Pharmacokinetics of Catechol Cephalosporins. The Effect of Incorporating Substituents into the Catechol Moiety on Pharmacokinetics in a Marmoset Model[1]

T. G. C. Bird,* J. C. Arnould, A. Bertrandie, and F. H. Jung

ICI Pharma, Centre de Recherches, Z.I. La Pompelle-B.P. 401, Chemin de Vrilly, 51064 Reims Cedex, France.
Received September 16, 1991

Two series of cephalosporins A and B have been synthesized, bearing at C-3′ catechols substituted with various electron withdrawing groups (Y) and differing links (X), and were evaluated for their in vitro antibacterial activity and their pharmacokinetics in marmosets. Compounds in series A, bearing an isobutyric oxime substituent, proved to be highly active against Gram-negative organisms and were especially noteworthy for showing long elimination phase (β) half-lives in marmosets. It was established that introduction of electron withdrawing substituents greatly increased the β half-lives of compounds (5, X = NHCO, Y = H, $t_{1/2}$ = 1.25 h, AUC = 27 mg/h per L; 11, X = NHCO, Y = 5-Cl, $t_{1/2}$ = 4.5 h, AUC = 638 mg/h per L) and that the nature of the link also influenced $t_{1/2}$, the highest values being obtained when X = NHCO and OCO. Acidities (pK_a values) of the substituted catechols were measured, and relationships between the acidities and half-lives were evaluated. Thus it was established that the more acidic catechols gave the longest half-lives (12, X = NHCO, Y = 2,5-Cl_2, $t_{1/2}$ = 8.2 h, AUC = 461 mg/h per L). Further elaboration of the catechol to bicyclic systems maintained good pharmacokinetics when the pK_a was sufficiently acidic.

Introduction

Cephalosporins, widely used antibacterial drugs for the treatment of infections, have long been studied and modified chemically because of the continuing need to produce increasingly targeted and effective therapy.[2] This is mostly due to evolving resistance of a wide range of bacteria conferred by new β-lactamases[3] or other mechanisms. Equally important has been the necessity to find agents effective against specific organisms such as *Pseudomonas aeruginosa* which have proved elusive to treatment by antibacterial drugs in the past.[4]

Consequently, many papers have been published concerning in vitro structure–activity relationship (SAR) studies in this field[5] and more recently several have described the exciting advances achieved with catechol β-lactams, in particular penicillins,[6] monobactams,[7] and cephalosporins.[8] These compounds display exceptional in vitro activity and β-lactamase stability notably against *Enterobacteriaceae* and *P. aeruginosa* (see Table I and accompanying publication[9]). Typical MIC values are in the range of 0.06–8 μg/mL against *P. aeruginosa* (for example see ref 10), and it has been recently shown that these outstandingly low MIC's are due to subversion of the *tonB*-dependent iron transport process.[11]

However, relatively few reports have been published in the literature dealing with detailed pharmacokinetic structure–activity relationships of cephalosporins.[12] Equally, even less is disclosed on the pharmacokinetics of catechol cephalosporins; there is for example the work of Mochida[13] and Glaxo,[14] and some data have been published

(1) A preliminary account of this work has been presented: Arnould, J. C.; Bertrandie, A.; Bird, T. G. C.; Jung, F. H.; Lohmann, J. J. Pharmacokinetics of Substituted Catechol Cephalosporins in Marmosets. 29th Interscience Conference on Antimicrobial Agents and Chemotherapy, Houston, TX, 1989; Abstract 473.

(2) For a concise summary, see: Newall, C. E.; Hallam, P. D. β-Lactam Antibiotics: Penicillins and Cephalosporins. In *Comprehensive Medicinal Chemistry*; Hansch, C., Ed.; Pergamon Press: Oxford, 1990; Vol. 2, Chapt. 9.2, pp 609–654.

(3) Livermore, D. M. Mechanisms of Resistance to Cephalosporin Antibiotics. *Drugs* 1987, *34* (Suppl. 2), The Cephalosporin Antibiotics, seminar-in-print, pp 64–88.

(4) van Klingeren, B. An in Vitro Comparison of New Cephalosporins with Special Reference to *Pseudomonas aeruginosa*. *J. Antimicrob. Chemother.* 1981, *8* (Suppl. B), 97–105.

(5) An evaluation of the literature can be found in, for example: Sassiver, M. L.; Lewis, A. Structure-Activity Relationships among Semisynthetic Cephalosporins. In *Structure-Activity Relationships among the Semisynthetic Antibiotics*; Perlman, D., Ed.; Academic Press: New York, 1977; pp 87–233.

(6) (a) Ohi, N.; Aoki, B.; Shinozaki, T.; Moro, K.; Noto, T.; Nehashi, T.; Okazaki, H.; Matzunaga, I. Semisynthetic β-Lactam Antibiotics. I. Synthesis and Antibacterial Activity of New Ureidopenicillin Derivatives having Catechol Moieties. *J. Antibiot.* 1986, *39*, 230–241. (b) Basker, M. J.; Frydrych, C. H.; Harrington, F. P. Antibacterial Activity of Catecholic Piperacillin Analogues. *J. Antibiot.* 1989, *42*, 1328–1330 and references cited therein.

(7) Brewer, H.; Bissachi, B. S.; Drossard, J. M.; Ermann, P.; Koster, W. H.; Kronenthal, P.; Kuester, P.; Linder, K. R.; Straub, H.; Treuner, U. D.; Zahler, R. Structure-Activity Relationships among Sulfonylaminocarbonyl Activated Monobactams leading to SQ 83,360. 25th Interscience Conference on Antibacterial Agents and Chemotherapy, Minneapolis, MN, 1985; Abstract 371.

(8) (a) Katzu, K.; Kitoh, K.; Inoue, M.; Mitsuhashi, S. In Vitro Antibacterial Activity of E-0702, a New Semisynthetic Cephalosporin. *Antimicrob. Agents Chemother.* 1982, *22*, 181–185. (b) Branch, C. L.; Basker, M. J.; Finch, S. C.; Guest, A. W.; Harrington, F. P.; Kaura, A. C.; Knott, S. J.; Milner, P. H.; Pearson, M. J. Studies on Semi-Synthetic 7α-Formamidocephalosporins. *J. Antibiot.* 1987, *40*, 646–651. (c) Mochizuki, H.; Yamada, H., Oikawa, Y.; Murakami, K.; Ishiguro, J.; Kosozume, H.; Aizawa, N.; Mochida, E. Bactericidal Activity of M14659 Enhanced in Low-Iron Environments. *Antimicrob. Agents Chemother.* 1988, *32*, 1648–1654.

(9) Detailed in vitro data of the classes of compounds discussed here are given in the accompanying publication: Synthesis and Structure–Activity Relationships of Cephalosporins with C-3′ Catechol containing residues, Jung, F. H. et al.

(10) Weissberger, B. A.; Abruzzo, G. K.; Fromtling, R. A.; Gill, C.; Ponticas, S.; Valliant, M. E.; Shungu, D. L.; Gadebusch, H. H. L-658310 a New Injectable Cephalosporin. I. In Vitro Antibacterial Properties. *J. Antibiot.* 1989, *42*, 795–806.

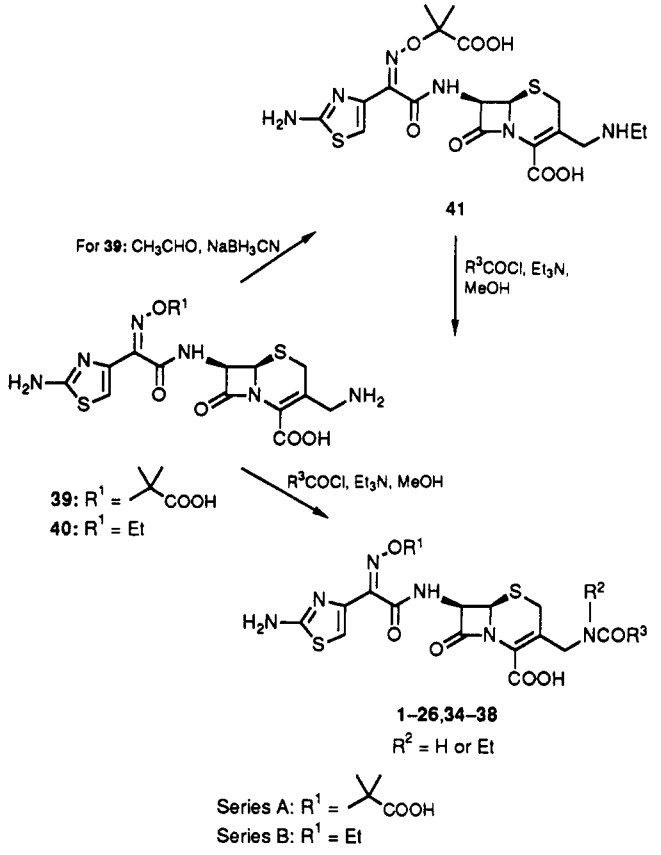
(11) (a) Curtis, N. A. C.; Eisenstadt, R. L.; East, S. J.; Cornford, R. J.; Walker, L. A.; White, A. J. Iron-Regulated Outer Membrane Proteins of *Escherichia coli* K-12 and Mechanism of Action of Catechol-Substituted Cephalosporins. *Antimicrob. Agents Chemother.* 1988, *32*, 1879–1886. (b) Watanabe, N.-A.; Nagasu, T.; Katsu, K.; Kitoh, K. E-0702 a New Cephalosporin is Incorporated into *Escherichia coli* Cells via the *tonB*-Dependent Iron Transport System. *Antimicrob. Agents Chemother.* 1987, *31*, 497–504.

(12) For reviews, see: Harding, S. M. Pharmacokinetics of the Third Generation Cephalosporins. *Am. J. Med.* 1985, *79*, 21–24. Noble, J. T.; Barza, M. Pharmacokinetic Properties of the Newer Cephalosporins. A Valid Basis for Drug Selection? *Drugs* 1985, *30*, 175–181.

(13) Mochizuki, H.; Oikawa, Y.; Yamada, H.; Kusakabe, S.; Shiihara, T.; Murakami, K.; Kato, K.; Ishiguro, J.; Kosuzume, H. Antibacterial and Pharmacokinetic Properties of M14659 a New Injectable Semisynthetic Cephalosporin. *J. Antibiot.* 1988, *41*, 377–391.

0022-2623/92/1835-2643$03.00/0

Scheme I



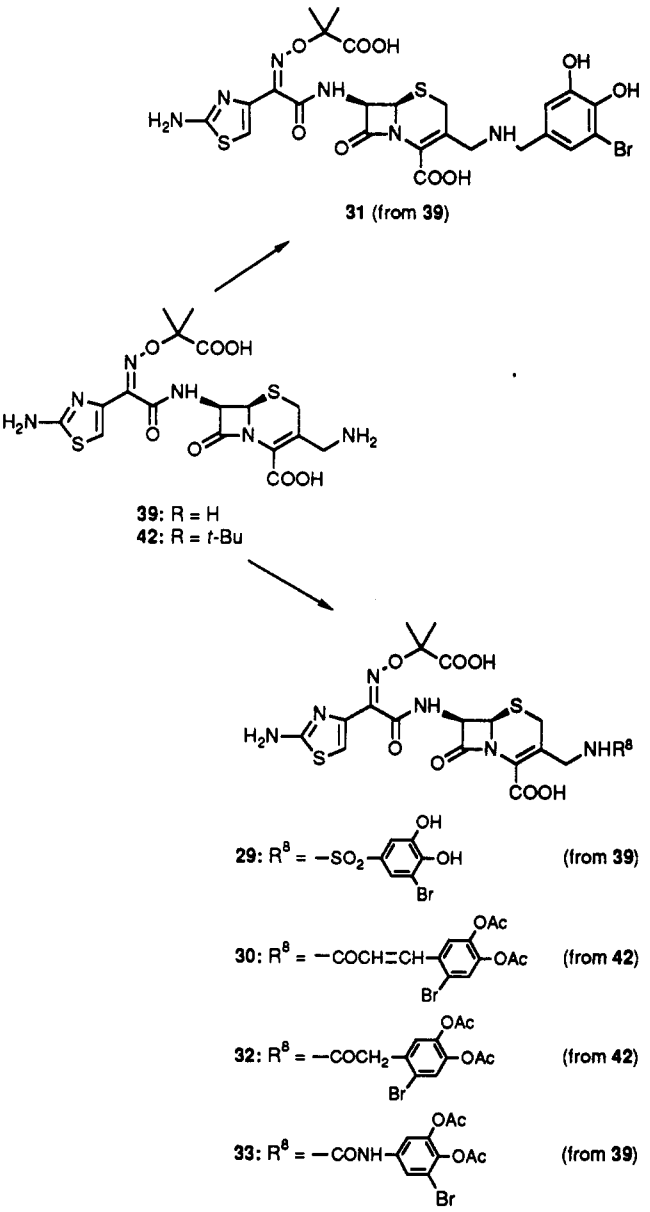
on catechol-type monobactams.[15]

Previous to the work described in this paper, studies in this laboratory seeking antibacterial agents with high activity against Gram-negative organisms, in particular *P. aeruginosa*, and with retained activity against organisms that are stably derepressed with regard to Type I β-lactamase production (e.g. *Enterobacter cloacae* P99+) led to the syntheses of cephalosporins of novel structural types A and B (Y = H) attached by various links (X) to an unsubstituted catechol.[9]

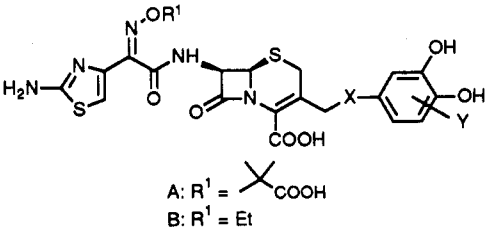
It was found that different links (X) were compatible with maintaining high activity, which was slightly modified when the oxime substituent was changed from an acid (ceftazidime type A) to a neutral group (B). Compounds **5** and **15** in Table I show the activity of two typical nonsubstituted catechol cephalosporins. Full details of in vitro structure–activity relationships are given in the accompanying publication.[9]

We decided that it was important to determine the effect of introducing substituents (Y) into the catechol ring since by varying electronic and π values of the substituents it would be possible to control the acidity (pK_a) of the catechol OH's and also vary the lipophilicity of the molecules

Scheme II



overall. These changes in physical characteristics were consequently expected to influence the spectrum of activity. This was in fact noted, and is shown in Table I where it is seen that the bromo catechol **1** and the bicyclic compound **4** are more active than the nonsubstituted analogue—but most surprising were the profound effects on pharmacokinetic parameters that were observed. A previous report on in vivo effects of substituting catechols in the penicillin series has been made,[16] but no pharmacokinetic data were given.

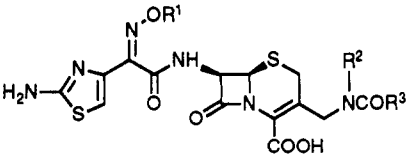


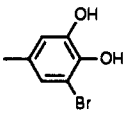
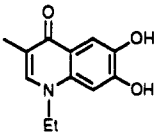
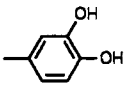
(14) Mackay, J. A.; Harding, S. M.; Palmer, J. L.; Evans, G. L. Volunteer Studies with GR 69153 a Novel Cephalosporin. 29th Interscience Conference on Antimicrobial Agents and Chemotherapy, Houston, TX, 1989; Abstract 351.

(15) (a) Clark, J. M.; Whitney, R. R.; Olsen, S. J.; Weinberg, D. S.; Dalvi, M.; Bonner, D. P.; Sykes, R. B. SQ 83360 Animal Studies with a Novel Monobactam. 25th International Conference on Antimicrobial Agents and Chemotherapy, Minneapolis, MN, 1985; Abstract 373. (b) Treuner, U. D.; Errmann, S.; Jendrzejewski, S.; Straub, H. Monobactams Having Potent Antipseudomonal Activity. 29th International Conference on Antimicrobial Agents and Chemotherapy, Houston, TX, 1989; Abstract 236.

(16) Ohi, N.; Aoki, B.; Kuroki, T.; Matsumoto, M.; Kojima, K.; Nehashi, T. Semisynthetic β-Lactam Antibiotics. *J. Antibiot.* **1987**, *40*, 22–28.

Table I. In Vitro Activities of Representative Catechol Cephalosporins[a]



compd	R^1	R^2	R^3	*Pseudomonas aeruginosa* 18SH[b]	*Pseudomonas aeruginosa* PU21	*Enterobacter cloacae* P99+[b]	*Enterobacter cloacae* P99−[c]	*Escherichia coli* DC0[d]	*Escherichia coli* DC2[e]	*Staphylococcus aureus* Oxford	*Staphylococcus aureus* 147N[f]
1		H		0.015	≤0.008	2	0.03	≤0.008	≤0.008	16	16
2	Et	H	*g*	4	0.06	8	0.015	≤0.008	≤0.008	2	4
3		Et	*g*	0.06	0.03	1	0.25	0.015	0.015	32	32
4	*g*	H		≤0.008	0.06	0.25	0.06	≤0.008	0.008	16	16
5	*g*	H		0.06	0.015	16	0.125	≤0.008	0.008	8	16
15	Et	H	*g*	64	0.25	64	0.5	0.06	0.008	2	4
ceftazidime				16	1	32	0.125	0.125	0.06	4	8

[a] Agar dilution method using an inoculum of 10^4 colony forming units in IST growth medium, data are MIC values in μg/mL. [b] Constitutive derepressed Type I β-lactamase producer. [c] Inducible Type I β-lactamase producer. [d] Parent organism. [e] Permeability mutant. [f] Penicillin-sensitive *S. aureus*. [g] Same as above.

In this paper we describe the synthesis of a new class of cephalosporins bearing at C-3′ substituted catechol moieties and report that in addition to highly interesting in vitro properties these compounds can show very unusual pharmacokinetics in marmosets and that these pharmacokinetics are partially governed by the catechol pK_a.

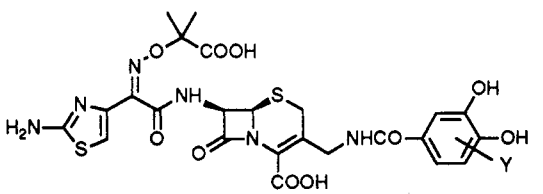
Chemistry

Two series of cephalosporins have been synthesized, one with isobutyric acid oximes (ceftazidime types, structure A) the other with neutral oximes (B). In each of these two broad series, different substituted catechols were attached via a primary amide link (Tables II and III); in addition in the isobutyric acid series A the nitrogen of the amide was further substituted (Table IV), widely differing links were made (Table V), and bicyclic catechol systems were prepared (Table VI).

Crucial for the syntheses of all the amide linked compounds were the C-3′-aminocephalosporins **39** and **40** and the *N*-ethyl analogue **41** which were synthesized from the appropriate (azidomethyl)cephalosporins[17] (Scheme I). These amines were reacted, without further protection, with the acid chlorides of the appropriate substituted diacetyl catechols in MeOH–triethylamine, and the amides thus formed were deprotected in situ by raising the pH to 8.0–8.5 with aqueous ammonia to give the final compounds, **1–26** and **34–38**.

The amine **39** or its *tert*-butyl ester analogue **42** was used in the preparation of compounds **29–33** as shown in Scheme II. Compound **28** was prepared from the known (chloromethyl)cephalosporin **43**[18] by a Wittig reaction (Scheme III).

Table II. Effect of pK_a on Half-Life in the Amidic Substituted Catechol Series



compd	Y	pK_a	$t_{1/2}$ (h)	AUC (mg/h per L)
5	H	8.5	1.25 ± 0.1	27.2 ± 2.5
6	5-OH	8.35	0.9 ± 0.1	51.9 ± 4.8
7	6-Br	8.1	0.9 ± 0.2	13.4 ± 3.3
8	6-F	7.8	1.6 ± 0.0	78.0 ± 7.5
9	2-Cl	7.65	2.9 ± 0.4	131.0 ± 29.3
10	5-F	7.45	3.5 ± 0.6	116.8 ± 18.9
11	5-Cl	7.0	4.6 ± 0.5[a]	638.3 ± 74.6[a]
1	5-Br	6.9	2.9 ± 0.75[b]	137.1 ± 18.9[b]
12	2,5-Cl_2	6.3	8.2 ± 1.3[a]	461.0 ± 61.7[a]
13	5-CN	5.5	5.3 ± 1.4[a]	394.5 ± 58.1[a]
14	5-NO_2	5.5	2.9 ± 0.8	181.7 ± 45.6
ceftazidime[c]			0.7	14

[a] Mean values from two tests, quoting the greatest standard errors observed. [b] Mean values from four tests, quoting the greatest standard error observed. [c] Used as comparator, mean values from four tests.

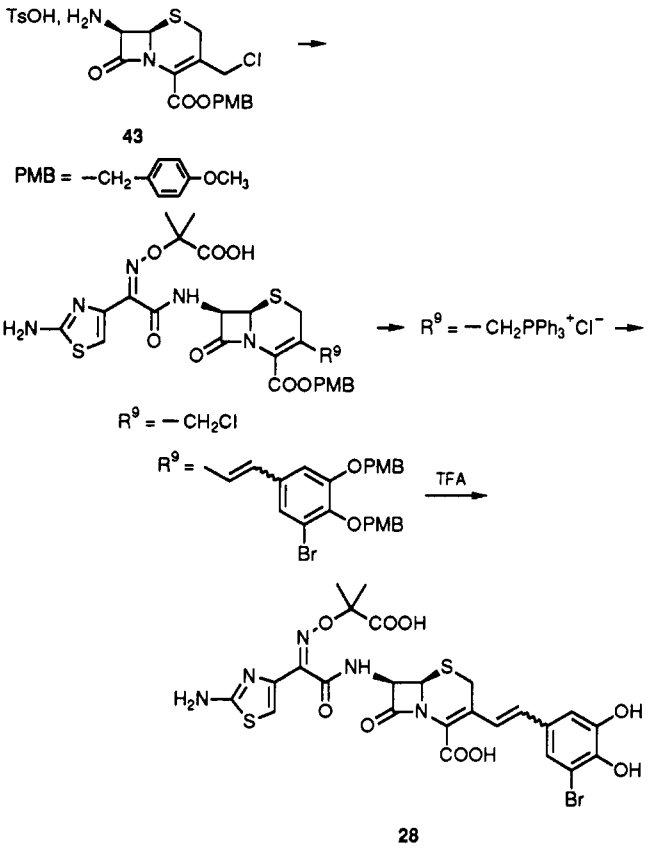
Pharmacokinetic Evaluation

Pharmacokinetic properties in marmosets (serum concentration, elimination half-life, and urinary excretion) and human serum protein binding were studied for these compounds. The marmosets were dosed iv (three animals per study) at 3 mg/kg, and serum concentrations were

(17) (a) Murphy, C. F.; Webber, J. A. Alteration of the Dihydrothiazine Ring Moiety. In *Cephalosporins and Penicillins, Chemistry and Biology*; Flynn, E. H., Ed.; Academic Press: New York, 1972; p 162. (b) Imperial Chemical Industries PLC European Patent 127992A2, 1984; *Chem. Abstr.* **1985**, *102*, 220651c. (c) Fujisawa G.B. Patent 2103205A, 1982; *Chem. Abstr.* **1983**, *98*, 197894x.

(18) Obtained from Otsuka Pharmaceuticals.

Scheme III



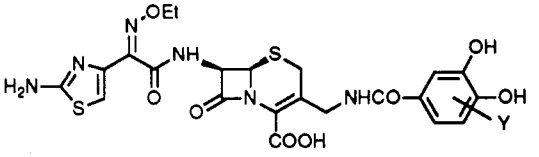
measured by bioassay over 3 h; this permitted the area under the serum concentration curve (AUC) and elimination half-lives for the β-phase to be calculated. Pharmacokinetic parameters for the different series are given in Tables II–VI.

Half-Life. Introduction of substituents into the catechol moiety was investigated initially in a series of cephalosporins with isobutyric acid oximes at C-7 and an unsubstituted amide link at C-3′ (A, X = NHCO); the first compound to be tested was the 5-bromo derivative **1**, and we were surprised and delighted to discover the significant difference in its pharmacokinetics (**1**, $t_{1/2}$, 2.8 h; AUC, 137 mg/h per L) compared with the unsubstituted analogue (**5**, $t_{1/2}$, 1.2 h; AUC, 27 mg/h per L). This result provoked us to undertake a thorough investigation of catechol substitution which has led to the discovery of the role of the pK_a of the catechol moiety.

Table II shows that, in general, electron withdrawing groups (halogens, nitrile, or nitro) have a profound effect on increasing half-lives and AUC values when introduced in the 2- and 5-positions and a less pronounced effect for the 6-position, and that the effect is compounded in the 2,5-dichloro compound **12**. To determine whether it is the acidity of the phenolic catechol groups which influences half-life, the pK_a values of the compounds were measured and are also shown in Table II. Acidity did appear to account for higher $t_{1/2}$'s, but the very high value for the dichloro derivative **12** (8.2 h) suggested that other factors such as steric interference could also be involved in modifying the metabolism/elimination process. It is an established phenomenon that certain cephalosporins which are substituted at C-3′ with acid-bearing groups do have unusually long half-lives. Particular examples are ceftriaxone[19] (Roche), YM-13115,[20] and cefodizime[21] (Hoechst).

When the oxime substituent was changed from an isobutyric acid to a neutral ethyl group, as shown in Table III, the trend to longer half-lives with lower pK_a values was inapparent since this structural change greatly reduced all $t_{1/2}$ and AUC values. A similar effect is seen in Table IV where the isobutyric acid oxime was retained but where the amide linking group was substituted on the nitrogen by ethyl. Here no compound showed a half-life longer than 1.6 h, the most interesting compound again being the most acidic 5-nitro **26**. This amide substitution was tested since there are other advantages to be gained in so doing; in particular, stability to β-lactamases can be improved.[9]

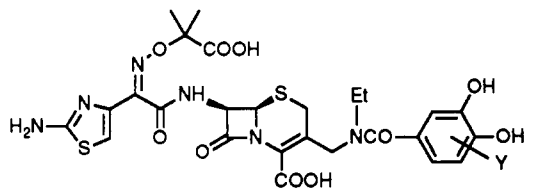
Table III. Effect on Half-Life of Replacing the Isobutyric Acid Oxime Substituent by Ethyl



compd	Y	pK_a	$t_{1/2}$ (h)	AUC (mg/h per L)
15	H	8.5	0.8 ± 0.1	4.3 ± 0.05
16	6-Br	6.9	0.35 ± 0.1	2.0 ± 0.4
17	5-F	7.4	0.65 ± 0.1	25.0 ± 2.65
18	5-Cl	6.9	1.3 ± 0.1	57.6 ± 4.2
2	5-Br	6.9	0.7 ± 0.1	17.0 ± 0.5
19	2,5-Cl_2	6.9	1.4 ± 0.1	54.5 ± 0.9
20	5-CN	5.5	1.2 ± 0.2	107.0 ± 7.3
21	5-NO_2	5.5	0.8 ± 0.1	48.9 ± 1.7
cefotaxime[a]			0.5	46.7

[a] See footnote *c* in Table II.

Table IV. Effect of Amide Link *N*-Ethyl Substitution on Half-Life



compd	Y	pK_a	$t_{1/2}$ (h)	AUC (mg/h per L)
22	6-F	8.25	0.6 ± 0.05	7.6 ■ 0.4
23	5-F	7.85	0.5 ■ 0.0	10.9 ± 1.6
24	5-Cl	7.4	0.9 ± 0.2	16.4 ± 4.1
3	5-Br	7.3	1.0 ± 0.15	21.8 ± 4.75
25	5-CN	5.8	1.0 ± 0.1	41.0 ± 3.2
26	5-NO_2	5.8	1.6 ± 0.4	49.2 ± 17.6

It was important to determine whether the primary amide link is the optimal choice, and so a number of compounds with differing links were prepared and are shown in Table V. The only alternative with similar pharmacokinetics was the urea (**33**) although ester (**27**) and vinyl (**28**) replacements showed quite similar characteristics without being better than the amide.

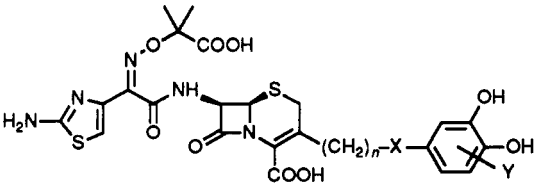
Finally, a more widespread investigation of catechol structural variation was carried out by preparing a number of bicyclic catechol systems of highly different structural

(19) 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.

(20) Matsui, H.; Komiya, M.; Ikeda, C.; Tachibana, A. Comparative Pharmacokinetics of YM-13115, Ceftriaxone and Ceftazidime in Rats, Dogs and Rhesus Monkeys. *Antimicrob. Agents Chemother.* **1984**, *26*, 204–207.

(21) 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.

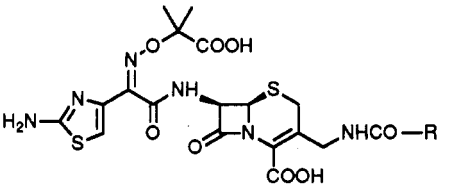
Table V. Influence of Varying the Nature of the Link X on Half-Life

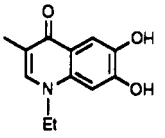
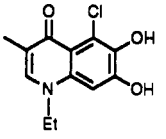
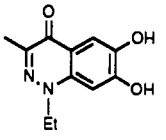
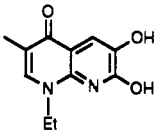
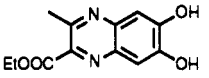
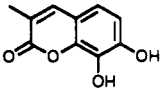


compd	X	*n*	Y	pK_a	$t_{1/2}$ (h)	AUC (mg/h per L)
1	-NHCO-	1	5-Br	6.9	2.9 ± 0.75	137.1 ± 18.9
27	-OCO-	1	5-Br	6.5	1.7 ± 0.1	67.7 ± 4.1
28	-CH=CH-	0	5-Br	7.7	1.5 ± 0.1	61.6 ± 1.9
3	-NEtCO-	1	5-Br	7.3	1.0 ± 0.15	21.8 ± 4.75
29	$-NHSO_2-$	1	5-Br	6.4	1.4 ± 0.2	98.5 ● 14.3
30[a]	-NHCOCH=CH-	1	6-Br		1.0 ± 0.2	24.2 ± 5.8
31	$-NHCH_2-$	1	5-Br		0.7 ± 0.2	4.4 ± 0.7
32[a]	$-NHCOCH_2-$	1	6-Br		0.7 ± 0.05	1.6 ± 0.2
33[a]	-NHCONH-	1	5-Br		2.8 ± 0.2	122.3 ± 6.8

[a] These compounds were tested as the diacetoxy derivatives of the catechol.

Table VI. Replacement of the Substituted Catechol by Bicyclic Catechol Systems



compd	R	pK_a	$t_{1/2}$ (h)	AUC (mg/h per L)
4		7.2	3.5 ± 1.0	238.5 ± 90.5
34		7.0	5.7 ± 2.9	645.7 ± 251.4
35		6.5	2.2 ● 0.2	129.8 ± 5.1
36		5.6	2.8 ± 0.4	137.3 ± 14.7
37		6.0	4.6 ± 0.7	287.0 ± 37.9
38		*a*	3.95 ± 1.35	243.9 ± 6.2

[a] Not measured.

types in which the catechols were acidic (pK_a < 7). The simple amide link was used and here, as is seen in Table VI, compounds with long half-lives and high AUC values were detected.

Urinary Excretion and Serum Protein Binding. The urinary recovery of compounds over 27 h dosed at 3 mg/kg per marmoset was generally relatively modest and compounds exhibited high binding to human serum protein (Table VII gives values for representative compounds). There was relatively little difference for either parameter between nonsubstituted (compound **5**) and substituted catechols (**1**, **12**, **13**, and **14**); so although high serum protein binding was consistent with an extended serum half-life it was insufficient in this case to explain the significant difference we observed in half-lives between nonsubstituted and substituted catechol cephalosporins. Biliary excretion in rats was measured (details not given) but was highly variable within the series.

It is anticipated that catechol cephalosporins in general could be metabolized by the enzyme catechol-*O*-methyl transferase (COMT) and that the longer half-life substituted catechols either were poorer substrates[16] or inhibited

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

compd	serum protein binding[a,b]	urinary recovery[c]
1	99.2	13.9
4	99.0	17.5
5	96.3	44.8
12	98.7	16.2
13	96.7	8.7
14	97.5	47.3
35	97.8	26.9
38	99.6	6.9
ceftriaxone	94.5 (92.8 in marmoset blood)	25.4

[a] In human blood (%). [b] Besides ceftriaxone, some other closely related compounds not covered in this paper have been measured in marmoset blood and there was no difference between marmosets and humans. [c] In marmosets dosed at 3 mg/kg, over 27 h (%).

Table VIII. In Vivo Antibacterial Activity[a]

compd	PD_{50} (mg/kg)
1	0.024
11	0.004
12	0.003
13	0.013
4	0.01
ceftazidime[b]	1.0–6.0[c]

[a] Challenge of 10^4 bacteria with Ps. aeruginosa (strain 101 010) IP into groups of four mice. [b] Used as comparator in all tests. [c] Range of values over 20 tests.

its action (ref 22 gives examples of nitrocatechols as inhibitors of COMT). The metabolic fate of compound 4 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 marmosets and rats.

In Vivo Antibacterial Activity. The excellent in vitro activities of these compounds is reflected in their in vivo antibacterial activity demonstrated in a standard mouse protection model. Representative results are shown by examples in Table VIII which are compounds also giving the most interesting pharmacokinetics.

Conclusion

It is remarkable, in an area where predictions are difficult to generate, that the incorporation of a substituted catechol residue at C-3′ of cephalosporins can give rise to compounds with unusually prolonged elimination phase life-lives specifically when the substituents are electron withdrawing and thus generate sufficiently acidic catechols. Hence controlling the pK_a of the catechol can modulate important properties in this series, although other factors must be involved since varying the links between the cephalosporin and the acidic catechols can cause a reduction of this effect. For example, the reason for the detrimental effect of N-substitution remains unclear, and equally it is difficult to explain the difference in half-lives between ethyl- and isobutyric acid oximes in terms of catechol pK_a's.

Experimental Section

Chemical Synthesis. General. Melting points were determined on a Reichert Jung microscope and are uncorrected. ^1H NMR spectra were recorded on a 90-MHz JEOL FX 90Q or a 300-MHz Bruker 300 AC nuclear magnetic resonance spectrometer. IR spectra were taken as liquid films or KBr pellets on a Perkin-Elmer 781 spectrophotometer. Mass spectra were obtained on a VG 7250 SA mass spectrometer. Analytical HPLC chromatography was carried out on a Shimadzu LC 6A apparatus using Nucleosil C18 5-μm columns, and preparative medium pressure chromatography was carried out using Mitsubishi HP 20 SS resin with an eluant of $MeOH/H_2O$ containing 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′-(Aminomethyl)cephalosporins **39** and **40** are known in the cephalosporin literature,[17] and their syntheses together with intermediate **41** are described in the accompanying paper.[9] In addition the syntheses of compounds **2**, **4**, **5**, **15**, **24**, **25**, **27**, **29**, and **37** are given in the same paper.[9]

C-3′-Carboxamido Cephalosporins. General Procedure A Used for Compounds 1, 3, 6–14, 16–23, and 26. Triethylamine (1.0 mmol) was added rapidly to a stirred suspension of the appropriate 7-substituted 3-(aminomethyl)ceph-3-em-4-carboxylic acid (0.5 mmol) **39**, **40**, or **41** in methanol (10 mL) at 0 °C, followed by the appropriately substituted diacetoxybenzoyl chloride (0.5 mmol). After about 2 h the mixture was diluted with an equal volume of water and the pH adjusted to and maintained at 8.5 with aqueous ammonia to hydrolyze the acetoxy groups and to obtain the dihydroxy derivative. The mixture was acidified to pH 3.5 with aqueous 2 N HCl and evaporated to dryness. It was purified on a Diaion HP 20 SS resin column using methanol/water mixtures of increasing proportions of methanol and containing acetic acid (1%). Evaporation and freeze-drying of the appropriate fractions gave the following final products.

(1). Starting materials were **39** and 3-bromo-4,5-diacetoxybenzoyl chloride. The yield of **1** was 45%: ^1H NMR (DMSO-d_6/CD_3COOD/TFA-*d*) 1.5 (s, 6 H, *gem*-dimethyl), 3.5 (m, 2 H, SCH_2), 4.3 (m, 2 H, CH_2NH), 5.15 (d, 1 H, J = 5 Hz, H6), 5.8 (d, 1 H, J = 5 Hz, H7), 7.0 (s, 1 H, thiazole H), 7.3 (d, 1 H, J = 2 Hz), 7.5 (d, 1 H, J = 2 Hz).

(3). Starting materials were **41** and 3-bromo-4,5-diacetoxybenzoyl chloride. The yield of **3** was 22%: ^1H NMR (DMSO-d_6/CD_3COOD/TFA-*d*) 1.05 (t, 3 H, J = 6 Hz, CH_2CH_3), 1.55 (s, 6 H, *gem*-dimethyl), 3.0–3.8 (m, 4 H, CH_2CH_3, SCH_2), 4.45 (s, 2 H, CH_2NH), 5.2 (d, 1 H, J = 5 Hz, H6), 5.8 (d, 1 H, J = 5 Hz, H7), 6.85 (d, 1 H, J = 2 Hz), 7.0 (d, 1 H, J = 2 Hz), 7.1 (s, 1 H, thiazole H).

(6). Starting materials were **39** and 3,4,5-triacetoxybenzoyl chloride. Three equivalents of triethylamine were used. The yield of **6** was 73%: ^1H NMR (DMSO-d_6/CD_3COOD/TFA-*d*) 1.55 (s, 6 H, *gem*-dimethyl), 3.6 (m, 2 H, SCH_2), 4.3 (m, 2 H, CH_2NH), 5.15 (d, 1 H, J = 5 Hz, H6), 5.8 (d, 1 H, J = 5 Hz, H7), 6.85 (s, 2 H), 7.05 (s, 1 H, thiazole H).

(7). Starting materials were **39** and 2-bromo-4,5-diacetoxybenzoyl chloride. The yield of **6** was 45%: ^1H NMR (DMSO-d_6/CD_3COOD/TFA-*d*) 1.55 (s, 6 H, *gem*-dimethyl), 3.6 (m, 2 H, SCH_2), 4.25 (m, 2 H, CH_2NH), 5.15 (d, 1 H, J = 5 Hz, H6), 5.8 (d, 1 H, J = 5 Hz, H7), 6.85 (s, 1 H), 6.95 (s, 1 H), 7.05 (s, 1 H, thiazole H).

(8). Starting materials were **39** and 4,5-diacetoxy-2-fluorobenzoyl chloride. Three equivalents of triethylamine were used. The yield of **8** was 35%: ^1H NMR (DMSO-d_6/CD_3COOD/TFA-*d*) 1.55 (s, 6 H, *gem*-dimethyl), 3.6 (m, 2 H, SCH_2), 4.3 (m, 2 H, CH_2NH), 5.15 (d, 1 H, J = 5 Hz, H6), 5.8 (d, 1 H, J = 5 Hz, H7), 6.6 (d, 1 H, J = 2 Hz), 7.05 (s, 1 H, thiazole H), 7.2 (d, 1 H, J = 2 Hz).

(9). Starting materials were **39** and 2-chloro-3,4-diacetoxybenzoyl chloride. Three equivalents of triethylamine were used. The yield of **9** was 84%: ^1H NMR (DMSO-d_6/CD_3COOD/TFA-*d*) 1.5 (s, 6 H, *gem*-dimethyl), 3.5 (m, 2 H, SCH_2), 4.2 (m, 2 H, CH_2NH), 5.15 (d, 1 H, J = 5 Hz, H6), 5.8 (d, 1 H, J = 5 Hz, H7), 6.75 (s, 2 H), 7.05 (s, 1 H, thiazole H).

(10). Starting materials were **39** and 3,4-diacetoxy-5-fluorobenzoyl chloride. Three equivalents of triethylamine were used. The yield of **10** was 36%: ^1H NMR (DMSO-d_6/CD_3COOD/TFA-*d*) 1.55 (s, 6 H, *gem*-dimethyl), 3.55 (m, 2 H, SCH_2), 4.3 (m, 2 H, CH_2NH), 5.15 (d, 1 H, J = 5 Hz, H6), 5.8 (d, 1 H, J = 5 Hz, H7), 7.05–7.15 (m, 3 H, thiazole H and aryls).

(22) Nissinen, E.; Linden, I.-B.; Schultz, E.; Kaakkola, S.; Mannisto, P. T.; Pohto, P. Inhibition of Catechol-O-Methyl Transferase Activity by Two Novel Disubstituted Catechols in the Rat. *Eur. J. Pharmacol.* **1988**, *153*, 263–269.

(11). Starting materials were **39** and 3-chloro-4,5-diacetoxybenzoyl chloride. Three equivalents of triethylamine were used. The yield of **11** was 64%: ^{1}H NMR (DMSO-d_6/CD_3COOD/TFA-*d*) 1.55 (s, 6 H, *gem*-dimethyl), 3.5 (m, 2 H, SCH_2), 4.3 (m, 2 H, CH_2NH), 5.15 (d, 1 H, J = 5 Hz, H6), 5.8 (d, 1 H, J = 5 Hz, H7), 7.1 (s, 1 H, thiazole H), 7.3 (d, 1 H, J = 2 Hz), 7.4 (d, 1 H, J = 2 Hz).

(12). Starting materials were **39** and 3,4-diacetoxy-2,5-dichlorobenzoyl chloride. Three equivalents of triethylamine were used. The yield of **12** was 65%: ^{1}H NMR (DMSO-d_6/CD_3COOD/TFA-*d*) 1.55 (s, 6 H, *gem*-dimethyl), 3.6 (m, 2 H, SCH_2), 4.3 (m, 2 H, CH_2NH), 5.15 (d, 1 H, J = 5 Hz, H6), 5.8 (d, 1 H, J = 5 Hz, H7), 7.0 (s, 1 H, thiazole H), 7.05 (s, 1 H).

(13). Starting materials were **39** and 3-cyano-4,5-diacetoxybenzoyl chloride. Three equivalents of triethylamine were used. The yield of **13** was 42%: ^{1}H NMR (DMSO-d_6/CD_3COOD/TFA-*d*) 1.55 (s, 6 H, *gem*-dimethyl), 3.5 (m, 2 H, SCH_2), 4.3 (m, 2 H, CH_2NH), 5.15 (d, 1 H, J = 5 Hz, H6), 5.8 (d, 1 H, J = 5 Hz, H7), 7.05 (s, 1 H, thiazole H), 7.55 (m, 2 H).

(14). Starting materials were **39** and 3,4-diacetoxy-5-nitrobenzoyl chloride. Three equivalents of triethylamine were used. The yield of **14** was 73%: ^{1}H NMR (DMSO-d_6/CD_3COOD/TFA-*d*) 1.55 (s, 6 H, *gem*-dimethyl), 3.55 (m, 2 H, SCH_2), 4.35 (m, 2 H, CH_2NH), 5.15 (d, 1 H, J = 5 Hz, H6), 5.8 (d, 1 H, J = 5 Hz, H7), 7.1 (s, 1 H, thiazole H), 7.6 (d, 1 H, J = 2 Hz), 8.0 (d, 1 H, J = 2 Hz).

(16). Starting materials were **40** and 2-bromo-4,5-diacetoxybenzoyl chloride. The yield of **16** was 37%: ^{1}H NMR (DMSO-d_6/CD_3COOD/TFA-*d*) 1.3 (t, 3 H, J = 6 Hz, OCH_2CH_3), 3.6 (m, 2 H, SCH_2), 4.0–4.6 (m, 4 H, OCH_2CH_3, CH_2NH), 5.15 (d, 1 H, J = 5 Hz, H6), 5.75 (d, 1 H, J = 5 Hz, H7), 6.85 (s, 1 H), 6.95 (s, 1 H, thiazole H), 7.0 (s, 1 H).

(17). Starting materials were **40** and 3,4-diacetoxy-5-fluorobenzoyl chloride. Three equivalents of triethylamine were used. The yield of **17** was 28%: ^{1}H NMR (DMSO-d_6/CD_3COOD/TFA-*d*) 1.3 (t, 3 H, J = 6 Hz, OCH_2CH_3), 3.5 (m, 2 H, SCH_2), 4.0–4.4 (m, 4 H, OCH_2CH_3, CH_2NH), 5.15 (d, 1 H, J = 5 Hz, H6), 5.7 (d, 1 H, J = 5 Hz, H7), 7.0–7.2 (m, 3 H).

(18). Starting materials were **40** and 3-chloro-4,5-diacetoxybenzoyl chloride. The yield of **18** was 39%: ^{1}H NMR (DMSO-d_6/CD_3COOD/TFA-*d*) 1.25 (t, 3 H, J = 6 Hz, OCH_2CH_3), 3.25–3.75 (m, 2 H, SCH_2), 4.0–4.6 (m, 4 H, OCH_2CH_3, CH_2NH), 5.15 (d, 1 H, J = 5 Hz, H6), 5.75 (d, 1 H, J = 5 Hz, H7), 7.0 (s, 1 H, thiazole H), 7.3 (d, 1 H, J = 2 Hz), 7.35 (d, 1 H, J = 2 Hz).

(19). Starting materials were **40** and 3,4-diacetoxy-2,5-dichlorobenzoyl chloride. The yield of **19** was 42%: ^{1}H NMR (DMSO-d_6/CD_3COOD/TFA-*d*) 1.25 (t, 3 H, J = 6 Hz, OCH_2CH_3), 3.55 (m, 2 H, SCH_2), 4.0–4.5 (m, 4 H, OCH_2CH_3, CH_2NH), 5.15 (d, 1 H, J = 5 Hz, H6), 5.75 (d, 1 H, J = 5 Hz, H7), 6.95 (s, 1 H, thiazole H), 7.0 (s, 1 H).

(20). Starting materials were **40** and 3-cyano-4,5-diacetoxybenzoyl chloride. The yield of **20** was 28%: ^{1}H NMR (DMSO-d_6/CD_3COOD/TFA-*d*) 1.25 (t, 3 H, J = 6 Hz, OCH_2CH_3), 3.5 (m, 2 H, SCH_2), 4.2 (m, 4 H, OCH_2CH_3, CH_2NH), 5.2 (d, 1 H, J = 5 Hz, H6), 5.7 (d, 1 H, J = 5 Hz, H7), 7.0 (s, 1 H, thiazole H), 7.55 (m, 2 H).

(21). Starting materials were **40** and 3,4-diacetoxy-5-nitrobenzoyl chloride. The yield of **21** was 21%: ^{1}H NMR (DMSO-d_6/CD_3COOD/TFA-*d*) 1.25 (t, 3 H, J = 6 Hz, OCH_2CH_3), 3.4–3.8 (m, 2 H, SCH_2), 4.0–4.6 (m, 4 H, OCH_2CH_3, CH_2NH), 5.1 (d, 1 H, J = 5 Hz, H6), 5.75 (d, 1 H, J = 5 Hz, H7), 6.95 (s, 1 H, thiazole H), 7.6 (d, 1 H, J = 2 Hz), 7.95 (d, 1 H, J = 2 Hz).

(22). Starting materials were **41** and 4,5-diacetoxy-2-fluorobenzoyl chloride. Three equivalents of triethylamine were used. The yield of **22** was 62%: ^{1}H NMR (DMSO-d_6/CD_3COOD/TFA-*d*) 1.0 (t, 3 H, J = 6 Hz, CH_2CH_3), 1.55 (s, 6 H, *gem*-dimethyl), 3.0–3.6 (m, 4 H, CH_2CH_3, SCH_2), 4.45 (m, 2 H, CH_2N), 5.2 (d, 1 H, J = 5 Hz, H6), 5.8 (d, 1 H, J = 5 Hz, H7), 6.6 (d, 1 H, J = 2 Hz), 6.7 (d, 1 H, J = 2 Hz), 7.1 (d, 1 H, thiazole H).

(23). Starting materials were **41** and 3,4-diacetoxy-5-fluorobenzoyl chloride. Three equivalents of triethylamine were used. The yield of **23** was 39%: ^{1}H NMR (DMSO-d_6/CD_3COOD/TFA-*d*) 1.05 (t, 3 H, J = 6 Hz, CH_2CH_3), 1.55 (s, 6 H, *gem*-dimethyl), 3.0–3.6 (m, 4 H, CH_2CH_3, SCH_2), 4.45 (m, 2 H, CH_2N), 5.2 (d, 1 H, J = 5 Hz, H6), 5.85 (d, 1 H, J = 5 Hz, H7), 6.65 (m, 2 H), 7.1 (d, 1 H, thiazole H).

(26). Starting materials were **41** and 3,4-diacetoxy-5-nitrobenzoyl chloride. Three equivalents of triethylamine were used. The yield of **26** was 36%: ^{1}H NMR (DMSO-d_6/CD_3COOD/TFA-*d*) 1.05 (t, 3 H, J = 6 Hz, CH_2CH_3), 1.55 (s, 6 H, *gem*-dimethyl), 3.0–3.3 (m, 4 H, CH_2CH_3, SCH_2), 4.45 (m, 2 H, CH_2N), 5.2 (d, 1 H, J = 5 Hz, H6), 5.85 (d, 1 H, J = 5 Hz, H7), 7.05 (s, 1 H), 7.1 (s, 1 H, thiazole H), 7.4 (s, 1 H).

Preparation of Benzoyl Chlorides. The benzoyl chlorides used in the above reactions were prepared by the following general method and were used directly: Thionyl chloride (5 mL) was added to the appropriate diacetoxybenzoic acid (1 mmol), the reaction mixture heated under reflux for 20 min, the remaining thionyl chloride evaporated under vacuum, and the resulting crude mixture taken up in toluene. Evaporation of the solvent gave the acid chloride in quantitative yield.

The diacetoxybenzoic acids were prepared as follows: Concentrated H_2SO_4 (1 drop) was added to a solution of the appropriate dihydroxybenzoic acid (1 mmol) in acetic anhydride (1 mL), and the mixture was stirred at 25 °C for 3 h. The mixture was poured onto ice/water and extracted with CH_2Cl_2. The organic layer was washed with water and brine and was dried over $MgSO_4$. Evaporation gave the diacetoxybenzoic acid.

The following dihydroxybenzoic acids were used: 3-Bromo-4,5-dihydroxybenzoic acid[23] (for compounds **1**, **3**); 3,4,5-Trihydroxybenzoic acid[24] (gallic acid) (for compound **6**); 2-Bromo-4,5-dihydroxybenzoic acid[25] (for compounds **7**, **16**); 4,5-Dihydroxy-2-fluorobenzoic acid[26] (for compounds **8**, **22**); 2-Chloro-3,4-dihydroxybenzoic acid[27] (for compound **9**); 3,4-Dihydroxy-5-fluorobenzoic acid[27] (for compounds **10**, **17**, **23**); 3-Chloro-4,5-dihydroxybenzoic acid[27] (for compounds **11**, **18**); 2,5-Dichloro-3,4-dihydroxybenzoic acid[16] (for compounds **12**, **19**); 3-Cyano-4,5-dihydroxybenzoic acid[28] (for compounds **13**, **20**); 3,4-Dihydroxy-5-nitrobenzoic acid[29] (for compounds **14**, **21**, **26**).

C-3′-Carboxamido Cephalosporins. General Procedure B Used for Compounds 34, 35, and 36. The appropriate dihydroxyquinolone carboxylic acid (1 mmol) was solubilized in CH_2Cl_2 (10 mL) by the addition of trimethylsilyl chloride (6.0 mmol) and triethylamine (6.0 mmol) at room temperature. After 2 h, $SOCl_2$ (1.1 mmol) was added to the solution which was stirred for a further 2 h. This solution was then added to a cooled −10 °C solution of cephalosporin **39** (1 mmol) in DMF (20 mL) and triethylamine (3.0 mmol). The mixture was stirred at −10 °C for 30 min, the solvents were evaporated, and the residual solid was purified on a Diaion HP 20 SS resin column using methanol/water mixtures of increasing proportions of methanol and containing acetic acid (1%). Evaporation and freeze-drying of the appropriate fractions gave the following final products.

(34). The starting material was 5-chloro-1,4-dihydro-6,7-dihydroxy-1-ethyl-4-oxoquinoline-3-carboxylic acid.[30] The yield

(23) Pschorr, R. Bromination of m-Hydroxy Benzaldehyde, Vanillin and Homovanillic acid. *Justus Liebigs Ann. Chem.* **1912**, *391*, 29–35.

(24) Obtained from Aldrich.

(25) Tomita, M.; Kondo, Y.; Tanaka, S. Rearrangement of the Bromine atom in the Demethylation of Bromomethoxybenzoic Acid. II. 6-Bromoveraric acid. *J. Pharm. Soc. Jpn.* **1956**, *76*, 1119–1122.

(26) Made by standard techniques of oxidation and deprotection of the known 3,4-dimethoxy-6-fluorobenzaldehyde. Furlano, D. C.; Kirk, K. L. An Improved Synthesis of 4-Fluoroveratrole. Efficient Route to 6-Fluoroveratraldehyde and 6-Fluoro-D,L-DOPA. *J. Org. Chem.* **1986**, *51*, 4073–4075.

(27) Walsch, T. A.; Ballon, D. P. Halogenated Protocatechuates as Substrates for Protocatechuate Dioxygenase from *Pseudomonas cepacia*. *J. Biol. Chem.* **1983**, *258*, 14413–14421.

(28) Made by deprotection by standard techniques of the known 3-cyano-5-hydroxy-4-methoxybenzoic acid. Borchardt, R. T.; Huber, J. A.; Houston, M. Catechol O-Methyltransferase. 10. 5-Substituted 3-Hydroxy-4-methoxybenzoic Acids (Isovanillic Acids) and 5-Substituted 3-Hydroxy-4-methoxybenzaldehydes (Isovanillins) as Potential Inhibitors. *J. Med. Chem.* **1982**, *25*, 258–263.

(29) Traxler, P.; Ghisalba, O. A Genetic Approach to the Biosynthesis of the Rifamycin-Chromophore in *Nocardia mediterranei*. V. Studies on the Biogenetic Origin of 3-Substituents. *J. Antibiot.* **1982**, *35*, 1361–1366.

of **34** was 12%: ^{1}H NMR (DMSO-d_6/CD_3COOD/TFA-*d*) 1.35 (t, 3 H, J = 5 Hz, CH_2CH_3), 1.5 (s, 6 H, *gem*-dimethyl), 3.6 (m, 2 H, SCH_2), 4.0–4.6 (m, 4 H, CH_2CH_3, CH_2NH), 5.15 (d, 1 H, J = 5 Hz, H6), 5.8 (d, 1 H, J = 5 Hz, H7), 7.05 (s, 1 H, thiazole H), 7.1 (s, 1 H), 8.65 (s, 1 H); MS m/e $(M - H)^-$ 748.

(35). The starting material was the dibenzoyl diester of 1,4-dihydro-6,7-dihydroxy-1-ethyl-4-oxocinnoline-3-carboxylic acid.[31] The yield of **35** was 7%: ^{1}H NMR (DMSO-d_6/CD_3COOD/TFA-*d*) 1.5 (m, 9 H, CH_2CH_3, *gem*-dimethyl), 3.65 (m, 2 H, SCH_2), 4.0–4.8 (m, 4 H, CH_2CH_3, CH_2NH), 5.15 (d, 1 H, J = 5 Hz, H6), 5.8 (d, 1 H, J = 5 Hz, H7), 7.05 (s, 1 H, thiazole H), 7.2 (s, 1 H), 7.55 (s, 1 H).

(36). The starting material was 1,4-dihydro-6,7-dihydroxy-1-ethyl-4-oxo-1,8-naphthyridine-3-carboxylic acid.[32] The yield of **36** was 27%: ^{1}H NMR (DMSO-d_6/CD_3COOD/TFA-*d*) 1.35 (t, 3 H, J = 5 Hz, CH_2CH_3), 1.5 (s, 6 H, *gem*-dimethyl), 3.6 (m, 2 H, SCH_2), 4.0–4.6 (m, 4 H, CH_2CH_3, CH_2NH), 5.15 (d, 1 H, J = 5 Hz, H6), 5.8 (d, 1 H, J = 5 Hz, H7), 7.0 (s, 1 H, thiazole H), 7.65 (s, 1 H), 8.8 (s, 1 H); MS m/e $(M - H)^-$ 715.

C-3′-Carboxamido Cephalosporins. General Procedure C Used for Compound 38. *N*-Methylmorpholine (20.0 mmol) was added rapidly to a stirred suspension of 3-(aminomethyl)-7-[2-(aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]-acetamido]ceph-3-em-4-carboxylic acid (10 mmol) (**39**) in a mixture of water (60 mL) and acetonitrile (60 mL) at 15 °C followed by 7,8-diacetoxy-2-oxo-2*H*-1-benzopyran-3-carbonyl chloride (20 mmol). After 1 h the mixture was diluted with an equal volume of water and the pH adjusted to and maintained at 8.5 with aqueous ammonia to hydrolyze the acetoxy groups and to obtain the dihydroxy derivative. The mixture was acidified to pH 3.5 with aqueous 2 N HCl and evaporated to dryness. It was purified on a Diaion HP 20 SS resin column using methanol/water mixtures of increasing proportions of methanol and containing acetic acid (1%). Evaporation and freeze-drying of the appropriate fractions gave **38** (34%): ^{1}H NMR (DMSO-d_6/CD_3COOD/TFA-*d*) 1.45 (s, 3 H, CH_3), 1.5 (s, 3 H, CH_3), 3.6 (dd, 2 H, J = 18 Hz, SCH_2), 4.0–4.5 (m, 2 H, CH_2NH), 5.1 (d, 1 H, J = 5 Hz, H6), 5.8 (d, 1 H, J = 5 Hz, H7), 6.85 (d, 1 H, J = 7 Hz ArH), 7.0 (s, 1 H, thiazole H), 7.2 (d, 1 H, J = 7 Hz, ArH), 8.7 (s, 1 H); MS m/e $(M - H)^-$ 687.

7,8-Diacetoxy-2-oxo-2*H*-1-benzopyran-3-carbonyl chloride was prepared as for the above diacetoxybenzoyl chlorides from 7,8-diacetoxy-2-oxo-2*H*-1-benzopyran-3-carboxylic acid.[33]

Preparation of Differently Linked Compounds 28, 30–33. 7-[2-(Aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]acetamido]-3-[2-(3-bromo-4,5-dihydroxyphenyl)-ethenyl]ceph-3-em-4-carboxylic Acid (28). A mixture of 7-[2-(aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]acetamido]-4-[[(4-methoxybenzyl)oxy]carbonyl]-3-[(triphenylphosphonio)methyl]ceph-3-em chloride (5 g, 5.3 mmol) and 3-bromo-4,5-bis[(4-methoxybenzyl)oxy]benzaldehyde (3.3 g, 5 mmol) in THF (50 mL) and sufficient aqueous sodium carbonate (2 N) to maintain the pH at 9 was stirred for 4 h at room temperature. The mixture was diluted with water (50 mL) and extracted with ethyl acetate. The crude product was purified by chromatography on SiO_2 using a mixture of CH_2Cl_2/ethyl acetate (7:3) as eluent, and the material thus obtained (1.6 g) was dissolved directly in a mixture of trifluoroacetic acid (4 mL) and anisole (2 mL). After 2 h the mixture was evaporated and the residue triturated with Et_2O. It was purified on a Diaion HP 20 SS resin column using a methanol/water mixture (11:9) and containing acetic acid (1%). Evaporation and freeze-drying of the appropriate fractions gave **28** (145 mg, 15%) as a 2:1 mixture trans/cis: ^{1}H NMR (DMSO-d_6/CD_3COOD/TFA-*d*) 1.6 (s, 6 H, *gem*-dimethyl), 3.2–3.6 (m, SCH_2, cis), 3.6–4.1 (m, SCH_2, trans), 5.25 (d, 1 H, J = 5 Hz, H6), 5.8 (d, 1 H, J = 5 Hz, H7), 6.35 (d, J = 11 Hz, ethylene cis), 6.45 (d, J = 11 Hz, ethylene cis), 6.7–7.0 (m, 2 H, ArH), 7.05 (s, 1 H, thiazole H), 7.3 (d, J = 17 Hz ethylene trans); MS m/e $(M - H)^-$ 667.

The starting phosphonium chloride was prepared as follows: A solution of 4-methoxybenzyl 7-amino-3-(chloromethyl)ceph-3-em-4-carboxylate[18] (3.4 g, 9 mmol) and 2-mercaptobenzothiazolyl 2-(aminothiazol-4-yl)-2-[[2-(*tert*-butoxycarbonyl)isopropoxy]imino]acetate[34] (4.4 g, 9 mmol) in CH_2Cl_2 (50 mL) was stirred for 1 h. The solvent was evaporated and the crude product purified by chromatography on SiO_2 using a mixture of CH_2Cl_2/ethyl acetate (4:1) as eluent. The intermediate (chloromethyl)cephalosporin thus obtained (5.6 g, 8.4 mmol) was heated under reflux for 2 h with a mixture of triphenylphosphine (2.2 g, 8.4 mmol) and sodium iodide (300 mg) in ethyl acetate (100 mL). The mixture was cooled, and the precipitated phosphonium salt (5.3 g, 69%) was filtered and used directly as described above.

C-3′-Carboxamido Cephalosporins. General Procedure D Used for Compounds 30 and 32. Triethylamine (1.0 mmol) was added rapidly to a stirred suspension of *tert*-butyl 3-(aminomethyl)-7-[2-(aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]acetamido]ceph-3-em-4-carboxylate (0.7 mmol) (**42**) in DMF (15 mL) at 0 °C, followed by the appropriate acid chloride (2 mmol). After 2 h the mixture was diluted with an equal volume of water and extracted with CH_2Cl_2 and the organic phase dried and concentrated. The crude cephalosporin was dissolved at 0 °C in 90% TFA (10 mL) and the reaction stirred at 0 °C for 2 h. The mixture was concentrated, and the gum thus obtained was triturated with Et_2O to give a solid which was purified on a Diaion HP 20 SS resin column using acetonitrile/water mixtures of increasing proportions of acetonitrile and containing acetic acid (1%). Evaporation and freeze-drying of the appropriate fractions gave the following final products.

(30). The starting material was 2-bromo-4,5-diacetoxy-(*E*)-cinnamyl chloride. The yield of **30** was 10%: ^{1}H NMR (DMSO-d_6/CD_3COOD/TFA-*d*) 1.45 (s, 6 H, *gem*-dimethyl), 2.3 (s, 6 H, $COCH_3$), 3.5 (m, 2 H, SCH_2), 4.05–4.45 (m, 2 H, CH_2NH), 5.15 (d, 1 H, J = 5 Hz, H6), 5.85 (d, 1 H, J = 5 Hz, H7), 6.7 (d, 1 H, J = 14 Hz), 6.8 (s, 1 H, thiazole H), 7.6 (s, 1 H), 7.65 (d, 1 H, J = 14 Hz), 7.7 (s, 1 H); MS m/e $(M + H)^+$ 809.

(32). The starting material was 2-bromo-4,5-diacetoxyphenylacetyl chloride. The yield of **32** was 20%: ^{1}H NMR (DMSO-d_6/CD_3COOD/TFA-*d*) 1.5 (s, 6 H, *gem*-dimethyl), 2.2 (s, 6 H, $COCH_3$), 3.5 (m, 2 H, SCH_2), 3.6 (s, 2 H, $COCH_2$), 3.95–4.25 (m, 2 H, CH_2NH), 5.1 (d, 1 H, J = 5 Hz, H6), 5.8 (d, 1 H, J = 5 Hz, H7), 6.85 (s, 1 H, thiazole H), 7.25 (s, 1 H), 7.5 (s, 1 H); MS m/e $(M + H)^+$ 797.

The diacetoxy acid chlorides were prepared by acetylation and chlorination as described above of the following dihydroxy acids: 2-bromo-4,5-dihydroxy-(*E*)-cinnamic acid;[35] 2-bromo-4,5-dihydroxyphenylacetic acid.[36]

7-[2-(Aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]acetamido]-3-[[*N*-(3-bromo-4,5-dihydroxybenzyl)amino]methyl]ceph-3-em-4-carboxylic Acid (31). 3-Bromo-4,5-dihydroxybenzaldehyde[23] (669 mg, 1.38 mmol) was added rapidly to a stirred suspension of 3-(aminomethyl)-7-[2-(aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]-acetamido]ceph-3-em-4-carboxylic acid (10 mmol) (**39**) in a mixture of water (3 mL) and methanol (22 mL) at 0 °C, followed by sodium cyanoborohydride (95 mg, 1.5 mmol). After 1 h DMF (8 mL) was added to obtain a solution. After 3 h the mixture was evaporated to dryness. It was purified on a Diaion HP 20 SS resin column using methanol/water mixtures of increasing proportions of methanol and containing acetic acid (1%). Evaporation and freeze-drying of the appropriate fractions gave **31** (26%): ^{1}H NMR (DMSO-d_6/CD_3COOD/TFA-*d*) 1.55 (s, 3 H, CH_3), 1.6 (s, 3 H, CH_3), 3.7 (m, 2 H, SCH_2), 3.9 (m, 2 H, CH_2NH), 4.0 (s, 2 H,

(30) Frank, J.; Rakoczy, P.; Radics, L.; Gacs-Baitz, E. Some Anomalous Reactions of 4-Quinolone-5-diazonium Salts. *J. Heterocycl. Chem.* **1981**, *18*, 985–990.

(31) Made by deprotection by standard techniques of commercial Cinoxacin (1-ethyl-1,4-dihydro-4-oxo[1,3]dioxolo[4,5-*g*]-cinnoline-3-carboxylic acid; Lilly).

(32) Imperial Chemical Industries PLC European Patent 341990 A2, 1989; *Chem. Abstr.* **1989**, *113*, 40328w.

(33) Boehm, T.; Schumann, G. The Coumarin Group. II. Synthesis of Certain Coumarin Aldehydes; the Catalytic Hydrogenation of Acid Chlorides. *Arch. Pharm.* **1933**, *271*, 490–513.

(34) Obtained from Lonza.

(35) Prepared from 3,4-dimethoxybenzaldehyde[24] by bromination, Knoevenagel condensation, and deprotection by standard techniques. Anal. ($C_9H_7BrO_4$·0.25H_2O) C, H.

(36) Prepared from 3,4-dimethoxyphenylacetic acid[24] by bromination and deprotection by standard techniques. Anal. ($C_8H_7BrO_4$) C, H.

$NHCH_2$), 5.2 (d, 1 H, J = 5 Hz, H6), 5.95 (d, 1 H, J = 5 Hz, H7), 6.95 (d, 1 H, J = 2 Hz, ArH), 7.1 (s, 1 H, thiazole H), 7.15 (d, 1 H, J = 2 Hz, ArH); MS m/e $(M - H)^-$ 685.

7-[2-(Aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]acetamido]-3-[[[*N*-(3-bromo-4,5-diacetoxyphenyl)amino]formamido]methyl]ceph-3-em-4-carboxylic Acid (33). A solution of 3-bromo-4,5-diacetoxyphenyl isocyanate (314 mg, 1 mmol) in acetonitrile (2 mL) was added rapidly to a stirred suspension of 3-(aminomethyl)-7-[2-(aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]acetamido]ceph-3-em-4-carboxylic acid (**39**) (691 mg, 1 mmol) in a mixture of diisopropylethylamine (259 mg, 2 mmol) and acetonitrile (10 mL) under argon at 25 °C. After 4 h the mixture was evaporated to dryness. The residue was purified by preparative chromatography on a Dynamax column using an acetonitrile/water mixture (3:7) containing trifluoroacetic acid (1%). Evaporation and freeze-drying of the appropriate fractions gave **33** (15%): ^1H NMR (DMSO-d_6/CD_3COOD/TFA-d) 1.45 (s, 3 H, CH_3), 1.46 (s, 3 H, CH_3), 2.2 (s, 3 H, $COCH_3$), 2.25 (s, 3 H, $COCH_3$), 3.5–3.65 (m, 2 H, SCH_2), 3.84–4.15 (m, 2 H, CH_2NH), 5.1 (d, 1 H, J = 5 Hz, H6), 5.85 (d, 1 H, J = 5 Hz, H7), 6.85 (s, 1 H, thiazole H), 7.3 (d, 1 H, ArH), 7.6 (d, 1 H, ArH); MS m/e $(M - H)^-$ 796.

3-Bromo-4,5-diacetoxyphenyl isocyanate was prepared as follows: Trimethylsilyl azide (0.6 mL) was added dropwise, during 5 min, to a solution of 3-bromo-4,5-diacetoxybenzoyl chloride (1.41 g) (see above) in toluene at 100 °C, and the mixture was heated at this temperature for 4 h. The solvent was evaporated to give a gum (1.27 g) which was used directly as above.

Biological Studies. 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.[11]

Marmoset Pharmacokinetic Test. Animals were obtained from 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 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 assuming that the antimicrobial activity detected in the assay is related to the test compound concentration. 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.

Mouse Protection Test. Male mice (Alpk: ApfCD-1 strain), weighing 18–20 g, were obtained from the ICI Barriered Animal Breeding Unit. The mice were infected intraperitoneally with 10 × LD_{50} of the challenge organism suspended in hog gastric mucin (Sigma). Five groups of four mice were used per compound. The animals were given a single subcutaneous dose of compound within minutes of the infection, and the experiment was terminated on day 4 postinfection. The PD_{50} values were computed by logit analysis from the numbers of survivors over the range of doses tested.

Acknowledgment. We thank Dr. T. D. Hennessey, Dr. J. R. Edwards, Mr. R. G. Wilson, and colleagues for the determination of pharmacokinetics in marmosets, and Dr. G. M. Davies, Dr. D. H. Davies, Dr. D. M. Hollinshead, and their colleagues for the syntheses of compounds **30–34** and **38** described in this paper.

Registry No. **1**, 119734-73-5; **2**, 119734-78-0; **3**, 119734-38-2; **4**, 122233-83-4; **5**, 119786-58-2; **6**, 119734-60-0; **7**, 119734-72-4; **8**, 119734-11-1; **9**, 119734-31-5; **10**, 119734-34-8; **11**, 119734-30-4; **12**, 119734-61-1; **13**, 119761-15-8; **14**, 119734-80-4; **15**, 119734-66-6; **16**, 119734-68-8; **17**, 119734-13-3; **18**, 119760-97-3; **19**, 119734-14-4; **20**, 119734-27-9; **21**, 119734-76-8; **22**, 119760-95-1; **23**, 119734-10-0; **24**, 119734-33-7; **25**, 119734-32-6; **26**, 119734-29-1; **27**, 141555-09-1; (*Z*,*E*)-**28**, 141555-10-4; (*Z*,*Z*)-**28**, 141583-27-9; **29**, 119733-90-3; **30**, 119733-98-1; **31**, 141555-11-5; **32**, 119760-93-9; **33**, 141555-12-6; **34** f, 122233-86-7; **35**, 122234-17-7; **36**, 141555-13-7; **37**, 141555-14-8; **38**, 122234-33-7; **39**, 96628-33-0; **40**, 96629-31-1; **41**, 115309-14-3; **42**, 141555-15-9; 7-[2-(aminothiazol-4-yl)-2-[(*Z*)-(1-carboxy-1-methylethoxy)imino]acetamido]-2-[[(4-methoxybenzyl)oxy]-carbonyl]-3-[(triphenylphosphonio)methyl]ceph-3-em chloride, 141555-16-0; 3-bromo-4,5-diacetoxybenzoyl chloride, 122306-86-9; 3,4,5-triacetoxybenzoyl chloride, 70475-59-1; 2-bromo-4,5-diacetoxybenzoyl chloride, 141555-17-1; 4,5-diacetoxy-2-fluorobenzoyl chloride, 141555-18-2; 2-chloro-3,4-diacetoxybenzoyl chloride, 141555-19-3; 3,4-diacetoxy-5-fluorobenzoyl chloride, 141555-20-6; 3-chloro-4,5-diacetoxybenzoyl chloride, 141555-21-7; 3,4-diacetoxy-2,5-dichlorobenzoyl chloride, 127345-53-3; 3-cyano-4,5-diacetoxybenzoyl chloride, 137419-43-3; 3,4-diacetoxy-5-nitrobenzoyl chloride, 112057-05-3; 5-chloro-1,4-dihydro-6,7-dihydroxy-1-ethyl-4-oxoquinoline-3-carboxylic acid, 80104-60-5; 1,4-dihydro-6,7-dihydroxy-1-ethyl-4-oxocinnoline-3-carboxylic acid, 141555-22-8; 1,4-dihydro-6,7-dihydroxy-1-ethyl-4-oxo-1,8-naphthyridine-3-carboxylic acid, 127980-49-8; 7,8-diacetoxy-2-oxo-2*H*-1-benzopyran-3-carbonyl chloride, 84738-42-1; 2-bromo-4,5-diacetoxy-(*E*)-cinnamyl chloride, 141555-23-9; 2-bromo-4,5-diacetoxyphenylacetyl chloride, 141555-24-0; 3-bromo-4,5-dihydroxybenzaldehyde, 16414-34-9; 3-bromo-4,5-diacetoxyphenyl isocyanate, 141555-25-1; 3-bromo-4,5-bis[(4-methoxybenzyl)-oxy]benzaldehyde, 141555-26-2.