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Synthetic Approaches to 2-Methoxycysteine containing Peptides. The conversion of [(5S)-5-amino-5-carboxy-2-oxapentanoyl]-2-methoxy-(2S)-cysteinyl-(2R)-valine into 10-Oxa-6α-methoxyisopenicillin N by the Enzyme Isopenicillin N Synthase

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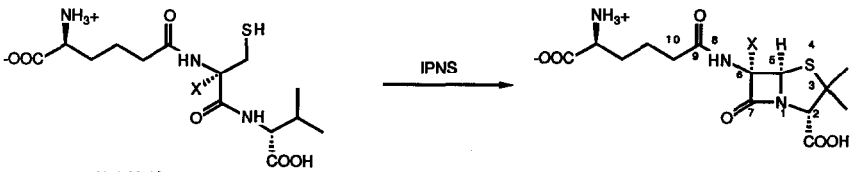
Abstract Synthetic approaches to peptides containing 2-methoxycysteine and the direct enzymic synthesis of 10-oxa-6α-methoxyisopenicillin N (17) from N-[(5S)-5-amino-5-carboxy-2-oxapentanoyl]-2-methoxy-(2S)-cysteinyl-(2R)-valine are described.

Over the past few years, investigations have shown that the enzyme IPNS (Isopenicillin N synthase), responsible for the conversion of the tripeptide [(5S)-5-amino-5-carboxy-pentanoyl]-(2R)-cysteinyl-(2R)-valine* (1a) into Isopenicillin N (2a) is not totally substrate specific.[1] The feeding of variants of the natural substrate for this enzyme has provided an array of novel β-lactam containing compounds.[1] Structural variants of the valine moiety of (1a) in particular have provided the most pronounced variety of β-lactam containing products. Through this work, insight into the mechanistic action of the IPNS enzyme has began to emerge.[1] As a complement to this continuing investigation attention was directed towards structural variants of the cysteinyl moiety of (1a).[2] Of particular interest was the possible IPNS mediated conversion of N-[(5S)-5-amino-5-carboxypentanoyl]-2-methoxy-(2S)-cysteinyl-(2R)-valine (1b) into the corresponding 6α-methoxyisopenicillin N (2b). In addition to providing information on the tolerance of IPNS for substrates containing functionality at the 2-position of the cysteinyl moiety, the successful enzymatic conversion of (1b) into (2b) would constitute the first step towards the direct enzymatic preparation of cephamycin type antibiotics from the tripeptide level. The presence of an α-methoxy group, particularly at the 7-position of cephalosporin derivatives, provides compounds (cephamycins) possessing β-lactamase resistance as well as retaining good antibiotic properties.[3]



(1a) X=H
(1b) X=OCH_3
(1c) X=CH_3

(2a) X=H
(2b) X=OCH_3
(2c) X=CH_3

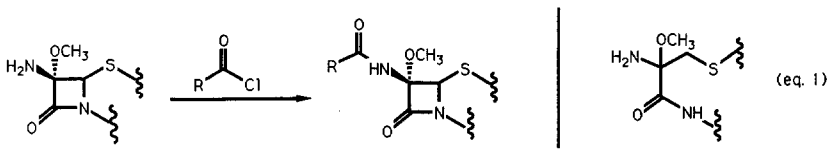
The successful conversion of (1b) into (2b) would require the IPNS enzyme to tolerate the presence of a bulky methoxy substituent at a site usually occupied by a sterically less demanding hydrogen. The enzyme would also have to be able to carry out the penicillin ring forming processes in the presence of the electronic influence of the methoxy group. In addition the first carbon-hydrogen bond broken in formation of the penicillin ring system[1d]

* δ-(L-α-aminoadipoyl)-L-cysteinyl-D-valine.

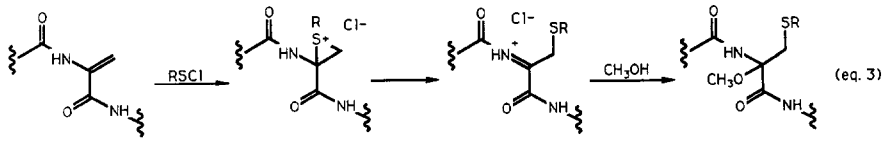
would be directly α to the electronegative methoxy group, also a potential leaving group.

Before undertaking the more difficult synthesis of the methoxy tripeptide (1b), the steric requirements of the IPNS enzyme were briefly examined using the methyl analogue (1c). As described in a previous report [(5S)-5-amino-5-carboxypentanoyl]-2-methyl-(2R)-cysteinyl-(2R)-valine (1c) upon incubation with IPNS (5 I.U./mg substrate) provided 6α-methyl-isopenicillin N (2c) in about 60% yield.[2b] This result suggested that IPNS was tolerant towards sterically more demanding substituents at the 2-position of the cysteinyl moiety and encouraged synthesis of the methoxy tripeptide (1b).

At the commencement of this work 2-methoxy cysteinyl containing peptides had not been reported in the literature and the stability of such compounds was uncertain. Natural products containing other 2-alkoxy-amino acids are known however,[4] the cephamycins perhaps being the most recognized examples. The commercial importance of the cephamycins have provided the impetus for development of a number of methods for the introduction of the 7α-methoxy group.[3] There are also a few reports in the literature dealing with the specific preparation of protected 2-alkoxy amino acids,[5] including the preparation of N-acetyl-S-methyl-2-methoxy cysteine ethyl ester.[5c] Most of the existing methods, however, are not directly applicable to the preparation of peptides containing 2-methoxycysteine. Unlike cephamycin syntheses where the 4-membered β-lactam ring imparts stability to an amino-methoxy intermediate thereby allowing facile acylation of the amine (eq.1),[3] the analoguous acyclic form is unstable. Also approaches that rely on the N-chlorination of the amide bond followed by elimination to the acylimine and trapping with methanol[3,4,5a] (eq.2) suffer from lack of selectivity when more than one peptide bond is present. As 2-methoxycysteine is not a known compound and would not be expected to be a stable species in standard peptide coupling reactions, it seemed inevitable that the methoxy group would have to be introduced at the peptide level.



One attractive approach envisaged for the preparation of 2-methoxycysteinyl peptides involved the reaction of a suitable didehydroalaninyl peptide with an appropriate sulfenyl chloride in the presence of methanol (eq.3). Because of the enamine nature of the didehydroalaninyl double bond, it was predicted that an episulfonium salt intermediate[6] would likely isomerize to an acyliminium salt which could then be trapped with methanol.

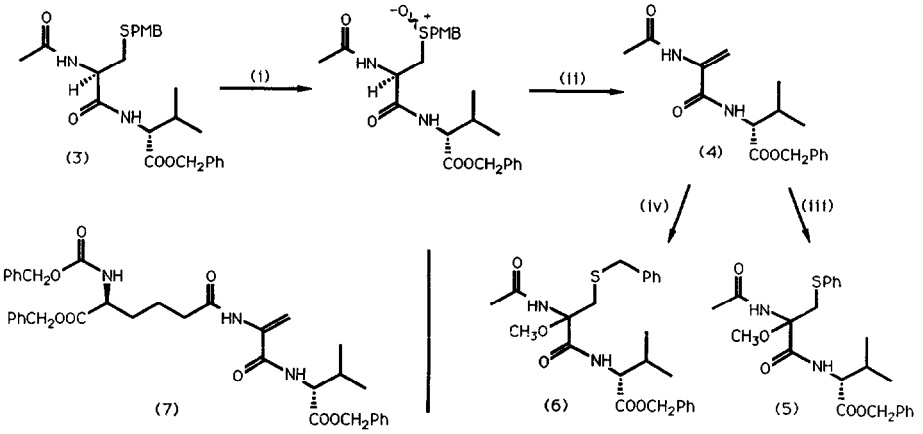


Before this idea could be tested, a general and efficient procedure for the preparation

of didehydroalaninyl peptides was required. There are numerous methods for the preparation of didehydroamino acids and this subject has been well reviewed.[7] The most common approach involves the dehydration of serine derivatives through hydroxyl activation. However, one of the most economical and direct approaches involves the oxidation and subsequent sulfoxide elimination of S-alkylcysteine containing peptides.[8] This approach allows the formation of polypeptides incorporating, relatively inexpensive cysteine derivatives as opposed to the more expensive serine derivatives and the advantage that the S-protecting group of the cysteine does not have to be removed to allow for subsequent didehydroalanine formation.

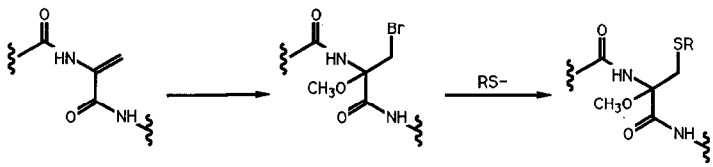
To examine the proposed route to 2-methoxycysteinyl peptides, the model compound N-acetyldidehydroalaninyl-(2R)-valine benzyl ester (4) was prepared (scheme 1). The N-acetyl group of (4) was intended as a simple model for the aminocarboxypentanoyl moiety of the target tripeptide (1b). S-p-methoxybenzyl-(2R)-cysteine was converted to its corresponding N-acetyl derivative (87%) and then coupled with the tosyl ammonium salt of (2R)-valine benzyl ester (EEDQ, Et_3N) to provide compound (3) (86%). Reaction of (3) with one equivalent of m-CPBA and gently refluxing the resulting sulfoxide mixture in xylene afforded the didehydroalaninyl peptide (4) (78%). In a preliminary evaluation, compound (4) was reacted with phenylsulfenyl chloride[9] in the presence of methanol. This produced the methoxycysteinyl peptide (5) (87%) as a roughly equal mixture of two diastereomers which could be readily separated by chromatography. The n.m.r. spectra of (5) were consistent with the assigned regiochemistry and the mass spectra of each showed [MH^+-MeOH] as the base peak. The formation of a near equal ratio of diastereomers was not surprising as little face selectivity in the electrophilic addition of the sulfenyl chloride would be expected. Though this result was encouraging, the preparation of (5) was not directly applicable to the synthesis of the target tripeptide (1b) because there are no facile means of cleaving the sulfur-phenyl bond in (5) while retaining the integrity of the rest of the molecule.[10]

Attention was then directed towards finding a sulfenylchloride that could produce a 2-methoxycysteinyl peptide containing a sulfur protecting group that could be removed by standard chemical procedures. The use of benzylsulfenyl chloride seemed a likely candidate. However, unlike phenylsulfenyl chloride, the relatively unstable benzylsulfenyl chloride was found to be reactive towards methanol of the reaction mixture. Attempts to generate an intermediate in the absence of methanol followed by subsequent trapping with methanol led only to the isolation of uncharacterized byproducts. Optimization of the reaction conditions, however, eventually led to the isolation of the S-benzyl-2-methoxy-cysteinyl peptide diastereomers (6) (38%). This result prompted the application of this reaction to the didehydroalaninyl peptide (7) derived from fully protected [(5S)-5-amino-5-carboxypentanoyl]-(2R)-cysteinyl-(2R)-valine.[11] Unfortunately, essentially none of the desired 2-methoxy-cysteinyl peptide could be isolated from this reaction. Likely the additional functionality of the aminocarboxypentanoyl moiety slows down the rate of addition of the benzylsulfenyl chloride to the didehydroalaninyl moiety enough to favour various side reactions.



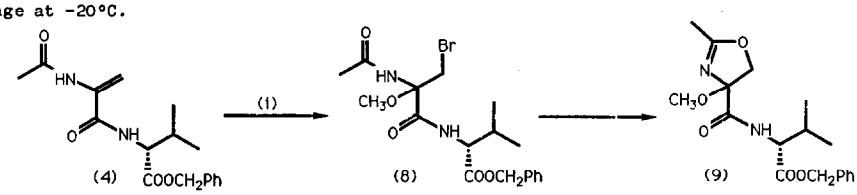
Scheme 1. Reagents: (i) mCPBA, CH_2Cl_2; (ii) Δ, xylene, 78%; (iii) PhSCl, CH_2Cl_2-MeOH, 87%; (iv) $PhCH_2SCl$, CH_2Cl_2-MeOH, 38%. PMB = p-methoxybenzyl.

A variety of different sulfenyl chlorides were examined including acetylsulfenyl chloride,[12] diethylaminosulfenyl chloride,[13] and methoxycarbonylsulfenyl chloride,[14] but none of these reagents were useful for the preparation of the desired 2-methoxycysteinyl peptides. Reluctantly, another approach for the preparation of these compounds had to be developed. It was envisaged that if a 3-bromo-2-methoxyalaninyl derivative could be made from the corresponding didehydroalaninyl peptide, it might be possible to displace the bromine with a suitable thiolate anion (eq.4).



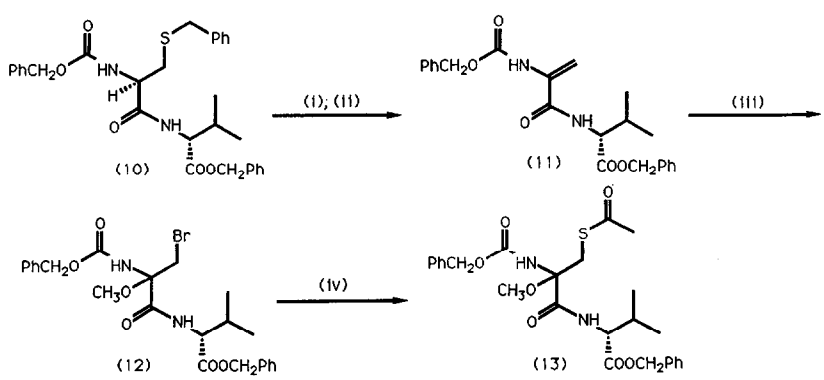
(eq. 4)

To test this hypothesis, the didehydroalaninyl peptide (4) was reacted with bromine in the presence of methanol to provide the 3-bromo-2-methoxyalaninyl peptide (8) (78%) as a 1:1 mixture of diastereomers. This mixture could be separated by chromatography, but on reacting either of the two diastereomers with any form of thio-nucleophile, the only product isolable was the oxazoline (9) (scheme 2). In fact, compound (8) slowly decomposed to (9) even on storage at -20°C.



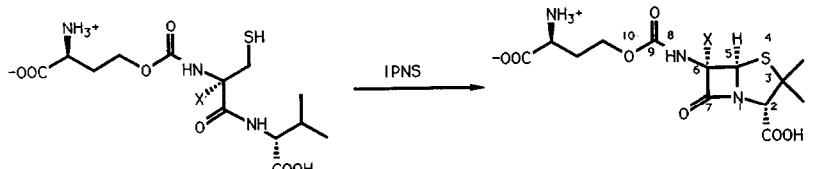
Scheme 2. Reagents: (i) Br_2, CH_2Cl_2-MeOH, (78%).

The formation of the oxazoline (9) is analogous to the classic problem of azlactone formation that can occur during peptide coupling when an amide protecting group is used on the nitrogen.[15] This problem was classically solved by using a urethane protecting group on the nitrogen as opposed to an amide; the presence of the extra oxygen of the urethane greatly diminishes the nucleophilicity of the carbonyl oxygen. It was therefore proposed that substitution of a urethane protecting group for the amide protecting group on the nitrogen of the 3-bromo-2-methoxyalaninyl moiety of (8) might allow thiolate displacement of the bromide to compete with oxazoline formation. To examine this question, the dipeptide (10)[11] was converted into the didehydroalaninyl peptide (11) (80%) and then reacted with bromine in the presence of methanol to provide (12) as a 1:1 mixture of diastereomers (91%). These two isomers were separated by chromatography and the first isomer to elute was reacted with the potent nucleophile potassium thioacetate.[16] This isomer reacted to provide the desired S-acetyl-2-methoxycysteinyl peptide (13) in just over 30% yield (scheme 3). This reaction did produce uncharacterized byproducts though no oxazoline containing products were detected. The product (13) could be purified by conventional chromatography on silica gel.



Scheme 3. Reagents: (i) mCPBA, CH_2Cl_2; (ii) Δ, xylene, 80%; (iii) Br_2, CH_2Cl_2-MeOH, 91%; (iv) $KSCOCH_3$, DMF, 40°C, 5 h, 31%.

The success of this reaction was somewhat diminished by the realization that a urethane group on the nitrogen of the 2-methoxycysteinyl moiety could not be used in the preparation of the desired methoxy tripeptide (1b). Any urethane group would have to be removed in order to attach the necessary aminocarboxypentanoyl functionality, and as was mentioned previously (eq.1), acyclic methoxy-amino species are not stable entities. However, previous investigations in this laboratory[17] had shown that the urethane analogue of the natural tripeptide (1a), compound (14), was a reasonable substrate for the IPNS enzyme and provided 10-oxaisopenicillin N(15) in good yield (41%, 10 I.U./mg substrate) (eq.5). This gave reason to believe that the methoxy analogue (16) could be prepared and this substrate could act as an alternate means of evaluating the tolerance of IPNS for substrates containing a 2-methoxycysteinyl moiety. If (16) were to be a substrate for IPNS, the resulting product should be 10-oxa-6α-methoxyisopenicillin N(17).

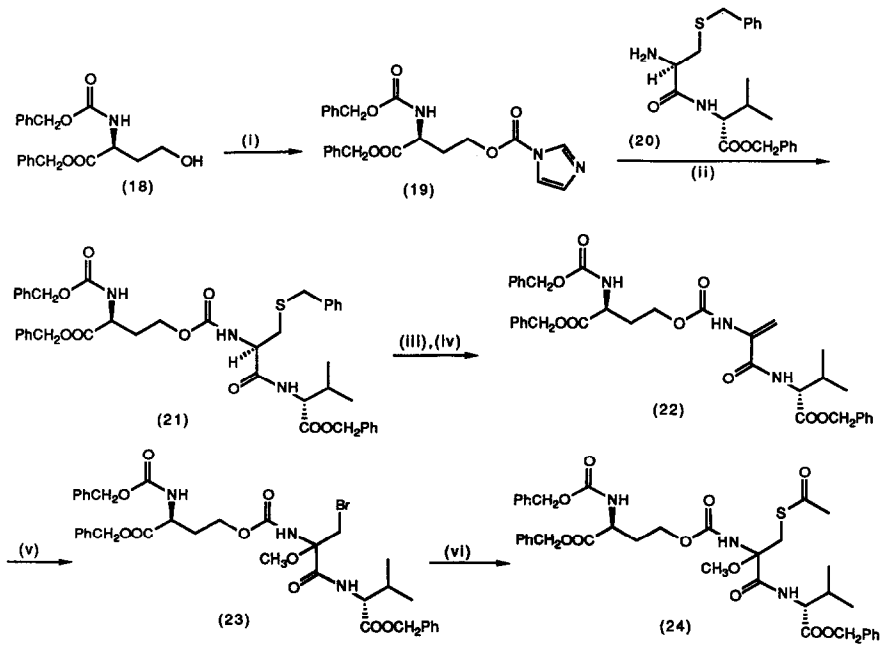


(14) X=H
(16) X=OCH_3

(15) X=H
(17) X=OCH_3

(eq. 5)

Thus the protected homoserine derivative (18)[18] was reacted with carbonyldiimidazole to provide the imidazolide (19) (96%) (scheme 4). The coupling of (19) with the cysteinylvaline derivative (20)[11] afforded the tripeptide analogue (21) (80%). The didehydroalaninyl peptide (22) was then prepared as before by treating (21) with one equivalent of mCPBA and then heating the resulting sulfoxide mixture in xylene (85%). Subsequent treatment of (22) with bromine in the presence of methanol resulted in a good yield of (23) (83%). This equal mixture of diastereomers could be separated by careful chromatography, but of course at this point, the stereochemistry at the 2 position of the 3-bromo-2-methoxy alaninyl moiety of each diastereomer was not known. Each isomer of (23) was reacted separately with potassium thioacetate to provide the S-acetyl-2-methoxycysteinyl peptide (24), each in over 30% yield. This yield was acceptable considering that the nucleophilic displacement had occurred at a neopentyl centre of a molecule rich in alternative electrophilic centres.



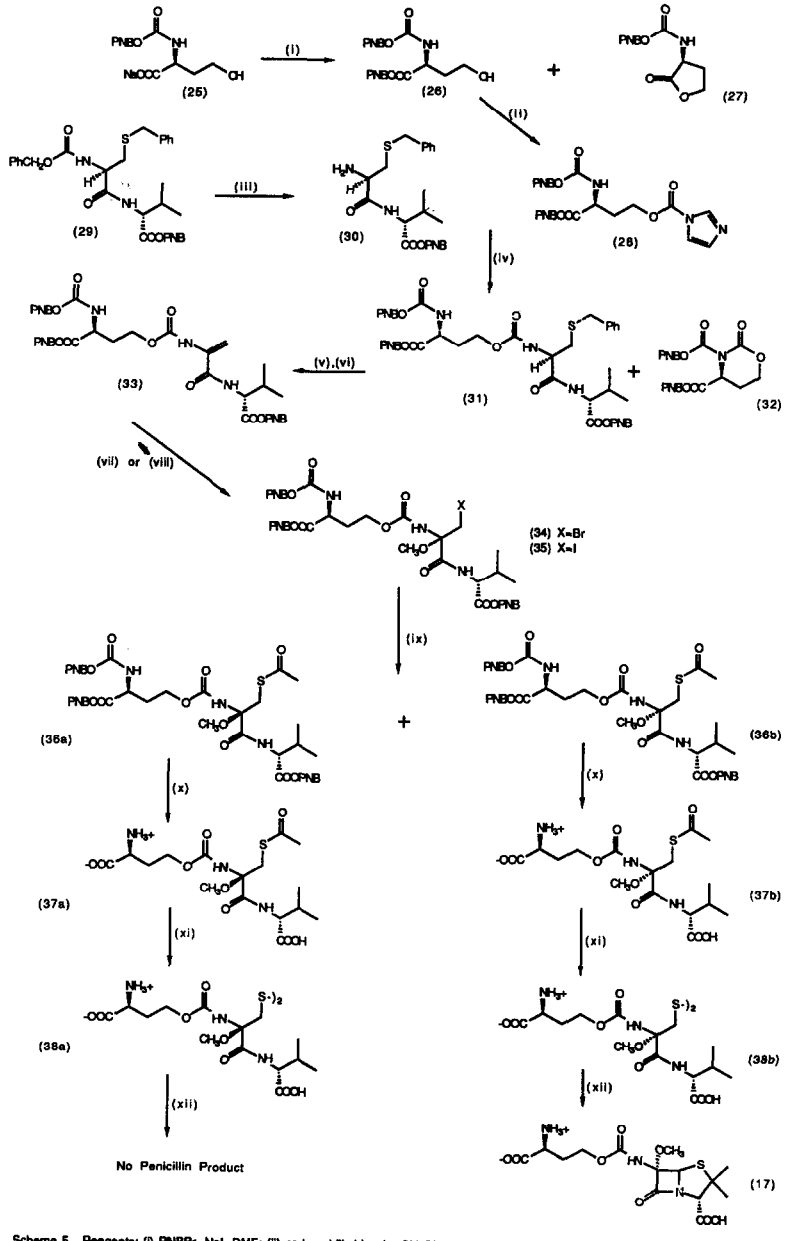
Scheme 4. Reagents: (i) carbonyldiimidazole, CH_2Cl_2, 96%; (ii) DMAP, CH_3CN, 80%; (iii) mCPBA, CH_2Cl_2; (iv) Δ, xylene, 85%; (v) Br_2, CH_2Cl_2-MeOH, 83%; (vi) $KSCOCH_3$, DMF, 40°C, 5 h, >30%.

With the successful isolation of each of the diastereomers of (24), attention now had to be directed towards the removal of the various protective groups. Unfortunately, reaction of (24) under dissolving metal reduction conditions (Na,NH_3) or various hydrogenolysis conditions failed to produce isolable amounts of any desired deprotected product. At this point, however, we were unsure as to whether the corresponding deprotected products were inherently unstable or whether the established deprotection conditions required to remove the benzyl groups were too harsh for the presumed sensitive 2-methoxycysteinyl functionality. Hoping for the latter explanation, it was decided to repeat the synthesis of (24), this time protecting the acid and amino groups with p-nitrobenzyl derived protective groups. These types of protective groups were reported to be readily removed under mild hydrogenolysis conditions.[19]

The repeated synthesis, however, produced a new set of problems. The first of these was that the corresponding protected homoserine derivative (26) was far more difficult to prepare than the corresponding derivative (18). The attempted preparation of (26) frequently led to the isolation of copious quantities of the homoserine lactone derivative (27). After considerable experimentation it was found best to convert (2R)-homoserine into the homoserine sodium salt derivative (25) (scheme 5) This material had to be pure and dry in order for efficient p-nitrobenzylation of the carboxylate salt. It proved very difficult to isolate the free alcohol derivative (26) pure because of its tendency to lactonize to (27), so impure samples of (26) were quickly treated with carbonyldiimidazole. The desired imidazolide (28) was isolated by chromatography in yields up to 63% from the sodium salt (25) along with the lactone (27) and p-nitrobenzyloxycarbonylimidazole. Any recovered lactone (27) could be readily purified by recrystallization from methanol and then converted back into the sodium salt (25) by treatement with one equivalent of sodium hydroxide in methanol. N-benzyloxy-carbonyl-S-benzyl-(2R)-cysteine and (2R)-valine p-nitrobenzyl ester were coupled (EEDQ) to afford the protected dipeptide (29) (84%). The urethane protecting group was removed with 48% HBr in acetic acid and the resulting amine product (30) (87%) was coupled with the imidazolide (28) in the presence of DMAP. The resulting coupled product (31) could be isolated in 70% yield after chromatography. A byproduct was also isolated from the crude reaction mixture corresponding to the cyclic urethane (32). Compound (31) was then converted into the didehydroalaninyl peptide (33) (84%) in the usual manner and (33) was subsequently reacted with bromine in the presence of methanol to provide a 1:1 mixture of diastereomeric 3-bromo-2-methoxycysteinyl peptides (34) (91%). Unlike the bromide isomers (23), the mixture (34) was inseparable by conventional chromatography. More significantly, treatment of (34) with potassium thioacetate failed to produce isolable amounts of the desired 2-methoxy-S-acetylcysteinyl peptides (36). This initially disconcerting problem was solved by preparing the corresponding iododerivatives (35). Treatment of (33) with N-iodosuccinimide in the presence of methanol afforded (35), also as a 1:1 mixture of diastereomers inseparable by normal chromatogrpahy (87%). The increased leaving group ability of the iodine facilitated the potassium thioacetate displacement reaction and the S-acetyl-2-methoxycysteinyl peptide mixture (36) could now be isolated in 38% yield. This mixture was subjected to chiral HPLC to afford each of the diastereomers (36a) and (36b) pure.

With pure samples of each of the two diastereomers of (36) in hand, protecting group removal was investigated. Stirring each isomer of (36) separately under an atmosphere of hydrogen (10% Pd/C, THF-H_2O 1:1) provided, after reverse phase HPLC, the two deprotected derivatives (37a) and (37b). These two products were treated with 1N aqueous ammonia to remove the S-acetyl groups and the resulting free thiols were oxidized to the corresponding disulfides (38) by bubbling oxygen through the ammonia solution. Both isomers (38a) and (38b) were isolated in yields greater than 50% from the corresponding isomer of (36). The successful isolation of both isomers of (38) proved that free 2-methoxycysteinyl peptides were indeed stable isolable compounds.

At this stage the stereochemistry at the 2 position of the cysteinyl moiety of (38a) and (38b) was still not known. Thus both isomers of (38) were incubated with purified extracts of IPNS[20] and the appropriate cofactors (L-ascorbic acid, DTT, O_2, and $FeSO_4$). The substrate corresponding to the second component of (36) to elute from the chiral HPLC column proved an excellent substrate for the IPNS enzyme producing 10-oxo-6-α-methoxyisopenicillin N (17) in 51% yield. (30 I.U./mg substate). This material was isolated in pure form from the crude incubation mixture by reverse phase HPLC and provided NMR and mass spectral data consistent with the proposed structure. The other diastereomer on incubation with IPNS failed to produce any NMR detectable β-lactam containing product.



Scheme 5. Reagents: (i) PNBBr, NaI, DMF; (ii) carbonyldiimidazole, CH_2Cl_2, 63%; (iii) 48% HBr, HOAc-CH_2Cl_2; NaOH, 87%, *(iv)* DMAP, CH_3CN, 70%; (v) mCPBA, CH_2Cl_2; (vi) Δ, xylene, 84%; (vii) Br_2, CH_2Cl_2-MeOH, 91%; (viii) NIS, MeOH-CH_2Cl_2, 87%; (ix) $KSCOCH_3$, DMF, 40°C, 5 h, 38%; (x) H_2, Pd-C, THF-H_2O, 18°C, 16 h; (xi) 1N NH_3(aq.), O_2, >50%; (xii) IPNS (30 I.U./mg), L-Ascorbic Acid, DTT, $FeSO_4$, O_2. PNB = p-nitrobenzyl

On the basis of the established stereochemical requirements of IPNS [1,2b] in regards to the 2-position of the cysteinyl moiety, the diastereomer that was a substrate for the enzyme was assigned the stereochemistry of compound (38b). The failure of diastereomer (38a) to afforded any penicillin product on incubation is consistent with the IPNS enzymes stereochemical requirement at this center.

This work has shown a partial solution for the preparation of 2-methoxycysteinyl containing peptides and demonstrated that these compounds are relatively stable entities. The successful enzymatic conversion of (38b) into (17) demonstrates a significant tolerance of the IPNS enzyme for substituents at the 2-position of the cysteinyl moiety.

Experimental

All reagents and solvents were purified and/or dried according to standard procedures.[21] Organic solutions were concentrated on a Buchi rotovapor and all isolated products were held under vacuum (< 1 mm Hg) until constant weight. Organic extracts were dried over $MgSO_4$. TLC was carried out on Merck Kieselgel 60 F_{254} plates visualizing with UV and 10% w/v ammonium molybdate (VI) tetrahydrate in 2N H_2SO_4. Flash chromatography was carried out using Merck silica gel 60 (230-400 Mesh). Melting points were determined using a Buchi 510 apparatus and are uncorrected. Infrared spectra were recorded on a Perkin Elmer 1750 FTIR Spectrometer and only medium (m) and strong (s) bands between 4000 - 1450 cm^{-1} were reported. Proton NMR spectra were recorded on either a Bruker AM 250 (250 MHz), WH 300 (300 MHz) or AM 500 (500 MHz) spectrometer. Spectra run in $CDCl_3$ were referenced to residual $CHCl_3$ (7.27 ppm) while those run in D_2O were referenced to sodium 3-trimethylsilylpropionate-2,2,3,3-d_4 (TSP) (0 ppm). The abbreviations s singlet, d doublet, t triplet, q quartet, m multiplet, and br broad are used and coupling constants are reported to the nearest 0.5 Hz. Carbon NMR spectra were recorded on a Bruker AM 500 (125 MHz) spectrometer and δC values are references to $CDCl_3$ (77.0 ppm). ^{13}C assignments were made possible by DEPT experiments. Mass spectra (m/e) were recorded on VG Micromass 16F, 30F, or ZAB-1F spectrometers.

N-Acetyl-S-p-methoxybenzyl-(2R)-cysteinyl-(2R)-valine benzyl ester (3).

A solution of the ammonium chloride salt of S-p-methoxybenzyl-(2R)-cysteine (1.37 g, 4.93 mmol) in 1:1 dioxane-H_2O (20 mL) was cooled to 0°C and an aqueous solution of 2N NaOH (5.0 mL, 10.0 mmol) was added. To the resulting solution was added dropwise simultaneously a solution of acetyl chloride (355 μL, 5.0 mmol) in dioxane (5 mL) and an aqueous solution of 1N NaOH (5.0 mL, 5.0 mmol). The mixture was stirred at room temperature for 30 min, then diluted with water (30 mL), and extracted with ether (2 x 20 mL). The aqueous phase was acidified to ~ pH 2 with 2N HCl and the resulting mixture was extracted with ethyl acetate (3 x 20 mL). The combined organic phases were dried and concentrated to provide N-acetyl-S-p-methoxybenzyl-(2R)-cysteine as a very pale yellowish solid (1.20 g, 87%). To a room temperature solution of the above material (731 mg, 2.58 mmol) in dry CH_2Cl_2 (60 mL) was added, under an argon atmosphere, the ammonium tosylate salt of (2R)-valine benzyl ester (1.03 g, 2.71 mmol), triethylamine (380 μL, 2.71 mmol) and EEDQ (700 mg, 2.84 mmol). The mixture was stirred for 21 h, then diluted with ethyl acetate (120 mL), and extracted with saturated $NaHCO_3$ (2 x 30 mL), 1M aqueous HCl (2 x 30 mL) and brine (25 mL). The organic phase was dried and concentrated to afford crude (3) as a pale yellow solid (1.2 g). This material could be further purified by flash chromatography (silica, eluent ethyl acetate-hexane 1.5:1) (1.05 g, 86%) and recrystallization from heptane. TLC (ethyl acetate-hexane 1:1) R_f 0.20; mp 102 - 104°C; $[\alpha]_D^{\circ}$ -3.1° (C 0.54, $CHCl_3$); $\nu_{max.}$ ($CHCl_3$) 3420m, 3315m, 1740s, 1660s, 1610m, 1585m, 1510s; δH (300 MHz, $CDCl_3$) 0.87, 0.92 (2 x 3H, d, J 7 Hz, $CHMe_2$), 1.97 (3H, s, CH_3CO), 2.15 - 2.26 (1H, m, $CHMe_2$), 2.67 (1H, dd, J 14, 7.5 Hz, $CHCH_2S$), 2.89 (1H, dd, J 14, 5.5 Hz, $CHCH_2S$), 3.76 (2H, s, SCH_2Aryl), 3.80 (3H, s, OCH_3), 4.52 - 4.59 (2H, m, $HNCHCO_2$), 5.13 and 5.21 (2H, ABq, J 12 Hz, OCH_2Aryl), 6.31 (1H, br.d, J 7 Hz, NH), 6.80 (1 H, br.d, J 8.5 Hz, NH), 6.86, 7.28 (2 x 2H, d, J 8.5 Hz, CH_3OAryl), 7.31 - 7.40 (5H, m, Aryl); m/e (NH_3CI) 473 (MH^+, base). Found (%): C, 63.56; H, 7.02; N, 5.84. $C_{25}H_{32}N_2O_5S$ requires C, 63.54; H, 6.82; N, 5.93.

General Procedure 1. Preparation of Didehydroalaninylpeptides.

A solution of the cysteinyl peptide (1.0 mmol) in CH_2Cl_2 (25 mL) was cooled to 0°C. A solution of mCPBA (1.0 mmol) in CH_2Cl_2 (12 mL) was added dropwise over 15 min. The mixture was stirred at room temperature for 30 min, and then washed with saturated aqueous $NaHCO_3$ (2 x 30 mL) and brine (20 mL). The organic phase was dried and concentrated to afford the crude sulfoxide mixture, usually as a relatively insoluble white solid. This material was used without further purification in the next reaction. The crude sulfoxide mixture was suspended in Na dried xylene (140 mL) and the mixture was heated in a 150°C oil bath for 4 h under an argon atmosphere. The xylene was removed under vacuum and the orange-red residue was flash chromatographed (silica) to provide the didehydroalaninyl peptide.

N-Acetyldidehydroalaninyl-(2R)-valine benzyl ester (4).

General procedure 1 was followed to provide (4) (78%) as a clear colorless viscous liquid. TLC (ethyl acetate-hexane 1.5:1) R_f 0.35; $\nu_{max.}$ ($CHCl_3$) 3440m, 3390m, 1735s, 1700s,

1660s, 1630s, 1495s; δ_H (300 MHz, $CDCl_3$) 0.90, 0.95 (2 x 3H, d, J 7 Hz, $CHMe_2$), 2.12 (3H, s, CH_3CO), 2.19 - 2.30 (1H, m, $CHMe_2$), 4.64 (1H, dd, J 8.5, 5 Hz, $HNCHCO_2$), 5.17 and 5.23 (2H, ABq, J 12 Hz, OCH_2Aryl), 5.31 (1H, br.s, $C=CH_2$), 6.51 (1H, d, J 1.5 Hz, $C=CH_2$), 6.64 (1H, br.d, J 8.5 Hz, NH), 7.35 - 7.41 (5H, m, Aryl), 8.01 (1H, br.s, NH); m/e (NH_3CI) 318 (MH^+, base).

N-Acetyl-S-phenyl-2-methoxy-(2R/S)-cysteinyl-(2R)-valine benzyl ester (5).

A solution of the didehydropeptide (4) (27 mg, 0.085 mmol) in dry CH_2Cl_2 (1.5 mL) and dry methanol (0.6 mL) was cooled to -78°C. Phenylsulfenylchloride[9] (8 µL, 0.09 mmol) was added neat via a syringe and the reaction mixture was allowed to warm to room temperature. After 30 min, the solvent was evaporated and the pale yellow viscous residue was directly flash chromatographed (silica, eluent ethyl acetate) to provide (5) as a mixture of two diastereomers. The first diastereomer to elute provided a white solid (21.5 mg, 55%). TLC (ethyl acetate) R_f 0.4; mp 112 - 113°C; $\nu_{max.}$ ($CHCl_3$) 3395m, 1740s, 1680s, 1490s; δ_H (300 MHz, $CDCl_3$) 0.89, 0.96 (2 x 3H, d, 7 Hz, $CHMe_2$), 1.77 (3H, s, CH_3CO), 2.16 - 2.28 (1H, m, $CHMe_2$), 3.14 (3H, s, CH_3O), 3.43, 4.10 (2 x 1H, d, J 14 Hz, CH_2S), 4.52 (1H, dd, J 8.5, 4.5 Hz, $HNCHCO_2$), 5.13 and 5.24 (2H, ABq, J 12 Hz, OCH_2Aryl), 6.87 (1H, br.s, NH), 6.93 (1H, br.d, J 9 Hz, NH) 7.17 - 7.45 (10H, m, Aryl); m/e (NH_3CI) 459 (MH^+, 25%), 427 (MH^+-MeOH, base). The second diastereomer to elute also provided a white solid (12.5 mg, 32%): TLC (ethyl acetate) R_f 0.25; mp 103-105°C; $\nu_{max.}$ ($CHCl_3$) 3395m, 1740s, 1680s, 1490s; δ_H (300 MHz, $CDCl_3$) 0.89, 0.95 (2 x 3H, d, J 7 Hz, $CHMe_2$), 1.85 (3H, s, CH_3CO), 2.18 - 2.30 (1H, m, $CHMe_2$), 3.21 (3H, s, CH_3O), 3.42, 3.94 (2 x 1H, d, J 14 Hz, CH_2S), 4.45 (1H, dd, J 9, 4.5 Hz, $HNCHCO_2$), 5.13 and 5.20 (2H, ABq, J 12 Hz, OCH_2Aryl), 6.83 (1H, br.s, NH), 7.09 (1H, br.d, J 9 Hz, NH), 7.15 - 7.43 (10H, m, Aryl); m/e (NH_3CI) 459 (MH^+, 50%), 427 (MH^+-MeOH, base).

N-Acetyl-S-benzyl-2-methoxy-(2R/S)-cysteinyl-(2R)-valine benzyl ester (6).

A solution of benzyl disulfide (250 mg, 1.0 mmol) in dry CH_2Cl_2 (2.0 mL) was cooled to 0°C and sulfuryl chloride (80 µL, 1.0 mmol) was added neat under an argon atmosphere. After 1 h at 0°C, the mixture was cooled to -78°C and a solution of the didehydropeptide (4) (30 mg, 0.094 mmol) in CH_2Cl_2-MeOH (1:1, 0.4 mL) was added via a syringe. The reaction mixture was allowed to warm to room temperature. After 30 min. the solvent was removed and the yellow viscous residue was flash chromatographed (silica, eluent ethyl acetate-hexane 4:1) to provide (6) as a mixture of diastereomers. The first isomer to elute provided a white solid (8 mg, 18%). TLC (ethyl acetate-hexane 4:1) R_f 0.3; mp 103 - 105°C; $\nu_{max.}$ ($CHCl_3$) 3395m, 1740s, 1680s, 1490s; δ_H (300 MHz, $CDCl_3$) 0.87, 0.94 (2 x 3H, d, J 7 Hz, $CHMe_2$), 2.05 (3H, s, CH_3CO) 2.20 - 2.31 (1H, m, $CHMe_2$), 3.19 (3H, m, CH_3O), 3.24 and 3.30 (2H, ABq, J 14 Hz, CCH_2S), 3.75 and 3.84 (2H, ABq, J 13 Hz, SCH_2Aryl), 4.57 (1H, dd, J 9, 4.5 Hz, $HNCHCO_2$), 5.13 and 5.24 (2H, ABq, J 12 Hz, OCH_2Aryl), 6.94 (1H, br.s, NH) 7.08 (1H, br.d, J 9 Hz, NH) 7.21 -7.37 (10H, m, Aryl); m/e (NH_3CI) 473 (MH^+, 10%), 441 (MH^+-MeOH, base); Found (%): C, 63.24; H, 7.06; N, 5.57; $C_{25}H_{32}N_2O_5S$ requires: C, 63.53; H, 6.77; N. 5.92. The second isomer to elute provided a clear colourless viscous gum (9 mg, 20%). TLC (ethylacetate-hexane 4:1) R_f 0.2; $\nu_{max.}$ ($CHCl_3$) 3390m, 1740s, 1680s, 1490s; δ_H (300 MHz, $CDCl_3$) 0.93, 0.99 (2 x 3H, d, J 7 Hz, $CHMe_2$), 2.02 (3H, s, CH_3CO), 2.22 - 2.32 (1H, m, $CHMe_2$), 3.13 (2H, s, CCH_2S), 3.27 (3H, s, CH_3O), 3.69 and 3.76 (2H, ABq, J 13 Hz, SCH_2Aryl), 4.56 (1H, dd, J 9, 4.5 Hz, $HNCHCO_2$), 5.14 and 5.21 (2H, ABq, J 12 Hz, OCH_2Aryl), 6.74 (1H, br.s, NH), 7.20 - 7.37 (11H, m, Aryl and NH); m/e (NH_3CI) 473 (MH^+, 25%), 441 (MH^+-MeOH, base).

General Procedure 2. Preparation of 2-methoxy-3-bromoalaninyl peptides.

A solution of the didehydroalaninyl peptide (0.50 mmol) in dry CH_2Cl_2 (15 mL) and dry methanol (0.70 mL) was cooled to -10°C and a solution of bromine (0.50 mmol) in dry CH_2Cl_2 (0.50 mL) was added dropwise via a syringe. The mixture was stirred at -10°C for 20 min, the solvent was removed, and the reddish viscous residue was flash chromatographed (silica).

N-Acetyl-3-bromo-2-methoxy(2R/S)-alaninyl-(2R)-valine benzyl ester (8).

General Procedure 2 was followed to provide (8) as a mixture of diastereomers. Both isomers were relatively unstable and were stored immediately at -20°C. The first diastereomer to elute provided a white solid (43%). TLC (ethyl acetate) R_f 0.35; mp 89-91°C; $\nu_{max.}$ ($CHCl_3$) 3395m, 1740s, 1680s, 1490s; δ_H (300 MHz, $CDCl_3$) 0.89, 0.99 (2 x 3H, d, J 7 Hz, $CHMe_2$), 2.09 (3H, s, CH_3CO), 2.25 - 2.36 (1H, m, $CHMe_2$), 3.23 (3H, s, CH_3O), 3.70, 4.36 (2 x 1H, d, J 10.5 Hz, CH_2Br), 4.60 (1H, dd, J 9, 4.5 Hz, $HNCHCO_2$), 5.14 and 5.25 (2H, ABq, J 12 Hz, OCH_2Aryl), 6.91 - 6.97 (2H, br.m, NH), 7.32 - 7.40 (5H, m, Aryl). The second isomer to elute provided a clear slightly yellowish gum (35%). TLC (ethyl acetate) R_f 0.25; $\nu_{max.}$ ($CHCl_3$) 3395m, 1740s, 1685s, 1490s; δ_H (300 MHz, $CDCl_3$) 0.91, 0.97 (2 x 3H, d, J 7 Hz, $CHMe_2$), 2.10 (3H, s, CH_3CO), 2.21 - 2.32 (1H, m, $CHMe_2$), 3.32 (3H, s, CH_3O), 3.68, 4.18 (2 x 1H, d, J 11 Hz, CH_2Br), 4.58 (1H, dd, J 9, 4.5 Hz, $HNCHCO_2$), 5.15 and 5.23 (2H, ABq, J 12 Hz, OCH_2Aryl), 6.85 (1H, br.s, NH), 7.03 (1H, br.d, J 9 Hz, NH), 7.33 - 7.38 (5H, m, Aryl).

N-Benzyloxycarbonyldidehydroalaninyl-(2R)-valine benzyl ester (11).

General procedure 1 was followed to provide (11) as a clear colorless gum (80%). TLC (hexane-ethyl acetate 3:1) R_f 0.35; $\nu_{max.}$ ($CHCl_3$) 3440m, 3390m, 1735s, 1670s, 1635s, 1590m, 1500s; δ_H (300 MHz, $CDCl_3$) 0.89, 0.93 (2 x 3H, d, J 7 Hz, $CHMe_2$), 2.18 - 2.29 (1H, m, $CHMe_2$), 4.64 (1H, dd, J 8.5, 4.5 Hz, $HNCHCO_2$), 5.14 - 5.25 (5H, m, OCH_2Aryl, $C=CH_2$), 6.15 (1H, d, J 1.5 Hz, $C=CH_2$), 6.59 (1H, br.d, J 8.5 Hz, NH), 7.30 - 7.41 (10H, m, Aryl), 7.50 (1H, br.s, NH); m/e (NH_3CI) 411 (MH^+, base).

N-Benzyloxycarbonyl-3-bromo-2-methoxy-(2R/S)-alaninyl-(2R)-valine benzyl ester (12). General procedure 2 was followed to provide (12) as a mixture of diastereomers. The first isomer to elute provided a white solid (47%). TLC (hexane-ethyl acetate 2.5:1) R_f 0.35; mp 85-87°C; $\nu_{max.}$ ($CHCl_3$) 3400m, 1735s, 1695s, 1490s; δ_H (300 MHz, $CDCl_3$) 0.89, 1.00 (2 x 3H, d, 7 Hz, $CHMe_2$), 2.25 - 2.36 (1H, m, $CHMe_2$), 3.23 (3H, s, CH_3O), 3.68 (1H, d, J 11 Hz, CH_2Br), 4.26 (1H, br.d, J 11 Hz, CH_2Br), 4.61 (1H, dd, J 9, 4.5 Hz, $HNCHCO_2$), 5.13 and 5.24 (2H, ABq, J 12 Hz, OCH_2Aryl), 5.14 (2H, s, OCH_2Aryl), 6.41 (1H, br.s, NH), 6.93 (1H, br.d, J 9 Hz, NH), 7.31 - 7.40 (10H, m, Aryl); m/e (NH_3CI) 540, 538 (MNH_4^+, base), 508, 506 (MNH_4^+-MeOH, 40%); Found (%); C, 55.53; H, 5.75; N, 5.12; $C_{24}H_{29}BrN_2O_6$ requires: C, 55.29; H, 5.61; N, 5.37. The second isomer to elute provided a white solid (44%). TLC (hexane-ethyl acetate 2.5:1) R_f 0.30; mp 113-115°C; $\nu_{max.}$ ($CHCl_3$) 3405m, 1740s, 1695s, 1490s; δ_H (300 MHz, $CDCl_3$) 0.91, 0.96 (2 x 3H, d, J 7 Hz, $CHMe_2$), 2.20 - 2.31 (1H, m, $CHMe_2$), 3.31 (3H, s, CH_3O), 3.66 (1H, d, J 11 Hz, CH_2Br), 4.11 (1H, br.d, J 11 Hz, CH_2Br), 4.59 (1H, dd, J 9, 4.5 Hz, $HNCHCO_2$), 5.14 (2H, s, OCH_2Ar), 5.16 and 5.23 (2H, ABq, J 12 Hz, OCH_2Ar), 6.28 (1H, br.s, NH), 7.03 (1H, br.d, J 9 Hz, NH), 7.31 - 7.41 (10H, m, Aryl); m/e (NH_3CI) 540, 538 (MNH_4^+, base) 508, 506 (MNH_4^+ - MeOH, 60%).

General Procedure 3. Preparation of S-acetyl-2-methoxycysteinyl peptides.

The potassium thioacetate required in this reaction was prepared as follows. A solution of KOH (433 mg, 7.72 mmol) in dry methanol (12.0 mL) was cooled to 0°C and thioacetic acid (555 µL, 7.76 mmol) was added dropwise under an argon atmosphere. The resulting yellow reaction mixture was stirred at room temperature for 10 min. and the solvent was carefully evaporated. The resulting yellow solid was washed with dry THF (3 x 3 mL) and then dry CH_2Cl_2 (2 x 3 mL) under argon. This produced, after pumping overnight, a white fluffy solid which was stored at -20 to -10°C under argon. A solution of the 3-halo-2-methoxyalaninyl peptide (0.20 mmol) in dry DMF (0.35 mL) was transferred to a 1 dram vial equipped with a stir bar, septum, and argon needle. A solution of potassium thioacetate (0.60 mmol) in dry DMF (0.25 mL) was added and the mixture was stirred at 35-40°C for 5 h. The dark red mixture was poured into ethyl acetate (40 mL) and extracted with water (3 x 10 mL) and brine (10 mL). The organic phase was dried and concentrated to afford a reddish viscous residue which was directly flash chromatographed (silica).

N-Benzyloxycarbonyl-S-acetyl-2-methoxy-(2R/S)-cysteinyl-(2R)-valine benzyl ester (13). General procedure 3 was followed, using the first isomer to elute of the diastereomeric mixture (12), to provide (13) as a clear colorless gum (31%). TLC (hexane-ethyl acetate 2:1) R_f 0.3; $\nu_{max.}$ ($CHCl_3$) 3400m, 1735s, 1695s, 1485s; δ_H (300 MHz, $CDCl_3$) 0.90, 0.93 (2 x 3H, d, 7 Hz, $CHMe_2$), 2.20 - 2.31 (1H, m, $CHMe_2$), 2.31 (3H, s, CH_3COS), 3.18 (3H, s, CH_3O), 3.58 (1H, br.d, J 14 Hz, CH_2S), 3.93 (1H, d, J 14 Hz, CH_2S), 4.50 (1H, dd, J 8.5, 4.5 Hz, $HNCHCO_2$), 5.11 and 5.23 (2H, ABq, J 12 Hz, OCH_2Aryl), 5.13 (2H, s, OCH_2Aryl), 6.34 (1H, br.s, NH), 6.97 (1H, br.d, J 8.5 Hz, NH), 7.31 - 7.37 (10H, m, Aryl); m/e (NH_3CI) 534 (MNH_4^+, base), 502 (MNH_4^+ - CH_3OH, 90%), 485 (MH^+ - CH_3OH, 40%).

N-Benzyloxycarbonyl-O-carbonylimidazole-(2S)-homoserine benzyl ester (19). To a solution of N-benzyloxycarbonyl-S-homoserine benzyl ester (18)[10] (1.82 g, 73% pure 3.86 mmol) in dry CH_2Cl_2 (20 mL) was added carbonyldiimidazole (1.29 g, 7.95 mmol) in one portion under an argon atmosphere. The mixture was stirred at room temperature for 20 h, then the solvent was removed, and the yellowish viscous residue was flash chromatographed (silica, eluent ethyl acetate-hexane 1.2:1) to provide N-benzyloxycarbonyl-S-homoserine lactone (425 mg), an impurity in the starting material, and the desired product (19) as a clear colorless gum (1.62 g, 96%). TLC (ethyl acetate-hexane 1.5:1) R_f 0.35; $[\alpha]_D^{\beta}$° 5.8° (c 0.055, $CHCl_3$); $\nu_{max.}$ ($CHCl_3$) 3425m, 1765s, 1730s, 1610m, 1530s; δ_H (300 MHz, $CDCl_3$) 2.17 - 2.27, 2.38 - 2.47 (2 x 1H, m, CH_2CH_2O), 4.50 (2H, t, J 6 Hz, CH_2CH_2O), 4.57 - 4.63 (1H, m, $HNCHCO_2$), 5.06 -5.20 (4H, m, OCH_2Aryl), 5.53 (1H, br.d, J 7.5 Hz, NH), 7.05 (1H, s, Imid H), 7.27 - 7.36 (11H, m, Aryl and Imid H), 8.10 (1H, s, Imid H); m/e (NH_3CI) 438 (MH^+, 10%), 330 (MH^+-$PhCH_2OH$, 35%), 91 (base).

[(5S)-5-Benzyloxycarbonylamino-5-benzyloxycarbonyl]-2-oxapentanoyl-S-benzyl-(2R)-cysteinyl-(2R)-valine benzyl ester (21). To a solution of the N-benzyloxy-O-carbonylimidazole-(2S)-homoserine benzyl ester (19) (1.46 g, 3.34 mmol) and S-benzyl-(2R)-cysteinyl-(2R)-valine benzyl ester (20)[11] (1.34 g, 3.34 mmol) in dry acetonitrile (2.5 mL) was added dimethylaminopyridine (410 mg, 3.35 mmol). The mixture was stirred under argon at 45°C for 24 h and then at room temperature for 36 h. The remaining solvent was pumped off, the residue was dissolved in hexane-ethyl acetate-CH_2Cl_2 1:1:1, and then flash chromatographed (silica, eluent hexane-ethyl acetate 1.2:1) to provide (21) as a white solid (2.06 g, 80%). TLC (hexane-ethyl acetate 1.2:1) R_f 0.4; mp 125 - 127°C; $[\alpha]_D^{\beta}$° -3.1° (c 0.10, $CHCl_3$); $\nu_{max.}$ ($CHCl_3$) 3425m, 1730s, 1680m, 1500s; δ_H (250 MHz, $CDCl_3$) 0.86, 0.91 (2 x 3H, d, J 7 Hz, $CHMe_2$), 2.05 - 2.30 (3H, m, $CHMe_2$ and CH_2CH_2O), 2.67 - 2.90 (2H, m, $CHCH_2S$), 3.73 (2H, s, SCH_2Aryl), 4.10 - 4.27 (3H, m, CH_2CH_2O and NHCH), 4.53 - 4.59 (2H, m, NHCH), 5.39, 5.61, 6.72 (3 x 1H, br, NH), 7.18 - 7.39 (20H, m, Aryl); m/e (FAB+) 770 (MH^+); Found (%): C, 65.34; H, 6.31; N, 5.26. $C_{42}H_{47}N_3O_9S$ requires: C, 65.52; H, 6.15; N, 5.46.

[(5S)-5-Benzyloxycarbonylamino-5-benzyloxycarbonyl]-2-oxapentanoyldidehydroalaninyl-(2R)-valine benzyl ester (22).

General procedure 1 was followed to provide (22) as a clear colourless gum (85%). TLC (hexane-ethyl acetate 1.5:1) R_f 0.35; $[\alpha]_D^{25}$° -10.5° (c 0.047, $CHCl_3$); $\nu_{max.}$ ($CHCl_3$) 3430m, 3390m, 1735s, 1670m, 1635m, 1500s; δ_H (250 MHz, $CDCl_3$) 0.90, 0.93 (2 x 3H, d, J 7 Hz, $CHMe_2$), 2.06 - 2.35 (3H, m, $CHMe_2$ and CH_2CH_2O), 4.13 - 4.29 (2H, m, CH_2CH_2O), 4.49 - 4.60 (1H, m, $HNCHCH_2$), 4.64 (1H, dd, J 8.5, 4.5 Hz, HNCHCH), 5.08 - 5.28 (7H, m, C=CH_2 and OCH_2Aryl), 5.50 (1H, br.d, J 8 Hz, NH), 6.07 (1H, d, J 1.5 Hz, C=CH_2), 6.61 (1H, br.d, J 8.5 Hz, NH), 7.27 - 7.45 (16H, m, Aryl and NH); m/e (FAB+) 646 (MH^+).

[(5S)-5-Benzyloxycarbonylamino-5-benzyloxycarbonyl]-2-oxapentanoyl-3-bromo-2-methoxy-(2R/S)-alaninyl-(2R)-valine benzyl ester (23).

General procedure 2 was followed to provide (23) as a mixture of diastereomers (83%) which could be separated by flash chromatography (silica). The first isomer to elute provided a white solid which could be recrystallized from methanol. TLC (hexane-ethyl acetate 1.5:1) R_f 0.25; mp 87 - 89°C; $[\alpha]_D^{25}$° 7.5° (c 0.085, $CHCl_3$); $\nu_{max.}$ ($CHCl_3$) 3405m, 1735s, 1695s, 1480s; δ_H (250 MHz, $CDCl_3$) 0.90, 0.96 (2 x 3H, d, J 7 Hz, $CHMe_2$), 2.03 - 2.36 (3H, m, $CHMe_2$ and CH_2CH_2O), 3.27 (3H, s, CH_3O), 3.61, 4.04 (2 x 1H, d, J 11 Hz, CH_2Br), 4.05 - 4.27 (2H, m, CH_2CH_2O), 4.49 - 4.62 (2H, m, $HNCHCO_2$), 5.10 - 5.26 (6H, m, OCH_2Aryl), 5.54 (1H, br.d, J 9 Hz, NH), 6.21 (1H, br.s, NH), 7.02 (1H, br.d, J 7.5 Hz, NH), 7.28 - 7.42 (15H, m, Aryl); m/e (FAB+) 758, 756 (MH^+); Found (%): C, 57.35; H, 5.55; N, 5.28; $C_{36}H_{43}BrN_3O_{10}$ requires: C, 57.07; H, 5.72; N, 5.55. The second isomer to elute provided a colourless cloudy gum. TLC (hexane-ethyl acetate 1.5:1) R_f 0.25; $\nu_{max.}$ ($CHCl_3$) 3425m, 1735s, 1695s, 1495s; δ_H (250 MHz, $CDCl_3$) 0.88, 0.98 (2 x 3H, d, J 7 Hz, $CHMe_2$), 1.97 - 2.35 (3H, m, $CHMe_2$ and CH_2CH_2O), 3.22 (3H, s, CH_3O), 3.63 (1H, d, J 11 Hz, CH_2Br), 4.05 - 4.27 (3H, m, CH_2CH_2O and CH_2Br), 4.51 - 4.64 (2H, m, $HNCHCO_2$), 5.11 - 5.27 (6H, m, OCH_2Aryl), 5.51 (1H, br, NH), 6.27 (1H, br.s, NH), 6.94 (1H, br.d, J 9 Hz, NH) 7.28 - 7.42 (15H, m, Aryl); m/e (FAB+) 758, 756 (MH^+), 726, 724 (MH^+ - CH_3OH).

[(5S)-5-Benzyloxycarbonylamino-5-benzyloxycarbonyl]-2-oxapentanoyl-S-Acetyl-2-methoxy-(2R/S)-cysteinyl-(2R)-valine benzyl ester (24).

Each of the diastereomeric bromides (23) were separately converted into the corresponding thioacetyl compounds (24) following general procedure 3. The diastereomer corresponding to the first isomer of (23) to elute was isolated as a clear colourless gum (35%). TLC (hexane-ethyl acetate 1.5:1) R_f 0.25; $\nu_{max.}$ ($CHCl_3$) 3405m, 1735s, 1695s, 1490s; δ_H (250 MHz, $CDCl_3$) 0.89, 0.93 (2 x 3H, d, J 7 Hz, $CHMe_2$), 2.02 - 2.31 (3H, m, $CHMe_2$ and CH_2CH_2O), 2.28 (3H, s, CH_3COS), 3.17 (3H, s, CH_3O), 3.43 (1H, br.d, J 14 Hz, CH_2S), 3.90 (1H, d, J 14 Hz, CH_2S), 4.08 - 4.20, 4.22 - 4.29 (2 x 1H, m, CH_2CH_2O), 4.47 - 4.61 (2H, m, $HNCHCO_2$), 5.09 - 5.25 (6H, m, OCH_2 Aryl), 5.55 - 5.72 (1H, br, NH), 6.19 (1H, br.s, NH), 6.97 (1H, br.d, J 8.5 Hz, NH), 7.28 - 7.42 (15H, m, Aryl); m/e (FAB^+) 752 (MH^+), 720 (MH^+-MeOH). The second diastereomer corresponding to the second isomer of (23) to elute was also isolated as a clear colourless gum (31%). TLC (hexane-ethyl acetate 1.2:1) R_f 0.4; $\nu_{max.}$ ($CHCl_3$) 3425m, 1735s, 1695s, 1500s; δ_H (250 MHz, $CDCl_3$) 0.89, 0.93 (2 x 3H, d, J 7 Hz, $CHMe_2$), 2.01 - 2.30 (3H, m, $CHMe_2$ and CH_2CH_2O), 2.25 (3H, s, CH_3COS), 3.21 (3H, s, CH_3O), 3.44, 3.72 (2 x 1H, d, J 14 Hz, CH_2S), 4.06 - 4.27 (2H, m, CH_2CH_2O), 4.47 - 4.60 (2H, m, $NHCHCO_2$), 5.10 -5.26 (6H, m, OCH_2Aryl), 5.46 - 5.60 (1H, br, NH), 6.22 (1H, br.s, NH), 7.00 - 7.15 (1H, br, NH), 7.28 - 7.41 (15H, m, Aryl); m/e (FAB^+) 752 (MH^+), 720 (MH^+-MeOH).

N-Benzyloxycarbonyl-S-benzyl-(2R)-cysteinyl-(2R)-valine p-nitrobenzyl ester (29).

To a solution of (2R)-valine-p-nitrobenzyl ester (1.65 g, 6.54 mmol) in dry CH_2Cl_2 (40 mL) was added successively N-benzyloxycarbonyl-S-benzyl-(2R)-cysteine (2.26 g, 6.54 mmol) and EEDQ (1.62 g, 6.54 mmol). The mixture was stirred at room temperature for 1 day under argon, the solvent was evaporated and the residue was dissolved in ethyl acetate (120 mL). This solution was extracted with aqueous 5% $NaHCO_3$ (2 x 30 mL), aqueous 1N HCl (2 x 30 mL) and brine (20 mL). The organic phase was dried and the solvent removed to provide crude product. This material was further purified by precipitating from boiling heptane (80 mL) and enough ethyl acetate to effect dissolution. (29) was isolated as an amorphous white solid (3.20 g, 84%). TLC (hexane-ethyl acetate 1:1) R_f 0.6; mp 134 - 135°C; $[\alpha]_D^{25}$° -5.8° (c 0.05, $CHCl_3$); $\nu_{max.}$ ($CHCl_3$) 3420m, 1740s, 1725s, 1680m, 1610m, 1525s, 1495s; δ_H (250 MHz, $CDCl_3$) 0.86, 0.93 (2 x 3H, d, J 7 Hz, $CHMe_2$), 2.10 - 2.28 (1H, m, $CHMe_2$), 2.74 (1H, dd, J 14, 7 Hz, $CHCH_2S$), 2.92 (1H, dd, J 14, 5.5 Hz, $CHCH_2S$), 3.75 (2H, s, SCH_2Aryl), 4.26 - 4.35 (1H, m, $CHCH_2S$), 4.58 (1H, dd, J 8.5, 5 Hz, HNCHCH), 5.13, 5.24 (2 x 2H, s, OCH_2Aryl), 5.59 (1H, br.d, J 7 Hz, NH), 6.65 (1H, br.d, J 8.5 Hz, NH), 7.22 - 7.39 (10H, m, Bn-Aryl), 7.50, 8.21 (2 x 2H, d, J 8.5 Hz, PNB-Aryl); m/e (FAB^+) 580 (MH^+); Found (%): C, 62.06; H, 5.53; N, 6.95. $C_{30}H_{33}N_3O_7S$ requires: C, 62.16; H, 5.73; N, 7.25.

S-Benzyl-(2R)-cysteinyl-(2R)-valine p-nitrobenzyl ester (30).

A solution of the dipeptide (29) (1.8 g, 3.1 mmol) in CH_2Cl_2 (7.0 mL) was cooled to 10°C and 48% HBr in acetic acid (7.0 mL) was added. After 1 h, the solvents were removed and the viscous dark yellow residue was dissolved in a mixture of CH_2Cl_2 (75 mL) and water (25 mL). 2N aqueous NaOH was added to this mixture until the pH of the aqueous phase indicated ~12.

The organic phase was washed with water (20 mL) and brine (20 mL), then dried and concentrated to afford crude product. This material was flash chromatographed (silica, eluent ethyl acetate) to provide (30) as a pale yellowish gum (1.2 g, 87%). TLC (ethyl acetate) R_f 0.3; $[\alpha]_D^{25}$ -24° (c 0.15, $CHCl_3$); $\nu_{max.}$ ($CHCl_3$) 3365m, 1745s, 1670s, 1608m, 1525s; δ_H (250 MHz, $CDCl_3$) 0.91, 0.96 (2 x 3H, d, J 7 Hz, $CHMe_2$), 2.13 - 2.30 (1H, m, $CHMe_2$), 2.68 (1H, dd, J 14, 8.5 Hz, $CHCH_2S$), 2.94 (1H, dd, J 14, 4 Hz, $CHCH_2S$), 3.48 (1H, dd, J 8.5, 4 Hz, $CHCH_2S$), 3.69 (2H, s, SCH_2Aryl) 4.53 (1H, dd, J 9, 5 Hz, $HNCHCH$), 5.24 (2H, s, OCH_2Aryl), 7.20 - 7.32 (5H, m, Bn-Aryl), 7.50, 8.19 (2 x 2H, d, J 8.5 Hz, PNB-Aryl); m/e (FAB$^+$) 446 (MH^+).

N-p-Nitrobenzyloxycarbonyl-(2S)-homoserine sodium salt (25).

To a solution of (2S)-homoserine (1.0 g, 8.4 mmol) in water (20 mL) and dioxane (28 mL) was added an aqueous solution of 1N NaOH (8.4 mL, 8.4 mmol). To this mixture was added dropwise, simultaneously, a solution of p-nitrobenzyl chloroformate (2.36 g, 10.9 mmol) in dioxane (7 mL) and an aqueous solution of 1N NaOH (11.0 mL, 11.0 mmol). The mixture was stirred at room temperature for 45 min occasionally insuring that the reaction mixture remained basic. Water (80 mL) was added, and the resulting mixture was extracted with CH_2Cl_2 (3 x 25 mL). The aqueous phase was acidified to pH 2 with 1N HCl and extracted with ethyl acetate (2 x 40 mL). The combined organic layers were washed with water (20 mL) and brine (20 mL) and then dried and concentrated to provide N-p-nitrobenzyloxycarbonyl-(2S)-homoserine as a pale yellowish glass (2.3 g, 91%). This unstable material was dissolved in methanol (30 mL) and the solution was cooled to 0°C. A solution of aqueous 1N NaOH (7.5 mL, 7.5 mmol) was added dropwise. The mixture was diluted with water (80 mL) and extracted with ethyl acetate (2 x 25 mL). The aqueous phase was freeze dried and the resulting pale yellow solid (25) was dried over P_2O_5 (2.33 g). δ_H (300 MHz, D_2O) 1.79 - 1.93 (1H, m, CH_2CH_2OH), 2.01 - 2.12 (1H, m, CH_2CH_2OH), 3.63 - 3.74 (2H, m, CH_2CH_2OH), 4.04 (1H, dd, J 9.5, 4.5 Hz, $HNCHCO_2$), 5.20 and 5.26 (2H, ABq, J 14 Hz, OCH_2Aryl), 7.58, 8.23 (2 x 2H, d, J 8.5 Hz, Aryl); m/e (FAB$^+$) 343 (MNa^+).

N-p-Nitrobenzyloxycarbonyl-(2S)-homoserine p-nitrobenzyl ester (26).

To a solution of N-p-nitrobenzyloxycarbonyl-(2S)-homoserine sodium salt (25) (2.33 g, 7.27 mmol) in dry DMF (18 mL) was quickly added p-nitrobenzyl bromide (2.50 g, 11.6 mmol, recrystallized from heptane) and dry sodium iodide (360 mg). This mixture was immediately immersed in a 65°C oil bath and stirred for 10 min. under argon. The mixture was stirred for an additional 1.5 h at room temperature and then poured into ethyl acetate (150 mL) and water (50 mL). The organic layer was washed with water (30 mL), 5% aqueous $Na_2S_2O_3$ (30 mL), and brine (30 mL) and then dried and concentrated to afford a yellow viscous residue containing the desired product (26) along with N-p-nitrobenzyloxycarbonyl-(2S)-homoserine lactone (27), p-nitrobenzyl alcohol and excess p-nitrobenzyl bromide. This mixture was quickly flash chromatographed (silica, eluent CH_2Cl_2-ethyl acetate 1:1) to provide a purer sample of (26) as a white solid (2.15 g). This material still contained some lactone (27) and p-nitrobenzyl alcohol. Further purification was difficult and not practical yield wise as (26) has a strong tendency to lactonize. The lactone (27) could be efficiently recrystallized from methanol and recycled to the sodium salt (25) by treatment with 1 equivalent of NaOH in methanol. Compound (26): TLC (CH_2Cl_2-ethyl acetate 1:1) R_f 0.3; δ_H (250 MHz, $CDCl_3$) 1.79 -1.93, 2.14-2.28 (2 x 1H, m, CH_2CH_2OH), 2.41 (1H, dd, J 6.5, 5 Hz, OH, disappears on D_2O exchange), 3.62 - 3.84 (2H, m, CH_2CH_2OH), 4.64 (1H, ddd, J 7.5, 7.5, 5 Hz, $HNCHCO_2$), 5.23, 5.30 (2 x 2H, s, OCH_2Aryl), 5.87 (1H, br.d, J 7.5 Hz, NH), 7.51, 7.53, 8.22, 8.24 (4 x 2H, d, J 8.5 Hz, Aryl). Lactone (27):TLC (CH_2Cl_2-ethylacetate 1:1) R_f 0.4; mp 149 - 151°C; $[\alpha]_D^{25}$ -6.3° (c 0.053, $CHCl_3$); $\nu_{max.}$ ($CHCl_3$) 3430m, 1785s, 1610m 1525s; δ_H (300 MHz, $CDCl_3$) 2.25 (1H, dddd, J 11.5, 11.5, 11.5, 9 Hz, CH_2CH_2O), 2.76 - 2.87 (1H, m, CH_2CH_2O), 4.28 (1H, ddd, J 11.5, 9.5, 5.5 Hz, CH_2CH_2O), 4.34 - 4.38 (2H, m, CH_2CH_2O and $HNCHCO_2$), 5.24 (2H, s, OCH_2Aryl), 5.43 (1H, br, NH), 7.53, 8.23 (2 x 2H, d, J 8.5 Hz, Aryl); m/e (NH_3CI) 298 (MNH_4^+), 281 (MH^+); Found (%): C, 51.23; H, 4.25; N, 9.69. $C_{12}H_{12}N_2O_6$ requires C, 51.43; H, 4.67; N, 9.99.

N-p-Nitrobenzyloxycarbonyl-O-carbonylimidazole-(2S)-homoserine p-nitrobenzyl ester (28).

To a solution of the impure alcohol (26) (2.05 g) from the previous reaction in dry CH_2Cl_2 (60 mL), was added carbonyldiimidazole (1.1 g, 6.8 mmol) in one portion. The mixture was stirred at room temperature under argon for 30 min. The solvent was removed and the pale yellow viscous residue was flash chromatographed (silica, eluent CH_2Cl_2-ethyl acetate 1.5:1). Early fractions contained the lactone (27) and N-p-nitrobenzyloxycarbonylimidazole while later fractions provided pure (28) as a pale yellow foam (2.3 g, 63% from sodium salt (25)). TLC (CH_2Cl_2-ethyl acetate 1.5:1) R_f 0.3; $[\alpha]_D^{25}$ 10.6° (c 0.15 $CHCl_3$); $\nu_{max.}$ 3425m, 1765s, 1730s, 1610m, 1530s; δ_H (250 MHz, $CDCl_3$) 2.19 - 2.34, 2.40 - 2.54 (2 x 1H, m, CH_2CH_2O), 4.54 (2H, t, J 6 Hz, CH_2CH_2O), 4.59 - 4.69 (1H, m, $HNCHCO_2$), 5.15 - 5.31 (4H, m, OCH_2Aryl), 5.72 (1H, br.d, J 8 Hz, NH) 7.05 (1H, dd, J 1.5, 1 Hz, Imid H), 7.37, 8.08 (2 x 1H, br.s, Imid H), 7.47, 7.49, 8.19, 8.21 (4 x 2H, d, J 8.5 Hz, Aryl); m/e (FAB$^+$) 528 (MH^+); Found (%): C, 52.02; H, 4.13; N, 12.75. $C_{23}H_{21}N_5O_{10}$ requires 52.38; H, 4.01; N, 13.28.

[(5S)-5-p-Nitrobenzyloxycarbonyl-5-p-nitrobenzyloxycarbonylamino]-2-oxapentanoyl-S-benzyl-(2R)-cysteinyl-(2R)-valine p-nitrobenzyl ester (31).

To a solution of the imidazolide (28) (630 mg, 1.19 mmol) and the amine (30) (534 mg, 1.19 mmol) in dry acetonitrile (1.5 mL) was added dimethylaminopyridine (146 mg, 1.19 mmol). The mixture was stirred at 40-45°C under argon for 2 days. The solvent was pumped off and the residue was flash chromatographed (silica, eluent CH_2Cl_2-ethyl acetate 5:1). Early fractions provided the cyclic urethane (32) as white solid (120 mg) while later fractions provided the

desired product (31) as a glassy pale yellowish solid (760 mg, 70%). Product (31): TLC (CH_2Cl_2-ethyl acetate 5:1) R_f 0.35; mp 147 - 149°C; $[\alpha]_D^{\beta}$° 1.2° (c 0.072, $CHCl_3$); $\nu_{max.}$ ($CHCl_3$) 3425m, 1730s, 1680s, 1610m, 1525s; δ_H (300 MHz, $CDCl_3$) 0.88, 0.94, (2 x 3H, d, J 7 Hz, $CHMe_2$), 2.10 - 2.30 (3H, m, $CHMe_2$ and CH_2CH_2O), 2.69 (1H, dd, J 14, 7.5 Hz, $CHCH_2S$), 2.81 - 2.92 (1H, br.m, $CHCH_2S$), 3.76 (2H, s, SCH_2Aryl), 4.15 - 4.29 (2H, m, HNCH), 4.53 - 4.59 (1H, m, HNCH), 5.20, 5.24, 5.28 (3 x 2H, s, OCH_2Aryl), 5.48, 5.69, 6.63 (3 x 1H, br, NH), 7.20 - 7.49 (5H, m, Bn-Aryl), 7.48 - 7.52, 8.18 - 8.22 (2 x 6H, m, PNB-Aryl); m/e (FAB^+) 905 (MH^+); Found (%): C, 55.86; H, 4.75; N, 8.94. $C_{42}H_{44}N_6O_{15}S$ requires: C, 55.75; H, 4.90; N, 9.29. Cyclic urethane (32): TLC (CH_2Cl_2-ethyl acetate 5:1) R_f 0.7; mp 143-145°C; $\nu_{max.}$ ($CHCl_3$) 1750s, 1740s, 1610m, 1525s; δ_H (250 MHz, $CDCl_3$) 2.11 - 2.43 (2H, m, CH_2CH_2O), 4.21 - 4.48 (2H, m, CH_2CH_2O), 4.55 - 4.65 (1H, m, $HNCHCO_2$), 5.20 - 5.28 (4H, m, OCH_2Aryl), 5.56 (1H, br.d, J 8 Hz, NH), 7.50, 7.52, 8.20, 8.22 (4 x 2H, d, J 8.5 Hz Aryl-H); m/e (FAB^+) 560 (MH^+).

[(5S)-5-p-Nitrobenzyloxycarbonylamino-5-p-nitrobenzyloxycarbonyl]-2-oxapentanoyl didehydroalaninyl-(2R)-valine p-nitrobenzyl ester (33).

General procedure 1 was followed to provide (33) as a pale yellowish foam (84%). TLC (CH_2Cl_2-ethyl acetate 5:1) R_f 0.45; $[\alpha]_D^{\beta}$° -14° (c 0.043, $CHCl_3$); $\nu_{max.}$ ($CHCl_3$) 3435m, 3390m, 1735s, 1670m, 1635m, 1605m, 1525s, 1500s; δ_H (300 MHz, $CDCl_3$) 0.93, 0.97 (2 x 3H, d, J 7 Hz, $CHMe_2$), 2.14 - 2.34 (3H, m, $CHMe_2$ and CH_2CH_2O), 4.22 - 4.29 (2H, m, CH_2CH_2O) 4.53 -4.63 (1H, m, $HNCHCH_2$), 4.63 (1H, dd, J 8.5, 5.5 Hz, HNCHCH), 5.18 - 5.30 (7H, m, OCH_2Aryl and $C{=}CH_2$), 5.59 (1H, br.d, J 8 Hz, $HNCHCH_2$), 6.07 (1H, d, J 2 Hz, $C{=}CH_2$), 6.54 (1H, d, J 8.5 Hz, HNCHCH), 7.34 (1H, s, NH), 7.49 - 7.56, 8.19 - 8.27 (2 x 6H, m, Aryl), δ_C (50 MHz, $CDCl_3$) 17.7, 18.9 (2 x CH_3), 31.0, 31.1 (CH_2CH_2O and $CHMe_2$), 51.4, 57.8 (2 x NCH), 61.0 (CH_2CH_2O), 65.4, 65.6, 65.8 (3 x OCH_2Aryl), 99.6 ($C{=}CH_2$), 123.8, 123.9, 128.1, 128.5, 128.7 (5 x ArylCH), 134.0 ($C{=}CH_2$), 142.5, 143.8, 147.6, 147.9 (4 x Aryl C), 153.1, 155.8 (2 x NCO_2), 163.9, 171.6, 171.8 (NCO and 2 x CO); m/e (FAB+) 780 (MH^+); Found (%): C, 54.04; H, 4.61; N, 10.76. $C_{35}H_{36}N_6O_{15}$ requires: C, 53.85; H, 4.61, N, 10.46%.

[(5S)-5-p-Nitrobenzyloxycarbonylamino-5-p-nitrobenzyloxycarbonyl]-2-oxapentanoyl-3-bromo-2-methoxy-(2R/S)-alaninyl-(2R)-valine p-nitrobenzyl ester (34).

General procedure 2 was followed to provide (34) as a pale yellowish foam (91%). TLC (CH_2Cl_2-ethyl acetate 5:1) R_f 0.3; $\nu_{max.}$ ($CHCl_3$) 3420m, 1740s, 1695m, 1610m, 1525s; δ_H (250 MHz, $CDCl_3$) 1:1 mixture of diastereomers with characteristic signals at 3.26 and 3.30 (2 x 3H, s, CH_3O); 6.18 and 6.25 (2 x 1H, br.s, NH); all other signals were overlapping multiplets; m/e (FAB+) 893, 891 (MH^+), 861, 859 (MH^+-MeOH).

[(5S)-5-p-Nitrobenzyloxycarbonylamino-5-p-nitrobenzyloxycarbonyl]-2-oxapentanoyl-3-iodo-2-methoxy-(2R/S)-alaninyl-(2R)-valine p-nitrobenzyl ester (35).

To a solution of the didehydroalaninyl peptide (33) (350 mg, 0.45 mmol) in dry CH_2Cl_2 (1.5 mL) and dry methanol (9.0 mL) was added a very quickly prepared solution of N-iodosuccinimide (106 mg, 0.47 mmol) in dry methanol (1.5 mL). The mixture was stirred at room temperature for 20 min under argon and then poured into ethyl acetate (120 mL). Extraction with water (30 mL), 5% aqueous $Na_2S_2O_3$ (30 mL) and brine (30 mL) followed by drying and concentrating afforded crude (35) as a yellow viscous gum. This material was flash chromatographed (silica, eluent CH_2Cl_2-ethyl acetate 4:1) to provide pure (35) as a pale yellowish foam (365 mg, 87%). TLC (CH_2Cl_2-ethyl acetate 4:1) R_f 0.45; $\nu_{max.}$ 3425m, 1740s, 1695m, 1610m, 1525s; δ_H (300 MHz, $CDCl_3$) 1:1 mixture of diastereomers with characteristic signals at 3.26 and 3.30 (2 x 3H, s, CH_3O), 6.10 and 6.18 (2 x 1H, br.s, NH); all other signals were overlapping multiplets; m/e (FAB^+) 939 (MH^+), 907; Found (%): C, 46.07; H, 4.19; N, 8.95. $C_{36}H_{39}IN_6O_{16}$ requires C, 45.93; H, 4.15; N, 8.52.

[(5S)-5-p-Nitrobenzyloxycarbonylamino-5-p-nitrobenzyloxycarbonyl]-2-oxapentanoyl-S-acetyl-2-methoxy-(2R/S)-cysteinyl-D-valine-p-nitrobenzyl ester (36).

General procedure 3 was followed to provide after chromatography (silica, eluent CH_2Cl_2-ethyl acetate 4:1), the diastereomeric mixture (36) as a yellowish foam (38%). The two diastereomers were separated by HPLC (D-3,5-dinitrobenzoylphenylglycine bonded aminosilica semi-prep Pirkle column, eluent benzene-ethyl acetate 7:1). The first diastereomer to elute provided (36a) as a colourless foam (17.5%). TLC (CH_2Cl_2-ethyl acetate 4:1) R_f 0.25; $[\alpha]_D^{\beta}$° -7.1° (c 0.066, $CHCl_3$); $\nu_{max.}$ ($CHCl_3$) 3425m, 1740s, 1700s, 1610m, 1525s; δ_H (250 MHz, $CDCl_3$) 0.92, 0.97 (2 x 3H, d, J 7 Hz, $CHMe_2$), 2.27 (3H, s, CH_3COS), 2.07 - 2.34 (3H, m, $CHMe_2$ and CH_2CH_2O), 3.25 (3H, s, CH_3O), 3.42, 3.72 (2 x 1H, d, J 14.5 Hz, CH_2S), 4.11 - 4.29 (2H, m, CH_2CH_2O), 4.50 (1H, dd, J 8.5, 5 Hz, HNCHCH), 4.52 - 4.63 (1H, m, $HNCHCH_2$), 5.21 (2H, s, OCH_2Aryl), 5.30 (4H, s, OCH_2Aryl) 5.63 - 5.71 (1H, br, NH), 6.25 (1H, br.s, NH), 7.04 - 7.10 (1H, br, NH), 7.50 - 7.58, 8.20 - 8.27 (2 x 6H, m, Aryl); δ_C (125 MHz, $CDCl_3$) 17.8, 19.0 (2 x $CHMe_2$), 30.2 (CH_3COS), 31.2 ($CHMe_2$ and CH_2CH_2O), 33.8 (CH_2S), 51.3 (CH_3O), 51.6 (HNCH) 58.0 (HNCH), 61.2 (CH_2CH_2O), 65.5, 65.6, 65.8 (3 x OCH_2Aryl), 86.8 (CH_3OC), 123.8, 123.9, 128.1, 128.5, 128.6 (5 x ArylCH), 142.2, 142.4, 143.5, 147.7, 147.8 147.9 (6 x Aryl C), 153.5, 155.5 (2 x $HNCO_2$), 168.2, 170.8, 171.3 ($HNCO$, 2 x CO_2), 194.8 (SCO); m/e (FAB^+) 887 (MH^+), 885 (MH^+-MeOH). The second diastereomer to elute provided (36b) as a colourless foam (15.5%). TLC (CH_2Cl_2-ethyl acetate 4:1) R_f 0.25; $[\alpha]_D^{\beta}$° 11° (c 0.066, $CHCl_3$); $\nu_{max.}$ ($CHCl_3$) 3405m, 1740s, 1695s, 1610m, 1525s; δ_H (250 MHz, $CDCl_3$) 0.92, 0.95 (2 x 3H, d, J 7 Hz, $CHMe_2$),

2.12 - 2.33 (3H, m, $CHMe_2$ and CH_2CH_2O), 2.33 (3H, s, CH_3COS), 3.21 (3H, s, CH_3O), 3.42 (1H, br.d, J 14 Hz, CH_2S), 3.87 (1H, d, J 14 Hz, CH_2S), 4.07 - 4.18, 4.22 - 4.34 (2 x 1H, m, CH_2CH_2O), 4.50 (1H, dd, J 8.5, 5 Hz, HNCHCH) 4.52 - 4.62 (1H, m, $HNCHCH_2$), 5.21 - 5.34 (6H, m, OCH_2Aryl), 5.79 - 5.83 (1H, br, NH), 6.15 (1H, br.s, NH), 6.96 - 7.00 (1H, br.d, J 8.5 Hz, NH), 7.49 - 7.56, 8.19 - 8.26 (2 x 6H, m, Aryl); δ_C (125 MHz, $CDCl_3$) 17.8, 19.0 (2 x $CHMe_2$), 30.2 (CH_3COS), 31.0 ($CHMe_2$), 31.2 (CH_2CH_2O), 33.8 (CH_2S), 51.2 (CH_3O), 51.5, 57.8 (2 x HNCH), 61.0 (CH_2CH_2O), 65.6, 65.8 (2 x OCH_2Aryl), 86.9 (CH_3OC), 123.7, 123.8, 123.9, 128.0, 128.5, 128.8 (6 x ArylCH), 142.3, 143.5, 147.7, 147.9 (4 x ArylC), 153.4, 155.5 (2 x $HNCO_2$), 168.7, 170.9, 171.4 (HNCO and 2 x CO_2), 194.2 (SCO); m/e (FAB^+) 887 (MH^+), 855 (MH^+-MeOH).

[(5S)-5-Amino-5-carboxy-2-oxapentanoyl]-S-acetyl-2-methoxy-(2R)-cysteinyl-(2R)-valine (37a).

Palladium on charcoal (10%, 50 mg) was prehydrogenated and a solution of the protected peptide (36a) (15 mg, 0.017 mmol) in THF (0.7 mL) was added followed by water (0.7 mL). The mixture was stirred vigorously under an atmosphere of H_2 for 16 h. The reaction mixture was filtered through a small column of celite (2 x 0.5 cm), rinsing with additional THF-water 1:1. The eluent (3mL) was diluted with water (5 mL), extracted with ethyl acetate (2 x 3 mL) and the aqueous layer was concentrated to provide crude (37a). This material was subjected to reverse phase HPLC (ODS semiprep. column, eluent 10 mM aqueous NH_4HCO_3-methanol 7:1) to provide pure (37a) (retention time 6 min, flow 4 ml/min). δ_H (500 MHz, D_2O) 0.91, 0.94 (2 x 3H, d, J 7 Hz, $CHMe_2$), 2.00 - 2.10 (1H, m, $CHMe_2$), 2.17 - 2.22, 2.24 - 2.32 (2 x 1H, m, CH_2CH_2O), 2.35 (3H, s, CH_3COS), 3.30 (3H, s, CH_3O), 3.40, 3.55 (2 x 1H, d, J 14.5 Hz, CH_2S), 3.81 - 3.84 (1H, m, $HNCHCH_2$), 3.99 (1H, d, J 5.5 Hz, HNCHCH), 4.22 (2H, t, J 5.5 Hz, CH_2CH_2O); m/e (FAB+) 438 (MH^+), 406 (MH^+ - MeOH).

[(5S)-5-Amino-5-carboxy-2-oxapentanoyl]-S-acetyl-2-methoxy-(2S)-cysteinyl-(2R)-valine (37b).

The same procedure as for (36a) was followed. The crude product was purified by HPLC (ODS semiprep. column, eluent 10mM aqueous NH_4HCO_3-methanol 7:3) to provide pure (37b) (retention time 8.5 min, flow 4 mL/min). δ_H (500 MHz, D_2O) 0.93, 0.95 (2 x 3H, d, J 7 Hz, $CHMe_2$), 2.06 - 2.29 (3H, m, $CHMe_2$ and CH_2CH_2O), 2.40 (3H, s, CH_3COS), 3.28 - 3.40 (3H, br, CH_3O), 3.54, 3.61 (2 x 1H, J 14 Hz, CH_2S), 3.76 - 3.82 (1H, br, $HNCHCH_2$), 4.06 (1H, d, J 5.5 Hz, NHCHCH), 4.14 -4.24 (2H, br, CH_2CH_2O); m/e (FAB^+) 438 (MH^+), 406 (MH^+-MeOH).

[(5S)-5-Amino-5-carboxy-2-oxapentanoyl]-2-methoxy-(2R)-cysteinyl-(2R)-valine disulfide (38a).

The pure sample of the thioacetyl peptide (37a) was dissolved in 1N aqueous NH_3 (2 mL) and O_2 was bubbled through this solution for 1.5 h. The mixture was freeze dried to provide (38a) (3.3 mg, TSP calibrated, 51%) contaminated with a trace of acetamide. δ_H (500 MHz, D_2O) 0.94, 0.96 (2 x 3H, d, J 7 Hz, $CHMe_2$), 2.03 - 2.13 (1H, m, $CHMe_2$), 2.17 - 2.23, 2.28 - 2.34 (2 x 1H, m, CH_2CH_2O), 3.19 (1H, d, J 14.5 Hz, CH_2S), 3.32 (3H, s, CH_3O), 3.38 (1H, d, J 14.5 Hz, CH_2S), 3.84 - 3.87 (1H, m, $HNCHCH_2$), 4.08 (1H, d, J 5.5 Hz, HNCHCH), 4.19 - 4.24 (2H, m, CH_2CH_2O); m/e (FAB^+) 789 (MH^+).

[(5S)-5-Amino-5-carboxy-2-oxapentanoyl]-2-methoxy-(2S)-cysteinyl-(2R)-valine disulfide (38b).

The same procedure as for (37a) was followed to give (3.6 mg, TSP calibrated, 54%). The disulfide (38b) could be further purified by reverse phase HPLC (ODS semiprep. column, eluent 10 mM aqueous NH_4HCO_3-methanol 5:3, retention time 9.5 min, flow 4 mL/min.), 2.8 mg. δ_H (500 MHz, D_2O) 0.96, 0.98 (2 x 3H, d, J 7 Hz, $CHMe_2$), 2.12 - 2.33 (3H, m, $CHMe_2$ and CH_2CH_2O), 3.30 - 3.47 (4H, br, CH_3O and CH_2S), 3.57 (1H, d, J 15.5 Hz, CH_2S), 3.82 - 3.91 (1H, br, $HNCHCH_2$), 4.10 (1H, d, J 5.0 Hz, HNCHCH), 4.12 -4.26 (2H, br, CH_2CH_2O); m/e (FAB^+) 789 (MH^+).

[(5S)-5-Amino-5-carboxy-2-oxapentanoyl]-(2R)-cysteinyl-(2R)-valine disulfide (14a).

A solution of the protected peptide (21) (72 mg, 0.094 mmol) in dry THF (2 mL) under an argon atmosphere was cooled to -78°C and liquid ammonia (50 mL, distilled from sodium metal) was introduced. The cold bath was removed and very small pieces of sodium metal were introduced until the dark blue color persisted for 2 min. The excess sodium was consumed by the addition of a few drops of a solution of bromobenzene in THF (10%). The solvents were evaporated to dryness under a stream of argon and the yellow residue was partitioned between water (10 mL) and ethyl acetate (5 mL). The aqueous phase was washed with additional ethyl acetate (2 x 3 mL) and then freeze dried to provide a yellowish solid, crude thiol (14). This material was dissolved in 0.5 N aqueous NH_3 (4 mL) and oxygen was bubbled through this solution for 1.5 h. Freeze drying this mixture provided crude (14a) which was further purified by reverse phase HPLC (ODS semiprep column, eluent 10 mM aqueous NH_4HCO_3-methanol 2:1, retention time 9 min., flow 4 mL/min.). This provided pure (14a) as a white solid (25 mg,TSP calibration, 73%). δ_H (500 MHz, D_2O) 0.89, 0.93 (2 x 3H, d, J 7 Hz, $CHMe_2$), 2.09 - 2.37 (3H, m, $CHMe_2$ and CH_2CH_2O), 3.04 (1H, dd, J 14, 8.5 Hz, CH_2S), 3.23 (1H, br.dd, J 14, 5.5 Hz, CH_2S), 3.75 - 3.95 (1H, m, $HNCHCH_2$), 4.10 (1H, d, J 6 Hz, HNCHCH), 4.27 (2H, t, J 6 Hz, CH_2CH_2O), 4.56 (1H, dd, J 8.5, 5.5 Hz, $CHCH_2S$); m/e (FAB^+) 729 (MH^+).

10-Oxoisopenicillin N (15).

To an IPNS enzyme preparation[20] in 50 mM aqueous NH_4HCO_3 (3.4 mL, 42 I.U., 66 mg of protein) was added aqueous solutions of L-ascorbic acid (0.2 mL, 50 mM), DTT (0.2 mL, 100 mM) and $FeSO_4$ (0.2 mL, 5 mM). This mixture was added to the disulfide (14a) (3.8 mg, 5.2 μmol). The resulting solution was divided into two 2 mL pots and the pots were shaken at 270 cpm at 27°C for 1 h. After 25 min, additional DTT solution (40 μL, 100 mM) was added. The protein

was precipitated by the addition of acetone (10 mL). After centrifugation (15000 rpm), the supernatant was concentrated to provide the crude penicillin (17) (1.6 mg by NMR calibration of the β-lactam hydrogens at δ 5.41 and δ 5.57 against a known quantity of TSP, 41%). The crude product was purified by reverse phase HPLC (ODS analytical column, eluent 10 mM aqueous NH_4HCO_3, retention time 10 min, flow 1.5 mL/ min). The penicillin (15) was susceptible to ammonolysis of the β-lactam ring and was isolated after HPLC (0.71 mg) contaminated with trace amounts of the β-lactam opened compound. δ_H (500 MHz, D_2O) 1.53, 1.64 (2 x 3H, s, CMe_2), 2.13 - 2.32 (2H, m, CH_2CH_2O), 3.81 - 3.85 (1H, m, $HNCHCH_2$), 4.24 (1H, s, $CHCMe_2$), 4.27 (2H, t, J 5.5 Hz, CH_2CH_2O), 5.41 (1H, d, J 4 Hz, β-lactam ring H), 5.57 (1H, d, J 4 Hz, β-lactam ring H); m/e (FAB$^+$) 362 (MH^+). (15) was not active against Staphylococcus aureus N.C.T.C. 6571 at a concentration of 50μg/100μL * (100 μL sample).

6α-Methoxy-10-oxoisopenicillin N (17).

The disulfide (38b) (1.3 mg, 3.3 μmol) was incubated under identical conditions as was the disulfide (14a) to provide crude methoxy penicillin (17) (0.66 mg by NMR calibration of the β-lactam hydrogen at δ 5.55 with a known quantity of TSP, 51%). This material was purified by HPLC (ODS analytical column, eluent 25 mM aqueous NH_4HCO_3, retention time 8.5 min, flow 1 mL/min) two times to provide pure methoxy penicillin (17) (0.36 mg). δ_H (500 MHz, D_2O) 1.51, 1.56 (2 x 3H, s, CMe_2), 2.13 - 2.20, 2.24 - 2.36 (2 x 1H, m, CH_2CH_2O), 3.55 (3H, s, CH_3O), 3.81 - 3.85 (1H, m, $HNCHCH_2$), 4.24 - 4.31 (2H, m, CH_2CH_2O), 4.31 (1H, s, $CHCMe_2$), 5.55 (1H, s, β-lactam H); N.o.e. experiments performed on the penicillin (17) were consistent with such stereochemical assignments. Thus irradiation of OMe gave n.O.e (1%) to 5-H but none to 2-H. M/e (FAB$^+$) 392 (MH^+), 360 (MH^+ - MeOH). (17) was not active against Staphylococcus aureus N.C.T.C. 6571 at a concentration of 150 μg/100μL* (100μL sample).

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