# SYNTHESIS OF $^{14}$C-LABELLED LATAMOXEF (6059-S), A POTENT ANTIBACTERIAL

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## SUMMARY

Latamoxef (I) was labelled with $^{14}$C for metabolic and pharmacological studies.

The carbon-14 label was introduced into the C-1 position and the C-2 position of the amido side chain to prepare (II) and (III) in overall yields of 10.6 and 13.5% based on barium carbonate-$^{14}$C, respectively.

Key Words: Latamoxef, Moxalactam, 6059-S, 1-Oxacephem derivative, Antibacterial agent, $^{14}$C

## INTRODUCTION

7β-[2-Carboxy-2-(4-hydroxyphenyl)acetylamino]-7α-methoxy-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]-1-oxa-1-dethia-3-cephem-4-carboxylic acid (1) [I, Latamoxef, Moxalactam, 6059-S (disodium salt)] is a novel third-generation β-lactam antibiotic exhibiting an expanded gram-negative spectrum and an excellent β-lactam stability (2). Latamoxef, prepared and developed in our laboratories, is the first β-lactam antibiotic having an unnatural 1-oxacephem nucleus which has been marketed. In relation with preclinical studies of latamoxef, $^{14}$C-labelled compound (II) was prepared first for metabolic and pharmacological studies (3). During these studies, (II) was found to lose $CO_2$-$^{14}$C on drastic acid hydrolysis of carcasses of experimental animals and,

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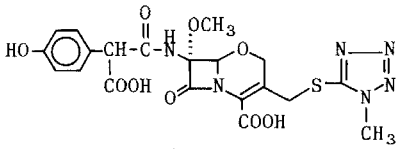
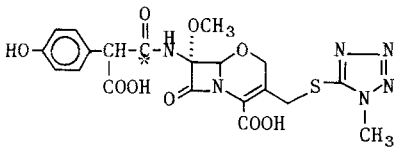


(I)



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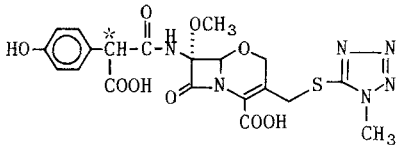
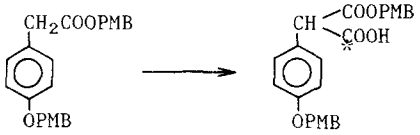


(III)

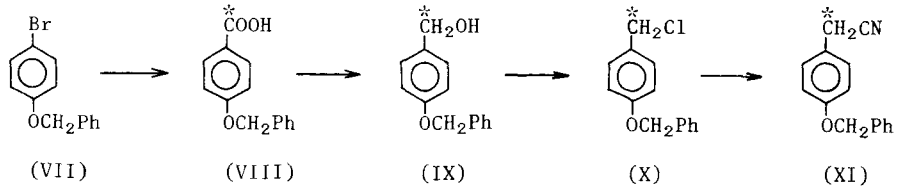
therefore, a more stable compound (III) became necessary. This note describes the synthesis of (II) and (III).

Half ester (V) used for synthesis of the $^{14}C$-labelled compound (II) was prepared easily by deprotonation of (IV) (1) with lithium diisopropylamide and subsequent carboxylation with carbon dioxide-$^{14}C$ generated from barium carbonate-$^{14}C$. On the other hand, half ester (VI) needed for the synthesis of the $^{14}C$-labelled compound (III) was obtained by multi-step synthesis as described in the following. The Grignard reagent prepared from 4-benzyloxy-1-bromobenzene (VII) (4) was carboxylated with carbon dioxide-$^{14}C$ to produce 4-benzyloxybenzoic acid (carboxy-$^{14}C$) (VIII). This acid was converted into 2-(4-benzyloxyphenyl)acetonitrile-2-$^{14}C$ (XI) on lithium aluminum hydride reduction to give alcohol (IX), chlorination to give (X) and subsequent treatment with potassium cyanide. Hydrolysis of (XI) and catalytic hydrogenation of the resulting (XII) afforded 2-(4-hydroxyphenyl)acetic acid-2-$^{14}C$ (XIII) as pure crystals, mp 150-152°, 64 mCi in 64% radiochemical yield based on barium carbonate-$^{14}C$. Alkylation of (XIII) with p-methoxybenzyl chloride gave 2-[4-(4-methoxybenzyloxy)phenyl]acetic acid-2-$^{14}C$ 4-methoxybenzyl ester (XIV), which was carboxylated on successive treatments with lithium diisopropylamide and carbon dioxide to give the

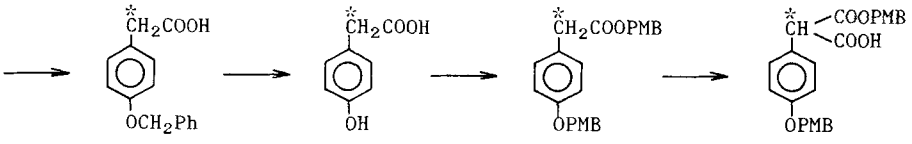



(IV) (V)

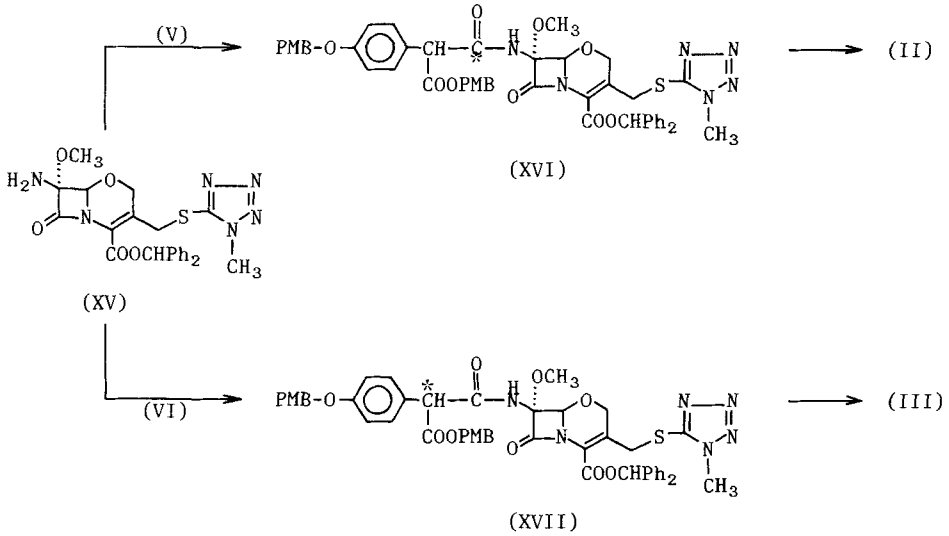
PMB = $CH_2$–C$_6$H$_4$–$OCH_3$




(VII) (VIII) (IX) (X) (XI)




(XII) (XIII) (XIV) (VI)



half ester (VI).

Coupling of diphenylmethyl 7β-amino-7α-methoxy-3-[1-methyl-1H-tetrazol-5-yl)thiomethyl]-1-oxa-1-dethia-3-cephem-H-carboxylate (XV) (1) with acid chlorides prepared from (V) and (VI) in the presence of pyridine yielded amides (XVI) and (XVII), respectively, which after chromatographic purification, were deprotected by treatment with trifluoroacetic acid in anisole to give the desired labelled compounds (II) and (III) in overall radiochemical yields of 13.2 and 13.5% based on barium carbonate-$^{14}C$, respectively.

## EXPERIMENTAL

Radioactivity determination was carried out with Aloka liquid Scintillation Spectrometer 672. Radiochemical purity of every labelled compound was measured by t.l.c.-autoradiogram and liquid scintillation counting.

2-(4-Methoxybenzyloxycarbonyl)-2-[4-(4-methoxybenzyloxy)-phenyl]acetic acid-1-$^{14}C$ (V) -- To a solution of diisopropylamine (0.7 ml, 4.85 mmol) in anhydrous tetrahydrofuran (25 ml) placed in a vacuum manifold Grignard apparatus was added dropwise a 15% solution of n-butyllithium in n-hexane (2.5 ml, 5 mmol) with stirring at -30° under a nitrogen atmosphere and the stirring was continued for 30 min at the same temperature. Then, a solution of 4-methoxybenzyl 2-[4-(4-methoxybenzyloxy)phenyl]-acetate (IV) (1.96 g, 5 mmol) in anhydrous tetrahydrofuran (16 ml) was added to the resulting solution over a period of 50 min at -60°. The mixture was stirred at the same temperature for 10 min and at -10° for 30 min. Carbon dioxide-$^{14}C$, derived from barium carbonate-$^{14}C$ (100 mCi, 989 mg, 5 mmol) and 60% perchloric acid (8 ml), was introduced into the above reaction mixture cooled in a liquid nitrogen bath and the stirring was continued at -60° for 30 min. Unreacted carbon dioxide was collected by

cooling in a liquid nitrogen bath and again introduced into the reaction mixture. The reaction mixture was acidified to pH 1 with 2N sulfuric acid, diluted with water and extracted with ether. The extract was washed with water, dried ($Na_2SO_4$) and evaporated to leave an oil, which was purified by silica gel column chromatography. The fractions containing the desired product were combined and evaporated to give crude (V) as a crystalline solid. Recrystallization from methylene chloride and peteroleum ether gave (V) as pure crystals (23.6 mCi, 514 mg, 1.18 mmol), mp 136.5-138.5°, in 23.6% radiochemical yield.

4-Benzyloxybenzoic acid (carboxy-$^{14}C$) (VIII) -- A Grignard reagent was prepared from 4-benzyloxy-1-bromobenzene (3.68 g, 14 mmol) and magnesium (312 mg, 13 mmol) in anhydrous tetrahydrofuran (30 ml) placed in a vacuum manifold Grignard apparatus under a nitrogen atmosphere. Carbon dioxide-$^{14}C$, derived from barium carbonate-$^{14}C$ (100 mCi, 1.97 g, 10 mmol) and 60% perchloric acid (8 ml), was introduced into the Grignard reagent cooled in a liquid nitrogen bath and the mixture was stirred at -56° for 20 min. Unreacted carbon dioxide-$^{14}C$ was collected by cooling in a liquid nitrogen bath and again introduced into the mixture, which was then stirred at -56° for 30 min. The reaction mixture was acidified to pH 1 with 2N sulfuric acid and extracted with ether. The ether layer was extracted with 10% sodium bicarbonate solution. The aqueous layer was acidified to pH 1 and extracted with ether-methanol (5:1). The extract was washed with a sodium chloride solution, dried ($Na_2SO_4$) and evaporated to leave a crystalline residue, which was recrystallized from methanol giving pure 4-benzyloxybenzoic acid (carboxy-$^{14}C$) (VIII) (78 mCi, 1.792 g, 7.8 mmol), mp 194-197°, in 78% radiochemical yield.

1-(4-Benzyloxyphenyl)methanol-1-$^{14}C$ (IX) -- To a solution of lithium aluminium hydride (890 mg, 26 mmol) in anhydrous

tetrahydrofuran (30 ml) was added dropwise over a 20 min period a solution of (VIII) (78 mCi, 1.792 g, 7.8 mmole) in anhydrous tetrahydrofuran (30 ml) with stirring under gentle reflux and the refluxing was continued for 2.0 hr. To the resulting mixture, ether (30 ml) and water (5 ml) were added with stirring and cooling in an ice bath. The precipitate which formed was filtered off. The filtrate was evaporated and the residue was dissolved in ether (50 ml). The solution was washed with water, dried ($Na_2SO_4$) and evaporated to dryness to give almost pure (IX) (77 mCi, 1.64 g, 7.7 mmol) as a crystalline solid, mp 84-86°, in 99% radiochemical yield.

1-(4-Benzyloxyphenyl)methyl-1-$^{14}$C chloride (X) -- To a solution of (IX) (77 mCi, 1.64 g, 7.7 mmol) in ether (50 ml) was added conc. hydrochloric acid (20 ml) with stirring. After stirring at room temperature for 1 hr, the reaction mixture was poured into ice water and extracted with ether. The extract was washed twice with water, dried ($Na_2SO_4$) and evaporated *in vacuo* to leave crystalline (X) (76.2 mCi, 1.76 g, 7.62 mmol) in 99% radiochemical yield. This material was used in the next step without further purification.

2-(4-Benzyloxyphenyl)acetonitrile-2-$^{14}$C (XI) -- To a solution of sodium cyanide (457 mg, 9.3 mmol) and sodium iodide (80 mg, 0.53 mmol) in dry dimethylformamide (4 ml) was added a solution of (X) (76.2 mCi, 1.76 g, 7.62 mmol) in dimethylformamide (8 ml) with stirring at 55°. After being stirred for 2 hr at the same temperature, the mixture was concentrated *in vacuo* at 55° to about 2 ml, poured into ice water, and extracted with benzene. The extract was washed with water, dried ($Na_2SO_4$) and evaporated to dryness to leave a crystalline residue. Recrystallization from benzene-hexane gave (XI) as pure crystals (71 mCi, 1.59 g, 7.1 mmol), mp 67-68°, in 93% radiochemical yield.

2-(4-Benzyloxyphenyl)acetic acid-2-$^{14}$C (XII) -- A solution of

(XI) (71 mCi, 1.59 g, 7.1 mmol) in 10% potassium hydroxide (1:1 water-ethanol, 30 ml) was heated under reflux for 6.5 hr. The reaction mixture was poured into ice water, washed twice with ether, acidified with 2N sulfuric acid, and extracted with ether. The extract was washed with water, dried ($Na_2SO_4$) and evaporated to dryness giving (XII) as crystals, mp 121-123° (69 mCi, 1.67 g, 6.9 mmol), in 97% radiochemical yield.

2-(4-Hydroxyphenyl)acetic acid-2-$^{14}C$ (XIII) -- To a solution of (XII) (69 mCi, 1.67 g, 6.9 mmol) in 95% ethanol (34 ml) was added 5% palladium-carbon (340 mg) and the mixture was hydrogenated at room temperature until 197 ml of hydrogen was absorbed. The catalyst was filtered off and the filtrate was evaporated to leave a residue, which was recrystallized from ethyl acetate:benzene (1:4) to give (XIII) as pure crystals (64 mCi, 973 mg, 6.4 mmol), mp 150-152°, in 92% radiochemical yield.

4-Methoxybenzyl 2-[(4-methoxybenzyloxy)phenyl]acetate-2-$^{14}C$ (XIV) -- A mixture of (XIII) (64 mCi, 973 mg, 6.4 mmol), anhydrous potassium carbonate (1920 mg, 19.2 mmol), potassium iodide (2.30 g, 15.4 mmol), 4-methoxybenzyl chloride (2.42 g, 15.4 mmol) and anhydrous acetone (9 ml) was heated under reflux with stirring for 8 hr. The reaction mixture was poured into ice water and extracted with ether. The extract was washed with water, dried ($Na_2SO_4$) and evaporated to leave a crystalline residue. Recrystallization from methylene chloride-ether-hexane gave (XIV) as colorless crystals (45 mCi, 1.78 g, 4.5 mmol), mp 90-92°, in 70% radiochemical yield.

2-(4-Methoxybenzyloxycarbonyl)-2-[4-(4-methoxybenzyloxy)phenyl]-acetic acid-2-$^{14}C$ (VI) -- To a solution of diisopropylamine (0.76 ml, 5.4 mmol) in tetrahydrofuran (21 ml) cooled at -8° was added under nitrogen a 1.74 M n-hexane solution of n-butyllithium (3.10 ml, 5.4 mmol). To the resulting solution stirred at -5° for 20 min was added at -55° a solution of (XIV) (45 mCi, 1.78 g, 4.5

mmol) in dry tetrahydrofuran (8.5 ml) over a period of 20 min. The mixture was stirred at -17° for 10 min, cooled to -55°, and then mixed with crushed dry ice (ca 5 g) to give a white, pasty mixture. After concentration in vacuo, the mixture was diluted with water, acidified to pH 2 with 2N hydrochloric acid and extracted with ethyl acetate. The organic extract was washed with water, dried ($Na_2SO_4$) and evaporated in vacuo to give a colorless crystalline solid, which was recrystallized from methylene chloride-n-hexane to give pure (VI) as crystals (36 mCi, 1.57 g, 3.6 mmol), mp 136.5-138.5°, in 80% radiochemical yield.

Diphenymethyl 7β-{2-(4-methoxybenzyloxycarbonyl)-2-[4-(4-methoxybenzyloxy)phenyl]acetylamino-1-$^{14}$C}-7α-methoxy-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]-1-oxa-1-dethia-3-cephem-4-carboxylate (XVI) -- To a stirred mixture of half ester (V) (10 mCi, 390 mg, 0.9 mmol), pyridine (225 mg, 2.8 mmol), and dry methylene chloride (5.0 ml) was added phosphorus oxychloride (137 mg, 0.9 mmol) with stirring at -23° and the stirring was continued at -7° for 20 min. The resulting mixture was added to a suspension of diphenylmethyl 7β-amino-7α-methoxy-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]-1-oxa-1-dethia-3-cephem-4-carboxylate (XV) (405 mg, 0.8 mmol) in pyridine (225 mg, 2.8 mmol) and dry methylene chloride (8 ml) at -23°. After being stirred at -7° for 25 min, the reaction mixture was poured into a stirred mixture of 2N sulfuric acid (10 ml), ice water (30 g) and ethyl acetate. The organic layer was washed with a cold 3% sodium bicarbonate solution, dried ($Na_2SO_4$) and evaporated in vacuo below 40° to leave an oil (590 mg). The oily product was chromatographed (Merck Lobar column-size B; benzene-ethyl acetate 2:1, 6 ml/min). Fractions containing (XVI) were collected and the solvent was removed in vacuo to give pure (XVI) (5.6 mCi, 459 mg, 0.5 mmol) in 56% radiochemical yield.

Diphenymethyl 7β-{2-(4-methoxybenzyloxycarbonyl)-2-[4-(4-methoxybenzyloxy)phenyl]acetylamino-2-$^{14}C$}-7α-methoxy-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]-1-oxa-1-dethia-3-cephem-4-carboxylate (XVII) -- In the same way as described above, half ester (VI) (13.2 mCi, 578 mg, 1.32 mmol) was reacted with (XV) (642 mg, 1.3 mmol) to afford pure (XVII) (8.2 mCi, 760 mg, 0.82 mmol) in 62% radiochemical yield.

7β-[2-Carboxy-2-(4-hydroxyphenyl)acetylamino-1-$^{14}C$]-7α-methoxy-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]-1-oxa-1-dethia-3-cephem-4-carboxylic acid (II) -- To a solution of (XVI) (5.6 mCi, 459 mg, 0.5 mmol) in anhydrous anisole (3 ml) was added aluminum trichloride (490 mg, 3.6 mmol) with stirring at -25° and the stirring was continued at the same temperature for 50 min. The mixture was diluted with ethyl acetate chilled at -5° and poured into a stirred mixture of 2N-sulfuric acid, ice water and ethyl acetate. The organic layer was separated and extracted with a 3% sodium bicarbonate solution. The aqueous layer was acidified to pH 1 with 2N hydrochloric acid and extracted with ethyl acetate. The extract was washed with a sodium chloride solution, dried ($Na_2SO_4$) and evaporated in vacuo below 40° to leave a residue (276 mg). The residue dissolved in dry methyl ethyl ketone (0.3 ml) was gradually diluted with dry methylene chloride (15 ml) to form a precipitate, which was washed twice with methylene chloride (15 ml) by decantation and finally filtered off to give a white amorphous powder. Drying in vacuo at room temperature gave almost pure (II) (4.5 mCi, 0.37 mmol) in 80% radiochemical yield. This material was physicochemically identical with an authentic sample of the unlabelled compound.

7β-[2-Carboxy-2-(4-hydroxyphenyl)acetylamino-2-$^{14}C$]-7α-methoxy-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]-1-oxa-1-dethia-3-cephem-4-carboxylic acid (III) -- In the same way as described above, (XVII) (10.9 mCi, 1.01 g, 1.09 mmol) was deprotected to

give (III) (9.3 mCi, 858 mg, 0.93 mmol) in 85% radiochemical yield.

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