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Design, Synthesis, and Biological Evaluation of a Series of β-Lactam-Based Prodrugs

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Abstract—By use of pro-dual-drug concept the synthesis of 6-β-[(*R*)-2-(clavaminio-9-*N*-yl)-2-(4-hydroxyphenylacetamido)]penicillanic acid (**10**), 6-β-[(*R*)-2-(amino)-2-(4-(clavulano-9-*O*-yl)phenylacetamido)]penicillanic acid (**13**), (*Z*)-4-[2-(amoxycillin-4-*O*-yl)ethylidene]-2-(clavulano-9-*O*-yl)-3-methoxy-$\Delta^{\alpha,\beta}$-butenolide (**19**), and 3-[(amoxicillin-4-*O*-yl)methyl]-7-(phenoxyacetamido)-(1-oxo)-3-cephem-4-carboxylic acid (**23**) was accomplished. Unlike penicillin G, ampicillin, or amoxicillin, these four heretofore undescribed compounds **10**, **13**, **19**, and **23** showed notable activity against β-lactamase (βL) producing microorganisms, *Staphylococcus aureus* A9606, *S. aureus* A15091, *S. aureus* A20309, *S. aureus* 95, *Escherichia coli* A9675, *E. coli* A21223, *E. coli* 27C7, *Pseudomonas aeruginosa* 18S-H, and *Klebsiella pneumoniae* A20634 TEM. In comparison with amoxicillin (**9**), α-amino-substituted compound **10** and butenolide derivative **19** showed a broadened spectrum of antibacterial activity; yet they were found to be less active than **13** and **23**. Like clavulanic acid (**7**) or cephalosporin-1-oxide (**21**), the newly synthesized compounds **10**, **13**, **15**, **16**, **19**, or **23** functioned as potent inhibitors of various bacterial βLs.

Introduction

Since the discovery of penicillin, β-lactam antibiotics have been the most important family of antibacterial agents. They exert certain biological activity by acylating serine residues of transpeptidases, where the cross-linking of peptidoglycans does not take place.[1] Bacteria become resistant to antibiotics either through genetic mutations or by acquiring resistant genes from other bacteria.[2] The rapid development and spread of mechanisms of bacterial resistance, however, are making virtually all β-lactam antibiotics obsolete.[2] The main cause of bacterial resistance to β-lactam antibiotics is the β-lactamases (βLs), which are related in evolutionary terms to transpeptidases.[3] There are three ways to overcome the destructive action of a βL. The first is to alter the structure of the β-lactam, rendering it insensitive to hydrolysis by the βL while maintaining its potency as an antibiotic.[4] It was often found that molecules more resistant to the βL were also less good as antibiotics, since at least some of the enzymes of cell-wall biosynthesis are acylated by β-lactam antibiotics at a unique serine residue in a peptide that shows convincing homology[5] with the serine residue involved in acyl-enzyme formation by the βL.[6] The second approach includes dual actions cephems, which can kill bacteria by two different mechanisms for example release of quinolones acting on bacterial DNA gyrase.[7] However, other antibiotics (i.e., quinolones) display more toxic side effects than β-lactam antibiotics. The third strategy

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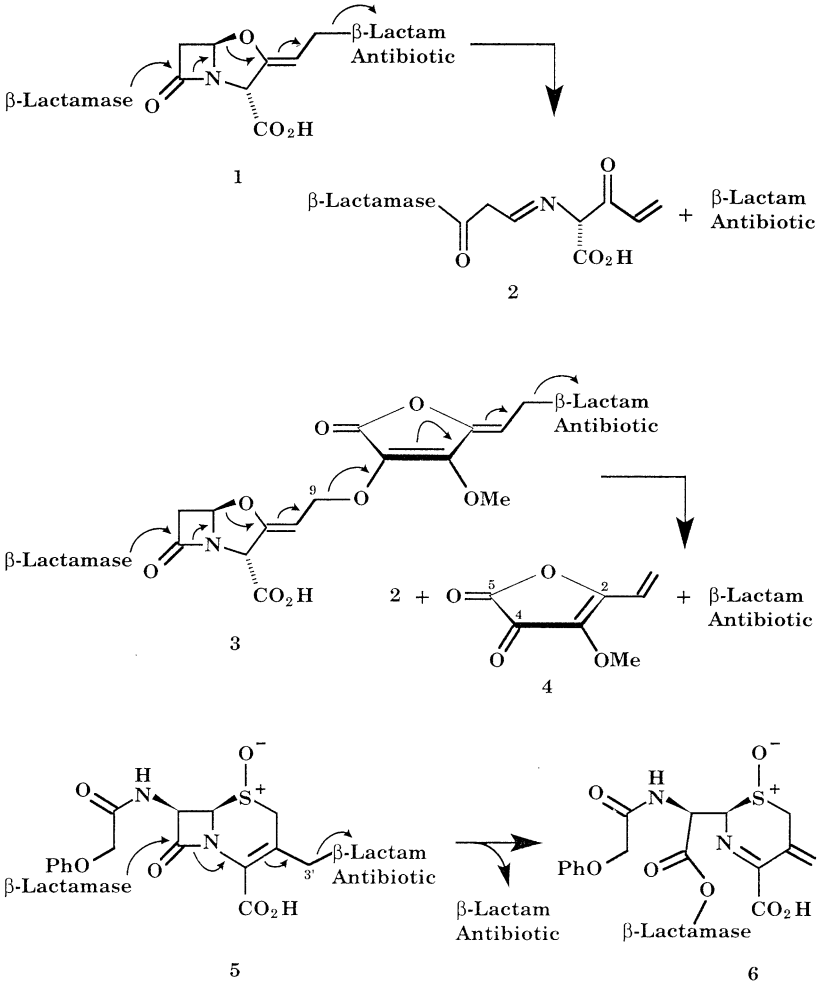
uses a reagent [i.e., clavulanic acid (**7**)] that incapacitates the βL, in synergy with a β-lactam antibiotic that would otherwise be rapidly destroyed by the enzyme.[8–10] Indeed, a combination of amoxicillin (**9**) and clavulanic acid (**7**), 'Augmentin', is now in clinical use. The finding of clavulanate was quickly followed by reports of the discovery and synthesis of a number of other reagents[10] (i.e., 3′-[substituted]-7-(phenoxyacetamido)-(1-oxo)-3-cephem-4-carboxylic acid)[11] having powerful inhibitory properties toward βL.

The voyage of a combination of a β-lactam antibiotic with a βL inhibitor inside Gram-negative bacteria starts with their cross via the outer membrane by passive diffusion through channels formed by the 'porin' proteins.[12] These channels do present some barrier to free access to the periplasm. After penetration through the bacterial cell wall, the antibiotic and the inhibitor must cross the periplasm on their way to the target enzymes.[13] There is no doubt, however, that there would be a difference in the penetration capability of these two independent moving molecules, β-lactam antibiotic and βL inhibitor, toward the periplasm-containing βL as well as the inner membrane enzymes that are responsible for the biosynthesis of the cell wall. If the bacterium carries the gene for the synthesis of a βL, then the periplasm may contain several thousand copies of this enzyme.[3] In addition, the major functional difference between the transpeptidase and the βL is that the latter have acquired the ability to deacylate.[3] As such, hydrolytic destruction of the antibiotic may occur even after acylation of the βL. Consequently, a change in strategy for the killing of resistant bacteria that make a βL is needed. This may include the block of the hydrolytic deacylation catalyzed by the βL, the postponement of this deacylation process, or the inhibition of the βL just prior to the acylation of the transpeptidase.

As shown in Scheme 1, ring opening of the β-lactam nucleus would occur when clavulanates **1** and **3** or cephalosporin **5** react with a βL. Consequently, the substituent attached at the C-9 position of **1** and **3** or at the C-3′ position of **5** is eliminated depending on the nature of the substituent.[14–23] These βL acylation reactions by a serine residue release the β-lactam antibiotic, which inhibits the transpeptidation reactions catalyzed by penicillin binding proteins (PBPs). As such, compounds **1**, **3**, and **5** would act as a targeted prodrug for the antibacterial agent.

Herein we report the synthesis of a new class of β-lactams that have antibacterial as well as βL inhibitory properties.



Scheme 1. A novel counterattack strategy against resistant strains of pathogenic bacteria.

They include clavulanate derivatives of amoxicillin **10**, **13**, and **19** as well as amoxicillin-containing cephalosporin **23**.

Chemistry

Synthesis of penicillin derivatives of clavaminic acid 10 and clavulanic acid 13 (Scheme 2)

Reaction of clavulanic acid (**7**) with methanesulfonyl chloride and pyridine in CH_3CN at 25 °C afforded 9-chloro-9-deoxyclavulanic acid (**8**) in 75% yield.[24a,24b] Amoxicillin (**9**) was silylated with trimethylsilyl chloride and then condensed with the trimethylsilyl ester of **8** in the presence of Et_3N at 25 °C to give the desired conjugate **10** in 80% yield. For the synthesis of prodrug **13**, amoxicillin **9** was first converted to its protected derivative **11** (65%) with diphenylmethyl chloride. Then, condensation of **11** with the trimethylsilyl ester of **8** in the presence of K_2CO_3 in CH_3CN at 25 °C led to the desired intermediate **12** in 70% yield. Removal of the diphenylmethyl group from **12** by use of CF_3CO_2H–anisole in CH_2Cl_2 gave the bifunctional target compound **13** in 85% yield.

Synthesis of penicillin-containing butenolide derivative of clavulanic acid 19 (Scheme 3)

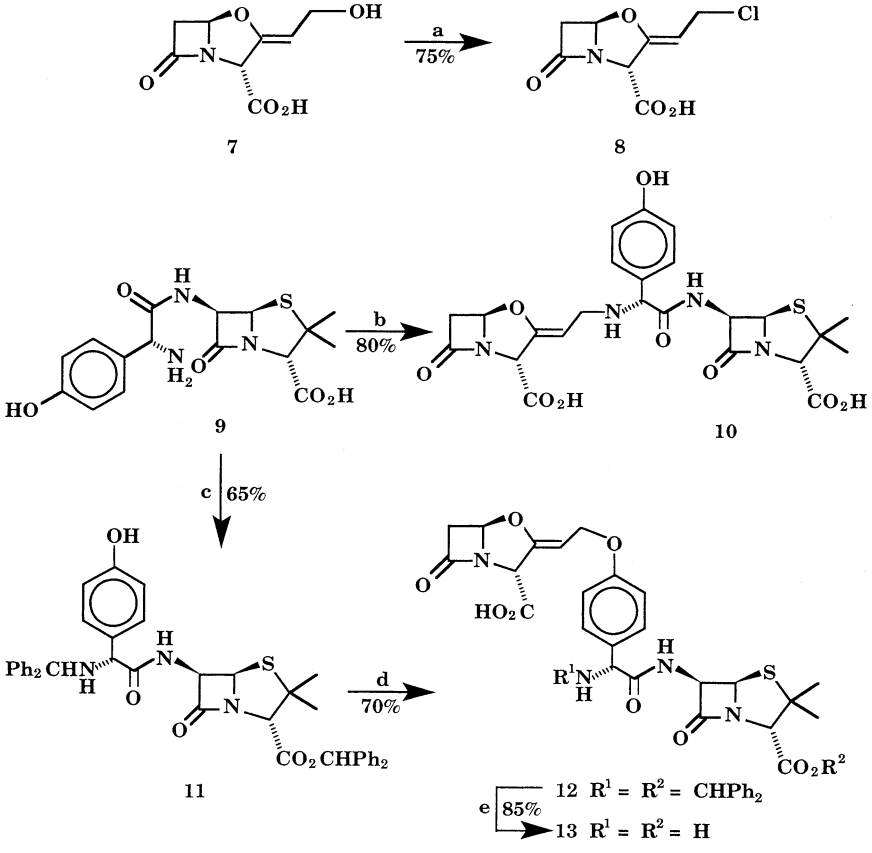
Compound **19** was synthesized in five steps. We treated butenolide **14**[24,25] with trimethylsilyl ester of **8** in the presence of K_2CO_3 in CH_3CN at 25 °C to produce clavulanate derivative **15** in 90% yield. Reaction of **15** with methanesulfonyl chloride and pyridine in CH_3CN at 25 °C afforded the corresponding chloro compound **16** in 76% yield.[24] Silylation of **16** with trimethylsilyl chloride in the presence of Et_3N at 25 °C produced trimethylsilyl ester derivative **17**. Without isolation, **17** was subsequently reacted with amoxicillin derivative **11** to give the desired intermediate **18** in 73% overall yield. Treatment of **18** with CF_3CO_2H–anisole in CH_2Cl_2 afforded the prodrug **19** in 80% yield.

Synthesis of cephalosporin 3′-amoxicillin ether 23 (Scheme 4)

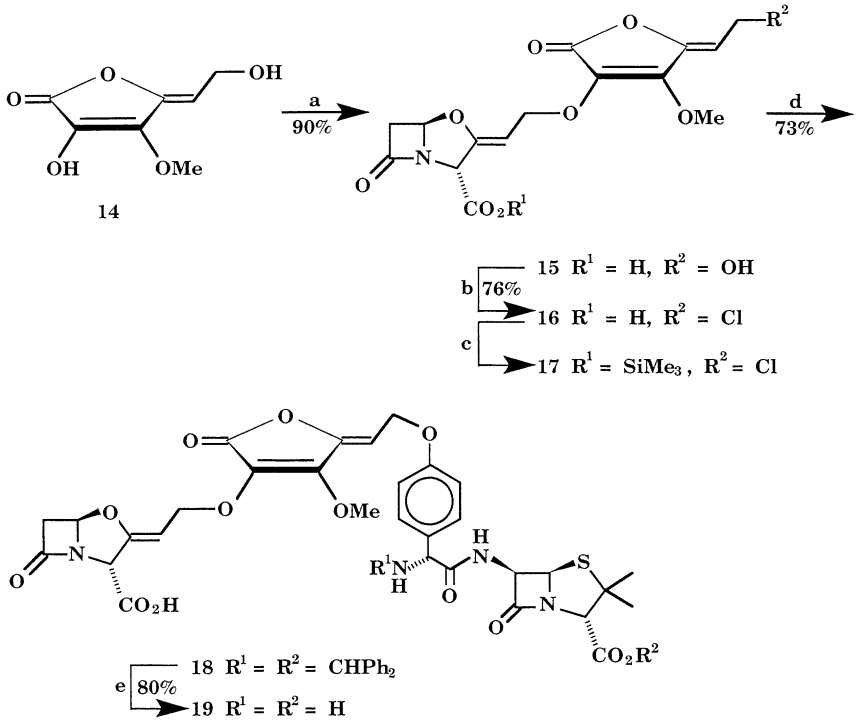
Alkylation of amoxicillin derivative **11** with 3′-iodocephalosporin **20**[7,26] in the presence of K_2CO_3 in CH_3CN at 25 °C produced the conjugate **22** in 75% yield. Conversion of **22** to pro-dual-drug **23** (87% yield) was accomplished by use of CF_3CO_2H–anisole in CH_2Cl_2 at 25 °C.

Lipophilicity, solubility, and stability studies

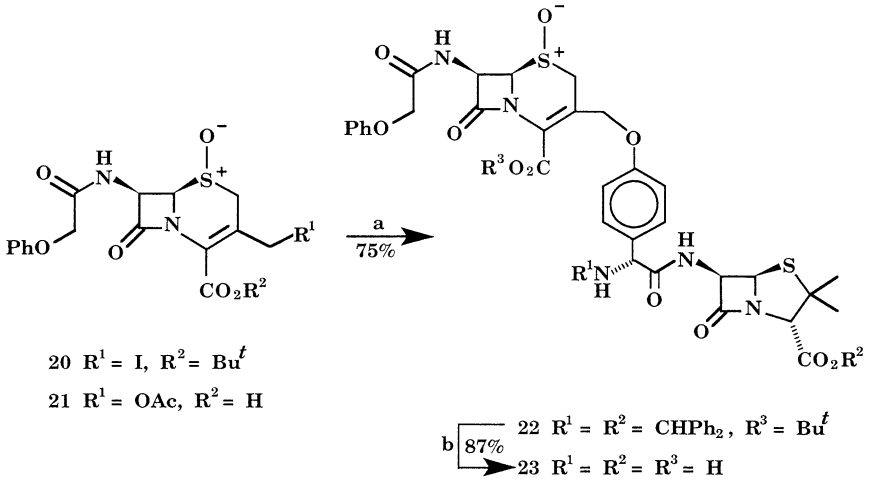
Lipophilicity and water solubility were determined by the distribution between 1-octanol and water according to the methods reported by Baker et al.[11,27] Conjugates **10**,**13**, **15**, **19**, and **23** were observed to exhibit much higher lipophilicity than that exhibited by clavulanic acid (**7**), amoxicillin (**9**), and 3′-[acetyloxymethyl]-7-(phenoxyacetamido)-(1-oxo)-3-cephem-4-carboxylic acid



Scheme 2. Reagents and conditions: (a) $MeSO_2Cl$, pyridine, CH_3CN, 25 °C, 24 h; (b) (1) Me_3SiCl, Et_3N, CH_3CN, 25 °C, 1 h; (2) trimethylsilyl ester of **8**, Et_3N, 25 °C, 6 h; (c) Ph_2CHCl, Et_3N, CH_3CN, 25 °C, 3 h; (d) K_2CO_3, trimethylsilyl ester of **8**, CH_3CN, 25 °C, 13 h; (e) CF_3CO_2H–anisole, CH_2Cl_2, 25 °C, 30 min.



Scheme 3. Reagents and conditions: (a) trimethylsilyl ester of **8**, K_2CO_3, CH_3CN, 25 °C, 24 h; (b) $MeSO_2Cl$, pyridine, CH_cCN, 25 °C, 20 h; (c) Me_3SiCl, Et_3N, CH_3CN, 25 °C, 1 h; (d) K_2CO_3, **11**, CH_3CN, 25 °C, 15 h; (e) CF_3CO_2H-anisole, CH_2Cl_2, 25 °C, 20 min.



Scheme 4. Reagents and conditions: (a) K_2CO_3, **11**, CH_3CN, 25 °C, 13 h; (b) CF_3CO_2H–anisole, CH_2Cl_2, 25 °C, 1.5 h.

(**21**). The solubility of **10**,**13**, **15**,**19**, and **23** in water was also found to be more compare to that of the parent molecules **7**, **9**, or cephalosporin-1-oxide (**21**) (see Table 1). Unlike clavulanate-containing chlorobutenolide **16** that was converted to its hydroxylated derivative **15** (15.0 min), compounds **10**, **13**, **15**, **19**, and **23** were found to be stable at physiological pH for >2 days as judged by HPLC and ^{1}H NMR studies. At pH = 9.5, however, the β-lactam ring of clavulanate moiety in **10**, **13**, **15**, and **19**, as well as the β-lactam ring of cephalosporin component in **23** decomposed within 7.0 min. After neutralization of the basic solution, amoxicillin (**9**) was isolated in about 60% yield and characterized by ^{1}H NMR. In the case of **15** or **19**, (*Z*)-4-(2-hydroxyethylidenyl)-2-hydroxy-3-methoxy-$\Delta^{\alpha,\beta}$-butenolide (**14**) was also isolated in about 55% yield.

Biological Results

Enzymatic hydrolysis study of clavulanic acid 7, amoxicillin 9, penicillin–clavaminic acid conjugate 10, penicillin–clavulanic acid conjugate 13, clavulanate-containing butenolide 15, clavulanate-containing amoxicillin derivative 19, and cephalosporin–amoxicillin conjugate 23 by ^{1}H NMR

Phosphate buffer solution (pD = 7.2) was used for ^{1}H NMR study of βLs catalyzed hydrolysis.[11,28] Minimum amount of βLs necessary for hydrolysis of clavulanic acid (**7**) was used in all cases. In the presence of βLs from *Staphylococcus aureus* 95, *S. aureus* A9606, *Escherichia coli* A9675, *E. coli* 27C7 *Pseudomonas aeruginosa* 18S-H, and *Klebsiella pneumoniae* A20634 TEM, the ^{1}H NMR spectra of clavulanic acid (**7**), amoxicillin

Table 1. Solubility in water and lipophilicity of β-lactams

Compd	Solubility in water (mg/mL)	Solubility in 1-octanol (mg/mL)	Log *P* (1-octanol/water)[a]
7	4.62	0.27	−1.23
9	3.96	0.008	−2.69
10	4.96	1.88	−0.42
13	4.72	1.79	−0.42
15	11.31	0.71	−1.20
19	4.68	3.46	−0.13
21	4.31	0.17	−1.40
23	5.04	2.13	−0.37

[a]Partition coefficients were calculated as $P = [\text{substrate}]_{1\text{-octanol}}/[\text{substrate}]_{H_2O}$.

(**9**), and cephalosporin-1-oxide (**21**) showed the β-lactam ring opening, the spectra of conjugates **10**, **13**, and **23** exhibited the appearance of the free amoxicillin (**9**), the spectrum of clavulanate-containing butenolide **15** showed the liberation of butenolide **14**, and the spectrum of prodrug **19** changed rapidly to that of the eliminated compounds **9** and **14**. In the control experiments, in the absence of βLs, **7**, **9**, **10**, **13**, **15**, **19**, **21**, and **23** were stable to hydrolysis for >2 days.

Antibacterial activity

We carried out the screening experiments for antibacterial activities of the penicillin–clavaminic acid conjugate **10**, penicillin–clavulanic acid conjugate **13**, clavulanate-containing amoxicillin derivative **19**, and cephalosporin–amoxicillin conjugate **23**. Amoxicillin (**9**),[29] a mixture of **9** and clavulanic acid (**7**) (1:1 W/W),[7b,29] ampicillin,[29] and penicillin G[29,30] were used as the reference compounds. The experiments were performed in vitro[31,32] against different strains of five pathogenic microorganisms up to 128 μg/mL. The results are summarized in Table 2.

β-Lactamase inhibitory property

We tested the βLs inhibitory[33] properties of the penicillin–clavaminic acid conjugate **10**, penicillin–clavulanic acid conjugate **13**, clavulanate-containing amoxicillin derivative **19**, and cephalosporin–amoxicillin conjugate **23**. Clavulanic acid (**7**) and cephalosporin-1-oxide (**21**) were used in vitro as the reference compounds. The results are shown in Table 3.

Discussion

βLs catalyze the hydrolysis of β-lactam antibiotics, deactivating them. These enzymes are behind the most widespread resistance mechanism to drugs of the penicillin and cephalosporin families.[34,35] To inhibit these enzymes, β-lactam-based inhibitors such as clavulanic acid (**7**) or cephalosporin-1-oxide (**21**) have been introduced.[8,11] Amoxicillin/clavulanate, augmentin, is a broad-spectrum antibiotic for the treatment of a wide range of bacterial infections.[9] The clavulanate component inhibits βL and protects amoxicillin from βL-mediated inactivation, thereby maintaining amoxicillin's antibacterial activity.[9] The exposure of bacteria to βL inhibitors over evolutionary time,[36] however, has allowed bacteria to rapidly acquire resistance to these anti-resistance drugs.[37] In addition, it is important that inhibitor and antibiotic achieve sufficiently high concentrations at the site of infection at about the same time, to ensure that treatment is successful. By applying the dual targeting approach,[7] we first combined amoxicillin (**9**) with clavulanic acid (**7**) or with cephalosporin-1-oxide (**21**) to produce novel antibacterial agents **10**, **13**, or **23** (see Schemes 2 and 4). We also combined amoxicillin (**9**) with clavulanic acid (**7**) via a butenolide linker to produce prodrug **19** (see Scheme 3). These conjugates displayed superior lipophilicity relative to their parent drugs (see Table 1), and exhibited pronounced activity against βL-producing organisms, *S. aureus* A9606, *S. aureus* A15091, *S. aureus* A20309, *S. aureus* 95, *E. coli* A9675, *E. coli* A21223, *E. coli* 27C7, *P. aeruginosa* 18S-H, and *K. pneumoniae* A20634 TEM (Table 2). These newly synthesized compounds **10**, **13**, **19**, or **23** also exhibited βLs inhibitory property com-

Table 2. Minimum inhibitory concentrations[a] of novel β-lactams **10**, **13**, **19**, **23**, and the reference compounds penicillin G (pen G), ampicillin (ampn), clavulanic acid (**7**), amoxicillin (**9**), as well as a 1:1 (W/W) mixture of **7** and **9** against pathogenic microorganisms in vitro

Microorganism	pen G	ampn	**7**	**9**	**7**+**9**	**10**	**13**	**19**	**23**
S. aureus FDA 209P	0.67	0.56	>128	0.93	0.58	1.81	1.02	2.99	1.79
S. aureus A9606[b]	>128	>128	>128	>128	2.27	0.75	0.34	0.82	0.25
S. aureus A15091[b]	>128	>128	>128	>128	3.01	0.87	0.25	1.07	0.19
S. aureus A20309[b]	>128	>128	>128	120	1.98	0.55	0.07	0.89	0.10
S. aureus 95[b,c]	>128	>128	>128	>128	3.09	1.07	0.87	1.23	0.69
E. coli ATCC 39188	3.65	3.42	>128	4.76	2.98	4.01	3.02	3.53	2.14
E. coli A9675[b]	128	97.0	>128	72.1	1.79	0.68	0.03	0.50	0.08
E. coli A21223[b]	>128	>128	>128	93.4	1.35	0.16	0.06	0.34	0.07
E. coli 27C7[b]	>128	>128	>128	>128	2.48	0.75	0.07	0.90	0.10
P. aeruginosa 1101–75	>128	>128	>128	>128	8.74	7.32	6.24	5.65	4.85
P. aeruginosa 18S-H[b]	>128	>128	>128	>128	6.05	1.01	0.42	1.30	0.06
S. typhi O-901	>128	>128	>128	>128	5.93	8.53	4.37	6.34	3.71
K. pneumoniae NCTC 418	>128	>128	>128	>128	2.45	3.10	2.15	5.21	1.79
K. pneumoniae A20634 TEM[b]	>128	>128	>128	>128	1.98	0.47	0.09	0.71	0.12

[a]The values of minimum inhibitory concentrations (μg mL^{-1}), obtained as the average of duplicate determinations, represent the lowest concentrations of antibiotics required to prevent visible growth of microorganisms. 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.[32]
[b]β-Lactamase-producing organism.
[c]Methicillin resistant organism.

Table 3. Minimum protective concentrations[a] of novel β-lactams **10**, **13**, **19**, **23**, as well as the reference compounds clavulanic acid (**7**) and cephalosporin-1-oxide (**21**) against bacterial βLs

βL from	7	10	13	19	21	23
S aureus A9606	0.53	1.29	0.76	1.87	0.97	1.05
S. aureus 95	0.71	2.05	1.04	2.24	1.10	1.27
E. coli A9675	4.08	6.31	4.72	3.08	2.08	2.99
E. coli 27C7	1.40	2.87	1.57	1.75	0.97	1.20
P. aeruginosa 18S-H	3.08	5.20	4.02	6.21	1.68	2.11
K. pneumoniae A20634 TEM	0.20	1.45	0.39	2.03	0.94	1.03

[a]The values of minimum protective concentrations (μg mL^{-1}), obtained as the average of duplicate determinations, represent the lowest concentration of compounds required to protect an indicator, 3-[*E*-(2,4-dinitro)styryl]-(6*R*,7*R*)-7-(2-thienylacetamido)-3-cephem-4-carboxylic acid, from hydrolysis by βLs under standard test conditions[33] within 35 min. The hydrolysis of indicator was evidenced by the appearance of a distinct red color.

parable to that of the parent molecule, clavulanic acid (**7**) or cephalosporin-1-oxide (**21**) (Table 3). They underwent hydrolysis by βLs to liberate their amoxicillin component, as evidenced by their notable values of the minimum protective concentrations (MPC) against the β-lactamases of *S. aureus* A9606, *S. aureus* 95, *E. coli* A9675,*E. coli* 27C7, *P. aeruginosa* 18S-H, and *K. pneumoniae* A20634 TEM. Therefore, conjugates **10**, **13**, **19**, and **23** exhibited 'augmentin-like' activity against resistant strains of pathogenic microorganisms (see Table 2).

Conclusions

To combat resistant strains of pathogenic microorganisms, clavulanic acid (**7**) was attached to amoxicillin (**9**) at either the α-amino or the phenolic hydroxy group to afford the corresponding conjugates **10** and **13**, respectively. Similarly, attachment of amoxicillin (**9**) to the cephalosporin-1-oxide (**21**) at the C-3′ position afforded antibiotic **23**. Clavulanic acid (**7**) was also conjugated with amoxicillin (**9**) through a butenolide linker to produce antibacterial agent **19**. These compounds exhibited notable MPC values against the βLs of different bacterial species. Their antibacterial activity was found to be better than amoxicillin/clavulanic acid, 'Augmentin', against βL producing microorganisms, *S. aureus* A9606, *S. aureus* A15091, *S. aureus* A20309, *S. aureus* 95, *E. coli* A9675, *E. coli* A21223, *E. coli* 27C7, *P. aeruginosa* 18S-H, and *K. pneumoniae* A20634 TEM.

Experimental

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 (Switzerland) or Sigma (St. Louis, USA). Solvents, including dry ether and tetrahydrofuran (THF), were obtained by distillation from the sodium ketyl of benzophenone under nitrogen. Other solvents, including chloroform, dichloromethane, ethyl acetate, and hexanes were distilled over CaH_2 under nitrogen. Absolute methanol and ethanol were purchased from Merck (Germany) and used as received.

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*, D_2O, and dimethylsulfoxide-d_6 were used as solvent; 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. UV spectroscopy was carried out using an HP8452A diode array spectrophotometer. Mass spectra were carried out on a VG 70–250 S mass spectrometer. 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). 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.

Determination of solubility in water

Each compound, **7**, **9**, **10**, **13**, **15**, **19**,**21**, and **23** (70 mg) was agitated in a 25-mL volumetric flask with phosphate buffer (0.10 M, pH 6.9, 5.0 mL) for 10 min. This solution was filtered from undissolved solid through a sintered glass funnel (4.0–5.5 mesh ASTM) and the concentration of the solution was determined by UV absorbance (Table 1).

Determination of partition coefficients (lipophilicity)

A solution of each compound, **7**, **9**, **10**, **13**, **15**, **19**,**21**, and **23** (10 mL) in phosphate buffer (0.10 M) possessing an UV absorbance at 215–276 nm was shaken with 1-octanol (10 mL) in a separatory funnel for 1.5 h. The layers were separated and their concentrations were determined by an UV spectrophotometer. The partition coefficient was calculated as $P = [S]_{1\text{-octanol}}/[S]_{H_2O}$ (Table 1).

Enzymatic hydrolysis in phosphate buffer (pD 7.2)–(^{1}H NMR study)

Each compound, **7**, **9**, **10**, **13**, **15**, **19**,**21**, and **23**, (0.10 mmol) was dissolved in 0.10 M deutrated phosphate buffer (4.0 mL, pD 7.2) at 25 °C. The ^{1}H NMR spectra were taken at this temperature and then 12.0 nM of βL (from *S. aureus* 95, *S. aureus* A9606, *E. coli* A9675, *E. coli* 27C7 *P. aeruginosa* 18S-H, or *K. pneumoniae* A20634 TEM) was added. The ^{1}H NMR spectra at 25 °C were taken at various times. After 8 min, the spectra of clavulanic acid (**7**) and cephalosporin-1-oxide

(**21**) revealed 100% β-lactam ring opening. In each case, the spectrum of amoxicillin (**9**) showed approximately 50% hydrolysis of the substrate within 25–40 min. After 15 min, the spectra of conjugates **10**, **13**, and **23** exhibited the appearance of the free amoxicillin (**9**) quantitatively. The spectrum of clavulanate-containing butenolide **15** revealed the β-lactam ring opening of the clavulanate moiety and 100% appearance of the butenolide **14** within 20 min. Finally, the spectrum of prodrug **19** completely changed, within 30 min, to that of the eliminated compounds **9** and **14**. The solutions were extracted, individually, with MeOH/AcOH/$CDCl_3$ (2:1:1, 5×6.0 mL) to remove amoxicillin (**9**) (>90% yield), which was found to be identical with an authentic sample.

Antibacterial activity test

The serial broth dilution method was used to study the antibiotic activity.[31,32] 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 (Table 2).

β-Lactamase inhibitory property test

An established procedure[33] was used to study the βLs inhibitory property. Results are summarized in Table 3.

9-Chloro-9-deoxyclavulanic acid (8). To a solution of clavulanic acid (**7**) (1.99 g, 9.99 mmol) in CH_3CN/pyridine (2:1, 90 mL) was added $MeSO_2Cl$ (2.52 g, 22.0 mmol). The reaction mixture was stirred at 25 °C for 24 h. The solution was concentrated under reduced pressure and EtOAc (160 mL) was added. The EtOAc solution was washed with water (2×100 mL). Then, it was dried over $MgSO_4$ (s) and filtered. Evaporation under reduced pressure and purification of the residue by use of column chromatography (EtOAc) gave **8** (1.63 g, 7.49 mmol) as a foam in 75% yield: R_f (EtOAc) 0.27; IR (CH_2Cl_2) ν 3418–3295 (OH), 1804 (β-lactam), 1698 (C=C), 1652 (C=O) cm^{-1}; UV (EtOH) λ_{max} 220 (ε 8 800); ^1H NMR ($CDCl_3/D_2O$) δ 3.12 (dd, $J=17.5$, 1.0 Hz, 1H, $C_{6\beta}H$), 3.49 (dd, $J=17.5$, 3.4 Hz, 1H, $C_{6\alpha}H$), 4.07 (dd, $J=7.5$, 6.1 Hz, 2H, C_9H_2), 4.85 (ddd, $J=7.5$, 6.1, 1.3 Hz, 1H, C_8H), 4.93 (d, $J=1.3$ Hz, 1H, C_3H), 5.79 (dd, $J=3.4$, 1.0 Hz, 1H, C_5H); MS m/z 217 (M^+, Cl-cluster). Anal. calcd for $C_8H_8NO_4Cl$: C, 44.16; H, 3.71; N, 6.44; Cl, 16.29. Found: C, 44.27; H, 3.70; N, 6.53; Cl, 16.41.

6-β-[(*R*)-2-(Clavaminio-9-*N*-yl)-2-(4-hydroxyphenylacetamido)]penicillanic acid (10). To a suspension of amoxicillin (**9**) (3.25 g, 8.90 mmol) in CH_3CN (120 mL) and Et_3N (3.60 g, 35.6 mmol) was added Me_3SiCl (2.90 g, 26.7 mmol). The reaction mixture was stirred at 25 °C for 1 h. In another flask, compound **8** (1.93 g, 8.90 mmol) in CH_3CN (90 mL) and Et_3N (1.80 g, 17.8 mmol) was similarly silylated with Me_3SiCl (0.98 g, 9.00 mmol). Then, the reaction mixture-containing trimethylsilyl ester of **8** was added to the first flask-containing silylated derivative of **9**. After stirring at 25 °C for 6 h, the solution was concentrated under reduced pressure. Purification of the residue by use of column chromatography (EtOAc/MeOH 8.5:1.5) afforded **10** (3.89 g, 7.12 mmol) in 80% yield: mp 189–191 °C (decomp); R_f (EtOAc) 0.13; IR (CH_2Cl_2) ν 3500–3200 (OH, NH), 1797 (β-lactam), 1778 (β-lactam), 1690 (C=C), 1680 (amide), 1652 (C=O), 1643 (C=O) cm^{-1}; UV (EtOH) λ_{max} 228, 275 (ε 11 050, 2 357); ^1H NMR (DMSO-d_6/D_2O) δ 1.38 (s, 3H, CH_3), 1.49 (s, 3H, CH_3), 3.10 (dd, $J=17.9$, 0.9 Hz, 1H, $C_{6\beta}H$), 3.50 (dd, $J=17.9$, 3.5 Hz, 1H, $C_{6\alpha}H$), 3.68 (dd, $J=7.0$, 5.9 Hz, 2H, C_9H_2), 4.18 (s, 1H, C_3H), 4.56 (s, 1H, NCHCON), 4.79 (ddd, $J=7.0$, 5.9, 1.1 Hz, 1H, C_8H), 4.90 (d, $J=1.1$ Hz, 1H, C_3H), 5.38 (d, $J=4.5$ Hz, 1H, C_5H), 5.54 (d, $J=4.5$ Hz, 1H, C_6H), 5.82 (dd, $J=3.5$, 0.9 Hz, 1H, C_5H), 7.10 (AB q, $J=8.2$ Hz, 2H, Ph), 7.25 (AB q, $J=8.2$ Hz, 2H, Ph); MS m/z 546 (M^+). Anal. calcd for $C_{24}H_{26}N_4O_9S$: C, 52.74; H, 4.79; N, 10.25; S, 5.87. Found: C, 52.63; H, 4.82; N, 10.29; S, 5.91.

Diphenylmethyl 6-β-[(*R*)-2-(diphenylmethylamino)-2-(4-hydroxyphenylacetamido)]penicillanate (11). To a solution of amoxicillin (**9**) (2.01 g, 5.50 mmol) in CH_3CN (80 mL) and Et_3N (1.11 g, 11.0 mmol) was added Ph_2CHCl (2.23 g, 11.0 mmol). The reaction mixture was stirred at 25 °C for 3 h. To this solution was added EtOAc (200 mL). The EtOAc solution was washed with water (3×150 mL). Then, it was dried over $MgSO_4$ (s) and filtered. Evaporation under reduced pressure and purification of the residue by use of column chromatography ($CHCl_3$/EtOAc 1:1) gave **11** (2.50 g, 3.58 mmol) in 65% yield: mp 169–170 °C (decomp); R_f (EtOAc) 0.21; IR (CH_2Cl_2) ν 3370–3225 (OH, NH), 1771 (β-lactam), 1750 (ester), 1682 (amide), cm^{-1}; UV (EtOH) λ_{max} 230, 276 (ε 10,960, 1350); ^1H NMR ($CDCl_3/D_2O$) δ 1.42 (s, 3H, CH_3), 1.54 (s, 3H, CH_3), 4.37 (s, 1H, C_3H), 4.87 (s, 1H, NCHCON), 5.40 (d, $J=4.1$ Hz, 1H, C_5H), 5.57 (d, $J=4.1$ Hz, 1H, C_6H), 5.88 (s, 1H, $NCHPh_2$), 6.97 (s, 1H, $CHPh_2$), 7.04 (AB q, $J=8.0$ Hz, 2H, Ph), 7.23 (AB q, $J=8.0$ Hz, 2H, Ph), 7.30–7.50 (m, 20H, 4 Ph); MS m/z 697 (M^+), 530 ($M^+ - Ph_2CH$), 363 ($M^+ - 2\ Ph_2CH$).

Diphenylmethyl 6-β-[(*R*)-2-(diphenylmethylamino)-2-(4-(clavulano-9-*O*-yl)phenylacetamido)]penicillanate (12). To a solution of **11** (6.96 g, 9.98 mmol) in CH_3CN (150 mL) was added K_2CO_3 (4.97 g, 36.0 mmol). The mixture was stirred at 25 °C. After 30 min, an CH_3CN (50 mL) solution of trimethylsilyl ester of **8** (1.08 g, 9.98 mmol of **8** was used) was added, and the reaction mixture was stirred at 25 °C for 13 h. Then, the solution was filtered and 2% aqueous HCl (200 mL) was added to the filtrate. After addition of EtOAc (300 mL), the organic layer was separated and washed with water (3×150 mL). Then, it was dried over $MgSO_4$ (s) and filtered. Evaporation under reduced pressure and purification of the residue by use of column chromatography (EtOAc) afforded **12** (6.14 g, 6.99 mmol) in 70% yield: mp 156–158 °C; R_f (EtOAc) 0.19; IR (CH_2Cl_2) ν 3450–3220 (OH, NH), 1802 (β-lactam), 1780 (β-lactam), 1752 (ester), 1697 (C=C), 1690 (amide), 1650 (C=O) cm^{-1}; UV (EtOH) λ_{max} 206, 230, 276 (ε 765, 12 000, 2876); ^1H NMR (DMSO-d_6/D_2O) δ 1.39 (s, 3H, CH_3), 1.55 (s, 3H, CH_3), 3.11 (dd, $J=18.0$, 0.8 Hz, 1H, $C_{6\beta}H$), 3.50 (dd,

$J=18.0$, 3.0 Hz, 1H, $C_{6\alpha}H$), 4.41 (dd, $J=7.3$, 6.2 Hz, 2H, C_9H_2), 4.48 (s, 1H, C_3H), 4.83 (s, 1H, NCHCON), 4.90 (ddd, $J=7.3$, 6.2, 1.3 Hz, 1H, C_8H), 5.08 (d, $J=1.3$ Hz, 1H, C_3H), 5.41 (d, $J=4.0$ Hz, 1H, C_5H), 5.56 (d, $J=4.0$ Hz, 1H, C_6H), 5.84 (dd, $J=3.0$, 0.8 Hz, 1H, C_5H), 5.86 (s, 1H, $NCHPh_2$), 6.98 (s, 1H, $CHPh_2$), 7.01 (AB q, $J=8.0$ Hz, 2H, Ph), 7.20 (AB q, $J=8.0$ Hz, 2H, Ph), 7.25–7.54 (m, 20H, 4 Ph); MS m/z 878 (M^+), 711 ($M^+ - Ph_2CH$), 544 ($M^+ - 2\ Ph_2CH$).

6-β-[(*R*)-2-(Amino)-2-(4-(clavulano-9-*O*-yl)phenylacetamido)]penicillanic acid (13). To a solution of **12** (3.95 g, 4.50 mmol) in CH_2Cl_2 (50 mL) was added anisole (0.22 g, 2.0 mmol) and CF_3CO_2H (2.85 g, 25.0 mmol). The mixture was stirred at 25 °C for 30 min. Then, it was concentrated under reduced pressure; the residue was treated with 1% methanolic ammonia (20 mL), and then evaporated to dryness. Purification of the residue by use of column chromatography (EtOAc/EtOH 8.0:2.0) afforded **13** (2.09 g, 3.83 mmol) in 85% yield: mp 182–184 °C (decomp); R_f (EtOAc) 0.10; IR (CH_2Cl_2) ν 3540–3200 (OH, NH, NH_2), 1800 (β-lactam), 1776 (β-lactam), 1695 (C=C), 1680 (amide), 1650 (C=O), 1640 (C=O) cm^{-1}; UV (EtOH) λ_{max} 229, 275 (ε 10,767, 3010); ^1H NMR (DMSO-d_6/D_2O) δ 1.40 (s, 3H, CH_3), 1.50 (s, 3H, CH_3), 3.11 (dd, $J=18.0$, 0.9 Hz, 1H, $C_{6\beta}H$), 3.50 (dd, $J=18.0$, 3.3 Hz, 1H, $C_{6\alpha}H$), 4.41 (dd, $J=7.6$, 6.3 Hz, 2H, C_9H_2), 4.17 (s, 1H, C_3H), 4.50 (s, 1H, NCHCON), 4.90 (ddd, $J=7.6$, 6.3, 1.0 Hz, 1H, C_8H), 4.88 (d, $J=1.0$ Hz, 1H, C_3H), 5.40 (d, $J=4.0$ Hz, 1H, C_5H), 5.54 (d, $J=4.0$ Hz, 1H, C_6H), 5.80 (dd, $J=3.3$, 0.9 Hz, 1H, C_5H), 7.07 (AB q, $J=8.1$ Hz, 2H, Ph), 7.24 (AB q, $J=8.1$ Hz, 2H, Ph); CIMS m/z 547 ($M^+ + 1$). Anal. calcd for $C_{24}H_{26}N_4O_9S$: C, 52.74; H, 4.79; N, 10.25; S, 5.87. Found: C, 52.85; H, 4.88; N, 10.30; S, 5.75.

(*Z*)-4-[2-(Hydroxyethylidene)]-2-(clavulano-9-*O*-yl)-3-methoxy-$\Delta^{\alpha,\beta}$-butenolide (15). To a solution of **14** (1.72 g, 9.99 mmol) in CH_3CN (110 mL) was added K_2CO_3 (5.24 g, 38.0 mmol). The mixture was stirred at 25 °C. After 30 min, an CH_3CN solution of trimethylsilyl ester of **8** (2.17 g, 9.99 mmol of **8** was used) was added, and the reaction mixture was stirred at 25 °C for 24 h. Then, it was filtered into 2% aqueous HCl (170 mL). After addition of EtOAc (300 mL), the organic layer was separated and washed with saline (100 mL). Then, it was dried over $MgSO_4$ (s) and filtered. Evaporation under reduced pressure and purification of the residue by use of column chromatography (EtOAc/MeOH 7.5:2.5) gave **15** (1.88 g, 8.99 mmol) in 90% yield: mp 123–124 °C; R_f (EtOAc) 0.12; IR (nujol) ν 3360–3200 (OH), 2940 (C_5H), 1807 (β-lactam), 1778 (C=O), 1691 (C=C), 1653 (C=C), 1642 (C=O) cm^{-1}; UV (EtOH) λ_{max} 208, 227 (ε 9100, 5298); ^1H NMR (DMSO-d_6/D_2O) δ 3.15 (dd, $J=17.7$, 1.2 Hz, 1H, $C_{6\beta}H$), 3.49 (dd, $J=17.7$, 3.6 Hz, 1H, $C_{6\alpha}H$), 4.10 (s, 3H, C_3–OCH_3), 4.45 (d, $J=8.0$ Hz, 2H, CH_2), 4.57 (dd, $J=8.5$, 6.0 Hz, 2H, C_9H_2), 4.85 (ddd, $J=8.5$, 6.0, 1.1 Hz, 1H, C_8H), 4.93 (d, $J=1.1$ Hz, 1H, C_3H), 5.36 (t, $J=8.0$ Hz, 1H, =CH), 5.79 (dd, $J=3.6$, 1.2 Hz, 1H, C_5H); MS m/z 353 (M^+). Anal. calcd for $C_{15}H_{15}NO_9$: C, 50.99; H, 4.28; N, 3.96. Found: C, 50.87; H, 4.27; N, 3.82.

(*Z*)-4-[2-(Chloroethylidene)]-2-(clavulano-9-*O*-yl)-3-methoxy-$\Delta^{\alpha,\beta}$-butenolide (16). To a solution of **15** (1.46 g, 6.98 mmol) in CH_3CN/pyridine (2:1, 70 mL) was added $MeSO_2Cl$ (1.72 g, 15.0 mmol). The reaction mixture was stirred at 25 °C for 20 h. The solution was concentrated under reduced pressure, and EtOAc (160 mL) was added. The EtOAc solution was washed with water (100 mL). Then, it was dried over $MgSO_4$ (s) and filtered. Evaporation under reduced pressure and purification of the residue by use of column chromatography (EtOAc) gave **16** (1.29 g, 5.30 mmol) in 76% yield: mp 102–104 °C; R_f (EtOAc) 0.24; IR (nujol) ν 3297–3250 (OH), 2952 (C_5H), 1810 (β-lactam), 1779 (C=O), 1695 (C=C), 1654 (C=C), 1648 (C=O) cm^{-1}; UV (EtOH) λ_{max} 210, 227 (ε 8430, 6287); ^1H NMR (DMSO-d_6/D_2O) δ 3.17 (dd, $J=18.0$, 1.2 Hz, 1H, $C_{6\beta}H$), 3.49 (dd, $J=18.0$, 3.5 Hz, 1H, $C_{6\alpha}H$), 4.08 (s, 3H, C_3–OCH_3), 4.31 (d, $J=7.5$ Hz, 2H, CH_2), 4.60 (dd, $J=8.1$, 5.9 Hz, 2H, C_9H_2), 4.85 (ddd, $J=8.1$, 5.9, 0.8 Hz, 1H, C_8H), 4.93 (d, $J=0.8$ Hz, 1H, C_3H), 5.36 (t, $J=7.5$ Hz, 1H, =CH), 5.80 (dd, $J=3.5$, 1.2 Hz, 1H, C_5H); MS m/z 371 (M^+, Cl-cluster). Anal. calcd for $C_{15}H_{14}NO_8Cl$: C, 48.47; H, 3.80; N, 3.77; Cl, 9.54. Found: C, 48.52; H, 3.89; N, 3.80; Cl, 9.46.

(*Z*)-4-[((2-Diphenylmethyl 2-*N*-Diphenylmethylamoxicillinate)-4-*O*-yl)ethylidene]-2-(clavulano-9-*O*-yl)-3-methoxy-$\Delta^{\alpha,\beta}$-butenolide (18). To a solution of **16** (1.43 g, 5.90 mmol) in CH_3CN (50 mL) and Et_3N (1.26 g, 12.5 mmol) was added Me_3SiCl (0.67 g, 6.20 mmol). The reaction mixture was stirred at 25 °C. After 1 h, it was added to a solution of **11** (4.11 g, 5.90 mmol) in CH_3CN (90 mL) containing K_2CO_3 (2.48 g, 18.0 mmol). The mixture was stirred at 25 °C for 15 h. Then, it was filtered and 2% aqueous HCl (200 mL) was added to the filtrate. After addition of EtOAc (350 mL), the organic layer was separated, washed with water (3×100 mL), dried over $MgSO_4$ (s), and filtered. Evaporation under reduced pressure and purification of the residue by use of column chromatography (EtOAc) afforded **18** (4.45 g, 4.31 mmol) in 73% yield: mp 146–148 °C; R_f (EtOAc) 0.25; IR (CH_2Cl_2) ν 3550–3200 (OH, NH), 2945 (C_5H), 1810 (β-lactam), 1779 (β-lactam), 1776 (C=O), 1750 (ester), 1695 (C=C), 1655 (C=C), 1685 (amide), 1648 (C=O) cm^{-1}; UV (EtOH) λ_{max} 212, 230, 275 (ε 10,500, 9164, 3945); ^1H NMR (DMSO-d_6/D_2O) δ 1.42 (s, 3H, CH_3), 1.57 (s, 3H, CH_3), 3.13 (dd, $J=18.5$, 0.9 Hz, 1H, $C_{6\beta}H$), 3.51 (dd, $J=18.5$, 3.8 Hz, 1H, $C_{6\alpha}H$), 4.14 (s, 3H, C_3-OCH_3), 4.32 (d, $J=7.5$ Hz, 2H, CH_2), 4.50 (dd, $J=7.1$, 6.0 Hz, 2H, C_9H_2), 4.75 (s, 1H, C_3H), 4.80 (s, 1H, NCHCON), 4.84 (ddd, $J=7.1$, 6.0, 1.2 Hz, 1H, C_8H), 4.89 (d, $J=1.2$ Hz, 1H, C_3H), 5.28 (t, $J=7.5$ Hz, 1H, =CH), 5.45 (d, $J=4.3$ Hz, 1H, C_5H), 5.58 (d, $J=4.3$ Hz, 1H, C_6H), 5.84 (dd, $J=3.8$, 0.9 Hz, 1H, C_5H), 5.87 (s, 1H, $NCHPh_2$), 6.96 (s, 1H, $CHPh_2$), 7.10 (AB q, $J=7.8$ Hz, 2H, Ph), 7.27 (AB q, $J=7.8$ Hz, 2H, Ph), 7.30–7.59 (m, 20H, 4 Ph); MS m/z 865 ($M^+ - Ph_2CH$), 698 ($M^+ - 2\ Ph_2CH$).

(*Z*)-4-[2-(Amoxicillin-4-*O*-yl)ethylidene]-2-(clavulano-9-*O*-yl)-3-methoxy-$\Delta^{\alpha,\beta}$-butenolide (19). To a solution of **18** (5.68 g, 5.50 mmol) in CH_2Cl_2 (250 mL) was added anisole (0.22 g, 2.0 mmol) and CF_3CO_2H (2.85 g, 25.0 mmol). The mixture was stirred at 25 °C for 30 min.

Then, it was concentrated under reduced pressure, the residue was treated with 1% methanolic ammonia (35 mL), and then evaporated to dryness. Purification of the residue by use of column chromatography (EtOAc/EtOH 8.5:1.5) afforded **19** (3.08 g, 4.40 mmol) in 80% yield: mp 179–181 °C (decomp); R_f (EtOAc) 0.16; IR (CH_2Cl_2) ν 3600–3170 (OH, NH, NH_2), 2950 (C_5H), 1806 (β-lactam), 1776 (β-lactam), 1774 (C=O), 1691 (C=C), 1645 (C=C), 1679 (amide), 1648 (C=O) cm^{-1}; UV (EtOH) λ_{max} 209, 227, 275 (ε 10,100, 10,530, 3879); ^1H NMR (DMSO-d_6/D_2O) δ 1.39 (s, 3H, CH_3), 1.53 (s, 3H, CH_3), 3.20 (dd, $J=17.9$, 1.0 Hz, 1H, $C_{6\beta}H$), 3.53 (dd, $J=17.9$, 3.2 Hz, 1H, $C_{6\alpha}H$), 4.15 (s, 3H, C_3–OCH_3), 4.30 (d, $J=7.4$ Hz, 2H, CH_2), 4.54 (dd, $J=7.7$, 5.8 Hz, 2H, C_9H_2), 4.30 (s, 1H, C_3H), 4.53 (s, 1H, NCHCON), 4.78 (ddd, $J=7.7$, 5.8, 1.2 Hz, 1H, C_8H), 4.92 (d, $J=1.2$ Hz, 1H, C_3H), 5.25 (t, $J=7.4$ Hz, 1H, =CH), 5.39 (d, $J=3.8$ Hz, 1H, C_5H), 5.55 (d, $J=3.8$ Hz, 1H, C_6H), 5.78 (dd, $J=3.2$, 1.0 Hz, 1H, C_5H), 7.05 (AB q, $J=7.9$ Hz, 2H, Ph), 7.22 (AB q, $J=7.9$ Hz, 2H, Ph); CIMS m/z 701 ($M^+ + 1$). Anal. calcd for $C_{31}H_{32}N_4O_{13}S$: C, 53.14; H, 4.60; N, 8.00; S, 4.58. Found: C, 53.19; H, 4.64; N, 7.98; S, 4.63.

3-[((Diphenylmethyl 2-*N*-diphenylmethylamoxicillinate)-4-*O*-yl)methyl]-7-(phenoxyacetamido)-(1-oxo)-3-cephem-4-*tert*-butylcarboxylate (22). To a solution of **11** (3.42 g, 4.90 mmol) in CH_3CN (85 mL) was added K_2CO_3 (2.10 g, 15.0 mmol). After 10 min, to the stirred reaction mixture was added **20** (2.62 g, 4.90 mmol) and further stirred at 25 °C for 13 h. It was then filtered. The filtrate was diluted with EtOAc (160 mL) and aqueous HCl solution (1%, 170 mL). The organic layer was separated, washed with H_2O (3×150 mL), dried over $MgSO_4$ (s), filtered, and concentrated under reduced pressure. Purification of the residue by use of silica gel column chromatography with EtOAc as eluant gave **22** (4.10 g, 3.68 mmol) in 75% yield: mp 208–210 °C (decomp); R_f (EtOAc) 0.22; IR (CH_2Cl_2) 3340–3210 (NH), 1790 (β-lactam), 1773 (β-lactam), 1730–1759 (ester), 1680 (amide), 1675 (amide) cm^{-1}; UV (EtOH) λ_{max} 240, 275 (ε 8730, 2998); ^1H NMR (DMSO-d_6/D_2O) δ 1.40 (s, 3H, CH_3), 1.48 (s, 9H, $(CH_3)_3C$), 1.56 (s, 3H, CH_3), 3.82 (d, $J=16.8$ Hz, 1H, HCSO), 3.95 (d, $J=16.8$ Hz, 1H, HCSO), 4.27 (br s, 2H, CH_2), 4.39 (s, 1H, C_3H), 4.61 (br s, 2H, OCH_2CO), 4.85 (s, 1H, NCHCON), 5.30 (d, $J=5.0$ Hz, 1H, C_6H), 5.53 (d, $J=4.3$ Hz, 1H, C_5H), 5.61 (d, $J=4.3$ Hz, 1H, C_6H), 5.80 (d, $J=5.0$ Hz, 1H, C_7H), 5.89 (s, 1H, $NCHPh_2$), 6.97 (s, 1H, $CHPh_2$), 7.01 (AB q, $J=8.1$ Hz, 2H, Ph), 7.20 (AB q, $J=8.1$ Hz, 2H, Ph), 7.26–7.53 (br s, 25H, 5 Ph); MS m/z 948 ($M^+ - Ph_2CH$), 781 ($M^+ - 2\ Ph_2CH$), 724 ($M^+ - 2\ Ph_2CH - C_4H_9$).

3-[(Amoxicillin-4-*O*-yl)methyl]-7-(phenoxyacetamido)-(1-oxo)-3-cephem-4-carboxylic acid (23). To a solution of **22** (4.13 g, 3.70 mmol) in CH_2Cl_2 (200 mL) was added anisole (0.22 g, 2.0 mmol) and CF_3CO_2H (2.85 g, 25.0 mmol). The mixture was stirred at 25 °C for 1.5 h. Then, it was concentrated under reduced pressure, the residue was treated with 1% methanolic ammonia (20 mL), and then evaporated to dryness. Purification of the residue by use of column chromatography (EtOAc/EtOH 8.0:2.0) afforded **23** (2.34 g, 3.22 mmol) in 87% yield: mp 220–223 °C (decomp); R_f (EtOAc) 0.08; IR (nujol) 4850–2100 (OH, NH, NH_2), 1788 (β-lactam), 1770 (β-lactam), 1681 (amide), 1670 (amide) 1655–1630 (C=O) cm^{-1}; UV (EtOH) λ_{max} 238, 275 (ε 8900, 3080); ^1H NMR (DMSO-d_6/D_2O) δ 1.38 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 3.80 (d, $J=16.8$ Hz, 1H, HCSO), 3.91 (d, $J=16.8$ Hz, 1H, HCSO), 4.30 (br s, 2H, CH_2), 4.07 (s, 1H, C_3H), 4.58 (br s, 2H, OCH_2CO), 4.75 (s, 1H, NCHCON), 5.25 (d, $J=5.0$ Hz, 1H, C_6H), 5.48 (d, $J=3.6$ Hz, 1H, C_5H), 5.59 (d, $J=3.6$ Hz, 1H, C_6H), 5.77 (d, $J=5.0$ Hz, 1H, C_7H), 7.07 (AB q, $J=8.0$ Hz, 2H, Ph), 7.19 (AB q, $J=8.0$ Hz, 2H, Ph), 7.34 (br s, 5H, Ph); CIMS m/z 728 ($M^+ + 1$). Anal. calcd for $C_{32}H_{33}N_5O_{11}S_2$: C, 52.81; H, 4.57; N, 9.62; S, 8.81. Found: C, 52.96; H, 4.61; N, 9.70; S, 8.85.

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