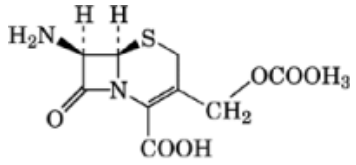
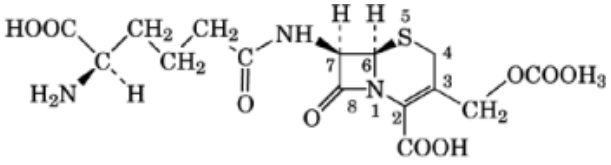
Kirk-Othmer Encyclopedia of Chemical Technology.

CEPHALOSPORINS

The cephalosporins, a subgroup of β-lactam antibiotics, consist of a 4-membered lactam ring fused through the nitrogen and the adjacent tetrahedral carbon atom to a second heterocycle forming a 6-membered dihydrothiazine ring. Other structural features common to all the cephalosporins are a carboxyl group on the dihydrothiazine ring on the carbon next to the ring nitrogen and a functionalized amino group on C-7, the carbon of the β-lactam ring opposite the nitrogen. These features are evidenced in 7-aminocephalosporanic acid [957-68-6] (7-ACA), $C_{10}H_{12}N_2O_5S$ **1**. Cephalosporins, like all β-lactam antibiotics, exert their antibacterial effect by interfering with the synthesis of the bacterial cell wall. These antibiotics tend to be "irreversible" inhibitors of cell wall biosynthesis and they are usually bactericidal at concentrations close to their bacteriostatic levels. Cephalosporins are widely used for treating bacterial infections. They are highly effective antibiotics and have low toxicity.



(**1**)



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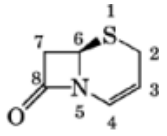


(**3**)

1. Nomenclature and Stereochemistry

Chemical Abstracts indexes cephalosporins as 5-thia-1-azabicyclo[4.2.0]oct-2-enes. Using this system then, cephalosporin C [61-24-5], $C_{16}H_{21}N_3O_8S$, **2** is 3-[(acetyloxy)methyl]-7-[(5-amino-5-carboxy-1-oxopentyl)amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. Such names are too cumbersome for general use. One simplification defines the ring system of the cephalosporins as cepham **3** (1). Hence cephalosporins become 3-acetoxymethyl-7-acylamino-3-cephem-4-carboxylic acids. In this widely used nomenclature system, the numbering, as shown in structure **3**, differs from that used by *Chemical Abstracts* shown in structure **2**. In both systems, however, the important C-7, C-6, and C-3 positions remain unchanged. The cephem numbering system **3** is used herein.

The largest group of cephalosporins are derived from the cephem nucleus containing a 3-(substituted)methyl group. It is sometimes useful to designate the methyl group as C-3′. Thus, for example, a 3-acetoxymethyl, present in structures **1** and **2**, becomes the 3′-acetoxy functionality. Another simplification in nomenclature is the use of the term cephalosporanic acid (2) as the parent name. Although convenient, this system is somewhat restrictive for cephalosporins in which the 3-acetoxymethyl grouping has been replaced. The clinically important cephalosporins all have a generic name which, by convention, starts with ceph- or cef-.

Structures of the naturally occurring cephalosporins, cephamycins, and the 7-formamido cephalosporins are as indicated in Tables 1 and 2. The amide bearing carbon, C-7, has the *R*-configuration; the nonplanar fused rings are folded along the C-6 to ring nitrogen bond; and the configuration of the fused rings is such that the hydrogen on C-6 is cis to the hydrogen (or the methoxyl or formamido moiety) on C-7. Thus the absolute stereochemistry is 6*R*:7*R*. The stereochemistry of substituents attached to the rings is designated using α and β. For cephalosporins, the hydrogens at C-7 and C-6 are α, the acylamino group at C-7 is β; in the cephamycins, the C-7 methoxyl is α. These stereochemical relationships and the absolute stereochemistry are also essential for antibacterial activity in the synthetic and semisynthetic cephalosporins. Antibacterial activity is lost whenever either or both of the asymmetric centers are inverted.

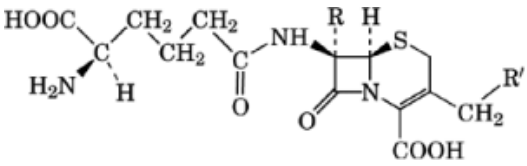
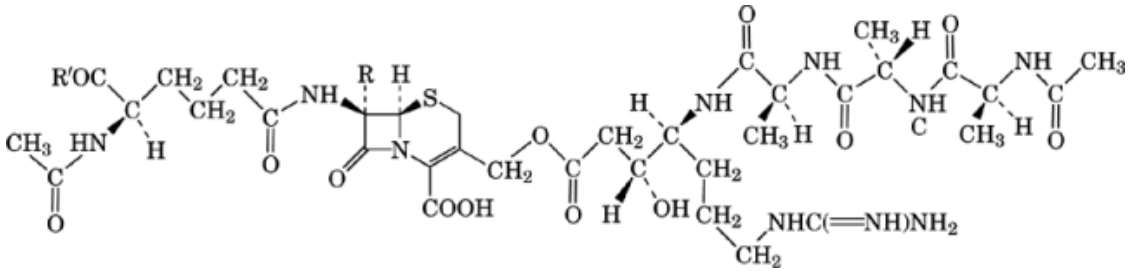
2. Discovery and Historical Development

In 1945 it was noted that a strain of *Cephalosporium acremonium* produced antibiotic material that was active against gram-negative as well as gram-positive organisms (18). The main hydrophilic component, originally called cephalosporin N, and active against both gram-negative and gram-positive organisms was found, in fact, to be a penicillin and was renamed penicillin N. A second hydrophilic antibiotic, present only in minute amounts in the original fermentation mixture and stable to acidic hydrolysis, was named cephalosporin C **2** (19). Chemical studies demonstrated that, like penicillin N, cephalosporin C contained a fused β-lactam ring system and a D-α-aminoadipamido substituent. Additional experiments led to the correct structure which was confirmed by x-ray crystallographic studies (4–6, 20).

All cephalosporins found in nature (Tables 1 and 2) have the D-α-aminoadipic acid 7-acyl side chain (21). All of these compounds can be classified as having rather low specific activity. A substantial amount of the early work in the cephalosporin area was unsuccessfully directed toward replacing the aminoadipic acid side chain or modifying it appropriately by fermentation or enzymatic processes (6, 22). A milestone in the development of cephalosporins occurred in 1960 with the discovery of a practical chemical process to remove the side chain to afford 7-ACA **1** (1). Several related processes were subsequently developed (22, 23). The ready availability of 7-ACA opened the way to thousands of new semisynthetic cephalosporins. The cephalosporin structure offers more opportunities for chemical modification than does that of penicillins. There are two side chains that especially lend themselves to chemical manipulation: the 7-acylamino and 3-acetoxymethyl substituents.

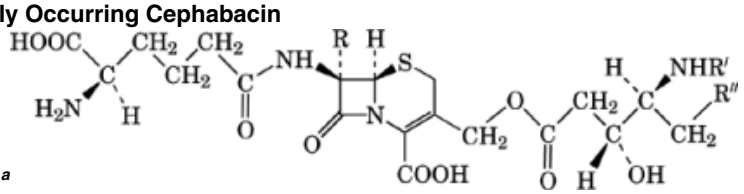
The cephamycins, described almost simultaneously by two different groups (7, 11) in 1971, are produced by the *Streptomyces* species and have the same basic cephalosporin ring system (Table 1) (24). The natural

Table 1. Naturally Occurring Cephalosporins

Named	CAS Registry Number	Molecular formula	R	R′	Ref.
					
deacetoxycephalosporin C	[26924-74-3]	$C_{14}H_{19}N_3O_8S$	—H	—H	3
deacetylcephalosporin C	[1476-46-6]	$C_{14}H_{19}N_3O_7S$	—H	—OH	3
cephalosporin C	[61-24-5]	$C_{16}H_{12}N_2O_5S$	—H	—$OCOCH_3$	(4–6)
O-carbamoyldeacetylcephalosporin C	[32178-83-9]	$C_{15}H_{19}N_4O_8S$	—H	—$OCONH_2$	7
3′-methylthiodeacetoxycephalosporin C (F_1)	[60890-86-0]	$C_{15}H_{21}N_3O_6S_2$	—H	—SCH_3	8
3′-sulfothiodeacetoxycephalosporin C (F_2)	[65580-83-8]	$C_{14}H_{19}N_3O_9S_3$	—H	—SSO_2OH	9
C43-219	[60857-71-8]	$C_{19}H_{28}N_4O_8S_2$	—H	—$SC(CH_3)_2CH(NH_2)CO_2H$	10
7α-methoxycephalosporin C	[32178-82-8]	$C_{17}H_{23}N_3O_9S$	—OCH_3	—$OCOCH_3$	7
cephamycin C	[34279-55-1]	$C_{16}H_{22}N_4O_9S$	—OCH_3	—$OCONH_2$	(7, 11)
cephamycin A	[34279-78-2]	$C_{25}H_{29}N_3O_{14}S_2$	—OCH_3	—$OCOC(OCH_3)$═$CHC_6H_4OSO_2OH$-*p*	11
cephamycin B	[34729-77-1]	$C_{25}H_{29}N_3O_{11}S$	—OCH_3	—$OCOC(OCH_3)$═CHC_6H_4OH-*p*	11
Takeda C2801X	[62851-50-7]	$C_{25}H_{29}N_3O_{12}S$	—OCH_3	—$OCOC(OCH_3)$═$CHC_6H_3(OH)_2$-*m,p*	12
SF-1623	[56487-86-6]	$C_{15}H_{21}N_3O_{10}S_3$	—OCH_3	—SSO_2OH	13
					
SQ 28,516	[92121-46-5]	$C_{36}H_{55}N_{11}O_{15}S_1$	—NHCHO	—OH	14
SQ 28,517	[92131-67-4]	$C_{36}H_{56}N_{12}O_{14}S_1$	—NHCHO	—NH_2	14

products have the 7β-D-α-aminoadipamido substituent and a methoxy substituent instead of a hydrogen in the 7α-position. These cephamycins have only weak antibacterial activity, but the methoxy group imparts good β-lactamase stability to these compounds. Semisynthetic analogues (25) have been obtained either by chemically introducing a methoxy group at the 7α-position of the cephalosporin ring system or by manipulating the 3- and 7β-substituents of the natural cephamycins (26, 27). Some 7α-methoxylated cephalosporins have also been reported which have amino acid or oligopeptides at the C-3 position (Table 2) (17). The compounds are designated herein as cephabacin M (from *ceph*em *a*ntibiotics of *bac*terial orig*in* with the M designating a 7-methoxyl substituent).

Table 2. Naturally Occurring Cephabacin



Cephalosporins[a]

Name	CAS Registry Number	Molecular formula	R	R′	R″	Refs.
cephabacin F_1 or chitinovorin A	[95041-98-8]	$C_{26}H_{41}N_9O_{11}S$	—NHCHO	-L-Ala	$—CH_2CH_2NHC(=NH)NH_2$	15 16
cephabacin F_2 or chitinovorin B	[95722-76-2]	$C_{29}H_{46}N_{10}O_{12}S$	—NHCHO	-L-Ala-L-Ala	$—CH_2CH_2NHC(=NH)NH_2$	15 16
cephabacin F_3	[95042-00-5]	$C_{32}H_{51}N_{11}O_{13}S$	—NHCHO	-L-Ala-L-Ala-L-Ala	$—CH_2CH_2NHC(=NH)NH_2$	15
cephabacin F_4	[95042-01-6]	$C_{26}H_{41}N_9O_{12}S$	—NHCHO	-L-Ser	$—CH_2CH_2NHC(=NH)NH_2$	15
cephabacin F_5	[95042-02-7]	$C_{29}H_{46}N_{10}O_{14}S$	—NHCHO	-L-Ser-L-Ser	$—CH_2CH_2NHC(=NH)NH_2$	15
cephabacin F_6	[95042-03-8]	$C_{32}H_{51}N_{11}O_{15}S$	—NHCHO	-L-Ser-L-Ser-L-Ala	$—CH_2CH_2NHC(=NH)NH_2$	15
cephabacin F_7	[95230-87-8]	$C_{26}H_{41}N_7O_{12}S$	—NHCHO	-L-Ser	$—CH_2CH_2CH_2NH_2$	15
cephabacin F_8	[95230-88-9]	$C_{29}H_{46}N_8O_{14}S$	—NHCHO	-L-Ser-L-Ser	$—CH_2CH_2CH_2NH_2$	15
cephabacin F_9	[95230-89-0]	$C_{32}H_{51}N_9O_{15}S$	—NHCHO	-L-Ser-L-Ser-L-Ala	$—CH_2CH_2CH_2NH_2$	15
cephabacin H_1	[95042-07-2]	$C_{25}H_{40}N_8O_{10}S$	—H	-L-Ala	$—CH_2CH_2NHC(=NH)NH_2$	15
cephabacin H_2	[95042-08-3]	$C_{28}H_{45}N_9O_{11}S$	—H	-L-Ala-L-Ala	$—CH_2CH_2NHC(=NH)NH_2$	15
cephabacin H_3	[95042-09-4]	$C_{31}H_{50}N_{10}O_{12}S$	—H	-L-Ala-L-Ala-L-Ala	$—CH_2CH_2NHC(=NH)NH_2$	15
cephabacin H_4	[95042-10-7]	$C_{25}H_{40}N_8O_{11}S$	—H	-L-Ser	$—CH_2CH_2NHC(=NH)NH_2$	15
cephabacin H_5	[95042-11-8]	$C_{28}H_{45}N_9O_{13}S$	—H	-L-Ser-L-Ser	$—CH_2CH_2NHC(=NH)NH_2$	15
cephabacin H_6	[95042-12-9]	$C_{31}H_{50}N_{10}O_{14}S$	—H	-L-Ser-L-Ser-L-Ala	$—CH_2CH_2NHC(=NH)NH_2$	15
cephabacin M_1	[99332-96-4]	$C_{31}H_{50}N_8O_{13}S$	$—OCH_3$	-L-Val-L-Orn	$—CONH_2$	17
cephabacin M_2	[99313-76-5]	$C_{34}H_{55}N_9O_{15}S$	$—OCH_3$	-L-Val-L-Orn-L-Ser	$—CONH_2$	17
cephabacin M_3	[99313-75-4]	$C_{37}H_{60}N_{10}O_{16}S$	$—OCH_3$	-L-Val-L-Orn-L-Ser-L-Ala	$—CONH_2$	17
cephabacin M_4	[99313-74-3]	$C_{41}H_{69}N_{11}O_{15}S$	$—OCH_3$	-L-Val-L-Orn-L-Val-L-Orn	$—CONH_2$	17
cephabacin M_5	[99332-95-3]	$C_{44}H_{74}N_{12}O_{17}S$	$—OCH_3$	-L-Val-L-Orn-L-Val-L-Orn-L-Ser	$—CONH_2$	17
cephabacin M_6	[99313-73-2]	$C_{47}H_{79}N_{13}O_{18}S$	$—OCH_3$	-L-Val-L-Orn-L-Val-L-Orn-L-Ser-L-Ala	$—CONH_2$	17

[a] Structure as shown where R, R′, and R″′ are as indicated.

In 1984 a new class of naturally occurring cephalosporins containing a 7α-formamido group, was isolated (14–16). These compounds (Table 2) called chitinovorins(**14**) (16) or cephabacin F (15) where F designates a formamido group in the 7-position, also have amino acids or oligopeptides at the C-3 position. An analogous 7α-hydrogen series, named cephabacin H, containing the same oligopeptides at C-3 has also been isolated (15). The derivatives all have weak antibacterial activity. The 7α-formamido group imparts good β-lactamase stability (15) as is the case for the cephamycins. Chemical manipulation afforded a series of semisynthetic analogues having excellent broad-spectrum activity (28–30).

3. Biogenesis

The biosynthesis of cephalosporins and penicillins (Fig. 1) both start from the amino acids and proceed via δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine [32467-88-2] (LLD-ACV) (**9**), $C_{14}H_{25}N_3O_6S$, (31–34), often referred to as the

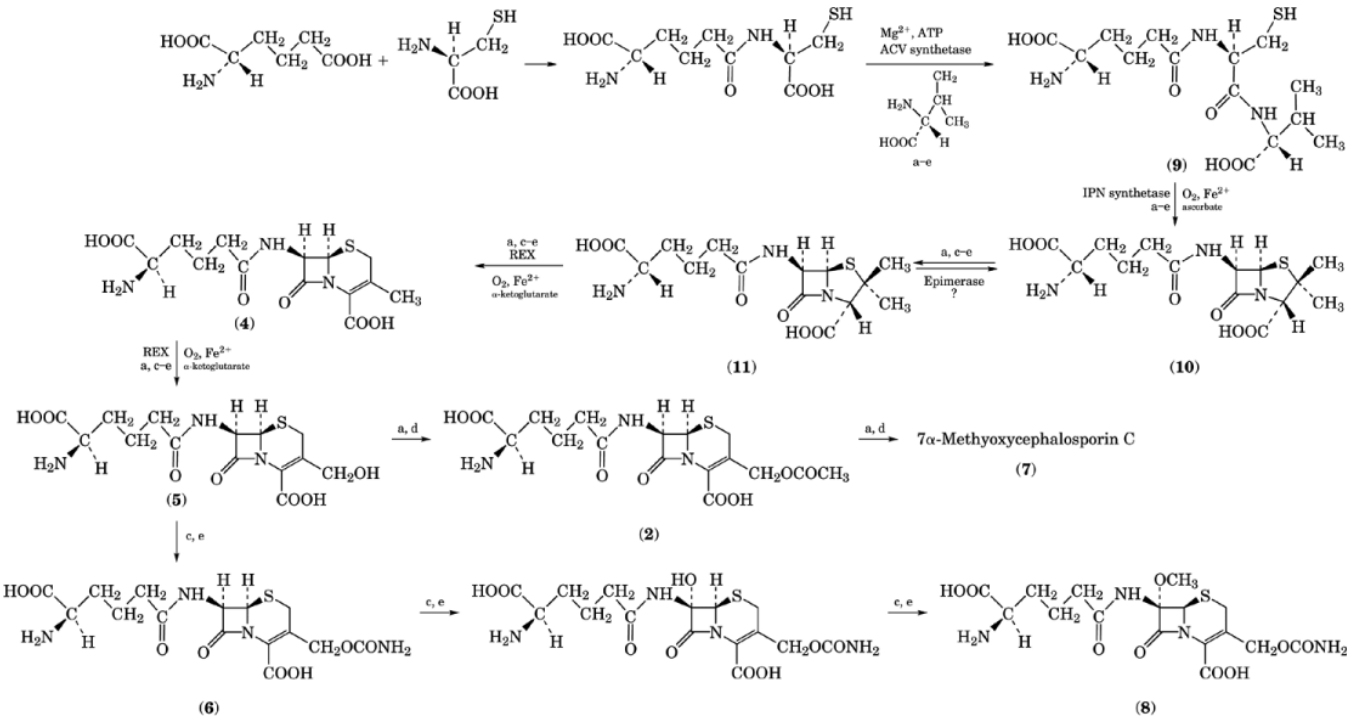


Fig. 1. Biosynthesis of cephalosporins and cephamycins. a, *Cephalosporium acremonium*; b, *Penicillium chrysogenum*; c, *Streptomyces clavuligerus*; d, *Streptomyces lipmanii*; e, *Streptomyces wadayamensis*; REX is a ring expansion enzyme (deacetoxycephalosporin C synthethase).

Arnstein tripeptide (35). Because LLD-ACV is not transported into intact cells, a cell-free system was required to determine that this intermediate is the precursor of the penicillins (36). The cell-free system, obtained fron *C. acremonium*, converts LLD-ACV into isopenicillin N [525-94-0] $C_{14}H_{21}N_3O_6S$ (**10**). Isopenicillin N (IPN) synthetase, the enzyme which catalyses this conversion, requires oxygen, Fe^{2+}, a reducing agent such as ascorbate, and a thiol group such as that of dithiothreitol for high activity (37, 38). Isopenicillin N synthetase is present in *P. chrysogenum* and in species of *Streptomyces* as well as in *C. acremonium* (39, 40). In the *Cephalosporium* species and the *Streptomyces* species, an epimerase is present that converts (**10**) into penicillin N [58678-43-6] (**11**), $C_{14}H_{21}N_3O_6S$, (41, 42) which then undergoes a ring expansion to deacetoxycephalosporin C (**4**) (43–46). The ring expansion enzyme (REX) from *C. acremonium* appears to be bifunctional (47); it also catalyzes (48, 49) the subsequent hydroxylation of (**4**) to deacetylcephalosporin C (**5**), itself a precursor of cephalosporin C **2** (50). The introduction of the methoxyl group is also a two-step process involving molecular oxygen (51, 52). Using cephalosporin C **2** or the corresponding carbamoyl derivative (**6**) as a substrate, another dioxygenase catalyzes the incorporation of a 7α-hydroxy function (53). The resulting 7α-hydroxycephalosporin is then methylated using *S*-adenosylmethionine to form the corresponding 7α-methoxycephalosporin (53). The details of these steps are discussed in depth in the literature (21, 39, 42, 48, 54, 55). Rapid advances in this area have been made possible by the successful cloning and expression of isopenicillin N synthetase (IPNS) and ring expansion-hydroxylase (REX) (39, 48, 56, 57).

Table 3. Properties of Cephalosporins[a]

Name	pK_a[b]	β-Lactam ir stretching frequency, cm^{-1}[c]	Uv absorption, λ max, nm (ϵ, $cm^{-1}M^{-1}$)[d]	Nmr absorption, ppm[e] H-7[f]	H-6[g]	$J_{6,7}$, Hz[h]
7-ACA	1.75, 4.63	1806	261(8500)	5.53d 4.83d	5.13d	4.5
cephalosporin C	<2.6, 3.1, 9.8	1780	260(8900)	5.66d	5.15d	4.7
cephalothin	5.0	1760	265(9000)	5.70d	5.14d	4.5
cephalexin	5.2, 7.3	1775	260(7750)	6.10d	5.45d	4.2
cephamycin C	4.2, 5.6, 10.4[i]	1770	264(6900) 242(5700)		5.19s	

[a] Refs. (7, 11, 58–62, 64, 65).
[b] Values for aqueous solutions unless otherwise noted, either by direct determination or by extrapolation from mixed solvents.
[c] Nujol mull.
[d] In aqueous solution.
[e] In D_2O relative to external standard tetramethyl silane (TMS); s = singlet, d = doublet.
[f] Range 5.23–6.21.
[g] Range 4.24–5.46.
[h] $J_{6,7}(cis) = 4 - 5$ Hz, $J_{6,7}(trans) = 1.5 - 2$ Hz, $J_{7-} = 8 - 11$ Hz.
[i] In 66% DMF.

4. Physical Properties

Most cephalosporin antibiotics are white, off-white, tan, or pale yellow solids that are usually amorphous, but can sometimes be obtained crystalline. The cephalosporins do not usually have sharp melting points, but rather decompose upon heating at elevated temperatures. The acid strength, pK_a, of the carboxyl group on the dihydrothiazine ring depends on environment (58). Representative pK_a values are given in Table 3, as are other physical properties.

One of the distinguishing physical characteristics of the cephalosporins is the infrared stretching frequency of the β-lactam carbonyl. This absorption occurs at higher frequencies ($1770 - 1815$ cm^{-1}) than those of either normal secondary amides ($1504 - 1695$ cm^{-1}) or ester carbonyl groups ($1720 - 1780$ cm^{-1}). The nuclear magnetic resonance signals of the protons attached to the β-lactam ring provide information about ring integrity, the attachments to the ring, and the relative stereochemistry of substituents in these positions. These protons form an ABX system with the vicinal proton on the side-chain amide nitrogen (Table 3) (58–62). The cephalosporins display uv absorption maxima at about 260 nm, which is at a longer wavelength than expected for a normal α,β-unsaturated amide, usually about 220–230 nm. This shift may result from the presence of low lying sulfur atom d-orbitals (63).

5. Chemical Properties

Much of the chemical reactivity of the β-lactam antibiotics is associated with the β-lactam moiety. The geometry and the accompanying increased ring strain results in very little, if any, amide-resonance stabilization leading to a marked increase in chemical reactivity when compared to a normal amide. In fact, in many instances the reactivity of the lactam carbonyl is analogous to that of a carboxylic acid anhydride. Fused β-lactam antibiotics are readily attacked by nucleophiles with resultant ring opening and loss of biological activity. The cephalosporins are more resistant to ring opening than the penicillins. For example, although alcohols readily attack the penicillin β-lactam, cephalosporins are sufficiently stable to permit the use of methanol as a recrystallization solvent.

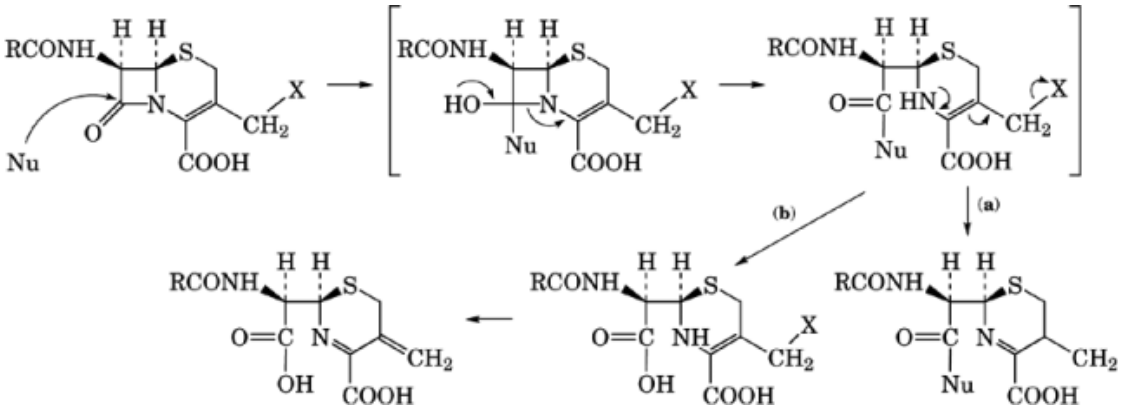


Fig. 2. Cephalosporin β-lactam reactivity where Nu is a nucleophile and X is a leaving group; (**a**) path followed for nucleophiles; (**b**) when Nu is the serine OH of an enzyme (ie, $_{\text{Nu=Enz-Ser-OH}}$) deacylation may precede expulsion of the leaving group and both pathways (**a**) and (**b**) may be observed.

Cephalosporins are attacked by nucleophiles such as alkoxide or hydroxylamine, but because of the nature of the dihydrothiazine ring, products equivalent to the penicilloic or penilloic acids derived from the penicillins under similar conditions, are not obtained. Rather, studies indicate involvement of the 3′-substituent as a leaving group (66). Thus 3-deacetoxycephalosporins are found to be more stable than cephalosporins having 3′-substituents that can be readily lost. The 3′-substituents exert an electronic influence on the chemical reactivity of the β-lactam carbonyl group (67, 68) which can be correlated with antibiotic activity within a series (69, 70) and it has been claimed that cephalosporin antibacterial activity is related to the ease of C-3′ group expulsion (71). The deacetoxy analogues generally show lower levels of activity *in vitro* than their 3′-functionalized analogues.

It has also been proposed that reactivity is enhanced because nucleophilic attack on the β-lactam carbonyl is concerted with expulsion of the leaving group (69). However, evidence is now available which strongly suggests that in alkaline hydrolysis and alcoholysis fission of the cephalosporin β-lactam C–N bond occurs before that of the C-3′ leaving group (71–73). This would seem to be the case for interaction of cephalosporins with β-lactamase enzymes (72, 74). Thus cephalosporin reactivity is probably best represented by the sequence outlined in Figure 2. In the case where the attacking nucleophile is part of the active site of an enzyme (serine hydroxyl), the acyl–enzyme complex may break down before expulsion of the leaving group or may expel the leaving group to afford a new acyl–enzyme complex (72). The chemistry of cephalosporins, as utilized in synthetic processes and manufacturing, such as deacylation and acylation of the C-7 amino, esterification of the carboxyl, modification at C-3, and introduction of functionality into the C-7α position are discussed later. A discussion of other aspects of cephalosporin chemistry, such as deamination or epimerization at C-7, reactions at sulfur, isomerization of and addition to the double bond, and modification on the allylic methylene group at C-2, can be found in leading reviews (75, 76).

6. Biological Properties

The clinical effectiveness of the cephalosporins depends on a number of properties. The antibiotic must inhibit, or preferably kill, bacteria at acceptable concentrations of the drug (*in vitro* activity); it must be capable of achieving host serum and tissue levels greater than those required to inhibit the pathogenic organism; and the selective toxicity profile must allow for safe administration to the host. Much of the chemical work that

has been done on the cephalosporins has been directed toward modifying and improving the antibacterial and pharmacokinetic properties of these substances. Pharmacokinetic properties include the efficiency and rate of adsorption, the rate of metabolism and excretion, tissue distribution, serum binding, and serum and tissue inactivation. Chemical studies are thus closely coupled to extensive biological evaluation which examines *in vitro* and *in vivo* antibacterial activity expressed as minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and protective effectiveness in laboratory animals (PD_{50} or ED_{50}) as well as pharmacokinetic characteristics. New compounds are also tested for the ability to resist inactivation by β-lactamases (penicillinases, cephalosporinases) produced by bacteria.

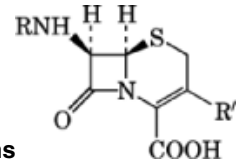


Table 4. Oral Cephalosporins

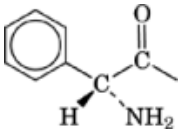
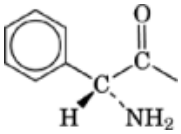
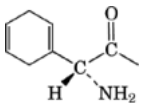
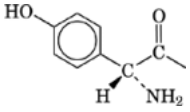
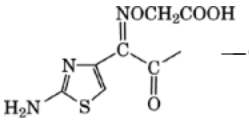
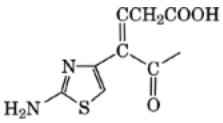
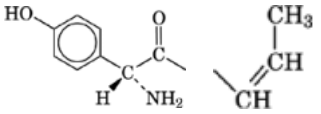
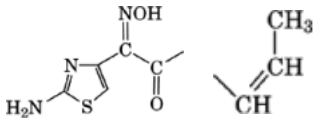
Generic name	CAS Registry Number	Molecular formula	Generation	Trade name	Company	Structure number	R	R′	Year[a]	Refs.
cephalexin	[15686-71-2]	$C_{16}H_{17}N_3O_4S$	[1]	Keflex	Lilly	**12**		—CH_3	1970	(77, 89)
cefaclor	[53994-73-3]	$C_{15}H_{14}N_3O_4S_1Cl$	[2]	Ceclor	Lilly	**13**		—Cl	1979	(77, 90)
cephradine	[3882-53-3]	$C_{16}H_{19}N_3O_4S$	[1]	Velosef Anspor	Squibb SKF-Beecham	**14**		—CH_3	1972	(77, 91–93)
cefadroxil	[50370-12-2]	$C_{16}H_{17}N_3O_5S$	[1]	Duricef Ultracef	Mead-Johnson Bristol-Myers Squibb	**15**		—CH_3	1980	(77, 94, 95)

Table 4. ***Continued***

Generic name	CAS Registry Number	Molecular formula	Generation	Trade name	Company	Structure number	R	R′	Year[a]	Refs.
cefixime	[79350-37-1]	$C_{16}H_{15}N_5O_7S_2$	[2–3]	Suprax Cefspan[b]	Lederle Fujisawa	**16**		—CH=CH$_2$	1989 1987[c]	96
ceftibuten	[97519-39-6]	$C_{15}H_{14}N_4O_6S_2$	[2–3]	Sch 39720 7432-S	Schering Shionogi	**17**		—H	Ph-III	97
cefprozil	[92665-29-7]	$C_{18}H_{19}N_3O_5S$	[2]	BMY28100	Bristol-Myers Squibb	**18**			Ph-III	98
—	[107888-46-0]	$C_{15}H_{15}N_5O_6S_2$	[2–3]	BMY28232	Bristol-Myers Squibb	**19**			Pre-Cl	99

[a] Year is year of launch in the U.S. market unless indicated. Pre − Cl = preclinical, Ph III = phase III clinical trials (status as of Feb. 1990).
[b] Japanese trade name.
[c] Launch date in Japan.

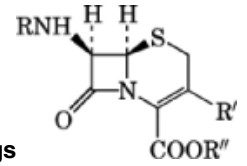
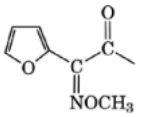
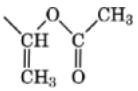
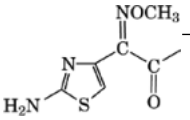
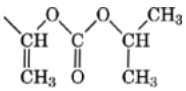
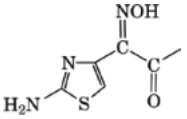
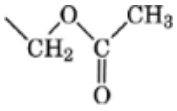


Table 5. Oral Cephalosporins-Prodrugs

Generic name[a]	CAS Registry Number	Molecular formula	Trade name	Company	Structure number	R	R′	R″	Year[b]	Refs.
cefuroxime-axetil [2]	[64544-07-6]	$C_{20}H_{22}N_4O_{10}S_1$	Ceftin	Glaxo Roche	**20**		—CH_2OCONH_2		1988	100
cefpodoxime-proxetil [2–3]	[87239-81-4]	$C_{21}H_{27}N_5O_9S_2$	Banan	Upjohn Sankyo	**21**		—CH_2OCH_3		Ph-III	101
[2–3]	[107888-50-6]	$C_{18}H_{19}N_5O_7S_2$	BMY28271	Bristol-Myers Squibb	**22**				Pre-Cl	(99, 102)

[a] Drug generation is given in brackets.
[b] Year is year of launch in the U.S. market unless indicated. Pre − Cl = preclinical and Ph III = phase III clinical trials (status as of Feb. 1990).

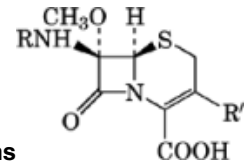


Table 6. Cephamycins

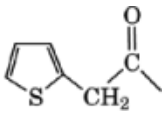
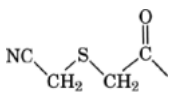
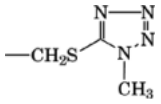
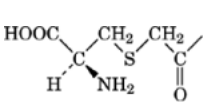
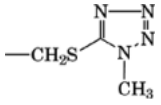
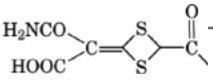
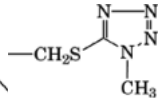
Generic name[a]	CAS Registry Number	Molecular formula	Trade name	Company	Structure number	R	R′	Year[b]	Refs.
cefoxitin [2]	[35607-66-0]	$C_{15}H_{15}N_3O_6$	Mefoxin	Merck	**23**		—CH_2OCONH_2	1978	103

Table 6. ***Continued***

Generic name[a]	CAS Registry Number	Molecular formula	Trade name	Company	Structure number	R	R′	Year[b]	Refs.
cefmetazole [2]	[56796-20-4]	$C_{14}H_{15}N_7O_4S_3$	Zefazone Cefmetazon[c]	Upjohn Sankyo	**24**			1989 1980[d]	104
cefminox [2]	[75221-13-0]	$C_{15}H_{19}N_7O_6S_3$	Meicelin[c]	Meiji Seika Bristol-Myers Squibb	**25**			1987[d]	105
cefotetan [2]	[69712-56-7]	$C_{16}H_{15}N_7O_7S_4$	Ceftan Apacef[c] Yamatetan	Stuart Yamanouchi	**26**			1986 1984[d]	106

[a] Drug generation is given in brackets.
[b] Year is year of launch in the U.S. market unless indicated.
[c] Japanese trade name.
[d] Launch date in Japan.

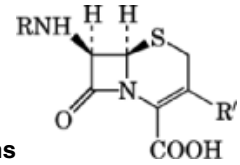


Table 7. Parenteral Cephalosporins

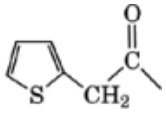
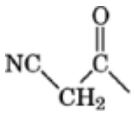
Generic name[a]	CAS Registry Number	Molecular formula	Trade name	Company	Structure number	R	R′	Year[b]	Refs.
cephalothin [1]	[153-61-7]	$C_{16}H_{16}N_2O_6S_2$	Keflin	Lilly	**27**		$-CH_2OCOCH_3$	1965	(87, 107)
cephacetrile [1]	[23239-41-0]	$C_{13}H_{13}N_3O_6S$	Celospor	Ciba	**28**		$-CH_2OCOCH_3$	1979[c]	(87, 108)

Table 7. ***Continued***

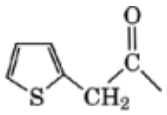
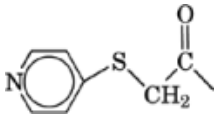
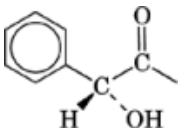
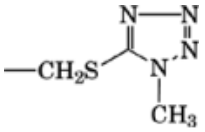
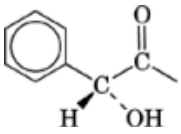
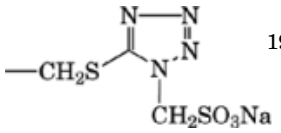
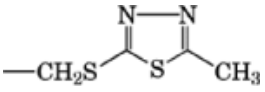
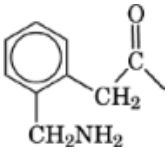
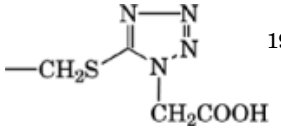
Generic name[a]	CAS Registry Number	Molecular formula	Trade name	Company	Structure number	R	R′	Year[b]	Refs.
cephalothin [1]	[153-61-7]	$C_{16}H_{16}N_2O_6S_2$	Keflin	Lilly	**27**		—CH_2OCOCH_3	1965	(87, 107)
cephapirin [1]	[21593-23-7]	$C_{17}H_{17}N_3O_6S_2$	Cefadyl	Bristol-Myers Squibb	**29**		—CH_2OCOCH_3	1974	(87, 91, 109)
cefamandole [2]	[34444-01-4]	$C_{11}H_{18}N_6O_5S_2$	Mandol	Lilly	**30**			1978	(87, 110)
cefonicid [2]	[61270-58-4]	$C_{18}H_{17}N_6O_8S_3Na$	Monicid	SKF-Beecham	**31**			1984	(87, 111)
cefazolin [1]	[25953-19-9]	$C_{14}H_{14}N_8O_4S_3$	AncefKefzol	SKF-BeechamLilly	(32)			1973	(87, 112)
ceforanide [2]	[40851-79-4]	$C_{20}H_{19}N_7O_6S_2$	Precef	Bristol-Myers Squibb	**33**			1984	(87, 113)

Table 7. ***Continued***

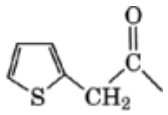
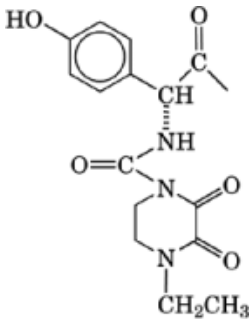
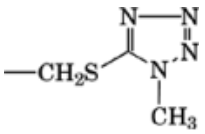
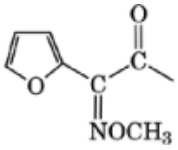
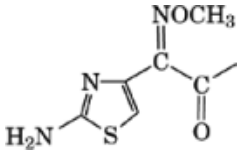
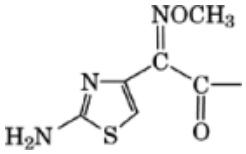
Generic name[a]	CAS Registry Number	Molecular formula	Trade name	Company	Structure number	R	R′	Year[b]	Refs.
cephalothin [1]	[153-61-7]	$C_{16}H_{16}N_2O_6S_2$	Keflin	Lilly	**27**		—CH_2OCOCH_3	1965	(87, 107)
cefoperazone [3]	[62893-19-0]	$C_{25}H_{27}N_9O_8S_2$	Cefobid	PfizerRoerig	**34**			1983	(87, 114)
cefuroxime [2]	[55268-75-2]	$C_{16}H_{16}N_4O_8S$	Zinacef Kefurox	GlaxoLilly	**(35)**		—CH_2OCONH_2	1983	(87, 115)
cefotaxime [3]	[63527-52-6]	$C_{16}H_{17}N_5O_7S_2$	Claforan	Hoechst-Roussel	**(36)**		—CH_2OCOCH_3	1981	(87, 116)
ceftizoxime [3]	[68401-81-0]	$C_{13}H_{13}N_5O_5S_2$	Cefizox	SKF-Beecham	**37**		—H	1983	(87, 117)

Table 7. ***Continued***

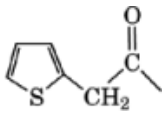
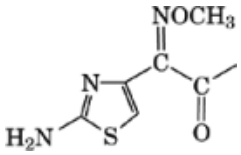
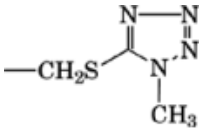
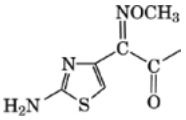
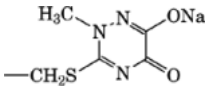
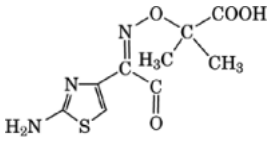
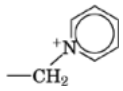
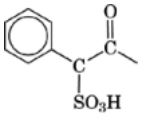
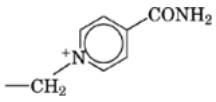
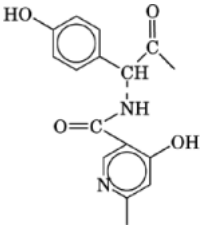
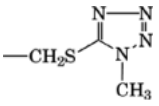
Generic name[a]	CAS Registry Number	Molecular formula	Trade name	Company	Structure number	R	R′	Year[b]	Refs.
cephalothin [1]	[153-61-7]	$C_{16}H_{16}N_2O_6S_2$	Keflin	Lilly	**27**		—CH_2OCOCH_3	1965	(87, 107)
cefmenoxime [3]	[65085-01-0]	$C_{16}H_{17}N_9O_5S_3$	Cefmax Bestcall[d]	Abbott Takeda-Roche	**(38)**			1987[e] 1983[f]	(87, 118)
ceftriaxone [3]	[73384-59-5]	$C_{18}H_{17}N_8O_7S_3Na$	Rocephin	Roche	**(39)**			1985	(87, 119)
ceftazidime [3]	[72558-82-8]	$C_{22}H_{22}N_6O_7S_2$	Fortaz Tazicef Tazidime	GlaxoSKF-BeechamLilly	**40**			1985	(87, 120)
cefsulodin	[52152-93-9]	$C_{22}H_{20}N_4O_8S_2$	Cefomonil	Takeda-Abbott	**41**			1986[e]	121
cefpiramide [3]	[70797-11-4]	$C_{25}H_{24}N_8O_7S_2$	Cefpiran Sepatren[d] Suncefal[d]	Wyeth-Ayerst Sumitomo Yamanouchi Banyu	**42**			1989[e] 1985[f] 1985[f]	122

Table 7. ***Continued***

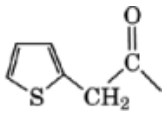
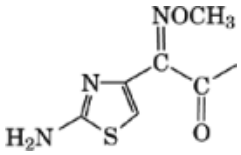
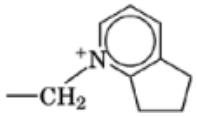
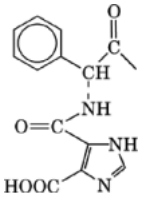
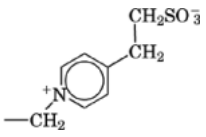
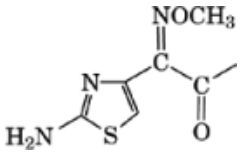
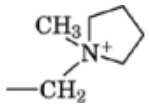
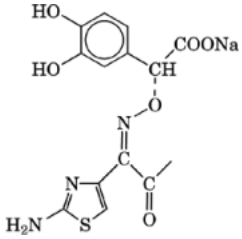
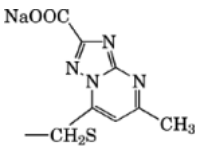
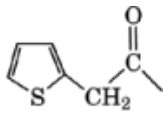
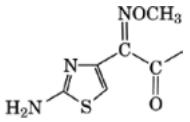
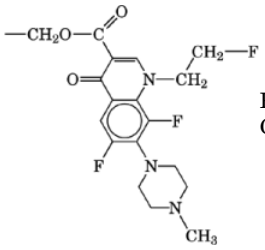
Generic name[a]	CAS Registry Number	Molecular formula	Trade name	Company	Structure number	R	R′	Year[b]	Refs.
cephalothin [1]	[153-61-7]	$C_{16}H_{16}N_2O_6S_2$	Keflin	Lilly	**27**		—CH_2OCOCH_3	1965	(87, 107)
cefpirome [3–″4″]	[84957-29-9]	$C_{22}H_{22}N_6O_5S_2$	HR810	Hoechst-Roussel	**(43)**			Ph-III	123
cefpimizole [3]	[84572-33-8]	$C_{28}H_{26}N_6O_{10}S_2$	U-63,196 Ajicef[d] Renilan[d]	UpJohn Ajinomoto Mochida	**44**			Ph-III1987[f] 1987[f]	124
cefepime [3–″4″]	[88040-23-7]	$C_{19}H_{24}N_6O_5S_2$	BMY28142	Bristol-Myers Squibb	**(45)**			Ph-II	125
[3–″4″]	[106772-82-1]	$C_{28}H_{21}N_9O_{11}S_3Na_2$	M14659	Mochida	**46**			Pre-Cl	126

Table 7. ***Continued***

Generic name[a]	CAS Registry Number	Molecular formula	Trade name	Company	Structure number	R	R′	Year[b]	Refs.
cephalothin [1]	[153-61-7]	$C_{16}H_{16}N_2O_6S_2$	Keflin	Lilly	**27**		$-CH_2OCOCH_3$	1965	(87, 107)
[″4″]	[115622-58-7]	$C_{31}H_{31}N_8O_8S_2F_3$	Ro 23-9424	Roche	**(47)**			Pre-Cl	127

[a] Drug generation is given in brackets.
[b] Year is year of launch in the U.S. market unless indicated. Pre − Cl = preclinical, Ph III = phase III clinical trials, and Ph II = phase II clinical trials (status as of Feb. 1990).
[c] Launch date in Italy.
[d] Japanese trade name.
[e] New drug application (NDA) filing or approval.
[f] Launch date in Japan.

7. Classification

As of 1991, there were approximately fifty different cephalosporins in clinical use or at an advanced stage of evaluation and development (77–79). Cephalosporins may be classified for convenience by their clinical pharmacology, β-lactamase resistance, chemical structure, or their antibacterial spectrum. The most common classification, which is somewhat arbitrary, divides the cephalosporins into three groups or "generations" (Tables 4, 5, 6, 7, 8), based primarily on their antibacterial spectrum (80, 81). First-generation cephalosporins are characterized by good gram-positive activity and modest to weak gram-negative activity. They are usually active against aerobic gram-positive cocci, with the exception of enterococci, methicillin-resistant *Staphylococcus aureus*, and *Staphylococcus epidermidis*, and are also active against some gram-negative bacilli such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*, and most anaerobic cocci and bacilli other than *Bacteroides fragilis* (80–83).

The second-generation cephalosporins have a broader spectrum and generally are more active against aerobic gram-negative organisms than the first-generation cephalosporins. Most second-generation cephalosporins have some activity against indole-positive *Proteus*, *Enterobacter* (except *E. cloacae*), and *Haemophilus influenzae*. Two agents included in this group, cefotetan (**26**) and cefoxitin (**23**), which are both cephamycin analogues, have significant activity against *Bacteroides fragilis* (80–83).

Third-generation cephalosporins have an expanded gram-negative spectrum and are the most active against enteric gram-negative bacilli, including penicillinase-producing strains, as well as *Serratia* and

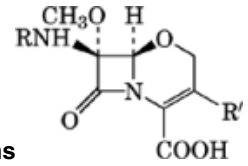
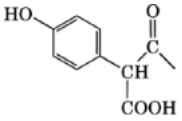
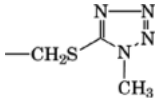
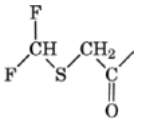
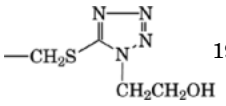


Table 8. Oxadethiacephalosporins

Generic name	CAS Registry Number	Molecular formula	Generation	Trade name	Company	Structure number	R	R′	Year[a]	Refs.
moxalactam latamoxef	[64952-97-2]	$C_{20}H_{20}N_6O_8S$	[3]	Moxam	Lilly	**48**			1981[b]	128
flomoxef	[99665-00-6]	$C_{15}H_{18}N_6O_6S_2F_2$	[3]	Flumarin[a]	Shionogi	**49**			1988[c]	129

[a] Japanese trade name.
[b] Year is year of launch in the U.S. market unless indicated.
[c] Launch date in Japan.

Citrobacter. They are also highly active against *Haemophilus influenzae* and *Neisseria gonorrhoeae*, *Streptococcus pneumoniae* and *pyogenes*, moderately active against *Pseudomonas aeruginosa* and anaerobes, but somewhat less active against staphylococci than the first-generation cephalosporins (80–84). A subset of the third-generation cephalosporins has somewhat improved, albeit still not ideal, activity against *Pseudomonas aeruginosa*. This group includes cefoperazone **34**, cefpiramide **42**, ceftazidime **40**, and cefsulodin **41** (80), although cefsulodin is relatively inactive against most other organisms. Cefixime **16**, a new oral cephalosporin touted as the first oral third-generation cephalosporin, has a spectrum between those of the second- and third-generation compounds. It has enhanced activity against *Enterobacteriaceae*, and against most common respiratory and urinary tract pathogens, but lacks activity against *Staphylococcus aureus*, enterococci, and *Pseudomonas aeruginosa*. Cefixime **16** would be better compared to its direct competition in the oral market, cefaclor **13**, and the oral prodrug cefuroxime axetil **20**.

Some other compounds tentatively labeled as examples of the as yet undefined "fourth-generation" have appeared in the literature, but these are probably best thought of as third-generation cephalosporins, having slight advantages over earlier examples of this group. This group includes cefpirome **43**, cefepime **45**, and others undergoing clinical trials.

It should be noted that the classification into generations is not chronological, and so some second-generation compounds came to the market relatively recently, after the third-generation was firmly established. Within each class there is also a further classification as to whether the compounds are administered orally or parenterally. A contemporary variation of the generation classification has been proposed, but has not found widespread use (85). Another proposal groups cephalosporins according to clinical indication or application (86).

The structures of selected cephalosporins on the U.S. market, or in the final stages of development, are shown in Tables 4, 5, 6, 7, 8 (see also (78, 87)). For every cephalosporin which has made it to the marketplace,

Table 9. Cephalosporin In Vitro Antibacterial Activity, μg / mL, and Pharmacokinetic Data[a,b]

	(12)	(27)	(13)	(31)	(35)	(16)	(36)	(39)	(40)	(23)	(48)	(47)
Antibacterial activity												
Staph. aureus[c]	2	0.5	1	4	1	32	2	4	4	2	8	1
Strep. pyogenes	0.5	0.12	0.25	0.5	0.03	0.25	0.03	0.03	0.12	1	1	0.06
Strep. pneumoniae	2	0.12	1	1	0.12	0.5	0.12	0.25	0.25	2	1	<0.06
E. coli[d]	4	4	1	1	1	0.25	0.03	0.12	0.12	4	0.12	0.06
Kleb. pneumoniae[e]	4	4	1	4	2	0.12	0.03	0.06	0.12	2	0.12	0.06
Pr. mirabilis[d]	8	2	1	0.5	1	0.03	0.03	0.03	0.06	2	0.12	0.25
Proteus sp (ind+)	>128	>128	>128	16	8		0.12	0.12	0.12	4	0.25	0.12
Enterobacter sp[f]	>128	>128	>128	32	16	64	0.12	0.25	0.25	>128	0.12	0.06
Citrobacter sp	>128	64	>128		8		0.25	0.5	0.5		0.25	0.12
Serratia marcescens	>128	>128	>128	64	64		0.12	0.25	0.12	16	0.25	<0.12
Ps. aeruginosa	>128	>128	>128	>128	>128	>128	32	32	2	>128	16	4
H. influenzae	8	4	1	1	0.5	0.03	0.03	0.03	0.12	2	0.12	0.03
B. fragilis[g]	64	64	>128	128	32		32	64	64	4	4	128
N. gonorrhoeae[c]	2	0.5	0.5	1	0.06	0.06	<0.01	<0.01	0.06	0.5	0.06	<0.01
Pharmacokinetic data												
plasma half life, h	0.8	0.6	0.6	4.0	1.3	0.7	1.2	8.0	1.8	0.8	2.5	
protein bound, %	10	65	25	98	35	25	35	95	17	70	50	
urinary excretion, %	90	70[h]	60[i]	96	95	65	70[h]	65	85	80	80	
biliary excretion, %	0.3	0.03	0.05	1	0.5		1	35	<3	<2	20	

[a] Refs. (77, 80, 81, 130–132). Cefixime Ref. 96; Ro 24-9424 Ref. 127.
[b] Note that values are approximated from references because specific strains are not specified.
[c] Penicillin resistant.
[d] Ampicillin sensitive.
[e] Cefazolin sensitive.
[f] Cefotaxime sensitive.
[g] Penicillin sensitive.
[h] Plus 30% deacetylcephalosporin.
[i] ±30% inactive.

literally thousands of analogues were synthesized in order to establish the structure-activity profile and allow selection of a clinical candidate. In addition to these compounds, there is a tremendous number of cephalosporin compounds currently at various stages of development. A more extensive listing of the newer cephalosporins under preclinical or clinical evaluation may be found in a number of reviews (79, 88).

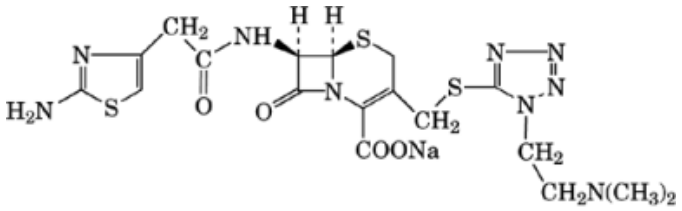
7.1. In Vitro Antibacterial Activity and Structure-Activity Relationships

The *in vitro* antibacterial activity of any particular cephalosporin is a combination of the degree and type of activity at the target site, the ease with which it can penetrate to the target, and the ability to resist the attack of destructive enzymes. The nature and complexity of the biochemical target(s) for β-lactam antibiotics is fairly well-established and the mechanisms of penetration are also understood to some extent. However, many factors are involved, including pharmacokinetic and pharmacodynamic properties, and the antibiotic may not perform as predicted. The *in vitro* antibacterial activity of a representative selection of the cephalosporins given in Tables 4, 5, 6, 7, 8 against a variety of bacterial organisms is depicted in Table 9. More extensive comparative listings of the antibacterial activity of cephalosporins may be found in the literature (77, 80, 81, 130–132).

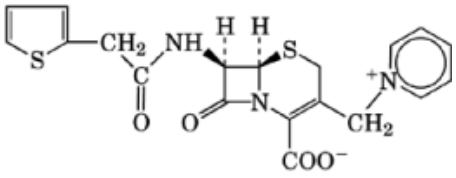
Structure-activity relationships can be inferred by comparison of the antibacterial properties of the clinical agents and related compounds. Different acyl side chains can result in significant changes in the antibacterial activity, both with respect to potency and to breadth of spectrum. The highest activities are observed when the

acylamino side chain at C-7 is a substituted acetic acid. Homologation of the acetic acid moiety lowers activity dramatically as exemplified by the naturally occurring cephalosporins which all have weak activity. Compared to the penicillins, there is somewhat more latitude for the type of substituent(s) attached to the α-carbon of the side chain. The phenylacetyl and phenoxyacetyl side chains are less effective in conferring activity on the cephalosporins than the pencillins. More effective are acetic acid side chains attached to heterocyclic rings, such as thiophene, pyridine, tetrazole, furan, and aminothiazole. Much smaller and nonaromatic substituents can also confer high levels of *in vitro* activity. Such substituents include the cyano, methylthio, methylsulfonyl, and trifluoromethylthio moieties. The aromatic ring seems to only be associated with effective activity when there is a second substituent on the α-carbon of the acyl group. The effect of this second substituent on the cephalosporin activity is different to what is observed for the penicillins. The carboxyl and sulfonic acid groups do not particularly improve activity overall for the cephalosporins, whereas the amino and hydroxy group seem effective in this regard. A second substituent on the α-carbon introduces a new chiral center. The D-configuration (*R*) is preferred for the phenylglycine and mandelic acid derived side chains as in both cases the L-configuration (*S*)-analogue is considerably less active.

That the aminothiazole group imparts high potency to the cephalosporins against gram-negative bacteria was first demonstrated in 1977 with the synthesis of cefotiam [61625-34-2] (SCE-963), $C_{18}H_{22}N_9O_4Na$ **50** (133). The oxime group in cefuroxime **35** and most of the aminothiazoyl containing cephalosporins is especially effective in further increasing potency and conferring resistance to certain β-lactamases. A marked stereochemical preference is observed: the syn-isomer where the alkoxy and acyl groups are cis to each other, is considerably more active than the corresponding antiisomer.



(50)



(51)

One of the principal deficiencies of the older cephalosporins was the lack of resistance to β-lactamases. Compounds with improved β-lactamase resistance have one or more of the following characteristics: a second, monovalent substituent on the α-carbon of the C-7 acyl group such as found in cefamandole **30** and cefoperazone **34**; a syn-oxime substituent, eg, cefuroxime **35** and ceftriaxone **39**; a methoxy, or formamido, substituent on the β-lactam ring at the 7α-position such as in cefoxitin **23**. However, these various substituents have different effects, and increased resistance to one enzyme does not indicate resistance to all β-lactamases. The 7α-methoxy, or formamido, group is the most effective in imparting a broad spectrum of resistance to β-lactamases, followed by acyl side-chain oxime ether substituents. Thus cefuroxime **35**, the first oximino–cephalosporin, has a β-lactamase resistance spectrum similar to that of cefotaxime **36** whereas the less resistant cefamandole **30** is similar to cefoperazone **34**.

The nature of the C-3 substituent influences the pharmacokinetic properties, as well as the intrinsic antibacterial activity. Analogues having a 3-methyl substituent generally have a low level of antibacterial activity except when the 7-acyl group is D-mandelic acid, D-phenylglycine, or a related moiety. Thus an α-hydroxyl or amino group on the side chain compensates, at least in part, for the lack of a nucleophilic leaving group on the C-3 methyl. The 7-phenylglycine analogues were the only known cephalosporins that combined *in vitro* activity and efficient oral adsorption. However, more recent work has shown that a C-3 vinyl substituent permits reasonable oral adsorption even for the oximino–aminothiazole side chain, eg, cefixime **16**, and BMY28232 **19**, and it appears likely that other C-3 substituents will be found to facilitate oral adsorption. Changes in the 3′-position are thought to primarily effect pharmacologic activity. However some improvement in antibacterial activity can also result from such substitution. Of particular note is the improvement seen using pyridinium and other quaternary-ammonium moieties, especially against the gram-positive organisms, and certain heterocyclic thio groups. A number of the newer cephalosporins, such as M14659 **46**, have been rationally designed to take advantage of the so called *ton B* mediated iron transport system (*vide infra*) and show considerably improved activity against *Enterobacteriaceae* and *Pseudomonas aeruginosa* in particular (126).

Another interesting approach to obtaining potent, broad-spectrum activity has been reported (127). The "dual-action" antibacterial concept involves incorporation of two moieties having complimentary antibacterial modes of action into the same molecule, and uses the mode of action of one part to release the second antibacterial at the site of action. This approach is exemplified in Ro 23–9424 **47** (127), which uses the mode of action and reactivity of the cephalosporin moiety (Fig. 2) to release the quinolone portion.

7.2. Intrinsic Activity and Biochemical Targets

β-Lactam antibiotics affect sensitive bacteria by inhibiting late stages in the biosynthesis of their cell wall peptidoglycan (134–136). The cell wall contains a matrix of complex macromolecules that provide rigidity and mechanical stability by way of cross-linked latticeworklike structures. The peptidoglycan is one of these polymers, and is made up of glycan strands (consisting of alternating *N*-acetylglucosamine and *N*-acetylmuramic acid units) which are interconnected by peptide cross-links in a three-dimensional array. The last step in the construction of the peptidoglycan cross-link involves a transpeptidase-catalyzed reaction in which an amide bond is formed. In the case of the peptidoglycan from *Escherichia coli* (type A1 γ-peptidoglycan) the cross-linking-peptide chain is a pentapeptide attached to the *N*-acetylmuramic acid residue of the glycan repeating subunit via the lactic acid side chain. The third amino acid in the pentapeptide is *meso*-diaminopimelic acid (DAP) which is anchored into the peptide via its L-center. The cross-linking step, referred to as transpeptidation, which the β-lactam antibiotics inhibit, is the process whereby the amino group of the D-center of *meso*-DAP forms an amide link to the penultimate D-alanine of another peptide with concomitant expulsion of the final D-alanine residue. Not all peptide chains are involved in the formation of cross-links, in *E. coli* the extent of cross-linking is about 33%, and the noncross-linked chains may be modified by the removal of the terminal D-alanine unit without transpeptidation. The enzyme responsible for this "pruning" is a D,D-carboxypeptidase (135, 136).

β-Lactam antibiotics inhibit the cross-linking process by forming a covalent acyl-enzyme complex with transpeptidase or carboxypeptidase (Fig. 2). A serine hydroxyl function in the active site of these enzymes is implicated in the formation of the acyl-enzyme bond. The stability of the acyl-enzyme bond in the case of the transpeptidases is such that for all intents and purposes the enzyme is irreversibly inhibited. The carboxypeptidases, although intrinsically more sensitive to the β-lactam antibiotics in general, are reversibly inhibited because the acyl-enzyme bond is readily hydrolyzed to regenerate free enzyme (135, 136).

Cell wall biosynthesis is a dynamic process and during cell division new cell wall must be synthesized (135–137). Thus there must also be some cleavage of certain peptide cross-links and/or glycan chains to allow for new cell growth and division. Hence the picture is rather more complicated than the basic mechanism just described and there is a group of enzymes which has an affinity for β-lactam antibiotics. These enzymes, the

Table 10. Penicillin Binding Proteins (PBPs) of *E. coli*[a]

PBP	Mol wt	Number per cell[b] (%)	Proposed *in vitro* functions	Effects of inactivation
1A	92,000	200 (6.5)	transglycosylase, minor transpeptidase, can partially compensate for loss of 1B activity; essential for cylindrical cell wall synthesis	rapid lysis if both 1A and 1B are inactivated
1B	90,000	250 (8)	transglycosylase, major transpeptidase of cell elongation; essential for cylindrical cell wall synthesis	rapid lysis
2	66,000	20 (0.7)	maintenance of cylindrical rod shape; requires transpeptidase?	spherical cell formation
3	60,000	50 (1.6)	transglycosylase–transpeptidase required septum cross-wall synthesis	nonseptate cells; filament formation
4	49,000	110 (3.6)	secondary transpeptidase, D,D-carboxypeptidase, or D,D-endopeptidase acting on maturing peptidoglycan	controls transpeptidation delayed transpeptidation is absent
5	42,000	1800 (59)	D,D-carboxypeptidase	none apparent
6	40,000	600 (19.6)	D,D-carboxypeptidase	none apparent

[a] Refs. (134–136), and 138.
[b] Number in parenthesis is the % of the total PBPs per cell.

Table 11. Selective Binding of Cephalosporins to the PBPs of *E. coli*[a, b]

Antibiotic	PBP-1A	PBP-1B	PBP-2	PPB-3	PBP-4	PBP-5	PBP-6
cephalexin (12)	4	240	250	8	30	250	250
cephalothin (27)	<0.25	16	37	1	60	125	130
cephaloridine (51)	0.25	2.5	50	8	17	250	250
cefotaxime (36)	0.05	0.7	5	0.05	30	50	50
cefoxitin (23)	0.1	3.9	>250	5.8	7.0	0.6	0.9

[a] Refs. 134, 136, and 138.
[b] Concentration required for 50% inhibition of binding of [^{14}C]pencillin G, μg/mL.

so-called penicillin-binding proteins (PBPs), are embedded in the inner, or plasma, membrane. These PBPs are designated according to decreasing molecular weight (Table 10) and are species specific. Hence PBP1 of *Escherichia coli* may not have anything in common with PBP 1 of any other organism. There are invariably multiple PBPs varying in number (from 3 in *gonococci* to 10 in *Escherichia coli*), molecular weight, and number of copies per cell. In the case of Escherichia coli there are seven important PBPs, of which four, namely 1A, 1B, 2, and 3 are considered essential for cell viability (134–136, 138).

In a general sense all cephalosporins are thought to form a covalent acyl-enzyme complex involving the β-lactam carbonyl and a serine hydroxyl in the enzyme active site. However, as shown by competitive binding experiments, cephalosporins differ in their affinities for the various PBPs and it is well-established that preferential binding to the various PBPs produces different morphological effects (135–138). These effects are summarized in Table 10. Preferential binding to PBP 1B of *Escherichia coli*, as exhibited by cephaloridine [50-59-9] **51**, $C_{19}H_{17}N_3O_4S_2$, produces rapid lysis with little malformation of the cells. Septum cross-wall formation is controlled by PBP 3, and cephalosporins, such as cefuroxime **35**, that primarily inhibit this protein leave growing cells incapable of cell division. Each cephalosporin inhibits the various proteins to varying extents, and the intrinsic activity depends largely on the concentration of the compound required to saturate the most susceptible PBP. The 7α-methoxycephalosporins have considerable affinity for the nonessential PBPs 4, 5, and 6, whereas cephalosporins with no 7α-substituent bind very little if at all to these proteins. The binding of selected cephalosporins to the PBPs of *Escherichia coli* is detailed in Table 11.

7.3. Resistance

Resistance to the cephalosporins may result from the alteration of target pencillin-binding sites (PBPs), decreased permeability of the bacterial cell wall and outer membrane, or by inactivation via enzyme mediated hydrolysis of the lactam ring (80, 81, 138–140). This resistance can be either natural or acquired. Although resistance is often attributed specifically to one of these factors, in reality it reflects the interplay of several factors. In most instances, however, resistance results from the production of a β-lactamase enzyme, which opens the β-lactam ring as depicted in Figure 2.

Bacteria produce chromosomally and R-plasmid (resistance factor) mediated β-lactamases. The plasmid-mediated enzymes can cross interspecific and intergeneric boundaries. This transfer of resistance via plasmid transfer between strains and even species has enhanced the problems of β-lactam antibiotic resistance. Many species previously controlled by β-lactam antibiotics are now resistant. The chromosomal β-lactamases are species specific, but can be broadly classified by substrate profile, sensitivity to inhibitors, analytical isoelectric focusing, immunological studies, and molecular weight determination. Individual enzymes may inactivate primarily penicillins, cephalosporins, or both, and the substrate specificity predetermines the antibiotic resistance of the producing strain. Some β-lactamases are produced only in the presence of the β-lactam antibiotic (inducible) and others are produced continuously (constitutive).

Because of the highly permeable nature of the cell wall of gram-positive organisms, they produce β-lactamases which are not only found throughout the cell wall, but also in the extracellular environment. Hence the extracellular β-lactamases can act on the antibiotic before the cell is entered. Gram-negative organisms produce cell-bound β-lactamases which reside in the periplasmic space. Thus, for gram-negative bacteria, the antibiotic must penetrate the outer cell membrane/wall before coming in contact with a β-lactamase (80, 139, 140).

In general, the first-generation cephalosporins are readily hydrolyzed by the inducible, chromosomally mediated β-lactamases, and to a lesser extent, by the plasmid mediated enzymes such as the common TEM enzymes. Second- and third-generation cephalosporins have greater stability to hydrolysis by these better enzymes, although the emergence of resistant organisms during therapy using second- and third-generation cephalosporins is an increasingly serious medical problem (141). Resistance is most frequently reported in cases involving species which characteristically possess inducible β-lactamase enzymes (such as the so-called "overproducers" *Enterobacter* and *Serratia* (142). Although it has also been proposed that some β-lactamases may also protect the enzyme-producing organisms from the effects of certain, mostly third-generation, cephalosporins by "trapping" or binding the drugs without actually hydrolyzing them (141, 143), experiments have cast doubts as to the validity of this proposal (144).

Resistance can also arise when target enzymes, ie, the PBPs, and in particular the transpeptidases, are modified. Target-mediated cephalosporin resistance can involve either a reduced affinity for an existing PBP, or the acquisition of a supplementary, β-lactam insensitive PBP (139).

The third general mechanism for resistance is permeability barriers, or inaccessibility of the target because of transport problems. In gram-positive organisms, the PBPs are located outside of the cytoplasmic membrane and problems from permeability barriers is minimal. However the situation with gram-negative bacteria is considerably more complex, and penetration to the target can be a problem.

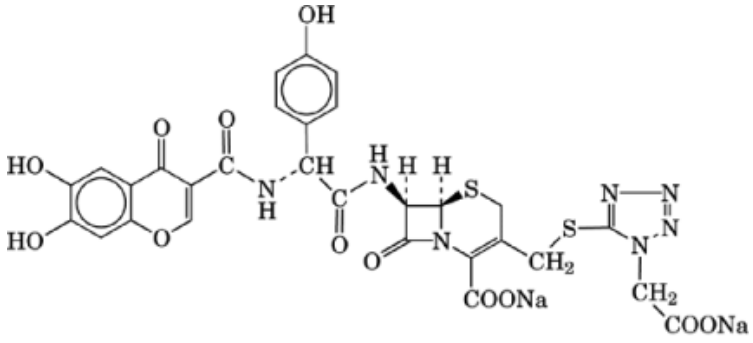
7.4. Transport and Cell Penetration

One of the causes of bacterial resistance to the cephalosporins is poor transport of the antibiotic through the outer membrane of gram-negative bacteria. This lipid-bilayer membrane carries receptor proteins for the recognition and transport of essential nutrients, but provides an effective barrier to large molecules. In the case of the cephalosporins there can be a considerable difference between the concentration required to inhibit intact cells and the concentrations required to saturate the target enzymes in broken cell preparations. For

example, the concentration of cefoxitin **23** required to inhibit the growth of whole organisms is ca 100-fold that needed to saturate the essential PBPs, whereas using cefuroxime **35** the minimum inhibitory concentration (MIC) is only four times the concentration required to saturate the PBPs (145).

Many small molecules can penetrate the outer cell membrane by diffusion through channels created by outer-membrane proteins called porins. Porin channels are implicated in the transport of cephalosporins because cells deficient in porins are much more impermeable than are cells that are rich in porins. The porins appear to function as a molecular sieve, allowing molecules of relatively low molecular weight to gain access to the periplasmic space by passive diffusion. In enterobacteria, a clear correlation exists between porin quantity and cephalosporin resistance, suggesting that the outer membrane is the sole barrier to permeability. However, such a relationship is not clearly defined for *Pseudomonas aeruginosa*, where additional barriers may be involved (139, 144, 146).

Bacteria have evolved efficient acquisition systems to meet specific needs. In the case of the transport of iron, an essential trace element, an iron-chelating ligand (siderophore) is used to sequester the iron, and this siderophore-iron complex is then recognized by specific receptors on the outer membrane. This is known as the *ton B*-dependent iron transport pathway because it appears to be under the general control of the *ton B* gene in *Escherichia coli*. The most common siderophores are catechols, α-hydroxy carboxylic acids or *N*-alkylated hydroxamic acids. In an attempt to exploit the iron-transport pathway a number of cephalosporins have been made containing catechol or the isosteric 3-hydroxypyrid-4-one group (147–150). These compounds, such as E-0702 [77768-57-1] **52**, $C_{29}H_{21}N_7O_{12}S_2Na_2$, (148) and M14659 **46** (150), have good activity against *Pseudomonas aeruginosa* and *Enterobacteriaceae* and experiments do indeed implicate the iron-transport mechanism in the ability of these catechol cephalosporins to penetrate to their targets.



(**52**)

7.5. Pharmacokinetics

The pharmacokinetic properties of the cephalosporins depend to a large extent on the substituent at C-3. The 3′-acetoxy group is metabolized in the body to the less active 3′-alcohol. Most other substituents, including the 3′-carbamate, are metabolically stable. Most cephalosporins are eliminated rapidly, having serum half-lives of 1–2 hours. A number of the clinically useful cephalosporins contain the 1-*N*-substituted tetrazol-5-ylmercaptomethyl moiety in the C-3 position, eg, cefoperazone **34**, moxalactam **48**, cefmenoxime **38**, cefamandole **30**, and cefotetan **26**. However, these compounds do not all have identical pharmacokinetic properties because there is always some interaction with, or contribution from, the 7-acyl group in determining the overall pharmacokinetic profile. When the tetrazoylmercaptomethyl 3′-substituent bears an acidic group such as found in cefonicid **31** or ceforanide **33**, the rate of urinary excretion is reduced and higher, longer-lasting serum levels are obtained. Ceftriaxone **(39)**, having an acidic group in its C-3 substituent, has extremely long-lasting serum levels. It appears that acidic C-3 substituents are associated with a very high level of serum binding which may

contribute to the long-lasting serum levels. Most cephalosporins, both parenteral and oral, are renally excreted by glomerular filtration (free unbound drug) or tubular secretion (total drug). Some cephalosporins are readily secreted and have renal clearances via tubular secretion essentially independent of protein binding; others are eliminated predominantly by filtration, eg, ceftazidime **40**, and exhibit renal clearance that is highly dependent on free drug concentration. A few of the cephalosporins, such as cefoperazone **34** and ceftriaxone **(39)** are also eliminated by biliary excretion. In such cases of dual excretion modes, the biliary excretion increases when renal function is impaired and, conversely, renal excretion increases when biliary excretion is impaired (80, 151, 152).

8. Manufacture and Chemical Synthesis

At present all of the cephalosporins are manufactured from one of four β-lactams, cephalosporin C **2**, penicillin V [87-08-1], penicillin G [113-98-4], and cephamycin C (**8**), which are all produced in commercial quantities by fermentation (87). The manufacturing process consists of three steps: fermentation, isolation, and chemical modification.

8.1. Fermentation

The commercial β-lactam antibiotics which act as starting material for all of the cephalosporins are produced by submerged fermentation. The organisms used for the commercial production of the penicillins and cephalosporins are mutants of *Penicillin chrysogenum* and *Cephalosporium acremonium*, respectively (3, 153, 154). Both are true fungi (eucaryotes). In contrast, the cephamycins are produced by certain species of procaryotic *Streptomyces*, including *Streptomyces clavuligerus* and *Streptomyces lipmanii* (21, 103).

Superior penicillin producing cultures are capable of producing in excess of 30 mg/mL of penicillin G (154). Cephalosporin producing strains, however, generally grow poorly and cephalosporin C production is not as efficient as is that of penicillin. Factors such as strain maintenance, strain improvement, fermentation development, inoculum preparation, and fermentation equipment requirements are discussed in the literature (3, 154).

The β-lactam antibiotics are produced by secondary metabolic reactions that differ from those responsible for the growth and reproduction of the microorganism. In order to enhance antibiotic synthesis, nutrients must be diverted from the primary pathways to the antibiotic biosynthetic sequences. Although most media for the production of penicillins and cephalosporins are similar, they are individually designed for the specific requirements of the high yielding strains and the fermentation equipment used.

A typical fermentation medium for penicillin production contains lactose, corn steep liquor, and calcium carbonate (3, 153, 154). In most industrial processes the carbohydrate source, glucose, beet molasses, or lactose, is continuously added to the fermentation. The rate of glucose addition must be carefully monitored, by pH or rate of oxygen depletion, because the synthesis of penicillin is markedly reduced in the presence of excess glucose. Alternative sources of nitrogen, such as cottonseed, peanut, linseed, or soybean meal, have been substituted for corn steep liquor. Other ingredients that help to optimize penicillin production are monopotassium phosphate, for additional phosphorus and pH control, and sodium, ammonium or magnesium sulfate, for additional sulfur. Media for the large scale fermentative production of cephalosporin C generally contains fish meal, peanut meal or soybean meal, corn steep liquor, beet molasses, lard oil or methyl oleate, glucose, and methionine (155). In the cephalosporin medium, lipids, rather than glucose, provide the main source of carbon and energy. The sulfur for cephalosporin C is derived more efficiently from methionine than from sulfate. The yields of cephalosporin C are potentiated by both methionine and methyl oleate. The media and fermentation conditions for cephamycin C production are briefly discussed (3, 103).

8.2. Isolation

Isolation procedures rely primarily on solubility, adsorption, and ionic characteristics of the β-lactam antibiotic to separate it from the large number of other components present in the fermentation mixture. The penicillins are monobasic carboxylic acids which lend themselves to solvent extraction techniques (154). Pencillin V, because of its improved acid stability over other penicillins, can be precipitated directly from broth filtrates by addition of dilute sulfuric acid (154, 156). The separation process for cephalosporin C is more complex because the amphoteric nature of cephalosporin C precludes direct extraction into organic solvents. This antibiotic is isolated through the use of a combination of ion-exchange and precipitation procedures (157). The use of neutral, macroporous resins such as XAD-2 or XAD-4, allows for a more rapid elimination of impurities in the initial steps of the isolation (158). The isolation procedure for cephamycin C also involves a series of ion exchange treatments (103).

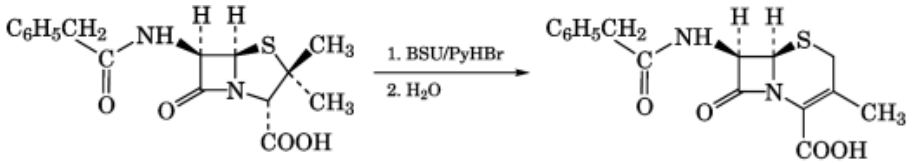
8.3. Chemical Modification

The chemistry and synthetic strategies used in the commercial synthesis of cephalosporins have been reviewed (87) and can be broadly divided into: (*1*) Selection of starting material; penicillin precursors must be rearranged to the cephalosporin nucleus; (*2*) cleavage of the acyl side chain of the precursor; (*3*) synthesis of the C-7 and C-3 side-chain precursors; (*4*) acylation of the C-7 amino function to introduce the desired acylamino side chain; (*5*) introduction of the C-3 substituent; and (*6*) protection and/or activation of functional groups that may be required.

For a viable commercial process, the selection of materials and the choice of synthetic route is governed primarily by cost, not by overall yield. The selection of starting material is dictated usually by the desired C-3 substituent. For cephalosporins containing 3-acetoxymethyl or 3-(substituted)methyl such as 3-thiomethyl and 3-aminomethyl derived moieties, the most direct synthetic route is from cephalosporin C, whereas pencillin V or G is the preferred starting material for the synthesis of the C-3 methyl cephalosporins. The three chemical transformations (*3*), (*5*), and (*6*) can potentially be carried out in a variety of ways, the precise sequence being determined by a balance of competing factors such as cost and optimization of yield (87).

8.3.1. Substituents at C-3

8.3.1.1. 3-Methyl Cephalosporins. Direct reduction of the 3′-acetoxy group of compounds derived from cephalosporin C, either by catalytic hydrogenation (159) or by acid catalyzed reduction using trialkyl-silanes (160), leads to 3-methyl cephalosporins (87). However, the most economical route to this class of compounds involves the acid-catalyzed rearrangement of penicillin sulfoxides (161, 162). Since the disclosure of this rearrangement in 1963, a tremendous number of papers dealing with the nature of the catalyst, solvent, optimal reaction parameters, and the reaction mechanism have appeared and been thoroughly reviewed (87, 163–165). In addition, over twenty U.S. patents dealing with this process have been issued (87). The process requires that the carboxylic acid be protected, and high yields can be obtained by the correct choice of protecting group, solvent, and acid catalyst. The relatively stable *p*-nitrobenzyl, trichloroethyl, and benzhydryl esters of pencillin V or G sulfoxide undergo ring expansion best in either 1,4 dioxane or 1,1,2-trichloroethane with pyridine salts of phosphoric acid as the catalyst (166). This affords the cephalosporin with a carboxylic acid protecting group suitable for subsequent C-7 side-chain manipulation. Alternatively the carboxylic acid protecting group can be chosen such that deprotection occurs during isolation of the ring-expanded product. The best example is the use of trimethylsilyl protection. Thus reaction of penicillin G sulfoxide [34104-15-9] **53**, $C_{16}H_{18}N_2O_5S$, with pyridinium hydrobromide (PyHBr) and bistrimethylsilylurea [18297-63-7] (BSU) in toluene followed by hydrolysis of the silyl ester affords the cephalosporin [27255-72-7] **54** in 97% yield (87, 167).



(53) (54)

8.3.1.2. 3′-Heteroatom Cephalosporins. The most direct synthetic route to this class of compounds utilizes cephalosporin C **2** as the starting material, and thus requires the introduction of a new 3′-substituent. The 3′-acetoxy group can be readily displaced using a variety of nitrogen, sulfur, and carbon nucleophiles under both aqueous and nonaqueous conditions (75, 87, 168). However, oxygen nucleophiles do not readily displace the acetoxy group. These displacement reactions have been extensively studied, and tend to follow S_N kinetics (75, 168). In fact under aqueous conditions, mechanistic studies show that displacement occurs via an S_N process with carboxylate stabilization of the intermediate carbonium ion (75, 87, 169, 170). These displacement reactions generally cannot be carried out on cephalosporin esters, lactones, or sulfoxides.

Nitrogen nucleophiles used to diplace the 3′-acetoxy group include substituted pyridines, quinolines, pyrimidines, triazoles, pyrazoles, azide, and even aniline and methylaniline if the pH is controlled at 7.5. Sulfur nucleophiles include alkylthiols, thiosulfate, thio and dithio acids, carbamates and carbonates, thioureas, thioamides, and most importantly, from a biological viewpoint, heterocyclic thiols. The yields of the displacement reactions vary widely. Two general approaches for improving 3′-acetoxy displacement have been reported. One approach involves initial, or *in situ*, conversion of the acetoxy moiety to a more facile leaving group. The other approach utilizes Lewis or Brønsted acid activation (87).

Conversion to a more facile, sulfur-derived, leaving group can be achieved by treatment with sodium thiosulfate or salts of thio and dithio acids (75, 87). Under anhydrous conditions, boron tribromide converts the 3′-acetoxy group to a bromide whereas trimethylsilyl iodide gives good yields of the 3′-iodide (87, 171, 172). These 3′-halides are much more reactive, even when the carboxyl group is esterified, and can be displaced readily by cyano and by oxygen nucleophiles (127).

Methane sulfonic acid, trifluoroacetic acid, hydrogen iodide, and other Brønsted acids can facilitate 3′-acetoxy displacement (87, 173). Displacement yields can also be enhanced by the addition of inorganic salts such as potassium thiocyanate and potassium iodide (174). Because initial displacement of the acetoxy by the added salt does not appear to occur, the role of these added salts is not clear. Under nonaqueous conditions, boron trifluoride complexes of ethers, alcohols, and acids also facilitate displacement (87,175).

An alternative to the displacement of a 3′-acetoxy group for entry into the 3′-heteroatom cephalosporins is functionalization of a 3-methyl cephalosporin. The low cost of the 3-methyl cephalosporins, derived from penicillin via the Morin rearrangement, makes this approach potentially competitive and attractive. Allylic bromination, using *N*-bromosuccinimide [128-08-5] (NBS) and photochemical initiation, of the esters of 3-methyl-Δ-3-cephem sulfoxides affords the desired 3′-bromo compound (87, 176, 177), although this reaction does not appear to work on the corresponding sulfide (75). Bromination can be carried out with a variety of substituents on the C-7 amino, but can suffer from significant bromination at C-2. However, the competing bromination at C-2 appears to be related to the nature of the substituent on the C-7 amino. Of particular note, the trichloroacetylamino group at C-7 completely suppresses the undesired C-2 bromination (176). Figure 3 outlines a particularly interesting application of this chemistry. Overbrominating, to afford a mixture of the 3′-monobromo compound [71543-00-5] (**55**) and corresponding C-2,3′-dibromo compound [71542-99-9] (**56**) is deliberate. This mixture is then treated with trimethylphosphite to effect selective reduction at C-2, and the resulting 3′-monobromo cephalosporin (**55**) can then be subjected to nucleophilic displacement (87, 178).

8.3.1.3. 3-Hydroxymethyl Cephalosporins. The 3-hydroxymethyls are a special case of the 3′-heteroatom cephalosporins. These deacetylcephalosporins serve as precursors for the 3′-carbamoyl cephalosporins, such as

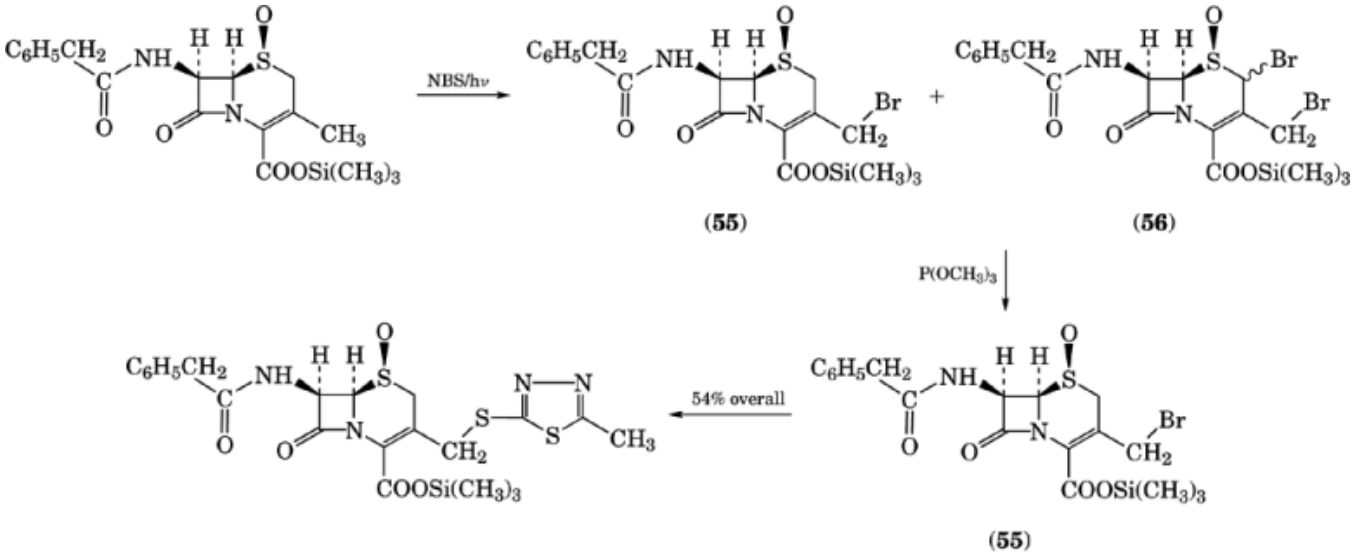


Fig. 3. Allylic bromination at C-3′; NBS=*N*–bromosuccinimide.

cefuroxime **(35)** and cefoxitin **23**. Treatment of a cephalosporin acid or ester to hydrolytic deacylation under basic conditions often suffers from the problems of double bond migration and/or β-lactam cleavage. However, deacylation of 7-aminocephalosporanic acid (7-ACA) **1**, derived from cephalosporin C **2** by treatment with sodium hydroxide at 0°C affords the corresponding 3′-hydroxy compound in good yield (64%) (87, 179, 180). This deacylation can also be achieved by enzymatic hydrolysis, using a variety of esterases and substrates (75, 179–182). The mild selective conditions of enzyme catalyzed cleavage make this the process of choice for industrial production and such a process using a yeast, *Rhodotorula rubra* as the source of esterase activity is employed in the commercial synthesis of cefuroxime **(35)** (87, 182).

8.3.1.4. 3-Methylene Cephalosporins and 3-Heteroatom Cephalosporins. A number of clinically important cephalosporins have a hydrogen or a heteroatom at C-3 instead of a methyl or substituted methyl, requiring removal of the C-3 methyl from either of the commercially available starting materials. Removal is conveniently achieved via ozonolysis of an exocyclic methylene group, and a number of routes have been developed to produce such 3-methylene cephalosporins from both penicillins and cephalosporins derived from cephalosporin C. Industrially, the most viable routes to 3-methylenecephams are via penicillins, and thus the penicillins serve as the precursors for the C-3 heteroatom cephalosporins (87). This chemistry has been reviewed rather extensively, along with the subsequent conversion into heteroatom substituted cephalosporins (164, 183–185). One of these routes takes advantage of advances in penicillin sulfoxide chemistry and involves the rearrangement of the sulfoxide to the 3-exomethylenecepham. This is exemplified in the synthesis of cefaclor (**13**) shown in Figure 4 (185, 186).

8.3.2. 7β-Substituents

8.3.2.1. C-7 Side-Chain Cleavage. 7-ACA **1**, the key intermediate for the synthesis of a large number of the clinical cephalosporins, cannot be made by fermentation, nor can it be readily obtained by enzymatic removal (187) of the aminoadipic acid side chain from cephalosporin C. Because substantial quantities of 7-ACA are used, an efficient method for the chemical cleavage of the acyl side chain of cephalosporin C was sought. The first efficient method involved diazotization using nitrosyl chloride, followed by hydrolysis or alcoholysis,

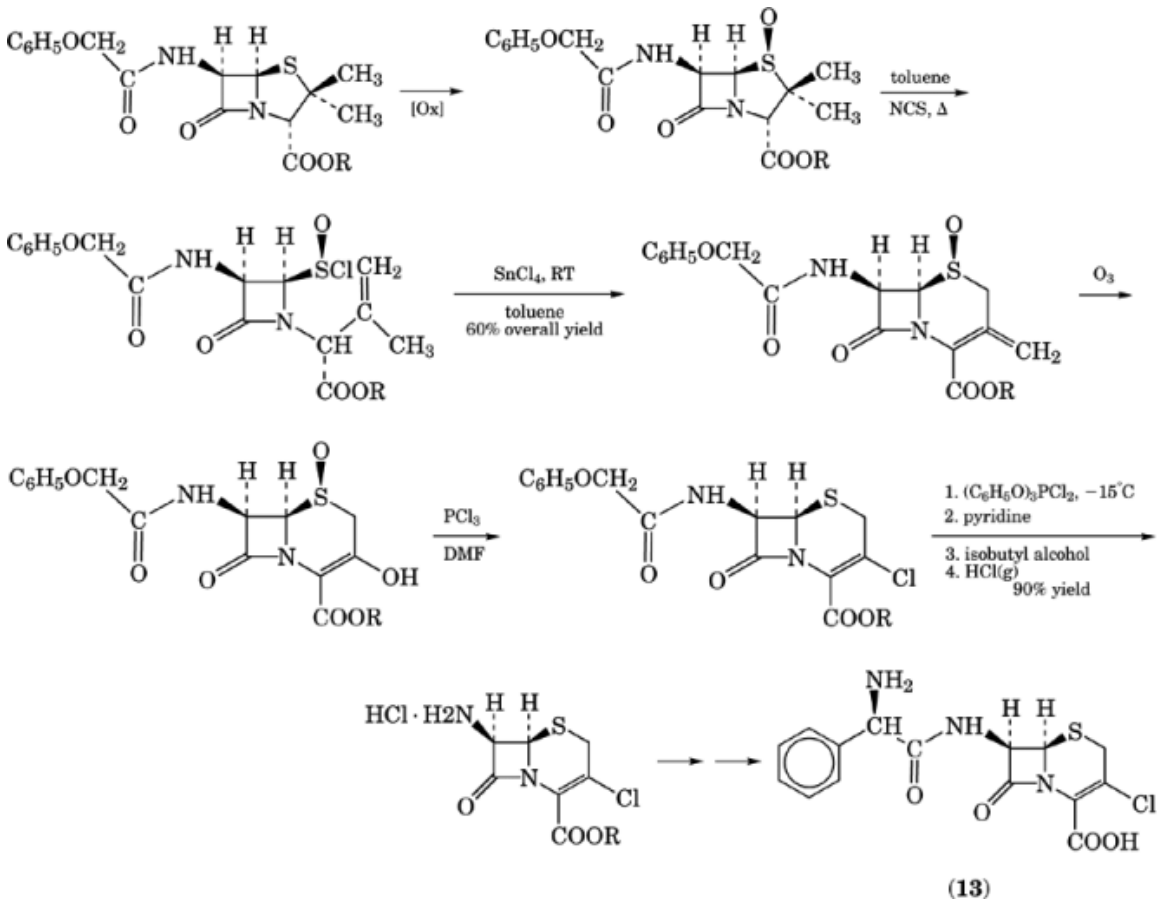
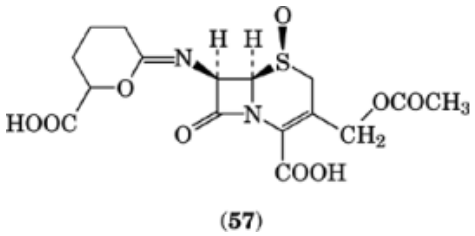


Fig. 4. Synthesis of cefaclor (**13**) where DMF is dimethylformamide.

and gave 7-ACA in 50% yield (22, 23, 87, 188). The reaction proceeds via the formation of the cyclic imino ether **57** which is easily hydrolyzed under very mild conditions.



(**57**)

Subsequently, a general procedure for amide side-chain cleavage was developed that involves initial conversion of the amide linkage into an imino chloride, achieved under anhydrous conditions using phosphorus trichloride, or more preferably phosphorus pentachloride. Alcoholysis to the imino ether, followed by hydrolysis affords 7-ACA (22, 23, 87, 189). The process requires protection of both the free carboxyl and the amino group. This can be achieved either by reaction with acetyl chloride before cleavage or by silylation of the anhydrous

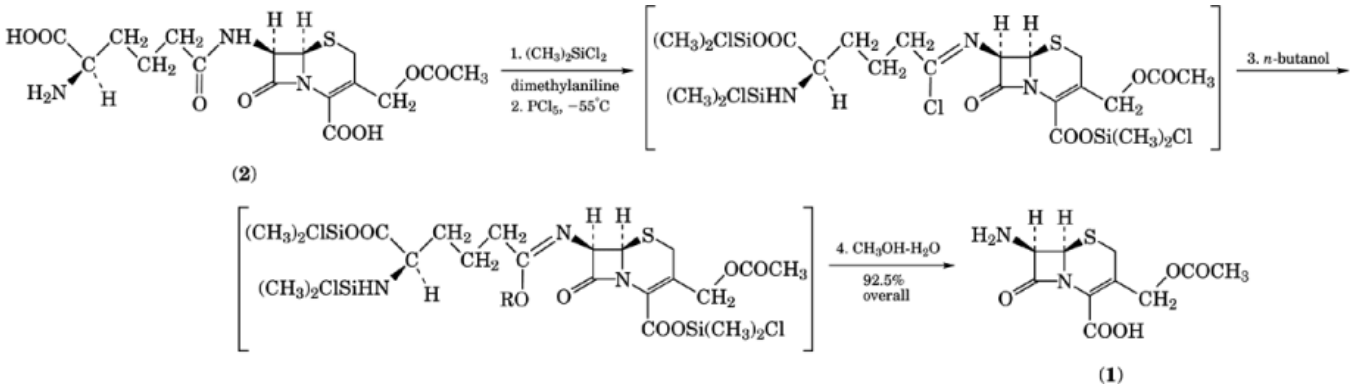


Fig. 5. C-7 side-chain cleavage.

sodium salt as shown in Figure 5. In the latter case, the overall yield of 7-ACA is 92.5%. A procedure for the removal of the acyl-amino side chain of cephalosporin C, using an immobilized enzyme has been reported (187). However, this process requires either the prior removal, or protection, of the adipoyl side-chain amino group, before enzymatic cleavage of the amide bond can be effected.

8.3.3. C-7 Amino Acylation

Essentially all the known methods for amine acylation, used in the peptide field, have been applied to cephalosporins. These procedures generally involve acyl group activation prior to the acylation (76). Commercially, the most common activation probably involves either mixed anhydride or acid chloride formation (87). Acylations via acid chloride activation are usually carried out under Schotten-Baumman conditions eg, aqueous acetone, 0°C, sodium bicarbonate, pH 7–8. A wide variety of active esters have also been used in cephalosporin chemistry, especially heterocyclic-thio esters and *o*-acyl substituted hydroxylamines, either preformed or generated *in situ*, as well as coupling methods employing dicyclohexylcarbodiimide (DCC) and similar condensing agents. The use of active esters in the acylation step is exemplified by the synthesis of ceftriaxone **(39)**. In order to circumvent the poor solubility of 7-ACA in organic solvents, prior formation of a soluble derivative, usually an ester, is required for most activation methods using nonaqueous conditions. Silylation of 7-ACA meets this requirement and the silylated moiety has the advantage of easy removed during the workup (87). Other commonly used esters include benzhydryl, *t*-butyl, and less frequently *p*-nitrobenzyl, *p*-methoxybenzyl, trichloroethyl, and allyl. However, the use of esters in the acylation step, under conditions requiring an organic base, often leads to the problematic isomerization of the ring double bond (75).

8.3.4. 7α-Substituents

A large number of cephalosporins with a variety of substituents in the 7α-position have been prepared and their biological properties and antibacterial activities evaluated. The substituents introduced into the 7α-position include groups such as CH_3—, CH_3CH_2—, —CH_2OH, —CH_2F, —CH_2NH_2, —COOH, —CHO, —$COCH_3$, —OCH_3, —OCH_2CH_3, —OCH_2CN, —$OCH_2CH_2OCH_3$, —SCH_3, —CN, —Br, —SCH^3, —$CH(COOCH_2CH_3)^2$, —N_3, —NHCHO, —$PO(OCH_3)_2$, and —SCH_3 (25–30). Of these 7 α-substituted cephalosporins, only those with the methoxyl and formamido group have interesting antibacterial activity. A number of synthetic routes to such compounds have been described and reviewed (27). The key steps for the introduction of the 7α-substituent in three of these approaches to the 7α-methoxy substituted cephalosporins are depicted in Figure 6 (27). Figure 6e outlines the introduction of the 7α-formamido substituent (28–30).

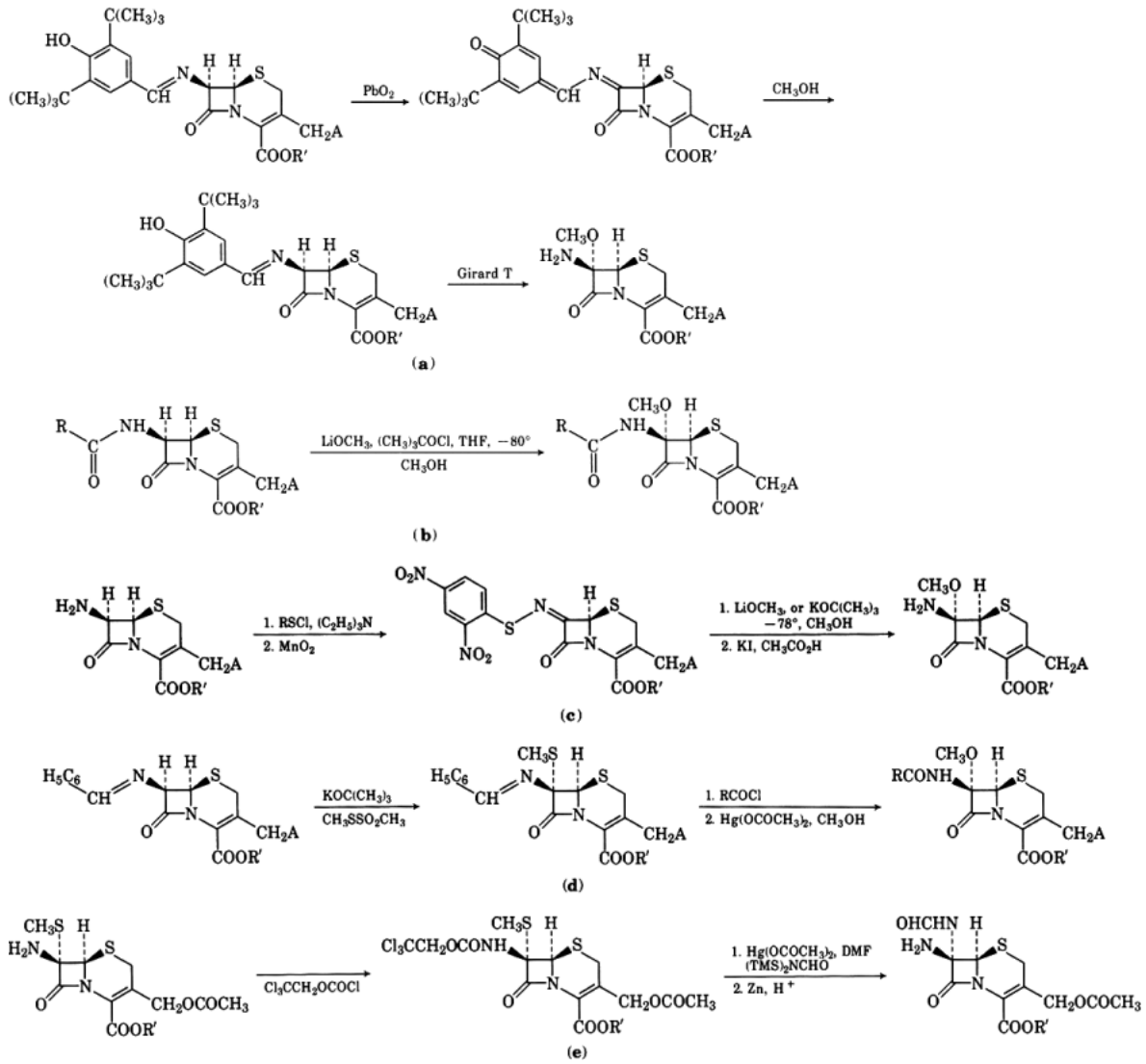


Fig. 6. Synthetic routes to (**a**)–(**d**) 7α-methoxycephalosporins and (**e**) 7α-formamidocephalosporins where THF=tetrahydrofuran, and DMF=dimethylformamide, and TMS=trimethylsilane.

8.4. Synthetic Strategy

For a commercial process the deciding factor in a synthetic strategy is overall cost, although other factors, such as patent rights and material and facility availability also play a role. Generally the goal is to introduce the more expensive materials as late in the total synthesis as possible (87), and to use a convergent rather than a long linear approach. These factors generally favor complete construction of side chains before attachment to the cephalosporin nucleus and most routes follow this approach. The variety of routes to some of the more common, newer C-3 and C-7 side chains have been compared in a review (87). The side chains may also be built up on the cephalosporin nucleus, generally 7-ACA, and furthermore, the sequence by which side chains

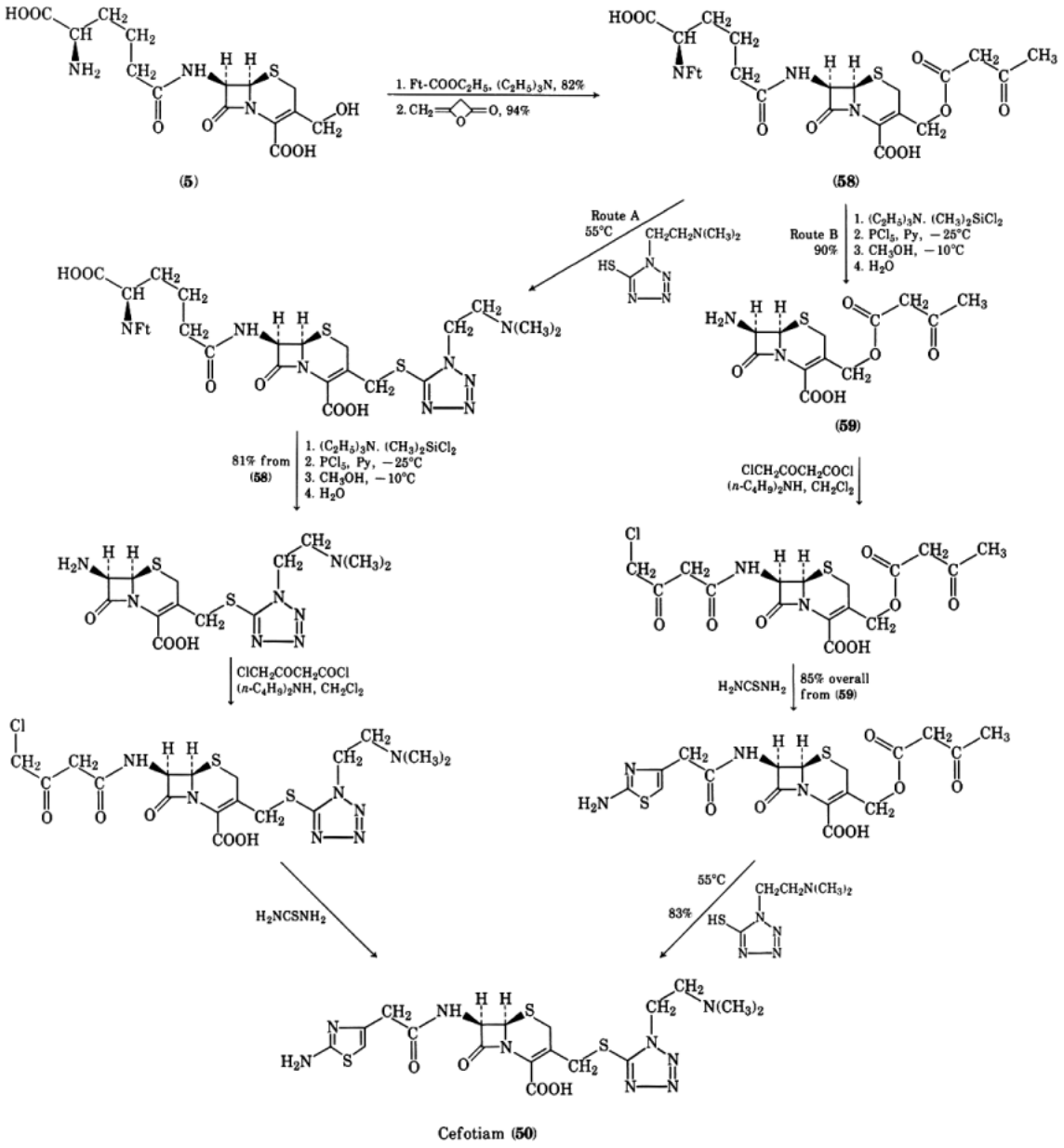


Fig. 7. Synthesis of cefotiam (**50**) where Ft is phthalimido and Py is pyridine, C_5H_5N.

are either built up or introduced intact, can also be varied. The syntheses of cefotiam **50**, ceftazidime **40**, and ceftriaxone **(39)** are described by the reaction sequences given in Figures 7, 8, 9, respectively. These are not necessarily the commercial, or the most efficient, routes.

The schemes depicted in Figure 7 contrast two complimentary approaches to cefotiam **50** in which the timing of the introduction of the C-3 substituent differs. In Route A the heterocyclic thiol C-3 substituent is introduced even before the removal of the aminoadipoyl acyl side chain. The acetonylacetyl C-3 substituent was

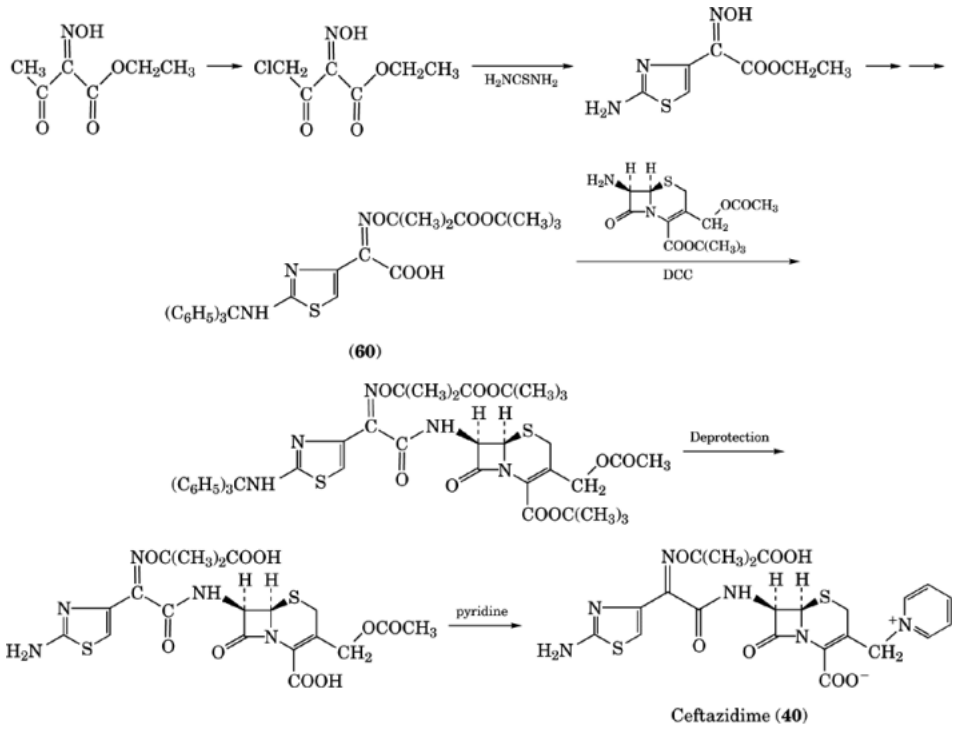


Fig. 8. Synthesis of ceftazidime (**40**); DCC=*N,N*−dicyclohexyl−carbodiimide.

introduced because it gave considerably higher yields and cleaner product in the nucleophilic displacement step than the corresponding acetoxy, and the starting material, deacetylcephalosporin C (**5**) was readily available from a fermentation process (190, 191).

In the synthesis of ceftazidime **40** (Fig. 8), the protected, preassembled aminothiazole side chain [68672-66-2] (**60**) is coupled to a protected 7-ACA first and the C-3 displacement step carried out last. By way of contrast, in the synthesis of ceftriaxone **(39)** (Fig. 9), the preformed C-3 substituent is introduced onto the cephalosporin nucleus in the first step and then the acyl-amino side chain is introduced. This last step is noteworthy for two reasons in that it demonstrates the use of an activated thio ester in the coupling step and that no protecting group chemistry is required (192, 193).

The substitution of oxygen for sulfur, as found in the 1-oxadethiacephems such as moxalactam **48**, introduces a new synthetic problem in that the oxadethiacephem nucleus is not naturally occurring. Synthetic routes to the oxadethiacephems have been investigated fairly extensively (194, 195) and in all the routes published the key step is the stereocontrolled introduction of the ring oxygen function. The synthesis of moxalactam **48**, depicted in Figure 10, efficiently retains all the carbons of its starting material, 6-aminopenicillanic acid [551-16-6] (6-APA) 10, $C_8H_{12}N_2O_2S$ (194–196).

9. Nuclear Analogues of Cephalosporins

In the search for improved antibacterials not only has the effect produced by the variation of the C-7 amido side chain and the 3′ substituent been studied, but so also has the more synthetically challenging question of

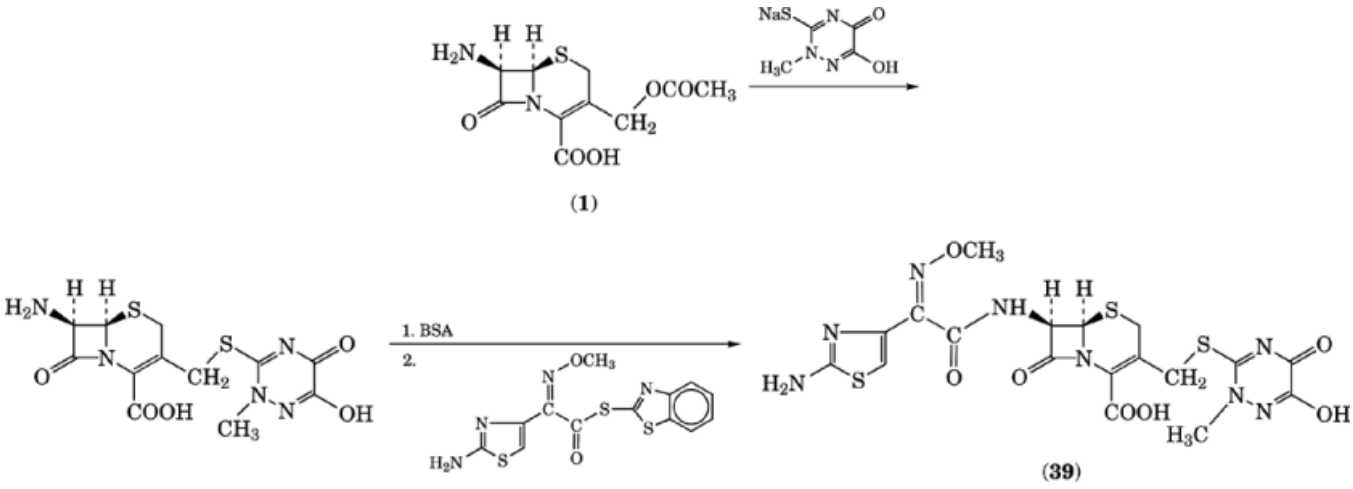
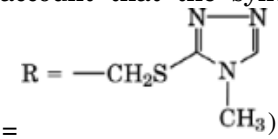


Fig. 9. Synthesis of ceftriaxone (**39**) where BSA is *N,O*-bis (trimethylsilyl) acetamide.

the effect of changes in the cephem nucleus (194, 197, 198). Nuclear analogues have been studied since the early 1970s but only the oxacephem class has reached the marketplace.

9.0.1. Oxadethiacephalosporins

The first compound in which the sulfur of a cephalosporin was replaced by oxygen was 1-oxa-dethiacephalothin [54214-83-4] **64**, $C_{16}H_{16}N_2O_7S$, (199) which is approximately twice as active as cephalothin **27** taking into account that the synthetic material was racemic. The oxygen analogue [62504-53-4], (**65**, X = OH, R = ) of cefamandole **30** was as good as, or better than, cefamandole itself, except for activity against *Proteus mirabilis* (200). Other studies have demonstrated that the 1-oxa-dethiacephalosporins are for the most part comparable to, or slightly better than, the cephalosporin counterpart in antibacterial activity, although there are some exceptions, such as 1-oxa-dethiacephalexin [66204-07-7] (**65**, X = NH_2, R = H), $C_{15}H_{15}N_3O_5$, which was inactive (201). Both the 1-oxa-dethiacephalosporins having a mandelamido C-7 side chain and either a methyl (**65**, X = OH, R = CH_3) or a hydrogen (**65**, X = OH, R = H) at C-3 had properties comparable to cephalexin **12** (201). The effect of various substituents at C-3, C-7 β, acylamino side chains, and C-7α, methoxy and hydrogen, in the 1-oxa-dethiacephalosporin system have been extensively studied (194). These structure-activity investigations led to the development of moxalactam **48** (194, 195). The substitution of oxygen for sulfur in moxalactam **48** affords a several-fold increase in intrinsic activity, but introduces the synthetic challenge of the oxacephem nucleus.

The antibacterial spectrum of moxalactam (Table 9) is similar in breadth and potency to that of cefotaxime **(36)**. Hence, moxalactam **48** is classified with the third-generation cephalosporins. In general 1-oxacephalosporins are considerably more susceptible to β-lactamases than their sulfur counterparts (194). However, moxalactam is highly resistant to a broad spectrum of β-lactamases owing to the effect of the 7α-methoxy group (194, 195).

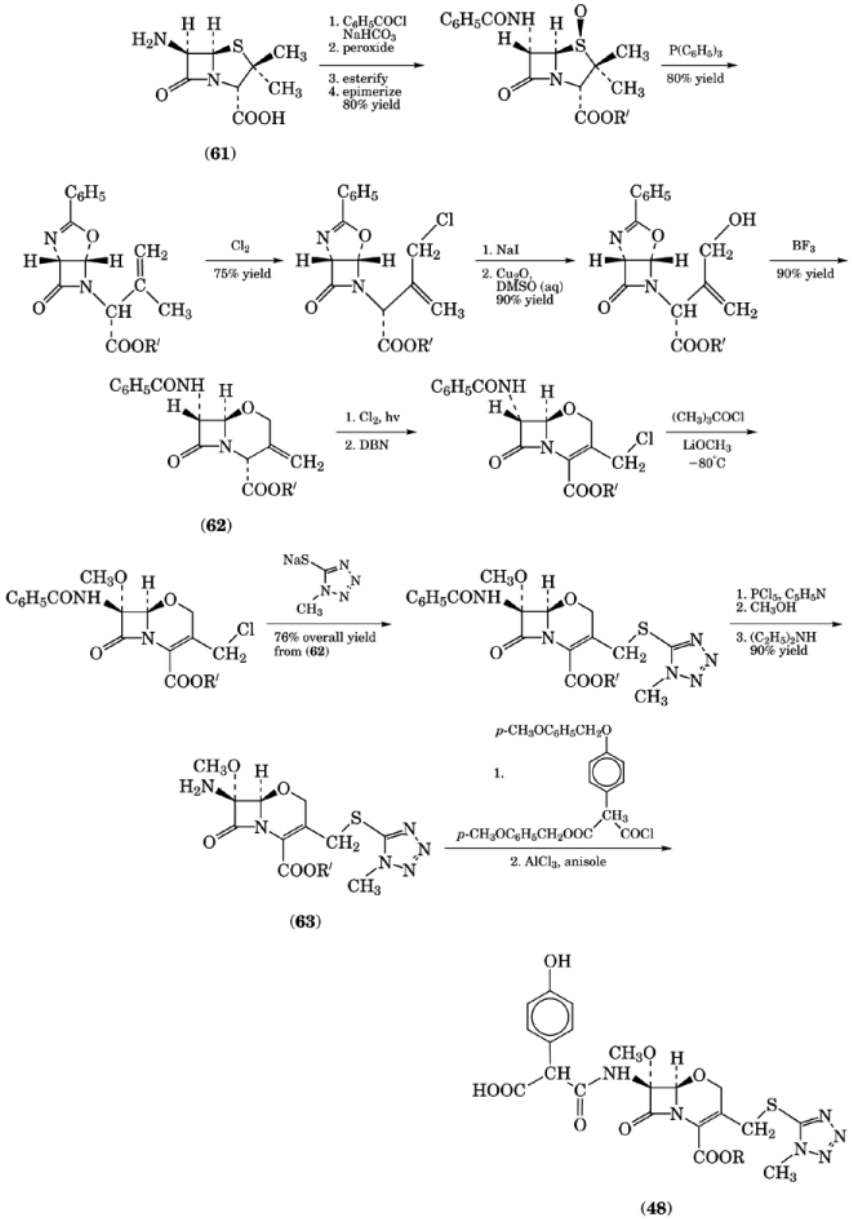
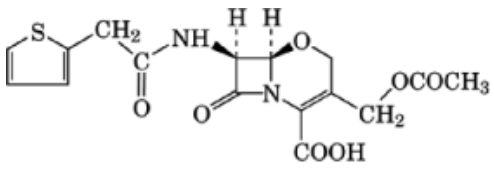
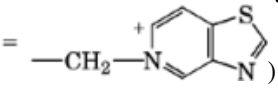
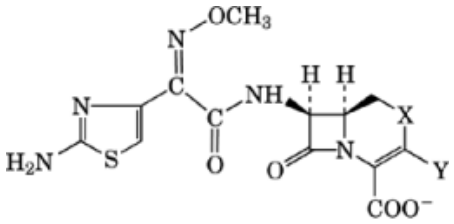


Fig. 10. Synthesis of moxalactam (**48**) from 6-aminopenicillanic acid (6-APA) where R′ is $CH(C_6H_5)_2$, DMSO is dimethyl sulfoxide and DBN is 1,5-diazabicyclo[4.3.0]non-5-ene. The overall yield of (**63**) is 26% from 6-APA in the 13 steps shown.

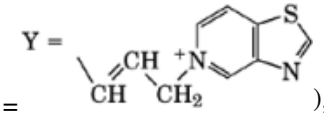
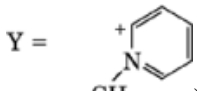


9.0.2. Isocephems

1-Dethia-2-thia-cephalosporins, more conveniently known as isocephems, have attracted interest mostly from a synthetic perspective (197), but analogues have been found, eg, (**66**, X = S, Y = ) which exhibit good antibacterial activity *in vitro* (198). Structure-activity studies in the series clearly demonstrate the need for a polar, preferably positively charged potential

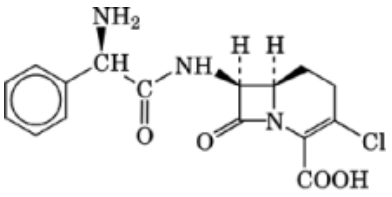


(**66**)

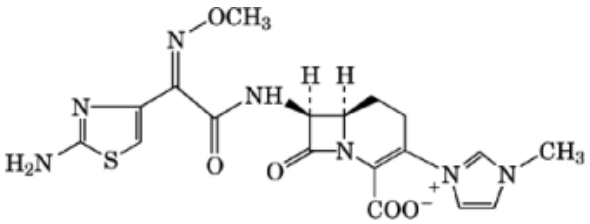
leaving group in the 3′-position to obtain good antibiotic activity. The insertion of a *z*-double bond between the 3- and 3′-position of the isocephems, eg, [121037-46-5] (**66**, X = S, Y = ), profoundly effects the antibacterial potency (198, 202) especially for the gram-positive bacteria where the improvement in potency can be 10-fold. The corresponding 2-oxa-isocephems have also been investigated, eg, [123581-13-5] (**66**, X = O, Y = ), but were found to not be quite as potent as the corresponding isocephem and more susceptible to β-lactamase inactivation (198).

9.0.3. Carbacephems

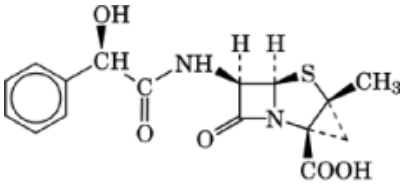
The carbacephems in which the sulfur group is replaced by a methylene, were introduced in the 1970s (197, 200, 203, 204). Generally these 1-carba analogues possess antibacterial activity that is similar to the cephalosporin counterpart (197, 204), and have the advantage of possessing greater chemical stability (204). Thus, for example, locarbacef [76470-66-1] (LY163892 or KT3777) **67**, $C_{11}H_{16}N_2O_4Cl$, has antibacterial activity similar to that of cefaclor **13** (205), but is 140 times more stable than cefaclor in pH 7.4 buffer (206). Furthermore, human pharmacokinetic studies demonstrate that locarbacef has a longer half life and better oral bioavailability than cefaclor (207). A new series of 3-acyl-1-carbacephems with promising activity and pharmacokinetic properties has been reported (208). Carbacephems having an iminoaminothiazole acyl side chain and a quaternary nitrogen group directly attached to the ring in the C-3 position, eg, LY258360 [125615-06-7] **68**, $C_{18}H_{19}N_7O_5S$, are reported to exhibit improved potency compared to the third-generation cephalosporins, with the exception of their poor activity against *Pseudomonas* (209).



(**67**)



(**68**)



(**69**)

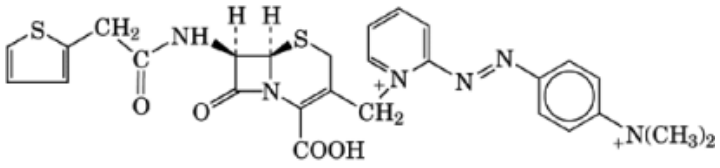
9.0.4. α-Cyclopropylpenams

Although at first glance (2,3)-α-methylenepenams such as [109279-36-9] **69**, look like penicillins, these compounds can be considered as nuclear analogues of the cephalosporins. The profile of their antibacterial activity and their mode of action support this view. The penicillin nucleus in these compounds is locked in the open conformation, which is the proposed biologically active form for pencillins. However, the absolute configuration at the carboxylic acid bearing carbon (C-2) is opposite to the naturally occurring penicillins, and places the carboxyl group in a position similar to that found in cephalosporins. Thus the compounds exhibit an antibacterial profile closer to those of their cephalosporin analogues than to that of the penicillin counterpart. Consistent with this, the compounds in this series show a marked selectivity for PBP-3 of *Escherichia coli*, and are more susceptible to cephalosporinases than penicillinases (210).

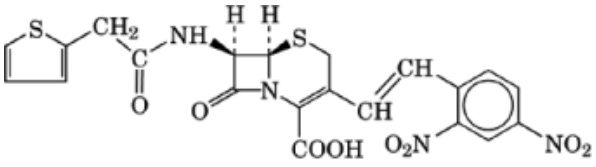
9.1. Cephalosporins with Special Properties

9.1.1. Chromogenic Cephalosporins

A 3-substituted pyridinium cephalosporin, known as PADAC [77449-91-3] **70**, $C_{27}H_{26}N_6O_4S_2$, is purple in color but on hydrolysis or treatment with β-lactamase releases the 3′-pyridinium group with concomitant loss of the purple color (211). PADAC is an example of the chromogenic cephalosporins which are useful in studying interactions with β-lactamases. Another example is nitrocefin [41906-86-9] **71**, $C_{21}H_{16}N_4O_8S_2$, which is light yellow in solution, λ_{max} = 386 nm. On hydrolysis, or treatment with β-lactamase, the color changes to a deep red, λ_{max} = 482 nm, but unlike the situation with PADAC the nitrocefin ′ group is not expelled (212) (see Chromogenic materials).



(70)



(71)

9.2. Uses

The cephalosporins are used for treating infectious diseases of bacterial origin in both humans and animals (80, 132, 213–218). First-generation cephalosporins such as cephalothin **27** and cephalexin **12** are the most active against staphylococci and nonenterococcal streptococci and are effective alternatives to the penicillins in patients with endocarditis, osteomyelitis, septic arthritis, and cellulitis (213). They are especially useful for treating patients who are allergic to the pencillins or who have mixed infections from gram-positive and gram-negative bacteria. Although these drugs have proved useful in treating infections such as bacteremias, urinary tract infections, and pneumonias, caused by gram-negative bacilli, their use as single agents in this regard is not recommended, because activity against gram-negative organisms is somewhat weak and unpredictable. The first-generation cephalosporins have been widely used for prophylaxis in cardiovascular, orthopedic, biliary, pelvic, and intra-abdominal surgery (132, 214). Cefazolin (32), which has a longer half-life than the other first-generation compounds, is the first-generation agent of choice for surgical prophylaxis (132, 213, 214).

Cefuroxime **(35)** is effective against community-acquired pneumonia in which ampicillin-resistant *Haemophilus influenzae* is the probable etiologic agent. Cefoxitin **23** is used to treat mixed aerobic–anaerobic infections including pelvic infections, intra-abdominal infections, and nosocomial aspiration pneumonia. Cefonicid **31**, because of its long half-life has been used in a once-a-day regimen to treat a variety of mild to moderate infections including community-acquired pneumonias, urinary tract infections, and infections of the skin and soft tissue (132, 215).

Whereas third-generation cephalosporins do have some coverage against gram-positive infections, they are not the agents of choice. Similarly, most community-acquired infections are better treated with drugs other than the third-generation cephalosporins. The treatment of meningitis is an important exception (132, 216). Cefotaxime **(36)**, ceftriaxone **(39)**, and ceftazidime **40** have proven effective in treating meningitis, especially in children where *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Neisseria meningitidis* are the leading etiologic agents. In meningitis caused by enteric gram-negative bacilli (community-acquired or nosocomial) the third-generation cephalosporins are clearly the drugs of choice. Ceftazidime **40** has even been used to treat patients with *Pseudomonas aeruginosa* meningitis with an 80% response rate.

The third-generation cephalosporins are effective in the treatment of bacteremias, pneumonias, urinary tract infections, intra-abdominal infections, and skin and soft tissue infections. Because of its long half-life, cetriaxone **(39)** has been used for outpatient treatment of skin and soft tissue infections. Single dose ceftriaxone **(39)** has also been effectively used in the treatment of urinary tract infections (132, 215). Ceftriaxone is now the agent of choice for treating uncomplicated urethral, anorectal, or pharyngeal gonorrhea, and gonococcal opthalmia, including infections caused by penicillin-resistant strains (132, 217). There is no clearly defined

role that cephalosporins play in the treatment of syphilis, mycoplasma or clamydial infections, or bacterial vaginosis, but ceftriaxone may be effective in the treatment of chancroid.

10. Toxicity

The cephalosporins generally cause few side effects (80, 132, 219–221). Thrombophlebitis occurs as a result of intravenous administration of all cephalosporins. Hypersensitivity reactions related to the cephalosporins are the most common side effects observed, but these are less common than found with the penicillins. Clinically only about 5–10% of patients with allergic reactions to the penicillins manifest the same reactions to the cephalosporins, and data would contradict any true cross-reactivity to cephalosporins in patients who have previously reacted to penicillin (80, 132, 219).

Although immediate reactions of anaphylaxis, bronchospasm, and urticaria have been reported, most commonly patients exhibiting an adverse reaction develop a maculopapular rash, usually after several days of therapy. They may also develop fever and eosinophilia (80, 219). Cefoperazone **34** and ceftriaxone **(39)**, having greater biliary excretion than other cephalosporins, are associated with an increased risk of diarrhea, which may be caused by selection of cytotoxin producing stains of *Clostridium difficile* (219).

Generally, nephrotoxicity is not a problem. Some cephalosporins, especially those with the 3-methylthiotetrazole side chain, such as moxalactam **48**, show a tendency to promote bleeding. This appears to be due to a reduction in the synthesis of prothrombin and can be a problem especially in elderly patients, patients with renal insufficiency, or patients suffering from malnutrition (219). The same side chain seems to promote a disulfiramlike reaction in patients consuming alcohol following a cephalosporin dose (80, 219).

10.1. Economic Aspects

Cephalosporins first entered the marketplace in 1964, when cephalothin **27** and cephaloridine **51**, which are both injectable, were launched. By the late 1970s, the injectable cephalosporins had become important therapeutic agents in the hospitals. Also in 1964 the first oral cephalosporin, cephaloglycin [3577-01-3], $C_{18}H_{19}N_3O_6S$, was launched only to be displaced by the end of the year by cephalexin **12**. For years cephalexin was the leading oral cephalosporin on the market. It has since been displaced by cefaclor **13**. With the advent of the more β-lactamase stable cephalosporins such as cefoxitin **23** and cefuroxime **(35)**, and the more potent agents such as cefotaxime **(36)** and other third-generation compounds, cephalosporins now dominate the antibiotic market worldwide.

On a worldwide basis, total pharmaceutical sales for 1983 were $71 billion, antibiotic sales totaled $9.2 billion, and cephalosporins accounted for $1.2 billion, or 13% of the antibacterial market (87). By 1986 the worldwide antibiotic market was valued at around $11 billion and cephalosporins represented approximately 41% of the total. Marketing projections for antibiotics from 1986 to 1991 show an average annual growth rate of 3.7% to $13,144 million. Cephalosporin sales are projected to reach 40% or $5,234 million (222). Worldwide, in 1988, the top selling cephalosporin was the oral cephalosporin Ceclor **13** which had 1988 sales of $605 million and projected 1989 sales of $696 million. The second best selling, and top parenteral, cephalosporin was Rocephin **(39)** which had 1988 sales of $475.8 million and projected sales of $597 million for 1989. The third cephalosporin on the list was Claforan **(36)** which had 1988 sales of $376.2 million and a projected decline in sales for 1989 to $353 million as it is displaced by the newer cephalosporins (223).

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