

Enzymatic Ring Expansion of Penicillins to Cephalosporins: Side Chain Specificity

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Structural variants on the acylamino-side chains of penicillins as substrates for the ring expansion enzyme from *Cephalosporium acremonium* CO728 show that a six carbon chain terminating in a carboxy group permits efficient conversion into cephems with the exception of δ -(L- α -aminoadipoyl) [5-(5S)-amino-5-carboxypentanoyl].

The biosynthesis of cephalosporins involves a ring expansion of penicillin N (**1**, R = a) to deacetoxycephalosporin C (DAOC) (**2**, R = a).¹ The δ -(α -aminoadipoyl) side chain of (**1**) is D-configured, deriving from the L-side chain of isopenicillin N,² the first formed penicillin.³ We have purified the ring expansion activity from *Cephalosporium acremonium* CO728⁴ and used it to assess the side chain specificity of this important enzymatic step. Thus a series of penicillins were exposed to this purified activity and their conversions into cephems monitored by ¹H n.m.r. spectroscopy, bioassay, and steady-state initial rate measurements based on the generation of increased u.v. absorption at λ_{max} 260 nm, characteristic of the dihydrothiazine moiety of cephalosporins.⁵ In those cases where the efficiency of the conversion permitted we isolated and characterised the so-formed cephalosporins. The results are shown in Table 1. In the case of (**1**, R = b) the product, active against *Escherichia coli* both in the presence and absence of β -lactamase 1 (from *Bacillus cereus*), was purified by h.p.l.c. [reverse phase ODS column, acetonitrile: water (1:40)] to give the cephem (**2**, R = b), δ_{H} (D₂O, 500 MHz)[†] 1.77 (3H, s, 3-Me), 3.03, 3.35 (2H, ABq, *J* 18 Hz, 4-H), 3.53, 3.58 (2H, ABq, *J* 15 Hz, COCH₂), 4.88 (1H, d, *J* 4.5 Hz, β -lactam-H), 5.36 (1H, d, *J* 4.5 Hz, β -lactam-H), and 7.23–7.35 (4H, 2 \times m, ArH), *m/z* (fast atom bombardment) 360 (MH⁺, 45%), which was identical to a synthetically prepared sample.[‡]

Similarly with (**1**, R = c) the product was purified by h.p.l.c. (reverse phase ODS column, 7.5 mM NH₄HCO₃) to yield the cephem (**2**, R = c), δ_{H} (D₂O, 500 MHz)[†] 1.46–1.53 (4H, m, [CH₂]₂CH₂CO), 1.79 (3H, s, 3-Me), 2.06–2.10 (2H, m, CH₂CO), 2.22–2.30 (2H, m, CH₂CO), 3.12, 3.47 (2H, ABq, *J* 13 Hz, 4-H), 5.44 (1H, d, *J* 4.5 Hz, β -lactam-H), and 5.96 (1H, d, *J* 4.5 Hz, β -lactam-H), which was identical to an authentic sample. The dimethyl ester of (**2**, R = c) obtained (diazomethane) from the enzymatic reaction gave *m/z* (NH₃ desorption chemical ionisation) 388 (MNH₄⁺, 32%) and 371 (MH⁺, 34%), identical to an authentic sample.[§] This result is in contrast to that of Kupka *et al.*⁶ who reported, using a protoplast lysate from *C. acremonium* CW-19, containing ring

expansion activity, that 'carboxy n-butyl penicillin' [which we assume to be (**1**, R = c)] did not give cephalosporin products (by bioassay or h.p.l.c.).

It is of some interest that the *m*-carboxyphenylacetyl side chain of (**1**, R = b) provides a 'rigid' version of the adipoyl side chain of (**1**, R = a) and (**1**, R = c), *cf.* Figure 1.

Of the other penicillins (**1**, R = d–k) we tested as substrates for the ring expansion activity only the γ -(D-glutamyl) penicillin (**1**, R = d) gave a low conversion into a cephem product detectable by ¹H n.m.r. spectroscopy and

Table 1. Side chain specificity of the ring expansion of penicillins to cephalosporins.^a

Conversion		Initial velocity / $\mu\text{mol min}^{-1} \times 10^6$ % (rel. to 1 , R = a)	No conversion		
R	R		R		
a		3.88	100	f	
b		1.93	50	g	
c		1.56	40	h	
d		0.54	14	j	
e		0.13	3	k	H

^a Conditions: [substrate] = 1 mM; [enzyme] = 4 μM ; cofactors FeSO₄, O₂, α -ketoglutarate, ascorbate, dithiothreitol; buffer: Tris-HCl, pH 7.5; temp. 30 °C.

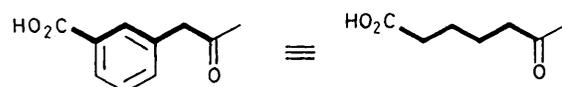
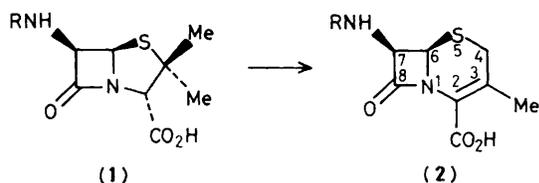


Figure 1



[†] Referenced to sodium [2,2,3,3-²H₄]-3-trimethylsilylpropanoate (TSP) = 0.00 p.p.m.

[‡] Prepared by hydrogenation (Pd/C, H₂, 20 °C) of the bis-*p*-nitrobenzyl ester of (**2**, R = b).

[§] Prepared by hydrogenation (Pd/C, H₂, 20 °C) of the bis-*p*-nitrobenzyl ester of *N*-adipoyl-7-aminodeacetoxycephalosporin C.

antibacterial activity in the presence of β -lactamase 1. However, using the more sensitive spectrophotometric assay, based on the observation of the 260 nm chromophore of the products (2) we found that both the γ -(*D*-glutamyl) penicillin (1, R = d) and the glutaryl penicillin (1, R = e) were poor substrates (see Table 1).

In conclusion these studies indicate that a six carbon-*N*-acyl side chain, terminating in a carboxy group, permits reasonable penicillin into cephem conversion by the ring expansion enzyme. Although we have found a broadly similar requirement for the isopenicillin N synthetase enzyme,^{7,8} the ring expansion enzyme differs in its inability to process isopenicillin N (1, R = f) bearing the δ -(*L*- α -aminoadipoyl) side chain.⁹

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