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SYNTHESIS OF CEPHEMS BEARING OLEFINIC SULFOXIDE SIDE CHAINS AS POTENTIAL β-LACTAMASE INHIBITORS

Vittorio Farina,*[1] Sheila I. Hauck, and Raymond A. Firestone

Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, P.O.Box 5100, Wallingford CT 06492-7660 USA

Abstract: Several cephems bearing vinyl sulfoxide and sulfone moieties at C-3 were prepared by the Stille coupling of a 3-trifloxy cephem with stannanes. The sulfoxides were designed to be suicide lactamase inhibitors. None of the cephems described in this study functioned as an enzyme inhibitor, but two derivatives displayed interesting biological activity against Methycillin - resistant *S. Aureus*. Copyright © 1996 Elsevier Science Ltd

Resistance to β-lactam antibiotics is a major clinical problem in antibacterial chemotherapy. [2] A well-understood mechanism of resistance is derived from the ability of bacteria to produce β–lactamases, a class of enzymes that efficiently hydrolyze, and therefore inactivate, β-lactam antibiotics. One of the several possible approaches has traditionally been the administration of a lactamase inhibitor together with the antibiotic. [3]

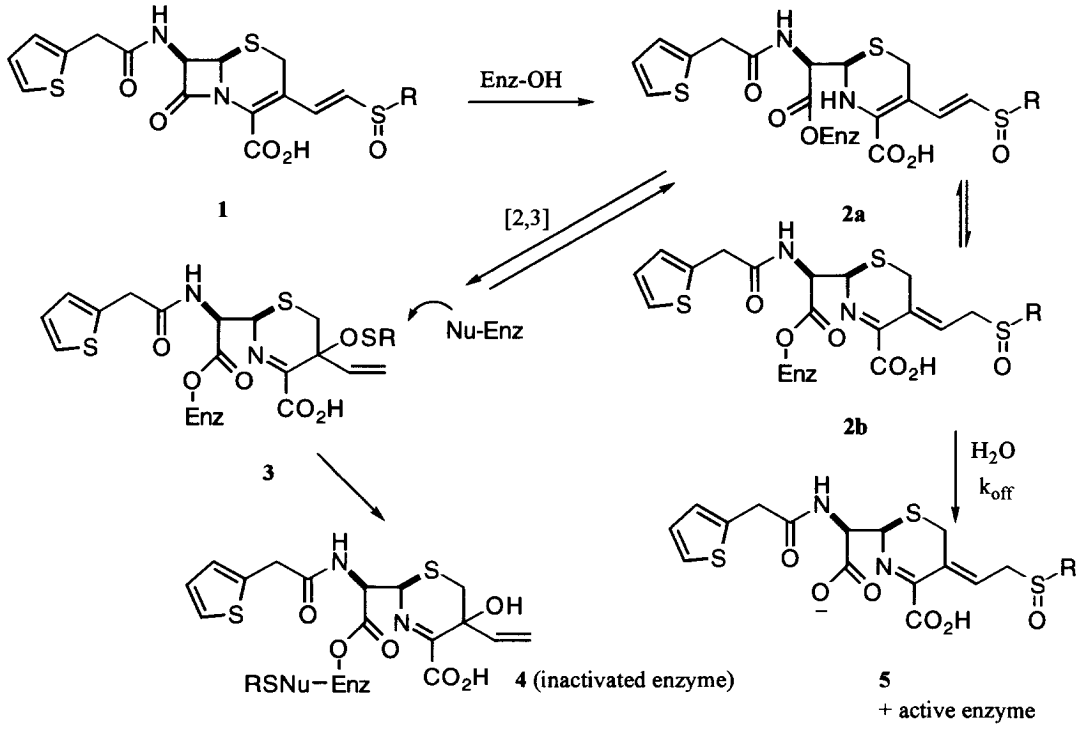
In connection with our interest in the synthesis of novel cephalosporins with unusual side-chains,[4] we thought it would be desirable to develop antibiotics that could also inactivate β-lactamases in an irreversible fashion.

Our approach to irreversible enzyme inhibition is based on a concept introduced by Firestone and Walsh.[5] This involves the generation, at the active site of the enzyme, of an allylic sulfoxide. The latter is in rapid equilibrium, via a [2,3] sigmatropic shift, with the corresponding allylic sulfenate, a powerful electrophile that has been shown to irreversibly sulfenylate crucial nucleophilic residues at the active site.[5]

Our candidate as β-lactamase inhibitor is therefore represented by **1** (Scheme 1). Processing of **1** by the β-lactamase will yield **2b,** since allylic sulfoxides are more stable than vinylic ones (e.g. **2a**), [6] and this should undergo the desired sigmatropic shift to yield **3**, leading to enzyme inactivation. In order for this mechanism to be operative, of course, the [2,3] shift and the sulfenylation steps must be able to kinetically compete with the "off" reaction, i.e. the hydrolysis step leading to **5** (or its allylic sulfenate counterpart). Since no quantitative data are available on the rate of [2,3] shift at an enzyme active site, we decided it would be worthwhile to test the idea experimentally.

The method we used to prepare the desired inhibitors is the Stille coupling, involving alkenyl stannanes and 3-trifloxycephems.[4] Sulfoxide-containing stannane **8** was prepared from bis-stannane **6** according to the method of Keck,[7] albeit in very low yield. The sulfone-containing congener **9** was similarly prepared (Scheme 2). We envisioned preparing a few 3-sulfonylvinyl cephems as control compounds: if activity is a consequence of the ability of these compounds to undergo the [2,3] shift, sulfones should be much less active than sulfoxides.

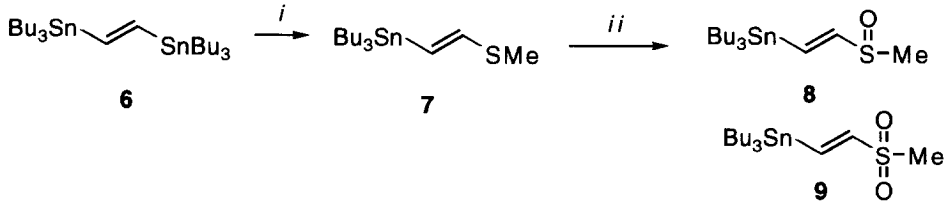
Scheme 1



Another issue that we decided to address is the absolute configuration at sulfur. Racemic sulfoxides such as **8** would lead, upon coupling, to a mixture of diastereomers, since the cephem nucleus has two chiral centers.

Recognizing that different diastereomeric sulfoxides may bind the enzyme active site with different affinity, we decided to develop a route to enantiomerically pure β-sulfinyl alkenyl stannanes. Thus, both enantiomers of **10** were prepared in >95% ee from the corresponding vinyllithium compound and and commercially available (Aldrich) (-)-menthyl (R)-*p*-toluenesulfinate and (+)-menthyl (S)-*p*-toluenesulfinate (Scheme 3).

Scheme 2



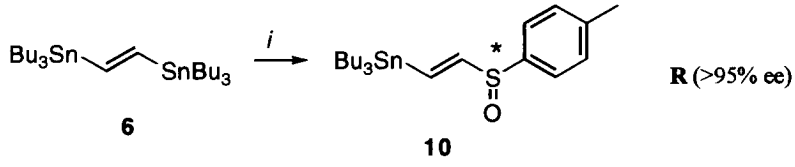
6 **7** **8** **9**

Conditions: (i) *n*-BuLi, THF, -78 °C, then MeSSMe , rt (30%); (ii) MCPBA (1.25 equiv), CH_2Cl_2, 0 °C to rt (35% of **8** and 27% of **9**).

The enantiomeric purity was established by chiral HPLC,[8] while the absolute configuration at sulfur was assigned by assuming the customary inversion of configuration.[9]

Finally, it was deemed necessary to include an aryl sulfoxide bearing an electron-withdrawing group on the ring, since this moiety had given the best result in previous studies.[5] Stannane **15** was therefore prepared by our previously described method.[10]

Scheme 3



6 **10**

Conditions: (i) *n*-BuLi, -78 °C, then (-)-menthyl (S)-*p*-toluenesulfinate, -78 °C to rt (29%).

All stannanes underwent smooth coupling with triflate **11** (Scheme 4 and Table 1). The reaction, as previously reported, proceeded rapidly at room temperature provided the palladium ligand was tri(2-furyl)phosphine (TFP).[4,11]

Interestingly, the reaction was substantially accelerated by zinc chloride (we employed two equivalents). The reaction was also stereospecific with respect to the configuration at sulfur. In particular, the (R) enantiomer of **10** produced **12a** as a single diastereomer, while in the case of **12b, d** two diastereomers were produced in a 1:1 ratio (no separation was performed and these sulfoxides were evaluated microbiologically as a mixture).

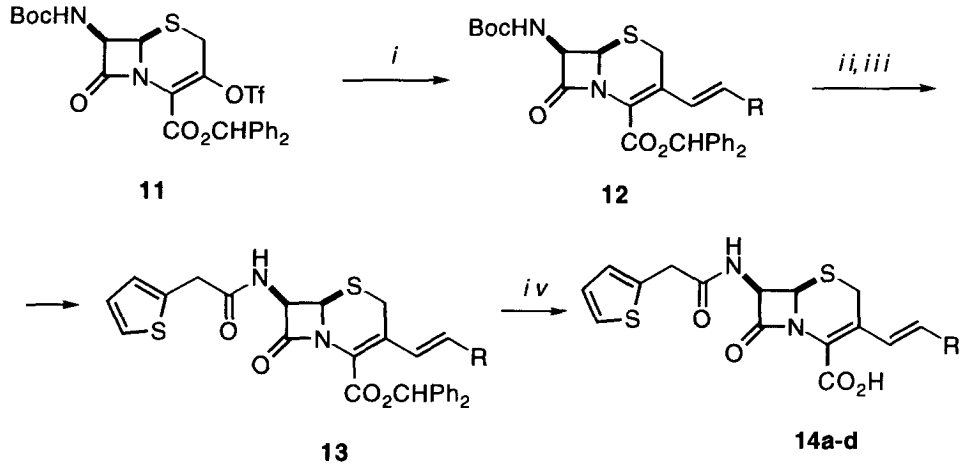
Derivatives **12** were converted into compounds with a biologically appropriate side-chain by deprotection at C-7 with *p*-toluenesulfonic acid, and reacylation of the crude 7-aminocephem to yield **13** in fair yield. Removal of the DPM blocking group afforded the cephem 4-carboxylates **14** in good yields. All yields are reported in Table 1.

Microbiological evaluation of derivatives **14a-d** surprisingly showed that the most active compound is sulfone **14c**. Also unexpected is the rather poor activity of **14d,** which should have been the most active compound according to the original hypothesis. Some of the key microbiological data are shown in Table 2.

The above compounds were also tested as possible inhibitors against a panel of typical β-lactamases (TEM-1, PC1, P99), and none of them was found to be an inhibitor. Indeed, all were good substrates and were rapidly hydrolyzed.

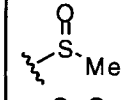
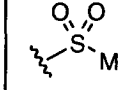
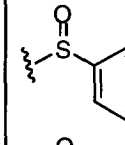
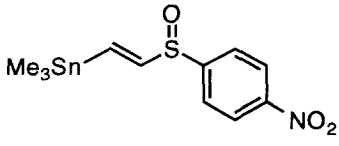
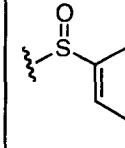
The lack of inhibition may be due to fast processing of intermediate **2** by the lactamase, in competition with the [2,3] shift, i.e. the k_{off} (Scheme 1) may be too fast for the sigmatropic shift to compete ($k_{off} >> k_1$). In this case, the above strategy may be workable if the [2,3] shift can be made faster by suitable manipulation of the substituents.[12] Another possibility is that the enzyme active site does not possess a nucleophilic residue in the vicinity of the allylic sulfenate.

Scheme 4



Conditions: (i) Stannane (1.1 equiv), 1% Pd_2dba_3, 4% TFP, $ZnCl_2$ (2 equiv), NMP, rt; (ii) *p*-TsOH (2 equiv), MeCN, rt; (iii) Thienylacetyl chloride (1.5 equiv), THF, H_2O, $NaHSO_3$ (2 equiv); (iv) CF_3CO_2H, PhOMe, 5 min, 0 °C.

Table 1. *Synthesis of 3-substituted cephems-4-carboxylates: Isolated yields.*

Stannane	12	13	14	R
8	**12b** (93%)	**13b** (47%)	**14b** (96%)	
9	**12c** (85%)	**13c** (66%)	**14c** (93%)	
10	**12a** (88%)	**13a** (37%)	**14a** (59%)	
 **15**	**12d** (82%)	**13d** (65%)	**14d** (89%)	

On the other hand, compounds **14b, c** displayed very interesting biological activity against a number of Methicillin-Resistant (MR) *S. Aureus* strains, and showed enhanced binding to PBP-2A (a Penicillin-Binding Protein) which is the target associated with Methicillin resistance. These observations suggest that cephems with this type of side chains should be studied more thoroughly as a new class of antibacterial agents. The preparation

of further members of this class, as well as more complete biological data on cephem **14c**, will be reported separately.[13]

Table 2. *Minimum Inhibitory Concentrations (MIC), in μg/ml, of the new cephems vs. selected organisms.*

ORGANISM	A No.	COMPOUND				
		Methicillin	**14a**	**14b**	**14c**	**14d**
S. Aureus	A9537	0.5	0.25	0.03	0.06	0.5
S.Aureus (MR)	A20699	32	16	16	4	64
S.Aureus (MR)	CEF1198	32	8	4	0.5	n.d.
E.Coli	A15119	>128	4	1	0.5	64
K.Pneumoniae	A9432	>128	2	0.5	0.5	64

Typical Cross-Coupling Procedure: Preparation of **12c**:

Triflate **11**[3] (476 mg, 0.7745 mmol) in dry *N*-Methylpyrrolidinone (NMP, 5 mL) was treated under Ar with tri(2-furyl)phosphine (7.4 mg, 0.04 equiv), tris(dibenzylideneacetone)dipalladium (7.3 mg, 0.02 equiv Pd), and zinc chloride (200 mg, 1.467 mmol). After stirring at rt for 15 min, the yellow solution was treated with (*E*)-2-methylsulfonylethenyltributyltin, **9** (327.3 mg, 0.828 mmol) in NMP(1 mL). The solution was stirred at rt for a further 48 h period, then it was diluted with water, and extracted with ethyl acetate. The organics were washed with water three times, then with brine, dried, and evaporated. The crude product was dissolved in acetonitrile, and further washed with hexanes three times. After evaporation, the crude product was purified by chromatography (50% ethyl acetate in hexane), to yield **12c** as a colorless foam (376.8 mg, 85.2%).

All new compounds were characterized by ^{1}H NMR and mass spectroscopy, as well as by elemental analysis and/or accurate mass determination.

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13. Control experiments showed that all tested compounds are stable under the assay conditions for the duration of the experiments.

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