Nuclear analogs of β-lactam antibiotics. XIII. Structure activity relationships in the isocephalosporin series

TERRENCE W. DOYLE, JAMES L. DOUGLAS, BERNARD BELLEAU, TERRY T. CONWAY, C. F. FERRARI, DONALD E. HORNING, GARY LIM, BING-YU LUH, ALAIN MARTEL, MARCEL MENARD, AND L. R. MORRIS
Bristol Laboratories of Canada, 100 Industrial Boulevard, Candiac, P.Q., Canada J5R 1J1

AND

MARTIN MISIEK
Research Division, Bristol Laboratories, P.O. Box 657, Syracuse, NY 13201, U.S.A

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This paper is dedicated to Prof. Raymond U. Lemieux on the occasion of his 60th birthday

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The general synthetic scheme to nuclear analogs of cephalosporins described in previous papers of this series is reviewed and the synthesis of a series of sidechain derivatives is given. Thus, systems of the following type were formed: carbacephems (A), 2-isocephems (B), *N*-2-isocephems (C), and *O*-2-isocephems (D). The *in vitro* microbiological activity of these compounds (with appropriate side-chains attached) is given, with comparison to analogous cephalosporins. Systems of type A, B, and C were only prepared with a 3-H or CH_3 substituent and had modest antibacterial activity. The *O*-2-isocephems (D) were prepared with a wide variety of sidechains at 3 and 7 and were found to have biological activity quite comparable to the cephalosporins. A more detailed biological examination (both *in vitro* and *in vivo*) of the *O*-2-isocephem analog (**19*i***) of cephalothin was made.

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On passe en revue le schéma de synthèse générale des analogues nucléaires des céphalosporines décrit antérieurement dans cette série et on rapporte la synthèse des dérivés de la chaîne latérale. On a ainsi préparé les systèmes suivants: carbacéphèmes (A), isocéphèmes-2 (B), *N*-isocéphèmes-2 (C) et *O*-isocéphèmes-2 (D). On donne l'activité microbiologique *in vitro* de ces composés (avec les chaînes latérales appropriées) par comparaison avec les céphalosporines analogues. On a préparé les composés de types A, B et C avec des substituants H-3 où CH_3 et ils ont une activité antibactérienne modérée. On a préparé les *O*-isocéphèmes-2 (D) ayant une grande variété de chaînes latérales en positions 3 et 7 et on leur a trouvé une activité tout à fait comparable à celle des céphalosporines. On a réalisé un examen biologique (*in vitro* et *in vivo*) plus détaillé de l'analogue *O*-isocéphème-2 (**19*i***) de la céphalothine.

[Traduit par le journal]

While literally thousands of semisynthetic penicillins and cephalosporins, obtained by manipulation of the C6 (or C7) amide side chains or the C3 side chain in the cephalosporins, are known, it is only relatively recently that β-lactam antibiotics extensively modified in the nucleus have been reported (for a recent review see ref. 1). Whether natural or synthetic in origin, these analogs have shattered many of the notions regarding the requirements for optimal activity which were current when we started our work. Our initial goals were rather modest ones, being to first examine whether or not it was necessary to retain the sulfur atom at position one in the cephalosporin ring **1** (Fig. 1) (X = S, Y = CH_2), and secondly if the heteroatom could be transposed to position 2 (X = CH_2, Y = O, S, N, etc.). In the previous papers of this series we have reported the syntheses of a variety of nuclear analogs of the cephalosporins in which the sulfur atom has been replaced by variously substituted carbon atoms and position two by various heteroatoms, as well as retaining a carbon atom

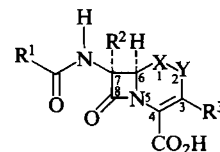

FIG. 1

(e.g. X has been CH_2, CHCl, $CH(CH_3)$, C=O, and Y has been O, S, SO, SO_2, NCO_2Et, NCH_3, CH_2, C=O, CHOR, CHBr, in Fig. 1) (2*a*–*l*). While this work was in progress, a number of other workers reported the syntheses of cephalosporin analogs having the type of substitutions in which we were interested. Lowe and co-workers (3) have reported the syntheses of the 2-isocepham and 2-isocephem systems (X = CH_2, Y = S), as have Bryan and co-workers (4). The latter workers have also reported the syntheses of a 1,2-benzofused cephalosporin (5) and several cephams incorporating two nitrogen atoms at positions 2 and 3 as well as a carbonyl at position 1 (6). A large series of

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1-oxadethiacephalosporins has been synthesized and these are reported to have quite good antibacterial activity (7). In addition to these cephalosporins, a number of papers have appeared which describe the total syntheses of a number of other β-lactam nuclear analogs, i.e., *N*-2-isopenams (8), 2-isopenams (9), clavulanic acid (10), oxapenems (11), penems (12), and thienamycin (13). In this paper we wish to more fully describe the biological activities of the compounds we have reported earlier (2) and to describe the synthesis and biological activities of a number of additional analogs.

Chemistry

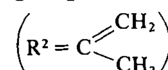
Initially our goal was the development of a general stereoselective synthesis which would provide access to a variety of isocephalosporins. As the work proceeded, the following general considerations evolved.

1. The substituted β-lactam would be synthesized first in a stereospecific manner so as to provide an amide at the eventual C7 position which would be *cis* to the second ring.
2. The substituent on the nitrogen atom of the initially-formed β-lactam would be used to form the second ring.[1]
3. The attached carbon atom at C4 in the initial azetidinone would be sp^2 hybridized so as to minimize problems inherent in the synthetic scheme chosen to form the β-lactam.

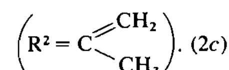
O-2-Isocephems

The synthetic scheme utilized for the majority of analogs we have synthesized may be illustrated by the synthesis of **8***a* ($R^1 = CH_3$) (Scheme I) (2*b*). Condensation of the amine **2** ($R^1 = CH_3$ throughout) with cinnamaldehyde gave **3** ($R^2 = CH=CH\phi$) which on treatment with azidoacetyl chloride in the presence of triethylamine gave the *cis* β-lactam **4***a* in good yield.[2] Ozonolysis of **4***a* gave **4***b* which was reduced by sodium borohydride to **4***c*. Compound **4***c* was converted to its mesylate **4***d* and hydrolysed to give a key intermediate **5***a*. When **5***a* was treated with base, cyclization occurred to the bicyclic β-lactam **6***a*. Reduction of **6***a* to the amine **7***a*, coupling to phenoxyacetic acid using EEDQ (14),[3] and hydrogenolysis of the benzyl ester gave the desired acid **8***a* ($R^1 = CH_3$, $R^2 = R^3 = H$). The syntheses of **8***b*–*d* were carried out similarly (2*b*) using the appropriately substituted amines **2**, or in the case of **8***b* starting from **9** (2*a*).

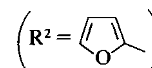
In the case of the *O*-2-isocephems carrying substituents at position 1, we employed either methacrylaldehyde or furfural as the aldehyde in the Schiff base forming step. Thus, treatment of **3**



with azido acetyl chloride and TEA gave the β-lactam **4**

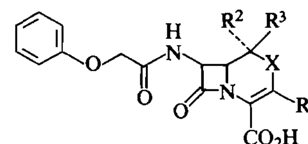


Ozonolysis followed by sodium borohydride reduction gave two diastereoisomeric alcohols (**4**, $R^2 = \overset{*}{C}HOHCH_3$) which were separated by chromatography and carried through the same sequence of reactions to yield **8***e* and **8***f*. From the 4-furyl β-lactam **4**



was obtained **8***g* (2*d*) (Fig. 2).

One goal was the production of analogs having a 3-acetoxymethyl group in place of the 3-methyl group of **8***a*. Initially we had intended to produce these compounds via functionalization of **8***a* or **6***a*. A number of attempts to functionalize the 3-methyl group using NBS or other free radical oxidizing



Compound	R^1	R^2	R^3	X
8*a*	CH_3	H	H	O
8*b*	H	H	H	O
8*c*	$CH_2\phi$	H	H	O
8*d*	$CH_2CH_2\phi$	H	H	O
8*e*	CH_3	CH_3	H	O
8*f*	CH_3	H	CH_3	O
8*g*	CH_3	—O—		O
8*h*	$CH_2CO_2CH_3$	H	H	O

FIG. 2

[1]Initially we attempted to use a variety of nitrogen blocking groups which we hoped to remove selectively, e.g. trityl, benzhydryl, and benzyl. The results were poor and the alternate approach of incorporating the *N*-blocking group in the final structure was decided on. The group at SKF has successfully utilized the first approach in their synthesis of the 2-isocephem system (4).

[2]The stereoselectivity of this and all other cycloadditions we have carried out in the course of our work has been very high, there being no detectable amounts of *trans* isomer formed.

[3]*N*-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline has been used extensively in the course of our studies for coupling sidechains to the nuclei.

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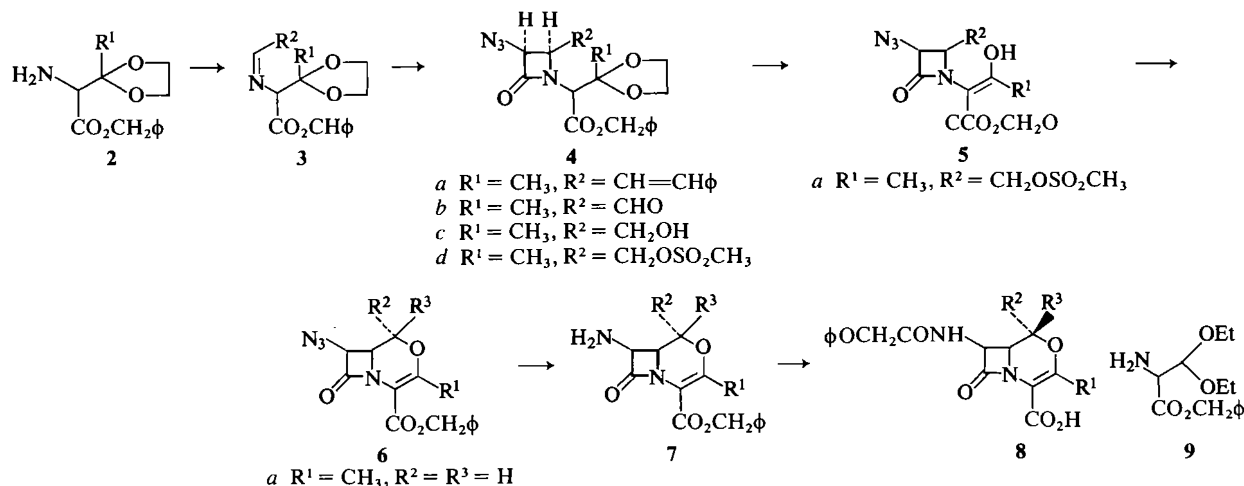


SCHEME 1

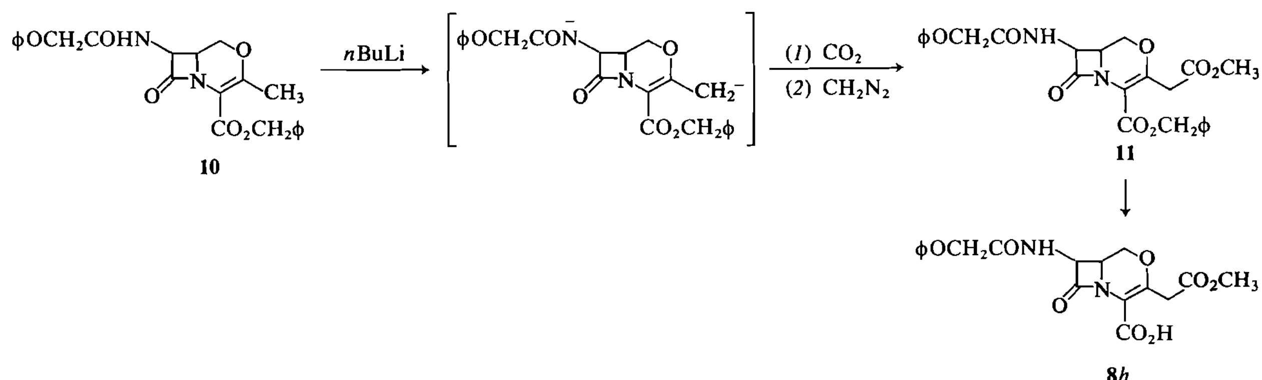
agents failed (2*h*).[4] As part of this study, the treatment of the benzyl ester of **8***a* with a variety of strong bases, followed by treatment with electrophiles, was examined. For the most part, this resulted in the decomposition of starting material and no isolable products or in recovery of the starting material unchanged. Treatment of **10** with *n*-butyllithium followed by quenching with carbon dioxide resulted in the formation of a mixture of carboxylic acids, which was further treated with diazomethane to yield the methyl ester **11** in 3.5% overall yield from **10**. Hydrogenolysis of **11** gave the carboxylic acid **8***h* (Scheme 2).

In view of the disappointing yield of **11**, further attempts to prepare the desired 3-acetoxymethyl compounds via direct functionalization of **6***a*, **8***a*, or **10** were abandoned. Routes based on amines such as **2** where $R^1 = CH_2OR$ also failed due to the fact that the presence of an α hetero atom rendered the subsequent hydrolysis of the ketals impossible without concomitant destruction of the β-lactam.[5] Fortunately, at about this time we discovered an alternate route to the desired compounds (2*h*). As part of our strategy to utilize the enol **5***a* to synthesize various other heteroatom substituted cephalosporins, the conversion of **5***a* to the dimesylate **12***a* or the mesylate triflate **12***b* had been carried out. This conversion was normally carried out by treating a mixture of the enol **5***a* plus either methane sulfonyl choride or triflic anhydride with one equivalent of triethylamine (TEA). On one occasion an excess of TEA was used and upon workup, instead of the desired **12**, compound **13** was obtained (Scheme 3). While **13** was unstable, it could be readily handled in solution. Halogenation of **13** with either bromine or iodine gave **14** as a mixture of geometrical isomers in excellent yields. When **14** was treated with potassium acetate in DMF, the desired 3-acetoxymethyl-*O*-2-isocephem **15***a* (R^1 = Bz) was produced in good yield. Attempted displacement of the 3-acetoxymethyl group by thiols failed. The hydrolysis of **15***a* to **15***c* did not proceed in good yield. Consequently, the solvolysis of **14***b* (R^1 = PNB) was carried out using potassium formate to yield **15***b* which could be hydrolysed to **15***c*. Treatment of **15***c* with sodium cyanate in trifluoroacetic acid gave the carbamate **15***d*. Mesylation of **15***c* gave **15***e* which was used to prepare **16***a–c* by displacement. While the displacements using the thiolates proceeded in good yield, that using 1-methyltetrazol-5-ol gave **16***c* in 22.5% yield. Compound **16***a* could also be prepared directly from **14***a* (R^1 = PNB). Thus, treatment of **14***a* with one equivalent of 1-methyltetrazole-5-thiol and TEA in CH_2Cl_2 gave **14***c* which was treated with potassium acetate in DMF to give **16***a*.

With compounds **6***a*, **15***a*, **15***d*, **16***a–c* readily available, the conversion of these to suitably substituted cephalosporin analogs was attempted next. We used two general methods to accomplish this goal. Reduction of the azide using hydrogen sulfide – triethylamine proceeded in good yield to give the amines **17***a*, *b* and **17***d–g*. In the case of **15***a* an alternate scheme was used. Hydrogenolysis of **15***a* over platinum accomplished both reduction of the azide and cleavage of the benzyl ester simultaneously to yield **17***c*. Compounds **17***a*, *b*, *d–g* were coupled to the appropriate carboxylic acids using EEDQ. Thus, from **17***a* we obtained **18***a–d*, from **17***b* compounds **18***e–g*, from **17***d* compound **18***h*, from **17***e* compounds **18***i–l*, from **17***f* compound **18***m*, and from **17***g* compound **18***n* (Scheme

[4]The SKF group has reported the successful bromination of the 2-isocephem system with NBS (4).

[5]M. Menard, unpublished results.

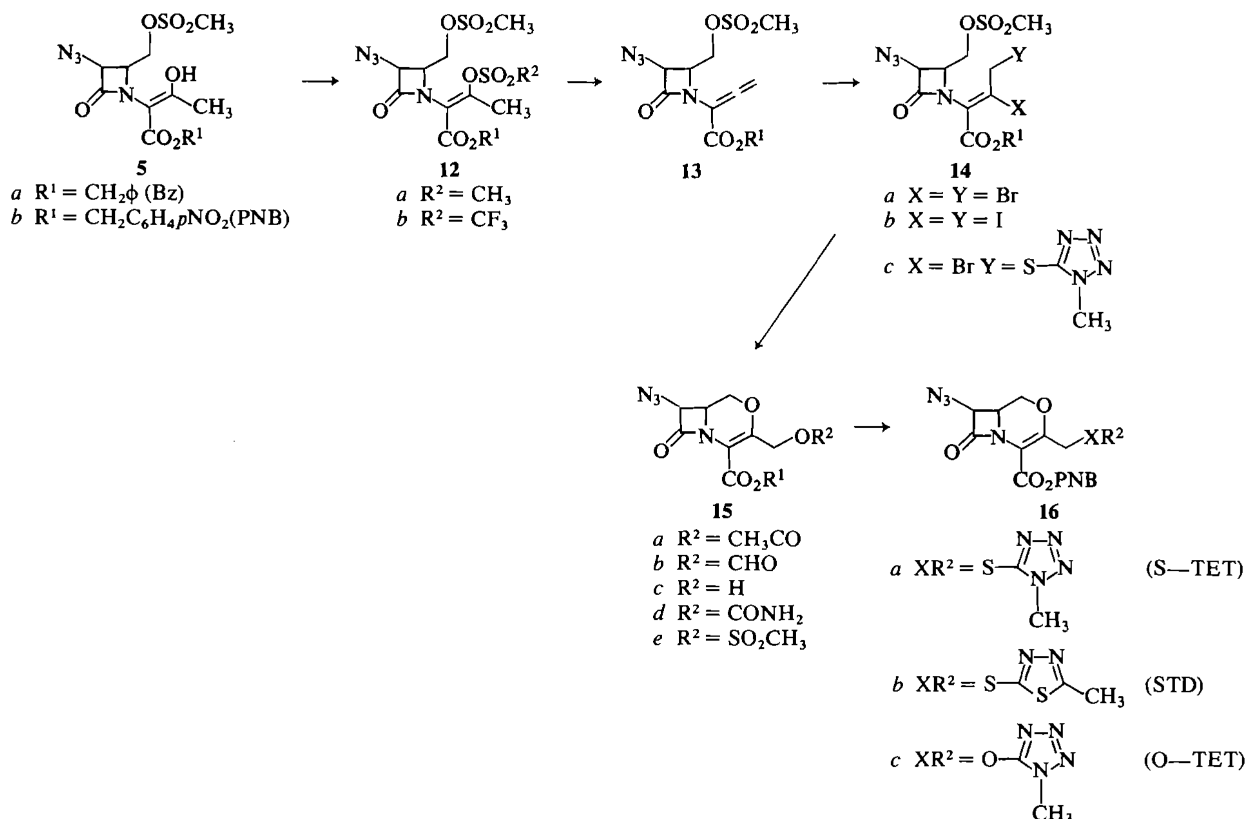


SCHEME 2

4), Hydrogenolysis of the benzyl or p-nitrobenzyl esters proceeded smoothly to give the corresponding carboxylic acids. In the cases of **18***b*, **18***c*, **18***e*, the secondary blocking groups on the sidechains were simultaneously deblocked. The azido function of **18***l* was reduced simultaneously with PNB removal to give **19***r*. A number of the final products were obtained via direct coupling of the acid chloride or mixed anhydrides to the amino acid. Thus, **17***c* was coupled with 4-chloromethylphenylacetyl chloride to give **20**. Reaction of **20** with thiourea gave **19***m*. Similarly, 2-(1-carbomethoxypropen-2-yl-aminomethyl)phenylacetic acid was coupled to **17***c* or **21** via the mixed anhydride procedure to yield **19***j* and **19***e*, respectively (Scheme 5). Compounds **19***k* and **19***l* were prepared via the method of Crast *et al.* (15).

2-Isocephems, N*-2-Isocephems, and Carbacephems*

The facile ring closure of the enol mesylate **5***a* to give the *O*-2-isocephem system **6***a* encouraged us to further explore the chemistry of **5***a* with a view to preparing isocephalosporins having other hetero-



SCHEME 3

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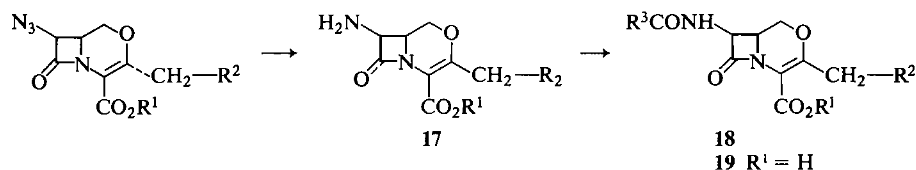
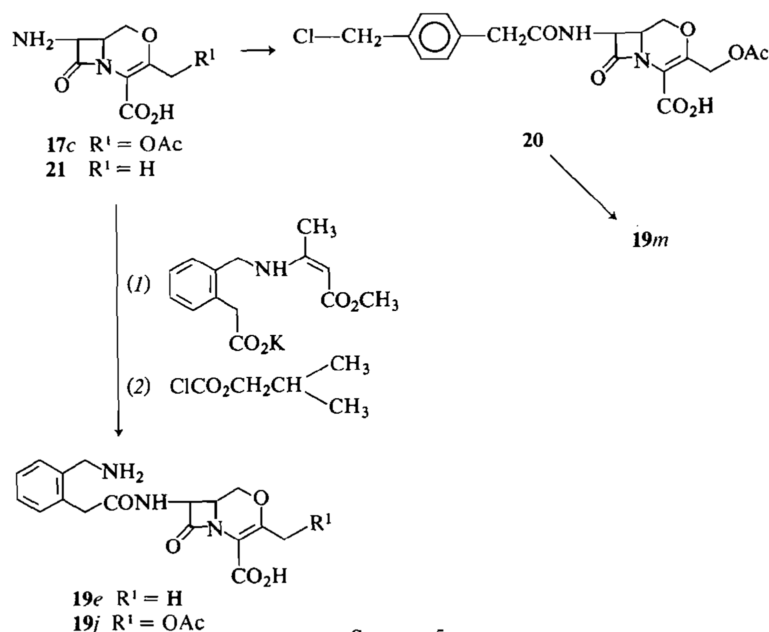


Compound	R^1	R^2	R^3
17*a*	Bz	H	—
17*b*	Bz	OAc	—
17*c*	H	OAc	—
17*d*	PNB	$OCONH_2$	—
17*e*	PNB	S-TET	—
17*f*	PNB	S-TD	—
17*g*	PNB	O-TET	—
18*a*	Bz	H	ϕCH_2
18*b*	Bz	H	ϕCH(NHCBz)
18*c*	Bz	H	$\phi CH(CO_2Bz)$
18*d*	Bz	H	ϕOCH_2
18*e*	Bz	OAc	ϕCH(NHCBz)
18*f*	Bz	OAc	$NCCH_2$
18*g*	Bz	OAc	ϕOCH_2
18*h*	PNB	$OCONH_2$	(2-thienyl)—CH_2
18*i*	PNB	S-TET	ϕOCH_2
18*j*	PNB	S-TET	ϕCHOH
18*k*	PNB	S-TET	(2-thienyl)—CH_2
18*l*	PNB	S-TET	ϕCHN_3
18*m*	PNB	S-TD	(2-thienyl)—CH_2
18*n*	PNB	O-TET	(2-thienyl)—CH_2
18*o*	H	H	ϕOCH_2
19*a*	—	H	ϕCH_2
19*b*	—	H	$\phi CHNH_2$
19*c*	—	H	$\phi CHCO_2H$
19*d*	—	H	(2-thienyl)—CH_2
19*e*	—	H	o-(NH_2CH_2)C_6H_4—CH_2
19*f*	—	OAc	ϕOCH_2
19*g*	—	OAc	$\phi CHNH_2$
19*h*	—	OAc	$CNCH_2$
19*i*	—	OAc	(2-thienyl)—CH_2
19*j*	—	OAc	o-(NH_2CH_2)C_6H_4—CH_2
19*k*	—	OAc	(4-pyridyl)—SCH_2
19*l*	—	OAc	CH_3—N^+(pyridinium-4-yl)—SCH_2
19*m*	—	OAc	$(H_2N)_2C^+$—SCH_2—C_6H_4—CH_2
19*n*	—	$OCONH_2$	(2-thienyl)—CH_2
19*o*	—	S-TET	ϕOCH_2
19*p*	—	S-TET	(2-thienyl)—CH_2
19*q*	—	S-TET	ϕCHOH
19*r*	—	S-TET	$\phi CHNH_2$
19*s*	—	STD	(2-thienyl)—CH_2
19*t*	—	O-TET	(2-thienyl)—CH_2

SCHEME 4

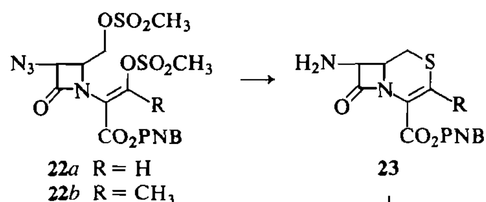
atoms at position 2. A number of attempts were made to prepare the ene thiol directly from the enol with no success. Consequently the enols were converted to their mesylates **22***a* or **22***b* as described earlier. Treatment of the dimesylate with hydrogen sulfide – triethylamine (Scheme 6) gave the desired 2-isocephem systems **23***a* and **23***b*. These were converted to their amides **24***a* and **24***b*. Compound **24***b* was converted by oxidation into the sulfoxide **24***c* and the sulfone **24***d*. Treatment of **24***c* with *tert*-butyl hypochlorite gave **24***e* (2*g*).

Similarly, treatment of **25***a* with monomethylamine (Scheme 7) or with ammonia followed by ethyl chloroformate gave the *N*-2-isocephems **26***a* and **26***b*. These were converted to **27***a* and **27***b*, respectively, in the usual fashion (2*f*).



SCHEME 5

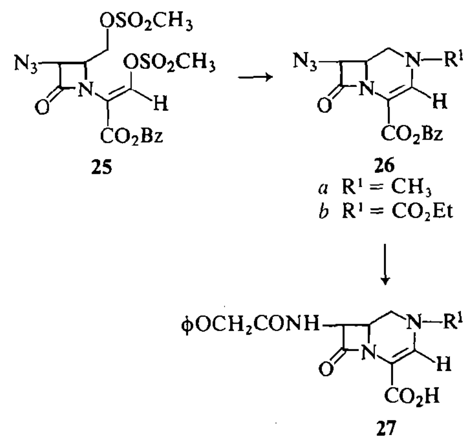
Encouraged by the successful syntheses of **24** and **27**, we next examined the reactions of **12***b* with various carbon nucleophiles (Scheme 8). Treatment of **12***b* with the sodium salts of dimethyl, dibenzyl, and di-*tert*-butyl malonate gave the cyclized products of **28***a*, *b*, and *c*, respectively. Compounds **28***a* and *b* were converted to compounds **30***a–e* (2*j*). When **28***c* (R = *tert*-butyl) was treated with acid and the resultant dicarboxylic acid **28** (R = H) decarboxylated there was obtained the Δ^2-cephalosporin analog **29**. This was converted to **30***d* as described earlier (2*k*). Starting from **29** we have also synthesized compounds **30***e–j* (2*l*).



24

	R^1	R^2	X	Y
a	H	H	—	—
b	CH_3	H	—	—
c	CH_3	H	O	—
d	CH_3	H	O	O
e	CH_3	Cl	O	—

SCHEME 6



SCHEME 7

7-Methoxy Isocephalosporins

In view of the reported broadening of the antibacterial spectrum of cephalosporins by substitution with a 7-α-methoxy group (16), we decided to prepare 7-methoxy analogs of the 2-iso and the *O*-2-isocephems (2*i*). The method of Spitzer and Goodson (17) was chosen to introduce a thiomethyl group (Scheme 9). Thus, **31***a* (A = R^2 = H, R^3 = PNB, X = S) was treated with LDA followed by methoxycarbonylmethyl disulfide to give **31***b* (A = SCH_3, R^2 = H, R^3 = PNB, X = S). The Schiff base was hydrolysed to the amine which was acylated. Treatment of the sulfide with mercuric acetate in methanol (18) followed by hydrogenolysis of the

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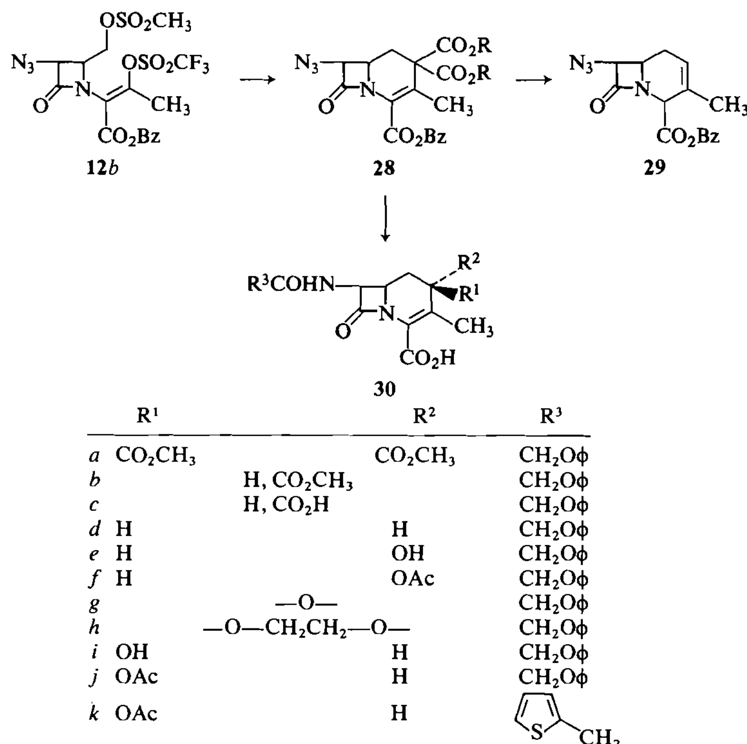


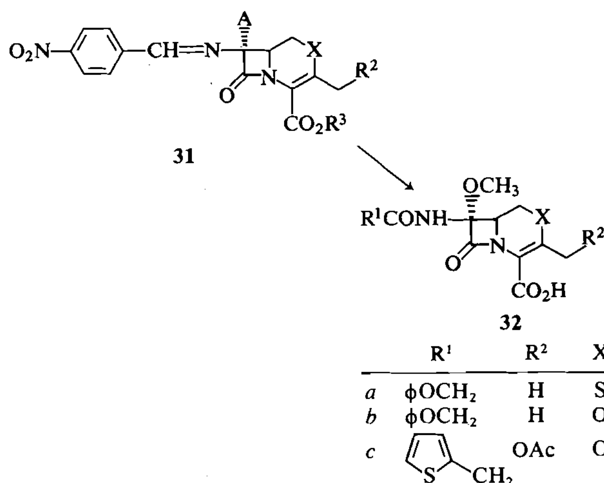
	R^1	R^2	R^3
a	CO_2CH_3	CO_2CH_3	$CH_2O\phi$
b	H, CO_2CH_3		$CH_2O\phi$
c	H, CO_2H		$CH_2O\phi$
d	H	H	$CH_2O\phi$
e	H	OH	$CH_2O\phi$
f	H	OAc	$CH_2O\phi$
g	—O—		$CH_2O\phi$
h	—O—CH_2CH_2—O—		$CH_2O\phi$
i	OH	H	$CH_2O\phi$
j	OAc	H	$CH_2O\phi$
k	OAc	H	(thienyl) CH_2

SCHEME 8

ester group gave **32***a* ($R^1 = \phi OCH_2$, R^2 = H, X = S). Compounds **32***b* and **32***c* were prepared similarly.

Biological Activity

In vitro antibiotic activities for the cephalosporin analogs prepared were determined by a two-fold serial dilution assay in Difco nutrient broth by the method of Pursiano *et al.* (19). Results in terms of minimum inhibitory concentrations (MIC) in μg/mL are given in Tables 1–4. Bacterial strains utilized included gram positive organisms (*Streptococcus pyogenes*, *Staphylococcus aureus* Smith), a β-lactamase producing gram positive organism (*S. aureus* BX 1633), gram negative organisms (*Escherichia coli* Juhl, *Klebsiella pneumoniae* 9977, *Proteus mirabilis*), β-lactamase producing gram negative organisms (*E. coli* 9675, *K. pneumoniae* 15130, *P. morganii*, *Enterobacter cloacae*), and *Pseudomonas aeruginosa*. None of the compounds tested were active against *Pseudomonas* so it is not included in the tables. In the tables the effects of changing substituents at the 1, 2, 3, and 7 positions are demonstrated. As well, comparisons to cephalosporins are made.



	R^1	R^2	X
a	ϕOCH_2	H	S
b	ϕOCH_2	H	O
c	(thienyl) CH_2	OAc	O

SCHEME 9

The limited amount of data in Table 1 demonstrates that substitution at position 1 seems to be deleterious to antibiotic activity. In particular, compare the lower activity of **8***f* or **8***g* with **8***a* and **24***e* with **24***c*.

Table 2 demonstrates the effect of varying the 2 substituent on antibiotic activity. For example, the nuclear analogs **8***a*, **24***a*, **24***b*, **27***b*, and **30***d* show that the 2-O analog is most active while the 2-S, CH_2, and 2-NCO_2Et analogs are of reduced activity but roughly equivalent to one another. However, oxidation of the 2-S gives sulfoxide **24***c* and sulfone **24***d* with close to the activity of **8***a*. Similarly, the 2-keto analog **30***g* has activity comparable to **8***a*. Except for the 2-β-acetoxy analog **30***j*, other sub-

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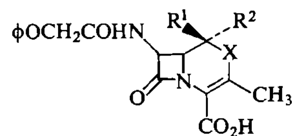
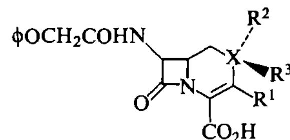
TABLE 1. Effect of substituents at C1 on antibacterial activity



Compound	X	R¹	R²	*S. pyogenes*	*S. aureus* Smith	*S. aureus* BX1633	*E. coli* Juhl	*E. coli* 9675	*K. pneumoniae* 9977	*K. pneumoniae* 15130	*Proteus mirabilis*	*Proteus morganii*	*Enterobacter cloacae*
8*a*	O	H	H	0.5	0.5	32	63	>125	32	>125	16	>125	63
8*f*	O	CH_3	H	10	>125	>125	>125	>125	>125	>125	>125	>125	>125
8*g*	O	H	CH_3	10	>10	63	>125	>125	>125	>125	>125	>125	>125
8*h*	O	O		>125	>125	>125	>125	>125	>125	>125	>125	>125	>125
24*c*	SO	H	H	5	2.5	16	125	>125	63	>125	63	>125	125
24*e*	SO	Cl	H	8	16	63	>125	>125	>125	>125	>125	>125	>125

TABLE 2. Effects of substituents at position 2 on antibacterial activity



Compound	X	R¹	R²	R³	*S. pyogenes*	*S. aureus* Smith	*S. aureus* BX1633	*E. coli* Juhl	*E. coli* 9675	*K. pneumoniae* 9977	*K. pneumoniae* 15130	*Proteus mirabilis*	*Proteus morganii*	*Enterobacter cloacae*
8*a*	O	H	—	—	0.6	0.6	>125	63	>125	32	>125	63	>125	—
27*b*	NCO_2Et	H	—	—	2	8	125	>125	>125	>125	>125	>125	>125	>125
24*a*	S	H	—	—	2	2	16	>125	>125	125	>125	>125	>125	125
8*a*	O	CH_3	—	—	0.5	0.5	32	63	>125	32	>125	16	>125	63
24*b*	S	CH_3	—	—	16	32	63	>125	>125	>125	>125	>125	>125	>125
24*c*	S	CH_3	O	—	5	2.5	16	125	>125	63	>125	63	>125	125
24*d*	S	CH_3	O	O	2	4	63	>125	>125	125	>125	63	>125	>125
30*a*	C	CH_3	CO_2CH_3	CO_2CH_3	16	>125	>125	>125	>125	>125	>125	>125	>125	>125
30*b*	C	CH_3	H, CO_2CH_3		16	>32	63	>125	>125	>125	>125	>125	>125	>125
30*c*	C	CH_3	H, CO_2H		125	>125	125	>125	>125	>125	>125	>125	>125	>125
30*d*	C	CH_3	H	H	4	4	8	>125	>125	>125	>125	>125	>125	>125
30*e*	C	CH_3	OH	H	8	16	32	>125	>125	>125	>125	>125	>125	>125
30*f*	C	CH_3	OAc	H	125	125	>125	>125	>125	>125	>125	>125	>125	>125
30*g*	C	CH_3	O		0.13	0.5	125	>125	>125	63	>125	32	>125	>125
30*h*	C	CH_3	$O(CH_2)_2O$		16	16	>125	>125	>125	>125	>125	>125	>125	>125
30*i*	C	CH_3	H	OH	4	8	32	>125	>125	>125	>125	>125	>125	>125
30*j*	C	CH_3	H	OAc	0.5	2	4	>125	>125	>125	>125	>125	>125	>125

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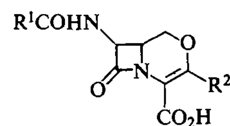
TABLE 3. Effect of substituents at C-3



Compound	R^1	R^2	*S. pyogenes*	*S. aureus* Smith	*S. aureus* BX1633	*E. coli* Juhl	*E. coli* 9675	*K. pneumoniae* 9977	*K. pneumoniae* 15130	*Proteus mirabilis*	*Proteus morganii*	*Enterobacter cloacae*
8*b*	ϕOCH$_2$	H	0.6	0.6	>125	63	>125	32	>125	63	>125	—
8*a*		CH$_3$	0.5	0.5	32	63	250	32	500	16	500	63
8*c*		CH$_2$ϕ	0.25	0.25	>125	Inactive						
8*d*		(CH$_2$)$_2$ϕ	0.03	0.06	>125	Inactive						
8*h*		CH$_2$CO$_2$CH$_3$	0.06	0.25	>125	125	>125	63	>125	32	>125	>125
19*f*		CH$_2$OAc	0.13	1	125	16	125	16	>125	4	>125	63
9*o*		CH$_2$STET	0.13	0.25	16	4	32	2	32	2	63	32
19*b*	ϕCHNH$_2$	CH$_3$	0.13	1	63	4	32	4	8	8	63	4
19*g*		CH$_2$OAc	0.06	1	8	1	4	1	8	0.5	32	2
19*r*		CH$_2$STET	0.25	4	125	4	16	1	16	2	16	8
19*d*	(2-thienyl)CH$_2$	CH$_3$	0.25	0.5	16	16	63	4	32	4	>125	8
19*i*		CH$_2$OAc	0.03	0.5	2	8	63	1	63	1	>125	16
19*n*		CH$_2$OCONH$_2$	0.25	0.5	8	16	125	2	32	2	>125	16
19*p*		CH$_2$STET	0.016	0.13	2	1	16	0.25	8	0.13	32	1
19*s*		CH$_2$STD	0.008	0.06	2	4	23	0.5	32	0.5	32	4
19*t*		CH$_2$OTET	0.06	0.25	125	32	125	2	63	2	125	16

stituents at the 2-carbon (**30**a–c, e, f, h) generally produced lessened activity. Thus it would appear that electron donation to the $\Delta^{3,4}$ double bond decreases antibiotic activity, and steric bulk at the 2 position also decreases activity.

Since the *O*-2-isocephem nucleus appeared to have the greatest potential for high antibiotic activity, an extensive series of 3 and 7 substituted analogs was prepared. Table 3 shows the effect of varying substituents at position 3. Increasing hydrophilic character at 3 (**8***a*, **8***c*, **8***d*) leads to an increase in activity against non-β-lactamase producing gram positive organisms but loss of activity against other organisms. For the 3-methylene substituted compounds, the order of activity is (with minor exceptions) as follows: S-tetrazole more active than S-thiadiazole, more active than acetoxy, more active than O-tetrazole, same as carbamate, more active than H. These are similar to the trends seen with cephalosporin 3-sidechains (20). The effect of varying 7-sidechains can be seen in Tables 3 and 4. In general, the phenylglycine group confers the highest activity followed by the thienylacetyl and the groups of compounds **19***m*, *j*, *k*, and *l*, with the phenoxyacetyl, cyanoacetyl, mandeloyl, and phenylmalonyl groups conferring the lowest activity. It should be noted that compounds with the phenylglycine sidechain (except for **19***b*) had poor chemical stability. These trends in activity seen with the various 7-sidechains parallel the trends seen with the cephalosporins with some exceptions. Notably the mandeloyl sidechain confers much poorer activity on the O-isocephem than one would expect from the corresponding cephalosporins.

A direct comparison between *O*-2-isocephems and cephalosporins with the same side-chains is given in Table 4. With certain combinations of sidechains, the *O*-2-isocephem is more active (**8***a*, **19***a*, *d*, *p*, *s*), with others the cephalosporin is more active (**19***h*, *j*, *k*, *l*, *m*, *q*), while the rest are more or less equivalent. With regard to individual strains of bacteria, the following generalizations can be made: the cephalosporins are more active than *O*-2-isocephems against staphylococci and β-lactamase producing strains of *E. coli*, *Klebsiella*, and *Proteus*. Thus it would seem that the *O*-2-isocephem and cephalosporin nucleii have about the same inherent activity but that the *O*-2-isocephem nucleus is more susceptible to β-lactamases. The 7-methoxy substituent appears to give some protection against β-lactamases but overall activity is reduced considerably (2*i*).

More extensive microbiological testing was carried out with several *O*-2-isocephems. Compound **19***i* (2*h*) (the cephalothin analog) can be taken as typical. Data comparing **19***i* (resolved) with cephalothin are presented in Fig. 3. Both are highly active against *S. aureus* with cephalothin being slightly better. Against the gram negative organisms, **19***i* is superior for the more sensitive strains, but this advantage is reduced for the more resistant strains (presumably which include many β-lactamase producers).

Table 5 shows the *in vivo* activity of compound **19***i* compared to cephalothin. Blood levels in mice are essentially equivalent. Both provide high protection for mice experimentally infected with *Streptococcus pyogenes* with **19***i* being somewhat superior.

In summary, various nuclear modifications of cephalosporins (carba, 2-iso, *N*-2-iso, *O*-2-iso) were found to have antibiotic activity. More extensive testing of *O*-2-isocephem analogs, both *in vitro* and *in vivo*, showed these compounds to have antibiotic activity similar to cephalosporins with only minor differences in detail.

Experimental

General

The ir spectra were recorded on a Unicam SP-200G spectrometer. The nmr spectra were determined on a Varian A60-A spectrometer using tetramethylsilane as an internal standard. Melting points were uncorrected and were determined on a Gallenkamp melting point apparatus. The analyses were performed by Micro-Tech Laboratories, Skokie, IL. Many of the final compounds were relatively unstable and were best submitted for microbiological testing without purification. Such products usually did not give satisfactory elemental analyses. However, spectral data indicated that they were ≥90% pure in most cases (unless otherwise indicated below). Extraction solutions were dried over sodium sulfate. All chromatographic purifications were carried out on activity III silica gel by the dry column technique.

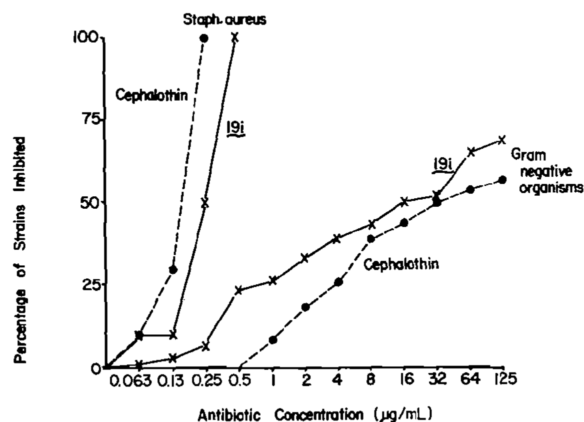


FIG. 3. Antibiotic susceptibility of 10 strains of *Staphylococcus aureus* and 54 strains of gram negative organisms to cephalothin and **19***i* in Mueller–Hinton medium. (Five strains each of *Enterobacter aerogenes* and *Ent. cloacae*, 10 strains each of *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*, 5 strains each of *Proteus rettgeri* and *P. vulgaris*, 4 strains of *P. morganii*.)

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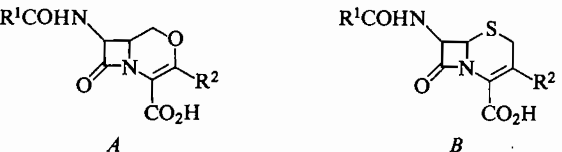
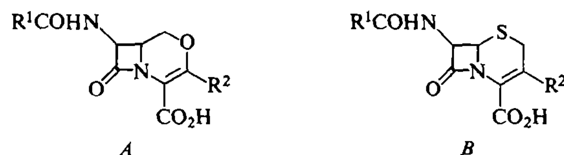
TABLE 4. Comparison of *O*-2-isocephems to cephalosporins



Compound	A / B	R^1	R^2	*S. pyogenes*	*S. aureus* Smith	*S. aureus* BX1633	*E. coli* Juhl	*E. coli* 9675	*K. pneumoniae* 9977	*K. pneumoniae* 15130	*Proteus mirabilis*	*Proteus morganii*	*Enterobacter cloacae*
8*a*	A	ϕOCH_2	CH_3	0.5	0.5	32	63	> 125	32	> 125	16	> 125	63
	B			2	1	2	> 125	> 125	> 125	> 125	> 125	> 125	> 125
19*a*	A	ϕCH_2	CH_3	0.5	1	32	125	> 125	16	125	16	> 125	> 125
	B			0.6	2.5	—	250	> 250	32	> 250	32	> 250	> 250
19*d*	A	2-thienyl-CH_2	CH_3	0.25	0.5	16	16	63	4	32	4	> 125	8
	B			1	2	32	125	> 125	16	125	16	> 125	16
19*b*	A	ϕ-CH(NH_2)	CH_3	0.13	1	63	4	32	4	8	8	63	4
Cephalexin	B			0.3	1.3	4	8	16	4	16	4	> 125	4
19*e*	A	2-(NH_2CH_2)$C_6H_4CH_2$	CH_3	0.08	0.6	32	16	32	16	32	32	125	8
	B			0.3	1.3	8	32	63	32	63	32	> 125	—
19*c*	A	ϕ-CH(CO_2H)	CH_3	8	16	> 125	125	125	63	> 125	63	125	63
	B												
19*f* (2*h*)	A	ϕOCH_2	CH_2OAc	0.13	1	125	16	125	16	> 125	4	> 125	63
	B			0.016	0.062	—	> 100	—	100	—	—	—	
19*i*	A	2-thienyl-CH_2	CH_2OAc	0.03	0.5	2	8	63	1	63	1	> 125	16
Cephalothin	B			0.06	0.13	0.25	16	63	0.25	16	0.5	> 125	2
19*g*	A	ϕCH(NH_2)	CH_2OAc	0.06	1	8	1	4	1	8	0.5	32	2
Cephaloglycine	B			0.13	0.5	2	2	4	1	2	1	63	1
19*h*	A	$NCCH_2$	CH_2OAc	0.25	2	8	32	125	16	125	32	> 125	63
Cephacetrile	B			0.13	0.5	1	8	16	8	16	4	> 125	8
19*m*	A	4-[$(NH_2)_2C^+$-S-CH_2]$C_6H_4CH_2$	CH_2OAc	0.016	0.13	8	8	63	0.5	16	1	> 125	8
	B			0.004	0.008	0.13	4	32	0.13	4	0.13	> 125	1
19*j*	A	2-(NH_2CH_2)$C_6H_4CH_2$	CH_2OAc	0.03	0.5	125	4	8	2	8	4	32	2
	B			0.03	0.13	0.25	1	4	1	2	1	63	0.5

TABLE 4 (*Concluded*)



Compound	A/B	R¹	R²	*S. pyogenes*	*S. aureus* Smith	*S. aureus* BX1633	*E. coli* Juhl	*E. coli* 9675	*K. pneumoniae* 9977	*K. pneumoniae* 15130	*Proteus mirabilis*	*Proteus morganii*	*Enterobacter cloacae*
19*k*	A	N(ring)—SCH_2	CH_2OAc	0.13	1	63	32	125	2	>125	4	>125	32
Cephapirin	B			0.008	0.13	1.3	16	125	1	8	1	>125	8
19*f*	A	CH_3—N(+)(ring)—SCH_2	CH_2OAc	0.13	1	125	32	125	4	63	4	>125	32
	B			0.004	0.13	0.5	2	16	2	4	1	>125	8
19*q*	A	ϕCH(OH)	S-TET	0.25	2	16	8	63	4	63	2	32	32
Cefamandole	B			0.03	0.25	2	1	8	0.5	4	0.25	4	2
19*p*	A	(thienyl)—CH_2	S-TET	0.016	0.13	2	1	16	0.25	8	0.13	32	1
	B			0.06	0.13	0.5	4	16	1	16	1	63	2
19*s*	A	(thienyl)—CH_2	S-TD	0.008	0.06	2	4	32	0.5	32	0.5	32	4
	B			0.008	0.016	0.016	4	16	2	32	1	>125	8
19*r*	A	ϕCH(NH_2)	S-TET	0.25	4	125	4	16	1	16	2	16	8
	B			0.13	1	16	2	4	1	4	1	16	1

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TABLE 5. *In vivo* activity

Compound	Blood levels[a] (minutes after administration) (μg/mL) 15	30	60	PD_{50}[b] (mg/kg)
19*i*	8.9	5.6	<2	0.5
Cephalothin	7.3	2.4	<2	3

[a]Average mouse blood levels after intramuscular administration of 10 mg/kg body weight.
[b]Protective effect after administration in mice experimentally infected with *Streptococcus pyogenes*. PD_{50} is drug dose per treatment protecting 50% of infected mice from death; mice treated 1 and 3.5 h after infection.

All cephalosporins used for comparison were either from the Bristol Laboratories (Syracuse) reference collection or were prepared from 7-ACA or 7-ADCA by the same method as used to prepare the corresponding *O*-2-isocephem.

The following abbreviations are used: THF, tetrahydrofuran; DMF, dimethylformamide.

7-β-Phenoxyacetamido-3-carbomethoxymethyl-Δ³-O-2-isocephem-4-carboxylic Acid 8h

A solution of benzyl 7-β-(aminophenoxyacetoyl)-3-methyl-Δ³-*O*-2-isocephem-4-carboxylate **10** (2.11 g, 5 mmol) in 100 mL of THF was cooled to −70°C under a slow nitrogen stream. A solution of 1.66 *M* butyllithium (6.34 mL, 10.5 mmol) was slowly added keeping the reaction temperature at −70°C. A slow stream of dry carbon dioxide gas was then introduced into the reaction mixture, the cooling bath was removed, and the carbon dioxide introduction continued until the reaction temperature reached 25°C.

The reaction mixture was poured into 200 mL of 10% hydrochloric acid, saturated with sodium chloride, and extracted three times with diethylether (150 mL portions). The combined extracts were washed three times with brine, dried (anhydrous sodium sulfate), and evaporated *in vacuo* to give 2.09 g of a yellow gum. This gum was partitioned between diethylether and 10% sodium bicarbonate solution three times. The bicarbonate solutions were then washed with diethylether (2 × 50 mL) and with methylene chloride (2 × 50 mL). The bicarbonate solution was then acidified to pH 2 with concentrated hydrochloric acid and extracted three times with methylene chloride (100 mL portions). The methylene chloride extracts were washed twice with brine, dried (anhydrous sodium sulfate), and evaporated *in vacuo* to give 0.31 g of a colorless gum. This gum was used as such in the next step.

A solution of diazomethane in diethylether was slowly added to a solution of benzyl 7-β-(aminophenoxyacetoyl)-3-carboxymethylene-Δ³-*O*-2-isocephem-4-carboxylate (0.88 g) in 100 mL of diethylether, until a permanent yellow color (excess diazomethane) was produced. The reaction mixture was then stirred at room temperature for 10 min. The reaction was acidified with 10% hydrochloric acid and extracted twice with 100 mL portions of diethylether. The extract was washed with 10% sodium bicarbonate solution (2 × 75 mL), with brine (100 mL), dried (anhydrous sodium sulfate), and evaporated *in vacuo* to give 0.72 g of crude product which was dry column chromatographed over 36 g of activity III silica gel. Elution with chloroform gave a fraction containing 240 mg of methyl ester **11**. The nmr and ir spectra are in agreement with the assigned structure. This material was used as such in the next step.

A mixture of benzyl 7-β-(aminophenoxyacetoyl)-3-carbomethoxymethylene-Δ³-*O*-2-isocephem-4-carboxylate **11** (147 mg), 10% Pd–C (100 mg), 25 mL of ethanol (USP), and 15 mL of THF was hydrogenated in a Parr hydrogenator at 17 psig for 1 h. The catalyst was filtered off and the filtrate evaporated to dryness *in vacuo* to give 87 mg of a white foam, **8***h*.

The potassium salt of acid **8***h* was prepared by dissolving the foam in a small amount of methylisobutylketone and adding a saturated solution of potassium 2-ethylhexanoate in butanol. The resultant precipitate was filtered off and washed with methylisobutylketone and then diethylether; mp 139–144°C (dec.) (cor.).

*p-Nitrobenzyl 7-β-Azido-3-formyloxymethyl-Δ³-O-2-isocephem-4-carboxylate 15*b

A solution of di-iodide **14***b* (6.6 g, 9.6 mmol) in 100 mL of DMF plus 0.1 mL of water was maintained at 0°C while powdered potassium formate (2.54 g, 30 mmol) was added. The cooling bath was removed and the mixture was stirred vigorously for 5 h. The mixture was added to 100 mL of ice water. The aqueous mixture was extracted with CH_2Cl_2 (100 + 50 mL). The combined organic extracts were washed with dilute aqueous NaCl (5 × 100 mL), dried, and the solvent was evaporated *in vacuo* to give the crude product **15***b* (5.3 g) as a brown oil. The product was used as such in the subsequent reaction. The product could be purified by chromatography on silica gel. Elution with Et_2O/EtOAc 3:1 gave pure **15***b*, mp 104–105°C.

*p-Nitrobenzyl 7-β-Azido-3-hydroxymethyl-Δ³-O-2-isocephem-4-carboxylate 15*c

A solution of compound **15***b* (5.3 g, 13 mmol), acetone (53 mL), water (26 mL), and 12 *M* HCl (3.2 mL) was stirred at 28°C for 7 h, then mixed with water (100 mL) and extracted with CH_2Cl_2 (60 + 25 + 15 mL). The combined extracts were washed with water containing a little NaCl (4 × 100 mL) dried, and the solvent was evaporated *in vacuo* to give a brown oil, 3.6 g. The product was purified by chromatography on silica gel (90 g). Elution of the column first with Et_2O, then with Et_2O/EtOAc 3:1 gave a product which was recrystallized from acetone/Et_2O to give alcohol **15***c*, 950 mg (17.5% yield from compound **14***b*), mp 147–148°C.

*p-Nitrobenzyl 7-β-Azido-3-carbamoyloxymethyl-Δ³-O-2-isocephem-4-carboxylate 15*d

A mixture of compound **15***c* (375 mg, 1.0 mmol), benzene (15 mL), sodium cyanate (130 mg, 2.0 mmol), and trifluoroacetic acid (0.16 mL, 2.1 mmol) was stirred for 2 h at 23°C. Water (25 mL) and EtOAc (15 mL) were added to the mixture and it was shaken and separated. The aqueous layer was extracted with another 15 mL of EtOAc. The combined organic phases were washed with water and saturated NaCl (30 mL each), dried, and the solvent was evaporated *in vacuo* to give carbamate **15***d* as an amorphous solid, 400 mg (95% yield).

*p-Nitrobenzyl 7-β-Azido-3-[5-thio(1-methyl)tetrazolyl]-methyl-Δ³-O-2-isocephem-4-carboxylate 15*e

A solution of methanesulfonyl chloride (0.50 mL, 6.5 mmol) in 10 mL of CH_2Cl_2 was added dropwise to a stirred solution of compound **15***c* (2.41 g, 6.43 mmol), Et_3N (0.97 mL, 7.0 mmol), and CH_2Cl_2 (75 mL) at −10°C. The solution was maintained at −10°C for ½ h and 23°C for 1 h, then washed with 5% HCl, 2% $NaHCO_3$, and water (85 mL each). The solvent was evaporated *in vacuo* to give mesylate **15***e* as an amorphous solid, 2.86 g (98% yield).

*p-Nitrobenzyl 7-β-Azido-3-[5-thio(1-methyl)tetrazolyl]-methyl-Δ³-O-2-isocephem-4-carboxylate 16*a, *p-Nitrobenzyl 7-β-Azido-3-[2-thio(5-methyl)-1,3,4-thiadiazolyl]methyl-Δ³-O-2-isocephem-4-carboxylate 16*b, *and p-Nitrobenzyl 7-β-Azido-3-[5-oxo(1-methyl)tetrazolyl]methyl-Δ³-O-2-isocephem-4-carboxylate 16*c

A solution of mesylate **15***e* (4.53 g, 10 mmol), Et_3N (1.4 mL, 10 mmol), CH_2Cl_2 (90 mL), and 10 mmol of 1-methyltetrazole-

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TABLE 6

Starting material	Product	Reaction time (h)	Yield (%)	Melting point (°C)	Anal.	Purification[a]
17_a_	**18**_a_	2	100			
17_a_	**18**_b_	16				cc Et_2O/Pet. ether
17_a_[b]	**18**_c_	16	30	152–157	CHN	cc Et_2O/Hexane
17_b_	**18**_e_	16				cc Et_2O
17_b_	**18**_f_	24	56			cc Et_2O/EtOAc
17_d_	**18**_h_	18	29	95–98		cc Et_2O/EtOAc
17_e_	**18**_i_	2	67			cc Et_2O/CH_2Cl_2
17_e_	**18**_j_[c]	16	92			—
17_e_	**18**_k_	2	52	165–168		R Et_2O/CH_2Cl_2
17_e_	**18**_l_	16	93	132–134	CHN	Trit Et_2O
17_f_	**18**_m_	2	55	127–129		cc EtOAc R Et_2O/CH_2Cl_2
17_g_	**18**_n_	16	91			—

[a]Either column chromatography (cc) or recrystallization (R).
[b]Preparation of sidechain; ref. 21.
[c]Prepared using **17**_e_ and mandeloyl carbonate but no EEDQ.

TABLE 7

Product	Starting material	Solvent mixture[a]	Reaction conditions[b]	Catalyst[c]	Yield (%)	Melting point (°C)	Anal.
19_a_	**18**_a_	A	A	A	69	197–198	CHN
19_b_	**18**_b_	BCD	B	B	100	—	—
19_c_	**18**_c_	BC	B	B	100	—	—
19_g_	**18**_e_	BCD	B	B	95	—	—
19_h_	**18**_f_	B	C	C	73	146–149	CHN
19_n_	**18**_h_	CED	D	C	12.5	—	—
19_o_	**18**_i_	AB	D	A	37	—	—
19_p_	**18**_k_	CED	D	C	18.5	182–184	CHN
19_q_	**18**_j_	CED	C	C	45	—	—
19_r_	**18**_l_	CED	D	C	67	—	—
19_s_	**18**_m_	CED	D	C	39	100–105	CHNS
19_t_	**18**_n_	CED	D	C	32	190–192	CHN

[a]Solvents used: A, THF; B, EtOH; C, EtOAc; D, aqueous HCl; E, _n_-BuOH.
[b]A, 1 atm for 10 min; B, 1 atm until uptake completed; C, 3 atm for 1 h; D, 3 atm for 3–4 h.
[c]A, 10% Pd/C.B, 30% Pd/Celite. C, 20% $Pd(OH)_2/C$.

TABLE 8

Product	Procedure	Yield (%)	Melting point (°C)	Anal.
19_d_	Same method as for **19**_i_ (2_h_)	50	—	CHNS
19_e_	Below	13	—	CHN
19_m_	Below	52	—	—
19_j_	Below	27	—	—
19_h_	Same method as for corresponding cephalosporin (15)	73	115–120 (dec.)	—
19_l_	Same method as for corresponding cephalosporin (15)	38	165–170 (dec.)	—

5-thiol, 2-methylthiadiazole-5-thiol, or 1-methyltetrazole-5-ol was stirred at 23°C for 16 h. In the cases of the first two, the solution was washed with 5% HCl and water (100 mL each), dried, and the solvent was evaporated _in vacuo_ to give the product.

The tetrazole **16**_a_ was recrystallized from EtOAc, 3.70 g (78% yield), mp 150–152°C.

The thiadiazole **16**_b_ was obtained as an oil, 4.65 g (95% yield).

In the case of **16**_c_, the solvent was evaporated _in vacuo_, replaced by $CHCl_3$ (500 mL), Et_3N (10 mmol), 1-methyltetrazole-5-ol (10 mmol), and the solution was heated under reflux for 20 h. The solution was washed and concentrated (in the manner for **16**_a_ and **16**_b_) to give crude **16**_c_. This material was purified by chromatography on silica gel (730 g) and was eluted

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from the column with Et_2O/EtOAc 3:1. It was recrystallized from EtOAc to give **16*c***, 1.07 g (22.5% yield), mp 174–176°C (dec.).

p-Nitrobenzyl 7-β-azido-3[5-thio(1-methyl) tetrazolyl]methyl-Δ³-O-2-isocephem-4-carboxylate **16a** *from* **14b**

The dibromide **14*a*** (R^1 = PNB) was prepared in exactly the same manner as the corresponding benzyl ester (2*h*). 1-Methyltetrazole-5-thiol (565 mg, 4.85 mmol) was added to a solution of dibromide **14*a*** (2.90 g, 4.85 mmol) in 35 mL of CH_2Cl_2 at 0°C. A solution of Et_3N (0.67 mL, 4.85 mmol) in 7 mL of CH_2Cl_2 was added and the solution was stirred at 23°C for 30 min. The solution was washed with water (2 × 40 mL), dried, treated with charcoal, and filtered. The solvent was evaporated *in vacuo* to give compound **14*c***, 2.70 g, in about 30% purity.

Potassium acetate (835 mg, 8.54 mmol) was added to a solution of **14*c*** (2.70 g) in 27 mL of DMF at 0°C. The mixture was stirred at 23°C for 18 h, then diluted with 80 mL of CH_2Cl_2 and washed with 1% NaCl solution (6 × 80 mL). The organic phase was dried and absorbed onto silica gel (7.5 g) which was placed on a silica gel column (30 g). The column was eluted with Et_2O then with Et_2O/CH_2Cl_2 1:1. Evaporation of the solvent from the appropriate fractions gave compound **16*a***, 344 mg (15% yield), identical in all respects to that prepared above.

Azide Reductions using Hydrogen Sulfide – Triethylamine *17*d–g

A solution of equimolar amounts of the azido compound and triethylamine in CH_2Cl_2 (~10% solution) was cooled to 0°C and saturated with H_2S. After 1 to 1.5 h the solution was acidified (10% HCl), the CH_2Cl_2 was evaporated *in vacuo*, and the mixture was extracted with CH_2Cl_2. The CH_2Cl_2 extract was filtered to remove elemental sulfur and the solvent was evaporated *in vacuo* to give product **17*e*** (86% yield), **17*f*** (85% yield), or **17*g*** (61% yield). Compound **17*d*** was obtained in 100% yield simply by evaporation of the solvent *in vacuo* after the H_2S addition. These crude products were used as such in subsequent reactions.

EEDQ Coupling Reactions

A solution of equimolar amounts of EEDQ, the acid to be coupled, and the appropriate amine in methylene chloride (from 5–20 wt% solutions) was let stand at room temperature (~23°C) for the specified time (see Table 6). The resulting solution was washed with dilute solutions of HCl, $NaHCO_3$, and NaCl. The organic layers were dried over Na_2SO_4 and the solvent evaporated at reduced pressure.

Ester Removal by Hydrogenolysis

The hydrogenolysis of the various benzyl and *p*-nitrobenzyl esters to yield the desired acids was carried out as follows: a solution of x mg of the ester in 100 × mL of solvent was hydrogenated under the conditions shown in Table 7. When the reaction was complete the catalyst was removed by filtration. For those products carrying a phenylglycyl side chain one equivalent of HCl was added to the hydrogenation mixture. The solution was evaporated at reduced pressure to give the product as its hydrochloride salt. For non-basic products the reaction solution was extracted with aqueous sodium bicarbonate. The aqueous extracts were acidified and back extracted into EtOAc. The solvent was evaporated at reduced pressure to yield the product.

*Compounds 19*d, e, j, k, l, m

These compounds were prepared from **17*c*** or **21** as described in Table 8.

Compound **17*c*** and 4-chloromethylphenylacetyl chloride were reacted by the method used to prepare **19*i*** (2*h*) to give amide **33**. A solution of **33** (635 mg, 1.5 mmol) in 6 mL of acetone was mixed with a solution of thiourea (120 mg, 1.6 mmol) in 3 mL of acetone and maintained at 23°C for 72 h. The precipitate was collected by filtration and washed with acetone to give **19*m***, 397 mg, in about 80% purity.

To a suspension of potassium 2-(1-carbomethoxypropen-2-ylaminomethyl)phenylacetate (1.2 g, 38 mmol) in 23 mL of tetrahydrofuran was added 3 drops of dimethylbenzylamine. The mixture was cooled to −40°C and isobutyl chloroformate (520 mg, 38 mmol) was added. After 5 min, the solution was added to a cooled (0°C) solution of compound **17*c*** or **21** (1.8 mmol) and *N*-methylmorpholine (0.71 mL) in 13 mL of water. The solution was stirred for 1 h at 0°C, then acidified to pH 5.2 with hydrochloric acid. Ethyl acetate was added and the mixture was stirred for 1 h at 23°C. The product was collected by filtration and dried *in vacuo*. Compound **19*e*** was obtained analytically pure while **19*j*** was obtained in about 80% purity.

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