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THE ENZYMATIC RING EXPANSION OF PENICILLINS TO CEPHALOSPORINS : SIDE CHAIN SPECIFICITY

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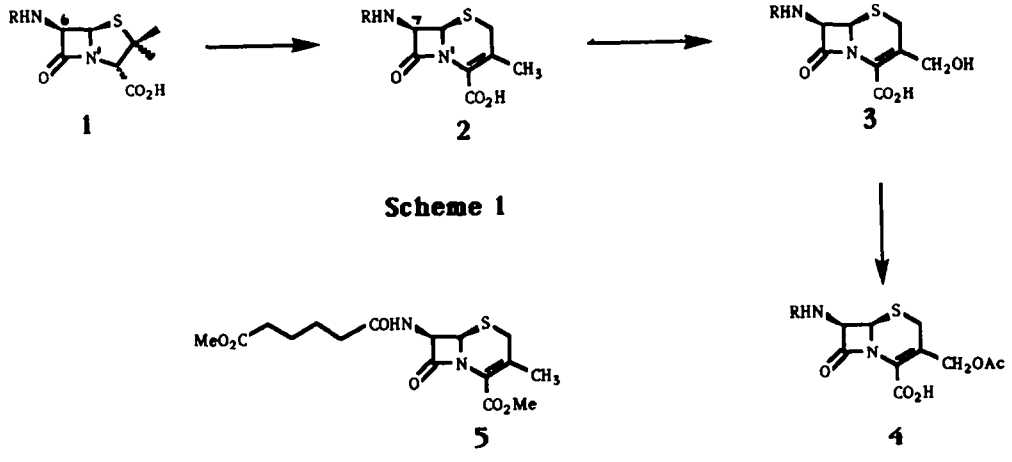
Abstract: Structural variants of the acylamino side chain of penicillins have been tested as substrates for Deacetoxy/Deacetyl Cephalosporin C Synthetase from C.acremonium CO 728. A six carbon chain terminating in a carboxyl group was found to permit efficient ring expansion to cephems, with the exception of δ-(L-α-aminoadipoyl).[1]

The biosynthesis of the cephalosporin nucleus, involves the ring expansion of penicillin N (1, R=a), to deacetoxycephalosporin C (DAOC) (2, R=a).[2] The δ-(α-aminoadipoyl) side chain of 1 is D-configured, deriving from the L-side chain of isopenicillin N (1, R=f),[3] the first formed penicillin.[4] Subsequent hydroxylation of DAOC (2, R=a) gives deacetylcephalosporin C (DAC) (3, R=a), which is then acetylated to yield cephalosporin C (4, R=a). Recently we have described the purification and characterisation of an enzyme catalysing both DAOC Synthetase and Hydroxylase activities, from Cephalosporium acremonium.[5] In this paper we describe the use of partially purified extracts from C.acremonium to assess the side chain specificity of the ring expansion step catalysed by this important enzyme.

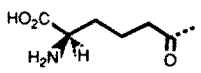
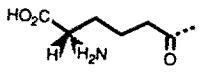
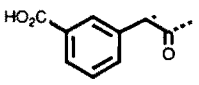
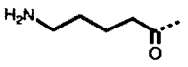
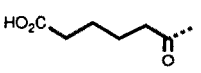
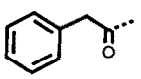
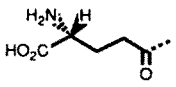
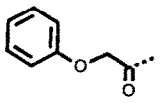
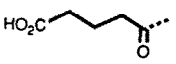
Thus a series of penicillins were exposed to the partially purified enzyme and their conversions to cephems monitored by ^{1}H nmr, bioassay and steady state initial rate measurements, based on the generation of increased U.V. absorption at λ_{max} 260nm, characteristic of the dihydrothiazine moiety of cephems.[6] The results are shown in Table 1. In cases where the efficiency of the conversion permitted, the product cephems were isolated and characterised.



Scheme 1

In the case of (1, R=b) the product, active against Escherichia coli, both in the presence and absence of β-lactamase I (from Bacillus cereus), was purified by h.p.l.c. to give the cephem (2, R=b).

Similarly with (1, R=c) the product was purified by h.p.l.c. to yield the cephem (2, R=c). Acidification of an aqueous solution of (2, R=c), followed by extraction with ethyl acetate and methylation (diazomethane) gave the dimethyl ester (5).

Conversion				No Conversion	
R		Initial Velocity /μmoles min^{-1} x10^4	%(rel. to 1(R = a)	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

Conditions: [Substrate] = 1mM; [Enzyme] = 4μm; Cofactors = $FeSO_4$, O_2, α-ketoglutarate, ascorbate, DTT; Buffer = TRIS.HCl, pH = 7.5; Temp. = 30°C.

Table 1. Side Chain Specificity of the Ring Expansion of Penicillins to Cephalosporins

This result contrasts with that of Kupka et at[7], who reported, using a protoplast lysate from C.acremonium CW-19, containing DAOC Synthetase/Hydroxylase activity, that "carboxy n-butyl penicillin" [which we assume to be (1, R=c)] did not give cephem products (by bioassay or h.p.l.c.). It is of 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 Fig.1.

Of the other penicillins (d-k) we tested as substrates for ring expansion activity only the γ-(D-glutamyl) penicillin (1, R=d) gave a low conversion to a cephem product, as detected by ^1H n.m.r. (500 MHz) and antibacterial activity in the presence of β-lactamase. However, using the more sensitive spectrophotometric assay[6] we found that both the γ-(D-glutamyl) penicilin (1, R=d) and the glutaryl penicillin (1, R=e) were poor substrates (Table 1).

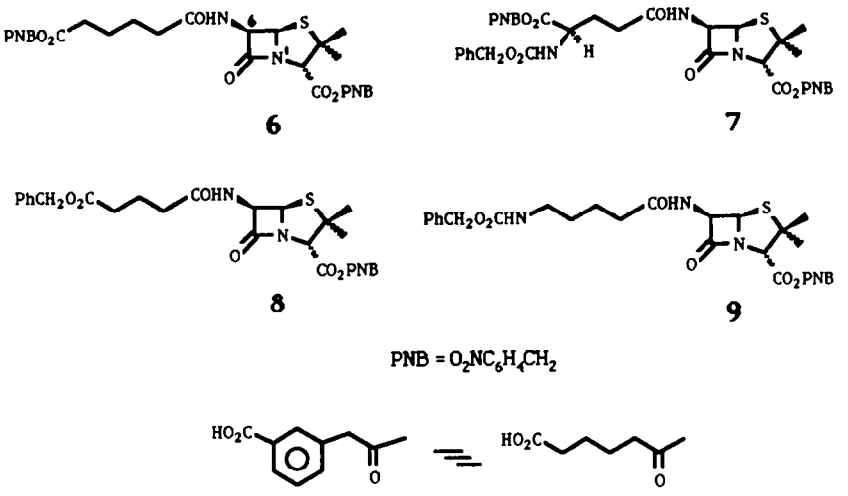


Fig. 1

We conclude these studies indicate that a six carbon N-acyl side chain, terminating in a carboxyl group, permits reasonable penam to cephem conversion by DAOC Synthetase/Hydroxylase. Although we have found a broadly similar requirement Isopenicillin N Synthetase enzyme,[8,9] the DAOC synthetase enzyme differs in it inability to process Isopenicilin N (1, R=f), bearing the δ-(L-α-aminoadipoyl) side chain.[10]

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Synthesis of Penicillins and Cephems

Penicillin N (1, R=a), Isopenicillin N (1, R=f) and (1, R=b) were prepared by published procedures.[5,11,12] Penicillins G (1 R=h), V (1, R=j) and 6-aminopenicillanic acid (1, R=k) were gifts from Eli Lilly & Co. Penicillins (1, R=c), (1, R=d) and (1, R=e) and cephems (2, R=b) and (2, R=c) were prepared as described below.

GENERAL EXPERIMENTAL

Standard chemical procedures as previously reported were used.[13] Melting points were recorded on a Büchi 510 apparatus and are uncorrected. Infra red spectra were recorded on a Perkin Elmer 681 spectrophotometer. ^{1}H n.m.r. spectra were either recorded at 300 MHz on a Bruker WH300 spectrometer or at 500 MHz on a Bruker AM500 spectrometer. ^{13}C n.m.r. were recorded at 62.85 MHz on a Bruker AM250 spectrometer. Mass spectra in the electron impact, or chemical ionisation modes were recorded on a VG Micromass 16F spectrometer. Samples requiring desorption chemical ionisation or fast atom bombardment were run on VG Micromass 30F or ZAB 1F spectrometers.

H.p.l.c. of (i) crude incubation mixtures were carried out using a Waters M-6000 A pump, a UK-6 injector or a Rheodyne 7125 injector and a PYE Unicam LC3 UV detector; (ii) penicillin substrates, using dual Gilson 303 pumps, a Rheodyne 7125 injector and a Gilson holochrome set at 220 nm.

Protocol for Incubations with DAOC Synthetase/Hydroxylase

The enzyme was obtained after purification as a suspension in 50 mmolar, pH 7.4 TRIS-HCl buffer (ca. 0.5-1.0 I.U./ml).[5] For the incubation of 0.2 mg of substrate, the following procedure was used: To a solution containing iron (II) sulphate (4.2×10^{-1} mg, 1.5×10^{-3} mmol) and L-ascorbic acid (4.3 mg, 2.4×10^{-2} mmol) in water (3 ml) was added α-ketoglutarate (5.3 mg) and the pH of the mixture then adjusted to pH 7.6 with aqueous sodium hydroxide (100 mmolar). This co-factor solution (250 µl) was then added to a solution of the enzyme in 50 mmolar, pH 7.4 TRIS-HCl buffer (1.65 ml, ca 1 I.U.) and the mixture pre-incubated at 270 rpm at 27°C for 2 minutes. The substrate in water (0.1 ml) was then added and the mixture incubated for 2 hours. The reaction was terminated by the addition of acetone (5 ml) and the precipitate separated by centrifugation (10,000 rpm, 10 minutes). The supernatant was then decanted and the acetone and water removed in vacuo (< 30°C) to give the crude product.

(2S,5R,6R)-1-Aza-3,3'-dimethyl-6-(5-p-nitrobenzyloxycarbonylpentanamido)-7-oxo-4-thiabicyclo-[3.2.0]heptane-2-carboxylic acid p-nitrobenzyl ester (6)

To a solution of 6-aminopenicillanic acid p-nitrobenzyl ester (172 mg, 0.50 mmol) in dichloromethane (10 ml) were added 1-ethoxycarbonyl-2-ethoxy-dihydroquinoline (124 mg, 0.50 mmol), adipic acid mono-p-nitrobenzyl ester[14] (140 mg, 0.50 mmol) and anhydrous sodium sulphate (5 mg). The reaction mixture was stirred for 24 hours, after which it was evaporated to dryness. The residue was dissolved in ethyl acetate (50 ml), washed with 2N hydrochloric acid (25 ml), aqueous sodium bicarbonate solution (25 ml) and brine (25 ml), dried (Na_2SO_4), filtered and evaporated to dryness. Chromatography [flash silica (diethylether/dichloromethane)]gave (6) (168 mg, 55%) as a colourless oil. t.l.c. [diethylether/dichloromethane, 1:1] Rf 0.4; δ_H (300 MHz, $CDCl_3$) 1.56 (3H, s, 3-CH_3), 2.12 (3H, s, 3-CH_3), 1.68-1.73 (4H, m, $CH_2CH_2CH_2CO$), 2.26-2.30 (2H, m, CH_2CO), 2.41-2.46 (2H, m, CH_2CO), 4.49 (1H, s, 2-H), 5.21 (2H, ca s, CH_2Ar), 5.28, 5.29 (2H, ABq, J 13 Hz, CH_2Ar), 5.54 (1H, d, J 4 Hz, 5-H), 5.73 (1H, dd, J 9, 4 Hz, 6-H), 6.10 (1H, d, J 9 Hz, NH), 7.49-7.56 (4 H, m, Ar-H), 8.21-8.28 (4H, m, Ar-H). m/e (electron impact) 614 (M^+). [Found C, 54.89; H, 5.03; N, 9.36; $C_{28}H_{30}SN_4O_{10}$ requires C, 54.71; H, 4.92; N, 9.12%].

(2S,5R,6R)-1-Aza-3,3'-dimethyl-6-(5-carboxypentanamido)-7-oxo-4-thiabicyclo[3.2.0]heptane-2-carboxylic acid (1, R=c)

To a solution of (6) (160 mg, 0.17 mmol) in tetrahydrofuran (15 ml) was added a solution of sodium bicarbonate (29 mg, 0.34 mmol) in water (15 ml) and 10% palladium on charcoal (100 mg). The reaction mixture was hydrogenated for 1 hour (at 1 atmosphere), after which it was filtered (celite). The filtrate was washed with ethyl acetate (20 ml) and evaporated to dryness to give (1, R=c) (27 mg, 46%). δ_H (300 MHz, D_2O) 1.35-1.55 (4H, m, $CH_2CH_2CH_2CO$), 1.36 (3H, s, 3-CH_3), 1.48 (3H, s, 3-CH_3), 2.05-2.32 (4H, 2 x m, CH_2CO), 4.08 (1H, s, 2-H) and 5.29, 5.39 (2 x 1H, 2 x d, J 4 Hz, 5-H, 6-H). m/e (fast atom bombardment) 345 (MH^+). (1, R=c) showed antibacterial activity against Staphylococcus aureus NCTC 6571.

(2S,5R,6R)-1-Aza-3,3'-dimethyl-6-(4-N-benzyloxycarbonylbutanamido)-7-oxo-4-thiabicyclo[3.2.0]-heptane-2-carboxylic acid p-nitrobenzyl ester (8)

To a solution of 6-aminopenicilanic acid p-nitrobenzylester (647 mg, 1.91 mmol) in dichloromethane (12 ml) were added 1-ethoxycarbonyl-2-ethoxy-dihydroquinoline (474 mg, 1.92 mmol), glutaric acid mono-benzyl ester[14] (376 mg, 1.92 mmol) and anhydrous sodium sulphate (20 mg). The reaction mixture was stirred for 36 hours, after which it was evaporated to dryness. The residue was dissolved in ethyl acetate (50 ml), washed with 2N hydrochloric acid (25 ml), aqueous sodium bicarbonate solution (25 ml) and brine (25 ml), dried (Na_2SO_4), filtered and evaporated to dryness. Chromatography [flash silica (ethyl acetate/hexane)] gave (8) as a foam (523 mg, 49%). t.l.c. [ethyl acetate/hexane, 1:10] Rf 0.35; ν_{max} ($CHCl_3$) 1788 s, 1750 s, 1742 s, 1689 s, 1528 s, 1504 m, 1351 s and 1182 s cm^{-1}; δ_H (60 MHz, $CDCl_3$) 1.34(3H, s, 3-CH_3), 1.56(3H, s, 3-CH_3), 1.72-2.45[6H, m, $(CH_2)_4$], 4.39(1H, s, 2-H), 5.02(2H, s, CH_2Ar), 5.11(2H, s, CH_2Ar), 5.38-5.72(2H, m, 5-H, 6-H), 6.20 (1H, d, J 8 Hz, NH), 7.35 (2H, d, J 8.5 Hz, CH_2ArNO_2) and 8.04 (2H, d, J 8.5 Hz, CH_2ArNO_2). m/e (desorption chemical ionistion) 556 (MH^+, 5%), 160 (42%). [Found C, 58.24; H, 5.45; N, 7.32; $C_{27}H_{29}SN_3O_8$ requires C, 58.36; H, 5.26; N, 7.56%].

(2S,5R,6R)-1-Aza-3,3'-dimethyl-6-(4-carboxybutanamido)-7-oxo-4-thiabicyclo[3.2.0]heptane-2-carboxylic acid (1, R=e)

To a solution of (8) (118 mg, 0.21 mmol) in tetrahydrofuran (5 ml) was added a solution of sodium bicarbonate (18 mg, 0.21 mmol) in water (5 ml) and 10% palladium on charcoal (200 mg). The reaction mixture was hydrogenated for 3 hours (at 1 atmosphere), after which it was filtered (celite). The filtrate was washed with ethyl acetate (2 x 20 ml) and evaporated to dryness to give (1, R=e) (39 mg, 56%). δ_H (500 MHz, D_2O) 1.39(3H, s, 3-CH_3), 1.51(3H, s, 3-CH_3), 1.70-1.77(2H, m, CH_2CH_2CO), 2.07-2.11(2H, m, CH_2CO), 2.20-2.23(2H, m, CH_2CO), 4.12 (1H, s, 2-H) and 5.34, 5.43(2 x H, 2 x d, J 4 Hz, 5-H, 6-H); m/e (fast atom bombardment) 331 (MH^+).

(2S,5R,6R)-1-Aza-3,3'-dimethyl-6-(5-N-benzyloxycarbonylaminopentanamido)-7-oxo-4-thiabicyclo-[3.2.0]heptane-2-carboxylic acid p-nitrobenzylester (9)

To a solution of 6-aminopenicillanic acid (1.26 g, 3.73 mmol) in dichloromethane (20 ml) were added 1-ethoxycarbonyl-2-ethoxy-dihydroquinoline (923 mg, 3.73 mmol), N-benzyloxycarbonyl aminovaleric acid (831 mg, 3.73 mmol) and anhydrous sodium sulphate (10 mg). The reaction mixture was stirred for 24 hours, after which it was evaporated to dryness. The residue was dissolved in ethyl acetate (100 ml), washed with 2N hydrochloric acid (50 ml), aqueous sodium bicarbonate solution (50 ml) and brine (50 ml), dried (Na_2SO_4), filtered and evaporated to dryness. Chromatography [flash silica (ethyl acetate/hexane)] gave (9) (986 mg, 45%) as a colourless oil. t.l.c. [ethyl acetate/hexane, 1:10] Rf 0.40; ν_{max} ($CHCl_3$) 2935 m, 1788 s, 1720 bs, 1525 s, 1351 s, 1182 s and 912 m cm^{-1}; δ_H (60 MHz, $CDCl_3$) 1.33-1.86[6H, m, $(CH_2)_3CH_2N$], 1.45(3H, s, 3-CH_3), 1.63(3H, s, 3-CH_3), 2.13-2.48(2H, m, CH_2CO), 3.02-3.48(2H, m, CH_2N), 4.45(1H, s, 2-H), 5.01, 5.23(2 x 2H, 2 x ca s, CH_2Ar), 5.68(1H, d, J 4 Hz, 5-H), 5.75(1H, dd, J 8, 4 Hz, 6-H), 6.39(1H, bd, J 8 Hz, NH), 7.28-7.36(6H, m, Ar-H and NH), 7.47(2H, d, J 8-5 Hz, Ar-H) and 8.38(2H, d, J 8.5 Hz, Ar-H).

N-Benzoyloxycarbonyl-5-aminovaleric acid

5-Aminovaleric acid (1.50 g, 12.8 mmol) was dissolved in tetrahydrofuran (13 ml) and aqueous sodium hydroxide (1 molar, 13 ml) and cooled to 0°C. Solutions of benzylchloroformate (85% w/w, 2.83 g, 14.1 mmol) in tetrahydrofuran (13 ml) and aqueous sodium hydroxide (1 molar, 13 ml) were then added simultaneously (over ca 20 minutes). The reaction mixture was allowed to warm to room temperature and stirred for a further 1 hour, after which ether (50 ml) was added. the aqueous layer was separated, re-washed with ether (50 ml), acidified to pH 2 (2N hydrochloric acid) and extracted with ethyl acetate (3 x 50 ml). The organic extracts were dried (Na_2SO_4) and evaporated to dryness. Crystallisation (ethyl acetate/hexane) gave the title compound (2.45 g, 76%). m.p. 98-99°C (from ethyl acetate/hexane); ν_{max} ($CHCl_3$) 3475 w, 1712 bs and 1515 cm^{-1}; δ_H (60 MHz, $CDCl_3$) 1.40-1.73(4H, m, $CH_2CH_2CH_2CO$), 2.25(2H, ca t, J 6 Hz, CH_2CO), 2.88-3.28(2H, m, NCH_2), 5.08(2H, s, CH_2Ar), 5.73-5.96(1H, bm, NH), 7.21-7.28(5H, m, Ar-H) and 9.80(1H, bs, CO_2H). δ_C (62.85 MHz, $CDCl_3$) 21.8(t, 1 x $(CH_2)_2CH_2CO$), 29.3(t, 1 x $(CH_2)_2CH_2CO$), 33.4(t, CH_2N), 40.6(t, CH_2Ar), 128.1,

128.5(Ar-C), 136.5(s, ipso Ar-C), 158.5(s, CO), 178.3(s, CO). m/e (Chemical ionisation) 252 (MH^+, 25%), 208 (MH^+-CO_2, 40%), 91 (100%).

2S,5R,6R)-1-Aza-3,3'-dimethyl-6-(5-aminopentanamido)-7-oxo-4-thiabicyclo[3.2.0]heptane-2-carboxylic acid (1, R=g)

To a solution of (9) (288 mg, 0.49 mmol) in tetrahydrofuran (7.5 ml) was added a solution of sodium bicarbonate (41 mg, 0.49 mmol) in water (7.5 ml) and 10% palladium on charcoal (300 mg). The reaction mixture was hydrogenated for 3 hours (at 1 atmosphere), after which it was filtered (celite). The filtrate was washed with ethyl acetate (2 x 20 ml) and evaporated to dryness to give (1, R=g) (69 mg, 47%). δ_H (500 MHz, D_2O) 1.35(3H, s, 3-CH_3), 1.47(3H, s, 3-CH_3), 1.48-1.66(4H, m, CH_2CH_2CO), 2.11-2.15(2H, m, CH_2CO), 2.82-2.87(2H, m, CHN), 4.07(1H, s, 2-H), 5.31, 5.38(2 x 1H, 2 x d, J 4 Hz, 5-H, 6-H); m/e (fast atom bombardment) 316 (MH^+).

Incubation of (1, R=b) with DAOC Synthetase/Hydroxylase

(1, R=b) (2 mg) was incubated with DAOC Synthetase/Hydroxylase following the general procedure. The product was isolated by h.p.l.c. of the crude incubation mixture:

6R,7R)-1-Aza-3-methyl-7-(m-carboxyphenylacetyl)-8-oxo-5-thiabicyclo[4.2.0]oct-2-ene carboxylic acid (2, R=b)

δ_H (500 MHz, D_2O) 1.77(3H, s, 3-CH_3), 3.06, 3.37(2H, ABq, J 18 Hz, SCH_2), 3.57, 3.63(2H, ABq, J 15 Hz, CH_2Ar), 4.89 and 5.39(2 x 1H, 2 x d, J 4.5 Hz, 6-H, 7-H), 7.31-7.43(2H, m, Ar-H), 7.74-7.77(2H, m, Ar-H); m/e (fast atom bombardment) 399 ($M+Na^+$). (2, R=b) showed antibacterial activity against Escherichia coli ESS. in the presence and absence of β-lactamase I from Bacillus cereus. Purification: h.p.l.c. details as for the isoltion of (2, R=c) except: Mobile phase: 2% CH_3CN/10mM ammonium bicarbonate; Retention time: 8.5 minutes.

Incubation of (1, R=c) with DAOC Sytnetase/Hydroxylase

(1, R=c) (2 mg) was incubated with DAOC Synthetase/Hydroxylase following the general procedure. The product was isolated by h.p.l.c. of the crude incubation mixture:

(6R,7R)-1-Aza-3-methyl-7-(5-carboxypentanamido)-8-oxo-5-thiabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (2, R=c)

δ_H (D_2O, 500 MHz) 1.46-1.53(4H, m, $CH_2CH_2CH_2CO$), 1.79(3H, s, 3-CH_3), 2.06-2.10, 2.22-2.30(4H, 2 x m, CH_2CO), 3.12, 3.47(2H, ABq, J 13 Hz, 7-H) and 5.44, 5.96 (2 x 1H, 2 x d, J 4.5 Hz, 6-H, 7-H). Purification: Reverse phase octadecylsilane h.p.l.c. (250 x 4.6 mm column); λ_{max} = 220 nm; Mobile phase: 50 mM ammonium bicarbonate (aqueous); Flow rate: 1ml min^{-1}; Retention time: 6.1 min. Bioassay: positive against S.aureus. (2, R=c) was derivatised using the following procedure: the product as obtained fom h.p.l.c. was dissolved in 2N hydrochloric acid (2 ml) and extracted with ethyl acetate (2 x 10 ml). Excess diazomethane in ether was then added to the organic extracts. The solution was stirred for 10 minutes and was evaporated to dryness to give the crude diester (5). δ_H (500 MHz, $CDCl_3$) 1.43-1.71 (4H, m, $CH_2CH_2CH_2CO$), 1.70(3H, s, 3-CH_3), 2.29-2.35(4H, m, 2 x CH_2CO), 3.24, 3.50(2H, ABq, J 18 Hz, 4-H), 3.68, 3.85(6H, 2 x s, 2 x OCH_3), 4.98(1H, d, J 4.5 Hz, 6-H), 5.79 (1H, dd, J 8.5, 4.5 Hz, 7-H), 6.18 (1H, d, J 8.5 Hz, NH); m/e (desorption chemical ionisation) 388 (32%, MNH_4^+), 371 (34, MH^+).

(2S,5R,6R)-1-Aza-3,3'-dimethyl-6-[4R)-4-(benzyloxycarbonylamino)-5-(p-nitrobenzyloxycarbonyl)-butanamido]-7-oxo-4-thiabicyclo[3.2.0]heptane-2-carboxylic acid p-nitrobenzyl ester (7)

To a solution of 6-aminopenicillanic acid p-nitrobenzyl ester (153 mg, 0.44 mmol) in dichloromethane (5 ml) were added 1-ethoxycarbonyl-2-ethoxy-dihydroquinoline (110 mg, 0.44 mmol), protected glutamic acid[15] (183 mg, 0.44 mmol) and anhydrous sodium sulphate (5 mg). The reaction mixture was stirred for 36 hours, after which it was evaporated to dryness. The residue was dissolved in ethyl acetate (50 ml), washed with 2N hydrochloric acid (25 ml), aqueous sodium bicarbonate solution (25 ml) and brine (25 ml), dried (Na_2SO_4), filtered and evaporated to dryness. Chromatography [flash silica (ethyl acetate/ dichloromethane)] gave (7) (112 mg, 34%) as a colourless oil. t.l.c. [ethyl acetate/dichloromethane, 1:4] Rf 0.4; ν_{max} (neat) 1786 s, 1750 bs, 1622 s, 1526 s, 1348 s, 1182 s, 940 m and 908 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 1.43(3H, s, 3-CH_3), 1.64(3H, s, 3-CH_3), 1.95-2.10(1H, m, CH_2CH_2CHN), 2.15-2.48(3H, m, CH_2CH_2), 4.38-4.50(1H, m, $NCHCO_2$), 4.49(1H, s, 2-H), 5.12(2H, ca s, CH_2Ar), 5.29(2H, ABq, J 13.5 Hz, CH_2Ar), 5.50(1H, d, J 4 Hz, 5-H), 5.71(1H, dd, J 9.5, 4 Hz, 6-H), 6.66(1H, d, J 9.5 Hz, NH), 7.29-7.32(5H, m, $CH_2C_6H_5$), 7.45-7.58(5H, m, $C_6H_4NO_2$, NH), 8.15-8.28(4H, m, $C_6H_4NO_2$). δ_C (62.85 MHz, $CDCl_3$) 27.0 (q, CH_3), 28.1(t, CH_2CH_2CO), 53.4(d), 58.8(d), 64.9(t), 65.7(t), 65.9(t) 67.2(t), 68.0(d), 70.4(d), 123.8-148.0(12 lines, Ar-C), 156.2, 171.2, 171.5, 173.6(5 x s, CO). m/e (desorption chemical ionisation) 735 (MH^+).

(2S,5R,6R)-1-Aza-3,3'-dimethyl-6-[(4R)-4-amino-4-carboxybutanamido]-7-oxo-4-thiabicyclo[3.2.0]-heptane-2-carboxylic acid (1, R=d)

To a solution of (7) (75 mg, 0.10 mmol) in tetrahydrofuran (5 ml) was added a solution of sodium bicarbonate (8.5 mg, 0.1 mmol) in water (5 ml) and 10% palladium on charcoal (50 mg). The reaction mixture was hydrogenated for 5 hours (at 1 atmosphere), after which it was filtered (celite). The filtrate was washed with ethyl acetate (2 x 25 ml) and evaporated to dryness to give crude (1, R=d), which was purified by reverse phase octadecylsilane h.p.l.c. (250 x 9.4 mm column); λ_{max} = 220 n.m.; to give pure 1 (R=f) (29 mg, 84%). Retention time 8.5 min; δ_H (500 MHz, D_2O) 1.33(3H, s, 3-CH_3), 1.44(3H, s, 3-CH_3), 1.89-1.96(2H, m, CH_2CH_2CO), 2.27-2.32(2H, m, CH_2CH_2CO), 3.53-3.55(1H, m, $NCHCH_2$), 4.04(1H, s, 2-H) and 5.30-5.37(2 x 1H, 2 x d, J 4 Hz, 5-H, 6-H) m/e (fast atom bombardment) 346 (MH^+).

REFERENCES

1. Part of this work has been published in a preliminary form: J.E. Baldwin, R.M. Adlington, J.B. Coates, M.J. Crabbe, J.W. Keeping, G. Knight, T. Nomoto, C.J. Schofield and H.-H. Ting, J.Chem.Soc., Chem.Commun., 1987, 374.

2. J.E. Baldwin, P.D. Singh, M. Yoshida, Y. Sawada, and A.L. Demain, Biochem.J., 1980, 186, 889.

3. J.E. Baldwin, J.W. Keeping, P.D. Singh, and C.A. Vallejo, Biochem.J., 1981, 194, 649.

4. J.E. Baldwin in "Recent Advances in the Chemistry of β-Lactam Antibiotics" (3rd. Internatinal Symposium), eds. A.G. Brown and S.M. Roberts, Royal Society of Chemistry Special Publication, No. 52, 1985, p.62.

5. J.E. Baldwin, E.P. Abraham, R.M. Adlington, J.B. Coates, M.J. Crabbe, N.P. Crouch, J.W. Keeping, G.C. Knight, C.J. Schofield, H.-H. Ting, C.A. Vallejo and T. Willis, Biochem.J., in press.

6. J.E. Baldwin and M.J. Crabbe, FEBS Letts., in press.

7. J. Kupka, Y-Q. Shen, S. Wolfe and A.L. Demain, FEMS Microbiol. Letts., 1983, 16, 1.

8. J.E. Baldwin, E.P. Abraham, G.A. Bahadur, B. Chakravarti, B.P. Domayne-Hayman, L.D. Field, S.L. Flitsch, G.S. Jayatilake, A. Spakovskis, H.-H. Ting, N.J. Turner, R.L. White, and J.J. Usher, J.Chem.Soc., Chem.Commun., 1984, 1225.

9. J.E. Baldwin, E.P. Abraham, G.L. Burge, and H.-H. Ting, J.Chem.Soc., Chem.Commun., 1985, 1908.

10. (a) J. Kupka, Y.-Q. Shen, S. Wolfe and A.L. Demain, Can.J.Microbiol., 1983, 29, 488; (b) A. Scheidigger, M.T. Kuenzi, and J. Neush J.Antibiot., 1984, 37, 522.

11. J.E. Baldwin, S.R. Herchen, and P.D. Singh, Biochem. J., 1980, 186, 881.

12. J.E. Baldwin, R.M. Adlington, M.J. Crabbe, T. Nomoto, and C.J. Schofield, J.Chem.Soc., Chem.Commun., submitted for publication.

13. J.E. Baldwin, R.M. Adlington, R.H. Jones, C.J. Schofield, C. Zaracostas and C.W. Greengrass, Tetrahedron, 1986, 42, 4879.

14. G.N. Freidlin, S.M. Zhenodanova, A.P. Chukus, and N.V. Fomina, Zh.Obsch.Khim., 1963, 33, 934.

15. J.S. Morley, J.Chem.Soc., Perkin I, 1967, 2410.