Expedited Articles

Syntheses of New Isodethiaazacephems as Potent Antibacterial Agents

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Received August 14, 1998

New isodethiaazacephems (±)-**3**, (±)-**4**, and (±)-**10** as well as the 4-sulfonylated isodethiaazacepham (±)-**5** were synthesized by chemical methods and found to possess biological activity against five pathogenic microorganisms in vitro. The mesylate and the triflate functionalities in (±)-**3** and (±)-**4**, acting as effective leaving groups, enhanced remarkably the biological activity in comparison with the parent 3-hydroxyisodethiaazacephem (±)-**10**. The mode of action related to (±)-**3** and (±)-**4** can be explained by a [1,4]-elimination process.

Introduction

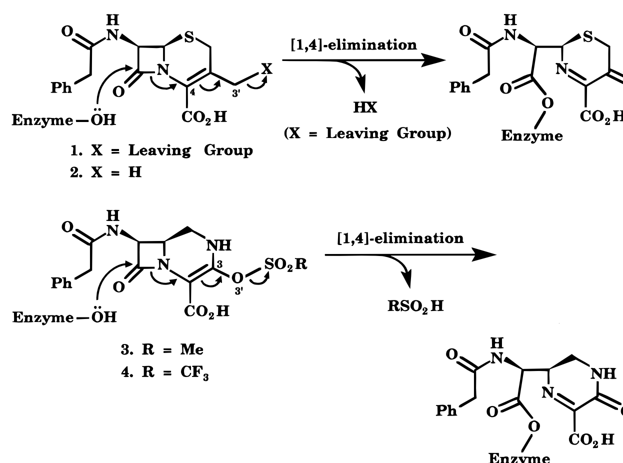
β-Lactam antibiotics exert certain biological activity by acylating serine residues of transpeptidases[1] so that the cross-linking of peptidoglycans does not take place.[1,2] As shown in Scheme 1, ring opening of the β-lactam nucleus would occur when cephalosporins (**1**) react with enzymes responsible for the cell wall synthesis of bacteria. Consequently, the substituent at the C-3′ position is liberated.[3−7] When the eliminated species possesses excellent leaving ability, cephalosporins (**1**) may exhibit profound antibacterial activity. Accordingly, we designed and synthesized unprecedented isodethiaazacephems (±)-**3** and (±)-**4** (see Scheme 1). We believe that the sulfone moiety at the O-3′ position of (±)-**3** and (±)-**4** could act as a leaving group and thus enhance the antibacterial activity in comparison with that of the parent 3-hydroxyisodethiaazacephem (±)-**10**.

Recognizing the feasibility of 1,4-elimination in β-lactam antibiotics as shown in Scheme 1, we also considered the possibility of 1,2-elimination occurring in cepham sulfone (±)-**5** (see Scheme 2). The newly designed compound (±)-**5** bears a leaving group at the C-4 position; the elimination process could also be initiated by bacterial enzymes. Results from our antibacterial tests suggest that the mode of action resulting from novel cephem sulfonates (±)-**3** and (±)-**4** as well as sulfone (±)-**5** can come from the leaving ability of their sulfone moieties.

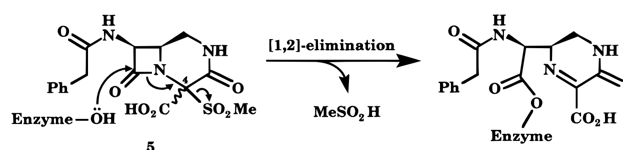
Results

Synthesis of β-Lactams (±)-3, (±)-4, (±)-5, and (±)-10. For the synthesis of isodethiaazacephems (±)-**3** and (±)-**10**, we treated racemic β-lactam mesylate **6**[8] with NaN_3 in DMF at room temperature to give azido

Scheme 1. Mode of Action of Cephalosporins with Transpeptidases



Scheme 2. Proposed Mechanism for Reaction between a Cepham Sulfone and Transpeptidases



β-lactam **7** in 90% yield (Scheme 3). Catalytic hydrogenation of **7** by use of Pd/C and H_2 (30−35 psi) in EtOAc at room temperature resulted in the reduction of the azide moiety and spontaneous formation of isodethiaazacephem **9** in 94% yield. Debenzylation of **9** by use of $PdCl_2$ and H_2 (60 psi) in EtOH produced the target isodethiaazacephem (±)-**10** in 50% yield.

An alternative way to obtain **9** from azido β-lactam **7** involved two steps. Chlorination of **7** with CF_3SO_2Cl in Et_3N and CH_2Cl_2 produced chloride **8** in 90% yield.[9] Consequent reduction of **8** by use of Pd/C and H_2 (30−35 psi) in EtOAc gave the desired compound **9** in 87% yield (Scheme 3). On the other hand, reaction of **8** with H_2S in Et_3N and CH_2Cl_2 produced a mixture of isode-

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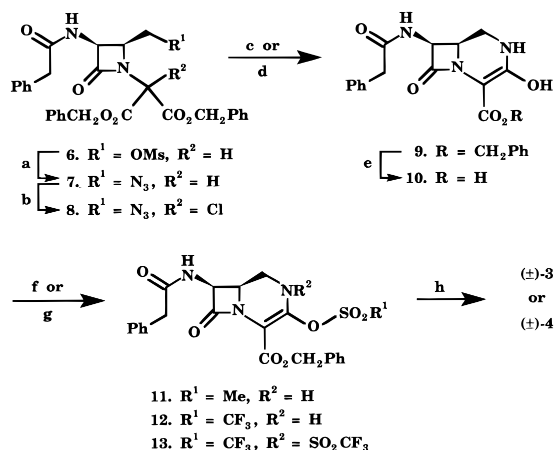
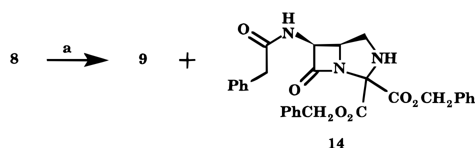
10.1021/jm980476h CCC: $15.00 © 1998 American Chemical Society
Published on Web 11/19/1998

Scheme 3. Synthesis of Isodethiaazacephems **3** and **4**[a]



[a] Reagents: (a) NaN_3, DMF, rt (90%); (b) CF_3SO_2Cl, Et_3N, CH_2Cl_2, 0 °C → rt (90%); (c) Pd/C, H_2, EtOAc, rt; **7** → **9** (94%); (d) Pd/C, H_2, EtOAc, rt; **8** → **9** (87%); (e) $PdCl_2$, H_2, EtOH, rt (50%); (f) $MeSO_2Cl$, pyridine, CH_2Cl_2, 15 °C; **9** → **11** (45%); (g) CF_3SO_2Cl, pyridine, CH_2Cl_2, 15 °C; **9** → **12** (30%) + **13** (10%); (h) $PdCl_2$, H_2, EtOH, rt; **11** → **3** (35%), **12** → **4** (30%).

Scheme 4. Alternative Way To Synthesize Isodethiaazacephem **9**[a]



[a] Reagents: (a) H_2S, Et_3N, CH_2Cl_2, rt (55%); **8** → **9** (15%) + **14** (40%).

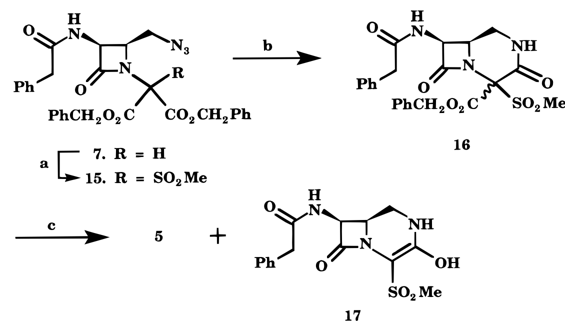
thiaazacephem **9** (15%) and isodethiaazapenam **14** (40%) as shown in Scheme 4.

We attached a sulfonyl group onto the cephem nucleus of **9** by mesylation with $MeSO_2Cl$ in pyridine and CH_2Cl_2 to give the 3-mesyloxy *β*-lactam **11** in 45% yield (Scheme 3). It was then hydrogenated with $PdCl_2$ in EtOH at 60 psi of H_2 to produce the desired isodethiaazacephem (±)-**3** in 35% yield. Moreover, we treated *β*-lactam **9** with CF_3SO_2Cl in pyridine and CH_2Cl_2 to afford a 3:1 mixture of trifluoromethanesulfonates **12** and **13** in 40% overall yield. Catalytic reduction of **12** with $PdCl_2$ in EtOH at 60 psi of H_2 gave the target isodethiaazacephem (±)-**4** in 30% yield.

For the synthesis of isodethiaazacepham (±)-**5** bearing a methylsulfonyl group at the C-4 position, we treated racemic azido *β*-lactam **7** with $MeSO_2Cl$ in Et_3N and CH_2Cl_2 (Scheme 5). Sulfone **15**, generated in 85% yield, was treated with H_2 (30−35 psi) and Pd/C in EtOAc to give bicyclic *β*-lactam **16** in 90% yield through sequential reduction and lactamization. Upon further reduction with H_2 at 60 psi in the presence of $PdCl_2$ and EtOH, compound **16** was converted to a mixture of the desired 4-substituted isodethiaazacepham (±)-**5** in 20% yield and the decarboxylated product (±)-**17** in 50% yield.

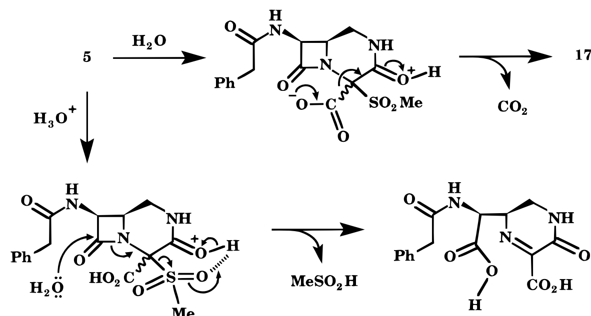
Solubility and Stability of *β*-Lactams (±)-3, (±)-4, (±)-5, (±)-10, and (±)-17 in Water. We found that the solubility in water was 21 and 27 mg/mL for isodethiaazacephems (±)-**3** and (±)-**4**, respectively; they were stable at physiological pH for 6 and 4 days, respectively. At pH 1.0, the *β*-lactam rings in (±)-**3** and (±)-**4** survived for ~4 and ~2 h, respectively; yet at pH 12, they were destructed within 5−10 min. On the other hand, 3-hydroxyisodethiaazacephem (±)-**10** was highly soluble (35 mg/mL) in a phosphate buffer (0.10 M, pH 6.8) and was stable at least for 2 months.

Scheme 5. Synthesis of Isodethiaazacepham **5**[a]



[a] Reagents: (a) $MeSO_2Cl$, Et_3N, CH_2Cl_2, 0 °C (85%); (b) Pd/C, H_2, EtOAc, rt (90%); (c) $PdCl_2$, H_2, EtOH, rt; **16** → **5** (20%) + **17** (50%).

Scheme 6. Decarboxylation and Destruction of *β*-Lactam **5** at Physiological or Acidic pH



Isodethiaazacepham (±)-**5**, highly soluble (32 mg/mL) in a phosphate buffer (0.10 M, pH 6.8), underwent decarboxylation gradually to give (±)-**17** at room temperature within 6 days (Scheme 6). In a basic solution with pH 12, the decarboxylation also occurred to (±)-**5** within 20 min; in an acidic solution with pH 1.0, the *β*-lactam ring in (±)-**5** was destructed within 3 h. The *β*-lactam ring in (±)-**17**, however, was destructed at pH 1.0 within 2 days.

In another series of assays, we dissolved isodethiaazacephems (±)-**3** and (±)-**4** as well as isodethiaazacepham (±)-**5** in distilled water (5.0 mg/mL). The pH value of the aqueous solutions was kept initially at about 4.0 for (±)-**3** and (±)-**4** and at about 2.0 for (±)-**5**. The pH values of the aqueous solutions of (±)-**3** and (±)-**4** changed to ~1 within 3 and 2 days, respectively. We found that the change in pH was accompanied by the destruction of the *β*-lactam rings in (±)-**3** and (±)-**4**, as detected by IR spectroscopy, as well as the liberation of $MeSO_2H$ and CF_3SO_2H, respectively. On the other hand, the pH value of an aqueous solution of (±)-**5** changed from 2.0 to ~6.0 within 5−6 days. This change was accompanied by the gradual production of (±)-**17** through a decarboxylation process (see Scheme 6).

Biological Activity. We tested the antibacterial activity of the synthesized *β*-lactams (±)-**3**, (±)-**4**, (±)-**5**, (±)-**10**, (±)-**11**, (±)-**12**, and (±)-**17** as well as the reference compounds cefotaxime,[10,11] penicillin G,[12] and 7-(*β*-phenylacetamido)-3′-desacetoxycephalosporanic acid ((+)-**2**)[6] in vitro against five pathogenic microorganisms.

Table 1. Minimal Inhibitory Concentrations[a] (μg/mL) of Synthetic β-Lactams as Well as the Reference Compounds Cefotaxime, Penicillin G, and Cephalosporin (+)-**2**

compound	*S. aureus* FDA 209P	*E. coli* ATCC 39188	*S. typhi* O-901	*Ps. aeruginosa* 1101–75	*K. pneumoniae* NCTC 418
cefotaxime	0.080	0.25	20.30	62.35	10.25
penicillin G	0.40	2.30	>128	>128	>128
(+)-**2**	0.64	13.13	24.50	100.0	2.98
(±)-**3**	0.070	0.95	1.20	4.38	0.68
(±)-**4**	0.010	0.090	0.68	1.15	0.24
(±)-**5**	48.50	97.17	65.30	120.0	51.02
(±)-**10**	29.50	94.68	>128	>128	>128
(±)-**11**	>128	>128	>128	>128	>128
(±)-**12**	>128	>128	>128	>128	>128
(±)-**17**	>128	>128	>128	>128	>128

[a] Obtained by the serial broth dilution method. The values represent the average of triplicate determinations.

The doses used were as high as 128 μg/mL. The results are summarized in Table 1.[13]

Discussion

By following the newly proposed enzyme-initiated 1,2-elimination as shown in Scheme 2, we found that mesylated β-lactam (±)-**5** indeed exhibited some antibacterial activity (Table 1). The lack of significant activity is due to its instability in the testing media: (±)-**5** was gradually degraded to the biologically inactive compound (±)-**17**. A carboxyl group at the C-4 position of cephalosporins (**1**) is essential for recognition by the target enzymes,[14] such as penicillin-binding proteins (PBPs). Compound **17** cannot interact with the target enzymes because it lacks such a functionality.

Results from biological tests reveal promising antimicrobial activities for the enol sulfonate β-lactams (±)-**3** and (±)-**4**. In contrast, enol β-lactam (±)-**10** exhibited much lower activity. Nevertheless, all of these three β-lactams must be substrates of PBPs.[14] Thus, our results indicate the importance of mesylate and triflate functionalities at the C-3 position on the biological activity of cephalosporins. On the other hand, their benzyl ester derivatives (±)-**11** and (±)-**12** did not exhibit antibacterial activity (Table 1) although they both possess an excellent SO_2R leaving group. Thus, both the chemical reactivity and the recognition capability of a substrate by the target enzymes are essential for its biological activity, as expected.

In conclusion, the antibacterial activity of cephalosporins is enhanced substantially by possessing a potential leaving group at the C-3′ position.[3,4,15–19] The trifluoromethane sulfone unit in (±)-**4** is a better leaving group than the methane sulfone unit in (±)-**3**. Thus, antibacterial activity is more potent for (±)-**4** than (±)-**3**, as observed. This is in agreement with our hypothesis regarding their mode of action in biological systems (Scheme 1).

Experimental Section

General Methods. For anhydrous reactions, glassware was dried overnight in an oven at 120 °C and cooled in a desiccator over anhydrous $CaSO_4$ or silica gel. Reagents were purchased from Fluka Chemical Co. Solvents, including chloroform, dichloromethane, dimethylformamide, ethyl acetate, hexanes, and pyridine, were distilled over CaH_2 under nitrogen. Absolute ethanol was purchased from Merck and used as received. Solid magnesium sulfate (i.e., $MgSO_4$ (s)) from Aldrich was used for drying reaction products after workup. Reactions were carried out in nitrogen atmosphere; the apparatus was evacuated and filled with dry nitrogen at least three times.

Melting points were obtained with a Büchi 510 melting point apparatus. Infrared (IR) spectra were recorded on a Beckman IR-8 spectrophotometer. The wavenumbers reported are referenced to the 1601 cm^{-1} absorption of polystyrene. Proton NMR spectra were obtained on a Varian XL-300 (300-MHz) spectrometer. Chloroform-*d* and D_2O were used as solvents; Me_4Si (δ 0.00 ppm) was used as an internal standard. All NMR chemical shifts are reported as δ values in parts per million (ppm), and coupling constants (J) are given in hertz (Hz). The splitting pattern abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quartet; br, broad; m, unresolved multiplet due to the field strength of the instrument; and dd, doublet of doublets. Microanalyses were performed on a Perkin-Elmer 240-B microanalyzer. Purification on silica gel refers to gravity column chromatography on Merck silica gel 60 (particle size 230–400 mesh), packed in glass column (35 g of silica gel/gram of crude material). Analytical TLC was performed on precoated plates purchased from Merck (silica gel 60 F_{254}). Compounds were visualized by use of UV light, I_2 vapor, or 2.5% phosphomolybdic acid in ethanol with heating.

(±)-Dibenzyl 2-[*cis*-4-(Azidomethyl)-2-oxo-3-(phenylacetamido)-1-azetidinyl]malonate (7). To a solution containing (±)-**6** (2.97 g, 4.99 mmol) in DMF (40 mL) was added NaN_3 (1.30 g, 20.0 mmol). The solution was stirred at room temperature for 48 h and then partitioned between Et_2O (100 mL) and water (100 mL). The organic layer was washed with water (4 × 80 mL), dried over $MgSO_4$ (s), filtered, and concentrated under reduced pressure. The crude product was purified by use of column chromatography ($CHCl_3$ as eluant) to give (±)-**7** (2.43 g, 4.49 mmol) as a foam in 90% yield: ^1H NMR ($CDCl_3$) δ 3.59 (s, 2H), 3.68–3.88 (m, 2H), 4.10–4.28 (m, 1H), 5.27 (s, 4H), 5.32 (s, 1H), 5.34 (dd, J = 8.5, 5.0, 1H), 6.99 (d, J = 8.5, 1H), 7.15–7.45 (m, 15H); IR (CH_2Cl_2) 3405, 2100, 1768, 1740, 1680 cm^{-1}. Anal. ($C_{29}H_{27}N_5O_6$) C, H, N.

(±)-Dibenzyl 2-[*cis*-4-(Azidomethyl)-2-oxo-3-(phenylacetamido)-1-azetidinyl]-2-chloromalonate (8). To a solution of (±)-**7** (2.70 g, 4.99 mmol) in CH_2Cl_2 (50 mL) was added Et_3N (0.61 g, 6.0 mmol). Trifluoromethanesulfonyl chloride (0.86 g, 5.1 mmol) in CH_2Cl_2 (5.0 mL) was added dropwise to the reaction mixture at 0 °C over 5.0 min. After the mixture was warmed to room temperature, it was concentrated to dryness, and then Et_2O was added. The ethereal layer was washed with water (2 × 50 mL), dried over $MgSO_4$ (s), and treated with charcoal. After filtration, evaporation, and purification by use of column chromatography ($CHCl_3$ as eluant), β-lactam (±)-**8** (2.59 g, 4.49 mmol) was obtained in 90% yield as a foam: ^1H NMR ($CDCl_3$) δ 3.58 (s, 2H), 3.70–3.91 (m, 2H), 4.15–4.30 (m, 1H), 5.21 (s, 2H), 5.32 (s, 2H), 5.36 (dd, J = 8.0, 5.0, 1H), 7.00 (d, J = 8.0, 1H), 7.20–7.38 (m, 15H); IR (CH_2Cl_2) 3410, 2110, 1790, 1750, 1682 cm^{-1}. Anal. ($C_{29}H_{26}N_5O_6Cl$) C, H, N, Cl.

Benzyl (6*RS*,7*SR*)-3-Hydroxy-8-oxo-7-(phenylacetamido)-1,4-diazabicyclo[4.2.0]oct-2-ene-2-carboxylate ((±)-9). Method A: β-Lactam (±)-**7** (2.70 g, 4.99 mmol) in EtOAc (80

mL) was hydrogenated under 30−35 psi of H_2 in the presence of Pd/C (10%, 40.0 mg, 0.0400 mmol) at room temperature for 30 min. After filtration and evaporation, the crude foam was chromatographed (EtOAc as eluant) to give (±)-**9** (1.91 g, 4.69 mmol) as a foam in 94% yield: ^{1}H NMR ($CDCl_3$) δ 2.50−2.68 (br, 1H), 2.85−3.02 (br, 1H), 3.21−3.45 (m, 2H), 3.55 (s, 2H), 4.18−4.43 (m, 1H), 5.09 (dd, J = 8.0, 4.5, 1H), 5.15 (s, 2H), 6.38 (d, J = 8.0, 1H), 7.31−7.42 (m, 10H); IR (CH_2Cl_2) 3500−3200, 1787, 1740, 1725, 1680 cm^{-1}. Anal. ($C_{22}H_{21}N_3O_5$) C, H, N.

Method B: β-Lactam (±)-**8** (575 mg, 0.998 mmol) in EtOAc (50 mL) was hydrogenated under 30−35 psi of H_2 in the presence of Pd/C (10%, 20.0 mg, 0.020 mmol) at room temperature for 30 min. After filtration and evaporation, the crude foam was chromatographed (EtOAc as eluant) to give (±)-**9** (354 mg, 0.868 mmol) as a foam in 87% yield.

(6*RS*,7*SR*)-3-Hydroxy-8-oxo-7-(phenylacetamido)-1,4-diazabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid ((±)-10). A solution of (±)-**9** (0.41 g, 1.0 mmol) in EtOH (35 mL) was hydrogenated on $PdCl_2$ (150 mg, 0.846 mmol) at 60 psi of H_2 at room temperature for 3.0 h. It was then filtered and concentrated under reduced pressure. The crude product was recrystallized from EtOAc to afford pure (±)-**10** (0.16 g, 0.50 mmol) in 50% yield: mp 140−142 °C; ^{1}H NMR ($CDCl_3$/DMSO-d_6/D_2O) δ 3.20−3.41 (m, 2H), 3.50 (s, 2H), 4.20−4.40 (m, 1H), 5.05 (d, J = 5.0, 1H), 7.30−7.58 (m, 5H); IR (Nujol) 3650−3155, 1781, 1725, 1680 cm^{-1}. Anal. ($C_{15}H_{15}N_3O_5$) C, H, N.

Dibenzyl (5*RS*,6*SR*)-7-Oxo-6-(phenylacetamido)-1,3-diazabicyclo[3.2.0]heptane-2,2-dicarboxylate ((±)-14). Triethylamine (0.12 g, 1.2 mmol) was added to a solution of (±)-**8** (0.58 g, 1.0 mmol) in CH_2Cl_2 (50 mL) at 0 °C, and then H_2S was bubbled into the solution for 15 min. The solution was allowed to stand at room temperature for 2.0 h while evolution of N_2 gas was observed. The solution was purged with N_2 gas for 30 min, which was then washed with water (4 × 50 mL), dried over $MgSO_4$ (s), and concentrated under reduced pressure. The crude product was purified by use of column chromatography (EtOAc as eluant) to afford (±)-**14** (0.21 g, 0.40 mmol) as a foam in 40% yield. Further elution of the column with EtOAc gave (±)-**9** (61 mg, 0.15 mmol) as a foam in 15% yield. For (±)-**14**: ^{1}H NMR ($CDCl_3$) δ 2.85−3.29 (m, 2H), 3.31−3.46 (br, 1H), 3.53 (s, 2H), 4.29−4.52 (m, 1H), 5.31 (dd, J = 8.0, 4.5, 1H), 5.20 (s, 2H), 5.21 (s, 2H), 7.16−7.46 (m, 16H); IR (CH_2Cl_2) 3450−3300, 1786, 1749, 1682 cm^{-1}. Anal. ($C_{29}H_{27}N_3O_6$) C, H, N.

Benzyl (6*RS*,7*SR*)-3-[(Methylsulfonyl)oxy]-8-oxo-7-(phenylacetamido)-1,4-diazabicyclo[4.2.0]oct-2-ene-2-carboxylate ((±)-11). To a solution containing (±)-**9** (4.07 g, 9.99 mmol) and pyridine (2.80 g, 35.4 mmol) in CH_2Cl_2 (86 mL) was added $MeSO_2Cl$ (1.15 g, 10.0 mmol). After the solution was stirred at 15 °C for 5.0 h, it was washed with water (100 mL), dried over $MgSO_4$ (s), and concentrated under reduced pressure. Purification of the residue by use of column chromatography (EtOAc as eluant) gave (±)-**11** (2.18 g, 4.50 mmol) as a foam in 45% yield: ^{1}H NMR ($CDCl_3$) δ 2.66−2.80 (br, 1H), 2.99 (s, 3H), 3.22−3.45 (m, 2H), 3.55 (s, 2H), 4.10−4.41 (m, 1H), 5.05 (dd, J = 9.0, 5.0, 1H), 5.23 (s, 2H), 6.40 (d, J = 9.0, 1H), 7.30−7.50 (m, 10H); IR (CH_2Cl_2) 3450−3250, 1788, 1750, 1730, 1680 cm^{-1}. Anal. ($C_{23}H_{23}N_3O_7S$) C, H, N, S.

(6*RS*,7*SR*)-3-[(Methylsulfonyl)oxy]-8-oxo-7-(phenylacetamido)-1,4-diazabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid ((±)-3). A solution of (±)-**11** (0.500 g, 1.03 mmol) in EtOH (50 mL) was hydrogenated with H_2 at 60 psi on $PdCl_2$ (150 mg, 0.846 mmol) at room temperature for 3.0 h. The solution was then filtered and concentrated under reduced pressure. Purification of the residue by use of column chromatography (EtOAc/EtOH (9:1)) gave (±)-**3** (0.14 g, 0.36 mmol) in 35% yield: mp 115−117 °C; ^{1}H NMR ($CDCl_3$/D_2O) δ 2.98 (s, 3H), 3.20−3.43 (m, 2H), 3.52 (s, 2H), 4.12−4.42 (m, 1H), 5.10 (d, J = 5.0, 1H), 7.25−7.48 (m, 6H); IR (CH_2Cl_2) 3460−3100, 1787, 1710, 1700, 1680 cm^{-1}. Anal. ($C_{16}H_{17}N_3O_7S$) C, H, N, S.

Benzyl (6*RS*,7*SR*)-3-[[(Trifluoromethyl)sulfonyl]oxy]-8-oxo-7-(phenylacetamido)-1,4-diazabicyclo[4.2.0]oct-2-ene-2-carboxylate ((±)-12) and Benzyl (6*SR*,7*SR*)-3-[[(Trifluoromethyl)sulfonyl]oxy]-8-oxo-7-(phenylacetamido)-4-[(trifluoromethyl)sulfonaza]-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylate ((±)-13). To a solution containing (±)-**9** (4.07 g, 9.99 mmol) and pyridine (2.80 g, 35.4 mmol) in CH_2Cl_2 (80 mL) was added CF_3SO_2Cl (1.69 g, 10.0 mmol) in CH_2Cl_2 (5.0 mL). After the solution was stirred at 15 °C for 5.0 h, it was washed with water (100 mL), dried over $MgSO_4$ (s), and concentrated under reduced pressure. Purification of the residue by use of column chromatography (EtOAc as eluant) gave (±)-**12** (1.62 g, 3.00 mmol) as a foam in 30% yield. Further elution of the column with EtOAc afforded (±)-**13** (0.67 g, 1.0 mmol) as an oil in 10% yield. For (±)-**12**: ^{1}H NMR ($CDCl_3$) δ 2.96−3.10 (br, 1H), 3.24−3.48 (m, 2H), 3.54 (s, 2H), 4.21−4.49 (m, 1H), 5.04 (dd, J = 9.0, 5.0, 1H), 5.25 (s, 2H), 6.48 (d, J = 9.5, 1H), 7.25−7.48 (m, 10H); IR (CH_2Cl_2) 3455−3250, 1792, 1752, 1735, 1680 cm^{-1}. Anal. ($C_{23}H_{20}F_3N_3O_7S$) C, H, F, N, S. For (±)-**13**: ^{1}H NMR ($CDCl_3$) δ 3.45−3.78 (m, 2H), 3.58 (s, 2H), 4.26−4.51 (m, 1H), 5.06 (dd, J = 8.0, 4.5, 1H), 5.36 (s, 2H), 6.60 (d, J = 8.0, 1H), 7.30−7.50 (m, 10H); IR (CH_2Cl_2) 3350−3300, 1798, 1754, 1739, 1678 cm^{-1}.

(6*RS*,7*SR*)-3-[[(Trifluoromethyl)sulfonyl]oxy]-8-oxo-7-(phenylacetamido)-1,4-diazabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid ((±)-4). A solution of (±)-**12** (0.54 g, 1.0 mmol) in EtOH (50 mL) was hydrogenated with H_2 at 60 psi on $PdCl_2$ (150 mg, 0.846 mmol) at room temperature for 3.0 h. The solution was then filtered and concentrated under reduced pressure. Purification of the residue by use of column chromatography (EtOAc/EtOH (9:1)) gave (±)-**4** (135 mg, 0.300 mmol) in 30% yield: mp 100−102 °C; ^{1}H NMR ($CDCl_3$/D_2O) δ 3.21−3.43 (m, 2H), 3.55 (s, 2H), 4.13−4.44 (m, 1H), 5.02 (d, J = 5.0, 1H), 7.30−7.50 (m, 5H); IR (CH_2Cl_2) 3465−3100, 1790, 1720, 1710, 1680 cm^{-1}. Anal. ($C_{16}H_{14}F_3N_3O_7S$) C, H, F, N, S.

(±)-Dibenzyl 2-[*cis*-4-(Azidomethyl)-2-oxo-3-(phenylacetamido)-1-azetidinyl]-2-mesylmalonate (15). To a solution containing (±)-**7** (5.41 g, 9.99 mmol) and Et_3N (1.05 g, 10.4 mmol) in CH_2Cl_2 (80 mL) was added dropwise $MeSO_2Cl$ (1.15 g, 10.0 mmol) in CH_2Cl_2 (10 mL). After the solution was stirred at 0 °C for 1.0 h, it was washed with water (100 mL), dried over $MgSO_4$ (s), and concentrated under reduced pressure. Purification of the residue by use of column chromatography ($CHCl_3$ as eluant) afforded (±)-**15** (5.26 g, 8.49 mmol) in 85% yield: mp 114−115 °C; ^{1}H NMR ($CDCl_3$) δ 3.39 (s, 3H), 3.61 (s, 2H), 3.69−3.95 (m, 2H), 4.15−4.30 (m, 1H), 5.12 (s, 2H), 5.13 (s, 2H), 5.35 (dd, J = 8.0, 5.0, 1H), 6.98 (d, J = 8.0, 1H), 7.40−7.70 (m, 15H); IR (CH_2Cl_2) 3410, 2100, 1790, 1751, 1680 cm^{-1}. Anal. ($C_{30}H_{29}N_5O_8S$) C, H, N, S.

Benzyl (2*RS*,6*RS*,7*SR*)- and (2*SR*,6*RS*,7*SR*)-2-(Methylsulfonyl)-3,8-dioxo-7-(phenylacetamido)-1,4-diazabicyclo-[4.2.0]octane-2-carboxylate (Diastereoisomeric Mixture, (±)-16). A solution of (±)-**15** (3.10 g, 5.00 mmol) in EtOAc (200 mL) was hydrogenated on 10% Pd/C (40 mg, 0.041 mmol) at 30−35 psi of H_2 at room temperature for 1.5 h. After filtration and condensation, the crude foam was crystallized from Et_2O to give (±)-**16** (2.19 g, 4.50 mmol) in 90% yield: mp 135−137 °C; ^{1}H NMR ($CDCl_3$) δ 3.41 (s, 3H), 3.35−3.61 (m, 2H), 3.58 (s, 2H), 4.20−4.45 (m, 1H), 4.96 (dd, J = 8.0, 4.5, 1H), 5.14 (s, 2H), 6.40−7.10 (br, 2H), 7.35−7.63 (m, 10H); IR (CH_2Cl_2) 3415−3405, 1791, 1745, 1682, 1668 cm^{-1}. Anal. ($C_{23}H_{23}N_3O_7S$) C, H, N, S.

(2*RS*,6*RS*,7*SR*)- and (2*SR*,6*RS*,7*SR*)-2-(Methylsulfonyl)-3,8-dioxo-7-(phenylacetamido)-1,4-diazabicyclo[4.2.0]-octane-2-carboxylic Acid (Diastereoisomeric Mixture, (±)-5) and (6*RS*,7*SR*)-3-Hydroxy-8-oxo-7-(phenylacetamido)-1,4-diazabicyclo[4.2.0]oct-2-ene-2-methyl Sulfone ((±)-17). A solution of (±)-**16** (0.49 g, 1.0 mmol) in EtOH (40 mL) was hydrogenated with H_2 at 60 psi on $PdCl_2$ (150 mg, 0.846 mmol) at room temperature for 4.0 h. The solution was then filtered and concentrated under reduced pressure. Purification of the residue by use of column chromatography (EtOAc as eluant) gave (±)-**17** (0.18 g, 0.50 mmol) as a foam in 50% yield. Further elution of the column with a mixture of EtOAc and EtOH (4:1) afforded (±)-**5** (80 mg, 0.20 mmol)

in 20% yield. For (±)-**5**: mp 160−166 °C; ^{1}H NMR ($CDCl_3$/D_2O) δ 3.20−3.42 (m, 2H), 3.19 (s, 3H), 3.55 (s, 2H), 4.15−4.40 (m, 1H), 5.13 (d, J = 5.0, 1H), 7.23 (br s, 5H); IR (CH_2Cl_2) 3400−3120, 1780, 1700, 1680, 1670 cm^{-1}. For (±)-**17**: ^{1}H NMR ($CDCl_3$) δ 2.60−2.90 (br, 2H), 3.22−3.48 (m, 2H), 3.50 (s, 3H), 3.59 (s, 2H), 4.17−4.42 (m, 1H), 5.13 (dd, J = 8.0, 4.5, 1H), 6.95 (d, J = 8.0, 1H), 7.30 (br s, 5H); IR (CH_2Cl_2) 3500−3200, 1789, 1727, 1680 cm^{-1}. Anal. ($C_{15}H_{17}N_3O_5S$) C, H, N, S.

Antibacterial Activity Tests. The serial broth dilution method was used to study the antibiotic activity.[13] The inocula were prepared by use of the heart infusion broth (Difco Laboratories) to make 10^{-4} dilutions of the overnight cultures. Tubes of the seeded antibiotic-containing media were incubated at 37 °C for 20 h. The lowest concentration of antibiotic that prevented visible growth of microorganisms was then determined.

Acknowledgment. We thank the National Science Council of the Republic of China and Academia Sinica for financial support of this work. We are also grateful to Prof. Sunney I. Chan for helpful discussions.

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JM980476H