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Dipolar Cycloaddition of Novel 6-(Nitrileoxidomethyl) Penam Sulfone: An Efficient Route to a New Class of β-Lactamase Inhibitors

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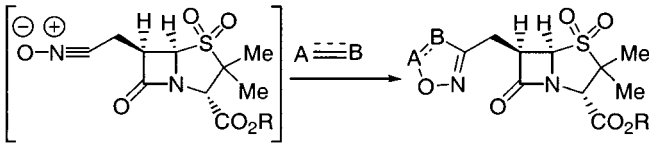
ABSTRACT



6-(Nitrileoxidomethyl) penam sulfone intermediate was prepared in a few steps starting from commercially available (+)-6-aminopenicillanic acid. This intermediate underwent smooth 1,3-dipolar cycloaddition reactions with various alkenes and alkynes to give cycloadducts in moderate to good yields. By this new method, several potent β-lactamase inhibitors were synthesized. The regio- and stereoselectivity outcomes of the cycloaddition process are also discussed.

The emergence of wide-spread resistance to antibacterial drugs in recent years has created an upsurge of activity in this area.[1] Production of β-lactamase enzymes by microbes is the most common mechanism of resistance to β-lactam antibiotics.[2] These enzymes efficiently hydrolyze the amide bond of the β-lactam ring to give products that are devoid of antibacterial activity. Therefore, the administration of a β-lactamase inhibitor along with an antibiotic represents an important strategy for combating the resistance against these drugs.[3]

Sulbactam (**1**)[4] and tazobactam (**2**)[5] (Figure 1) are commercially available β-lactamase inhibitors currently being used with an antibiotic in this context. These inhibitors have potent activity against class A enzymes but are not effective inactivators of class B and C enzymes.[5] These inhibitors have limited use due to considerable turnover of the substrate before irreversible inactivation of the enzyme occurs. Even though the mechanism of β-lactamase catalysis is not fully understood,[6] it has been shown that the penam sulfone **1**

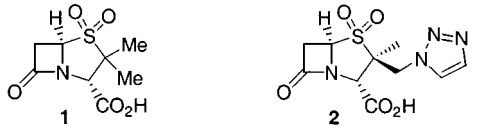


Figure 1. Commercially available β-lactamase inhibitors.

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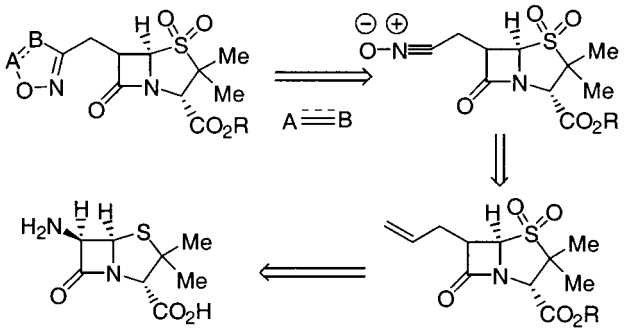
reacts as a branched chain inhibitor with the TEM β-lactamase enzyme from *Escherichia coli*.[7]

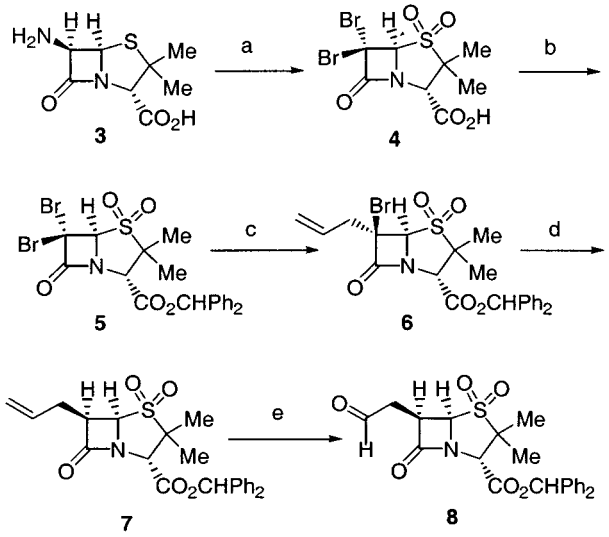
In connection with our interest in finding a broad-spectrum β-lactamase inhibitor, introduction of a novel side chain adjacent to the β-lactam carbonyl was considered. Molecular modeling studies of the above known inhibitors with several β-lactamase enzyme structures revealed that there is ample space in the active site to accommodate larger substituents at the C6-position of these compounds. Therefore, a properly introduced C6-side chain might improve the binding to the active site of the enzyme. This qualitative binding may slow the hydrolysis of the β-lactam nucleus and thereby increase the inhibitory activity. The design was to introduce an isoxazole or isoxazoline heterocycle at the 6-position of the β-lactam ring via a methylene bridge. Several groups have studied such modifications to the β-lactam core structure.[8] In most cases, the desired side chain was prepared and then coupled with the β-lactam ring in an aldol-type transformation or by employing the Wittig reaction. Herein, we report an efficient methodology to introduce isoxazole and isoxazoline heterocycles to the penam sulfone nucleus via a common intermediate (Scheme 1).

Scheme 1



The target molecules in the present study are derived from 1,3-dipolar cycloaddition of the corresponding penam sulfone nitrile oxide with the appropriate alkene or alkyne (Scheme 1). The precursor to the key intermediate nitrile oxide comes from the ozonolysis of the corresponding allylpenicillanate sulfone, which was prepared from the 6-aminopenicillanic acid (Scheme 2).[9] This strategy allowed us to employ a large arsenal of commercially available alkenes and alkynes for the cycloaddition process. The dipolar cycloaddition occurs with diversely substituted alkene and alkyne partners,[10] which allows us to build a comprehensive SAR profile of these compounds against the β-lactamase enzymes.

Scheme 2[a]



[a] Reagents and conditions: (a) (i) $Br_2/NaNO_2/H_2SO_4$; (ii) $KMnO_4/H_3PO_4$ (for two steps 56%); (b) Ph_2CHN_2/acetone (95%); (c) allyl tribytyltin/AIBN/toluene (72%); (d) Bu_3SnH/AIBN/toluene (75%); (e) $O_3/CH_2Cl_2/Me_2S$ (85%).

To this end, amino acid **3** was converted to dibromide **4** by following a procedure described by Volkman et al.[11] in 56% overall yield. Acid **4** was protected using diphenyldiazomethane to give compound **5**. Radical-mediated C-allylation with allyltributyltin followed by tin hydride reduction in the presence of a catalytic amount of AIBN gave compound **7** as the sole diastereoisomer.[8a,9] Under these conditions, tin reagents approach from the less-hindered 6α-face to effect the observed stereochemistry. Ozonolysis of compound **7** at −78 °C led to the desired aldehyde **8** in excellent yield.

Having obtained the aldehyde, the stage was set to carry out the key nitrile oxide formation and the subsequent dipolar cycloaddition reactions. An initial attempt to convert aldehyde **8** to aldoxime **9** using Et_3N gave a poor yield of **9**. However, treatment of compound **8** with hydroxylamine salt in the presence of sodium acetate gave aldoxime **9** in 90% yield. While compound **9** was chemoselectively formed, it was a mixture of *E* and *Z* isomers. Standard chlorination of **9** with *N*-chlorosuccinamide[12] gave hydroxamoyl chloride **10** as a single (*E*)-isomer in 83% yield (Scheme 3).

Initial attempts to effect dipolar cycloadditions using Et_3N as the base gave reasonable yields of cycloadducts only with methyl acrylate and phenyl vinyl sulfone. Poor yields were obtained especially with alkynes. Given the opportunity, the putative nitrile oxide intermediate can undergo dimerization and other potential decomposition pathways.[10] Therefore, it is possible that the basicity of the amine and the lower reactivity of the alkynes led mostly to the decomposition of

(6) For a comprehensive account on the mechanism, see; Silverman, R. B. In *Mechanism-Based Enzyme Inactivation: Chemistry and Enzymology*; CRC press: Boca Raton, 1988.

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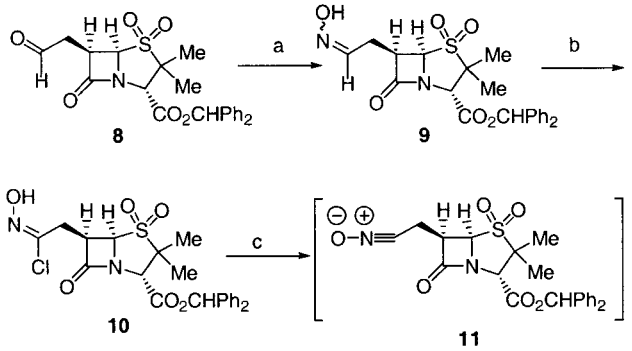
Scheme 3[a]



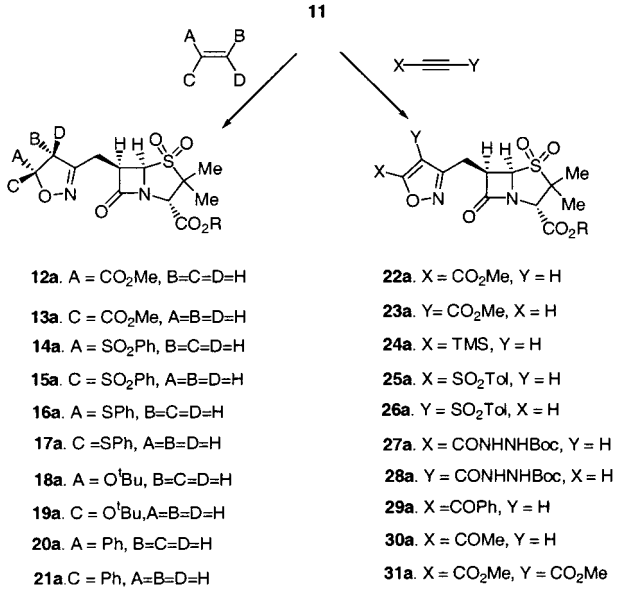
[a] Reagents and conditions: (a) $NH_2OH{\cdot}HCl/NaOAc/EtOH/H_2O$ (90%); (b) NCS/DMF (83%); (c) $(Bu_3Sn)_2O$/benzene.

11. In contrast, bis(tributyltin)oxide, a neutral reagent,[13] was found to be a superior reagent for dehydrochlorination, giving better yields of cycloadducts in almost all cases. For example, when methyl propiolate was used as the trapping agent, the corresponding cycloadduct was obtained in 56% yield with bis(tributyltin)oxide as opposed to 20% yield with Et_3N.

Thus, the treatment of nitrile oxide **11** with a series of alkenes and alkynes gave the corresponding dipolar cycloadducts in moderate to good yields regardless of their electronic nature.[14] Reaction of **11** with methyl acrylate gave the isoxazoline cycloadducts in 50% yield as a 1:1 mixture of diastereoisomers **12a** and **13a**. As expected, no regioisomers were formed. A similar reactivity pattern was observed with phenyl vinyl sulfone to give the diastereoisomeric cycloadducts **14a** and **15a** in 52% yield. The cycloaddition proceeded smoothly with electron-rich dipolarophiles such as phenyl vinyl sulfide and *tert*-butyl vinyl ether, giving the corresponding cycloadducts in 60% and 68% yields, respectively. In both cases, mixtures of diastereoisomers at the 5-position of the isoxazoline ring were obtained with complete regioselectivity. Styrene also participated well in this process, giving the cycloaddition products in 54% yield as a 1:1 mixture of diastereoisomers, **20a** and **21a** (Scheme 4).

Although the alkenes used in this process gave 5-substituted isoxazoline penam sulfones with complete regioselectivity, certain alkynes gave both 5- and 4-substituted isoxazole derivatives. The cycloaddition reaction between methyl propiolate and the dipole **11** gave a 6:1 mixture of regioisomeric products in 56% yield. In this case, 5-isoxazole isomer **22a** was the major one. In contrast, when *p*-tolyl ethynyl sulfone was used as the trapping agent, the major isomer obtained was the 4-isoxazole substituted penam sulfone **26a** in a 2:1 ratio. The assigned regiochemistry of the products follows from their ^1H NMR spectra. The relative positions of the isoxazole ring hydrogens of the regioisomeric products **25a** and **26a** are well separated with an ~2 ppm chemical shift value. A similar reaction with *N*′-propynoyl-

Scheme 4[a]



[a] Series a: R = $CHPh_2$ (esters).

hydrazinecarboxylic acid *tert*-butyl ester[15] reagent gave a 1:1 mixture of regioisomers **27a** and **28a**. Methyl and phenyl propargyl ketones as well as TMS−acetylene gave exclusively 5-substituted products **30a**, **29a**, and **24a**, respectively, in good yields. The reaction proceeded smoothly with disubstituted alkyne, DMAD, giving the cycloaddition product **31a** in 52% yield. The regiochemical outcomes observed in these examples can be rationalized by FMO considerations.[16] Depending on the size of the coefficients, a favorable HOMO−LUMO interaction between the dipole and the dipolarophile leads to the observed regioselectivity. In general, dipole (LUMO)−dipolarophile (HOMO) interaction seems to be dominant for alkenes whereas both dipole (HOMO) and dipole (LUMO) become important for alkynes depending upon the nature of the substituent. This is apparent from the dramatic selectivity difference between methyl propiolate and *p*-tolyl ethynyl sulfone.

The acid derivatives of some of these compounds were evaluated for their inhibitory activity against three representative β-lactamase enzymes from different enzyme classes (Table 1).[17] First, the benzhydryl esters were converted to the corresponding acids (series b: R = H) by catalytic hydrogenolysis (H_2, Pd−C, EtOAc). The inhibitory activity of the acids were compared with the reference compounds, sulbactam and tazobactam. Our objective was a compound with a sub-micromolar IC_{50} against at least class A and class C enzymes, which are the most common β-lactamase enzymes found in bacteria. Table 1 presents the IC_{50} data

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(17) For details about the assay and experimental procedure, see: Bush, K.; Macalintal, C.; Rasmussen, B. A.; Lee, V. J.; Yang, Y. *Antimicrob. Agents Chemother*. **1993**, *37*, 851.

Table 1. In Vitro Inhibition Data of Selected Compounds

	IC_{50} (nM)[b]		
compd[a]	TEM-1 (class A)	CcrA (class B)	AmpC (class C)
12b	3800	4300	7100
13b	2300	25000	53000
14b	310	1400	6300
15b	31	6300	4800
22b	1000	6000	860
26b	20000	370	9800
29b	660	2200	2500
31b	2700	330	5100
sulbactam	1400	>400000	66000
tazobactam	60	>400000	48000

[a] Series b: R = H (acids). [b] Determined graphically from six different concentrations of the inhibitor.

for the best compounds of this series. Overall, all the compounds showed better activity against CcrA and AmpC enzymes as compared to the reference compounds. Compounds **14b**, **15b**, **22b**, and **29b** are more potent compounds than sulbactam against all three enzymes. Substitution at the 6-position of the isoxazole ring has dramatically increased the CcrA enzyme activity as exemplified by **26b** and **31b**. On the other hand, phenylsulfonyl substitution at the 5-position of the isoxazoline ring gave the most potency against TEM-1 enzyme as is evident from compounds **14b** and **15b**. The β-isomer **15b** was 10-fold more active than the corresponding α-isomer **14b** and showed a 2-fold improved activity as compared to tazobactam against TEM-1 enzyme. Only compound **22b** nearly met our criterion. However, the fact that individual compounds achieved much higher potency compared to the reference compounds suggests that further efforts within this series is warranted.

In summary, a new strategy has been developed to introduce a variety of heterocycles via a novel 6-(nitrileoxidomethyl) penam sulfone intermediate. Some of the compounds made by this methodology showed potent in vitro activity against several β-lactamase enzymes.

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Supporting Information Available: Full experimental details and characterization data for compounds **4**−**31**. This material is available free of charge via the Internet at http://pubs.acs.org.

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