An Effective Substitute for α-Aminoadipic Acid in the Enzymatic Synthesis of Penicillins

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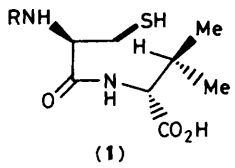
meta-Carboxyphenylacetylcysteinylvaline has been demonstrated to be a highly efficient substrate for the enzyme isopenicillin N synthetase with similar Michaelis constants and maximum velocity parameters to the natural substrate δ-(L-α-aminoadipoyl)-L-cysteinyl-D-valine.

Previous studies in this laboratory have demonstrated that structural variations of the α-aminoadipoyl moiety in the natural substrate δ-(L-α-aminoadipoyl)-L-cysteinyl-D-valine (ACV) (**1a**) of isopenicillin N synthetase (IPNS) require a six-carbon or equivalent chain terminating in a carboxy function to be effective substrates. Thus L- or D-α-aminoadipoyl-(**1a**), (**1b**) and adipoyl-(**1c**) cysteinylvalines were efficient substrates,[1] but phenylacetyl-(**1d**) and phenoxyacetylcysteinylvaline (**1e**) were converted only at very slow rates into (**2d**) and (**2e**), respectively.[2] As penicillins with such arylamido side chains generally possess potent antibacterial activity, their *direct* enzymatic synthesis from tripeptides would obviate the *in vivo* deacylation–reacylation processes of the natural pathway. In order to increase the efficiency of the enzymatic synthesis, we proposed that the *m*-carboxyphenylacetyl moiety should represent a conformationally rigid form of the required *transoid* 6-carbon chain terminating in a carboxy function (Scheme 1) and therefore be an effective substrate. Thus the *m*-carboxyphenylacetylcysteinylvaline tripeptide (**1f**) was synthesised from 3-(benzyloxycarbonyl)-phenylacetic acid (from isophthalic acid in 5 steps) and

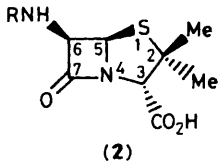
Table 1.[a]

Substrate	K_m/mM	v_{max}/μmol min^{-1}
(**1a**)	0.16	1.64
(**1d**)	0.9	2.5×10^{-3}
(**1f**)	0.8	0.8

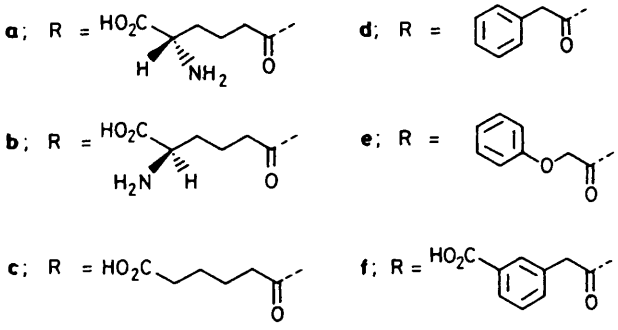
[a] *Conditions:* pH 7.7, 27 °C. A coupled enzyme assay with β-lactamase and pH stat titration[6] was used, and parameters determined by computer analysis of an integrated Michaelis–Menten equation.[7]



(1)



(2)



Scheme 1

S-*p*-methoxybenzylcysteinylvaline benzhydryl ester following standard coupling procedures[3] and deprotection[4] (CH_2Cl_2, anisole, $AlCl_3$, 0—20 °C). Incubations of (**1f**) (1.4 mg) with highly purified IPNS (3.5 I.U.) from *Cephalosporium acremonium* CO 728[5] and standard co-factors gave, after purification by h.p.l.c. [reverse phase octadecylsilane column, acetonitrile: 10 mM ammonium hydrogen carbonate (3 : 97) as eluant] the *m*-carboxyphenylacetylpenam (**2f**) (70%, n.m.r. integration calibration against internal standard), δ_H (300 MHz, HOD, $Me_3SiCD_2CD_2CO_2Na$ = 0.00) 1.30 (3H, s, 2-Me), 1.38 (3H, s, 2-Me), 3.55, 3.62 (2H, ABq, *J* 15 Hz, CH_2Ar), 4.07 (1H, s, 3-H), 5.25, 5.35 (2H, 2 × d, *J* 4 Hz, 5,6-H), 7.32—7.40 and 7.69—7.73 (2 × 2H, 2 × m, aryl-H); *m*/*z* (positive argon fast atom bombardment) 379 (MH^+). The sample was identical (h.p.l.c., ^1H n.m.r.) to an authentic synthetic sample {from 3-(*p*-nitrobenzyloxycarbonyl)phenylacetic acid and 6-aminopenicillanic acid, *p*-nitrobenzyl ester and standard coupling procedures followed by deprotection [H_2 (1 atm), Pd on carbon, $NaHCO_3$ (1 equiv.), H_2O, tetrahydrofuran, 3 h]}, and showed *ca.* 75% relative antibacterial activity towards *Staphylococcus Aureus* N.C.T.C. 6571 when compared to penicillin G.

Table 1 shows a comparison of steady-state kinetic parameters K_m and v_{max} for tripeptides (**1a**), (**1d**), and (**1f**) with IPNS. As v_{max} (= $k_{cat}[E]_0$) represents the lower limit on the rate constants for catalysis, these results suggest that the catalytic event(s) for the *m*-carboxyphenylacetyl tripeptide (**1f**) occur much more rapidly [and with similar magnitude to the natural substrate (**1a**)] than for phenylacetyl tripeptide (**1d**). This is despite similar apparent dissociation constants for enzyme-bound species (represented by K_m) for the two tripeptides (**1d**) and (**1f**). These results may be interpreted as implying that once bound, the *m*-carboxy function of (**1f**) helps to orient the tripeptide into an optimal conformation for ring closure at the catalytic site.

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