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Electrophilic Sulfenylation in a Stereocontrolled Synthesis of Protected (*2R,3R*)-3-Mercaptoaspartic Acid from L-Aspartic Acid

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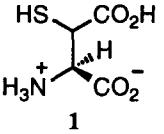
Abstract: *A novel electrophilic sulfenylating agent, (2,4-dimethoxybenzylthio)-4-methylphenyl sulfonate (13) was developed to give thiols bearing an acid labile protecting group. This was subsequently used to prepare in a stereocontrolled manner, a protected form of (2R,3R)-3-mercaptoaspartic acid which was incorporated into a tripeptide.* Copyright © 1996 Published by Elsevier Science Ltd

We have previously reported the behaviour of many modified versions of the Arnstein tripeptide δ-(L-aminoadipoyl)-L-cysteinyl-D-valine towards the penicillin forming enzyme isopenicillin N synthase. As a part of our continuing interest in this field, we required the incorporation of the unnatural amino acid, 3-mercaptoaspartic acid (MAsp) **1** (Figure 1) as a modified L-cysteinyl residue in such a tripeptide. We have recently described in a preliminary report[1], a stereocontrolled synthesis of this amino acid in a protected form, the optimisation and full details of which we describe here along with the use of the amino acid in a synthesis of the required tripeptide.

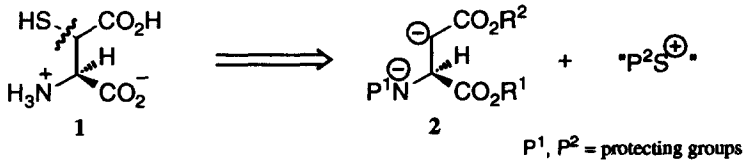


1

Figure 1

Prior to our work, **1** had been prepared in racemic form as its disulfide from 3-hydroxyaspartic acid[2] however for our biosynthetic studies, we required material of known absolute stereochemistry at the α-centre.

Amongst the well known methods for modification of proteinogenic amino acids, our group has previously demonstrated stereoselective alkylation at the β-position of aspartic acid *via* dianion **2**[3] which suggested to us, a possible synthesis of **1** by electrophilic sulfenylation of **2** (Figure 2).



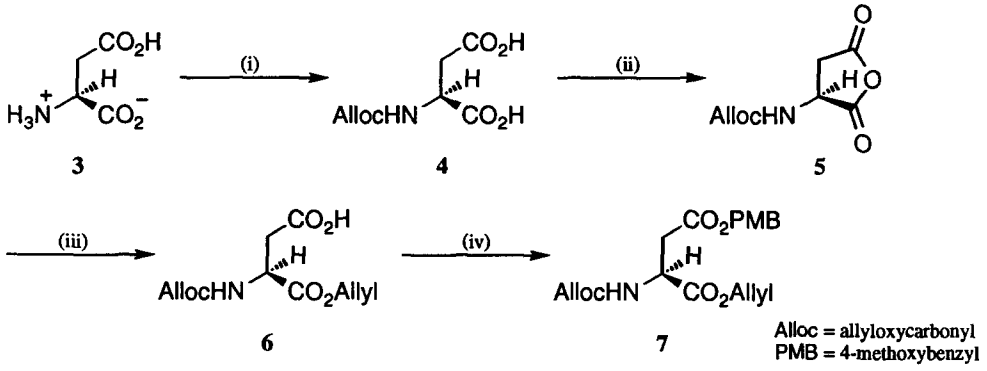
1 **2**

P^1, P^2 = protecting groups

Figure 2

A method for electrophilic sulfenylation of enolates had been reported using methyl methanethiosulfonate as the sulfenylating agent[4] but this gave rise to methyl sulfides which could not be readily cleaved to the free thiol. We therefore wished to develop an electrophilic sulfenylating agent to produce protected thiols which could be readily revealed by acidolytic cleavage.

We firstly required a suitably protected aspartic acid derivative **7** which was prepared by standard methodologies. L-Aspartic acid **3** was *N*-protected with allyl chloroformate[5] under Schotten-Baumann conditions. This gave **4** in 71% yield (based on allyl chloroformate) which was quantitatively cyclised to the corresponding *N*-protected anhydride **5** by treatment with acetic anhydride. The anhydride was opened regioselectively to the mono-α-ester **6**[6] with allyl alcohol, the unwanted β-ester being removed by pH controlled 2-phase extraction followed by silica gel chromatography. A 4-methoxybenzyl ester was introduced to the β-carboxyl group by coupling of **6** with 4-methoxybenzyl alcohol under standard diimide mediated conditions[7] (Scheme 1). This gave the necessary orthogonal carboxyl protection in diester **7**.

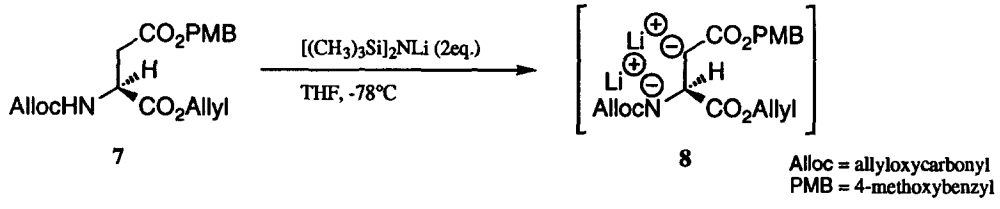


3 **4** **5** **6** **7**

Scheme 1

Reagents and conditions: (i) allyl chloroformate (0.9eq.), Na_2CO_3, (2.7eq.), H_2O, 0°C to RT. (79% from allyl chloroformate); (ii) Ac_2O (2.7eq.), THF, 50-60°C (100%); (iii) allyl alcohol, RT. (52%); (iv) 4-methoxybenzyl alcohol (1.1eq.), DCCI (1eq.), DMAP (0.05eq.), CH_2Cl_2, RT. (87%).

Dianion **8** was formed from **7** at -78°C in THF using 2 equivalents of lithium bis(trimethylsilylamide) (Scheme 2).



7 **8**

Scheme 2

A variety of electrophilic sulfenylating agents were used to trap **8** and other dianions **2** with different amino and carboxyl protecting groups. Quenching of dianion **2** (P = phenoxyacetyl, R^1 = allyl, R^2 = benzyl) with thiosulfonate **9** (prepared in 23% yield from dibenzyldisulfide by hydrogen peroxide oxidation) gave

protected 3-mercaptoaspartic acid derivative **10** (Figure 3) with high diastereoselectivity[8] in good yield (77%). Using standard reductive cleavage conditions (Na / NH_3(l)), we experienced problems with removal of the *S*-benzyl group. Turning to the acid labile 4-methoxybenzyl group[9] necessitated the use of thiosulfonate **11** which was prepared by treatment of 4-methoxybenzyl chloride with potassium 4-methylbenzenethiosulfonate. An improved yield of 96% was obtained in the sulfenylation of **2** (P = phenoxyacetyl, R^1 = allyl, R^2 = benzyl) to give **12** (Figure 3) which was again formed with high diastereoselectivity[8]. Problems were however experienced with acidolytic cleavage (hydrogen fluoride / pyridine or trifluoroacetic acid) of the *S*-(4-methoxybenzyl) protecting group from derived peptides, complex product mixtures being obtained.

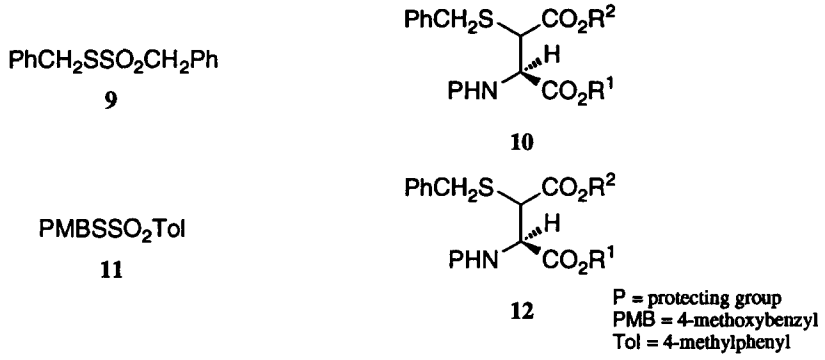
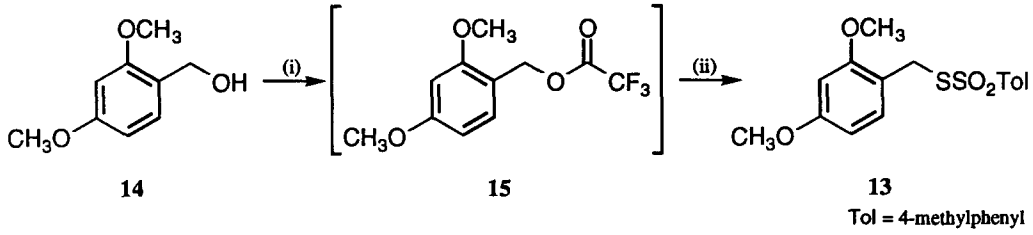


Figure 3

Reasoning that an increase in the number of mesomerically electron donating groups attached to the "benzyl" type sulfur protecting group would increase its acid lability, we turned to crystalline thiosulfonate **13**, derived from 2,4-dimethoxybenzyl alcohol **14** *via* its trifluoroacetate ester **15** (Scheme 3).

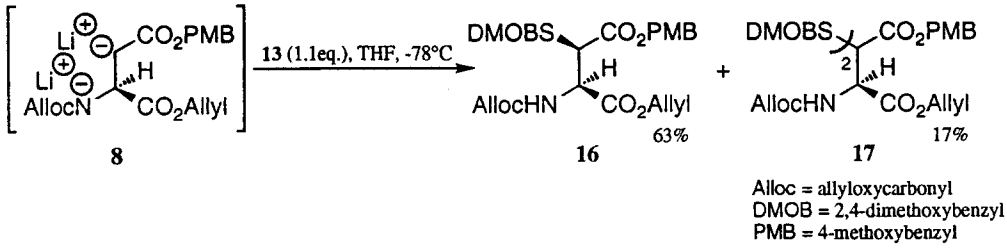


Scheme 3

Reagents and conditions: (i) $(CF_3CO)_2O$ (1eq.), Et_3N (1.15eq.), CH_2Cl_2, 0°C; (ii) K^+·SSO_2Tol (1eq.), $(CH_3)_2CO$, 0°C to RT. (34% over 2 steps).

Dianion **8** was quenched with thiosulfonate **13** to give two readily separable products, monosulfenylated material **16** and disulfenylated product **17** in 63% and 17% isolated yields respectively (Scheme 4). **16** was shown to be a single diastereoisomer with the configuration of the newly generated stereogenic centre being proved by x-ray crystallographic analysis[10] (Figure 4).

To explain the formation of **16** as the sole monosulfenylated product, we have suggested an essentially planar dianion **8** (Figure 5) with preferential attack of the sulfenylating agent on the least hindered face as shown[1].



Scheme 4

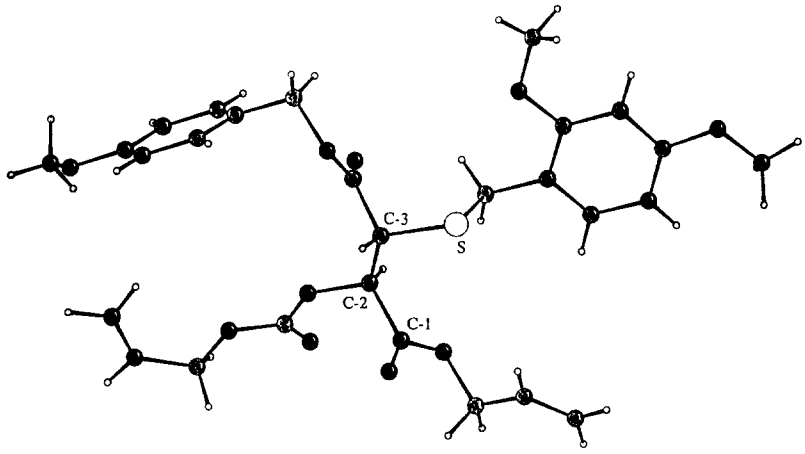


Figure 4

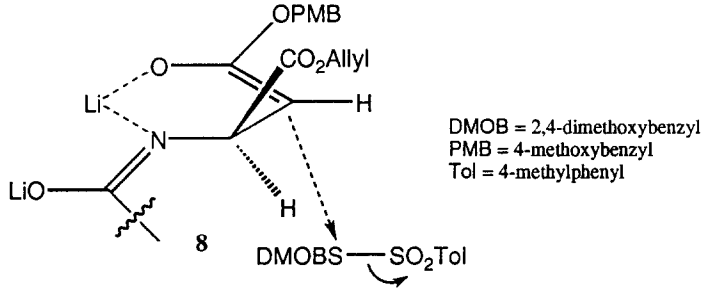
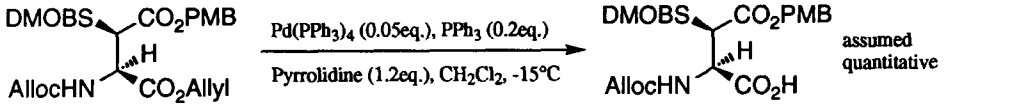


Figure 5

Selective cleavage of the allyl ester in **16** could be achieved despite the Alloc *N*-protecting group normally being cleaved under identical conditions[11]. **16** was treated with 5mol% palladium (0) / triphenylphosphine in the presence of pyrrolidine at -15°C, the free acid **18** being used in subsequent steps without further purification / characterisation (Scheme 5).

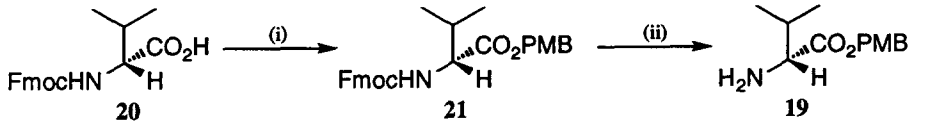
The free acid **18** was coupled in high efficiency (90%) to D-valine-(4-methoxybenzyl) ester **19** (prepared from *N*-(9-fluorenylmethoxycarbonyl)-D-valine **20** *via* diprotected derivative **21** (Scheme 6)) using standard DCCI / HOBT mediated coupling conditions (Scheme 7) giving protected dipeptide **22**.



16 **18**

Alloc = allyloxycarbonyl
DMOB = 2,4-dimethoxybenzyl
PMB = 4-methoxybenzyl

Scheme 5

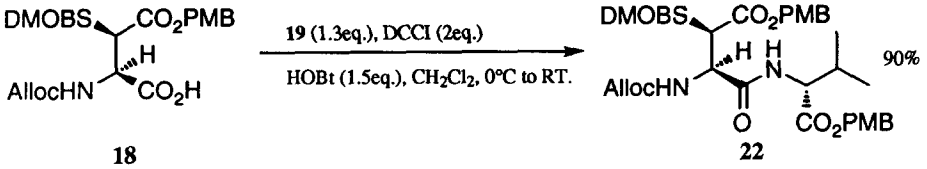


20 **21** **19**

Fmoc = 9-fluorenylmethoxycarbonyl
PMB = 4-methoxybenzyl

Scheme 6

Reagents and conditions: (i) 4-methoxybenzyl alcohol (1eq.), EDCI (1.1eq.), pyridine (1eq.), CH_2Cl_2, 0°C (44%); (ii) Et_2NH / CH_2Cl_2 (1:1v/v) (excess), 0°C (assumed quantitative).

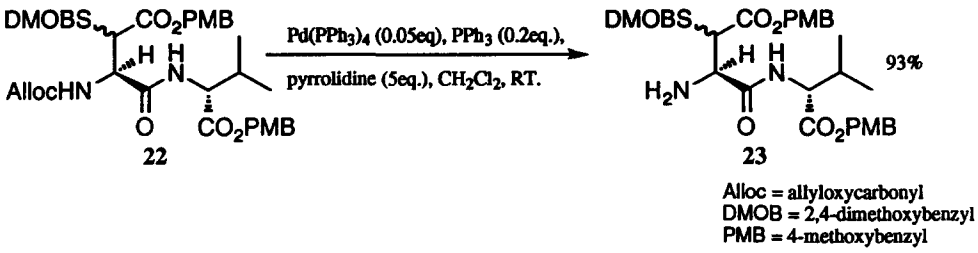


18 **22**

Alloc = allyloxycarbonyl
DMOB = 2,4-dimethoxybenzyl
PMB = 4-methoxybenzyl

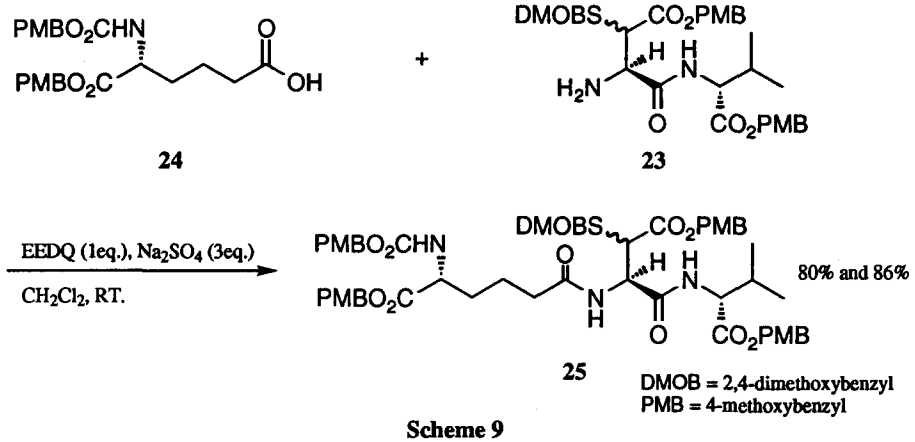
Scheme 7

Attempts to remove the *N*-Alloc protecting group from **22** unfortunately resulted in total loss of stereochemical integrity at the 3-position of the MAsp residue in free amine **23** (Scheme 8). (This suggested significant acidity of the proton at this position which is also consistent with isolation of disulphenylated **17** in the earlier sulfenylation step (Scheme 4)). A 1:1 mixture of diastereoisomers of **23** was obtained in high (93%) yield. For our intended biosynthetic experiments, we in fact needed both diastereoisomers of **23** and fortunately, they were readily separable by silica gel chromatography.



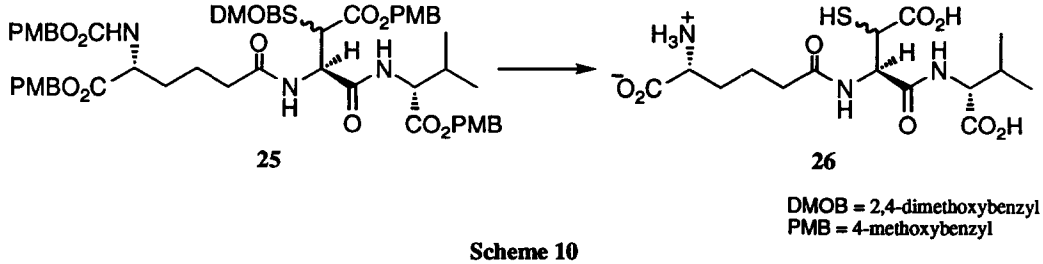
Scheme 8

An EEDQ mediated coupling reaction was then used to introduce a diprotected δ-(L-α-aminoadipoyl) residue *via* acid **24**[12] for both diastereoisomers of **23** giving fully protected tripeptides **25** in good (80% and 86%) yields (Scheme 9).



Scheme 9

Global deprotection to the required diastereoisomeric tripeptides was then achieved using trifluoroacetic acid / anisole / mercury (II) trifluoroacetate[13], the free thiols **26** being liberated by treatment of the resulting mercury (II) salt with hydrogen sulfide (Scheme 10).



Scheme 10

Reagents and conditions: (i) CF_3CO_2H / anisole (5:1v/v, excess), $Hg(O_2CCF_3)_2$ (1.5eq.), 0°C; (ii) $H_2S(g)$, H_2O, RT. (45-81%).

We were not able to determine the stereochemistry at C-3 in the two tripeptides **26**, neither tripeptide being converted by isopenicillin N synthase[14] to a β-lactam containing product showing antibiotic activity towards *S. aureus* in a "hole plate" assay.

In summary, we have demonstrated a stereocontrolled synthesis of a protected form of 3-mercaptoaspartic acid **16** *via* electrophilic sulfenylation of a β-aspartyl enolate. This involved the development of a novel sulfenylating agent (2,4-dimethoxybenzylthio)-4-methylphenyl sulfonate **13** which gives rise to protected thiols from anions. The free thiol can be later revealed by mild acidolysis.

Acknowledgements

The authors wish to thank the Japan Society for the Promotion of Sciences for Research Abroad for funding to N. S., Glaxo-Wellcome for a fellowship to M. E. W., James Bartleet for carrying out x-ray crystallographic work and the EPSRC mass spectrometry service (Swansea) for high resolution mass spectra.

Experimental

Melting points were obtained using a Büchi 510 capillary apparatus and are uncorrected.

Specific optical rotations were determined with a Perkin-Elmer 241 automatic polarimeter with a cell of path length 1dm. Concentrations are given in g/100ml.

Infrared spectra were recorded using a Perkin-Elmer 1750 Fourier transform spectrometer with major absorbances only being quoted.

^{1}H NMR spectra were recorded at 200, 300 and 500MHz using Varian Gemini 200, Brüker AC200, Brüker WH300, Brüker AM500 and Brüker AMX500 instruments. For ^{1}H spectra recorded in $CDCl_3$ or D_2O, chemical shifs are quoted in parts per million and are referenced to the residual solvent peak. The following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Coupling constants are quoted to the nearest 0.5Hz.

Low resolution mass spectra were recorded on V.G. Micromass ZAB 1F (FAB / CI / DCI), V.G. Masslab 20-250 (CI / DCI) and V.G. Bio-Q (Electrospray) instruments as appropriate with only molecular ions, fragments from molecular ions and other major peaks being reported.

Flash chromatography was carried out using Sorbsil™ C60 (40-63mm, 230-40 mesh) silica gel as stationary phase. Thin layer chromatography was carried out on aluminium and glass backed plates pre-coated with Merck silica gel 60 F_{254} which were visualised by quenching of u.v. fluorescence or by staining with iodine vapour or 10% w/v ammonium molybdate in 2M sulfuric acid (followed by heat) as appropriate.

All solvents and reagents were purified by standard techniques reported in Perrin, D. D.; Armarego, W. L. F., *Purification of Laboratory Chemicals*, 3rd edition, Pergamon Press, Oxford, **1988** or used as supplied from commercial sources as appropriate. 40-60 Petroleum ether (40-60 PE) and 60-80 Petroleum ether (60-80 PE) refer to the fractions of light petroleum ether boiling between 40-60°C and 60-80°C respectively. Solvents were removed under reduced pressure using a Büchi R110 Rotavapor fitted with a water or dry ice condenser as necessary.

***N*-Allyloxycarbonyl-L-aspartic acid (4)**

Allyl chloroformate (10.9ml, 103mmol) was added slowly to a stirred solution of L-aspartic acid (**3**) (15.0g, 113mmol) and sodium carbonate (31.8g, 300mmol) in water (400ml) at 0°C. The mixture was stirred for 12h during which time, it was allowed to slowly attain room temperature. The reaction mixture was washed with ethyl acetate (2 x 500ml) and the separated aqueous phase was acidified to pH 1 by addition of concentrated hydrochloric acid. The resulting suspension was extracted with several portions of ethyl acetate (1000ml), the combined extracts being dried (Na_2SO_4), filtered and evaporated *in vacuo* to give *N*-allyloxycarbonyl-L-aspartic acid (**4**) as a colourless solid (17.5g, 79% from allyl chloroformate); m.p. 137-138°C; $[\alpha]_D^{21}$ -49.8 (c 1.005, H_2O); υ_{max}/cm^{-1} (KBr disc) 3316, 3200-2500, 1708, 1546; δ_H (200MHz; $CDCl_3$: CD_3OD, 9:1v/v) 2.76 (1H, dd, *J* 17.5, 4.5Hz, CH_2CO_2H), 2.96 (1H, dd, 17.5, 4.5Hz, CH_2CO_2H), 4.40-4.60 (5H, complex, CH_2=CHCH_2, 2 x CO_2H, NCHCO$_2$H), 5.13-5.31 (2H, complex, CH_2=CHCH_2), 5.85 (1H, m, CH=CH_2); *m/z* (DCI, NH_3) 235 ($[M+NH_4]^+$), 218 (MH^+); (Found MH^+ 218.0665, $C_8H_{12}NO_6$ requires 218.0665).

***N*-Allyloxycarbonyl-L-aspartic anhydride (5)**

N-Allyloxycarbonyl-L-aspartic acid (**4**) (17.0g, 78.3mmol) was dissolved in tetrahydrofuran (80ml) by heating under reflux. The resulting solution was cooled to 50-60°C and acetic anhydride (20ml, 212mmol) was added with stirring at this temperature being continued for 5h. The cooled reaction mixture was evaporated *in vacuo* to give *N*-allyloxycarbonyl-L-aspartic anhydride (**5**) as a pale yellow oil (17.0g, assumed quantitative) which was used without further purification; υ_{max}/cm^{-1} ($CHCl_3$) 3691, 3455, 2393, 2361, 1873, 1795, 1719, 1650, 1603; δ_H (200MHz; $CDCl_3$) 3.04 (1H, dd, *J* 18, 7Hz, CH_2CO_2), 3.30 (1H, dd, *J* 18, 10Hz, CH_2CO_2), 4.50-4.70 (3H, complex, CH_2=CHCH_2, NCHCO$_2$), 5.18-5.37 (2H, complex, CH_2=CHCH_2), 5.88 (1H, m, CH=CH_2), 6.29 (1H, br d, *J* 7Hz, NH); *m/z* (Probe CI, NH_3) 200 (MH^+); (Found MH^+ 200.0559, $C_8H_{10}NO_5$ requires 200.0559).

***N*-Allyloxycarbonyl-L-aspartic acid-α-allyl ester (6)**

N-Allyloxycarbonyl-L-aspartic anhydride (**5**) (17.0g, 85mmol) was dissolved in allyl alcohol (50ml, 250mmol) and the resulting solution was stirred at room temperature for 3 days. The excess allyl alcohol was evaporated *in vacuo* and the residue was dissolved in ethyl acetate (200ml). The resulting solution was extracted with aqueous sodium bicarbonate solution (0.3M, 3 x 150ml) and finally with saturated aqueous sodium bicarbonate solution (200ml). The first and second extracts were contaminated with some β-ester and were discarded. The remaining two fractions were combined, acidified to pH 2 with saturated aqueous sodium bisulfate solution and extracted with ethyl acetate (2 x 500ml). The combined extracts were dried (Na_2SO_4), filtered and evaporated *in vacuo* and the residue was purified by flash chromatography on silica gel (eluting with a gradient from 99:1v/v dichloromethane : methanol to 95:5v/v dichloromethane : methanol) to give *N*-allyloxycarbonyl-L-aspartic acid-α-allyl ester (**6**) as a pale yellow oil (11.5g, 52%); R_f 0.4 (9:1v/v CH_2Cl_2 : CH_3OH); $[\alpha]_D^{26}$ +18.3 (c 1.48, $CHCl_3$); υ_{max}/cm^{-1} ($CHCl_3$) 3690, 3600, 3500, 3440, 3400-2800, 1718, 1650; δ_H (200MHz; $CDCl_3$) 2.91 (1H, dd, *J* 17.5, 4.5Hz, CH_2CO_2H), 3.09 (1H, dd, *J* 17.5, 4.5Hz, CH_2CO_2H), 4.58-4.69 (5H, complex, 2 x CH_2CH=CH_2, NCHCO$_2$), 5.21-5.34 (4H, complex, 2 x CH_2=CH), 5.81 (1H, d, *J*

8.5Hz, NH), 5.85-5.95 (2H, complex, 2 x CH=CH_2), 7.80-8.40 (1H, br s, CO_2H); *m/z* (Probe CI, NH_3) 275 ($[M+NH_4]^+$), 258 (MH^+); (Found MH^+ 258.0978, $C_{11}H_{16}NO_6$ requires 258.0978).

***N*-Allyloxycarbonyl-L-aspartic acid-α-allyl ester-β-(4-methoxybenzyl) ester (7)**

To a stirred solution of *N*-allyloxycarbonyl-L-aspartic acid-α-allyl ester (**6**) (2.80g, 10.9mmol) and 4-methoxybenzyl alcohol (1.50g, 12mmol) in dichloromethane (40ml) at 0°C was added dicyclohexylcarbodiimide (2.25g, 10.9mmol) and 4-*N*,*N*-dimethylaminopyridine (66.5mg, 0.54mmol). The resulting mixture was allowed to slowly attain room temperature and stirring was continued for 48h. Ethyl acetate (100ml) was added and the resulting precipitate was removed by filtration, the filtrate then being evaporated *in vacuo*. The residue was purified by flash chromatography on silica gel (eluting with dichloromethane) to give *N*-allyloxycarbonyl-L-aspartic acid-α-allyl ester-β-(4-methoxybenzyl) ester (**7**) as a pale yellow oil (3.58g, 87%); R_f 0.6 (3:2v/v 60-80 PE : EtOAc); $[\alpha]_D^{25}$ +14.8 (c 1.585, $CHCl_3$); υ_{max}/cm^{-1} ($CHCl_3$) 3691, 3600, 3434, 1728, 1650, 1614; δ_H (200MHz; $CDCl_3$) 2.87 (1H, dd, *J* 17, 4.5Hz, CH_2CO_2Ar), 3.07 (1H, dd, *J* 17, 4.5Hz, CH_2CO_2Ar), 3.82 (3H, s, CH_3O), 4.55-4.70 (5H, complex, 2 x $CH_2CH{=}CH_2$, $NCHCO_2$), 5.07 (2H, s, CO_2CH_2Ar), 5.17-5.38 (4H, complex, 2 x $CH_2{=}CH$), 5.70-6.03 (3H, complex, 2 x $CH{=}CH_2$, NH), 6.80 (2H, m, Ar-H), 7.28 (2H, m, Ar-H); *m/z* (Probe CI, NH_3) 395 ($[M+NH_4]^+$), 378 (MH^+); (Found MH^+ 378.1553, $C_{19}H_{24}NO_7$ requires 378.1553).

(2,4-Dimethoxybenzylthio)-4-methylphenyl sulfonate (13)

To a stirred solution of 2,4-dimethoxybenzyl alcohol (**14**) (1.68g, 10mmol) and triethylamine (1.6ml, 11.5mmol) in dichloromethane (20ml) at 0°C was added dropwise, trifluoroacetic anhydride (1.4ml, 10mmol). After stirring for 5min at this temperature, a solution of potassium 4-methylbenzenethiosulfonate (2.26g, 10mmol) in acetone (20ml) was added and after stirring the mixture for 18h at room temperature, the solvents were removed *in vacuo*. The residue was dissolved in diethyl ether (25ml), the resulting solution being washed with water (2 x 25ml), dried (Na_2SO_4) and evaporated to dryness *in vacuo*. The residue was recrystallised from ethyl acetate : 40-60 petroleum ether to give (2,4-dimethoxybenzylthio)-4-methylphenyl sulfonate (**13**) as a white, crystalline solid (1.15g, 34%); m.p. 90-93°C; R_f 0.7 (3:2v/v 60-80 PE : EtOAc); (Found: C, 57.1; H, 5.7. $C_{16}H_{17}O_4S_2$ requires C, 56.8; H, 5.4%); υ_{max}/cm^{-1} (film on KBr disc) 2965, 1725, 1610, 1583, 1503, 1318, 1209, 1133; δ_H (200MHz; $CDCl_3$) 2.46 (3H, s, CH_3Ar), 3.73 (3H, s, CH_3OAr), 3.79 (3H, s, CH_3OAr), 4.22 (2H, s, CH_2Ar), 6.35 (2H, complex, Ar-H), 7.03 (1H, d, *J* 8Hz, Ar-H), 7.32 (2H, d, Ar-H), 7.80 (2H, d, *J* 8Hz, Ar-H); *m/z* (CI, NH_3) 183, 151, 91.

(2*R*,3*R*)-*N*-Allyloxycarbonyl-*S*-(2,4-dimethoxybenzyl)-3-mercaptoaspartic acid-α-allyl ester-β-(4-methoxybenzyl) ester (16) and 2*R*-*N*-Allyloxycarbonyl-*S*,*S'*-*bis*-(2,4-dimethoxybenzyl)-3-dimercaptoaspartic acid-α-allyl ester-β-(4-methoxybenzyl) ester (17)

To a stirred solution of 1,1,1,3,3,3-hexamethyldisilazane (5.78ml, 27mmol) in tetrahydrofuran (60ml) at 0°C was added a solution of *n*-butyllithium (2.3M solution in hexanes, 8.25ml, 19mmol). After stirring at this temperature for 10min, the mixture was cooled to -78°C and a solution of *N*-allyloxycarbonyl-L-aspartic

acid-α-allyl ester-β-(4-methoxybenzyl) ester (**7**) (3.58g, 9.5mmol) in tetrahydrofuran (40ml) was added dropwise. The stirred mixture was maintained at -30°C for 2h and then re-cooled to -78°C and a solution of (2,4-dimethoxybenzylthio)-4-methylphenyl sulfonate (**13**) (3.53g, 10.5mmol) in tetrahydrofuran (40ml) was added. After stirring at this temperature for 1h, the reaction mixture was poured into saturated aqueous ammonium chloride solution (50ml) and the mixture obtained was extracted with diethyl ether (2 x 300ml). The combined extracts were washed with water (200ml) and brine (200ml) and were dried (Na_2SO_4), filtered and evaporated *in vacuo*. The residue was purified by flash chromatography on silica gel (eluting with a gradient from 95:5v/v 60-80 petroleum ether : ethyl acetate to 7:3v/v 60-80 petroleum ether : ethyl acetate). Fraction 1 gave (2*R*,3*R*)-*N*-allyloxycarbonyl-*S*-(2,4-dimethoxybenzyl)-3-mercaptoaspartic acid-α-allyl ester-β-(4-methoxybenzyl) ester (**16**) as a colourless, crystalline solid (3.36g, 63%); m.p. 62-63°C; R_f 0.5 (3:2v/v 60-80 PE : EtOAc); $[\alpha]_D^{25}$ +110.5 (c 0.475, $CHCl_3$); υ_{max}/cm^{-1} ($CHCl_3$) 3691, 3606, 3430, 1729, 1650, 1614; δ_H (500MHz; $CDCl_3$) 3.77, 3.80, 3.82 (3 x 3H, 3 x s, 3 x CH_3OAr), 3.84, 3.88 (2H, ABq, J_{AB} 13Hz, CH_2S), 3.98 (1H, d, *J* 4.5Hz, $SCHCO_2$), 4.53 (2H, d, *J* 5.5Hz, $CH_2CH=CH_2$) 4.59 (2H, m, $CH_2CH=CH_2$), 4.80 (1H, dd, *J* 10, 4.5Hz, $NCHCO_2$), 5.06-5.34 (6H, complex, CO_2CH_2Ar, 2 x $CH_2=CH$), 5.75-5.95 (3H, complex, 2 x $CH=CH_2$, NH), 6.39-6.43 (2H, m, Ar-H), 6.87-6.90 (2H, m, Ar-H), 7.09 (1H, d, *J* 8Hz, Ar-H), 7.27-7.30 (2H, m, Ar-H); *m/z* (DCI, NH_3) 560 (MH^+); (Found MH^+ 560.1954, $C_{28}H_{34}NO_9S$ requires 560.1954). Fraction 2 gave 2*R*-*N*-allyloxycarbonyl-*S*,*S*'-*bis*-(2,4-dimethoxybenzyl)-3-dimercaptoaspartic acid-α-allyl ester-β-(4-methoxybenzyl) ester (**17**) as a pale yellow oil (1.21g, 17%); R_f 0.45 (3:2v/v 60-80 PE : EtOAc);$[\alpha]_D^{21}$ +6.03 (c 0.78, $CHCl_3$); υ_{max}/cm^{-1} ($CHCl_3$) 3690, 1728, 1613; δ_H (500MHz; $CDCl_3$) 3.789, 3.790, 3.793, 3.808 (15H, complex, 5 x CH_3OAr), 3.83, 4.10 (2H, ABq, J_{AB} 11.5Hz, CH_2S), 3.84, 3.95 (2H, ABq, J_{AB} 11.5Hz, CH_2S), 4.59 (4H, complex, 2 x $CH_2CH=CH_2$), 5.10, 5.18 (2H, ABq, J_{AB} 12Hz, CO_2CH_2Ar), 5.19-5.33 (5H, complex, $NCHCO_2$, 2 x $CH_2=CH$), 5.80-5.95 (2H, complex, 2 x $CH=CH_2$), 6.24 (1H, d, *J* 10Hz, NH), 6.39-6.43 (4H, m, Ar-H), 6.85-6.90 (2H, m, Ar-H), 7.09 (1H, d, *J* 8Hz, Ar-H), 7.12 (1H, d, *J* 8Hz, Ar-H), 7.33-7.47 (2H, m, Ar-H); *m/z* (FAB) 764 ($[M+Na]^+$).

***N*-(9-Fluorenylmethoxycarbonyl)-D-valine-(4-methoxybenzyl) ester (21)**

To a stirred solution of *N*-(9-fluorenylmethoxycarbonyl)-D-valine (**20**) (5.07g, 15mmol) and 4-methoxybenzyl alcohol (2.07g, 15mmol) in dichloromethane (100ml) at 0°C were added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (3.17g, 16.5mmol) and pyridine (1.22ml, 15mmol). After 1h at 0°C and a further 3h stirring at room temperature, the reaction mixture was washed with aqueous phosphate buffer (1M, pH 7, 2 x 50ml) and brine (50ml) and the separated organic phase was dried (Na_2SO_4), filtered and evaporated *in vacuo*. The crude product was recrystallised from ethyl acetate - 40-60 petroleum ether to give *N*-(9-fluorenylmethoxycarbonyl)-D-valine-(4-methoxybenzyl) ester (**21**) as white needles (3.00g, 44%); m.p. 123-124°C; R_f 0.4 (CH_2Cl_2); $[\alpha]_D^{25}$ +6.3 (c 1.00, CH_2Cl_2); (Found: C, 73.05; H, 6.6; N, 3.0. $C_{28}H_{29}NO_5$ requires C, 73.2; H, 6.4; N, 3.05%); υ_{max}/cm^{-1} (film on KBr disc) 3351, 2963, 1724, 1613, 1516; δ_H (200MHz; $CDCl_3$) 0.87 (3H, d, *J* 7Hz, CH_3CH), 0.95 (3H, d, *J* 7Hz, CH_3CH), 2.19 (1H, m, CH_3CHCH_3), 3.82 (3H, s, CH_3OAr), 4.24 (1H, t, *J* 7Hz, $CHCH_2OCONH$), 4.32-4.43 (3H, complex, CH_2OCONH, $NHCHCO_2$), 5.09 (1H, d, *J* 12Hz, $CHCO_2CH_2Ar$), 5.18 (1H, d, *J* 12Hz, $CHCO_2CH_2Ar$), 5.34 (1H, d, *J* 9Hz, NH), 6.90 (2H, d, *J* 9Hz, Ar-H), 7.28-7.81 (10H, complex, Ar-H); *m/z* (+ve FAB) 460 (MH^+), 179, 121.

(2*R*,3*R*)-*N*-Allyloxycarbonyl-*S*-(2,4-dimethoxybenzyl)-β-(4-methoxybenzyl)-3-mercaptoaspartyl-D-valine-(4-methoxybenzyl) ester (22)

To a stirred solution of (2*R*,3*R*)-*N*-allyloxycarbonyl-*S*-(2,4-dimethoxybenzyl)-3-mercaptoaspartic acid-α-allyl ester-β-(4-methoxybenzyl) ester (**16**) (1.19g, 2.13mmol) and pyrrolidine (214μl, 2.56mmol) in dichloromethane (10ml) at -15°C were added triphenylphosphine (112mg, 0.43mmol) and tetrakis(triphenylphosphine)palladium (0) (124mg, 0.11mmol). After stirring at this temperature for 30min, water (3ml) and acetonitrile (50ml) were added and the resulting mixture was extracted with 40-60 petroleum ether (3 x 250ml). The separated acetonitrile layer was evaporated *in vacuo* and the residue dissolved in dichloromethane (30ml) containing D-valine-(4-methoxybenzyl) ester (**19**) [prepared by deprotection of *N*-(9-fluorenylmethoxycarbonyl)-D-valine-(4-methoxybenzyl) ester (**21**) (1.27g, 2.77mmol) with diethylamine : dichloromethane (1:1v/v, 20ml)] at 0°C. 1-Hydroxybenzotriazole (431mg, 3.19mmol) and dicyclohexylcarbodiimide (878mg, 4.26mmol) were added and the resulting mixture was stirred for 24h during which time it was allowed to attain room temperature. The solids produced were removed by filtration and the filtrate was evaporated *in vacuo*, the residue being purified by flash chromatography on silica gel (eluting with a gradient from 85:15v/v 60-80 petroleum ether : ethyl acetate to 7:3v/v 60-80 petroleum ether : ethyl acetate) to give (2*R*,3*R*)-*N*-allyloxycarbonyl-*S*-(2,4-dimethoxybenzyl)-β-(4-methoxybenzyl)-3-mercaptoaspartyl-D-valine-(4-methoxybenzyl) ester (**22**) as a white solid (1.41g, 90%); m.p. 92-93°C; R_f 0.4 (3:2v/v 60-80 PE : EtOAc); $[\alpha]_D^{21}$ +104.2 (c 0.445, $CHCl_3$); υ_{max}/cm^{-1} ($CHCl_3$) 3690, 3600, 3413, 2380, 2361, 1728, 1679, 1613; δ_H (500MHz; $CDCl_3$) 0.82 (3H, d, *J* 7Hz, CH_3CH), 0.87 (3H, d, *J* 7Hz, CH_3CH), 2.13 (1H, m, CH_3CHCH_3), 3.776, 3.78, 3.80, 3.81 (14H total, 4 x s, 4 x CH_3OAr, CH_2S), 3.91 (1H, d, *J* 4Hz, $SCHCO_2$), 4.46 (1H, dd, *J* 8.5, 4.5Hz $NHCHCO_2$ of Val), 4.51-4.59 (2H, m, $CH_2CH=CH_2$), 4.66 (1H, dd, *J* 8.5, 4.5Hz, $AllocNHCHCO_2$), 5.03-5.32 (6H, complex, 2 x CH_2Ar, $CH_2=CH$), 5.86-5.94 (1H, m, $CH=CH_2$), 6.21 (1H, d, *J* 8.5Hz, NH), 6.37-6.43 (2H, m, Ar-H), 6.86-6.90 (4H, m, Ar-H), 7.05 (1H, d, *J* 8.5Hz, Ar-H), 7.22 (1H, d, *J* 8.5Hz, NH), 7.26-7.30 (4H, m, Ar-H); *m/z* (FAB) 761 ($[M+Na]^+$).

2*R*-*S*-(2,4-Dimethoxybenzyl)-β-(4-methoxybenzyl)-3-mercaptoaspartyl-D-valine-(4-methoxybenzyl) ester ((+)-(23)) and ((-)-(23))

To a stirred solution of (2*R*,3*R*)-*N*-allyloxycarbonyl-*S*-(2,4-dimethoxybenzyl)-β-(4-methoxybenzyl)-3-mercaptoaspartyl-D-valine-(4-methoxybenzyl) ester (**22**) (1.20g, 1.63mmol) and pyrrolidine (678μl, 8.14mmol) in dichloromethane (10ml) at room temperature was added triphenylphosphine (85.4mg, 0.33mmol) and tetrakis(triphenylphosphine)palladium (0) (94.2mg, 81.5μmol). After stirring at this temperature for 15min, the solvent was removed *in vacuo* and the residue was dissolved in acetonitrile (100ml). The resulting solution was washed with 40-60 petroleum ether (3 x 250ml) and the separated acetonitrile phase was evaporated *in vacuo* the residue being purified by flash chromatography on silica gel (eluting with a gradient from 7:3v/v 60-80 petroleum ether : ethyl acetate to 2:3v/v 60-80 petroleum ether : ethyl acetate). This gave 2*R*-*S*-(2,4-dimethoxybenzyl)-β-(4-methoxybenzyl)-3-mercaptoaspartyl-D-valine-(4-methoxybenzyl) ester (**23**) as a 1:1 mixture of diastereoisomers (overall 987mg, 93%); (+)-(**23**); white solid; m.p. 104-105°C; R_f 0.2 (3:2v/v 60-80 PE : EtOAc); $[\alpha]_D^{22}$ +144.1 (c 0.80, $CHCl_3$); υ_{max}/cm^{-1} ($CHCl_3$ 3691, 3600, 3374, 1729, 1673, 1613; δ_H (500MHz; $CDCl_3$) 0.84 (3H, d, *J* 7Hz, CH_3CH), 0.88 (3H, d, *J* 7Hz, CH_3CH), 1.76 (2H, br s, NH_2), 2.13 (1H, m, CH_3CHCH_3), 3.54 (1H, d, *J* 4.5Hz, H_2NCHCO), 3.78, 3.80,

3.81 (12H total, 3 x s, 4 x CH_3OAr), 3.84 (2H, s, CH_2S), 4.07 (1H, d, *J* 4.5Hz, $SCHCO_2$), 4.47 (1H, dd, *J* 9, 5Hz, $NHCHCO_2$ of Val), 5.03 (2H, dd, *J* 15, 12Hz, CH_2Ar), 5.15 (2H, dd, *J* 12, 5Hz, CH_2Ar), 6.38-6.42 (2H, m, Ar-H), 6.85-6.89 (4H, m, Ar-H), 7.08 (1H, d, *J* 8Hz, Ar-H), 7.26-7.31 (4H, m, Ar-H), 7.88 (1H, d, *J* 9Hz, NH); *m/z* (electrospray) 655 (MH^+); (-)-(**23**); pale yellow oil; R_f 0.25 (3:2v/v 60-80 PE : EtOAc); $[\alpha]_D^{22}$ -18.6 (c 0.695, $CHCl_3$); υ_{max}/cm^{-1} ($CHCl_3$) 3691, 3606, 3375, 1730, 1674, 1613; δ_H (500MHz; $CDCl_3$) 0.86 (3H, d, *J* 7Hz, CH_3CH), 0.90 (3H, d, *J* 7Hz, CH_3CH), 1.76 (2H, br s, NH_2), 2.18 (1H, m, CH_3CHCH_3), 3.77, 3.78, 3.79, 3.81 (14H total, 4 x s, 4 x CH_3OAr, CH_2S), 3.85 (1H, d, *J* 6Hz, H_2NCHCO), 3.93 (1H, d, *J* 6Hz, $SCHCO_2$), 4.51 (1H, dd, *J* 9, 5Hz, $NHCHCO_2$ of Val), 5.03-5.14 (4H, complex, 2 x CH_2Ar), 6.37-6.43 (2H, m, Ar-H), 6.84-6.89 (4H, m, Ar-H), 7.10 (1H, d, *J* 8Hz, Ar-H), 7.26-7.31 (4H, m, Ar-H), 7.81 (1H, d, *J* 9Hz, NH); *m/z* (electrospray) 655 (MH^+).

***N*-(α-(4-Methoxybenzyl)-*N*-(4-methoxybenzyloxycarbonyl)-δ-L-α-aminoadipoyl)-2*R*-*S*-(2,4-dimethoxybenzyl)-β-(4-methoxybenzyl)-3-mercaptoaspartyl-D-valine-(4-methoxybenzyl) ester ((+)-(25)) and ((-)-(25))**

To a stirred solution of 2*R*-*S*-(2,4-dimethoxybenzyl)-β-(4-methoxybenzyl)-3-mercaptoaspartyl-D-valine-(4-methoxybenzyl) ester ((+)-(**23**)) (300mg, 0.46mmol) and α-(4-methoxybenzyl)-*N*-(4-methoxybenzyloxycarbonyl)-L-α-aminoadipic acid (**24**) (205mg, 0.46mmol) in dichloromethane (10ml) at room temperature was added anhydrous sodium sulfate (196mg, 1.38mmol) followed by 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (119mg, 0.48mmol). After stirring at this temperature for 48h, the solvent was removed *in vacuo* and the residue was partitioned between ethyl acetate (200ml) and sarurated aqueous sodium bicarbonate (50ml). The separated organic phase was further extracted with aqueous hydrochloric acid (4%, 50ml) and brine (50ml) and was dried (Na_2SO_4), filtered and evaporated *in vacuo*. The residue was purified by flash chromatography on silica gel (eluting with a gradient from 3:2v/v 60-80 petroleum ether : ethyl acetate to 1:1v/v 60-80 petroleum ether : ethyl acetate) to give *N*-(α-(4-methoxybenzyl)-*N*-(4-methoxybenzyloxycarbonyl)-δ-L-α-aminoadipoyl)-2*R*-*S*-(2,4-dimethoxybenzyl)-β-(4-methoxybenzyl)-3-mercaptoaspartyl-D-valine-(4-methoxybenzyl) ester ((+)-(**25**)) as a white solid (429mg, 86%); m.p. 104-105°C; R_f 0.65 (2:3v/v 60-80 PE : EtOAc); $[\alpha]_D^{22}$ +50.5 (c 0.64, $CHCl_3$); υ_{max}/cm^{-1} ($CHCl_3$) 3691, 3600, 3470, 1719, 1682, 1614; δ_H (500MHz; $CDCl_3$) 0.80 (3H, d, *J* 7Hz, CH_3CH), 0.87 (3H, d, *J* 7Hz, CH_3CH), 1.57-1.71, 1.83-1.85, 2.05-2.17 (7H total, complex, $(CH_2)_3$, CH_3CHCH_3), 3.73, 3.76, 3.78, 3.79, 3.80, 3.81 (6 x 3H, 6 x s, 6 x CH_3OAr), 3.73-3.81 (3H, complex, CH_2S, $SCHCO_2$), 4.33 (1H, m, $NHCHCO_2$ of α-aminodipoyl), 4.42 (1H, dd, *J* 8.5, 4.5Hz, $NHCHCO_2$ of Val), 4.85 (1H, dd, *J* 8, 3.5Hz, NHCHCONH), 4.92-5.24 (8H, complex, 4 x CH_2Ar), 5.64 (1H, d, *J* 8.5Hz, NH), 6.36-6.40 (2H, m, Ar-H), 6.82-6.90 (8H, complex, Ar-H), 7.03 (1H, d, *J* 8.5Hz, Ar-H), 7.10 (1H, d, *J* 8Hz, NH), 7.21 (1H, d, *J* 8.5Hz, NH), 7.24-7.32 (8H, complex, Ar-H); *m/z* (FAB) 1104.7 ($[M+Na]^+$). ((-)-(**25**)) was prepared as for ((+)-(**25**)) above using 2*R*-*S*-(2,4-dimethoxybenzyl)-β-(4-methoxybenzyl)-3-mercaptoaspartyl-D-valine-(4-methoxybenzyl) ester ((-)-(**23**)) (340mg, 0.51mmol), α-(4-methoxybenzyl)-*N*-(4-methoxybenzyloxycarbonyl)-L-α-aminoadipic acid (**24**) (232mg, 0.52mmol), anhydrous sodium sulfate (222mg, 1.56mmol) and 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (135mg, 0.55mmol) in dichloromethane (5ml). Work-up followed by chromatography yielded *N*-(α-(4-methoxybenzyl)-*N*-(4-methoxybenzyloxycarbonyl)-δ-L-α-aminoadipoyl)-2*R*-*S*-(2,4-dimethoxybenzyl)-β-(4-methoxybenzyl)-3-mercaptoaspartyl-D-valine-(4-methoxybenzyl) ester ((-)-(**25**)) as a

colourless solid (449mg, 80%); m.p. 87-88°C; R_f 0.65 (2:3v/v 60-80 PE : EtOAc); $[\alpha]_D^{22}$ -53.3 (c 0.48, $CHCl_3$); υ_{max}/cm^{-1} ($CHCl_3$) 3690, 3600, 3430, 1730, 1685, 1613; δ_H (500MHz; $CDCl_3$) 0.80 (3H, d, *J* 7Hz, CH_3CH), 0.85 (3H, d, *J* 7Hz, CH_3CH), 1.59-1.71, 1.81-1.83, 2.02-2.13 (7H total, complex, $(CH_2)_3$, CH_3CHCH_3), 3.73, 3.76, 3.77, 3.78, 3.79 (20H total, 5 x s, CH_2S, 6 x CH_3OAr), 3.86 (1H, d, *J* 8.5Hz, $SCHCO_2$), 4.31 (1H, m, $NHCHCO_2$ of α-aminoadipoyl), 4.38 (1H, dd, *J* 8.5, 4.5Hz, $NHCHCO_2$ of Val), 4.84 (1H, dd, *J* 8.5, 8Hz, NHCHCONH), 4.93-5.11 (8H, complex, 4 x CH_2Ar), 5.63 (1H, d, *J* 8Hz, NH), 6.07 (1H, d, *J* 7Hz, NH), 6.38-6.42 (2H, m, Ar-H), 6.82-6.89 (8H, complex, Ar-H), 7.08 (1H, d, *J* 8Hz, Ar-H), 7.18-8.33 (9H, complex, NH, Ar-H); *m/z* (FAB) 1104.8 ($[M+Na]^+$).

L-(δ-Aminoadipoyl)-2*R*-3-mercaptoaspartyl-D-valine ((+)-(26)) and ((-)-(26))

To a stirred solution of *N*-(α-(4-methoxybenzyl)-*N*-(4-methoxybenzyloxycarbonyl)-δ-L-α-aminoadipoyl)-2*R*-*S*-(2,4-dimethoxybenzyl)-β-(4-methoxybenzyl)-3-mercaptoaspartyl-D-valine-(4-methoxybenzyl) ester ((+)-(**25**)) (40mg, 37μmol) in a mixture of trifluoroacetic acid (500μl) and anisole (100μl) at 0°C was added mercury (II) trifluoroacetate (23.7mg, 56μmol) and stirring was continued at this temperature for 1h. The trifluoroacetic acid was removed *in vacuo* below room temperature and the residue was triturated with ethyl acetate (4 x 20ml). The final suspension was centrifuged and the solid product was suspended in water (10ml) and hydrogen sulfide gas was passed through the mixture for 15min. After centrifugation, the resulting solution was filtered through a Celite® pad and freeze-dried to give L-(δ-aminoadipoyl)-2*R*-3-mercaptoaspartyl-D-valine ((+)-(**26**)) as a grey solid (15.6mg, 81% as a CF_3CO_2H salt); $[\alpha]_D^{21}$ +4.33 (c 0.30, H_2O); δ_H (500MHz; D_2O) 0.99 (3H, d, *J* 7Hz, CH_3CH), 1.01 (3H, d, *J* 7Hz, CH_3CH), 1.67-1.80, 1.85-1.96, 2.20-2.27, 2.37-2.45 (7H, total, complex, CH_3CHCH_3, $(CH_2)_3$), 3.81 (1H, d, *J* 8.5Hz, NHCHCONH), 3.87 (1H, t, *J* 6.5Hz, H_2NCHCO_2H), 4.29 (1H, d, *J* 6Hz, $CONHCHCO_2H$), 4.92 (1H, d, *J* 8.5Hz, $SCHCO_2H$); *m/z* (electrospray) 408 (MH^+). L-(δ-Aminoadipoyl)-2*R*-mercaptoaspartyl-D-valine ((-)-(**26**)) was prepared as for ((+)-(**26**)) above *N*-(α-(4-methoxybenzyl)-*N*-(4-methoxybenzyloxycarbonyl)-δ-L-α-aminoadipoyl)-2*R*-*S*-(2,4-dimethoxybenzyl)-β-(4-methoxybenzyl)-3-mercaptoaspartyl-D-valine-(4-methoxybenzyl) ester ((-)-(**25**)) (40mg, 37μmol), trifluoroacetic acid (500μl), anisole (100μl) and mercury (II) trifluoroacetate (23.7mg, 56μmol). Work-up gave L-(δ-aminoadipoyl)-2*R*-3-mercaptoaspartyl-D-valine ((-)-(**26**)) as a grey solid (8.6mg, 45% as a CF_3CO_2H salt); $[\alpha]_D^{21}$ -51.6 (c 0.275, H_2O); δ_H (500MHz; D_2O) 0.99 (3H, d, *J* 7Hz, CH_3CH), 1.02 (3H, d, *J* 7Hz, CH_3CH), 1.75-1.87, 1.94-2.04, 2.23-2.29, 2.46-2.55 (7H total, complex, CH_3CHCH_3, $(CH_2)_3$), 3.91 (1H, t, *J* 6.5Hz, H_2NCHCO_2H), 4.02 (1H, d, *J* 8Hz, HNCHCONH), 4.31 (1H, d, *J* 6Hz, $CONHCHCO_2H$), 4.97 (1H, d, $SCHCO_2H$); *m/z* (electrospray) 408 (MH^+).

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