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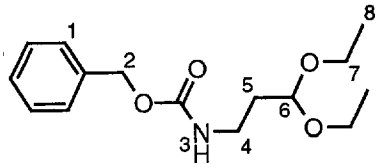
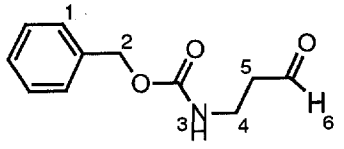
General Methods

Unless otherwise indicated, ^{1}H-NMR spectra were recorded on a 250MHz Bruker spectrometer. 400MHz spectra were recorded on a Varian VXR 400 spectrometer. Spectra are referenced internally to residual protio solvent signals. Data for ^{1}H are reported as follows : chemical shift (δ ppm), multiplicity (s = singlet, b = broad, m = multiplet, d = doublet, t = triplet, q = quartet) coupling constant, integration, and where appropriate, assignment. Infra-red spectra were recorded on a Perkin-Elmer 1700 series spectrometer. Thermospray mass spectra were recorded on an HP5989B Engine, using ammonia as carrier gas, with the filament on in positive ion mode. Electrospray mass spectra were recorded on a Micromass Autospec Voltage Spec in positive ion mode using PEG/PEG-NH_3 conditions. Thin layer chromatography (tlc) was performed on Polygram Sil G/UV_{254} pre-coated plastic sheets and visualised by uv or potassium permanganate in dilute sodium carbonate. Flash chromatography was carried out on Merck 9385 silica gel 60.



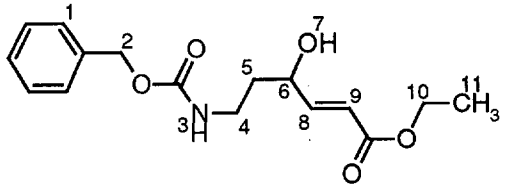
3,3-Diethoxypropylcarbamic acid benzyl ester. Benzyl chloroformate (143mL, 1mol) in DCM (200mL) was added with stirring over 1h to 3-aminopropanal diethylacetal (147g, 1mol) in DCM (800mL) and 2M Na_2CO_3 (1000mL). After a further 1h, *N*-(2-aminoethyl)piperazine (10mL) was added. After ½h, the organic layer was separated and washed with 20% aqueous citric acid (2x250mL) and 8% aqueous $NaHCO_3$ (500mL). After drying ($MgSO_4$), solvent removal *in vacuo* gave a pale yellow oil (266g, 95%).
^{1}H-NMR (250MHz, $CDCl_3$) 7.40-7.23 (m, 5H, H-1), 5.28-5.18 (bm, 1H, H-3), 5.10 (s, 2H, H-2), 4.55 (t, $J_{6\text{-}5}$ = 5.6Hz, H-6), 3.72-3.58 (m, 2H, H-7), 3.55-3.42 (m, 2H, H-7'), 3.38-3.24 (m, 2H, H-4), 1.87-1.77 (m, 2H, H-5), 1.21 (t, $J_{8\text{-}7,7'}$ = 7Hz, 6H, H-8).



(4)

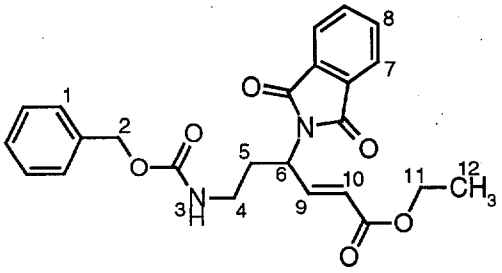
3-Oxopropylcarbamic acid benzyl ester (4). (3,3-Diethoxy-propyl)-carbamic acid benzyl ester (261g, 0.93mol), pyridinium tosylate (233.4g, 0.93mol) acetone (2000mL) and water (500mL) were warmed to 45°C for 1½h when tlc showed absence of starting material. The acetone was removed *in vacuo* and the residue diluted with

EtOAc (2000mL) and water (500mL). The organic layer was separated and washed with brine (500mL), dried ($MgSO_4$) and the solvent removed *in vacuo* to afford the aldehyde **4** as a white solid (176.6g, 92%). Mpt 47-53°C.
^{1}H-NMR (250MHz, $CDCl_3$) 9.78 (s, 1H, H-6), 7.34 (m, 5H, H-1), 5.33-5.19 (bs, 1H, H-3), 5.08 (s, 2H, H-2), 3.47 (q, $J_{4\text{-}5,\,4\text{-}3}$ = 6Hz, 2H, H-4), 2.72 (t, $J_{5\text{-}4}$ = 6Hz, 2H, H-5).



(6)

(E)-6-Benzyloxycarbonylamino-4-hydroxyhex-2-enoic acid ethyl ester (6). Dry DMF (3000mL) was purged with argon for 40min whilst cooling to 0°C (ice/MeOH cooling bath). With continual argon purging the aldehyde **4** (112.34g, 0.543mol), *trans*-3-iodoethylacrylate **5** (239.50g, 1.06mol) and nickel(II) chloride (1.06g, 8.1mmol) (1.5mol %) were then added followed by rapid addition (30sec) of solid chromium(II) chloride (2x100g, 1.63mol). The temperature rose to 32°C over 10 min. After removing the cooling bath, the mixture was stirred vigorously at ambient temperature for 6h. After cooling to 17°C (ice bath) the reaction was quenched with 1M lithium chloride solution (6000mL) with the temperature rising to 28°C. The mixture was then washed with hexane (4x3000mL) and the product extracted with 1:1 EtOAc:Et_2O (4x3000mL). These combined extracts were washed with 1M LiCl solution (4x2000mL), dried ($MgSO_4$) and the solvent removed *in vacuo* to afford a pale yellow oil identified as desired product (123g, 70%) with no significant impurities by ^{1}H-NMR.
A small sample was purified by flash column chromatography on silica 9385 eluting with Et_2O:hexane 2:1 to afford analytically pure material.
^{1}H-NMR (250MHz, $CDCl_3$) 7.35 (m, 5H, H-1) 6.93 (dd, $J_{8\text{-}9}$ = 16Hz, $J_{8\text{-}6}$ = 4Hz, 1H, H-8), 6.03 (dd, $J_{9\text{-}8}$ = 16Hz, $J_{9\text{-}6}$ = 2Hz, 1H, H-9), 5.21-5.08 (bm, 1H, H-3), 5.10 (s, 2H, H-2), 4.42-4.32 (m, 1H, H-6), 4.19 (q, $J_{10\text{-}11}$ = 7Hz, 2H, H-10), 3.62-3.43 (m, 2H, H-7, H-4), 3.31-3.18 (m, 1H, H-4'), 1.86-1.71 (m, 1H, H-5), 1.69-1.55 (m, 1H, H-5'), 1.28 (t, $J_{11\text{-}10}$ = 7Hz, 3H, H-11).
IR (film) ν_{max} 3367, 2981, 2942, 1703, 1659, 1535, 1303, 1267 cm^{-1}
Assay. Found: C, 62.62; H, 7.10; N, 4.43%
$C_{16}H_{21}NO_5$ requires C, 62.53; H 6.89; N, 4.56%



(7)

(E)-6-Benzyloxycarbonylamino-4-(1,3-dioxo-1,3-dihydroisoindol-2-yl)-hex-2-enoic acid ethyl ester (7). The alcohol **6** (53g, 0.17mol), triphenyl phosphine (95.2g, 0.36mol), phthalimide (53.3g. 0.36mol) and THF (910mL) were mixed and cooled under N_2 to 10°C. Diethylazodicarboxylate (56.9mL, 0.36mol) in THF (57mL) was added dropwise over 1h. After approximately half of the addition, a solution was formed. After stirring at room temperature overnight the solvent was removed *in vacuo* and the residue dissolved in *t*-butylmethyl ether (TBDME) (265mL). After cooling to 5°C for 1h triphenylphosphine oxide precipitated out of solution and was then filtered off and washed with further cold TBDME (270mL). To the filtrate was added further TBDME (500mL) and hexane (100mL) and the mixture washed with 1:1 H_2O:DMF (6x500mL). After drying ($MgSO_4$), solvent removal *in vacuo* afforded a viscous yellow oil (119g). This was purified by flash chromatography on silica gel eluting with hexane:EtOAc (65:35) affording pure material as a colourless oil (25.38g, 34%). There were numerous impure fractions which can be purified on repeated chromatography.

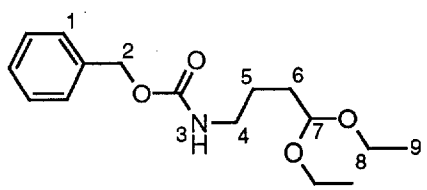
Tlc 65:35 hexane: EtOAc (run twice) $R_f = 0.50$

^{1}H-NMR (250MHz, $CDC1_3$) 7.88-7.68 (m, 4H, H-7,8), 7.35 (m, 5H, H-1), 7.15 (dd, $J_{9\text{-}10}$ = 16Hz, $J_{9\text{-}6}$ = 7Hz, 1H, H-9), 5.94 (dd, $J_{10\text{-}9}$ = 16Hz, $J_{10\text{-}6}$ = 1Hz, 1H, H-10), 5.12-4.94 (m, 4H, H-2, H-3, H-6), 4.17 (q, $J_{11\text{-}12}$ = 7Hz, 2H, H-11), 3.42-3.19 (m, 1H, H-4), 3.15-2.94 (m, 1H, H-4'), 2.49-2.33 (m, 1H, H-5), 2,24-2.07 (m, 1H, H-5'), 1.26 (t, $J_{12\text{-}11}$ = 7Hz, 3H, H-12).

IR (film) ν_{max} 3370, 2982, 2939, 1774, 1713, 1659, 1530, 1468, 1386, 1335, 1261 cm^{-1}

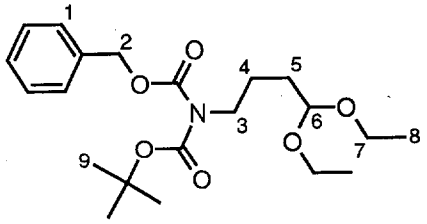
Assay. Found: C, 65.71; H, 5.79; N, 6.70%

$C_{24}H_{24}N_2O_6$ requires C, 66.04; H, 5.54; N, 6.42%

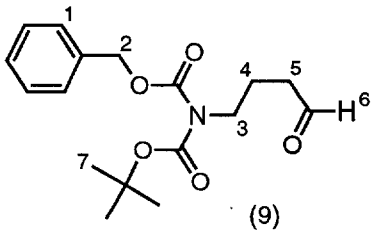


4,4-Diethoxybutylcarbamic acid benzyl ester. A solution of benzylchloroformate (780mL, 5.46mol) in dichloromethane (510mL) was added over 2h to a vigorously stirred mixture of 4-aminobutyraldehyde diethylacetal (910mL, 5mol) in dichloromethane (3000mL) and aqueous sodium carbonate (1M, 3000mL) with iced

water cooling. After a further 1h, when gas evolution had ceased, *N*-(2-aminoethyl)piperazine (40mL, 0.3mol) was added and stirring was continued for 1.5h. The organic layer was separated and washed with aqueous citric acid (1M, 2x2000mL) and saturated aqueous sodium bicarbonate (2000mL). After drying ($MgSO_4$), solvent removal *in vacuo* gave a yellow oil (1.5kg, 100%).
Tlc ether $R_f = 0.45$
^{1}H-NMR (250MHz, $CDCl_3$) 7.40-7.28 (m, 5H, H-1), 5.10 (s, 2H, H-2), 4.93 (bs, 1H, H-3), 4.48 (t, $J_{7-6} = 5.5$Hz, 1H, H-7), 3.70-3.40 (m, 4H, H-8), 3.21 (q, J = 6Hz, 2H, H-4), 1.70-1.50 (m, 4H, H-5,6), 1.20 (t, $J_{9-8} = 7$Hz, H-9).



Benzyloxycarbonyl-4,4-diethoxybutylcarbamic acid tert-butyl ester. A solution of di-*t*-butyldicarbonate (1380mL, 6mol) in ethyl acetate (1500mL) was added over 4.5h to a stirred mixture of (4,4-diethoxy-butyl)-carbamic acid benzyl ester (840mL, 3mol), triethlamine (420mL, 3mol) and 4-(dimethylamino)-pyridine (370g, 3mol) in ethyl acetate (1500mL) maintaining the internal temperature at 22-25°C with water bath cooling. After 14h, when tlc indicated consumption of starting material, the mixture was cooled to 12°C and dilute hydrochloric acid (2M, 2000mL) was added over 40min. The organic layer was separated and washed with dilute hydrochloric acid (1M, 2000mL) and water (2000mL). After drying ($MgSO_4$), solvent removal *in vacuo* gave a red oil (1.55kg, 100%). An aliquot (20g) was purified by column chromatography on silica gel (350g) with ether:hexane (1:1) as eluent. Concentration of the appropriate fractions afforded a pale yelow oil (17.68g, 88%).
Tlc ether:hexane (1:1) $R_f = 0.43$
^{1}H-NMR (250MHz, $CDCl_3$) 7.42-7.30 (m, 5H, H-1), 5.21 (s, 2H, H-2), 4.45 (t, J_{6-5} = 5.5Hz, 1H, H-6), 3.70-3.38 (m, 6H, H-3,7), 1.70-1.55 (m, 4H, H-4,5), 1.48 (s, 9H, H-9), 1.19 (t, $J_{8-7} = 7$Hz, 6H, H-8).
IR (liquid film) ν_{max} 2976, 2932, 2879, 1792, 1747, 1698, 1456, 1370, 1339, 1279, 1208, 1132, 1065, 985, 857, 780, 751, 699 cm^{-1}.
Assay. Found C, 63.8; H, 8.4; N, 3.5%
$C_{21}H_{33}NO_6$ requires C, 63.8; H, 8.4; N, 3.5%



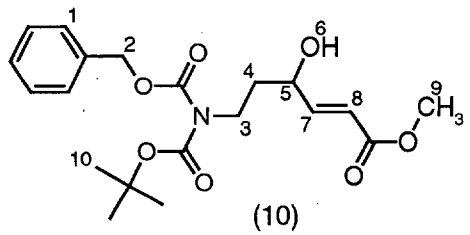
(9)

Benzyloxycarbonyl-3-formylpropylcarbamic acid tert-butyl ester (9). A solution of pyridinium *p*-toluenesulfonate (7.5g, 30mmol) in water (100mL) was added to a solution of benzyloxycarbonyl-(4,4-diethoxy-butyl)-carbamic acid tert-butyl ester (118.6g, 0.3mol) in acetone (400mL). The resulting mixture was warmed to 50°C and maintained at that temperature until tlc indicated consumption of starting material (usually about 5.5h). The acetone was removed *in vacuo* and the aqueous residue was extracted with diethyl ether (1000mL). The organic layer was washed with water (200mL). After drying ($MgSO_4$) solvent removal *in vacuo* gave an orange oil which was purified by flash column chromatography on silica gel (2kg) with ether:hexane (1:1) as eluent. Concentration of the appropriate fractions afforded a colourless oil (76.9g, 80%)

Tlc ether:hexane (1:1) $R_f = 0.31$

^{1}H-NMR (250MHz, $CDCl_3$) 9.70 (s, 1H, H-6), 7.43-7.30 (m, 5H, H-1), 5.22 (s, 2H, H-2), 3.69 (t, $J_{3-4} = 7$Hz, 2H, H-3), 2.46 (t, $J_{5-4} = 7$Hz, 2H, H-5), 1.91 (quintet, $J_{4-3,5} = 7$Hz, 2H, H-4), 1.48 (s, 9H, H-7).

IR (liquid film) ν_{max} 2979, 1790, 1751, 1724, 1698, 1456, 1370, 1342, 1294, 1154, 1119, 855, 780, 751, 699 cm^{-1}.

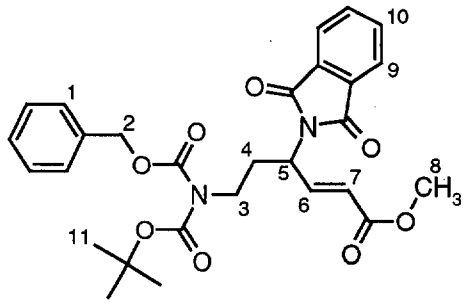


(10)

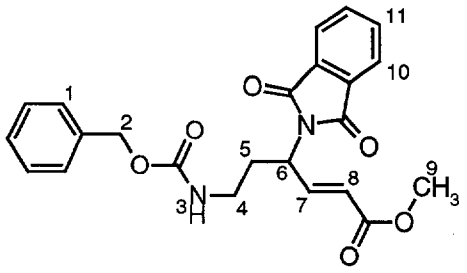
(E)-6-(Benzyloxycarbonyl-tert-butoxycarbonylamino)-4-hydroxy-hex-2-enoic acid methyl ester (10). A solution of the crude aldehyde **9** (482g, 1.5mol) in dry acetonitrile (950mL) was added over 3.5h to a stirred solution of methylphenylsulfinyl acetate (248g, 1.25mol) and piperidine (150mL, 1.5mol) in dry acetonitrile (1900mL) under nitrogen. After 17h at room temperature the solvent was removed *in vacuo* and the residue was dissolved in ethyl acetate (3000mL). The organic solution was washed sequentially with dilute hydrochloric acid (1M, 2x1500mL), saturated aqueous sodium bicarbonate (1000mL) and saturated aqueous brine (500mL). After drying, solvent removal *in vacuo* gave an orange oil. This was purified by column chromatography on silica gel (10kg) with a graded elution of hexane:ethyl acetate starting with 6:1 (30L) and progressing through 3:1 (16L) and 2:1 (18L) to 1:1. Concentration of the appropriate fractions afforded an orange oil (343g, 70%).

Tlc hexane:ethyl acetate (1:1) $R_f = 0.41$

^{1}H-NMR (250MHz, $CDCl_3$) 7.42-7.34 (m, 5H, H-1), 6.91 (dd, J_{7-8} = 15.5Hz, J_{7-5} = 4.2Hz, 1H, H-7), 6.09 (dd, J_{8-7} = 15.5Hz, J_{8-5} = 2Hz, 1H, H-8), 5.23 (s, 2H, H-2), 4.28 (m. 1H, H-5), 3.85 (m, 2H, H-3), 3.64 (s, 3H, H-9), 3.39 (d, J_{6-5} = 4Hz, 1H, H-6), 1.90 (m, 1H, H-4), 1.68 (m, 1H, H-4'), 1.46 (s, 9H, H-10).
IR (KBr diffuse reflectance) ν_{max} 3505, 2981, 1790, 1738, 1731, 1714, 1693, 1681, 1454, 1370, 1277, 1216, 1155, 771, 698 cm^{-1}.
Assay. Found: C, 60.67; H, 6.98; N, 3.77%
$C_{20}H_{27}NO_7$ requires C, 61.06; H, 6.92; N, 3.56%



(E)-6-(Benzyloxycarbonyl-tert-butoxycarbonylamino)-4-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-hex-2-enoic acid methyl ester. A solution of diethylazodicarboxylate (120mL, 0.76mol) in dry tetrahydrofuran (250mL) was added over 2.5h to a stirred mixture of triphenylphosphine (200g, 0.76mol), phthalimide (112g, 0.76mol) and the alcohol **10** (298.85g, 0.75mol) in dry tetrahydrofuran (1250mL) at 4-6°C under nitrogen. After stirring at 4°C for 2h the mixture was allowed to warm to room temperature over 16h. The solvent was removed *in vacuo* and the residue was treated with cold (6°C) *t*-butylmethylether (1000mL). The insoluble triphenylphosphine oxide was filtered off and washed with more cold *t*-butylmethyl ether (200mL). The filtrate was concentrated *in vacuo* and the residual oil was purified by column chromatography on silica gel (9kg) with hexane:ethyl acetate (2:1) as eluent. Concentration of the appropriate fractions gave a viscous oil plus some solid. This was treated with an ethyl acetate:hexane (1:1) mixture (1000mL). Concentration of the filtrate afforded pure material as a yellow oil (332g, 85%).
Tlc hexane:ethyl acetate (2:1) R_f = 0.23
^{1}H-NMR (250MHz, $CDCl_3$) 7.88-7.70 (m, 4H, H-9,10), 7.38-7.28 (m, 5H, H-1), 7.13 (dd, J_{6-7} = 15Hz, J_{6-5} = 6.5Hz, 1H, H-6), 5.93 (dd, J_{7-6} = 15Hz, J_{7-5} < 1Hz, 1H, H-7), 5.18 (s, 2H, H-2), 4.92 (m, 1H, H-5), 3.82-3.78 (m, 5H, H-8,3), 2.51 (m, 1H, H-4), 2.29 (m, 1H, H-4'), 1.42 (s, 9H, H-11).
IR (KBr diffuse reflectance) ν_{max} 2990, 1774, 1717, 1384, 1368, 1287, 1254, 1214, 1172, 1155, 1130, 1087, 720 cm^{-1}.
MS (Electrospray, NH_3) Found MNH_4^+: 540.234957
$C_{28}H_{34}N_3O_8^+$ requires 540.234591 (0.7ppm).



(11)

(E)-6-Benzyloxycarbonylamino-4-(1,3-dioxo-1,3-dihydroisoindol-2-yl)-hex-2-enoic acid methyl ester (11). Trifluoroacetic acid (174mL, 2.26mol) was added over 12.5min to a stirred solution of (E)-6-(benzyloxycarbonyl-tert-butoxycarbonyl-amino)-4-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-hex-2-enoic acid methyl ester (296g, 0.566mol) in dry dichloromethane (3000mL) at 5-6°C under nitrogen. After 2h, when tlc indicated consumption of starting material, the mixture was quenched by the addition of aqueous sodium carbonate (1M, 1000mL) over 20 min, **(caution-gas evolution).** Once the gas evolution had ceased the layers were separated. The organic solution was washed with saturated aqueous sodium bicarbonate (500mL) and saturated brine (500mL). After drying (Na_2SO_4) solvent removal *in vacuo* gave a viscous yellow gum which crystallised on standing to a white solid (235.5g, 98.5%) with mpt 78-79°C.

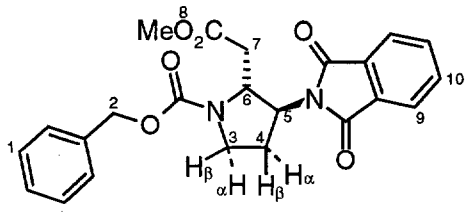
Tlc hexane:ethyl acetate (3:2) $R_f = 0.23$

^{1}H-NMR (250MHz, $CDCl_3$) 7.88-7.70 (m, 4H, H-10,11), 7.40-7.26 (m, 5H, H-1), 7.16 (dd, J_{7-8} = 15Hz, J_{7-6} = 6Hz, 1H, H-7), 5.95 (d, J_{8-7} = 15Hz, 1H, H-8), 5.12-4.93 (m, 4H, H-2,3,6), 3.73 (s, 3H, H-9), 3.35 (m, 1H, H-4), 3.07 (m, 1H, H-4'), 2.40 (m, 1H, H-5), 2.17 (m, 1H, H-5').

IR (KBr diffuse reflectance) ν_{max} 3366, 3030, 2953, 1771, 1723, 1713, 1698, 1694, 1537, 1384, 1254, 1175, 760, 755, 720 cm^{-1}.

Assay. Found: C, 65.2; H, 5.2; N, 6.6%

$C_{23}H_{22}N_2O_6$ requires C, 65.4; H, 5.25; N, 6.6%



(12)

Trans-3-(1,3-dioxo-1,3-dihydroisoindol-2-yl)-2-methoxycarbonylmethylpyrrolidine-1-carboxylic acid benzyl ester (12). Sodium hydride (60% in mineral oil 2.3g, 57.5mmol) was added in one portion to a stirred solution of **11** (97.2g, 0.23mol) in dry tetrahydrofuran (1500mL) at 4°C under nitrogen. After 2h, when tlc indicated consumption of starting material, the reaction was quenched by adding a water tetrahydrofuran mixture (1:4, 50mL). The resulting mixture was poured into water

and brine (2:3, 700mL) and the organic layer was separated. The aqueous layer was extracted with ethyl acetate (2x500mL). The organic solutions were combined and washed with saturated brine (500mL). After drying ($MgSO_4$) solvent removal *in vacuo* gave an opaque yellow gum (81g, 83%). A portion of the gum (8.5g) was triturated twice with a diethyl ether:hexane (1:1) mixture (20mL and 10mL) to afford a white solid (5.79g) with mpt 98-99°C.

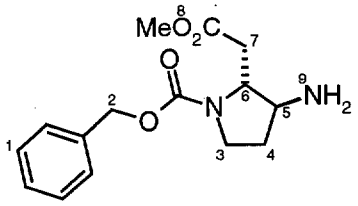
Tlc hexane:ethyl acetate (3:2) $R_f = 0.23$

^{1}H-NMR (400MHz, DMSO, 90°C) 7.85-7.83 (m, 4H, H-9,10), 7.38-7.31 (m, 5H, H-1), 5.12 (s, 2H, H-2), 4.73 (dt, $J_{5\text{-}4_\alpha} = 7.5$Hz, $J_{5\text{-}4_\beta} = 7.5$Hz, $J_{5\text{-}6} = 5.5$Hz, 1H, H-5), 4.47 (quintet, $J_{6\text{-}7} = 8.5$Hz, $J_{6\text{-}5} = 5.5$Hz, $J_{6\text{-}7'} = 4$Hz, 1H, H-6), 3.82 (ddd, $J_{3_\alpha\text{-}3_\beta} = 10.5$Hz, $J_{3_\beta\text{-}4_\beta} = 8.5$Hz, $J_{3_\beta\text{-}4_\alpha} = 5$Hz, 1H, H-3β), 3.47 (s, 3H, H-8), 3.42 (dt, $J_{3_\alpha\text{-}3_\beta} = 10.5$Hz, $J_{3_\alpha\text{-}4_{\alpha,\beta}} = 8$Hz, 1H, H-3α), 2.86 (dd, $J_{7'\text{-}7} = 15.5$Hz, $J_{7'\text{-}6} = 4$Hz, 1H, H-7'), 2.68 (dd, $J_{7\text{-}7'} = 15.5$Hz, $J_{7\text{-}6} = 8.5$Hz, 1H, H-7), 2.35 (m, 1H, H-4β), 2.20 (m, 1H, H-4α). Structural assignments are made from COSY and nOe experiments. The assignments of H-4α and H-4β are based on observation of a strong nOe between H-5 and H-4α and a weak nOe between H-5 and H-4β. Other strong nOe's observed are H-4α to H-3α, H-4β to H-3β, H-5 to H-7 and weaker nOe's are H-3α toH-5, H-5 to H-6, H-4β to H-6, H-5 to H-7'.

IR (KBr diffuse reflectance) ν_{max} 1770, 1737, 1721, 1712, 1694, 1415, 1384, 1359, 1211, 1169, 1120, 720 cm^{-1}.

Assay. Found: C, 64.6; H, 5.2; N, 6.5%

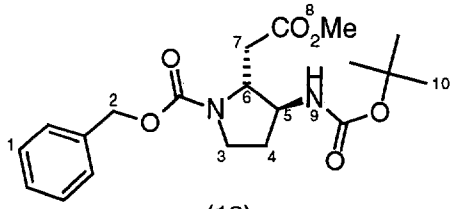
$C_{23}H_{22}N_2O_6.0.3H_2O$ requires C, 64.6; H, 5.3; N, 6.5%



Trans-3-amino-2-methoxycarbonylmethylpyrrolidine-1-carboxylic acid benzyl ester. Hydrazine hydrate (60mL of 55% hydrazine 1.06mol) was added to a stirred solution of **12** (448.2g, 1.06mol) in ethanol (2500mL) at room temperature under nitrogen. The resulting mixture was heated under reflux for 3.5h whereupon tlc indicated consumption of starting material. The mixture was allowed to cool to room temperature and the precipitate was filtered off. The filtrate was concentrated *in vacuo* and the residue was partitioned between ethyl acetate (1000mL) and dilute hydrochloric acid (1M, 700mL). The aqueous extracts were combined,washed with ethyl acetate (1000mL) then carefully neutralised with aqueous sodium hydroxide (2M, 900mL) and finally aqueous sodium carbonate (1M, 200mL). The aqueous solution was extracted with ethyl acetate (3x1500mL). These extracts were combined, washed with saturated brine (750mL) and dried (Na_2SO_4). Solvent removal *in vacuo* afforded a brown oil (239.9g, 77%).

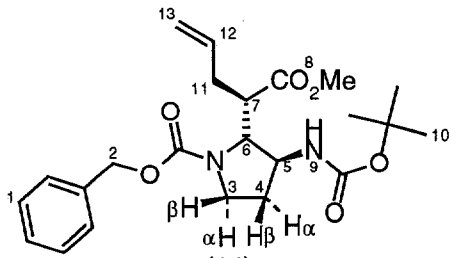
Tlc ethyl acetate:ethanol (19:1) $R_f = 0.11$

^{1}H-NMR (250MHz, $CDCl_3$) 7.40-7.28 (m, 5H, H-1), 5.20-5.06 (m, 2H, H-2), 3.95-3.85 (m, 1H, H-6), 3.72-3.57 (m, 1H, H-5), 3.68 (s, 3H, H-8), 3.53-3.41 (m, 2H, H-3), 3.02-2.73 (m, 1H, H-7), 2.42-2.26 (m, H1, H-7'), 2.15-1.99 (m, 1H, H-4), 1.74-1.60 (m, 1H, H-4'), 1.46 (bs, 2H, H-9).
IR (KBr diffuse reflectance) ν_{max} 3233, 3065, 3034, 2953, 2897, 1693, 1681, 1650, 1556, 1537, 1454, 1418, 1362, 1209, 1116, 700 cm^{-1}.
MS (Electrospray, NH_3) Found MH^+: 293.149175
$C_{15}H_{21}N_2O_4^+$ requires 293.150132 (3.3ppm error).



(13)

Trans-3-tert-butoxycarbonylamino-2-methoxycarbonylmethylpyrrolidine-1-carboxylic acid benzyl ester (13). A solution of di-*t*-butyldicarbonate (69g), 0.316mol) in dry acetonitrile (500mL) was added over 30 min to a stirred solution of trans-3-amino-2-methoxycarbonylmethyl-pyrrolidine-1-carboxylic acid benzyl ester (80.77g, 0.276mol) and triethylamine (44mL, 0.316mol) in dry acetonitrile (950mL) at room temperature under nitrogen. After 24h, when tlc indicated consumption of starting material, the solvent was removed *in vacuo* and the residue was partitioned between dilute hydrochloric acid (1M, 650mL) and ethyl acetate (1300mL). The aqueous layer was re-extracted with ethyl acetate (650mL). The organic extracts were combined and washed with saturated brine (500mL). After drying ($MgSO_4$) solvent removal *in vacuo* gave a red brown oil which was purified by column chromatography on silica gel (1.2kg) with hexane:ethyl acetate (2:1) as eluent. Concentration of the appropriate fractions gave a pale yellow oil which crystallised on standing. Trituration of the solid with a hexane:diethyl ether (4:1) mixture (250mL) afforded a white solid (81.2g, 87%) with mpt 73-74°C.
Tlc hexane: ethyl acetate (2:1) $R_f = 0.21$
^{1}H-NMR (400MHz, DMSO, 100°C) 7.39-7.25 (m, 5H, H-1), 6.78 (bs, 1H, H-9), 5.08 (d, $J_{2\text{-}2'}$ = 13Hz, 1H, H-2), 5.05 (d, $J_{2'\text{-}2}$ = 13Hz, 1H, H-2'), 3.95 (m, 1H, H-6), 3.89 (m, 1H, H-5), 3.56 (s, 3H, H-8), 3.55 (m, 1H, H-3), 3.33(ddd, J = 11, 8, 4.5Hz, 1H, H-3'), 2.61 (dd, J = 15, 5Hz, 1H, H-7), 2.53 (dd, J = 15, 7.5Hz, 1H, H-7'), 2.07 (m, 1H, H-4), 1.78 (m, 1H, H-4'), 1.40 (s, 9H, H-10).
IR ($CHCl_3$ solution) ν_{max} 3442, 2949, 1729, 1698, 1500, 1455, 1418, 1368, 1163, 1119, 983 cm^{-1}.
Assay. Found: C, 61.2; H, 7.4; N, 7.1%
$C_{20}H_{28}N_2O_6$ requires C, 61.2; H, 7.2; N, 7.1%



(14)

rel-(2R,3S)-3-tert-Butoxycarbonylamino-2-(1R-methoxycarbonylbut-3-enyl)-pyrrolidine-1-carboxylic acid benzyl ester (14). A solution of lithium bis(trimethylsilyl)amide in tetrahydrofuran (1M, 80mL) was added over 65 min to a stirred solution of **13** (9.81g, 25mmol) in dry tetrahydrofuran (54mL) and dry 1,3-dimethyl-3,4,5,6-tetrahydro-2-(1H)-pyrimidone (120mL) mixture at -69°C under nitrogen. After 1h at -70°C allyl iodide (2.8mL, 30.6mmol) was added over 5min at -69°C and the resulting mixture was stirred at -70°C for 2h. The reaction was quenched by adding saturated aqueous ammonium chloride (20mL) over 10 min and allowing the mixture to warm to 0°C. Water (20mL) was added and the resulting mixture was extracted with ethyl acetate (4x100mL). The extracts were combined, concentrated *in vacuo* and partitioned between toluene (200mL) and water (100mL). The organic phase was washed with water (2x100mL) and saturated brine (50mL). After drying (Na_2SO_4), solvent removal *in vacuo* gave an oil which was purified by column chromatography on silica gel (700g) with hexane:ethyl acetate (7:3) as eluent. Concentration of the appropriate fractions gave a colourless oil (7.7g, 71%) which solidified on standing.

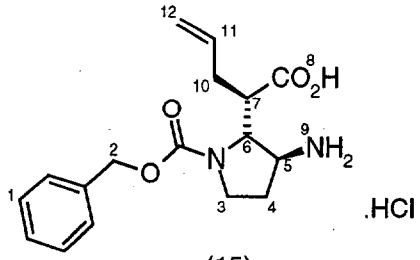
Tlc hexane:ethyl acetate (7:3) $R_f = 0.28$

^{1}H-NMR (400MHz, DMSO, 100°C) 7.38-7.26 (m, 5H, H-1), 6.88 (bs, 1H, H-9), 5.71 (m, 1H, H-12), 5.11 (d, $J_{2\text{-}2'}$ = 12.5Hz, 1H, H-2), 5.08 (d, $J_{2'\text{-}2}$ = 12.5Hz, 1H, H-2'), 5.01 (m, 1H, H-13), 4.95 (m, 1H, H-13'), 4.09 (m, 1H, H-5), 3.90 (dd, J = 5.5, 2.5Hz, 1H, H-6), 3.63 (m, 1H, H-3β), 3.59 (s, 3H, H-8), 3.22 (ddd, J=10.5, 9, 5Hz, 1H, H-3α), 2.90 (m, 1H, H-7), 2.39 (m, 1H, H-11), 2.25 (m, 1H, H-11'), 1.97 (m, 1H, H-4α), 1.74 (m, 1H, H-4β), 1.40 (s, 9H, H-10). Structural assignments are made from COSY, HMQC and nOe experiments. The assignments of H-4α and H-4β are based on observation of a strong nOe between H-5 and H-4α and a weak nOe between H-5 and H-4β. Further strong nOe's observed were H-4α to H-3α, H-9 to H-4β, H-7 to H-4α. A weak nOe is observed between H-3α and H-4β. No nOe is observed for H-9 to H-4α.

IR (KBr diffuse reflectance) ν_{max} 3341, 2978, 1731, 1708, 1683, 1520, 1436, 1414, 1364, 1348, 1243, 1196, 1166, 1116 cm^{-1}.

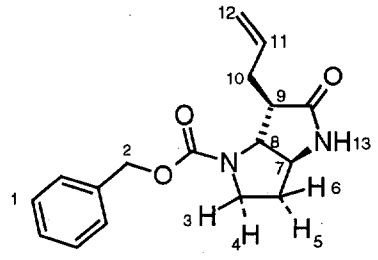
Assay. Found: C, 63.87; H, 7.68; N, 6.83%

$C_{23}H_{32}N_2O_6$ requires C, 63.87; H, 7.46; N, 6.48%



(15)

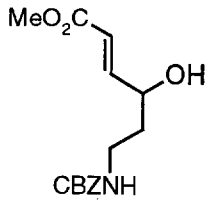
rel-(2R,3S)-3-Amino-2-(1R-carboxybut-3-enyl)-pyrrolidine-1-carboxylic acid benzyl ester hydrochloride (15). Aqueous potassium hydroxide (2M, 400mL) was added to a solution of the ester **14** (32.17g, 74mmol) in ethanol (400mL) and the resulting mixture was stirred at 55°C under nitrogen for 5h when tlc indicated consumption of starting material. The ethanol was removed *in vacuo* and the resulting mixture was acidified to *ca* pH2 with dilute hydrochloric acid (2M, 400mL). The product was extracted into ethyl acetate (3x500mL) and the combined extracts dried ($MgSO_4$). Solvent removal *in vacuo* afforded a colourless foam (29g, 93%).
The foam (29g, 69mmol) was dissolved in a solution of hydrogen chloride in dioxan (4M, 300mL) and the mixture was stirred at room temperature under nitrogen for 3h when tlc indicated consumption of starting material. The solvent was removed *in vacuo* and the residual solid was triturated with diethyl ether (2x80mL) to give a white solid (22g, 90%) with mpt 158-159°C.
^{1}H-NMR (400MHz, DMSO) 13.50-11.50 (bs, 1H), 9.20-8.00 (bs, 3H), 7.44-7.31 (m, 5H), 5.85-5.62 (m, 1H), 5.20-4.93 (m, 4H), 4.12 (d, J = 6.5Hz, 1H), 3.75-3.57 (m, 2H), 3.48-3.30 (m, 1H), 2.82-2.65 (m, 1H), 2.40-2.00 (m, 4H). Warming to 105°C did not lead to significant simplification of the spectrum.
IR (KBr diffuse reflectance) ν_{max} 3403, 2953, 2612, 1712, 1693, 1681, 1667, 1650, 1454, 1416, 1346, 1215, 1184, 1122, 917, 698 cm^{-1}.
Assay. Found: C, 55.5; H, 6.6; N, 8.0; H_2O, 2.6%
$C_{17}H_{22}N_2O_4$. 1.05 HCl. 0.5 H_2O requires C, 55.8; H, 6.6; N, 7.7%



(1)

rel-(3aS,6R,6aR)-6-Allyl-5-oxohexahydropyrrolo[3,2-b]pyrrole-1-carboxylic acid benzyl ester (1). 2-Chloro-1-methylpyridinium iodide (3g, 11.4mmol) was added in one portion to a stirred solution of the hydrochloride **15** (2.73g, 7.5mmol) and *N,N*-diisopropylethylamine (1.3mL, 7.5mmol) in dry dichloromethane (1250mL) at room

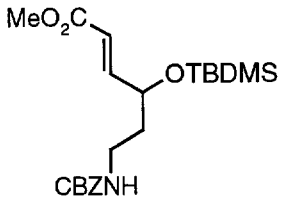
temperature under nitrogen. After 1h more *N,N*-diisopropylethlamine (2.6mL, 15mmol) was added and the mixture was stirred at room temperature under nitrogen for a further 20h. The solution was washed with dilute hydrochloric acid (0.1M, 2x75mL) and water (75mL). After drying (Na_2SO_4) solvent removal *in vacuo* in the presence of silica gel (5g) gave a brown solid. The silica gel was applied to a silica column (120g) which was eluted with ethyl acetate:hexane (3:1). Concentration of the appropriate fractions gave a yellow solid which was triturated twice with ether (20mL and 10mL) to afford a pale cream solid (1.66g, 74%) with mpt 159-160°C.
Tlc ethyl acetate:hexane (3:1) $R_f = 0.18$
^{1}H-NMR (750MHz, DMSO, 90°C) 7.86 (bs, 1H, H-13), 7.37-7.30 (m, 5H, H-1), 5.76 (m, 1H, H-11), 5.09 (s, 2H, H-2), 4.96-4.92 (m, 2H, H-12), 3.72 (dd, J_{3-4} = 11Hz, J_{3-6} = 10Hz, 1H, H-3), 3.63 (ddd, J_{4-3} = 11Hz, J_{4-6} = 10Hz, J_{4-5} = 6.5Hz, 1H, H-4), 3.19 (m, 1H, H-7), 3.04 (dd, J_{8-7} = 11.5Hz, J_{8-9} = 10Hz, 1H, H-8), 2.59 (m, 1H, H-10), 2.56 (m, 1H, H-10'), 2.54 (m, 1H, H-9), 2.10 (m, 1H, H-5), 1.69 (m, 1H, H-6). Structural assignments are made from HMQC and nOe experiments. The assignments of H-5 and H-6 is based on observation of a strong nOe between H-7 and H-5, and a weak nOe between H-7 and H-6. Further strong nOe's observed were H-6 and H-8, H-6 and H-3, H-5 and H-4, H-7 and H-4. A weak nOe is observed between H-3 and H-8.
IR (KBr diffuse reflectance) ν_{max} 3291, 3075, 2984, 2897, 1720, 1713, 1703, 1694, 1682, 1454, 1434, 1386, 1360, 1326, 1229, 1133, 1116, 918, 760, 704 cm^{-1}.
Assay. Found: C, 67.6; H, 7.0; N, 9.4%
$C_{17}H_{20}N_2O_3$ requires C, 68.0; H, 6.7; N, 9.3%



16

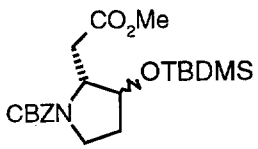
(E)-6-Benzyloxycarbonylamino-4-hydroxyhex-2-enoic acid methyl ester (16). Trifluoroacetic acid (55mL, 0.71mol) was added over 10 min to a stirred solution of the carbamate **10** (71.4g, 0.18mol) in dry dichloromethane (1L) at 4°C under nitrogen. After 2h, when tlc indicated consumption of starting material, saturated aqueous sodium bicarbonate (400mL) was added and the mixture was stirred for 1h. The layers were separated and the organic layer was washed with saturated aqueous sodium bicarbonate (400mL), water (400mL) and saturated brine (200mL). After drying ($MgSO_4$) solvent removal *in vacuo* gave an orange oil (75g). This was purified by column chromatography on silica gel (300g) with ether:hexane initially (1:1) and progressing through (2:1) to (3:1) as eluents. Concentration of the appropriate fractions afforded **16** as a colorless oil (42.77g, 80%)
Tlc ethyl acetate:hexane (1:1) $R_f = 0.23$
^{1}H-NMR (250MHz, $CDCl_3$) 7.38-7.30 (bs, 5H), 6.93 (dd, J = 15.5, 4Hz, 1H), 6.10 (d, J = 15Hz, 1H), 5.20 (bt, 1H), 5.09 (s, 2H), 4.35 (m, 1H), 3.72 (s, 3H), 3.48 (m,1H), 3.25 (m, 1H), 1.78 (m, 1H), 1.62 (m, 1H).

MS (Electrospray, NH_3) Found MH^+: 294.135055
$C_{15}H_{20}NO_5^+$ requires 294.134148 (3.1ppm error).
Assay. Found: C, 61.4; H, 6.5; N, 5.1%
$C_{15}H_{19}NO_5$ requires C, 61.1; H, 6.5; N, 4.8%



17

(E)-6-Benzyloxycarbonylamino-4-(tert-butyl-dimethylsilanyloxy)-hex-2-enoic acid methyl ester (17). ^{t}-Butyldimethylsilyl chloride (253g, 1.68mol) was added over 20 min to a stirred solution of unpurified alcohol **16** (410g, 1.4mol) and imidazole (190g, 2.8mol) in dry dimethylformamide (1.3L) under nitrogen at room temperature. After 18h, when tlc indicated consumption of starting material, the reaction was quenched with saturated aqueous ammonium chloride (4.2L) with external cooling and then extracted with ethyl acetate (3x1.6L). The extracts were combined and washed with aqueous lithium chloride (1M, 2.1L), water (1.9L) and saturated brine (1L). After drying ($MgSO_4$) solvent removal *in vacuo* gave an orange oil (615g). This was purified by column chromatography on silica gel (6kg) using hexane:ether, starting with (6:1) and proceeding to (1:1) via (3:1) and (2:1), as eluent. Concentration of the appropriate fractions afforded **17** as a colorless oil (501.8g, 88%)
Tlc ether:hexane (1:1) $R_f = 0.26$
^{1}H-NMR (250MHz, $CDCl_3$) 7.35-7.25 (bs, 5H), 6.88 (dd, J = 14.5, 4Hz,1H), 5.97 (dd, J = 14.5, 1.5Hz, 1H) 5.10 (bt, 1H), 5.05 (s, 2H), 4.43 (m, 1H), 3.70 (s, 3H), 3.24 (m, 2H), 1.74 (m, 2H), 0.88 (s, 9H), 0.03 (s, 6H).
MS (Electrospray, NH_3) MH^+ 408 (50%), MNH_4^+ 425 (100%).
Assay. Found: C, 62.1; H, 8.0; N, 3.7%
$C_{21}H_{33}NO_5Si$ requires C, 61.9; H, 8.2; N, 3.4%



18 3:2
trans:cis

3-(tert-Butyldimethylsilanyloxy)-2-methoxycarbonylmethylpyrrolidine-1-carboxylic acid benzyl ester (18). Sodium hydride (2.5g of 60% in mineral oil, 62.5mmol) was added over 3 min to a stirred solution of the urethane **17** (255.7g, 0.62mol) in dry tetrahydrofuran (3.4L) at 4°C under nitrogen. After 5 min the cooling

bath was removed and the reaction was allowed to warm to 12°C over 30 min whereupon tlc indicated consumption of starting material. The mixture was re-cooled to 8°C over 5 min and was quenched by the addition of saturated aqueous ammonium chloride (850mL). The layers were separated and the aqueous layer was extracted with ethyl acetate (2x680mL). The organic solutions were combined and washed with saturated brine (2x500mL). After drying ($MgSO_4$), solvent removal *in vacuo* afforded the pyrrolidines **18** as an orange oil (246.4g, 97%).

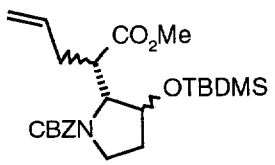
Tlc ether:hexane (1:1) $R_f = 0.43$

^{1}H-NMR (250MHz, 55°C, $CDCl_3$) 7.35-7.20 (m, 5H), 5.07 (s, 0.33 x 2H, *cis*), 5.03 (s, 0.66 x 2H, *trans*), 4.42 (m, 0.33 x 1H, *cis*), 4.19 (m, 1H), 3.93 (m, 0.66 x 1H, *trans*), 3.59 (s, 0.66 x 3H, *trans*), 3.54 (s, 0.33 x 3H, *cis*), 3.51-3.32 (m, 2H), 2.74-2.53 (m, 1.33H), 2.28 (dd, 0.66 x 1H, *trans*), 2.10-1.90 (m, 1H), 1.84-1.68 (m, 1H), 0.85 (s, 9H), 0.06 (s, 6H).

IR ($CHCl_3$ solution) ν_{max} 2940, 2931, 2897, 2858, 1734, 1704, 1471, 1418, 1359, 1159, 1104, 838cm^{-1}.

Assay. Found: C, 62.1; H, 7.9; N, 3.4%

$C_{21}H_{33}NO_5Si$ requires C, 61.9; H, 8.1; N, 3.4%

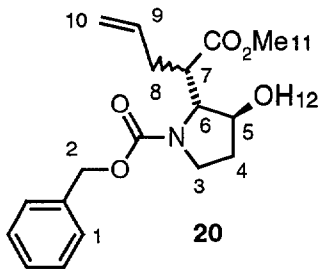


19

Trans-3-(tert-butyldimethylsilanyloxy)-2-(1-methoxycarbonyl-but-3-enyl)-pyrrolidine-1-carboxylic acid benzyl ester (19). Lithium bis(trimethylsilyl)amide (1M in THF) (720mL, 0.72mol) was added dropwise over 1h to a stirred solution of crude methyl esters **18** (246g, 0.6mol) in dry THF (2.4L) under nitrogen at -70°C. After 1h, allyl bromide (57mL, 0.66mol) was added dropwise over 5 min, and the reaction mixture allowed to warm to room temperature overnight. After quenching with saturated ammonium chloride solution (1L), the product was extracted with EtOAc (2L). The combined extracts were washed with brine (1L), dried ($MgSO_4$) and concentrated *in vacuo* to afford crude product **19** as an orange oil (294.34g).

Tlc EtOAc:hexane (3:1) $R_f = 0.71$

^{1}H-NMR (250MHz, $CDCl_3$) 7.39-7.27 (m, 5H), 5.84-5.54 (m, 1H), 5.19-4.93 (m, 4H), 4.35-4.19 (m, 1H), 4.08-3.92 (m, 1H), 3.82-3.29 (m, 5H), 3.06-2.75 (m, 1H), 2.62-2.16 (m, 2H), 2.16-1.70 (2H), 0.84 (s, 9H), 0.10 (s, 6H).



20

Trans-3-hydroxy-2-(1-methoxycarbonylbut-3-enyl)-pyrrolidine-1-carboxylic acid benzyl ester (20). Tetrabutylammonium fluoride (1M in THF) (1.3L, 1.3mol) was added over ½h to a solution of crude silyl ethers **19** (591.3g, *ca* 1.2mol) in dry THF (3L) at room temperature under nitrogen. After 2h, the solvent was removed *in vacuo* and the residue diluted with water (1L) and extracted with EtOAc (1x2L, 2x1L). The combined extracts were dried ($MgSO_4$) and concentrated *in vacuo* to afford **20** as a brown oil (498g).

Tlc EtOAc:hexane (3:1) $R_f = 0.42$

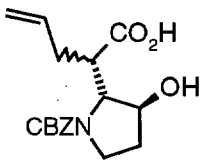
A sample (8.84g) was purified by flash chromatography on silica gel eluting with EtOAc:cyclohexane 2:3 to afford a 3:2 (by ^{1}H-NMR) mixture of β- and α-allyl hydroxy-esters **20** (4.2g).

^{1}H-NMR (400MHz, DMSO, 90°C) 7.39-7.26 (m, 5H, H-1), 5.78-5.62 (m, 1H, H-9β,α), 5.11 (d, $J_{2\text{-}2'}$ = 13Hz, 1H, H-2α), 5.10 (s, 2H, H-2β), 5.05 (d, $J_{2'\text{-}2}$ = 13Hz, 1H, H-2α), 5.02-4.92 (m, 2H, H-10β,α), 4.84 (m, 1H, H-12β), 4.79 (m, 1H, H-12α), 4.19-4.10 (m, 1H, H-5β,α), 5.89 (m, 1H, H-6β,α), 3.60-3.50 (m, 1H, H-3β,α), 3.58 (s, 3H, H-11β), 3.54 (s, 3H, H-11α), 3.36-3.24 (m, 1H, H-3'β,α), 2.79-2.67 (m, 1H, H-7β,α), 2.42-2.10 (m, 2H, H-8β,α), 2.10-2.03 (m, 1H, H-4β,α), 1.80-1.71 (m, 1H, H-8'β,α).

MS (Electrospray, NH_3) MH^+ 334 (100%)

Assay. Found: C, 64.7; H, 6.9; N, 4.5%

$C_{18}H_{23}NO_5$ requires C, 64.8; H, 6.9; N, 4.2%



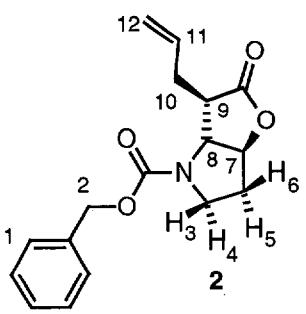
21

Trans-2-(1-carboxybut-3-enyl)-3-hydroxypyrrolidine-1-carboxylic acid benzyl ester (21). A suspension of lithium hydroxide monhydrate (71.4g, 1.7mol) in water (680mL) was added to the crude esters **20** (239g, *ca* 0.72mol) in THF (5L) under N_2. This was stirred at 60°C for 18h when tlc showed absence of starting material. After cooling, the mixtures were diluted with water (1L) and washed with ether (1x2L, 2x1L), and the aqueous layer acidified to pH1 with 2M HC1. This was then extracted with EtOAc (2x2L) and the combined extracts washed with brine (750mL) and dried

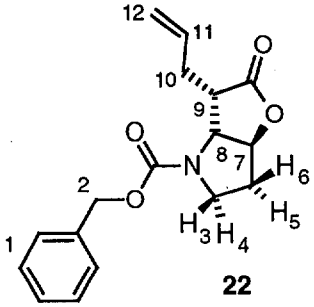
($MgSO_4$) and concentrated *in vacuo* to give the crude hydroxy-acid **21** (125.15g) as a 3:2 (by ^{1}H-NMR) mixture of β:α allyl isomers.

Tlc Et_2O $R_f = 0.30$ (streak)

^{1}H-NMR (250MHz, $CDCl_3$, 55°C), 7.38-7.26 (m, 5H), 6.40-5.60 (m, 3H), 5.14 (s, 2Hβ), 5.12 (s, 2Hα), 5.10-4.93 (m, 2H), 4.38 (m, 1H), 4.14-4.04 (m, 1H), 3.78-3.64 (m, 1H), 3.46-3.34 (m, 1H), 3.18-3.04 (bs, 1Hα), 2.76-2.64 (m, 1Hβ), 2.55-2.23 (m, 2H), 2.18-1.97 (m, 1H), 1.96-1.81 (m, 1H).



2



22

Rel-(3R,3aR,6aS)-3-allyl-2-oxohexahydrofuro[3,2-b]pyrrole-4-carboxylic acid benzyl ester (2) and rel-(3S,3aR,6aS)-3-allyl-2-oxohexahydrofuro[3,2-b]pyrrole-4-carboxylic acid benzyl ester (22). 2,4,6-Trichlorobenzoyl chloride (14.5mL, 92.8mmol) was added to a solution of crude hydroxy-acid **21** (24.77g, 77.5mmol) and triethylamine (14.5mL, 104.2mmol) in dichloromethane (1.5L) under nitrogen. After 3h, tlc showed absence of starting material. After diluting with toluene (0.5L), the solution was added over 4h to a refluxing solution of dimethylaminopyridine (41.5g, 0.34mol) in toluene (4.5L). After refluxing for a further 45min, the mixture was allowed to cool overnight. After concentration *in vacuo*, the residue was treated with 1M hydrochloric acid (1L) and extracted with EtOAc (3x1L). The combined extracts were washed with 1M hydrochloric acid (1L), brine (500mL), dried ($MgSO_4$) and concentrated *in vacuo* to afford the crude product as a brown oil (52.2g) with β:α allyl ratio of *ca* 3:2 by ^{1}H-NMR. Crude material from 4 runs was combined (193g) and purified by flash chromatography on silica (8kg) eluting with Et_2O:hexane 1:1 to 2:1. Pure β product **2** (16.28g, 17%) as a white solid, pure α product **22** (18.44g, 19%) as a waxy solid, and mixed fractions 4:1 β:α **2:22** (24.32g, 26%) were thus isolated. Trituration of the mixed fractions with Et_2O (20mL), gave further pure β product **2** (15.74g). The β-product can also be recrystallised from hexane:EtOAc 3:1.

Data for 2.

Tlc Et_2O:hexane (1:1) $R_f = 0.16$

^{1}H-NMR (400MHz, DMSO, 60°C) 7.39-7.29 (m, 5H, H-1), 5.76 (m, 1H, H-11), 5.10 (s, 2H, H-2), 5.02-4.94 (m, 2H, H-12), 4.05 (ddd, $J_{7\text{-}6} = 12.5$Hz, $J_{7\text{-}8} = 10$Hz, $J_{7\text{-}5} = 5.5$Hz, 1H, H-7), 3.79 (dd, $J_{3\text{-}4} = 10.5$Hz, $J_{3\text{-}6} = 10$Hz, 1H, H-3), 3.63 (m, 1H, H-4), 3.33 (dd, $J_{8\text{-}9} = 12.5$Hz, $J_{8\text{-}7} = 10$Hz, 1H, H-8), 3.10 (m, 1H, H-9), 2.70-2.50 (m, 2H, H-10), 2.72 (m, 1H, H-5), 2.00 (m, 1H, H-6). Structural assignments are based on HMQC, COSY and nOe experiments. The assignment of H-5 is based on a strong nOe

between H-7 and H-5. Other strong nOe's observed are H-3 and H-8; H-3 and H-6; H-4 and H-5; H-4 and H-7; H-6 and H-8; H-7 and H-9.
IR ($CHBr_3$ solution) ν_{max} 3676, 2959, 2923, 2900, 1791, 1701, 1432, 1388, 1359, 1324, 1033, 925, 753 cm^{-1}.
Assay. Found: C, 67.4; H, 6.7; N, 4.4%
$C_{17}H_{19}NO_4$ requires C, 67.8; H, 6.4; N, 4.6%

Data for 22.
Tlc Et_2O:hexane (1:1) $R_f = 0.11$
^{1}H-NMR (400MHz, DMSO, 60°C) 7.35-7.27 (m, 5H, H-1), 5.27 (m, 1H, H-11), 5.09 (d, $J_{2\text{-}2'}$ = 12.5Hz, 1H, H-2), 5.06-4.90 (m, 2H, H-12), 5.02 (d, $J_{2'\text{-}2}$ = 12.5Hz, 1H, H-2'), 4.33 (ddd, $J_{7\text{-}6}$ = 11.5Hz, $J_{7\text{-}8}$ = 10.5Hz, $J_{7\text{-}5}$ = 5Hz, 1H, H-7), 3.80 (dd, $J_{3\text{-}4}$ = 11.5Hz, $J_{3\text{-}6}$ = 9.5Hz, 1H, H-3), 3.67 (dd, $J_{8\text{-}7}$ = 11.5Hz, $J_{8\text{-}9}$ = 7Hz, 1H, H-8), 3.54 (ddd, $J_{4\text{-}3}$ = 11.5Hz, $J_{4\text{-}5}$ = 10.5Hz, $J_{4\text{-}6}$ = 6.5Hz, 1H, H-4), 2.90 (m, 1H, H-9), 2.30 (m, 2H, H-10), 2.23 (m, 1H, H-5), 2.01 (m, 1H, H-6). Structural assignments are based on HMQC, COSY, HMBC and nOe experiments. The assignment of H-5 is based on a strong nOe between H-7 and H-5. Other strong nOe's observed are H-4 and H-5; H-4 and H-7; H-6 and H-8; H-7 and H-10; H-8 and H-9.
IR ($CHBr_3$ solution) ν_{max} 3675, 2991, 2961, 2899, 1790, 1703, 1428, 1382, 1353, 1325, 1027, 925, 772, 753 cm^{-1}.
Assay. Found: C, 67.7; H, 6.7; N, 4.7%
$C_{17}H_{19}NO_4$ requires C, 67.8; H, 6.4; N, 4.6%

Trans-2-(1-carboxybut-3-enyl)-3-hydroxypyrrolidine-1-carboxylic acid ethyl ester (23). The ethyl carbamate may be prepared in an analogous to that described for the benzyl carbamate **2**. The hydroxy-acid (0.16g, 0.62mmol) in dry dichloromethane (15mL) was added portionwise over 5h to a stirred refluxing suspension of 2-chloro-1-methylpyridinium iodide (0.67g, 2.59mmol) and triethylamine in dry dichloromethane (235mL). After refluxing for a further 2h, the mixture was cooled to room temperature and concentrated *in vacuo*. After trituration with ether (60mL), the organic solution was concentrated *in vacuo* and the residue purified by chromatography eluting with ether:hexane 2:1 to afford **23** (0.048g, 32%).
^{1}H-NMR (400MHz, $CDCl_3$) 5.87-5.78 (m, 1H), 5.30-5.09 (m, 2H), 4.18 (q, J = 7Hz, 2H), 3.94-3.82 (m, 2H), 3.74-3.66 (m, 1H), 3.34-3.27 (m, 1H), 2.98-2.76 (m, 3H), 2,40-2.32 (m, 1H), 2.08-1.96 (m, 1H), 1.30 (t, J = 7Hz, 3H).
IR ($CHCl_3$ solution) ν_{max} 1785, 1704 cm^{-1}
Assay. Found: C, 60.4; H, 7.2; N, 5.7%
$C_{12}H_{17}NO_4$ requires C, 60.2; H, 7.1; N, 5.9%

Rel-(3aS,6R,6aR)-6-allyl-4-methanesulfonyl-5-oxohexahydropyrrolo[3,2-b]pyrrole-1-carboxylic acid benzyl ester (24). The lactam **1** (0.309g, 1.03mmol)was dissolved in dry THF (18mL) and cooled to –70°C. Lithium hexamethyldisilazide (1.3mL, 1.3mmol, 1M in THF) was added, and after 5min, the cooling bowl was

replaced with an ice (5°C). After 20min, the mixture was recooled to –70°C and mesyl chloride (0.2mL, 2.17mmol) was added and the mixture stirred for 75min. After quenching with saturated ammonium chloride solution (25mL), and extracting with EtOAc (3x25mL) the combined extracts were concentrated *in vacuo* and purified by flash chromatography eluting with hexane:EtOAc 2:1 to afford **24** as a white solid(0.264g, 67%).
^{1}H-NMR (250MHz, $CDCl_3$) 7.39-7.32 (bs, 5H), 5.84-5.62 (bs, 1H), 5.17-5.01 (m, 4H), 3.91-3.69 (m, 2H), 3.61-3.48 (m, 1H), 3.35-3.23 (m, 1H), 3.25 (s, 3H), 2.94-2.70 (m, 3H), 2.62-2.51 (m, 1H), 2.11-1.93 (m, 1H).
IR (KBr diffuse reflectance) ν_{max} 1768, 1709 cm^{-1}
Assay. Found: C, 57.0; H, 6.1; N, 7.3; S, 8.4%
$C_{18}H_{22}N_2O_5S$ requires C, 57.1; H, 5.9; N, 7.4; S, 8.5%

Rel-(3aS,6R,6aR)-6-allyl-4-(naphthalene-1-sulfonyl)-5-oxohexahydro-pyrrolo[3,2-b]pyrrole-1-carboxylic acid benzyl ester (25). The lactam **1** (0.035g, 0.12mmol) was dissolved in THF (1.5mL) under nitrogen and cooled to 5°C. NaH (0.007g, 0.17mmol) (60% in oil) was then added. After 30min, 1-naphthylsulphonyl chloride (0.040g, 0.17mmol) was added. After 2h, tlc showed complete reaction. The mixture was quenched with saturated ammonium chloride solution (5mL) and extracted with EtOAc (3x10mL). The combined extracts were washed with brine (10mL), dried (MgSO4), and the solvent removed *in vacuo*. Flash chromatography eluting with hexane:EtOAc 4:1 gave **25** (0.045mg, 75%).
^{1}H-NMR (250MHz, $CDCl_3$) 8.69 (d, J = 8Hz, 1H), 8.47 (d, J = 7Hz, 1H), 8.15 (d, J = 8Hz, 1H), 7.97 (d, J = 7Hz, 1H), 7.73-7.58 (m, 3H), 7.33 (bs, 5H), 5.65-5.45 (m, 1H), 5.10 (s, 2H), 5.03-4.87 (m, 2H), 3.90-3.70 (m, 2H), 3.64-3.50 (m, 1H), 3.29-3.18 (m, 1H), 2.78-2.55 (m, 4H), 2.21-2.02 (m, 1H).
IR ($CHCl_3$ solution) ν_{max} 1759, 1703 cm^{-1}
Assay. Found: C, 65.9; H, 5.3; N, 5.5%
$C_{27}H_{26}N_2O_5S$ requires C, 66.1; H, 5.3; N, 5.7%

Enzyme Assays

Materials and Methods
Assay plate reader was a Biotek™ EL-340, using KinetiCalc™ software. The data from each assay were analysed using Activity Base™ and XL-fit™. IC_{50} data were generated using a four parameter fit, equation: $Y = A + (B - A)/1 + ((X / C)^D)$ where Y = inhibition; X = concentration of inhibitor; C = IC_{50}; D = Hill coefficient; A and B are fixed at 0% and 100% respectively. The fit is actually two parameter given the constraints.

Human Neutrophil Elastase was purchased from Calbiochem (cat. no. 324681, batch B18370) and solubilised with saline to 100µg/ml (stored at -20°C, freeze/thawed before use). Human Pancreatic Chymotrypsin was purchased from Calbiochem (cat.

no. 230900, batch 091290) and solubilised with 1mM HCl to 50µg/ml (stored at -20°C). Human Thrombin-α was purchased from Sigma (cat. no. T-6759) and solubilised with water to 10µM (stored at -20°C). N-Methoxysuccinyl-Ala-Ala-Pro-Val-p-nitroanilide was purchased from Sigma (cat. no. M-4765, batch 77H5800, stored as solid at -20°C). Methoxysuccinyl-Arg-Pro-Tyr-p-nitroanilide was purchased from Quadratech (cat. no. S-2586, batch X3088) solubilised with water to 1.78mM (stored 4°C). N-p-Tosyl-Gly-Pro-Lys-p-nitroanilide was purchased from Sigma (cat. no. T-6140) and solubilised with water to 10mM (stored at 4°C).

Human Neutrophil Elastase Assay Protocol: *0 Minute Pre-incubation.* A serial dilution of compound (see below) was incubated for fifteen minutes at 30°C (to reach assay temperature) with 50mM Tris/HCl (pH 8.6); 150mM NaCl; with MeO-succ-Ala-Ala-Pro-Val-pNA (600µM, 2.5% DMSO). After pre-incubation, the reaction was started with human neutrophil elastase (HNE, 12.18nM, in water) and evolution of product (p-nitroanilide) was read spectrophotometrically at 405nm for 20 minutes at 30°C. Blank (0 minutes) and end point (15 minutes) data were used to calculate IC_{50}. *40 Minute Pre-incubation.* A serial dilution of compound (see below) was incubated with 50mM Tris/HCl (pH 8.6); 150mM NaCl; HNE (12.18nM, in water) at 30°C for 40 minutes. After pre-incubation, the reaction was started with MeO-succ-Ala-Ala-Pro-Val-pNA (600µM, 2.5% DMSO) and evolution of product (p-nitroanilide) was read spectrophotometrically at 405nm for 20 minutes at 30°C. Blank (0 minutes) and end point (15 minutes) data were used to calculate IC_{50}.

Dilutions and concentrations are shown below: (**23** concentration was a 1:2 dilution series of 100µM top concentration in 10% DMSO with 15min preincubation).

Compound	**Assay Concentration Range (µM, 10% DMSO)**	
	0 minute Pre-incubation	**40 minute Pre-incubation**
1	500 – 0.976	500 – 0.976
22	100 – 0.195	10 – 0.0195
2	1 – 0.00195	1 – 0.00195
24	100 – 0.195	10 – 0.0195
25	100 – 0.195	10 – 0.0195
L-694,458	1 – 0.00195	1 – 0.00195

Human Pancreatic Chymotrypsin Assay Protocol. A serial dilution of compound (1:2 dilution series of 100µM top concentration in 10% DMSO) was pre-incubated with 50mM Tris/HCl (pH 8.4), 150mM NaCl, 25mM $CaCl_2$ and human pancreatic chymotrypsin (0.1µg/ml) at 30°C for 15 minutes. The reaction was started with MeOsucc-Arg-Pro-Tyr-p-nitroanilide (0.178mM, in water) and evolution of product

(p-nitroanilide) was read spectrophotometrically at 405nm for 20 minutes at 30°C. Blank (0 minutes) and end point (15 minutes) data were used to calculate IC_{50}.

Human Thrombin Assay Protocol. A serial dilution of compound (1:2 dilution series of 100μM top concentration in 1% DMSO) was pre-incubated with 50mM Hepes (pH7.4); 150mM NaCl; 5mM $CaCl_2$; 0.1% Peg 8000 and human thrombin (1nM) at room temperature for 15 minutes. The reaction was started with *N*-p-Tosyl-Gly-Pro-Lys-p-nitroanilide (100μM, in water) and evolution of product (p-nitroanilide) was read spectrophotometrically at 405nm for 10 minutes. Blank (0 minutes) and end point (10 minutes) data were used to calculate IC_{50}.

Human Cathepsin G Assay Protocol. A serial dilution of compound (1:2 dilution series of 100μM top concentration in 10% DMSO) was preincubated with human cathepsin G (10nM) in 50mM Hepes, 150mM NaCl at pH 7.2 for 15min at 30°C. Remaining enzyme activity was then assessed by measuring the rate of hydrolysis of MeOSucc-Ala-Ala-Pro-Phe-p-nitroanilide (3.5mM) followed for 15min at 30°C at 405nm.

Stability of (23) to aqueous solutions at pD 0.8 and 9.3. 0.02M DCl solution and borax buffer (pD 0.8 and 9.3) were prepared using D_2O. **23** (2mg) was dissolved in 1:1 buffer:MeCN and transferred to a 0.05mm path length CaF_2 cell and the IR absorbance at ν_{max} = 1785cm^{-1} monitored at 20°C. Spectra were recorded every 5min for the first hour and then at 24h. At pD 9.3, a new band at 1575cm^{-1} (carboxylate ion) appeared as the 1785cm^{-1} band disappeared.

Human plasma and whole blood stabilities of 2 and 25. Each compound was incubated at 37°C in freshly collected human plasma and blood at an initial concentration of 50μM, and disappearance of parent monitored by HPLC.