SYNTHESIS OF ^{14}C-LABELED 7α-METHOXYCEPHALOSPORIN DERIVATIVE (^{14}C-CEFOTETAN)

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SUMMARY

[Caboxamido-^{14}C]Cefotetan, 7β-[4-(carbamoyl carboxymethylene)-1,3-dithietan-2-yl][^{14}C]carboxamido-7α-methoxy-3-(1-methyl-1H-tetrazol-5-yl)thiomethyl-3-cephem-4-carboxylic acid (IX), a new cephamycin derivative, was synthesized from bromo[1-^{14}C]acetic acid for metabolic studies. The results of duplicate preparation of ^{14}C-Cefotetan are described. In the second synthesis, a new improved method was adopted. The overall radiochemical yields were 21.5 % and 26.7 %, at specific activity of 44.5 μCi/mg and 53.3 μCi/mg respectively.

Keywords: Carbon-14,[7β- ^{14}C]Carboxamido-cephamycin derivative, Cefotetan, Antibacterial agent, Dithietan, Isothiazole

INTRODUCTION

Cefotetan, 7β-[4-(carbamoyl carboxymethylene)-1,3-dithietan-2-yl]-carboxamido-7α-methoxy-3-(1-methyl-1H-tetrazol-5-yl)thiomethyl-3-cephem-4-carboxylic acid (I) synthesized in our laboratory, is a new cephamycin derivative having a broad spectrum, especially strong antibacterial activity against gram-negative organisms and against many resistant strains to the other cephalosporins.[1a,b,c] ^{14}C-Labeled Cefotetan was required in order to investigate the metabolic fate of Cefotetan.[2] We synthesized carboxamido-^{14}C-labeled Cefotetan from commercially available bromo[1-^{14}C]acetic acid by the reactions given in Scheme I.

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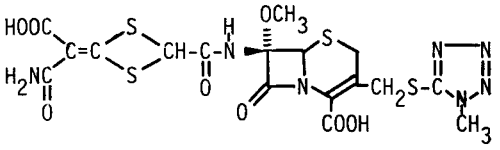
RESULTS AND DISCUSSION

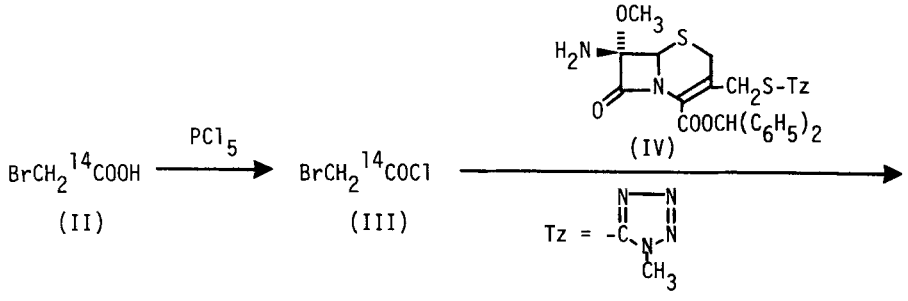
In the cold experiments, use of convenient methods for bromoacetamidation of diphenylmethyl 7β-amino-7α-methoxy-3-(1-methyl-1H-tetrazol-5-yl)thiomethyl-3-cephem-4-carboxylate(IV),[3] i.e. mixed anhydride method, active ester method, dicyclohexylcarbodiimide method, gave the bromoacetamide of IV in poor or insufficient yield. By dropwise addition of bromoacetyl chloride to IV in acid chloride method, the desired amide was obtained in good yield, but the reverse addition of IV to the acid chloride provided it in low yield.

Bromo[1-^{14}C]acetic acid (II) was treated with phosphorous pentachloride in dichloromethane to afford bromo[1-^{14}C]acetyl chloride (III). Reaction of IV with III in the presence of pyridine at low temperature gave diphenylmethyl-7β-bromo[carbonyl-^{14}C]acetamido-7α-methoxy-3-(1-methyl-1H-tetrazol-5-yl)-thiomethyl-3-cephem-4-carboxylate (V) in good yield based on II. Diphenylmethyl group of V was removed by treatment with trifluoroacetic acid and anisole in dichloromethane under ice-cooling to obtain 7β-bromo[carbonyl-^{14}C]acetamido-7α-methoxy-3-(1-methyl-1H-tetrazol-5-yl)thiomethyl-3-cephem-4-carboxylic acid (VI) in 89.5% yield. Addition of trisodium 4-carboxy-3-hydroxy-5-mercaptoisothiazole (VII)[1a] to VI diluted with cold VI afforded 7β-(4-carboxy-3-hydroxyisothiazol-5-yl)thio[carbonyl-^{14}C]acetamido-7α-methoxy-3-(1-methyl-1H-tetrazol-5-yl)thiomethyl-3-cephem-4-carboxylic acid (VIII), which was converted to 7β-[4-(carbamoyl craboxymethylene)-1,3-dithietan-2-yl]-[^{14}C]carboxamido-7α-methoxy-3-(1-methyl-1H-tetrazol-5-yl)thiomethyl-3-cehpem-4-carboxylic acid (^{14}C-Cefotetan, IX) by adjusting to pH 7.6 - 8.2 and standing overnight without isolation of VIII. By usual work up and recrystallization, pure IX was given in 42.1% yield from VI.

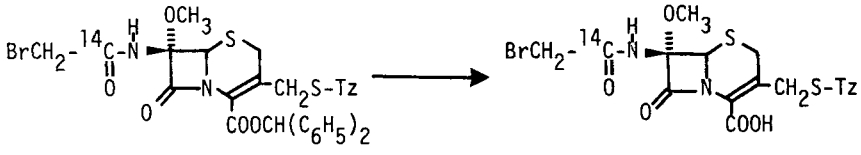
The synthesis of ^{14}C-labeled Cefotetan was done twice. In the second preparation minor modifications were adopted in aspects of recent improvement of method for synthesis of Cefotetan and easier handling of labeled compounds (see experimental part). Overall radiochemical yield, chemical yield and batch analysis data in two experiments were summarized in Table I.



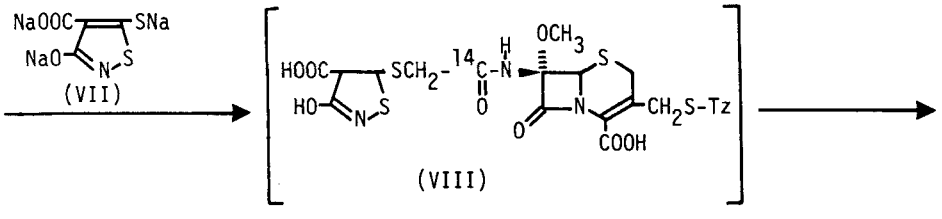
(I) Cefotetan



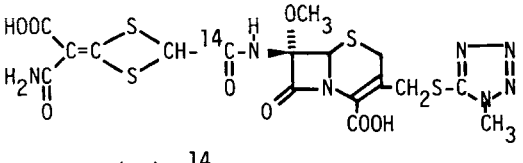
(II) (III) (IV)



(V) (VI)



(VII) (VIII)



(IX) ^{14}C-Cefotetan

Scheme I

Table I. Overall radiochemical yield, chemical yield and batch analysis data of ^{14}C-Cefotetan

	Run 1	Run 2
Radiochemical yield	21.5 %	26.7 %
Chemical yield	25.7 %	28.8 %
Specific activity	44.5 μCi/mg	53.3 μCi/mg
Radiochemical purity*	96.3 %	97 %
Chemical purity**	98.1 %	99 %

* determined by radio-TLC and radio-HPLC. Solvent system, Rf value and retention time were described experimental section.

** determined by HPLC with external standard method.

EXPERIMENTAL

Radioactivity determination was carried out with a Tri-Carb Liquid Scintillation Spectrometer 3380 or 3255 (Packerd). Thin layer chromatography (TLC) was scanned with a Radio-TLC Scanner LB 2723 (Berthold). High performance liquid chromatography (HPLC) was performed with a model 440 (Waters) and a HPLC Radioactivity Monitor LB 503 (Berthold). Determination of pH was carried out with a pH meter HM-20E (Toa). For TLC precoated silica gel plates (60 F_{254}, Merck), for column chromatography silica gel (Wakogel C-200) and prepacked silica gel (Lobar® size B, Merck) were used.

Bromo[1-^{14}C]acetyl chloride (III)-----To the ampoule containning 20 mCi (53.2 mg) of bromo[1-^{14}C]acetic acid (II, Batch 40, Amersham International Limited, Amersham, England) were added phosphorous pentachloride (96 mg) and dry dichloromethane (1 ml). The mixture was stirred for 1 hr at room temperature protecting from moisture with anhydrous calcium chloride. Thus obtained solution of III was used without further purification.

Diphenylmethyl 7β-bromo[carbonyl-^{14}C]acetamido-7α-methoxy-3-(1-methyl-1H-tetrazol-5-yl)thiomethyl-3-cephem-4-carboxylate (V)-----To a cooled mixture of diphenylmethyl 7β-amino-7α-methoxy-3-(1-methyl-1H-tetrazol-5-yl)thiomethyl-3-cephem-4-carboxylate (197.5 mg, IV),[3] pyridine (340.5 μl) and dry dichloromethane (1 ml) was added dropwise the above solution of III through a stainless steel tube at -60° with stirring. After 20 min at -30°, to the reaction mixture were added saturated aq. $NaHCO_3$ (10 ml) and ethyl acetate (40 ml). After vigorous shaking, the organic layer was separated and washed with saturated aq. $NaHCO_3$ (10 mlx2), water (10 ml), 1N hydrochloric acid (10 ml), water (10 ml), saturated aq. $NaHCO_3$ (10 ml) and saturated aq. NaCl (10 ml) successively. The organic layer was dried ($MgSO_4$) and the solvent was evaporated in vacuo to give crude V, which was purified by silica gel column chromatography. To the top of column (silica gel, 6 g/benzene) the crude V was charged with benzene (2 ml) and eluted with benzene/ethyl acetate (9:1 v/v). The fractions from 120 ml to 300 ml, which showed single spot of V on TLC (Rf=0.5, benzene/ethyl acetate, 2:1 v/v), were concentrated under reduced pressure to obtain pure V as an oil, followed by crystallization from benzene (1 ml) standing overnight at room temperature. Resulting crystals were collected by filtration and dried over phosphorous pentoxide in vacuo. Yield : 185.5 mg.

In the second experiment, III prepared also from bromo[1-^{14}C]acetic acid (20 mCi = 55.1 mg, Batch 46, Amersham International Limited, England) was added through a teflon tube instead of a stainless steel tube. The crude V was purified on Lobar® column chromatography eluted with n-hexane/ethyl acetate (1:1 v/v, 20 ml/min) with monitor at 280 nm. Yield : 217 mg (Radiochemical yield : 75.3% based on II).

7β-Bromo[carbonyl-^{14}C]acetamido-7α-methoxy-3-(1-methyl-1H-tetrazol-5-yl)thiomethyl-3-cephem-4-carboxylic acid (VI)-----a) With trifluoroacetic acid ; To a solution of V (185.5 mg), anisole (0.6 ml) and

dichloromethane (3 ml) cooled in an ice bath was added dropwise trifluoroaceticacid with stirring below 10°. After 2 hr at the same temperature the reaction mixture was evaporated in vacuo, and a waxy residue was triturated with ether (6 ml). The precipitates were collected by filtration, washed with ether (6 mlx2), and dried over phosphorous pentoxide in vacuo overnight. A white powder of VI was obtained. Yield : 110.1 mg.

b) With dry hydrogen chloride; The solution of V (217 mg) diluted with cold V (109 mg) in dichloromethane (4 ml) was stirred under dry HCl atomosphare at -33° - -30° (bath temp.) for 3hr. After standing overnight at -32°, the solvent and excess HCl were removed under reduced pressure below -50°. The residue was completely dried over phosphorous pentoxide in vacuo to give white solid (245 mg). This solid was a mixture of VI and diphenylmethyl chloride.

7β-[4-(Carbamoyl Carboxymethylene)-1,3-dithietan-2-yl][^{14}C]carboxamido-7α-methoxy-3-(1-methyl-1H-tetrazol-5-yl)thiomethyl-3-cephem-4-carboxylic acid, ^{14}C-Cefotetan (IX)-----a) To the solution of diluted VI (110.1 mg with cold VI=81.4 mg) in methanol (2.5 ml), were added water (5 ml) and 1N $NaHCO_3$ (0.7 ml) below 10°. To the resulting mixture (pH 8.2), were added dropwise alternately a solution of trisodium 4-carboxy-3-hydroxy-5-mercaptoisothiazole [1a] (130 mg/1.3 ml) and 1N HCl at pH 7.0-9.0 during 1 hr with stirring below 10°. The reaction mixture was maintained at pH 7.6-8.2 for 3 hr by adding HCl at room temperature. After standing overnight, the reaction mixture was filtered by suction using Millipore® filter, and the funnel was washed with water (5 ml). The combined filtrates (ca. 16 ml) were saturated with NaCl, mixed with sec-butanol/ethyl acetate (1:3 v/v, 16 ml), and adjusted to pH 1 with 10% HCl under stir. After shaking well, the organic layer was separated and the aqueous layer was extracted with the same solvent (16 ml) once more. The combined organic layers were washed with saturated aq. NaCl (7 mlx2) and extracted with 0.1N $NaHCO_3$ (5 ml, 2.5 ml, 2.5 ml). The

combined water layers were washed with ether and adjusted to pH 1 with 10% HCl. The resulting precipitates were collected by filtration,washed with water (2 ml) and dried over phosphrous pentoxide in vacuo to give crude ^{14}C-Cefotetan (IX) as a white powder. Yield : 141.9 mg. The crude IX (141.9 mg) was dissolved in methanol/chloroform (4:1 v/v, 0.7 ml) and stirred at room temperature until crystallization began. After standing for 2 hr, ethanol(0.3 ml) was added and the crystals were collected by filtration, washed with ethanol (0.2 mlx5), and dried over phosphorous pentoxide in vacuo to give pure ^{14}C-Cefotetan (IX). Yield : 96.8 mg (42.1% from VI). TLC : Rf value of ^{14}C-Cefotetan = 0.72, ethyl acetate/acetic acid/water (10:7:3, v/v/v). HPLC : Retention time of IX = 3.4 min, on LiChrosorb® RP-18 (Merck), 4ϕ x 150 mm, 0.1M NaH_2PO_4/methanol (78:22 v/v).

b) To the mixture (245 mg) of VI and diphenylmethyl chloride described above, was added 0.2N $NaHCO_3$ (5 ml). After stirring for 10 min, the resulting mixture was washed with ether (2 mlx2) and the aqueous layer was adjusted to pH 6.2 - 6.4 by passing of CO_2 gas. Tripotassium 4-carboxy-3-hydroxy-5-mercaptoisothiazole[4] in water (137 mg/0.5 ml) was added dropwise to this solution at pH 7.7 - 8.9 under ice-cooling in a period of 10 min. Then passing of CO_2 gas was stopped and the reaction mixture was adjusted to pH 8.5 adding 10% Na_2CO_3. The reaction mixture was maintained at pH 8.5 $\pm$ 0.1 for 2 hr at 0°, then was adjusted to pH 8.0 with CO_2. The reaction vessel was stoppered tightly and allowed to stand at 0° for 65 hr. The reaction mixture was saturated with NaCl (ca. 2.4 g), mixed with methyl ethyl ketone (MEK) and adjusted to pH 1.7 with 10 % HCl at 0° with stirring. The organic layer was separated and the aqueous layer was extracted with MEK (2 mlx2). The combined organic layers were evaporated in vacuo to give crude IX, which was purified by Lobar® column chromatography. A Lobar® column connected in series with precolumn (silica gel, 3g) was washed with a mixture (1000 ml) of chloroform/methanol/ N-HCl-ethanol (100:9:1 v/v/v,), chloroform/methanol (10:1 v/v, 300 ml) and dimethyl formamide (DMF, 0.5 ml). The crude IX dissolved in

DMF (1 ml) and the washings of the vessel with DMF (0.5 mlx2) were charged to the precolumn and eluted with chloroform /methanol (10:1 v/v, 24 min, followed by chloroform/ methanol, 10:2 v/v, flow rate = 10 ml/min) monitoring at 280 nm. The eluates from 24 min to 40 min were collected and evaporated under reduced pressure. The residue was crystallized from methanol to obtain pure IX. Yield: 37.7% from V. TLC : a) Rf value of IX = 0.43, ethyl acetate/acetic acid/water (4:2:1 v/v/v). b) Rf value of IX = 0.26, chloroform/methanol/formic acid (80:20:2 v/v/v). HPLC : Retention time of IX = 3.2 min, on LiChrosorb® RP-18 (Merck), 4 ϕ x 150 mm, 0.05M KH_2PO_4/methanol (85:15 v/v).

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