C]thiadiazol-3-yl)-2-fluoromethoxyiminoacetic acid (II).
(RS) - [*(E)* - 3 - [*(6R,7R)* -7-Amino- 2 - carboxy - 8 - oxo- 5- thia-1-azabicyclo[4.2.0]oct-2-ene-3-yl]-2-propenyl](carbamoylmethyl) ethylmethylammonium hydroxide inner salt (III) was acylated with the above acid using the $POCl_3$-DMF method, to afford the title compound (IV); [^{14}C]E1077, having a specific activity of 3.36 MBq/mg, was obtained in 50.3 % overall radiochemical yield, with a radiochemical purity of more than 97.1 %.

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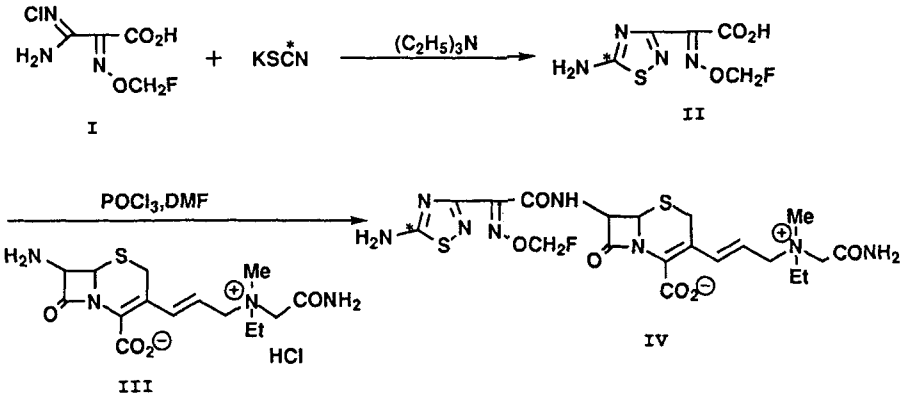
SYNTHETIC PATHWAY



INTRODUCTION

E1077 is a novel parenteral cephalosporin with a wide spectrum and potent antibacterial activity against aerobic and 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. This paper describes the synthesis of the ^{14}C-labelled E1077 which was required for pharmacokinetic studies.

RESULTS AND DISCUSSION

The reaction of potassium [^{14}C]thiocyanate with *(Z)* - 2 - (*N*-chloro amidino) - 2 - fluoromethoxyiminoacetic acid (I) produced *(Z)*-2-((5-amino-1,2,4-[5-^{14}C] thiadiazol - 3 - yl)-2-fluoromethoxyimino acetic acid (II). The acid, activated by the $POCl_3$-DMF system, was condensed with amine (III), and the product was purified through an ODS column, to produce [^{14}C] E1077 as an aqueous solution.

The product yield was measured by HPLC against a nonlabelled E1077 standard. The overall chemical yield from potassium thiocyanate was 50.3%. All experimental conditions were optimized using non-radioactive materials.

EXPERIMENTAL

Potassium [^{14}C]thiocyanate with a specific activity of 2.15 GBq /mmol was purchased from Amersham Japan. All chemicals used in the synthesis were purchased, and were used without purification. All solvents were either distilled or were of analytical reagent quality.

The high performance liquid chromatography used consisted of a Waters Model 510 pump, Jasco UVIDEC-100-VI UV detector set at 295nm and a TOSOH RS-8000 radioisotope detector with CP-8080 modulator. Analysis of purity and determination of amount were performed on a reverse - phase column(YMC A-301-3 S3 120A, 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 10.9 min at a flow rate of 1 ml / min.

Thin layer chromatography (TLC) was carried out on silica gel plates (Merck 60F254, 20 x 20 cm) using either of two developing systems: A. acetone : water : acetic acid : 28% 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). Liquid scintillation counting was performed with an Aloka Model LSC-900 liquid scintillation spectrometer.

(Z)-2-(5-amino-1,2,4-[5-^{14}C]thiadiazol-3-yl)-2-fluoromethoxy imino acetic acid (II), 1:1 mixture with isopropylether (IPE).

In a 200 ml round - bottom flask , potassium [^{14}C] thiocyanate (11.12 GBq, 512 mg) was dissolved in methanol (20 ml)

containing triethylamine (627mg). 2 - (*N* - Chloroamidino) - 2 -(fluoromethoxyimino)acetic acid (I, 1.124 g) was added and the reaction mixture was stirred at room temperature for 14 hours. After removal of the solvent in vacuo, the residue was dissolved in 4*N* sulfuric acid (1.7ml) - water (15ml) while being cooled in an ice bath. The mixture was extracted with ethyl acetate (50 ml x 6). The combined organic layers were dried over anhydrous $MgSO_4$ and then treated with charcoal.

The solvent was evaporated in vacuo, and the residual syrup was dissolved in acetone, then IPE was added. The solvent was evaporated in vacuo, and the residue was triturated with IPE. The resulting solid was collected by filtration, washed with IPE, and dried over P_2O_5 in vacuo to afford (II) as the 1:1 mixture with IPE (1.408 g, 84 %).

(-)-*(RS)*-[*(E)*-3-[*(6R,7R)*-7-[*(Z)*-2-(5-amino-[5-^{14}C]-1,2,4-thiadiazol-3-yl) - 2-fluoromethoxyiminoacetamide] -2-carboxy-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-3-yl]-2-propenyl] (carbamoylmethyl)ethylmethylammonium hydroxide inner salt (IV, [^{14}C]E1077)

To a stirred solution of dimethylformamide (0.23 ml) and tetrahydrofuran (THF, 2.4 ml) cooled in an ice bath, was added phosphoryloxychloride (0.20 ml). The mixture was stirred at ice bath temperature for 30 min. The above mixture was added to a stirred solution of the acid (II, mixture with IPE, 640 mg) in THF (6.0 ml) at -20°C , and stirring was continued at -20°C for 15 min. The above mixture was then added to a solution of *(RS)* - [*(E)* - 3 - [*(6R,7R)*-7-Amino-2-carboxy-8-oxo-5- thia-1-azabicyclo[4.2.0]oct-2 - ene - 3 - yl] - 2 - propenyl] (carbamoylmethyl) ethylmethyl ammonium hydroxide inner salt(III, 776 mg) and anhydrous sodium acetate (1.74 g) in water - ethanol (15 ml - 3 ml) at -20°C , and stirring was continued at -20°C for a further 10 min.

The solvent was evaporated at < 40°C in vacuo. The residue was dissolved in water (15 ml), and placed on an ODS column (YMC, SH-343-7, 20 x 250mm). The column was eluted first with water (250 ml), then with 0.8% THF-water. The fraction containing the desired compound was concentrated at < 40°C under reduced pressure to a volume of 10 ml, and purified again using the ODS column under the same conditions to afford IV, (756.4 mg based on non labeled compound), as a 10 ml solution, 2.54 GBq, 50.3% radiochemical yield based on potassium [^{14}C]thiocyanate. The specific activity was 3.36 MBq/mg (50.5 mCi/mmol). The radiochemical purity was 98.6% by HPLC analysis and 97.9%(A),97.1%(B) respectively by TLC analysis (A: Rf = 0.769 , B: Rf = 0.472). The chemical purity was 99.8% by HPLC analysis. The [^{14}C]E1077 solution was kept at -80°C prior to it being used for pharmacokinetic studies.

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