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A Total Synthesis of the Natural Enantiomer of the Gastroprotective Natural Products AI-77-B and Amicoumacin C Hydrochloride.[1]

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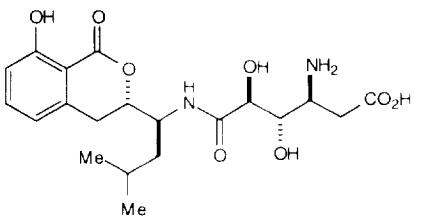
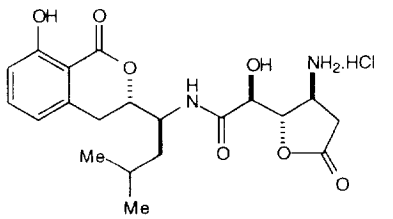
Abstract: A total synthesis of the natural enantiomer of AI-77-B and amicoumacin C hydrochloride is described.

AI-77-B **1** is the major component of a group of closely related compounds isolated from the culture broth of *Bacillus pumilus* AI-77.[2] This same compound was also isolated independently from *Bacillus pumilus* BN-103 and named amicoumacin B, but **1** and those compounds produced by these organisms are generally known as AI-77's rather than amicoumacins.[3] The biological activity of AI-77-B is of some interest, as it exhibits potent antiulcerogenicity against stress ulcers whilst being non-central suppressive, non-anticholinergic, and non-antihistaminergic.[4] Other members of this family of natural products also possess such gastroprotective activity, but of particular interest is lactone **2**, known as amicoumacin C (Scheme 1). Unlike AI-77-B this hydrochloride has been reported to possesses oral activity, affording 44 percent protection at an oral dose of 25 mg/kg.[4c] In view of the structures and biological activities of these compounds we became interested in developing a relatively short total synthesis of AI-77-B and amicoumacin C as its HCl salt.



AI-77-B
1

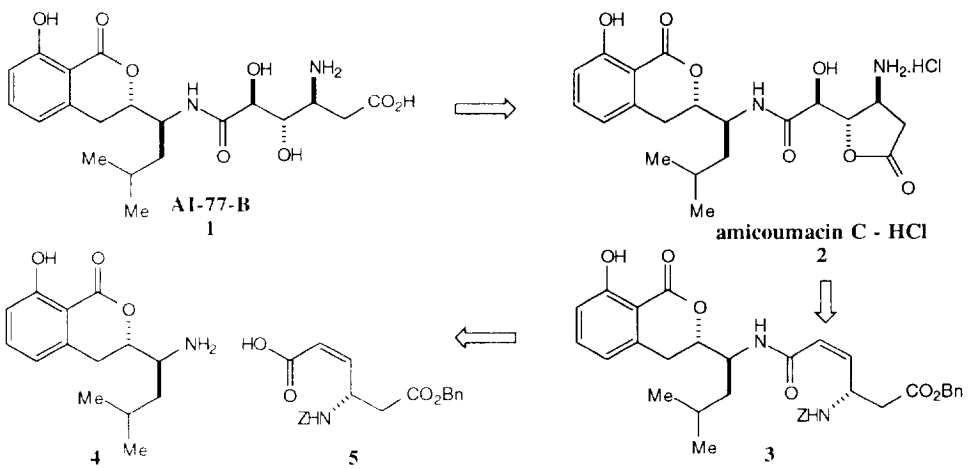


Amicoumacin C hydrochloride
2

Scheme 1

The AI-77 family of natural products has elicited a significant amount of synthetic work, concentrating mainly on the 'amino acid' unit and resulting in two other total syntheses to date.[5] In both of these, complete fragments corresponding to the 'Eastern' and 'Western' units were assembled and coupled together. Our synthetic strategy was guided by the desire to reduce the number of steps and protecting groups involved in the synthesis of this class of compound, to use cheap and readily available enantiomerically pure starting materials, and which could produce analogues and stereoisomers. Our synthetic strategy is outlined in Scheme 2. The

hydrolysis of amicoumacin C is known to produce AI-77-B,[3c] and we planned to generate a protected version of **2** by dihydroxylation of the *Z* enoate **3**. The late introduction of the 1,2-diol functionality avoids the necessity for protection-deprotection steps involving these hydroxyl groups, but the diastereoselectivity of this step was difficult to predict given the relatively complex structure of the substrate and the acyclic nature of the alkene. The dihydroxylation precursor **3** would be derived from peptide coupling of the Eastern and Western fragments **4** and **5**, both of which could originate from the natural enantiomers of aspartic acid and leucine respectively.

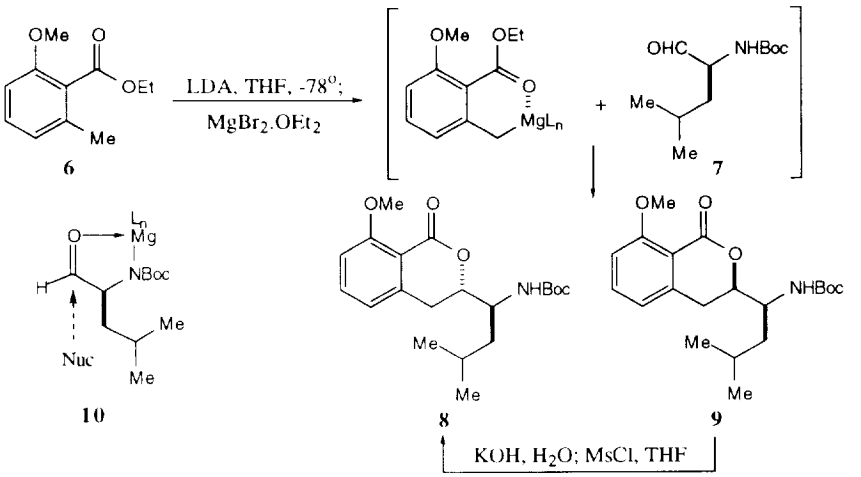


Scheme 2

Synthesis of the Western Fragment 4

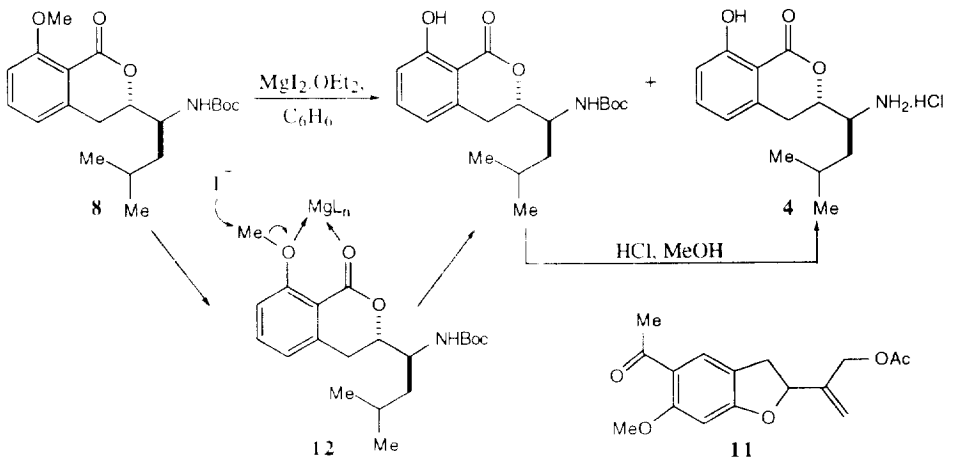
The synthesis of this fragment was similar to that used in the original total synthesis and depends on the addition of the anion of **6** to Boc-leucinal **7** (Scheme 3).[5a,b] Extensive investigations into this addition reaction demonstrated that it was difficult to achieve high reproducible yields and stereoselectivity with a readily removable protecting group on nitrogen. In our hands, the best compromise was as follows. The anion generated from **6** by treatment with LDA was treated with magnesium bromide etherate, and the cold solution added to aldehyde **7**. Acidification (to ensure complete lactonization) and chromatography provided the stereoisomeric lactones **8** and **9** in a ratio of ~2.2:1 and a combined yield of 33 percent. The lactone **9** possessing the wrong stereochemistry for a synthesis of AI-77-B and amicoumacin C could be converted quantitatively into the desired lactone **8** by a one-pot sequence involving opening of the lactone with KOH followed by mesylation (Scheme 3). In this way a reproducible 33 percent yield of pure lactone **8** could be obtained.

The modest diastereoselectivity observed in this reaction is consistent with previous reports and corresponds to chelation control in the addition of the nucleophile to aldehyde **7** represented by **10** (Scheme 3).[5a,b,c] Although (presumably) some of the anion of **6** acts as a base in deprotonation of **7** to produce the chelated aldehyde represented in **10**, the use of two or more equivalents of this anion did not increase the yield of lactones **8** and **9**.



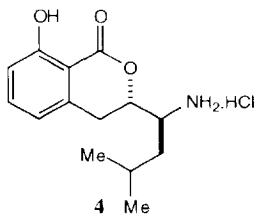
Scheme 3

Deprotection of the phenol by demethylation of **8** was achieved by treatment with magnesium iodide. This step was modelled on a report of an analogous reaction in which this reagent was used to effect a selective demethylation of **11** (Scheme 4).[6] Exposure of our lactone **8** to similar conditions provided the desired phenol along with some of the Western fragment **4** (after isolation as the hydrochloride salt) in which the Boc group had been lost, in a combined yield of 88 percent. No attempt was made to suppress this Boc cleavage as N-deprotection was the subsequent step, the combined yield of the Western fragment **4** from lactone **8** being 87 percent, corresponding to an overall conversion of ~29 percent from Boc-leucinal **7** to the desired fragment **4** as the hydrochloride. This mild *O*-demethylation is thought to be aided by strong chelation of the magnesium between the lactone carbonyl group and the oxygen of the methoxy group which activates the latter towards nucleophilic displacement by iodide as represented by **12** (Scheme 4).

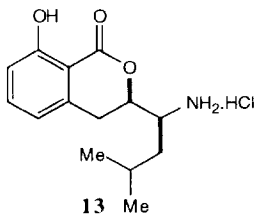


Scheme 4

The Western fragment **4** possessed physical data (optical rotation, ^{1}H nmr spectrum, and melting point) in good agreement with the material obtained by degradation of the natural product.[3a,4b] A sample of the lactone **9** with the unnatural stereochemistry was converted to the corresponding hydrochloride **13**, which possessed physical data (optical rotation, ^{1}H nmr spectrum, and melting point) quite different from **4**, confirming the relative and absolute stereochemistry of the synthetic materials. The diacetate derived from **4** also exhibited physical properties consistent with those reported for a sample derived from AI-77-B.[2c]



m.p. 209-212°C (dec.)
$[\alpha]_D$ -55.6 (c=1.08, MeOH)

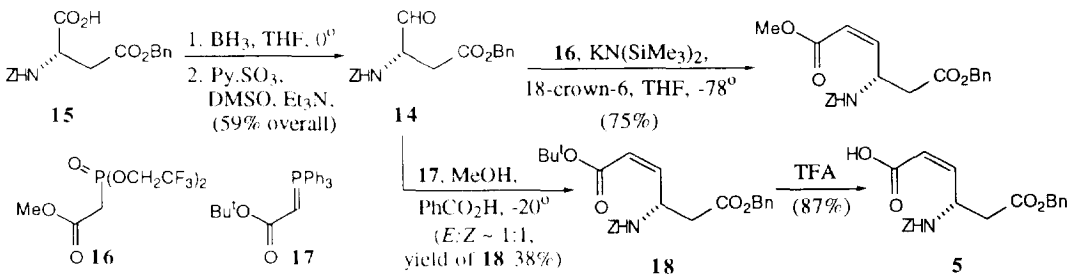


m.p. 200-203°C (dec.)
$[\alpha]_D$ +36.5 (c=1.11, MeOH)

Scheme 5

Synthesis of the Eastern Fragment 5

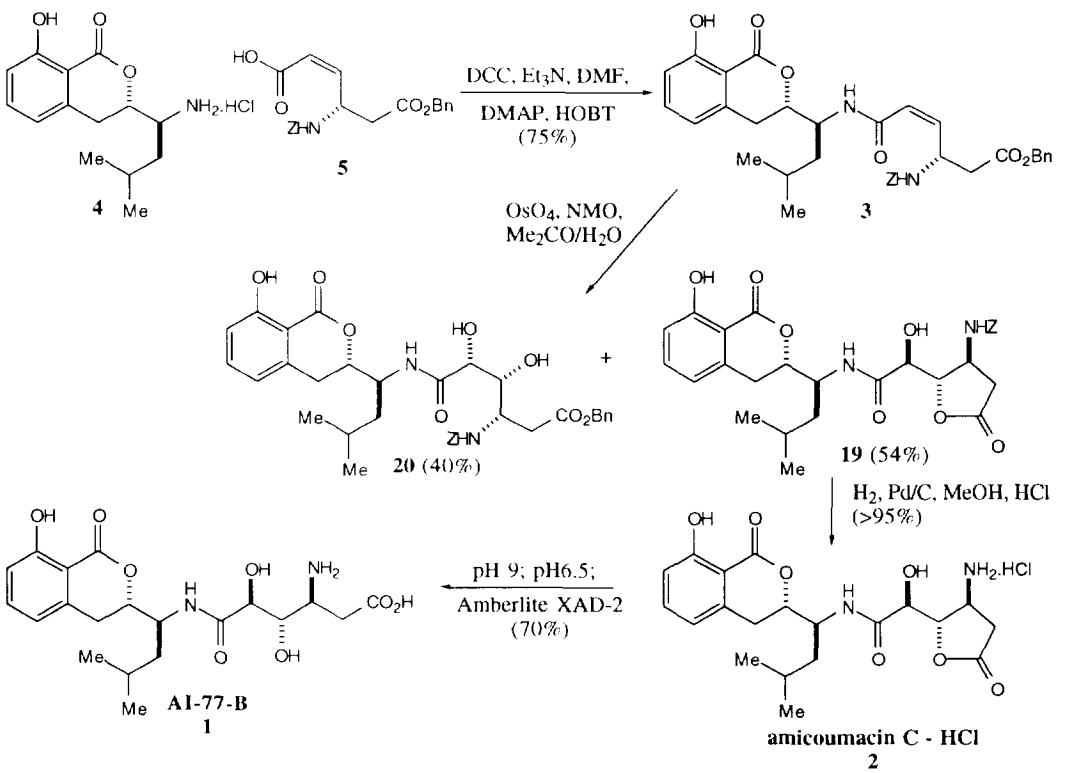
This simple enoate was prepared from aldehyde **14**, obtained in 59 percent overall yield by standard transformation of the commercially available protected aspartic acid derivative **15** (Scheme 6).[7] The next step involved Wittig olefination of this aldehyde to produce a *Z* enoate with an ester group suitable for selective deprotection to the desired acid **5**. Olefination with the *Z* selective trifluoroethyl phosphonate[8] **16** gave exclusively (>20:1) the desired olefin geometry, but the methyl ester group in the product could not be hydrolyzed selectively. The most convenient solution was found to be to carry out the olefination using the stabilized phosphorane **17** in methanol at -20°. Although this reaction exhibits little *E/Z* selectivity, the *E* and *Z* isomers can be separated by chromatography, and on scales above 2g the desired enoate **18** crystallizes directly out of the reaction mixture and can be isolated in ~35% yield by filtration. Selective deprotection of the *tert*-butyl ester then provided the desired fragment **5**.



Scheme 6

Synthesis of AI-77-B and Amicoumacin B

The two fragments **4** and **5** were coupled in high yield without the need for protection of the phenolic hydroxyl group to provide amide **3**, the substrate for dihydroxylation (Scheme 7). Dihydroxylation with catalytic osmium tetroxide using *N*-methylmorpholine *N*-oxide as the stoichiometric oxidant proved to be the most effective method. Under these conditions the diol with the 'natural' stereochemistry converted into the corresponding lactone **19** *in situ* whereas the 'unnatural' diol **20** was reluctant to lactonize. Attempts to improve the diastereoselectivity using Sharpless' 'original' catalysts (dihydroquinine 4-chlorobenzoate and dihydroquinidine 4-chlorobenzoate) did not improve the diastereoselection and under these conditions the 'natural' diol did not lactonize *in situ*. This *in situ* lactonization made separation of the products of dihydroxylation very simple, routine flash column chromatography easily separating diol **20** from the desired lactone **19**.



Scheme 7

Deprotection of lactone **19** in the presence of HCl provided the lactone hydrochloride **2** which corresponds to the hydrochloride salt of the natural product amicoumacin C.[9] Careful hydrolysis followed by de-salting with Amberlite XAD-2 provided the natural product AI-77-B **1**, identical to an authentic sample.[10] The 'unnatural' diol **20** was also converted to the corresponding diastereoisomer of AI-77-B, which possessed quite different physical properties to those of the natural product.

In conclusion we have carried out a total synthesis of the natural enantiomer of the natural gastroprotective agent AI-77-B in eight steps from commercially available acid **15**, nine steps from Boc-valine, or ten from ethyl acetoacetate and crotonaldehyde, the precursors to **6**.

Acknowledgements: We thank the SERC and Wellcome for a CASE studentship (R.A.W.), and Dr. D.M. Miller (Wellcome) and Professor E.J. Thomas (Manchester) for invaluable discussions.

Experimental

Infra-red absorption spectra were recorded on a Perkin-Elmer 1710 Fourier-transform spectrophotometer. The spectra were recorded either as a thin film (liquid and oil samples) or Nujol mull (solid samples). High field ^{1}H n.m.r. spectra were recorded on a Bruker AC-300 (300 MHz) in deuterochloroform unless otherwise stated. Chemical shifts are quoted in δ and followed by the integration value, the signal multiplicity, J value(s) and proton assignment. The following abbreviations have been used to describe the signal multiplicity: br(broad), s(singlet), d(doublet), t(triplet), q(quartet), m(multiplet). Low resolution mass spectra (E.I. and C.I.) were recorded on a Finnigan 4500 and high resolution mass spectra (E.I., C.I. and F.A.B.) were measured on a Kratos Concept. Melting points were determined using an "Electrothermal" apparatus and are uncorrected. Elemental analysis were carried out by the analytical chemistry department, Wellcome Research Ltd., Beckenham, Kent. Optical rotations were measured using an AA-10 monochromatic 589 nm polarimeter (Optical Activity Ltd.). Concentrations are expressed in grams per 100 ml of solvent. Thin layer chromatography was performed using Whatmann or Macherey-Nagel glass-backed plates. The plates were visualised by use of ultraviolet light, iodide or one of the following reagents: ethanolic phosphomolybdic acid, ethanolic vanillin, aqueous potassium permanganate or ethanolic ninhydrin. Silica gel (particle sizes 0.040-0.063 mm) supplied by E.M. Merck was employed for flash chromatography.[11] Diethyl ether ("ether") and tetrahydrofuran were distilled from sodium/benzophenone ketyl immediately prior to use. Dichloromethane was distilled from calcium hydride prior to use. Chloroform, toluene, benzene, acetonitrile, dimethyl sulphoxide, dimethylformamide, triethylamine and diisopropylamine were distilled from calcium hydride and stored over 4Å molecular sieves. Pyridine was distilled from calcium hydride and stored over potassium hydroxide pellets. Methanol and ethanol ('Analar' grade) were routinely dried by standing over 3Å molecular sieves overnight. Reactions were routinely carried out under an argon or nitrogen atmosphere. *n*-Butyl lithium (~ 1.5M in hexane) was supplied by Lithco Corporation and was standardised by titration using diphenylacetic acid as indicator.

N-(*tert*-Butyloxycarbonyl)-(S)-2-amino-4-methyl-1-pentanol

Borane-tetrahydrofuran complex (1M in THF, 10 ml, 10.00 mmol) was added dropwise over 1 hour to a stirred solution of *N*-Boc-L-leucine (1 g, 4.01 mmol) in dry THF (5 ml) at 0°C. The mixture was stirred for a further hour at room temperature before re-cooling in an ice bath and dropwise addition of a 10% acetic acid in methanol solution (3 ml, *care, effervescence!*). The solvent was removed *in vacuo* to give a colourless oil which was dissolved in ethyl acetate (20 ml). The organic solution was washed with water (20 ml), 2M

hydrochloric acid (20 ml), saturated sodium bicarbonate solution (20 ml) and saturated brine (20 ml). The organic phase was then dried ($MgSO_4$) and concentrated *in vacuo* to give a colourless oil. This was purified by flash chromatography to give the title compound as a colourless oil, 854 mg, 91% [R_f ~ 0.37 (20% ethyl acetate in petrol)].

δ_H 0.90 (6H, d, J = 7.5 Hz, -CH(C<u>H</u>$_3$)$_2$); 1.22-1.28 (2H, m, H-3a and H-3b); 1.39 (9H, s, ^{t}Bu); 1.55-1.57 (1H, m, H-4); 2.94 (1H, br s (exchangeable with D_2O), -OH); 3.45 (1H, dd, J = 11.0, 6.0 Hz, H-1a); 3.60 (1H, dd, J = 11.0, 3.0 Hz, H-1b); 3.60-3.72 (1H, m, H-2), 4.64 (1H, br d, J = 8 Hz, N-H).

ν_{max} (thin film); 3346 cm^{-1} (-OH and -NH); 1690 cm^{-1} (N-CO-O).

m/z (C.I., NH_3); 218 (M + H$^+$); high resolution measured at 218.1759; $C_{11}H_{24}NO_3^+$ requires 218.1756.

$[\alpha]_D$ = -27.9° (c = 2.08, MeOH); lit.[7] $[\alpha]_D$ = -27.4° (c = 2.03, MeOH).

N-(*tert*-Butyloxycarbonyl)-(S)-2-amino-4-methyl-1-pentanal 7

A solution of pyridine-sulphur trioxide complex (1.77 g, 11.10 mmol) in dry DMSO (4 ml) was added to a vigorously stirred solution of *N*-Boc-(S)-2-amino-4-methyl-pentanol (242 mg, 1.11 mmol) and triethylamine (1.13 g, 11.1 mmol) in dry DMSO (3 ml). A cooling bath (10°C) was applied during the addition. The mixture was then stirred at room temperature for 20 minutes before pouring into ice/water (60 ml). The mixture was then extracted with ether (3 x 20 ml), the combined organic extracts then being washed with 10% citric acid solution (3 x 30 ml), water (3 x 30 ml), saturated sodium bicarbonate solution (30 ml) and saturated brine (30 ml) before being dried ($MgSO_4$) and concentrated *in vacuo*. the desired aldehyde **7** was obtained as a pale yellow oil, 240 mg, 99% [R_f ~ 0.5 (20% ethyl acetate/petrol)].

δ_H 0.96 (6H, d, J = 7.5 Hz, CH(C<u>H</u>$_3$)$_2$); 1.28-1.35 (1H, m, H-3a); 1.41 (9H, s, ^{t}Bu); 1.5-1.8 (2H, m, H-3b, H-4); 4.13-4.24 (1H, m, H-2); 4.96 (1H, d, J = 8 Hz, N-H); 9.53 (1H, s, -C<u>H</u>O).

ν_{max} (thin film); 3350 cm^{-1} (N-H); 1720 cm^{-1} (sh, -CHO); 1699 cm^{-1} (N-CO-O).

m/z (C.I. NH_3); 216 (M + H$^+$).

$[\alpha]_D$ = -48.8° (c = 1.07, MeOH), lit.,[12] $[\alpha]_D$ = -45.7° (c = 1, MeOH).

(1'S,3S)-3-[1'-(*tert*-Butyloxycarbonylamino)-3'-methylbutyl]-3,4-dihydro-1-oxo-8-methoxy-1H-2-benzopyran 8

A solution of ethyl 2-methoxy-6-methylbenzoate[13] **6** (536 mg, 2.79 mmol) in dry THF (5 ml) was added dropwise to a solution of LDA [1.05 eq., generated from diisopropylamine (354 mg, 3.50 mmol) and *n*-butyl lithium (2.21 ml of a 1.32 M solution in hexanes] in dry THF (5 ml) at -78°C. A bright red colour appeared on addition of the ester. The mixture was stirred at -78°C for 15 minutes before the addition of magnesium bromide etherate (1.65 ml of 2.62 M solution in diethyl ether, 4.38 mmol). The mixture was stirred for a further 15 minutes and was then cannulated into a solution of aldehyde **7** (545 mg, 2.53 mmol) in dry THF (5 ml) at -78°C, the bright red colouration disappearing upon contact with the aldehyde solution. The mixture was stirred for a further 30 minutes before addition of 1M hydrochloric acid (20 ml). The mixture was then stirred at room temperature for 2 hours before extracting with ethyl acetate (2 x 20 ml). The combined organic extracts

were washed with saturated sodium bicarbonate solution (30 ml) and brine (30 ml), dried ($MgSO_4$) and concentrated to give a yellow oil. This was purified by flash chromatography (eluting with 20% ethyl acetate/petrol) to give the desired product **8** as a pale yellow oil, 240 mg, 23% [R_f ~ 0.33 (33% ethyl acetate/petrol)].

δ_H 0.90 (6H, d, J = 7 Hz, $CH(C\underline{H}_3)_2$); 1.33-1.47 (1H, m, H-2'a); 1.40 (9H, s, ^tBu); 1.57-1.65 (2H, m, H-2'b, H-3'); 2.73 (1H, dd, J = 16, 2 Hz, H-4a*); 3.06 (1H, dd, J = 16, 11.5 Hz, H-4b*); 3.81-3.86 (1H, m, H-1'); 3.91 (3H, s, Ar-O<u>Me</u>); 4.34 (1H, d with slight further splitting, J = 11.5 Hz, H-3); 4.80 (1H, br d J = 10 Hz, N-H); 6.79 (1H, d, J = 8 Hz, H-5**); 6.88 (1H, d, J = 8 Hz, H-7**); 7.41 (1H, t, J = 8 Hz, H-6) (* and ** - assignments may be reversed).

ν_{max} (thin film); 3351 cm^{-1} (N-H); 1711 cm^{-1} (carbamate and δ-lactone).

m/z (C.I., NH_3); 381 ($M + NH_4^+$); 364 ($M + H^+$); high resolution measured at 364.2133; $C_{20}H_{30}NO_5^+$ requires 364.2124.

$[\alpha]_D$ = -122.8° (c = 2.02, $CHCl_3$).

Further elution from the column gave the (1'S,3R) diastereoisomer **9**, 104 mg, 10% [R_f ~ 0.28 (33% ethyl acetate/petrol)].

δ_H 0.92 (3H, d, J = 7 Hz, CH-C<u>H</u>$_3$); 0.96 (3H, d, J = 7 Hz, CH-C<u>H</u>$_3$); 1.41 (9H, s, ^tBu); 1.4-1.75 (3H, m, H-2'a, H-2'b, H-3'); 2.77 (1H, dd, J = 15.5, 1.5 Hz, H-4a); 2.99 (1H, dd, J = 15.5, 11.0 Hz, H-4b); 3.80-3.90 (1H, m, H-1'); 3.92 (3H, s, Ar-O<u>Me</u>); 4.39 (1H, br d, J = 11 Hz, H-3); 4.78 (1H, br d, J = 9.5 Hz, N-H); 6.79 (1H, d, J = 7 Hz, H-5*); 6.89 (1H, d, J = 7 Hz, H-7*); 7.43 (1H, t, J = 7 Hz, H-6) (* - assignments may be reversed).

ν_{max} (thin film); 3350 cm^{-1} (N-H); 1716 cm^{-1} (carbamate and δ-lactone).

m/z (C.I., NH_3); 381 ($M + NH_4^+$); 364 ($M + H^+$); high resolution measured at 364.2132; $C_{20}H_{29}NO_5^+$ requires 364.2124.

$[\alpha]_D$ = -3.5° (c = 2.30, $CHCl_3$).

Inversion of Stereochemistry, Conversion of 9 into 8

A solution of lactone **9** (357 mg, 1.03 mmol) and potassium hydroxide (67 mg, 1.25 mmol) in ethanol/water (3:1, 4 ml) was heated to reflux for 3 hours after which time no starting material remained. The mixture was then concentrated to dryness and the solid residue azeotroped with benzene (3 x 10 ml), providing the crude potassium salt as an off-white solid. This was suspended in dry THF (15 ml) and cooled to 0°C with stirring before addition of triethylamine (432 mg, 4.27 mmol) and methanesulphonyl chloride (380 mg, 3.32 mmol). The mixture was then stirred at 0°C for 1 hour before addition of 2M aqueous sodium hydroxide solution (2.4 ml, 4.8 mmol) and water (4 ml). The mixture was then refluxed for 1 hour before cooling and acidifying to pH 1 with 1M hydrochloric acid. The mixture was then extracted with ethyl acetate (2 x 25 ml), the combined organic extracts being dried ($MgSO_4$) and concentrated *in vacuo* to give the (1'S,3S) lactone **8**, 356 mg, ~100%.

(1'S,3S)-3-[1'-(*tert*-Butyloxycarbonylamino)-3'-methylbutyl]-3,4-dihydro-8-hydroxy-1-oxo-1H-2-benzopyran

Iodine crystals (140 mg, 1.10 mmol) were added in small portions over one hour to a stirred suspension of magnesium (27 mg, 1.15 mmol) in dry benzene (0.65 ml) and dry ether (0.3 ml). The mixture was stirred for a further hour after the final addition until the iodine colour had disappeared. A solution of the methoxy compound **8** (280 mg, 0.83 mmol) in dry benzene (1.5 ml) was then added and the mixture was refluxed for 1 hour after which it was cooled and poured into 1M hydrochloric acid (10 ml). The product was extracted with diethyl ether (2 x 15 ml), the combined organic phases then being washed with saturated sodium bicarbonate solution (20 ml) and saturated brine (20 ml) before drying ($MgSO_4$) and evaporation *in vacuo*. The crude product was purified by flash chromatography (eluting with 5% ethyl acetate/petrol) to give the title compound as a colourless oil, 156 mg, 58% [R_f ~ 0.38 (10% ethyl acetate/petrol)].

δ_H 0.92 (6H, d, J = 6.5 Hz, CH-(C<u>H</u>$_3$)$_2$); 1.33-1.47 (1H, m, H-2'a*) 1.40 (9H, s, ^{t}Bu); 1.62-1.78 (2H, m, H-2'b*, H-3'*); 2.80 (1H, dd, J = 16.5, 2.5 Hz, H-4a); 3.11 (1H, dd, J = 16.5, 12.5 Hz, H-4b); 3.89-3.97 (1H, m, H-1'); 4.52 (1H, d with slight further splitting, J = 12.5 Hz, H-3); 4.73 (1H, br d, J = 10 Hz, N-H); 6.68 (1H, d, J = 7.5 Hz, H-5**); 6.83 (1H, d, J = 7.5 Hz, H-7**); 7.38 (1H, t, J = 7.5 Hz, H-6); 10.81 (1H, s, Ar-O<u>H</u>) (* and ** - these assignments may be reversed).

ν_{max} (thin film); 3315 cm^{-1} (N-H); 1675 cm^{-1} (H-bonded δ-lactone).

m/z (C.I., NH_3); 367 (M + NH_4^+); high resolution measured at 367.2232; $C_{19}H_{27}NO_5$ + NH_4^+ requires 367.2233.

$C_{19}H_{27}NO_5$	requires:	C, 65.31%; H, 7.79%; N, 4.01%.
	found:	C, 65.42%; H, 8.03%; N, 3.77%.

$[\alpha]_D$ = -80.0° (c = 2.00, $CHCl_3$).

Basification of the acid extract with 1M sodium hydroxide solution followed by chloroform extraction recovered the free amine. (i.e. the product of demethylation and Boc removal). This was isolated by drying the chloroform extract ($MgSO_4$), addition of 3M HCl in methanol and evaporation which gave the hydrochloride salt **4**, 72 mg, 30% (See below for data).

(1'S,3R)-3-[1'-(*tert*-butyloxycarbonylamino)-3'-methylbutyl]-3,4-dihydro-8-hydroxy-1-oxo-1H-2-benzopyran

This compound was prepared using the same procedure as that used for its diastereoisomer but using **9** in place of **8**, yield 56% [R_f ~ 0.31 (10% ethyl acetate/petrol)].

δ_H 0.92 (3H, d, J = 7.5 Hz, CH-C<u>H</u>$_3$); 0.95 (3H, d, J = 7.5 Hz, CH-C<u>H</u>$_3$); 1.42 (9H, s, ^{t}Bu); 1.42-1.51 (2H, m, H-2'a, H-2'b); 1.64-1.76 (1H, br m, H-3'); 2.84 (1H, dd, J = 16, 2.5 Hz, H-4a); 3.02 (1H, dd, J = 16, 11.5 Hz, H-4b); 3.83-3.92 (1H, m, H-1'); 4.56 (1H, d with slight further splitting, J = 11.5 Hz, H-3); 4.78 (1H, br d, J = 9 Hz, N-H); 6.67 (1H, d, J = 8 Hz, H-5*); 6.85 (1H, d, J = 8 Hz, H-7*); 7.38 (1H, d, J = 8 Hz, H-6); 10.85 (1H, s, Ar-O<u>H</u>) (* - assignments may be reversed).

ν_{max} (thin film); 3315 cm^{-1} (N-H); 1675 cm^{-1} (H-bonded δ-lactone).

m/z (C.I., NH_3): 367 ($M + NH_4^+$); high resolution measured at 367.2239; $C_{19}H_{27}NO_5 + NH_4^+$ requires 367.2233.

$[\alpha]_D$ = - 48.0° (c = 2.00, $CHCl_3$).

As above the amine product from removal of the Boc group was isolated from the acid extract as its hydrochloride salt **13**, 29%.

(1'S,3S)-3-(1'-amino-3'-methylbutyl)-3,4-dihydro-8-hydroxy-1-oxo-1H-2-benzopyran hydrochloride 4

A solution of (1'S,3S)-3-[1'-(*tert*-Butyloxycarbonylamino)-3'-methylbutyl]-3,4-dihydro-8-hydroxy-1-oxo-1H-2-benzopyran (88 mg, 0.25 mmol) in dry methanol (1 ml) was treated with 3M HCl in methanol (2 ml) at 0°C for four hours. The solution was then concentrated *in vacuo* to give the desired product as a pale yellow solid. The solid was triturated with dry ether (3 ml) to leave the product **4** as a crystalline solid, 71 mg, 99%.

m.p. 209-212°C (dec.), lit.[4b] m.p. 210°C (dec.).

δ_H (CD_3OD); 0.98 (3H, d, J = 6.5 Hz, CH-C<u>H</u>$_3$); 1.00 (3H, d, J = 6.5 Hz, CH-C<u>H</u>$_3$); 1.55-1.60 (2H, m, H-2'a, H-2'b); 1.61-1.85 (1H, m, H-3'); 3.04-3.20 (2H, m, H-4a, H-4b); 3.57 (1H, dt, J = 8, 5 Hz, H-1'); 4.70 (1H, dt, J = 11, 5 Hz, H-3); 6.82 (1H, d, J = 7.5 Hz, H-5*); 6.87 (1H, d, J = 7.0 Hz, H-7*); 7.48 (1H, dd, J = 7, 7.5 Hz, H-6) (* - assignments may be reversed).

ν_{max} (nujol mull); 3200-2800 cm^{-1}, br (-NH_3^+, -OH); 1678 cm^{-1} (H-bonded δ-lactone).

m/z (F.A.B.); 250 (R-NH_3^+); high resolution measured at 250.1458; $C_{14}H_{20}NO_3^+$ requires 250.1443.

$[\alpha]_D$ = -55.6° (c = 1.08, MeOH) lit.[3a] $[\alpha]_D$ = -58.5 (c = 0.5, MeOH).

(1'S,3R)-3-(1'-Amino-3'-methylbutyl)-3,4-dihydro-8-hydroxy-1-oxo-1H-2-benzopyran hydrochloride 13

A solution of (1'S,3R)-3-[1'-(*tert*-Butyloxycarbonylamino)-3'-methylbutyl]-3,4-dihydro-8-hydroxy-1-oxo-1H-2-benzopyran (85 mg, 0.24 mmol) in dry methanol (1 ml) was treated with 3M HCl in methanol (2 ml) and the mixture stirred at room temperature for 3 hours. The mixture was concentrated to give a pale yellow solid. Trituration with dry ether (4 ml) gave **13** as a solid, 69 mg, 99%; m.p. 200-203°C (dec.).

δ_H (CD_3OD); 0.97 (3H, d, J = 6.4 Hz, CH-C<u>H</u>$_3$); 1.00 (3H, d, J = 6.4 Hz, CH-C<u>H</u>$_3$); 1.5-1.8 (3H, m, H-2'a, H-2'b, H-3'); 2.96 (1H, dd, J = 16.2, 3.2 Hz, H-4a); 3.08 (1H, dd, J = 16.2, 13.0 Hz, H-4b); 3.61-3.67 (1H, m, H-1'); 6.83 (1H, d, J = 8.7 Hz, H-5*); 6.86 (1H, d, J = 8.8 Hz, H-7*); 7.47 (1H, t, J = 8.7 Hz, H-6) (*these assignments may be reversed).

ν_{max} (nujol mull); 3333 cm^{-1}, (br, -NH_3^+, -OH); 1682 cm^{-1} (H-bonded δ-lactone).

m/z (F.A.B.); 250 (R-NH_3^+); high resolution measured at 250.1444; $C_{14}H_{20}NO_3^+$ requires 250.1443.

$[\alpha]_D$ = +36.5° (c = 0.11, MeOH).

(1'S,3S)-8-Acetoxy-3-[1'-(acetylamino)-3'-methylbutyl]-3,4-dihydro-1-oxo-1H-2-benzopyran

A solution of amine hydrochloride **4** (30 mg, 0.11 mmol) and 4-dimethylaminopyridine (~2 mg) in dry pyridine (0.5 ml) was treated with acetic anhydride (54 mg, 0.53 mmol) and the mixture stirred overnight at room temperature. The mixture was then concentrated *in vacuo*. The oily residue was dissolved in ethyl acetate (4 ml) and the solution washed with water (2 x 5 ml), saturated copper(II) sulphate solution (3 x 5 ml) and saturated brine (5 ml). The organic phase was then dried ($MgSO_4$) and concentrated to give a yellow oil. This was purified by flash chromatography (eluting with 50% ethyl acetate/petrol) to give the title compound as a colourless oil, 32 mg, 90% [R_f ~ 0.30 (50% ethyl acetate/petrol)].

δ_H 0.91 (3H, d, J = 6 Hz, CH-C$\underline{H}_3$); 0.92 (3H, d, J = 6 Hz, CH-C$\underline{H}_3$); 1.30-1.46 (1H, m, H-2'a); 1.53-1.67 (1H, m, H-3'); 1.67-1.77 (1H, m, H-2'b); 1.95 (3H, s, N-COC$\underline{H}_3$); 2.35 (3H, s, OOCC$\underline{H}_3$); 2.90 (1H, dd, J = 17.0, 3.5 Hz, H-4a); 3.07 (1H, dd, J = 17.0, 11.0 Hz, H-4b); 4.3.96-4.08 (1H, m, H-1'); 4.51 (1H, d with slight further splitting, J = 11 Hz, H-3); 5.70 (1H, br d, J = 9.5 Hz, N-H); 7.01 (1H, d, J = 8 Hz, H-5*); 7.12 (1H, d, J = 8 Hz, H-7*); 7.51 (1H, t, J = 8 Hz, H-6) (* - assignments may be reversed). δ_H (CD_3OD); 0.89 (3H, d, J = 8 Hz, CH-C$\underline{H}_3$); 0.93 (3H, d, J = 8 Hz, CH-C$\underline{H}_3$); 1.32-1.41 (1H, m, H-2'a); 1.56-1.64 (1H, m, H-3'); 1.66-1.76 (1H, m, H-2'b); 1.93 (3H, s, N-COC$\underline{H}_3$); 2.25 (3H, s, O-COC$\underline{H}_3$); 2.90 (1H, dd, J = 18, 4 Hz, H-4a); 2.99 (1H, dd, J = 18, 13 Hz, H-4b); 4.23 (1H, br dt, J = 11.5, 4 Hz, H-1'); 4.49 (1H, dt, J = 11.5, 4 Hz, H-3); 7.04 (1H, d, J = 9.5 Hz, H-5*); 7.22 (1H, d, J = 9.5 Hz, H-7*); 7.58 (1H, t, J = 9.5 Hz, H-6) (* - assignments may be reversed).

ν_{max} (thin film): 3298 cm^{-1} (N-H); 1770 cm^{-1} (ester); 1728 cm^{-1} (δ-lactone); 1655 cm^{-1} (amide).

m/z (F.A.B.); 334 $(M + H)^+$; high resolution measured at 334.1644; $C_{18}H_{24}NO_5^+$ requires 334.1654.

$[\alpha]_D$ = -158.0° (c = 2.0, $CHCl_3$).

(1'S,3R)-8-Acetoxy-3-[1'-(acetylamino)-3'-methylbutyl]-3,4-dihydro-1-oxo-1H-2-benzopyran

A solution of amine hydrochloride **13** (30 mg, 0.11 mmol) and 4-dimethyl-aminopyridine (~2 mg, catalytic amount) in dry pyridine (0.5 ml) was treated with acetic anhydride (54 mg, 0.53 mmol) and stirred overnight at room temperature. The mixture was then concentrated *in vacuo* to give an oil which was partitioned between water (5 ml) and ethyl acetate (10 ml). The organic phase was separated and washed with water (3 x 10 ml), saturated copper(II) sulphate solution (3 x 10 ml) and saturated brine (10 ml). The organic phase was then dried ($MgSO_4$) and concentrated *in vacuo* to give a pale yellow oil which was purified by flash chromatography (eluting with 50% ethyl acetate/petrol) to give the title compound as a colourless oil, 30 mg, 85% [R_f ~ 0.27 (50% ethyl acetate/petrol)].

δ_H 0.89 (3H, d, J = 6.5 Hz, CH-C$\underline{H}_3$); 0.91 (3H, d, J = 6.5 Hz, CH-C$\underline{H}_3$); 1.37-1.46 (1H, m, H-2'a); 1.51-1.60 (1H, m, H-2'b); 1.59-1.70 (1H, m, H-3'); 1.98 (3H, s, N-COC$\underline{H}_3$); 2.32 (3H, s, O-COC$\underline{H}_3$); 2.84 (1H, dd, J = 15.5, 3 Hz, H-4a); 3.05 (1H, dd, J = 15.5, 13 Hz, H-4b); 4.16-4.20 (1H, m, H-1'); 4.48 (1H, dt, J = 13, 3 Hz, H-3); 5.92 (1H, br d, J = 9 Hz, N-H); 7.01 (1H, d, J = 8 Hz, H-5*); 7.09 (1H, d, J = 8 Hz, H-7*); 7.50 (1H, t, J = 8 Hz, H-6) (* - assignments may be reversed). δ_H (CD_3OD); 0.88 (3H, d, J = 6 Hz,

CH-C$\underline{H}_3$); 0.98 (3H, d, J = 6 Hz, CH-C$\underline{H}_3$); 1.48-1.52 (2H, m, H-2'a, H-2'b); 1.58-1.70 (1H, m, H-3'); 1.95 (3H, s, N-COC$\underline{H}_3$); 2.25 (3H, s, O-COC$\underline{H}_3$); 2.96-3.08 (2H, m, H-4a, H-4b); 4.18 (1H, dt, J = 8.5, 5.5 Hz, H-1'); 4.33 (1H, dt, J = 9.5, 5.5 Hz, H-3); 7.05 (1H, d, J = 8 Hz, H-5*); 7.23 (1H, d, J = 8 Hz, H-7*); 7.58 (1H, t, J = 8 Hz, H-6) (* - assignments may be reversed).

ν_{max} (thin film); 3239 cm^{-1} (N-H); 1770 cm^{-1} (ester); 1729 cm^{-1} (δ-lactone); 1656 cm^{-1} (amide).

m/z (F.A.B.); 334 (M + H^+); high resolution measured at 334.1649; $C_{18}H_{24}NO_5^+$ requires 334.1654.

$[\alpha]_D$ = -27.2° (c = 2.5, $CHCl_3$).

(S)-*N*-(Benzyloxycarbonyl)-2-amino-3-(benzyloxycarbonyl)-1-propanal 14

A solution of pyridine-sulphur trioxide complex (5.31 g, 33.40 mmol) in dry DMSO (18 ml) was added dropwise over 10 minutes to a *vigorously* stirred and cooled (10-15°C) solution of (S)-*N*-(benzyloxycarbonyl)-2-amino-3-(benzyloxycarbonyl)-1-propanol (1.91 g, 8.77 mmol) and triethylamine (3.38 g, 33.40 mmol) in dry DMSO (15 ml). The cooling bath was then removed and the reaction mixture stirred vigorously at room temperature for 20 minutes before being poured into ice/water (250 ml). The aqueous mixture was then extracted with diethyl ether (3 x 100 ml). The combined extracts were washed with 10% aqueous citric acid (3 x 100 ml), water (3 x 100 ml), saturated sodium bicarbonate solution (100 ml) and saturated brine (100 ml) before being dried ($MgSO_4$), and concentrated *in vacuo* to give **14** as a pale yellow oil which crystallised on standing, 1.94 g, 97% [R_f ~ 0.35 (30% ethyl acetate/petrol)]. A sample was recrystallised from diethyl ether/light petrol to give a colourless crystalline solid, m.p. 89.5-91.5°.

δ_H 2.89 (1H, dd, J = 18, 5.5 Hz, H-3a); 3.04 (1H, dd, J = 18, 5.5 Hz, H-3b); 4.43 (1H, dt, J = 9, 5.5 Hz, H-2); 5.10 (2H, s, PhC$\underline{H}_2$O); 5.12 (2H, s, PhC$\underline{H}_2$O); 5.94 (1H, br d, J = 9 Hz, N-H); 7.32 (5H, s, Ph-); 7.34 (5H, s, Ph-); 9.63 (1H, s, C$\underline{H}$O).

ν_{max} (nujol mull); 3363 cm^{-1} (N-H); 1735 cm^{-1} (-CO_2R); 1699 cm^{-1} (H-bonded -CHO, N-CO-O).

m/z (C.I., NH_3); 359 (M + NH_4^+); 342 (M + H^+); high resolution measured at 359.1611; $C_{19}H_{19}NO_5$ + NH_4^+ requires 359.1607.

$[\alpha]_D$ = -16.2° (c = 0.99, MeOH).

(S)-(Z)-*N*-(Benzyloxycarbonyl)-4-aminohex-2-enedioic acid, 1-*tert*-butyl 6-benzyl diester 18

(*tert*-Butyloxycarbonyl)methylene triphenylphosphorane (685 mg, 1.82 mmol) was added in one portion to a stirred solution of the aldehyde **14** (540 mg, 1.58 mmol) and benzoic acid (19 mg, 0.16 mmol) in dry methanol (10 ml) at -20°C. Stirring was continued until all the phosphorane had dissolved (10 minutes) and the homogeneous mixture was then transferred to a freezer (~ -20°C). The mixture was left at this temperature for 3 days before warming to room temperature and evaporation of the solvent to give a yellow oil. This was purified by careful flash chromatography to give **18** as a crystalline solid [R_f ~ 0.26 (17% ethyl acetate/petrol)]. Recrystallisation from diethyl ether/ petrol (b.p. 40-60°C) gave needles, 218 mg, 38% m.p. 102.5-103.5°C.

δ_H 1.45 (9H, s, ^tBu); 2.80 (1H, dd, J = 16.5, 5 Hz, H-5a); 2.93 (1H, dd, J = 16.5, 5 Hz, H-5b); 5.06 (2H, s, PhC$\underline{H_2}$O); 5.09 (2H, s, PhC$\underline{H_2}$O); 5.40-5.49 (1H, m, H-4); 5.69 (1H, d, J = 11.7 Hz, H-2); 5.79 (1H, br d, J = 7.5 Hz, N-H); 6.20 (1H, dd, J = 11.7, 8 Hz, H-3); 7.31 (10H, s, 2 x Ph).

ν_{max} (nujol mull); 3386 cm^{-1} (N-H); 1736 cm^{-1} (-CO_2R); 1699 cm^{-1} (α,β-unsaturated ester, carbamate).

m/z (C.I., NH_3); 457 (M + NH_4^+); 440 (M + H^+); high resolution measured at 457.2336; $C_{25}H_{29}NO_6$ + NH_4^+ requires 457.2338.

$C_{25}H_{29}NO_6$	requires:	C, 68.32%; H, 6.65%; N, 3.19%.
	found:	C, 68.56%; H, 6.75%; N, 3.13%.

$[\alpha]_D$ = +34.0° (c = 2.12, $CHCl_3$).

Further elution from the flash chromatography column gave the *E*-isomer [R_f ~ 0.24 (17% ethyl acetate/petrol)]. Recrystallisation from diethyl ether/petrol (b.p. 40-60°C) gave needles, 212 mg, 37%, m.p. 72-74°C.

δ_H 1.45 (9H, s, ^tBu); 2.65-2.73 (2H, m, H-5a, H-5b); 4.69-4.77 (1H, m, H-4); 5.09 (4H, s, 2 x PhC$\underline{H_2}$O); 5.57 (1H, br d, J = 9 Hz, N-H); 5.86 (1H, dd, J = 15.6, 1.7 Hz, H-2); 6.76 (1H, dd, J = 15.6, 5.1 Hz, H-3); 7.31 (10H, s, 2 x Ph).

ν_{max} (nujol mull); 3395 cm^{-1} (N-H); 1735 cm^{-1} (-CO_2R); 1708 cm^{-1} (α,β-unsaturated CO_2R); 1699 cm^{-1} (N-CO-O).

m/z (C.I., NH_3); 457 (M + NH_4^+); high resolution measured at 457.2348; $C_{25}H_{29}NO_6$ + NH_4^+ requires 457.2338.

$C_{25}H_{29}NO_6$	requires:	C, 68.32%; H, 6.65%; N, 3.19%.
	found:	C, 68.16%; H, 6.69%; N, 3.01%.

$[\alpha]_D$ = +8.4° (c = 2.37, $CHCl_3$).

(S)-(Z)-*N*-(Benzyloxycarbonyl)-4-aminohex-2-enedioic acid, 1-methyl, 6-benzyl ester

Potassium *bis*(trimethylsilyl)amide (0.5M solution in toluene, 0.55 ml, 0.27 mmol) was added dropwise to a stirred solution of *bis*(2,2,2-trifluoroethyl)methoxycarbonylmethylphosphonate (83 mg, 0.26 mmol) and 18-crown-6/acetonitrile complex (397 mg, 1.30 mmol) in dry THF (5 ml) at -78°C. The mixture was stirred at -78°C for 30 minutes before the addition of a solution of the aldehyde **14** (56 mg, 0.26 mmol) in dry THF (1 ml). The mixture was stirred at -78°C for a further 30 minutes before addition of saturated aqueous ammonium chloride solution (10 ml). The mixture was then extracted with ether (3 x 10 ml), and the combined organic extracts washed with 1M hydrochloric acid (20 ml) saturated sodium bicarbonate solution (20 ml) and saturated brine (20 ml), before being dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by flash chromatography (eluting with 17% ethyl acetate/petrol) to give the title compound as a colourless oil, 49 mg, 75% [R_f ~ 0.31 (17% ethyl acetate/petrol)].

δ_H 2.79-2.88 (2H, m, H-5a, H-5b); 3.90 (3H, s, -CO_2Me); 5.08 (2H, s, PhCH_2O); 5.10 (2H, s, PhCH_2O); 5.46-5.53 (1H, m, H-4); 5.81 (1H, d, J = 11.5 Hz, H-2); 5.85 (1H, br d, J = 8 Hz, N-H); 6.31 (1H, dd, J = 11.5, 7 Hz, H-3); 7.30 (5H, s, Ph).

ν_{max} (thin film): 3312 cm^{-1} (N-H); 1737 cm^{-1} (-CO_2R); 1715 cm^{-1} (α,β-unsaturated -CO_2R); 1695 cm^{-1} (N-CO-O).

m/z (C.I., NH_3): 415 ($M + NH_4^+$); 398 ($M + H^+$); high resolution measured at 398.1619; $C_{22}H_{24}NO_6^+$ requires 398.1604.

$[\alpha]_D$ = +23.7° (c = 1.86, $CHCl_3$).

(S)-(Z)-*N*-(Benzyloxycarbonyl)-4-aminohex-2-enedioic acid, 6-benzyl ester 5

Trifluoroacetic acid (2 ml) was added dropwise to a stirred solution of *tert*-butyl ester **18** (550 mg, 1.51 mmol) in dry dichloromethane (3 ml) at 0°C. The mixture was then allowed to warm to room temperature before stirring for 2 hours. Evaporation of the solution and crystallisation of the solid residue from ethyl acetate/petrol (b.p. 60-80°C) gave **5** as a crystalline solid, 405 mg, 87%, m.p. 103-106°C.

δ_H 2.79-2.91 (2H, m, H-5a, H-5b); 5.07 (2H, s, PhCH_2O); 5.09 (2H, s, PhCH_2O); 5.43-5.52 (1H, m, H-4); 5.83 (1H, d, J = 11.5 Hz, H-2); 5.94 (1H, br d, J = 8.2 Hz, N-H); 6.36 (1H, dd, J = 11.5, 8.4 Hz, H-3); 7.31 (10H, s, 2 x Ph); 7.53 (1H, br s, OH).

ν_{max} (nujol mull): 3358 cm^{-1} (NH); 3324 cm^{-1} (OH); 1724 cm^{-1} (-CO_2R); 1694 cm^{-1} (-CO_2H, N-CO-O).

m/z (F.A.B.): 384 ($M + H^+$), high resolution measured at 384.1422; $C_{21}H_{22}NO_6^+$ requires 384.1447.

$C_{21}H_{21}NO_6$: requires: C, 65.78%; H, 5.52%; N, 3.65%.

found: C, 65.45%; H, 5.56%; N, 3.57%.

$[\alpha]_D$ = +27.2° (c = 2.06, $CHCl_3$).

(1'S,3S,4"S)-(Z)-*N*-[1'-(3,4-Dihydro-8-hydroxy-1-oxo-1H-2-benzopyran-3-yl)-3'-methylbutyl]-4"-[(benzyloxycarbonyl)amino]-5"-(benzyloxycarbonyl)-pent-2-enamide 3

Dicyclohexylcarbodiimide was added to a solution of the hydrochloride **4** (45 mg, 0.16 mmol), carboxylic acid **5** (48 mg, 0.16 mmol) and 1-hydroxy-benzotriazole (27 mg, 0.20 mmol) in dry DMF (1 ml). The mixture was stirred at room temperature for 18 hours before diluting with ethyl acetate. The resulting mixture was filtered through celite (to remove the precipitate of dicyclohexyl urea) before washing sequentially with water (10 ml), 1M hydrochloric acid (10 ml), saturated sodium bicarbonate solution (10 ml) and brine (10 ml). The organic phase was dried ($MgSO_4$) and concentrated *in vacuo* to give a pale yellow oil. This was purified by flash chromatography (eluting with 30% ethyl acetate/petrol) to give the **3** as a colourless oil, 63 mg, 75% [R_f ~ 0.31 (17% ethyl acetate/petrol)].

δ_H 0.92 (3H, d, J = 6.6 Hz, CH-CH_3); 0.93 (3H, d, J = 6.4 Hz, CH-CH_3); 1.42-1.50 (1H, m, H-2'a); 1.60-1.80 (2H, m, H-2'b, H-3'); 2.70-2.90 (3H, m, H-4a, H-5"a, H-5"b); 3.12 (1H, br t, J = 13.5 Hz, H-4b); 4.33-4.42 (1H, m, H-1'); 4.58 (1H, d with slight further splitting, J = 12.6 Hz, H-3); 5.01 (2H, s,

PhC$\underline{H}_2$O); 5.06 (2H, s, PhC$\underline{H}_2$O); 5.27-5.37 (1H, m, H-4"); 5.80 (1H, d, J = 11.6 Hz, H-2"); 5.91 (1H, d, J = 8.2 Hz, N-H); 5.99 (1H, t, J = 11.1 Hz, H-3"); 6.69 (1H, d, J = 7.2 Hz, H-5*); 6.76 (1H, d, J = 8.9 Hz, N-H); 6.85 (1H, d, J = 8.4 Hz, H-7*); 7.28 (5H, s, Ph); 7.29 (5H, s, Ph); 7.37 (1H, t, J = 8 Hz, H-6); 10.82 (1H, s, Ar-O$\underline{H}$) (* - assignments may be reversed).

ν_{max} (thin film); 3323 cm^{-1} (N-H); 1723 cm^{-1} (-CO_2R); 1700 cm^{-1} (N-CO-O); 1673 cm^{-1} (H-bonded δ lactone, amide).

m/z (F.A.B.); 615 (M + H^+); high resolution measured at 615.2692; $C_{35}H_{39}N_2O_8^+$ requires 615.2706.

$C_{35}H_{38}N_2O_8$	requires:	C, 68.39%; H, 6.23%; N, 4.56%.
	found:	C, 68.55%; H, 6.42%; N, 4.67%.

$[\alpha]_D$ = -46.0° (c = 2.00, $CHCl_3$).

Further elution from the flash chromatography column gave the *E*-isomer as a crystalline solid, 8.5 mg, 10%, m.p. 174-177°C [R_f ~ 0.20 (17% ethyl acetate/petrol)].

δ 0.92 (3H, d, J = 6.4 Hz, CH-C$\underline{H}_3$); 0.93 (3H, d, J = 6.5 Hz, CH-C$\underline{H}_3$); 1.39-1.48 (1H, m, H-2'a); 1.54-1.70 (1H, m, H-2'b); 1.74-1.81 (1H, m, H-3'); 2.64-2.73 (2H, m, H-5"a, H-5"b); 2.80 (1H, dd, J = 16.6, 3 Hz, H-4a); 3.00 (1H, dd, J = 16.6, 13 Hz, H-4b); 4.41 (1H, td, J = 9.8, 4.5 Hz, H-1'); 4.59 (1H, d with slight further splitting, J = 13 Hz, H-3); 4.69-4.76 (1H, m, H-4"); 5.06 (4H, s, 2 x PhC$\underline{H}_2$O); 5.61 (1H, d, J = 9.2 Hz, N-H); 5.67 (1H, d, J = 9.8 Hz, N-H); 5.88 (1H, d, J = 15.2 Hz, H-2"); 6.66 (1H, d, J = 7.3 Hz, H-5*); 6.79 (1H, dd, J = 15.2, 5.1 Hz, H-3"), 6.86 (1H, d, J = 8.4 Hz, H-7*); 7.30 (10H, s, 2 x Ph); 7.40 (1H, t, J = 7.9 Hz, H-6); 10.78 (1H, s, Ar-O$\underline{H}$).

ν_{max} (nujol mull); 3335 cm^{-1} (N-H); 3250 cm^{-1}(N-H); 1738 cm^{-1} (ester); 1714 cm^{-1} (carbamate); 1670 cm^{-1} (H-bonded δ-lactone); 1627 cm^{-1} (amide).

m/z (F.A.B.); 615 (M + H^+); high resolution measured at 615.2679; $C_{35}H_{39}N_2O_8^+$ requires 615.2706.

$C_{35}H_{38}N_2O_8$	requires:	C, 68.39%; H, 6.23%; N, 4.56%.
	found:	C, 68.01%; H, 6.61%; N, 4.77%.

(1'S,2"R,3S,3"R,4"S)-*N*-[1'-(3,4-dihydro-8-hydroxy-1-oxo-1H-2-benzo-pyran-3-yl)-3'-methylbutyl]-4"-[(benzyloxycarbonyl)amino]-5"-(benzyloxycarbonyl)-2",3"-dihydroxypentamide 20

Osmium tetroxide (0.66 ml of a 5% solution in *tert*-butanol, 13 μmol) was added to a stirred solution of alkene **3** (400 mg, 0.65 mmol) and *N*-methylmorpholine-*N*-oxide (114 mg, 0.65 mmol) in acetone (9 ml) and water (1 ml). The mixture was stirred at room temperature for 18 hours before addition of sodium metabisulphite (1 g, 5.26 mmol) and stirring for a further 30 minutes. The mixture was then filtered and the filter cake washed with ethyl acetate (2 x 10 ml). The combined filtrate was concentrated to give a pale yellow oil. This was dissolved in ethyl acetate (20 ml) and the solution washed with 1M hydrochloric acid (20 ml) before drying ($MgSO_4$) and evaporation *in vacuo*. Flash chromatography then gave the diol **20** as a colourless oil, 170 mg, 40% [R_f ~ 0.31 (30% ethyl acetate/petrol)].

δ_H 0.92 (3H, d, J = 6.5 Hz, CH-C$\underline{H}_3$); 0.95 (3H, d, J = 6.6 Hz, CH-C$\underline{H}_3$); 1.45 (1H, ddd, J = 13.9, 9.0, 4.8 Hz, H-2'a); 1.57-1.72 (1H, m, H-3'); 1.78-1.89 (1H, m, H-2'b); 2.59-2.72 (2H, m, H-5"a, H-5"b); 2.78 (1H, dd, J = 16.4, 3.0 Hz, H-4a); 2.96 (1H, dd, J = 16.4, 11.9 Hz, H-4b); 3.58 (1H, d, J = 9.6 Hz, H-3"); 3.73 (1H, dd, J = 9.6, 5.0 Hz, H-2"); 4.30 (1H, br td, J = 9.2, 3.5 Hz, H-1'); 4.39 (1H, br q, J = 7.3 Hz, H-4"); 4.58 (1H, d with slight further splitting, J = 11.8 Hz, H-3); 5.02 (1H, br s, exchangeable with D_2O, C-3" OH); 5.12 (4H, m, 2 x PhC$\underline{H}_2$O); 5.24 (1H, d, J = 4.7 Hz, exchangeable with D_2O, C-2" OH); 5.64 (1H, br d, J = 8 Hz, N-H); 6.68 (1H, d, J = 7.4 Hz, H-5); 6.86 (1H, d, J = 8.4 Hz, H-7); 7.18 (1H, br d, J = 10 Hz, N-H); 7.28 (5H, m, Ph); 7.29-7.37 (5H, m, Ph); 7.39 (1H, t, J = 7.9 Hz, H-6); 10.82 (1H, s, Ar-O$\underline{H}$) (* - assignments may be reversed).

ν_{max} (thin film): 3350 cm^{-1} (OH and NH); 1735 cm^{-1} (-CO_2R); 1685 cm^{-1} (H-bonded δ-lactone, amide, carbamate).

m/z (F.A.B.): 649 (M + H^+); high resolution measured at 649.2760; $C_{35}H_{41}N_2O_{10}^+$ requires 649.2760.

$[\alpha]_D$ = -31.8° (c = 2.01, $CHCl_3$).

Further elution from the flash chromatography column gave the γ-lactone **19** as a crystalline solid 190 mg, 54%, m.p. 237-240°C [R_f ~ 0.35 (50% ethyl acetate/petrol)].

δ_H 0.72 (3H, d, J = 6.5 Hz, CH-C$\underline{H}_3$); 0.78 (3H, d, J = 6.5 Hz, CH-C$\underline{H}_3$); 1.28-1.37 (1H, m, H-2'a*); 1.47-1.52 (1H, m, H-2'b*); 1.66-1.78 (1H, m, H-3'*); 2.34 (1H, dd, J = 18, 3 Hz, H-5"a); 2.66 (1H, dd, J = 16.5, 2.6 Hz, H-4a); 2.8-3.0 (2H, m, H-5"b, H-4b); 4.14-4.22 (2H, m, H-1', H-4"); 4.34 (1H, dd, J = 5.6, 2.1 Hz, H-2"); 4.50 (1H, dt, J = 10.6, 0.5 Hz, H-3); 4.79 (1H, t, J = 2.6 Hz, H-3"); 4.87 (1H, d, J = 12.0 Hz, PhC$\underline{H}$aHbO); 4.95 (1H, d, J = 12.0 Hz, PhCHa$\underline{H}$bO); 6.13 (1H, br d, J = 5.1 Hz, N-H); 6.57 (2H, d, J = 7.1 Hz, H-5**, N-H); 6.74 (1H, d, J = 7.3 Hz, H-7**); 7.24 (5H, s, Ph); 7.28 (1H, t, J = 7.2 Hz, H-6); 10.72 (1H, s, Ar-O$\underline{H}$) (* and ** - assignments may be reversed).

ν_{max} (nujol mull): 3412 cm^{-1} (N-H); 3395 cm^{-1} (N-H); 3175 cm^{-1} (O-H); 1767 cm^{-1} (γ-lactone); 1730 cm^{-1} (carbamate); 1683 cm^{-1} (H-bonded δ-lactone); 1660 cm^{-1} (amide).

m/z (F.A.B.): 541 (M + H^+); high resolution measured at 541.2189; $C_{28}H_{33}N_2O_9^+$ requires 541.2186.

Amicoumacin C Hydrochloride 2

A suspension of the *N*-Cbz protected-lactone **19** (110 mg, 0.20 mmol) and palladium black (~ 10 mg) in ethanol (5 ml) was stirred vigorously under a hydrogen atmosphere for 2 hours, after which all the starting material had dissolved and T.L.C. showed complete reaction. The reaction mixture was filtered through pre-washed celite before concentrating to give **2** as a crystalline solid, 94 mg, 100%, m.p. 140-145°C (lit.[6] m.p. 131-133°C) [R_f ~ 0.25 (10% methanol/chloroform)].

δ_H (CD_3OD) 0.87 (3H, d, J = 6.4 Hz, CH-C$\underline{H}_3$); 0.93 (3H, d, J = 6.5 Hz, CH-C$\underline{H}_3$); 1.38 (1H, ddd, J = 14.5, 9.5, 3.5 Hz, H-2'a); 1.58-1.70 (1H, m, H-2'b*); 1.71-1.82 (1H, m, H-3'*); 2.53 (1H, dd, J = 18.7, 1.7 Hz, H-5"a); 2.82-3.08 (2H, m, H-4a, H-4b); 3.17 (1H, dd, J = 18.7, 8.8 Hz, H-5"b); 4.11-4.22 (2H, m, H-1', H-4"); 4.43 (1H, d, J = 2.8 Hz, H-2"); 4.67 (1H, dt, J = 9.5, 5.2 Hz, H-3); 6.77 (1H, d, J = 7.3 Hz, H-5**); 6.81 (1H, d, J = 8.4 Hz, H-7**); 7.43 (1H, dd, J = 8.4, 7.3 Hz, H-6) (* and ** - assignments may be reversed).[14]

ν_{max} (nujol mull); 3400-3000 cm^{-1} (-NH_3^+, -OH); 1788 cm^{-1} (γ-lactone); 1671 cm^{-1} (H-bonded δ-lactone, amide).

m/z (F.A.B.); 407 ($C_{20}H_{27}N_2O_7^+$); high resolution measured at 407.1819; $C_{20}H_{27}N_2O_7^+$ requires 407.1818.

$[\alpha]_D$ = -71.1° (c = 0.34, MeOH).

AI-77-B 1

A solution of the γ-lactone hydrochloride **2** (30 mg, 67.8 μmol) in methanol (2 ml) was taken to pH 9 by dropwise addition of 0.1M sodium hydroxide solution. The mixture was maintained at this pH by dropwise addition of 0.1M NaOH solution until T.L.C. showed no remaining starting material (approx. 6 hours). The mixture was then acidified to pH 6.5 (by dropwise addition of 0.1M HCl in methanol before concentrating to give a solid residue. This was purified by passing down a column of Amberlite XAD-2 resin, the column was washed first with water (20 ml) followed by methanol (20 ml), fractions containing only **1** were combined and concentrated to give AI-77-B **1** as a colourless oil, 20 mg, 70%.

δ_H (CD_3OD) 0.89 (3H, d, J = 6.4 Hz, CH-C<u>H</u>$_3$); 0.93 (3H, d, J = 6.5 Hz, CH-C<u>H</u>$_3$); 1.36-1.45 (1H, m, H-2'a); 1.66 (1H, m, H-3'); 1.78 (1H, m, H-2'b); 2.4-2.6 (2H, m, H-5"a, H-5"b); 2.87 (1H, dd, J = 16.7, 3.1 Hz, H-4a); 3.04 (1H, dd, J = 16.7, 12.2 Hz, H-4b); 3.52-3.61 (1H, m, H-4"); 3.86-3.92 (1H, m, H-3"); 4.09 (1H, d, J = 6.8 Hz, H-2"); 4.22-4.38 (1H, m, H-1'); 4.54-4.64 (1H, m, H-3); 6.74 (1H, d, J = 7.2 Hz, H-5*); 6.79 (1H, d, J = 8.3 Hz, H-7*); 7.40 (1H, t, J = 7.9 Hz, H-6) (* - assignments may be reversed).

ν_{max} (thin film); 3400-3000 cm^{-1} (-NH_3^+, -OH); 1670 cm^{-1} (δ-lactone, amide, amino acid).

m/z (F.A.B.); 425 (M + H^+); high resolution measured at 425.1910; $C_{20}H_{29}N_2O_8^+$ requires 425.1924.

$[\alpha]_D$ = -70.0° (c = 0.49, MeOH); lit.[5a] $[\alpha]_D$ = -72.0° (c = 0.07, MeOH).

2",3"-*epi* AI-77-B

Palladium black (3 mg) was added to a solution of diol-ester **20** (29 mg, 44.8 μmol) in dry ethanol (0.5 ml). The mixture was then stirred vigorously under a hydrogen atmosphere for 4 hours before filtering through pre-washed celite and concentrating to give the title compound as a crystalline solid, 19 mg, 100%. m.p. 140-145°C (dec.).

δ_H (CD_3OD); 0.87 (3H, d, J = 6.3 Hz, CH-C<u>H</u>$_3$); 0.93 (3H, d, J = 6.5 Hz, CH-C<u>H</u>$_3$); 1.40 (1H, ddd, J = 13.4, 9.5, 3.9 Hz, H-2'a); 1.60-1.80 (2H, m, H-2'b, H-3'); 2.55-2.68 (2H, m, H-5"a, H-5"b); 2.92 (1H, dd, J = 16.6, 3.2 Hz, H-4a); 3.09 (1H, dd, J = 16.6, 11.5 Hz, H-4b); 3.74-3.80 (1H, m, H-4"); 3.90 (1H, t, J = 2.6 Hz, H-3"); 4.25 (1H, d, J = 2.6 Hz, H-2"); 4.29 (1H, dt, J = 11.0, 4.0 Hz, H-1'); 4.57 (1H, dt, J = 12.0, 3.7 Hz, H-3); 6.74 (1H, d, J = 7.5 Hz, H-5*); 6.79 (1H, d, J = 8.3 Hz, H-7*); 7.40 (1H, dd, J = 8.3, 7.5 Hz, H-6) (* - assignments may be reversed).

ν_{max} (nujol mull); 3400-3000 cm^{-1} (-NH_3^+, -OH); 1670 cm^{-1} (H-bonded δ-lactone, amide, amino acid).

m/z (F.A.B.); 425 (M + H^+); high resolution measured at 425.1930; $C_{20}H_{29}N_2O_8^+$ requires 425.1924.

$[\alpha]_D$ = -57.9° (c = 0.97, MeOH).

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