Synthesis and Structure-Activity Relationships of Cephalosporins with C-3′ Catechol-Containing Residues

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Cephalosporins with new catechol substituents at C-3′ have been synthesized, including novel compounds with C-3′ carbon–carbon bonds. Many of these compounds have high potency against Gram-negative bacteria, in particular against resistant strains like *Pseudomonas aeruginosa*. Structure–activity relationships are discussed in terms of their dependence on the pK_a of the C-3′ catechol and also in terms of steric and conformational factors of the C-3′ substituent. The best overall properties were found in compounds with a bulky and/or conformationally restricted acidic C-3′ catechol.

The introduction of a catechol moiety at C-6 of penicillins,[1,2] C-7[3-6] and C-3′[7] of cephalosporins, as well as at N-1 or C-3 of monobactams,[8] has been shown recently to give compounds with vastly improved potency against Gram-negative bacteria, especially the most resistant strains, such as *Pseudomonas aeruginosa*.

The influence of the C-3′ substituents of cephalosporins on their biological properties, level of activity, spectrum and pharmacokinetics is well known.[9,10] At the onset of our work only the C-3′ quaternary ammonium family of catechol cephalosporins had been studied.[7] We have been seeking cephalosporins with improved biological properties to include activity against *P. aeruginosa*, better β-lactamase stability, and improved pharmacokinetics. We have thus focused on the C-3′ position as a point of attachment for novel catechol substituents. We report here novel structure–activity findings for these compounds.

Chemistry

N-Linked C-3′ Substituents. The C-3′-(aminomethyl)cephalosporins **1a–e**,[11,12] key intermediates in our syntheses, have been obtained by reduction of the corresponding C-3′-(azidomethyl)cephalosporins **2**[11-13] using Raney nickel in TFA. Reductive amination of acetaldehyde with the amine **1a** gave the N-substituted secondary amine **3a** (Scheme I).

The amides **4–21**, **23–25** were obtained by acylation of the corresponding amines **1a–e** and **3a**, respectively. Condensation of 3,4-diacetoxyphenyl isocyanate with **1a** gave urea **22**. Reaction of **1a** with the corresponding sulfonyl chlorides led to the sulfonamides **26** and **27** (Scheme I).

O-Linked C-3′ Substituents. The cephalosporin esters **28** have been obtained by acylation of the corresponding 3-(hydroxymethyl)cephalosporin[14] in the presence of (dimethylamino)pyridine, and transformed into the desired compounds **30** and **31** via the amino ester **29**. Treatment of **28** with trifluoroacetic acid gave the free acid **32** (Scheme II).

C-Linked C-3′ Substituents. We have found that catechol reacts readily with 7-aminocephalosporanic acid in the presence of boron trifluoride etherate with direct introduction of the catechol group in C-3′ to give **33**. No prior Δ^3 to Δ^2 double bond isomerization is necessary under these conditions for a successful condensation.[15-17] The

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(10) Webber, J. A.; Wheeler, W. J. Antimicrobial and pharmacokinetic properties of newer penicillins and cephalosporins. In *Chemistry and Biology of β-Lactam Antibiotics*; Morin, R. B., Gorman, M., Eds.; Academic Press: New York, 1982; Vol. 1, pp 371–436.
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amino acid **33** has been taken through to the final compound **34** following Scheme III.

Interestingly, the keto esters **35** and **36** can also be obtained by a boron trifluoride etherate promoted condensation of the corresponding benzoylacetates on 7-aminocephalosporanic acid[17] (Scheme IV). In the case of **36**, spontaneous in situ loss of the *tert*-butyl ester under the Lewis acid conditions, followed by a decarboxylation, gave the ketone **37**. Both compounds **37** and **35** gave the desired final compounds **38** and **39** via standard procedures (Scheme IV).

Structure–Activity Discussion

Antibacterial Spectrum. A common feature of most of the compounds shown in Tables I, II, III, and IV is their outstanding activity against Gram-negative organisms, especially *P. aeruginosa*. Their high potency is due to the presence of a catechol in the C-3′ substituent, which allows these molecules to penetrate exceptionally well, by a novel route, into the periplasmic space of Gram-negative bacteria. This is well demonstrated by their activities against the isogenic pairs of *P. aeruginosa* 799WT/799/61 (Tables I, II, III, IV) and the isogenic pairs of *Escherichia coli* DC0/DC2 (Tables III, IV). The parent organisms, 799WT, DC0, and the permeability mutants, 799/61, DC2, of these two organisms differ mainly by the absence of an intact outer membrane in the permeability mutant, hence allowing the antibacterial to reach their target in an unrestricted fashion in these latter strains. The ratio of the minimum inhibitory concentration (MIC) of the parent to the permeability mutant allows a reasonable estimation of the ability of an antibacterial to penetrate intact *E. coli* or *P. aeruginosa*. The precise mechanism of this illicit transport has been studied extensively in our laboratory.[18] It was thus shown that this transport is *ton B*-dependent, the known functionality of the *ton B* gene product concerning the transport of iron-chelated siderophores in *E. coli*. More specifically, it was also demonstrated that the transport of catechol cephalosporins into bacteria occurs via the *Fiu* and *Cir* iron-regulated outer membrane proteins, since only mutant strains lacking simultaneously these two outer membrane proteins were resistant to the catechol cephalosporins.[18]

The nature of the C-3′ link has been varied extensively and was found to influence the spectrum, but to have little effect on the penetration through the outer membrane of Gram-negative bacteria, see for instance compounds **8**, **30**, **27** (Table III), **4**, and **31** (Table IV). Activity against *Staphylococcus aureus* remains typical of the third generation cephalosporins (Tables III, IV). Neutral oxime substituents give compounds with more Gram-positive activity than compounds with acidic oxime substituents, for example compounds **9** and **4**; **13** and **8**, **10**, **11** and **12** (Table IV). Interestingly, the thienylacetyl C-7 side chain gave compounds where the "catechol effect" is much less expressed than in the oximido aminothiazole cephalosporins, compare compounds **32** and **31** (Table IV). Catechol cephalosporins with the catechol group protected as diacetates as in **32**, always gave identical MIC's to the free catechol, demonstrating the enzymatic/hydrolytic instability of catechol acetates under our in vitro test conditions. This is also well documented in the literature.[32]

β-Lactamase Stability. We have found that the β-lactamase stability of these compounds is dependent on a number of physicochemical parameters of the C-3′ substituent:

The pK_a of the C-3′ catechol has a direct influence on the β-lactamase stability of these compounds (Table I); within a series, increasing the acidity of the catechol leads generally to more stable compounds, as highlighted by the examples of Table I.

Conformational factors also play an important role in the β-lactamase stability of the compounds. The C-3′ amidic NH of the cephalosporins **14**, **15**, and **18** is strongly hydrogen bonded to the carbonyl of the quinolone ring (δ 10.4 ppm in DMSO-d_6, compared to 8.3 ppm for normal amidic compounds). This situation leads to a planar conformation of the C-3′ group, which might explain the observed good enzymatic stability of this family of compounds (Table I). It is striking that structurally related compounds **16** and **17** (Table II) where such a H-bond cannot exist, have poorer enzymatic stability than that of cephalosporins **14**, **15**, and **18**.

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(19) Kessler, R. E.; Bies, M.; Buck, R. E.; Chisholm, D. R.; Pursiano, T. A.; Tsai, Y. H.; Misiek, M.; Price, K. E.; Leitner, F. Comparison of a new cephalosporin, BMY28142, with other broad-spectrum β-lactam antibiotics. *Antimicrob. Agents Chemother.* **1985**, *27*, 207–216.

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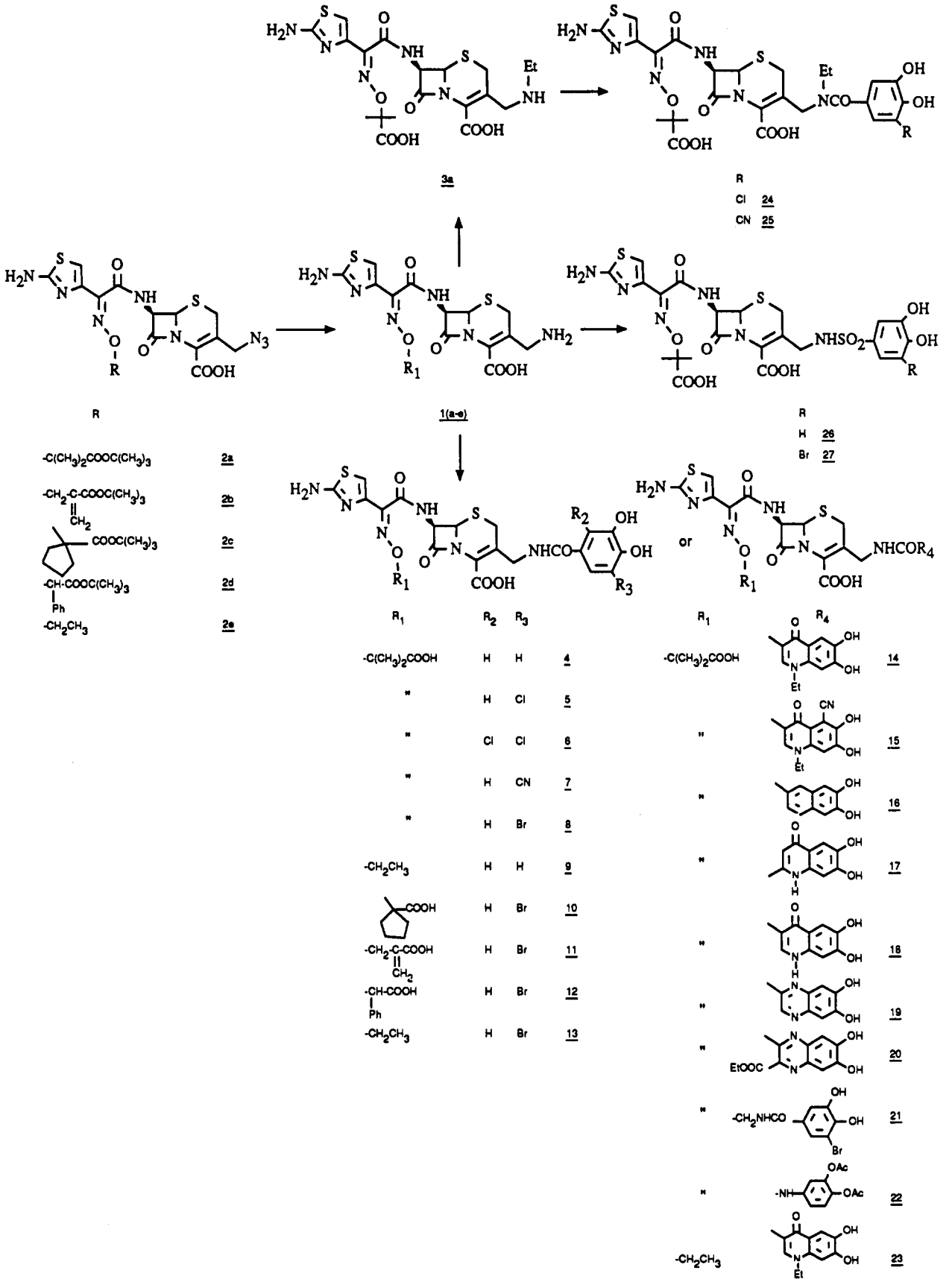
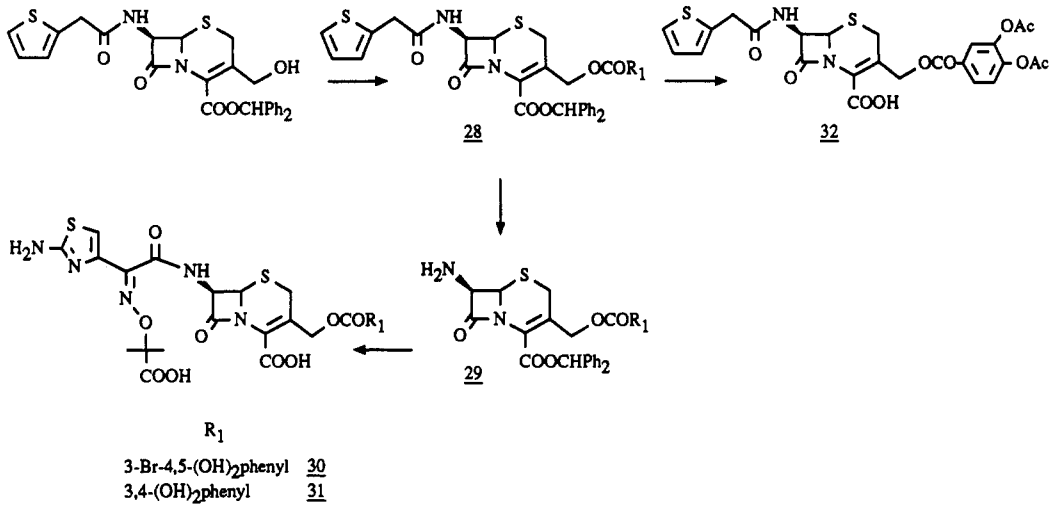
Scheme I

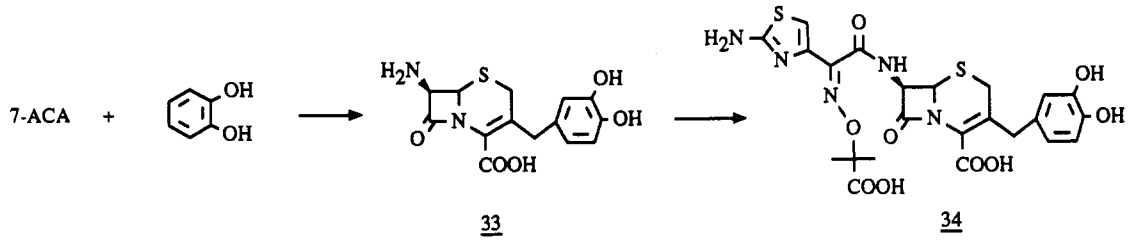


Steric factors also contribute significantly to the overall β-lactamase stability in these series. Substitution of the C-3′ nitrogen, **24**, **25**, or introduction of steric hindrance in the vicinity of this C-3′ nitrogen, **20**, improves the β-lactamase stability in these compounds (Table II). It is known that cephalosporins with C-3′-charged sp^3-hybridized substituents, such as the bulky quaternary ammonium group, are quite stable to β-lactamases.[19] It is of interest to note that compound **39**, with a neutral, sp^3 hybridized, C-3′ carbon atom substituent, also has exceptional β-lactamase stability (Table II). The lactamase-stable bulky sulfonamides **26** and **27** with a partially sp^3 hybridized C-3′

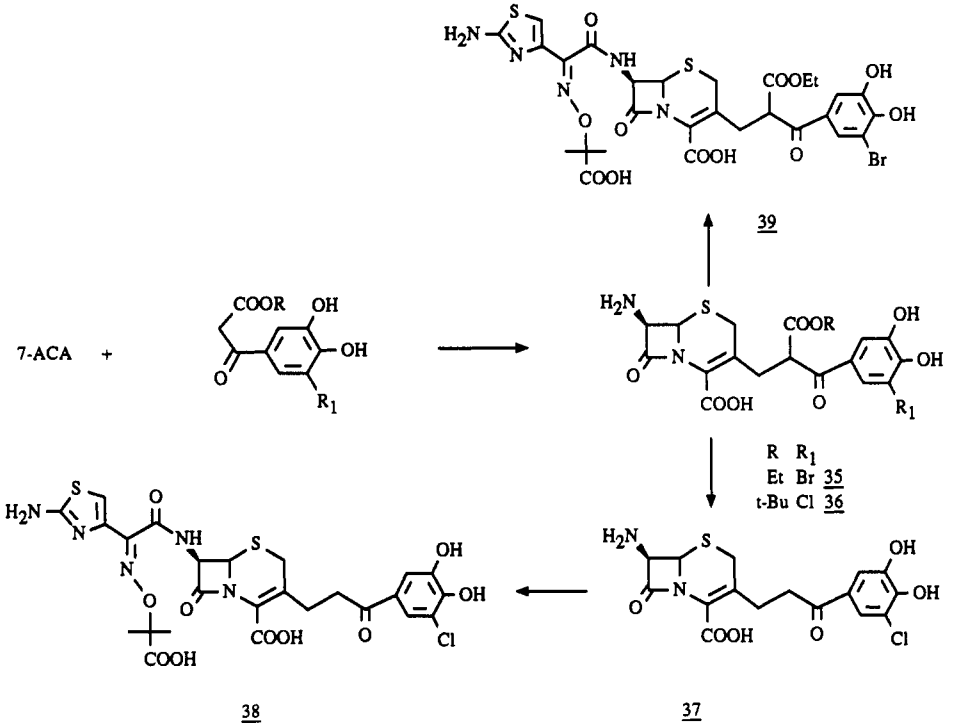
Scheme II



Scheme III



Scheme IV



nitrogen atom[20,21] also fall into this category of compounds (Table I).

The oxime substituent of the C-7 side chain influences β-lactamase stability. Within a series, neutral oxime substituents gave compounds will less lactamase stability than compounds with acidic oxime substituents; compare for instance cephalosporins 9 and 4 (Table IV).

Pharmacokinetics, Metabolism, and Serum Protein Binding. It is a known phenomenon that certain cephalosporins substituted in C-3′ with acid-bearing groups can have unusually long half-lives; ceftriaxone,[33] YM-13115,[34] and cefodizime[35] are representative examples of such compounds. We have found a similar relationship in the

(33) Beskid, G.; Christenson, J. G.; Cleeland, R.; Delorenzo, W.; Trown, P. W. *In vivo* activity of ceftriaxone (Ro 13-9904), a new broad spectrum semisynthetic cephalosporin. *Antimicrob. Agents Chemother.* **1981**, *20*, 159–167.

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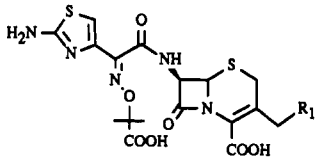
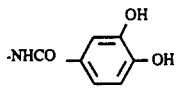
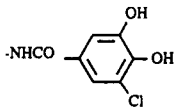
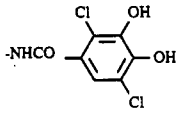
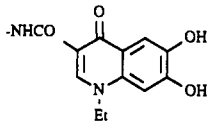
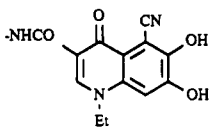
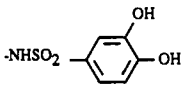
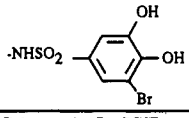
Table I. pK_a of C-3′ Catechol and β-Lactamase Stability[a]



compd	R_1	pK_a	*Pseudomonas aeruginosa* 18S[e]	18SH[b]	799WT[c]	799/61[d]	*Enterobacter cloacae* P99–[e]	P99+[b]	I+[e] 401029	DR[b] 401108	*Pseudomonas stuartii* I+[e] 442019	DR[b] 442049	*Citrobacter freundii* I+[e] 382010	DR[b] 382031
4[f]		8.50	0.008	0.06	0.008	0.008	0.125	16	0.125	>128	0.25	1	0.03	32
5[f]		7.00	0.008	0.03	0.008	0.008	0.06	4	0.06	16	0.03	2	0.008	4
6		6.30	0.008	0.015	0.008	0.008	0.03	4	0.008	4	0.06	1	0.008	2
14[f]		7.20	0.008	0.008	0.008	0.008	0.06	0.25	0.06	1	0.25	8	0.008	0.125
15		<5.50	0.008	0.008	0.008	0.008	0.015	0.015	0.015	0.5	0.008	–	0.008	0.03
26[f]		8.00	0.015	0.125	0.008	0.008	0.125	2	0.03	2	0.5	8	0.015	2
27[f]		6.45	0.015	0.015	0.008	0.008	0.015	0.25	0.015	1	0.03	2	0.008	0.5

[a] MIC in μg/mL, IST growth medium, inoculum 10^4 cfu per spot. [b] Constitutive derepressed type I lactamase producer. [c] Parent organism. [d] Permeability mutant. [e] Inducible type I lactamase producer. [f] Tested twice or more.

C-3′ catechol cephalosporin series. Thus, compounds **5**, **6**, and **14** with acidic catechol functions in C-3′, leading to over 50% ionization at physiological pH, possess long half-lives and large AUC's (area under the curve), compared to the parent compound **4**.[36] Compounds **24**, **30**, and **13** emphasize also the importance of the nature of the C-3′ link and the oxime substituent for long half-lives[36] (Table V).

The urinary recovery of the compounds of Table VI over 27 h dosed at 3 mg/kg per marmoset was generally relatively modest, indicating the existence of other ways of elimination for these compounds. Biliary excretion in rats was measured (details not given) but was highly variable within a series.

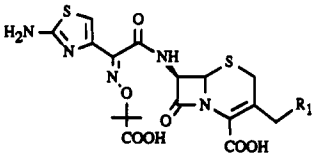
It is anticipated that catechol cephalosporins in general could be metabolized by the enzyme catechol-*o*-methyl transferase and that the longer half-life and the more active compounds could either be poorer substrates[37] or could inhibit its action. The metabolic fate of compound **14** was studied in detail. We found that this compound was not metabolized in marmoset or rat blood, and only one inactive metabolite was detected by HPLC in the urine of marmoset and rat. It underwent rapid metabolism in rat bile to give at least two biologically inactive metabolites. The development of this compound was hampered by evidence of cardiac toxicity in dogs (effect on blood pressure and heart rate). In conclusion, the introduction of a substituted catechol residue at C-3′ of the cephalosporin nucleus can lead to compounds possessing exceptional in vitro antibacterial activity against Gram-negative bacteria, in particular *P. aeruginosa*. We also found that an important biological property, the lactamase stability, is dependent on the pK_a of the C-3′ catechol. Steric and conformational factors of the C-3′ substituent play an important role in this respect. The best lactamase stability was found in compounds with bulky and/or conformationally restricted acidic C-3′ catechols. The pharmacokinetics of these compounds are also influenced by the pK_a

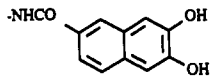
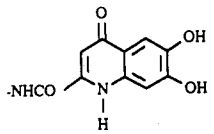
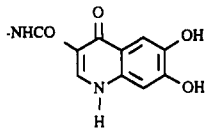
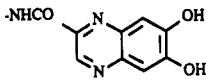
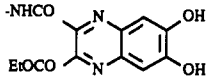
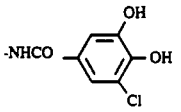
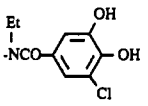
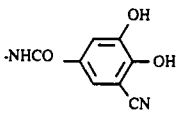
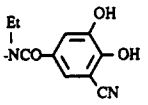
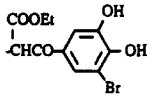
(35) Skully, B. E.; Jules, K.; Neu, H. C. *In vitro* activity and β-lactamase stability of cefodizime, an aminothiazolyl iminomethoxy cephalosporin. *Antimicrob. Agents Chemother.* **1983**, *23*, 907–913.

(36) A full discussion of the structure–activity relationships between the pK_a of the catechol group and the pharmacokinetics of C-3′ catechol cephalosporins are given in the following article. Bird, T. G. C. et al. *J. Med. Chem.*

(37) Ohi, N.; Aoki, B.; Kuroki, T.; Matsumoto, M.; Kojima, K.; Nehashi, T. Effect on antibacterial activity and COMT-susceptibility of chlorine introduction into the catechol nucleus of 6-[(R)-2-[3-(3,4-dihydroxybenzoyl)-3-(3-hydroxy-propyl)-1-ureido]-2-phenylacetamido]penicillanic acid. *J. Antibiot.* **1987**, *40*, 22–28.

Table II. Steric Factors and β-Lactamase Stability[a]



		P. aeruginosa				*E. cloacae*				*P. stuartii*		*C. freundii*	
compd	R_1	18S[e]	18SH[b]	799WT[c]	799/61[d]	P99-[e]	P99+[b]	I+[e] 401029	DR[b] 401108	I+[e] 442019	DR[b] 442049	I+[e] 382010	DR[b] 382031
16		0.008	0.125	0.03	0.008	0.125	16	0.125	>128	0.25	8	0.008	16
17		0.008	0.125	0.008	0.008	0.06	2	0.06	16	0.5	4	0.008	16
18		0.008	0.008	0.008	0.008	0.03	0.06	0.03	1	0.03	2	0.008	0.125
19		0.008	0.008	0.008	0.008	0.03	0.5	0.03	8	0.25	8	0.008	1
20		0.008	0.015	0.008	0.008	0.03	0.06	0.03	0.5	0.06	2	0.008	0.06
5		0.008	0.03	0.008	0.008	0.06	4	0.03	16	0.03	2	0.008	4
24		0.008	0.06	0.008	0.008	0.125	1	0.015	1	0.25	8	0.008	1
7		0.008	0.06	0.008	0.008	0.06	2	0.03	8	0.06	4	0.008	2
25		0.008	0.125	0.008	0.008	0.06	1	0.015	1	0.25	16	0.008	1
39		0.008	0.008	0.008	0.008	0.125	0.25	0.03	0.25	0.125	0.5	0.008	0.25

[a] MIC in μg/mL, IST growth medium, inoculum 10^4 cfu per spot. [b] Constitutive derepressed type I lactamase producer. [c] Parent organism. [d] Permeability mutant. [e] Inducible type I lactamase producer.

of the C-3′ catechol. Some compounds, with negatively charged catechols at C-3′ at physiological pH, have long half-lives and large AUC's.

Experimental Section

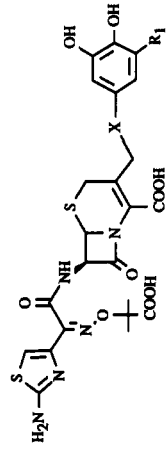
IR spectra were taken as liquid films or KBr pellets on a Perkin-Elmer 781 spectrophotometer. ^{1}H NMR spectra were recorded on a Jeol FX90Q or a Bruker 300AC spectrometer. FAB mass spectra were obtained on a VG 7250 SA mass spectrometer. Analytical HPLC chromatography was carried out on Shimadzu LC6A apparatus using Nucleosil C18 5-μm columns, eluent MeOH/H_2O with 1% AcOH. Preparative medium pressure chromatography was carried out using the Mitsubishi HP20SS resin, eluent MeOH/H_2O with 1% AcOH.

The pK_a values were measured at 25 °C on solutions of 2–3 mg of accurately weighed cephalosporin in 100 μL of DMSO diluted to 3 mL with aqueous 0.1 M KCl by titration with 0.1 N NaOH. The titration and pH of the solution were automatically recorded using a Radiometer RTS 822 titration apparatus, and the pK_a's thus graphically determined from the half-neutralization points. C-3′-(azidomethyl)cephalosporins **2a–e** were obtained by condensation of 7-amino-3-(azidomethyl)ceph-3-em-4-carboxylic acid with the corresponding C-7 side chains using the standard procedures of cephalosporin chemistry.[12] For the synthesis of carboxamidocephalosporins **5–8** see the following paper.[22]

Biological Studies and MIC Determinations. MIC values were determined by agar dilution at an inoculum of about 10^4 colony forming units in Iso-Sensitest (IST) growth medium (Oxoid Ltd., London, England) and recorded after overnight incubation at 30 °C as the lowest antibiotic concentration inhibiting visible bacterial growth.

Marmoset Pharmacokinetic Test. Animals were obtained from the ICI marmoset breeding unit and weighed about 350 g. Groups comprised three male or three female animals but were never of mixed sexes. A presample of blood was taken on the day before the experiment. The compounds were prepared as solutions in 0.05 M phosphate buffer, pH 7, and the animals were dosed at 3 mg/kg intravenously and then housed in metabowls for the

Table III. Nature of C-3′ Link, Spectrum, and Enzymatic Stability[a]



compd	X	R_1	*P. aeruginosa*					*E. cloacae*			*P. stuartii*		*C. freundii*		*E. coli*		*S. aureus*	
			18S[c]	18SH[b]	799WT[c]	799/61[d]	PU21	P99-[e]	P99+[b]	I+[e] 401029	DR[b] 401108	I+[e] 442019	DR[b] 442049	I+[e] 382010	DR[b] 382031	DC0[c]	DC2[d]	Oxford[f]	147N[f]
8	$-CH_2NHCO-$	Br	0.008	0.015	0.008	0.008	0.008	0.03	2	0.015	8	0.25	4	0.008	1	0.008	0.008	16	16
30	$-CH_2OCO-$	Br	0.008	0.03	0.008	0.008	0.008	0.03	2	0.03	>128	0.03	2	0.008	4	0.008	0.008	8	8
27	$-CH_2NHSO_2-$	Br	0.015	0.015	0.008	0.008	0.015	0.015	0.25	0.015	1	0.03	2	0.008	0.5	0.008	0.008	128	128
21	$-CH_2NHCOCH_2NHCO-$	Br	0.008	0.5	0.008	0.008	0.015	0.06	8	0.06	>32	0.125	4	0.015	8	0.015	0.015	8	16
5	$-CH_2NHCO-$	Cl	0.008	0.03	0.008	0.008	0.008	0.06	4	0.06	16	0.03	2	0.008	4	0.008	0.008	16	16
38	$-(CH_2)_2CO-$	Cl	0.008	0.015	0.008	0.008	0.015	0.03	1	0.03	>32	0.03	1	0.008	2	0.008	0.008	16	16
34	$-CH_2-$	H	0.03	0.125	0.06	0.125	0.25	4	32	0.5	32	2	8	0.25	16	0.125	0.03	8	16
22	$-CH_2NHCONH-$	H	0.125	0.125	0.125	0.125	0.125	1	16	0.5	64	2	16	0.25	8	0.06	0.015	64	64
ceftazidime			2	16	0.125	0.008	1	0.125	32	0.125	32	0.25	4	0.125	128	0.125	0.06	4	8

[a] MIC in μg/mL IST growth medium, inoculum 10^4 cfu per spot. [b] Constitutive derepressed type I lactamase producer. [c] Parent organism. [d] Permeability mutant. [e] Inducible type I lactamase producer. [f] Penicillin-sensitive *S. aureus*.

duration of the experiment. They were bled from the tail vein at 0, 15, 30, 60, 120, and 180 min into Microtainers (Beckton Dickenson) for serum samples. The samples were assayed in triplicate by an agar diffusion method using Mueller Hinton agar and *E. coli* ESS (A8341207). Zones of inhibition were measured using electronic calipers connected to an IBM PC and concentrations computed from a standard curve Lin Reg Analysis. Half-life and AUC values were then computed.

Serum Protein Binding Assay. Samples of compounds at 50 μg/mL in human serum were prepared in duplicate and allowed to equilibrate with shaking at 37 °C for 15 min before loading into an Amicon ultrafiltration membrane cone, Type CF25, and centrifuging. The centrifugates were assayed in triplicate by the agar diffusion method described for the marmoset pharmacokinetic test in the previous paragraph. The percentages of binding were calculated.

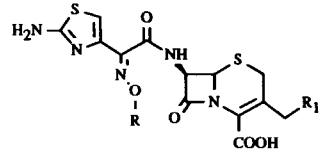
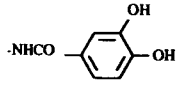
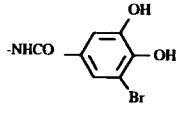
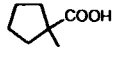
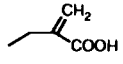
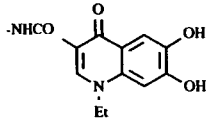
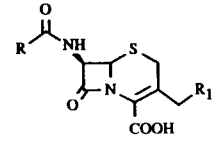
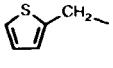
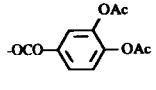
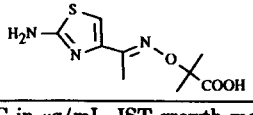
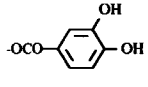
Urinary Recovery in Marmosets. Groups of three male or three female ICI marmosets were housed individually in metabolism cages. Solutions of the compounds were prepared in 0.05 M phosphate buffer, pH 7, and the animals were dosed at 3 mg/kg intravenously followed by an oral dose of 3 mL of water (to stimulate urine production). Urine was collected into solid carbon dioxide-cooled containers to freeze samples, during the periods of 0–4, 4–8, and 8–24 h, and the animals were given a further oral dose of water at each collection point. After 24 h, the metabowls were washed into a collecting vessel, and a final sample was collected over 24–27 h. Samples were assayed in triplicate by the agar diffusion method described for the marmoset pharmacokinetic test. The percentages of recovery, relative to dose, were calculated from the cumulative product of concentrations and volumes.

C-3′-(Aminomethyl)cephalosporins (1a–e): General Procedure. These compounds are known in the cephalosporin literature[11,12] but were obtained in our work by the following convenient procedure. Cephalosporins **2a–e** (1 equiv) were solubilized in TFA at room temperature. Prolabo Raney nickel (about 50% suspension in water and 1.5 times the weight of cephalosporin) was added to the solution in small portions, and the reaction mixture was stirred at room temperature for about 1 h. The progress of the reduction was monitored by HPLC. At the end of the reaction, the Raney nickel was filtered off through Celite and washed with water and methanol, and the combined solutions were evaporated under vacuum. The crude reaction product was purified by medium pressure chromatography. Yields were typically around 30–40% after purification. The physical and spectral properties of the compounds were in agreement with their structure and published data.

7-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]acetamido]-3-[(ethylamino)methyl]ceph-3-em-4-carboxylic Acid (3a). Acetaldehyde (88 μL, 1.6 mmol) is added slowly to a solution of **1a** (970 mg, 1.4 mmol) in anhydrous MeOH (100 mL) in presence of Et_3N (195 μL, 1.4 mmol) and $NaBH_3CN$ (88 mg, 1.4 mmol). The reaction mixture is stirred at room temperature for 0.5 h. The solvent is evaporated and the residue purified by preparative HPLC on a Whatman ODS3 column, eluent H_2O/MeOH/AcOH, 90:10:1 to give **3a**, 257 mg (36%): ^{1}H NMR (DMSO-d_6/CD_3COOD/TFA-d) δ ppm 1.3 (t, J = 7 Hz, 3 H), 1.6 (s, 6 H), 2.8–3.2 (m, 2 H), 3.7–4.1 (m, 4 H), 5.3 (d, J = 4.5 Hz, 1 H), 6 (d, J = 4.5 Hz, 1 H), 7.2 (s, 1 H); MS (+FAB) 513 $(M + H)^+$.

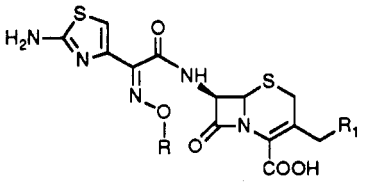
C-3′-Carboxamido and C-3′-Sulfonamido Cephalosporins 4, 9–13, 16, and 24–27: General Procedure. The acid chloride or bromide (1.1 equiv) was added to a stirred solution of cephalosporin 1 (1 equiv) in MeOH or DMF (for the sulfonamido cephalosporins) at 0 °C in the presence of Et_3N (3 equiv). The mixture was stirred at this temperature for 1–3 h, the progress of the reaction being followed by HPLC. At the end of the reaction, an equal volume of water was added. The pH of the solution was adjusted to 8.5 with diluted ammonia, and removal of the acetate group from the catechol was followed by HPLC as the mixture was stirred at this pH for 1–3 h. At the end of the deprotection, the pH was adjusted to 6 with 2 N HCl, the solvents were evaporated under vacuum, and the residue was purified by medium pressure chromatography. The yields of the purified products were in the range of 20–70% (see supplementary material).

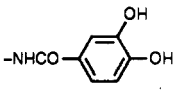
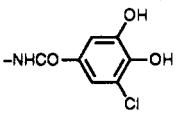
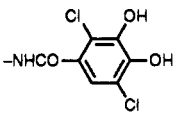
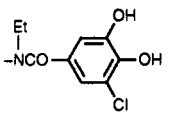
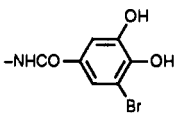
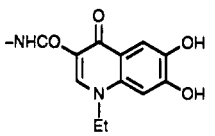
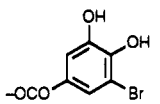
Table IV. C-7 Side Chain, Spectrum, and β-Lactamase Stability[a]

			P. aeruginosa					*E. cloacae*				*P. stuartii*		*C. freundii*		*E. coli*		*S. aureus*	
compd	R	R_1	18S[e]	18SH[b]	799WT[c]	799/61[d]	PU21	P99–[e]	P99+[b]	I+[e] 401029	DR[b] 401108	I+[e] 442019	DR[b] 442049	I+[e] 382010	DR[b] 382031	DC0[c]	DC2[d]	Oxford[f]	147N[f]
																			
4	$-C(CH_3)_2COOH$		0.008	0.06	0.008	0.008	0.015	0.125	16	0.125	>128	0.25	1	0.03	16	0.008	0.008	8	16
9	$-CH_2CH_3$	*g*	0.03	64	0.06	0.015	0.25	0.5	64	0.25	128	0.5	1	0.06	>128	0.06	0.008	2	4
8	$-C(CH_3)_2COOH$		0.008	0.015	0.008	0.008	0.008	0.03	2	0.015	8	0.125	4	0.008	1	0.008	0.008	16	16
10		*g*	0.015	0.008	0.008	0.008	0.015	0.03	2	0.03	16	0.03	2	0.008	4	0.008	0.008	16	32
11		*g*	0.008	0.05	0.008	0.008	0.03	0.06	8	0.06	>32	0.03	2	0.008	16	0.008	0.008	16	16
12		*g*	0.008	0.03	0.015	0.008	0.015	0.06	2	0.03	>128	0.06	0.25	0.008	4	0.008	0.008	16	16
13	$-CH_2CH_3$	*g*	0.008	4	0.015	0.008	0.06	0.015	8	0.03	128	0.125	4	0.008	16	0.008	0.008	2	4
14	$-C(CH_3)_2COOH$		0.008	0.008	0.03	0.008	0.06	0.06	0.25	0.06	1	0.25	8	0.008	0.125	0.008	0.008	16	16
23	$-CH_2CH_3$	*g*	0.008	0.25	0.015	0.008	0.06	0.03	1	0.015	32	0.06	2	0.008	0.5	0.008	0.008	2	1
																			
32			>128	>128	>128	0.125	>128	16	>128	128	–	64	>128	16	>128	0.5	0.03	0.03	0.06
31			0.008	0.125	0.008	0.008	0.3	0.5	4	0.06	>128	0.125	4	0.03	8	0.008	0.008	4	8

[a] MIC in μg/mL, IST growth medium, inoculum 10^4 cfu per spot. [b] Constitutive derepressed type I lactamase producer. [c] Parent organism. [d] Permeability mutant. [e] Inducible type I lactamase producer. [f] Penicillin sensitive S. aureus. [g] Same as above.

Table V. Pharmacokinetics of C-3′ Catechol Cephalosporins in Marmosets



compd	R	R_1	pK_a	$t_{1/2}$ (h)	AUC (mg/h per L)
4	$-C(CH_3)_2COOH$		8.5	1.25 ● 0.09	27.2 ± 2.52
5	*c*		7.0	4.57 ● 0.46[a]	638.3 ± 74.6
6	*c*		6.3	8.22 ● 1.3	461.0 ● 61.7
24	*c*		7.4	0.87 ● 0.19	16.4 ● 4.1
13	$-CH_2CH_3$		6.9	0.67 ● 0.04	16.97 ● 0.5
14	$-C(CH_3)_2COOH$		7.2	3.47 ± 0.96	238.5 ± 90.5
30	*c*		6.5	1.68 ± 0.12	67.7 ● 4.1
ceftazidime[b]				0.7	14

[a] Mean values from two tests, quoting the greatest standard error observed. [b] Used as a comparator in all tests, mean values from a large number of tests. [c] Same as above.

Table VI. Serum Protein Binding and Urinary Recovery of Selected Compounds

compound	serum protein binding[a]	urinary recovery[b]
4	96.3	44.8
6	98.7	16.2
8	99.0	17.5
14	99.2	13.9

[a] In human blood (%). [b] In marmosets, dosed at 3 mg/kg, over 27 h (% of administered dose).

C-3′-Carboxamido Cephalosporins 17, 19: General Procedure. The activated esters of the 1,4-dihydro-6,7-dihydroxy-4-oxoquinoline-2-carboxylic acid and 6,7-dihydroxyquinoxaline-2-carboxylic acid (1 equiv) were obtained by reaction with *N*-hydroxybenzotriazole (1.1 equiv) and dicyclohexylcarbodiimide (1.1 equiv) in DMF at room temperature for 2 and 1 h, respectively. The precipitated dicyclohexylurea was filtered off, and the resulting solution was added to a solution of **1a** (0.8 equiv) in DMF in the presence of 4 equiv of Et_3N. The mixture was stirred at room temperature for 16 and 2 h, respectively, the DMF was evaporated, and the residue purified by medium pressure chromatography to give the desired compounds in 5% and 40% yield, respectively (see supplementary material).

C-3′-Carboxamido Cephalosporins 14, 15, 18, 20, 23: General Procedure. The heterocyclic carboxylic acids (1 equiv) were carefully dried by azeotropic removal of water, followed by drying over P_2O_5 under vacuum, were polysilylated in CH_2Cl_2 by reaction with trimethylsilyl chloride (3.2 equiv) and Et_3N (3.2 equiv) over 2 h at room temperature. Thionyl chloride (1.1 equiv) was added to the solution, and the resulting mixture was stirred at room temperature for 2 h. This solution was then added to a solution of C-3′-aminocephalosporin **1a** in DMF with Et_3N (3 equiv) at 0 °C. The mixture was stirred at this temperature for 30 min, the solvents were evaporated, and the residual solid was purified by medium pressure chromatography; yields are in the range of 20–40% (see supplementary material).

7-(2-Thienylacetamido)-3-[[(3,4-diacetoxybenzoyl)oxy]-methyl]ceph-3-em-4-carboxylic Acid (32) and 7-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]acetamido]-3-[[(3-bromo-4,5-dihydroxybenzoyl)oxy]-methyl]ceph-3-em-4-carboxylic Acid (30). 4-DMAP (125 mg, 1 mmol, 0.2 equiv) and Et_3N (1.04 mL, 7.5 mmol, 1.5 equiv) were added to a solution of diphenylmethyl 7-(2-thienylacetamido)-3-(hydroxymethyl)ceph-3-em-4-carboxylate[14] (2.7 g, 5 mmol, 1 equiv) and 3-bromo-4,5-diacetoxybenzoyl chloride[22] 2.5 g, 7.5 mmol, 1.5 equiv) in anhydrous CH_2Cl_2 at 0 °C. The mixture was stirred at 0 °C for 10 min, washed with water and diluted HCl, and dried to give **28** (R_2 = 3-bromo-4,5-diacetoxyphenyl), 4.1 g (100%): ^1H NMR ($CDCl_3/CD_3COOD$) δ ppm 2.25 (s, 3 H), 2.35 (s, 3 H), 3.25–3.75 (m, 2 H), 3.8 (s, 2 H), 5 (d, J = 4.5 Hz, 1 H), 5.8 (d, J = 4.5 Hz, 1 H), 4.9–5.3 (m, 2 H), 6.9 (s, 1 H), 7 (m, 2 H), 7.25 (m, 11 H), 7.35 (d, J = 2 Hz, 1 H), 8.05 (d, J = 2 Hz, 1 H).

PCl_5 (2.04 g, 10 mmol, 2 equiv) was added to a solution of **28** (R_2 = 3-bromo-4,5-diacetoxyphenyl) (4 g, 4.9 mmol, 1 equiv) in anhydrous CH_2Cl_2 and pyridine (1.6 mL, 20 mmol, 4 equiv) at −20 °C. The mixture was stirred under argon for 15 min at −20 °C and stirred at room temperature for 1.5 h. The solution was again cooled to −20 °C, butane-1,3-diol (2.1 mL, 5 equiv) in CH_2Cl_2 (20 mL) was added, and the mixture was allowed to reach room

temperature over 1 h. The solvent was evaporated, and the residue was purified by chromatography over SiO_2 (eluent CH_2Cl_2/MeOH 98/2). The combined fractions were acidified with a solution of hydrochloric acid in ether and evaporated to give **29** hydrochloride (R_2 = 3-bromo-4,5-diacetoxyphenyl), 2.9 g (83%): ^1H NMR ($CDCl_3/CD_3COOD$) δ ppm 2.25 (s, 3 H), 2.3 (s, 3 H), 3.25–3.75 (m, 2 H), 5.05 (s, 2 H), 4.75–5.25 (m, 2 H), 6.95 (s, 1 H), 7.25 (m, 10 H), 7.75 (d, J = 2 Hz, 1 H), 8.05 (d, J = 2 Hz, 1 H).

29 (R_1 = 3-bromo-4,5-diacetoxyphenyl) free base (1.9 g, 2.7 mmol, 1 equiv) was reacted with 2-benzothiazolyl 2-(2-aminothiazol-4-yl)-2-[[1-*tert*-butoxycarbonyl)-1-methylethoxy]imino]-thioacetate (1.3 g, 2.7 mmol, 1 equiv) in CH_2Cl_2 (30 mL) at room temperature for 1 h. The crude product was chromatographed over SiO_2, eluent CH_2Cl_2MeOH 98/2, to give diphenylmethyl 7-[2-(2-aminothiazol-4-yl)-2-[[(*Z*)-1-(*tert*-butoxycarbonyl)-1-methylethoxy]imino]acetamido]-3-[[(3-bromo-4,5-diacetoxybenzoyl)oxy]methyl]ceph-3-em-4-carboxylate, 1.8 g (68%): ^1H NMR ($CDCl_3$) δ ppm 2.25 (s, 3 H), 2.35 (s, 3 H), 3.25–3.75 (m, 2 H), 5.05 (d, J = 4.5 Hz, 1 H), 4.9–5.4 (m, 2 H); 6 (dd, J = 4.5, 9 Hz, 1 H), 6.9 (s, 1 H), 6.95 (s, 1 H), 7.15–7.4 (m, 10 H), 7.75 (d, J = 2 Hz, 1 H), 8.05 (d, J = 2 Hz, 1 H).

The previous compound (1.65 g, 1.7 mmol) was treated with a 3:1 mixture of TFA/anisole (4 mL) at room temperature for 1 h, and the solvents were evaporated. The residue was dissolved in MeOH/H_2O 1:1, and the pH was adjusted to 8.5 by addition of diluted ammonia; at the end of the reaction, the pH was acidified to 5 and the solution evaporated. After medium pressure chromatography purification, **30** was obtained, 488 mg (42%): ^1H NMR (DMSO-d_6/CD_3COOD/TFA-d) δ ppm 1.55 (s, 6 H), 3.55 (d, J = 18 Hz, 1 H), 3.8 (d, J = 18 Hz, 1 H), 4.9 (d, J = 12.5 Hz, 1 H), 5.25 (d, J = 12.5 Hz, 1 H), 5.2 (d, J = 4.5 Hz, 1 H), 5.85 (d, J = 4.5 Hz, 1 H), 7.05 (s, 1 H), 7.4 (d, J = 2.5 Hz, 1 H), 7.5 (d, J = 2.5 Hz, 1 H); IR (KBr) 1770 cm^{-1}; MS (+FAB) 700 $(M + H)^+$.

28 (R_1 = 3,4-diacetoxyphenyl) (100 mg, 0.13 mmol) was treated with a 2:1 TFA/anisole mixture (1.5 mL) at room temperature for 0.5 h. The solvents were evaporated, and the residue was precipitated by a mixture of ether/pentane to give **32**, 43 mg (56%): ^1H NMR (DMSO-d_6/CD_3COOD/TFA-d) δ ppm 2.25 (s, 6 H), 3.5–3.9 (m, 4 H), 5 (d, J = 12.5 Hz, 1 H), 5.3 (d, J = 12.5 Hz, 1 H), 5.1 (d, J = 4.5 Hz, 1 H), 5.7 (d, J = 4.5 Hz, 1 H), 6.8–8 (m, 6 H); IR (KBr) 1780 cm^{-1}; MS (+FAB) 575 $(M + H)^+$.

7-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]acetamido]-3-[[(3,4-dihydroxybenzoyl)oxy]methyl]ceph-3-em-4-carboxylic Acid (31). This compound was obtained following the same approach as for compound **30**. The intermediates and compound **31** have the following spectral data.

28 (R_2 = 3,4-diacetoxyphenyl): ^1H NMR (DMSO-d_6/CD_3COOD) δ ppm 2.29 (s, 6 H), 3.8–4 (m, 4 H), 4.9 (d, J = 12.5 Hz, 1 H), 5.2 (d, J = 12.5 Hz, 1 H), 5.2 (d, J = 4.5 Hz, 1 H), 5.85 (d, J = 4.5 Hz, 1 H), 7 (m, 2 H), 7.2–8 (m, 15 H).

29 (R_2 = 3,4-diacetoxyphenyl): ^1H NMR (DMSO-d_6/CD_3COOD)/TFA-d) δ ppm 2.28 (s, 6 H), 3.7–4 (m, 2 H), 5 (d, J = 12.5 Hz, 1 H), 5.3 (d, J = 12.5 Hz, 1 H), 5.3 (s, 2 H), 7 (s, 1 H), 7.2–8 (m, 13 H).

Diphenylmethyl 7-[2-(2-aminothiazol-4-yl)-2-[(*Z*)-[1-(*tert*-butoxycarbonyl)-1-methylethoxy]imino]acetamido]-3-[[(3,4-diacetoxybenzoyl)oxy]methyl]ceph-3-em-4-carboxylate: ^1H NMR (DMSO-d_6/CD_3COOD) δ ppm 1.35 (s, 9 H), 1.41 (s, 6 H), 2.28 (s, 6 H), 3.5–3.9 (m, 2 H), 4.9 (d, J = 12.5 Hz, 1 H), 5.2 (d, J = 12.5 Hz, 1 H), 5.25 (d, J = 4.5 Hz, 1 H), 5.8 (d, J = 4.5 Hz, 1 H), 6.71 (s, 1 H), 6.9 (s, 1 H), 7.8 (m, 28 H).

31: ^1H NMR (DMSO-d_6/CD_3COOD/TFA-d) δ ppm 1.6 (s, 6 H), 3.4–3.9 (m, 2 H), 4.9 (d, J = 12.5 Hz, 1 H), 5.3 (d, J = 12.5 Hz, 1 H), 5.2 (d, J = 4.5 Hz, 1 H); 5.9 (d, J = 4.5 Hz, 1 H), 6.8 (d, J = 8.3 Hz, 1 H), 7.04 (s, 1 H), 7.35 (dd, J = 8.3, 1.7 Hz, 1 H), 7.45 (s, 1 H); IR (KBr) 1770 cm^{-1}; MS (+FAB) 622 $(M + H)^+$.

7-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]acetamido]-3-[(3,4-dihydroxyphenyl)-methyl]ceph-3-em-4-carboxylic Acid (34). 7-ACA (5 g, 1 equiv) and catechol (2 g, 1 equiv) in acetonitrile (50 mL) and BF_3·Et_2O (15 mL) were stirred at room temperature for 3 h. The solvent was evaporated and the residue was purified over medium pressure chromatography to give 7-amino-3-[(3,4-dihydroxyphenyl)-methyl]ceph-3-em-4-carboxylic acid **33** (3.5 g, 60%): ^1H NMR (DMSO-d_6/CD_3COOD/TFA-d) δ ppm 3.1–4.3 (m, 4 H), 5.04 (d, J = 4.8 Hz, 1 H), 5.2 (d, J = 4.8 Hz, 1 H), 6.4–7 (m, 3 H).

Compound **33** (100 mg, 1 equiv) was solubilized in anhydrous CH_2Cl_2 (3 mL) with BSA (385 μL, 5 equiv) under nitrogen for 3 h at room temperature. 2-[2-(Tritylamino)thiazol-4-yl]-2-[1-(*tert*-butoxycarbonyl)-1-methylethoxy)imino]acetyl chloride (177 mg, 1 equiv) in CH_2Cl_2 (3 mL) was added to the previous solution at 0 °C. After 5 min, the solvent was evaporated, and the residue was treated with a mixture of TFA/H_2O 5:1 at room temperature for 2 h. After evaporation of the solvents, the residue was purified by preparative HPLC Whatman ODS3 to give **34**, 40 mg (22%): ^1H NMR (DMSO-d_6/CD_3COOD/TFA-d) δ ppm 1.53 (s, 6 H), 3.–4.1 (m, 4 H), 5.17 (d, J = 4.5 Hz, 1 H), 5.76 (d, J = 4.5 Hz, 1 H), 6.15–6.9 (m, 3 H), 7.06 (s, 1 H); MS (+FAB) 578 $(M + H)^+$.

7-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]acetamido]-3-[3-(3-chloro-4,5-dihydroxyphenyl)-3-oxopropyl]ceph-3-em-4-carboxylic Acid (38). 3-Chloro-4,5-dihydroxybenzoic acid[22] (1.88 g, 10 mmol, 1 equiv) was silylated with trimethylsilyl chloride (3.47 g, 32 mmol, 3.2 equiv) and Et_3N (3.23 g, 32 mmol, 3.2 equiv) in CH_2Cl_2 (80 mL) at room temperature for 1.5 h. Thionyl chloride (1.31 g, 11 mmol, 1.1 equiv) and Et_3N (1.1 g, 11 mmol, 1.1 equiv) were added, and the mixture was stirred at room temperature for 1 h. A suspension of the sodium salt of *tert*-butyl acetoacetate in ether (300 mL), prepared from *tert*-butyl acetoacetate (12.6 g, 80 mmol, 8 equiv) and NaH (1.9 g, 80 mmol, 8 equiv), was added at room temperature and the mixture stirred for 30 min. After concentration and SiO_2 chromatography (CH_2Cl_2/ether, 90:10), 600 mg of adduct were obtained. This compound was treated with sodium acetate (200 mg) in *tert*-butanol (50 mL) at reflux, monitoring the reaction by TLC. At the end of the reaction, the solvent was evaporated and the product purified by SiO_2 chromatography (eluent: CH_2Cl_2, AcOH) to obtain *tert*-butyl 2-(3-chloro-4,5-dihydroxybenzoyl)-acetate, 200 mg (7%): ^1H NMR ($CDCl_3/CD_3COOD$) δ ppm 1.4 (s, 9 H), 3.85 (s, 2 H), 7.35 (d, J = 2 Hz, 1 H), 7.5 (d, J = 2 Hz, 1 H).

tert-Butyl 2-(3-chloro-4,5-dihydroxybenzoyl)acetate (161 mg, 0.56 mmol, 1 equiv) was reacted with 7-ACA (153 mg, 0.56 mmol, 1 equiv) in sulfolane (1.5 mL) in presence of BF_3/Et_2O (0.7 mL). After 4 h at room temperature, water (10 mL) was added and the mixture kept for 12 h at 5 °C. The product was then purified by medium pressure chromatography to obtain 7-amino-3-[3-(3-chloro-4,5-dihydroxyphenyl)-3-oxopropyl]ceph-3-em-4-carboxylic acid (**37**), 100 mg (45%): ^1H NMR (CD_3COOD/TFA-d) δ ppm 3.1–3.5 (m, 4 H), 3.7 (s, 2 H), 5.1 (d, J = 4.5 Hz, 1 H), 5.35 (d, J = 4.5 Hz, 1 H), 7.55 (d, J = 2 Hz, 1 H), 7.65 (d, J = 2 Hz, 1 H).

Compound **37** (100 mg, 0.25 mmol, 1 equiv) in DMF (2 mL) in the presence of Et_3N (70 μL, 2 equiv) was stirred at room temperature with 2-benzothiazolyl 2-(2-aminothiazol-4-yl)-2-[[1-(*tert*-butoxycarbonyl)-1-methylethoxy]imino]thioacetate (120 mg, 0.25 mmol, 1 equiv). After completion of the condensation, the solvent was evaporated and the crude was treated with TFA (2 mL). The product was purified by medium pressure chromatography to obtain **38**, 24 mg (15%): ^1H NMR (DMSO-d_6/CD_3COOD/TFA-d) δ ppm 1.5 (s, 3 H), 1.55 (s, 3 H), 2.5–2.8 (m, 2 H), 3.1 (m, 2 H), 3.55 (d, J = 18 Hz, 1 H), 3.65 (d, J = 18 Hz, 1 H), 5.15 (d, J = 4.4 Hz, 1 H), 5.8 (d, J = 4.4 Hz, 1 H), 7.1 (s, 1 H), 7.35 (d, J = 2 Hz, 1 H), 7.5 (d, J = 2 Hz, 1 H); IR (KBr) 1760 cm^{-1}; MS (+FAB) 652 $(M + H)^+$.

7-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]acetamido]-3-[3-(3-bromo-4,5-dihydroxyphenyl)-2-(ethoxycarbonyl)-3-oxopropyl]ceph-3-em-4-carboxylic Acid (39). 3-Bromo-4,5-dimethoxybenzaldehyde[23] (10 g, 1 equiv) was dissolved in THF (100 mL) and cooled to −78 °C. MeLi (37 mL, 1.5 equiv) was slowly added to the solution. The mixture was allowed to reach room temperature, hydroylzed with water (50 mL), dried, concentrated, and purified by SiO_2 chromatography (eluent: AcOEt/petroleum ether) to give 1-(3-bromo-4,5-dimethoxyphenyl)-1-ethanol, 7.64 g (72%): ^1H NMR (DMSO-d_6) δ ppm 1.35 (s, 3 H), 3.73 (s, 3 H), 3.84 (s, 3 H), 4.5–4.8 (m, 1 H), 7.04 (s, 1 H), 7.12 (s, 1 H).

This alcohol (7.6 g) was dissolved in acetone (300 mL). Jones reagent (20 mL) was added with stirring. After 20 min at room temperature, 2-propanol (35 mL) was added, the mixture filtered through Celite, the organic phase evaporated, and the residue dissolved in ether, washed with water, and dried to give 3-

bromo-4,5-dimethoxyacetophenone, 6.8 g (90%): ^{1}H NMR (DMSO-d_6) δ ppm 2.56 (s, 3 H), 3.82 (s, 3 H), 3.9 (s, 3 H), 7.54 (s, 1 H), 7.78 (s, 1 H).

3-Bromo-4,5-dimethoxyacetophenone (6.8 g, 1 equiv) was dissolved in diethyl carbonate (70 mL), sodium hydride (2.6 g, 2 equiv) was added, and the mixture was stirred at 80 °C for 3 h. The reaction mixture was then poured into water (200 mL) and acetic acid (6.5 mL), extracted with ether, and purified by chromatography over SiO_2 to give ethyl 3-(3-bromo-4,5-dimethoxyphenyl)-3-oxopropionate, 5 g (58%): ^{1}H NMR ($CDCl_3$) δ ppm 1.26 (t, J = 7 Hz, 3 H), 3.9 (s, 3 H), 3.91 (s, 3 H), 3.93 (s, 2 H), 4.22 (q, J = 7 Hz, 2 H), 7.48 (s, 1 H), 7.7 (s, 1 H).

Ethyl 3-(3-bromo-4,5-dimethoxyphenyl)-3-oxopropionate (5 g, 1 equiv) was dissolved in CH_2Cl_2 (50 mL) and boron tribromide (4.2 mL, 3 equiv) in CH_2Cl_2 (25 mL) was added at 0 °C. After 1 h, the solvent was evaporated, and the residue was poured into iced water. After extraction with ethyl acetate, drying, and concentration, the crude product was purified by SiO_2 chromatography (eluent: ethyl acetate/petroleum ether) to obtain ethyl 3-(3-bromo-4,5-dihydroxyphenyl)-3-oxopropionate, 3.18 g (70%): ^{1}H NMR ($CDCl_3$) δ ppm 1.25 (t, J = 7 Hz, 3 H), 3.89 (s, 2 H), 4.21 (q, J = 7 Hz, 2 H), 7.46 (d, J = 1.5 Hz); 7.65 (d, J = 1.5 Hz, 1 H).

Ethyl 3-(3-bromo-4,5-dihydroxyphenyl)-3-oxopropionate (760 mg, 1 equiv) was reacted with 7-ACA (680 mg, 1 equiv) in sulfolane (4 mL) and BF_3/Et_2O (1.5 mL) for 2 h at room temperature. Water (4 mL) and AcOH (0.5 mL) were then added, and stirring was continued for an additional 2 h at room temperature. The product was purified by preparative HPLC (Amicon C-18, 15 μm). 7-Amino-3-[2-(ethoxycarbonyl)-3-(3-bromo-4,5-dihydroxyphenyl)-3-oxopropyl]ceph-3-em-4-carboxylic acid (**35**), 196 mg (16%), was obtained as a mixture of diastereoisomers: ^{1}H NMR (DMSO-d_6/TFA-d) δ ppm 1.1 (t, J = 7 Hz, 1 H), 2.8–3.5 (m, 2 H), 3.56 (d, J = 18 Hz, 1 H), 3.7 (d, J = 18 Hz, 1 H), 4.1 (q, J = 7 Hz, 2 H), 4.6–4.9 (m, 1 H), 5.0–5.2 (m, 2 H), 7.4 (d, J = 1.5 Hz, 1 H), 7.67 (d, J = 1.5 Hz, 1 H).

2-Benzothiazolyl 2-(2-aminothiazol-4-yl)-2-[[1-(*tert*-butoxycarbonyl)-1-methylethoxy]imino]thioacetate (167 mg, 1 equiv) was added to **35** (180 mg, 1 equiv) in DMF (5 mL) and Et_3N (48 μL, 1 equiv), and the mixture was stirred at room temperature for 3 h. The solvent was evaporated, the residue was treated with TFA (5 mL) for 1 h, and the product was purified by medium pressure chromatography; **39** (20 mg, 8%) was obtained as a mixture of diastereoisomers: ^{1}H NMR (DMSO-d_6/CD_3COOD/TFA-d) δ ppm 1.05 (t, J = 7 Hz, 3 H), 1.52 (s, 6 H), 2.6–3.4 (m, 2 H), 3.5–3.8 (m, 2 H), 4.0 (q, J = 7 Hz, 2 H), 4.6–4.7 (m, 1 H), 5.0 and 5.1 (2 d, J = 4.5 Hz, 1 H), 5.7 and 5.8 (2 d, J = 4.5 Hz, 1 H), 7.03 and 7.06 (2 s, 1 H), 7.35 and 7.39 (2 d, J = 1.5 Hz, 1 H), 7.6 and 7.65 (2 d, J = 1.5 Hz, 1 H); IR (KBr) 1770 cm^{-1}; MS (+FAB) 769 $(M + H)^+$.

7-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]acetamido]-3-[[(3,4-diacetoxyphenyl)ureido]methyl]ceph-3-em-4-carboxylic Acid (22). A suspension of 3,4-diacetoxybenzoyl chloride[25] (12.8 g, 50 mmol) in toluene (50 mL) was heated to 108 °C to give a clear, colorless solution. Trimethylsilyl azide (7.5 mL, 56 mmol) was added over 5 min, and the reaction was stirred under reflux for 16 h. The mixture was cooled, and solvent was removed under reduced pressure to give 3,4-diacetoxyphenyl isocyanate as a yellow oil, 12.31 g (100%). This oil was used without further purification: ^{1}H NMR ($CDCl_3$) δ ppm 2.16 (s, 6 H), 6.8–6.88 (m, 2 H), 7.02 (d, J = 9 Hz, 1 H); IR 2275 cm^{-1} (N=C=O).

Bis(trimethylsilyl)acetamide (411 mg, 2 mmol) was added to a stirred suspension of 3-(aminomethyl)-7-[2-(2-aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]acetamido]ceph-3-em-4-carboxylic acid (484 mg, 1 mmol) in acetonitrile (10 mL) under argon. The mixture was stirred for 10 min, 3,4-diacetoxyphenyl isocyanate (235 mg, 0.92 mmol) in acetonitrile (2 mL) was added over 2 min, and the mixture was left to stir for 4 h. The solvent was removed under reduced pressure to give a residual yellow foam (812 mg). This was dissolved in aqueous dimethylformamide and subjected to chromatography on Diaion HP20 resin, gradient-eluting with water to 40% acetonitrile to give **22**, 84 mg (11%): ^{1}H NMR (DMSO-d_6/CD_3COOD) δ ppm 1.44 (s, 3 H), 1.46 (s, 3 H), 2.2 (s, 3 H), 2.22 (s, 3 H), 3.5 (d, J = 18 Hz, 1 H), 3.65 (d, J = 18 Hz, 1 H), 3.85 (d, J = 14 Hz, 1 H), 4.18 (d, J = 14 Hz, 1 H), 5.12 (d, J = 5 Hz, 1 H), 5.83 (d, J = 5 Hz, 1 H), 6.73 (s, 1 H), 7.08 (m, 2 H), 7.43 (m, 1 H); MS (+FAB) 720 $(M + H)^+$.

7-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]acetamido]-3-[[[(3-bromo-4,5-dihydroxybenzoyl)glycyl]amino]methyl]ceph-3-em-4-carboxylic Acid (21). *N*-(*tert*-Butyloxycarbonyl)glycine (437 mg, 2.5 mmol) was dissolved in DMSO (4 mL) and the solution treated with *N*-hydroxysuccinimide (316 mg, 2.75 mmol, 1.1 equiv) and DCCI (515 mg, 2.5 mmol). The mixture was stirred for 2 h at room temperature during which time dicyclohexylurea precipitated. This mixture was then added, during 2 min, to a solution of **1a** (1.21 mg, 2.5 mmol) in DMSO (6 mL) and Et_3N (1.04 mL, 7.5 mmol, 3 equiv). The mixture was stirred at room temperature for 3 h before being diluted with H_2O (10 mL). The pH of the solution was adjusted to 3 with glacial acetic acid, the insoluble solid filtered off, and the filtrate subjected to chromatography on reverse-phase C-18 silica (Dynamax column, eluting with H_2O/CH_3CN/TFA 75:25:0.1). The appropriate fractions were collected, and the desired product 7-[2-(2-aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]acetamido]-3-[[[*N*-(*tert*-butyloxycarbonyl)glycyl]amino]methyl]ceph-3-em-4-carboxylic acid was isolated by freeze-drying, 0.54 g (34%): ^{1}H NMR (DMSO-d_6/HOAC) δ ppm 1.33 (s, 9 H), 1.47 (s, 3 H), 1.49 (s, 3 H), 3.3–3.6 (m, 4 H), 3.94 (d, 14 Hz, 1 H), 4.22 (d, J = 14 Hz, 1 H), 5.1 (d, J = 4 Hz, 1 H), 5.84 (d, J = 4 Hz, 1 H), 6.82 (s, 1 H), 8.0 (t, J = 6 Hz, 1 H), 9.45 (d, J = 9 Hz, 1 H); MS (-FAB) 640 $(M - H)^-$.

7-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]acetamido]-3-[[[*N*-(*tert*-butyloxycarbonyl)glycyl]amino]methyl]ceph-3-em-4-carboxylic acid (108 mg, 0.169 mmol) was dissolved in TFA (1 mL) and stirred at room temperature for 30 min before being evaporated under reduced pressure to give an oil. This oil was then dissolved in DMF (1.5 mL) and Et_3N (200 μL) added to give a solution which was treated at 0 °C with a solution of the 3-bromo-4,5-diacetoxybenzoyl chloride[22] (1 equiv) in DMF. After stirring at 0 °C for 10 min, the ice bath was removed, and the mixture was slowly allowed to attain room temperature. HPLC analysis after 1 h at room temperature confirmed that all cephalosporin had been consumed.

The solvent was removed under reduced pressure, the residue diluted with H_2O (3 mL), and $(NH_4)_2CO_3$ (150 mg) added. The mixture was stirred at room temperature for 1 h before being stored at 5 °C overnight. The mixture was then stirred at room temperature for 4 h, acidified to pH 3 with glacial acetic acid, and then subjected to chromatography on Dynamax C-18 preparative reverse-phase silica column (eluting with H_2O/CH_3CN/TFA, 80:20:0.1). The appropriate fractions were collected, combined, and freeze-dried to give a white solid (31 mg). This solid was subjected to further chromatography on the Dynamax column (eluting with H_2O/CH_3OH/HOAC, 70:30:1). The appropriate fractions were again collected, combined, and freeze-dried to give a white solid, 18 mg (14%): ^{1}H NMR (DMSO-d_6/TFA-d) δ ppm 1.52 (s, 3 H), 1.55 (s, 3 H), 3.4 (d, J = 20 Hz, 1 H), 3.55 (d, J = 20 Hz, 1 H), 3.81 (s, 2 H), 4.01 (dd, J = 17, 9 Hz, 1 H), 4.26 (dd, J = 17, 9 Hz, 1 H), 5.14 (d, J = 5 Hz, 1 H), 5.81 (dd, J = 9, 5 Hz, 1 H), 7.08 (s, 1 H), 7.32 (d, J = 1 Hz, 1 H), 7.58 (d, J = 1 Hz, 1 H), 8.12 (t, J = 3 Hz, 1 H), 9.62 (d, J = 9 Hz, 1 H); MS (-FAB) 756 $(M - H)^-$.

Deprotection of Methoxy Ethers to Free Catechols. 6,7-(Methylenedioxy)-1-ethyl-4-quinolone-3-carboxylic acid,[27] or 5-cyano-6,7-(methylenedioxy)-1-ethyl-4-quinolone-3-carboxylic acid, or 6,7-(methylenedioxy)-4-quinolone-3-carboxylic acid[27] was treated with a large excess of neat BBr_3 at reflux for 24 h. The BBr_3 was evaporated and the residue hydrolyzed with ice and purified by medium pressure chromatography (yields 30–80%).

6,7-Dimethoxyquinoxaline-2-carboxylic acid[28] (3 g, 12.8 mmol) was treated at reflux for 7 h with hydrobromic acid (46%). The reaction mixture was evaporated, and the product was precipitated in water, filtered, washed, and dried to give the deprotected quinoxaline, 2.3 g (88%).

6,7-Diacetoxy-2-naphthoyl Chloride. 2-Carboxy-6,7-diacetoxynaphthalene was obtained by acylation[30] of 2-carboxy-6,7-dihydroxynaphthalene.[29] The acid chloride was obtained in quantitative yield by treatment with neat $SOCl_2$ at reflux for 10 min.

3-Bromo-4,5-diacetoxybenzenesulfonyl Chloride. Barium 3,4-dihydroxybenzenesulfonate[28] (59 g, 0.23 mmol) was solubilized in 500 mL AcOH, and bromine (15 mL) in AcOH (270 mL) was added over 3 h to the solution, which was stirred for 20 h at room temperature. The solvent was evaporated, and the residue was purified by medium pressure chromatography to give 3-bromo-4,5-dihydroxybenzenesulfonic acid, 47 g (76%): ^{1}H NMR (DMSO-d_6/TFA-d) δ ppm 8.14 (d, J = 2 Hz, 1 H), 8.22 (d, J = 2 Hz, 1 H).

3-Bromo-4,5-diacetoxybenzenesulfonic acid was obtained by treatment of the catechol (12 g, 44.6 mmol) with Ac_2O (20 mL) and a catalytic amount of H_2SO_4.[30] After medium pressure chromatography, pure compound was obtained, 14.9 g (95%): ^{1}H NMR (DMSO-d_6) δ ppm 2.31 (s, 6 H), 7.51 (d, J = 2 Hz, 1 H), 7.75 (d, J = 2 Hz, 1 H).

3-Bromo-4,5-diacetoxybenzenesulfonic acid (1.33 g, 3.77 mmol, 1 equiv) in CH_3CN (2 mL) and sulfolane (2 mL) was treated successively with DMA (100 μL), Et_3N (1.05 mL, 7.55 mmol), and PCl_5 (1.4 mL, 15.25 mmol, 4 equiv) at 70 °C for 1 h. The reaction mixture was cooled to 0 °C, hydrolyzed, and extracted with CH_2Cl_2. The pure 3-bromo-4,5-diacetoxybenzenesulfonyl chloride was obtained after chromatography over SiO_2 (eluent: CH_2Cl_2), 800 mg (57%): ^{1}H NMR ($CDCl_3$) δ ppm 2.33 (s, 3 H), 2.39 (s, 3 H), 7.9 (d, J = 2 Hz, 1 H), 8.15 (d, J = 2 Hz, 1 H).

5-Cyano-1-ethyl-6,7-(methylenedioxy)-4-quinolone-3-carboxylic Acid. 5-Amino-1-ethyl-6,7-(methylenedioxy)-4-quinolone-3-carboxylic acid[24] (2.76 g, 1 equiv) was dissolved in AcOH (20 mL) at room temperature and treated with nitrosylsulfuric acid, HO_3SONO (1.9 g, 1.5 equiv). The yellow diazonium salt was filtered off and washed with ether (3.7 g, yield 96%). This salt was added in small portions to a solution of $NaCu(CN)_2$ (prepared from NaCN (1.75 g, 1 equiv) and CuCN (1.6 g, 0.5 equiv) in 10 mL water). The mixture was stirred for 2 h at room temperature, the precipitate was filtered and treated with 2 N HCl, and the residue filtered and washed with DMF to obtain the title compound, 1.2 g (45%): ^{1}H NMR (DMSO-d_6/AcOH-d) δ ppm 1.25–1.5 (m, 3 H), 4.4–4.7 (m, 2 H), 6.5 (s, 1 H), 7.85 (s, 1 H), 8.95 (s, 1 H).

3-(Carbethoxy)-6,7-dihydroxyquinoxaline-2-carboxylic Acid. 1,2-Diamino-4,5-dimethoxybenzene[31] (2.7 g, 16 mmol) was treated with tartaric acid (1.8 g, 8 mmol) for 10 min at 60 °C in water. HCl was bubbled into a cooled mixture for 30 min. The precipitate was collected, washed with water, and dried, 550 mg (13%): ^{1}H NMR (DMSO-d_6/TFA-d) δ ppm 4.01 (s, 6 H), 7.52 (s, 2 H).

The deprotection of 2,3-dicarboxy-6,7-dimethoxyquinoxaline (1.2 g, 4.3 mmol) was carried out using neat BBr_3 (25 mL) as in the case of the quinolone carboxylic acids. Purification by medium pressure chromatography gave pure 2,3-dicarboxy-6,7-dihydroxyquinoxaline, 870 mg (80%): ^{1}H NMR (DMSO-d_6/TFA-d) δ ppm 7.35 (s).

2,3-Dicarboxy-6,7-dihydroxyquinoxaline (1.1 g, 4.4 mmol) was refluxed in Ac_2O (30 mL) for 30 min. The solvent was evaporated and the residue heated in anhydrous EtOH at reflux for 10 min to give 6,7-diacetoxy-3-(carbethoxy)quinoxaline-2-carboxylic acid, 1.59 g (100%): ^{1}H NMR (DMSO-d_6/TFA-d) δ ppm 1.35 (t, J = 7 Hz, 3 H), 2.36 (s, 6 H), 4.4 (q, J = 7 Hz, 2 H), 8.16 (s, 2 H).

6,7-Diacetoxy-3-(carbethoxy)quinoxaline-2-carboxylic acid (1.59 g, 4.2 mmol) was suspended in water (50 mL), the pH adjusted to 8–8.5 with diluted NaOH, the mixture stirred at room temperature for 3 h, and the pH adjusted to 2 with diluted HCl. The water was evaporated and the residue extracted with MeOH to give 6,7-dihydroxy-3-(carbethoxy)quinoxaline-2-carboxylic acid, 1.22 g (100%): ^{1}H NMR (DMSO-d_6/TFA-d) δ ppm 1.32 (t, J = 7 Hz, 3 H), 4.35 (q, J = 7 Hz, 3 H), 7.38 (s, 1 H), 7.4 (s, 1 H).

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Registry No. **1a**, 122234-43-9; **1b**, 141613-21-0; **1c**, 141613-22-1; **1d**, 141613-23-2; **1e**, 96629-31-1; **2a**, 124299-99-6; **2b**, 141613-24-3; **2c**, 141613-25-4; **2d**, 141613-26-5; **2e**, 141613-27-6; **3a**, 115309-14-3; **4**, 119786-58-2; **5**, 119734-30-4; **6**, 119734-61-1; **7**, 119761-15-8; **8**, 141634-75-5; **9**, 119734-66-6; **10**, 119734-53-1; **11**, 141613-28-7; **12**, 141613-29-8; **13**, 119734-78-0; **14**, 122233-83-4; **15**, 122233-85-6; **16**, 119733-53-8; **17**, 122234-11-1; **18**, 122233-81-2; **19**, 122256-29-5; **20**, 141555-14-8; **21**, 141613-30-1; **22**, 141613-31-2; **23**, 122256-27-3; **24**, 119734-33-7; **25**, 119734-32-6; **26**, 119786-56-0; **27**, 119733-90-3; **28**, 141613-32-3; **28** (R_1 = 3,4-diacetoxyphenyl), 119735-54-5; **29**, 141613-33-4; **29** (R_1 = 3,4-diacetoxyphenyl), 118679-75-7; **29**·HCl, 141613-34-5; **30**, 141555-09-1; **31**, 118680-12-9; **32**, 119733-54-9; **33**, 103544-88-3; **34**, 113886-58-1; **35** (isomer 1), 141613-35-6; **35** (isomer 2), 141613-36-7; **36**, 141613-37-8; **37**, 141613-38-9; **38**, 141613-39-0; **39** (isomer 1), 141613-40-3; **39** (isomer 2), 141613-41-4; 7-ACA, 957-68-6; *o*-HOC_6H_4OH, 120-80-9; H_3CCOCH^-COOBu-*t*-Na^+, 64770-14-5; β-lactamase, 9073-60-3; 6,7-dihydroxy-1-ethyl-4-oxoquinoline-3-carboxylic acid, 18465-39-9; 5-cyano-6,7-dihydroxy-1-ethyl-4-oxoquinoline-3-carboxylic acid, 122234-64-4; 6,7-dihydroxy-4-oxo-1*H*-quinoline-2-carboxylic acid, 122234-44-0; 6,7-dihydroxy-4-oxo-1*H*-quinoline-3-carboxylic acid, 70393-87-2; 6,7-dihydroxyquinoxaline-2-carboxylic acid, 122234-89-3; 6,7-dihydroxy-3-(carbethoxy)quinovaline-2-carboxylic acid, 141613-42-5; diphenylmethyl 7-(2-thienylacetamido)-3-(hydroxymethyl)ceph-3-em-4-carboxylate, 29126-13-4; 3-bromo-4,5-diacetoxybenzoyl chloride, 122306-86-9; 2-benzothiazolyl 2-(2-aminothiazol-4-yl)-2-[[1-(*tert*-butoxycarbomyl)-1-methylethoxy]imino]thioacetate, 89604-92-2; diphenylmethyl 7-[2-(2-aminothiazol-4-yl)-2-[[(*Z*)-1-(*tert*-butoxycarbonyl)-1-methylethoxy]imino]acetamido]-3-[[(3-bromo-4,5-diacetoxybenzoyl)oxy]methyl]ceph-3-em-4-carboxylate, 141613-43-6; diphenylmethyl 7-[2-(2-aminothiazol-4-yl)-2-[(*Z*)-[1-(*tert*-butoxycarbonyl)-1-methylethoxy]imino]acetamido]-3-[[(3,4-diacetoxybenzoyl)oxy]methyl]ceph-3-em-4-carboxylate, 141613-44-7; *tert*-butyl 2-(3-chloro-4,5-dihydroxybenzoyl)acetate, 141613-45-8; 3-bromo-4,5-dimethoxybenzaldehyde, 6948-30-7; 1-(3-bromo-4,5-dimethoxyphenyl)-1-ethanol, 5293-21-0; 3-bromo-4,5-dimethoxyacetophenone, 141613-46-9; ethyl 3-(3-bromo-4,5-dimethoxyphenyl)-3-oxopropionate, 141613-47-0; 3-(3-bromo-4,5-dihydroxyphenyl)-3-oxopropionate, 141613-48-1; 3,4-diacetoxyphenyl isocyanate, 118708-55-7; 7-[2-(2-aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]acetamido]-3-[[[*N*-(*tert*-butyloxycarbonyl)glycyl]amino]methyl]ceph-3-em-4-carboxylic acid, 141613-49-2; 3,4-diacetoxybenzoyl chloride, 57929-25-6; *N*-(*tert*-butyloxycarbonyl)glycine, 4530-20-5; 6,7-(methylenedioxy)-1-ethyl-4-quinolone-3-carboxylic acid, 14698-29-4; 5-cyano-6,7-(methylenedioxy)-1-ethyl-4-quinolone-3-carboxylic acid, 141613-50-5; 6,7-(methylenedioxy)-4-quinolone-3-carboxylic acid, 19746-58-8; 6,7-dimethoxyquinoxaline-2-carboxylic acid, 33311-24-9; 6,7-diacetoxy-2-naphthoyl chloride, 112057-13-3; 2-carboxy-6,7-dihydroxynaphthalene, 113458-95-0; barium 3,4-dihydroxybenzenesulfonate, 141613-51-6; 3-bromo-4,5-dihydroxybenzenesulfonic acid, 119735-59-0; 3-bromo-4,5-diacetoxybenzenesulfonic acid, 141613-52-7; 3-bromo-4,5-diacetoxybenzenesulfonic chloride, 119735-46-5; 5-amino-1-ethyl-6,7-(methylenedioxy)-4-quinolone-3-carboxylic acid, 51624-54-5; 5-diazonium-1-ethyl-6,7-(methylenedioxy)-4-quinolone-3-carboxylic acid sulfate, 80104-52-5; 1,2-diamino-4,5-dimethoxybenzene, 27841-33-4; tartaric acid, 87-69-4; 2,3-dicarboxy-6,7-dimethoxyquinoxaline, 1770-39-4; 2,3-dicarboxy-6,7-dihydroxyquinoxaline, 122234-55-3; 6,7-diacetoxy-3-(carbethoxy)quinoxaline-2-carboxylic acid, 141613-53-8; 2-[2-(tritylamino)thiazol-4-yl]-2-[(1-(*tert*-butoxycarbonyl)-1-methylethoxy)imino]acetyl chloride, 91622-14-9; 3-chloro-4,5-dihydroxybenzoic acid, 87932-49-8.

Supplementary Material Available: A table containing ^{1}H-NMR data for C-3′-carboxamido and -sulphonamido cephalosporins (compounds **4**, **9**, **10–20**, **23–27**) (1 page). Ordering information is given on any current masthead page.