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STEREOSPECIFIC SYNTHESIS OF DEALANYLALAHOPCIN†

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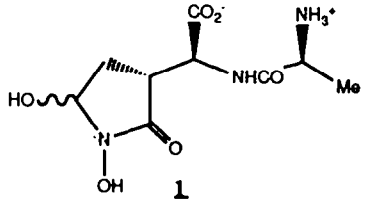
Abstract: The first synthesis of the novel α-amino acid dealanylalahopcin starting from (L)-aspartic acid is described.

Introduction

The dipeptide antibiotic alahopcin (B-52653) 1 was isolated by Higashide *et al.* from a culture of *Streptomyces albulus*.[1] Previous to this a new antimetabolite of (L)-proline, nourseimycin, had been discovered which was later shown to be identical to alahopcin 1 from its physical and spectroscopic data.[2,3] The antibiotic 1 was found to be active against a wide range of both Gram -positive and -negative bacteria with especially strong activity against *Staphylococcus aureus* 4R, previously an antibiotic resistant mutant. Alahopcin 1 was also found to inhibit prolyl collagen hydroxylase *in vivo* and *in vitro* and to have a stimulatory effect on the production of bacterial α-amylase in mice.[1]



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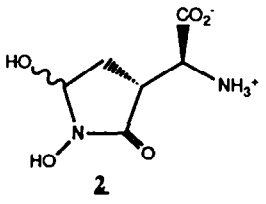


2

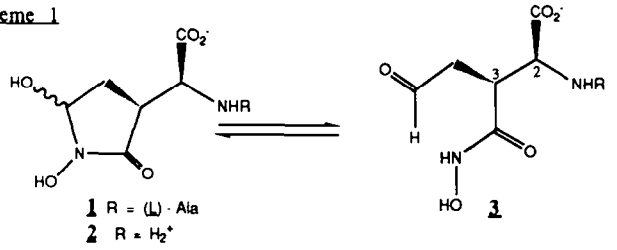
During the initial fermentation experiments producing alahopcin 1, a new α-amino acid 2 was discovered, which had low antibacterial activity (~1% c.f. alahopcin 1) but showed similar prolyl collagen hydroxylase and related enzyme inhibition (~60% c.f. alahopcin 1). Enzymatic hydrolysis (using α-amino acid hydrolase) of alahopcin 1 also yielded this new α-amino acid 2 and (L)-alanine, and thus 2 was named dealanylalahopcin (B-52653-C).[4] A degradative study was performed from which the structures of alahopcin 1, and hence dealanylalahopcin 2 were deduced.[3] To date, however, no synthetic routes to these compounds have been published to confirm their structures and configurations.

† This article is dedicated to Professor W. D. Ollis on the occasion of his 65th birthday and retirement. We would like to express our thanks to Professor Ollis for his continuing interest in our research over many years

Retrosynthetic Analysis

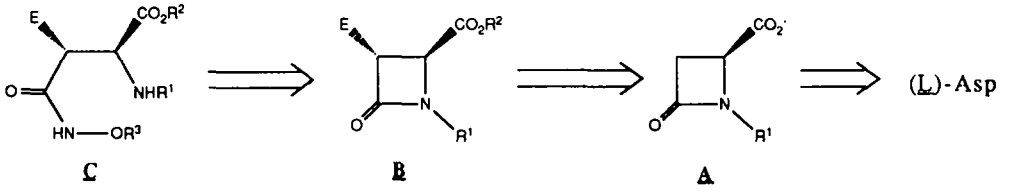
The *N*-hydroxy-γ-lactam system of 1 and 2 is in equilibrium with a ring opened form 3 which can be seen to be a stereospecifically β-functionalised (L)-aspartic acid derivative† (scheme 1)

Scheme 1



From this observation a retrosynthetic analysis to 1 and 2 from (L)-aspartic acid was devised (scheme 2). Stereospecific *'trans'* allylation, A to B, followed by β-lactam opening by a hydroxylamino nucleophile, B to C, would provide both the key stereochemical relationships at C-2 and C-3 as well as the required constitutional form of 3, and hence 1 and 2.

Scheme 2



Stereoselective alkylations of a number of monocyclic β-lactams have been reported,[6] and we chose as a key synthon (4S)-*N*-(*t*-butyldimethylsilyl)azetidin-2-one-4-carboxylic acid 4, a compound successfully *'trans'* alkylated [LDA (2.2eq.), CH_3CHO, THF] by Reider and Grabowski in their synthesis of (±)-thienamycin.[6c] 4 can be obtained from (L)-aspartic acid *via* a Grignard-mediated β-lactam ring closure,[7] a procedure previously shown to occur without racemisation.[8] Preferential attack at the β-lactam carbonyl bond with a hydroxylamino nucleophile was anticipated, if careful choice of protecting groups R^1, R^2 of synthon B, was taken. However competitive epimerisation at either (or both) chiral centres during such an opening could not, without experimentation, be ruled out.

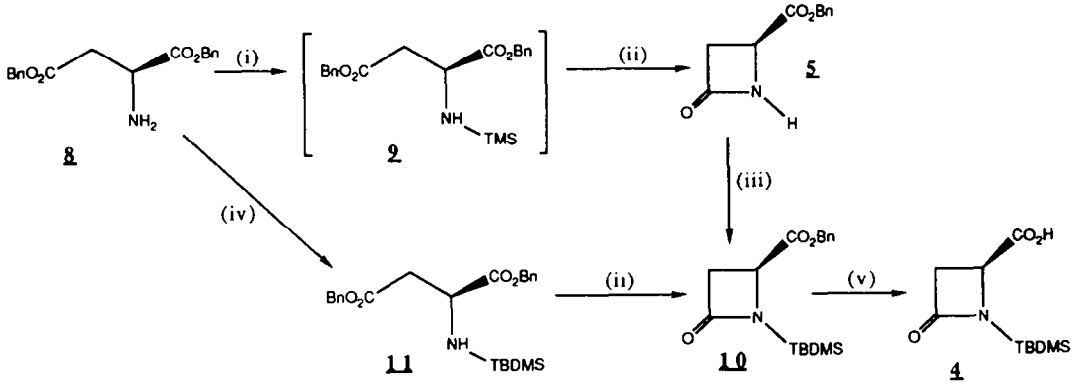
Synthesis of dealanylalahopcin (2)

(L)-aspartic acid 6 was converted to crystalline (L)-aspartic acid dibenzyl ester *p*-toluenesulphonate 7,[9] which was extracted ($K_2CO_{3(aq)}$/EtOAc) to provide the free amine 8.

† Previously reported β-alkylations of (L)-aspartic acid derivatives are generally not completely stereospecific and would not provide easy access to the β-hydroxamic acid moiety.[5]

Salzmann's[6a] cyclisation procedure to the β-lactam 5 involved the *in situ* formation of (L)-*N*-(trimethylsilyl)dibenzyl aspartate 9 (TMSCl, Et_3N, Et_2O), from 8, however 9 proved to be very moisture sensitive requiring that the necessary removal of the co-produced triethylamine.hydrochloride be carried out under an inert atmosphere.§ Treatment of the so obtained *N*-silylated species 9 with *t*-butyl magnesium chloride (*t*-BuMgCl) led to the formation of 5 in 69% yield from 7. The β-lactam 5 was then *N*-protected (TBDMSCl, Hunig's base, DCM)[10] to yield 10 in 92% (scheme 3). An alternative and more direct route to 10 was to form (L)-*N*-(*t*-butyldimethylsilyl)dibenzyl aspartate 11 by reacting the free amine 8 with *N*-methyl-*N*-(*t*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) in acetonitrile.[11] Treatment of this with *t*-BuMgCl yielded 10 in 75%, from 7, with identical spectral (^1H NMR, ^{13}C NMR and IR) and physical data ($[\alpha]_D^{20}$), to that produced previously by Salzmann's route.[6a] Conversion of this *N*-protected β-lactam 10 to the requisite free acid 4 (90%) was easily carried out by hydrogenolysis (H_2, 10% Pd/C, THF) (scheme 3).

Scheme 3.



i) TMSCl, Et_3N, Et_2O, 0°C; ii) ^{t}BuMgCl, Et_2O, 0°C

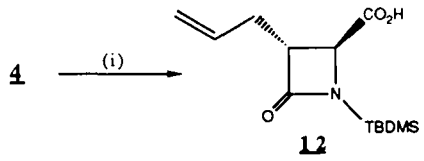
iii) TBDMSCl, Hunig's base, DCM, r.t.; iv) MTBSTFA, MeCN, r.t.

v) H_2, 10% Pd/C, THF, r.t.

In order to generate the correct carbon framework found in 1, 2, and 3 the azetidinone 4 required stereospecific alkylation with an electrophile which could easily be converted into the necessary methylene aldehyde, seen in 3. Thus, the protected free acid 4 was treated with LDA (2.2eq.) and the resulting dianion quenched with allyl bromide to yield, after workup, 12 (scheme 4). The ^1H NMR of the crude reaction mixture showed only one set of resonances attributable to a β-lactam and the stereochemistry of 12 was assigned as *'trans'*, based upon the observed couping constant of 2.5Hz for $J_{3H\text{-}4H}$. In previous reports,[6b,c] $J_{3H\text{-}4H}$ for a *'trans'* β-lactam system was found to be in the range 1-3Hz, whereas for a *'cis'* orientation a coupling of 5-8Hz was observed. The required methylene aldehyde of 3 should be subsequently available by oxidative cleavage of the olefinic function in 12.

§ In the previous report[6a] of this procedure a full experimental account was not given.

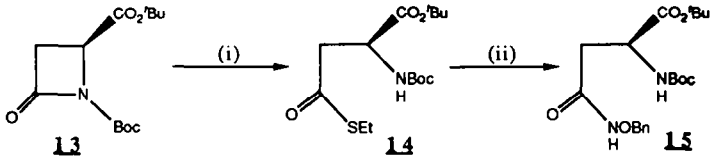
Scheme 4.



i) LDA (2.1eqs.), allyl bromide (2.2eqs), THF, 0°C

With the carbon skeleton in place it was necessary to investigate the cleavage of the β-lactam amide bond by a hydroxylamino nucleophile (e.g. *O*-benzyl hydroxylamine) to generate the hydroxamic acid moiety. Before carrying out reactions on an allylated azetidinone, a number of model ring opening reactions were carried out on β-lactams not substituted at C-3 (e.g. 13). From these early reactions it was found that a sterically demanding ester function was needed to inhibit products formed from reactions at the ester carbonyl, and that the efficiency of ring opening was promoted if the β-lactam nitrogen was protected and also activated by a *t*-butoxycarbonyl group.[12] The addition of a catalytic amount of sodium ethanethiolate (10%mol.eq.) was also seen to promote[13] the formation of the desired hydroxamic acid product 15 presumably by initial β-lactam thiolysis to a sterically more accessible thiolester 14 (scheme 5).

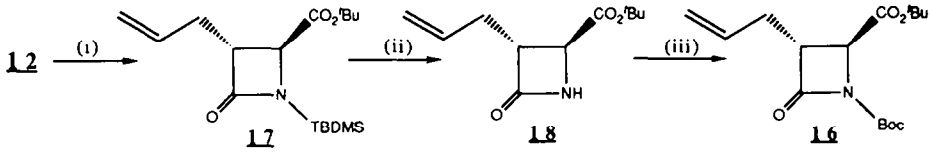
Scheme 5.



i) EtS^-Na^+, THF; ii) NH_2OBn, THF, reflux

Thus, it was found necessary to construct (3R,4S)-*t*-butyl *N*-(*t*-butoxycarbonyl)azetidin-2-one-3-allyl-4-carboxylate 16 from 12 (scheme 6). Firstly, a mild esterification procedure[14] was used to form the *t*-butyl ester 17 (68% from 4, *via* 12) using *O*-(*t*-butyl)trichloroacetimidate and catalytic boron trifluoride etherate in DCM:cyclohexane (1:2), at 0°C. Removal of the *N*-TBDMS group (CsF, MeOH) gave 18 which was then directly *N*-Boc protected[15] [di-(*t*-butyl)dicarbonate, cat. DMAP, acetonitrile] to give 16 in 85% yield from 17 (scheme 6).

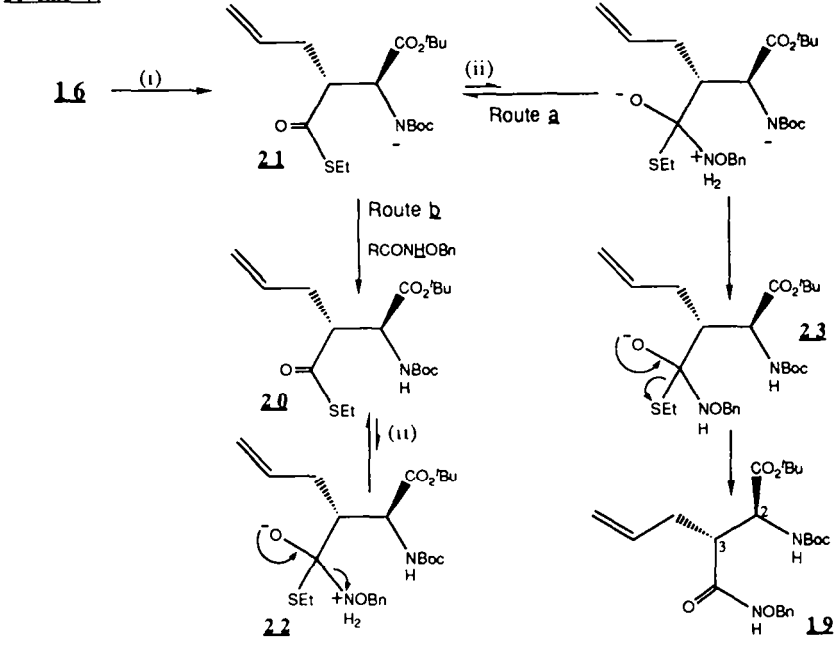
Scheme 6.



i) *O*-(*t*-butyl)trichloroacetimidate, cat. $BF_3.Et_2O$, DCM:cyclohexane (1:2), 0°C
ii) CsF, MeOH, r.t., iii) Boc_2O, cat. DMAP, MeCN

The ring opening of 16 was initially tried under the optimal conditions developed for the non allylated model system 13 (scheme 5). However these conditions gave only low yields (<20%) of the desired product 19 in addition to the formation of the thiolester 20 (~10%). After a series of reactions, conditions for the ring opening of 16 were optimised to give a 78% yield of (2S,3R)-*t*-butyl 2-(*t*-butoxycarbonylamino)-3-[(*O*-benzyl)hydroxyaminocarbonyl]hex-5-enoate 19, and 9% (2S,3R)-*t*-butyl 2-(*t*-butoxycarbonylamino)-3-(ethylthiocarbonyl)hex-5-enoate 20. To obtain this yield it was necessary to effect a slow addition of a solution of potassium *t*-butoxide in THF (~0.8eq.) to the solution of the *N*-Boc protected azetidinone 16, sodium ethanethiolate (10%mol.eq.), and *O*-benzyl hydroxylamine (1.5eq.) in THF heated under reflux. The purpose of the potassium *t*-butoxide was to deprotonate the hydroxamic ester proton of the product 19 which presumably prevented protonation of the *O*-benzyl hydroxylamine, thus maintaining it's nucleophilicity. In addition, if this acidic hydroxamic ester proton (pKa ~10)[16] were not neutralised it may protonate the initial ring opened thiolester anion 21. The so-formed 20 may no longer be able to acylate the *O*-benzyl hydroxylamine, as the tetrahedral intermediate 22 could not be deprotonated intramolecularly as required to form 23, and the reverse reaction 22 to 20 would now dominate (scheme 7, routes a *vs* b). The requirement for the addition of the basic reagent $K^{+-}O^tBu$ to obtain this optimal yield for the ring opening of 16 did not appear to lead to epimerisation at C-2 or C-3, of 19, as no evidence of diastereomers were detected from the ^1H (200MHz) or ^{13}C NMR's of the crude ring opened products.

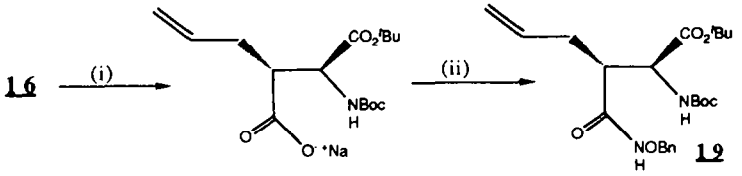
Scheme 7.



i) $Na^+{}^-SEt$; ii) NH_2OBn

The ring opening of 16 was also observed using sodium deuteroxide (2eq.) in D_2O:THF Subsequent DCC coupling of the free acid group with *O*-benzyl hydroxylamine.hydrochloride, at pH 4, gave 19 although with poor efficiency (30%) (scheme 8). Hydroxamic acid 19 formed by this route (scheme 8) was found to be identical by ^{1}NMR and specific optical rotation to 19 derived *via* scheme 7, and was shown to be formed without significant deuterium incorporation at C-2 or C-3 (*m/z* and ^{1}H NMR). This result is consistent with formation of 19 without racemisation *via* the higher yielding scheme 7.

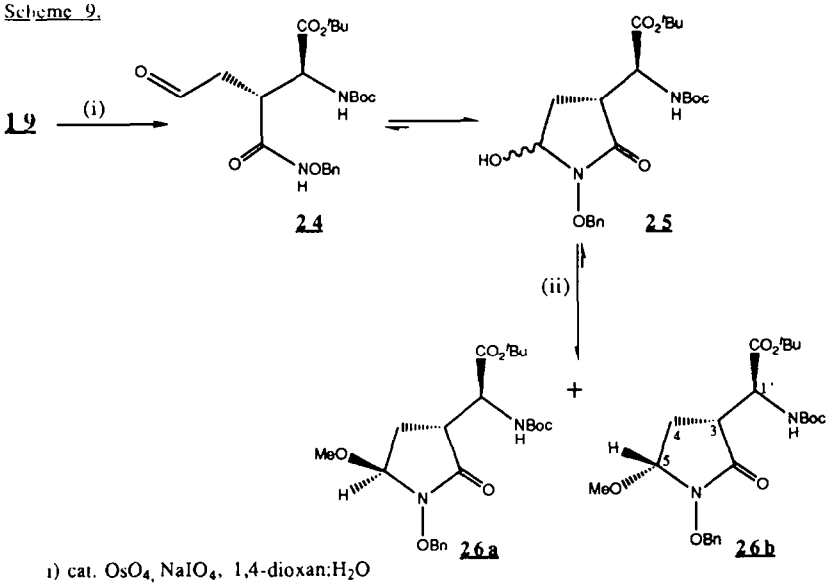
Scheme 8.



i) NaOD (2eq.), D_2O:THF (1:10); ii) $Cl^-{}^+H_3NOBn$, DCC, pH 4, H_2O:THF (~1:1)

With the acyclic species 19 in hand it was necessary to form the heterocyclic ring of 1 and 2. Cleavage of the terminal olefin to form an aldehyde was carried out [cat. OsO_4, $NaIO_4$, 1,4-dioxan:water (1:1)] to yield 24 which was found to cyclise *in situ* to the hydroxylactam 25, with both epimers at C-5 seen in the crude ^{1}H NMR in a ratio of ~1:1. This hydroxylactam moiety would be susceptible to the reductive conditions required later to remove the benzyl protecting group and thus 25 was protected as the δ-methoxylactams 26a,b (MeOH, cat. TFA). Both epimers of 26a,b were obtained in a 6:1 ratio (5S:5R) (assignments made from the results of a series of nOe irradiations) in 68% yield from the acyclic hexenoate 19 (scheme 9).

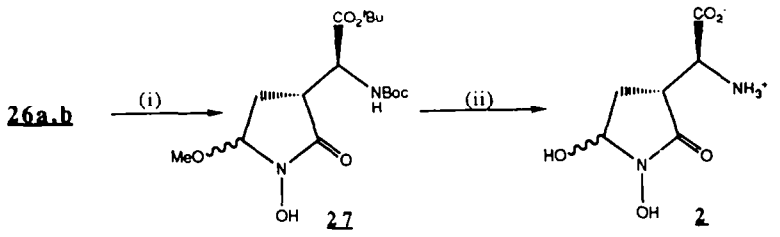
Scheme 9.



i) cat. OsO_4, $NaIO_4$, 1,4-dioxan:H_2O
ii) MeOH, cat. TFA

A two stage deprotection procedure was then employed to release dealanylalahopcin 2. Firstly the benzyl group was removed (H_2, 10% Pd/C, MeOH), in 75% to give 27. Deprotection using aqueous acid [1,4-dioxan:1N HCl (1:1), 18hrs] gave 2 in 65% yield, *m/z* 191 (FAB^+) (scheme 10). After ion exchange the ^1H (fig. 1.) and ^{13}C spectral data obtained for the synthetic dealalanylalahopcin 2 were in agreement with the previously published spectral data,[3,4] and measurement of the optical rotation for the synthesised compound gave values for $[\alpha]_D^{20}$ consistent with those published {$[\alpha]_D^{20}$ + 45.6° (c=0.88, H_2O), $[\alpha]_D^{20}$ + 56.2° (c=0.4, 0.1N HCl); lit.,[4] $[\alpha]_D^{20}$ + 50.8° (c=0.5, H_2O), $[\alpha]_D^{20}$ + 55.6° (c=1, 0.1N HCl)}.§

Scheme 10.



i) H_2, cat. Pd/C, MeOH; ii) 1,4-dioxan:1M HCl, 18hrs

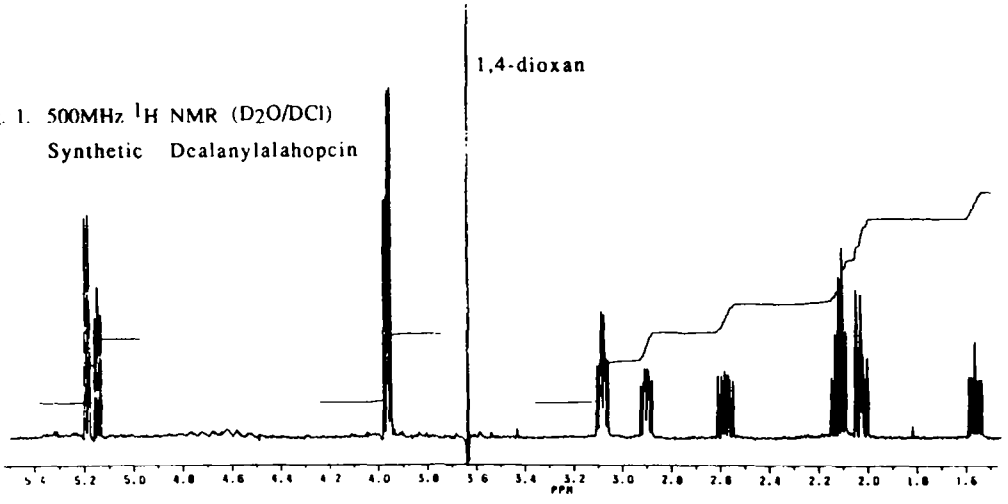


fig. 1. 500MHz ^1H NMR (D_2O/DCl)
Synthetic Dealanylalahopcin

In conclusion, the first synthesis of dealanylalahopcin 2 has been achieved. Noteworthy points of the synthetic approach are the stereospecific '*trans*' allylation (>95%) of the β-lactam anion derived from 4 and the use of the so-formed β-lactam as a stereospecifically β-alkylated (L)-aspartic acid synthon. This synthesis also confirms the original structural assignment of 2 which was based upon a degradative study.[3] The synthesis of alahopcin 1 is a current objective.

§ Note added in proof. Synthetic dealanylalahopcin has now been shown to be identical (500MHz NMR, individual and mixed samples) with an authentic sample gratefully received from Takeda Chemical Industries, Osaka, Japan.

Experimental Section

Infrared (IR) spectra were recorded on either a Perkin-Elmer 681 or Perkin-Elmer 1710 FT-IR spectrometers with only selected absorptions being reported. Nuclear magnetic resonance (NMR) spectra were recorded on Bruker AM-500, Bruker WH-300, and Varian Gemini-200 spectrometers. Spectra were taken using $CDCl_3$ as solvent with chemical shifts quoted in parts per million (δ p.p.m.) using the residual solvent peak as an internal reference except when stated otherwise. Coupling constants (*J*) are quoted to the nearest 0.5Hz. ^{13}C spectra were run using DEPT editing, except when otherwise stated. Mass spectra were recorded on a V. G. Micromass ZAB 1F (DCI), a V. G. 20-250 (DCI/CI/FAB$^+$) or a V. G. TRIO 1 (GCMS) spectrometers. Optical rotation were recorded on a Perkin-Elmer 241 polarimeter, at 20°C with a pathlength of 1dm with concentrations given in g/100ml. Melting points were obtained using a Buchi 510 capillary melting point apparatus and are uncorrected. Microanalyses were performed within the Dyson Perrins.

Flash chromatography was accomplished on silica gel using Sorbsil™ C60. Thin layer chromatography was performed on aluminium sheets pre-coated with Merck silica gel 60 F_{254}, plates being visualised with UV (254nm) or 10%w/v ammonium molybate in 2M sulphuric acid, followed by heat. Amino acids were located on t.l.c. by 3%w/v ninhydrin in ethanol.

All solvents were distilled before use; tetrahydrofuran (THF) from sodium/benzophenone ketyl, acetonitrile, dichloromethane (DCM) and *t*-butanol from calcium hydride. 'Petrol' refers to that fraction of light petroleum ether boiling between 40-60°C. Triethylamine and diisopropylamine were distilled from calcium hydride before use. Allyl bromide was washed with 1M sodium hydrogen carbonate, dried (Na_2SO_4), and distilled before use. *O*-benzyl hydroxylamine was extracted from it's hydrogen chloride using sodium hydrogen carbonate. *n*-Butyl lithium was standardised by titration using 1,3-diphenylacetone-*p*-tosylhydrazone at -78°C.[17] Lithium diisopropylamide was prepared when required using the procedure of Ireland *et al.*.[18] *O*-(*t*-butyl)trichloroacetimidate was prepared by the method of Jackson *et al.*.[14] Sodium ethanethiolate was prepared by the action of sodium hydride on ethanethiol (1.1equivalent) in THF (10ml/mmol). All other reagents were used as obtained from commercial sources.

(L)-*Dibenzyl aspartate* 8

(L)-aspartic acid dibenzyl ester *p*-toluenesulphonate 7[9] (40.98g, 84.5mmol) {m.p. 157-158°C (from MeOH/Et_2O) (lit.,[9] 158-160°C); $[\alpha]_D^{20}$ +0.9° (c=1, MeOH) [lit.,[9] +1.0° (c=1.5, MeOH)]} and potassium carbonate (34.97g, 253mmol) were dissolved in distilled water (500ml); this solution was then extracted with ethyl acetate (3x250ml). The organic layer was washed with saturated brine (500ml) and dried ($MgSO_4$) whence removal of ethyl acetate yielded 8 (26.24g, 90%) as a pale yellow oil; ν_{max} ($CHCl_3$) 1740(s, C=O), 1500(w), 1455(w), and 1170(m); δ_H(200MHz) 2.05 (2H, br s, NH_2), 2.73-2.96 (2H, m, 3-H), 3.82-3.92 (1H, m, 2-H), 5.13 & 5.16 (2x2H, 2xs, 2xPh-CH_2-O), and 7.33-7.37 (10H, m, Ph); δ_C(50.3MHz) 38.7 (3-C), 51.2 (2-C), 66.6 & 67.1 (2xPh-CH_2-O), 127.0-128.7 (Ph), 135.6 & 135.7 (2xPh *ipso* C), and 171.2 & 174.2 (2xC=O).

(4S)-*Benzyl azetidin-2-one-4-carboxylate* 5

To a solution of (L)-dibenzyl aspartate 8 (12.6g, 40mmol) in diethyl ether (55ml) at 0°C, trimethylchlorosilane (5.8ml, 46mmol) was added followed, after 15 minutes, by triethylamine (6.4ml, 46mmol) with the immediate formation of a white precipitate. After 1 hour the resulting suspension was filtered, under an inert atmosphere, and the filtrate diluted with diethyl ether (100ml). Dropwise addition, over 45mins, of *t*-butylmagnesium chloride (23.0ml of a 2M solution in diethyl ether, 46mmol) yielded a suspension in a pale yellow oil which was allowed to warm to room temperature and stir overnight. The mixture was again cooled to 0°C and 2N HCl saturated with NH_4Cl (80ml) was carefully added, the organic layer was separated and the aqueous phase washed with DCM (2x60ml). The organic portions were then washed with saturated $NaHCO_3$ (100ml), saturated brine (100ml), dried ($MgSO_4$), and combined. Removal of the solvent yielded a white dispersion in a pale yellow oil. Crystallisation from DCM yielded 5 (5.60g, 69%) as white flakes m.p. 138-139°C (from DCM) (lit.,[6a] 141-143°C (from $CHCl_3$)); R_f 0.5 (EtOAc: DCM 4:1) (Found C, 64.40; H, 5.40; N, 6.70. Calc. for $C_{11}H_{11}O_3N$ C, 64.39; H, 5.40; N, 6.82%); $[\alpha]_D^{20}$ -42.6° (c=2.05, $CHCl_3$) (lit.,[6a] $[\alpha]_D^{20}$ -43.4° (c=3.28, $CHCl_3$)); ν_{max} ($CHCl_3$) 3450(m), 1780(s, β-lactam C=O), 1748(m, ester C=O), 1350(w), 1265(m), and 1045(w); δ_H(200MHz) 3.09 (1H, ddd, *J* 2, 3, 15 Hz, 3-H), 3.35 (1H, ddd, *J* 1, 6, 15 Hz, 3-H), 4.22 (1H, dd, *J* 3, 6 Hz, 4-H), 5.22 (2H, s, Ph-C$\underline{H}_2$-O), 6.11 (1H, br s, NH), and 7.34-7.42 (5H, m, Ph); δ_C(50.3MHz) 43.3 (3-C), 47.2 (4-C), 67.4 (Ph-$\underline{C}H_2$-O), 128.6-128.8 (Ph), 135.1 (Ph *ipso* C), and 166.8 & 171.2 (2xC=O); *m/z* [DCI(NH_3)] 223 (MNH_4^+, 100%), 206 (MH^+, 43), 178 (47), 108 (36), and 91 ($C_7H_7^+$, 54).

(4S)-*Benzyl* N-*(t-butyldimethylsilyl)azetidin-2-one-4-carboxylate* 10

(4S)-Benzyl azetidin-2-one-4-carboxylate 5 (6.15g, 30mmol), *t*-butylchlorodimethylsilane (5.20g, 34.5mmol) in anhydrous DCM (50ml) and *N,N'*-diisopropylethylamine (7.84ml, 45mmol) in anhydrous DCM (25ml) were mixed and stirred at room temperature for 24 hours. Removal of the solvent led to a pale yellow solid in an orange oil which was washed with diethyl ether (3x50ml) until the washings were colourless. Combination, and then removal of the diethyl ether, led to 10 (8.83g, 92%), as a pale brown oil, which could be directly used in the next reaction. A small amount (250mg) was separated and purified by flash chromatography [SiO_2 (25g); eluting with petrol:diethyl ether 1:1] to yield a colourless oil, R_f 0.3 (petrol:Et_2O 1:1); (Found: C, 64.00; H, 8.22; N, 4.05. $C_{17}H_{25}O_3NSi$ requires C, 63.92; H, 7.89; N, 4.38%.); $[\alpha]_D^{20}$ -61.2° (c=1.55, $CHCl_3$); ν_{max}($CHCl_3$) 2955(m), 2860(m), 1746(s, C=O), 1290(m), 1260(m), 1180(m), 840(m), and 825(m); δ_H(200MHz) 0.07 & 0.25 (2x3H, 2xs, Si-C$\underline{H}_3$), 0.94 (9H, s, Si-C(C$\underline{H}_3$)$_3$), 3.07 (1H, dd, *J* 2, 15 Hz, 3-H), 3.34 (1H, dd, *J* 6, 15 Hz, 3-H), 4.08 (1H, dd, *J* 2, 6 Hz, 4-H), 5.19 (2H, s, Ph-C$\underline{H}_2$-O), and 7.35 (5H, s, Ph); δ_C(50.3MHz) -6.6 & -6.2 (2xSi-$\underline{C}H_3$), 18.3 (Si-$\underline{C}$(CH$_3$)$_3$), 25.95 (Si-C($\underline{C}$H$_3$)$_3$), 43.8 (3-C), 48.7 (4-C), 67.2 (Ph-$\underline{C}H_2$-O), 128.8 (Ph), 135.1 (Ph *ipso* C), and 171.1 & 172.2 (2xC=O); *m/z* [CI(NH_3)] 337 (MNH_4^+, 7%), 320 (MH^+, 100), 292 (15), 108 (27), and 91 ($C_7H_7^+$, 46).

(4S)-N-(t-Butyldimethylsilyl)azetidin-2-one-4-carboxylic acid 4

To a suspension of 10% Pd/C (640mg) in THF (40ml) was added (4S)-benzyl *N*-(*t*-butyldimethylsilyl)azetidin-2-one-4-carboxylate 10 (8.50g, 26.6mmol) which was then placed under a balloon of hydrogen and stirred for 24 hours. The suspension was filtered through a plug of Celite® and the volatile components were removed to yield a pale brown solid. Crystallisation from diethyl ether/petrol yielded 4 as fine white needles (5.84g, 87% from 5), m.p. 147-148°C (from Et_2O/petrol); (Found: C, 52.21; H, 8.65; N, 5.87. $C_{10}H_{19}O_3NSi$ requires C, 52.37; H, 8.35; N, 6.11%); $[\alpha]_D^{20}$ -71.7° (c=1.03, $CHCl_3$) (lit.,[6c] $[\alpha]_D^{20}$ -74° (c=1.0, $CHCl_3$)); $\nu_{max}(CHCl_3)$ 1755(s, β-lactam C=O), 1725(m, ester C=O), 1290(m), 1260(m), and 1180(m); δ_H(200MHz) 0.17 & 0.32 (2x3H, 2xs, 2xSi-C$\underline{H}_3$), 0.97 (9H, s, Si-C(C$\underline{H}_3$)$_3$), 3.15 (1H, dd, *J* 3, 15 Hz, 3-H), 3.43 (1H, dd, *J* 6, 15 Hz, 3-H), 4.09 (1H, dd, *J* 3, 6 Hz, 4-H), and 7.95(1H br s, $CO_2\underline{H}$); δ_C(50.3MHz) -6.6 & -6.2 (2xSi-$\underline{C}H_3$), 18.3 (Si-$\underline{C}$(CH$_3$)$_3$), 25.9 (Si-C(C$\underline{H}_3$)$_3$), 43.6 (3-C), 48.6 (4-C), and 172.4 & 176.6 (2xC=O); *m/z* [DCI(NH_3)] 247 (MNH_4^+, 100%), 230 (MH^+, 50), 202 (27), and 130 (56).

(L)-N-(t-Butyldimethylsilyl)dibenzyl aspartate 11

A solution of dibenzyl aspartate 8 (661mg, 2.1mmol), MTBSTFA (1.48ml, 6.3mmol), and *t*-butylchlorodimethylsilane (31.6mg, 0.21mmol) in acetonitrile (10ml) was stirred for 30 minutes. The solvent was then removed and the residual mobile oil placed under vacuum (1mmHg) for 18 hours, after which time excess silylating reagent had been removed to yield 11 (875.3mg, 98%) as a pale yellow mobile oil; δ_H (200MHz) -0.02 & 0.00 (2x3H, 2xSi-C$\underline{H}_3$), 0.90 (9H, s, Si-C(C$\underline{H}_3$)$_3$), 2.62-2.88 (2H, m, 3-H), 3.82-3.95 (1H, m, 2-H), 5.02-5.18 (4H, m, 2xPh-C$\underline{H}_2$-O), and 7.27-7.35 (5H, m, Ph); δ_C (50.3MHz) -5.3 & -5.2 (2xSi-$\underline{C}H_3$), 0.9 (Si-$\underline{C}$(CH$_3$)$_3$), 25.9 (Si-C(C$\underline{H}_3$)$_3$), 41.8 (3-C), 52.6 (2-C), 66.4 & 66.8 (2xPh-$\underline{C}H_2$-O), 128.4-128.7 (Ph), 135.8 (Ph *ipso* C), and 170.9 & 174.7 (2xC=O); *m/z* [DCI(NH_3)] 428 (MH^+, 100%), 370 (18), 314 (20), 108 (18), and 91 ($C_7H_7^+$).

Ring closure of 11

The crude *N*-(*t*-Butyldimethylsilyl)dibenzyl aspartate 11 (875.3mg, 2.05mmol) was dissolved in diethyl ether (20ml) and cooled to 0°C. Dropwise addition of *t*-butylmagnesium chloride (1.13ml of a 2M solution in diethyl ether, 2.26mmol) then led to the formation of a yellow suspension. After warming to room temperature and stirring overnight saturated NH_4Cl (20ml) was carefully added and stirring continued for 10 minutes. Separation of the organic portion followed by washing with water (20ml), brine (30ml), and drying ($MgSO_4$) and subsequent removal led to a yellow oil. Flash chromatography [SiO_2 (40g); eluting with petrol:diethyl ether 1:1] yielded 10 (490mg, 75% from 7) as a colourless oil, $[\alpha]_D^{20}$ -60.8° (c=1.0, $CHCl_3$); with identical spectroscopic data (^1H, ^{13}C NMR, and *m/z*) to that produced *via* 9.

(3R,4S)-N-(t-Butyldimethylsilyl)azetidin-2-one-3-allyl-4-carboxylic acid 12

Freshly prepared LDA (42.6mmol) was added dropwise to (4S)-*N*-(*t*-butyldimethylsilyl)azetidin-2-one-4-carboxylic acid 4 (4.647g, 20.3mmol) dissolved in THF (25ml) at 0°C. The solution was stirred for 15 minutes then allyl bromide (3.51ml, 44.7mmol) was added, resulting in the formation of a deep red solution which faded to pale yellow over 5 minutes. After stirring at 0°C for 2 hours, 1M $KHSO_4$ (200ml) and ethyl acetate (200ml) were added, the organic layer being separated and then washed with 1M $KHSO_4$ (200ml), saturated brine (2x200ml) and dried ($MgSO_4$). Removal of the solvent yielded 12 as a pale yellow solid (5.19g, 95%), which required no purification to be used in subsequent reactions. A small portion was recrystallised (diethyl ether/petrol) to yield white needles, m.p. 112-114°C (from Et_2O/petrol) (Found C, 57.80; H 8.59; N, 5.30. $C_{13}H_{23}O_3NSi$ requires C, 57.96; H, 8.61; N, 5.21%); $[\alpha]_D^{20}$ -68.6° (c=1.0, $CHCl_3$); ν_{max} ($CHCl_3$) 3500(w), 1750(s, β-lactam C=O), 1720 (m, acid C=O), 1285(m), 1255(m), 840(m), and 825(m); δ_H(200MHz) 0.13 & 0.31 (2x3H, 2xs, Si-C<u>H</u>3), 0.96 (9H, s, Si-C(C<u>H</u>3)3), 2.49-2.57 (2H, m, C<u>H</u>2-CH=CH2), 3.39-3.47 (1H, m, 3-H), 3.81 (1H, d, *J* 2.5 Hz, 4-H), 5.14-5.24 (2H, m, CH2-CH=C<u>H</u>2), 5.75-5.89 (1H, m, CH2-C<u>H</u>=CH2), and 8.10 (1H, br s, -CO2H); δ_C(50 3MHz) -6.6 & -6.1 (2xSi-<u>C</u>H3), 18.3 (Si-<u>C</u>(CH3)3), 26.0 (Si-C(<u>C</u>H3)3), 32.3 (<u>C</u>H2-CH=CH2), 53.8 & 56.9 (3-C & 4-C), 118.7 (CH2-CH=<u>C</u>H2), 133.2 (CH2-<u>C</u>H=CH2), and 174.2 & 177.2 (2xC=O); *m/z* [CI(NH3)] 384 ($MHTBDMS^+$, 92%), 287 (MNH_4^+, 88), 270 (MH^+, 100), 242 (80), 169 (38), 130 (50), and 74 (18).

(3R,4S)-t-Butyl N-(t-butyldimethylsilyl)azetidin-2-one-3-allyl-4-carboxylate 17

To a solution of the crude (3R,4S)-*N*-(*t*-butyldimethylsilyl)azetidin-2-one-3-allyl-4-carboxylic acid 12 (3.17g, 11mmol) in anhydrous DCM:cyclohexane (1:1) (64ml) at 0°C was added boron trifluoride etherate (20μl). This was immediately followed by the slow addition of *O*-(*t*-butyl)trichloroacetimidate (4.79g, 22mmol) in 30ml cyclohexane, over 30 minutes. After stirring for a further 20 minutes while warming to room temperature solid $NaHCO_3$ (0.5g) was added. Filtration through Celite® and removal of the volatiles yielded a dark brown suspension. Flash chromatography [SiO_2 (250g), graded elution 4:1 to 1:1 petrol:diethyl ether] yielded 17 (2.43g, 68% from 4) as a colourless oil, Rf 0.5 (petrol:Et_2O 4:1) (Found C, 62.40; H, 9.73; N, 4.75. $C_{17}H_{31}NO_3Si$ requires C, 62.73; H, 9.60; N, 4.30%); $[\alpha]_D^{20}$ -51.1° (c=0 85, $CHCl_3$); ν_{max} ($CHCl_3$) 1740(s, C=O), 1370(m), 1295(m), 1255(m), 1155(s), 842(m), and 825(m); δ_H (200MHz) 0.09 & 0.29 (2x3H, 2xs, Si-C<u>H</u>3), 0.96 (9H, s, Si-C(C<u>H</u>3)3), 1.46 (9H, s, C(C<u>H</u>3)3), 2.42-2.53 (2H, m, C<u>H</u>2-CH=CH2), 3.19-3.27 (1H, m, 3-H), 3.65 (1H, d, *J* 2.5 Hz, 4-H), 5.11-5.19 (2H, m, CH2-CH=C<u>H</u>2), and 5.74-5.88 (1H, m, CH2-C<u>H</u>=CH2), δ_C(50.3MHz) -6.6 & -6.1 (2xSi-<u>C</u>H3), 18.2 (Si-<u>C</u>(CH3)3), 26.0 & 27.7 (2xC(<u>C</u>H3)3), 32.4 (<u>C</u>H2-CH=CH2), 55.0 & 56.6 (3-C & 4-C), 81.8 (<u>C</u>(CH3)3), 117.8 (CH2-CH=<u>C</u>H2), 133.7 (CH2-<u>C</u>H=CH2), and 171.4 & 173.8 (2xC=O); *m/z* [DCI(NH3)] 326 (100%, MH^+), and 270 (7).

(3R,4S)-t-*Butyl azetidin-2-one-3-allyl-4-carboxylate* 18

To a solution of *t*-butyl *N*-(TBDMS)azetidin-2-one-3-allyl-4-carboxylate 17 (2.88g, 8.88mmol) in methanol (100ml) was added caesium fluoride (2.16g, 13.3mmol) and the solution was stirred for 1 hour. The solvent then was removed and the residue was taken into DCM (100ml), washed with water (2x100ml), brine (150ml) and dried ($MgSO_4$). Evaporation of the DCM afforded crude 18 (1.80g, 90%) as a white powder, which was used without purification in subsequent reactions; ν_{max} ($CHCl_3$) 3420(w), 1772(s, β-lactam C=O), 1735(m, ester C=O), 1370(m), 1235(m), 1155(m); δ_H(200MHz) 1.49 (9H, s, $C(CH_3)_3$), 2.41-2.66 (2H, m, CH_2-CH=CH_2), 3.39-3.48 (1H, m, 3-H), 3.81 (1H, d, *J* 2 Hz, 4-H), 5.11-5.26 (2H, m, CH_2-CH=CH_2), 5.75-5.96 (1H, m, CH_2-CH=CH_2), 6.19 (1H, br s, NH); δ_C(50.3MHz) 27.7 ($C(CH_3)_3$), 32.0 (CH_2-CH=CH_2), 53.5 (3-C), 82.3 (4-C), 82.3 ($C(CH_3)_3$), 117.7 (CH_2-CH=CH_2), 133.6 (CH_2-CH=CH_2), 169.7 & 170.5 (2xC=O; *m/z* [CI(NH_3)] 229 (MNH_4^+, 100%), 212 (MH^+, 58), 184 (50), 173 (37), 128 (30), and 84 (57).

(3R,4S)-t-*Butyl* (N-t-*butoxycarbonyl*)*azetidin-2-one-3-allyl-4-carboxylate* 16

t-Butyl azetidin-2-one-3-allyl-4-carboxylate 18 (0.49g, 2.3mmol), di-(*t*-butyl)dicarbonate (1.00g, 4.6mmol), and 4-dimethylaminopyridine (12mg, 0.23mmol) were stirred in acetonitrile at 30°C for 24 hours. The solvent was removed and the residue taken into DCM (20ml) and the resulting solution washed with 1M $KHSO_4$ (2x20ml), sat. $NaHCO_3$ (20ml), saturated brine (20ml), and dried ($MgSO_4$). Removal of the solvent yielded a pale yellow solid which was purified by flash chromatography [SiO_2 (15g), eluting with 4:1 diethyl ether: petrol] to yield 16 (610mg, 85%) as a white amorphous solid, (Found C, 61.54; H, 8.16; N, 4.30. $C_{16}H_{23}O_5N$ requires C, 61.72; H, 8.09, N, 4.50%) R_f 0.4 (Et_2O:petrol 7:3); $[\alpha]_D^{20}$ -49.8° (c=0.5, $CHCl_3$); ν_{max} ($CHCl_3$) 1813(s, β-lactam C=O), 1727(s, ester C=O), 1370(s), 1355(m), 1340(m), 1325(s), 1150(s); δ_H(200MHz) 1.40 & 1.42 (2x9H, 2xs, $C(CH_3)_3$), 2.39-2.52 (2H, m, CH_2-CH=CH_2), 3.09-3.17 (1H, m, 3-C), 3.91 (1H, d, *J* 3 Hz, 4-C), 5.06-5.15 (2H, m, CH_2-CH=CH_2), and 5.62-5.79 (1H, m, CH_2-CH=CH_2); δ_C(50.3MHz) 27.7 ($C(CH_3)_3$), 31.6 (CH_2-CH=CH_2), 54.0 & 55.9 (3-C & 4-C), 82.5 & 83.5 (2x$C(CH_3)_3$), 118.4 (CH_2-CH=CH_2), 132.7 (CH_2-CH=CH_2), 147.1 (NC(O)-O^{t}Bu), and 165.4 & 168.2 (2xC=O); *m/z* [CI(NH_3)] 329 (MNH_4^+, 75%), 273 (50), 229 (MNH_4^+-^{t}Boc, 100), 212 (MH^+-^{t}Boc, 82), 173 (58), 156 (25), 128 (21), 82 (21), and 57 ($C_4H_9^+$, 38).

(2S,3R)-t-*butyl* 2-(t-*butoxycarbonylamino*)-3-[(O-*benzyl*)*hydroxyaminocarbonyl*] *hex-5-enoate* 19

To a solution of (3R,4S)-*t*-butyl *N*-(*t*-butoxycarbonyl)azetidin-2-one-3-allyl-4-carboxylate 16 (100mg, 0.32mmol) in THF (10ml) was added a suspension of ethanethiolate (0.1eq. in THF). After stirring for 30 minutes at 30°C *O*-benzyl hydroxylamine (59mg, 0.46mmol) in THF (5ml) and potassium *t*-butoxide (3mg, 0.024mmol) in THF (0.5ml) were added and the solution heated to reflux. Reflux was maintained for 5 hours during which time further potassium *t*-butoxide (28mg,

0.23mmol) in THF (5ml) was added. Heating under reflux was continued for a further 30 minutes and the resulting solution was allowed to cool to room temperature diluted with diethyl ether (25ml) and washed with sat. NH_4Cl (2x35ml), brine (35ml) and dried ($MgSO_4$) to yield a pale yellow oil. Purification by flash chromatography [SiO_2 (25g), eluting with 2:1 petrol:diethyl ether] gave *(2S,3R)-t-butyl 2-(t-butoxycarbonylamino)-3-(ethylthiocarbonyl)hex-5-enoate* 20; as a colourless oil (11mg, 9%), R_f 0.7 (Et_2O:petrol 1:1); $[\alpha]_D^{20}$ +47.5° (c=1.36, $CHCl_3$); ν_{max} ($CHCl_3$) 3430(m), 1735(m), 1715(s), 1670(m), 1500(s), 1370(s), 1155(s); δ_H(200MHz) 1.22 (3H, t, *J* 7 Hz, SCH_2CH_3), 1.43 (18H, 2xs, 2x9H, $2xC(CH_3)_3$), 2.23-2.56 (2H, m, CH_2-CH=CH_2), 2.85 (2H, q, *J* 7 Hz, SCH_2CH_3), 3.17-3.28 (1H, m, 3-H), 4.37 (1H, dd, *J* 4, 10 Hz, 2-H), 5.06-5.15 (2H, m, CH_2-CH=CH_2), 5.51 (1H, br d, *J* 10 Hz, N*H*^{t}Boc), and 5.71-5.88 (1H, m, CH_2-C*H*=CH_2); δ_C(50.3MHz) 14.4 (SCH_2CH_3), 23.3 (SCH_2CH_3), 27.8 & 28.1 ($2xC(CH_3)_3$), 33.8 (CH_2-CH=CH_2), 54.0 & 54.4 (2-C & 3-C), 79.7 & 82.3 ($2xC(CH_3)_3$), 118.4 (CH_2-CH=CH_2), 134.0 (CH_2-CH=CH_2), 156.0 (N*C*(O)O^{t}Bu), and 170.0 & 201.8 (2xC=O); *m/z* [CI(NH_3)] 374 (MH^+, 37%), 318 (41), 279 (57), 274 (MH^+-^{t}Boc, 100), 262 (67), 218 (60), and 172 (55), and *(2S,3R)-t-butyl 2-(t-butoxycarbonylamino)-3-[(O-benzyl)hydroxyaminocarbonyl]hex-5-enoate* 19, as a white powder (108mg, 78%), (Found C, 63.76; H, 8.19; N, 6.54. $C_{23}H_{34}O_6N_2$ requires C, 63.58; H, 7.89; N, 6.54%); R_f 0.2 (Et_2O:petrol 1:1); $[\alpha]_D^{20}$ -24.3° (c=1.0, $CHCl_3$); ν_{max} ($CHCl_3$) 3400(m), 1710(s, C=O), 1500(s), 1455(m), 1370(s), and 1155(s); δ_H(CD_3CN, 200MHz) 1.42 & 1.43 (18H, 2xs, 2x9H, $2xC(CH_3)_3$), 2.30-2.33 (2H, m, CH_2-CH=CH_2), 2.56-2.59 (1H, m, 3-H), 4.15 (1H, dd, *J* 4, 10 Hz, 2-H), 4.80 (2H, s, $NOCH_2Ph$), 5.06-5.16 (2H, m, CH_2-CH=CH_2), 5.67-5.80 (1H, m, CH_2-C*H*=CH_2), 6.13 (1H, br d, *J* 10 Hz, N*H*^{t}Boc), 7.40 (5H, s, Ph), and 9.54 (1H, br s, N(*H*)OBn); δ_C(CD_3CN, 50.3MHz) 27.1 & 27.5 ($2xC(CH_3)_3$), 33.9 (CH_2-CH=CH_2), 42.7 & 55.0 (2-C & 3-C), 78.0 ($NOCH_2Ph$), 79.2 & 81.8 ($2xC(CH_3)_3$), 117.7 (CH_2-CH=CH_2), 128.6-129.3 (Ph), 134.2 (CH_2-CH=CH_2), 156.2 (N*C*(O)O^{t}Bu), and 170.1 & 170.6 (2xC=O); *m/z* [DCI(NH_3)] 435 (MH^+, 51%), 379 (65), 335 (MH^+-^{t}Boc, 28), 323 (71), 279 (33), 173 (31), 127 (40), 108 (35), and 91 ($C_7H_7^+$,100).

Sodium deutroxide ring opening of 16

To a solution of 16 (75mg, 0.24mmol) in THF (10ml) was added D_2O (1ml) and sodium deutroxide (0.25ml of a 2M solution in D_2O, 0.5mmol). The resultant solution was stirred for 3hrs, then the THF was removed and the remaining solution freeze-dried. The resultant solid was taken into THF (4ml) and a solution of *O*-benzylhydroxylamine.hydrochloride (80mg, 0.5mmol) in water (6ml) was added. After stirring for 5 minutes a solution of dicyclohexylcarbodiimide (103mg, 0.5mmol) in THF (1ml) was added and the apparent pH adjusted to 4 by the addition of 2M HCl. The mixture was further stirred for 24hrs, the pH being maintained at 4 by further addition of 2M HCl, resulting in the formation of a precipitate. This precipitate was filtered off and washed with ethyl acetate (3x10ml). The organic portion was then dried ($MgSO_4$), and evaporated to yield a pale yellow sticky solid. Flash chromatography [SiO_2 (25g), eluting with 4:1 petrol:diethyl ether) yielded 19 (34.0mg, 30%) $[\alpha]_D^{20}$ - 23.8 (c=1.05, $CHCl_3$); *m/z* 435 (MH^+, 100), 436 (M^++2, 35), and (M^++3, 5%) with identical specroscopic data to that obtained for 19 by thiolate mediated ring opening (IR, ^{1}H NMR, ^{13}C NMR).

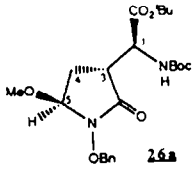
(3R)-3-[(1'S)-t-butyl 1'-(t-butyloxycarbonylamino)acetate]-1-benzyloxy-5-hydroxy-2-oxopyrrolidine 25

To a solution of (2S,3R)-*t*-butyl 2-(*t*-butoxycarbonylamino)-3-[(*O*-benzyl)hydroxyaminocarbonyl] hex-5-enoate 19 (195mg, 0.45mmol) in water: 1,4-dioxan 1:1 (20ml) was added osmium tetroxide (1 grain); the solution became dark brown over 30 minutes. Sodium metaperiodate (295mg, 1.38mmol) was added over a period of 90 minutes; after this time the solution had become pale brown. Diluting with ethyl acetate (40ml), washing with water (2x40ml) and evaporation of the ethyl acetate yielded 25 as a pale orange oil (200mg, >100%), as a mixture of epimers, which was used directly in the subsequent reaction, R_f 0.45 (Et_2O); ν_{max} ($CHCl_3$) 3590(br w), 3420(br w), 1715(s, C=O), 1500(m), 1455(w), 1395(w), 1370(m), 1250(m), and 1155(s); δ_H(200MHz) 1.45 (2x9H, 2xs, 2xC(CH_3)$_3$), 2.39-2.55 (1/2H, m, 4-H), 3.00-3.14 (1/2H, m, 4-H), 3.25-3.46 (1H, m, 4-H; both epimers), 4.04-4.11 (1H, m, 3-H), 4.28-4.41 (1H, m, 1'-H; both epimers), 4.83-4.88 (1H, m, 5-H; both epimers), 5.01 & 5.04 (2H, ABq, J_{AB} 5 Hz, O-CH_2Ph), 5.21 (1/2H, d, *J* 10 Hz, NH^{t}Boc), 5.32 (1/2H, d, *J* 10 Hz, NH^{t}Boc), and 7.35-7.48 (5H, m, Ph); *m/z* [DCI(NH_3)] 437 (MH^+, 28%), 419 (22), 381 (30), 363 (29), 257 (30), 157 (31), and 91 ($C_7H_7^+$, 100).

(3R)-3-[(1'S)-t-butyl 1'-(t-butyloxycarbonylamino)acetate]-1-benzyloxy-5-methoxy-2-oxopyrrolidine 26

The crude (3R)-3-[(1'S)-*t*-butyl 1'-(*t*-butyloxycarbonylamino)acetate]-1-benzyloxy-5-hydroxy-2-oxopyrrolidine 25 (200mg) was dissolved in anhydrous methanol (20ml) containing a catalytic amount of TFA (0.5ml of a solution of TFA (200µl) in methanol(10ml)), the solution being stirred at 30°C for 72 hours. Excess triethylamine (0.5ml) was then added and the volatile components were evaporated to yield a pale brown suspension. Flash chromatography [SiO_2 (25g); eluting with petrol: diethyl ether 2:1] yielded the two epimers of 26, as colourless oils, [138.5mg (major epimer, 26a; 118mg, minor epimer, 26b; 20.5mg, 68% from 19], (Found, major epimer, C, 61.32; H, 7.74; N, 6.21. $C_{23}H_{34}O_7N_2$ requires C, 61.32; H, 7.61; N, 6.22%); R_f's 0.7 (major epimer), 0.6 (minor epimer) (Et_2O); ν_{max} ($CHCl_3$) both epimers 1715(s, C=O), 1500(m), 1370(m), 1105(s), and 1085(m); δ_H(C_6D_6, 500MHz) major epimer 1.38 & 1.42 (2x9H, 2xs, 2xC(CH_3)$_3$), 1.68-1.72 & 1.91-1.96 (2x1H, 2xm, 4-H), 3.00 (3H, s, OCH_3), 3.38 (1H, dt, *J* 3, 9 Hz, 3-H), 4.14 (1H, d, *J* 6 Hz, 5-H), 4.58 (1H, dd, *J* 3, 8.5 Hz, 1'-H), 4.75 & 4.84 (2H, ABq, J_{AB} 9 Hz, OCH_2Ph), 5.45 (1H, br d, *J* 8.5 Hz, NH^{t}Boc), and 7.20-7.35 (5H, m, Ph); minor epimer 1.38 & 1.45 (2x9H, 2xs, 2xC(CH_3)$_3$), 1.66-1.72 & 1.78-1.84 (2x1H, 2xm, 4-H), 2.92 (1H, dt, *J* 4, 11 Hz, 3-H), 3.08 (3H, s, OCH_3), 4.14 (1H, dd, *J* 4, 7 Hz, 5-H), 4.69 (1H, dd, *J* 5, 7 Hz, 1'-H), 4.83 & 5.08 (2H, ABq, J_{AB} 10Hz, OCH_2Ph), 5.56 (1H, d, *J* 8 Hz, NH^{t}Boc), and 7.16-7.40 (5H, m, Ph); δ_C(125.8MHz) major epimer 27.5 (4-C), 27.9 & 28.3 (2xC(CH_3)$_3$), 40.4 (3-C), 53.4 (1'-C), 56.6 (OCH_3), 77.7 (OCH_2Ph), 80.1 & 82.7 (2xC(CH_3)$_3$), 88.4 (5-C), 128.5-129.7 (Ph), 135.0 (Ph *ispo* C), 142.5 (NHC(O)O^{t}Bu), and 169.1 & 169.2 (C=O); *m/z* [DCI(NH_3)] both epimers 451 (MH^+, 100%), 419 (20), 395 (43), 351 (MH^+-^{t}Boc, 18), 339 (26), 295 (20), 114 (15), and 91 (52);

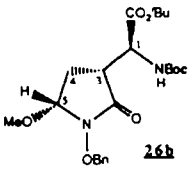
selected nOe's (major epimer):



26a

IRRADIATION ppm	nOe ENHANCEMENT (%) 4-H3	4-Hα	3-H	5-H	1'-H
1.70	1.70	1.93	3.38	4.14	4.58
1.70	-	17	14.5	5	
1.93	16	-	3	15	6
3.38	7	<1	-	1	12
4.14	<1	8	<1	-	

selected nOe's (minor epimer):



26b

IRRADIATION ppm	nOe ENHANCEMENT (%) 4-Hβ	4-Hα	3-H	5-H	1'-H
1.68	1.68	1.82	2.92	4.14	4.69
1.68	-		20	19	6
1.82		-	14	13	9
2.92	5	2.5	-	3	15
4.14	4	2	3	-	

(3R)-3-[(1'S)-t-butyl 1'-(t-butyloxycarbonylamino)acetate]-1-hydroxy-5-methoxy-2-oxopyrrolidine **27**

To a solution of the two epimers of (3R)-3-[(1'S)-*t*-butyl 1'-(*t*-butyloxycarbonylamino)acetate]-1-benzyloxy-5-methoxy-2-oxopyrrolidine **26** (55mg, 0.12mmol) in methanol (10ml) was added solid sodium hydrogen carbonate (5mg) and 10% Pd/C (15mg). The resulting suspension was stirred under a balloon of hydrogen for 2.5 hours. The catalyst was removed by filteration through Celite® and the volatile components evaporated to yield a pale brown oil. Purification by flash chromatography [SiO_2 (10g); eluting with diethyl ether) yielded **27** (32.1mg, 75%) as a colourless oil, R_f 0.2 (Et_2O); δ_H(500MHz) major epimer;1.45 & 1.48 (2x9H, 2xs, 2xC(CH_3)$_3$), 2.07-2.18 (2H, m, 4-H), 3.40-3.43 (1H, m, 3-H), 3.52 (3H, s, OC**H**$_3$), 4.40 (1H, dd, *J* 3, 9 Hz, 1'-H), 4.93 (1H, dd, *J* 1, 6 Hz, 5-H), and 5.31 (1H, br s, N**H**-^{t}Boc); minor epimer 1.46 & 1.50 (2x9H, 2xs, 2xC(CH_3)$_3$), 1.92-1.99 & 2.41-2.50 (2x1H, 2xm, 4-H), 3.17-3.23 (1H, m, 3-H), 3.60 (3H, s, OC**H**$_3$), 4.41 (1H, dd, *J* 3, 8 Hz, 1'-H), 4.91 (1H, dd, *J* 5, 12 Hz, 5-H), 5.59 (1H, d, *J* 8 Hz, N**H**-^{t}Boc); δC(125.8MHz) major epimer 27.5 (4-C), 27.9 & 28.3 (2xC(**C**H$_3$)$_3$), 40.7 (3-C), 53.8 (1'-C), 56.9 (O**C**H$_3$), 80.2 & 82.8 (2x**C**(CH$_3$)$_3$), 89.1 (5-C), and 156.2, 169.1 & 169.2 (3xC=O); *m/z* [DCI(NH_3)] 361 (MH^+, 43%), 305 (100), 289 (38), 257 (55), 249 (60), 157 (41), and 111 (78).

(3R)-3-[(1'S)-(amino)acetate]-1,5-dihydroxy-2-oxopyrrolidine
(Dealanylalahopcin) 2

(3R)-3-[(1'S)-*t*-butyl 1'-(*t*-butyloxycarbonylamino)acetate]-1-hydroxy-5-methoxy-2-oxopyrrolidine **27** (52.3mg, 0.145mmol) was disolved in 1,4-dioxan (4ml) and 1N hydrochloric acid (4ml) was added. The solution was stirred for 18 hours, whereupon the solvents were evaporated to yield a brown solid of crude **2**, as the hydrochloride salt (19.8mg); R_f 0.15 (*n*-BuOH:AcOH:H_2O (4:1:2)); $[\alpha]_D^{20}$ + 54.8 (c=1, H_2O). Purification by ion exchange [Dowex 50W-X8(H) resin, desalting with water and eluting with 2M ammonia solution], collecting the first 50ml of eluant, followed by freeze drying led to **2** as a pale brown solid (18.5mg, 65%), $[\alpha]_D^{20}$ + 45.6° (c= 0.88, H_2O), +56.2 (c=0.4,

0.1N HCl) [lit.,[4] $[\alpha]_D^{20}$ + 50.8° (c=0.5, H_2O), $[\alpha]_D^{20}$ + 55.6° (c=1, 0.1N HCl]; δ_H(D_2O/DCl, referenced to 1,4-dioxan @ δ 3.63, 500MHz) 1.53-1.59 & 2.55-2.61 (2x1H, 2xm, 4-H, minor epimer), 2.00-2.05 & 2.09-2.15 (2x1H, 2xm, 4-H, major epimer), 2.88-2.92 (1H, m, 3-H, minor epimer), 3.06-3.11 (1H, m, 3-H, major epimer), 3.95-3.98 (2x1H, m, 1'-H, both epimers), 5.14 (1H, dd, *J* 5, 7 Hz, 5-H, minor epimer), 5.19 (1H, d, *J* 7 Hz, 5-H, major epimer); δ_C(D_2O/DCl, referenced to 1,4-dioxan, 125.8MHz) 28.6 & 28.7 (4-C), 38.5 & 39.1 (3-C), 54.7 (1'-C), 81.7 & 82.1 (5-C), and 169.1, 170.0 & 170.7 (C=O); (both ^1H and ^{13}C NMR are in agreement with literature values[3,4]); *m/z* (FAB$^+$) 191 (MH$^+$).

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