Table II. Pharmacological Data[a]

no.	nicotine TE	nicotine D	pentylene-tetrazole (P)	bicucullin (B)	γ-butyro-lactone (γ-B)	hypoxic stress (HS)	yohimbine (Y)	oxo-tremorine (OX)	apomorphine gnawing (AG)
3	0.11	0.13	0.9	0.7	1.6	5	12.5	5.3	5.3
4	1.4	1.6	5.6	40	6	>50	>50	>50	>50
8[c]	1.0	1.1	2.5	2	0.5	10	>50	>50	>50
8[d]	0.63	0.63	b	3	2	>50	>50	>50	>50
9	8	8	8.8	17.7	5.3	10.5	>50	14.9	>50
12	0.025	0.025	0.08	1	0.4	>50	>50	>50	>50
13	0.002	0.002	0.14	b	b	2.2	b	b	b
14	0.09	0.1	0.45	0.7	0.4	4	>100	>100	>100
15	1.3	1.3	4.0	10	1	>50	>50	>50	>50
16	0.89	1.4	3.2	6	5	30	>100	>100	>100
17	3.6	3.6	32	40	10	>50	>50	>50	>100
18	1.0	1.1	b	7	2	40	>50	>50	>50
19	0.4	0.5	3.7	>25	3.7	>30	25	25	>25
20	1.1	1.3	8.8	>50	5.3	10	>50	>25	>50
21	0.9	1.1	6.3	14.9	2.2	>50	>50	>50	7.4
25	b	b	0.3	3.1	0.2	3.1	>25	12.5	>25
26	0.8	0.8	4.4	>12.5	12.5	7	>12.5	10.5	>12.5
27	1.4	1.4	14.9	>50	25	9	>50	>50	>50
28	40	40	>50	>50	50	>50	>50	>50	>50
29	0.5	0.56	6.3	12.5	2	20	>25	>25	>30
30[e]	0.28	0.28	0.1	2.6	0.035	0.2	>50	>50	>50
31[f]	7.0	7.0	>50	21	18	>50	4	1	1

[a] Values are ED_{50} values expressed in mg/kg. [b] Not tested. [c] HCl salt. [d] Free base. [e] Diazepam, obtained from Hoffman-LaRoche, Inc. [f] Imipramine hydrochloride.

A solution of 3-(diethylamino)propyl chloride (1.64 g, 0.011 mol) in DMF (50 mL) was then added to the hot solution, and heating was continued for 1.5 h. The mixture was concentrated in vacuo, and the residue was mixed with water and extracted with $CHCl_3$. The extract was washed with brine, dried (Na_2SO_4), and concentrated. The residue was chromatographed on silica gel (400 g) with 5% MeOH-95% $CHCl_3$. The product thus obtained was dissolved in EtOAc and acidified with ethanolic hydrogen bromide. The salt was recrystallized from MeOH-EtOAc to give 2.91 g of **20**, mp 234-238 °C. The analytical sample had mp 230-236 °C.

8-Chloro-2,4-dihydro-2-[2-(4-methyl-1-piperazinyl)-ethyl]-6-phenyl-1*H*-*s*-triazolo[4,3-*a*][1,4]benzodiazepin-1-one (9). Procedure H. A stirred solution of **10** (3.73 g, 0.01 mol), KI (3.32 g, 0.02 mol), and 1-methylpiperazine (2.0 g, 0.02 mol) in dry DMF (30 mL) was heated at 60 °C for 6 days, under N_2. The solution was mixed with brine and extracted with CH_2Cl_2. The extract was washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was crystallized from EtOAc and recrystallized from CH_2Cl_2-EtOAc-Skelly B to give 0.71 (mp 118-125 °C) and 0.37 g (mp 120-125 °C) (soften at 115 °C) of **9**. The analytical sample had mp 118-125 °C.

Pharmacology. Methods. Carworth Farms male, albino mice (CF-1) weighing 18-22 g were used for all studies reported here. The test compounds were dissolved or suspended in 0.25% aqueous methylcellulose solution and administered intraperitoneally to groups of four or six mice per dose at multiple dose levels distributed at 0.3 log intervals. Procedures for measuring the effect of test compounds on the antagonism of nicotine-induced tonic-extensor convulsions (TE) and death (D), bicucullin-induced tonic-extensor convulsions (B), pentylenetetrazole-induced clonic convulsions (P), and oxotremorine-induced hypothermia (OX); on the potentiation of γ-butyrolactone-induced sleep (γ-B), apomorphine-induced gnawing (AG), and yohimbine-induced toxicity in aggregated mice (Y); and on the prolongation of hypoxic survival time (HS) have been described previously.[1,5,16] ED_{50} values were calculated by the method of Spearman and Karber.[17]

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Orally Active Cephalosporins and Penicillins

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A number of orally active cephalosporins and penicillins with interesting biological activity were synthesized. Two of these, 7-[[[3,4-(methylenedioxy)phenyl]glycyl]amino]deacetoxycephalosporanic acid and 7-[[2-(2,3-dihydro-5-benzofuranyl)glycyl]amino]deacetoxycephalosporanic acid were considerably more active than cephalexin both in vitro and in vivo against staphylococcal and streptococcal infections.

In our search for an orally active cephalosporin, we became involved with a series of β-lactam antibiotics in which some compounds exhibited interesting microbiological activity. The compounds synthesized differ from the usual penicillins, such as ampicillin and carbenicillin, and cephalosporins, such as cephalexin and cefamandole,

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Scheme I

Scheme II

6, W = CH_2
8, W = CH_2O

5, W = CH_2
7, W = CH_2O

in the substitution on the aromatic ring of the side chain. The compounds presented were synthesized using several different, but related, amino acids, as well as two hydroxy acids and one diacid.

The amino acids used for coupling to the parent β-lactam nuclei were prepared as follows: 2,3-(Methylenedioxy)phenylglycine (**1**) was prepared from piperonal (**2**) according to Scheme I.

1,2-(Methylenedioxy)phenylglycine (**3**) was prepared by the same route as **1** using the isomeric aldehyde **4** as starting material. 2-(2,3-Dihydro-5-benzofuranyl)glycine (**5**) was synthesized from dihydrobenzofuran **6** according to the method of Ben-Ishai.[1] 2-(1,4-Benzodioxan-6-yl)glycine (**7**) was also prepared as in Scheme II with benzodioxane (**8**) being the starting material. Hydroxy acids **9** and **10** were prepared by diazotization of the corresponding amino acids **1** and **5** with sodium nitrite (Schemes I and II).

Acid **11** was prepared according to Scheme III from 3,4-(methylenedioxy)phenylacetonitrile (**12**).

Since the configuration at the asymmetric carbon of the side chain at C-6 of penicillins and C-7 of cephalosporins must be *R* for potent activity to occur,[2] the above amino

Scheme III

acids were resolved. Resolutions of acids **1**, **5**, and **7** were carried out using binaphthylphosphoric acid as the resolving agent.[3] No attempts were made to resolve acids **3**, **9**, **10**, and **11**. Compounds **12b**, **14b**, **19b**, **20–22**, **23b**, **26b**, and **27** were prepared using the corresponding resolved amino acids.

To obtain the compounds listed in Tables I and II, the above prepared acids were first coupled to the cephalosporin nucleus. Deprotection then gave the desired structures. One or more of the following methods of Scheme IV were used to prepare molecules **12** through **27**. Synthesis of **28** was achieved according to Scheme V.

Biological Results and Discussion

First considering compounds with the (methylenedioxy)phenylglycyl side chain, comparisons of **12b** with **17**, and **13** with **18**, showed that a broader spectrum of activity was found with the methylenedioxy ring in the 3,4 position than in the 2,3 position (Table III). For purposes of comparison, one can assume that the *S* isomer is essentially inactive.[2]

It is well-known that compounds substituted at the 3 position by certain heterocyclic thiomethyl groups often are intrinsically more active against Gram-negative organisms than analogues having a methyl or acetoxymethyl group in that position.[4] Not surprisingly, this was found to be true in this series of cephalosporins in which a selected number of derivatives were prepared. In the series with the 3,4-(methylenedioxy)phenylglycyl side chain, the most active compound, **16b**, had a tetrazolylthiomethyl group in that position (Table III). As shown in Table III, the in vitro spectrum of activity and potency of compounds with the 3,4-(methylenedioxy)phenylglycyl side chain and the analogous compounds bearing the 2-(2,3-dihydro-5-benzofuranyl)glycyl side chain were quite similar.

Two compounds were prepared as racemic mixtures with substituted mandelic acid side chains in the 7 position (**24** and **25**). These did not display particularly interesting activity in vitro (Table III), which also was reflected by

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Scheme IV

method A

method B

method C

method D

[a] EEDQ = *N*-(ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline. [b] BSA = bis(trimethylsilyl)acetamide.

Scheme V

[a] EEDQ = *N*-(ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline.

their activity in vivo (data not presented).

As shown in Table IV, the penicillins **26** and **27** were essentially comparable to amoxicillin in vitro against Gram-positive organisms but less active against Gram-negative organisms. Similarly, compound **28**, an analogue of carbenicillin, was as active as the latter against Gram-positive organisms but less active against Gram-negative organisms.

Cephalosporins with the 7-aminodeacetoxycephalosporanic acid nucleus, as well as those with thio heterocyclic groups in the 3 position, were tested in vivo for efficacy against lethal mouse infections (Tables V and VI) and were compared to cephalexin in separate tests. Compounds **12b** and **19b** were superior to cephalexin against both the staphylococcal and streptococcal infections when the compounds were administered either subcutaneously or orally. Compound **23b** was superior to cephalexin against the streptococcal infections and essentially comparable to cephalexin against the staphylococcal infections. None of these three compounds was more potent than cephalexin against Gram-negative organisms in vitro and, therefore, the results obtained with **12b** as compared to cephalexin against *Escherichia coli* and *Salmonella schottmuelleri* were not surprising. Because of the good activity of **12b** and **19b** against staphylococcal and streptococcal infections in mice, we view these compounds with interest.

Table VI shows that of the thio heterocyclic compounds tested in vivo, **16b** was the most potent when administered subcutaneously. However, in three out of four comparative

Table I

no.	confign[g]	R	X	acid used	method	formula	anal.
12a	R,S						
12b	R		H	1	A	$C_{17}H_{17}N_3O_6S \cdot 1.5H_2O$	C, H, N, S
12c	S		H	1	A, B	$C_{17}H_{17}N_3O_6S \cdot H_2O$	C, H, N
			H	1	A[a]		
13a	R,S		OAc	1	A	$C_{19}H_{19}N_3O_8S \cdot 2H_2O$	C, H, N, S
14a	R,S			1	A	$C_{20}H_{19}N_5O_6S_3 \cdot 2H_2O$	C, H, N
14b	R			1	A	$C_{20}H_{19}N_5O_6S_3 \cdot HCl \cdot H_2O$	C, H, N, S
14c	S			1	A[d]		h
15a	R,S			1	A[e]		h
15b	R			1	A[f]		81%[i]
15c	S			1	A[f]		h
16a	R,S			1	A[b]		h
16b	R			1	A[c]		91%[i]
16c	S			1	A[c]		h
17	R,S		H	3	A	$C_{17}H_{17}N_3O_6S \cdot CF_3CO_2H$	C, H, N
18	R,S		OAc	3	A	$C_{19}H_{19}N_3O_8S \cdot CF_3CO_2H$	C, H, N
19a	R,S		H	5	A		h
19b	R		H	5	A, B	$C_{18}N_{19}N_3O_5S \cdot CF_3CO_2H$	C, H, N
20	R		OAc	5	A	$C_{20}H_{21}N_3O_7S \cdot CF_3CO_2H$	C, H, N
21	R			5	A	$C_{21}H_{21}N_5O_5S_3 \cdot CF_3CO_2H$	C, H, N
22	R			5	A		73%[i]
23a	R,S		H	7	C		h
23b	R		H	7	C	$C_{18}H_{19}N_3O_6S \cdot H_2O$	C, H, N
24	R,S		OAc		D	$C_{19}H_{17}N_2O_9SNa \cdot 0.5H_2O$	C, H, N
25	R,S		OAc		D		87%[i]

[a] Isolated from **12a** by preparative high-pressure LC. [b] Prepared from **13** by solvolysis with i. [c] Isolated from **16a** by preparative high-pressure LC. [d] Isolated from **14a** by preparative high-pressure LC. [e] Prepared from **13** by solvolysis with ii. [f] Isolated from **15a** by preparative high-pressure LC. [g] Configuration at asymmetric carbon in the side chain. [h] Not analyzed. [i] Chromatographic purity as determined by high-pressure LC.

studies, the results obtained by oral as compared to subcutaneous administration indicated that the compound was not well absorbed orally. With respect to the other compounds tested, the ratios of the ED_{50} values obtained by subcutaneous and oral administration indicated that they were better adsorbed than **16b**.

The peak antibiotic levels in serum and the peak times were determined for selected compounds given to the mouse orally. As shown in Table VII, compounds **12b** and **19b** were rapidly absorbed by the mouse and levels produced were comparable to that of cephalexin. On the other hand, compounds **14b** and **15b** were more slowly absorbed as compared to cephalexin and produced much lower peak levels.

As judged by in vivo studies, penicillins **26** and **27** appeared to be well absorbed and were quite comparable to amoxicillin in efficacy against staphyloccal and streptococcal infections in mice when administered either orally or subcutaneously. These compounds did not display the potency of amoxicillin against Gram-negative bacteria (Table VIII). However, this conceivably could be a clinical advantage over amoxicillin in treating staphylococcal or

Table II

no.	config[a]	R	X	acid used	method	formula	anal.
26a	*R,S*			1	A	$C_{17}H_{19}N_3O_6S \cdot 0.5H_2O$	C, H, N
26b	*R*			1	B	$C_{17}H_{19}N_3O_6S \cdot 1.5H_2O$	C, H, N
27	*R*			5	B	$C_{18}H_{21}N_3O_5S \cdot 0.5H_2O$	C, H, N
28	*R,S*			11		$C_{18}H_{16}N_2O_8S \cdot 2Ca \cdot H_2O$	C, H, N

[a] See footnote *g*, Table I.

Table III. Activity in Vitro of Cephalosporins with Ring-Substituted Phenylacyl Side Chains and Cephalexin and Cephalothin

	minimal inhibitory concentration, μg/mL;[a] organism:[b]										
compd	*S.a.* (R)	*S.a.* (S)	*S.f.*	*S.pn.*	*S.p.*	*En.a.*	*En.c.*	*E.c.*	*K.p.*	*P.m.*	*S.s.*
12b	3.1	0.4	>100	1.6	0.8	>100	>100	25	25	>100	12.5
12c	nt[c]	25	>100	50	12.5	nt	nt	>100	>100	>100	>100
13	6.2	3.1	200	0.4	0.2	>200	>200	50	25	100	12.5
14b	3.1	0.2	50	0.1	0.1	25	25	12.5	6.2	>100	1.6
14c	nt	6.2	>100	0.8	0.8	nt	nt	>100	>100	>100	50
15b	0.8	0.4	50	0.2	0.2	50	50	12.5	3.1	50	1.6
15c	nt	6.2	>100	nt	1.6	nt	nt	>50	>50	>50	50
16b	1.6	0.2	50	0.1	0.05	3.1	6.2	3.1	3.1	25	0.8
17	6.2	3.1	>200	6.2	0.4	>200	>200	>200	>200	>200	100
18	3.1	3.1	200	0.2	0.2	>200	>200	100	50	100	25
19a	3.1	1.6	>200	3.1	0.4	>200	>200	100	50	>200	50
19b	1.6	0.4	>100	0.4	0.2	>100	>100	50	25	>100	12.5
20	3.1	0.8	50	0.1	0.1	100	100	25	6.2	50	3.1
21	3.1	0.8	100	⩽0.03	0.05	25	>100	12.5	6.2	>100	6.2
22	1.6	0.4	100	0.1	0.1	25	50	12.5	6.2	100	3.1
23a	6.2	0.8	>200	1.6	0.8	>200	>200	200	200	>200	100
23b	3.1	0.8	>100	0.8	0.4	>100	>100	>100	100	>100	50
cephalexin	3.1-6.2	0.8-1.6	⩾100	1.6-3.1	0.4-0.8	25-50	25-50	6.2-12.5	3.1-6.2	12.5-25	3.1-6.2
24	3.1	0.8	>200	0.1	0.1	>200	>200	25	25	50	6.2
25	3.1	0.8	>200	0.1	0.1	>200	>200	50	50	50	12.5
cephalothin	0.8	0.2	100	0.1	0.1	100	>100	12.5	3.1	12.5	3.1

[a] Determined by serial twofold dilution of compound in Mueller–Hinton agar or trypticase soy broth and inoculation of the agar surface or the broth with an appropriately diluted 18- to 24-h broth culture. Agar plates and broth cultures were incubated at 37 °C for 17 h, and the lowest concentration causing complete or virtually complete inhibition of visible growth was considered to be the minimal inhibitory concentration. [b] *S.a.* (R), *Staphylococcus aureus* (benzylpenicillin resistant); *S.a.* (S), *Staphylococcus aureus* (benzylpenicillin sensitive); *S.f.*, *Streptococcus faecalis*; *S.pn.*, *Streptococcus pneumoniae*; *S.p.*, *Streptococcus pyogenes*; *En.a.*, *Enterobacter aerogenes*; *En.c.*, *Enterobacter cloacae*; *E.c.*, *Escherichia coli*; *K.p.*, *Klebsiella pneumoniae*; *P.m.*, *Proteus mirabilis*; *S.s.*, *Salmonella schottmuelleri*. [c] nt, not tested.

Table IV. Activity in Vitro of Compounds 26–28, Amoxicillin, and Carbenicillin

compd	minimal inhibitory concentration, μg/mL;[a] organism:[b]											
	S.a. (R)	*S.a.* (S)	*S.f.*	*S.pn.*	*S.p.*	*En.a.*	*En.c.*	*E.c.*	*K.p.*	*P.m.*	*S.s.*	*P.a.*
26	100	0.1	3.1	≤0.03	≤0.03	>100	>100	25	6.2	25	1.6	>100
27	100	0.05	3.1	≤0.03	≤0.03	>100	>100	25	6.2	12.5	1.6	>100
amoxicillin	100	0.1	1.6	≤0.03	≤0.03	>100	>100	6.2	0.8	0.4	0.4	>100
28	12.5	0.8	>100	0.2	0.8	50	100	50	100	3.1	50	3.1
carbenicillin	25	0.4	>100	0.4	0.1	6.2	6.2	6.2	6.2	0.8	6.2	0.8

[a] Determined by serial twofold dilution of compound in Mueller-Hinton agar and inoculation of the agar surface with an appropriately diluted 18- to 24-h broth culture. Agar plates were incubated at 37 °C for 17 h, and the lowest concentration causing complete or virtually complete inhibition of visible growth was considered to be the minimal inhibitory concentration. [b] See footnote *b*, Table III; *P.a.*, *Pseudomonas aeruginosa.*

Table V. Activity of Compounds 12b, 19b, 23b, and Cephalexin against Mouse Infections

compd	ED_{50}, (mg/kg)/dose;[a] organism:[b]										
	S.a. (R):	*S.a.* (S)		*S.pn.*		*S.p.*		*E.c.*		*S.s.*	
	po[c]	sc[c]	po	sc	po	sc	po	sc	po	sc	po
12b	3.1	0.45	0.61	26	19	1.4	1.0	15	28	33	37
cephalexin	8.9	1.5	2.6	38	28	3.2	3.1	4.2	5.2	6.7	13
19b	7.0	0.63	0.67	10	8.6	0.34	0.37	nt[d]	nt	nt	nt
cephalexin	10	1.5	1.4	27	31	1.2	1.5	nt	nt	nt	nt
23b	8.3	1.5	0.5	14	15	0.81	1.0	nt	nt	nt	nt
cephalexin	5.8	1.5	1.4	27	31	1.2	2.0	nt	nt	nt	nt

[a] Male albino CD-1 mice weighing 20 (±1) g were infected by intraperitoneal injection of a bacterial suspension to produce uniformly lethal infections. Groups of ten mice each were treated subcutaneously with aqueous solutions or suspensions of appropriate concentrations of antibiotics at 1 and 4 h after infection. The number of mice in each group surviving the challenge for 4 days was recorded and the ED_{50} (the dose in mg/kg required to protect 50% of the infected mice) determined by the method of Reed and Muench [*Am. J. Hyg.*, 27, 493 (1938)]. [b] See footnote *b*, Table III. [c] po, compound administered per os; sc, compound administered subcutaneously. [d] nt, not tested.

Table VI. Activity of Compounds 14b, 15b, 13b, 21, 22, and Cephalexin against Mouse Infections

compd	ED_{50}, (mg/kg)/dose;[a] organism:[b]									
	S.a. (s)		*S.pn.*		*S.p.*		*E.c.*		*S.s.*	
	sc[c]	po[c]	sc	po	sc	po	sc	po	sc	po
14b	0.29	0.8	0.79	1.3	0.23	0.84	10.6	33.9	8	40
cephalexin	0.10	0.31	34	25	1.7	1.3	6.2	12.2	13	10
15b	0.13	0.7	4.1	5.0	0.32	0.41	9.1	24.8	8.1	12.5
cephalexin	0.19	0.16	67.3	42.0	3.6	2.8	6.2	19.8	16.9	20.4
16b	0.31	>2	0.74	>10	0.14	0.37	2.1	nt[d]	<3.1	16
cephalexin	0.84	1.0	34	28	1.0	2.9	3.4	nt	7.4	20
21	0.88	1.8	0.89	1.6	0.44	0.78	9.3	20	23	31
cephalexin	0.98	3.4	37	37	4.5	3.7	4.4	6.1	21	13
22	nt	nt	nt	nt	0.19	0.27	11	19	30	>40
cephalexin	nt	nt	nt	nt	nt	3.6	15	18	40	16

[a] See footnote *a*, Table V. [b] See footnote *b*, Table IV. [c] See footnote *c*, Table V. [d] nt, not tested.

streptococcal infections caused by sensitive organisms.

Experimental Section

Melting points were determined on a Hoover melting point apparatus and are uncorrected. All compounds prepared had spectral data consistent with their assigned structures. IR spectra were recorded on a Perkin-Elmer Model 337 instrument. NMR spectra were recorded on a Varian EM 360 spectrometer using Me_4Si as internal standard.

3,4-(Methylenedioxy)phenylglycine (1; Scheme I). A mixture of 300 g (2.0 mol) of piperonal, 260 g (4.0 mol) of potassium cyanide, 768 g (8.0 mol) of ammonium carbonate, 3 L of ethanol, and 3 L of water was refluxed for 2 h. The reaction mixture was cooled in an ice bath and concentrated hydrochloric acid was added to a final pH of 6.7 with evolution of HCN. The precipitated product was filtered, washed with water, and dried in vacuo at 60 °C to give 326 g (74% yield) of 5-[3,4-(methylenedioxy)phenyl]hydantoin. A mixture of 220 g (1.0 mol) of the latter, 1260 g (4.0 mol) of $Ba(OH)_2{\cdot}8H_2O$, and 5 L of water was stirred and refluxed for 18 h. The hot reaction mixture was filtered and the solid washed with hot water. The combined

Table VII. Peak Serum Levels in Mice following 40 mg/kg Oral Doses of Cephalosporins[a]

compd	dose, μg/mL	peak time, min
12b[b]	47	10
14b[c]	11.8	30
14b[d]	6.9	30
15b[e]	12.5	20
19b[b]	39	10
cephalexin[b]	43	15

[a] Compounds were administered by gavage to CD-1 male mice weighing 25 (±1) g. Four mice were bled from the orbital cavity for each sampling time (5, 10, 15, 20, 30, 40, 60, and 90 min), the blood was pooled and the serum assayed by an agar diffusion procedure using *Sarcina lutea* ATCC 9341 as the assay organism. [b] Compound was dissolved and/or suspended in H_2O. [c] Compound was administered as the sodium salt dissolved in H_2O. [d] Compound was administered as the hydrochloride salt dissolved in H_2O. [e] Compound was dissolved in dilute aqueous $NaHCO_3$ solution containing 10% Tween 80.

Table VIII. Activity of Compounds 26, 27, and Amoxicillin against Mouse Infections

	ED$_{50}$, (mg/kg)/dose;[a] organism:[b]											
	S.a. (M202)[c]		*S.a.* (M240)		*S.pn.* (D137)		*S.pn.* (D815)		*S.p.* (St139)		*S.p.* (St 141)	
compd	sc[d]	po[d]	sc	po	sc	po	sc	po	sc	po	sc	po
26	0.53	0.25	<0.063	0.32	0.16	0.35	0.078	0.25	0.06	0.29	0.13	0.22
27	0.89	0.41	0.063	0.4	0.14	0.22	0.078	0.16	0.053	0.084	0.07	0.11
amoxicillin	0.62	0.62	0.074	0.31	0.14	0.25	0.05	0.13	0.063	0.073	0.06	0.08

[a] See footnote *a*, Table V. [b] See footnote *b*, Table III. [c] Merrell Research Center strain designation in parentheses; both strains of *Staphylococcus aureus* are benzylpenicillin sensitive.

filtrates were mixed with 50 g of charcoal, stirred for 2 h, and filtered through Celite. The pH of the filtrate was adjusted to 6 by the addition of hydrochloric acid. The mixture was cooled for 16 h at 5 °C and filtered. After washing the solid with cold water, it was dried first in air and then over P_2O_5 in vacuo to give 138 g of **1** in 35% yield, mp 252–254 °C.

2,3-(Methylenedioxy)phenylglycine (3). Amino acid **3** was prepared in the same manner as **1** using isomeric aldehyde **4** as starting material, mp 198–200 °C. Anal. ($C_9H_9NO_4{\cdot}0.5H_2O$) C, H, N.

2-(2,3-Dihydro-5-benzofuranyl)glycine (5; Scheme II). A mixture of 4.8 g (40 mmol) of dihydrobenzofuran and 9.0 g (40 mmol) of 2-hydroxy-*N*-(benzyloxycarbonyl)glycine was stirred in a 200-mL solution of 9:1 HOAc–concentrated H_2SO_4 for 0.5 h at room temperature. The reaction mixture was poured into 100 mL of water and extracted three times with 75 mL of EtOAc. The organic extracts were combined, dried over $MgSO_4$, and evaporated to dryness to give 14.2 g (88%) of a white solid. This was recrystallized from CH_2Cl_2/hexane to give 11.75 g of a white crystalline material, mp 119–121 °C. A portion of the latter (4.0 g, 12.2 mM) was dissolved in a mixture of 100 mL of MeOH/200 mL of H_2O and hydrogenolized over 1 g of 10% Pd/C on a Paar shaker at 40 psi for 4 h. The catalyst was removed by filtration through Celite and washed five times with 50 mL of a 1:1 methanol–water mixture. The combined filtrates were evaporated to dryness to give 1.37 g (60%) of the desired compound as a white solid: mp 225–227 °C; NMR (Me_2SO-d_6) δ 6.84 (m, 3, aromatic), 4.88 (m, 1 H, CH), 4.41 (t, 2 H, OCH_2), 3.00 (t, 2 H, CH_2).

2-(1,4-Benzodioxan-6-yl)glycine (7). A mixture of 6.8 g (50 mmol) of 1,4-benzodioxane and 11.3 g (50 mmol) of 2-hydroxy-*N*-(benzyloxycarbonyl)glycine was added to 330 mL of a 10% H_2SO_4 in HOAc solution at room temperature. The reaction was allowed to proceed for 4 h at room temperature before being poured into 1 L of ice–water. This aqueous mixture was extracted three times with 250 mL of EtOAc. The combined EtOAc extracts were back-washed three times with 100 mL of water and then shaken with 300 mL of aqueous Na_2CO_3. After washing the Na_2CO_3 solution with EtOAc, it was acidified to pH 1 with concentrated HCl. This was then extracted four times with 100 mL of CH_2Cl_2. The combined organic extracts were washed once with water, dried over $MgSO_4$, filtered, and evaporated to dryness in vacuo to give an off-white foam, which was crystallized from CH_2Cl_2/hexane to give 8.8 g (68%) of *N*-(benzyloxycarbonyl)-3,4-(1′,4′-dioxanyl)phenylglycine. This was dissolved in 800 mL of absolute MeOH and, after the addition of 0.12 g of 10% Pd/C, hydrogenated on a Parr shaker at 40 psi for 4 h. At this point, 2 equiv of Et_3N was added along with 100 mL of water in order to solubilize the precipitated zwitterion. The catalyst was removed by filtration through Celite. The filtrate was acidified to pH 4 with concentrated HCl, at which point the zwitterion precipitated from the solution. After cooling in ice for 0.5 h, the desired product (**7**) was removed by filtration, washed with 2-propanol and ether, and then dried in vacuo to give 2.49 g of amino acid (**7**) in 64% yield: NMR (CD_3CN/TFA) δ 7.04 (s, 3 H, aromatic), 5.11 (s, 1 H, *H*>NH_2), 4.41 (s, 4 H, OCH_2CH_2O).

3,4-(Methylenedioxy)mandelic Acid (9). To a mixture of 2.0 g (10.2 mmol) of **1** and 20 mL of HOAc was added dropwise 50 mL of water containing 1.4 g (20.4 mmol) of $NaNO_2$. After complete addition the reaction mixture was heated to 65 °C for 4 h. This mixture was then diluted with 200 mL of water and extracted four times with 100 mL of EtOAc. The combined organic extracts were dried over $MgSO_4$, filtered, and evaporated to dryness in vacuo to give 1.3 g of **9** as a white solid: 65% yield; mp 160–162 °C; IR (KBr) 1720 cm^{-1} (CO_2H); NMR (Me_2SO-d_6) δ 7.00 (m, 3 H, aromatic), 6.04 (s, 2 H, OCH_2O), 5.03 (s, 1 H, CH). Anal. ($C_9H_8O_5$) C, H.

2-(2,3-Dihydro-5-benzofuranyl)-2-hydroxyacetic Acid (10). This hydroxy acid was prepared in a similar manner using **5** as the starting material: NMR (Me_2SO-d_6) δ 7.02 (m, 4 H, aromatic and OH), 5.02 (s, 1 H, CH), 4.57 (t, 2 H, OCH_2), 3.22 (t, 2 H OCH_2CH_2).

2-Carboxy-3,4-(methylenedioxy)phenylacetic Acid *p*-Methoxybenzyl Ester (11). A solution of 3.0 g (30 mmol) of diisopropylamine in 30 mL of anhydrous THF was stirred under N_2 at -15 °C. Then, 12.5 mL of 2.4 M *n*-BuLi in hexane solution was added and stirring continued an additional 15 min. To this solution was added 20 mL of anhydrous THF containing 7.75 g (25.8 mmol) of 3,4-(methylenedioxy)phenylacetic acid *p*-methoxybenzyl ester over about 0.5 h. The solution turned dark orange. A strong stream of CO_2 was then passed through the reaction mixture for 0.5 h, after which time it was poured into 300 mL of ice-cold dilute aqueous $NaHCO_3$ and washed three times with 100 mL of chloroform. The aqueous layer was carefully acidified to pH 2 with 10% aqueous HCl before being extracted three times with 100 mL of ether. The organic extracts were dried over $MgSO_4$, filtered, and evaporated to dryness in vacuo to give 6.2 g of an orange oil which crystallized on standing: 70% yield; NMR ($CDCl_3$) δ 9.73 (s, 1 H, CO_2H), 7.03 (m, 7 H, aromatic), 5.92 (s, 2 H, OCH_2O), 5.17 (s, 2 H, CH_2-Ph), 4.64 (s, 1 H, -$CHCO_2H$), 3.85 (s, 3 H, OCH_3).

Resolution of (±)-2-(2,3-Dihydro-5-benzofuranyl)glycine (5). A mixture of 2.7 g (13.99 mmol) of (*R*,*S*)-2-(2,3-dihydro-5-benzofuranyl)glycine and 3.5 g (10 mmol) of (+)-binaphthylphosphoric acid (BPA) was refluxed in 25 mL of methanol for 0.5 h. After the mixture was cooled to 0 °C, 2.76 g (5.08 mmol) of white solid was removed by filtration. The latter was slurried in 50 mL of methanol and treated with 0.691 g (5.08 mmol) of sodium acetate trihydrate and refluxed for 1 h. The hot solution was filtered and the solid was washed with hot methanol. After drying, 880 mg of white crystals was recovered, $[\alpha]^{20}_D$ -131° (*c* 10, 0.1 N HCl). Amino acids **1** and **7** were resolved similarly.

Coupling Procedures. In the general methods an actual example is described.

Method A (Illustrated by Synthesis of 14b). Preparation of Protected Amino Acid. A solution of 19.5 g (0.1 mol) of (*R*)-(–)-3,4-(methylenedioxy)phenylglycine was stirred in 500 mL of a 1:1 water–dioxane mixture with 40.4 g (0.4 mol) of Et_3N and 17 mL of *tert*-butoxycarbonyl azide for 30 h at room temperature under argon. After dilution with 300 mL of water, the solution was adjusted to pH 2, and this aqueous mixture was extracted three times with 200 mL of EtOAc. The organic extracts were dried over $MgSO_4$, filtered, and evaporated to give 27.7 g (94% yield) of (*R*)-(–)-*N*-(*tert*-butoxycarbonyl)-3,4-(methylenedioxy)-phenylglycine as a white crystalline solid.

Preparation of Mixed Anhydride. A mixture of 4.7 g (0.016 mol) of *N*-(*tert*-butoxycarbonyl)-3,4-(methylenedioxy)phenylglycine, 60 mL of anhydrous THF, and 1.62 g (0.016 mol) of Et_3N was stirred and cooled to -30 °C. A solution of 1.85 g (0.017 mol) of ethyl chloroformate in 10 mL of anhydrous THF was added to the stirred reaction mixture at -20 to -30 °C over an 8-min period. The mixture was stirred at -30 °C for 0.5 h and then allowed to increase in temperature to -6 °C.

Coupling Reaction. A mixture of 5.0 g (0.0145 mol) of 7-amino-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)thio]methyl]cephalosporanic acid, 60 mL of water, and 1.83 g (0.018 mol) of Et_3N was stirred to form a solution. The reaction was cooled to +4 °C and added to the above stirred mixed anhydride solution at -3 to -7

°C during an 8-min period. The reaction mixture was stirred at −2 to −4 °C for 2.5 h and allowed to warm to 0 °C during the next 0.5 h and then to 14 °C during the following hour.

The stirred reaction mixture was treated with 15 mL of water, then with 11 mL of acetic acid, followed by 75 mL of EtOAc and filtered through Celite to clarify. The EtOAc layer was separated, and the aqueous layer was extracted twice with 50-mL portions of EtOAc. The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated in vacuo to give an oil. The latter was stirred for 10 min with 300 mL of dry ether, cooled in an ice bath for 10 min, and then filtered to give 8.1 g (90%) of *N*-(*tert*-butoxycarbonyl)-protected cephalosporin.

Removal of the Boc Group. An 8.0-g sample of the above Boc derivative was dissolved in 140 mL of nitromethane and cooled to −60 °C and HCl was slowly bubbled through with stirring for 7 min. This mixture was stirred at −5 °C for an additional 5 min and filtered, and the solid was washed twice with acetone and twice with ether. After drying in vacuo, 6.8 g of the HCl salt was recovered. This was dissolved in 30 mL of methanol and filtered, and the filtrate was diluted with 325 mL of 0.1 N HCl with stirring. After 30 min, the product started to crystallize. The mixture was then cooled at −5 °C for 20 min, filtered, washed with acetone and ether, and dried in vacuo to give 3.9 g (50% yield) of **14b**: mp 175–176 °C dec; IR 1780 (β-lactam), 1695 cm^{-1} (amide); NMR (HCO_2H) δ 7.17 (s, 3 H, aromatic), 6.17 (s, 2 H, OCH_2O), 5.89 (q, 1 H, C_7H), 5.49 (s, 1 H, *H*>NH_2), 5.25 (d, 1 H, C_6 H), 4.51 (s, 2 H, CH_2-S-thiadiazole), 4.41 (q, 2 H, C_2 H_2), 2.82 (s, 3 H, CH_3).

Method B (Illustrated by Synthesis of 26b). Preparation of Protected Amino Acid by the Dane Salt Procedure.[5] To 3 L of absolute ethanol was added 14.5 g of sodium (0.63 g-atom), which was then stirred until reaction was complete. (*R*)-(−)-3,4-(Methylenedioxy)phenylglycine (**1**; 117 g, 0.6 mol) and ethyl acetoacetate (78 g, 0.6 mol) were added and the stirred mixture was refluxed for 16 h.

The hot reaction mixture was filtered and the filtrate concentrated in vacuo. The residual solid weighed 193 g. This was refluxed for 3 h in 3 L of acetone. The reaction mixture was filtered and the filtrate cooled at 5 °C for 24 h. The solid was removed by filtration, washed with cold acetone, and dried at 60 °C for 24 h in vacuo to give 161 g (82% yield) of Dane salt.

Coupling Reaction. A solution of 1.1 g (5.09 mmol) of 6-aminopenicillanic acid (6-APA) in 8 mL of water, 0.75 mL of Et_3N, and 2 mL of acetone was prepared and chilled in an ice bath. Then, 1.65 g (5.02 mM) of the above prepared Dane salt was dissolved in 15 mL of anhydrous acetone and 1 drop of *N*-methylmorpholine. To this solution was added slowly, under N_2 and at −20 °C, 0.4 mL of ethyl chlorocarbonate in 5 mL of acetone. This mixture was stirred for an additional 10 min before the solution of 6-APA prepared previously was added in one portion at −30 °C and under N_2. The reaction mixture was stirred at −10 °C for 0.5 h and then allowed to warm to room temperature. The solution was adjusted to pH 2 with dilute HCl and held there for 10 min before being extracted twice with 15 mL of EtOAc. The aqueous layer was adjusted to pH 5 with 1 N NaOH, layered with 10 mL of methyl isobutyl ketone, and chilled overnight at 4 °C. The formed crystals were removed by filtration, washed with acetone, and dried in vacuo to give 800 mg (40% yield) of penicillin (**26b**).

Method C (Illustrated by the Synthesis of 23b). Preparation of Protected Amino Acid. *N*-(*tert*-Butoxycarbonyl)-(2*R*)-2-(1,4-benzodioxan-6-yl)glycine (**7**) was prepared as described in method A.

Coupling Reaction. A solution of 1.55 g (5 mmol) of protected amino acid and 1.24 g (5 mmol) of *N*-(ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline (EEDQ) in 30 mL of CH_2Cl_2 was stirred at room temperature for 15 min. To this clear solution was added 1.35 g (5 mmol) of 7-aminodeacetoxycephalosporanic acid *tert*-butyl ester and another 20 mL of CH_2Cl_2. This resulting yellow mixture was stirred at room temperature overnight. The reaction mixture was diluted with 100 mL of CH_2Cl_2, washed three times with 30 mL of 0.1 N HCl and two times with saturated $NaHCO_3$, dried over $MgSO_4$, filtered, and evaporated to dryness in vacuo to give 2.32 g (~100% yield) of a light yellow foam.

Removal of Protecting Groups. The above 2.32 g of diprotected cephalosporin was treated with 10 mL of anhydrous trifluoroacetic acid (TFA) at room temperature for 10 min. At this time, the reaction mixture was poured into 400 mL of dry ether, and the precipitated salt was removed by filtration: 1.9 g (88% yield) of the TFA salt of **23b** was recovered; ^1H NMR (CD_3CN/TFA) δ 7.80 (d, 1 H, NH), 7.04 (s, 3 H, aromatic), 5.78 (q, 1 H, C_7 H), 5.16 (s, 1 H, *H*>NH_2), 5.09 (d, 1 H, C_6 H), 4.38 (s, 4 H, OCH_2CH_2O), 3.47 (q, 2 H, C_2 H_2), 2.17 (s, 3 H, CH_3).

Method D (Illustrated by Synthesis of 24). Preparation of Mixed Anhydride. A mixture of 1.65 g (8.5 mmol) of 2-(2,3-dihydro-5-benzofuranyl)-2-hydroxyacetic acid (**10**), 0.86 g (8.5 mmol) of Et_3N, and 1.73 g (8.5 mmol) of bis(trimethylsilyl)acetamide (BSA) was refluxed in 50 mL of anhydrous THF for 2 h. The reaction mixture was cooled to −10 °C and 1.16 g (8.5 mmol) of isobutyl chloroformate was added at that temperature. The reaction was allowed to proceed for 20 min.

Coupling Reaction. A solution containing 2.31 g (8.5 mmol) of 7-ACA and 0.86 g (8.5 mmol) of Et_3N in 35 mL of water and 15 mL of THF was added dropwise to the above prepared mixture at −10 °C under N_2. After complete addition, the reaction was allowed to proceed at −5 °C for 0.5 h and then at room temperature for 1 h. The reaction mixture was then concentrated in vacuo, diluted with 50 mL of water, treated with 30 mL of saturated aqueous $NaHCO_3$, and washed twice with 50 mL of ether. The aqueous extracts were layered with EtOAc, adjusted to pH 1.5 with 1 N HCl, and extracted three times with 75 mL of EtOAc. The organic fractions were combined, washed with 100 mL of EtOAc, dried over $MgSO_4$, filtered, and evaporated to dryness to give 3.5 g (88% yield) of a light yellow foam. This foam was converted to its sodium salt by treatment with 5 mL (10 mmol) of sodium 2-ethylhexanoate in methanol solution. Anhydrous ether (200 mL) was added to precipitate the desired material, **24**: high-pressure LC (Waters' C_{18} column, 40% MeOH in 0.05 M Et_3N/formic acid solution) indicated the presence of two diastereoisomers; IR 1760 (β-lactam), 1680 (amide), 1600 (COO^-) cm^{-1}; NMR (Me_2SO-d_6) δ 6.98 (m, 3 H, aromatic), 5.56 (s, 1 H, C_7 H), 5.01 (m, 3 H, C_6 H, CH_2OAc), 4.58 (t, 2 H, OCH_2CH_2), 2.27 (m, 4 H, OCH_2CH_2, C_2 H_2), 1.98 (s, 3 H, $OCOCH_3$).

6-[[α-Carboxy-3,4-(methylenedioxy)phenyl]acetamido]penicillanic Acid (28). α-Carboxy-3,4-(methylenedioxy)phenylacetic acid *p*-methoxybenzyl ester (**11**; 1.35 g, 4 mmol) was coupled to 1.38 g (4 mmol) of 6-aminopenicillanic acid *p*-methoxybenzyl ester by the EEDQ coupling procedure (method C) to give, after chromatography on silica gel with 1:1 hexane-$CHCl_3$, 1.8 g (68% yield).

The di-*p*-methoxybenzyl ester (475 mg) was dissolved in 50 mL of a 1:1 dioxane-water solution and hydrogenolized over 475 mg of 10% Pd/$CaCO_3$ for 14 h. The catalyst was removed by filtration through Celite. The filtrate was diluted with water and basified with dilute aqueous $NaHCO_3$. This basic aqueous layer was washed with chloroform before being acidified to pH 2 with 1 N HCl. This was then extracted three times with 50 mL of EtOAc, dried ($MgSO_4$), filtered, and evaporated to give 87 mg of white solid penicillin **28**.

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