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Synthesis and reactivity with β-lactamases of a monobactam bearing a retro-amide side chain

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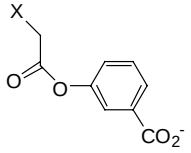
Abstract—The monobactam sodium 3-benzylcarbamoyl-2-oxo-1-azetidinesulfonate, bearing a retro (vs classical β-lactam)-amide side chain, has been synthesized and the kinetics of its reaction with typical β-lactamases studied. The new compound is generally a poorer substrate than the analogous compound with a normal side chain but its formation of a transiently stable complex with a class C β-lactamase sustains the retro-amide side-chain concept.

β-Lactamases catalyze the hydrolysis of β-lactam antibiotics and are thereby responsible for much of the bacterial resistance toward these drugs. At present, the most important β-lactamases in medicine are the serine-based classes A, C, and D.[1] Much research has been and is still directed toward the discovery of new molecular entities that react with the active site of these enzymes and thus may serve as lead compounds in antibiotic development.

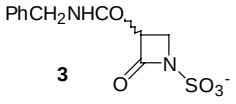
Recently, we showed that acyclic depsipeptides such as **1**, containing an atypical retro-amide side chain, reacted covalently with typical β-lactamases.[2] In some cases, these reactions were more rapid than those of analogous compounds containing the normal amide side chain such as **2** (and as found in the classical β-lactams).

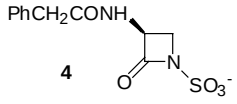


1: X = $PhCH_2NHCO$-

2: X = $PhCH_2CONH$-

It was clearly of interest, therefore, to examine the effect of this side-chain reorientation on the reactivity of β-lactams themselves. Although a small number of such compounds have been described in the literature,[3] their reactions with any relevant enzymes have not. In this communication, we report the synthesis of the retro-amide monobactam **3** and describe its reactivity with typical class A, C, and D β-lactamases. The classical monobactam **4** was also studied to allow a direct comparison of the side-chain effects.





The synthesis of **3** is shown in outline in Scheme 1. Thus, the method of Southwick[4] was applied to *p*-methoxybenzylamine to yield the dioxopyrrolidine **5**. Acid hydrolysis and decarboxylation afforded **6**. Then, the Rapoport periodate-induced rearrangement[4c,5] led to the ring-contracted β-lactam **7**, which has a carboxylic acid group at C-3. The coupling of **7** with benzylamine readily afforded the amide **8**. Oxidative removal of the *N*-*p*-methoxybenzyl-protecting group[6] yielded the 1-unsubstituted azetidinone **9**. Finally, sulfation[7] of the ring NH of the β-lactam **9** yielded the racemic monobactam **3**, which was isolated as the sodium salt.[8] The monobactam **4** was obtained from Bristol-MyersSquibb.

The reactions of **3** and **4** in aqueous buffer were monitored spectrophotometrically at 240 nm ($\Delta\varepsilon = 75\ cm^{-1}\ M^{-1}$). The spontaneous hydrolysis of **3** and **4** in 100 mM MOPS, pH 7.5, was quite slow, with rate constants of 6.7×10^{-6} and $1.1 \times 10^{-5}\ s^{-1}$, respectively, and

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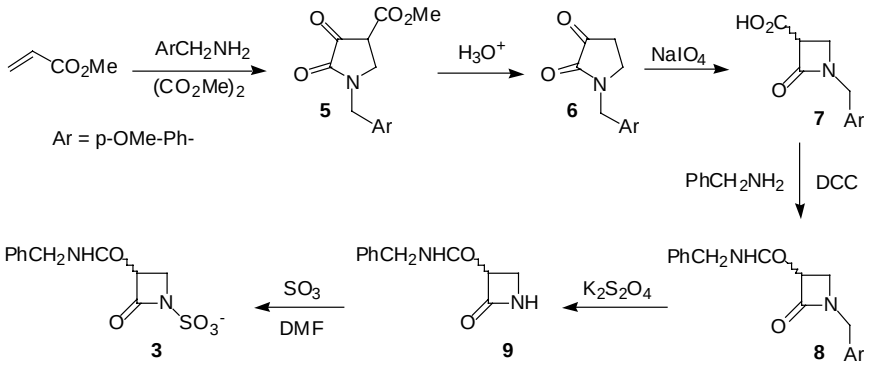


Scheme 1. Synthesis of **3**.

thus could be ignored in initial rate measurements with the various enzymes. Steady state parameters for the hydrolysis of **3** and **4**, catalyzed by several β-lactamases and derived from the initial rate measurements, are given in Table 1.

Compound **4** had been prepared[9] in the reactive 3β-amido configuration shown in the structural diagram above. Compound **3**, however, was a racemic mixture of enantiomers, as derived from the synthesis of Scheme 1. A ^{1}H NMR experiment with **3** showed that proton exchange with solvent and hence epimerization at C3 was faster than the enzyme-catalyzed reactions. It was assumed, in accordance with precedent,[10] that the 3β-enantiomer reacted with β-lactamases, while the 3α-enantiomer was inert. Under these conditions, the real K_m for the reactive 3β-enantiomer is half the apparent value.[10c] The K_m and k_{cat}/K_m values of Table 1 include this adjustment.

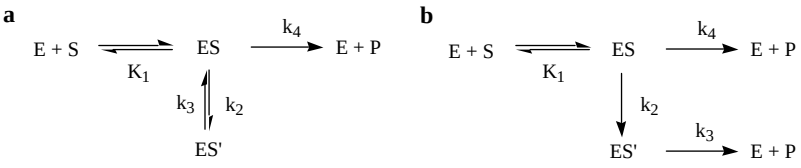
The reaction of **3** with the *Enterobacter cloacae* P99 β-lactamase differed qualitatively from that with the other enzymes in that it involved a slowly equilibrating side branch (Schemes 2a or b, which could not be distinguished by the data at hand).

The smallest rate constant, k_3, was obtained from a return of activity experiment, where aliquots of a reaction mixture of **3** and the enzyme were diluted into a solution of a good substrate at saturating concentration (cephalothin, 200 μM) and the return of activity was monitored spectrophotometrically. A value of 0.013 s^{-1} was thus obtained for k_3. The other rate constants were obtained from total progress curve analysis, where k_3 was fixed at the value obtained as described above. In both experiments, the data were analyzed by the Dynafit program.[11] Values of the other constants thus obtained (Scheme 2a) were $K_1 = 0.21 \pm 0.04$ mM, $k_2 = 0.023 \pm 0.002$ s^{-1}, and $k_4 = 2.2 \pm 0.15$ s^{-1}; steady state parameters were then calculated to be $k_{cat} = k_4/(1 + k_2/k_3) = 0.82$ s^{-1} and $K_m = K_1/(1 + k_2/k_3) = 0.075$ mM. Analysis in terms of Scheme 2b yielded the same values for the steady state parameters k_{cat} and K_m.

Table 1. Steady state kinetics parameters for the hydrolysis of **3** and **4** by β-lactamases

Enzyme[a]	k_{cat} (s^{-1})[b]		K_m (mM)[c]		k_{cat}/K_m ($s^{-1}M^{-1}$)[c]	
	3	**4**	**3**	**4**	**3**	**4**
P99	0.82[d]	410 ± 90	0.075[d]	1.0 ± 0.4	1.1×10^4	4.1×10^5
TEM	43 ± 1	1600 ± 570	2.5 ± 0.2	6.8 ± 0.4	1.7×10^4	2.4×10^5
PC1	0.46 ± 0.03	0.38 ± 0.01	0.30 ± 0.03	0.07 ± 0.02[e]	1.5×10^3	5.8×10^3
OXA-1	1.6 ± 0.2	6.0 ± 0.1	2.3 ± 0.4	0.029 ± 0.001[f]	7.0×10^2	2.1×10^5

[a] P99, the class C β-lactamase of *Enterobacter cloacae* P99; TEM, the class A β-lactamase from the TEM-2 plasmid; PC1, the class A β-lactamase of *Staphylococcus aureus* PC1; OXA-1, the class D β-lactamase from *Escherichia coli*.
[b] Reaction conditions.[15]
[c] The values for **3** were adjusted as described in the text.
[d] See text.
[e] Determined from competitive inhibition of nitrocefin hydrolysis.
[f] Determined from competitive inhibition of cephalothin hydrolysis.



Scheme 2.

The results of Table 1 show that, in general, **3** is a poorer substrate of the enzymes tested than is **4**. It is also noticeable that, even in cases where enzyme deacylation is usually rate-determining in the k_{cat} step (P99, PC1, and OXA-1 β-lactamases), there is no evidence of formation of a tightly bound acyl-enzyme intermediate (low K_m), although this phenomenon is evident in the parameters for the PC1 and OXA-1 enzymes with **4**. It seems, therefore, that the normal amide group in the side chain interacts considerably more favorably with these enzymes. Crystal structures indicate that the normal amide side chain of β-lactam substrates generally forms hydrogen bonds with an asparagine carboxamide NH and a protein backbone CO,[12] but modeling suggested that the retro-amide of **3** might also be able to interact in a favorable way.[2] Although the latter may be true in some cases with acyclic substrates, it does not appear to be true with β-lactams.

The most interesting feature of the results appears to be the observation that the retro-amide promotes or allows partition of a complex of **3** with the P99 β-lactamase, most likely the acyl-enzyme intermediate, into a more inert complex ($t_{1/2}$ = 54 s). This phenomenon is not unusual with β-lactamases,[13] although it is not generally seen with the simple benzyl side chain. It is possible that incorporation of a third generation side chain into **3** might generate much more inert complexes and thus effective inhibitors. Neither **3** nor **4** inhibited the *Streptomyces* R61 DD-peptidase or *Escherichia coli* pbp5 to any significant extent although, again, this situation may also be changed by the presence of appropriate side chains, as in aztreonam, for example.[14]

Acknowledgments

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8. White powder (47% yield); mp > 250 °C; R_f (acid form) 0.24 (ethyl acetate/methanol, 85:15); ^{1}H NMR (D_2O) δ 3.84 (d, J = 4.0 Hz, 2H), 4.29 (t, J = 4.0 Hz, 1H), 4.45 (s, 2H), 7.36–7.41 (m, 5H); ^{13}C NMR (D_2O) δ 46.03, 46.36, 56.16, 129.99, 130.28, 131.57, 140.27, 165.74, 170.18; Anal. Calcd for C, 41.91; H, 3.84; N, 8.89. Found: C, 41.69; H, 3.78; N, 8.98.
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15. Stock solutions of **3** and **4** were prepared in DMSO. The buffer employed for enzyme kinetics was 100 mM MOPS, pH 7.5, except in the case of the OXA-1 enzyme where 50 mM $NaHCO_3$ was also included. The reaction temperature was 25 °C and reaction solutions contained 5% DMSO. The enzymes were obtained as previously described.[2]