Cephalosporin 3′-Phloroglucide Esters and 7-(Phloroglucidamido)cephalosporins as Novel Antibacterial Agents

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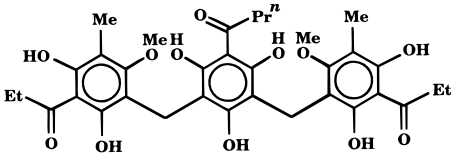
Two series of new phloroglucide derivatives were synthesized that possessed antibacterial activities. The first series includes cephalosporin 3′-phloroglucide esters **19** and **20**, which were obtained by condensation of cephalosporin **16** with bioactive phloroglucides **14** and **15**, respectively. They exhibited a dual mode of antibacterial action. In comparison with cephalosporins **26** and **27**, bearing an acetoxy unit at the C-3′ position, the bifunctional cephalosporins **19** and **20** showed a broadened spectrum of activity. Results from the consistent valence force field (CVFF) calculations indicate that the most stable conformational isomer of phenolic acid **14**, holding a *cis*−*syn*−*syn* geometry, possessed a cavity. It provides an ideal environment to accommodate metal ions of holoenzymes. Phenolic keto acid **15**, however, possessed a *trans*−*anti*−*syn* conformation, which allowed chelation between metal ions and the phenolic hydroxyl groups as well as the carbonyl functionalities. Our biological results show that the cavity formed in phloroglucides plays an important role. The second series includes 7-(phloroglucidamido)cephalosporins **24** and **25**, which were synthesized by condensation of cephalosporin **21** with **14** and **15**, respectively. Results from the CVFF calculations indicate that cephalosporin **24** also possessed a cavity. Unlike cephalosporin 3′-phloroglucide esters **19** and **20**, cephalosporins **24** and **25** were found resistant to β-lactamases from *Staphylococcus aureus* 95 and *Pseudomonas aeruginosa* 18S-H. These new compounds, however, showed notable activities against *S. aureus* FDA 209P, *S. aureus* 95, *Candida albicans*, *P. aeruginosa* 1101−75, and *P. aeruginosa* 18S-H.

Introduction

The presence of essential functional groups with a suitable spatial arrangement for chelation with metal ions of enzymes is a significant feature common to several classes of antibiotics.[1] They include trisaspidinol (**1**),[2] cryptosporin (**2**),[3] and tetracycline (**3**).[4] These



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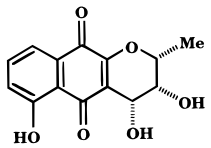

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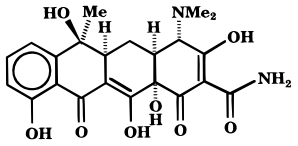

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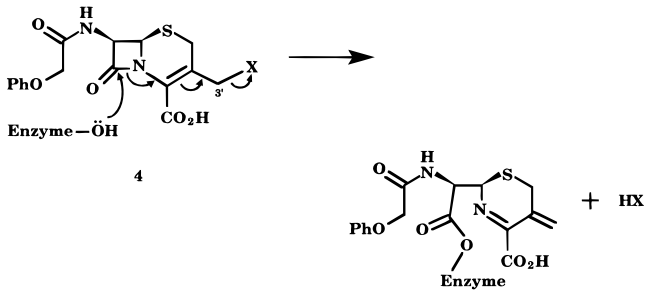
compounds exhibit various degrees of activity against Gram-positive bacteria as well as other microorganisms.[2−4] Our previous studies on the structure−activity relationship of phloroglucide analogs reveal that the presence of halogen atoms is essential for their antibacterial activity.[5−8]

Scheme 1



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β-Lactam antibiotics exert certain biological activity by acylating serine residues of transpeptidases, in which the cross-linking of peptidoglycans does not take place.[9] As shown in Scheme 1, ring opening of the β-lactam nucleus would occur when cephalosporins (**4**) react with bacterial enzymes. Consequently, the substituent attached at the C-3′ position is liberated.[10−17] When the eliminated species possesses antibacterial activity, cephalosporins (**4**) would exhibit a dual mode of action.[18−20] Recently, Albrecht et al.[21−23] have reported that attachment of antibacterial quinolones to the C-3′ position of cephalosporins gives a class of new compounds with a broadened spectrum of antibacterial activities.

Herein we report the synthesis and antibacterial properties of four classes of new phloroglucides. They include symmetrical phloroglucides **9** and **10**, unsymmetrical phloroglucide analogs **14** and **15**, phloroglucides attached to cephalosporins at the C-3′ position (i.e.,

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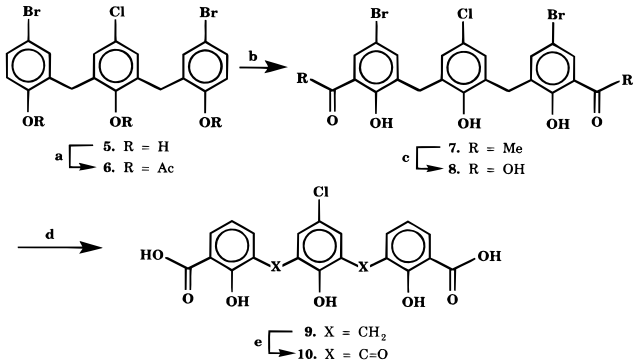
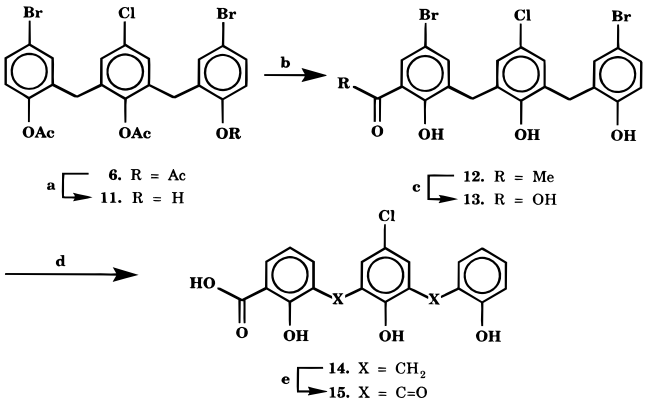
Scheme 2[a]



[a] Reagents: (a) Ac_2O, H_2SO_4 (cat.); (b) $AlCl_3$; (c) (1) NaOI, (2) HCl; (d) Zn, KOH (aq); (e) (1) Ac_2O, (2) CrO_3, (3) NaOH, (4) HCl.

Scheme 3[a]



[a] Reagents: (a) NaOH, THF; (b) $AlCl_3$; (c) (1) NaOI, (2) HCl; (d) Zn, KOH (aq); (e) (1) Ac_2O, (2) CrO_3, (3) NaOH, (4) HCl.

19 and **20**), and 7-(phloroglucidamido)cephalosporins (i.e., **24** and **25**).

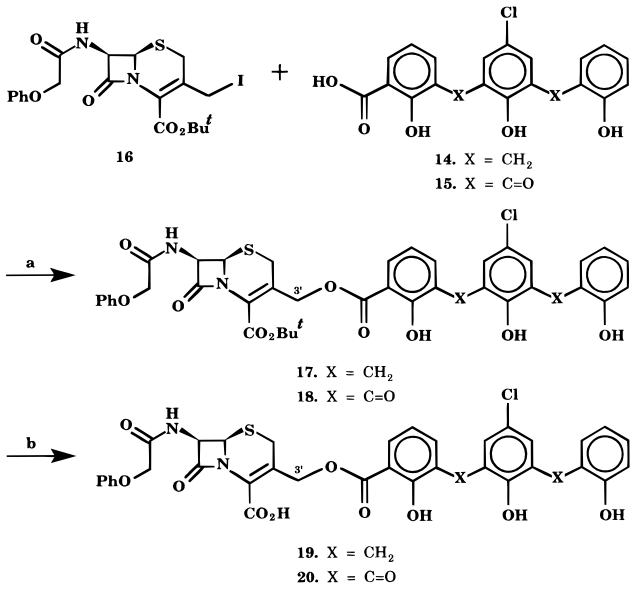
Results

Synthesis of Phloroglucide Analogs 9, 10, 14, and 15 (Schemes 2 and 3). For the preparation of symmetrical phloroglucide derivatives **9** and **10**, we acetylated triol **5**[7] to produce triacetate **6** (98% yield, Scheme 2). Upon treatment of **6** with $AlCl_3$, acetyl migration took place to give phenone **7** in 75% yield. Oxidative degradation of **7** with sodium hypoiodite afforded phenolic acid **8** in 90% yield. Debromination of **8** with Zn and KOH gave the phloroglucide analogs **9** in 97% yield. We then oxidized **9** with CrO_3 in acetic anhydride to afford the corresponding phenone acid **10** in 75% yield after hydrolytic workup.

For the synthesis of unsymmetrical phloroglucide analogs **14** and **15**, we hydrolyzed acetate **6** with NaOH (aq) in THF to give diacetate phenol **11** in 98% yield (Scheme 3). Treatment of **11** with $AlCl_3$ produced phenone **12** (70% yield) through acetyl migration. Oxidation of **12** with sodium hypoiodite afforded a 90% yield of phenolic acid **13**. Debromination of **13** with Zn and KOH produced the phloroglucide derivative **14** in 87% yield. Compound **14** was oxidized to phenolic ketone **15** in 73% yield by sequential use of a mixture of CrO_3 and acetic anhydride, NaOH (aq), and HCl (aq).

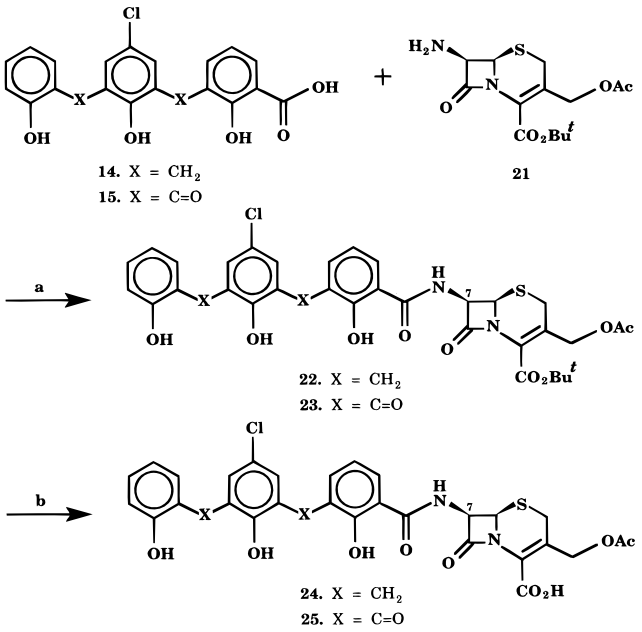
Synthesis of Cephalosporin 3′-Phloroglucide Esters 19 and 20 (Scheme 4). We condensed 3′-iodocephalosporin **16**[21,24] with the sodium salt of phloroglucidic acid **14** to produce the desired ester **17** in 95%

Scheme 4[a]



[a] Reagents: (a) $NaHCO_3$, DMF; (b) CF_3CO_2H, CH_2Cl_2.

Scheme 5[a]



[a] Reagents: (a) (1) $Me_3SiNHSiMe_3$, $(NH_4)_2SO_4$ (cat.), (2) EEDQ, THF; (b) CF_3CO_2H, CH_2Cl_2.

yield (Scheme 4). Then removal of the *tert*-butyl group from **17** by use of CF_3CO_2H in CH_2Cl_2 gave the bifunctional target compound **19** in 90% yield.

Furthermore, we treated 3′-iodocephalosporin **16** with phenolic keto acid **15** and $NaHCO_3$ in DMF to provide the desired intermediate **18** in 90% yield (Scheme 4). Removal of the *tert*-butyl group from **18** by use of CF_3CO_2H in CH_2Cl_2 gave the bifunctional target molecule **20** in 94% yield.

Synthesis of 7-(Phloroglucidamido)cephalosporins 24 and 25 (Scheme 5). To condense carboxylic acid **14** with amine **21**, we first silylated phloroglucidic acid **14** with 1,1,1,3,3,3-hexamethyldisilazane at reflux by using $(NH_4)_2SO_4$ as a catalyst (Scheme 5). The resultant compound was then treated with 7-aminocephalosporin **21** and 2-ethoxy-1-(ethoxycarbonyl)-1,2-dihydroquinoline (EEDQ) in THF to afford amide **22** in 80%

Table 1. The Minimized Energies of Possible Stable Conformational Isomers of Phloroglucides **14**, **15**, and **28** Obtained by the CVFF Calculations

entry	compound	conformation	minimized energy (kcal/mol)[a]
1	**14**	*cis*−*syn*−*syn*	111.8986
2	**14**	*trans*−*syn*−*syn*	112.6013
3	**14**	*cis*−*syn*−*anti*	114.0316
4	**14**	*trans*−*syn*−*anti*	117.4551
5	**14**	*cis*−*anti*−*syn*	116.1644
6	**14**	*trans*−*anti*−*syn*	114.5202
7	**14**	*cis*−*anti*−*anti*	115.0033
8	**14**	*trans*−*anti*−*anti*	117.9318
9	**15**	*cis*−*syn*−*syn*	175.6486
10	**15**	*trans*−*syn*−*syn*	175.3755
11	**15**	*cis*−*syn*−*anti*	171.6754
12	**15**	*trans*−*syn*−*anti*	171.4053
13	**15**	*cis*−*anti*−*syn*	169.5517
14	**15**	*trans*−*anti*−*syn*	169.4483
15	**15**	*cis*−*anti*−*anti*	175.8334
16	**15**	*trans*−*anti*−*anti*	175.1267
17	**28**	*cis*−*syn*−*syn*	110.4575
18	**28**	*trans*−*syn*−*syn*	124.4224
19	**28**	*cis*−*syn*−*anti*	122.3947
20	**28**	*trans*−*syn*−*anti*	125.7400
21	**28**	*cis*−*anti*−*anti*	124.2391
22	**28**	*trans*−*anti*−*anti*	128.9658

[a] The maximum derivatives are less than 0.0004 kcal/(mol Å).

yield. Removal of the *tert*-butyl group from **22** by use of CF_3CO_2H in CH_2Cl_2 provided 7-(phloroglucidamido)-cephalosporin **24** in 83% yield. Similarly, we were able to condense phenolic keto acid **15** with 7-aminocephalosporin **21** to produce 7-(phloroglucidamido)cephalosporin **25** via **23** in 70% overall yield (Scheme 5).

Conformations of Phloroglucides 14, 15, 24, and 28 Revealed by the Consistent Valence Force Field (CVFF) Calculations. We carried out the CVFF calculations on phenolic acid **14**, phenolic keto acid **15**, cephalosporin **24**, and phenol **28**[7] by using a Silicon Graphics workstation. Our goal was to obtain their possible conformations with thermal stability.

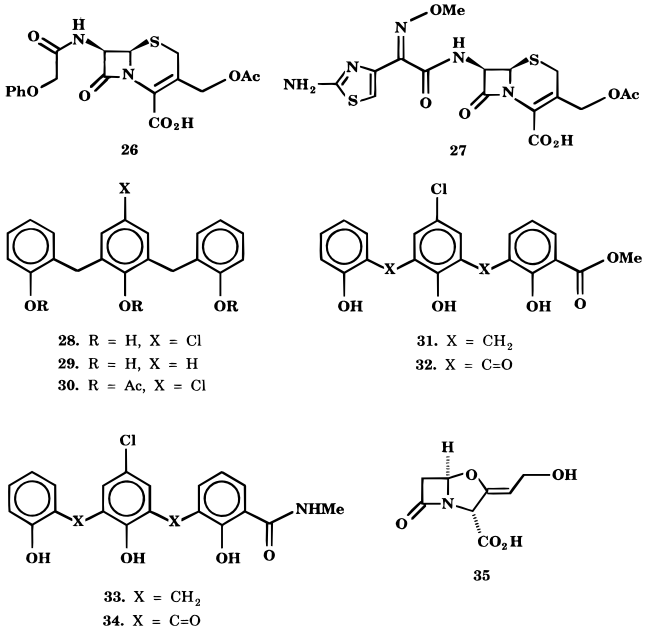
A molecule is designated as *cis* when the first and the third aromatic ring reside on the same side of the plane defined by the middle aromatic ring and as *trans* when on the opposite side. In addition, the designation *syn* indicates that the two hydroxyl groups in adjacent aromatic rings are in close proximity and *trans* for the two hydroxyl groups in the opposite sides. Accordingly, we obtained the minimized energy of eight possible conformational isomers for phenolic acid **14**, eight isomers for phenolic keto acid **15**, and six isomers for phenol **28**. The data are listed in Table 1.

We found that the most stable conformational isomers of phenolic acid **14** (entry 1 of Table 1) and phenol **28** (entry 17) held a *cis*−*syn*−*syn* geometry. Each of these two isomers provided an ideal conformation to form hydrogen bondings between the phenolic hydroxyl groups. The shape of the entire molecules and the location of hydroxyl groups allowed **14** and **28** to establish a cavity, as shown in parts a and b of Figure 1, respectively. Such a geometry created a perfect environment for chelation with metal ions.

On the other hand, we found that the most stable isomer of phenolic keto acid **15** possessed a *trans*−*anti*−*syn* conformation (entry 14), as shown in Figure 1c,d. Different from **14** and **28** was the phenolic keto acid **15** possessing two bridging carbonyl groups, which formed a hydrogen bond with the adjacent hydroxyl groups. Although the location of the four hydrogen bonds in **15** allows it to chelate with metal ions, its geometry greatly differs from that of **14** and **28**.

Furthermore, we found that the most stable conformational isomer of 7-(phloroglucidamido)cephalosporin **24** possessed a *cis*−*syn*−*syn* geometry (see Figure 1e). The spatial arrangement of the three hydroxyl groups, one amide carbonyl functionality, and the sulfur atom in the cephem moiety allows the formation of a cavity in **24**. This cavity enables molecule **24** to act as an ideal host for metal ions, such as Zn^{2+}, as shown in Figure 1f,g.

Biological Activity. We carried out the screening experiments in vitro for antibacterial activities of the phloroglucide analogs **5**, **6**, **9**, **10**, **14**, **15**, **28**, **29**[6]−**34**, bifunctional cephalosporins **19** and **20**, and 7-(phloroglucidamido)cephalosporins **24** and **25**.[25,26] Cephalosporins **26** and **27** were used as the reference compounds. The results are summarized in Table 2.



Moreover, we tested the β-lactamase inhibitory properties of cephalosporin phloroglucide esters **19** and **20**, 7-(phloroglucidamido)cephalosporins **24** and **25**, and cephalosporins **26** and **27**. Clavulanic acid (**35**) was also used in vitro as the reference compound.[27] The results are summarized in Table 3.

Discussion

A direct relationship exists between the chelating abilities of some antibiotics and their bacteriostatic action.[28,29] Nonhalogenated triol **29** and trihalogenated triol **5** possess the same skeleton as that of the biologically active compound **28**. Nevertheless, we found that the antimicrobial activities of **5** and **29** were either lost or decreased dramatically. Furthermore, we added $FeCl_3$ to an ethanolic solution of phloroglucides **5**, **28**, or **29**. The solution turned violet because of chelation between the organic species and the Fe^{3+} ions. In contrast, after the hydroxyl groups in **5** and **28** were protected by an acetyl group, the resultant acetates **6** and **30** did not show chelating ability or biological activity. These results indicate that the chelating capability of the model compound **28** and the presence

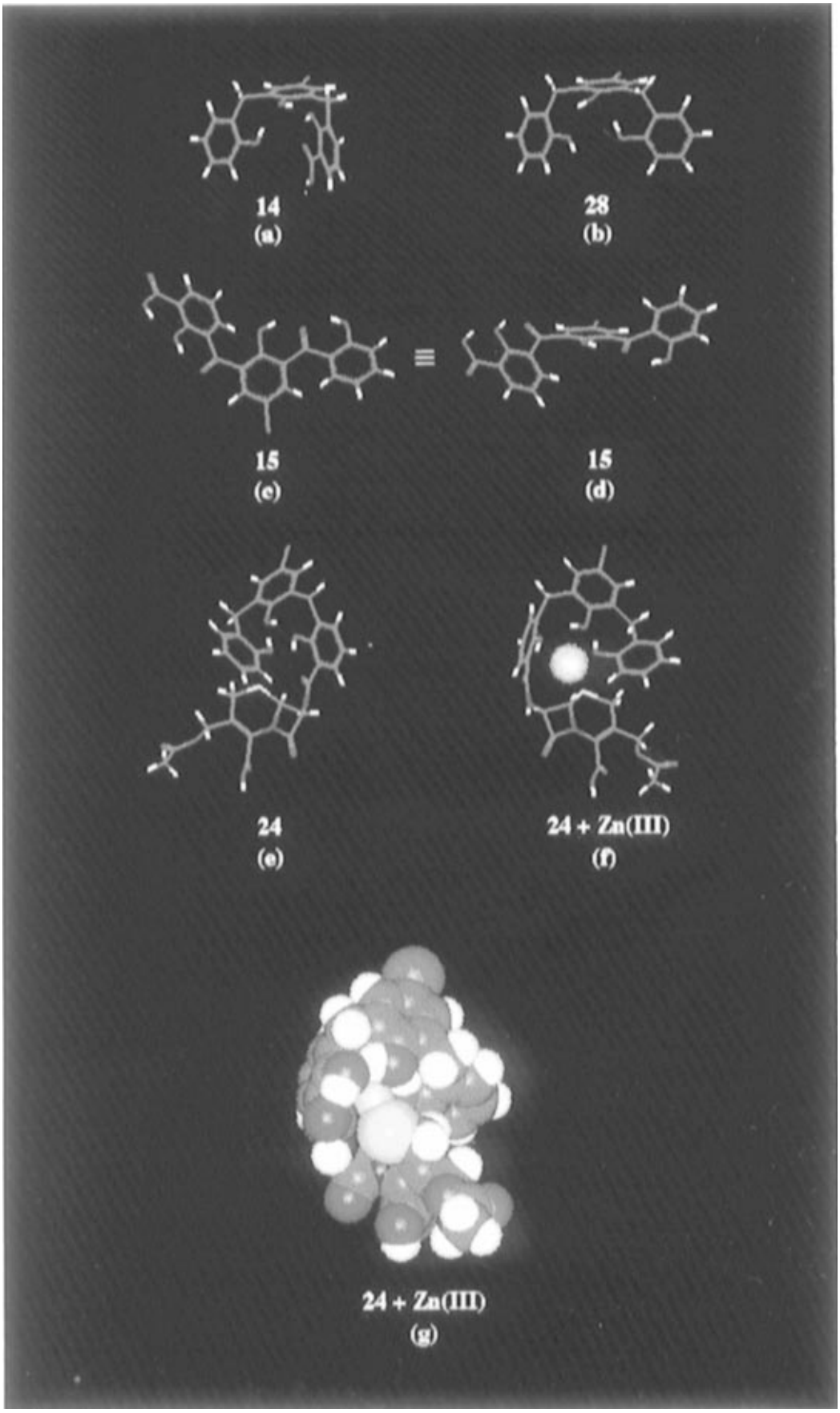


Figure 1. The conformational isomers with the lowest energy obtained by the CVFF calculations: (a) phenolic acid **14** with a *cis*−*syn*−*syn* geometry and a cavity created by three hydroxyl and one carboxylic groups; (b) phenol **28** with a *cis*−*syn*−*syn* geometry and a cavity created by three hydroxyl groups; (c) and (d) phenolic keto acid **15** with a *trans*−*anti*−*syn* geometry but without a cavity; (e) 7-(phloroglucidamido)cephalosporin **24** with a cavity created by three hydroxyl groups, one amide carbonyl functionality, and a sulfur atom; (f) chelation of a Zn^{2+} ion (the yellowish gray ball) by cephalosporin **24** with a rear view; and (g) The CPK space filling molecular model to present the complexation of **24** with a Zn^{2+} ion (the yellowish gray ball).

Table 2. Minimum Inhibitory Concentrations[a] (μg/mL) of Phloroglucide and Cephalosporin Analogs against Microorganisms

	S. aureus			*P. aerureus*	
compd	FDA 209P	95[b,c]	*C. albicans*	1101−75	18S-H[b]
5	45.00	48.95	115.00	39.38	36.71
6	>145	>145	>145	>145	>145
9	3.25	4.15	16.51	0.86	1.32
10	18.25	22.18	19.12	2.54	3.06
14	0.86	0.43	1.43	0.02	0.10
15	3.67	0.98	3.70	0.17	0.26
19	0.41	1.20	5.86	0.98	1.24
20	0.90	2.10	6.47	1.37	3.13
24	0.35	0.28	28.31	0.28	0.45
25	0.78	0.45	14.23	0.79	0.38
26	0.70	>145	>145	>145	>145
27	1.70	40.60	>145	69.86	135
28	1.20	0.58	2.06	0.070	0.18
29	>145	>145	>145	>145	>145
30	>145	>145	>145	>145	>145
31	2.98	4.65	117.43	1.25	1.56
32	14.69	15.32	125.30	5.78	4.30
33	7.35	6.63	114.20	3.28	4.70
34	18.15	17.76	119.56	6.03	8.32

[a] The lowest concentrations of antibiotics needed for prevention of visible growth of microorganisms, reported as the average values of duplicate determinations. Minimum inhibitory concentrations (MIC) values obtained by use of an agar dilution method whereby organisms were deposited onto medicated agar plates by the replication device of Steers et al.[26] [b] β-Lactamase-producing organism. [c] Methicillin-resistant organism.

Table 3. Minimum Protective Concentrations (μg/mL) of Cephalosporin Analogs against β-Lactamases[a]

	β-lactamase of			β-lactamase of	
compd[b]	*S. aureus* 95	*P. aeruginosa* 18S-H	compd[b]	*S. aureus* 95	*P. aeruginosa* 18S-H
19	7.08	6.74	**26**	1.35	0.97
20	4.81	5.98	**27**	2.72	1.03
24	95.20	99.78	**35**	0.38	3.00
25	88.68	93.45			

[a] The average values of duplicate determinations and the ability of compounds to inhibit the hydrolysis of 3-[*E*-(2,4-dinitro)styryl]-(6*R*,7*R*)-7-(2-thienylacetamido)-3-cephem-4-carboxylic acid by β-lactamases from *S. aureus* 95 and *P. aeruginosa* 18S-H. Minimum protective concentrations (MPC) values were determined by the procedure of O'Callaghan et al.[27] and are the lowest concentrations of β-lactams needed to protect the indicator from hydrolysis by β-lactamases under standard test conditions within 40 min. The hydrolysis of indicator was evidenced by the appearance of a distinct red color. [b] All compounds were stable (>20 h) in the absence of β-lactamases at 37 °C in a phosphate buffer solution (pH 6.5), except for clavulanic acid (**35**). The β-lactam ring in **35** was destructed within 13 h.

of the chlorine atom in phloroglucide **28** influence its antibacterial activity.

Symmetrical phloroglucide derivative **9** bearing two carboxylic acid units was found to be less active than the model compound **28** toward pathogenic microorganisms (Table 2). Unsymmetrical phloroglucide **14**, having one carboxylic acid unit at the C-3′ position, however, exhibited more potent antimicrobial activity than **28**. When the methylene bridges in phloroglucides **9** and **14** were converted to carbonyl functionalities as in the phenolic keto acids **10** and **15**, their antimicrobial property decreased. In comparison with dicarboxylic acid derivative **10**, monocarboxylic acid **15**, however, exhibited greater potency.

Results from our CVFF calculations indicate that molecule **15** with cross-conjugation was not planar (see parts c and d of Figure 1). It could chelate effectively with metal ions because the chelation would involve an unsaturated six-membered ring with considerable resonance character.[30] Upon chelation of **15** with Cu^{2+} ions, we observed a notable peak shift from 1648 to 1615 cm^{-1} for the carbonyl stretching in the IR spectra. This shift is attributed to the weakening of the C=O bond by chelation.[31] Acetylation of the phenolic hydroxyl groups in **15** resulted in a loss of the chelating ability and thus its biological activity.

Phenolic keto acid **15**, however, showed a lower efficacy than phenolic acid **14** and phenol **28** against pathogenic microorganisms (see Table 2). Phloroglucides **14** and **28** possess a cavity in their most stable conformational isomers (see parts a and b of Figure 1), which allowed them to act as ideal hosts for metal ions (i.e., cofactors) of holoenzymes.

Consequently, we conclude that the chelation between metal ions from a holoenzyme and the phenolic hydroxyl groups in **14** and **28** results in significant antibacterial activities. Those activities were more potent than those from the chelation between metal ions and the phenolic hydroxyl groups as well as the carbonyl functionalities in **15**. Thus the cavity formed in phloroglucides plays an essential role in biological system.

In comparison with the reference compounds **26** and **27**, cephemphloroglucidic esters **19** and **20** possessed a broadened spectrum of antibacterial activity in vitro. On the other hand, **19** and **20** showed greater antibacterial activity than the corresponding phloroglucides **14** and **15** against *Staphylococcus aureus* FDA 209P, yet less activity against other pathogenic microorganisms (Table 2). Nevertheless, the activity added to the spectrum of the parent cephalosporin parallels the activity of the phloroglucide component in each assay.

To support the argument that esters **19** and **20** are dual-action antibiotics, we evaluated the antibacterial activity of simple esters **31** and **32** as well as the corresponding amides **33** and **34**.[32] These derivatives exhibited lower efficacy than phloroglucides **14** and **15** as well as the bifunctional cephalosporins **19** and **20** (see Table 2). Therefore the contribution to the biological activities of esters **19** and **20** came from both phloroglucide and cephalosporin moieties. This pattern of activity reveals that the corresponding phloroglucides **14** and **15** were released in situ from bifunctional cephalosporins **19** and **20**, respectively.

On the other hand, 7-(phloroglucidamido)cephalosporins **24** and **25** are not expected to exhibit a dual mode of action, yet they showed notable antibacterial activity (Table 2). 7-(Phloroglucidamido)cephalosporin **24** possessed a sulfur atom, which assisted the most stable conformational isomer of **24**, as shown in Figure 1e, to form a cavity. For chelation with metal ions, such as Zn^{2+},[33] the environment is better for compound **24** (see Figure 1f,g) than phenolic acid **14** and phenol **28**. Accordingly, compound **24** exhibited potent phloroglucide-like antibacterial activity (Table 2).

Most β-lactamases inactivate β-lactam antibiotics through hydrolysis of their β-lactam ring.[9] Therefore a potential leaving group attached at the C-3′ position of a cephalosporin (e.g., **19** and **20**) may be eliminated upon treatment with a β-lactamase (Scheme 1).

To substantiate our antimicrobial results, we tested the β-lactamase inhibitory properties of bifunctional cephalosporins **19** and **20** and 7-(phloroglucidamido)-cephalosporins **24** and **25**, as well as cephalosporins **26**

and **27** (Table 3). Cephalosporin phloroglucide esters **19** and **20** underwent hydrolysis to liberate their phloroglucide components, as evidenced by their notable values of the minimum protective concentration (MPC) against the β-lactamases of *S. aureus* 95 and *Pseudomonas aeruginosa* 18S-H. Therefore, cephemphloroglucidic esters **19** and **20** exhibited phloroglucide-like antibacterial activity (Table 2). Cephalosporins **26** and **27** were also susceptible to hydrolysis by β-lactamases. As a result, they did not show significant activity against β-lactamase-producing microorganisms (i.e., *S. aureus* 95). 7-(Phloroglucidamido)cephalosporins **24** and **25** did not exhibit notable β-lactamase inhibitory property (Table 3). Thus their pronounced antimicrobial activity (Table 2) is mainly due to their stability toward β-lactamases (Table 3).

Conclusions

Four classes of new phloroglucide antibiotics were synthesized and their structure−activity relationship was studied. Those compounds include symmetrical phloroglucide analogs **9** and **10**, unsymmetrical phloroglucide analogs **14** and **15**, bifunctional cephalosporins **19** and **20** possessing a phloroglucide moiety at the C-3′ position, and 7-(phloroglucidamido)cephalosporins **24** and **25**.

The CVFF computational results indicate that the most stable conformations of the biologically active phloroglucides **14**, **24**, and **28** possessed a *cis*−*syn*−*syn* geometry and **15** held a *trans*−*anti*−*syn* geometry. Phenolic acid **14**, cephalosporin **24**, and phenol **28** have a cavity in their molecules for chelation with metal ions of bacterial holoenzymes, which differs from phenolic keto acid **15**. Thus they exhibited more potent antibacterial activity than **15**.

Results from the biological assay indicate that cephemphloroglucidic esters **19** and **20** acted as effective substrates for β-lactamases of certain bacterial species. Thus their antibacterial spectrum came from phloroglucides liberated from the bifunctional molecules. This conclusion was supported by the low antibacterial activity of simple esters of **14** and **15** (i.e., **31** and **32**) as well as simple amides of **14** and **15** (i.e., **33** and **34**). Moreover, new cephalosporins **24** and **25** were designed to possess phloroglucidamide chains attached to the C-7 position. They showed potent antimicrobial activities and were found resistant to β-lactamases from *S. aureus* 95 and *P. aeruginosa* 18S-H.

Experimental Section

General. For anhydrous reactions, glassware was dried overnight in an oven at 120 °C and cooled in a desiccator over anhydrous $CaSO_4$(s) or silica gel. Reagents purchased from Fluka Chemical Co., 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 and used as received. Enzymes were obtained from the Shiraz Saadi Hospital.

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. Dimethyl sulfoxide-d_6 was used as solvent; Me_4-Si (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; dd, doublet of doublets; dm, doublet of multiples. 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.

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 individually) were used for model building and energy minimization, respectively. The energies for all conformations were minimized with the CVFF[34] until the maximum derivative was less than 0.0004 kcal/(mol Å).

2-Acetoxy-1,3-bis(2-acetoxy-5-bromobenzyl)-5-chlorobenzene (6). To a solution of **5** (4.99 g, 10.0 mmol) in acetic anhydride (50 mL) was added one drop of concentrated H_2SO_4. The mixture was heated at reflux for 2.0 h. After cooling, it was poured into cold water (400 mL) and the solution was allowed to stand at room temperature for 15 h. The precipitate was filtered off, washed with water (100 mL), and dried over P_2O_5 in a vacuum oven at 35 °C. Purification by use of column chromatography (silica gel, CH_2Cl_2 as eluant) gave **6** (6.12 g, 9.80 mmol) as a white crystal in 98% yield: mp 146−148 °C; ^{1}H NMR (DMSO-d_6) δ 2.25 (s, 9 H, 3 × CH_3), 3.61 (s, 4 H, 2 × CH_2), 6.92−7.35 (m, 8 H, 3 × ArH); IR (CH_2Cl_2) 1748 (esters) cm^{-1}. Anal. ($C_{26}H_{21}Br_2ClO_6$) C, H, Br, Cl.

2,6-Bis(3-acetyl-5-bromo-2-hydroxybenzyl)-4-chlorophenol (7). Anhydrous $AlCl_3$ (15.0 g, 112 mmol) in a flask (250 mL) was heated in an oil bath at 115 °C for 15 min and stirred with a glass rod. Compound **6** (6.94 g, 11.1 mmol) was added into this reaction flask and the mixture was kept at 150−155 °C for 20 min. After cooling, it was added to a mixture of crushed ice (500 g) and concentrated HCl solution (37%, 100 mL). A yellow precipitate was obtained within 6.0 h. It was then filtered, washed with water (100 mL), and dried over P_2O_5 in a vacuum oven at 35 °C to give the crude product **7** (6.12 g, 10.5 mmol) in 95% yield. Crystallization from EtOH gave pure product **7** (4.85 g, 8.33 mmol) as a needle crystal in 75% yield: mp 205−208 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 2.18 (s, 6 H, 2 × CH_3), 3.60 (s, 4 H, 2 × CH_2), 7.00−7.30 (m, 6 H, 3 × ArH); IR (KBr) 3300−3430 (OH), 1650 (C═O), 1168, 1360, 1200, 780 cm^{-1}; MS m/z 582 (M^+, Cl, Br clusters). Anal. ($C_{24}H_{19}Br_2ClO_5$) C, H, Br, Cl.

2,6-Bis(5-bromo-3-carboxy-2-hydroxybenzyl)-4-chlorophenol (8). Compound **7** (1.28 g, 2.20 mmol) was dissolved in aqueous NaOH solution (2.0 N, 50 mL). A solution of iodine (4.00 g, 15.8 mmol) and potassium iodide (10.0 g, 60.2 mmol) in water (20 mL) was added to the reaction mixture. The solution was stirred and warmed in a water bath for 30 min. Iodoform was removed by filtration and $NaHSO_3$ (15 g) was added to the filtrate. The yellow precipitate was formed by addition of concentrated HCl solution (27 mL). It was then filtered off, washed with water (2 × 100 mL), and dried over P_2O_5 in a vacuum oven at 35 °C. The crude product was dissolved in 10% aqueous $NaHCO_3$ solution (50 mL) and treated with charcoal. After filtration, the solution was acidified with 37% aqueous HCl solution (16 mL) to afford **8** (1.16 g, 1.98 mmol) in 90% yield: mp >250 °C (dec); ^{1}H NMR (DMSO-d_6/D_2O) δ 3.60 (s, 4 H, 2 × CH_2), 6.80−7.35 (m, 6 H, 3 × ArH); IR (KBr) 3250−3500 (OH, CO_2H), 1626 (C═O), 1211, 995 cm^{-1}. Anal. ($C_{22}H_{15}Br_2ClO_7$) C, H, Br, Cl.

2,6-Bis(3-carboxy-2-hydroxybenzyl)-4-chlorophenol (9). To an aqueous solution of KOH (40%, 55 mL) containing **8** (5.87 g, 10.0 mmol) was added Zn dust (5.00 g, 76.5 mmol). The mixture was heated at reflux for 5.0 h. After acidification with 20% aqueous HCl solution, phenol **9** (4.16 g, 9.70 mmol) was obtained in 97% yield. Compound **9** was sublimed at 190−195 °C under 0.03 Torr: mp >260 °C (dec); ^{1}H NMR (DMSO-d_6/D_2O) δ 3.60 (s, 4 H, 2 × CH_2), 6.82−7.30 (m, 8 H, 3 × ArH);

IR (KBr) 3280−3500 (OH, CO_2H), 1620 (C═O), 1211, 994 cm^{-1}; MS *m/z* 428 (M^+, Cl clusters). Anal. ($C_{22}H_{17}ClO_7$) C, H, Cl.

2,6-Bis(3-carboxy-2-hydroxybenzoyl)-4-chlorophenol (10). Compound **9** (0.43 g, 1.0 mmol) was heated at reflux in acetic anhydride (40 mL) for 10 h. After cooling, CrO_3 (0.50 g, 5.0 mmol) was added at room temperature over a period of 30 min. The reaction mixture was stirred at room temperature for 2.0 h and heated at reflux for 3.0 h. Subsequently the brown solution turned to green. Then the solution was condensed under reduced pressure and the residue was treated with 5% aqueous NaOH solution (15 mL). After 1.0 h, it was filtered and the filtrate was acidified with concentrated HCl solution to afford a precipitate. Crystallization from a mixture of Et_2O and MeOH (1:1) gave pure **10** (0.34 g, 0.75 mmol) as a pale yellow crystal in 75% yield: mp 145−147 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 6.81−7.28 (m, 8 H, 3 × ArH); IR (KBr) 3200−3420 (OH, CO_2H), 1610−1630 (C═O), 1168, 1590, 1490, 1210, 995 cm^{-1}; MS *m/z* 456 (M^+, Cl clusters). Anal. ($C_{22}H_{13}ClO_9$) C, H, Cl.

2-Acetoxy-1-(2-acetoxy-5-bromobenzyl)-3-(5-bromo-2-hydroxybenzyl)-5-chlorobenzene (11). To a solution of **6** (6.12 g, 9.80 mmol) in THF (70 mL) was added 1% aqueous solution of NaOH (0.39 g, 9.75 mmol) at 0 °C within 30 min. The reaction mixture was stirred at the same temperature for 1.0 h and then was allowed to stand at room temperature for 1.5 h. After evaporation of THF and acidification with 10% aqueous HCl solution (12 mL), phenol **11** (5.60 g, 9.60 mmol) was recrystallized from EtOH as a white crystal in 98% yield: mp 190−192 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 2.26 (s, 6 H, 2 × CH_3), 3.61 (s, 4 H, 2 × CH_2), 6.92−7.35 (m, 8 H, 3 × ArH); IR (CH_2Cl_2) 3300−3350 (OH), 1740 (esters) cm^{-1}. Anal. ($C_{24}H_{19}Br_2ClO_5$) C, H, Br, Cl.

2-(3-Acetyl-5-bromo-2-hydroxybenzyl)-6-(5-bromo-2-hydroxybenzyl)-4-chlorophenol (12). Compound **12** (3.78 g, 6.99 mmol) was prepared in 70% yield from **11** (5.82 g, 9.99 mmol) and $AlCl_3$ (15.0 g, 112 mmol) by the method used for the synthesis of **7** from **6**. For **12**: mp 185−186 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 2.18 (s, 3 H, CH_3), 3.60 (br s, 4 H, 2 × CH_2), 7.03−7.32 (m, 7 H, 3 × ArH); IR (KBr) 3300−3400 (OH), 1650 (C═O), 1169, 1360, 1210, 780 cm^{-1}. Anal. ($C_{22}H_{17}Br_2ClO_4$) C, H, Br, Cl.

2-(5-Bromo-3-carboxy-2-hydroxybenzyl)-6-(5-bromo-2-hydroxybenzyl)-4-chlorophenol (13). Compound **13** (5.15 g, 9.49 mmol) was synthesized in 95% yield from **12** (5.40 g, 9.99 mmol) by the same method used for the preparation of **8** from **7**. For **13**: mp 200 °C (dec); ^{1}H NMR (DMSO-d_6/D_2O) δ 3.61 (br s, 4 H, 2 × CH_2), 6.65−7.30 (m, 7 H, 3 × ArH); IR (KBr) 3250−3500 (OH, CO_2H), 1626 (C═O), 1215, 990 cm^{-1}. Anal. ($C_{21}H_{15}Br_2ClO_5$) C, H, Br, Cl.

2-(3-Carboxy-2-hydroxybenzyl)-6-(2-hydroxybenzyl)-4-chlorophenol (14). Compound **14** (3.34 g, 8.69 mmol) was prepared in 87% yield from **13** (5.42 g, 9.99 mmol), 40% KOH solution (50 mL), and Zn dust (5.00 g, 76.5 mmol) by the same method used for the synthesis of **9** from **8**. Compound **14** was sublimed at 160−165 °C under 0.03 Torr: mp 198−200 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 3.60 (s, 4 H, 2 × CH_2), 6.84−7.29 (m, 9 H, 3 × ArH); IR (KBr) 3300−3500 (OH, CO_2H), 1620 (C═O), 1211, 994 cm^{-1}; MS *m/z* 384 (M^+, Cl clusters). Anal. ($C_{21}H_{17}ClO_5$) C, H, Cl.

2-(3-Carboxy-2-hydroxybenzoyl)-6-(2-hydroxybenzoyl)-4-chlorophenol (15). Compound **15** (1.50 g, 3.64 mmol) was prepared from **14** (1.92 g, 4.99 mmol) in 73% yield by the same method used for the synthesis of **10** from **9**. For **15**: mp 127−130 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 6.70−7.49 (m, 9 H, 3 × ArH); IR (KBr) 3210−3430 (OH, CO_2H), 1610−1630 (C═O), 1169, 1600, 1495, 1210, 995 cm^{-1}; MS *m/z* 412 (M^+, Cl clusters). Anal. ($C_{21}H_{13}ClO_7$) C, H, Cl.

***tert*-Butyl 3-[[[3-[5-Chloro-2-hydroxy-3-(2-hydroxybenzyl)benzyl]-2-hydroxybenzoyl]oxy]methyl]-7-(phenoxyacetamido)-3-cephem-4-carboxylate (17).** A solution of **16** (5.30 g, 9.99 mmol), **14** (3.86 g, 10.0 mmol), and $NaHCO_3$ (2.52 g, 30.0 mmol) in DMF (150 mL) was stirred under N_2 for 4.0 h. The solvent was evaporated under reduced pressure and the residue was taken up in EtOAc (200 mL) and washed with 1% aqueous HCl solution (100 mL) and water (150 mL). The organic layer was dried over $MgSO_4$ (s), filtered, and evaporated. Purification by use of silica gel column chromatography with EtOAc as the eluant afforded **17** (7.57 g, 9.50 mmol) as a white crystal in 95% yield: mp 155−157 °C; ^{1}H NMR (DMSO-d_6) δ 1.49 (s, 9 H, 3 × CH_3), 3.61 (br s, 4 H, 2 × CH_2), 3.70, 3.80 (AB, J_{gem} = 19 Hz, 2 H, CH_2S), 4.63 (s, 2 H, OCH_2-CO), 4.86, 5.10 (AB, J_{gem} = 14 Hz, 2 H, CH_2O), 5.17 (d, J = 4.9 Hz, 1 H, HC(6)), 5.77(dd, J = 4.9 and 8.5 Hz, 1 H, HC(7)), 6.60−7.35 (m, 18 H, NH + 3 × OH + 4 × ArH); IR (Nujol) 3300−3400 (OH, NH), 1790 (β-lactam), 1745 (ester), 1725 (ester), 1680 (amide) cm^{-1}. Anal. ($C_{41}H_{39}N_2O_{10}SCl$) C, H, N, S, Cl.

***tert*-Butyl 3-[[[3-[5-Chloro-2-hydroxy-3-(2-hydroxybenzoyl)benzoyl]-2-hydroxybenzoyl]oxy]methyl]-7-(phenoxyacetamido)-3-cephem-4-carboxylate (18).** Compound **18** (7.33 g, 8.99 mmol) was prepared from **16** (5.30 g, 9.99 mmol), **15** (4.13 g, 10.0 mmol), and $NaHCO_3$ (2.52 g, 30.0 mmol) in 90% yield by the same method used for the preparation of **17**. For **18**: mp 138−140 °C; ^{1}H NMR (DMSO-d_6) δ 1.50 (s, 9 H, 3 × CH_3), 3.72, 3.83 (AB, J_{gem} = 19 Hz, 2 H, CH_2S), 4.60 (s, 2 H, OCH_2CO), 4.99, 5.29 (AB, J_{gem} = 15 Hz, 2 H, CH_2O), 5.18 (d, J = 5.0 Hz, 1 H, HC(6)), 5.79 (dd, J = 5.0 and 9.5 Hz, 1 H, HC(7)), 6.20 (br, 3 H, 3 × OH), 6.90−7.50 (m, 14 H, 4 × ArH), 8.30 (d, J = 9.5 Hz, 1 H, NH); IR (Nujol) 3290−3370 (OH, NH), 1792 (β-lactam), 1750 (ester), 1730 (ester), 1688 (amide), 1620 (C═O) cm^{-1}. Anal. ($C_{41}H_{35}N_2O_{12}SCl$) C, H, N, S, Cl.

3-[[[3-[5-Chloro-2-hydroxy-3-(2-hydroxybenzyl)benzyl]-2-hydroxybenzoyl]oxy]methyl]-7-(phenoxyacetamido)-3-cephem-4-carboxylic Acid (19). A solution of **17** (3.93 g, 4.99 mmol) in CF_3CO_2H (35%) in CH_2Cl_2 (45 mL) was stirred at room temperature for 13 h. The solution was condensed under reduced pressure and then CCl_4 (30 mL) was added to the residue and evaporated. The resultant solid was crystallized from a mixture of EtOH and ether (2:1) to afford pure **19** (3.28 g, 4.49 mmol) as a white crystal in 90% yield: mp 196−198 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 3.60 (br s, 4 H, 2 × CH_2), 3.68, 3.79 (AB, J_{gem} = 19 Hz, 2 H, CH_2S), 4.65 (s, 2 H, OCH_2CO), 4.95, 5.21 (AB, J_{gem} = 14 Hz, 2 H, CH_2O), 5.15 (d, J = 5.1 Hz, 1 H, HC(6)), 5.76 (d, J = 5.1 Hz, 1 H, HC(7)), 6.80−7.35 (m, 14 H, 4 × ArH); IR (Nujol) 3300−3450 (OH, NH, CO_2H), 1785 (β-lactam), 1720 (ester), 1685 (amide), 1610 (C═O) cm^{-1}. Anal. ($C_{37}H_{31}N_2O_{10}SCl$) C, H, N, S, Cl.

3-[[[3-[5-Chloro-2-hydroxy-3-(2-hydroxybenzoyl)benzoyl]-2-hydroxybenzoyl]oxy]methyl]-7-(phenoxyacetamido)-3-cephem-4-carboxylic Acid (20). Compound **20** (3.21 g, 4.23 mmol) was prepared from **18** (3.66 g, 4.49 mmol) in 94% yield by the method used for the preparation of **19**. For **20**: mp 180−182 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 3.69, 3.81 (AB, J_{gem} = 19 Hz, 2 H, CH_2S), 4.61 (s, 2 H, OCH_2CO), 4.93, 5.20 (AB, J_{gem} = 14 Hz, 2 H, CH_2O), 5.20 (d, J = 5.0 Hz, 1 H, HC(6)), 5.77 (d, J = 5.0 Hz, 1 H, HC(7)), 6.96−7.53 (m, 14 H, 4 × ArH); IR (Nujol) 3270−3400 (OH, NH, CO_2H), 1788 (β-lactam), 1725 (ester), 1680 (amide), 1620−1635 (C═O) cm^{-1}. Anal. ($C_{37}H_{27}N_2O_{12}SCl$) C, H, N, S, Cl.

***tert*-Butyl 7-[3-[5-Chloro-2-hydroxy-3-(2-hydroxybenzyl)benzyl]-2-hydroxybenzamido]cephalosporanate (22).** To a suspension of compound **14** (1.67 g, 4.34 mmol) in 1,1,1,3,3,3-hexamethyldisilazane (50 mL) was added ammonium sulfate (0.300 g, 2.27 mmol). The reaction mixture was heated at reflux for 5.0 h. The solvent was evaporated and the residue was dissolved in dry THF (40 mL). β-Lactam **21** (1.44 g, 4.39 mmol) and 2-ethoxy-1-(ethoxycarbonyl)-1,2-dihydroquinoline (1.23 g, 4.97 mmol) were then added, and the solution was stirred at room temperature for 24 h. It was then partitioned between EtOAc (100 mL) and 3% aqueous HCl solution (70 mL). The organic layer was washed with water (100 mL), dried over $MgSO_4$ (s), filtered, and condensed. Purification by use of silica gel column chromatography with EtOAc as eluant gave **22** (2.41 g, 3.47 mmol) as a white crystal in 80% yield: mp 147−149 °C; ^{1}H NMR (DMSO-d_6) δ 1.50 (s, 9 H, 3 × CH_3), 2.22 (s, 3 H, CH_3CO), 3.59 (br s, 4 H, 2 × CH_2), 3.69, 3.80 (AB, J_{gem} = 18 Hz, 2 H, CH_2S), 4.80, 5.22 (AB, J_{gem} = 13 Hz, 2 H, CH_2O), 5.20 (d, J = 5.1 Hz, 1 H, HC(6)), 5.86 (dd, J = 5.1 and 10 Hz, 1 H, HC(7)), 6.58 (br, 3 H, 3 × OH), 6.89−7.40 (m, 9 H, 3 × ArH), 9.20 (d, J = 10 Hz, 1 H, NH); IR

(Nujol) 3300−3350 (NH, OH), 1785 (β-lactam), 1750 (ester), 1735 (ester), 1655 (amide) cm^{-1}. Anal. ($C_{35}H_{35}N_2O_9SCl$) C, H, N, S, Cl.

***tert*-Butyl 7-[3-[5-Chloro-2-hydroxy-3-(2-hydroxybenzoyl)benzoyl]-2-hydroxybenzamido]cephalosporanate (23).** Compound **23** (2.76 g, 3.82 mmol) was prepared in 88% yield from **21** (1.44 g, 4.39 mmol) and **15** (1.79 g, 4.34 mmol) by the method used for the preparation of **22** from **21** and **14**. For **23**: mp 136−138 °C; ^{1}H NMR (DMSO-d_6) δ 1.48 (s, 9 H, 3 × CH_3), 2.23 (s, 3 H, CH_3CO), 3.70, 3.81 (AB, J_{gem} = 19 Hz, 2 H, CH_2S), 4.82, 5.29 (AB, J_{gem} = 13 Hz, 2 H, CH_2O), 5.10 (d, J = 4.9 Hz, 1 H, HC(6)), 5.90 (dd, J = 4.9 and 9.5 Hz, 1 H, HC(7)), 6.90−7.50 (m, 12 H, 3 × OH + 3 × ArH), 9.50 (d, J = 9.5 Hz, NH); IR (Nujol) 3250−3350 (NH, OH), 1790 (β-lactam), 1750 (ester), 1735 (ester), 1656 (amide), 1625 (C═O) cm^{-1}. Anal. ($C_{35}H_{31}N_2O_{11}SCl$) C, H, N, S, Cl.

7-[3-[5-Chloro-2-hydroxy-3-(2-hydroxybenzyl)benzyl]-2-hydroxybenzamido]cephalosporanic Acid (24). Compound **24** (1.77 g, 2.77 mmol) was synthesized in 83% yield from **22** (2.32 g, 3.33 mmol) by the method used for the synthesis of **19** from **17**. For **24**: mp 189−192 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 2.23 (s, 3 H, CH_3CO), 3.60 (br s, 4 H, 2 × CH_2), 3.70, 3.81 (AB, J_{gem} = 19 Hz, 2 H, CH_2S), 4.81, 5.22 (AB, J_{gem} = 13 Hz, 2 H, CH_2O), 5.21 (d, J = 4.9 Hz, 1 H, HC(6)), 5.80 (d, J = 4.9 Hz, 1 H, HC(7)), 6.90−7.39 (m, 9 H, 3 × ArH); IR (Nujol) 3260−3400 (NH, OH, CO_2H), 1780 (β-lactam), 1730 (ester), 1650 (amide), 1615 (C═O) cm^{-1}. Anal. ($C_{31}H_{27}N_2O_9SCl$) C, H, N, S, Cl.

7-[3-[5-Chloro-2-hydroxy-3-(2-hydroxybenzoyl)benzoyl]-2-hydroxybenzamido]cephalosporanic Acid (25). Compound **25** (1.02 g, 1.53 mmol) was prepared in 80% yield from **23** (1.38 g, 1.91 mmol) by the method used for the preparation of **20** from **18**. For **25**: mp 173−175 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 2.22 (s, 3 H, CH_3CO), 3.68, 3.79 (AB, J_{gem} = 19 Hz, 2 H, CH_2S), 4.80, 5.20 (AB, J_{gem} = 13 Hz, 2 H, CH_2O), 5.20 (d, J = 5.1 Hz, 1 H, HC(6)), 5.85 (d, J = 5.1 Hz, 1 H, HC(7)), 6.90−7.40 (m, 9 H, 3 × ArH); IR (Nujol) 3250−3400 (NH, OH, CO_2H), 1785 (β-lactam), 1730 (ester), 1652 (amide), 1615−1625 (C═O) cm^{-1}. Anal. ($C_{31}H_{23}N_2O_{11}SCl$) C, H, N, S, Cl.

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