Theoretical Study of the Reaction from 6-Methylidene Penem to Seven-Membered Ring Intermediates

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Received: January 26, 2007; In Final Form: March 19, 2007

A sort of β-lactamase inhibitor, 6-methylidene penem can inhibit both class A and class C serine β-lactamase. Its inhibition mechanism involves yielding a seven-membered ring intermediate after acylation of the serine. Density functional theory (DFT) method was used on the molecular model to determine the mechanism of producing the seven-membered ring intermediate. Solvent effects were considered via polarizable continuum model (PCM). Moreover, a water-assisted process was considered in the hydrogen transfer process. The results show that the seven-membered ring intermediate can be obtained via two possible mechanisms, namely, concerted mechanism and stepwise mechanism. In stepwise mechanism, a new thiirane intermediate which has never been reported was found. The product of stepwise mechanism, **e**, has five tautomerics, and they can be tautomerized by hydrogen transfer.

Introduction

β-Lactam antibiotics are currently the most widely used antimicrobial agents by virtue of their high efficiency, broad spectrum, and low toxicity. However, their massive misuse has caused bacterial strains to become resistant. The primary defensive mechanism of the bacteria involves the production of β-lactamases. These β-lactamases can be divided into four classes on the basis of the activity site differences, and class A and C β-lactamases are the most commonly encountered clinically. They can inactivate β-lactam antibiotics through an acylation−deacylation mechanism.[1−3] An approach to combat resistance is to coadminister the β-lactam with a β-lactamase inhibitor. β-Lactamase inhibitor can bind to β-lactamases in an irreversible (e.g., so-called "suicidal substrates" such as clavulanic acid) or a reversible manner with low turnover rates (e.g., third-generation cephalosporins), consequently protecting the antibiotics.[4,5] Inhibitors that are presently available (such as clavulanic acid, sulbactam, and tazobactam) are generally effective against the class A enzymes,[6] and there is mcuh experimental evidence about their actions.[7−10] However, their usefulness is rapidly declining with the appearance of mutant forms, and they display little inhibition of the class C enzymes.[11,12] So, it is desirable to develop a more effective inhibitor that is able to inhibit all classes of the serine-reactive β-lactamases.

A new kind of β-lactamase inhibitors, 6-methylidene penems, have appeared,[13,14] which were found to exhibit significant inhibitory activity against both class A and C serine β-lactamases. They have a heterocyclic substituent at the C6 position with a Z configuration. The heterocyclic substituent can be triazolyl (Figure 1 BRL42715) or [6′5]-fused double ring (Figure 1, **a**) and so forth. Their structure, activity, and inhibit mechanism have been explored in many experiments.[15−20] The experimental results suggest that after acylation of the serine, a nucleophilic attack by the departing thiolate on the C6′ atom yields a novel seven-membered ring which has different stereochemistry on C6′. The intermediate can stabilize the acyl ester bond to hydrolysis, and they have three tautomeric forms, 2,6′-dihydro-, 4, 6′-dihydro-, and 6, 6′-dihydro-1,4-thiazepines. However, the detailed processes of these reactions still need to be discussed.

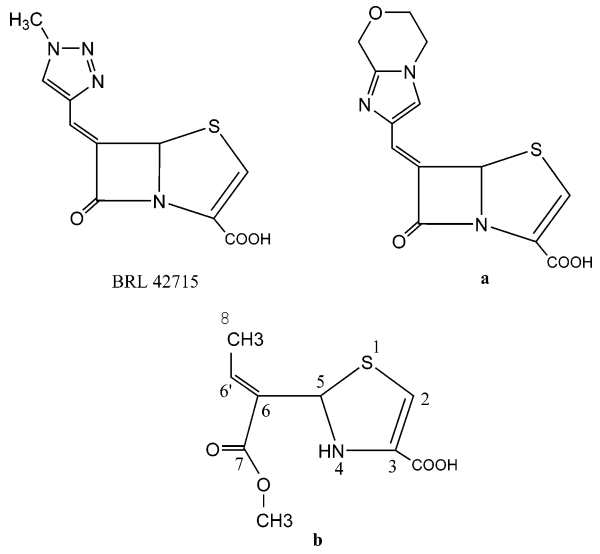

Figure 1. Chemical Structure of methylene penem inactivators.

Nukaga et al.[16] trapped the dihydrothiazepine intermediate by cryocrystallography. They concluded that ring opening of the five-membered thiazole ring produced a reactive linear thiolate species, and it underwent a seven-endo-trig cyclization to produce thiazepine ring. However, there is no evidence of linear thiolate, and they consider that the linear species is not stabilized in class A and class C binding sites.

Michaux et al.[19] investigated the stereoselective mechanism of action of the inhibitor and lactamases. They suggested that enzyme structure affected the complex configuration. In class C 908R β-lactamase, the triazolyl cycle of BRL42715 was able to stack with Tyr221, and the complex configuration was S, while the heterocyclic double ring of **a** (Figure 1) in class C extended-spectrum GC1 β-lactamase could not stack with

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10.1021/jp070685q CCC: $37.00
Published on Web 05/04/2007

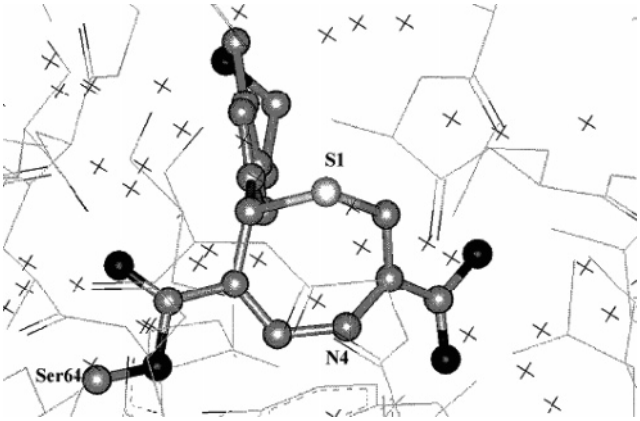


Figure 2. The conformation of seven-membered ring intermediate in 1ONH.

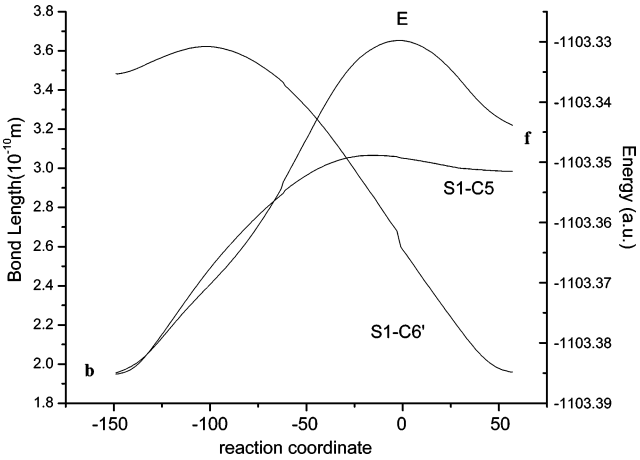


Figure 3. The profiles of the changes of the energy and the bond lengths of IRC of concerted mechanism.

tyrosine residue, which made the binding site more flexible, and so the configuration of the complex was R.

In this work, we studied the reaction mechanism of a novel methylidene penem **a** (Figure 1) which inhibited a class C β-lactamase GC1. The study was based on a quantum chemical modeling. Here, we described the form of a seven-membered ring complex and tautomerism between intermediates.

2. Models and Methods

In the structure of **a** with GC1 (PDB entry 1ONH),[19] the absolute configuration at C6′ is R (Figure 2). On the basis of this structure, we built a model **b** (Figure 1) as the initial reactant. To predigest calculation, the heterocyclic double ring was replaced by methyl, and the ser64 binding with inhibitor was also replaced by methoxy. In this model, the lactam ring was open, which resulted from ser64 attacking lactam carbonyl. There have been many works on the open lactam ring,[21−27] and so we do not consider this process here.

The geometries of reactants, intermediates, transition states, and products were fully optimized using density functional theory B3LYP[28] at 6-31G* levels. All energies were calculated including zero-point vibrational energy (ZPE). Frequencies of all stationary points were carried out at B3LYP/6-31G* levels. Reactants, intermediates, and products have no imaginary frequencies and each transition state has only one imaginary frequency. IRC[29] calculations at B3LYP/6-31G* levels were performed to confirm these transition states. Considering the solvent effect, the energies of all structure in water were calculated by using polarizable continuum model (PCM)[30] method at the B3LYP/6-31G* levels. The standard dielectric constant of water implemented in the Gaussian program was employed. These calculations have been carried out using the GAUSSIAN03[31] package of programs.

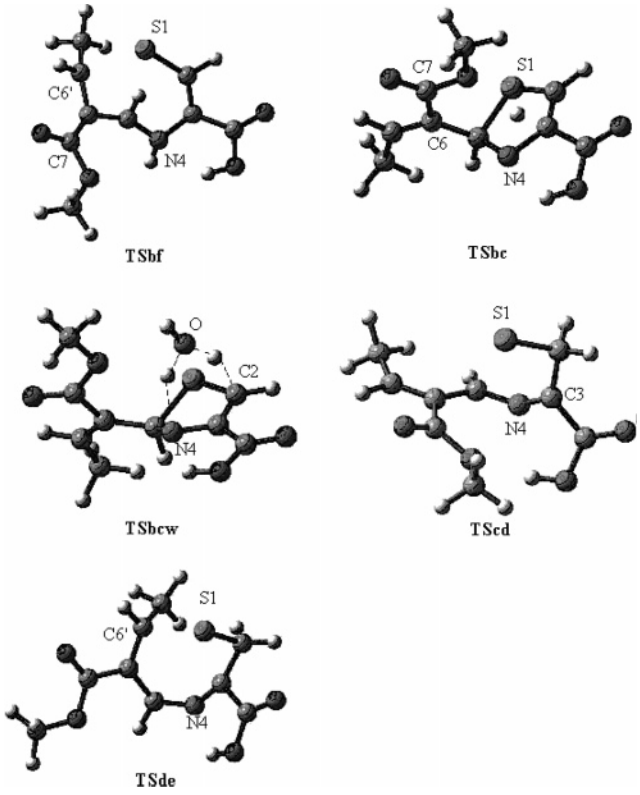


Figure 4. The transition states in concerted and stepwise mechanisms.

To calculate the energy barrier of a water-assisted process, obtaining the energy of reactants and products in each reaction step combined with water is required. Because we adopt a simple model, direct optimization on these hydrates will cause unsuitable changes of the model. (However, in transition state, there is no problem because molecular water is a bridge of H migration which is fixed between two groups.) Therefore, we define the energies of these hydrates as

$$E_{XW} = E_X + E_W - E_H$$

In the formula, E_X is energy of anhydrous compound, E_W is energy of one water molecule (−76.38779 au in B3LYP/6-31G* level), and E_H is hydrogen bond energy which combines with a water molecule. Here, E_H is different according to the atom which links the hydrogen bond. In our paper, the atoms C2 and N4 are involved, so we evaluate the hydrogen bond energy of $(CH3)_2NH-OH_2$ (0.00433au) and $(CH3)_2CH_2-OH_2$ (0.00172 au) to be E_H approximately, respectively.

3. Results and Discussion

Cartesian coordinates of optimized reactants, transition states, and products are listed in the Supporting Information.[32] Energies of all stationary points are summarized in Table 1 and Table 3. The results showed that the solvent effect decreased the energy of each configuration, but the effects on the changes of relative energies were not the same. However, compared with the decreased energy barriers arising from the water-assisted process, they are quite small. Thus, more attention is paid to catalysis for the action of water in the reactions. The main geometric parameters are listed in Table 2 and Table 4.

TABLE 1: Energy of the Reactants, Intermediates, Transition States, and the Hydrates in Concerted and Stepwise Mechanism

	E + ZPE[c]	E_{XW}[a]	rel energy	E + ZPE (PC M)	E_{XW}[a] (PCM)	rel energy (PC M)
b	−1103.19212		0	−1103.21365		0
tsbc	−1103.08635		66.37	−1103.10577		67.70
c	−1103.20668		−9.14	−1103.22521		−7.36
tscd	−1103.13508		35.79	−1103.16899		28.02
d	−1103.17236		12.40	−1103.19249		13.28
tsde	−1103.14873		27.23	−1103.16802		28.63
e	−1103.20145		−5.85	−1103.21871		−3.18
tsbf	−1103.13262		37.34	−1103.15918		34.18
f	−1103.14819		27.57	−1103.16863		28.25
bw		−1179.58424	0[b]		−1179.60577	0[b]
tsbcw	−1179.51685		42.28	−1179.54206		39.98
cw		−1179.59619	−7.50		−1179.61472	−5.61

[a] E_{XW} is the energy of hydrates. [b] In water-assisted hydrogen transfer, the relative energy of **bw** is 0. [c] E, ZPE, and E_{XW} in hartrees and relative energies in kcal/mol.

TABLE 2: Main Geometric Parameters in Concerted and Stepwise Mechanism

	S1−C5	N4−C5	S1−C6′	C3N4C5	S1−X2−X3−N4	C3−N4−X5−X6	N4−X5−X6−X7	C8−X6′−C6−C5
b	1.930	1.456	3.807	107.9	−2.4	−157.1	51.9	1.5
tsbc	1.977	1.441	3.820	111.1	5.0	−112.5	47.6	−1.1
c	1.892	1.453	3.825	114.9	−4.5	−123.8	55.3	1.3
tscd	3.142	1.303	4.139	137.1	63.4	−67.9	−0.4	−6.3
d	3.326	1.279	4.066	125.3	116.4	−3.0	−50.5	−5.7
tsde	3.426	1.308	2.429	130.7	110.4	7.9	−135.3	54.0
e	3.322	1.389	1.835	129.0	58.8	−20.4	−171.0	106.8
tsbf	3.056	1.341	2.639	111.5	7.6	−119.4	−18.8	57.7
f	3.030	1.412	1.919	101.1	2.9	−85.5	−26.0	62.5

TABLE 3: Energy of the Reactants, Intermediates, Transition States, and Hydrates in Tautomerism of Intermediates

(A) The Energy of the Seven-Membered Ring Intermediate

	E + ZPE[b]	rel energy	E + ZPE (PCM)	rel energy (PCM)
e	−1103.20145	0	−1103.21871	0
g1	−1103.19782	2.28	−1103.22058	−1.17
g2	−1103.19479	4.18	−1103.21759	0.70
h1	−1103.20284	−0.88	−1103.22226	−2.22
h2	−1103.20042	0.65	−1103.22171	−1.88

(B) The Energy of Transition State and Intermediate Hydrates

	E + ZPE	E_{XW}[a]	rel energy	E + ZPE (PC M)	E_{XW}[a] (PCM)	rel energy (P CM)
ew		−1179.59096	0		−1179.60822	0
tseg1	−1179.52838		39.27	−1179.56348		28.07
tseg2	−1179.51778		45.92	−1179.55277		34.80
g1w		−1179.58994	0.64		−1179.61270	−2.81
g2w		−1179.58691	2.54		−1179.60971	−0.93
tsg1h1	−1179.52433		41.81	−1179.56353		28.04
tsg2h2	−1179.52309		42.59	−1179.56185		29.10
h1w		−1179.59235	−0.87		−1179.61177	−2.23
h2w		−1179.58993	0.65		−1179.61122	−1.88

[a] E_{XW} is the energy of hydrates. [b] E, ZPE, and E_{XW} in hartrees and relative energies in kcal/mol.

TABLE 4: Main Geometric Parameters in Tautomerism of Intermediates

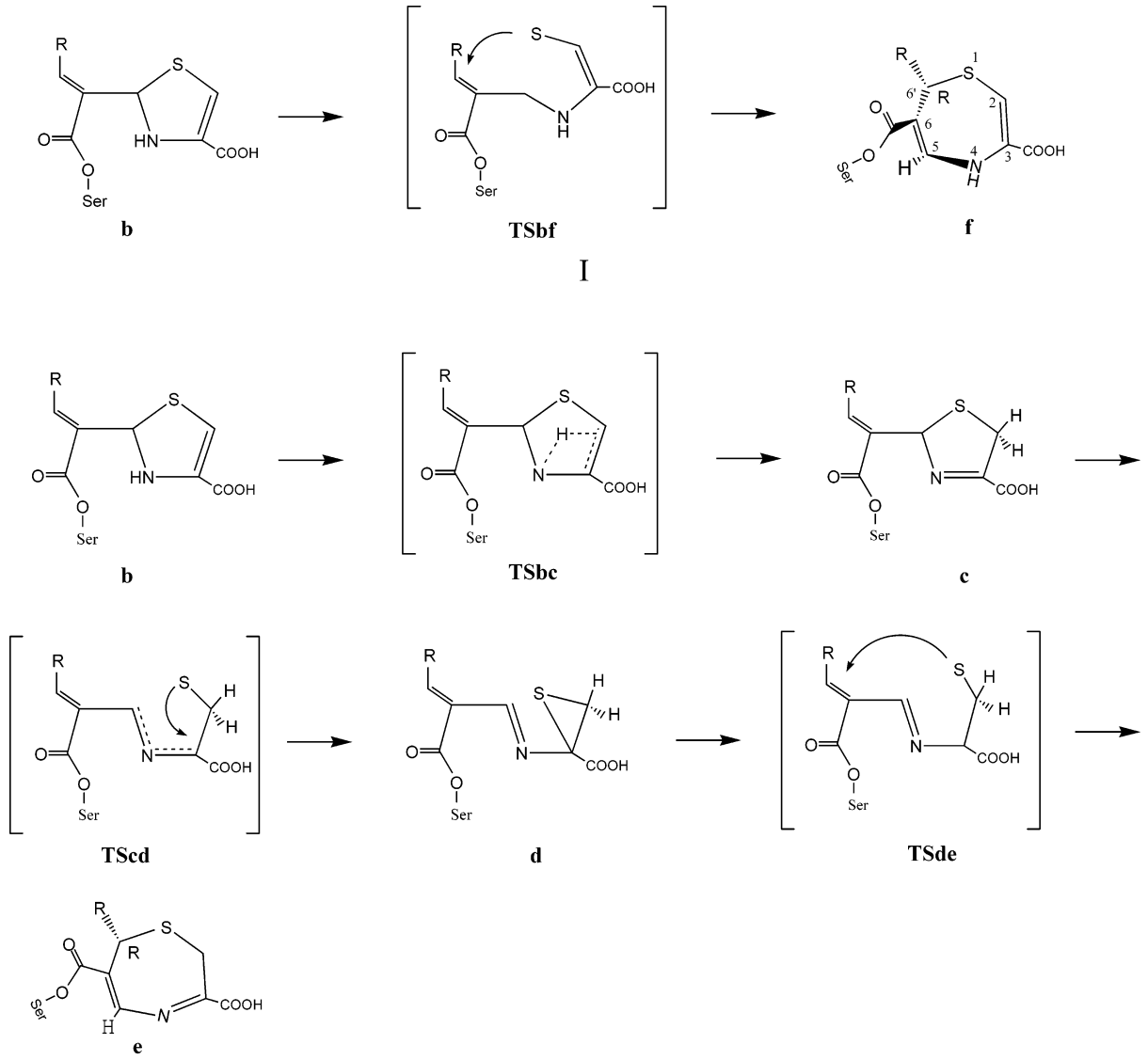
	C2−C3	C3−N4	N4−C5	C5−C6
e1	1.493	1.286	1.389	1.361
e2	1.493	1.286	1.389	1.361
tseg1	1.387	1.365	1.379	1.364
tseg2	1.382	1.364	1.371	1.375
g1	1.349	1.414	1.379	1.355
g2	1.354	1.406	1.378	1.356
tsg1h1	1.353	1.411	1.341	1.406
tsg2h2	1.355	1.399	1.345	1.408
h1	1.360	1.397	1.278	1.518
h2	1.360	1.393	1.280	1.516

3.1. Reactant. Reactant **b** was optimized. The opened lactam ring was not a plane structure because of the rotation of C5−C6, and the dihedral angle C7−C6−C5−N4 is 51.9° (Table 2). The relative energy of **b** is 0 kcal/mol.

3.2. Mechanisms of Producing Seven-Membered Ring Intermediate. There are two possible mechanisms from **b** to seven-membered ring intermediate: concerted and stepwise. These two mechanisms are shown in Scheme 1, where I represents concerted mechanism and II represents stepwise mechanism.

3.2.1. Concerted Mechanism. Reactant **b** can evolve with cleavage of S1−C5 bond through transition-state **tsbf**, and the activation energy of this process is 37.34 kcal/mol. To verify the mechanism farther, we carried out IRC calculation on **tsbf**, and the changes of the bond lengths of S1−C5 and S1−C6′ along with the energy are shown in Figure 3. While the length of S1−C5 gradually increases, the length of S1−C6′ has no change in the beginning but a distinct decrease later. It indicates that the processes of S1 leaving from C5 and attacking C6′ are simultaneous, and a seven-membered thiazepine ring is formed. So, this process is concerted.

SCHEME 1: The Reaction Mechanism of Seven-Membered Ring Intermediate Form Where I Represents Concerted Mechanism and II Represents Stepwise Mechanism



The product of the concerted process is seven-membered ring intermediate **f**, and the relative energy of **f** is 27.57 kcal/mol. There are two double bond C2−C3 and C5−C6 in the structure of **f**. On the C5−C6 double bond, C7 and N4 are in cis, and the dihedral angle C7−C6−C5−N4 is −26.0°, which makes the entire seven-membered ring foldaway. This conformation is different from the one in 1ONH.[19] In 1ONH, C7 and N4 are in cis, which makes the entire seven-membered ring and C7 side chain present a plane conformation.

3.2.2. Stepwise Mechanism. In reactant **b**, C2−C3 is a double bond, so the H atom on N4 can transfer to C2, which produces an imine **c**. The transition state of this process is **Tsbc**, and the energy barrier is 66.37 kcal/mol. The energy barrier is so high that the reaction cannot proceed. However, this process can proceed in virtue of water assistance, namely, a water molecule can be the bridge of hydrogen transfer of hydrogen acting as the acceptor and the donor (Figure 4). The transition state of this process is **Tsbcw**, and the energy barrier is 42.28 kcal/mol. It is obvious that the energy barrier can be decreased in water assistance process.

Compared with **b**, the conformation of **c** changes little. Just the dihedral angle C3−N4−C5−C6 becomes −123.8° from −157.1°, which made the entire structure tighter. The relative energy of **c** is −9.14 kcal/mol, which is more stable than **b**.

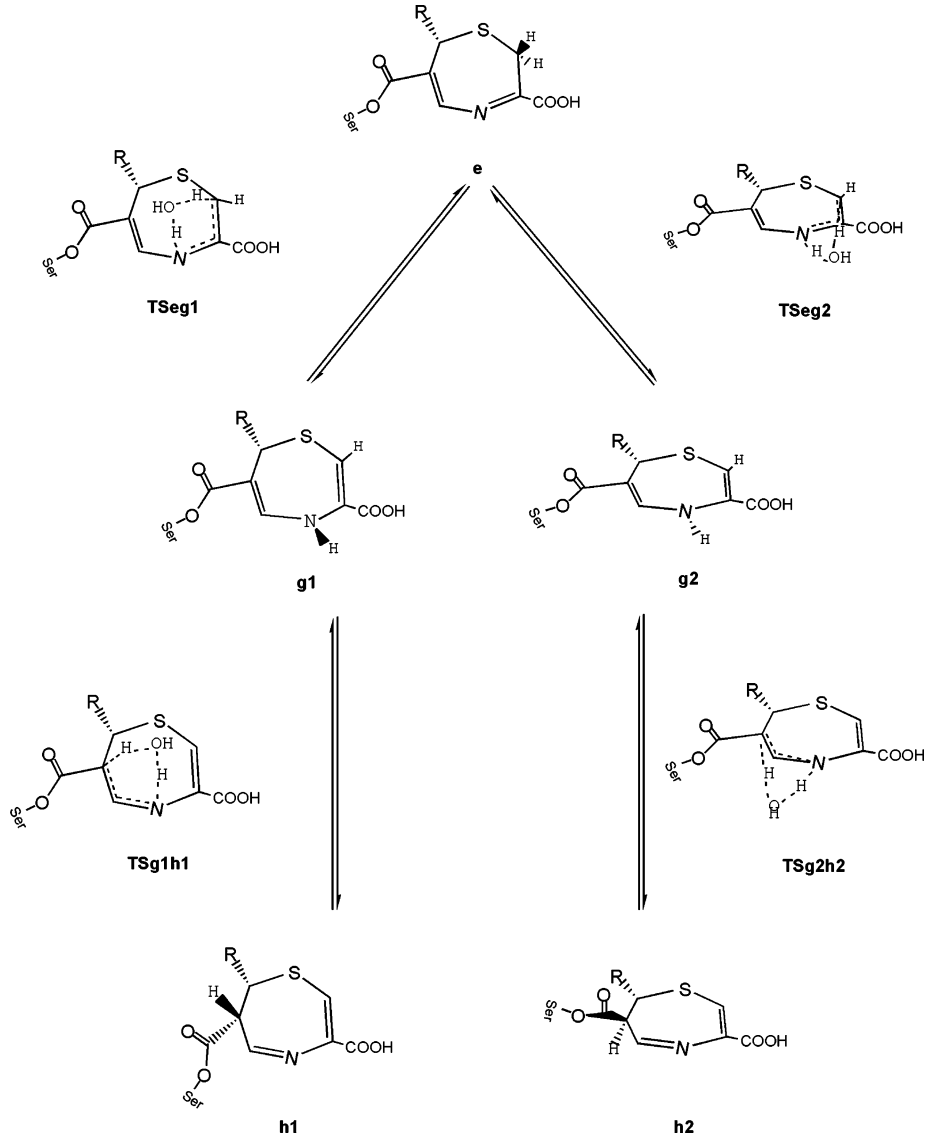
c can also evolve with cleavage of the S1−C5 bond. The energy barrier of this process is 44.93 kcal/mol. Different from **b**, S1 attacks C3 instead of C6′ after it leaves C5, and this reaction led to a thiirane intermediate **d**.

The transition state that connects **c** with **d** is **tscd**. In **tscd**, the dihedral angle C3−N4−C5−C6 becomes −67.9° from −123.8°, which shows that the original butterfly-shaped conformation is gradually closing. The dihedral angle S1−C2−C3−N4 becomes 63.4° from 4.5°, which indicates that S1 rotates around C2−C3 after leaving C5.

Compound **d** possesses a triangular thiirane group. This intermediate has not been reported before. In **d**, the dihedral angle C3−N4−C5−C6 is −3.0°, namely, these four atoms are close to be a plane. **d** can evolve with cleavage of S1−C3 bond through transition-state **tsde**, and this process exhibits a low-energy barrier of 14.83 kcal/mol. IRC calculation on **tsde** led to a seven-membered ring intermediate **e**. It is obvious that the leaving S1 attacks C6′ after S1−C3 cleavage.

e is an imine intermediate, and it possesses two double bonds C3−N4 and C5−C6. On the C5−C6 double bond, C7 and

SCHEME 2: Tautomerism between the Seven-Membered Ring Intermediates



N4 are in trans, and the dihedral angle C7−C6−C5−N4 is −171.0°, which is different from **f**. The conformation of **e** is unfolded, which is similar to the one in 1ONH (Figure 2). The relative energy of **e** is −5.85 kcal/mol, which is more stable than **f**.

3.3. Tautomerism. The seven-membered ring intermediate has three tautomeric forms, namely, 2,6′-dihydro-, 4,6′-dihydro-, and 6,6′-dihydro-1,4-thiazepines, which have been reported.[16] We consider that the tautomerism is caused by ring-endo-hydrogen transfer. Here, we start from stepwise reaction product **e** and describe all possible hydrogen transfer processes (Scheme 2).

As stated above, **e** is an unfolded conformation, and the seven-membered ring is nearly a plane. There are two hydrogen atoms on C2, and they locate above and below the plane. They can both transfer to N4 without steric hindrance. Because of the far distance, the hydrogen transfer need to in virtue of water assistance, and the transition states are **tseg1** and **tseg2**. The energy barriers of these two processes are 39.27 kcal/mol and 45.92 kcal/mol, respectively. The enamine products obtained by these two processes are **g1** and **g2**, respectively. Their relative energies are 2.28 kcal/mol and 4.18 kcal/mol, respectively. Their conformations have an appreciable difference, which is the H position on N4. When hydrogen transfer occurs above the plane, the hydrogen attacks N4 above. Thus, in **g1**, the H on N4 is above the plane. The other way around, if hydrogen transfer is below the plane, the hydrogen attacks N4 below. So, in **g2**, the H on N4 is below the plane.

Reversely, **g1**and **g2** can yield **e** through **tseg1** and **tseg2**. The energy barriers are 38.63 kcal/mol and 43.38 kcal/mol, respectively. Besides, the H on N4 also can be transferred to C6 because of the neighboring C5−C6 double bond. Similarly, this process also needs water assistance. The H in **g1** is above the plane, and so it only transfers above the plane through the transition-state **tsg1h1** to yield **h1**. The H in **g2** is below the plane, and so it only transfers below the plane through the transition-state **tsg2h2** to yield **h2**. The energy barriers of the two processes are 41.17 kcal/mol and 40.05 kcal/mol, respectively. **h1** and **h2** are imine products, and their relative energies are −0.88 kcal/mol and 0.65 kcal/mol, respectively. Their difference is the transferred hydrogen position on C6. In **h1**, the absolute configuration on C6 is R, while it is S in **h2**. Reversely, **h1**and **h2** can yield **g1** and **g2** through **tsg1h1** and **tsg2h2**, respectively. The energy barriers are 42.68 kcal/mol and 41.94 kcal/mol, respectively.

In these tautomeric forms, **e** is 2,6′-dihydro-1,4-thiazepine, **g1** and **g2** are 4,6′-dihydro-1,4-thiazepine, and **h1** and **h2** are 6,6′-dihydro-1,4-thiazepine.

4. Conclusions

We have studied the reaction mechanism of a novel methylidene penem **a** which inhibited a class C β-lactamase GC1 by quantum chemical modeling. We described the form of seven-membered ring complex in detail. The seven-membered ring intermediate can be obtained via two possible mechanisms, and two different products **e** and **f** are obtained. According to the energetic barriers, the stepwise mechanism is more feasible. In this mechanism, a new thiirane intermediate which has never been reported was found. In organisms, these reactions occur in water, and so the solvent effect must be considered when water is regarded as the solvent, and our studies show that the solvent effect has little affect on the mechanism of producing the seven-membered ring intermediate. Moreover, the water molecules can assist the hydrogen transfer and can decrease the energy barrier greatly.

The 2,6′-dihydro-1,4-thiazepine **e** can get other tautomerics, namely, 4,6′-dihydro and 6,6′-dihydro-1,4-thiazepine through hydrogen transfer, and both the 4,6′-dihydro and 6,6′-dihydro-1,4-thiazepines have two conformations. In these tautomerics, the 6,6′-dihydro-1,4-thiazepine is the most stable by our results.

Acknowledgment. The research work was supported by the National Natural Science Foundation of China (No. 20373034).

Supporting Information Available: Table containing standard orientation of all stationary points. This material is available free of charge via the Internet at http://pubs.acs.org.

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(32) Standard orientation of all stationary points are given as Supporting Information.