Synthesis of 1-[^{14}C]Methyl-1H-tetrazole-5-thiol ([^{14}C]NMTT) and [NMTT-[^{14}C]]Latamoxef

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SUMMARY

The title compounds were prepared for metabolic studies, with the ^{14}C labelling being made at methyl of the 1-methyl-1H-tetrazol-5-ylthio (NMTT) group in overall radiochemical yields of 26% and 22% based on barium [^{14}C]carbonate, respectively.

Key words: Latamoxef, NMTT, ^{14}C, Antibacterial agent, 1-Methyl-tetrazole-5-thiol.

INTRODUCTION

7β-[2-Carboxy-2-(4-hydroxyphenyl)acetamido]-7α-methoxy-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]-1-oxa-1-dethia-3-cephem-4-carboxylic acid (latamoxef) (1) has been widely used therapeutically as a representative third-generation β-lactam antibiotic exhibiting an expanded gram-negative spectrum and excellent β-lactam stability.[1] Previously, we have reported synthesis of two [^{14}C]latamoxef derivatives labelled at the 2-carboxy group and the amide carbonyl group in the 7β side chain, respectively.[2] Recently, it has been reported that cephem antibiotics having a (1-methyl-1H-tetrazol-5-yl)thiomethyl group at C–3 are associated with hypoprothrombinemia in rats[3] which may arise from inhibition of vitamin-K epoxide reductase activity by the metabolite, 1-methyl-1H-tetrazole-5-thiol (NMTT) (2).[4] In order to conduct in vivo metabolic studies of the thiol 2 liberated from the parent antibiotic 1, sodium 1-[^{14}C]methyl-1H-tetrazole-5-thiolate ([^{14}C]NMTT sodium salt) (3) and [NMTT-^{14}C]latamoxef disodium salt (4) were required.

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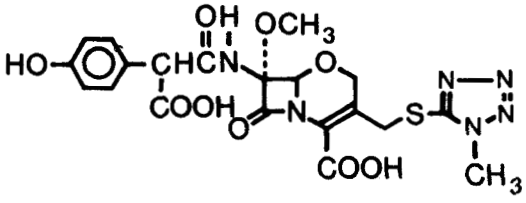

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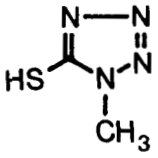

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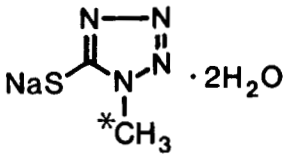

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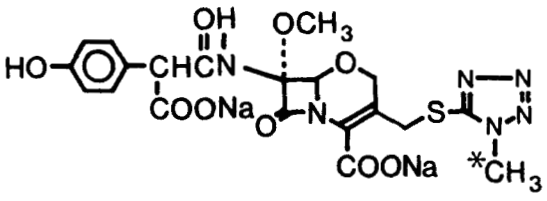

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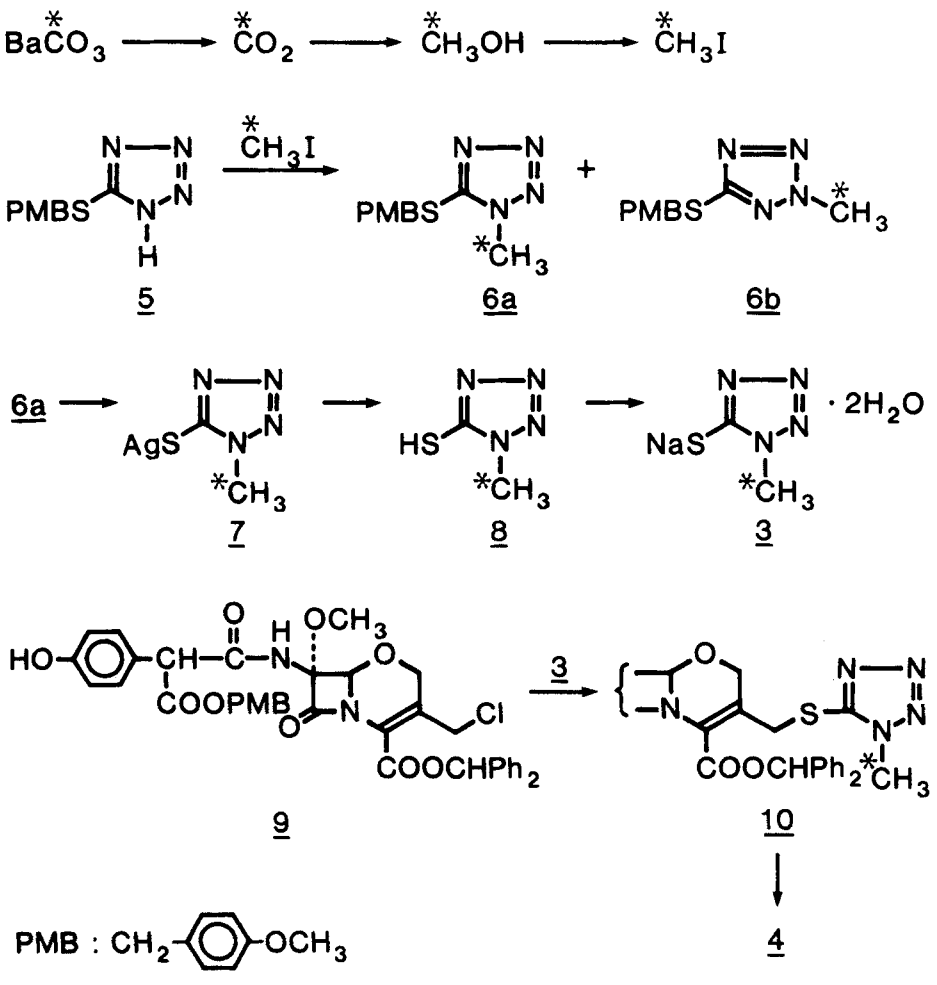
The ^{14}C labelling step in synthesis of [^{14}C]NMTT sodium salt (3) consists of methylation of PMB thio ether 5 with [^{14}C]methyl iodide prepared by the known method[5,6] from barium [^{14}C]carbonate via [^{14}C]methanol, giving [^{14}C]NMTT PMB thio ether 6a and its isomer 6b. Deprotection of 6a with silver perchlorate gave [^{14}C]-NMTT (8). The thiol 8 was purified in the form of sodium salt 3 by recrystallization after treatment with sodium methoxide. A pure material of [^{14}C]NMTT sodium salt (3) (56.3 μCi/mg, 9.81 mCi/mmol) was obtained in 26% overall yield based on barium [^{14}C]carbonate. The conventional method[7] for preparing NMTT by treatment of sodium azide with methyl isothiocyanate or methyl N-methyldithiocarbamate was not applied because of more tedious preparation of the N-[^{14}C]methyl reagents from barium [^{14}C]carbonate. Coupling of the thiolate 3 with 3-chloromethyl-1-oxa-cephem derivative 9 yielded [^{14}C]latamoxef diester 10 in 93% yield. Deprotection of 10 with aluminum trichloride in anisole gave [^{14}C]latamoxef which underwent neutralization with sodium bicarbonate followed by lyophilization to give [NMTT-^{14}C]latamoxef disodium salt (4) having a specific activity of 21.4 μCi/mg (12.1 mCi/mmol) in 94% yield from 10. An overall yield of 4 based on barium [^{14}C]-carbonate is 22%.



EXPERIMENTAL

[^{14}C]Methanol

According to a modification of the method of Cox et al.,[5] [^{14}C]carbon dioxide generated from barium [^{14}C]carbonate (154 mCi, 1.97 g, 10 mmol) was introduced into a suspension of lithium aluminum hydride (1.60 g, 42 mmol) in diethylene glycol diethyl ether (65 ml) and the mixture stirred for 1 hr at room temperature and for 2 hr at 75°C. Addition of tetrahydrofulfuryl alcohol (35 ml) followed by vacuum distillation (70 mmHg) at 110°C with bubbling a slow nitrogen stream gave [^{14}C]-methanol (146 mCi, 305 mg, 9.5 mmol) in 95% radiochemical yield.

[^{14}C]Methyl iodide

[^{14}C]Methyl iodide was prepared by the method of Ronzio et al.[6] A solution of

[^{14}C]methanol (146 mCi, 305 mg, 9.5 mmol) in 55% hydrogen iodide (40 ml) was heated at 40-90°C for 30 min. Distillation of the reaction mixture with passing a slow nitrogen stream and redistillation of the distillate in vacuum line gave [^{14}C]methyl iodide (146 mCi, 1.35 g, 9.5 mmol) in quantitative yield.

1-[^{14}C]Methyl-5-[4-methoxyphenyl)methylthio]-1H-tetrazole (6a)

To a stirred solution of 5-[(4-methoxyphenyl)methylthio]tetrazole (5) (2.33 g, 10.5 mmol) in dimethylformamide (10 ml) was added sodium hydride (250 mg, 10.4 mmol) at 0°C. After stirring for 30 min, a solution of [^{14}C]methyl iodide (146 mCi, 1.35 g, 9.5 mmol) in dimethylformamide (2.0 ml) was added dropwise. The stirring was continued for 45 min at 0°C and for 3 hr at room temperature. The mixture was diluted with ethyl acetate (30 ml), washed with water (10 ml), dried with sodium sulfate and evaporated in vacuo leaving a yellow viscous oil (2.30 g), which was chromatographed on silica gel (Merck Lobar column size B, elution with benzene-ethyl acetate) to give pure 6a (63.2 mCi, 969 mg, 4.1 mmol) as a yellow viscous oil in 43.3% radiochemical yield and the isomer 6b (1.19 g).

Silver 1-[^{14}C]Methyl-1H-tetrazole-5-thiolate (7)

To a stirred solution of the PMB thio ether 6a (63.2 mCi, 969 mg, 4.1 mmol) in methanol (14 ml) was added dropwise a solution of silver perchlorate (1.04 g, 5 mmol) in methanol (4 ml) at room temperature. After stirring for 2 hr, white crystals which precipitated were collected by filtration, washed with methanol (2 ml) and ether (10 ml) subsequently, and dried in vacuo to give crystalline 7 (63.2 mCi, 1.07 g, 4.1 mmol), m.p. above 250°C.

Sodium 1-[^{14}C]methyl-1H-tetrazole-5-thiolate dihydrate (3)

Gaseous hydrogen sulfide was introduced for 15 min through a capillary tube into a solution of the silver salt 7 (63.2 mCi, 1.07 g, 4.1 mmol) in methanol (20 ml) at a rate of 10 ml/min at 0°C. After being allowed to stand for 2 hr at room temperature, the reaction mixture was filtered to remove silver sulfide and the filtrate was evaporated in vacuo to dryness to leave crude [^{14}C]NMTT (8) as a yellow solid residue (470 mg). To the residue dissolved in methanol (10 ml) was added dropwise a solution of sodium methoxide (270 mg, 5 mmol) in methanol (2 ml) at room temperature. After being stirred for 2 hr, the mixture was evaporated in vacuo to dryness to leave a crystalline residue, which was recrystallized from isopropanol-

water (12 : 1) to give pure 3 (22.2 mCi, 394 mg, 2.26 mmol), m.p. 92-97°C, as white prisms in 35.1% radiochemical yield. A crude sample of 3 (purity: 95%) (18.3 mCi, 324 mg, 1.86 mmol) also was obtained from the mother liquor in 29% radiochemical yield.

Diphenylmethyl 7α-methoxy-7β-[2-[(4-methoxyphenyl)methoxycarbonyl]-2-(4-hydroxyphenyl)acetamido]-3-[(1-[^{14}C]methyl-1H-tetrazol-5-yl)thiomethyl]-1-oxa-1-dethia-3-cephem-4-carboxylate (10)

To a stirred solution of [^{14}C]NMTT sodium salt (3) (30 mCi, 522 mg, 3.0 mmol) and tetra-n-butylammonium bromide (70 mg, 0.22 mmol) in water (5.0 ml) was added dropwise a solution of diphenylmethyl 3-chloromethyl-7α-methoxy-7β-[2-[(4-methoxyphenyl)methoxycarbonyl]-2-(4-hydroxyphenyl)acetamido]-1-oxa-1-dethia-3-cephem-4-carboxylate (9)[8] (2.62 g, 3.6 mmol) in methylene chloride (20 ml) at room temperature. After stirring for 4.5 hr, the mixture was poured into ice-water (30 ml) and extracted with ethyl acetate (100 ml). The extract was washed with water (30 ml), dried with sodium sulfate and evaporated in vacuo below 35°C to leave a viscous oily residue (2.6 g). The residue was crystallized from ethyl acetate containing 0.1% pyridine, and the crystals were recrystallized from ethyl acetate to give 10 (28.2 mCi, 2.19 g, 2.71 mmol), m.p. 102-104°C, as white crystals of 98.2% radiochemical purity. The radiochemical yield is 90.4%.

7β-[2-Carboxy-2-(4-hydroxyphenyl)acetamido]-7α-methoxy-3-[(1-[^{14}C]methyl-1H-tetrazol-5-yl)thiomethyl]-1-oxa-1-dethia-3-cephem-4-carboxylic acid disodium salt ([^{14}C]latamoxef disodium salt) (4)

To a stirred mixture of ester 10 (15.1 mCi, 1.17 g, 1.45 mmol), methylene chloride (2.2 ml) and anisole (5.8 ml) was added dropwise a solution of aluminum trichloride (1.06 g, 7.95 mmol) in anisole (6.0 ml) at -20°C. After stirring for 1 hr, the mixture was diluted with cold ethyl acetate (25 ml) and poured into a stirred mixture of ice-water (10 ml), ethyl acetate (25 ml) and 1N-hydrochloric acid (15 ml) at 0°C. The organic layer was separated, mixed with water and adjusted to pH 6 with 10% sodium bicarbonate to extract acid substances. The aqueous layer was adjusted to pH 3.7, washed with ethyl acetate to remove impurities, acidified to pH 1.7 and reextracted with ethyl acetate (30 ml). The ethyl acetate extract was washed with sodium chloride solution, dried with sodium sulfate and evaporated in vacuo below

30°C to leave a colorless solid residue (712 mg) which underwent neutralization with 2% sodium bicarbonate to pH 6 followed by lyophilization to give 4 (14.3 mCi, 670 mg, 1.19 mmol) in 94% radiochemical yield (22% overall radiochemical yield based on barium [^{14}C]carbonate). The specific activity of 4 was 21.43 μCi/mg, and the radiochemical purity was 97.4%.

Radioactivity was determined with an Aloka liquid scintillation counter LSC-672. Radiochemical purity of every [^{14}C]-labelled compound was measured by T.L.C.-autoradiogram and liquid scintillation counting. [^{14}C]-Labelled compounds, 3, 4, 6a and 10, were identified with the corresponding authentic samples by comparison of T.L.C., m.p. and IR or NMR spectra. The following table shows the data on T.L.C. determination using Merck pre-coated silica gel plates:

Compd. No.	Plate No.	Solvent system*	Plate No.
3 & 8	5715	$AcOC_2H_5$-AcOH-H_2O (32 : 1 : 1)	0.55
4	5746	$AcOC_2H_5$-AcOH-H_2O (3 : 1 : 1)	0.39
6a & 6b	5715	C_6H_6-$AcOC_2H_5$ (7 : 1)	0.29 & 0.43
10	5715	C_6H_6-$AcOC_2H_5$ (1 : 1)	0.30

* Ac: CH_3CO

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8. The 3-chloromethyl derivative 9 was prepared from the corresponding 7β-phenylacetamide compound[9] by the side chain cleavage followed by acylation.

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