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SYNTHESIS OF 2(*R*)-[3(*S*)-ACYLAMINO-2-OXO-1-AZETIDINYLOXY]-ACETIC ACIDS

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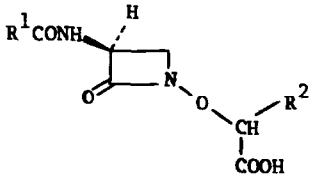
Abstract—The title compounds when constructed from α-aminoxyacetic esters coupled with N-benzyloxycarbonyl-L-serine in the presence of N-ethoxycarbonyl-2-ethoxy-1, 2-dihydroquinoline gave modest and variable yields of L-2-(benzyloxyformamido)-3-hydroxypropionamidooxy acetates. Alternative coupling with N-benzyloxycarbonyl-L-serine 3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl ester gave high yields of these acetates and cyclisation using triphenyl phosphine and dimethyl azodicarboxylate gave the fully protected monocyclic β-lactams. In the phenylacetic acid series hydrogenolytic deprotection led to decomposition. However, in the phenylpropionic series the hydrogenolysis product was sufficiently stable to allow acylation by the thiol ester route to the title compound.

Our interest in the synthesis of the title compounds (I) arose from investigations of the properties of a new class of antibacterial agents, the phosphonopeptides.[1]



I

Alafosfalin, L-alanyl-L-1-aminoethylphosphonic acid, had exhibited antibacterial synergy, *in vitro* and *in vivo* when used in combination with ampicillin, mecillinam and cephalexin.[2] By contrast, L-norvalyl-L-1-aminoethylphosphonic acid, showed different antibacterial synergy in combination with Nocardicin A (M. J. Hall*, W. J. Lloyd, D. Westmacott and P. Angehrn In press; *Antimicrobial Agents and Chemotherapy*).

Total synthesis of simplified variants of Nocardicin A (II) was therefore initiated. Our synthesis of a series of 3(*S*) - acylamino - 1[(phenyl)(1H - tetrazol - 5 - yl)amino] - 2 - azetidinones has been reported recently.[3] Our work in the "hydroxylamine" series (I) is reported herein.

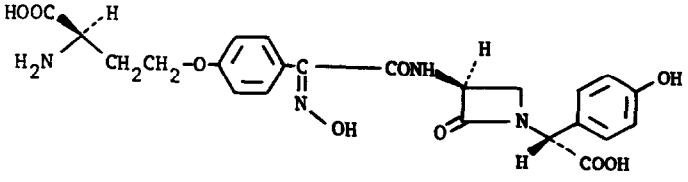
In an attempt to synthesise monocyclic β-lactams having higher reactivity than Nocardicin A (II) we set out to prepare compounds I which could be derived from hydroxylamines. Cyclisation of appropriate L-serine derivatives to N-hydroxy-β-lactams using triphenyl phosphine and diethyl azodicarboxylate had previously been described,[4] e.g. III, IV → V, VI and we were able to repeat these reactions.

Hydrogenolysis of VI gave VII.[4b] Treatment of VI with trifluoroacetic acid gave VIII and the by-product IX. Reaction of VI with toluenesulphonic acid gave X. β-Lactam nucleus VIII was further characterised by coupling with N-benzyloxycarbonyl-L-norvaline XI to give protected peptide XII. Hydrogenolysis of XII gave only the ring expanded product XIII.

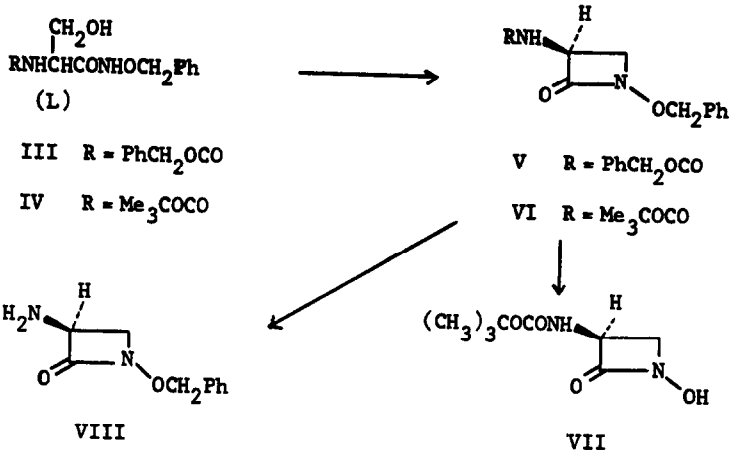
An attempt to synthesise precursors of the title compounds I by reacting VII with ethyl mandelate in a Mitsunobu reaction[5] failed to give XIV. No satisfactory reaction was obtained between models compounds N-hydroxybenzyl carbamate XV and ethyl α-chlorophenyl acetate XVI, 2-cyano-4-nitrochlorobenzene XVII or 2-ethoxycarbonyl-4-nitrochlorobenzene XVIII.

In an alternative approach to title compounds I, the β-lactam was constructed at a later stage as shown in Scheme 1. Carrying out the sequence from L-phenylalanine XIX [R = $PhCH_2$ (L)], gave the inverted intermediate XXIV [R = $PhCH_2$ (D)] in the yields shown.[6] Coupling of XXIV with N-benzyloxycarbonyl-L-serine XXV, using the N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) method, gave XXVI in 54% yield. This XXVI underwent Mitsunobu reaction[5] to give a 70% yield of β-lactam XXVII after chromatography. The precursor [XX, R = Ph (D, L)] of a closer analogue of Nocardicin A II could not be obtained as shown in Scheme 1, but the racemic material was easily obtained by bromination of phenylacetic acid. (In this instance racemisation would probably occur on nucleophilic displacements.)

As was expected, the model methyl ester XXVII could not be hydrolysed without destruction of the β-lactam ring and the synthesis of alternative esters was necessary. Benzyl esters XXVIII and XXIX were prepared as toluenesulphonic acid salts in high yields (> 85%) from XXIII [R = $PhCH_2$(D) and R = Ph (D, L)] using the conventional Dean and Stark azeotrope method.



Nocardicin A II



(L)

III R = $PhCH_2OCO$

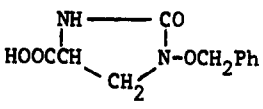
IV R = Me_3COCO

V R = $PhCH_2OCO$

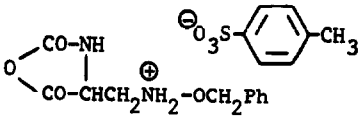
VI R = Me_3COCO

VIII

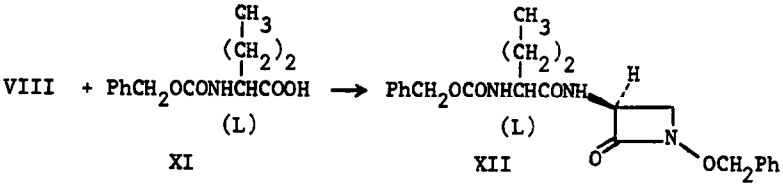
VII



IX

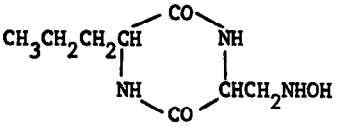


X

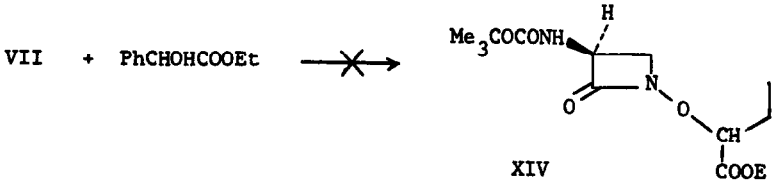


XI

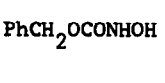
XII



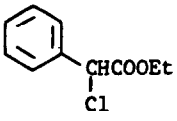
XIII



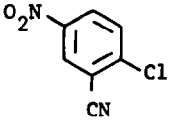
XIV



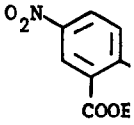
XV



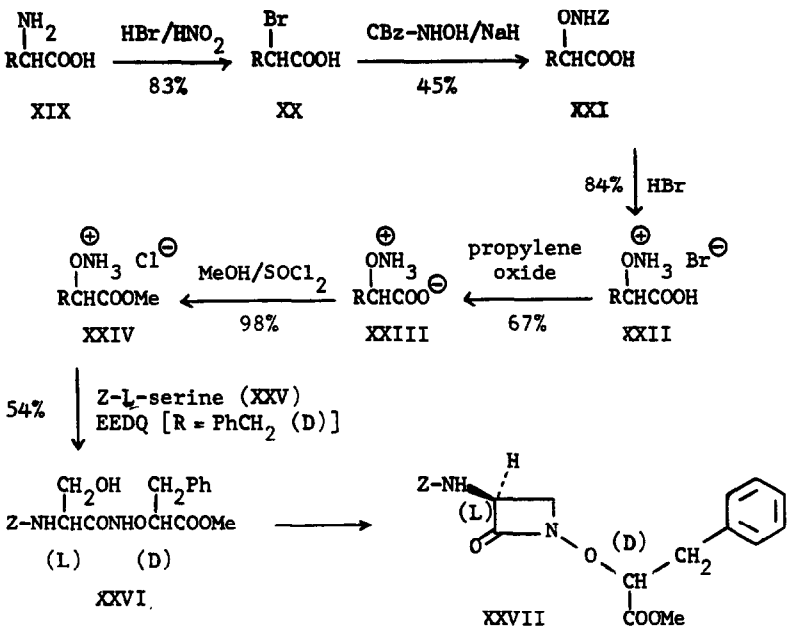
XVI



XVII



XVII

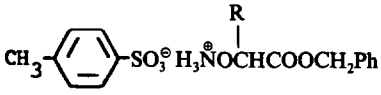


XIX XX XXI XXIV XXIII XXII XXVI XXVII

Scheme 1.

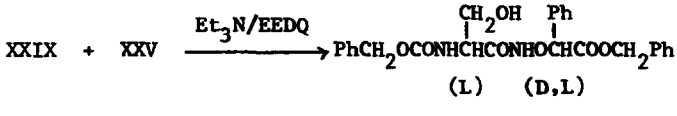
Coupling of XXIX with N-benzyloxycarbonyl-L-serine XXV in the presence of triethylamine and N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) gave a complex mixture which on chromatography gave fractions containing the required XXX as judged by proton NMR. The low yield and complex work up prompted us to re-examine the problem of synthesis of serine derivatives when the hydroxyl group was unprotected. Literature searches showed that active esters of type XXXI had been used[7] as additives in racemisation inhibitor studies on the dicyclohexylcarbodi-imide condensation of carboxyl and amino groups. They exhibited high carbonyl frequencies at 1810–1815 cm^{-1} and were stable to OH groups. Serine derivative XXXII had been synthesised though not examined in detail. The required heterocycle was obtained by the literature route[7] and reacted with Z-L-serine XXV and dicyclohexylcarbodiimide to give XXXII in 89% yield. Active ester XXXII was then reacted with XXIX to give XXX as the sole product. Cyclisation of XXX in a modified Mitsunobu reaction[3,5] gave the fully protected monocyclic β-lactam XXXIII. This compound proved unstable to extended chromatography on silica gel.

A rapid chromatographic procedure gave substantially pure XXXIII as a mixture of diastereoisomers (TLC, NMR). Attempted selective hydrogenolysis of this model compound in the presence of potassium bicarbonate, followed by *in situ* coupling[3] of XXXIV with active ester XXXV[8] failed to give any of the initial target compound XXXVI.

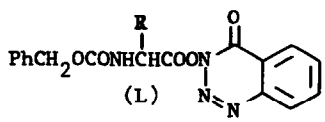


XXVIII [R = $PhCH_2$ (D)]

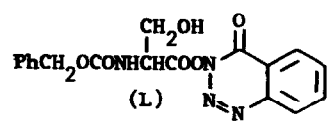
XXIX [R = Ph (D,L)]



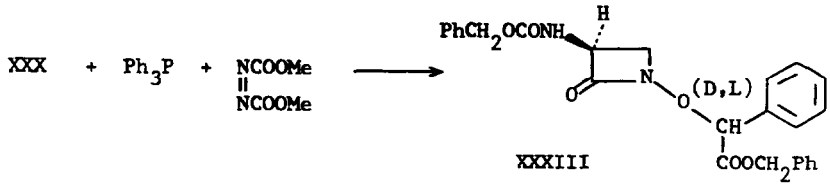
XXX



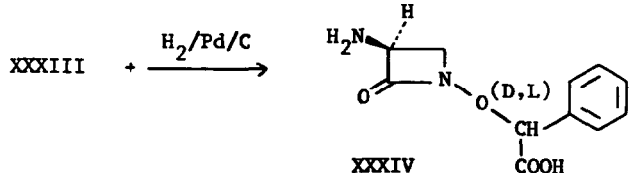
XXXI



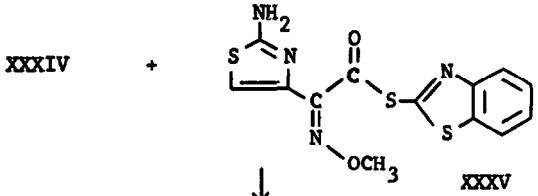
XXXII



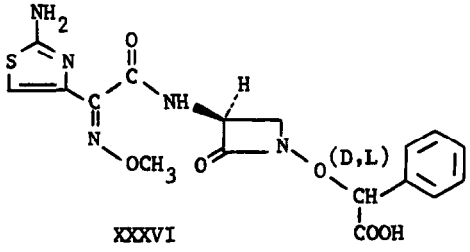
XXXIII



XXXIV



XXXV



XXXVI

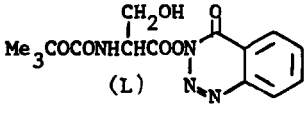
Hydrogenolysis of XXXIII to XXXIV in the presence of sodium acetate gave a mixture of five products (TLC) which on *in situ* reaction with XXXV gave no β-lactam (IR). Deprotection in the presence of acetic acid, see below, and coupling with XXXV similarly did not give any XXXVI.

Synthesis of alternative intermediates to XXXIII with t-butoxycarbonyl (BOC) protection of the amino function and *p*-methoxybenzyl or t-butyl ester protection of the carboxylic acid was therefore examined. These groups would allow trifluoroacetic acid deprotection, which had worked satisfactorily for VI to VIII above, and thus avoid possible hydrogenolytic cleavage of the benzylic 1-azetidinyloxy-acetic acid (N–O≋CH(Ph)-COOH) bond. Briefly, the BOC-L-seryl active ester XXXVII, which is analogous to XXXII was prepared by the same method and the initial reaction product was identified by the characteristic IR spectrum at 1822 cm^{-1} compared to 1810–1815 cm^{-1} reported[7] for XXXII above. This material could not be crystallised and characterised, as for XXXII. Direct coupling of the crude product XXXVII with XXXVIII [prepared as for XXIX above, but not isolated], as for XXIX + XXXII→XXX above, did not give any of the desired XXXIX. The relative instability of the active ester XXXVII was thought to be responsible for this result and this approach was therefore abandoned.

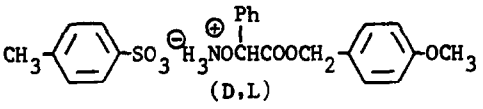
Coupling of the benzyl ester XXVIII [R = $PhCH_2$ (D)], derived from L-phenylalanine, with active ester XXXII gave XL as sole product in 90% yield.

Modified Mitsunobu cyclisation[3,5] gave monocyclic β-lactam XLI in 79% yield. Catalytic hydrogenolysis of XLI in ethanol gave the deprotected β-lactam XLII which was somewhat unstable in aqueous ethanol at ambient temperature. Attempted coupling of XLII with thiol ester XXXV, using triethylamine as base in dimethylformamide, gave only the trivial dimethylamide XLIII.

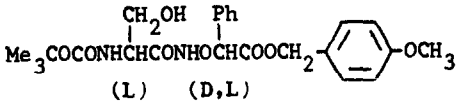
This result was surprising since this active ester



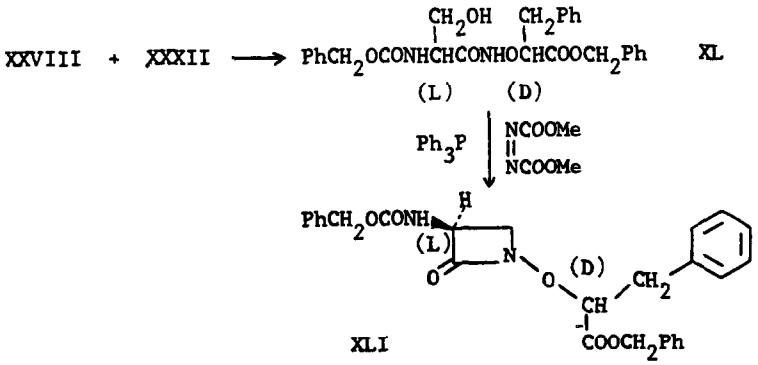
XXXVII



XXXVIII

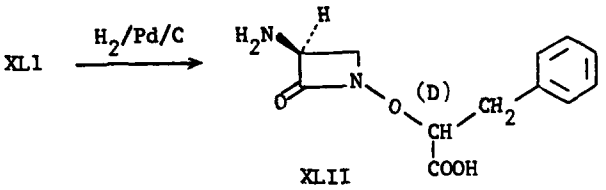


XXXIX

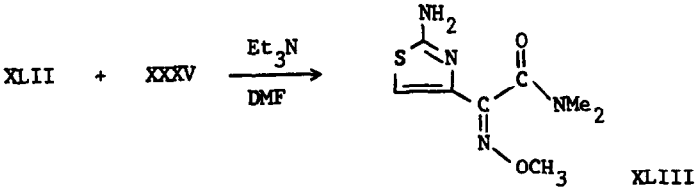


XL

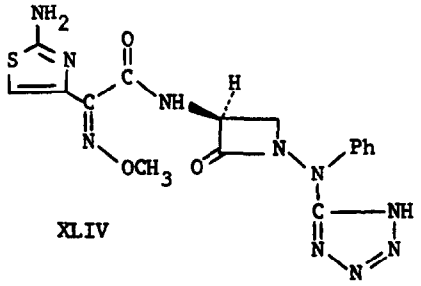
XLI



XLII



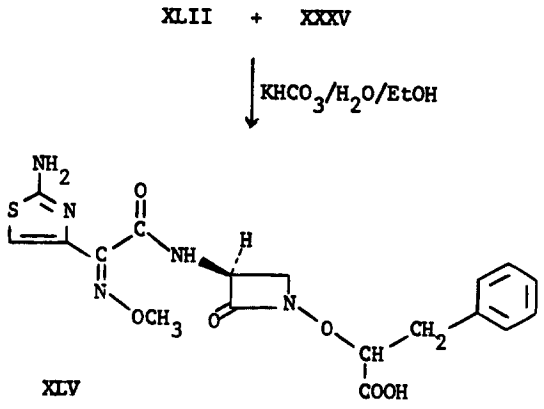
XLIII



XLIV

XXXV and conditions had previously led to analogous monocyclic β-lactam XLIV.[3] Coupling in aqueous-ethanol with potassium bicarbonate as base did however give the desired XLV. The stability problem with XLII was subsequently overcome by carrying out the hydrogenolysis in the presence of one mole proportion of acetic acid.

β-Lactams XLII and XLV were solids which could not be crystallised. The IR spectra showed characteristic β-lactam CO absorptions of 1815 cm^{-1} and 1790 cm^{-1} respectively. The ^1H NMR spectra of all the β-lactams prepared showed characteristic β-lactam resonances. The 300 MHz proton spectrum was particularly useful in the case of XLV, compared to the 100 MHz spectrum, and led to assignment of all chemical shifts and coupling constants. Fast atom bombardment mass



XLV

spectrometry[3,9,10] gave an $(M + H)^+$ ion for XLV and an accurate mass determination led to confirmation of its molecular composition.

EXPERIMENTAL

NMR spectra were recorded on a Bruker WM 300 and on a Varian XL 100/15 spectrometer and chemical shifts (δ) are presented in ppm from internal TMS. Mass spectra were obtained using a Kratos MS 902 mass spectrometer in the normal electron impact mode or fitted with a Kratos Fast Atom Bombardment (FAB) source. +ve and −ve ion FABMS were recorded. Samples were mixed with glycerol on the probe tip from acetone, dimethylformamide or dimethyl sulphoxide solutions. Source accelerating voltage 8 kV. Multiplier gain 10^5.

IR spectra were determined on a Pye–Unicam SP 1000 spectrometer and elemental analyses were carried out on a Perkin–Elmer Model 240 instrument. TLC was carried out using glass-supported silica gel 60 plates.

Optical rotations were measured on a Perkin–Elmer Model 141 polarimeter. Mps were recorded on a Buchi melting point apparatus and are uncorrected.

3(S)-*Amino*-1-*benzyloxy*-2-*azetidinone* (VIII). Compound VI[4c] (1.46 g, 5 mmol) was stirred in trifluoroacetic acid (6 ml) at room temp for 10 min when there was a vigorous evolution of gas. The mixture was evaporated *in vacuo* and the residual oil was triturated with ether to give 0.83 g of crude product, m.p. 126–128° (dec). Recrystallisation from a mixture of EtOAc (12 ml) and ether gave 0.43 g of VIII, m.p. 131.5–133°. (Found: C, 50.1; H, 4.6; N, 10.3. $C_{10}H_{12}N_2O_2.0.73TFA$ Requires: C, 50.2; H, 4.4; N, 10.2%); ν_{max} (KBr) 1760, 1780 cm^{-1}; δ ^1H (DMSO-d_6) 3.52 (1H, dd, H-4), 3.78 (1H, t, H-4), 4.36 (1H, dd, H-3), 5.00 (2H, s, $PhCH_2O$), 7.3 (5H, m, Ar), 8.8 (3H, broad, NH_3^+).

1-(*Benzyloxy*)-2-*oxo*-4-*imidazolidinecarboxylic acid* (IX). Compound VI[4c] (5.8 g, 20 mmol) was stirred with trifluoroacetic acid (25 ml) for 5 min and the mixture was evaporated *in vacuo* and re-evaporated toluene. The residue was crystallised from a mixture of EtOAc (20 ml) and ether (60 ml) to give 2.9 g of VIII. 0.93 TFA salt. The mother liquors were evaporated and triturated with ether to give 1.2 g of a product having m.p. 168–171° (dec). Recrystallisation from a mixture of EtOAc (25 ml) petroleum ether, b.p. 40–60° (40 ml) gave 0.85 g of product assigned structure IX. (Found: C, 55.5; H, 5.2; N, 11.8. $C_{11}H_{12}N_2O_4$ Requires: C, 55.9; H, 5.1; N, 11.9%); ν_{max} (KBr) 1713 cm^{-1} (no β-lactam); δ ^1H (DMSO-d_6) 3.38 (1H, q, 1H, $CHCH_2N$), 3.54 (1H, t, $CHCH_2N$), 4.06 (1H, dd, $CHCH_2$), 4.88 (2H, s, OCH_2Ph), 7.38 (5H, m Ar), 7.62 (1H, s, NH). The resonance at 7.62 was eliminated by deuterium exchange.

4-[(*Benzyloxyamino*)*methyl*]-2,5-*oxazolidinedione* p-*tosylate* (X) Compound VI[4c] (0.585 g, 2 mmol) was dissolved in ether (30 ml) and treated with toluene-4-sulphonic acid monohydrate (0.36 g, 1.9 mmol) in ether (20 ml). There was no evolution of gas. After 10 min a white solid was precipitated, 0.21 g, m.p. 124–6°. Evaporation of the filtrat gave a further 0.52 g, m.p. 112–116°. (Found: C, 52.5; H, 4.8; N 7.0 $C_{18}H_{20}N_2O_7S$ Requires: C, 52.9; H, 4.9; H, 6.9%); ν_{max}(KBr 1780 (strong), 1840, 1860 cm^{-1}; δ ^1H (DMSO-d_6), 2.3 (3H, s p-tosyl CH_3), 3.4 (1H, q, $CHCH_2NH_2$), 3.57 (1H, t, $CHCH_2NH_2$ 4.1 (1H, dd, $CHCH_2$), 4.87 (2H, s, OCH_2Ph), 7.15 (2H, d, tosy Ar), 7.38 (5H, m, Ph), 7.55 (2H, d, tosyl Ar). On these data th product was assigned structure X.

Benzyl-[1(S)-[(1-*benzyl*-2-*oxo*-3(S)-*azetidinyl*)*carbamoyl butyl*]-*carbamate* (XII). N-Benzyloxycarbonyl-L-norvaline (3 g, 12 mmol) and VIII (3 g, 10 mmol) were stirred i CH_2Cl_2 (25 ml). The suspension was cooled to −10° an Et_3N (1.7 ml, 12 mmol) was added dropwise. After 10 mi a soln of N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinolin (EEDQ) in CH_2Cl_2 (15 ml) was added rapidly. The mixtur was stirred for 1 hr at −10° then overnight at room temp The resulting clear soln was washed with 15% $KHCO_3$ a (3 × 20 ml) and then with water (20 ml), dried ov Na_2SO_4 and evaporated to give a white solid. This wa triturated with ether and filtered to give 3.6 g of XII, m.p 147–149°. A sample of 0.5 g was recrystallised from EtOA (15 ml) to give 0.41 g of pure XII, m.p. 149–150°. (Found: C, 64.2 H, 6.25; N, 9.9. $C_{23}H_{27}N_3O_5$ Requires: C, 64.9; H, 6.4; N, 9.9%) ν_{max} ($CHCl_3$) 1677, 1707, 1770 cm^{-1}, $[\alpha]_D^{20}$ − 9.1° (c = 1%, MeOH) *m/e* 425 (M); δ ^1H ($CDCl_3$) 0.87 (3H, t, CH_2CH_3), 1.34 (2H, m CH_2CH_3), 1.62 (2H, m, $CH_2CH_2CH_3$), 3.19 (1H, dd, H-4 o lactam), 3.50 (1H, t, H-4), 4.15 (1H, m, $NHCHCO$), 4.60 (1H, m H-3), 4.93 (2H, s, OCH_2Ph), 5.08 (2H, s, OCH_2Ph), 5.43 (1H broad doublet, NH), 7.32 (5H, m, Ar), 7.37 (5H, m, Ar). Th resonance at 5.43 was sharpened to a distinct double by deu terium exchange.

3-[(*Hydroxyamino*)*methyl*]-6-*propyl*-2,5-*piperazinedione* (XIII) The XII (3 g, 7 mmol) prepared above was suspended i MeOH, 10% Pd–C catalyst (0.5 g) was added and a soda lime tra was fitted. The mixture was hydrogenated for 2 hr when 14 mmo had been absorbed. Catalyst was filtered off and the filtrate wa evaporated to give 1.02 g of crude product, m.p. 331–335° (dec) Recrystallisation from a mixture of MeOH (15 ml) and water (3 ml gave 0.49 g of product having m.p. 343–345° (dec). (Found: C 44.3; H, 7.1; N, 19.0. $C_8H_{15}N_3O_8$ Requires: C, 47.7; H, 7.5; N 20.9%, but ratio correct for $C_8H_{15}N_3O_8$); ν_{max} 1680 cm^{-1} (n β-lactam); *m/e* 201 $(M)^+$; δ ^1H (D_2O) 0.93 (3H, t, CH_2CH_3), 1.4 (2H, m, CH_2CH_3), 1.82 (2H, m, $CH_2CH_2CH_3$), 3.31 (2H, m $CHCH_2NHOH$), 4.15 (1 H, m, $CHCH_2CH_2$), 4.37 (1 H), m $CHCH_2NHOH$). On the basis of these data the product wa assigned structure XIII.

Methyl D-*alpha*-[[L-2-(*benzyloxyformamido*)-3-*hydroxypropionamido*]-*hydrocinnamate* (XXVI). Methyl D-2-aminoxy-3-phenylpropionate hydrochloride[b] (5.8 g, 25 mmol) and N-benzyloxycarbonyl-L-serine (6.0 g, 25 mmol) were stirred with CH_2Cl_2 (125 ml) and cooled to −5°. Et_3N (3.5 ml, 25 mmol) and a soln of EEDQ (7.4 g, 30 mmol) in CH_2Cl_2 (25 ml) was added a −5°. The mixture was stirred for 1 hr at −5° then overnight a room temp. The resulting clear soln was washed with wate (2 × 60 ml) and ice-cold 2N H_2SO_4 (3 × 30 ml) and the aqueous

layers were back washed with CH_2Cl_2 (30 ml). The combined organic extracts wree washed with 15% $KHCO_3$ (2 × 30 ml) and again back-washed with CH_2Cl_2 (30 ml). The combined CH_2Cl_2 extracts were dried over Na_2SO_4, filtered and evaporated to give 5.7 g (54%) of XXVI, m.p. 133–135°. Recrystallisation from EtOAc gave pure XXVI, m.p. 138–140°. (Found: C, 60.3; H, 5.9; N, 6.8. $C_{21}H_{24}N_2O_7$ Requires: C, 60.6; H, 5.8; N, 6.7%), $[\alpha]_D^{20}$ + 30.7°(c = 1%, MeOH); *m/e* 416 $(M)^+$; δ ^{1}H ($CDCl_3$) 3.18 (2H, d, $CHCH_2Ph$), 3.64 (3H, s, COOMe), 3.74 (2H, m, CH_2OH), 4.18 (1H, m, $CHCH_2OH$), 4.70 (1H, t, $CHCH_2Ph$), 5.06 (2H, s, $PhCH_2O$), 5.94 (1H, d, NH), 7.20 (5H, m, Ar), 7.27 (5H, m, Ar).

Methyl D - *alpha*[3(S) - (*benzyloxyformamido*) - 2 - *oxo* - 1 - *azetidinyl*]*oxy*]-*hydrocinnamate* (XXVII). Triphenylphosphine (2.9 g, 11 mmol) was stirred in CH_2Cl_2 (50 ml) at room temp and a soln of dimethyl azodicarboxylate (1.6 g, 11 mmol) in CH_2Cl_2 (10 ml) was added rapidly dropwise. The XXVI (4.2 g, 10 mmol) prepared above was added and the mixture was stirred overnight. The mixture was stirred with water (25 ml) for 1 hr and the solvent phase was washed with water (2 × 25 ml). The organic extract was dried over Na_2SO_4 and evaporated to give 7.2 g of an oil. This material was chromatographed on a column of Kieselgel (150 g, 46 × 3.2 cm) in 50% EtOAc-petrol to give 3.85 g of product as an oil. Trituration with ether gave 2.6 g of pure XXVII, m.p. 80–82° (66%). (Found: C, 63.2; H, 5.4; N, 7.0. $C_{21}H_{22}N_2O_6$ Requires: C, 63.3; H, 5.6; N, 7.0%); ν_{max} ($CHCl_3$) 1790 cm^{-1} (β-lactam), 1730 cm^{-1} (ester); *m/e* 399$(M+H)^+$, $[\alpha]_D^{20}$ +34.7° (c = 1%, MeOH); δ ^{1}H ($CDCl_3$) 3.01 (2H, d, $CHCH_2Ph$), 3.39 (1H, dd, H-4 *Trans*), 3.68 (3H, s, COOMe), 3.75 (1H, t (part obscured by COOMe), H-4 *cis*), 4.52 (1H, m, H-3), 4.72 (1H, t, $CHCH_2Ph$), 5.09 (2H, s, OCH_2Ph), 5.38 (1H, d, NH), 7.24 (5H, m, Ar), 7.32 (5H, m, Ar). Treatment of the ether filtrate with petrol gave a further 0.18 g (4.5%) of XXVII, m.p. 76–78°.

*Benzyl-*D*-2-aminoxy-3-phenylpropionate* p-*toluenesulphonate* (1:1) (XXVIII). A mixture of D-aminoxyphenylpropionic acid[6] (9.05 g, 50 mmol) (XXIII, R = $PhCH_2$, D), toluenesulphonic acid monohydrate (10.5 g 55 mmol), benzyl alcohol (60 ml) and toluene (140 ml) was refluxed for 2 hr under a Dean and Stark water separator. The mixture was cooled and evaporated *in vacuo* and the partially solid residue was treated with ether (300 ml) and refrigerated. The solid was filtered off to give 19.3 g (87%) of pure XXVIII, m.p. 163–166°. (Found: C, 62.0; H, 5.6; N, 3.1. $C_{23}H_{25}NO_6S$ Requires: C, 62.3; H, 5.7; N, 3.2%), $[\alpha]_D^{20}$ + 50.8°(*c* = 1%, MeOH); *m/e* 271 (M for free base); δ ^{1}H (DMSO-d_6) 2.0 (3H, s, tosyl-CH_3), 3.06 (2H, d, $CHCH_2Ph$), 4.94 (1H, t, $CHCH_2Ph$), 5.09 (2H, s, OCH_2Ph), 6.98–7.5 (14H, m, Ar), 8.14 (3H, broad, $\overset{+}{N}H_3$). The resonance at 8.14 was eliminated by deuterium exchange.

Benzyl D,L-α-*aminoxy-phenylacetate* p-*toluenesulphonate* (1:1) (XXIX). A mixture of α-D,L-aminoxyphenylacetic acid[12] [XXIII, R = Ph (D,L)] (8.35 g, 50 mmol), 4-toluenesulphonic acid monohydrate (10.5 g, 55 mmol) and benzyl alcohol (60 ml) was refluxed under a Dean and Stark trap for 1.8 hr. Evaporation and trituration of the residue with ether gave 18.3 g (85%) of pure XXIX, m.p. 175–177°. (Found: C, 61.25; 5.5; N, 3.25. $C_{22}H_{23}NO_6S$ Requires: C, 61.5; H, 5.4; N, 3.3%); *m/e* 257 (M for free base); δ ^{1}H (DMSO-d_6) 2.24 (3H, s, tosyl CH_3), 5.19 (2H, s, OCH_2Ph), 5.63 (1H, s, CHPh), 6.99–7.56 (14H, m, Ar), 8.7 (3H, broad, $\overset{+}{N}H_3$). The resonance at 8.7 was eliminated by deuterium exchange.

Benzyl D,L-2-[L-2-(*benzyloxyformamido*)-3-*hydroxypropionamidooxy*]-*phenyl acetate* (*XXX*). The *p*-tosyl compound XXIX (8.6g, 20 mmol) was stirred in CH_2Cl_2 (150 ml) and cooled to −15°. Et_3N (2.8 ml, 20 mmol) was added to give an almost clear soln. The active ester[7] XXXII (7.7 g, 20 mmol) was added. The mixture was stirred for 1 hr at −10°, then overnight at room temp. The solid by-product [2.2 g, m.p. 192–4° (dec)] was filtered off and the filtrate was washed with N HCl (2 × 50 ml), water (50 ml), 15% $KHCO_3$ (3 × 50 ml). The aqueous layers were back-washed with CH_2Cl_2 and the combined organic extracts were dried over Na_2SO_4, filtered and evaporated to give the desired XXX as a gum, 9.1 g 95%). δ ^{1}H ($CDCl_3$) 3.76 (2H, m, CH_2OH), 4.31 (1H, m, $CHCH_2OH$), 5.14 (2H, s, OCH_2Ph), 5.24 (2H, s, OCH_2Ph), 5.58 (1H, s CHPh), 5.90 (1H, broad doublet, NH), 7.22 (15H, m, Ar). This material was used without further purification in the next step to give XXXIII.

Benzyl [1-[DL-*alpha*-(*benzyloxycarbonyl*)*benzyloxy*]-2-*oxo*-3 (S)-*azetidinyl*]-*carbamate* (XXXIII). Triphenyl phosphine (5.8 g, 22 mmol) was stirred in CH_2Cl_2 (100 ml) at 25° as a soln of dimethyl azodicarboxylate (3.2 g, 22 mmol) in CH_2Cl_2 (20 ml) was added. The XXX (9.1 g, *ca* 20 mmol) prepared as above, was added in a soln of CH_2Cl_2 (10 ml) during a period of 2 min. The mixture was stirred for 6 hr then stood overnight. The mixture was then stirred with water (50 ml) for 1 hr and the organic layer was washed with water (5 × 50 ml). The aqueous layers were back-washed with CH_2Cl_2 (50 ml). The combined organic extract was dried over Na_2SO_4 and evaporated to give *ca* 14 g of a gum. This was dissolved in 40 ml of a mixture of EtOAc (40 parts) and petroleum ether (b.p. 40–60°) (60 parts). After standing for 1 hr the resulting triphenylphosphine oxide by-product (2.6 g, m.p. 148–157°) was filtered off. The filtrate was chromatographed on Kieselgel (300 g) in EtOAc-petroleum ether (b.p. 40–60°) (6:4) solvent ratio) elution being complete within 3 hr. Total β-lactam containing fractions (ν_{max} 1785 cm^{-1}) amounted to 5.98 g (65%). A middle cut of 0.98 g was pure XXXIII. (Found: C, 67.4; H, 5.9; N, 5.9. $C_{26}H_{24}N_2O_6$ Requires: C, 67.8; H, 5.25; N, 6.1%); *m/e* (electron impact) 460 $(M)^+$, *m/e* 460.1641 ($C_{26}H_{24}N_2O_6$ = 460.1632, 0.2%); 432 $(M-CO)^+$, *m/e* 432.1684 ($C_{25}H_{24}N_2O_5$ = 432.1683, 0.4%); 91 $(PhCH_2)^+$, 100%, *m/e* (FAB) 461 $(M+H)^+$, 7%; 91 $(PhCH_2)^+$, 100%; δ ^{1}H ($CDCl_3$) 3.33 and 3.46 (1H, 2 × dd, H-4 diastereoisomers), 3.74 (1H, m, H-4 diastereoisomers), 4.54 (1H, m, H-3), 5.10 (2H, s, $PhCH_2OCO$), 5.20 and 5.23 (2H, 2s, $COOCH_2Ph$ diastereoisomers), 5.57 and 5.60 (1H, 2s, CHPh diastereoisomers), 7.23 (15H, m, Ar), 7.58 and 7.70 (1H, m, NH).

Benzyl D - 2 - [L - 2 - (*benzyloxyformamido*) - 3 - *hydroxypropionamido-oxy*]-*phenylpropionate* (XL). The *p*-tosyl salt XXVIII (8.9 g, 20 mmol) prepared above was stirred in CH_2Cl_2 (150 ml) at −10°. Et_3N (2.8 ml, 20 mmol) and then active ester XXXII (7.7 g, 20 mmol) were added. Soln was effected within 30 min. A cream coloured solid then precipitated. The mixture was stirred at room temp overnight and the solid by-product was filtered off [2.1 g, m.p. 193–5° (dec)]. The filtrate was washed with water (50 ml), N HCl (2 × 50 ml), water and finally 15% $KHCO_3$ (3 × 50 ml). The $KHCO_3$ was back-washed with CH_2Cl_2 (50 ml). The combined organic extract was dried over Na_2SO_4, filtered and evaporated to give a solid. Trituration with ether and filtration gave 7.4 g (75%) of pure XL, m.p. 118–121°. (Found: C, 65.4; H, 5.7; N, 5.7. $C_{27}H_{28}N_2O_7$ Requires: C, 65.8; H, 5.7; N, 5.7%), $[\alpha]_D^{20}$ + 43.7° (*c* = 1%, MeOH); *m/e* 493 $(M+H)^+$; δ ^{1}H ($CDCl_3$) 3.14 (2H, d, $CHCH_2Ph$), 3.74 (2H, m, CH_2OH), 4.18 (1H, m, $CHCH_2OH$), 4.79 (1H, t, $CHCH_2Ph$), 5.08 (4H, s, 2 × OCH_2Ph), 5.84 (1H, d, NH), 7.22 (15 H, m, Ph).

Evaporation of the mother liquors and re-evaporation with EtOAc, followed by trituration with ether gave a further 1.55 g (15.6%) of XL, m.p. 108–111°, total yield 90%.

Benzyl [1-[1(R)-(*benzyloxycarbonyl*)*phenethyloxy*]-2-*oxo*-3(S)-*azetidinyl*]-*carbamate* (XLI). Triphenyl phosphine (2.88 g, 11 mmol) was stirred in CH_2Cl_2 (30 ml) at room temp. Dimethyl azodicarboxylate (1.6 g, 11 mmol) in CH_2Cl_2 (10 ml) was added rapidly dropwise when the temp rose from 19 to 26°. The benzyl ester XL (5.0 g, 10 ml), prepared as above, in CH_2Cl_2 was added rapidly dropwise. The mixture was stirred overnight. Water (25 ml) was added and the mixture was stirred for 1 hr. The organic layer was washed with water (2 × 25 ml) which was back-washed with CH_2Cl_2 (25 ml). The combined CH_2Cl_2 extract was dried over Na_2SO_4, filtered and evaporated and re-evaporated with EtOAc. The residue was dissolved in ether (30 ml) and cooled to give 5.15 g of crude solid A.

The mother liquors on evaporation gave an oil which partly solidified; solid B. Crude solid A was treated with 60% EtOAc–petrol (100 ml) and filtered to give 2.2 g of pure XLI, m.p. 108–110°. (Found: C, 68.6; H. 5.6: N, 5.9. $C_{27}H_{26}N_2O_6$ Requires: C, 68.3; H, 5.5; N, 5.9%), $[\alpha]_D^{20}$ + 31.2° (*c* = 0.2%, MeOH); ν_{max} ($CHCl_3$) 1790 cm^{-1}; 1730 cm^{-1}; *m/e* 475 $(M+H)^+$; δ ^{1}H($CDCl_3$) 3.11 (2H, d, $CHCH_2Ph$), 3.34 (1H, dd, H-4 *trans*), 3.67 (1H, t, H-4 *cis*), 4.46 (1H, m, H-3), 4.75 (1H, t, $CHCH_2Ph$), 5.07 (2H, s, OCH_2Ph), 5.11 (2H, s,

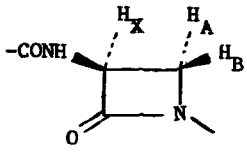
OCH_2Ph), 5.46 (1H, broad doublet, NH), 7.28 (15H, m, Ph).

Crude solid B was combined with the filtrate from the pure XLI obtained above and chromatographed on Kieselgel (100 g) in 60% EtOAc–petrol (b.p. (40–60°) to give a further 1.55 g of pure XLI, m.p. 110–112°. Total yield of pure XLI was 3.8 g (79%).

2(R)-[3(S) - *Amino* - 2 - *oxo* - 1 - *azetidinyloxy*] - 3 - *phenylpropionic acid* (XLII). Protected β-lactam XLI (0.48 g, 1.0 mmol) was taken up in EtOH (30 ml) and 10% Pd–C catalyst (20 mg) in EtOH (10 ml) was added. The mixture was hydrogenated at ambient temp and pressure for *ca* 3 hr. Fresh catalyst was added and the reaction was continued for a further 1.5 hr. Catalyst was filtered off and washed well with EtOH and then with water. The combined filtrate was evaporated to give 0.16 g m.p. 164–170° (dec) of crude product (Crop 1). Further extraction of the catalyst with EtOH gave 0.06 of similar product. Trituration of Crop 1 with acetone (10 ml) gave 0.075 g of pure product XLII, m.p. 167–170° (dec). (Found: C, 56.1; H, 5.5; N, 10.75. $C_{12}H_{14}N_2O_4.0.5H_2O$ Requires: C, 55.6; H, 5.8; N, 10.8%), $[\alpha]_D^{20}$ + 51.7° (*c* = 0.2%, H_2O); ν_{max} (nujol) 1815 cm^{-1}; *m/e* 250.0968 ($C_{12}H_{14}N_2O_4$ = 250.0952), *m/e* (Fast Atom Bombardment) 251 $(M+H)^+$ (37%), 91 $(PhCH_2)^+$, (100%); δ ^{1}H(DMSO-d_6) 3.06 (2H, m, $CHCH_2Ph$), 3.18 (1H, m, H-4), 3.76 (2H, m, H-4 and H-3), 4.60 (1H, t, $CHCH_2Ph$), 7.28 (5H, m, Ph).

2(R) - [1(S) - [2 - (2 - *Amino* - 4 - *thiazolyl*) - 2(Z) - (*methoxyimino*) *acetamido*] - 2 - *oxo* - 1 - *azetidinyloxy*] - 3 - *phenylpropionic acid* (XLV). The deprotected β-lactam XLII (1.0 mmol) was freshly generated as a soln in aqueous EtOH as described above and was cooled to 0°. $KHCO_3$ (0.1 g, 1.0 mmol) was added with stirring. The active ester[8] XXXV (0.35 g, 1.0 mmol) was added and the mixture was stirred and slowly allowed to come to room temp then stirred overnight. The solvent was evaporated to give a gum which was partitioned between CH_2Cl_2 (20 ml) and water (20 ml). The organic layer was washed with water (2 × 10 ml) and the aqueous fractions were back extracted with CH_2Cl_2 (2 × 10 ml). TLC on silica gel, eluting with n-BuOH (12):CH_3COOH (3):H_2O (5), showed that the product was only to be found in the original aqueous extract. This soln (pH 8–9) was carefully acidified with 5N HCl to pH 2–3 to give a cream coloured ppt which was filtered off (0.075 g). The filtrate was evaporated to give 0.31 g of a gummy solid which was triturated with EtOH and filtered to give 0.086 g of XLV, m.p. *ca* 215° (dec). This material was a single spot in the above TLC system, having R_f 0.6. ν_{max} (nujol) 1790 cm^{-1}. Elemental analysis showed that XLV ($C_{18}H_{19}N_5O_6S$) was contaminated with KCl but had an elemental ratio of $C_{18.0}$; $H_{19.7}$; $N_{4.94}$. *m/e* (FAB, +ve ion mode) 434 $(M+H)^+$, 50%; 126 ($C_4H_4N_3S$), 100%. *m/e* (FAB, −ve ion mode) 432 $(M-H)^-$, *m/e* 432.1007, ($C_{18}H_{18}N_5O_6S$ = 432.0978); δ ^{1}H (DMSO-d_6) at 300 MHz 3.07 (2H, 8 lines, $CHCH_2Ph$), 3.46 (1H, dd, H-4), 3.80 (1H, t, H-4), 3.83 (3H, s, $NOCH_3$), 4.70 (1H, 4 lines, $CHCH_2Ph$), 4.77 (1H, 8 lines, H-3), 6.71 (1H, s, thiazolyl ring), 7.22 (2H, s, NH_2), 7.29 (5H, m, Ph), 9.18 (1H d, NH).

Irradiation at the 3.07 resonance reduced the 4.70 resonanc from 4 lines to a singlet, confirming the assignments. Irradiatio at the 4.77 resonance reduced both the 3.46 and 3.80 resonance to doublets. Irradiation at 3.46 gave J_{BX} = 5.5 Hz and at 3.80 gav J_{AX} = 2.2 Hz, J_{AB} = 4.75 Hz and J_{NH-CH} = 8.04 Hz.



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