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STEREOCONTROLLED ENANTIOSPECIFIC SYNTHESIS OF ANTICAPSIN

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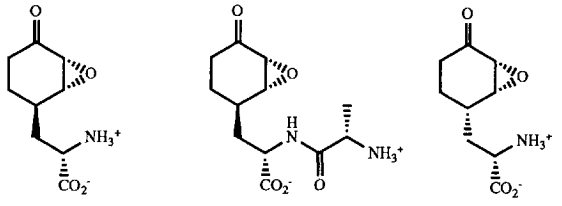
Abstract: A stereocontrolled enantiospecific synthesis of the revised structure of anticapsin (3) is described. The carbonyl group of anticapsin has also been observed to be markedly electrophilic, showing a high propensity for hydration and enolisation. A possible rôle for the hydrated form of anticapsin during the inhibition of glucosamine synthetase is proposed.

The antimycotic amino acid anticapsin has been shown to be a potent irreversible inhibitor of glucosamine synthetase,[1-3] an enzyme which is essential to the synthesis of the peptidoglycan and chitin cell walls of bacteria and fungi respectively. Whilst anticapsin has a substantial antifungal action, it has only a limited antibacterial effect since it is not generally incorporated into bacterial cells. In contrast, the dipeptide bacilysin, comprising anticapsin and L-alanine, is readily transported into microbial cells by means of a dipeptide permease system (so called 'illicit transport') and anticapsin liberated by the action of intracellular peptidases.[4,5] The resulting inhibition of glucosamine synthetase leads to a weakening of the cell wall and subsequent cell lysis due to the high internal osmotic pressure.[6] The literature until 1993 depicts anticapsin and bacilysin as the *trans*-structures (**1**) and (**2**) respectively.



(**1**) (**2**) (**3**)

Figure 1

We have recently reported[7] the revised structure of anticapsin (**3**) correcting the previous erroneous structural assignment (**1**). This finding has also been made independently by Wild, who has recently described the synthesis of anticapsin (**3**), bacilysin, and the related compounds chloro- and bromotetaine.[8,9]

Our synthetic route to anticapsin (**3**) (Figure 2) commences with the enantiospecific elaboration of chiral ester (**4**) to alcohol (**6**) *via* the known lactone (**5**). This was achieved by utilising chemistry previously developed by Ohno[10,11] for the synthesis of the enantiomer of (**6**). Alcohol (**6**), protected as its *tert*-butyldiphenylsilyl ether (**7**), was saponified using the anhydrous hydroxide equivalent, potassium

trimethylsilanolate.[12] Use of this reagent was preferable to conventional alkaline hydrolysis which was very sluggish, with unreacted ester (**7**) remaining even after several days at reflux. Radical decarboxylation of the resulting carboxylic acid (**8**) using the thiohydroxamic ester method of Barton[13,14] afforded the bis-silyl ether (**9**), subsequently transformed to the iodide (**12**) in three steps, *via* primary alcohol (**10**) and mesylate (**11**). Alkylation of (**12**) with a higher order bislactim ether[15] lithium cyanocuprate afforded the coupled product (**13**) in 71% yield with only minimal competing elimination (12%). Use of the higher order lithium cyanocuprate was essential to effect this alkylation since we found that the corresponding lower order reagent affords the coupled product (**13**) only in very low yield.[7] The use of the lithium azaenolate was discounted, since we had found in an earlier experiment that this reacted with the enantiomer of (**12**) predominantly with elimination (91%)[7] of the iodide.

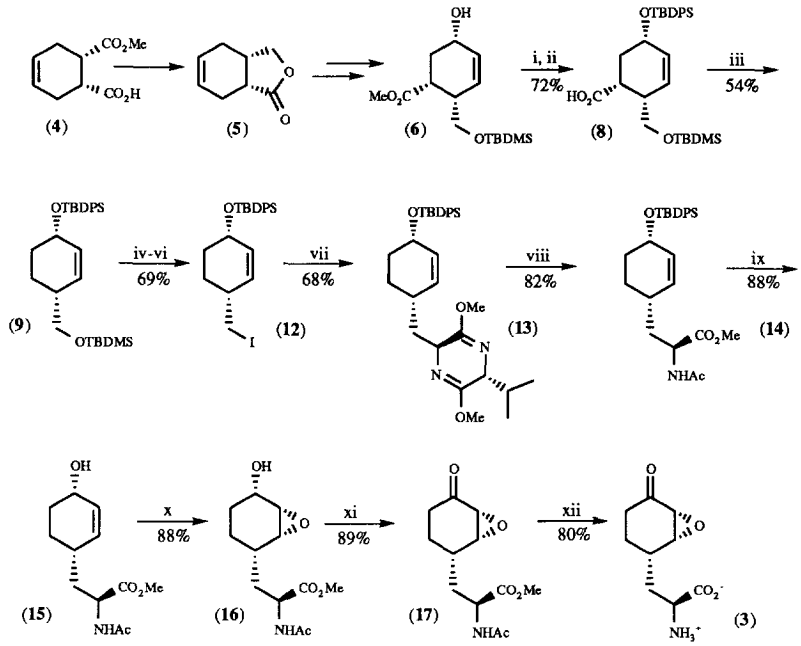


Figure 2

Reagents and conditions: i,TBDPSCl, imidazole, DMF; ii, $KOSiMe_3$, benzene, reflux 1.5h, acidic work-up [NH_4Cl (sat. aq. soln.)]; iii, (a) oxalyl chloride, DMF (cat.), toluene -5°C to 10°C 45 mins; (b) sodium 2-mercapto pyridine-*N*-oxide, DMAP (cat.), benzene, 30 mins at r.t. followed by the addition of *tert*-dodecanethiol (5 equiv.), *hν* (200W tungsten lamp) 20°C-30°C 2.5h; iv, $TsOH.H_2O$ (cat.), THF-H_2O, 24h, r.t.; v, MsCl, pyridine, 19h, r.t.; vi, NaI, acetone, reflux 21h; vii, (2R)-2,5-dihydro-2-isopropyl-3,6-dimethoxypyrazine (2 equiv.), *n*BuLi (2 equiv.), THF, -78°C; CuCN (1 equiv.), 2 mins 0°C, -78°C then -21°C; (**12**) (1 equiv.) -21°C 24h; viii, (a) 0.25M (aq) HCl (6 equiv.), CH_3CN 100min r.t.; (b) acetic anhydride, pyridine 1.75h r.t.; ix, NH_4F, MeOH, 50°C 18h; x, mCPBA, $CHCl_3$, 2h, r.t.; xi, TPAP (cat.), N-methyl morpholine-N-oxide, CH_3CN, 1h, r.t.; xii, (a) pronase E, phosphate buffer [≈2:3 ratio of 0.1M KD_2PO_4 and 0.1M Na_2DPO_4 in D_2O] pH 7.5 30°C 3h; (b) acylase I from *sp. aspergillus* immobilised on eupergrit C, phosphate buffer [≈2:3 ratio of 0.1M KD_2PO_4 and 0.1M Na_2DPO_4 in D_2O] pH 7.5 30°C 30h then cellulose chromatography (80% aqueous propan-2-ol as eluant).

The bislactim ether (**13**) was smoothly converted in four steps to (**17**), which had nmr data identical with that reported for authentic anticapsin *N*-acetyl methyl ester.[16] A key stereochemical feature of this sequence was a *cis* directed epoxidation[17,18] (step x, Figure 2) furnishing (**16**) as the sole product. Deprotection of (**17**)

was achieved by the sequential application of the enzymes pronase E[19,20] and acylase I[21] from *species aspergillus*. The synthetic anticapsin (**3**) was obtained clean and had spectroscopic data (^{1}H NMR, IR, MS, CD and $[\alpha]_D$) consistent with natural anticapsin obtained from Eli Lilly and Co. The positive Cotton effect observed in the CD spectrum (Figure 3) is indicative of the epoxide configuration depicted in structure (**3**) on the basis of the reverse octant rule.[22]

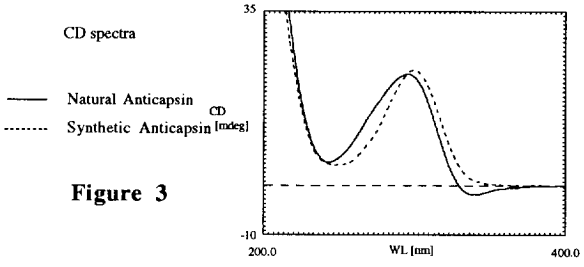
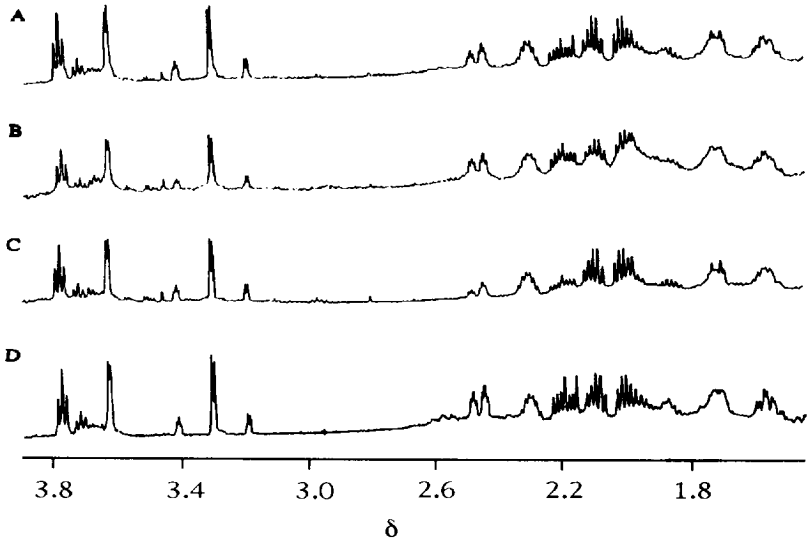


Figure 3

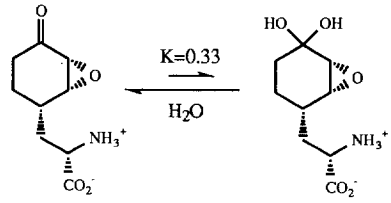
A comparison of synthetic and natural anticapsin by 500MHz ^{1}H NMR spectroscopy is illustrated in Figure 4.



A: Doped Spectrum (Natural and Synthetic) 500MHz ^{1}H NMR
B: Synthetic Anticapsin (After freeze drying from H_2O at pH 7.5)
C: Synthetic Anticapsin (After step xii, Figure 2)
D: Natural Anticapsin

Figure 4

We believe the satellite peaks at δ 3.72 (t, *J* 7.0Hz) consistent with an α-proton, and δ 3.41 (*ca.* t, *J* 4.0Hz), 3.18 (d, *J* 4.0Hz) consistent with epoxide protons are due to the hydrate (**18**) which would be in equilibrium with anticapsin (**3**) in aqueous solution (Figure 5). The equilibrium constant was estimated at 0.33 from integration of the ^{1}H NMR spectrum.



(3) (18)

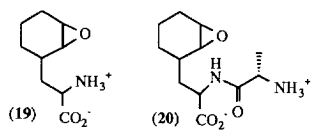
Figure 5

Our evidence for hydration stems from a more extensive NMR study of anticapsin *N*-acetyl methyl ester (**17**). Using $CDCl_3$ as solvent, the 500MHz ^1H NMR consisted of two doublets in the epoxide region and a single α-proton. On changing the solvent to D_2O, high field satellite peaks of the α and epoxide protons [δ_H 4.44 (dd, *J* 5.0, 10.0Hz) and δ_H 3.36 (*ca.* t, *J* 4.0Hz), 3.20 (d, *J* 4.0Hz)] were observed, which were of analogous intensity to those seen for the free amino acid (**3**). In addition, this phenomenon was found to be completely reversible. The 125MHz ^{13}C NMR spectrum of (**17**) taken in D_2O had a minor signal at δ 92.4 highly characteristic[23] of the carbon atom in a *gem*-diol; this signal was absent when the spectrum was recorded in $CDCl_3$. Wild has also observed these satellite peaks in the ^1H NMR of both anticapsin and bacilysin, similarly attributing them to hydration of the ketone.[8,9]

The electrophilic nature of the carbonyl group in anticapsin (**3**) was confirmed by its ease of enolisation. Thus it was observed that a solution of anticapsin in D_2O buffered at pH 7.5 (as part of the deprotection sequence xii, Figure 2) incorporated deuterium at the methylene centre adjacent to the carbonyl group, resulting in a decreased intensity of the multiplets centred at δ 2.44, and 2.16 (Figure 4, Spectrum C). After freeze drying from water at pH 7.5 these multiplets increased in intensity, as expected (Figure 4, Spectrum B). In fact, complete incorporation of deuterium occurred on standing anticapsin in deuterated phosphate buffer at pH 7.5 for 2-3h.

Discussion on the biological activity of anticapsin

Anticapsin is the most effective inhibitor of glucosamine synthetase known to date, as determined from inhibition studies utilising enzymes derived from both bacterial[24] and fungal[3] sources. Borowski has synthesised[25] the amino acid (**19**) and dipeptide (**20**), analogues of anticapsin (**3**) and bacilysin (**2**) which lack the ketone moiety (Figure 6), and has compared the ability of these compounds to inhibit glucosamine synthetase activity in cell free extracts derived from *E. coli, Sacch. cerevisae* and *Candida albicans* relative to bacilysin and anticapsin (Table 1).[26]



(19) (20)

Figure 6

Compound	Concentration causing 50% inhibition (μM)		
	E. coli K-12	*Sacch. cerevisae*	*C. albicans*
Anticapsin	5	6	6
(19)	330	400	200
Bacilysin	16	18	20
(20)	500	1000	100

Table 1

Thus although the keto group is not essential for inactivation of the amidotransferase, its absence does significantly decrease the activity of the inhibitor. This finding taken in conjunction with our observation that anticapsin is easily hydrated, suggests the keto moiety may play a significant rôle in the mechanism of inhibition. The crucial step in the biosynthesis of glucosamine-6-phosphate has been identified as sulphydryl attack by the active site thiol on the carboxyamide group of glutamine, resulting in the concommitant formation of thioester (**21**) and ammonia which is then presumed to subsequently react with the ketone moiety in the open chain form of fructose-6-phosphate.[27] The product, glucosamine-6-phosphate, is obtained following Amadori rearrangement of imine (**22**), whilst hydrolysis of thioester (**21**) regenerates the active site thiol residue (Figure 7).

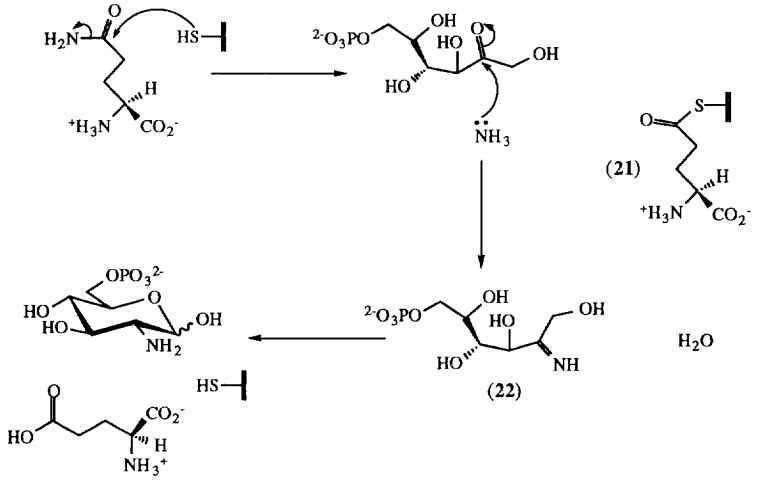


Figure 7

A theoretical study by Tempczyk and Dauter suggests the most likely spatial arrangement of the reactants at the active site is that depicted in Figure 8 (**A**), the structure being stabilised by hydrogen bonded interactions.[28] During enzyme inhibition by anticapsin, the *gem*-diol (**18**) may be crucial in maintaining the optimal disposition of reactants, by way of hydrogen bonding analagous to that proposed for the natural substrate L-glutamine (Figure 8 (**B**)).

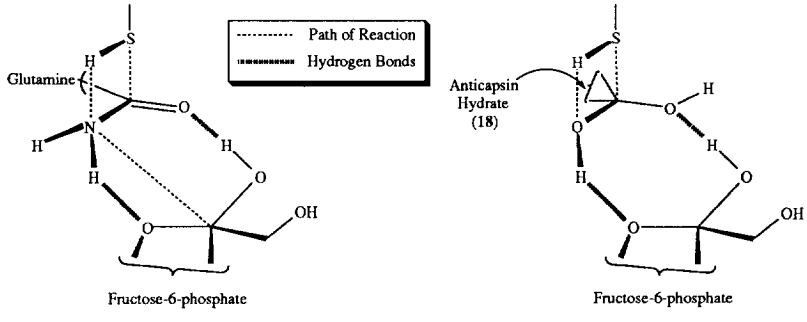


Figure 8

This would be in accord with the observation that anticapsin behaves as a glutamine analogue and that the acceleration of inactivation caused by fructose-6-phosphate is associated to ordered binding of first fructose-6-phosphate and then anticapsin.[29] Reaction is envisaged to proceed by way of nucleophilic addition of the proximate sulphydryl group[30] to the reactive *keto* form of anticapsin (**3**) in equilibrium with hydrate (**18**), resulting in formation of hemithioketal (**23**). This subsequently rearranges, leaving the active site thiol irreversibly alkylated (Figure 9).

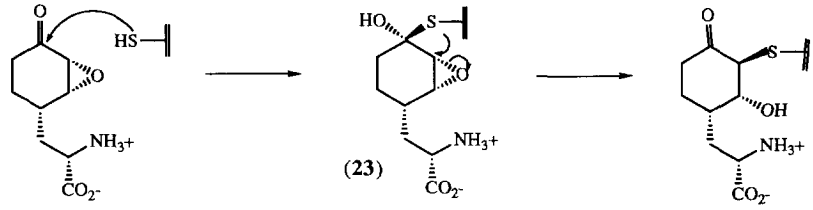


Figure 9

Chmara has pointed out that the exceptional efficacy of anticapsin as an enzyme inactivator cannot be explained just by the high affinity to the enzyme binding site and chemical reactivity of the moiety participating in the formation of a covalent bond. It was proposed that proximity and orientation effects due to the specific structure of anticapsin occurred upon binding of the inhibitor to the enzyme glutamine binding site.[29] The rôle we have assigned to the *gem*-diol (**18**) (Figure 8 (**B**)) is consistent with this postulate. Alternatively a bimolecular hydrate derived from fructose-6-phosphate and the *keto* form of anticapsin (**3**) may be involved.

In summary, we have developed a first stereocontrolled asymmetric synthesis of anticapsin (**3**), thus enabling revision of the previously reported incorrect structure. The electrophilic nature of the carbonyl group of anticapsin has been revealed by its propensity for hydration and enolisation. Such electrophilicity may play an important and hitherto unconsidered rôle in the mechanism of inactivation of glucosamine synthetase by anticapsin.

Acknowledgements

We thank the SERC for a quota award (to M.B.M.), Eli Lilly and Co. for the gift of a sample of natural anticapsin, and Prof. H. Wild for informing us of his work. We also thank Dr J. Robertson, Dr V. Lee and Dr A.T. Russell for useful discussions, Dr M.E. Wood for assistance with high field NMR, and Dr A. Rodger for recording the CD spectra.

EXPERIMENTAL SECTION

Infrared (IR) spectra were recorded on a Perkin-Elmer 781 spectrometer with only selected absorptions being reported. Absorption maxima are reported in cm^{-1}. Optical rotations were measured with a Perkin-Elmer 241 polarimeter at 20°C with a pathlength of 1dm. Concentrations (c) are reported in g/100ml. Nuclear magnetic resonance (NMR) spectra were recorded on either a Varian Gemini-200 or a Bruker AM-500 spectrometer. Spectra were taken using the indicated solvent with chemical shifts quoted in parts per million (δ p.p.m.) using the residual solvent peak as an internal reference. Coupling constants (*J*) are quoted to the nearest 0.5Hz. ^{13}C spectra were recorded on a Varian Gemini-200 spectrometer or a Bruker AM-500 spectrometer,

using DEPT editing when indicated. Mass spectra were recorded on a V.G. Micromass ZAB 1F (IBEI/EI/DCI), a V.G. 20-250 (DCI/CI), a V.G. TRIO 1 (GCMS), or a V.G. BIO-Q (Electrospray) spectrometer, with only molecular ions and major fragment peaks being reported. Melting points were obtained using a Büchi 510 capillary melting point apparatus and are uncorrected. Microanalyses were performed in the Dyson Perrins Laboratory.

Flash chromatography was accomplished on silica gel using Sorbsil™ C60. Dry flash chromatography was performed using Merck silica gel 60 HF_{254}. Cellulose chromatography was accomplished using Fluka microcrystalline cellulose Avicel PH-101. Preparative plate chromatography (PLC) was carried out on glass plates (20cm x 20cm) coated with silica gel (Blend 41) and with a Kieselgel band. Thin layer chromatography was performed on aluminium sheets pre-coated with either Merck silica gel 60 F_{254} or Merck cellulose, plates being visualised by either the quenching of u.v. fluorescence (λ_{max}=254nm) or by staining with 5% w/v phosphomolybdic acid in 95% ethanol or 10% w/v ammonium molybdate in 2M sulphuric acid, followed by heat. Amino acids were visualised by staining with 4% w/v ninhydrin in ethanol, followed by heat.

All solvents were distilled before use; tetrahydrofuran (THF) was obtained dry and oxygen free by distillation under nitrogen from sodium/benzophenone ketyl. 'Petrol' refers to the fraction of light petroleum ether boiling between 40-60°C. Solvents were evaporated at 30°C or below on a Büchi R110 Rotavapor. *n*-Butyllithium was standardised by titration using 1,3-diphenylacetone-*p*-tosylhydrazone at -78°C.[31] All other reagents were used as obtained from commercial sources.

(1R,2S,5S) Methyl-2-[(tert-butyldimethylsilyloxy)methyl]-5-(tert-butyldiphenylsilyloxy)cyclohex-3-en-1-carboxylate **(7)**. To a stirred solution of the alcohol (**6**) (5.20g, 17.3mmol) and imidazole (2.40g, 35mmol) in dry DMF (37ml) at 0°C under an argon atmosphere was added a precooled solution of TBDPSCl (5.23g, 19mmol) in dry DMF (20ml) *via* cannula. The mixture was allowed to warm to room temperature over 3-4h, poured into water (400ml) and extracted with ethyl acetate (3 x 300ml). The combined organic extracts were thoroughly washed with water (3 x 500ml) , dried ($MgSO_4$) and the solvent removed *in vacuo*. Flash chromatography (SiO_2; 15:1 petrol/ether as eluant) afforded (1R,2S,5S)-methyl 2-[(*tert*-butyldimethylsilyloxy)methyl]-5-(*tert*-butyldiphenylsilyloxy)cyclohex-3-en-1-carboxylate (**7**) (8.95g, 96%) as a colourless oil: (Found: C, 69.00; H, 8.97. $C_{31}H_{46}O_4Si_2$ requires C, 69.10; H, 8.60%); $[\alpha]_D^{20}$+67.7 (c 1.0, $CHCl_3$); $\nu_{max.}$ (thin film) 3070 (m, Ar-H), 3050 (m, Ar-H), 2958 (s, C-H), 2928 (s, C-H), 2858 (s, C-H), 1742 (s, C=O), 1652 (w, C=C), 1590 (w), 1429 (s), 1255 (s) and 701 (s); δ_H (200MHz, $CDCl_3$) 0.06 (6H, s, $Si(C\underline{H}_3)_2$), 0.91 (9H, s, $Me_2SiC(C\underline{H}_3)_3$), 1.08 (9H, s, $Ph_2SiC(C\underline{H}_3)_3$), 1.58-2.69 (4H, m, $C\underline{H}_2C\underline{H}(CO_2Me)C\underline{H}$), 3.58-3.74 (5H, m, $CO_2C\underline{H}_3$ and $C\underline{H}_2OTBDMS$), 4.21-4.33 (1H, m, $C\underline{H}OSi$), 5.64 (2H, s, $\underline{H}C{=}C\underline{H}$), 7.32-7.50 (6H, m, aromatic protons), and 7.62-7.78 (4H, m, aromatic protons); δ_C (50.4MHz, DEPT, $CDCl_3$) 174.2 ($\underline{C}$=O), 136.1, 134.5, 134.3, 133.1, 129.8, 128.5 and 127.8 ($H\underline{C}{=}\underline{C}H$ and aromatic carbons), 68.6 ($\underline{C}HOSi$), 63.7 ($\underline{C}H_2OSi$), 51.4 ($CO_2\underline{C}H_3$), 39.6 and 39.2 ($\underline{C}H$), 30.4 ($\underline{C}H_2$), 26.9 and 25.9 (2 x $C(\underline{C}H_3)_3$), 19.0 and 18.3 (2 x $\underline{C}(CH_3)_3$), and -5.6 ($Si(\underline{C}H_3)_2$); *m/z* [CI] 539 (MH^+, 57%), 523 (39), 284 (26), 283 (100) and 151 (18).

(1R,2S,5S)-2-[(tert-Butyldimethylsilyloxy)methyl]-5-(tert-butyldiphenylsilyloxy)cyclo-hex-3-en-1-carboxylic acid **(8)**. To a solution of the ester (**7**) (8.60g, 16.0mmol) in dry distilled benzene (29ml) under an argon atmosphere was added potassium trimethylsilanolate (6.70g, 52mmol) and the resulting solution heated at reflux for 1.5h. The mixture was poured into saturated aqueous ammonium chloride solution (350ml) and extracted with ether (2 x 400ml). The combined organic extracts were dried ($MgSO_4$) and evaporated *in vacuo*.

Dry flash chromatography (SiO_2; 1:1 petrol/ether→neat ether as eluant) afforded (1R,2S,5S)-2-[(*tert*-butyldimethylsilyloxy)methyl]-5-(*tert*-butyldiphenyl-silyloxy)cyclohex-3-en-1-carboxylic acid (**8**) (6.30g, 75%) as a colourless oil: $[\alpha]_D^{20}$-16.0 (c 1.0, $CHCl_3$); $\nu_{max.}$ (thin film) 3700-2300 (br, m, O-H), 3070 (s, Ar-H), 3049 (s, Ar-H), 2958 (vs, C-H), 2931 (vs, C-H), 2859 (vs, C-H), 1708 (vs, C=O), 1652 (m, C=C), 1590 (w), 1429 (s), 1252 (s) and 700 (s); δ_H (200MHz, $CDCl_3$) (Displays rotamer peaks) 0.01-0.19 (6H, m, $Si(C\underline{H}_3)_2$), 0.84-0.99 (9H, m, $Me_2SiC(C\underline{H}_3)_3$), 1.10 (9H, s, $Ph_2SiC(C\underline{H}_3)_3$), 1.66-2.98 (4H, m, $C\underline{H}_2C\underline{H}(CO_2H)C\underline{H}$), 3.58-3.77 (2H, m, $C\underline{H}_2OTBDMS$), 4.18-4.33 (1H, m, $C\underline{H}OSi$), 5.61-5.73 (2H, m, $\underline{H}C=C\underline{H}$), 7.32-7.51 (6H, m, aromatic protons), and 7.63-7.79 (4H, m, aromatic protons); δ_C (50.4MHz, $CDCl_3$) (Displays rotamer peaks) 182.3 ($\underline{C}$=O), 136.0, 135.0, 134.5, 134.2, 129.9, 129.8, 129.6, 128.3, 127.9, and 127.7 ($H\underline{C}=\underline{C}H$ and aromatic carbons), 65.8 ($\underline{C}HOSi$), 64.1 ($\underline{C}H_2OSi$), 40.4, 38.0, and 34.2 ($\underline{C}H$ and $\underline{C}H_2$), 26.8, 26.5 and 25.9 (2 x $C(\underline{C}H_3)_3$), 19.0 and 18.2 (2 x $\underline{C}(CH_3)_3$), and -5.7 ($Si(\underline{C}H_3)_2$); *m/z* [DCI] 525 (MH^+, 5%), 383 (18), 286 (MNH_4^+-TBDPSOH, 82), 269 (MH^+-TBDPSOH, 100), 253 (28), 216 (32), 196 (70), 168 (29), 136 (52), and 91 (65).

(1S,4S)-4-[(tert-Butyldimethylsilyloxy)methyl]-1-(tert-butyldiphenylsilyloxy)cyclohex-2-ene (**9**). To a stirred solution of the acid (**8**) (6.10g, 11.6mmol) in dry distilled toluene (270ml) at -5°C under an argon atmosphere was added DMF (4-5 drops) followed by oxalyl chloride (1.48ml, 17mmol) in one portion *via* syringe. The reaction vessel was allowed to warm to 10°C over the course of 30mins and the solution subsequently degassed at this temperature for 15mins. The toluene was removed *in vacuo* to afford crude (1R,2S,5S)-2-[(*tert*-butyldimethylsilyloxy)methyl]-5-(*tert*-butyldiphenylsilyloxy)cyclohex-3-en-1-carboxylic acid chloride as a pale yellow oil, which was immediately taken on to the next step.

The acid chloride was dissolved in dry degassed benzene (47ml) and transferred *via* cannula to a stirred suspension of the sodium salt of 2-mercapto pyridine-N-oxide (2.1g, 14mmol) and DMAP (0.15g, 1.2mmol) in dry degassed benzene (47ml) under an argon atmosphere. The reaction flask was wrapped in aluminium foil to exclude light from the vessel and left to stir under an argon atmosphere for 30 mins. The foil was removed to reveal the bright yellow colour of the derived thiohydroxamic ester, and the flask charged with *tert*-dodecanethiol (14ml, 59mmol) and immersed in a water bath at 20°C. The reaction mixture was subjected to white light photolysis (200W tungsten lamp), periodically changing the water bath so as to maintain the temperature between 20-30°C. Photolysis was stopped after *ca.* 2.5h, the solution being essentially colourless after this time. The solution was washed with water (120ml), dried ($MgSO_4$) and the solvent removed *in vacuo*. The residue was purified by flash chromatography (SiO_2; neat petrol→30:1 petrol/ether as eluant) to afford (1S,4S)-4-[(*tert*-butyldimethylsilyloxy)methyl]-1-(*tert*-butyldiphenylsilyloxy)cyclohex-2-ene (**9**) (3.00g, 54%) as a colourless oil: (Found: C, 72.23; H, 9.04. $C_{29}H_{44}O_2Si_2$ requires C, 72.44; H, 9.22%); $[\alpha]_D^{20}$-19.2 (c 1.0, $CHCl_3$); $\nu_{max.}$ (thin film) 3072 (m, Ar-H), 3050 (m, Ar-H), 3028 (m, Ar-H), 2932 (vs, C-H), 2860 (vs, C-H), 1649 (w, C=C), 1590 (w), 1429 (s), 1258 (s), 1111 (vs), 1077 (vs), 836 (s) and 701 (vs); δ_H (200MHz, $CDCl_3$) 0.11 (6H, s, $Si(C\underline{H}_3)_2$), 0.98 (9H, s, $Me_2SiC(C\underline{H}_3)_3$), 1.12 (9H, s, $Ph_2SiC(C\underline{H}_3)_3$), 1.41-1.89 (4H, m, $(C\underline{H}_2)_2$), 2.19 (1H, *ca* t, *J* 8.0Hz, $C\underline{H}CH_2OTBDMS$), 3.57 (2H, d, *J* 8.0Hz, $C\underline{H}_2OTBDMS$), 4.23 (1H, br s, $C\underline{H}OSi$), 5.69 (2H, s, $\underline{H}C=C\underline{H}$), 7.36-7.52 (6H, m, aromatic protons), and 7.70-7.81 (4H, m, aromatic protons); δ_C (50.4MHz, $CDCl_3$) 136.1, 134.8, 131.4, 130.7, 129.7, and 127.7 ($H\underline{C}=\underline{C}H$ and aromatic carbons), 66.6 and 66.5 ($\underline{C}HOSi$ and $\underline{C}H_2OSi$), 38.4 ($\underline{C}H$), 30.0 ($\underline{C}H_2$), 26.9 and 25.9 (2 x $C(\underline{C}H_3)_3$), 21.0 ($\underline{C}H_2$), 19.1 and 18.3 (2

x $\underline{C}(CH_3)_3$), and -5.5 ($Si(\underline{C}H_3)_2$); *m/z* [CI] 498 (MNH_4^+, 2%), 242 (MNH_4^+-TBDPSOH, 23), 225 (MH^+-TBDPSOH, 100), 171 (34), and 93 (78).

(1S,4S)-1-(tert-Butyldiphenylsilyloxy)-4-(hydroxymethyl)cyclohex-2-ene (**10**). To a solution of the silyl ether (**9**) (2.79g, 5.81mmol) in a 20:1 mixture of THF/water (56ml) was added toluene-4-sulphonic acid monohydrate (0.27g, 1.4mmol) and the solution stirred for 24h at ambient temperature. The solvent was removed under reduced pressure and the cloudy residue purified by flash chromatography (SiO_2; 5:1 petrol/ether as eluant) to afford (1S,4S)-1-(*tert*-butyldiphenylsilyloxy)-4-(hydroxymethyl)cyclohex-2-ene (**10**) (2.00g, 94%) as a colourless oil: (Found: C, 75.34; H, 8.28. $C_{23}H_{30}O_2Si$ requires C, 75.36; H, 8.53%); $[\alpha]_D^{20}$-36.7 (c 1.0, $CHCl_3$); $\nu_{max.}$ (thin film) 3660-3100 (br, s, OH), 3071 (m, Ar-H), 2932 (s, C-H), 2859 (s, C-H), 1590 (w), 1429 (s), 1258 (s), 1110 (vs), 1021 (s), 821 (m) and 701 (s); δ_H (200MHz, $CDCl_3$) 1.07 (9H, s, $C(C\underline{H}_3)_3$), 1.43-1.86 (4H, m, $(C\underline{H}_2)_2$), 2.12-2.27 (1H, m, $C\underline{H}CH_2OH$), 3.60 (2H, d, *J* 6.5Hz, $C\underline{H}_2OH$), 4.17-4.26 (1H, m, $C\underline{H}OSi$), 5.60-5.79 (2H, m, $\underline{H}C=C\underline{H}$), 7.33-7.50 (6H, m, aromatic protons), and 7.66-7.76 (4H, m, aromatic protons); δ_C (50.4MHz, DEPT, $CDCl_3$) 136.0, 134.7, 132.3, 130.1, 129.8 and 127.7 ($H\underline{C}=\underline{C}H$ and aromatic carbons), 66.4 ($\underline{C}H_2OH$), 66.2 ($\underline{C}HOSi$), 38.2 ($\underline{C}H$), 30.0 ($\underline{C}H_2$), 26.9 ($C(\underline{C}H_3)_3$), 21.0 ($\underline{C}H_2$), and 19.1 ($\underline{C}(CH_3)_3$); *m/z* [CI] 384 (MNH_4^+, 6%), 367 (MH^+, 3), 289 (22), 274 (35), 216 (22), 196 (61), 128 (MNH_4^+-TBDPSOH, 26), and 93 (MH^+-(TBDPSOH+H_2O), 100).

(1S,4S)-1-(tert-Butyldiphenylsilyloxy)-4-[(methanesulphonyloxy)methyl]-cyclohex-2-ene (**11**). To a stirred solution of alcohol (**10**) (1.80g, 4.92mmol) in dry pyridine (22ml) at 0°C was added methanesulphonyl chloride (0.75ml, 9.7mmol) dropwise *via* syringe. The solution was allowed to warm to ambient temperature over 1h and left to stir for a further 18h. The pyridine was removed *in vacuo* and the residual solid taken up in ether (200ml). The ether was shaken with saturated aqueous copper sulphate solution (2 x 150ml) and then brine (1 x 150ml), dried ($MgSO_4$) and evaporated *in vacuo* to afford (1S,4S)-1-(*tert*-butyldiphenylsilyloxy)-4-[(methanesulphonyloxy)methyl]cyclohex-2-ene (**11**) (2.18g, 99%) as a pale yellow oil, which was used without further purification: $[\alpha]_D^{20}$ -23.9 (c 1.0, $CHCl_3$); $\nu_{max.}$ (thin film) 3072 (m, Ar-H), 2931 (s, C-H), 2860 (s, C-H), 1590 (w), 1470 (m), 1428 (s), 1360 (s), 1175 (s), 820 (s) and 702 (s); δ_H (200MHz, $CDCl_3$) 1.09 (9H, s, $C(C\underline{H}_3)_3$), 1.52-1.87 (4H, m, $(C\underline{H}_2)_2$), 2.36-2.52 (1H, m, $C\underline{H}CH_2OS$), 3.05 (3H, s, $C\underline{H}_3SO_3$), 4.15 (2H, d, *J* 6.5Hz, $C\underline{H}_2OS$), 4.17-4.27 (1H, m, $C\underline{H}OSi$), 5.56-5.82 (2H, m, $\underline{H}C=C\underline{H}$), 7.33-7.52 (6H, m, aromatic protons), and 7.64-7.76 (4H, m, aromatic protons); δ_C (50.4MHz, $CDCl_3$) 136.0, 134.6, 134.4, 133.4, 129.9, 127.8 and 127.7 ($H\underline{C}=\underline{C}H$ and aromatic carbons), 72.3 ($\underline{C}H_2OS$), 65.7 ($\underline{C}HOSi$), 37.3 ($\underline{C}H_3SO_3$), 35.2 ($\underline{C}H$), 29.5 ($\underline{C}H_2$), 26.9 ($C(\underline{C}H_3)_3$), 20.8 ($\underline{C}H_2$), and 19.1 ($\underline{C}(CH_3)_3$); *m/z* [CI] 462 (MNH_4^+, 12%), 349 (27), 277 (21), 206 (MNH_4^+-TBDPSOH, 42), 196 (36), 110 (MNH_4^+-(TBDPSOH+CH_3SO_3H), 25), and 93 (MH^+-(TBDPSOH+CH_3SO_3H), 100).

(1S,4S)-1-(tert-Butyldiphenylsilyloxy)-4-iodomethylcyclohex-2-ene (**12**). A solution of the mesylate (**11**) (0.67g, 1.51mmol) and sodium iodide (0.5g, 3.1mmol) in acetone (12.2ml) was heated at reflux for 21h. The reaction vessel was allowed to cool to room temperature and ether (40ml) added. The organic solution was washed with water (2 x 40ml) and saturated aqueous sodium thiosulphate solution (1 x 40ml), then dried ($MgSO_4$) and concentrated *in vacuo*. Flash chromatography (SiO_2; 3:1 petrol/ether as eluant) afforded (1S,4S)-1-(*tert*-butyldiphenylsilyloxy)-4-iodomethylcyclohex-2-ene (**12**) (0.53g, 74%) as a colourless oil: $[\alpha]_D^{20}$-38.9 (c 1.0, $CHCl_3$); $\nu_{max.}$ (thin film) 3073 (m, Ar-H), 2932 (s, C-H), 2861 (s, C-H), 1590 (w), 1470 (m), 1428 (s), 1112 (s), 1080 (s), 1007 (s), 822 (s) and 741 (s); δ_H (200MHz, $CDCl_3$) 1.10 (9H, s, $C(C\underline{H}_3)_3$), 1.48-1.87 (4H,

m, ($C\underline{H}_2$)$_2$), 2.19-2.34 (1H, m, $C\underline{H}CH_2I$), 3.20 (2H, d, *J* 6.5Hz, $CH_2\underline{I}$), 4.13-4.21 (1H, m, $C\underline{H}OSi$), 5.59-5.76 (2H, m, $\underline{H}C{=}C\underline{H}$), 7.33-7.51 (6H, m, aromatic protons), and 7.67-7.78 (4H, m, aromatic protons); δ_C (50.4MHz, DEPT, $CDCl_3$) 136.1, 134.7, 134.6, 131.8, 129.8 and 127.8 ($H\underline{C}{=}\underline{C}H$ and aromatic carbons), 65.7 ($\underline{C}HOSi$), 37.7 ($\underline{C}H$), 29.9 ($\underline{C}H_2$), 26.9 ($C(\underline{C}H_3)_3$), 24.9 ($\underline{C}H_2$), 19.1 ($\underline{C}(CH_3)_3$), and 12.5 ($\underline{C}H_2I$); *m/z* [CI] 494 (MNH_4^+, 14%), 477 (MH^+, 5), 196 (28), 112 (29), and 95 (100).

***(2S,5R)-2-{[(1'S,4'S)-1'-(tert-Butyldiphenylsilyloxy)cyclohex-2'-en-4'-yl]methyl}-2,5-dihydro-5-isopropyl-3,6-dimethoxypyrazine* (13).** A solution of *n*-butyllithium in hexane (0.48ml of a 2.27**M** solution, 1.09mmol) was added dropwise *via* syringe to a stirred solution of (2R)-2,5-dihydro-2-isopropyl-3,6-dimethoxypyrazine (0.20g, 1.09mmol) in THF (1.5ml) at -78°C under an argon atmosphere. After 5mins the yellow solution was transferred *via* a teflon cannula to a stirred slurry of copper (I) cyanide (0.049g, 0.55mmol) in THF (1.5ml) at -78°C under argon. The reaction mixture was warmed to 0°C to facilitate dissolution of the copper (I) cyanide (*ca.* 90secs) affording the cyanocuprate as a tan coloured solution which was then immediately recooled to -78°C. A solution of iodide (**12**) (0.17g, 0.36mmol) in THF (1.5ml) was precooled to -20°C and then added *via* syringe to the cyanocuprate. The reaction vessel was warmed to -21°C and stirred under argon at this temperature for 24h. The reaction was quenched by the addition of a 1:9 mixture of concentrated aqueous ammonia/saturated aqueous ammonium chloride (10ml) then ether (60ml) was added. The organic layer was thoroughly washed (3 x 50ml of a 1:9 mixture of concentrated aqueous ammonia/saturated aqueous ammonium chloride), dried ($MgSO_4$) and evaporated *in vacuo*. Flash chromatography (SiO_2; 40:1 petrol/ether as eluant) afforded the title compound (**13**) (0.13g, 68%) as a colourless oil: $[\alpha]_D^{20}$-16.6 (c 1.0, $CHCl_3$); $\nu_{max.}$ (thin film) 3073 (m, Ar-H), 2860 (s, C-H), 1693 (s, C=N), 1429 (s), 1239 (s), 1197 (m), 1110 (s), 910 (m), 821 (s) and 611 (s); δ_H (200MHz, $CDCl_3$) 0.69 (3H, d, *J* 7.5Hz, $CHC\underline{H}_3$), 1.00-1.11 (12H, m, $C(C\underline{H}_3)_3$ and $CHC\underline{H}_3$), 1.51-1.98 (7H, m, $C\underline{H}_2C\underline{H}_2C\underline{H}C\underline{H}_2$) 2.19-2.38 (1H, m, $C\underline{H}(CH_3)_2$), 3.67 and 3.71 (2 x 3H, 2 x s, $OC\underline{H}_3$), 3.91-4.10 (2H, m, 2-C$\underline{H}$ and 5-C$\underline{H}$), 4.12-4.21 (1H, m, $C\underline{H}OSi$), 5.57 (2H, s, $\underline{H}C{=}C\underline{H}$), 7.31-7.42 (6H, m, aromatic protons), and 7.65-7.75 (4H, m, aromatic protons); δ_C (50.4MHz, DEPT, $CDCl_3$) 164.7 ($\underline{C}$=N), 163.5 ($\underline{C}$=N), 136.1, 134.9, 129.7 and 127.7 ($H\underline{C}{=}\underline{C}H$ and aromatic carbons), 66.3 ($\underline{C}HOSi$), 60.6 (5-$\underline{C}H$), 53.7 (2-$\underline{C}H$), 52.3 ($O\underline{C}H_3$), 40.2 ($\underline{C}H_2CHN$), 31.6 ($\underline{C}H(CH_3)_2$ and allylic $\underline{C}H$), 30.3 ($\underline{C}H_2$), 26.9 ($C(\underline{C}H_3)_3$), 23.9 ($\underline{C}H_2$), 19.1 ($\underline{C}(CH_3)_3$), 19.0 and 16.5 ($CH(\underline{C}H_3)_2$); *m/z* [CI] 533 (MH^+, 25%), 277 (100), 141 (20), and 95 (17).

N-*Acetyl-β-[(1S,4S)-1-(tert-butyldiphenylsilyloxy)cyclohex-2-en-4-yl]-(S)-alanine methyl ester* (14). A solution of the bislactim ether (**13**) (0.34g, 0.64mmol) in a mixture of dilute aqueous hydrochloric acid (0.25**M**, 16ml, 6 equiv.) and acetonitrile (19.7ml) was stirred at room temperature for 100mins. Ethyl acetate (80ml) was added and the mixture was thoroughly washed with a solution of saturated aqueous sodium bicarbonate (2 x 90ml). The organic layer was dried ($MgSO_4$) and the solvent removed *in vacuo* to afford crude β-[(1S,4S)-1-(*tert*-butyldiphenylsilyloxy)cyclohex-2-en-4-yl]-(S)-alanine methyl ester as a pale yellow oil which was immediately used in the next step.

The amino acid methyl ester was placed under an argon atmosphere and dissolved in dry pyridine (1.2ml). Acetic anhydride (1.2ml, 12.7mmol) was added dropwise *via* syringe and the solution stirred at ambient temperature for 1.75h. The solvent was removed *in vacuo* and the residue taken up in ethyl acetate (50ml). The organic solution was washed with saturated aqueous copper sulphate (2 x 40ml) and brine (1 x 40ml), dried ($MgSO_4$), and the solvent removed *in vacuo*. Flash chromatography (SiO_2; 40:1 petrol/ether→1:1

petrol/ethyl acetate as eluant) afforded the title compound (**14**) (0.25g, 82%) as a colourless oil: $[\alpha]_D^{20}$-7.8 (c 1.0, $CHCl_3$); $\nu_{max.}$ (thin film) 3500-3140 (br, m, N-H), 3071 (m, Ar-H), 2860 (vs, C-H), 1740 (vs, ester C=O), 1656 (br, vs, amide C=O), 1429 (vs), 1240 (vs), 821 (m), 740 (m), and 702 (s); δ_H (200MHz, $CDCl_3$) 1.06 (9H, s, $C(CH_3)_3$), 1.43-1.83 (7H, m, $CH_2CH_2CHCH_2$), 2.02 (3H, s, CH_3CONH), 3.75 (3H, s, CO_2CH_3), 4.16 (1H, br s, CHOSi), 4.66-4.80 (1H, m, α-proton), 5.46-5.68 (2H, m, HC=CH), 6.08 (1H, d, *J* 8.5Hz, NH), 7.32-7.49 (6H, m, aromatic protons), and 7.62-7.75 (4H, m, aromatic protons); δ_C (50.4MHz, $CDCl_3$) 173.8 (C=O), 170.3 (C=O), 136.0, 134.7, 134.5, 132.8, 130.9, 129.7 and 127.6 (HC=CH and aromatic carbons), 65.9 (CHOSi), 52.3 (OCH_3), 50.2 (α-carbon), 38.3 ($C=CCHCH_2$), 31.8 (CH_2), 29.9 (CH_2), 26.8 ($C(CH_3)_3$), 23.6 (CH_2), 23.0 (CH_3CO), and 19.0 ($C(CH_3)_3$); *m/z* [DCI] 480 (MH^+, 42%), 450 (73), 448 (71), and 224 (MH^+-TBDPSOH, 100).

N-*Acetyl-β-[(1S,4S)-1-hydroxycyclohex-2-en-4-yl]-(S)-alanine methyl ester* (**15**). A solution of silyl ether (**14**) (45mg, 0.09mmol) and ammonium fluoride (50mg, 1.35mmol) in methanol (1.8ml) was heated at 50°C for 18h. The crude reaction mixture was adsorbed onto silica gel and subjected to flash chromatography (SiO_2; neat ethyl acetate as eluant) to afford *N*-acetyl-β-[(1S,4S)-1-hydroxycyclohex-2-en-4-yl]-(S)-alanine methyl ester (**15**) (20mg, 88%) as a colourless oil: $[\alpha]_D^{20}$+19.2 (c 1.0, $CHCl_3$); $\nu_{max.}$ (thin film) 3700-3000 (br, s, N-H and O-H), 2938 (s, C-H), 2860 (s, C-H), 1742 (s, ester C=O), 1656 (br, s, amide C=O), 1435 (m), and 1376 (m); δ_H (200MHz, $CDCl_3$) 1.20-1.89 (7H, m, $CH_2CH_2CHCH_2$), 2.01-2.27 (4H, m, CH_3CONH and OH), 3.75 (3H, s, CO_2CH_3), 4.10-4.21 (1H, m, CHOH), 4.67-4.80 (1H, m, α-proton), 5.61-5.89 (2H, m, HC=CH), and 6.11 (1H, d, *J* 8.5Hz, NH); δ_C (50.4MHz, $CDCl_3$) 173.8 (C=O), 170.5 (C=O), 134.3 and 130.2 (HC=CH), 64.3 (CHOH), 52.5 (OCH_3), 50.1 (α-carbon), 38.5 (CH_2), 32.0 ($C=CCHCH_2$), 29.8 (CH_2), 23.3 (CH_2), and 23.0 (CH_3CO); *m/z* [CI] 224 (MH^+-H_2O, 12%), 175 (24), 160 (15), and 158 (100).

N-*Acetyl-β-[(1S,2R,3S,4R)-2,3-epoxy-4-hydroxycyclohex-1-yl]-(S)-alanine methyl ester* (**16**) A solution of olefin (**15**) (18mg, 0.075mmol) and mCPBA (45mg, 0.26mmol) in chloroform (0.3ml) was stirred at room temperature for 2h. The crude reaction mixture was adsorbed onto silica gel and purified by flash chromatography (SiO_2; neat ethyl acetate→9:1 ethyl acetate/methanol as eluant). The desired oxirane (**16**) (17mg, 88%) was obtained as a colourless oil: $[\alpha]_D^{20}$+30.1 (c 1.0, $CHCl_3$); $\nu_{max.}$ (thin film) 3600-3100 (br, vs, N-H and O-H), 2958 (s, C-H), 1742 (vs, ester C=O), 1655 (br, vs, amide C=O), 1549 (s), 1437 (m), 1374 (m), 1260 (s), and 796 (m); δ_H (200MHz, $CDCl_3$) 1.21-1.99 (7H, m, $CH_2CH_2CHCH_2$), 2.03 (3H, s, CH_3CONH), 3.27 (1H, t, *J* 4.0Hz, epoxide proton), 3.39 (1H, t, *J* 4.0Hz, epoxide proton), 3.76 (3H, s, CO_2CH_3), 4.00-4.10 (1H, m, CHOH), 4.71 (1H, *ca.* q, *J* 8.0Hz, α-proton), and 6.18 (1H, d, *J* 8.0Hz, NH); δ_C (50.4MHz, $CDCl_3$) 173.6 (C=O), 170.5 (C=O), 65.6 (CHOH), 58.0 and 56.3 (epoxide carbons), 52.5 (OCH_3), 50.4 (α-carbon), 35.5 (CH), 30.3 (CH_2), 27.9 (CH_2), 23.0 (CH_3CO), and 22.4 (CH_2); *m/z* [CI] 258 (MH^+, 63%), 240 (MH^+-H_2O, 100), 226 (59), 224 (80), 210 (57), 132 (90), and 60 (95).

N-Acetyl-β-[(1S,2R,3R)-2,3-epoxy-4-oxocyclohex-1-yl]-(S)-alanine methyl ester (**17**). To a solution of (**16**) (17mg, 0.066mmol) stirring in dry acetonitrile (0.5ml) under an argon atmosphere was added NMO (12mg, 0.10mmol) and powdered 4Å molecular sieves (*ca.* 32mg). After 5mins TPAP (4mg, 0.01mmol) was added and the solution left to stir at ambient temperature and under argon for 1h. The crude reaction mixture was adsorbed onto silica gel and subjected to flash chromatography (SiO_2; neat ethyl acetate as eluant). (**17**) (15mg, 89%) was obtained as a colourless oil: $[\alpha]_D^{20}$+111.2 (c 1.0, $CHCl_3$); $\nu_{max.}$ (thin film) 3650-3140 (br, vs, N-H),

2958 (vs, C-H), 1728 (br, vs, ester C=O and ketone C=O), 1659 (br, vs, amide C=O), 1540 (br, s), 1439 (s), 1373 (s), and 861 (m); δ_H (500MHz, $CDCl_3$) 1.62-2.20 (6H, m, $CH_2CHCH_2CH(H)C=O$), 2.04 (3H, s, CH_3CONH), 2.50 (1H, dt, *J* 18.0, 4.5Hz, $CH_2CH(H)C=O$), 3.23 (1H, d, *J* 4.0Hz, CH), 3.41 (1H, d, *J* 4.0Hz, CH), 3.77 (3H, s, CO_2CH_3), 4.74-4.78 (1H, m, α-proton), and 6.14 (1H, d, *J* 8.0Hz, NH); δ_C (125MHz, HETCORR, $CDCl_3$) 205.4 (C=O), 172.9 (C=O), 170.0 (C=O), 58.5 (CH), 55.5 (CH), 52.6 (OCH_3), 50.1 (α-carbon), 36.8, 35.6, and 31.0 (CH_2 and CH), 23.1 (CH_3CO), and 21.9 (CH_2); *m/z* [CI] 273 (MNH_4^+, 18%), and 256 (MH^+, 100).

NMR Data for (17) using D_2O as solvent: δ_H (500MHz, D_2O) 1.50-2.26 (6H, m, $CH_2CHCH_2CH(H)C=O$), 2.00 (3H, s, CH_3CONH), 2.46 (1H, dt, *J* 18.0, 4.0Hz, $CH_2CH(H)C=O$), 3.32 (1H, d, *J* 4.0Hz, CH), 3.60 (1H, d, *J* 4.0Hz, CH), 3.73 (3H, s, CO_2CH_3), 4.50 (1H, dd, *J* 9.5, 6.0Hz, α-proton). Additional minor signals attributable to the *gem*-diol *N*-acetyl-β-[(1S,2R,3R)-4,4-dihydroxy-2,3-epoxycyclohex-1-yl]-(S)-alanine methyl ester were observed at: δ_H 1.99 (s, CH_3CONH), 3.20 (d, *J* 4.0Hz, CH), 3.36 (*ca.* t, *J* 4.0Hz, CH), 3.72 (s, CO_2CH_3), and 4.44 (dd, *J* 5.0, 10.0Hz, α-proton); δ_C (125MHz, HETCORR, D_2O) 210.3 (C=O), 175.0 (C=O), 60.1 (CH), 56.0 (CH), 53.8 (OCH_3), 51.4 (α-carbon), 36.1, 34.7, and 30.8 (CH_2 and CH), 22.4 (CH_3CO), and 21.2 (CH_2). Additional minor signals attributable to the *gem*-diol were observed at: δ_C 92.4 ($C(OH)_2$), 58.8 (CH), and 58.7 (CH).

***(S)-β-[(1S,2R,3R)-2,3-Epoxy-4-oxocyclohex-1yl]alanine* (3) {Anticapsin}.** To a solution of anticapsin *N*-acetyl methyl ester (**17**) (26mg, 0.10mmol) in deuterated phosphate buffer [≈2:3 ratio of 0.1**M** KD_2PO_4 and 0.1**M** Na_2DPO_4 in D_2O, pH 7.5] (2ml) was added pronase E (12mg), and the solution stirred at 30°C and under an argon atmosphere for 3h. Complete hydrolysis of the methyl ester was confirmed by ^1H NMR analysis of a portion of the reaction mixture. This aliquot was returned to the reaction vessel and acylase I from *sp. aspergillus* [immobilised on eupergrit C]* (30mg) added. The solution was incubated at 30°C with efficient stirring for 18h. Analysis of the reaction mixture by ^1H NMR suggested that deprotection was incomplete, so an additional quantity of acylase I (30mg) was added and the solution incubated for a further 12h at 30°C. ^1H NMR analysis now indicated that hydrolysis was complete. The solution was freeze dried to afford a colourless solid which was subjected to column chromatography (cellulose; 1:4 water/propan-2-ol as eluant). The ninhydrin positive fractions were pooled and the propan-2-ol removed *in vacuo*. The aqueous solution so obtained was freeze dried to afford anticapsin (**3**) (16mg, 80%) as a colourless amorphous solid: $[\alpha]_D^{20}$+45 (c 0.1, H_2O) [Lit.[32] $[\alpha]_D^{25}$+125 (c 1, H_2O); Lit.[33] $[\alpha]_D^{25}$+21 (c 0.2, H_2O); Natural anticapsin (in our hands) $[\alpha]_D^{20}$+51 (c 0.1, H_2O)]; $\nu_{max.}$ (KBr disk) 3700-2600 (br, vs, N-H, O-H, and C-H), 1714 (m, C=O), 1638 (br, vs, N-H), 1408 (m), and 1086 (vs); δ_H (500MHz, D_2O) 1.48-2.11 (4H, m) and 2.23-2.31 (1H, m) ($CH_2CHCH_2CH_2C=O$), 2.12-2.21 (1H, m, $CH_2CH(H)C=O$), 2.44 (1H, dt, *J* 18.5, 4.0Hz, $CH_2CH(H)C=O$), 3.29 (1H, d, *J* 4.0Hz, CH), 3.62 (1H, d, *J* 4.0Hz, CH), 3.77 (1H, t, *J* 7.0Hz, α-proton). Additional minor signals attributable to the *gem*-diol (S)-β-[(1S,2R,3R)-4,4-dihydroxy-2,3-epoxycyclohex-1-yl]alanine (**18**) were observed at: δ_H 3.72 (t, *J* 7.0Hz, α-proton), 3.41 (*ca.* t, *J* 4.0Hz, CH), and 3.18 (d, *J* 4.0Hz, CH); δ_C (125MHz, D_2O) 210.4 (C=O), 175.3 (C=O), 59.6 (CH), 56.1 (CH), 53.5 (α-carbon), 36.0 ($CH_2C=O$), 35.1, 30.5, and 21.7 (CH_2 and CH). Additional minor signals consistent with the *gem*-diol (**18**) were observed at: δ_C 58.9, and 58.2 (epoxide carbons); *m/z* [Electrospray] 200 (MH^+, 100).

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* Purchased from the Fluka chemical company. The resin was thoroughly washed with D_2O prior to use.

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