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Synthesis of ^{14}C-Labelled Cefclidin (E1040)

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SUMMARY

Cefclidin (E1040), a new injectable cephalosporin with potent antipseudomonal activity, was synthesized labelled with carbon-14, starting from bromo[1-^{14}C]acetic acid according to the method illustrated in Scheme 1, 2. [^{14}C] Cefclidin, having a specific activity of 3.43MBq/mg (water content 15.3%, which was based on water content of non labelled compound prepared by the same procedure), was obtained in 2% overall radiochemical yield, with a radiochemical purity of more than 99.1%.

Key Words: [^{14}C] Cefclidin, injectable cephalosporin, Pseudomonas aeruginosa, 4-carbamoyl[3-^{14}C]quinuclidine

INTRODUCTION

Cefclidin is a new parenteral cephalosporin with a broad spectrum of activity both *in vivo* and *in vitro* (1). When compared with ceftazidime (2) or cefmenoxime *in vitro*, against *Pseudomonas aeruginosa*, *Enterobacter cloacae* and *Citrobacter freundii*, it has greater activity (3). The comparative therapeutic efficacy of the compound in experimental infections was consistent with its relative

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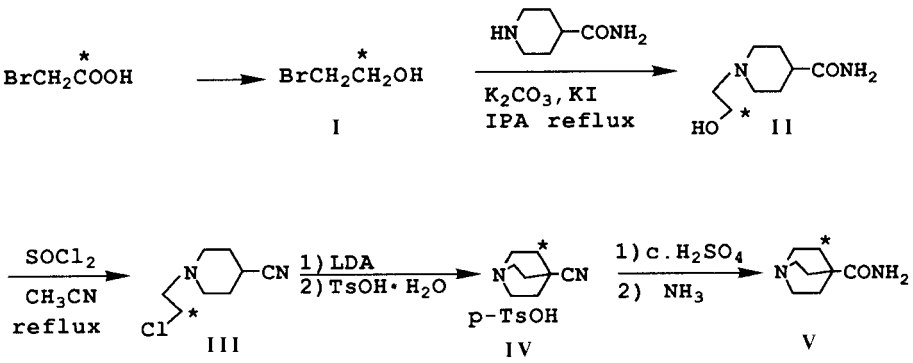
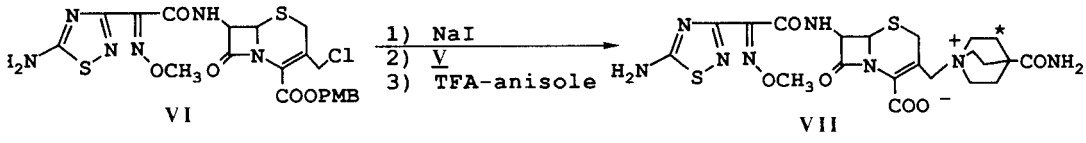
activity *in vitro* (4). This report describes the synthesis of the ^{14}C-labelled cephalosporin for metabolism and pharmacokinetic studies.

Scheme 1



Scheme 2



* = position of label

TFA; Trifluoroacetic acid
PMB; p-Methoxybenzyl

RESULTS AND DISCUSSION

Bromo[1-^{14}C]acetic acid was converted into bromoethanol (I) on borane-dimethyl sulfide complex. Treatment of (I) with isonipecotamide followed by chlorination and dehydration of the resulting alcohol (II) afforded nitrile (III), which was treated with

lithium diisopropylamide to give a toluenesulfonate salt (IV) of 4-cyanoquinuclidine. Hydrolysis of (IV) afforded 4-carbamoyl[3-^{14}C]quinuclidine (V), 5.25 GBq in 7% radiochemical yield based on bromo[1-^{14}C]acetic acid. p-Methoxybenzyl (6R,7R)-7-[2-(5-amino-1,2,4-thiadiazol-3-yl)-(Z)-2-methoxyiminoacetamido]-3-chloromethyl-3-cephem-4-carboxylate (VI) was treated with sodium iodide to afford the iodide, which was, without isolation, allowed to react (V) to give the quaternary salt. Removal of the protecting group was carried out by the use of trifluoroacetic acid and anisole, and the product passed through an ODS column and then crystalized with EtOH, to produce a crystalline product (water content 15.3%, which was based on water content of non labelled compound prepared by the same procedure). The overall chemical yield from bromo[1-^{14}C]acetic acid was 2%. All experimental conditions were optimized using non-radioactive materials.

EXPERIMENTAL

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

High performance liquid chromatography (HPLC) analysis was performed on a Waters Model 590 equipped with a UV-detecter (254 nm, JASCO UVIDEC 100-III), a Shimadzu Chromatopac C-RIA injector and an ODS column (YMC-A312, 6 x 150 mm) using a mobile phase of H_2O:CH_3OH:$AcONH_4$ (850:150:1, v/v/w). The retention time value was 4.0 min at a flow rate of 1.5 mL/min. Liquid scintillation counting was performed with an Aloka Model LSC-3500 liquid scintillation spectrometer.

Bromo[1-^{14}C]ethanol (I)

Bromo[1-^{14}C]acetic acid (5.02 g, 72.37 GBq), in dry Et_2O (80 mL) was stirred at RT under an atmosphere of nitrogen. Borane-dimethyl

sulfide complex (30.8 mL) in dry Et_2O (140 mL) was added. The mixture was heated under reflux for 4h. The mixture was cooled to 0°C then treated with water (41 mL) dropwise and then with conc. HCl (26.6 mL). NaCl (56.5 g) was added and the aqueous layer extracted with Et_2O (2 x 100 mL). Most of the solvent was removed using a vigreaux column. The mixture was subjected to flash column chromatography on silica eluting with Et_2O:pentane (2:1). Pure compound (I) was isolated (1.41 g, 22.57 GBq, 31%)

1-[3-^{14}C]Hydroxyethylisonipecotamide (II)

(I) (1.41 g, 22.57 GBq) in isopropyl alcohol (23 mL) was treated with the isonipecotamide (1.54 g), anhydrous K_2CO_3 (1.7 g), and KI (45 mg). The mixture was heated at reflux for 12h with stirring. The hot solution was filtered through celite. The filter pad was washed with hot isopropyl alcohol (2 x 15 mL). The solvent was removed *in vacuo*. CH_3CN (100 mL) was added and the solvent again removed *in vacuo*. Compound (II) was obtained (2 g, 22.2 GBq, 98%).

1-[3-^{14}C]Chloroethyl-4-cyanopiperidine (III)

(II) (2 g, 22.2 GBq) was treated with dry CH_3CN (40 mL). The mixture was stirred at 0°C and then treated with thionyl chloride (6.5 g). the mixture was heated at reflux for 5h. The solvent was removed *in vacuo*. Isopropyl alcohol (30 mL) was added and again the solvent was removed *in vacuo*. The residue was treated with CH_2Cl_2 (80 mL) and saturated $NaHCO_3$ (80 mL) at 0°C. The aqueous layer was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic layers were washed with brine (50 mL), and dried ($MgSO_4$). The material was subjected to flash column chromatography on silica gel eluting with CH_2Cl_2:MeOH (100:1). Compound (III) was isolated (1.25 g, 13.62 GBq, 61%).

4-Cyano[3-^{14}C]quinuclidine. TsOH (IV)

Lithium diisopropylamide mono (tetrahydrofuran) (1.5 M solution in cyclohexane) (5.3 mL) in dry tetrahydrofuran (42 mL) was stirred at -78°C under an atmosphere of nitrogen. (III) (1.25 g, 13.62 GBq) was added to the above solution in dry tetrahydrofuran (5 mL). The mixture was stirred for 30 minutes at -78°C and for 30 minutes at 0°C. The solvent was removed *in vacuo*. CH_2Cl_2 (60 mL) was added and the mixture washed with saturated K_2CO_3 (20 mL). The aqueous layer was extracted with CH_2Cl_2 (2 x 20 mL). The combined organic layers were washed with brine (15 mL) and dried ($MgSO_4$). The solvent was removed *in vacuo*. The residue was subjected to flash column chromatography on silica gel eluting with CH_2Cl_2:MeOH:Et_3N (50:4:1). Pure compound [free base of compound (IV)] was obtained (490 mg, 7.22 GBq, 53%). This materials was dissolved in $CHCl_3$ (10 mL) and cooled to 0°C. The mixture was then treated with p-toluenesulfonic acid monohydrate (634 mg) in EtOH (2 mL). The solvent was removed *in vacuo* to give compound (IV) (1.1 g, 7.22 GBq).

4-Carbamoyl[3-14C]quinuclidine (V)

(IV) (1.1 g, 7.22 GBq) was treated with cold (0°C) conc. H_2SO_4 (1.4 mL). The mixture was stirred and heated at 60°C for 2h. The mixture was cooled 0°C and treated with 1,2-dimethoxyethane (10 mL). The mixture was stirred at 0°C for 30 minutes. The mixture was filtered and the solid washed with 1,2-dimethoxyethane (10 mL) and diisopropyl ether (5 mL). Compound (V) was obtained as sulfate (5.92 GBq). The product (5.92 GBq) was dissolved in EtOH (15 mL) at 0°C. The mixture was treated with a sample of cold (0°C) ammonia in EtOH (6%, 15 mL). The mixture was stirred at 0°C for 1h. The mixture was filtered and the solid washed with EtOH (2 x 15 mL). The solvent

from the mother liquor was removed *in vacuo*. Acetone (40 mL) was added and the mixture stirred at 0°C for 10 minutes. The mixture was filtered through celite. The filter pad was washed with EtOH (50 mL). The solvent from the acetone mother liquor was removed *in vacuo*. The resulting solid was triturated with acetone (2 mL) and (5 mL). The solid was dissolved in EtOH (20 mL). The solvent from the combined EtOH solutions of compound (V) (20 mL + 50 mL) was removed *in vacuo* to give compound (V) (447 mg, 5.25 GBq, 73%).

(6R,7R)-7-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-(Z)-2-methoxyiminoacetamido]-3-[4-(carbamoyl-1-[3-^{14}C]quinuclidinio)methyl]ceph-3-em-4-carboxylate (VII, [^{14}C]cefclidin)

A mixture of the 3-chloromethyl derivative (VI) (1.43 g) and NaI (530 mg) in acetone (7 mL) was stirred at RT for 1h. The mixture was treated with EtOAc (70 mL). The mixture was washed with 10% aq. $Na_2S_2O_3$ (2 x 10 mL), brine (10 mL) and dried ($MgSO_4$). The filtrate was treated with acetonitrile (9 mL) and cooled to 0°C. A solution of EtOAc (14 mL) and MeOH (7 mL) of (V) (3.52 GBq) was added dropwise over 30 minutes at 0°C thereto. The resulting precipitate was collected by filtration. This compound was suspended in anisole (8.1 mL). After ice cooling trifluoroacetic acid (20 mL) was added dropwise thereto. The mixture was stirred for 20 minutes at 0°C. The mixture was treated with CH_2Cl_2 (10 mL) and diisopropyl ether (75 mL). The resulting precipitate was collected by filtration, and the solid washed with CH_2Cl_2 (10 mL) and diisopropyl ether (5 mL). The solid was dissolved in 30% MeOH-H_2O (70 mL). The solution was adjusted to pH 5 by the addition of AcONa.3H_2O (1.25 g). The mixture was filtered. The filter pad was washed with 30% MeOH-H_2O (30 mL). The filtrate was removed *in vacuo*. The residue was dissolved in water (10 mL), and placed on an ODS column containing

133 g of ODS in water. The column was first eluted with H_2O (600 mL), and then with 1% CH_3OH-H_2O (300 mL), 1.23% CH_3OH-H_2O (300 mL) , 1.3% CH_3OH-H_2O (100 mL), 1.4% CH_3OH-H_2O (100 mL), 1.5% CH_3OH-H_2O (100 mL). The fraction containing the desired compound was concentrated at reduced pressure to a volume of 5 mL. C_2H_5OH (10 mL) was added to the solution, and the resulting white suspension was then stirred, with ice cooling. An additional portion of C_2H_5OH (10 mL) was added to the mixture and stirred for 30 min at 0~5°C. The solid produced was collected by filtration, washed with cold 90% C_2H_5OH-H_2O (10 mL), then C_2H_5OH (10 mL), and then was air-dried at room temperature overnight to afford the amide (VII), (394.3 mg) as a white powder, water content 15.3%, which was based on water content of non labelled compound prepared by the same procedure, 1.15 GBq, 2% radiochemical yield based on bromo[1-^{14}C]acetic acid. The specific activity was 3.43 MBq/mg (51.2 mCi/mmol) . The radiochemical purity was more than 99.1% by HPLC analysis. The chemical purity was 98.3% by HPLC analysis.

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