Concept of Counterattack Reagents: Intramolecular Counterattack Strategy in the Synthesis of Biologically Active Isopenams

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Abstract: A novel strategy was developed for the synthesis of isopenams in high yields. The strategy involves use of the intramolecular counterattack process in the conversions of (±)-**5** →(±)-**11**, (±)-**6** →(±)-**12**, and (±)-**19** →(±)-**11**. Catalytic hydrogenation of (±)-**11** afforded isopenam (±)-**13**, which possessed notable antimicrobial activities. Oxidation of (±)-**13** with $KMnO_4$ gave sulfone (±)-**15**, which functioned as a potent inhibitor of various bacterial β-lactamases. Sulfone (±)-**15** exerted a great synergistic effect on antimicrobial agent (±)-**13**. Results from the CVFF calculations of the C-2 β-epimer of isopenam **13** (i.e., **23**) and the corresponding sulfone derivative **24** indicate the existence of a severe electronic repulsion between the β-lactam carbonyl and the C-2 carboxyl groups. Steric interaction also exists between the C-6 amide side chain and the C-2 carboxylic acid moiety in **23** and **24**. These interactions, however, do not exist in the corresponding α-epimers **13** and **15**.

Keywords: antimicrobial agents · counterattack · drug research · isopenam · lactams · reaction mechanisms

Introduction

Application of a counterattack reagent strategy in organic synthesis allows two transformations or more to be accomplished in one flask.[1] In the first transformation, the reagent is attacked by a reactant to give a stable intermediate. In the second transformation, a moiety produced from this originally consumed reagent counterattacks the intermediate to afford the desired product. The concept of counterattack reagents has been utilized in the performance of various chemical transformations.[2–5] Use of this method can simplify chemical conversions and minimize manipulations. With these advantages in mind, we developed an intramolecular counterattack process for efficient synthesis of isopenams (±)-**13** and (±)-**15**. These compounds were found to possess important biological activities.

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Results

For the synthesis of isopenams (±)-**13** and (±)-**15**, we treated racemic monocyclic β-lactam **1**[6] with KSCOMe in DMF to produce the corresponding thioester **3** in 90% yield (Method A in Scheme 1). Chlorination of thioester **3** with CF_3SO_2Cl[7] in Et_3N generated chloride **5** in 90% yield. Treatment of **5** with piperidine afforded isopenam **11** in 90% yield (Method C). By the same procedures, we successfully accomplished the transformations of diastereomeric monocyclic β-lactam **2**[8] →**4** (95%) →**6** (95%) →**12** (95%) in an excellent yield for each step.

Finally, we debenzylated **11** and **12** with H_2 at 45 °C in the presence of Pd/C and MeOH to give the corresponding carboxylic acids (±)-**13** and (±)-**14**, respectively, in 55% yield. Oxidation of sulfides **13** and **14** with $KMnO_4$ in aqueous acetic acid solution gave the corresponding sulfones (±)-**15** (40%) and (±)-**16** (50%), respectively. The low yields for the formation of compounds **13**–**16** could be attributed to the susceptibility of their β-lactam rings toward nucleophilic attack by methanol or water. This was confirmed by the lack of the β-lactam ring in the corresponding residues, as evidenced by IR spectroscopy.

To support the proposed mechanism for the transformations of **5** →**11** and **6** →**12**, we performed the following control experiments. Treatment of chloride **5** with *n*BuSH in a mixture of CH_2Cl_2 and Et_3N afforded sulfide **18** in 60% yield (Method F in Scheme 2). Performance of the same reaction in

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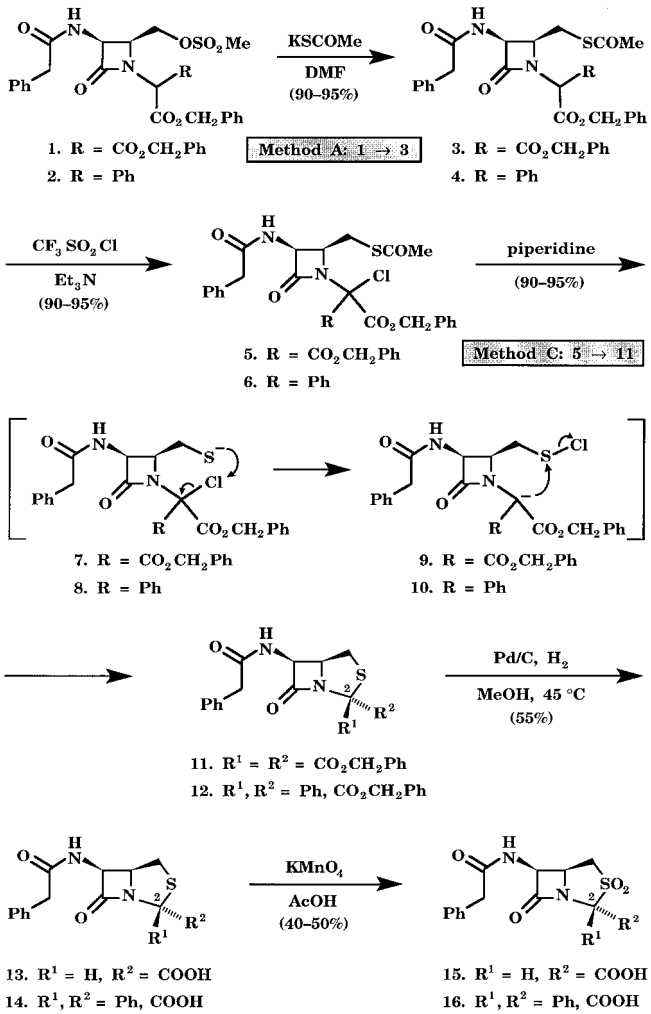
the absence of Et_3N gave dechlorinated product **3**, isolated in 85% yield (Method B), and *n*BuSCl. Subsequent treatment of this mixture with Et_3N afforded **18** in 46% yield. Furthermore, reaction of pure **3** with *n*BuSCl and Et_3N also generated **18** in 46% yield (Method G in Scheme 2).

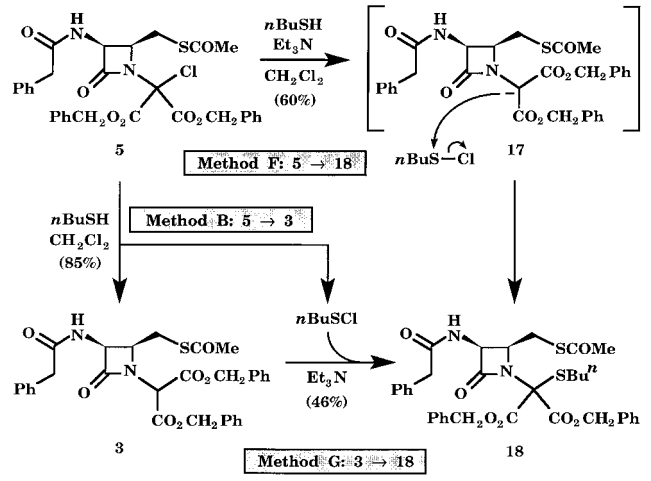
To investigate the mechanism of an intramolecular cyclization process, we mesylated racemic malonate **3** with $MeSO_2Cl$ in Et_3N (Scheme 3). The corresponding sulfone thioester **19** was obtained in 98% yield. Deacetylation of sulfone thioester **19** in DMF with piperidine in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) at 60°C produced isopenam (±)-**11** in 80% yield (Method D). In the absence of DBU, sulfone thioester **19** was deacetylated by piperidine at 25°C to give mercaptomesylate **22** (96% yield). This reaction involved a protonation step of anion **21** by a 1-acyl piperidinium species generated in situ. In a separate reaction, we cleaved the S–SO_2Me bond in **22** through an intramolecular cyclization by use of DBU in DMF at 60°C (Method E). Accordingly, isopenam (±)-**11** was isolated in 78% yield.

With a Silicon Graphics workstation, we carried out the CVFF calculations on the isopenam **13**, its sulfone derivative **15**, and their corresponding β-epimers **23** and **24**. Our goal was to justify the exclusive formation of the α-epimers **13** and **15**. The thermodynamically most stable forms for compounds **13**, **15**, **23**, and **24** are shown in Figure 1. We found that the distance between the lactam carbonyl oxygen and the carboxyl oxygen atoms in the β-epimer **23** was 3.69 Å (see Figure 1). It is significantly shorter than 4.09 Å in the α-epimeric isopenam **13**. The distances between the lactam carbonyl oxygen and the carboxyl oxygen atoms in the α-epimer **15** and the β-epimer **24** were 4.34 Å and 3.78 Å, respectively (see Figure 1). We obtained these values by the same calculation method.

In the Figure 1, the blue ball on the top center side represents the C-6 amide nitrogen atom; the two red balls



Scheme 1. Synthesis of isopenams **13**–**16** involving an intramolecular counterattack process.



Scheme 2. Counterattack strategy in the conversion of chloride **5** to sulfide **18**.

反撲試劑之觀念：應用「分子內反撲策略」合成具生物活性之異青黴素類化合物

Abstract in Chinese:

本文揭示合成異青黴素類化合物之新策略，該策略包括應用「分子內反撲過程」將 (±)-**5** 轉換為 (±)-**11**，(±)-**6** 轉換為 (±)-**12**，及 (±)-**19** 轉換為 (±)-**11**。化合物 (±)-**13** 經與高錳酸鉀發生氧化反應，而得碸化合物 (±)-**15**，其可有效地做為不同細菌的β-內醯胺酶之抑制劑。碸 (±)-**15** 亦可與抗菌劑 (±)-**13** 混合使用，導致產生極佳之"協同效應"。

由 CVFF 分子模擬計算結果顯示，異青黴素類化合物 **13** 之碳-2位置之β-差向異構物(即化合物 **23**)及其相對應之碸衍生物 **24**，在其β-內醯胺之羰基團及碳-2羧基團之間存在極強之電子排斥力。在化合物 **23** 及 **24** 之碳-6醯胺側鏈及碳-2羰酸基之間亦存有立體相互作用力。但該等作用力並不存在於其α-差向異構物 **13** 和 **15** 中。

關鍵字：抗菌、反撲、異青黴素、β-內醯胺、β-內醯胺酶、抑制劑

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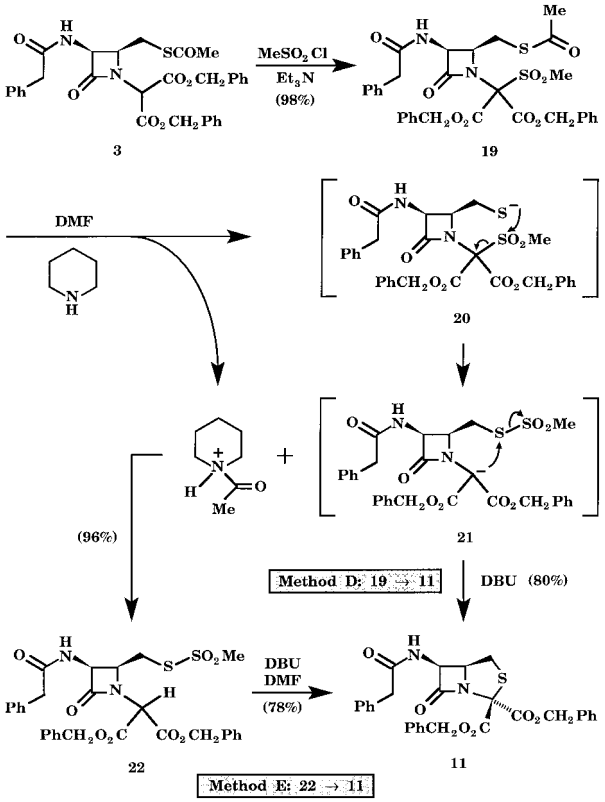


Scheme 3. Intramolecular counterattack strategy in the transformation of thioester **3** to isopenam **11**.

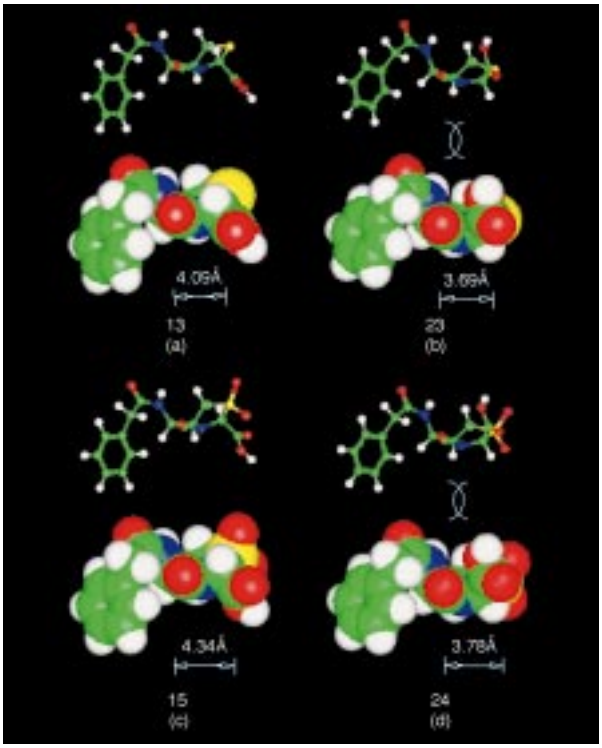


Figure 1. The structures represented by ball-and-stick as well as CPK space filling molecular models are those with the lowest energy obtained by the CVFF calculations. The green balls represent carbon atoms, white for hydrogen, red for oxygen, blue for nitrogen, and yellow for sulfur: a) isopenam **13** with an α-carboxylic group at the C-2 position; b) isopenam **23** with a β-carboxylic group at the C-2 position; c) isopenam sulfone **15** with an α-carboxylic group at the C-2 position; and d) isopenam sulfone **24** with a β-carboxylic group at the C-2 position.

attached to the same green (not yellow) atom on the right side represent the C-2 carboxylate oxygen atoms. We observe severe steric interaction between the C-6 amide side chain and the C-2 carboxylic acid moiety in β-epimers **23** and **24**. Such interaction is absent in the α-epimeric isopenam **13** and isopenam sulfone **15**. On the basis of these two factors, **13** and **15** are thermodynamically more stable than **23** and **24** by 3.50 and 2.73 kcal mol^{-1}, respectively.

Discussion

By examining the reaction conditions and products of the control experiments shown in Scheme 2, we believe that *n*BuS$^-$, generated from *n*BuSH and Et_3N in the first reaction, attacks chloride **5** to give *n*BuSCl and carbanion **17**. Regarded as a nucleophile, this carbanion then counterattacks *n*BuSCl, generated in situ, to produce sulfide **18**. A similar mechanism can be applied to cyclizations of **5** →**11** and **6** →**12** (Scheme 1): upon deacetylation with piperidine, thioesters **5** and **6** would give the corresponding sulfide anions **7** and **8**. The thiolate moiety of each then attacks the corresponding chlorine atoms in **7** and **8** intramolecularly to produce carbanions **9** and **10**, respectively. Subsequently, intramolecular cyclizations occur in **9** and **10** by cleavage of the weak S–Cl bond to afford the corresponding thiazolidines **11** and **12**, respectively.

Feasibility of the intramolecular counterattack process is further confirmed in a related conversion (i.e., **19** →**11**) as shown in Scheme 3. Upon reaction with piperidine, sulfone thioester **19** affords sulfide **20**. Intramolecular transfer of the sulfone moiety then takes place in **20** to give the mercaptomesylate carbanion **21**, which has an ideal geometry to allow intramolecular cyclization to occur through the favorable 5-*Exo-Tet* mode.[9] Our experimental results about the successful conversion of **22** to **11** by DBU provide more evidence to support the mechanism.

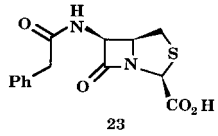
In the transformation of **5** →**18** in the presence of *n*BuSH and Et_3N as shown in Scheme 2, chloride **5** can be regarded as a counterattack reagent. By analogy, the conversions of **5** →**11**, **6** →**12** (Scheme 1), and **19** →**11** (Scheme 3) under basic conditions involve an intramolecular counterattack process. The α-chloro ester moieties in **7** and **8** as well as the sulfone malonate moiety in **20** are first attacked intramolecularly by the sulfide moieties, which are generated by deacetylation of the corresponding thioesters **5**, **6**, and **19**. The resultant carbanions **9**, **10**, and **21** then counterattack the S–Cl or the S–SO_2Me unit to form the thiazolidine ring in **11** and **12**.

Biological activity: We carried out the screening experiments for antimicrobial activities[10, 11] of β-lactams (±)-**13**–(±)-**16**, a mixture of (±)-**13** and (±)-**15** (1:1 w/w), as well as the reference compounds ampicillin (**25**), amoxicillin (**26**), penicillin G (**27**), and clavulanic acid (**28**). The experiments were performed in vitro against different strains of five pathogenic microorganisms up to 128 μg mL^{-1}. The results are summarized in Table 1. Furthermore, we tested the β-lactamase inhibitory[12] properties of β-lactams (±)-**13**–(±)-**16**. Clavulanic acid (**28**) was used in vitro as the reference compound. The results are shown in Table 2.

Table 1. Minimum inhibitory concentrations[a] of synthetic β-lactams (±)-**13**, (±)-**14**, (±)-**15**, a 1:1 (w/w) mixture of (±)-**13** and (±)-**15**, and (±)-**16** as well as the reference compounds ampicillin (**25**), amoxicillin (**26**), penicillin G (**27**), and clavulanic acid (**28**).

Microorganism	(±)-**13**	(±)-**14**	(±)-**15**	(±)-**13** + (±)-**15**	(±)-**16**	**25**	**26**	**27**	**28**
S. a. FDA 209P	0.10	> 128	12.5	0.060	> 128	0.33	0.78	0.40	> 128
S. a. A9606[b]	1.20	> 128	16.7	0.20	> 128	> 128	> 128	> 128	> 128
S. a. A15091[b]	0.92	> 128	8.85	0.47	> 128	> 128	> 128	120	> 128
S. a. A20309[b]	1.70	> 128	15.3	0.70	> 128	100	112	> 128	> 128
S. a. 95[b,c]	3.25	> 128	16.6	0.98	> 128	> 128	> 128	> 128	> 128
E. c. ATCC 39188	0.76	> 128	8.93	0.17	> 128	2.50	3.60	2.30	> 128
E. c. A9675[b]	2.56	> 128	7.85	1.20	> 128	70.3	57.1	100	> 128
E. c. A21223[b]	1.77	> 128	8.72	0.89	> 128	125	80.5	128	> 128
S. t. O-901	35.0	> 128	62.9	15.0	> 128	> 128	> 128	> 128	> 128
P. a. 1101-75	128	> 128	65.8	45.0	> 128	> 128	> 128	> 128	> 128
P. a. 18S-H[b]	100	> 128	85.0	37.0	> 128	> 128	> 128	> 128	> 128
K. p. NCTC 418	98.4	> 128	> 128	35.0	> 128	> 128	> 128	> 128	> 128
K. p. A20634 TEM[b]	68.0	> 128	100	23.5	> 128	> 128	> 128	> 128	> 128

[a] The values of minimum inhibitory concentrations ($\mu g\,mL^{-1}$), obtained as the average of triplicate determinations, represent the lowest concentrations of antibiotics required to prevent visible growth of *Staphylococcus aureus* (*S. a.*), *Escherichia coli* (*E. c.*), *Salmonella typhi* (*S. t.*), *Pseudomonas aeruginosa* (*P. a.*), and *Klebsiella pneumoniae* (*K. p.*). These values were obtained by use of an agar dilution method whereby organisms were deposited onto medicated agar plates by the replication device of Steers et al.[15] [b] β-Lactamase-producing organism. [c] Methicillin resistant organism.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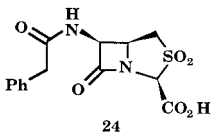
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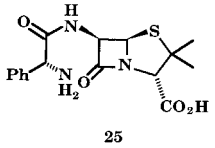
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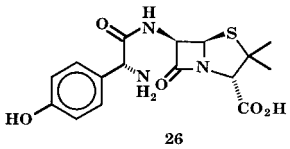
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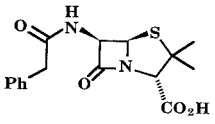
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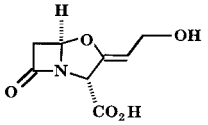
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Table 2. β-Lactamase inhibitory properties of synthetic β-lactams (±)-**13**–(±)-**16** as well as natural clavulanic acid (**28**) against bacterial β-lactamases.

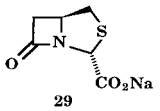
	Minimum protective concentrations[a] [$\mu g\,mL^{-1}$]			
	S. a.	*E. c.*	*P. a.*	*K. p.*
(±)-**13**	74.1	60.6	58.9	68.5
(±)-**14**	> 128	> 128	> 128	> 128
(±)-**15**	0.88	3.15	2.74	0.98
(±)-**16**	126	114	128	> 128
28	0.56	5.12	3.00	0.17

[a] The values of minimum protective concentrations, obtained as the average of triplicate determinations, represent the ability of compounds to inhibit the hydrolysis of an indicator, 3-[*E*-(2,4-dinitro)styryl]-(6*R*,7*R*)-7-(2-thienylacetamido)-3-cephem-4-carboxylic acid by β-lactamases from *S. aureus* A9606 (*S. a.*), *E. coli* A9675 (*E. c.*), *P. aeruginosa* 18S-H (*P. a.*), and *K. pneumoniae* A20634 TEM (*K. p.*). The values of minimum protective concentrations, determined by the procedure of O'Callaghan et al.,[12] represent the lowest concentrations of inhibitors required to protect the indicator from hydrolysis by β-lactamases under standard test conditions within 30 min. The hydrolysis of indicators was evidenced by the appearance of a distinct red color.

Isopenam (±)-**13**, which has an identical skeleton to penicillin antibiotics at the C-2 position, exhibited notable antimicrobial activities but low β-lactamase inhibitory properties. Isopenam sulfone (±)-**15**, however, showed excellent minimum protective concentration (MPC) values against the β-lactamases of different bacterial species. It also exhibited moderate minimum inhibitory concentration (MIC) against some pathogenic microorganisms.

The β-lactamase inhibitory properties of (±)-**15** allowed it to exert a large synergistic effect on antimicrobial agent (±)-**13**. Thus a 1:1 mixture of (±)-**13** and (±)-**15** exhibited notable antimicrobial activity (Table 1). The oxidation of the sulfur atom in isopenam (±)-**13** to the corresponding sulfone derivative (±)-**15** led to significant changes in antimicrobial as well as β-lactamase inhibitory properties.

In contrast to isopenam (±)-**13** (see Table 1), isopenam **29** does not inhibit the growth of the bacteria *S. aureus*.[13] Moreover, isopenam sulfone (±)-**15** exhibited β-lactamase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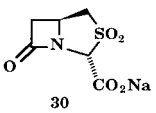
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inhibitory properties (Table 2), yet isopenam sulfone **30** has no effect upon the β-lactamase from *P. aeruginosa*. These results indicate that the presence of a *cis*-oriented acylamino group at the C-6 position is necessary for the biological activity of isopenams[14] and isopenam sulfones. It should be noted that our synthetic β-lactams were in a racemic form, whereas natural β-lactam antibiotics are single stereoisomers.[15] Thus, only one half of the minimum inhibitory or protective concentrations would be necessary for each of the desired single enantiomer of **13**–**16**.

β-Lactams (±)-**14** and (±)-**16** possess a phenyl functionality at the C-2 position. The quaternary carbon center therein makes these two β-lactams biologically inactive either as antimicrobial agents (Table 1) or β-lactamase inhibitors (Table 2).

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Conclusion

A new type of reaction mechanism was established for the construction of a heterocyclic ring fused with a β-lactam nucleus. In the formation of the thiazolidine ring in isopenams **11** and **12** from the corresponding thioesters **5** and **6** under basic conditions, the sulfide unit in intermediates **7** and **8** attacked the corresponding chloromalonate and chlorophenylacetate moieties, respectively. An intramolecular cyclization occurred subsequently in the resultant intermediates **9** and **10** to form the corresponding isopenams **11** and **12**. The entire reaction thus went through an intramolecular counterattack process. The thioesters **5** and **6** acted as counterattack reagents. The same mechanism was responsible for the transformation of sulfone thioester **19** to isopenam **11** via sulfide **20** and malonate anion **21**. Formation of isopenam **11** from **19** through intramolecular cyclization indicates that **19** functioned as an intramolecular counterattack reagent in the presence of a base.

Our CVFF computational results indicate that α-epimers isopenam **13** and isopenam sulfone **15** were thermodynamically more stable than the corresponding β-epimers. They possessed the same epimeric configuration as that of penicillins at the C-2 position.

Isopenam (±)-**13** showed notable antimicrobial activities and its sulfone derivative (±)-**15** exhibited potent β-lactamase inhibitory properties. Moreover, a mixture of isopenam (±)-**13** and isopenam sulfone (±)-**15** in a ratio of 1:1 (w/w) was found to possess remarkable activities against different strains of pathogenic microorganisms in vitro.

Experimental Section

General: Chemicals were purchased from Fluka. Reagent-grade solvents were distilled and then stored over molecular sieves 4 Å. Products were isolated by use of column chromatography (Merck silica gel 60, particle size 230–400 mesh ASTM), packed in glass column (35 g of silica gel per gram of crude material). Analytical thin-layer chromatography (TLC) analyses were performed on precoated plates (silica gel 60 F_{254}), purchased from Merck. Melting points were carried out with Büchi 510 apparatus. Proton NMR spectra were obtained on a Bruker-WH 90 or a Varian-XL 200 spectrometer, which was used to determine the ratios (1:1 to 1:1.2) of all diasteromeric isomers. Infrared spectra were measured on a Beckman IR-8 spectrophotometer. Microanalysis data were obtained by use of a Perkin–Elmer 240B microanalyzer.

Computation was performed on a Silicon Graphics IRIS CRIMSON/Elan workstation. The *Builder* and *Discover* modules of Insight II (Biosym Technologies, versions 2.3.5 and 2.9.5) were used for model building and energy minimization, respectively. The energies for all conformations were minimized with the consistent valence forcefield (CVFF)[16] until the maximum derivative was less than 0.0004 kcal mol^{-1} Å^{-1}.

Dibenzyl-(3*RS*,4*RS*)-2-(4-acetylthiomethyl-2-oxo-3-phenylacetamido-1-azetidinyl) malonate (3)

Method A: KSCOMe (2.28 g, 19.9 mmol) was added to a solution of (±)-**1**[6] (5.94 g, 9.99 mmol) in DMF (80 mL). The mixture was stirred at room temperature under N_2 for 15 h. Then EtOAc (100 mL) was added, and the mixture was washed with water (5 × 100 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The crude product was purified by column chromatography ($CHCl_3$ as eluant) to give (±)-**3** (5.17 g, 8.99 mmol) as a foam in 90% yield. ^{1}H NMR ($CDCl_3$): δ = 2.34 (s, 3H; CH_3CO), 2.67–2.93 (br, 2H; CH_2S), 3.62 (s, 2H; CH_2CO), 4.11–4.28 (m, 1H; HC(4)), 5.23 (s, 4H; 2 × CH_2O), 5.33 (s, 1H; CH), 5.39 (dd, ^3J(H,H) = 8.5, 5.0 Hz, 1H; HC(3)), 7.02 (br s, 1H; NH), 7.42 (br s, 15H; 3 × C_6H_5); IR (CH_2Cl_2): $\tilde{\nu}$ = 3405 (NH), 1775 (β-lactam), 1740 (ester), 1732 (thioester), 1680 (amide) cm^{-1}; $C_{31}H_{30}N_2O_7S$ (574.7): calcd C 64.79, H 5.26, N 4.87, S 5.58; found C 64.70, H 5.13, N 4.72, S 5.60.

Method B: *n*BuSH (187 mg, 2.10 mmol) was added to a solution of (±)-**5** (1.22 g, 2.01 mmol) in CH_2Cl_2 (10 mL) at room temperature. After 30 min, the solution was concentrated and the residue was purified by column chromatography ($CHCl_3$ as eluant) to afford (±)-**3** (980 mg, 1.70 mmol) in 85% yield.

Benzyl(3*RS*,4*RS*)-2-(4-acetylthiomethyl-2-oxo-3-phenylacetamido-1-azetidinyl)-2-phenylacetates [(±)-4]: Compounds (±)-**4** (4.90 g, 9.49 mmol) were prepared in 95% yield from (±)-**2**[8] (5.36 g, 9.99 mmol) and KSCOMe (2.28 g, 19.9 mmol) by the same procedure for the conversion of **1** → **3**. ^{1}H NMR ($CDCl_3$): δ = 2.35 (s, 3H; CH_3CO), 2.65–2.90 (br, 2H; CH_2S), 3.60 (s, 2H; CH_2CO), 3.99–4.20 (m, 1H; HC(4)), 5.19 (s, 2H; CH_2O), 5.50 (dd, ^3J(H,H) = 9.0, 4.8 Hz, 1H; HC(3)), 5.70 (s, 1H; CH), 6.90–7.59 (m, 16H; NH, 3 × C_6H_5); IR (CH_2Cl_2): $\tilde{\nu}$ = 3400 (NH), 1770 (β-lactam), 1750 (ester), 1730 (thioester), 1680 (amide) cm^{-1}; $C_{29}H_{28}N_2O_5S$ (516.6): calcd C 67.42, H 5.46, N 5.42, S 6.21; found C 67.30, H 5.45, N 5.51, S 6.11.

Dibenzyl(3*RS*,4*RS*)-2-(4-acetylthiomethyl-2-oxo-3-phenylacetamido-1-azetidinyl)-2-chloromalonate (5): CF_3SO_2Cl (0.86 g, 5.1 mmol) in CH_2Cl_2 (5.0 mL) was added to a solution containing (±)-**3** (2.87 g, 4.99 mmol) and Et_3N (0.61 g, 6.0 mmol) in CH_2Cl_2 (50 mL) at 0 °C over a period of 5.0 min. The reaction mixture was stirred and allowed to warm to room temperature within 1.0 h. The solvent was evaporated to dryness under reduced pressure, and then Et_2O (50 mL) was added. The ethereal solution was washed with water (2 × 50 mL), dried over $MgSO_4$, and treated with charcoal. After filtration, evaporation, and then purification by use of column chromatography ($CHCl_3$ as eluant), (±)-**5** (2.74 g, 4.49 mmol) was obtained as a foam in 90% yield. ^{1}H NMR ($CDCl_3$): δ = 2.35 (s, 3H; CH_3CO), 2.68–2.92 (br, 2H; CH_2S), 3.61 (s, 2H; CH_2CO), 4.12–4.29 (m, 1H; HC(4)), 5.16, 5.18 (2s, 4H; 2 × CH_2O), 5.30 (dd, ^3J(H,H) = 9.0, 5.0 Hz, 1H; HC(3)), 7.04 (d, ^3J(H,H) = 9.0 Hz, 1H; NH), 7.39 (br s, 15H; 3 × C_6H_5); IR (CH_2Cl_2): $\tilde{\nu}$ = 3410 (NH), 1791 (β-lactam), 1750 (ester), 1730 (thioester), 1680 (amide) cm^{-1}; $C_{31}H_{29}N_2O_7SCl$ (609.1): calcd C 61.13, H 4.80, N 4.60, S 5.26, Cl 5.82; found C 61.12, H 4.81, N 4.71, S 5.30, Cl 5.89.

Benzyl(3*RS*,4*RS*)-2-(4-acetylthiomethyl-2-oxo-3-phenylacetamido-1-azetidinyl)-2-chloro-2-phenylacetates [(±)-6]: Compounds (±)-**6** (4.05 g, 7.34 mmol) were prepared in 95% yield from (±)-**4** (4.00 g, 7.74 mmol), Et_3N (0.94 g, 9.3 mmol), and CF_3SO_2Cl (1.30 g, 7.90 mmol) by the same procedure for the conversion of **3** → **5**. ^{1}H NMR ($CDCl_3$): δ = 2.30 (s, 3H; CH_3CO), 2.61–2.90 (br m, 2H; CH_2S), 3.60 (s, 2H; CH_2CO), 4.12–4.30 (m, 1H; HC(4)), 5.17 (s, 2H; CH_2O), 5.31 (dd, ^3J(H,H) = 9.5, 5.0 Hz, 1H; HC(3)), 6.80 (d, ^3J(H,H) = 9.5 Hz, 1H; NH), 7.10–7.65 (m, 15H; 3 × C_6H_5); IR (CH_2Cl_2): $\tilde{\nu}$ = 3405 (NH), 1790 (β-lactam), 1750 (ester), 1730 (thioester), 1680 (amide) cm^{-1}; $C_{29}H_{27}N_2O_5SCl$ (551.1): calcd C 63.21, H 4.94, N 5.08, S 5.82, Cl 6.43; found C 63.20, H 4.90, N 5.17, S 5.70, Cl 6.33.

Dibenzyl(5*RS*,6*RS*)-7-oxo-6-(phenylacetamido)-3-thia-1-azabicyclo-[3.2.0]heptane-2,2-dicarboxylate (11): *Method C*: Piperidine (0.85 g, 1.0 mmol) was added to a solution containing (±)-**5** (0.61 g, 1.0 mmol) in CH_2Cl_2 (3.0 mL) at room temperature. The mixture was stirred for 1.5 h, diluted with CH_2Cl_2 (20 mL) and washed with 2% aqueous HCl solution (20 mL) and water (20 mL). The organic layer was dried over $MgSO_4$, concentrated under reduced pressure, and then purified by use of column chromatography ($CHCl_3$ as eluant) to give (±)-**11** (0.48 g, 0.90 mmol) in 90% yield. ^{1}H NMR ($CDCl_3$): δ = 2.71–3.10 (m, 2H; CH_2S), 3.52 (s, 2H; CH_2CO), 4.31–4.57 (m, 1H; HC(5)), 5.08, 5.12 (2s, 4H; 2 × CH_2O), 5.22 (dd, ^3J(H,H) = 8.0, 4.5 Hz, 1H; HC(6)), 6.81–7.01 (br, 1H; NH), 7.30–7.51 (m, 15H; 3 × C_6H_5); IR (CH_2Cl_2): $\tilde{\nu}$ = 3410 (NH), 1785 (β-lactam), 1745 (ester), 1670 (amide) cm^{-1}; $C_{29}H_{26}N_2O_6S$ (530.6): calcd C 65.65, H 4.94, N 5.28, S 6.04; found C 65.59, H 4.88, N 5.30, S 6.14.

Method D: Piperidine (0.85 g, 1.0 mmol) and DBU (0.15 g, 0.99 mmol) was added to a solution containing (±)-**19** (0.65 g, 1.0 mmol) in DMF (5.0 mL). The mixture was stirred at 60 °C for 3.0 h. After cooling, Et_2O (50 mL) was added, and the mixture was washed with 2% aqueous HCl solution (20 mL) and water (5 × 40 mL). The organic layer was dried over $MgSO_4$, concentrated under reduced pressure, and then purified by use of column chromatography ($CHCl_3$ as eluant) to give (±)-**11** (0.42 g, 0.80 mmol) in 80% yield.

Method E: DBU (0.15 g, 0.99 mmol) was added to a solution containing (±)-**22** (0.61 g, 1.0 mmol) in DMF (5.0 mL). The mixture was heated at 60 °C for 2.0 h. After cooling, Et_2O (50 mL) was added, and the mixture was washed with water (5 × 40 mL). The organic layer was dried over $MgSO_4$, concentrated under reduced pressure, and then purified by use of column chromatography ($CHCl_3$ as eluant) to give (±)-**11** (0.41 g, 0.78 mmol) in 78% yield.

Benzyl(5*RS*,6*RS*)-7-oxo-6-(phenylacetamido)-3-thia-1-azabicyclo[3.2.0]-heptane-2-phenyl-2-carboxylates [(±)-12]: Compounds (±)-**12** (2.08 g, 4.40 mmol) were prepared in 95% yield from (±)-**6** (2.55 g, 4.63 mmol) and piperidine (0.394 g, 4.63 mmol) by the same procedure for the conversion of **5**→**11**. ^{1}H NMR ($CDCl_3$): $\delta = 2.70-3.10$ (m, 2H; CH_2S), 3.62 (br s, 2H; CH_2CO), 3.91–4.20 (m, 1H; HC(5)), 4.90, 5.00 (2 s, 2H; CH_2O), 5.20 (dd, $^3J(H,H) = 8.0$, 4.5 Hz, 1H; HC(6)), 6.81 (br s, 1H; NH), 7.22–7.45 (m, 15H; $3 \times C_6H_5$); IR (CH_2Cl_2): $\tilde{\nu} = 3405$ (NH), 1781 (β-lactam), 1740 (ester), 1685 (amide) cm^{-1}; $C_{27}H_{24}N_2O_4S$ (472.6): calcd C 68.63, H 5.12, N 5.93, S 6.78; found C 68.51, H 5.20, N 5.88, S 6.70.

(2*RS*,5*SR*,6*SR*)-7-Oxo-6-(phenylacetamido)-3-thia-1-azabicyclo[3.2.0]-heptane-2-carboxylic acid (13): A solution of (±)-**11** (2.65 g, 4.99 mmol) in MeOH (100 mL) containing 1% aqueous $NaHCO_3$ (15 mL) was hydrogenated over Pd/C (10%, 1.50 g, 1.41 mmol) and 60 psi of H_2 at 45 °C for 5.0 h. The mixture was filtered and then acetic acid (20 mL) was added to the residue. The solvent was removed under reduced pressure, and then the residue was purified by use of column chromatography (EtOAc as eluant) to give (±)-**13** (0.84 g, 2.7 mmol) in 55% yield. M.p. 141–143 °C; ^{1}H NMR ([D$_6$]DMSO): $\delta = 2.72-3.12$ (m, 2H; CH_2S), 3.50 (s, 2H; CH_2CO), 4.21–4.28 (m, 1H; HC(5)), 4.88 (s, 1H; CH), 5.17 (dd, $^3J(H,H) = 9.0$, 4.5 Hz, 1H; HC(6)), 6.91 (br s, 1H; NH), 7.38 (s, 5H; C_6H_5), 7.80–8.50 (br, 1H; CO_2H); IR (Nujol): $\tilde{\nu} = 3500-3300$ (NH, CO_2H), 1779 (β-lactam), 1705 (acid), 1669 (amide) cm^{-1}; $C_{14}H_{14}N_2O_4S$ (306.3): calcd C 54.89, H 4.61, N 9.14, S 10.47; found C 54.81, H 4.50, N 9.20, S 10.39.

(5*RS*,6*RS*)-7-Oxo-6-(phenylacetamido)-3-thia-1-aza-bicyclo[3.2.0]hep-tane-2-phenyl-2-carboxylic acids [(±)14]: Compounds (±)-**14** (1.05 g, 2.75 mmol) were prepared in 55% yield from (±)-**12** (2.36 g, 4.99 mmol) and Pd/C (10%, 1.50 g, 1.41 mmol) by the same procedure for the conversion of **11**→**13**. M.p. 149–151 °C; ^{1}H NMR ($CDCl_3 + D_2O$): $\delta =$ 2.83–3.31 (m, 2H; CH_2S), 3.51 (s, 2H; CH_2CO), 3.89–4.15 (m, 1H; HC(5)), 5.14, 5.17 (2 d, $^3J(H,H) = 5.0$ Hz, 1H; HC(6)), 7.31 (br s, 5H; C_6H_5), 7.44 (br s, 5H; C_6H_5); IR (CH_2Cl_2): $\tilde{\nu} = 3650-3150$ (OH, NH), 1779 (β-lactam), 1702 (acid), 1680 (amide) cm^{-1}; $C_{20}H_{18}N_2O_4S$ (382.4): calcd C 62.81, H 4.74, N 7.32, S 8.38; found C 62.79, H 4.60, N 7.22, S 8.40.

(2*RS*,5*SR*,6*SR*)-7-Oxo-6-(phenylacetamido)-3-dioxothia-1-azabicyclo-[3.2.0]heptane-2-carboxylic acids (15): A solution of $KMnO_4$ (316 mg, 2.00 mmol) in H_2O (2.0 mL) was added to a solution of (±)-**13** (306 mg, 0.999 mmol) in glacial acetic acid (8.0 mL) at 25 °C. The reaction mixture was treated dropwise with 30% H_2O_2 (3.0 mL) until the solution became clear. The solution was poured into water (25 mL) and stored at 10 °C for 2.0 h. The crystalline sulfone was collected, washed with H_2O (20 mL), and dried under reduced pressure over P_2O_5 to give (±)-**15** (135 mg, 0.400 mmol) in 40% yield. M.p. 145–147 °C; ^{1}H NMR ([D$_6$]DMSO): $\delta =$ 3.08–3.42 (br, 2H; CH_2SO_2), 3.61 (s, 2H; CH_2CO), 4.26–4.31 (m, 1H; HC(5)), 5.12 (s, 1H; CH), 5.26 (dd, $^3J(H,H) = 9.6$, 5.0 Hz, 1H; HC(6)), 7.01 (br s, 1H; NH), 7.40 (s, 5H; C_6H_5), 7.61–8.01 (br, 1H; CO_2H); IR (Nujol): $\tilde{\nu} = 3350-3000$ (NH, CO_2H), 1795 (β-lactam), 1700 (acid), 1675 (amide) cm^{-1}; $C_{14}H_{14}N_2O_6S$ (338.4): calcd C 49.70, H 4.17, N 8.28, S 9.48; found C 49.55, H 4.20, N 8.30, S 9.52.

(5*RS*,6*RS*)-7-Oxo-6-(phenylacetamido)-3-dioxothia-1-azabicyclo[3.2.0]-heptane-2-phenyl-2-carboxylic acids [(±)16]: Compounds (±)-**16** (207 mg, 0.499 mmol) were prepared in 50% yield from (±)-**14** (382 mg, 0.999 mmol), glacial acetic acid (8.5 mL), and $KMnO_4$ (316 mg, 2.00 mmol) by the same procedure for the conversion of **13**→**15**. ^{1}H NMR ([D$_6$]DMSO): $\delta = 3.10-3.41$ (br, 2H; CH_2SO_2), 3.60 (br s, 2H; CH_2CO), 4.21–4.35 (m, 1H; HC(5)), 5.29 (dd, $^3J(H,H) = 9.0$, 4.9 Hz, 1H; HC(6)), 6.90 (br s, 1H; NH), 7.30 (s, 5H; C_6H_5CCON), 7.41–7.60 (m, 5H; C_6H_5), 7.62–8.03 (br, 1H; CO_2H)); IR (Nujol): $\tilde{\nu} = 3350-3010$ (NH, CO_2H), 1795 (β-lactam), 1705 (acid), 1672 (amide) cm^{-1}; $C_{20}H_{18}N_2O_6S$ (414.4): calcd C 57.96, H 4.38, N 6.76, S 7.74; found C 57.87, H 4.30, N 6.66, S 7.80.

Dibenzyl(3*RS*,4*RS*)-2-(4-acetylthiomethyl-2-oxo-3-phenylacetamido-1-azetidinyl)-2-butylthiomalonate (18): *Method F*: A solution containing (±)-**5** (610 mg, 1.00 mmol), 1-butanethiol (92.0 mg, 1.02 mmol), and Et_3N (105 mg, 1.04 mmol) in CH_2Cl_2 (15 mL) was stirred at room temperature under N_2 for 1.0 h. The solution was then washed with water (2 × 15 mL), dried over $MgSO_4$, and concentrated under reduced pressure. Purification of the residue by use of column chromatography (CH_2Cl_2 followed by $CHCl_3$ as the eluant) gave (±)-**18** (398 mg, 0.600 mmol) in 60% yield. ^{1}H NMR ($CDCl_3$): $\delta = 0.94$ (t, $^3J(H,H) = 6.0$ Hz, 3H; CH_3), 1.39–1.78 (m, 4H; CH_2CH_2), 2.35 (s, 3H; CH_3CO), 2.49–3.05 (m, 4H; $CH_2S +$ CH_2SCO), 3.59 (s, 2H; CH_2CO), 4.08–4.30 (m, 1H; HC(4)), 5.30, 5.34 (2 s, 4H; $2 \times CH_2O$), 5.31 (dd, $^3J(H,H) = 8.0$, 5.0 Hz, 1H; HC(3)), 7.08 (br s, 1H; NH), 7.45 (br s, 15H; $3 \times C_6H_5$); IR (CH_2Cl_2): $\tilde{\nu} = 3410$ (NH), 1772 (β-lactam), 1743 (ester), 1732 (thioester), 1679 (amide) cm^{-1}; $C_{35}H_{38}N_2O_7S_2$ (662.8): calcd C 63.42, H 5.78, N 4.23, S 9.67; found C 63.31, H 5.79, N 4.09, S 9.51.

Method G: *n*BuSCl (127 mg, 1.02 mmol) and Et_3N (105 mg, 1.04 mmol) was added to a solution containing (±)-**3** (575 mg, 1.00 mmol) in CH_2Cl_2 (15 mL). After the solution was stirred at room temperature under N_2 for 1.0 h, it was washed with water (2 × 15 mL), dried over $MgSO_4$, concentrated under reduced pressure, and then purified by use of column chromatography (CH_2Cl_2 followed by $CHCl_3$ as the eluant) to afford (±)-**18** (305 mg, 0.46 mmol) in 46% yield.

Dibenzyl(3*RS*,4*RS*)-2-(4-acetylthiomethyl-2-oxo-3-phenylacetamido-1-azetidinyl)-2-methanesulfonylmalonate (19): $MeSO_2Cl$ (1.15 g, 10.0 mmol) in CH_2Cl_2 (5.0 mL) was added dropwise to a solution of (±)-**3** (5.74 g, 9.99 mmol) and Et_3N (2.02 g, 20.0 mmol) in CH_2Cl_2 (80 mL). The solution was stirred at 0 °C for 1.0 h and then washed with water (100 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The crude product was purified by use of column chromatography ($CHCl_3$ as eluant) to give (±)-**19** (6.39 g, 9.79 mmol) in 98% yield. M.p. 112–114 °C; ^{1}H NMR ($CDCl_3$): $\delta = 2.38$ (s, 3H; CH_3CO), 2.65–2.83 (br, 2H; CH_2S), 3.39 (s, 3H; CH_3SO_2), 3.63 (s, 2H; CH_2CO), 4.12–4.30 (m, 1H; HC(4)), 5.11, 5.13 (2 s, 4H; $2 \times CH_2O$), 5.31 (dd, $^3J(H,H) = 9.5$, 5.0 Hz, 1H; HC(3)), 7.01 (br s, 1H; NH), 7.32–7.51 (m, 15H; $3 \times C_6H_5$); IR (CH_2Cl_2): $\tilde{\nu} = 3410$ (NH), 1790 (β-lactam), 1752 (ester), 1730 (thioester), 1679 (amide) cm^{-1}; $C_{32}H_{32}N_2O_9S_2$ (652.7): calcd for C 58.88, H 4.94, N 4.29, S 9.82; found C 58.90, H 5.01, N 4.39, S 9.78.

Dibenzyl(3*RS*,4*RS*)-2-(4-methanesulfonylthiomethyl-2-oxo-3-phenylacet-amido-1-azetidinyl)malonate (22): A solution containing (±)-**19** (3.26 g, 4.99 mmol) and piperidine (4.25 g, 49.9 mmol) in DMF (25 mL) was stirred at room temperature under N_2 for 10 h. After addition of EtOAc (100 mL) to quench the reaction, the mixture was washed with water (5 × 100 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The crude product was purified by use of column chromatography ($CHCl_3$ as eluant) to give (±)-**22** (2.93 g, 4.79 mmol) in 96% yield. M.p. 118–120 °C; ^{1}H NMR ($CDCl_3$): $\delta = 2.81-3.20$ (m, 2H; CH_2S), 2.89 (s, 3H; CH_3SO_2), 3.61 (s, 2H; CH_2CO), 4.14–4.31 (m, 1H; HC(4)), 5.22 (s, 4H; $2 \times CH_2O$), 5.27 (s, 1H; CH), 5.40 (dd, $^3J(H,H) = 8.0$, 5.0 Hz, 1H; HC(3)), 7.08 (d, $^3J(H,H) =$ 8.0 Hz, 1H, NH), 7.45 (br s, 15H; $3 \times C_6H_5$); IR (CH_2Cl_2): $\tilde{\nu} = 3400$ (NH), 1778 (β-lactam), 1748 (ester), 1680 (amide) cm^{-1}; $C_{30}H_{30}N_2O_8S_2$ (610.7): calcd C 59.00, H 4.95, N 4.59, S 10.50; found C 59.12, H 5.00, N 4.48, S 10.51.

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