SYNTHESIS OF A RADIOLABELLED NEW ORAL CEPHALOSPORIN, E1101

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SUMMARY

The new broad-spectrum oral antibacterial agent, E1101, has been labelled in the C-2 position of the aminothiazole ring using [^{14}C]thiourea. The cephalosporin analog was synthesized in 5 steps and is suitable for metabolism and disposition studies.

Key Words: Oral cephalosporin, Antibacterial agent, ^{14}C-Carbon, Radiolabelled

INTRODUCTION

E1101(1) is being developed as a new broad antibacterial spectrum oral cephalosporin. As E1100 exhibited well-balanced and potent antibacterial activity but poor oral absorbability, extensive modification at the C-4 carboxylic acid to several esters was carried out, which led us to find a compound with satisfactory bioavailability, namely E1101. In other words, E1101 is a type of prodrug esters, which is hydrolyzed by an esterase in the intestinal tract of humans to yield the biologically active compound, E1100, in a similar manner to that of other prodrugs such as CFTM-POM (2), CPDX-PR (3), and ME1207 (4). As an analog labelled with [^{14}C] was required for metabolite and drug disposition studies, [^{14}C]E1101, which was labelled at C-2 position of the aminothiazole ring was prepared using commercially available [^{14}C]thiourea as the source of [^{14}C]

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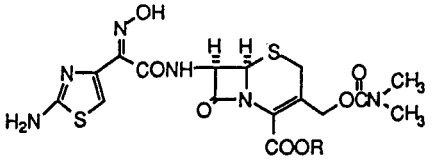
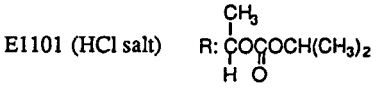
labelled carbon. In this paper, we describe the synthetic method for the preparation of (*RS*)-1-(isopropoxycarbonyloxy)ethyl (+)-(6*R*,7*R*)-7-[(*Z*)-2-(2-[2-^{14}C]aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-(*N*,*N*-dimethylcarbamoyloxy)methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate monohydrochloride ([^{14}C]E1101).





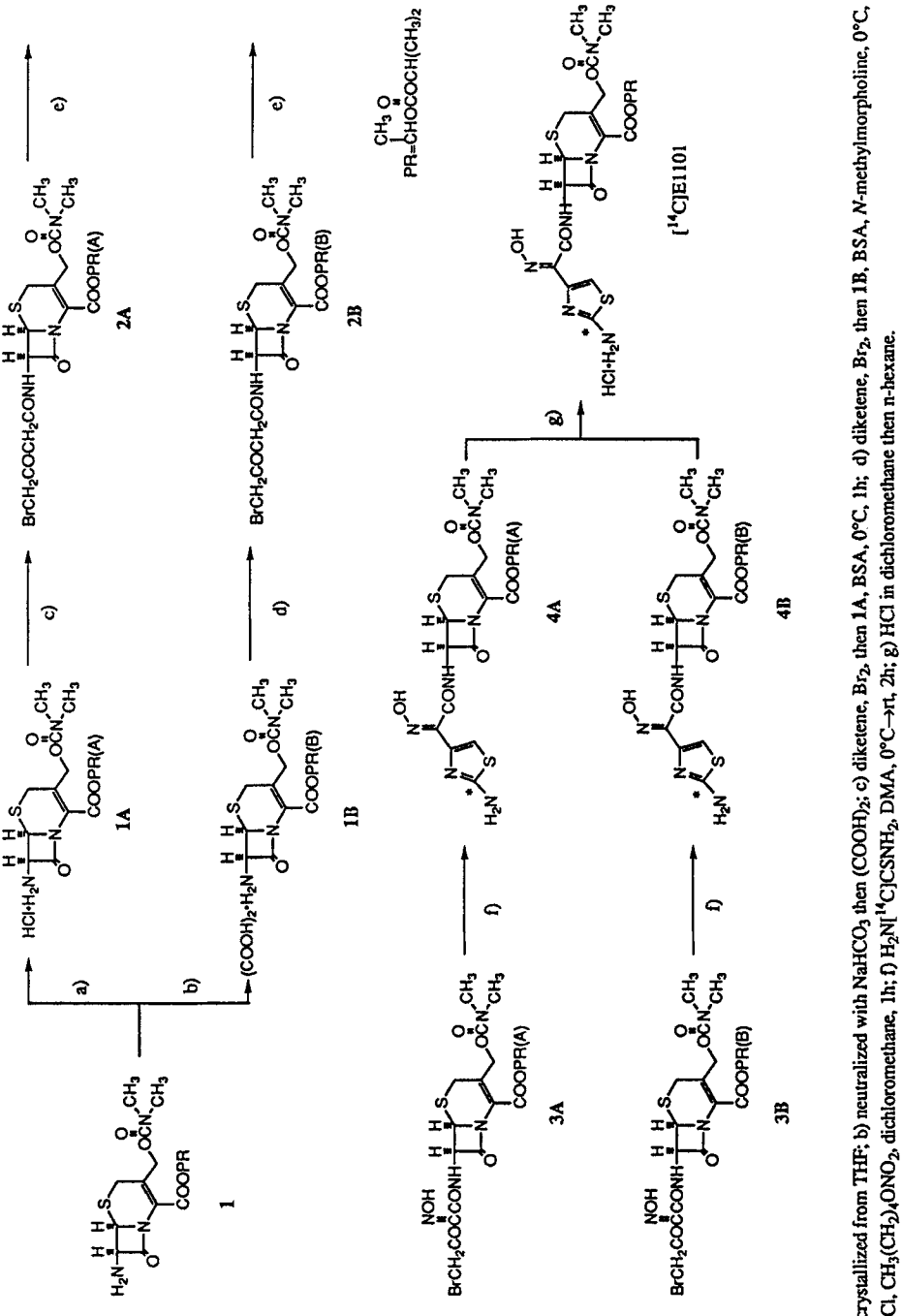
E1101 (HCl salt) R: $CH(CH_3)COCOCH(CH_3)_2$

E1100 R: Na

Results and Discussion

E1101 has been developed as a *ca.*1:1 mixture of diastereomers, because both diastereomers exhibited almost the same oral absorbability. However, it was found to be extraordinary difficult to obtain to high-quality [^{14}C]E1101 as a diastereomeric mixture by conventional methods such as crystallization or column chromatography; crystallization gave either isomer rich mixture, and column chromatography isolation yielded the Δ^2 isomers as concomitant by-products. Therefore, as shown in Scheme 1, each diastereomer was separated and converted into the free form of E1101 using a similar method to that described by Y. Inamoto (5). A 1:1 mixture of (aminothiazole-[2-^{14}C])-E1101 was then prepared as the hydrochloride salt. This route was advantageous because the key intermediates **1A** and **1B** as well as isomers of our target compounds **4A** and **4B** were purifiable by crystallization and, eventually, the high-quality racemic [^{14}C]E1101 was yielded by the combination of both purified **4A** and **4B** in the process of hydrochloric acid salt formation.

Firstly, diastereomeric 7-amino-3-cephem (**1**), which was prepared by a known procedure (6), was separated into component isomers through crystallization. Since the stereochemistry at the ester



a) crystallized from THF; b) neutralized with $NaHCO_3$ then $(COOH)_2$; c) diketene, Br_2, then **1A**, BSA, 0°C, 1h; d) diketene, Br_2, then **1B**, BSA, *N*-methylmorpholine, 0°C, 1h; e) AcCl, $CH_3(CH_2)_4ONO_2$, dichloromethane, 1h; f) $H_2N[^{14}C]CSNH_2$, DMA, 0°C→rt, 2h; g) HCl in dichloromethane then n-hexane.

Scheme 1

moiety of **1** is unclear, the more polar isomer was termed A isomer and the less polar isomer was termed B isomer. Treatment of **1** in THF gave crystals of A-rich isomer (**1A**:**1B**=98:2) in 45% yield. The filtrate was neutralized and then B-rich isomer **1B** was isolated as the oxalate salt (**1A**:**1B**=18:82) in 45% yield.

Secondly, amino cephem **1A** was coupled in the presence of *N*,*O*-bis(trimethylsilyl)acetamide (BSA) with 4-bromoacetoacetylbromide which is readily prepared from diketene and bromine to give crude **2A** which was purified by chromatography to afford **2A** in 55% yield. **2A** was transformed by treatment with acetyl chloride (1.1 eq) and isoamyl nitrite (1.3 eq) to the hydroxyimino cephem **3A** in 72% yield. Treatment of **3A** with [^{14}C]thiourea in *N*,*N*-dimethylacetamide gave crude desired compound, which was purified by crystallization using methanol-diisopropyl ether to furnish **4A**-rich mixture (**4A**:**4B**=98:2) in 52% yield .

Amino cephem **1B** was coupled with 4-bromoacetoacetylbromide in the presence of BSA and *N*-methylmorpholine (1 eq) to give **2B** (55%), which was converted to hydroxyimino cephem **3B** (53%). Similar treatment of **3B** with [^{14}C]thiourea and recrystallization from acetonitrile was carried out to give labelled **4B** in 86% yield (**4A**:**4B**=5:95). Finally, both isomers **4A** and **4B** were dissolved in dichloromethane under ice-cooling and n-hexane was added dropwise to this mixture to give [^{14}C]E1101 (60.8 Ci/mmol) as a *ca.*1:1 diastereomer mixture. The radiochemical and chemical purities of [^{14}C]E1101 were measured as 97.7% and 98.2% by HPLC, respectively.

Experimental

[^{14}C]Thiourea was purchased from Amersham International plc. Evaporation was performed under reduced pressure at less than 30 ℃ and the reaction was monitored by HPLC. Melting points were determined using a Yamato MP21. ^{1}H NMR spectra were measured at 400Hz on a Varian UNITY 400 spectrometer. Chemical shifts are quoted in δ (ppm) downfield from tetramethylsilane (TMS) as

an internal standard, coupling constants (*J*) are given in Hz. The following abbreviation are used: s=singlet, d=doublet, q=quartet, ABq=AB quartet, m=multiplet. Infrared spectra were recorded on a Hitachi 260-30 or a Nicolet 205 FT-IR spectrometer. HPLC analysis was performed using a Waters 510 pump, a Waters 712 WISP autoinjector, a JASCO 875 UV detector, a TOSO RS-8000 radiodetector, and a TOSO-CP8080 integrator. Separations were accomplished at room temperature with a C-18 column (150 x 4.6 mm) manufactured by YMC Co. THF=tetrahydrofuran, EtOAc=ethyl acetate, IPE=diisopropyl ether, DMA=*N*,*N*-dimethylacetamide.

Separation of **1** to yield isomers (**1A**) and (**1B**)

1-(Isopropoxycarbonyloxy)ethyl (6*R*, 7*R*)-7-Amino-3-(*N*,*N*-dimethylcarbamoyloxy)methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate Monohydrochloride A-rich isomer (**1A**)

1 (9.5 g, 20.3 mmol) was added to a solution of THF (145 mL), the mixture was stirred at room temperature for 1 h, and crystals appeared gradually thereafter. To this mixture, IPE (180 mL) was added and the mixture was stirred for a further 13 h. Crystals were collected by filtration and washed with a 1:1 mixture of THF-IPE (30mL) and dried under reduced pressure to afford 4.3 g of **1A**-rich isomer (**1A**:**1B**=98:2) in 45% yield. (The ratio of **1A**:**1B** was determined by HPLC in the following conditions; mobile phase: CH_3CN : 0.1% aqueous ammonium acetate solution = 30 : 70 (v/v); flow rate: 2.0 mL/ min ; UV 254 nm; retention times (min): 14.2 (**1A**), 15.9 (**1B**).)

mp. 157—158 ℃ (dec). IR (nujol): 1787, 1755, 1716 cm^{-1}. ^1H NMR for **1A** (CD_3OD) δ: 1.27 (3H, d, *J*=7 Hz, $CH(C\underline{H}_3)_2$), 1.29 (3H, d, *J*=7 Hz, $CH(C\underline{H}_3)_2$), 1.53 (3H, d, *J*=5 Hz, CH_3), 2.91 (3H, s, CH_3), 2.94 (3H, s, CH_3), 3.68 and 3.77 (2H, ABq, *J*=18 Hz, CH_2), 4.86 and 5.19 (2H, ABq, *J*=14 Hz, CH_2), 4.82—4.94 (1H, m, CH), 5.14 (1H, d, *J*=5 Hz, CH), 5.25 (1H, d, *J*=5 Hz, CH), 6.94 (1H, q, *J*=5 Hz, CH).

1-(Isopropoxycarbonyloxy)ethyl (6*R*,7*R*)-7-Amino-3-(*N*,*N*-dimethylcarbamoyloxy)methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate Oxalate B-rich isomer (**1B**)

The filtrate of **1A** described above was concentrated and then dissolved in a mixture of EtOAc (150 mL) and water (72 mL). The mixture was adjusted with aqueous $NaHCO_3$ solution to pH 5.5 under ice cooling, and the organic layer was separated. The ethyl acetate solution was washed with brine (63 mL), and then dried over magnesium sulfate. After the solid was filtered off, IPE (95 mL) was added to the filtrate and the mixture was warmed to 30 ℃. To this mixture, a solution of oxalic acid (1.23 g, 14 mmol) in EtOAc (24.5 mL) was added and the mixture was stirred for 17h. The precipitate was collected by filtration and dried under reduced pressure to yield 4.76 of **1B**-rich isomer (**1A**:**1B**=14:86) in 45%.

mp. 138—139 ℃ (dec). IR (nujol): 1816, 1771, 1699, 1627 cm^{-1}. ^1H NMR for **1B** (CD_3OD) δ: 1.28 (6H, d, *J*=6 Hz, $CH(CH_3)_2$), 1.53 (3H, d, *J*=6 Hz, CH_3), 2.91 (3H, s, CH_3), 2.93 (3H, s, CH_3), 3.61 and 3.74 (2H, ABq, *J*=18 Hz, CH_2), 4.80—4.92 (2H, m, CH_2), 5.01 (1H, d, *J*=5 Hz, CH), 5.08 (1H, d, *J*=5 Hz, CH), 6.84 (1H, q, *J*=6 Hz, CH).

1-(Isopropoxycarbonyloxy)ethyl (6*R*,7*R*)-7-(4-Bromo-3-oxobutyrylamino)-3-(*N*,*N*-dimethylcarbamoyloxy)methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate A-rich isomer (**2A**)

1M bromine dichloromethane solution (5.5 mL, 5.5 mmol) was added to a solution of diketene (0.46 g, 5.5 mmol) in dichloromethane (5 mL) in a dry-ice acetonitrile bath. This mixture was added to a mixture of **1A** (2.34 g, 5.0 mmol) and BSA (2.7 mL, 11 mmol) in THF (24 mL) at the same temperature, and the resultant mixture was stirred for 45 min. To this mixture, EtOAc (200 mL) and water (100 mL) were added and the organic layer was separated. The extract was washed with water (100 mL), then brine (100 mL), and dried over magnesium sulfate. It was then concentrated under reduced pressure. Purification of the residue by silica gel chromatography (n-hexane : EtOAc = 9:1) yielded **2A**-rich isomer as an amorphous solid (2.3 g, 77%).

IR (nujol): 1789, 1760, 1694, 1681 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.30 (3H, d, J=6 Hz,$CH(C\underline{H}_3)_2$), 1.32 (3H, d, J=6 Hz, $CH(C\underline{H}_3)_2$), 1.58 (3H, d, J=6 Hz, CH_3), 2.92 (6H, s, CH_3), 3.46 (0.5H, d, J=19 Hz, CH_2), 3.48 (0.5H, d, J=19 Hz, CH_2), 3.57 (1H, d, J=19 Hz, CH_2), 3.70 and 3.77 (0.5H, ABq, J=18 Hz, $COCH_2CO$ (keto)), 3.84 (0.5H, s, $BrCH_2$ (enol)), 4.02 (0.5H, s, $BrCH_2$ (keto)), 4.86—5.24 (3.5H, m, CH_2, CH, OH), 5.09 (0.5H, s, $COCH_2CO$ (enol)), 6.80—6.90 (1H, m, CH), 6.00 (0.5H, d, J=9 Hz, NH), 7.01 (1H, q, J=6 Hz, CH), 7.40 (0.5H, d, J=10 Hz, NH).

1-(Isopropoxycarbonyloxy)ethyl (6*R*, 7*R*)-7-(4-Bromo-3-oxo-2-hydroxyiminobutyrylamino)-3-(*N*,*N*-dimethylcarbamoyloxy)methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate A-rich isomer (3 A)

A stirred solution of **2 A** (960 mg, 1.62 mmol) in dichloromethane (10 mL) was cooled to 0 ℃. To the mixture, acetyl chloride (0.14 mL, 1.78 mmol) was added and isoamyl nitrite (0.26 g, 2.16 mmol) was successively added. The mixture was stirred at the same temperature for 2 h and EtOAc (150 mL) was then added. The resultant mixture was washed with water (50 mLx2) then brine, and dried over magnesium sulfate. It was concentrated under reduced pressure to afford crude product **3 A**. The residue was purified by chromatography (dichloromethane: EtOAc= 9:1) to give **3 A**-rich isomer (720 mg, **3 A**:**3 B**=97:3, 72%) as an amorphous solid. (The ratio of **3 A**:**3 B** was determined by HPLC in the following conditions; mobile phase: 50% CH_3CN-0.5% $HClO_4$; flow rate: 2.0 mL/ min ; UV 254 nm; retention times (min): 5.55 (**3 A**), 5.92 (**3 B**).)

IR (nujol): 1789, 1756, 1681 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.31 (3H, d, J=6 Hz, $CH(C\underline{H}_3)_2$), 1.33 (3H, d, J=6 Hz, $CH(C\underline{H}_3)_2$), 1.59 (3H, d, J=6 Hz, CH_3), 2.93 (6H, s, CH_3), 3.53 and 3.60 (2H, ABq, J=19 Hz, CH_2), 4.54 (2H, s, CH_2), 4.88 and 5.22 (2H, ABq, J=14 Hz, CH_2), 4.90—4.95 (1H, m, CH), 5.03 (1H, d, J=5 Hz, CH), 5.85 (1H, dd, J=5 Hz, 9 Hz, CH), 7.13 (1H, q, J=6 Hz, CH), 9.40 (1H, d, J=9 Hz, NH)

1-(Isopropoxycarbonyloxy)ethyl (6*R*,7*R*)-7-(4-Bromo-3-oxobutyrylamino)-3-(*N*,*N*-dimethylcarbamoyloxy)methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate B-rich isomer (**2 B**)

1M dichloromethane solution of bromine (5.5 ml, 5.5 mmol) was added dropwise to a solution of diketene (0.46 g, 5.5 mmol) in dichloromethane (5 ml) in a dry-ice acetonitrile bath. This mixture was added to a solution of **1 B** (2.64 g, 5.0 mmol), *N*-methylmorpholine (1.0 g, 10 mmol) and BSA (2.7 ml, 11 mmol) in THF (24 ml) at the same temperature. After 1 h, a mixture of EtOAc (250 mL) and water (100 mL) was added and the organic layer was separated, washed and dried over magnesium sulfate then evaporated. The residue was purified by silica gel chromatography (dichloromethane: EtOAc= 9:1) to give **2 B**-rich isomer (1.63 g) in 55% yield as an amorphous solid. IR (nujol): 1789, 1756, 1708, 1646 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.31 (3H, d, J=6 Hz, $CH(C\underline{H}_3)_2$), 1.32 (3H, d, J=6 Hz, $CH(C\underline{H}_3)_2$), 1.57 (3H, d, J=6 Hz, CH_3), 2.92 (3H, s, CH_3), 2.93 (3H, s, CH_3), 3.49 and 3.59 (2H, ABq, J=16 Hz, CH_2), 3.72 and 3.78 (0.67H, ABq, J=9 Hz, $COCH_2CO$ (keto)), 3.85 (0.33H, s, $BrCH_2$ (enol)), 4.01 (0.67H, s, $BrCH_2$ (keto)), 4.85—5.02 (3H, m, CH_2, CH), 5.13 (1H, d, J=14 Hz, CH_2), 5.23 (0.33H, s, CH (enol)), 5.31 (0.33H, s, OH (enol)), 5.84—5.93 (1H, m, CH), 6.03 (0.33H, d, J=9 Hz, NH), 6.91 (1H, q, J=6 Hz, CH), 7.40 (0.67H, d, J=10 Hz, NH).

1-(Isopropoxycarbonyloxy)ethyl (6*R*,7*R*)-7-(4-Bromo-3-oxo-2-hydroxyiminobutyrylamino)-3-(*N*,*N*-dimethylcarbamoyloxy)methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate B-rich isomer (**3 B**)

Acetyl chloride (0.285 mL, 3.6 mmol) was added to a solution of **2 B** (1.96 g, 3.3 mmol) in dichloromethane (20 mL) at 0 ℃ and isoamyl nitrite (0.53 g, 4.3 mmol) was successively added to the reaction mixture. After 1 h, a mixture of EtOAc (150 mL) and water (100 mL) was added and the organic layer was separated. The extract was washed with water then brine, and dried over

magnesium sulfate. It was then evaporated. The residue was purified by silica column chromatography (dichloromethane: AcOEt= 9:1) to give 1.1 g of **3 B**-rich isomer (**3 A**:**3 B**=9:91) in 53% yield as crystals.

mp. 147—149 ℃ (dec). IR (nujol): 1783, 1731, 1707, 1687 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.33 (3H, d, J=6 Hz, $CH(C\underline{H}_3)_2$), 1.34 (3H, d, J=6 Hz, $CH(C\underline{H}_3)_2$), 1.61 (3H, d, J=6 Hz, CH_3), 2.95 (6H, s, CH_3), 3.56 and 3.64 (2H, ABq, J=18 Hz, CH_2), 4.56 (2H, s, CH_2), 4.97 and 5.22 (2H, ABq, J=14 Hz, CH_2), 4.86—4.95 (1H, m, CH), 5.08 (1H, d, J=5 Hz, CH), 5.90 (1H, dd, J=5 Hz, 9 Hz, CH), 6.93 (1H, q, J=6 Hz, CH), 9.46 (1H, d, J=9 Hz, NH)

1-(Isopropoxycarbonyloxy)ethyl (6*R*,7*R*)-7-[(*Z*)-2-([2-^{14}C]Aminothiazole-4-yl)-2-hydroxyiminoacetamido]-3-(*N*,*N*-dimethylcarbamoyloxy)methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate A-rich isomer (**4 A**)

[^{14}C]Thiourea (65.6 mg, 0.84 mmol, 50 mCi, 60 mCi/mmol) was dissolved in DMA (4 mL). Half of the mixture (2 mL) was added to a solution of **3 A** (263 mg, 0.42 mmol) in DMA (3 mL) at 0 ℃ and the mixture was stirred for 10 min at the same temperature and for further 2h at room temperature. The mixture was diluted with EtOAc (50 mL) and washed with a solution of $NaHCO_3$ (35 mg) in water (30 mL), then twice with water (10 mL), then brine (10 mL) and dried over magnesium sulfate. The filtrate was concentrated under reduced pressure, methanol (2.6 mL) was added and IPE (11 mL) was then added to the residue. The mixture was stirred for 15 min and the precipitate was collected by filtration to give the first crop. This solid was redissolved in methanol (2 mL) and IPE (12 mL) was then added to the mixture which was stirred for 15 min. The precipitate was collected by filtration and dried under reduced pressure to give **4 A**-rich isomer (132 mg, **4 A**:**4 B**=98:2, 52%) as an amorphous solid.

mp. 140—141℃ (dec). IR (nujol): 1791, 1766, 1690, 1662 cm^{-1}. ^1H NMR (CD_3OD) δ: 1.27 (3H, d, J=6 Hz, $CH(C\underline{H}_3)_2$), 1.29 (3H, d, J=6 Hz, $CH(C\underline{H}_3)_2$), 1.54 (3H, d, J=6 Hz, CH_3), 2.90 (3H,

s, CH_3), 2.93 (3H, s, CH_3), 3.56 and 3.69 (2H, ABq, J=19 Hz, CH_2), 4.78 and 5.14 (2H, ABq, J=14 Hz, CH_2), 4.82—4.96 (1H, m, CH), 5.18 (1H, d, J=5 Hz, CH), 5.91 (1H, d, J=5 Hz, CH), 6.75 (1H, s, thiazole-H), 6.94 (1H, q, J=6 Hz, CH)

1-(Isopropoxycarbonyloxy)ethyl (6*R*,7*R*)-7-[(*Z*)-2-([2-^{14}C]Aminothiazole-4-yl)-2-hydroxyiminoacetamido]-3-(*N*,*N*-dimethylcarbamoyloxy)methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate B-rich isomer (4 B)

The rest of the [^{14}C]thiourea solution in DMA (4 mL) was added to the solution of **3 B** (263 mg, 0.42 mmol) in DMA (2.5 mL) under ice cooling and the mixture was stirred at the same temperature for 1.5 h. This mixture was diluted with EtOAc (50 mL) and the organic layer was washed with a solution of $NaHCO_3$ (35 mg, 43 mmol) in water (10 mL), then with water, then brine, and dried over magnesium sulfate. The filtrate was concentrated under reduced pressure and 12 mL of acetonitrile was added to the residue and the mixture was stirred for 15 min. Crystals were collected by filtration and washed with IPE (50 mL) to afford 217 mg of **4 B**-rich isomer (**4 A**:**4 B**=5:95) in 86% yield as crystals.

mp. 141—142 ℃ (dec). IR (nujol): 1793, 1760, 1687, 1668 cm^{-1}. ^{1}H NMR (CD_3OD) δ: 1.28 (6H, d, J=6 Hz, $CH(CH_3)_2$) , 1.54 (3H, d, J=6 Hz, CH_3), 2.91 (3H, s, CH_3), 2.93 (3H, s, CH_3), 3.56 and 3.71 (2H, ABq, J=18 Hz, CH_2), 4.80—4.95 (2H, m, CH_2), 5.07 (1H, d, J=14 Hz, CH), 5.21 (1H, d, J=5 Hz, CH), 5.95 (1H, d, J=5 Hz, CH), 6.75 (1H, s, thiazole-H), 6.85 (1H, q, J=6 Hz, CH).

1-(Isopropoxycarbonyloxy)ethyl (6*R*,7*R*)-7-[(*Z*)-2-([2-^{14}C]aminothiazole-4-yl)-2-hydroxyiminoacetamido]-3-(*N*,*N*-dimethylcarbamoyloxy)methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate monohydrochloride ([^{14}C]E1101)

4 B (134 mg, 0.22 mmol) was dissolved in dichloromethane (2.6 mL) and **4 A** (132 mg, 0.22 mmol) was then added. 4N HCl ethyl acetate solution (0.11 mL, 0.44 mmol) was added to this mixture at 0

℃, and then n-hexane (8 mL) was added dropwise to the mixture. After 15 min, the precipitate was collected by filtration, washed with n-hexane (20mL), then dried under reduced pressure to give [^{14}C]E1101 (279 mg, 52%, total radioactivity 26.64 mCi, specific activity 60.8 mCi/mmol, radiochemical purity=97.7%)

HPLC conditions (1); mobile phase: 30% THF-0.5%$HClO_4$; flow rate: 2.0 mL/min; UV 254 nm; retention times (min): 7.17 (**4A**) and 8.97 (**4B**). HPLC conditions (2); mobile phase: 20% THF-0.5%$HClO_4$; flow rate: 2.0 mL/min; UV 254 nm; retention times (min): 31.78 (**4A**) and 45.77 (**4B**).

^{1}H NMR (d_6-DMSO) δ: 1.24 (3H, d, J=6 Hz, $CH(C\underline{H}_3)_2$), 1.27 (3H, d, J=6 Hz, $CH(C\underline{H}_3)_2$), 1.49 (3H, d, J=5 Hz, CH_3), 2.93 (6H, s, CH_3), 3.58 and 3.69 (2H, ABq, J=18 Hz, CH_2), 4.79 and 4.90 (2H, ABq, J=13 Hz, CH_2), 4.79 (1H, sep, J=6Hz, CH), 5.22 (1H, d, J=5 Hz, CH), 5.85 (1H, dd, J=5 Hz, 8 Hz, CH), 6.83 (1H, s, thiazole-$\underline{H}$), 6.79 (0.5H, q, J=5 Hz, CH), 6.85 (0.5H, q, J=5 Hz, CH), 9.07 (2H, s, NH_2), 9.68 (1H, d, J=8 Hz, NH).

References

1. S. Negi, M. Yamanaka, I. Sugiyama, Y. Komatsu, M. Sasho, A. Tsuruoka, A. Kamada, I. Tsukada, R. Hiruma, K. Katsu, and Y. Machida. *J. Antibiotics* **47**, 1507 (1994).
2. H. Sadaki, H. Imaizumi, T. Inaba, T. Hirakawa, Y. Murotani, Y. Watanabe, S. Minami, and I. Saikawa. *Yakugaku Zasshi*, **106**, 129 (1986).
3. K. Fujimoto, S. Ishihara, H. Yanagisawa, J. Ide, E. Nakayama, H. Nakao, S. Sugawara, and M. Iwata. *J. Antibiotics* **40**, 370 (1987).
4. K. Sakagami, K. Atsumi, A. Tamura, T. Yoshida, K. Nishikawa, and S. Fukatsu. *J. Antibiotics* **43**, 1047 (1990).
5. Y. Inamoto, T. Chiba, T. Kamimura, and T. Takaya. *J. Antibiotics* **41**, 828 (1988).
6. S. Negi, M. Yamanaka, I. Sugiyama, Y. Komatsu, A. Tsuruoka, I. Tsukada, and N. Minami. *Chem. Pharm. Bull.* (submitted)