Epimerization Induced by a Remote Cationic Center in Potent New Carbapenems

Hidenori AZAMI,* David BARRETT,[a] Toshiyuki CHIBA,[a] Akihiko FUJIKAWA,[b] Kazuo SAKANE,[a] and Fumiyuki SHIRAI[a]

New Drug Research Laboratories[a] and Basic Research Laboratories,[b] Fujisawa Pharmaceutical Co., Ltd., 2–1–6 Kashima, Yodogawa-ku, Osaka 532, Japan. Received June 14, 1996; accepted September 2, 1996

A new, potent 1β-methylcarbapenem (FR21751) containing a novel pyridiniomethylpyrrolidine side chain has been synthesized, and was found to undergo epimerization at the pyrrolidine C-2 position. To investigate this isomerization, we evaluated the epimerization rate by HPLC at various pH values in aqueous solution and the deuterium exchange rate by ^{1}H-NMR spectroscopy in buffered D_2O solution. The rate of this epimerization was greater at high pH (≧6), and deuterium exchange occurred only at the benzylic position of the pyridine ring. The results can be interpreted in terms of a mechanism involving anionic and acyclic intermediates. We synthesized the postulated acyclic intermediate of this epimerization independently and demonstrated its cyclization to give a mixture of four diastereomers (6a, 9), in support of our proposed mechanism.

Key words 1β-methylcarbapenem; epimerization; acyclic intermediate

The introduction of carbapenem antibiotics, in the form of the imipenem–cilastatin combination, into clinical practice led to a significant advance in the treatment of hospital-acquired pathogens.[1] However, problems concerning the spectrum of activity, stability, and toxicity of imipenem remained. In our group, research to find new carbapenems has led to the discovery of FR21818.[2] In the course of studies on the structure–activity relationships of this compound, we have discovered a new compound FR21751 (**6b**) that has an excellent biological profile, comparable to that of FR21818,[2] but was found to display an unexpected tendency to undergo epimerization in aqueous solution at the pyrrolidine C-2 position.

Epimerization of asymmetric centers generally occurs by a deprotonation mechanism. In the present case, deprotonation at the C-2 position would require a strong base and an electron-withdrawing group at the N-1 position.[3] However, **6b** was found to undergo an extremely facile epimerization in aqueous solution at ambient temperature. In this note, we report the synthesis and epimerization in aqueous solution of FR21751, and propose a mechanism.

Synthesis of Target Compounds FR21751 (**6b**) and its epimeric mixture (**6a**) were synthesized by the route shown in Fig. 1. The thiobenzoate **1** was synthesized by a similar method to that described for the synthesis of FR21818,[2] in 9 steps from hydroxyproline. Deacylation to give the thiol **2**, followed by coupling with **3**[4] afforded the protected carbapenem **4** in 47% yield. Elaboration to the final antibacterial agent was accomplished by quaternary salt formation and deprotection steps. In the deprotection step, we discovered that, depending on the reaction conditions and the purification method, a variable mixture of epimers was obtained. By using morpholine as an allyl trap in the Pd-catalyzed deprotection,[5] ion-exchange with Amberlyst A-26 (Cl^-), and then octadecyl silane (ODS) resin column chromatography for purification, we obtained a 1 : 1 epimeric mixture, **6a** (21%). However, when tri-*n*-butyltin hydride (Bu_3SnH)[5] was used as the trapping agent in the presence of acetic acid (AcOH) (weakly acidic conditions), FR21751 (**6b**) was obtained almost exclusively as a single isomer (37% yield, epimer ratio >99 : 1 by HPLC) after precipitation and recrystallization twice. These results strongly suggest that basic conditions are required to facilitate epimerization. The epimers of **6a** were separated by preparative HPLC to give **6b** and **6c** with *ca.* 90% purity (HPLC).

Epimerization and Proposed Mechanism Many reports of carbapenem derivatives with a 2-substituted pyrrolidine ring have appeared.[2,6] However, epimerization at the pyrrolidine ring has not been noted. Thus, we became interested in this novel epimerization, and investigated its mechanism by measuring its rate and also the rate of deuterium exchange. The epimerization of **6b** was measured at various pH values using HPLC. The results (Fig. 2) indicate that FR21751 (**6b**) was not epimerized in weakly acidic buffer solution (pH 5.2), but was readily epimerized at higher pH (≧6). That is, the epimerization does not occur under acidic conditions. This is in agreement with the observations during the synthesis and purification of **6a** and **6b**.

Next we examined the epimerization in a deuterated phosphate buffer solution (pH 7.0) by ^{1}H-NMR (Fig. 3). A comparison of the initial spectrum *A* (incubation time = 0 h) and the final one *D* (incubation time = 48 h), clearly indicates that only epimerization reaction and deuterium exchange occurred during this time. The tendency of carbapenems to undergo intermolecular dimerization and other processes is well known,[7] but we saw no evidence for those. Inspection of the NMR spectra confirmed the occurrence of epimerization at the C-2 asymmetric center. This is shown by the variation of Hc between spectra *A* and *D*. A detailed comparison of the integration of Ha in spectra *A* and *D* indicates that deuterium exchange occurred at the benzylic protons of the pyridine ring. On the other hand, Hb was not deuterated at all. These results suggest that an anionic intermediate at the benzylic position of the pyridine ring is produced in the course of epimerization.

The 2-(4-pyridyl)ethyl group is a well known protect-

* To whom correspondence should be addressed.

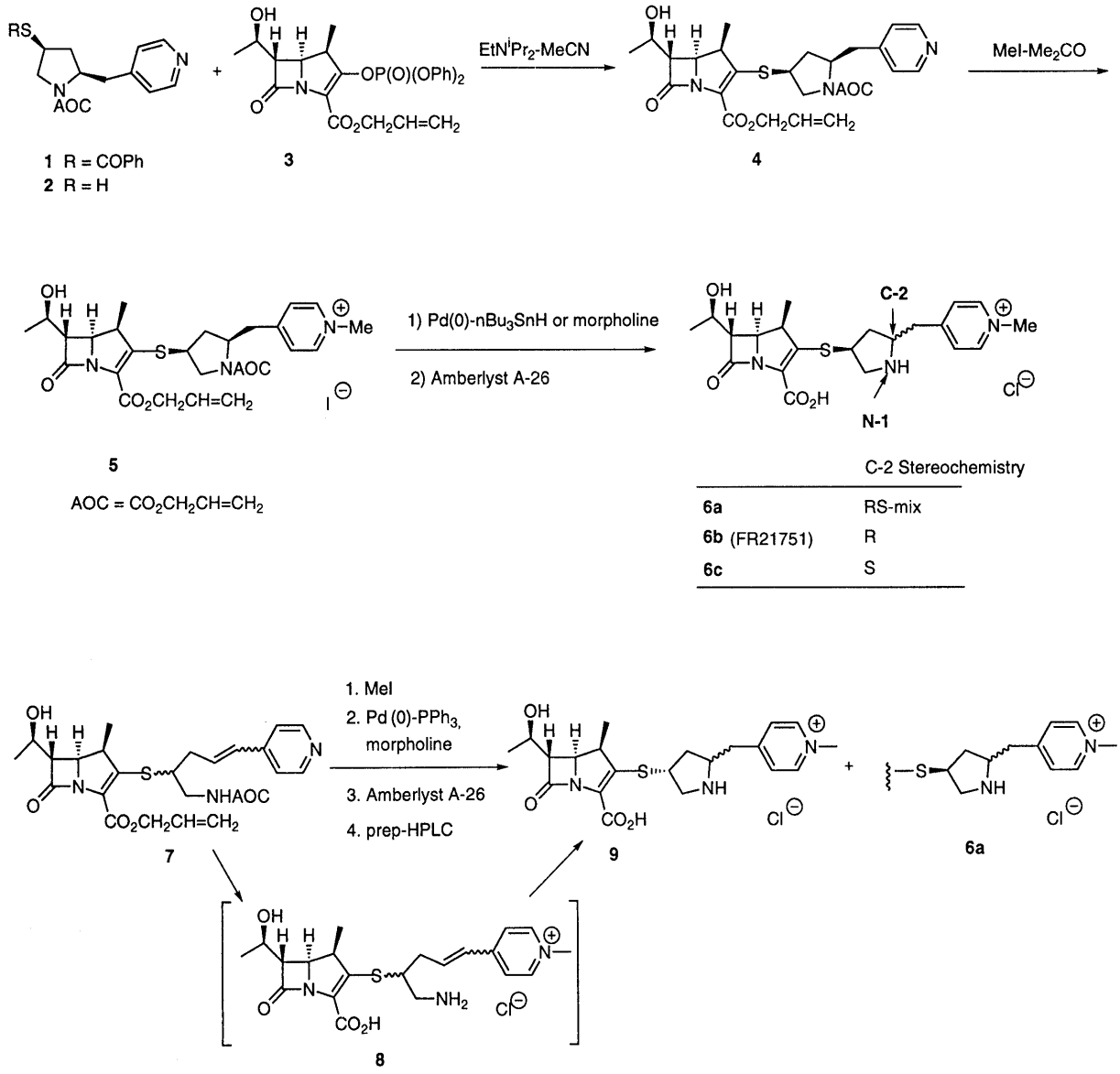

Fig. 1. Synthetic Scheme for FR21751 (**6b**) and Related Compounds (**6a**, **9**)

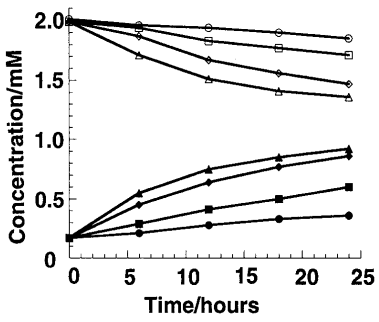

Fig. 2. Epimerization of FR21751 (**6b**) at Various pH Values

Concentrations of **6b** and **6c** were determined by HPLC. Concentrations of **6b** at pH 5.2 (○), pH 5.7 (□), pH 6.3 (◇), pH 6.7 (△), and **6c** at pH 5.2 (●), pH 5.7 (■), pH 6.3 (◆), pH 6.7 (▲) are shown. Measurements under basic conditions (pH >7.0) were not attempted due to the instability of the carbapenem skeleton.

ing group for heterocyclic NH groups.[8] Deprotection proceeds by a two-step process, involving quaternary salt formation and mild base-mediated β-elimination, leading to a 1-methyl-4-vinylpyridinium salt and the regenerated heterocyclic NH groups. This suggests a plausible mechanism for our epimerization (Fig. 4). There are two possible routes leading to the acyclic intermediate B from **6b** or **6c**. One is a stepwise route *via* the anionic intermediates A and A′, and the other is a concerted one. The first step of the stepwise route is a pH-dependent deprotonation of the benzylic position facilitated by the increase in acidity associated with quaternary salt formation, and the stabilization of the negative charge by delocalization. The second step is pyrrolidine ring opening by C–N bond cleavage to give the acyclic intermediate B. Another possibility for producing the acyclic intermediate B is *via* a concerted route, but this essentially only differs in the timing of bond breakage. The final step is ring closure of intermediate B to afford **6b** and **6c** by intramolecular cyclization of the amine to the olefin, the olefin presumably being activated due to conjugation with the electron-withdrawing 4-pyridinium group, to produce the pyrrolidine ring.

This type of epimerization mechanism has been proposed for 1-methyl-2-(2-oxopropyl)piperidine, involving an acyclic intermediate and a 1,4-conjugate addition

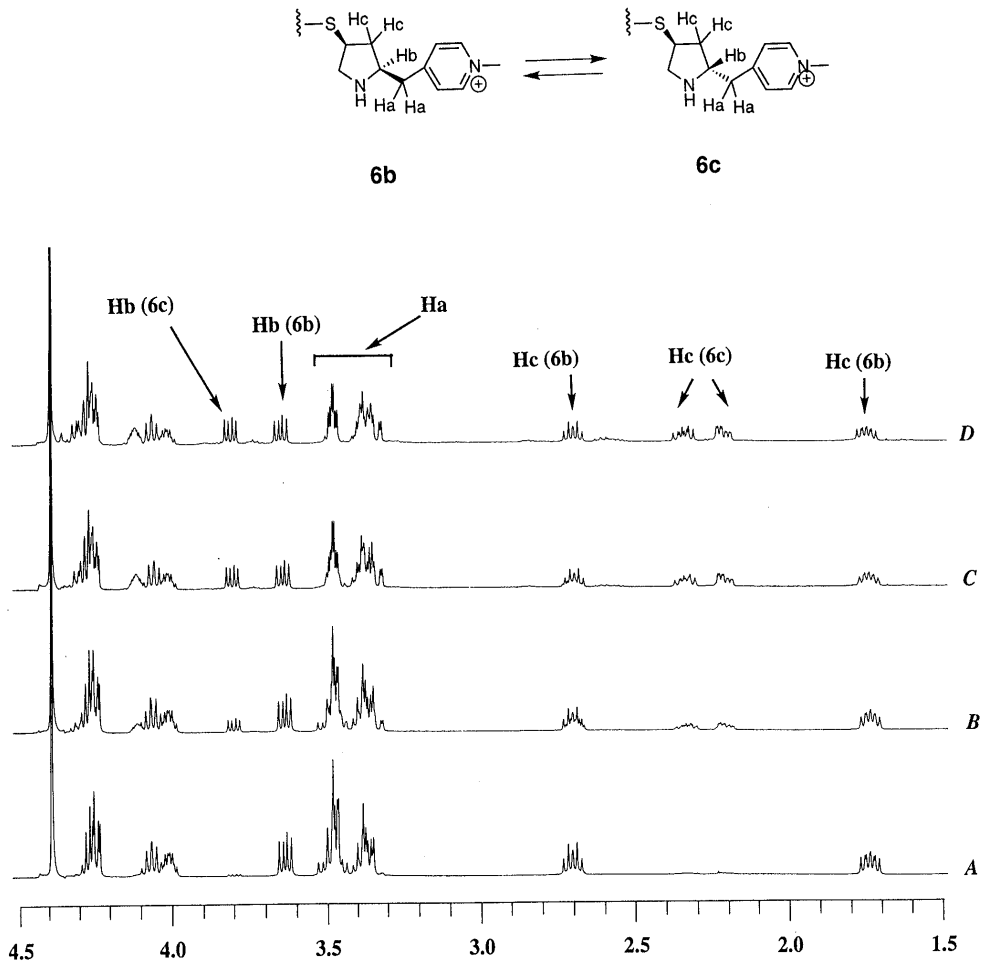

Fig. 3. Deuterium Exchange Reaction Followed by ^{1}H-NMR (500 MHz)

Time-dependent changes in the NMR signal of FR21751 (**6b**) are shown in Charts *A* (0 h), *B* (6 h), *C* (24 h) and *D* (48 h).

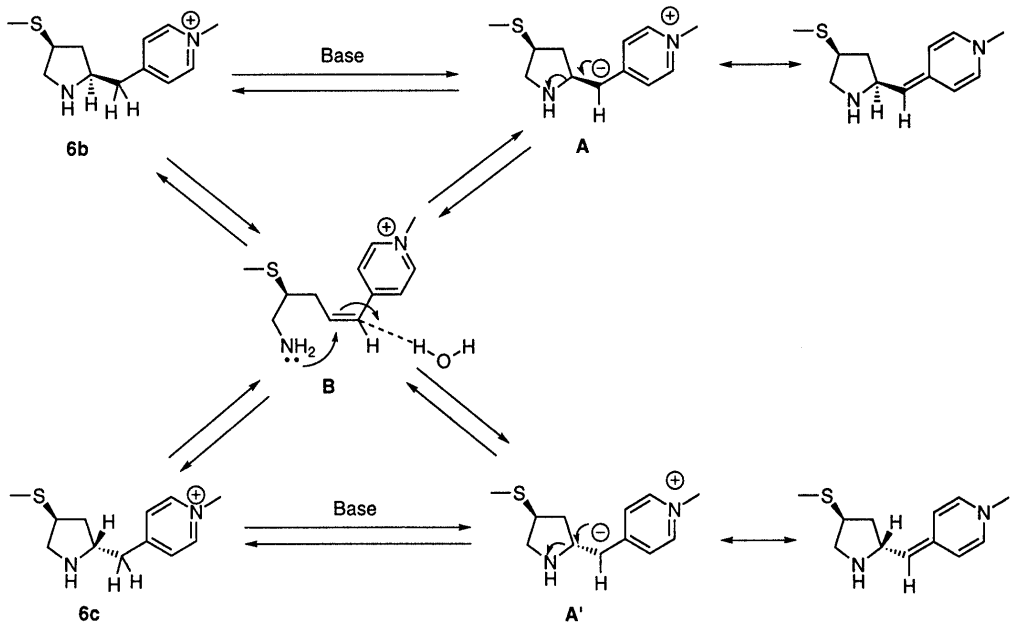

Fig. 4. Proposed Mechanism of Epimerization

reaction of an amine with an α,β-unsaturated carbonyl group,[9] but we know of no example of addition reactions of amines to a 1-methyl-4-vinylpyridinio group. Essentially, this epimerization was induced by a remote cationic center.

To support our proposal regarding the mechanism of epimerization, we prepared the acyclic compound (**7**) by a similar method to that described for the synthesis of **5**, in the expectation that quaternary salt formation, followed by deprotection would lead to a cyclized product (Fig. 1).

As expected, quaternary salt formation, deprotection and purification led to cyclized product as a mixture of four diastereomers (**6a**, **9**). This result indicates that the final step of our proposed mechanism is feasible.

In conclusion, we present a possible mechanism for the epimerization of the novel 1β-methylcarbapenem FR21751 (**6b**), based on epimerization and deuterization experiments. We also describe cyclization of the acyclic compound (**7**) to **6a** and **9**, in support of our proposal.

Experimental

Reagents used in this study were obtained from commercial sources. Reaction solvents were of the highest grade available. The mobile phase for HPLC analysis employed HPLC-grade acetonitrile (MeCN) and buffer solutions were prepared with water (H_2O) which had been deionized by a Milli-Q system and filtered (Millipore membrane filter). IR spectra were recorded on a Horiba FT-210. ^{1}H-NMR spectra were recorded at 200 MHz on a Bruker AC200P or at 500 MHz on a Bruker AMX-500 in $CDCl_3$, DMSO-d_6 or D_2O (chemical shifts are reported in ppm (δ units) downfield from tetramethylsilane or sodium 4,4-dimethyl-4-silapentanesulfonate (DSS)). FAB-MS were recorded on a Finnigan MAT TSQ-70. The purity of the final carbapenem was determined by analytical HPLC, using a system consisting of a Shimadzu SPD-6A (254 nm), LC-6A pumps, a SIL-6B auto injector, a SCL-6B analysis system, and a CR-5A. An ODS 80TMCTR (Tosoh) analytical column and guard column were used (flow rate 1.0 ml/min, detection at 254 nm, mobile phase, phosphate buffer (pH 3.0) : MeCN = 97 : 3).

Allyl (4*R*,5*S*,6*S*)-3-[(2*R*,4*S*)-1-Allyloxycarbonyl-2-(4-pyridinylmethyl)-pyrrolidin-4-yl]thio-6-[(1*R*)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (4) A solution of **1** (315 g, 0.82 mol) in MeCN (1.4 l) was treated slowly with sodium methoxide (28% solution in methanol) (158 ml, 0.82 mol) at -20 °C. The mixture was stirred at 0—5 °C for 10 min, the reaction was quenched with AcOH (47.5 ml, 0.83 mol), and the whole was diluted with ethyl acetate (EtOAc) (7 l), and washed with H_2O (2 l × 2) and brine (2 l). The organic layer was dried over magnesium sulfate ($MgSO_4$) and evaporated under reduced pressure to give the crude thiol **2** as an oil. A solution of **2** in MeCN (300 ml) and diisopropylethylamine (EtN^iPr_2) (179 ml, 1.03 mol) was added to a solution of **3**[4] (0.69 mol) in MeCN (1.0 l) at 0—5 °C. The mixture was then allowed to stand at 0—5 °C overnight, poured into H_2O (3 l), and extracted with EtOAc (3 l × 3). The combined organic layers were dried ($MgSO_4$), evaporated under reduced pressure, and purified by column chromatography (SiO_2 5 kg, acetone (Me_2CO): dichloromethane (CH_2Cl_2) = 3 : 7) to give 171.3 g (47%) of **4** as an amorphous solid. IR (CH_2Cl_2) cm^{-1}: 1755, 1685. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.23 (d, 3H, J = 7.4 Hz), 1.35 (d, 3H, J = 6.2 Hz), 1.60—1.78 (m, 1H), 2.25—2.40 (m, 1H), 2.70—4.25 (m, 10H), 4.62 (br d, 2H, J = 5.8 Hz), 4.62—4.85 (m, 2H), 5.23—5.49 (m, 4H), 5.88—6.04 (m, 2H), 7.17 (br s, 2H), 8.52 (br d, 2H, J = 5.2 Hz).

(4*R*,5*S*,6*S*)-3-{(2*RS*,4*S*)-2-[4-(1-Methylpyridinio)methyl]pyrrolidin-4-yl}thio-6-[(1*R*)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]-hept-2-ene-2-carboxylic Acid, Chloride (6a) Methyl iodide (2.3 ml, 37.4 mmol) was added to a solution of **4** (3.94 g, 7.47 mmol) in Me_2CO (20 ml). After standing at room temperature overnight, the mixture was evaporated to give a crude salt **5** as a brown amorphous solid (4.94 g, *ca.* 100%) that was used immediately in the next reaction. Compound **5** was dissolved in a mixture of tetrahydrofuran (THF) (18 ml) and ethanol (EtOH) (37 ml), then triphenylphosphine (Ph_3P) (200 mg, 0.76 mmol), morpholine (1.63 ml, 18.7 mmol) and tetrakistriphenylphosphinepalladium (0) (Pd(0)) (340 mg, 0.3 mmol) were added successively. Stirring at room temperature for 20 min gave a precipitate that was collected by filtration. The obtained precipitate was dissolved in H_2O (150 ml), and the solution was washed with EtOAc (50 ml × 2) and CH_2Cl_2 (50 ml × 5). The pH was adjusted to 4.0 with 1 N HCl, then the aqueous layer was applied to an Amberlyst A-26 (Cl^-) column (40 ml). The eluate was concentrated under reduced pressure to *ca.* 50 ml and the concentrate was chromatographed on ODS (100—200 mesh, Fuji-Davison) (300 ml). The column was washed with 1 N phosphate buffer solution (pH 6.86) (600 ml), and eluted with 1 N phosphate buffer–MeCN (97 : 3—95 : 5). Product-containing fractions (TLC) were concentrated to a small volume and re-applied to an ODS column (300 ml). The column was washed with H_2O (900 ml), and eluted with H_2O–MeCN (4 : 6). Product-containing fractions were adjusted to pH *ca.* 4.5 and treated with Amberlyst A-26 (Cl^-). The obtained solution was concentrated and lyophilized to give an epimeric mixture, **6a** (**6b** : **6c** = 1 : 1), as a white amorphous powder (680 mg, 21%). IR (Nujol) cm^{-1}: 1740. ^{1}H-NMR (200 MHz, D_2O) δ: 1.21 (d, 3H, J = 7.1 Hz), 1.30 (d, 3H, J = 6.3 Hz), 1.43—1.56 (m, 0.5H, *R*-isomer), 2.07—2.15 (m, 1H, *S*-isomer), 2.51—2.65 (m, 0.5H, *R*-isomer), 2.98—3.96 (m, 8H), 4.19—4.28 (m, 2H), 4.35 (s, 3H), 7.96 (d, 2H, J = 6.4 Hz), 8.68 (d, 2H, J = 6.4 Hz). MS (FAB) m/z 418 ($MH^+ - Cl^-$).

(4*R*,5*S*,6*S*)-3-{(2*R*,4*S*)-2-[4-(1-Methylpyridinio)methyl]pyrrolidin-4-yl}thio-6-[(1*R*)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]-hept-2-ene-2-carboxylic Acid, Chloride (6b) A solution of **5** (172 g, 0.26 mol) and Ph_3P (13.6 g, 0.052 mol) was prepared in a mixture of THF (680 ml) and EtOH (680 ml), then AcOH (44.6 ml, 0.78 mol), Bu_3SnH (210 ml, 0.78 mol) and a solution of Pd(0) (12.0 g, 0.01 mol) in a mixture of THF (320 ml) and EtOH (320 ml) were added successively. The mixture was stirred at room temperature for 10 min, then the precipitate was collected by filtration, washed with EtOAc (500 ml) and CH_2Cl_2 (500 ml) and dissolved in H_2O (2.5 l). The solution was washed with EtOAc (1.5 l), adjusted to pH *ca.* 4.3 and applied to an Amberlyst A-26 (Cl^-) column (500 ml). The H_2O eluate was concentrated to *ca.* 1.0 l and treated with isopropanol (3.0 l). The mixture was stirred at room temperature for 1 h, then the precipitate was collected, washed with EtOH (1.0 l) and dried under reduced pressure. The crude product was further purified by recrystallization twice from a mixture of H_2O and EtOH to give pure **6b** as a white powder (43.6 g, 37%), purity (HPLC): 99%. IR (KBr) cm^{-1}: 1749, 1718. ^{1}H-NMR (200 MHz, D_2O) δ: 1.21 (d, 3H, J = 7.1 Hz), 1.30 (d, 3H, J = 6.3 Hz), 1.43—1.56 (m, 1H), 2.51—2.65 (m, 1H), 2.98—3.96 (m, 8H), 4.19—4.28 (m, 2H), 4.35 (s, 3H), 7.96 (d, 2H, J = 6.4 Hz), 8.68 (d, 2H, J = 6.4 Hz). MS (FAB) m/z 418 ($MH^+ - Cl^-$). *Anal.* Calcd for $C_{21}H_{28}ClN_3O_4S \cdot 1.8H_2O$, C, 52.63%, H, 6.65%, N, 8.77%. Found, C, 52.69%, H, 6.53%, N, 8.73%.

Allyl (4*R*,5*S*,6*S*)-3-[(2*RS*,4*EZ*)-1-Allyloxycarbonylamino-5-(4-pyridinyl)penta-4-ene-2-yl]thio-6-[(1*R*)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (7) IR (neat) cm^{-1}: 1774, 1720. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.17—1.35 (m, 6H), 1.70—4.20 (m, 8H), 4.56—4.86 (m, 4H), 5.19—5.49 (m, 5H), 5.83—6.04 (m, 2H), 6.35—6.46 (m, 1H), 7.12—7.27 (m, 3H), 8.49 (d, 2H, J = 6.0 Hz). MS (FAB) m/z 528 (MH^+).

Deprotection of the Acyclic Carbapenem 7 The carbapenem **7** (1.26 g) was quaternized and deprotected by the morpholine method in a similar manner to that described for the preparation of **6a** from **5**. A mixture of carbapenems **9** and **6a** was thus obtained (90 mg). This mixture was separated by preparative HPLC (H_2O–MeCN (92 : 8), flow rate 70 ml/min) to give two fractions (fraction 1, 2 components, retention time 9.0 min and 9.9 min; fraction 2, retention time 14 min). Each fraction was adjusted to pH 4.0, passed through an Amberlyst A-26 (Cl^-) column (1.3 ml) and lyophilized to give **9** (fraction 1, 12 mg, 1.1%) and **6a** (fraction 2, 25 mg, 2.3%).

(4*R*,5*S*,6*S*)-3-{(2*RS*,4*R*)-2-[4-(1-Methylpyridinio)methyl]pyrrolidin-4-yl}thio-6-[(1*R*)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]-hept-2-ene-2-carboxylic Acid, Chloride (9) IR (KBr) cm^{-1}: 1755. ^{1}H-NMR (200 MHz, D_2O) δ: 1.20 (d, 3H, J = 7.2 Hz), 1.27 (d, 3H, J = 6.4 Hz), 1.80—2.00 (m, 0.5H), 2.2—2.4 (m, 1H), 2.6—2.8 (m, 0.5H), 3.30—3.95 (m, 7H), 4.00—4.40 (m, 3H), 4.36 (s, 3H), 7.94 (d, 1H, J = 6.8 Hz), 7.98 (d, 1H, J = 6.9 Hz), 8.73 (br d, 2H, J = 6.8 Hz). The ^{1}H-NMR spectrum of **6a** (1 : 1 mixture by HPLC) was identical with that of the sample obtained from **5**. Coinjection with an authentic sample confirmed the identity of **6a**.

HPLC Separation of the Epimers of 6a Epimers of the carbapenem **6a** (1 : 1) were separated by using preparative HPLC (YMC Packed Column, D-ODS-15C, S-15, 120A) (phosphate buffer (pH 6.5)–MeCN (95 : 5), flow rate 50 ml/min). Two fractions were obtained (fraction 1, retention time 15 min; fraction 2, retention time 17 min). Each fraction was immediately adjusted to pH *ca.* 4.5, evaporated to remove the organic solvent, and then re-applied to the HPLC column. The column was washed with H_2O (20 min), then eluted with H_2O–MeCN (4 : 6). The product-containing fractions were adjusted to pH *ca.* 4.5 and treated with Amberlyst A-26 (Cl^-). The obtained solution was evaporated, and lyophilized to give pure **6c** (from fraction 1) and pure **6b** (from fraction 2). The ^{1}H-NMR spectrum of **6b** was identical to that of the material obtained by deprotection of **5** with Pd(0)–Bu_3SnH. **6c** showed the following signals. ^{1}H-NMR (200 MHz, D_2O) δ: 1.22 (d, 3H, J = 7.2 Hz), 1.30 (d, 3H, J = 6.4 Hz), 2.07—2.15 (m, 2H), 2.98—3.95 (m, 8H),

4.19—4.28 (m, 2H), 4.34 (s, 3H), 7.94 (d, 2H, J=6.6 Hz), 8.67 (d, 2H, J=6.6 Hz).

HPLC Analytical Study of Epimerization of 6b at Various pH Values HPLC was performed using the above analytical system (flow rate 1.2 ml/min, mobile phase, CH_3CN–phosphate buffer (pH 3.0)=2:98). FR21751 (**6b**) (HPLC purity *ca.* 92%) was dissolved at a concentration of 2.2 mM in 0.05 M phosphate buffer at various pH values (phosphate buffer solutions were prepared by mixing aqueous solutions of KH_2PO_4 and Na_2HPO_4). The sample solution was then incubated at 20 °C.

Deuterium Exchange Experiment FR21751 (**6b**) was dissolved at a concentration of 44 mM in 0.05 M sodium deuterium phosphate buffer ($NaH_2PO_4+Na_2HPO_4$ in D_2O) (pH 7.0) and incubated at 20 °C. The extent of deuterium exchange of the sample solution was measured by ^{1}H-NMR (500 MHz, Bruker AMX-500).

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