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The Synthesis of Aryl Substituted Analogues of Phenoxyacetyl-L-cysteinyl-D-valine and Phenylacetyl-L-cysteinyl-D-valine. Application to the Photoaffinity Labeling of Isopenicillin N Synthetase.

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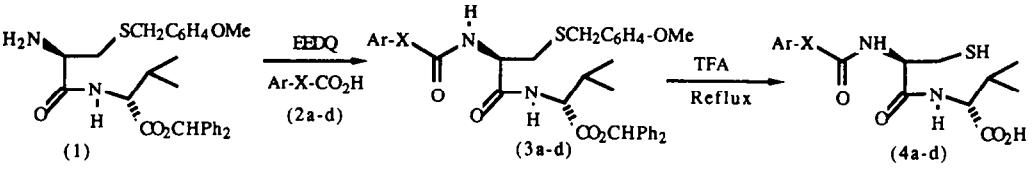
Abstract

The acetylaryldipeptides (4a) - (4b) have been synthesised in high yield, and found to be converted to the corresponding penicillins (5a) -(5d) by Isopenicillin N Synthetase (IPNS). Of these, the m-substituted analogue (4c) was converted most efficiently. The synthesis of a potential photoaffinity label for IPNS which incorporates this molecular framework, 2-[3-(3-trifluoromethyl-3H-diazirin-3-yl)phenoxy]acetyl-S-carbomethoxysulfenyl-L-cysteinyl-D-valine (21), is described.

During studies of the mechanism of penicillin biosynthesis[1], it has been shown that phenoxyacetyl-L-cysteinyl-D-valine and phenylacetyl-L-cysteinyl-D-valine were converted by IPNS to Penicillin V and G in 0.04 and 0.12% conversion yield respectively[2]. The Michaelis constant (K_m) and maximum velocity (V_{max}) parameters determined for these substrate conversions, indicated that the K_m values were comparable to that of the natural substrate L,L,D-ACV, while the V_{max} values were considerably smaller, implying that the substrates were binding to the enzyme active site, but that the catalytic events leading to the formation of the β-lactam products were retarded. Recent work in these laboratories[3] has demonstrated that m-carboxyphenylacetyl-L-cysteinyl-D-valine is converted by IPNS to a bioactive[4] penicillin in a significantly higher yield. We were interested in gaining more information concerning the effect of substituents of the aromatic ring on bioconversion efficiencies, and undertook the synthesis of m- and p-acetyl analogues of both phenoxyacetyl- and phenylacetylcysteinylvaline.

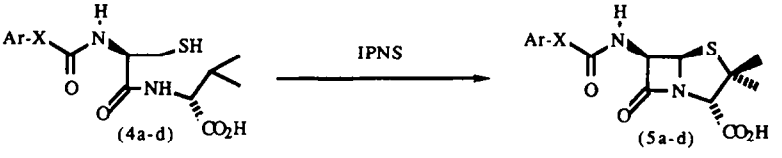
Scheme 1



	-X-	Ar
(2a), (3a), (4a)	$-CH_2-$	m-$CH_3COC_6H_4$
(2b), (3b), (4b)	$-CH_2-$	p-$CH_3COC_6H_4$
(2c), (3c), (4c)	$-OCH_2-$	m-$CH_3COC_6H_4$
(2d), (3d), (4d)	$-OCH_2-$	p-$CH_3COC_6H_4$

The acetyldipeptides 4(a) - (d) (Scheme 1) were readily obtained by EEDQ coupling of the protected dipeptide (1)[5] with the appropriately substituted phenylacetic acid or phenoxyacetic acid (2(a) - (d)), followed by trifluoroacetic acid (TFA) deprotection. Incubations of 4(a) - (d) with IPNS[6] indicated that bioactive[5] products were formed (Scheme 2). Assuming similar bioactivities to Penicillin V, the m-acetyl analogues (4a) and (4c) were converted in approximately 5.5 times greater yield (0.11 and 0.17%) than the p-acetyldipeptides (4b) and (4d) (0.02 and 0.03% respectively), and the conversion yields of both Penicillin V analogues (5c) and (5d) were 1.5 times greater than those of the Penicillin G analogues (5a) and (5b). Thus, an aromatic side chain bearing a two-carbon non-acidic substituent could be converted to the corresponding penicillin by IPNS, but without the rate enhancement seen with a m-carboxyl group. The greatest conversion was obtained with dipeptide (4c) having the m-acetylphenoxyacetyl side chain.

Scheme 2



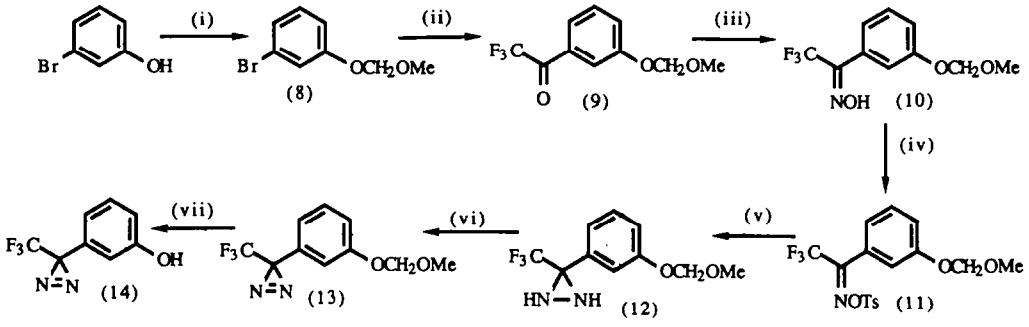
Dipeptide	-X-	Ar	Penicillin	Conversion Yield (%)
(4a)	$-CH_2-$	m-$CH_3COC_6H_4$	(5a)	0.11
(4b)	$-CH_2-$	p-$CH_3COC_6H_4$	(5b)	0.02
(4c)	$-OCH_2-$	m-$CH_3COC_6H_4$	(5c)	0.17
(4d)	$-OCH_2-$	p-$CH_3COC_6H_4$	(5d)	0.03

Although the low conversions of these arylcysteinylvaline analogues limited any potential synthetic applications, the small V_{max} parameters determined for the conversions yielding Pen V and G indicated that these analogues could be useful in enzyme inhibition studies. An appropriately modified substrate, which contains a functional group capable of forming a covalent bond with a residue at the enzyme active site, would be useful for the identification of the region of IPNS in contact with the side chain, and, consequently, give some understanding to those factors affecting side-chain binding and specificity. Although simple alkylating agents have been used to identify active site residues (e.g. of chymotrypsin[7]), the application of such chemical affinity labels to studies of IPNS was precluded by the existence of free sulfhydryl groups in the substrate. However, photoaffinity labeling, in which a chemically reactive species is generated at the enzyme active site by irradiation of a suitable substrate, was a viable option.

Photoaffinity labeling has been used to probe interactions between biological ligands and their receptors[8], and in particular, it has found extensive application to the identification of the active site of enzymes, and to those regions of enzymes required for substrate binding[9]. Diazirines[10] are finding increasing application as photoaffinity labels because of their synthetic accessibility, and chemical and thermal inertness. Trifluoromethyldiazirines[9c,11] have been found to be particularly useful, since photolysis by near-UV radiation is efficient, leading to reactive carbenes, without the possibility of internal rearrangements of hydrogens β- to the carbene centre. We therefore undertook the synthesis of 2-[3-(3-trifluoromethyl-3H-diazirin-3-yl)phenoxyacetyl]-L-cysteinyl-D-valine (6) to evaluate its potential as a photoaffinity label for IPNS.

The synthesis of the dipeptide (6) is outlined in Schemes 3 and 4. We chose as the key step a phenolic displacement on the iodoacetyldipeptide (7) because this allowed an independent synthesis of the diazirine-bearing aromatic side chain, and also to allow the ready incorporation of a ^{14}C label (from iodo-^{14}C-acetic acid) which will ultimately be required in any photoaffinity labeling experiments. Although several methods of preparation of aryl diazirines have been reported in the literature[9], we used a modification of the methods of Brunner[11a], Nassal[11c] and Bayley[11e]. Thus, trifluoroketone (9) was readily obtained in 70% yield by treatment of bromoacetal (8) with n-butyllithium, followed by methyl trifluoroacetate[12]. Formation of oxime (10), tosyl oxime (11), diaziridine (12) and diazirine (13) proceeded as described[11e], and deprotection[13] gave 3-(3-hydroxyphenyl)-3-trifluoromethyl-3H-diazirine (14) in 10% overall yield from m-bromophenol.

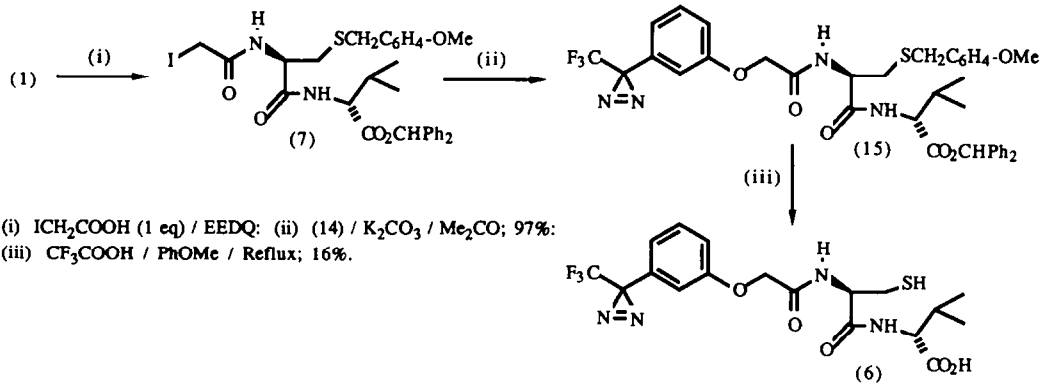
Scheme 3



(i) $ClCH_2OMe$ / NaH / DMF; 88%: (ii) (a) BuLi, (b) CF_3COOMe; 70%: (iii) NH_2OH / C_5H_5N; 99%: (iv) TsCl / C_5H_5N; 85%: (v) NH_3 /Et_2O; 73%: (vi) Ag_2O: (vii) 3M aq. HCl / AcOH; 54%.

Treatment of iodoacetyldipeptide (7) and phenol (14) with potassium carbonate/acetone gave dipeptide (15), which could be deprotected in only low yield (16%) with refluxing TFA/anisole. Although suitable for the acetyl compounds (3a) - (3d), this method of deprotection proved to be troublesome with (15) due to the limited stability of the diazirine functionality under the reaction conditions, and we began a search for a milder, higher-yielding method which was compatible with the diazirine group.

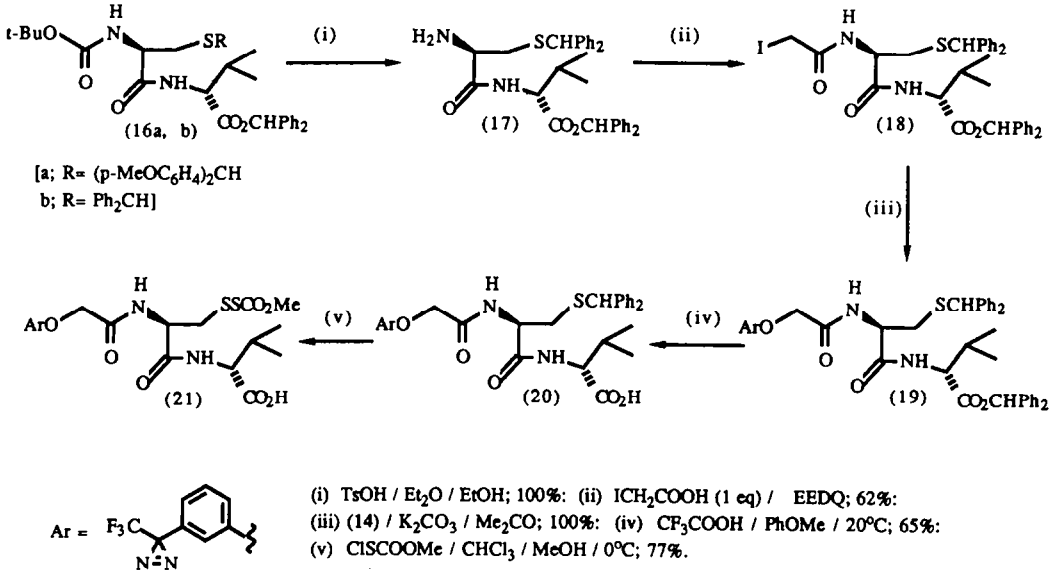
Scheme 4



(i) ICH_2COOH (1 eq) / EEDQ: (ii) (14) / K_2CO_3 / Me_2CO; 97%: (iii) CF_3COOH / PhOMe / Reflux; 16%.

Initial attempts concentrated on a more acid-labile S-protecting group, since refluxing TFA is required to remove the S-p-methoxybenzyl group[14]. The S-bis(p-methoxyphenyl)methyldipeptide (16a) (Scheme 5) was found to be too acid sensitive to be useful. The S-benzhydryl compound (16b) was sufficiently stable to allow conversion to dipeptide (19) using the same methods already described. Treatment of (19) with cold TFA/anisole gave complete benzhydryl ester cleavage, but no S-deprotection. We were able to effect S-deprotection in the following manner. Certain thioethers have been reported[15] to be converted to the S-sulfenylthiocarbonate derivatives in good yield on treatment with methoxycarbonylsulfenylchloride in chloroform/-methanol at room temperature, and in separate experiments we found that the trifluoromethyl-diazirine group was stable to this reagent. Reaction of dipeptide (20) under similar conditions gave the desired disulfide (21), which could be readily purified by chromatography in 77% yield. Under the reductive conditions of the enzyme incubation (dithiothreitol present), S-sulfenyl-thiocarbonates react to give the free thiol[15], so that the conversion of thioether (20) to disulfide (21) is equivalent to a deprotection.

Scheme 5



Incubation of this dipeptide (21) with IPNS[6] gave a bioactive[4], β-lactamase sensitive product in 0.26% yield. We are currently investigating the applicability of dipeptide (21) to the photoaffinity labeling of IPNS, and will report the details of this work in due course.

EXPERIMENTAL

Melting points were determined with a Buchi 510 capillary apparatus and are uncorrected. Optical rotations were recorded on a Perkin-Elmer 241 Polarimeter. I.R. spectra were obtained in $CHCl_3$ solution, or as Nujol mulls, and were recorded on a Perkin-Elmer 681 spectrophotometer; broad (br), weak (w), medium (m), and strong (s) bands are reported. U.V. spectra were recorded on a Perkin-Elmer 555 UV-Vis spectrophotometer, and solution solvents are stated in parenthesis. ^1H n.m.r. spectra were recorded on Bruker WH 300 MHz, AM 250 MHz or Varian 60 MHz NMR spectrometers using tetramethylsilane as internal standard. ^{13}C n.m.r. spectra were recorded at 62.85 MHz on a Bruker AM 250 spectrometer using $CDCl_3$ = 77.0 p.p.m. as internal reference. Only selected ^{13}C n.m.r. signals are assigned. Multiplicities are recorded as b (broad peak), s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Mass spectra were recorded on VG Analytical Ltd. ZAB1F or MM30F Mass spectrometers [for Ammonia Desorption Chemical Ionisation (NH_3 DCI), positive argon Fast Atom Bombardment (FAB), Electron Impact (EI) or Field Desorption (FD)]. Microanalyses were performed by Mrs. V. Lamburn, Dyson Perrins Laboratory, University of Oxford. All starting materials, reagents and solvents were purified and dried unless otherwise stated.

m-Acetylphenylacetic acid (2a) was obtained by the method of Papa et.al.[16] as a white solid from water, m.p. 101° (lit.[16] 106°). δ($CDCl_3$/$(CD_3)_2SO$) 2.55 (3H, s, CH_3), 3.62 (2H, s, CH_2), 7.35 -7.48 and 7.79 - 7.83 (4H, m, ArH).

p-Acetylphenylacetic acid (2b) was obtained by the method of Papa et.al.[16] as a white solid from benzene, m.p. 116-7° (lit.[16] 119°). δ($CDCl_3$/$(CD_3)_2SO$) 2.51 (3H, s, CH_3), 3.58 (2H, s, CH_2), 7.32 (2H, AA'BB' system, $J_{AB}+J_{AB'}$ =8.2 Hz, ArH m- to $COCH_3$), 7.83 (2H, AA'BB' system, $J_{AB}+J_{AB'}$ = 8.2 Hz, ArH o-to $COCH_3$).

m-Acetylphenoxyacetic acid (2c) was obtained by the method of Hayes and Branch[17] as a white solid from benzene/light petroleum, m.p. 115-6° (lit.[18] 117°). δ($CDCl_3$/$(CD_3)_2SO$) 2.40 (3H, s, CH_3), 4.45 (2H, s, OCH_2), 6.95 (1H, m, ArH), 7.15 - 7.43 (3H, m, ArH).

p-Acetylphenoxyacetic acid (2d) was purchased from Lancaster Synthesis Ltd.

General Method for the Synthesis of Dipeptides (3a) - (3d). The peptides were obtained by stirring the appropriate acid ((2a) - (2d)) (1 equivalent) and S-(p-methoxybenzyl)-L-cysteinyl-D-valine benzhydryl ester[5] (1) (1 equivalent) in dichloromethane with EEDQ (1 equivalent) at room temperature overnight. The solvent was removed, the residue dissolved in ethyl acetate, washed with saturated sodium bicarbonate solution, 10% citric acid solution, and dried over sodium sulfate. The solvent was removed, and the product purified by flash chromatography[19] (10% ethyl acetate/dichloromethane).

The following compounds were prepared according to the above general method:

N-(m-Acetylphenyl)acetyl-L-(S-p-methoxybenzylcysteinyl)-D-valine benzhydryl ester (3a) was obtained in 93% yield as a colourless foam, m.p. 55-7°. $C_{39}H_{42}N_2O_6S$ requires C, 70.2; H, 6.35; N, 4.20%. Found: C, 70.2; H, 6.5; N, 4.0%. $[\alpha]_D^{\delta}$° = -5.6° (c 0.7, $CHCl_3$). R_f = 0.3 (10% EtOAc/CH_2Cl_2). δ($CDCl_3$) 0.74 (3H, d, J = 6.9 Hz, Me), 0.86 (3H, d, J = 6.9 Hz, Me), 2.20 - 2.24 (1H, m, $CH\,Me_2$), 2.57 (3H, s, $COCH_3$), 2.63 - 2.86 (2H, m, AB of ABX), 3.58 (2H, s, CH_2CO), 3.69 (2H, s, SCH_2Ph), 3.76 (3H, s, OMe), 4.55 - 4.66 (2H, m, α Cys and α Val), 6.50 (1H, d, J = 7.5 Hz, CONH), 6.77 -6.83 (3H, m, AA'BB' system, J_{AB} + $J_{AB'}$ = 8.5 Hz, ArH o- to OMe, and CONH), 6.88 (1H, s, Ph_2CH), 7.22 (2H, AA'BB' system, J_{AB} + $J_{AB'}$ = 8.5 Hz, ArH m- to OMe), 7.27 - 7.52 (12H, m, ArH), 7.85 (2H, m, ArH o- to $COCH_3$). ^{13}C nmr δ($CDCl_3$) 17.2 (q, Me), 19.0 (q, Me), 26.6 (q, COMe), 31.1 (d, βC Val), 33.4 (t, βC Cys), 35.8 (t, SCH_2Ph), 43.0 (t, CH_2CO), 52.4 (d, αC), 55.2 (q, OCH_3), 57.3 (d, αC), 78.0 (d, Ph_2CH), 114.0 (d, ArC), 126.9, 127.2, 127.3, 128.0, 128.1, 128.5, 128.8, 129.0, 129.1, 129.5, 129.7, 130.0, 133.9, 135.0, 137.6, 139.3, 139.6 (ArC), 158.7 (s, 4°C of ArOMe), 170.0 and 170.4 (3 x s, 2 x CONH and C(O)O), 197.7 (s, CH_3CO). ν($CHCl_3$) 3400m, 3010m, 1740m, 1680s, 1610m, 1510s, 1490m, 1250s. m/z (FD) 666 (M^+).

N-(p-Acetylphenyl)acetyl-L-(S-p-methoxybenzylcysteinyl)-D-valine benzhydryl ester (3b) was obtained in 62% yield as a colourless foam, m.p. 120-2°C. $C_{39}H_{42}N_2O_6S$ requires C, 70.2; H, 6.35; N, 4.2%. Found: C, 70.4; H, 6.3; N, 4.2%. $[\alpha]_D^{\delta}$° = -4.4° (c 1.6, $CHCl_3$). R_f = 0.3 (10% EtOAc/CH_2Cl_2). δ($CDCl_3$) 0.74 (3H, d, J = 6.8 Hz, Me), 0.86 (3H, d, J = 6.8 Hz, Me), 2.20 - 2.23 (1H, m, Me_2CH), 2.57 (3H, s, CH_3CO), 2.62 - 2.86 (2H, m, AB of ABX), 3.56 (2H, s, CH_2CO), 3.70 (2H, s, SCH_2Ph), 3.76 (3H, s, OCH_3), 4.54 - 4.66 (2H, m, α Cys and α Val), 6.48 (1H, d, J = 7Hz, CONH), 6.75 - 6.85 (3H, AA'BB' system, J_{AB} + $J_{AB'}$ = 8.5 Hz, ArH o- to OMe, and CONH), 6.88 (1H, s, Ph_2CH), 7.18 - 7.40 (14H, m, ArH), 7.88 (2H, AA'BB' system, J_{AB} + $J_{AB'}$ = 7.5 Hz, ArH o- to $COCH_3$). ^{13}C nmr ($CDCl_3$) 17.2 (q, Me), 19.0 (q, Me), 26.5 (q, COMe), 31.1 (d, βC Val), 33.5 (t, βC Cys), 35.8 (t, SCH_2Ph), 43.1 (t, CH_2CO), 52.3 (d, αC), 55.2 (q, OCH_3), 57.3 (d, αC), 78.0 (d, Ph_2CH), 114.0 (d, ArC), 126.9, 127.3, 128.0, 128.2, 128.5, 128.8, 129.5, 129.7, 130.0, 136.1, 139.3, 139.5, 139.8 (ArC), 158.8 (s, 4° ArC), 170.0 and 170.4 (3 x s, 2 x CONH and C(O)O), 197.5 (s, $COCH_3$). ν($CHCl_3$) 3400w, 3000m, 2960m, 1740m, 1680s, 1610m, 1570s, 1490s, 1360m, 1300m, 1270s, 1250s, 1180 cm^{-1}. m/z (FAB) 667 (M^++1, <1%), 499(1), 168(20), 167(100), 121(65).

N-(m-Acetylphenoxy)acetyl-L-(S-p-methoxybenzylcysteinyl)-D-valine benzhydryl ester (3c) was obtained as a colourless oil in 27% yield. $C_{39}H_{42}N_2O_7S$ requires C, 68.6, H, 6.2; N, 4.1%. Found: C, 68.9; H, 6.2; N, 4.7%. $[\alpha]_D^{\delta}$° = +13.3° (c 0.8, $CHCl_3$). R_f = 0.3 (10% EtOAc/CH_2Cl_2). δ($CDCl_3$) 0.80 (3H, d, J = 6.8 Hz, Me), 0.91 (3H, d, J = 6.8 Hz, Me), 2.26 -2.28 (1H, m, $CH\,Me_2$),

2.59 (3H, s, $COCH_3$), 2.69 - 2.92 (2H, m, AB of ABX), 3.76 (5H, s, OMe and SCH_2Ph), 4.47 (1H, d, J = 14.8 Hz, OCH_α), 4.56 (1H, d, J = 14.8 Hz, OCH_β), 4.61 - 4.71 (2H, m, α Cys and α Val), 6.80 (1H, d, CONH), 6.83 (2H, AA' BB' system, $J_{AB} + J_{AB'}$ = 7.5 Hz, ArH o-to OMe), 6.90 (1H, s, Ph_2CH), 7.10 - 7.63 (17H, m, ArH and CONH). ^{13}C nmr ($CDCl_3$) 17.3 (q, Me), 19.1 (q, Me), 26.6 (q, COMe), 31.2 (d, βC Val), 33.4 (t, βC Cys), 35.9 (t, SCH_2Ph), 52.0 (d, αC), 55.2 (q, OCH_3), 57.4 (d, αC), 67.3 (t, OCH_2CO), 78.0 (d, Ph_2CH), 113.9, 114.1, 119.8, 122.3, 126.9, 127.3, 128.0, 128.1, 128.5, 129.7, 129.9, 130.1 (ArC), 138.8, 139.3 and 139.5 (4° ArC), 157.3 and 158.8 (4° ArC) 167.8, 169.7 and 170.5 (3 x s, 2 x CONH and C(O)O), 197.3 (s, COMe). ν($CHCl_3$) 3400m, 3000m, 1735m, 1680s, 1605m, 1585m, 1510s, 1435m, 1360m, 1270s cm^{-1}. m/z (FD) 683 (M^+ + 1).

N-(p-Acetylphenoxy)acetyl-L-(S-p-methoxybenzylcysteinyl)-D-valine benzhydryl ester (3d) was obtained as a colourless oil in 73% yield. $C_{43}H_{42}N_2O_7S$ requires C, 68.6; H, 6.2; N, 4.1%. Found: C, 68.9; H, 6.2; N, 4.3%. $[\alpha]_D^{\delta}$ = + 16.6° (c 1.2, $CHCl_3$). R_f = 0.3 (10% EtOAc/CH_2Cl_2). δ($CDCl_3$) 0.79 (3H, d, J = 6.8 Hz, Me), 0.91 (3H, d, J = 6.8 Hz, Me), 2.24 (1H, m, Me_2CH), 2.57 (3H, s, CH_3CO), 2.66 - 2.91 (2H, m, AB of ABX), 3.77 (5H, s, OMe and SCH_2Ph), 4.51 (1H, d, J = 14.8 Hz, OCH_α), 4.55 (1H, d, J = 14.8 Hz OCH_β), 4.60 - 4.64 (1H, m, α Val), 4.69 (1H, m, αCys), 6.72 (1H, d, CONH), 6.83 (2H, AA'BB' system, $J_{AB} + J_{AB'}$ = 7.5 Hz, ArH o- to OMe), 6.89 (1H, s, Ph_2CH), 6.96 (2H, AA'BB' system, $J_{AB} + J_{AB'}$ = 8.0 Hz, ArH o- to OCH_2), 7.23 -8.23 (13H, m, ArH and CONH); 7.93 (2H, AA'BB' system, $J_{AB} + J_{AB'}$ = 8.0 Hz, ArH o- to COMe). ^{13}C nmr δ($CDCl_3$) 17.2 (q, Me), 19.0 (q, Me), 26.2 (q, CH_3CO), 31.2 (d, βC Val), 33.5 (t, βC Cys), 35.8 (t, SCH_2Ph), 51.9 (d, αC), 55.1 (q, OCH_3), 57.4 (d, αC), 67.0 (t, OCH_2), 78.0 (d, Ph_2CH), 114.0 (d, ArC), 114.4 (d, ArC), 120.9, 126.4, 126.8, 127.3, 127.7, 128.0, 128.1, 128.4, 129.4, 129.6, 130.0, 130.6, 131.5, 135.9 (ArC), 139.3 and 139.5 (s, 4° ArC), 150.3 (s, ArC), 158.7 (s, 4° ArC), 160.6 (s, 4° ArC), 167.5, 169.6 and 170.4 (3 x s, 2 x CONH and C(O)O), 196.4 (s, CH_3CO). ν($CHCl_3$) 3400w, 3000w, 1735m, 1675s, 1600s, 1510s, 1250s, 1175s, 1145w, 1030w cm^{-1}. m/z (NH_3 DCI) 684(M^++2, 1%), 683(M^++1,2), 547(3), 473(4), 321(10), 167(65), 154(10), 137(100), 121(25).

General Method For The Deprotection of Compounds (3a) - (3d)

The protected peptides (2a), (2b), (2c) and (2d) (35 - 50 mg) were dissolved in redistilled TFA (2 - 5 ml) and anisole (40 mg), and gently refluxed for 15 min. under an argon atmosphere. The solution was cooled, and the solvent removed, and any remaining TFA azeotroped out using toluene. The product was dissolved in ethyl acetate, extracted into a saturated aqueous solution of sodium bicarbonate, which was acidified with hydrochloric acid (1M), and extracted with ethyl acetate. The solution was dried over sodium sulfate, and the solvent removed. The crude material was purified by flash chromatography (10% MeOH/$CHCl_3$), and used directly in incubation experiments with IPNS[6].

The following compounds were prepared by the above general method:

(m-Acetylphenyl)acetyl-L-cysteinyl-D-valine (4a) was obtained in 88% yield as a colourless solid, m.p. 165°, $[\alpha]_D^{\delta}$ = -30.9° (c 0.2, MeOH). δ($CDCl_3$/CD_3OD) 0.81 (3H, d, J = 6.9 Hz, Me), 0.87 (3H, d, J = 6.9 Hz, Me), 1.49 (1H, t, J = 10 Hz, SH), 2.08 - 2.15 (1H, m, $CHMe_2$), 2.53 (3H, s, $COCH_3$), 2.76 - 2.81 (2H, m, AB of ABX), 3.59 (2H, s, CH_2CO), 4.35 (1H, m, αH), 4.60 (1H, t, J = 5.8 Hz, αH), 7.34 - 7.39 (1H, m, ArH), 7.45 - 7.48 (1H, m, ArH), 7.76 - 7.82 (2H, m, ArH). ν_{max} (Nujol) 1735m, 1680m, 1650s, 1610s, 1600s cm^{-1}. m/z (FAB) 381 (M^++1, 8%), 133(85), 118(35), 117(18), 115(16), 91(35), 76(55), 72(100), 57(20), 55(40), 44(30), 43(100), 41(50), 39(55), 31(25).

(p-Acetylphenyl)acetyl-L-cysteinyl-D-valine (4b) was obtained in 96% yield as a colourless solid, $[\alpha]_D^{\delta}$ = -35.8° (c 0.4, MeOH). δ($CDCl_3$/CD_3OD) 0.73 (3H, d, J = 6.9 Hz, Me), 0.79 (3H, d, J = 6.9 Hz, Me), 2.00 - 2.07 (1H, m, Me_2CH), 2.45 (3H, s, $COCH_3$), 2.69 (2H, d, J = 6.1 Hz, AB of ABX), 3.51 (2H, s, OCH_2), 4.24 (1H, m, αH), 4.46 (1H, t, J = 6.0 Hz, αH), 7.26 (2H, AA'BB' system, $J_{AB} + J_{AB'}$ = 8.3 Hz, ArH m- to $COCH_3$), 7.77 (2H, AA'BB' system, $J_{AB} + J_{AB'}$ = 8.3 Hz, ArH o- to $COCH_3$). ν_{max} (Nujol) 3300m, 1730s, 1680s, 1650s, 1610s, 1530m, 1260m, 1200w cm^{-1}. m/z (FAB) 381 (M^+ + 1), 380(M^+).

(m-Acetylphenoxy)acetyl-L-cysteinyl-D-valine (4c) was obtained in 77% yield as a colourless solid, $[\alpha]_D^{\delta}$ = -5.03° (c 1.0, MeOH). δ($CDCl_3$/CD_3OD) 1.07 (3H, d, J = 6.8 Hz, Me), 1.09 (3H, d, J = 6.8 Hz, Me), 1.67 (1H, t, J = 9.0 Hz, SH), 2.22 - 2.28 (1H, m, $CHMe_2$), 2.66 (3H, s, $COCH_3$), 2.73 - 2.97 (2H, m, AB of ABX), 3.30 (1H, d, J = 14.5 Hz, OCH_α), 4.15 (1H, d, J = 14.5 Hz, OCH_β), 4.47 - 4.52 (1H, m, αH), 4.71 - 4.78 (1H, m, αH), 6.74 (1H, dd, CONH), 7.39 (1H, dd, ArH), 7.59 (1H, d, J = 7.4 Hz, ArH), 7.75 (1H, m, ArH), 7.90 (1H, d, J = 8.1 Hz, ArH), 8.47 (1H, d, J = 8.2 Hz, CONH). ν_{max} (Nujol) 3300w, 1730m, 1690s, 1660s, 1645s, 1540s, 1290m, 1270m, 1210w cm^{-1}. m/z (FD) 397(M^++1, 4%), 396 (M,2), 252(15), 149(18), 121(25), 118(22), 91(25), 88(20), 77(20), 76(20), 72(90), 57(22), 56(25), 44(20), 43(100), 42(25), 41(70), 39(90), 31(45).

(p-Acetylphenoxy)acetyl-L-cysteinyl-D-valine (4d) was obtained in 93% yield as a colourless solid, $[\alpha]_D^{\delta}$ = -4.4° (c - 0.8, MeOH). δ($CDCl_3$/CD_3OD) 0.97 (3H, d, J = 6.9 Hz, Me), 1.00 (3H, d, J = 6.8 Hz, Me), 1.63 (1H, t, J = 8.0 Hz, SH), 2.22 - 2.28 (1H, m, $CHMe_2$), 2.55 (3H, s, $COCH_3$), 2.86 - 3.02 (2H, m, AB of ABX), 4.52 - 4.63 (3H, m, αH and OCH_2), 4.90 - 4.97 (1H, m, αH), 6.95 (2H, AA'BB' system, $J_{AB} + J_{AB'}$ = 8.9, ArH o- to OCH_2), 7.36 (1H, d, J = 8.5 Hz, CONH), 7.71 (1H, d, J = 8.2 Hz, CONH), 7.93 (2H, AA'BB' system, $J_{AB} + J_{AB'}$ = 8.9 Hz, ArH o- to $COCH_3$). ν_{max} (Nujol) 1730m, 1650s, 1600s cm^{-1}.

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1-Bromo-3-methoxymethoxybenzene (8).

A solution of m-bromophenol (10 g, 0.058 mol) in dry dimethylformamide (40 ml) was treated with sodium hydride (50% oil dispersion, 3.1 g, 1.1 eq.) at 20°C. The solution was stirred for 2 h, and chloromethylmethyl ether (5.2 g, 0.064 mol) was added dropwise, and the solution stirred overnight. The mixture was carefully diluted with water, and extracted with ether. The ether layer was washed with water, dried over sodium sulfate, and the solvent removed, to give a yellow liquid. The crude material was distilled (Kugelrohr) to give (8) as a colourless liquid (11 g, 88 %), b.p. 100 - 120°/1 mmHg (lit[20] b.p. 125-30°/7 mmHg). δ(CDCl$_3$) 3.4 (3H, s, OCH$_3$), 5.1 (2H, s, OCH$_2$O), 6.8 - 7.4 (4H, m, ArH). m/z (EI) 218 (M$^+$+2, 10%), 216 (M$^+$,8), 44 (100).

1-(3-Methoxymethoxyphenyl)-2,2,2-trifluoroethanone (9).

To a solution of protected bromophenol (8) (2 g, 9.3 mmol) in THF (30 ml) at -78°C was added BuLi (5 ml, 1.1 equiv.) and the solution stirred for 1 h. A solution of methyl trifluoroacetate (1.3 g, 10.2 mmol) in THF (10 ml) was added at -78°C and the mixture stirred for 1 h.[12] A solution of HCl (10M, 2 ml) in methanol (5 ml) at -78°C was added, and the mixture warmed to 0°C, diluted with ether, and washed with water. The solution was dried over sodium sulfate, and the solvent removed. The crude material was distilled (Kugelrohr) to give (9) as a colourless liquid (1.5 g, 70%), b.p. 90 - 110°/1 mmHg. $C_{10}H_9F_3O_3$ requires C, 51.3; H, 3.87%. Found: C, 51.5; H, 4.14%. δ(CDCl$_3$) 3.5 (3H, s, OMe), 5.2 (2H, s, OCH$_2$O), 7.3 - 7.9 (4H, m, ArH). ^{13}C nmr δ(CDCl$_3$) 56.2 (q, OMe), 94.5 (t, OCH$_2$O), 114.3 (s, CF$_3$), 116.3 (s, ArC), 117.3 (d, ArC), 118.9 (s, ArC), 123.6 (d, ArC), 123.7 (d, ArC), 130.2 (d, ArC), 157.7 (s, CO). ν(CHCl$_3$) 1720s, 1600m, 1580m, 1490m, 1450m, 1440m, 1340w, 1240s, 1200s, 1160s, 1080m, 1020m, 980s, 970s cm^{-1}. m/z (EI) 234 (M$^+$, 7%), 45 (100).

1-(3-Methoxymethoxyphenyl)-2,2,2-trifluoroethanone oxime (10).

Ketone (9) (2 g, 8.5 mmol) and hydroxylamine hydrochloride (0.7 g, 10.1 mmol) were refluxed in a solution of pyridine (20 ml) and absolute ethanol (10 ml) for 4 h. The solvent was removed in vacuo, the residue dissolved in ether, and the solution washed with water, and dried with sodium sulfate. The solvent was removed to give the crude material, which was purified by flash chromatography (5% EtOAc/CH$_2$Cl$_2$). The product (10) was obtained as a colourless liquid (2.1 g, 99%). $C_{10}H_{10}F_3NO_3$ requires C, 48.2; H, 4.1; N, 5.6%. Found: C, 48.3; H, 4.3; N, 5.9%. δ(CDCl$_3$) 3.5 (3H, s, OMe), 5.2 (2H, s, OCH$_2$O), 7.2 (4H, s, ArH), 9.2 and 9.4 (1H, syn- and anti-OH). ^{13}C nmr δ(CDCl$_3$) 56.1 (q, OCH$_3$), 94.5 (t, OCH$_2$O), 116.4 (s), 116.8 (s), 118.3 (d), 118.4 (d), 122.0 (d), 127.2 (s), 129.7 (d), 131.3 (s), 157.1 (s, C=O). ν(CHCl$_3$), 3560s, 3300b, 1600m, 1580s, 1490s, 1380s, 1240s, 1150s, 1080s, 1010s, 990s, 970s. m/z (EI) 249 (M$^+$, 8%), 45(100).

O-(p-Toluenesulfonyl)-1-(3-methoxymethoxyphenyl)-2,2,2-trifluoroethanone oxime (11)

Oxime (10) (2.1g, 8.4 mmol) in pyridine (30 ml) was treated with p-toluenesulfonyl chloride (2.4 g, 12.6 mmol) and the mixture refluxed for 2 h. The solvent was removed in vacuo, the residue dissolved in ether, washed with water, dried over sodium sulfate, and the solvent removed. The crude material was purified by flash chromatography (25% CH$_2$Cl$_2$/light petroleum then 50% CH$_2$Cl$_2$/light petroleum) to give (11) as a colourless liquid (2.9 g, 85%). $C_{17}H_{16}F_3NO_5S$ requires C, 50.6; H, 4.0; N, 3.5%. Found: C, 50.4; H, 4.1; N, 3.4%. δ(CDCl$_3$) 2.49 (3H, s, CH$_3$), 3.49 (3H, s, OCH$_3$), 5.19(2H, s, OCH$_2$O), 7.00 - 7.06 (2H, m, ArH), 7.19 - 7.23 (1H, m, ArH), 7.36 - 7.42 (3H, m, ArH), 7.90 (2H, AA'BB' system, J_{AB}+$J_{AB'}$ = 8.0 Hz, ArH o-to OSO$_2$). ^{13}C nmr δ(CDCl$_3$) 21.5 (q, OCH$_3$), 55.9 (q, Me), 94.5 (t, OCH$_2$O), 116.3 (d), 119.4 (d), 121.5 (d), 121.7 (s), 125.5 (s), 129.1 (d), 129.8 (d), 129.9 (d), 131.2 (s), 146.1 (s), 157.3 (s, C=N). ν(CHCl$_3$) 1600m, 1580m, 1480m, 1390s, 1340m, 1250s, 1190s, 1180s, 1150s, 1090m, 1050m, 1000m, 990m, 890m cm^{-1}. m/z (NH$_3$ DCI) 421 (M$^+$ + NH$_4^+$,100%), 266(15), 234(12).

3-(3-Methoxymethoxyphenyl)-3-(trifluoromethyl)diaziridine (12).

Tosyl oxime (11) (2.9 g, 7.2 mmol) was dissolved in dry ether (30 ml), cooled to -78°C, and ammonia (-20 ml) added. The mixture was stirred at -78°C for 8 h, and the ammonia allowed to evaporate overnight. The residue was diluted with ether, washed with water, dried over sodium sulfate, and the solvent removed. The crude material was purified by flash chromatography (1% EtOAc/CHCl$_3$ then 5% EtOAc/CHCl$_3$), to give a colourless liquid (12) (1.3 g, 73%). $C_{10}H_{11}F_3N_2O_2$ requires C, 48.4; H, 4.5; N, 11.3%. Found: C, 48.6; H, 4.5; N, 11.2%. δ(CDCl$_3$) 2.26 (1H, d, NH), 2.79 (1H, d, NH), 3.49 (3H, s, OMe), 5.20 (2H, s, OCH$_2$O), 7.11 -7.15 (1H, m, ArH), 7.25 - 7.37 (3H, m, ArH). ^{13}C nmr δ(CDCl$_3$) 56.0 (q, OCH$_3$), 94.5 (t, OCH$_2$O), 116.2 (d), 117.9 (d), 121.3 (s), 121.4 (d), 125.6 (s), 129.9 (d) 133.1 (s), 157.4 (s,NCN). ν(CHCl$_3$) 3280m, 1600m, 1560s, 1490m, 1450m, 1390m, 1370m, 1300m, 1175s, 1140s, 1080s, 1020s, 990m, 950m, 920m cm^{-1}. λ$_{max}$ (MeOH) 214 nm (log ε 3.68), 271 (3.01), 278 sh (2.95). m/z 248 (M$^+$, 8%), 217(8), 216(8), 45(100).

3-(3-Methoxymethoxyphenyl)-3-(trifluoromethyl)-3H-diazirine (13).

Sodium hydroxide (0.32 g, 8.0 mmol) in water (8 ml) was added dropwise to a boiling solution of silver nitrate (1.4 g, 8.2 mmol) in water (20 ml). The precipitated Ag$_2$O was filtered, washed with water, acetone, and ether, and used immediately.

Diaziridine (12) (500 mg, 2.0 mmol) was dissolved in ether (20 ml) and stirred with the freshly prepared Ag$_2$O (1.9 g, 8.2 mmol) in the dark for 3 h. The solution was filtered through Celite, and the solvent removed, to give (13) as a yellow oil. The crude material was purified

by flash chromatography (5% EtOAc/light petroleum) to give (13) as a yellow liquid. δ($CDCl_3$) 3.48 (3H, s, OCH_3), 5.19 (2H, s, OCH_2O), 6.84 - 6.87 (2H, m, ArH), 7.07 - 7.13 (1H, m, ArH), 7.27 - 7.35 (1H, m, ArH). ^{13}C nmr δ($CDCl_3$) 56.1 (q, OCH_3), 94.5 (t, OCH_2O), 114.8 (d), 117.4 (d), 119.9 (d), 119.9 (s), 124.3 (s), 130.0 (d), 130.2 (s), 157.6 (s, diazirine C). ν($CHCl_3$) 1605s, 1580s, 1490s, 1450m, 1340s, 1250s, 1200s, 1150s, 1080s, 1020s, 960s cm^{-1}. λ_{max} (cyclohexane) 220 nm (log ε3.87), 274 (2.69), 352 (2.40). m/z (EI) 246 (M^+, 2%), 218(6), 215(2), 45(100).

3-(3-Hydroxyphenyl)-3-trifluoromethyl-3H-diazirine (14).

Diazirine (13) (500 mg, 2.0 mmol) was stirred with a mixture of glacial acetic acid (8 ml) and HCl (2M, 2 ml)[13] in the dark for 3h. The solution was diluted with ether, washed with water, saturated aqueous sodium bicarbonate solution, water and dried over sodium sulfate. The crude material was purified by flash chromatography (75% CH_2Cl_2/light petroleum), to give a pale yellow liquid (14) (220 mg, 54%) that crystallised at 0°C. Upon exposure to air/light this compound rapidly turned a deeper yellow in colour. δ($CDCl_3$) 5.17 (1H, s, OH), 6.67 (1H, bs, H2), 6.73 (1H, d, J = 8.0 Hz, H4), 6.87 (1H, dd, J''' = 2.5 Hz, J' = 8.1 Hz, H6), 7.27 (1H, dd, J = 8.0 Hz, J' = 8.1 Hz, H5). ^{13}C nmr δ($CDCl_3$) 113.6 (d), 116.8 (d), 118.9 (d), 124.3 (s), 130.3 (d), 130.9 (s), 155.8 (s, diazirine C). ν($CHCl_3$) 3590m, 1610m, 1600m, 1590s, 1495w, 1450m, 1350m, 1275s, 1160s, 980m, 840m cm^{-1}. λ_{max} (cyclohexane) 220 nm (log ε 3.83), 276(3.30) 352 (2.46). m/z (EI) 202 (M^+, 5%), 175(9), 174(100), 173(23), 146(10), 145(60), 127(13), 126(9), 125(9), 105(12), 96(25), 95(7), 77(9), 75(8), 51(16), 50(8).

Iodoacetyl-L-(S-p-methoxybenzyl)cysteinyl-D-valine benzhydryl ester (7).

A mixture of iodoacetic acid (150 mg, 0.81 mmol) and S- p-methoxybenzyl-L-cysteinyl-D-valine benzhydryl ester[5] (1) (370 mg, 0.73 mmol) in dichloromethane (10 ml) was stirred overnight with EEDQ (200 mg, 0.81 mmol). The solvent was removed, the residue dissolved in ethyl acetate, washed with saturated sodium bicarbonate solution, 10% citric acid solution, and water, and dried over sodium sulfate, and the solvent removed. The crude product was purified by flash chromatography (10% EtOAc/CH_2Cl_2) to give (7) as a white solid. $C_{31}H_{33}IN_2O_5S$ requires C, 55.2; H, 5.23; N, 4.15%. Found: C, 55.2; H, 5.26; 3.95%. δ($CDCl_3$) 0.80 (3H, d, J = 6.9 Hz, Me), 0.90 (3H, d, J = 6.9 Hz, Me), 2.27 (1H, m, $CHMe_2$), 2.64 - 2.71 and 2.85 - 2.95 (2H, m, AB of ABX), 3.63 (2H, d, ICH_2), 3.75 (2H, s, SCH_2Ph), 3.78 (3H, s, OMe), 4.49 (1H, m, αH), 4.68 (1H, m, αH), 6.71 (1H, d, J = 8.8 Hz, CONH), 6.80 (1H, d, J = 6.1 Hz, CONH), 6.85 (2H, d, AA'BB' system, $J_{AB}+J_{AB'}$ = 8.7 Hz, ArH o- to OMe), 6.91 (1H, s, $CHPh_2$), 7.25 - 7.38 (12H, m, ArH). ^{13}C nmr δ($CDCl_3$) -1.54 (t, ICH_2), 17.4 (q, Me), 19.1 (q, Me), 31.2 (d, βC Val), 33.4 (t, βC Cys), 35.9 (t, SCH_2Ph), 52.9 (d, αC), 55.2 (q, OMe), 57.4 (d, αC), 78.0 (d, Ph_2CH), 114.0 (d, ArC), 126.9, 127.3, 128.0, 128.1, 128.5, 130.0, 130.1 (all ArC), 139.3 (s, ArC), 139.4 (s, ArC), 158.7 (s, ArC), 167.5, 169.8 and 170.4 (3 x s, 2 x CONH and C(O)O). ν($CHCl_3$) 3400m, 3300m, 3010s, 2960s, 1740s, 1670s, 1610m, 1510s, 1465m, 1455m, 1370m, 1300s, 1180s, 1030m. m/z (FD) 674 (M^+).

[3-(3-Trifluoromethyl-3H-diazirin-3-yl)phenoxy]acetyl-L-(S-p-methoxybenzyl)cysteinyl-D-valine benzhydryl ester (15).

Diazirinophenol (14) (90 mg, 0.45 mmol) and dipeptide (7) (300 mg, 0.45 mmol) and potassium carbonate (170 mg, 1.23 mmol) were stirred in acetone (6 ml) in the dark overnight. The solvent was removed and the crude material purified by flash chromatography (75% CH_2Cl_2/light petroleum then 10% EtOAc/CH_2Cl_2) to give (15) as a colourless oil (320 mg, 95%). $[\alpha]_D^{20}$ = + 7.7° (c 0.9, MeOH). δ($CDCl_3$) 0.81 (3H, d, J = 6.9 Hz, Me), 0.93 (3H, d, J = 6.9 Hz, Me), 2.28 (1H, m, Me_2CH), 2.70 -2.91 (2H, m, AB of ABX), 3.77 (5H, s, OMe and SCH_2Ph), 4.43 (1H, d, J = 14.7 Hz, OCH_α), 4.49 (1H, d, J = 14.7 Hz, OCH_β), 4.66 - 4.73 (2H, m, αCys and αVal), 6.66 -6.98 and 7.18 - 7.45 (21H, m, ArH and 2 x CONH and Ph_2CH). ^{13}C nmr δ($CDCl_3$) 17.3 (q, Me), 19.1 (q, Me), 31.2 (d, βC Val), 33.5 (t, βC Cys), 35.8 (t, SCH_2Ph), 51.9 (d, αC), 55.1 (q, OMe), 57.3 (d, αC), 67.1 (t, OCH_2), 78.0 (d, Ph_2CH), 113.4, 114.0, 115.0, 115.7, 119.7, 120.7, 124.1, 126.8, 127.3, 128.0, 128.1, 128.4, 129.6, 130.0, 130.3, 130.8, 139.3, 139.5 (all ArC, and CF_3), 157.2 (s), 158.7 (s), 167.6, 169.7 and 170.5 (3 x s, 2 x CONH and C(O)O). ν($CHCl_3$) 3400m, 3010m, 2960m, 1740s, 1670s, 1610s, 1585m, 1510s, 1475m, 1455m, 1440m, 1300m, 1250s, 1180s, 1160s, 1030m cm^{-1}. λ_{max} (MeOH) 276 nm (log ε3.64), 348 (2.4).

[3-(3-Trifluoromethyl-3H-diazirin-3-yl)phenoxy]acetyl-L-cysteinyl-D-valine (6).

To dipeptide (15) (100 mg, 0.13 mmol) was added anisole (50 mg) and freshly distilled TFA (5 ml), and the solution purged with argon for 15 min, and then gently refluxed for 15 min. The mixture was cooled, the solvent removed *in vacuo* and dried at high vacuum. The residue was dissolved in ethyl acetate, and extracted with saturated sodium bicarbonate. The aqueous layer was acidified, and extracted with ethyl acetate. The organic phase was dried over sodium sulfate, and the solvent removed. The crude material was purified by flash chromatography (5% MeOH/CH_2Cl_2 then 30% MeOH/CH_2Cl_2) to give (6) as a white solid (12.4 mg, 16%). δ($CDCl_3$/CD_3OD) 0.86 (3H, d, J = 6.8 Hz, Me), 0.92 (3H, d, J = 6.8 Hz, Me), 1.30 (1H, m, SH), 2.13 (1H, m, $CHMe_2$), 2.87 - 2.90 (2H, m, AB of ABX), 4.13 (d, αH), 4.50 (2H, s, OCH_2), 4.64 (1H, m, αH), 6.71 (1H, s, H2), 6.81 (1H, d, J = 8.0 Hz, H4), 6.96 (1H, dd, J''' = 2.2 Hz, J' = 8.2 Hz, H6), 7.30 (1H, dd, J = 8.0 Hz, J' = 8.2 Hz, H5).

N-(t-Butyloxycarbonyl)-S-(diphenylmethyl)-L-cysteine.

To S-(diphenylmethyl)-L-cysteine[21] (1.0 g, 3.5 mmol) in water (10 ml) and dioxan (5 ml) and aqueous sodium hydroxide (1M, 35 ml), was added with stirring and cooling di-t-butyldicarbonate (0.89 g, 4.1 mmol)[22]. The mixture was stirred overnight at room temperature, and the dioxan removed *in vacuo*. The aqueous solution was diluted with water, and extracted with ethyl acetate. The aqueous phase was acidified with 5% citric acid solution, and extracted with ethyl acetate.

The combined extracts were dried over sodium sulfate, and the solvent removed, to give a colourless oil, which was purified by conversion to the dicyclohexylamine salt[23] ($[\alpha]_D^{\beta}$ = +6.7° (c 1, $CHCl_3$), lit.[23] $[\alpha]_D^{\beta}$ = +6.4° (c 0.9, $CHCl_3$)). N-t-Butyloxycarbonyl-S-(diphenylmethyl)-L-cysteine was obtained as a colourless oil by washing the above salt in ethyl acetate solution with citric acid solution[23](1.2 g, 88%), $[\alpha]_D^{\beta}$ = -6.6° (c 1.7, MeOH) (lit.[23] $[\alpha]_D^{\beta}$ = -6.6° (c 1, EtOH)). δ($CDCl_3$) 1.50 (9H, s, 3 x Me), 2.81 - 2.94 (2H, m, CH_2S), 4.60 (1H, m, αH), 5.30 (1H, s, $CHPh_2$), 5.41 (1H, d, CONH), 6.78 (1H, bs, COOH), 7.17 - 7.37 (6H, m, ArH), 7.40 - 7.48 (4H, m, ArH). m/z (FAB) 388 (M^+ + 1, <1%), 387 (M^+, <1), 386 (<1), 332(1), 330(1), 288(1), 286(1), 210(2), 168(23), 167(100), 165(10), 57(50).

N-(t-Butyloxycarbonyl)-S-(diphenylmethyl)-L-cysteinyl-D-valine benzhydryl ester (16b).

A mixture of the above diprotected cysteine (0.35 g, 0.90 mmol) and valine benzhydryl ester (0.25 g, 0.88 mmol) and EEDQ (0.3 g, 1.2 mmol) in dichloromethane (10 ml) was stirred overnight. The solvent was removed, the residue dissolved in ethyl acetate, washed with saturated sodium bicarbonate solution, 10% citric acid solution, dried over sodium sulfate, and the solvent removed. The crude material was purified by flash chromatography (2% ethyl acetate/dichloromethane) to give (16b) as a colourless foam (600 mg, 100%). $C_{39}H_{44}N_2O_5S$ requires C, 71.7, H, 6.8; N, 4.3%. Found: C, 71.1; H, 7.0; N, 4.2%. $[\alpha]_D^{\beta}$ = +3.6° (c 0.8, $CHCl_3$). δ($CDCl_3$) 0.76 (3H, d, J = 6.7 Hz, Me), 0.89 (3H, d, J = 6.7 Hz, Me), 1.45 (9H, s, 3 x Me), 2.24 (1H, m, Me_2CH), 2.77 (2H, d, AB of ABX), 4.27 (1H, bs, αVal), 4.65 - 4.69 (1H, m, αCys), 5.23 (1H, bs, CONH), 5.24 (1H, s, $SCHPh_2$), 6.68 (1H, d, J = 8.8 Hz, CONH), 6.92 (1H, s, Ph_2CHOOC), 7.2 -7.52 (20H, m, ArH). ^{13}C nmr δ($CDCl_3$) 17.2 (q, Me), 19.0 (q, Me), 28.2 (q, 3 x Me), 31.2 (d, βC Val), 31.4 (s, Me_3C), 34.3 (t, βC Cys), 53.8 and 54.2 and 57.1 (all d, 2 x αC and $SCHPh_2$), 77.9 (d, Ph_2CH), 126.5, 126.9, 127.3, 128.0, 128.1, 128.3, 128.4, 128.5, 128.6 (ArC), 139.4, 139.6, 140.79 and 140.83 (all s, 4 x 4° ArC), 155.3, 170.4 and 170.6 (3 x s, 3 x Carbonyl Carbons). ν($CHCl_3$) 3420w, 3010w, 2970w, 1730s, 1710s, 1680s, 1500s, 1370m, 1160s cm^{-1}. m/z 653 (M^++1, 1%), 652(M^+,1), 596(1), 484(2), 429(4), 387(3), 385(3), 190(2), 183(3), 168(18), 167(100), 144(16).

S-(Diphenylmethyl)-L-cysteinyl-D-valine benzhydryl ester (17).

The above protected dipeptide (16) (300 mg, 0.46 mmol) was treated with p-toluenesulfonic acid (158 mg, 0.92 mmol) in ether (1 ml) and ethanol (1 ml)[24]. The solution was stirred at room temperature for 15 min., the solvent removed in vacuo, and the residue kept under water pump vacuum for 30 min. The residue was redissolved in ether/ethanol, and the process repeated until t.l.c (10% ethyl acetate/dichloromethane) indicated that no starting material (16) remained.

The residue was dissolved in dichloromethane, washed with saturated sodium bicarbonate, dried over sodium sulfate, and the solvent removed, to give a colourless oil, S-(diphenylmethyl)-L-cysteinyl-D-valine benzhydryl ester (17) (270 mg, 100%). δ($CDCl_3$) 0.80 (3H, d, J = 6.8 Hz, Me), 0.90 (3H, d, J = 6.8 Hz, Me), 2.21 - 2.32 (1H, m, Me_2CH), 2.55 -2.63 and 2.92 - 2.98 (2H, AB of ABX), 3.42 - 3.47 (1H, m, α Val), 4.59 - 4.64 (1H, m, α Cys), 5.17 (1H, s, $SCHPh_2$), 6.88 (1H, s, Ph_2CH), 7.18 - 7.37 (22H, m, ArH and NH_2), 7.78 (1H, d, J = 7.5 Hz, CONH). m/z (NH_3 DCI) 553 (M^+, 5%), 415(20), 168(20), 167(100), 144(10). The amine (17) was used immediately without further purification.

Iodoacetyl-L-(S-diphenylmethyl)cysteinyl-D-valine benzhydryl ester (18).

Amine (17) (270 mg, 0.49 mmol) was treated with iodoacetic acid (110 mg, 0.59 mmol) and EEDQ (181 mg, 0.73 mmol) in dichloromethane (10 ml) and stirred overnight at room temperature. The solvent was removed, the residue dissolved in ethyl acetate, washed with saturated sodium bicarbonate, 10% citric acid solution, dried over sodium sulfate and the solvent removed. The crude product was purified by flash chromatography (CH_2Cl_2 then 5% EtOAc/CH_2Cl_2) to give (18) as a colourless oil, which solidified on standing (217 mg, 62%), m.p. 129-30°. $C_{36}H_{37}IN_2O_4S$ requires C, 60.0; H, 5.2; N, 3.9%. Found: C, 59.9; H, 5.0; N, 3.7%. $[\alpha]_D^{\beta}$ = -9.2° (c 1.4, $CHCl_3$). δ($CDCl_3$) 0.78 (3H, d, J = 6.8 Hz, Me), 0.89 (3H, d, J = 6.8 Hz, Me), 2.20 - 2.28 (1H, m, $CHMe_2$), 2.69 - 2.90 (2H, m, AB of ABX), 3.60 (2H, s, ICH_2), 4.48 - 4.54 (1H, m, αVal), 4.63 - 4.68 (1H, m, αCys), 5.30 (1H, s, $SCHPh_2$), 6.59 (1H, d, J = 8.8 Hz, CONH), 6.79 (1H, d, J = 7.5 Hz, CONH), 6.91 (1H, s, Ph_2CHOOC), 7.21 - 7.47 (20H, m, ArH). ^{13}C nmr δ($CDCl_3$) -1.6 (t, ICH_2), 17.4 (q, Me), 19.1 (q, Me), 31.3 (d, βC Val), 34.4 (t, βC Cys), 52.9 and 54.6 and 57.4 (all d, 2 x αC and $SCHPh_2$), 78.0 (d, Ph_2CH), 127.0, 127.4, 128.1, 128.2, 128.3, 128.4, 128.5, 128.7 (ArC), 139.3 and 139.5 and 140.86 and 140.93 (all s, 4 x 4° ArC), 167.4 and 169.7 and 170.4 (3 x s, 2 x CONH and C(O)O). ν($CHCl_3$) 3400w, 3000m, 2960m, 1740s, 1670s, 1590 cm^{-1}. m/z 721 (<5%, M^+), 553 (<5), 533 (<6), 167(100), 166(8), 165(25), 152(12).

2-[3-(3-Trifluoromethyl-3H-diazirin-3-yl)phenoxy]acetyl-L-(S-diphenylmethyl)cysteinyl-D-valine benzhydryl ester (19).

The iodoacetyldipeptide (18) (220 mg, 0.31 mmol) and 3-(3-hydroxyphenyl-3-trifluoromethyl)-3H-diazirine (14) (84 mg, 0.42 mmol) were stirred with potassium carbonate (115 mg, 0.83 mmol) in acetone (5 ml) overnight at room temperature with protection from light. The solvent was removed, and the crude product purified by flash chromatography (1-2% ethyl acetate/dichloromethane), the give the dipeptide (19) as a colourless oil (250 mg, 100%). $[\alpha]_D^{\beta}$ = -3.0° (c 1, $CHCl_3$). δ($CDCl_3$) 0.82 (3H, d, J = 6.8 Hz, Me), 0.93 (3H, d, J = 6.8 Hz, Me), 2.25 - 2.34 (1H, m, Me_2CH), 2.81 -2.89 (2H, m, AB of ABX), 4.32 (1H, d, J_{α} = 14.8 Hz, OCH_{α}), 4.48 (1H, d, J_{β} = 14.8 Hz, OCH_{β}), 4.70 - 4.81 (2H, m, αCys and αVal), 5.33 (1H, s, $SCHPh_2$), 6.70 - 6.73 and 6.89 - 6.95 and 7.19 -7.48 and 7.62 (27H, m, 24 x ArH and 2 x CONH and Ph_2CHOOC). ^{13}C nmr δ($CDCl_3$) 17.3 (q, Me), 19.0 (q, Me) 31.2 (d, βC Val), 34.4 (t, βC Cys), 52.0 and 54.6 and 57.6 (all d, 2 x αC and $SCHPh_2$), 67.1 (t, OCH_2CO), 78.2 (d, Ph_2CH), 113.6, 115.8, 116.9, 118.2, 119.9, 120.4, 124.2, 126.9, 127.4, 127.5, 128.1, 128.2, 128.3, 128.5, 128.6, 128.7, 130.1, 130.4, 130.6, 131.0, 139.3,

139.5, 140.7, 140.8, 156.7, 157.2 (ArC and diazirine Carbon and CF_3), 168.2 and 169.9 and 170.5 (3 x s, 2 x CONH and C(O)O). ν($CHCl_3$) 3400s, 3000m, 2800m, 1740s, 1670s, 1610m, 1590s, 1410s, 1390s, 1350m, 1260s, 1200s, 1150s, 700s cm^{-1}. λ_{max} (MeOH) 274 nm (log ε 3.68), 352 (2.39).

2-[3-(3-Trifluoromethyl-3H-diazirin-3-yl)phenoxy]acetyl-L-(S-diphenylmethyl)cysteinyl-D-valine (20).

To the above tripeptide (19) (100 mg, 0.13 mmol) was added anisole (8.4 mg, 0.078 mmol) and TFA (2 ml), and the mixture stirred until all the starting material had been consumed (10 min.), as indicated by t.l.c. (CH_2Cl_2). The solvent was removed *in vacuo*, and the residue treated twice with toluene. The crude product was dried at high vacuum, and then purified by flash chromatography (5 - 10% MeOH/CH_2Cl_2), to give the acid (20) as a colourless oil (53 mg, 65%). δ($CDCl_3$) 0.89 (3H, s, Me), 0.92 (3H, s, Me), 2.09 - 2.27 (1H, m, Me_2CH), 2.65 - 2.83 (2H, m, AB of ABX), 4.27 - 4.57 (4H, m, OCH_2 and αCys and αVal), 5.09 (1H, bs, CONH), 5.33 (1H, s, $SCHPh_2$), 6.65 (1H, s, H2), 6.83 (1H, d, J = 7.5 Hz, H4), 6.90 (1H, bd, J = 7.5 Hz, H6), 7.06 - 7.81 (13H, m, ArH and COOH and CONH). ^{13}C nmr δ($CDCl_3$) 17.7 (q, Me), 19.1 (q, Me), 31.1 (d, βC Val), 34.8 (t, βC Cys), 52.3 and 53.9 and 58.0 (all d, 2 x αC and $SCHPh_2$), 67.3 (t, OCH_2CO), 113.5 (d), 116.0 (d), 119.8 (s), 120.4 (d), 124.1 (s), 127.4, 128.3, 128.4, 128.5, 128.6, 130.3, 130.9, 140.6, 140.7, 157.2 (ArC and diazirine Carbon and CF_3), 168.9, 170.2 and 175.9 (3 x s, 2 x CONH and C(O)). ν($CHCl_3$) 3400b, 1725m, 1665s, 1610m, 1585m, 1530m, 1490m, 1435m, 1285m, 1260s, 1160s cm^{-1}. λ_{max} (MeOH) 268 nm (log ε 3.26), 350 (2.70). m/z (FAB) 651 (M^++Na, 23%), 623 (M^++Na-N_2, 1), 168(17), 167(100), 166(10), 165(22), 152(15).

2-[3- (3-Trifluoromethyl-3H-diazirin-3-yl)phenoxy]acetyl-S-carbomethoxysulfenyl-L-cysteinyl-D-valine (21).

A solution of the above acid (20) (250 mg, 0.40 mmol) in chloroform (5 ml) and methanol (2.5 ml) at 0°C was treated with redistilled methoxycarbonylsulfenyl chloride (69 mg, 0.54 mmol), and the mixture stirred for 2 h. The solvent was removed *in vacuo*, and dried under high vacuum. The crude product was purified by flash chromatography (5-10% MeOH/CH_2Cl_2), to give the acid (21) as a colourless oil (170 mg, 77%). $[\alpha]_D^{\circ}$ = -54.1° (c 1, $CHCl_3$). δ($CDCl_3$) 0.96 (3H, d, Me), 0.99 (3H, d, Me), 2.23 (1H, m, $CHMe_2$), 3.14 - 3.37 (2H, m, βCys), 3.89 (3H, s, OMe), 4.26 - 4.53 (3H, m, OCH_2 and αH), 4.92 (1H, bs, αH), 6.65 (1H, bs, H2), 6.83 (1H, d, J = 8.0 Hz, H4), 6.90 (1H, dd, J''' = 2.2 Hz, J' = 8.2 Hz, H6), 7.29 (1H, dd, J = 8.0 Hz, J' = 8.2 Hz, 7.84 (1H, bs, CONH (slow exch. D_2O)), 7.98 (1H, bs, COOH (fast exch. D_2O)). ^{13}C nmr δ($CDCl_3$) 17.6 (q, Me), 19.3 (q, Me), 30.7 (d, βC Val), 41.8 (t, βC Cys), 52.5 (d, αC), 55.8 (q, OCH_3), 58.6 (d, αC), 67.4 (t, OCH_2), 113.6 (d), 116.0 (d), 119.8 (s) 120.4 (d), 124.1 (s), 130.3 (d), 130.9 (s), 157.4 (s), 169.2 and 169.6 and 170.9 and 176.2 (4 x s, 2 x CONH and 2 x C(O)O). ν($CHCl_3$) 3400w, 3300w, 2980w, 1720s, 1665s, 1610m, 1580m, 1530m, 1430m, 1340m, 1290m, 1260s, 1160s cm^{-1}. λ_{max} ($CHCl_3$) 228 nm (log ε 3.77), 274 (3.20), 358 (2.67).

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REFERENCES

1. For a review of this work, see: J.E. Baldwin, Proceedings of the 3rd International Symposium on Recent Advances in the Chemistry of β-Lactam Antiobiotics, Eds. A.G. Brown and S.M. Roberts, The Royal Society of Chemistry, 1985, p.62.

2. J.E. Baldwin, E.P. Abraham, G.L. Burge and H.-H. Ting, J.Chem.Soc., Chem.Commun., 1985, 1808.

3. J.E. Baldwin, R.M. Adlington, M.J.C. Crabbe, G.C. Knight, T. Nomoto, and C.J. Schofield, J.Chem.Soc., Chem.Commun., 1987, accepted for publication.

4. Microbiological assays were performed by the "holed plate" assay method using the test organism Staphylococcus Aureus N.C.T.C. 6571.

5. J.E. Baldwin and G.L. Burge, unpublished results.

6. Incubation method: To a mixture of the dipeptide (1 mg) in NH_4HCO_3 (1.5 ml, 50mM), dithiothreitol (50μl, 500 mM), ascorbate (50 μl, 50 mM), TRIS buffer (pH 7.4, 50 mM) and ferrous sulfate (50 μl, 50 mM) was added IPNS (6-8 IU in NH_4HCO_3 (1.5 - 2.2 ml, 50 mM)), the mixture divided into two vials and shaken (27°, 260 rpm) for 25 min. Acetone (~5 ml) was added, the supernatant collected after centrifugation, and used directly for microbiological assay.[4]

7. G. Schoellmann and E. Shaw, Biochemistry, 1963, 2, 252.

8. (a) J.R. Knowles, Acc.Chem.Res., 1972, 5, 155; (b) H. Bayley and J.R. Knowles, Methods Enzymol., 1977, 46, 69; (c) H. Bayley, "Photogenerated Reagents in Biochemistry and Molecular Biology", 1983, Elsevier, Amsterdam.

9. (a) R. Ray, S.A. Holick, N. Hanafin, and M.F. Holick, Biochemistry, 1986, 25, 4729; (b) A.B. Frey, G. Kreibich, A. Wadhera, L. Clarke and D.J. Waxman, ibid., 1986, 25, 4797; (c) S. Chen, T.D. Lee, K. Legesse, and J.E. Shively, ibid., 1986, 25, 5391; (d) G. Hegyi, L. Szilagyi and M. Elzinga, ibid., 1986, 25, 5793; (e) S.C. Kowalczykowski, ibid., 1986, 25, 5872; (f) G.R. Banks and S.G. Sedgwick, ibid., 1986, 25, 5882.

10. For reviews of the chemistry of diazirines, see: (a) E. Schmitz, Angew.Chem.,Int.Ed.Engl., 1964, 3, 333; (b) E. Schmitz, in "Advances in Heterocyclic Chemistry", 1963, 2, 83 and 1979, 24, 63; (c) H.W. Heine, in "Heterocyclic Compounds" (Ed. A. Hassner) Vol. 42, Part 2, p.547; (d) E. Schmitz, in "Comprehensive Heterocyclic Chemistry" (Eds. A.R. Katritzky and C.W. Rees), Vol. 7, Part 5, p.195.

11. (a) J.F. Brunner, H. Senn, and F.M. Richards, J.Biol.Chem., 1980, 255, 3313; (b) J.F. Brunner and F.M. Richards, J.Biol.Chem., 1980, 255, 3319; (c) M. Nassal, Liebigs Ann. Chem., 1983, 1510; (d) M. Nassal, J.Am.Chem.Soc., 1984, 106, 7540; (e) L.B. Shih and H. Bayley, Anal.Biochem., 1985, 144, 132.

12. L.S. Chen, G.J. Chen, and C. Tamborski, J.Fluorine.Chem., 1981, 18(2), 117.

13. P. Leblanc and G.E. Gerber, Can.J.Chem., 1984, 62, 1767.

14. T.W. Greene, "Protective Groups in Organic Synthesis", 1981, Wiley Interscience, New York.

15. R.G. Hiskey, N. Muthukumaraswamy and R.R. Vunnam, J.Org.Chem., 1975, 40, 950.

16. D. Papa, E. Schwenk, and A. Klingsberg, J.Am.Chem.Soc., 1946, 68, 2133.

17. N.V. Hayes and G.E.K. Branch, J.Am.Chem.Soc., 1943, 65, 1555.

18. Q.F. Soper, C.W. Whitehead, O.K. Behrens, J.J. Corse and R.G. Jones, J.Am.Chem.Soc., 1948, 70, 2849.

19. W.C. Still, M. Kahn, and A. Mitra, J.Org.Chem., 1978, 43, 2923.

20. E. Ohki, S. Oida, Y. Ohashi, A. Yoshida, K. Kamoshita, and H. Takagi, Chem.Pharm.Bull., 1974, 22, 1014.

21. I. Photaki, J. Taylor-Papadimitriou, C. Sakarellos, P. Mazarakis and L. Zervas, J.Chem.Soc.(C), 1970, 2683.

22. L. Moroder, A. Hallett, E. Wuensch, O. Keller and G. Wersin, Hoppe-Seyler's Z.Physiol.Chem., 1976, 357, 1651.

23. R.G. Hiskey, L.M. Beacham, V.G. Matl, J.N. Smith, E.B. Williams, A.M. Thomas, and E.T. Wolters, J.Org.Chem., 1971, 36, 488.

24. J. Goodacre, R.J. Ponsford, and I. Stirling, Tetrahedron Lett., 1975, 3609.