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A COMPARISON OF THE ANTIBACTERIAL AND β-LACTAMASE INHIBITING PROPERTIES OF PENAM AND (2,3)-β-METHYLENEPENAM DERIVATIVES

THE DISCOVERY OF A NEW β-LACTAMASE INHIBITOR. CONFORMATIONAL REQUIREMENTS FOR PENICILLIN ANTIBACTERIAL ACTIVITY†

D. D. KEITH,* J. TENGI, P. ROSSMAN, L. TODARO and M. WEIGELE
Chemical Research Department, Hoffmann-La Roche Inc., Nutley, NJ 07110, U.S.A.

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Abstract—The antibacterial potencies of **2a** and **4** are shown to be diminished considerably from their penam analogues, penicillin G (**1a**) and mecillinam (**3**). Despite this, **2a** is a substrate for bacterial β-lactamases, and compounds **6a**, **8** and **10** were found to be β-lactamase inhibitors.

Penicillin-binding protein (PBP) studies indicate that penicillin G and mecillinam have much greater affinity for these enzymes than the (2,3)-β-methylenepenam analogues. Based on a comparison of hydrolytic stabilities, it is proposed that the change in biological properties is due to conformational differences between the two types of penam nuclei. The cyclopropyl methylene of **2a** and **4** blocks the side chain from forming an oxazolone with the β-lactam carbonyl. Hence, activation of the β-lactam is prevented and the molecules are rendered less active. We thus conclude that **19** is the biologically active conformation of penicillin antibacterials, and further suggest that the interaction of such antibiotics with their bacterial enzyme targets involves intermediates such as **25–27**.

For nearly forty years penicillin G and related penams have proven to be of enormous value for the treatment of bacterial infections. During much of this time pharmaceutical chemists have made many structural modifications on the penicillins in their search for materials with better therapeutic properties. Improvements have included broader or more selective spectra, increased potency and increased stability to β-lactamases.[1] In addition there has been a large amount of work done to elucidate the mode of action exerted by these β-lactams on bacterial cell wall synthesizing enzymes.[2] Despite the intense interest in these compounds over the years, there remains much to be learned about the fundamental factors which influence their antibacterial action, and in particular, about the structural and conformational requirements for activity. In this paper we present our work on penam analogs containing the (2,3)-β-methylenepenam nucleus. Deductions made possible by a comparison of the biological, physical and chemical properties of these compounds with their penam counterparts led to the synthesis of several new β-lactamase inhibitors and, most importantly, resulted in certain conclusions concerning the specific conformation of the thiazolidine ring which is necessary for good antibacterial activity of the penicillin molecule. Our results also provide new details concerning the participation of the acylamino side chain in the event which leads to the inactivation of β-lactam sensitive enzymes.

SYNTHESES

Except for penicillin G(**1a**) and mecillinam (**3**), which were obtained from commercial sources, the chemicals used in this study were synthesized. Their preparation is discussed in the following section.

The methyl ester of penicillin G (**1b**) was made from commercial material by esterification with diazomethane. The penams **5a**,[3] **7**,[4] and **9**[5] were prepared according to methods reported in the literature. Esterification of **5a** with diazomethane gave **5b**.

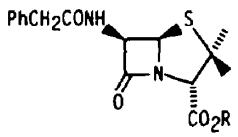
In the (2,3)-β-methylenepenam series, the cyclopropyl analog **2a** of penicillin G and the amino acid **11** (Scheme 1) were also prepared according to literature procedures, following the very excellent work reported by Kamiya *et al.*[6] Esterification of **2a** with diazomethane provided **2b**. Compounds **2a** and **11** were then used to make the other (2,3)-β-methylenepenams as shown in Scheme 1.

The (2,3)-β-methylenepenam analog **4** of mecillinam (**3**) was made by treating amino acid **11**[6] with the imminium chloride **12**, obtained from the reaction of N-formylhexamethyleneimine and oxalyl chloride (Sequence A, Scheme 1).

Amino acid **11**[6] was used again in the synthesis of (2,3)-β-methylenepenam sulfone (**6a**) (Sequence B, Scheme 1). Diazotization of **11** in the presence of hydrogen bromide gave the α-bromide **13**, which was purified as its diphenylmethyl ester and then converted back to the acid by treatment with trifluoroacetic acid. Removal of the bromine was accomplished by catalytic hydrogenation yielding the penam analog **14**. Oxidation of **14** with potassium permanganate resulted in sulfone **6a**, and esterification with diazomethane gave **6b**. This method is essentially the same as the one reported for the synthesis of **5a**.[3]

Chlorosulfone **8** was prepared as shown in Sequence C, Scheme 1. Esterification of **2a**[6] with diphenyl diazomethane followed by nitrosation with dini-

†We dedicate this paper to the memory of Dr. Willy Leimgruber (1930–81), our late friend and Director of Chemical Research.



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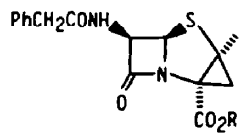

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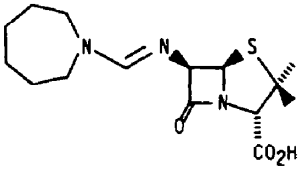

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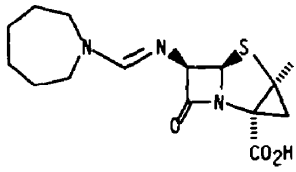

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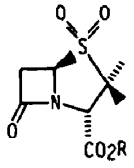

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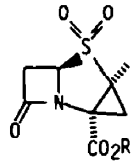
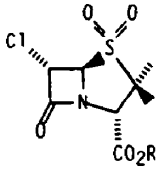


6a; R=H-
6b; R=CH_3-



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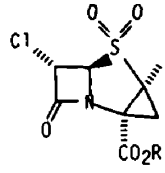

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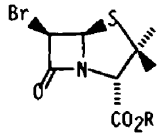

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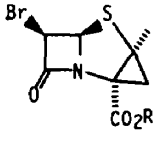

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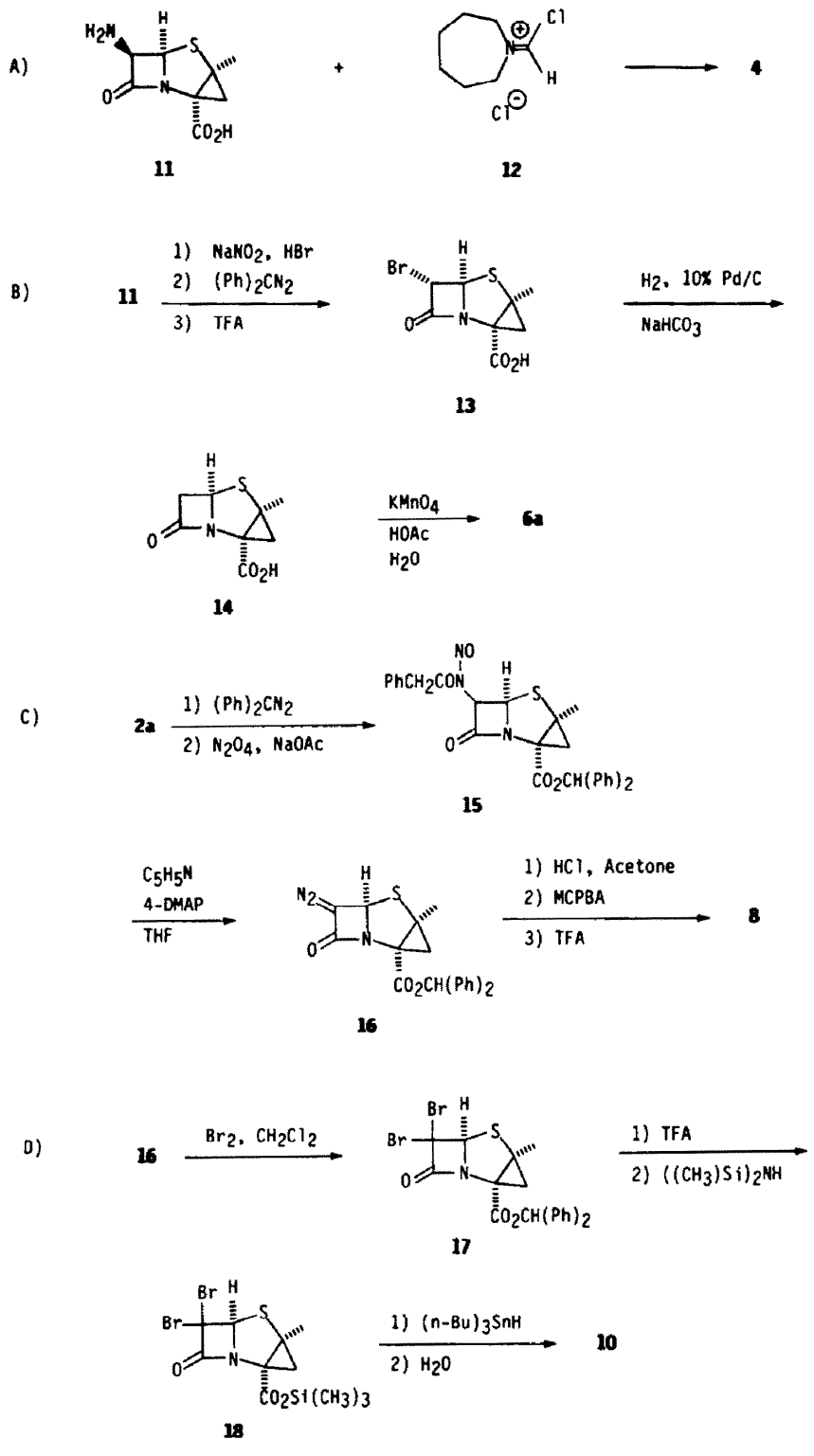
trogen tetroxide produced the N-nitrosoamide **15**. The diazo compound **16** was formed from the nitrosoamide by treatment with pyridine in the presence of a catalytic amount of 4-dimethylaminopyridine (4-DMAP). Chlorination of **16** was effected with hydrogen chloride in acetone, and the resultant chloride converted to chlorosulfone **8** by oxidation with *m*-chloroperbenzoic acid (MCPBA) and ester removal mediated by trifluoroacetic acid (TFA).

Diazo compound **16** found use again in the production of the 6-β-bromide **10** (Sequence D, Scheme 1). Bromination of **16** gave dibromide **17** which was converted to the silyl ester **18** by sequential treatment with TFA and hexamethyldisilazane. Selective bromide reduction with tri-n-butyltin hydride followed by ester hydrolysis gave **10**. This method is the same as the one used for the synthesis of **9**.[5]

A COMPARISON OF PROPERTIES: PENAM VS (2,3)-β-METHYLENEPENAM

(a) *Antibacterial potency*. While investigating various derivatives of the (2,3)-β-methylenepenams, we noted that these compounds possessed greatly reduced antibacterial potency compared to their penam counterparts. This effect is illustrated in Table 1 where the minimum inhibitory concentrations (MICs) of penicillin G (**1a**) and mecillinam (**3**) are compared to the corresponding MICs of cyclopropyl analogs **2a** and **4**. The focus of subsequent sections will be on isolation of the reasons for these differences.

(b) *β-Lactamase susceptibility and inhibition*. Despite the poor antibacterial effectiveness of compound **2a**, it proved to be a substrate for several β-lactamases. Thus, the MIC (125 μg/mL) of this



Scheme 1.

compound against *Micrococcus luteus* PCI is significantly higher in the presence of various β-lactamases (e.g. 500 μg/mL in the presence of cephalosporinase from *Enterobacter cloacae* and > 1000 μg/mL in the presence of the TEM-1 β-lactamase from *E. coli* and the penicillinase from *Staphylococcus aureus*).[7] These results suggest that (2,3)-β-methylenepenam analogs of known penam-derived β-lactamase inhibitors might also be β-lactamase inhibitors.

The (2,3)-β-methylenepenams **6a**, **8** and **10** are analogs of the known β-lactamase inhibitors **5a**,[3] **7**[4] and **9**[5]. The new compounds were also found to be inhibitors of various β-lactamases with activities

Table 1. A comparison of antibacterial properties: the penam nucleus vs the (2,3)-β-methylenepenam nucleus

Compound	Minimum inhibitory concentration (MIC) μg/mL[a,b]			
	S.aureus[c]	*B.subtilis*[d]	*E.coli*[e]	*P.vulgaris*[f]
1a (Pen G)	0.03	0.015	125.	31.3
2a (Pen G cyclopropylog)	15.7	1.9	>1000	>1000
3a (Mecillinam)	62.5	31.3	0.12	0.12
4a (Mecillinam cyclopropylog)	>1000	1000	>1000	>1000

a) The MICs were determined by E. Lasala and D. Pruess of Hoffmann-La Roche Inc., Nutley, NJ
b) Agar-diffusion well method in Antibiotic Medium #1 was used.
c) *Staphylococcus aureus* ATCC 6538P
d) *Bacillus subtillis* NRRL 558
e) *Escherichia coli* ATCC 27856
f) *Proteus vulgaris* ATCC 6380

Table 2. A comparison of β-lactamase inhibitors[a]

Compounds	I_{50}[b] (μM)		
	β-Lactamase from		
	Staphylococcus aureus	*Escherichia coli* RI	*Enterobacter cloacae*
5a (Sulbactam)	2.6	1.4	12
6a (Sulbactam cyclopropylog)	6.7	1.1	69
Sodium clavulanate	0.056	0.042	192

a) These data were determined by D. Pruess, Microbiology Department, Hoffmann-La Roche Inc., Nutley, NJ
b) I_{50} is the concentration necessary to inhibit the rate of nitrocefin hydrolysis by 50%. The test compound is preincubated with enzyme for 20 min at 30° and pH 7. Chromogenic cephalosporin substrate, nitrocefin, is added and its initial rate of hydrolysis is recorded spectrophotometrically by reading absorption at 482 nm. Three enzyme preparations were employed: a) the inducible penicillinase from *Staphylococcus aureus* 1059B, b) the constitutive broad-spectrum TEM type beta-lactamase mediated by the resistance transfer factor R1 in *Escherichia coli* 1268, and c) the type Ia cephalosporinase from *Enterobacter cloacae* purchased from Miles Laboratories.

ranging from essentially the same as (in the case of **6a**), to somewhat less than their penam counterparts (**8** and **10**).[7] Compound **5a** is the broad-spectrum β-lactamase inhibitor sulbactam, which is currently being developed in the clinic.[8] A comparison of the I_{50} values shown by **5a** and **6a** against some β-lactamases is given in Table 2. Values for sodium clavulanate, another clinically useful β-lactamase inhibitor,[9] are included for reference.

The results of tests done with several strains of β-lactamase producing bacteria are shown in Table 3. As can be seen from these data, the ability of the new β-lactamase inhibitor **6a** to protect the β-lactam antibacterials mecillinam and ampicillin from the β-lactamases produced by the listed bacteria is about equal to that of sulbactam and sodium clavulanate.

(c) *Penicillin-binding protein studies.* It has been shown that penicillins interfere in the terminal step of bacterial cell wall biosynthesis. They do this by acylation (*via* the β-lactam CO) of membrane associated enzymes (e.g. transpeptidases) which effect the cross-linking of linear peptidoglycan, thereby preventing the formation of the peptide bridges which give structural strength to the cell wall.[2] An assay developed recently permits study of the interaction of β-lactams with proteins of the bacterial cytoplasmic membrane.[10,11] It is accepted that certain of these penicillin-binding proteins (PBPs) can be equated with the penicillin-sensitive enzymes (PSEs), which play essential roles in the normal growth of the bacterial cell.[12] Accordingly, the binding of β-lactams **1a**, **2a**, **3** and **4** by the PBPs of *E. coli* following the Spratt system was examined.[11] The results of this study are shown in Table 4. It is evident from the data

Table 3. Protection afforded mecillinam and ampicillin by β-lactamase inhibitors

Organism	Minimum inhibitory concentration (MIC) μg/mL[a,b]			
		Mecillinam plus		
	Mecillinam	**5a** (Sulbactam) 8 μg/mL	**6a** (Sulbactam cyclopropylog) 8 μg/mL	Sodium clavulanate 4 μg/mL
E. cloacae P99	0.03	<0.008	<0.008	<0.008
E. coli 7289	32	8	0.5	0.06
E. coli K12R1	1	0.18	0.06	0.13
E. coli ST323	4	0.13	0.06	0.06
S. marcescens S5[c]	4	0.25	2	1
P. aeruginosa 5700[d]	>128	>128	>128	>128
		Ampicillin plus		
	Ampicillin	**5a** (Sulbactam) 8 mg/mL	**6a** (Sulbactam cyclopropylog) 8 μg/mL	Sodium clavulanate 4 μg/mL
E. cloacae P99	>100	100	>100	>100
E. coli 7289	>100	>100	50	25
E. coli K12R1	100	12.5	6.25	12.5
E. coli ST323	100	ND[e]	12.5	3.12
S. marcescens S5	100	12.5	50	100
P. aeruginosa 5700	>100	100	>100	>100

a) The MICs were determined by E. Squires and J. Christenson of the Department of Chemotherapy, Hoffmann-La Roche Inc., Nutley, NJ
b) A standard serial dilution method was used utilizing Mueller-Hinton agar. The β-lactamase inhibitors were used at inactive concentrations and the β-lactam antibiotic titrated in the presence of an inactive concentration of inhibitor.
c) Serratia marcescens S5.
d) Pseudomonas aeruginosa 5700.
e) Not determined.

Table 4. The binding of compounds **1a**–**4** by the penicillin-binding proteins of *E. coli* KN126.[a,b]

Compound	Conc.[b] (μg/mL)	Effect on PBPs[c]	MIC (μg/mL)[d]
1a (Pen G)[e]	----	----	6.0
2a (Pen G cyclopropylog)	1200	4 complete 3 in part	1200
3 (Mecillinam)	5.4	2 complete	0.18
4 (Mecillinam cyclopropylog)	1000	2 complete 1A,1B, 3 in part	1000

a) This study was carried out by R.B. Wright and S.D. Makover, Microbiology Department, Hoffmann-La Roche Inc., Nutley, NJ.
b) The residual binding of [^{14}C] penicillin G by the penicillin-binding proteins was measured after preincubation of membranes with various concentrations of unlabelled **2a**-**4** for 10 min at 30° as described by Spratt.[12] The qualitative effects of the highest concentrations of **2a**-**4** are noted under "Blocked PBPs".
c) For example, after pretreatment with **2a** at a concentration of 1200 μg/mL, there is no residual binding of [^{14}C] penicillin G by PBP 4, there is some residual binding by PBP 3 and the binding of [^{14}C] penicillin G by the other PBPs is unaffected.
d) Minimum inhibitory concentration.
e) In this system the concentrations of penicillin G required to reduce [^{14}C] penicillin G binding by 50% are 5.0 μg/mL or less for proteins 1-6, except for protein 5 which requires a concentration of 18 μg/mL.

Table 5. A comparison of physical properties of the β-lactams: the penam nucleus vs the (2,3)-β-methylenepenam nucleus

	Compound	C=O Stretching Frequency cm^{-1}[a]	Bond Lengths[b,c] Å OC-N	C=O	Altitude of Pyramid,[b,d] Å	Sum of Bond Angles Around Nitrogen in degrees[b]
1b	(Pen G methyl ester)[e]	1782	1.39	1.19	0.44	332
			1.39	1.21	0.43	333
			1.38	1.20	0.43	333
2b	(Pen G cyclopropylog methyl ester)[e]	1792	1.38	1.21	0.39	337
			1.40	1.19	0.41	335
5b	(Sulbactam methyl ester)	1802	1.40	1.19	0.39	337
6b	(Sulbactam cyclopropylog methyl ester)	1807	1.40	1.18	0.35	342

a) Measured in $CHCl_3$ solution.
b) Determined from the X-ray analyses of **1b**, **2b**, **5b**, and **6b**.
c) Maximum standard deviation ±0.008 Å.
d) Having N-1 as apex and C-2, C-5, and C-7 as base (for numbering, see Fig. 1).
e) The unit cell of the crystal of **1b** has three independent molecules while the cell of **2b** has two. Therefore, there are three values and two values, respectively, given for the parameters of these molecules which were determined from the X-ray data.

that the (2,3)-β-methylenepenams **2a** and **4** do not successfully compete with their penam counterparts in the binding assay. They may either be excluded from the active site, or once in the site, they may not be capable of covalent interaction.

(d) *Physical properties.* In past studies, certain physical measurements of β-lactam compounds have been used to predict their chemical reactivity and their antibacterial activity.[13a,b] Such parameters include the IR stretching frequency of the β-lactam CO (higher frequencies equal greater reactivity/activity),[14] the bond lengths of the β-lactam (more reactive/active β-lactams have shorter CO bonds and longer C–N bonds),[15] the height of the pyramid having the β-lactam N at its apex and the three atoms attached to the N as its base (higher pyramids equal greater reactivity/activity),[15] and the sum of the angles around the β-lactam N (smaller sums equal greater reactivity/activity).[15] A comparison of these parameters for some of the penams and their cyclopropyl analogs might provide insight into the reasons for the differences between them. To aid in this task, single-crystal X-ray analyses were performed on the methyl esters **1b**, **2b**, **5b** and **6b**. The comparison is shown in Table 5.

On balance, the two types of rings have similar characteristics. Whereas the IR stretching frequencies of the cyclopropyl analogs **2b** and **6b** are slightly higher than those of the corresponding penams **1b** and **5b**, the height of the pyramid (as defined above) is slightly more and the sum of the angles around N slightly less for the penams. The bond lengths of the CO and C–N bonds are approximately the same in each case. On this basis, one would predict that these β-lactams should exhibit similar chemical reactivities and similar antibacterial activities.

(e) *Chemical reactivity.* The carboxylic acid on the thiazolidine ring of penicillins is necessary for antibacterial activity.[16] On the chance that the cyclopropane ring might have an effect on this activity by influencing the acidity of the carboxylic acid attached to it, the pKa's of **1a**, **2a**, **5a** and **6a** were determined. The ring has essentially no effect on acidity as can be seen from the pKa values (Table 6).

Table 6. A comparison of chemical reactivities of the β-lactams: the penam nucleus vs the (2,3)-β-methylenepenam nucleus

	pKa[a]	Chemical Half-life,[b] h
1a (Pen G)	4.2[c]	110
2a (Pen G cyclopropylog)	4.3[c]	>500[e]
5a (Sulbactam)	4.3[d]	290
6a (Sulbactam cyclopropylog)	4.3[d]	337

a) These are "apparent pKas" since the titrations were done in aqueous organic solvent mixtures.
b) In phosphate buffer, pD 7.4, at 38°C.[16] The potassium salts of **1a**, **2a**, **5a**, and **6a** were used.
c) Determined in 50% ethanol/water.
d) Determined in 66% *N,N*-dimethylformamide/water.
e) After 500 h under these conditions, **2a** had suffered ~26% degradation.

The hydrolytic reactivity of β-lactams has been related to biological activity, the more reactive compounds being better antibacterials.[13a] Thus, some of the penams and cyclopropylpenams were examined under hydrolytic conditions identical to those used in a recent study of other β-lactams.[17]

The reaction rates of the compounds were monitored by ^{1}H NMR spectroscopy in deuterated buffer solution (Table 6). There was found to be a marked difference in the chemical stability of penicillin G (**1a**) and its cyclopropyl analog, **2a**. The half-life of **1a** was 110 hr while only 26% of **2a** had decomposed after 500 hr. Interestingly, sulbactam (**5a**) and its analog, **6a**, are more similar in reactivity, having half-lifes of 290 hr and 337 hr respectively. Both are more stable than **1a**.

Although product studies were not carried out, it was shown (in the case of **1a**, **5a** and **6a**) from the IR spectra that the hydrolysis reaction involved loss of the β-lactam CO.

(f) *The conformations of penicillin G* (**1a**) *and its* (2,3)-β-*methylenepenam analog* **2a**. The structures of **1** and **2** differ only in the substitution pattern of the thiazolidine ring. Thus, while considering conformational differences, the emphasis will be on that part of the molecule.

The 5-membered ring of the penicillin nucleus can assume two conformations. In one (see **19** in Fig. 1) the C6–C10 distance is 5.0 Å and the C7-N1-C2-C8 torsion angle, which defines the position of the carboxylic acid relative to the β-lactam CO, is approximately 120° (Table 7). This is the conformation which obtains in the crystal of **1b**.

The C6 to C10 distance in the second conformation is 3.6 Å and the C7-N1-C2-C8 torsion angle is 160° (see **20** in Fig. 1 and Table 7). We refer to the first mentioned conformation as "open" and the second as "closed" because of the relative shielding effect C10 has on the concave side (β-face) of the bicyclic ring system in each arrangement. For example, an approaching nucleophile would be blocked by C10

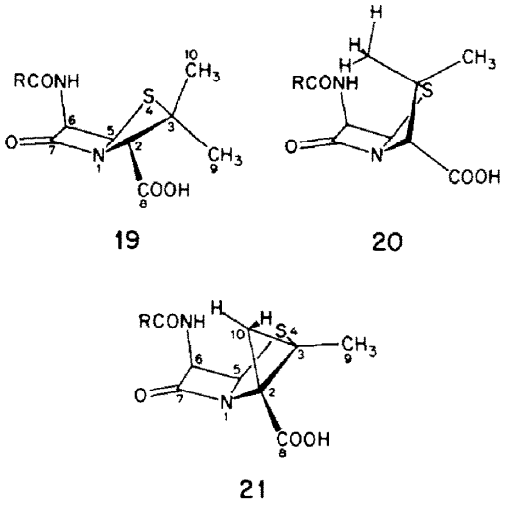


Fig. 1. The structures of the two penicillin conformations, "open" (**19**) and "closed" (**20**), and of the conformationally rigid (2,3)-β-methylene analog **21**. They were drawn from Dreiding models, each from the same perspective. The numbering of atoms is non-standard and is used for the purpose of clarity only.

from taking an ideal "approach vector"[18] to the β-lactam CO from the *endo* face of the bicyclic ring system when the penam is in the "closed" conformation. In the "open" conformation C10 would have no effect on an approaching nucleophile. These conclusions have been drawn from the study of "Dreiding" models and their consequence will become apparent in the Discussion section.

The (2,3)-β-methylenepenam nucleus of **2** is conformationally rigid with a C6 to C10 distance of 3.9–4.0 Å (see **21** in Fig. 1 and Table 7), which places C10 in a position similar to the position of C10 in the "closed" conformation, **20**, of the penam nucleus.

Table 7. Comparison of the C6–C10 distances and the carboxylic acid/β-lactam carbonyl torsion angles: the penam nucleus vs the (2,3)-β-methylenepenam nucleus

Compound	C6-C10 Distance,[a] Å	Carboxylic Acid/β-Lactam Torsion Angle in degrees[b]
1b[c,d] (Pen G, open conformation, see **19**)	5.0 5.1 5.0	125 122 117
1b[e] (Pen G, closed conformation, see **20**)	3.6	160
2b[c,d] (Pen G cyclopropylog, conformationally fixed, see **21**)	4.0 3.9	119 120

a) This measurement represents the distance between C6 and C10 following the numbering scheme given in Fig. 1.

b) This angle is the torsion angle for the bonds between atoms C7/N1/C2/C8 following the numbering scheme given in Fig. 1.

c) The unit cell of **1b** has three independent molecules while the cell of **2b** has two. Therefore, there are three and two values, respectively, given for these measurements.

d) Determined from the X-ray analyses of **1b** and **2b**.

e) These values were determined by measurements made on Dreiding models.

The C7-N1-C2-C8 torsion angle, on the other hand, is about 120°, as in the "open" conformation, **19**.

DISCUSSION

The large difference in the antibacterial activity shown by penicillin G (**1a**) and mecillinam (**3**) vs their (2,3)-β-methylenepenam analogs **2a** and **4** is quite striking and difficult to explain at first glance, given the close structural relationship of these compounds. The poor binding of **2a** and **4** by the PBPs of *E. coli* (Table 4) indicates the reason for their low activity is fundamental in nature. The molecules **2a** and **4** are either excluded from the enzyme receptor sites (e.g. because of conformation), or once bound, they do not acylate the enzyme to make the attachment irreversible.[2,19] The greater stability of **2a** compared to **1a** towards hydrolysis in buffer solution supports the latter possibility, since it seems quite probable that the reason for the difference in chemical reactivity is the same as the reason for the difference in antibacterial activity.

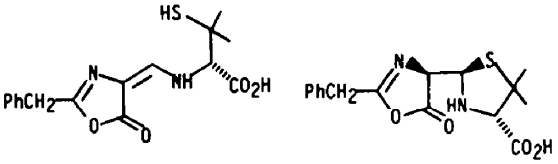
The cyclopropyl analogs differ from their penam counterparts only in the hybridization of some sigma bonds and in the conformational rigidity imposed by the 3-membered ring. The changes in sigma bond hybridization are not likely to be the source of a major difference in reactivity of the β-lactams. In the first place, the physical properties of each type β-lactam are very similar (Table 5), and furthermore, the cyclopropane has very little effect on the acidity of the thiazolidine carboxylic acid (Table 6). We are thus left with only conformational factors to explain the different reactivities of **1a** and **2a**.

Unlike the penams which can exist in the "open" or "closed" conformations, **19** and **20**, the (2,3)-β-methylenepenams are locked in a unique conformation, **21** (Fig. 1). In this arrangement the carboxyl group, C8, is in a position similar to the carboxylic acid in the "open" penam conformation, **19**, while the cyclopropyl methylene, C10, is in a position similar to the β-Me group, C10, in the "closed" penam conformation, **20**.

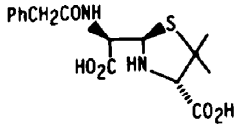
As pointed out previously,[15,20,21] the position of the carboxylic acid in β-lactam antibiotics can vary considerably without loss of activity. Thus, the torsion angle, C7-N1-C2-C8, which defines the position of the carboxylic acid *vis-a-vis* the β-lactam CO, is 120° and 160°, respectively, in the two conformations available to penicillins (**19**, **20** and Table 7). Values for the analogous torsion angle in the biologically active cephalosporins range from 30° to 60°.[15] Since the conformational requirements placed on the carboxyl group of the antibiotic for substrate recognition by the enzyme are apparently not too restrictive, it is unlikely that its position in the (2,3)-β-methylenepenams has a major effect on their antibacterial activity.

On the other hand C10, both in the "closed" penam conformation **20** as well as in the analog **21**, is in a position to have a dramatic effect on the reactivity of the β-lactam CO. While access to the *exo* face of the ring system remains relatively unaffected, C10 prevents nucleophilic attack on the CO from the *endo* face by blocking the ideal "approach vector".[18] The consequence of this is the subject of the next few paragraphs.

The role of the phenylacetylamino substituent in the hydrolysis of penicillin G (**1a**) in water or aqueous buffer solution at various pH values has been discussed at length.[22] Although the situation is not completely straightforward, the importance of benzylpenicillenic acid (**22**) is unambiguous,[22a-c] and in addition, the involvement of 2 - phenylmethyl - 4,2' - (4' - carboxy - 5',5' - dimethylthiazolidinyl)oxazol - 5 - one (**23**) seems only slightly less certain.[22b,c] Subsequent nucleophilic attack on these oxazolones occurs readily (particularly on **23**, we suspect),[23] and where water is the nucleophile, penicilloic acid (**24**)



22 **23**



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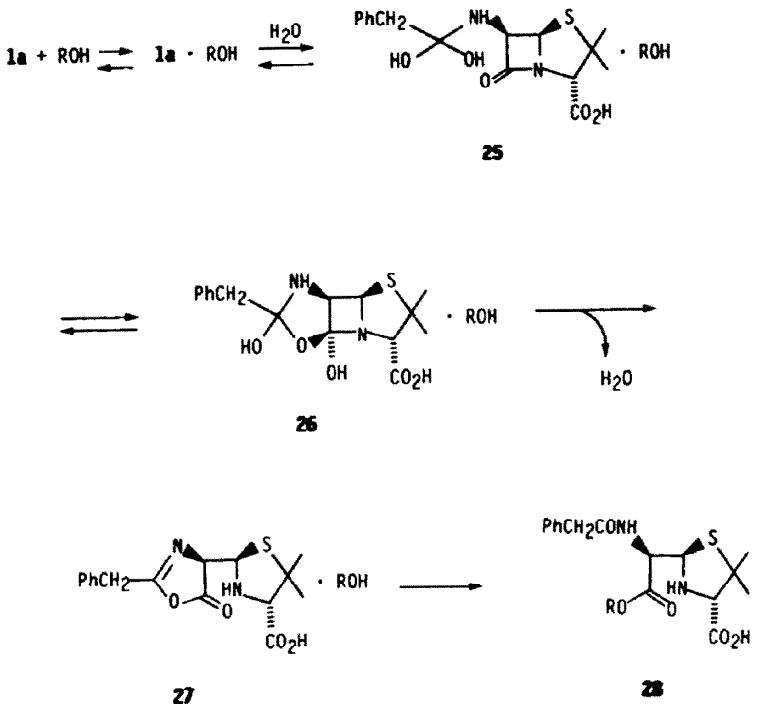
results.[22a-c] Thus, the β-lactam CO of **1a** is activated for further reaction by β-face nucleophilic attack of the amide group in the 6-position, resulting in oxazolone formation. It is quite clear that C10 in the (2,3)-β-methylenepenam analog **21** prevents this activation from occurring by blocking the approach path of the nucleophilic amide O. The result is considerable stability towards hydrolysis for the cyclopropyl analog **2a** when compared to penicillin G (**1a**), a molecule which can assume the "open" conformation with C10 far removed from the site of action (Tables 6 and 7 and Fig. 1).

As one would predict from this, sulbactam **5a** and its cyclopropyl analog **6a**, both of which have no acylamino substituent, are much more similar in their stability towards hydrolysis than **1a** and **2a** (Table 6). Furthermore, each is more stable than penicillin G (**1a**). The fact that they are both more reactive than the (2,3)-β-methylenepenam **2a** probably reflects the inductive effect of the sulfone on the β-lactam.

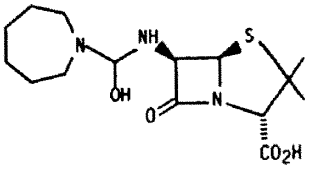
We suggest that *activation of the β-lactam carbonyl is of paramount importance also for antibacterial activity*. Thus, penicillin G (**1a**) which can assume the "open" conformation is a potent antibacterial while the analog **2a** with its "approach vector" blocked, has greatly reduced activity (Table 1). It may even be that the remaining activity is due to competitive inhibition by **2a** of the penicillin sensitive enzymes (PSEs).

We further suggest that an attractive mechanism which depicts the interaction of a penicillin with its enzyme target is shown in Scheme 2 (where ROH is a PSE). Thus, hydration of the amide side chain of the antibiotic leads to an intermediate **25** in which a newly generated nucleophilic function is ideally lo-



Scheme 2.

cated for intramolecular attack on the β-lactam CO. Further transformation of **26** to a highly reactive oxazolone **27**[24] sets the stage for the irreversible acylation of the PSE, as in **28**. The amidino β-lactams, mecillinam (**3**) and its cyclopropyl analog **4**, which also show a large difference in antibacterial potency, fit the above hypothesis in an analogous manner, involving a hydrated intermediate of type **29**. Of course activation by oxazolone formation



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could as well be achieved by attack of an enzyme-bound nucleophile on the amide or amidino side chain.

In contrast to published reports,[20,21] our results indicate that the "open" penicillin conformation **19** is the biologically active one. Our results also indicate, that in spite of significant homologies,[25] there are basic differences in the way penicillin sensitive enzymes and β-lactamases handle the β-lactam function.

EXPERIMENTAL

General M.ps were taken on a Kofler hot stage apparatus (Reichert) and are uncorrected. IR spectra were recorded on Digilab FTS 14 spectrophotometer. ^{1}H NMR spectra were obtained on Varian T-60, XL-100 and XL-200 instruments. Chemical shifts are reported in ppm downfield from TMS. Mass spectra were obtained on a CEC-110 mass spectrometer. Rotations were measured on a Perkin–Elmer 141 polarimeter. Elemental analyses were carried out under the supervision of Dr. Scheidl (of our Microanalytical Laboratory).

SYNTHESES

(A) *The preparation of* **4**—*Sequence A, Scheme* 1

2S - (2α,4α,6α,7β) - 7 - [(*Hexahydro* - 1H - *azepin* - 1 - *yl*)*methyleneamino*] - 4 - *methyl* - 8 - *oxo* - 5 - *thia* - 1 - *azatricyclo*[4.2.0.0.^{2,4}]*octane* - 2 - *carboxylic acid* (**4**). (1) A soln consisting of 0.36 mL (2.9 mmol) N-formylhexamethylene imine in 5.0 mL $CHCl_3$ (filtered through Alumina Woelm® B-Super I) was cooled to −15° with stirring under an atmosphere of argon. To this soln was added dropwise 0.25 mL (2.9 mmol) oxalyl chloride. Effervescence was noted during the addition. The resultant soln was stirred at −10° for 1 hr and then used in Step 2.

(2) A suspension consisting of 0.558 g (2.6 mmol) **11**[6] and 8.0 mL $CHCl_3$ (filtered through Alumina Woelm® B-Super I) was stirred under an atmosphere of argon. To the mixture was added dropwise 0.66 mL (5.3 mmol) chlorotrimethylsilane. The resultant mixture was stirred 15 min and cooled to 10°. At this point 0.74 mL (5.3 mmol) Et_3N was added dropwise to the stirred suspension. The resultant soln was allowed to warm to ambient temp and stirred for 15 min. It was then cooled to −65° in a dry ice/acetone bath and the soln from step 1 was added dropwise *via* syringe. During the addition the external bath was kept at −65° to −60°, while the internal temp rose to −50°. After the addition was complete, 0.74 mL (5.3 mmol) Et_3N was added to the mixture, and the resultant soln stirred at −65° for 1 hr. The mixture was allowed to warm to −10° and concentrated with exclusion of moisture on a rotary evaporator and then under high vacuum. The solid obtained was triturated under argon with 2 × 20 mL of ether (passed through Alumina Woelm® B-Super I). To the combined ether filtrates was added 10 mL water and the two layers

were shaken vigorously. They were separated and the ether layer extracted with a second 10 mL portion of water. The combined water extracts were placed on a rotary evaporator to remove traces of ether, filtered, and freeze-dried. The resultant solid was slurried with ether and collected by filtration to yield 0.456 g of the title compound: IR (KBr) 3500–2500 (broad), 1780, 1693, 1630, 1600 cm^{-1}; NMR (D_2O) δ 8.37 (s, 1H, –CH=N–), 6.75 and 5.74 (AB quartet, 2H, J = 4 Hz, H6 and H7), 3.8–4.2 (m, 4H, 2 × CH_2N), 2.57 (AB quartet, 2H, J = 12 Hz, cyclopropyl CH_2), 1.9–2.4 (m with s at 2.08, 11H, –$(CH_2)_4$– and CH_3–).

Attempts to further purify this compound were not successful.

(B) *The preparation of* **6a**—*Sequence B, Scheme* 1

2S - (2α,4α,6α,7α) - 7 - *Bromo* - 4 - *methyl* - 8 - *oxo* - 5-*thia* - 1 - *azatricyclo*[4.2.0.$0^{2,4}$] - *octane* - 2 - *carboxylic acid diphenylmethyl ester*. A soln consisting of 1.74 g (8.1 mmol) of **11**,[6] 4.56 g (44.3 mmol) NaBr and 22 mL 2N H_2SO_4 was cooled to −5°. To this was added dropwise 0.954 g (13.8 mmol) $NaNO_2$ in 5 mL water. The reaction was stirred at 0° for 30 min, allowed to warm to 15°, and extracted with two 30 mL portions CH_2Cl_2. The combined CH_2Cl_2 extracts were dried over Na_2SO_4, filtered, and the filtrate treated with a slight excess freshly prepared diphenyl diazomethane in CH_2Cl_2. After stirring for 15 min, the reaction was concentrated on the rotary evaporator to yield an amber foam.

The foam was chromatographed on silica gel 60 (70–230 mesh) using EtOAc (1)/cyclohexane(9) to elute. The appropriate fractions were combined and concentrated to yield 0.86 g (24%) of the title compound as a yellow foam: NMR ($CDCl_3$) δ 7.3 (s, 10H, 2 Ph–), 6.91 (s, 1H, $(Ph)_2CH$–), 5.93 (d, 1H, H6), 4.53 (d, 1H, H7), 2.35 and 2.05 (AB-quartet, 2H, CH_2), 1.53 (s, 3H, CH_3–).

Diphenyl diazomethane. A pressure bottle was charged with 5.88 g (30 mmol) benzophenone hydrazone, 12.0 g of activated MnO_2 and 60 mL CH_2Cl_2. After shaking for 6½ hr, the mixture was filtered and the deep wine-red filtrate, which contained the diphenyl diazomethane, was stored under argon at 10° until used.

2S - (2α,4α,6α,7α) - 7 - *Bromo* - 4 - *methyl* - 8 - *oxo* - 5-*thia* - 1 - *azatricyclo* - [4.2.0.$0^{2,4}$]*octane* - 2 - *carboxylic acid* (**13**). A soln consisting of 0.86 g (1.94 mmol) of 7-α-bromo tricyclic β-lactam diphenyl methyl ester and 4.2 mL anisole was cooled to 0° and 22 mL trifluoroacetic acid was added at once. The resultant soln was kept at 0° for 1 hr. The then dark amber soln was concentrated *in vacuo* and the residue chromatographed on silica gel (70–230 mesh). The column was eluted with EtOAc (25)/EAW-632 (1). EAW-632 is a soln consisting of EtOAc (6)/AcOH (3)/water (2). The appropriate fractions were combined and concentrated *in vacuo* to yield 0.494 g (92%) of **13** as a light amber oil: NMR ($CDCl_3$) δ 10.5 (broad absorption, 1H, –CO_2H), 5.93 (d, 1H, H6), 4.58 (d, 1H, H7), 2.4 and 2.1 (AB-quartet, 2H, CH_2), 1.73 (s, 3H, CH_3–).

2S - (2α,4α,6α) - 4 - *Methyl* - 8 - *oxo* - 5 - *thia* - 1 - *azatricyclo*[4.2.0.$0^{2,4}$] - *octane* - 2 - *carboxylic acid* (**14**). A mixture consisting of 0.494 g (1.78 mmol) of **13**, 0.75 g (8.9 mmol) $NaHCO_3$, 0.5 g 10% Pd/C and 50 mL water was stirred under H_2 at ambient temp and atmospheric pressure for 2 hr. The mixture was filtered and the pH of the filtrate adjusted to 2.0 with 2N HCl. The aqueous soln was extracted twice with 60 mL EtOAc. The extracts were combined, washed with brine, dried over Na_2SO_4, and concentrated *in vacuo* to yield 0.183 g (52%) white solid. Recrystallization from MeOH/ether/petroleum ether gave **14** as a pure, white, crystalline solid: m.p. 153–157°; IR (KBr) 3170, 1775, 1718, 1673 cm^{-1}; NMR ($CDCl_3$) δ 5.87 (q, 1H, H6), 3.34 (q, 1H, –CH_2CO–), 2.71 (q, 1H, –CH_2CO–), 2.19 and 1.91 (AB-quartet, 2H, cyclopropyl CH_2), 1.63 (s, 3H, CH_3–). (Found: C, 48.12; H, 4.59; N, 6.95. Calc for $C_8H_9NO_3S$: C, 48.23; H, 4.55; N, 7.03%).

2S - (2α,4α,6α) - 4 - *Methyl* - 8 - *oxo* - 5 - *thia* - 1 - *azatricyclo*[4.2.0.$0^{2,4}$] - *octane* - 2 - *carboxylic acid* 5,5-*dioxide* (**6a**). A soln consisting of 0.158 g (1 mmol) $KMnO_4$, 0.12 mL (2.2 mmol) AcOH, and 3 mL water was cooled to 0°. To the cooled, stirred mixture was added dropwise at a rate to maintain the temp between 0° and 5° soln consisting of 0.1 g (0.5 mmol) of **14**, 0.042 g (0.5 mmol) $NaHCO_3$ and 2 mL water. The mixture was stirred at 5° for 20 min after addition was complete. Excess permanganate was destroyed by the addition of $NaHSO_3$. The mixture was then filtered through Celite, and the pH of the filtrate was adjusted to 2 with 2N HCl. The aqueous soln was extracted twice with 25 mL portions EtOAc. The combined extracts were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to yield 0.092 g (79%) white solid. Recrystallization from MeOH/ether/petroleum ether gave **6a**: m.p. 177 179°; $[\alpha]_D^{25}$ + 200.6° (*c* 1.0206, MeOH); IR (KBr) 3750, 1770, 1730, 1310 cm^{-1}; NMR ($CDCl_3$) δ 5.12 (q, 1H, H6), 3.45 (q, 1H, –CH_2CO–), 2.98 (q, 1H, –CH_2CO–), 2.17 (AB-quartet, 2H, cyclopropyl CH_2), 1.58 (s, 3H, CH_3–). (Found: C, 41.75; H, 4.04; N, 6.31; S, 13.87. Calc for $C_8H_9NO_5S$: C, 41.56; H, 3.92; N, 6.06; S, 13.87%).

(C) *The preparation of* **8**—Sequence C, Scheme 1

2S - (2α,4α,6α,7β) - 4 - *Methyl* - 8 - *oxo* - 7 - *phenylacetylamino* - 5 - *thia* - 1 - *azatricyclo* - [4.2.0.$0^{2,4}$]*octane* - 2-*carboxylic acid diphenylmethyl ester*. A magnetically stirred soln consisting of 6.25 g (18.8 mmol) of **2** and 325 mL CH_2Cl_2 was treated dropwise with CH_2Cl_2 soln of diphenyl diazomethane until the red color just persisted. The resultant soln was stirred 1 hr, the excess diazo compound discharged by the addition of glacial AcOH, and the mixture concentrated *in vacuo* to yield a thick oil. Chromatography of the residue on a Waters Prep LC/System 500A using two silica gel cartridges and EtOAc (1)/hexane (2) as eluent gave, after concentration of the appropriate fractions and drying of the residue under high vacuum, 12.9 g (85%) of the title compound as a foam. A small portion was crystallized from EtOAc/ether/petroleum ether: m.p. 125–128°; $[\alpha]_D^{25}$ + 202.5° (*c* 0.9922, $CHCl_3$); IR (KBr) 3385, 1788, 1727, 1690, 1665 cm^{-1}; NMR ($CDCl_3$) δ 7.3 (m, 15H, 3 Ph–), 6.89 (s, 1H, Ph_2CH–), 6.17 (d, 1H, J = 5 Hz, H5), 6.14 (broad d, 1H, J = 9 Hz, NH), 5.48 (dd, 1H, J = 5 and 9 Hz, H6), 3.58 (s, 2H, $PhCH_2$–), 2.19 and 1.95 (AB quartet, 2H, cyclopropyl CH_2), 1.49 (s, 3H, CH_3–); mass spectrum, *m/z* 167. (Found: C, 70.10; H, 5.31; N, 5.70; S, 6.43. Calc for $C_{29}H_{26}N_2O_4S$: C, 69.86; H, 5.26; N, 5.62; S, 6.43%).

This material was used directly in the next reaction to make **15**.

2S - (2α,4α,6α,7β) - 4 - *Methyl* - 8 - *oxo* - 7 - (*phenylacetyl*)*nitrosamino* - 5 - *thia* - 1 - *azatricyclo*[4.2.0.$0^{2,4}$]-*octane* - 2 - *carboxylic acid diphenylmethyl ester* (**15**). To a magnetically stirred mixture under argon and consisting of 18.5 g (226 mmol) NaOAc suspended in 85 mL dry CH_2Cl_2 was added at once 108 mL of a 1.05 M soln of N_2O_4 in dry CH_2Cl_2. To prepare the N_2O_4 soln, an amount of the gas (*extremely toxic, insidious*) was condensed under argon in a vessel using ice water for cooling. The collected liquid was loosely stoppered and rapidly weighed. It was then diluted to an appropriate volume with CH_2Cl_2 and used directly for nitrosation.

The resultant stirred mixture was cooled to −5° (ice/acetone) and a soln consisting of 9.7 g (19 mmol) of diphenylmethyl ester described in the last section, and 85 mL dry CH_2Cl_2 was added dropwise so as to keep the temp below 0°. The stirring and cooling were continued and additional portions (total 214 ml) of N_2O_4 soln were added at intervals of 30 min. After addition was complete, the reaction was stirred 30 min and 344 mL sat $NaHCO_3$ aq was added cautiously to destroy the excess N_2O_4, and the layers were separated. The aqueous layer was extracted with 250 mL CH_2Cl_2, and the combined organic layers washed with water, dried over Na_2SO_4 and concentrated *in vacuo* to give 10.2 g yellow foam. This material was crystallized from EtOAc/hexane yielding 8.44 g (82%) of **15** as a yellow powder: m.p. 126–129°; $[\alpha]_D^{25}$ + 200.6 (c 1.0232, $CHCl_3$); IR (KBr) 1808, 1740, 1725 cm^{-1}; NMR ($CDCl_3$) δ 7.1–7.6 (m, 15H, 3 Ph–), 6.92 (s, 1H, $(Ph)_2CH$–), 5.96 and 5.56 (AB

quartet, 2H, J = 4 Hz, H6 and H7), 4.49 (s, 2H, $PhCH_2$–), 2.97 and 2.23 (AB quartet, 2H, J = 7 Hz, cyclopropyl CH_2), 1.50 (s, 3H, CH_3C); mass spectrum, *m/e* 167. (Found: C, 65.86; H, 4.76; N, 7.94; S, 5.83. Calc for $C_{29}H_{25}N_3O_5S$: C, 66.02; H, 4.78; N, 7.96; S, 6.08%).

2S - (2α,4α,6α) - 7 - *Diazo* - 4 - *methyl* - 8 - *oxo* - 5 - *thia* - 1 - *azatricyclo*[4.2.0.0^{2,4}] - *octane* - 2 - *carboxylic acid diphenylmethyl ester* (**16**). A soln consisting of 10 g (19 mmol) of **15**, 2.6 mL (32.2 mmol) pyridine, 0.39 g (3.22 mmol) 4-N,N-dimethylaminopyridine and 375 mL dry THF (passed through a column of basic Alumina-Woelm® B-super 1) was stirred magnetically at ambient temp under argon for 3½ hr. The mixture was concentrated *in vacuo* and the residue chromatographed on a Waters Prep LC/System 500A using two silica gel cartridges and eluting with EtOAc (1)/hexane (11). Concentration of the appropriate fractions gave 3.9 g (53%) of **16** as yellow needles: m.p. ~ 70° dec; IR (KBr) 2100, 1767, 1717 cm^{-1}; NMR ($CDCl_3$) δ 7.3 (m, 10H, 2 Ph–), 6.92 (s, 1H, Ph_2CH–), 6.63 (s, 1H, H6), 2.25 and 1.93 (AB quartet, 2H, cyclopropyl CH_2), 1.52 (s, 3H, CH_3–).

This material decomposes slowly at room temperature.

2S - (2α,4α,6α,7α) - 7 - *Chloro* - 4 - *methyl* - 8 - *oxo* - 5 - *thia* - 1 - *azatricyclo*[4.2.0.0^{2,4}] - *octane* - 2 - *carboxylic acid diphenylmethyl ester*. A soln consisting of 3.01 g (7.68 mmol) of **16** and 60 mL acetone was cooled under argon to 0° with an ice/acetone bath. To the magnetically stirred soln was added dropwise over a 2 min period a soln consisting of 0.65 mL conc HCl in 3 mL acetone. Effervescence was observed, and the resultant soln was stirred at 0° for 30 min. The mixture was poured into 1.2 L EtOAc and the organic soln washed with 100 mL sat $NaHCO_3$ aq, 250 mL brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on a Waters Prep LC/System 500A using two silica gel cartridges and eluting with EtOAc (1)/hexane (10). Concentration *in vacuo* of the appropriate fractions and drying of the resultant residue under high vacuum gave 1.72 g (56%) of the title compound: NMR ($CDCl_3$) δ 7.4 (m, 10H, 2 Ph–), 7.0 (s, 1H, Ph_2CH–), 5.90 and 4.53 (AB quartet, 2H, J = 1 Hz, H6 and H7), 2.35 and 2.1 (AB quartet, 2H, J = 7 Hz, cyclopropyl CH_2), 1.53 (s, 3H, CH_3–).

This material was used as is in the next experiment.

2S - (2α,4α,6α,7α) - 7 - *Chloro* - 4 - *methyl* - 8 - *oxo* - 5 - *thia* - 1 - *azatricyclo*[4.2.0.0^{2,4}] - *octane* - 2 - *carboxylic acid diphenylmethyl ester* 5,5 - *dioxide*. A soln consisting of 1.72 g (4.3 mmol) crude chloro compound (see the previous experiment) and 50 mL $CHCl_3$ was cooled to 0° (ice/acetone). To this cooled, stirred soln was added dropwise over 45 min another soln consisting of 1.75 g (8.6 mmol) *m*-chloroperbenzoic acid (85% pure, Aldrich) in 60 mL $CHCl_3$. After stirring for an additional 45 min, the mixture was poured into 500 mL EtOAc and the resultant soln washed with 3 × 100 mL sat $NaHCO_3$ aq and 100 mL brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to yield 2.52 g foam. Initial spectral and TLC characterization indicated the material was a mixture consisting mainly of the sulfoxide. Therefore, the material was redissolved in 50 mL $CHCl_3$ and stirred at ambient temp for 15 hr with an additional 0.85 g (4.2 mmol) *m*-chloroperbenzoic acid. The reaction was processed as described above and the product chromatographed on a Waters Prep LC/System 500A using two silica gel cartridges and eluting with EtOAc (1)/hexane (10). The appropriate fractions were combined and concentrated *in vacuo* to yield 1.57 g (84%) of the title compound. Precipitation of a small portion from ether/petroleum ether gave a powder: m.p. 58–63°; IR (KBr) 1817, 1743, 1335 cm^{-1}; NMR ($CDCl_3$) δ 7.4 (m, 10H, 2 Ph–), 7.0 (s, 1H, Ph_2CH–), 5.05 and 4.85 (AB quartet, 2H, J = 1 Hz, H6 and H7), 2.53 and 2.32 (AB quartet, 2H, J = 7 Hz, cyclopropyl CH_2), 1.59 (s, 3H, CH_3–); mass spectrum, *m/z* 431, 183, 167. (Found: C, 58.94; H, 4.34; N, 3.35; Cl, 7.90; S, 6.92. Calc for $C_{21}H_{18}ClNO_5S$: C, 58.40; H, 4.20; N, 3.24; Cl, 8.21; S, 7.42%).

The high value for the C analysis and the low values for Cl and S are due to a trace of hydrocarbon impurity present from the method used for purification, and which was very difficult to remove. The crude sulfoxide was used as is in the next step.

2S - (2α,4α,6α,7α) - 7 - *Chloro* - 4 - *methyl* - 8 - *oxo* - 5 - *thia* - 1 - *azatricyclo*[4.2.0.0^{2,4}] - *octane* - 2 - *carboxylic acid* 5,5 - *dioxide* (**8**). To a cooled (−5°–0°) stirred soln under argon consisting of 1.37 g (3.17 mmol) chlorosulfone diphenylmethyl ester (see previous experiment) and 5.85 mL anisole was added 27.5 mL cold trifluoroacetic acid. The resultant soln was stirred at a temp between −5° and 0° for 5 hr. It was then concentrated *in vacuo* and the residue taken up in 100 mL EtOAc. The EtOAc soln was washed with 100 mL brine (1)/water (1) and 2 × 100 mL sat $NaHCO_3$ aq. The combined $NaHCO_3$ extracts were acidified to pH 2 with conc HCl. The aqueous mixture was then extracted with 2 × 100 mL EtOAc. The combined EtOAc extracts were washed with 2 × 100 mL brine, dried over Na_2SO_4 and concentrated *in vacuo*. Drying under high vacuum yielded 0.9 g foam. This material could be precipitated from EtOH (trace)/ether/petroleum ether to yield 0.36 g (43%) of the title compound as a solid: IR (KBr) 3575, 3495, 2600–2500, 1796, 1720, 1337 cm^{-1}; NMR ($CDCl_3$ + d_6-DMSO) δ 5.01 and 4.85 (AB quartet, 2H, J = 1 Hz, H6 and H7), 2.55 and 2.34 (AB quartet, 2H, J = 8 Hz, cyclopropyl CH_2), 1.75 (s, 3H, CH_3–). (Found: C, 37.29; H, 3.77; N, 4.80. Calc for $C_8H_8ClNO_5S \cdot 0.15$ EtOH·0.1 EtOAc: C, 37.13; H, 3.47; N, 4.98%).

The presence of 0.15 mol EtOH and 0.1 mol EtOAc in the microanalytical sample was confirmed by ^{1}H NMR.

(D) *The preparation of* **10** *–sequence D, Scheme* 1

2S - (2α,4α,6α) - 7,7 - *Dibromo* - 4 - *methyl* - 8 - *oxo* - 5 - *thia* - 1 - *azatricyclo*[4.2.0.0^{2,4}] - *octane* - 2 - *carboxylic acid diphenylmethyl ester* (**17**). A stirred soln of 3.9 g (9.96 mmol) of **16** in 100 mL dry CH_2Cl_2 was cooled under argon to −5° in an ice/acetone bath. To the cooled soln was added dropwise 25 mL of 0.31M Br_2 in dry CH_2Cl_2. After TLC indicated that the reaction was complete, the mixture was concentrated *in vacuo* and the residue chromatographed on a Waters Prep LC/System 500A using two silica gel cartridges and eluting with EtOAc (1)/hexane (15). The appropriate fractions were combined and concentrated *in vacuo* to yield 3.3 g (63%) of **17** as a beige foam. Crystallization from EtOAc/ether/petroleum ether gave an analytically pure sample: m.p. 92–96°; $[\alpha]_D^{25}$ + 198.15° (*c* 1.0815, $CHCl_3$); IR (KBr) 1803, 1728, 758, 698 cm^{-1}; NMR ($CDCl_3$) δ 7.3 (m, 10H, 2 Ph–), 6.9 (s, 1H, Ph_2CH–), 6.25 (s, 1H, H6), 2.25 and 1.94 (AB quartet, 2H, cyclopropyl CH_2), 1.5 (s, 3H, CH_3–). (Found: C, 48.27; H, 3.09; N, 2.87; S, 6.37. Calc for $C_{21}H_{17}Br_2NO_3S$: C, 48.21; H, 3.27; N, 2.68; S, 6.13%).

2S - (2α,4α,6α) - 7,7 - *Dibromo* - 4 - *methyl* - 8 - *oxo* - 5 - *thia* - 1 - *azatricyclo*[4.2.0.0^{2,4}] - *octane* - 2 - *carboxylic acid trimethylsilyl ester* (**18**). (1) A soln consisting of 2.35 g (4.99 mmol) of **17** and 8.2 mL anisole was cooled with stirring under argon to −5° in an ice/acetone bath. To the cooled soln was added 40 mL cooled trifluoroacetic acid. The reaction was stirred 5 hr with the temp between 0° and −10°. The trifluoroacetic acid was removed *in vacuo* and the residue dissolved in 200 mL EtOAc. The resultant soln was washed with 150 mL water (1)/brine (1) and extracted with 2 portions (100 mL and 50 mL) sat $NaHCO_3$ aq. The combined aqueous layers were washed with EtOAc and acidified to pH 1 with conc HCl. The acidified soln was extracted with 2 × 100 mL EtOAc. The combined organic extracts were washed with brine and dried over Na_2SO_4. Concentration and drying under high vacuum gave a residue weighing 1.8 g. The material was dissolved in 300 mL ether and the resultant soln stirred with 3.5 g activated charcoal for 3 hr. The mixture was filtered through Celite and the filtrate concentrated and dried under high vacuum to yield 1.37 g (86%) of the free acid.

(2) A soln of 0.393 g (1.1 mmol) dibromo acid and 1.5 mL hexamethyldisilazane in 10 mL $CHCl_3$ (filtered through a column of basic Alumina-Woelm® B-super 1) was heated at reflux with stirring under argon for 2 hr. The reaction was

allowed to cool and was concentrated *in vacuo* with exclusion of moisture to yield **18** as a crystalline solid: NMR ($CDCl_3$) δ 6.23 (s, 1H, H6), 2.18 and 1.85 (AB quartet, 2H, cyclopropyl CH_2), 1.6 (s, 3H, CH_3–), 0.28 (s, 9H, $(CH_3)_3Si$–).

2S - (2α,4α,6α,7β) - 7 - *Bromo* - 4 - *methyl* - 8 - *oxo* - 5-*thia* - 1 - *azatricyclo*[4.2.0.0^{2,4}] - *octane* - 2 - *carboxylic acid* (**10**), as its sodium salt. The ester **18** (0.393 g, 1.10 mmol) was taken up in 6 mL dry toluene and 0.29 mL distilled (b.p. 74–75°/0.1 mm) tri-n-butyltin hydride was added. The soln was heated at 62–66° for 40 min. The mixture was cooled to ambient temp, diluted with EtOAc and the resultant soln washed with water. The organic layer was then concentrated and the residue dissolved in 5 mL MeOH (1)/water (5). To this mixture was added 10 mL of 1% $NaHCO_3$ aq. The mixture was stirred 15 min, concentrated to remove MeOH and washed twice with 20 mL portions EtOAc. The aqueous layer was then freeze-dried. The residue was chromatographed on a column of Diaion HP-20 (40 mL, height 17 cm). The column was developed with water and 3 mL fractions were collected. The appropriate fractions were combined and freeze-dried to yield 42 mg of the Na salt of **10** as a white solid: IR (KBr) 1780, 1603, 1455, 1425, 1405 cm^{-1}; NMR (D_2O) δ 6.22 and 5.38 (AB quartet, 2H, J = 4 Hz, H6 and H7), 2.14 and 1.77 (AB quartet, 2H, J = 7 Hz, cyclopropyl CH_2), 1.62 (s, 3H, CH_3–). (Found: C, 30.67; H, 2.82; N, 4.41. Calc for $C_8H_7BrNO_5SNa \cdot 0.75\ H_2O$: C, 30.63; H, 2.72; N, 4.47%).

In addition to **10**, the compound with both bromines removed and the 7-α-bromo compound were isolated from the Diaion HP-20 column.

X-Ray analyses

The structures of **1b**, **2b**, **5b** and **6b** were solved by X-ray analysis. All four substances are methyl esters and were made routinely by treatment of an EtOAc soln of the corresponding acid with ethereal diazomethane.[26] The X-ray intensity data for all four compounds were measured on a Hilger–Watts diffractometer (Ni-filtered Cu Kα radiation, 0–20 scans, pulse height discrimination).

2S - (2α,5α,6β) - 3,3 - *Dimethyl* - 7 - *oxo* - 6 - *phenylacetylamino* - 4 - *thia* - 1 - *azabicyclo* - [3.2.0]*heptane* - 2 - *carboxylic acid methyl ester* (**1b**). The compound was crystallized from EtOAc/benzene/petroleum ether: m.p. 97–105°; $[\alpha]_D^{25}$ + 170.45 (*c* 0.6700, $CHCl_3$); IR ($CHCl_3$) 3420, 1787, 1751, 1677, 1508 cm^{-1}; NMR ($CDCl_3$) δ 7.2–7.4 (m, 5H, Ph–), 6.08 (broad d, 1H, J = 11 Hz, NH), 5.65 (dd, 1H, J = 5 and 11 Hz, H6), 5.49 (d, 1H, J = 5 Hz, H5), 4.38 (s, H, H2), 3.76 (s, 3H, $-CO_2CH_3$), 3.64 (s, 2H, $PhCH_2$–), 1.44 (s, 6H, 2 CH_3–); mass spectrum, *m/z* 348, 234, 114. (Found: C, 58.53; H, 5.67; N, 8.06. Calc for $C_{17}H_{20}N_2O_4S$: C, 58.60; H, 5.79; N, 8.04%).

Crystals of **1b** are of space group $P2_12_12_1$, a = 7.637 (1), b = 17.224 (4), c = 41.593 (8) Å, and d_{calc} = 1.269 g cm^{-3} for Z = 12 ($C_{17}H_{20}N_2O_4S$, M = 348.42). The size of crystal used for data collection was approx. 0.04 × 0.35 × 0.75 mm; the data were corrected for absorption. Of the 4210 independent reflections for $\theta < 57°$, 3175 were considered to be observed [$I > 2.5\sigma(I)$]. The structure was solved by a multiple-solution procedure[27] and was refined by block-diagonal least squares in which the matrix was partitioned into three blocks. One reflection which was strongly affected by extinction was excluded from the final refinement and difference map. In the final refinement, anisotropic thermal parameters were used for the H atoms and isotropic temp factors were used for the H atoms. The atoms were included in the structure factor calculations but their parameters were not refined. The final discrepancy indices are R = 0.040 and wR = 0.037 for the remaining 3174 observed reflections. The final difference map has no peaks greater than ± 0.2 e A^{-3}.

The unit cell contains three independent molecules not related by crystallographic symmetry. The conformations of the three independent molecules are similar, except for the orientation of the benzyl moeity.

2S - (2α,4α,6α,7β) - 4 - *Methyl* - 8 - *oxo* - 7 - *phenylacetylamino* - 5 - *thia* - 1 - *azatricyclo* - [4.2.0.0^{2,4}]*octane* - 2-*carboxylic acid methyl ester* (**2b**). The compound was crystallized from ether/petroleum ether: m.p. 150–153°; $[\alpha]_D^{25}$ + 260.1° (*c* 1.0400, $CHCl_3$); IR ($CHCl_3$) 3420, 1792, 1732, 1680, 1510 cm^{-1}; NMR ($CDCl_3$) δ 7.2–7.5 (m, 5H, Ph–), 6.13 (m, 2H, NH and H6), 5.45 (dd, 1H, J = 5 and 11 Hz, H7), 3.78 (s, 3H, $-CO_2CH_3$), 3.59 (s, 2H, $PhCH_2$–), 2.18 and 1.93 (AB quartet, J = 9 Hz, cyclopropyl CH_2), 1.63 (s, 3H, CH_3–); mass spectrum, *m/z* 346, 176, 172, 171. (Found: C, 58.74; H, 5.24; N, 8.11; S, 9.28. Calc for $C_{17}H_{18}N_2O_4S$: C, 58.95; H, 5.24; N, 8.09; S, 9.26%).

Crystals of **2b** are of space group $P2_1$, a = 11.759 (5), b = 17.073 (7), c = 9.566 (4) Å, β = 113.67 (3)°, and d_{calc} = 1.308 g cm^{-3} for Z = 4 ($C_{17}H_{18}N_2O_4S$, M = 346.40). The size of the crystal used for data collection was approximately 0.08 × 0.15 × 0.8 mm; the data were corrected for absorption. Of the 2480 independent reflections for $\theta < 57°$, 2218 were considered to be observed [$I > 2.5\sigma(I)$]. The structure was solved by a multiple-solution procedure[27] and was refined by full-matrix least squares. In the final refinement, anisotropic thermal parameters were used for the nonhydrogen atoms and isotropic temperature factors were used for the H atoms. The H atoms were included in the structure factor calculations but their parameters were not refined. The final discrepancy indices are R = 0.039 and wR = 0.041 for the 2218 observed reflections. The final difference map has no peaks greater than ± 0.3 e A^{-3}.

The unit cell contains two independent molecules not related by crystallographic symmetry. The conformations of the two independent molecules are similar.

2S,5R - 3,3 - *Dimethyl* - 7 - *oxo* - 4 - *thia* - 1 - *azabicyclo*[2.2.0]*heptane* - 2 - *carboxylic acid methyl ester* 4,4 - *dioxide* (**5b**). The compound was crystallized from EtOAc/ether/petroleum ether: m.p. 118–120°; $[\alpha]_D^{25}$ + 205.3° (*c* 0.9642, $CHCl_3$); IR ($CHCl_3$) 1802, 1761, 1328, 1120 cm^{-1}; NMR ($CDCl_3$) δ 4.63 (m, 1H, H5) 4.41 (s, 1H, H2), 3.86 (s, 3H, $-CO_2CH_3$), 3.48 (m, 2H, $-CH_2$–), 1.62 (s, 3H, CH_3–), 1.43 (s, 3H, CH_3–). (Found: C, 43.79; H, 5.31; N, 5.65; S, 13.23. Calc for $C_9H_{13}NO_5S$: C, 43.72; H, 5.30; N, 5.66; S, 12.97%).

Crystals of **5b** are of space group $P2_12_12_1$, a = 6.794 (3), b = 10.072 (3), c = 16.447 (4) Å, and d_{calc} = 1.459 g cm^{-3} for Z = 4 ($C_9H_{13}NO_5S$, M = 247.26). The size of the crystal used for data collection was approx. 0.08 × 0.08 × 0.4 mm; the data were corrected for absorption. Of the 909 independent reflections for $\theta < 57°$, 839 were considered to be observed [$I > 2.5\sigma(I)$]. The structure was solved by a multiple-soln procedure[27] and was refined by full-matrix least squares. Five reflections which were strongly affected by extinction were excluded from the final refinement and difference map. In the final refinement, anisotropic thermal parameters were used for the H atoms and isotropic temp factors were used for the H atoms. The H atoms were included in the structure factor calculations but their parameters were not refined. The final discrepancy indices are R = 0.030 and wR = 0.033 for the remaining 834 observed reflections. The final difference map has no peaks greater than ± 0.2 e A^{-3}.

2S - (2α,4α,6α) - 4 - *Methyl* - 8 - *oxo* - 5 - *thia* - 1 - *azatricyclo*[4.2.0.0^{2,4}]*octane* - 2 - *carboxylic acid methyl ester* 5,5 - *dioxide* (**6b**). The compound was crystallized from EtOAc/ether: m.p. 142–144°; $[\alpha]_D^{25}$ + 218.9° (*c* 0.9963, $CHCl_3$); IR ($CHCl_3$) 1807, 1737, 1330 cm^{-1}; NMR ($CDCl_3$) δ 4.81 (m, 1H, H6), 3.84 (s, 3H, $-CO_2CH_3$), 3.3 (m, 2H, $-COCH_2$–), 2.5 and 2.25 (AB quartet, 2H, J = 10 Hz, cyclopropyl CH_2), 1.69 (s, 3H, CH_3–). (Found: C, 44.34; H, 4.46; N, 5.56; S, 13.25. Calc for $C_9H_{11}NO_5S$: C, 44.08; H, 4.52; N, 5.71; S, 13.07%).

Crystals of **6b** are of space group $P2_12_12_1$, a = 7.574 (2), b = 9.972 (4), c = 14.172 (4) Å, and d_{calc} = 1.522 g cm^{-3} for Z = 4 ($C_9H_{11}NO_5S$, M = 245.25). The size of the crystal used for data collection was approximately 0.14 × 0.15 × 0.7 mm; the data were corrected for absorption. Of the 864 independent reflections for $\theta < 57°$, 841 were considered to be observed [$I > 2.5\sigma(I)$]. The structure was solved by a

multiple-solution procedure[27] and was refined by full-matrix least squares. Five reflections which were strongly affected by extinction were excluded from the final refinement and difference map. In the final refinement, anisotropic thermal parameters were used for the non-H atoms and isotropic temp factors were used for the H atoms. The H atoms were included in the structure factor calculations but their parameters were not refined. The final discrepancy indices are R = 0.033 and wR = 0.042 for the remaining 836 observed reflections. The final difference map has no peaks greater than ± 0.3 e A^{-3}.

Apparent pKa determinations

The apparent pKa's of **1a** and **2a** (as their K salts) were determined by potentiometric titration with 1N HCl in 50% EtOH/water soln. The apparent pKa of **1a** was determined to be 4.17 and **2a**, 4.26. Similar results were obtained by titrating the free acids with 0.5 N KOH (**1a**: pKa = 4.05; **2a**: pKa = 4.16).

The apparent pKa's of **5a** and **6a** were determined by potentiometric titration with 0.1 N KOH in 66% N,N-dimethylformamide/33% water soln. The apparent pKa's of **5a** and **6a** were both found to be 4.3.

Stability determinations of **1a**, **2a**, **5a** *and* **6a**

Deuterated "biological buffer solution" was prepared as described.[17] The buffer had pD 7.4. Solns containing ~ 10 μ mol of β-lactam compound (as its K salt) and 0.3 mg of t-BuOH (internal standard) in 0.5 mL of deuterated buffer were kept in NMR tubes at 38°. At appropriate time intervals the tubes were shaken and the spectrum recorded on the XL-200 (100 scans). From the integral ratios between the H-5 (**1a**, **5a**) proton or H6 (**2a**, **6a**) proton and the t-Bu resonance the half-life was determined.

Although product studies were not done, it was shown that the decompositions involved loss of the β-lactam CO. This was done by examining the IR spectra of the freeze-dried NMR samples.

Note added in proof: In a recent publication, N. C. Cohen discusses the geometric requirements which must be met for β-lactams to possess antibacterial activity. As we do, Cohen concludes that **19** is the active conformation of penicillins: N. C. Cohen, *J. Med. Chem.* **26**, 259 (1983).

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