SYNTHESIS OF 7-[α-(2-AMINO-[2-^{14}C]THIAZOL-4-YL)-α-(Z)-METHOXYIMINOACETAMIDO]-3-(1-METHYLPYRROLIDINIO)METHYL-3-CEPHEM-4-CARBOXYLATE HYDROCHLORIDE ([^{14}C]CEFEPIME HYDROCHLORIDE)

R.T. Standridge and J.E. Swigor
Bristol-Myers Squibb Company
Pharmaceutical Research Institute
Syracuse, New York 13221-4755 U.S.A.

SUMMARY

The title compound 9 was prepared by the route outlined in Scheme I. [^{14}C]Thiourea (1) was condensed with ethyl 4-bromo-3-oxo-2-methoxyiminoacetate (2), providing ethyl 2-(2-amino-4-[2-^{14}C]thiazolyl)-2-methoxyiminoacetate (3), as the pure Z-isomer. Saponification gave the amino acid 4; this was reacted with 1-hydroxybenzotriazole to give the activated ester 5. Condensation *in situ* with 7-amino-3-(1-methylpyrrolidinio) methyl-3-cephem-4-carboxylate (6) yielded the product as the pure sulfate salt (7).

Treatment of 7 with base provided the zwitterion 8, isolated as the stable N-methyl-2-pyrrolidinone adduct. An aqueous solution of the adduct was converted to the crystalline title compound, [^{14}C]Cefepime hydrochloride hydrate (9), with hydrochloric acid/acetone. Radiochemical purity was 99.0% and specific activity, 34.2 μCi/mg. Overall yield from [^{14}C]thiourea was 18%.

Key Words: Antibiotic, cephalosporin, Cefepime, 7-[α-(2-amino-[2-^{14}C]thiazol-4-yl)-α-(Z)-methoxyiminoacetamido]-3-(1-methylpyrrolidinio) methyl-3-cephem-4-carboxylate.

INTRODUCTION

7-[α-(2-Aminothiazol-4-yl)-α-(Z)-methoxyiminoacetamido]-3-(1-methylpyrrolidinio)methyl-3-cephem-4-carboxylate hydrochloride (Cefepime hydrochloride hydrate) is a broad-spectrum, injectable cephalosporin antibiotic prepared in these laboratories[1] and currently in clinical evaluation.

In order to thoroughly profile the absorption and metabolism of this compound, ^{14}C-labelled material was required. Preparation of a [^{14}C]-N-methyl compound, 7-[α-(2-aminothiazol-4-yl)-α-(Z)-methoxyiminoacetamido]-3-(1-[^{14}C]- methylpyrrolidinio)methyl-3-cephem-4-carboxylate sulfate, has

0362-4803/93/080759-07$08.50

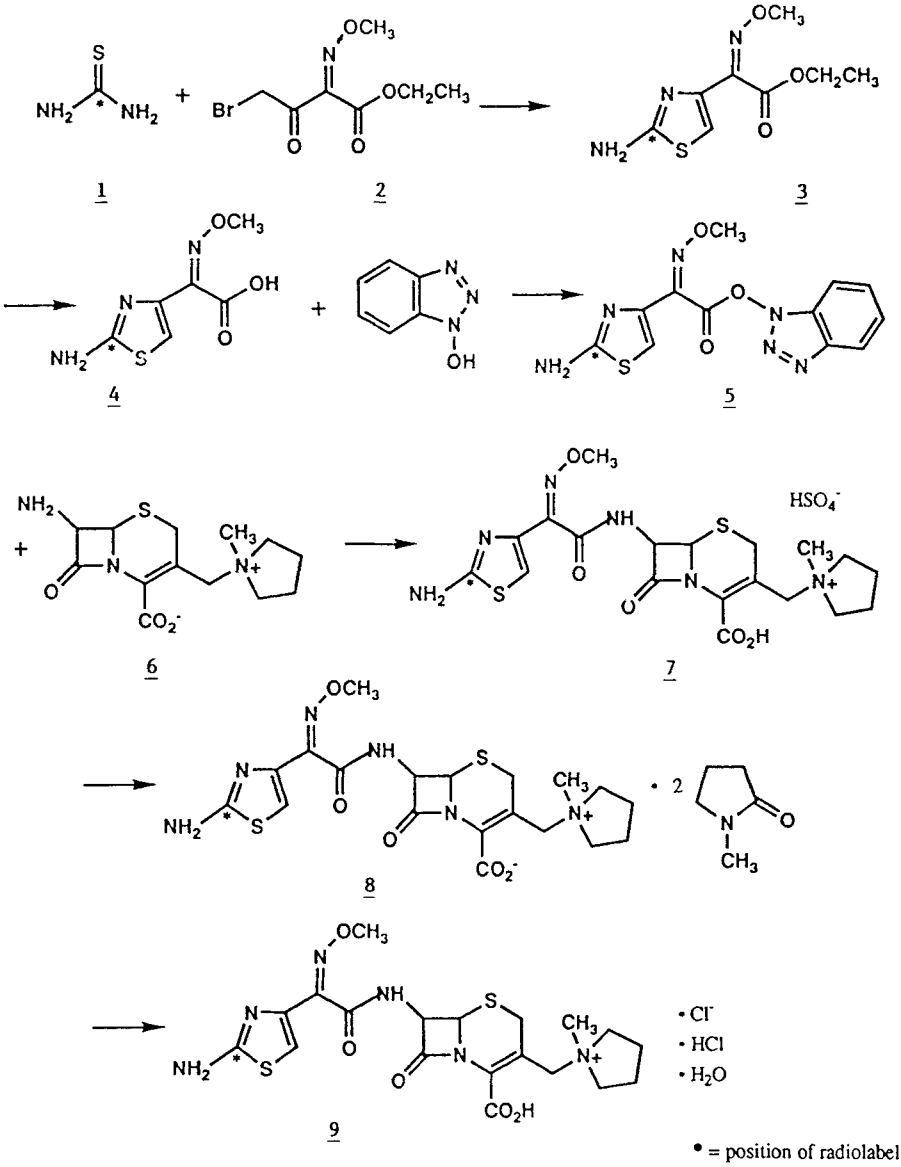
Received 9 March, 1993
Revised 28 March, 1993

previously been described by us.[2] To provide increased stability, the label was desired in the thiazole ring. The synthetic route is outlined in Scheme I.

DISCUSSION

Condensation of [^{14}C]thiourea (1) with ethyl 4-bromo-3-oxo-2-methoxyiminoacetate[4] (2) in aqueous ethanol at 20°C gave ethyl 2-(2-amino-[2-^{14}C]thiazol-4-yl)-2-methoxyiminoacetate[5] (3) exclusively as the Z-isomer. Employment of other conditions resulted in generation of some E-isomer.

Scheme I



Saponification gave a high yield of the corresponding amino acid 4. Treatment of 4 and 1-hydroxybenzotriazole with dicyclohexylcarbodiimide yielded the activated ester 5. This was not isolated but condensed in situ with 7-amino-3-(1-methylpyrrolidinio)methyl-3-cephem-4-carboxylate (6), obtained by titrating the corresponding hydriodide[6] to its isoelectric point. The product was purified by precipitation from aqueous solution as the slightly water-soluble sulfate salt 7. This removed numerous contaminants, including excess 6, 1-hydroxy-benzotriazole, and the Δ2-isomer of 7.

For most purposes the more water-soluble hydrochloride is preferred. This requires generation of the zwitterion, which is of limited stability, and removal of sulfate. These requirements were met by treatment of 7 with triethylamine in excess N-methyl-2-pyrrolidinone, a procedure developed by Kaplan and co-workers.[7] The product was obtained as the stable solid diadduct 8, while triethylamine sulfate remained in solution. Addition of hydrochloric acid/acetone to an aqueous solution of 8 caused separation of the crystalline product, [^{14}C]Cefepime hydrochloride hydrate 9. Radiochemical purity was 99.0% and specific activity, 34.2 μCi/mg. Overall yield from [^{14}C]thiourea was 15%.

Experimental procedures were optimized with non-radiolabelled materials prior to the use of radioisotopes.

EXPERIMENTAL

Nmr spectra were obtained on a Bruker 360 MHz or 400 MHz instrument, with tetramethylsilane as an external reference. All products gave nmr spectra consistent with structure. Radioactivity was measured by a Beckmann LS9000 liquid scintillation spectrometer. Radiochemical purities were determined by HPLC.

High Performance Liquid Chromatography (HPLC)

Instrumentation: Waters model 600 E solvent delivery system, WISP model 712 autosampler, model 484 UV detector and model 745B data processor. Method: Column, Waters 3.9 x 150 mm stainless steel; stationary phase, Waters C18 4μ (Nova-Pak); mobile phase: System A, 60:40 acetonitrile:water; System B, 0.01 M aqueous sodium 1-pentanesulfonate: acetonitrile 94:6, adjusted to pH 4; flow rate, 1.0 ml/minute isocratic; detector 254 nm.

Thin Layer Chromatography (TLC)

Plates, 250 μ silica gel (Analtech); mobile phase: System A, ether; System B, methanol:dichloromethane 40:60, visualization, 254 nm UV.

Ethyl 2-(2-amino-4-[2-^{14}C]thiazolyl)-2-(Z)-methoxyiminoacetate (3)

A 50 ml flask was charged with thiourea (0.237 g) and [^{14}C]thiourea (0.138 g) of sp act = 55 mCi/mmole (total, 0.375 g (4.93 mmoles), sp act = 20.3 mCi/mmole). The thiourea was rinsed in with water (2.8 ml) and 95% ethanol (1.39 ml) was added. The mixture was stirred magnetically and after a few minutes a slightly cloudy solution was obtained.

The reaction mixture was placed under a static nitrogen atmosphere and a solution of ethyl 4-bromo-3-oxo-2-methoxyiminoacetate[4] (2) (1.26 g, 4.98 mmoles) in 95% ethanol (1.26) ml) was added dropwise, cooling the reaction mixture as necessary to keep the temperature 18-20°. The solution was washed in with a little 95% ethanol and the reaction mixture was stirred at ambient temperature for 1 hour. An oil which initially separated had mostly dissolved by 1 hour.

A pH probe was introduced and saturated sodium bicarbonate solution was added to pH 6.2. There was some foaming and a solid separated. The solid was filtered and washed with water (3 portions). The filter cake was dried under vacuum (room temperature, 1 mm, phosphorus pentoxide) to give 0.822 g (73% yield) of the title compound as a yellow solid. TLC (system A), Rf = 0.4. There was no E-isomer (Rf = 0.8). HPLC (system A) showed retention time = 1.4 minutes, purity 85 area %.

2-(2-Amino-4-[2-^{14}C]thiazolyl)-2-(Z)-methoxyiminoacetic acid (4)

To a magnetically stirred solution of ethyl 2-(2-amino-4-[2-^{14}C]thiazolyl)-2-(Z)-methoxyiminoacetate (3) (0.882 g, 3.60 mmoles) in methanol (7 ml) was added 1 N sodium hydroxide solution (7.2 ml, 7.2 mmoles). The mixture was stirred and refluxed. After a few minutes an amber solution was obtained.

After 4.5 hours the methanol was removed under reduced pressure at 40°. The aqueous solution was stirred at 2° and 5% hydrochloric acid solution (5.1 ml, 7.2 mmoles) was added dropwise; after a few minutes a solid separated. A pH probe was introduced and the pH (initially 1.5) was adjusted to 2.7 with sodium bicarbonate solution; the mixture was stirred in the cooling bath for 15 minutes and then filtered. The filter cake was washed with 3 portions each of pH 2.9 water, acetonitrile, and finally ether. The solid was air-dried and then dried in vacuum to give the title compound (0.440 g, 61% yield) as a tan powder. TLC (system B) showed a single zone, Rf = 0.4. HPLC (system A) showed retention time = 0.94 minute, purity 100 area %.

7-[α(2-Amino-[2-^{14}C]thiazol-4-yl)-α-(Z)-methoxyiminoacetamido]-3-(1-methylpyrrolidinio)methyl-3-cephem-4-carboxylate sulfate (7) ([^{14}C]-Cefepime sulfate)

A. 1-Hydroxybenzotriazole ester of 2-(2-amino-4-[2-^{14}C]thiazolyl)-2-(Z)-methoxyiminoacetic acid (5)

To a magnetically stirred solution, under nitrogen, of dicyclohexylcarbodiimide (0.454 g, 2.20 mmoles) in anhydrous tetrahydrofuran (THF) (5 ml) was added 1-hydroxybenzotriazole (0.297 g, 2.20 mmoles). Stirring for 15 minutes gave a clear solution.

To this stirred solution was added 2-(2-amino-4-[2-^{14}C]thiazolyl)-2-(Z)-methoxyiminoacetic acid (4) (0.440 g, 2.20 mmoles), washing in with anhydrous THF (2.7 ml). The suspension was stirred, under nitrogen, at ambient temperature for 1.5 hours. The suspension was then filtered and the filter cake (dicyclohexylurea) was washed (two 1 ml portions of THF). The combined filtrate and washings were stored in a sealed flask and used as soon as possible in step B.

B. 7-[α-(2-Amino-[2-^{14}C]thiazol-4-yl)-α-(Z)-methoxyiminoacetamido]-3-(1-methylpyrrolidinio)methyl-3-cephem-4-carboxylate sulfate (7)

A stirred suspension of 7-amino-3-(1-methylpyrrolidinio)methyl-3-cephem-4-carboxylate hydriodide[6] (6) (0.778 g, 1.83 mmoles) in water (8 ml) was cooled to 10°. Sodium hydroxide solution (2 N) was added dropwise just to solution (pH 6.0, pH probe). The active ester THF solution from part A (2.20 mmoles) was added in 5 portions, adjusting the pH to about 6.8 with 2 N sodium hydroxide solution after each addition. After the second addition the cooling bath was removed and after the final addition (rinsed in with THF (1 ml)) the temperature was 18°C. The reaction mixture was stirred at ambient temperature for 2 hours, adding 2 N sodium hydroxide solution as required to maintain the pH at about 6.5.

The reaction mixture was filtered and the solid was washed with water (2 ml). The combined filtrate and washing were extracted with methyl iso-butyl ketone (23 ml). The layers were separated and the organic layer was washed with water (1.9 ml). To the combined aqueous solutions was added 2 N sulfuric acid (4.58 ml, 4.58 mmoles). The pH of the solution was 1.45. The solution was seeded with non-radiolabelled Cefepime sulfate, scratched, and incubated at 5°C.

After 20 hours the solid was filtered and washed with 5°C 0.5 N sulfuric acid (two 2 ml portions) and then with acetone (three 2 ml portions). The solid was air-dried and then stirred for 1 hour suspended in acetone (12 ml). The solid was again filtered, washed with acetone (three portions), and air-dried. Drying in vacuum gave the title compound

as a light beige solid (0.603 g, 57% yield). HPLC (system B) showed a retention time of 5.2 minutes and a purity of 99.7 area %.

7-[α-(2-Amino-[2-^{14}C]thiazol-4-yl)-α-(Z)-methoxyiminoacetamido]-3-(1-methylpyrrolidinio)methyl-3-cephem-4-carboxylate dihydrochloride hydrate (9) ([^{14}C]Cefepime hydrochloride

A. 7-[α-(2-Amino-[2-^{14}C]thiazol-4-yl)-α-(Z)-methoxyiminoacetamido]-3-(1-methylpyrrolidinio)methyl-3-cephem-4-carboxylateN-methyl-2-pyrrolidinone adduct (8)

A suspension of 7-[α-(2-amino-[2-^{14}C]thiazol-4-yl)-α-(Z)-methoxyiminoacetamido]-3-cephem-4-carboxylate sulfate (7) (0.603 g, 1.04 mmoles) in N-methyl-2-pyrrolidinone (12.2 ml) was stirred magnetically at room temperature and triethylamine (0.32 ml, 2.29 mmoles) was added dropwise by syringe through a rubber septum. The reaction mixture was stirred for 30 minutes. The rubber septum was then removed, seed crystals of adduct were added, and the flask was sealed with a glass stopper. Stirring at ambient temperature was continued for 18 hours.

A rubber septum was reattached. The suspension was placed under a static nitrogen atmosphere and was stirred at 50°C for 1 hour. After stirring the reaction mixture for an additional 2 hours at ambient temperature, the solid was filtered and the filter cake was washed with N-methyl-2-pyrrolidinone (two 1 ml portions) and anhydrous ether (two 5 ml portions). Drying in vacuum gave the title compound (0.573 g, 81% yield) as an off-white powder. HPLC showed retention time and purity conforming to (7).

B. 7-[α-(2-Amino-[2-^{14}C]thiazol-4-yl)-α-(Z)-methoxyiminoacetamidol-3-(1-methylpyrrolidinio)methyl-3-cephem-4-carboxylatedihydrochloride hydrate (9)

To a magnetically stirred solution of 7-[α-(2-amino-[2-^{14}C]-thiazol-4-yl)-α-(Z)-methoxyiminoaceamido]-3-(1-methylpyrrolidinio)methyl-3-cephem-4-carboxylate N-methyl-2-pyrrolidinone adduct (8) (0.573 g, 0.884 mmole) in water (2.5 ml), contained in a 40 ml centrifuge tube, was added all at once a 1:9 solution of concentrated hydrochloric acid: acetone (2.32 ml, 2.72 mmoles).

The solution was stirred for a few minutes and then acetone (15.2 ml) was added. A solid separated. Stirring was continued for 20 minutes. The stirrer was removed and the mixture was incubated at 5°C for 30 minutes.

The mixture was centrifuged for 5 minutes and the mother liquor was pipetted off. The solid was washed with acetone (two 7.5 ml portions) in

a like manner. The wet residue was then freed of excess solvent in a stream of nitrogen and dried in vacuum. The title compound 9 (0.427 g, 89% yield) was obtained as an off-white solid. HPLC showed retention time identical to that of reference standard. Radiochemical purity was 99% and the specific activity, 34.2 μCi/mg. The overall yield from [^{14}C]thiourea was 15%.

ACKNOWLEDGEMENT

The authors are grateful to Mr. Murray A. Kaplan and Dr. Donald G. Walker for many fruitful discussions and helpful suggestions during the course of this work.

REFERENCES

1. "Program and Abstracts of the 23rd Interscience Conference on Antimicrobial Agents and Chemotherapy", American Society of Microbiology, Las Vegas, Nevada, October 24-26, 1983.

2. Swigor, J.E. and Pittman, K.A. - J. Labelled Comp. Radiopharm. 24:15 (1987).

3. Obtained from New England Nuclear, specific activity = 55 mCi/mmole.

4. Heymes, R. and Lutz, A. - U.S. Patent 4,152,432, Example 3A, step B.

5. Bucourt, R., Heymes, R., Lutz, A., Penasse, L., and Perronet, J. - Tetrahedron, 34, 2233 (1978).

6. Obtained from Bristol-Myers Squibb Industrial Division, Syracuse, New York.

7. Kaplan, M.A., Bogardus, J.B., and Lipper, R.A. - U.S. patent 4,680,389.