for 74 was carried out by mixing 0.2 mL of plasma with 0.8 mL of 0.026 M NH_4OAc in 82% MeOH and for 67 by mixing 0.2 mL of plasma with 0.8 mL of MeOH. The samples were allowed to stand for 10 min and centrifuged, and aliquots of the clarified supernatants were injected into the HPLC chromatographic system.

At 5 min following iv administration of 1a at 1, 3, and 10 mg/kg, mean plasma concentrations of 1a were 0.28 ± 0.01, 0.79 ± 0.16, and 4.6 ± 3.3 μg/mL, respectively. Those of the corresponding carboxylic acid metabolite, 32, were 0.15 ± 0.04, 0.56 ± 0.06, and 1.23 ± 0.31 μg/mL, respectively (n = 3, each consisting of pooled blood from three mice). Following oral administration of 67 at 300 mg/kg, mean plasma concentrations of parent drug were below the minimum quantifiable level (MQL) of 0.068 μg/mL at time points ranging from 0.25 to 8.0 h, whereas a mean maximum concentration (C_{max}) of 5.04 ± 0.74 μg/mL of the acid metabolite 74 was achieved at 1 h. The concentration of this metabolite at 8 h, the last time point examined, was still 2.7 ± 0.4 μg/mL (n = 3, each consisting of pooled blood from three mice). Mean C_{max} values of the parent drug achieved following oral administration of 30 at 30, 100, 300, and 1000 mg/kg were below the MQL, 0.34 ± 0.10, 1.44 ± 0.32, and 1.52 ± 0.27 μg/mL of the parent drug, respectively, and of the acid metabolite were 4.1 ± 0.13, 10.3 ± 0.78, 18.6 ± 2.3, and 17.6 ± 1.3 μg/mL of the acid metabolite 34, respectively. (These experiments were performed utilizing HPLC conditions which did not distinguish between the enantiomers of 34.)

Acknowledgment. We thank the following people for their valuable technical assistance and contributions to this project: John Alexander, Michael Alexander, Craig Behn, Stephen Clemans, Greg Daley, Marion Drozd, Charles Hutchins, Kurt Josef, Edward Maliski, Gloria Martinson, Ruthann McGarry, Lynn McNaughton, Peter Pareene, and Alicia Todaro.

Supplementary Material Available: X-ray crystallographic data for compounds 1a and 1b (12 pages). Ordering information is given on any current masthead page.

Synthesis and Structure–Activity Relationship of New Cephalosporins with Amino Heterocycles at C-7. Dependence of the Antibacterial Spectrum and β-Lactamase Stability on the pK_a of the C-7 Heterocycle

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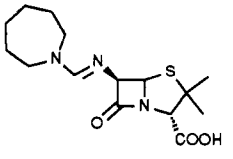
Cephalosporins with new aminobenzimidazole and aminoimidazoline heterocycles at C-7 have been synthesized starting with versatile C-7 isocyanide dihalide synthons. The aminobenzimidazoles have a broad spectrum of antibacterial activity, including Gram-positive and Gram-negative organisms, but possess limited β-lactamase stability. In contrast, the aminoimidazolines have a narrow spectrum of antibacterial activity, limited to Gram-negative strains only, but possess outstanding β-lactamase stability. Structure–activity relationships are discussed in terms of their dependence on the pK_a of the C-7 amino heterocycle, basic C-7 residues giving cephalosporins with exceptional β-lactamase stability.

Modification of the C-6 and C-7 acylamino residue in penicillins and cephalosporins is still, after decades of work, a very active and fruitful area of investigation.[1] Introduction of nonamidic C-6, C-7 substituents on penicillins and cephalosporins has also been performed over the years.[2] Variable levels of biological activity have been found, but in general, this approach has only been moderately successful. Indeed, all the therapeutically useful cephalosporins have acylamino C-7 chains. This is also true for the penicillins, with only one exception, mecillinam, a C-6 amidino penicillin.[3]



MECILLINAM

An intriguing feature of mecillinam is its highly selective affinity for penicillin binding protein 2 (PBP2), in contrast to amidic penicillins or cephalosporins which display a much broader pattern of affinity for the various PBPs.[4]

We were attracted by the interesting possibility that β-lactam antibacterials with an original mode of action could be devised by introduction of nonamidic C-6 or C-7 substituents in penicillins or cephalosporins. Cephalosporins with C-7 amino heterocycles of various basicities were selected as our initial targets.

Chemistry

Isocyanide Dihalide Chemistry. Isocyanide dihalides are well-known, powerful electrophiles, easily prone to a variety of nucleophilic displacement reactions leading to monocyclic or polycyclic heterocycles.[5]

(1) *Structure-Activity Relationships Among the Semisynthetic Antibiotics*; Perlman, D., Ed.; Academic Press: New York, 1977. *Chemistry and Biology of β-Lactam Antibiotics*; Morin, R. B., Gorman, M., Ed.; Academic Press: New York, 1982; Vol. 1, p 371.
(2) *Topics in Antibiotics*; Sammes, P. G., Ed.; Ellis Horwood Ltd.: Chichester, 1980, Vol. 4, p 218.
(3) Lund, F.; Tybring, L. *Nature, New Biol.* **1972**, *135*, 236.
(4) *Chemistry and Biology of β-Lactam Antibiotics*; Morin, R. B., Gorman, M., Ed.; Academic Press: New York, 1982, Vol. 3, p 209.
(5) Kühle, E. *Angew. Chem., Int. Ed. Engl.* **1969**, *8*, 20. *The Chemistry of Cyanates and their Thio Derivatives*; S. Patai ed., John Wiley and Sons: New York, 1977, Part 2, 969.
(6) Martin, A. F.; Morris, J. J.; Page, M. J. *J. Chem. Soc., Chem. Commun.* **1976**, 495.
(7) Beecham US Patent 3996235, 1976; *Chem. Abstr.* **1977**, *86*, 155672v.
(8) Jen, T.; Frazee, J., Hoover, J. R. E. *J. Org. Chem.* **1973**, *38*, 2857.

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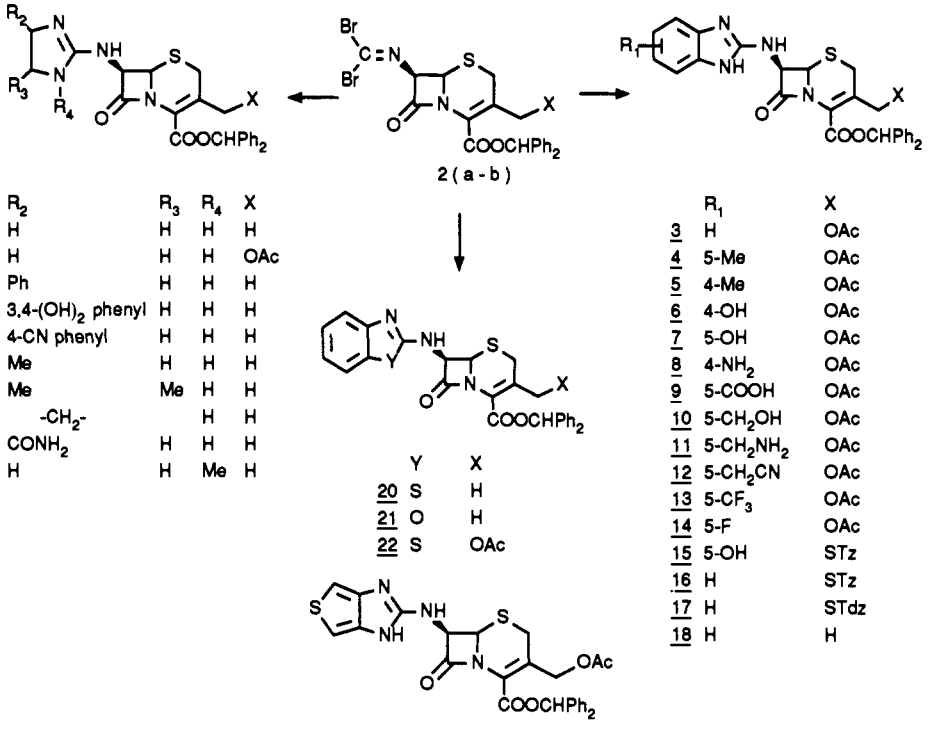
Scheme I

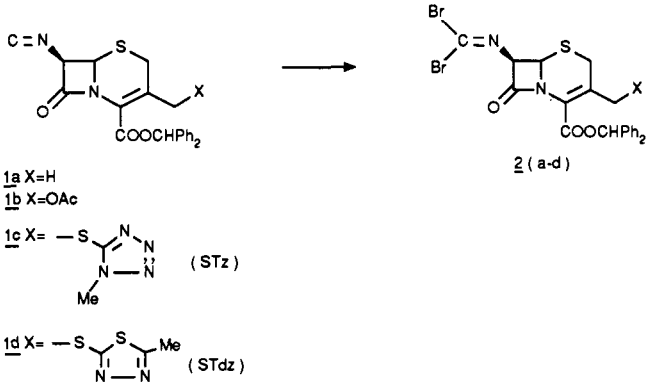


	R_2	R_3	R_4	X
23	H	H	H	H
24	H	H	H	OAc
25	Ph	H	H	H
26	3,4-$(OH)_2$ phenyl	H	H	H
27	4-CN phenyl	H	H	H
28	Me	H	H	H
29	Me	Me	H	H
30	$-CH_2-$		H	H
31	$CONH_2$	H	H	H
32	H	H	Me	H

	Y	X
20	S	H
21	O	H
22	S	OAc

	R_1	X
3	H	OAc
4	5-Me	OAc
5	4-Me	OAc
6	4-OH	OAc
7	5-OH	OAc
8	4-NH_2	OAc
9	5-COOH	OAc
10	5-CH_2OH	OAc
11	5-CH_2NH_2	OAc
12	5-CH_2CN	OAc
13	5-CF_3	OAc
14	5-F	OAc
15	5-OH	STz
16	H	STz
17	H	STdz
18	H	H

We have found that 7-isocyano cephalosporins **1a–d**[10] can be easily halogenated at low temperature to give the versatile isocyanide dihalides **2a–d** in good yield (>80%). These novel intermediates are reasonably stable at room temperature and can be kept unchanged at low temperatures (−18 °C) for months.

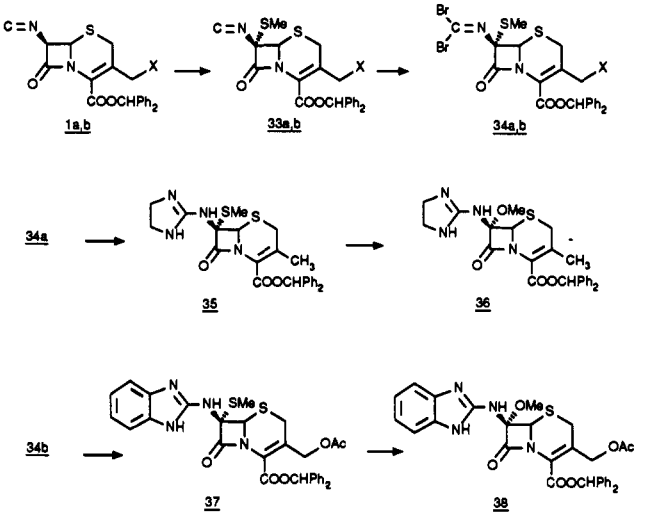


1a X=H
1b X=OAc
1c X= (STz)
1d X= (STdz)

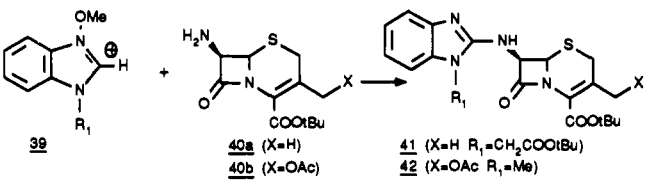
Thus, even with strongly basic amines, like ethylenediamines which are known to cleave the β-lactam ring system[6] easily, an almost exclusive attack on the isocyanide dibromide function could be achieved at low temperature (−78 °C). Scheme I illustrates some of the transformations which have been carried out successfully with these isocyanide dibromides.

7-α-OMe analogues of some of our compounds have been synthesized via the known 7-α-methylthio isonitriles[7] **33a,b** (Scheme II). These compounds were obtained with a markedly improved yield (50%) and with fewer byproducts (2- and/or 4-methylthiolation products etc.) using a cuprous oxide promoted reaction of the isonitrile with *S*-methylsulfenyl *O*-methyl thiocarbonate (MeSSCOOMe) instead of the published potassium carbonate/DMF procedure.[7] The reaction with the diamine was carried out as in the 7-α unsubstituted series and was followed by SMe–OMe exchange and deprotection[8] (Scheme II).

Scheme II



N-Substituted Benzimidazolylamino and Imidazolinylamino Cephalosporins. N-Substituted imidazoline **32** was obtained by reaction of *N*-methylethylenediamine with the corresponding 7-isocyanide dibromide **2a** under the usual conditions. In the benzimidazole series we used a direct condensation between cephalosporin esters **40a,b** and *N*-methoxybenzimidazolium salts **39**.[9] This reaction occurs smoothly in MeOH at room temperature to give benzimidazoles **41** and **42**.



(9) Takahashi, S.; Kano, H. *Chem. Pharm. Bull.*, **1964**, *12*, 783.
(10) Glaxo Ger. Offen. 2337105, 1974; *Chem. Abstr.* **1974**, *80*, 108550*v*.

Table I. Antibacterial Activity of Benzimidazolylamino Cephalosporins and Heterocyclic Analogues

organisms[a]	compounds (free acids)											
	3	4	5	6	7	8	48	49	45	9	10	11
S. pyogenes A1	2	1	8	2		40	1	4	1	8	1	0.5
S. aureus A6	2	2	4	2	4	40	2	8	1	32	8	4
S. aureus A4[h]	8	16	8	32	16	>200	32	256	200	256	64	32
E. coli A8	2	2	16	1	0.12	40	0.25	1	40	16	0.5	0.12
K. pneumonia A24	1	4	8	8	2	>200	0.5	32	40	32	1	1
E. cloacae A13	≥128	256	≥128	>256	128	>200	256	>256	40	>256	>256	256
S. marcescens A16	64	128	≥128	256	256	>200	256	>256	>200	>256	>256	>256
P. mirabilis A18	4	4	8	16	0.5	200	32	8	40	32	8	32
P. vulgaris Pv9	64	256	≥128	128	32		128	128			128	64
P. aeruginosa A21	≥128	>256	≥128	>256	>256	>200	>256	>256	>200	>256	>256	>256
E. cloacae P99^{-b}	2	2	32	1	0.12	4	0.06	2	64	4	0.5	0.12
E. cloacae P99$^{+b,c}$	≥128	256	≥128	>256	128	>200	32	>256	>200	>256	>256	64
E. coli TEM X3[b,d]	2	4	32	32	1	128	1	16	>200	16	4	2
E. coli X4[b,f]	0.25	1	8	0.5	0.12	8	0.06	1	>200	8	0.5	0.12
K. aerogenes X5L[b,e]	≥128	128	≥128	256	64	>200	8	>256	>200	>256	128	32
K. aerogenes X6[b,f]	2	4	16	1	0.5	8	0.12	2	>200	16	1	0.25
E. coli DC0[b,f]	0.5	1	8	0.5	0.12	4	0.06	1	128	8	0.5	0.12
E. coli DC2[b,g]	<0.03	0.06	0.25	0.12	0.016	1	0.03	0.12	8	0.25	0.06	0.06

[a] MIC in μg/mL; Jewell and Permain growth medium; inoculum, 10^5 cfu per spot. [b] Inoculum, 10^4 cfu per spot. [c] Constitutive type I lactamase producer. [d] TEM I type lactamase producer. [e] Type IV lactamase producer. [f] Parent organism. [g] Permeability mutant. [h] Lactamase producer.

Imidazolinylamino, Benzimidazolylamino Cephalosporins by Direct Nucleophilic Displacement. 2-Chlorobenzimidazoles or 2-chloroimidazolines cannot be condensed under mild conditions with 7-amino cephalosporin esters. High temperatures and vigorous conditions are generally required to condense these chloroheterocycles with amines.[11]

We found that 2-chloro-5-nitrobenzimidazole, 2-chloroimidazoline, and 2-chloroimidazopyridines ([4,5-*b*] and [4,5-*c*]) can be condensed smoothly under acid catalysis with 7-amino cephalosporin esters (pK_a 4.5); the charged heterocycle and the free base **40a–d** are the reactive species in the condensation (Scheme III).

Antibacterial Properties, Structure–Activity Discussion

Benzimidazolylamino Cephalosporins. The antibacterial properties of some of the compounds synthesized are shown in Tables I–III. Compounds **7** and **15** display a level of activity comparable to cefotaxime against *Staphylococci* and some of the Gram-negative organisms. Their activity against *Streptococcus pyogenes*, *Serratia marcescens*, and *Proteus vulgaris* is inferior to that of cefotaxime. The β-lactamase stability against the R TEM type enzymes of *Escherichia coli* X3 is good (Table I). They are unstable however to the type I enzymes of *Enterobacter cloacae* P99$^+$ and type IV enzymes of *Klebsiella aerogenes* X5L. In general, they penetrate only imperfectly through the outer membrane of *E. coli*, as can be seen by comparing their activity against the *E. coli* DC0 with their activity against the permeability mutant *E. coli* DC2 (Table I).

The nature of the substituent on the aromatic ring has little influence on the intrinsic activity against the DC2 organism. More important seems to be the position of the substituent. Thus, for a given substituent, the C-5 substituted derivatives always lead to more active molecules than the C-4 substituted ones, which are generally only poorly active compounds, for instance **6**, **7**, **8**, **48**. Few N-substituted cephalosporins have been synthesized and they were always much less active than their N-unsubstituted counterparts (Table I). 7-α-Methoxybenzimidazole **38** was completely devoid of antibacterial activity (Table I). 7-α-Methoxylation is known to decrease considerably the affinity of cephalosporins for PBP2.[12] This result indicates that the primary PBP target of this class of compounds could well be PBP2, which was confirmed later by competitive displacement experiments.[13] The heterocylic analogues **46** and **47** showed less activity than the parent unsubstituted benzimidazole (Table I). Replacement of the C-3′ acetoxy group by S-heterocyclic substitutents leads to somewhat more potent compounds, as is usually found in the amidic series (Table I).

Table II displays comparative biological properties of some benzimidazolylamino, benzothiazolylamino, and benzoxazolylamino cephalosporins. It is interesting to note that although Gram-positive activity is usually similar in the three series, the benzoxazoles and benzothiazoles are substantially less active against Gram-negative organisms than the corresponding benzimidazoles. A hypothetical but attractive explanation can be formulated in which the spectral differences are correlated with the differences in pK_a (and hence to a different percentage of protonated form present in the medium at physiological pH) of the C-7 substituent. The structure–activity discussion in the imidazoline series will emphasize this point even further and in a future paper on 7-imidazolylamino cephalosporin we will present additional evidence of the importance of the pK_a of the C-7 side chain on the spectrum and other biological properties of this class of compounds.

Imidazolinylamino Cephalosporins. The antibacterial properties of representative examples of this family of compounds are displayed in Table III. The antibacterial activity is now strictly confined to Gram-negative organisms. Thus, even imidazolines substituted by a lipophilic group, such as **25**, show no antistaphylococcal nor antistreptococcal activity.

Imidazoline nitrogen and C-7-α-OMe substitution are also detrimental to biological activity in this series (**32**, **36**), which implies a similar mode of action to the benzimidazoles, i.e. PBP2 is probably the primary target of these compounds. A pronounced inoculum effect, probably related to this particular mode of action, is seen with some *Proteus* species (example **30**, Table III).

The great hydrophilicity of these compounds allows

(11) Huger, A.; Kebrle, J.; Rossi, A.; Hoffmann, K. *Helv. Chim. Acta* **1961**, *44*, 1273.

(12) Curtis, N. A. C.; Ross, G. W.; Boulton, M. G. *J. Antimicrob. Chemother.* **1979**, *5*, 391.

(13) Nolan, R. D., I.C.I. Pharmaceuticals. Unpublished results.

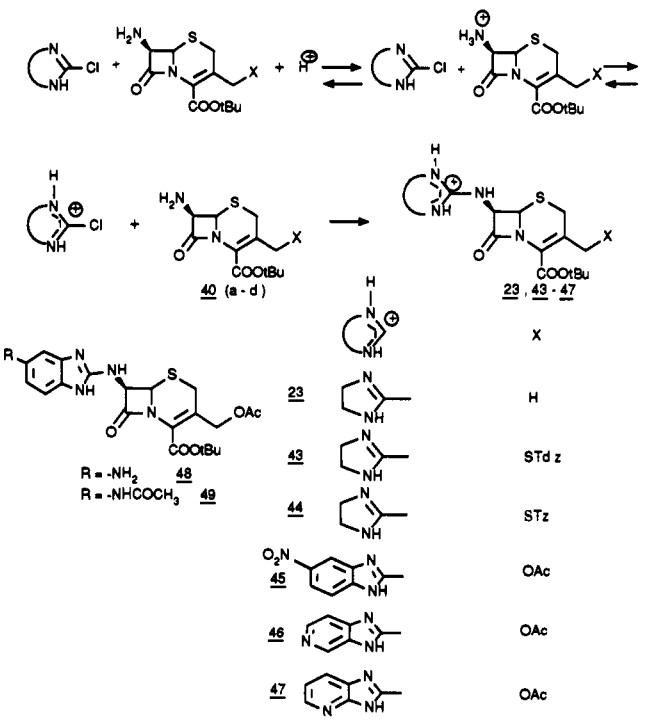
12	13	14	15	16	17	38	41	42	19	46	47	cefotaxime	mecillinam
0.5	1	2	0.5	0.5	0.5	>256	>256	32	2	8	4	0.03	16
1	2	2	2	4	1	>256	>256	32	4	256	2	4	16
256	128	16	16	128	8	>256	>256	>256	32	256	64	32	>256
0.5	32	4	0.25	0.5	1	>256	>256	128	8	8	2	0.25	0.06
0.5	32	64	4	0.5	1	>256			16	32	2	0.06	0.03
>256	>256	>256	>256	>128	≥128	>256	>256	>256	>256	32	256	256	0.25
64	>256	256	64	64	≥128	>256	>256	>256	>256	>256	>256	0.25	128
64	128	16	0.5	0.25	4	>256	>256	32	32	16	32	0.06	32
>256	>256	>256	16	8	≥128					>256	>256	0.5	64
>256	>256	>256	>256	>128	≥128	>256	>256	>256	>256	>256	>256	128	>256
1	32	4	0.25	0.5	1	>256	>256	64	1	2	1	0.03	0.03
256	>256	256	32	64	≥128	>256	>256	>256	>256	>256	>256	32	0.06
4	32	4	1	4	4	>256	>256	64	4	32	4	0.06	1
0.25	32	1	0.12	0.5	0.5	>256	>256	32	0.5	2	0.5	0.06	0.03
128	>256	256	64	128	≥128	>256	>256	256	128	>256	256	4	4
0.5	64	4	0.25	2	2	>256	>256	256	2	8	1	0.06	0.12
0.5	32	1	0.12	0.5	0.5	>256	>256	64	1	4	0.5	0.03	0.03
<0.03	0.12	0.12	<0.03	0.016	<0.03	>256	>256	0.25	0.06	0.5	0.5	0.002	0.004

Table II. Comparative Antibacterial Activity of Benzimidazolylamino (**3**, **18**), Benzoxazolylamino (**21**), and Benzothiazolylamino (**20**, **22**) Cephalosporins

	compounds (free acids)				
organisms[a]	**18**	**20**	**21**	**22**	**3**
S. pyogenes A1	40	40	200	1	2
S. aureus A6	40	2	8	0.5	2
S. aureus A5	8	1	8	0.5	2
E. coli A8	8	>1000	>1000	200	0.25
E. cloacae A13	8	>1000	1000	200	0.5
S. marcescens A16	200	>1000	>1000	1000	200
P. mirabilis A18	8	1000	1000	200	8
P. aeruginosa A21	>1000	>1000	>1000	>1000	>1000

[a] MIC in μg/mL: Jewell and Permain growth medium: inoculum, 10^2 cfu per spot.

Scheme III



them to penetrate well through the outer membrane of *E. coli*, in contrast to most benzimidazoles. Strangely, the usual improvement in antibacterial activity seen by replacement of the C-3 Me by C-3 CH_2OAc or CH_2–S–Het does not occur in this series. This is probably due to their chemical instability ($t_{1/2}$ of **24**, **43**, **44** in phosphate buffer, pH 7, at 37 °C being 2, 0.4, and 0.2 h, respectively). This is in contrast to benzimidazoles, which are stable molecules ($t_{1/2}$ > 2 days under identical conditions). Another interesting feature of these compounds resides in their very good β-lactamase stability against all classes of lactamases (Table III). The basic nature of the C-7 amino imidazoline substituent, largely protonated at physiological pH, might again explain these properties.

Thus, high Gram-negative activity has only been found, so far, in penicillins or cephalosporins having a degree of positive charge on their C-6 or C-7 side chain, i.e. aminobenzimidazoles (pK_a = 5–6), aminoimidazolines (pK_a = 8-10), amidines[14] ($pK_a \approx 8$), or aminopyridinium[15] substituents (pK_a > 11).

On the other hand, this property is not necessary for Gram-positive activity, which is found among weakly basic heterocycles such as aminobenzothiazoles (pK_a 2.5) or aminobenzoxazoles (pK_a = 1.8), cyanoamidines,[16] heterocycles of medium basicity such as the aminobenzimidazoles, and substituents of high basicity such as amidines.[14]

In the cephalosporin series, very good β-lactamase stability is restricted to C-7 substituents of high basicity such as aminoimidazolines or aminopyridinum salts (conjugated

(14) *Recent Advances in the Chemistry of β-Lactam Antibiotics*; Elks, J., Ed.; The Chemical Society: London, 1977, p 25.
(15) Hannah, J., Johnson, C. R.; Wagner, A. F.; Walton, E. *J. Med. Chem.* **1982**, *25*, 457.
(16) Petersen, H. J. *J. Med. Chem.* **1974**, *17*, 101.

Table III. Antibacterial Activity of Imidazolinylamino Cephalosporins

organisms[a]	compounds (free acids)															
	23	24	43	44	25	27	26	28	29	30	30[b]	31	36	32	cefotaxime	mecillinam
S. pyogenes A1	128	32	16	128	256	64	>256	>256	>256	128		>256	>256	>200	0.03	16
S. aureus A6	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	>200	4	16
S. aureus A4[i]	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	>200	32	>256
E. coli A8	2	32	4	2	2	4	4	128	32	2	1	32	>256	40	0.25	0.06
K. pneumonia A24	4	64	4	2	4	16	>256	>256	16	1	1	16	>256	40	0.06	0.03
E. cloacae A13	4	64	64		2	>256	>256	>256	32	4	2	>256	>256	>200	256	0.25
S. marcescens A16	>256	256	64	32		16	>256	>256	>256	4	4	>256	>256	>200	0.25	128
P. mirabilis A18	256	256	>256	>256	>256	>256	>256	>256	>256	>256	16	>256	>256	>200	0.06	32
P. vulgaris Pv9	>256	>256	64		>256		>256	>256	>256	256	4				0.5	64
P. aeruginosa A21	>256	>256	>256	>256	>256	>256	>256	>256	>256	256	128	>256	>256	>200	128	>256
E. cloacae P99[-c]	2	32	8	1	1	4	2	2	4	1		4	>256	64	0.03	0.03
E. cloacae P99[+c,d]	4	64	16	8	2	8	4	2	4	2		8	>256	128	32	0.06
E. coli TEM X3[c,e]	2	32	8	1	1	4	2	2	4	1		8	>256	128	0.06	1
E. coli X4[c,g]	2	32	4	1	1	4	2	2	4	1		8	>256	128	0.06	0.03
K. aerogenes X5L[c,f]	4	64	16	2	8	8	8	40	32	2		16	>256	>200	4	4
K. aerogenes X6[c,g]	4	64	8	1	2	8	4	8	16	2		16	>256	128	0.06	0.12
E. coli DC0[c,g]	4	32	8	8	1	2	4	8	4	1		8	>256	128	0.03	0.03
E. coli DC2[c,h]	2	2	1		0.5	2	2	4	4	0.5		2	>256	32	0.002	0.004

[a] MIC in μg/mL; Jewell and Permain growth medium; inoculum, 10^5 cfu per spot. [b] Inoculum, 10^2 cfu per spot. [c] Inoculum, 10^4 cfu per spot. [d] Constitutive type I lactamase producer. [e] TEM I type lactamase producer. [f] Type IV lactamase producer. [g] Parent organism. [h] Permeability mutant. [i] Lactamase producer.

base).[15] Residues of medium basicity such as the aminobenzimidazoles show stability only against the TEM type enzymes.

Conclusions

Active antibacterials can be obtained by the introduction of basic amino heterocycles in the C-7 position of cephalosporins. Thus cephalosporins with a 7-aminobenzimidazole residue are chemically stable molecules with a broad spectrum of activity against Gram-positive and Gram-negative organisms. They have, however, no useful activity against *Pseudomonas* organisms, have only an average β-lactamase stability, and show an inoculum effect with certain organisms. The cephalosporins with a 7-aminoimidazoline substituent are chemically less stable molecules with a narrow spectrum of activity directed toward the Gram-negative organisms exclusively. They also have no useful activity against *Pseudomonas* and show an inoculum effect with some Gram-negative organisms. However they possess a very good degree of β-lactamase stability. The dependence of the antibacterial spectrum and the β-lactamase stability on the pK_a of the C-7 substituent is an interesting feature of this series and constitutes a novel discovery in cephalosporin and penicillin structure–activity relationships.

These results clearly point toward the imidazolylamino cephalosporins as being a class of compounds with interesting potential.

Intermediate pK_a's can be anticipated in the imidazole series, and fine tuning of the pKa of the C-7 substituent should be much easier than it has been in the benzimidazole or imidazoline series, allowing a better exploration of our various pKa hypotheses. This work will be reported in a future paper.

Experimental Section

IR spectra (not reported) were recorded as liquid films or KBr pellets on a Perkin-Elmer 781 spectrophotometer and were fully consistent with the assigned structure. ^{1}H NMR spectra were recorded on a 90-MHz JEOL FX90Q, a Varian EM390, or a Perkin-Elmer R12 spectrometer.

The free acids of the compounds described in Tables I–III were usually hygroscopic solids, which on analysis proved to be mixtures of zwitterion, hydrobromide, and trifluoroacetate. Meaningful microanalyses were therefore difficult to obtain. IR and ^{1}H NMR have been used to establish the structures of our compounds (Tables V and VI of the supplementary material).

The precursor isonitriles **1a–d** have been obtained according to a published procedure.[10]

Synthesis of the Isocyanide Dibromides (2a–d, 34a,b). General Procedure. Isonitrile **1a–d** (1 equiv) was dissolved in anhydrous toluene (1 mmol in 40 mL of toluene) at −78 °C. A solution of Br_2 (1 equiv) in a few milliliters of CCl_4 (1 mmol in 3 mL CCl_4) was slowly added over 0.5 h to the isonitrile solution. The reaction can be monitored by the initial disappearance of the orange of the bromine, which persists at the end of the reaction. Slightly less than 1 equiv was usually required. The temperature of the reaction was then allowed to reach ambient temperature and the solvent evaporated.

The crude isocyanide dibromide was usually pure enough to be used in the next step. It can, however, be purified by low temperature (−20 °C) silica gel chromatography (CH_2Cl_2–Et_2O 98:2). Physical properties of the isocyanide dibromides thus obtained are shown in Table IV (supplementary material). **2a**: ^{1}H NMR (60 MHz, $CDCl_3$) δ 2.1 (s, 3 H), 3.4, 3.7 (AB, J = 18 Hz, 2 H), 4.92, 5.14 (AB, J = 4.6 Hz, 2 H), 6.9 (s, 1 H), 7.3 (m, 10 H).

Procedure A. Synthesis of 7-Benzimidazolylamino Cephalosporins 3–18 and Heterocyclic Variants 19–22. Isocyanide dibromide (1 equiv) was dissolved in freshly distilled THF (1 mmol in 20–30 mL of THF). The *o*-phenylenediamine (2 equiv) was added to the solution at room temperature. The reaction was monitored by TLC and usually took between 2 and 24 h for completion. The solvent was then evaporated, and the

was purified by silica gel chromatography (CH_2Cl_2–MeOH) at low temperature (−20 °C/0 °C). The purified product was deprotected in a mixture of TFA–anisole (1:1) at room temperature for 30 min. The solvent was evaporated, and the residual oil dissolved in a mixture of CH_2Cl_2–MeOH and precipitated in ether. The solid was recovered by filtration and dried in vacuo (physical data in Table V, supplementary material). **3**: ^{1}H NMR (90 MHz, DMSO-d_6) δ 2.04 (s, 3 H), 3.82 (m, 2 H), 4.76, 5.07 (AB, 2 H, J = 13 Hz), 5.28, 5.84 (AB, 2 H, J = 4.5 Hz), 6.8–7.7 (m, 4 H).

***tert*-Butyl 3,4-Diaminobenzoate.** 3,4-Diaminobenzoic acid (10 g, 66 mmol) was dissolved in dioxane (100 mL) and concentrated H_2SO_4 (10 mL). The solution was cooled to 0 °C and isobutylene (50 mL) was added. The mixture was shaken in a pressure flask at room temperature overnight. Water (200 mL) was then added to the reaction mixture, the pH was adjusted to 10 with 10 N NaOH, the aqueous phase was extracted three times with chloroform (100 mL) and the combined extracts were dried and concentrated to give ester (2.5 g, 18%).

3,4-Diaminobenzyl Alcohol. Commercial 3,4-dinitrobenzyl alcohol (1.98 g, 10 mmol) in ethanol (20 mL) was hydrogenated with 10% Pd/C (100 mg) for 2 h. The catalyst was removed by filtration and the crude diaminobenzyl alcohol (1.36 g, 99%) was used as described in the next step.

1-[[(*tert*-Butoxycarbonyl)amino]methyl]-3,4-diaminobenzene. 4-Chloro-3-nitrobenzonitrile was obtained by nitration of 4-chlorobenzonitrile (50 g, 360 mmol) in concentrated H_2SO_4 (150 mL) with fuming HNO_3 (100 mL) at 10–15 °C for 2 h. After pouring in iced water, filtration, and washing of the solid with water, 45 g (68 %) of compound was obtained (mp 97–98 °C). 4-Amino-3-nitrobenzonitrile was obtained by reaction of 4-chloro-3-nitrobenzonitrile (19 g, 0.1 mmol) with saturated methanolic ammonia (900 mL) at room temperature for 3 days. The solvent was evaporated and the solid recrystallized from MeOH–H_2O to give 10.3 g (63%) of compound (mp 159–162 °C). Reduction of this compound (500 mg, 6 mmol) with BH_3/THF 1 M (24 mmol) at 10 °C for 2 h gave, after workup and extraction with ether, 350 mg (50%) of crude amine, which was used directly in the next step. The amine (350 mg, 10 mmol) was dissolved in dioxane (10 mL), 2-[[(*tert*-butoxycarbonyl)oxy]imino]-2-phenylacetonitrile (258 mg, 10 mmol) was added to the solution which was stirred at room temperature overnight. After removal of the solvent, the crude product was purified by silica gel chromatography (CH_2Cl_2–ether 9:1). Final hydrogenation of this compound (200 mg, 7.5 mmol) over Pd/C (10% in a 1:1 THF–EtOH solution, 6 mL) for 5 h gave the title compound (180 mg) in quantitative yield.

1-(Cyanomethyl)-3,4-diaminobenzene. 4-Amino-3-nitrobenzyl cyanide[27] (6 g, 33.8 mmol) was dissolved in EtOH (100 mL) and reduced by catalytic hydrogenation over Pd/C 10% (3.5 g) for 1.5 h. After filtration of the catalyst and removal of the solvent, diamine was obtained (3.7 g, 73%).

Procedure B. Synthesis of 3-(Acetoxymethyl)-7-[(5-nitrobenzimidazol-2-yl)amino]ceph-3-em-4-carboxylic Acid (45), 3-(Acetoxymethyl)-7-[(imidazo[4,5-*b*]pyridin-2-yl)-amino]ceph-3-em-4-carboxylic Acid (46), and 3-(Acetoxymethyl)-7-[(imidazo[4,5-*c*]pyridin-2-yl)amino]ceph-3-em-4-carboxylic Acid (47). *tert*-Butyl 3-(acetoxymethyl)-7-aminocephalosporanate (**40b**; 4.78 g, 14.6 mmol) in DMF (14 mL) was heated at 50 °C for 20 h with *p*-toluenesulfonic acid monohydrate (3.04 g, 16 mmol) and 2-chloro-5-nitrobenzimidazole (6.32 g, 32 mmol). The solvent was removed under vacuum, and the residue chromatographed over silica gel at −20 °C (CH_2Cl_2–AcOEt 70:30) to give **45** (1.6 g, 22%).

Similarly, **40b** (800 mg, 2.4 mmol) in DMF (2 mL) was heated at 90 °C for 2 h with 2-chloroimidazo[4,5-*b*]pyridine hydrochloride[25] (300 mg, 1.6 mmol). The solvent was removed under vacuum and the residue chromatographed over silica gel (CH_2Cl_2–MeOH 95:5) to give **46** (140 mg, 14%). **40b** (1.7 g, 5.2 mmol) in DMF (3 mL) was heated at 70 °C for 3 h with 2-chloroimidazo[4,5-*c*]pyridine hydrochloride (760 mg, 4 mmol) to give, after removal of the solvent and silica gel chromatography (CH_2Cl_2–MeOH 95:4) **47** (160 mg, 7%). The final deprotections were carried out as in procedure A (physicochemical data in Table V, supplementary material).

Synthesis of 3-(Acetoxymethyl)-7-[(5-aminobenzimidazol-2-yl)amino]ceph-3-em-4-carboxylic Acid (48). Nitrobenzimidazole **45** (1.5 g, 3.07 mmol) in solution in THF–MeOH 1:1 (30 mL) was reduced with a 15% $TiCl_3$ solution (18.4 mL, 18.4 mmol) in water at 0 °C (18 mL). At the end of the reaction the medium was neutralized with $NaHCO_3$ (10% solution) and evaporated, and the mixture purified by SiO_2 chromatography (CH_2Cl_2–MeOH–AcOH, 97:2:1 to 78:14:8) to give ester (650 mg, 46%). The final deprotection was carried out as in procedure A. (^{1}H NMR in Table V, supplementary material).

Synthesis of 3-(Acetoxymethyl)-7-[[5-(acetylamino)-benzimidazol-2-yl]amino]ceph-3-em-4-carboxylic Acid (49). 5-Aminobenzimidazole (**48**; 521 mg, 0.9 mmol) was dissolved in CH_2Cl_2 (100 mL) and AcOH (2 mL). Acetic anhydride (92 mg, 0.9 mmol) was added to the solution at 0 °C. After a few minutes at 0 °C, the solvent was evaporated under vacuum and the residual oil was purified by silica gel chromatography at −20 °C (CH_2Cl_2–MeOH–AcOH 96:2:2). Cephalosporin ester (220 mg, 39%) was thus obtained. The final deprotection was carried out as in procedure A (^{1}H NMR in Table V, supplementary material).

2-Chloro-5-nitrobenzimidazole. Fuming HNO_3 (65 mL) was added over 10 min to a solution of 2-chlorobenzimidazole (4.57 g, 30 mmol) in concentrated H_2SO_4 (32 mL) at −20 °C. The reaction mixture was stirred at 0 °C for 1 h, poured onto ice, and neutralized with concentrated ammonia. The pale yellow solid (5.2 g, 91%) was recovered and dried under vacuum.

2-Chloroimidazo[4,5-*c*]pyridine. 2-(Methylthio)imidazo-[4,5-*c*]pyridine[26] (3.5 g) was dissolved in concentrated HCl (3 mL) at −10 °C. Chlorine was bubbled through the solution for 0.75 h. The solution was allowed to reach 5 °C, poured onto ice, and neutralized to pH 7 with NH_4OH. The solid thus obtained was dissolved in aqueous sodium hydroxide at pH 10 and precipitated by addition of HCl to pH 5.5 (1.8 g, 55%).

Procedure C. Synthesis of N-Substituted Benzimidazolylamino Cephalosporins (41 and 42). **40a** (135 mg, 0.5 mmol) and *N*-methoxybenzimidazolium bromide (**39**) (R_1 = CH_2COO-*t*-Bu) (171 mg, 0.5 mmol) were solubilized in methanol (1 mL) and stirred at room temperature for 48 h. The solvent was evaporated, and the residue purified by crystallization from ether–2-propanol 1:1 to give **41**, 100 mg (52%) (mp 196–198 °C). Similarly, 40b (328 mg, 1 mmol) and *N*-methoxybenzimidazolium iodide (**39**;[9] R_1 = Me) (290 mg, 1 mmol) were solubilized in methanol (2 mL) and stirred at room temperature for 3 days. The solvent was evaporated, and the residue purified by silica gel chromatography (CH_2Cl_2–MeOH–AcOH 98:1:1) to give **42** (250 mg). The pure esters thus obtained were deprotected as in procedure A (physicochemical data in Table V, supplementary material).

***tert*-Butyl 3-Methoxy-1-benzimidazolium Acetate Bromide Salt (39). (R_1 = CH_2COO-*t*-Bu).** *N*-methoxybenzimidazole[23] (296 mg, 2 mmol) and *tert*-butyl bromoacetate (390 mg, 2 mmol) were stirred at room temperature overnight. The crude salt obtained after trituration with ether (340 mg, 50%) was used without further purification in the next step.

Synthesis of Diphenylmethyl 3-(Acetoxymethyl)-7-(benzimidazol-2-ylamino)-7-methoxyceph-3-em-4-carboxylate (38). Isonitrile **1b** (8 g, 17.7 mmol) was dissolved in anhydrous THF (12 mL). Red Cu_2O (260 mg) and MeOOCSSMe[7] (4 mL, 39.8 mmol) were added to the reaction mixture, which was kept under argon at 45 °C for 3.5 h. At the end of the reaction the Cu_2O was filtered off and the residue purified by silica gel

(17) Trani, A.; Bellasio, E. *J. Heterocycl. Chem.* **1973**, *11*, 257.
(18) Matier, W. L.; Owens, D. A.; Comer, W. T. *J. Med. Chem.* **1973**, *16*, 901.
(19) Fitzgerald, R. J. *Inorg. Chem.* **1969**, *8*, 2254.
(20) Staab, H. A.; Vögtle, F. *Chem. Ber.* **1965**, 2691.
(21) Poduska, K.; Rudinger, J.; Sorm, F. *Collect. Czech. Chem. Commun.* **1955**, *20*, 1174.
(22) Worrall, D. E. Organic Synthesis; Wiley: New York, 1941; Collect. Vol. I, p 413.
(23) Takahashi, S.; Kano, H. *Chem. Pharm. Bull.* **1963**, *11*, 1375.
(24) Mozingo, R.; Harris, S. A.; Wolf, D. E.; Hoffhine, C. E.; Easton, N. R.; Folkers, K. *J. Am. Chem. Soc.* **1945**, *67*, 2092.
(25) Dornow, A.; Hahmann, O. *Arch. Pharm.* **1957**, *20*, 290.
(26) Barlin, G. B. *J. Chem. Soc. B* **1966**, 285.
(27) Gabriel, S. *Chem. Ber.* **1882**, *15*, 834.

chromatography (AcOEt–cyclohexane, 3:10) at low temperature, giving the desired compound **33b** (4 g, 46%). Δ^2-Isomer (700 mg) was also isolated. Compound **37** was obtained according to general procedure A. Cephalosporin **37** (1.1 g, 1.6 mmol) was dissolved in anhydrous MeOH (40 mL), DMF (2 mL), and CH_2Cl_2 (6 mL) at −15 °C. Pyridine (290 μL, 3.5 mmol) was added to the cold solution, followed by $HgCl_2$ (432 mg, 1.9 mmol) solubilized in DMF. The reaction mixture was stirred for 1 h between −15 and 0 °C, filtered through Celite, and saturated with H_2S at 0 °C. Mercuric sulfide was filtered off, the organic phase was concentrated, and the residue was dissolved in a small amount of MeOH and precipitated in ether to give **38** (780 mg, 78%).

Deprotection of ester **38** to the free acid was carried out as in procedure A: ^{1}H NMR (90 MHz, DMSO-d_6) δ 2.02 (s, 3 H), 3.35, 3.60 (AB, 2 H, J = 16.5 Hz), 3.65 (s, 3 H), 4.85, 5.15 (AB, 2 H, J = 13.9 Hz), 5.35 (s, 1 H), 7.2, 7.6, (m, 4 H).

Procedure D. Synthesis of 7-Imidazolinylamino Cephalosporins 23-32. Ethylenediamine (1 mmol in 10 mL of THF, 2 equiv) was added to a solution of isocyanide dibromide (1 mmol in 50 mL of THF, 1 equiv) at −78 °C. After 1 h at −78 °C, 2 equiv of TFA was added to the cold solution to protonate any residual free amino group, the temperature was then allowed to become ambient. The solvent was evaporated, and the crude product was purified by low temperature (−20 °C) silica gel chromatography (CH_2Cl_2–MeOH). The purified product was deprotected with TFA/anisole under the usual conditions (procedure A) (physicochemical data in Table VI, supplementary material).

Procedure E. Synthesis of 7-Imidazolinylamino Cephalosporins 23, 43, and 44. 2-Chloroimidazolinehydrochloride[17] (1 equiv) and 7-aminocephalosporanate (1 equiv) were stirred in acetonitrile (1 mmol in 10 mL) at room temperature for 8–24 h. The mixture was then evaporated to dryness and the residue purified by low-temperature silica gel chromatography (CH_2Cl_2–MeOH). The deprotection of the ester was carried out as usual (procedure A) (physicochemical data in Table VI, supplementary material). **23**: ^{1}H NMR (90 MHz, DMSO-d_6) δ 2.08 (s, 3 H), 3.38, 3.65 (AB, 2 H, J = 18 Hz), 3.65 (s, 4 H), 5.12, 5.52 (AB, 2 H, J = 4.5 Hz).

Synthesis of Diphenylmethyl 7-(Imidazol-2-ylamino)-7-methoxy-3-methylceph-3-em-4-carboxylate (36). The synthesis was carried out according to general procedure D, starting with the isocyanide dibromide **34a**. The SMe–OMe exchange reaction was carried out as described for the synthesis of **38**. The residual oil, after elimination of all mercuric salts, was crystallized from MeOH–Ether (mp 140–143 °C). The deprotection of ester **36** to the free acid was carried out as in procedure A: ^{1}H NMR (DMSO-d_6) δ 2.15 (s, 3 H), 3.3 (m, 2 H), 3.45 (s, 3 H), 3.65 (s, 4 H), 5.15 (s, 1 H).

1-(4-Cyanophenyl)-1,2-diaminoethane. A clear solution of hydroxylamine, prepared from NH_2OH·HCl (1.91 g, 27 mmol) and NaOH (1 g, 25 mmol) in MeOH (12.5 mL) after filtration of precipitated NaCl, was added to a solution of 4-cyano-β-nitrostyrene[22] (3.73 g, 21 mmol) in ethanol (12.5 mL). After 15 min the mixture was evaporated to dryness and extracted with $CHCl_3$–MeOH 9:1. The nitrohydroxylamine crystallizes from the organic solution (2.03 g, 44%).

The nitrohydroxylamine (3.1 g, 15 mmol) dissolved in MeOH (200 mL) was hydrogenated over Raney Ni (1.8 g) at 50 psi and room temperature until hydrogen (4 equiv) was taken up. The Raney Ni was then filtered off. The solvent was evaporated, and the residual red oil triturated with CH_2Cl_2 to give a light brown solid of the title compound (1.7 g, 70%).

Acknowledgment. We are grateful to Dr. J. C. Arnould for the synthesis of compounds **19**, **46**, and **47**. We also thank Dr. T. D. Hennessey and his colleagues for performing the biological evaluation.

Supplementary Material Available: Table IV listing physical parameters of isocyanide dibromides (^{1}H NMR, microanalysis) and Tables V and VI giving ^{1}H NMR data on benzimidazolylamino cephalosporins and imidazolinylamino cephalosporins, respectively, together with literature references for the synthesis of the starting materials (4 pages). Ordering information is given on any current masthead page.