New Straightforward Synthesis and Characterization of a Unique 1β-Methylcarbapenem Antibiotic Biapenem Bearing a σ-Symmetric Bicyclotriazoliumthio Group as the Pendant Moiety

Toshio Kumagai,*[1a] Satoshi Tamai,[1a] Takao Abe,[1a] Hiroshi Matsunaga,[1a] Kazuhiko Hayashi,[1a] Ikuo Kishi,[1a] Motoo Shiro,[1b] and Yoshimitsu Nagao*[1c]

The Chemical and Formulation Research Laboratories, Lederle (Japan), Ltd., Kashiwa-cho, Shiki, Saitama 353-8511, Japan, Rigaku Corporation, 3-9-12 Matsubara-cho, Akishima-shi, Tokyo 196-8666, Japan, and Faculty of Pharmaceutical Sciences, The University of Tokushima, Sho-machi, Tokushima 770-8505, Japan

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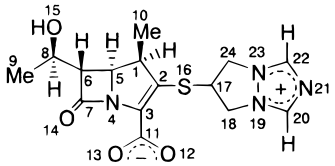
Biapenem **1**, (1*R*,5*S*,6*S*,)-2-[(6,7-dihydro-5*H*-pyrazolo[1,2-*a*][1,2,4]triazolium-6-yl)thio]-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylate, is a new non-natural 1β-methylcarbapenem antibiotic which exhibits a wide range of antibacterial activity, remarkable chemical stability, and extensive stability against human renal dehydropeptidase-I. Mercaptobicyclotriazolium chloride **2** useful for the pendant moiety of **1** was successfully synthesized starting from hydrazine hydrate **3** along an economically available synthetic route. The thiol **2** was efficiently exploited for an expeditious synthesis of biapenem **1**. Characterization (crystal structure, nonbonded S- - -O interaction, conformational analysis, and CH- - -O hydrogen bonds) of **1** was investigated by its X-ray crystallographic, ^{1}H NMR, and deuteration experiment analyses.

Introduction

Since the development of a remarkable non-natural 1β-methylcarbapenem antibiotic by a Merck Sharp & Dohme research group,[2] there have been several reports on new characteristic 1β-methylcarbapenems, such as Meropenem,[3a] biapenem,[3b] lenapenem,[3c] and others.[3b,d,4a,b] These 1β-methylcarbapenems are promising as new-generation non-natural β-lactam antibiotics because of their excellent biological and chemical behavior.[3,4] We have developed a unique biapenem **1**[3b] bearing a σ-symmetric (6,7-dihydro-5*H*-pyrazolo[1,2-*a*][1,2,4]triazolium-6-yl)thio ("bicyclotriazolium"thio) group at C2 as the pendant moiety that is strikingly different from that of other 1β-methylcarbapenems.[3a,c] It is generally known that the C2-pendant moiety of the carbapenem antibiotics plays an important role in their antibacterial activity, chemical stability, and stability against renal dehydropeptidase-I (DHP-I).[5] Biapenem **1** exhibits a wide range of strong antibacterial activity, remarkable chemical stability, and extensive stability against human renal DHP-I.[4c,d] In the earlier synthesis of **1**, we adopted a roundabout synthetic route by which the desired bicyclotriazolium moiety was constructed after introduction of 4-mercaptopyrazolidine bis-*N*-*p*-nitrobenzyloxycarbonyl derivative[6] onto the 1β-methylcarbapenem skeleton **14**.[3b] We now describe an alternative straightforward synthesis of biapenem **1**[7] and its characterization based on X-ray crystallographic and ^{1}H NMR analyses and a deuteration experiment.



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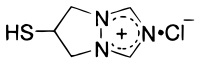

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Results and Discussion

In designing the pendant molecule, a new heterocyclic compound, mercaptobicyclotriazolium chloride **2**, was chosen on the basis of the following consideration. Namely, we wished to establish a reaction cascade (*vide infla*) for construction of this particular heterocycle by treatment of a pyrazolidine derivative with ethyl formimidate hydrochloride. Thus, we attempted to define an economically viable synthesis of **2** starting from hydra-

(1) (a) Lederle (Japan). (b) Rigaku Corporation. (c) The University of Tokushima.

(2) Shih, D. H.; Baker, F.; Cama, L.; Christensen, B. G. *Heterocycles* **1984**, *21*, 29.

(3) (a) Sunagawa, M.; Matsumura, H.; Inoue, Y.; Fukasawa, M.; Kato, M. *J. Antibiotics* **1990**, *43*, 519. (b) Nagao, Y.; Nagase, Y.; Kumagai, T.; Matsunaga, H.; Abe, T.; Shimada, O.; Hayashi, T.; Inoue, Y. *J. Org. Chem.* **1992**, *57*, 4243. (c) Ohtake, N.; Okamoto, O.; Mitomo, R.; Kato, Y.; Yamamoto, K.; Haga, Y.; Fukatsu, Y.; Nakagawa, S. *J. Antibiot.* **1997**, *50*, 598. (d) Wildonger, K. J.; Ratcliffe, R. W. *J. Antibiot.* **1993**, *46*, 1866.

(4) (a) Guthikonda, R. N.; Cama, L. D.; Quesada, M.; Woods, M. F.; Salzmann, T. N.; Christensen, B. G. *J. Med. Chem.* **1987**, *30*, 871. (b) Kim, C. U.; Luh, B. Y.; Misco, P. F.; Hichicock, M. J. *J. Med. Chem.* **1989**, *32*, 601. (c) Ubukata, K.; Hikida, M.; Yoshida, M.; Nishiki, K.; Furukawa, Y.; Tashiro, K.; Konno, M.; Mitsuhashi, S. *Antimicrob. Agents Chemother.* **1990**, 994. (d) Hikida, M.; Kawashima, K.; Nishiki, K.; Furukawa, Y.; Nishizawa, K.; Saito, I.; Kuwao, S. *Antimicrob. Agents Chemother.* **1992**, 481.

(5) Andrus, A.; Baker, F.; Bouffard, F. A.; Cama, L. D.; Christensen, B. G.; Guthikonda, R. N.; Heck, J. V.; Johnston, D. B. R.; Leanza, W. J.; Ratcliffe, R. W.; Salzmann, T. N.; Schmitt, S. M.; Shih, D. H.; Shah, N. V.; Wildonger, K. J.; Wilkening, R. R. In *Recent Advances in the Chemistry of β-Lactam Antibiotics*; Roberts, S. M., Brown, A. G., Eds.; The Royal Society of Chemistry; London, 1984; pp 86−99.

(6) Kumagai, T.; Tamai, S.; Abe, T.; Nagase, Y.; Inoue, Y.; Nagao, Y. *Heterocycles* **1994**, *37*, 1521.

(7) (a) Kumagai, T.; Matsunaga, H.; Machida, R.; Nagase, Y.; Hikida, M.; Nagao Y. *Japan Kokai Tokkyo Koho* (Japanese Patent), 1989, JP S64−25779. (b) Abe, T.; Tamai, S.; Nagase, Y. *Japan Kokai Tokkyo Koho* (Japanese Patent), 1992, JP H4−230267. (c) Abe, T.; Tamai, S.; Nagase, Y. *Japan Kokai Tokkyo Koho* (Japanese Patent), 1992, JP H4−230286. A Merck research group[3d] also achieved an expeditious synthesis of biapenem **1** utilizing our cyclization procedure[3b,7] for the bicyclotriazolium derivative.

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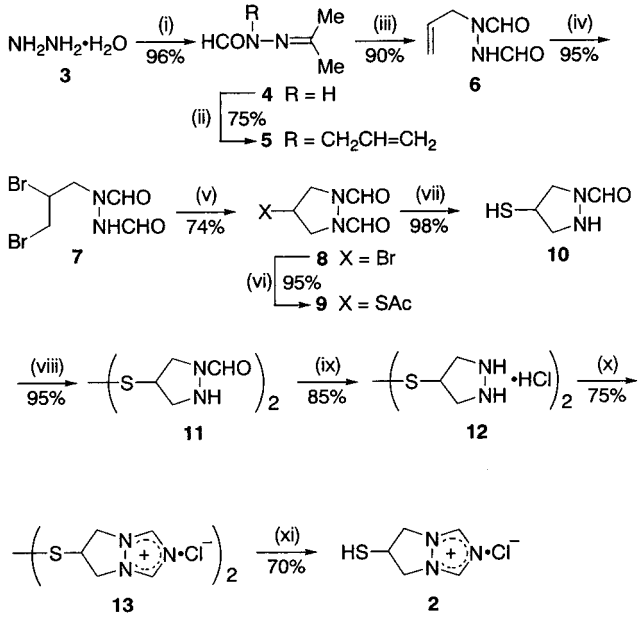
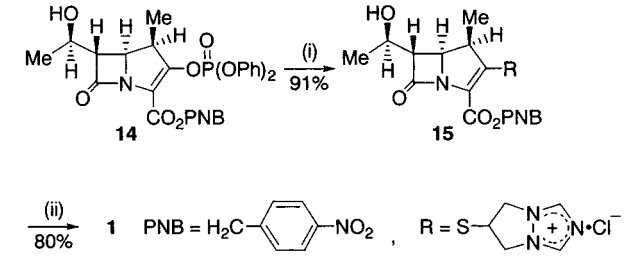
Scheme 1[a]



[a]Key: (i) HCO_2Et, EtOH, -5 °C → rt / acetone, rt; (ii) allyl bromide, K_2CO_3, AcOEt, 80 °C; (iii) HCO_2H, 80 °C; (iv) Br_2, LiBr, CH_2Cl_2, MeOH, 0 °C; (v) K_2CO_3, AcOEt, 40 °C; (vi) AcSK, AcOEt, 40 °C; (vii) KOH, MeOH, 0 °C / HCO_2H, 0 °C; (viii) $FeCl_3$, air, MeOH, rt; (ix) *c*.HCl, MeOH, rt; (x) EtOCH=NH•HCl, $KHCO_3$, H_2O, 0 °C; (xi) Bu_3P, THF, H_2O, 0 °C.

Scheme 2[a]



[a]Key: (i) compound **2**, *i*-Pr_2NEt, MeCN, acetone, DMF; (ii) Zn dust, 0.35 M phosphate buffer solution (pH 5.6).

zine hydrate **3** and an expeditious synthesis of biapenem **1**, as shown in Schemes 1 and 2. Treatment of **3** with HCO_2Et and then acetone gave crystalline compound **4** in 96% yield. Allylation of **4** with allyl bromide employing K_2CO_3 in AcOEt at 80 °C followed by deprotection of ketimine **5** with excess HCO_2H at 80 °C afforded bisformylated allylhydrazine **6** in 68% yield from **4**. Bromination of **6** with Br_2 in the presence of catalytic LiBr in CH_2Cl_2−MeOH at 0 °C followed by cyclization of the resultant dibromide **7** with K_2CO_3 in warm AcOEt furnished cyclic diformyl product **8** as colorless prisms in 70% yield from **6**. Substitution reaction of **8** with potassium thioacetate in warm AcOEt gave crystalline acetylthiolate **9** (95% yield) which was submitted to methanolysis in MeOH containing KOH followed by acidification with a small excess of HCO_2H to obtain thiol **10** in 98% yield. Air oxidation of **10** with catalytic $FeCl_3$ in MeOH followed by deformylation of the resultant disulfide **11** with concd HCl in MeOH gave pyrazolidine disulfide dihydrochloride **12** as colorless prisms in 81% yield from **10**. Then the pyrazolidine derivative **12** was subjected to the reaction cascade toward the desired

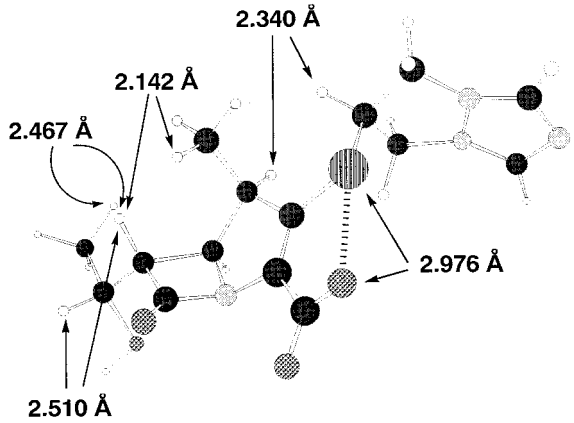


Figure 1. Computer-generated drawing of **1** derived from the X-ray coordinates.

bicyclotriazolium derivative **13**, which has been previously reported by our group.[3b,7] Namely, the compound **12** was allowed to react with ethyl formimidate hydrochloride[3b,7] in the presence of $KHCO_3$ in water at 0 °C, and the crude product was purified on a Dowex 50W-X4 column with 50% MeOH and 6 N HCl−MeOH (1:1) to furnish a crystalline compound bicyclotriazolium disulfide dichloride **13** in 75% yield. An aqueous solution of **13** was treated with a solution of Bu_3P in THF at 0 °C, and the reaction mixture was repeatedly washed with AcOEt. After evaporation of the resultant water layer in vacuo, the crude product was crystallized in isopropyl alcohol to give mercaptobicyclotriazolium chloride **2** as the hydrate compound ($C_5H_8N_3SCl•H_2O$) in 70% yield.

Subsequently, the mercaptobicyclotriazolium moiety was introduced to the 1β-methylcarbapenem skeleton in a Michael type reaction manner, as shown in Scheme 2.[3b] The compound **14**, obtained by our earlier synthetic method,[3b] was treated with **2** in the presence of diisopropylethylamine in a solution of MeCN−acetone−DMF (1:1:0.1) at 0 °C to afford thioether **15** as colorless needles in 91% yield. Finally, deprotection of the *p*-nitrobenzyl group (PNB) of **15** was efficiently performed by our method[8] employing Zn dust as follows. Treatment of **15** with excess Zn dust in 0.35 M phosphate buffer solution (pH 5.6) at room temperature followed by the usual workup[3b,8] of the reaction mixture gave the desired biapenem[3b] in 80% yield.

Successful recrystallization of **1** from aqueous EtOH furnished excellent crystals [colorless needles; mp 210−218 °C (decomp); $[\alpha]^{20}_D$ −33.7° (*c* 0.5, water)] that were then submitted to X-ray analysis. The crystallographic structure of **1** is depicted in Figure 1. The conformation of C6-hydroxyethyl group of **1** is different from that of imipenem[9a] and meropenem.[9b] An intramolecular nonbonded O12- - -S16 interaction ("close contact") is recognized in the crystalline structure of **1**.[10] This O12- - -S16 close contact must be common even in other β-lactam antibiotics.[9a,b,11,12] To learn the conformation of C2 and C6 side chain groups of **1** in D_2O, a nuclear Overhauser effect (NOE) experiment [^{1}H NMR analysis (400 MHz)]

(8) Kumagai, T.; Abe, T.; Fujimoto, Y.; Hayashi, T.; Inoue, Y.; Nagao, Y. *Heterocycles* **1993**, *36*, 1729.

(9) (a) Ratcliffe, R. W.; Wildonger, K. J.; Michele, L. D.; Douglas, A, W.; Hajdu, R.; Goegelman, R. T.; Springer, J. P.; Hishfield, J. *J. Org. Chem.* **1989**, *54*, 653. (b) Yanagi, K.; Takeuchi, Y.; Sunagawa, M. *Acta Crystallogr.* **1992**, *C48*, 1737. (c) Takeuchi, Y.; Inoue, T.; Sunagawa, M. *J. Antibiot.* **1993**, *46*, 827.

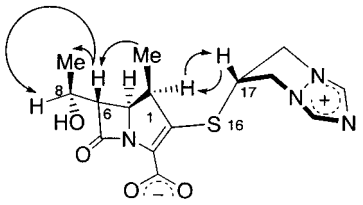
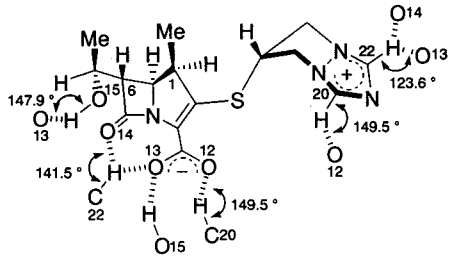


Figure 2. Selected NOE enhancement for **1**.



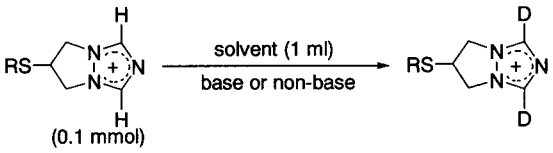
acceptor (A)	doner (D)	A---H / Å	< A---H—D / deg
O13--------H—O15		2.03	147.9
O12--------H—C20		2.25	149.5
O14--------H—C22		2.22	141.5
O13--------H—C22		2.28	123.6

Figure 3. Possible intermolecular hydrogen bonds in the crystal structure of **1**.

was performed. Selected NOE enhancements are illustrated in Figure 2. Interestingly, an NOE enhancement between H1 and H8 was confirmed on the spectrum of meropenem by Sumitomo Pharm. Co. research group[9c] but was not recognized on that of biapenem **1**. On the other hand, an NOE enhancement between H6 and H8 was observed on the spectrum of **1** but was not on that of meropenem.[9c] Thus, the predominant conformation of C6-hydroxyethyl and C2-bicyclotriazoliumthio groups in the molecule **1** in D_2O may be similar to that in the crystalline structure (Figure 1). Particularly, the conformational stability of the bicyclotriazoliumthio group at C2 may be caused by the intramolecular nonbonded S- - -O interaction due to n (O12) σ^* (S16−C17) orbital overlap.[10]

Interestingly, four kinds of possible intermolecular hydrogen bonds (O13- - -H−O15, O12- - -H−C20, O14- - -H−C22, and O13- - -H−C22) were suggested in the crystal structure of **1**. The interatomic distances (A- - -H: ca. 2.03−2.28 Å) between the specific acceptor atom (A: O12, O13, or O14) and hydrogen atom (H) of the specific donor (D: O15, C20, or C22) are unusually shorter than the sum (2.72 Å) of van der Waals radii between oxygen and hydrogen atoms, as shown in Figure 3. The angles (<A- - -H−D: 123.6−149.5°) are also sufficiently large for the hydrogen bonding described above.

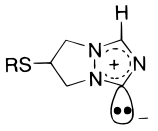
Table 1. Deuteration Experiments of the 6,7-Dihydro-5*H*-pyrazolo[1,2-*a*][1,2,4]triazolium Moiety



compound	conditions: base (mmol)	solvent	time	temp, °C	percent deuteration (%)[a]
1	Et_3N (0.005)	D_2O	2−5 min	rt	100
1	none	D_2O	24 h	rt	0
1	none	D_2O	12 h	50	50
13	Et_3N (0.1)	CD_3OD	2−5 min	rt	100
13	none	D_2O	24 h	rt	50
13	none	D_2O	144 h	rt	100
13	none	D_2O	12 h	50	95
15	Et_3N (0.005)	CD_3OD	2−5 min	rt	67
15	Et_3N (0.005)	CD_3OD	20 min	rt	100
15	Et_3N (0.1)	CD_3OD	2−5 min	rt	100

[a] Each percent deuteration was determined by ^1H NMR analysis.

In order to get an insight into the acidity of both the C20 and C22 hydrogen atoms of the bicyclotriazolium moiety, deuteration experiments (200 MHz ^1H NMR analysis) on compounds **1**, **13**, and **15** were carried out. The results of these experiments are listed in Table 1. When these compounds were exposed to CD_3OD or D_2O containing 1 mol equiv or 5 mol % of Et_3N at room temperature for 2−5 or 20 min, 100% deuteration of both C20 and C22 hydrogens was recognized. Surprisingly, the similar deuteration proceeded even without Et_3N at room temperature or 50 °C. The easy deuteration of the C20 and C22 hydrogens can be explained by the contribution of ylide **16** to the mechanism of the deuteration reaction.



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In conclusion, we have established useful, practical, and large-scale synthetic procedures for the preparation of mercaptobicyclotriazolium chloride **2** and biapenem **1** and clarified some interesting physical and chemical characteristics of **1**.

Experimental Section

General Methods. All reactions were monitored by thin-layer chromatography. Melting points are uncorrected. The column chromatographic separations were performed on Wako gel C-200 (particle size 74−149 μm). Optical rotations and IR and ^1H NMR (270 MHz) spectra were recorded at Medical Research Laboratories, Lederle (Japan), Ltd. Elemental analyses and ^1H NMR (200 and 400 MHz) and all mass (MS, HRMS, FAB, and SIMS) spectra were recorded at Faculty of Pharmaceutical Sciences, The University of Tokushima.

1-Formyl-2-isopropylidenehydrazine (4). To a solution of hydrazine hydrate (377 g, 7.54 mol) in EtOH (760 mL) was added dropwise ethyl formate (726 mL, 9 mol) at −5 °C over

(10) The nonbonded O12- - -S16 atoms distance (2.976 Å) is significantly shorter than the sum (3.35 Å) of the van der Waals radii of oxygen and sulfur atoms. In addition, a linear relationship between the O12 atom and the S16−C17 σ bond and a plane conformation of the O12−C11−C3−C2−S16 moiety can support the O12- - -S16 close contact. See, Kucsman, A.; Kapovits, I. *Organic Sulfur Chemistry: Theoretical and Experimental Advances*; Bernardi, F., Csizmadia, I. G., Mangini, A., Eds.; Elsevier Scientific Publishing Co.: Amsterdam, 1985; pp 191−245.

(11) Nagao, Y.; Abe, T.; Shimizu, H.; Kumagai, T.; Inoue, Y. *J. Chem. Soc., Chem. Commun.* **1989**, 821.

(12) The intramolecular nonbonded S- - -O interactions recognized in several drugs have been reported by Y. Nagao et al. Nagao, Y.; Hirata, T.; Goto, S.; Sano, S.; Kakei, A.; Iizuka, K.; Shiro, M. *J. Am. Chem. Soc.* **1998**, *120*, 3104.

1 h. The mixture was stirred at −5 °C for 30 min and at room temperature for 14 h. The reaction mixture was poured into acetone (1011 mL, 15 mol) over 30 min and then stirred at room temperature for 30 min. The resultant solution was evaporated to dryness in vacuo to give **4** (721 g, 96% yield) as colorless needles: mp 68−69 °C (EtOH); IR (KBr) ν_{max} 3200, 1690, 1640 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 1.92 (s, 3H), 2.00 (s, 3H), 8.65 (d, 1H, J = 9.9 Hz), 9.75 (d, 1H, J = 9.9 Hz); HRMS calcd for $C_4H_8N_2O$ MW 100.0637, found m/z 100.0637 (M^+).

1-Allyl-1-formyl-2-isopropylidenehydrazine (5). To a solution of **4** (254 g, 2.54 mol) in EtOAc (762 mL) were added allyl bromide (330 mL, 3.81 mol) and powdered K_2CO_3 (877 g, 6.35 mol). The mixture was stirred at 80 °C for 5 h. After cooling to room temperature, the reaction mixture was filtered, and the filtrate was concentrated in vacuo. The oily residue was purified by vacuum distillation to give **5** (266.7 g, 75% yield) as a colorless oil. Compound **5** was found to be a mixture of conformational isomers (3:1) due to the N−CHO bond rotation: bp 60 °C/4 mmHg; IR ($CHCl_3$) ν_{max} 1660 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 1.74 (s, 0.75H), 1.86 (s, 2.25H), 1.99 (s, 0.75H), 2.13 (s, 2.25H), 4.09 (d, 1.5H, J = 6.3 Hz), 4.23 (d, 0.5H, J = 6.3 Hz), 5.17−5.37 (m, 2H), 5.66−5.91 (m, 1H), 7.93 (s, 0.25H), 7.97 (s, 0.75H); HRMS calcd for $C_7H_{12}N_2O$ MW 140.0950, found m/z 140.0974 (M^+).

1-Allyl-1,2-diformylhydrazine(6). A solution of ketimine **5** (210 g, 1.5 mol) in formic acid (420 mL) was stirred at 80 °C for 15 h followed by evaporation of the solvent in vacuo. The residue was chromatographed on a silica gel column with EtOAc to give **6** (173 g, 90%) as a colorless oil: IR ($CHCl_3$) ν_{max} 3400, 1720, 1685 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 4.13−4.18 (m, 2H), 5.23−5.38 (m, 2H), 5.66−5.90 (m, 1H), 8.01−8.32 (m, 3H); HRMS calcd for $C_5H_8N_2O_2$ MW 128.0586, found m/z 128.0598 (M^+).

1-(2,3-Dibromopropyl)-1,2-diformylhydrazine (7). To a solution of **6** (154 g, 1.2 mol) in CH_2Cl_2 (750 mL) were added a solution of $LiBr\cdot H_2O$ (124 g, 1.2 mol) in MeOH (250 mL) and a solution of Br_2 (199 g, 1.26 mol) in CH_2Cl_2 (250 mL) at 0 °C, and the mixture was stirred at 0 °C for 10 min. To the reaction mixture were successively added a suspension of $NaHCO_3$ (396 g) in H_2O (250 mL) and saturated aqueous Na_2SO (250 mL). After separation of the CH_2Cl_2 layer, the aqueous layer was extracted with EtOAc (3 × 500 mL). The CH_2Cl_2 and EtOAc extracts were combined and dried over $MgSO_4$ followed by evaporation of the solvent in vacuo. The residue was chromatographed on a silica gel column with EtOAc−hexane, (3:1) to give **7** (326 g, 95%) as a colorless oil: IR ($CHCl_3$) ν_{max} 1715, 1690 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 3.60−4.50 (m, 5H), 8.10−8.30 (m, 2H), 8.77 (s, 1H); HRMS calcd for $C_5H_8N_2O_2$-Br_2 MW 285.8954, found m/z 285.8969 (M^+).

4-Bromo-1,2-diformylpyrazolidine (8). To a solution of dibromide **7** (214 g, 0.75 mol) in dry EtOAc (1070 mL) was added powdered anhydrous K_2CO_3 (207 g, 1.5 mol), and then the mixture was stirred at 40 °C for 6 h. After filtration of the mixture, the filtrate was evaporated in vacuo. The residue was chromatographed on a silica gel column with EtOAc−hexane (3:1) to give **8** (114 g, 74%) as colorless prisms: mp 83−84 °C (EtOAc); IR ($CHCl_3$) ν_{max} 1700 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 3.80−4.40 (m, 4H), 4.60−4.80 (m, 1H), 8.50 (s, 2H); Anal. Calcd for $C_5H_7N_2O_2Br$: C, 29.01; H, 3.41; N, 13.53. Found: C, 28.95; H, 3.43; N, 13.75.

4-(Acetylthio)-1,2-diformylpyrazolidine (9). A mixture of bromide **8** (89 g, 0.43 mol) and potassium thioacetate (72 g, 0.63 mol) in EtOAc (450 mL) was stirred at 40 °C for 6 h. After filtration of the reaction mixture, the filtrate was evaporated in vacuo. The residue was chromatographed on a silica gel column with EtOAc−hexane (3:1) to give **9** (83 g, 95%) as colorless prisms: mp 47−48 °C (EtOH); IR ($CHCl_3$) ν_{max} 1700, 1695 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 2.37 (s, 3H), 3.50−3.60 (m, 2H), 4.00−4.20 (m, 3H), 8.42 (s, 2H); Anal. Calcd for $C_7H_{10}N_2O_3S$: C, 41.58; H, 4.98; N, 13.85. Found: C, 41.51; H, 5.03; N, 14.10.

1-Formyl-4-mercaptopyrazolidine (10). To a solution of acetylthiolate **9** (50.5 g, 0.25 mol) in MeOH (180 mL) was added a solution of KOH (14 g, 0.25 mol) in MeOH (130 mL) at 0 °C, and then the mixture was stirred at 0 °C for 10 min followed by addition of formic acid (12 mL, 0.26 mol). After filtration of the mixture, the filtrate was evaporated in vacuo. The residue was chromatographed on a silica gel column with EtOAc to give **10** (32.3 g, 98%) as a colorless oil: IR ($CHCl_3$) ν_{max} 1665 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 1.85 (d, 1H, J = 5.6 Hz), 2.82−2.91 (m, 1H), 3.19−3.29 (m, 2H), 3.69−3.79 (m, 1H), 3.98 (dd, 1H, J = 7.9 and 12.2 Hz), 8.44 (s, 1H); HRMS calcd for $C_4H_8N_2OS$ MW 132.0357, found m/z 132.0345 (M^+).

Bis(1-formylpyrazolidin-4-yl) Disulfide (11). To a solution of thiol **10** (33 g, 0.25 mol) in MeOH (330 mL) was added a solution of $FeCl_3$ (405 mg, 2.5 mmol) in MeOH (40 mL) at 0 °C, and air was bubbled into the solution at room temperature for 2 h. After evaporation of the reaction mixture in vacuo, the residue was chromatographed on a silica gel column with CH_2Cl_2−EtOH (9:1) to give **11** (31 g, 95%) as a colorless oil: IR ($CHCl_3$) ν_{max} 1665 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 3.10−4.00 (m, 10H), 8.42 (s, 2H),; HRMS calcd for $C_8H_{14}N_4O_2S_2$ MW 262.0558, found m/z 262.0531 (M^+).

Bis(4-pyrazolidinyl) Disulfide Dihydrochloride (12). A solution of disulfide **11** (31 g, 0.119 mol) in concd HCl (40 mL, 0.48 mol) and MeOH (400 mL) was stirred at room temperature for 6 h to give a precipitate. The desired precipitate was filtered off, and the filtrate was evaporated to dryness in vacuo. The solid residue was suspended with a small amount of MeOH, and then the precipitate was filtered off. The combined precipitate was dried in vacuo to give **12** (28 g, 85%) as colorless prisms: mp 194 °C (aq MeOH); IR (KBr) ν_{max} 3200, 3000−2500 cm^{-1}; ^1H NMR (D_2O, 270 MHz) δ 3.39 (dd, 4H, J = 3.8 and 13.0 Hz), 3.59 (dd, 4H, J = 6.8 and 13.0 Hz), 3.94−4.02 (m, 2H); FAB MS m/z 207 [(M − 2Cl − H)$^+$]; FAB HRMS calcd for $C_6H_{17}N_4S_2Cl_2$ MW − 2Cl − H 207.0738, found m/z 207.0716 [(M − 2Cl − H)$^+$].

Bis(6,7-dihydro-5*H*-pyrazolo[1,2-*a*][1,2,4]triazolium-6-yl) Disulfide Dichloride (13). To a solution of **12** (28 g, 0.1 mol) and $KHCO_3$ (20 g, 0.2 mol) in H_2O (700 mL) was added ethyl formimidate hydrochloride (109 g, 1 mol) at 0 °C, and the mixture was stirred at 0 °C for 10 min. After adjusting to pH 2 with 6 N HCl, the acidic reaction mixture was evaporated to dryness in vacuo. The solid residue was suspended with MeOH (420 mL) and then the undesired precipitate was filtered off. The filtrate was evaporated in vacuo to give an oily residue. The residue was chromatographed on a DOWEX-X4 (H^+ type) resin column with MeOH−H_2O (1:1) and then 6 N HCl−MeOH (1:1) to give **13** (26.5 g, 75%) as colorless prisms: mp 183 °C (decomp) (MeOH); ^1H NMR (D_2O, 270 MHz) δ 4.70−4.85 (m, 6H), 4.85−5.00 (m, 4H), 8.90 (s, 4H); FAB MS m/z 317 [(M − Cl)$^+$]; FAB HRMS calcd for $C_{10}H_{14}N_6S_2$-Cl_2 MW − Cl 317.0410, found m/z 317.0433 [(M − Cl)$^+$].

6,7-Dihydro-6-mercapto-5*H*-pyrazolo[1,2-*a*][1,2,4]-triazolium Chloride (2). To a solution of **13** (17.7 g, 0.05 mol) in THF (90 mL) and H_2O (90 mL) was added *n*-Bu_3P (20.2 g, 0.1 mol) at 0 °C, and then the mixture was stirred at 0 °C for 1 h. After removal of THF in vacuo, the resultant aqueous solution was washed with EtOAc followed by concentration in vacuo. The residue was chromatographed on a Diaion SP-207 resin column with H_2O to give **2** (12.4 g, 70%) as colorless prisms: mp 127−128 °C (decomp) (*n*-PrOH); IR (KBr) ν_{max} 2400 cm^{-1}; ^1H NMR (D_2O, 270 MHz) δ 4.20−4.35 (m, 2H), 4.70−4.85 (m, 3H), 8.67 (s, 2H); FAB MS m/z 142 [(M − Cl)$^+$]; FAB HRMS calcd for $C_5H_8N_3SCl$ MW − Cl 142.0439, found m/z 142.0486 [(M − Cl)$^+$]; Anal. Calcd for $C_5H_8N_3SCl\cdot H_2O$: C, 30.69; H, 5.15; N, 21.48. Found: C, 30.51; H, 4.76; N, 21.58.

***p*-Nitrobenzyl (1*R*,5*S*,6*S*)-2-[(6,7-dihydro-5*H*-pyrazolo-[1,2-*a*][1,2,4]triazolium-6-yl)thio]-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylate Chloride (15).** To a suspension of *p*-nitrobenzyl (1*R*,5*R*,6*S*)-2-(diphenylphosphoryloxy)-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylate **14** (5.95 g, 10 mmol) and thiol **2** (2.3 g, 13 mmol) in a mixture of MeCN (18 mL), acetone (18 mL), and DMF (1.8 mL) was added diisopropylethylamine (2.1 g, 16.3 mmol) at 0 °C, and the mixture was stirred at 0 °C for 2 h to afford a precipitate. The desired precipitate was filtered off and washed with CH_2Cl_2 followed by dryness in vacuo to give **15** (4.74 g, 91%) as colorless needles: mp 163−168 °C (decomp)

(DMF−CH_2Cl_2); $[\alpha]^{25}_D$ +55.4° (*c* 0.5, H_2O); IR (KBr) ν_{max} 1760, 1695 cm^{-1}; ^1H NMR (CD_3OD, 270 MHz) δ 1.40 (d, 3H, J = 7.3 Hz), 1.43 (d, 3H, J = 6.3 Hz), 3.53 (dd, 1H, J = 3.0 and 6.3 Hz), 3.70 (dq, 1H, J = 7.3 and 9.6 Hz), 4.26 (quint, 1H, J = 6.3 Hz), 4.48 (dd, 1H, J = 3.0 and 9.6 Hz), 4.81 (d, 1H, J = 12.2 Hz), 4.82 (d, 1H, J = 12.2 Hz), 5.18−5.31(m, 3H), 5.46 (d, 2H, J = 13.9 Hz), 7.78 (d, 2H, J = 8.9 Hz), 8.30 (d, 2H, J = 8.9 Hz), 9.16 (s, 1H), 9.18 (s, 1H); FAB MS *m*/*z* 486 $[(M - Cl)^+]$; FAB HRMS calcd for $C_{22}H_{24}N_5O_6SCl$ MW − Cl 486.1447, found *m*/*z* 486.1425 $[(M - Cl)^+]$. Anal. Calcd for $C_{22}H_{24}N_5O_6SCl\cdot^3/_2H_2O$: C, 48.13; H, 4.96; N, 12.76. Found: C, 48.44; H, 5.25; N, 12.92.

(1*R*,5*S*,6*S*)-2-[(6,7-Dihydro-5*H*-pyrazolo[1,2-*a*][1,2,4]-triazolium-6-yl)thio]-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylate (1). To a solution of thioether **15** (1.042 g, 2 mmol) in 0.35 M phosphate buffer solution (pH 5.6) (35 mL) was added zinc dust (8.3 g), and then the mixture was stirred at room temperature for 1 h. The insoluble materials were filtered off through a Celite pad, and the filtrate was adjusted to pH 5.5 with 0.1 N HCl followed by concentration in vacuo. The oily residue was chromatographed on a Diaion SP-207 resin column with isopropyl alcohol−H_2O (5:95) to give **1** (0.56 g, 80%) as colorless needles: mp 210−218 °C (decomp) (H_2O−EtOH); $[\alpha]^{20}_D$ −33.7° (*c* 0.5, H_2O); IR (KBr) ν_{max} 1750, 1600 cm^{-1}; ^1H NMR (D_2O, 270 MHz) δ 1.26 (d, 3H, J = 7.3 Hz), 1.30 (d, 3H, J = 6.6 Hz), 3.41 (dq, 1H, J = 7.3 and 9.6 Hz), 3.53 (dd, 1H, J = 3.0 and 5.9 Hz), 4.27 (dq, 1H, J = 5.9 and 6.6 Hz), 4.31 (dd, 1H, J = 3.0 and 9.6 Hz), 4.71−4.80 (m, 2H), 4.98 (m, 1H), 5.04−5.15 (m, 2H), 9.02 (s, 1H), 9.04 (s, 1H); SIMS *m*/*z* 351 $[(M + 1)^+]$. Anal. Calcd for $C_{15}H_{18}N_4O_4S$: C, 51.42; H, 5.18; N, 15.99. Found: C, 51.09; H, 5.18; N, 15.96.

General Procedure for Deuteration Experiments of the Compounds 1, 13, and 15. To a solution of each compound (0.1 mmol) in CD_3OD (1 mL) or D_2O (1 mL) was added Et_3N (0.005 or 0.1 mmol). The mixture was allowed to stand for a time indicated in Table 1 at room temperature and then the D-content of C20 and C22 hydrogens in each sample was determined by the 200 MHz ^1H NMR analysis at room temperature. The deuteration experiments and the D-content analysis for **1** and **13** in the absence of Et_3N were similarly carried out at room temperature or 50 °C, as described above. All experimental results are recorded in Table 1.

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Supporting Information Available: ^1H NMR spectra for new compounds which were not characterized by combustion analysis and X-ray characterization data for biapenem **1**, including tables of experimental details, atomic coordinates, anisotropic displacement parameters, bond lengths, bond angles, torsion angles, nonbonded contacts out to 3.60 Å, intermolecular hydrogen bonds, ORTEP drawing, and crystal structure (36 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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