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N-Thiolated Bicyclic and Monocyclic β-Lactams

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This paper is dedicated to the memory of a friend and colleague, Prof. Raymond Castle, for his many contributions to the field of heterocyclic chemistry.

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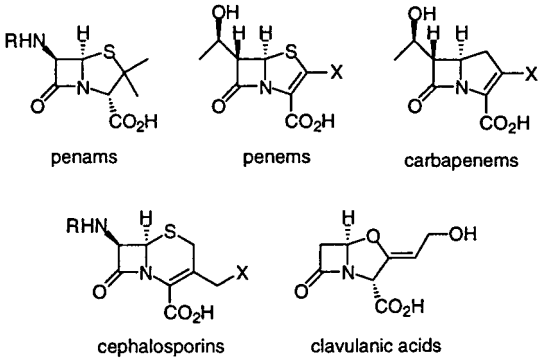
Abstract—In this study, we describe the synthesis and features of β-lactam ring systems having an alkylthio substituent on the lactam nitrogen center. The sulfur group acts to enhance the electrophilic character of the lactam carbonyl through electron withdrawal, and in bicyclic systems, reduces pyramidalization of the nitrogen center. Despite their electrophilic nature, these ring systems are chemically stable towards hydrolysis in aqueous media, but can be cleaved at the N–S bond by reducing agents such as triphenylphosphine. *N*-Methylthio substituted lactams favor a conformation having the sulfur–carbon bond of the SMe group aligned orthogonally with respect to the ring, with a facile interconversion between the cis and trans rotamers. These *N*-methylthio substituted lactams show potent antimicrobial behavior towards *Staphylococcus aureus*, including drug-resistant forms, and are not hydrolyzed by β-lactamases. From the data presented, there is a strong suggestion that these lactams may operate through a chemical and biological mechanism of action that is different from all previous classes of β-lactam drugs.

Introduction

For over 50 years, the β-lactam antibiotics have provided a powerful line of defense against a wide variety of bacterial infections.[3] These potent antibacterial agents have helped control not only the spread of life-threatening illnesses, but have also prevented the onset of opportunistic infections in immune-deficient patients. Among the many different families of β-lactam drugs now known, the vast majority have a fused bicyclic framework. The structures of the most common families are shown below.



The β-lactam antibacterials act by blocking the final step in the biosynthesis of the bacterial cell wall.[4] As a bacterium grows, a series of covalent crosslinks must be formed between adjoining peptidoglycan strands within the cell wall to avoid leakage or catastrophic rupture of the bacterium. These crosslinks are stitched together by transpeptidase enzymes in the cell membrane, through the replacement of a terminal D-alanine unit on one peptidoglycan strand with a glycine residue on a neighboring peptidoglycan chain. The initial cleavage of the D-alanine residue by the transpeptidase occurs by way of a nucleophilic addition of an active site serine onto the amide functionality, in the manner illustrated in Scheme 1. The resulting enzyme-linked peptidoglycan is then converted to the crosslinked material in a subsequent amidation step, which releases the serine for further catalysis.

The β-lactam drugs have an unusual ability to irreversibly block this process by acylating the serine hydroxyl group to create a stable enzyme-drug adduct that is catalytically inactive (see Scheme 2).[5] The end result is that there is a deficiency in the number of crosslinked residues within the cell wall, which makes the bacterium structurally deformed and prone to rupture.

The antimicrobial effects of the β-lactams are related to the location of the lactam nitrogen, which for bicyclic or multicyclic systems is in all cases at the site of ring fusion. This

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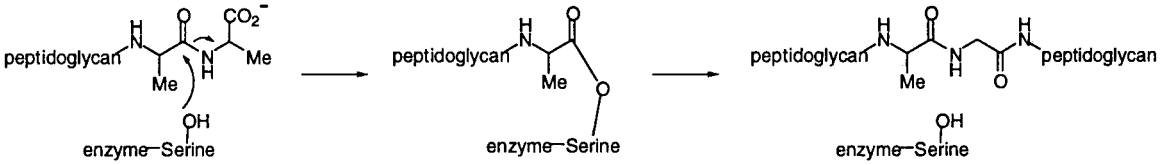
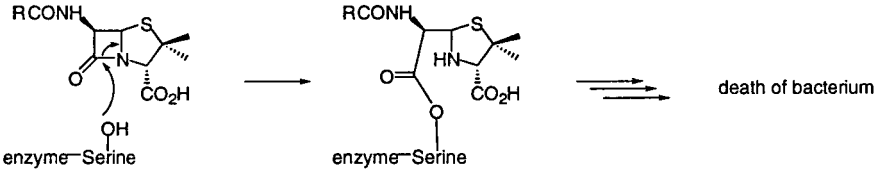


Scheme 1.



Scheme 2.

feature ensures that the nitrogen center is sufficiently pyramidalized to perturb the planarity and resonance stabilization of the lactam functionality. Consequently, the β-lactam ring within these *N*-fused structures has pronounced reactivity toward nucleophilic ring opening. Our laboratory became interested in exploring the properties of bicyclic systems in which the β-lactam ring has been reorganized so that the lactam nitrogen occupies the other site of ring fusion, where it is directly attached to the sulfur atom.[6] This paper describes the results of our computational, synthetic, and microbiological studies on bicyclic, and related monocyclic, β-lactams having an alkylthio group on the lactam nitrogen.

Computational studies

Semi-emperical calculations performed in our laboratories indicate that the N–S fused penam (below) has about the same thermodynamic stability as the classical penam ring.

A similar result is obtained for the two penem isomers (Fig. 1). The N–S fused systems do have slightly less

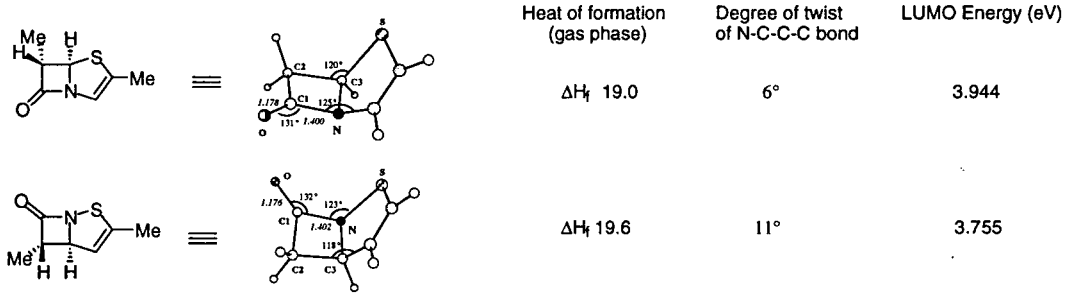


Figure 1.

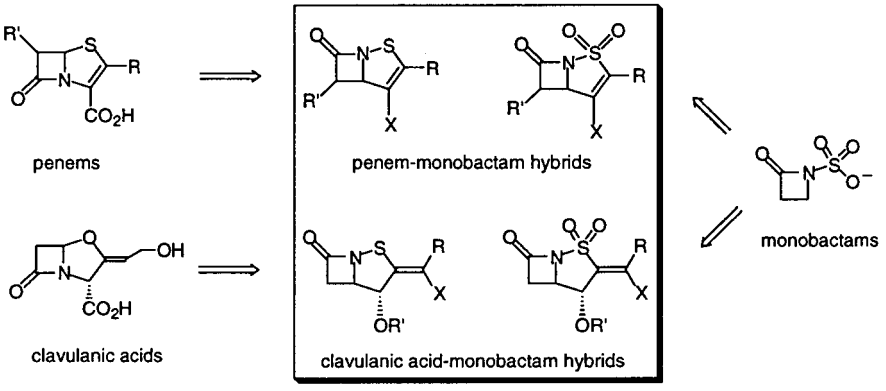
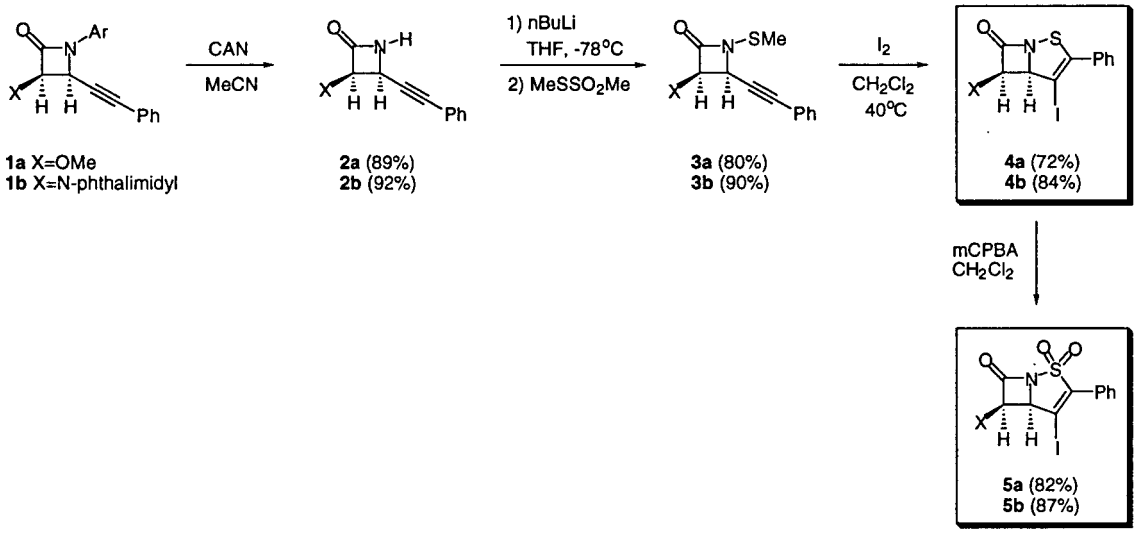


Figure 2.



Scheme 3.

pyramidalization at nitrogen which is reflected by the smaller angle between the rings (125° versus 123°). Ab initio calculations indicate that the N–S fused penem ring is also somewhat more twisted about the N–**C**–**C**–C bond than the normal penem ring (11° versus 6°, respectively), and has a lower LUMO energy. These data are in line with early structural studies[7] on *N*-fused bicyclic lactams which show that the carbonyl stretching frequency within the β-lactam ring increases as a function of increasing ring strain.[7]

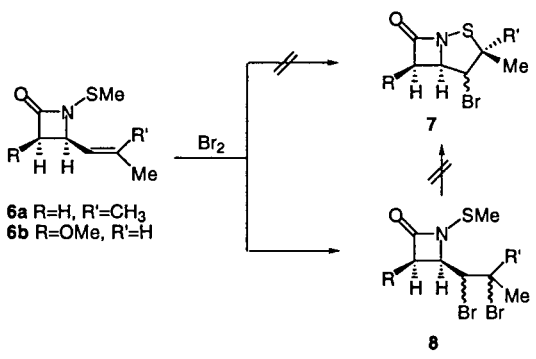
Synthetic studies

In order to study the chemical properties of these N–S fused heterocycles, we developed synthetic approaches to 'inversely fused' penem and clavulanic acid structures shown in the box in Fig. 2. These structures can formally be considered to be hybrids of the penem/monobactam and penem/clavulanic acid families of antibiotics, and offer a bonding arrangement found in only one previously reported β-lactam structure.[8]

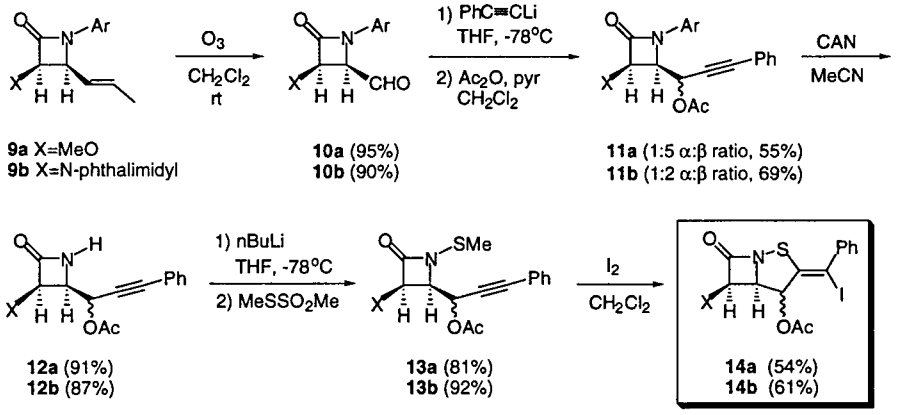
The first types of rings we examined are penem-type compounds **4** and **5**, which could be prepared in a few steps from alkynyl azetidinones **1** (Ar═4-MeOPh).[6] The aryl nitrogen protecting group of lactams **1** could be removed with ceric ammonium nitrate, and reaction of lactams **2** with *n*-BuLi followed by methyl methanethiosulfonate gave the desired *N*-methylthio β-lactams **3** in high overall yield. Despite some initial concerns over the potential difficulty in closing to the 1,2-thiazine ring via a halocyclization process, the reaction of **3** with iodine in refluxing methylene chloride solution proceeded to give penems **4** cleanly. The C═O stretching band near 1790 cm^{-1} in the infrared spectrum of compounds **4** confirmed the powerful electron-withdrawing influence of the sulfur–nitrogen linkage on the lactam carbonyl. These bicyclic sulfenamides can be subsequently oxidized to the sulfonamides **5** whose C═O stretching absorption occurs at around 1800 cm^{-1} (Scheme 3).

The finding that the halocyclization of *N*-acyl sulfenamides **3** occurs only at an elevated reaction temperature (40°C) reflects the increase in ring strain of the bicyclic product. This is illustrated even more dramatically in the halogenation of *alkenyl* sulfenamides **6**, which fail to cyclize but instead yield the olefin bromination products **8** (Scheme 4). These undesired dibromides could not be transformed to the thiazine **7** under any of the conditions we tried.

On the other hand, we were able to construct clavulanic acid-type ring systems **14** using this halocyclization approach. The *N*-methoxythio lactam precursors **13** were prepared in several steps from β-lactam **9**. Thus, ozonolysis of the alkene side chain in **9** gave aldehydes **10**, which enabled the acetylenic side chain to be incorporated by lithium acetylide addition (Scheme 5). The reaction of lithium phenylacetylide with **10** occurs stereoselectively to give the secondary alcohol adducts, which after acetylation afforded acetates **11**. One of the interesting features of the acetylide incorporation was the stereochemistry, which follows from Felkin–Ahn addition (Fig. 3, left). However, in the case of methoxy-substituted aldehyde **10a**, the ratio of α:β acetate products **11a** can be inverted by conducting the acetylide addition in the presence of $MgCl_2$. We speculate that the $MgCl_2$-promoted addition occurs preferentially by approach of the acetylide from the less hindered face of a chelated magnesium complex, with the metal ion being



Scheme 4.



Scheme 5.

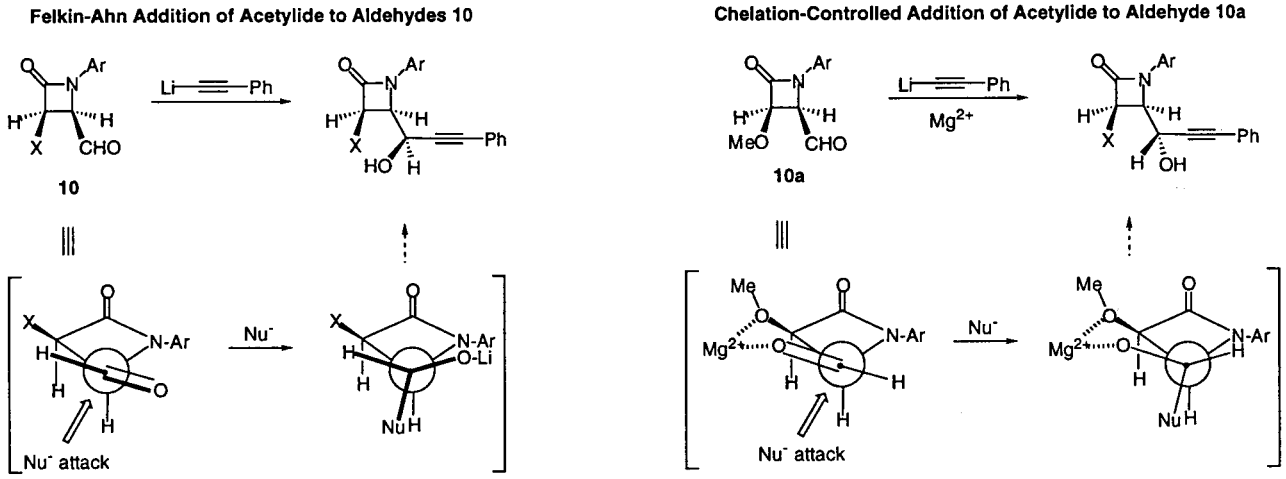
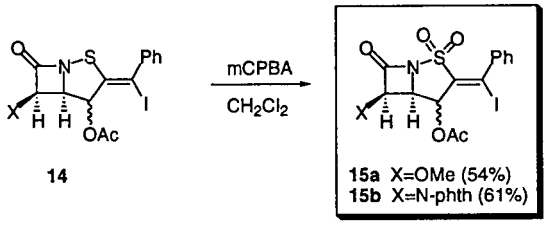


Figure 3.

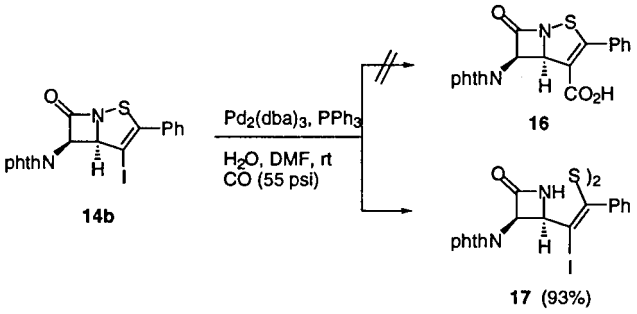
coordinated to the oxygens of the methoxy and aldehyde moieties (Fig. 3, right).

Deprotection of the *N*-aryl group of **11** and attachment of the methylthio moiety led to iodocyclization substrate **13**, which underwent the anticipated ring closure to **14** with iodine at room temperature. The six contiguous and uniquely heterosubstituted carbon centers within the backbone of **14** is a highly unusual pattern. The electrophilicity of the carbonyl center in **14**, which is enhanced ($\upsilon_{C=O}$ 1780 cm^{-1}) by having the nitrogen center at the site of ring fusion and bonded to the sulfur atom, can be further elevated by oxidation to sulfonamides **15** ($\upsilon_{C=O}$= 1800 cm^{-1}) (Scheme 6).

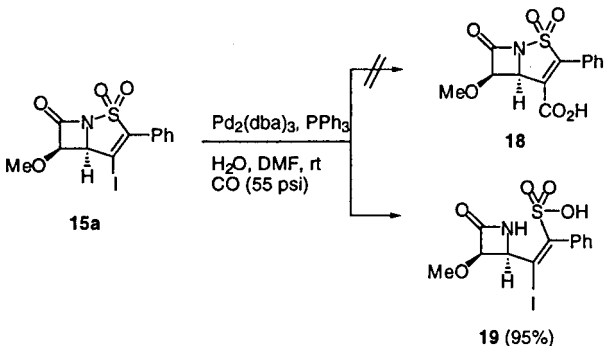


Scheme 6.

Despite their highly electrophilic nature, all of the N–S fused bicyclic lactams we prepared (**4**, **5**, **14**, and **15**) are highly resistant to hydrolysis over a wide pH range (pH 1 to pH 10) and can be easily purified by silica gel chromatography. However, the nitrogen–sulfur bond is prone to rupture under certain conditions. For instance, attempts to execute palladium-catalyzed Stille and carbonylation reactions to exchange the iodide in **14b** for other side chains gave only the ring-opened disulfide **17** (Scheme 7). This product arises from reductive cleavage of the nitrogen–sulfur ring bond by PPh_3 in the media. Sulfonamide derivative **15a** experienced the same fate, yielding the ring-opened



Scheme 7.



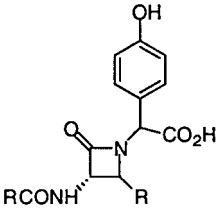
Scheme 8.

sulfinic acid **19** while trying to prepare carboxylic acid **18** (Scheme 8).

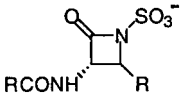
Each of the β-lactam compounds in this study were screened for antimicrobial properties against a variety of common strains of Gram-positive and Gram-negative bacteria. None of the bicyclic compounds showed any appreciable antibacterial effects. It is not clear whether more highly functionalized ring derivatives would possess biologically activity, but we decided to not pursue this question further. Instead, our interest became focused on the *N*-methylthio monocyclic lactams **3a** and **6b**, which during routine testing were found to exert powerful growth inhibition properties against *Staphylococcus aureus*. This unexpected observation prompted us to explore these monocyclic compounds in more detail.

Studies on monocyclic β-lactam antibiotics

In the late 1970s and early 1980s, the first classes of monocyclic β-lactam antibacterial agents were isolated from natural sources. The discovery of the nocardicins[9] and monobactams[10] demonstrated for the first time that β-lactams do not require a conformationally constrained bicyclic structure to have antibacterial properties.

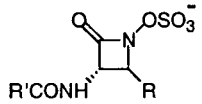


nocardicins

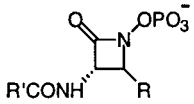


monobactams

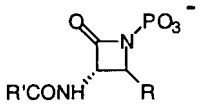
Following these reports, subsequent investigations were carried out on monocyclic lactams having various types of anionic heterosubstituents on the nitrogen center (see structures below).[11]



N-sulfato lactams



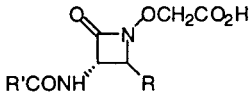
N-phosphato lactams



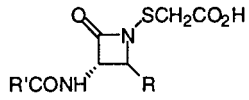
N-phosphono lactams

These synthetic analogues were observed to display a broad spectrum of activity against aerobic Gram-negative bacteria but little or no activity against Gram-positives such as *Staphylococcus aureus*.

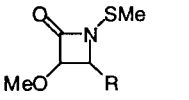
In 1985, Marvin Miller's laboratory at Notre Dame compared the oxamazins, monocyclic β-lactams having an OCH_2CO_2H group on the lactam nitrogen, to their sulfur analogues, the thiamazins.[12] While the oxamazins are strong antimicrobial agents, the thiamazins are devoid of antibacterial activity.



oxamazins
(active)



thiamazins
(inactive)



N-thiomethoxy lactams
(active)

This remarkable difference in biological properties appears to be due to the longer N–S bond compared to the N–O bond of the oxamazins, which prevents a proper fit of the thiamazin lactam within the active site of the transpeptidase enzyme. Miller has also pointed out that the carbonyl stretching frequencies of the oxamazins are consistently 10–20 cm^{-1} higher than those of the thiamazins, falling in the range of other active β-lactams such as the penicillins, cephalosporins, and monobactams.

Until now, antibacterial activity in β-lactams has only been associated with those compounds that have an ionizable group on the nitrogen that can lie within 3.6 Å of the β-lactam carbonyl carbon. Given this well-known structural requirement, it is totally surprising that our *N*-methylthio lactams possess potent antimicrobial activity, and that this activity is *Staphylococcus*-specific. In fact, the striking similarity our lactams have in structure to the *biologically-inactive* thiamazins would suggest that they should be incapable of having any antibacterial properties at all. The β-lactam drugs are typically employed as water-soluble salts, which enables them to act on the outer periphery of the cell. Our *N*-methylthio lactams on the other hand are highly lipophilic and have limited solubility in aqueous media. Like the thiamazins previously described by Miller, *N*-methylthio lactams **3** and **6** are stable from pH 1–10.[12] These compounds fail to react with glutathione, NaN_3, NaCN, or alcohols, and are impervious toward enzymatic degradation by penicillinases under conditions in which penicillin G is rapidly hydrolyzed. Each of the *N*-methylthio β-lactams are also stable

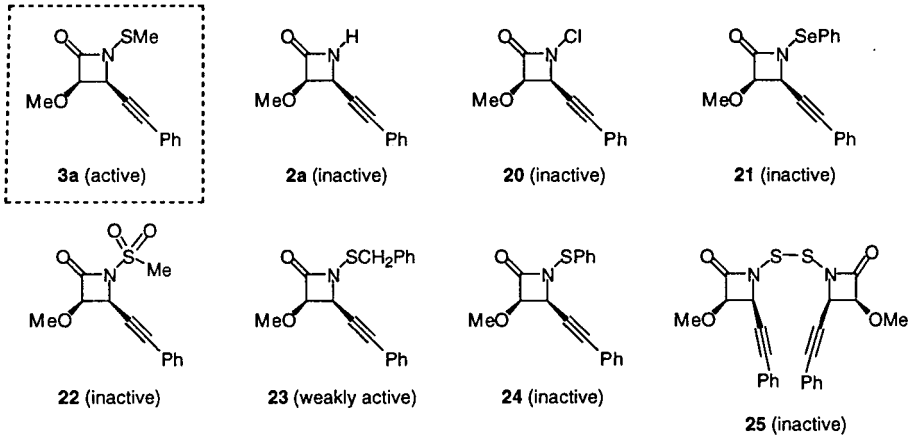


Figure 4.

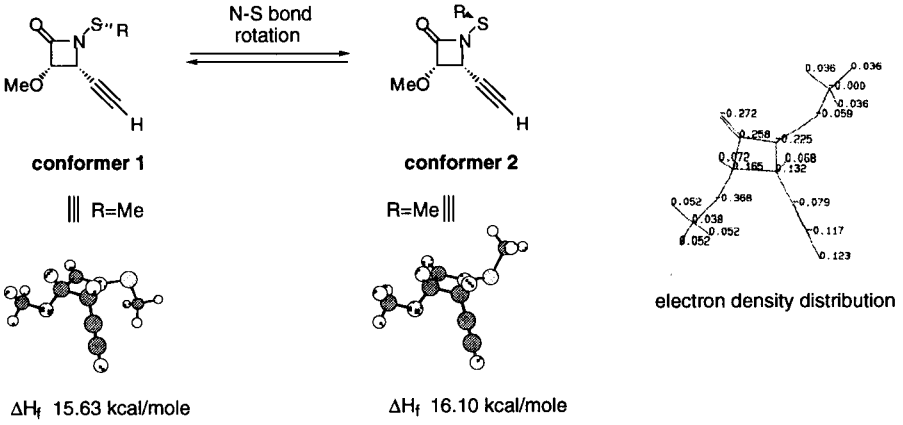
during column chromatography. All of these findings provide grounds for us to propose that our *N*-methylthio lactams are not functioning in the same manner, or even on the same group of bacterial enzymes, as the known β-lactam drugs.

We are now trying to understand how the ring substituents affect antimicrobial behavior of these lactams. From N–H compound **2**, a number of *N*-substituted analogues (**20**–**25**) (Fig. 4) were prepared to explore the importance of the *N*-methylthio group on activity. Upon screening for antimicrobial susceptibilities of these compounds, it became clear that any changes to the S-methyl group results in loss of antibacterial properties. The N–H compound is completely inactive, as are the chloro (**20**), Se-phenyl (**21**), methanesulfonyl (**22**), S-phenyl (**24**), and disulfide (**25**) derivatives. Only the S-benzyl compound **23** shows any antimicrobial activity, which is sharply reduced relative to that of lactam **3a**. Therefore, the *N*-methylthio substituent is required for biological activity.

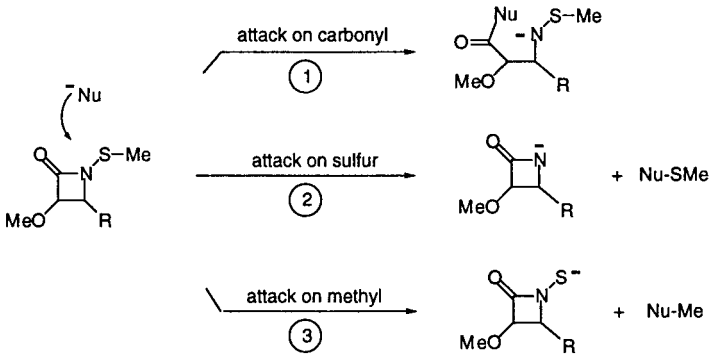
Conformational studies of *N*-alkylthio lactams

To gain a better grasp on the chemical behavior of these new monocyclic lactams, computational studies were performed to assess the conformational biases of the *N*-methylthio moiety. It is known that sulfenamides can have significant barriers to rotation around the sulfur–nitrogen single bond, generally in the range of 9–23 kcal/mol.[13] Miller and his colleagues have observed this behavior in their studies on the thiamazins, which have a barrier to sulfur–nitrogen bond rotation as high as 12 kcal/mol.[12] The sulfur–carbon bond of the S-acetic acid group prefers to lie orthogonal to the ring atoms in both of the ground state rotameric forms. Low temperature NMR experiments indicated that both conformers are present in solution at −20°C. In the case of our SMe lactams, ab initio studies reveal that the sulfur–carbon bond of the *N*-thiomethoxy group also prefers to be oriented orthogonally to the ring, with the '*cis*' rotamer being about 1 kcal/mol more stable than the '*trans*' form. The barrier for *cis*–*trans* rotamerization is very small, less than 8 kcal/mol. This low barrier to N–S bond rotation was confirmed by variable temperature proton NMR studies of compound **3a**, which show a single average solution structure at temperatures as low as −50°C. The computed electron density distribution for the '*trans*' rotamer is shown (Scheme 9), suggesting that the sulfur and carbon centers in the S-methyl group are both electroneutral in character.

We are now extending these computations to predict how a potential nucleophile (such as an enzyme) might react with



Scheme 9.



Scheme 10.

the *N*-methylthio lactam. These structures differ from normal β-lactams in that there are at least three sites for nucleophilic attack: (1) addition to the β-lactam carbonyl, (2) substitution on the thiomethyl sulfur center, or (3) S_N2 displacement at the thiomethyl carbon atom (Scheme 10). Of these processes, only the first pathway (ring opening) is precedented in the β-lactam literature. Experiments to deduce whether the compounds are operating through one of these modes are underway.

Experimental

Procedures for the preparation and purification of lactams **1**–**6**, **8**–**15**, **17**, **19**, and disulfide **25** are previously described.[6a]

General procedure for the preparation of *N*-derivatized β-lactams 20–24

To a solution of **2a** (1 mmol) in 25 mL of anhydrous THF at −78°C is added a 1.6 M solution of *n*-butyllithium in hexanes (1 mmol). After 30 min, a solution of the electrophile (6.6 mmol) in 10 mL of THF is added and the reaction mixture is stirred for 12 h with warming to room temperature. The mixture is poured into 5% aqueous NH_4Cl (50 mL) and extracted with CH_2Cl_2 (3×50 mL). The combined organic layers were dried over $MgSO_4$, filtered, and evaporated. Flash chromatography of the crude mixture afforded the *N*-derivatized lactam (except for the N–Cl derivative **20**, which due to its high reactivity was used for testing immediately without isolation or further purification).

21. 0.14 g (95%); colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71 (m, 2H), 7.31 (m, 8H), 4.85 (d, J=4.5 Hz, 1H), 4.69 (d, J=4.5 Hz, 1H), 3.60 (s, 3H). IR (thin film) 1750 cm^{-1} (β-lactam C=O). ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.9, 133.5, 132.8, 132.6, 130.2, 129.8, 129.5, 128.9, 122.7, 89.8, 87.6, 83.0, 59.2, 54.5.

22. 0.25 g (99%); colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, J=7.2 Hz, 2H), 7.34 (m, 3H), 5.13 (d, J=4.8 Hz, 1H), 4.84 (d, J=4.8 Hz, 1H), 3.63 (s, 3H), 3.27 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.0, 132.8, 130.3, 129.2, 124.5, 90.8, 85.2, 80.0, 60.0, 52.6, 43.6. Anal. calcd for $C_{13}H_{13}NO_4S$: C, 55.90; H, 4.69. Found: C, 55.55; H, 4.73.

23. 0.35 g (97%); colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (m, 2H), 7.32 (m, 8H), 4.61 (d, J=4.3 Hz, 1H), 4.34 (d, J=12.2 Hz, 1H), 4.19 (d, J=4.3 Hz, 1H), 3.99 (d, J=12.2 Hz, 1H), 3.54 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.8, 135.9, 132.6, 130.2, 129.7, 129.5, 129.3, 129.1, 128.6, 89.7, 86.7, 82.6, 59.3, 56.1, 43.5. Anal. calcd for $C_{19}H_{17}NO_2S$: C, 70.56; H, 5.30. Found: C, 70.83; H, 5.54.

24. 0.18 g (67%); colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (d, J=7.2 Hz, 2H), 7.28 (m, 8H), 4.82 (d, J=4.8 Hz, 1H), 4.74 (d, J=4.8 Hz, 1H), 3.61 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.8, 136.5, 134.5, 132.5, 129.9, 129.6, 129.0, 128.9, 122.5, 90.4, 87.0, 82.0, 59.5, 56.0. Anal. calcd for $C_{18}H_{15}NO_2S$: C, 69.88; H, 4.89. Found: C, 70.13; H, 5.07.

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