^{14}C-Labeling of a Novel Carbapenem Antibiotic SM-7338

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SUMMARY

(1R,5S,6S)-2-[(3S,5S)-5-Dimethylaminocarbonylpyrrolidin-3-ylthio]-6-[(1R)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylic acid trihydrate (SM-7338), a novel 1β-methyl carbapenem antibiotic, was labeled with carbon-14 at the C3 position of the carbapenem nucleus for use in metabolic studies. The synthesis was achieved according to the scheme illustrated in Fig. 3. Selective esterification of [2-^{14}C]malonic acid (2) with 4-nitrobenzyl bromide (3) gave its monoester (4). Condensation of the magnesium salt of 4 with the imidazolide derived from the azetidinone carboxylic acid (5) provided the β-keto ester (6), which was desililated with hydrochloric acid to give the alcohol (7). Treatment of 7 with carboxybenzenesulfonyl azide followed by decomposition of the resulting diazo intermediate (8) in the presence of a rhodium catalyst produced the bicyclic keto ester (9). Reaction of 9 with diphenyl chlorophosphate in the presence of N,N-diisopropylethylamine and subsequent displacement reaction of the vinyl phosphate (10) with mercaptopyrrolidine (11) gave bis-protected SM-7338 (12). Catalytic hydrogenolysis of 12 afforded [carbapenem-3-^{14}C]-SM-7338 (1). The overall yield of 1 was 5.8% from 2.

Key words: ^{14}C-labeling, carbapenem antibiotic, carbene insertion, bicyclic keto ester

INTRODUCTION

SM-7338[1] is a new synthetic 1β-methyl carbapenem antibiotic. Since the discovery of thienamycin[2], carbapenem antibiotics have attracted considerable interest because of their broad spectra of anti-

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bacterial activity as well as β-lactamase stability. Thienamycin and related naturally occurring carbapenems, however, suffer serious disadvantages that they are chemically and biologically unstable and readily metabolized by renal dehydropeptidase-I (DHP-I)[3]. Therefore, the design of a chemically and biologically stable carbapenem antibiotic has been the current focus of structural modification work. Recently, the syntheses of 1β-methyl substituted carbapenem carboxylic acids, a new class of synthetic β-lactam antibiotics, were reported[4], and noticeable improvements in the chemical and biological properties have been achieved while retaining their excellent activities. In our intensive efforts to develop antibiotics of this new type, SM-7338 has received a lot of attention. In the course of the evaluation, it was required to synthesize radioactive SM-7338 labeled with carbon-14 at the carbapenem nucleus for use in metabolic and pharmacokinetic studies. In this paper, we wish to report the synthesis of [carbapenem-3-^{14}C]SM-7338.

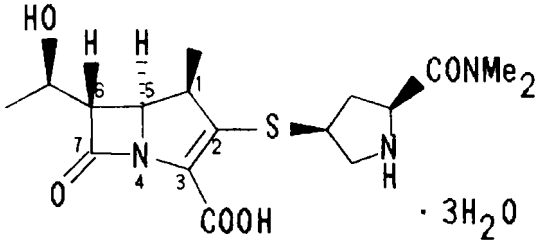

Fig. 1 Structure of SM-7338

RESULTS AND DISCUSSION

There were two problems to be addressed in the execution of the retrosynthesis: (1) the chemical lability of the intact carbapenem nucleus, which suggested that construction of the bicyclic system should be delayed as late as possible in the synthetic scheme; (2) stereochemistry in devising the synthetic sequence, which recommended that incoporation of the label should be achieved onto the unlabeled chiral building block possessing desired configuration. With these problems in mind, we formulated the retrosynthetic scheme illustrated in Fig. 2. Among several approaches so far devloped for the construction of the carbapenem nucleus[5,6,7], the

carbene insertion reaction to produce a bicyclic keto ester by the C3-N bond formation would provide a mild route to the bicyclic system. It was considered, therefore, preferable to utilize the chain extention of the acid (5') for the preparation of the bicyclic keto ester (9'). The acid (5') had the desired stereochemistry at the 4 chiral centers of the carbapenem key precursor, and the reaction between the radiolabeled monoester (4') and 5' would not influence the absolute configurations.

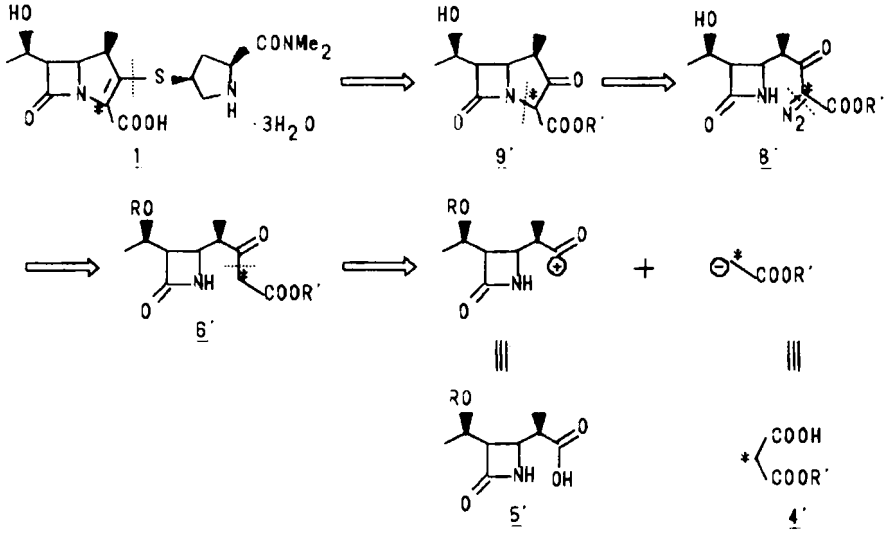

Fig. 2 Retrosynthetic analysis of [carbapenem-3-^{14}C]SM-7338

The synthetic strategy ultimately employed for our approach is shown in Fig. 3. The incorporation of the label was achieved by employing Masamune's method[8]. The monoester (4) was obtained by esterification of [2-^{14}C]malonic acid (2) with 4-nitrobenzyl bromide (3). It was found that the yield of 4 was markedly dependent on the molar ratio of 2 and 3. Reaction of 2 with an equimolar amount of 3 produced a considerable amount of diester. A reasonable yield (47%) was attained when 2 in a slight excess was treated with 3 in the presence of triethylamine. The monoester (4) was converted to the magnesium salt by treating with magnesium ethoxide and the resulting salt was condensed with the imidazolide derived from the azetidinone carboxylic acid (5) to give the keto ester (6) in 47% yield.

Treatment of 6 with methanolic hydrochloric acid effected the removal of the silyl protecting group to give the alcohol (7) in 86% yield. Diazotization of 7 with carboxybenzenesulfonyl azide in the presence of triethylamine provided the cyclization precursor (8) in 93% yield.

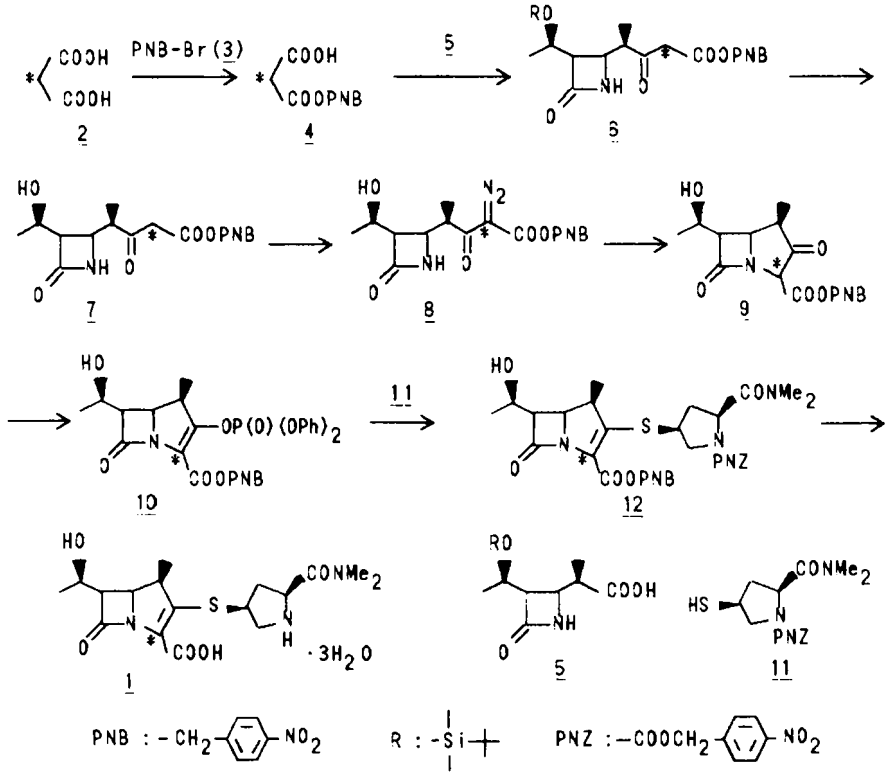

Fig. 3 Synthetic procedure of [carbapenem-3-^{14}C]SM-7338

Conversion of 8 to the bis-protected SM-7338 (12) was carried out in one pot. Thus, generation of the carbene intermediate occurred upon treatment of 8 with rhodium (II) octanoate catalyst to produce the highly strained bicyclic keto ester (9). Due to the instability of the product, 9 was **further reacted without purification.**

After concentration of the reaction mixture, 9 was treated with diphenyl chlorophosphate in the presence of N,N-diisopropylethylamine to provide the vinylphosphate (10). Displacement reaction of 10 with mercaptopyrrolidine (11) gave bis-protected SM-7338 (12) in 58% yield from 9. The final deprotection of 12 was accomplished by catalytic hydrogenolysis. Purifica-

tion of the crude product by column chromatography and subsequent lyophilization provided SM-7338 in anhydrous form. The resulting anhydrous SM-7338 was converted to the exceptionally stable trihydrate (1) in aqueous tetrahydrofuran. The yield of 1 was 56% from 12, and the specific activity was found to be 1.41 GBq/mmol.

EXPERIMENTAL

Radioactivity was measured by a TRI-CARB 460 liquid scintillation counter (Packard Instrument Co., USA) by using Permafluor I (Packard) as the counting medium. Radio-thin layer chromatography (RTLC) was carried out on a Silica Gel 60 F_{254} (Merck) unless otherwise mentioned, and the radioactivity on the plate was determined by a JTC-601 Radiochromanyzer (Aloka, Japan). Radio-high performance liquid chromatogrphy (RHPLC) was conducted on a LC-3A high performance liquid chromatograph (Shimadzu Co., Ltd., Japan) equipped with a SPD-2A UV detector (Shimadzu Co.) and RLC-551 Radioanalyzer (Aloka). An infrared spectrum (IR) was measured by a IR-810 grating infrared spectrophotometer (Jasco Co., Ltd., Japan), and the characteristic absorptions (ν_{max}) were reported in cm^{-1}. A proton nuclear magnetic resonance spectrum (NMR) was determined on a JNM FX-100 spectrometer (JEOL Ltd., Japan), and the chemical shifts (δ) were quoted in ppm downfield from tetramethylsilane as the internal standard. A mass spectrum was obtained on a Hitachi DF/GC/MS M-80B and DPS M-0101 (3 kV) spectrometer (Hitachi Ltd., Japan).

4-Nitrobenzyl [2-^{14}C]malonate (4)

To a stirred solution of the acid (2) (21.5 GBq, 1.61 g, 15.5 mmol) in anhydrous acetonitrile (15.0 ml) was added dropwise triethylamine (1.88 ml, 13.5 mmol) at room temperature under a nitrogen atmosphere. After the addition, 4-nitrobenzyl bromide (3) (2.92 g, 13.5 mmol) was added, and the mixture was heated at reflux for 3h. The mixture was cooled, acidified with 5% hydrochloric acid and extracted with ethyl acetate. The organic phase was extracted with 5% sodium hydrogen carbonate. After acidification, the aqueous phase was extracted with benzene. The extract was dried over anhydrous sodium sulfate and evaporated to give 4 (10.2 GBq, 47.4%) as a

pale yellow solid. Radiochemical purity 90% on RTLC (ethyl acetate/benzene/acetic acid=50/50/1 v/v/v, Rf=0.20). NMR: 3.56 (2H, s), 5.33 (2H, s), 7.51-8.30 (4H, m).

(3S,4R)-3-[(1R)-1-t-Butyldimethylsilyloxyethyl]-4-[(1R)-1-methyl-3-(4-nitrobenzyloxycarbonyl)-2-oxo[3-^{14}C]propyl]azetidin-2-one (6)

To a solution of the monoester (4) (10.2 GBq, 1.76 g, 7.34 mmol) in anhydrous tetrahydrofuran (5.6 ml) was added magnesium ethoxide (420 mg, 3.67 mmol), and the mixture was stirred at room temperature for 2 h. The mixture was filtered, and the filtrate was added dropwise to anhydrous diisopropyl ether (34.0 ml). The mixture was stirred for 2 h and the resulting precipitate was filtered, washed with diisopropyl ether and dried under reduced pressure to give the magnesium salt. A mixture of the azetidinonecarboxylic acid (5) (1.84 g, 6.10 mmol) and carbonyldiimidazole (1.20 g, 7.41 mmol) in anhydrous acetonitrile (29.0 ml) was stirred for 30 min under a nitrogen atmosphere to give a solution of the imidazolide. To this solution was added the magnesium salt described above, and the mixture was heated at 65 ℃ for 3.5 h. After dilution with ice-cold 5% hydrochloric acid, the mixture was extracted with ethyl acetate. The extract 1 h. After dilution with ethyl acetate, the mixture was washed with 0.1M potassium phosphate and saturated sodium chloride solution successively and dried over anhydrous sodium sulfate. Evaporation of the solvent gave a residue, which was chromatographed on silica gel with ethyl acetate/benzene (2/1 v/v) to give 8 (3.85 GBq, 93.2%) as a pale yellow solid. Radiochemical purity 98% on RTLC (ethyl acetate, Rf=0.27). NMR (δ, ppm, $CDCl_3$): 1.23 (3H, d, J=6.3 Hz), 1.30 (3H, d, J=6.3 Hz), 2.60 (1H, d, J=3 Hz), 2.90 (1H, dd, J=7.2, 1.0 Hz), 3.64-3.86 (2H, m), 4.00-4.20 (1H, m), 5.37 (2H, s), 6.12 (1H, s), 7.42-7.58 (2H, m), 8.08-8.29 (2H, m). IR (ν_{max}, cm^{-1}, nujol): 3350, 2150, 1750, 1720.

4-Nitrobenzyl (1R,5S,6S)-2-[(3S,5S)-5-dimethylaminocarbonyl-1-(4-nitrobenzyloxycarbonyl)pyrrolidin-3-ylthio]-6-[(1R)-1-hydroxyethyl]-1-methyl[3-^{14}C]carbapen-2-em-3-carboxylate (12)

Under a nitrogen atmosphere, the diazo keto ester (8) (3.85 GBq, 1.08 g, 2.77 mmol) was dissolved in anhydrous n-hexane (4.0 ml) and an-

hydrous ethyl acetate (12.0 ml) and maintained at gentle reflux. To this solution was added rhodium (II) octanoate (6 mg), and the mixture was refluxed for an additional 30 min. After cooling, the mixture was concentrated under reduced pressure to afford the bicyclic keto ester (9), which was used for the next step without further purification.

To a solution of the keto ester (9) in anhydrous acetonitrile (11.0 ml) was added N,N-diisopropylethylamine (0.72 ml, 4.16 mmol) and diphenyl chlorophosphate (0.87 ml, 4.16 mmol) at 0 ℃, and the mixture was stirred at the same temperature for 1 h to give the vinyl phosphate (10). After cooling to -35 ℃, N,N-diisopropylethylamine (0.72 ml, 4.16 mmol) and mercaptopyrrolidine (11) (1.41 g, 3.98 mmol) were added, and the mixture was stirred at -30 ℃ for 1.5 h. After dilution with dichloromethane (30 ml) and ether (60 ml), the organic phase was separated and washed with ice-cold saturated sodium chloride solution. The aqueous phase was extracted with dichloromethane/ether (1/2 v/v). The combined organic extracts was washed with ice-cold 0.1M potassium phosphate, ice-cold saturated sodium chloride solution and dried over anhydrous sodium sulfate. Evaporation of the solvent and purification of the crude product by column chromatography on silica gel with ethyl acetate/acetone (1/1 v/v) gave 12 (2.25 GBq, 58.4%) as a pale yellow solid. Radiochemical purity 96% on RTLC (ethyl acetate/acetone=1/1 v/v, Rf=0.17). NMR (δ, ppm, $CDCl_3$): 1.19-1.39 (6H, m), 1.72-2.07 (3H, m), 2.93-3.11 (7H, m), 3.20-3.58 (3H, m), 3.99-4.32 (3H, m), 4.60-4.80 (1H, m), 5.15-5.52 (4H, m), 7.36-7.62 (4H, m), 8.17-8.27 (4H, m). IR (ν_{max}, cm^{-1}, nujol): 3400, 1770, 1710.

(1R,5S,6S)-2-[(3S,5S)-5-Dimethylaminocarbonylpyrrolidin-3-ylthio]-6-[(1R)-1-hydroxyethyl]-1-methyl[3-^{14}C]carbapen-2-em-3-carboxylic acid trihydrate (SM-7338) (1)

In the presence of 10% palladium on carbon (1.65 g), bis-protected SM-7338 (12) (2.25 GBq, 1.13 g, 1.62 mmol) in tetrahydrofuran (20.0 ml) and 0.6M morphorinopropanesulfonic acid (MOPS) (12.0 ml) was hydrogenated under atomospheric pressure at room temperature for 7 h. The catalyst was filtered off and washed with 0.1M MOPS solution. The filtrate was concentrated under reduced pressure to remove tetrahydrofuran. The resulting aqueous solution was washed with dichloromethane, concentrated again to a

volume around 5 ml. The residual solution was applied to column chromatography on high porous polymer (MCI GEL CHP-20P, Mitsubishi Chemical Industries Ltd., Japan) with water and water/tetrahydrofuran (99/1 v/v) at 5 ℃. The fractions containing SM-7338 was collected and lyophilized to afford SM-7338 in anhydrous form. The anhydrous product was dissolved in water (3.9 ml) and stirred at 0 ℃ for 1 h. After the dropwise addition of tetrahydrofuran (11.7 ml), the mixture was stirred at the same temperature for 1 h. The precipitate was filtered, washed with tetrahydrofuran and dried under reduced pressure (100-110 Torr) to give 1 (1.25 GBq, 395 mg, 55.6%) as a colorless solid. Radiochemical purity 99% on RTLC (Silica Gel 60 WF_{254S}, acetonitrile/water/acetic acid=10/5/1 v/v/v, Rf=0.37; RP-8 F_{254S}, water/dioxane=10/1 v/v, Rf=0.58) and radio-HPLC (column SUMIPAX ODS A-212, 6 mmφ x 15 cm, 5 μm; mobile phase 5mM phosphate buffer (pH 7.0)/ was washed with ice-cold 0.1M phosphate buffer (pH 7.4), 5% sodium hydrogen carbonate and saturated sodium chloride solution, successively. After drying over anhydrous sodium sulfate, the solvent was evaporated to give a residue, which was chromatograhed on silica gel with ethyl acetate/benzene (3/7 v/v). Evaporation of the main fractions afforded 6 (4.78 GBq, 46.8%). Radiochemical purity 98% on RTLC (ethyl acetate/benzene=1/1 v/v, Rf=0.25). NMR (δ, ppm, $CDCl_3$): 0.063 (6H, s), 0.87 (9H, s), 1.11-1.26 (6H, s), 2.82-3.00 (2H, m), 3.64 (2H, s), 3.78-3.95 (1H, m), 4.02-4.26 (1H, m), 5.27 (2H, s), 6.08 (1H, s), 7.41-7.54 (2H, m), 8.15-8.24 (2H, m). IR (ν_{max}, cm^{-1}, neat): 3250, 2950, 2850, 1760, 1720.

(3S,4R)-3-[(1R)-1-Hydroxyethyl]-4-[(1R)-1-methyl-3-(4-nitrobenzyloxycarbonyl)-2-oxo[3-^{14}C]propyl]azetidin-2-one (7)

A mixture of the keto ester (6) (4.78 GBq, 1.65 g, 3.44 mmol) and 6N hydrochloric acid (0.95 ml) in methanol (10.0 ml) was stirred at room temperature for 4 h. After neutralization with saturated sodium hydrogen carbonate solution, the mixture was extracted with ethyl acetate. The extract was washed with ice-cold 0.1M potassium phosphate and ice-cold saturated sodium chloride solution. After drying over anhydrous sodium sulfate, the solvent was evaporated to afford 7 (4.13 GBq, 86.4%) as a pale yellow oil. Radiochemical purity 98% on RTLC (ethyl acetate, Rf= 0.16). NMR (δ, ppm, $CDCl_3$): 1.25 (3H, d, J=7.1 Hz), 1.28 (3H, d, J=6.1

Hz), 2.82-3.00 (2H, m), 3.67 (2H, s), 3.82 (1H, dd, J=6.6, 2.2 Hz), 4.02-4.18 (1H, m), 5.25 (2H, s), 6.25 (1H, s), 7.40-7.58 (2H, m), 8.12-8.24 (2H, m). IR (ν_{max}, cm^{-1}, nujol): 3450, 3230, 1740, 1700.

(3S,4R)-3-[(1R)-1-Hydroxyethyl]-4-[(1R)-1-methyl-3-(4-nitrobenzyloxy-carbonyl)-3-diazo-2-oxo[3-^{14}C]propyl]azetidin-2-one (8)

Under a nitrogen atmosphere, to a solution of the alcohol (7) (4.13 GBq, 1.08 g, 2.97 mmol) in anhydrous acetonitrile (6.2 ml) was added carboxybenzenesulfonyl azide (913 mg, 4.02 mmol) and triethylamine (0.62 ml, 4.47 mmol) at 0 ℃, and the mixture was stirred at the same temperature for methanol=5/1 v/v; flow rate 1.0 ml/min; detectors UV (220 nm) and radio-detector; temperature room temperature; retention time 13.7 min). NMR (δ, ppm, methanol-d_4): 1.19 (3H, d, J=7.5 Hz), 1.27 (3H, d, J=6.2 Hz), 1.58-1.90 (1H, m), 3.00 (3H, s), 3.06 (3H, s), 3.58-4.21 (5H, m). IR (ν_{max}, cm^{-1}, nujol): 3400, 1760. Mass spectrum (SIMS, m/z): 384 (M^+-$3H_2O$+1), 386 (M^+-$3H_2O$+3).

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