Quaternary Heterocyclylamino β-Lactams: A Generic Alternative to the Classical Acylamino Side Chain

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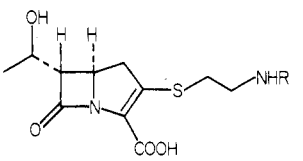
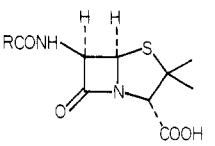
6β-[(1-Substituted-4-pyridinio)amino]penam-3-carboxylates and 7β-[(1-substituted-4-pyridinio)amino]ceph-3-em-4-carboxylates have been found to be interesting new classes of antibacterial β-lactams, readily available by S_N2 Ar coupling of fluoro-substituted quaternized pyridines and appropriate amino lactam carboxylic acids. Compared to penicillin G, the penam **12c** exhibited a spectrum extended to Gram-negative species, such as *Escherichia, Shigella, Klebsiella* and *Enterobacter*, offset by a loss of potency against Gram-positive species. Excluding *Pseudomonas*, many examples of the cephems showed excellent activity against the above Gram-negative organisms, and in some cases, such as **15i**, the spectrum included good performance against the staphylococci and streptococci. With *Serratia* and many *Proteus* species, there was an adverse inoculum and medium effect which was not observed in the good Gram-positive reach of the cephem series.

The recent discoveries of thienamycin (**1a**),[1,2] the epithienamycins,[3,4] the olivanic acids,[5-10] the PS 5-7 series,[11-13] and the carpetimycins[14,15] have stimulated a new wave of structure-activity speculation in the field of β-lactam antibiotics. In contrast, the classical acylamino prerequisite for substantial antibacterial activity in penicillins (**2**) and cephalosporins (**3**) has allowed only one exception: the substituted amidine class best represented by mecillinam (**4**).[16,17]



1a, R = H
b, R = $COCH_3$
c, R = CH=NH



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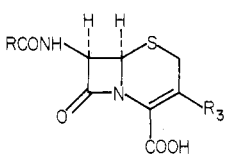

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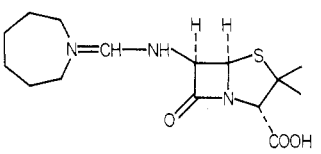

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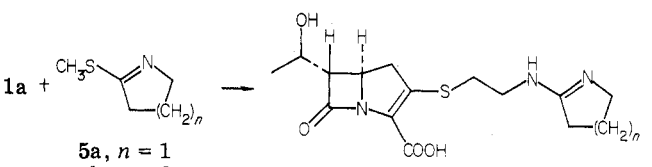
In these laboratories, **1a** was considered to be too unstable to be used directly in the clinic, and an extensive program of derivatization was launched with the prime objective of finding a stable analogue which retained the outstanding broad spectrum of the natural product. A major pathway of decomposition in aqueous solution was thought to be polymerization by self-acylation in a manner similar to the polymerization of ampicillin.[18] Thereafter, diminution of the nucleophilic character of the cysteamine side chain was a productive line of research which led to the *N*-formimidoyl derivative (**1c**),[19] a stable crystalline compound more active than **1a**.

Many other thienamycin amidines were prepared, including the cyclic examples **6a** and **6b**.



5a, $n = 1$
b, $n = 3$

6a, $n = 1$
b, $n = 3$

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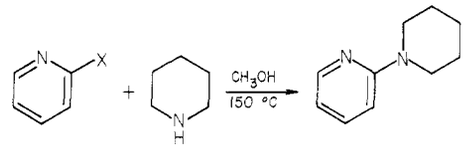
Table I. Antibacterial Activity[a] of Thienamycin Derivatives against Aerobic Organisms

organism	MB no.	6a	6b	8	10a	10b	1b	1c
Straphylococcus aureus	2314	38 (40)	41 (42)	42.5 (41.5)	42 (40)	36 (35.5)	39 (41)	42 (42)
Streptococcus pyogenes	2874	40 (40)	37.5 (40)	37 (39)	37.5 (40)	35.2 (37.7)	37 (38)	43 (43)
Escherichia coli	2891	26.5 (26.5)	29 (29.5)	25.5 (26.5)	27.5 (27)	28.5 (29)	31 (28)	31.2 (31)
Shigella sp.	2880	32 (32)	31 (33)	29 (29)	29.7 (28)	29.5 (29.5)	34.5 (33)	30.5 (29.5)
Salmonella sp.	826	28 (29)	27 (29)	27.5 (28.2)	28 (27.5)	26 (28)	30.5 (29)	29.5 (28)
Enterobacter aerogenes	2828	27 (24)	26.5 (25.5)	29 (25)	29 (25.7)	27 (25)	30 (25)	29.2 (26)
Klebsiella sp.	2921	24 (24.5)	24 (25.5)	26 (25.5)	27 (26)	24 (24.7)	28 (25.5)	26.5 (25.5)
Serratia marcessans	2855	25.5 (25)	24.5 (25.5)	26 (25)	26.5 (26)	25 (25)	28 (25)	24.5 (23)
Morganella morganii	2834	24.5 (24.5)	24.5 (24)	27 (24.7)	26 (24)	26.5 (24)	28.5 (24.5)	27 (25)
Pseudomonas aeruginosa	2835	25.5 (27)	20 (27)	23.7 (26.5)	28 (26.5)	11 (27)	12 (26.5)	30.5 (28.7)
Pseudomonas aeruginosa	3350	25.5 (26)	16 (24.5)	17.5 (26)	24.5 (25.7)	11.2 (25.2)	10 (25)	31 (27)

[a] 25 μg/disk; zone diameters in millimeters; a 2-mm difference in zone diameter corresponds approximately to a 2 times difference in activity; values in parentheses are for thienamycin (1a). We are grateful to Ms. J. Kahan of MSDRL for these assays.

The progression from the Δ^1-pyrroline to the Δ^1-tetrahydroazepine caused a precipitous selective drop in antipseudomonal activity (Table I) to the extent that the Δ^1-tetrahydropyridine analogue (n = 2) would probably have been an uninteresting addition to the series. There remained, however, the intriguing aromatic variation to be considered.

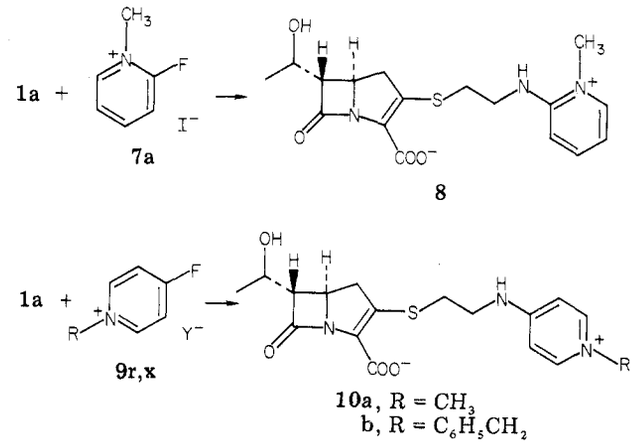
The chemical sensitivity of **1a** had placed a premium on reagents which avoided extremes of time, temperature, and pH, so that direct bond formation from the cysteamine nitrogen to pyridine was not feasible by reactions such as that between piperidine and the halopyridines.[20]



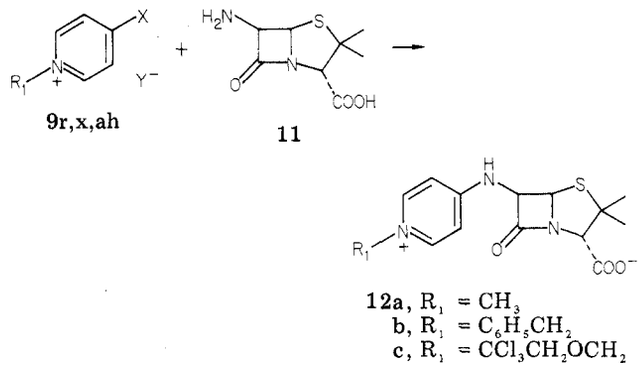
X = F: 10^3k = 0.271 L mol^{-1} s^{-1}
X = Cl: 10^3k = 0.019 L mol^{-1} s^{-1}

Fortunately, the increase in activity of 2- and 4-halopyridines toward nucleophiles by quaternizing the ring nitrogen has been known for many years.[21,22] Quantitative kinetic treatment[23-25] of this S_N2 Ar process demonstrated that N-methylation afforded >10^6-fold increase in reactivity over the parent halopyridine, and within the halogen series, fluorine is outstanding by a factor of >10^2. The ortho reagents were consistently more reactive than the para isomers, and again the disparity was greatest for the fluoro analogues. The use of such reagents to prepare many functional groups, such as esters, amides, sulfides, halides, and lactones, and to induce intramolecular cyclizations and rearrangements has been extensively studied[26] but always with respect to end products which did not incorporate the quaternary heterocycle.

Scheme I



Scheme II



With thienamycin, reaction with 2-fluoro-1-methylpyridinium iodide (**7a**)[25] and 4-fluoro-1-methylpyridinium iodide (**9r**)[24] in aqueous solution at room temperature and pH 8.5 rapidly formed the zwitterionic N-heterocyclic

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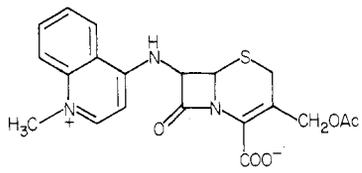
derivatives 8 and **10a**. Compound **10a** was as active as *N*-formimidoylthienamycin (**1c**), except for *Pseudomonas*. This skewed antibacterial spectrum was even more pronounced for the ortho analogue 8 and was reminiscent of the activity profile of *N*-acetylthienamycin (**1b**) (Scheme I).

With this *N*-acyl equivalence in mind, the coupling reaction was extended to 6-aminopenicillanic acid (**11**), to the phenylglycyl side chain of ampicillin, and then to a wide range of reagents, **9r–al** (Table III), and 7β-aminocephalosporanic acids, **13a–m** (Table IV) (Schemes II and III). The products were **12a–c** (Table V), **14a–c** (Table VI), and **15a–am** (Tables VII, VIII, and IX). For Tables V, VI, VIII, and IX, see paragraph at the end of the paper concerning supplementary material.

By exactly analogous reactions were prepared the 1′-methyl-4′-quinolinio- (**16**), 1′-methyl-2′-pyrimidinio- (**17**),



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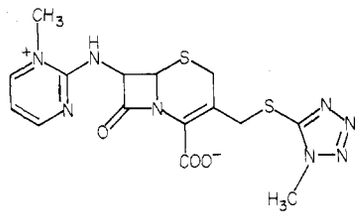

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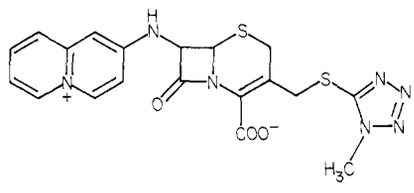

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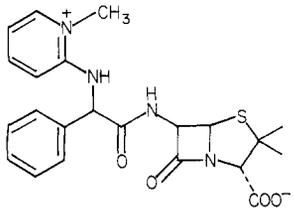

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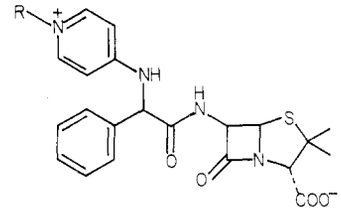
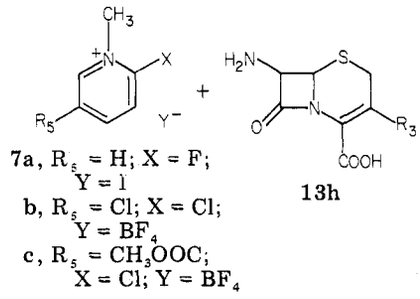


20a, R = CH_3
b, R = $CH_2C_6H_5$

and 2′-quinoliziniocephalosporin (**18**) (Table X); 1′-methyl-2′-pyridinio- (**19**), 1′-methyl-4′-pyridinio- (**20a**), and 1′-benzyl-4′-pyridinioampicillin (**20b**) (Table XI). The additional reagents were 4-chloro-1-methylquinolinium iodide,[38] 2-fluoro-1-methylpyrimidinium fluoroborate, and 2-fluoroquinolizinium bromide. For Tables X and XI, see paragraph at the end of the paper concerning supplementary material.

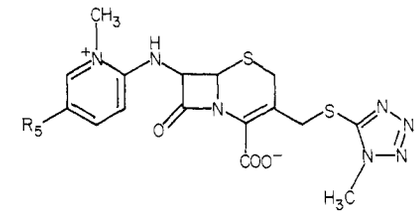
Chemistry. (a) Quaternization of Halo Heterocycles. Conditions were taken from two extensive reviews.[39,40] Only very active alkylating reagents reacted

Scheme III

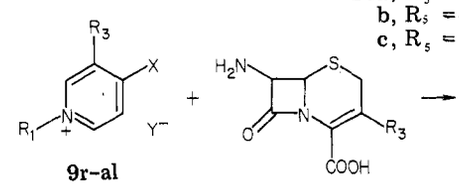


7a, R_5 = H; X = F; Y = I
b, R_5 = Cl; X = Cl; Y = BF_4
c, R_5 = CH_3OOC; X = Cl; Y = BF_4

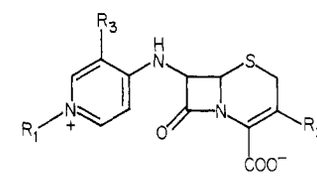
13h



14a, R_5 = H
b, R_5 = Cl
c, R_5 = CH_3OOC

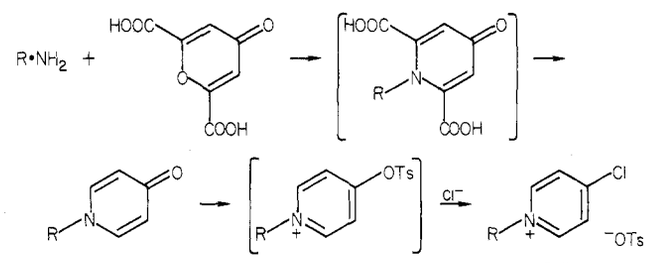


9r-al **13a-m**



15a-am

Scheme IV



9d,f,g

with the weakly basic chloro and fluoro heterocycles. In the pyridine series, the range of substituents could be extended by converting primary amines to 1-substituted 4-pyridones,[41] followed by reaction with *p*-toluenesulfonyl chloride to form 1-substituted 4-chloropyridinium tosylates[22] (Scheme IV).

The quaternary haloheterocycles were reactive compounds and, particularly in the case of the fluoro analogues, were difficult to free completely from the pyridones, which were readily formed by moisture. The pyridones, however, and the related quaternary chloro, fluoro, and amino heterocycles displayed highly individual NMR spectra, which allowed quantitative assessment of the reagents and products. Since the pyridones took no part in the coupling reaction, the crude (>70% to >95%) halo reagents were used directly, and their β-lactam derivatives were isolated chromatographically in a highly purified state. The

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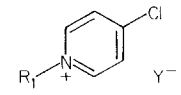
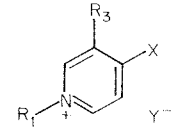
Table II. Quaternary 4-Chloropyridinium Precursors (9a–q)



				^{1}H NMR (Varian T60), δ			
	R_1	Y^-	solvent[a]	N^+CH[b]	$(H_3 + H_5)$[c]	$(H_2 + H_6)$[c]	other assignments
a[d]	CH_3	I	A	4.35 (s, 3 H)	8.12	8.76	
b	CH_3 (and $R_3 = CH_3$)	I	B	4.33 (s, 3 H)	8.35 (d, 1 H, J = 7 Hz, H_5)	9.18 (s, 1 H, H_2)	2.49 (s, 3 H, $R_3 = CH_3$), 8.94 (d, 1 H, J = 7 Hz, H_6)
c	CH_3CH_2	BF_4	A	4.63 (q, 2 H, J = 7.5 Hz)	8.14	8.83	1.63 (t, 3 H, J = 7.5 Hz, CH_3)
d	$(CH_3)_2CH$	OTs	B	4.20 (m, 1 H)	8.43	9.27	1.61 [d, 6 H, J = 7 Hz, $(CH_3)_2$], 2.33 (s, 3 H, CH_3), 7.15 (d, 2 H, J = 8 Hz, Ar), 7.57 (d, 2 H, J = 8 Hz, Ar)
e	$C_6H_5CH_2$	Br	A	5.83	8.15	8.89	7.54 (s, 5 H, Ar)
f	$C_6H_5CH_2CH_2$	OTs	B	4.83 (t, 2 H, J = 8 Hz)	8.30	9.02	2.30 (s, 3 H, CH_3), 3.25 (t, 2 H, J = 8 Hz, CH_2), 7.08 (d, 2 H, J = 8 Hz, Ar), 7.25 (s, 5 H, Ar), 7.42 (d, 2 H, J = 8 Hz, Ar)
g	2-$CH_3(C_6H_5)$	OTs	A		8.31	8.90	2.16 (s, 3 H, CH_3), 2.37 (s, 3 H, CH_3), 7.36 (d, 2 H, J = 7 Hz, Ar), 7.37–7.67 (m, 4 H, Ar), 7.70 (d, 2 H, J = 7 Hz, Ar)
h	4-$CH_3O(C_6H_4)CH_2$	Br	B	5.80	8.28	9.18	3.73 (s, 3 H, OCH_3), 6.92 (d, 2 H, J = 9 Hz, Ar), 7.44 (d, 2 H, J = 9 Hz, Ar)
i	3,4-$(CH_3O)_2(C_6H_3)CH_2$	Br	A	5.81	8.22	8.98	3.94 [s, 6 H, $(OCH_3)_2$]
j	4-$CN(C_6H_4)CH_2$	Br	B	6.00	8.41	9.27	7.70 (d, 2 H, J = 7.5 Hz, Ar), 7.93 (d, 2 H, J = 7.5 Hz, Ar)
k	4-$F(C_6H_4)CH_2$	I	B	5.82	8.36	9.20	7.27 (dd, 2 H, J_{HH} = 9 Hz, J_{HF} = 9 Hz, Ar $H_{3'}$ + $H_{5'}$), 7.64 (dd, 2 H, J_{HH} = 9 Hz, J_{HF} = 6 Hz, Ar $H_{2'}$ + $H_{6'}$)
l	CH_3OCH_2	Br	A	5.85	8.21	8.91	3.51 (s, 3 H, OCH_3)
m	$C_6H_5CH_2OCH_2$	Br	B	6.32	8.54	9.45	4.90 (s, 2 H, Ar CH_2), 7.47 (s, 5 H, Ar)
n	$CCl_3CH_2OCH_2$	Br	A	6.23	8.30	9.08	4.58 (s, 2 H, CCl_3CH_2)
o	$CF_3CH_2OCH_2$	Br	A	6.10	8.26	9.00	4.36 (q, 2 H, J_{HF} = 8.5 Hz, CF_3CH_2)
p	CH_3CH_2O	Cl	A		8.35	9.21	1.52 (t, 3 H, J = 7.5 Hz, CH_3), 4.81 (q, 2 H, J = 7.5 Hz, CH_2O)
q	4-$NO_2(C_6H_4)CH_2OCH_2$	Br	A	6.25	8.35	9.13	5.08 (s, 2 H, Ar CH_2), 7.67 (d, 2 H, J = 9 Hz, Ar), 8.22 (d, 2 H, J = 9 Hz, Ar)

[a] A = D_2O; B = Me_2SO-d_6. [b] s, 2 H. [c] d, 2 H, J = 7 Hz, unless otherwise designated. [d] Reference 23.

Table III. Quaternary 4-Halopyridinium Reagents (9r–al)

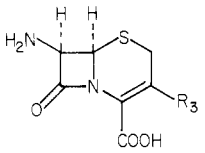


						^{1}H NMR (Varian T60), δ			
	R_1	R_3	Y^-	X	solvent[a]	N^+CH[b]	$(H_3 + H_5)$[c]	$(H_2 + H_6)$[d]	other assignments
r[e]	CH_3	H	I	F	A	4.37 (s, 3 H)	7.82	8.84	
s	CH_3	CH_3	I	F	B	4.30 (s, 3 H)	8.05 (dd, 1 H, J = 7 & 7 Hz, H_5)	9.11 (m, 2 H)	2.39 (s, 3 H, CH_3)

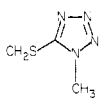
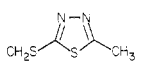
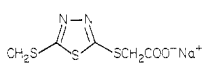
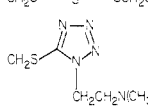
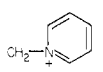
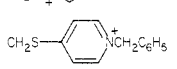
t	CH_3	CH_3OOC	BF_4	Cl	A	4.42 (s, 3 H)	8.23 (d, 1 H, J = 7 Hz, H_5)	9.33 (d, 1 H, J = 2 Hz, H_2)	4.05 (s, 3 H, OCH_3), 8.84 (dd, 1 H, J = 7 & 2 Hz, H_6)
u	CH_3CH_2	H	BF_4	F	A	4.68 (q, 2 H, J = 7 Hz)	7.94 (poorly resolved)	8.96	1.63 (t, 3 H, J = 7 Hz, CH_3)
v	$(CH_3)_2CH$	H	OTs	F	B	5.05 (m, 1 H, J = 6.5 Hz)	8.18	9.33	1.60 [d, 6 H, J = 6.5 Hz, $(CH_3)_2$], 2.30 (s, 3 H, Ar CH_3), 7.12 (d, 2 H, J = 8 Hz, Ar), 7.52 (d, 2 H, J = 8 Hz, Ar)
w	$CH_3CH_2OCOCH_2$	H	Br	Cl	B	5.88	8.59 (d, 2 H, J = 7 Hz)	9.31 (d, 2 H, J = 7 Hz)	1.31 (t, 3 H, J = 7.5 Hz, CH_3), 4.31 (q, 2 H, J = 7.5 Hz, OCH_2)
x	$C_6H_5CH_2$	H	Br	F	A	6.04	8.29	9.58	7.42–7.70 (m, 5 H, Ar)
y	$C_6H_5CH_2CH_2$	H	OTs	F	A	4.85 (t, 2 H, J = 6 Hz)	8.55	8.65	2.38 (s, 3 H, CH_3), 3.30 (t, 2 H, J = 6 Hz, CH_2), 7.27 (m, 5 H, Ar), 7.35 (d, 2 H, J = 8 Hz, Ar), 7.72 (d, 2 H, J = 8 Hz, Ar)
z	2-$CH_3(C_6H_4)$	H	OTs	F	A		8.04	9.02	2.14 (s, 3 H, tolyl CH_3), 2.37 (s, 3 H, tosyl CH_3), 7.33 (d, 2 H, J = 8 Hz, Ar), 7.67 (d, 2 H, J = 9 Hz, Ar), 7.33–7.68 (m, 4 H, Ar)
aa		H	Br	Cl	A	5.95	8.23 (d, 2 H, J = 7 Hz)	8.94 (α, 2 H, J = 7 Hz)	6.63 (dd, 1 H, J = 3 & 2 Hz, H_4), 6.95 (d, 1 H, J = 3 Hz, H_3), 7.70 (d, 1 H, J = 2 Hz, H_2)
ab	4-$CH_3O(C_6H_4)CH_2$	H	Br	F	A	5.89	8.02	9.13	3.94 (s, 3 H, OCH_3), 7.15 (d, 2 H, J = 9 Hz, $H_{3'}$ + $H_{5'}$), 7.63 (d, 2 H, J = 9 Hz, $H_{2'}$ + $H_{6'}$)
ac	3,4-$(CH_3O)_2(C_6H_3)CH_2$	H	Br	F	A	5.92	8.01	9.20	4.00 (s, 3 H, OCH_3), 4.03 (s, 3 H, OCH_3), 7.05–7.43 (m, 3 H, Ar)
ad	4-$CN(C_6H_4)CH_2$	H	Br	F	B	6.22	8.66 (poorly resolved)	9.62	7.56 (d, 2 H, J = 8 Hz, Ar), 8.24 (d, 2 H, J = 8 Hz, Ar)
ae	4-$F(C_6H_4)CH_2$	H	I	F	A	5.81	7.89 (not resolved)	8.99 (3 d, 2 H, J = 7, 5, & 2 Hz)	7.24 (dd, 2 H, J_{HH} = 9 Hz, J_{HF} = 5 Hz, Ar), 7.48 (dd, 2 H, J_{HH} = 9 Hz, J_{HF} = 3 Hz, Ar)
af	CH_3OCH_2	H	Br	F	A	5.88	8.23	9.10	3.42 (s, 3 H, CH_3)
ag	$C_6H_5CH_2OCH_2$	H	Br	F	B	6.03	8.27	9.36	4.71 (s, 2 H, Ar CH_2), 7.32 (s, 5 H, Ar)
ah	$CCl_3CH_2OCH_2$	H	Br	F	A	6.17	8.11	9.12	4.54 (s, 2 H, CH_2CCl_3)
ai	$CF_3CH_2OCH_2$	H	Br	F	A	6.10	8.09	9.14	4.36 (q, 2 H, J = 8.5 Hz, CH_2CF_3)
aj	$CCl_3CH_2OCH_2$	$(CH_3)_3COOC$	Br	Cl	B	5.92	7.26 (d, 1 H, J = 7 Hz, H_5)	9.00 (d, 1 H, J = 2 Hz, H_2)	1.57 (s, 9 H, t-Bu), 4.57 (s, 2 H, CH_2CCl_3), 8.56 (dd, 1 H, J = 7 & 2 Hz, H_6)
ak	CH_3CH_2O	H	Cl	F	B		8.35	9.83	1.41 (t, 3 H, J = 7 Hz, CH_3), 4.76 (q, 2 H, J = 7 Hz, CH_2)
al	4-$NO_2(C_6H_4)CH_2OCH_2$	H	Br	F	B	6.26	8.33	9.51	5.00 (s, 2 H, Ar CH_2), 7.73 (d, 2 H, J = 9 Hz, $H_{3'}$ + $H_{5'}$), 8.13 (d, 2 H, J = 9 Hz, $H_{2'}$ + $H_{6'}$)

[a] A = D_2O; B = Me_2SO-d_6. [b] s, 2 H. [c] dd, 2 H, J_{HH} = 7 Hz, J_{HF} = 7 Hz. [d] dd, 2 H, J_{HH} = 7 Hz, J_{HF} = 5 Hz, unless otherwise designated. [e] Reference 24.

Table IV. 3-Substituted 7-Aminocephalosporanic Acids



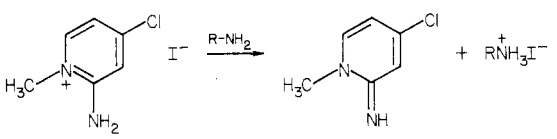
13a-m

	R_3	ref
a	CH_2OAc	27
b	CH_2OH	28
c	CH_2N_3	29
d	$CH_2SCSOCH_2CH_3$	30
e	CH_3	31
f	Cl	32
g	H	33
h		34
i		34
j		35
k		36
l		37
m		

products were characterized by high-field NMR spectra.

(b) Formation of Quaternary Amino Heterocycles. The reaction was of quite general application to primary and secondary amino acids forming zwitterionic heterocyclylaminocarboxylates in aqueous solution in the pH range 6–8.5 at room temperature. Due to the relatively weak nucleophilic character of the bicyclic amino lactams, it was necessary in all but a few cases to use the fluoro reagents to effect successful coupling. For example, no reaction occurred between **13a** and 2-chloro-1-methylpyrimidinium trifluoromethanesulfonate[42] or between **13h** and **9e**. Substitution of the fluoro analogues in both cases led to the desired products **17** and **15i**. Most of the fluoro reagents in Table III were prepared from the corresponding chloro quaternaries (Table II) by exchange with KF in dimethylformamide under anhydrous conditions. The exceptions were **7a** and the pyrimidine, where the fluoro heterocycles were directly alkylated.

The products of the KF exchange are listed in Table III with the same counterion as in the starting material. In practice, since an excess of fluoride ion was used to displace chloride, the counterion was probably a mixture of all possible species. Simple amines reacted with even the chloro quaternaries with the greatest ease, except for 2-amino-4-chloro-1-methylpyridinium iodide, which was simply deprotonated to the iminopyridone.

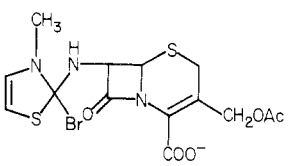


Despite instantaneous reaction with diethylamine, 1,3-dimethyl-2-fluoropyridinium iodide was too hindered to react with **16a** and at pH 7.5 was solvolyzed to 1,3-dimethyl-2-pyridone.

The quaternary halo heterocycles were increasingly rapidly solvolyzed to the corresponding pyridones at pH >8, but this loss in an unbuffered situation was self-limiting, since it generated an equivalent of HF. The reaction is best carried out in aqueous solution by the addition of base from an automatic burette controlled by a pH meter. The reaction also succeeded in the presence of a tertiary base in nonaqueous solvents, the best of which was Me_2SO under strictly anhydrous conditions: any water present very rapidly solvolyzed the reagent. The most extreme example was 2-fluoro-1-methylpyrimidinium tetrafluoroborate, where only the pyrimidinone NMR spectrum could be measured in either D_2O or Me_2SO-d_6. Nonetheless, successful coupling was achieved to **13h** by repetitive addition of the solid fluoro reagent under standard aqueous conditions at pH 7. The less reactive chloro analogue[42] was also solvolyzed instantly in Me_2SO-d_6, was 30% converted to 1-methyl-2-pyrimidinone in D_2O in 10 min at room temperature, and did not react with **13h**.

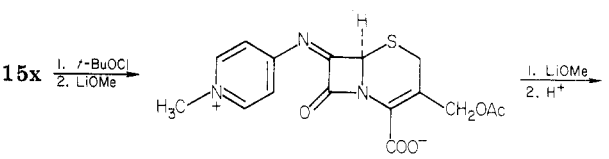
The need for nonaqueous conditions arose from the very poor water solubility of 4-fluoro-1-(4-fluorobenzyl)-pyridinium iodide (**9ae**).

Attempts to form a 7β-(2-thiazolioamino)cephalosporin using **13a** and 2-bromo-3-methylthiazolium bromide were unsuccessful: the required intermediate (**21**) allows the alternative expulsion of thiolate, which in conjunction with a β-lactam can lead to polymerization.

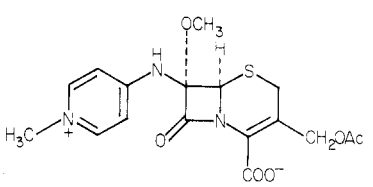


21

Entry to the cephamycin series was achieved by direct 7α-methoxylation of **15x** by the *tert*-butyl hypochlorite/lithium methoxide process[43,44] without the need for ester formation.



22



23

Intermediate **22** with a fully developed positive charge is probably a better acceptor of methoxide anion at position 7 than is the acylamine intermediate in conventional cephalosporins.

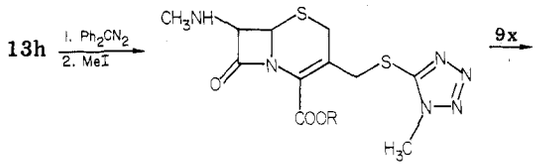
After an unsuccessful attempt had been made to alkylate directly the secondary amine function in **15a** using *O*-methyl-1,3-diisopropylurea, substrate **13h** was converted

(42) Commerically available.

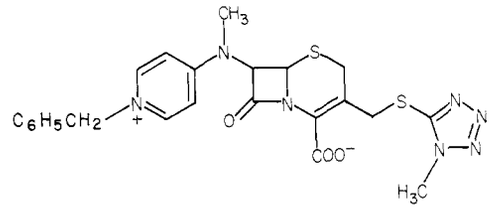
(43) J. E. Baldwin and F. J .Urban, *J. Am. Chem. Soc.*, **95**, 2401 (1973).

(44) G. A. Koeppel and R. E. Koehler, *J. Am. Chem. Soc.*, **95**, 2403 (1973).

to the benzhydryl ester (**24**) and then indiscriminately methylated, and the mixture of primary, secondary (**25a**), and tertiary amino esters was chromatographically separated. The ester group in **25a** was cleaved in the usual manner, and the *N*-methylamino acid (**25b**) was coupled with the fluoro reagent (**9x**) to yield **26**.

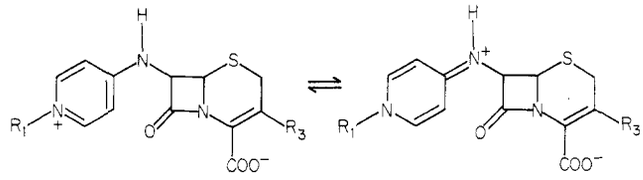


25a, R = $CH(C_6H_5)_2$
b, R = H



26

NMR Spectra of 7β-(4-Pyridinioamino)ceph-3-em-4-carboxylates (Tables VIII and IX). The greater complexity of the pyridinium absorbance in Me_2SO-d_6 compared to that in D_2O indicated that the iminopyridone (**27**) is the dominant structure in that medium. In D_2O the broad singlet for $H_{2'}$ and $H_{6'}$ resolved to a normal doublet on raising the temperature of the solution.



15 **27**

Antibacterial Properties and Structure–Activity Relationships. Activity in the penicillin series, Table XII, showed for **12c** a significant displacement of the penicillin G spectrum toward Gram-negative species of *Escherichia*, *Shigella*, *Salmonella*, *Klebsiella*, and *Enterobacter*, offset by a loss in Gram-positive potency. No activity was observed for the *Proteus*, *Pseudomonas*, or *Serratia* species tested. Compound **12c** was notably superior to mecillinam (**4**).

The hybrid examples **19** and **20a**, derived from ampicillin, showed generally parallel activities (Table XII) with no advantage over the parent antibiotic. Moreover, **20b** was markedly inferior to **20a**.

Although considerably less potent, the first of the new quaternary heterocyclylaminocephems (**15x**) exhibited a similar spectrum to cephalothin with indications of improved activity against *Proteus vulgaris*. The structure offered many degrees of freedom in exploring the limits of the new lead (Tables VII, XIII and XIV), beginning with replacement of the 3-acetoxy group with the metabolically stable methyltetrazolylthio unit. Compound **15a** was considerably improved in potency and spectrum, with *Escherichia*, *Enterobacter*, *Proteus*, and *Serratia* species now susceptible. The isomeric **14a** was much less active, which deemphasized research interest in the ortho series. Of the two other ortho examples, **14b** was made more active against Gram-negative bacteria by chloro substitution in the pyridine ring but not to the level of the unsubstituted **15a**. The ester group in **14c** caused complete deactivation. Compound **15b**, the 7-epimer of **15a**, was also inactive.

In view of the importance of the cephamycin family of antibiotics, it was a matter of considerable disappointment to find that the conversion of **15x** to the 7α-methoxy derivative (**23**) gave an almost inactive compound. Equally disconcerting was the observation that the new series was susceptible to media effects, which were first seen with **15a** and *Serratia* species. When these organisms were tested in tryptocase soy agar, the minimum inhibitory concentration rose to 128 μg/mL. Later it was found that this phenomenon in varying degree was demonstrable with most Gram-negative but not Gram-positive species.

A consequence of this effect was a discrepancy between the in vitro MIC values and the results of in vivo mouse protection assays, particularly with the *Proteus* species. Better correlation was achieved when the in vitro assay was based entirely on tryptocase soy agar as the growth medium, at first using 10^5 (Table XIV) and finally 10^4 cfu (colony forming units) per inoculum spot (Table XIV).

Relative to **15a**, attachment of a methyl group to position 3′ of the pyridine ring to form **15c** caused a 4- to 16-fold decrease in activity, whereas the change from N^+CH_3 to $N^+CH_2CH_3$ improved potency 2 to 4 times. The $N^+CH(CH_3)_2$ analogue **15f**, however, was very similar to **15a**. Substitution of an electronegative group in position 3′ caused almost total deactivation in **15d** and **15u**, and conversion of the *tert*-butyl ester to the sodium carboxylate in **15v** still gave an essentially inactive compound. This also held true for **15ai** where the cephem position 3 substituent was methylthiadiazolylthiomethyl.

Ester and sodium carboxylate functions were better tolerated in groups attached to the quaternary nitrogen or in the cephem position 3 substituent, as is shown with **15g**, **15h** (Table XIII), and **15aj** (Table XIV). The latter, indeed, is one of the best of the series, falling into the subgroup of N^+-benzyl analogues, of which **15i** was overall the most attractive example. Subsequently, **15i** was prepared on a large scale, and its biological properties were examined in detail.

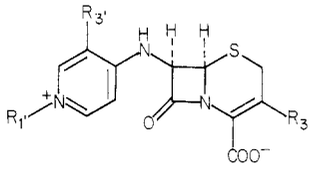
Insertion of an additional methylene between the quaternary nitrogen and the aromatic ring caused loss of Gram-negative activity in **15j**, and the isomeric **15k**, where there is a direct bond from nitrogen to the aromatic ring, was even more extensively deactivated. Conversion of **15i** to the tertiary amine **26** gave an almost inactive compound. The *N*-benzyl variants **15l–p** were all comparable to **15i** in spectrum but, with the exception of the furan, were much less soluble in water (<1 mg/mL).

With the quaternary nitrogen substituent held constant as benzyl, variation at the cephem position 3 led to several comparable (**15aa**, **15ah**, **15ak**, **15al**, and **15am**) but to no superior antibacterial agents. The bispyridinium examples showed particularly good Gram-positive activity.

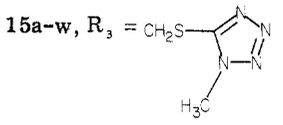
Deacetylation of **15aa** to form **15ab** markedly reduced activity against *Salmonella*, *Klebsiella*, and *E. aerogenes* but improved the *P. vulgaris* value. The azide **15ac** was 8-fold less active than **15i** against *Klebsiella* and *P. morganii*, and the dithiocarbonate **15ad** was generally 8- to 16-fold deactivated toward Gram-negative species. The cefaclor analogue **15af** was more active than its cephalexin counterpart **15ae**, particularly against *E. coli*, *Enterobacter* species, and *P. vulgaris*, but it was still 8 to 16 times less active than **15i**. The improvement in antibacterial properties found in certain 7β-(acylamino)cephems[45] when

(45) T. Kamimura, Y. Matsumoto, N. Okada, Y. Mine, and M. Nishida, S. Goto, and S. Kuwahara, *Antimicrob. Agents Chemother.*, **16**, 540 (1979).

Table VII. 7β-(4'-Pyridinioamino)ceph-3-em-4-carboxylates

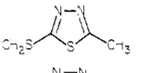
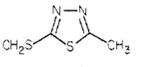
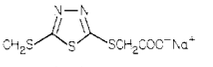
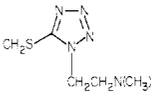
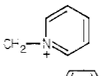
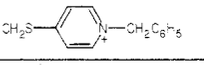


15a-am



15a-w, R_3 = CH_2S-(1,2,3-triazole)-CH_3

	$R_{1'}$	$R_{3'}$	isolation procedure	yield, %
a	CH_3	H	C	27
b[a]	CH_3	H	C	21
c	CH_3	CH_3	C	35
d	CH_3	CH_3OOC	C	36
e	CH_3CH_2	H	C	71
f	$(CH_3)_2CH$	H	C	56
g	$CH_3CH_2OOCCH_2$	H	C	12
h	$Na^{+-}OOCCH_2$	H		80[b]
i	$C_6H_5CH_2$	H	A/crystallized	69
j	$C_6H_5CH_2CH_2$	H	A	59
k	2-$CH_3(C_6H_4)$	H	A/C	48
l		H	C	13
m	4-$CH_3O(C_6H_4)CH_2$	H	A	89
n	3,4-$(CH_3O)_2(C_6H_3)CH_2$	H	A/C	20
o	4-$CN(C_6H_4)CH_2$	H	A	29
p[c]	4-$F(C_6H_4)CH_2$	H		27
q	CH_3OCH_2	H	C	16
r	$C_6H_5CH_2OCH_2$	H	A	7
s	$CCl_3CH_2OCH_2$	H	A	80
t	$CF_3CH_2OCH_2$	H	C	38
u	$CCl_3CH_2OCH_2$	$(CH_3)_3COOC$	A	43
v	$CCl_3CH_2OCH_2$	$Na^{+-}OOC$		60[d]
w	CH_3CH_2O	H	C	18

	$R_{1'}$	$R_{3'}$	R_3	isolation procedure	yield, %
x	CH_3	H	CH_2OAc	B	91
y	$CCl_3CH_2OCH_2$	H	CH_2OAc	C	7
z	4-$NO_2(C_6H_4)CH_2OCH_2$	H	CH_2OAc	A	69
aa	$C_6H_5CH_2$	H	CH_2OAc	A/crystallized	31[e]
ab	$C_6H_5CH_2$	H	CH_2OH	C	30
ac	$C_6H_5CH_2$	H	CH_2N_3	A	84
ad	$C_6H_5CH_2$	H	$CH_2SCSOCH_2CH_3$	A	48
ae	$C_6H_5CH_2$	H	CH_3	C	56
af	$C_6H_5CH_2$	H	Cl	C	30
ag	$C_6H_5CH_2$	H	H	C	54
ah	$C_6H_5CH_2$	H		A/C	5[f]
ai	$CCl_3CH_2OCH_2$	$Na^{+-}OOC$		A[g]	24
aj	$C_6H_5CH_2$	H		C	14
ak	$C_6H_5CH_2$	H		A	14
al	$C_6H_5CH_2$	H		B	41
am	$C_6H_5CH_2$	H		A	91

[a] **15b** is the 7α epimer of **15a**. Both were isolated from the same reaction mixture. [b] Prepared from **15g**. [c] Reaction solvent was Me_2SO. [d] Prepared from **15u**. [e] **15aa** was difficult to recrystallize but readily formed a 1:1 crystalline complex with Me_2SO. [f] The product was leached from the dark tarry precipitate with water at 65 °C and the extract was chromatographed. [g] The *tert*-butyl ester separated from solution and was cleaved as for **15v**.

position 3 is unsubsituted did not carry over into the new series: **15ag** was generally about 4 times less active than **15i**.

Compound **15q** was the first example of the alkoxymethyl subgroup on the pyridine nitrogen and was between **15a** and **15i** in activity. The benzyloxymethyl analogue **15r** was 8-fold deactivated against *E. coli* and lost all activity against *P. vulgaris*. When the phenyl unit of **15r** was replaced with the other highly lipophilic trichloro and trifluoro groups, the very active **15s** and **15t** derivatives were obtained. Comparative mouse protection studies, however, showed **15i** clearly more active against *S. pyogenes* and *M. morganii*. The trifluoroethoxymethyl quaternary was notable for its good water solubility in contrast

Table XII. Antibacterial Activity[a] of Pyridinium Aminopenams and Ampicillins against Aerobic Organisms

organism	MB no.	12a	12b	12c	pen. G[b]	4	19	20a	amp.[b]
S. pyogenes	3124[c]	1.56	0.25		<0.39		<0.39	<0.39	<0.09
S. faecalis	2864[c]	>100	128	128	1.56	>100	12.5	12.5	6.25
Staphylococcus aureus	2868[d]	>100	64	>128	12.5	>100	3.12	3.12	25
E. coli	2891[d]	50	4	0.5	>100	12.5	>100	>100	>100
Shigella sp.	2880	12.5	0.25	<0.06	6.25	12.5	25	25	6.25
Salmonella schottmuelleri	2837	0.78	0.125	<0.06	3.12	12.5	12.5	6.25	1.56
Klebsiella pneumoniae	2882	100	16	0.5	25	100	>100	>100	50
Enterobacter cloacae	2646[c]	>100	4	0.5	>100		>100	>100	>100

[a] MIC in μg/mL; inoculum 10^5 cfu per spot; Tryptocase soy agar as growth medium. [b] pen. G = penicillin G; amp. = ampicillin. [c] Except for 12b and 12c, these organisms were tested with Mueller Hinton agar. [d] β-Lactamase producer.

Table XIII. Antibacterial Activity[a] of 7β-(2′-Pyridinioamino)- and 4′-(Pyridinioamino)ceph-3-em-4-carboxylates against Aerobic Organisms

organism	MB no.	14a	14b	14c	15a	15b	15c	15d	15e	15f	15g	15h	15i	15j
S. pyogenes	3124	32	32	16	2	32	32	32	1	1	<0.5	2	0.015	<0.5
S. faecalis	2864	>128	>128	>128	>128	>128	>128	>128	>128	128	>128	>128	128	>128
Staphylococcus aureus	2868[b]	>128	>128	>128	64	>128	>128	>128	64	32	16	32	4	4
E. coli	2891[b]	64	2	>128	0.5	>128	8	>128	<0.5	0.5	2	4	0.06	4
Shigella sp.	2880	32	8	>128	1	>128	16	>128	<0.5	0.5	<0.5	2	<0.004	<0.5
Salmonella schottmuelleri	2837	>128	4	128	8	>128	128	128	4	0.125	2	4	<0.004	1
K. pneumoniae	2882	128	64	>128	8	64	16	>128	1	16	4	8	0.25	1
Enterobacter aerogenes	2906[b]	16	>128	>128	0.5[c]	16[c]	8[c]	>128	0.5[c]	128	32	32	8	64
E. cloacae	2646[b]	>128	>128	>128	1	>128	16	>128	2	32	16	64	1	8
P. Mirabilis	2830[b]	128[d]	>128	>128	8[d]	>128[d]	16[d]	>128[d]	2[d]	128	128	64	32	>128
P. vulgaris	2829[b]	0.5[d]	1	>128	0.25[d]	4[d]	1[d]	>128[d]	<0.06[d]	<0.06	<0.5	1	1	>128
Morganella morganii	2833[b]	>128[d]	>128	>128	4[d]	>128[d]	16[d]	>128[d]	0.5[d]	>128	>128	>128	16	>128
Ps. aeruginosa	2835[b]	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
Serratia marcescens	2852[b]	>128	>128	>128	1[c]	64[c]	>128	>128	>128	>128	>128	>128	64	>128

organism	15k	15l	15m	15n	15o	15q	15r	15s	15t	15x	15y	15aa	15ae	15ag
S. pyogenes	0.25	0.03	<0.06	0.125	0.125	0.125		<0.06	<0.06	2			0.06	
S. faecalis	>128	128	>64	>64	>128	>128	>128	>128	>128	>128	128	128	128	128
Staphylococcus aureus	32	2	8	8	32	16	16	2	4	64	8	4	64	16
E. coli	2	0.06	0.25	0.5	0.25	0.125	1	<0.06	<0.06	>128	<0.06	0.5	4	1
Shigella sp.	1	0.0075	0.5	0.25	<0.06	0.5	1	<0.06	<0.06	64	<0.06	<0.06	2	<0.06
Salmonella schottmuelleri	2	0.0075	0.125	0.5	0.125	1	1	<0.06	<0.06	8	<0.06	<0.06	0.5	<0.06
K. pneumoniae	128	0.06	0.5	0.5	1	1	2	0.125	1	32	<0.06	<0.06	32	<0.06
Enterobacter aerogenes	>128	8	4	8	8	64	128	8	4	>128	32	16	128	64
E. cloacae	>128	8	8	64	8	32	16	0.125	<0.06	>128	0.125	4	64	8
P. mirabilis	>128	16	16	>64	128	64	>128	64	>128	>128	>128	>128	>128	>128
P. vulgaris	2	<0.25	<0.06	0.25	16	<0.06	128	<0.06	4	<0.5[d]	32	0.125	>128	0.125
Morganella morganii	>128	>128	16	32	128	>128	>128	32	>128	>128	>128	>128	>128	>128
Ps. aeruginosa	>128	128	>64	>64	>128	>128	>128	>128	>128	>128	>128	>128	>128	128
Serratia marcescens	>128	128	64	64	128	>128	>128	64	128	>128	>128	>128	>128	>128

[a] MIC in μg/mL; tryptocase soy agar growth medium unless otherwise designated; inoculum 10^5 cfu per spot. [b] β-Lactamase producer. [c] Nutrient agar growth medium; comparable values for 15i were <0.06 μg/mL (2906); <1 μg/mL (2852). [d] Mueller–Hinton growth medium; comparable values for 15i were 0.5 (2830); 0.5 (2829); and <0.06 μg/mL (2833).

Table XIV. Antibacterial Activity[a] of 7β-(4′-Pyridinioamino)ceph-3-em-4-carboxylates against Aerobic Organisms

organism	MB no.	15i	15p	15u	15v	15w	15z	15ab	15ac	15ad
S. faecalis	2864	128	128	>128	>128	>128	>128	>128	>128	>128
Staphylococcus aureus	2865	0.25	0.5	128	>128	8	2	0.5	0.5	1
S. aureus	2868[b]	4	1	>128	>128	>128	8	16	2	4
E. coli	2891[b]	<0.06	<0.06	128	64	0.125	1	0.5	0.25	1
Salmonella	3860	1	4	>128	>128	128	128	>128	1	4
K. pneumoniae	4005	8	8	>128	>128	128	128	>128	64	>128
Enterobacter aerogenes	2828[b]	2	1	>128	>128	32	>128	128	8	>128
E. cloacae	2646	0.25	0.125	>128	>128	1	64	1	1	4
P. mirabilis	2830	32	64	>128	>128	>128	>128	>128	>128	>128
P. vulgaris	2829[b]	0.125	0.25	>128	>128	0.25	1	<0.06	<0.06	8
Morganella morganii	2833[b]	16	32	>128	>128	>128	>128	>128	>128	>128
Pseudomonas	2835	>128	>128	>128	>128	>128	>128	>128	>128	>128

15af	15ah	15ai	15aj	15ak	15al	15am	4	cephalothin	cephaloridine
>128	64	>128	>128	128	128	64	>128	8	64
2	0.25	64	1	0.5	0.125	<0.06	8	0.015	0.25
128	2	>128	4	4	0.5	0.25	>128	0.06	4
0.25	<0.06	128	<0.125	<0.06	<0.06	<0.06	<0.06	>128	>128
128	0.125	>128	0.25	2	2	4	0.125	2	0.5
64	16	>128	1	4	32	32	16	0.25	0.25
8	16	>128	0.5	0.5	2	1	64	32	8
4	0.125	>128	0.05	0.25	0.125	<0.06	0.125	>128	>128
>128	128	>128	64	128	>128	128	>128	32	16
<0.125	0.25	16	<0.125	0.125	0.25	<0.06	0.5	128	>128
>128	128	>128	16	64	>128	>128	>128	>128	>128
>128	>128	>128	128	>128	64	>128	>128	>128	>128

[a] MIC in μg/mL; tryptocase soy agar growth medium; inoculum 10^4 cfu per spot. [b] β-Lactamase producer.

to the sparingly soluble **15i** (2 mg/mL) and **15s** (<1 mg/mL). In the last alkoxymethyl example **15z**, the *p*-nitro group restored action lost by **15r** against *P. vulgaris* but at the cost of deactivation toward *Salmonella* and *Klebsiella* species. The only N^+-alkoxy derivative prepared, **15w**, was found to be very similar to the N^+-alkyl analogue **15e** in its antibacterial profile.

A very limited survey of other heterocyclylamino β-lactams showed that the 1-methyl-4-quinolinium analogue **16** was considerably less active than **15x**, which is in accord with the deactivating effect of electron-withdrawing groups attached to the pyridine ring. The almost totally inactive 1-methyl-2-pyrimidinium example **17** also conforms to this classification. However, the general complexity of structure–activity relationships could be seen in the deactivation of **15a** by the mildly electron-donating methyl group of **15c** and in a return to the thienamycin series where the change from *N*-methyl (**10a**) to *N*-benzyl (**10b**) markedly reduced antibacterial properties, particularly against *Pseudomonas*. The bridgehead nitrogen quaternary **18** was as active as **15a** against *S. aureus*, *E. coli*, and *P. vulgaris*, which suggests that superior properties may be found in the many heterocyclic systems not yet attached to β-lactams.

In extensive mouse protection studies, **15i** was found to be comparable to cefotaxime (HR756), with the exception of lower potency against *Klebsiella pneumonia* 4005 and higher potency against *Enterobacter cloacae* 2646. Compound **15i** was equal to the best performance of mecillinam against various strains of *E. coli*, including the potent β-lactamase producer 2891, and in addition protected mice against infection with strains of *Proteus* and the Gram-negative *Streptococci* and *Staphylococci*.

In conclusion, the best of the above quaternary heterocyclylaminocephemcarboxylates, **15i**, exhibits quite broad spectrum antibacterial properties. It is bactericidal, resistant to lactamase (though less than the cephamycins), active in vivo, nontoxic, stable, crystalline, and easy to prepare. It has the disadvantage of having only trace activity against *Pseudomonas*, an inoculum and medium effect, low aqueous solubility, poor oral absorption, and low urinary recovery. Detailed in vitro and in vivo evaluation of **15i** as a representative of a new class of β-lactam antibiotics will shortly be published from these laboratories by our colleagues in microbiology.

Experimental Section

IR spectra were taken as mulls in Nujol on a Perkin-Elmer 267 spectrometer and showed β-lactam absorbance in the range 1770 to 1780 cm^{-1} for both penicillins and cephalosporins. UV spectra were measured in aqueous solution using either a Perkin-Elmer 202 or Carey 15 spectrophotometer. The dominant feature of the UV spectra was the chromophore of the quaternary amino heterocycle, such as in the simple penicillin **12a** [λ_{max} 283 nm (ϵ 23 000)]. Typical modification by substituents may be illustrated by the cephalosporins **15a** [λ_{max} 280 nm (ϵ 36 000)], **15i** [λ_{max} 282 nm (ϵ 39 000)], and **15x** [λ_{max} 280 nm (ϵ 28 000)]. NMR spectra were obtained from either a Varian T60 or SC300 instrument in D_2O using DSS (deuterated sodium 2,2-dimethyl-2-silapentane-5-sulfonate) as an internal standard or in Me_2SO-d_6 for sparingly water-soluble products with $(CD_3)_4Si$ as internal standard. Control of reaction pH was achieved by use of an autoburette (ABU II), titrator (TTT 60) and pH meter (PHM 61) assembly made by Radiometer (Copenhagen). Analytical HPLC was carried out in a system consisting of a 0.4 × 25 cm Waters μ-Bondapak C_{18} reverse-phase column in aqueous 5–15% tetrahydrofuran, using a Waters M-6000A pump and a Schoeffel Spectroflow SF-770 variable UV monitor.

***N*-(3,4,5,6-Tetrahydro-2*H*-azepin-7-yl)thienamycin (6b).** 7-(Methylthio)-3,4,5,6-tetrahydro-2*H*-azepine (73 μL, 0.55 mmol) was added by syringe to a solution of thienamycin (100 mg, 0.37 mmol) in aqueous 50% tetrahydrofuran (2.5 mL) at 22 °C containing sodium bicarbonate (31 mg, 0.37 mmol). A stream of nitrogen was bubbled through the mixture to sweep out methyl mercaptan byproduct. After 3 h, the mixture was extracted with ether and the aqueous phase was chromatographed over a 2.2 × 28 cm column of amberlite XAD-2 resin, prepared in distilled deionized water. Using the same solvent, 4-mL fractions were collected and monitored by UV (254 nm). All unreacted thienamycin was washed out by fraction 40. The solvent was then changed to 10% THF in distilled deionized water, which eluted the product in fractions 62–73. The best fractons were selected by analytical reverse-phase HPLC and then combined and lyophilized to give a colorless powder: yield 25 mg (19%); IR ν 1765 cm^{-1}; UV (H_2O) λ_{max} 301 nm (ϵ 5870); NMR (D_2O) δ 1.27 (d, 3

H, J = 6 Hz, CH_3), 1.75 [br s, 6 H, $(CH_2)_3$], 2.60–2.77 (m, 2 H, CH_2), 2.85–3.24 (m, 4 H, CH_2 and SCH_2), 3.34–3.56 [m, 6 H, $(NCH_2)_2$ and H_6], 4.10–4.34 (m, 2 H, H_5 and CHOH).

In an exactly analogous manner using 2-(methylmercapto)-Δ′-pyrroline was prepared *N*-(Δ′-pyrrolin-2-yl)thienamycin (**6a**): yield 18 mg (15%); IR 1764 cm^{-1}; UV (H_2O) λ_{max} 299 nm (ϵ 4300).

***N*-(1-Methyl-2-pyridinio)thienamycin (8).** Thienamycin (91 mg, 0.33 mmol) was dissolved in distilled, deionized water (2.5 mL) at 22 °C. The solution was magnetically stirred and automatically titrated to pH 8.5 with aqueous 1 N NaOH. A solution of 4-fluoro-1-methylpyridinium iodide[25] (96 mg, 0.40 mmol) in water (0.5 mL) was added, causing rapid response from the autoburette to maintain pH 8.5. After 10 min, the pH was adjusted to 7.0, and the solution was chromatographed over a 3 × 44 cm column of amberlite XAD-2 resin prepared in distilled deionized water. After all unreacted thienamycin was washed out, the solvent was changed to 2% THF in water, which eluted the product. Reverse-phase HPLC identified the correct fractions and showed also by spiking that a minor contaminant was *N*-methyl-2-pyridone. Lyophilization gave **8** as a pale yellow powder: yield 63 mg (52%); IR ν 1764 cm^{-1}; UV (H_2O) λ_{max} 233 nm, (ϵ 13 300), 305 (14 960); NMR (D_2O/DSS) (SC300) δ 1.26 (d, 3 H, J = 6 Hz, CH_3), 3.07 (m, 2 H, H_4), 3.23 (m, 2 H, H_{11}), 3.35 (dd, 1 H, H_6), 3.79 (m, 2 H, H_{12}), 3.82 (s, 3 H, N^+CH_3), 4.12 (m, 1 H, H_5), 4.22 (m, 1 H, H_8), 6.96 (m, 1 H, $H_{5'}$), 7.24 (d, 1 H, $H_{3'}$), 7.96 (m, 2 H, $H_{4'}$ + $H_{6'}$).

Similarly were prepared **10a** [yield 65 mg (49%); IR ν 1761 cm^{-1}; UV (H_2O) λ_{max} 281 nm (ϵ 19 600); NMR (D_2O/DSS) (SC300) δ 1.24 (d, 3 H, J = 6 Hz, CH_3), 3.01 (m, 2 H, H_4), 3.19 (m, 2 H, H_{11}), 3.30 (dd, 1 H, H_6), 3.67 (br s, 2 H, H_{12}), 3.87 (s, 3 H, N^+CH_3); 4.07 (m, 1 H, H_5), 4.18 (m, 1 H, H_8), 6.81 (br s, 1 H, $H_{3'}$ or $H_{5'}$), 6.97 (d, 1 H, J = 7 Hz, $H_{5'}$ or $H_{3'}$), 7.84 (d, 1 H, J = 7 Hz, $H_{2'}$ or $H_{6'}$), 7.92 (d, 1 H, J = 7 Hz, $H_{6'}$ or $H_{2'}$)] and **10b** [yield 33 mg (17%); IR ν 1762 cm^{-1}; UV (H_2O) λ_{max} 282 nm (ϵ 20 500); NMR (D_2O/DSS) (SC300) δ 1.27 (d, 3 H, J = 6 Hz, CH_3), 3.04 (m, 2 H, H_{11}), 3.20 (m, 2 H, H_4), 3.29 (dd, 1 H, J = 6 and 2 Hz, H_6), 3.66 (m, 2 H, H_{12}), 4.09 (dt, 1 H, J = 8 and 2 Hz, H_5), 4.19 (dq, 1 H, J = 6 and 6 Hz, H_8), 5.35 (s, 2 H, N^+CH_2), 6.87 (d, 1 H, J = 7 Hz, $H_{3'}$ or $H_{5'}$), 7.00 (d, 1 H, J = 7 Hz, $H_{5'}$ or $H_{3'}$), 7.46 (m, 5 H, Ar), 8.01 (d, 1 H, J = 7 Hz, $H_{2'}$ or $H_{6'}$), 8.13 (d, 1 H, J = 7 Hz, $H_{6'}$ or $H_{2'}$).

2,5-Dichloro-1-methylpyridinium Tetrafluoroborate (7b). Trimethyloxonium tetrafluoroborate (1.00 g, 6.8 mmol) was added to a magnetically stirred solution of 2,5-dichloropyridine (1.00 g, 6.8 mmol) in CH_2Cl_2 (5 mL) at 22 °C. After 1 h, the supernatant was decanted off from an oily precipitate, which then crystallized on evaporation at 40 °C (1 mm) to form colorless, compact prisms: yield 1.61 g (95%); mp 70–90 °C; NMR (D_2O/DSS) (T60) δ 4.42 (s, 3 H, N^+CH_3), 8.18 (d, 1 H, J = 9 Hz, H_3), 8.59 (dd, 1 H, J = 9 and 2 Hz, H_4), 9.14 (d, 1 H, J = 2 Hz, H_6).

Similarly, methyl 4-chloronicotinate[46] and methyl 6-chloronicotinate[47] were converted to **9t** (77% yield; NMR, see Table III) and **7c** (84% yield; NMR (D_2O/DSS) δ 4.09 (s, 3 H, CH_3O), 4.53 (s, 3 H, N^+CH_3), 8.35 (d, 2 H, J = 8 Hz, H_5), 9.06 (dd, 1 H, J = 8 and 2 Hz, H_4), 9.60 (d, 2 H, J = 2 Hz, H_2).

4-Chloropyridine. 4-Chloropyridine was regenerated from the hydrochloride by neutralization and CH_2Cl_2 extraction of the aqueous solution at 0 °C. The free base remains as a mobile colorless oil for several weeks at −25 °C but polymerizes in a few days at room temperature.

4-Chloro-1-ethylpyridinium Tetrafluoroborate (9c). A solution of 4-chloropyridine (5.90 g, 0.05 mol) and triethyloxonium tetrafluoroborate (9.87 g, 0.05 mol) in anhydrous acetonitrile (20 mL) was left at 22 °C for 20 h. Yellow insoluble material was filtered off and discarded. The filtrate was evaporated at 60 °C (1 mm) to give an off-white solid: yield 10.16 g (64%); mp 70–98 °C; NMR, see Table II.

1-Benzyl-4-chloropyridinium Bromide (9e). 4-Chloropyridine (33 g, 0.29 mol) and benzyl bromide (198 g, 1.16 mol) were mixed at 0 °C, and the solution was left at this temperature for 40 h, becoming a mixture of crystals and dark red tar. The addition of toluene (200 mL) slowly dissolved the tar. The mixture was filtered; the solid was washed with toluene, dried at 25 °C (1 mm), and obtained as pale yellow crystals: yield 66.3 g (80%); mp 155–170 °C; NMR, see Table II.

Similarly, using 4-methoxybenzyl bromide[48] (in benzene), 3,4-dimethoxybenzyl bromide[49] (in benzene), 4-cyanobenzyl bromide[42] (in DMF/ether), and 2-(bromomethyl)furan[50] (in ether) were prepared **9h** (46%), **9i** (50%), **9j** (58%), and **9aa** (20%), respectively: NMR, see Table II.

4-Chloro-1-(4-fluorobenzyl)pyridinium Iodide (9k). A solution of 4-chloropyridine (3.30 g, 29 mmol) and 4-fluorobenzyl iodide[51] (7.63 g, 32 mmol) in anhydrous acetonitrile (20 mL) was left at 0 °C for 40 h. Filtration then gave a black crystalline powder, which was repeatedly extracted with hot acetonitrile (5 × 50 mL). Evaporation of the combined filtrates resulted in dark yellow crystals: yield 5.54 g (55%); mp 177–185 °C; NMR, see Table II.

4-Chloro-1-(2-methylphenyl)pyridinium Tosylate (9f). A solution of 1-(2-methylphenyl)-4-pyridone[41] (1.00 g, 5.40 mmol) and recrystallized *p*-toluenesulfonyl chloride (1.03 g, 5.40 mmol) in anhydrous toluene (10 mL) was boiled under reflux for 5 min and then cooled to 20 °C. The toluene was decanted from the insoluble oily product, which became a pale brown glass, 2.03 g, after heating to 50 °C (1 mm): NMR, see Table II.

Similarly were prepared **9d** and **9g**: NMR, see Table II. The necessary pyridones were derived by the process in ref 41. 1-Isopropyl-4-pyridone: NMR (acetone-d_6) (T60) δ 1.37 [d, 6 H, J = 7 Hz, $(CH_3)_2$], 4.30 (m, 1 H, J = 7 Hz, NCH), 6.09 (d, 2 H, J = 8 Hz, H_3 + H_5), 7.77 (d, 2 H, J = 8 Hz, H_2 + H_6). 1-(2-Phenethyl)-4-pyridone: NMR ($CDCl_3$) δ 3.02 (t, 2 H, J = 7 Hz, Ar CH_2), 3.99 (t, 2 H, J = 7 Hz, NCH_2), 6.29 (d, 2 H, J = 8 Hz, H_3 + H_5), 7.09 (d, 2 H, J = 8 Hz, H_2 + H_6), 6.95–7.35 (m, 5 H, Ar).

4-Chloro-1-(methoxymethyl)pyridinium Bromide (9l). A solution of methoxymethyl bromide (4.40 g, 36 mmol) in anhydrous ether (10 mL) was added to a magnetically stirred solution of 4-chloropyridine (2.00 g, 18 mmol) in anhydrous ether (20 mL) at 22 °C, at once forming a precipitate. The mixture was filtered after 30 min, and the solid was washed with ether and then dried in vacuo to give colorless crystals: yield 3.83 g (92%); NMR, see Table II.

Similarly, using benzyloxymethyl bromide,[52] 2,2,2-trichloroethoxymethyl bromide [by the same process[52] (53%); bp 84–86 °C (11 mm); NMR ($CDCl_3$) (T60) δ 4.28 (s, 2 H, CCl_3CH_2), 5.83 (s, 2 H, CH_2Br)], 2,2,2-trifluoroethoxymethyl bromide [by the same process[52] (71%); bp 59–62 °C (180 mm); NMR ($CDCl_3$) (T60) δ 4.00 (q, 2 H, J = 8 Hz, CF_3CH_2), 5.67 (s, 2 H, CH_2Br)], and 4-nitrobenzyloxymethyl bromide [by the same process[52] but not distilled (>90%); NMR ($CDCl_3$) (T60) δ 4.95 (s, 2 H, Ar CH_2), 5.93 (s, 2 H, CH_2Br), 7.67 and 8.33 (AB q, 4 H, J = 9 Hz, Ar)] were prepared **9m** (33%), **9n** (84%), **9o** (87%), and **9q** (73%); for NMR, see Table II.

***tert*-Butyl 4-Chloronicotinate.** *O-tert*-Butyl-1,3-diisopropylisourea[53] (5.19 g, 26 mmol) was dissolved in a suspension of 4-chloronicotinic acid (4.10 g, 26 mmol) in CH_2Cl_2 (40 mL) at 22 °C, and the mixture was magnetically stirred overnight. Solids were then filtered off, and the solution was evaporated at 40 °C (0.1 mm) to give yield an oil, which slowly crystallized: yield 2.20 g (39%); NMR ($CDCl_3$) (T60) δ 1.71 [s, 9 H, $C(CH_3)_3$], 7.47 (d, 1 H, J = 5 Hz, H_5), 8.64 (d, 1 H, J = 5 Hz, H_6), 9.05 (s, 1 H, H_2).

3-(*tert*-Butoxycarbonyl)-4-chloro-1-[(2′,2′,2′-trichloroethoxy)methyl]pyridinium Bromide (9aj). 2,2,2-Trichloroethoxymethyl bromide (750 mg, 3.1 mmol) was added to a solution of *tert*-butyl 4-chloronicotinate (641 mg, 3.0 mmol) in CH_2Cl_2 (10

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mL) at 22 °C. The mixture was stirred for 4 h, and then the insoluble product was filtered off and dried in vacuo: yield 510 mg (37%); NMR, see Table III.

4-Chloro-1-ethoxypyridinium Chloride (9p). 4-Chloro-1-ethoxypyridinium tetrafluoroborate[54] was dissolved in distilled deionized water and put on a Dowex 1-X2 ion-exchange column prepared in the chloride cycle. The product was eluted with water and the solution was evaporated at 40 °C (1 mm) to give **9p**: NMR, see Table II.

1-Benzyl-4-fluoropyridinium Bromide (9x). A mixture of 1-benzyl-4-chloropyridinium bromide (**9e**; 10.00 g, 35 mmol) and powdered anhydrous KF[42] (6.12 g, 105 mmol) was heated at 60 °C (1 mm) for 30 min to remove traces of moisture. DMF (30 mL) sequentially dried[55] by 3A molecular sieves was added to the mixed solids at 22 °C to form a very dark solution/suspension, which was magnetically stirred with exclusion of moisture for 2 h, progressively lightening in color. The mixture was rapidly filtered, solids were washed with dry DMF (10 mL), and the combined DMF filtrates were rotoevaporated at 60 °C (0.1 mm) to a red-brown viscous oil (13.42 g), which slowly crystallized at room temperature. By NMR, this product was 65% **9x**, 5% 1-benzyl-4-pyridone, and 30% DMF and was used directly in coupling reactions. Material of this quality is stable indefinitely in a sealed flask at room temperature. On another occasion, more prolonged heating to reduce the DMF content gave a product 78% **9x**, 9% pyridone, and 13% DMF, which then slowly crystallized over 6 days with complete metasthesis to 1-benzyl-4-bromopyridinium fluoride, a much less reactive reagent: NMR (Me_2SO-d_6) (T60) δ 5.97 (s, 2 H, NCH_2), 7.40–7.60 (m, 5 H, Ar), 8.13 (d, 2 H, J = 7.5 Hz, $H_{3'}$ + $H_{5'}$), 9.47 (d, 2 H, J = 7.5 Hz, $H_{2'}$ + $H_{6'}$).

The other fluoro reagents of Table III were prepared by the above process for **9x**.

2-Fluoro-1-methylpyrimidinium Tetrafluoroborate. Trimethyloxonium tetrafluoroborate (754 mg, 5.1 mmol) was added to a magnetically stirred solution of 2-fluoropyrimidine[56] (500 mg, 5.1 mmol) in CH_2Cl_2 (4 mL) at 22 °C. The suspension of sparingly soluble reagent gradually changed in appearance and after 3 h the mixture was filtered. The solid was washed with CH_2Cl_2, dried at 22 °C (0.1 mm), and obtained as colorless microneedles: yield 1.02 g (100%); mp 98–102 °C dec. In water the product solvolyzes instantly to 1-methyl-2-pyrimidinone hydrofluoride: NMR (D_2O/DSS) (T60) δ 3.83 (s, 3 H, CH_3), 7.05 (dd, 1 H, J = 6.2 and 6.2 Hz, H_5), 8.77 (dd, 1 H, J = 6.2 and 2.7 Hz, H_4), 8.96 (dd, 1 H, J = 6.2 and 2.7 Hz, H_6), shifted in neutral solution to 3.70 (s, 3 H, CH_3), 6.84 (dd, 1 H, J = 6.5 and 5.1 Hz, H_5), 8.50 (dd, 1 H, J = 6.5 and 2.5 Hz, H_4), 8.70 (dd, 1 H, J = 5.1 and 2.5 Hz, H_6).

4-[[(2-Carboxy-8-oxo-7β-amino-5-thia-1-azabicyclo[4.2.0]-oct-2-en-3-yl)methyl]thio]-1-benzylpyridinium Hydroxide Inner Salt Chloride (13m). 7-Aminocephalosporanic acid (**13a**;[27] 787 mg, 2.9 mmol) was dissolved in water (30 mL) containing $NaHCO_3$ (487 mg, 5.8 mmol) at 25 °C. A solution of 1-benzyl-1*H*-pyridine-4-thione[57] (1.17 g, 5.8 mmol) in acetonitrile (30 mL) was added, and the solution was heated at 60 °C with stirring for 24 h. Acetonitrile was removed under reduced pressure, and a precipitate of unreacted thione (500 mg) was filtered off. The filtrate was acidified to pH 3.7 with 2.5 N HCl, forming a precipitate of unreacted 7-aminocephalosporanic acid which was filtered off. The aqueous acidic filtrate was freeze-dried, yielding 1.08 g (90%): NMR (D_2O/DSS/$NaHCO_3$) (SC300) δ 3.43 and 3.70 (AB q, 2 H, J = 18 Hz, C_2 H), 4.13 and 4.41 (AB q, 2 H, J = 13 Hz, C_3 CH_2S), 4.75 (d, 1 H, J = 5 Hz, H_6), 5.02 (d, 1 H, J = 5 Hz, H_7), 5.63 (s, 2 H, NCH_2), 7.41–7.57 (m, 5 H, Ar), 7.82 (d, 2 H, J = 7 Hz, $H_{3'}$ + $H_{5'}$), 8.53 (d, 2 H, J = 7 Hz, $H_{2'}$ + $H_{6'}$).

General Method for the Reaction of Amino Acids and Quaternary Haloheterocycles. A solution of the quaternary haloheterocycle (0.6–1.0 mmol) in water (0.5 mL) is added to a magnetically stirred solution of the amino acid (0.5 mmol) in water (2.5 mL) at pH 7 and 22 °C. Aqueous 1 N NaOH is automatically added from a burette to maintain pH 7 (range 6–8.5 depending on $pK_{a/b}$). Reaction times are usually only a few minutes but may in certain cases be several hours, especially when the halogen to be displaced is chlorine.

Procedure A. Some products are sparingly soluble and can be filtered off. Other reaction mixtures become very dark and tarry and are extracted with ethyl acetate to remove byproducts such as the pyridone from the solvolyzed reagent. The aqueous pH 7 phase containing the zwitterionic product is then worked up by one of two chromatographic procedures.

Procedure B. The aqueous phase is added to a 3 × 45 cm column of amberlite XAD-2 resin prepared in distilled deionized water, and elution is continued with the same solvent using an LKB fraction collector, drop counter, and UV (λ 254 nm) monitor. When the eluate absorbance returns to near base-line values (0.5–1 L), the solvent is changed to 1 to 5% tetrahydrofuran in distilled deionized water, which elutes the product. Fractions are examined in greater detail by analytical reverse-phase HPLC, which selects the best to be combined and lyophilized to yield the product as an amorphous powder.

Procedure C. The more recent availability of preparative μC_{18} columns permits direct reverse-phase chromatography of the aqueous pH 7 reaction solution. Excellent separation can be achieved by use of the large μ-Bondapak, Chromegaprep and Partisil M9 columns, injecting up to 50 mg of solute at one time in the above system and collecting the appropriate fraction for lyophilization.

The Waters Prep/LC System 500 in the reverse-phase mode can handle several grams as a single injection, but the coarser C_{18} packing material (37–75 μm) has much less resolving power than the μC_{18} packing.

4-[[2-Carboxy-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]-methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-7β-yl]-amino]-1-benzylpyridinium Hydroxide Inner Salt (15i). 7β-Amino-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]ceph-3-em-4-oic acid (**13h**;[34] 120 g, 0.366 mol) was suspended in water (1.2 L at 22 °C, pH 2.8). With vigorous mechanical stirring of the solution 2.5 N NaOH (146 mL, 0.366 mol) was added at a rate not exceeding pH 8.5. The solid was very slow to dissolve, and after 90 min traces of material in the suspension were filtered off. To the filtrate at pH 7 was added in one portion a solution of 1-benzyl-4-fluoropyridinium bromide (**9x**; 163 g of 78% reagent, 0.475 mol) in water (250 mL), causing the pH to fall to 5.0. With very rapid stirring, 2.5 N NaOH (175 mL, 0.438 mol) was added at a rate not exceeding pH 7 in the mixture. The product began to crystallize during the addition of base, and a powerful stirring motor was essential to homgenize the slurry to avoid local concentration of base and extensive decomposition. All the base was added in 30 min. The mixture was stirred for an additional 20 min and then filtered. The solid was washed with water (2 × 500 mL) and with acetone (2 × 500 mL), then the filter cake was suspended in acetone (1 L) at 50 °C, and water (1.5 L) at 65 °C was gradually added. The solution was treated with activated charcoal (20 g), filtered, and rotoevaporated at 45 °C (140 mm) to remove most of the acetone. The crystalline slurry was cooled to 15 °C and filtered. The solid was washed as before, dried to constant weight, and obtained as pale brown microneedles: yield 125 g (69%); mp 162–164 °C dec. Recrystallization gave 111 g of pure **15i** with the same melting point: IR ν 1780 cm^{-1}; UV (H_2O) λ_{max} 282 nm (ϵ 39 000); NMR, see Tables VIII and IX. Anal. ($C_{22}H_{21}N_7O_3S_2$) C, H, N, S.

4-[[3-[[(1-Methyltetrazol-5-yl)thio]methyl]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-7β-yl]amino]-1-(4-fluorobenzyl)pyridinium Hydroxide Inner Salt (15p). Triethylamine (212 μL, 1.52 mmol), dried over 4A molecular sieves, was added to a magnetically stirred suspension of **13h** (250 mg, 0.76 mmol) in sieve-dried dimethyl sulfoxide (2 mL) at 23 °C, forming a pale brown solution. Compound **9ae** (390 mg of 78% purity, 0.91 mmol) was added, and after 10 min the very dark solution was repeatedly extracted with ether (4 × 20 mL). The residual brown gum was extracted with water (3 × 2 mL) at 65 °C with filtration from much black tar. Lyophilization of the aqueous extracts gave a brown powder (234 mg). An aliquot (40 mg) was dissolved in aqueous 30% THF (2 mL) and injected onto a Partisil M9 ODS-2 μC_{18} reverse-phase column (0.96 × 50 cm)

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using aqueous 5% THF as eluant at 8 mL/min. The correct fraction appeared at 96 min, whereupon the eluant was changed to aqueous 10% THF to complete the collection at 117 min (168 mL). Concentration at 30 °C (0.1 mm) to 50 mL and then lyophilization yielded a pale yellow powder: yield 19 mg; IR ν 1776 cm^{-1}; UV (H_2O) λ_{max} 282 nm (29 900); NMR, see Table IX.

4-[[3-(Acetoxymethyl)-2-carboxy-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-7β-yl]amino]-1-methylpyridinium Hydroxide Inner Salt (23). A solution of **15x** (200 mg, 0.55 mmol) in anhydrous MeOH (5 mL) was cooled to −68 °C under dry nitrogen, and a 1.5 N solution of lithium methoxide in methanol (1.83 mL, 2.75 mmol) was added in 1 min by hypodermic syringe through a rubber septum to the magnetically stirred mixture. After 2 min, *tert*-butyl hypochlorite (79 μL, 0.66 mmol) was added in the same manner. The solution was stirred for 15 min more at −68 °C, then acetic acid (315 μL, 9 mmol) was added, and the solvent was removed at −68 °C to 20 °C (0.2 mm). The residual gum was dissolved in water (20 mL) and lyophilized to yield a pale brown foam, 405 mg. Isolation procedure B gave 136 mg of a mixture of **23** and **15x**. Isolation procedure C, using a 0.96 × 50 cm Chromegaprep μC_{18} reverse-phase column and aqueous 0.5% THF at 3.0 mL/min, then eluted **23** at 48–66 min, followed by **15x** at 100–200 min with 30 mg column loading. Lyophilization gave **23** as a colorless foam: yield 82 mg (38%); IR ν 1774 cm^{-1}; UV λ_{max} (H_2O) 277 nm (ϵ 28 800); NMR (D_2O/DSS) (Varian SC300) δ 2.09 (s, 3 H, CH_3O), 3.33 and 3.67 (AB q, 2 H, J = 18 Hz, C_2 H), 3.55 (s, 3 H, 7α, OCH_3), 4.06 (s, 3 H, N^+CH_3), 4.72 and 4.87 (AB q, 2 H, J = 13 Hz, C_3 CH_2O), 5.39 (s, 1 H, H_6), 7.31 (d, 2 H, J = 8 Hz, $H_{3'}$ + $H_{5'}$), 8.27 (d, 2 H, J = 8 Hz, $H_{2'}$ + $H_{6'}$).

Similarly **15x**, 27 mg (14%), was recovered.

4-[[2-Carboxy-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-7β-yl]amino]-3-carboxy-1-[(2,2,2-trichloroethoxy)methyl]pyridinium Hydroxide Inner Salt Sodium Salt (15v). A solution of **15u** (50 mg) in anisole (0.3 mL) was diluted with trifluoroacetic acid (3.0 mL) at 22 °C. After 5 h, the solution was evaporated at 22 °C (0.1 mm), and the residue was triturated with ether (3 × 0.5 mL). The insoluble material was suspended in water (15 mL) and titrated to pH 7 with 0.1 N NaOH. The solution was extracted with ether (5 mL) and the aqueous phase was lyophilized to give **15v**: yield 28.6 mg (60%); NMR, see Table IX.

7β-Amino-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]ceph-3-em-4-oic Acid Benzhydryl Ester (24). A suspension of **13h** (10.0 g, 30 mmol) and *p*-toluenesulfonic acid monohydrate (4.18 g, 22 mmol) in MeOH (400 mL) was stirred at 25 °C for 3 h, and then unreacted **13h** (3.0 g) was filtered off. The filtrate was evaporated at 40 °C (0.1 mm), and the residual oil was dissolved in peroxide-free dioxane (200 mL). Solid diazodiphenylmethane was added in portions to the stirred solution at 25 °C until a pink color persisted for 20 min, whereupon the solution was evaporated as above. The residual oil was dissolved in EtOAc (200 mL), was washed successively with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl solutions, then dried over $MgSO_4$, filtered, and again evaporated. The crude product was chromatographed over a column of silica gel (600 g, E. Merck), eluting first with $CHCl_3$ and then with $CHCl_3$/EtOAc (2:1), to give **24**, which was recrystallized from $CHCl_3$/Et_2O/petroleum ether: yield 4.0 g (38%); NMR ($CDCl_3$) (SC300) δ 3.70 and 3.77 (AB q, 2 H, J = 19 Hz, C_2 H), 3.85 (s, 3 H, NCH_3), 4.24 and 4.39 (AB q, 2 H, J = 13 Hz, C_3 CH_2S), 4.82 (d, 1 H, J = 5 Hz, H_6), 4.96 (d, 2 H, J = 5 Hz, H_7), 6.97 (s, 1 H, $CHPh_2$), 7.28–7.50 (m, 10 H, Ar).

7β-(Methylamino)-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]ceph-3-em-4-oic Acid Benzhydryl Ester (25a). A solution of **24** (2.00 g, 4 mmol) and methyl iodide (0.25 mL, 4 mmol) in dimethylformamide (40 mL) was stirred at 25 °C for 10 h. More methyl iodide (0.20 mL) was added, and after an additional 10 h the solution was evaporated at 50 °C (0.1 mm). The residue was dissolved in EtOAc (50 mL), washed successively with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl solutions, then dried over $MgSO_4$, filtered, and evaporated. The crude product was chromatographed over a column of silica gel (90 g, E. Merck) in $CHCl_3$/EtOAc (2:1), eluting in sequence the *N,N*-dimethyl analogue (520 mg, 25%), the desired monomethyl product **25a** (118 mg, 5.8%), and unreacted **24** (664 mg, 34%). Compound **25a** was further purified by thin-layer chromatography over silica gel using $CHCl_3$/EtOAc (1:1) to give 78 mg (4%): NMR ($CDCl_3$) (SC300) δ 2.60 (s, 3 H, 7β-NCH_3), 3.69 and 3.78 (AB q, 2 H, J = 18 Hz, C_2 H), 3.86 (s, 3 H, NCH_3), 4.24 and 4.39 (AB q, 2 H, J = 13 Hz, C_2 H); 4.59 (d, 1 H, J = 5 Hz, H_6), 4.96 (d, 1 H, J = 5 Hz, H_7), 6.96 (s, 1 H, $CHPh_2$), 7.28–7.50 (m, 10 H, Ar). The NMR of the *N,N*-dimethyl analogue showed a further upfield shift of the H_6 proton to δ 4.14.

7β-(Methylamino)-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]ceph-3-em-4-oic Acid (25b). A solution of **25a** (78 mg, 0.154 mmol) in anisole (0.5 mL) was diluted with trifluoroacetic acid (2 mL) at −15 °C. After 15 min at this temperature, the solution was evaporated at −15 to 0 °C (0.1 mm). The residue was redissolved in anisole (0.5 mL) and again evaporated, giving a gum which was triturated with ether (3 × 2 mL) and dried at 25 °C (0.1 mm) to give a colorless powder: yield 50 mg (95%); NMR (Me_2SO-d_6) (SC300) δ 2.41 (s, 3 H, 7β-NCH_3), 3.58 and 3.76 (AB q, 2 H, J = 18 Hz, C_2 H), 3.94 (s, 3 H, NCH_3), 4.21 and 4.37 (AB q, 2 H, J = 13 Hz, C_3 CH_2S), 4.68 (d, 1 H, J = 5 Hz, H_6), 5.01 (d, 1 H, J = 5 Hz, H_7).

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Supplementary Material Available: Full ^1H NMR data (Varian SC 300 spectrometer) for compounds **12a–c** (Table V), **14a–c** (Table VI), **15a–am** (Tables VIII and IX), **16–18** (Table X), **19**, and **20a,b** (Table XI) (10 pages). Ordering information is given on any current masthead page.